Bio-crystallography at high pressures

Piezophilic adaptation

- For more than 60 % of the biosphere: P > 10 MPa

- P can reach 100 MPa in the deepest trenches

A new approach to understand:

- Protein aggregates
(Alzheimer disease, Parkinson disease, Huntington syndrome)
- Conformationnal changes
(Prion diseases)

* High-pressure studies on protein aggregates and amyloid fibrils
Kim YS, Randolph TW, Seefeldt MB, Carpenter JF (2006) Methods Enzymol. 413, pp 237

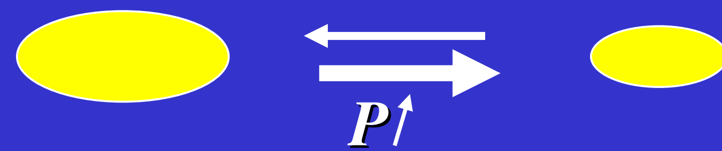
* Observation of intermediate states of the human prion protein by high pressure NMR spectroscopy
Kachel N, Kremer W, Zahn R, Kalbitzer HR (2006) BMC Struct Biol. 6, pp 16

* Hydration and packing effects on prion folding and beta-sheet conversion. High pressure spectroscopy and pressure perturbation calorimetry studies
Cordeiro Y, Kraineva J, Ravindra R, Lima LM, Gomes MP, Foguel D, Winter R, Silva JL (2004) J Biol Chem. 279, pp 32354

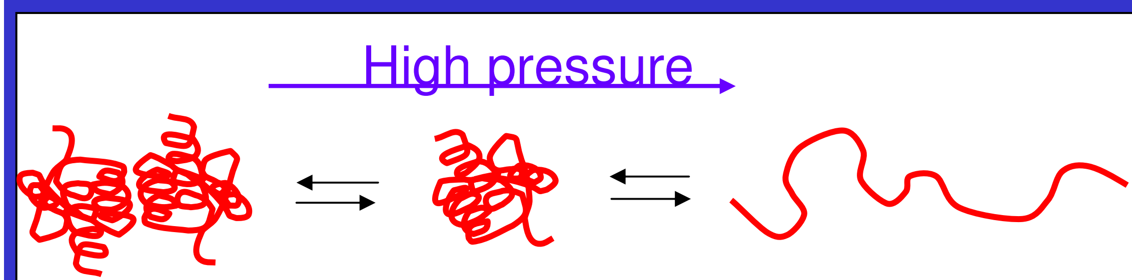
* High pressure induces scrapie-like prion protein misfolding and amyloid fibril formation
Torrent J, Alvarez-Martinez MT, Harricane MC, Heitz F, Liautard JP, Balny C, Lange R (2004) Biochemistry 43, pp 7162

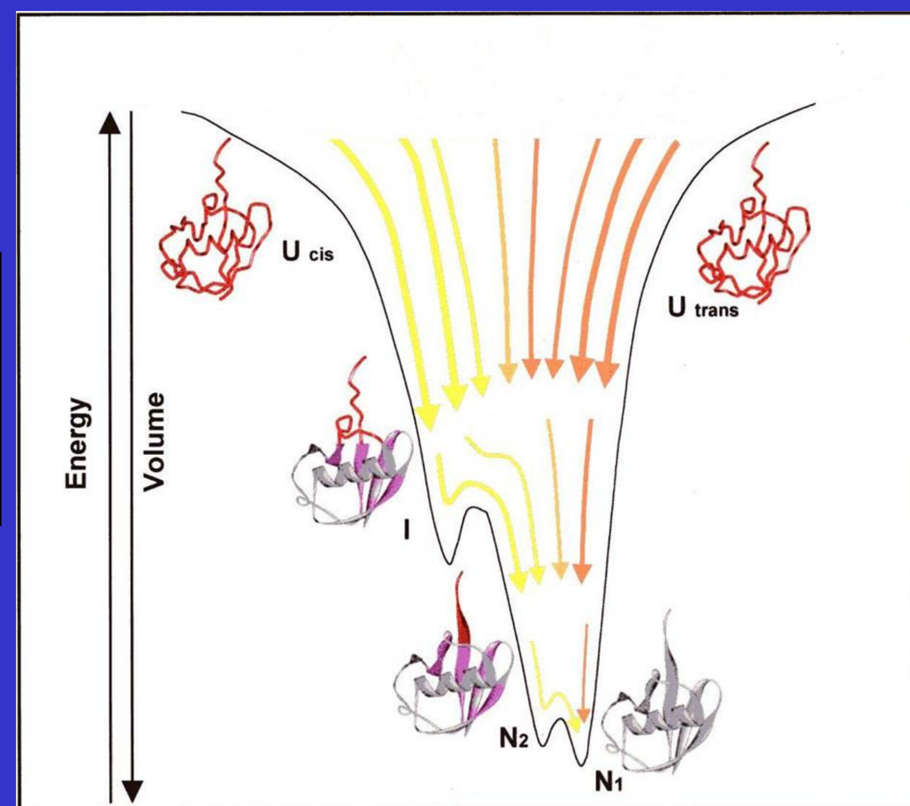
* Alternative prion structural changes revealed by high pressure
Torrent J, Alvarez-Martinez MT, Heitz F, Liautard JP, Balny C, Lange R (2003) Biochemistry 42, pp 1318

Le Châtelier principle:



Energy landscape of a protein:





- **Before 2001, bio-crystallography under high pressure was still in its infancy…**

  - **1 kbar structure of hen egg-white lysozyme by Kundrot & Richards (1987)**
  - **1.5 kbar structure of sperm whale myoglobin (Urayama et al. 2002)**

  ⟶ Both studies were performed using a polycrystalline beryllium cell (Kundrot & Richards, 1986)

  - **Use of diamond anvil cell (DAC) for protein crystallography by Katrusiak & Dauter (1996). Measurement of cell parameters.**

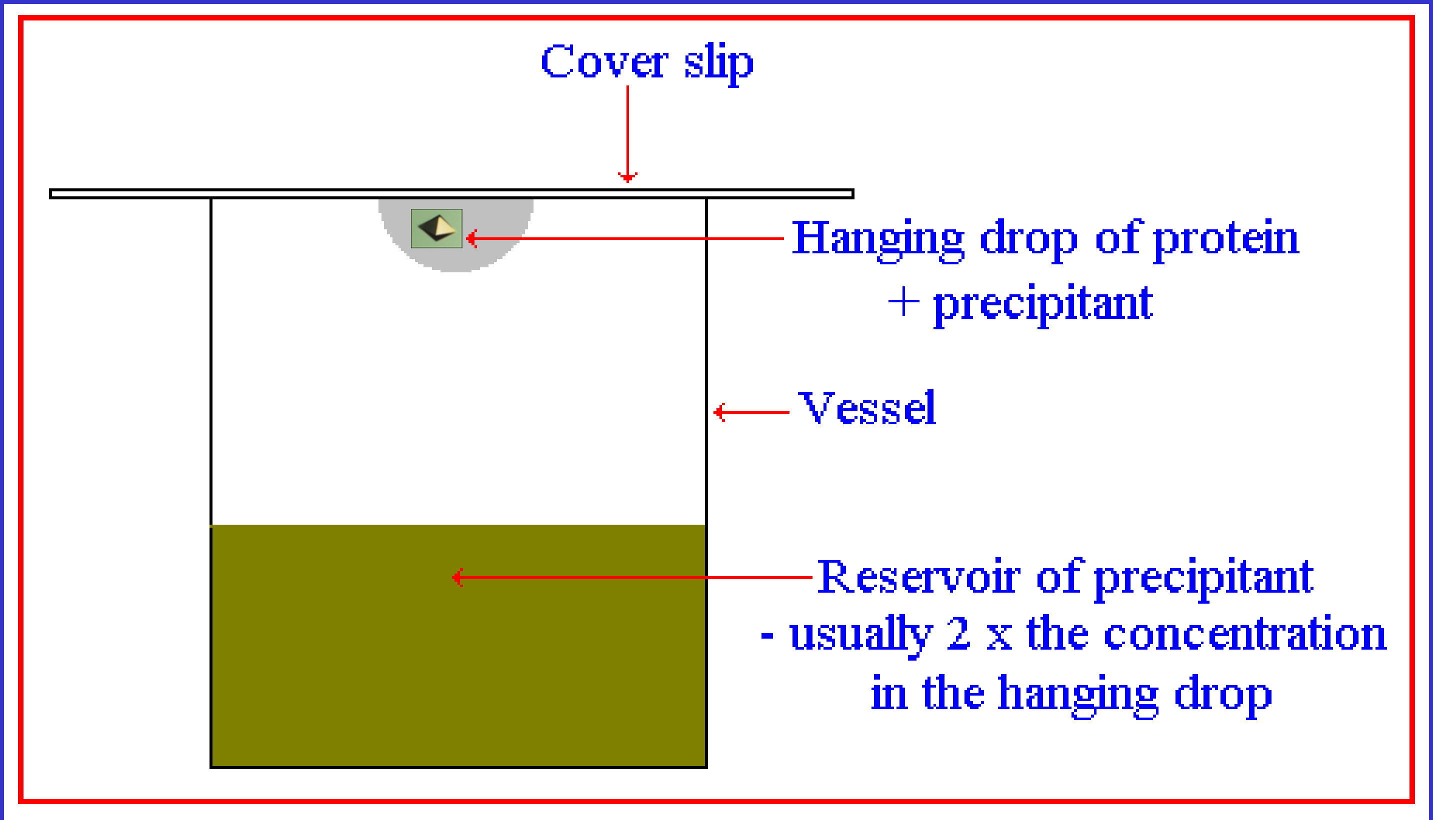
- **Still a lack of structural information under high pressure**

