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

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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-EH3	**Date of experiment**: from:04/07/2003 to: 05/07/2003	**Date of report**: 29/08/03
Shifts: 3	**Local contact(s)**: Dr Elena MICOSSI	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

I. Llorens (student), P. Meyer (Post-doc), **B. Golinelli (research assistant), S. Mouilleron (student), L. Renault (research assistant),** LEBS, Bat 34, CNRS UPR9063, 1 av. de la Terrasse, Gif-Sur-Yvette, France

Report:

I. Llorens, P. Meyer (1.15 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) YIR029w (target 205)

Spacegroup $P3_221$ a= b=109A; c=190A.
Resolution 2.2A.
Completion 100%
Rsym=6.7%

This orf codes for an allantoicase, an enzyme associated with the purine metabolism. It breaks down allantoin into glyoxylate and urea and thus is involved in the use of purine as a secondary source of nitrogen. We have collected a wavelength SAD SeMet dataset which allowed the resolution of the structure to 2.2A resolution.

2) DNA topoisomerase VI.

During this beamtime allocation, we were also able to test the diffraction power of many crystals of the DNA topoisomerase VI from the archaebacteria *S. shibatae*. One of these crystals diffracted to 4A but due to radiation damage, we could not collect a full dataset.

B. Golinelli (research assistant), S. Mouilleron (student) (0.8 shift): Structural study of a pyridoxal-P catalytic antibody complexed with different substrates

A 2.3 Å full data set of a pyridoxal-P catalytic antibody complexed with a D-ala-pyridoxal-P substrate analogue (space group P21, a=63.1, b=81.1, c=79.3, β=90.3) was recorded. The completion is 99.9 % (99.9 % in the last shell), Rsym=9.1 % (59.2 % in the last shell). The structure has been solved by molecular replacement and contains the ligand. The structure has been refined to R=25% Rfree=29.8%. A 2.2 Å full data set of the same catalytic antibody complexed with a L-ala-pyridoxal-P substrate analogue was recorded. The crystal was partially twinned ans is currently being processed. Different types of crystals of glucosamine-6-P synthase in complex with two substrate analogs have also been tested.

L. Renault (1.05 shift) : 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-EH3 different small crystals obtained in presence of different constructs of the small GTP-binding protein Arf1 and the Arf-GEF Sec7 domain of Arno and in different purification and crystallization conditions. We have collected two data sets to 1.86 Å and 1.46 Å on two different crystalline forms. The first crystal belongs to $P2_12_12_1$ space group and gives an overall Rsym=8.7% (an Rsym = 42.4% in the last resolution shell from 1.88 to 1.86 Å) with an overall completeness of 97.1% (83.6% in the last resolution shell from 1.88 to 1.86 Å). The second crystal belongs to $P3_221$ space group and gives an overall Rsym=5.9% (an Rsym = 46.2% in the last resolution shell from 1.47 to 1.46 Å) with an overall completeness of 99.3% (97.0% in the last resolution shell from 1.47 to 1.46 Å). Solutions for molecular replacement have been obtained with Arf1Δ17 and the Sec7 domain of ARNO as search models confirming the presence of the proteins complex in both crystal forms. Refinement is in process.