1 Biological Membranes

1.1 Introduction

Biological membranes maintain the spatial organization of life. The first living cells felt the need to be enveloped by a selectively permeable barrier to protect and shield the set of their life-sustaining chemical transformations from changes in the environment. This envelope, which encloses all living cells, is the plasma membrane. The plasma membrane prevents undesirable agents from entering the cell, while keeping needed molecules on its inside. To function effectively, it must also selectively pass molecules, ions, and signals from one side to the other. Its permeability properties ensure that essential molecules such as glucose, amino acids, and lipids readily enter the cell, metabolic intermediates remain in the cell, and waste compounds leave the cell. The organization of the metabolic activity of the cell requires an additional compartmentalization in its inside, via specialized subunits called organelles, which are again enveloped by a membrane and carry out specific functions. The aqueous solution inside the organelles often contains solutes that are different from those of the solution directly contained in the cell (the cytosol), and the organelle membranes maintain such a difference.

Since all membranes are interposed between two aqueous media, the strategy underlying their function involves creating a hydrophobic barrier, which is formed by a thin lipid layer. The water-soluble compounds present both within cells and organelles as well as outside of them are not soluble in the lipid medium of the membrane, and pass slowly or not at all through it. The lipid material is particularly convenient for a number of reasons. Thus, it assembles spontaneously into two juxtaposed lipid monolayers that generate a highly hydrophobic region in their interior, while exposing a relatively hydrophilic surface to the aqueous solutions bathing the two sides of the membrane. Moreover, the lipid bilayer so formed is highly fluid and allows an easy incorporation of different biomolecules capable of spanning it and of transferring molecules, energy, or information across it in a selective way. Finally, the hydrophobic interior of the lipid bilayer, thanks to its low dielectric constant, concentrates by far the majority of the electric potential difference between the two sides of the membrane, that is, the transmembrane potential. The change of the transmembrane potential
over time, often induced by some external stimulus, modulates the function of many biomolecules incorporated in the membrane.

Life, similar to all other processes in our universe, obeys the laws of physics and chemistry. Consequently, all biological processes may occur only if they are accompanied by a decrease in the corresponding Gibbs energy, in accordance with the second law of thermodynamics. Some biological processes, when taken separately and out of their context, may seem to proceed with an increase in Gibbs energy. However, an attentive examination of their context reveals unavoidably that they are intimately coupled to some other process that proceeds with a decrease in Gibbs energy, such that the combination of the two is still characterized by a decrease in Gibbs energy. This coupling finds its justification in the thermodynamics of irreversible processes.

The thickness of the plasma membrane is much smaller than the radius of a cell, allowing us to treat the membrane surface as if it were planar; this simplifies the mathematics to a significant extent. To understand in depth the structure and function of biological membranes, it is also essential to understand and apply the principles of physical chemistry. In particular, the fundamental role played by the transmembrane potential in modulating the function of the biomolecules incorporated in the membrane allows us to consider and treat the membrane as a proper electrified interface. Hence, to understand the function of biological membranes and the properties of their experimental models, called biomimetic membranes, a knowledge of some basic principles of electrochemistry and of the most significant electrochemical techniques is required. The purpose of this work is to construct a coherent thermodynamic and electrochemical framework to achieve this goal. The role of the electrochemical foundations and techniques for the investigation of processes of biological relevance was first recognized in the 1980s, when Gutmann and Keyzer (1986) coined the name of bioelectrochemistry to denote this area of science.

In addition to membrane processes, bioelectrochemistry deals with the investigation of the properties and functions of water-soluble biomolecules, usually by adsorbing them on surface-modified and derivatized electrodes and by studying electron transfer reactions between them and the electrode (Bartlett, 2008; Alkire et al., 2011). However, the electrochemical behavior of biomolecules at electrodes does not necessarily pertain to bioelectrochemistry: this is true only if it provides some useful piece of information on the role played by these molecules in biological processes. These aspects of bioelectrochemistry are beyond the scope of this book. Bioelectrochemistry has also many applications in practical devices such as biosensors and biofuel cells.

1.2 The Biological Membranes

To stay alive, all living things need biological membranes (briefly, biomembranes). Biomembranes are thin layers that form the outer boundary of living cells, separating their inside (the cytoplasm) from their outside (the extracellular
The cytoplasm comprises the cytosol (a gel-like substance enclosed within the cell membrane) and a number of substructures called organelles, which are also enclosed by a membrane. The membrane enclosing a cell is called plasma membrane. One important component of biological membranes consists of two monolayers (leaflets) of lipid molecules (Fig. 1.1). Lipid molecules are amphiphilic, that is, they have a hydrophobic section (the hydrocarbon tail) and a hydrophilic section (the polar head or headgroup). In biomembranes, the two lipid monolayers are oriented with the hydrocarbon tails directed toward each other and the polar heads turned toward the aqueous solutions that bath the two sides of the membrane. The resulting lipid bilayer, about 6 nm thick, is a matrix that incorporates different proteins performing a variety of functions.

