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 original report should be sent, together with 5 reduced (A4) copies, to the User Office.

In addition, please send a copy of your file as an e-mail attachment to reports@esrf.fr**,** using the number of your experiment to name your file. This will enable us to process your report for the ESRF Annual Report.

Reports accompanying requests for additional beam time

If your report is to support **a new proposal**, the orginal report form should be sent with the new proposal form, and a copy of your report should be attached to each copy of your proposal. 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.

Postal address: User Office, ESRF, B.P. 220, F-38043 GRENOBLE Cedex, France
Street address: 6 rue Jules Horowitz, F-38043 GRENOBLE Cedex
Telephone: +33 (0)4 7688 2552; Fax: +33 (0)4 7688 2020; e-mail: useroff@esrf.fr

- bear in mind that the report will be reduced to 71% of its original size. A type-face such as "Times", 14 points, with a 1.5 line spacing between lines for the text, produces a report which can be read easily.

ESRF	**Experiment title:** St. Andrews Dundee BAG	**Experiment number**: LS-1951
Beamline: ID29	**Date of experiment**: from: 16th July 2001 to:17th July 2001	**Date of report**: 21st August 2001
Shifts: 3	**Local contact(s)**: Dr. Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

William N. Hunter*, Charles S. Bond*, Lauris E. Kemp*, Matthew Shaw*

Report:

4-diphosphocytidyl-2C-methyl-D-erythritol synthetase.

The priority was a MAD experiment on a potential mercury derivative of this enzyme in the orthorhombic form ($P2_12_12$) with unit cell of 54 x 115 x 88Å. A XANES scan gave a typical mercury edge and two wavelengths (λ_1 1.064Å for f"; λ_2 1.0088Å for f') were selected to record the measurements using a ϕ, $\phi + 180^0$ strategy. Data were recorded to 2.7Å resolution, approx. 98% complete, redundancy of 4 with Rsym of 7.9 and 5.4% for λ_1 and λ_2 respectively. At present we have identified potential heavy atom sites from anomalous difference Pattersons and are trying different phasing strategies. We have noted a lack of good isomorphism even for just the native crystals and are searching for an appropriate native data set that might optimise the phasing.

UDP-galactose epimerase from *Trypanosoma brucei*.

The larger crystals (0.5mm) of this enzyme proved difficult to cryo-protect leading to high mosaic spread and a decrease in the resolution of diffraction. After trying several crystals a switch was made to a much smaller, well-formed orthorhombic block (0.25 x 0.05 x 0.05 mm) which provided 100 ^{0} of data before radiation damage became apparent. Resolution limit 1.95Å, 87% complete overall, 100% to 2.3Å. Rsym 6.7%. Weak in-house data to 2.4Å had been used to solve the structure by molecular replacement however the low sequence

homology of the search model, about 30% with the human enzyme, combined with four subunts per asymmetric unit (about 1400 amino acids) was complicating refinement. Acquiring a high-resolution data set has greatly helped the analysis with improved statistics for the molecular replacement and improved electron density maps. Refinement is continuing.

Hjc Studies of DNA/metal complexes in a resolvase

Attempts to produce crystals of native Hjc containing the mechanistically essential divalent ions have so far failed. Success can only be determined by collecting complete data on candidate crystals, and is thus time consuming. This pursuit has, however, led to the collection of increased resolution data (to 1.8Å) for native Hjc which has led to clarification of some disorder found in electron density maps of the published structure (2.2Å resolution; Bond *et al.* PNAS 2001, **98**, 5509. Hexagonal $P6_122$ with a = b = 53.1, c = 209.7Å, Rsym 7% (33% at 1.8%), completeness: 90.1% (58.1% in outer bin). The size of the unique axis means that small oscillation ranges are required when measuring the data.

Diffraction tests were unsuccessful for Hjc-Holliday junction co-crystals and further work to prepare samples is underway. The crystals are fragile and small rendering in-house characterisation effectively impossible.

The remaining time was used to test numerous samples which have given small crystals that are unsuitable for in-house experiments:

1. Potential lead and zinc derivatives of 4-diphosphocytidyl-2C-methyl-D-erythritol synthetase obtained by co-crystallisation. All give poor diffraction.

2. Needles of a bifunctional enzyme implicated in the biosynthesis of the bacterial cell wall. Weak diffraction to 3.5Å evident from one set of conditions which will now be followed up.

3. Se-Met MobB crystals recently obtained. Crystals are twinned and diffract to near 3.0Å. These will need to be improved prior to a full MAD experiment.

In general ID29 is an easy-to-use station, although some aspects of automation from BM14 are missed. We note that documentation is not as rigorously explicit is that found, for example on ID14-EH2. Specifically the instructions for moving the monochromator led us to uncalibrating it by moving it in two different programs and we lost some time because of this mistake. The printer queue on the linux box next to the printer has not worked on the two occasions we have used ID29 which is inconvenient when trying to solve structures on the station.