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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-function studies of a highly active, highly selective and thermolabile nuclease from shrimp	**Experiment number**: MX-1063
Beamline:	**Date of experiment**: from: 10.12.09, 17.00 to: 11.12.09, 01.00	**Date of report**: 13.04.10
Shifts:	**Local contact(s)**: Dr. Mats Ökvist	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

This experiment was run together with experiments MX-1060 and MX-1065

Aims of the experiment and scientific background

DNase from shrimp has a very high specific activity, estimated to be 30 times higher than bovine DNase I, which is widely used in modern biotechnology. In addition, shrimp DNase is heat labile, and it has a particularly strong preference for the hydrolysis of double-stranded DNA (dsDNA). Shrimp DNase can therefore be used to specifically degrade dsDNA, leaving single stranded DNA (ssDNA) essentially intact. The higher catalytic efficiency, together with the reduced thermal stability, has made the enzyme an important component in kits used in molecular biology.

The shrimp DNase consists of about 400 amino acids where the C-terminal part of the sequence resembles other endonucleases with a NUC domain. Nucleases with NUC domains are active towards both DNA (dsDNA and ssDNA) and RNA. The closest structural homologue is of a nuclease from an *Anabaena* Sp., but the sequence identity is only 29% over 130 aligned residues. The N-terminal part of the shrimp DNase sequence also shows low sequence similarity (20% over 140 aligned residues) to the structure of human dead-box Rna-helicase.

Because of the low sequence similarity to other proteins, the structure of shrimp Dnase have to be solved by experimental phasing

Results

Crystals of DNnase had been soaked in solutions containing various heavy atoms. Data were eventually collected on two crystals soaked in bromine, one crystal soaked in zink and one crystal soaked in iridium. The presence of the heavy atoms were confirmed by energy scans.

The Zn and the two Br derivatives diffracted to about 2.7 – 3.0 Å while the Ir derivative diffracted to 1.86 Å. The crystals belonged to the orthorhombic space group $P2_12_12$ with cell parameters of about 176 x 47 x 50 $Å^3$. The lengths of the b- and c-axes varied slightly between the derivatives. R_{sym} for the Zn and Br derivatives were in the order 8 – 10%, for the Ir derivative it was 5%. The multiplicity of the anomalous signals for the Br and Zn derivatives were in the order of 4 – 8, but no significant anomalous signal was observed for the Ir derivative. The position of the Zn and Br atoms could be located, but further model building has not been possible yet. Attempts to perform phase extension using the high resolution Ir data has been tried but without success.

Other (valid for all experiments; MX1060, MX1063 and MX1065)

Essentially all of the allocated beamtime was spent analysing proteins in the beamtime applications. Only very little time was spent testing other proteins for diffraction. These were crystals of a uracil-dna-glycosylase (UDG) complex, two different kinases and two metallo-β-lactamases (the latter was relevant for experiments MX1061 and MX1062).

The UDG crystal diffracted to about 1.7Å, but peaks were split giving poor statistics and the structure could not be solved.

One of the kinases was p38 from a new species (Atlantic salmon). The crystal diffracted to 2.7 Å, belonged to space group $P2_1$ with cell parameters of 67.78 x 100.93 x 67.89 $Å^3$, b=98.15, and R_{sym} was 5.4%. The structure has been solved, and further analysis of the structure is in progress.. The other kinase was a complex between PKA and two different inhibitors. Both crystals diffracted beyond 2 Å, and belonged to space group $P2_12_12_1$ with cell parameters of about 72 x 75 x 80 $Å^3$. Rsym was about 7-8%. The structures have been solved and the inhibitors are identified. Further analysis is in progress.

Two crystals of metallo-β-lactamases were tested for diffraction, and were found to diffract beyond 2Å. One crystal belonged to space group P43 and the other to P61. The structures have now been solved, and are currently being further refined and analysed.