- **DAC + ultra-short-wavelength radiation**

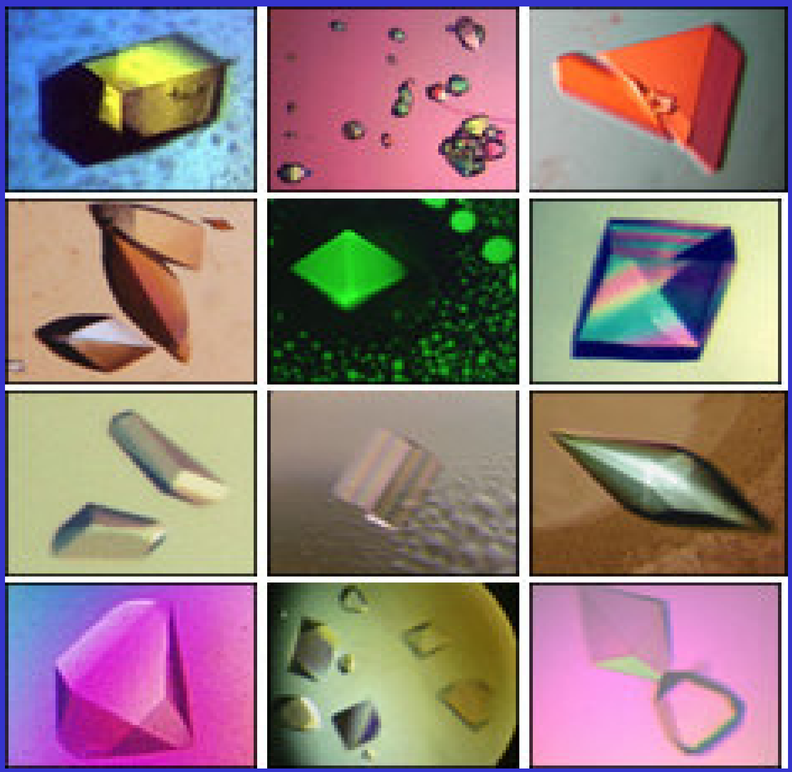
  ⟶ Data collection on crystals of biological macromolecules under high pressure (beyond 1 GPa)

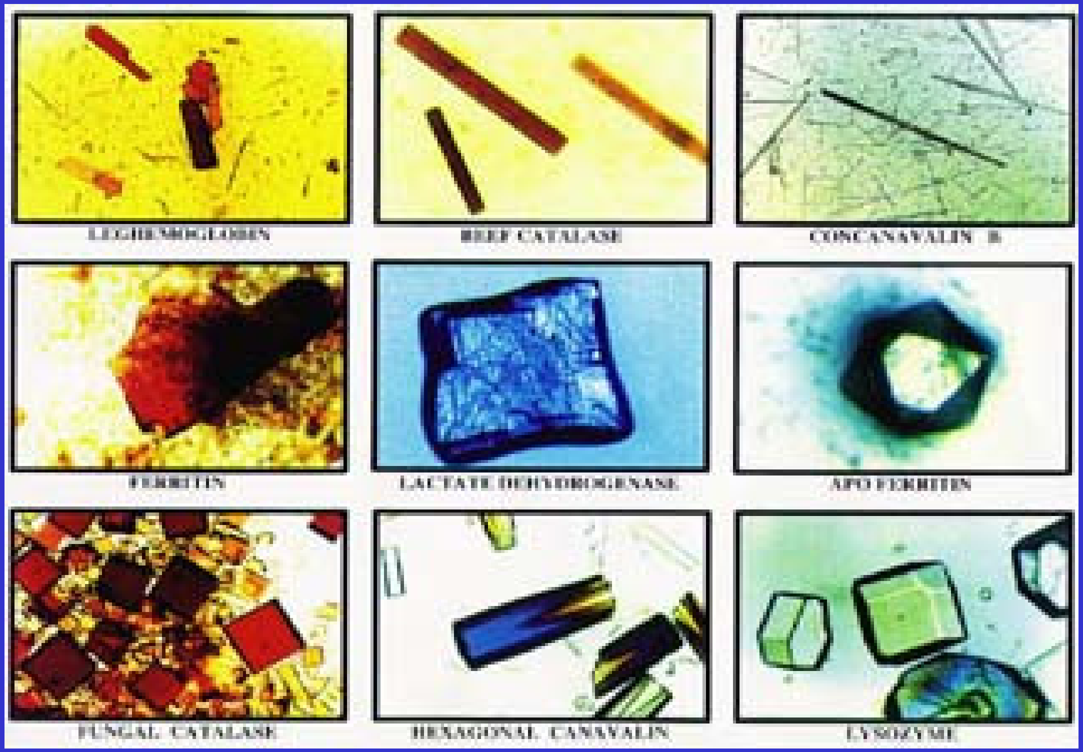
Macromolecular crystals

- Our samples are crystals which are difficult to grow, fragile and highly radiation sensitive. They need to be kept in equilibrium with the liquid solution (the mother liquor) in which they grow.



