Supporting Information

Tyrosine Monomer Nanocrystal as a Potent Ice Recrystallization Inhibition Activity

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1. Materials

Homo-oligopeptides (pentamer of L-phenylalanine) and amino acid (L-phenylalanine [≥98%], L-tyrosine [≥98%], and 3,4-dihydroxy-L-phenylalanine [≥98%]) were purchased from AnyGen Co., Ltd. (Gwangju, South Korea) and Sigma-Aldrich (St. Louis, Mo, USA), respectively. Polystyrene nanoparticles (PSNPs; nanosphere size standards: 31 ± 3 nm, 51 ± 3 nm, 61 ± 4 nm, 100 ± 6 nm, and 203 ± 5 nm) and micro particles size standards (994 ± 15 nm) were obtained from Thermo Fisher Scientific Inc. (Waltham, MA, USA). Reagents including 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP, ≥99%), poly(vinyl alcohol) (80% hydrolyzed, Mw 9,000–10,000), poly(ethylene glycol) (PEG; Mw 3,500–4,500), styrene (≥99%), and Amicon® Ultra centrifugal filters (0.5 mL, 3.0 kDa MWCO) were purchased from Sigma-Aldrich. Circular cover glasses (SciLab®, Φ 12 mm, 0.13–0.16 mm thick) were obtained from SciLab Korea Co., Ltd. (Seoul, Korea). Dulbecco's Modified Eagle's medium (DMEM), phenol red-free medium (colorless DMEM), and fetal bovine serum (FBS) were obtained from HyClone (Waltham, MA, USA). Dulbecco's phosphate-buffered saline (DPBS) was purchased from Mediatech Inc. (Manassas, VA, USA). Penicillin–streptomycin and 0.25% trypsin-EDTA were purchased from Gibco (Grand Island, NY, USA). CellTiter 96® AQueous One Solution Cell Proliferation Assay (MTS assay) was purchased from Promega (Madison, WI, USA). The CoroNa™ Green Sodium Indicator (C36676) was obtained from Thermo Fisher Scientific Inc.

2. Calculations of the number of particles.

To calculate the difference in particle counts of polystyrene nanoparticles (PSNPs) in a solution of the same mass (e.g., 1 mg/mL), the following process can be followed by considering the volume difference and density according to the size of the particles.

$$V = \frac{4}{3}\pi r^3$$

Assuming spherical particles and a polystyrene density of **1.05 g/cm³**, the volume (V) and mass (m) of a single particle are calculated as follow:

For 1000 nm PSNP (r = 500 nm = 5.0×10^{-7} m):

$V_{1000} = 5.24 \times 10^{-19}$ m³

$m_{1000} = 1.05 \times 10^{3}$ kg/m³ $\times 5.24 \times 10^{-19}$ m³ $= 5.5 \times 10^{-13}$ g

$N_{1000} = 1.0 \times 10^{-3}$ g / 5.5×10^{-13} g/particle ≈ 1.82×10^{9} particles/mL

For 30 nm PSNP (r = 15 nm = 1.5×10^{-8} m):

$V_{30} = 1.41 \times 10^{-23}$ m³

$m_{30} = 1.05 \times 10^{3}$ kg/m³ $\times 1.41 \times 10^{-23}$ m³ $= 1.48 \times 10^{-17}$ g

$N_{30} = 1.0 \times 10^{-3}$ g / 1.48×10^{-17} g/particle ≈ 6.76×10^{13} particles/mL

Thus, the 30 nm PSNPs solution contains approximately:

$$\frac{N_{30}}{N_{1000}} \approx 3.7 \times 10^{4} \text{ times more particles}$$

