Cell Biology
Lecture 5
Part I

Membrane structure

Sesilja Aranko

8.11.2023

Alberts • Johnson • Lewis • Morgan • Raff • Roberts • Walter

Molecular Biology of the Cell

Sixth Edition

Chapter 10
Membrane Structure

Pages: 566-582, 588-593

Course overview – Tentative schedule

Date	Lecture		Chapters & Topics	Assignments
25.10.	1	Part 1	*Course overview,* DNA, Chromosomes, Genome, Ch. 4	
27.10.	2 -G		Replication, Repair, Recombination, Ch. 5	
1.11.	3		From DNA to protein, Ch. 6	
3.11.	4		Control of gene expression, Ch. 7	
8.11.	5	Part 2	Membrane structures, Ch. 10 Membrane transport, Ch. 11	**Assignment I (Essay) Draft I** (8.11.)
10.11.	6 -G		Intracellular compartments and protein sorting, Ch. 12	
15.11.	7		Intracellular compartments and protein sorting, Ch. 12 **Susanna Mäkinen, Solar Foods**	**Assignment II – Draft I (15.11.)**
17.11.	8		Membrane Traffic, Ch. 13 **iGEM team 2023**	*+iGEM intro*
22.11.	9	Part 3	Cell signalling, Ch. 15	**Assignment II – Peer review** (22.11.)
24.11.	10 -G		Cell signalling, Ch. 15	**Assignment I (Essay) Draft II** (24.11.)
29.11.	11		Cell cycle, Ch. 17 **Jere Weltner, Folkhälsan**	
1.12.	12		Apoptosis, Ch. 18	**Assignment II – final version** (1.12.)
7.12.	EXAM		December 7th	
8.12.	Final version essay		December 8th	**Assignment I (Essay) Final version** (8.12.) Aim at finishing before exam date. Use last days for polishing.

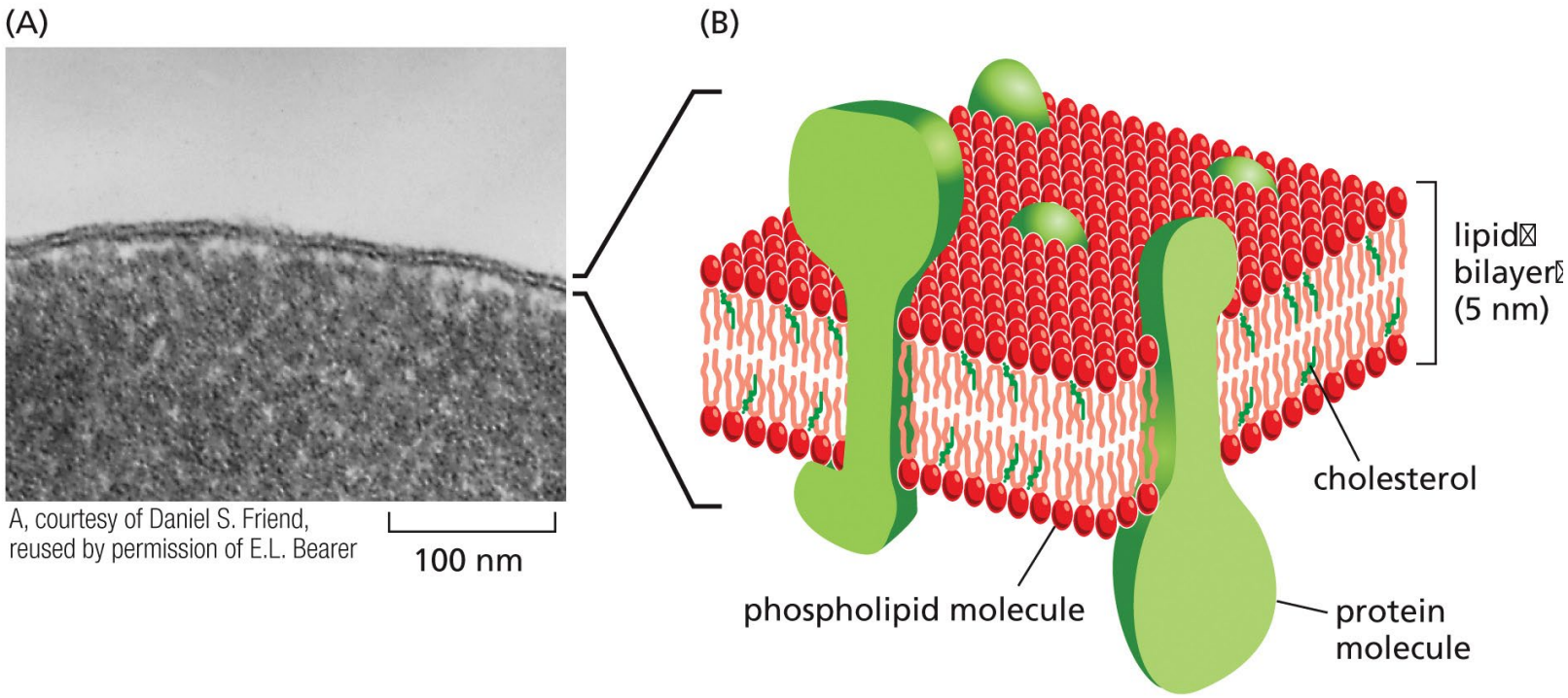
Learning outcomes

Can explain what are the key components of the membranes and what are their roles in both defining the boundaries of cells and organelles and allowing signal and molecules.

Topics

1. The lipid bilayer ~50%

2. Membrane proteins ~50%



Membranes…

- Enclose the cell, defining the *boundaries* and maintaining the *differences* between cytosol and environment.
- Inside eukaryotic cells membranes maintain the differences between the contents of the *organelles and the cytoplasm*
- Plasma membrane also contains *proteins* that act as *sensors*.
- Lipids act as primary and *secondary messengers*.

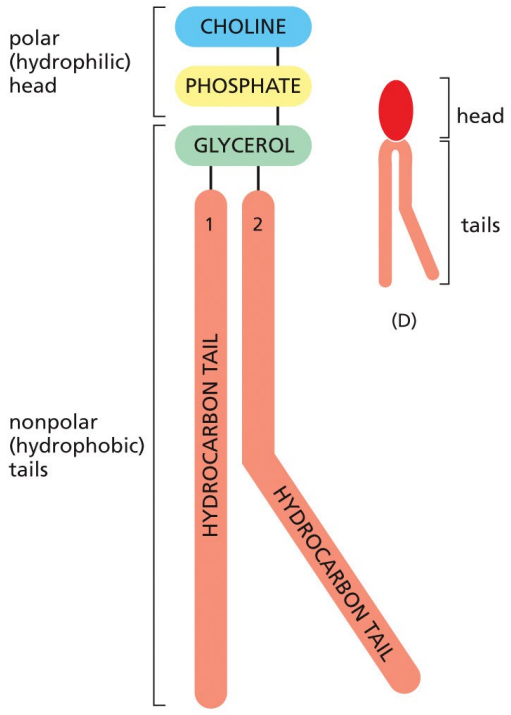
Membranes…

- Important for:
  - Energy conversation
  - Transport of small molecules (part II today)
  - Cell signalling (Lectures 9 and 10)
  - Cell adhesion

- Intracellular compartments and trafficking
  - Lectures 6-8

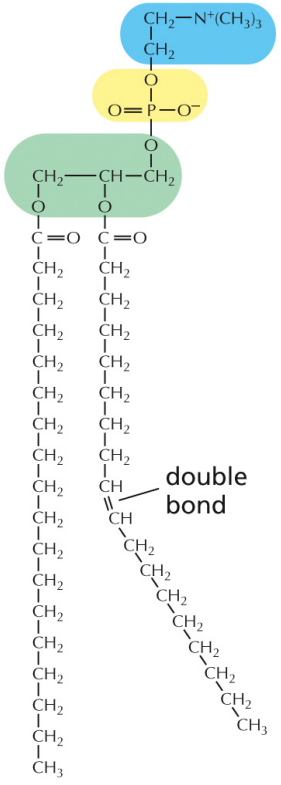
The Lipid Bilayer

- Glycerophospholipids, Sphingolipids, and Sterols Are the Major Lipids in Cell Membranes



(D)

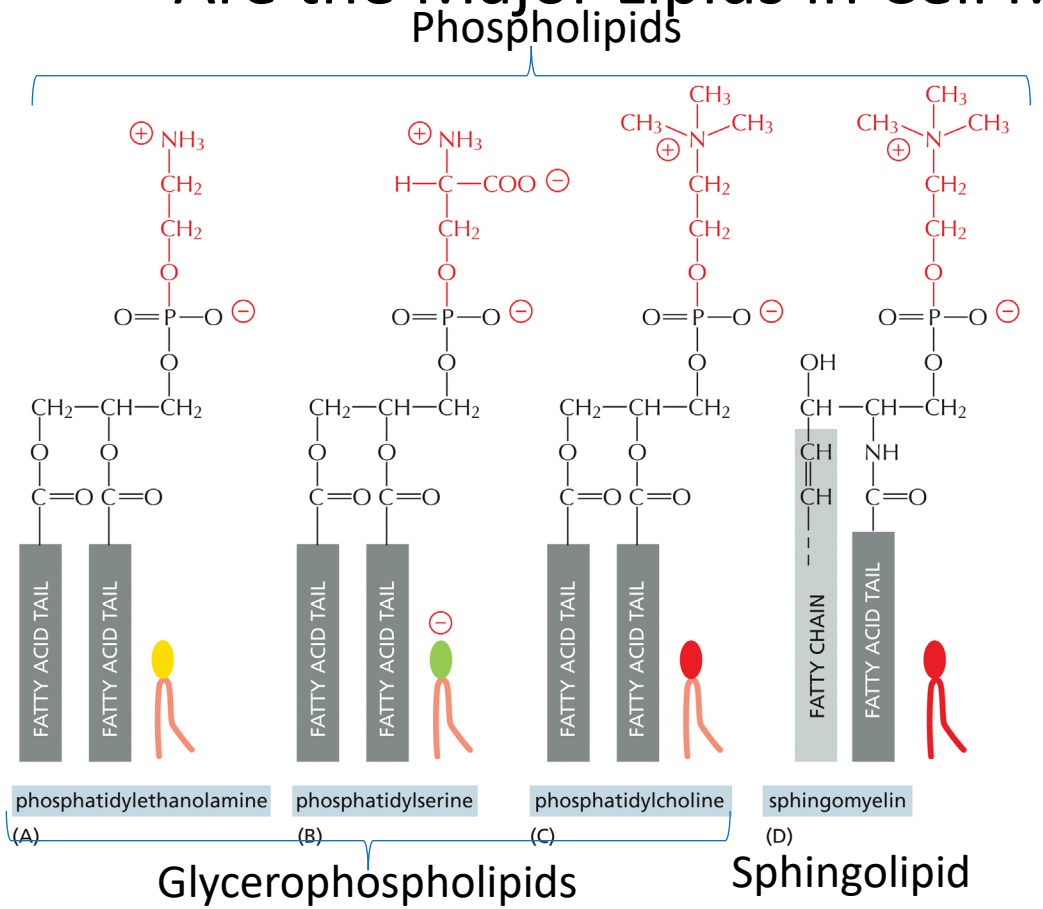
Amphiphilic structure

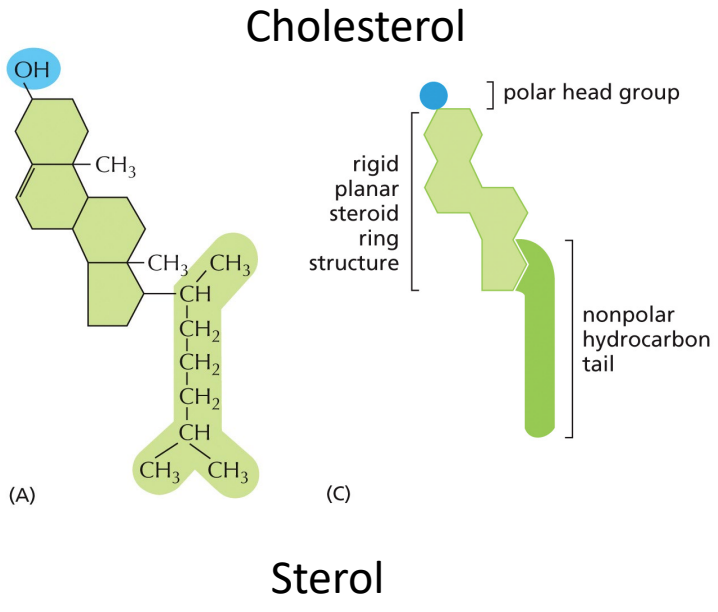


Phosphatidylcholine, a **typical phospholipid molecule**

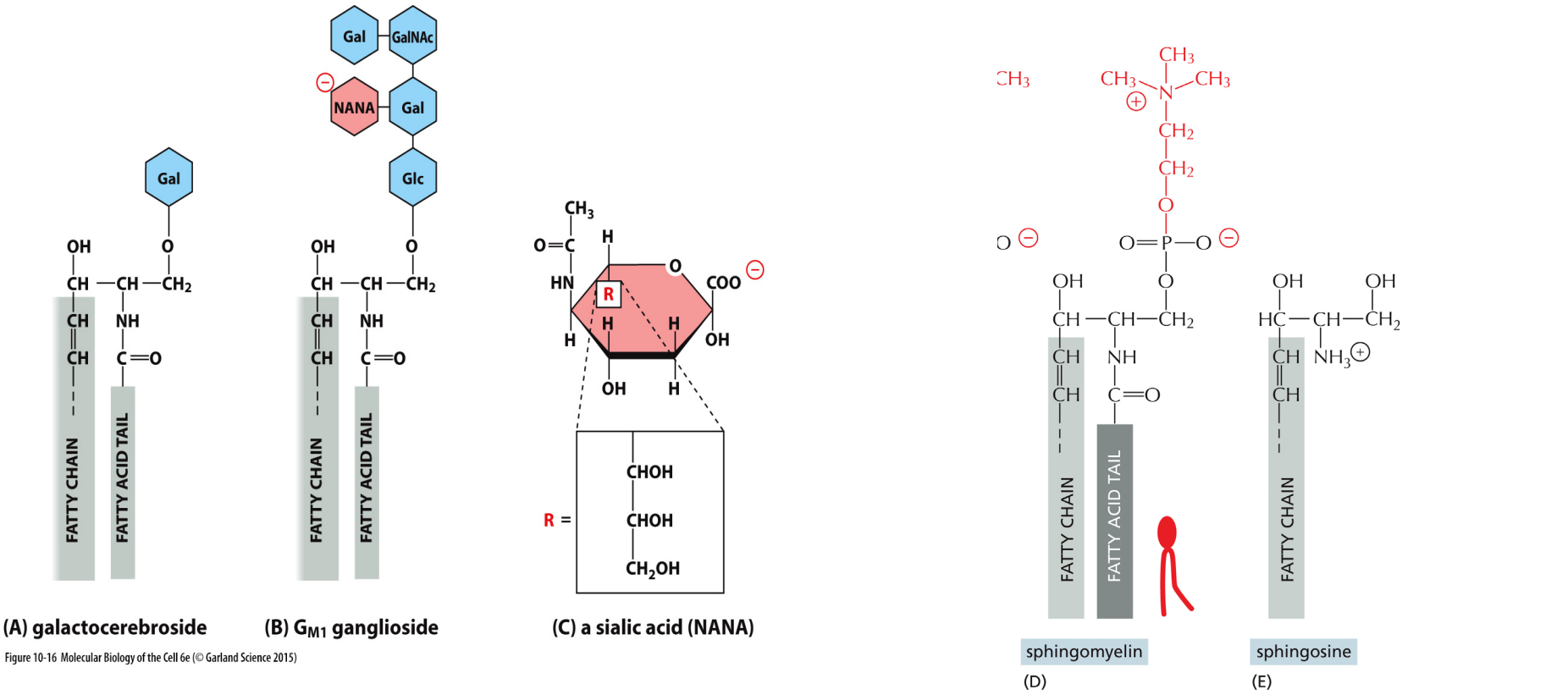
The Lipid Bilayer

- Glycerophospholipids, Sphingolipids, and Sterols Are the Major Lipids in Cell Membranes





