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Extreme makeover: the incredible cell membrane adaptations of extremophiles to harsh environments

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The existence of life beyond Earth has long captivated humanity, and the study of extremophiles—organisms surviving and thriving in extreme environments—provides crucial insights into this possibility. Extremophiles overcome severe challenges such as enzyme inactivity, protein denaturation, and damage of the cell membrane by adopting several strategies. This feature article focuses on the molecular strategies extremophiles use to maintain the cell membrane's structure and fluidity under external stress. Key strategies include homeoviscous adaptation (HVA), involving the regulation of lipid composition, and osmolyte-mediated adaptation (OMA), where small organic molecules protect the lipid membrane under stress. Proteins also have direct and indirect roles in protecting the lipid membrane. Examining the survival strategies of extremophiles provides scientists with crucial insights into how life can adapt and persist in harsh conditions, shedding light on the origins of life. This article examines HVA and OMA and their mechanisms in maintaining membrane stability, emphasizing our contributions to this field. It also provides a brief overview of the roles of proteins and concludes with recommendations for future research directions.

1. Introduction

The question of whether life exists beyond Earth is a captivating inquiry that has intrigued humanity for centuries. While the vastness of the universe suggests the potential for extraterrestrial life, the immense distances involved in space exploration

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make direct contact seem dauntingly remote. However, the study of extremophiles presents a fascinating avenue for exploring this possibility.[1–4] Extremophiles are organisms that survive and thrive in environments considered "extreme" by conventional standards, such as scorching heat, freezing cold, *bone-crushing* pressure, high salinity, high radiation, acidic or alkaline conditions, and low water conditions.[5–14] By studying the survival strategies of extremophiles, scientists gain valuable insights into how life can adapt and thrive in seemingly inhospitable conditions, offering clues about the origins of life.

The extremophiles face severe challenges at different extreme conditions, such as low enzyme activity, mechanical damage of cellular subunits by tiny ice crystals, drop down in the transcription and translation rate, cold and heat denaturation of proteins, disruption of the molecular structure of the cell membrane, reduction of cell membrane fluidity, loss of membrane barrier function, *etc.* To combat these challenges, the extremophiles must adapt to these extreme conditions. Two different types of adaptations are known: genotypic or phenotypic[15–17] While genotypic adaptation occurs over an evolutionary timescale, phenotypic adaptation takes place within the lifetime of the organism and can have timescales ranging from minutes to days. Studying these strategies, which the extremophiles adopt to survive and thrive in extreme conditions, is important in various areas of science and technology, starting from the fundamental quest for the origins of life to the food processing and probiotic industries[18–27]

Fig. 1 presents a schematic representation showing different types of extremophiles, which survive and thrive in various extreme conditions. The extremophiles exhibit a diverse array of survival strategies tailored to their respective extreme environments. For instance, thermophiles have evolved specialized enzymes and proteins that remain stable at high temperatures, allowing them to thrive in hydrothermal vents or geothermal springs.[28–31] Conversely, psychrophiles have mechanisms to prevent cellular freezing in frigid environments, such as polar regions or deep-sea trenches.[32,33] Halophiles flourish in environments with high salt concentrations, employing adaptations to regulate osmotic pressure and mitigate the damaging effects of salt on cellular structures.[34–36] Acidophiles[21] and alkaliphiles[37] adopt suitable strategies so that they are capable of surviving in environments with extreme pH levels, such as the use of proton efflux proteins.[38–42] The barophiles, which thrive in high-pressure environments such as the deep sea, adopt strategies to combat high-pressure stress by morphological, physiological, and molecular evolutions.[43–45] Some extremophiles even exhibit remarkable tolerance to radiation, earning them the label of radio-tolerant organisms.[45–48] These organisms have mechanisms to repair DNA damage caused by high levels of radiation exposure, enabling them to persist in radioactive environments.

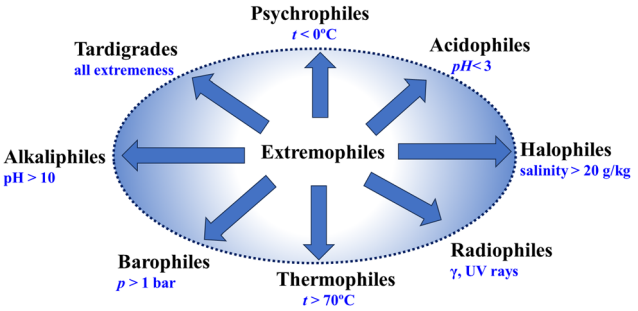

Fig. 1 Schematic diagram showing the classification of different types of extremophiles.

In this feature article, we focus on the molecular-level understanding of the adaptive strategies employed by extremophiles to maintain the packing density and fluidity of their cell membranes under various external stresses. The cell membrane, composed primarily of lipids and proteins, is a crucial component of the cell, providing essential functions. The packing density of lipids in the membrane indicates how closely the lipid molecules are arranged within the lipid bilayer, while fluidity refers to the ease with which these lipids can diffuse within the membrane. The packing density and fluidity of lipid membranes are severely challenged by extreme conditions. The extremophilic organisms must adopt effective strategies to combat the stress and retain the packing density and fluidity of the lipid membrane for several reasons:

(i) Barrier function: the appropriate barrier function of the cell membrane requires optimum fluidity, since higher or lower values of the latter may severely impact the barrier function.

(ii) Cell communication: some transmembrane proteins transmit signals between the exterior and interior of the cell, which requires appropriate fluidity of the cell membrane to enable these proteins to move and interact effectively with the signaling molecules.[49–51]

(iii) Transport of molecules: both active and passive transport of water and other molecules through the cell membrane are strongly impacted by a change of membrane fluidity.[52–56]

(iv) Cell shape and stability: the cell shape and stability are strongly affected by the packing density of the lipid membrane,

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preventing collapse or bursting due to osmotic pressure changes.[57]

(v) Cell recognition and adhesion: some membrane proteins aid in cell recognition and adhesion, essential for tissue formation and immune response.[58–60] These are strongly influenced by the change of membrane packing density and fluidity.

Among various strategies that are adopted by extremophiles, we will primarily discuss here the two most important ones: (i) homeoviscous adaptation (HVA)[61–65] and (ii) osmolyte-mediated adaptation (OMA)[66–75] HVA is the regulation of the lipid composition of the cell membrane to keep the membrane structure and fluidity intact against external stress. HVA can occur *via* remodeling of both the lipid acyl chain and lipid head groups. On the other hand, OMA refers to the process by which osmolytes, small organic molecules that regulate osmotic pressure and maintain the structure and function of cellular components, stabilize lipid membranes by protecting their packing density and fluidity against environmental stress. We will also briefly discuss the direct and indirect roles of proteins in stabilizing lipid membranes under stressed conditions.

2. Homeoviscous adaptation (HVA) for protecting the cell membrane

HVA was first observed by Sinensky *et al.*[76] in *Escherichia coli* (*E. coli*) bacteria surviving at higher growth temperatures. To date, several studies have shown a similar mode of adaptation in different classes of extremophiles and even in mammalian cell membranes.[4,77–91] Therefore, adopting the theory of HVA is a universal paradigm of membrane adaptation to extreme growing environments. In this section, we detail the mechanism of HVA and its impact on the lipidomics of the cell membrane, providing sufficient examples from both simulation and experimental studies.

