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Perspective

Bioinspired Ice-Binding Materials for Tissue and Organ Cryopreservation

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ABSTRACT: Cryopreservation of tissues and organs can bring transformative changes to medicine and medical science. In the past decades, limited progress has been achieved, although cryopreservation of tissues and organs has long been intensively pursued. One key reason is that the cryoprotective agents (CPAs) currently used for cell cryopreservation cannot effectively preserve tissues and organs because of their cytotoxicity and tissue destructive effect as well as the low efficiency in controlling ice formation. In stark contrast, nature has its unique ways of controlling ice formation, and many living organisms can effectively prevent freezing damage. Ice-binding proteins (IBPs) are regarded as the essential materials identified in these living organisms for regulating ice nucleation and growth. Note that controversial results have been reported on the utilization of IBPs and their mimics for the cryopreservation of tissues and organs, that is, some groups revealed that IBPs and mimics exhibited unique superiorities in tissues cryopreservation, while other groups showed detrimental effects. In this perspective, we analyze possible reasons for the controversy and predict future research directions in the design and construction of IBP inspired ice-binding materials to be used as new CPAs for tissue cryopreservation after briefly introducing the cryo-injuries and the challenges of conventional CPAs in the cryopreservation of tissues and organs.

1. INTRODUCTION

Via completely stopping all metabolic actions at cryogenic temperatures, cryopreservation enables unlimited storage times of tissues and organs without deterioration, which can bring revolutions to medicine and medical science (Figure 1a). For example, breakthroughs in tissue and organ cryopreservation can transform organ transplantation via making more organs available, decreasing costs, improving transplant outcomes, and mitigating risks.[1] Breakthroughs in tissue engineering and regenerative medicine require advances in organ and tissue cryopreservation to enable batch manufacturing and distribution as well as quality control of tissue products.[2] Ballooning costs in drug discovery could be greatly reduced if currently used animal models could be replaced by effectively cryopreserved human tissue models.[3] Advances in the cryopreservation of healthy and pathological tissues and organs enable biobanks[4] to provide high-quality and data-rich biosamples for developing human biomolecular atlas[5] as well as revealing the pathological processes of major and serious diseases.[6,7]

Currently, almost all of the donated tissues and organs can only be preserved for some hours at 4−8 °C with the state-of-the-art static cold storage technology, leading to an unfortunately high discard rate; for instance, donated hearts can be typically preserved for only ∼4 h with a discard rate as high as 70% (Figure 1b).[8] In the United States, if 10% of donated hearts currently discarded could be transplanted, the number of additional hearts made transplantable would be equal with that of waiting-list patients who currently die or are too sick for a transplant before receiving one.[1] Recognizing the great importance of tissue and organ cryopreservation, many organizations and science agencies including the National Institutes of Health, the US Commerce Department, US Department of Defense, the US government's Multi-Agency Tissue Engineering Science working group,[9] Pan-European Biobanking, and Biomolecular Resources Research Infrastructure[10] have identified cryopreservation of tissues and organs as a key priority.

The main challenge for tissue and organ cryopreservation is uncontrolled ice formation, which induces mechanical damages and osmotic pressure stress, causing irreversible destructions to the intra- and intercellular structures.[11,12] Therefore, cryoprotective agents (CPAs) must be used to control the ice nucleation/growth so that the destruction of the tissues and organs during the cryopreservation can be prevented or at least minimized. However, tissues and organs are large in volume, containing a rich variety of cells and complex extracellular components in comparison with cells;[9] consequently, ice nucleation and growth in tissues and organs during the cryopreservation is very complicated and challenging to control. Therefore, it is not yet possible to cryopreserve almost all human tissues and organs, although quite a few cells can currently be successfully cryopreserved.[1,11,13]

In stark contrast, some living organisms, such as polar fish and Alaska wood frog, can effectively protect themselves from freeze

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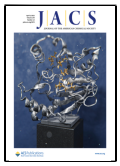

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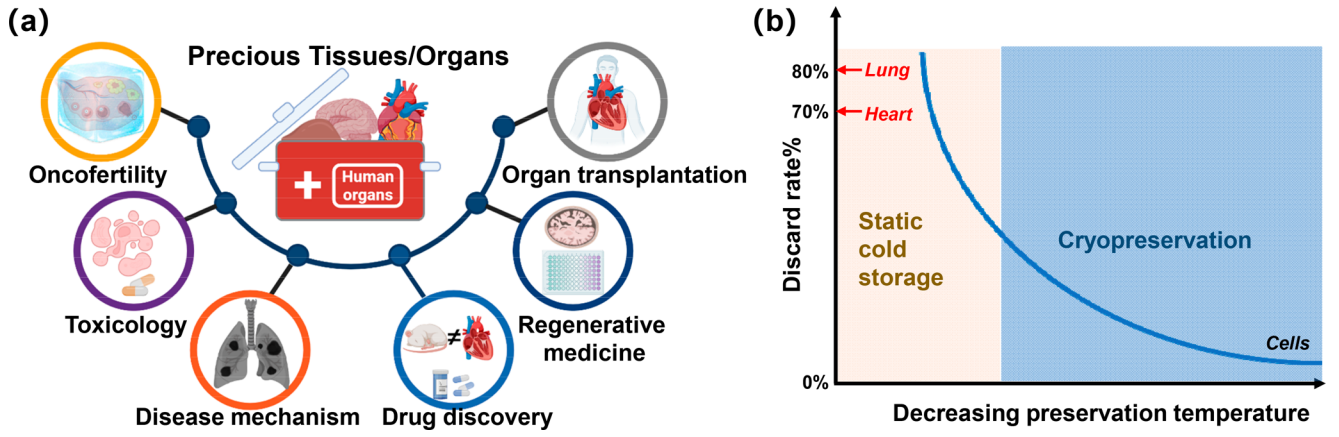

Figure 1. Urgent needs for the cryopreservation of tissues and organs. (a) Major areas with the need of cryopreserving tissues and organs.[1] Created with BioRender.com. (b) Relatively high storage temperatures of the state-of-the-art static cold storage limit the storage duration and increase the discard rate of solid organs,[8] which can be greatly reduced by the successful cryopreservation at much lower temperatures.

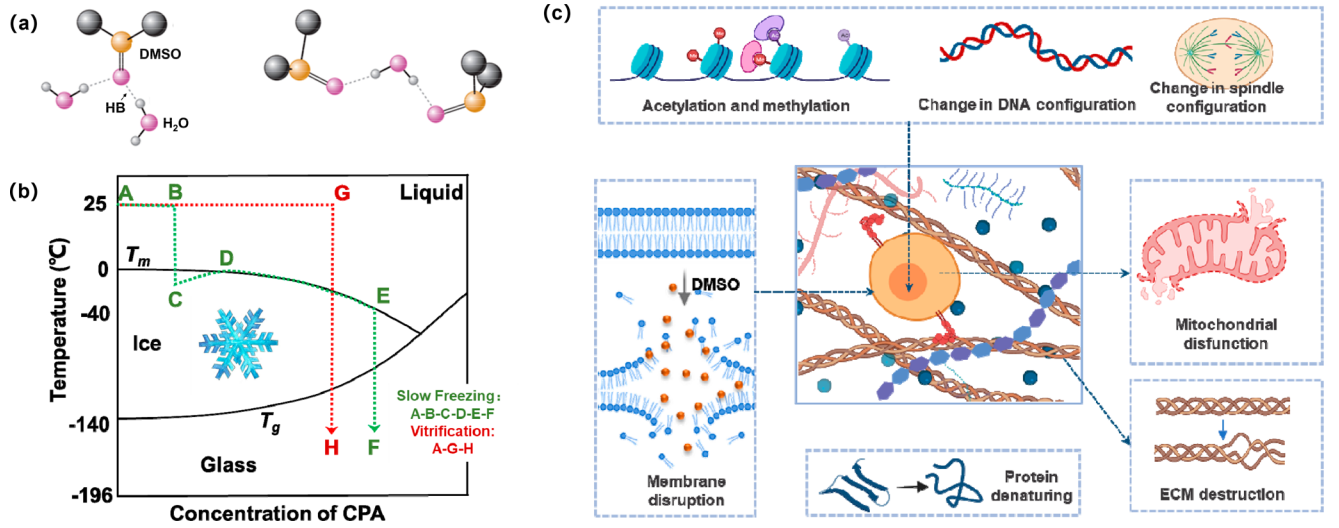

Figure 2. (a) Typical HBs formed in DMSO-water mixtures. Reproduced with permission from ref 27. Copyright 1992 American Chemical Society. (b) Supplemented phase diagram of CPA/water mixtures, and the pathways of cryopreservation by slow freezing (A−B−C−D−E−F) and vitrification (A−G−H). During slow freezing, a moderate concentration of CPA is loaded (A−B), and extracellular ice initiates at C and then propagates and grows (C−D−E), during which the intracellular water flows out and extracellular CPA moves in. When the concentration of CPA inside the cells is high enough to achieve intracellular vitrification, the frozen sample is finally transferred into liquid nitrogen for long-term storage (E−F). The vitrification method uses a high concentration of CPA (A−G), which substitutes a large fraction of intracellular water. As such, the vitrification of the solutions both inside and outside the cells is achieved (G−H). Reproduced with permission from ref 34. Copyright 2021 Springer Nature. (c) The detrimental effect of DMSO in cryopreservation ranges from molecular structure to tissue integrity. Created with BioRender.com.

damage because of their unique strategies in regulating ice nucleation and growth,[14,15] and it has been recognized that ice-binding proteins (IBPs) are the most efficient materials for controlling ice formation in these living organisms. The past decade has witnessed rapid progress in imaging, sequencing, omics approaches, and others, which allow us to understand and intervene in physiology from the tissue and organ level down to the molecular level.[13] With these cutting-edge techniques, it is now possible to reveal the exact mechanisms of IBPs in controlling ice formation in organisms like polar fish and Alaska wood frog, so as to guide the creation of new biocompatible IBPs-inspired ice-binding materials (IBMs), which will certainly provide a completely new avenue for the cryopreservation of tissues and organs.

In this perspective, we will first give an overview of the ice-induced injuries during the cryopreservation of tissues and organs. Then, we will analyze why conventional CPAs that succeeded in cryopreserving cells failed in most cases for cryopreserving tissues. After that, IBPs, which are isolated from living organisms that can survive severe cold and even freezing, will be introduced, and their working mechanisms will be detailed. Specifically, a summary will be given on recent endeavors in utilizing the IBPs for the cryopreservation of tissues and organs, which surprisingly showed controversial results.[16−18] Special emphasis will be placed on the analysis of possible reasons for this controversy, which sheds light on future research directions in designing and utilizing IBMs for the cryopreservation of tissues and organs.

2. ICE-INDUCED CRYOINJURIES AND CONVENTIONAL CRYOPROTECTANTS

Uncontrolled ice nucleation and growth in the tissues and organs is a major challenge for cryopreservation. The freeze of intracellular water results in severe mechanical damage inside

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the cells, such as disruption of the microtubules, organelles, and cell nucleus,[19−21] all of which are fatal to cells. The uncontrolled freezing of the extracellular water induces severe mechanical damage to the samples, including the rupture of the cell membranes, the breakage of the intercellular connection, and damage to vessel walls.[22,23] Moreover, when extracellular ice forms, the osmotic pressure outside the cells increases correspondingly, which leads to severe dehydration and shrinkage of the cells as well as excessive salt accumulation.[22−24] As such, cell connections could be broken, and the excessive salt accumulation could lead to protein destabilization. In the thawing process, small ice crystals recrystallize into larger ones as the temperature increases, which exacerbates the mechanical injuries both inside and outside the cells.[25] When the temperature raises close to the melting point, the extracellular space becomes rapidly hypotonic with the melting of large ice crystals, and the osmotic pressure drives water moving into the cells, which can lead to their swelling and even rupture. All these ice-induced cryoinjuries to the cells, intercellular connections, and blood vessels may lead to the loss of the functions of tissues and organs. As such, it is crucially important and at the same time extremely challenging to prevent uncontrolled ice formation during the cryopreservation, and breakthroughs in cryopreservation are highly dependent on the discovery of new CPAs that can effectively regulate the nucleation and growth of ice crystals.

Conventional CPAs include permeable molecules such as alcohols, sulfoxides, and amides, and nonpermeable molecules such as sugars, sugar alcohols, and polymers,[26] all of which have outstanding hydrogen bonds (HBs) forming capabilities. For example, dimethyl sulfoxide (DMSO), the most widely used CPA, can form HBs with water molecules, as illustrated in Figure 2a,[27] which are stronger than the HBs formed among water molecules. As the fraction of DMSO increases, the proportion of DMSO-water HBs increases correspondingly, leading to a dramatic decrease in the translational mobility of water molecules.[28] The slowing down of water diffusion delays the formation and growth of ice embryos, which depresses the freezing point and promotes ice-free glass formation, that is, the so-called vitrification.[29] Vitrification allows water to reach cryogenic temperatures without forming ice crystals. Without CPAs, glass formation can be achieved only if the cooling rate is as fast as 10^7 °C/s, which is almost impossible for large tissues and organs. The presence of CPAs with an increasing concentration can decrease the ice nucleation temperature and increase the glass transition temperature, thus the chance of ice formation is greatly decreased (Figure 2b).[30] In summary, conventional CPAs depress ice nucleation temperature following the colligative rule, that is, it is concentration dependent and promotes the vitrification of water (glass water) by forming HBs with water molecules.[31−33]

If conventional CPAs are to be used in the tissue and organ cryopreservation, a very high CPA concentration is required to reach uniform CPA distribution in the heterogeneous and large tissues. When conventional CPAs are used in a high dosage, the CPA toxicity becomes non-negligible and even becomes the dominant injury. Indeed, it has been experimentally revealed that DMSO has a detrimental effect on the multilevel structures of the tissues (Figure 2c): (1) On the molecular level, the histone acetylation,[35] global DNA methylation, proteins denaturation, and change in DNA conformation have been reported; (2) on the subcellular level, negative effects include the membrane disruption, change in spindle configuration,[35] mitochondrial disfunction[36] and so on; and (3) on the level of cell connections, the destruction of extracellular structures may break the cell junctions and invalidate the cell communications.[37,38] All these detrimental effects may directly affect the function of the tissues and organs after cryopreservation. As such, better cryopreservation strategies for tissues and organs should focus on two aspects, that is, more efficacy in ice control during the freezing/thawing processes and greatly reducing or completely replacing toxic CPAs with nontoxic alternatives.[39]

3. STRATEGIES IN NATURE: TO FREEZE OR NOT TO FREEZE

3.1. Noncolligative Ice Controlling Strategy. Many organisms in nature can survive supercooling and even freezing in subzero habitats (Figure 3a). Fishes living in the polar regions

