Biophysical properties of lipids and dynamic membranes

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Abstract

The lipid bilayer is a three-dimensional assembly with a rich variety of physical features that modulate cell signaling and protein function. Even though it is globally fluid, the large number of different amphipathic molecules that make up the cell membrane have different physical-chemical properties that have to compromise and coordinate within the self-assembled bilayer they share. As a result, lateral and transverse forces within the membrane are significant and rapidly change as the membrane is bent or stretched and as new constituents are added, removed, or chemically modified. Many of these physical effects have an impact on functions critical to cell structure and function.

Introduction
The lipid bilayer of a cell membrane might appear to be a passive film that blocks flow of water and solutes and in which the truly regulatory elements - proteins - are inserted. But the variety of lipids, their controlled spatial organization, and many other data suggest that they play an active role in cell function, and much of this role depends on the biophysical properties of the membrane. The appearance of a self-assembled vesicle, perhaps formed by isoprene derivatives \(^1\) was a turning point in evolution because it allowed macromolecules and other solutes to be enclosed, separated, and concentrated in a small volume distinct from that of its surroundings. The more complex functions such as selective entry or exit of solutes, and transmitting or transducing signals from the outside to the inside of cells is generally ascribed primarily or entirely to proteins that use the lipid bilayer only as an amphipathic film in which to insert. However, despite their small size, there is evidence that lipids play many roles in cell biology, some of which depend on their physical state.

**Transverse lipid asymmetry**

The bilayer in a typical eukaryotic cell has a thickness of 5 nm and can have a continuous surface area of hundreds of square microns, containing \(>10^8\) individual lipids. The chemical compositions of the inner (facing the cytoplasm) and outer leaflets of the plasma membrane and those of internal organelles, are very different. Significant metabolic energy goes into maintaining this bilayer asymmetry, in part through the function of ATP consuming aminophospholipid translocases \(^2\) that retrieve phosphatidylserine (PS) and other anionic phospholipids from the outer leaflet so that this face of the cell contains almost exclusively zwitterionic phospholipids whereas the inner
leaflet is highly enriched in acidic lipids. Loss of this asymmetry to expose PS on the outer surface is often a sign of injury leading to activation of blood coagulation or of cell apoptosis and can be triggered by numerous factors including agents that promote lateral sequestration of inner leaflet polyphosphoinositides. Intriguingly, cancer cells and vascular endothelial cells in tumors also expose PS, causing augmented coagulation and thrombosis in cancer patients. Acidic phospholipids create not only a high negative surface charge density but also provide a highly acidic environment, with a surface pH of approx. 5.2 estimated for a membrane containing 20 mol% of PS, for instance. Such membranes provide an environment that is dramatically different from bilayers composed of zwitterionic lipids and cholesterol, for instance, and have been shown to trigger the formation of amyloid-type fibers by a number proapoptotic, cytotoxic, and antimicrobial proteins and peptides.

Lateral lipid asymmetry

The lipid bilayer is also heterogeneous laterally, with various descriptions of this asymmetry as evidence of rafts or other domains. The basis for lateral mixing/demixing is in the lipid-lipid interactions, which are manifested in the so-called phase diagrams. Along these lines the lateral segregation of cholesterol-induced microdomains in sphingomyelin bilayers and other mixed lipid systems was demonstrated already in the early investigations soon after development of the fluid mosaic model of lipid membranes, substantiating similar conclusions reached on the basis of studies on cellular membranes. An early model of a lipid membrane domain, now often called a raft, shown in Figure 1, was based in part on differential changes in spectra
obtained from membrane probes with preference for ordered or disordered phases caused when saturated or unsaturated fatty acids, or other membrane active molecules were added to the cell membrane. The importance of cholesterol and sphingolipids in stabilizing outer leaflet domains and in the formation of detergent-insoluble lipid fractions has also been demonstrated for lipid domain formation. Studies comparing cholesterol to its metabolic and probably evolutionary precursor lanosterol point to the unique enhanced ability of cholesterol to stabilize the so-called liquid ordered membrane phase which is the basis for raft formation.

