**Supplementary Information**

**Figure S1 – Model of PbINP prior to solvated MD simulation.**  
A) The tetra-peptide xxxS and GYGS loops were manually built to connect the TQTA and SLTA β-strands of the altered β-roll from alkaline protease.  
B) The constructed 16-aa loop was duplicated, changed to the appropriate PbINP residues, and then aligned to the β-strands of the ensuing loop. This was done eight times in total, producing a β-helical structure of PbINP residues 217-345.
Figure S2 – RMSD plot of the 5-ns MD simulation and Ramachandran plot of averaged structure.  

A) The C\textsubscript{α} RMSD (nm) of the protein was plotted as a function of time (ns).  

B) Ramachandran plot of the energy-minimized average structure from the final 3 ns of the 5-ns MD simulation. All residues are represented as black squares, except glycine, which is represented by black triangles.
**Figure S3 – Stereo view of water-bridged glutamine ladder.** A glycine substitution typically interrupts the glutamine ladder every third loop in the model. This produces a void in the structure that is filled by a water molecule that bridges adjacent glutamine residues. Hydrogen bonds made by the water are shown by dashed lines.
Figure S4 – RMSD plots of the PbINP monomer and dimer simulated at 310 K for 10 ns. The C$_{\alpha}$ RMSD (nm) of both the monomer (black) and dimer (green) were plotted as a function of time (ns). The dimer remained stable throughout the entire trajectory, while the C terminus of the monomer began to unravel at the ca. 4-ns point.
Figure S5 – Anti-parallel dimer of PbINP. A) PbINP aligned as an anti-parallel dimer prior to the start of a solvated 10-ns MD simulation. The unsatisfied rank of Ser and Tyr hydroxyl groups from adjacent chains are indicated. B) Energy-minimized average structure of PbINP following the solvated 10-ns MD simulation. While the structure was stable, its flatness was spoiled due to a large twist that developed. When PbINP is aligned as a parallel dimer, no twisting occurs, and this maintains the molecule's flatness, a characteristic of all ice-binding proteins.
Figure S6 – Anchored clathrate waters on the IBS of *MpAFP_RIV*.  

A) Chain B of *MpAFP_RIV* is shown in surface mode, with carbons coloured light blue, oxygens red, and nitrogens dark blue. The water electron density (black mesh) is contoured at $\sigma=6$.  

B) The X-ray crystal structure of *MpAFP_RIV* chain B (carbons coloured white) aligned to chain B from the MD simulation (carbons coloured light blue). Waters identified by crystallography are shown as red spheres, while waters built into the electron density (contoured at $\sigma=6$) are shown as light blue spheres. Ca$^{2+}$ ions are represented as green spheres.