EUROPEAN SYNCHROTRON RADIATION FACILITY

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 via the User Portal:

https://wwws.esrf.fr/misapps/SMISWebClient/protected/welcome.do

Reports supporting requests for additional beam time

Reports can 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:** Crystal structure of a eukaryotic DNA polymerase	**Experiment number**: MX1342
Beamline: ID23-2	**Date of experiment**: from: 21 Sep 2011 to: 22 Sep 2011	**Date of report**: 23 Sep 2011
Shifts: 2	**Local contact(s)**: Tobias Karl	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Matthew Hogg, Umeå University

Report:

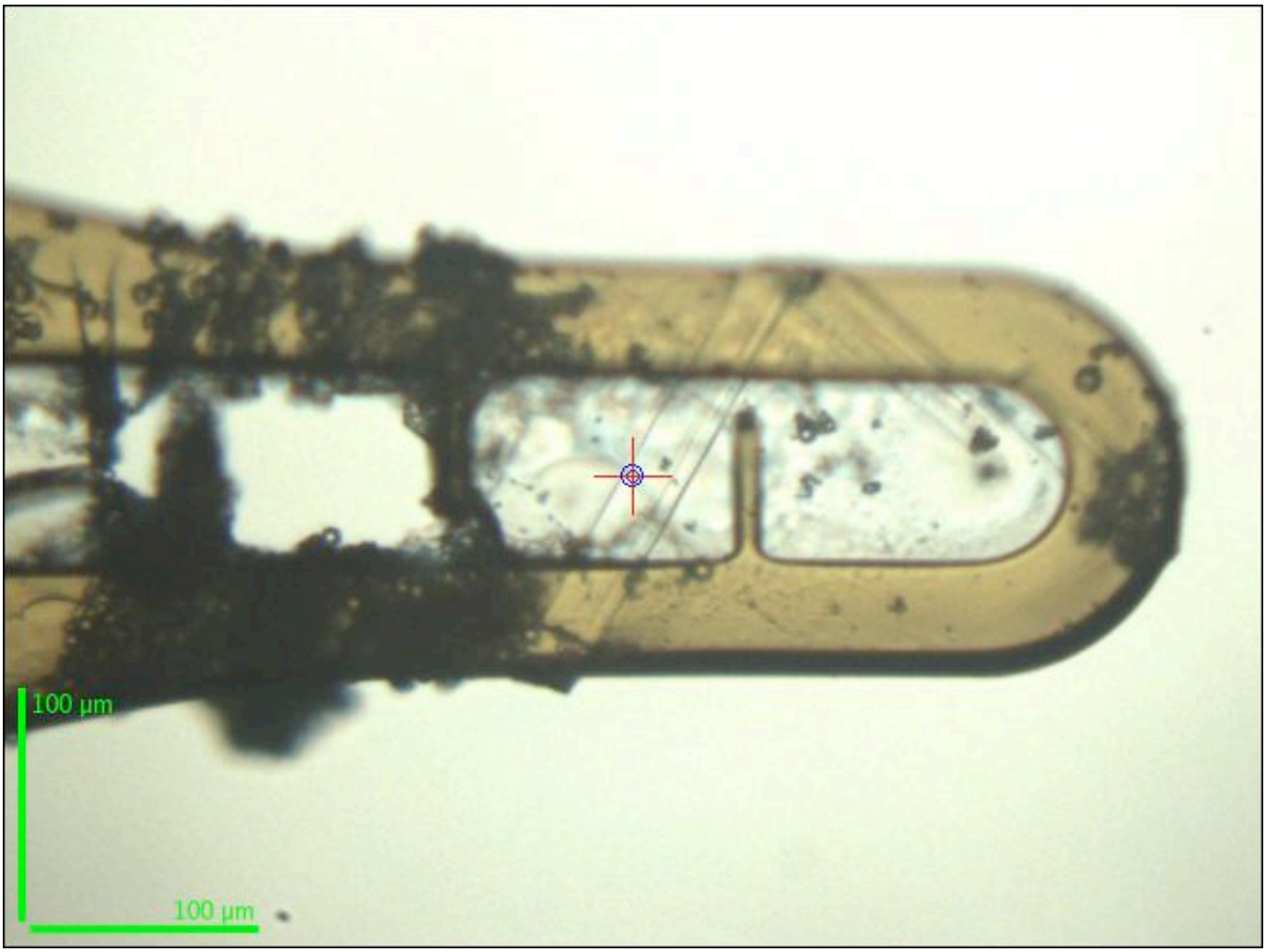
With the beamtime allocated for project number MX1342, we were able to collect initial diffraction data from a screen of 50 unique crystals. These crystals were frozen with a broad range of cryprotectants, heavy atom derivatives, soak times and crystal mounting devices. The results ranged from heavy ice rings and/or no diffraction to the very last crystal tested that showed diffraction to greater than 4Å. The observed diffraction is the first seen for this polymerase. The microfocus beamline was critical to obtaining our results due to the fact that our crystals are thin plates with maximum dimensions of approximately 100 x 15 x 5 microns and have so far proven impossible to grow apart from clusters of needles or thin plates. We were thus able to focus the beam onto individual crystals within the drop and could test different regions of individual crystals.