Glycolipid molecules



(A) galactocerebroside (B) G_{M1} ganglioside (C) a sialic acid (NANA)

Figure 10-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

- Sugar-containing lipids
- Sugar groups added to the lipid in the lumen of Golgi apparatus
- Made from sphingosine

Glycolipid molecules

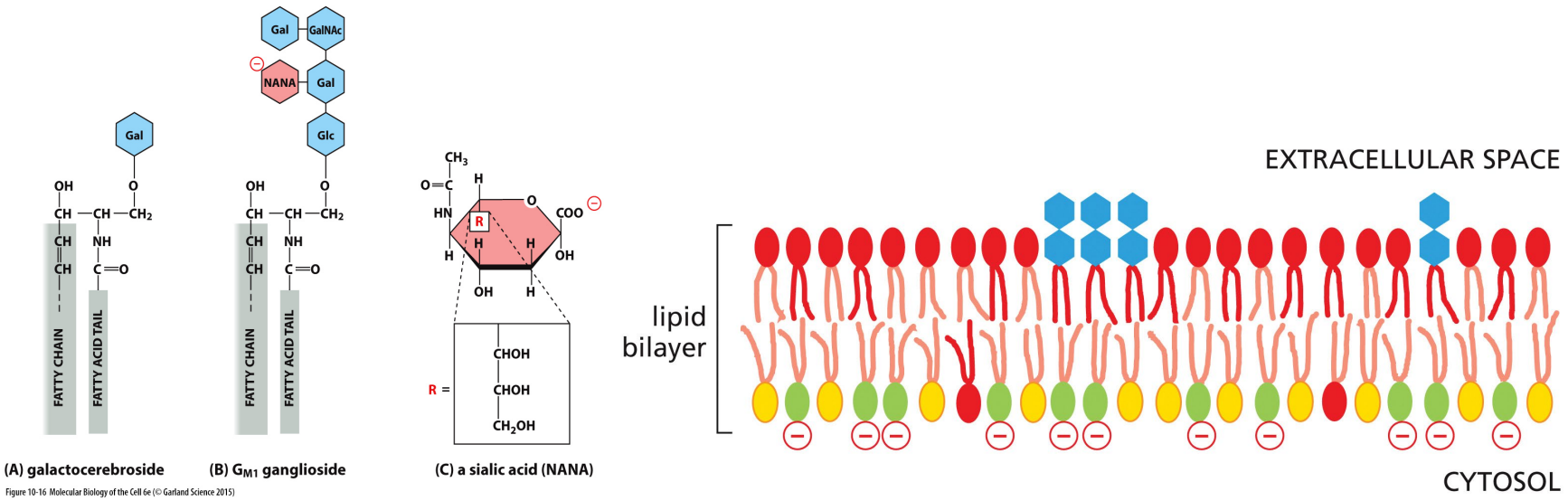
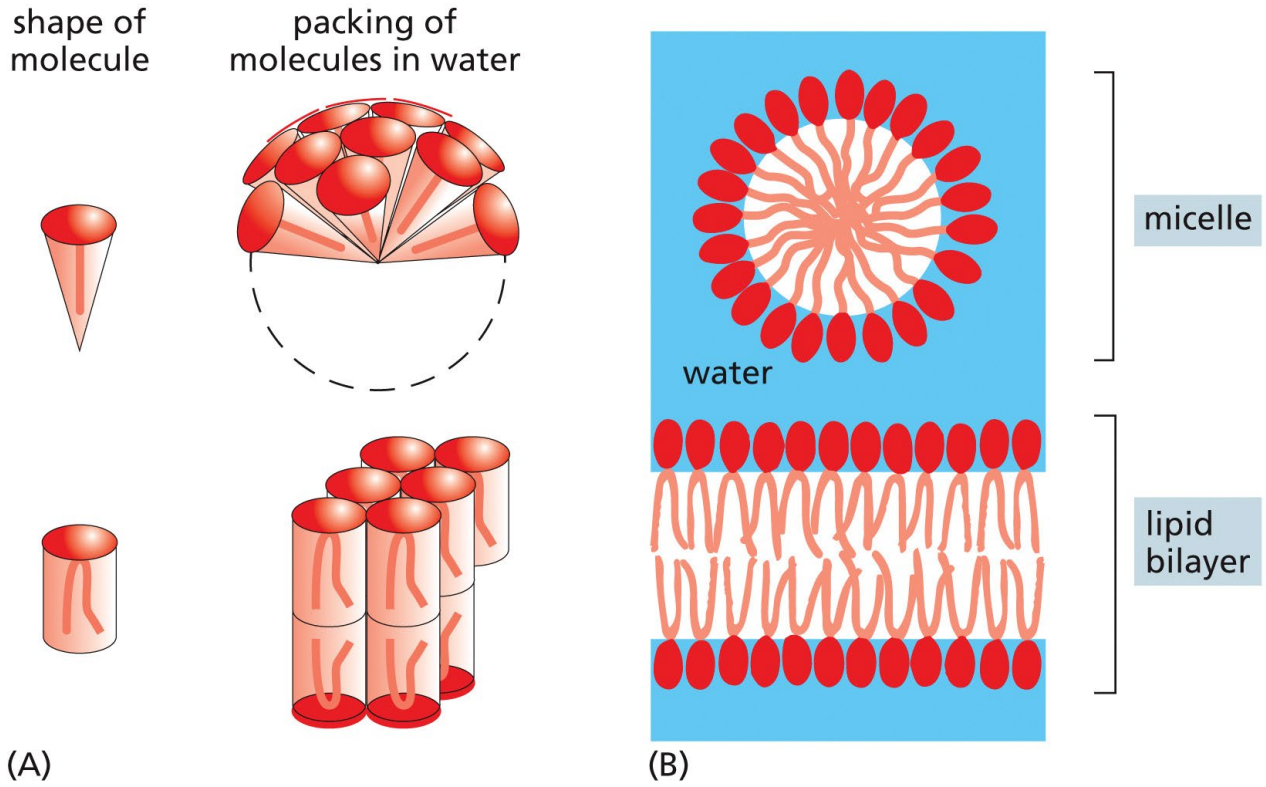


Figure 10-16 Molecular Biology of the Cell 6e (© Garland Science 2015)

- Exclusively found in monolayer *facing away* from the cytoplasm
- Glycolipids tend to self-associate via their sugars
- Role in, e.g., protecting cells and recognition of cells

The Lipid **Bi**layer

Phospholipids Spontaneously Form Bilayers

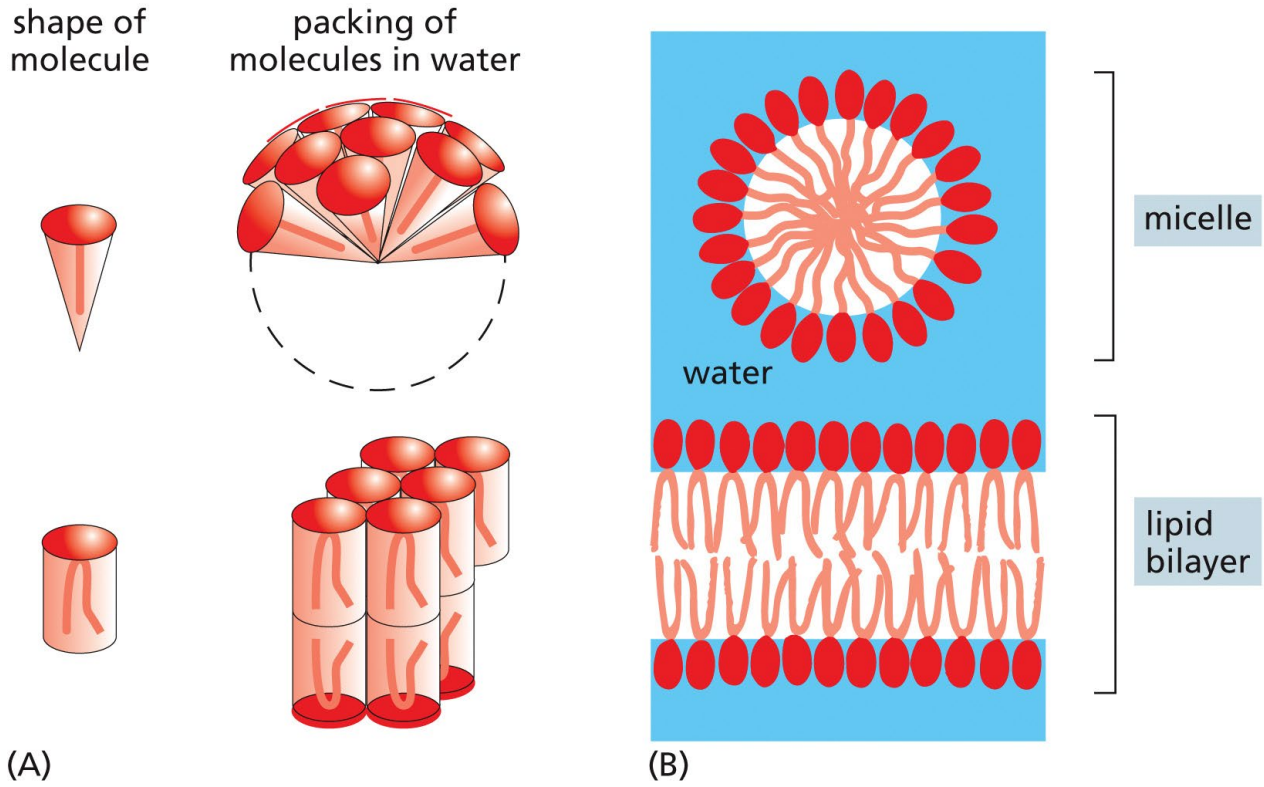


Hydrophobic tails buried in interior

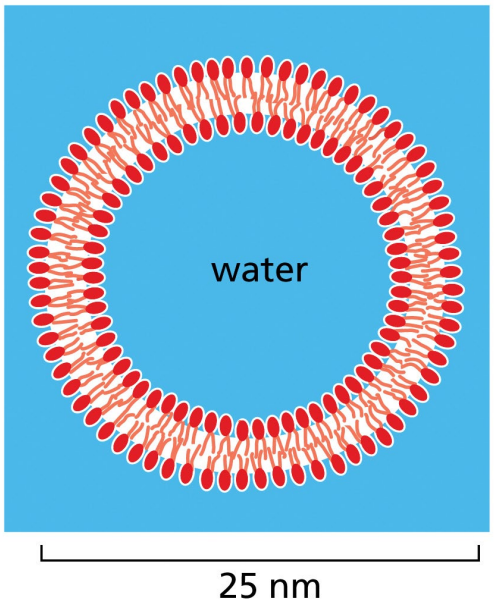
The Lipid **Bi**layer

Note: only weak interactions!

Phospholipids Spontaneously Form Bilayers



Liposomes

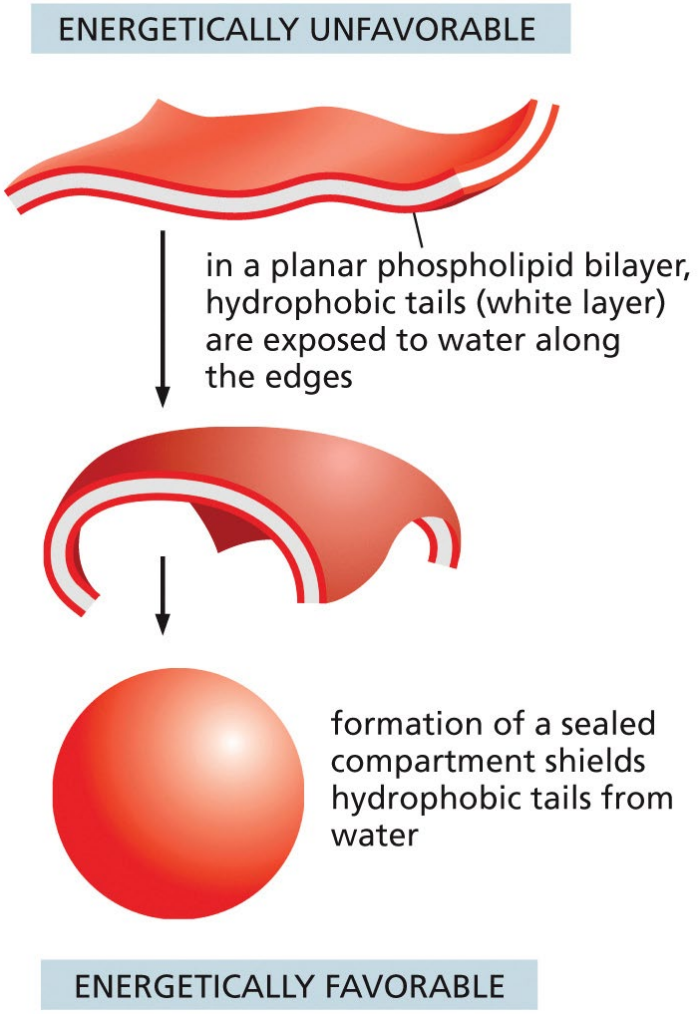


Hydrophobic tails buried in interior

The Lipid Bilayer

- No free edges exposed
- Spontaneous formation of sealed compartments
- Self-healing!

- *Note: fusion of two liposomes is not spontaneous – requires fusion proteins (lecture 8)*

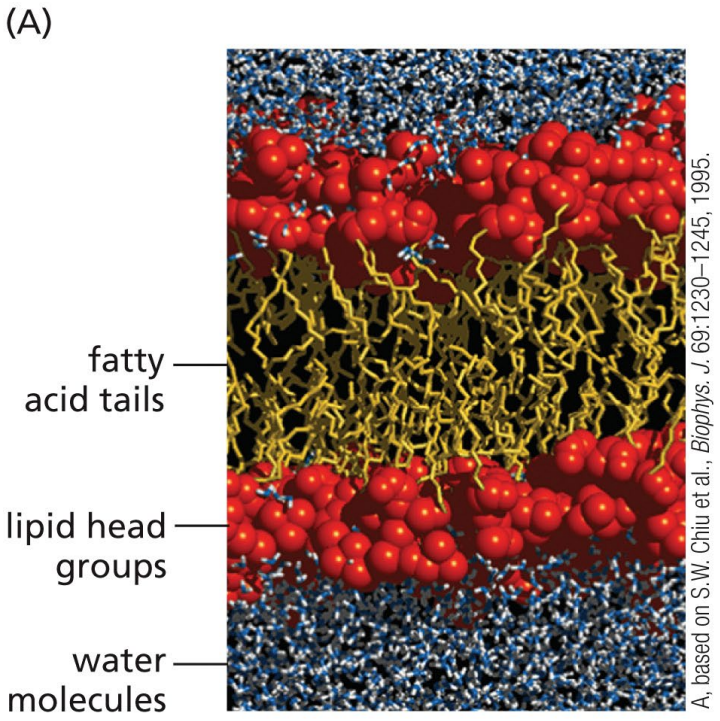


The Lipid Bilayer

The Lipid Bilayer Is a **Two**-dimensional **Fluid**

Moving freely within the **mono**layer

Self-heeling

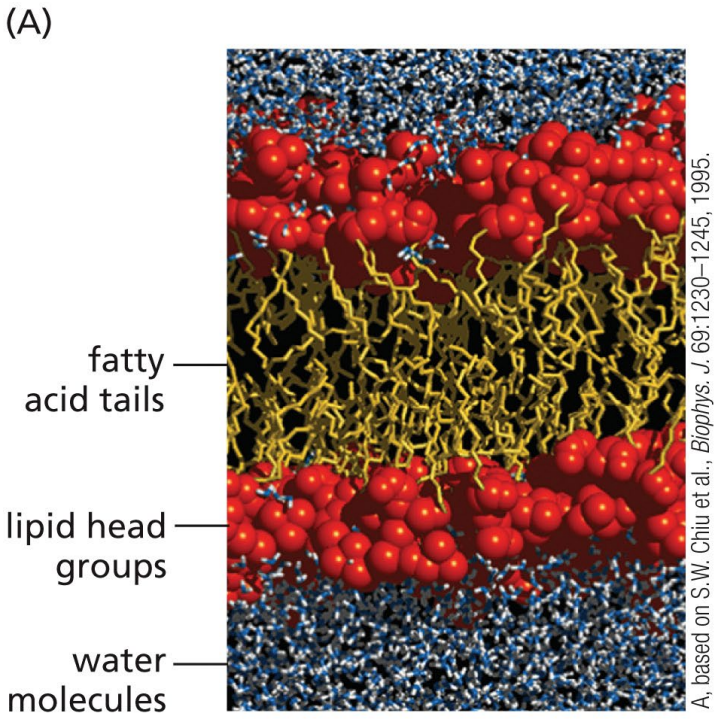


Fluidity and properties controlled by fraction of saturated hydrocarbon chains and cholesterol

