Supplementary Information

Design, Synthesis and Antifreeze Properties of Biomimetic Peptoid Oligomers

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1. Material and methods

1.1 Materials.

Dichloromethane, N-hydroxyethyl phthalimide, methylamine solution, ethylamine solution, propylamine, butylamine, methanol and ethanol were purchased from Sinopharm Chinese Reagent Company. Bromoacetic acid, acetone, trifluoroacetic acid, acetonitrile, dimethyldichlorosilane, 3,4-dihydro-2H-pyran, ether, n-hexane, pyridine 4-methylbenzenesulfonate, N,N'-dimethylformamide, hydrazine hydrate, triisopropylsilane(TIS), rink amide resin (0.3-0.8mmol·g^{-1}), 4-methylpiperidine, sodium chloride and N,N'-diisopropylcarbodiimide were purchased from Aladdin. All reagents and solvents were used without further purification.

1.2 Synthesis of peptoid oligomers

The synthesis of peptoid oligomers was conducted on the basis of the method previously reported by Zuckermann.[1] The ethylhydroxyamine were prepared according to the method published by M.Rajesh.[2] Firstly, the peptoid synthesis tube was silanized for 30 min in the silicone solution ($C_2H_6Cl_2Si$ /DCM= 5%/95%, v/v), rinsed by dichloromethane and methanol, and dried at room temperature. 12 mL N,N'-dimethylformamide (DMF) was added to swell the rink amide resin (600 mg) for 10 min. DMF was removed through vacuum filtration to obtain the swollen resin. In order to remove the protective Fmoc group. 6 mL 4-methylpiperidine solution (4-methylpiperidine/DMF=1:4, v/v) were added to react with the resin for 2 min under a stream of N_2 gas, and draining. Another 6 mL 4-methylpiperidine solution were added to react with the resin for 12 min and drained. 12 mL DMF solution was added to rinse the resin under nitrogen bubbles, then DMF was removed through vacuum filtration (5×30 s). 6 mL bromoacetic acid solution (0.6 M in DMF) and 516 μL N, N'-diisopropylcarbodiimide (DIC) were added to acylate resin for 20 min, then removed through vacuum filtration. The resin was washed by 12 mL DMF (5×30 s). 6 mL primary amine submonomer solution (1 M in DMF) was added to conduct substitution reaction for 30 min with the resin obtained in the previous step. Then 12 mL DMF was added to wash the resin (5×30 s). After the process was repeated six times, the crude samples were obtained. When the final substitution reaction was completed, DMF (5×30 s) and DCM (3×30 s) was used to wash resin sequentially. Peptoid oligomers were cleaved by trifluoroacetic acid cleavage solution (H_2O/TIS/TFA=2.5/2.5/95, v/v/v) for 2 hours. Then the exceed cleavage solution was removed by N_2. The separated sample was dissolved in 1 mL acetonitrile/water (1/1, v/v) solution and characterized by LC-MS (Thermo LXQ) performed on a C18 column (AnPu,4.6mm×250mm, 5 μm). The analysis was conducted by a solvent gradient (5–95% acetonitrile/water with 0.1% TFA) over 45 min, at a flow rate of 500 μL min^{-1}). Subsequently, the prepared Reversed-phase high performance liquid chromatography (RP-HPLC) (chromatographic column was SHIMADZU Inertsil OSD-SP,4.6mm×250mm, 5 μm) was used to purify and lyophilize the oily products. The pure

products were identified by Electrospray ionization tandem liquid chromatography mass spectrometry (ESI-LC- MS) analysis.

1.3 Nanoliter Osmometer Experiments

A nanoliter osmometer with the precision of 0.01℃ was used to measure the growth rate and morphology of ice crystals at different temperature. The relative humidity, temperature and droplets were accurately controlled. In order to avoid the influence of ambient air condensation and other factors during the experiment, the chamber was purged with dry purified nitrogen (99.99%, 25°C).[3] Briefly, the submicroliter peptoid solution was injected into a six-hole plate filled with immersion oil. The droplet was quickly cooled to about -20 ℃(v=40℃/min) and froze. The temperature was slowly increased to the melting temperature (T_m) and then drop down to freezing temperature (T_f) slowly. When the ice crystal just began to grow, ice growth was immediately recorded by digital camera. At least five snapshots were taken during the growth process to acquire the growth rate. Ice crystal growth rate was the elongation of a single ice crystal divided by the time spent in the growth process. The elongation was measured by Image J software. The corresponding experiments were repeated at least three times for all three concentrations (1 $mg \cdot mL^{-1}$, 5 $mg \cdot mL^{-1}$, 10 $mg \cdot mL^{-1}$) of seven different samples, and the final average value of each ΔT was calculated.