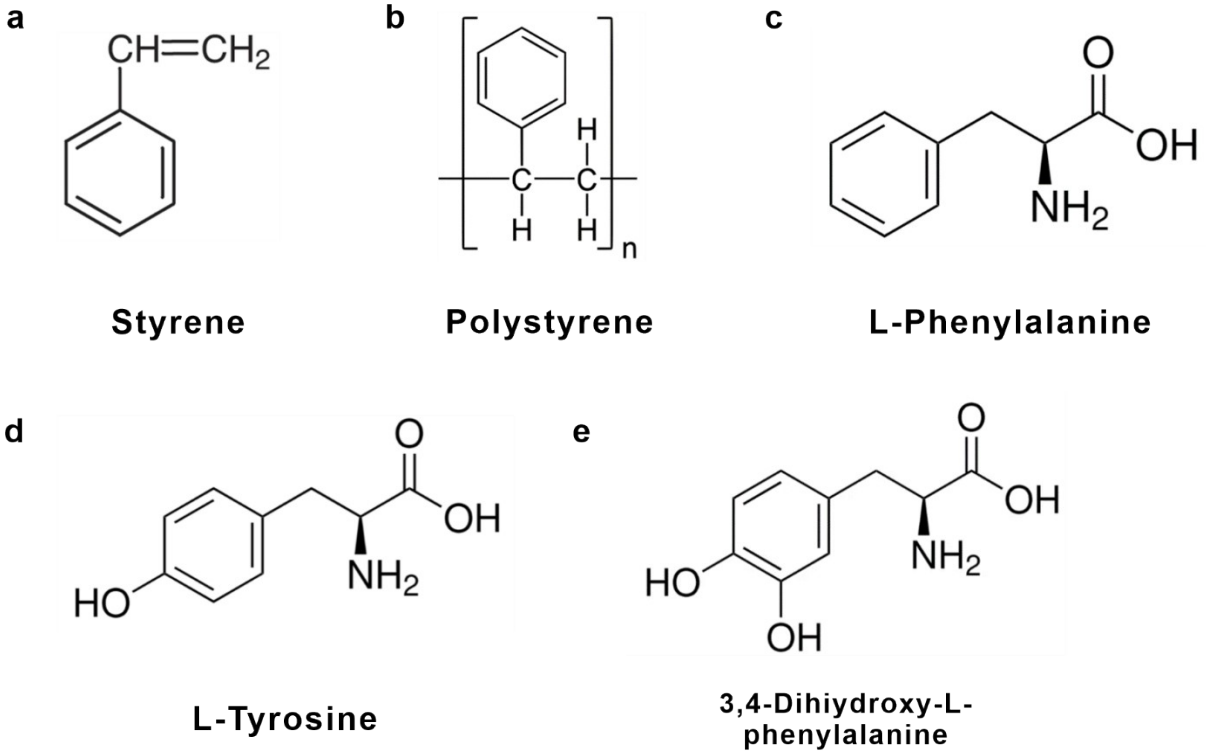

Fig. S1. Chemical structures of the substances used in this study.

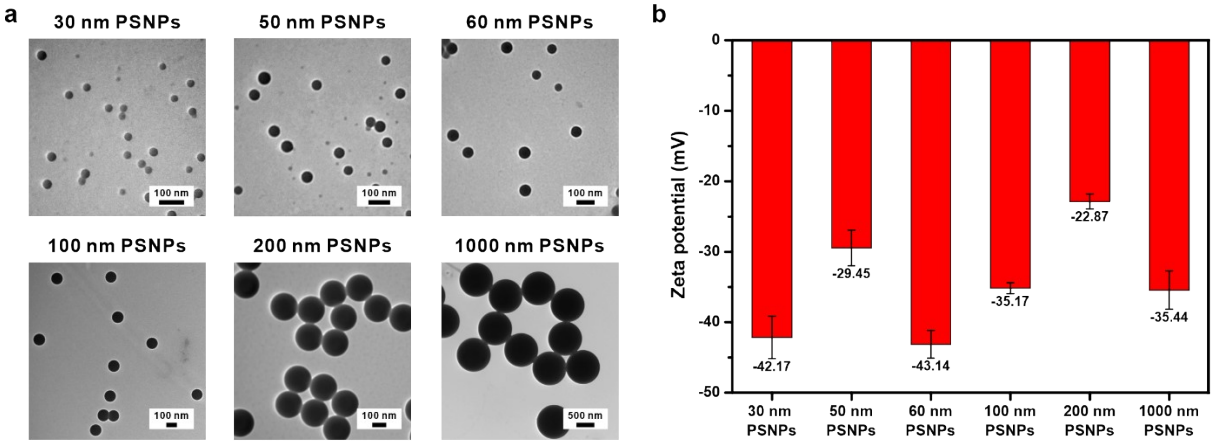

Fig. S2. (a) TEM images and (b) zeta potential analysis of polystyrene nanoparticles (30, 50, 60, 100, 200, and 1000 nm).

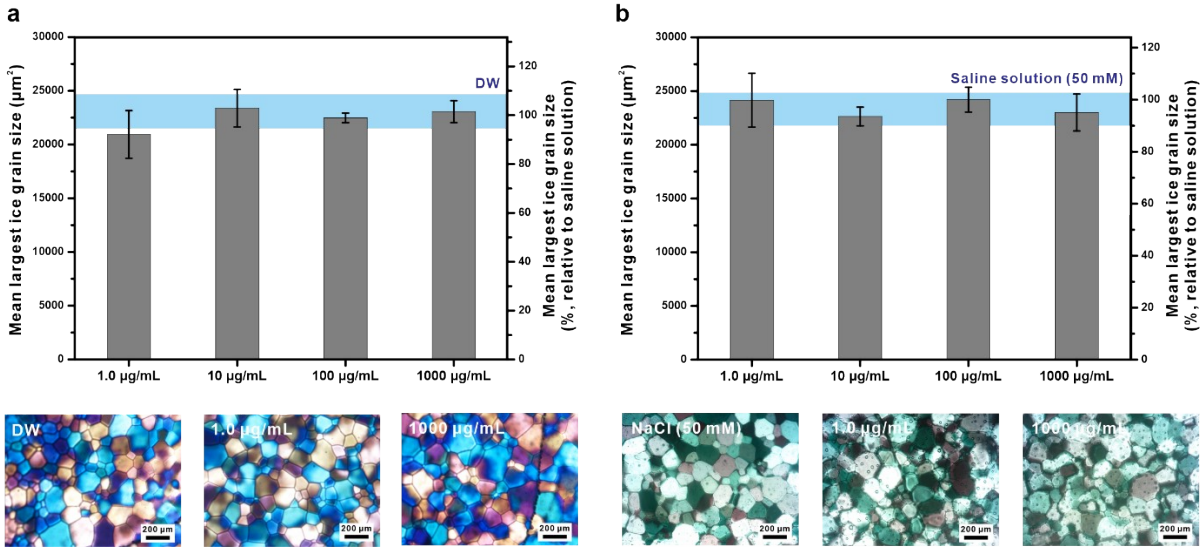

Fig. S3. IRI activity of styrene monomer at various concentrations (concentration: 1.0, 10, 100, and 1000 μg/mL) in (a) DW or (b) saline ([NaCl]=50 mM).

