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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:** **Block Allocation Group (Portugal)**	**Experiment number**: LS-1667
Beamline: ID14-1	**Date of experiment**: from: 29-04-00 to: 02-05-00	**Date of report**: 18-08-00
Shifts: 6	**Local contact(s)**: Hassan BELRHALI	*Received at ESRF:*

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Report:

ATP Sulfurylase from *Desulfovibrio desulfuricans* ATCC 27774 (native):

A data set was collected to 2.60 Å resolution. Overall statistics: R-merge = 6.4 %, completeness = 88.2 %, I/σ(I) = 18.0, Redundancy =6.0. Highest resolution shell statistics (2.69 to 2.60 Å): R-merge = 28.1 %, completeness = 83.6 %, I/σ (I) = 5.2, Redundancy = 6. Heavy atom derivatives are being searched.

'Split-Soret' cytochrome *c* from *Desulfovibrio desulfuricans* D31 (native):

Two data sets were collected. Because crystals diffracted very poorly on a rotating anode generator at home, cryo conditions were difficult to establish in the home laborartory. Therefore, the first data set was collected from a crystal at room temperature from 35.0 to 5.0 Å resolution. The overall statistics are R-merge = 7.1%, completeness = 93.9 %, I/σ (I) = 4.5, redundancy = 3.9. Highest resolution shell statistics (5.22 to 5.00 Å) are R-merge = 52.6 %, completeness = 95.4 %, I/sigma(I) = 1.1, redundancy = 3.9. A second data set was collected from 35 to 3.6 Å resolution, from a frozen crystal after finding suitable cryoprotecting conditions. Overall statistics: R-merge = 7.3 %, completeness = 98.5 %, I/σ(I) = 8.4, redundancy = 6.9. Highest resolution shell statistics (3.76 to 3.60 Å): R-merge = 26.9 %, completeness = 96.1 %, I/σ (I) = 2.7, redundancy = 7.2. The room temperature crystal was found to belong to space group $P2_12_12$ or $P2_12_12_1$ (no 00l reflections were recorded) with unit cell parameters a= 98.86, b= 96.78, c=364.89 Å, while the frozen crystal was found to belong to the tetragonal crystal system, space group $P4_12_12$ or $P4_32_12$ with unit cell parameters a= 93.98, c=349.108 Å. It is not known whether these crystals truly belong to different crystal forms or a phase transition to a higher symmetry space group was induced by the flash cooling process. The very long c axis limited the resolution of the data that could be recorded on ID14-1. A search for other crystal forms is in

progress, and a further data collection on these crystals will be tried on ID14-2 or ID14-4 whenever beam time on these lines becomes available. These data will be also used to solve the structure of this protein, using as model the three-dimensional structure of a similar protein *from Desulfovibrio desulfuricans* ATCC 27774.

Calcium binding protein from *Desulfovibrio gigas*:
This protein was isolated from the natural microorganism, and has a M.W. of about 45 kDa. Its N-terminal shows significant homology with the calcium binding 'finger' found in calmodulins. Several data sets were collected on different crystals of this protein, as detailed below:

Crystal	Space group	Cell (Å)	Resolution	% R-merge	% complete	I/σ(I)	Redundancy
Native 1 ID14-2	$P6_1$ or $P6_5$	a=50.25 c=247.73	1.45 Å (1.54-1.42)	5.4 (32.0)	96.4 (95.0)	14.2 (4.4)	4.5 (2.8)
Native 2	$P6_1$ or $P6_5$	a=50.36 c=247.89	2.05 Å (2.10-2.05)	5.9 (27.0)	99.6 (98.0)	25.1 (3.5)	4.1 (2.4)
Samarium derivative 1	$P6_1$ or $P6_5$	a=50.04 c=247.77	2.70 Å (2.80-2.70)	8.7 (27.0)	96.8 (83.0)	11.7 (2.1)	3.2 (2.1)
Samarium derivative 2	$P6_1$ or $P6_5$	a=49.81 c=247.53	2.90 Å (3.00-2.90)	6.5 (16.7)	94.4 (94.4)	14.8 (8.0)	4.0 3.2
Europium derivative 1	$P6_1$ or $P6_5$	a=49.45 c=247.44	3.60 Å (3.73-3.60)	4.2 (5.5)	89.1 (83.5)	16.4 (10.1)	2.8 1.5

The long unit cell axis (nearly 250 Å) combined with the increase in crystal mosaicity upon flash cooling limited the maximum resolution of the data that could be collected on ID14-1. Data set Native 1 was measured on ID14-2 taking advantage of two spare shifts that were made available to us during this experiment. Of the 'derivative' data sets, Samarium 1 was obtained as a co-crystal and the initial results from a difference Patterson were encouraging. However, we haven't yet managed to interpret it. The other 'derivatives' were soaks prepared at the ESRF and the difference Patterson maps were nearly featureless, indicating very little or no heavy atom incorporation into the crystals.

'Hybrid Cluster protein' from *Desulfovibrio desulfuricans* (native):
Crystals of this protein were obtained and flash frozen under anaerobic conditions from an anaerobically purified sample. A data set was collected to 1.6 Å resolution on ID14-2, taking advantage of two spare shifts that were made available to us during this experiment. The crystal was found to be a random twin but by closing the slits to 50 μm and by finding a starting crystal orientation where one of the twin components was predominant, its diffraction pattern could be indexed and the data processed satisfactorily. The need to minimise the risk of spatial overlap between spots coming from the different twin components limited the maximum resolution of the data set to 1.6 Å. Also, some decay was apparent towards the end of the data collection. Crystal system is triclinic, space group P1 with cell parameters a=57.40 b=61.66 c=72.14 Å α=82.8 β=73.7 γ=87.4°. Overall statistics: R-merge = 6.8 %, completeness = 96.9 %, I/σ(I) = 7.4, redundancy = 2.3. Highest resolution shell statistics (1.67 to 1.60 Å): R-merge = 10.7 %, completeness = 95.8 %, I/σ(I) = 6.1, redundancy = 2.3. The structure was solved by the 'molecular replacement' method and is being refined with 'CNS'. Current R-factor is 20.1 % and R-free is 20.7 %.