1.4 Ice Recrystallization Inhibition (IRI) Experiments

In this experiment, ice recrystallization inhibition was conducted by the improved splay assay method. The apparatus equipped with a Linkam cryostage (C194) and a Nikon polarized optical microscope (LV100ND, Japan). The device was sealed to ensure a certain humidity (about 46%).[4] A glass sheet was put in the Linkam cryostage. The purified peptoid was dissolved in PBS buffer at concentrations of 1.0 $mg \cdot mL^{-1}$, 5.0 $mg \cdot mL^{-1}$ and 10.0 $mg \cdot mL^{-1}$. Droplets of peptoid solutions (10 μL) were dropped from a fixed height (h=1.5m) to the glass sheet (pre-cooled to -60 ℃) to obtain a thin solid ice film. Then the temperature was increased to -6℃ (v=5 ℃/ min), and the frozen sample was kept at -6℃ for 30 min to permit recrystallization and evaluate IRI activity. The size and morphology of ice crystals during recrystallization were observed in situ by microscope. In the next 20 min, microphotographs of the sample were recorded through a digital camera (Nikon Y-TV55, Japan). The defined grain size was obtained when the two largest orthogonal dimensions across the ice grain surface were measured. The statistical method of mean largest grain size (MLGS) was used. The grain sizes of all ice crystals were measured by Image J software. Ten ice crystals with the largest grain size were selected and their average values were taken, in order to quantitatively evaluate ice recrystallization activity. For each sample, the corresponding procedure was repeated at least three times. In general, the PBS buffer was used as the reference sample, the recrystallization inhibition effect was characterized by the percentage of the MLGS of the recrystallized sample to the standard PBS buffer.

1.5 Cytotoxicity Experiments

The cytotoxicity test (Fig. S1) is an important characterization for biological evaluation to ensure the safety of the material.[5] Herein, the Cell Counting Kit-8 (CCK-8) was employed to test the cytotoxicity of peptoid hexamers.[6] The mouse fibroblasts (L929) were seeded in 96-well plates at a density of 1～2×10^4 cells/well and then incubated in the regular medium (RPMI-1640 with 10% FBS) for 24 h. The peptoid oligomers were dissolved in the regular medium to form peptoid solutions at various concentrations of 0 (the negative control),1.0 mg·mL^{-1}, 5.0 mg·mL^{-1} and 10.0 mg·mL^{-1}. The solutions were filtered to remove bacteria through a filter. The cells were incubated in 100 μL of the above media containing peptoid oligomers for 24 h. After cleaning with PBS solution, 100 μL of the regular medium and 10 μL CCK-8 were added to each well and incubated for 1～4 h, then the Optical Density (OD) at 450 nm was measured (BioTek microplate reader, USA). The relative cell viability was calculated as follows:

$$\text{Relative cell viability (\%)} = \frac{(OD_{treated} - OD_{blank})}{(OD_{negative} - OD_{blank})} \times 100\%$$

OD_{blank} was the average OD of the blank control group, $OD_{negative}$ was the average OD of the negative control group, and $OD_{treated}$ was the average OD of the experimental group.

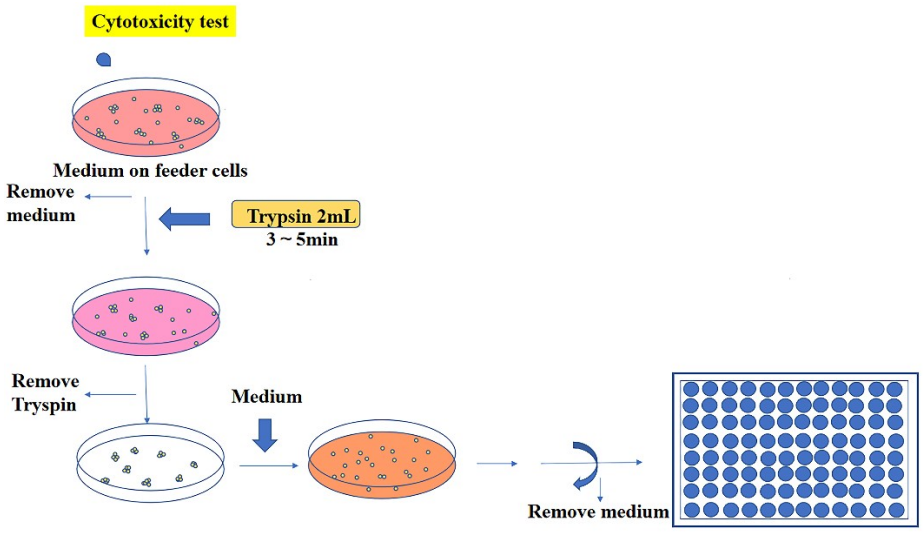
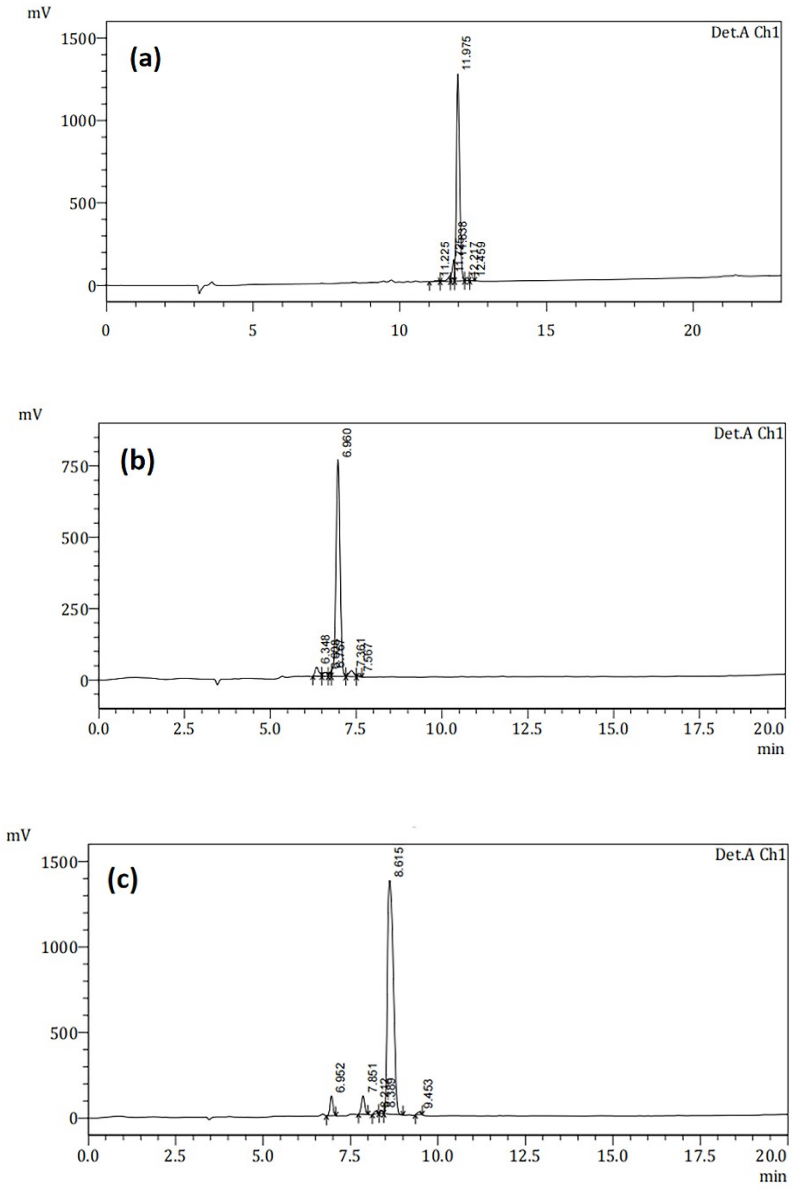


