

Rhodopsin: The hydrogen atom of membrane biophysics

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ABSTRACT Membranes possess characteristic lipidomes that are preserved by homeostatic regulation, even as environmental conditions change. Technological advancements in lipidomics instrumentation have revealed that altering this composition can produce significant physiological effects and can influence protein function. As lipidomics has expanded our view of membrane diversity, a key question remains: which membrane features must be maintained by cells to ensure proper protein function? Here, we focus on key membrane properties such as asymmetry, packing, and elasticity and highlight cases in which lipid composition modulates protein function. We find that curvature stress is a likely target of such regulation and accounts for the gradual changes in protein activity observed across lipid series that differ systematically in their physical properties. Curvature stress arises when there is a difference between the actual (mean) and preferred (spontaneous) curvature of a membrane. The magnitude of the stress depends on the amount of deformation and the membrane bending rigidity, both of which depend on lipid packing. These properties are further modulated by composition and number asymmetry between the two leaflets. Throughout these studies, rhodopsin has played a pivotal role in uncovering these principles due to its natural abundance and spectroscopic accessibility, enabling experiments that would be difficult or impossible with other membrane proteins. We therefore consider rhodopsin as the hydrogen atom of membrane biophysics in recognition of its unparalleled significance as a model system, in analogy to how the hydrogen atom provides the foundation for atomic theory. Because rhodopsin uniquely permits precise measurements of conformational equilibria, it remains a powerful system for dissecting how lipid composition and asymmetry give rise to membrane curvature adaptation.

SIGNIFICANCE To complement lipidomics studies and guide future research in membrane biophysics, both conceptual frameworks and model systems are required. Rhodopsin offers a promising system for investigating lipid-protein interactions, owing to its distinctive spectroscopic features and high natural abundance in retinal disk membranes. The ability to directly measure conformational states of rhodopsin using electronic and vibrational spectroscopy makes it one of the premier examples of how membrane asymmetry and curvature stresses alter protein function. Competition between polyunsaturated fatty acids and cholesterol has likely evolved to regulate membrane properties through curvature homeostasis.

INTRODUCTION

Membrane proteins are involved in some of the most fundamental cellular processes and are strongly influenced by their lipid environment—a complex mixture whose collec-

tive properties shape the physical behavior of the membrane (1). The composition of this mixture is tightly regulated, at the expense of substantial metabolic effort from the cell (2). Such an energetic investment suggests the importance of maintaining a specific lipid composition for proper cellular function. Indeed, numerous studies have demonstrated that variations in lipid composition can alter membrane properties. Asymmetry in the number or types of lipids between the two membrane leaflets can alter molecular packing (3), which affects membrane properties such as bending

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rigidity, curvature, and cholesterol distribution (4–6). Cholesterol also modulates membrane properties, especially bending rigidity and packing, typically exerting effects opposite to those of lipids containing polyunsaturated fatty acid (PUFA) chains (7–9), as illustrated in the visual system (10,11). Cells seem to exploit the differing properties of such lipids to preserve constant lipid packing even as environmental conditions fluctuate, termed homeostasis (12–14). Although lipid composition clearly tunes biophysical properties of the membrane, the mechanisms by which these properties influence protein function are still not well understood.

Current lipidomics research attempts to explain this regulation by relating changes in lipid composition to physiological function and diseases (15). This has provided important insights, and it now opens the door for building a deeper conceptual framework to understand how lipid composition affects protein function. One of the earliest frameworks proposed to explain lipid-protein interactions in cellular membranes was the fluid mosaic model (FMM). It arose from measurements of the rotational and translational diffusion of rhodopsin (16–18), which led to the ideas of weak lipid-protein interactions and membrane fluidity that form the foundation of the model. Such measurements were possible due to the covalently bound chromophore of rhodopsin, retinal, which imparts many useful spectroscopic properties. For example, if rhodopsin is photobleached with polarized light, then only retinal at certain orientations will be photoselected, leading to an induced dichroism. This photoinduced dichroism decays rapidly, indicating that rhodopsin with its bound retinal chromophore must be able to freely rotate around an axis perpendicular to the membrane (16). Similarly, if rhodopsin on one side of a retinal rod cell is photobleached, then it temporarily loses the ability to absorb light. Within a few seconds, the photobleached area spreads across the rod cell, indicating translational diffusion of rhodopsin within the membrane (17,18). This photobleaching recovery has since become a widely applicable method in cellular biophysics.

While the FMM was invaluable as a foundational framework, highlighting that membranes are dynamic and laterally mobile, subsequent work has revealed that its central assumption of a purely two-dimensional fluid with weak in-plane interactions does not fully capture the complexity of real membranes. In response, models such as the flexible surface model (FSM) (19–21), the mattress model (22), and lipid rafts (23) were developed to capture the physical interactions and out-of-plane forces that lie beyond the scope of the original FMM. These newer frameworks have, in turn, shaped ongoing efforts to understand how collective membrane properties, arising from the geometry of individual lipids, influence protein activity, a question that continues to be explored using rhodopsin (24). Rhodopsin has a lipidome that has been shown to be required for visual signaling (10,11). How the naturally asymmetric membrane

composition of rhodopsin affects conformational changes required for function has also been explored (25). Work showing the opposing effects of PUFAs and cholesterol on rhodopsin function is an extreme but illustrative case of how the geometry of individual lipids can regulate collective membrane properties (20,25–31). Rhodopsin has been used to illustrate how protein conformation and function are influenced by elastic forces (out-of-plane coupling), particularly through the lamellar-to-nonlamellar phase transition characteristic of certain lipids. This understanding has given rise to the concept of curvature homeostasis (or homeocurvature adaptation), which suggests that the membrane has evolved to maintain a functional level of curvature stress even under varying environmental conditions (20,32,33). Even then, the role of out-of-plane curvature forces are only now beginning to be understood in regard to lipid-protein interactions.

Throughout these developments, rhodopsin has remained a central and irreplaceable experimental system for membrane biophysics (Fig. 1). Just as the hydrogen atom forms the foundation of our understanding for atomic theory from which molecular theory is derived, rhodopsin is the basis for many foundational concepts in biophysics from which broader principles of lipid-protein interactions have emerged. This is due to the covalently bound retinal chromophore whose optical properties change with protein conformation, enabling highly sensitive real-time spectroscopic measurements of the structural state of rhodopsin. Early measurements of its rotational and translational diffusion in aligned rod outer segment (ROS) membranes provided some of the first experimental demonstrations of lateral mobility, helping to establish the dynamic membrane behavior central to the FMM. Other studies have shown that membranes of a specific thickness favor the active conformation of rhodopsin (19,35), demonstrating the effect of hydrophobic matching that is the foundational assumption of the mattress model. Rhodopsin has been used to show how curvature stress of the membrane explains the gradual increase in protein activity observed across a series of lipids that differ systematically in their physical properties, leading to the formulation of the FSM (36). Together, these contributions position rhodopsin not only as a historic foundational system but also as a continuing benchmark for evaluating emerging models of membrane structure and function. Indeed, rhodopsin finds itself the namesake of the largest family of G-protein-coupled receptors (GPCRs). They include medically important receptors such as the β -adrenergic receptors, opioid receptors, and GPCRs implicated in cancer. All of these share a highly conserved activation mechanism, and as such, any findings with rhodopsin extend to these other GPCRs (37), with major implications for understanding and improving human health.

