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The Physics of Flash Cooling of Biosolutions: Competition Between Ice Glass and Ice Crystal Phases.

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Successful flash cooling of protein crystals requires inhibition of hexagonal ice formation both inside the crystal and in the liquid surrounding it. This is usually accomplished by adding cryoprotectants to the growth or harvest solutions. Excessive cryoprotectant concentrations can cause crystal damage and can degrade diffraction quality. We have measured the boundary between amorphous ice and hexagonal ice produced by flash cooling in liquid nitrogen as a function of both cryoprotectant concentration and liquid volume, for several common cryoprotectants. Required cryoprotectant concentrations to achieve amorphous ice decrease strongly with liquid volume of the sample. For typical crystal sizes these concentrations are a factor of two smaller than those of a previous study using large liquid volumes [1].

Recent experiments suggest that differences in thermal contraction between internal solvent and the protein lattice may be an important source of flash-cooling-induced disorder. We have designed and constructed an apparatus that allows measurement of flash cooled densities of microliter volumes. Using this apparatus, we have measured the slow and flash cooled densities as a function of cryoprotectant concentration for a variety of common cryoprotectant mixtures. Together, these results provide for the first time a solid quantitative basis for the design of cryoprotection and flash cooling protocols.

1. E.F. Garman, E.P.Mitchell, *J.Appl.Cryst.*, 1996, **29**, 584-587