Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Electronic Supplementary Information

Do Antifreeze Proteins Generally Possess the Potential to Promote Ice Growth?

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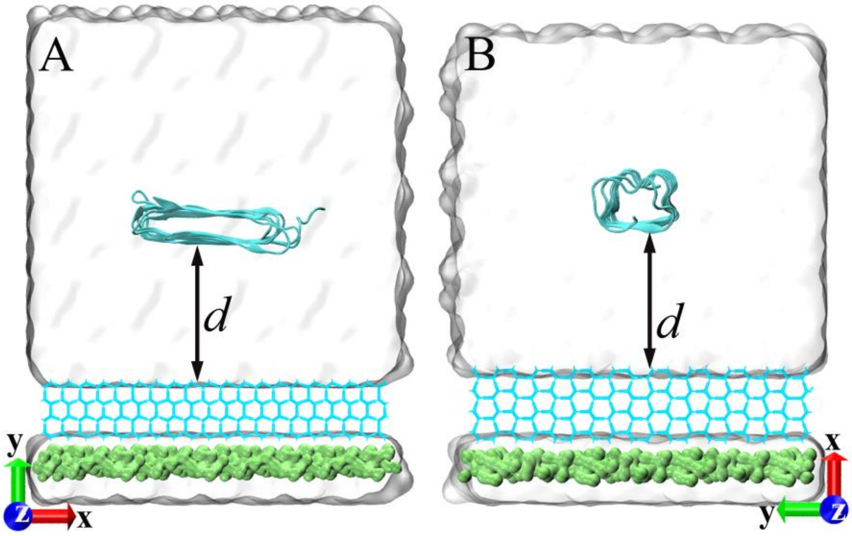
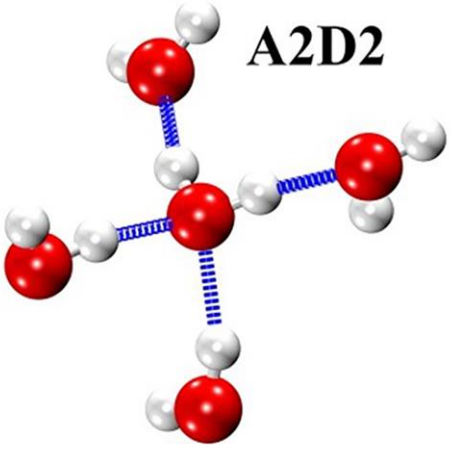


Scheme S1. Molecular models built in this study. *d* is the distance between the IBS and ice crystals. (A) AFPs in Group 1 are placed on the basal surface of ice (*d* =1.0 nm for assemblies 3−5, *d* =2.5 nm for assemblies 14−16). (B) AFPs in Group 2 are placed on the prismatic surface of ice (*d* =1.0 nm for assemblies 9−11, *d* =2.5 nm for assemblies 17−19). Water molecules in the green region below the ice crystals were restrained in a 0.5 nm thick disordered layer. Protein is shown by NewCartoon. Water is shown by QuickSurf in white, and ice is shown by both HBonds and Lines in light blue.



Scheme S2. Diagram of a water molecule with hydrogen bond structure of A2D2.

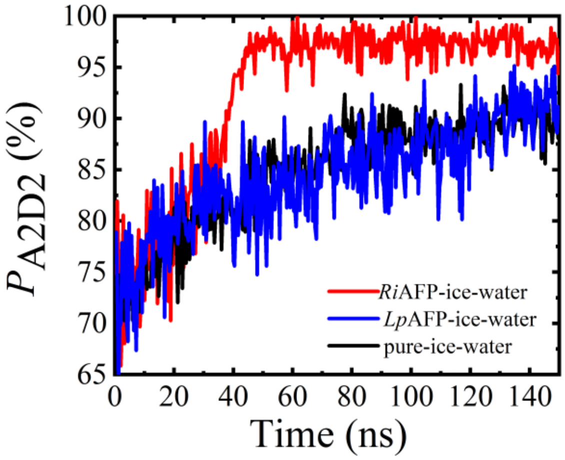


Fig. S1 Time evolution of P_{A2D2} in the designated regions. The distance between the IBS of AFP and the basal plane of ice is 2.6 nm.