Biomembranes form a highly selective barrier between the inside and the outside of living cells. They are highly insulating to inorganic ions, and large ion concentration gradients can be maintained across them. The permeability and structural properties of biological membranes are sensitive to the chemical nature of the membrane components and to events that occur at the interface or within the bilayer. For example, biomembranes provide the environmental matrix for proteins that specifically transport certain ions and other molecules, for receptor proteins and for signal transduction molecules. The lipid and protein portions of biomembranes are also sensitive to the presence of lipophilic perturbants, that is, molecules with a high affinity for lipids. Anesthetics, for example, readily partition into lipid membranes, altering their electrical and permeability characteristics. The various responses observed in biomembranes are concentration dependent, usually very rapid and reversible, and frequently dependent upon the transmembrane potential.

**Figure 1.1** Schematic picture of a plasma membrane, showing the bimolecular layer of lipid molecules (including cholesterol), integral proteins spanning the lipid bilayer, peripheral proteins, filaments of cytoskeleton (the cellular “scaffolding” present in the cytoplasm), as well as glycoproteins, which expose their covalently attached oligosaccharide chains (glycans) to the extracellular fluid. Source: https://commons.wikimedia.org/wiki/File:Cell_membrane_detailed_diagram_de.svg.
Proteins are composed of linear chains of polymers of amino acids (polypeptide chains) linked by amide bonds, called peptide bonds. Some of the proteins (the structural proteins) simply support the texture of the membrane. A more important group of proteins (the functional proteins) participates directly in membrane processes such as flow of matter, energy, or information. Some proteins (the integral proteins) are embedded in the lipid bilayer with the hydrophobic section of their polypeptide chains, and protrude from the bilayer surface into one or both the adjacent aqueous solutions with the extrinsic, more hydrophilic section of the chains. Other proteins (the peripheral proteins) are weakly bound to the surface of the bilayer by electrostatic interactions or by hydrogen bonds, and interact with the polar heads of the lipid or with the integral membrane proteins. These are globular proteins, namely proteins in which the polypeptide chain folds spontaneously in a way that removes the hydrophobic sections from contact with the aqueous solution, burying them in the interior of the protein; conversely, the hydrophilic sections remain on the surface of the protein, where they form hydrogen bonds with water and between themselves. These proteins are water soluble. Some of them (e.g., cytochrome c, plastocyanin, ferredoxin) contain electrophilic metal ions and exchange electrons with the integral proteins. The majority of redox proteins, namely proteins containing one or more redox sites, have no biological function when taken alone; rather, they are associated with other redox proteins.

Short linear chains of polymers of amino acids linked by peptide bonds are called peptides. The distinction between peptides and proteins is not well defined. This distinction is often based on the number of amino acid residues. Peptides are considered to have less that 50 residues, polypeptides from 50 to 100 residues, and proteins more than 100 residues. However, according to a different classification, even polypeptide chains of less than 50 residues are referred to as proteins, provided they have a well-defined secondary structure, consisting of regularly repeating conformations of their polypeptide backbone. This is the case of sarcolipin (with 31 residues) or phospholamban (with 51 residues), which modulate the function of the calcium pump \(\text{Ca}^{2+}\)-ATPase of the sarcoplasmic reticulum. Many small peptides have biological activity and important functions.

1.2.1 The Lipids and the Lipid Bilayer

Lipids form the basic structure of biological membranes in/on which other components may be anchored. Nonetheless, the overall average weight composition of biological membranes is only 40% lipid and 60% protein, with a considerable variation in this proportion, depending on the membrane type. Lipids are amphiphilic compounds consisting of a relatively small hydrophilic head bound to two long hydrocarbon tails. They can be classified into derivatives of glycerol (glycerolipids), derivatives of sphingosine (sphingolipids), and sterols.

In glycerolipids, the glycerol is usually esterified by two fatty acids and by phosphoric acid. Without further esterification, one obtains phosphatidic acid (PA). However, the phosphate group of PA almost always undergoes a further esterification with ethanolamine, choline, or serine, giving rise to
**Figure 1.2** Structures of dioleoylphosphatidylethanolamine (DOPE), dioleoylphosphatidylcholine (DOPC), dioleoylphosphatidylserine (DOPS), and diphytanoylphosphatidylcholine (DPhPC).

*phosphatidylethanolamine* (PE), *phosphatidylcholine* (PC), and *phosphatidylserine* (PS) (Fig. 1.2). At physiological pH, both PE and PC are neutral, albeit zwitter-ionic, while PS carries a negative charge. The variously esterified phosphate group constitutes the hydrophilic polar head of the glycerolipid.

