ESRF	**Experiment title:** Application of ultra-short wavelengths for high pressure and conventional macromolecular crystallography	**Experiment number**: MX384/MX421
Beamline: ID27	**Date of experiment**: from: 26/06/2005 to: 01/07/2005 (MX384) from: 14/12/2005 to: 19/12/2005 (MX421)	**Date of report**: 01/03/2006
Shifts: 12 + 15	**Local contact(s)**: Mohamed Mezouar / Iain Oswald	*Received at ESRF:*

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Report:

Report on MX384 experiment as well as first run of the LTP project MX421 and exploitation of previous experiments.

In similar previous experiments, we have opened the field of macromolecular crystallography at high hydrostatic pressure up to about 1000-1500 MPa (100 MPa = 1 kbar) through the combination of ultra-short wavelength X-rays from an undulator on the ESRF ID30 beamline, a diamond anvil cell (DAC) and a large image plate detector [1, 2]. During the MX384 run, we have adapted our various protocols and data collection conditions to the new high pressure beamline ID27.

1- Psychropiezophilic enzyme

We have undertaken the study of proteins from Psychropiezophilic bacteria. These proteins are adapted both to low temperature (around 0°C) and high pressure (0.1-100 MPa) encountered in cold deep sea.
Our first priority was the study of alpha-amylase from *Alteromonas haloplanctis*, a bacteria living in symbiosis with the plancton of Antarctic Ocean. As the strain of *A. haloplanctis* lives in Antarctic Ocean at various depths, this enzyme may be used to study the influence of pressure on the 3D structure. High resolution information at standard conditions on the native enzyme are available [3]. But up to now, we have faced troubles in getting high-quality reproducible crystals sufficiently stable to be loaded in the DAC.
We have thus studied a second protein, a cellulase, from *Pseudoalteromonas haloplanctis.* More precisely we have studied the catalytic domain of this enzyme for which the structure has been recently determined at 1.4 Å resolution [3] using cryo-conditions (space group $P2_12_12_1$, a = 44.1 Å; b = 78.9 Å; c = 135.4 Å). Two data sets were recorded at 1.8 Å resolution. One data set was obtained at ambient pressure and the second at a pressure of 1750 bars. Both data sets were recorded at ambient temperature leading to a direct comparison of the two structures. Refinements were performed with CNS and lead in both cases to low residual R-factors (R

and Rfree of about 16% and 20 % respectively). Comparison of the two structures would be achieved in a couple of months.

2- Structural analysis of urate oxidase

Urate oxidase is a tetrameric enzyme of around 136 kDa involved in purine degradation. The structure of urate oxidase from *Aspergillus flavus* complexed with its competitive inhibitor, 8-azaxanthine, has been solved at high resolution [4,5]. The crystals belong to the orthorhombic space group I222 space group with a = 80.3 Å, b = 96.3 Å and c = 105.6 Å. There is one monomer per asymmetric unit, with a solvent content of around 60%. The homotetrameric enzyme is globular with an external size of 50 Å x 50 Å, enclosing a tunnel 50 Å long and 12 Å in diameter. The tetramer is made of two dimers stacked face to face, related by a crystallographic two-fold axis. The dimer consists of an $\alpha_8\beta_{16}$ barrel with an antiparallel β-sheet of 16 sequential strands, the eight helices forming the exterior of the barrel.

We have studied the high pressure behaviour of urate oxidase by X-ray crystallography. These crystals are especially sensitive to high pressure compared to other crystals we have already characterized [1,6]. Indeed diffraction disappears at about 180 MPa. Thus, the high pressure structure of urate oxidase was recorded at 140 MPa and compared to the ambient pressure one [7]. The crystal structure analysis shows that we have captured the onset of dissociation of the tetrameric assembly.

3- Evaluation of the Flat Panel detector

During the MX421 run, we have evaluated the capacity of the new Flat Panel detector from MAR Research to collect data on macromolecules using a beam energy of 37 keV as done during our high pressure measurement. Data were recorded on lysozyme crystals. A 1.9 Å data set was recorded on a 10° range with an angular step of 1° leading to an overall Rsym of 5% for 45% completeness. These results are very promising.

References

[1]. Fourme R., Ascone I, Kahn R., Mézouar M., Bouvier P., Girard E., Tianwei Lin. & Johnson J. E. "Opening the high-pressure domain beyond 2 kbar to protein and virus crystallography." (2002) *Structure* **10**, 1409-1414 ; *ESRF Highlights* (2002).

[2]. Fourme R, Girard E, Kahn R, Dhaussy AC, Mézouar M, Colloc'h N & Ascone I. "High-Pressure Macromolecular Crystallography (HPMX): Status and prospects." (2006) *Biochim Biophys Acta*. (In Press)

[3]. Violot S., Aghajari N., Czjzek M., Feller G., Sonan G. K., Gouet P., Gerday C., Haser R. & Receveur-Bréchot V. "Structure of a Full Length psychrophilic cellulose from *Pseudoalteromonas haloplanctis* revealed by X-ray Diffraction and Small Angle X-ray Scattering." (2005) *J. Mol. Biol.* **348(5)**, 1211-1224.