Here, we discuss the role of lipid composition in regulating protein function. From lipidomics studies alone, it

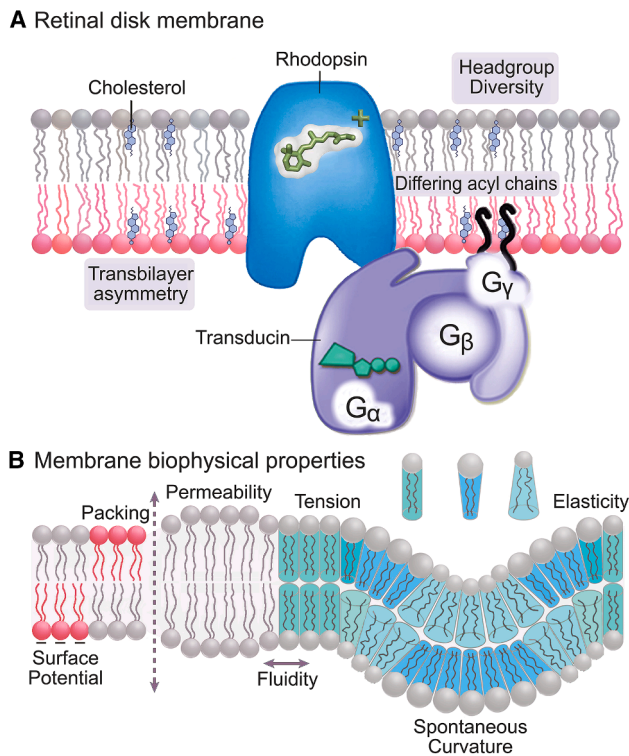


FIGURE 1 Visual rhodopsin illustrates collective biophysical properties relevant to lipidomics and lipid diversity in biomembranes. (A) Rhodopsin, a G-protein-coupled receptor (GPCR) found in retinal disk membranes (RDMs), serves to activate the heterotrimeric G-protein transducin comprising G α , G β , and G γ subunits, which begins the signal transduction cascade responsible for vision in dim light (34). (Note that the proteins are for illustrative purposes only and are not to scale.) After photoactivation, rhodopsin adopts an equilibrium between the preactive metarhodopsin I (MI) and active metarhodopsin II (MII) conformational states, which can be distinguished spectroscopically. This allows for measurements of how lipid-protein interactions alter protein function. (B) Lipid membranes are complex and dynamic mixtures that possess a number of biophysical properties capable of altering membrane protein activity. Tight regulation of the lipidome allows for the biophysical properties of the membrane to remain nearly constant, even under changing environmental conditions, a process termed homeostasis. Rhodopsin exhibits a tightly regulated lipidome shown to be required for visual signaling, allowing for exploration of which membrane properties regulate protein function.

is unclear whether the observed effects of compositional changes on physiological function are due to specific lipid-protein interactions or material properties of the membrane system. However, lipid composition appears to be controlled by the cell to maintain the lamellar-to-nonlamellar phase transition within a narrow temperature range (19,20,32,33). The curvature free energy of the membrane may potentially explain this phenomenon; a certain value of curvature stress may confer some evolutionary fitness due to its role in controlling protein function, requiring cells to regulate their lipid composition to maintain that value, as illustrated by rhodopsin (20,21,24,35,38). Curvature stress can arise from lipid packing, which itself is affected by lipid composition, especially the competing effects of polyunsat-

urated lipids and cholesterol. Compositional and lipid number asymmetry can further affect the curvature free energy by creating differential packing in the two leaflets (5). The role of curvature stress in regulating protein function has also been proposed for mechanosensitive channels, ion channels, and gramicidin, suggesting the widespread applicability of this idea (39–43). Here, we explore the hypothesis that in addition to chemical or allosteric lipid-protein interactions, the tightly regulated lipid composition gives rise to specific mechanical properties, particularly curvature free energy, that in turn regulate membrane protein function.

CELLS AND ORGANELLES HAVE CHARACTERISTIC LIPIDOMES

Different cellular environments have unique, tightly regulated lipid compositions (1,2,44,45), and even different leaflets of the same membrane can differ in composition (1,3,46–48). Such lipid diversity manifests as differences in acyl chain length and saturation, as well as head group charge and size. These subtle structural variations lead to significant differences in the physical properties of individual lipids, which collectively influence the behavior of cell membranes. Because lipid composition is crucial for maintaining cell homeostasis, particularly in response to changing temperatures or pressures, it is tightly regulated by lipid biosynthetic pathways. Chemical properties of lipids, both endogenously and exogenously obtained, can be changed to maintain the lamellar phase and to control the physicochemical membrane properties that best suit the requirements of cells (49,50). The specific mechanisms underlying lipid composition homeostasis are complex, poorly understood, and require further investigation (51). Still, there has been a recent surge of lipidomics research focused on identifying and quantifying lipid species, made possible by technological advancements in lipidomics instrumentation. These technological developments have brought lipids and their compositional diversity into the spotlight and helped fill in gaps in classical models. However, on their own, such advances have not fundamentally altered the prevailing conceptual framework. Instead, the paradigm began to shift away from the FMM due to the influence of models such as the FSM (20,52), mattress model (22), and raft-like nanodomains (23). The introduction of the FSM in the 1980s (19–21) was particularly influential because it was the first to propose that collective membrane mechanics, not specific lipid-protein interactions, could bias the conformational equilibrium of a protein. In particular, it was noted that “the occurrence of phosphoethanolamine headgroups and polyunsaturated acyl chains in retinal rod disk membranes may influence the curvature free energy of the bilayer,” and thereby facilitate rhodopsin activation (21). Together, these models helped move the field beyond the depiction of the membrane by the FMM as a weakly interacting two-dimensional fluid. Despite these

developments, how lipid composition affects protein function remains an open question.

One of the key chemical differences in lipids is acyl tail unsaturation (Fig. 2 A), which is linked to lipid packing and elasticity as well as membrane fluidity (57,58). The addition of a CH=CH bond within the acyl tail introduces steric hindrances because of its molecular geometry, causing lateral packing defects and increased area per lipid (57,59). The area per lipid and lipid packing describe the space occupied by an individual lipid molecule and directly control how tightly lipids pack within the membrane assembly. Consequently, increasing the area per lipid decreases the lipid packing and vice versa, because each individual molecule requires more space to avoid unfavorable interactions like steric clashes (60). Decreased lipid packing increases the free volume in the membrane arising from the packing defects, allowing for greater bending than would be allowed in a fully saturated bilayer (60). As a result of these physical properties, the degree of acyl tail unsaturation within the membrane (Fig. 2 B) directly affects its bending rigidity and fluidity (9). Other important areas of chemical variation are the head groups and tail length. Head groups determine the charge on the hydrophilic end of the phospholipid, which controls how the membrane interacts with external proteins and ligands through electrostatic interactions (61). Head groups also vary in size, which plays a direct role in lipid packing and spontaneous curvature of the membrane. Larger headgroups decrease the lipid packing (38,62) and favor positive spontaneous curvature (where the membrane maximizes the head group area while bunching up the hydrophobic lipid tails) (63). Smaller head groups, as in the case of phosphatidic acid, plasmalogens, and phosphatidylethanolamine (PE), increase the lipid packing and favor negative curvature (61,64). Acyl tail length has long been shown to control bilayer thickness, area per lipid, and membrane fluidity (62,65,66). The area per lipid increases as acyl tails lengthen because of how the membrane distributes the increased volume from the additional methylene groups (66).

Lipidomic analysis was first applied to rhodopsin-containing membranes by employing electroretinography to quantify voltage changes associated with rod cell activity (10,11). These studies demonstrated that dietary manipulation of retinal lipid composition produced measurable alterations in electroretinogram responses, thereby establishing, for the first time, a direct link between membrane lipid composition and physiological function, a relationship made accessible by the unique phototransductive properties of rhodopsin (10,11). It has since been shown that altering membrane composition affects protein activity in the case of the (Ca²⁺-Mg²⁺)-ATPase and mannosyltransferase II (39,40,67). These investigations of membrane proteins are paralleled by analogous studies of membrane-bound peptides, and together point to common effects of membrane composition on function (43,68). On a broader biological

scale, lipid composition has been shown to affect microbial growth, with bacterial cell size dictated by acyl chain length and unsaturation (69). Together, these findings suggest that cells evolved a tightly regulated membrane lipid compositions to finely tune protein activity and physiological function (1,2).

LIPID COMPOSITION GOVERNS PROTEIN CONFORMATION AND FUNCTION

There are two primary ways lipid composition may regulate protein activity: through specific, ligand-like interactions or via nonspecific, collective properties of the membrane. Specific interactions have been demonstrated for numerous proteins, including the Na⁺/K⁺-ATPase (70), Akt (also known as protein kinase B) (71,72), Kir channels (73,74), electron transport chain complexes (75–77), and GPCRs such as the β_1 -adrenergic receptor (78) and rhodopsin (79–81). Specific lipid-protein interactions have been reviewed in detail elsewhere (82,83). While such examples are well documented, specific lipid-protein interactions alone cannot fully account for the gradual increases in protein activity observed across a series of lipids that differ systematically in their physical properties.

