	Experiment title: High pressure crystallography	**Experiment number**: MI 514
Beamline: ID30	**Date of experiment**: from: February 28 to March 5 2001	**Date of report**: April 10 2001
Shifts: 15	**Local contact(s)**: M. Mezouar	*Received at ESRF:*

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Report: In spite of their potential, high-pressure protein crystallography (HPPX) studies have been very scarce and only one 3D structure of a protein crystal under high pressure (0.1 GPa) has been published (Kundrot & Richards,1987, J. Mol. Biol. 193, 157). We have developed on beamline ID30 the instrumentation and the associated methodology for the collection of angle-dispersive diffraction data from protein crystals submitted to high hydrostastic pressure. The instrument makes use of intense x-rays emitted by two collinear undulators, and combines a membrane-driven diamond-anvil cell mounted on a two-axis goniometer and a MAR345 imaging plate scanner.

Several crystals of two proteins, hen egg-white lysozyme (tetragonal polymorph, or tHEWL) and bovine Superoxide dismutase (SOD) were loaded in the cell and compressed gradually up to 0.915 and 1 GPa respectively. High quality diffraction pictures were obtained using an ultra-short wavelength (λ = 0.3738 Å). Due to the condition of hydrostaticity, data collection must proceed at room temperature; in order to alleviate relatively fast degradation under irradiation, the crystal was displaced by 50 μm steps every 20 images. Using this strategy, diffraction data sets were recorded at 0.36, 0.58 and 0.69 GPa from tHEWL crystals. Sets of structure amplitudes of good quality in terms of signal-to-noise ratio, completeness and R_{sym} values were derived from these data (tables 1 & 2). Data sets at 0.36 and 0.58 GPa are essentially complete and readily usable for detailed structural studies ; the set at 0.69 GPa needs to be completed. The compressibility of tHEWL has been determined from unit cell parameters determined at 24 different pressures up to 0.880 GPa

(fig.3). The pressure-induced loss of crystalline order of a tHEWL crystal above 0.880 GPa has been captured through a series of diffraction pictures. Overall, the experiment was quite succesful and open new possibilities in HPPX.

Publication: One article has been submitted and another one is being prepared.
High-pressure protein crystallography (HPPX): Instrumentation, methodology and results of data collection on lysozyme crystals.
R Fourme, R. Kahn, M. Mezouar, E. Girard, C. Hoerentrup and I. Ascone
Submitted to J. Synchrotron Rad.

Pressure (GPa)	Crystal	Number of images	Resolution (Å)	R_{sym} (%)	Completeness (%)	Multiplicity
10^{-4}	2	46	2.24	8.4	55. 7	4.5
0.300	2	58	2.00	6.2	67.9	4.0
0.300	4	57	2.12	6.5	89.7	3.1
0.350	3	54	1.90	5.1	63.5	4.2
0.580	3	54	2.12	7.9	61.2	4.3
0.580	4	56	2.27	9.7	88.1	3.1
0.690	3	39	2.58	9.1	64.8	2.8
0.690	4	12	2.10	7.2	55.7	1.2

Table 1. tHEWL crystals. Summary of processing of individual diffraction data sets. Values between brackets refer to the corresponding values in the highest resolution shell.

Pressure (GPa)	Crystals	Resolution (Å)	R_{merge} (%)	Completeness (%)	Multiplicity
0.300	2, 4	2.09	8.5	91.0	6.0
0.580	3, 4	2.23	8.2	99.5	5.2
0.690	3, 4	1.90	7.5	75.7	4.4

Table 2. Merging between data sets taken at the same pressure on different crystals.

