Theoretical and experimental study of antifreeze protein AFP752, trehalose and dimethyl sulfoxide cryoprotection mechanism: correlation with cryopreserved cell viability – Supplementary material

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Supplementary Material

Expression and purification of recombinant TrxA-ApAFP752 fusion protein

The recombinant plasmid pET32b-*Apafp752*[1] was transformed into *Escherichia coli* Rosetta-gami 2(DE3) or BL21 (DE3)pLysS competent cells (Novagen). A single transformed colony was used to inoculate 25 mL of Luria-Bertani (LB) medium containing 0.3 mM ampicillin and cultured overnight at 37 °C and shaken at 225 rpm. 4 mL of the overnight culture was transferred into 1 L of fresh LB medium with 0.3 mM ampicillin and grown at 37 °C and 225 rpm until the optical density at 600 nm (OD_{600}) reached 0.6-0.7. The culture was induced with 1 mL of 400 mM isopropanol-1-thio-β-D-galactopyranoside (IPTG), to cause over-expression of TrxA-ApAFP752, for 8 hours at 25 °C. The cells were harvested by centrifugation (20 min, 8700 g, 4 °C) and stored at -80 °C until purification.
The cell pellet was resuspended with ice-cold binding buffer (EDTA-free Halt™ protease inhibitor cocktail (Fisher), 50 mM sodium phosphate, 150 mM NaCl, 20 mM imidazole and Benzonase nuclease (Millipore) at pH 8.0) and lysed using a French press (1300 psi). The lysate was collected and centrifuged (20 min., 27200 g, 4 °C) to remove the cell debris. The supernatant was filtered through a 0.22 μm syringe-driven filter (Millipore) and concentrated to 5 mL before being purified by a Fast Protein Liquid Chromatographer (FPLC; GE Healthcare ÄKTA purifier 900) equipped with a HisTrap HP Ni-NTA column (GE Healthcare). The sample was loaded into the Ni-NTA column, washed with washing buffer

(50 mM sodium phosphate, 150 mM NaCl, 20 mM imidazole at pH 8.0), and eluted with the same buffer containing 500 mM imidazole through a gradient elution. The fractions were pooled and dialysed in sodium phosphate buffer (50 mM sodium phosphate, 150 mM NaCl at pH 8.0) for 2 hours, then switched to fresh buffer and left overnight. The purity of the sample was analysed by 12.5% SDS-PAGE and stained with Coomassie blue; TrxA-ApAFP752 has a molecular weight of 27 kDa and was compared to a stained protein ladder (BioRad). Additional purification steps were taken to obtain a pure sample and SDS-PAGE was used after each step to analyse the purity. The sample was divided into three fractions that were each concentrated to 1 mL and run through a Superdex 75 10/300 GL size exclusion column (GE Healthcare) equilibrated with sodium phosphate buffer. Fractions that were still not pure were run through a Ni-NTA column and dialysed for a second time.

Once the desired purity was obtained, the sample was buffer exchanged into a $K_xH_YPO_4$ buffer (50 mM potassium phosphate, 20 mM NaCl, and 1 mM NaN_3 at pH 8.0). The concentration was estimated using UV-Visible spectrophotometry at 280 nm with the calculated extinction coefficient of 19,575 $M^{-1}cm^{-1}$. The sample was concentrated to 1 mL and lyophilized overnight. For further experiments, the protein was rehydrated as needed and buffer exchanged.

