

	Experiment title: Study of GFP under high pressure	Experiment number: MX911
Beamline: ID27	Date of experiment: from: 12/02/2010 to: 16/02/2010	Date of report: 20/03/2010
Shifts: 12	Local contact(s): Pierre Bouvier	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): * Roger Fourme – Synchrotron SOLEIL, L’Orme des Merisiers, Saint Aubin, BP48, 91192 Gif sur Yvette Cedex * Richard Kahn – Institut de Biologie Structurale, 41 Jules Horowitz, 38027 Grenoble * Eric Girard - Institut de Biologie Structurale, 41 Jules Horowitz, 38027 Grenoble * Anne-Claire Dhaussy - C.R.I.S.M.A.T., Université de Caen, 6 Boulevard du Marechal Juin, 14050 Caen cedex * Philippe Oger – ENS Lyon - 46, allée d'Italie, 69364 Lyon cedex 7		

High Pressure Macromolecular Crystallography project (HPMX).

This session on ID27 was dedicated to the crystallographic study of GFP (Green Fluorescent Protein) crystals under high pressure, following preliminary experiments in Jan 2009 (MX759). Participants were E. Girard, A.-C. Dhaussy, R. Kahn and R. Fourme with Pierre Bouvier as staff member. The clone of the protein, an enhanced GFP (EGFP) with mutations F64L, S65T and H231L, was supplied by Philippe Oger (ENS Lyon, France) who also participated to the session. The goals of this study are the following: i) to solve and refine the 3D structure of this EGFP at several pressures ; (ii) to record the variation of fluorescence emission as a function of pressure ; and (iii) to correlate fluorescence variation with small pressure-induced changes in the chromophore and neighbouring protein and water structures.

The crystal structure in space group $C222_1$ is unprecedented, in connection with the very high content of MPD in the crystallization solution. Crystals can withstand pressures in excess of 1 GPa, which means that the secondary and tertiary structures of EGFP are preserved in this

range. Crystals degrade above. Pressure induces an elastic compression of the protein. No other conformation was detected, which is coherent with the fact that EGFP has (as far as we know) a single function, light emitting. The experiment was performed at ultra-short wavelength using a MAR165 CCD detector. Data were recorded at room temperature. Sets at 4 pressures (Pamb, 160, 350 and 780 MPa) were essentially complete and a fifth one (860 MPa) was recorded with a lower completeness (85%). The useful resolution was 0.185 nm. Ambient-pressure structure was solved by molecular replacement and refined ($R = 0.16$, $R_{\text{free}} = 0.18$), providing high quality electron densities in particular in the chromophore region. Refinement of the high-pressure structures is in progress. Overall, the beam quality was excellent and high quality data were obtained. The fluorescence emission of single crystals in the diamond anvil-cell was recorded using a Raman spectrometer. Study of another GFP (citrine) was performed by Gruner et al. at Cornell University using high pressure cryocooling (Barstow et al., 2008, PNAS [105](#), 13362-13366) with data collection at atmospheric pressure. We have performed the first single crystal analysis of GFP under high pressure. The detailed analysis of structural variations induced by pressure is underway.

Other HPMX results

During the last few months, besides review articles in *Ann. Rev. Biophys*¹ and *High Press. Res*², there are several publications in press giving structural results on urate oxidase³ and superoxide dismutase⁴ and the determination by HPMX of the intrinsic isothermal compressibility of « dry » proteins and DNA models⁵.

Technical developments

As we are interested in trapping higher energy conformers of proteins, we have developed a pneumatic diamond anvil cell (ELSA) oriented toward pressure and temperature cycling and annealing. The new cell has been designed and constructed in collaboration with Betsa (Nangis, France) and tests have been performed (submitted⁶). All experiments during this session were performed with this cell.

Publications 2009-2010

A. articles in peer-reviewed journals

1. Roger Fourme, Eric Girard, Richard Kahn, Anne-Claire Dhaussy, Isabella Ascone. (2009). Advances in high-pressure biophysics : Status and prospects of macromolecular crystallography. *Ann. Rev. Biophys.* 38, 153-171
2. Roger Fourme, Eric Girard, Richard Kahn, Thierry Prangé, Anne Claire Dhaussy, Mohamed Mezouar, Isabella Ascone. High-resolution structures and properties of biomolecules under high pressure probed by x-ray crystallography. (2010) *High Press. Res.* 30(1), 10-103.
3. Eric Girard, Stéphane Marchal, Javier Perez, Stéphanie Finet, Richard Kahn, Roger Fourme, Guillaume Marassio, Anne-Claire Dhaussy, Thierry Prangé, Marion Giffard, Fabienne Dulin, Françoise Bonneté, Reinhard Lange, Jacques H. Abraini, Mohamed Mezouar, Nathalie Colloc'h. Structure-function perturbation and dissociation of tetrameric urate oxidase by high hydrostatic pressure. In press in *Biophys. J*
4. Isabella Ascone, Carmelinda Savino, Richard Kahn, Roger Fourme. (2009). Crystal and molecular structure of Cu,Zn superoxide dismutase at 0.57 GPa: protein flexibility investigated at high pressure. In press in *J. Appl. Cryst.*
5. Isabella Ascone, Richard Kahn, Eric Girard, Thierry Prangé, Anne-Claire Dhaussy, Mohamed Mezouar, Nikodem Ponikwicki, Roger Fourme. Isothermal compressibility of macromolecular crystals and macromolecules derived from high-pressure x-ray crystallography. In press in *J. Appl. Cryst.*
6. Eric Girard, Roger Fourme, Romain Cieurko, Jacques Joly, Florian Bouis, Pierre Legrand, Jeroen Jacobs, Anne-Claire Dhaussy, Jean-Luc Ferrer, Mohamed Mezouar and Richard Kahn. Macromolecular crystallography at high pressure with pneumatic diamond-anvil cells handled by a 6-axis robotic arm. *Submitted to J. Appl. Cryst.*