Mobility of phospholipids in a bilayer

The Lipid Bilayer Is a **Two**-dimensional **Fluid**

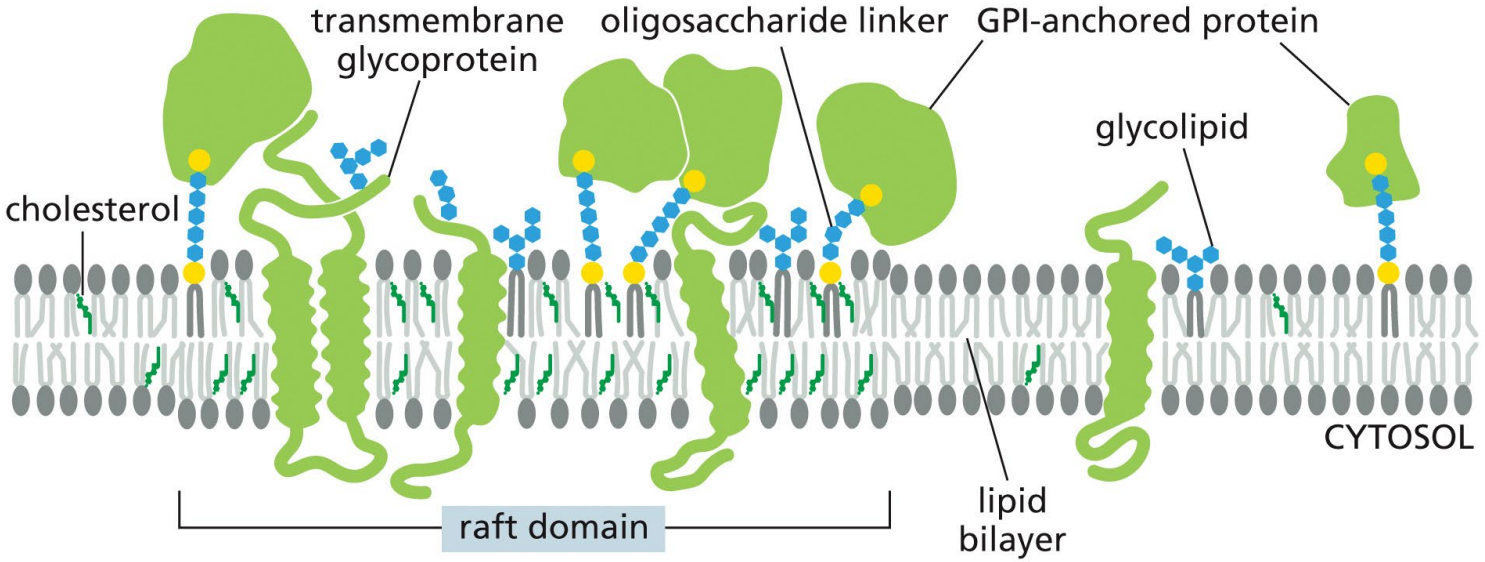
Moving from one monolayer to another assisted by *flippases*



Flippases important also for creation of asymmetry between two monolayers, e.g., cytosolic and extracellular side of PM

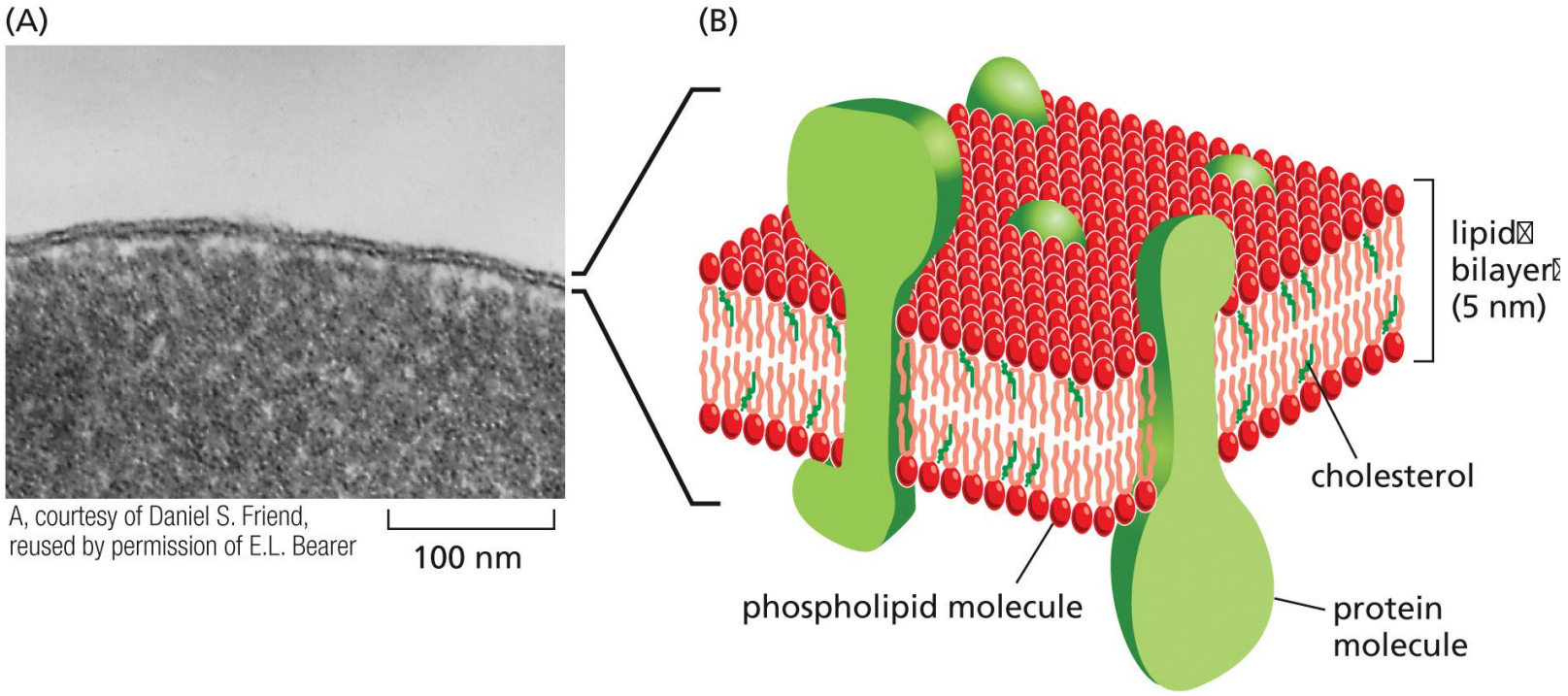
The Lipid Rafts

- Despite their fluidity, lipid bilayers can form **domains of different compositions, lipid rafts.**
- Weak protein-protein, protein-lipid and lipid-lipid interactions reinforce one another to partition the interacting components
  - Restricts mobility of lipids and membrane associated proteins



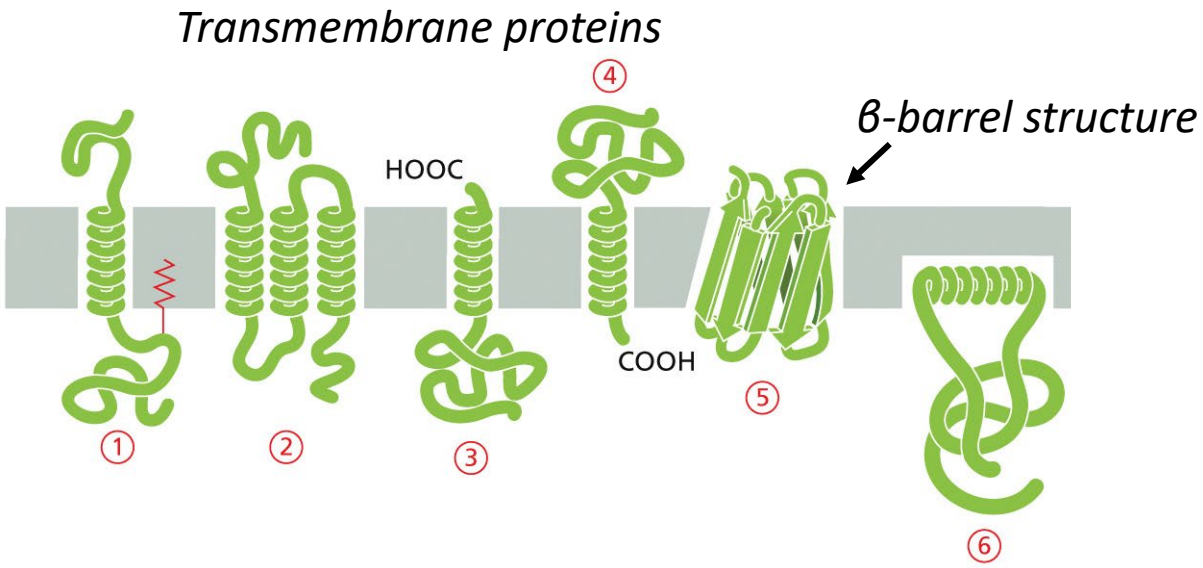
Membrane Proteins

→ Functionality of membranes



Membrane Proteins

- Membrane proteins can be associated with the lipid bilayer in various ways.

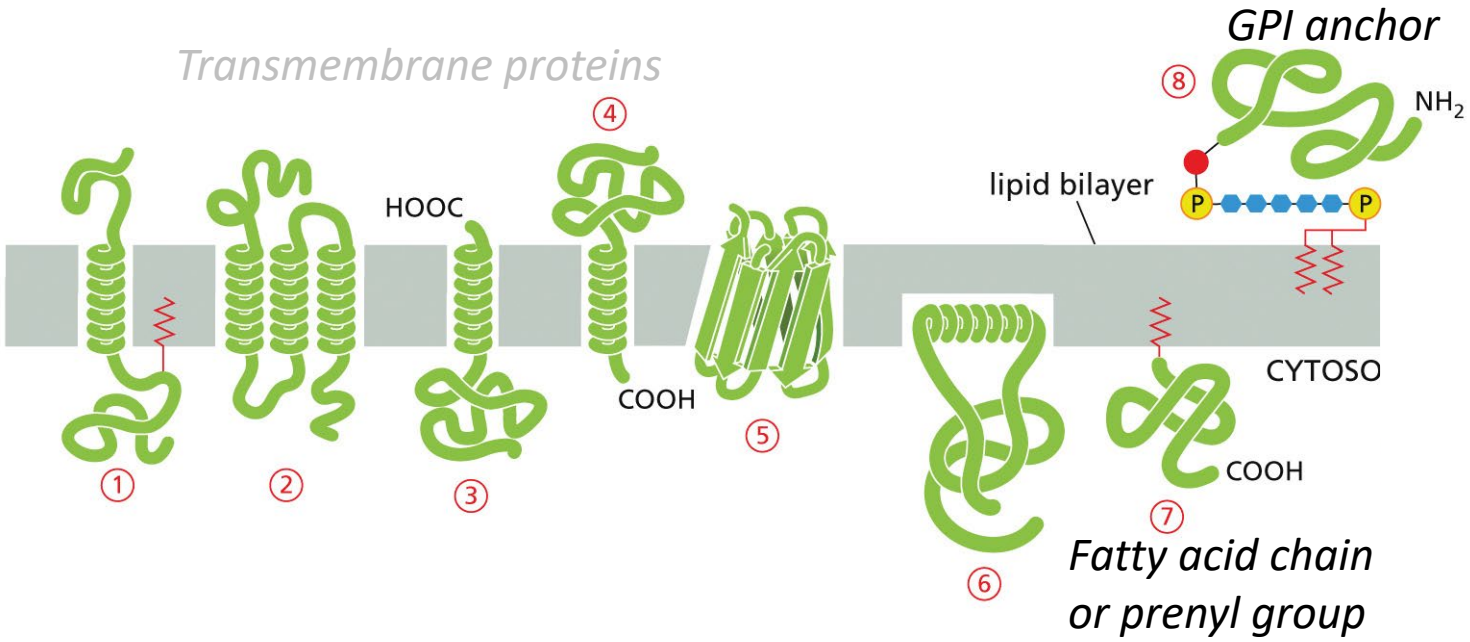


E.g., cell surface receptors

- Can transfer information or small molecules across the membrane

Membrane Proteins

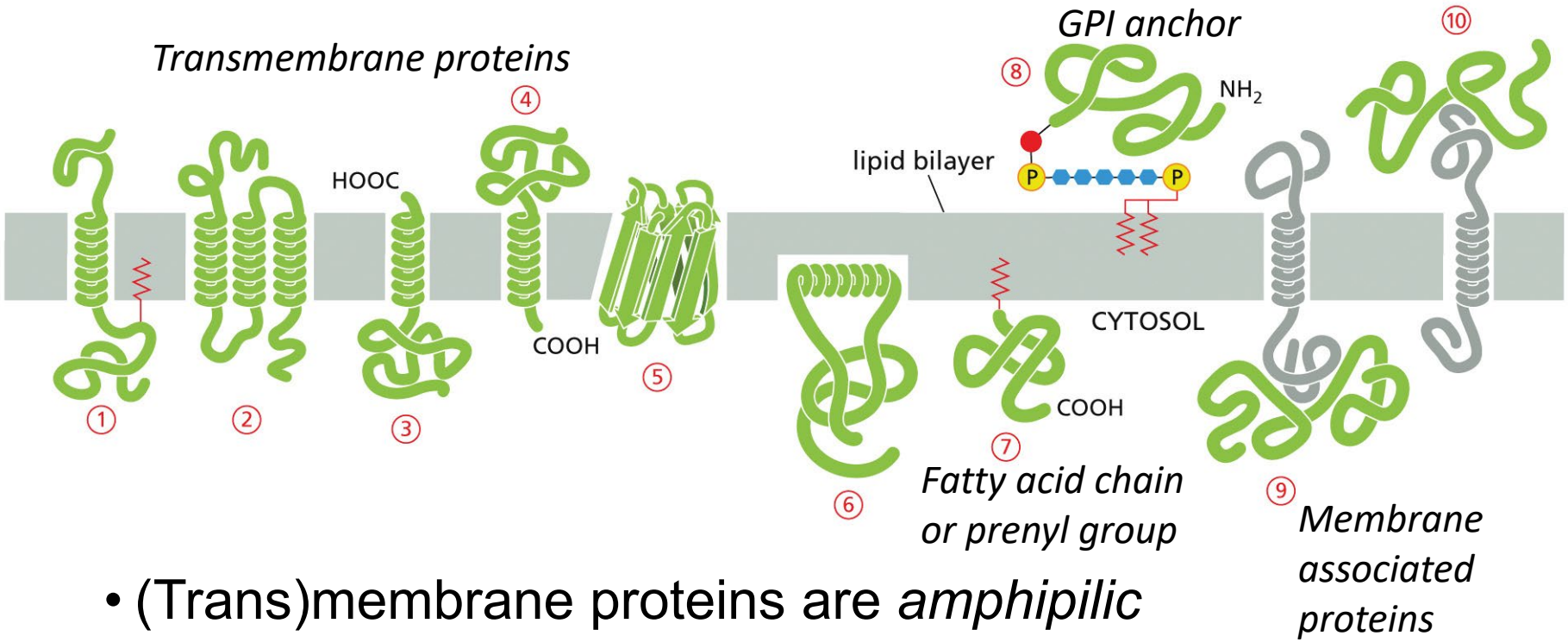
- Membrane proteins can be associated with the lipid bilayer in various ways.
- Can be facing both “inside” and “outside”.



Typically, proteins that can move from membrane-bound to cytosol aa a response to a signal.

