In the course of our programme on high-pressure macromolecular crystallography at ID30, we he now performed two series of 15x8-hour shifts. We combine the use of X-rays of ultra-short wavelength emitted by undulators with a diamond anvil cell (DAC) and a large imaging plate area detector (MAR345).

I- First series

We performed first experiments on crystals of two proteins, hen egg-white lysozyme (tetragonal crystals, tHEWL) and Cu,Zn superoxide dismutase (SOD), which are monomeric and dimeric respectively. We used radiation of wavelength 0.37 Å. Compressibility measurements were performed up to 8.2 and beyond 10 kbar respectively on the tetragonal and orthorhombic forms of HEWL and SOD. Data were collected on tHEWL crystals. Best results from individual crystals were obtained for a crystal at 3.5 kbar (resolution 1.9 Å, multiplicity 4.2, R_{sym} 0.051). Merged data sets with high completeness were obtained at 3.5 and 5.8 kbar, and a set at 6.9 kbar with lower completeness. Using these data, the three structures were refined using the SHELXL program at resolution of at least 2 Å. The instrumentation, methodology and a summary of results on tHEWL were reported [1]. A data set with 60 % completeness was also collected for SOD.

II- Second series

II-1 Protein crystals

Measurements on crystals of tHEWL and SOD have been completed with quite significant improvements. We used harder radiation (0.33 Å) in order to maximise efficiency of the image plate while decreasing the noise due to Compton scattering from diamonds. Thinner diamonds with larger culets were mounted, thus improving both maximum crystal-size and angular opening of the cell.

Structure amplitudes were measured on two tHEWL crystals at 7.0 kbar. We believe that quality of these data is a milestone in high-pressure crystallography (two crystals, results for merged data: resolution 1.6 Å, R_{sym} 0.057, multiplicity 7.2, completeness 0.93). Derived structure amplitudes have been used for refinement with CNS and a detailed analysis of the 7.0-kbar structure is currently well advanced. For SOD, data have been collected on crystals with complementary orientations and are only partially processed.

II-2 CPMV crystals

We have performed studies of the first crystallised assembly under high pressure, particles of cowpea mosaic virus (CPMV). CPMV is a member of the *Comovirus* family, a group of icosahedral plant viruses similar to mammalian *Picornaviruses*. Three types of virus particles can be isolated as distinct bands in ultra-centrifugation. The band on the top of the gradient (T) contains no RNA (empty capsids); the middle (M) component contains the smaller RNA2 and the bottom (B) component the larger RNA1. For the initial structure determination, crystals with a rhombic dodecahedral symmetry were obtained and the space group I23 (a = 317 Å) was assigned. A structure at with a mixture of M and B components and individual M and B components was refined at 2.8 Å resolution, leading to structures of three closely similar nucleoprotein particles. The roughly 300 Å capsid is similar to the *Picornavirus* capsid displaying a pseudo T = 3 (P = 3) surface lattice. The three beta-sandwich domains adopt two orientations, one with the long axis radial and the other two with the long axis tangential in reference to the capsid sphere. T = 3 viruses display one or the other of these two orientations.

In crystallisation drops at normal temperature and pressure, only a fraction (about 10 %) of the rhombic dodecahedral CPMV crystals conform strictly to the characteristics of the body centred I23 space group. In most crystals, weak reflections with h + k + l = 2n + 1 (odd reflections) are present, which is prohibited in the body centred I23 space group. P23 space group was tentatively assigned, which agreed with the presence of odd reflections but not with the packing criteria of the virus particles. The intensities of odd reflections vary from one crystal to another and their intensity distribution as a function of resolution is abnormal. The resolution is much lower than in I23 crystals. Disorder in crystals and the occurrence of odd reflections are attributed to the deviation from ideal packing with a superposition of a pseudo-symmetric superstructure on the I23 lattice. Going from I23 to P23 might involve a transition with both a displacement component and a rotation by about 7.6° of the virus particles with respect to the position in I23 structure.

High-pressure experiments were performed with crystals from the M component of CPMV, grown at La Jolla, packaged in capillaries and sent at the ESRF. These crystals had the canonical rhombic dodecahedral habit. In a preliminary test, one crystal was selected, mounted in a diamond cell and submitted to an increasing pressure. Material and methods were essentially as previously described [1]. The standard stabilisation solution was used as compression medium. Observation of the crystal with a low power microscope did not reveal any cracking or morphology change up to a pressure in excess of 3.5 kbar, which prompted us

to collect some diffraction data. For this purpose, another crystal with dimensions about 150 μm was mounted in the diamond cell. The X-ray wavelength was adjusted at 0.3305 Å. A single 0.3° oscillation photograph was recorded at atmospheric pressure, then at 1.1, 2.0 and 3.3 kbar. For all images, indexation in a cubic cell was readily performed with DENZO.

At 1 bar and 1.1 kbar, images were indexed in P23 but not in I23. Results from visual inspection of the image at 1.1 kbar and analysis with DENZO are summarised in table 1. High mosaicity, modest resolution and diffuse scattering all indicate that the P23 crystal is poorly ordered, with a special type of disorder revealed by the abnormal distribution of diffracted intensities.

At 3.3 kbar, a picture was first recorded at a crystal-to-detector distance of 1400 mm, which limited resolution to 2.7 Å on the edge of the imaging plate. The same picture was repeated after setting the detector at 900 mm in order to determine the maximum resolution. Both look strikingly different with respect to the 1.1-kbar picture. Bragg spots can be indexed in space group I23 and extend to about 2.6Å, with spots visible up to about 2.2 Å. Diffuse scattering is either not visible or very weak. On integration with DENZO, most reflections are flagged as completely recorded, which reveals a low mosaicity. The variation of intensities as a function of resolution is normal. Both visual observations and results obtained from analysis of images (table1) indicate that I23 crystals are highly ordered. *It can be concluded that pressure induces a phase transition from a disordered P23 crystal diffracting to low resolution to a highly ordered I23 crystal diffracting to high resolution*.

A final image was recorded at 4.4 kbar. At this pressure, the virus crystal no longer diffracts, probably due to pressure-induced denaturation.

At 3.3 kbar, the cell volume is reduced by 2.2% with respect to the structure atmospheric pressure. The average compressibility from 1 bar to 3.3 kbar is 0.666 $kbar^{-1}$, about two-thirds of the average compressibility of tHEWL from 1 bar to 10 kbar (0.98 $kbar^{-1}$) [1] **.**

Conclusion

The possibility of obtaining accurate structural information under high pressure opens a wealth of possibilities such as exploration of sub-states, study of interactions between molecules and between sub-units, and detection of the onset of pressure-induced denaturation. Furthermore, high pressure might become a standard tool to improve order in macromolecular crystals, either by favouring more ordered packing or by restricting amplitudes of atomic motions in regions which are disordered at atmospheric pressure.

References

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Tables

Pressure	1.1 kbar	3.3 kbar
Space group	P23	I23
Resolution ($I/\sigma > 1$)	3.9 Å	2.6 Å
$\langle I/\sigma \rangle$ variation	abnormal	normal
	decay 25-5.4 Å increase 5.4-4.5 Å decay 4.5-3.9 Å	
mosaicity	0.35°	0.10°
diffuse scattering	strong	weak

Table 1: Results from visual observation and analysis of diffraction pictures of CPMV crystals at 1.1 and 3.3 kbar.