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: | Experiment number: |
|---|---|---|
| | DUNDEE-ST.ANDREWS BAG | LS-2178 |

| Beamline: | Date of experiment: | | | Date of report: |
|---|---|---|---|---|
| ID14EH2 | from: 08/05/2002 | to: | 09/05/2002 | 25/07/2002 |
| **Shifts:** | **Local contact(s):** | | | *Received at ESRF:* |
| 1 | Joanne McCarthy | | | |

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

**Charles Bond***

**School of Life Sciences, University of Dundee**

## Report:

## * Hje, a second Holliday junction resolvase from Sulfolobus solfataricus.

Following up on work from the previous BAG on Hjc, we have obtained crystals of Hje, a second resolvase from S. solfataricus which has homology to Hjc. The structure is of interest has Hje and Hjc have quite different DNA cutting patterns. Restriction enzymes in general utilise differing arrangements of monomers in a dimer to vary the cutting pattern, but sequence analysis suggests that Hjc and Hje should have a conserveddimer interface. Time on EH2 was used to obtain a good quality native dataset prior to the expected (and successful) structure solution on EH4 on the next day 09/05/2002 (see separate report).

Data: Data to 2.1 A resolution were collected from a crystal of dimensions 0.2x0.2x0.4mm grown at pH 8.0 from a solution containing tris buffer, $Li_2SO_4$ PEG 4000 and ethylene glycol. The data were of high quality (Rsym 4.0%) allowing confirmation of the spacegroup as $P6_{1/5}$. This knowledge prepared us fully for the subsequent MAD experiment, but the data has become redundant due to the recent collection of higher resolution data.

## * Mutants of Hjc, a Holliday junction resolvase.

Also following up BAG work, we observed that the same conspicuously exposed Ile residue in Hjc was responsible for crystal contacts in both hexagonal and cubic crystal forms, and that this might explain concentration-dependent inhibition of Hjc's activity and cause its 'hypercrystallisability' (poorly diffracting crystals grow within minutes in over half of the Hampton conditions). We rationally designed a mutant, delta62-63 with the view that this should remove this interface with the minimum structural disturbance. Indeed crystals grow more slowly in a new primitive monoclinic spacegroup which makes screening for protein-DNA complex crystals more straightforward.

Data: Highly redundant data to 2.2 A resolution were collected on crystals of dimensions 0.1x0.1x0.5mm in spacegroup $P2_{(1)}$. This data is not very clean, possibly due to crystal splitting, however judicious data processing followed by molecular replacement (AMORE) using the native Hjc dimer has produced an interpretable map in $P2_1$ showing disruption of the relevant part of the structure. Refinement/analysis is underway.

## * Hjc-DNA cocrystals.

Numerous crystals of Hjc cocrystallised with double-stranded DNA, single-stranded DNA or Holliday junction DNA were screened. Two crystals diffracted well enough for full data collection, but both were in previously characterised native Hjc crystal forms (P6122 and I23) and neither contained any DNA. This work is ongoing and use of BAG time for screening the sometimes poorly diffracting crystals is beneficial in leading us towards much desired crystals of a complex.