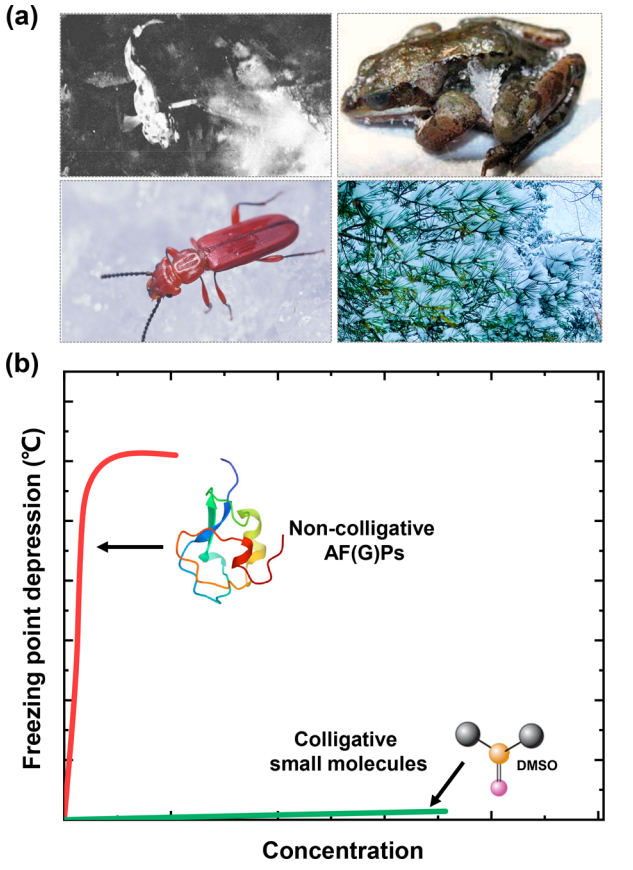

Figure 3. (a) Typical living organisms that survive in subzero environments: Antarctic fish. Reproduced with permission from ref 40. Copyright 1969 American Association for the Advancement of Science. Wood frog reproduced with permission from ref 41. Copyright 2017 The American Physiological Society. Insects *Cucujus clavipes* reproduced with permission from ref 42. Copyright 2011 Springer Nature; and plants (a pine tree covered with snow). (b) Freezing points as a function of the molar concentration for aqueous solutions of colligative small molecules (DMSO) and noncolligative AF(G)Ps (glycoprotein 3).[46]

are constantly exposed to freezing water;[40] wood frogs in Alaska can revive well in spring after frozen in winter;[41] some insects larvae can even survive at temperatures as low as −100 °C;[42,43] and many plants can endure extreme cold during winter.[44] To maintain the supercooled state or to survive the frozen state, these organisms have developed various strategies,[45] with

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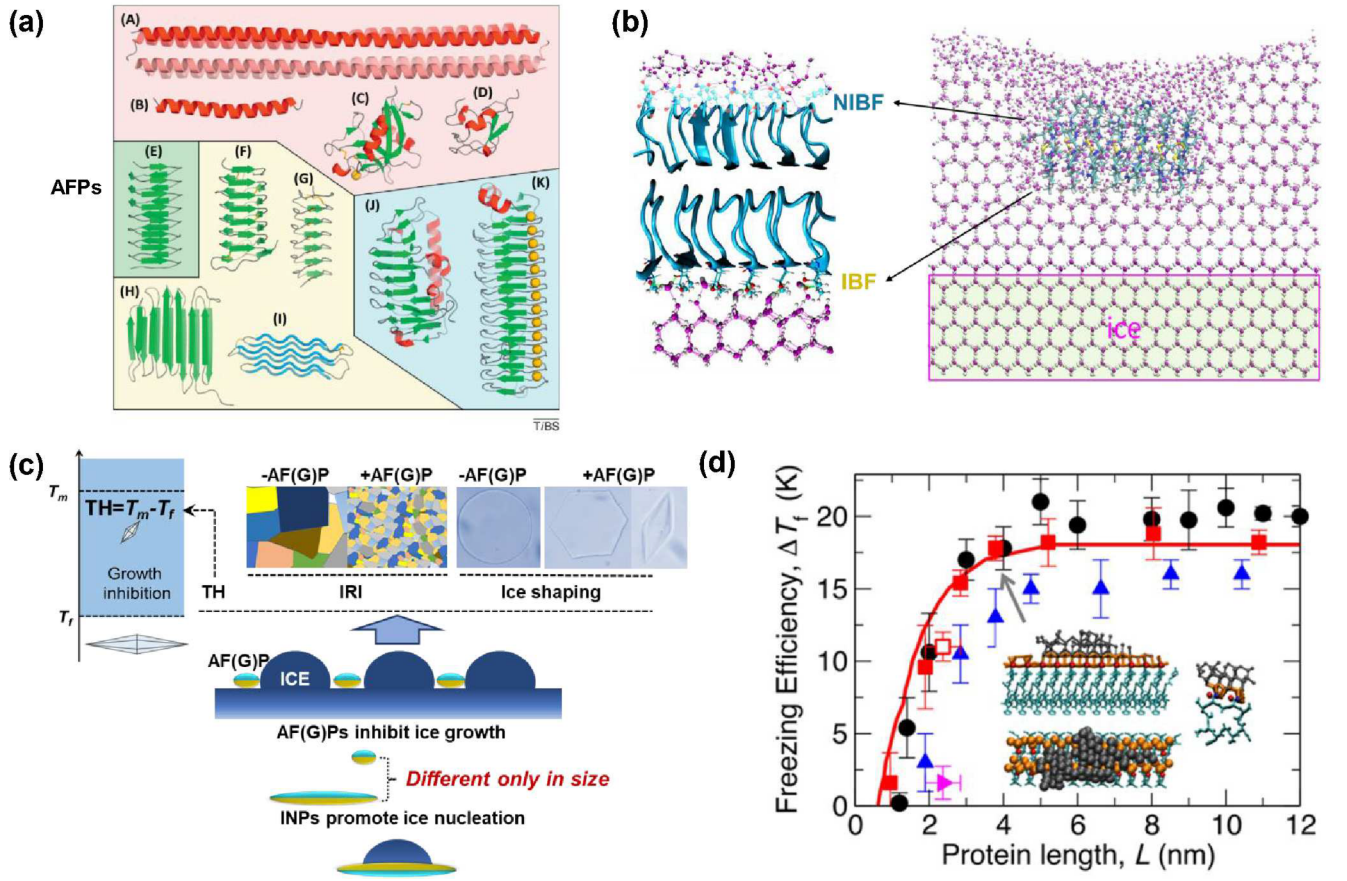

Figure 4. (a) Structures of some typical AFPs found in different species living in extreme cold habitats. Reproduced with permission from ref 57. Copyright 2014 Elsevier Ltd. (b) Side view of water molecules atop the IBF and the NIBF, and a typical side view snapshot of the simulation result with the IBF of *Tm*AFP in contact with ice (carbon, nitrogen, oxygen, and hydrogen atoms are represented in cyan, blue, red, and white spheres, respectively). Reproduced with permission from ref 49. Copyright 2016 National Academy of Sciences. (c) AF(G)Ps can depress the freezing point (TH), inhibit the ice recrystallization (IRI), and shape ice crystals;[58] the size difference between INPs and AF(G)Ps leads to their completely different activity in controlling ice nucleation. (d) Ice nucleation efficiency of model proteins as a function of the length L of the ice-binding site. Symbols indicate the $\Delta T_f(L)$ computed in molecular simulations with *Ps*INP (blue ▲), *Tm*INP (red ■), *Alcohol*INP (black ●), and *Tm*AFP (magenta right ▲). Inset: Views of the critical ice nucleus on the 5 nm long *Tm*INP; orange shows the anchored clathrate, and gray shows the rest of the ice nucleus. Reproduced with permission from ref 59. Copyright 2019 American Chemical Society.

antifreeze (glyco)proteins (AF(G)Ps) being found as a special type of material due to the unique capability in controlling ice nucleation/growth. The first discovered AF(G)P is isolated from Antarctic fishes by DeVries and Wohlschlag.[40] They found that purified glycoprotein is about 500 times more effective than conventional CPAs, such as DMSO, galactose, and sodium chloride, which colligative depress the freezing temperature (Figure 3b),[46] that is, AF(G)Ps can depress freezing much more efficiently in a noncolligative way.

3.2. Antifreeze (Glyco)protein (AF(G)P). Since the first discovery of AF(G)Ps in the blood of Antarctic fishes in 1969,[40] various types of AF(G)Ps with diverse structures ranging from the α-helix to β-sheet and random coils have been identified in the biological kingdom (Figure 4a).[47] The AF(G)Ps generally work in a noncolligative manner with the magnitude of freezing point depression being protein specific. Some of them possess more impressive freezing point depression capabilities. For example, the AFP isolated from *Tenebrio molitor* (*Tm*AFP)[48] can depress the freezing point by 2 °C at 10 μM, which is 100,000 times more effective than the colligative salt on the molar base.[47] Inspired by their outstanding ice controlling properties, the ice-inhibiting mechanism of AF(G)Ps has been intensively investigated.

The widely accepted ice-inhibiting mechanism of AF(G)Ps is the adsorption−inhibition mechanism. It is proposed that the AF(G)Ps comprise two distinct faces with or without ice-binding affinity (Figure 4b), that is, the ice-binding face (IBF) and the non-ice-binding face (NIBF).[49] Molecular dynamics (MD) simulations showed that the water molecules atop the IBF display an ordered hexagonal ice-like structure, which is attributed to the periodic spatial arrangement of the HB-forming groups. In contrast, no ordered water structure has been observed atop the NIBF due to the irregular arrangement of the hydrophilic groups. Further MD simulation (Figure 4b, right) reveals that the IBF binds preferably to the ice surface, while the disordered interfacial water layer atop the NIBF hinders the further growth of the ice crystals, which results in the so-called adsorption−inhibition.

The adsorption of AF(G)Ps on ice lattice affects the ice growth from three important aspects as illustrated in the top row of Figure 4c. Due to the adsorption−inhibition and the formation of the curved ice surface, the freezing temperature (T_f) is reduced due to the Kelvin effect.[50] At the same time, the

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melting temperature (T_m) is not obviously affected or only slightly elevated.[51] The difference between T_m and T_f is termed as thermal hysteresis (TH), that is, TH = $T_m - T_f$.[52] As such, the living organisms can avoid fatal ice growth and survive when the temperature is within the TH gaps. Based on the TH values, AF(G)Ps can be classified as moderately active and hyperactive, the former includes AF(G)Ps from plants and fishes whose TH values are commonly <2 °C;[53] while the latter includes AF(G)Ps that derived from insects or bacteria with TH values as high as 2−13 °C.[54,55]

Another beneficial effect of AF(G)Ps is their ice recrystallization inhibition (IRI) activity. Without AF(G)Ps, large ice crystals grow at the expense of small ones, which results in an increase in the mean crystal size and a decrease in the total number of crystals, thereby minimizing the free energy of the system, a process known as ice recrystallization.[56] This process is undesirable since the growth of large ice crystals aggravates the mechanical injuries; and at the same time, as the ice-to-liquid ratio generally increases in the initial period of recrystallization, the exclusion of solutes accompanying ice growth results in the increase in the concentration of solute molecules and leads to more severe osmotic stress.[56] By binding with AF(G)Ps, the ice recrystallization can be greatly inhibited (Figure 4c), and the formed ice crystals remain small and numerous so that the blood circulation is not blocked, and the integrity of cells and tissues is not destructed by ice crystals.

The ice-shaping effect is an indicator that testified the existence of AF(G)Ps. The ice-shaping effect of AF(G)Ps results from their different affinity toward different crystallographic planes.[16] The planes adsorbed with AF(G)Ps are prohibited from further growth, while the unadsorbed planes grow preferentially, endowing ice with certain crystal shapes (Figure 4c). Theoretically, such angular shaped ice crystals are only a side effect of the adsorption of the AF(G)Ps, but do not favor the survival of living organisms. The possible beneficial effect of ice shaping in the cold evolution is still unknown.

3.3. Ice Nucleation Proteins. Another protein that can regulate ice formation is the ice nucleation protein (INP), which initiates ice nucleation at a relatively higher subzero temperature to prevent deep supercooling.[60] If nucleation occurs in a deeply supercooled temperature, the formed ice crystals tend to grow faster and more dendritic; meanwhile, the temperature following ice nucleation will rise close to the melting point of the solution before decreasing rapidly to the environment temperature due to the sudden release of the latent heat, which leads to sharp local temperature gradients. As a result, the fast and disruptive ice growth, the accompanying accumulation of salts, and the temperature shock (the sudden drop/raise of the temperature due to latent heat release) can cause more severe tissue damage.[61,62] With the INPs, ice formation at deep supercooling can be effectively prevented and the related injuries can be alleviated.

Although the effect of INPs is completely opposite to that of the AF(G)Ps on the ice formation, both INPs and AF(G)Ps can bind effectively to the ice surface; therefore, these two types of proteins are also termed as IBPs. It has been proved that these two extraordinary families of proteins, more specifically INP and hyperactive AFPs, actually share very similar repeat units, that is, both INPs and hyperactive AFPs have tandem arrays of amino acids as β-helix that can organize water molecules into the ice-like clathrate structures;[63] and the determining factor of ice inhibition or promotion between INPs and hyperactive AFPs is the size (Figure 4c). Molinero and co-workers[59] used MD simulations to elucidate the effect of the length (L) of IBPs on the nucleating efficiency of IBPs, including both INP and AFP. They proved that the ice nucleation efficiency of model proteins is a function of the length of the IBF. Figure 4d presents the ice nucleation efficiencies as a function of the L of the binding faces for the bacterial INP (*Ps*INP), the *Tm*INP made by stacking of ice-binding loops of the antifreeze protein *Tm*AFP, and the rigid fragments of alcohol monolayer, *Alcohol*INP, with the same width and lattice mismatch to ice. The three IBFs display the same qualitative behavior: ΔT_f is zero for very short molecules, then increases sharply, and finally plateaus. Wang and co-workers[64] prepared a series of narrow-sized graphene oxide (GO) nanosheets to experimentally investigate the effect of the size of nanosheets on the nucleation of water droplets. It was shown that ice nucleation occurs only above a certain size of GO that varies with the degree of supercooling of the water droplets. Inferred from the experimental data and theoretical calculations, it was concluded that the critical size of GO reflects the size of the critical ice nucleus; only when the size of GO is larger than that of the critical ice nucleus can the ice form; otherwise, the ice nucleus is pinned at the periphery of the GO, leading to a much higher free-energy barrier for nucleation and consequently freezing does not occur. As such, they used the GO to probe the critical nucleus size for ice formation, which had previously only been explored theoretically and through simulations due to its transient and nanoscale nature.

The IBPs, including both INPs and AF(G)Ps, are crucial ingredients for the survival of organisms living in subzero habitats. Some species can survive in subzero environments by preventing the formation of apparent ice crystals, and these organisms are referred as "freeze avoidant". The existence of AF(G)Ps is crucial for their survival, as AF(G)Ps bind to the possible ice nucleation sites and the existing tiny ice embryos to prevent the freezing of the body fluid; consequently, ice-induced injuries are avoided.[46,65] Some other species can survive when the body fluid is frozen and are referred to as "freezing tolerant". The coexistence of INPs and AF(G)Ps is critical, as the INPs initiate the ice nucleation at a relatively higher subzero temperature; meanwhile, the AF(G)Ps bind to the formed ice embryos to inhibit their uncontrolled growth and alleviate the accompanying ice-related injuries.[66−68]

4. THE CONTROVERSIAL EFFECT OF IBPS IN CRYOPRESERVATION

Inspired by the IBPs in effectively controlling ice nucleation/growth and protecting living organisms from ice-related damages, continuous investigations have been carried out for utilizing IBPs in the cryopreservation of tissues and organs. Similar to living organisms that survive freezing damage via "freezing avoidance" or "freezing tolerance", preservation of tissues and organs with IBPs can be categorized into avoiding-freezing and tolerating-freezing as detailed below.