Current problems in studying lipid domains in cell membranes, with emphasis on the technical challenges that limit visualization of these small domains and in the conceptual challenges to relate equilibrium phase diagrams of pure systems to small, transient domains in the cell, have been discussed in several recent reviews. A recent advance is the use of atomic force microscopy to visualize domains of chemically unmodified lipids in supported membranes because of the height difference between domains, but significant challenges remain to apply this technique to intact cells. A crucial unknown in cell membrane domains is whether their formation is driven entirely by the physical chemical principles that drive self-assembly of amphiphiles in water, or whether transient chemical interactions - hydrogen bonding or specific ionic attractions - also are important. The potential for specificity in domain formation of selected lipids is suggested by models of cholesterol -phosphatidyl choline pair formation in the outer leaflet and hydrogen bonding networks possible for a small number of rare lipids involved in signaling, such as phosphoinositides and phosphatidic acid, in the
inner leaflet. Differentiating specific lipid interactions from steric, hydrophobic, dipolar, and electrostatic interactions common to them all may help resolve some of the uncertainty whether, how, and when lipid domains form.

**Lateral membrane pressures**

The conformation of amphiphilic molecules is always a compromise of free energies of their hydrophobic and hydrophilic parts. In a lipid bilayer this compromise results in neither the hydrophobic nor hydrophilic part of the phospholipid being in the lowest energy configuration it would take if it were not tied to its chemically incompatible partner. The hydrophilic head groups at the surface of the membrane are crowded together more tightly than they would be if free in solution. This frustration is evident when a headgroup such as IP$_3$ is liberated from the membrane by a phospholipase and diffuses into the cell interior to activate its cytoplasmic targets. In contrast, the hydrophobic acyl chains are generally stretched out more than they would be without their hydrophilic anchors. The end to end distance of, for example, a 16 carbon chain in a dipalmitoyl phosphatidyl choline bilayer is much longer than the end to end distance of hexadecane in bulk, and the loss of entropy due to straightening out the chain results in a significant lateral pressure within the lipid bilayer that varies with the depth into the bilayer (see Box 1). Decades of structural and theoretical work have provided quantitative estimates for how much different regions of the phospholipid acyl chains deviate from a random configuration$^{36}$, and this deviation results in a lateral pressure gradient throughout the lipid bilayer$^{37}$. 
Even though the bilayer as a whole may be in a stable state, or near equilibrium, each part of it is highly stressed. In general the hydrophobic/hydrophilic interface exerts interfacial tension, due to the hydrophobic effect minimizing contacts of the hydrocarbon parts with the aqueous phase, which is balanced by the steric repulsion between the headgroups and entropic intermolecular repulsion between the acyl chains in the monolayer leaflets, exerting lateral pressure that would tend to compress proteins embedded within. As the forces acting on the system are confined to very narrow zones of only few Ångstroms within the bilayer the prevailing pressures can be ultimately very high, reaching hundreds of atm. These lateral stresses, which depend sensitively on lipid composition, curvature, pH, divalent cations, drugs, and binding to proteins is increasingly considered in models for how transmembrane proteins, especially those that respond to force, can alter their configurations when they are stimulated \(^\text{38}\). A schematic diagram showing how changes in lateral pressures activate or inactivate transmembrane proteins through structural changes is shown in Figure 2. Similar conformational changes can be produced by transverse forces due to hydrophobic mismatch \(^\text{39}\), as illustrated in Figure b.

**Membrane stress and antimicrobial agents**

The physical aspects of the cell membrane are increasingly recognized to be crucial for understanding the function of antimicrobial peptides and other agents. Antimicrobial peptides represent the first line of defense, innate immunity, against invading microorganisms by all eukaryotic cells, from plants to human. They vary greatly in
primary and secondary structure but have in common a net positive charge and
amphiphilicity, with a hydrophobic face that allows their partitioning into the interface of
cell membranes. Although the precise mechanisms of these agents are not known, they
are unlikely to require specific receptors in the bacteria they kill, since most of them have
very broad specificities, often killing Gram-positive and Gram-negative bacteria with
similar efficiency. Further, peptides composed of D-amino acids are equally effective as
the natural L-peptides. This apparent lack of specificity is likely to be important for
their function since attacks on the generic physical properties of the membrane cannot be
evaded by mutations that alter specific membrane components, and bacteria are much
less capable of developing resistance to antimicrobial peptides than to antibiotic agents
that attack specific bacterial proteins. Indeed antimicrobial agents need not be peptides,
and some organisms such as shark express cationic steroids such as squalamine that
have antimicrobial efficiency and broad antibacterial spectra similar to those of cationic
peptides. One model to explain the strong but selective membrane destabilizing effects
of antimicrobial agents is shown in Figure 3. Cationic amphiphilic antimicrobial
molecules dock to the anionic surface of bacteria, changing the lateral pressure as the
structures at the hydrophilic-hydrophobic interface are altered. Eventually, the forces
stabilizing the interface are weakened until the bilayer loses integrity and allows
uncontrolled flow of solutes across the membrane.

