The fine-tuning of cell membrane lipid bilayers accentuates their compositional complexity

Tamir Dingjan | Anthony H. Futerman

Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot, Israel

Correspondence
Anthony H. Futerman, Department of Biomolecular Sciences, Weizmann Institute of Science, Rehovot 7610001, Israel. Email: tony.futerman@weizmann.ac.il

This essay is dedicated to the memory of Dr. Richard E. Pagano (1944–2010) who first taught me (AHF) to appreciate the beauty of cell membrane lipid bilayers.

Abstract
Cell membranes are now emerging as finely tuned molecular systems, signifying that re-evaluation of our understanding of their structure is essential. Although the idea that cell membrane lipid bilayers do little more than give shape and form to cells and limit diffusion between cells and their environment is totally passé, the structural, compositional, and functional complexity of lipid bilayers often catches cell and molecular biologists by surprise. Models of lipid bilayer structure have developed considerably since the heyday of the fluid mosaic model, principally by the discovery of the restricted diffusion of membrane proteins and lipids within the plane of the bilayer. In reviewing this field, we now suggest that further refinement of current models is necessary and propose that describing lipid bilayers as “finely-tuned molecular assemblies” best portrays their complexity and function. Also see the video abstract here: https://www.youtube.com/watch?v=ddkP-QRZTI8

KEYWORDS
bilayer, cell membrane, fine-tuning, lipid complexity, molecular assembly

INTRODUCTION
Fine-tuning in cosmology and physics is the idea that certain parameters of the universe must occur within very stringent limits in order to support life (Figure 1).[1] These parameters include the sensitive dependencies of the values of certain physical parameters, conditions in the early universe, and the physical laws, all of which are fine-tuned to a remarkable degree. Among the pioneers in developing the concept of fine-tuning were Drs. John D. Barrow and Freeman Dyson, both of whom recently passed away. Barrow made many contributions to the fields of matter and antimatter, black holes, dark matter, and the origin of galaxies, although he is arguably best known for his book “The Anthropic Cosmological Principle.”[2] Indeed, an obituary to Barrow in Scientific American stated that “a truly great scientist not only makes significant technical contributions but also reshapes a discipline’s conceptual landscape.”[3]

In the current essay, we propose that the time is ripe for a shift in the conceptual landscape concerning lipid bilayers in cell membranes. We will suggest that current models of lipid bilayer structure and function all fall far short inasmuch as they do not take into account the unexpected compositional complexity of the lipid (and protein) constituents of membranes (Figure 2). We will propose that lipid bilayers can best be described as “finely-tuned molecular assemblies.” To support this suggestion, we will consider three properties of lipid bilayers that appear to fulfill the criteria of fine-tuning, namely their composition, the distribution of lipids within and across bilayers, and the specific interactions of membrane lipids with membrane proteins.

Abbreviations: CoA, coenzyme A; ER, endoplasmic reticulum; ESI-MS/MS, electrospray ionization tandem mass spectrometry; GPCR, G protein-coupled receptor; GSLs, glycosphingolipids; MALDI, matrix-assisted laser desorption/ionization; MD, molecular dynamics; MS, mass spectrometry; PA, phosphatidic acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PM, plasma membrane; PS, phosphatidylserine; SM, sphingomyelin; TGN, trans-Golgi network; TLC, thin layer chromatography; TMD, transmembrane domain

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Higher tolerance

Membrane lipid composition

Bilayer distribution

Lipid-protein interactions

Lower tolerance

Cosmological

Physical sciences

Biological sciences

FIGURE 1 Tolerance and fine-tuning in the physical and biological sciences. Cosmology has unearthed extraordinarily low tolerance for the fundamental physical constants. It is much more difficult to obtain precise quantification for values that might support the concept of fine-tuning in the physical sciences, due to the multiple interactions (specifically non-covalent interactions) that sustain complex biological systems, and due to the limitations of currently available experimental tools to measure an appropriate readout. Biological systems, such as membrane lipid bilayers, likely show a range of tolerances that are less sensitive to change than the numerical values quantified in cosmology and physics. The best example of fine-tuning with respect to lipid bilayers is likely to be the specific, Ångström-resolution interactions between lipids and the transmembrane domains of proteins, while the other two parameters discussed herein may display a somewhat higher tolerance to change.

