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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:** Crystallographic Studies of Stringent Response Proteins	**Experiment number**: LS-1894
Beamline: ID29, ID14-3	**Date of experiment**: from: ID29: 03 June to: 04 June 2001 ID14-3: 29 September to: 30 September 2001	**Date of report**: 01 September, 2001
Shifts: 6	**Local contact(s)**: ID29: Gordon Leonard ID14-3: Elsepeth GORDON	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

The current proposal has the last part of the allocated beamtime scheduled on September 29, 2001. Thus the submitted report is by necessity of deadlines preliminary. The proposal has focus on detailed study of mechanisms and structural features of proteins related to bacterial stringent response. This system involves several different proteins. We have until now only collected data (ID29) on two of the targets, namely elongation factor G(EF-G) in complex with ppGpp and a seleno methionine MAD

dataset on guanosine pentaphosphate phosphohydrolase (GPP). Data collection was in both cases influenced by problems in beamline operation and a significant amount of time lost due to inability to open the front end. The quality of the EF-G:ppGpp crystals and the collected data, which extended only to about 4Å, was realized to be too low to pursue refinement. Naturally the sequence of GPP contains only two methionines of which one is the starting N-terminal methionine. To improve the expected signal in the planned SeMet MAD experiment at ID29 we had produced crystals of double methionine mutant. The crystals, which belong to space group P212121, had unit cell parameters: a=50, b=70 and c=90 Å. Despite this relative similarity to crystals of the native – for which data had previously been collected to 1.8 Å – we experienced problems reproducing diffraction to high resolution. Data were collected to 2.9 Å from a crystal that showed clear signal for incorporation of selenium. The Rmerge in the 3.0-2.9 Å shell was 23% with 91% completeness at the peak wavelength. After the experiment it was realized, however, that the data had obviously not been collected in an optimal fashion. The mosfilm strategy option was used to find the best starting angle, which would ensure that all anomalous data was collected in minimum time. A 90 degree wedge of data was collected with poor redundancy before progressing to acquisition of inflection point and remote wavelength data. A later attempt to return to the exact position of the peak for further data collection failed. Patterson methods were used to localize 3 out of the 4 seleno methionine residues but all attempts to improve phasing using both SHARP, SOLVE and various density modification procedures have failed to give electron density maps that would allow tracing of the structure. We are currently gearing up for an effective data collection at ID 14 1 on September 29. A triple seleno methionine mutant of GPP has been prepared and well diffracting crystals are available. Further, a new crystal form has been obtained. We look forward to the visit.