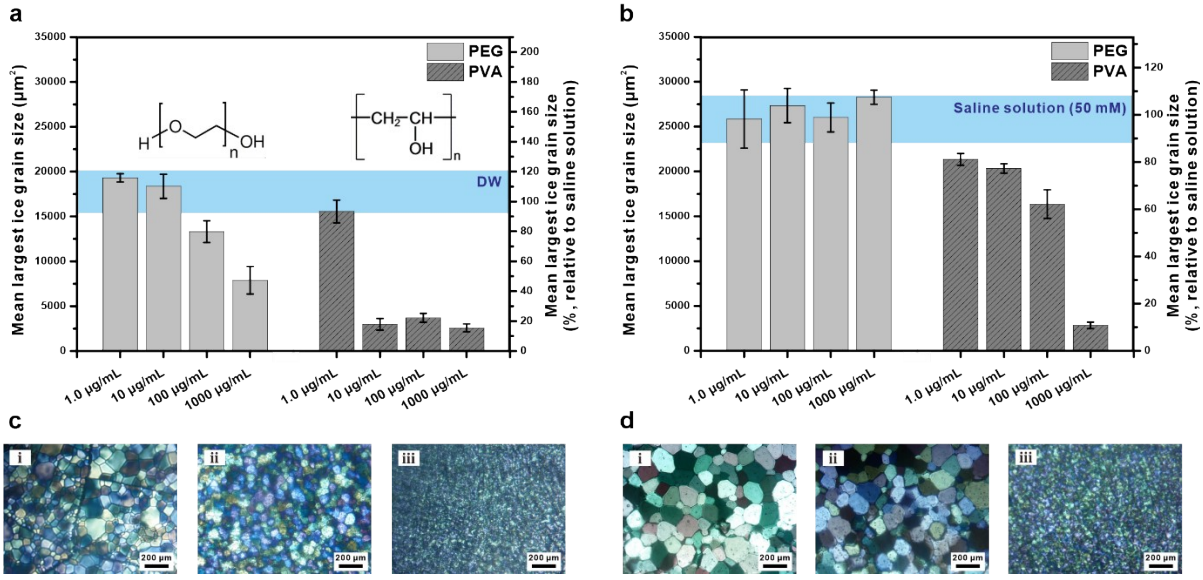

Fig. S4. IRI activity of PEG and PVA in (a) DW or (b) saline ([NaCl]=50 mM). (c) Photos of ice grains (i) DW, (ii) PEG (1.0 mg/mL), and (iii) PVA (1.0 mg/mL) in DW. (d) Photos of ice grains (i) saline solution, (ii) PEG (1.0 mg/mL), and (iii) PVA (1.0 mg/mL) in saline.

