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The effects of molecular and nanoscopic additives on phospholipid membranes

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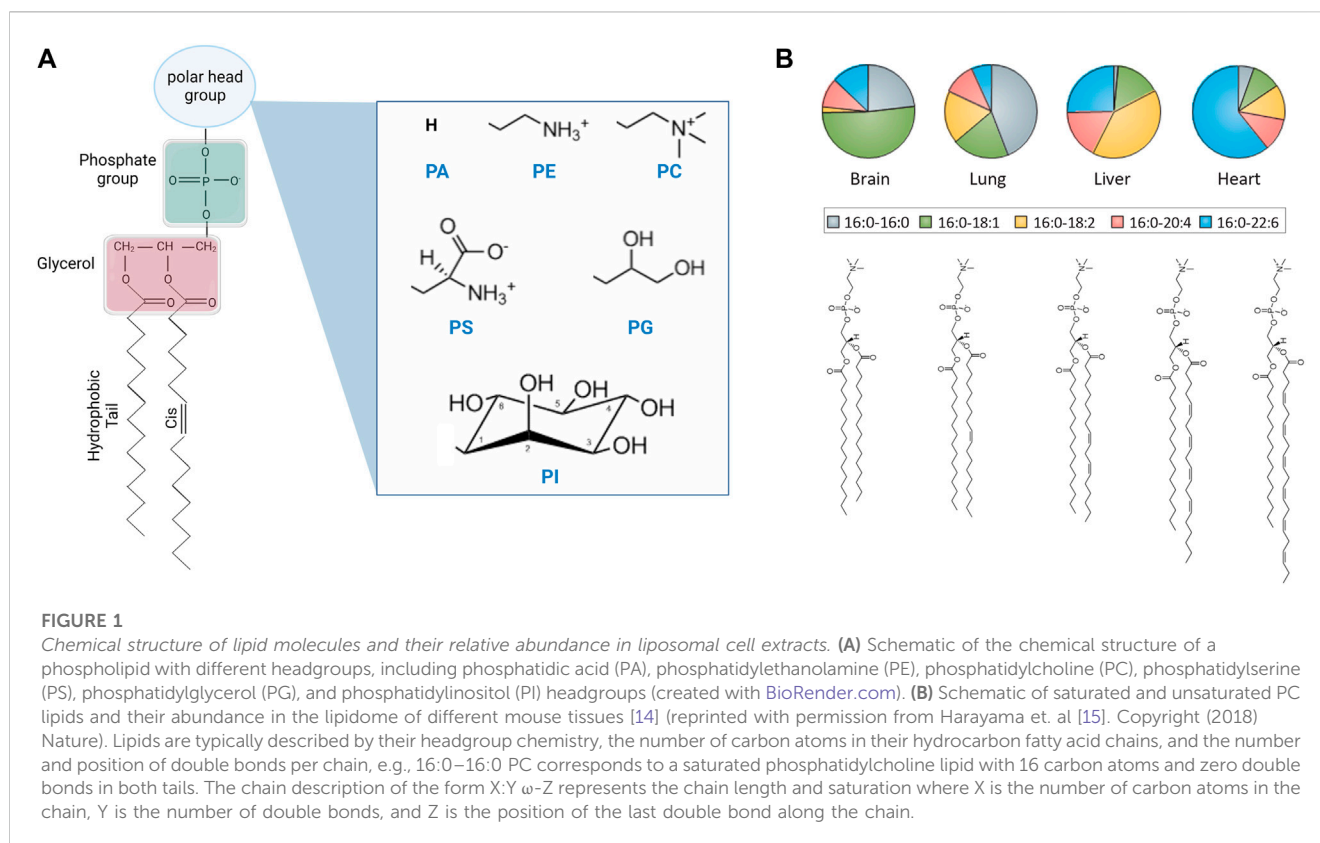
Lipid bilayers—the main matrix of cell membranes—are a paradigm of soft molecular assemblies whose properties have been evolutionarily optimized to satisfy the functional requirements of cells. For instance, lipid bilayers must be rigid enough to serve as the protective barrier between cells and their environment, yet fluid enough to enable the diffusion of proteins and molecular clusters necessary for biological functions. Inspired by their biological multifunctionality, lipid membranes have also been used as a central design element in many practical applications including artificial cells, drug nanocarriers, and biosensors. Whether biological or synthetic, lipid membranes often involve molecular or nanoscopic additives that modulate the membrane properties through various mechanisms. Hence, how lipid membranes respond to additives has justifiably drawn much attention in recent years. This review summarizes findings and observations on different classes of additives and their effects on structural, thermodynamic, elastic, and dynamical membrane properties that are central to biological function or synthetic membrane performance. The review primarily focuses on phospholipids as a major component of cell membranes and a widely used lipid type in synthetic membrane designs.

KEYWORDS

sterols, drug molecules, proteins and peptides, nanoparticles, molecular packing, bending rigidity, fluidity, phase transitions

Introduction

Lipid bilayers are the primary structure of the plasma membranes of living cells, playing a crucial role as the barrier between the cytosol and the extracellular environment and the first line of cellular defense against pathogens and foreign particles. These 3–4 nm thick membranes are formed of dynamic, fluid self-assemblies of lipids—amphiphilic molecules characterized by hydrophilic heads and hydrophobic fatty acid tails [1]. The properties of lipid membranes dictate various cellular functions, including molecular transport, protein recruitment, signal transduction, and maintaining a stable intracellular environment for biochemical reactions. This multifunctionality has inspired the use of synthetic and biomimetic lipid membranes in numerous practical applications, including artificial cells [2–6], molecular and therapeutic nanocarriers [7–10], as well as biosensing and biosorting platforms [11–13]. Whether biological or synthetic, lipid membranes have to meet important yet often contradictory requirements. For instance, they should maintain reasonable mechanical integrity while also providing a fluid environment for membrane components to diffuse and interact. Therefore, understanding the physical properties of lipid membranes is central to every aspect of their biological function and their practical applications.

FIGURE 1
Chemical structure of lipid molecules and their relative abundance in liposomal cell extracts. **(A)** Schematic of the chemical structure of a phospholipid with different headgroups, including phosphatidic acid (PA), phosphatidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylserine (PS), phosphatidylglycerol (PG), and phosphatidylinositol (PI) headgroups (created with BioRender.com). **(B)** Schematic of saturated and unsaturated PC lipids and their abundance in the lipidome of different mouse tissues [14] (reprinted with permission from Harayama et. al [15]. Copyright (2018) Nature). Lipids are typically described by their headgroup chemistry, the number of carbon atoms in their hydrocarbon fatty acid chains, and the number and position of double bonds per chain, e.g., 16:0−16:0 PC corresponds to a saturated phosphatidylcholine lipid with 16 carbon atoms and zero double bonds in both tails. The chain description of the form X:Y ω-Z represents the chain length and saturation where X is the number of carbon atoms in the chain, Y is the number of double bonds, and Z is the position of the last double bond along the chain.

Lipids constitute a large class of molecules that vary in their head group chemistry, tail length, and tail unsaturation (see Figure 1) [16]. In cells, lipid chemical structures have evolved to meet different functional requirements, and accordingly they exist in varying concentrations in the membranes of different organelles and across different cellular tissues [15, 17, 18]. In fact, mammalian membranes include over 1,000 unique lipid species which play various roles in biological function. In this review article, we primarily focus on phospholipids, which constitute a major lipid component of mammalian plasma cell membranes and are widely used in synthetic lipid membrane applications. Phospholipids are characterized by a phosphate headgroup and two hydrophobic fatty acid chains. Although over half of the phospholipids in the plasma membrane have a phosphatidylcholine (PC) headgroup, the small fraction that do not are still required in vital cell processes and play a critical role in locally modifying the membrane properties [17]. This diversity of chemical structures has positioned phospholipids as attractive molecular candidates in numerous applications requiring synthetic membranes with biomimetic functionality.

Besides phospholipids, biological and synthetic lipid membranes typically host other molecules or additives that impart specific functions. For example, cholesterol—an abundant molecular component in mammalian plasma cell membranes—is known to regulate membrane compartmentalization and cell signaling, two essential processes in cell survival [19, 20]. In synthetic and liposomal membranes, cholesterol is often used as a membrane stabilizer and a regulator for lateral lipid organization [21–24]. Membrane proteins are another functionally critical constituent of cell membranes and synthetic membrane mimics. How

proteins interact with their host lipid membranes has direct effects on human health and disease [25, 26] and on the functional properties of synthetic membranes and artificial cell communication [27, 28]. In addition to biological molecules, cell membranes are often exposed to small synthetic additives, such as small drug molecules, diagnostic nanoparticles, and environmental cytotoxins [29–31]. Interestingly, studies show compelling evidence that cell membranes often adapt to changes in molecular uptake or environmental conditions by modifying their lipid composition to maintain specific membrane properties that are necessary for function [32–34], a process known as homeoviscous adaptation [35–37]. Replicating this adaptive behavior in artificial cells or utilizing it in molecular biosensing or therapeutic approaches would be transformative. Therefore, understanding how lipid membranes respond to additives is necessary in uncovering adaptive cellular mechanisms and emulating them in the design of lipidic membranes as configurable soft materials.

Importantly, such applications strongly rely on the mode of incorporation of different additives in lipid membranes and the magnitude of their perturbation of the membrane physical properties. Knowledge of the molecular mechanisms that underly the interaction of additives with their host membranes is central to the functional assignment of additives and their informed use in artificial and biomimetic membrane systems. To describe these mechanisms and identify emerging rules for soft matter interactions of molecular and nanoscopic additives, we will discuss the partitioning of different types of additives into lipid membranes and their affinity to different lipid phases within the lateral lipid organization [38–40]. This in turn will be mapped to

trends of additive-induced effects on the structural, thermodynamic, or elastic properties of phospholipid membranes. The obtained trends will elucidate general design rules that could accelerate the development of artificial membrane technologies with targeted functionality and tunability.

To this end, we will summarize how different classes of molecular and nanoscopic additives—including sterols, fatty acids, proteins/peptides, drug molecules, polymers, and nanoparticles—affect the membrane structure, function, and dynamics and how these properties can be obtained using various characterization techniques. In each section, we will discuss the effects of these additives on physical membrane descriptors associated with biological functions or synthetic membrane performance and that are commonly ascribed to molecular self-assemblies. This includes structural properties described by the molecular packing (expressed in terms of the area per lipid (A_L) [41] or average area per molecule [42]) and the membrane thickness (typically expressed in terms of the hydrophobic thickness of the fatty acid chain region (D_C), the head-to-head thickness (D_{HH}), or the total thickness (D_B) spanning the entire membrane) [41]. Dynamic properties are described in the context of membrane fluidity and fluctuations, including molecular diffusivity [43–45], membrane viscosity [46, 47], and collective fluctuations associated with elastic membrane properties [48–56] such as the area compressibility modulus (K_A) and the bending rigidity modulus (κ). Thermodynamic membrane properties are described in terms of the Gibbs free energy [57] and phase transitions [58–60] as well as partition and diffusion coefficients [43]—which describe the interactions between molecular additives and their host lipid membrane. Understanding these interactions on a molecular level and mapping them onto physical membrane observables are necessary for elucidating the role of additives in membrane function and designing lipid membranes with tunable functionality.

Amphiphilic additives

Cholesterol and other sterols

Cholesterol (Chol) is an abundant component of mammalian plasma membranes and a common additive in synthetic and liposomal membranes. Accordingly, cholesterol has drawn much attention across various fields of research [61–63] due to its distinct role in membrane structure, rigidity, and fluidity [64, 65]. How cholesterol modulates physical membrane properties is largely determined by where and how it incorporates into the lipid membrane. In membranes with compatible lipid tail lengths, nuclear magnetic resonance (NMR) and neutron diffraction studies show that cholesterol orients itself in an upright position against lipid tails (see Figure 2A), i.e., parallel to the bilayer normal [66, 71]. However, when the hydrophobic thickness of the host lipid membrane becomes smaller than the hydrophobic thickness of cholesterol, cholesterol starts to exhibit tilted orientations and can even sequester between the two membrane leaflets in very thin lipid membranes [66, 72–74]. The dependence of cholesterol's orientation on bilayer composition has also been observed in molecular dynamics (MD) simulations on membranes formed of saturated lipids (i.e., lipids with no double

bonds in the fatty acid chains) and unsaturated lipids (with one or more double bonds in the fatty acid chains) [75–77]. For example, MD simulations show that cholesterol segregates neatly into the hydrocarbon region of SOPC (18:0–18:1) membranes up to a concentration of around 30 mol%, but above this threshold cholesterol is driven towards the center of the bilayer and its tilt angle relative to the bilayer normal increases (Figure 2B) [68]. Similarly, in membranes composed of polyunsaturated DAPC (di20:4 ω-6), doped with 10 mol% DMPC (14:0–14:0), a non-negligible fraction of cholesterol is found to reside between the monolayer leaflets (i.e., perpendicular to the bilayer normal) in DAPC-rich domains, whereas an upright orientation of cholesterol is observed in the DMPC-rich regions [78]. This is in line with cholesterol's stronger affinity to saturated hydrocarbon chains compared to polyunsaturated lipids. Indeed, other studies on DAPC-cholesterol membranes show that while only 5 mol% of fully saturated DMPC (14:0–14:0) is required to pull cholesterol back up into its upright position, it takes 50 mol% of POPC (16:0–18:1) to achieve the same effect [79, 80].