Sphingolipids are based on the backbone of sphingosine, a long-chain alcohol containing a hydrocarbon tail, an amino group that may attach a fatty acyl group yielding ceramide, and a C-1 hydroxyl group (Fig. 1.3). Esterification of the C-1 hydroxyl group with phosphoric acid and esterification of the latter with choline yields sphingomyelin, one of the most important lipids of this class. On the other hand, if the C-1 hydroxyl group of sphingosine binds to a sugar residue by a β-glycosidic linkage, it gives rise to a glycosphingolipid. Sphingolipids are particularly abundant in the tissues of the nervous system. Sterols are rigid, compact molecules derived from a system of four fused saturated rings (Fig. 1.4). The major sterol in the plasma membrane of all mammalian cells is cholesterol. Plant membranes do not contain cholesterol, but contain other sterols, such as ergosterol.

Glycerophospholipids and sphingolipids dispersed in an aqueous phase tend to spontaneously form lipid bilayers. In the absence of a solid support, these bilayers have a spherical shape and consist of multilamellar aggregates in which stacked lipid bilayers are separated by water layers (multilamellar vesicles). Excess water and rapid stirring may break these aggregates giving rise to unilamellar vesicles, which consist of a single spherical lipid bilayer (Fig. 1.5). The term liposome is used to denote both multi- and unilamellar vesicles. Micelles are a different type of lipid aggregates in which the hydrocarbon tails of the lipid molecules converge.
Figure 1.3 (A) Basic building blocks of sphingolipids; ceramide consists of the combination of the two differently highlighted portions, with \( R = H \). (B) Structure of the predominant form of egg sphingomyelin.

Figure 1.4 Structures of cholesterol and ergosterol.

Lipid bilayers are formed when the overall cross-sectional area of the two hydrocarbon tails of the lipid molecule is almost equal to that of the polar heads. Thus, lysophospholipids, which have only one hydrocarbon tail, cannot form lipid bilayers, because the head is appreciably larger than the tail. Instead, they tend to form micelles in aqueous solution. Lipid bilayer formation is accompanied by a decrease in the Gibbs energy, \( G = H - TS \), and, hence, occurs spontaneously. The decrease in the entropy \( S \), due to the increased order of lipid molecules in the bilayer, is more than compensated for by the increased translational entropy of the water molecules escaped from direct contact with the lipid. Moreover, the attractive van der Waals interactions between adjacent lipid molecules within the bilayer and the increase in the number of hydrogen bonds of the water molecules on the center of the aggregate, while their polar heads are exposed to the aqueous solution.
escaped from contact with the lipid contribute in decreasing the enthalpy $H$ of the system.

A lipid bilayer can exist in a solid-ordered ($s_o$) state, called gel state, if the temperature is low enough. The gel state is anisotropic, tightly packed, and has limited lateral mobility and axial rotation of the hydrocarbon tails. As temperature is increased, a melting temperature $T_m$ is reached at which a phase transition from the $s_o$ state to a liquid-crystalline state takes place. The latter state is isotropic, loosely packed, and has a high degree of lateral mobility and axial rotation. However, even in this state, the lipid molecules do not easily pass from one leaflet of the bilayer to the other, due to the high resistance opposed by the hydrophobic core of the bilayer to the penetration of the hydrophilic polar head, as required by this flip-flop movement.

Lipids with one or more carbon–carbon double bonds (unsaturated lipids) have low $T_m$ values, because each double bond produces a kink in the acyl chain, creating extra free space within the bilayer, disrupting its regular packing, and imparting additional flexibility to the hydrocarbon tails. On the other hand, lipids without carbon–carbon double bonds (saturated lipids) have melting temperatures that may exceed room temperature, if the length of their hydrocarbon tails is long enough. Longer hydrocarbon tails increase the van der Waals interactions between adjacent lipid molecules, decreasing the lipid mobility. Increasing the length of a saturated hydrocarbon tail by one carbon usually increases the melting temperature of a saturated lipid by 10°C or less. In particular, saturated phospholipids with hydrocarbon tails longer than 14 carbons are in the $s_o$ state at room temperature, while those with fewer than 14 are in the liquid-crystalline state. Thus, dipalmitoylphosphatidylcholine and palmitoylsphingomyelin, with saturated, 16-carbon hydrocarbon tails, have a $T_m$ value of 41°C, while dioleoylphosphatidylcholine, with two 18-carbon monounsaturated tails, has a $T_m$ value of $-20^\circ$C. However, DPhPC (Fig. 1.2),
albeit saturated, forms liquid-crystalline bilayers at room temperature and does not exhibit a detectable gel to liquid-crystalline phase transition from $-120^\circ C$ to $+120^\circ C$, thanks to the presence of the methyl groups regularly distributed along the two hydrocarbon tails. Due to its high fluidity and resistance to oxidation, it is frequently employed in the preparation of biomimetic membranes.

