Chapter 7

Membranes: Their Structure, Function, and Chemistry
Membranes: Their Structure, Function, and Chemistry

- **Membranes** define the boundaries of a cell, and its internal compartments

- Membranes play multiple roles in the life of a cell
Figure 7-1A

(a) Rat pancreas cells

- Secretory granules
- Plasma membranes
- Nuclear envelope
- Nucleus
- Rough ER
- Mitochondria

5 μm
The Functions of Membranes

• 1. Define boundaries of a cell and organelles and act as permeability barriers

• 2. Serve as sites for biological functions such as electron transport

• 3. Possess transport proteins that regulate the movement of substances into and out of cells and organelles
The Functions of Membranes (continued)

4. Contain protein molecules that act as receptors to detect external signals

5. Provide mechanisms for cell-to-cell contact, adhesion, and communication
Models of Membrane Structure: An Experimental Approach

• The development of electron microscopy in the 1950s was important for understanding membrane structure

• The fluid mosaic model is thought to be descriptive of all biological membranes

• The model envisions a membrane as two fluid layers of lipids with proteins within and on the layers
Overton and Langmuir: Lipids Are Important Components of Membranes

• In the 1890s Overton observed the easy penetration of lipid-soluble substances into cells and concluded that the cell surface had some kind of lipid “coat” on it

• Langmuir studied phospholipids and found that they were *amphipathic* and reasoned that they must orient on water with the hydrophobic tails away from the water
Gorter and Grendel: The Basis of Membrane Structure Is a Bilayer

- In 1925, these two physiologists extracted lipids from red blood cells and spread the lipids in a monolayer on a water surface.
- The film on the water was twice the surface area of the blood cells, suggesting that lipids on the cell surface consisted of two layers.
- They suggested that the most favorable structure would be a lipid bilayer, with the nonpolar regions of the lipids facing inward.
Figure 7-3C

(c) Lipid bilayer

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Davson and Danielli: Membranes Also Contain Proteins

- Davson and Danielli showed that the bilayer alone could not account for all properties of membranes, especially
  - surface tension
  - solute permeability
  - electrical resistance

- They suggested that proteins are present in membranes, as thin sheets, coating the lipids
Robertson: All Membranes Share a Common Underlying Structure

- Using electron microscopy, biologists could verify the presence of membranes around cells and organelles

- A trilaminar structure, visible under the TEM, was observed for all membranes, leading to the suggestion of a common membrane structure, called the *unit membrane*
Figure 7-4

Intercellular space

Cell 1

Cell 2
Further Research Revealed Major Shortcomings of the Davson–Danielli Model

- Electron microscopy revealed that there was not enough space to either side of the bilayer for an additional layer of protein

- The Davson–Danielli model also did not account for the chemical distinctiveness of particular types of membranes, especially the protein/lipid ratio
Table 7-1

<table>
<thead>
<tr>
<th>Membrane</th>
<th>Protein</th>
<th>Lipid</th>
<th>Carbohydrate</th>
<th>Protein/Lipid Ratio</th>
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<tbody>
<tr>
<td>Plasma membrane</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Human erythrocyte</td>
<td>49</td>
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<td>Mammalian liver cell</td>
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<td>Amoeba</td>
<td>54</td>
<td>42</td>
<td>4</td>
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<tr>
<td>Myelin sheath of nerve axon</td>
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<td>79</td>
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<td>0.23</td>
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<td>Nuclear envelope</td>
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<td>3.54</td>
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<td>Gram-positive bacterium</td>
<td>75</td>
<td>25</td>
<td>0</td>
<td>3.00</td>
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Additional challenges to the Davson–Danielli model

- Membranes are susceptible to digestion by *phospholipases*, suggesting that membrane lipids are exposed.

- Scientists were unable to isolate “surface” proteins from membranes unless organic solvents or detergents were used.
Singer and Nicholson: A Membrane Consists of a Mosaic of Proteins in a Fluid Lipid Bilayer

• The **fluid mosaic bilayer** model accounts for all the inconsistencies with previous models

• The model has two key features

  – A *fluid* lipid bilayer

  – A *mosaic* of proteins attached to or embedded in the bilayer
Figure 7-3F

(f) Fluid mosaic model

Singer and Nicolson
Unwin and Henderson

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Three classes of membrane proteins

1. *Integral membrane proteins* are embedded in the lipid bilayer due to their hydrophobic regions.

2. *Peripheral proteins* are hydrophilic and located on the surface of the bilayer.