The possibility that lateral pressures in the membrane due to lipid packing can alter
protein function and affect cellular signals including anesthetic effects is reinforced by
recent findings that some peptides hypothesized to alter ion channel activity by binding
the eukaryotic cell channel protein are also potent antibacterial peptides\textsuperscript{44}. While it is possible that these small peptides have specific and distinct protein ligands on both prokaryotic and eukaryotic cells, a common effect elicited by changes in membrane biophysics is also plausible. On the other hand different biological effects of stereoisomers of steroids\textsuperscript{45, 46} suggest that separating bilayer effects from specific binding to chiral elements in proteins will be complicated.

**Elasticity of the membrane**

The cell membrane resists deformation, and the magnitude of this resistance to forces applied in various directions is characterized by several elastic constants.

**Shear deformation and viscous flow**

Shear deformations within the plane of the fluid bilayer, which is usually presumed to be the primary or only phase present in eukaryotic cells, meet no elastic resistance since the lipids and the transmembrane proteins can flow past each other. An underlying protein mesh such as the spectrin-actin network endows the membrane with resistance to shear, and the composite of 2-D protein network and lipid bilayer together determine the remarkable viscoelastic properties of erythrocytes and other cells\textsuperscript{47}. The lack of resistance to shear places limits on how forces can be applied by motor proteins at the membrane surface. For example, myosin\textsuperscript{1, 48, 49}, kinesin\textsuperscript{50, 51}, and other motor proteins have specific binding sites for phosphoinositides or other acidic lipids suggesting that these lipids may anchor them to the plasma membrane or the surface of a vesicle. Such
an anchor may suffice to transport a vesicle within the cytoplasm as the motor walks along its track, but it is less clear if this mechanism can be used to displace the plasma membrane with respect to the cytoskeleton. Without a shear elastic modulus, the lipid part of the membrane might provide resistance and therefore allow movement of the cytoskeleton if the motor moved rapidly enough for the viscous resistance to be significant, but a slow movement would result in passive flow of the lipid to which the motor is anchored, with no relative motion of the cytoskeleton. This scenario is changed if the anchoring lipid is bound within a larger structure or sequestered within a rigid domain.

Membrane bending

Even without proteins, lipid bilayer membranes resist stretching and bending with elastic constants that are physiologically relevant. Bending is characterized by two curvatures $c_1$ and $c_2$, or equivalently radii of curvature $R_1 = 1/c_1$ and $R_2 = 1/c_2$ in orthogonal directions. These two curvatures define two quantities, a mean curvature $H = (c_1 + c_2) / 2$ and a Gaussian curvature $K = c_1 c_2$. The mean and Gaussian curvatures at each point of the membrane define its topology, which in addition to lamellar membranes can include a wide variety of forms, many of which are found in biology. A striking feature of the chemical composition of cell membranes is that many if not most of its lipid constituents are, by themselves, unable to form planar bilayer membranes. Phosphatidylcholine and phosphatidylserine are the common constituents of the outer and inner leaflets, respectively of eukaryotic cell plasma membrane and do form flat or gently curved planar membranes in vitro, but phosphatidylethanolamine, cholesterol, and other abundant
cellular lipids, and important rarer lipids such as phosphoinositides, diacylglycerol, ceramides, and lysophospholipids cannot form bilayers except when mixed with other lipids (see Figure a). The presence of these lipids in planar membranes destabilizes them, and indeed this destabilization appears to be essential to the biological function of membranes and for their ability to undergo vesicle budding, fusion, and other shape transformations. Therefore, local accumulation of these lipids in specialized domains will have mechanical as well as biochemical consequences.

