CHAPTER **ELEVEN** **11**

Membrane Structure

A living cell is a self-reproducing system of molecules held inside a container. That container is the **plasma membrane**—a protein-studded, fatty film so thin that it cannot be seen directly in the light microscope. Every cell on Earth uses such a membrane to separate and protect its chemical components from the outside environment. Without membranes, there would be no cells, and thus no life.

The structure of the plasma membrane is simple: it consists of a two-ply sheet of lipid molecules about 5 nm—or 50 atoms—thick, into which proteins have been inserted. Its properties, however, are unlike those of any sheet of material we are familiar with in the everyday world. Although it serves as a barrier to prevent the contents of the cell from escaping and mixing with molecules in the surrounding environment (**Figure 11–1**), the plasma membrane does much more than that. If a cell is to survive and grow, nutrients must pass inward across the plasma membrane, and waste products must make their way out. To facilitate this

THE LIPID BILAYER

MEMBRANE PROTEINS

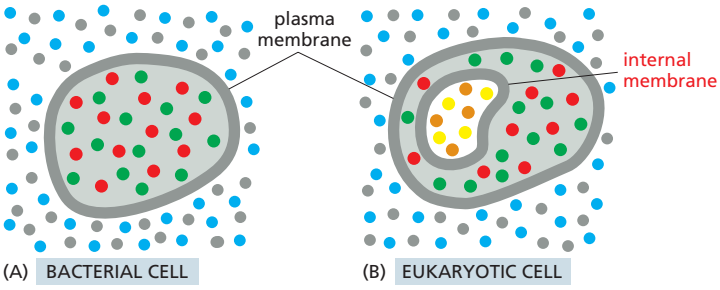


Figure 11–1 Cell membranes act as selective barriers. The plasma membrane separates a cell from its surroundings, enabling the molecular composition of a cell to differ from that of its environment. (A) In some bacteria, the plasma membrane is the only membrane. (B) In addition to a plasma membrane, eukaryotic cells also have internal membranes that enclose individual organelles. All cell membranes prevent molecules on one side from freely mixing with those on the other, as indicated schematically by the colored dots.

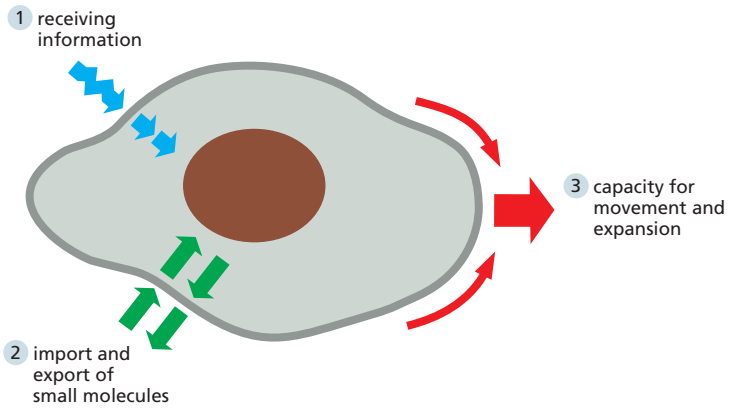


Figure 11–2 The plasma membrane is involved in cell communication, import and export of molecules, and cell growth and motility. (1) Receptor proteins in the plasma membrane enable the cell to receive signals from the environment; (2) channels and transporters in the membrane enable the import and export of small molecules; (3) the flexibility of the membrane and its capacity for expansion allow the cell to grow, change shape, and move.

exchange, the membrane is penetrated by highly selective channels and transporters—proteins that allow specific, small molecules and ions to be imported and exported. Other proteins in the membrane act as sensors, or receptors, that enable the cell to receive information about changes in its environment and respond to them in appropriate ways. The mechanical properties of the plasma membrane are equally impressive. When a cell grows, so does its membrane: this remarkable structure enlarges in area by adding new membrane without ever losing its continuity, and it can deform without tearing, allowing the cell to move or change shape (**Figure 11–2**). The membrane is also self-healing: if it is pierced, it neither collapses like a balloon nor remains torn; instead, the membrane quickly reseals.

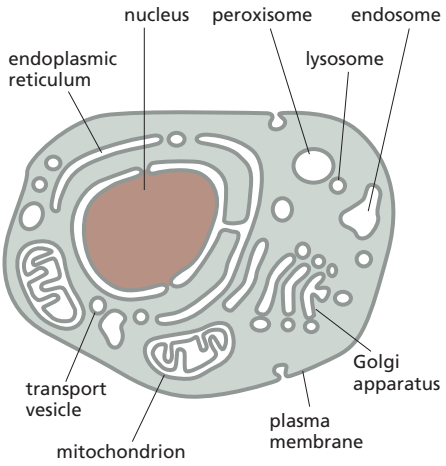


Figure 11–3 Internal membranes form many different compartments in a eukaryotic cell. Some of the main membrane-enclosed organelles in a typical animal cell are shown here. Note that the nucleus and mitochondria are each enclosed by two membranes.

As shown in Figure 11–1, many bacteria have only a single membrane—the plasma membrane—whereas eukaryotic cells also contain *internal membranes* that enclose intracellular compartments. The internal membranes form various organelles, including the endoplasmic reticulum, Golgi apparatus, endosomes, and mitochondria (**Figure 11–3**). Although these internal membranes are constructed on the same principles as the plasma membrane, they differ subtly in composition, especially in their resident proteins.

Regardless of their location, all cell membranes are composed of lipids and proteins and share a common general structure (**Figure 11–4**). The lipids are arranged in two closely apposed sheets, forming a *lipid bilayer* (see Figure 11–4B). This lipid bilayer serves as a permeability barrier to most water-soluble molecules, while the proteins embedded within it carry out the other functions of the membrane and give different membranes their individual characteristics.

In this chapter, we consider the structure of biological membranes and the organization of their two main constituents: lipids and proteins. Although we focus mainly on the plasma membrane, most of the concepts we discuss also apply to internal membranes. The functions of cell membranes, including their role in cell communication, the transport of small molecules, and energy generation, are considered in later chapters.

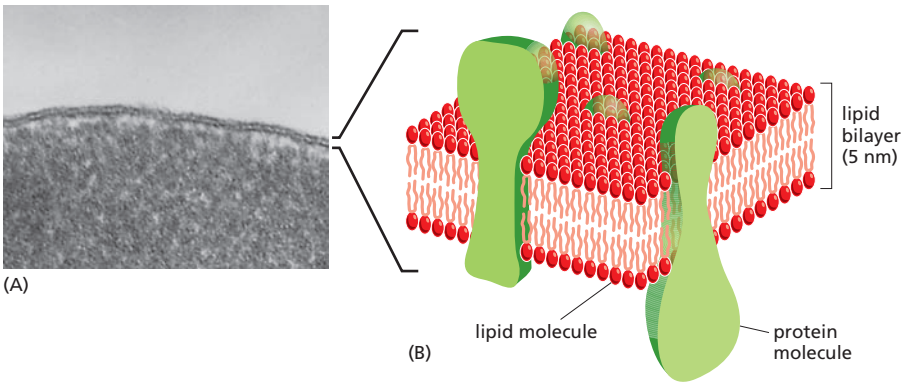


Figure 11–4 A cell membrane consists of a lipid bilayer in which proteins are embedded. (A) An electron micrograph of a plasma membrane of a human red blood cell seen in cross section. In this image, the proteins that extend from either side of the bilayer form the two closely spaced dark lines indicated by the brackets; the thin, white layer between them is the lipid bilayer. (B) Schematic drawing showing a three-dimensional view of a cell membrane. (A, by permission of E.L. Bearer.)

THE LIPID BILAYER

Because cells are filled with—and surrounded by—water, the structure of cell membranes is determined by the way membrane lipids behave in a watery (aqueous) environment. Lipid molecules are not very soluble in water, although they do dissolve readily in organic solvents such as benzene. It was this property that scientists exploited in 1925, when they set out to investigate how lipids are arranged in cell membranes.

Using benzene, investigators extracted all the lipids from the plasma membranes of purified red blood cells. These lipids were then spread out in a film on the surface of a trough filled with water, like an oil slick on a puddle. Using a movable barrier, the researchers then pushed the floating lipids together until they formed a continuous sheet only one molecule thick. When the investigators measured the surface area of this monolayer, they found that it occupied twice the area of the original, intact cells. Based on this observation, they deduced that, in an intact cell membrane, lipid molecules must double up to form a bilayer—a finding that had a profound influence on cell biology.

In this section, we take a closer look at this **lipid bilayer**, which constitutes the fundamental structure of all cell membranes. We consider how lipid bilayers form, how they are maintained, and how their properties establish the general properties of all cell membranes.

Membrane Lipids Form Bilayers in Water

The lipids found in cell membranes combine two very different properties in a single molecule: each lipid has a hydrophilic ("water-loving") head and a hydrophobic ("water-fearing") tail. The most abundant lipids in cell membranes are the **phospholipids**, which have a phosphate-containing, hydrophilic head linked to a pair of hydrophobic, hydrocarbon tails (**Figure 11–5**). For example, **phosphatidylcholine**, one of the most abundant phospholipids in the membranes of animals and plants, has the small molecule choline attached to a phosphate group as its hydrophilic head (**Figure 11–6**).

Phospholipids are not the only membrane lipids that are **amphipathic**, a term used to describe molecules with both hydrophilic and hydrophobic parts. Cholesterol, which is found in animal cell membranes, and *glycolipids*, which have sugars as part of their hydrophilic head, are also amphipathic (**Figure 11–7**).

Having both hydrophilic and hydrophobic parts plays a crucial part in driving lipid molecules to assemble into bilayers in an aqueous environment.

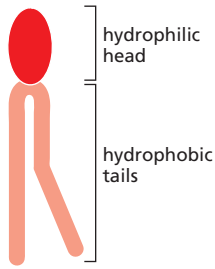


Figure 11–5 Cell membranes are packed with phospholipids. A typical membrane phospholipid molecule has a hydrophilic head and two hydrophobic tails.

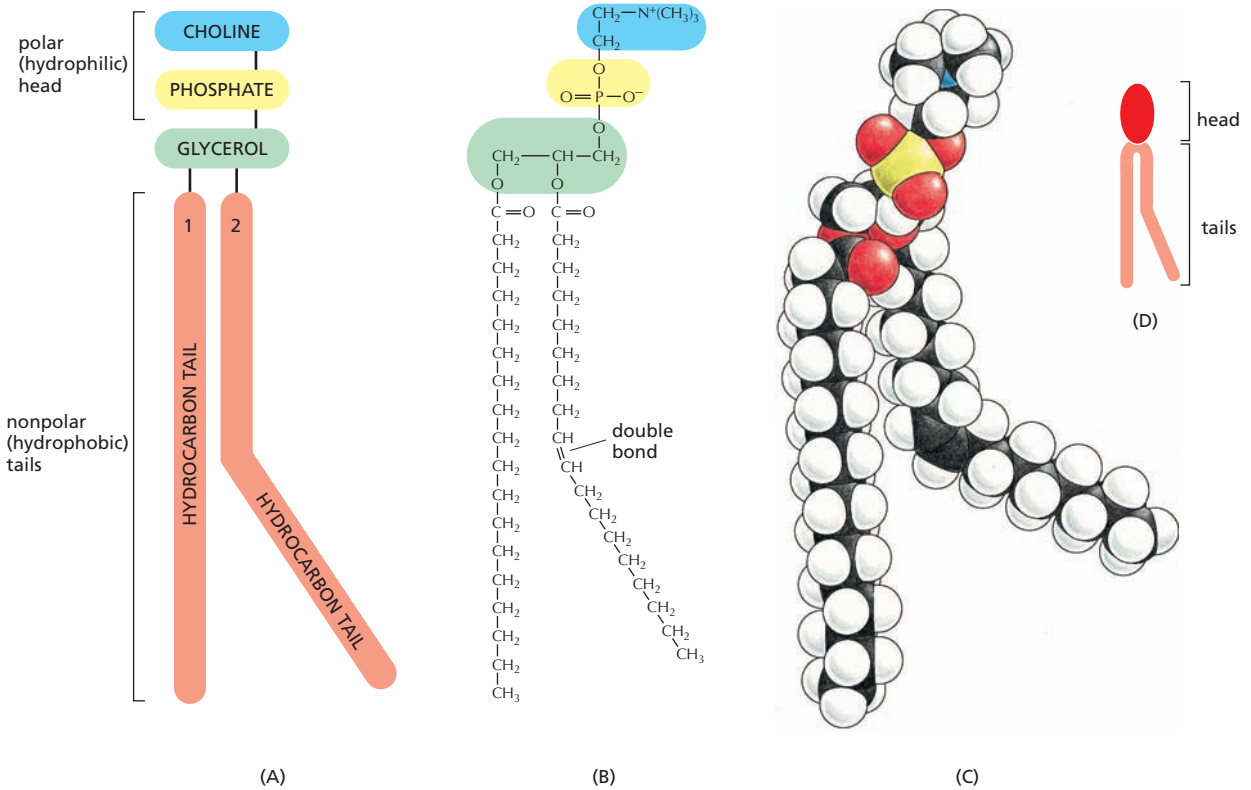


Figure 11–6 Phosphatidylcholine is the most common phospholipid in cell membranes. It is represented schematically in (A), as a chemical formula in (B), as a space-filling model in (C), and as a symbol in (D). This particular phospholipid is built from five parts: the hydrophilic head, which consists of *choline* linked to a *phosphate* group; two *hydrocarbon chains*, which form the hydrophobic tails; and a molecule of glycerol, which links the head to the tails. Each of the hydrophobic tails is a *fatty acid*—a hydrocarbon chain with a carboxyl (–COOH) group at one end; glycerol attaches via this carboxyl group, as shown in (B). A kink in one of the hydrocarbon chains occurs where there is a double bond between two carbon atoms. (The "phosphatidyl" part of the name of a phospholipid refers to the phosphate–glycerol–fatty acid portion of the molecule.)

