



High pressure and conventional macromolecular crystallography using ultra-short wavelengths. The case of the Cornell ERL source.

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N306



SOLEIL. 2.75 GeV Third Generation SR source with topping-up. 15 km South of Paris.

Status: e- beam stored at 2.75 GeV on June 2nd 2006. 25 BL under construction.
Commissioning of 12 BL begin in 2006. External Users in 2007.

HPMX: a new field

Steps in instrumentation

1. Laboratory source

+ Beryllium cell (Kundrot & Richards, 1986) pressure limit 2kbar, crystal not visible, scattering/diffraction from polycrystalline Be

*Results: 3D structure of HEWL (hen egg-white) lysozyme at 1 kbar (Kundrot & Richards, 1987).
3D structure of met-myoglobin (Urayama, Phillips & Gruner, 2002)*

2. Second generation SR source, conventional wavelength

+ DAC (Katrusiak & Dauter, EMBL Hamburg, 1996)

Result: compressibility of HEWL single crystals up to 10 kbar

3. Third generation SR source, ultra-short wavelength

+ DAC (Fourme *et al.*, 2001)

Results: Fully-fledged HPMX up to 12-15 kbar

Steps in science

Initially:

Doubts about scientific interest of HPMX beyond life adaptation to extreme conditions
Discouraging effect of increased complexity of data collection.

Now:

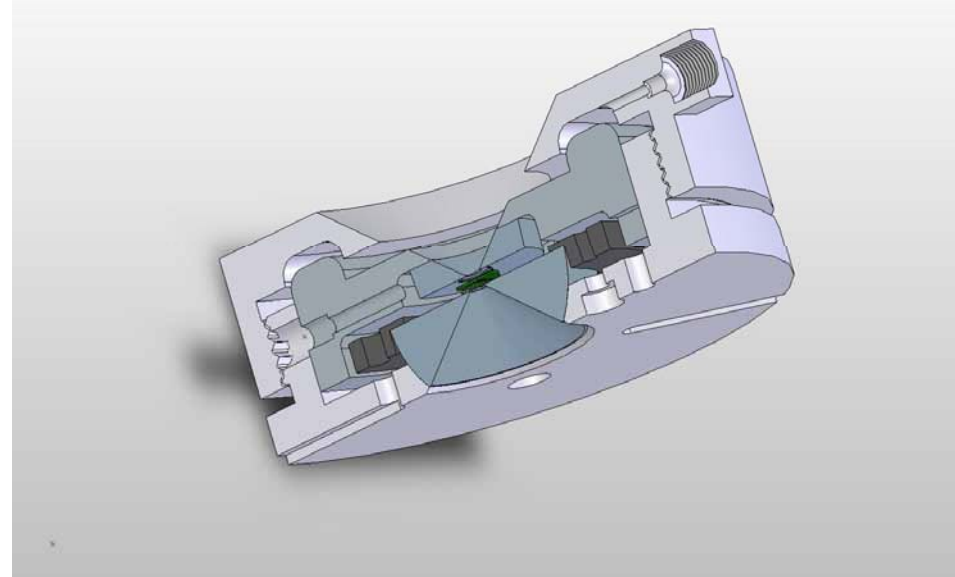
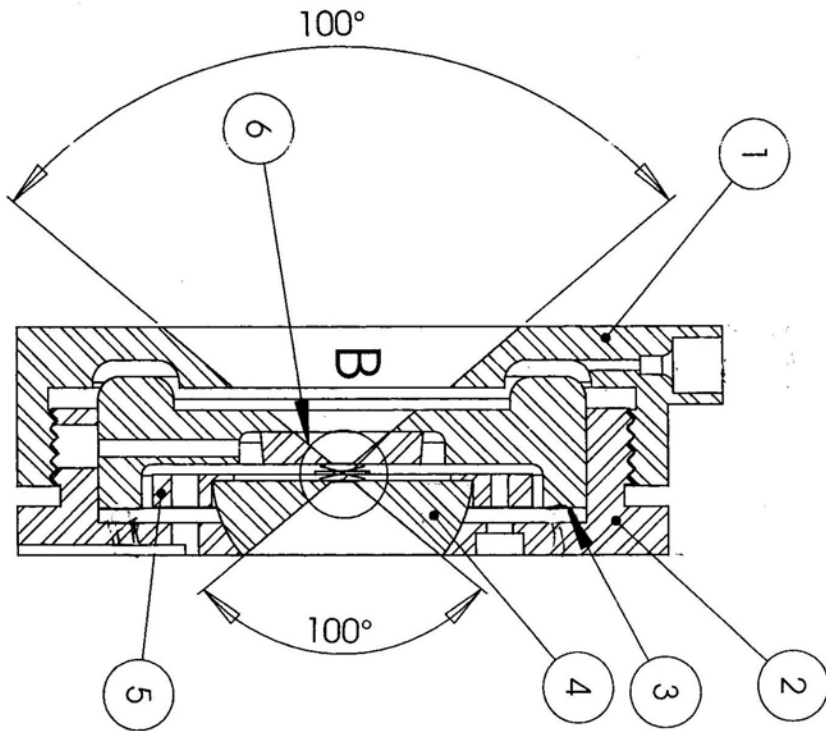
New domain for MX