An increment in the internal pressure of the bilayer hydrocarbon region by lipids such as diacylglycerol or phosphatidylethanolamine with unsaturated chains increases the tendency for the membrane to curl, while the membrane remains lamellar. Such a state is called ‘frustrated’ as these lipids increase the membrane’s tendency to adopt a negative curvature while the lamellar state remains. One of the key enzymes in cellular signaling cascades, protein kinase C, a peripheral membrane protein, can be activated by this membrane stress. As another example, a novel type of a peripheral lipid-protein interaction, ‘extended-lipid anchorage’ has been described for cytochrome c, in which high internal pressure in the membrane hydrocarbon region promotes movement of the acyl chain to the membrane surface and further into a hydrophobic cavity inside this protein, thus establishing a hydrophobic lipid-protein interaction in the absence of intercalation of the protein into the bilayer. The above studies demonstrate that the activity of membrane proteins can be regulated (i) by direct lipid-protein interactions, with specific lipids acting as allosteric effectors, and (ii) by lipids influencing the
physical state of the membrane. Obviously, these two mechanisms are not mutually exclusive for a given lipid.

Membrane negative curvature also promotes membrane fusion. After two opposing membranes are brought together by specific proteins overcoming barriers due to undulations and steric protrusions, rapid fusion for PS containing bilayers can be triggered by \( \text{Ca}^{2+} \), which causes dehydration of the headgroup of this phospholipid and causes its subsequent effective shape to promote membrane negative spontaneous curvature. These latter two factors are known to be critical determinants in the events favoring the merging of adhering bilayers. Physical aspects of bilayer fusion have been extensively reviewed elsewhere (e.g.\(^5\)).

Enzymes acting on membrane lipids can have pronounced consequences not only in causing changes in the lateral distribution of lipids but also in producing changes in the 3-D organization of membranes. Along these lines removal of the phosphocholine headgroup of sphingomyelin by sphingomyelinase (SMase) yields ceramide, a lipid with very different physicochemical properties. While sphingomyelin in the absence of cholesterol is miscible in phosphatidylcholine, ceramide has a profound tendency for segregation into microdomains, driven by intermolecular hydrogen bonding\(^5\). This results in tight packing and reduced trans->gauche isomerization of the hydrocarbon chains, with augmented bending rigidity. As ceramide has a tendency to promote the formation of the inverted hexagonal phase, the domains enriched in ceramide form cusps. Experiments using microinjection of SMase on the surface of giant vesicles composed of
phosphatidylcholine and sphingomyelin have demonstrated the formation of smaller vesicles consistent with this model. More specifically formation of either endocytotic vesicles into the internal cavity of giant liposomes or shedding of vesicles from the outer surface of the substrate liposome following the action of SMase on the external or internal leaflet of the giant liposome was seen\(^\text{56}\). These results show how reorganization of cellular membranes can be driven without ATP, simply by inducing enzymatically a phase transition of the membrane lipids.

**Membrane stretching**

Lipid bilayers strongly resist stretching because increasing the average distance between head groups increases exposure of the hydrophobic domain to water. Some membranes, such as the plasma membrane of leukocytes, have much greater surface area than needed to enclose the cell volume and so deformation of the membrane does not lead to bilayer stretching. However, internal organelle membranes are often near the limit where further deformation is elastically resisted, and this resistance can influence shape changes. For example, the membrane of the endoplasmic reticulum is pulled into tubes by motors that run along microtubules\(^\text{57}\). This process has recently been reproduced in vitro with a minimal set of proteins and purified lipids\(^\text{58,59}\). As shown in Figure 4, thin tubes can be pulled out of a large vesicular reservoir that may mimic the tubes pulled out of the ER. An important finding of this study is that the force needed to pull out a pure lipid tube is on the order of 50 pN, and therefore requires coordinate pulling by multiple motors. The membrane resistance to deformation can be sufficiently large to stall the motors, stop tube elongation, and in some cases lead to elastic recoil of the tube. Therefore,
regulation of tube extension in vivo could be regulated by changes in membrane tension as efficiently as by regulation of the motor proteins that pull on the tubes. Changes in the lipid composition of the ER, the binding of peripheral membrane proteins that alter surface tensions, or changes in internal pressure can all impact the rate and direction of tube extension even without a change in the number of bound or activated motor proteins. Conversely, the mechanical work of pulling out a tube changes the lipid composition within the tube compared to that in the parent vesicle 60. The lipid lateral packing also bears on the control of the activity of membrane proteins, with several enzymes and mechanosensitive ion channels exhibiting an optimum of membrane lateral packing density required for activity. The equilibrium lateral pressure $\pi_e$ of biomembranes has been estimated at approximately 33-35 mN/m, which is already inhibitory for some phospholipases, for instance. Subjecting the membrane to tension (for example due to osmotic swelling decreasing lateral pressure) lowers $\pi_e$, enabling phospholipase A2 to exert catalytic activity 61. This example demonstrates a fundamental role for the membrane physical state in controlling the activity of a peripheral membrane protein, a mechanical force being directly converted into a biochemical signal, membranes thus acting as an osmotic response element.

