EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

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|---|---|---|
| ESRF | **Experiment title:**<br>Monomeric and Dimeric Reaction Centers of spinach Photosystem II and Determination of structures of the enzyme KDO8P synthase from e. coli and the hyperthermophile Aquifex pyrophilus in the presence of substrates and analogs | **Experiment number**:<br>MX-146 and MX-147 |
| **Beamline**:<br>ID14-1 | **Date of experiment**:<br>from: 28/11/03 to: 29/11/03 | **Date of report**:<br>8/5/2004 |
| **Shifts:**<br>3 | **Local contact(s)**:<br>Dr. Stephanie Monaco | *Received at ESRF:* |


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

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Ms. Valeria Rukhman*

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Mr. Radion Vainer


## Report: MX-146

1) Photosystem II
   a. Monomeric and dimeric reaction center from spinach. Five crystals of different forms were examined for quality and effectiveness of possible cryo-protection agents. Diffraction was poor (~10Å) in all cases, however short incubations with parrafin oil gave the best results.
   b. MntC – the manganese solute binding protein (SBP) of the MntABC transport system. Four complete data sets were collected. These data were used as native stes in connection with previously collected MAD data, affording structure determinatino and refinement. The MntC crystallized in space group P3121, with a trimer in the asymmetric unit, which is different then previously determined SBPs. We have refined the structure to an $R/R_{free}$ of 0.25/0.31, and are in the process of refinement completion and preperation of the manuscript. Fig. 1 shows the electron density (8σ) surrounding the manganese in the binding site and the positions of the four liganding residues.


Fig. 1. $Mn^{+2}$ binding site in the MntC protein. Electron density is contoured at 1.5 and 8σ (cyan and red respectively)

## Report: MX-147

1) KDO8P synthase from *E. coli*. We collected three full data sets of the enzyme in the following conditions:
    a. In the presence of both substrates A5P and PEP. The crystals were soaked at 4°C and then flash frozen. Data was collected to 2.9Å. The structure was solved by molecular replacement, however the substrates could not be visualized.
    b. In the presence of the substrate PEP, following crystal stabilizatin with glutaraldehyde (GA). The KDO8PS crystals are problematic due to their inherent instability upon addition of substrates. We attempted to stabilize the proteins by a short treatment with GA, however the treatment induced a high degree of mosaic spread.
    c. In the presence of the product KDO8P. A full data set to 2.7Å was collected. The positin of the product was located in the structure as seen in fig. 2. This structure, along with a number of other KDO8PS structures have been described in a paper recently submitted to JBC: Crystallographic analysis of initial modes of ligand binding in *Escherichia coli* KDO8P synthase Radion Vainer, Valery Belakhov, Emilia Rabkin, Apurba Sau, Cristina Furdui, Karen S. Anderson, Timor Baasov and Noam Adir.

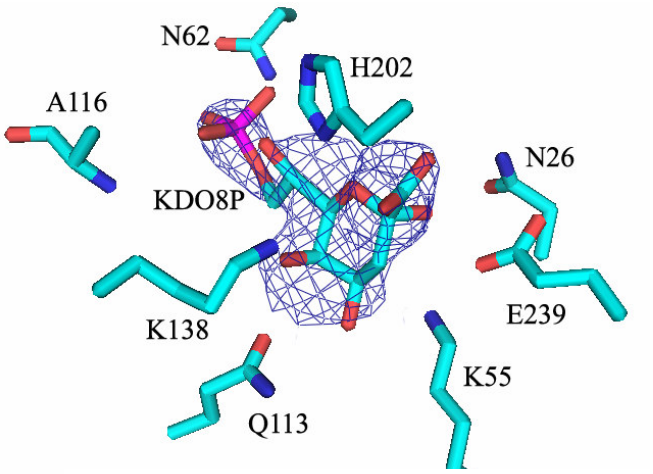


Fig. 2. Binding of the product KDO8P in the KDO8PS active site. Fo-Fc omit electron density map contoured at 1.5σ. Backbone atoms have been removed for clarity.

2) KDO8PS from the hyperthermophile *A. pyrophilus.* A number of trial crystals were examined, cro- protection conditions were found, and a data set to 3.5Å was collected. We have used these results to now improve the crystal quality, and we have now collected data to 2.2Å and structure determination is under way.