

BCHM 421/422 – 2020/2021

Project #2 Outline: Some plant bacteria produce 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 and damage the plant tissues the bacteria might gain easier access to nutrients. But what is the molecular mechanism these INPs use to nucleate ice, and what is their relationship to antifreeze proteins? Our model for INP suggests that it might resemble a giant antifreeze protein, and organize enough surface-bound waters to start ice freezing.

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Project Title: Structure-function relationships in ice nucleation proteins

Keywords (3-5):

1. Recombinant protein expression
2. Ice nucleation
3. Protein engineering
4. Site-directed mutagenesis
5. Fluorescence microscopy

Project Goals: Produce a series of truncations, extensions and mutations in the INP protein tagged with GFP. Characterize their activity in ice nucleation assays when expressed on the surface of *E. coli*. Test the hypothesis that INPs use their repeating Thr-X-Thr motifs to organize water into an ice nucleus on the surface of the protein. Study the relationship between INP size and nucleation temperature

Experimental Approaches: INP variants will be designed at the DNA level and expressed in *E. coli*. Their production and accumulation on the bacterial outer surface will be observed by GFP tagging using fluorescence microscopy. Ice nucleation assays will be performed on a programmable cooling stage. Sections of the INP gene will be redesigned and ligated into place to test a series of hypotheses on INP structure-function relationships.

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](#)