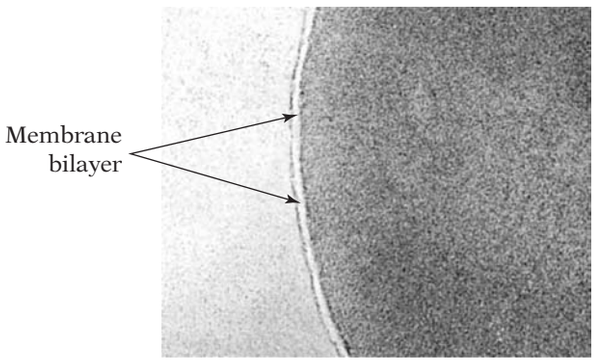
1 Introduction



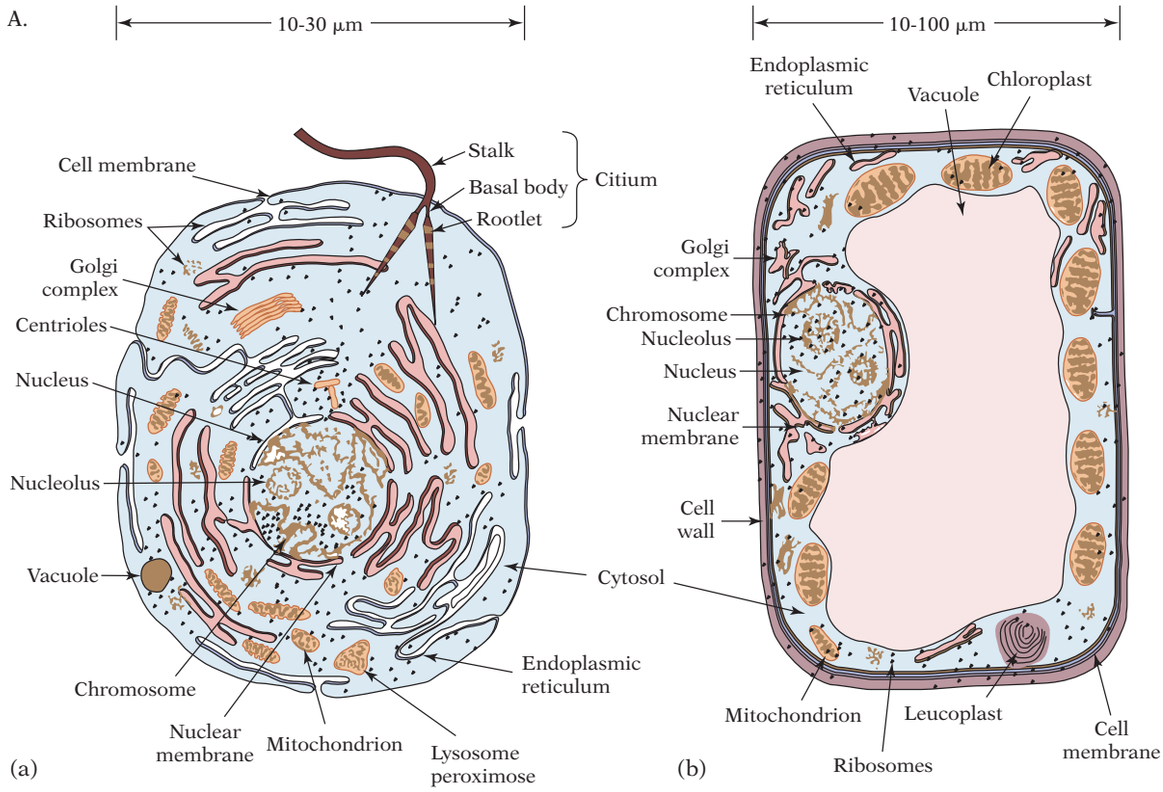
The plasma membrane delineates a cell, defining its borders and determining what can and cannot enter the cytoplasm. Its trilaminar appearance is revealed by electron microscopy after staining with osmium tetroxide. From Nelson, D. L., and M. M. Cox (eds.), *Lehninger Principles of Biochemistry*, 4th ed., W. H. Freeman, 2005, p. 369. © 2005 by W. H. Freeman and Company. Used with permission.

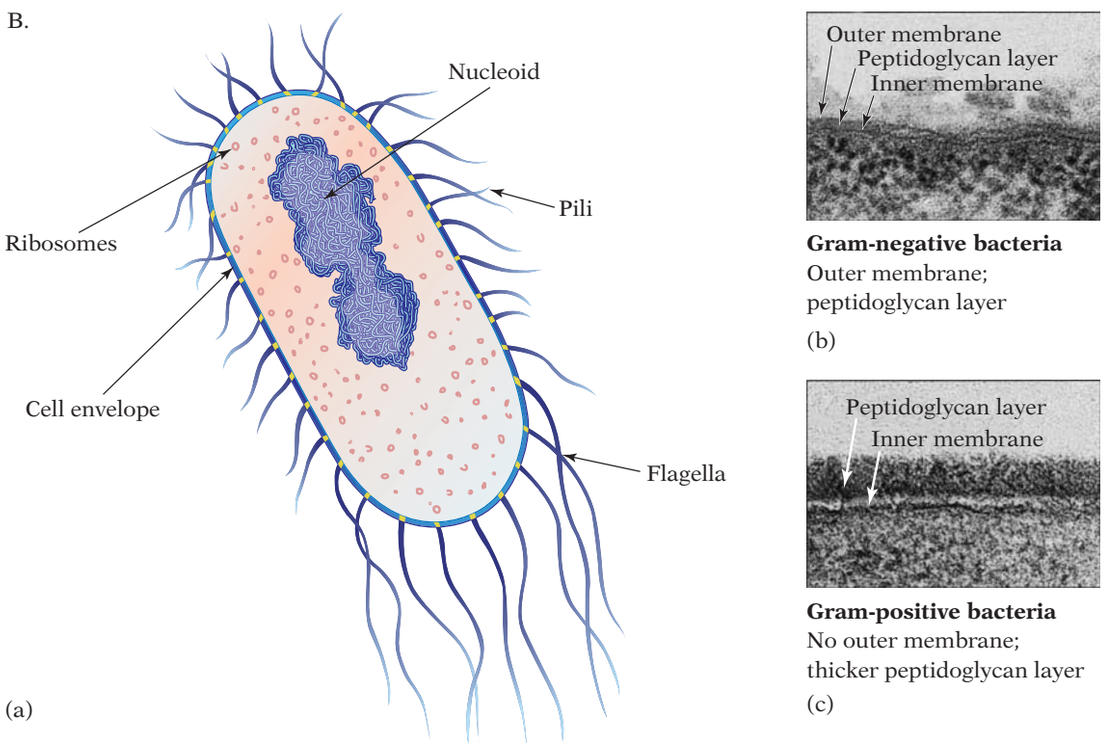
Essential for the compartmentalization that defines cells and organisms, biomembranes are fundamental to life. Early membranes played a crucial role in the origin of life as the structures that defined what stayed in and what was kept out of primordial cells. In addition to their compartmentalization function, membranes provide modern cells with energy derived from chemical and charge gradients, organize and regulate enzyme activities, facilitate the transduction of information, and even supply substrates for biosynthesis and for signaling molecules. Some membranes have specialized functions; for example, the brush border membrane lining the intestines absorbs nutrients, the myelin surrounding nerves functions as insulation, and the rod cell membrane of the eye captures light. While prokaryotes either have one cell membrane (Gram positive) or have inner and outer membranes in the cell envelope (Gram negative), eukaryotic cells have many membranes (Figure 1.1). In addition to the plasma membrane, eukaryotes have membranes surrounding the nucleus, organelles such as mitochondria, chloroplasts in plants, lysosomes, and of course the membrane-based endoplasmic reticulum (ER), Golgi apparatus, and other vesicles involved in intracellular transport. Even some viruses have membrane envelopes. In spite of this variety, much can be generalized about the structure and function of biomembranes.