The upright positioning of cholesterol along lipid tails induces tighter molecular packing or condensing of fluid phospholipid membranes, a phenomenon explained by the umbrella model whereby the phospholipid headgroups shield the non-polar cholesterol from unfavorable interactions with the aqueous medium [81]. Although this condensing effect has been observed across various phospholipid membranes with different degrees of chain unsaturation [82–84], the extent to which cholesterol affects molecular packing depends on the degree of lipid chain unsaturation and the position of the double bond along the acyl chain [85, 86]. Notably, the ordering of lipids by cholesterol often results in an increased thickness of the lipid membrane, as observed by numerous techniques including small angle neutron and X-ray scattering (SANS/SAXS) [82], neutron and X-ray diffraction [84, 87], and ^{2}H-NMR [88, 89]. For example, ^{2}H-NMR studies on lipid membranes with varying degrees of chain unsaturation show an increase in quadrupolar splitting with increasing cholesterol content, indicating greater acyl chain ordering (see example in Figure 2C) [89]. Similar studies have illustrated that cholesterol-induced thickening of membranes decreases with lipid chain unsaturation, such that DMPC (14:0–14:0) membranes exhibit the strongest thickening with cholesterol, followed by POPC (16:0–18:1) and DOPC (18:1–18:1) membranes [90]. These observations are in agreement with scattering experiments [69, 91–93] as well as ^{2}H-NMR studies and MD simulations [68, 69, 94]. Importantly, the area-per-molecule values calculated from the membrane thickness agree well with direct measurements of molecular packing in Langmuir monolayer studies [75–77, 95–100]. These observations are consistent with the excess Gibbs free energy, ΔG_{ex}, obtained from Langmuir pressure area isotherms (see Figure 2D), illustrating the role of molecular interactions between lipids and cholesterol in determining the membrane structural properties [75–77]. In such measurements, more negative ΔG_{ex} values indicate stronger attractions between lipids and cholesterol. Here, we note that the condensing effect of cholesterol on fluid membranes, as discussed above, is contrary to its effects on saturated lipid membranes *in the gel state*, where the addition of cholesterol is known to disrupt chain packing and eventually causes a transition from a gel-like to a fluid-like membrane state [101].
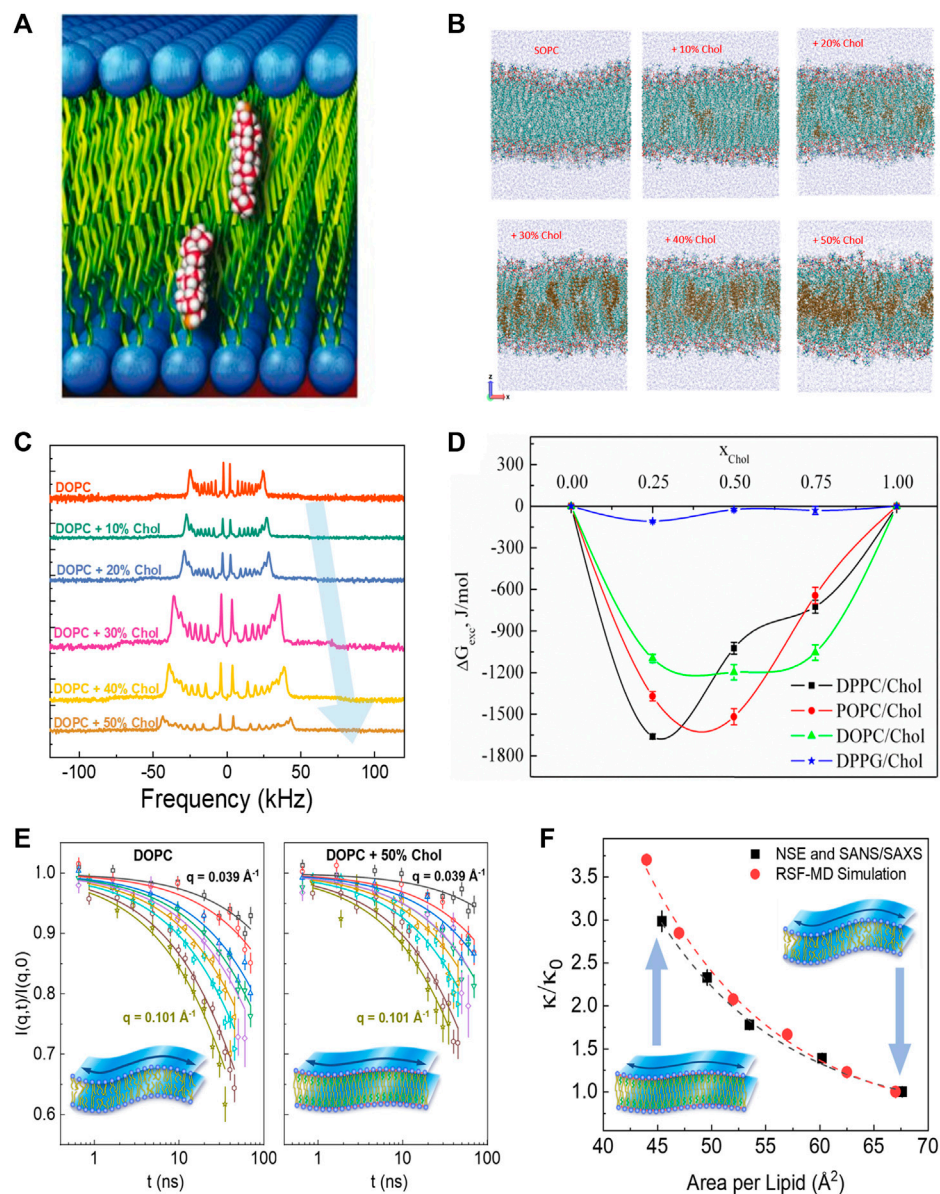
FIGURE 2
Effects of cholesterol on membrane structure and dynamics. **(A)** Schematic of cholesterol's upright orientation in lipid membranes, with its hydroxyl group near the lipid-water interface [66] (reproduced with permission from Kinnun et al. [67]. Copyright (2021) Frontiers). **(B)** MD simulations of SOPC bilayers with increasing cholesterol concentration (reprinted with permission from Ivanova et al [68] Copyright (2023) MDPI). **(C)** Solid-state ^{2}H NMR spectra illustrating cholesterol-induced acyl-chain ordering in multilamellar dispersion of DOPC, indicated by the increase in quadrupolar splitting with increasing cholesterol content (reprinted with permission from Chakraborty et al [69]. Copyright (2020) National Academy of Sciences). **(D)** Excess free energy of mixing (ΔG_{exc}) vs cholesterol molar fraction (χ_{Chol}) in PC/cholesterol lipid monolayers showing the differential affinity of cholesterol to lipids with varying chain unsaturation and headgroup charge (reprinted with permission from Jurak et al [70]. Copyright (2013) American Chemical Society). **(E)** Intermediate scattering functions, $I(q,t)/I(q,0)$, measured by neutron spin-echo on DOPC liposomes with 0 mol% (left) and 50 mol% (right) of cholesterol showing clear slowdown in membrane fluctuations in cholesterol-rich DOPC membranes. **(F)** Dependence of cholesterol-induced bending rigidity in DOPC membranes on corresponding changes in the area per lipid. **(E,F)** are reprinted with permission from Chakraborty et al [69]. Copyright (2020) National Academy of Sciences.

Differences in cholesterol-lipid affinity are a critical factor in the incorporation of cholesterol into various phospholipid membranes, especially in synthetic or liposomal phospholipid membranes requiring high cholesterol concentrations for additional stability. In such applications, cholesterol loading into the membrane is dictated by cholesterol's solubility limit, which strongly depends on the phospholipid headgroup, e.g., phosphatidylcholine (PC) vs

phosphatidylethanolamine (PE) or phosphatidylserine (PS) [81, 102, 103]. These observations highlight differences in cholesterol interactions with phospholipids of various chain or headgroup structures and are confirmed by a range of techniques including SANS [104], NMR [105], fluorescence microscopy [106–108] Langmuir isotherms [109, 110] and MD simulations [111]. This differential affinity of cholesterol to lipids with saturated vs

unsaturated tails or PC vs PE headgroups has important consequences on cholesterol partitioning across different lipid phases in mixed lipid membranes [112–114]. Such concepts have been frequently used in model lipid membranes mimicking the lateral organization of cell membranes [108, 115–117], of high importance in artificial cell designs mimicking signaling pathways. Specifically, in lipid membranes containing saturated and unsaturated PC lipids, cholesterol was initially thought to preferentially partition into regions rich in saturated lipids, resulting in lipid phase separation into a liquid-ordered (L_O) phase that is rich in saturated lipids and cholesterol and a liquid-disordered (L_D) phase that is predominantly formed by unsaturated and/or short chain lipids [118, 119]. However, recent MD simulations suggest a different partitioning mechanism, in which saturated lipids form highly ordered domains surrounded by regions of unsaturated lipids and cholesterol [60]. Nonetheless, and regardless of the partitioning mechanism, the introduction of cholesterol in phospholipid membranes containing saturated and unsaturated lipids typically results in lipid phase separation into domains with different packing states, with tighter lipid packing in the L_O domains and looser packing in the L_D regions.

As expected, such changes in molecular packing due to cholesterol have important implications in the fluidity and permeability of lipid membranes [120–123], with consequences both in biological function and in applications [124, 125]. For example, liposomes rich in cholesterol have been regularly used in drug delivery applications where the addition of cholesterol results in reduced leakage, higher retention, and extended release of the drug load [126, 127]. Cholesterol also plays a significant role in solute partitioning into lipid membranes depending on lipid-cholesterol interactions (discussed earlier) with more pronounced effects for large solutes, membranes with less chain unsaturation, and membranes with smaller phospholipid headgroups [128]. In cell membranes, cholesterol-induced molecular packing can significantly impact the folding of membrane proteins and resultant biological function [129]. Similarly, changes in lipid packing by cholesterol have been associated with changes in the elastic membrane properties. For example, micropipette aspiration studies [130] have demonstrated that the inclusion of cholesterol in lipid membranes leads to an increase in the area stretch modulus, as corroborated by recent MD simulations [131, 132]. Interestingly, similar correlations between molecular packing and the area compressibility modulus, K_A, have been observed in lipid membranes with different degrees of chain unsaturation [133]. These results align with earlier Langmuir monolayer compression studies by Smaby et al. [42] showing that K_A of lipid monolayers decreases with chain unsaturation and increases with cholesterol concentration irrespective of chain unsaturation. Comparable results have been reported in other Langmuir monolayer studies [76, 77, 95] as well as MD simulations [132]. These observations all point to the importance of molecular packing as a key physical descriptor of lipid membranes.

Despite the well-documented effects of cholesterol on lipid packing and lateral compressibility in common phospholipid membranes (particularly PC lipids), its effects on the bending rigidity of these membranes is still a topic of current debate. For example, earlier studies using diffuse X-ray scattering, flicker spectroscopy, and electrodeformation have reported that

cholesterol has different stiffening effects on membranes with varying degrees of chain unsaturation [134, 135]. In these studies, cholesterol was found to significantly stiffen saturated DMPC (14:0–14:0) membranes, moderately stiffen mono-unsaturated SOPC (18:0–18:1) membranes, and have no effect on the bending rigidity of di-monounsaturated DOPC (18:1–18:1) membranes. However, a recent study using neutron spin-echo (NSE) spectroscopy, ^{2}H-NMR relaxometry, and MD simulations showed that the bending fluctuations of DOPC membranes experience significant slowdown on nanosecond timescales with the addition of cholesterol (Figure 2E) [57]. More importantly, the observed increase in the bending rigidity modulus κ closely followed the measured changes in the area-per-lipid induced by cholesterol (Figure 2F). These findings also align with the role of cholesterol in maintaining cell stability against lysis and its use as a stabilizing molecule in liposomal drug delivery applications [126, 136, 137]. Such observations have important consequences in biological or synthetic membranes composed of saturated and unsaturated lipids with cholesterol, resulting in phase-separation into L_O and L_D phases, also known as raft formation. Indeed, neutron spectroscopy studies have reported that in such membranes the L_D phase exhibits a lower κ than the L_O phase [138]—in agreement with the lipid packing dependence of the two phases.

In liposomal applications, while cholesterol has been frequently used in stabilizing liposomes and reducing premature drug release [139, 140], it tends to rapidly exchange with cell membranes and lipoproteins—eventually resulting in compromised liposomal stability. An alternative workaround is to use synthetic molecules formed by the conjugation of sterols to lipid acyl chains, also known as sterol modified lipids (SMLs) [141]. As intended, SMLs prevent the exchange of free sterols out of liposomal membranes while maintaining the structural and mechanical properties of the liposome [141]. Studies of SMLs in saturated DMPC (14:0–14:0) and DPPC (16:0–16:0) lipid bilayers using NMR, Langmuir pressure-area isotherms, SANS, and neutron reflectometry show an increase in membrane thickness and concomitant decrease in the mean molecular area with the addition of SMLs [142, 143]. More importantly, liposomes containing SMLs show remarkable decrease in leakage over extended time compared to traditional liposomes [141], further emphasizing the interdependence of molecular packing, stability, and leakage in lipid membranes.