**Conclusion**

The biophysical features of the cell membrane are increasingly recognized to be important control elements in cell signaling and membrane protein function. A few examples where physical effects may be as important as specific biochemical reactions in
the function of the cell membrane are discussed here. However, a separation between
physical and chemical events in the membrane is subtle. Nearly any chemical change in
the membrane caused by lipid hydrolysis, trafficking or sequestration in the membrane
has a physical consequence manifested, for example, as a change in pressure, or
curvature. Likewise, mechanical work done on the membrane to bend or expand it will
cause redistribution of the hundreds of distinct lipid species that form the bilayer. It
seems likely that physical and chemical features have evolved together to form the
complexity of interactions responsible for cell function.

**Figure Legends**

**Figure 1**  Organization of structurally distinct domains within the plasma membrane.
Lipids with saturated chains are hypothesized to preferentially partition into ordered
domains (darker regions) whereas lipids with unsaturated chains partition into disordered
domains. From ref. 21.

**Figure 2.**  Activation of a transmembrane protein by changes in bilayer lateral stresses.
A slice through a bilayer membrane containing an intrinsic protein is viewed in two
different conformational states, r and t. At right, the cross-sectional area profile A(z) of
each of the two states is plotted as a function of depth z within the membrane of thickness
h. Each conformation, for example, an open or closed channel, is in mechanical
equilibrium with the forces exerted by the membrane lipids. As these forces change, due
to such effects as curvature, redistribution or enzymatic modification, the probability of
being in state r or s will also change. From ref\textsuperscript{37}.

**Figure 3.** Model of membrane destabilization by a cationic antimicrobial agent. Some
antimicrobial agents that do not appear to form specific pores, are hypothesized to
function by a “carpet” mechanism. A single cationic amphiphile with positive charges
denoted as small protruding circles docks electrostatically on the negatively charged
surface of the bacterium. Each such binding event alters the structure and local
electrostatic charge of the hydrophilic part of the membrane interface and may affect the
interfacial tension, but need not affect membrane permeability. Additional binding
events eventually lead to sufficient membrane destabilization that the bilayer integrity is
lost and the permeability barrier breaks down. From ref\textsuperscript{62}.

**Figure 4.** Shape changes in a membrane vesicle pulled by molecular motors. (A)
Confocal side-view image of a membrane tube pulled out of a lipid vesicle by kinesin
motor proteins bound to the membrane by biotin-avidin links and translocating along
microtubules at the bottom surface (B) Schematic representation of the geometry and
attachment sites at the tip of the tube. (C, D) molecular linkages and arrangement of
motors at the tip of the tube. From ref\textsuperscript{59}.  

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References


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**Box 1. Forces acting within the cell membrane**

**Membrane tension**

The cell membrane tends to maintain a specific lipid packing density, and therefore an optimal surface pressure on the order of 40 mN/m. Increasing the lipid spacing by osmotic swelling, for example, is strongly resisted, and leads to rupture when the membrane is strained slightly above its optimal packing. Compression within the plane of the membrane would also be resisted, but the membrane will buckle out of plane before significant compression occurred.

