	Experiment title: BAG-LEBS-2007-2	Experiment number: MX-669
Beamline: ID29	**Date of experiment**: from: 28/01/2008 at 8:30 to: 29/01/2008 at 8:00	**Date of report**: 28/2/06
Shifts: 6	**Local contact(s)**: **Dr. Gianlucca CROCI**	*Received at ESRF:*

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Report:

Nicolas Leulliot* (Assistant professor, Univ. Paris-Sud), Mark Brooks* (CNRS, post-doc)**: yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project (1.2 shift)**

1) Tpa1

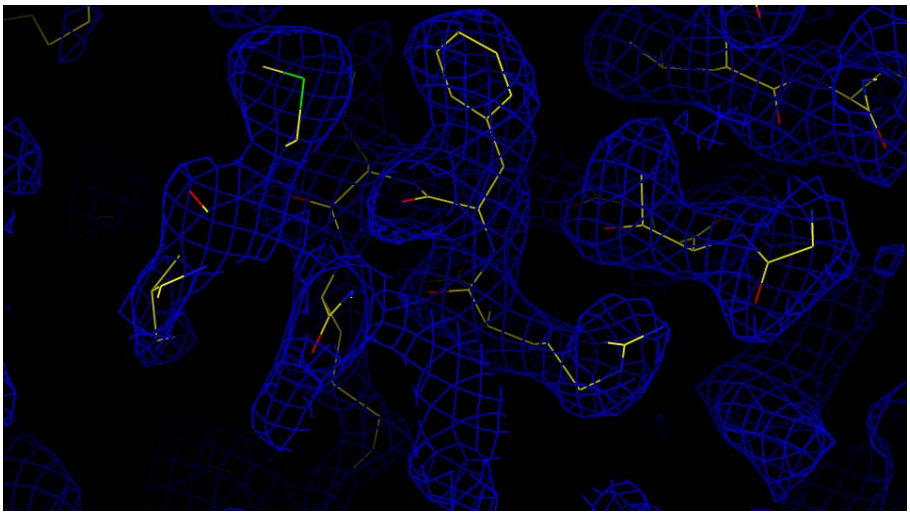
The deletion of the gene encoding for yeast Tpa1 protein strongly affects translation termination, deadenylation and mRNA stability, suggesting a role in the control of gene expression at the level of translation. The Tpa1 protein is a component of a ribonucleotidic complex bound to the 3'-end of mRNAs. The knowledge of its 3D structure might help to decipher the precise function of Tpa1. Crystals of native and selenomethionlylated protein have been grown and diffract to 3Å. However, they suffer from serious anisotropy and weak anomalous signal.

2) V28

Spacegroup: P21 a=50A b=59A c=84A
Resolution: 2.7
Completion= 100%
Rsym=10%

Lately, phages infecting hyperthermophilic and acidophilic archaea (Sulfolobus, Acidianus) have been discovered in geothermally heated hot aquatic environments. They belong to a new class unrelated to other phages. 90% of their genes have no homologues in sequences databases suggesting unknown mechanisms for their biological functions. With the aim of deciphering this new branch of life at a molecular level, we have launched a multidisciplinary project for systematic expression and structure/function determination of all proteins from Acidianus Filamentus phages (AFV) associating structural, biochemical and genetical

approaches. The strategy consists in expression, purification and structure determination of 60 proteins from 2 archaeophages. In a situation where little information is deductible from sequence, 3D structure will lead to hypothesis about the molecular function. V28 is a 12kD protein from AFV6 which is homologue to CopG a transcription factor from a plasmid from *E.coli*, involved in copies number control. We collected a data set on a SeMet labelled crystal at the Se k-edge. The quality of anomalous signal and diffraction pattern allowed us to solve the structure (see experimental electron density map). The structure is currently rebuilt and refined against a 2.3A native dataset.



During this session, several crystals were tested, the best diffracted to 4Å resolution. Considering the sets previously collected, no data was collected.

P. Roblin, L. Renault (0.7 shifts): structural studies of complexes between actin and actin-nucleators and of GTP-dependent oligomerization of GBP1.

structural studies of complexes between actin and actin-nucleators.

Spire is known to regulate polarity of both oocytes and embryos during Drosophila development. Spire accelerates actin filament assembly by promoting de novo filament nucleation via its N-terminal half which contains four WASP homology 2 (WH2) domains (Quinlan M. E. at al. (2005) Drosophila Spire is an actin nucleation factor. Nature 433, 382-8). In addition the four Spire WH2 domains inhibit actin filament disassembly from filament pointed end. How a nucleating activity may emerge from the cooperation of several WH2 domains in Spire proteins is not understood.

We have initiated structural studies with several constructs containing from one to four WH2 domains of Spire alone and in complex with actin to enlighten the role of the different domains in the mechanism of actin nucleation, filament formation and the different properties of Spire proteins towards each filament end. We have tested on ID29 different crystal forms that we have obtained recently with a construct of Spire in complex with several actin monomers in different nucleotide-states, a few with inhibitors preventing actin polymerization. A few crystals were crystals of small molecules. The best protein crystal form diffracted at the most to 7 Å on ID29 preventing the structure to be currently solved. With a unit cell dimension of 450 Å, the crystals have an hexagonal symmetry with 6 to 12 complexes per assymetric unit. Further crystallization improvements are required to solve the structure.

structural studies of the GTP-dependent oligomerization of GBP1.

Gamma-induced Guanylate-binding proteins are 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. As a first model

we target the poorly understood function of human GBP1 of 68 kDa which relays antiviral and anti-angiogenic effects in cells with mechanisms not understood 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.). Using ESRF beamlines, we have recently solved the first structural basis for the catalytic machinery of the Nterminal GTPase domain of GBPs which has both a GTPase and GDPase activity (Ghosh A et al., (2006) How Guanylate Binding Proteins achieve assembly stimulated processive cleavage of GTP to GMP. Nature, 440, 101). We are focusing now on the possible cross-talk between GBP1 different domains upon nucleotide-dependent oligomerization by analysing conformational changes between the different states of full-length GB1-GTP dimers, full length GBP1-GDP/AlF tetramers and full length GBP1-GDP monomers.

To understand the link between oligomerization, membrane-anchoring, and GTPase/GDPase activity we have produced myristoylated-GBP1 and obtained different crystal forms without nucleotide. With ID29 beam time, we have collected a native data set at 2.5 Å resolution. Analysis showed the presence of pseudo-merohedral twinning with a twinning fraction close to 50%, which emulates orthorhombic symmetry. We have solved the phasing by molecular replacement but electron density maps did not show the membrane-anchoring region and the pseudo-merohedral twinning with a twinning fraction close to 50% makes the assignement of the monoclinic space group ambiguous. Refinement is in process to know if the membrane-anchoring region is organized and linked to nucleotide-binding region.

Solange Morera *, Sylvie Nessler*, Kelvin Eckert*, Jakub Gruczszyk* : structural and functional analysis of protein kinases and helicases in conjunction with the Hsp90 machinery and of complexes with specific inhibitors for therapeutic purposes (1.1 shift)

We tested a lot of crystals. Crystals of AP and Rvb diffracted very poorly, no data collection was done. We collected 3 datasets on CapB and AMADH:

- a 3.5 A and a 4 A resolution datasets of bacterial tyrosine kinase CapB. We had no time to process the data yet. We will do it soon.
- A hight resolution dataset (2.4 A) of AMADH (aminoaldehydr dehydrogenase from Pisum sativum). The structure is under refinement.