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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:** Structure studies of MopE, AfalkA, Rac proteins, Dnase and Spektrin	**Experiment number**: MX419
Beamline:	**Date of experiment**: from: 02.05.05 to: 03.05.05	**Date of report**: 14.06.05
Shifts:	**Local contact(s)**: Andrew McCarthy	*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.

RESULTS

Structure studies of proteins involved in copper homeostasis
Copper is an essential micronutrient for all living organisms but is toxic at elevated levels. It is therefore important for the cell to keep a tight control on the level of copper ions. MopE is a protein that is involved in this process. To our knowledge, MopE has no homologous protein with known structure. Native crystals of MopE have previously been grown, and data have been collected to ca 2.2 Å. Copper binds to the protein, but the anomalous signal was too weak to phase the structure. New crytals were grown for this experiment where MopE was co-crystallized with millimolar concentrations of various heavy atom derivatives (Au, Pt, Pd, Yb and Hg). All heavy atoms give a different morphology of the crystal, and this led us to belive that the metals bind to the protein.
The crystals of the Au and Hg derivatives were of suitable diffraction quality, and 2 MAD datasets were collected on the Au derivatives, and one MAD dataset on the Hg derivative. All crystals diffracted to ca 2.0 Å. Both crystal forms belonged to the C2 space group, but the cell parameters were different. The Au derivative had cell parameters of 112 x 101 x 44 $Å^3$, β=109, and the Hg derivative had cell parameters of 65 x 101 x 54 $Å^3$, β=97. The Au derivatives had overall Rmerge values of ca 8%, I/σI of ca 4, 100% completeness and 99% anomalous completeness. A weak anomalous signal was present in one of the crystals, but pasing has not been successful yet. Indexing of the Hg derivative was difficult, and refinement of the cell turned out to be unstable. The Hg data have therefore not been possible to process.

Structure-function-relation studies of methylpurine DNA glycosylase (AfalkA) from Archaeoglobus fulgidus
AfalkA is a DNA glycosylase from the hyperthermophilic archaebacterium *Archaeoglobus fulgidus*. AfalkA is involved in the repair of DNA and removes alkylated bases from DNA. The enzyme is a homologue to *Escherichia coli* 3-methyladenine DNA glycosylase II. Data was collected to 1.9 Å on allocated beam time, but the structure could not be solved by molecular replacement. The structure has later been solved by SAD on a Hg derivative in a collaborative effort involving members of the MX-Group at and in-house-research time at the ESRF. The crystals belonged to space group P21, with cell parameters of 69.49 x 49.96 x 105.79 $Å^3$, β=107.43. SAD data to 1.8 Å was collected on the peak, and 400 degrees resulted in a multiplicity of ca 8, R-merge of 8.4% and 99% completeness. The structure was solved by SHELXD, SHARP, DM and ARP/wARP. Data was further collected on the native structure. The native crystals diffracted to 1.75 Å and the cell parameters were approximately the same as the derivative. R-merge was 4.5% and completeness was ca 94%. Refinement of the structure is in progress and present R-factor is 20.6% and R-free 23.6%

Structure study of RAC GTPases from Arabidopsis thaliana
GTP-ases generally acts as “molecular switches” in cellular processes in eukaryotic organisms. The function of RAC GTP-ase in *Arabidopsis thaliana* is not known, but it is expected to be involved in the defence of plants. Diffraction has previously been observed to 2.5 Å, but this time none of the crystals were of diffraction quality.

Structure studies of Dnase from shrimp
DNnase from shrimp has a strong preference for the hydrolysis of double stranded DNA and a very high specific activity. MAD data have previously been collected on a potential W-derivative, but no anomalous signal was found. No new derivatives have been obtained, and no crystals were tested.

Structure study of spectrin
Spectrin is a major component of the cytoskeleton in multicellular eukaryotes, and it associates with actin filaments and forms cross-links within the network of cortical cytoskeleton that is thought to confer strength and flexibility to the cells.
The small crystals diffracted to ca 3 Å. The cell could not be indexed due to icing. Unfortunately the crystal did not survive cryo annealing.

Other:

Biological adaptation to extreme temperatures: see also MX-372

Isocitrate dehydrogenase (IDH) from various species is used as a model system to study how organisms, on the molecular, have adapted to extreme environmental conditions. Data has been collected previously on IDH from two species, and crystals of IDH on two more specied has recently been obtained. One of the crystals diffracted to 2.6 Å. The crystals belonged to space group P21 with cell parameters of ca 56.10 x 106.86 x 154.21, β=93.62. R-merge was ca 8% and the data was 97% complete. The strucrure has been solved by molecular replacement identifying two molecules in the asymmetric unit. Refinement of the structure is in progress.

Structure-function studies of a nuclease from the cold adapted Vibrio salmonicida: see also MX-235 (grouped allocation MX-233 – 235 and MX-253)

The nuclease from the cold adapted *Vibrio salmonicida* is used as a model system to study how enzymes have adapted to cold environments in addition to being the target of a study on DNA/RNA hydrolyzing enzymes.

Crystals of the nuclease was obtained from 0.1 M Tris pH 8.25, 30% PEG 3350, 0.2 M Na-acetate. The crystals diffracted to 1.2 Å and belong to space group P21 with cell parameters of 42.08 x 44.95 x 51.74, β=92.65. A complete data set was collected 1.5 Å, and 75% to 1.2 Å. Limitations in the detector – crystal distance is the reason for the lower completeness in the high resolution shells. R-merge was 4.8%. The structure could be solved by molecular replacement using a homologous structure from *Vibrio vulnificus* as search model. Rebuilding of the structure and refinement is in progress.