Novel dimeric beta-helical model of an ice nucleation protein with bridged active sites

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Ice nucleation proteins (INPs) allow water to freeze at high subzero temperatures. How they function at the molecular level is unknown. Here we have predicted a beta-helical fold for the INP produced by the bacterium *Pseudomonas borealis* that uses internal serine and glutamine ladders for stabilization. The model also predicts a mode of dimerization that will allow for multimerization, which could explain the aggregation-dependence of INP activity. Both sides of the *Pb*INP have tandem arrays of amino acids that can organize waters into the ice-like clathrate structures seen on antifreeze proteins. Dimerization dramatically increases the 'ice-active' surface area of the protein by doubling its width, increasing its length, and presenting identical ice-forming surfaces on both sides of the protein. We suggest that this allows sufficient anchored clathrate waters to align on the INP surface to nucleate freezing. As *Pb*INP is highly similar to all known bacterial INPs, we predict its fold and mechanism of action will apply to these other INPs.

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