Mechanism of HVA

The first step of HVA is sensing the changes in the cell membrane due to external stress with the help of sensor proteins.[1,4,92–94] The changes in the local environment include the drastic alteration of the membrane fluidity, membrane curvature, and packing density of the lipids. Although significant progress has been made in recognizing potential sensory mechanisms, understanding their molecular mechanism and how they collaborate to uphold the physicochemical characteristics of cellular membranes remains largely elusive.[95,96] There is a debate on what changes in membrane properties are sensed by sensor proteins. As per one school of thought, sensor proteins exhibit sensitivities to the membrane fluidity (measured by the lateral diffusion D_{xy} of the lipids).[97–102] On the contrary, a more recent work[4] proposed that instead of the fluidity of the membrane, the sensor protein can sense lipid-packing density to initiate a homeostatic response.[103] Through the integration of molecular dynamics simulations with experimental approaches, the authors discovered a notable sensitivity of the transcriptional regulator Mga2 (a transcriptional regulator found in yeast, specifically *Saccharomyces cerevisiae*) to variations in the abundance, positioning, and arrangement of double bonds within lipid acyl chains. This revelation offers insights into the molecular principles governing membrane adaptation.

The second step is as follows. Once the sensor proteins detect changes in membrane properties, they undergo conformational changes of the protein. These changes activate downstream signaling pathways, known as signal transduction. The nature of these pathways can vary depending on the organism and the specific sensor proteins involved. The signaling cascade often involves the activation of specific transcription factors.[104] In the case of yeast (*Saccharomyces cerevisiae*), for example, the transcriptional regulator Mga2 is sensitive to changes in membrane lipid composition.[105] Upon activation, these transcription factors move to the nucleus, where they bind to promoters of genes involved in lipid metabolism. This initiates transcription, producing mRNA, which is translated into proteins in the cytoplasm. These proteins include enzymes for lipid synthesis and modification, as well as other proteins crucial for membrane integrity.[106]

In the third step, newly synthesized enzymes and proteins adjust the cell membrane's lipid composition. They may increase unsaturated fatty acids for fluidity or alter lipid headgroups to adjust membrane curvature, restoring optimal membrane properties for proper cellular function.

The outcome of HVA on the lipid composition

The alterations in the fatty acid profile of the phospholipids in some of the extremophiles are shown in Fig. 2. Under subzero temperatures, high pressure, and dehydration the membrane undergoes a phase transition to an ordered gel state triggered by the tight packing of membrane lipids.[68,107–116] This phase transition is fatal to the organism and is avoided by timely modifications in the lipid types. At low temperatures and high pressure, lipids with low melting temperatures (T_g) including monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), branched fatty acids (methyl branching), short-chain fatty acids, hydroxylated fatty acids, and polar headgroups are incorporated in a higher proportion.[4,13,77–91,117–119] Unlike the *trans* double bond, the presence of kinks in the *cis* double bond pushes the lipids apart, decreasing the order of packing and avoiding the fluid-to-gel phase transition. For example, micrococcus cryophilus bacterium extracted from a temperature regime of −30 °C to −40 °C shows that 97% of the lipids are with unsaturated fatty acids (UFAs).[120] Together with the changes in unsaturation, these species also set the best example for the shortening of fatty acids chain length with a lowering of growth temperature. The actual biochemical process of cold-induced shortening of the acyl chains is still not clearly understood. However, overactivity of some molecules, such as acetyl-CoA or malonyl-CoA in the cells during stress suggests their roles in the modification of the lipidome.[121–124] Some psychrophiles show an increased abundance of lipids with short-chain (carbon length less than 12) and branched fatty acids.[125–128] A study[129] on

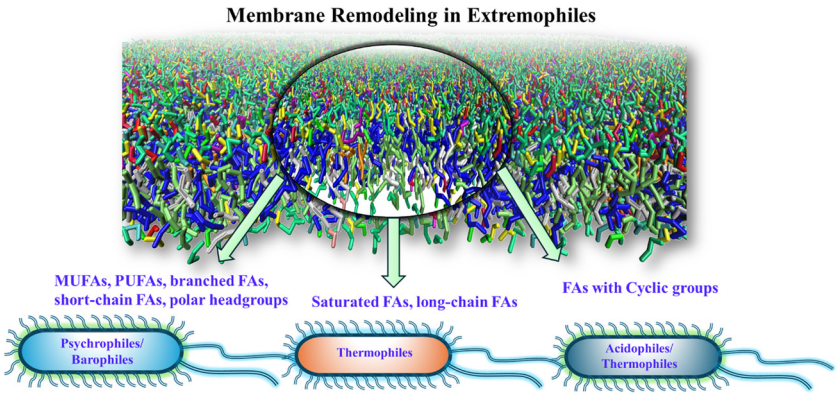

Fig. 2 Schematic representation of the membrane remodeling occurring in the fatty acid profile of lipids in different classes of extremophiles.

Pseudomonas syringae (antarctic psychrotrophic bacterium) showed an increased level of hydroxylated fatty acids in the lipopolysaccharides (LPS) of the outer membrane when exposed to a temperature of 4 °C.

Remodeling of the lipid headgroup is also observed to be an effective adaptive strategy to maintain the structure and fluidity of the lipid membrane.[130,131] A recent study by Chwastek *et al.*[91] reported a headgroup-specific remodeling in *Methylobacterium extorquens*, a soil-based and plant-associated Gram-negative bacterium. The diurnal temperature drops to 6 °C and gradually increases the bacteria's PC/PE lipid ratio. An increased amount of lysophospholipids (LPS) is also reported in some psychrophilic yeast.[121] These are inverted conical shapes and have been shown to disrupt the membrane packing and increase fluidity in response to cold.

Cyclisation of fatty acids in response to temperature and pH is also reported in some species although not widely documented to date.[132] The cyclase enzyme synthesizes these derivatives from the unsaturated acyl chains. Like the MUFAs, the cyclic groups also maintain the membrane fluidity by introducing *gauche* defects and affect the packing of lipids. This is captured in the bacterial membrane of *Lactococcus lactis* when cultivated at lower fermentation temperature.[133] The cyclopropanation of unsaturated fatty acids aids in perturbing the membrane rigidification upon freeze-drying and storage. The response of membrane lipids of *R. erythropolis* cells,[134] *E. coli*,[135] and *Salmonella enterica serovar typhimurium* (*S. typhimurium*)[136] with increased content of cyclopropane FAs follows the extremity in pH levels. Cyclopropane rings aid the survival of *E. coli* under acid shock and freeze-drying.

Along with the modifications in the lipid compositions, studies also report the role of sterols (cholesterol and hopanoids) in extremophiles.[137,138] The increased expression of cholesterol biosynthetic genes in fishes is related to the temperature variations across seasons.[139] At low temperatures, an increase in cholesterol can hinder the fluid-to-gel phase transition of the membrane through the cholesterol-induced condensation effect as in 'lipid rafts'. The packing of cholesterol between the acyl chains of lipids perturbs the ordered packing of lipids and thereby the gel phase.