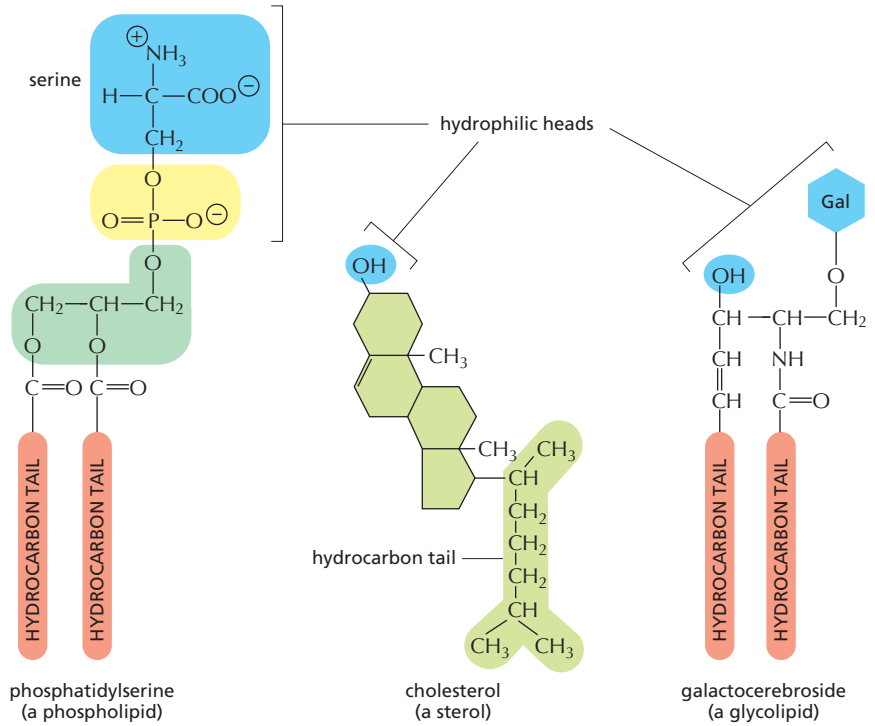


Figure 11–7 Different types of membrane lipids are all amphipathic. Each of the three types shown here has a hydrophilic head and one or two hydrophobic tails. The hydrophilic head is serine phosphate (shaded *blue* and *yellow*) in phosphatidylserine, an –OH group (*blue*) in cholesterol, and the sugar galactose plus an –OH group (both *blue*) in galactocerebroside. See also Panel 2–4, pp. 72–73.

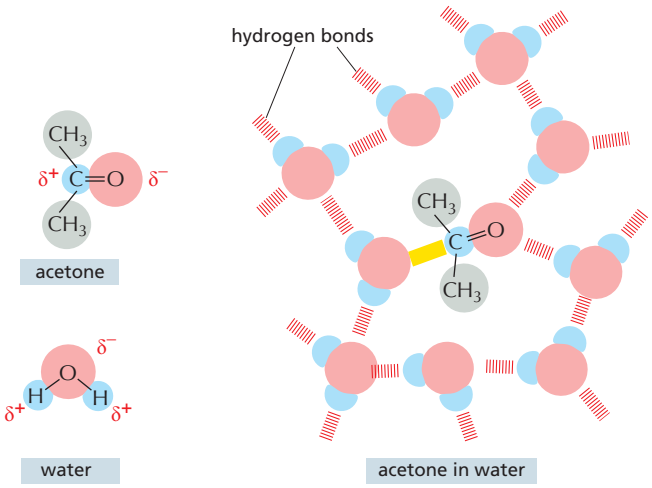


Figure 11–8 A hydrophilic molecule attracts water molecules. Both acetone and water are polar molecules: thus acetone readily dissolves in water. Polar atoms are shown in *red* and *blue*, with δ^- indicating a partial negative charge, and δ^+ indicating a partial positive charge. Hydrogen bonds (*red*) and an electrostatic attraction (*yellow*) form between acetone and the surrounding water molecules. Nonpolar groups are shown in *gray*.

As discussed in Chapter 2 (see Panel 2–2, pp. 68–69), hydrophilic molecules dissolve readily in water because they contain either charged groups or uncharged polar groups that can form electrostatic attractions or hydrogen bonds with water molecules (**Figure 11–8**). Hydrophobic molecules, by contrast, are insoluble in water because all—or almost all—of their atoms are uncharged and nonpolar; they therefore cannot form favorable interactions with water molecules. Instead, they force adjacent water molecules to reorganize into a cagelike structure around them (**Figure 11–9**). Because this cagelike structure is more highly ordered than the rest of the water, its formation requires free energy. This energy cost is minimized when the hydrophobic molecules cluster together, limiting their contacts with the surrounding water molecules. Thus, purely hydrophobic molecules, like the fats found in the oils of plant seeds and the adipocytes (fat cells) of animals (**Figure 11–10**), coalesce into large **fat droplets** when dispersed in water.

QUESTION 11–1

Water molecules are said "to reorganize into a cagelike structure" around hydrophobic compounds (e.g., see Figure 11–9). This seems paradoxical because water molecules do not interact with the hydrophobic compound. So how could they "know" about its presence and change their behavior to interact differently with one another? Discuss this argument and, in doing so, develop a clear concept of what is meant by a "cagelike" structure. How does it compare to ice? Why would this cagelike structure be energetically unfavorable?

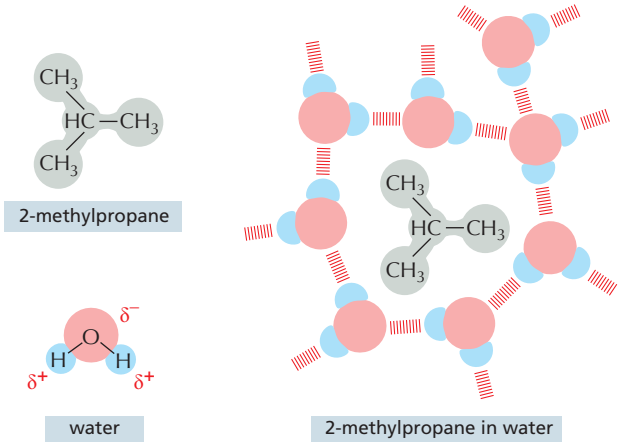


Figure 11–9 A hydrophobic molecule tends to avoid water. Because the 2-methylpropane molecule is entirely hydrophobic, it cannot form favorable interactions with water. This causes the adjacent water molecules to reorganize into a cagelike structure around it, to maximize their hydrogen bonds with each other.

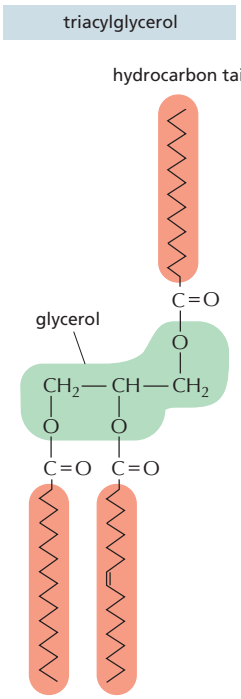


Figure 11–10 Fat molecules are entirely hydrophobic. Unlike phospholipids, triacylglycerols, which are the main constituents of animal fats and plant oils, are entirely hydrophobic. Here, the third hydrophobic tail of the triacylglycerol molecule is drawn facing upward for comparison with the structure of a phospholipid (see Figure 11–6), although normally it is depicted facing down (see Panel 2–5, pp. 74–75).

Amphipathic molecules, such as membrane lipids (see Figure 11–7), are subject to two conflicting forces: the hydrophilic head is attracted to water, while the hydrophobic tails shun water and seek to aggregate with other hydrophobic molecules. This conflict is beautifully resolved by the formation of a lipid bilayer—an arrangement that satisfies all parties and is energetically most favorable. The hydrophilic heads face water on both surfaces of the bilayer, while the hydrophobic tails are shielded from the water within the bilayer interior, like the filling in a sandwich (**Figure 11–11**).

The same forces that drive the amphipathic molecules to form a bilayer help to make the bilayer self-sealing. Any tear in the sheet will create a free edge that is exposed to water. Because this situation is energetically unfavorable, the molecules of the bilayer will spontaneously rearrange to eliminate the free edge. If the tear is small, this spontaneous rearrangement will exclude the water molecules and lead to repair of the bilayer, restoring a single continuous sheet. If the tear is large, the sheet may begin to fold in on itself and break up into separate closed vesicles. In either case, the overriding principle is that free edges are quickly eliminated.

The prohibition on free edges has a profound consequence: the only way an amphipathic sheet can avoid having free edges is to bend and seal, forming a boundary around a closed space (**Figure 11–12**). Therefore, amphipathic molecules such as phospholipids necessarily assemble into self-sealing containers that define closed compartments—from vesicles and organelles to entire cells. This remarkable behavior, fundamental to the creation of a living cell, is essentially a by-product of the nature of membrane lipids: hydrophilic at one end and hydrophobic at the other.

The Lipid Bilayer Is a Flexible Two-dimensional Fluid

The aqueous environment inside and outside a cell prevents membrane lipids from escaping from the bilayer, but nothing stops these molecules from moving about and changing places with one another within the plane of the membrane. The lipid bilayer therefore behaves as a two-dimensional fluid, a fact that is crucial for membrane function and integrity (**Movie 11.1**; "laser tweezers" are explained in **Movie 11.2**).

At the same time, the lipid bilayer is also flexible—that is, it is able to bend. Like fluidity, flexibility is important for membrane function, and it

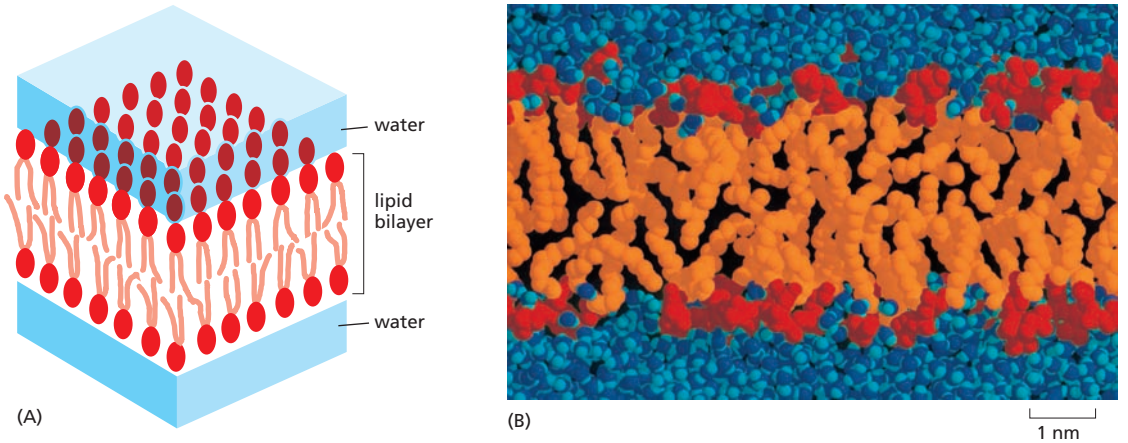


Figure 11–11 Amphipathic phospholipids form a bilayer in water. (A) Schematic drawing of a phospholipid bilayer in water. (B) Computer simulation showing the phospholipid molecules (*red* heads and *orange* tails) and the surrounding water molecules (*blue*) in a cross section of a lipid bilayer. (B, adapted from R.M. Venable et al., *Science* 262:223–228, 1993.)

sets a lower limit of about 25 nm to the vesicle diameter that cell membranes can form.

The fluidity of lipid bilayers can be studied using synthetic lipid bilayers, which are easily produced by the spontaneous aggregation of amphipathic lipid molecules in water. Pure phospholipids, for example, will form closed, spherical vesicles, called *liposomes*, when added to water; these vesicles vary in size from about 25 nm to 1 mm in diameter (**Figure 11–13**).

Using such simple synthetic bilayers, investigators can measure the movements of the lipid molecules in a lipid bilayer. These measurements reveal that some types of movement are rare, while others are frequent and rapid. Thus, in synthetic lipid bilayers, phospholipid molecules very rarely tumble from one half of the bilayer, or monolayer, to the other. Without proteins to facilitate the process, it is estimated that this event, called "flip-flop," occurs less than once a month for any individual lipid molecule under conditions similar to those in a cell. On the other hand, as the result of random thermal motions, lipid molecules continuously exchange places with their neighbors within the same monolayer. This exchange leads to rapid lateral diffusion of lipid molecules within the plane of each monolayer, so that, for example, a lipid in an artificial bilayer may diffuse a length equal to that of an entire bacterial cell (~2 μm) in about one second.

Similar studies show that individual lipid molecules not only flex their hydrocarbon tails, but they also rotate rapidly about their long axis—some reaching speeds of 500 revolutions per second. Studies of whole cells—and of isolated cell membranes—indicate that lipid molecules in cell membranes undergo the same movements as they do in synthetic bilayers. The movements of membrane phospholipid molecules are summarized in **Figure 11–14**.