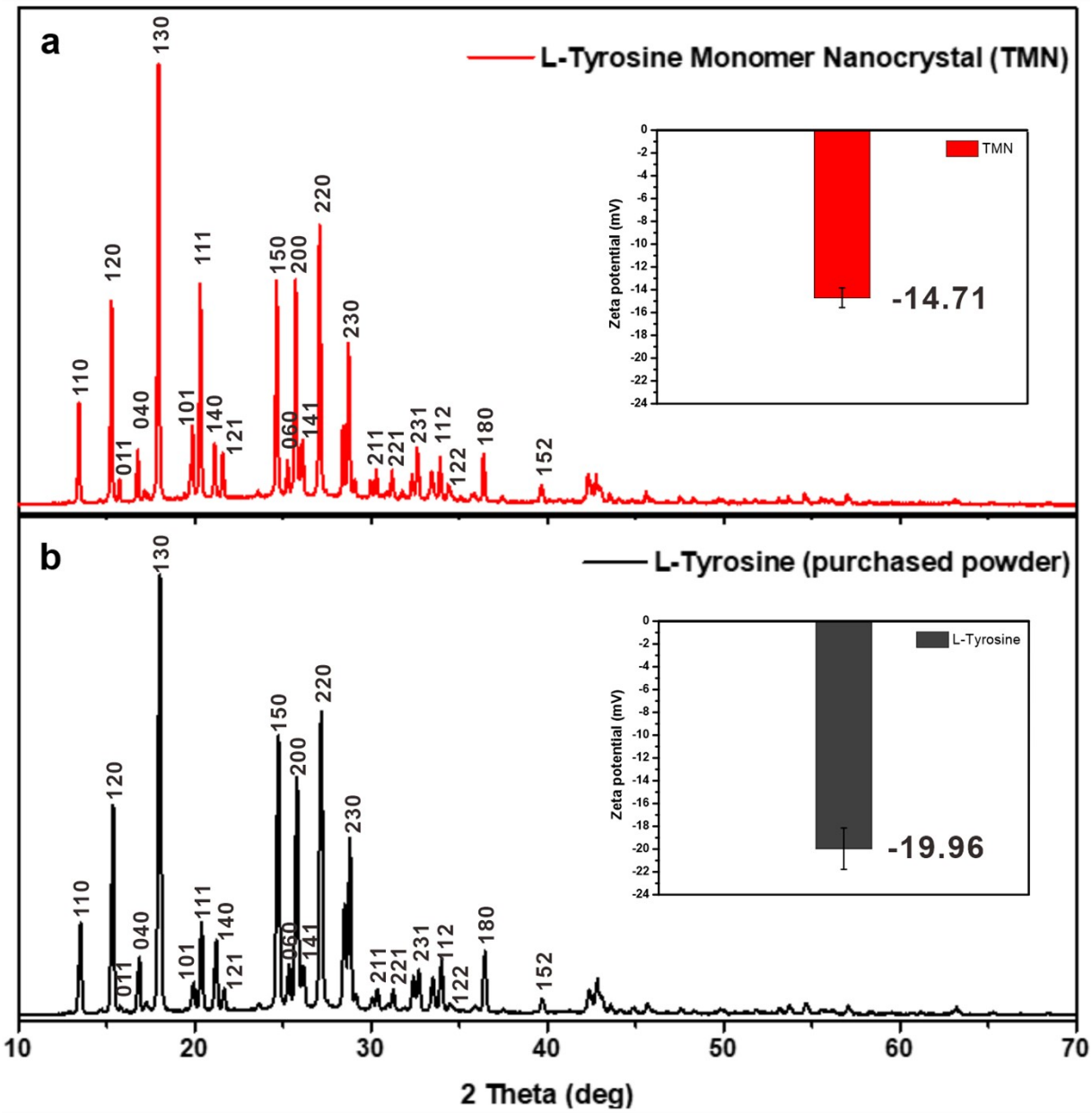

Fig. S5. XRD patterns and zeta potentials of (a) TMN and (b) L-Tyrosine (purchased powder).

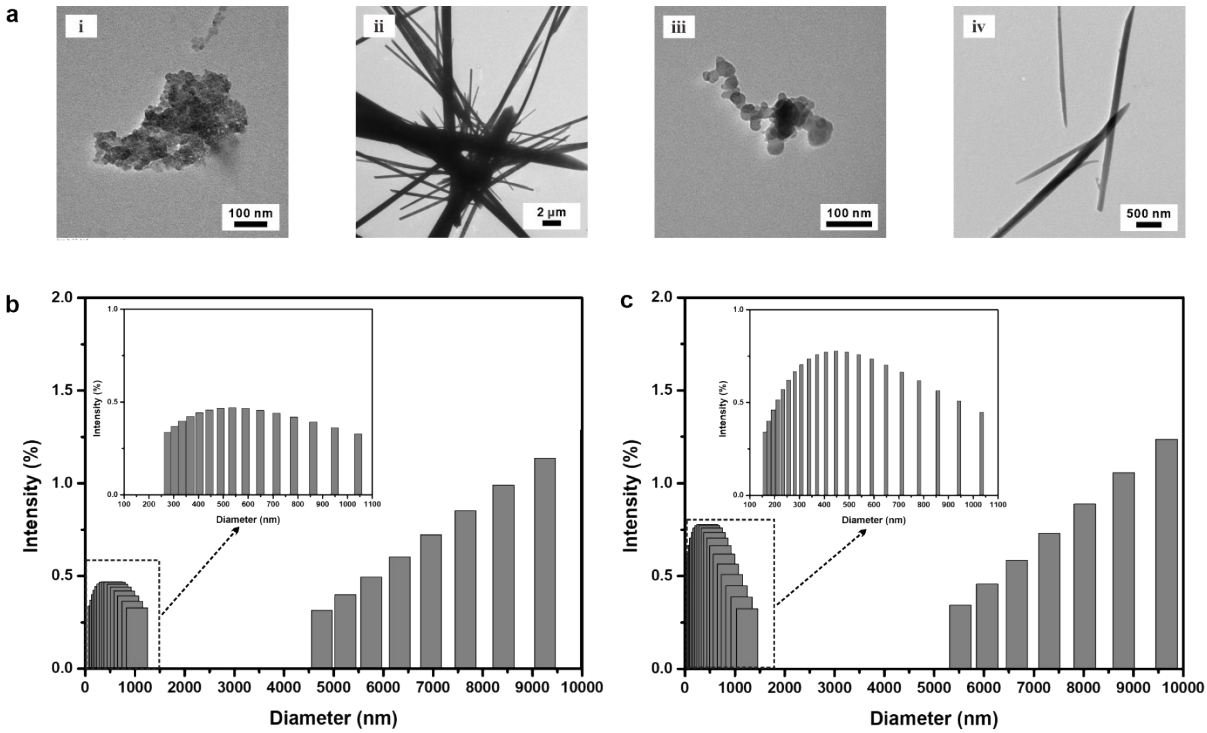

Fig. S6. (a) TEM images of (i) nano-sized TMN, (ii) micro-sized TMN, (iii) nano-sized L-Tyrosine (purchased), and (iv) micro-sized L-Tyrosine (purchased). (b) Measured size distribution of TMN (0.45 mg/mL). (c) Measured size distribution of L-Tyrosine (0.45 mg/mL).

