

## **THE TECHNOLOGY**

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A method to cryo-cool protein crystals under high pressure has been developed. This simple method involves pressurizing a protein crystal with noble gas which results in dramatic improvement in the collection of high resolution data, and eliminates the need to use cryoprotectants. The method can easily be extended to produce high-quality crystals from which high resolution amplitude and phase information can be simultaneously obtained by single anomalous diffraction (SAD).

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## **THE PRODUCT** High Pressure Cryo-Cooler™

HPC-201 cryocooling was developed as an alternative method for cryopreservation of macromolecular crystals. The method requires the preservation of crystal hydration as the crystal is pressurized with dry helium gas. Previously, crystal hydration was maintained either by coating crystals with a mineral oil or by enclosing crystals in a capillary that was filled with crystallization mother liquor. These methods are not well suited to weakly diffracting crystals because of the relatively high background scattering from the hydrating materials. With this method the specimen is kept hydrated via vapor diffusion in a shielding capillary while it is being pressure cryocooled. After cryocooling, the shielding capillary is removed to reduce background X-ray scattering. It is shown that, compared to previous crystalhydration methods, the new hydration method produces superior crystal diffraction with little sign of crystal damage. Using the HPC-201, a weakly diffracting protein crystal may be properly pressure cryocooled with little or no addition of external cryoprotectants, and significantly reduced background scattering that can be observed from the resulting sample. Beyond the applications for macromolecular crystallography, it is shown that the HPC-201 has great potential for the preparation of non-crystalline hydrated biological samples for coherent diffraction imaging with future X-ray sources.

