EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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:** Structure-function studies of isocitrate dehydrogenase, receptor for activated C-kinase (RACK1), alcaline phosphatase (TAB5) and lactate oxidase	**Experiment number**: MX372
Beamline:	**Date of experiment**: from: 26.11.04 to: 27.11.04	**Date of report**: 15.02.05 *Received at ESRF:*
Shifts:	**Local contact(s)**: Stéphanie Monaco	

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Report:

Background

The protein crystallography laboratory at the University of Tromsø has been regular user of 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.

RESULTS

Biological adaptation to extreme temperatures

Icocitrate dehydrogenase from *Pyrococcus furiosu*s (IDH1) and *Desulfotalea psychrophila* (IDH6) can be crystallized from 0.4 M NH_4HPO_4 and 50 mM $MgSO_4$ (IDH1) and 1.7M ammonium sulfate, 0.1M Tris pH7.4 and 0.1 M Mg_2SO_4 (IDH6). Both proteins give plate like crystals up to 0.4 x 0.5 x 0.05 mm^3.

Data were collected on native IDH1, native IDH6, IDH1 with NAD cofactor and IDH6 with isocitrate substrate.
The best native IDH1 diffracted to 2.5 Å and belonged to space group C2 with cell parameters of 99.4 x 58.0 x 79.6 mm^3, β=113.2. R_{sym} was 8%, completeness was 95.2% and $I/\sigma I$ was 6.4. The structure has been solved by molecular replacement to an R-factor of 29.4% (R-free=34%). The protein is now subjected to structural analysis.
IDH1 crystals were also soaked with NAD and data was collected. The crystals diffracted up to 2.7 Å. The data could be indexed to a space group isomorphous to the native crystals, but could not be processed.
Native IDH6 diffracted to 1.75Å, and belong to the triclinic space group with cell parameters of 59.34 x 73.28 x 126.41, α=98.90, β=98.98, γ=113.876. R_{sym} was 4.7%, $I/\sigma I$ was 9.9 and completeness 86.3%. The structure has been solved with molecular replacement, identifying three molecules in the asymmetric unit. Model building and refinement is in progress.
IDH6 was cocrystallized with substrate (isocitrate). The crystal morphology changed, and the crystals diffracted to 2.4 Å. IDH6 with isocitrate belong to space group C2221 with cell parameters of 58.49 x 121.29 x 272.25 mm^3. We believe the structure is solved by molecular replacement, identifying two molecules in the asymmetric unit. Further analysis of this structure is in progress.

Structure studies of Receptor for Activated C-Kinase (RACK 1)

The crystals of this protein did not diffract.

Crystallographic studies of the Antarctic alkaline phosphatase TAB5

Several crystals were tried and one crystal diffracting to 2.0 Å was found. SAD data was colledted close to the Zn edge. Space group is P222 with cell parameters of 55.88 x 174.30 x 70.14 mm^3. R_{sym} was 16.5%, $I/\sigma I$ was 4.3, completeness and anomalous completeness were 99.9% and multiplicity was 15. Attempts to phase the protein is in progress.

Crystallographic studies of lactate oxidase from Aerococcus viridans

No crystals of sufficient diffracting quality were found.