Before describing these properties in more detail, a brief overview of the history of lipid bilayers may be helpful to set the scene. The first evidence that cells were surrounded by a "plasma membrane" was obtained by Pfeffer, who demonstrated that this membrane acted as a barrier to the passage of water and of solutes. A few years later, in 1899, Overton observed that membrane permeability of solutes was determined by their polarity, and thus suggested that membranes are a solid "oil-like" barrier with a non-polar interior. Next, in 1925, Gorter and Grendel suggested that lipids are arranged in a "bilayer." A scheme of the molecular organization of lipid bilayers was proposed by Davson and Danielli in 1935, who suggested that proteins coat the surface of the lipid bilayer, in what was known as the "unit membrane" hypothesis. Robertson appeared to confirm this hypothesis in 1959 by electron microscopy when he detected two electron dense bands (suggested to be the proteins) separated by an electron lucent band (the lipids). It was only in the 1960s and 1970s that the currently accepted model of lipid bilayers was formulated by Singer and Nicolson, whose "mosaic model" was later refined together with Nicolson, to become the "fluid mosaic model" in which "the hydrophobic fatty acyl chains of the lipids and a large fraction of the nonpolar amino acid residues of the integral proteins are sequestered in the membrane interior," while "the ionic and saccharide groups of the lipids are in direct contact with water." Critically, membrane lipids and proteins were free to diffuse in the plane of the lipid bilayer (hence the "fluid" mosaic model; Figure 3), implying that the tertiary structure and precise composition of the lipid bilayer was ultimately of little importance, as long as the bilayer maintained sufficient fluidity to permit diffusion. The fluid mosaic model was a true shift in the conceptual landscape of understanding the structure and function of lipid bilayers.

While the structural basis of the fluid mosaic model remains essentially valid nearly 50 years later, it has undergone substantial evolution in the past couple of decades principally due to the discovery of regions of lateral heterogeneity within the plane of the lipid bilayer, leading to revision of the notion of free diffusion of membrane components. Although lateral heterogeneity has been known since the 1970s, when such domains were observed in liposomes (artificial lipid bilayers) made of two or more different lipids, the extent and range of lateral heterogeneity and interactions between specific lipids and proteins has only been fully appreciated in the past few years (Figure 3).

Another crucial finding that needs to be taken into account is the discovery that the composition of membrane lipids is much more complex than once thought. This has come about mainly from technical advances in detecting and quantifying lipids. Lipidomics (herein described as "systems-level identification and quantitation of thousands of pathways and networks of cellular lipids, molecular species and their interactions with other lipids, proteins and other moieties in vivo") has revealed an enormous combinatorial complexity of lipid species: the LIPID MAPS Structure Database (https://www.lipidmaps.org; accessed January 18, 2021) documents 45,045 individual chemical structures and the LIPID MAPS In-Silico Structure Database suggests that >1,100,000 potential lipid structures may exist in nature. Clearly, not all of these lipids are found in lipid bilayers, although 8,000 lipid species have been identified, for instance, in whole human platelets, and up to 400 in isolated plasma membranes (PMs). with these numbers likely to substantially increase as the sensitivity of the mass spectrometry (MS) techniques used to analyze the lipidome increases. Do each of these individual lipid species have a specific function, or is it the collective properties of the sum of the lipids that determines the biochemical and biophysical properties of lipid bilayers, and if so, what functional constraints are determined by lipid composition and by changes in lipid composition?
FIGURE 2  Combinatorial complexity of lipids in cell membranes and the distribution of lipids with different levels of saturation across the two halves of the bilayer. (A) Eukaryotic membranes consist of three major lipid classes, namely glycerolipids, sphingolipids, and sterols. The structural complexity of the first two classes is indicated by the range of headgroups (purple), backbones (blue), and acyl chains (orange) that combine to yield the cellular lipidome. The head groups normally found in sphingolipid structures are indicated by a dashed line. Two examples of typical lipid structures are shown on the right (boxes), where SM (N-stearoyl-D-erythro-sphingosylphosphorylcholine) consists of a d18:1 sphingoid long-chain base backbone to which a C18:0 fatty acid is N-acylated, and PS (1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine), which consists of a glycerol backbone to which two different fatty acids are esterified, one of which is saturated (16:0) and one unsaturated (18:1). (B) The distribution of the number of fatty acid unsaturations across the inner and outer leaflets of the erythrocyte plasma membrane, by lipid type. Coloring corresponds to lipid head group colors in Figure 3. The number of unsaturations clearly differs between the outer and inner leaflets. Adapted from [17]

Moreover, although “lipid asymmetry” has been known since the 1970s,[20] it is now apparent that the leaflets of the lipid bilayer vary much more significantly than previously thought, with each half having a completely different lipid composition,[17] such that each leaflet displays distinctive rates and extents of fluidity. Thus, membrane properties are much more dependent on their lipid composition than once thought and lipid bilayers are far more than the sum of their individual parts. Whether it is appropriate to describe them as “finely-tuned molecular assemblies” (Figure 3; see also Box 1) is the subject of the detailed discussion below.
MEMBRANE LIPID BILAYER COMPOSITION EXHIBITS PROPERTIES OF FINE TUNING

Membranes are critical in all kingdoms of life but the focus of the subsequent discussion will be on eukaryotic cell membranes and on their three major lipid classes, namely glycerolipids, sphingolipids, and sterols (Figure 2). Membranes of course also contain many proteins with recent estimates suggesting that about 25% (~5000 proteins) of the human genome encodes membrane proteins. Later, we will discuss how the transmembrane domains (TMDs) of these proteins perfectly match, in terms of hydrophobic length and helical surface area, the asymmetric distribution of lipids on each side of the bilayer.

What are the main lipid classes found in cellular membranes?

