

Supporting information:

Bottom-up design of efficient antifreeze peptides

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1. Supporting figures

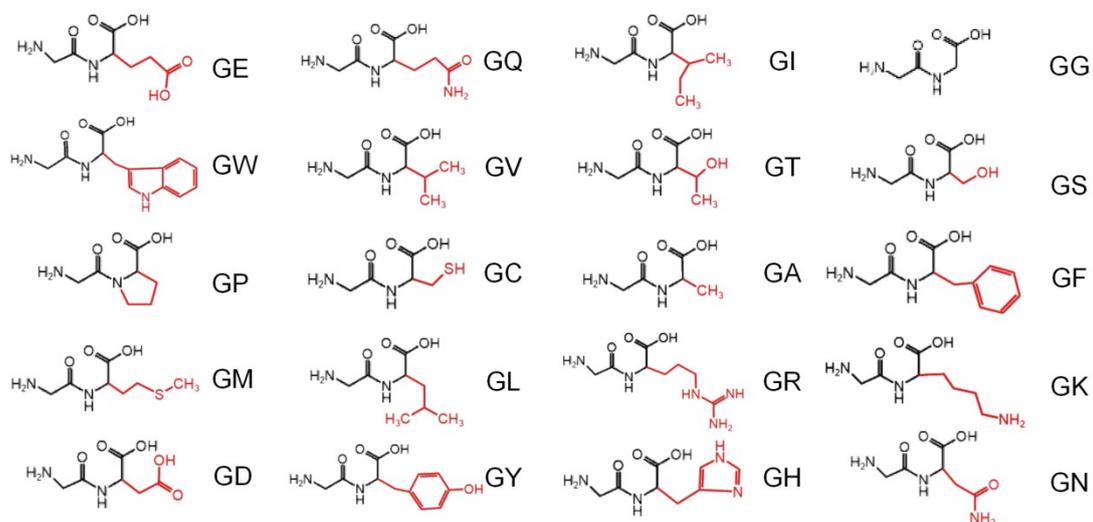


Fig S1. The structures of GX dipeptides. The amino acid residue at the N-terminal of the dipeptide is glycine, while the residue at the C-terminal is one of the 20 standard natural amino acids.

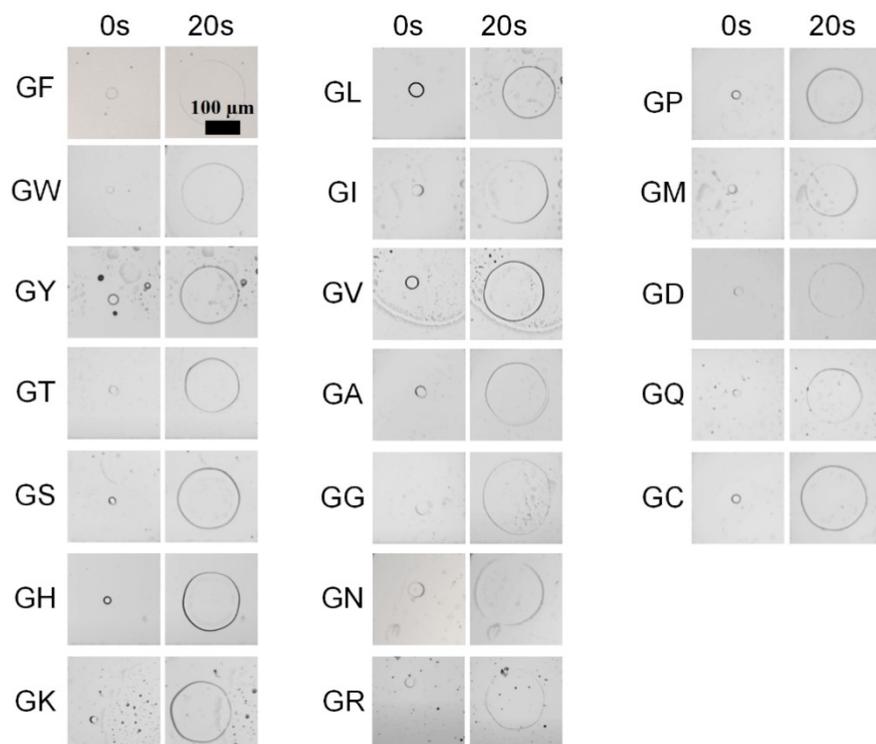


Fig S2. Growth processes of single ice crystals in dipeptide solutions, the concentration was 1%.