In many biomembranes, lipid bilayers consist of mixtures of lipids with $T_m$ values higher and lower than room temperature. In this case, the lipid bilayer is often not homogeneous, but consists of lipid microdomains immersed in a lipid matrix. Typically, gel-phase microdomains, enriched in the high-$T_m$ saturated components, are surrounded by a liquid-crystalline phase enriched in the low-$T_m$ unsaturated components (Brown and London, 1998; 2000). The presence of cholesterol in these lipid mixtures has a disordered effect on the gel phase and an ordering effect on the liquid-crystalline phase. More precisely, in the liquid-crystalline phase cholesterol decreases the axial rotation of acyl chains and, at concentrations above 30 mol%, it also decreases lateral diffusion. This is due to its nonpolar rigid ring system, which allows the cholesterol molecule to fit into the kink of the unsaturated hydrocarbon tails; this increases van der Waals contact and decreases the membrane fluidity. Conversely, cholesterol increases lateral diffusion and axial rotation in the gel-phase microdomains, causing them to become isotropic (Parasassi et al., 1995). This is revealed by a morphological investigation of these lipid mixtures by two-photon excitation fluorescence microscopy (Bagatolli, 2006). Thus, gel-phase microdomains have irregular shapes due to their anisotropic structure. Conversely, the microdomains in the presence of cholesterol have a roundish shape, because they are isotropic similar to the surrounding liquid-crystalline phase. In other words, cholesterol causes the microdomains to pass from the gel phase to the so-called liquid-ordered ($l_o$) phase. This phase is considered as liquid, thanks to its sufficient lateral diffusion, and ordered because it is more tightly packed than the liquid-crystalline phase, due to the still modest axial rotation of its acyl chains. These $l_o$ microdomains are called lipid rafts (Pike, 2004) (Fig. 1.6). In contraposition with the $l_o$ phase, the liquid-crystalline phase is often referred to as the liquid-disordered ($l_d$) phase.

The effect of cholesterol on gel-phase microdomains is again ascribable to its nonpolar rigid ring system, which prevents close contact between the saturated hydrocarbon tails, acting as an impurity that lowers the $T_m$ of the microdomain. The dual role of cholesterol in animal plasma membranes serves to maintain the fluidity of the membrane constant through fluctuations in temperature. Lipid rafts are considered to regulate the membrane function in eukaryotic cells, namely cells containing a nucleus, as distinct for prokaryotic cells, which do not contain it (Simons and Ikonen, 1997). They are involved in cell signaling and molecular trafficking. A number of peripheral proteins tend to associate with lipid rafts, thus communicating more easily between themselves.

The lipid composition of biomembranes, albeit constant for a given cell type, varies notably in passing from one cell to another. Membranes that do not contain cholesterol, such as the inner and outer membranes of mitochondrion, do not form lipid rafts and are often in the $l_d$ state. Lipids are distributed asymmetrically
between the inner and outer leaflets of the bilayer, thanks to the very slow flip-flop movement. For example, the plasma membrane of human red blood cells (the *erythrocytes*) contains sphingomyelin and PC mainly in the outer leaflet, and PE and the negatively charged PS exclusively in the inner leaflet.

1.2.2 The Membranes of Cells and Organelles

The cytoplasm, enclosed within the plasma membrane, is a highly organized system concerned with the activity of the cell (Fig. 1.7). The plasma membrane separates this highly organized system from the relative chaos that exists outside the cell. To maintain and increase its high degree of organization and to respond to its environment, the cell requires a continuous exchange of matter, energy, and information with its surroundings, which takes place across the plasma membrane. Analogous exchanges also take place across other important membranes that envelope several organelles; these subunits reside inside eukaryotic cells and are suspended in the cytosol, the aqueous medium of the cytoplasm (Rawn, 1989).

The nucleus is an organelle that contains more than 95% of the cell genetic material, organized as multiple long linear *deoxyribonucleic acid* (DNA) molecules, and is the control center of eukaryotic cells. The nucleus is enclosed by a double membrane called *nucleus envelope*, which allows the movement of proteins and *ribonucleic acid* (RNA) from the nucleus to the cytoplasm. DNA replication and RNA transcription, the first steps in the expression of genetic information, take place in the nucleus.

Another important organelle, the *mitochondrion*, is the site of the major part of the energy production of *aerobic* cells, that is, cells that require oxygen for their function. It is enveloped by an unfolded outer membrane and by an inner membrane folded into membrane curtains called *cristae*. The aqueous solution enclosed by the inner membrane is called *matrix*, whereas the region enclosed between the inner and outer membranes is called *intermembrane space.*
The matrix is the site of glycolysis and the citric acid cycle, which consist of numerous enzymatic oxidation reactions of carbohydrates, amino acids, and fatty acids. The final product of many of these reactions is the reduced form of nicotinamide adenosine dinucleotide (NADH). The inner mitochondrial membrane incorporates a system of three multiprotein electron-transport chain complexes that carry electrons from NADH to molecular oxygen across the membrane (cf. Section 3.4.2), according to the net reaction:

\[
\text{NADH} + \frac{1}{2}\text{O}_2 + \text{H}^+ \rightarrow \text{H}_2\text{O} + \text{NAD}^+ \tag{1.1}
\]