In contrast to such ligand-like binding, collective interactions arise from the overall properties of the lipid bilayer and are chemically nonspecific. One of the early frameworks linking lipid properties to protein regulation was the mattress model (22), which proposed that membrane deformation increases when there is hydrophobic mismatch between the transmembrane region of a protein and the surrounding lipids, incurring an energetic penalty (84–86). This concept of hydrophobic matching has since been expanded to explain why certain proteins and lipids partition into thicker, liquid-ordered microdomains, often referred to as raft-like regions. These microdomains are thought to arise from the preferential packing of saturated lipids with cholesterol, which produces a thicker and more ordered bilayer that better accommodates proteins with longer transmembrane regions (23,89–92). Such lipid-driven sorting is proposed to generate dynamic nanodomains ranging from 10 to 100 nm in size (93,94). The idea of raft-like domains has been reviewed in greater detail elsewhere (93–97).

The general principle—that local bilayer deformation can modulate protein energetics—recognizes that distortion of the lipid environment is likely to influence protein interactions and may affect proteins that undergo transmembrane conformational changes during activity (98). However, classical hydrophobic matching models are limited by their simplification of the membrane, considering only acyl chain length and neglecting the fact that lateral pressure, tension, and compression vary with depth in the bilayer. These depth-dependent pressure profiles generate curvature stress, which can bias the conformational equilibrium of

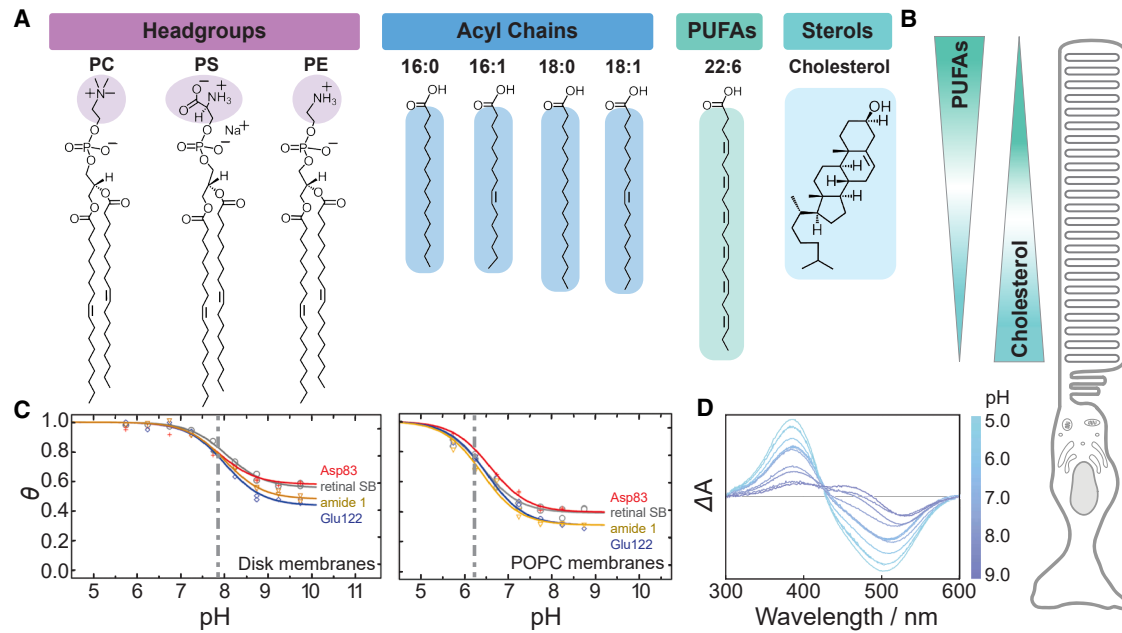


FIGURE 2 Lipidomic studies investigate the effects of RDM lipid diversity on rhodopsin function. (A) Structures of the primary lipids found in RDMs. As these lipids have different lateral pressure profiles, their relative abundance modulates membrane properties. Phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS) lipids are the backbone of the RDM bilayer and control the spontaneous curvature and average charge of the membrane. The length of the acyl chains controls membrane thickness and hydrophobic matching. Polyunsaturated fatty acids (PUFAs) and cholesterol have opposing effects on the bending rigidity and lipid packing of the membrane. (B) Schematic illustration showing how the concentration of cholesterol decreases with the age of the disks, while the concentration of PUFAs increases (53). (C) The fraction of rhodopsin in the active MIII state (θ) measured as a function of pH in native RDMs and 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC) membranes (using Fourier-transform infrared (FTIR) and UV-visible spectroscopy) reveals how lipid diversity strongly affects rhodopsin photochemical activity. Reversible shifting of its activation equilibrium occurs in various recombinant membrane environments. Bands due to Asp83, Glu122, and amide I are markers in FTIR spectroscopy experiments that indicate conformation changes in rhodopsin associated with activation. The protonation status of the retinal Schiff base is monitored with UV-visible spectroscopy under the same conditions as the FTIR experiments. The dotted gray line indicates the approximate pK_a values: disk membranes ($pK_a = 7.8$) and POPC membranes ($pK_a = 6.0$) with an estimated error of ± 0.2 (54). (D) Representative difference spectra of rhodopsin in RDM as a function of pH showing the activation of rhodopsin increases under more acidic conditions. (C) Adapted with permission from (55). Copyright 2010, American Chemical Society. (D) Adapted from Hewage (56).

membrane proteins and affect their function (86). The FSM and related elastic theories extend the idea of hydrophobic matching by incorporating bilayer elasticity, spontaneous curvature, leaflet asymmetry, and lateral pressure profiles, providing a more complete description of how membrane physical properties regulate protein structure and function (3). Regardless of the mechanism by which lipids influence protein activity, the lipids immediately surrounding a protein can differ in composition from those in the bulk membrane (99–102). These annular lipids exhibit reduced mobility and slower exchange rates with the surrounding bilayer (103–106). As it is entropically unfavorable for specific lipids to be concentrated around a protein, there must be some enthalpic bonus from interactions with the protein, which could be due to either allosteric or collective interactions. For instance, if the active conformation of a protein requires the surrounding membrane to adopt negative curvature, then lipids that prefer negative curvature would be favored near the protein.

One of the best-documented examples of lipid composition affecting protein function is rhodopsin (Fig. 2 C), as the equilibrium between the preactive and active conforma-

tions of rhodopsin can be measured spectroscopically (Fig. 2 D). Unlike other systems that infer protein activity indirectly by measuring substrate or product concentrations, utilizing this unique property of rhodopsin allows for the effect of lipid composition on protein conformation to be directly measured in real time. Reconstituting rhodopsin into various lipid environments has shown that lipids with high acyl tail unsaturation, acyl tails roughly 20 carbons long (Fig. 3 A–D), and small head groups favor the active conformation (Fig. 4 A–D). Membranes filled with these kinds of lipids favor the expanded active form of the receptor compared with the more compressed preactive conformation (35,86,108,109). These differences in lipid composition and the membrane properties that result pave the road for the FSM, an alternative to the FMM, which reflects the biophysical properties of lipids and how they affect membrane proteins like rhodopsin. Using rhodopsin as a model system, we can understand how lipid composition impacts membrane proteins, particularly other GPCRs (e.g., opioid and β -adrenergic receptors) and interrogate further how these physical properties regulate membrane proteins.