GENERAL FEATURES OF MEMBRANES

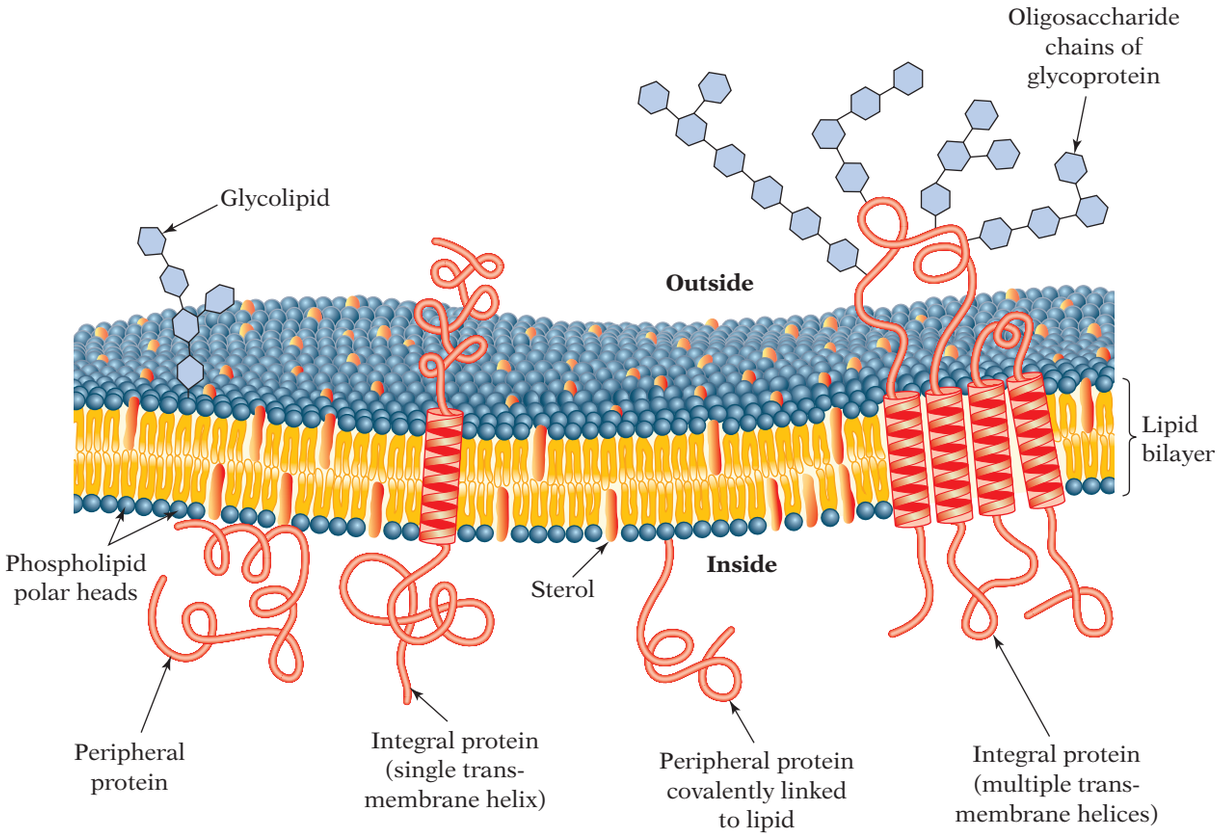
Biological membranes consist of lipids, proteins, and carbohydrates (Figure 1.2). The lipid components include glycerophospholipids (also called phospholipids), sphingolipids, and sterols. The basic unit of the membrane is a bilayer formed by phospholipids and sphingolipids organized in two layers with their polar headgroups along the two surfaces and their acyl chains forming the nonpolar domain in between. Embedded in the lipid bilayer are integral membrane proteins, which cannot be removed without disrupting the membrane. Most of these proteins have one or more transmembrane (TM) segments, and they interact closely with nearby lipids as well as other proteins. In addition, there are peripheral membrane proteins that associate at the surface of the membrane and lipid-anchored proteins that are held into the membrane by covalently attached fatty acids or lipids. Although carbohydrate membrane constituents serve important functions, these hydrophilic moieties are always on the portions of glycoproteins and glycolipids external to the membrane bilayer. Such glycoconjugates deserve detailed consideration on their own and are not covered in this book.

Membranes are responsible for the selective permeability of cell envelopes that enables cells to take





1.1. A variety of types of membranes. **A**. Plasma membrane and intracellular membranes in eukaryotic cells are shown in a diagram based on thin section electron micrographs of generalized animal (a) and plant (b) cells illustrating the plasma membrane and membrane-bound organelles. Redrawn from Jain, M. K., and R. C. Wagner, *Introduction to Biological Membranes*, 2nd ed., Wiley, 1988, p. 2. © 1998. Reprinted with permission from John Wiley & Sons, Inc. **B**. Bacterial membranes are shown in a diagram of a bacterial cell (a) and in thin section electron micrographs of the cell envelope of Gram-negative (b) and Gram-positive (c) bacteria. Redrawn from Nelson, D. L., and M. M. Cox (eds.), *Lehninger Principles of Biochemistry*, 4th ed., W. H. Freeman, 2005, p. 6. © 2005 by W. H. Freeman and Company. Used with permission.



