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Supporting information for article:

Solvent flows, conformation changes and lattice reordering in a cold protein crystal

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Table S1 Number of cubic apoferritin crystals measured at each temperature and glycerol concentration that remained ice-free for long enough to determine the unit cell parameter, Wilson B-factor and mosaicity in a fully cooled state.

The number of crystals at room temperature refers to the total number of crystals used in this analysis. Data from these crystals is used to generate Figs. 3(a), 3(b), and 4.

Temperature (K)	Number of Apoferritin Crystals Examined			
	Glycerol (% *v/v*)			
	0%	10%	20%	40%
180	8	5	3	4
190	-	-	5	-
200	6	3	10	3
210	-	-	7	4
220	7	8	13	7
230	-	-	7	6
240	7	5	4	4
260	5	3	2	6
RT	61	35	58	34

Table S2 Total number of crystals where a structure could be determined in a fully cooled state without post-cooling expansion.

Data from these crystals is used for the protein and solvent cavity volumes in Fig. 3 (c-f).

Temperature (K)	Glycerol (% *v/v*)			
	0%	10%	20%	40%
180	4	1	1	-
200	4	-	1	1
210	-	-	1	1
220	3	3	2	1
230	-	-	2	1
240	3	-	-	1
260	2	1	-	1
RT	4	3	4	1

Table S3 Total number of crystals that showed post-cooling expansion and for which the unit-cell parameter, mosaicity, and expansion time-scale was determined.

Data from these crystals is used for Figs. 6-8.

Temperature (K)	Number of Apoferritin Crystals Examined			
	Glycerol (% *v/v*)			
	0%	10%	20%	40%
220	3	-	2	-
230	-	-	2	1
240	6	3	3	3
260	2	2	2	4

Table S4 Total number of glycerol free apoferritin crystals at each temperature where a structure could be determined before or after cold unit cell expansion, according to whether they showed residue GLN82 in the "*in*" or "*out*" conformation.

Data from these crystals is used for Figs. 9-11 and S5.

Temperature	*In* Conformation		*Out* Conformation	
	Contracted	Expanded	Contracted	Expanded
180	–	–	4	–
200	–	–	4	–
220	–	–	3	3
240	–	3	3	1
260	2	2	–	–
Room-Temperature	2		2	