4.1. Preservation by Avoiding-Freezing. Subzero preservation can considerably extend the viable preservation time of organs compared with the standard hypothermic preservation, as the metabolic rate decreases exponentially with the temperature.[69] For example, the preservation time of rat livers is <12 h by hypothermic preservation at +4 °C but can be prolonged to about 4 days when effectively stored at −6 °C.[70,71]

To realize avoiding-freezing subzero preservation, the freezing point of the body fluid can be depressed either colligatively by increasing the concentration of the colligative agents in the preservation solution or noncolligatively using AF(G)Ps. It

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Table 1. Controversial Effects of AF(G)Ps in the Cryopreservation of Tissues and Organs

Methods	Controversial outcomes		Determining factors
Freeze-avoiding	Promote supercooling	Initiate ice nucleation	1. Concentration of AF(G)Ps 2. Type of AF(G)Ps 3. Composition and concentration of other CPAs 4. Features of tissues and organs 5. Specific preservation protocols
Freeze-tolerating	Ice recrystallization inhibition Inhibit devitrification	Sharp ice crystals due to ice shaping Ice burst growth	

requires about 10 wt % sodium chloride (NaCl) to depress the freezing point of water to −6 °C, which is 10 times more than that of the physiological solution (equivalent to 0.90 wt % NaCl). Obviously, such a high salt concentration affects adversely the function of cells and tissues, thus simply increasing the solute concentration is of limited application. It is therefore obvious that the addition of AF(G)Ps provides an effective way to depress the freezing point noncolligatively by the Kelvin effect as noted above, at the same time, without greatly increasing the concentration.[46,72]

AFP III, which is isolated from Antarctic fish and can reduce noncolligatively the freezing temperature of their body fluid by 1.2 °C,[73] is the most frequently used AF(G)Ps for subzero preservation.[74] Using the mixture of the University of Wisconsin (UW) preservation solution and AFP III, rat hearts preserved at −1.3 °C can be protected from freezing damages. In contrast, all rat hearts preserved at −1.3 °C without the addition of AFP III completely lost their function due to the formation of ice.[72] By using a hyperactive insect AFP (*Tm*AFP), whose TH is as high as 5.5 °C, rat kidneys were stored at −4.4 °C with the Carolina Rinse (CR) preservation solution.[75] In this way, the preservation time of rat kidneys was prolonged to 72 h, which is three times longer than that stored at 4 °C. Utilization of the *Dendroides* antifreeze proteins (*D*AFPs), whose TH is as high as 10 °C, enables ice-free storage at temperatures down to −7 °C.[76] However, it was reported that AF(G)Ps are not always effective in promoting supercooling as expected. Wang and co-workers[77] studied the application of AFGPs for the perfusion preservation of rat hearts via exposure to −1.4 °C for 3−5 h. They reported that the presence of AFGPs increased the ice-related injuries due to the formation of destructive needle-like ice at high AFGP concentrations. There are even reports suggesting that the AFGPs promote intracellular freezing of rat cardiomyocytes at high subzero temperatures.[78] A consensus of beneficial effects of AF(G)Ps in promoting subzero preservation by preventing apparent ice formation is missing.

4.2. Preservation by Tolerating-Freezing. By mimicking the survival principles of freeze-tolerant species, that is, allowing the formation of ice crystals but controlling their nucleation sites, their size, shape and growth rate, so as to mitigate the ice-related injuries, the tissues and organs can be cryopreserved by programmed slow freezing.[79] Rubinsky and co-workers[80] managed to freeze rat livers by closely mimicking freeze-tolerant species: Rat livers were infused by glycerol and AFGP isolated from North Atlantic Cod, and then ice nucleation was intrigued at a higher subzero temperature of −3 °C and kept for 6 h. On subsequent *ex vivo* reperfusion, these livers produced bile and had a well-preserved histological morphology, compared to livers frozen in the absence of AFGP. When the AF(G)Ps are used in the presence of ice crystals, the IRI activity instead of the TH activity ensures the protective effect. For example, it has been found that the moderately active *Le*AFP protects mouse ovarian tissue more effectively than the hyperactive *Ff*IBP, whose TH value is ∼10 times higher, in slow freezing preservation.[81] However, contradicting effects of AF(G)Ps in the slow freezing are also widely reported. Both positive and negative outcomes were reported when using AFP I and AFP III in the embryos' slow-freezing preservation. It has also been reported that improved viability outcomes are observed at low AFP concentrations but not at high concentrations.[82] Indeed, both in the preservation of cells and tissues, higher concentrations of AF(G)Ps usually lead to a decrease in the post-thaw survival of the preserved biosamples compared with that of low concentration ones.[83]

Some studies have also reported beneficial effects of AF(G)Ps in the preservation of tissues via vitrification,[84] and some underlying protection mechanisms have been proposed: (1) decrease in the freezing temperature and control of the ice crystal shape;[85] (2) inhibition of ice recrystallization and devitrification during thawing;[86] (3) protection of the plasma membrane;[87] and (4) reduce reactive oxygen species production in the vitrification−warm process.[88] Some investigations also reported that the addition of AF(G)Ps in the preservation solutions can also reduce the amount of conventional CPA needed for vitrification. However, there are also some reports revealing that the addition of AF(G)Ps induces more severe ice injuries possibly due to the bursting growth of ice crystals beyond the TH gap of the used AF(G)Ps.

A survey of the research about using AF(G)Ps for the cryopreservation has made it clear that the effects of AF(G)Ps in the cryopreservation are complicated and, sometimes, even controversial. As summarized in Table 1, the AF(G)Ps can both promote and depress supercooling in the preservation of tissues and organs at high subzero temperatures. When tissues and organs are aimed to be preserved in a freeze-tolerant manner, both ice growth inhibition and ice burst growth with sharp ice edges have been reported. Moreover, the beneficial effect of AF(G)Ps is found to be dependent on a variety of factors, including the concentration, the type of AF(G)Ps, the composition and concentration of other cryoprotectants, the features of the tissues and organs, and the specific preservation protocols. Because of the reported controversial effects of the AF(G)Ps on the cryopreservation of tissues and organs, further investigation on the mechanisms of AF(G)Ps on controlling ice formation during the cryopreservation of tissues and organs is urgently needed so that breakthroughs can be achieved.

5. THE CONTROVERSIAL EFFECT OF IBPS IN ICE REGULATION

Reported controversial effects of IBPs in the cryopreservation of tissues and organs can be reconciled when considering the following two facts: (1) The molecular structure and aggregation behavior of IBPs differ greatly for different IBPs, which certainly affects the capability of IBPs in controlling ice

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formation; and (2) the native environments differ dramatically from the *in vitro* diluted solutions and are distinct for different tissues and even the same tissue at different locations, which can have a completely different effect on the activity of the IBPs in regulating ice nucleation and growth.

5.1. The Effect of the Molecular Structure and Aggregation of AF(G)Ps in Ice Regulation. *5.1.1. AF(G)Ps in Ice Inhibition.* According to the adsorption−inhibition mechanism, the activity of AF(G)Ps in controlling ice formation is closely related to their efficacy of binding with the ice surfaces. The AF(G)Ps have a Janus feature, containing an IBF and a NIBF. MD simulations revealed that water molecules atop the IBF display an ordered hexagonal ice-like structure,[49] which facilitates the recognition and binding of the AF(G)Ps to ice surface by lowering the energy barrier for binding. Meanwhile, the disordered water structure around NIBF helps prevent the adsorbed protein from becoming engulfed by ice due to the increased energy barrier for converting the disordered interfacial water into an ordered ice-like one; this leads to the formation of curvatures on the ice surface, which in turn facilitates the decrease of freezing point following the Kelvin law.[89] As shown in the left illustration of Figure 5a, only when the IBFs of AF(G)Ps bind to the ice surface can the growth of ice crystals be effectively inhibited.

Because of the Janus feature of the IBPs, it is critically important whether the IBFs of the AF(G)Ps are exposed to the ice surface available for binding to ice. If the NIBFs are exposed, they do not have the capability for binding to ice surface, thus losing the freezing point depression activity. Two possible situations with exposed or buried IBFs are as follows:

(1) Components binding with the IBFs or NIBFs (Figure 5a, middle). It has been reported that the presence of large groups attached to the AFP (fusion proteins) of the non-ice-binding regions increases the area covering the ice surfaces, which leads to an increased ice surface curvature and, consequently, an increase in TH values.[91] However, if the IBFs are covered by other components with the NIBFs being exposed, the IBPs fail to bind to the ice surfaces with the loss of the TH activity.

(2) Formation of multimers or aggregates with the IBFs or NIBFs exposed to ice surfaces (Figure 5a, right). Davies and co-workers genetically fused AFPs to a 24-subunit protein cage, resulting in a multimer containing 12 AFPs with their IBFs being exposed to the ice surface, which exhibited freezing point depression >50-fold greater than monomeric AFPs. This high efficiency is explained by the more curved ice surfaces formed by adsorbing the AFP multimers, as shown in Figure 5a, right column.[90] Similar multimer-enhanced TH activities have been widely observed in nature[93] and synthetic systems.[94] On the other hand, if all the IBFs are buried inside the multimer, the freezing depression capability will surely be lost.

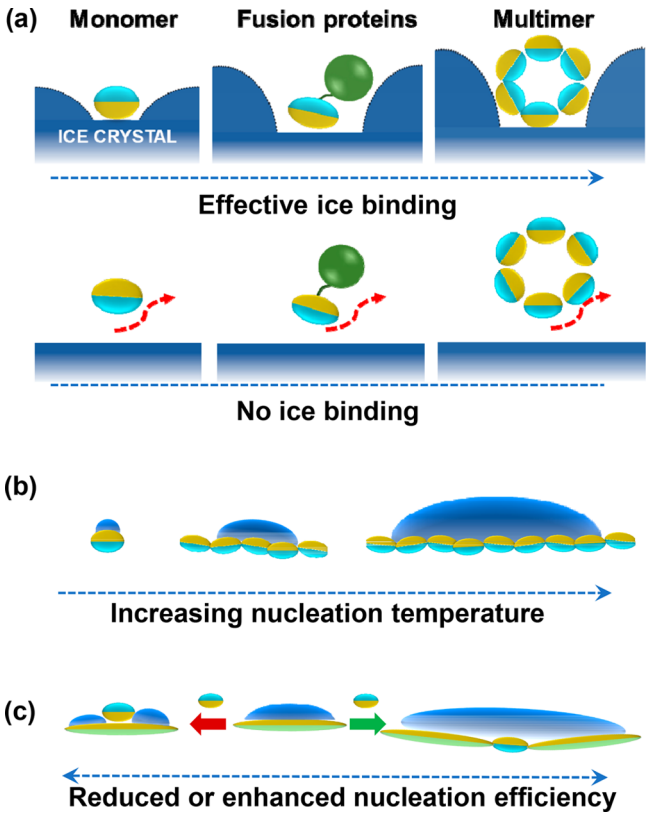

Figure 5. Controversial effects of AF(G)Ps in regulating ice formation. (a) The Janus feature of AF(G)Ps and the exposure or burying of the IBFs by molecular interactions and aggregation can greatly influence the activity of AF(G)Ps in controlling ice growth.[90,91] (b) Larger lateral size of AF(G)P aggregates induces the increase in ice nucleation temperature. (c) The adsorption of AF(G)Ps on or between INPs can either inhibit or promote nucleation activity.[92]

As AF(G)Ps are amphiphilic, having a relatively hydrophobic IBF and a relatively hydrophilic NIBF,[95] AF(G)Ps tend to form aggregates when dissolved in water, with the hydrophobic IBFs buried inside and the hydrophilic NIBFs exposed.[96] Interestingly, such aggregates were observed to enhance but not reduce the TH values of the AF(G)Ps.[96] A possible reason is the dynamic nature of the aggregates, which aggregate and disaggregate all the time, and the thermodynamic equilibrium changes in the presence of ice crystals. It is also noteworthy to emphasize that the investigation about the aggregation behaviors of AF(G)Ps is far from satisfactory, and sometimes contradictory results are reported even for the same AF(G)P.[96−98] All these seemingly contradictory effects of AFPs contribute to the observed controversial results when using AFPs in the cryopreservation of tissues and organs.

5.1.2. Effect of Aggregates on Ice Nucleation. The way AF(G)Ps aggregate can also affect greatly ice nucleation.[96] As revealed by recent investigations, the larger the lateral size of the AF(G)P aggregates, the higher the ice nucleation temperature can be (Figure 5b).[59,99] Also, Wilson and co-workers[100] reported that for the AFP I, ice nucleation is enhanced rather than hindered when the concentration is above ∼8 mg/mL, possibly because of the formation of AFP I aggregates at high concentrations. A recent report by Koop and co-workers showed that both AFP III and *Tm*AFP can also trigger ice nucleation at higher concentrations.[99] Note that the effect of AF(G)P aggregates in promoting ice nucleation is highly dependent on the surfaces of AF(G)Ps and aggregates in contact with water; that is, if IBFs of AF(G)Ps are in contact with water, ice nucleation is promoted; otherwise promotion of ice nucleation may not be obvious.[49]

It is widely reported that AF(G)Ps can effectively deactivate the ice nucleation sites via binding to potential ice nucleation sites, as illustrated in Figure 5c, left.[101,102] Interestingly, a recent investigation by Meister and co-workers revealed that such an effect is protein specific,[92] as they discovered that larger