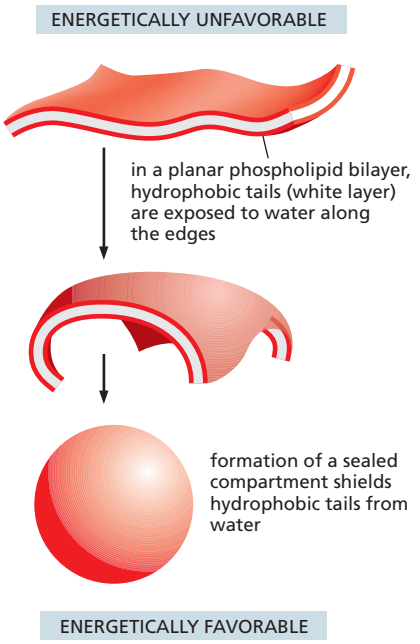


Figure 11–12 Phospholipid bilayers spontaneously close in on themselves to form sealed compartments. The closed structure is stable because it avoids the exposure of the hydrophobic hydrocarbon tails to water, which would be energetically unfavorable.

The Fluidity of a Lipid Bilayer Depends on Its Composition

The fluidity of a cell membrane—the ease with which its lipid molecules move within the plane of the bilayer—is important for membrane function and has to be maintained within certain limits. Just how fluid a lipid bilayer is at a given temperature depends on its phospholipid composition and, in particular, on the nature of the hydrocarbon tails: the closer and more regular the packing of the tails, the more viscous and less fluid the bilayer will be.

Two major properties of hydrocarbon tails affect how tightly they pack in the bilayer: their length and the number of double bonds they contain. A shorter chain length reduces the tendency of the hydrocarbon tails to interact with one another and therefore increases the fluidity of the bilayer. The hydrocarbon tails of membrane phospholipids vary in length between 14 and 24 carbon atoms, with 18 or 20 atoms being the most common. For most phospholipids, one of these hydrocarbon tails contains only single bonds between its adjacent carbon atoms, whereas the other tail includes one or more double bonds (see Figure 11–6). The chain that harbors a double bond does not contain the maximum number of hydrogen atoms that could, in principle, be attached to its carbon backbone; it is thus said to be **unsaturated** with respect to hydrogen. The

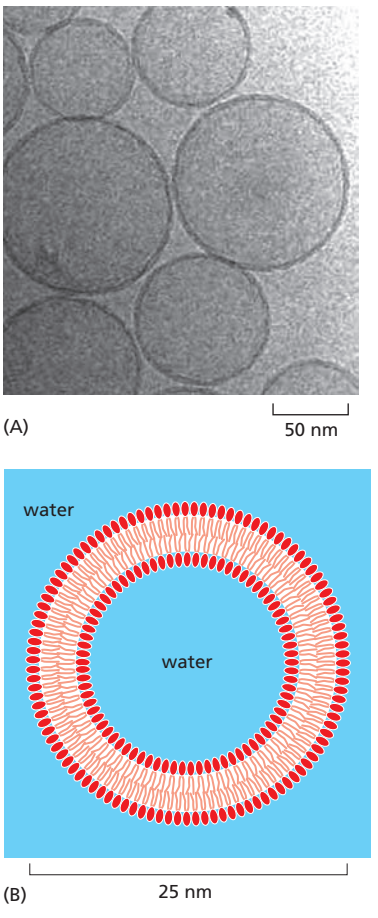


Figure 11–13 Pure phospholipids can form closed, spherical liposomes. (A) An electron micrograph of phospholipid vesicles, or liposomes. (B) A drawing of a small, spherical liposome seen in cross section. (A, courtesy of Jean Lepault.)

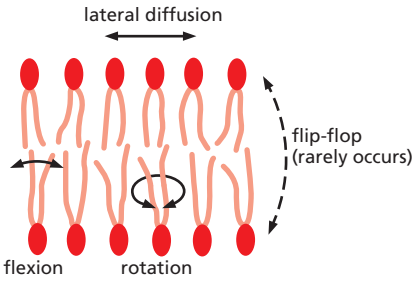


Figure 11–14 Membrane phospholipids move within the lipid bilayer. Because of these motions, the bilayer behaves as a two-dimensional fluid, in which the individual lipid molecules are able to move in their own monolayer. Note that lipid molecules do not move spontaneously from one monolayer to the other.

QUESTION 11–2

Five students in your class always sit together in the front row. This could be because (A) they really like each other or (B) nobody else in your class wants to sit next to them. Which explanation holds for the assembly of a lipid bilayer? Explain. Suppose, instead, that the other explanation held for lipid molecules. How would the properties of the lipid bilayer be different?

hydrocarbon tail with no double bonds has a full complement of hydrogen atoms and is said to be **saturated**. Each double bond in an unsaturated tail creates a small kink in the tail (see Figure 11–6), which makes it more difficult for the tails to pack against one another. For this reason, lipid bilayers that contain a large proportion of unsaturated hydrocarbon tails are more fluid than those with lower proportions.

In bacterial and yeast cells, which have to adapt to varying temperatures, both the lengths and the degree of saturation of the hydrocarbon tails in the bilayer are adjusted constantly to maintain a membrane with a relatively consistent fluidity: at higher temperatures, for example, the cell makes membrane lipids with tails that are longer and that contain fewer double bonds. A similar trick is used in the manufacture of margarine from vegetable oils. The fats produced by plants are generally unsaturated and therefore liquid at room temperature, unlike animal fats such as butter or lard, which are generally saturated and therefore solid at room temperature. To produce margarine, vegetable oils are "hydrogenated": this addition of hydrogen removes their double bonds, making the oils more solid and butterlike at room temperature.

In animal cells, membrane fluidity is modulated by the inclusion of the sterol **cholesterol**. This molecule is present in especially large amounts in the plasma membrane, where it constitutes approximately 20% of the lipids in the membrane by weight. With its short and rigid steroid ring structure, cholesterol can fill the spaces between neighboring phospholipid molecules left by the kinks in their unsaturated hydrocarbon tails (**Figure 11–15**). In this way, cholesterol tends to stiffen the bilayer, making it less flexible, as well as less permeable. The chemical properties of membrane lipids—and how they affect membrane fluidity—are reviewed in **Movie 11.3** and **Movie 11.4**.

For all cells, membrane fluidity is important for a number of reasons. It enables many membrane proteins to diffuse rapidly in the plane of the bilayer and to interact with one another, as is crucial, for example, in cell signaling (discussed in Chapter 16). It permits membrane lipids and proteins to diffuse from sites where they are inserted into the bilayer after their synthesis to other regions of the cell. It ensures that membrane molecules are distributed evenly between daughter cells when a cell divides. And, under appropriate conditions, it allows membranes to fuse with one another and mix their molecules (discussed in Chapter 15). If biological

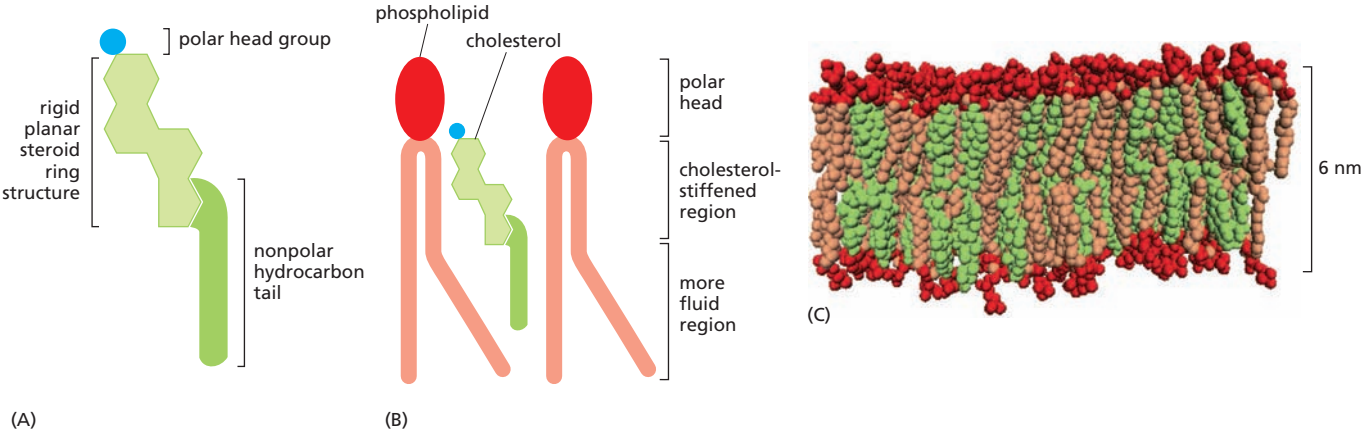
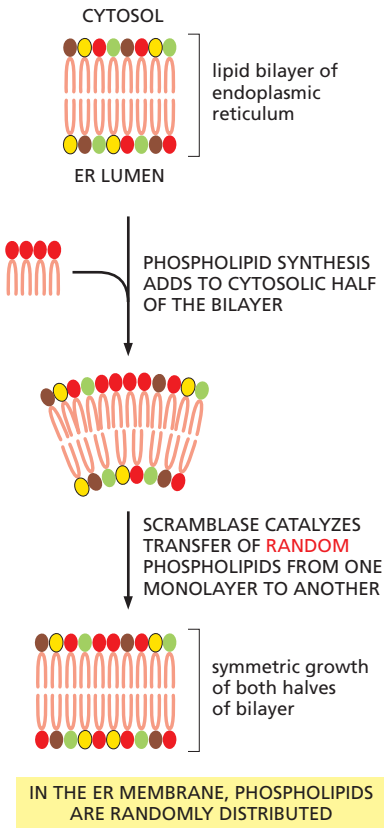


Figure 11–15 Cholesterol tends to stiffen cell membranes. (A) The shape of a cholesterol molecule. The chemical formula of cholesterol is shown in Figure 11–7. (B) How cholesterol fits into the gaps between phospholipid molecules in a lipid bilayer. (C) Space-filling model of the bilayer, with cholesterol molecules in *green*. Although the nonpolar hydrocarbon tail of cholesterol is shown in *green*—to visually distinguish it from the hydrocarbon tails of the membrane phospholipids—in reality, the hydrophobic tail of cholesterol is chemically equivalent to the hydrophobic tails of the phospholipids. (C, from H.L. Scott, *Curr. Opin. Struct. Biol.* 12:495–502, 2002.)



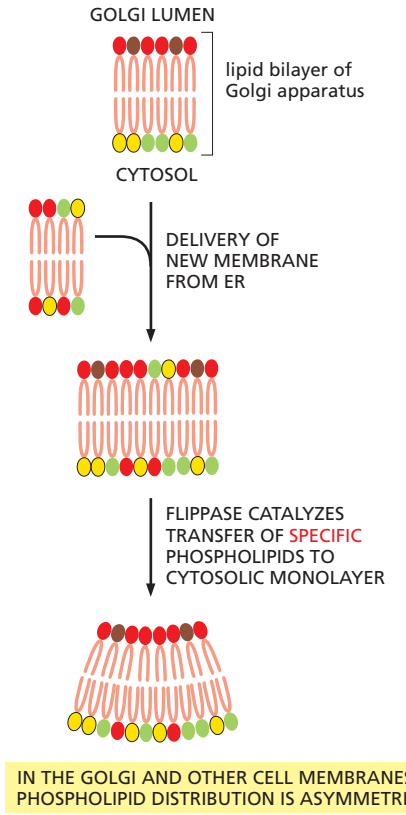


Figure 11–16 Newly synthesized phospholipids are added to the cytosolic side of the ER membrane and then redistributed by transporters that transfer them from one half of the lipid bilayer to the other. Biosynthetic enzymes bound to the cytosolic monolayer of the ER membrane (not shown) produce new phospholipids from free fatty acids and insert them into the cytosolic monolayer. Transporters called scramblases then randomly transfer phospholipid molecules from one monolayer to the other, allowing the membrane to grow as a bilayer in which the two leaflets even out continuously in size and lipid composition.

membranes were not fluid, it is hard to imagine how cells could live, grow, and reproduce.

Membrane Assembly Begins in the ER

In eukaryotic cells, new phospholipids are manufactured by enzymes bound to the cytosolic surface of the *endoplasmic reticulum* (*ER*). Using free fatty acids as substrates (see Panel 2–5, pp. 74–75), these enzymes deposit the newly made phospholipids exclusively in the cytosolic half of the bilayer.

Despite the unbalanced addition of newly made phospholipids, cell membranes manage to grow evenly. So how do new phospholipids make it to the opposite monolayer? As we saw in Figure 11–14, flip-flops that move lipids from one monolayer to the other rarely occur spontaneously. Instead, phospholipid transfers are catalyzed by a *scramblase*, a type of transporter protein that removes randomly selected phospholipids from one half of the lipid bilayer and inserts them in the other. (Transporters and their functions are discussed in detail in Chapter 12.) As a result of this scrambling, newly made phospholipids are redistributed equally between each monolayer of the ER membrane (**Figure 11–16**).

Some of this newly assembled membrane will remain in the ER; the rest will be used to supply fresh membrane to other compartments in the cell, including the Golgi apparatus and plasma membrane (see Figure 11–3). We discuss this dynamic process—in which membranes bud from one organelle and fuse with another—in detail in Chapter 15.