In addition to cholesterol, there is a considerable number of related sterols that are similar in chemical structure but differ in the number of double bonds, planar roughness, or alkyl chain (see Figure 3A). The sterol group originates from the triterpenoid squalene, which is the precursor molecule to sterols found in higher order organisms [146]. For example, ergosterol, sitosterol and stigmasterol are commonly found in plants and fungi [147] and are then taken up into mammalian cells through dietary means. Understanding how such sterols affect the physical properties of phospholipid membranes is necessary for gaining a full grasp of their biological role and potential applications [148]. Ergosterol, for example, is known to induce lipid ordering in fungal membranes leading to denser packing, reduced lateral diffusion, increased membrane thickness, and higher membrane stiffness due to the conformational restrictions on adjacent lipids [149–151]. However, X-ray diffraction studies by Hung et al. show that the condensing effect of ergosterol on PC lipid membranes strongly depends on lipid
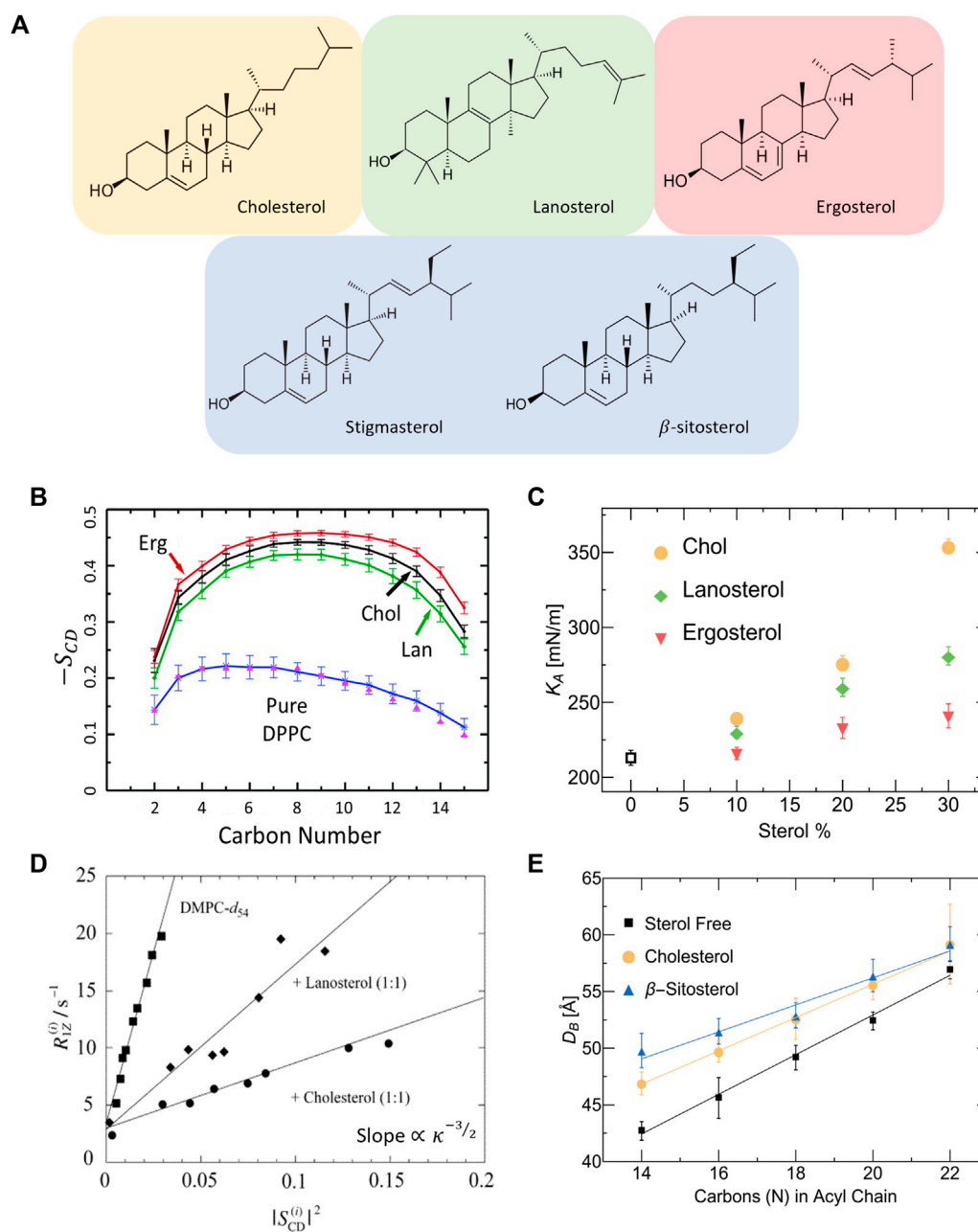
FIGURE 3
Effect of different sterols on PC lipid membranes. **(A)** Schematic of the chemical structures of sterols found in animal cells (tan), fungi and protozoa (red), plants (blue), and the precursor molecule to animal and fungal steroids, lanosterol (green). **(B)** Simulation results on DPPC show that ergosterol and lanosterol respectively induce stronger and weaker ordering of DPPC chains compared to cholesterol, illustrated by the order parameter S_{CD} of carbon atoms along the sn-2 chain (reproduced with permission from Cournia et al. [153]. Copyright (2007) American Chemical Society). **(C)** Micropipette aspiration studies of giant vesicles show changes in the area compressibility moduli of POPC membranes as a function of sterol concentration. In contrast to panel **(B)** ergosterol exhibits a weaker effect the membrane lateral compressibility, K_A, compared to lanosterol and cholesterol (reproduced with permission from Henriksen et al. [148]. Copyright (2006) Cell Press). **(D)** Relaxation rate R1z vs segmental order parameter $|S_{CD}|^2$ obtained from solid-state ^{2}H-NMR studies, showing that cholesterol increases the bending rigidity of DMPC membranes to a larger extent than lanosterol, leading to a greater stabilization of the liquid ordered phase (reprinted with permission from Martinez et al. [145]. Copyright (2004) American Chemical Society). **(E)** Differences in bilayer thickness, D_B, of di-N:1 PC with cholesterol and β-sitosterol show a stronger condensing effect of sitosterol on bilayers with shorter chain lengths with little differences in membranes with longer chain lengths (reproduced with permission from Gallová et al. [144]. Copyright (2008) Elsevier).

chain unsaturation and the sterol tilt angle, resulting in different condensing and membrane thickening effects compared to cholesterol [152].

Differences in sterol effects on lipid membranes have also been observed in MD simulations on saturated DMPC and DPPC membranes, showing that ergosterol orders the lipid chains to a

larger extent than cholesterol while lanosterol (the parent sterol) shows a weaker ordering effect (see Figure 3B) [153, 154]. Interestingly, micropipette aspiration studies on the three sterols in monounsaturated POPC (16:0–18:1) membranes show that the area expansion modulus increases significantly with the addition of cholesterol and to a lesser extent with lanosterol and ergosterol (see Figure 3C) [148]. Remarkably, these effects are commensurate with the respective sterol-induced ordering of lipids. The effects of sterols on membrane properties have also been investigated in several NMR studies reporting that, compared to cholesterol, ergosterol causes increased ordering of the hydrocarbon chains of DMPC [148, 153, 155] and DPPC [154] up to 30 mol% sterol concentration. In comparison, lanosterol was found to result in reduced condensing [77] and stiffening effects [145, 148] compared to cholesterol in DMPC membranes (see Figure 3D). For details of NMR investigations of lipid membrane with various sterols, the reader is referred to other focused reviews [156].

Other changes in sterol chemistry are also known to result in non-trivial changes in membrane structure and dynamics which can affect basic cellular functions. For example, plant sterols have been found to increase the bilayer thickness in a similar fashion to cholesterol [149]. Stigmasterol and sitosterol were specifically found to increase lateral packing density and bilayer thickness in both DMPC (14:0–14:0) and POPC (16:0–18:1) membranes [147]. By comparing the effects of cholesterol and β-sitosterol, SANS measurements show that they have different condensing effects on membranes with varying chain lengths, with β-sitosterol causing a more pronounced thickening (or condensing) effect in lipid membranes with shorter chains (Figure 3E) [144]. Importantly for synthetic membrane applications, small differences in sterol structures have been found to result in drastic differences in their solubility limits in phospholipid membranes containing saturated and unsaturated PC lipids [157]. This can potentially influence the degree to which different sterols can be used in modifying membrane properties. These observations point to the importance of understanding the effects of sterol structure on the properties of membranes with different phospholipid compositions and sterol content.
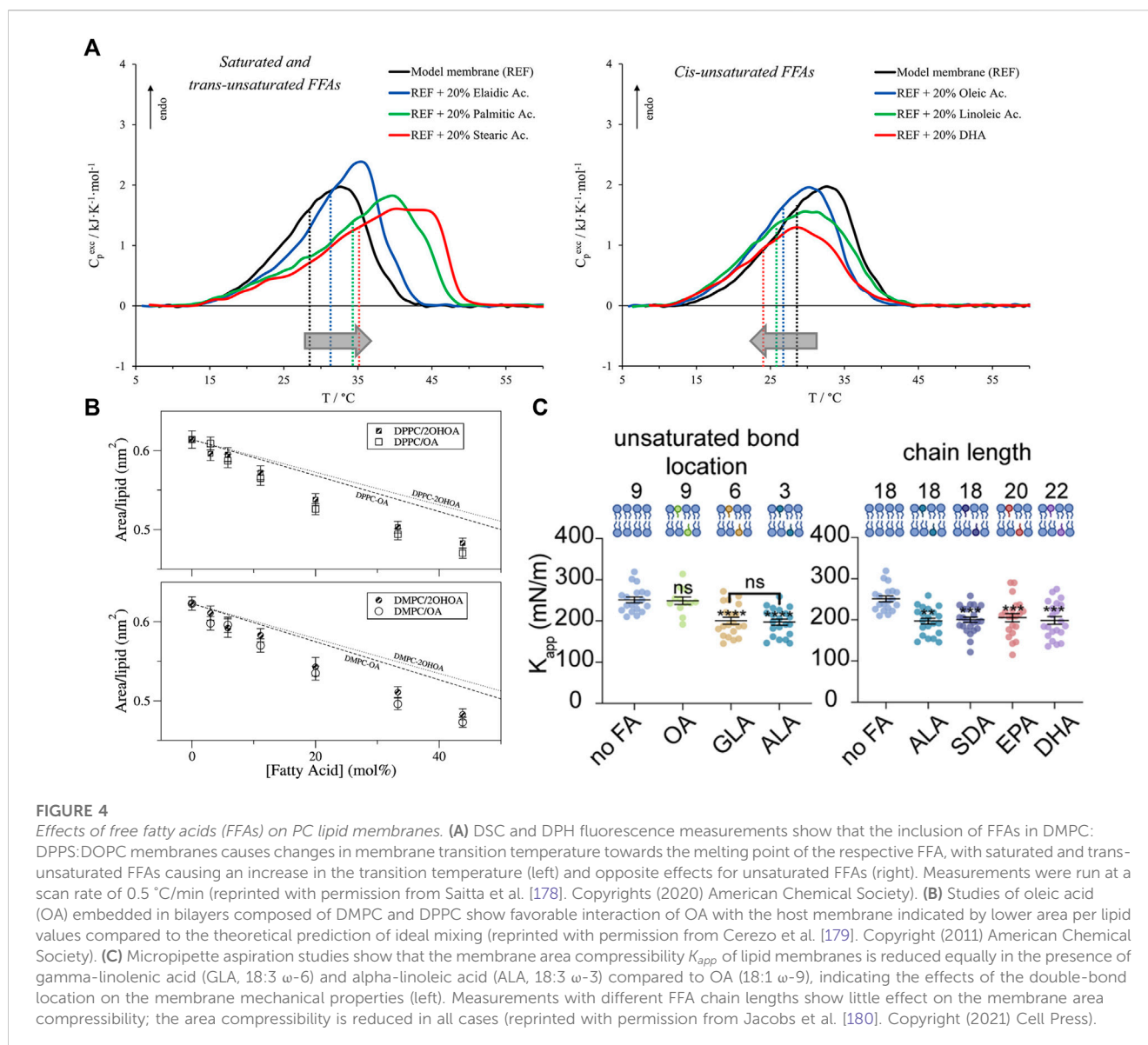
Free fatty acids (FFAs)

Free Fatty Acids (FFAs) are known as an important energy source for cells and cellular tissues. They are classified according to their aliphatic chain length and degree of chain unsaturation, i.e., saturated fatty acids (SFAs) with no double bonds, unsaturated fatty acids (UFAs) containing one double bond, and polyunsaturated fatty acids (PUFAs) containing two or more double bonds. For a detailed review on natural and synthetic fatty acids, we refer to Ibarguren et al. [158]. FFAs are primarily taken in through diet and are known to perturb the membranes of cells they interact with [159]. For instance, the high intake of oleic acid (18:1 ω-9) in olive-oil rich Mediterranean diet has been linked to a reduction in blood pressure through regulation of cell membrane properties and protein signaling [160]. In this review, we focus on the physical effects of FFAs on lipid membranes studied with various techniques including differential scanning calorimetry (DSC) [161–164], fluorescence spectroscopy [165, 166], electron spin resonance

[167, 168], light scattering [169], NMR [170], and other approaches. These studies report that the interaction and incorporation of FFAs with phospholipid membranes happens within minutes [171, 172]. More importantly, structural differences in the chain length, degree of unsaturation, location of double bond, and cis/trans isomerization can significantly change the membrane properties [173, 174].