3. *Lipid-anchored proteins* are hydrophilic and attached to the bilayer by covalent attachments to lipid molecules embedded in the bilayer.
(b) An integral membrane protein with multiple α-helical transmembrane segments is shown below. Many integral membrane proteins of the plasma membrane have carbohydrate side chains attached to the hydrophilic segments on the outer membrane surface.
The fluid nature of the bilayer

- Lipids in the bilayer are in constant motion
- Proteins are also able to move laterally within the membrane, though some are anchored to internal structural elements
- Anchored proteins have restricted mobility
Unwin and Henderson: Most Membrane Proteins Contain Transmembrane Segments

- Most integral membrane proteins have one or more hydrophobic segments that span the lipid bilayer

- These *transmembrane segments* anchor the protein to the membrane

- *Bacteriorhodopsin* was the first membrane protein shown to possess this structural feature
Recent Findings Further Refine Our Understanding of Membrane Structure

• Membranes are
  – not homogenous, but freely mixing
  – ordered through dynamic microdomains called lipid rafts

• Most cellular processes that involve membranes depend on structural complexes of specific lipids and proteins
Membrane Lipids: The “Fluid” Part of the Model

- Membrane lipids are important components of the “fluid” part of the fluid mosaic model

- Membranes contain several types of lipids
Membranes Contain Several Major Classes of Lipids

- The fluid mosaic model of membrane structure retains the lipid bilayer of earlier models.
- However, there is a greater diversity and fluidity of lipids than originally thought.
- The main classes of membrane lipids are phospholipids, glycolipids, and sterols.
Phospholipids

- **Phospholipids** are the most abundant lipids in membranes.

- They include the glycerol-based **phosphoglycerides** and the sphingosine-based **sphingolipids**.

- The kinds and relative proportions of phospholipids vary greatly among types of membranes.
Glycolipids

- **Glycolipids** are formed by the addition of carbohydrates to lipids

- Some are glycerol-based and some are sphingosine-based; the **glycosphingolipids**

- The most common glycosphingolipids are **cerebrosides** and **gangliosides**
Cerebrosides and gangliosides

- Cerebrosides are *neutral glycolipids*; each molecule has an uncharged sugar as its head group

- A ganglioside has an oligosaccharide head group with one or more negatively charged sialic acid residues

- Cerebrosides and gangliosides are especially prominent in brain and nerve cells
(b) **GLYCOLIPIDS**

Cerebrosides  
(galactocerebrosides shown)  
Gangliosides

![Sphingosine](image)

Galactose

Fatty acid

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(a) Monogalactosyldiacylglycerol (MGDG)

(b) Digalactosyldiacylglycerol (DGDG)
Sterols

- The membranes of most eukaryotes contain significant amounts of sterols.

- The main sterol in animal cell membranes is cholesterol, which is needed to stabilize and maintain membranes.

- Plant cell membranes contain small amounts of phytosterols, whereas fungal cell membranes contain ergosterol, similar to cholesterol.
Figure 7-6C

(c) STEROLS

- Cholesterol (shown)
- Campesterol
- Sitosterol
- Stigmasterol
- Ergosterol
- Hopanoids

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Video: Space-filling model of phosphatidylcholine
Thin-Layer Chromatography Is an Important Technique for Lipid Analysis

- Lipids can be isolated, separated, and studied using nonpolar solvents such as acetone and chloroform.

- **Thin-layer chromatography** is used to separate different kinds of lipids based on their relative polarities.

- A glass plate is coated with silicic acid and lipids are spotted onto a position near the bottom of the plate called the *origin*. 
Principle of separation of lipids via TLC

• A nonpolar organic solvent moves up the plate by capillary action, taking different lipids with it to varying degrees.

• Nonpolar lipids have little affinity for the silicic acid on the plate, and so move readily with the solvent, near the solvent front.

• Polar lipids will interact variably (depending on how polar they are) with the silicic acid, and their movement will be slowed proportionately.
Figure 7-9

(a) TLC plate

(b) Solvent front

Cholesterol

Less polar

More polar

PE

PC

PS

Solvent system

Origin
Fatty Acids Are Essential to Membrane Structure and Function

- *Fatty acids* are components of all membrane lipids except the sterols

- Their long hydrocarbon tails provide a barrier to diffusion of polar solutes

- The sizes of membrane fatty acids range between 12–20 carbons long, which is optimal for bilayer formation and dictates the usual thickness of membranes (6–8 nm)
Fatty acids vary in degree of saturation

- Fatty acids vary considerably in the presence and number of double bonds

- *Palmitate* (16C) and *stearate* (18C) are common saturated fatty acids

- *Oleate* (one double bond) and *linoleate* (two double bonds), are both 18C unsaturated fatty acids
### Table 7-2