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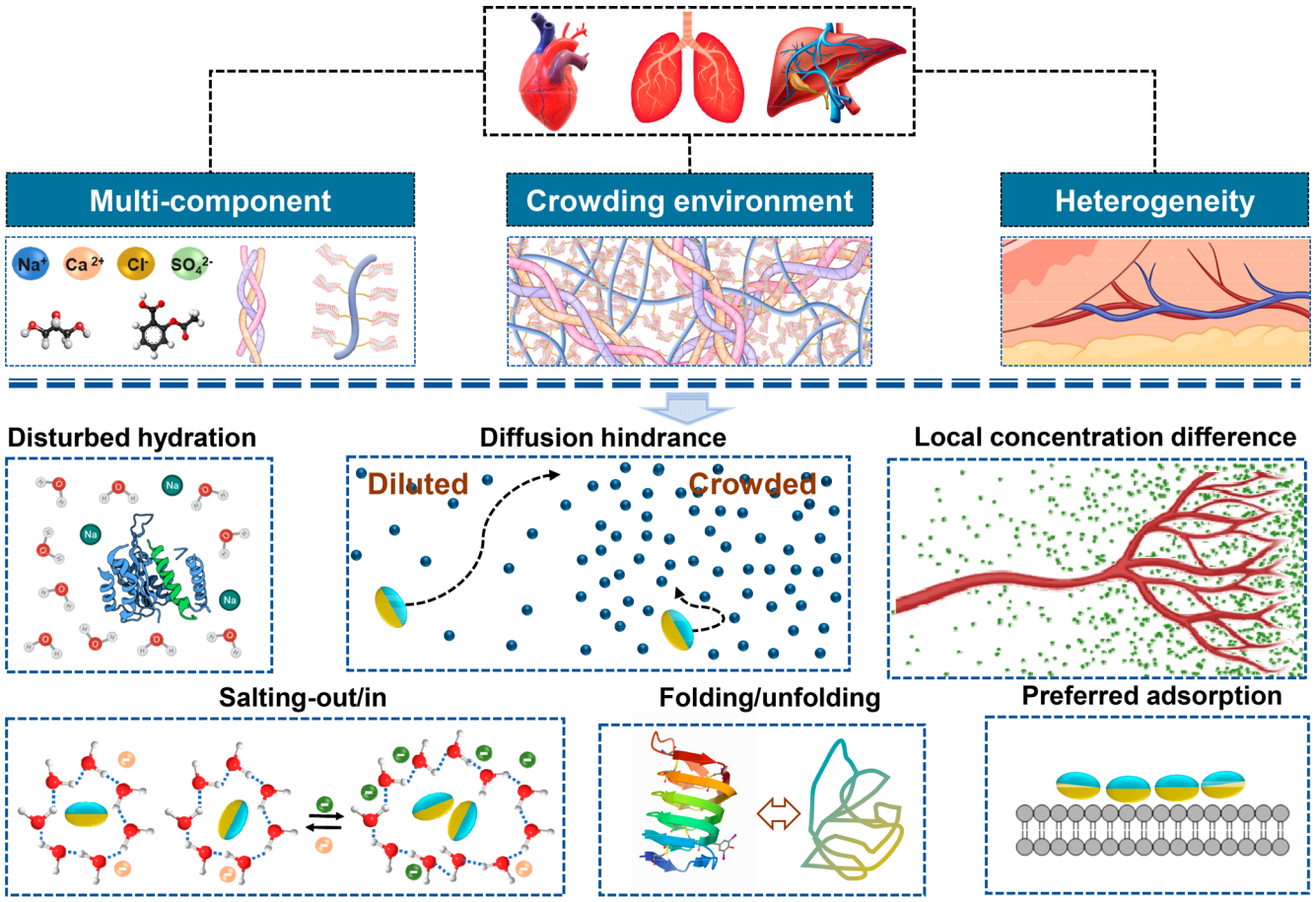

Figure 6. Multicomponent, crowded, and heterogeneous nature of the microenvironments inside different tissues and organs affects the conformation, aggregation, and distribution of IBPs, which may profoundly affect their ice regulation activities in the cryopreservation of tissues and organs. Created with BioRender.com.

AF(G)Ps, such as larger AFGP1−5 isoforms, can act as bridges to bring together INPs leading to much larger INP aggregates; consequently, ice nucleation is greatly facilitated rather than inhibited due to the increased size of the ice nucleation sites (Figure 5c).[92]

5.2. Influence of the Microenvironments of Tissues on the Activity of IBPs. In addition to the complicated molecular structure and aggregation behaviors of the IBPs, the complexity of the structure and composition of tissues and organs can inevitably influence the ice regulating properties of IBPs, which can also account for their controversial effect in the cryopreservation of tissues and organs. As summarized in Figure 6, the fluid within the tissues and organs differs from the dilute solution in three important aspects. First, the *in vivo* environment of tissues and organs is a multicomponent system, which contains both organic and inorganic ingredients, including cationic/anionic ions, small-molecule metabolites, and biomacromolecules such as collagen, proteins, and hyaluronans.[103] Interaction of these individual molecules with IBPs can affect the activity of IBPs in controlling ice formation. Second, the total concentration of these soluble molecules in the tissues and organs is usually very high, occupying a substantial fraction of the total volume of the medium.[104] Such an environment exerts surprisingly large effects on the thermodynamics and kinetics of processes such as intermolecular associations and self-aggregation.[104] Third, different types of cells and extracellular components in different tissues and organs form varying and sometimes completely different heterogeneous microenvironments with a rich variety of interfaces. These interfaces can also affect the adsorption, aggregation, and local concentration of IBPs and, consequently, their ice controlling activity.

Inorganic ions and small organic osmolytes are ubiquitous in the interstitial fluid in the tissues and organs. It has been reported that these inorganic/organic small molecules can disturb the hydration layer of IBPs and thus affect the adsorption of IBPs on the ice surfaces and consequently the TH values (Figure 6).[105−108] The inorganic salts induce salting-out/in effects on IBPs following the Hofmeister series, which may promote cluster formation[109,110] and enhance the adsorption of IBPs on the ice surfaces, inducing an increase in the TH activity. However, there are also studies reporting the inactivation of AFGPs via complexing the carbohydrate *cis*-hydroxyl groups with borate.[111] The small organic osmolytes, especially the seasonal regulated ones, may have a pronounced effect on the TH activity of the AF(G)Ps. It has been reported that citrate can increase the TH value of *Dendroides canadensis* AFP from 1.2 to 6.8 °C, which is nearly 6-fold higher.[112] Other solutes that can increase the TH value by about 4-fold are succinate, malate, aspartate, glutamate, and ammonium sulfate. Glycerol, sorbitol, alanine, and ammonium bicarbonate are reported to increase the TH value by approximately 3-fold. Actually, among the survival strategies of living organisms in subzero habitats, the production of a high concentration of organic osmolytes, for example, glucose, glycerol, and urea, is also very important. These organic

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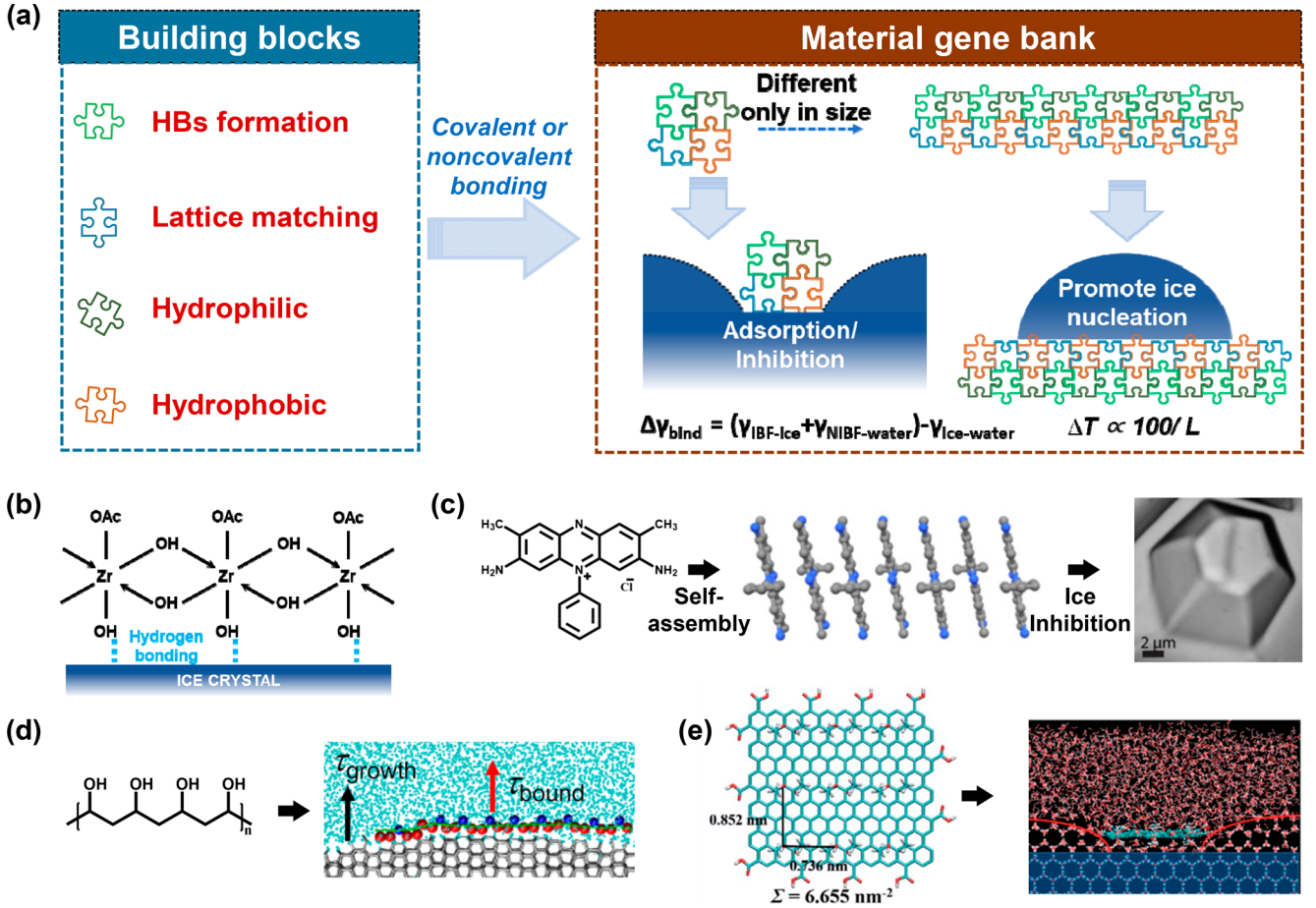

Figure 7. (a) Guiding lines of construction of IBMs for controlling ice formation. (b) Proposed mechanism for ice recognition by ZrAc. Reproduced with permission from ref 137. Copyright 2012 American Chemical Society. (c) The adsorption of safranin-O on ice crystals by supramolecular stacking. Reproduced with permission from ref 138. Copyright 2016 American Chemical Society. (d) The binding of poly(vinyl alcohol) (PVA) on the ice crystals by repeated hydroxyl groups. Reproduced with permission from ref 139. Copyright 2020 American Chemical Society. (e) Ice adsorption of two-dimensional graphene derivatives. Reproduced with permission from ref 140. Copyright 2020 Royal Society of Chemistry.

metabolites may work synergistically with the IBPs in these living organisms to facilitate freeze-avoidance or freeze-tolerance.[107,113−115]

Another feature of the tissues and organs is that they are highly crowded with various inorganic/organic components.[116,117] It is well-known that the level of crowding can easily alter various biological processes.[117−120] Here, we want to emphasize three important aspects (Figure 6): (1) Protein diffusion in the crowded *in vivo* environment is found to be slowed down to ∼20% of that in the dilute media solely due to hydrodynamic interactions,[121] as the AF(G)Ps have to diffuse to the ice surface before effective binding. If the diffusion of AF(G)Ps is slower than their binding on the ice surfaces, diffusion dominates the adsorption−inhibition process.[122] (2) Depletion force induced aggregation or adsorption: Gibson and co-workers[123] reported that the depletants shift the equilibrium toward ice binding, hence enabling a higher IRI activity at lower concentrations. (3) Crowding is a parameter that affects the conformational isomerization of the proteins,[104,119,120,124] including the regularity of the function groups and the secondary structures of the proteins, which are crucially important to ensure the ice affinities of IBPs.[125]

Tissues and organs are ensembles of cells and extracellular matrices. Consequently, there are always interfaces that lead to preferential adsorption and aggregation of IBPs on the interfaces. Meanwhile, the local concentration of IBPs could differ greatly due to the existence of interfaces. If the INPs are enriched in certain locations, ice preferentially grows there. Note that even for the freeze-tolerant species, ice forms in some specific locations. It is highly possible that the ice nucleation in particular sites is a result of adaptive evolution. For example, INPs are often found in the blood vessels,[126,127] preferentially initiating ice nucleation there, because the formation of ice crystals in the lumen of blood vessels is less detrimental. As such, the presence of a rich variety of interfaces leads to the difference in the local concentration of IBPs, which could be beneficial for cryopreservation if the distribution can be regulated.

As the effectiveness of IBPs in controlling ice formation and protecting living organisms from freezing damage always depends on the microenvironments the IBPs locate, a full understanding of the working mechanisms of IBPs in the *in vivo* native environment must be achieved before IBPs and mimics can be used for the cryopreservation of tissues and organs. The last decades have witnessed the fast development of high-resolution and *in vivo* real-time detection techniques in crowded biological environments. For example, researchers have been able to determine the high-resolution three-dimensional structure of proteins inside crowded biological environments for the first time by combining "in-cell" nuclear magnetic resonance spectroscopy and the cutting-edge computational

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algorithms.[128,129] Imaging techniques such as the ambient mass spectrometric imaging,[130] single-molecule localization microscopy,[131,132] and chemical imaging technique[133] are becoming ever-more powerful tools for quantitatively visualizing macromolecules in the complex *in vivo* environment. Fluorescence correlation and cross-correlation spectroscopies have seen a greater rise in the last decades revealing information about flow velocity, protein−protein binding, ligand−receptor affinity, and transport in living organisms.[134−136] Linking IBPs with fluorescent molecules, the dynamics of IBPs in their *in vivo* surroundings can be determined. It is therefore possible to reveal the conformation, aggregation, distribution, and dynamics of IBPs in the crowded *in vivo* environment and discover the specific ice nucleation sites and the ice growth behavior in tissues and organs. As such, a correlation between the structure and the function of IBPs in the tissues and organs can be established that will guide the application of IBPs in the cryopreservation of human tissues and organs.

6. ICE-BINDING MATERIALS AS CPA FOR THE CRYOPRESERVATION OF TISSUES AND ORGANS

6.1. Building the Library of Bioinspired Ice Binding Materials. As the natural IBPs are specifically designed to work synergistically within their *in vivo* microenvironments, so as to protect the insects, fishes, and amphibians, for example, beetle *Cucujus clavipes*,[43] polar fishes,[40] and wood frogs,[126,127] these proteins may not be suitable for the cryopreservation of the tissues and organs of human beings; as such, it is urgently necessary for chemists and materials scientists to design and construct a library of materials that can effectively control ice formation. In the past decade, many IBMs mimicking IBPs have been synthesized, which have already displayed great potential for the cryopreservation of cells. Based on the current understanding of the mechanisms of IBPs, we summarize the designing principles of the IBMs in the following sections (Figure 7).

6.1.1. HB Forming Groups. Without any exceptions, the IBMs with good ice affinities include HB forming groups both on the IBFs and NIBFs, such as hydroxyl, carboxyl, and amino groups. It was reported that the lifetime of the HBs between water molecules and the IBFs is longer compared with that on the NIBFs,[141] and this elongated HBs lifetime on the IBF surface is correlated with their capability in binding to the ice surfaces. Replacing the threonine to tyrosine on the IBFs decreases the HBs lifetime to be comparable with the NIBF, which results in 90% loss of the TH activity of *Tm*AFP. The amino acid residues frequently discovered on the IBFs of IBPs include alanine, glycine, proline, threonine, cysteine, and glutamines,[52,142] and this may guide the selection of ice-binding moieties in constructing IBMs.