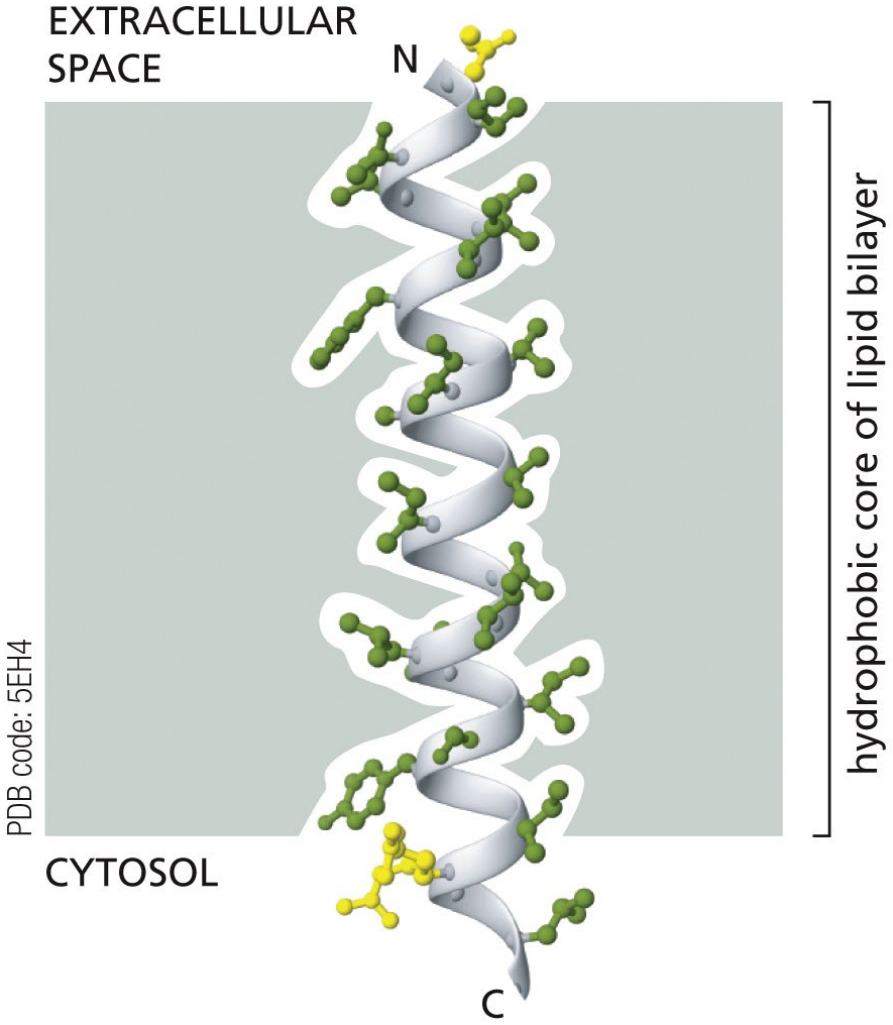
Membrane Proteins

- Membrane proteins can be associated with the lipid bilayer in various ways.
- Can be facing both “inside” and “outside”.



- (Trans)membrane proteins are *amphipilic*

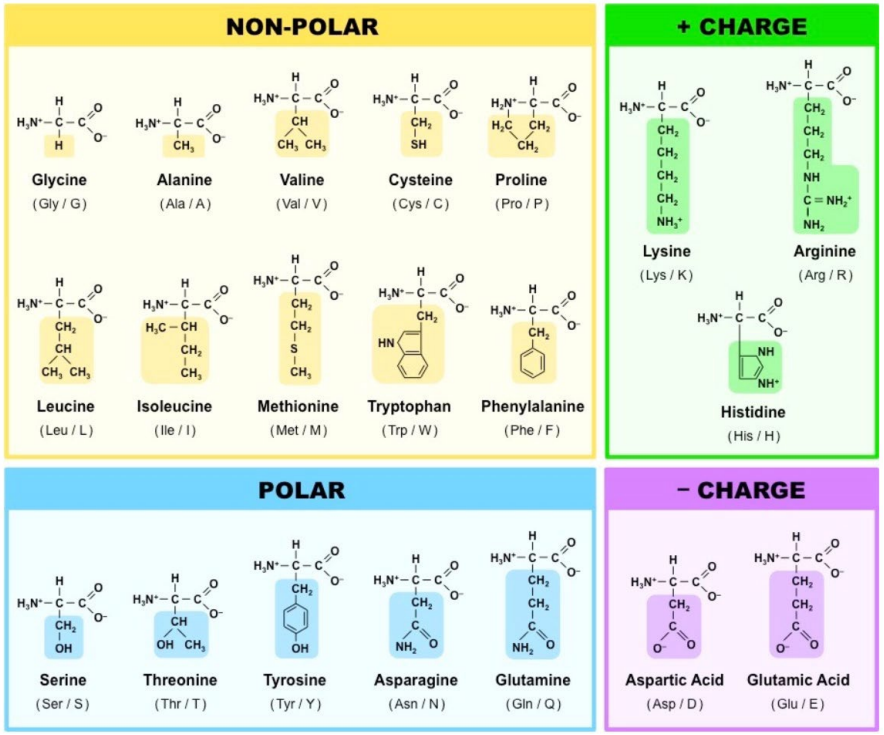
Membrane-spanning segment



- In most transmembrane proteins, the polypeptide chain crosses the lipid bilayer in an α-helical conformation
- Membrane-spanning region(~20-30 aa) composed largely of nonpolar side-chains.

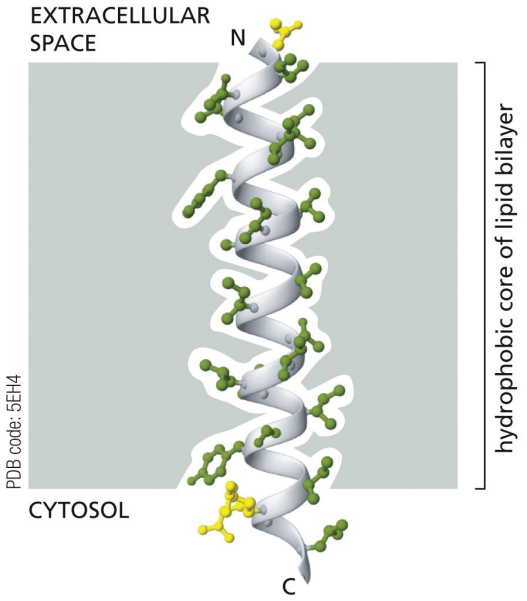
Monomeric single-pass transmembrane proteins span a membrane with a single α helix that has characteristic chemical properties in the region of the bilayer. Which of the three 20-amino—acid sequences listed below is the most likely candidate for such a transmembrane segment? Explain the reasons for your choice.

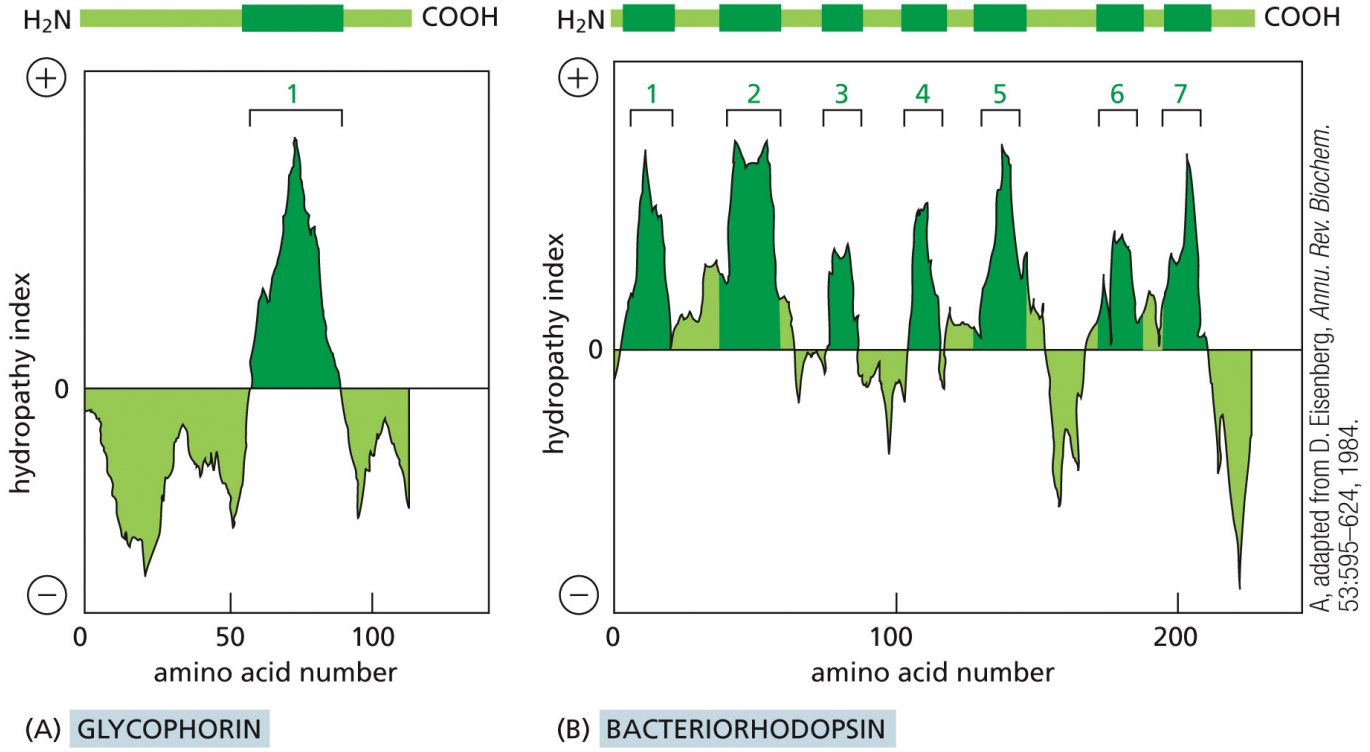
A. I T L I Y F G V M A G V I G T I L L I S
B. I T P I Y F G P M A G V I G T P L L I S
C. I T E I Y F G R M A G V I G T D L L I S



Membrane Proteins

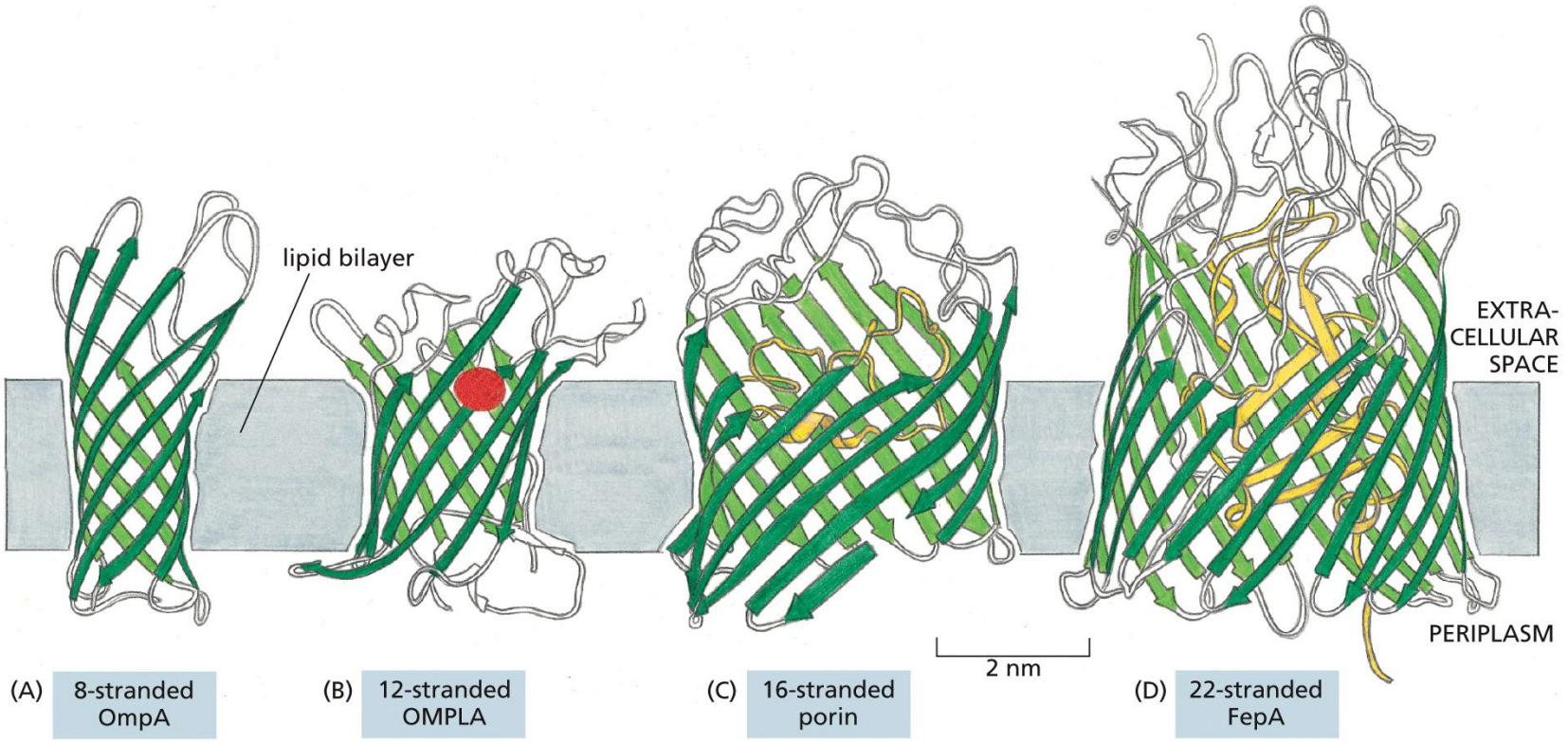
Topology of proteins can be predicted from the primary amino acid sequence.



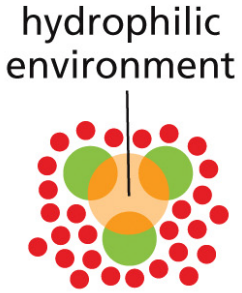


β Barrels

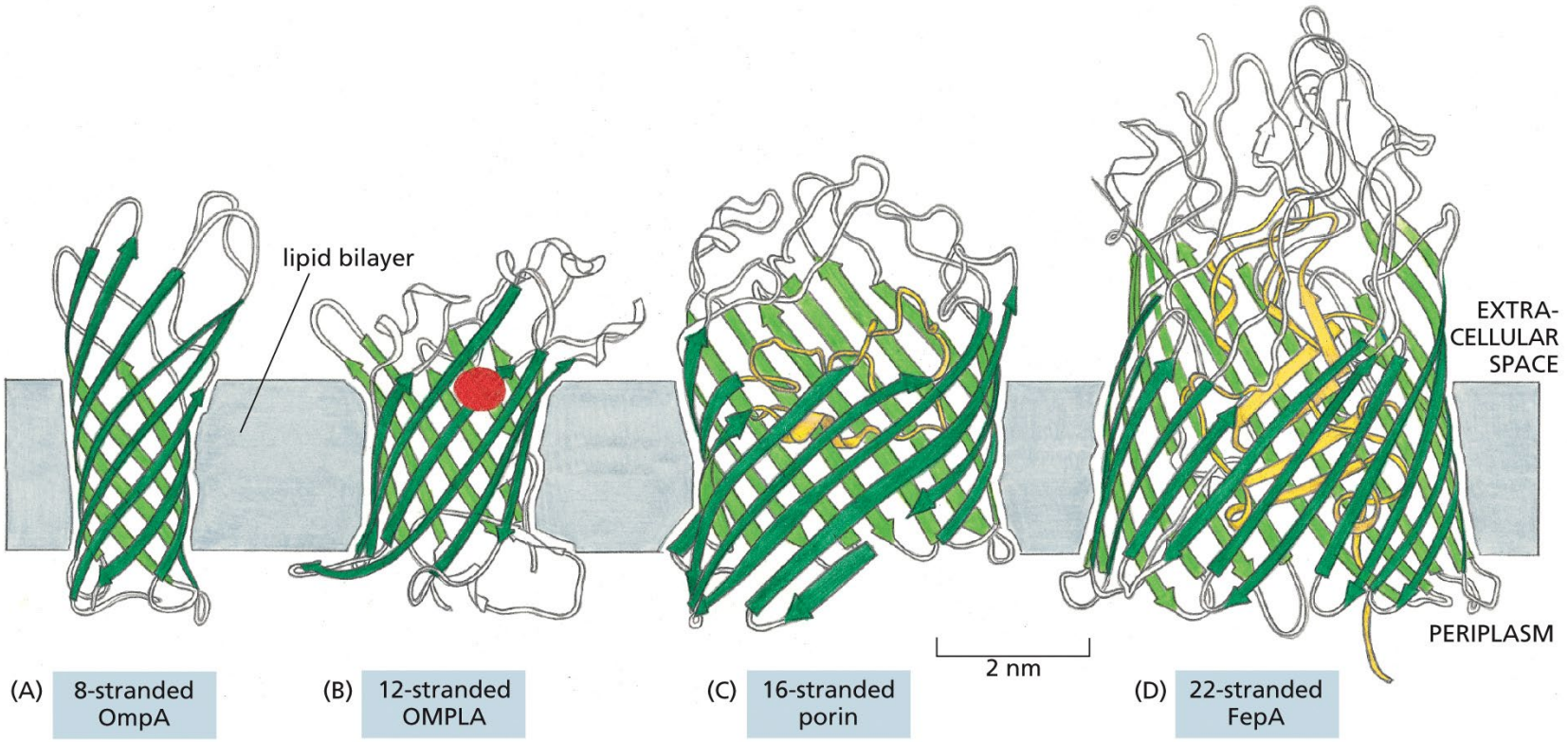
- Some β barrels form large channels.
- Typical in bacteria and cell organelles originating from those.



