Brain freeze

Antifreeze proteins promise new developments in ice cream production and organ preservation. Some claim that life itself evolved from these cool little nanoplayers. Scientists ponder the icy implications.

By Anita Martin

The Vikings believed that in the beginning, there was fire and there was ice. Across the yawning void, frost met flames and from those first thawing drops sprang life.

Although Charles Darwin might object, Ido Braslavsky, assistant professor of physics and astronomy at Ohio University, has reason to believe that the ancient Norsemen may have been on to something.

Braslavsky, a member of the university’s Nanoscale and Quantum Phenomena Institute, focuses his nanoscale research on, among other things, antifreeze proteins. These mini-aminos simultaneously represent the salvation of polar fishes, the future of organ transplants and ice cream, and may have something to do with the origin of life itself.

Curb your crystallization

During frosty winter months, we rely on our human ingenuity for mechanical mobility. A little neon green ethylene glycol antifreeze in the car radiator and we’re on our way.

Meanwhile, fishes, insects and plants from Canadian winter rye to Arctic sea flounder to Alaskan budworms have developed their own immunities to ice. Without antifreeze proteins, unseasonable frosts ruin crops and artic waters turn live game into frozen flounder. Yet with these secret weapons, wildlife can survive and even bypass the winter chill. Although each of the species claims a distinct version of antifreeze proteins, the basic function is the same: antifreeze proteins inhibit and control ice formation.

“Antifreeze proteins are unique because they’re a product of their environment,” explains Natalya Petaya, a post doctorate from Germany who assists Braslavsky’s antifreeze research, “but one thing common to all is that they arrest the growth of ice crystals.”
The mechanism used by antifreeze proteins varies by method and intensity. Pertaya and Braslavsky study proteins from two creatures: the eelpout fish (a Scandinavian delicacy) and the Canadian budworm.

The antifreeze proteins bind themselves to ice, thereby forcing them to grow with high curvature. This trick can actually lower the freezing point of ice from one to six degrees Celsius. The result: water molecules stay in liquid form below the normal freezing point.

**Artful analysis**

Because the proteins themselves span only about five nanometers, their icy interactions can be pretty obscure. Pertaya and Braslavsky use fluorescent molecules from a green jellyfish to shed some light on the process.

“All activity happens on the nanometer scale,” Braslavsky says, “but we must look through the global, macroscopic scale.”

To do so, their collaborator, Peter Davies from Queen's University, marks the antifreeze proteins with fluorescent jellyfish molecules. These luminous proteins are added to water.

Next, the water is dropped between glass slides. The incandescent droplets are alternately frozen and thawed while Pertaya observes the growth patterns.

Pertaya demonstrates the crystalline shapes with the pride of an artist. “The water without the proteins will freeze round, into a sphere,” she says. “But the fish protein makes this: a bi-pyramid.” On Pertaya’s computer monitor, a slender white diamond shape extends two sharp points.

The budworm protein, the hardier, more powerful antifreeze, reveals more delicate tastes in crystal design. When the budworm water droplet freezes, ornate spindles stretch into snowflake formation.

**Frozen goods**

Beyond their aesthetic appeal, antifreeze proteins promise medical and commercial implications. The ability of antifreeze proteins to prevent cell damage in some crops, even after they freeze and thaw, could lead to safer, more efficient blood and organ preservation.
Tissues preserved for transplant, for example, may undergo some damage due to recrystallization. Recrystallization occurs when changes in temperature and pressure cause ice to thaw slightly and refreeze into larger crystal structures. Imagine what happens to milkshake popped in the freezer for later. Antifreeze proteins prevent this process.

Tharp and Young, a scientific business consulting partnership, affirms that “low levels of antifreeze proteins (a.k.a. ‘ice structuring proteins’) in ice cream can reduce ice crystal growth rate substantially and control the shape of ice crystals in ways that provide unique textural and structural compounds.”

But before Ben and Jerry come out with a budworm and eelpout crunch, antifreeze proteins may find other agricultural functions. Genetic introduction of antifreeze proteins may grow orange trees resistant to Florida’s occasional frost, or boost Canada’s salmon market.

**Dawn of life**

Antifreeze proteins, due to their simple design and great polar profusion, are cited often as evidence of Darwin’s theory of evolution. Even Michael Behe, a leading “intelligent design” theorist concedes that “antifreeze proteins in fish and plants may indeed be explained by a Darwinian mechanism.”

But the theory of evolution begs a beginning. If current complexity developed out of former, simpler design, there had to be a point of origin. According to Braslavsky, antifreeze proteins may have been early players in the game of life.

One of the early prebiotic ideas focused on RNA, a molecule that both carries information and self-replicates.

“But before you can self-replicate, there have to be other steps,” Braslavsky says. “What came before RNA? How do you develop something that supports complexity to the point where you can self-replicate?”

Some, including Braslavsky, suggest crystals played a role. Crystals like ice, clay and minerals grow in a self-assembled pattern. “It’s almost as though they self-replicate,” he says.

If developing prebiotic material could bind to and interact with crystals, as antifreeze proteins do with ice, the self-replicating crystals could act as a mold from which the material could evolve its own self-assembly.
In *Origins of Life and Evolution of the Biosphere*, Vladimir Tolstoguzov agrees that “both antifreezing and ice nucleating biopolymers could be very old and evidence of the importance of conjugates for the emergence of life.”

“It’s a thought,” Braslavsky says, “a new hypothesis that combines many new hypothoses.” He hopes to develop ways to test that thought in the future.

Regardless of prebiotic origins, antifreeze proteins contribute to the Darwinism of ideas – a must for the evolution of science. Meanwhile, these little guys are finding their niche, whether in Northerly crop beds, medical operations or just around the corner in your grocer’s freezer.