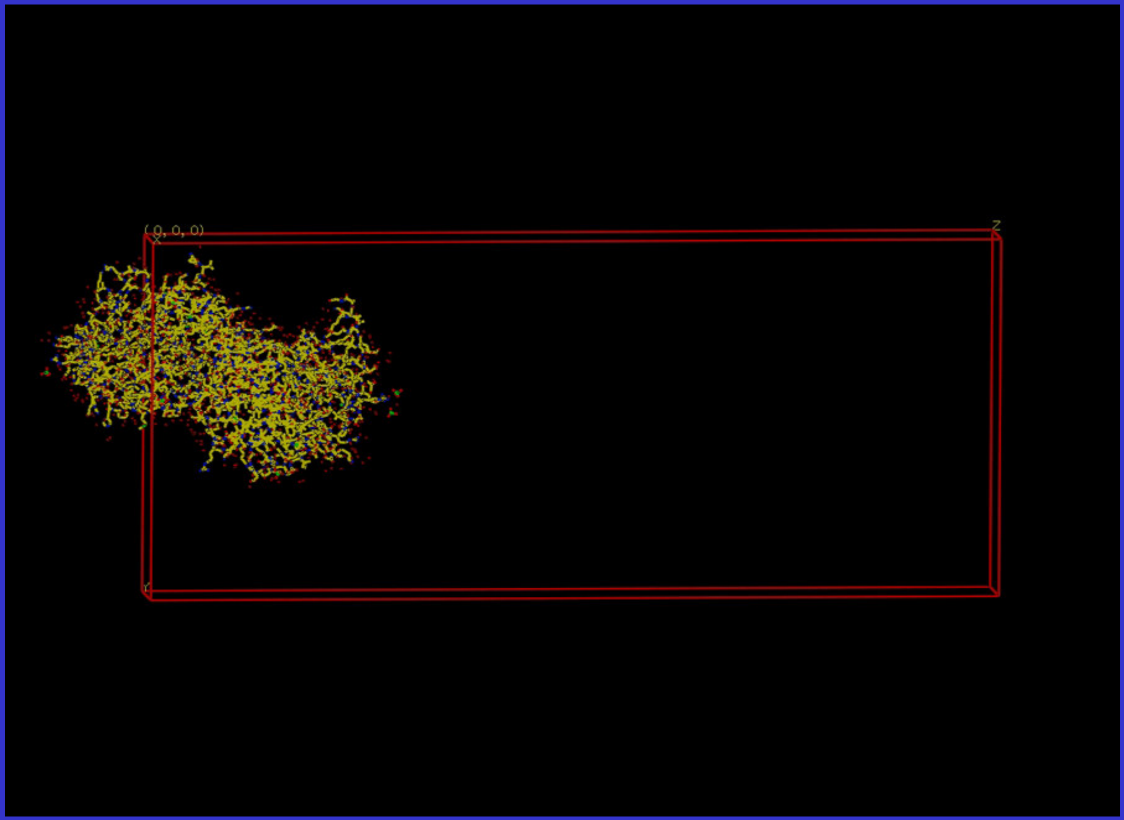
Macromolecular crystals



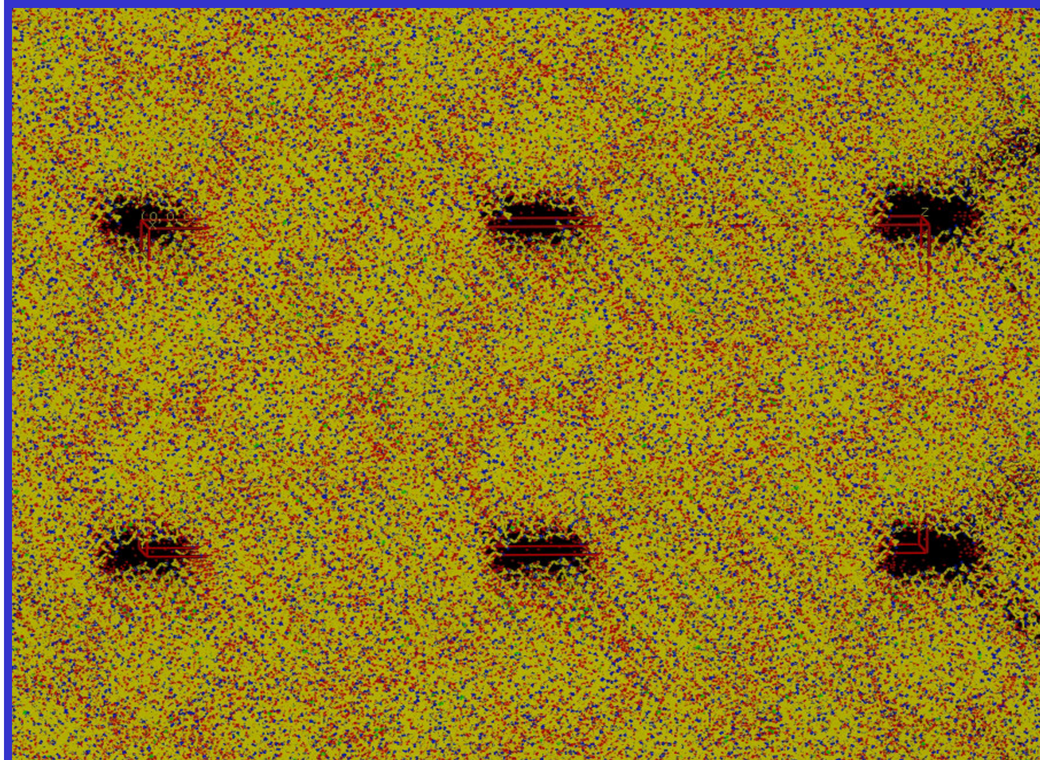


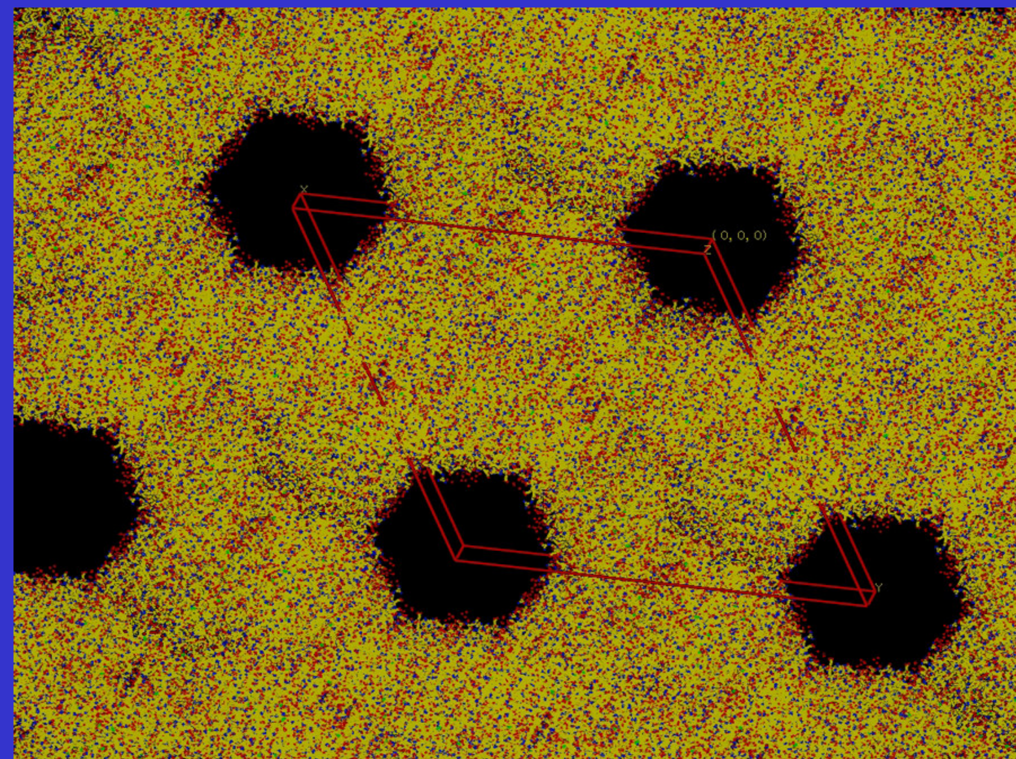
Macromolecular crystals

- **These crystals contain from 30 % to 80 % of solvent (water, salts, other molecules present in the crystallizing solution).**
- **The liquid phase in the sample and the mother liquor in which the crystal is bathed communicate through channels within the crystal structure. Compression is truly hydrostatic.**



Macromolecular crystals





Macromolecular crystals

- This semi-liquid/semi-solid state is:
  - Sufficiently rigid so the long range order may be extremely good
  - Sufficiently plastic allows annealing and accommodation of large variations of specific volume (if the pressure variation is smooth and relatively slow).

Conclusion

Macromolecular crystals are well adapted to high pressure studies!

Experimental conditions

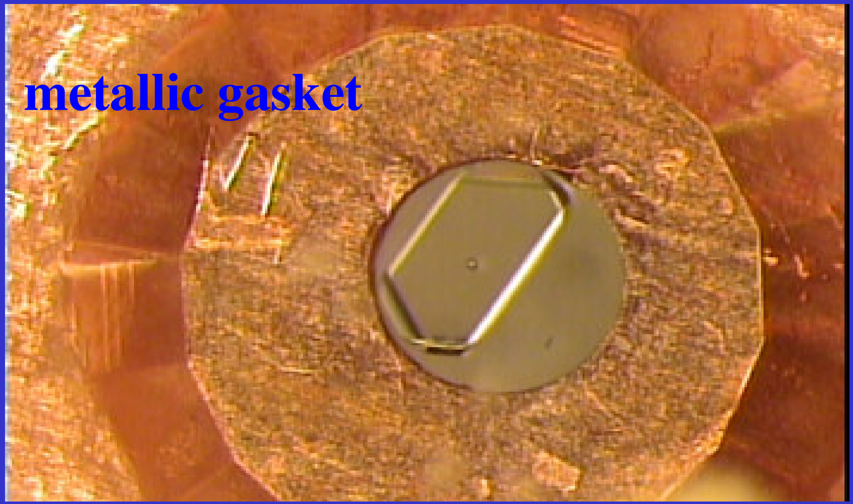
- Wavelength: λ = 0.3301 Å (Ba K-edge)
- Crystal-to-detector: 600 - 1600 mm
- Exposure time: 30 - 90 s/image