**Structures of Some Common Fatty Acids Found in Lipid Bilayers**

<table>
<thead>
<tr>
<th>Name of Fatty Acid</th>
<th>Number of Carbon Atoms</th>
<th>Number of Double Bonds</th>
<th>Structural Formula</th>
<th>Space-Filling Model</th>
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<td><strong>Unsaturated</strong></td>
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<td>Linoleate</td>
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Membrane Asymmetry: Most Lipids Are Distributed Unequally Between the Two Monolayers

Figure 7-10

Transverse diffusion ("flip-flop")

Rotation

Lateral diffusion
Figure 7-11

Measuring lipid mobility with FRAP

Unlabeled cell surface
Cell surface molecules labeled with fluorescent dye
Laser beam bleaches an area of the cell surface
Fluorescent-labeled molecules diffuse into bleached area
Rate of diffusion of fluorescence into bleached area measured over time
Membranes Function Properly Only in the Fluid State

- Membrane fluidity changes with temperature, decreasing as temperature falls and vice versa.

- Every lipid bilayer has a characteristic transition temperature $T_m$, the temperature at which it becomes fluid.

- This change of state is called a phase transition, in this case from solid to liquid.

- Below the $T_m$, any functions that rely on membrane fluidity will be disrupted.
(a) Normal membrane. When the temperature of a typical membrane preparation is increased slowly in a calorimeter chamber, a peak of heat absorption marks the gel-to-fluid transition temperature, $T_m$.  

![Graph showing heat absorption vs. temperature with $T_m = 28^\circ C$ between gel and fluid phases.](image)
(b) Membranes enriched in unsaturated or saturated fatty acids. Membranes from cells grown in media enriched in the unsaturated fatty acid oleate (left) are more fluid than normal membranes (lower $T_m$). Membranes from cells grown in media enriched in the saturated fatty acid stearate (right) are less fluid than normal membranes (higher $T_m$).
Effects of Fatty Acid Composition on Membrane Fluidity

• Fluidity of a membrane depends mainly on the fatty acids that it contains

• The length of fatty acid chains and the degree of saturation both affect the fluidity of the membrane

• Long-chain and saturated fatty acids have higher $T_m$s, whereas short-chain and unsaturated fatty acids have lower $T_m$s
(a) Effect of chain length on the melting point
(b) Effect of unsaturation on the melting point
(a) Lipids with saturated fatty acids pack together well in the membrane

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(b) Lipids with a mixture of saturated and unsaturated fatty acids do not pack together well in the membrane
Effects of Sterols on Membrane Fluidity

• Membrane fluidity is influenced by sterols

• The intercalation of rigid cholesterol molecules into a membrane decreases its fluidity and increases the $T_m$

• However, cholesterol also prevents hydrocarbon chains of phospholipids from packing together tightly and so reduces the tendency of membranes to gel upon cooling

• Therefore cholesterol is a fluidity buffer
Figure 7-15A

(a) Cholesterol in plasma membrane

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Other effects of sterols on membranes

• Sterols decrease the permeability of membranes to ions and small polar molecules

• This is likely because they fill spaces between the hydrocarbon chains of phospholipids

• This effectively blocks the routes that ions and small molecules would take through the membrane
Figure 7-15B

(b) Bonding of cholesterol to phospholipid

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Most Organisms Can Regulate Membrane Fluidity

- Most organisms can regulate membrane fluidity by varying the lipid composition of the membranes.
- This is most important in poikilotherms, organisms that cannot regulate their body temperature.
- Poikilotherms use homeoviscous adaptation, compensating for changes in temperature by altering the length and degree of saturation of fatty acids in their membranes.
Lipid Rafts Are Localized Regions of Membrane Lipids That Are Involved in Cell Signaling

• Localized regions of membrane lipids in association with specific proteins are called *lipid microdomains*, or *lipid rafts*

• These are dynamic, changing composition as lipids and proteins move into and out of them

• Lipid rafts in the outer monolayer of animal cells have elevated levels of cholesterol and glycosphingolipids and are less fluid than the rest of the membrane
Lipid raft formation (continued)

- Lipid rafts contain actin-binding proteins, suggesting that the cytoskeleton may play a role in their formation and organization.

- Depleting cholesterol from a membrane, or disrupting the actin cytoskeleton, can both interfere with the targeting of proteins to rafts.
Functions of lipid rafts

- Lipid rafts are thought to have roles in detecting and responding to extracellular signals.