The net effect of this respiratory electron-transport chain is the generation of an electrochemical potential gradient of protons across the inner mitochondrial membrane; the driving force that moves protons from the matrix to the intermembrane space is sometimes referred to as the proton-motive force. The three multiprotein complexes are also referred to as the proton-pumping oxidoreductases. The protons pumped into the intermembrane space move back into the matrix spontaneously along an integral protein embedded in the inner mitochondrial membrane, called ATP synthase, which exploits part of the Gibbs energy involved in this spontaneous proton flow to synthesize adenosine $S'$-triphosphate (ATP). This highly energetic molecule powers several cellular processes, such as $\text{H}^+$, $\text{Na}^+$, $\text{K}^+$, and $\text{Ca}^{2+}$ pumping across membranes, by dissociating into adenosine
$S'$-diphosphate (ADP) and inorganic phosphate, briefly denoted by $P_i$. A respiratory electron-transport chain similar to that present in the inner mitochondrial membrane is also found in the plasma membrane of bacteria. The respiratory electron-transport chain is a typical example of energy flow across membranes.

Other membranes present in eukaryotic cells are those enveloping the *rough endoplasmic reticulum*, which form a network through the cell and merge with the outer membrane of the nucleus envelope (Fig. 1.7). They are coated with *ribosomes*, large complexes of RNA and proteins that catalyze the formation of proteins from individual amino acids, using messenger RNA as a template. The *smooth endoplasmic reticulum* has no attached ribosomes and is in charge of lipid production. It is associated with a membrane system consisting of sets of flattened sacs called the *Golgi complexes*, where proteins are provided with carbohydrate or lipid moieties, yielding glycoproteins or lipoproteins.

Muscle fiber cells contain a specialized form of endoplasmic reticulum called *sarcoplasmic reticulum*, a network of pockets that acts as a calcium reservoir, where the calcium concentration is from three to four orders of magnitude higher than in the cytoplasm. Excitation by a nerve impulse changes the electric potential across the membrane of the muscle fiber cell, inducing the sarcoplasmic reticulum to release Ca$^{2+}$ ions into the cytoplasm (called the *sarcoplasm* in muscle cells) and promoting muscle contraction. The calcium pump Ca$^{2+}$-ATPase, an integral protein embedded in the membrane of the sarcoplasmic reticulum and powered by ATP, drives Ca$^{2+}$ ions from the sarcoplasm back into the lumen of the sarcoplasmic reticulum, promoting muscle relaxation. Cells also contain vesicles filled with several enzymes, called *lysosomes*. They digest excess of worn-out organelles and food particles, and engulf viruses or bacteria. When a cell dies, its lysosomes rupture, and the enzymes so released hydrolyze the components of the dead cell.

The shape of cells is maintained by a protein scaffolding consisting of a network of different structures, called *cytoskeleton*, contained within the cytoplasm of all cells; it is made up of three types of protein filaments: *actin filaments* (also called microfilaments), *intermediate filaments*, and *microtubules*. This ordered network of filaments forms a band just beneath the plasma membrane; besides providing mechanical support to the plasma membrane, it has additional functions, such as moving organelles within the cell or linking transmembrane proteins (especially receptors) to cytoplasmic proteins.

The structure of plant cells is in many ways similar to that of animal cells. However, plant cells also contain specialized organelles called *chloroplasts*, which are the site of green-plant photosynthesis (Fig. 1.8). The chloroplast is enveloped by a double membrane that surrounds an aqueous solution called *stroma*. This contains a continuous membrane called *thylakoid membrane*, which is highly folded into flattened, sac-like vesicles, called *grana* (singular: *granum*), and encloses an internal space (the *lumen*). An electron-transport chain, consisting of a sequence of redox proteins embedded in the thylakoid membrane, absorbs light and couples the photon energy with a redox reaction. This process transfers electrons from water to the oxidized form of nicotinamide adenine dinucleotide phosphate (NADP$^+$) yielding its reduced form, NADPH, and molecular oxygen.
Such a reaction is accompanied by an increase in Gibbs energy and, therefore, it could not occur spontaneously without coupling with light absorption. Even this electron-transport chain generates an electrochemical potential gradient of protons across the thylakoid membrane. The spontaneous proton flow from the stroma to the lumen along an ATP synthase embedded in the thylakoid membrane is exploited by this protein to synthesize ATP from ADP, similar to that in mitochondrial respiration. This is a further example of energy flow across membranes.

The vacuoles of plant and fungal cells are analogous to animal lysosomes in their relatively low pH and in their content of enzymes (called proteinases or peptidases), which hydrolyze the peptide bonds of the polypeptide chains of proteins. The smooth flat membrane that surrounds them is called tonoplast.

