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The Role of Cryoprotectants in the Successful Cryogenic Cooling of Protein Crystals

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Protein crystals for high resolution X-ray structure determination are typically cooled to ∼100K prior to X-ray exposure to minimize crystal damage from the ionizing radiation. However, cooling itself can damage the crystal. This cooling-induced damage can be minimized by adding small molecules, called cryoprotectants, to the disordered phase that permeates the interstices between the protein molecules in the crystal lattice. The cryoprotectants appear to minimize cooling-induced damage by adjusting the thermal contraction of the disordered phase to match the thermal contraction of the protein crystal lattice. We will discuss our recent experiments in characterizing the thermal contraction of cryoprotective solutions in order to develop predictive capabilities for potential cryoprotectants. We measured the thermal contraction caused by binary solutions between water and 20 different cryoprotectants. At a concentration of 50% (w/w), these solutions contracted over a range of 0-15%. To develop predictive capabilities we looked for easily measured physical properties of the pure cryoprotectants that were well correlated with the observed thermal contraction. While viscosity, vapor pressure, molecular weight and the water-octanol partitioning were all reasonably correlated with the thermal contraction, the best predictor of the thermal contraction of the cryosolution was the number of hydroxyls in the cryoprotective molecule.