Although different cellular and intracellular membranes vary in their lipid composition, an approximation of the distribution of the three lipid classes in cell membranes is that glycerophospholipids comprise ~50%, sphingolipids comprise ~30%, and sterols ~20%. All of these lipids are amphipathic, that is, they contain a hydrophilic head group in direct contact with water or other polar moieties, and a hydrophobic core that forms the “oil-like” interior first suggested by Overton. While each lipid class follows this general rule, they differ widely in their chemical composition (Figure 2). Thus, glycerolipids are based on a glycerol backbone to which various head groups and fatty acyl chains are attached, sphingolipids contain a sphingoid long-chain base (which itself is generated from a fatty acyl coenzyme A [CoA]) to which a fatty acid is N-acylated, and sterols consist of an almost completely saturated tetracyclic hydrocarbon scaffold typically bearing a single polar hydroxyl head group. A number of other lipids are not associated with cellular membranes (e.g., triacylglycerols found mainly in lipid droplets); herein we only discuss lipids that reside in membrane lipid bilayers.

These three major membrane lipid classes have been known for many decades. However, it is only in the past couple of decades that lipid biochemists have moved on from the classical analytical
Box 1. Biophysical and biochemical characteristics of plasma membrane (PM) lipid bilayers that reflect their fine-tuning. Each characteristic listed below is referred to in the text and in Figure 3

a. Distinct lipid composition of the PM and of intracellular membranes.
b. Lateral heterogeneity of lipid species within the plane of the bilayer, resulting in domains enriched in specific lipids, often cholesterol and sphingolipids.
c. Tighter packing of lipids in lateral domains than in the surrounding regions of the bilayer.
d. Lipids in domains contain longer acyl chains than in the surrounding bilayer, resulting in a greater hydrophobic width.
e. Asymmetric distribution of lipid classes between bilayer leaflets.
f. Asymmetric distribution of lipid acyl chain unsaturation between bilayer leaflets.
g. Asymmetric lipid packing between bilayer leaflets, resulting in a less fluid, more tightly packed outer leaflet, and a fluid, looser packed inner leaflet.
h. Protein helices spanning the bilayer are asymmetric in their surface area, matching the difference in lipid packing between leaflets.
i. The width of transmembrane domains closely matches the local lipid environment.
j. Some membrane proteins bind specifically to individual lipid species.

How many structurally distinct lipid species are found in a typical eukaryotic cell membrane bilayer?

This question, which may appear trivial to a non-lipid aficionado, is actually not that easy to address because of difficulties in isolating pure cellular membranes. In contrast, there is significant data available on whole cell lipidomes, which include lipids in membrane bilayers but also in intracellular stores. Whole cell lipidomes display major differences between tissues,[29] cell types,[30] and between individuals.[15] the former presumably reflects functional differences between different cell types in specific tissues whereas the latter reflects person-to-person differences, comparable to that known in, for instance, gene expression and single nucleotide polymorphisms[34] between individuals. Thus, between two individuals, approximately 84% of the platelet whole cell lipidome is common.[15]

In terms of compositional differences between different cell types, cells in the brain are perhaps the best example. Lipid composition differs between myelin, axonal membranes, and white matter,[35] the levels and types of gangliosides (acidic GSLs that contain sialic acid) differ between neurons and astrocytes,[36] and neurons and glia differ with respect to their ceramide composition (the dominant ceramide fatty acid in neurons is C18, while in glia it is C24).[37] Finally, the cellular lipidome is dynamic inasmuch as it changes during development and also in response to external stimuli, such that lipids, including but not limited to the phosphoinositides and sphingolipids, rapidly turnover in signaling pathways, both to generate second messengers and also to modify the biophysical properties of the membrane. This means that lipidomic analysis of a tissue or of a specific cell type may vary widely depending on the physiological state of the cell at the time of the analysis. Studies over the past decade or so using isolated PMs (which are easier to isolate than intracellular membranes) suggest between 70 and 400 unique lipid species,[16–18,38–40] with the number increasing as the resolution of MS machines increases. Of these studies, the lipid composition of the erythrocyte PM is...
perhaps of most use in illustrating the number of specific lipid species in any one bilayer, where ~400 unique lipid species have been detected.[17]

How tolerant are cellular bilayers to change with respect to their lipid composition?

While these numbers help determine the complexity of a typical PM lipid bilayer, they do not address the tolerance of any particular membrane, or of intracellular membranes, to changes in their lipid composition. However, analysis of lipid composition along the secretory pathway reveals that lipid composition changes from the endoplasmic reticulum (ER) to the Golgi apparatus to the PM, and even to the lysosome, and that the composition of these intracellular organelles is maintained in multiple cell types in multiple species; presumably this reflects functional differences between these different intracellular membranes.[43] Thus, in yeast, the sphingolipid and sterol content is 27 mole percent in the trans-Golgi network (TGN), while secretory vesicles budding off from the TGN contain 53 mole percent of these lipids and the PM 82 mole percent.[42] The membranes of the secretory pathway also differ in other parameters: PM bilayers are thicker than the Golgi apparatus[43] and display tighter lipid packing and more negative charge.[44] Indeed, differences in membrane composition along the secretory pathway play a critical role in the sorting of proteins to different membrane compartments (Box 1a).[45]

