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 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.

	Experiment title: St Andrews – Dundee BAG	Experiment number: LS2178
Beamline: ID14 EH2	**Date of experiment**: from: 21.06.02 to: 22.06.02	**Date of report**: 14.08.02
Shifts: 3	**Local contact(s)**: Dr. Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. William Hunter*
Dr Magnus Alphey*
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Report:
We were originally scheduled on ID29 to carry out MAD data collection but due to problems with that station were given time on ID14 EH2. An added complication was the failure of FEDEX to transport our cryo-stored samples to ESRF. Fortunately we travelled with many samples taken for screening and as a backup we also had put down crystallisation conditions on our previous trip to ESRF.

The bifunctional methylerythritol phosphate cytidyltransferase – methylerythritol cyclodiphosphate synthase (MECS).
After exhaustive crystallisation trials with a lot of work done on the home lab we can now reproducably get ordered crystals. Previously, native crystals only diffracted to between 3 and 3.5 Å, SeMet samples only between 4 and 5 Å at ESRF. Time was used to screen all the crystals, to collect several datasets and we have now obtained significant improvements to our data.

Native data now extends to 2.3 Å, 98% complete, Rsym 6.8% with redundancy of 19. Unit cell is a=b=108.8, c=161.3 Å, space group $P6_322$. The enzyme contains zinc and we have tried to locate the metal from the weak anomalous signal but so far without success. The SeMet derivative now extends to 2.9 Å, 100% complete, Rsym 10.3% with redundancy of 38. Unit cell a=b=107.7, c=161.0 Å, space group $P6_322$. Attempts to derive phases using a SAD or SIRAS approach have so far failed and we now plan a MAD experiment targeting the SeMet protein.

Complex of methylerythritol cyclodiphosphate synthase with a novel inhibitor.

The crystals of this sample are frequently twinned, numerous crystals were tried, two datasets measured one of which has processed satisfactorily to 2.10 Å. 100% complete, Rsym 8.3% with redundancy of 4. Unit cell a=88.8, b=54.2, c=118.3, $\alpha=\gamma=90$, $\beta= 95.2^{o}$, space group $P2_1$. Structure solved by molecular replacement and refinement is nearing completion with current R and Rfree of 21 and 26% respectively. The inhibitor is well ordered in the active site.

Thioredoxin from *Trichomonas vaginalis*.
As part of a new project to study redox metabolism in this parasite we have generated crystals of one component of the pathway, the thioredoxin. Crystals are thin rods often clumped together but we were eventually able to isolate a fragment that gave data to 2.0 Å. Unit cell a=34.2, b=34.1, c=50.9 Å, $\alpha=70^{o}$, $\beta= 73^{o}$, $\gamma=88^{o}$, space group P1. Rsym 3.5%, 91% complete with redundancy of near 2. Data awaits use in molecular replacement calculations.

Mercaptopyruvate sulfurtransferase (MST) from *Leishmania major*.
In a new project to investigate aspects of sulfur utilization in parasitic trypanosomatids we are studying MST. Unit cell =b=109.6, c=67.3 Å, space group $P4_22_12$, 100% complete to 2.2 Å, Rsym 4.8% with redundancy of 8. A SAD approach has solved this structure based on the Se anomalous signal. 12 Se sites were located and gave a FOM of 0.3 which improved to 0.8 after density modification. The map was manually interpreted and refinement of the SeMet form is nearing completion. Current R and Rfree of 23 and 29% respectively. Unexpectedly we see covalent modification of the active site cysteines and note that the C terminal extension not present in homologues, forms a separate domain not seen in this class of enzyme. The functional significance of these observations will now be further investigated.