3. Protecting lipid membrane *via* HVA: insights from molecular dynamics simulations

Computer simulation studies can provide a molecular perspective on HVA under stress. However, these studies are less common than experimental ones. Here, we review the insights gained from simulation-based studies to date, including work from our own group.

Role of unsaturation

The tuning of saturated/unsaturated lipids under sub-zero temperature on a bacteria *Leeuwenhoekiella aequorea* isolated from Antarctica was studied by Singh *et al.*[90] The study reported a percentage profile of different fatty acid chains in bacteria over a temperature range of 15 to −20 °C involving a gradual reduction of temperature and freeze–thaw cycles. They analyzed the fatty acid profiles of three characteristic states of the bacteria: (i) I_0, where the bacterial strains are incubated at standard growth conditions (15 °C) for 5 days, (ii) B_{FT}, where the temperature is gradually reduced to 4 °C and (iii) P_{FT}, where the system is quickly frozen to −20 °C and thawed back to 4 °C. In the I_0 state, almost 70% of the lipids are saturated fatty acids (SFAs), while the remaining portion (∼30%) is unsaturated fatty acids (UFAs). This trend has reverted in both B_{FT} and P_{FT} systems, where more than 85% of the lipids are UFAs. Scrutinizing the profile, it was observed that the abundant 18 : 0 SFA gets converted to 18 : 2 UFA at lower temperatures. To elucidate how the homeoviscous adaptation protects the cell membrane during cold stress, our group[112] undertook a project to simulate a model lipid membrane of the bacteria in question. With some approximations, symmetric lipid bilayers were modeled and simulated over a temperature range from 250 K to 300 K using molecular dynamics (MD) simulation techniques, as depicted in Fig. 3. The study described the importance of the experimentally reported fatty acid remodeling with a reduction of temperature for the retention of membrane packing density and fluidity, the key for cell survival. This study provided a molecular basis of the observations that the I_0 state of the lipid

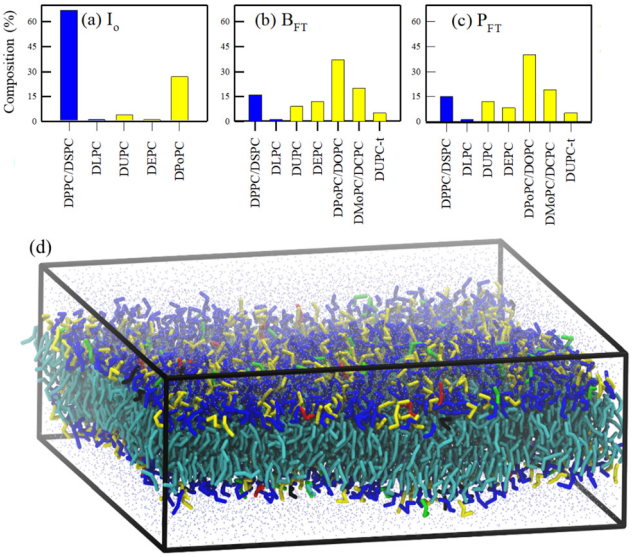

Fig. 3 Lipid profiles (saturated lipids: blue; unsaturated lipids: yellow) of eight different lipid membranes (a)–(c). (d) A snapshot of the equilibrated I_0 membrane. The head groups of 5 different lipids are color-coded as blue (DPPC/DSPC) red (DLPC), yellow (DPoPC), green (DUPC), and black (DEPC). The tails of all the lipids are represented by cyan color. CG water beads are represented by points. Reprinted with permission from ref. 112 Copyright {2020}American Chemical Society.

membrane, effective for cell survival at or above 280 K, becomes unsuitable below this temperature due to a fluid–gel phase transition. At temperatures near the phase transition, a phase separation occurs, with the unsaturated lipids forming a fluid-like phase and the saturated lipids forming a gel-like phase, along with a strong correlation between the leaflets. Our research also suggested an increasing role of the desaturase enzyme in cold adaptation as temperatures drop. By comparing the properties of five intermediate membranes, we outlined the gradual progression toward homeoviscous adaptation.

The cold adaptation mechanism of marine algae has gained significant interest owing to their extensive applications. The aquatic environment's diurnal and seasonal temperature variations remarkably influence the lipid composition of marine algae's thylakoid membrane (thylakoid-LBM).[140–144] These membranes are involved in photosynthesis and are composed of galacto- and sulfolipids with UFAs in their acyl chain. A recent study by Manna and coworkers[145] revealed the purpose of membrane complexity in the thylakoid membrane of a commercially relevant Red algae *Gracilaria corticata* through atomistic simulations. The membrane composition at optimal growth conditions (25–30 °C) is modeled and further simulated over a temperature spectrum of 10 °C to 40 °C, at intervals of 5 °C. In between 25 °C and 30 °C they observed the ordered gel and disordered fluid domains of saturated and unsaturated lipids, respectively, in the multicomponent lipid mixture. Furthermore, a phase transition from fluid L_α phase to gel L_β phase was observed below 10 °C. Fig. 4 shows the phase of the membrane across different temperatures they studied.

This agrees with the phase separation observed in a binary/ternary/quaternary mixture of saturated and unsaturated lipids.

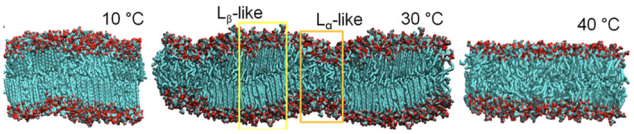

Fig. 4 Representative snapshots of side views of the thylakoid-LBM, the L_β phase at 10 °C and the L_α phase at 40 °C. A snapshot with its periodic image shows the L_β/L_α coexistence in the membrane with a wave-like surface at 30 °C. Reprinted with permission from ref. 145 Copyright {2023} American Chemical Society.

The preferential interaction between lipids of the same acyl chain type drives the phase separation in the thylakoid-LBM. However, a segregation of lipids based on the acyl chain type is not observed in the membrane. The lipids with UFAs retain the fluidity of the membrane, whereas those with SFA get rigidified. The study concluded that the increased concentration of PUFAs can avoid the otherwise happening membrane rigidification under cold stress.

Role of polar headgroups

An experimental study by Chwastek *et al.*[91] reported a headgroup-based lipidomic remodeling of a soil-based and plant-associated bacterium *Methylobacterium extorquens* under diurnal temperature variations. Almost 11 out of the total 25 lipid types undergo major remodeling across four temperatures: 30, 20, 13, and 6 °C. Lipids distributed across five headgroup types: phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylglycerol (PG), phosphatidylethanolamine (PE), and cardiolipin (CL) are reported in the bacterium. An idiosyncratic adaptation strategy with tuning of the PE and PC class of lipids was reported remarkably at 6 °C. Under low temperatures (6 °C), the PE lipids are replaced with the PC class of lipids. Interestingly the bacterium did not have any saturated lipids even at the higher temperature regime (30 °C).