β Barrels



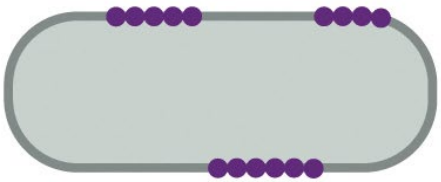
- Inside of the channel, lumen, can be hydrophilic.
- Loops protruding inside further regulate the size and properties of the channel.



Membrane proteins as complexes

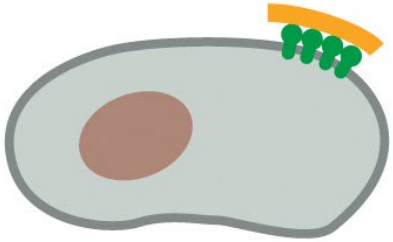
- Membrane proteins often function as large complexes.
- Cells can confine proteins and lipids to specific domains within a membrane.

Self-assembly into large aggregates.

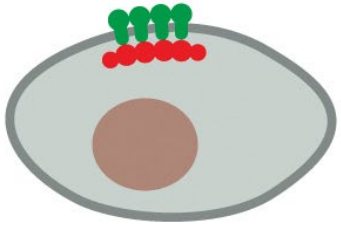


(A)

Tethered by interactions with assemblies of macromolecules .

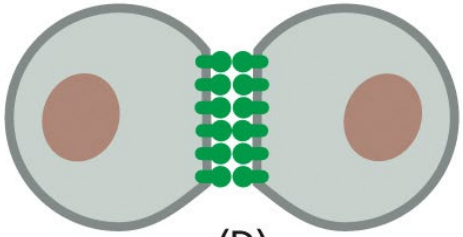


(B)



(C)

Interact with proteins on the surface of another cell.

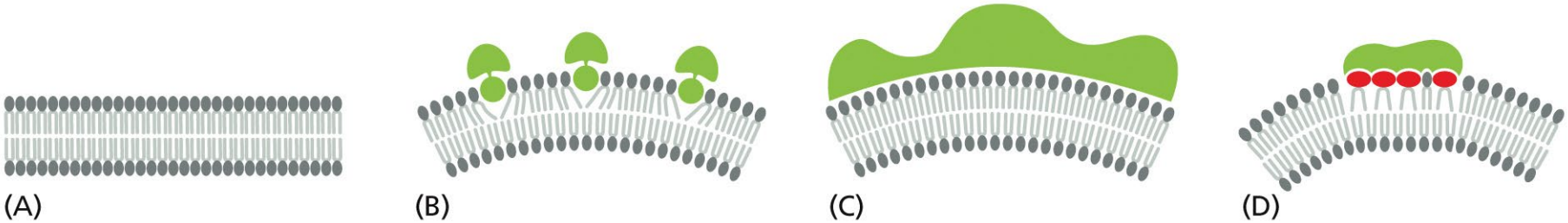


(D)

Membrane bending proteins deform bilayers

- Cell membranes assume many different shapes: flat sheets, tubules, round vesicles, fenestrated sheets
- Membrane shape is controlled dynamically
- A crucial par in producing membrane deformation is played by membrane-bending proteins which control local membrane bending

Membrane-bending proteins deform bilayers



Adapted from W.A. Prinz and J.E. Hinshaw, *Crit. Rev. Biochem. Mol. Biol.* 44:278–291, 2009

A) Bilayer without protein bound.

B) A hydrophobic region of the protein can insert as a wedge into one monolayer to pry lipid head groups apart. Such regions can either be amphiphilic helices as shown or hydrophobic hairpins.

C) The curved surface of the protein can bind to lipid head groups and deform the membrane or stabilize its curvature.

D) A protein can bind to and cluster lipids that have large head groups and thereby bend the membrane.

Summary, part I

- Glycerophospholipids, Sphingolipids, and Sterols Are the Major Lipids in Cell Membranes
- The Lipid Bilayer Is a Two-dimensional Fluid
- Membrane Proteins Include Trans-membrane Proteins, Proteins Anchored To Membranes Via e.g. GPI-linker And Membrane Associated Proteins
- Transmembrane Proteins Are Amphiphilic, Transmembrane Segments Consisting Of Largely Non-polar Residues
- Cells can confine proteins and lipids to specific domains within a membrane.

Cell Biology
Lecture 5
Part II

Membrane transport

Sesilja Aranko

8.11.2023

Intracellular Compartments and Protein Sorting

Sesilja Aranko

15.11.2023

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Molecular Biology of the Cell

Sixth Edition

Chapter 11

Membrane Transport of Small Molecules and the Electrical Properties of Membranes

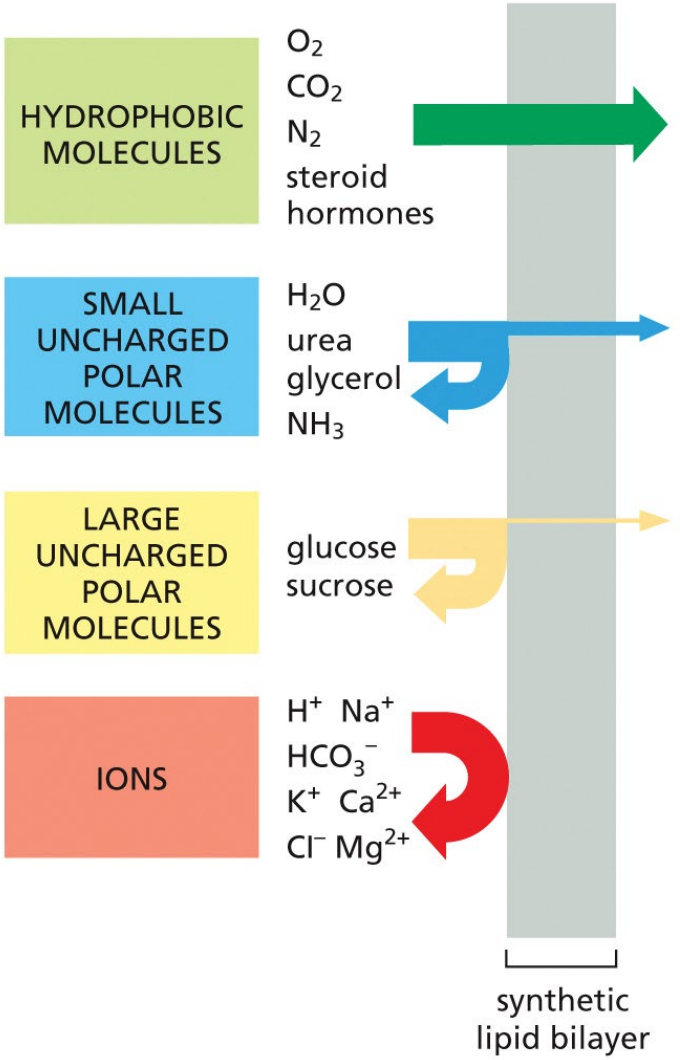
Pages: 597-618, 620-625

Learning outcomes

- Can describe the principles of passive and active transport.
- Can explain how transporters and channels differ and what are their mechanisms based on.

The relative permeability of a synthetic lipid bilayer to different classes of molecules

- The *smaller* the molecule and, the less strongly it associates with water (=the more **hydrophobic** it is), the more rapidly the molecule diffuses across the bilayer
- Protein-free lipid bilayers are **impermeable to ions**
- Impermeability allows creating **concentration differences**, that can be used for:
  - Transport
  - Signalling



Comparison of inorganic ion concentrations inside and outside a typical mammalian cell

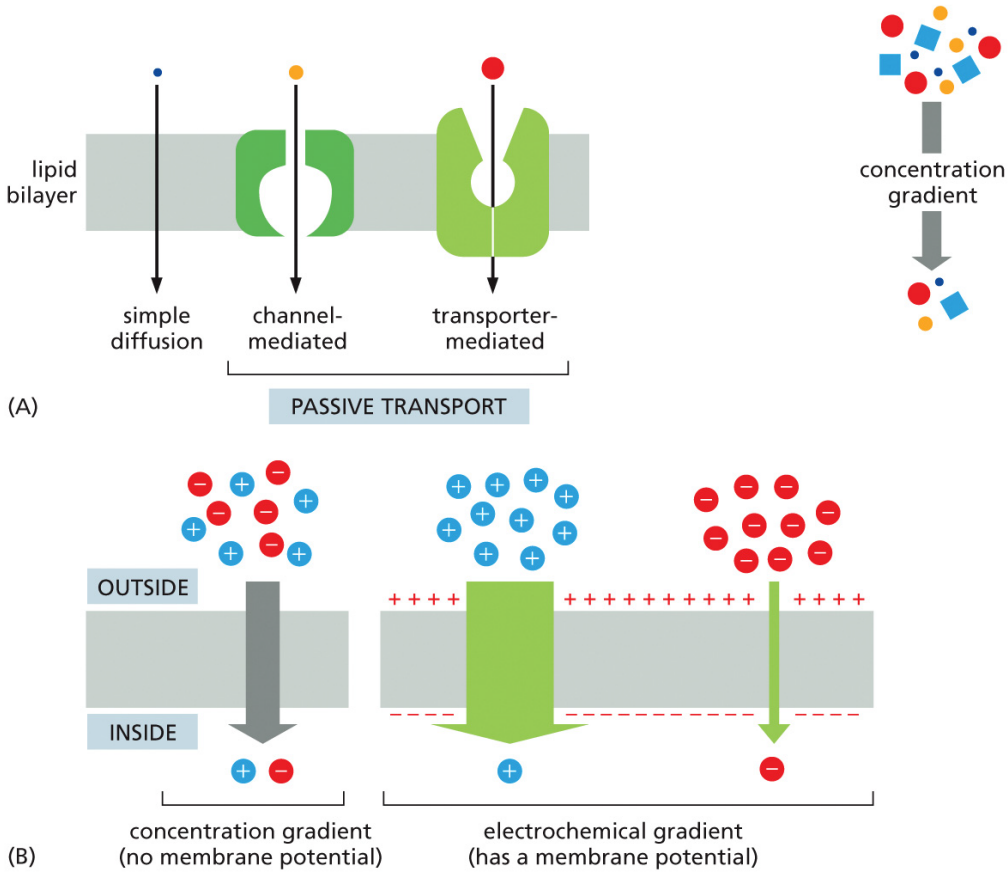
TABLE 11–1 A Comparison of Inorganic Ion Concentrations Inside and Outside a Typical Mammalian Cell*

Component	Cytoplasmic concentration (mM)	Extracellular concentration (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions		
Cl^-	5–15	110

*The cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions: although there is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, both ions are mostly bound to other substances (such as proteins, free nucleotides, RNA, etc.) and, for Ca^{2+}, stored within various organelles (such as the endoplasmic reticulum and mitochondria).

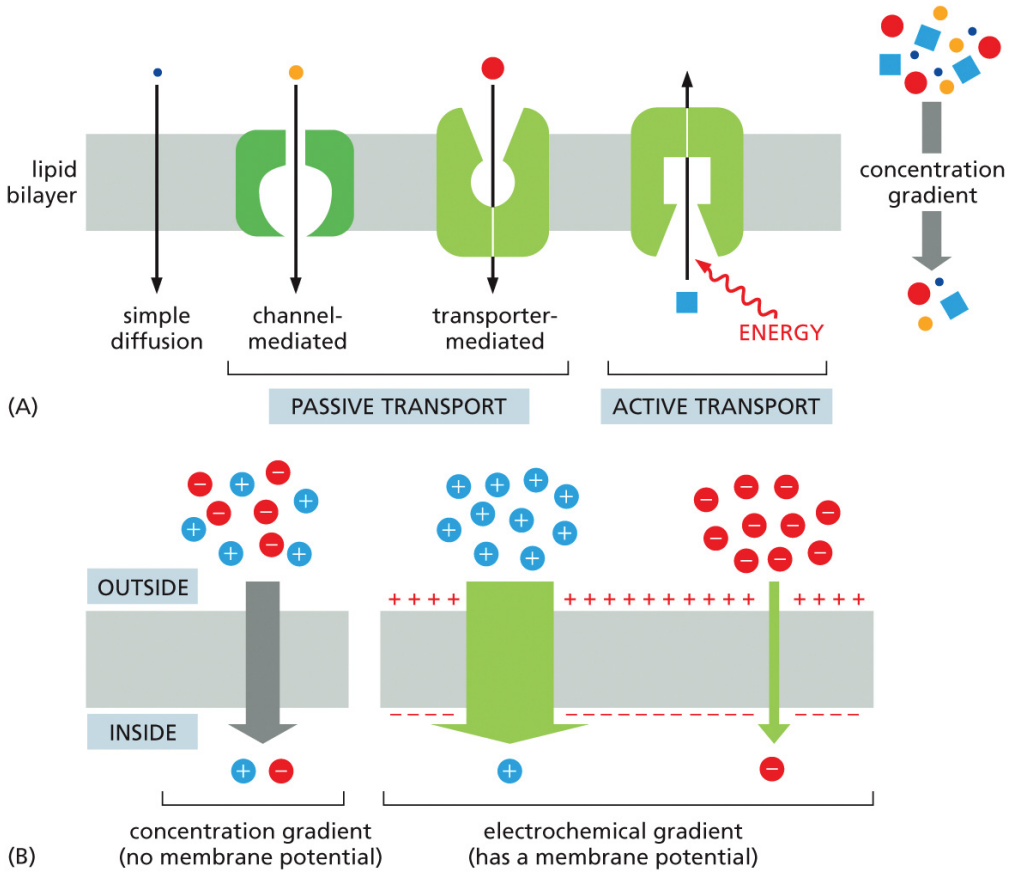
Concentration differences across the membrane

- **Passive transport** is driven by *concentration gradient* and possible electrical potential difference (*membrane potential*) together: **electrochemical gradient**

