EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

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.

	Experiment title: Structural characterization of reaction intermediates in the O2 activation mechanism of an extradiol ring-cleaving dioxygenase trapped in crystallo	**Experiment number**: MX 840
Beamline: 14-1	**Date of experiment**: from: 06.12.2008 to: 07.12.2009	**Date of report**: 01.05.2009
Shifts: 3	**Local contact(s)**: Ganesh Natrajan	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr Arwen Pearson, University of Leeds, UK*

Dr Elena Kovaleva, University of Leeds, UK*

Prof. John Lipscomb, University of Minnesota, USA

Report:

This project aims to determine the molecular details of the oxygen activation, insertion and ring cleavage reactions catalysed by the mononuclear iron(II)-dependent 2,3-homoprotocatechuate dioxygenase. We are using a structural enzymology approach which aims to cryo-trap normally transient species for structure determination. In particular, we have identified enzyme mutants that either perturb the acid-base chemistry of the active site or alter protein dynamics and subsequently the subunit reactivity of this enzyme. We are using these mutants in combination with alternate substrate series with varied electronic properties to allow the detailed structural characterization of every important step in the oxygen activation process of a non-heme iron containing enzyme for the first time.

We were able to collect 26 high-resolution diffraction datasets (1.3-2.2A) during our beamtime. The resulting electron density maps are of excellent quality and are readily interpretable. Some very well diffracting crystals enabled us to record data to 1.3A resolution.

To date all the data has been processed and initial electron density maps have been calculated. We are now in the process of refining protein structures as well as structures of the ligands cryo-trapped in the active sites under specific reaction conditions. These structures are correlated with the single crystal UV/Vis and Raman spectroscopic data that was collected at the Cryobench Laboratory as part of the same trip.

Initial examinations of the electron density maps, particularly those at very high resolution have revealed interesting details in the active site. However, these data require careful refinement and chemical correlation before conclusions can be drawn. Some preliminary results obtained during this experiment have been presented at the Metals in Biology GRC meeting this January. They have also provided key preliminary data for a 4-year research grant application submitted to the BBSRC in January.