Fig. S1 The process of cytotoxicity test.

2. Supplementary data

2.1 HPLC data of purified peptoids



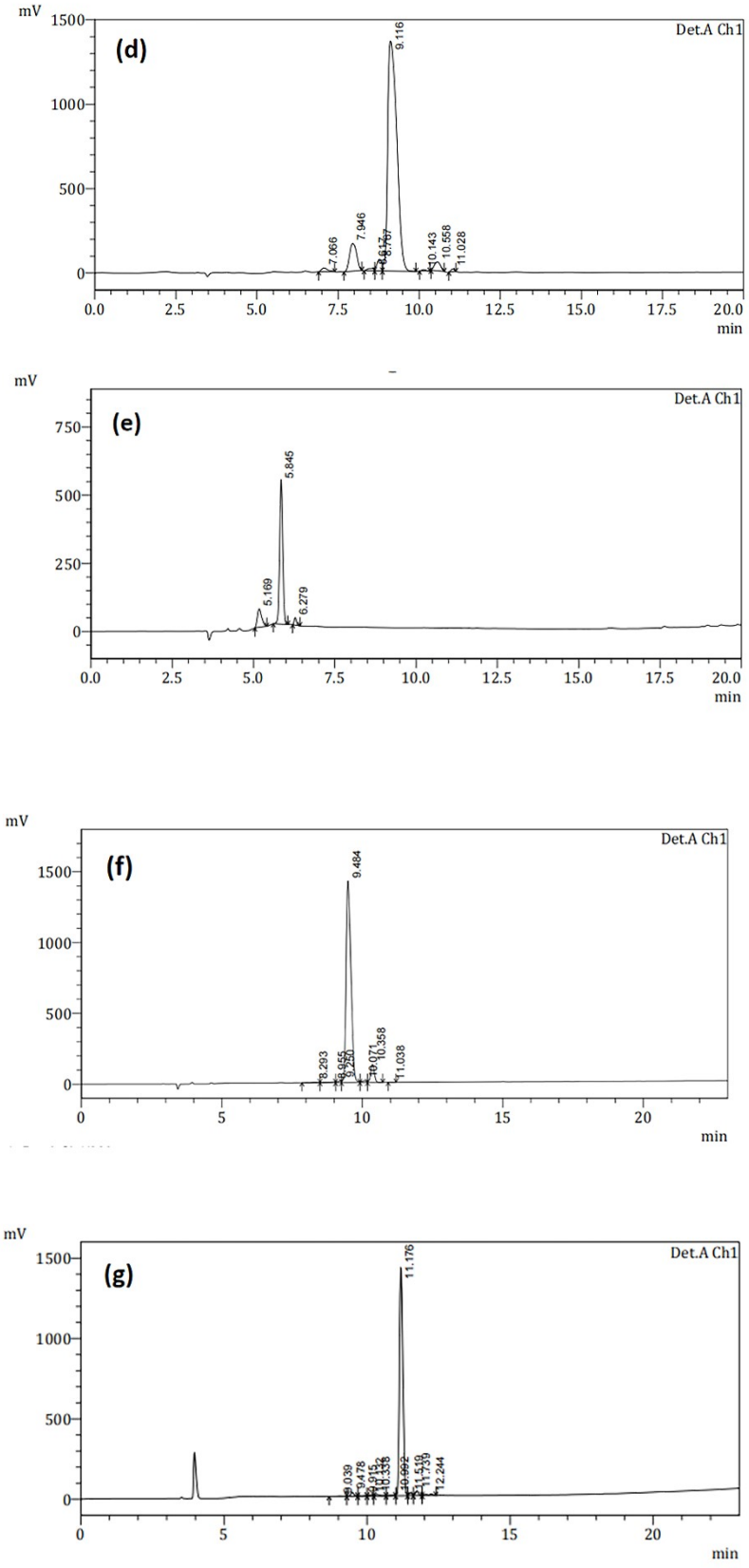
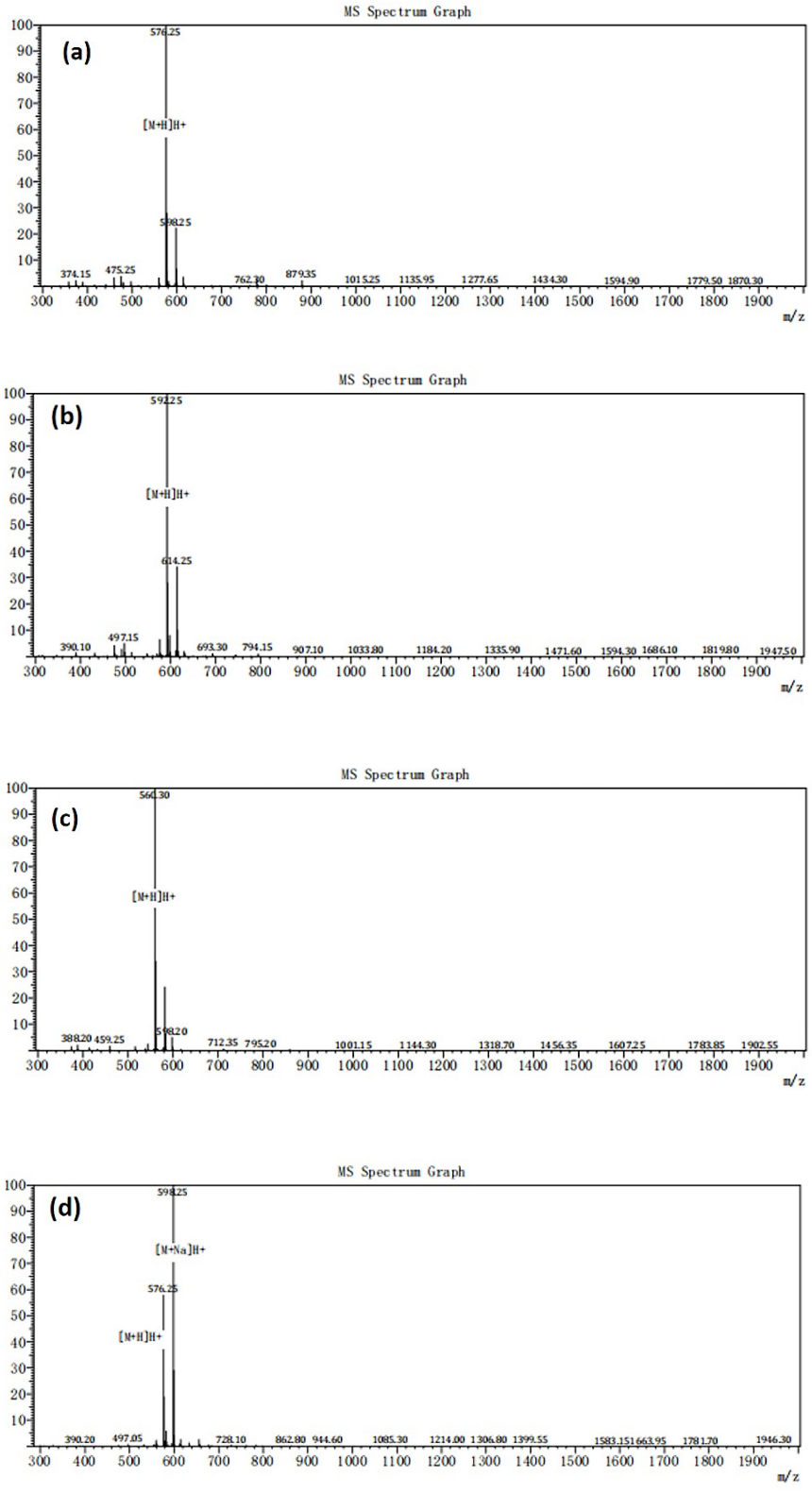


Fig. S2 HPLC traces of purified peptoids, detecting at 220 nm. (a) P-$(Net)_3$-$(Nhe)_3$; (b) P-[Net-$(Nhe)_2]_2$; (c) P-[$(Net)_2$-$Nhe]_2$; (d) P-(Net-$Nhe)_3$; (e) P-(Nme-$Nhe)_3$; (f) P-(Npr-$Nhe)_3$;(g) P-(Nbu-$Nhe)_3$.