Certain Phospholipids Are Confined to One Side of the Membrane

Most cell membranes are asymmetric: the two halves of the bilayer often include strikingly different sets of phospholipids. But if membranes emerge from the ER with an evenly assorted set of phospholipids, where does this asymmetry arise? It begins in the Golgi apparatus.

The Golgi membrane contains another family of phospholipid-handling transporters, called *flippases*. Unlike scramblases, which move random phospholipids from one half of the bilayer to the other, flippases remove specific phospholipids from the side of the bilayer facing the exterior space and flip them into the monolayer that faces the cytosol (**Figure 11–17**).

Figure 11–17 Flippases help to establish and maintain the asymmetric distribution of phospholipids characteristic of animal cell membranes. When membranes leave the ER and are incorporated in the Golgi, they encounter a different set of transporters called flippases, which selectively remove phosphatidylserine (*light green*) and phosphatidylethanolamine (*yellow*) from the noncytosolic monolayer and flip them to the cytosolic side. This transfer leaves phosphatidylcholine (*red*) and sphingomyelin (*brown*) concentrated in the noncytosolic monolayer. The resulting curvature of the membrane may help drive subsequent vesicle budding.

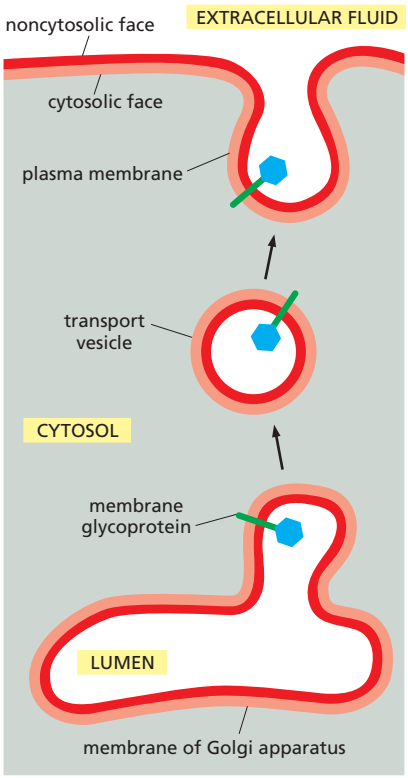


Figure 11–18 Membranes retain their orientation during transfer between cell compartments. Membranes are transported by a process of vesicle budding and fusing. Here, a vesicle is shown budding from the Golgi apparatus and fusing with the plasma membrane. Note that the orientations of both the membrane lipids and proteins are preserved during the process: the original cytosolic surface of the lipid bilayer (*pink*) remains facing the cytosol, and the noncytosolic surface (*red*) continues to face away from the cytosol, toward the lumen of the Golgi and the transport vesicle—or toward the extracellular fluid. Similarly, the glycoprotein shown here (*blue* and *green*) remains in the same orientation, with its attached sugar facing the noncytosolic side.

The action of these flippases—and of similar transporters in the plasma membrane—initiates and maintains the asymmetric arrangement of phospholipids that is characteristic of the membranes of animal cells. This asymmetry is preserved as membranes bud from one organelle and fuse with another—or with the plasma membrane. This means that all cell membranes have distinct "inside" and "outside" faces: the cytosolic monolayer always faces the cytosol, while the noncytosolic monolayer is exposed to either the cell exterior—in the case of the plasma membrane—or the interior space (*lumen*) of an organelle. This conservation of orientation applies not only to the phospholipids that make up the membrane, but also to any proteins that might be inserted in the membrane (**Figure 11–18**). This positioning is very important, as a protein's orientation within the lipid bilayer is crucial for its function (see Figure 11–20).

Among lipids, those that show the most dramatically lopsided distribution in cell membranes are the glycolipids, which are located mainly in the plasma membrane, and only in the noncytosolic half of the bilayer (**Figure 11–19**). The sugar groups of these membrane lipids face the cell exterior, where they form part of a continuous coat of carbohydrate that surrounds and protects animal cells. Glycolipid molecules acquire their sugar groups in the Golgi apparatus, where the enzymes that engineer this chemical modification are confined. These enzymes are oriented such that sugars are added only to lipid molecules in the noncytosolic half of the bilayer. Once a glycolipid molecule has been created in this way, it remains trapped in this monolayer, as there are no flippases that transfer glycolipids to the cytosolic side. Thus, when a glycolipid molecule is finally delivered to the plasma membrane, it displays its sugars to the exterior of the cell.

Other lipid molecules show different types of asymmetric distributions, which relate to their specific functions. For example, the inositol phospholipids—a minor component of the plasma membrane—have a special role in relaying signals from the cell surface into the cell interior (discussed in Chapter 16); thus they are concentrated in the cytosolic half of the lipid bilayer.

QUESTION 11–3

It seems paradoxical that a lipid bilayer can be fluid yet asymmetrical. Explain.

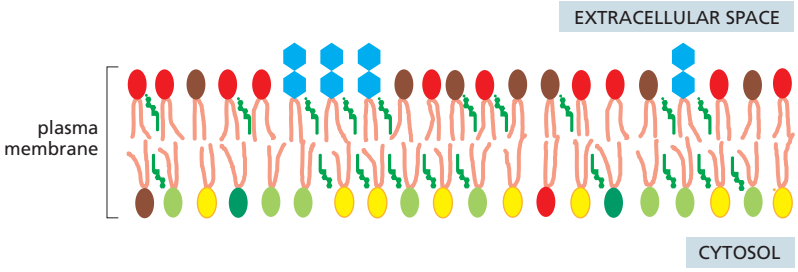


Figure 11–19 Phospholipids and glycolipids are distributed asymmetrically in the lipid bilayer of an animal cell plasma membrane. Phosphatidylcholine (*red*) and sphingomyelin (*brown*) are concentrated in the noncytosolic monolayer, whereas phosphatidylserine (*light green*) and phosphatidylethanolamine (*yellow*) are found mainly on the cytosolic side. In addition to these phospholipids, phosphatidylinositols (*dark green* head group), a minor constituent of the plasma membrane, are shown in the cytosolic monolayer, where they participate in cell signaling. Glycolipids are drawn with hexagonal *blue* head groups to represent sugars; these are found exclusively in the noncytosolic monolayer of the membrane. Within the bilayer, cholesterol (*green*) is distributed almost equally in both monolayers.

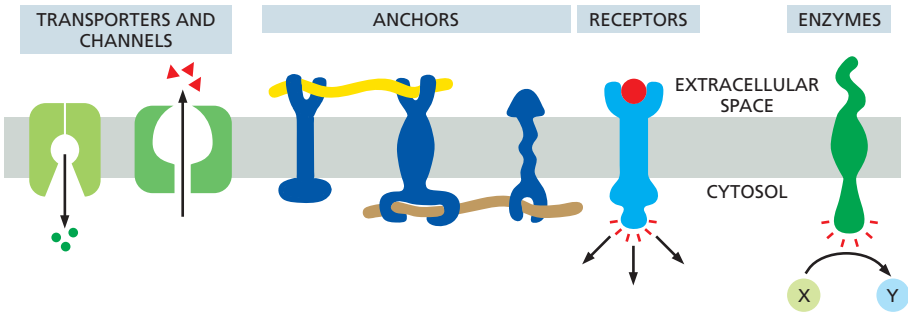


Figure 11–20 Plasma membrane proteins have a variety of functions. They transport molecules and ions, act as anchors, detect signals, or catalyze reactions.

MEMBRANE PROTEINS

Although the lipid bilayer provides the basic structure of all cell membranes and serves as a permeability barrier to the hydrophilic molecules on either side of it, most membrane functions are carried out by **membrane proteins**. In animals, proteins constitute about 50% of the mass of most plasma membranes, the remainder being lipid plus the relatively small amounts of carbohydrate found on some of the lipids (glycolipids) and many of the proteins (glycoproteins). Because lipid molecules are much smaller than proteins, however, a cell membrane typically contains about 50 times the number of lipid molecules compared to protein molecules (see Figure 11–4B).

Membrane proteins serve many functions. Some transport particular nutrients, metabolites, and ions across the lipid bilayer. Others anchor the membrane to macromolecules on either side. Still others function as receptors that detect chemical signals in the cell's environment and relay them into the cell interior, or work as enzymes to catalyze specific reactions at the membrane (**Figure 11–20** and **Table 11–1**). Each type of cell membrane contains a different set of proteins, reflecting the specialized functions of the particular membrane. In this section, we discuss the structure of membrane proteins and how they associate with the lipid bilayer.

TABLE 11–1 SOME EXAMPLES OF PLASMA MEMBRANE PROTEINS AND THEIR FUNCTIONS

Functional Class	Protein Example	Specific Function
Transporters	Na^+ pump	actively pumps Na^+ out of cells and K^+ in (discussed in Chapter 12)
Ion channels	K^+ leak channel	allows K^+ ions to leave cells, thereby influencing cell excitability (discussed in Chapter 12)
Anchors	integrins	link intracellular actin filaments to extracellular matrix proteins (discussed in Chapter 20)
Receptors	platelet-derived growth factor (PDGF) receptor	binds extracellular PDGF and, as a consequence, generates intracellular signals that direct the cell to grow and divide (discussed in Chapters 16 and 18)
Enzymes	adenylyl cyclase	catalyzes the production of the small intracellular signaling molecule cyclic AMP in response to extracellular signals (discussed in Chapter 16)

Membrane Proteins Associate with the Lipid Bilayer in Different Ways

Although the lipid bilayer has a uniform structure, proteins can interact with a cell membrane in a number of different ways.

- Many membrane proteins extend through the bilayer, with part of their mass on either side (**Figure 11–21A**). Like their lipid neighbors, these *transmembrane proteins* are amphipathic, having both hydrophobic and hydrophilic regions. Their hydrophobic regions lie in the interior of the bilayer, nestled against the hydrophobic tails of the lipid molecules. Their hydrophilic regions are exposed to the aqueous environment on either side of the membrane.
- Other membrane proteins are located almost entirely in the cytosol and are associated with the cytosolic half of the lipid bilayer by an amphipathic α helix exposed on the surface of the protein (**Figure 11–21B**).
- Some proteins lie entirely outside the bilayer, on one side or the other, attached to the membrane by one or more covalently attached lipid groups (**Figure 11–21C**).
- Yet other proteins are bound indirectly to one face of the membrane or the other, held in place only by their interactions with other membrane proteins (**Figure 11–21D**).

Proteins that are directly attached to the lipid bilayer—whether they are transmembrane, associated with the lipid monolayer, or lipid-linked—can be removed only by disrupting the bilayer with detergents, as discussed shortly. Such proteins are known as *integral membrane proteins*. The remaining membrane proteins are classified as *peripheral membrane proteins*; they can be released from the membrane by more gentle extraction procedures that interfere with protein–protein interactions but leave the lipid bilayer intact.

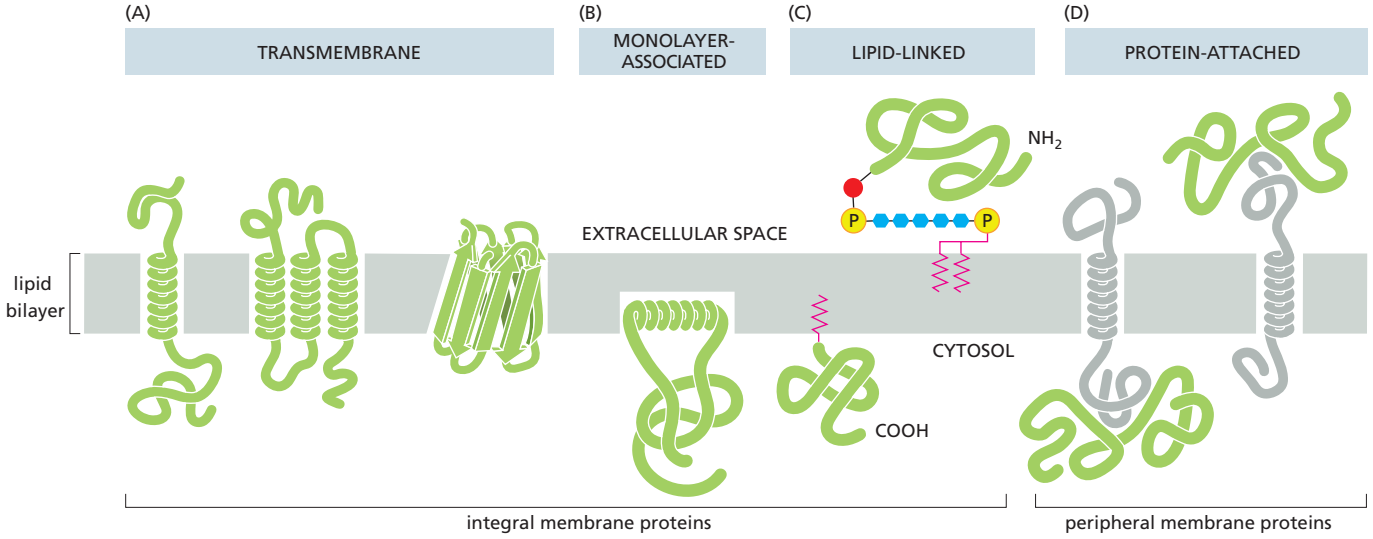


Figure 11–21 Membrane proteins can associate with the lipid bilayer in different ways. (A) Transmembrane proteins can extend across the bilayer as a single α helix, as multiple α helices, or as a rolled-up β sheet (called a β barrel). (B) Some membrane proteins are anchored to the cytosolic half of the lipid bilayer by an amphipathic α helix. (C) Others are linked to either side of the bilayer solely by a covalently attached lipid molecule (*red* zigzag lines). (D) Many proteins are attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins. (A–C) are examples of integral membrane proteins; the proteins shown in (D) are considered peripheral membrane proteins.