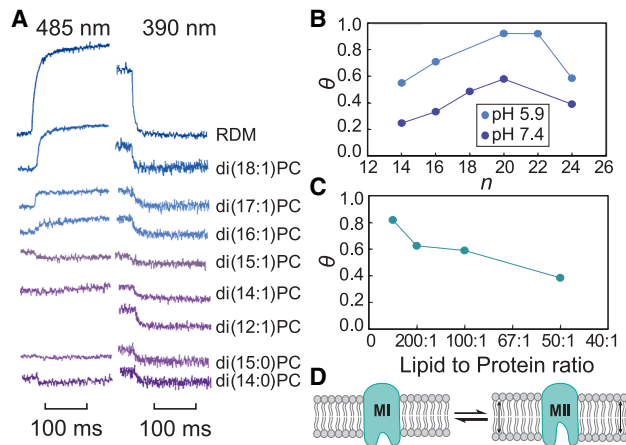


FIGURE 3 Membrane lipid packing and hydrophobic matching affect rhodopsin photochemical function. (A) Flash photolysis transients showing increasing acyl chain length and polyunsaturation in PC lipid recombinants increase the activation of rhodopsin. Photomultiplier tube voltages correspond to the number of photons transmitted through the sample. After light activation, increased voltages obtained at $\lambda = 485$ nm equate to loss of the MI state, while the decreased voltage at $\lambda = 390$ nm signifies appearance of the MII state. The fraction of rhodopsin in the active MII state (θ) is influenced by bilayer thickness and lipid packing properties in unsaturated PC lipid recombinants and is plotted against (B) increasing acyl chain carbon length (n) and (C) the lipid-to-protein ratio. (D) Increased hydrophobic matching and lipid packing increases rhodopsin activation. (A) Adapted with permission from Brown (88) Copyright 1997, Academic Press. Published by Elsevier. (B) and (C) Adapted and reanalyzed with permission from Botelho et al. (87). Copyright 2006, The Biophysical Society. Published by Elsevier.

ASYMMETRIC MEMBRANE COMPOSITION CREATES AN IMBALANCE OF FORCES

In addition to different cellular compartments having their own tightly regulated lipid composition, different leaflets of the same compartment can have their own unique lipid composition, creating an asymmetric membrane. The first discovery of this asymmetry was in red blood cells (46), where amino-phospholipids were found to be enriched in the cytoplasmic leaflet and choline-phospholipids enriched in the extracellular leaflet (110). It was shown that this asymmetry could alter membrane surface properties and affect physiological function, such as blood coagulation, apoptosis, and neuronal function (110,111). Membrane asymmetry is ancient and conserved, with proteins responsible for maintaining asymmetry appearing in all eukaryote genomes and even some bacteria (112–114). The exact composition and relative abundance of these lipids in each leaflet also varies based on cell type and species (46–48). The high conservation of membrane asymmetry, which varies with cell function, implies its importance. However, despite the great lengths to which cells go to maintain membrane asymmetry, it remains unclear how membrane asymmetry impacts protein function at the molecular level.

Asymmetric membranes are fairly stable, as it is highly unfavorable to move the hydrophilic heads of lipids into the hydrophobic membrane environment (115). Consequently, lipids require the assistance of proteins to move between the two leaflets. Lipid asymmetry is thought to be generated and maintained primarily through the action of three types of proteins: flippases, floppases, and scramblases. Flippases are active transporters, primarily P4-ATPases, and move from the extracellular to the cytosolic leaflet (116). Floppases are also active transporters, primarily ATP-binding cassette (ABC) transporters, that move lipids in the opposite direction, from the cytosolic leaflet to the extracellular leaflet (117). Scramblases allow lipids to nonspecifically diffuse down their concentration gradient and abolish membrane asymmetry (118). The emergence and diversification of P4-ATPases, ABC transporters, and scramblases appears to be tied to the rise of eukaryotic complexity (112). The expansion of these transporters parallels the evolution of specialized organelles and complex signaling pathways (111). Aside from proteins that regulate membrane asymmetry, there are certain lipids (119), especially cholesterol, that can rapidly switch between the two leaflets. This tendency makes quantifying the distribution of cholesterol quite difficult, and the actual distribution of cholesterol is highly debated. Some studies suggest up to 90% lies in the extracellular leaflet (6,8,120), while others suggest the complete opposite, that 70%–80% lies in the cytosolic leaflet (121–123). Even outside the extreme example of cholesterol, absolute leaflet-specific lipidomic quantification remains poorly resolved due to methodological limitations: direct physical separation of the two leaflets is not feasible, and current methods such as enzymatic digestion or cyclodextrin-mediated exchange can introduce perturbations or artifacts (124–126). These uncertainties underscore the need for improved strategies to accurately quantify lipid asymmetry in native membranes.

Compositional asymmetry naturally results in asymmetrical biophysical properties of the membrane. For instance, the inner leaflet typically contains more unsaturated lipids and is therefore less tightly packed than the extracellular leaflet, as shown by packing-sensitive dyes (113). This looser packing of the inner leaflet favors thicker transmembrane domains, and indeed, many membrane protein transmembrane domains have a higher surface area in the inner leaflet (113). Such packing differences may also underlie the distinct functional roles of the two leaflets: the outer leaflet is more tightly packed to act as a barrier, whereas the inner leaflet is less tightly packed to facilitate diffusion of signaling molecules. Structural asymmetry can also give rise to mechanical imbalances; differences in lipid number or packing between the two leaflets can cause differential stress, placing the leaflet with fewer lipids under tension and the one with more lipids under compression. This physical imbalance can alter the bending rigidity and spontaneous curvature of the bilayer (4,6,9). Differential stresses

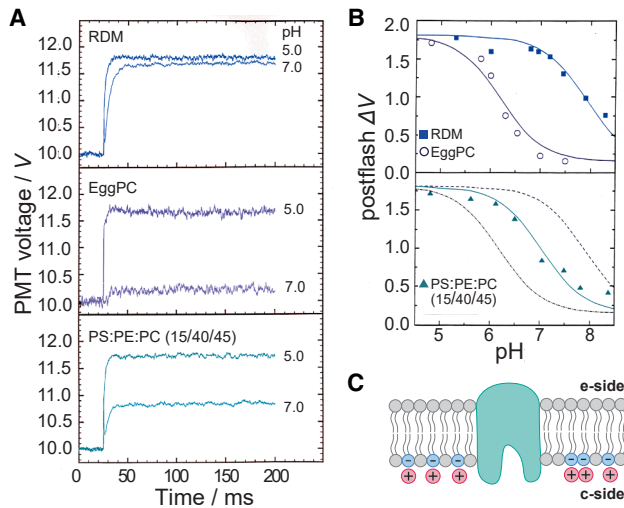


FIGURE 4 Native-like polar headgroups and polyunsaturated acyl chains are necessary for rhodopsin photochemical function. (A) Flash photolysis transients at $\lambda = 478$ nm demonstrate that varying headgroup composition strongly influences rhodopsin activation in recombinant membranes. At pH 7, EggPC (egg phosphatidylcholine) recombinants with non-native bulky headgroups have the lowest activation. Introducing native-like headgroups in PS:PE:PC recombinants leads to a partial recovery of activation. Native RDM, which has both native headgroups and PUFA chains, has the highest activity. At pH 5, all recombinants demonstrate complete activation, showing that the inactivation is reversible. (B) pH titration curves (measured through flash photolysis) showing activation of rhodopsin in various recombinant membranes, where right-shifted curves indicate greater rhodopsin activation. Native-like headgroups comprising PS:PE:PC lead to a reversible recovery of activation, but in the absence of PUFA chains, the activity remains lower than that of native RDM at all pH values. (C) Headgroup compositional effects on rhodopsin. Topologically the PS and PE lipids constitute the cytoplasmic side (c-side) while PC lipids compose the extracellular side (e-side) of RDMs. Notably, PS lipids are negatively charged and may interact with peripheral proteins, lipids, and ions, shifting their chemical properties and function (107). (A) and (B) Adapted with permission from Brown (88). Copyright 1997, Academic Press. Published by Elsevier.

can be reduced by cholesterol moving from the leaflet under compression to the one under tension (127). However, this movement alone is not sufficient to cancel out differential stress, as its partitioning is also affected by factors such as elastic stresses, solubility preferences, and entropy favoring a symmetric arrangement (5,8). However, despite recent progress in the field, the physical significance of membrane asymmetry remains an active area of study. If biophysical properties differ between the two leaflets, then proteins that span the membrane may be asymmetrically affected by those differences.