2.2 MS date verifying the masses of the synthesized peptoids



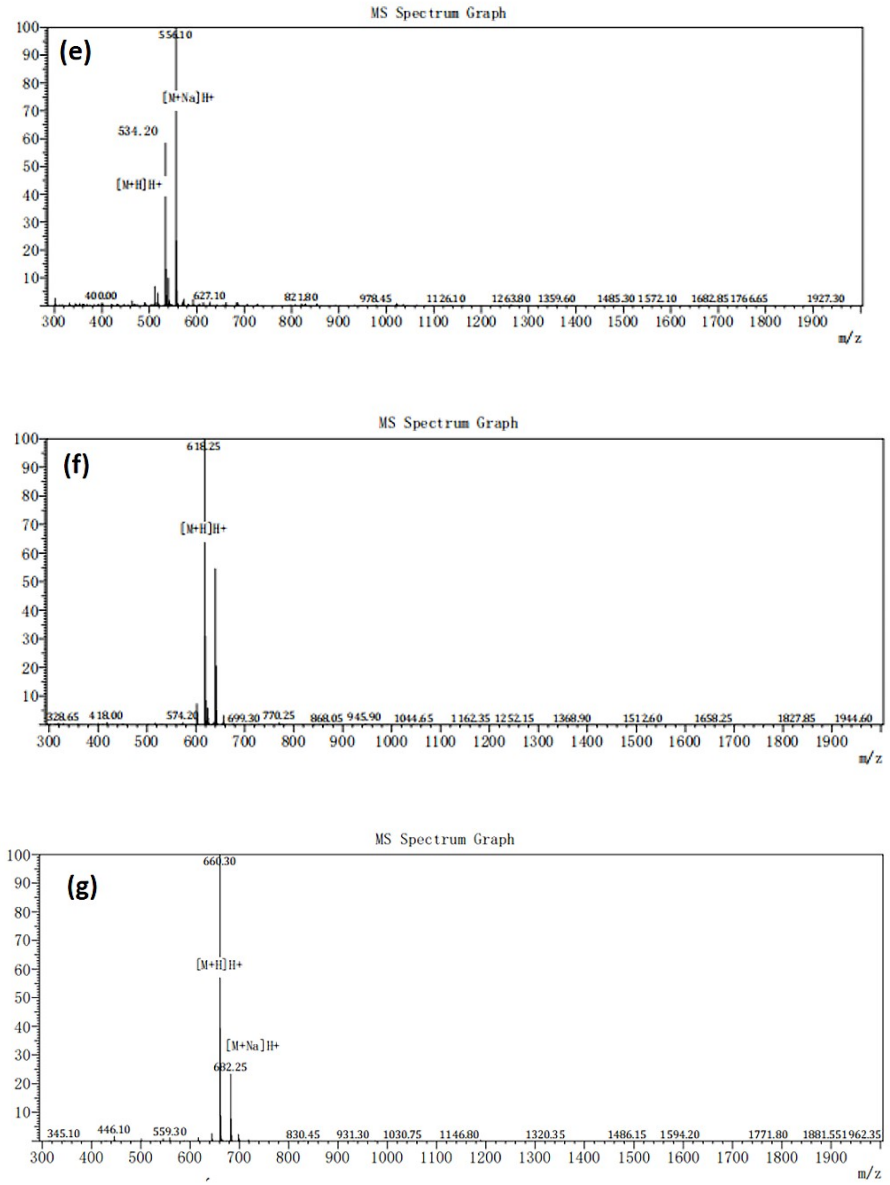


Fig. S3 Mass-spectrometry analysis for synthesized peptoids after purification. (a) P-$(Net)_3$-$(Nhe)_3$; (b) P-[Net-$(Nhe)_2$]$_2$; (c) P-[$(Net)_2$-Nhe]$_2$; (d) P-$(Net\text{-}Nhe)_3$; (e) P-$(Nme\text{-}Nhe)_3$; (f) P-$(Npr\text{-}Nhe)_3$; (g) P-$(Nbu\text{-}Nhe)_3$.

