

## **Engineering bacterial sialic acid synthase to have type III antifreeze protein activity**

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Type III antifreeze protein (AFP) is a 7-kDa, globular ice-binding protein with moderate thermal hysteresis activity. It was recently shown to have a compound ice-binding site where one surface of the more active isoforms binds to the primary prism plane of ice and an adjacent surface binds to a pyramidal ice plane. This AFP appears to have evolved from the C-terminal domain of the enzyme sialic acid synthase (SAS) by a process of gene duplication and divergence. Consistent with this origin, SAS from the ocean pout has the ability to bind and shape an ice crystal, but not to stop it from growing. To test our understanding of what structural elements constitute an ice-binding site and the mechanism by which it binds to ice, we have set out to engineer a bacterial SAS into an ice-binding protein. We started with SAS from *Neisseria meningitidis*, whose crystal structure has been solved. This wild-type bacterial SAS has no affinity for ice, but after making several mutations to improve flatness and hydrophobicity of the target surface we are starting to convert the SAS into an ice-binding protein judging by its ability to facet ice crystals. These mutated proteins are being monitored by X-ray crystallography to structurally visualize the engineering process and become aware of any potentially destabilizing changes to the underlying fold of the protein.

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