6.1.2. Arrangement of HB-Forming Groups Matches the Ice Lattice. The periodic arrangement of HB-forming groups which matches the periodicity of ice lattice is an important prerequisite for the effective binding of IBMs on the ice surfaces.[143,144] The single α-helix structure of fish type I AFP, which contains a linear-like array of regularly spaced threonine residues, enables lattice-matching regularity in one direction.[145] While the hyperactive insect AFPs, taking *Tm*AFP as an example, which owes six short β-strands form a remarkably flat β-sheet, have two-dimensional array of threonine side chains that makes a remarkably good match to the repeated spacing between oxygen atoms in the ice lattice on the primary prism plane.[146] The periodic arrangement of HB-forming groups of the IBMs can be achieved covalently or noncovalently, including coordination effect,[147] supramolecular assemblies,[138] polymerization,[148] and so on.[149,150]

6.1.3. Amphiphilic Nature. The requirement for the high activity in controlling ice growth is the full adsorption of the IBMs at the ice−water interface. It is a thermodynamically favorable process when the binding free energy per unit area of the IBMs to the ice surface, $\Delta\gamma_{bind} < 0$.[151] That is, the sum of interfacial energy of the IBF of the IBMs with ice ($\gamma_{IBF\text{-}ice}$) and that between NIBF of the IBMs and the water ($\gamma_{NIBF\text{-}water}$) is lower than the ice−water interfacial energy ($\gamma_{ice-water}$), written as $\Delta\gamma_{bind} = (\gamma_{IBF\text{-}ice} + \gamma_{NIBF\text{-}water}) - \gamma_{ice-water}$, and $\Delta\gamma_{bind} < 0$. Therefore, the efficient adsorption of IBMs requires both icephilic and hydrophilic natures of the IBMs. To endow the IBMs with proper icephilicity, the HB forming groups on the IBFs are usually arranged to match the ice lattice. In addition to HB forming groups, it is proposed that the addition of methyl groups[152] can enhance the icephilic nature of the IBFs due to the entropy gain in the dehydration process when the methyl groups nest in the cavities on the ice lattice.[152,153] Meanwhile, when the IBFs are bound on ice surfaces, it is crucially important that the NIBFs are not engulfed by ice. Therefore, the NIBFs should be an "ice-phobic" disorder where the interfacial water structures do not match with the ice lattice and create an energy barrier for converting the disordered interfacial water into an ordered ice-like one. At the same time, the NIBFs should also be hydrophilic to endow the IBMs with good solubility. The disordered arrangement of HBs forming groups and the introduction of charged groups are frequently used strategies to construct NIBFs.

By meeting the above-mentioned three criteria, IBMs with efficient ice-binding affinity are achieved, and ice growth can be inhibited. Note that the capability of IBMs in facilitating ice nucleation is highly dependent on the size of IBMs (right two roles in Figure 7a). Molinero and co-workers discovered for the first time via MD simulation that AFPs can greatly increase the ice nucleation temperature with an increase in the number of ice-binding loops.[59] Koop and co-workers proved experimentally that the AFPs can promote ice nucleation in a size-dependent manner, that is, larger AFPs promote ice formation at higher temperatures.[99] A direct size dependency of nanoparticles on promoting ice nucleation was reported in the GO systems.[64] It was found that the potency of GOs in promoting ice nucleation as measured by the nucleation supercooling (ΔT) is inversely proportional to the lateral size (L) of GOs, which may be extended to other nanoparticles.

So far, the developed IBMs range from small molecules to polymers and nanomaterials. The smallest ice-binding molecule found is an inorganic salt, that is, the zirconium acetate (ZrAc), which can form complexes with the hydroxyl groups periodically arranged to match with the ice lattice (Figure 7b).[147] Small organic molecule safranin-O can self-assemble by $\pi-\pi$ stacking to order amino groups matching with the ice lattices (Figure 7c).[138] It was interestingly reported that safranin-O not only has good activities of IRI and ice shaping but also shows an obvious thermal hysteresis property with the TH value as high as 0.6 °C. Polymers, which have adjustable repetitive functional groups and varying degrees of polymerization, are also promising IBMs. Poly(vinyl alcohol) (PVA) is the most active synthetic polymer with the most active IRI activity reported so far.[148] The spacing between the neighboring hydroxyl groups on PVA matches well with the lattice on the basal plane of the ice crystals and hence may explain its high IRI activity as proposed by Koop and co-

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workers.[154] Molinero and co-worker[139] reveal that the limiting step for the binding of flexible molecules such as PVA to ice is not the alignment of the molecule to the surface or the initiation of the binding but the propagation to reach its full binding potential (Figure 7d). Recently, it was reported that nanomaterials, which have fixed structures, precise and adjustable function groups, can also be designed to exhibit ice affinities.[149,150] MD simulations reveal that the modification of $-CH_3$ and $-OH$ groups similar to the IBFs of AFPs on the surface of graphene enables their effective adsorption on ice lattice (Figure 7e).[140] This also applies for the three-dimensional nanomaterials. It was found that the long-range arrangement of HB donors on the surface of a zirconium (Zr)-based metal organic framework can provide accurate matching with the ice crystal faces and has a good IRI activity.[155] Such IBP-inspired IBMs have received great success in cryopreservation of cell suspensions, including RBCs,[150,156] sperms, oocytes,[149,157] and cell monolayers.[158]

6.2. Specific Requirements of IBMs for the Cryopreservation of Tissues and Organs. Although some IBMs have already received great success in the cryopreservation of cells and hold great promise in tissue cryopreservation, additional requirements need to be met before using IBMs for the cryopreservation of tissues and organs due to the multicomponent and crowded environment of tissues and organs, as illustrated in Figure 8.

6.2.1. Biocompatibility. The biocompatibility of IBMs used for cryopreservation of tissues and organs should not only include the low cytotoxicity to the cells[39] but also no destructive effects to the structure of the tissues, including the extracellular matrix and the cell junctions, etc.[38,159,160] In addition, the IBMs should not induce rejection effects and, at the same time, should be easily removable after thawing. Major variables that could influence the biocompatibility include the chemical composition, the micro- (or nano)-structure, the crystallography, the hydrophobic−hydrophilic balance, the surface charge properties, the degradation products, and so on.[161] It is apparent that whether an IBM is biocompatible depends on various criteria, and a series of tests must be conducted to ensure that it meets the standards of use,[162] such as testing the effect of the IBMs on the morphology integrities by histological imaging and evaluate the metabolism of tissues and organs with the presence of IBMs.

6.2.2. Controllable Aggregation of IBMs in the Crowded In Vivo Environment. Controllable lateral alignment of the IBMs can be achieved by decorating noncovalent linking groups on the opposing positions of the IBMs, as illustrated in Figure 8, which promote their ice-inhibiting efficiency by enabling larger adsorption areas on the ice crystals. However, sometimes the aggregation of IBMs is undesirable due to the following two reasons. First, the aggregation of IBMs into large clusters may lead to an undesirably enhanced ice nucleation activity. Second, the complexation of IBMs with other components may bury the IBFs, which inactivate the ice-binding capability of the IBMs. Therefore, strategies for preventing undesired aggregation are also necessary when IBMs are applied in the cryopreservation of tissues,[163,164] especially when the IBMs are dispersed in the crowded interstitial space as the crowding effect may dramatically affect the aggregation propensity of the IBMs. Traditional strategies inhibiting aggregation include modifying the IBMs with moieties with charged repulsions or steric hindrance and introducing inert noninteracting macromolecules such as polyethylene glycol, polyethylenimine, galactose, and so on.[165] All of these strategies may also be applied in inhibiting the aggregation of IBMs in the tissues and organs.

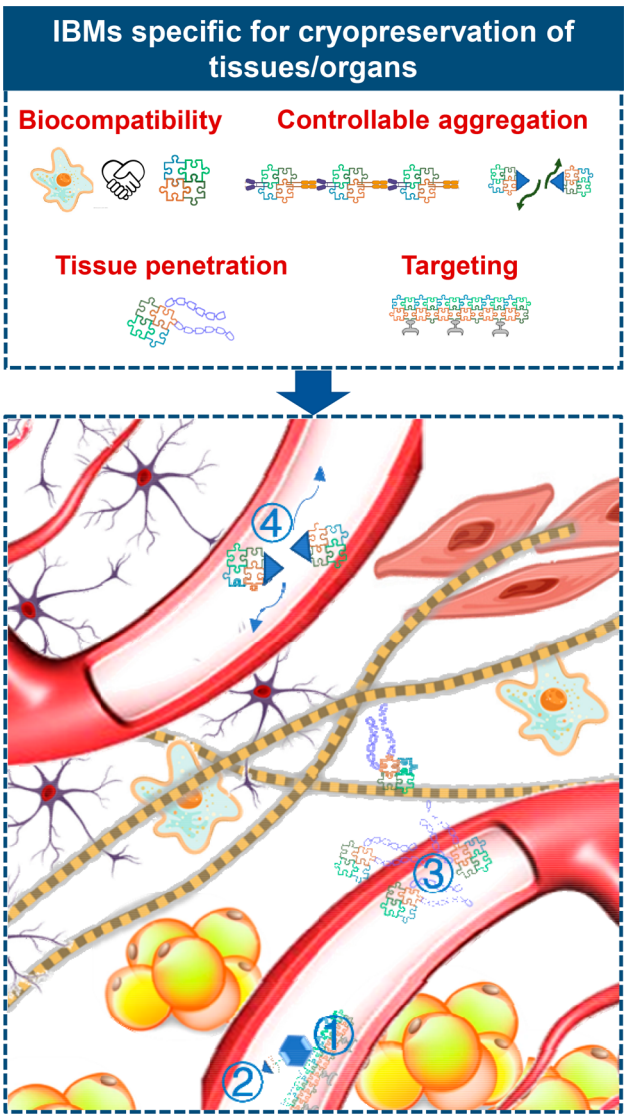

Figure 8. A scenario of ideal IBMs for the cryopreservation of tissues. Created with BioRender.com.

6.2.3. IBMs Can Facilely Permeate into the Tissues and Organs. The amount of IBMs permeated into the tissues/organs is crucial for appropriate protection of the cells in deep tissues and the intracellular structures. Tissue permeability is regulated by a molecular mechanism that involves the endothelial barrier, which has a high density of endothelial cells and adherens junctions and tight junctions between them, so as to control the extravasation of plasma and IBMs.[166] Mechanisms for enhancing the tissue permeability to macromolecules have been widely investigated in the field of drug delivery, and several strategies have been proposed.[167] For example, the cell-penetrating peptides (CPPs)[168] have been used to enhance the cell penetration ability of proteins, enzymes, mRNA, and polymers. Another approach to enhance tissue penetration is by using nanocarriers, such as polymeric complexes, superparamagnetic nanoparticles, quantum dots, dendrimers, and lipid nanoparticles.[169] Recently, it has been reported that nanoparticles fabricated by copolymers with dendritic arginine-rich CPPs, which mimic viral protein

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transduction domains, can realize not only robust cell entry but also vigorously penetration into deep tissues.[170] Therefore, grafting IBMs with cell penetrating peptides, carrying IBMs with nanostructures, or using dendritic-viral mimicking structures are promising approaches to enhance the permeability of IBMs to tissues and organs.

6.2.4. Targeting. Note that even for the freeze-tolerant species, ice forms only in some specific locations, such as the coelom of the wood frogs, the hemolymph,[171] malpighian tubules,[172] fat body cells,[172] muscle cell membranes, and epidermis cell membranes[173] of some insects. In wood frogs, which can survive after most of their body water freezes, about 25% of all ice is sequestered within the coelom and lymph sacs, and over half of the water in some organs is relocated to spaces where it freezes innocuously.[171] Therefore, ice only forms in less detrimental locations but not in ice-sensitive locations, in this way the survival rates of these frogs or insects are greatly increased. In human tissues and organs, there should also have so-called less detrimental spaces where ice formation is less destructive to the function of the tissues. Therefore, targeting of IBMs to less destructive locations, especially the ice nucleation materials, is of critical importance for the tissues to survive from freezing damages. Targeting strategies for molecules or nanoparticles based on small molecules, aptamers, peptides, antibodies, and cell-recognitions have been intensively investigated and well-reviewed in the field of targeted drug delivery,[174,175] which also provide references for targeting IBMs to specific locations in the tissues and organs. Among them, we emphasize here, a very promising approach to target IBMs by utilizing the homing peptide[176−178] that distributes in the blood vessels of specific tissues. It is reported that such peptides are capable of mediating selective localization of drugs, proteins, or nanocarriers to the blood vessels of specific tissues such as the white fat vasculature,[179] brains, and kidneys.[177] We believe that by targeting the IBMs to specific locations of tissues to initiate or inhibit ice formation, it is possible to mimic the survival of freeze-tolerant species and preserve tissues and organs at cryogenic temperature. The relatively safe locations for ice formation may include blood vessels, adipose tissue, muscles, etc., but there is no broad consensus on these arguments.[171−173] It is necessary to have a more in-depth study on the freezing tolerance of human tissues to ice crystals, so as to guide the distribution of ice crystals in tissues and organs during cryopreservation.

After building the antifreeze toolbox with IBMs designed specifically for the cryopreservation of tissues and organs, an ideal scenario of tissue cryopreservation is as follows: "The IBMs are perfused into the blood vessels of the organs. Along with the circulation of the IBMs, the ice nucleation IBMs are delivered to the targeted positions such as the blood vessel of specific tissues, ① in Figure 8. Therefore, ice crystals will form preferentially at these targeted positions. However, the ice crystals grow at a tardy rate and remain extremely small because of the existence of the ice-inhibiting IBMs, ② in Figure 8. At the same time, the IBMs with good tissue permeability will penetrate deep into the tissues and mask the possible ice nucleation sites or inhibit the growth of ice embryos in tissues that are sensitive to ice injuries, ③ in Figure 8. The IBMs will not aggregate unexpectedly into clusters due to the decoration of IBMs with local charge repulsion or steric repulsions, ④ in Figure 8. Therefore, the damage of ice formation to tissues/organs is minimized. In the thawing process, the size of the ice crystals is also controlled by the IBMs. As such, the tissues and organs maintain integrated histological morphology and good function in the whole cryopreservation process."

7. SUMMARY AND PERSPECTIVE

Tissue and organ shortage is among the greatest crises facing biomedicine today,[1] and success in cryopreservation of tissues and organs can substantially increase their availability; therefore, cryopreservation should be placed at the top of our scientific priority list.[12,180] The key challenge for the tissue and organ cryopreservation is the effective control of ice formation, which cannot be achieved by conventional CPAs widely employed for the cryopreservation of cells. Therefore, the discovery of new CPAs with high efficacy in regulating ice formation is required, which is indeed a mission for chemists. Understanding the mechanism of some living organisms in protecting themselves from freezing damage provides a unique solution to tackle the uncontrolled ice formation in tissues and organs during the cryopreservation, which is now highly possible due to rapid progress in imaging, sequencing, omics approaches, and others. It has already been revealed that IBPs are the essential materials these living organisms employ for regulating ice formation and consequently protect themselves from freezing damage. Therefore, building a library of IBPs inspired materials to be used as effective CPAs for controlling ice formation can lead to a completely new avenue for the cryopreservation of tissues and organs. Note that the construction of such an ice-binding material library specifically designed for the cryopreservation of tissues and organs requires coordinated cross-disciplinary research, so that multilevel structures and physiological functions before and after cryopreservation can be accurately assessed, and consequently, the design and synthesis of novel IBMs can have timely feedback. Meanwhile, the functions of tissues and organs after cryopreservation can be appropriately intervened with various newly developed technologies. In addition, rewarming techniques which use nanoparticles in combination with the electromagnetic field have also provided new approaches that enable fast and/or unified heating.[181,182] As the past decade has witnessed the great success of the cryopreservation of cells with IBMs, we, therefore, are confident that effective cryopreservation of tissues and organs with IBM as the CPA is an ambitious but achievable goal.