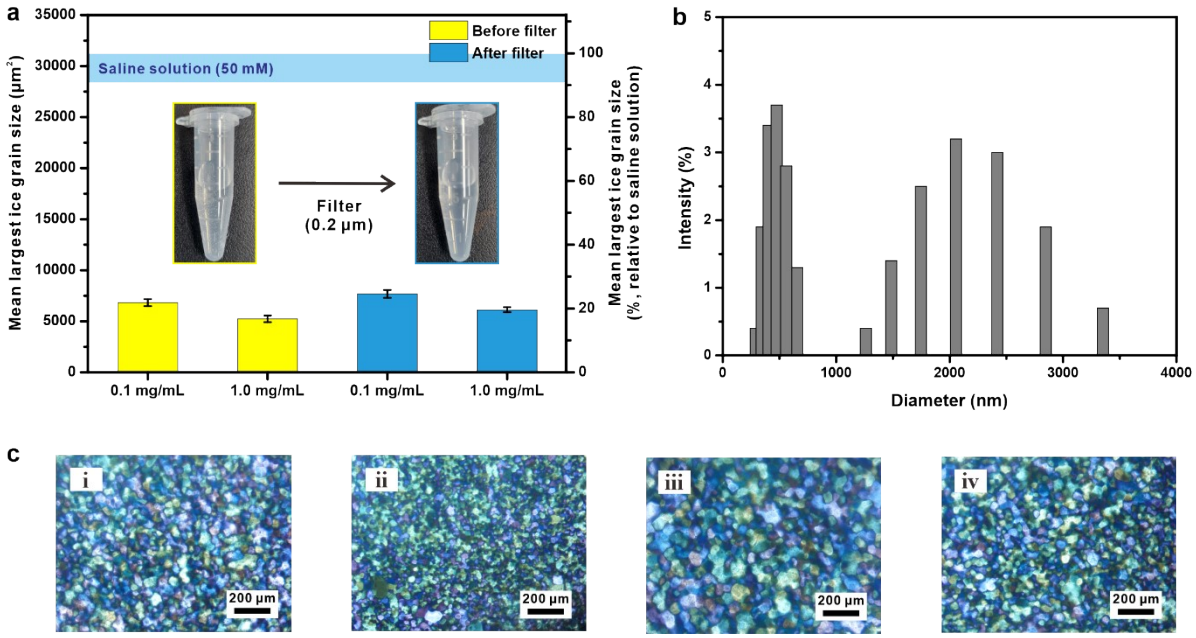

Fig. S7. (a) Mean largest ice grain size before and after filtering TMN solution in saline ([NaCl] = 50 mM). (b) Measured size distribution of TMN solution (1.0 mg/mL) after filtration. (c) Photos of ice grains (i) before filter TMN (0.1 mg/mL), (ii) before filter TMN (1.0 mg/mL), (iii) after filter TMN (0.1 mg/mL), and (iv) after filter TMN (1.0 mg/mL).

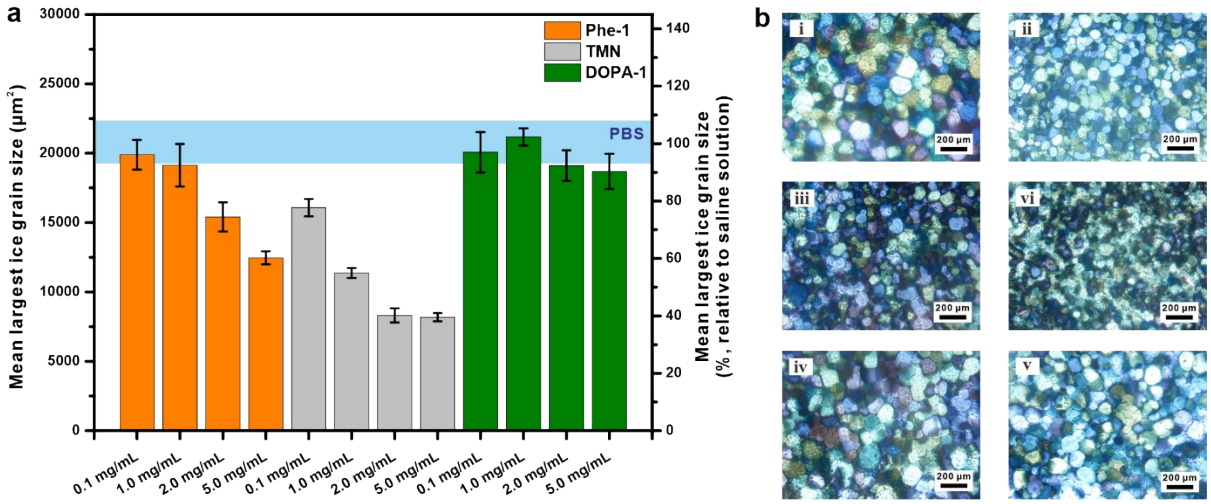

Fig. S8. (a) Measured mean largest ice grain size of PBS ([NaCl]=137 mM) with Phe-1, TMN, and DOPA-1. (b) Photos of ice grains (i) Phe-1 (0.1 mg/mL), (ii) Phe-1 (5.0 mg/mL), (iii) TMN (0.1 mg/mL), (iv) TMN (5.0 mg/mL), (v) DOPA-1 (0.1 mg/mL), and (vi) DOPA-1 (5.0 mg/mL).