### 1.3 The Proteins

A membrane incorporates a number of proteins, which are constituted by linear chains of polymers of amino acids. If a protein is hydrolyzed under accurately controlled conditions, it releases up to a maximum of 20 different amino acids. Since a genetic code is responsible for the formation of the amino acid chain, and since all organisms use the same genetic code, it is probable that evolution of life stared from a common ancestral cell. The conformation and function of a protein
are determined by its amino acid composition and by the sequence in which they are bound to form the polypeptide chain. This sequence constitutes the primary structure of the protein. Each protein amino acid has a central carbon, referred to as the $\alpha$-carbon, which is attached to four different groups: a basic amino group ($-$NH$_2$), an acidic carboxyl group ($-$COOH), a hydrogen atom, and a group called side chain, which characterizes one amino acid with respect to all others (Fig. 1.9).

Of the 20 protein amino acids, 11 have hydrophobic side chains (alkyl chains or aromatic rings, with the exclusion of cysteine and methionine, which also have a sulfur atom); 4 have hydrophilic side chains, due to the presence of a hydroxyl or carbamidic group ($\text{H}_2\text{N} = \text{C} = \text{O}$); 3 have positively charged side chains, due to the presence a charged nitrogen atom (lysine, arginine, and histidine); and 2 have a negatively charged chain due to the presence of a carboxyl group (aspartate and glutamate).

At physiological pH, amino acids are present in the zwitterionic form, in which the proton of the carboxyl group is donated to the amino group, giving rise to a neutral dipolar molecule. When amino acids join up in a chain, the $\alpha$-carboxylate group of one amino acid condenses with the $\alpha$-amino group of another, with loss of a water molecule and formation of a particular amide bond called peptide bond (Fig. 1.9). Formation of a sequence of peptide bonds generates a polypeptide, a long, continuous, and unbranched peptide, which is the structural element of proteins. The linked amino acids are referred to as the amino acid residues. The free amino group and the free carboxyl group at opposite ends of a polypeptide chain are called termini: amino terminus or N-terminus on one side, and carboxyl terminus or C-terminus on the opposite side. Both groups are ionized at pH 7. The side chains, denoted by $R_1$ and $R_2$ in Fig. 1.9, confer different properties to amino acids, and hence to the corresponding amino acid residues. Proteins consist of one or more polypeptides arranged in a biologically functional way and are often bound to cofactors, nonprotein chemical compounds that assist the protein in its transformations.

Integral proteins consist of polypeptide chains alternating, on the average, more hydrophobic and more hydrophilic sections. They are organized in the membrane with the hydrophobic sections embedded in the lipid bilayer, while the hydrophilic sections protrude from the lipid bilayer and are exposed to the two aqueous solutions that bath the membrane. The membrane organization is a
direct consequence of this partitioning between its two components: lipids and proteins. Since these components are not held together by chemical bonds, they are free to diffuse and move independently within the membrane plane. Clear experimental evidence in favor of protein mobility in membranes is obtained by labeling the protein with a fluorophore, in order to follow its movement. A short burst of intense excitation light is projected onto the membrane, destroying the fluorescence of the fluorophore molecules in a well-defined spot, a photochemical process called photobleaching. The gradual fluorescence recovery within the given spot is followed as a function of time, thus permitting an estimate of the diffusion coefficient of the protein. The model assuming free independent mobility of the various membrane components is termed fluid mosaic model, after Singer and Nicolson (1972).

Nowadays, the acknowledgment of the existence of ordered microdomains, called lipid rafts, within the more disordered lipid matrix, and of noncovalent bonds between plasma membrane proteins and the components of the cytoskeleton, has partly modified some concepts of the fluid mosaic model. In fact, not all components of a membrane are necessarily distributed in a totally homogeneous and casual way.

1.4 The Membrane Functions

Biological membranes mediate the interaction of the cells and organelles that they enclose with their environment. This mediation may give rise to three types of flows across membranes, namely flow of matter, flow of energy, and flow of information. The flow of matter involves the transport of molecules from one side of the membrane to the other (called translocation), and the movement of reactants and products of reactions catalyzed by membrane-bound enzymes. The flow of energy takes place across specialized membranes, that is, the inner mitochondrial membranes, the bacterial membranes, and the thylakoid membranes; here, the Gibbs energy released by the oxidation of nutrients or the energy of electromagnetic radiation can be exploited and stored in the form of an electrochemical potential gradient of protons and, exceptionally, of sodium ions, and ultimately, in the form of highly energetic molecules, such as ATP. The flow of information is restricted to some specialized membranes. They must be equipped with appropriate receptors for tactic stimuli, hormones, antigens, and any other piece of information that may arrive from the environment. They must also contain an enzymatic machinery capable of processing the information received, via the binding to a form recognizable by the intracellular components, where the final acceptor of the external stimulus is located.

The different types of flow across membranes are due to the various proteins embedded in the membrane. On the average, biomembranes contain more than one-half their weight of proteins, and it is this membrane component that endows them with a broad range of functional capabilities. A lipid bilayer lacking proteins is a mere diaphragm opposing an almost insurmountable potential energy barrier.
to any type of flow. It is, therefore, convenient to classify membrane proteins on the basis of their specific role. The proteins that span membranes may perform several diversified functions, and a single membrane protein may have multiple functions. These functions can be classified into several categories, which are discussed in the following sections.