  Unfocused X-ray Beam

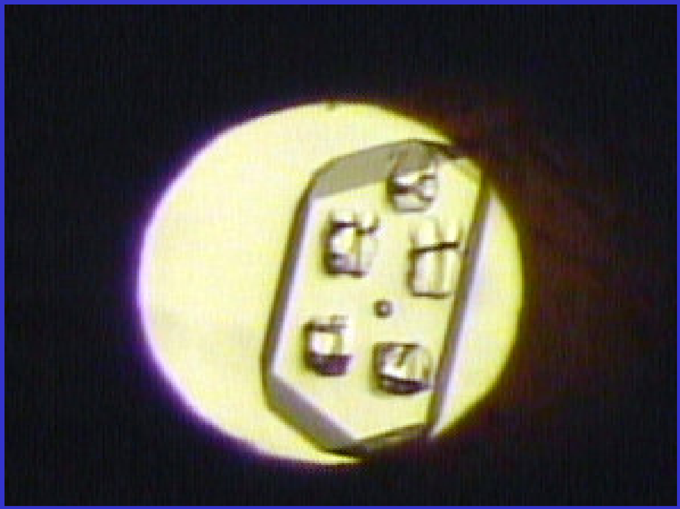
  Two in-vacuum undulators

- Cavity diameter: 400 μm
- Thickness: 150 - 200 μm

- Room temperature
- Beam size: 50 x 50 μm^2

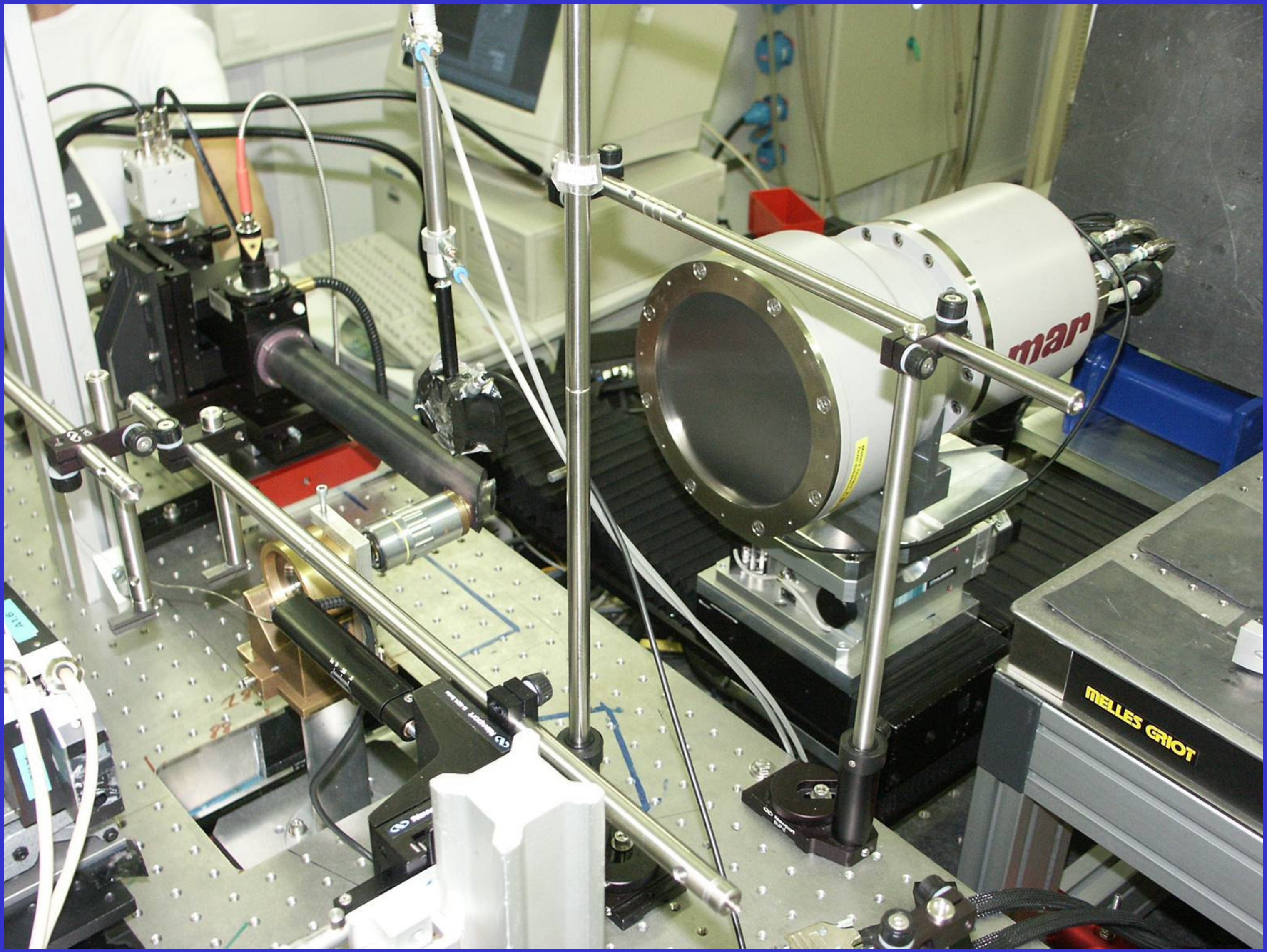


Fresh urate oxidase crystal



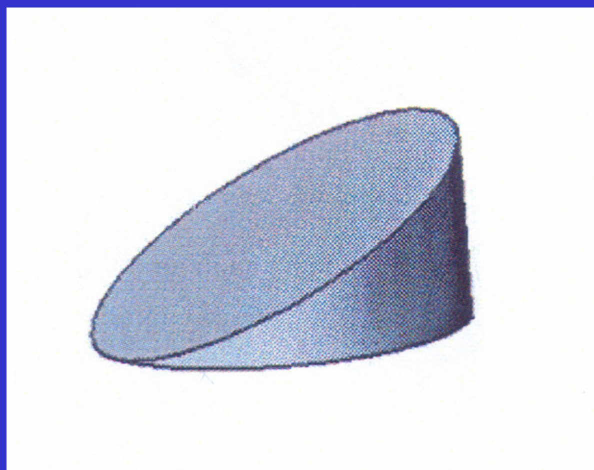
Crystal after multiple irradiations

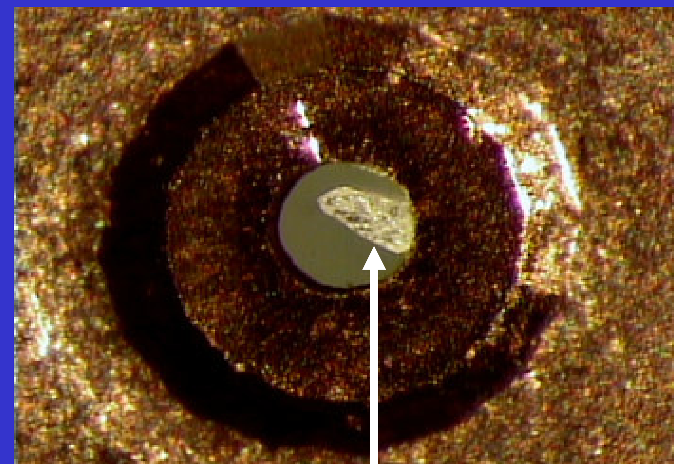
How to collect data on low symmetry crystals?



How to collect data on low symmetry crystals?