B. Articles in other journals and book chapters

7. Roger Fourme, Isabella Ascone, Gerald Kneller. (2009). New trends in high pressure biophysics. *Synchrotron Radiation News* 22(5) 39-41
8. Roger Fourme, Isabella Ascone, Mohamed Mezouar, Anne-Claire Dhaussy, Richard Kahn, Eric Girard. High pressure crystallography of biomolecules : recent achievements. I- Introduction, materials and methods. In *High Pressure Crystallography*. Editeur Elena Boldyreva et Przemyslaw Dera. NATO sciences series. Kluwer, Londres. In press
9. Roger Fourme, Isabella Ascone, Richard Kahn, Eric Girard. High pressure crystallography of biomolecules : recent achievements. II- Applications. In *High Pressure crystallography*. Editeurs Elena Boldyreva et Przemyslaw Dera. NATO science series. Kluwer, Londres. In press.

C. conferences and communications

C.1 Invited

Roger Fourme et al. HPMX studies of A-and B-DNA up to 2 GPa. Conference Proteins under Pressure, Santa Fe, USA, 21-25 Jan. 2008.

Roger Fourme et al. High pressure macromolecular crystallography : status, applications and prospects. 21th Congress of the IUCR, Osaka, Japon, 23-31 Aug. 2008.

Roger Fourme. From the early days of synchrotron radiation structural biology and Max Perutz to SOLEIL. UK China symposium on structural proteomics and Chair Symposium Max Perutz. Université de Liverpool, Grande Bretagne, 25-26 Sept. 2008.

R.oger Fourme. Recent developments in high-pressure macromolecular crystallography. High Pressure Synchrotron Science (HiPress) workshop, APS, Chicago, USA, 6-8 mai 2009.

Roger Fourme. High pressure crystallography of biomolecules : recent achievements. I-Introduction, materials and methods. II-Applications. (2 conférences). High Pressure Crystallography Course : from novel experimental approaches to applications in cutting-edge technologies. Erice, Italy, 4-14 Jun. 2009.

Thierry Prangé et al. Structural modifications of DNA under hydrostatic pressure up to 2 GPa. IUCr workshop on Advanced Crystallography at High-Pressure. Harbin, Chine, 20-22 Jul. 2009.

Roger Fourme et al. Status of high-pressure macromolecular crystallography. Application to the determination of crystal and molecular isothermal compressibilities. IUCr workshop on Advanced Crystallography at High-Pressure. Harbin, China, 20-22 Jul 2009.

Nathalie Colloc'h et al. Structure-function perturbation and dissociation of tetrameric urate oxidase by high hydrostatic pressure. IUCr workshop on Advanced Crystallography at High-Pressure. Harbin, Chine, 20-22 July 2009.

Roger Fourme et al. Crystal and molecular isothermal compressibilities determined by high-pressure macromolecular crystallography. Fifth International meeting Biomolecules under pressure. Regensburg, Allemagne. 3-5 Aug. 2009.

Eric Girard et al. High Pressure macromoleculer crystallography ISDSB2010. Orsay and Saint Aubin, France. 25-28 May 2010.

C.2 Contributed

Roger Fourme. Recent developments of high pressure macromolecular crystallography. 6eme Forum de technologie des hautes pressions, Réseau Hautes Pressions du CNRS. Les verrous technologiques dans l'expérimentation haute-pression. Batz sur mer, France, 6-10 Oct. 2008.

Eric Girard et al. Structure-function study of tetrameric urate oxidase studied with fluorescence spectroscopy, SAXS and x-ray crystallography under high hydrostatic pressure. XLVIIth EHPRG meeting. Paris, 6-11 Sep. 2009.

Isabella Ascone et al. Isothermal compressibility of macromolecular crystals and macromolecules: the case of Cu,Zn superoxide dismutase protein. XLVIIth EHPRG meeting. Paris, 6-11 sep. 2009.

C.3 Posters

Roger Fourme et al. A beamline for anomalous diffraction at SOLEIL : PROXIMA I. 21th Congress of the IUCR, Osaka, Japon, 23-31 Aug. 2008.

Isabella Ascone et al. Crystallographic study of Cu,Zn superoxide dismutase in extreme pressure conditions. 21th Congress of the IUCR, Osaka, Japan, 23-31 Aug. 2008.

Thierry Prange et al. X-ray structure of A- and B-DNA under high hydrostatic pressure (up to 2 GPa). 21th Congress of the IUCR Osaka, Japan, 23-31 Aug. 2008.

Roger Fourme et al. High-resolution structures and properties of biomolecules under high pressure probed by X-ray crystallography. XLVIIth EHPRG meeting. Paris, 6-11 Sep. 2009; SOLEIL Users Meeting, Saint Aubin. 20-21 Jan. 2010.

Roger Fourme et al. High-pressure macromolecular crystallography: instrumental developments and some applications. ESRF Users meeting (UM10) and PECS workshop. Grenoble, 9-11 Feb 2010.