	Experiment title: BAG-LEBS-2004-2	Experiment number: MX-350
Beamline: ID23-H1	**Date of experiment**: from: 19/02/2005 8h to: 21/02/2005 8h	**Date of report**: 22/2/05
Shifts: 6	**Local contact(s)**: Dr D. Nurizzo	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Lionel Trésaugues, Nicolas Leulliot (3 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) PH0168
The report has been included at the end of the text of the proposal.

2) Yeast YGL047w.
Spacegroup $P4_22_12$ a=b=168A; c=120A.
Resolution 3.3A.
Completion 100%
Rsym 10%

This essential yeast protein is required for the second step of dolichyl-linked oligosaccharide synthesis and is involved in N-linked glycosylation. It presents similarity to bacterial glycosyltransferases. We have tried to co-crystallise this protein with different sugars and crystals appeared in the presence of UDP-galactose. During this session, we have collected 2 MAD datasets from SeMet labelled protein crystals. Phasing assays are in progress.

3) Yeast Tpd3.
Spacegroup $P4_12_12$ a=b=130A; c=184.
Resolution 3.3A.

Completion 100%
Rsym 10%

The protein PP2A made of three subunits A, B and C has an important role in various cellular processes. The Tpd3 protein is the PP2A regulatory subunit A. During this session, we have collected a 3.3A dataset from crystal grown with a native protein. The structure of the human homolog is known (44% sequence identity) and indicates that conformational changes could occur, this dataset will allow us to test if the yeast structure can be solved by molecular replacement using the human structure as model or if SeMet labelling is needed.

4) Yeast Pst2.
Spacegroup F222 a=54A, b=126A; c=214A.
Resolution 2.4A.
Completion 96%
Rsym 12%
This 21kDa protein of unknown function has similarity to members of a family of flavodoxin-like proteins and shares 66% sequence identity with another yeast protein (YCP4) that we are currently studying but which does not have methionine residues. Resolution of this structure would help for the resolution of the YCP4 structure (4A dataset collected previously) and should provide helpful information on eukaryotic flavodoxins, proteins for which very little is known from a structural point of view. We have collected a 2.4A resolution MAD dataset as well as one native dataset. Phasing is currently under investigation.

Vincent CHAPTAL, Solange MORERA (2 shifts) : enymology of:

Hpr kinase/Phosphorylase (HprK/P):

HprK/P is a bifunctional enzyme that belongs to a new family of Ser/Thr kinases with a P-loop nucleotide binding site. We previously solved the structures of the native protein and its complex with Hpr. However, these two structures show HprK/P in its phosphorylase activity. We would like to get the kinase form of the enzyme using a mutant of HprK/P. We collected three dataset: the mutant alone, the mutant in complex with ATP and the mutant in complex with FBP, all around 3 Å resolution. The data are under process.

Yeast P-loop protein: YP6:

We started a systematic structural and functional analysis of yeast P-loop containing proteins of unknown function. The HPr K/P example suggests that some of them could also be protein kinases. We cloned 12 out of them and 5 soluble proteins (called YP4 to YP8) are under crystallization trials. We recently solved the structure of YP6 by SAD method. Here, we collected two datasets at 2 Å resolution:

YP6 in complex with ATP and YP6 in complex with CMP. The data are under process.

NDP kinase :

We tested several crystals of NDP kinase in complex with dioxolane-diphosphate without success. The diffraction quality was bad.

Guillaume HIBLE (1 shift): Structural study of a bacterial Guanylate 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 yield ADP and (d)GDP. In addition, antiviral prodrugs like Aciclovir, and anticancer prodrugs, are dependent of this enzyme 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 several crystals of two putative complexes between one bacterial GMPK and nucleotide analogues. We have tested small crystals (~ 20μm^3) for the first complex that diffracted at only 6 Å resolution. We have tested 15 crystals of the second complex that diffracted at 3.2 to 4 Å resolution, which allowed us to collect 3 incomplete datasets, as the crystal diffraction pattern exhibited high anisotropy. Actually these datasets should not lead to structure resolution as the quality of the data is limited. Indeed Rsym, I/? I and redundancy parameters are rather bad after merging of the three datasets. Improvements of the crystal quality for both complex is in progress.