IRG-Publishable summary for project 256364

Title: Freeze control in Food by Ice Binding Proteins

Freezing processes play a main role in food science. Storage of food in a frozen form has become one of the most common ways to elongate the self-life of many food products. Still, destructive processes during freezing and thawing limit the use of this method for recoverable storage. During the freezing of food materials, cell walls can be ruptured by ice crystals, or they can be separated by extra-cellular ice growth during the recrystallization process, in which ice crystal grows bigger on the expense of small crystals.

Antifreeze proteins (AFPs) are a remarkable group of proteins that facilitate survival of cold-adapted organisms at sub-zero temperatures. These proteins have the ability to bind to ice crystals and inhibit their growth to a certain level. Some of the AFP proteins are highly active and named hyperactive AFPs while others are called moderate AFPs. In frozen samples, damaging recrystallization processes are well inhibited by AFPs. These unique interactions of AFPs with ice crystals suggest their potential use in cryopreservation of food materials, cells, tissues, and organs. Our lab investigates the mechanism of action of AFP proteins on ice and promotes the potential use of AFPs in advancing cryopreservation technologies.

Two main tools were developed in our lab to study these proteins. A custom-made computer-controlled cold microscope stage system for activities measurements AFPs (This study has been published in the Journal of Visualized Experiments, Braslavsky and Drori, 2013), and a novel microfluidic device allowing temperature control of small ice crystal in a microscopic environment (Published in PNAS, Celik et al. 2013, and in R. Soc. Interface, Drori, et al, 2014). Using these devices and fluorescently labeled proteins, we explored the dynamic nature of the AFPs activity.

With these systems we study the way AFPs interact with ice. Ice crystals tend to be flat, with a plane that called basal plane, and has internal hexagonal symmetry in the perpendicular direction. We show that AFPs bind irreversibly to ice crystals (Celik et al. 2013), however, moderate AFPs are unable to bind to the basal plane of the crystal while hyperactive AFPS do bind to basal plane and they have different ice binding kinetics (Drori et al. 2014).

Another way to distinguish moderate from hyperactive AFPs is by observing ice shaping. We found that ice shapes in hyperactive AFPs solutions are actually formed during melting (Published in R. Soc. Interface, Bar-Dolev et al, 2012). Ice recrystallization inhibition (IRI) of AFPs is also an important issue that we investigate in our lab. (Related article in Plos One, Mizrahy et al, 2013). IRI and ice shaping, both involve in freezing damage of tissue and cells and their prevention will have great impact on cryopreservation of biological samples and in food industry.

Vitrification, an “ice free” method for cryopreservation, is the most desirable way to preserve biological matter, and yet in many systems, this method encounters some difficulties in achieving successful preservation. We examine the effect of AFPs on this process. Our preliminary results suggest that an ice growth inhibition activity of hyperactive AFP maintained at ultra low temperatures (i.e.-110 C), and thus might be effective in improving vitrification process.

Large quantities of AFPs are required for supporting cryopreservation investigations. We are currently in a process of developing methods for large scaling production of AFPs. We choose to express the proteins in bacterial system and the purification step will be based on the affinity to ice property of AFPs. In parallel a research on the effect of AFPs on lipid oxidation in meat, have been done in our lab. So far, significant effect of freezing on the level of lipid oxidation in minced meat was shown. Further investigation will reveal if AFPs administration expends this effect of freezing on lipid oxidation levels.

To Summarize, we study the basic mechanism of AFPs activity along with the development of their use in cryopreservation applications of food and other biomaterials.

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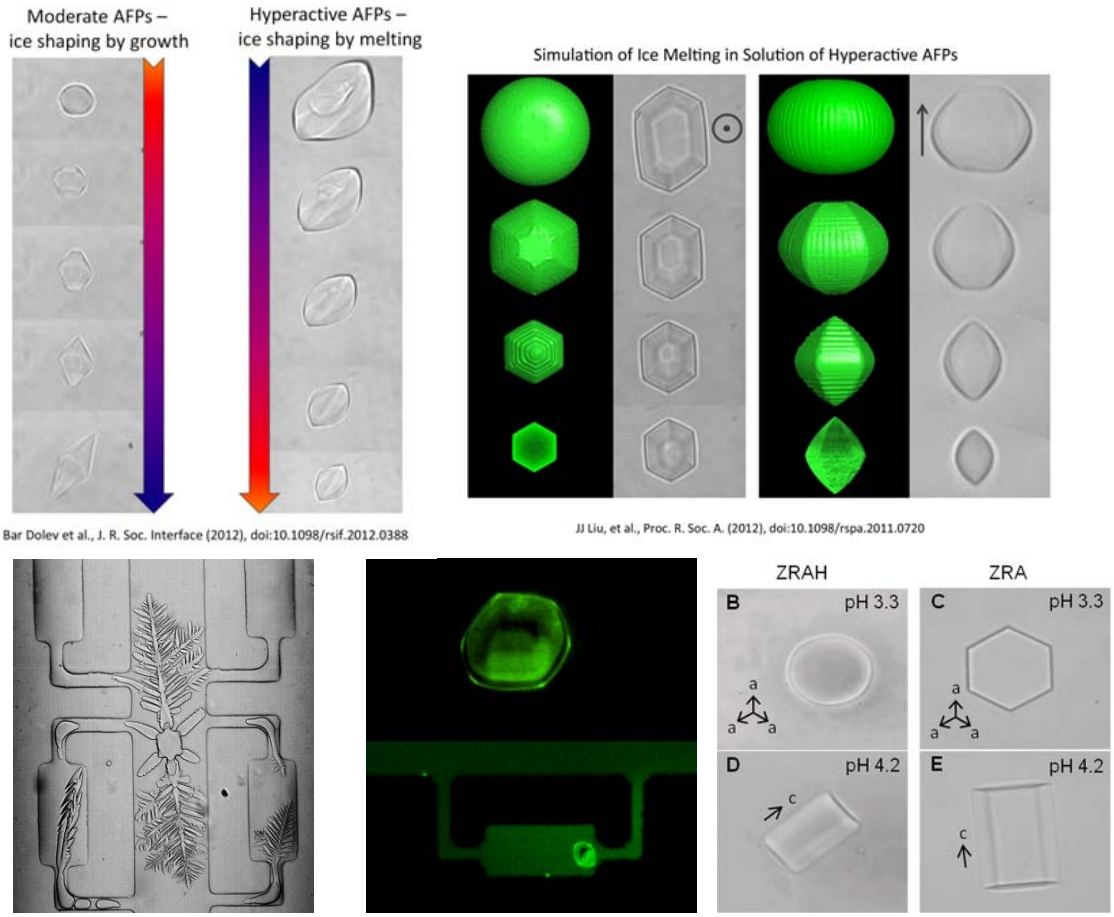

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