Changes in intracellular lipid composition between intracellular compartments are not limited to the secretory pathway. The inner mitochondrial membrane contains a novel lipid that is not found in any other organelle, namely cardiolipin, which consists of two phosphatidic acid (PA) moieties connected via a glycerol backbone, such that a single cardiolipin contains up to four fatty acyl chains. More than 150 different cardiolipin species have been profiled (with 1,307 cardiolipin structures listed by LIPID MAPS),[46] which vary in the number of acyl chains, the acyl chain length, and the degree of unsaturation. Moreover, a number of aspects of mitochondrial function, such as the exchange rate of the ATP/ADP carrier[47] or the function of respiratory chain complex IV,[48] show extremely low tolerance to changes in cardiolipin composition, and mitochondria from different tissues have a different cardiolipin composition, which reflects the different functions of the tissues. For instance, muscle mitochondria have a two-fold higher concentration of cardiolipin than liver mitochondria and possess more unsaturated cardiolipin species, bequeathing a higher capacity of oxidative phosphorylation.[49]

While analysis of lipid composition by MS depends upon the biochemical isolation of a specific membrane (although, as stated above, this will change in the coming years as new generations of MALDI machines provide better spatial resolution), the use of non-invasive biophysical tools, such as fluorescent lipid analogs and fluorescent probes, has revealed significant information about how changes in lipid composition affect bilayer properties. Thus, within the limits of resolution of the techniques used in biophysics, current data is consistent with the idea that relatively small changes in lipid composition can have a dramatic effect on bilayer properties, not least on the regions of nanoscale lateral heterogeneity,[50] found within the plane of lipid bilayers, colloquially known as "lipid rafts" or "lipid microdomains" (Box 1b).[51,52]

The bilayer composition affects lateral heterogeneity

Lateral domains first came to prominence with observations that phase separation could be observed in liposomes composed of sphingolipids and cholesterol,[53] where “clusters” of neutral GSLs were observed in phospholipid bilayers and in mammalian cell membranes,[54] moreover, upon addition of cholesterol, multiple phases were detected in liposomes containing glycerophospholipids and sphingomyelins (SMs).[55] Since then, this concept has evolved into the lipid raft hypothesis, which posits domains enriched in cholesterol and sphingolipids.[56] The association of a lipid with these domains is determined by a number of structural and chemical factors, such as favorable hydrogen-bonding interactions (for raft lipids, these are suggested to form between the sphingolipid amide proton and the cholesterol hydroxy group),[57] the size of the amphiphilic head group, and the degree of unsaturation of the acyl chains (Box 1c and d). These domains, while mainly studied in the PM, are now assumed to exist in a number of intracellular membranes, where they play critical functional roles. For instance, the ER bilayer contains ordered lipid domains at sites where it contacts other organelles;[58] Phosphatidylserine (PS) is also emerging as a lipid with the unique ability to form laterally heterogenous clusters in cooperation with sphingolipids and cholesterol,[59] with functional implications for the membrane localization of oncogenic proteins.[60]

What extent of change in lipid composition can be tolerated while maintaining regions of lateral heterogeneity? The limited resolution of analytical techniques used to study the biophysical properties of lipids in liposomes may not currently give an accurate answer to this question. However, some studies have indicated that changes as small as 1 mole percent may be sufficient. For example, while pure SM membranes are readily dissolved by detergents, the addition of 5 mole percent ceramide is sufficient to form detergent-resistant membranes.[61] The length of the ceramide acyl chain is also relevant for bilayer lateral heterogeneity: ceramides containing acyl chains longer than 12 carbons generate rigid ceramide-enriched domains at ceramide concentration as low as 1–5 mole percent.[62,63] Changes in the concentration of GM1 of up to 5 mole percent exerted a strong effect on the spacing of lipid headgroups within the bilayer in a ternary lipid mixture,[64] and changes in cholesterol concentrations of between 2 and 5 mole percent also have a significant effect.[65,66] Moreover, phase formation determined by a specific lipid composition impacts the binding of proteins to membranes. For instance, the sialic acid-binding Ig-like lectin 7 (Siglec-7) binds the disialyl ganglioside GD3 but does not recognize the same epitope when borne by phytoceramides (i.e., ceramides containing an additional hydroxyl group at the 4-position of the sphingoid base).[67] This altered recognition is due to the inability of phytoceramides to form clusters within the plane of the bilayer. Hence, altering ceramide
structure affects the lateral heterogeneity of the GD3 epitopes within the leaflet, which in turn affects their recognition by Siglec-7 and the susceptibility of the cell to natural killer lymphocytes. Thus, a minor change of ceramide composition impacts phase separation, with functional implications for cellular signaling pathways and immunity.