- For example, lipid rafts have roles in:
  - transport of nutrients and ions across membranes
  - binding of activated immune system cells to their microbial targets
  - transport of cholera toxin into intestinal cells
Caveolae

- *Caveolae*, small invaginations of the plasma membrane, are structurally related to lipid rafts

- They contain a cholesterol-binding protein called caveolin, and are enriched in cholesterol, sphingolipids, and lipid-anchored proteins

- Possible roles of caveolae: endocytosis, exocytosis, redox sensing, and regulation of airway function in the lungs
Membrane Proteins: the “Mosaic” Part of the Model

- The mosaic part of the fluid mosaic model includes lipid rafts and other lipid domains

- However, it is membrane proteins that are the main components
The Membrane Consists of a Mosaic of Proteins: Evidence from Freeze-Fracture Microscopy

- Support for the fluid mosaic model came from studies involving freeze-fracturing

- A bilayer or membrane is frozen and then hit sharply with a diamond knife

- The resulting fracture often follows the plane between the two layers of membrane lipid
(a) Separation of membrane monolayers. Notice how the fracture plane has passed through the hydrophobic interior of the membrane, revealing the inner surfaces of the two monolayers. Integral membrane proteins that remain with the outer monolayer are seen on the E (exoplasmic) face, whereas those that remain with the inner monolayer are seen on the P (protoplasmic) face.
(b) Surface view of monolayers. This sketch of a freeze-fractured membrane shows electron micrographs of the E and P faces from the plasma membrane of a mouse kidney tubule cell. Individual proteins imbedded in either face show up as small particles (TEMs).
(a) Erythrocyte plasma membrane
(b) Chloroplast membrane

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Freeze-fracture analysis of membranes

- When a fracture plane splits a membrane into its two layers, particles the size and shape of globular proteins can be seen.

- The $E$ surface is the exoplasmic face and the $P$ surface is the protoplasmic face.

- Artificial bilayers without added protein show no particles.
Figure 7-18

(a) Artificial bilayers without proteins

(b) Artificial bilayers with proteins

0.1 μm
Membranes Contain Integral, Peripheral and Lipid-Anchored Proteins

• Membrane proteins have different hydrophobicities and so occupy different positions in or on membranes

• This, in turn, determines how easily such proteins can be extracted from membranes

• Membrane proteins fall into three categories: *integral, peripheral, and lipid-anchored*
Figure 7-19

(a) Integral monotopic protein
(b) Singlepass protein
(c) Multipass protein
(d) Multi-subunit protein
(e) Peripheral membrane protein
(f) Fatty acid or isoprenyl anchor
(g) GPI anchor

Integral membrane proteins

Lipid-anchored membrane proteins
The erythrocyte plasma membrane

• The erythrocyte (red blood cell) membrane has been one of the most widely studied

• This is because of the wide availability of red blood cells and how easily plasma membrane can be isolated from them
Integral Membrane Proteins

• Most membrane proteins possess one or more hydrophobic regions with an affinity for the interior of the lipid bilayer

• These are *integral membrane proteins*, with hydrophobic regions embedded in the interior membrane bilayer

• They are difficult to remove from membranes by standard isolation procedures
Integral Membrane Proteins

• Some integral membrane proteins, called integral monotropic proteins, are embedded in just one side of the bilayer.

• However, most are transmembrane proteins that span the membrane and protrude on both sides.

• Transmembrane proteins cross either once (singlepass proteins) or several times (multipass proteins).
Transmembrane proteins

• Most transmembrane proteins are anchored to the lipid bilayer by one or more hydrophobic transmembrane segments

• In most cases, the polypeptide chain appears to span the membrane in an α-helical conformation about 20–30 amino acids long

• Some are arranged as a closed β sheet called a β barrel
Figure 7-21A

(a) Glycophorin

Carbohydrate side chain

H₃N⁺

OUTER SURFACE

INNER SURFACE

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(b) Bacteriorhodopsin
Peripheral Membrane Proteins

- Membrane proteins that lack discrete hydrophobic regions do not penetrate the lipid bilayer.

- These **peripheral membrane** proteins are bound to membrane surfaces through weak electrostatic forces and hydrogen bonds.

- Some hydrophobic residues play a role in anchoring them to the membrane surface.

- Peripheral membrane proteins are easily separated from membranes by changing pH or ionic strength.
Lipid-Anchored Membrane Proteins

• The polypeptide chains of lipid-anchored membrane proteins are located on the surfaces of membranes.

• They are covalently bound to lipid molecules embedded in the bilayer.