2.3 Optical images of ice crystal growth morphology

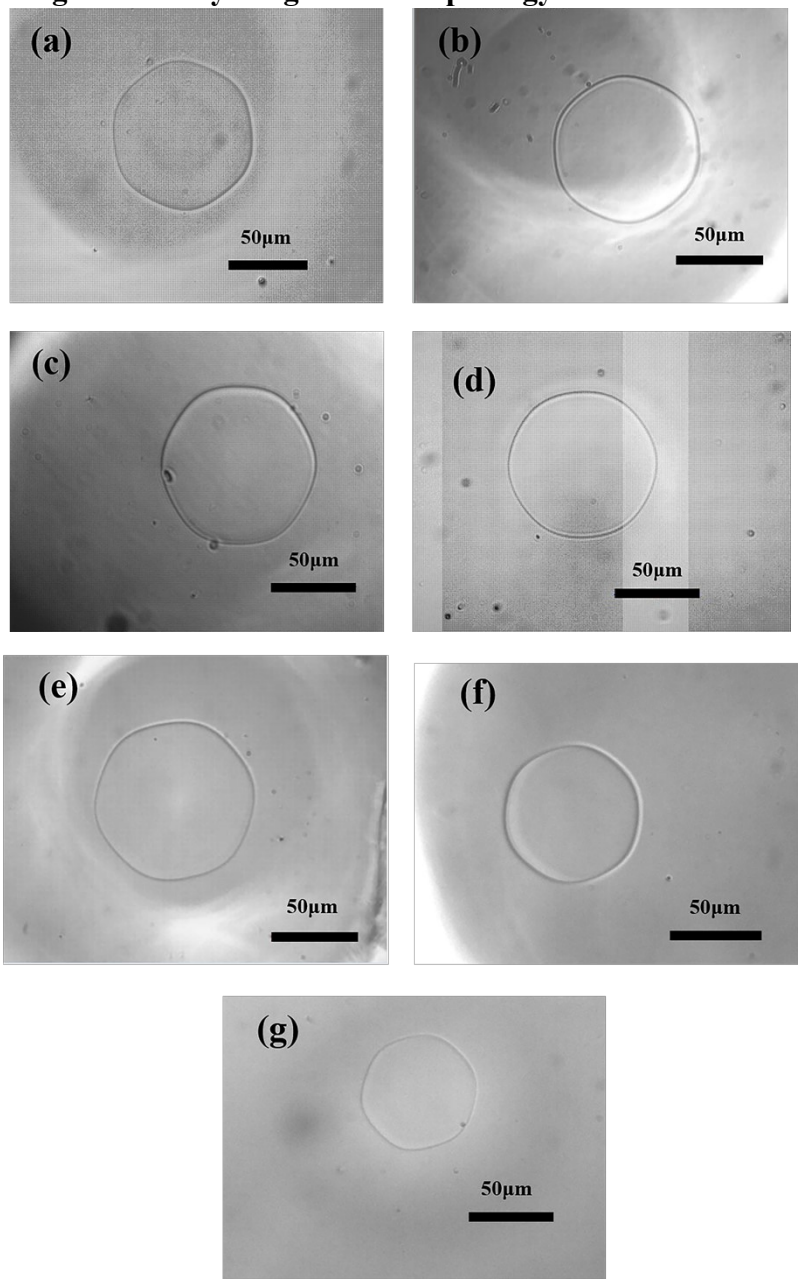


Fig. S4 Optical images of ice crystal morphology in peptoid solutions with different structures and DMSO solution at the concentration of 10.0 mg·mL^{-1} (ΔT = 0.08 ℃). (a) P-$(Net)_3$-$(Nhe)_3$; (b) P-[Net-$(Nhe)_2]_2$; (c) P-[$(Net)_2$-Nhe]$_2$; (d) P-(Net-Nhe)$_3$; (e)P-(Nme-Nhe)$_3$; (f) P-(Npr-Nhe)$_3$; (g) DMSO.