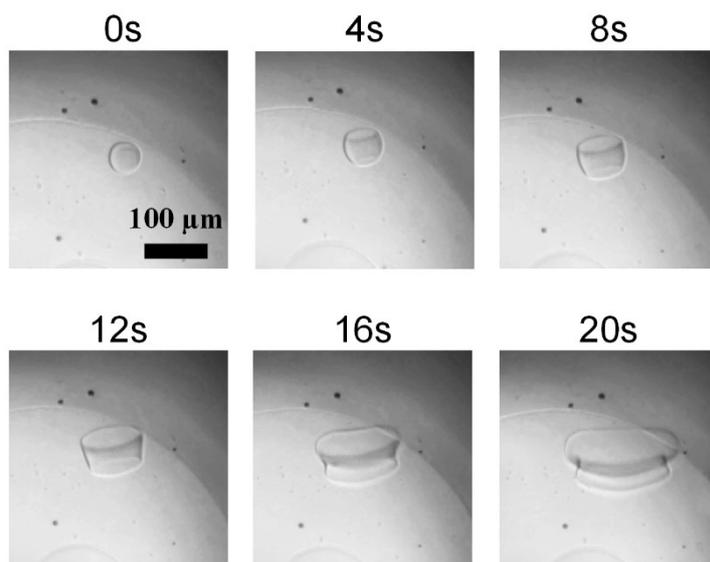


Fig S3. The image of side growth processes of single ice crystals in GE solution, the concentration was 1%.

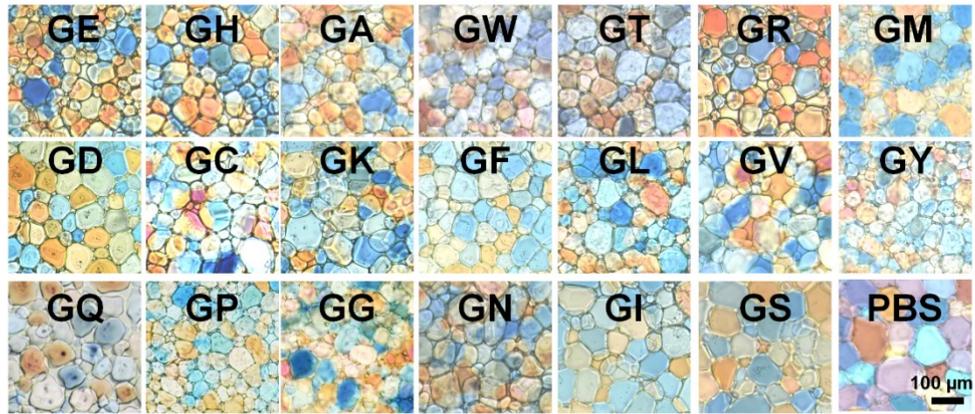


Fig S4. The cryomicrographs of ice crystals of dipeptides, the concentration was 1%.