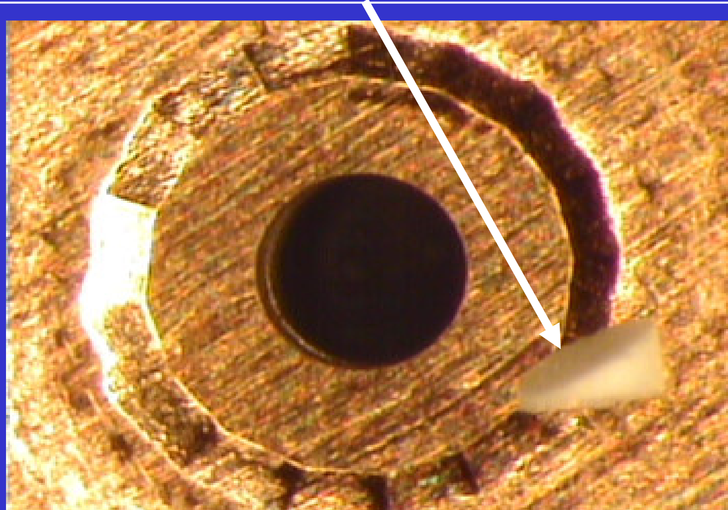
Solution 1: Tilt the crystal inside the compression chamber

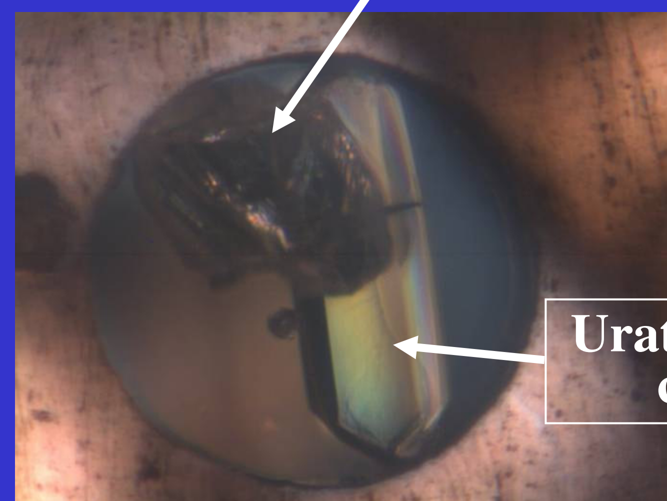




Micro machined BN bevelled plane

Diamond splinter





How to collect data on low symmetry crystals?

Does it work?

- Urate oxidase crystals: space group I222
- Use of a 62° useful aperture DAC & diamond splinter
- Data collected on 5 crystals, one being tilted with a diamond splinter

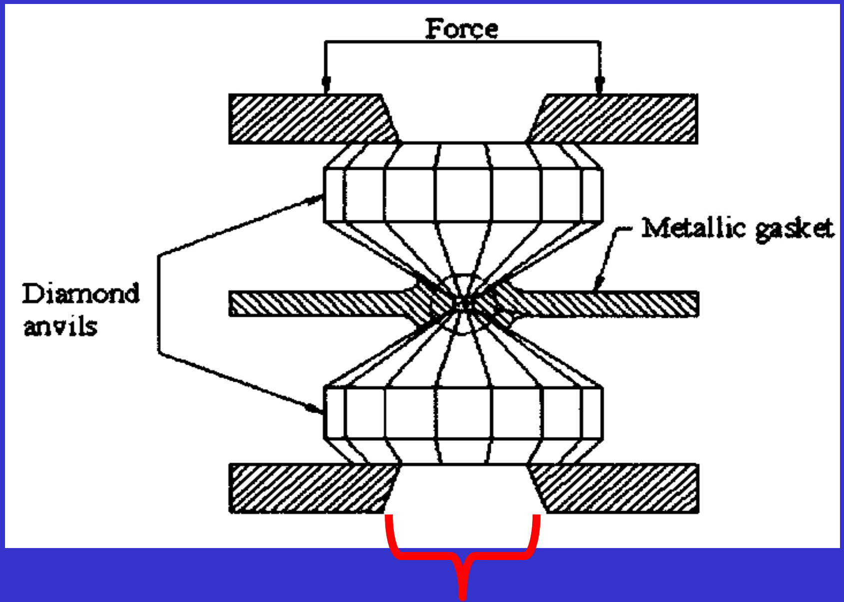
Data from the 2 best non-offset crystals	Data from the best non-offset crystal + the tilted one
↳ Data completeness: 85%	↳ Data completeness: 95%

Conclusion: it works !

- Increased data collection efficiency
- Less crystals to get a complete data set
- Decreased time dedicated to sample loading and pressure ramping

How to collect data on low symmetry crystals?

Solution 2: Increase the useful aperture of DAC



Limited useful aperture

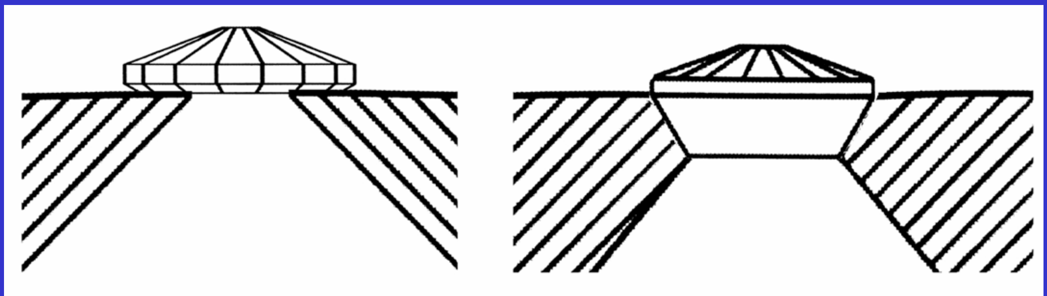
Past: 42°

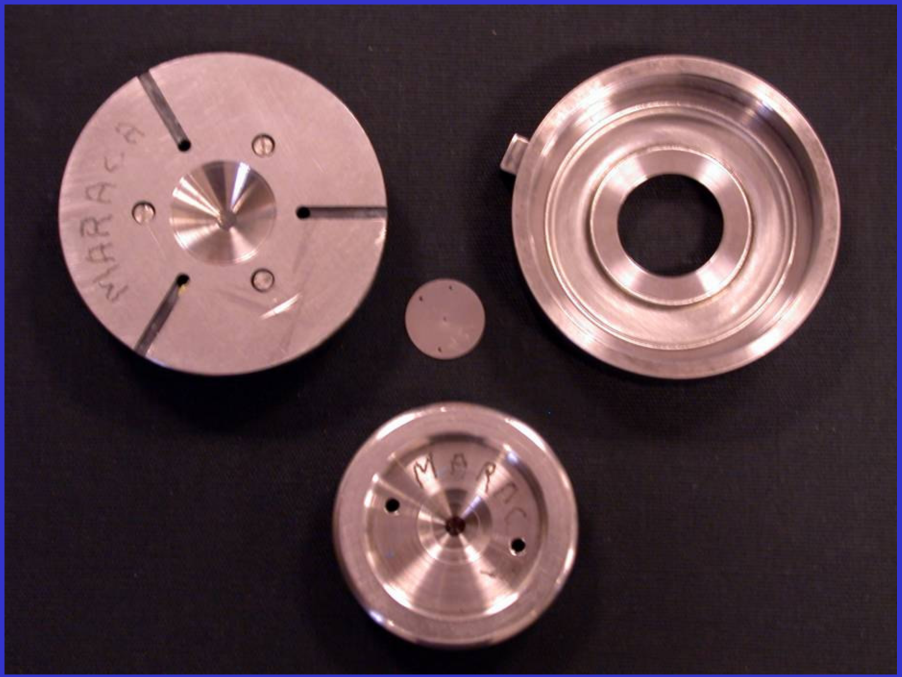
Present: 62°

New DAC: > 80°

How to collect data on low symmetry crystals?