A Polypeptide Chain Usually Crosses the Lipid Bilayer as an α Helix

All membrane proteins have a unique orientation in the lipid bilayer, which is essential for their function. For a transmembrane receptor protein, for example, the part of the protein that receives a signal from the environment must be on the outside of the cell, whereas the part that passes along the signal must be in the cytosol (see Figure 11–20). This orientation is a consequence of the way in which membrane proteins are synthesized (discussed in Chapter 15). The portions of a transmembrane protein located on either side of the lipid bilayer are connected by specialized membrane-spanning segments of the polypeptide chain (see Figure 11–21A). These segments, which run through the hydrophobic environment of the interior of the lipid bilayer, are composed largely of amino acids with hydrophobic side chains. Because these side chains cannot form favorable interactions with water molecules, they prefer to interact with the hydrophobic tails of the lipid molecules, where no water is present.

In contrast to the hydrophobic side chains, however, the peptide bonds that join the successive amino acids in a protein are normally polar, making the polypeptide backbone itself hydrophilic (**Figure 11–22**). Because water is absent from the interior of the bilayer, atoms that are part of the polypeptide backbone are thus driven to form hydrogen bonds with one another. Hydrogen-bonding is maximized if the polypeptide chain forms a regular α helix, and so the great majority of the membrane-spanning segments of polypeptide chains traverse the bilayer as α helices (see Figure 4–12). In these membrane-spanning α helices, the hydrophobic side chains are exposed on the outside of the helix, where they contact the hydrophobic lipid tails, while the atoms of the hydrophilic polypeptide backbone form hydrogen bonds with one another within the helix (**Figure 11–23**).

For many transmembrane proteins, the polypeptide chain crosses the membrane only once (see Figure 11–21A, *left*). Many of these *single-pass* transmembrane proteins are receptors for extracellular signals. Other transmembrane proteins function as channels, forming aqueous pores across the lipid bilayer to allow small, water-soluble molecules to cross the membrane. Such channels cannot be formed by proteins with a single transmembrane α helix. Instead, they usually consist of a series of α helices that cross the bilayer a number of times (see Figure 11–21A, *center*). For many of these *multipass* transmembrane proteins, one or more of the membrane-spanning regions are amphipathic—formed from α helices that contain both hydrophobic and hydrophilic amino acid side chains. These amino acids tend to be arranged so that the hydrophobic side chains fall on one side of the helix, while the hydrophilic side chains are concentrated on the other side. In the hydrophobic environment of the lipid bilayer, α helices of this type pack side by side in a ring, with the hydrophobic side chains exposed to the hydrophobic lipid tails and the hydrophilic side chains forming the lining of a hydrophilic pore

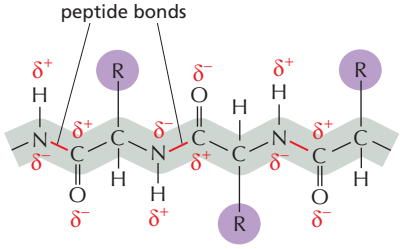


Figure 11–22 The backbone of a polypeptide chain is hydrophilic. The atoms on either side of a peptide bond (*red* line) are polar and carry partial positive or negative charges (δ^+ or δ^-). These charges allow these atoms to hydrogen-bond with one another when the polypeptide folds into an α helix that spans the lipid bilayer (see Figure 11–23).

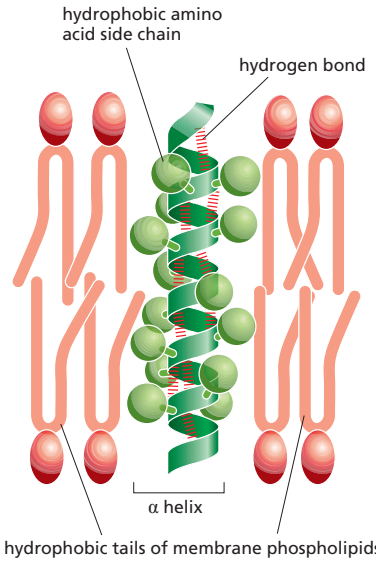


Figure 11–23 A transmembrane polypeptide chain usually crosses the lipid bilayer as an α helix. In this segment of a transmembrane protein, the hydrophobic side chains (*light green*) of the amino acids forming the α helix contact the hydrophobic hydrocarbon tails of the phospholipid molecules, while the hydrophilic parts of the polypeptide backbone form hydrogen bonds with one another (dashed *red* lines) along the interior of the helix. An α helix containing about 20 amino acids is required to completely traverse a cell membrane.

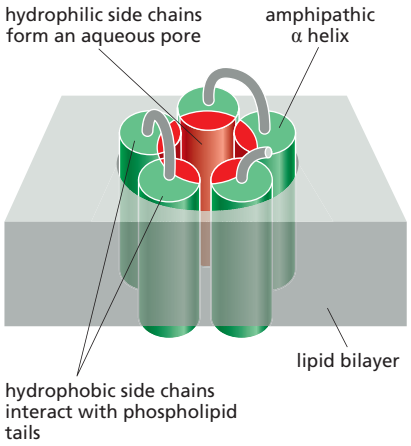


Figure 11–24 A transmembrane hydrophilic pore can be formed by multiple amphipathic α helices. In this example, five amphipathic transmembrane α helices form a water-filled channel across the lipid bilayer. The hydrophobic amino acid side chains on one side of each helix (*green*) come in contact with the hydrophobic lipid tails of the lipid bilayer, while the hydrophilic side chains on the opposite side of the helices (*red*) form a water-filled pore.

through the membrane (**Figure 11–24**). How such channels function in the selective transport of small, water-soluble molecules, especially inorganic ions, is discussed in Chapter 12.

Although the α helix is by far the most common form in which a polypeptide chain crosses a lipid bilayer, the polypeptide chain of some transmembrane proteins crosses the lipid bilayer as a β sheet that is rolled into a cylinder, forming a keglike structure called a β barrel (see Figure 11–21A, *right*). As expected, the amino acid side chains that face the inside of the barrel, and therefore line the aqueous channel, are mostly hydrophilic, while those on the outside of the barrel, which contact the hydrophobic core of the lipid bilayer, are exclusively hydrophobic. A striking example of a β-barrel structure is found in the *porin* proteins, which form large, water-filled pores in mitochondrial and bacterial outer membranes (**Figure 11–25**). Mitochondria and some bacteria are surrounded by a double membrane, and porins allow the passage of small nutrients, metabolites, and inorganic ions across their outer membranes, while preventing unwanted larger molecules from crossing.

QUESTION 11–4

Explain why the polypeptide chain of most transmembrane proteins crosses the lipid bilayer as an α helix or a β barrel.

Membrane Proteins Can Be Solubilized in Detergents

To understand a protein fully, one needs to know its structure in detail. For membrane proteins, this presents special problems. Most biochemical procedures are designed for studying molecules in aqueous solution. Membrane proteins, however, are built to operate in an environment that is partly aqueous and partly fatty, and taking them out of this environment to study in isolation—while preserving their essential structure—is no easy task.

Before an individual protein can be examined in detail, it must be separated from all the other cell proteins. For most membrane proteins, the first step in this purification process involves solubilizing the membrane with agents that destroy the lipid bilayer by disrupting hydrophobic associations. The most widely used disruptive agents are **detergents** (**Movie 11.5**). These small, amphipathic, lipidlike molecules differ from membrane phospholipids in that they have only a single hydrophobic tail (**Figure 11–26**). Because they have one tail, detergent molecules are shaped like cones; in water, these conical molecules tend to aggregate into small clusters called *micelles*, rather than forming a bilayer as do the phospholipids, which—with their two tails—are more cylindrical in shape.

When mixed in great excess with membranes, the hydrophobic ends of detergent molecules interact with the membrane-spanning hydrophobic regions of the transmembrane proteins, as well as with the hydrophobic tails of the phospholipid molecules, thereby disrupting the lipid bilayer and separating the proteins from most of the phospholipids. Because the other end of the detergent molecule is hydrophilic, these interactions draw the membrane proteins into the aqueous solution as protein–detergent complexes; at the same time, the detergent

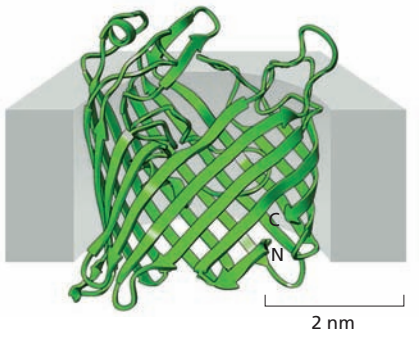


Figure 11–25 Porin proteins form water-filled channels in the outer membrane of a bacterium. The protein illustrated is from *E. coli*, and it consists of a 16-stranded β sheet curved around on itself to form a transmembrane water-filled channel. The three-dimensional structure was determined by x-ray crystallography. Although not shown in the drawing, three porin proteins associate to form a trimer with three separate channels.

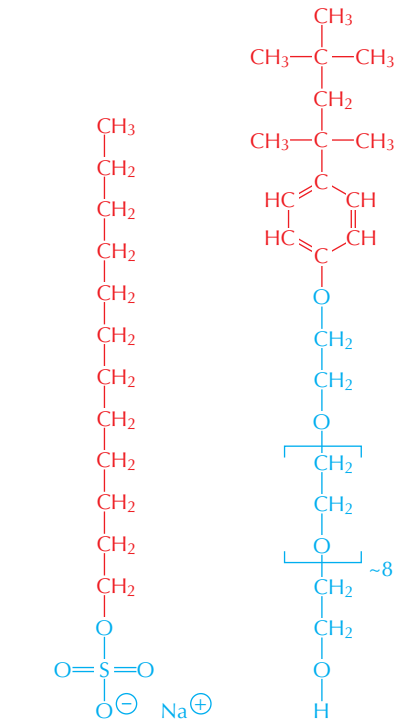


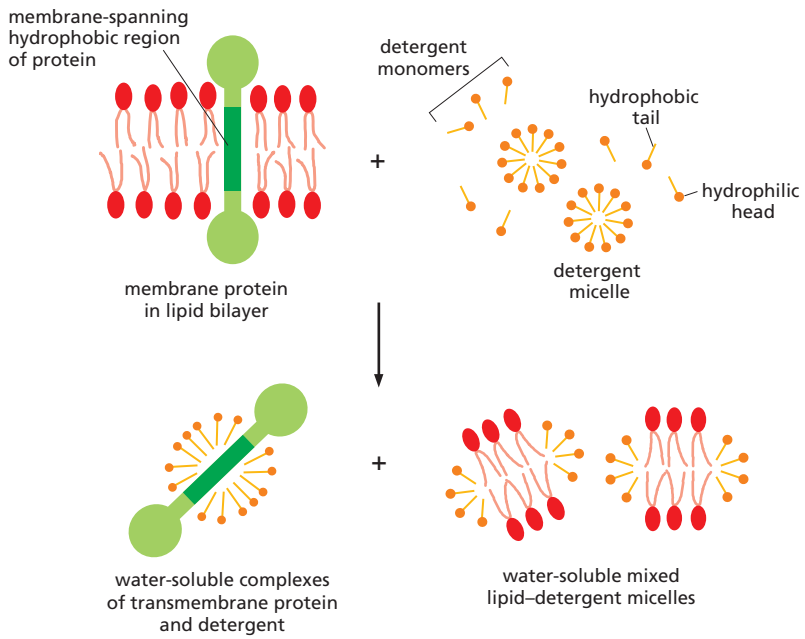
Figure 11–26 SDS and Triton X-100 are two commonly used detergents. Sodium dodecyl sulfate (SDS) is a strong ionic detergent—that is, it has an ionized (charged) group at its hydrophilic end (*blue*). Triton X-100 is a mild nonionic detergent—that is, it has a nonionized but polar structure at its hydrophilic end (*blue*). The hydrophobic portion of each detergent is shown in *red*. The bracketed portion of Triton X-100 is repeated about eight times. Strong ionic detergents like SDS not only displace lipid molecules from proteins but also unfold the proteins (see Panel 4–5, p. 167).

also solubilizes the phospholipids (**Figure 11–27**). The protein–detergent complexes can then be separated from one another and from the lipid–detergent complexes for further analysis.

We Know the Complete Structure of Relatively Few Membrane Proteins

For many years, much of what we knew about the structure of membrane proteins was learned by indirect means. The standard method for determining a protein's three-dimensional structure directly has been x-ray crystallography, but this approach requires ordered crystalline arrays of the molecule. Because membrane proteins have to be purified in detergent micelles that are often heterogeneous in size, they are harder to crystallize than the soluble proteins that inhabit the cell cytosol or extracellular fluids. Nevertheless, with recent advances in x-ray crystallography, along with powerful new approaches such as cryoelectron microscopy, the structures of an increasing number of membrane proteins have now been determined to high resolution (see Panel 4–6, pp. 168–169).

