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.

ESRF	**Experiment title:** STUDIES ON THE CYCLIC NUCLEOTIDE METABOLISM	**Experiment number:** 14-U-793
Beamline: BM14U	**Date of experiment:** from: 15.12.2005 to: 16.12.2005	**Date of report:** 27.2.2006 (re-sent 13.12.2007)
Shifts: 3	**Local contact(s):** Dr. Hassan BELRHALI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Dr. Marjetka Podobnik

Petra Draškovič, PhD student

Department of Biosynthesis and Biotransformation
National Institute of Chemistry Slovenia
Hajdrihova 19, 1000 Ljubljana
Slovenia

Report:

PROJECT SUMMARY REPORT was sent to tronel@embl-grenoble.fr in February 2005.
The electonic form was (re-) submitted on December 13, 2007. The results of the data collection at BM14UK beamline in December 2005 were published in the scientific paper (reference bellow).

ABSTRACT:
'Cyclic nucleotide monophosphate (cNMP) hydrolysis in bacteria and eukaryotes is brought about by distinct cNMP phosphodiesterases (PDEs). Since these enzymes differ in amino acid sequence and properties, they have evolved by convergent evolution. Cyclic NMP PDEs cleave cNMPs to NMPs, and the Rv0805 gene product is, to date, the only identifiable cNMP PDE in the genome of Mycobacterium tuberculosis. We have shown that Rv0805 is a cAMP/cGMP dual specificity PDE, and is unrelated in amino acid sequence to the mammalian cNMP PDEs. Rv0805 is a dimeric, Fe(3+)-Mn(2+) binuclear PDE, and mutational analysis demonstrated that the active site metals are co-ordinated by conserved aspartate, histidine and asparagine residues. We report here the structure of the catalytic core of Rv0805, which is distantly related to the calcineurin-like phosphatases. The crystal structure of the Rv0805 dimer shows that the active site metals contribute to dimerization and thus play an additional structural role apart from their involvement in catalysis. We also present the crystal structures of the Asn97Ala mutant protein that lacks one of the Mn(2+) co-ordinating residues as well as the Asp66Ala mutant that has a compromised cAMP hydrolytic activity, providing a structural basis for the catalytic properties of these mutant proteins. A molecule of phosphate is bound in a bidentate manner at the active site of the Rv0805 wild-type protein, and cacodylate occupies a similar position in the crystal structure of the Asp66Ala mutant protein. A unique substrate binding pocket in Rv0805 was identified by computational docking studies, and the role of the His140 residue in interacting with cAMP was validated through mutational analysis. This report on the first structure of a bacterial cNMP PDE thus significantly extends our molecular understanding of cAMP hydrolysis in class III PDEs.'

X-ray diffraction data obtained for both mutant proteins, D66A and N97A, were successully collected at BM14UK. The beamline is also acknowledged in the paper.

REFERENCE:
Shenoy AR, Capuder M, Draskovic P, Lamba D, Visweswariah SS, Podobnik M.
Structural and biochemical analysis of the Rv0805 cyclic nucleotide phosphodiesterase from Mycobacterium tuberculosis. J Mol Biol. 2007 Jan 5;365(1):211-25. Epub 2006 Oct 6.