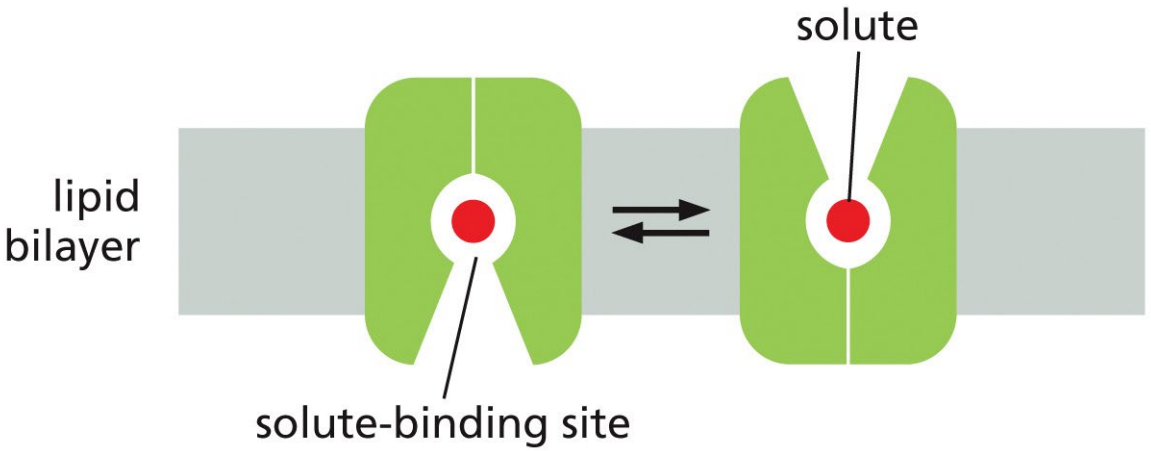


Concentration differences across the membrane

- **Passive transport** is driven by *concentration gradient* and possible electrical potential difference (*membrane potential*) together: **electrochemical gradient**
- **Active transport** is mediated by transporters *coupled to an energy source*



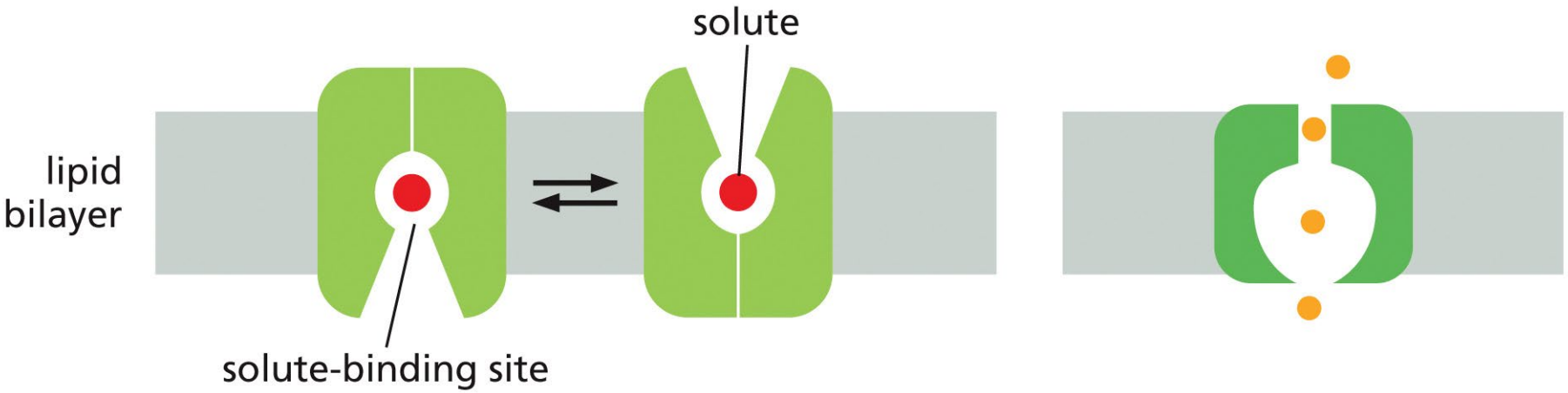
Transporters and Channels



(A) TRANSPORTER

- **A transporter** *alternates between two conformations*
- The solute-binding site of the transporter open to one side of the bilayer at the time
- Transfer of the solutes **actively** controlled

Transporters and Channels



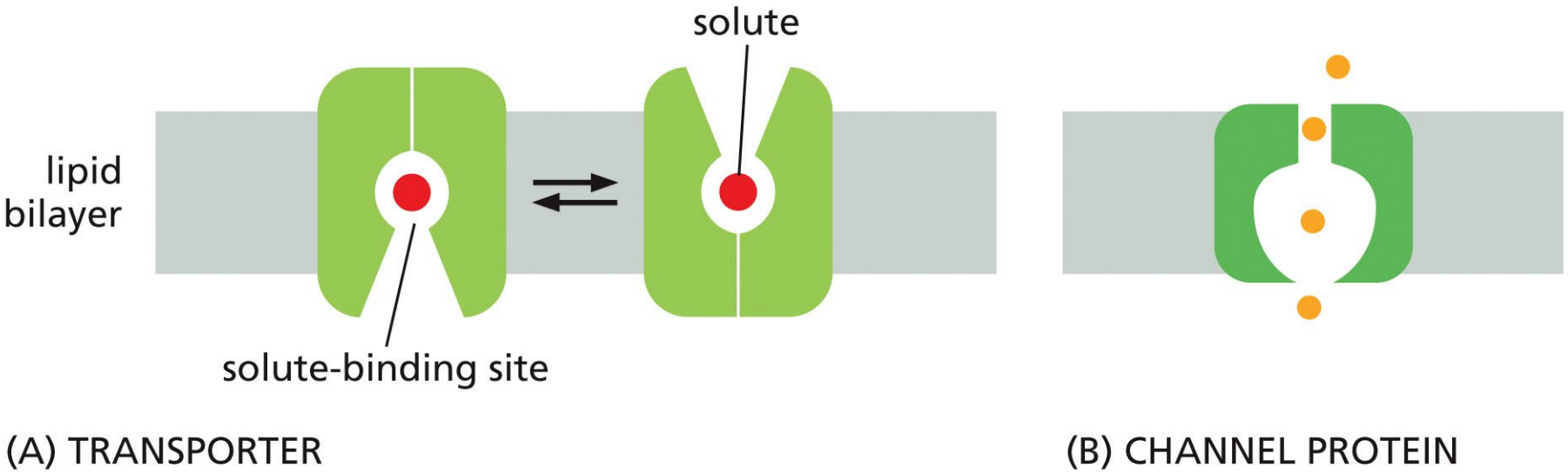
(A) TRANSPORTER

- **A transporter** *alternates between two conformations*
- The solute-binding site of the transporter open to one side of the bilayer at the time
- Transfer of the solutes can be **passive or actively** controlled

(B) CHANNEL PROTEIN

- **A channel protein** forms a pore across the bilayer through
- Specific solutes can **passively** diffuse.

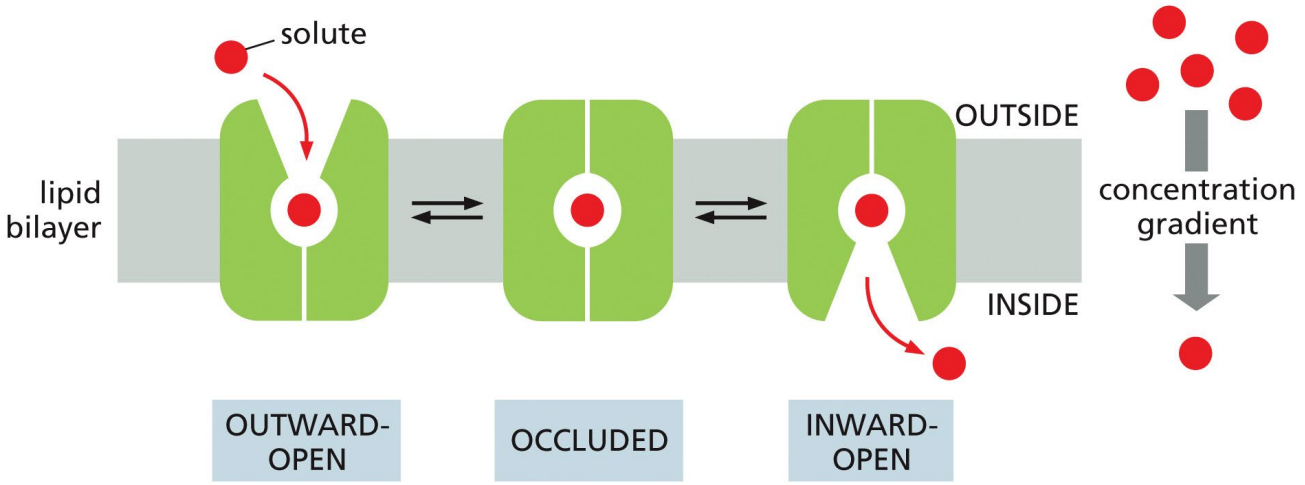
Transporters and Channels



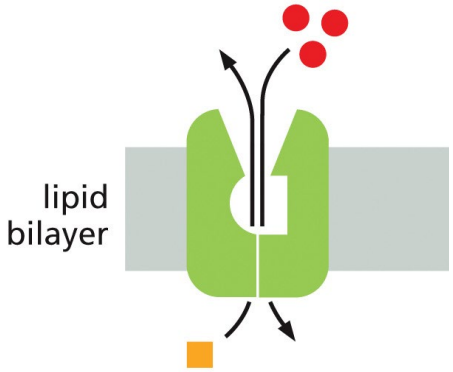
- Both classes are *membrane proteins* that form pathways inside, through which the solutes can move without interacting with the lipid bilayer

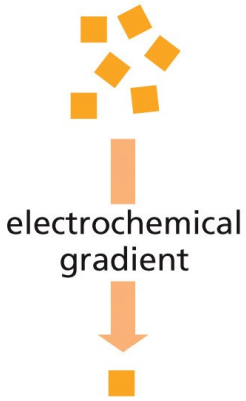
TRANSPORTERS AND **PASSIVE** MEMBRANE TRANSPORT

- **3 conformational states**: 1) outward-open state; 2) the occluded state; and 3) the inward-open state
- The transitions occur randomly, and solute does not need to be bound
- The higher the solute concentration the more solute binds to the transporter -> **net transport of solute down its concentration gradient** (or electrochemical gradient)



TRANSPORTERS AND **ACTIVE** MEMBRANE TRANSPORT

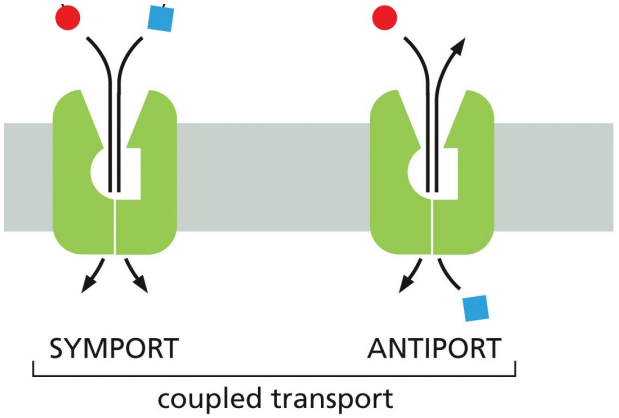




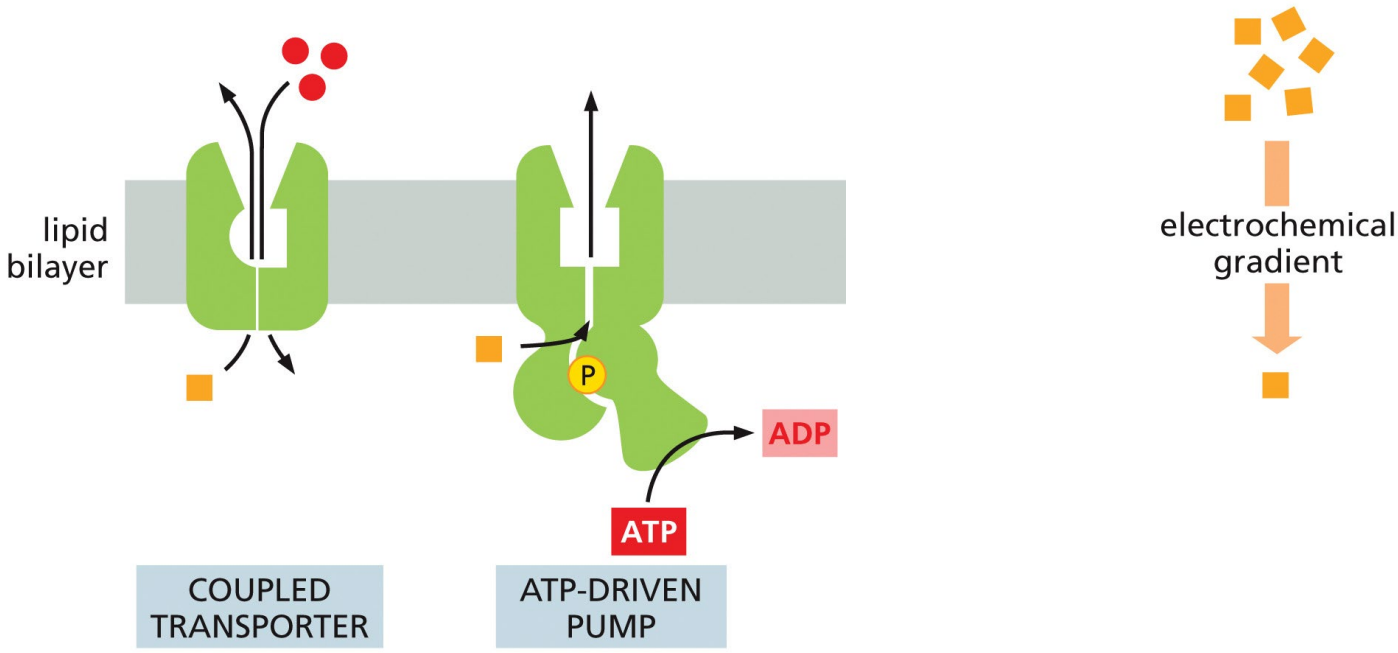
COUPLED TRANSPORTER

1. Uphill transport of one solute *coupled* to downhill transport of another.

Direction can be the same or different.