In summary, differences in lipid composition between different cell types and between the PM and intracellular membranes, along with the effect of altering lipid composition on the biophysical properties of lipid bilayers, strongly suggest, although do not unambiguously prove, that aspects of lipid bilayer composition are fine-tuned with a low tolerance to change. We suggest that it is likely that the development of better analytical and quantitative techniques to measure the effect of small changes in lipid composition (< 0.001 mole percent; the current limit of MS) will reveal that lipid composition is actually fine-tuned to an even higher degree than currently appreciated.

LIPID ASYMMETRY IS FINELY TUNED WITH LOW TOLERANCE TO CHANGE

While higher resolution analysis is still required to unequivocally determine the extent to which membrane lipid composition can be considered fine-tuned, there is far less ambiguity about fine-tuning with respect to lipid asymmetry across the two halves of the membrane bilayer (Figure 3 and Box 1e). Most lipids cannot undergo spontaneous transbilayer movement (colloquially known as “flip-flop”) due to the high energy barrier to diffusion of polar headgroups through the hydrophobic bilayer core, such that the half-life of spontaneous phospholipid flip-flop is in the order of hours to days.

Lipid asymmetry was first described by examining the distribution of lipid probes in bilayers and more commonly by the effect of enzymatic treatment on the lipid content on the outer leaflet of the bilayer. The accuracy of the latter was sometimes challenged since hydrolysis of lipids in the outer leaflet could cause the flipping of lipids from the inner to outer leaflet and thus confound results. However, these studies have held up remarkably well over time such that current data fully supports earlier conclusions, while adding a level of analytical sophistication with respect to lipid composition that was not previously available. Thus, the outer leaflet contains 94% of the sphingolipids (including 95% of the SM and 65% of the phosphatidylcholine (PC). The inner leaflet contains 94% of the phosphatidylethanolamine (PE), 88% of the phosphatidylserine (PS), 80% of the phosphatidic acid (PA), and 60% of the phosphatidylinositol (PI). This transbilayer distribution is reflected in the schematic of the lipid bilayer in Figure 3; note that the precise bilayer distribution of cholesterol is still a matter of discussion.

How does membrane asymmetry arise in cellular membranes?

Membrane asymmetry is essentially derived from two intracellular features: the topography of lipid synthesis and the activity of membrane lipid transporters. In terms of synthesis, sphingolipids are a good example. Complex sphingolipids, such as SM and most GSLs are synthesized on the luminal leaflet of the Golgi apparatus. This bilayer is subsequently externalized to become the outer leaflet of the PM via fusion of TGN-derived vesicles with the PM. Since SM contains a polar headgroup (phosphorylcholine), and GSLs contain bulky or polar sugars, they are unable to spontaneously redistribute between the two halves of the Golgi or PM bilayers. Concerning membrane lipid transporters, PS, which is overwhelmingly located at the inner leaflet of the PM, is the best example. The asymmetric distribution of PS relies upon the continuous removal of PS from the outer leaflet by P4 ATPases. The biosynthesis of PS, however, occurs on the ER luminal leaflet. If PS were to be transported in vesicles directly from the ER membrane to the PM, this would deposit all PS at the outer leaflet of the PM. The transport process is therefore more complex involving the exposure of small amounts of PS at the cytoplasmic leaflet of the ER where it is collected by lipid transport proteins. In this way, the cell closely manages the asymmetric distribution of PS. Irrespective of the mode of generation of lipid asymmetry, once established, the scarcity of spontaneous flip-flop cements the transbilar distribution of a particular lipid species.

Another level of sophistication in lipid asymmetry has recently been revealed by examining the extent of lipid saturation in each leaflet of the bilayer (Figures 2 and 3). The inner leaflet of the erythrocyte PM is twice as unsaturated as the outer leaflet (Box 1f). On average, outer leaflet lipids contain 1.6 double bonds per lipid compared to 3.4 for the inner leaflet. Moreover, lipids such as PC, which are relatively symmetrically distributed between the two halves of the bilayer (65% in the outer leaflet and 35% in the inner leaflet), display different levels of unsaturation between the leaflets. In the case of PC, the inner leaflet is more saturated than the outer leaflet (12% of inner leaflet PC lipids are fully saturated compared to 2% in the outer leaflet). This trend is opposite to the general distribution of lipid unsaturation in the PM with two-thirds of all lipids on the inner leaflet polyunsaturated, and the average number of unsaturations per lipid in the inner leaflet roughly twice that of the outer leaflet. Thus, the distribution of PC unsaturation is fine-tuned even with respect to other glycerophospholipids.

