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# 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:**<br>Proteins in lipid metabolism | **Experiment number**:<br>LS-2015 |
|---|---|---|
| **Beamline**:<br>ID29 | **Date of experiment**:<br>from: 14-11-2001 to: 15-11-2001 | **Date of report**:<br>28-2-2002 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Antoine Royant | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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**Report:**

**Department of Chemistry, Carlsberg Laboratory**

Experiment 1:
MAD data collection on Flocculin-Pt derivative. (2 hours)
Fluorescence scans of the back soaked crystals at ID29 confirmed the presence of platinum in the crystals. Data collection started at the peak but the crystal damage was to serious to collect more than 50% of the data for the first wavelength. The crystals diffracted to better than 2.5 Å on the first image but to worse than 5 Å when 50% of the data was collected.

Experiment 2:
MAD data collection on Flocculin-Ag derivative. (2 hours)
Fluorescence scans of the back soaked crystals at ID29 did not show any signal for Ag and data was only collected at one wavelength for this crystal. The data was the first complete native dataset ever collected on flocculin. The data is 99.3% complete to 2.6 Å.

Experiment 3:
MAD data collection on Flocculin-Au derivative. (2 hours)
Fluorescence scans of the back soaked crystals at ID29 indicated the presence of Au in the crystals. Data collection started at the peak, but the crystal suffered from radiation damage during the data collection and it was not possible to obtain data for more than one wavelength. The data was complete 99.9% to 3.0 Å with an anomalous completeness of 98.9%. It has been possible to determine the position of the Au atom using the program solve,

but the phasing power of the derivative is not good enough for model building neither by hand (after density modification in the program sharp) or by using the automated procedure in wARP.

Experiment 4:
Towards time-resolved crystallography of the KAS I decarboxylation-fatty acid elongation reaction. (5 hours)
Four complete data sets of the KAS I (Lys328Ala):dodecanoyl:malonyl-CoA ternary complex was collected to 1.7 Å. Previous data collection on this ternary complex has indicated that the decarboxylation reaction initiating the fatty acid elongation reaction is initiated when the crystals of the ternary complex is exposed to synchrotron radiation (ID14-4, LS-1757), probably due to radiation damage of the malonyl moiety. The data collections were initiated with crystal orientations separated by 20°. The quality of the fourth dataset had significantly worse scaling statistics than the other three datasets, and it was discharged. The other data sets were merged in batches of 20° (the first 20° from data set 1 was merged with first the first 20° from data set 2 and the first 20° from data set 3 etc.) to finally give us three (85-94% complete) data sets representing structures of the ongoing reaction merged over shorter time spans than when all data was collected on a single crystal. It is not possible to follow the reaction with spectroscopy and the 20° was chosen due to the time limit of the experiment, the need of complete data sets and the wish to follow the reaction from the start to the end. The time course of the reaction observed at ID29 was different than the one observed at ID14-4, however. The first 20° of data is too long a time span to trap the time zero ternary complex, and at time 60° the cleavage of the thio-ester bond between the fatty-acyl and the enzyme is not completed (the cleavage was observed in the ID14-4, one crystal data). To complete the experiment for time zero (the ternary complex) the data must be collected in even smaller exposure bins. New data can of course be merged with this data.

Protein Structure Group, University of Copenhagen

Experiment 1: Se-Met MAD data collection on SP. (6 hours)
The native sequence of sucrose phosphorylase had been mutated to provide additional methionine residues for Se-Met MAD phasing. Half of the seleno methionine protein stock was intensionally oxidized by hydrogen peroxide and both stocks crystallized. Fluorescence scans performed at beamline ID-29 confirmed the precense of selenium in these crystals. Unit cell parameters for native crystals, obtained in MAX lab-lund, Sweden, has never shown consistancy, but the space group P21 is the only space group obtained so far.
Data collection started at the peak wavelength and diffraction extended to below 2.5 Å on the first images. The spacegroup was P21 however, again the crystal suffered significantly from radiation damage. Data were collected at the peak wavelength from a 180 degrees rotation, but the decay was too serious to try the remote wavelength.
Four crystals where exposed with same result: early decay, within the first wawelenght. Further data collection was abandoned. Due to lacking data quality the selenium positions could not be located and our intension to obtain phase information not fulfilled.

Experiment 2: Se-Met MAD data collection on GPP. (4 hours)

The native sequence of guanosine pentaphosphate phosphohydrolase had been mutated to provide additional methionine residues for Se-Met MAD phasing. After production of seleno methionine protein it was intentionally oxidized by hydrogen peroxide and crystallized in a new crystal form. Fluorescence scans performed at beam line ID-29 confirmed the presence of selenium in the crystal. Unit cell parameters were as expected a=54.5, =83.5, and c=69.8 Å and =97.4° and agreed with the space group P21. Data collection started at the peak wavelength and diffraction extended to below 2.3 Å on the first images. However, it was soon evident that the crystal suffered significantly from radiation damage. Data were collected at the peak wavelength from a 180 degrees rotation before changing the to the remote wavelength. Unfortunately, the crystal decay was too serious even before finishing this second wavelength and further data collection was abandoned. Due to lacking data quality the selenium positions could not be located and our intension to obtain phase information not fulfilled.

Experiment 3: Se-Met MAD data collection on TF. (2 hours)
Native data on crystals of the ribosome-binding domain of trigger factor from E. coli had been collected previously to a resolution of 2 Å. The space group was R32 and the cell dimensions a=b=71.8 and c=257.6 Å. Work had been carried out to produce seleno methionine substituted crystals for the ID-29 data collection. To our disappointment we had to realize that crystals suitable for data collection were not available.