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Notes

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■ REFERENCES

(1) Giwa, S.; Lewis, J. K.; Alvarez, L.; Langer, R.; Roth, A. E.; Church, G. M.; Markmann, J. F.; Sachs, D. H.; Chandraker, A.; Wertheim, J. A.; Rothblatt, M.; Boyden, E. S.; Eidbo, E.; Lee, W. P. A.; Pomahac, B.; Brandacher, G.; Weinstock, D. M.; Elliott, G.; Nelson, D.; Acker, J. P.; Uygun, K.; Schmalz, B.; Weegman, B. P.; Tocchio, A.; Fahy, G. M.; Storey, K. B.; Rubinsky, B.; Bischof, J.; Elliott, J. A. W.; Woodruff, T. K.; Morris, G. J.; Demirci, U.; Brockbank, K. G. M.; Woods, E. J.; Ben, R. N.; Baust, J. G.; Gao, D.; Fuller, B.; Rabin, Y.; Kravitz, D. C.; Taylor, M. J.; Toner, M. The promise of organ and tissue preservation to transform medicine. *Nat. Biotechnol.* **2017**, *35*, 530−542.
(2) Messner, F.; Guo, Y.; Etra, J. W.; Brandacher, G. Emerging technologies in organ preservation, tissue engineering and regenerative medicine: a blessing or curse for transplantation? *Transpl. Int.* **2019**, *32*, 673−685.
(3) Dragunow, M. The adult human brain in preclinical drug development. *Nat. Rev. Drug Discovery* **2008**, *7*, 659−666.
(4) Baker, M. Building better biobanks. *Nature* **2012**, *486*, 141−146.
(5) Hu, B. C. The human body at cellular resolution: the NIH human biomolecular atlas program. *Nature* **2019**, *574*, 187−192.
(6) Muotri, A. R. The human model: changing focus on autism research. *Biol. Psychiatry* **2016**, *79*, 642−649.
(7) Weinstein, J. N; Collisson, E. A; Mills, G. B; Shaw, K. R M.; Ozenberger, B. A; Ellrott, K.; Shmulevich, I.; Sander, C.; Stuart, J. M The cancer genome atlas pan-cancer analysis project. *Nat. Genet.* **2013**, *45*, 1113−1120.
(8) Matas, A. J.; Smith, J. M.; Skeans, M. A.; Thompson, B.; Gustafson, S. K.; Stewart, D. E.; Cherikh, W. S.; Wainright, J. L.; Boyle, G.; Snyder, J. J.; Israni, A. K.; Kasiske, B. L. OPTN/SRTR 2013 Annual data report: kidney. *Am. J. Transplant.* **2015**, *15*, 1−34.
(9) Taylor, M. J.; Weegman, B. P.; Baicu, S. C.; Giwa, S. E. New approaches to cryopreservation of cells, tissues, and organs. *Transfus. Med. Hemother.* **2019**, *46*, 197−215.
(10) Coppola, L.; Cianflone, A.; Grimaldi, A. M.; Incoronato, M.; Bevilacqua, P.; Messina, F.; Baselice, S.; Soricelli, A.; Mirabelli, P.; Salvatore, M. Biobanking in health care: evolution and future directions. *J. Transl. Med.* **2019**, *17*, 172.
(11) Kaiser, J. New Prospects for Putting Organs on Ice. *Science* **2002**, *295*, 1015−1015.
(12) Dou, M.; Lu, C.; Rao, W. Bioinspired materials and technology for advanced cryopreservation. *Trends Biotechnol.* **2022**, *40*, 93−106.
(13) Friend, P. Strategies in Organ Preservation—A New Golden Age. *Transplatation* **2020**, *104*, 1753−1755.
(14) Zachariassen, K. E.; Hammel, H. T. Nucleating agents in the haemolymph of insects tolerant to freezing. *Nature* **1976**, *262*, 285−287.
(15) Storey, K. B.; Storey, J. M. Frozen and alive. *Sci. Am.* **1990**, *263*, 92−7.
(16) Tas, R. P.; Sampaio-Pinto, V.; Wennekes, T.; van Laake, L. W.; Voets, I. K. From the freezer to the clinic: Antifreeze proteins in the preservation of cells, tissues, and organs. *EMBO Rep* **2021**, *22*, No. e52162.
(17) Ustun, N. S.; Turhan, S. Antifreeze Proteins. In *Antifreeze Proteins*; Springer: Switzerland, 2020; Vol. *1*, pp 231−260.
(18) Chao, H.; Davies, P. L.; Carpenter, J. F. Effects of antifreeze proteins on red blood cell survival during cryopreservation. *J. Exp. Biol.* **1996**, *199*, 2071−2076.
(19) Muldrew, K.; McGann, L. E. Mechanisms of intracellular ice formation. *Biophys. J.* **1990**, *57*, 525−532.
(20) Hanna, C.; Sherman, J. K. Survival of rabbit corneal cells after the formation and dissolution of intracellular ice. *Cryobiology* **1971**, *8*, 46−72.
(21) Williams, L.; Hodson, S. Quench cooling and ice crystal formation in biological tissues. *Cryobiology* **1978**, *15*, 323−332.
(22) Leibo, S. P.; Mazur, P. The role of cooling rates in low-temperature preservation. *Cryobiology* **1971**, *8*, 447−452.
(23) Litvan, G. G. Mechanism of cryoinjury in biological systems. *Cryobiology* **1972**, *9*, 182−91.
(24) Pan, L.; Zhang, P.; Hu, F.; Yan, R.; He, M.; Li, W.; Xu, J.; Xu, K. Hypotonic Stress Induces Fast, Reversible degradation of the vimentin cytoskeleton via intracellular calcium release. *Adv. Sci. (Weinh)* **2019**, *6*, 1900865.
(25) Scudellari, M. Core concept: cryopreservation aims to engineer novel ways to freeze, store, and thaw organs. *Proc. Natl. Acad. Sci. U. S. A.* **2017**, *114*, 13060−13062.
(26) Fuller, B. J. Cryoprotectants: the essential antifreezes to protect life in the frozen state. *Cryoletters* **2004**, *25*, 375−388.
(27) Vaisman, I. I.; Berkowitz, M. L. Local structural order and molecular associations in water-DMSO mixtures. Molecular dynamics study. *J. Am. Chem. Soc.* **1992**, *114*, 7889−7896.
(28) Weng, L.; Elliott, G. D. Dynamic and thermodynamic characteristics associated with the glass transition of amorphous trehalose-water mixtures. *Phys. Chem. Chem. Phys.* **2014**, *16*, 11555−11565.
(29) Brüggeller, P.; Mayer, E. Complete vitrification in pure liquid water and dilute aqueous solutions. *Nature* **1980**, *288*, 569−571.
(30) Weng, L.; Stott, S. L.; Toner, M. Exploring Dynamics and Structure of Biomolecules, Cryoprotectants, and Water Using Molecular Dynamics Simulations: Implications for Biostabilization and Biopreservation. *Annu. Rev. Biomed. Eng.* **2019**, *21*, 1−31.
(31) Lovelock, J.; Bishop, M. Prevention of freezing damage to living cells by dimethyl sulfoxide. *Nature* **1959**, *183*, 1394.
(32) Yu, Z. W.; Quinn, P. J. Dimethyl sulfoxide: A review of its applications in cell biology. *Biosci. Rep.* **1994**, *14*, 259−81.
(33) Fahy, G. M.; Macfarlane, D. R.; Angell, C. A.; Meryman, H. T. Vitrification as an approach to cryopreservation. *Cryobiology* **1984**, *21*, 407−426.
(34) Huang, H.; He, X.; Yarmush, M. L. Advanced technologies for the preservation of mammalian biospecimens. *Nat. Biomed. Eng.* **2021**, *5*, 793−804.
(35) Kang, M.-H.; You, S.-Y.; Hong, K.; Kim, J.-H. DMSO impairs the transcriptional program for maternal-to-embryonic transition by altering histone acetylation. *Biomaterials* **2020**, *230*, 119604.
(36) Galvao, J.; Davis, B.; Tilley, M.; Normando, E.; Duchen, M. R.; Cordeiro, M. F. Unexpected low-dose toxicity of the universal solvent DMSO. *FASEB J.* **2014**, *28*, 1317−1330.
(37) Hanslick, J. L.; Lau, K.; Noguchi, K. K.; Olney, J. W.; Zorumski, C. F.; Mennerick, S.; Farber, N. B. Dimethyl sulfoxide (DMSO) produces widespread apoptosis in the developing central nervous system. *Neurobiol. Dis.* **2009**, *34*, 1−10.
(38) Best, B. P. Cryoprotectant Toxicity: Facts, Issues, and Questions. *Rejuvenation Res.* **2015**, *18*, 422−36.
(39) Chang, T.; Zhao, G. Ice Inhibition for Cryopreservation: Materials, Strategies, and Challenges. *Adv. Sci.* **2021**, *8*, 2002425.
(40) Devries, A. L.; Wohlschlag, D. E. Freezing Resistance in Some Antarctic Fishes. *Science* **1969**, *163*, 1073.
(41) Storey, K. B.; Storey, J. M. Molecular physiology of freeze tolerance in vertebrates. *Physiol. Rev.* **2017**, *97*, 623−665.
(42) Brockbank, K. G.; Campbell, L. H.; Greene, E. D.; Brockbank, M. C.; Duman, J. G. Lessons from nature for preservation of mammalian

cells, tissues, and organs. *In Vitro Cell. Dev. Biol. Anim.* **2011**, *47*, 210−217.
(43) Bennett, V. A.; Sformo, T.; Walters, K.; Toien, O.; Jeannet, K.; Hochstrasser, R.; Pan, Q.; Serianni, A. S.; Barnes, B. M.; Duman, J. G. Comparative overwintering physiology of Alaska and Indiana populations of the beetle Cucujus clavipes (Fabricius): roles of antifreeze proteins, polyols, dehydration and diapause. *J. Exp. Biol.* **2005**, *208*, 4467−77.
(44) Warren, G. J.; Thorlby, G. J.; Knight, M. R., The molecular biological approach to understanding freezing-tolerance in the model plant, Arabidopsis thaliana. In *Cell and Molecular Response to Stress*, Storey, K. B., Storey, J. M., Eds. Elsevier: Amsterdam, 2000; Vol. *1*, pp 245−258.
(45) Brockbank, K. G.; Campbell, L. H.; Greene, E. D.; Brockbank, M. C.; Duman, J. G. Lessons from nature for preservation of mammalian cells, tissues, and organs. *In Vitro Cell. Dev. Biol. Anim.* **2011**, *47*, 210−7.
(46) DeVries, A. L. Freezing resistance in fishes. *Fish physiology* **1971**, *6*, 157−190.
(47) Bar Dolev, M.; Braslavsky, I.; Davies, P. L. Ice-Binding Proteins and Their Function. *Annu. Rev. Biochem.* **2016**, *85*, 515−542.
(48) Liou, Y.-C.; Thibault, P.; Walker, V. K.; Davies, P. L.; Graham, L. A. J. B. A complex family of highly heterogeneous and internally repetitive hyperactive antifreeze proteins from the beetle Tenebrio molitor. *Biochemistry* **1999**, *38*, 11415−11424.
(49) Liu, K.; Wang, C.; Ma, J.; Shi, G.; Yao, X.; Fang, H.; Song, Y.; Wang, J. Janus effect of antifreeze proteins on ice nucleation. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 14739−14744.
(50) Raymond, J. A.; DeVries, A. L. Adsorption inhibition as a mechanism of freezing resistance in polar fishes. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 2589−2593.
(51) Celik, Y.; Graham, L. A.; Mok, Y.-F.; Bar, M.; Davies, P. L.; Braslavsky, I. Superheating of ice crystals in antifreeze protein solutions. *Proc. Natl. Acad. Sci. U. S. A.* **2010**, *107*, 5423.
(52) Barrett, J. Thermal hysteresis proteins. *Int. J. Biochem. Cell Biol.* **2001**, *33*, 105−117.
(53) Haji-Akbari, A. Rating antifreeze proteins: Not a breeze. *Proc. Natl. Acad. Sci. U. S. A.* **2016**, *113*, 3714−3716.
(54) Scotter, A. J.; Marshall, C. B.; Graham, L. A.; Gilbert, J. A.; Garnham, C. P.; Davies, P. L. The basis for hyperactivity of antifreeze proteins. *Cryobiology* **2006**, *53*, 229−239.
(55) Bredow, M.; Walker, V. K. Ice-binding proteins in plants. *Front. Plant Sci.* **2017**, *8*, 2153.
(56) Budke, C.; Koop, T., Inhibition of Recrystallization. In *Antifreeze Proteins: Biochemistry, Molecular Biology and Applications*; Ramløv, H., Friis, D. S., Eds.; Springer International Publishing: Cham, 2020; Vol. *2*, pp 159−184.
(57) Davies, P. L. Ice-binding proteins: a remarkable diversity of structures for stopping and starting ice growth. *Trends Biochem. Sci.* **2014**, *39*, 548−555.
(58) Voets, I. K. From ice-binding proteins to bio-inspired antifreeze materials. *Soft Matter* **2017**, *13*, 4808−4823.
(59) Qiu, Y.; Hudait, A.; Molinero, V. How size and aggregation of ice-binding proteins control their ice nucleation efficiency. *J. Am. Chem. Soc.* **2019**, *141*, 7439−7452.
(60) Duman, J. G. Antifreeze and ice nucleator proteins in terrestrial arthropods. *Annu. Rev. Physiol.* **2001**, *63*, 327−357.
(61) Zachariassen, K. E. Ice nucleating agents in cold-hardy insects. In *Water and Life*; Somero, G. N., Osmond, C. B., Bolis, C. L., Eds.; Springer Berlin Heidelberg: Berlin, 1992; pp 261−281.
(62) Morris, G. J.; Acton, E. Controlled ice nucleation in cryopreservation-a review. *Cryobiology* **2013**, *66*, 85−92.
(63) Garnham, C. P.; Campbell, R. L.; Walker, V. K.; Davies, P. L. Novel dimeric β-helical model of an ice nucleation protein with bridged active sites. *BMC Struct. Biol.* **2011**, *11*, 36.
(64) Bai, G.; Gao, D.; Liu, Z.; Zhou, X.; Wang, J. Probing the critical nucleus size for ice formation with graphene oxide nanosheets. *Nature* **2019**, *576*, 437−441.
(65) Zachariassen, K. E.; Kristiansen, E.; Pedersen, S. A.; Hammel, H. T. Ice nucleation in solutions and freeze-avoiding insects—homogeneous or heterogeneous? *Cryobiology* **2004**, *48*, 309−321.
(66) Duman, J. G. Antifreeze and ice nucleator proteins in terrestrial arthropods. *Annu. Rev. Physiol.* **2001**, *63*, 327−357.
(67) Hazlewood, C. F. A view of the significance and understanding of the physical properties of cell-associated water. In *Cell-Associated Water*; Drost-Hansen, W., Clegg, J. S., Eds.; Academic Press: New York, 1979; pp 165−259.
(68) Devries, A.; Cheng, C. Antifreeze proteins and organismal freezing avoidance in polar fishes. *Fish Physiol.* **2005**, *22*, 155−201.
(69) Robinson, W. R.; Peters, R. H.; Zimmermann, J. The effects of body size and temperature on metabolic rate of organisms. *Can. J. Zool.* **1983**, *61*, 281−288.
(70) Berendsen, T. A.; Bruinsma, B. G.; Puts, C. F.; Saeidi, N.; Usta, O. B.; Uygun, B. E.; Izamis, M.-L.; Toner, M.; Yarmush, M. L.; Uygun, K. Supercooling enables long-term transplantation survival following 4 days of liver preservation. *Nat. Med.* **2014**, *20*, 790−793.
(71) Bruinsma, B. G.; Berendsen, T. A.; Izamis, M.-L.; Yeh, H.; Yarmush, M. L.; Uygun, K. Supercooling preservation and transplantation of the rat liver. *Nat. Protoc.* **2015**, *10*, 484−494.
(72) Amir, G.; Rubinsky, B.; Horowitz, L.; Miller, L.; Leor, J.; Kassif, Y.; Mishaly, D.; Smolinsky, A. K.; Lavee, J. Prolonged 24-h subzero preservation of heterotopically transplanted rat hearts using antifreeze proteins derived from arctic fish. *Ann. Thorac. Surg.* **2004**, *77*, 1648−1655.
(73) Davies, P. L.; Hew, C. L. Biochemistry of fish antifreeze proteins. *FASEB J.* **1990**, *4*, 2460−2468.
(74) Naing, A. H.; Kim, C. K. A brief review of applications of antifreeze proteins in cryopreservation and metabolic genetic engineering. *3 Biotech* **2019**, *9*, 329.
(75) Tomalty, H. E.; Hamilton, E. F.; Hamilton, A.; Kukal, O.; Allen, T.; Walker, V. K. Kidney Preservation at Subzero Temperatures Using a Novel Storage Solution and Insect Ice-Binding Proteins. *Cryoletters* **2017**, *38*, 100−107.
(76) Brockbank, K. G. M.; Duman, J. D.; Chen, Z.; Greene, E. D.; Vu, H. M.; Campbell, L. H., Cell, Tissue, and Organ Preservation with Insect-Derived Antifreeze Peptides. In *Antifreeze Protein: Biochemistry, Molecular Biology and Applications*, Ramløv, H., Friis, D. S., Eds.; Springer International Publishing: Cham, 2020; Vol. *2*, pp 261−285.
(77) Wang, T.; Zhu, Q.; Yang, X.; Layne, J. R., Jr; Devries, A. L. Antifreeze glycoproteins from antarctic notothenioid fishes fail to protect the rat cardiac explant during hypothermic and freezing preservation. *Cryobiology* **1994**, *31*, 185−192.
(78) Mugnano, J. A.; Wang, T.; Layne, J. R., Jr; DeVries, A. L.; Lee, R. E., Jr Antifreeze glycoproteins promote intracellular freezing of rat cardiomyocytes at high subzero temperatures. *Am. J. Physiol.* **1995**, *269*, R474−R479.
(79) Bruinsma, B. G.; Uygun, K. Subzero organ preservation: the dawn of a new ice age? *Curr. Opin. Organ Transplant.* **2017**, *22*, 281−286.
(80) Rubinsky, B.; Arav, A.; Hong, J. S.; Lee, C. Y. Freezing of mammalian livers with glycerol and antifreeze proteins. *Biochem. Biophys. Res. Commun.* **1994**, *200*, 732−741.
(81) Kim, H.; Lee, J.; Hur, Y.; Lee, C.; Park, S.-H.; Koo, B.-W. Marine Antifreeze Proteins: Structure, function, and application to cryopreservation as a potential cryoprotectant. *Mar. Drugs* **2017**, *15*, 27.
(82) Campbell, L. H.; Brockbank, K. G.; Taylor, M. J. Two stage method for thawing cryopreserved cells. Patent US6596531B2, 2003.
(83) Wang, J.-H. A Comprehensive Evaluation of the Effects and Mechanisms of Antifreeze proteins during low-temperature preservation. *Cryobiology* **2000**, *41*, 1−9.
(84) Lee, J. R.; Youm, H. W.; Lee, H. J.; Jee, B. C.; Suh, C. S.; Kim, S. H. Effect of Antifreeze Protein on Mouse Ovarian Tissue Cryopreservation and Transplantation. *Yonsei Med. J.* **2015**, *56*, 778−784.
(85) Raymond, J. A.; Devries, A. L. Adsorption Inhibition as a Mechanism of Freezing Resistance in Polar Fishes. *Proc. Natl. Acad. Sci. U. S. A.* **1977**, *74*, 2589−2593.