Certain physiological processes have been shown to be triggered by a loss of membrane asymmetry, such as blood coagulation (128), macrophage recognition (129), or apoptosis (130), and lipid asymmetry may play a role in cancer (131) and thrombosis (132). The role of membrane asymmetry in physiological process has been reviewed further elsewhere (3,133). However, examples of membrane asymmetry directly affecting protein conformational

changes are far scarcer due to the difficulty of such measurements. The only example we are aware of where protein conformational changes have been measured as a function of membrane asymmetry is rhodopsin. Due to its covalently bound chromophore, which is sensitive to protein conformational changes, the active conformational fraction of rhodopsin can be directly measured, both in asymmetric and scrambled symmetric membranes. The retinal disk membranes (RDMs) where rhodopsin is located are naturally asymmetric, with phosphatidylserine (PS) and PE on the cytoplasmic face and phosphatidylcholine (PC) on the inside of the disks (topologically extracellular) (134–136). This asymmetry is inverted relative to that of typical plasma membranes, such as those of red blood cells (46) or other GPCR-containing cell membranes (137), because RDMs are formed by an endocytic process that reverses the membrane orientation (Fig. 5 A). Importantly, this process also flips the orientation of rhodopsin (such that it retains the same topological relationship to the membrane), making this lipid asymmetry a broader feature of GPCRs and other membrane proteins. Retinal disk membranes contain relatively little cholesterol, which results in a simpler system due to the elimination of the complex interactions it has with membrane properties and asymmetry. Despite what is expected based on the known physiological effect of membrane asymmetry, there is no significant difference in rhodopsin activation between symmetric and asymmetric membranes (Fig. 5 B, C) (138). This remains one of the few cases where the effect of membrane asymmetry on protein conformation has been directly measured, and this result warrants further consideration when constructing models of membrane asymmetry and its role in protein function.

POLYUNSATURATED LIPIDS AND CHOLESTEROL: OPPOSING INFLUENCES ON MEMBRANE PROPERTIES

Lipid composition plays a central role in regulating membrane properties, with the geometry of individual lipids determining their collective properties. Among the most influential factors determining membrane properties is the relative proportion of PUFAs and cholesterol, which together determine membrane mechanical properties, such as molecular packing and bending rigidity. As highlighted in Fig. 2 B, the concentration of PUFAs increases and the concentration of cholesterol decreases in disks further from their site of biogenesis in the cell body. The most abundant PUFA in RDMs is ω -3 docosahexaenoic acid (DHA; 22:6) (139,140), while the cholesterol content is relatively very low (141). Cholesterol increases the bending rigidity, thickens membranes, and reduces the area per lipid (7,9,142,143) by forming strong noncovalent interactions with neighboring phospholipids. Interactions between cholesterol and neighboring molecules have been suggested

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to promote the formation of raft-like nanodomains within the membrane, which have relatively high concentrations of cholesterol, sphingolipids, and certain membrane proteins (144). Notably, PUFAs have opposite effects on these properties, decreasing bending rigidity, decreasing molecular packing, and increasing the area per lipid (51,57,59,145–149). Such PUFA-containing lipids have multiple *cis* double bonds in their acyl chains, which destabilizes van der Waals interactions with neighboring lipids and induces packing defects in the bilayer (150). Because of these contradictory effects in bilayers, the presence of both cholesterol and PUFAs within a given lipid system results in a concentration-dependent competition between the two lipids over control of membrane properties (150–152).

Modulation of membrane properties by cholesterol and PUFAs has also been shown to alter transmembrane protein activity (1,36,51,150,153). Cholesterol directly modulates protein activity through both ligand-like and solvent-like interactions (154–156), and its effects on protein activity have been observed in ion channels, transporters, peptides, and GPCRs (155,157–161). Rhodopsin is a well-documented example of cholesterol affecting protein activity, where it was discovered to shift rhodopsin to the inactive state by enforcing entropic and enthalpic penalties on the active conformation (25,26). Similarly, polyunsaturated lipids regulate the activity of protein kinases, ion transporters, mechanosensitive channels, and GPCRs through altering curvature and electrostatic interactions, among other mechanisms (27–31). In contrast to cholesterol, PUFAs activate rhodopsin since their lateral pressure profile favors the expanded active state (36,35,162). Because cholesterol and PUFAs have opposite effects on rhodopsin activation, the concentration gradients of each lipid along the rod cell body are anticipated to be biologically significant. Within the retina, the ROS are oriented away from light entering the eye (163), so that the apical disks are furthest away from the actinic light. These apical disks are also most abundant in DHA and least abundant in cholesterol (164). This stark difference in concentrations could relate to rhodopsin activation, where the disks with the least exposure to light may compensate with increased activation from the lipid environment as a compensatory mechanism for the biologically necessary orientation of the rod cell. Further experimentation on how these compositional gradients affect rhodopsin function, and thus vision, may reveal new paradigms for the significance of tightly regulated lipid compositions. The opposite effects of cholesterol and PUFAs on the function of rhodopsin are extreme but illustrative examples of how certain lipids can alter protein conformational equilibria via membrane collective properties. Appreciable differences in lipid composition between membranes and their impact on protein activity are thus important areas of inquiry for understanding the biophysical regulation of membrane proteins.

EMERGENCE OF MEMBRANE ELASTICITY FROM LIPID PACKING

Biologically relevant membranes tend to be in the lamellar phase, which includes the gel and liquid-crystalline phases. It was originally thought that because cell membranes exist in these phases (165), the melting transition between them would be the most biologically relevant transition. However, it is now appreciated that lipids extracted from cell membranes tend to establish highly curved nonlamellar phases, such as the reversed hexagonal and cubic phases, suggesting the importance of curvature to biological function. This tendency is driven by the curvature free energy of the membrane and arises from a mismatch between the most favorable (or spontaneous) curvature and the actual curvature of lipid monolayers (20,138). This energy is counterbalanced by the lipid packing in such phases, requiring the acyl tails to stretch to maintain hydrophobic contact. Monolayer spontaneous curvature can thus be related to the lateral pressure profiles of the lipids—for example, the balance of attractive and repulsive forces generated by the polar headgroups and the nonpolar acyl chains. Repulsive forces generated by bulky acyl chains, together with the attractive forces from hydrogen bonding between the relatively small PE headgroups, lead to the formation of the reverse hexagonal phase. Highly unsaturated acyl tails, such as DHA found in the native membrane of rhodopsin, and small headgroups tend to favor the reverse hexagonal phase, and their existence as a lamellar phase in cells suggests a reservoir of energy that can be released upon assuming a more negative curvature at the lipid-protein interface. Studies have shown that the lamellar-to-nonlamellar phase transition is associated with function, both in microorganisms (32,33) and visual rhodopsin (19,20). In the case of rhodopsin, the curvature stress associated with nonlamellar forming lipids (referred to as frustration) provides a potent source of free energy that can drive the protein conformational changes.

Several techniques are key to our understanding of how curvature free energy depends on the membrane lipid composition. Solid-state nuclear magnetic resonance (NMR) spectroscopy and relaxation are established tools for studying structural and dynamic properties of membrane lipids associated with elasticity (142,166–174). These two types of NMR experiments are complementary, with spectra providing information about equilibrium properties such as interfacial area per lipid and phase transition energetics, and relaxation experiments providing information about dynamic properties such as bending rigidity. Lipid packing is described by the area per lipid and is calculated using the mean-torque model (175). Bending rigidity is directly related to lipid packing, and both are influenced by lipid unsaturation and the presence of sterols, such as cholesterol (9). Scattering-based methods—neutron (176) and X-ray scattering—are complementary to NMR spectroscopy and can measure structural properties such as the acyl chain length

