High Pressure Freezing of Macromolecular Crystals.

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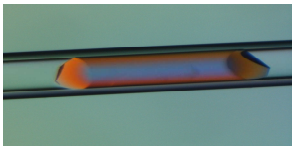
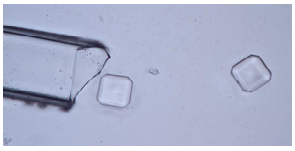
High pressure freezing allows sample vitrification without penetrative cryoprotectants. Macromolecular crystals are frozen directly in their mother liquor at 77 K and 210 MPa. The method is ideally suited for cryocooling large unit cell systems, such as virus crystals or membrane proteins, which are very difficult to cryoprotect.

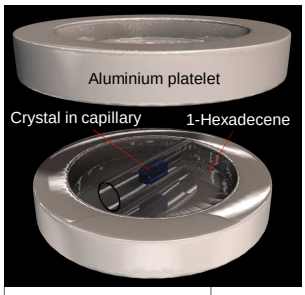
Motivation

Macromolecular crystals contain between 20 and 80% solvent (mainly water). Successful cryocooling of such crystals requires the use of cryoprotectants in order to suppress hexagonal ice formation and to convert the water to amorphous ice (vitrification). Finding optimal cryoconditions can be very time-consuming and the data quality is often degraded upon cryoprotection. High pressure freezing (HPF) allows sample vitrification without any cryoprotectants. It was our goal to develop a HPF protocol which is generally applicable to all types of macromolecular crystals including complex systems with large unit cells and/or high solvent content which represent very challenging targets for cryoprotection.

Experimental Setup







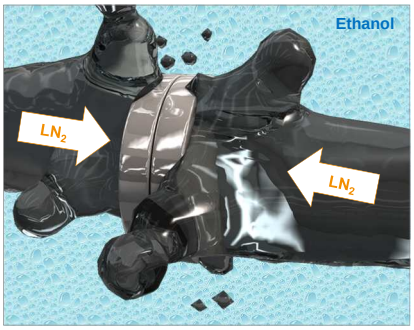
Assembly in HPF sample holder: quartz capillary with crystal in its mother liquor sandwiched between two aluminium plateletts.

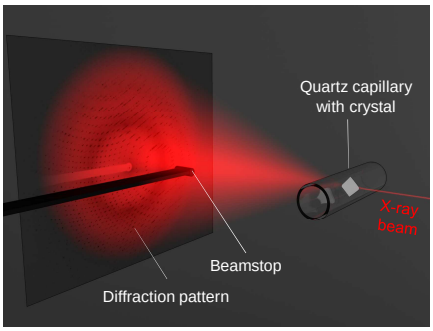
Crystallization

- Directly in quartz capillaries (by counter diffusion) or micro cellulose tubes (via dialysis)
- By vapour diffusion; subsequently, crystals are drawn in quartz capillaries

High Pressure Freezing

- Samples are **high pressure frozen at 77 K and 210 MPa**.
- Baltec HPM 010 high pressure freezer is commercially available and allows **fast sample cooling** with ~7000 K/s at the sample surface.
- Macromolecular crystals are **directly cryocooled in their mother liquor**.
- Sample manipulation after HPF is performed below 135 K to prevent hexagonal ice formation.

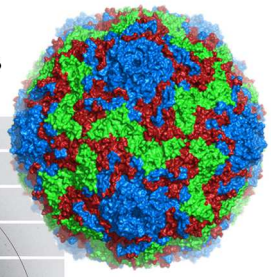




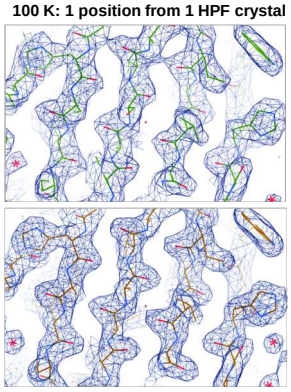
Results and Discussion

Bovine Enterovirus 2 (BEV2)

- Space group: *F*23
- $V = 8.10 \times 10^7$ Å^3
- Solvent content: ~60%
- Mosaic spread: 0.26°



100 K: 1 position from 1 HPF crystal



293 K: 76 positions from 28 crystals

The 'gain factor' in radiation tolerance for data collection at 100 K compared to room temperature measurements was more than 100.

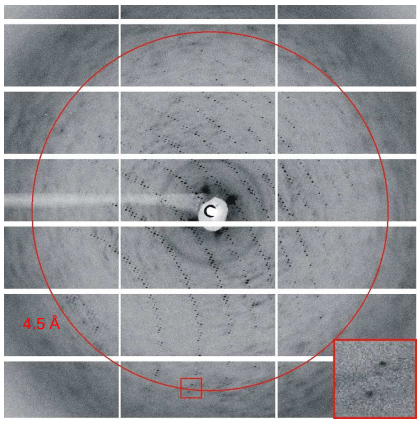
A. Burkhardt, A. Wagner, M. Warmer *et al.*, *Acta Cryst*. D69, 2013, 308–312.

Photosystem II (PSII)

- Space group: $P2_12_12_1$
- $V = 9.24 \times 10^6$ Å^3
- Solvent content: ~64%
- Mosaic spread: 0.22°

A. Burkhardt, M. Warmer, S. Panneerselvam *et al., Acta Cryst.* F68, 2012, 495–500.

J. Hellmich, M. Bommer, A. Burkhardt *et al.*, *Structure*, accepted for publication.



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