Aiming to unravel the effect of headgroup-based membrane tuning from a molecular perspective, a study from our group[146] modeled the membranes across all four temperatures 30, 20, 13, and 6 °C and named the membranes N30, N20, N13, and N6, respectively. These membranes are simulated at the respective temperatures. Fig. 5 shows the composition based on the headgroup for all four membranes. We performed cooling and heating simulations of the N30 and N6 membranes to understand how the membrane responds to abrupt temperature changes without an adaptation in its lipid profile. No phase transitions were marked in the membrane even at 6 °C. The study revealed that the membrane's properties are largely preserved through complex lipidome remodeling in response to both heat and cold stress. Specifically, the remodeling involves adjusting the acyl chain headgroups, fine-tuning the packing density and fluidity at various temperatures. At higher temperatures, lipids with headgroups that strongly interact become more prevalent, while at lower temperatures, lipids with headgroups that interact less strongly dominate the lipidome. This dynamic shift helps prevent disruptions to the lipid membrane caused by thermal stress. This study, therefore,

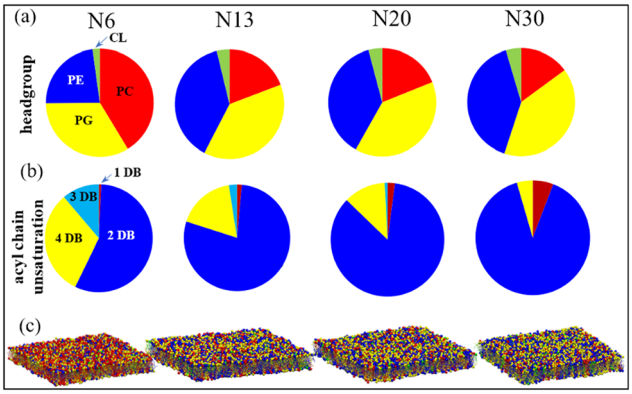

Fig. 5 Pie charts showing the lipidome compositions of four *native* membranes N6, N13, N20, and N30 at four temperatures based on (a) headgroups (PC: phosphatidylcholine, PE: phosphatidylethanolamine, PG: phosphatidylglycerol, CL: cardiolipin) and (b) unsaturation level (DB: double bond) of the acyl chains. (c) Simulation snapshots of the equilibrated native membranes, where the lipids are color-coded the same as the pie charts representing four different headgroups. The phosphate beads of headgroups are represented by spherical beads. Reprinted with permission from ref. 146 Copyright {2022} American Chemical Society.

unraveled the importance of headgroup remodeling during diurnal temperature variation of soil bacteria.

Role of cyclic groups

Analogous to unsaturated fatty acids, studies show the incorporation of cyclic fatty acids to maintain membrane fluidity. Our group[147] investigated the role of lipids with cyclopropane (CP) ring containing fatty acids on retaining the packing density of fluidity of the lipid membrane of *E. coli* bacteria with reduction of temperature. A realistic lipid bilayer of *E. coli* bacteria (containing 14 different types of lipids) was simulated using both coarse-grained and all-atom molecular dynamics simulations across a wide range of temperatures between 250 and 350 K. To address the question of why cyclopropane fatty acids are preferred over C–C single or C=C double bonds, a group of modified lipid membranes were also simulated by replacing cyclopropane (CP) groups with either single or double bonds. No differences were observed between the CP fatty acids with the unsaturated ones in coarse-grained resolution, suggesting that the CG model may not adequately represent the effects of cyclopropane-containing lipids. However, at the all-atom resolution, a noticeable difference emerged in membrane properties due to the presence of CPs and unsaturation, especially at lower temperatures. CPs are particularly effective at preventing close packing of membrane lipids, providing a rigid kink along the acyl chain that double bonds do not. Additionally, CPs not only inhibit the fluid-to-gel phase transition of the corresponding lipids but also prevent phase transitions in unsaturated lipids by interacting with various lipids in the membrane. This study helped in explaining why *E. coli* bacteria, which are vulnerable to freezing environments, utilize cyclopropanation of acyl chain double bonds. This explanation may also apply to other bacteria that adopt cyclopropanation as a survival strategy.

Role of hydroxylation and methyl branching

The cell membrane of Archaea includes lipids with isoprenoid carbon chains connected to glycerol by ether bonds. Of these, isoprenoid glycerol dialkyl glycerol tetraethers (GDGTs) are the widely reported membrane lipids. The methylation of GDGTs at low temperatures is reported as the fundamental HVA strategy of the Archaea class of microorganisms. The methyl modification of GDGTs modulates the fluidity and packing of the membrane under cold stress. Recent MD simulation studies by Naafs *et al.*[148] and Zhou *et al.*[149] provided insights into the structural changes in the membrane of archaeal cells in response to cold environments. The methyl groups hinder the packing of lipids and attenuate the phase transition changes in membrane fluidity. Their study extensively detailed the structural features including area per lipid, core thickness, core order, hydrogen bonding between lipid with lipid and lipid with water, and mobility of lipids compared between membranes with and without the methyl modifications. The study[148] compared tetra-, penta-, and hexa-methylated GDGTs with unmethylated ones at a constant temperature of 300 K and observed a consecutive increase in membrane fluidity and a decrease in membrane rigidity with the increase in degree of methylation. This observation supports the fact that in bacterial membranes the degree of methylation decreases with an increase in temperature owing to its cold adaptation.

4. Protecting lipid membrane *via* OMA: molecular level insights

Osmolytes, which are low molecular weight organic solutes, help in maintaining cell volume under stress and stabilize the membrane structure. Some examples are amino acids, sugars, polyols, methylamines, and urea *etc.* Often functioning as antioxidants[150] they support redox balance. These 'compatible solutes' do not interfere with macromolecules and can be regulated without disrupting cellular functions.[151,152] At physiological pH, osmolytes are typically neutral but can be anionic[153] in some bacteria and archaea, balanced by potassium ions. According to the compatibility, cells can use various osmolytes interchangeably for protection. Below, we discuss the use of various osmolytes for protecting the cell membrane under different stressed conditions and suggest possible mechanisms for the protection.

Dehydration stress

Dehydration can cause phase transition, lateral segregation, and membrane fusion, disrupting the membrane barrier functions and leading to cell injury. Some organisms produce fructans,[154] which stabilize the membranes by integrating into lipid headgroups, reducing leakage during freezing or drought. Cryptobiosis, another survival strategy, involves near-undetectable metabolic activity under extreme stress.[155,156] Anhydrobiosis, a type of cryptobiosis, occurs in extreme dehydration, and involves the production of different osmolytes.

Sugars are among the mostly abundant osmolytes observed in nematodes, tardigrades, bacteria, yeasts, mosses, fungi, pollens, seeds, and higher plants.[157–159] Interactions between sugars and cell membranes are believed to play a vital role in preserving cells during desiccation. Experimental techniques and molecular dynamics (MD) simulations have been instrumental in uncovering lipid–sugar interactions. Together, these methods have led to the formulation of three main hypotheses regarding OMA, as discussed below.