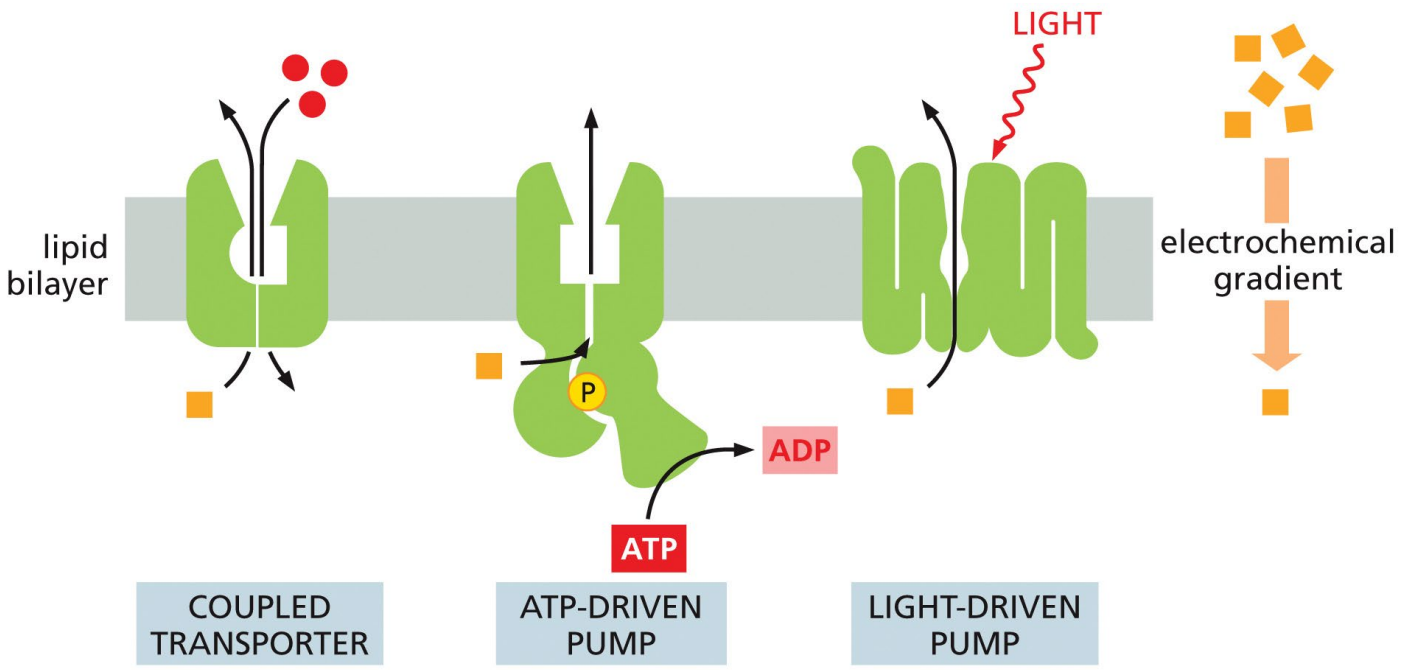


TRANSPORTERS AND **ACTIVE** MEMBRANE TRANSPORT



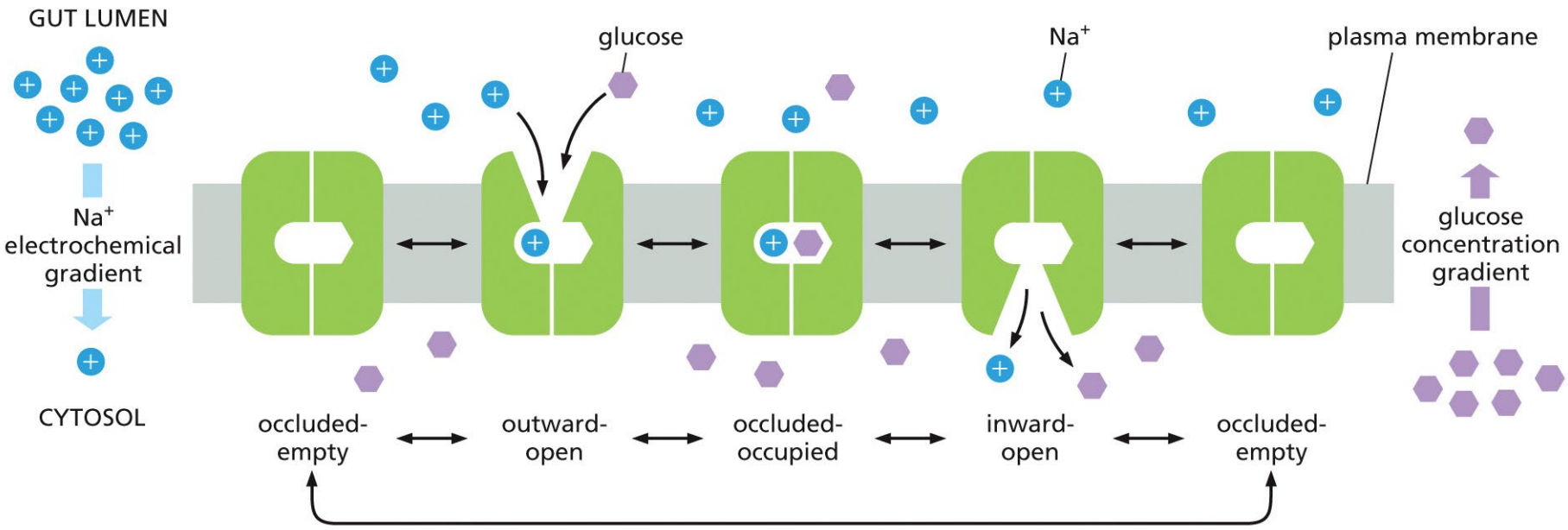
2. Energy from hydrolysis of ATP is used for transport

TRANSPORTERS AND **ACTIVE** MEMBRANE TRANSPORT



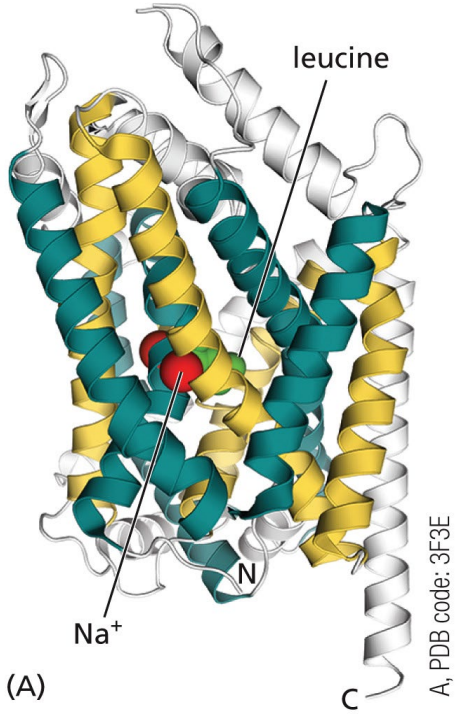
3. Energy from *light or redox-reactions*. In bacteria, archaea, and cell organelles with prokaryotic origin (mitochondria, chloroplasts).

MECHANISM OF GLUCOSE TRANSPORT FUELED BY AN NA^+ GRADIENT

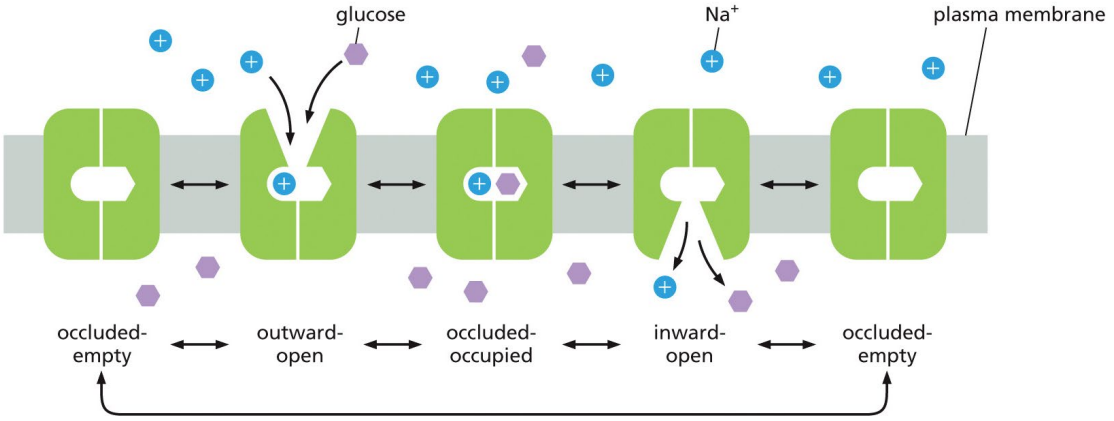


- Active transport is driven by ion-concentration gradient
  - Binding of Na^+ and glucose is **cooperative**
  - **Large electrochemical gradient of Na^+ is driving transport of glucose**

MECHANISM OF GLUCOSE TRANSPORT FUELED BY AN NA^+ GRADIENT



- Binding sites for the cosolutes in the center of the transporter
- Opens inwards only if both cosoluted bound



- Different ion concentration inside and outside cells are actively maintained

TABLE 11–1 A Comparison of Inorganic Ion Concentrations Inside and Outside a Typical Mammalian Cell*

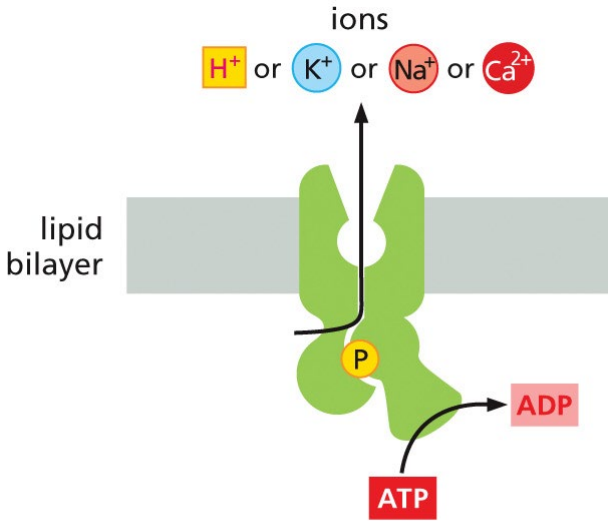
Component	Cytoplasmic concentration (mM)	Extracellular concentration (mM)
Cations		
Na^+	5–15	145
K^+	140	5
Mg^{2+}	0.5	1–2
Ca^{2+}	10^{-4}	1–2
H^+	7×10^{-5} ($10^{-7.2}$ M or pH 7.2)	4×10^{-5} ($10^{-7.4}$ M or pH 7.4)
Anions		
Cl^-	5–15	110

*The cell must contain equal quantities of positive and negative charges (that is, it must be electrically neutral). Thus, in addition to Cl^-, the cell contains many other anions not listed in this table; in fact, most cell constituents are negatively charged (HCO_3^-, PO_4^{3-}, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca^{2+} and Mg^{2+} given are for the free ions: although there is a total of about 20 mM Mg^{2+} and 1–2 mM Ca^{2+} in cells, both ions are mostly bound to other substances (such as proteins, free nucleotides, RNA, etc.) and, for Ca^{2+}, stored within various organelles (such as the endoplasmic reticulum and mitochondria).

→ ATP-driven pumps!

ATP-driven Pumps

- *Actively* maintain desired concentrations of ions and small molecules
- There are three classes of **ATP-driven** pumps

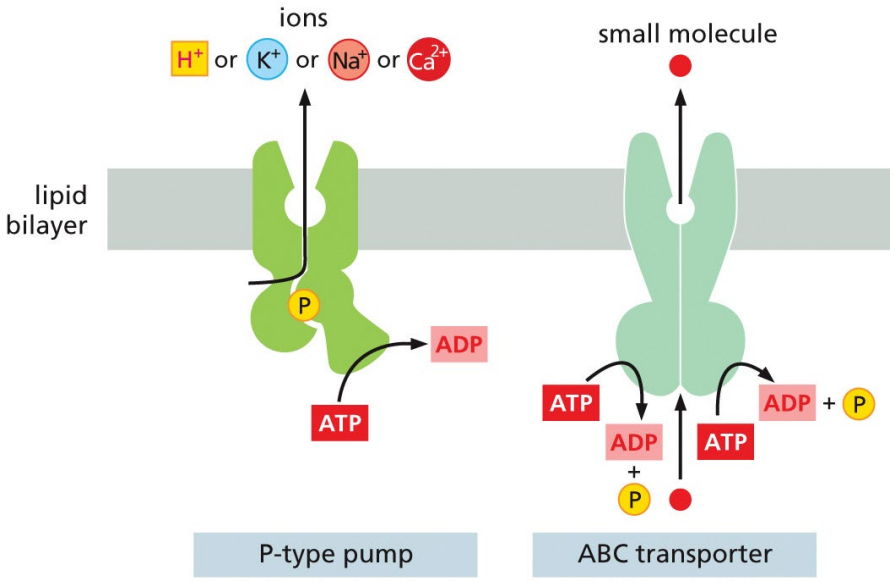


P-type pump

- Maintain gradients of Na+, K+. H+, and Ca+
- P-type, because phosphorylated during the cycle

ATP-driven Pumps

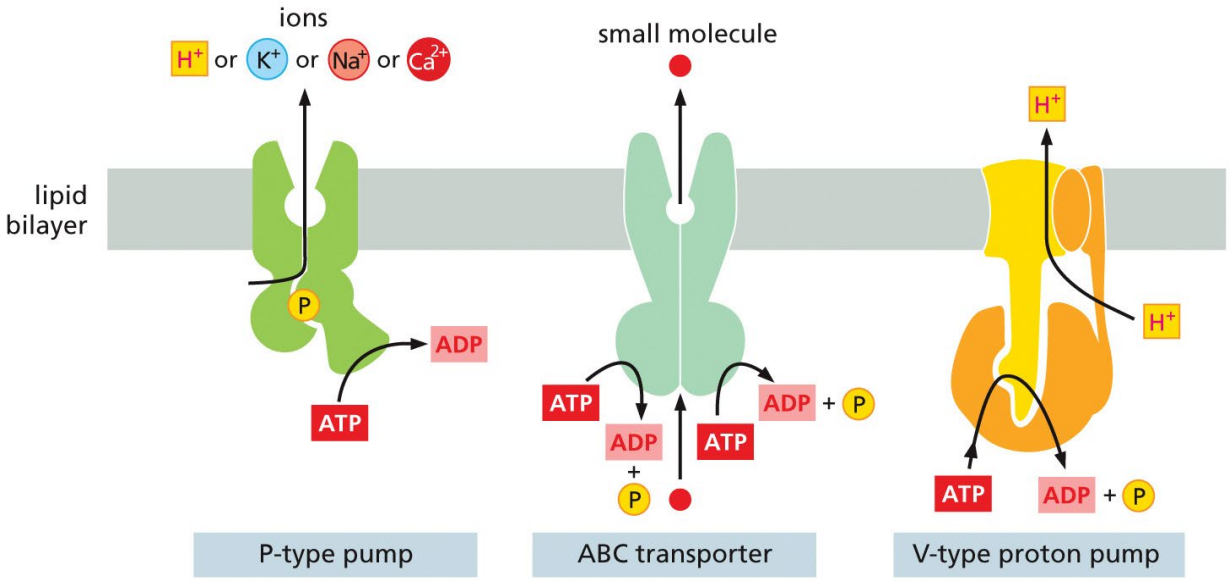
- *Actively* maintain desired concentrations of ions and small molecules
- There are three classes of **ATP-driven** pumps



- Pump small molecules

ATP-driven Pumps

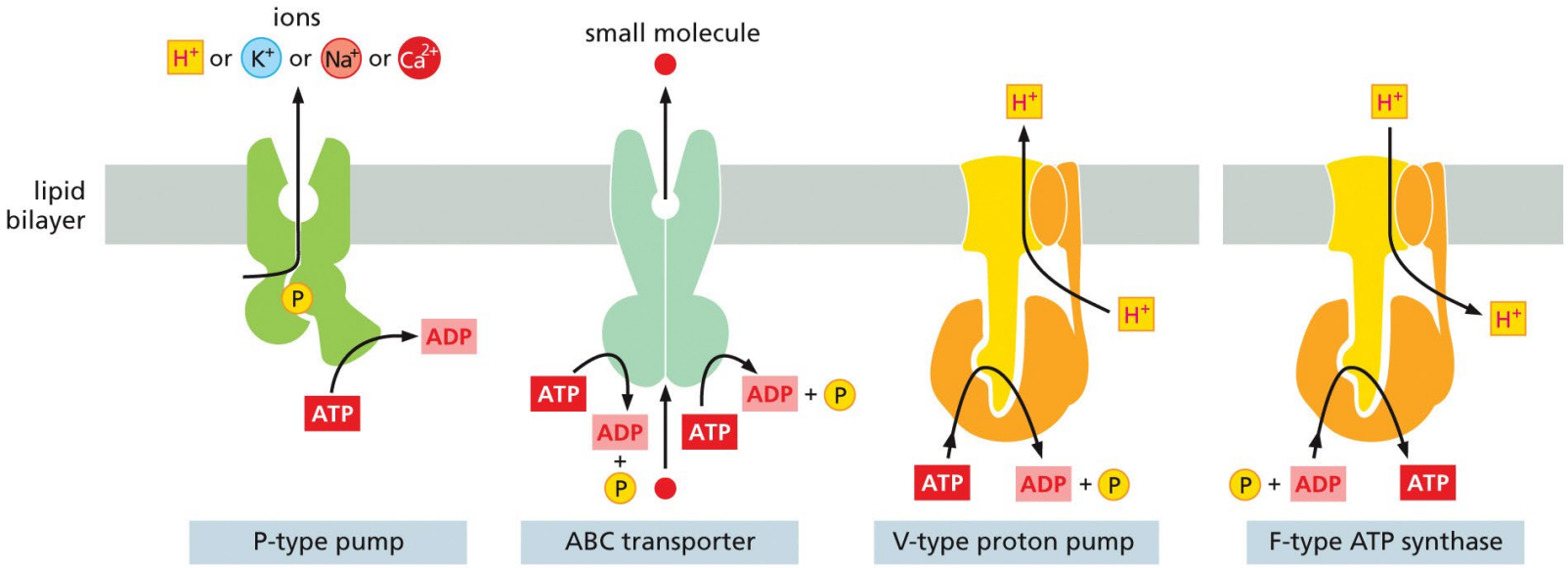
- *Actively* maintain desired concentrations of ions and small molecules
- There are three classes of **ATP-driven** pumps



