	Experiment title: BAG-LEBS-2005-2	Experiment number: MX-441
Beamline: ID14-EH1	**Date of experiment**: from: 17/09/2005 8h to: 19/09/2005 8h	**Date of report**: 24/02/05
Shifts: 6	**Local contact(s)**: Dr S. MONACO	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Mark BROOKS (3 shift) : 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) Yeast YGL047w.

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, several crystals have been tested but the resolution was too low to collect any datasets.

2) *P. abyssi Pab1159 protein.*

Spacegroup $P2_12_12_1$ a=61A; b=69A;c=73.5A.
Resolution 2.9A
Completion: 100 %.
Rsym=9.1%.

This protein of unknown function is conserved among the three kingdoms of life. From sequence analysis, it is predicted to be an endosialopeptidase. In order to get information on its function, we have purified and crystallized this protein from the archae *Pyrococcus abyssi*. We have previously collected a complete native dataset to 1.5A resolution. During this session, we have screened several crystals soaked with heavy metal derivatives and have collected one dataset from a mercury derivative. Data processing of this dataset did not revealed the presence of this derivative.

3) EVF protein.
Spacegroup $P2_12_12_1$ a=69A, b=86A; c=91A.
Resolution 2.7A.
Completion 97.8%
Rsym 8.9%

This 31kDa protein from the bacterium *Erwinia carotovora* is a virulence factor that affects *Drosophila melanogaster*. Nothing is known about the virulence mechanism of this protein. In collaboration with B. Lemaitre (CGM, CNRS, Gif/Yvette), we have undertaken the determination of the crystal structure of this protein to try to get insights into the function of this protein. During this run, we have been able to collect for the first time, a complete native dataset. As no structural homologues are known, SeMet labelling will be performed soon.

4) S. cerevisiae YLR016c protein.

This protein is a subunit of the heterotrimeric RES complex (for pre-mRNA REtention and Splicing), which is required for nuclear retention of unspliced pre-mRNAs. The sSubunits of this complex are not essential for viability but are required for efficient splicing *in vitro* and *in vivo*. Furthermore, inactivation of this complex causes pre-mRNA leakage from the nucleus. The presence of RES subunit homologues in numerous eukaryotes suggests that its function is evolutionarily conserved. We are collaborating with B. Séraphin's group (CGM, CNRS, Gif/Yvette) to determine the structure of this complex. To do so, we are studying in parallel the complex but also its isolated subunits. We have previously been able to collect a native dataset to 2.6A and during this session, we have tried to collect a higher resolution native dataset. Unfortunately, no crystals diffract to better than 2.8A.

Philip SIMISTER (2.5 shifts) : BIG1 Sec7 Guanine exchange factor of Arf small G protein:

The experiment went well. We tested nine crystals, the best of which diffracted to 2.3 Å; we collected a high and a low resolution pass. The processing statistics are listed in the table below showing the data quality. Despite sequence homology of ~40% to the best molecular replacement model, the solution could not be found with extensive attempts in MolRep nor AmoRe. The ß angle is close to 90°C but no improvement was found in P orthorhombic. We tried solving the MR with Phaser, which has yielded a better result. These new solutions are currently under investigation.

Data collection statistics		
Space Group		$P2_1$
Unit Cell	a (Å)	64.266
	b (Å)	36.777
	c (Å)	85.533
	ß (°)	89.797
Wavelength (Å)		0.934
Resolution limits (Å)		50.0 – 2.3 (2.42 – 2.3)
Reflections:	measured	86880
	unique	18123
Completeness (%)		99.7 (99.9)
R_{symm} (%)		7.6 (41.0)
I / σ		14.8 (4.2)

N.B. Values in parentheses are for the highest resolution shell.

Louis RENAULT (0.5 shift) : Structural study of innate immunity defense Guanylate binding proteins (GBPs):

Guanylate binding protein (GBP) 1 is a 67kDa multi-domain GTP-binding protein which relays antiviral and anti-angiogenic effects in cells with unknown mechanisms at the molecular level (Guenzi, E. et al. (2003) The guanylate binding protein-1 GTPase controls the invasive and angiogenic capability of endothelial cells through inhibition of MMP-1 expression. EMBO J. 22, 3772-82). GBP1 belongs to the family of dynamin-related GTPases which are multi-domain large GTPases characterized by nucleotide-dependent oligomerizations associated with high-turnover GTPase activities. Their mechanisms of regulation as molecular switch or mechano-chemical enzymes in key cellular pathways remain elusive (Praefcke, G. J. & McMahon, H. T. (2004) The dynamin superfamily: universal membrane tubulation and fission molecules? Nat Rev Mol Cell Biol 5, 133-47). Additionally GBP isoforms have the unique capacity among GTPase to hydrolyze GTP into GDP and GMP. As first model of Guanylate binding proteins we target the molecular mechanism of regulation of GBP1.

We tested on ID14-1 poorly diffracting crystals of the truncated GTPase domain of GBP1 in complex with GTP (68 kDa dimeric active state) or in complex with GDP (intermediate state) and collected a second and low resolution data set of the truncated GTPase domain of GBP1 in complex with GMP (monomeric product state) to confirm the absence of Mg^{2+} in GMP binding (Ghosh A., Praefcke G. J. K., Renault L.[#], Wittinghofer A.[#], Herrmann C. (2006) How Guanylate Binding Proteins achieve assembly stimulated processive cleavage of GTP to GMP. *Nature, in press*).