

Microfluidic devices for the investigation of ice-binding proteins

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In order to investigate ice-binding proteins (IBPs) and their binding kinetics with ice crystals, we developed novel microfluidic devices capable of precise local temperature control. We used microfluidic devices and fluorescence microscopy to demonstrate the basal plane affinities of hyperactive IBPs and to investigate the kinetics of the IBP - ice interaction. The first microfluidic device we used was a miniature cold finger that creates a sub millimeter ice lens in the middle of the main chamber in which the solution can be exchanged around ice crystals. We used the device to demonstrate the basal plane affinities of hyperactive IBPs. These experiments provided evidence that hyperactive IBPs adhere to the basal plane and this trait may contribute to their hyperactivity. We also found accumulation of the IBPs on the basal plane of ice over time. The second device we used was designed to allow the growth of an ice crystal (30-50 μm) in a small chamber and to permit exchange of the solution around the ice crystal by gentle flow and diffusion, thus minimizing disturbances at the crystal surface. We performed experiments in which ice crystals were incubated in IBP solution and then the solution was exchanged with a plain buffer. At this point, the reduction of the freezing point (thermal hysteresis activity) was measured. We consistently measured thermal hysteresis activity in IBP-depleted solutions which were more than 10 times higher than the expected activity in such low concentrations. The fact that ice crystals incubated in IBP solutions retained their activities after the exchange of the solution around them is of great importance in our current understanding of the concentration dependence of thermal hysteresis. These results strongly suggest that IBPs bind irreversibly to ice surfaces. We have demonstrated that the use of microfluidics in combination with fluorescence microscopy is a valuable technique to study the binding mechanisms of IBPs and their interaction with ice crystals.

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