EUROPEAN **S**YNCHROTRON **R**ADIATION **F**ACILITY

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:	Experiment number:
	Crystal structure of the Lactococcus lactis formamidopyrimidine-DNA glycosylase bound to a substrat analogue-containing DNA	**MX-407**
Beamline:	**Date of experiment**:	**Date of report**:
ID14-2	from: 15/11/2004 to: 16/11/2004	01/12/2004
Shifts:	**Local contact(s)**:	*Received at ESRF:*
3	Chloe ZUBIETA	

Names and affiliations of applicants (* indicates experimentalists):

Franck COSTE* (CNRS, CBM Orléans, IR2)

Report:

The aim of this experiment was to understand better the recognition mechanism of damaged bases by Fpg. To date, two models have been proposed for the recognition of the damaged bases. The first one was obtained in Verdine's lab and shows the recognition mode of the 8-oxoG lesion by the *Bacillus stearothermophilus (Bs)* protein. The second one was obtained in our lab and shows the recognition mode of the FapyG lesion by the *Lactococcus lactis (Ll)* enzyme. In contrast to the recognition of the 8-oxoG lesion which is bound with the glycosidic bond in a syn conformation, the FapyG lesion displays in the complex an anti conformation. In order to find out if this difference in the recognition mode is really significant or is just a crystallization artefact depending of the molecules used, we crystallized mutants of the *Ll* enzyme with 8-oxoG lesions containing DNA. In the same time, we did some soaking experiments with 8-oxoG or FapyG free bases and a complex between the *Ll* Fpg protein and an abasic site containing DNA.

During the 3 allocated shifts, 19 crystals were tested and 12 x-ray diffraction datasets with high redundancy were collected. More datasets could have been collected but some time was lost because the PC allocated to the crystals auto-alignment was not working properly. The results of this experiment are the following:

1°) concerning the crystals of the complex « 8-oxoG containing DNA/*Ll* Fpg mutants », none diffracted below 8Å resolution as we observed in-house.

2°) concerning the soaking experiments, after having solved the structures by molecular replacement, it seems that the free bases are not located in the active site of the protein as we expected. The free bases are not very solubles alone so we are going to do some co-crystallisation experiments.

Getting good diffracting crystals of a complex between the *Ll* Fpg protein and a 8-oxoG containing DNA seems to be quite tricky so to answer our question about the recognition

mode of DNA lesions by FPG we crystallized a FapyG containing DNA on a double mutants Fpg protein. We hope to get soon some synchrotron beamtime to solve the structure of this complex at high resolution.