Highlight of HP science with SR at ESRF

Crucial equipment on ID27 at ESRF

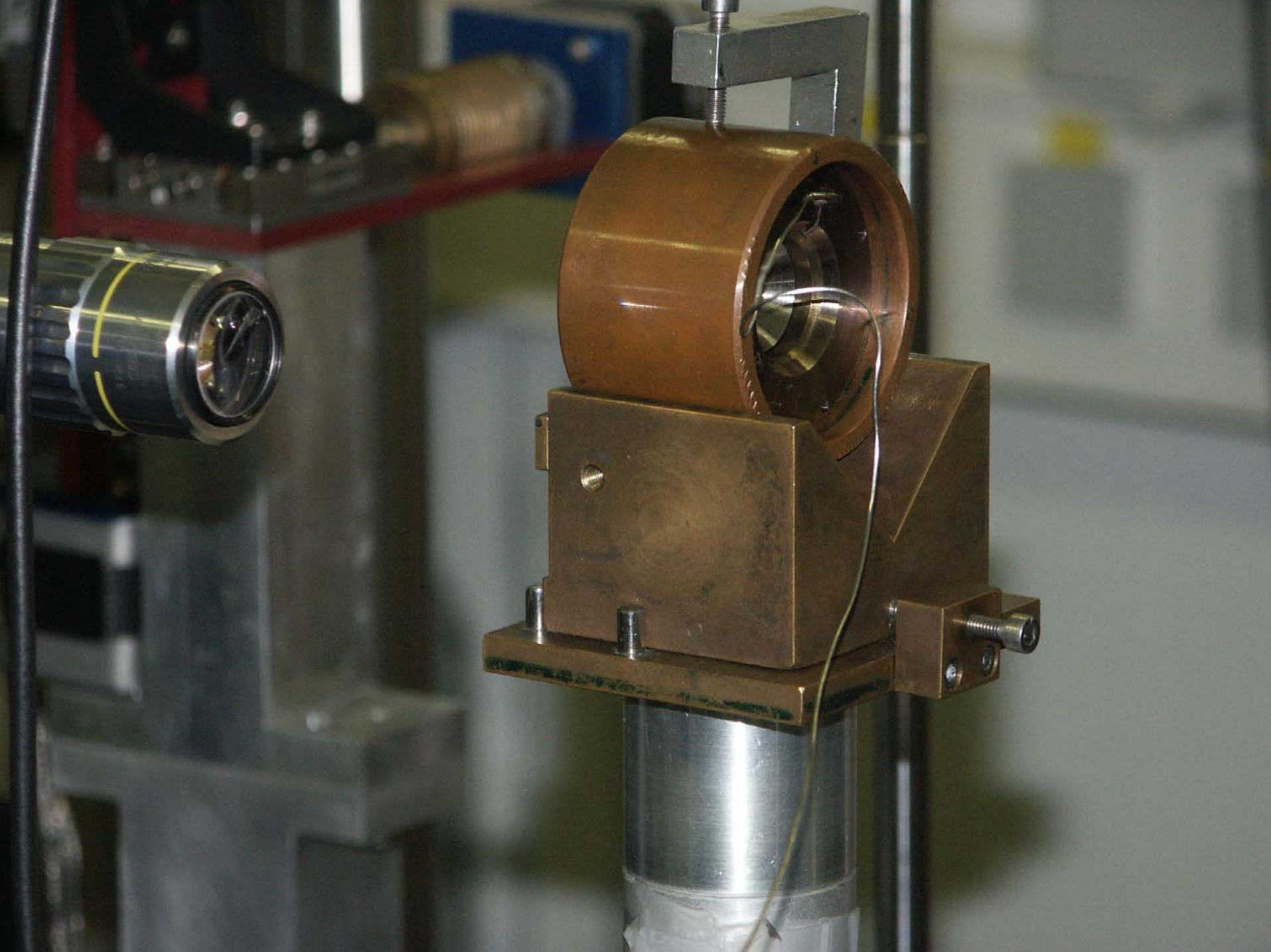
- **HP diamond cell:** cylinder/piston type, thrust generated by a toroidal membrane.
Two new DAC with aperture $> 90^\circ$
 - Thin diamonds (thickness 0.6 mm)
 - Conical diamonds (thickness 1.5 mm)Semi-automatic pressure ramping (to be tested soon)
- Two U23 undulators, unfocused radiation, ultra-short wavelength (0.331 Å)
- Large detector (MAR345 imaging plate. MAR Flat Panel tested).
- Pressure monitoring through the fluorescence of a ruby chip
- Goniometer: 3-circles, mounted on a stack of three accurate orthogonal translations. Adjustable slits : typically 50 μm x 50 μm .
- Software for data collection by the rotation method

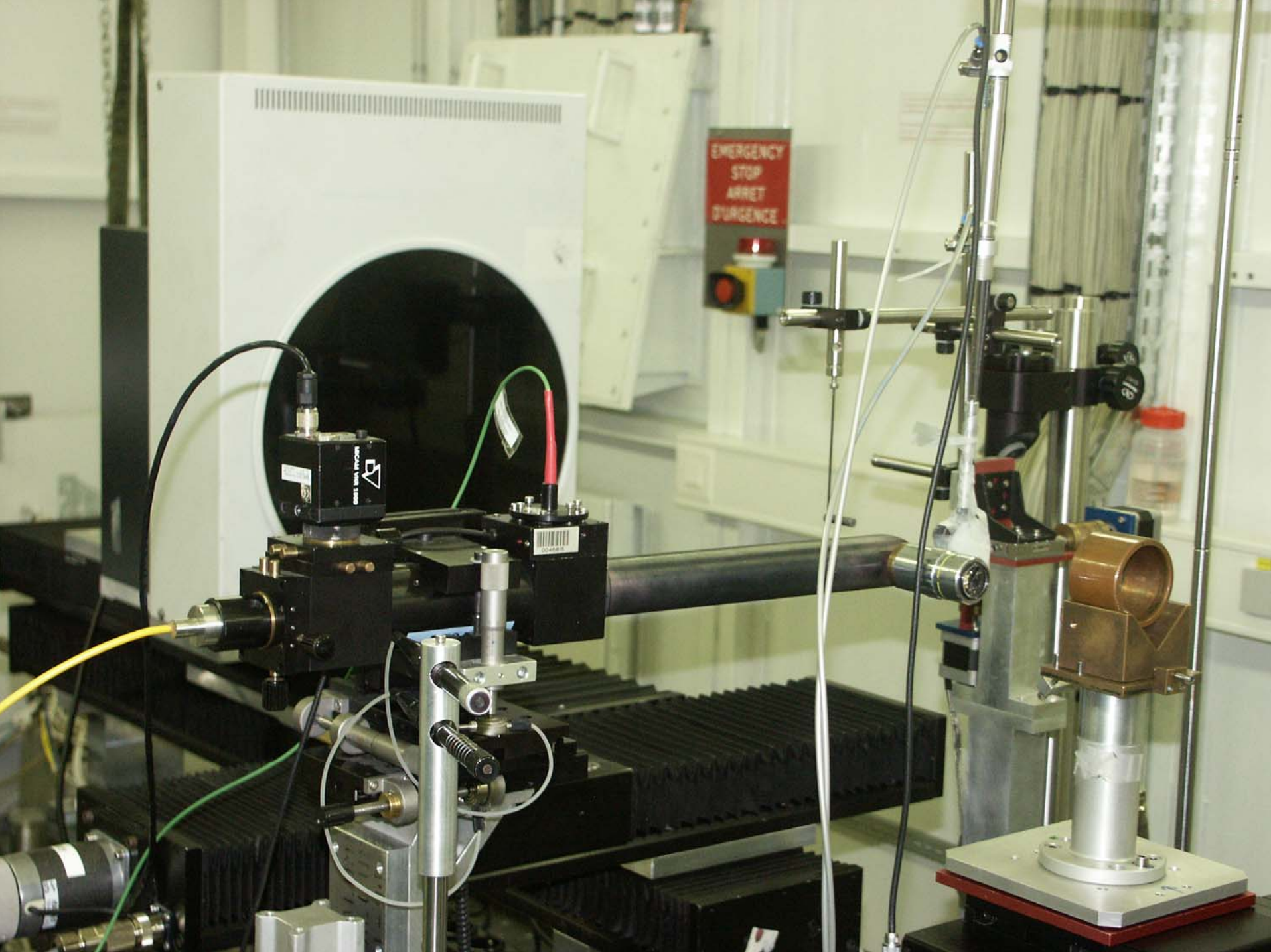
*R. Fourme, R. Kahn, M. Mezouar, E. Girard, C. Hörentrup, T. Prangé, I. Ascone
J. Synchrotron Rad. 6 (2001) 1149-1156.*

