BCHM 421/422 **2022-23**

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 nutrients. We hypothesize that the 130-kDa INPs have a surface that aligns many water molecules into a continuous ice-like pattern. When the number of organized waters reaches a critical threshold, they will initiate the freezing process. In the absence of a structure, we have developed a model for the INP using AlphaFold, which we will be testing here by mutagenesis and by analyzing the structure of individual domains.

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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 be directed at solving the structure of truncated versions of the INPs that could validate the AlphaFold model and determine the roles of each domain/region. In addition we will probe ways in which individual INPs might physically associate into oligomers.

Experimental Approaches: Truncated INP constructs and mutants 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:

Forbes et al., (2021) Deletion and mutation analyses of an ice nucleation protein reveal the importance of water-organizing motif continuity. Manuscript in preparation (available on request)

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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