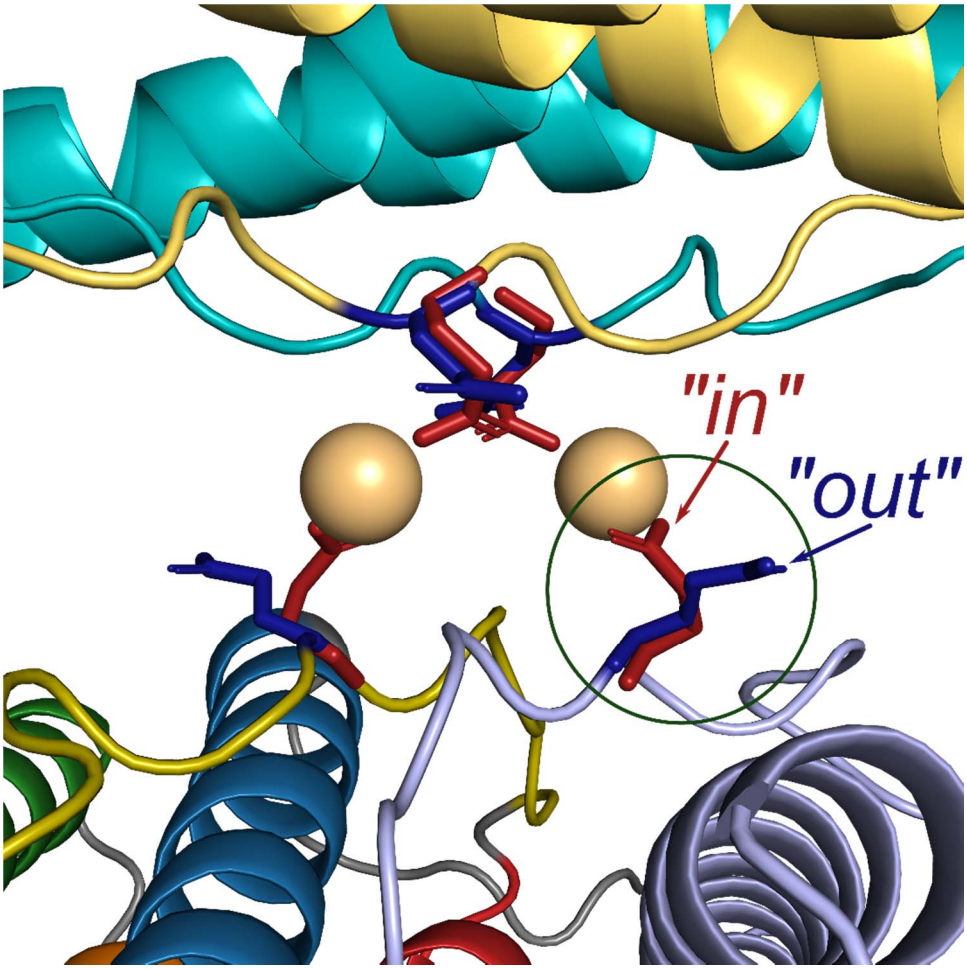

Figure S1 Interface between apoferritin shells, showing the region of crystal contact between two dimers along their BC loops (See Fig. 1). Residues ASP80 and GLN82 protrude from the BC loop and connect to a cadmium atom between the shells. ASP80 has well defined electron density and is always involved in the contact. GLN82 is found in either an '*in*' or '*out*' conformation in a given crystal. In the *in* conformation GLN82 is connected to the Cd atom with well-defined electron density. In the *out* conformation GLN82 protrudes outward into the solvent cavity and an additional water molecule coordinates with the Cd atom.

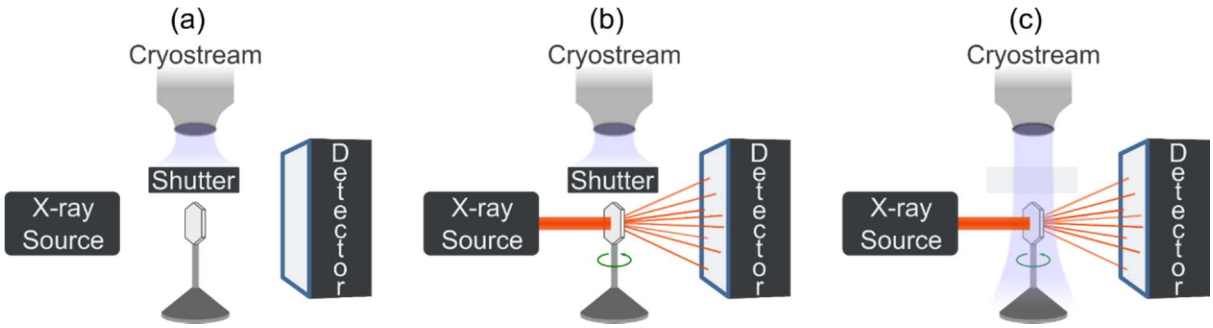

Figure S2 Experimental configuration used in time-resolved X-ray diffraction experiments at the Cornell High-Energy Synchrotron Source (CHESS). (a) A sample with its external solvent removed is placed in the X-ray beam path, (b) room temperature data is collected, and (c) the cryostream is unshuttered and data collected while the crystal is cooled.

