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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:** **Dundee St Andrews BAG**	**Experiment number**: LS-2087
Beamline: ID14-EH2	**Date of experiment**: from: 2 Feb to: 3 Feb	**Date of report**:
Shifts: 3	**Local contact(s)**: A. Royant	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Karen Mcluskey*, Ms Lauris Kemp*, Mr Mads Gabrielsen*

Report: Eight datasets on three projects were obtained.

2-C-Methyl –D-Erythritol 4-Phosphate Cytidyltransferase and 2-C-Methyl –D-Erythritol 2,4-Cyclodiphosphate Synthase bifunctional enzyme coded by the IspF/IspD gene.

Space group $P6_322$ a = 106, b = 106, c = 161Å
Two potential heavy atom derivatives were measured and highly redundant data obtained to also seek out the anomalous signal from a bound Zn.

Dataset 1. Crystal size 0.4 x 0.15 x 0.15 Diffraction limit 3.2Å
50º of data measured, 92% complete, Number of reflections 8560, Redundancy 6, Rsym 6.3% I/σI 20.

Dataset 2. Crystal size 0.3 x 0.10 x 0.10 Diffraction limit 3.0Å
82º of data measured, 100% complete, Number of reflections 11140, Redundancy 7, Rsym 6.9% I/σI 21.

Molecular replacement methods place the domain for the IspF protein but have so far failed to position the IspD structure recently solved using BAG time. Experimental phase information is now sought to solve this problem. Although we do not appear to have a heavy atom derivative the anomalous dispersion signal suggests a position for an ordered Zinc but the map based on combined phases is not yet good enough. We will now proceed to a MAD experiment targeting Zn or SeMet.

2-C-Methyl –D-Erythritol 2,4-Cyclodiphosphate Synthase coded by the IspF gene.
This structure was recently solved to high-resolution by MAD methods using ID29 in a previous BAG day (manuscript submitted). We are now characterising enzyme-ligand complexes. The previous crystals are orthorhombic with a trimer in the asy. unit but in the presence of new ligands and metal ions the symmetry changes to monoclinic space group $P2_1$ a = 55, b = 118, c = 88Å α =90 β = 90.2 γ = 90º. Data have been processed and molecular replacement/refinement calculations are underway. Samples suffered badly from radiation damage.

Dataset 1. Crystal size 0.05 x 0.3 x 0.5 Diffraction limit 2.2Å
125º of data used, 94% complete, Number of reflections 53475, Redundancy 1.4, Rsym 9% I/σI 6.
Dataset 2. Crystal size 0.05 x 0.2 x 0.4 Diffraction limit 2.0Å
110º of data used, 98% complete, Number of reflections 72737, Redundancy 2.1, Rsym 5% I/σI 15.
Dataset 3. Crystal size 0.05 x 0.2 x 0.4 Diffraction limit 2.3Å
110º of data used, 84% complete, Number of reflections 41192, Redundancy 2.6, Rsym 9% I/σI 10.
Dataset 4. Crystal size 0.05 x 0.3 x 0.4 Diffraction limit 2.4Å
105º of data used, 76% complete, Number of reflections 33428, Redundancy 2.4, Rsym 5% I/σI 13.

Molybopterin-guanine dinucleotide biosynthetic protein, MobB.

This protein has been crystallised in a monoclinic crystal form though the conditions are not reproducible and the samples are always mechanically twinned. We have only been able to get poorly ordered SeMet samples and have sought an alternative crystal form. Hexagonal blocks are now available for the native and SeMet proteins which diffract to 3.5 Å resolution in the home lab. The symmetry is 6/mmm with a = b = 237 c = 49Å. Two datasets have been measured which are not very isomorphous but the anomalous signal from the SeMet protein has shown a promising Patterson function. We will try to identify the Se positions from the data in hand and in addition now prepare for a MAD experiment to solve this structure.

Dataset 1. Native crystal size 0.2 x 0.2x 0.4 Diffraction limit 2.7Å
60º of data measured, 99% complete, Number of reflections 22119, Redundancy 6, Rsym 9% I/σI 16.
Dataset 2. SeMet crystal size 0.2 x 0.05 x 0.05 Diffraction limit 2.7Å
120º of data measured, 100% complete, Number of reflections 22777, Redundancy 6, Rsym 12% I/σI 12.

It was extremely useful to characterise these crystals on an undulator beamline in terms of when radiation damage is apparent and the experience will help us get the MAD experiments right.