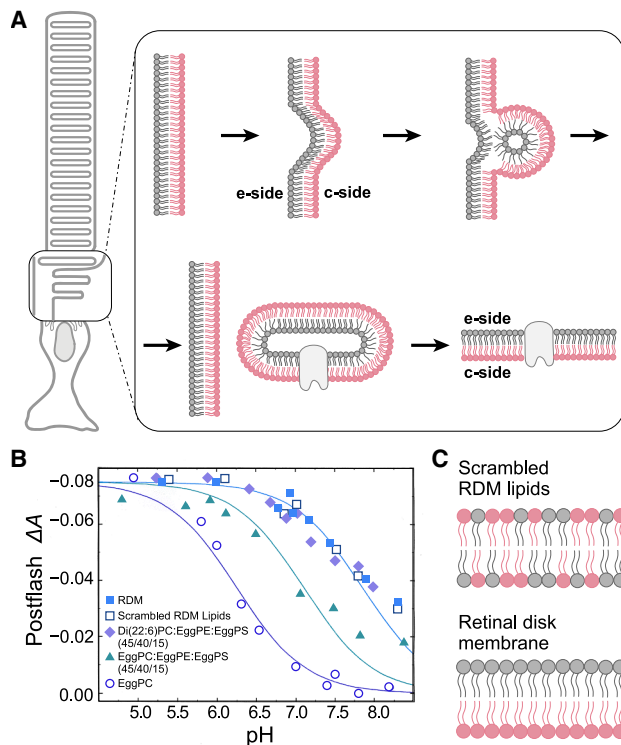


FIGURE 5 Origin of RDM asymmetry and its effects on rhodopsin activation. (A) The asymmetry of both the lipids and rhodopsin in RDMs is inverted from that of a typical cell due to their biogenesis via endocytosis. As such, rhodopsin maintains the same topological relationship to the membrane as other GPCRs. Pink represents the cytoplasmic facing side (c-side), while gray represents the topologically extracellular side (e-side). (B) Postflash change in absorbance (measured by flash photolysis at $\lambda = 478$ nm) as a function of pH, where rightward-shifted curves indicate higher rhodopsin activation. In both its asymmetric native RDM and in symmetric scrambled RDM lipids rhodopsin has similar photochemical activity, suggesting that leaflet asymmetry does not directly modulate protein function. (C) Illustration of how scrambling the RDM abolishes membrane asymmetry. (B) Adapted with permission from Brown (88). Copyright 1997, Academic Press. Published by Elsevier.

and interfacial area per lipid. The theory of X-ray scattering in the context of membrane elastic properties is reviewed in greater detail elsewhere (177–180). Recent progress in neutron scattering has involved neutron spin-echo spectroscopy (7,181–183), which is further enhanced by complementary molecular dynamics (MD) simulations (7,9,184). Combining these three methods (NMR, neutron scattering, and MD) delivers a more robust and unified approach. Just as in the case of NMR, the area per lipid found by scattering relates to both molecular packing and elasticity.

From the combination of these techniques, the picture that emerges is that lipid packing is the dominant factor affecting bilayer elasticity (7,9). More tightly packed lipids resulting from smaller headgroups such as PE give more rigid bilayers, while larger head groups such as PC (which includes additional methyl groups) lead to a reduction in bending energy (64). The energy required to bend the membrane is then a combination of the bending rigidity and how far the bilayer

is deformed from the spontaneous curvature. Native RDM lipids present an extreme example of this, containing both bulky PUFA chains and small PE headgroups. Such membranes exhibit a highly negative spontaneous curvature, resulting in a decreased bending energy penalty for the active state of rhodopsin (20,36,52,87,185,186). Cholesterol has the opposite effect, leading to tighter packing and greater bending rigidity, penalizing the formation of negative curvature around active rhodopsin (9,26,187,188). Experiments thus suggest that lipids influence mechanical properties emergent on short length scales, potentially creating micro- or nanodomains (7,189), as well as larger cellular scales (7,189) relevant to their role in curvature homeostasis (10,11,190).

ELASTIC DEFORMATION AS A REGULATOR OF PROTEIN ACTIVITY

Membrane curvature is a broad term describing collective motions within the bilayer, encompassing twist, splay, and bend deformations, that arise from asymmetric lateral pressures between the lipids, which can be approximated as a single elastic constant (75) (Fig. 6 A). Elasticity is closely connected with cell function, especially processes involving shape deformation or force production, such as endocytosis, cell adhesion, and trafficking (191–193). One of the best-studied systems are red blood cells, with mechanical properties arising from the interplay of cytoplasmic membranes with a spectrin-based cytoskeleton (194). The bending elasticity of the membrane also influences the activity of embedded proteins (86), where local bending energy (also referred to as curvature stress) favors the formation of specific protein conformations (Fig. 6 B). Membrane curvature stress is described by continuum models primarily based on the classical work by Canham (196) and Helfrich (197). The Helfrich model assumes the membrane as a two-dimensional elastic sheet without molecular details, with a principal curvature for each of the two axes $c_1 = 1/r_1$ and $c_2 = 1/r_2$ for the radii of curvature r_1 and r_2 , respectively. The free energy g_c required to deform the bilayer from its spontaneous curvature is then given by

$$g_c = \kappa_c(H - H_0)^2 + \bar{\kappa}K. \quad (1)$$

Here, κ_c is the bending rigidity of the bilayer, H is the average of the two principal curvatures of the membrane surface as given by $H = (1/2)(c_1 + c_2)$, and H_0 is the spontaneous curvature (196). The spontaneous curvature of a mixture is the weighted sum of the concentrations of the components of the mixture $H_0 = X_1H_0^{(1)} + X_2H_0^{(2)}$ (20,36,52). The second term of Equation 1 is the product of the Gaussian curvature modulus $\bar{\kappa}$ and the Gaussian curvature K . Together c_1 and c_2 characterize the mean curvature of the bilayer, with larger radii r_1 and r_2 corresponding to a less curved bilayer. The Gaussian curvature K is a product of

the two principal curvatures c_1 and c_2 and describes the membrane topology. If the membrane topology is fixed, the product $\bar{\kappa}K$ remains constant. In the case of a symmetric bilayer, the energy required to bend (deform) the membrane is primarily characterized by the bending modulus κ_c of the bilayer. The bending modulus κ_c remains the most readily quantifiable property describing membrane elasticity due to being accessible in spectroscopic and scattering experiments of the pure lipid systems, where topology of the membrane is assumed to be fixed. However, lipid bilayers are two-dimensional fluids that undergo bending fluctuations, so the Gaussian terms in Equation 1 also become important. There has been progress recently in determining $\bar{\kappa}$ computationally (198), but accessing these values experimentally remains a subject of intense research. Continuum models and modifications to the original Helfrich-Canham equations with respect to membrane protein interactions are reviewed elsewhere (199,200).

Although bending elasticity and curvature are proposed to be important mechanical properties, conventional models for membrane behavior tend to overlook the role of curvature stress in favor of other effects such as fluidity, lipid rafts, biomolecular condensates, and lipid mixtures having liquid-liquid phase transitions (201,202). To explain the interplay between lipid composition, bending energy, and protein activity, we first proposed the FSM in the 1980s to incorporate the effects of principal curvatures within the bilayer system (19). Under the FSM, curvature is expressed as an elastic force field based on the Helfrich formulation of bending energy (197), which is applied to lipid-protein interactions that occur both in-plane and out-of-plane (24,86). The most foundational concept of this model is that spontaneous curvature is an intrinsic property of the lipid bilayer. Curvature energy is then described in terms of the displacement of the mean membrane curvature from the spontaneous curvature, in addition to the effect of topology deformation represented by the Gaussian curvature (Equation 1) (86). In Fig. 6 C, data are shown for lipid recombinants of rhodopsin varying in the fraction of PE headgroups and polyunsaturated acyl tails, yielding different spontaneous curvatures. Plotting the metarhodopsin equilibrium constant ($\ln K$) against the inverse radius of spontaneous curvature ($R_0 \equiv 1/H_0$) yields an approximately linear relation, consistent with negative curvature at the proteolipid boundary, in striking agreement with the FSM. Unlike the more conventional FMM, the FSM approaches the mesoscopic collective motions of the membrane lipids from a material science perspective. Through application of physics to the archetypal membrane protein rhodopsin, the importance of curvature and elasticity as regulators of protein and membrane function can be understood.

