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**Supporting information for article:**

**Density and electron density of aqueous cryoprotectant solutions  
at cryogenic temperatures for optimized cryoprotection and  
diffraction contrast**

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**S1. Preparation of cryoprotective agent (CPA) solutions.**

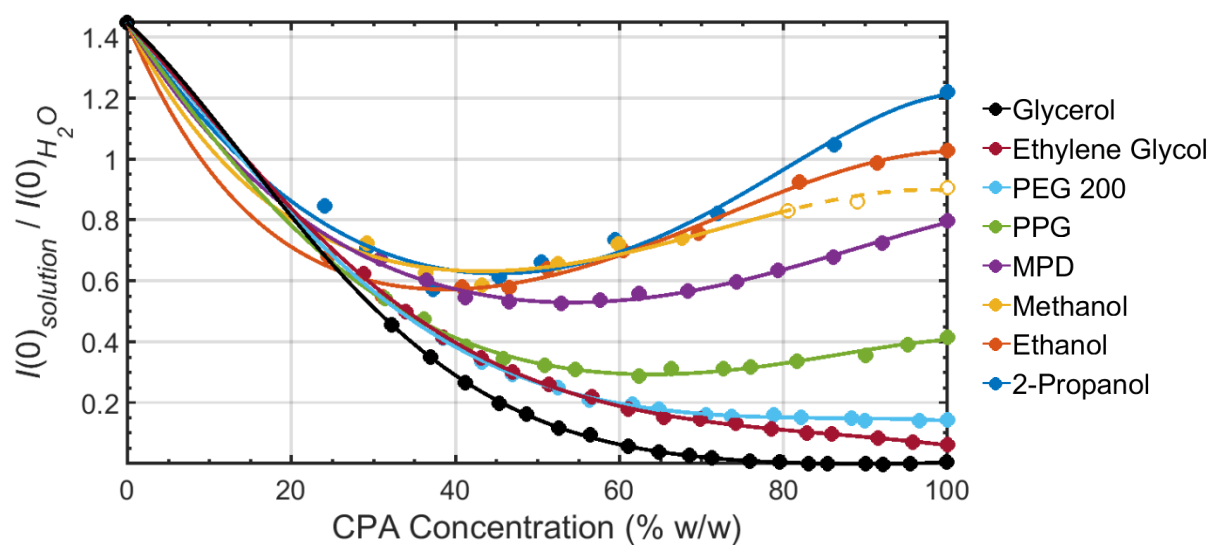
The cryoprotectants studied were anhydrous methanol from Macron Fine Chemicals; anhydrous ethanol from Decon Labs; 2-propanol from Macron Fine Chemicals; ethylene glycol from Mallinckrodt; glycerol from Fisher Chemical; 2-methyl-2,4-pentanediol (MPD) 99% from Sigma-Aldrich; polyethylene glycol (PEG) 200 from Sigma-Aldrich; and polypropylene glycol (PPG) 425 from Sigma Aldrich.

Cryoprotectant solutions were prepared by combining the desired masses of CPA and distilled deionized water. Masses were measured to an accuracy of  $\pm 5 \mu\text{g}$  using a Mettler Toledo AE240 analytical balance. Solutions were mixed using a Vortex-Genie 2T from Scientific Industries, Inc. until they were optically homogeneous. Drops were generated using 1 mL syringes with needle gauges ranging from 27-33.