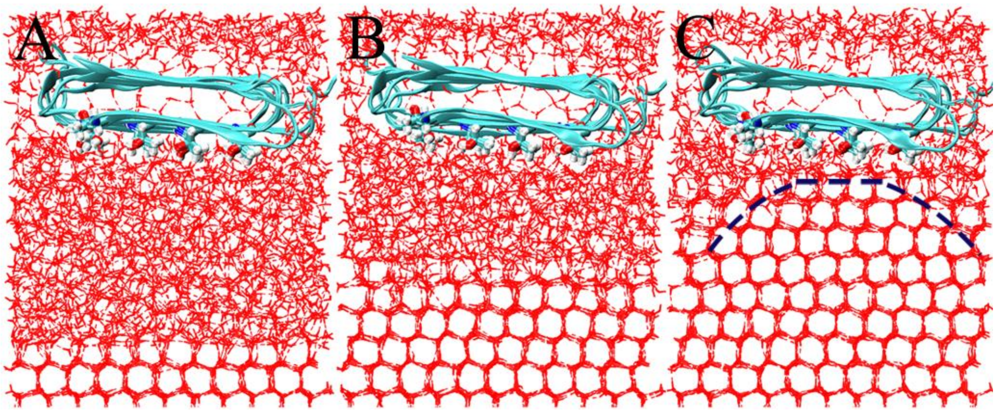


Fig. S2 (A, B, C) Snapshot structures of local growth of ice on the basal plane promoted by *Ri*AFP obtained from the MD trajectory, at 0, 22 and 46 ns, respectively.

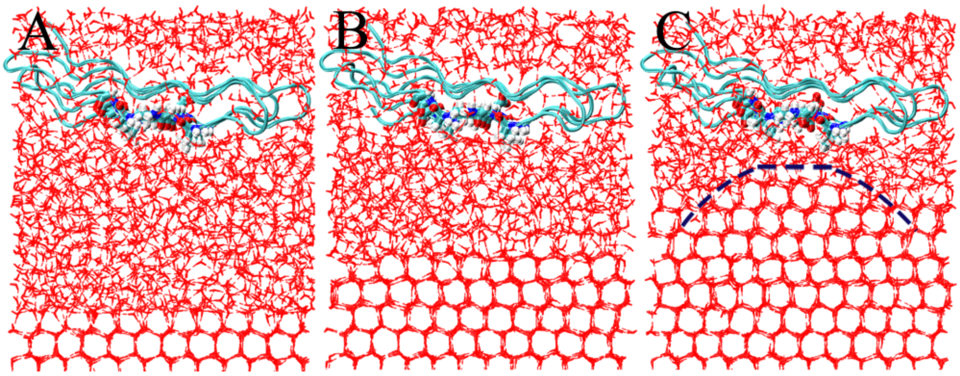


Fig. S3 (A, B, C) Snapshot structures of local growth of ice on the basal plane promoted by sfAFP obtained from the MD trajectory, at 0, 21 and 50 ns, respectively.