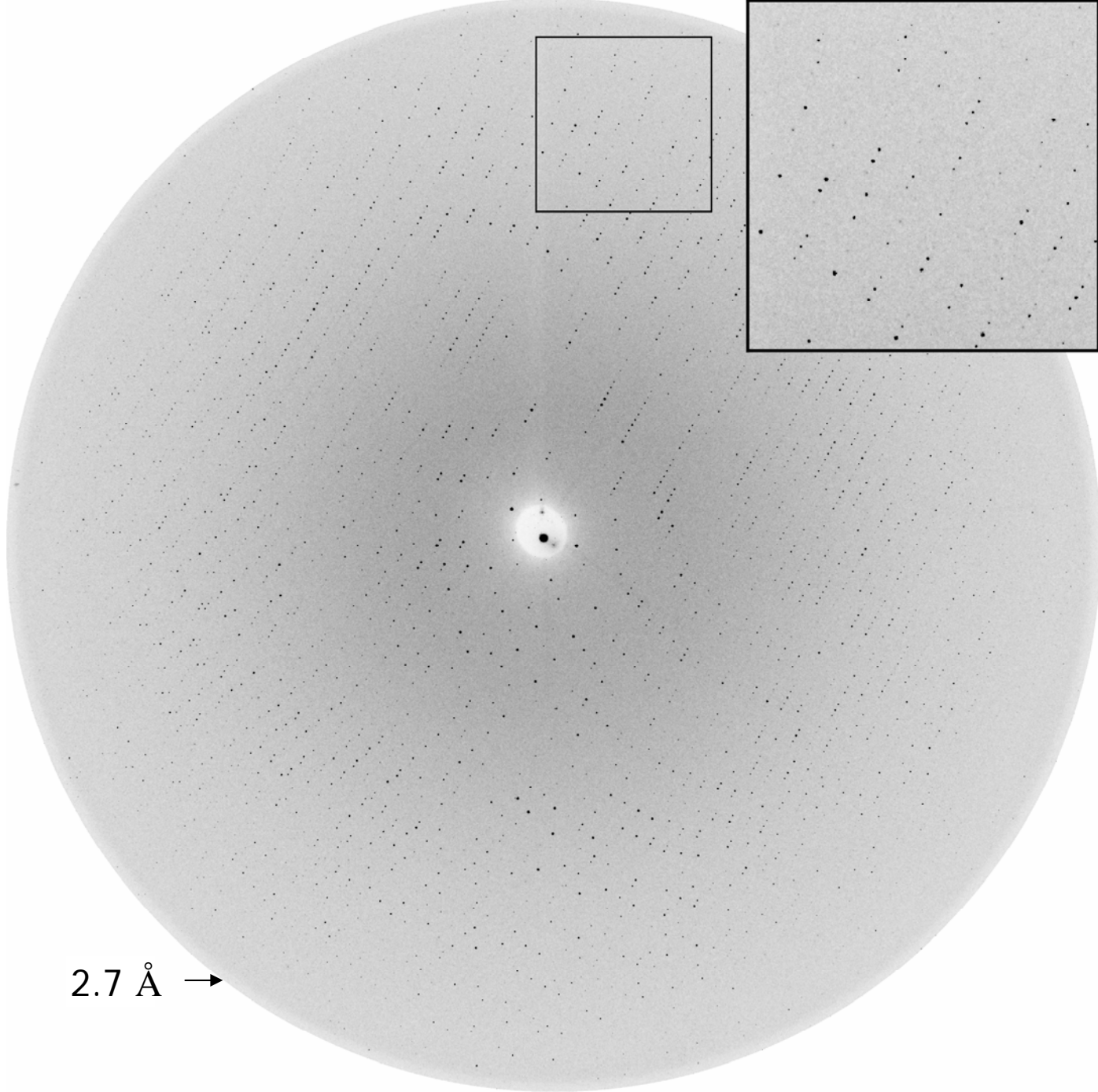


New DAC with large apertures

(collaboration J.C. Chervin & B. Couzinet, IMPMC Paris)