One example is bacteriorhodopsin, the structure of which first revealed exactly how α helices cross the lipid bilayer. **Bacteriorhodopsin** is a small protein found in large amounts in the plasma membrane of *Halobacterium halobium*, an archaean that lives in salt marshes. Bacteriorhodopsin acts as a membrane transport protein that pumps H^+ (protons) out of the cell. Each bacteriorhodopsin molecule contains a single chromophore, a light-absorbing, nonprotein molecule called *retinal*, that gives the protein—and



QUESTION 11–5

For the two detergents shown in Figure 11–26, explain why the blue portions of the molecules are hydrophilic and the red portions hydrophobic. Draw a short stretch of a polypeptide chain made up of three amino acids with hydrophobic side chains (see Panel 2–6, pp. 76–77) and apply a similar color scheme. Indicate which portions of your polypeptide would form hydrogen bonds with water.

Figure 11–27 Membrane proteins can be solubilized by a mild detergent such as Triton X-100. The detergent molecules (*gold*) are shown as both monomers and micelles, the form in which these molecules tend to aggregate in water. The detergent disrupts the lipid bilayer and interacts with the membrane-spanning hydrophobic portion of the protein (*dark green*). These actions bring the proteins into solution as protein–detergent complexes. As illustrated, the phospholipids in the membrane are also solubilized by the detergents, forming lipid–detergent micelles.

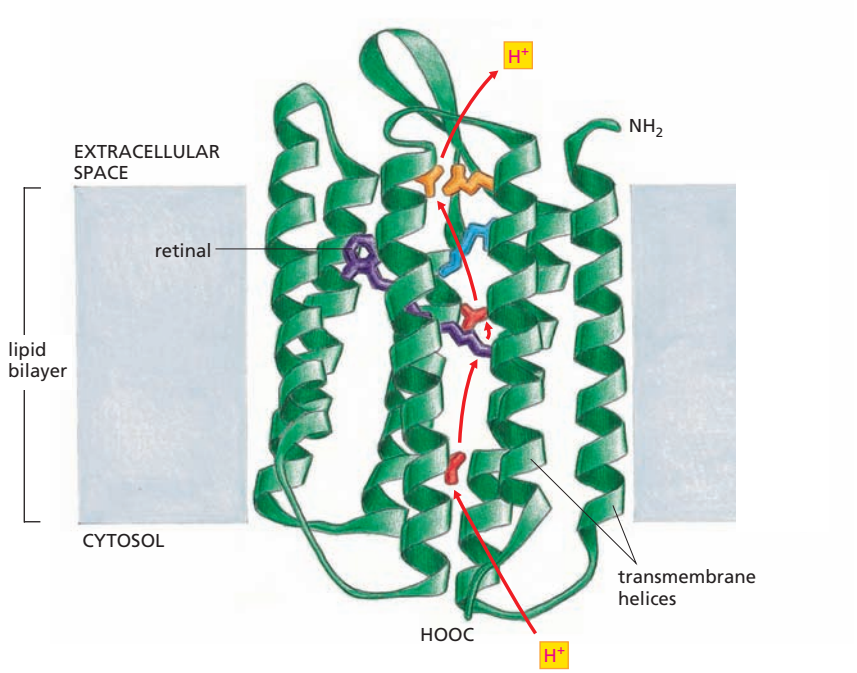


Figure 11–28 Bacteriorhodopsin acts as a proton pump. The polypeptide chain of this small protein (about 250 amino acids in length) crosses the lipid bilayer as seven α helices. The location of the retinal (*purple*) and the probable pathway taken by protons during the light-activated pumping cycle (*red* arrows) are highlighted. Strategically placed polar amino acid side chains—shown in *red*, *yellow*, and *blue*—guide the movement of the proton (H^+) across the bilayer, allowing it to avoid contact with the lipid environment. The retinal is then regenerated by taking up a H^+ from the cytosol, returning the protein to its original conformation—a cycle shown in **Movie 11.6**. Retinal is also used to detect light in our own eyes, where it is attached to a protein with a structure very similar to that of bacteriorhodopsin. (Adapted from H. Luecke et al., *Science* 286:5438 255–260, 1999.)

the entire organism—a deep purple color. When retinal, which is covalently attached to one of bacteriorhodopsin's transmembrane α helices, absorbs a photon of light, it changes shape. This shape change causes the surrounding helices to undergo a series of small conformational changes, which pump one proton from the retinal to the outside of the organism (**Figure 11–28**).

In the presence of sunlight, thousands of bacteriorhodopsin molecules pump H^+ out of the cell, generating a concentration gradient of H^+ across the plasma membrane. The cell uses this proton gradient to store energy and convert it into ATP, as we discuss in detail in Chapter 14. Bacteriorhodopsin is a *pump*, a class of transmembrane protein that actively moves small organic molecules and inorganic ions into and out of cells. We will discuss the action of other important transmembrane pumps in Chapter 12.

The Plasma Membrane Is Reinforced by the Underlying Cell Cortex

A cell membrane by itself is extremely thin and fragile. It would require nearly 10,000 cell membranes laid on top of one another to achieve the thickness of this paper. Most cell membranes are therefore strengthened and supported by a framework of proteins, attached to the membrane via transmembrane proteins. For plants, yeasts, and bacteria, the cell's shape and mechanical properties are conferred by a rigid *cell wall*—a fibrous layer of proteins, sugars, and other macromolecules that encases the plasma membrane. By contrast, the plasma membrane of animal cells is stabilized by a meshwork of filamentous proteins, called the **cell cortex**, that is attached to the underside of the membrane.

The cortex of the human red blood cell has a relatively simple and regular structure and has been especially well studied. Red blood cells are small and have a distinctive flattened shape (**Figure 11–29A**). The main component of their cortex is the dimeric protein *spectrin*, a long, thin, flexible rod about 100 nm in length. Spectrin forms a lattice that provides support for the plasma membrane and maintains the cell's biconcave shape. The spectrin network is connected to the membrane through intracellular

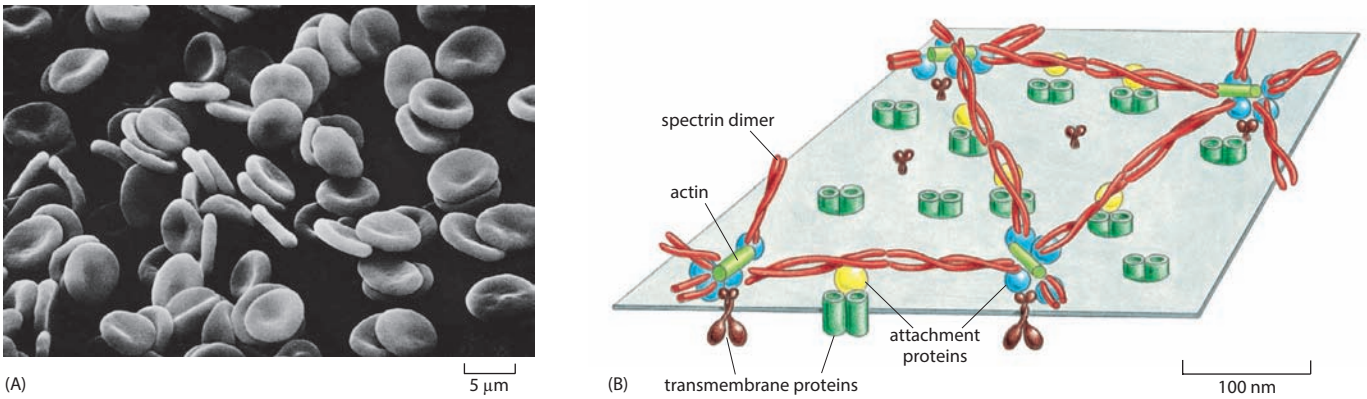


Figure 11–29 A cortex made largely of spectrin gives human red blood cells their characteristic shape. (A) Scanning electron micrograph showing human red blood cells, which have a flattened, biconcave shape. These cells lack a nucleus and other intracellular organelles. (B) In the cortex of a red blood cell, spectrin dimers (*red*) are linked end-to-end to form longer tetramers. The spectrin tetramers, together with a smaller number of actin molecules, are linked together into a mesh. This network is attached to the plasma membrane by the binding of at least two types of attachment proteins (shown here in *yellow* and *blue*) to two kinds of transmembrane proteins (shown here in *green* and *brown*). (A, courtesy of Bernadette Chailley.)

attachment proteins that link spectrin to specific transmembrane proteins (**Figure 11–29B** and **Movie 11.7**). The importance of this meshwork is seen in mice and humans that, due to genetic alterations, produce a form of spectrin with an abnormal structure. These individuals are anemic: they have fewer red blood cells than normal. The red cells they do have are spherical instead of flattened and are abnormally fragile.

Proteins similar to spectrin, and to its associated attachment proteins, are present in the cortex of most animal cells. But the cortex in these cells is especially rich in actin and the motor protein *myosin*, and it is much more complex than that of red blood cells. Whereas red blood cells need their cortex mainly to provide mechanical strength as they are pumped through blood vessels, other cells also use their cortex to selectively take up materials from their environment, to change their shape, and to move, as we discuss in Chapter 17. In addition, cells also use their cortex to restrain the diffusion of proteins within the plasma membrane, as we see next.

A Cell Can Restrict the Movement of Its Membrane Proteins

Because a membrane is a two-dimensional fluid, many of its proteins, like its lipids, can move freely within the plane of the bilayer. This lateral diffusion was initially demonstrated by experimentally fusing a mouse cell with a human cell to form a double-sized hybrid cell and then monitoring the distribution of certain mouse and human plasma membrane proteins. At first, the mouse and human proteins are confined to their own halves of the newly formed hybrid cell, but within half an hour or so the two sets of proteins become evenly mixed over the entire cell surface (**Figure 11–30**). We describe some other techniques for studying the movement of membrane proteins in **How We Know**, pp. 384–385.

The picture of a cell membrane as a sea of lipid in which all proteins float freely is too simple, however. Cells have ways of confining particular proteins to localized areas within the bilayer, thereby creating functionally specialized regions, or **membrane domains**, on the surface of the cell or organelle.

QUESTION 11–6

Look carefully at the transmembrane proteins shown in Figure 11–29B. What can you say about their mobility in the membrane?

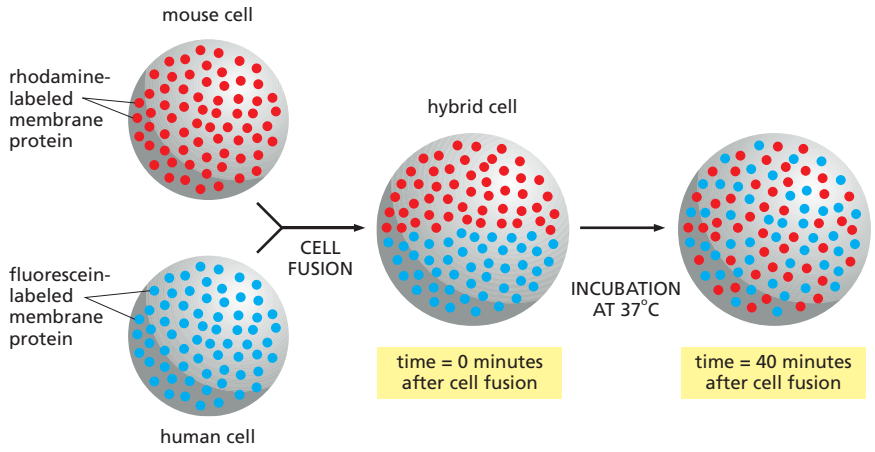


Figure 11–30 Formation of mouse–human hybrid cells shows that some plasma membrane proteins can move laterally in the lipid bilayer. When the mouse and human cells are first fused, their proteins are confined to their own halves of the newly formed hybrid-cell plasma membrane. Within a short time, however, the membrane proteins—and lipids—completely intermix. To monitor the movement of a selected sampling of the proteins, the cells are labeled with antibodies that bind to either human or mouse proteins; the antibodies are coupled to two different fluorescent tags—for example, rhodamine (*red*) and fluorescein (shown here in *blue*)—so they can be distinguished in a fluorescence microscope (see Panel 4–2, pp. 140–141). (Based on observations of L.D. Frye and M. Edidin, *J. Cell Sci.* 7:319–335, 1970.)

As illustrated in **Figure 11–31**, plasma membrane proteins can be tethered to structures outside the cell—for example, to molecules in the extracellular matrix or on an adjacent cell (discussed in Chapter 20)—or to relatively immobile structures inside the cell, especially to the cell cortex (see Figure 11–29B). Additionally, cells can create barriers that restrict particular membrane components to one membrane domain. In epithelial cells that line the gut, for example, it is important that transport proteins involved in the uptake of nutrients from the gut be confined to the *apical* surface of the cells (which faces the gut contents) and that other transport proteins—including those involved in the export of solutes out of the epithelial cell into the tissues and bloodstream—be confined to the *basal* and *lateral* surfaces (see Figure 12–17). This asymmetric distribution of membrane proteins is maintained by a barrier formed along the line where the cell is sealed to adjacent epithelial cells by a so-called *tight junction* (**Figure 11–32**). At this site, specialized junctional proteins form a continuous belt around the cell where the cell contacts its neighbors, creating a seal between adjacent plasma membranes (see Figure 20–22). Membrane proteins are unable to diffuse past the junction.

