Frozen frog–inspired material could improve cryopreservation of cells and organs

By Asher Jones
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Researchers have developed a material that reduces explosive growth of ice crystals at subzero temperatures, an advance that could enable better cold storage of living things for medicine and research.

Certain beetles and frogs can survive freezing solid in the wintertime, which inspired researchers to create the novel material, which they say could reduce damage to cells, tissues and organs during cryopreservation. The findings were described in a study published May 4 in *Biomacromolecules*.

Freezing harms cells in two main ways. The formation of sharp ice "like a knife or spear" can puncture and destroy them, said Jianjun Wang, study senior author and a professor in the Institute of Chemistry at the Chinese Academy of Sciences. Also, rapid freezing of water outside the cell causes the concentration of dissolved solutes to increase. As water rushes out of the cell to dilute this concentrated solution, the cell rapidly shrinks, a damaging process called osmotic shock.

One approach that cold-hardy organisms use to survive chilly climates is antifreeze proteins. Water usually freezes at 0 degrees Celsius (32 degrees Fahrenheit), but these proteins depress the temperature at which ice forms — though only to a point. Below a certain temperature, a sudden burst or explosive growth of ice crystals causes damage to animal cells.

"But some organisms can survive freezing. There is a frog; 65% of its body water freezes but it can still survive," Wang said. "For organisms to survive harsh winters, like the frog, there should be some special mechanism to prevent this sudden burst of ice crystals in the winter time."

Inspired by frozen frogs, Wang and his team sought to develop a material that would prevent this explosive growth of ice. In living things, the region outside cells known as the extracellular matrix is crowded with a network of molecules, including glycoproteins and collagens, that form loose molecular bonds, and this matrix may confine water within it and prevent it from freezing.

To mimic this extracellular matrix, the researchers made a solution of two components: a compound called polyethylene glycol and a salt called sodium citrate. At certain concentrations, these two compounds form droplets in which they become concentrated or crowded, forming networks similar to the extracellular matrix.

When antifreeze proteins were placed into a solution without these crowded droplets and cooled down, the solution showed explosive growth of sharp ice crystals. But when the crowded droplets were added to the solution, ice crystals grew very slowly.

"We found that the sudden burst of ice crystals can be completely inhibited," Wang explained.

Currently, the gold standard cryoprotectant for freezing animal cells is a compound called dimethyl sulfoxide, but this organic solvent can have toxic effects on cells and in patients, prompting researchers to look for alternative solutions.

Wang said his team's material could be used as a less-toxic approach to cryopreservation of cells and tissues for medical biobanks and storage of cells for treatments such as CAR T-cell therapy, which has been touted as a potential cure for cancer.

According to Wang, the work could also pave the way for cryopreservation of organs, which are currently stored at refrigerator temperatures, an advance that could aid the development of artificial organ transplants and extend the life of donated organs.

"The preservation time for donated organs is short. Almost 70% of donated hearts or donated lungs are discarded. That is a huge waste," Wang said. "If one can cryopreserve tissues or organs, that's a big win."

As for the possibility of cryopreserving human beings, Wang doesn't want to speculate. A handful of cryogenics companies have facilities that freeze people who have died, with the hope that future advances will enable them to be reanimated.

The study, "Bioinspired crowding inhibits explosive ice growth in antifreeze protein solutions," published May 4 in Biomacromolecules, was authored by Zhang Liu, Shenglin Jin, Shuo Liu and Zhiyuan He, Chinese Academy of Sciences; Yan Wang, Shihezi University; and Xia Zheng, Jun-Feng Xiang and Jianjun Wang, Chinese Academy of Sciences and University of Chinese Academy of Sciences.