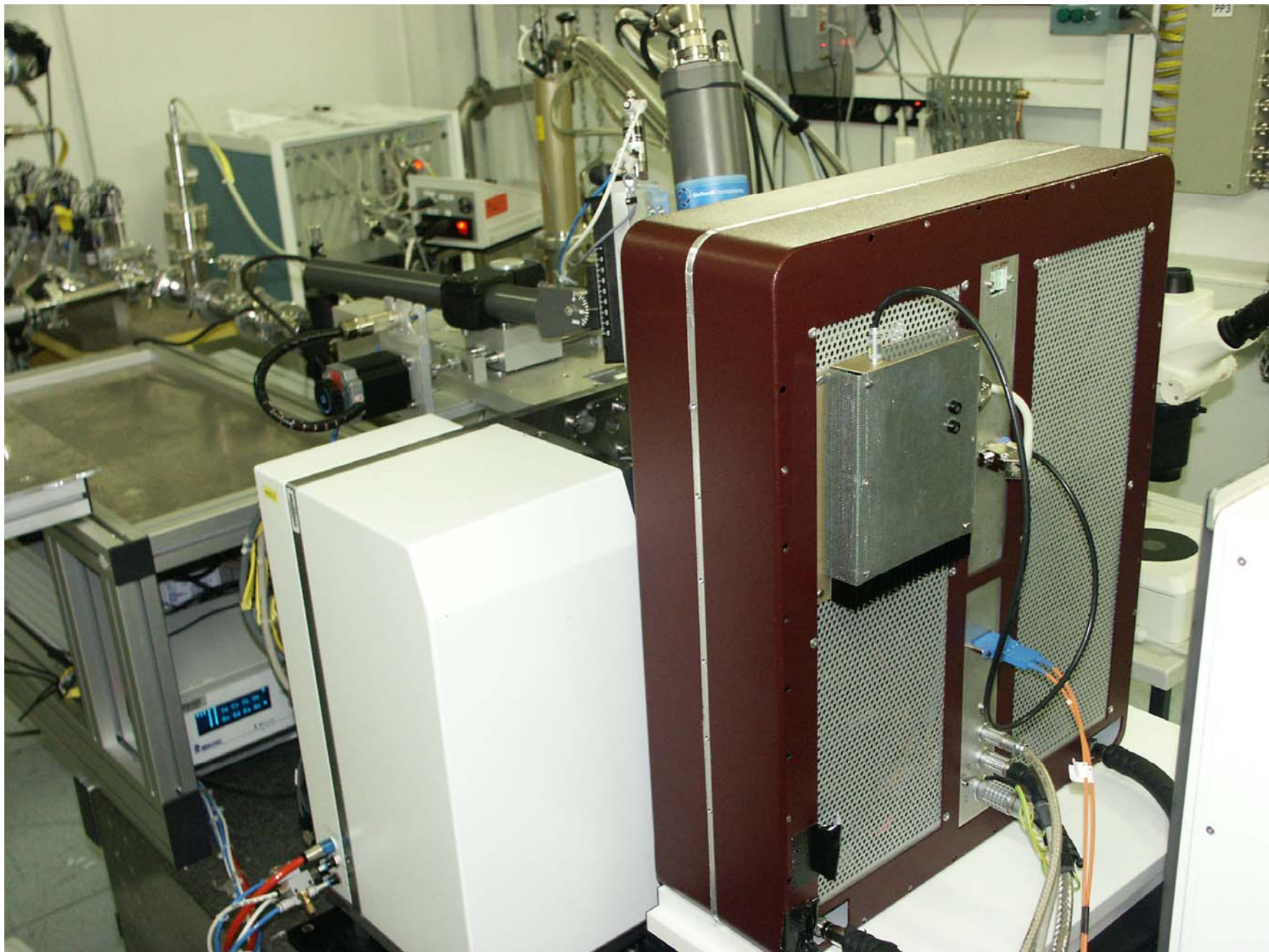




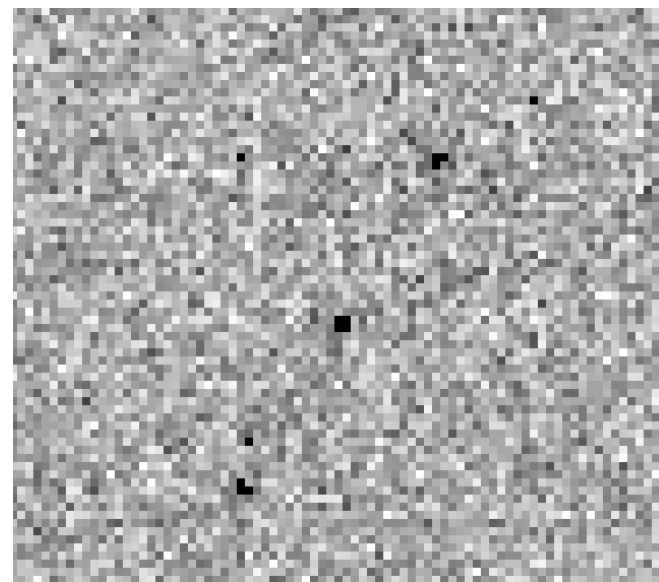
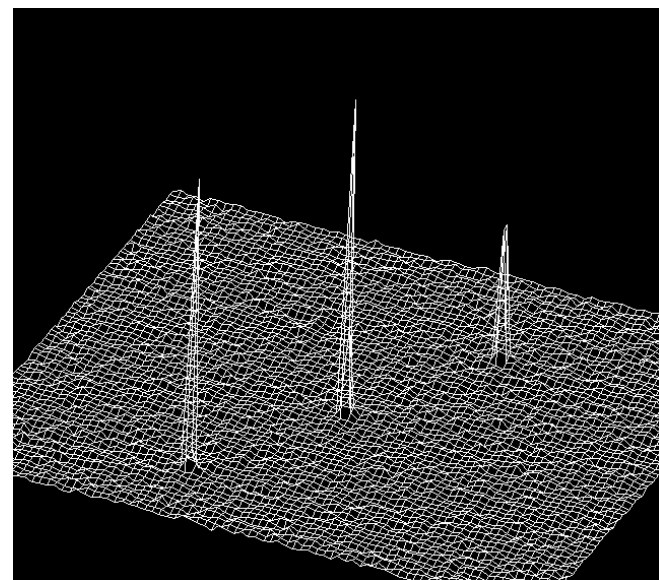
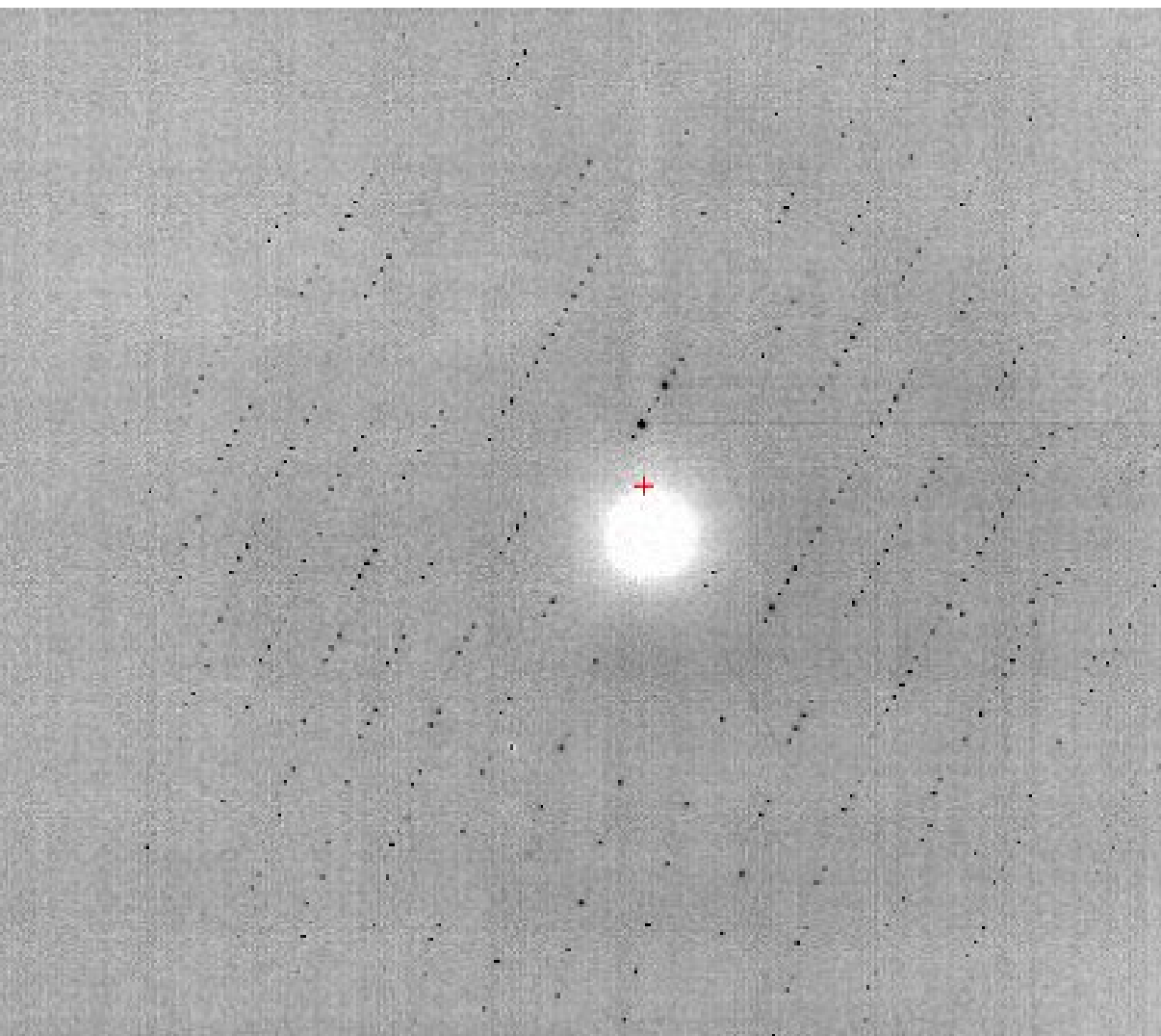
CpMV crystal
3.3 kbar
I23 space group

osc. 0.3°
exp. time 90 sec
MAR345 detector

2.7 Å →



R. Fourme, High Pressure Workshop, CHESS 2006



ESRF ID27 beamline, two U23 undulators, 6 GeV, 180 mA, parallel beam,
cross-section $50 \times 50 \mu\text{m}^2$, $\lambda = 0.0331 \text{ nm}$.

hen egg-white lysozyme crystal in DAC,
MAR Flat Panel,
crystal-to-detector distance 800 mm.
oscillation 1 degree, exposure 41 sec. (dec 2005)

Pros of ultra-short wavelengths...

- reduced absorption and extinction by diamonds and sample.

Absorption of the direct beam by the first diamond is constant for a narrow range of oscillation, and taken into account by the scaling procedure. Absorption of diffracted beams by the second diamond could be corrected by calculation of the absorption path for each reflection; at the present stage, no correction was applied as the maximum variation of transmission due to path length variations was small at $\lambda < 0.4 \text{ \AA}$.

Some reflections may be attenuated when the direct or diffracted beams are diffracted by diamonds. As the width of Bragg reflections for both protein crystals and diamonds is very small, prediction of relevant reflections and to what extent they were attenuated was difficult. However, the attenuated reflections were likely to be rejected during the data processing as the outliers.