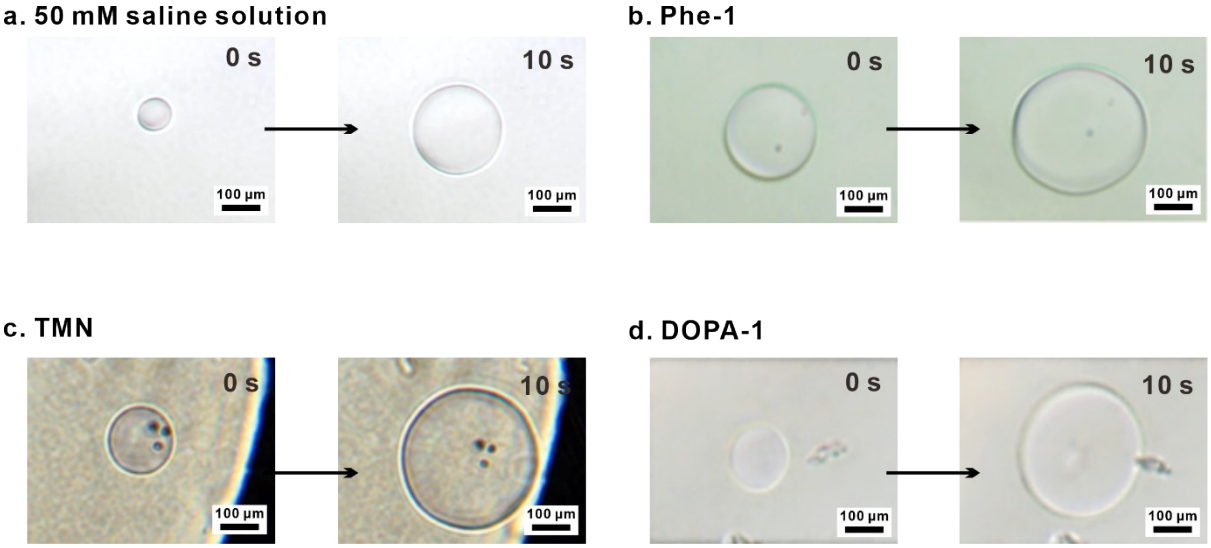

Fig. S9. Images of a single ice grain (0 and 10 s) in saline ([NaCl]=50 mM). (a) saline, (b) Phe-1 (1.0 mg/mL), (c) TMN (1.0 mg/mL), and (d) DOPA-1 (1.0 mg/mL).