[4]. Colloc'h N., El Hajji M., Bachet B., L'Hermite G., Schiltz M., Prange T., Castro B. & Mornon J.P., "Crystal structure of the protein drug urate oxidase-inhibitor complex at 2.05 A resolution." (1997) *Nat. Struc. Biol.* **4**, 947-952.

[5]. Retailleau P., Colloc'h N., Vivares D., Bonneté F., Castro B., El Hajji M., Mornon J.P., Monard G. & Prangé T., "Complexed and ligand-free high resolution structures of urate oxidase (Uox) from Aspergillus flavus : a reassignment of the active-site bonding mode." (2004) *Acta Cryst.*, D**60**, 453-462.

[6]. Girard E., Kahn R., Mezouar M., Dhaussy A-C., Lin T., Johnson J. E. & Fourme R. "The First Crystal Structure of a Macromolecular Assembly under High Pressure: CpMV at 330 MPa." (2005) *Biophysical Journal*, 88, 3562-3571.

[7]. Colloc'h N., Girard E., Dhaussy A.-C., Kahn R., Ascone I., Mezouar M. & Fourme R., "High pressure macromolecular crystallography: The 140-MPa crystal structure at 2.3 Å resolution of urate oxidase, a 135-kDa tetrameric assembly " (2006) *Biochim. Biophys. Acta* (In Press).

Publications

1 - "High-pressure protein crystallography (HPPX): instrumentation, methodology and results on lysozyme crystals"
R. Fourme, R. Kahn, M. Mezouar, E. Girard, C. Höerentrup, Prangé, T. & I. Ascone. (2001) J. Synchrotron Rad., **8**, 1149-1156.

2 - "Opening the high-pressure domain beyond 2kbar to protein and virus crystallography"
R. Fourme, I. Ascone, R. Kahn, M. Mezouar, P. Bouvier, E. Girard, J. E. Johnson & T. Lin. (2002) Structure, **10**, 1409-1414.

3 - "Protein and virus crystallography under High Hydrostatic Pressure"
R. Fourme, I. Ascone, R. Kahn, M. Mezouar, P. Bouvier, E. Girard, J. E. Johnson & T. Lin. (2002) ESRF Highlights.

4 - "New trends in macromolecular crystallography at high hydrostatic pressure"
R. Fourme, I. Ascone, R. Kahn, E. Girard, M. Mezouar, T. Lin & J. E. Johnson. (2003)
Advances in high pressure bioscience and biotechnology II: Proceedings of the 2nd International Conference on High Pressure Bioscience and Biotechnolog. September 16-19, 2002, Dortmund. Editor R. Winter, Springer Verlag, pp 161-170.

5 - "Using a quasi-parallel X-ray beam of ultrashort wavelength for high-pressure virus crystallography: implications for standard macromolecular crystallography"
R. Fourme, E. Girard, R. Kahn, I. Ascone, M. Mezouar, A.-C. Dhaussy, T. Lin. & J. E. Johnson (2003) Acta Cryst., D**59**, 1767-1772.

6 - "State of the art and prospects of macromolecular X-ray crystallography at high hydrostatic pressure "
R. Fourme, E. Girard, R. Kahn, I. Ascone, M. Mezouar, T. Lin. & J. E. Johnson. (2004)
'High Pressure Crystallography', Editors A. Katrusiak & M. F. Mac Millan, Kluwer, Dordrecht, pp 527-542.

7 - "A new dimension in structural biology: fully-fledged high-pressure macromolecular crystallography"
E. Girard, R. Kahn, I. Ascone, M. Mezouar, A.-C. Dhaussy, T. Lin, J. E. Johnson & R. Fourme. (2004) High Pressure Research, **24**, 173-182.

8 - "The First Crystal Structure of a Macromolecular Assembly under High Pressure: CpMV at 330 MPa."
E.Girard, R. Kahn, M. Mezouar, A-C. Dhaussy, T. Lin, J. E. Johnson & R. Fourme (2005) Biophysical Journal, **88**, 3562-3571.

9 - "High-Pressure Macromolecular Crystallography (HPMX): Status and prospects."
R. Fourme, E. Girard, R. Kahn, A.-C. Dhaussy, M. Mezouar, N. Colloc'h & I. Ascone (2006) Biochim. Biophys. Acta (In Press).

10 - "High-pressure macromolecular crystallography: The 140-MPa crystal structure at 2.3 Å resolution of urate oxidase, a 135-kDa tetrameric assembly."
N. Colloc'h, E. Girard, A.-C. Dhaussy, R. Kahn, I. Ascone, M. Mezouar & R. Fourme (2006) Biochim. Biophys. Acta (In Press).