- diffraction is confined within a narrow forward cone

at $\lambda = 0.33 \text{ \AA}$, the full opening angle $4\theta_{\max}$ is 25.3° at 1.5 \AA resolution; thus optimal use of useful aperture of DAC

- angular smearing of reflections reduced as θ is small (varies as $\tan\theta \delta\lambda/\lambda$)
- diffracted X-rays are nearly normal to detector
- radiation damage?

...combined with suitable X-ray beam and detector

- **Parallel beam** (as tightly collimated beam + small Bragg spots on the detector for high S/N) → *No focusing optics.*
- **High intensity** (as unfocused radiation + λ^2 -decrease of the scattering power) → *in vacuum undulator on a high energy storage ring such as ERSF.*
- **Optimal wavelength with Imaging plate detector: $\lambda = 0.331 \text{ \AA}$**
(on the high-energy side of the Ba K-absorption edge)
 - Stopping power of the BaFBr: Eu²⁺ sensitive layer is maximal (0.44) for elastic scattering
 - It is lower for the inelastic (Compton) scattering from diamonds.
The energy shift of Compton scattering is given by $\Delta E \cong -\frac{h^2}{2md^2}$.
 $\Delta E = 6 \text{ eV}$ at 5 \AA resolution, 16.7 eV at 3 \AA resolution, 66.8 eV at 1.5 \AA resolution. This is sufficient to produce a significant variation of the barium absorption coefficient. The signal-to-background ratio is improved where most useful, i.e. in the region of the diffraction pattern where signals are weakest.

... ensure diffraction data with high s/n ratio

- **Sharp Bragg reflections:** small size ($\delta x, \delta y$) and small angular extension ($\delta\omega$) due to combined effects of : crystal at room temperature with low mosaicity, parallel beam, small $\delta\lambda$, no extension for inclined beams, small angular smearing.
Interest of detector with
 - small point spread function (eg detector with direct conversion in amorphous Se, pixel detector)
 - fast readout, for fine ω -slicing or continuous rotation.
- **Large detector** for long crystal-to-detector distance, providing $1/d^2$ attenuation of parasitic scattering, fluorescence...

Interest of HPMX

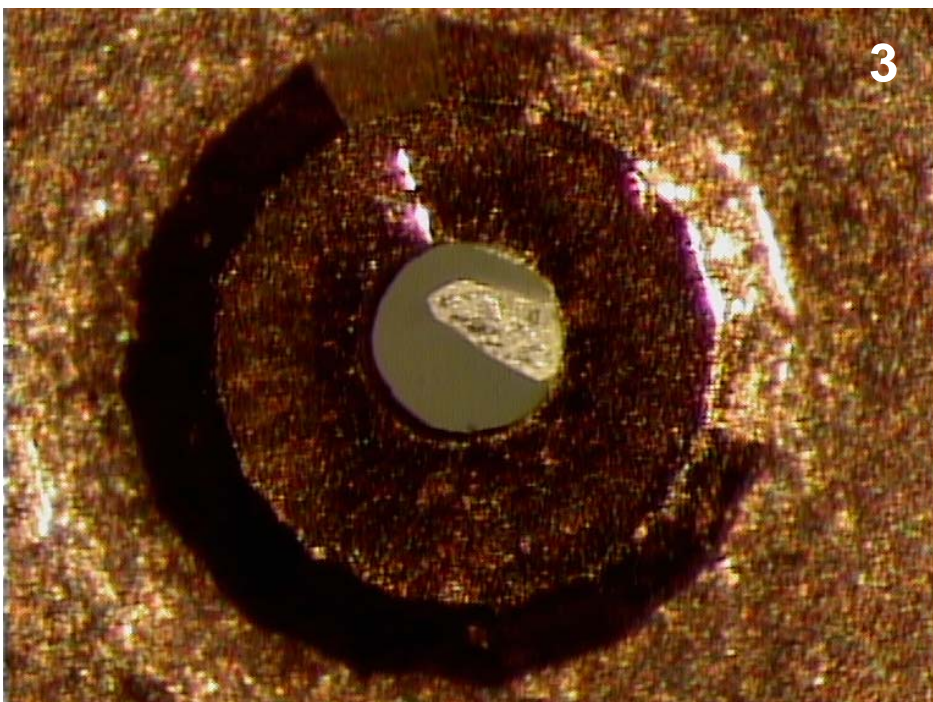
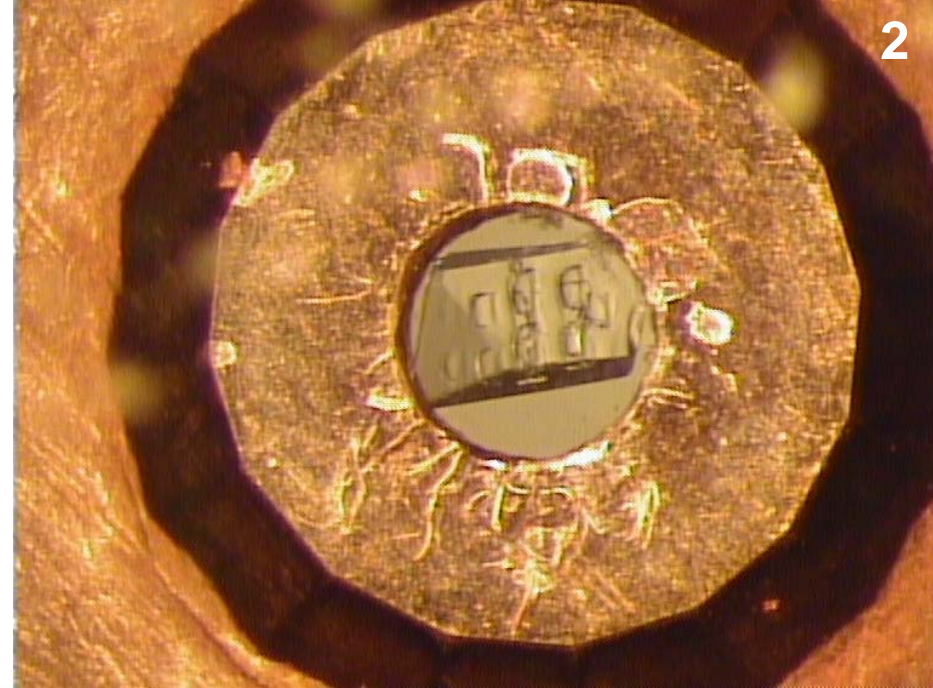
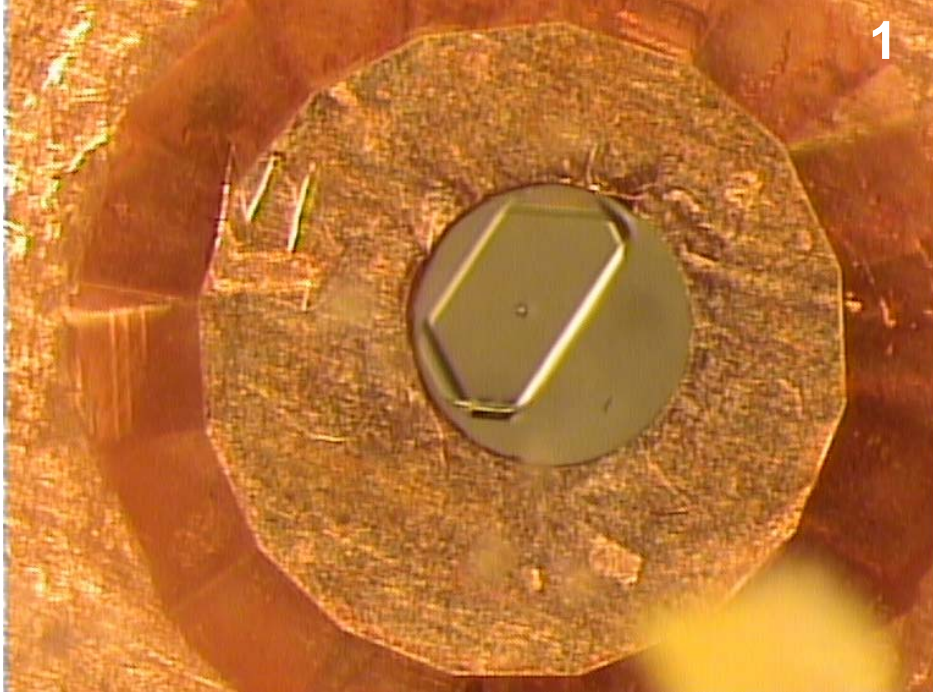
- 1. Pressure is a way to explore the energy landscape of macromolecules.**
It favours compact phases, and can accordingly modify the population of conformers in a protein solution (see NMR studies). The crystalline state is probably more selective than solution and may act to some extent as a conformation filter.
→ *Biologically relevant high-energy conformers may be amplified, trapped and their 3D structures solved.*
- 2. Molecular basis of life adaptation to extreme conditions**
- 3. Onset of pressure-induced dissociation of multimeric assemblies and protein unfolding/folding.**
- 4. Detailed analysis of interactions (H-bonds etc...), cavities**