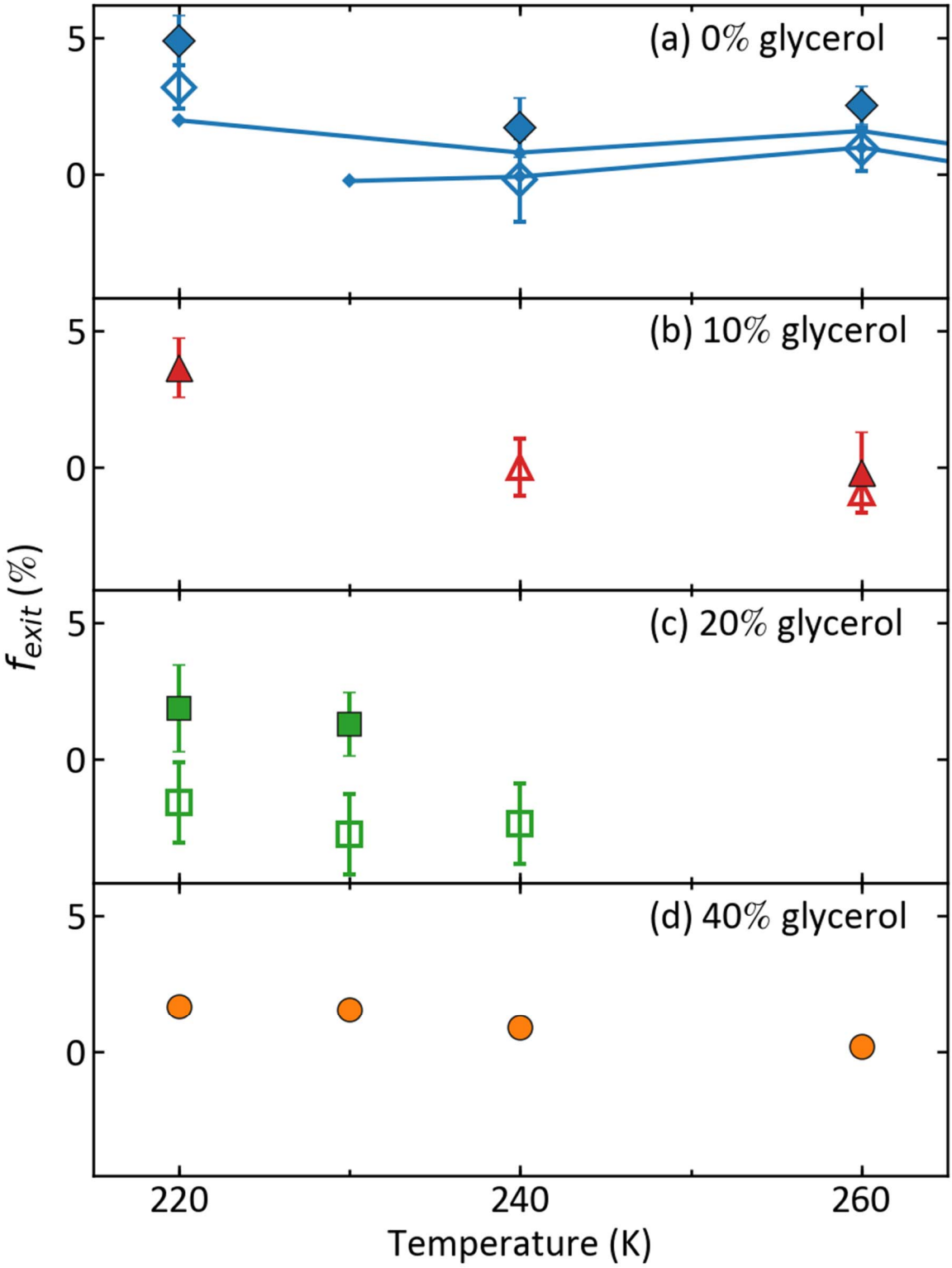

Figure S3 Estimates of the volume fraction of internal solvent present at room temperature that cannot be accommodated within the solvent cavity volume deduced from refined structures of the initial cold crystal (solid symbols) and the crystal after unit cell expansion (open symbols). Solid lines indicate results for crystals that were cooled at only 0.1 K/s. The total solvent volume at each temperature was estimated as described in (Moreau *et al.*, 2019) by assuming the first hydration layer does not contract and that the remaining solvent has the same contraction as the bulk liquid. Other

reasonable assumptions don't qualitatively change the results. This estimate neglects the effects of salts present in the crystallization solution (15% w/v ammonium sulfate, 2% w/v $CdSO_4$) on the solvent's volume change on cooling. This salt concentration suppresses the bulk freezing temperature by only 4 °C and should only modestly reduce the solvent volume expansion on cooling from room temperature to 220-260 K relative to that for pure water. For glycerol-containing solutions at the concentrations studied here, the effects of salts on solvent volume changes should be negligible. Unit cell expansion for crystals with 0% and 10% glycerol brings the excess solvent volume down to toward 0. Complete data sets acquired before and after expansion, needed to determine the change solvent volume, were not obtained for all glycerol concentrations and temperatures.