For example, long-chain saturated fatty acids increase the gel-to-fluid phase transition temperature of phospholipid bilayers (also known as the melting temperature, T_m), indicating an increase in the membrane structural order or lipid packing. On the other hand, short-chain or cis-unsaturated fatty acids—such as ARA (20:4 ω-6), EPA (20:5 ω-3), and DHA (22:6 ω-3)—decrease the T_m of saturated DPPC (16:0–16:0) membranes correlating with a fluidizing effect [175, 176]. Indeed, the increase in cis double bonds in the acyl chain of FFAs is generally assumed to lead to an increase in membrane fluidity, and hence membranes rich in DHA tend to be exceptionally fluid [177]. On the other hand, trans fatty acids such as elaidic acid (18:1 trans ω-9) increase the transition temperature of lipid membranes in a similar manner to saturated FFAs such as stearic acid (18:0) and palmitic acid (16:0) (see Figure 4A) [178].

As stated earlier, FFA-induced changes in membrane phase transitions are typically associated with a change in the membrane fluidity. For example, DPH fluorescence anisotropy studies show that increasing the degree of unsaturation of incorporated FFAs leads to decreased anisotropy and decreased resistance to detergent, indicating membrane fluidization [181]. But, in the presence of cholesterol, EPA has little to no effect on membrane fluidity compared to DHA [182]. Interestingly, X-ray diffraction studies have shown that neither DHA nor EPA have an effect on the headgroup-headgroup thickness of POPC bilayers but an increase in electron density in the hydrocarbon region was observed with EPA indicating tighter packing [183]. Increased packing was also found for DMPC or DPPC monolayers with free oleic acid based on area per lipid values that are lower than the weighted area average, indicating favorable FFA-lipid interactions [179] (Figure 4B). Here we note that changes in membrane fluidity are not always directly correlated with changes in membrane permeability. For example, earlier studies showed that the effect of FFAs (namely, monoglycerides) have on membrane permeability is due to both induced acyl chain disorder as well as their specific interaction with the lipid headgroup [184]. Naturally, the effects that FFAs have on the bilayer would depend on the ordered state of bilayer and the incorporated FFA. For example, the addition of myristic acid to DMPC (14:0–14:0) membranes was found to slightly reduce picosecond dynamics in the gel state but no differences were observed above T_m. This was explained by the free volume model; i.e., saturated straight myristic acid aligns parallel to the lipid chains and thus minimally changes the free volume in the chain region or the packing of the lipid headgroups [185].

Due to the high degree of chain conformations that PUFAs can explore, they can locally and globally modify membrane structural properties, thus impacting membrane elasticity [183]. For example, flicker spectroscopy shows that oleic acid decreases the bending rigidity modulus κ of DOPC (18:1–18:1) membranes, while EPA and DHA result in an increase in κ, indicating that PUFAs experience a competition between chain length and degree of chain unsaturation in modifying membrane rigidity [180]. Micropipette aspiration studies show that the presence of cholesterol in lipid membranes

FIGURE 4
Effects of free fatty acids (FFAs) on PC lipid membranes. **(A)** DSC and DPH fluorescence measurements show that the inclusion of FFAs in DMPC: DPPS:DOPC membranes causes changes in membrane transition temperature towards the melting point of the respective FFA, with saturated and trans-unsaturated FFAs causing an increase in the transition temperature (left) and opposite effects for unsaturated FFAs (right). Measurements were run at a scan rate of 0.5 °C/min (reprinted with permission from Saitta et al. [178]. Copyrights (2020) American Chemical Society). **(B)** Studies of oleic acid (OA) embedded in bilayers composed of DMPC and DPPC show favorable interaction of OA with the host membrane indicated by lower area per lipid values compared to the theoretical prediction of ideal mixing (reprinted with permission from Cerezo et al. [179]. Copyright (2011) American Chemical Society). **(C)** Micropipette aspiration studies show that the membrane area compressibility K_{app} of lipid membranes is reduced equally in the presence of gamma-linolenic acid (GLA, 18:3 ω-6) and alpha-linoleic acid (ALA, 18:3 ω-3) compared to OA (18:1 ω-9), indicating the effects of the double-bond location on the membrane mechanical properties (left). Measurements with different FFA chain lengths show little effect on the membrane area compressibility; the area compressibility is reduced in all cases (reprinted with permission from Jacobs et al. [180]. Copyright (2021) Cell Press).

can significantly affect to what extent PUFAs influence the apparent area compressibility modulus, K_{app}. For instance, while free fatty acids tend to decrease K_{app}, the presence of cholesterol can result in an increase in K_{app} depending on the degree of chain unsaturation of the FFAs added (see Figure 4C) [180]. Similar results have been seen by atomic force microscopy (AFM) demonstrating that plasma membranes enriched in EPA feature lower bending rigidity [186] whereas saturated margaric acid (17:0) increases the membrane bending rigidity [175]. When cholesterol was added, an increase in membrane stiffness was observed, but the stiffening extent decreased with increasing chain unsaturation, indicating the dominance of cholesterol's stiffening effect over PUFA-induced softening [175].

Interestingly, in comparing FFA effects on phospholipid membranes with PE headgroups Langer and coworkers argue that the partitioning of FFAs into lipid membranes depends more on the molecular packing than on the nature of the lipid headgroups [187]. For instance, due to their much smaller polar headgroup relative to their fatty acid chains, PE lipids have a

negative spontaneous curvature and thus they self-assemble into non-bilayer structures—typically resulting in hexagonal phases unless paired with other lipids or sterols. X-ray diffraction studies on C18 FAs (oleic, elaidic, and stearic acid) show that oleic acid (OA) causes concentration dependent alterations of the lipid self-assembly. More specifically, OA was found to induce a reduction of up to 20°C–23°C in the bilayer-to-hexagonal transition temperature of POPE (16:0–18:1) and DOPE (18:1–18:1) while elaidic and stearic acids did not markedly alter the membrane morphology. The above effects in PE membranes [188] as well as physiological cells [160] are attributed to the different molecular shape of OA with respect to their congeners, elaidic and stearic acids.

Other forms of fatty acids used in physiological processes include squalene typically utilized in the production of human sebum [189] and amine-conjugated free fatty acids used as molecular messengers in cells [190]. For instance, squalene, the precursor to sterols [146], was found via DSC to lower the main transition temperature and the fluid-bilayer to hexagonal phase

transition in 95:5 SOPE:POPC mixtures [191]. On the other hand, N-acyltaurines (NATs) have a fatty acid chain linked to a taurine headgroup and thus have a large headgroup to chain ratio, resulting in conical molecular geometry and a positive spontaneous curvature [192]. In a recent publication, Prakash et al. found that NATs easily intercalate into the membrane and can fluidize the membrane up to a maximum miscibility of approximately 50 mol% [193]. Other molecules like dioleoyl-glycerol (DOG), a molecule similar to DOPC (18:1–18:1) but lacking the phosphocholine headgroup motif, can significantly modify lipid packing in their host membranes. For example, when introduced in DOPC membranes, DOG resides in the center of the bilayer and interdigitates between the two membrane leaflets, resulting in an increase in the order parameter and membrane thickness, inducing curvature strain [192]. The absence of the headgroup in DOG can also induce curvature strain and create stress and packing defects in membranes. A molecule similar to DOG but lacking one of the oleic acid chains is monoolein (1-oleoyl-rac-glycerol), which due to its simple structure can adapt to complex membrane morphologies [194]. Indeed, monoolein was found to greatly increase the permeability of EggPC membranes as much as, if not more than, unsaturated FFAs which due to its conical shape [184].
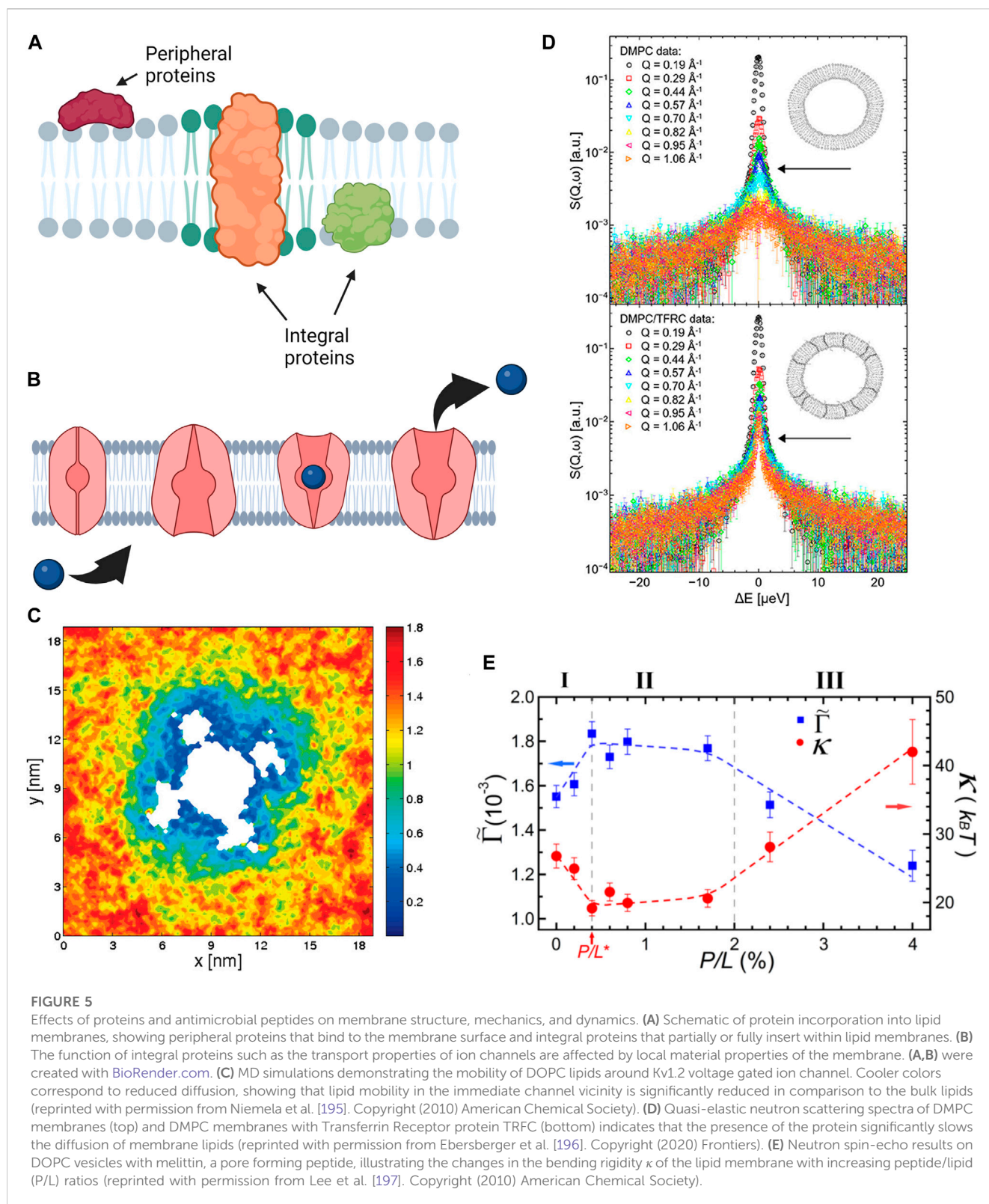
Peptides and proteins

Proteins are a major component of plasma cell membranes and an important design element in artificial cells and synthetic cell membrane mimics. Membrane proteins are mainly divided into two categories: peripheral proteins which adhere to the membrane surface and integral proteins that span partial or full membrane thickness (see Figure 5A). The focus of this section is to highlight the effects proteins (and peptides) on membrane properties including membrane thickness, intrinsic curvature, and elastic moduli [198]. Nevertheless, it is imperative to emphasize that the functions of membrane proteins are simultaneously regulated by their lipid environment [199–202]. For example, the lipid headgroup and fatty acid structure as well as cholesterol content of lipid membranes can significantly affect the binding affinity of proteins to membranes [203–205], their partitioning into the membrane-water interface [206, 207], and their folding into stable conformational states [208–210]. Other studies have shown that the activity of mechanosensitive proteins like Piezo1 [175], TRPV4 [186], and MscL [211] can be altered by the presence of PUFAs, becoming activated or inactivated depending on the fatty acid identity. Knowing that the activity of proteins is dictated by their conformational state, numerous examples have illustrated that conformational changes in membrane proteins tightly depend on the membrane material properties. Recent simulations by Soubias and coworkers show that the thickness of lipid membranes, determined by the cholesterol content, closely regulates the active state of G-protein-coupled receptor (GPCR) rhodopsin [212]. Similarly, the activity of mechanosensitive ion channels—which are responsible for regulating intracellular pressure—is closely determined by the elasticity and molecular packing of its immediate lipid membrane environment (see Figure 5B) [213]. Perozo et al. have shown that changes in the

membrane intrinsic curvature by the external addition of lyso-PC lipids plays a critical role in the conformations of MscL, generating significant asymmetry in the transbilayer pressure profile that can trap the channel in a fully open state [214]. More importantly, using electron paramagnetic resonance spectroscopy and spin labelling, they found that changes in the channel conformation are highly dynamic and require multiple transitions in the transmembrane helix domain of the channel protein [215].

Therefore, understanding the synergy of proteins and lipid membranes and their effects on structural and dynamical membrane properties is key to biological function [216, 217]. Emulating these functions often results in useful technologies when translated to synthetic lipid platforms, such as liposomes [218–220], droplet interface bilayers (DIBs) [221–223], and supported lipid bilayers [11, 224, 225]. For instance, alpha hemolysin (αHL), a protein that forms transmembrane pores, disrupts the membrane structure and eventually causes membrane rupture due to ion transport [226, 227]. While this is detrimental for cells, it nonetheless enables a variety of applications requiring transport of ions and molecules across the membrane, including biosensing [228], activation of genetic circuits [229], and engineered AND gates for controlled cargo release [230].

