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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-**1821**
Beamline: BM14	**Date of experiment**: from: 6 SEP 2000 to: 8 SEP 2000	**Date of report**: 2/3/01
Shifts: 6	**Local contact(s)**: Gordon Leonard	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

William Hunter*

Charles Bond*

Lauris Kemp*

Report:

The structure of Hjc

Homologous recombination is a means by which strand exchange can occur between homologous DNA sequences. The result of the nicking, strand invasion and rehybridisation is a branch point, a Holliday junction. These four-way DNA junctions must be resolved to produce two new DNA duplexes, a job performed by junction resolving enzymes. Hjc is a junction resolving enzyme found in the archaea. Crystallisation of Hjc from *Sulfolobus solfataricus* yielded crystals in strongly diffracting-hexagonal and weakly-diffracting cubic forms.

BAG time was used to collect complete native datasets of both forms and to successfully perform a MAD experiment on a SeMet derivative of the hexagonal form. The structure is in press with PNAS.

Hexagonal Form (P 6_1 2 2; a = 53 Å, c = 208 Å; native data to 2.15 Å)

See Table 1. Hexagonal crystals diffract to well beyond 2.15 Å, but the combination of the small MAR CCD and long **c** axis caused spot overlap at higher resolution. Were BM14 still part of the JSBG I would recommend replacement with a larger detector.

MAD experiment

Hjc contains one methionine. Crystals of a SeMet derivative of a mutant Hjc incorporating a further methionine grew to size suitable for a MAD experiment. A three wavelength MAD experiment was performed at the Se edge and the structure solved and built using SOLVE, RESOLVE and wARP. The relative ease with which structure solution and map inspection

was performed at the beamline is a credit to the JSBG. This kind of computational support is essential as it allows real time evaluation of the data.

Cubic Form (I 2 3, a = 143 Å; data complete to 3.6 Å)

Cubic crystals grow easily in a wide variety of conditions to a size of 1 mm, but never diffract to better than 7 Å in-house. The intense synchrotron beam allowed us to collect a dataset to 3.6 Å which was ultimately phased by molecular replacement with the model obtained from the hexagonal data. Although the data is unsuitable for refinement, it has provided some interesting insight into the intermolecular interactions of Hjc.

Table 1. Experimental Details

Data	Native	Selenomethionine derivative		
Space group		$P6_122$		
Cell Constants (Å)	*a* = 52.70		*a* = 52.85	
	c = 207.61		*c* = 208.30	
Wavelength (Å)	0.97626	0.97931	0.97961	0.93928
Resolution (Å)	20.0-2.15	20.0-2.40	20.0-2.40	20.0-2.20
Observations	121253	77229	61733	100170
Unique Reflexions	10061	7441	7308	9460
R_{sym} [§]	0.041 (0.105)	0.036 (0.095)	0.041 (0.112)	0.042 (0.125)
R_{anom} [§]		0.029 (0.064)	0.033 (0.085)	0.030 (0.117)
Completeness (%) [§]	99.2 (97.0)	99.2 (96.3) [¶]	94.7 (90.2) [¶]	94.8 (68.7) [¶]
$\langle I \rangle / \langle \sigma I \rangle$ [§]	36 (7.5)	13.6 (3.6)	13.1 (3.2)	9.9 (4.2)
Wilson B ($Å^2$)	25	27	27	28
FOM [‖] (SOLVE)			0.34	
FOM [‖] (RESOLVE)			0.64	
Refinement				
R-factor	0.220	Ramachandran Outliers (%)	3.6	
R-free (5%)	0.281	Cruickshank's DPI	0.217	
No. of atoms	1077	Average B-factor ($Å^2$)	35	
Protein	998	Protein	34	
Waters	79	Waters	39	

[§] Figures in parenthesis represent the highest resolution shell

[¶] Friedel pairs treated as separate

[‖] Figure of merit

We also used this beamtime to initiate studies on two proteins involved in the non-mevalonate pathway of isoprenoid biosynthesis. The enzyme DeOxy Xylulose Reductoisomerase (DOXR) catalyses the conversion of deoxyxylulose 5-phosphate to 2C-methyl-D-erthyritol-4-phosphate (MEP) and the metal ion dependent 2C-methyl-D-erthyritol-4-phosphate cytidyltransferase links MEP with CTP to give 4-diphosphocytidyl-2C-methyl D-erythritol.

DOXR gives orthorhombic blocks in space group $C222_1$, a = 101.5, b = 249.5 c = 133.1 Å and data (100% complete) to 2.6 Å were measured with an R-merge of 9%. MCT crystallises as tetragonal blocks in space group $P4_12_12$ or $P4_32_12$ with a = b = 73.5 c = 175.5 Å. Data (99.5% complete) to 2.4 Å have been recorded with an R-merge of 3%.

We attempted a MAD experiment on a Se-Met derivative of MCT and measured data at 3 wavelengths. Unfortunately, although we obtained a well defined XANES scan and the data

appear to be of good quality we have been unable to locate the 2 Se positions in the asy. unit. MCT requires a divalent metal ion for activity and we crystallised the protein in the presence of Mn^{2+} so also attempted to derive phase information from data measured at two wavelengths around the Mn edge. This experiment failed although we were encouraged that data could be obtained at the longer wavelengths used. We will now seek alternative derivatives to solve the MCT structure and seek to obtain Se-Met crystals of DOXR.