1.2. Membrane components. Membranes contain lipids, proteins, and carbohydrates as glycolipids and glycoproteins. Nelson, D. L., and M. M. Cox (eds.), *Lehninger Principles of Biochemistry*, 4th ed., W. H. Freeman, 2005, p. 372. © 2005 by W. H. Freeman and Company. Used with permission.

up many nutrients and exclude most harmful agents. The permeability properties are determined by both lipid and protein components of membranes. In general, the lipid bilayer is readily penetrated by nonpolar substances while proteins in the membrane make channels and transporters for ions and hydrophilic substances. This permeability barrier enables the membrane to maintain charge and concentration gradients that are critical to the cell's metabolism. The permeability barrier is maintained during activities such as cell division and exocytosis because the membrane is flexible and self-sealing.

Membranes are also very dynamic structures, with constant activity on their surfaces as well as constant movements in the bilayer, both in the transverse direction across the bilayer and the lateral direction in the plane of this two-dimensional matrix. The latter movements give rise to the fluid nature of the membrane and enable interactions among proteins and between proteins and lipids to provide temporal associations that are important to membrane functions.

Thanks to many, many scientists who have contributed to the enormous progress of the past decades, knowledge of the membrane goes beyond its basic architecture and properties to a multitude of details describing specific elements and functions. While the particular tools and approaches used by biochemists, biophysicists, geneticists, and cell biologists who study the membrane vary greatly, two paradigms* provide the framework for understanding their work. The starting point for understanding membrane structure is the hydrophobic effect. A far-reaching paradigm for many areas of chemistry, this principle governs the behavior of membrane components. The specific paradigm for membranes is the Fluid Mosaic Model, a description of membrane properties and organization that has endured for more than three decades.

A description of these paradigms and the classic work on which they are based will lay the groundwork for the rest of this book. Yet today the current paradigm is shifting because of new aspects of membrane organization that have risen to the forefront in the past few years. The importance of transient, specialized regions called membrane rafts affects the contemporary model of cell membranes. Finally, the organization of many membrane proteins into large assemblies that often involve molecules at the bilayer periphery and beyond

* Paradigms are scientific models. According to science philosopher Thomas Kuhn, the paradigms of a field of study shape it so thoroughly that they may be unacknowledged and even unobserved by its practitioners. Yet, they determine the assumptions and the tools with which those scientists operate daily.

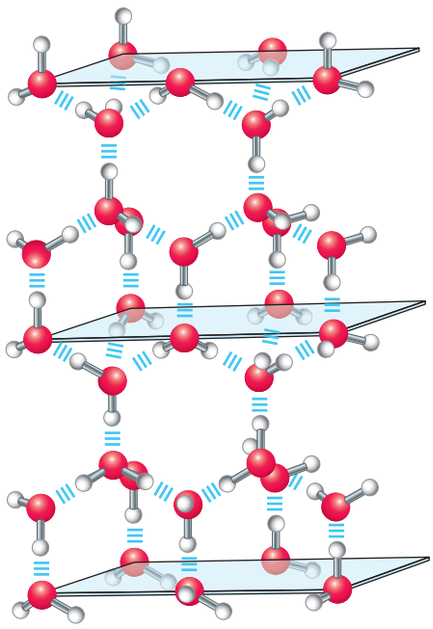
indicates that a more complex and comprehensive view is needed for future work.

PARADIGM 1: THE AMPHIPHILIC MOLECULES IN MEMBRANES ASSEMBLE SPONTANEOUSLY DUE TO THE HYDROPHOBIC EFFECT

All biomembranes contain amphiphilic lipid and protein constituents that have both polar and nonpolar parts, and this dual nature of its components is essential to membrane structure. Because proteins are simply polymers of amino acids, their polarity is a function of their amino acid composition; thus they have hydrophobic domains rich in residues with nonpolar side chains and hydrophilic domains generally lacking them. On the other hand, by classification a lipid is quite nonpolar because the definition of lipids is empirical: a lipid is a biological substance that is soluble in organic solvents and has poor solubility in water. Yet all lipids have hydrophilic domains, called their *headgroups*, even when the headgroup is simply a hydroxyl group, as in cholesterol. The structures of lipids vary considerably (as described in Chapter 2) but all provide the amphiphilicity* that leads to the formation of distinct phases in aqueous systems, in which the lipids aggregate spontaneously to form polar and nonpolar domains. Mixing a pure lipid with water can result in formation of monolayers, micelles, bilayers, hexagonal arrays, or cubic phases, depending on the nature of the lipid and the method of preparation.

The spontaneous formation of each type of lipidic aggregate depends on the structure and hydrophobicity of the lipid, but it is always driven by the structure of water. In ice each water molecule has four hydrogen bonds worth ~5 kcal/mol each (Figure 1.3). When ice melts ~85% of these hydrogen bonds are preserved, but of course in liquid water they are dynamic, with 10^{11}/sec positional changes. The extensive hydrogen bonding of water accounts for its special properties, such as its high boiling point and high dielectric constant (a measure of the extent to which it shields dissolved ions). It also provides the basis for the hydrophobic effect.

Insertion of a nonpolar molecule, such as a fatty acid with a long acyl chain, into liquid water reorders the water molecules closest to the hydrocarbon chain to form a hydrogen-bonded cage around the nonpolar moiety. Depending on the size of the nonpolar domain, there may be no net loss of hydrogen bonds so enthalpy does not necessarily have a strong effect. However, as the water molecules rearrange to form the cage around the nonpolar chains, their mobility is drastically reduced, resulting in a large loss of entropy. The best way to lower this entropic cost is to sequester the nonpolar moieties into large aggregates, thus reducing the total surface area of nonpolar material exposed to the aqueous layer and hence decreasing the number of immobilized water molecules. (This is possible because as a sphere increases in size, the volume increases as the cube of the radius while the surface area increases as only the square of the radius, with the result that a larger radius gives a smaller surface area-to-volume ratio.) The end result of this entropic driving force is the separation of the aqueous and lipid molecules into two phases or domains. The nonpolar domain may then be further stabilized by van der Waals forces between the close-packed acyl chains.