2.4 The growth rate(r) of ice crystals

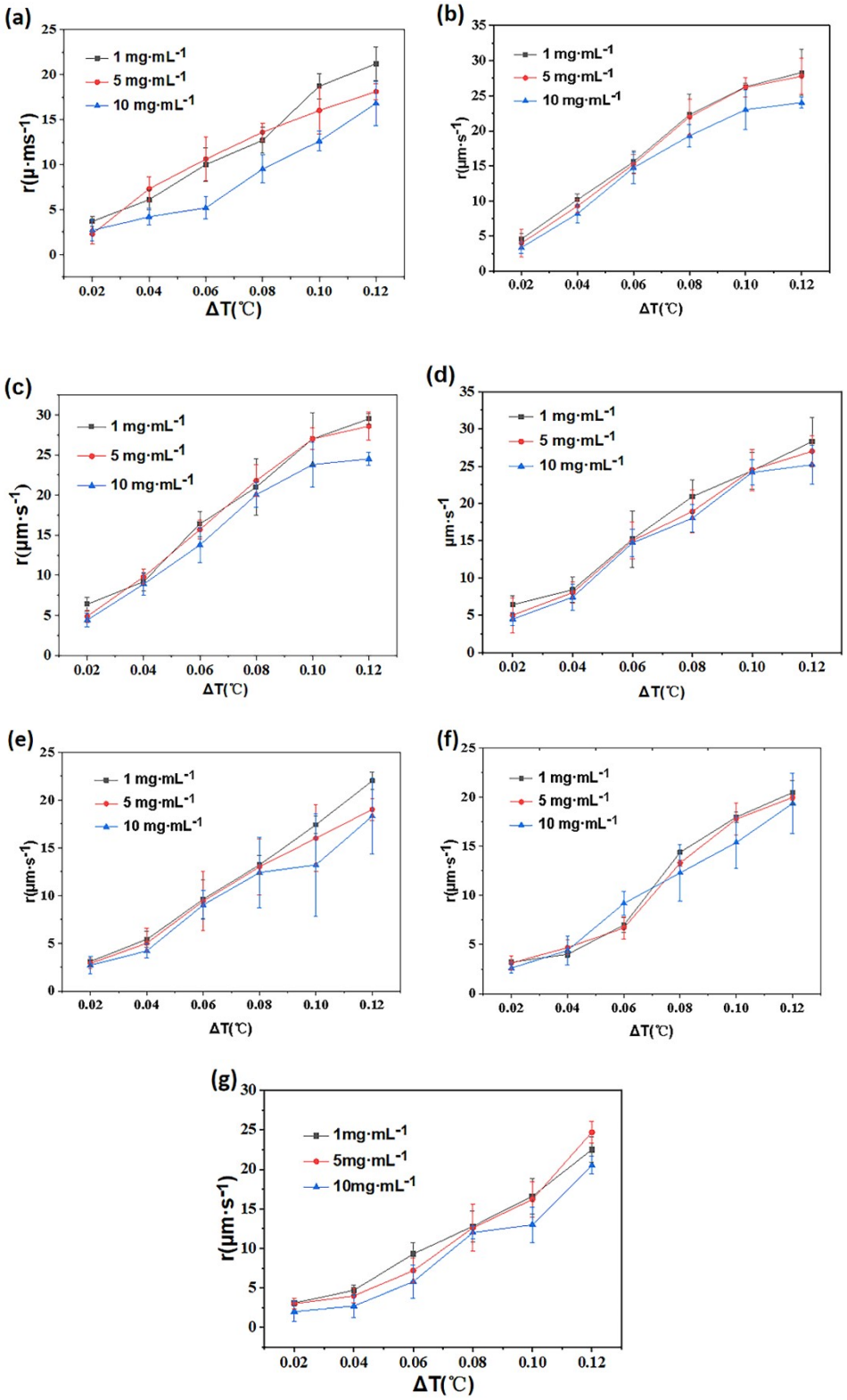


Fig. S5 The effect of peptoids at various concentrations on the growth rate(r) of ice crystals at different ΔT. (a) P-$(Net)_3$-$(Nhe)_3$; (b) P-[Net-$(Nhe)_2$]$_2$; (c) P-[$(Net)_2$-Nhe]$_2$; (d) P-$(Nme-Nhe)_3$; (e) P-$(Npr-Nhe)_3$; (f) P-$(Nbu-Nhe)_3$;(g) DMSO.