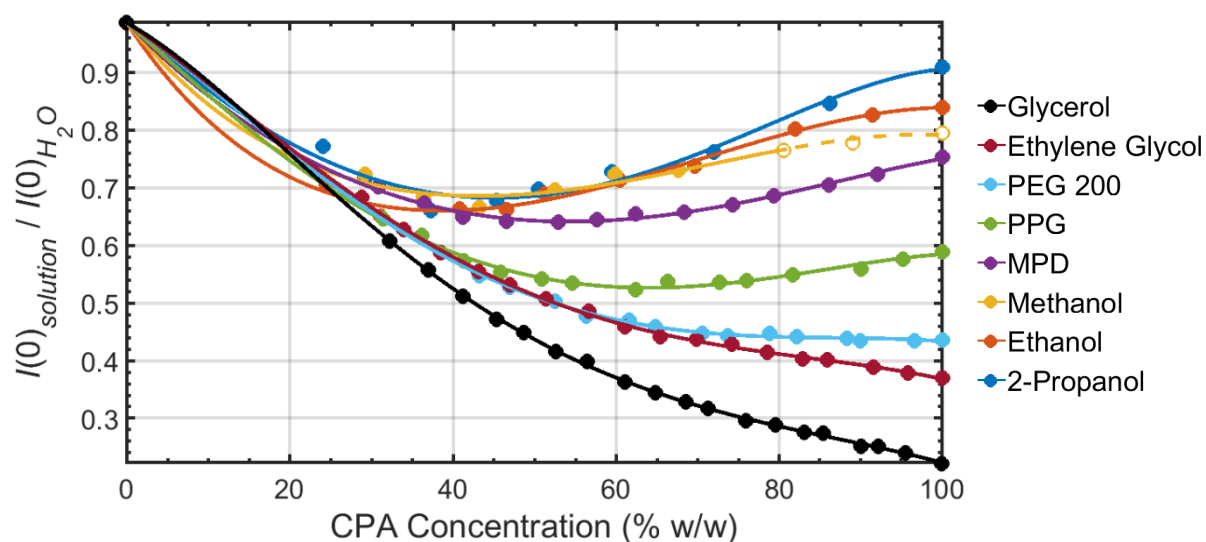
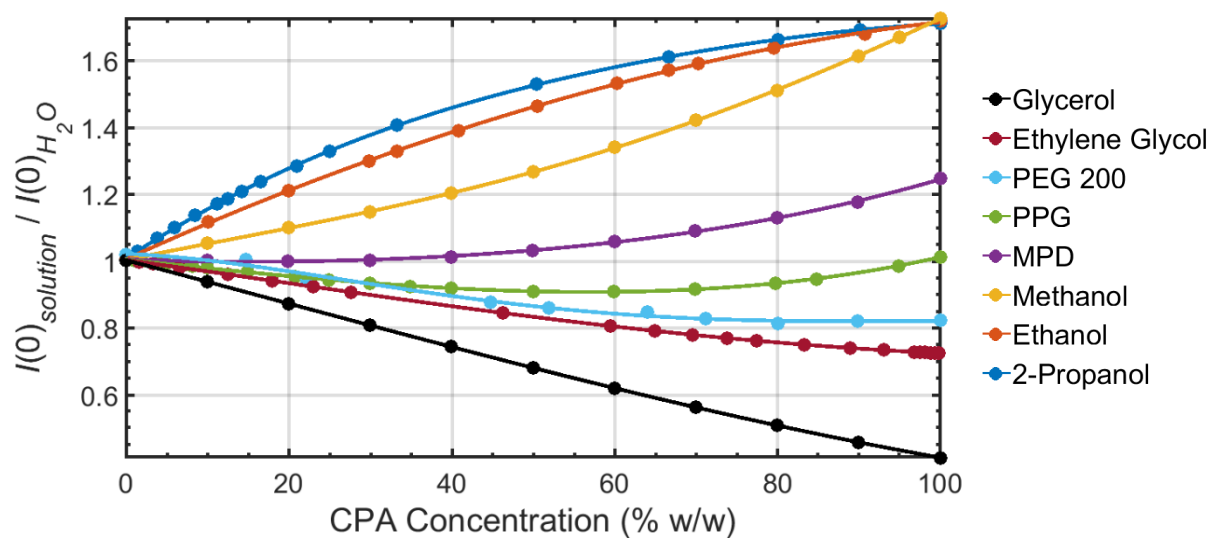
Uncertainties in final concentrations were somewhat larger for methanol, ethanol, and 2-propanol solutions, due to their volatility, than for the other CPAs. To minimize concentration errors, monoalcohol solutions were prepared in volumes of  $\sim 15 \text{ mL}$  and stored in  $\sim 15.5 \text{ mL}$  test tubes. Syringes for drop dispensing were filled leaving no air space, and their tips capped between measurements.

**Table S1** Parameter  $\beta$  in fits of Eq. 5 to data for critical cooling rates (K/s) vs. CPA concentration in % w/v from Warkentin et al., 2013.