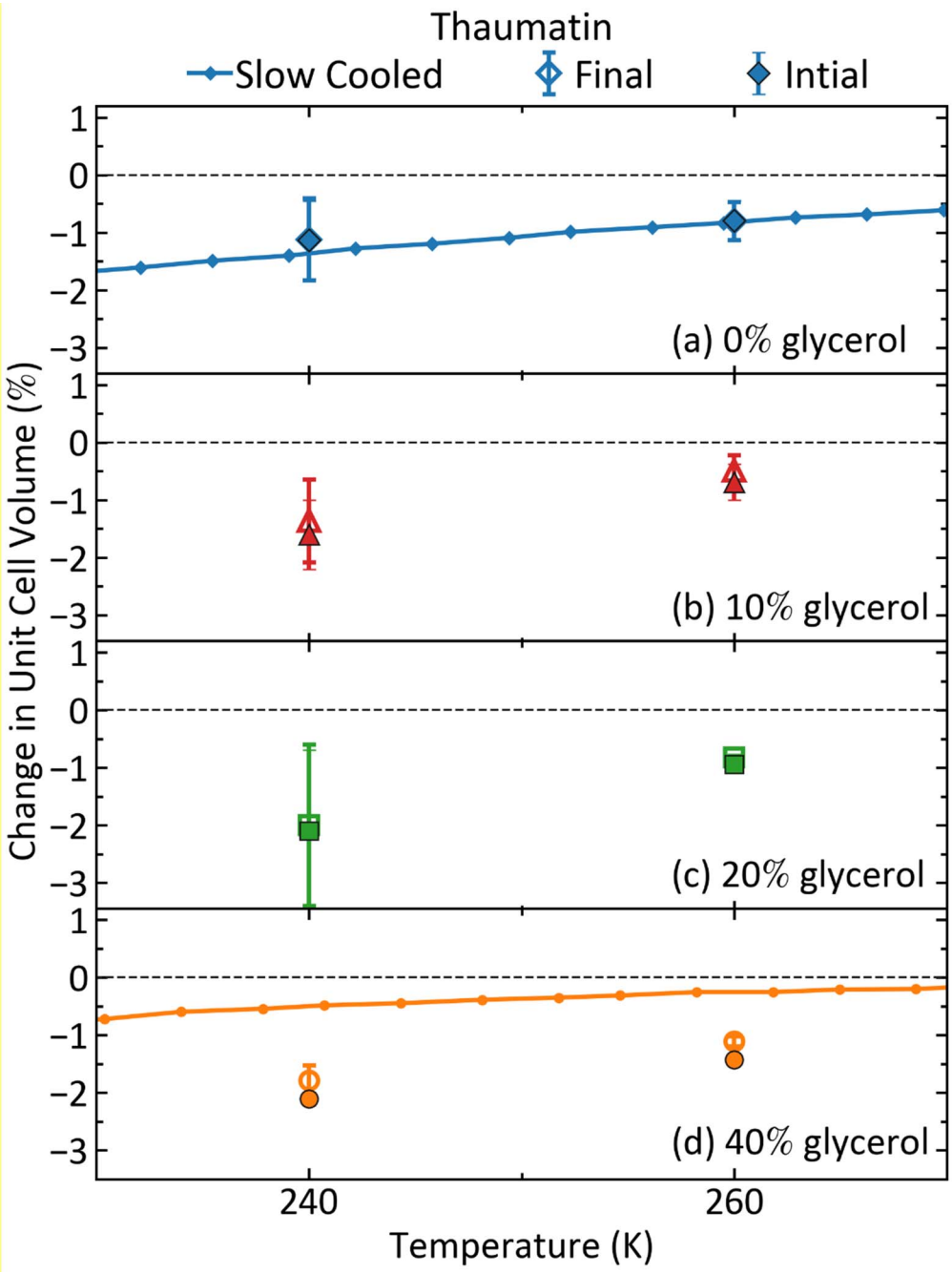

Figure S4 Change in unit cell volume relative to room temperature for thaumatin crystals that were slowly cooled at ~0.1 K/s (solid lines and small symbols) using data from (Warkentin & Thorne 2009), and that were abruptly cooled (in <1 s) to each temperature (large symbols), versus temperature. Closed and open symbols indicate unit cell volumes measured just after cooling had completed and at the completion of data collection (typically at t=40 s), respectively.