Equally important to advanced membrane applications is the binding of proteins to specific lipid domains for controlled functionality, as in the case of raft-forming membranes with coexisting L_O and L_D phases [60, 231, 232]. In fact, the selective partitioning of proteins into lipid domains with specific headgroup and fatty acid structures [233, 234] is a functional membrane feature that is of high interest in synthetic biology and technological membrane applications [235–237]. For example, the HIV GAG protein favors disordered lipids with unsaturated fatty acids on both chains, yet the addition of cholesterol which increases lipid packing was found to also increase GAG binding [204]. On the other hand, palmitoylated proteins such as those implicated in T-cell signaling [238–240] have a preferential interaction to the L_O phase whereas de-palmitoylated proteins have stronger interactions with the L_D phase due to the fatty acid residue acting as an anchor into the membrane [241]. As Lorent and Levental point out, while a handful of proteins have been identified to preferentially bind to or insert into L_O domains, the general mechanism of protein partitioning is yet to be established in complex *in vitro* or *in vivo* systems [242]. Indeed, this is the topic of ongoing investigations which will have far-reaching implications both in biological function and in constantly evolving membrane technologies.

One of the consequences of lipid-protein interactions is their effect on membrane phase transitions, i.e., their ability to influence the formation or disruption of distinct raft-like lipid domains that serve as a functional platform for various membrane processes [243]. For example, recent studies show that upon membrane association, α-helices of proteins are driven together by lipophobic effects which reduce the conformational freedom of lipids surrounding the α-helix complex, preventing them from taking part in gel-fluid phase transitions [244, 245]. Consequently, these bound proteins affect the diffusion of lipids within the membrane by acting as diffusion barriers [246]. Because lipid-protein interactions are highly dynamic in nature and span a

FIGURE 5
Effects of proteins and antimicrobial peptides on membrane structure, mechanics, and dynamics. **(A)** Schematic of protein incorporation into lipid membranes, showing peripheral proteins that bind to the membrane surface and integral proteins that partially or fully insert within lipid membranes. **(B)** The function of integral proteins such as the transport properties of ion channels are affected by local material properties of the membrane. **(A,B)** were created with BioRender.com. **(C)** MD simulations demonstrating the mobility of DOPC lipids around Kv1.2 voltage gated ion channel. Cooler colors correspond to reduced diffusion, showing that lipid mobility in the immediate channel vicinity is significantly reduced in comparison to the bulk lipids (reprinted with permission from Niemela et al. [195]. Copyright (2010) American Chemical Society). **(D)** Quasi-elastic neutron scattering spectra of DMPC membranes (top) and DMPC membranes with Transferrin Receptor protein TRFC (bottom) indicates that the presence of the protein significantly slows the diffusion of membrane lipids (reprinted with permission from Ebersberger et al. [196]. Copyright (2020) Frontiers). **(E)** Neutron spin-echo results on DOPC vesicles with melittin, a pore forming peptide, illustrating the changes in the bending rigidity κ of the lipid membrane with increasing peptide/lipid (P/L) ratios (reprinted with permission from Lee et al. [197]. Copyright (2010) American Chemical Society).

wide range of timescales down to picosecond (ps) and nanosecond (ns) timescales, a full understanding of their underlying mechanism is still missing. However, the development and application of high-resolution techniques has started to shed light on the molecular nature of these interactions and their effects on lipid membrane properties. For instance, NMR studies on rhodopsin – a protein

receptor responsible for dim light vision—show that increasing the mole fraction of rhodopsin in DMPC membranes causes an increase in the orientational order parameter of DMPC lipids and a corresponding increase in the measured gel-to-fluid transition [247]. Other studies using electron spin resonance (ESR) spectroscopy have been performed to inspect the effect of protein

inclusions on the mobility and rotational dynamics of lipids within host membranes [248]. ESR spectra reveal that the SecA protein found in *Escherichia coli* slows lipid mobility and eliminates the L_O-L_D transition [249].

Neutron spectroscopy and MD simulations are also regularly used in dynamic studies of lipid-protein interactions due to the compatibility of the length and time scales that they can access. For instance, MD simulations of voltage-gated ion channel, Kv1.2, embedded in POPC membranes show that the inclusion of protein significantly impacts the mobility of the lipids surrounding it (Figure 5C) [195]. Accordingly, the protein and the neighboring lipids form a dynamical complex which diffuses as a single unit within the membrane plane. Similarly, neutron spectroscopy studies have demonstrated that the inclusion of transferrin receptor protein (TFRC) in DMPC membranes (Figure 5D) leads to restricted lateral lipid mobility in the vicinity of the protein, as well as long-ranged lipid dynamics [196]. In more recent neutron spectroscopy studies, Kelley and coworkers showed that channel-forming peptides, gramicidin (dimer-forming) and alamethicin (membrane-spanning), have markedly different effects on nanosecond bending and thickness fluctuations of DMPC lipid membranes [250]. Their findings demonstrate that peptides cannot be simply treated as rigid objects in terms of their effects on membrane fluctuations. These examples illustrate the complexity of protein-membrane interactions and their manifestation on different spatial and temporal scales, from local dynamics to collective fluctuations.

An important class of peptides is antimicrobial peptides (AMPs) which target specific lipid compositions found in bacterial membranes, and thus have various uses in therapeutic, clinical, and agricultural applications. These peptides can destroy dormant antibiotic-resistant bacteria by lysing their membranes, leading to bacterial death [251]. As such, AMPs have gained increasing interest as a potential antibacterial treatment, especially with the rise of antibiotic-resistant bacteria. Their interactions with lipid membranes have been studied using numerous techniques, including most recently MD simulations [252, 253]. These studies show that depending on peptide concentration, lipid composition, and membrane mechanical properties, the peptide can change state from surface-bound to pore-forming [254]. Pore formation is dominated by the ability of the peptide to generate curvature strain [255]. In addition to pore formation, AMPs can modulate membranes in a detergent-like process, i.e., by solubilizing the membrane. An example of this was observed on NH125 which binds to the outer lipid monolayer through electrostatic forces. When it flips to the inner leaflet, it solubilizes the membrane leading to complete membrane destabilization and eventually cell death [251]. Other AMPs, including melittin [256] and alamethicin [257], have been observed to reduce the bilayer thickness with increasing peptide concentration until a critical value, corresponding to the onset of pore formation state [254]. These observations are consistent with neutron spectroscopy studies showing that melittin alters the bending rigidity of DOPC membranes in a concentration dependent manner (Figure 5E) [197]. At low melittin concentrations, the adsorption of melittin to the membrane surface disrupts lipid packing and causes a significant decrease in the bending rigidity. At a critical concentration, melittin forms pores in the membrane and the bending rigidity starts to exhibit a slight increase. At higher melittin concentrations, the membrane bending rigidity increases significantly

due to repulsive interpore interactions. Additionally, melittin displays an affinity to membranes displaying lower area compressibility moduli [258]. Not surprisingly, membrane budding and leakage due to melittin decreases upon the addition of cholesterol [259, 260] given the stiffening role of cholesterol described earlier. However, the maximum leakage correlates with intrinsic curvature of the specific lipid species within the membrane [259]. In addition, melittin has been shown to inhibit the gel-to-fluid phase transition in DMPC lipid membranes, while simultaneously enhancing lateral lipid mobility and chain flexibility [260]. In comparison, the introduction of alamethicin causes significant lipid disordering up to a critical concentration required pore formation, after which alamethicin stabilizes pore edges and reintroduces order [257].

AMPs are also dependent on lipid headgroup composition. For example, Zhao et al. demonstrated that temporin B and L, two related AMPs, readily insert into membranes composed of anionic PG headgroups and thus vesiculate SOPC:POPG liposomes but have no effect on pure SOPC (18:0–18:1) liposomes [261]. This suggests that negatively charged lipids reduce repulsion forces between peptides, inducing aggregation and leading to pore formation to relax leaflet asymmetry [261]. Another example can be found in the lysing mechanism of MSI-78 [262], a synthetic peptide mimicking the magainin family, as well as LL-37 [263] which were found via DSC to induce a positive curvature strain by increasing the transition temperature corresponding to the phase transition from a fluid bilayer to an inverted-hexagonal phase of POPE membranes. These changes can also be observed in shifts of the ^{31}P-NMR spectra even with small peptide to lipid concentrations (approximately 1:5000) [263]. Conversely, both polyphemusin I and PV5 were found to reduce the transition temperature indicating induced negative curvature strain [264]. These studies provide a better understanding of the physical observables of AMP interactions with lipid membranes and can facilitate treatment approaches that utilize peptide-induced structural and dynamical modifications of target membranes.

Furthermore, AMPs can modulate lipid distribution within the membrane plane or across the two membrane leaflets. In general, many antimicrobial compounds have multiple charged domains which can sequester oppositely charged lipids to induce lipid phase separation, thus resulting in phase boundary defects that can change the membrane permeability or alter the membrane stability and subsequently affecting bacterial function [265]. For example, aurein—a 13-amino acid antimicrobial peptide in the frog *Litoria* genus that exhibits high antibiotic efficacy—is found to induce significant lateral segregation in initially uniform lipid bilayers composed of zwitterionic lipids and anionic lipids. Interestingly, reduced lipid diffusion in the fluid phase was observed even at low aurein concentrations, making the membrane prone to additional stresses and defects that change membrane properties and impede membrane-related biological processes [266]. In asymmetric membranes with different leaflet compositions, AMPs have been observed to cause lipid flip-flop between the two membrane leaflets. In a recent study, using time-resolved SANS and selective lipid deuteration, Nguyen et al. illustrated that melittin significantly accelerates lipid flip-flop, resulting in complete scrambling of pre-formed asymmetric vesicles within a couple of hours [267]. Similar findings were observed in asymmetric membranes containing antimicrobial frog peptides L18W-PGLa and magainin

2, further relating lipid flip-flop to peptide translocation and membrane leakage [268]. These studies shed light on the mechanisms of AMP interactions with lipid membranes, illustrating how AMPs assume their antimicrobial potency with potential applications in clinical, agricultural, and food industries [269, 270].

Therapeutic compounds

Biogenic compounds

Drug-lipid interactions have a wide range of applications in various scientific disciplines from synthetic chemistry to pharmacology. These interactions often alter membrane structure and mechanics and subsequently dictate the effectiveness or design of the administered drug molecules, whether natural or synthetic [7, 271]. Among drug molecules, those produced by life forms are referred to as biogenic compounds. Examples of biogenic compounds include neurotransmitters and hormones, which play a major physiological role and therefore mimicking or modifying their chemical structures can be of benefit in future therapeutic approaches.
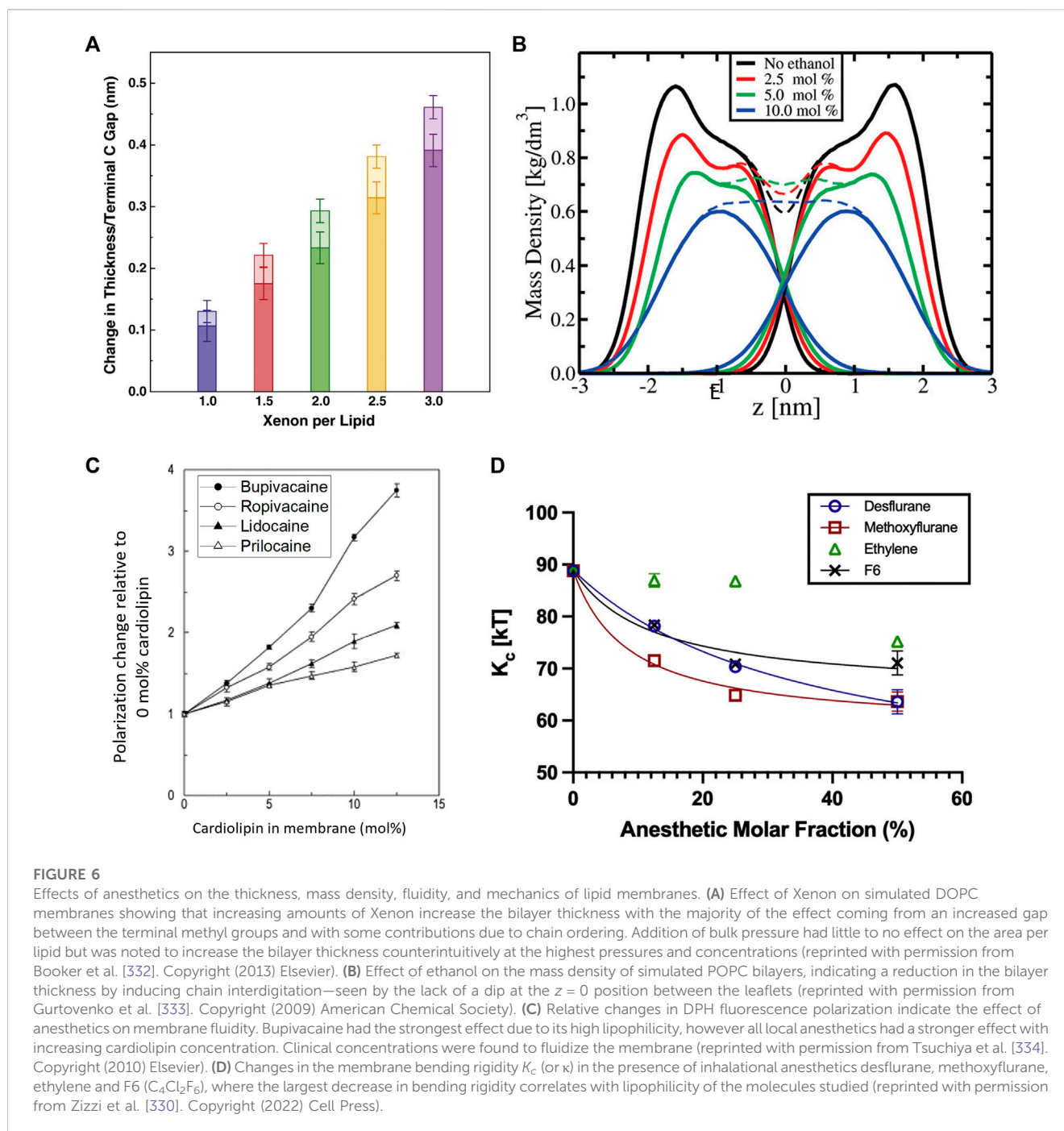
For example, melatonin—a hormone produced in the pineal gland of the human brain [272]—is generally used as supplement for regulating sleep but has many other therapeutic uses of relevance to health including the suppression of inflammations and tumors, cardio- and nervous system protection, and antioxidant activity [272–274]. Structurally, melatonin is known to partition into membranes at the interface between the headgroup and tail group regions of the bilayer, generally resulting in an increase in the area per lipid, a decrease in the bilayer thickness, and a subsequent increase in membrane permeability [275]. Indeed, melatonin is known to exhibit a high partition coefficient or association constant in lipid membranes, enabling it to permeate multilamellar vesicles and intracellular membranes [276]. Recent studies combining confocal microscopy, SANS, and DSC show that the incorporation of melatonin in phospholipid membranes, with compositions similar to pulmonary membranes, results in the stabilization of lipid domains implicated in membrane functions [277]. In phospholipid monolayers, melatonin is found to decrease the packing density in saturated DPPC (16:0–16:0) monolayers [278, 279] and di-unsaturated DOPC (18:1–18:1) monolayers [280], accompanied with a decrease in the area compressibility modulus K_A [278]. Similar observations are reported in MD simulations on phospholipid monolayers showing an overall fluidizing effect of melatonin that counterbalances the effects of cholesterol [278]. This fluidizing effect is also observed in DSC measurements on EggPC liposomes, showing a decrease in the main phase transition temperature with the introduction of melatonin [280]. However, high concentrations of melatonin (~30 mol%) in DMPC membranes have been found to result in a parallel alignment along lipid tails, resulting in increased lipid order [281]. Such physical changes in membranes by small naturally produced hormones, like melatonin, are critical to understanding the biological effects of similar drug molecules, developing improved drug designs, and tuning their membrane uptake.

