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:
	Crystal structure of ribosomal release factor 3 and fusidic-acid hypersensitive mutants of elongation factor G	MX-414
Beamline: ID29	**Date of experiment**: from: 04/3/2005 to: 05/3/2005	**Date of report**: 07/7/2005
Shifts: 3	**Local contact(s)**: William SHEPARD	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

RF3: We have studied crystals of RF3 from the mesophilic bacterium *Bacteroides (Dichelobacter) nodosus* in complex with GDP. The crystals are tiny, approximately 0.1 x 0.05 x 0.05 mm and we have so far been unable to improve their size The crystals diffract very weakly to 3.5 A at beamline I711 of the MAX-lab synchrotron and gave reasonable data to 3.0Å at beamline ID29 during this experiment. The space group is apparently $P2_12_12$. The cell dimensions are a = 55.7 Å, b = 66.1 Å, c = 153.7 Å, giving a solvent content of 48% and one molecule in the asymmetric unit. Crystalos were transported in the crystallization drops, but did not appear to have travelled well. We screened a large number of native crystals but obtained only one dataset, which proved very challenging to process. We then attempted to solve the structure using molecular replacement. However the best available model is elongation factor G from *Thermus thermophilus*, which is only 24% identical to *B. nodosus* RF3 along the whole 531-amino acid length of the latter. If one limits the alignment to the three GTPase domains (I/G, II and III, around 400 amino acids) thought to be common between the two enzymes, the identity is still only 29%, which is on the borderline for MR given the known flexibility of translational GTPases. None of our attempts to solve the structure using this model have so far been successful, even using state-of-the-art programs such as Phaser.

SeMet protein has been crystallized as for the native enzyme. We attempted data collection at ID29 but the non-frozen crystals did not appear to have travelled well. An edge scan revealed that they contained Se but they did not diffract beyond 8Å. We have improved crystal quality for the SeMet protein and in future will also "pre-freeze" a number of crystals, characterize them if possible at MAX-lab and send the best ones by courier. In view of the problems encountered with molecular

replacement, MAD or at the least SAD data from SeMet or some other derivative seem imperative for this project.

Other projects for which data was collected during this experiment:

Elongation factor G mutant T84A in complex with GDPCP: We have recently solved the structure of this mutant of elongation factor G (EF-G) in complex with GDPNP, which has revealed for the first time a GTP-like conformation of EF-G. However a peptide bond in the GTPase P-loop was seen to flip in order to hydrogen-bond to the imido nitrogen atom of GDPNP. This has not been observed in other translational GTPases. Thus in order to obtain additional independent information we collected data to 2.9 Å on crystals of the same mutant soaked with GDPCP, where this bond cannot be formed. The crystal structure revealed the same features as the GDPNP structure but without the peptide flip, as expected (Hansson, Singh, Gudkov, Liljas & Logan, *FEBS Letters,* in press)

Class II, coenzyme B12-dependent ribonucleotide reductase: two datasets to around 2.0 Å resolution were collected on crystals which had been soaked for short times with adenosylcobalamin in order to study the cofactor/enzyme complex and the mechanism for C-Co bond cleavage. Previous experiments with longer soaking times had resulted in cleavage of the C-Co bond before or during data collection. However no adenosylcobalamin was found in the active sites, showing that the soaking time was too short.

Class III, anaerobic ribonucleotide reductase: a dataset was collected to 3.1 Å resolution on the R291A mutant in order to study the possible mechanistic roles of completely conserved residues in the active site. The data were 96.1% complete with R_{merge} 7.2%. The structure showed significant movement in a loop containing an essential radical cysteine, demonstrating that Arg291 is important for pinning down this loop in the active site. A manuscript containing these data is in preparation.

*Tn*Bgl3B: We collected a preliminary native dataset to 2.6 Å resolution from crystals of this β-glucosidase from *Thermotoga neapolitana.* One of the main reasons for determining this structure is to provide a structural basis for engineering the enzyme for better synthesis of alkylglucosides. Crystals are small and despite extensive effort the size cannot be improved. They belong to space group $P2_12_12_1$ with cell dimensions a = 67.5 b = 106.2 c =175.5, giving a solvent content of 57.8% and two molecules in the asymmetric unit. The data are 94.2% complete with R_{merge}=10.1%. However experimental phase information is essential for this project, as there are only two other crystal structures available of members of the glycosyl hydrolase 3 family, and these have only 17% and 19% sequence identity in 400 of the 720 total amino acids constituting *Tn*Bgl3B.

An autoimmune antibody in complex with a collagen type II peptide: This antibody leads to collagen-induced arthritis in mice. We collected one dataset to 2.0 Å resolution in space group $P2_1$. R_{merge} was 13.7% and the data were 99% complete with an average I/s(I) = 7.8. The structure has been solved and refined.