1.3. Importance of hydrogen bonding in the structure of water. In ice, each water molecule forms four hydrogen bonds with its nearest neighbors. In liquid water at room temperature and atmospheric pressure, each water molecule has on average 3.4 hydrogen bonds. Redrawn from Nelson, D. L., and M. M. Cox (eds.), *Lehninger Principles of Biochemistry*, 4th ed., W. H. Freeman, 2005, p. 49.

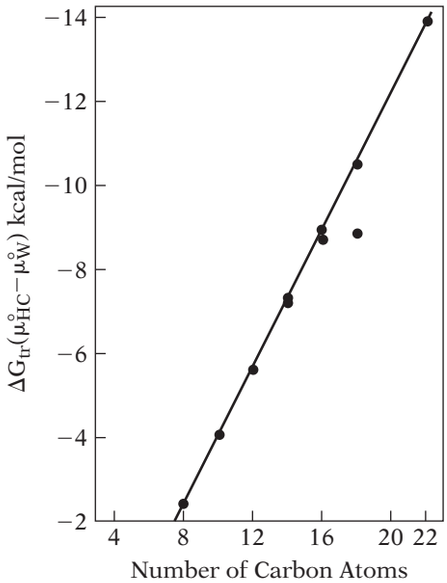
The hydrophobicity of a substance is traditionally measured by a partitioning experiment using two solvents, such as heptane and water. From the partition coefficient is calculated the $\Delta G_{transfer}$ for the solute of interest:

$$K_p = K_{eq} = [solute]_{H20}/[solute]_{heptane}$$
$$\Delta G_{tr} = -RT \ln K_{eq},$$

where K_p is the partition coefficient, K_{eq} is the equilibrium constant, and ΔG_{tr} is the free energy change for the transfer from heptane to water.

When the solutes are fatty acids with varying chain lengths, the energy cost is proportional to the chain length: a cost per CH_2 unit of 0.8 kcal/mol is derived

* *Amphiphilicity* means "having polar and nonpolar domains"; *amphiphilic* and *amphipathic* are used interchangeably.



1.4. The free energy of transfer of fatty acids from water to heptane is a function of the chain length. Fatty acids of varying lengths in n-heptane at 23°C to 25°C are equilibrated with dilute aqueous buffer and their activities (μ°) in each phase determined. The x-axis gives the number of carbon atoms, and the y-axis gives the free energies for transfer. Redrawn from Tanford, C., *The Hydrophobic Effect: Formation of Micelles and Biological Membranes*, 2nd ed., Wiley, 1979, p. 16. © 1979. Reprinted with permission from John Wiley & Sons, Inc.

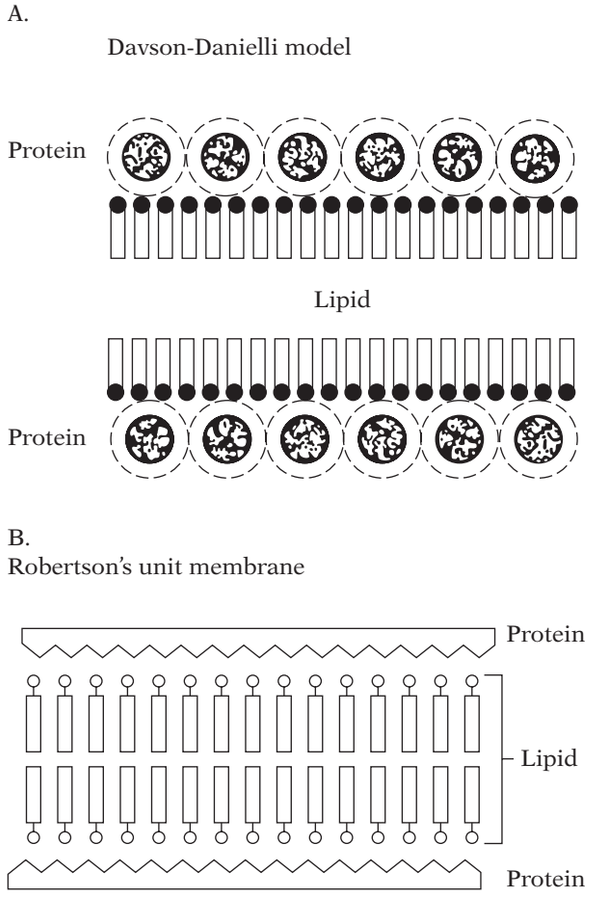
from the plot of ΔG_{tr} versus the chain length (Figure 1.4). Like other structures in biology, the aggregate structures of lipids are stabilized by the cooperative sum of many weak interactions. Thus the thermodynamic stability of the membrane bilayer maximizes water–water interactions outside and acyl chain interactions inside the nonpolar interior while minimizing water–acyl chain interactions that are entropically expensive.

The hydrophobic effect explains the energetics of membrane formation but does not address the basic structure of the biological membrane.