Other changes in membrane properties have also been observed with similar lipophilic neurohormones, which readily partition into lipid membranes through non-specific lipid interactions [282, 283]. For example, N-acetylserotonin is reported to increase the fluidity and area per molecule in phospholipid membranes, whereas serotonin is found to reduce the order parameter of the L_D phase but increases the order parameter of the L_O phase in a POPC:POPS mixture [279]. On the other hand, all-atom MD simulations of POPC:POPS mixtures found that natural psychedelics increase the area per lipid and decrease bilayer thickness [284]. These simulations also found that the hallucinogenic bufotenine causes the largest decrease in chain order parameter and largest modification of structural properties. In another study, low concentrations of psilocin, a psychoactive hallucinogen, were observed to decrease the transition temperature of DPPC:DPPS membranes in a manner similar to anesthetics [285]. In the same study, simulations of psilocin in a POPC:POPS mixture found an increase of 3–4 $\mathring{A}^2$ in the area per lipid and a reduction of the chain order parameter, indicating a stabilization of the fluid phase [285]. These studies shed light on the differential effect of small biogenic molecules in local lipid environments that exemplify the lipid heterogeneity in cell membranes. It is reasonable to assume that the changes in lipid packing and membrane elasticity induced by these small molecules could significantly impact the function of membrane proteins and subsequent cellular functions.

Other forms of biogenic compounds include phytochemicals, which are small natural bioactive molecules derived from plants and are part of the plant's immune system. Various phytochemicals have found pharmacological use and are known to play a protective role against several diseases and infections [286, 287]. Phenolic phytochemicals are particularly promiscuous in modifying cell functions and membrane protein activity due to their ability to readily partition into or permeate through lipid membranes. To explore this mechanism, recent MD simulations investigated five bioactive phenols of reported medicinal value—namely, curcumin from turmeric, EGCG from green tea, capsaicin from chili peppers, genistein from soybeans, and resveratrol from grapes [288]. Findings from these studies show that these compounds alter the membrane properties by localizing to the lipid-water interface, resulting in changes in the lateral pressure profiles. More importantly, the induced changes in membrane properties result in similar effects on membrane proteins suggesting that the mechanism underlying the biological activity of phenolic phytochemicals is due to their propensity in modifying membrane properties, rather than specific protein binding. Correlating changes in the physical membrane properties induced by such molecules paves the way to a better understanding of their effect on human health and informing future designs of therapeutic agents with optimized membrane interactions.

Pharmaceutical compounds

Pharmaceutical molecules are used across the world for various maladies, such as moderating blood pressure and pain relief [289,

FIGURE 6
Effects of anesthetics on the thickness, mass density, fluidity, and mechanics of lipid membranes. **(A)** Effect of Xenon on simulated DOPC membranes showing that increasing amounts of Xenon increase the bilayer thickness with the majority of the effect coming from an increased gap between the terminal methyl groups and with some contributions due to chain ordering. Addition of bulk pressure had little to no effect on the area per lipid but was noted to increase the bilayer thickness counterintuitively at the highest pressures and concentrations (reprinted with permission from Booker et al. [332]. Copyright (2013) Elsevier). **(B)** Effect of ethanol on the mass density of simulated POPC bilayers, indicating a reduction in the bilayer thickness by inducing chain interdigitation—seen by the lack of a dip at the $z = 0$ position between the leaflets (reprinted with permission from Gurtovenko et al. [333]. Copyright (2009) American Chemical Society). **(C)** Relative changes in DPH fluorescence polarization indicate the effect of anesthetics on membrane fluidity. Bupivacaine had the strongest effect due to its high lipophilicity, however all local anesthetics had a stronger effect with increasing cardiolipin concentration. Clinical concentrations were found to fluidize the membrane (reprinted with permission from Tsuchiya et al. [334]. Copyright (2010) Elsevier). **(D)** Changes in the membrane bending rigidity K_c (or κ) in the presence of inhalational anesthetics desflurane, methoxyflurane, ethylene and F6 ($C_4Cl_2F_6$), where the largest decrease in bending rigidity correlates with lipophilicity of the molecules studied (reprinted with permission from Zizzi et al. [330]. Copyright (2022) Cell Press).

290]. While some of these drug compounds target specific receptor proteins, others such as anti-depressants may directly affect the membrane properties [291]. Synthetically produced drugs have highly complex chemical structures, each with different functional groups that interact with the membrane in various ways. Here, we relate the effects of drugs in modifying membrane properties to their partitioning into lipid membranes [292]. As summarized below, literature on common classes of pharmaceutical compounds points to common observations of increased membrane fluidity, decreased molecular packing, and an overall softening of the membrane. However, deviations from these general observations can occur depending on whether a pharmaceutical compound resides in the

membrane hydrophobic core or partitions to the membrane-water interface [293, 294]. For example, azithromycin, a well-known synthetic antioxidant, has been shown to be effective in inhibiting viral entry across cell membranes [295] and preventing bacterial growth [296, 297]. In membranes, azithromycin interacts with the polar lipid headgroups of phospholipids and disrupts lipid order. AFM and optical microscopy studies show that it alters lateral lipid organization and the formation of lipid domains [298, 299]. These observations align with the propensity of azithromycin to decrease the membrane bending rigidity and area compressibility [299].

Other drug classes, like *sartan*s which are used for hypertension [289], display varied interactions with lipid membranes depending

on the lipid headgroup and acyl chain length. Many of these compounds reduce and broaden the main phase transition temperature in PC lipid membranes, indicating an induced decrease in lipid order [300, 301]. These findings align with observed changes in the lipid tilt angle [302] and with X-ray scattering studies reporting an increase in the area per lipid and a decrease in the membrane thickness [303]. This is also in agreement with other studies reporting amplified sartan-induced thermal fluctuations that uncouple the bilayer stacks [304, 305], most likely due to a softening of the membrane as expected from the reduction in the area per lipid.

Nonsteroid anti-inflammatory drugs (NSAIDs) are another class of commonly-used, over-the-counter medications for pain, inflammation, and fever [290]. To reach their target, the cyclooxygenase enzyme, NSAIDs must diffuse through cell membranes which can interfere with the membrane properties. For instance, aspirin [306]—the most common NSAID—has been shown to broaden and suppress the main phase transition of lipid membranes, resulting in overall membrane fluidization [307]. Scanning electron microscopy (SEM) studies show that red blood cells become blebby when exposed to aspirin or related compounds [308], suggesting membrane softening. Neutron spin-echo (NSE) measurements confirm that aspirin reduces the bending rigidity and increases lipid diffusion in DMPC membranes [307, 309]. Further studies using micropipette aspiration have demonstrated that aspirin-related compounds reduce the apparent area compressibility and bending rigidity in SOPC vesicles in a concentration dependent manner, concomitant with a decrease in the bilayer thickness and membrane tension [310].

Other studies on ibuprofen [311–313] and indomethacin [311] also show a shift in the main phase transition in DMPC lipid membranes to lower temperatures, indicating reduced lipid cooperativity typical of increased disorder and fluidity [311, 313]. Similar to aspirin, ibuprofen and indomethacin have also been demonstrated to lower the bending rigidity and area compressibility modulus of their host membranes [309, 314]. Analogous observations were reported on oxicams, another type of NSAIDs [315, 316]. These effects of NSAIDs on lipid membranes extend beyond the types discussed above. For example, acemetacin significantly reduces the cooperativity and phase transition in host lipid membranes [317]. Other NSAIDs like indomethacin influence phase coexistence in DPPC:Chol membranes and show preference to ordered domains induced by cholesterol [318]. Supporting studies found that indomethacin and acemetacin can influence chain packing in planar bilayers and destabilize the gel phase [317]. Similarly, aescin—another anti-inflammatory agent—lowers the main phase transition temperature of DMPC and simultaneously increases the area per lipid [319].

Studies on trifluoperazine (TFP), an antipsychotic, also show that it reduces the main phase transition temperature of DPPC membranes and completely eliminates the pretransition at concentrations as low as 1:100 TFP:DPPC [320]. Another antidepressant, fluoxetine (commonly known as Prozac) also broadens and shifts the main transition of DMPC membranes to lower temperatures and at a concentrations of 10 mM it completely suppresses the phase transition; however, less pronounced effects were observed in membranes with longer acyl chains like DPPC (16:0–16:0) and DSPC (18:0–18:0) [321]. All of these studies provide excellent examples of how pharmaceutical compounds modulate the physical properties of membranes in a way that is consistent with their partitioning mechanism and with structure-property relations of soft molecular assemblies.

Anesthetics and analgesics

In the very start of the 20th century, Meyer [38] and Overton [39] established a rule in predicting anesthetic strength. It was quite simple—the anesthetic potency of a specific chemical structure correlates linearly with partitioning from an aqueous to an organic phase. This correlation improves if the oil phase is substituted by octanol or even a lipid bilayer [322]. However, anesthetics vary wildly in chemical size, structure, and functional groups; how could such a diversity in molecules result in similar effects? One of the hypotheses is that anesthetics have a non-specific indirect mechanism of action via the membrane itself, in line with the pressure reversal effect [323, 324]. Other hypotheses support a direct effect on membrane receptors, whose function can also be significantly altered by local membrane properties as discussed earlier [325, 326].

In a dedicated review article on the lipid-centric vs protein-centric mechanism of action by anesthetics, Eckenhoff [327] drew examples to the fact that the effects seen on membrane properties were detectable at concentrations far above clinical concentrations, with the caveat that these model cell membranes may be poor approximations to real, highly complex biological systems. For instance, a commonly observed characteristic of anesthetics is their fluidizing effect, resulting in the reduction of the main phase transition at clinical concentrations in membrane mimics. In living systems, however, the plasma cell membrane is composed of hundreds of lipid species, proteins, and other molecules that prohibit a well-defined phase transition, rendering this descriptor of anesthetics invalid. Other studies have reported that clinical concentrations of volatile anesthetics like isoflurane inhibit the activity of voltage-gated sodium channels without affecting lipid bilayer properties, pointing to direct interactions with the channel protein instead of membrane-mediated interactions [328]. On the other hand, Cantor proposed a mechanism of action via changes in the lateral pressure profile of the membrane whereby anesthetic molecules modify the lateral pressure across the depth of the membrane, adjusting the preferred conformation of the protein [329]. These types of density profile calculations have become readily available in recent years due to increased computing power and as such MD simulations have become an invaluable tool for studying these systems.