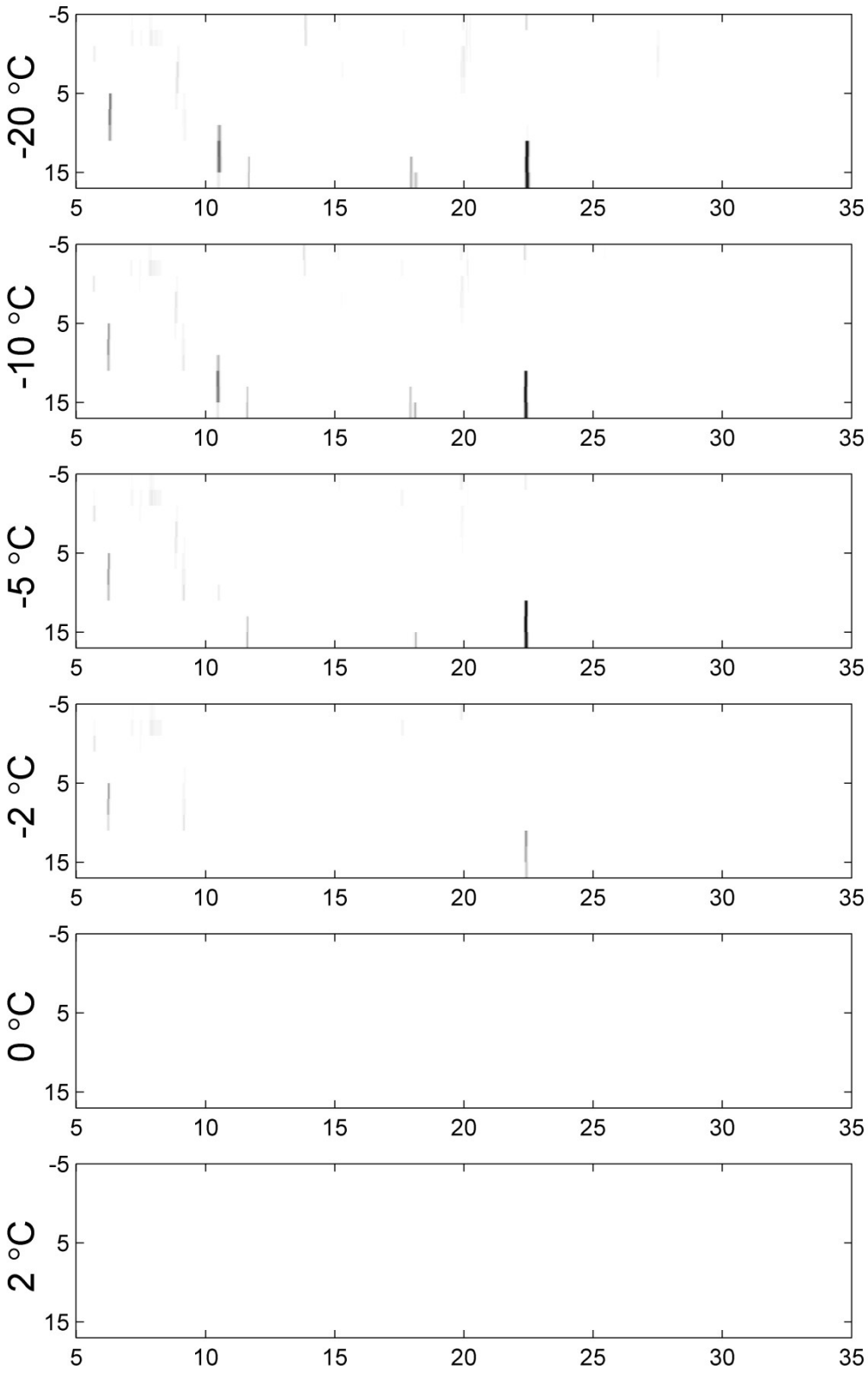

Fig. S1: Diffraction intensity maps in angular coordinates – pure water. The melting process (from top to bottom) of water crystals observed on 110 reflection intensities with various orientations.

