	Experiment title: BAG-LEBS-2007-2	Experiment number: MX-669
Beamline: ID23-1	**Date of experiment**: from 22/09/07-8:30 to 23/09/07-8:00	**Date of report**: 21/2/08
Shifts: 3	**Local contact(s)**: Dr **G. CIOCI**	*Received at ESRF:*

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Report:

Marc Graille*, Julien Henri*: yeast multi-protein complexes involved in DNA replication, ribosome biogenesis, mRNA quality control pathway and cell signalling and archeophage structural genomics project (1 shift)

1) *S. cerevisiae* Alg13
Spacegroup: P432 with a=b=c=120Å.
Resolution: 4A.
Completion= 100%
Rsym=10%

Alg13 is the catalytic component of UDP-GlcNAc transferase. This essential enzyme is required for the second step of dolichyl-linked oligosaccharide synthesis and hence is an interesting target for the development of antifungal drugs. Crystals diffracting to 3.3 Å have previously been obtained in two different space groups ($P4_12_12$ for the SeMet and P432 for the native). Despite extensive efforts, we could not obtain experimental phases to solve the structure of this protein. During this session, we have collected 2000° (wavelength= 1.9 Å) in order to try to solve this structure using sulphur anomalous signal (200 residues, 10 cysteines and methionines). For the moment, we have not been able to solve this structure using this dataset.

In parallel, we have collected a 360° dataset at the Gd edge from crystals soaked with Gd. No anomalous signal was observed indicating that no Gd ion was bound to the protein crystal.

2) *S. cerevisiae* Tpa1
Spacegroup: $P2_12_12_1$ with a= 105 Å, b=160 Å and c=210Å.
Resolution: 3.5A.
Completion= 97%
Rsym=14%

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.

During this run we collected a 3-wavelength MAD dataset (360° for the peak wavelength and 180° both for the inflexion and the remote) and a SAD dataset of 720° at the edge of selenium, on crystals grown from SeM-labelled protein. The resolution reached 3.5A for the latter but the crystal suffer from diffraction anisotropy. We have been able to localize Se sites but the anomalous signal is still too weak to allow experimental phases to be obtained so far.

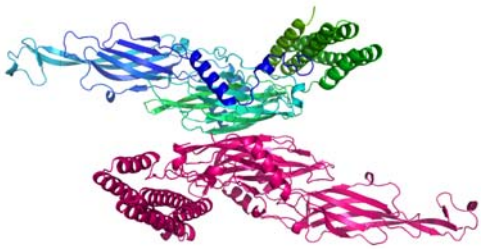
3) *Pyrococcus abyssi* Pab0255
Spacegroup C222; a= 95 Å, b=157 Å and c=46Å.
Resolution 4 Å.
Completion= 100%
Rsym=25%

The termination of replication in archaea remains poorly understood. The presence of the gene Pab0255 which exhibit a significant sequence homology with the bacterial tyrosine-recombinases Xer-C/D and the existence of a DIF-like sequence in the *P. abyssi* genome suggest some similarities between bacteria and archaea. Preliminary results support that Pab0255 could play a prominent role during replication termination in archaea. We have previously collected a 3.1 Å resolution dataset from crystals of native Pab0255 on ID23-EH2. During this session, we have collected a 180° SAD dataset at the Se K-edge from SeMet labelled protein crystals. The poor quality of the dataset did not allow us to solve the structure.

4) **V28**
Spacegroup: P21 a=50A b=59A c=86A
Resolution: 3
Completion= 98%
Rsym=6.4

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. The protein crystallizes as stacked plates and the diffraction quality is often poor. We collected a 2.5A data set on native crystal.

5) DHV35
Spacegroup: $P6_1$ a=b=133A c=164A
Resolution: 2.6
Completion= 99.1
Rsym= 12.8%



Ribbon representation of the DHV35 homodimer

DHV35 is a 70kD from the pT26-2 phage from Thermoccocus homologue to membrane proteins from other

archaea with unknown function. We collected a 2.6A data set that allowed us to phase the structure. The structure solved is a troncated version of the protein (soluble domain, 50kD). The quality of experimental data allowed us to build the protein. It consists in 3 adjacent domains from N to C terminus: one beta sandwich with 8 strands, one alpha beta beta sandwich with 8 strands and 3 helices and one 5 helix bundle. It is a novel fold and no function is directly deductible neither from sequence nor structure.

6) Acetyltransferase complex (MAK)

Space group $P6_422$
Resolution: 3.2 Å.
Completeness= 99.3%; Multiplicity = 3.2
Rsym=14.6%

Small crystals have been obtained of the catalytic subunit of a yeast complex involved in acetylation of the N-termini of certain proteins. A dataset diffracting to approximately 3.2 Å resolution was collected. A human homolog has subsequently used in a molecular replacement experiment to solve the structure, and a credible solution has been obtained. Experimental phases and/or higher resolution data would aid the successful refinement of the atomic model, however.

Moreover, during this session, diffraction tests have been performed on other proteins studied in our group but the crystals diffracted only weakly.