For instance, MD simulations on general inhalation anesthetics, like desflurane and methoxyflurane, show that they induce a significant increase in the area per lipid (A_L) of affected membranes [330]. Similarly, simulations on other anesthetics, such as sevoflurane and diethylether, also reveal an increase in A_L in DPPC (16:0–16:0) and DPPC:Chol membranes [331]. Interestingly, an increase in A_L, as well as an increase in membrane thickness, was found for Xenon [332] (Figure 6A) and other anesthetic noble gases [335]. On the other hand, local anesthetics like ethanol, also increase A_L but decrease the bilayer thickness, beside other effects on the membrane structure such as

inducing chain interdigitation and forming persistent non-bilayer structures at high enough concentrations (Figure 6B) [333]. Enflurane was also found to induce an interdigitated phase, greatly reducing the bilayer thickness due to only being able to partition into the headgroup region [336] with similar findings in DSC measurements on tetracaine in saturated membranes [337].

Not surprisingly, anesthetic-induced changes in molecular packing correlate with changes in the membrane fluidity. MD simulations illustrate that these additives induce similar effects to the chain order parameter as lipid melting, explaining the observed increase in fluidity [338]. Indeed, tetracaine [339] as well as sevoflurane and diethylether [331] are all found to fluidize lipid membranes, with a reduction of this effect upon application of high pressure (typically inducing higher lipid order). Notably, the effect that local anesthetics have on lipid membranes depends on lipid composition. For example, a study on biomimetic membranes with four different local anesthetics show that bupivacaine has the strongest effect on the membrane fluidity due to its high lipophilicity compared to the other anesthetics, especially in the presence of cardiolipin—an abundant lipid in mitochondrial membranes (Figure 6C) [334]. In fact, the striking disruption of cardiolipin (CL) membranes by bupivacaine can result in heavy leakage and complete rupture of cardiolipin vesicles [340]. At concentrations below clinical use, DPH fluorescence anisotropy studies show that lidocaine can fluidize pure DPPC liposomes but had a particularly strong impact on anionic lipids with a PS, PA, or PG headgroup with cardiolipin being affected the strongest [341]. The lipid dependence of the fluidizing effects of anesthetics was also observed in a study on dibucaine in various mixtures of POPC:POPE:CL, showing that the diffusion coefficient varies with lipid composition with an obvious dependence on cardiolipin [342]. Ethanol was found to have similar fluidizing effects on membranes, with a significant enhancement of lipid diffusion on short timescales [343]. This increase in membrane fluidity is commensurate with the observed increase in membrane thermal fluctuations and a reduction in the bending rigidity with ethanol [333] and other inhalational anesthetics [330] such as desflurane, methoxyflurane, and ethylene (Figure 6D).

As expected, anesthetic-induced changes in lipid packing also influence the thermodynamic properties of lipid membranes. For example, tetracaine, a local anesthetic which fluidizes lipid membranes, is found to lower the membrane gel-fluid transition temperature [344], with similar results found for lidocaine [345, 346] and dibucaine [342, 346]. Analogous observations were reported in DSC studies on alphaxalone, a general steroid anesthetic [347]. On the other hand, Xenon exhibits a reversible effect on the melting transition. It lowers the transition temperature over time, but upon venting heat capacity profiles recover to typical profiles of the pure membrane, as corroborated by simulations [332]. Anesthetics were also found to disrupt the phase transition of membranes into ordered-disordered lipid domains. In fact, the potency of lidocaine, dibucaine, and tetracaine has been correlated with their ability to disturb lipid domains and change membrane organization [348]. For example, due to the amphiphilic nature of tetracaine, it has the ability to significantly solubilize membranes and to further disrupt membrane structure by inducing pore formation and micron-scale tubules [337, 339]. These studies highlight the importance of the interaction of anesthetics with lipid membranes and their modulation of membrane properties in ways that can significantly influence cellular processes, potentially explaining their mode of action through modification of the membrane physical properties.

Industrial additives

Alkanes and alkanols

Straight chain alkanes and alkanols are chemically similar to the free fatty acids, discussed in a previous section, but they lack the carboxylic acid group of free fatty acids; yet one can still draw similarities in their effects. Notably, the polar hydroxyl group in alkanols gives them an amphiphilic character, in contrast to straight chain alkanes which are purely non-polar. This causes alkanes and alkanols to partition differently into lipid membranes depending on the polar chemistry as well as chain length, and thus leads to different effects on the host membrane. Alcohols generally incorporate in membranes with their hydroxyl group near the lipid headgroup and the chain intercalated with the lipid acyl chain [349], but those with short chains, such as ethanol [350–352], tend to partition to the lipid glycerol moiety while the shortest, methanol, is unable to penetrate at all into the chain region due to its comparably high polarity [353]. Ethanol has been found to displace water molecules from the phosphate headgroups of lipid membranes, thus increasing the headgroup area and decreasing bilayer thickness [354, 355]. Additionally, gas chromatography has demonstrated that ethanol has a partition coefficient in membranes with PG and PS headgroups nearly double that of PC headgroups [356]. Similar changes in membrane structure have been reported for short chain alcohols [357, 358] with a dependence on the alcohol concentration and chain length [359]. The membrane modifying potency of alcohols increases logarithmically with each additional methylene group and a cutoff effect occurs approximately when the length of the alkanol becomes longer than half of the acyl chain length [360, 361] corresponding to the most pronounced changes in membrane thickness [349]. Notably, a recent high-resolution SANS study of co-solvents on DMPC lipid membranes demonstrated that tetrahydrofuran (THF) exhibits two partition coefficients describing the fraction of THF that partitions to the membrane-water interface (or the lipid headgroup region) and the fraction of THF residing in the hydrophobic core of the membrane [362]. This two partition constant model was necessary for fitting the collected data, emphasizing the importance of accurately measuring solvent partitioning in lipid membranes for various applications.

Thickness changes may also occur by alkanol partitioning into the L_D phase of lipid membranes [363]. It should be noted that short alkanols, such as ethanol [343], have an affinity towards disordered domains with a partition coefficient that is 3–4 times larger than the L_O phase [364]. In contrast, longer alkanols prefer the ordered phase [363]. More interestingly, recent studies show that these differential effects of ethanol cause an increase in the interfacial tension of L_O-L_D domains, resulting in larger domain sizes with important implications in biofuel production [364]. SANS measurements [365] and simulations [366] have shown that longer chains have a condensing effect on DMPC (14:0–14:0) membranes, reducing the area per lipid. Other studies [367–370] have yielded contrasting

results, showing a decrease in thickness for longer alkanol chain lengths. Among these structural effects, it was also found that both linear and branched alcohols are able to induce an interdigitated phase at a critical concentration in saturated PC membranes [367–370]. This is attributed to the disruption of the hydrogen bonding between the headgroups, leading to decreased packing and interdigitation [371, 372]. Ethanol, in particular, can form non-bilayer globular structures from POPC [333] and DPPC [373] above concentrations of ~15% mol. Gurotvenko and Anwar point out that even though these concentrations are not found in typical physiological conditions, epithelial tissues come in contact with a localized, high concentration of ethanol when consuming hard alcohol [333].

In addition, alkanols and n-alkanes are known to generally broaden the main phase transition in phospholipid membranes [374]. However, the chain length plays an important role in the thermodynamics of lipid phase transitions. For instance, short to medium chains such as ethanol [358, 369], octane [375], and decane [375] decrease the main transition temperature while longer chains such as dodecane [282] and tetradecanol [358] increase the transition temperature. Fluorescence measurements explain these changes by assuming that alcohols have their own effective transition temperature, which is distinct from their respective bulk melting point, and thus act as a quasi-lipid when embedded in lipid membranes [376]. Similarly, n-alkanes exhibit dependence on the specific lipid headgroup but induce little to no observable change in the main transition temperature [375]. For PE membranes, alkanes reduce the transition temperature, T_H, from bilayer to inverted-hexagonal geometry [377], whereas short chain alkanols increase T_H and longer chains induce greater curvature strains leading to a lower T_H [375].
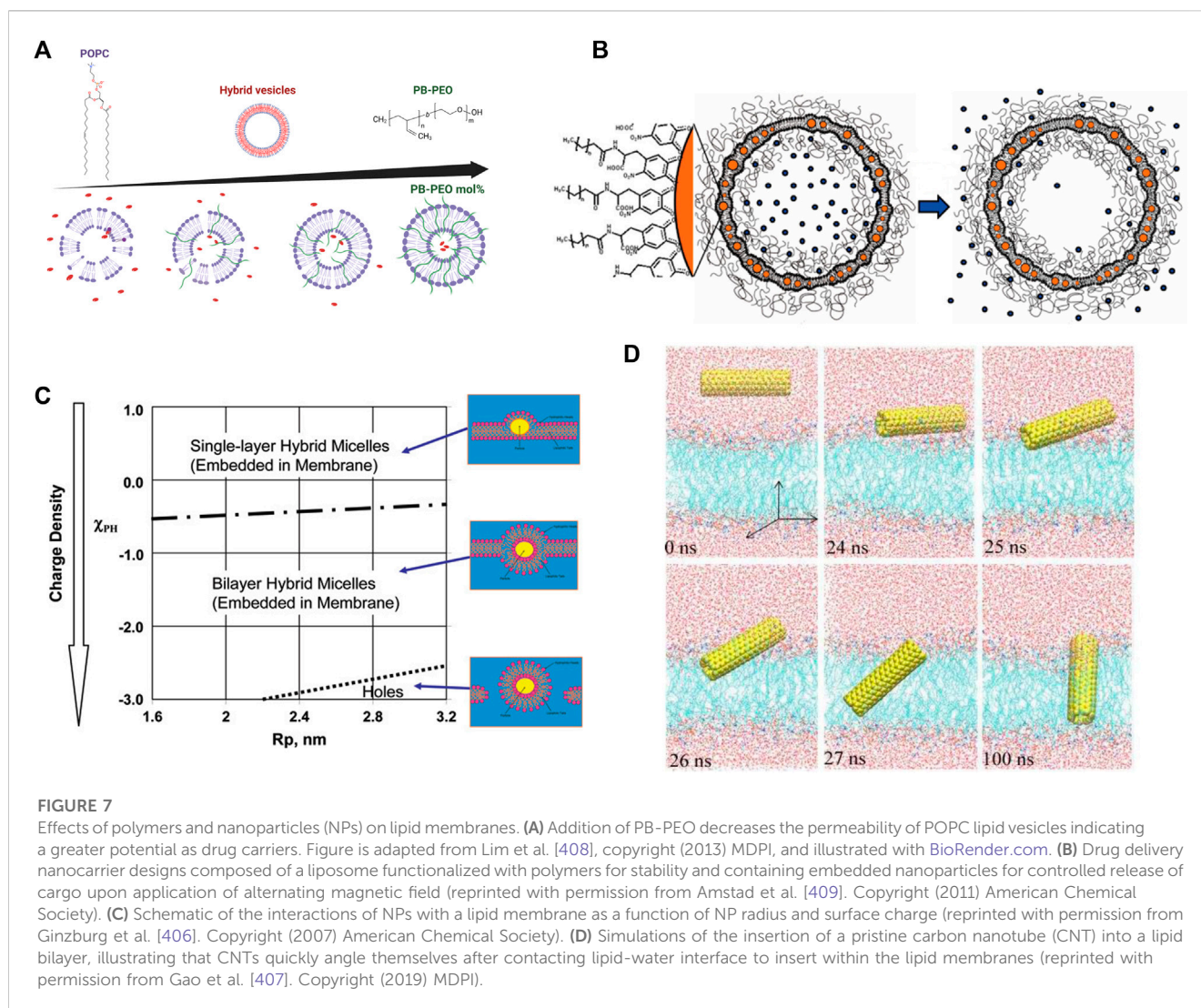
Furthermore, fluorescence studies [378] and simulations [343, 351, 366] show that ethanol increases the diffusion of membrane lipids, while longer chain alcohols tend to greatly reduce translational diffusion [366]. Complementary studies by ^{2}H-NMR [379], fluorescence assays [380], and simulations [358] have also reported observations of alcohols inducing order or disorder in lipid membranes depending on chain length. Notably, the addition of cholesterol to PC lipid membranes modifies the extent of chain disordering by ethanol [381]. Micropipette aspiration studies on SOPC (18:0–18:1) bilayers [355] and MD simulations on DMPC (14:0–14:0) membranes [358] both reported a reduction in the bending rigidity and membrane tension in the presence of ethanol [343, 366]. However, Ly and Longo found that alcohols with increasing chain length cause a larger decrease in the bending rigidity [357], in agreement with trends in the area compressibility modulus predicted by Traube's rule for alcohol surfactants at the water interface. Similar to ethanol, short chain alkanols have been found to decrease the bilayer interfacial tension, reducing the tension by up to 50% at membrane rupture [357]. For medium length alkanols, simulations show a decrease in the area compressibility modulus with increasing concentration of octanol [211] whereas long chain alkanes can stiffen [358] the membrane at high enough concentrations. Overall, short chains perturb the membrane until reaching a chain length "cut-off" where they then instead reinforce the membrane, increasing order, bilayer thickness, and bending rigidity [382].

Polymers

Natural polymers are present in almost all living organisms ranging from the extracellular matrix of animals and plant cell walls [1] to glucans and chitin that give fungi their structure [383] to the lipopolysaccharide layer of Gram-negative bacteria [384]. These biopolymers do exert effects on lipid membranes under various conditions. However, in their free form, they do not typically insert into the membrane but rather form coatings or mats in the membrane vicinity. Monolayer compression isotherms of DOPC on an aqueous subphase containing chitosan or hyaluronic acid display minor changes in molecular packing and monolayer compressibility, indicating minimal changes to the monolayer properties [385]. Therefore, despite the biological relevance of free biopolymers, we will not discuss them as a membrane additive. Instead, we focus here on bioderived or synthetic polymers that are conjugated to lipids or sterols or those that are designed to insert directly into lipid membranes, acting as a true additive.