1.4.1 Transport

The flow of molecules and ions across membranes is governed by sophisticated transport systems, usually integral membrane proteins, which play fundamental roles for the correct functioning of the cell. Thus, they regulate the cell volume and maintain the intracellular pH and ionic composition within a very narrow range to provide a favorable medium to the enzymatic activity; they allow the passage of molecules that are employed for energy production, or else as building blocks, and expel toxic substances; they generate electrochemical potential gradients essential for nerve and muscle excitability and for the transport of various substances.

The flow of substances across biological membranes, called membrane transport, may be categorized as either active or passive. In active transport, the transported species moves from a place where its electrochemical potential is lower to one where it is higher, namely against its electrochemical potential gradient. The active transport is an endergonic process, that is, a process characterized by an increase in Gibbs energy; as such, when taken separately, it cannot proceed spontaneously, as opposed to exergonic processes, that is, processes accompanied by a decrease in Gibbs energy. In fact, thermodynamics states that chemical processes may proceed only if they are accompanied by a decrease in their Gibbs energy. Thus, in order to proceed, an endergonic process must be coupled to an exergonic process, in such a way that the combined process is accompanied by a decrease in Gibbs energy. In other words, active transport requires the coupling to a primary energy source, such as light or chemical energy. In passive transport, the transported species moves from a place where its electrochemical potential is higher to one where it is lower, namely down its electrochemical potential gradient. Consequently, this type of transport is exergonic.

Membrane transport may also be classified on the basis of the number of different transported species and of the relative directions of flow. Transport is defined as uniport if it involves a single molecular species. The concerted and simultaneous movement of two species is distinguished between symport and antiport. The symport (also called cotransport) is the transport of two different molecular species in the same direction, whereas the antiport (also called countertransport) is the transport of two different molecular species in opposite directions. The proteins in charge of cotransport are called cotransporters, while those in charge of countertransport are called countertransporters. Ion transport across biological membranes may assume an electroneutral or electrogenic character. It is denoted as electroneutral or electrically silent if it does not give rise to a net flow of charge, as may be the case with the symport of ions of equal charge magnitude but opposite sign, or the antiport of ions of equal charge magnitude and sign. Conversely,
electrogenic ion transport is characterized by a net flow of charge and, therefore, generates a charge separation across the membrane through which it occurs.

Examples of passive transport are offered by a particular category of organic molecules, the ionophores. They are lipid-soluble molecules usually synthesized by microorganisms to transport ions across cell membranes. These molecules are frequently used to investigate transport phenomena, besides being important toxins or drugs. There are two broad classifications of ionophores: ion carriers and channel formers (also called pore formers). An ion carrier is a molecule that complexes a particular ion via hydrophilic groups lining the interior of its molecular structure (often carbonyl groups of its peptide chain), while it has a hydrophobic exterior. In this way, it shields the charge of the ion from the surrounding environment, thus facilitating its translocation across the hydrophobic interior of the membrane. A typical example is represented by valinomycin, consisting of a sequence of two amino acids and two carboxylic acids bound via amide and ester bridges, which is repeated three times to form a cyclic structure; it specifically cages K\(^+\) ions in the extracellular fluid and shuttles them into a cell across its plasma membrane.

Channel formers are molecules that form a pore whose interior is lined with a number of hydrophilic groups, while its exterior is hydrophobic and interacts attractively with the lipid molecules of the membrane. Channel formers span the whole membrane, allowing the ions that move within the pore to pass through at a rate several orders of magnitude higher than that of ions shuttled by ion carriers. They are usually formed by aggregation of a number of α-helical peptide monomers, which turn their hydrophilic side toward the lumen of the peptide bundle, giving rise to a hydrophilic ion channel. Incidentally, the α-helix is a common secondary structure of proteins with a right-hand-coiled conformation in which every backbone N − H group donates a hydrogen to the backbone C = O group of the amino acid four residues earlier (\(i + 4 \rightarrow i\) hydrogen bonding). The lumen of α-helices is too narrow to allow the passage of ions, even if desolvated. This is the reason why α-helical monomers must cluster to form an ion channel. A quite atypical, but extensively investigated, channel former is gramicidin, a linear peptide that transports monovalent cations. The diameter of the lumen of its helix is larger than that of α-helices, such that, differently than these latter, it allows the passage of ions through it. Since its length matches that of a single lipid monolayer, the gramicidin channel that spans a lipid bilayer is a dimer formed by two aligned monomeric units.

