	Experiment title: BAG-LEBS-2005-2	Experiment number: MX-441
Beamline: ID23-EH2	**Date of experiment**: from: 28/11/2005 8h to: 29/11/2005 8h	**Date of report**: 24/02/05
Shifts: 3	**Local contact(s)**: Dr E. Mitchell.	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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Report:

Mark BROOKS and Marc GRAILLE (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

This beam-time allocation on ID23-EH2 beamline, mainly dedicated to the study of microcrystals has been very helpful for us as it allowed bringing tiny crystals from several projects, that could not be tested on more "classical" beamlines. Some crystals did not exhibit diffraction or only limited (8A) resolution but for some projects (mentioned below), we observed diffraction up to 2.5A.

1) *E. coli* ColD-tRNA
Space group $P4_12_12$; a=b=63A; c=149A.
Resolution 4A.

The secreted toxin Colicin D (ColD) kills competing bacteria through an RNase activity. It is composed of 3 domains, involved either in translocation of the protein across the outer-membrane of the target bacteria, in binding to a cell-surface receptor or in the lethal function (specific cleavage of all bacterial arginyl tRNAs). In *E. coli*, this catalytic domain is inhibited by an immunity protein (ImmD), which is released upon entry into the cytoplasm of the target bacterium. As very few are known about colD

RNase active site, we are trying to crystallize the colD catalytic domain with a tRNA stem loop. Few tiny crystals have been obtained and have been tested during this session. Diffraction to 4A was observed and no dataset was collected. More efforts will be made to improve the size and the quality of the crystals.

2) Human fibronectin fragment.
Spacegroup $P3_121$ a=b=127A; c=61A.
Resolution 3.7A
Completion: 91 %.
Rsym=19.3%.

Human fibronectin is a dimeric glycoprotein found in extra-cellular matrixes and blood. It is involved several cellular processes such as wound healing and cell migration. Fibronectin is involved in tumor growth and as a consequence is a promising target for the development of anti-tumor drugs. This very long protein (23986 amino acids) contains a zinc metalloprotease localised in the gelatine binding domain. This fragment belongs to the same family as matrix metalloproteases MMP-2 and MMP-9. We are interesting in solving the structure of this fragment alone or bound to potent inhibitors in order to improve these inhibitors by drug design. We have tested small crystals obtained in the presence of a drug and observed diffraction up to 3A. A full dataset has been collected but due to crystal decay, the resolution of the complete dataset only reaches 3.7A. Efforts are being made to get larger crystals so as to collect data at higher resolution and to have a more detailed description of the drug binding site.

3) EVF protein.
Spacegroup $P2_12_12_1$ a=69A, b=86A; c=91A.
Resolution 1.9A.
Completion 100%
Rsym 7.6%

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. The structure of this protein has been solved previously on ID23-EH1 and refined to 2A resolution. The refinement has revealed the presence of a lipid covalently linked to a cysteine residue, hence allowing new assumption to be tested to unravel the molecular basis of the virulent effect of this protein. As this lipid cannot been clearly identified from the electron density, we have tried to collect a higher resolution dataset to help the better identification of this molecule. However, due to crystal decay, the resolution of the full dataset is 1.9A and hence insufficient to give clear information.

4) Virar9.
Spacegroup $P6_1$ a=b=87A, c=226A.
Resolution 2.5 A.
Completion 98%
Rsym 9%

This small protein (14kDa) of unknown function is encoded by an archaeophage. We have collected a native dataset to 2.5A during this session. However, experimental phases are needed to solve this structure. SeMet labelling has been done after the diffraction was observed during this run and crystallization of this SeMet protein is currently in progress.

5) *D. radiodurans* polymerase X (PolX).
Spacegroup $P2_12_12_1$ a=59A, b=68A, c=139A.
Resolution 3.5 A.
Completion 98%
Rsym 12.5%

Deinococcus radiodurans, a highly radioresistant bacterium able to mend hundreds of radiation-induced double-stranded DNA breaks, expresses a DNA polymerase belonging to the X family,

which is implicated in a variety of DNA repair processes in eukaryotes. This novel bacterial polymerase, named PolX(Dr), possesses a DNA polymerase activity that is stimulated by $MnCl_2$, a property of the X family DNA polymerases. Antibodies raised against PolX(Dr) recognized human pol lambda, rat pol beta and yeast Pol4 and, conversely, antibodies raised against these proteins recognized PolX(Dr). This immunological cross-reactivity suggests a high degree of structural conservation among the polymerases of the X family. Lack of PolX(Dr) reduced the rate of repair of double-stranded DNA breaks and increased cell sensitivity to gamma-rays. PolX(Dr) thus appears to play an important role in double-stranded DNA break repair in *D. radiodurans*. In collaboration with Suzanne Sommer's group (IGM, Université Orsay), we are interesting in determining the structure of this new class of DNA polymerase. We have obtained thin crystals that diffracted to 2.9A during this session. We have then collected one dataset to 3.5A, a limited resolution due to crystal decay.

6) Yeast YGL047w (Alg13).
Spacegroup P23 a=b=c=120A.
Resolution 3.3 A.
Completion 100%
Rsym 9.6%

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 one dataset to 3.3A resolution. As no accurate models are available to solve the structure by molecular replacement, SeMet labelling of this protein is in progress.

7) *S. cerevisiae* nicotinamidase Pnc1.
Spacegroup H3 a=b=304A; c=115A.
Resolution 3.9 A.
Completion 100%
Rsym 15.8%

This protein converts nicotinamide to nicotinic acid as part of the NAD(+) salvage pathway. It is required for life span extension by calorie restriction. Its expression responds to all known stimuli that extend replicative life span. We have previously solved the structure of this protein by molecular replacement and are trying to get crystals of this protein bound to an inhibitor. We have collected a full dataset during this run on a crystal initially diffracting to 3A but the resolution of the final dataset only reached 3.9A.