

## **Experimental observations of superheating of ice crystals in ice-binding protein solutions**

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Superheating of solids is defined as the absence of melting at temperatures higher than equilibrium melting point. Experimental observations of superheating of ice have been remarkably rare to due to the nature of the ice - water interface. Recently, we have quantitatively demonstrated that ice-binding proteins (IBPs) can prevent ice from melting; evidence of superheating. Raman spectra of crystals below and above the equilibrium melting point confirmed that the ice was superheated (Celik et al, PNAS 2010). Using successive cycles of cooling and warming, we also found it possible to repeatedly melt an ice crystal back to its original shape after it had been overgrown with ice. This repeatable process is likely due to melting inhibition by IBPs. By conjugating green fluorescent protein to IBPs we were able to visualize IBPs both on superheated ice crystals and as they diffuse into the solution upon reaching the maximum superheating temperature. These results strongly suggest that IBPs bind irreversibly to ice surfaces and that surface-adsorbed IBPs protect ice from melting. It is possible that melting inhibition plays a role in recrystallization inhibition as both the growth and the melting of ice crystals are inhibited.

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