All known processes of active transport are mediated by asymmetrically oriented integral proteins. These proteins are characterized by reaction cycles during which they undergo conformational transitions that modify the orientation and the affinity of the binding site toward the transported species. Active transport is classified into two main categories: primary and secondary. Primary active transport is powered by primary energy sources, such as ATP hydrolysis (transport ATPases), electron transport (e.g., proton-pumping oxidoreductases in the inner mitochondrial membrane), and light (e.g., bacteriorhodopsin and alorhodopsin).
These systems of primary active transport are called *ion pumps* and transport ions selectively, generating electrochemical potential gradients. In turn, these gradients provide the Gibbs energy required to activate several physiological processes that, taken separately, are endergonic. Secondary active transport refers to systems that exploit the Gibbs energy of electrochemical potential gradients to transport ions and neutral molecules against their own electrochemical potential gradient. Typical examples of secondary active transport are offered by cotransporters and countertransporters.

### 1.4.2 Signal Transduction

A membrane protein, called *receptor*, has a binding site with specific shape that fits the shape of a *chemical messenger*, for example, a hormone. The external messenger (the signaling molecule) induces a conformational change in the protein, known as receptor activation, allowing it to relay the message to the inside of the cell. Receptor activation is always the initial step, leading to the cell’s ultimate responses to the messenger. Signal transduction covers both signaling from the receptor located in the plasma membrane and signaling via molecules located within the cell. The movement of signals can be simple, similar to that associated with the class of acetylcholine receptor molecules. These receptors constitute channels that, upon ligand interaction, allow signals to be passed in the form of small ion movements, either into or out of the cell. These ion movements result in changes in the transmembrane potential of the cell that, in turn, propagates the signal along the cell. More complex signal transductions involve the coupling of ligand–receptor interactions to many intracellular events. These events include the addition of a phosphate group to a protein or another organic molecule (*phosphorylation*) by a protein in charge of this task (*kinase*). Protein phosphorylations change enzyme activities and protein conformations. The eventual outcome is an alteration in cellular activity and changes in the program of genes expressed within the responding cell, by regulating the initiation of transcription in the messenger RNA.

### 1.4.3 Cell–Cell Recognition

It is the ability of cells to distinguish one type of neighboring cell from another. This is important, for example, in the sorting of cells into tissues or in the rejection of a foreign cell by the immune system. Some proteins of the plasma membrane are *glycoproteins*, namely proteins having a short, branched chain of usually less than 15 sugar units, exposed to the extracellular side of the membrane. These chains are also covalently bound to lipids, forming molecules called *glycolipids*. The carbohydrates on the extracellular side of plasma membranes vary from one cell type to another. Quite often, a cell recognizes another cell by binding to the carbohydrate moieties of glycolipids or glycoproteins on the extracellular surface of the plasma membrane of the other cell.
1.4.4 Enzymatic Activity

A protein built into the membrane may act as an enzyme, catalyzing various reactions related to the plasma membrane, with its active site exposed to substances in the external solution. It can be considered as a transmembrane receptor that binds an extracellular ligand, causing enzymatic activity on the intracellular side of the membrane. It has an extracellular ligand-binding domain, a transmembrane helix, and an intracellular domain, which has a catalytic function. The signaling molecule binds to the receptor outside the cell and causes a conformational change in the catalytic functional group of the receptor, which is located inside the cell. In some cases, several enzymes in a membrane are organized as a team that carries out sequential steps of a metabolic pathway.

1.4.5 Intercellular Joining

Membrane proteins of adjacent cells may hook together in various kinds of junctions, such as gap junctions or tight junctions. A gap junction is a specialized intercellular connection between animal cells. It directly links the cytoplasm of two cells, allowing various molecules to pass freely between them. The first gap junction protein was isolated from the liver and is called CX32. A gap junction channel is composed of two connexons (or hemichannels), one per cell, which keep the two interacting plasma membranes at a distance of about 2–4 nm apart. When the connexons in the plasma membranes of two cells in contact are aligned, they form a continuous aqueous channel that joins the two cell interiors. Each connexon is formed from six four-pass transmembrane proteins called connexins. Dye-injection experiments suggest a functional pore diameter of about 1.5 nm, implying that coupled cells share their small molecules, such as inorganic ions, sugars, amino acids, subunits of nucleic acids such as DNA or RNA (the nucleotides), and vitamins, but not their macromolecules. This cell coupling has important functional implications.

A tight junction is composed of a branching network of strands that seals the plasma membranes of two adjacent cells. Each sealing strand consists of a long row of transmembrane adhesion proteins embedded in each of the two interacting plasma membranes. The extracellular domains of these proteins join directly to one another to occlude the intercellular space. The major transmembrane adhesion proteins are the claudins and occludins, which differ from one tight junction to another. Strands act independent from each other, and hence the sealing efficiency of tight junctions increases with an increase in the number of strands. Tight junctions hold the cells together. Moreover, by pinning adjacent membranes together, they prevent the lateral diffusion of integral membrane proteins, confining them to the particular portion of the plasma membrane (either the apical or the basal portion) where they are required to exert a specialized function. The epithelial tissue, which lines all internal and external body surfaces, is made up of cells closely packed and ranged in one or more layers, often held together by tight junctions.
References