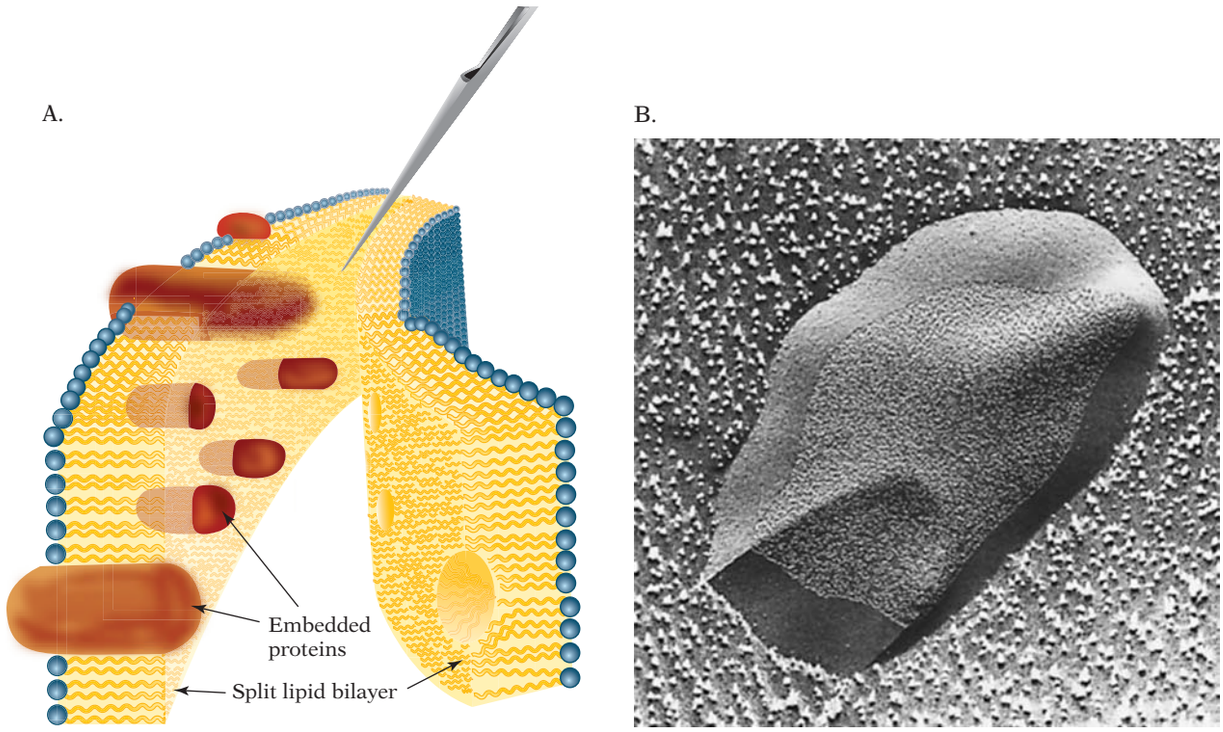
PARADIGM 2: THE FLUID MOSAIC MODEL DESCRIBES THE MEMBRANE STRUCTURE

While the Fluid Mosaic Model for the structure of membranes is now familiar to all life science students, the amazing unity it brought to a divided field is not apparent without an appreciation of its historical development. The earliest evidence for a lipid bilayer is attributed to Ben Franklin's calculation of the thickness of an olive oil film on pond water as 25 Å (2.5 nm). Then in 1925 Gorter and Grendel made surface area measurements for a compressed monolayer formed by acetone-extracted lipid from erythrocytes. Their conclusion that the monolayer area covered twice the surface area of the erythrocytes was correct, in spite of experimental errors that offset each other!

In 1935 Davson and Danielli used thermodynamic arguments along with measurements of surface tension and permeability to postulate a membrane structure that placed globular proteins on the outer surfaces of a membrane bilayer (Figure 1.5A). This model dominated



1.5. Early models for the structure of biological membranes. **A**. The Davson-Danielli membrane model with layers of globular proteins outside the lipid bilayer. © 1935. Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc. **B**. The unit membrane proposed by Robertson had the protein as β-sheets, still outside the lipid bilayer. © 1966. Reprinted with permission of Blackwell Publishing. **C**. In contrast, the Benson-Green model for the mitochondrial inner membrane showed protein particles that are solvated by lipids and are readily fractionated into complexes. © 1983 by Academic Press. Reprinted with permission from Elsevier. **A** and **B** redrawn from Gennis, R. B., *Biomembranes: Molecular Structure And Function*, Springer-Verlag, 1989, p. 8. **C** redrawn from Aloia, R. C., *Membrane Fluidity in Biology*, vol. I, Academic Press, 1983, p. 119.

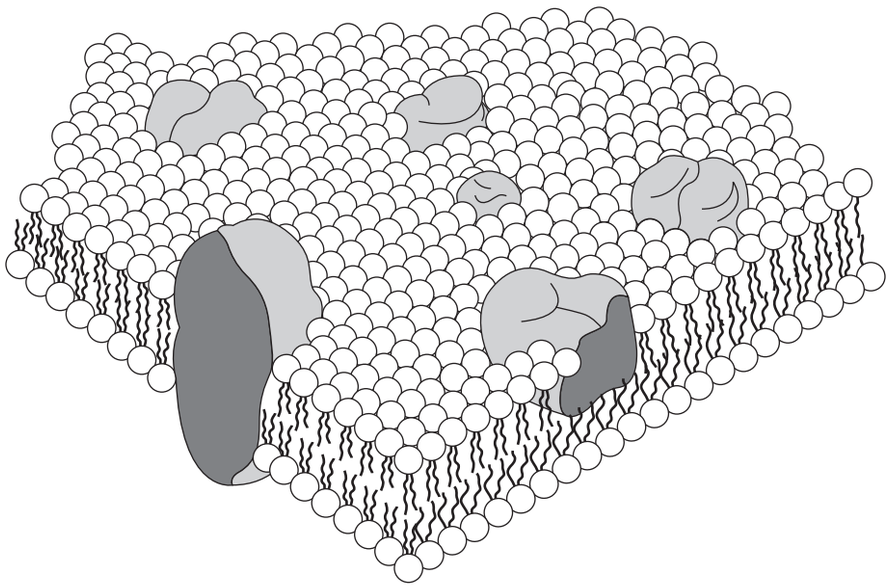


1.6. Visualization of the distribution of proteins in membranes. **A**. The freeze-fracture technique reveals the interior of a biological membrane by splitting a frozen membrane sample with a cold microtome knife. Redrawn from Voet, D., and J. Voet, *Biochemistry*, 3rd ed., John Wiley, 2004, p. 405. © 2004. Reprinted with permission from John Wiley & Sons, Inc. **B**. Electron microscopy of an erythrocyte plasma membrane split by freeze fracture shows the inner surface of the membrane is studded with embedded proteins. From Voet, D., J. Voet, and C. W. Pratt, *Fundamentals of Biochemistry* Upgrade Ed., John Wiley, p. 247. © 2002. Reprinted with permission of John Wiley and Sons and Vincent Marchesi, Yale University.

thinking about membrane structure for the next three decades, with modifications such as changing the protein conformation to extended β-sheets, and led to the concept of a "unit membrane" with a width of 6 to 8 nm, corresponding to the width of myelin sheath in x-ray diffraction measurements (Figure 1.5B). In 1959 Robertson argued that this unit membrane was common to all biological membranes, citing "railroad track" images from thin section electron microscopy (EM) of tissues stained with osmium tetroxide, which stained the phosphates of phospholipid headgroups and washed proteins out (see Frontispiece). Other staining techniques in use at the time, such as prior cross-linking with glutaraldehyde, produced images in which the full membrane was electron dense.

