

Project #2: Antifreeze proteins (AFPs) are thought to bind to ice by organizing surface waters on one side (the ice-binding site) that make a good enough match to ice to bind and freeze the protein to it. We would like to test some features of this hypothesis.

Supervisor: Peter L. Davies **TA:** Connor Scholl

Project Title: Structure-function relationships in ice-binding proteins

Keywords (3-5):

1. DNA cloning
2. Recombinant protein purification
3. Antifreeze assays
4. Site-directed mutagenesis
5. X-ray crystallography

Project Goals: Test the effect inactivating ice-binding site mutations have on the positioning of ice-like waters on an AFP to see if this can account for the loss of activity. Test the role of the opposite side of the protein (non-ice-binding site). If it is converted to an ice-binding surface will activity increase or decrease?

Experimental Approaches: Constructs coding for *Rhagium mordax* (beetle) AFP 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 antifreeze assays performed on a programmable cooling stage. Stable constructs will be put into crystallization trials to solve their structures by X-ray crystallography and look at surface waters. These structures will be compared to those of the parent antifreeze proteins to see how their water-organizing behaviour changes.

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](#)

Ye, Q., Eves, R., Campbell, R.L., Davies, P.L. (2020). Crystal structure of an insect antifreeze protein reveals ordered waters on the ice-binding surface. *Biochem J.* 2020 Sep 18; 477 (17): 3271-3286. doi: 10.1042/BCJ20200539. [PubMed: 32794579](#)

Scholl, C.L., Tsuda, S., Graham, L.A., Davies, P.L. (2021). Crystal waters on the nine polyproline type II helical bundle springtail antifreeze protein from *Granisotoma rainieri* match the ice lattice. *FEBS J.* 2021 Jan 18; Online ahead of print. doi: 10.1111/febs.15717. PubMed: [33460499](#)