https://doi.org/10.1021/jacs.2c00203
J. Am. Chem. Soc. 2022, 144, 5685−5701

(86) Carpenter, J. F.; Hansen, T. N. Antifreeze protein modulates cell survival during cryopreservation: mediation through influence on ice crystal growth. *Proc. Natl. Acad. Sci. U. S. A.* **1992**, *89*, 8953−8957.
(87) Rubinsky, B.; Arav, A.; Devries, A. L. The cryoprotective effect of antifreeze glycopeptides from antarctic fishes. *Cryobiology* **1992**, *29*, 69−79.
(88) Lee, H. H.; Lee, H. J.; Kim, H. J.; Lee, J. H.; Ko, Y.; Kim, S. M.; Lee, J. R.; Suh, C. S.; Kim, S. H. Effects of antifreeze proteins on the vitrification of mouse oocytes: comparison of three different antifreeze proteins. *Hum. Reprod.* **2015**, *30*, 2110−9.
(89) Nutt, D. R.; Smith, J. C. Dual function of the hydration layer around an antifreeze protein revealed by atomistic molecular dynamics simulations. *J. Am. Chem. Soc.* **2008**, *130*, 13066−13073.
(90) Phippen, S. W.; Stevens, C. A.; Vance, T. D. R.; King, N. P.; Baker, D.; Davies, P. L. Multivalent display of antifreeze proteins by fusion to self-assembling protein cages enhances ice-binding activities. *Biochemistry* **2016**, *55*, 6811−6820.
(91) DeLuca, C. I.; Comley, R.; Davies, P. L. Antifreeze proteins bind independently to ice. *Biophys. J.* **1998**, *74*, 1502−1508.
(92) Schwidetzky, R.; Kunert, A. T.; Bonn, M.; Pöschl, U.; Ramløv, H.; DeVries, A. L.; Fröhlich-Nowoisky, J.; Meister, K. Inhibition of bacterial ice nucleators is not an intrinsic property of antifreeze proteins. *J. Phys. Chem. B* **2020**, *124*, 4889−4895.
(93) Baardsnes, J.; Kuiper, M. J.; Davies, P. L. Antifreeze protein dimer: when two ice-binding faces are better than one. *J. Biol. Chem.* **2003**, *278*, 38942−38947.
(94) Can, O. z.; Holland, N. B. Conjugation of type I antifreeze protein to polyallylamine increases thermal hysteresis activity. *Bioconjugate Chem.* **2011**, *22*, 2166−2171.
(95) Davies, P. L.; Baardsnes, J.; Kuiper, M. J.; Walker, V. K. Structure and function of antifreeze proteins. *Philos. Trans. R. Soc. London B Biol. Sci.* **2002**, *357*, 927−35.
(96) Du, N.; Liu, X. Y.; Hew, C. L. Aggregation of antifreeze protein and impact on antifreeze activity. *J. Phys. Chem. B* **2006**, *110*, 20562−20567.
(97) Tsuda, S.; Yamauchi, A.; Khan, N.; Arai, T.; Mahatabuddin, S.; Miura, A.; Kondo, H. Fish-derived antifreeze proteins and antifreeze glycoprotein exhibit a different ice-binding property with increasing concentration. *Biomolecules* **2020**, *10*, 423.
(98) Salvay, A. G.; Gabel, F.; Pucci, B.; Santos, J.; Howard, E. I.; Ebel, C. Structure and interactions of fish Type III antifreeze protein in solution. *Biophys. J.* **2010**, *99*, 609−618.
(99) Eickhoff, L.; Dreischmeier, K.; Zipori, A.; Sirotinskaya, V.; Adar, C.; Reicher, N.; Braslavsky, I.; Rudich, Y.; Koop, T. Contrasting behavior of antifreeze proteins: ice growth inhibitors and ice nucleation promoters. *J. Phys. Chem. Lett.* **2019**, *10*, 966−972.
(100) Wilson, P. W.; Osterday, K. E.; Heneghan, A. F.; Haymet, A. D. J. Type I antifreeze proteins enhance ice nucleation above certain concentrations. *J. Biol. Chem.* **2010**, *285*, 34741−34745.
(101) Parody-Morreale, A.; Murphy, K. P.; Cera, E. D.; Fall, R.; DeVries, A. L.; Gill, S. J. Inhibition of bacterial ice nucleators by fish antifreeze glycoproteins. *Nature* **1988**, *333*, 782−783.
(102) Olsen, T. M.; Duman, J. G. Maintenance of the supercooled state in the gut fluid of overwintering pyrochroid beetle larvae, Dendroides canadensis : role of ice nucleators and antifreeze proteins. *J. Comp. Physiol., A* **1997**, *167*, 114−122.
(103) Woodard, H. Q.; White, D. R. The composition of body tissues. *British Journal of Radiology* **1986**, *59*, 1209−1218.
(104) Zimmerman, S. B.; Minton, A. P. Macromolecular crowding: biochemical, biophysical, and physiological consequences. *Annu. Rev. Biophys. Biomol. Struct.* **1993**, *22*, 27−65.
(105) Narayanan Krishnamoorthy, A.; Holm, C.; Smiatek, J. Local water dynamics around antifreeze protein residues in the presence of osmolytes: the importance of hydroxyl and disaccharide groups. *J. Phys. Chem. B* **2014**, *118*, 11613−11621.
(106) Meister, K.; Duman, J. G.; Xu, Y.; DeVries, A. L.; Leitner, D. M.; Havenith, M. The Role of sulfates on antifreeze protein activity. *J. Phys. Chem. B* **2014**, *118*, 7920−7924.
(107) Lewis, J. M.; Ewart, K. V.; Driedzic, W. R. Freeze resistance in rainbow smelt (Osmerus mordax): seasonal pattern of glycerol and antifreeze protein levels and liver enzyme activity associated with glycerol production. *Physiol. Biochem. Zool.* **2004**, *77*, 415−22.
(108) Miller, L. K. Physical and chemical changes associated with seasonal alterations in freezing tolerance in the adult northern Tenebrionid, Upis ceramboides. *J. Insect Physiol.* **1978**, *24*, 791−796.
(109) Suris-Valls, R.; Voets, I. K. The impact of salts on the ice recrystallization inhibition activity of antifreeze (glyco)proteins. *Biomolecules* **2019**, *9*, 347.
(110) Schwidetzky, R.; Lukas, M.; YazdanYar, A.; Kunert, A. T.; Pöschl, U.; Domke, K. F.; Fröhlich-Nowoisky, J.; Bonn, M.; Koop, T.; Nagata, Y.; Meister, K. Specific ion-protein interactions influence bacterial ice nucleation. *Chem.—Eur. J.* **2021**, *27*, 7402−7407.
(111) Ebbinghaus, S.; Meister, K.; Born, B.; DeVries, A. L.; Gruebele, M.; Havenith, M. Antifreeze glycoprotein activity correlates with long-range protein-water dynamics. *J. Am. Chem. Soc.* **2010**, *132*, 12210−12211.
(112) Li, N.; Andorfer, C. A.; Duman, J. G. Enhancement of insect antifreeze protein activity by solutes of low molecular mass. *J. Exp. Biol.* **1998**, *201*, 2243−51.
(113) Larson, D. J.; Middle, L.; Vu, H.; Zhang, W.; Serianni, A. S.; Duman, J.; Barnes, B. M. Wood frog adaptations to overwintering in Alaska: new limits to freezing tolerance. *J. Exp. Biol.* **2014**, *217*, 2193.
(114) Toxopeus, J.; Sinclair, B. J. Mechanisms underlying insect freeze tolerance. *Biol. Rev. Camb. Philos. Soc.* **2018**, *93*, 1891−1914.
(115) Duman, J. G.; Serianni, A. S. The role of endogenous antifreeze protein enhancers in the hemolymph thermal hysteresis activity of the beetle Dendroides canadensis. *J. Insect Physiol.* **2002**, *48*, 103−111.
(116) Dix, J. A.; Verkman, A. S. Crowding effects on diffusion in solutions and cells. *Annu. Rev. Biophys* **2008**, *37*, 247−63.
(117) Goldman, E. A new window on the cell's inner workings. *Science* **2002**, *298*, 1155−1157.
(118) Zhou, H.-X.; Rivas, G.; Minton, A. P. Macromolecular crowding and confinement: biochemical, biophysical, and potential physiological consequences. *Annu. Rev. Biophys.* **2008**, *37*, 375−397.
(119) Wang, Y.; Sarkar, M.; Smith, A. E.; Krois, A. S.; Pielak, G. J. Macromolecular crowding and protein stability. *J. Am. Chem. Soc.* **2012**, *134*, 16614.
(120) Ellis, R. J.; Minton, A. P. Cell biology: join the crowd. *Nature* **2003**, *425*, 27.
(121) Roosen-Runge, F.; Hennig, M.; Zhang, F.; Jacobs, R. M. J.; Sztucki, M.; Schober, H.; Seydel, T.; Schreiber, F. Protein self-diffusion in crowded solutions. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 11815.
(122) Can, Ö.; Holland, N. B. Utilizing avidity to improve antifreeze protein activity: a type III antifreeze protein trimer exhibits increased thermal hysteresis activity. *Biochemistry* **2013**, *52*, 8745−8752.
(123) Ishibe, T.; Congdon, T.; Stubbs, C.; Hasan, M.; Sosso, G. C.; Gibson, M. I. Enhancement of macromolecular ice recrystallization inhibition activity by exploiting depletion forces. *ACS Macro Lett.* **2019**, *8*, 1063−1067.
(124) Senske, M.; Törk, L.; Born, B.; Havenith, M.; Herrmann, C.; Ebbinghaus, S. Protein stabilization by macromolecular crowding through enthalpy rather than entropy. *J. Am. Chem. Soc.* **2014**, *136*, 9036−9041.
(125) Mittal, S.; Chowhan, R. K.; Singh, L. R. Macromolecular crowding: macromolecules friend or foe. *Biochim. Biophys. Acta* **2015**, *1850*, 1822−1831.
(126) Wolanczyk, J. P.; Storey, K. B.; Baust, J. G. Ice nucleating activity in the blood of the freeze-tolerant frog, Rana sylvatica. *Cryobiology* **1990**, *27*, 328−335.
(127) Storey, K. B.; Baust, J. G.; Wolanczyk, J. P. Biochemical modification of plasma ice nucleating activity in a freeze-tolerant frog. *Cryobiology* **1992**, *29*, 374−384.
(128) Tanaka, T.; Ikeya, T.; Kamoshida, H.; Suemoto, Y.; Mishima, M.; Shirakawa, M.; Güntert, P.; Ito, Y. High-resolution protein 3D structure determination in living eukaryotic cells. *Angew. Chem., Int. Ed.* **2019**, *131*, 7362−7366.