A challenge to the Davson-Danielli-Robertson model came with the application of freeze-fracture techniques: bumps visible by EM when the membrane was cleaved within the plane of the bilayer were attributed to embedded proteins (Figure 1.6). Support for the interpretation that the bumps were proteins came from their absence in membranes treated with proteases and in samples of myelin sheath, which has very little protein. In studies of the respiratory chain of mitochondria by Benson and later Green, mitochondrial inner membrane could be separated into lipoprotein subunits and reconstituted to regain activity. These results supported a model in which the lipid is solvent for embedded, globular proteins, consistent with EM images obtained after negative staining with heavy metals that showed subunits (not "railroad tracks") that were unaffected by lipid extraction prior to staining. Thus the Benson-Green subunit model was the antithesis of the Davson-Danielli-Robertson model (Figure 1.5C).

Today it is hard to realize the extent of controversy that occurred. As Singer and Nicolson wrote in 1972, "Some investigators who, impressed with the great diversity of membrane compositions and functions, do not think there are any useful generalizations to be made even about the gross structure of cell membranes . . ." Of course, their now-classic paper on membrane structure did present a general model for the structure of biomembranes – the Fluid Mosaic Model – which is included in every modern biochemistry and biology textbook (Figure 1.7). Their paper should be



1.7. The Fluid Mosaic Model proposed by Singer and Nicolson. The basic structure of the membrane is a lipid bilayer, with the fatty acyl chains from each leaflet forming a nonpolar interior. Intrinsic proteins are integral to the bilayer while extrinsic proteins are on its periphery. Redrawn from Singer, S. J., and G. L. Nicolson, *Science*. 1972, 175:720–731.

read in full, for it provides a beautiful example of examining all the biomembrane's properties conducive to testing with available techniques and summarizing the results in a consistent model.

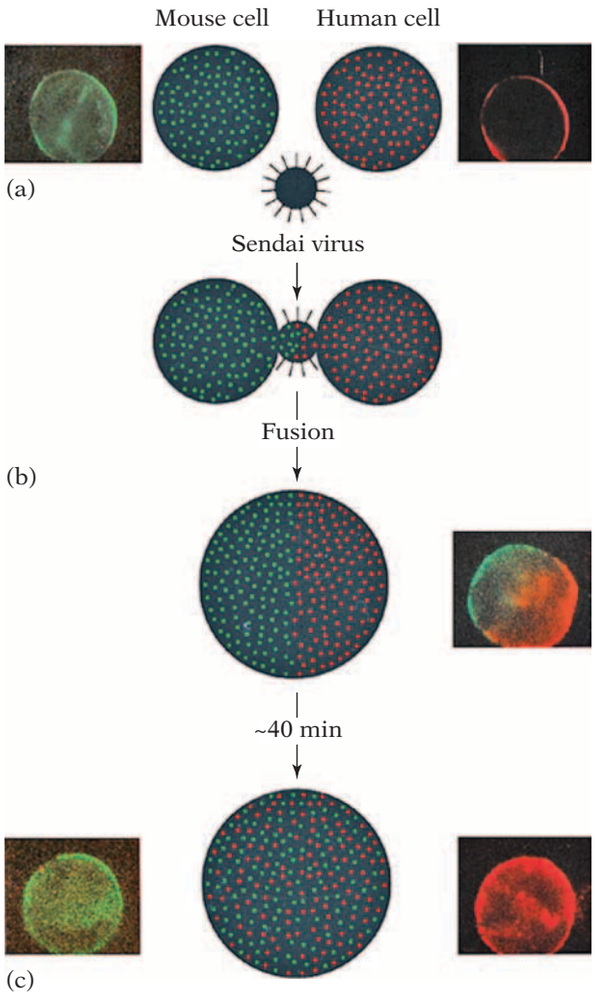
In addition to the thermodynamic principles and EM results discussed above, Singer and Nicolson emphasized the lateral mobility of membrane components. Significant lateral diffusion of membrane proteins had been demonstrated in the elegant Frye-Edidin experiment that followed the mixing of surface antigens in cell fusion experiments (Figure 1.8), and the rates of diffusion of lipids in the plane of the membrane were being measured by fluorescence techniques (discussed in Chapter 2). Singer and Nicolson also described the limited transverse mobility of lipids and the lack of it for proteins; the permeability barrier provided by the membrane; the structure of membrane proteins based on circular dichroism, x-ray diffraction, and labeling experiments (revealing them to be α-helical, globular, and membrane spanning); the assays of certain enzymes that require lipids for activity; and the phase transitions detected with differential calorimetry.

Based on these results, their Fluid Mosaic Model puts forth simple principles: the bulk of the lipid forms the bilayer, which provides the solvent for embedded proteins; most of the proteins are embedded and globular, termed *intrinsic* or *integral* membrane proteins. Some proteins are *extrinsic* (*peripheral*) as they can be removed by washes that change the pH or ionic strength. The bilayer, composed of two lipid layers, or leaflets, is fluid; in fact, it has the viscosity of olive oil, which allows lateral mobility of lipids and some protein components. It is mosaic in that proteins are scattered across it or on its surface. Both lipids and integral membrane proteins are amphipathic, allowing the nonpolar portions of proteins and lipids to interact and the polar portions of proteins and lipids to interact.

This widely accepted model for membrane structure is often abbreviated as a picture of integral proteins floating as icebergs in a sea of lipids, an oversimplification that denigrates the role of the lipids, whose diversity and polymorphic phases provide particular chemical activities as well as structural domains in that "sea," as the next section asserts. Furthermore, this simple picture obscures the wide variation in membrane composition (not overlooked in the original paper by Singer and Nicolson!). As Table 1.1 shows, the proportion of membrane components varies from ~80% lipid and ~20% protein (myelin) to ~75% protein and ~25% lipid (mitochondrial inner membrane). A rough calculation for the mitochondrial inner membrane suggests that these membranes have on the order of 100 lipid molecules per protein. Because it requires at least 40 to 50 lipid molecules to form a single belt of lipid around a protein, clearly this is not enough lipid to solvate individual proteins and provide a "sea" in which they float. So how does the mitochondrial inner membrane fit the model? First, the total protein given in Table 1.1 includes peripheral proteins. In the mitochondrial inner membrane over half the proteins are peripheral, leaving much less embedded in the lipid bilayer. Second, the protein–protein interactions between integral proteins

exclude bulk lipid; thus the lipid solvates the respiratory complexes, not each individual protein. No wonder scientists who concentrated on this membrane argued strongly for the subunit model!