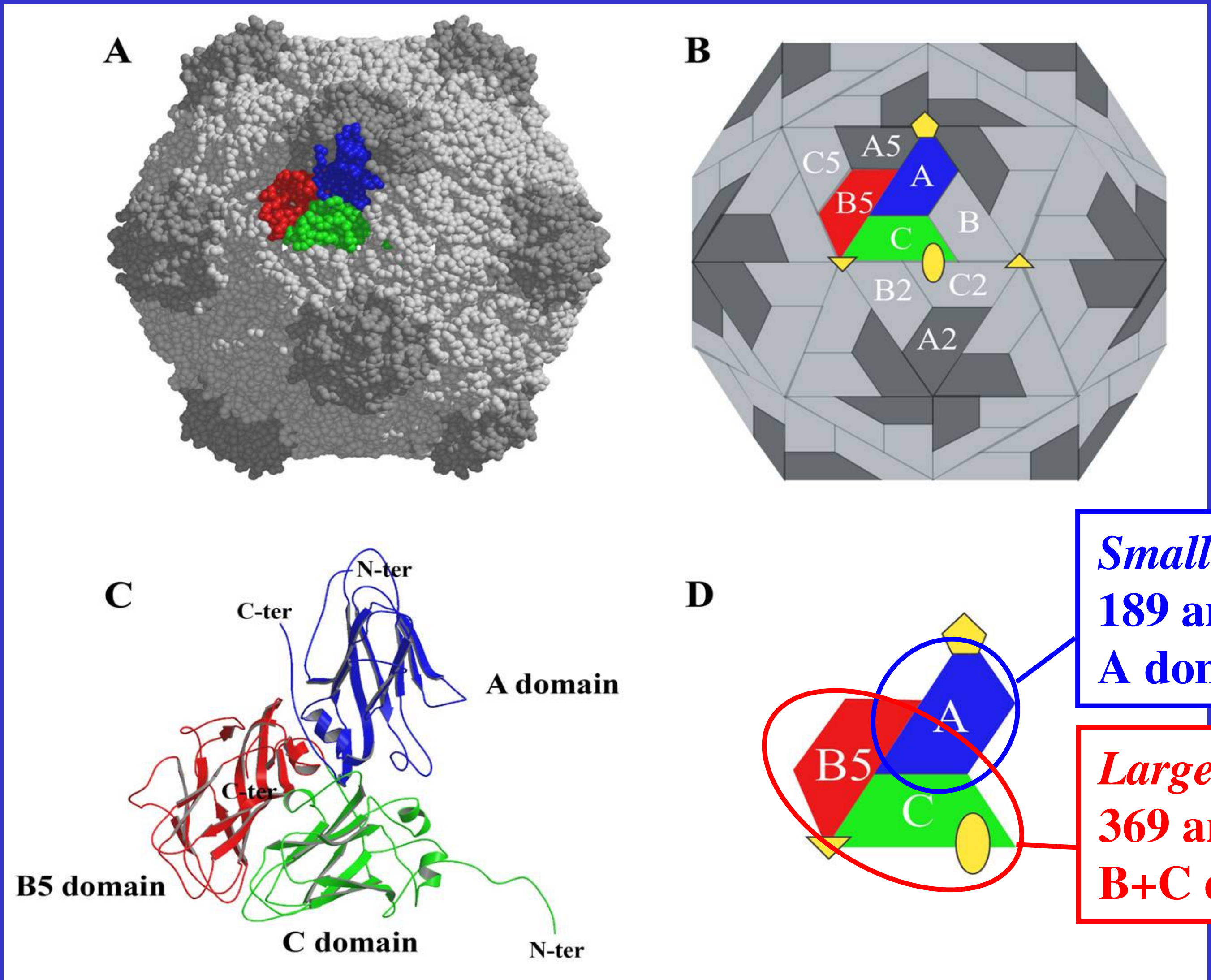
Solution 2: Increase the useful aperture of DAC





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

- **First crystallographic study of a complex macromolecular assembly: CpMV at 330 MPa**



Small protein (S)
189 amino acids
A domain

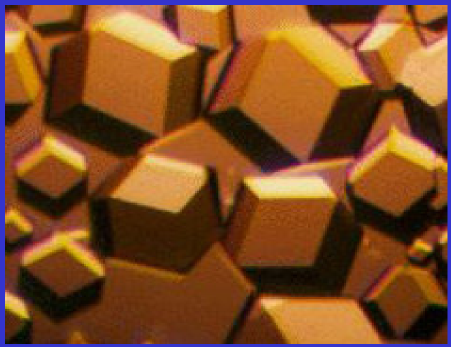
Large protein (L)
369 amino acids
B+C domains

Imperfection of cubic CpMV crystals at atmospheric pressure

- Only **about 10 %** of the CpMV crystals, grown in standard conditions, conform to the characteristics of cubic **I23** space group.
- The other crystals have **weak** reflections with indices of **h + k + l = 2n + 1** (odd reflections), which should be **absent** in the I23 space group.
- Space group **P23** was tentatively assigned to these crystals, which agrees with the presence of odd reflections **but not with the packing** criteria of the virus particles.
- The intensities of odd reflections **varies** from one crystal to another and their intensity increases from low to high resolution.