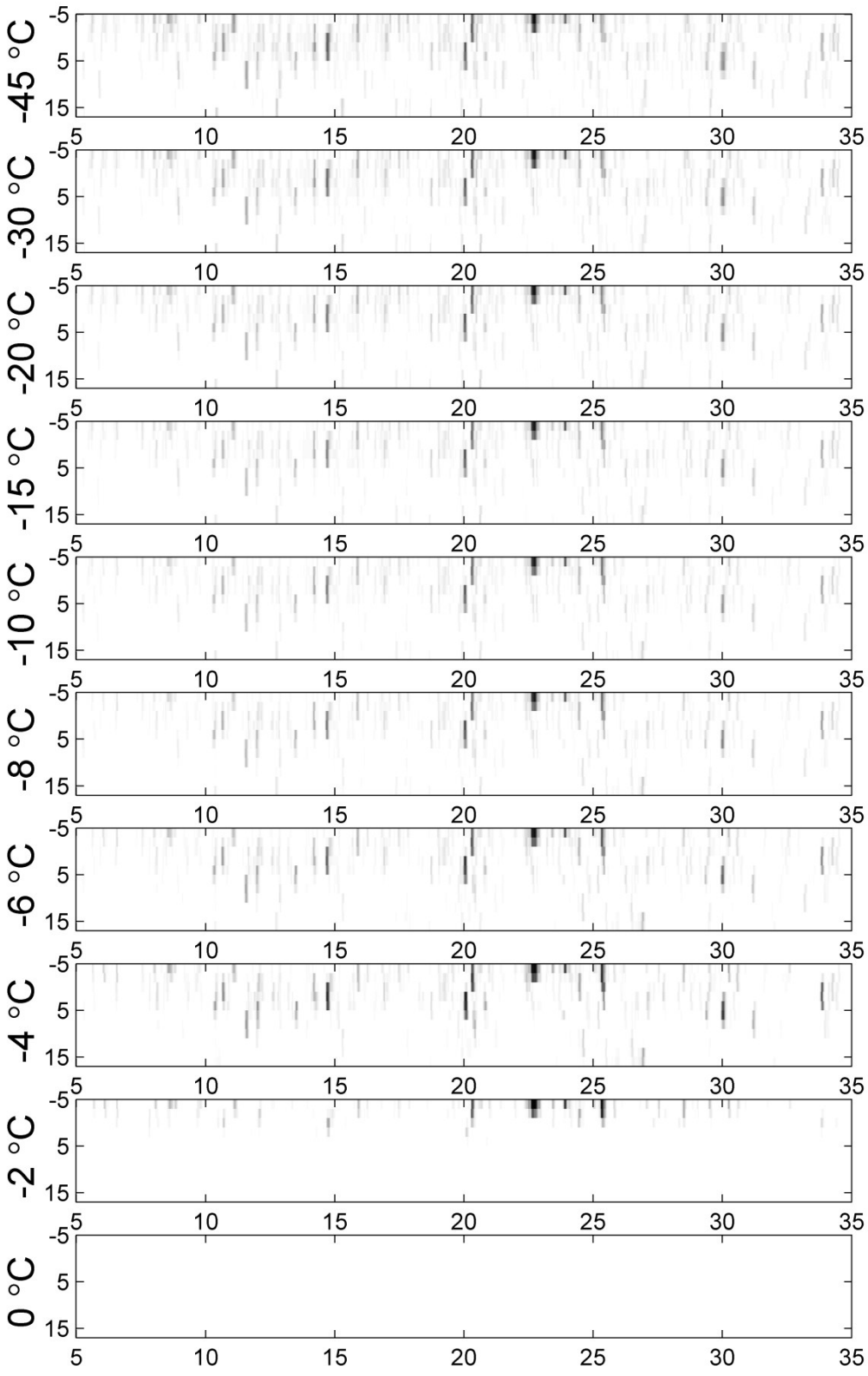

Fig. S2: Diffraction intensity maps in angular coordinates – 0.05% (w/w) AFP solution. The melting process (from top to bottom) of water crystals observed on 110 reflection intensities with various orientations.

