BCHM 421/422 – 2019/2020

Project Outline: Many organisms produce ice-binding proteins (IBPs) to stop ice growth. In freeze-resisting animals like fish and insects these serve in an antifreeze role; whereas in plants that can tolerate freezing the IBPs inhibit recrystallization of ice. IBPs have independently evolved on many occasions to have a diverse range of structures all with the same function of binding to ice. We would like to add to this diversity by prospecting for new IBPs to see if they have a common mechanism of action, and to find new candidates for biotechnological applications.

Supervisor: Peter L. Davies

Project Title: Discovery and characterization of new antifreeze proteins

Keywords:

1. Ice-affinity purification
2. Peptide sequencing
3. Transcriptomics
4. Recombinant protein
5. X-ray crystallography

Project Goals: Purify to homogeneity at least one novel antifreeze protein (AFP). Characterize its activity and biophysical properties. Determine sufficient peptide sequence information to recognize the sequence in a transcriptome library. Produce a recombinant version of the AFP and place in crystallization trials. Solve the AFP's crystal structure.

Experimental Approaches: Novel IBPs will be isolated from natural sources by ice-affinity purification. The IBP's antifreeze activity, mass, and amino acid composition will be determined, and its protein sequence established by tandem mass spectrometry. The gene for the IBP will be derived from transcriptome analysis of the tissue source. A codon-optimized gene for the IBP will be synthesized to produce recombinant protein in bacteria. The recombinant IBP will be purified, crystallized, and have its 3-D structure determined by X-ray crystallography.

References:

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Bar-Dolev, M., Braslavsky, I., Davies, P.L. (2016) Antifreeze protein function. Ann. Rev. Biochem. 85, 515-542. PubMed: 27145844