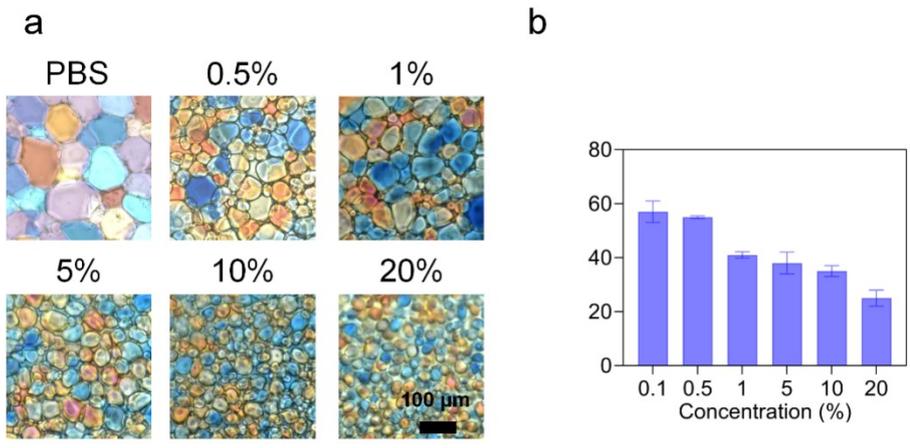


Fig S5. (a) Images of ice crystals with different GE concentrations. (b) Ice recrystallization inhibition activity of different GE concentrations.

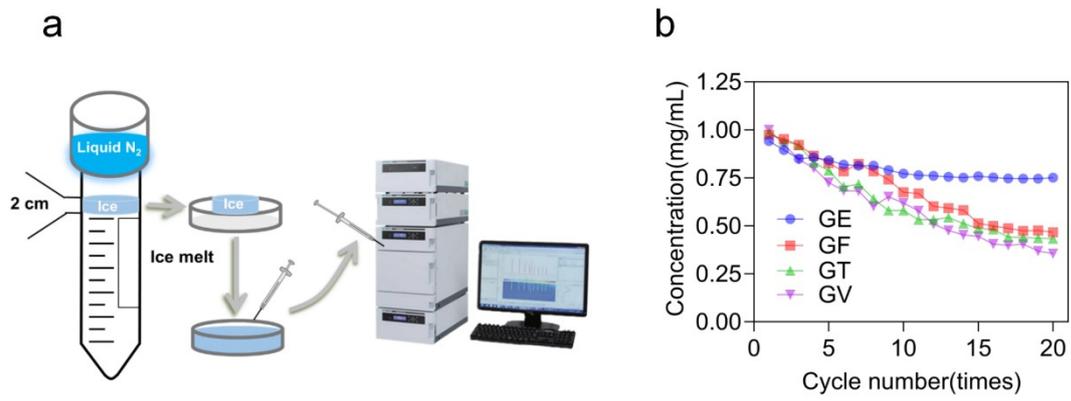


Fig S6. (a) Schematic diagram of ice affinity experiments. (b) Ice affinity tests for dipeptide solutions.

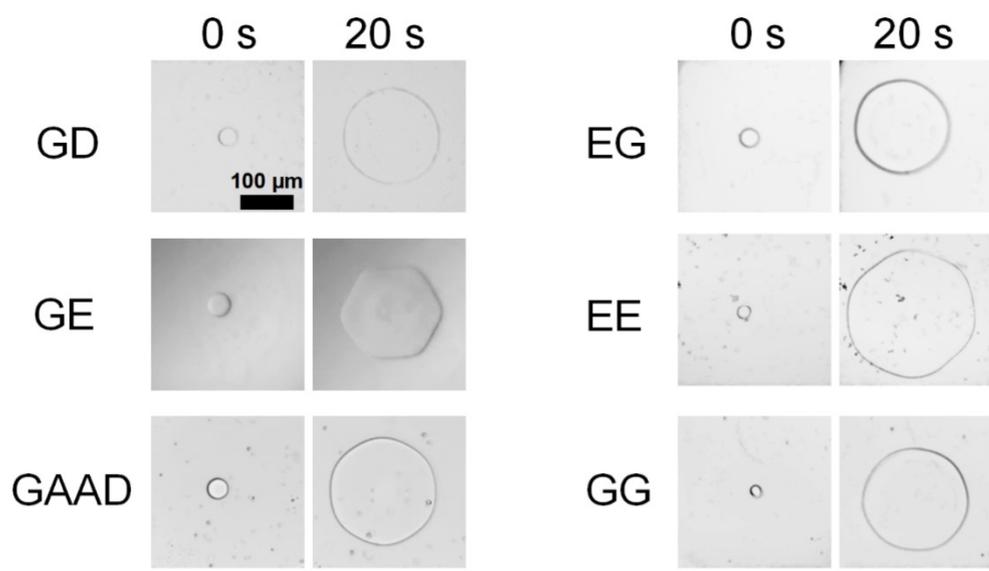


Fig S8 Growth processes of single ice crystals of GE and its analogues, the concentration was 1%.