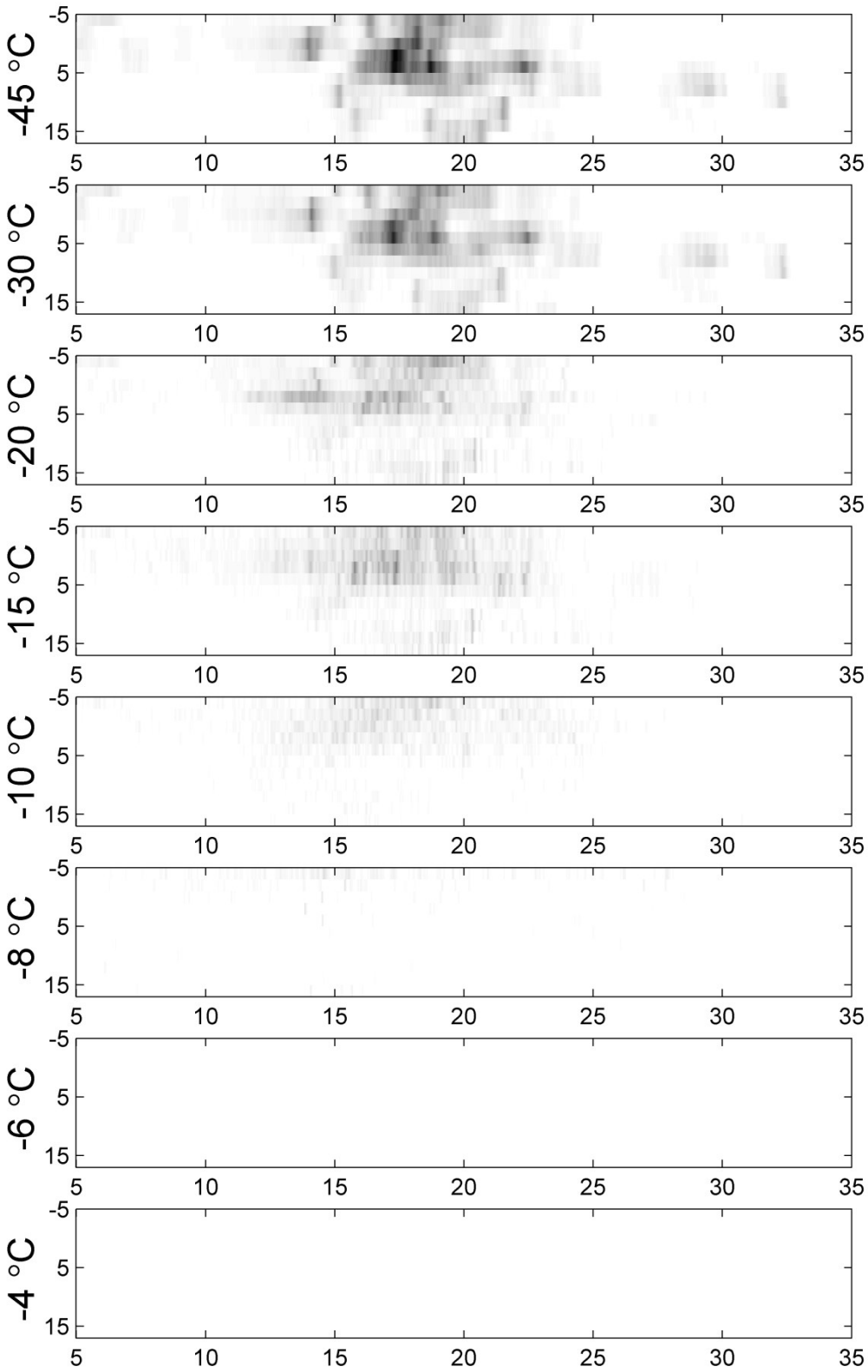

Fig. S3: Diffraction intensity maps in angular coordinates – 41% (w/w) trehalose solution. The melting process (from top to bottom) of water crystals observed on 110 reflection intensities with various orientations.