While much additional work has contributed support for the Fluid Mosaic Membrane, the uniform mixing of bilayer lipids has been challenged by experimental observations of lipid heterogeneity based on the physical measurements of phase separations, as well as the detection of membrane domains with separate functions. Today there is wide acceptance of a shift in the paradigm that allows membranes to have specialized microdomains called *lipid rafts*.



1.8. Diffusion of membrane components after cell fusion. Human and mouse antigens are labeled with red and green fluorescent markers, respectively. Virus-stimulated fusion of the mouse cell and human cell (a) produces a heterokaryon with both types of antigen on its surface (b). After 40 minutes, the red and green markers have fully intermingled (c). From Voet, D., and J. Voet, *Biochemistry*, 3rd ed., John Wiley, 2004, p. 4–5. © 2004. Reprinted with permission from John Wiley & Sons, Inc, and the Company of Biologists.

TABLE 1.1. Composition of membrane preparations by percent dry weight[a]

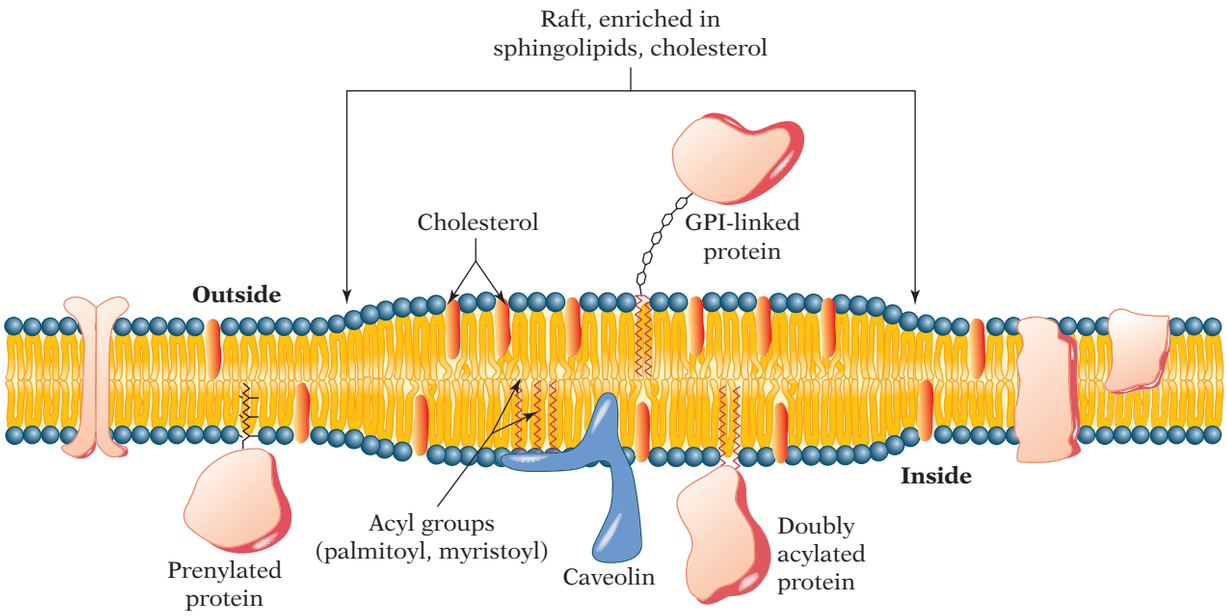
Source	Lipid	Protein	Cholesterol
Rat liver			
Plasma	30–50	50–70	20
Rough ER	15–30	60–80	6
Smooth ER	60	40	10
Inner mitochondria	20–25	70–80	<3
Outer mitochondria	30–40	60–70	<5
Nuclear	15–40	60–80	10
Golgi	60	40	8
Lysosomes	20–25	70–80	14
Rat brain			
Myelin	60–70	20–30	22
Synaptosome	50	50	20
Rat erythrocyte	40	60	24
Rat rod outer segment	50	40	<3
Escherichia coli	20–30	70	0
Bacillus subtilis	20–30	70	0
Chloroplast	35–50	50–65	0

[a] The percentages by weight of membrane preparations from various eukaryotic and prokaryotic sources are given.
ER, endoplasmic reticulum.
Source: Based on Jain, M. K., and R. C. Wagner, *Introduction to Biological Membranes*, 2nd ed. New York: Wiley, 1988, p. 34.

A SHIFT IN THE PARADIGM: BIOMEMBRANES HAVE LATERAL DOMAINS THAT FORM "RAFTS"

In addition to the wide variation in composition shown in Table 1.1, many biomembranes have protein-rich domains and other domains. In fact, some membranes are so rich in a particular protein, they contain quasi-crystalline arrays of that protein, such as bacteriorhodopsin in the purple membrane of halobacteria and porins in the outer membrane of Gram-negative bacteria (see "Bacteriorhodopsin" and "Porins" in Chapter 5). Furthermore, protein-rich domains often need particular lipid species, because some proteins require specific lipids in their boundary layer. The boundary layer of lipids, also called the annulus, is an old concept that is supported by much data from activity assays and electron spin resonance studies and more recently by x-ray structures (see "Protein–Lipid Interactions" in Chapter 4 and "Lipids Observed in X-ray Structures of Membrane Proteins" in Chapter 8). As Singer and Nicolson pointed out, specific lipid–protein interactions play important roles in the annulus. They did not anticipate that such interactions could extend the mosaic nature of the membrane to include functionally important lateral domains selective in terms of *both* protein and lipid components, which was unexpected in view of their emphasis on the fluidity of the bilayer.

Since 1972 a number of new techniques have been developed to measure the fluidity of model membranes.



1.9. Lipid rafts. Membranes have stable but transient microdomains that are enriched in cholesterol and sphingolipids, along with glycosylphosphatidylinositol (GPI)-linked proteins and proteins anchored by acyl groups. From Nelson, D. L., and M. M. Cox (eds.), *Lehninger Principles of Biochemistry*, 4th ed., W. H. Freeman, 2005, p. 385. © 2005 by W. H. Freeman and Company. Used with permission.