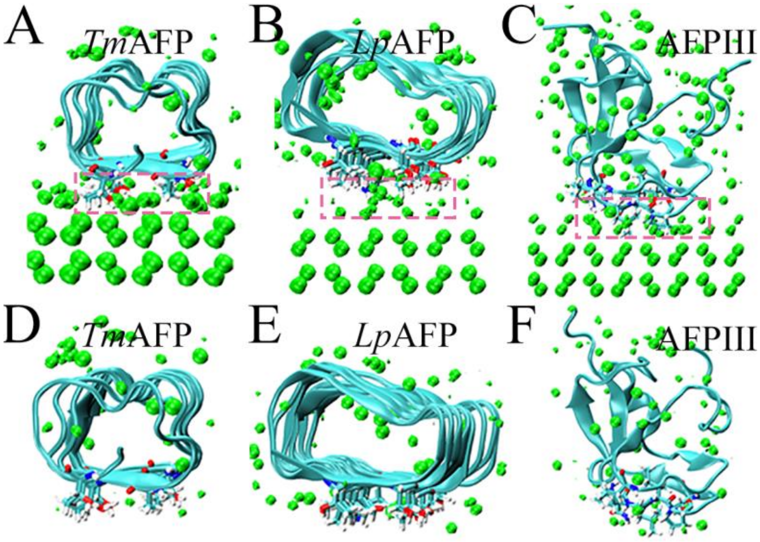


Fig. S4 Anchored clathrate water. (A, B, C) AFPs on the prismatic plane of ice. (D, E, F) AFPs in water. The pink frame is the binding area. The last 50 ns trajectory of each protein assembly is used for analysis.

Hydration water molecules of IBS

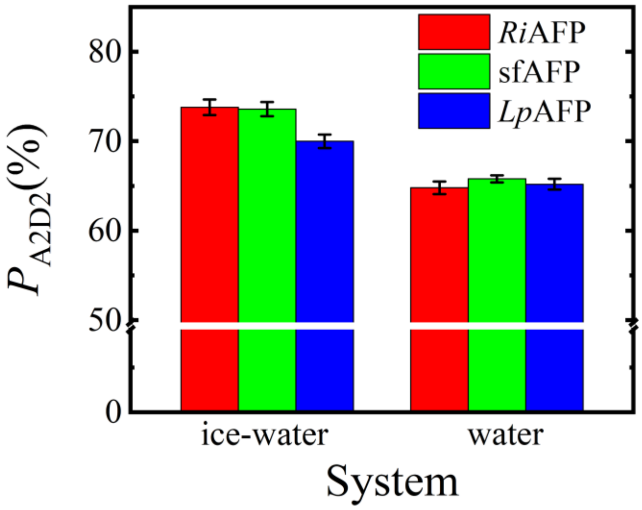


Fig. S5 Average value of P_{A2D2} of water molecules within 0.35 nm below the IBS of Group 1 (on basal plane), over the last 50 ns simulation trajectories (assemblies 14−16, 6−8).

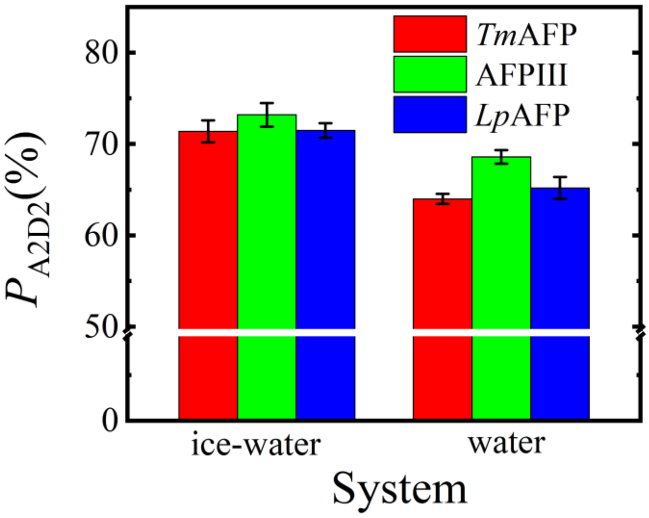


Fig. S6 Average value of P_{A2D2} of water molecules within 0.35 nm below the IBS of Group 2 (on prismatic plane), over the last 50 ns simulation trajectories (assemblies 17−19, 8, 12 and 13).

Thickness of interfacial water layer

First, we selected nine frames (structural snapshots) from a single run of the 0-30 ns MD trajectory of the growth on each ice plane. Display each structure using the OVITO software,[1] and identify water molecules in hexagonal and cubic ice with CHILL+ algorithm,[2] then manually determine the latest layer of grown ice, and then use OVITO to give the position of this layer of ice (see Figs. S7A and S8A), which is the position of the first vertical line of the corresponding density profile (see Figs. S7B and S8B).