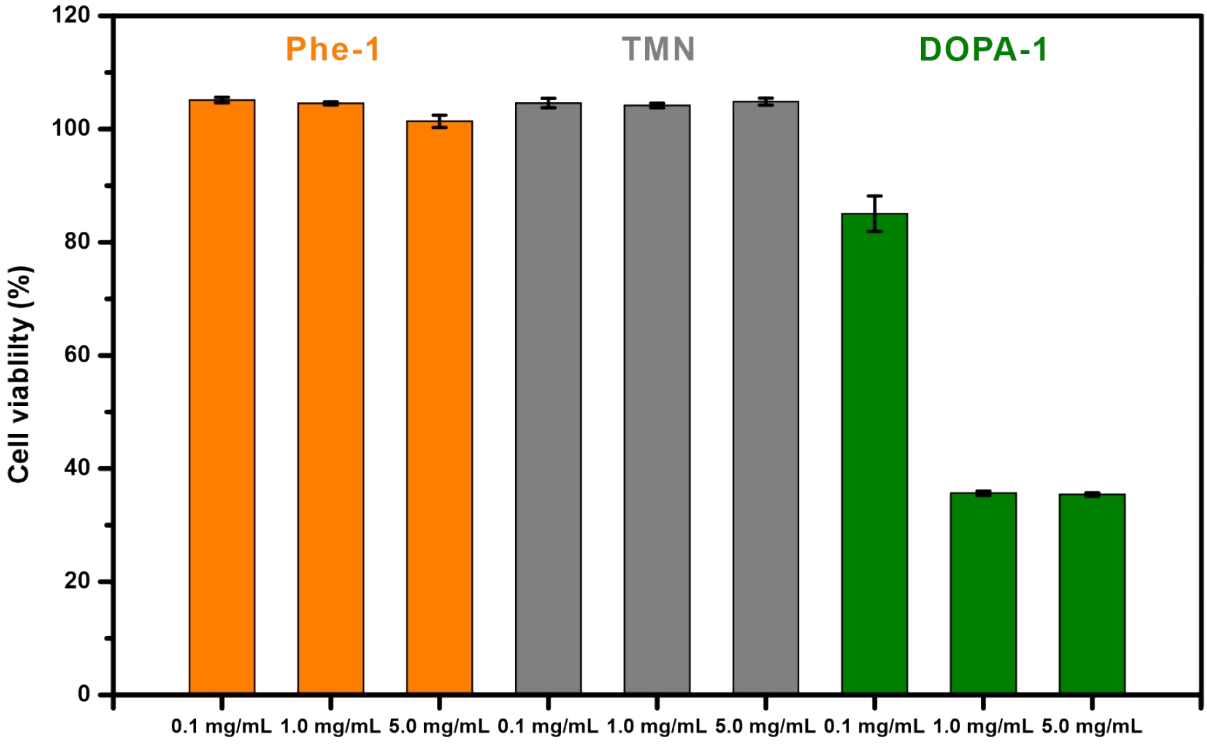

Fig. S10. Cell viability of Phe-1, TMN, and DOPA-1 at different concentrations (0.1, 1.0, and 5.0 mg/mL) in HSC-3 cells.

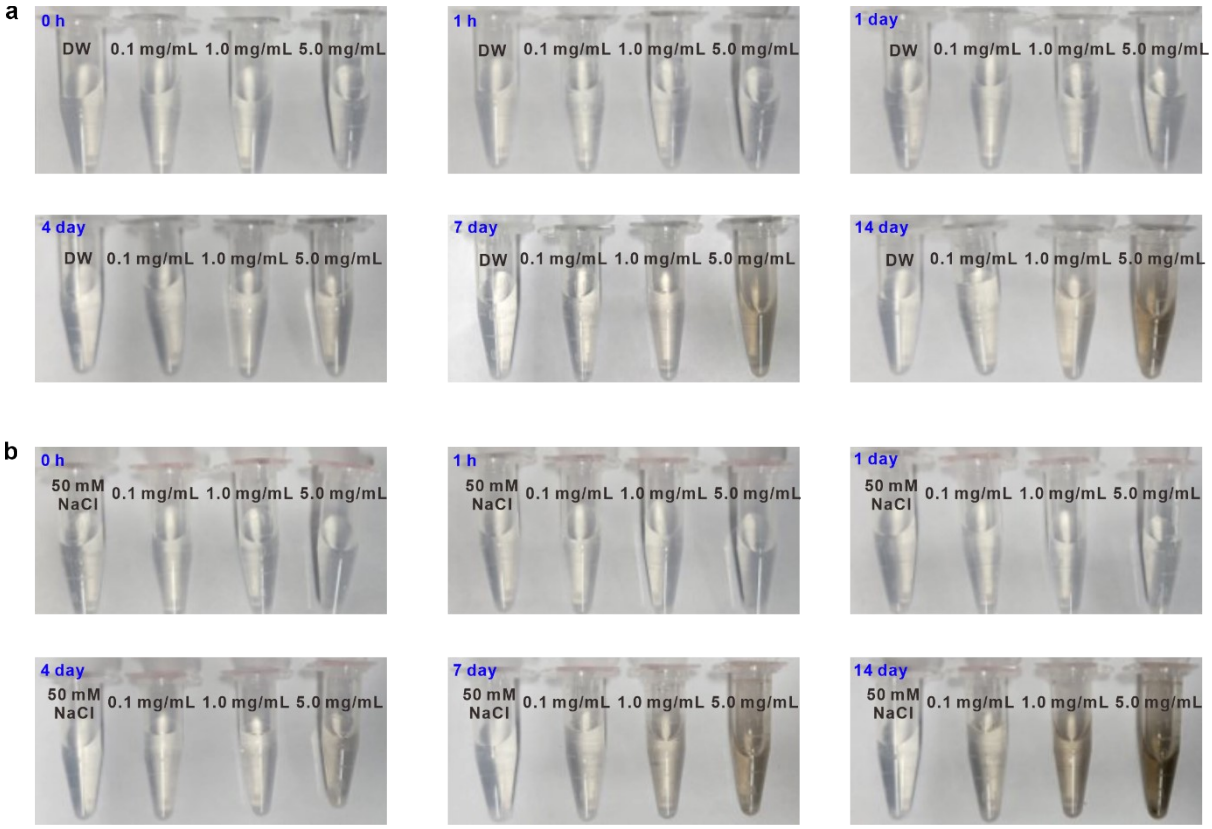

Fig. S11. Solvent color change due to autoxidation of DOPA-1. (a) Photographs of DOPA-1 maintained in DW for 14 days (at 0.1, 1.0, and 5.0 mg/mL). (b) Photographs of DOPA-1 cells maintained in 50 mM saline for 14 days (at 0.1, 1.0, and 5.0 mg/mL).