It has been proposed that cellular life has evolved to operate under a certain level of curvature stress, along with mechanisms to change or preserve the curvature stress under

various conditions, a process called curvature homeostasis (homeocurvature adaptation). Curvature stress does not necessarily imply physical curvature of the membrane, as even planar bilayers can be placed under stress without bending. This was originally shown in microorganisms (32,33) and in RDM containing rhodopsin (10,11), where the spontaneous curvature tends to remain the same regardless of the fatty acids provided. Moreover, flash photolysis experiments showed that the rhodopsin activation equilibrium is reversibly modulated in various lipid recombinants (Fig. 7 A), and that both the small PE headgroups and polyunsaturated acyl tails found in native RDMs are required for full rhodopsin activation (Fig. 7 B). These findings suggest that spontaneous curvature is an important regulated property that may explain the strong lipid-protein interactions observed in recombinant membrane systems. By exerting control over the composition of membrane lipids, such as head group size, acyl chain length, and unsaturation, the cell can maintain a functional level of membrane curvature stress (Fig. 7 C). Consequentially, protein activity is regulated as composition-induced curvature stress adjusts the energy landscape of proteins and favors specific conformations. Curvature stress has been found to regulate several membrane proteins, such as PC biosynthetic enzyme CTP:phosphocholine cytidyltransferase (203) and protein kinase C (204), along with peptides such as gramicidin A (43,205) and the antibiotic ion channel alamethicin (206). This concept is illustrated most clearly by rhodopsin, whose active conformation is favored by negative spontaneous monolayer curvature (35). Lipids with small headgroups, such as PE, shift the conformational equilibrium toward the active state. Similarly, PUFAs such as DHA also exhibit negative spontaneous curvature and promote rhodopsin activation (36,162). The indicated link between composition, curvature, and conformation is made possible by the favorable properties of rhodopsin, namely its native chromophore retinal, which is highly sensitive to conformational changes in the protein.

CURVATURE STRESS BIASES THE RHODOPSIN ENERGY LANDSCAPE

With the FSM as a conceptual framework and rhodopsin as a model system, we can investigate the direct contributions of curvature to protein conformational energetics. Among the unique advantages of the rhodopsin system is that we can directly measure the fraction of active rhodopsin in real time using flash photolysis based on ultraviolet-visible (UV-vis) spectroscopy. Reconstituting rhodopsin into synthetic bilayers allows for control over lipid composition and investigation into the impact of head group size, polyunsaturation, and acyl chain length on GPCR energetics. After the chromophore is exposed to light, isomerization triggers a series of conformational changes leading up to formation of the active conformation metarhodopsin II. Each state

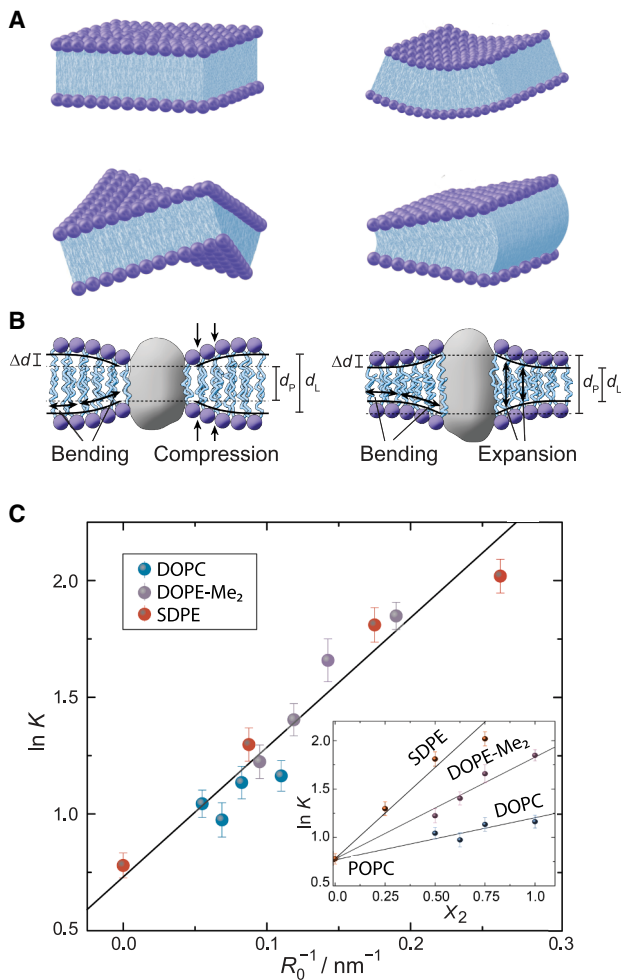


FIGURE 6 Flexible surface model explains balance of curvature and hydrophobic forces in membrane function. (A) Membrane curvature describes collective lipid motions within the bilayer—twist, splay, and bend deformations—that arise from asymmetric lateral pressures between the lipids (86). (Top, from left to right) Planar bilayer and splay deformation. (Bottom, from left to right) Twist and bend deformations. (B) The flexible surface model (FSM) predicts lipid-protein interactions are governed by a balance of forces between the lipid headgroups and acyl tails, which are influenced by the headgroup size, acyl chain length, and degree of unsaturation. Hydrophobic coupling of lipids causes local compression or expansion of the bilayer adjacent to the protein interface (d_p = protein hydrophobic thickness; d_L = mean lipid bilayer thickness; $\Delta d \equiv d_L - d_p$ or hydrophobic mismatch). Positive spontaneous curvature favors less extended protein conformations (left), and negative spontaneous curvature favors conformations that protrude from the lipid membrane (right). (C) Natural logarithm of the rhodopsin equilibrium constant ($\ln K$) in recombinant membrane systems versus the inverse radius of curvature (R_0^{-1}) explains how negative spontaneous curvature is correlated with increased activation. The inverse radius of curvature is related to the spontaneous curvature of the lipid mixture. (Inset) Graph of $\ln K$ against mole fraction (X_2) of DOPC, DOPE, DOPE, DOPE-Me₂, or SDPE lipids in POPC bilayers (see (185) for definitions), showing that rhodopsin activation depends on the average spontaneous curvature of the mixture (20,36,52). (A) Adapted with permission from Doole et al. (143). Copyright 2022, Springer Science Business Media. (B) Adapted with permission from Brown (38). Copyright 2012, American Chemical Society. (C) Adapted including error bars with permission from Brown et al. (195). Copyright 2017, Springer Nature Singapore.

forms its own basin of attraction in the rhodopsin energy landscape, with the energy associated with each state known to depend on the environment (207,208), as shown in Fig. 8. From work done with rhodopsin, we propose that curvature stress arising from the lipid composition impacts the energetics of protein conformations (36,88).

RHODOPSIN REVEALS MEMBRANE CURVATURE HOMEOSTASIS AS A UNIFYING PRINCIPLE

Current trends in biophysics and lipidomics have been pivotal for uncovering the role of lipid composition within any given biomembrane (1,15,51,209,210). Despite these recent technological advances, lipidomics research alone cannot explain the link between chemical composition and protein function. Membrane biophysics bridges this gap by examining how cells maintain the homeostasis of key material properties, particularly membrane curvature, which has become a central focus of the field (20,35,38,52,185,186,188,211). However, separating the individual contributions of these material properties remains challenging. At the molecular scale, lipid packing is physically related to the interfacial area per lipid, measurable by techniques such as NMR or scattering. The bending rigidity (elasticity) comes into play when one simplifies away molecular detail from a leaflet of the membrane, approximating it as an elastic sheet. However, the bending rigidity is still related to the underlying structure of the lipids, as the primary factor determining the bending rigidity is the lipid packing, with more tightly packed lipids resulting in a stiffer membrane (9,212). Building on this, curvature stress describes the energy required to bend a monolayer away from its spontaneous (preferred) curvature, which also depends on the molecular geometry of the lipids that comprise it. Although lipid composition determines the spontaneous curvature, the actual curvature adopted by a membrane also reflects additional energetic contributions, such as hydrophobic matching—driven by proteolipid solvation energy—and electrostatic forces.