Membrane asymmetry is biologically functional

The importance of lipid asymmetry can be ascertained by the effort that cells expend to maintain it. As mentioned above, unassisted lipid flip-flop between bilayer leaflets occurs with a half-life in the order of hours to days; in contrast, biological membranes show half-lives for flip-flop of seconds to minutes for certain lipids implicating mechanisms to facilitate flip-flop. The transit of lipids across the bilayer is accomplished by multiple classes of protein transporters. Flippases and floppases consume ATP to translocate lipids towards the inner or outer leaflets, respectively. Scramblases passively allow lipids to translocate in either direction. While flippases and floppases serve to maintain membrane asymmetry, scramblases equilibrate lipids between membrane leaflets. Together, these assist in controlling the asymmetric distribution of lipids within membranes. Mammals possess...
Membrane asymmetry contributes to the biophysical properties of cellular membranes

In addition to these functionally relevant cellular events, lipid asymmetry also comes to the fore when considering various biophysical properties of lipid bilayers, including how the two leaflets of the bilayer interact, by a process known as interleaflet coupling or interdigitation. In this process, an acyl chain from one leaflet of the bilayer protrudes (i.e., interdigitates) into the other leaflet. The number of carbon atoms in an acyl chain determines how deeply the chain extends into the apposing leaflet, controlling the degree of interdigitation. For this reason, lipids possessing long acyl chains (i.e., >C20) may contribute most significantly to interdigitation. While domains of restricted mobility have been experimentally observed in the outer leaflet, such domains are far more difficult to experimentally detect in the inner leaflet. However, the recent demonstration of asymmetry in lipid unsaturation (see above) along with molecular dynamics (MD) simulations of the fluidity of the two halves of the bilayer indicates that lipids in the outer leaflet pack more tightly and diffuse more slowly than those in the inner leaflet (Box 1g). This being the case, how might lipid interdigitation affect the biophysical properties of each half of the bilayer? In a liposome comprising four different lipid species, altering the lipid composition of one leaflet affects domain formation in the opposing leaflet. Domains of sizes up to 160 nm are coupled across the leaflets of liposomes and increase sharply with a 5 mole percent increase in SM, leading to the suggestion that domains move in register and are highly dependent on the lipid composition of each half of the bilayer. Small increases in the unsaturation of PC have been shown by MD simulations to alter domain size and interleaflet alignment in ternary systems. Finally, the length of the acyl chain in cholesterol esters affects interleaflet coupling and domain registration.

In summary, the extraordinary extent of lipid asymmetry and the impact of this property on a number of complex biological and biophysical events suggest that lipid asymmetry is fine-tuned, displaying an extremely low level of tolerance to change in lipid distribution across the two halves of the bilayer.

INTERACTIONS BETWEEN MEMBRANE PROTEINS AND LIPIDS ARE FINELY TUNED

Until now, the major focus has been on the unanticipated complexity of membrane lipids and their distribution in and within cell membranes. However, membranes also contain a wealth of proteins: between 20% and 30% of the human genome is estimated to encode transmembrane proteins, including transporters, enzymes, channels, pumps, and receptors. The protein content of the PM may be as much as one protein per 50 lipid molecules. Unlike the rather unspecific nature of lipid–protein interactions postulated by the fluid mosaic model, these interactions are now known to be highly specific, highly regulated, and strongly influenced by lipid composition and distribution.

How do membrane proteins and lipids interact?

In principle, there are a number of ways by which membrane proteins could interact with membrane lipids (Figure 4). First, membrane proteins could require a certain membrane environment, which gives rise to certain biophysical properties of the bilayer, such as fluidity; such an environment could be generated by the cumulative properties determined by the local lipid composition. Second, lipids could specifically bind to membrane proteins via highly conserved structural binding sites, thereby modifying biological function of the protein, either allosterically or directly. Both of these interactions have been known to occur for some time, although the extent and specificity of the latter has not been fully appreciated.

Examples abound of the specific membrane environment required for membrane protein function. The concept of “boundary lipids” was first suggested in the 1960s based on the co-purification of subsets of lipids with membrane proteins, leading to the notion that the proteins and lipids were in close proximity within the bilayer, and thus co-purified upon detergent solubilization. However, solubilization using detergents leads to scrambling of lipids, a process that could cause membrane proteins to associate with lipids in mixed micelles (i.e., lipid–detergent-protein mixtures) with which they do not normally associate. Subsequent to solubilization, proteins are often reconstituted in lipid mixtures or in liposomes, where some lipids are more effective than others in reconstituting protein activity. For instance, the Ca2+-ATPase from skeletal muscle sarcoplasmic reticulum shows the highest activity when reconstituted into PC bilayers of...
FIGURE 4  Modes of lipid–protein interactions in membranes. Upper left: TMDs of transmembrane proteins are asymmetric in their surface area, matching the difference in lipid packing between leaflets. The left-hand panel shows the skewed distribution of the ratio of TMD surface area between inner- and outer-leaflet resident regions of human single-pass TMDs. The solid line indicates the mean, while the two dashed lines indicate first and third quartiles. Adapted from [17]. The right-hand panel shows a section of the PM membrane bilayer illustrating a single-pass TMD helix with greater surface area in the inner leaflet compared to the outer leaflet. Upper right: An example of the binding of a specific sphingolipid, namely C18-SM, to the TMD of a membrane protein, p24; SM species of other chain lengths show much lower binding to p24 as measured by FRET measurements between a fluorescent lipid analog and the p24 transmembrane domain. The C18-SM gives an optimal balance between the dynamic volume of the lipid and the angle of the phosphorylcholine head group. Adapted from [111]. Lower panels: A number of examples of lipid–protein interactions that were discovered when a specific lipid was resolved in the crystal structure of a membrane protein. PDB accession codes are indicated as are the bound lipids. Models of lipids and proteins were prepared using PyMOL with hydrogen atoms omitted for clarity.