• Proteins bound to the inner surface of the plasma membrane are linked to fatty acids, or isoprenyl groups.
Types of lipid-anchored membrane proteins

- **Fatty acid-anchored membrane proteins** are attached to a saturated fatty acid, usually *myristic acid* (14C) or *palmitic acid* (16C)

- **Isoprenylated membrane proteins** are synthesized in the cytosol and then modified by addition of multiple *isoprenyl groups* (5C) usually *farnesyl* (15C) or *geranygeranyl* (20C) groups

- **GPI-anchored membrane proteins** are covalently linked to *glycosylphosphatidylinositol*
Proteins Can Be Separated by SDS-Polyacrylamide Gel Electrophoresis

- Membrane proteins must be solubilized and extracted from membranes so that they can be studied
- They are separated by electrophoresis
Figure 7-22

1. Membrane fragments are solubilized with sodium dodecyl sulfate (SDS), which coats the polypeptides and gives them a net negative charge.

2. A small sample of the solubilized polypeptides is placed into a well at the top of a gel of polyacrylamide that is held between two glass plates.

3. An electrical potential is applied across the gel, with the positively charged anode attached to the bottom of the gel.

4. This causes the negatively charged polypeptide molecules to move toward the bottom end of the gel, each forming a discrete band.

5. Each polypeptide moves down the gel at a rate that is inversely related to its size, with the smallest polypeptides reaching the bottom first.

6. The gel is stained with a dye that binds to polypeptides and makes them visible.

7. The polypeptide profile shown here is for the main membrane proteins of the human erythrocyte.

Completed gel

- Larger polypeptides
- Smaller polypeptides

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Additional techniques using electrophoresis

• Two-dimensional SDS - PAGE (polyacrylamide gel electrophoresis) separates proteins in two dimensions, first by charge and then by size

• Following electrophoresis, polypeptides can be identified by **Western blotting**

• In this technique proteins are transferred to a membrane and bound by specific antibodies
Determining the Three-Dimensional Structure of Membrane Proteins Is Becoming More Feasible

• *X-ray crystallography* can be used to determine the structure of proteins that can be isolated in crystalline form.

• Membrane proteins are hard to isolate and crystallize.

• An alternative approach called *hydropathic analysis* can be used.
Hydropathy Analysis

• The number and location of transmembrane segments in a membrane protein can be predicted if the protein sequence is known.

• A hydropathy (or hydrophobicity) plot is used for this.

• A computer program identifies clusters of hydrophobic residues, calculating a hydropathy index for successive “windows” along the protein.
(a) Hydropathy plot of connexin. The hydropathy index on the vertical axis is a numerical measure of the relative hydrophobicity of successive segments of the polypeptide chain based on its amino acid sequence.

(b) Transmembrane structure of connexin. Connexin has four distinct hydrophobic regions, which correspond to the four α-helical segments that span the plasma membrane.
Many Membrane Proteins Are Glycosylated

- **Glycoproteins** are membrane proteins with carbohydrate chains covalently linked to amino acid side chains

- The addition of a carbohydrate side chain to a protein is called **glycosylation**

- Glycosylation occurs in the ER and Golgi compartments
Figure 7-25A

(a) N-linked (to amino group of asparagine)
Figure 7-25B

(b) O-linked (to hydroxyl group of serine or threonine)
Figure 7-25C

(c) O-linked (to hydroxyl group of hydroxylysine or hydroxyproline)

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Carbohydrate chains attached to proteins

- Carbohydrate chains attached to peptides can be either straight or branched and range in length from 2 to about 60 sugar units
Figure 7-26A

(a) Common sugars found in glycoproteins

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Roles of glycoproteins

• Glycoproteins are most prominent in plasma membranes, where they play a role in cell-cell recognition

• The carbohydrate groups protrude on the outer surface of the cell membrane

• Lectins are plant proteins that bind specific sugar groups very tightly, and can be used to study membrane glycoproteins
Glycocalyx

• In animal cells, the carbohydrate groups of plasma membrane glycoproteins and glycolipids form a surface coat called a glycocalyx

• The carbohydrate groups on the cell surface are components of the recognition sites of membrane receptors involved in antibody-antigen reactions
Membrane Proteins Vary in Their Mobility

• Membrane proteins are more variable than lipids in their ability to move freely within the membrane.

• Some proteins can move freely, whereas others are constrained because they are anchored to protein complexes.
Membrane protein anchoring

- The most common restraint on mobility of membrane proteins is anchoring of such proteins to structures to one side of the membrane or the other.

- For example, many proteins of the plasma membrane are anchored to either cytoskeleton or to extracellular structures.