2.5 Micrographs of ice crystals

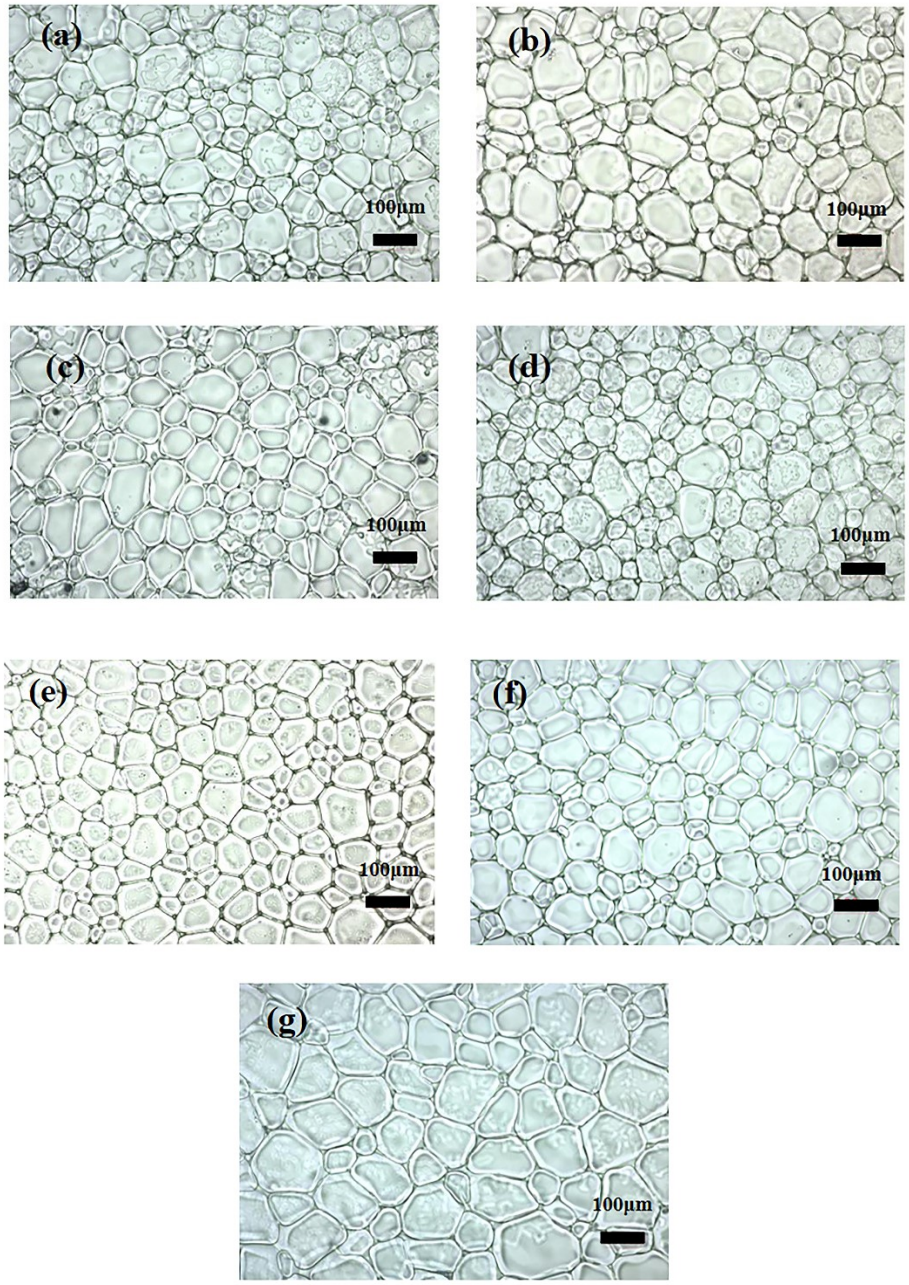


Fig. S6 Micrographs of ice crystals grown in 10 mg ·mL^{-1} solution of peptoid oligomers and DMSO solution. (a) P-$(Net)_3$-$(Nhe)_3$; (b) P-[Net-$(Nhe)_2]_2$; (c) P-[$(Net)_2$-Nhe]$_2$; (d) P-(Nme-Nhe)$_3$; (e) P-(Net-Nhe)$_3$; (f) P-(Npr-Nhe)$_3$; (g) DMSO.

2.6 Mean largest grain size percent of ice crystals