Acyl lengths from C16 to C20, the activity of the Na,K-ATPase peaks in bilayers containing cholesterol and PC with a C18 chain length; and modification of bilayer lipid composition affects the desensitization kinetics of the agonist response of the pentameric ligand-gated ion channel, ELIC. Presumably, these interactions affect the conformational equilibrium and activity of membrane proteins, although it is challenging to show that the same lipids used in liposomes in reconstitution experiments are proximally located to the proteins in intact cell membrane lipid bilayers. MS has recently begun to distinguish between loosely associated boundary lipids and more tightly associated lipids.

Functional allosteric interactions have also been widely documented between lipids and membrane proteins, and they can alter the interactions between proteins and their cognate agonists. Allosteric lipid interaction sites abound in multiple classes of membrane proteins, including ion channels, mechanosensors, and GPCRs. Many of these involve regulatory lipids that are found at relatively low levels (<1 mole percent) in membranes and contain a high degree of structural content, such as the phosphoinositides, in which the inositol ring can be phosphorylated at multiple positions. Phosphatidylinositol 4,5-bisphosphate (PIP2) can activate membrane protein channels (i.e., inward-rectifying potassium channels) by...
direct binding but the membrane environment or allostERIC presence of other lipids also contributes to channel activity.[106] The inward rectifier K+ channel Kir6.2 becomes sensitized to PIP2 when palmitoylated but an additional lipid acyl chain also contributes hydrophobic contacts to the PI binding site,[107] and the closely related Kir2.2 exhibits allosteric binding by PS at an additional, anionic lipid binding site.[106] PIP2 itself can act by different modes on different membrane proteins. For instance, PIP2 affects the interaction of the epithelial Na+ channel, ENaC, with cytoplasmic channel domains,[108] and allosterically increases the potency of agonists for TRPM8 (calcium-permeable ion channel responsible for cold sensation).[109] The interaction of more than one lipid with a particular membrane protein is consistent with the notion that modulation of membrane protein function by specific lipids is a highly complex process with functional significance. Thus, the acyl chain composition of diacylglycerol affects the binding affinities and kinetics of binding by protein kinase C,[110] such that stearoyl-arachidonoylglycerol (chain lengths of C18:0 and C20:4, respectively) and stearoyl-oleoylglycerol (C18:0 and C18:1) have an order of magnitude difference (300 nM vs. approx. 10 nM) in affinity for the protein kinase C C11a domain.

How fine-tuned are membrane protein–lipid interactions?

Evidence is also accumulating that indicates highly specific interactions between membrane proteins and membrane lipids (Box 1), largely based on the detection of particular lipids within the crystal structures of membrane proteins. These interactions are comparable to the sub-Ångström interactions documented in the ATP synthase (see below). One of the best examples is the binding of a specific SM of C18 chain length to the COPII protein p24,[111] in which the lipid-binding interaction was abolished by mutation of a single amino acid residue in the p24 carboxy terminal binding motif (Figure 4). More recent work has shown the binding of SM to p24 controls the dimerization of p24, which is required for formation of coat protein complexes.[112] In terms of atomic structures, cholesterol has been detected in the crystal structure of the sodium-potassium ATPase,[113] the beta 2 adrenergic receptor,[114] and in the smoothened receptor[115] carboxilipin has been detected in formate dehydrogenase[116] and in the bovine ADP/ATP carrier,[117] phosphatidylglycerol in the bacterial KcsA potassium channel[118] and PS in the P4 ATPase (Figure 4).[119] Moreover, binding sites for cholesterol in GPCRs are a frequent structural motif.[120]

A final aspect of lipid–protein interactions is the extraordinary correspondence between leaflet asymmetry and the structural asymmetry of TMDs in membrane proteins (Box 1h and i). This notion was first suggested in the 1990s by comparing Golgi apparatus and PM-resident TMDs; alpha-helices for Golgi TMDs were on average 7.5 Å shorter (corresponding to five amino acid residues) and contained more of the bulky amino acid phenylalanine.[121–123] Alpha helices in Golgi TMDs are also larger than those in PM TMDs.[124] Moreover, all single-pass TMD proteins in the human proteome were examined by a bioinformatics approach,[127] leading to the finding that the surface area of these TMDs is generally higher in the inner leaflet of the lipid bilayer than in the outer leaflet (Figure 4). Remarkably, this asymmetry matches the tighter packing of the outer leaflet lipids and the more fluid packing (i.e., more able to solvate proteins) of the lipids in the cytoplasmic leaflet. Moreover, by altering each half of the TMD, the intracellular localization of model peptides with different sequences (whereby the residues were more or less bulky) could be redirected to intracellular membranes whose lipid composition was more suitable.[127] This shows an astonishingly fine-tuned complementarity between bilayer asymmetry and the shape of membrane protein TMDs.

IS “FINE-TUNING” AN APPROPRIATE TERM TO USE IN BIOLOGY?