The density profile of water molecules for each structure is calculated by the g_density tool in GROMACS package.[3] After the first vertical line, the density peak gradually decays (see Figs. S7B and S8B). However, it is difficult to accurately determine the boundary between the interfacial water and bulk water with the density curve due to its large fluctuation. To address this issue, we calculated the standard deviation (*S*) of the density in a small window (window width is set to 0.4 nm, approximate the thickness of a layer of ice), then move the window step by step along the abscissa to get the fluctuation of *S* along the X- and Y-coordinate as shown in Figs. S7-S10. When *S* sharply decays to a plateau, it can be considered that the density of water in the plateau area is the density of bulk water, and the starting position of the plateau can, therefore, be used as the boundary between the interfacial water and bulk water.

The average *S* over the flat areas, which can be regarded as bulk water, is estimated to be around 110 kg/m^3, corresponding to the pink dotted line in each panel. Therefore, when *S* in each panel decays to 110 kg/m^3, the corresponding position is considered as the boundary between interface water and bulk water. This position is the position of the second vertical line in the corresponding density profile, and the distance between the two vertical lines is the thickness of the interfacial water, as shown in Figs. S7-S10. Based on these results, the average thickness of the ice/water interface of prismatic and basal surfaces is calculated to be 1.1±0.1 nm and 1.7±0.2 nm, respectively.

Note that the aforementioned results of each ice plane were obtained from a single run. Theoretically, it is possible that the details of the process of crystallization may be a bit different in independent runs, due to its randomness. More accurate results may require a large number of parallel runs. Here, our main conclusion is that the thickness of the ice/water interface of the basal plane is thicker than that of the prismatic plane, which is also agreement with the study of Todde et al.[4]

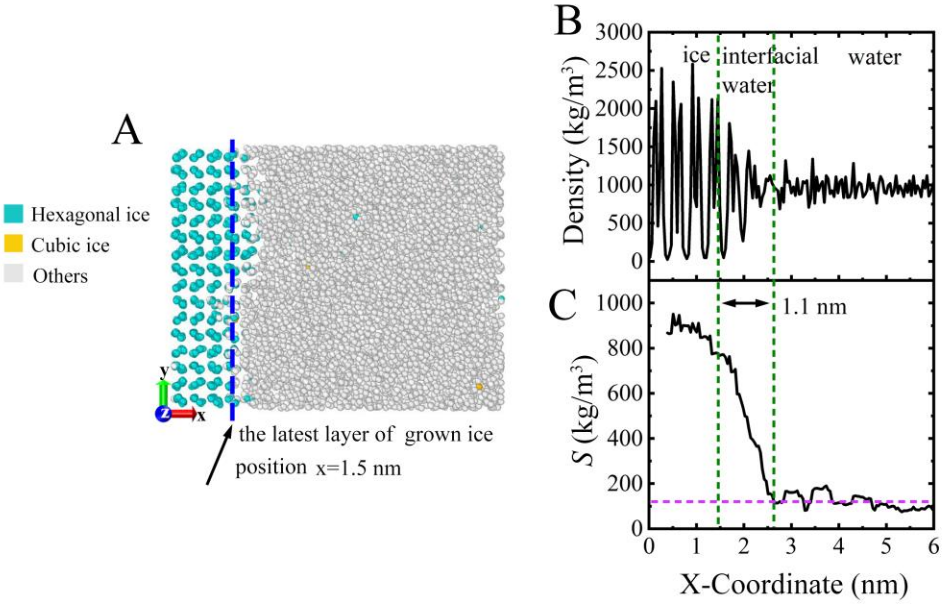


Fig. S7 (A) Water molecules with ice structure on the prismatic plane at 8 ns identified by CHILL+ algorithm,[2] displayed with OVITO.[1] (B) Density of water molecules changes with the X-coordinate. (C) Standard deviation (*S*) along the X-coordinate (perpendicular to the prismatic plane of ice).

