

E0060

Fast Vitrifying of Solutions Using for Protein Crystal Cryopreservation: Effects of Cryoprotectant Concentration and Cooling Rates. Matt Warkentin^a, Viatcheslav Berejnov^a, N.S. Husseini^b, O.A. Alsaied^c, R.E. Thorne^a, ^aPhysics Dept., Cornell Univ., USA, ^bApplied & Engineering Physics Dept., Cornell Univ., USA, ^cWeill Cornell Medical College, Doha, Qatar.

Successful flash cooling of protein crystals requires inhibition of crystalline 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 may cause the crystal damage and degrade diffraction quality. We have measured the phase boundary between amorphous ice and crystalline ice produced by flash cooling from $T=295$ K to $T=77$ K in liquid nitrogen as a function of both cryoprotectant concentration and liquid volume from \sim nL to 20 μ L^[1]. Fourteen common cryoprotectants were used: glycerol, methanol, isopropanol, sucrose, xylitol, dextrose, trehalose, ethylene glycol, PEG 200, PEG 2 000, PEG 20 000, DMSO, MPD, and NaCl. For most of the studied cryoprotectants, the critical concentration required to obtain amorphous ice decreases strongly with volume in the range from \sim 5 μ L to \sim 0.1 μ L, typically by a factor of two. By combining measurements of the critical concentration versus volume with cooling time versus volume for glycerol, we obtain the critical CPA concentration versus cooling rate during flash cooling. Our results provide a basis for more rational design of cryoprotective protocols, and should yield insight into the physics of glass formation in aqueous mixtures.

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[1] V. Berejnov, N. S. Husseini, O. A. Alsaied and R. E. Thorne, *J. Appl. Cryst.* (2006) (in press).