In this regard, the concentrations of PUFAs and cholesterol are key factors for regulating membrane properties, exerting opposing effects on spontaneous curvature and bending rigidity and allowing cells to tune membrane behavior through adaptive responses. Nevertheless, curvature stress is a universal feature of lipid bilayers; any membrane will experience stress when its actual curvature differs from the spontaneous curvature dictated by its lipidome. Asymmetry further alters this picture, as it considers that the opposing leaflet of the membrane may possess distinct physical properties that are determined by its own unique lipid composition. Differences in the amounts and types of lipids between leaflets create packing mismatches that place one side under compression and the other under tension, a physical imbalance that promotes membrane bending, altering the spontaneous curvature and bending rigidity of

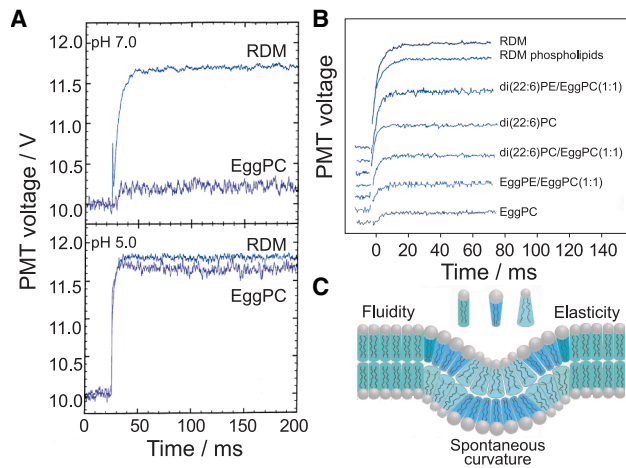


FIGURE 7 Phosphatidylethanolamine (PE) headgroups and polyunsaturated ω -3 docosahexaenoic acid (DHA) chains are sufficient for native-like rhodopsin activation equilibrium. (A) Flash photolysis transients at $\lambda = 478$ nm demonstrating that the activation equilibrium of rhodopsin is reversible under different environmental conditions. Rhodopsin is inactive in EggPC lipid recombinants at pH 7, whereas at alkaline pH, rhodopsin is almost fully active in both RDM and EggPC lipid recombinants. (B) Flash photolysis transients of rhodopsin lipid recombinants show the additive effects of unsaturated DHA chains and small PE headgroups on photochemical activity. Note that DHA chains or PE headgroups alone only result in partial rhodopsin activation, with increasing concentrations of DHA chains further increasing activity. Combining both PE headgroups and DHA chains adds their effects, resulting in the highest activity among recombinant membranes. (C) Illustration showing that headgroup composition and polyunsaturated acyl chains alter the average molecular shape of lipids and membrane curvature free energy. (A) and (B) Adapted with permission from Brown (88). Copyright 1997 Academic Press. Published by Elsevier.

the bilayer (3,5,8). The substantial energetic investment required to maintain membrane asymmetry underscores its importance for membrane protein function, yet not all aspects of asymmetry have a simple physical explanation, and its functional significance remains an active area of study.

Whether lipid-protein interactions were evolutionarily selected to be specific or instead arise from bulk lipid effects is still being debated (10,138,213,214), and visual rhodopsin has been central to efforts to resolve this question (10,138,213,214). Polar head groups and polyunsaturated chains have both been found to affect the activation equilibrium of rhodopsin, triggering visual signal amplification (20,186). In particular, the compositional abundance of PUFAs within the retina (136,139,140) and brain (215,216) suggests evolutionary selection for bulk lipid effects, since only a low concentration would be required if specific interactions were the sole mechanism (136,139,140). Pioneering lipidomics studies of rhodopsin (10) have been subsequently extended to the membrane level through biophysical work investigating head groups, electrostatic charge, acyl tail length, and curvature elasticity (88). The effects of head groups and acyl chains have been formulated in terms of elastic bilayer deformations through the FSM, which is an

alternative to other models (52). The interactions between rhodopsin and its lipid environment, as interpreted under the FSM, highlight the role of curvature stress in regulating protein activation. As such, we propose that lipid composition is maintained through curvature homeostasis to ensure a functional level of curvature stress (211).

We consider visual rhodopsin to be the hydrogen atom of membrane biophysics in recognition of its current and historical significance as a model membrane protein. Following the creative lead of Hans Frauenfelder (217), just as molecular structures are based on the atomic orbitals derived from the hydrogen atom, the foundational concepts of membrane biochemistry rely on pioneering studies of rhodopsin. It is highly abundant in retinal rod disk membranes, with a specific lipidome required for visual signal amplification. The covalently bound retinal chromophore allows rhodopsin to be studied with electronic spectroscopy (UV-vis) and to respond directly to light, distinguishing it from most other GPCRs. The ability to align retinal rods containing rhodopsin in a magnetic field allows its rotational and translation diffusion to be studied in the native membrane. Extending early lipidomic studies to recombinant lipid membranes containing rhodopsin has provided insights into the ongoing debate over whether protein function is governed primarily by chemically specific lipid-protein interactions or by broader biophysical membrane properties. Such studies have begun to illuminate the concept of curvature stress as a characteristic property that has undergone natural selection over the course of evolution. The current hypothesis is that it is maintained through homeocurvature adaptation (curvature homeostasis), which is distinct from the previously proposed homeoviscous adaptation (218–223). We have described how the specific retinal disk lipidome is related to two-way interactions involving rhodopsin and the retinal lipids in recombinant membrane systems based on *in vitro* work (88). Collective membrane properties arising from the lipid composition, native or otherwise, can control rhodopsin conformation and signaling, showing its importance for modeling general biophysical principles. Broader applicability of the insights obtained with visual rhodopsin includes photoactive caged ligands for other GPCRs, and because the rod cell is a neuronal cell, there is potential for future applications to optogenetics (224–227). Rhodopsin-lipid interactions involving effects on protein activity from altered elasticity can shed light on retinal disease pathology (213,228–232) and may underlie lipid metabolic disorders associated with essential fatty acid deficiency that merit future investigation.

Going forward, the role of curvature stress, as exemplified by visual rhodopsin, appears to be a unifying biochemical paradigm. Future efforts can investigate the extent to which curvature matching and other energetic contributions (hydrophobic matching, electrostatics, and specific molecular interactions) act on protein activity. Curvature stress as a membrane organizing principle warrants further investigation

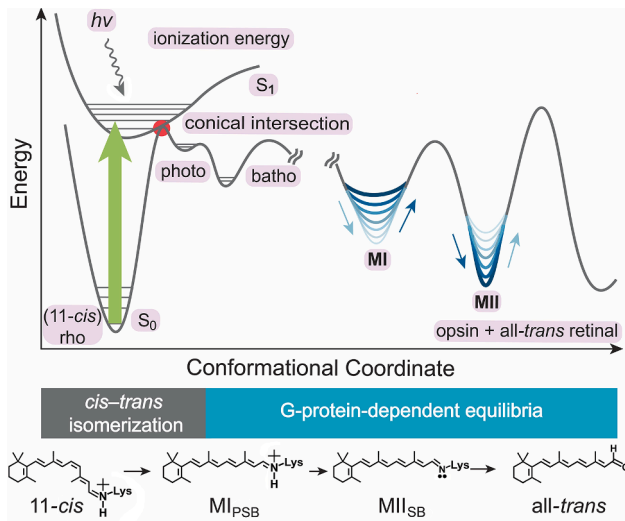


FIGURE 8 Energy landscape model describes how membrane lipids influence rhodopsin activation equilibrium. Upon absorption of a photon ($h\nu$), the retinal in rhodopsin isomerizes from 11-*cis* to all-*trans*, which is accompanied by excitation from the ground state (S_0) into an excited state (S_1). Following vertical excitation and vibrational relaxation, the excited state decays through a conical intersection (red) of the excited state potential surface and ground state potential energy surface. Rhodopsin proceeds through a series of intermediates, including bathorhodopsin (batho) and photorhodopsin (photo), before reaching an equilibrium between the preactive MI and active MII states. (Dark gray line) Energy landscape of rhodopsin as it appears in its native disk membranes. (Blue lines) Energy landscape of rhodopsin in recombinant lipid membranes, where darker lines indicate stabilization toward the active MII conformational state. Lipids with small headgroups or acyl tails with a high degree of unsaturation or greater chain length favor active MII and disfavor the preactive MI state.

for universality and comparison to electrostatics or hydration forces in globular protein function. Just as the development of an organism mirrors its evolutionary history, one can propose that the metabolic pathways for PUFAs and sterols reflect the evolutionary tree of these lipids, hinting at the specific lipid properties that were under selective pressure. By relating lipid composition to biological fitness, the role of curvature free energy can explain the effects of evolved sterols (lanosterol and cholesterol) (233) and adaptation to environmental variables (9–11,208,211,218). It is likely that lipid metabolic pathways have evolved to generate the biophysical properties needed for membrane function in the case of essential fatty acids and sterols. Often, these lipid pathways produce compensatory effects, thus conspiring in biomembranes to maintain homeostasis and establish life's rich diversity. How these foundational concepts of biophysics underlie current lipidomics research offers new insights to understanding the significance of evolved lipid compositions in cell biology.

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AUTHOR CONTRIBUTIONS

All authors wrote and edited the manuscript with discussion, review, and input from all contributors.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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