*R. Fourme, E. Girard, R. Kahn, A.C. Dhaussy, M. Mezouar, N. Colloc'h & I. Ascone
High Pressure Macromolecular Crystallography (HPMX): Status and prospects.
Biochimica et Biophysica Acta 1764 (2006), 384-390*

Crystal structure, evolution and stability under high pressure of urate oxidase, a 140 kDa tetramer

*N. Colloc'h, E. Girard, A.C. Dhaussy, R. Kahn, I. Ascone, M. Mezouar & R. Fourme
High Pressure Macromolecular Crystallography (HPMX): the 140 MPa crystal structure
at 2.3 Å resolution of urate oxidase, a 135 kDa tetrameric assembly.
Biochim. Biophys. Acta **1764** (2006) 391-397.*





- 1. Fresh plate-shaped urate oxidase crystal**
- 2. Crystal irradiated at 9 different positions**
- 3. Diamond chip introduced in the compression cavity. Forces different orientations of plate-shaped crystal with respect to diamond tips.**

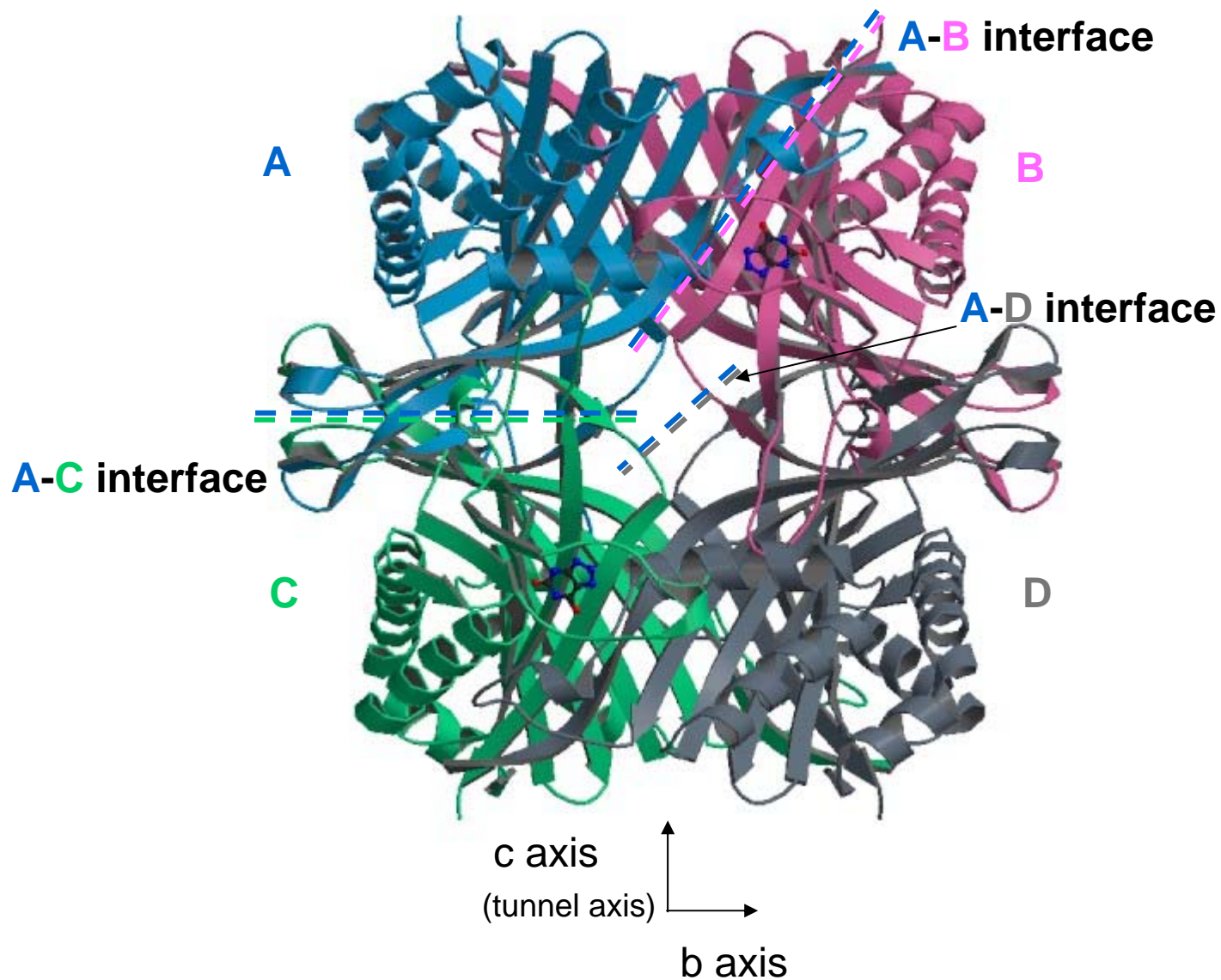
Urate oxidase-8 azaxanthin complex.

Space group I222

Data collection at 140 MPa and 21°C using 3 crystals.

d_{\min} (Å)	R_{merge}	I/sigma	Nmeas	Nref	Completeness (%)	Multiplicity
7.25	0.037	14.2	1809	516	90.8	3.3
5.12	0.040	15.6	4147	975	96.8	4.0
4.18	0.033	19.2	5301	1239	98.2	4.0
3.62	0.040	15.2	6487	1485	98.4	4.1
3.24	0.052	12.4	7385	1665	97.6	4.2
2.96	0.075	9.1	8447	1870	99.4	4.3
2.74	0.108	6.3	9182	2018	98.4	4.3
2.56	0.160	4.2	9937	2169	98.6	4.4
2.42	0.209	3.1	10673	2332	99.9	4.4
2.29	0.255	2.6	10575	2322	95.0	4.3
Over all:	0.068	9.2	73943	16591	97.8	4.2
	Refinement:		$R_{\text{cryst}} = 0.168$	$R_{\text{free}} = 0.215$		

Tetramer with interfaces



Evolution of urate oxidase under high pressure

Regular features

- Packing compression
- Strong reduction of cavity volume

Abnormal features

- For the « master » interfaces (A-B and A-C) in the tetramer:
 - Substantial and significant increase of hydrogen bond distances
 - Buried areas evolution: decrease for A-B, nearly unchanged for A-C
 - Van der Waals contacts hardly changed
- Increased remperature factor values (overall 27.0 instead of 23.7Å²)

→ The onset of dissociation of the tetramer has been captured.