(i) *Water-replacement hypothesis (WRH)*: sugars replace water molecules by forming favorable hydrogen bonds around polar and charged groups of lipids, stabilizing the original self-assembly structure of the lipid bilayer even in the absence of water.[68,158,160,161]

(ii) *Water-entrapment hypothesis*: saccharides concentrate residual water molecules near the lipid bilayer and thereby maintain solvation, which is necessary for retaining the structural and dynamical properties.[162–164]

(iii) *Vitrification hypothesis*: sugars in anhydrobiotic systems act as vitrifying agents, forming amorphous glasses that protect biological structures.[165–167] Vitrification reduces structural fluctuations and prevents mechanical disruption.

Note that the above three hypotheses are not only applicable for sugars but also valid for other osmolytes. Previous studies indicated that these hypotheses are not mutually exclusive and may collectively aid in protecting the cell membrane.[70,168–174]

Experiment and MD simulation suggested that the nature of interaction between lipid bilayers and sugars depends on the concentration of the sugar.[175–179] The latter can either bind to or be expelled from a lipid bilayer, depending on their concentration. At low concentrations, small sugars bind strongly to the bilayer, causing the membrane to become thinner and laterally expanded due to the accumulation of sugar at the interface.[176–178] At relatively larger concentration, sugars are gradually expelled from the membrane surface. This repulsive interaction helps counteract membrane thinning. This dual behavior of sugar–membrane interactions reconciles conflicting findings in earlier reports on how sugars modulate membrane properties.[178,180] Ref. 169 and 181 showed that in multicomponent lipid bilayers, lipid mixing is linked to sugar concentration.

In an MD simulation study by our group[70] we investigated how trehalose supports the survival of a realistic *Escherichia coli* (*E. coli*) bacterial membrane under severe dehydration. The hydration levels were varied from the fully hydrated state to severely dehydrated conditions. Fig. 6 illustrates that decreasing hydration levels lead to an increase in the packing density of the lipid bilayer measured by area per lipid and membrane thickness. However, unlike single-component lipid bilayers, the *E. coli* membrane exhibits only partial gel phase formation under reduced hydration. Trehalose plays a crucial role in stabilizing the lipid bilayer under low hydration conditions. By forming hydrogen bonds with lipids (WRH), trehalose prevents excessive lipid proximity and intra-lipid hydrogen bond formation. Importantly, trehalose does not necessarily enhance membrane fluidity but maintains the fluid phase by creating a viscous matrix at high sugar concentrations (vitrification hypothesis). In another work, we compared the effect of trehalose and sucrose on the dehydration-induced phase transition of the *E. coli* lipid membrane. The results show that both disaccharides exhibit nearly the same efficacy in stabilizing the membrane under severe desiccation.[171]

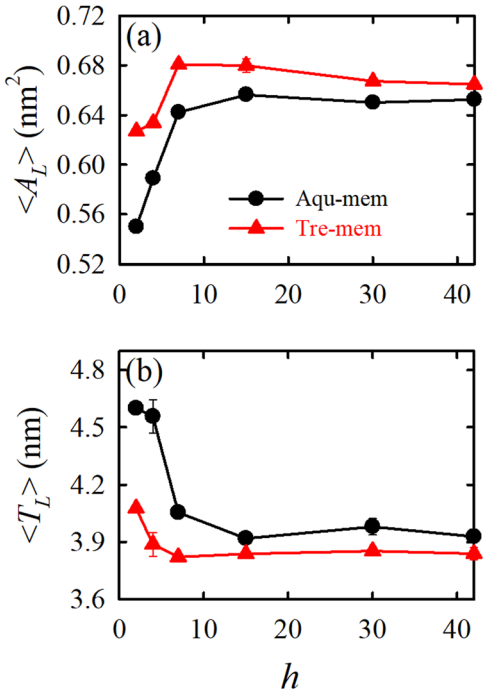

Fig. 6 The packing density parameters: (a) average area per lipid $\langle A_L \rangle$ and (b) average membrane thickness $\langle T_L \rangle$ for different hydration levels (h) for the *Aqu-mem* (without trehalose) and *Tre-mem* (with trehalose) systems. Error bars are calculated over three independent trajectory segments of the production run. Reprinted with permission from ref. 70 Copyright {2023} American Chemical Society.

In addition to sugar molecules, urea and trimethylamine-*N*-oxide (TMAO) serve as notable osmolytes that aid in stabilizing membranes during dehydration induced by osmotic stress, especially in marine organisms. Besides an experimental work by Schneck *et al.*,[69] our simulation[68] provided a thorough molecular level understanding on the impact of urea and TMAO on the dehydration-induced phase transition of lipid membranes from a fluid to a gel phase. TMAO counteracts urea's protein denaturation effects in deep-sea organisms. Fig. 7 shows that as the hydration level changes from fully hydrated (h = 35) to dehydrated (h = 4) conditions, the packing density of the membrane increases, leading to gel phase formation.

Urea prevents dehydration-induced changes, whereas TMAO induces a phase transition at lower hydration levels, causing a fluid-to-gel transition in the membrane. The normalized electron density profile (Fig. 8a) reveals that the TMAO density decreases earlier than urea, suggesting that urea penetrates the lipid headgroup region while TMAO stays excluded from the membrane interface. Radial distribution functions (Fig. 8b) and Kirkwood–Buff integrals (Fig. 8c) show that urea

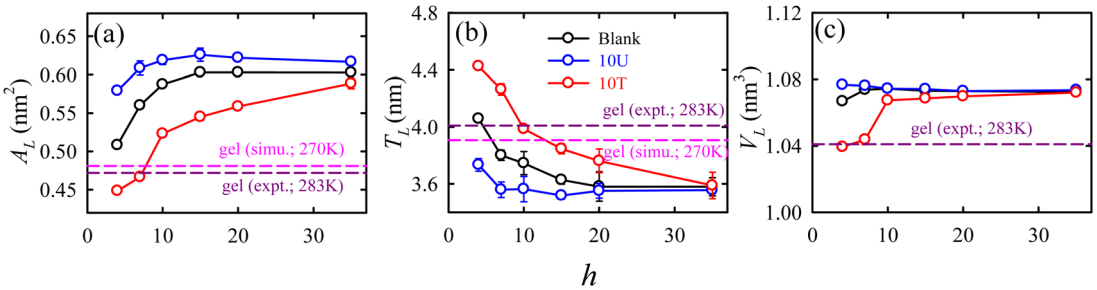

Fig. 7 The hydration level-dependent packing density parameters: (a) area per lipid A_L, (b) thickness T_L, and (c) volume per lipid V_L for different membrane systems: blank (black), 10U (blue), and 10T (red). The membrane systems with 10 wt% urea and 10 wt% TMAO are termed as 10U and 10T, respectively. "Blank" represents the membrane system without osmolytes. The error bars are the standard deviations over the average values calculated for three independent trajectory segments. The simulated[113] and experimental[182] values for the gel phase of DMPC, taken from the literature, are represented by horizontal dashed lines colour coded as pink and dark pink, respectively. Reprinted with permission from ref. 68 Copyright {2021} American Chemical Society.