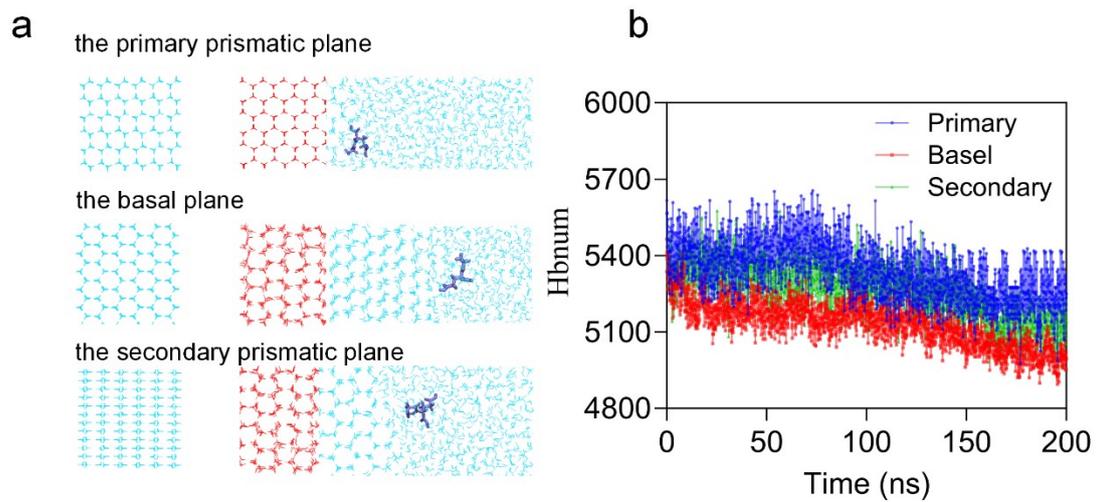


Fig S9. (a) Snapshots of the MD simulations for ice growing, the left side shows the base plane, the first prismatic plane and the secondary prismatic plane in contact with the water molecule, respectively. (b) The Hbnum values during simulations in GE solution.

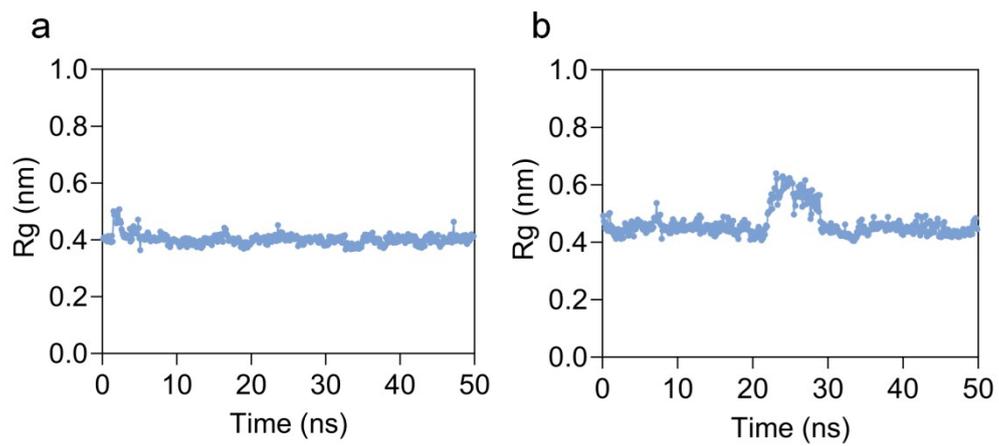


Fig S10. Rg of (GE)₂ and (GE)₃ (a) (GE)₂ (b) (GE)₃.