In the discussion above, we have frequently used the term “fine-tuning” when discussing the unexpected complexity of lipid bilayers and the multiple interactions between membrane components. Is it appropriate to use such terminology in the biological sciences, analogous to its more common use in cosmology and physics (Figure 1)?[124,125] While it is possible to measure and quantify physical constants to a very precise degree and to determine what degree of change in these constants is tolerated by living organisms, it is far more challenging to measure and quantify biological interactions—such as those between multiple lipid species and the TMD(s) of a protein—and to determine the tolerance of biological membranes to small changes in lipid structure or composition. With this caveat in mind, many examples of fine-tuning appear to exist in biology, and are intuitively evident upon the study of complex biological systems. Nevertheless, the term “fine-tuning” is used much less frequently in biology than in cosmology and physics, and even when it is used, the sense is that fine-tuning is used to describe “precisely or tightly regulated mechanisms,” or to define the number of constraints that can be applied before a deleterious effect is observed on the system under study (see, e.g.: [126–132]). A good example of the use of fine-tuning in biology is the ATP synthase (“Rate of hydrolysis in ATP synthase is fine-tuned by α-subunit motif controlling active site conformation”[126]), a large complex containing two rotary motors that harness the electrochemical potential across the mitochondrial inner membrane. Subtle, sub-Ångström conformational changes in the α subunit that control the position of an arginine residue (R373) in the catalytic site in the beta subunit play a crucial role in stabilizing the transition state; sub-Ångström changes in the side-chain result in loss of three orders of magnitude of catalytic activity.[133] This sensitivity is critical to the role of this arginine residue in controlling the rate of ATP hydrolysis, as confirmed experimentally [134–136] and by hybrid quantum mechanics/molecular mechanics calculations.[137,138]

The use of quantifiable values such as “sub-Ångström” reinforces the notion of tolerance in biological systems, that is, the range of variation permitted to maintain a specific property. In cosmology, tolerance is often applied to the relevant property that accommodates life; for example, the cosmological constant, whose value of 1.7 × 10−121 Planck units[139] is within a single order-of-magnitude from the
theoretical life-permitting upper bound.[140,141] Can similar arguments be made for tolerance with respect to fine-tuning in biology? The intuitive answer is yes, based on empirical observations of the extraordinary functional fitness of proteins, lipids, carbohydrates, and nucleic acids for life. However, the complexity of biological systems renders similar measurements and analyses much less amenable to experimental quantification, although the exponential rate of development of more accurate analytical tools is likely to help resolve this issue in the coming years. More specifically, many of the analytical tools in the membrane field (MS, optical, fluorescence, and electron microscopy) are limited in their spatial and temporal resolution.[142,143] As an example, MS can only detect overall lipid composition and cannot detect lipid asymmetry. This limitation is overcome by enzymatically modifying the lipids in the external leaflet, and subsequently comparing the lipidomic profiles of treated versus non-treated cells to assign lipids to the inner or outer leaflet of the PM. This situation is rapidly changing; for instance, details of lipid distribution in various tissues were only resolved after the development of MALDI and its application to tissue samples, an advance that has broached a new frontier in tissue-based (and presumably soon, cell-based) lipidomics.[144]

CONCLUSIONS AND PROSPECTS

The three properties of lipid bilayers discussed above (see Box 1), namely their composition, the distribution of lipids within and across bilayers, and the specific interactions of membrane lipids with membrane components, are entirely consistent with our suggestion that cell membrane lipid bilayers can be described as “finely-tuned molecular assemblies.” “Fine-tuning” refers to the low level of tolerance towards change for many of these properties, and “molecular assembly” refers to the unanticipated complexity of membrane bilayers and the multitude of specific interactions between members of the assembly. This description prompts testable hypotheses related to explicit aspects of bilayer composition and function. For instance, this concept suggests that lipid composition, even of low-abundance lipids, is of functional relevance for membrane function, and therefore changing composition, even by a small amount, should affect bilayer properties. Likewise, the relationship between membrane proteins and the lipids that bind to them could be experimentally examined by changing the lipid environment or altering the lipid-binding motif in the protein. Finally, altering lipid composition of each half of the bilayer is likely to affect biophysical properties, either in one half only or in both halves, if acyl chain interdigitation occurs (which in turn depends on lipid composition, namely the acyl chain length). While such experimental approaches could have been proposed based on earlier models of lipid bilayers, our fine-tuning model implies that considerably smaller changes than would have previously been anticipated are likely to have major effects on lipid bilayer properties and function.

Recently, a somewhat similar concept about the structure of membrane lipid bilayers was put forward,[145] with the suggestion that membrane lipids and proteins are part of a “molecular machine.” We fully encompass this idea but additionally highlight the sublime nature of cell membrane lipid bilayers (à la Newton who stated that “the universe is sublime”) and join Aristotle who proclaimed over two millennia ago that “in all things of nature there is something of the marvelous”; might we be so bold as to suggest that “in all aspects of lipid bilayers, there is something of the marvelous”?

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

ORCID

Tamir Dingjan https://orcid.org/0000-0001-5782-3161

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