Alternative 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 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.

Supervisor: Peter L. Davies **TA:** Jocelyn Lee

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.

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