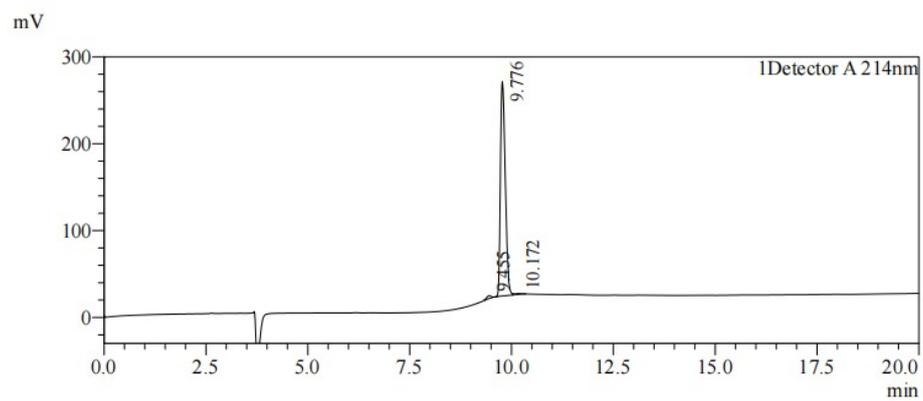


Fig S11. HPLC of $(GE)_2$

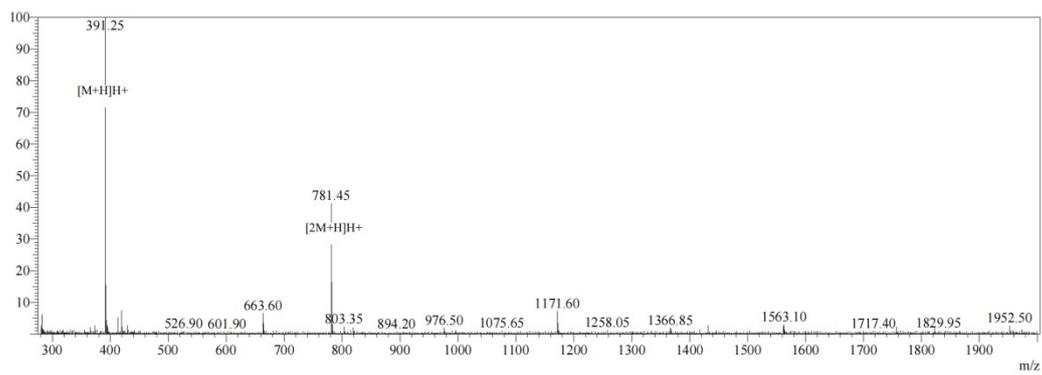


Fig S12 MS of $(GE)_2$

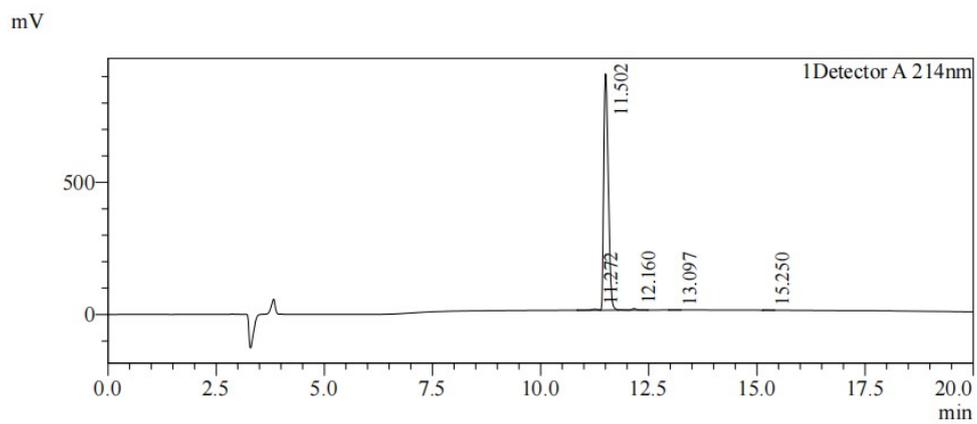


Fig S13. HPLC of $(GE)_3$

