**E**UROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

# 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:**<br>DrPCNA, a DNA polymerase III beta-subunit | **Experiment number**:<br>MX-1176 |
| **Beamline**:<br>ID 14-1 | **Date of experiment**:<br>from: 19.11.10, 09:30 to: 19.11.10, 17:00 | **Date of report**:<br>23.02.11 |
| **Shifts:**<br>1 | **Local contact(s)**:<br>Dr. Mats Ökvist | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

**Laila Niiranen*, Kenneth Johnson*, Hanna-Kirsti Leiros*, Ronny Helland**

Norwegian Structural Biology Centre
Department of Chemistry
University of Tromsø
9037 Tromsø
Norway

## Report:

This experiment was run as remote access.

## Aims of the experiment and scientific background

DNA polymerase III β-subunit, or Proliferating Cell Nuclear Antigen (PCNA) is a ring-shaped protein that encircles DNA. PCNA acts as a processivity factor, or sliding clamp, for a wide variety of proteins that act on DNA, including DNA polymerases, DNA ligase, endonucleases and glycosylases. PCNA is a dimer in bacteria, a homotrimer in eukaryotes and a heterotrimer in crenarchaea such as *Sulfolobus solfataricus*. So far, the structure of three bacterial PCNAs has been determined. The PCNA dimer forms a doughnut-shaped ring lined by 12 α-helices, with an opening big enough to accommodate dsDNA.

*Deinococcus radiodurans* PCNA (DrPCNA) is a 39.6 kDa protein with 362 residues that have a 29% sequence identity to *E. coli* PCNA and 28% to *Thermotoga maritima* PCNA. DrPCNA has been recombinantly expressed and purified with the aim to perform a comparative biochemical and structural study of PCNA and its interacting DNA repair pathway partners from the extremophilic radiation resistant *D. radiodurans* and the cold-adapted *Vibrio salmonicida*. The DrPCNA crystals diffracted to 3.6 Å resolution on the home-source, but the data was too weak for reliable space group determination. Even though the sequence similarity to other PCNAs is low, it is likely that molecular replacement can be used due to a common overall fold.

## Results

Numerous DrPCNA crystals were tested, but most of them diffracted only to 3.5 – 4 Å. Finally, a few crystals were found to diffract a little better, and three datasets were collected - the best was to 2.4 Å and was 94% complete. The crystals belonged to the triclinic space group P1 with cell parameters of 71.3 x 76.4 x 83.5 $Å^3$, a=103.5, b=107.1, c=111.0, and $R_{sym}$ was 5%. The structure was solved from the best 2.4 Å dataset by molecular replacement, revealing four monomers (two dimers) in the asymmetric unit. Refinement and analysis of the structure is now in progress.

Many (>10) crystals of antibiotic resistance protein NimB from *Bacteroides fragilis* (BfNimB) were also tested, and three full data sets were collected. The best was to 3.0 Å, the space group was found to be $P6_122$ or $P6_522$, and the data was complete. For this project we need phases and most likely bigger crystals in order to solve the structure, which is the aim for the next data collection time.

For another project data was collected on a mutant of the cold-active *Vibrio sp*. alkaline phosphatase (Helland R, Larsen RL, Asgeirsson B. 2009. The 1.4 Å crystal structure of the large and cold-active Vibrio sp. alkaline phosphatase. Biochim Biophys Acta. 1794:297-308). Here we got a complete low and a high resolution dataset from one crystal to 1.85 Å in a space group different than the published native structure, and the analysis of the structure is in progress.

The last project was on PtmG, a protein involved in the sialic acid pathway in *Aliivibrio salmonicida*, where we got a complete X-ray data set to 2.97 Å. The structure analysis is currently being worked at.

## Other

We encountered some problems at the start up of the remote access session, when we could not get mxCuBE to start. The addition of samples from ISpyB to mxCuBE had to be done by the local contact as it would not work from remote, and later on we had problems where the mouse would not work via the NX client. There was also a problem with the detector not working properly. About half-way in the shift a wavelength change at ID 14-4 halved the beam intensity. Towards the end of the shift the beam was lost for one hour as the whole synchrotron ring was down.

Nearly all of the allocated beam time was spent analysing the beamtime application protein DrPCNA. Time also allowed us to test three other proteins, all of which were safety declared. These were Vibrio alkaline phosphatase (Vap), BfNimB, and *Vibrio salmonicida* PtmG as described above in Results.