The Cell Surface Is Coated with Carbohydrate

We saw earlier that some of the lipids in the outer layer of the plasma membrane have sugars covalently attached to them. The same is true for most of the proteins in the plasma membrane. The great majority of these proteins have short chains of sugars, called *oligosaccharides*, linked to them; they are called *glycoproteins*. Other membrane proteins, the *proteoglycans*, contain one or more long polysaccharide chains. All of

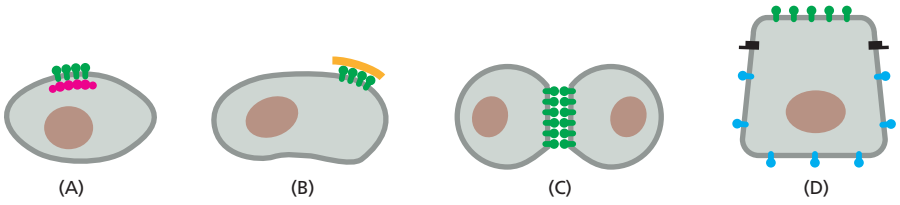


Figure 11–31 The lateral mobility of plasma membrane proteins can be restricted in several ways. Proteins can be tethered (A) to the cell cortex inside the cell, (B) to extracellular matrix molecules outside the cell, or (C) to proteins on the surface of another cell. (D) Diffusion barriers (shown as *black* bars) can restrict proteins to a particular membrane domain.

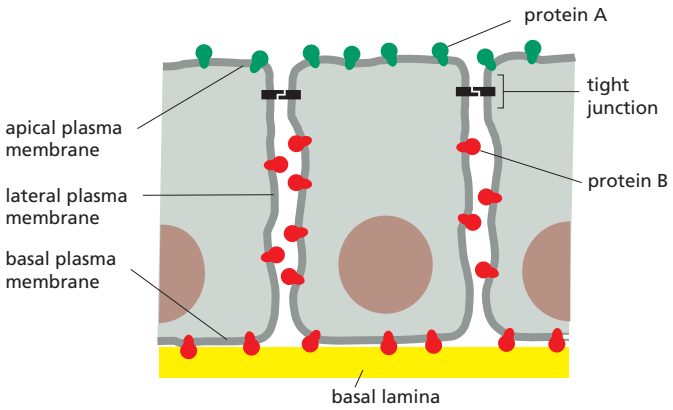


Figure 11–32 Membrane proteins are restricted to particular domains of the plasma membrane of epithelial cells in the gut. Protein A (*green*) and protein B (*red*) can diffuse laterally in their own membrane domains but are prevented from entering the other domain by a specialized cell junction called a tight junction. The basal lamina (*yellow*) is a mat of extracellular matrix that supports all epithelial sheets (discussed in Chapter 20).

the carbohydrate on the glycoproteins, proteoglycans, and glycolipids is located on the outside of the plasma membrane, where it forms a sugar coating called the *carbohydrate layer* or **glycocalyx** (Figure 11–33).

This layer of carbohydrate helps protect the cell surface from mechanical damage. And because the oligosaccharides and polysaccharides attract water molecules, they also give the cell a slimy surface, which helps motile cells such as white blood cells squeeze through narrow spaces and prevents blood cells from sticking to one another or to the walls of blood vessels.

Cell-surface carbohydrates do more than just protect and lubricate the cell, however. They have an important role in cell–cell recognition and adhesion. Transmembrane proteins called *lectins* are specialized to bind to particular oligosaccharide side chains. The oligosaccharide side chains of glycoproteins and glycolipids, although short (typically fewer than 15 sugar units), are enormously diverse. Unlike proteins, in which the amino acids are all joined together in a linear chain by identical peptide bonds, sugars can be joined together in many different arrangements, often forming elaborate branched structures (see Panel 2–4, pp. 72–73). Using a variety of covalent linkages, even three different sugars can form hundreds of different trisaccharides.

The carbohydrate layer on the surface of cells in a multicellular organism serves as a kind of distinctive clothing, like a police officer's uniform. It is characteristic of each cell type and is recognized by other cell types that

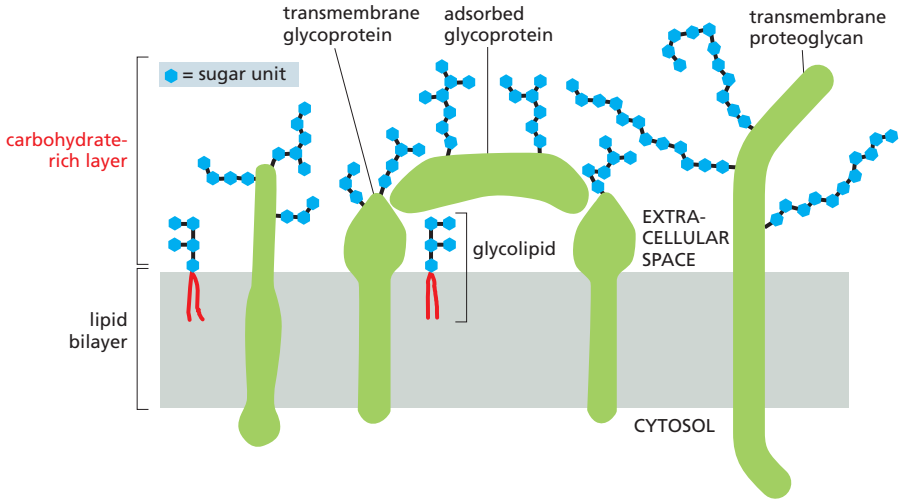


Figure 11–33 Eukaryotic cells are coated with sugars. This carbohydrate-rich layer is made of the oligosaccharide side chains attached to membrane glycolipids and glycoproteins, and of the polysaccharide chains on membrane proteoglycans. As shown, glycoproteins that have been secreted by the cell and then adsorbed back onto its surface can also contribute. Note that all the carbohydrate is on the external (noncytosolic) surface of the plasma membrane.

MEASURING MEMBRANE FLOW

An essential feature of the lipid bilayer is its fluidity, which is crucial for cell membrane integrity and function. This property allows many membrane-embedded proteins to move laterally in the plane of the bilayer, so that they can engage in the various protein–protein interactions on which cells depend. The fluid nature of cell membranes is so central to their proper function that it may seem surprising that this property was not recognized until the early 1970s.

Given its importance for membrane structure and function, how do we measure and study the fluidity of cell membranes? The most common methods are visual: simply label some of the molecules native to the membrane and then watch where they go. Such an approach first demonstrated the lateral movement of membrane proteins that had been tagged with labeled antibodies (see Figure 11–30). This experiment seemed to suggest that membrane proteins diffuse freely, without restriction, in an open sea of lipids. We now know that this image is not entirely accurate. To probe membrane fluidity more thoroughly, researchers had to invent more precise methods for tracking the movement of proteins within a membrane such as the plasma membrane of a living cell.

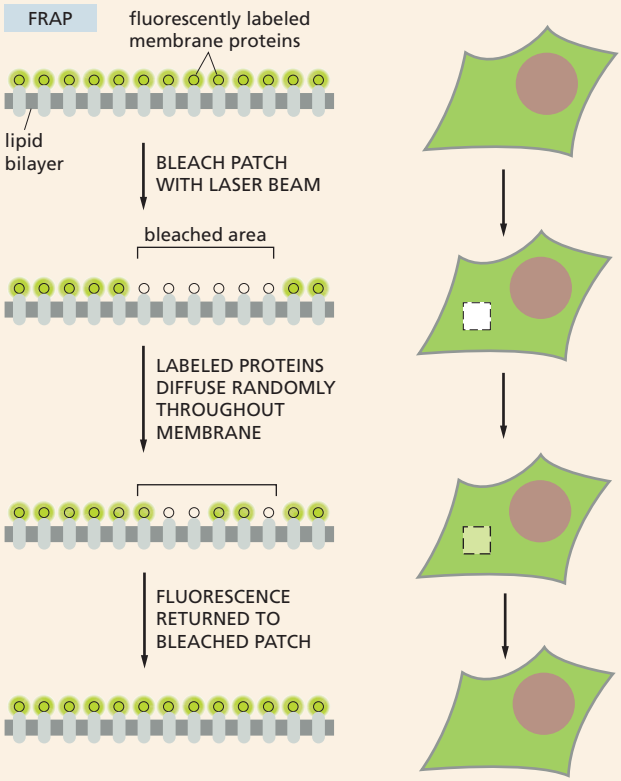
The FRAP attack

One such technique, called *fluorescence recovery after photobleaching* (*FRAP*), involves uniformly labeling the components of the cell membrane—its lipids or, more often, its proteins—with some sort of fluorescent marker. Labeling membrane proteins can be accomplished by incubating cells with a fluorescent antibody or by covalently attaching a fluorescent protein such as green fluorescent protein (GFP) to a membrane protein using the DNA techniques discussed in Chapter 10.

Once a protein has been labeled, a small patch of membrane is irradiated with an intense pulse of light from a sharply focused laser beam. This treatment irreversibly "bleaches" the fluorescence from the labeled proteins in that small patch of membrane, typically an area about 1 μm square. The fluorescence of this irradiated membrane is monitored in a fluorescence microscope, and the amount of time it takes for the neighboring, unbleached fluorescent proteins to migrate into the bleached region of the membrane is measured (**Figure 11–34**). The rate of this "fluorescence recovery" is a direct measure of the rate at which the protein molecules can diffuse within the membrane (**Movie 11.8**). Such experiments have revealed that, generally speaking, cell membranes are about as viscous as olive oil.

One-by-one

One drawback to the FRAP approach is that the technique monitors the movement of fairly large populations of proteins—hundreds or thousands—across a relatively large area of the membrane. With this technique



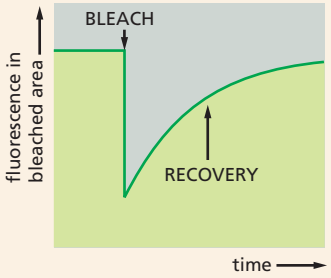


Figure 11–34 Photobleaching techniques such as FRAP can be used to measure the rate of lateral diffusion of a membrane protein. A specific type of protein can be labeled with a fluorescent antibody (as shown here) or tagged with a fluorescent protein, such as GFP. A small area of the membrane containing these fluorescent protein molecules is then bleached using a laser beam. As the bleached molecules diffuse away, and unbleached, fluorescent molecules diffuse into the area, the intensity of the fluorescence is recovered (shown here in side and top views). The diffusion coefficient is then calculated from a graph of the rate of fluorescence recovery: the greater the diffusion coefficient of the membrane protein, the faster the recovery.

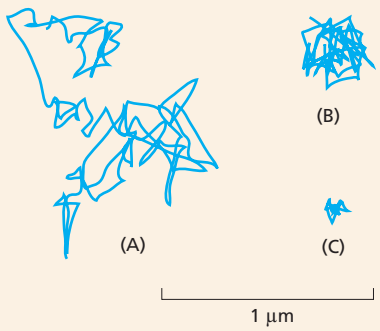


Figure 11–35 Proteins show different patterns of diffusion. Single-particle tracking studies reveal some of the pathways that single proteins follow on the surface of a living cell. Shown here are some trajectories representative of different kinds of proteins in the plasma membrane. (A) Tracks made by a protein that is free to diffuse randomly in the lipid bilayer. (B) Tracks made by a protein that is corralled within a small membrane domain by other proteins. (C) Tracks made by a protein that is tethered to the cytoskeleton and hence is essentially immobile. The movement of the proteins is monitored over a period of seconds.

it is impossible to track the motion of individual molecules, which can make analysis of the results difficult. If the labeled proteins fail to migrate into the bleached zone over the course of a FRAP study, for example, is it because they are immobile, essentially anchored in one place in the membrane? Or, alternatively, are they restricted to movement within a very small region—fenced in by cytoskeletal proteins—and thus only appear motionless?

To get around this problem, researchers have developed methods for labeling and observing the movement of individual molecules or small clusters of molecules. One such technique, dubbed *single-particle tracking* (*SPT*) *microscopy*, relies on tagging protein molecules with antibody-coated gold nanoparticles. The gold particles look like tiny black dots when seen with a light microscope, and their movement, and thus the movement of individually tagged protein molecules, can be followed using video microscopy.

From the studies carried out to date, it appears that membrane proteins can display a variety of patterns of movement, from random diffusion to complete immobility (**Figure 11–35**). Some proteins rapidly switch between these different kinds of motion.

Freed from cells

In many cases, researchers wish to study the behavior of a particular type of membrane protein in a synthetic lipid bilayer, in the absence of other proteins that might restrain its movement or alter its activity. For such studies, membrane proteins can be isolated from cells and the protein of interest purified and reconstituted in artificial phospholipid vesicles (**Figure 11–36**). The lipids allow the purified protein to maintain its proper structure and function, so that its activity and behavior can be analyzed in detail.

It is apparent from such studies that membrane proteins diffuse more freely and rapidly in artificial lipid bilayers than in cell membranes. The fact that most proteins show reduced mobility in a cell membrane makes sense, as these membranes are crowded with many types of proteins and contain a greater variety of lipids than an artificial lipid bilayer. Furthermore, many membrane proteins in a cell are tethered to proteins in the extracellular matrix, or anchored to the cell cortex just under the plasma membrane, or both (as illustrated in Figure 11–31).

Taken together, such studies have revolutionized our understanding of membrane proteins and of the architecture and organization of cell membranes.