Due to their versatile properties, synthetic polymers play an essential role in everyday life and have recently been incorporated in various designs of lipid membranes, often referred to as "polymer-lipid hybrid membranes". These hybrid membranes are attractive candidates for numerous applications such as controlled drug delivery [386, 387], biosensors [388–391], and artificial cells [392, 393]. Earlier studies on lipid-polymer hybrids, specially block copolymers, show that the thickness, permeability, and bending rigidity of membranes can be changed by controlling the properties of incorporated polymer, including the block length and hydrophilic to hydrophobic block ratio [394, 395]. Optimizing these properties is critical for designing successful targeted drug delivery approaches [396]. This affords adaptable polymer-liposome a great potential in the design of stable and long lasting controlled-release nanocarriers as a hybrid alternative to fluid liposomes and rigid polymersomes [397–399].

For instance, PEO-*b*-PCL-*b*-PEO, an amphiphilic triblock copolymer structured with a hydrophobic middle block and long hydrophilic end blocks, was shown to remarkably enhance the stability of DPPC (16:0–16:0) vesicles by increasing the pressure required for the onset of membrane lysis [400]. The membrane thickness and size polydispersity can be additionally altered by modifying the hydrophilic block fraction [401]. Other polymers including polyethylene glycol (PEG) [402–405] and polybutadiene-*b*-poly(ethylene oxide) (PB-b-PEO) have also been used in stable biocompatible drug delivery technologies (Figure 7A) [408], including most recent mRNA vaccines. Their incorporation into liposomes allows cyclic dosage, tunable release of both hydrophilic and hydrophobic drugs, and controlled release of therapeutic agents over extended periods. Polymer-lipid hybrid membranes also have widespread applications in medical devices and biomaterials. Polymers composed of 2-methacryloyloxyethyl phosphorylcholine (MPC) units are exceptional biomaterials for the design of artificial cell membrane structures with compatible bio-interfaces between artificial and biological systems [410]. For example, synthetic polymer vesicle nanoreactors composed of oligo(aspartic acid)-*b*-poly(propylene oxide)—with a negatively charged surface—are favorably permeable to cationic and neutral compounds and act as a synthetic molecular channel when inserted into lipid membranes [411]. This suggests that

FIGURE 7
Effects of polymers and nanoparticles (NPs) on lipid membranes. **(A)** Addition of PB-PEO decreases the permeability of POPC lipid vesicles indicating a greater potential as drug carriers. Figure is adapted from Lim et al. [408], copyright (2013) MDPI, and illustrated with BioRender.com. **(B)** Drug delivery nanocarrier designs composed of a liposome functionalized with polymers for stability and containing embedded nanoparticles for controlled release of cargo upon application of alternating magnetic field (reprinted with permission from Amstad et al. [409]. Copyright (2011) American Chemical Society). **(C)** Schematic of the interactions of NPs with a lipid membrane as a function of NP radius and surface charge (reprinted with permission from Ginzburg et al. [406]. Copyright (2007) American Chemical Society). **(D)** Simulations of the insertion of a pristine carbon nanotube (CNT) into a lipid bilayer, illustrating that CNTs quickly angle themselves after contacting lipid-water interface to insert within the lipid membranes (reprinted with permission from Gao et al. [407]. Copyright (2019) MDPI).

imparting the vesicle surface with anionic charge enhances the permeability and usage in biomedical materials and artificial cells.

Knowledge of how these polymers change the structure and dynamics of the lipid membrane is necessary to improve these applications and generate controllable membrane platforms. Previous studies have predicted that the insertion or grafting of polymer chains in lipid membranes can significantly impact the elasticity of lipid membranes and subsequent membrane-protein interactions [412]. These concepts were recently illustrated in liposomal studies, where the introduction of block-copolymers in liposomal membranes was shown to regulate membrane elasticity for optimal folding of mechanosensitive membrane proteins [413] and the insertion rates of natively folded peptides [414]. The inclusion of polymers in membranes has also been shown to impact membrane phase transitions. For example, the biocompatible polymers polyisobutylene (PIB) and poly(ethylene oxide) (PEO) completely disrupt the typical behavior of DPPC monolayers at the air/water interface, indicating a disruption to lipid packing [64, 415]. In DOPC, n-alkyl PEO was found to reduce the bending rigidity via transformation of the lamellar structure [416]. Other forms of

amphiphilic polymers have also been used to generate transient pores in lipid membranes [417], aiding in the design of hybrid lipid membranes with controllable transport properties.

Nanoparticles

Cell membranes frequently interact with small nanoscopic particles, or nanoparticles (NPs). For example, NPs present in the atmosphere from industrial processes or environmental pollution are known to partition into pulmonary membranes, causing lung complications and airway irritation [418]. A practical way to model the effect that various NPs have on the pulmonary surfactant, the monolayer lining the alveoli, is through the use of Langmuir lipid monolayers. This method has shown that silica NPs increase the area per lipid at any given surface pressure and simultaneously reduce the surface pressure required for collapse in DPPC:DOPC:Chol monolayers [419]. A similar result was seen for carbon NPs in DPPC:DPPG, but the degree to which the carbon NPs affect the monolayer depends on the value of the surface

pressure at the time when the NPs are deposited [420]. Other studies on saturated and unsaturated lipid monolayers composed of PC and PG headgroups show that cationic and anionic silver NPs have different effects depending on the lipid composition, charge mismatch, and surface pressure during NP insertion [421]. In a similar study on model PS monolayer composed of DPPC:DOPC, the addition of nanoparticles was found to change the surface pressure response to cyclic compression cycles simulating the respiratory rhythm. This study found that the total harmonic distortion increases in a pure DOPC monolayer, but in a DPPC: DOPC system NPs decrease the overall distortion, leading to a more linear response between area compression and surface pressure [422].

Nanoparticles also have novel uses in drug delivery applications, which still have shortcomings in controlled cargo release and exhibit a great increase in permeability around the lipid melting transition temperature. To circumvent these pitfalls, magnetic nanoparticles allow for controlled release of the drug by using timed magnetic field pulses as an external stimulus. This results in local magnetic heating in the vicinity of the NPs, raising the local membrane temperature beyond the melting transition, thereby increasing the permeability and releasing the encapsulated drug (Figure 7B) [409]. The incorporation of NPs in lipid membranes requires judicious surface functionalization; for instance, hydrophobic magnetic NPs are typically intended to heat their local lipid environment whereas hydrophilic NPs must heat the bulk aqueous medium to achieve the same release efficiency [409]. Hydrophobic cobalt ferrite NPs coated with a shell of oleic acid easily partition into the hydrocarbon region of a liposomal membrane. Interestingly, these particles cause slow release of cargo right after the application of a magnetic field due to pore formation but induce quicker release after a few hours due to increased membrane permeability [423]. On the other hand, superparamagnetic iron oxide NPs in bare, silica-coated, or charge-functionalized forms did not have any significant effect on lipid order, membrane fluidity, or phase transition when either incubated or encapsulated into PC liposomes [424]. These findings are important in understanding the biocompatibility of NPs and guiding future NP designs.

Liposomal leakage by nanoparticles is mainly due to the interactions between the lipid molecules and the functional group on the NP surface, with stronger effects of headgroup interactions compared to chain interactions. For example, a NP with a positive surface charge will interact much more strongly with negatively charged lipids compared to a NP with negative charge [425]. However, the NP itself plays a key role in membrane destabilization, i.e., free functional groups alone have no effect on leakage but can result in significant leakage when decorating a membrane-embedded NP. For example, in comparison to melittin, a pore forming protein discussed earlier, a single nanoparticle can cause the same degree of disruption that requires hundreds of melittin proteins [425].

Nanoparticle size plays a key role in NP partitioning into membranes and the induced disruption to the membrane structure. MD simulations show that a single gold NP alters the structural properties and fluidity of the membrane on a short and long range in a way that correlates with particle size [426]. Isothermal calorimetry studies show that the interaction of small NPs with DPPC:DPTAP liposomes is an entropically driven process favoring individual NP binding to the surface, while larger NPs

interact through an enthalpically driven process which favors NP aggregation [427]. This aggregation may lead to structural changes such as fusion of neighboring or encapsulated liposomes together [428]. Complementary TEM studies demonstrate that NP incorporation into membrane is size limited (maximum size of ~5 nm) [409], consistent with theoretical results [429] from a simple geometrical model which predicts whether a nanoparticle would partition into a membrane or cause micellar formation. Including the charge density of the NP into the model shows that, besides size, the charge strength on the NP surface is another determining factor in membrane partitioning or micellization (Figure 7C) [406]. In drug delivery liposomes, the NP size and surface functionalization should be optimized with the applied magnetic field strength [428], frequency, application time, and pulse sequence [409] to control the leakage rate and amount [423].

Other forms of environmental NP irritants include fullerenes, highly hydrophobic spherical nanoparticles composed of 60 carbon atoms with a diameter of approximately 1 nm. This class of molecules has a plethora of uses, both industrial and therapeutic [430, 431]. However, it is known to damage the plasma membrane through lipid peroxidation [432] which is an important health factor in the use of engineered nanomaterials in consumer applications. Fullerenes tend to sit off the bilayer center and have minimal changes on the local area per lipid or lipid chain stretching [433, 434]. However, due to this preferred displacement from the center of the bilayer, fullerene inclusion in membranes induces leaflet asymmetry and differential stresses that result in changes to the overall area compressibility, bilayer thickness, and bending rigidity [434]. Moreover, solid state NMR and FTIR studies show that fullerenes have an affinity towards lipids with an anionic headgroup, like those abundantly present in bacterial membranes, and therefore disturb chain packing and increase fluidity [435]. In monolayers, fullerenes were found to aggregate and greatly affect the stability and surface tension of monolayers formed of DPPC, a major component of the pulmonary surfactant [436].

Carbon nanotubes (CNTs) are another form of carbon-based nanoparticles and have been used as a synthetic analogue for porin, a protein involved in molecular exchange and transport across cell membranes. Short CNTs (10–20 nm in length) span the membrane (Figure 7D) and form pore sizes that can be engineered from tenths of a nanometer up to 10 nm in width, allowing for stable nanofluidic channels that have applications in transport, sensing, and filtration [437, 438]. The length and surface functionalization of CNTs can significantly impact the properties of their immediate lipid membrane environment, i.e., the annular lipid shell surrounding each CNT [439]. At high CNT densities, lipid-mediated interactions facilitate CNT diffusion and drive CNTs to assemble into clusters of 2D hexagonal arrays spaced apart by the annular lipid shells [440]. Other simulations found that while CNTs had virtually no effect on the structural properties of the membrane they cause slight ordering in neighboring lipid tails [407].

Conclusion and outlook

The interaction of lipid membranes with small molecules and nanoscopic additives is a common phenomenon in biological and synthetic membranes, offering a plethora of possibilities for tuning

membrane properties either through biological adaptation or through sophisticated engineering of molecules and nanoparticles. Whether biological or synthetic, how additives modulate the physical properties of lipid membranes is necessary to understanding the role of membranes as evolved and adaptive soft materials, and refining their designs for practical applications and advanced technologies. To date, numerous studies using experimental, theoretical, or computational approaches have contributed significant knowledge about the mechanisms by which different additives partition into lipid membranes and their subsequent effects on the membrane structural, elastic, thermodynamic, and dynamic properties. Nonetheless, a major gap still exists in combining these effects into unified physical laws or design rules to realize the potential of lipid membranes as functional platforms beyond trial-and-error approaches.

This review sheds light on the interdependence of partitioning of additives into phospholipid membranes and resultant physical membrane properties. By comparing findings across seemingly disparate classes of additives and phospholipid architectures, a more complete picture of molecular partitioning and structure-property relations starts to emerge. For example, a common observation is that additives that partition to the membrane-water interface, i.e., to the headgroup region of lipid membranes, tend to disrupt lipid packing and subsequently lower the phase transition temperatures, decrease the bending moduli, and increase lipid mobility. In contrast, additives that reside in the hydrophobic region of the lipid membrane in a way that induces higher lipid chain order generally have opposite effects. Put together, this shows a consistent picture in which additive-induced changes to the membrane structure are often coupled with changes to the physical membrane properties in an interdependent pattern. These observations point to the potential of establishing structure-property relations that unify the effects of additives and the design principles of lipid membranes as soft molecular assemblies. This interdependence is yet to be systematically investigated.

More importantly, exploring structure-property relations should be done across multiple spatiotemporal scales to identify emergent membrane properties that dictate function across different scales. Given the soft and highly dynamic nature of lipid membranes, how they respond to additives on fast and slow timescales could transform the nature of membrane-based technologies. Current and future investigations in these directions will help accelerate the development of artificial lipid membranes with functions that can be specifically tuned for therapeutic or technological purposes. With the advancement of characterization techniques, synthesis approaches, and membrane functionalization methods, lipid membranes and their interactions with biological and synthetic additives will continue to push the boundaries in technologies that improve human health, advance synthetic biology platforms, and further our knowledge of soft materials and their various applications.

Author contributions

TK and NBM contributed equally to collecting and summarizing existing literature. RA reviewed and edited the article. All authors contributed to the article and approved the submitted version.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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