The diffraction tests on all 50 crystals provided valuable insights into appropriate cryoprotectants, soak times and mounting techniques. We now have conditions that we believe will allow us to obtain more and better diffracting crystals for future experiments. Unfortunately, the best diffracting crystal was the last of 50 tested and so time did not allow a complete data set to be acquired, especially since the best diffraction was only observed with extended exposure times of ten seconds per image. We were, however, able to obtain unit cell and space group information from the recorded diffraction patterns from this crystal.

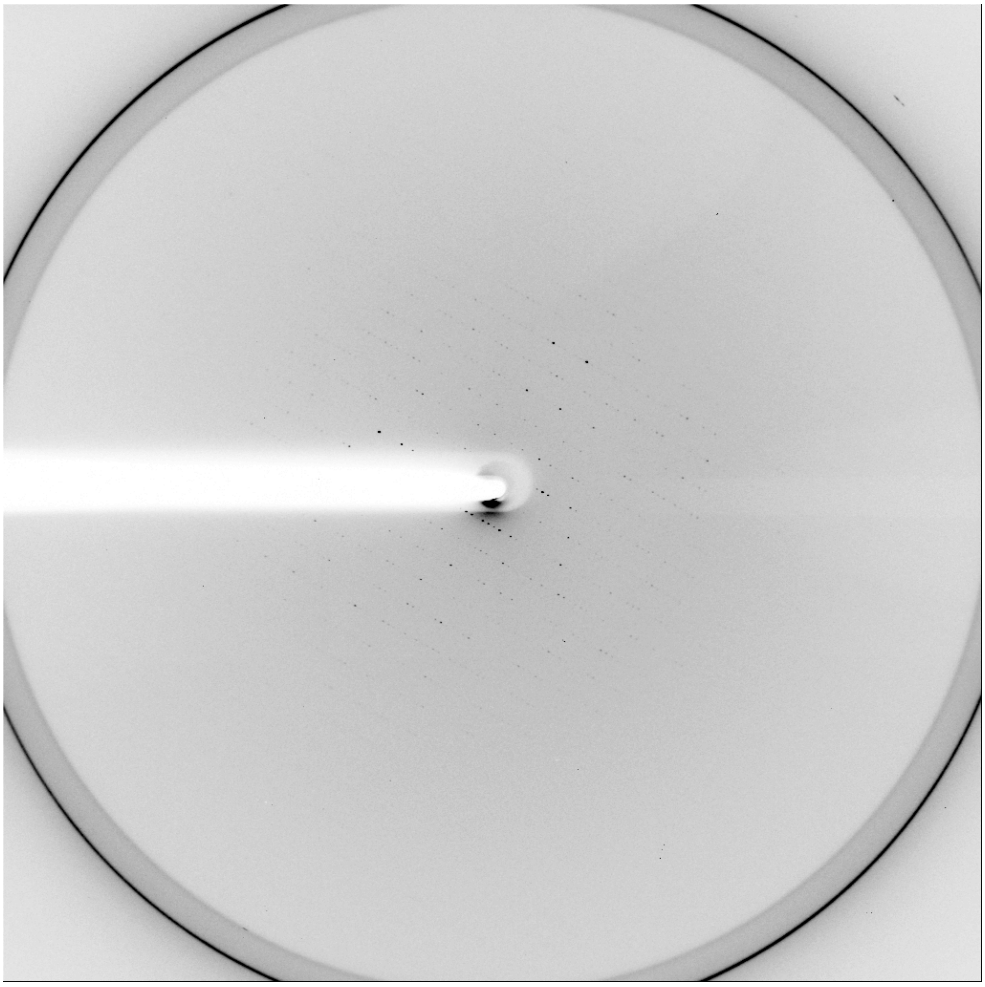
Using the knowledge gained from this experiment, which would have been unavailable to us otherwise, we will grow more crystals and freeze and mount them according to the best results obtained at the ID23-2 beamline. Knowing the best conditions for freezing and

mounting the crystals (as well as which crystal morphology seems to result in the best diffraction) will allow us to maximize the likelihood of obtaining usable heavy atom derivatives so as to solve the structure of our complex.

Attached below is an image of the best diffracting crystal and one of the resulting diffraction patterns. Note the flexibility of the crystal as it wraps around the loop like a length of ribbon. Also note that the crystal appears to split into several plates within the same mounting thus necessitating the use of the microfocus beamline to avoid hitting multiple objects with the beam. We plan to attempt to grow and mount individual crystals but the opportunity to explore crystallization experimental space is limited by the extreme difficulty in producing more protein. Thus we will likely succeed only in producing more crystals that grow as clusters of needles or thin plates and will require further beamtime with the microfocus capabilities of ID23-2.



Broad edge of the best diffracting crystal.



Diffraction pattern from the above crystal. This is, to our knowledge, the first diffraction pattern ever observed for this protein in complex with its substrates.