Space group: I23
Diffraction: 2.7 Å

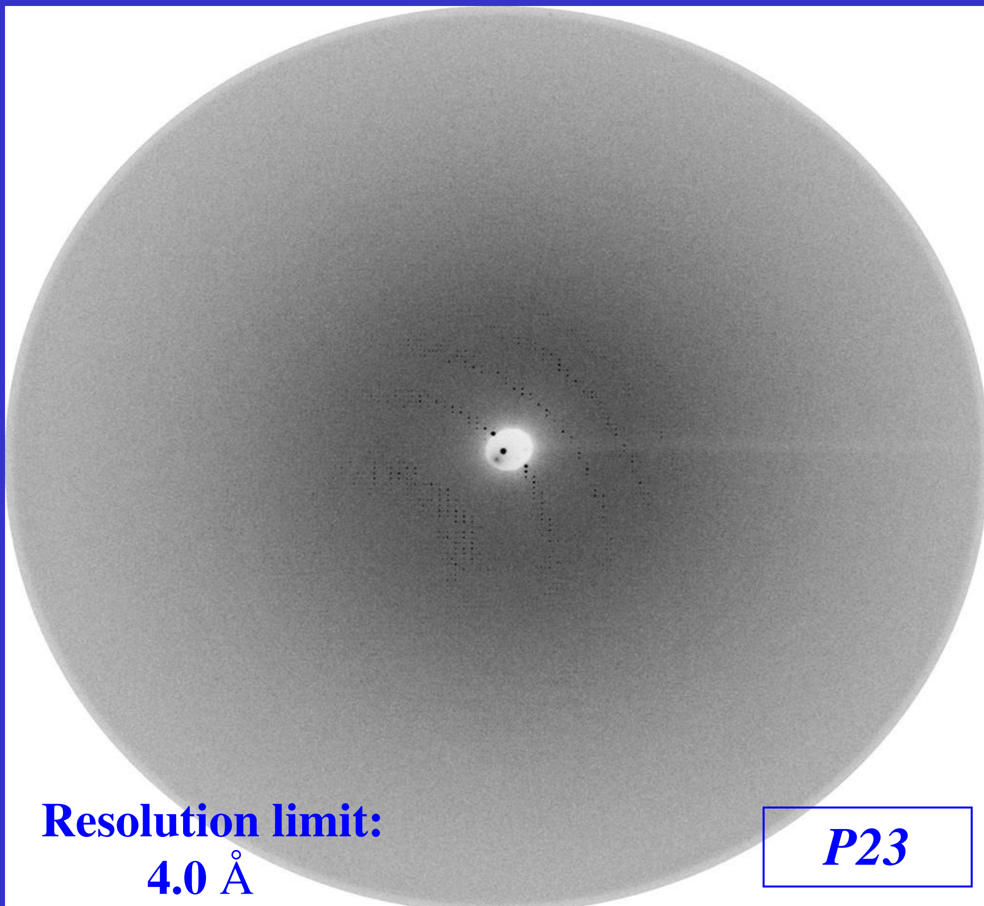
← 10 %

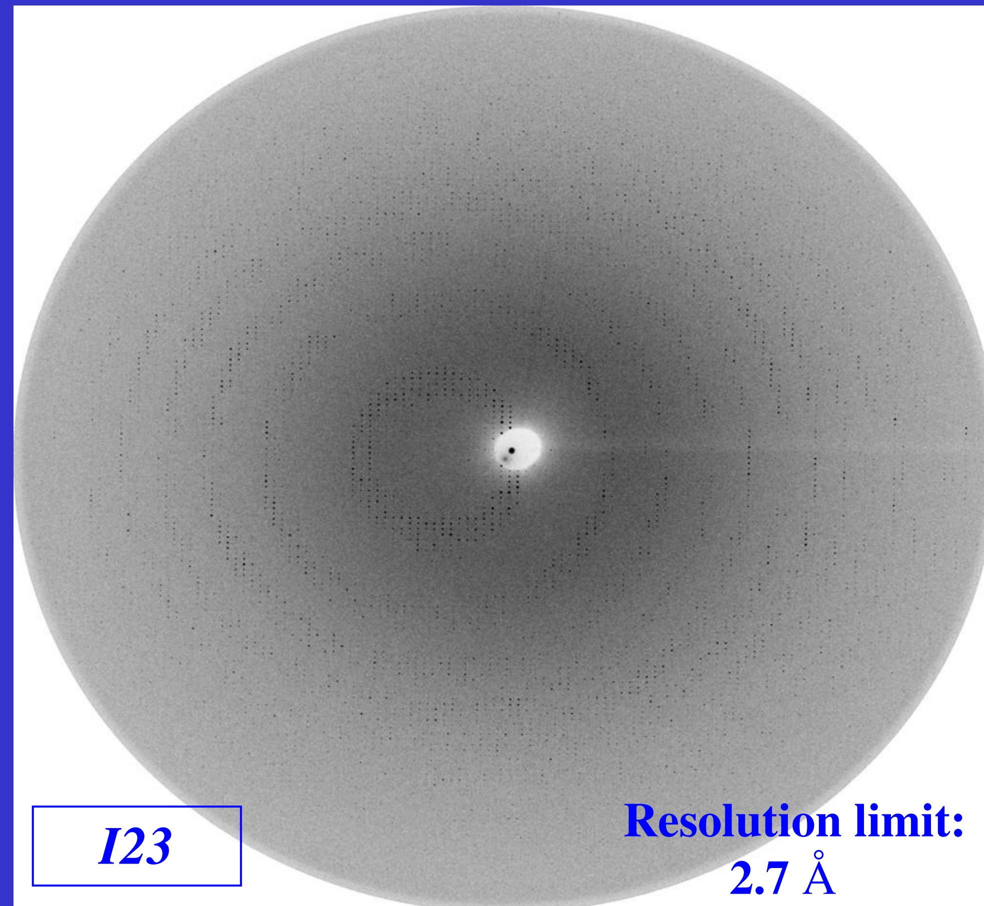


90 % →

Space group: P23
Diffraction: 4 Å

Disorder to order transition

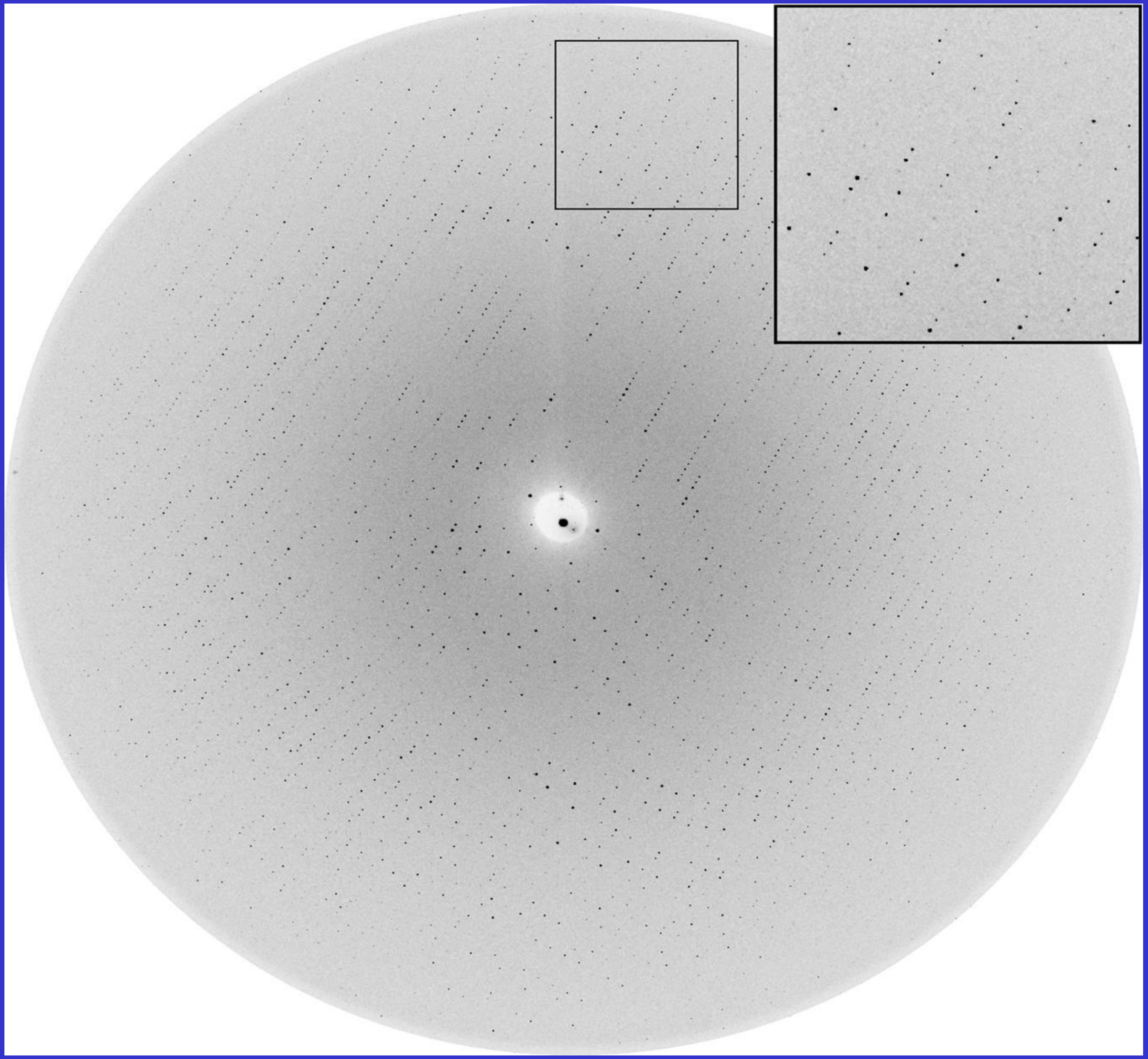




Applied pressure:
P < 230 MPa

Applied pressure:
P > 230 MPa

Disorder to order transition



Data collection

- *9 crystals, pressure 330 MPa*
- *Conditions:*

Exposure time:	80 seconds / image
$\Delta\phi$:	0.3° / image
Crystal to detector distance:	1400 mm

- *Statistics:*

Resolution:	50 - 2.8 Å	(2.95 - 2.80 Å)
Rsym:	14.9 %	(36.8 %)
I / σ(I):	4.9	(2.0)
Completeness:	91.2 %	(88.6 %)
Redundancy:	3.4	(3.1)

- *Refinement:*

Program:	CNS, NCS applied
R / Rfree:	16.1 % / 16.7 %

Structure comparison

- **Reduction of cell volume:** **3.4 %**
  - *Structure at ambient pressure (AP):* *a = 317 Å*
  - *Structure at high pressure (HP):* *a = 313.4 Å*
- **Reduction of the capsid volume:** **3.7 %**
- **RMS deviation between AP/HP structures:** **0.34 Å**