https://doi.org/10.1021/jacs.2c00203
J. Am. Chem. Soc. 2022, 144, 5685−5701

(129) Song, X.; Wang, M.; Chen, X.; Zhang, X.; Yang, Y.; Liu, Z.; Yao, L. Quantifying protein electrostatic interactions in cells by nuclear magnetic resonance spectroscopy. *J. Am. Chem. Soc.* **2021**, *143*, 19606−19613.
(130) Decelle, J.; Veronesi, G.; Gallet, B.; Stryhanyuk, H.; Benettoni, P.; Schmidt, M.; Tucoulou, R.; Passarelli, M.; Bohic, S.; Clode, P.; Musat, N. Subcellular chemical imaging: new avenues in cell biology. *Trends Cell Biol.* **2020**, *30*, 173−188.
(131) Coelho, S.; Baek, J.; Graus, M. S.; Halstead, J. M.; Nicovich, P. R.; Feher, K.; Gandhi, H.; Gooding, J. J.; Gaus, K. Ultraprecise single-molecule localization microscopy enables in situ distance measurements in intact cells. *Sci. Adv.* **2020**, *6*, No. eaay8271.
(132) Xu, F.; Ma, D.; MacPherson, K. P.; Liu, S.; Bu, Y.; Wang, Y.; Tang, Y.; Bi, C.; Kwok, T.; Chubykin, A. A.; Yin, P.; Calve, S.; Landreth, G. E.; Huang, F. Three-dimensional nanoscopy of whole cells and tissues with in situ point spread function retrieval. *Nat. Methods* **2020**, *17*, 531−540.
(133) Xiao, Y.; Deng, J.; Yao, Y.; Fang, L.; Yang, Y.; Luan, T. Recent advances of ambient mass spectrometry imaging for biological tissues: A review. *Anal. Chim. Acta* **2020**, *1117*, 74−88.
(134) Foo, Y. H.; Korzh, V.; Wohland, T. Fluorescence correlation and cross-correlation spectroscopy using fluorescent proteins for measurements of biomolecular processes in living organisms. In *Fluorescent Proteins II: Application of Fluorescent Protein Technology*, Jung, G., Ed. Springer Berlin Heidelberg: Berlin, 2012; pp 213−248.
(135) Dawes, M. L.; Soeller, C.; Scholpp, S. Studying molecular interactions in the intact organism: fluorescence correlation spectroscopy in the living zebrafish embryo. *Histochem. Cell Biol.* **2020**, *154*, 507−519.
(136) Wang, Z.; Marcu, O.; Berns, M. W.; Marsh, J. L. In *In vivo FCS measurements of ligand diffusion in intact tissues*; International Society for Optics and Photonics: Bellingham, WA, 2004; Vol. *5323*, pp 177−183.
(137) Deville, S.; Viazzi, C.; Guizard, C. Ice-structuring mechanism for zirconium acetate. *Langmuir* **2012**, *28*, 14892−14898.
(138) Drori, R.; Li, C.; Hu, C.; Raiteri, P.; Rohl, A. L.; Ward, M. D.; Kahr, B. A supramolecular ice growth inhibitor. *J. Am. Chem. Soc.* **2016**, *138*, 13396−13401.
(139) Naullage, P. M.; Molinero, V. Slow propagation of ice binding limits the ice-recrystallization inhibition efficiency of PVA and other flexible polymers. *J. Am. Chem. Soc.* **2020**, *142*, 4356−4366.
(140) Liu, X.; Geng, H.; Sheng, N.; Wang, J.; Shi, G. Suppressing ice growth by integrating the dual characteristics of antifreeze proteins into biomimetic two-dimensional graphene derivatives. *J. Mater. Chem. A* **2020**, *8*, 23555−23562.
(141) Meister, K.; Ebbinghaus, S.; Xu, Y.; Duman, J. G.; DeVries, A.; Gruebele, M.; Leitner, D. M.; Havenith, M. Long-range protein-water dynamics in hyperactive insect antifreeze proteins. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110*, 1617−1622.
(142) Chao, H.; Sonnichsen, F. D.; DeLuca, C. I.; Sykes, B. D.; Davies, P. L. Structure-function relationship in the globular type III antifreeze protein: identification of a cluster of surface residues required for binding to ice. *Protein Sci.* **1994**, *3*, 1760−1769.
(143) Jia, Z.; DeLuca, C. I.; Chao, H.; Davies, P. L. Structural basis for the binding of a globular antifreeze protein to ice. *Nature* **1996**, *384*, 285−288.
(144) Garnham, C. P.; Campbell, R. L.; Davies, P. L. Anchored clathrate waters bind antifreeze proteins to ice. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108*, 7363−7367.
(145) Sicheri, F.; Yang, D. S. Ice-binding structure and mechanism of an antifreeze protein from winter flounder. *Nature* **1995**, *375*, 427−31.
(146) Liou, Y.-C.; Tocilj, A.; Davies, P. L.; Jia, Z. Mimicry of ice structure by surface hydroxyls and water of a β-helix antifreeze protein. *Nature* **2000**, *406*, 322−324.
(147) Deville, S.; Viazzi, C.; Leloup, J.; Lasalle, A.; Guizard, C.; Maire, E.; Adrien, J.; Gremillard, L. Ice shaping properties, similar to that of antifreeze proteins, of a zirconium acetate complex. *PLoS One* **2011**, *6*, No. e26474.
(148) Knight, C. A.; Wen, D.; Laursen, R. A. Nonequilibrium antifreeze peptides and the recrystallization of ice. *Cryobiology* **1995**, *32*, 23−34.
(149) Geng, H.; Liu, X.; Shi, G.; Bai, G.; Ma, J.; Chen, J.; Wu, Z.; Song, Y.; Fang, H.; Wang, J. Graphene Oxide Restricts Growth and Recrystallization of Ice Crystals. *Angew. Chem., Int. Ed.* **2017**, *56*, 997−1001.
(150) Bai, G.; Song, Z.; Geng, H.; Gao, D.; Liu, K.; Wu, S.; Rao, W.; Guo, L.; Wang, J. Oxidized Quasi-Carbon Nitride Quantum Dots Inhibit Ice Growth. *Adv. Mater.* **2017**, *29*, 1606843.
(151) Jin, S.; Yin, L.; Kong, B.; Wu, S.; He, Z.; Xue, H.; Liu, Z.; Cheng, Q.; Zhou, X.; Wang, J. Spreading fully at the ice-water interface is required for high ice recrystallization inhibition activity. *Sci. China: Chem.* **2019**, *62*, 909−915.
(152) Mochizuki, K.; Molinero, V. Antifreeze Glycoproteins Bind Reversibly to Ice via Hydrophobic Groups. *J. Am. Chem. Soc.* **2018**, *140*, 4803−4811.
(153) Baardsnes, J.; Davies, P. L. Contribution of hydrophobic residues to ice binding by fish type III antifreeze protein. *Biochim. Biophys. Acta* **2002**, *1601*, 49−54.
(154) Budke, C.; Koop, T. Ice recrystallization inhibition and molecular recognition of ice faces by poly (vinyl alcohol). *ChemPhysChem* **2006**, *7*, 2601−2606.
(155) Zhu, W.; Guo, J.; Agola, J. O.; Croissant, J. G.; Wang, Z.; Shang, J.; Coker, E.; Motevalli, B.; Zimpel, A.; Wuttke, S.; Brinker, C. J. Metal-organic framework nanoparticle-assisted cryopreservation of red blood cells. *J. Am. Chem. Soc.* **2019**, *141*, 7789−7796.
(156) Mitchell, D. E.; Lovett, J. R.; Armes, S. P.; Gibson, M. I. Combining biomimetic block copolymer worms with an ice-inhibiting polymer for the solvent-free cryopreservation of red blood cells. *Angew. Chem., Int. Ed.* **2016**, *128*, 2851−2854.
(157) Qin, Q.; Zhao, L.; Liu, Z.; Liu, T.; Qu, J.; Zhang, X.; Li, R.; Yan, L.; Yan, J.; Jin, S.; Wang, J.; Qiao, J. Bioinspired l-proline oligomers for the cryopreservation of oocytes via controlling ice growth. *ACS Appl. Mater. Interfaces* **2020**, *12*, 18352−18362.
(158) Graham, B.; Bailey, T. L.; Healey, J. R. J.; Marcellini, M.; Deville, S.; Gibson, M. I. Polyproline as a minimal antifreeze protein mimic that enhances the cryopreservation of cell monolayers. *Angew. Chem., Int. Ed. Engl.* **2017**, *56*, 15941−15944.
(159) Eroglu, A. Alterations of the cytoskeleton and polyploidy induced by cryopreservation of metaphase II mouse oocytes. *Fertil. Steril.* **1998**, *69*, 944.
(160) Kang, M.-H.; You, S.-Y.; Hong, K.; Kim, J.-H. DMSO impairs the transcriptional program for maternal-to-embryonic transition by altering histone acetylation. *Biomaterials* **2020**, *230*, 119604.
(161) Williams, D. F. On the mechanisms of biocompatibility. *Biomaterials* **2008**, *29*, 2941−2953.
(162) Kirkpatrick, C. J.; Bittinger, F.; Wagner, M.; Köhler, H.; van Kooten, T. G.; Klein, C. L.; Otto, M. Current trends in biocompatibility testing. *Proc. Inst. Mech. Eng. H* **1998**, *212*, 75−84.
(163) Choi, S. I. A Simple Principle for Understanding the Combined Cellular Protein Folding and Aggregation. *Curr. Protein Pept. Sci.* **2020**, *21*, 3−21.
(164) Simpson, L. W.; Good, T. A.; Leach, J. B. Protein folding and assembly in confined environments: Implications for protein aggregation in hydrogels and tissues. *Biotechnol. Adv.* **2020**, *42*, 107573.
(165) Choi, S. I.; Seong, B. L. A conceptual framework for integrating cellular protein folding, misfolding and aggregation. *Life* **2021**, *11*, 605.
(166) Claesson-Welsh, L.; Dejana, E.; McDonald, D. M. Permeability of the Endothelial Barrier: Identifying and Reconciling Controversies. *Trends Mol. Med.* **2021**, *27*, 314−331.
(167) Sarmento, B. *Concepts and models for drug permeability studies: Cell and tissue based in vitro culture models*; Woodhead Publishing: Sawston, UK, 2015.
(168) Lindgren, M.; Hällbrink, M.; Prochiantz, A.; Langel, Ü. Cell-penetrating peptides. *Trends Pharmacol. Sci.* **2000**, *21*, 99−103.
(169) Chamundeeswari, M.; Jeslin, J.; Verma, M. L. Nanocarriers for drug delivery applications. *Environ. Chem. Lett.* **2019**, *17*, 849−865.

https://doi.org/10.1021/jacs.2c00203
J. Am. Chem. Soc. 2022, 144, 5685−5701

(170) Zhang, X.; Xu, X.; Li, Y.; Hu, C.; Zhang, Z.; Gu, Z. Virion-like membrane-breaking nanoparticles with tumor-activated cell-and-tissue dual-penetration conquer impermeable cancer. *Adv. Mater.* **2018**, *30*, 1707240.
(171) Duman, J. G.; Patterson, J. L.; Kozak, J. J.; DeVries, A. L. Isopiestic determination of water binding by fish antifreeze glycoproteins. *Biochim. Biophys. Acta* **1980**, *626*, 332−336.
(172) Mugnano, J.; Lee, R.; Taylor, R. Fat body cells and calcium phosphate spherules induce ice nucleation in the freeze-tolerant larvae of the gall fly Eurosta solidaginis (Diptera, Tephritidae). *J. Exp. Biol.* **1996**, *199*, 465−471.
(173) Tsumuki, H.; Konno, H. Tissue distribution of the ice-nucleating agents in larvae of the rice stem borer, Chilo suppressalis Walker (Lepidoptera: Pyralidae). *Cryobiology* **1991**, *28*, 376−381.
(174) Zhao, Z.; Ukidve, A.; Kim, J.; Mitragotri, S. Targeting strategies for tissue-specific drug delivery. *Cell* **2020**, *181*, 151−167.
(175) Arslan, F. B.; Atar, K. O.; Calis, S. J. I. J. o. P. Antibody-mediated drug delivery. *Int. J. Pharm.* **2021**, *596*, 120268.
(176) Aird, W. C. Endothelial cell heterogeneity. *Cold Spring Harb Perspect Med.* **2012**, *2*, a006429.
(177) Pasqualini, R.; Ruoslahti, E. Organ targeting In vivo using phage display peptide libraries. *Nature* **1996**, *380*, 364−366.
(178) Arap, W.; Kolonin, M. G.; Trepel, M.; Lahdenranta, J.; Cardo-Vila, M.; Giordano, R. J.; Mintz, P. J.; Ardelt, P. U.; Yao, V. J.; Vidal, C. I.; Chen, L.; Flamm, A.; Valtanen, H.; Weavind, L. M.; Hicks, M. E.; Pollock, R. E.; Botz, G. H.; Bucana, C. D.; Koivunen, E.; Cahill, D.; Troncoso, P.; Baggerly, K. A.; Pentz, R. D.; Do, K. A.; Logothetis, C. J.; Pasqualini, R. Steps toward mapping the human vasculature by phage display. *Nat. Med.* **2002**, *8*, 121−127.
(179) Hajitou, A.; Pasqualini, R.; Arap, W. Vascular targeting: recent advances and therapeutic perspectives. *Trends Cardiovasc. Med.* **2006**, *16*, 80−88.
(180) Ward, A.; Klassen, D. K.; Franz, K. M.; Giwa, S.; Lewis, J. K. Social, economic, and policy implications of organ preservation advances. *Curr. Opin. Organ Transplant.* **2018**, *23*, 336−346.
(181) Chiu-Lam, A.; Staples, E.; Pepine, C. J.; Rinaldi, C. Perfusion, cryopreservation, and nanowarming of whole hearts using colloidally stable magnetic cryopreservation agent solutions. *Sci. Adv.* **2021**, *7*, No. eabe3005.
(182) Manuchehrabadi, N.; Gao, Z.; Zhang, J.; Ring, H. L.; Shao, Q.; Liu, F.; McDermott, M.; Fok, A.; Rabin, Y.; Brockbank, K. G. M.; Garwood, M.; Haynes, C. L.; Bischof, J. C. Improved tissue cryopreservation using inductive heating of magnetic nanoparticles. *Sci. Transl. Med.* **2017**, *9*, No. eaah4586.

https://doi.org/10.1021/jacs.2c00203
J. Am. Chem. Soc. 2022, 144, 5685−5701