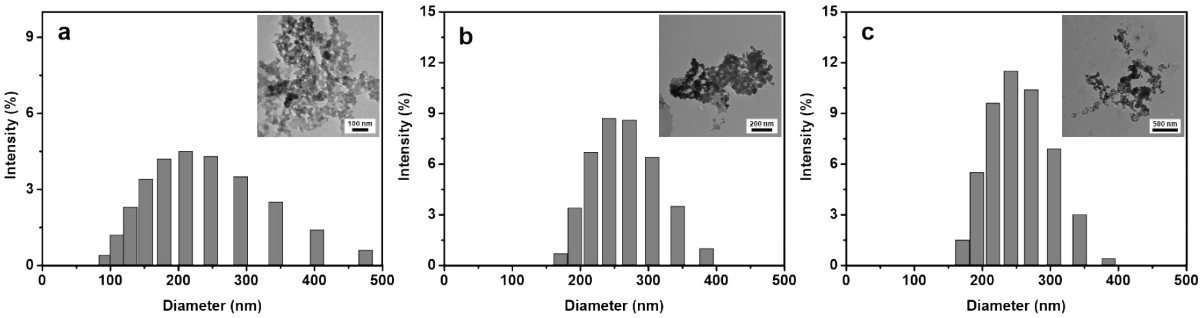

Fig. S12. Measured size distribution TMN solutions (0.45 mg/mL) in (a) 50 mM saline solution, (b) cryopreservation DMEM media, and (c) cryopreservation DMEM media + 1% DMSO.

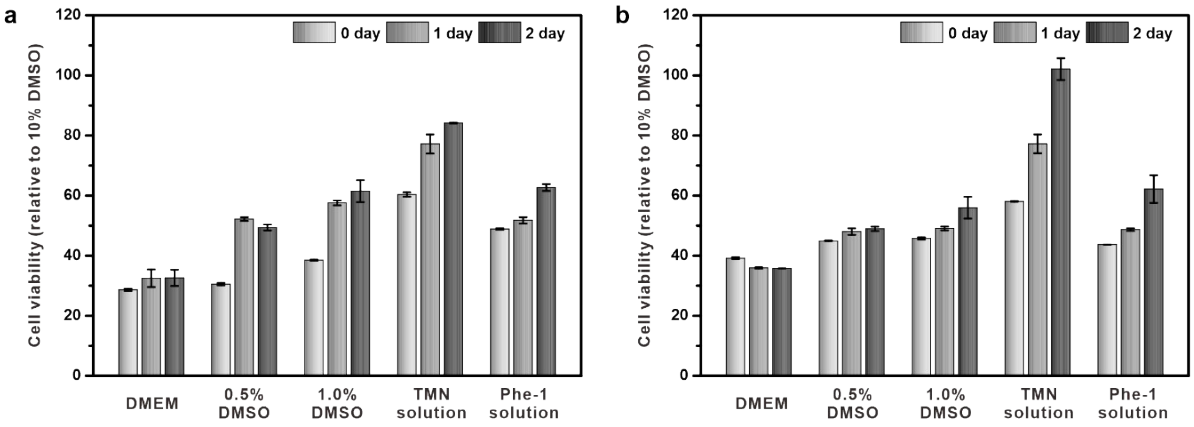

Fig. S13. Post-thaw proliferation of (a) HSC-3 and (b) RAW 264.7 cells over 2 days. TMN and Phe-1 solutions (0.45 mg/mL) in cryopreservation DMEM media (1% DMSO).

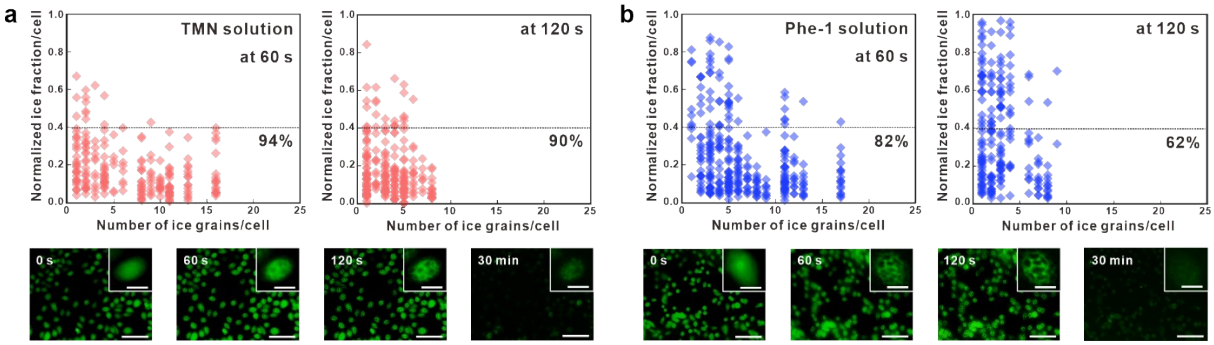

Fig. S14. Intracellular ice growth inhibition of TMN and Phe-1. Presents the number and proportion distribution of intracellular ice crystals observed at 60 and 120 s. (a) 0.45 mg/mL TMN solution treated cells. (b) 0.45 mg/mL Phe-1 solution-treated cells. Fluorescence images were acquired at individual time points (0 s, 60s, 120 s and 30 min, scale bar: 100 μm, inset image scale bar: 10 μm).