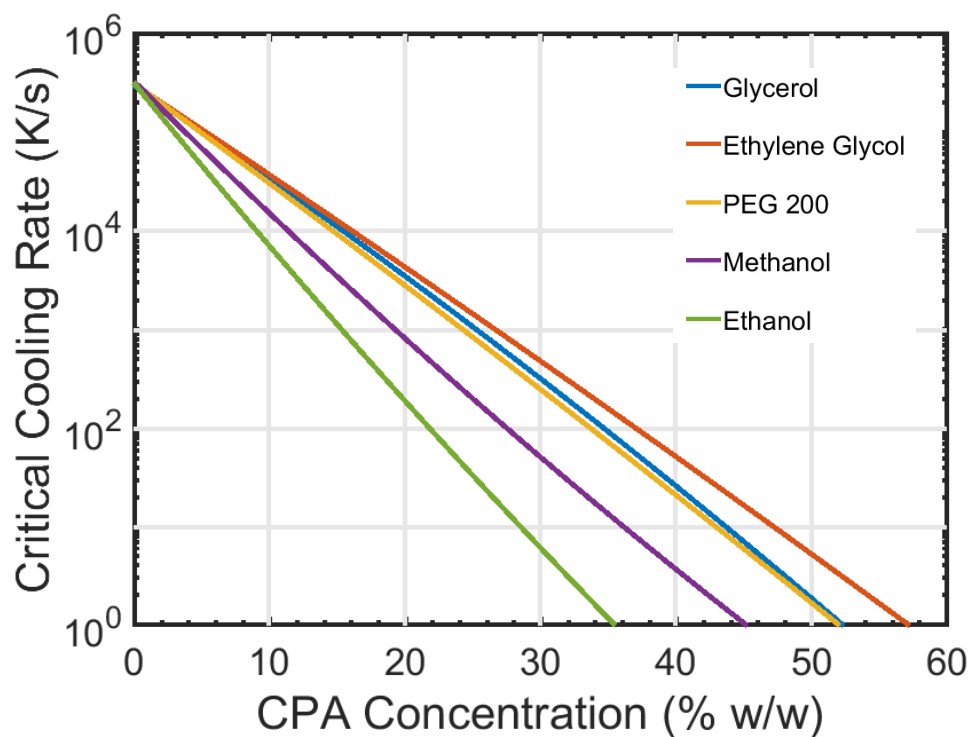
Cryoprotectant	$\beta$
ethanol	0.376
methanol	0.302
ethylene glycol	0.206
PEG 200	0.226
glycerol	0.213



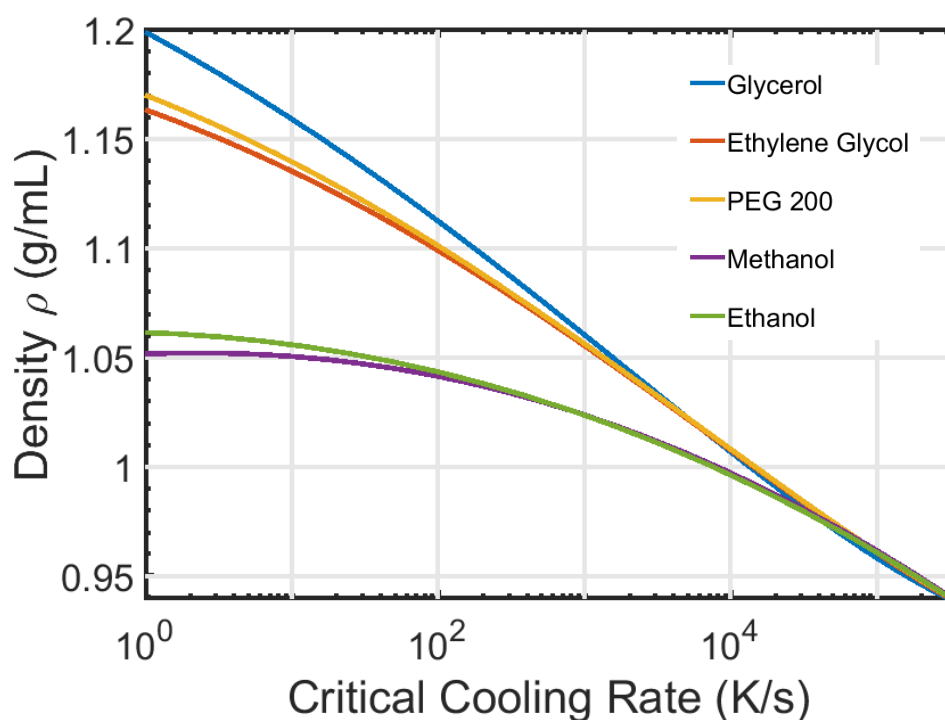
**Figure S1** Forward scattering of protein in a CPA solution at  $T = 77$  K (from the present data) normalized by the forward scattering of protein in pure water at  $T = 300$  K. This corresponds to a comparison of signal intensities in cryoSAXS and room temperature SAXS.