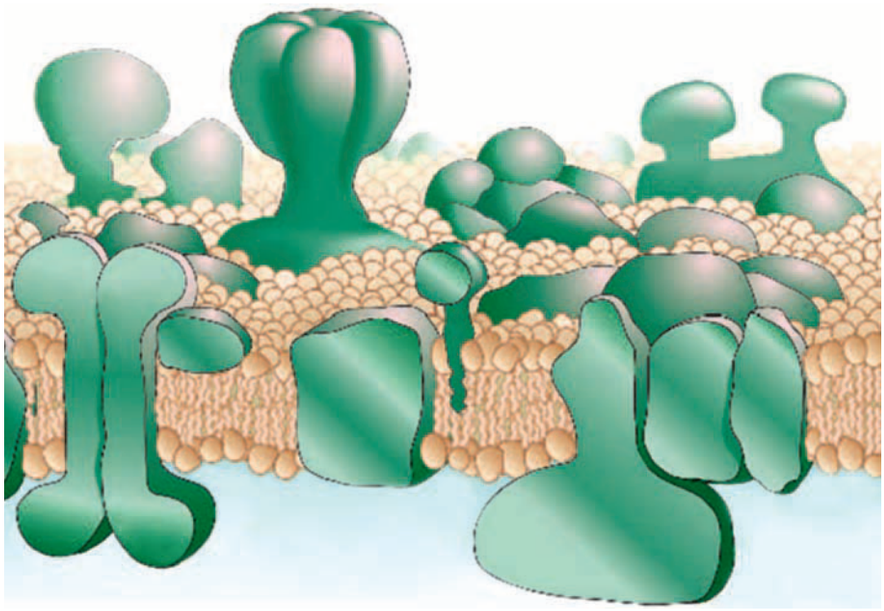
The physical definition of *fluidity* is the inverse of viscosity in an isotropic fluid, a liquid in which movement in all directions is equivalent. This definition does not directly apply to the membrane, which is highly anisotropic with a two-dimensional lipid bilayer as its base. Furthermore, the variation along the membrane normal (perpendicular to the bilayer) means the center is nearly isotropic, but a few angstroms away it is highly ordered, so position-dependent parameters are required. Therefore, measurements of membrane fluidity give results that depend on the method used, the probe for fluidity, and the conditions.

More recently, considerable lateral heterogeneity in lipid bilayers has been detected employing newer techniques such as fluorescence recovery after photobleaching, single-particle tracking, and now mass spectrometry imaging. Characterization of "liquid-ordered" microdomains in biological membranes indicates there are lateral domains with less fluidity, which form transient membrane "rafts" apart from the rest of the fluid bilayer (see "Organization of Bilayer Lipids" and "Lateral Domains and Lipid Rafts" in Chapter 2). Rafts are formed in the plasma membrane of many cell types as well as in many intracellular membranes. Although their composition varies, in general they are enriched in cholesterol and sphingolipids, which makes them thicker than the bulk membrane (Figure 1.9). They are also enriched with certain lipid-anchored proteins. Because many raft proteins are involved in signaling and trafficking, their transient associations have profound biological implications.

A VIEW FOR THE FUTURE: DYNAMIC PROTEIN COMPLEXES CROWD THE MEMBRANE INTERIOR AND EXTEND ITS BORDERS

Even with the addition of microdomains of different sizes, lifetimes, and functions, the model of the fluid mosaic membrane is incomplete. While the emphasis on lipid rafts focused attention on the lateral organization of the membrane, a variety of both old and new findings indicate the transverse organization across the plane of the membrane is complex as well. The new view of the membrane acknowledges variation in this transverse direction, encompasses layers outside the bilayer itself, and recognizes the activities going on at its borders.

The important activities occurring at the surfaces, along with striking differences across the bilayer, emphasize the significance of the third dimension of the membrane. Thus the membrane is more than a layer of proteins embedded in a lipid bilayer. Crucial functions are carried out by complexes involving interactions between integral and peripheral proteins at the interfaces. Many of the proteins are oligomers that operate in large assemblies in the membrane. Many large protein complexes operate in very close quarters in normally crowded biomembranes.



1.10. Peripheral proteins and complexes. Membranes encompass not only the bilayer but also peripheral proteins and, in the case of plasma membranes, the cytoskeleton. Typically crowded with proteins, membranes contain many complexes that extend beyond the bilayer. Engelman, D., *Nature*. 2005, 438:578–580. © 2005. Reprinted with permission of Macmillan Publishers Ltd.

To start to describe this complexity, researchers are mapping the microenvironments found along a line extending perpendicular to the plane of the bilayer at different sites along biological membranes. The asymmetry in lipid compositions of the inner and outer leaflets was detected long ago, yet new results show it is associated with complex patterns of lipid trafficking that can turn over components of the plasma membrane each hour. Also familiar for years has been the complex cytoskeleton on the internal surface of the eukaryotic plasma membrane that provides structural support and limits the mobility of some membrane proteins. In Gram-negative bacteria lipoproteins from the outer membrane provide a similar structural support through their links to the underlying peptidoglycan. These are stable structures at the borders of membranes. Gaining attention today are important transient associations with peripheral proteins along the surface of many biomembranes.

Even the picture of the lipid bilayer itself has been revised from the "lollipop" depiction of lipids in most drawings. Sophisticated analyses of diffraction data and computational modeling (described in Chapter 8) present a new picture of the bilayer in which the nonpolar domain, defined as the center that is free of water, is only about half of its thickness. Each interfacial region, made up of lipid headgroups and amphiphilic domains of proteins and containing some water molecules, contributes another quarter. Furthermore, these regions are the dynamic playgrounds for lipid-metabolizing enzymes and other proteins that insert into the bilayer (described in Chapter 4.)

The exterior surface of many cell membranes is crowded with peripheral proteins that interact at the interface of the bilayer. Many of these proteins have activities that are regulated by binding the membrane in dynamic cycles (see "Amphitropic Proteins" in Chapter 4). Their specific binding is mediated by highly conserved motifs and often by divalent cations. Thus, the focus of membrane research has expanded to include the membrane periphery as an additional important layer of the membrane.

Large complexes made up of integral membrane proteins and associated peripheral proteins carry out many functions of the membrane (see Chapter 11). The typical biomembrane is crowded with protein assemblies, many of which are tightly associated heterooligomers (Figure 1.10). Furthermore, some complexes are large enough to span two membranes, either from the same cell or organelle, seen in the double-membrane systems of Gram-negative bacteria and mitochondria; from different cells, such as at gap junctions; or from a cell and a virus, as observed in the fusion events enabling viral penetration. Future challenges in understanding membrane functions include characterizing these larger functional membrane complexes and must not neglect the essential activities at the bilayer interface and on its surface (see Chapter 12).