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High-Pressure Freezing of Protein Crystals

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Protein crystals can contain up to 90% of solvent (mainly water). Cryocooling of such crystals requires cryoprotectants like glycerol or ethylene glycol to suppress hexagonal ice formation and convert the water to amorphous ice (vitrification). Finding ideal cryoconditions can be very time and crystal consuming. Moreover, the crystal quality is often degraded upon cryocooling even if adequate cryoprotectants have been found.
A promising approach which allows sample vitrification without cryoprotectants is high-pressure freezing (HPF). This technique is well established in the field of electron microscopy for cryofixation of cells or tissue [1] and was recently optimized for protein crystals by our group [2].
The crystals are directly frozen in their mother liquor at 210 MPa and 77 K using a Baltec HPM 010 high-pressure freezer. First HPF trials were carried out on hen egg-white lysozyme and porcine insulin giving crystals of very good diffraction quality. In addition, a non-cryoprotected crystal of the membrane protein photosystem II (PSII) was successfully frozen for the first time. The HPF PSII crystal diffracted down to 4.5 Å and showed mosaic spreads of 0.22°. Thus, our HPF protocol is ideally suited for large unit cell systems with weak crystal contacts which are usually sensitive to osmotic shock and therefore difficult to cryoprotect.

1. H. Hohenberg et al., J. Microsc. 175, 24 (1994).
2. A. Burkhardt et al., Acta Cryst. F68, 495 (2012).

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