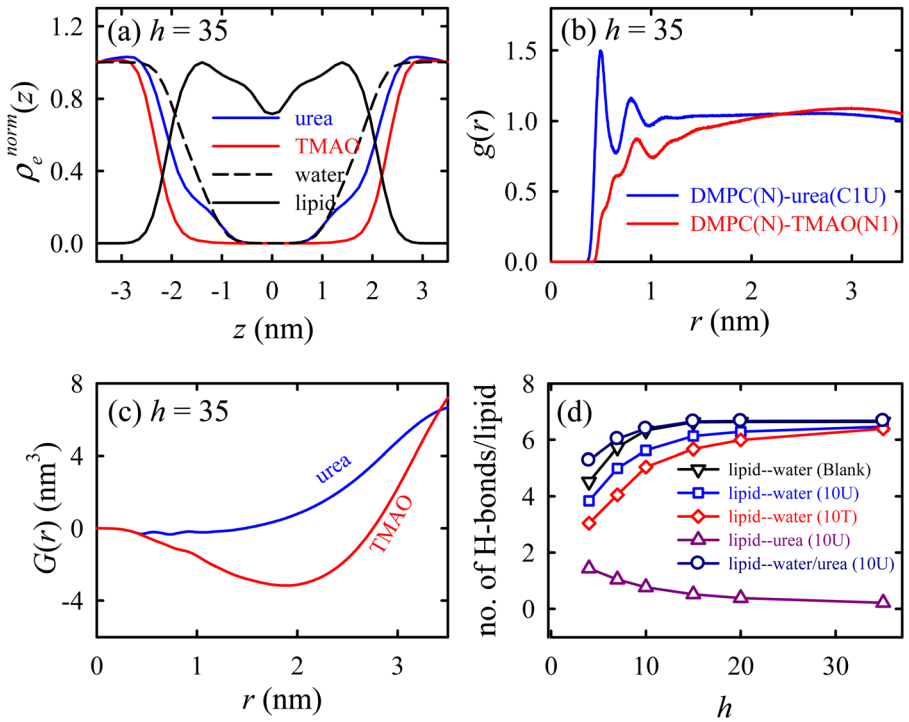

Fig. 8 Specific interactions of the osmolytes with the lipid membrane. (a) The normalized EDP ($\rho_e^{norm}(z)$) of the lipid, osmolytes, and water. (b) The radial distribution function $g(r)$ between the lipid and the osmolytes. (c) The Kirkwood–Buff integral (KBI) for the osmolytes as a function of distance r. (d) The number of lipid–water and lipid–urea H-bonds per lipid as functions of the hydration level h for different membrane systems. These results are for the Blank (without osmolytes), 10U (with urea), and 10T (with TMAO) membrane systems. Reprinted with permission from ref. 68 Copyright {2021} American Chemical Society.

has a strong interaction with lipid headgroups, unlike TMAO, which has a weaker affinity. Hydrogen bonding analysis (Fig. 8d) indicated that urea (both an H-bond donor and acceptor) forms favourable H-bonds with lipids, maintaining membrane fluidity. On the contrary, TMAO, with only H-bond accepting ability, primarily accumulates in bulk water rather than the membrane interface. Osmolyte mixtures (2 : 1 and 1 : 1 (urea : TMAO)[183]) show similar membrane properties to the system without osmolyte, suggesting that TMAO counteracts urea's effects by removing some urea molecules from the lipid membrane.

Cold stress

We have already discussed the importance of HVA of the cell membrane of psychrophiles in previous sections. These organisms can also protect their cellular components under cold stress *via* cryoprotectants.[184–187] Freeze-tolerant organisms synthesize various cryoprotectants, such as DMSO, glycerol, betaine, urea, TMAO, hydroxyectoine, sugars, proline, *etc.*, to combat the cold shock.[67,188–190] The impacts of these cryoprotectants on the lipid membrane at ambient conditions are also studied in detail using experiments and MD simulation techniques.[67,154,191–197] For example, a simulation study by

Smiatek *et al.*[198] demonstrated that hydroxyectoine impacts the properties of aqueous solutions containing DPPC lipid bilayers. Hydroxyectoine, acting as a kosmotrope, was preferentially excluded from the membrane surface, leading to increased surface pressure and stabilization of the membrane structure.

There are studies which focused on the protective action of these cryoprotectants on the lipid bilayer under cold stress.[67,188–190] Our group[113] investigated the effect of TMAO at different temperatures, particularly focusing on the fluid-to-gel phase transition of the lipid bilayer. Our findings indicate that TMAO has a more significant impact on the fluid phase of the membrane compared to the gel phase. Structural comparisons revealed that lipids with shorter acyl chains are more significantly influenced by TMAO. Furthermore, we assessed the gel-to-fluid phase transition temperature (T_m) using temperature annealing simulations. As shown in Fig. 9, T_m increases with higher TMAO concentrations, consistent with experimental observations.

Heat stress

The majority of osmolytes amassed by thermophiles and hyperthermophiles are negatively charged,[93,199–201] unlike mesophiles, which tend to accumulate neutral or zwitterionic compounds such as trehalose, glycerol, or proline.[202] This observation suggests that charged solutes may play a crucial role in protecting cells from heat-induced damage. Studies have shown that negatively charged solutes, such as phosphodiester compounds or carboxylic acids,[199] are particularly effective at enhancing protein stability. However, the use of these charged solutes requires the accumulation of positive counterions like potassium, which can be toxic to most mesophilic bacteria but not to hyperthermophiles, which have adapted to high salt concentrations.[93,200,201] Xerophilic fungi, which survive and thrive in extreme dehydrated conditions, use glycerol to counterbalance external osmotic pressure, but during heat shock, they switch to accumulating trehalose.[203,204] This switch is