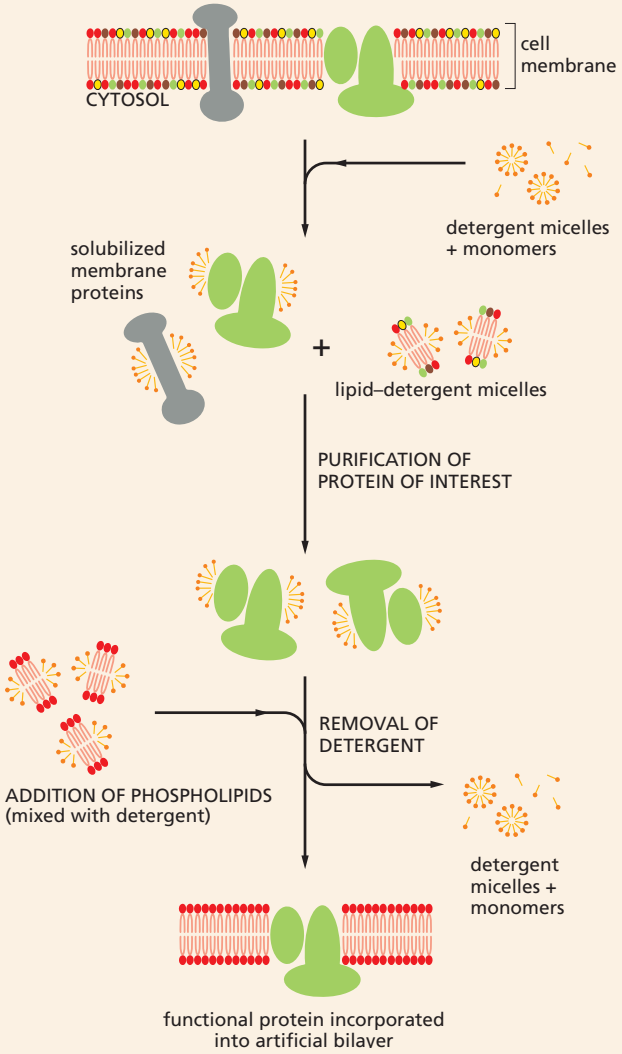


Figure 11–36 Mild detergents can be used to solubilize and reconstitute functional membrane proteins. Proteins incorporated into artificial lipid bilayers generally diffuse more freely and rapidly than they do in cell membranes.

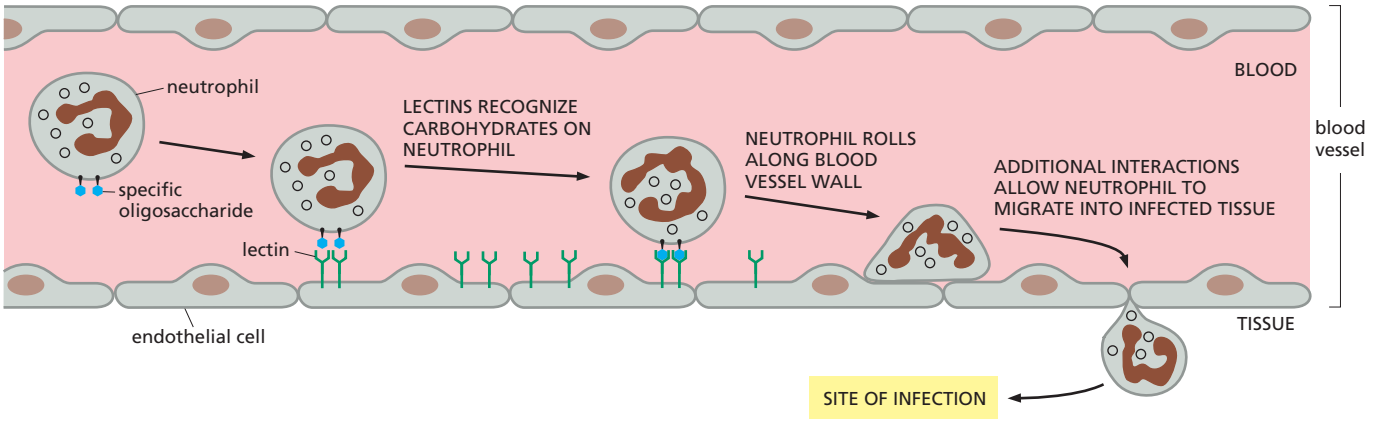


Figure 11–37 The recognition of cell-surface carbohydrates on neutrophils allows these immune cells to begin to migrate out of the blood and into infected tissues. Specialized transmembrane proteins (called lectins) are made by the endothelial cells lining the blood vessel in response to chemical signals emanating from a site of infection. These proteins recognize particular sugar groups carried by glycolipids and glycoproteins on the surface of neutrophils (a type of white blood cell, also called a leukocyte) circulating in the blood. The neutrophils consequently stick to the endothelial cells that line the blood vessel wall. This association is not very strong, but it leads to another, much stronger protein–protein interaction (not shown) that helps the neutrophil slip between the endothelial cells, so it can migrate out of the bloodstream and into the tissue at the site of infection (**Movie 11.9**).

interact with it. Specific oligosaccharides in the carbohydrate layer are involved, for example, in the recognition of an egg by sperm (discussed in Chapter 19). Similarly, in the early stages of a bacterial infection, carbohydrates on the surface of white blood cells called *neutrophils* are recognized by a lectin on the cells lining the blood vessels at the site of infection; this recognition causes the neutrophils to adhere to the blood vessel wall and then migrate from the bloodstream into the infected tissue, where they help destroy the invading bacteria (**Figure 11–37**).

ESSENTIAL CONCEPTS

- **Membranes enable cells to create barriers that confine particular molecules to specific compartments. They consist of a continuous double layer—a bilayer—of lipid molecules in which proteins are embedded.**
- **The lipid bilayer provides the basic structure and barrier function of all cell membranes.**
- **Membrane lipid molecules are amphipathic, having both hydrophobic and hydrophilic regions. This property promotes their spontaneous assembly into bilayers when placed in water, forming closed compartments that reseal if torn.**
- **There are three major classes of membrane lipid molecules: phospholipids, sterols, and glycolipids.**
- **The lipid bilayer is fluid, and individual lipid molecules are able to diffuse within their own monolayer; they do not, however, spontaneously flip from one monolayer to the other.**
- **The two monolayers of a cell membrane have different lipid compositions, reflecting the different functions of the two faces of the membrane.**
- **Cells that live at different temperatures maintain their membrane fluidity by modifying the lipid composition of their membranes.**
- **Membrane proteins are responsible for most of the functions of cell membranes, including the transport of small, water-soluble molecules across the lipid bilayer.**
- **Transmembrane proteins extend across the lipid bilayer, usually as one or more α helices but sometimes as a β sheet rolled into the form of a barrel.**
- **Other membrane proteins do not extend across the lipid bilayer but are attached to one or the other side of the membrane, either by noncovalent association with other membrane proteins, by covalent attachment of lipids, or by association of an exposed amphipathic α helix with a single lipid monolayer.**

- Most cell membranes are supported by an attached framework of proteins. An especially important example is the meshwork of fibrous proteins that forms the cell cortex underneath the plasma membrane.
- Although many membrane proteins can diffuse rapidly in the plane of the membrane, cells have ways of confining proteins to specific membrane domains. They can also immobilize particular membrane proteins by attaching them to intracellular or extracellular macromolecules.
- Many of the proteins and some of the lipids exposed on the surface of cells have attached sugar chains, which form a carbohydrate layer that helps protect and lubricate the cell surface, while also being involved in specific cell–cell recognition.

KEY TERMS

amphipathic
bacteriorhodopsin
cell cortex
cholesterol
detergent
fat droplet
glycocalyx
lipid bilayer
membrane domain
membrane protein
phosphatidylcholine
phospholipid
plasma membrane
saturated
unsaturated

QUESTIONS

QUESTION 11–7

Describe the different methods that cells use to restrict proteins to specific regions of the plasma membrane. Can a membrane with many of its proteins restricted still be fluid?

QUESTION 11–8

Which of the following statements are correct? Explain your answers.

A. Lipids in a lipid bilayer spin rapidly around their long axis.

B. Lipids in a lipid bilayer rapidly exchange positions with one another in their own monolayer.

C. Lipids in a lipid bilayer do not flip-flop readily from one lipid monolayer to the other.

D. Hydrogen bonds that form between lipid head groups and water molecules are continually broken and re-formed.

E. Glycolipids move between different membrane-enclosed compartments during their synthesis but remain restricted to one side of the lipid bilayer.

F. Margarine contains more saturated lipids than the vegetable oil from which it is made.

G. Some membrane proteins are enzymes.

H. The sugar layer that surrounds all cells makes cells more slippery.

QUESTION 11–9

What is meant by the term "two-dimensional fluid"?

QUESTION 11–10

The structure of a lipid bilayer is determined by the particular properties of its lipid molecules. What would happen if:

A. phospholipids had only one hydrocarbon tail instead of two?

B. the hydrocarbon tails were shorter than normal, say, about 10 carbon atoms long?

C. all of the hydrocarbon tails were saturated?

D. all of the hydrocarbon tails were unsaturated?

E. the bilayer contained a mixture of two kinds of phospholipid molecules, one with two saturated hydrocarbon tails and the other with two unsaturated hydrocarbon tails?

F. each phospholipid molecule were covalently linked through the end carbon atom of one of its hydrocarbon tails to a phospholipid tail in the opposite monolayer?

QUESTION 11–11

What are the differences between a phospholipid molecule and a detergent molecule? How would the structure of a phospholipid molecule need to change to make it a detergent?

QUESTION 11–12

A. Membrane lipid molecules exchange places with their lipid neighbors every 10^{-7} second. A lipid molecule diffuses from one end of a 2-μm-long bacterial cell to the other in

about 0.2 seconds. Are these two numbers in agreement (assume that the diameter of a lipid head group is about 0.5 nm)? If not, can you think of a reason for the difference?

B. To get an appreciation for the great speed of molecular diffusion, assume that a lipid head group is about the size of a ping-pong ball (4 cm in diameter) and that the floor of your living room (6 m × 6 m) is covered wall-to-wall with these balls. If two neighboring balls exchanged positions once every 10^{-7} second, what would their speed be in kilometers per hour? How long would it take for a ball to move from one side of the room to the opposite side?

QUESTION 11–13

Why does a red blood cell plasma membrane need transmembrane proteins?

QUESTION 11–14

Consider a transmembrane protein that forms a hydrophilic pore across the plasma membrane of a eukaryotic cell. When this protein is activated by binding a specific ligand on its extracellular side it allows Na^+ to enter the cell. The protein is made of five similar transmembrane subunits, each containing a membrane-spanning α helix with hydrophilic amino acid side chains on one surface of the helix and hydrophobic amino acid side chains on the opposite surface. Considering the function of the protein as a channel for Na^+ ions to enter the cell, propose a possible arrangement of the five membrane-spanning α helices in the membrane.

QUESTION 11–15

In the membrane of a human red blood cell, the ratio of the mass of protein (average molecular weight 50,000) to phospholipid (molecular weight 800) to cholesterol (molecular weight 386) is about 2:1:1. How many lipid molecules are there for every protein molecule?

QUESTION 11–16

Draw a schematic diagram that shows a close-up view of two plasma membranes as they come together during cell fusion, as shown in Figure 11–30. Show membrane proteins in both cells that were labeled from the outside by the binding of differently colored fluorescent antibody molecules. Indicate in your drawing the fates of these color tags as the cells fuse. Will the fluorescent labels remain on the outside of the hybrid cell after cell fusion and still be there after the mixing of membrane proteins that occurs during the incubation at 37°C? How would the experimental outcome be different if the incubation were done at 0°C?

QUESTION 11–17

Compare the hydrophobic forces that hold a membrane protein in the lipid bilayer with those that help proteins fold into a unique three-dimensional structure (described in Chapter 4, pp. 121–122 and pp. 127–128).

QUESTION 11–18

Predict which one of the following organisms will have the highest percentage of unsaturated phospholipids in its membranes. Explain your answer.

A. Antarctic fish

B. Desert snake

C. Human being

D. Polar bear

E. Thermophilic bacterium that lives in hot springs at 100°C.

QUESTION 11–19

Which of the three 20-amino-acid sequences listed below in the single-letter amino acid code is the most likely candidate to form a transmembrane region (α helix) of a transmembrane protein? Explain your answer.

A. I T L I Y F G N M S S V T Q T I L L I S

B. L L L I F F G V M A L V I V V I L L I A

C. L L K K F F R D M A A V H E T I L E E S

QUESTION 11–20

Figure Q11–20 shows the structure of triacylglycerol. Would you expect this molecule to be incorporated into the lipid bilayer? If so, which part of the molecule would face the interior of the bilayer and which would face the water on either side of the bilayer? If not, what sort of structure would these molecules form in the aqueous environment inside a cell?

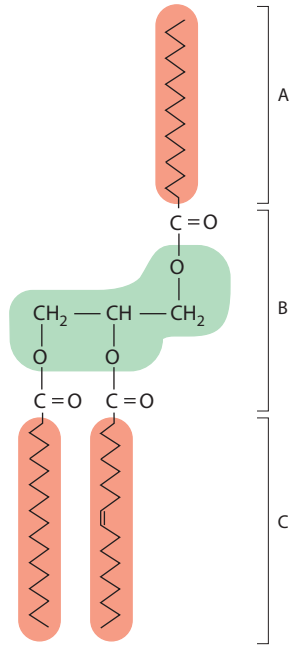


Figure Q11–20 triacylglycerol