The case for irreversible binding of ice-binding proteins to ice

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Ice-binding proteins (IBPs) include proteins that have the ability to stop ice crystal growth and inhibit ice recrystallization. IBPs do this by adsorbing to the surface of the ice and making the addition of water to the crystal unfavourable. Their surface adsorption causes a thermal hysteresis (TH) between the lowered freezing temperature and slightly elevated melting temperature. It has been argued that IBP binding must be irreversible because the ice crystals do not grow in the TH gap. However, this seems inconsistent with the observation that TH values are influenced by IBP concentration. We have made three recent experimental demonstrations that IBP binding to ice is indeed irreversible: i) photo-bleaching of GFP-tagged IBP residing on the surface of an ice crystal held in the TH gap shows that there is neither exchange nor overgrowth of the bleached IBP; ii) ice crystals bound by IBPs show a measurable resistance to melting (melting hysteresis) demonstrating that the IBPs remain surface-bound at temperatures above the equilibrium melting point; iii) using a temperature controlled microfluidics apparatus it is possible to entirely replace the IBP solution surrounding an IBP-bound ice crystal in the TH gap with buffer, without losing the bound IBP. In the case of hyperactive IBP these bound proteins prevented the crystal from growing in supercooled buffer. Recent experiments also show that the exposure time of a crystal to an IBP solution at a given concentration can change the TH activity up to 10-fold. It appears that IBPs have a slow on-rate for crystal binding. According to the anchored clathrate water hypothesis for the mechanism of IBP binding to ice, the ice-binding site of the IBP forms its ligand before merging with it. A slow on-rate might reflect the infrequency of having sufficient ice-like waters on the ice-binding site of the IBP for it to bind to ice. These results imply that IBP adsorption to the ice surface is irreversible and that TH is a function of the absorbed proteins on the surface and only indirectly a function of the concentration of IBPs in the solution.

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