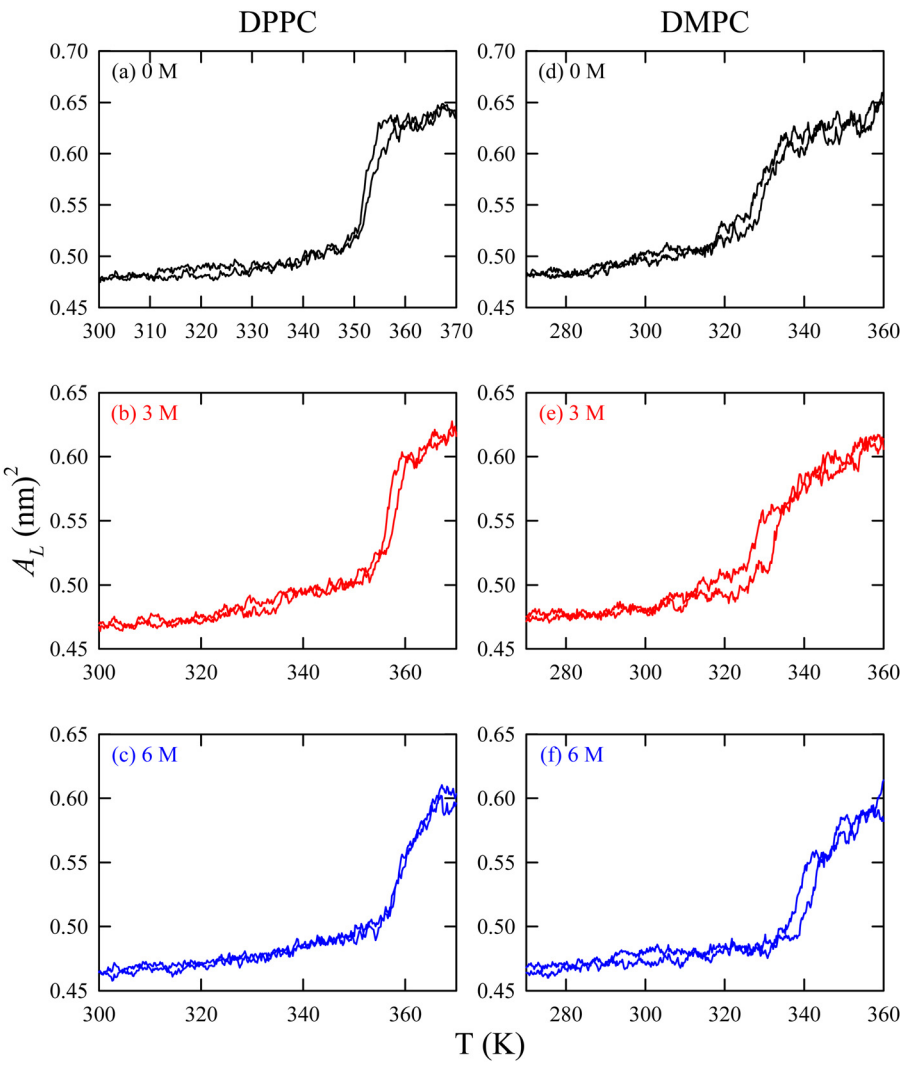

Fig. 9 Area per lipid *versus* temperature for DPPC ((a) 0 M TMAO, (b) 3 M TMAO, and (c) 6 M TMAO) and DMPC ((d) 0 M TMAO, (e) 3 M TMAO, and (f) 6 M TMAO) lipid membranes during heating from gel to fluid phases. A_L is different from $\langle A_L \rangle$ in the sense that the latter is a time-averaged quantity of A_L. Reprinted with permission from ref. 113 Copyright {2021} American Chemical Society.

accompanied by changes in membrane lipids. After heat shock, fungi show greater thermotolerance in glycerol than in salt media, suggesting a synergistic effect between glycerol and trehalose and a crucial role of osmolyte composition and membrane lipid changes in heat stress adaptation.[203,204]

Under heat stress, most genotypes displayed a notable accumulation of sugar and proline, alongside heightened activity of CAT, GPOX, and SOD.[205] Trehalose has been demonstrated to effectively stabilize the native structure of biological systems in extreme environments. An MD simulation work by Liu *et al.*[206] showed that as the temperature escalates from 303 K to 373 K, the presence of trehalose notably diminishes the disparity in membrane properties before and after heating, with this effect being more pronounced at higher trehalose concentrations. With an increase in trehalose concentration from 0 to 256, the relative variation upon heating decreases by approximately 46.6% for the area per lipid and 50.4% for bilayer thickness. This indicates that besides enhancing membrane structure at specific temperatures, trehalose also shields it from damage due to structural changes during abrupt temperature fluctuations. Furthermore, the underlying mechanism is attributed to the slow mobility and strong hydrogen bonding (H-bonding) ability of trehalose. These findings could offer valuable insights into the design of protective agents and processes.

Pressure stress

In the deep sea, high hydrostatic pressure disrupts the lipid membrane structure and fluidity. High pressure alters the lipid membrane's packing density, fluidity, and permeability, causing a phase transition from fluid to gel phase. To counteract this, certain solutes, termed 'piezolytes,'[207,208] accumulate under high-pressure conditions. In shallow-water marine organisms, trimethylamine-*N*-oxide (TMAO) is typically low or absent, except in ureosmotic fish like sharks. However, deep-sea teleost fishes and certain crustaceans and skates exhibit TMAO concentrations up to 300 mmol kg^{-1}, increasing with depth.[66,209,210] TMAO is known to stabilize the native structure of proteins. Deep-sea bacteria accumulate β-hydroxybutyrate in response to hydrostatic and osmotic pressure.

Some experimental studies extensively investigated the effect of pressure on the structure and phase behavior of various lipid bilayers.[211–213] The effect of osmolytes, such as TMAO and trehalose, on the lipid membrane under high pressure was studied by Winter and coworkers.[214,215] Their findings reveal that under high hydrostatic pressure, the lipid order parameters in fluid membranes increase slightly, causing minor lipid elongation. However, TMAO's significant impact on interlamellar spacing in lipid bilayers is largely unaffected by temperature and high pressure. Additionally, TMAO alters the lateral organization of heterogeneous model membranes, promoting the coalescence of lipid domains. This coalescence is likely due to increased line tension between liquid-ordered and disordered domains within raft-like lipid bilayer structures.

These experimental studies motivated us to elucidate the observations using the MD simulation technique.[216]

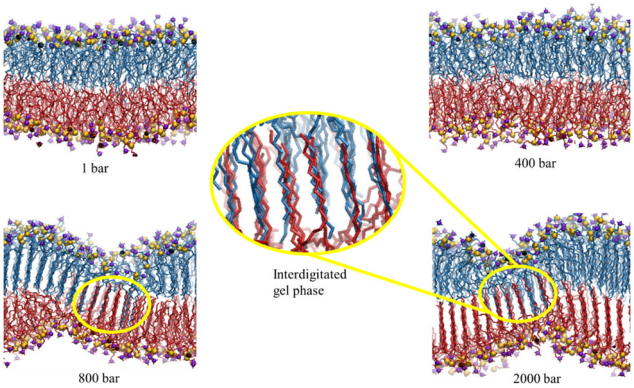

Fig. 10 The final configurations of the simulation trajectories of the DMPC membrane in the Blank membrane system (without the osmolytes) at 1, 400, 800, and 2000 bar. The hydrogen-atoms of lipid, water and osmolyte molecules are not shown for clarity. The nitrogen and phosphorous atoms of the lipid headgroup are color-coded as violet and yellow, respectively. The magnified version of the interdigitated gel phase is shown. The opposite leaflets are shown in different colors. Reprinted with permission from ref. 216 Copyright {2022}American Chemical Society.

We examined the pressure-induced phase transition of the lipid membranes from fluid-to-gel phase and the influence of two osmolytes, urea and TMAO. It was observed that neither solute can inhibit the pressure-induced phase transition of the membrane. Under high pressure, both solutes have negligible impact, and an interdigitated gel phase forms with reduced membrane thickness (Fig. 10). Urea maintains membrane fluidity at lower pressures through hydrogen bonds with lipid headgroups, while TMAO increases further dehydration. High pressure disrupts urea's hydrogen bonding, promoting the gel phase transition and expelling water and solutes from the membrane. These findings suggest that deep-sea organisms might use other strategies, like HVA, to protect their membranes under high pressure. Our findings align closely with the conclusions drawn by Schneck *et al.*[69,116] in their combined experimental and simulation investigations into the impact of urea and TMAO on lipid membranes.