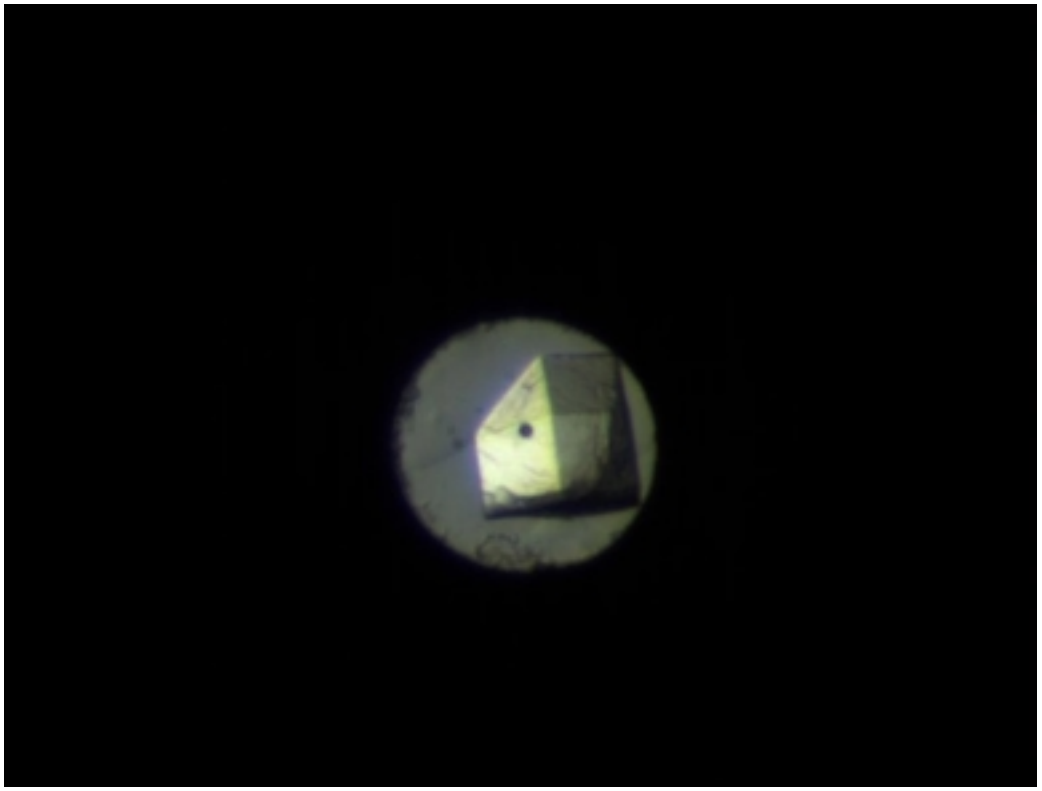

Fig. 1 photograph of a tHEWL crystal within a cavity machined in a steel gasket squeezed between diamond anvils. The black dot is a ruby chip used for pressure calibration.

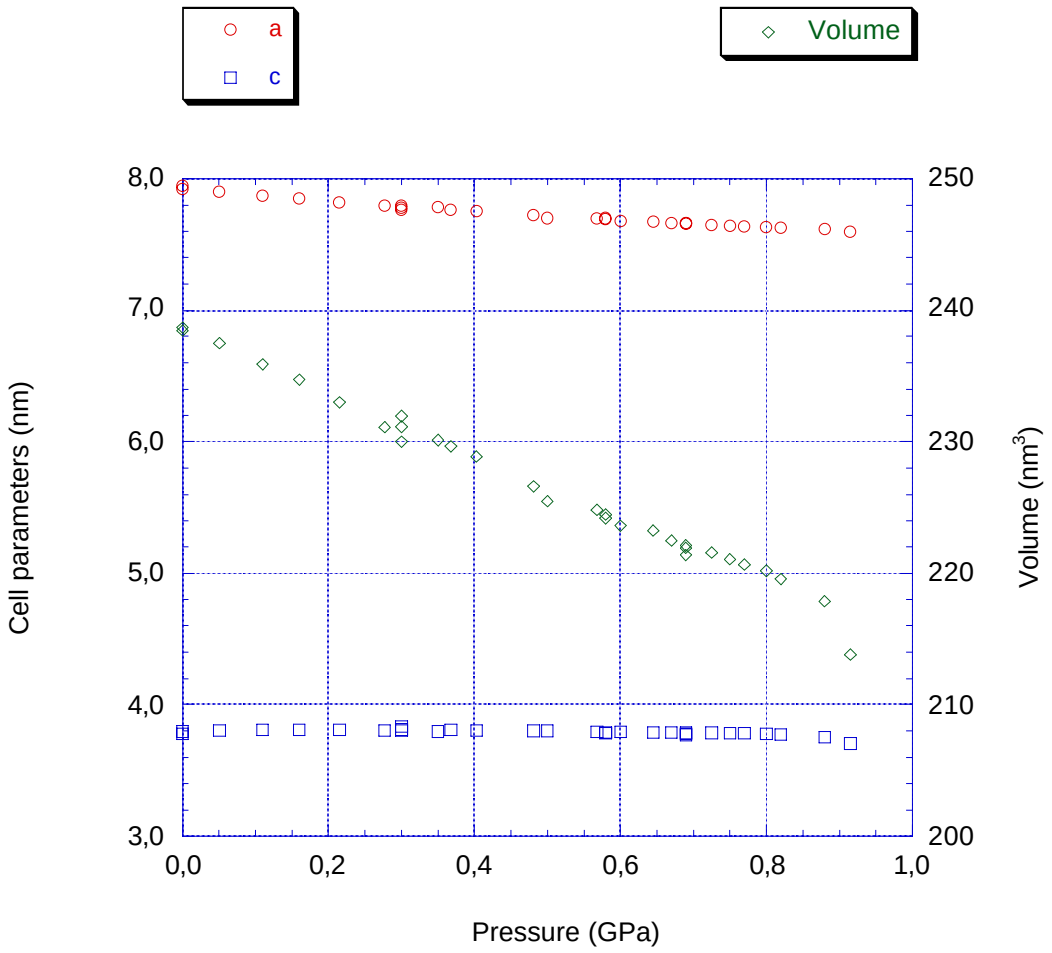

Fig. 2 Variation of cell parameters and cell volume of tHEWL as a function of pressure.