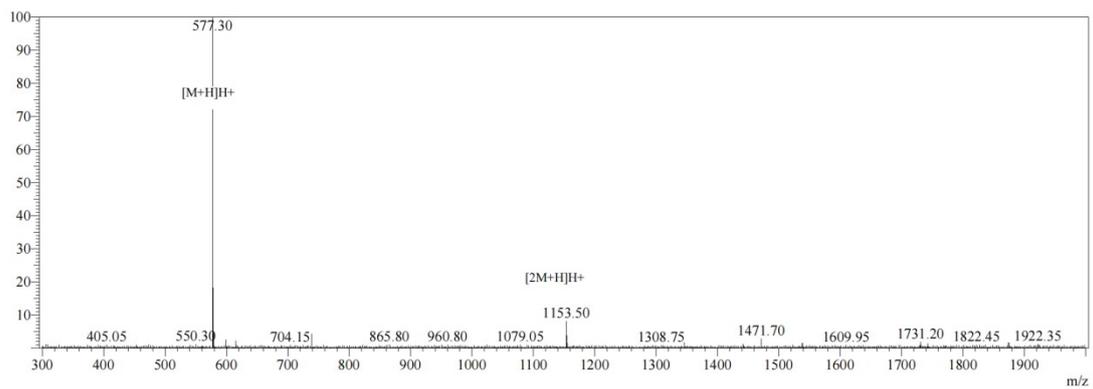


Fig S14. MS of $(GE)_3$

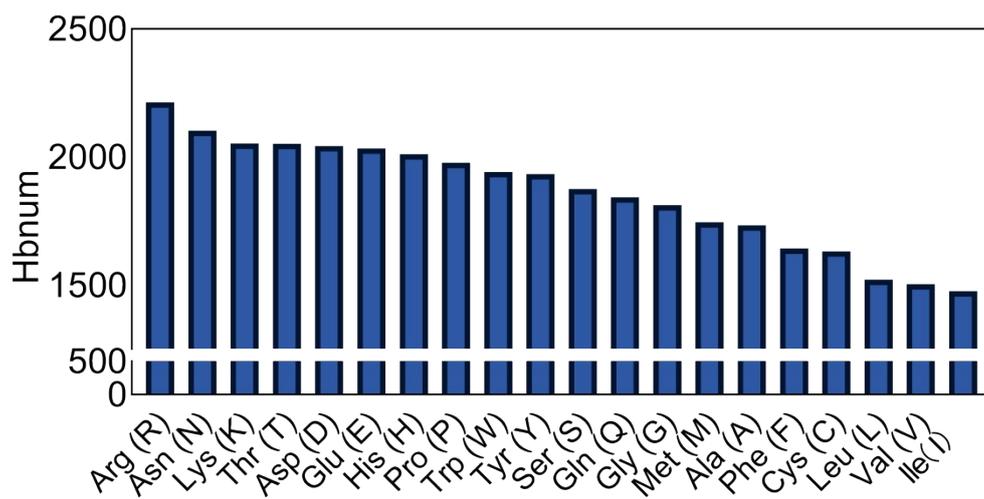


Fig S15. Hbond number in water environment of 20 types of natural amino acid.