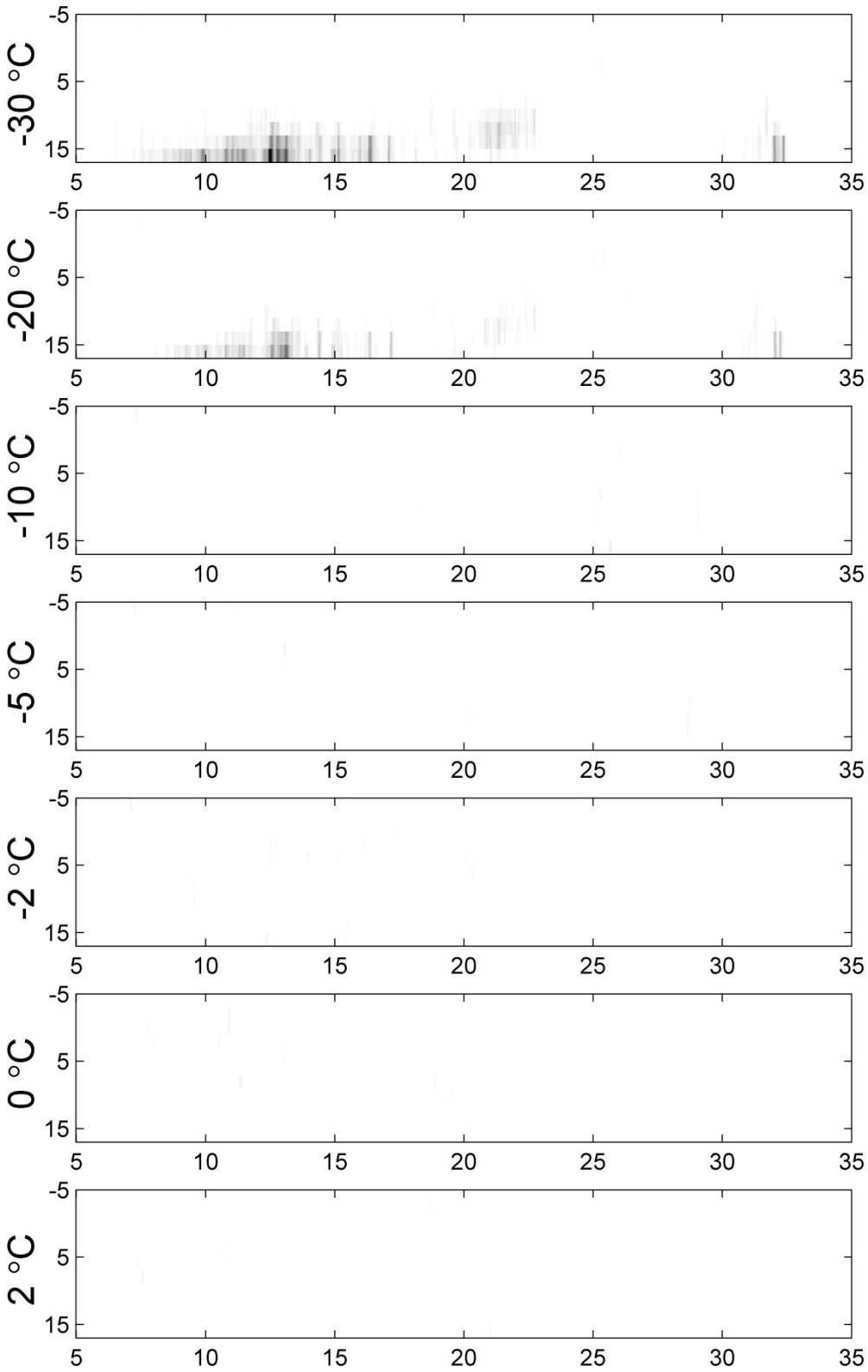

Fig. S4: Diffraction intensity maps in angular coordinates – 1% (w/w) AFP solution. The melting process (from top to bottom) of water crystals observed on 110 reflection intensities with various orientations.