- Complementary experiments by HP florescence and HP SAXS under way.

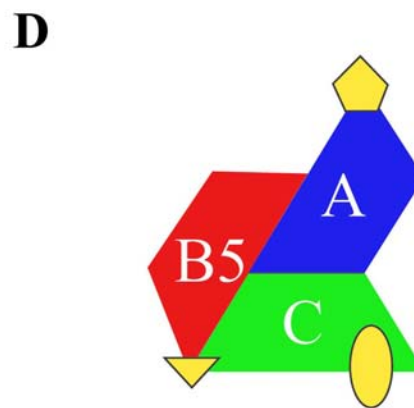
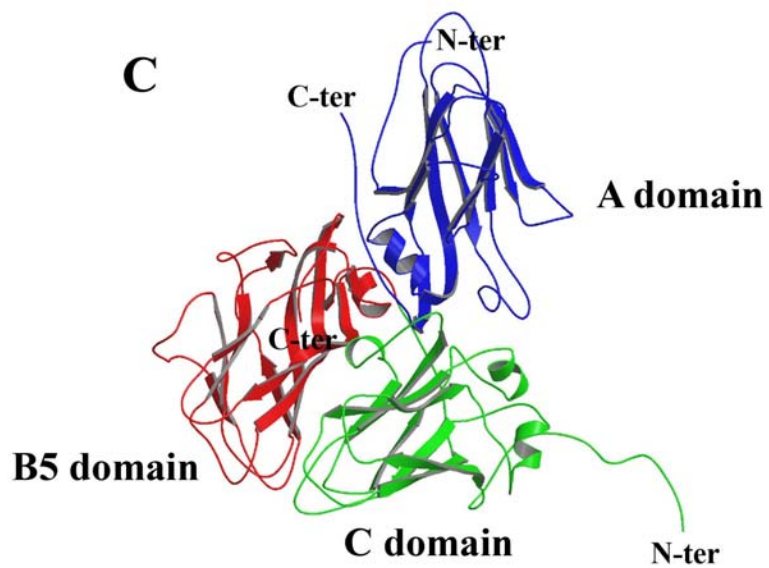
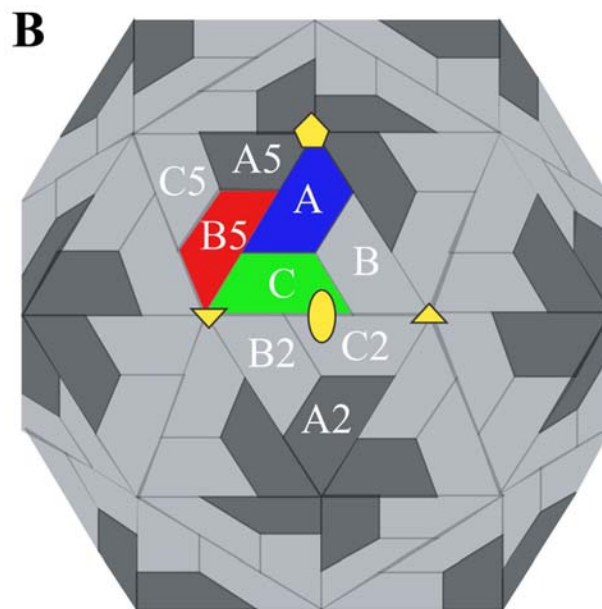
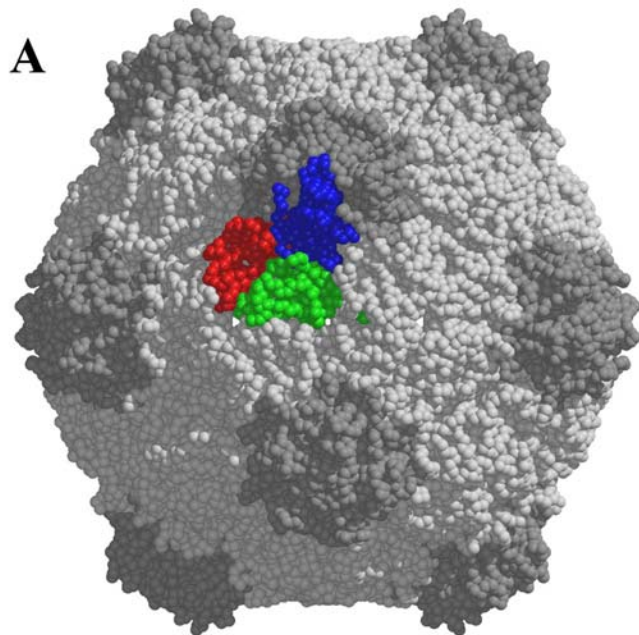
- Numerical simulations under way.

Crystal structure and evolution under high pressure of the viral capsid of a virus, CpMV

E. Girard, R. Kahn, M. Mezouar, A-C Dhaussy, T. Lin, J. E. Johnson & R. Fourme

The first crystal structure of a macromolecular assembly under high pressure: CpMV at 330 MPa.

Biophysical J. 88 (2005) 3562-3571.



Conditions

Crystal-to-detector distance : 1400 mm
Oscillation/frame: 0.3°
Exposure time: 80 sec. per frame
8 crystals at room temperature