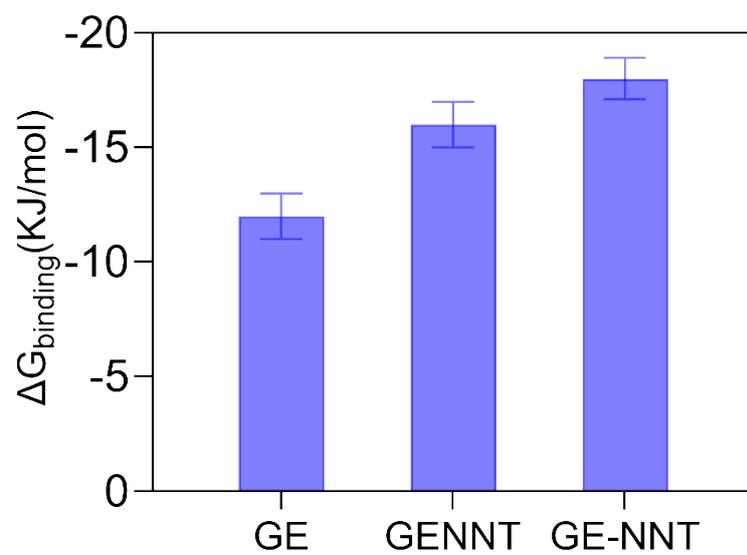


Fig S16. $\Delta G_{\text{binding}}$ of GE, GENNT and GE-NNT with ice. All-atom MD simulations using the CHARMM36 force field and TIP4P/Ice water model under controlled supercooled conditions (250 K, 1 atm).