**Shear stiffness**

Fluid bilayers have insignificant elastic resistance to shear deformation since the lipids are not bound to each other. Viscous resistance of bulk lipid is approximately an order of magnitude greater than that of the cytosol.

**Curvature and Bending stiffness.**

Membranes resist bending because changing local curvature alters both the headgroup spacing and the entropy of the hydrophobic chains. Bending stiffness is characterized by two bending moduli quantifying stiffness in the two orthogonal radii of curvature possible for a planar membrane. The bending stiffness is strongly dependent on the nature of the lipids and their spatial distribution. The default shape for most membrane constituents is not flat. Instead, each lipid shape that deviates from a cylinder (see Fig
a) contributes a spontaneous curvature to the membrane. Insertion or removal of lipids into either the inner or outer leaflet leads to area mismatches that also alter curvature.

**Figure a.** Effect of lipid shape on membrane spontaneous curvature. (A) Molecules that have an overall inverted conical shape, such as detergent molecules, lysophospholipids and polyphosphoinositides form structures with a positive curvature, such as micelles. (B) Cylindrical-shaped lipid molecules such as phosphatidyl choline and sphingomyelin preferentially form flat bilayer structures. (C) Lipid molecules having an overall conical shape, with a small hydrophilic cross-section form structures with a negative curvature. The local shape of a membrane depends on which lipids are present and on how they are spatially distributed. From ⁶³

**Electrostatic forces**

Eukaryotic cells have no cationic lipids, and no strong electrostatic attractions between lipids within the membrane. However, significant dipolar interactions occur between zwitterionic lipids in which the positive and negative charges are well separated. In the
inner leaflet of the plasma membrane, the presence of anionic lipids generates a
membrane potential, and in the absence of multivalent counterions, repulsive
electrostatic interactions within the plane of the bilayer occur. However, divalent metal
ions and multivalent ions including cationic proteins and polyamines can lead to
attractive interactions and lipid clustering.

Transverse forces
Membrane lipids have a limited hydrophobic length, and therefore a specified membrane
thickness. The optimal membrane thickness depends on the covalent chain length, the
degree of saturation, and the angle of tilt within the membrane. The transition from
higher to lower membrane profiles generates packing disorders that increase elastic
energy. Transmembrane proteins also have a specified length of hydrophobic contour
that may differ from the optimal hydrophobic thickness of the bilayer. This co-called
hydrophobic mismatch can lead to stretching or compression of both lipid and protein
within the membrane (see Figure b) or to tilting of transbilayer helixes to decrease the
hydrophobic height.
**Figure b.** Schematic representation hydrophobic mismatch between a membrane protein of hydrophobic length $d_P$ in a lipid bilayer in which the unperturbed hydrophobic thickness $d_L$ is smaller (top) or larger (bottom) than $d_P$. The influence of the protein extends over a certain distance from the protein surface and progressively vanishes so that the bilayer recovers its unperturbed thickness $d_L$. From 64, 65.

**Line tension**

When membrane lipids demix into domains, the border between domains results in lipid packing that is different from that inside and outside the domain, resulting from such effects as differences in height between the domains. The energy at the interface contributes to the parameters that determine domain size and stability.

**Lateral pressures**

An extensional pressure due to loss of chain entropy within the hydrophobic domain creates compressive forces within the bilayer, the magnitude of which depends on the distance into the center of the bilayer, the nature of the hydrophobic chains (e.g. saturated, unsaturated, single chains or sterols) and membrane curvature.

A compression force acts at the hydrophilic interface in order to crowd the headgroup close enough to minimize exposure of the hydrophobic chains to water. See Fig c.
Figure c. On the left are shown the forces that act on the lipid within the bilayer. Black lines representing the hydrophobic chains and blue dots the hydrophilic headgroup. At the right is shown the corresponding lateral pressure, $\pi(z)$ at different distances ($z$) across the bilayer thickness. Strong tensions at the interfaces are balanced by positive pressures through the interior, largest near the interfaces. The areas under the curves add to zero, and the membrane is globally at rest. The effects of hydrophobic mismatch due to insertion of a transmembrane protein are largely to move the hydrophilic-hydrophobic interface up or down, whereas the effect of curvature and chain unsaturation is to alter the pressures within the membrane interior. Adapted from $^{37,65}$. 
Figure 4