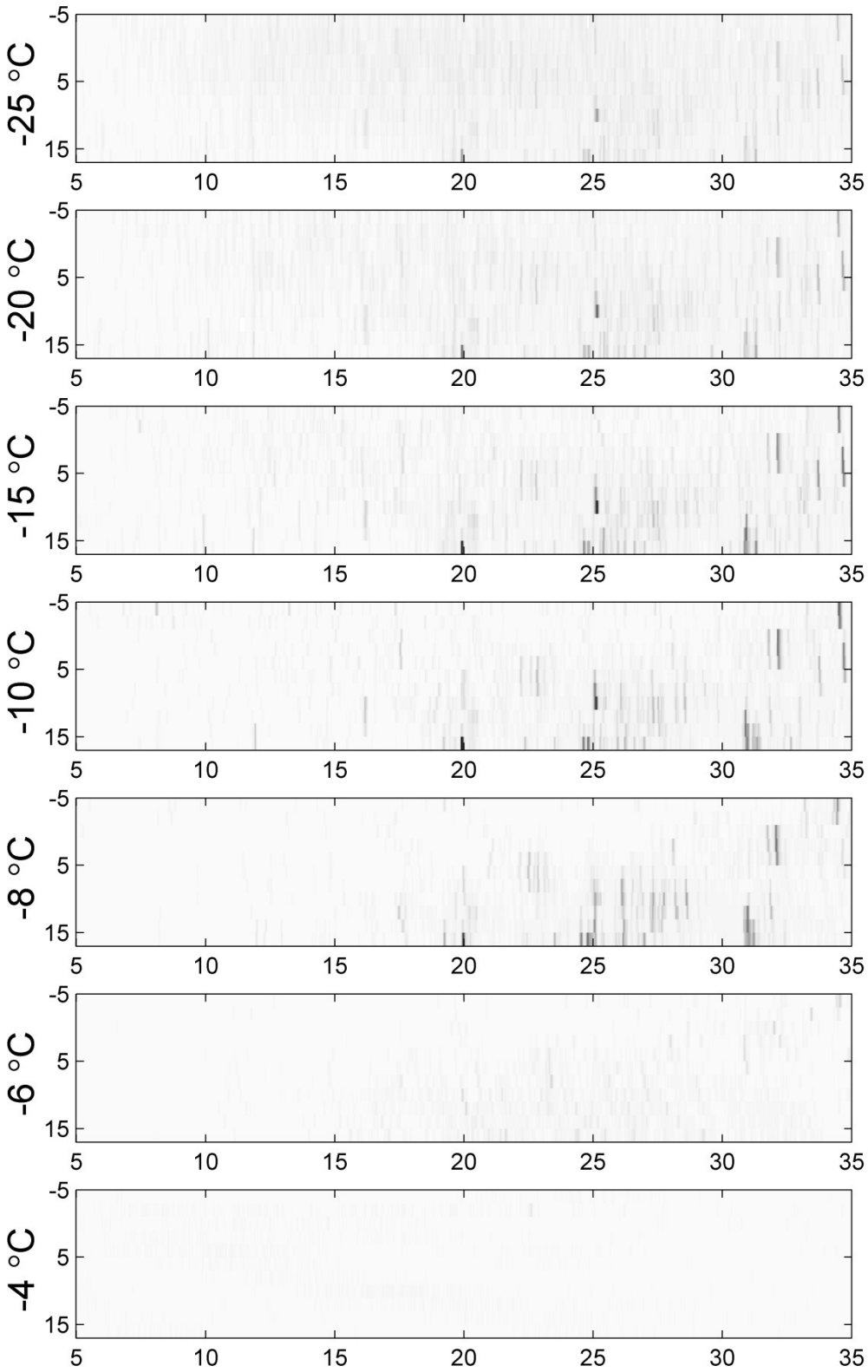

Fig. S5: Diffraction intensity maps in angular coordinates – 10% (w/w) DMSO solution. The melting process (from top to bottom) of water crystals observed on 110 reflection intensities with various orientations.

