

## **High Pressure Cooling of Protein Crystals without Cryoprotectants**

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The flash cooling of protein crystals is the best known method to effectively mitigate radiation damage in macromolecular crystallography. To prevent physical damage to crystals upon cooling, suitable cryoprotectants must usually be found, a process that is time-consuming and, in certain cases unsuccessful. Recently we have developed a novel method to cryocool protein crystals without the need for penetrative cryoprotectants. In the new method, each protein crystal is pressurized up to 200 MPa (2000 atm) in He gas at 10 °C. The crystal is then cryocooled under pressure and the pressure was released while the crystal is kept cooled at 77 K. Results are presented for two proteins that have been flash-cooled at ambient pressure and pressure-cooled, in all case without penetrating cryoprotectants. For glucose isomerase, the flash-cooled crystal diffracted to only 5.0 Å and mosaicity could not be estimated but the pressure-cooled one diffracted to 1.05 Å with 0.39° mosaicity. For thaumatin, the flash-cooled crystal diffracted to only 1.8 Å with 1.29° mosaicity but the pressure-cooled one diffracted to 1.15 Å with 0.11° mosaicity. The protein structures show that the structural perturbation by pressure is at the level of a few tenths of an angstrom, which is comparable to the typical structural changes always observed upon flash cooling at ambient pressure. A mechanism on the pressure cooling is proposed involving the dynamics of water at high pressure and high density amorphous (HDA) ice.

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