

BCHM 421/422 Project Outline

Project #2: 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 nutrients. We hypothesize that the 130-kDa INPs have a surface that aligns many water molecules into an ice-like pattern. When the number of organized waters reaches a critical threshold, they will initiate the freezing process.

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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. Protein engineering
5. Structural biology

Project Goals: Currently, there are no structures for the INPs - only a model for the central repetitive region. This project will be directed at solving the structure of truncated versions of the INPs that would help validate the model and determine the roles of the flanking N- and C-terminal regions.

Experimental Approaches: Truncated INP constructs will be designed at the DNA level and expressed in *E. coli*. The recombinant protein products will be purified and characterized for their stability and activity in ice nucleation assays performed on a programmable cooling stage. Stable constructs will be put into crystallization trials to solve their structures by X-ray crystallography. These structures will be compared to those of well-characterized antifreeze proteins to see if they have the same water-organizing mechanisms.

References:

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](#)

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](#)

Gurian-Sherman D., and Lindow, S. E. (1993) Bacterial ice nucleation: significance and molecular basis. *FASEB J.* 7, 1338-1343. [PubMed: 8224607](#)