

## **Macromolecular Crystal Annealing: Fact and Fiction**

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Macromolecular Crystal Annealing (MCA) is a technique that can significantly reduce the mosaicity of crystals that display increased mosaic spread because of flash cooling. The annealing procedure consists of transferring a flash-cooled crystal to a droplet of its original cryoprotectant solution at ambient temperature for approximately three minutes followed by re-flash-cooling to cryogenic temperature. The annealing process has been applied to crystals of many different macromolecules grown from different precipitants and using a variety of cryoprotectants. The method also has been applied to restore diffraction from flash-cooled crystals that were inadvertently partially warmed during transfer to or from cryogenic storage, to crystals that iced during data collection, and to characterize crystal diffraction prior to heavy-atom soak experiments. The favored annealing procedure and several variants will be described. Results will be discussed in relation to crystal mosaicity and radiation damage in flash-cooled macromolecular crystals.

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