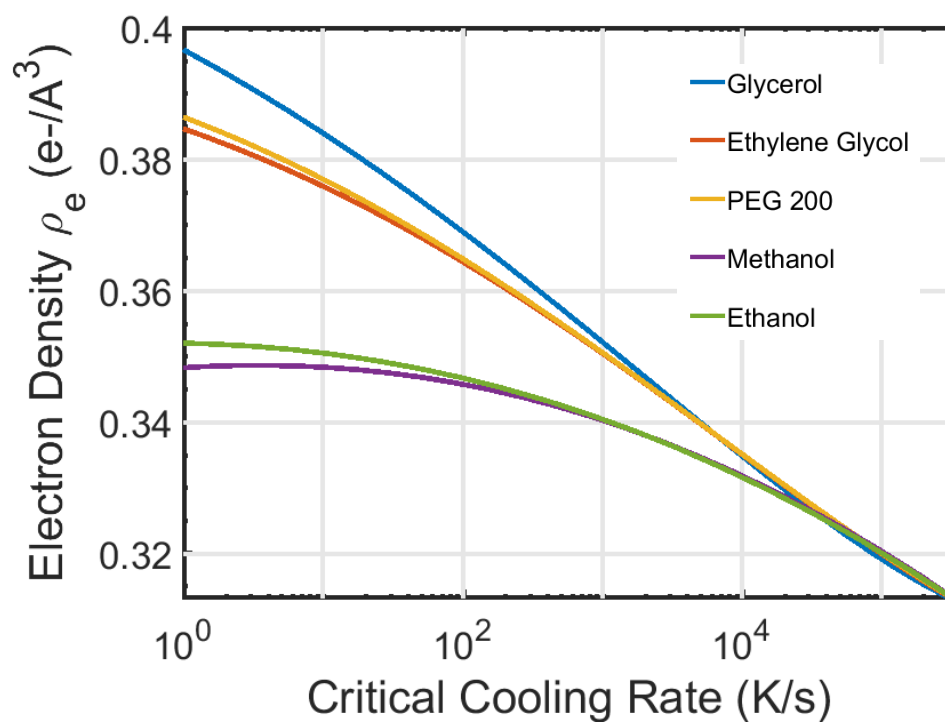
**Figure S2** Forward scattering of protein in a CPA solution (from the present data) normalized by the forward scattering of protein in pure water at (a)  $T = 300$  K and (b)  $T = 77$  K, calculated using Eq. 2, for nucleic acids with  $\rho_e \sim 0.55 \text{ e}^- / \text{\AA}^3$ .



**Figure S3** Critical cooling rate (K/s) vs. CPA concentration (% w/w), obtained by combining fits to data for critical cooling rate vs CPA concentration in % w/v (Warkentin, Sethna and Thorne, 2013) of Eq. 5 with parameter  $\beta$  given in Table S1, with fits to previous measurements of the room-temperature densities of each CPA solution shown in Fig. 1 (a) and given in Table 1.



(a)



(b)

**Figure S4** (a) Density  $\rho$  (g/mL) and (b) electron density  $\rho_e$  ( $e^-/\text{\AA}^3$ ) at  $T = 77$  K vs. critical cooling rate CCR (K/s), obtained by combining fits to the present density data versus CPA concentration in % w/w with fits to CCR versus CPA concentration in % w/w shown in Figure S3.