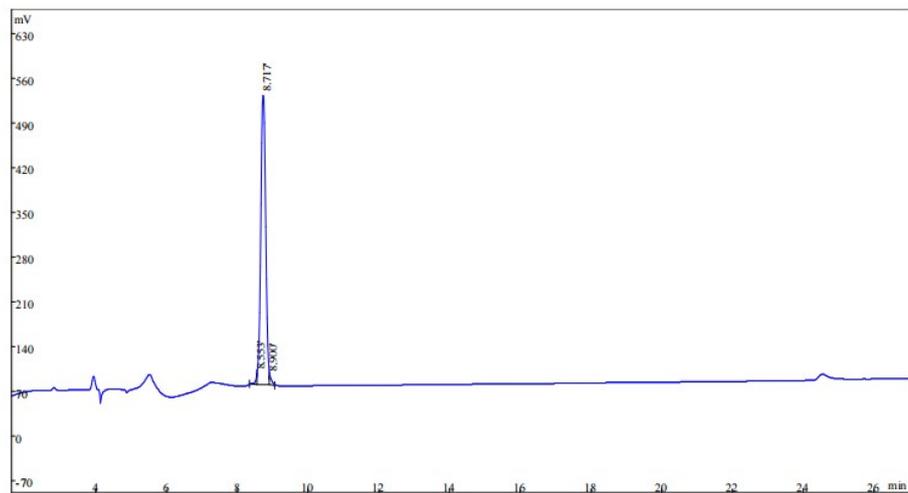


Fig S17. HPLC of GE-NNT

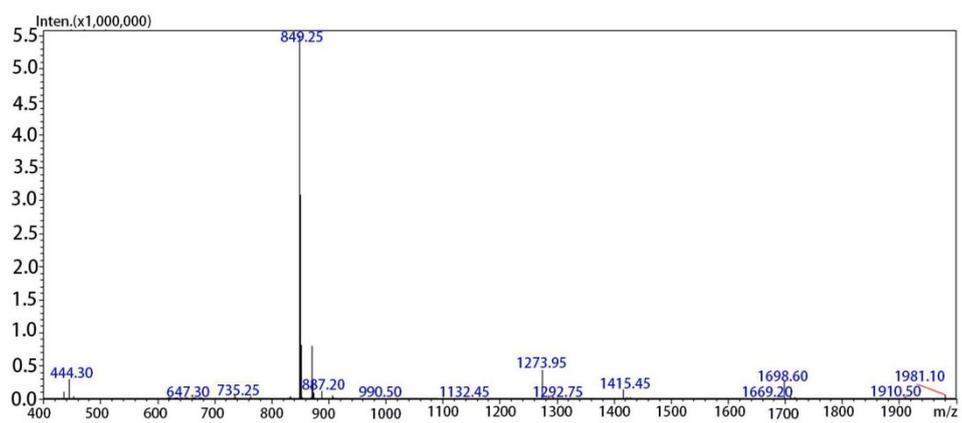


Fig S18. MS of GE-NNT

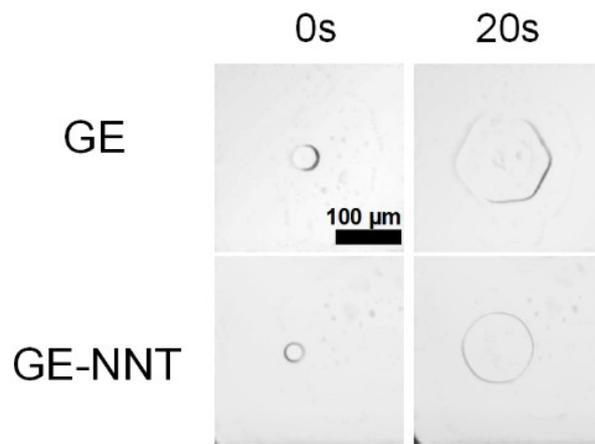


Fig S19. Growth processes of single ice crystals in GE and GE-NNT, the concentration was 1%.

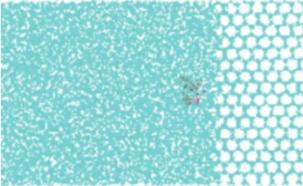
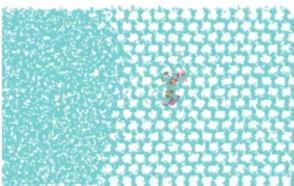
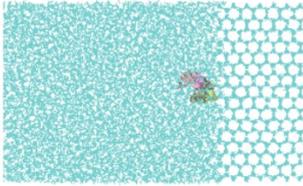
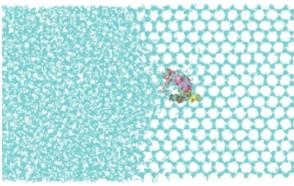
	0 ns	200 ns
GE		
GE-NNT		

Fig S20. Simulation of ice water environment of GE and GE-NNT

Table S1. H-bond number of 120 types of tripeptides

Tripeptide	Number	Tripeptide	Number	Tripeptide	Number
NNT	3985	ENR	3627	ERT	3142
REK	3972	RNK	3624	NKE	3137
EEK	3947	TER	3624	TKE	3135
NNE	3940	KKR	3615	REN	3133
NRK	3933	KTK	3614	ENT	3129
NRE	3930	RRN	3594	NET	3129
RER	3929	RRK	3571	EKN	3120
TTT	3924	ERK	3561	NTR	3119
RRR	3916	KTT	3533	KKT	3118
KNE	3915	NKN	3528	KNT	3118
REE	3897	KEN	3523	EKE	3108
EEE	3891	NEK	3522	RTR	3100
KNR	3890	NKT	3481	ETE	3096
KRT	3876	KEE	3473	EET	3073
RNR	3864	KRN	3472	RKN	3052
RTK	3862	TRK	3457	TTE	3039
TET	3858	TRT	3448	TNT	3031
ERR	3849	TNE	3437	ERE	3029
KRR	3847	TEE	3409	TTR	3025
NKK	3845	NEN	3399	ENK	3006
ENE	3832	RET	3397	RNT	3000
KER	3828	RRT	3386	TEN	2979
KRK	3828	KTE	3384	NRT	2975
TRE	3816	ENN	3384	NNR	2974
ETR	3810	KTR	3354	NTE	2955
TRN	3792	RKR	3339	KKK	2950
KKN	3772	RNN	3336	TTK	2938
TRR	3771	TKN	3318	EKR	2914
TKE	3766	NNK	3308	NKR	2894
EER	3766	RKT	3299	TNK	2869
RTE	3756	EKT	3285	KEK	2861
ETT	3753	TKR	3277	TEK	2857
TKK	3748	NRN	3260	TTN	2842
NTK	3739	ERN	3236	KTN	2826
TNN	3728	ETK	3221	KRE	2818
KKE	3724	RTN	3208	NTT	2816
NRR	3696	KNN	3203	RRE	2793
ETN	3685	RTT	3176	KET	2753
EKK	3647	RNE	3170	NER	2744
RKE	3637	NEE	3167	KNK	2721
RKK	3635	NTN	3163	TNR	2715
NNN	3633	EEN	3152		