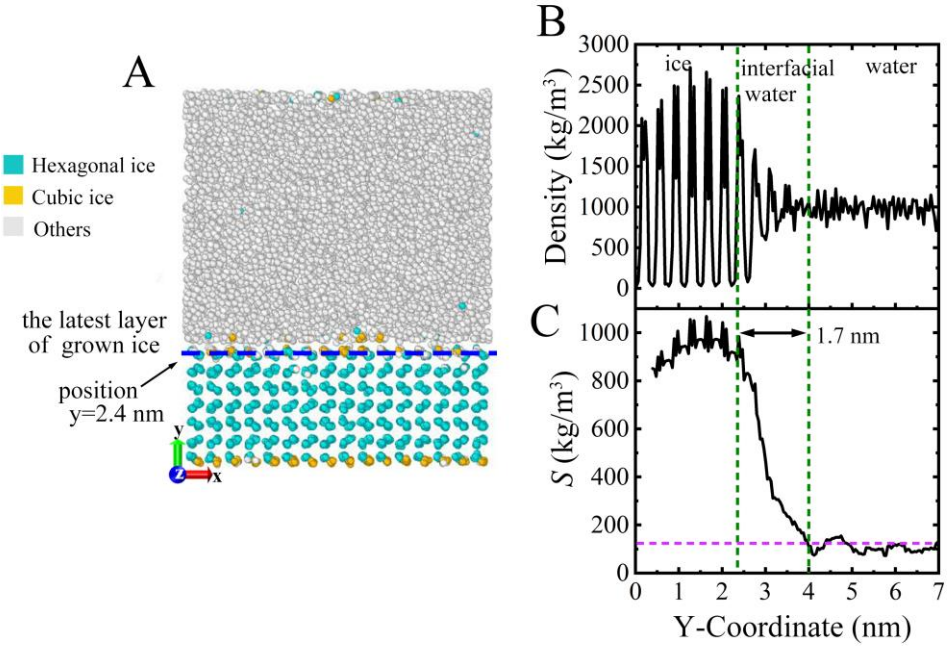


Fig. S8 (A) Water molecules with ice structure on the basal plane at 15 ns identified by CHILL+ algorithm,[2] displayed with OVITO.[1] (B) Density of water molecules changes with the Y-coordinate. (C) Standard deviation (*S*) along the Y-coordinate (perpendicular to the basal plane of ice).