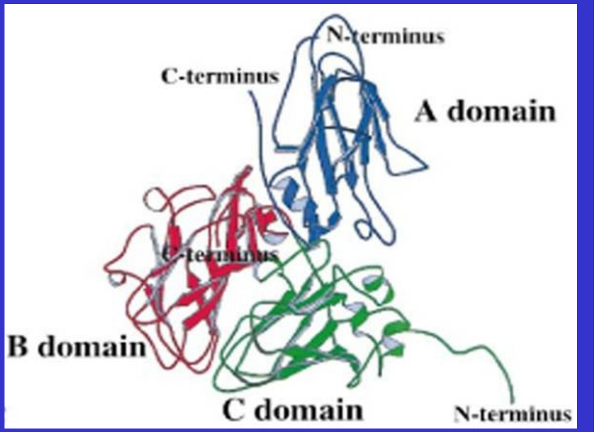
Ordered water molecules

Ambient-pressure structure (AP): 99

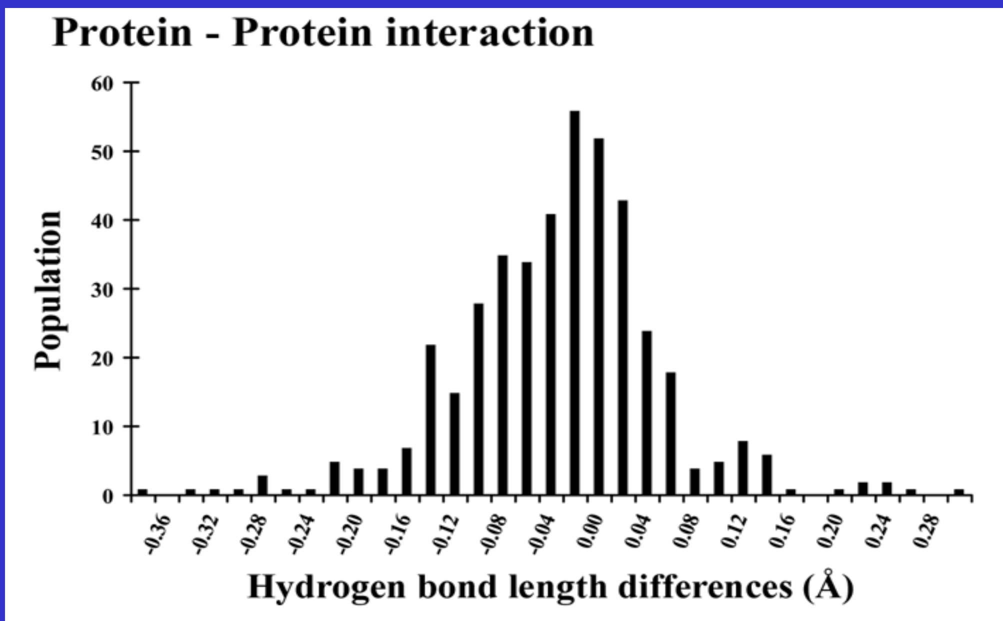
High-pressure structure (HP): 195

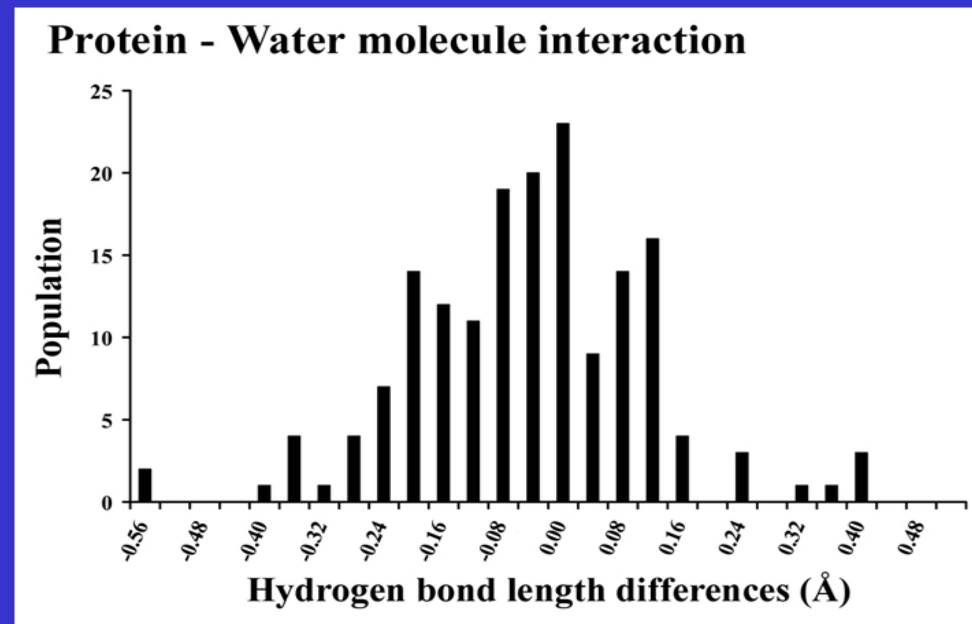
B factors

Mean B factor (Å^2)		HP structure	AP structure
Small and Large proteins		20.30	29.17
	Main chain	19.75	28.78
	Side chains	20.84	29.54
A domain		24.01	32.56
	Main chain	23.73	32.44
	Side chains	24.21	32.61
B5 domain		19.63	28.05
	Main chain	19.29	27.85
	Side chains	19.98	28.20
C domain		17.08	26.77
	Main chain	16.08	25.94
	Side chains	18.16	27.68
Water molecules		24.84	30.26



Evolution of hydrogen bond lengths





- **No. of measurements:**

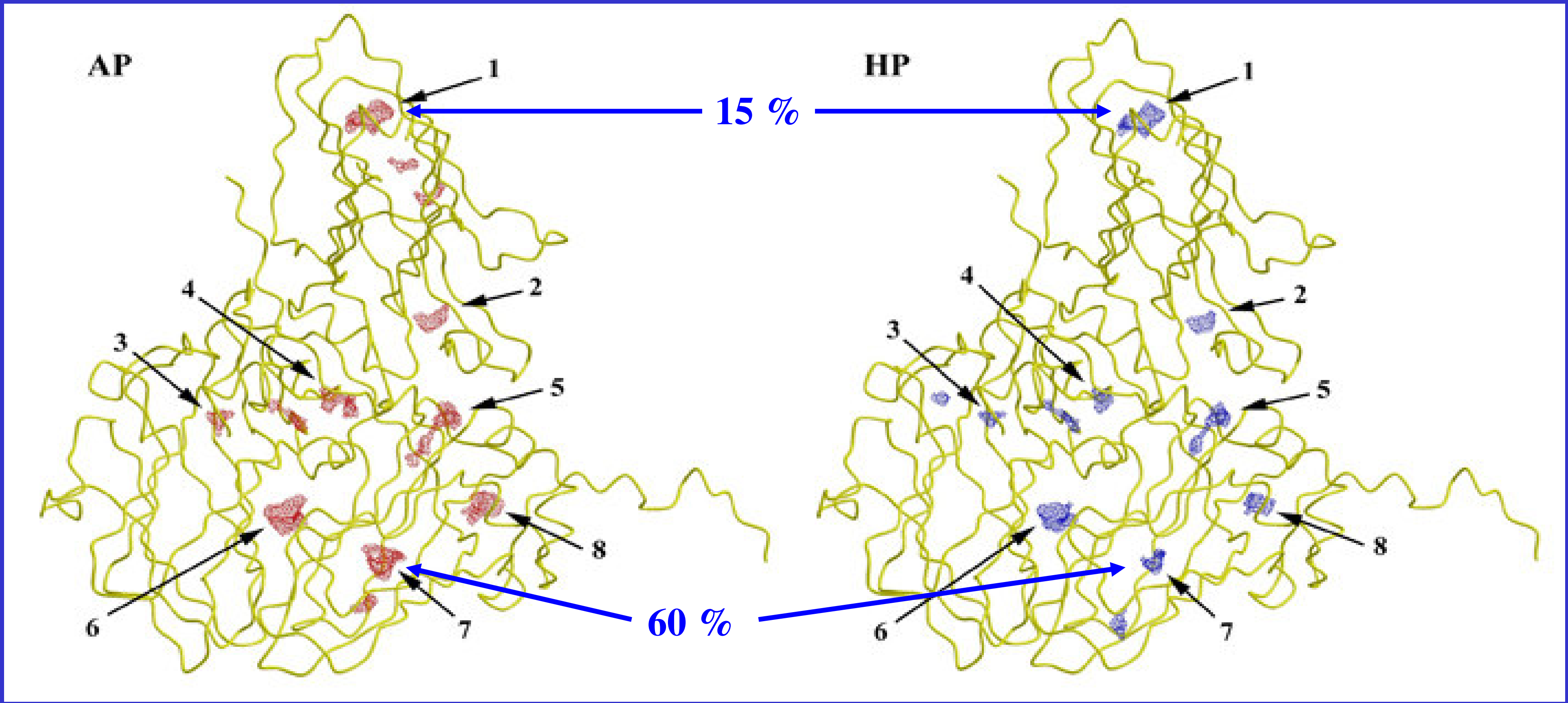
  428 hydrogen bonds *169 hydrogen bonds*

- **Mean value:**

  - 0.034 Å *- 0.048 Å*

Internal cavities

- Reduction of the number of cavities
- Reduction of the total cavity volume: about 40 %



Conclusion & Perspectives

- **Is the ordering effect of high pressure on crystals fairly general?**
  If yes, the implication for protein crystallography might be important

- **Pressure as a way to modify the Gibbs free energy: Exploration of phase transitions and protein sub-states.**

  Data collection on DHFR / Comparison with HP-NMR

- **Study of first steps of protein denaturation induced locally by pressure.**

  Data collection on hen-egg white lysozyme at different pressures
  (We already got a structure at 700 MPa)

Conclusion & Perspectives

- **Adaptation of life to high-pressure conditions**

  Structures of proteins from psychro/piezophilic bacteria
  Structures of DNA: A-form of DNA at 1.4 GPa

- **Accurate structural information for e.g. detailed analysis of interactions :**
  - between protein molecules within the crystal
  - between monomers of an oligomeric protein or components of a complex macromolecular assembly
  - water-water and water-molecules interactions

  Analysis of the Cu,Zn Superoxide Dismutase HP structure
  Analysis of the 140 MPa crystal structure of urate oxidase

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- Mohamed Mezouar - ID27 / ESRF - Grenoble
- Richard Kahn - Institut de Biologie Structurale - Grenoble
- Anne-Claire Dhaussy - CRISMAT - Caen
- Jean-Claude Chervin & Bernard Cousinet - IMPMC - Paris
- Nushin Aghajari & Richard Haser - Institut de Biologie et Chimie des Protéines - Lyon
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