Perturbing solutes

Certain organic osmolytes can counteract the disruptive effects of solutes that accumulate during osmotic stress and disrupt the macromolecules. Urea serves as one such disruptant and is counteracted by trimethylamine-*N*-oxide (TMAO).[68,69,183] Elevation in the concentrations of specific inorganic ions (such as Na^+ and K^+) above their typical intracellular levels can negatively impact both the catalytic rate and the apparent Michaelis constant, K_m of different enzymes found in plants and animals.[202] Consequently, high intracellular salt concentrations during osmotic stress are likely to impair metabolic function and the maintenance of proper transmembrane potentials. Methylamines have been observed to mitigate the disruptive effects of salts.[217] Methylated forms of glycine, such as sarcosine, dimethylglycine, and glycine betaine, can alleviate the inhibition of plant enzyme activity induced by NaCl, with

the level of protection increasing in correlation with the degree of methylation. For instance, the introduction of exogenous glycine betaine (GB) significantly enhanced the salt tolerance of common bean plants.[218,219] This enhancement was attributed to the augmentation of antioxidant defense mechanisms, encompassing both enzymatic (such as peroxidase, superoxide dismutase, and catalase) and non-enzymatic (like proline and glutathione) agents.[202] The salt tolerance induced by GB in common bean plants primarily relies on its osmoregulatory effect, with its antioxidant capacity playing a secondary role. GB results in a considerable reduction in Na^+ accumulation while concurrently promoting K^+ uptake, thereby maintaining a higher K^+/Na^+ ratio.[219]

5. Role of proteins in stabilizing lipid membranes under stress

Proteins play crucial roles in stabilizing lipid membranes under various stress conditions. In Section 2, we discussed how certain proteins aid in the homeoviscous adaptation of cell membranes, preventing the harmful effects of fluid-to-gel phase transitions in the lipid bilayer. Proteins also directly stabilize lipid membranes through interactions. Understanding these mechanisms provides insights into cellular adaptation and resilience. The protective roles of proteins on lipid membranes are summarized below with a few examples.

At low temperatures, proteins protect lipid membranes both directly and indirectly. Cold shock proteins (CSPs) are expressed in response to low temperatures and maintain membrane fluidity by directly interacting with the lipid bilayer. The best-characterized CSP is CspA, which is the primary CSP expressed in *Escherichia coli* when the temperature decreases.[220,221] CSPs facilitate the modification of lipid composition to ensure proper fluidity and functionality.[221,222] On the other hand, cryoprotective proteins indirectly protect cell lipid membranes under cold stress. Antifreeze proteins, a type of cryoprotective protein, prevent ice formation within intracellular water.[223–225] Experimental and simulation studies suggest that antifreeze proteins inhibit the growth and crystallization of ice by binding to ice crystals.[226–230] This inhibition protects membrane integrity in freezing conditions.

The detrimental effect of heat stress on lipid membranes can be mitigated by proteins. The heat shock proteins (HSPs) can assist in refolding denatured proteins and stabilizing protein structures associated with the membrane, maintaining membrane integrity.[231,232] HSP70, for example, binds to unfolding proteins and prevents aggregation, helping to preserve the overall stability of the membrane. Thermostable proteins are another class of proteins found in thermophiles that remain stable and functional at high temperatures. They can support membrane stability by maintaining their interactions with the lipid bilayer. These proteins often possess unique structural features that confer stability and enable them to operate under thermal stress.

Proteins play a crucial role in protecting lipid membranes under dehydration stress. Dehydrins, disordered proteins expressed in plants during embryogenesis and water-related stress, accumulate during dehydration and bind to phospholipids, preserving membrane structure. Acting as molecular chaperones, dehydrins stabilize macromolecules and membranes against dehydration-induced stress. Although the exact molecular function and structural mechanisms of dehydrins are not fully understood, evidence suggests that they protect cell membrane structure and dynamics.[233,234] For example, the cold-induced dehydrin Lti30 binds to membranes *via* its conserved K segments. This binding, regulated by pH and phosphorylation, shifts the membrane phase transition to lower temperatures, aiding in cold stress adaptation. Several models propose that dehydrins stabilize plasma and organelle membranes,[183,235–238] act as chaperones or cryoprotectants,[239] are hygroscopic to prevent complete dehydration,[240] and bind metal ions.[241] Dehydrins contain a high proportion of hydrophilic and charged amino acids and are characterized by repetitive K-segments. Studies on dehydrin Lti30 (K6) from *Arabidopsis thaliana* show that it strongly associates with anionic lipid membranes, where the disordered K-segments fold into α-helices on the membrane surface.[242–245] Aquaporins are the water channel proteins that regulate water transport, which can indirectly help in stabilizing membranes under desiccation. These channels manage water loss and maintain membrane hydration. They facilitate rapid water movement across the cell membrane, crucial for maintaining cell turgor and preventing desiccation damage.[246]

6. Concluding remarks

The study of extremophiles has unveiled remarkable adaptive strategies that enable organisms to thrive in some of Earth's most hostile environments. Through homeoviscous adaptation (HVA), these organisms meticulously regulate their lipid compositions to maintain the structural integrity and fluidity of their cell membranes amidst external stresses. By modulating lipid acyl chains and head groups, HVA ensures optimal barrier function, facilitates cell communication, regulates molecular transport, maintains cell shape and stability, and supports crucial cellular interactions such as recognition and adhesion. In parallel, osmolyte-mediated adaptation (OMA) employs small organic molecules to stabilize lipid membranes, protecting against fluctuations in osmotic pressure and environmental extremes. The insights gained from molecular-level studies of HVA and OMA not only deepen our understanding of extremophile biology but also offer insights into the broader potential for life in extreme environments, including extraterrestrial habitats.

Looking forward, future research on extremophiles is poised to advance our understanding in several key areas. First, continued exploration of novel extremophiles and their adaptive mechanisms will expand our knowledge of the limits of life and its adaptation strategies under extreme conditions.

This includes investigating extremophiles in unexplored terrestrial environments and potentially extraterrestrial habitats, leveraging advancements in technology and interdisciplinary approaches. Second, integrating omics technologies with structural biology and computational modeling will provide comprehensive insights into the molecular underpinnings of HVA and OMA. This holistic approach will elucidate how genetic, proteomic, and metabolomic adaptations synergistically contribute to membrane stability and function. Moreover, applying these insights to synthetic biology and biomaterials science holds promise for developing resilient biotechnological applications and materials that mimic extremophile strategies. Ultimately, by unraveling the adaptive strategies of extremophiles, we not only advance our scientific understanding but also inspire innovative solutions for sustainability, biotechnology, and the search for life beyond Earth.

Data availability

Data will be made available on request to the authors.

Conflicts of interest

The authors declare no competing financial interest.

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