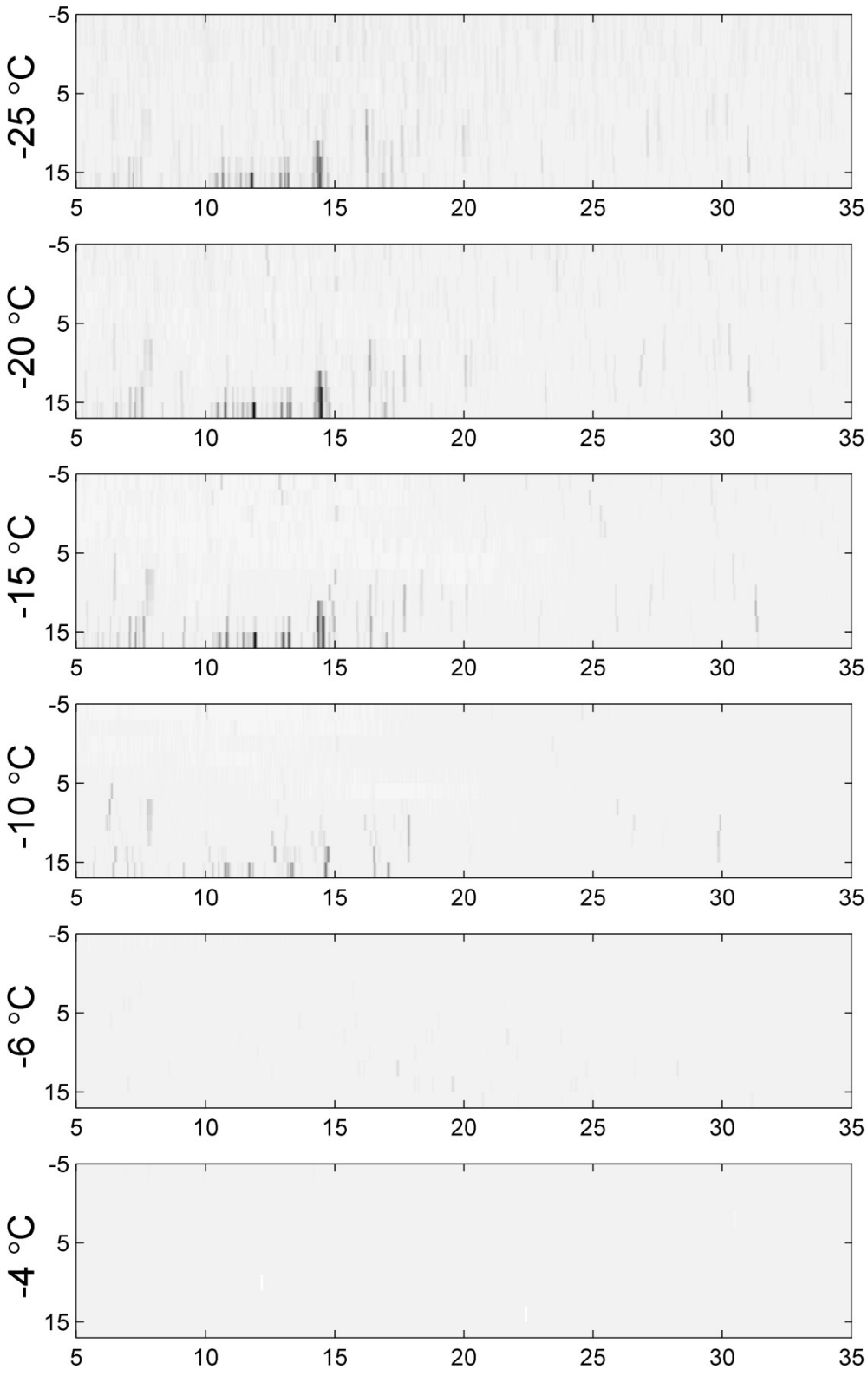

Fig. S6: Diffraction intensity maps in angular coordinates – 10% (w/w DMSO + 3.2% w/w trehalose solution. The melting process (from top to bottom) of water crystals observed on 110 reflection intensities with various orientations.

Table S1: The calculated interaction energies ΔE_{int}, (kcal/mol) of the various types of dimers with and without inclusion of BSSE corrections.

System	ΔE_{int}	ΔE_{int} *(BSSE)*
$[H_2O]_2$	-4.7	-3.9
$[(CH3)_2SO]_2$	-6.5	-5.5
$[(CH3)_2SO\ldots H_2O]$	-6.8	-5.9
$[C_{12}H_{22}O_{11}]_2$	-11.1	-7.0
$[C_{12}H_{22}O_{11}\ldots H_2O]$	-7.4	-6.2