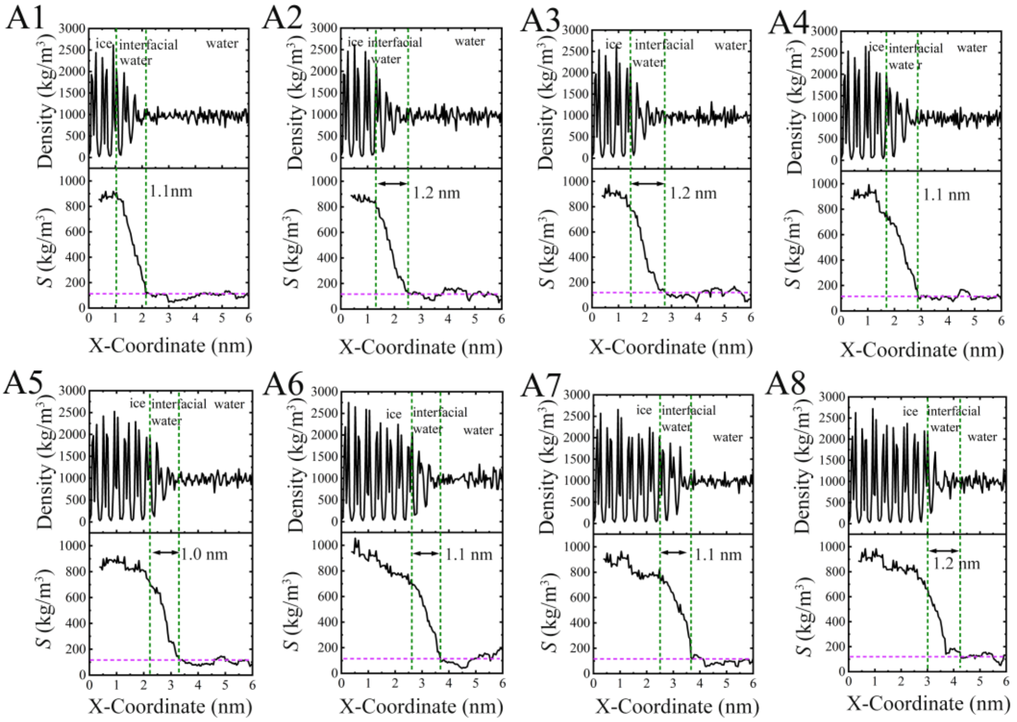


Fig. S9 (A1-A8) Density of water molecules and its standard deviation (*S*) along the X-coordinate (perpendicular to the prismatic plane of ice), at 3 ns, 5 ns, 7 ns, 12 ns, 16 ns, 20 ns, 24 ns and 28 ns, respectively.

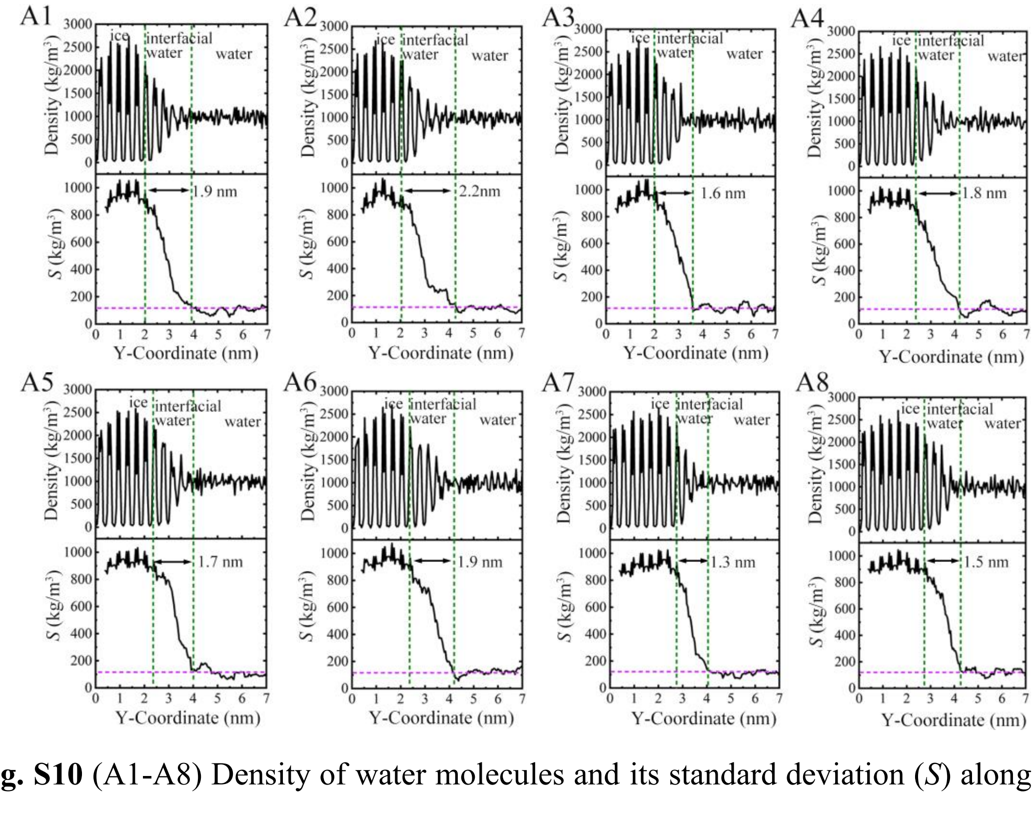


Fig. S10 (A1-A8) Density of water molecules and its standard deviation (*S*) along the

Y-coordinate (perpendicular to the basal plane of ice), at 4 ns, 7 ns, 10 ns, 18 ns, 21 ns, 24 ns, 27 ns and 29 ns, respectively.