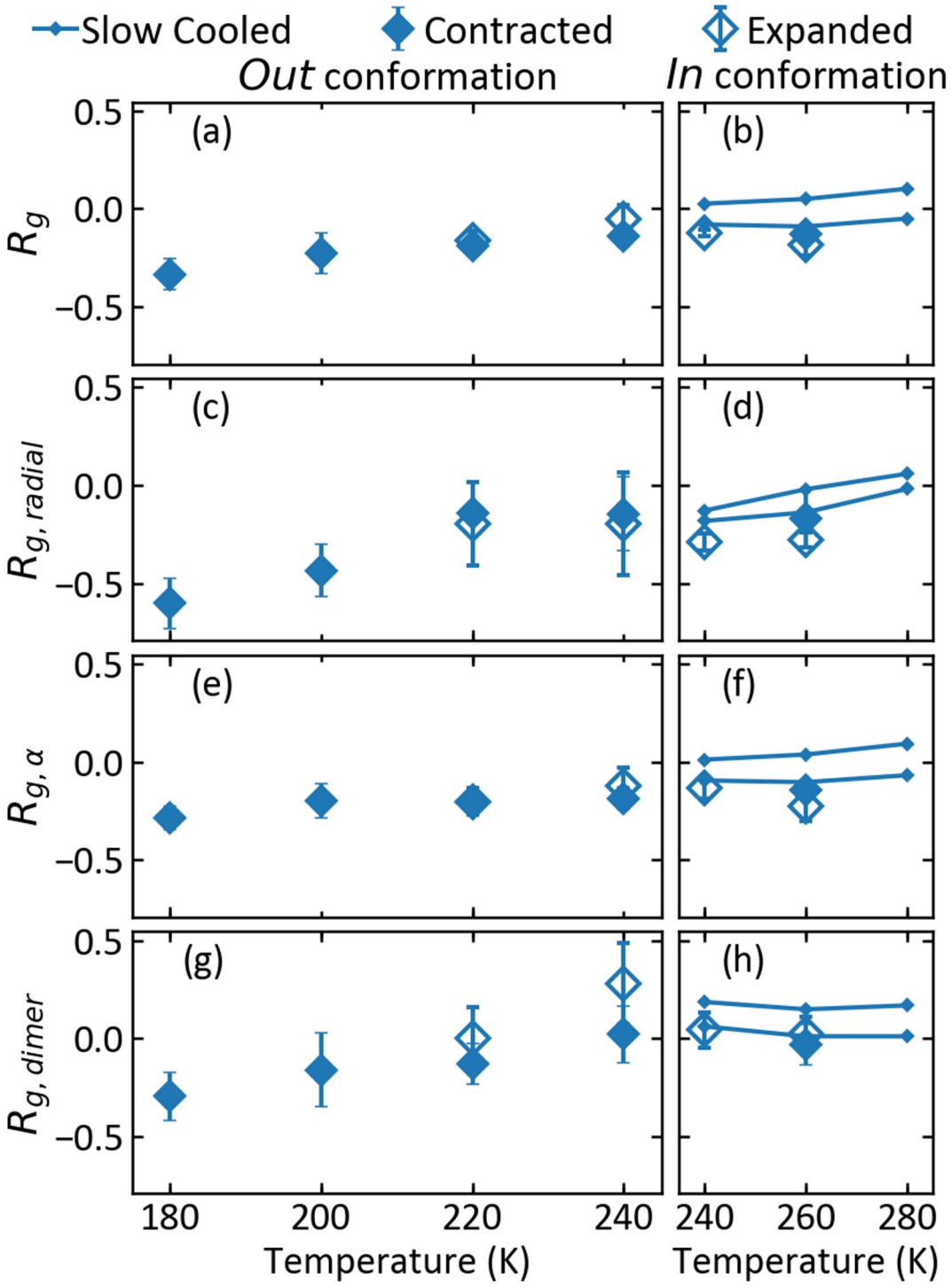

Figure S5 (a, b) The radius of gyration of a ferritin monomer and (c-h) its components along the axes shown in Fig. 1(d). The left and right panels show results obtained from crystals with GLN82 in the "*in*" and "*out*" configurations, respectively. Closed and open symbols indicate values determined just after cooling and after unit cell expansion had completed, respectively, and solid lines indicate results obtain for crystals that were slowly cooled.