

Project #1: Some plant-associated bacteria produce large ice-nucleating proteins (INPs) in their outer membrane that can initiate ice formation at high sub-zero temperatures (~ -2.0 °C). By causing frost to form, these temperature-stressed bacteria can damage plant tissues and gain access to their nutrients. We hypothesize that the 130-kDa INPs have a surface that aligns many water molecules into a continuous ice-like pattern. However, the INPs have to be organized into a protein superstructure in order to organize suffice ice-like waters to trigger the freezing process. We are using a variety of techniques to isolate these mega-Dalton complexes and deduce how the INP monomers assemble to form the superstructure.

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Project Title: Structure-function relationships in ice nucleation proteins

Keywords (3-5):

1. DNA Cloning
2. Recombinant protein purification
3. Ice nucleation
4. Site-directed mutagenesis
5. Structural biology

Project Goals: This project will involve isolating and characterizing INP superstructures that naturally form in the bacterial cytoplasm when the INPs are over-expressed. Models for the assembly of the INP superstructures will be proposed and tested by experimentation and AlphaFold predictions.

Experimental Approaches: INP superstructures produced in *E. coli* will be purified by affinity chromatography and differential centrifugation for characterization by electron microscopy and protein-protein cross-linking coupled to tandem mass spectrometry. Mutated and truncated INP constructs will be expressed in *E. coli* to see how alterations to the INPs affect their assembly into superstructures. Ice nucleation assays will be performed on a programmable cooling stage. Stable superstructures will be examined by cryo-Electron Microscopy to deduce how INPs pack together to form the complex.

References:

Forbes, J., Bissoyi, A., Eickhoff, L., Reicher, N., Hansen, T., Bon, C.G., Walker, V.K., Koop, T., Rudich, Y., Braslavsky, I., Davies, P.L. (2022). Water-organizing motif continuity is critical for potent ice nucleation protein activity. *Nature Communications*. 13(5019): 1-14.

Davies, P.L. (2014) Ice-binding proteins: a remarkable diversity of structures for stopping and starting ice growth. *Trends in Biochemical Sciences* 39(11), 548-555. [PubMed: 25440715](https://pubmed.ncbi.nlm.nih.gov/25440715/)

Garnham, C.P., Campbell, R.L., Walker, V.K., Davies, P.L. (2011) Novel dimeric beta-helical model of an ice nucleation protein with bridged active sites. BMC Structural Biology 11, 36. [PubMed: 21951648](#)