Hydrogen bond lifetime

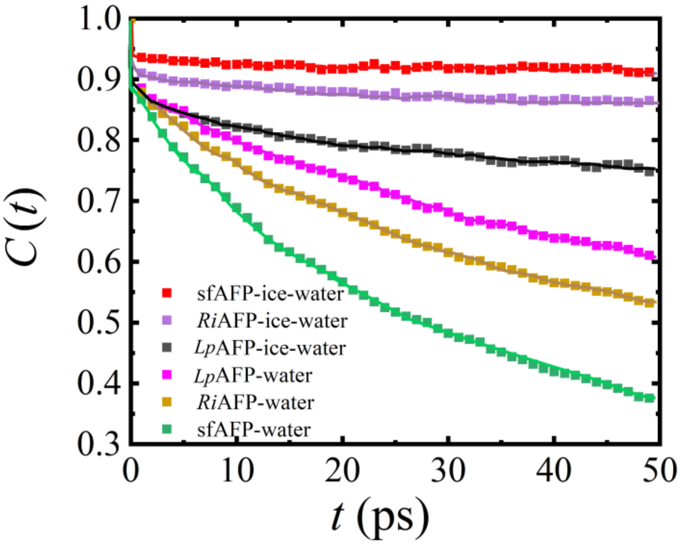


Fig. S11 Hydrogen bond lifetime correlation functions between water molecules and residues at the IBS of Group 1, when they are adsorbed on basal plane of ice crystals (assemblies 14−16) and in aqueous solution (assemblies 6−8).

To investigate why the ACW can stably exist, the hydrogen bond lifetime correlation functions $C(t)^5$ between the residues at the IBS and all water molecules when Group 1 were adsorbed on ice crystals (assemblies 14−16) and in aqueous solution (assemblies 6−8) was computed. It can reflect the time that the hydrogen bond can be maintained and is defined as follows

$$C(t)=\frac{<h(t)h(0)>}{<h(0)^2>}.$$

When there is a hydrogen bond between the residues at the IBS and all water molecules, $h(t)$ is 1; when it is absent, $h(t)$ is 0. The slower the decay rate of $C(t)$, the longer the hydrogen bond lifetime. The hydrogen bond lifetime is longer when binding to ice than that in water assemblies, which demonstrates that AFPs and ice crystals together stabilize the ACW through hydrogen bonds. Furthermore, a clear trend of retardation of the hydrogen bond dynamics in *Lp*AFP−ice−water assembly, indicating that the stability of hydrogen bond was poorer than that in *Ri*AFP/sfAFP−ice−water assembly, and the formation of hydrogen bonds relies on the regular sequence. The IBS of *Lp*AFP is considerably less repetitive than the hypAFPs.

Table S1 Mean residence times of CW.

Group	Ice plane	AFP	Residence time (ps, $d_{\mathrm{ice}} > 1.4$ nm)	Residence time (ps, $d_{\mathrm{ice}} = 1.4$ nm)	Residence time (ps, $d_{\mathrm{ice}} < 1.4$ nm)
1	basal plane	*Ri*AFP	116.2	134.3	257.9
		*sf*AFP	130.9	153.8	194.5
		*Lp*AFP	59.6	68.0	69.2
2	prismatic plane	*Tm*AFP	74.3	/	125.5
		*Lp*AFP	77.8	/	102.6
		AFPIII	/	/	/

HypAFPs were adsorbed on the basal surface, and the results indicate that when $d_{\mathrm{ice}} >$ 1.4 nm, CW molecules exchange frequently with the surrounding water molecules. When $d_{\mathrm{ice}} = 1.4$ nm, the residence time of CW becomes longer. When $d_{\mathrm{ice}} < 1.4$ nm, CW molecules are stable, which can also be observed from the MD trajectory. The stability of CW molecules inevitably leads to the stable existence of ACW. However, as for *Lp*AFP binding to the basal surface and the AFPs in Group 2 binding to the prismatic surface, the results indicate that CW molecules do not stably exist, suggesting ACW is unstable.

References

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