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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: **BAG-LEBS-ESRF-2003-1**	Experiment number: MX-60
Beamline: ID14-EH2	**Date of experiment**: from:02/08/2003 to: 04/08/2003	**Date of report**: 29/08/03
Shifts: 6	**Local contact(s)**: Dr. Cecile JAMIN	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

N. Fay (student), R. Melki(research director), M. Graille (Post-doc), P. Meyer (Post-doc), G. Hible (student), L. Renault (research assistant), V. Biou (research assistant), L. Guigou (student), T. Bizebard (research assistant), LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France

Corine Mas (student)*, Renaud Dumas (research assistant), CEA Grenoble

Report:

N. Fay + R. Melki (0.5 shifts): Structural/fibrils formation study of/by Ure2p

The [URE3] phenotype in yeast *Saccharomyces cerevisiae* is due to an altered form of Ure2p, a protein involved in nitrogen catabolism. To understand possible conformational changes at the origin of this structure-based inheritance, we previously solved the crystal structure of the Ure2p functional region [Bousset et al. (2001) *Structure 9*, 39-46]. We showed the protein to have a fold similar to that of the, class of glutathione *S*-transferases (GSTs). We also solved the crystal structures of the functional region of Ure2p (extending from residues 95-354) in complex with glutathione (GSH), the substrate of all GSTs, and two widely used GST inhibitors, namely, *S*-hexylglutathione and *S-p*-nitrobenzylglutathione [Bousset et al. (2001) Biochemistry. 40 :13564-7]. Ure2p assembles into fibrils. The protein looses its function upon assembly. X-ray fiber diffraction measurements allow us to access the packing of Ure2p molecules in the fibrils. Also, to determine the extent of changes in the conformation of Ure2p that lead to the loss of its function upon assembly.

X-ray fiber diffraction patterns of Ure2p fibrils assembled under different experimental conditions were collected. These patterns reveal in some cases cross beta sheet structures. This is part of an ongoing project [L., Bousset et al., (2003) J. Struct. Biol. 141, 132-142].

M. Graille + P. Meyer (1.65 shifts): yeast *Saccharomyces cerevisiae* Structural Genomics project

The systematic names of the genes are used. More details on every orf can be found on http://genomics.eu.org:targets.html

1) YGL148w (target 238).
Spacegroup I222 a= 58 Å, b=75 Å and c=153Å.
Resolution 2.25A.
Completion 89.6%
Rsym=9.5%

This orf codes for a chorismate synthase, an enzyme that catalyzes the last of the seven steps in the shikimate pathway, which is used in prokaryotes, fungi and plants for the biosynthesis of aromatic amino acids. Yeast chorismate synthase is a bifunctional enzyme: it first reduces flavin mononucleotide by NADPH and then catalyzes the 1,4-trans elimination of the phosphate group from 5-enolpyruvylshikimate-3-phosphate (EPSP) to form chorismate which can then be used in phenylalanine, tyrosine or tryptophan biosynthesis. The structure of this protein has recently been solved by SAD from SeMet data collected on BM30A beamline. In parallel, crystals containing various combinations of the three ligands have also been obtained. During this allocation time, we have collected a full dataset from a crystal grown in presence of the EPSP substrate. Unfortunately, difference Fourrier map revealed that the substrate is not bound to the protein in the crystal.

2) Corella virus ThyX protein.
Spacegroup $P2_12_12$ a=93A; b=69A; c=77A.
Resolution 2.14A.
Completion 96.2%
Rsym=8.5%

Thymidylate synthase are essential proteins in eukaryotes and procaryotes. However, many pathogenic bacteria and archebacteria are missing his essential gene. Recently a new gene, ThyX, complementing for thymidylate synthase was discovered. This gene is present in all organisms that are missing the classical thymidylate synthase. The activity of this gene was demonstrated by the group of Dr. Myllykallio at the IGM in Orsay. In collaboration with this group, we crystallized the ThyX protein from Corella virus. Crystals diffract to 5A on a rotating anode. A homologous structure was recently determined in another structural genomics project. We have collected two datasets to resolutions if 2.8 A and 2.14A, respectively. Molecular replacement trials are currently in progress to solve the structure of this protein.

3) Rnt1p.
Spacegroup C2.
Resolution 2.5 A.
Completion 94.3%
Rsym=7.1%

Rnt1p is the ortholog of the yeast ribonuclease III. It is made of 3 domains: the first one is involved in dimerisation, the second one has the catalytic activity and the third one binds to double stranded RNA (also named dsRBD). The dsRBD specifically recognises RNA hairpins with a AGNN loop. We have already solved the structure of this domain by NMR but this method failed to solve the structure of the dsRBD-RNA complex. We have then started the study of a little bit longer construct (20 C-terminal amino acids more) by X-ray crystallography. During this session, we have collected a 2.5A resolution dataset which allowed to solve the structure of the dsRBD complex by molecular replacement, using the NMR structure as search model. This structure is currently under refinement.

G. Hible + L. Renault (1.1 shifts): Structural study of Guanylate Monophosphate Kinase and of the GEF-catalysed activation of Arf small GTP-binding protein

Structural study of Guanylate Monophosphate Kinase

Guanylate Kinase (GMPK) is a nucleoside monophosphate kinase that is essential for the biosynthesis of GTP and dGTP by catalyzing the reversible phosphoryl transfer from ATP to (d)GMP to yeld ADP and (d)GDP. In addition, antiviral prodrugs like Aciclovir, and anticancer prodrugs, are dependent of this enzymze for their activation. Our aim is to characterize the catalytic intermediates and use the structures as models for the activation of antiviral prodrugs.