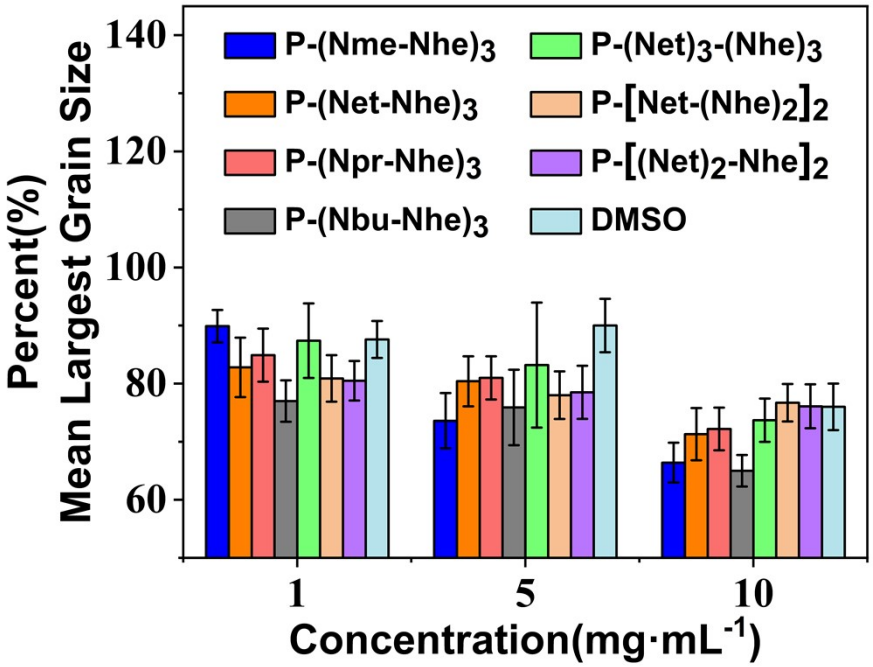


Fig. S7 Mean largest grain size (MLGS) is expressed as a percentage of PBS buffer.

2.7 The relative cell viability

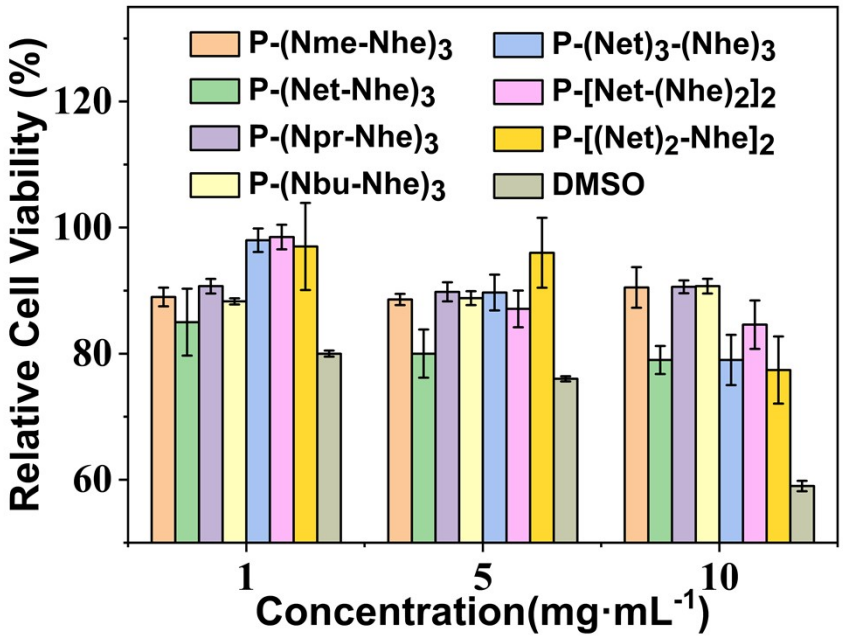


Fig. S8 The effect of DMSO and peptoid oligomers on relative cell viability.

3. References

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