Space group

cubic I23 **a = 313.38 Å**
260580 non-H atoms in the capsid

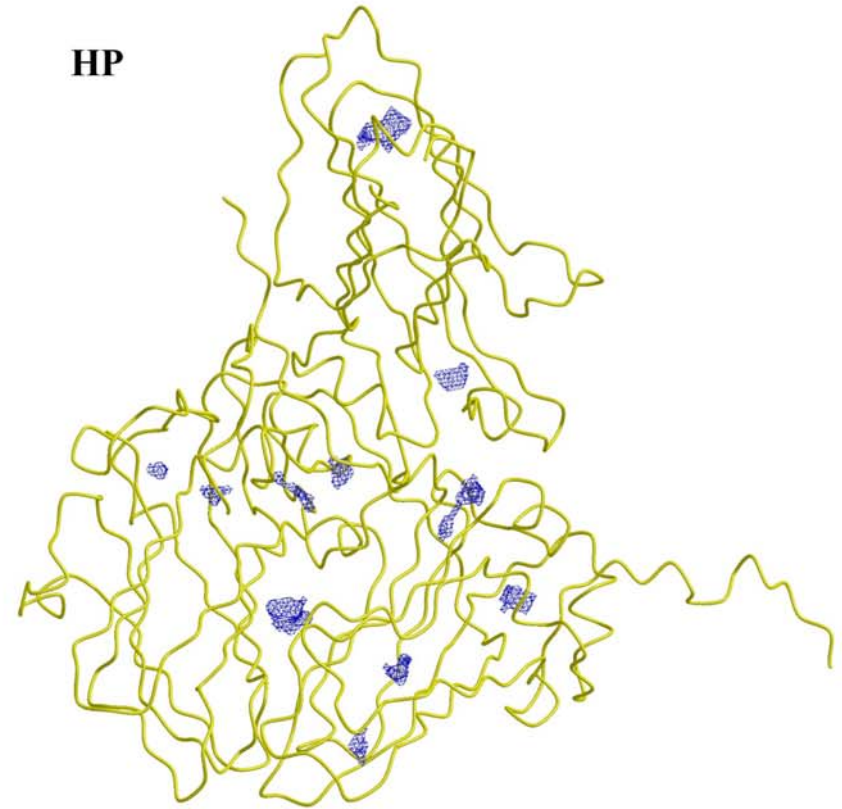
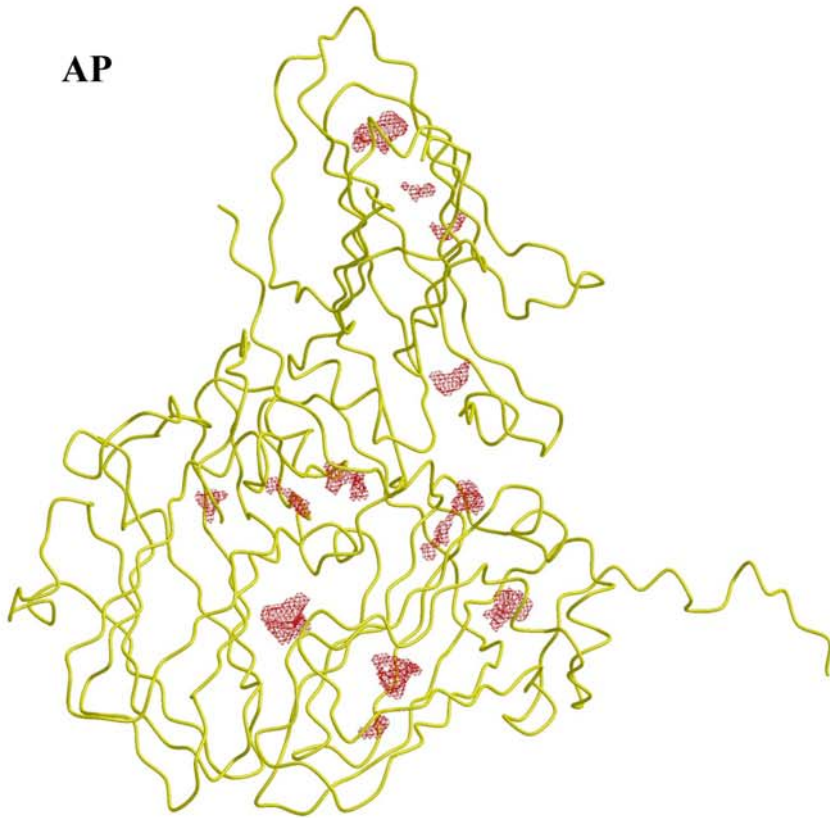
Data statistics

No. of observed reflections: 389239
No. of unique reflections: 113844
Resolution : 50 – 2,8 Å
I / σ (I) : 4.9
Completeness: 91.2 %
Redundancy : 3.4

Refinement

Program: CNS
4343 non-H atoms, 5 sulfate ions and 1 Cs ion.
R factor: 16.1 %
R free : 16.7 %

CpMV at 330 MPa : Results of data collection and refinement



The number of cavities within the L and S proteins is reduced.

The mean cavity volume reduction is 40%.

RESULTS

System	Aminoacids in asymmetric unit	Goals	Status
Hen egg-white Lysozyme (HEWL)	129	Structural evolution under HP.	Data collected at 700 and 650 MPa with refinement. New data to be collected at various pressures. Published in preliminary form [12].
Cowpea mosaic virus (CpMV)	5 x (189 + 369) = 2790	Disorder-to-order packing transition. Evolution of viral capsid proteins under HP.	Structure refined with 5-fold NCS at 330 MPa and AP. Published [13].
Urate oxidase from <i>A. flavus</i>	295	Evolution of 3D structure and tetrameric association under HP.	Structure refined at 140 MPa and AP. Onset of tetramer dissociation captured in crystal. Published [14].
Cellulase from <i>P. Haloplanktis</i>	2 x 293 = 586	Molecular basis of life adaptation to low temperature and HP.	3D-structures refined at 175 MPa and AP. <i>Coll. N. Aghajari, IBCP Lyon, France</i>
Bovine Cu,Zn superoxide dismutase (SOD)	2 x 150 = 300	Evolution of 3D structure and dimeric association under HP. Phase transition at the active site.	3D structure refined at 540 MPa.
Green Fluorescent Protein (GFP), wild type	230	Evolution of 3D structure and chromophore environment with pressure.	Data collection planned 2006. <i>Coll. P. Oger, ENS Lyon, France</i>
Ubiquitin	76	Trapping and 3D structure of high energy conformer.	Data collection planned 2006. <i>Coll. K. Akasaka, Kinki Univ, Japan</i>
Oligonucleotides	--	Evolution of 3D structure with pressure.	Data collection planned 2006. <i>Coll. T. Prangé, Paris 5 Univ, France</i>

Interest of HP and HPMX mode of data collection for standard MX

1. **Pressure tends to reduce entropy → a way to improve crystal order (instead of space shuttle?)**
2. **HPMX data collection suggests tracks to improve conventional data collection (in terms of S/N, data collection efficiency and accuracy)**

→ through ultra-short wavelengths, parallel beam geometry, long XtoD distances, multiple hit data collection on low mosaicity crystals at room temperature, detector with smaller PSF: new paradigm for data collection

R. Fourme, E. Girard, R. Kahn, I. Ascone, M. Mezouar, A.C. Dhaussy, T. Lin, J. E. Johnson

Results of data acquisition using a quasi-plane wave of ultra-short wavelength for high pressure virus crystallography. Possible implications for conventional data collection.

Acta Cryst. D **59** (2003) 1914-1922.

Interest of the Cornell ERL for HPMX

1. Monochromatic data collection

→ *Exploitation of high brilliance at short/ultrashort wavelengths*

At ESRF, exposures typically 30-300 sec/deg with parallel beam and irradiated volume of $50 \times 50 \times 100 \mu\text{m}^3$ at 0.33 \AA . With the ERL: data collection faster by several orders of magnitude and/or smaller samples.

Possible scheme:

Computer-driven translation of crystal by steps. For each sample position, continuous rotation of crystal with angular velocity 0.1-1 degree/second. Detector with very fast readout and reasonable DQE required.

2. Limited band-pass Laue data collection

→ *Exploitation of high brilliance continuous spectrum for ultrafast data acquisition*

To study transient substates, multimer dissociation, unfolding steps etc...

Interest of the Cornell ERL for general MX

1. Fast monochromatic data collection at ultra-short wavelengths

- **Crystal at atmospheric pressure** (similar to HPMX data collection but using crystals mounted in loops, either at room temperature or cryo-cooled)
 - Collect more accurate data
 - Increase Data Collection Efficiency (« taking the best from a given sample »).
- **Crystal at high pressure**
 - Pressure may improve order of existing crystals → better data.

2. Limited band-pass Laue data collection

- Kinetics

Conclusion: strong case for dedicated beamline(s)