We have investigated different nucleotides and analogues in complex with a bacterial GMPK (23,5kDa) and we have collected two datasets :

1) a complete dataset on a crystal of GMPK-ADPnP at 3,1Å resolution. The crystal belongs to space group $P4_32_12$ with unit cell parameters : a=b=108Å c=273Å, = = =90° (R-sym= 29,7% for the last resolution shell : from 3.11 to 3.10 Å). Estimation of the Matthews parameters give 6 to 8 molecules per asymmetric unit. Furthermore the crystal exhibit important merohedral twinning.

The model available is a monomere with only 39% identity to our protein and no solution for molecular replacement has been obtained so far. We are actually trying to improve cristal quality and to produce heavy atom derivatives in order to solve the structures by MAD or MIRAS.

2) a complete dataset on a crystal of GMPK-GDP-ADP at 2.45 Å resolution. The crystal belongs to space group $P6_3$ with unit cell parameters : a=b=68,2Å c=151,2Å, = =90° =120° (R-sym= 30% for the last resolution shell : from 2.50 to 2.45 Å). Estimation of the Matthews parameters give 2 molecules per asymmetric unit. Unfortunately the crystal appears to have an 38% estimated merohedral twinnig, thus leading to an inexploitable dataset.

We finally succeed to produce a crystal with the same unit cell parameters and space group, and no merohedral twinning (The crystallisation condition of the two crystal was identical exepted for the additive). We have collected a complete dataset on the beamline BM30-A at the ESRF at 2.0 Å resolution.

We have found a molecular replacement solution with AmoRe.We are now trying to refine the model in order to improve the electronic density map.

Study of the GEF-catalysed activation of Arf small GTP-binding protein

Arf G proteins functions as binary switches in regulating transport vesicle budding in endocytosis and exocytosis and phospholipase D activation by cycling between inactive cytosolic GDP-bound and active membrane-anchored GTP-bound states. Like many other regulatory G proteins, the conversion of Arf-GDP to Arf-GTP is intrinsically very slow and is catalyzed by a guanine nucleotide exchange factor (GEF) along a complex multi-step reaction which is poorly understood at the molecular level. This reaction involves binary and ternary complexes between G protein, guanine nucleotide, and GEF, that we try to trap for structural studies by mutations.

We have tested on ID14-EH2 different small crystals of the Arf-GEF Sec7 domain of Arno with different mutations. We have collected a single and slightly twinned data set to 1.7 Å. A full data set could not be obtained by lack of beam time on ID14-EH3 but we have been able to collect again on the same project later on BM30A. On BM3A we have collected a data set to 2.0 Å of middle quality (strong ice rings). Both data sets were therefore merged to obtain a full data set. The crystals belong to space group $P2_1$ and the merge data sets gives an overall Rsym=6.6% (an Rsym = 12.2% in the last resolution shell from 1.75 to 1.70 Å) with an overall completeness of 91.4% (65.5% in the last resolution shell from 1.75 to 1.70 Å). A molecular replacement solution has been obtained and refined to a final Rcryst=19.6% (Rcryst=24.4% in the last resolution shell from 1.75 to 1.70 Å) and a final Rfree=22.9% (Rfree=27.7% in the last resolution shell from 1.75 to 1.70 Å). The structure analysis is in process to understand the role of the mutants on the conformation and flexibility of the GEF-catalytic domain of ARNO.

V. Biou + C. Mas + R. Dumas (1.3 shifts): Structural study of threonine synthase

The experiment was run in 16 bunch mode. It followed one day at BM30 during which we were able to screen crystals, which we could not do in the lab because of their sizes.

We collected five data sets on crystals of wild type threonine synthase from plant. Those were new crystallisation conditions obtained using robot screening, and that allowed us to co-crystallise the protein with both its activator and its cofactor, which we had never been able to do before.

The best data set was interpretable to 3.2Å resolution.

Data processing with mosflm gave a triclinic Unit cell of 111.73Å, 111.73Å, 154Å, 90°, 90°,120°.
Space group determination gave the following Rsym :

Space group	Resolution limit	Rsym overall	At diffraction edge
P3	3.5	0.08	0.28
P321	3.2	0.107	0.67
P312	3.2	0.316	0.78
P6	3.5	0.35	0.84

L. Guigou (0.5 shifts): Structural study of the tRNA-binding protein p43

p43 is a tRNA-binding protein and is part of a large complex (1,5 MDa) comprising 9 enzymes of the Aminoacyl-tRNA synthetase family and three so-called auxiliary proteins, p43 being one of them. The cleavage of p43 by caspase-7, during apoptosis, leads to the release of EMAPII, that has lost the tRNA-binding properties of p43 but seems to possess a cytokine activity. We have isolated another short form of p43 that binds tRNA better than either full-length p43 and EMAP-II, and is much more soluble. Given the high affinity of this new p43-derivative, that we called EMAP-X; for tRNA, we tried and succeeded in obtaining crystals of this protein complexed to a RNA stem-loop, that mimics the acceptor domain of a tRNA.
Tests on a rotating anode have showed that those crystals diffract to at least 4 Angström, but they're both needle-shaped and twinned.
The time on ID14 has been used to test a little crystal (50 × 25 × 25 μm) that didn't look twinned. Unfortunately, no diffraction at all occured, probably because of a problem during the transfer of the crystal from the drop to the cryo-loop.

T. Bizebard (1 shift):Structural studies of bacterial ribonucleases of the RNase E/G family

During the allocated shift, various diffraction tests were made with crystals of the catalytic domain of *E.coli* Ribonuclease E (RNase E) on beamline ID14-2. These crystals only diffracted to very low resolution (> 10 Ångstroms) when exposed to the beam of the laboratory X-ray generator; at the ESRF, the diffraction limit could be increased to ca. 7 Ånsgstroms - an improvement insufficient to undertake a thorough study of this crystal form.