- Pumps H+ into organelles to acidify

ATP-driven Pumps

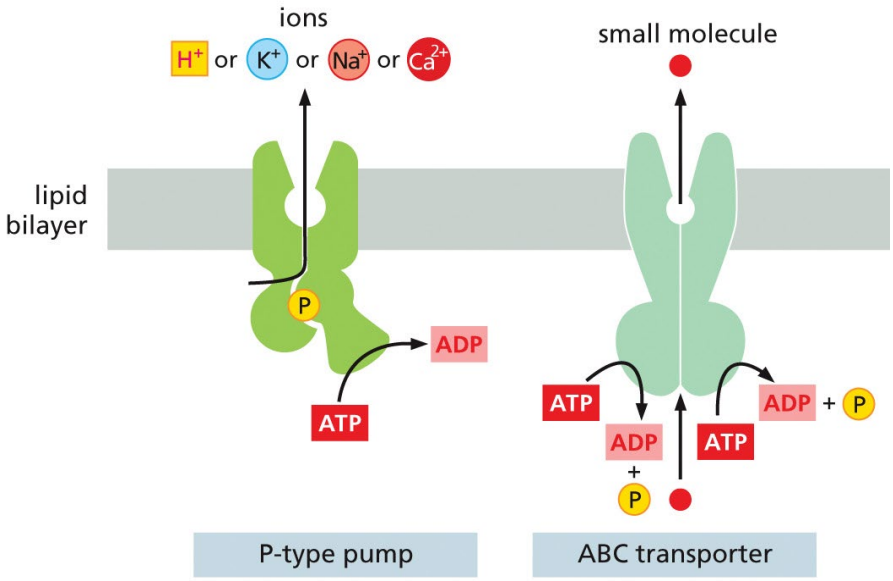
- *Actively* maintain desired concentrations of ions and small molecules
- There are three classes of **ATP-driven** pumps



- ATP **synthases**

ATP-driven Pumps

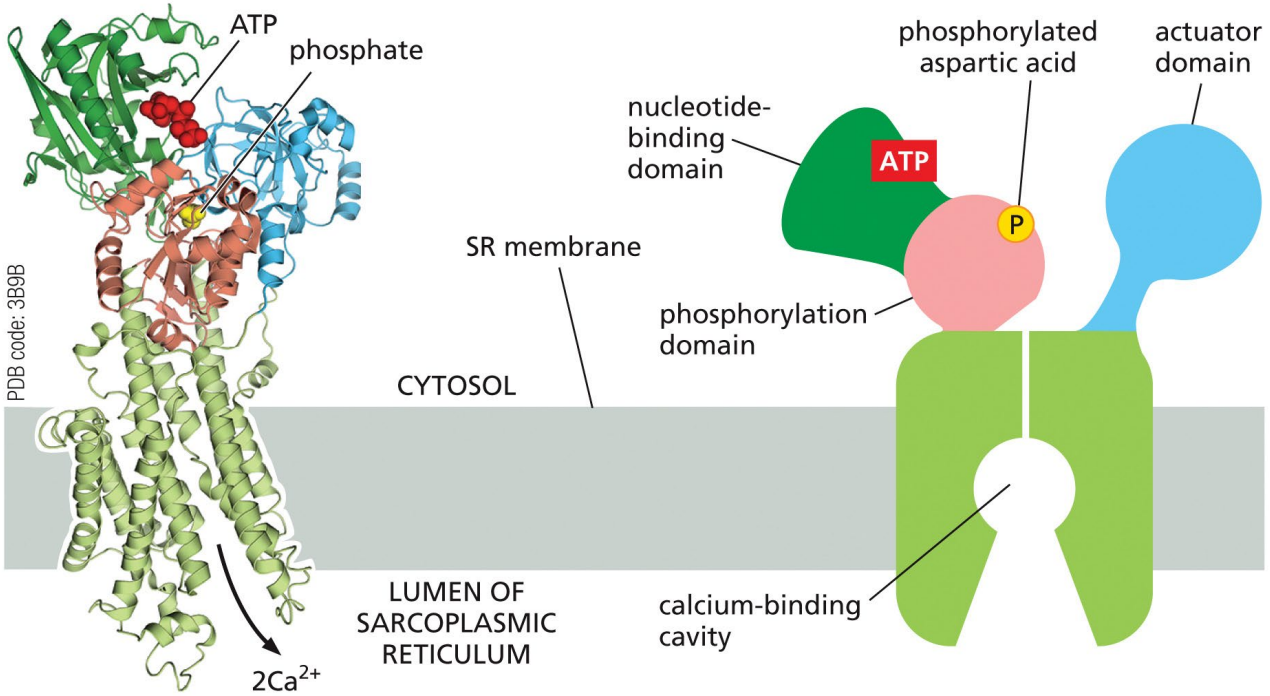
- *Actively* maintain desired concentrations of ions and small molecules
- There are three classes of **ATP-driven** pumps



- *Focus on these two*

P-TYPE ATPASE PUMPS

Component	Cytoplasmic concentration (mM)	Extracellular concentration (mM)
Ca^{2+}	10^{-4}	1–2



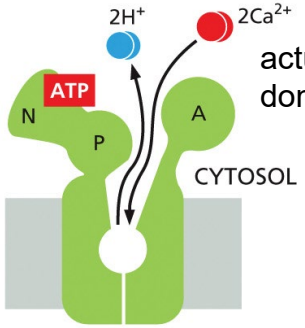
- A P-type ATPase pumps Ca^{2+} into the sarcoplasmic reticulum in muscle cells

P-TYPE ATPASE PUMPS

- The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump

nucleotide-binding domain (N)

phosphorylation domain (P)

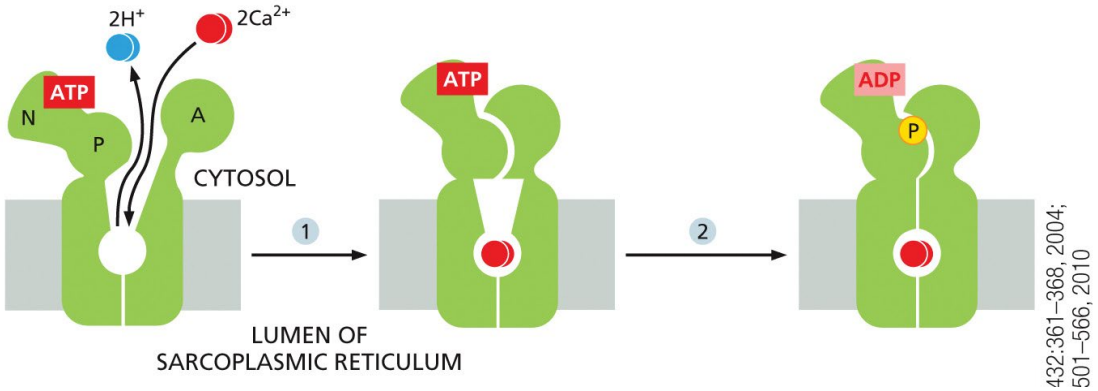


actuator domain (**A**)

- ATP-bound, Ca2+ binding sites accessible only form the cytosol

P-TYPE ATPASE PUMPS

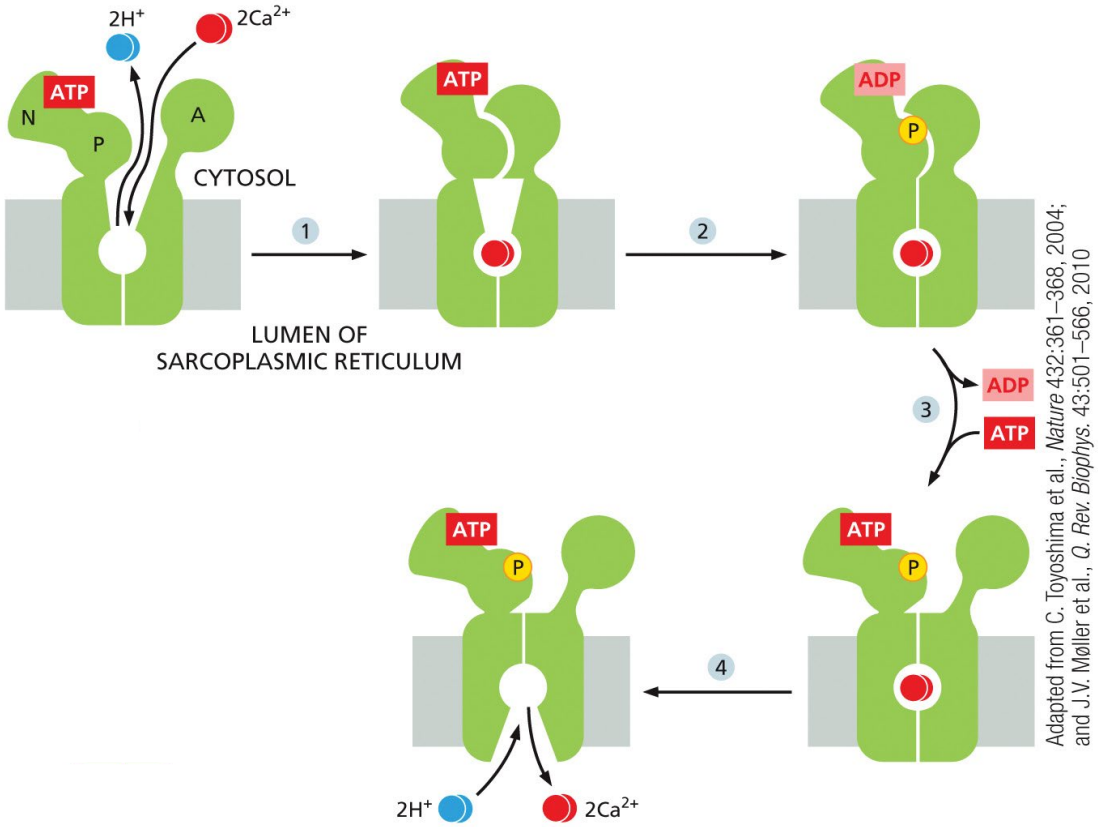
- The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump



- Binding of Ca2+ triggers conformational changes leading to closing of the passageway and transfer of a phosphate from ATP to an aspartate in the ATPase

P-TYPE ATPASE PUMPS

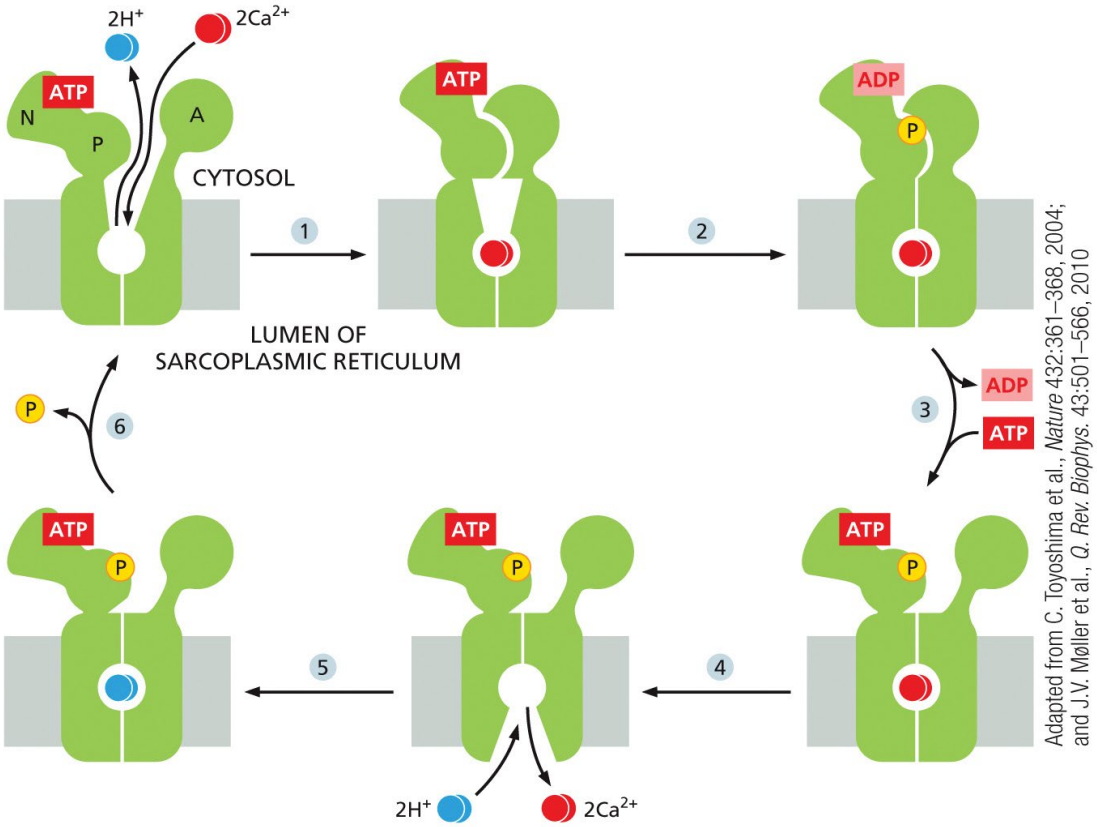
- The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump



- ADP is replaced with ATP which trigger another conformational change and opening of the lumen side pathway

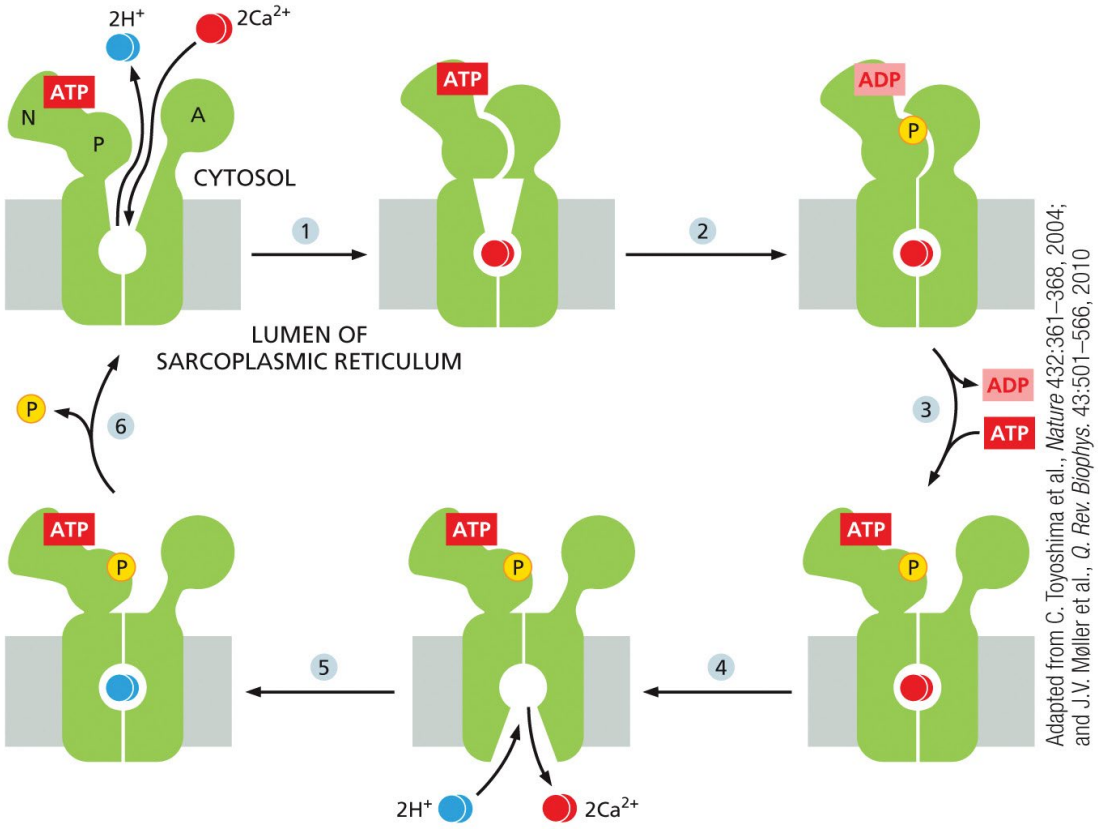
P-TYPE ATPASE PUMPS

- The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump



- H+ ions + H2O occupy space instead of Ca2+ -> closing of the passageway
- Labile phosphorylated Asp is hydrolyzed-> original conformation

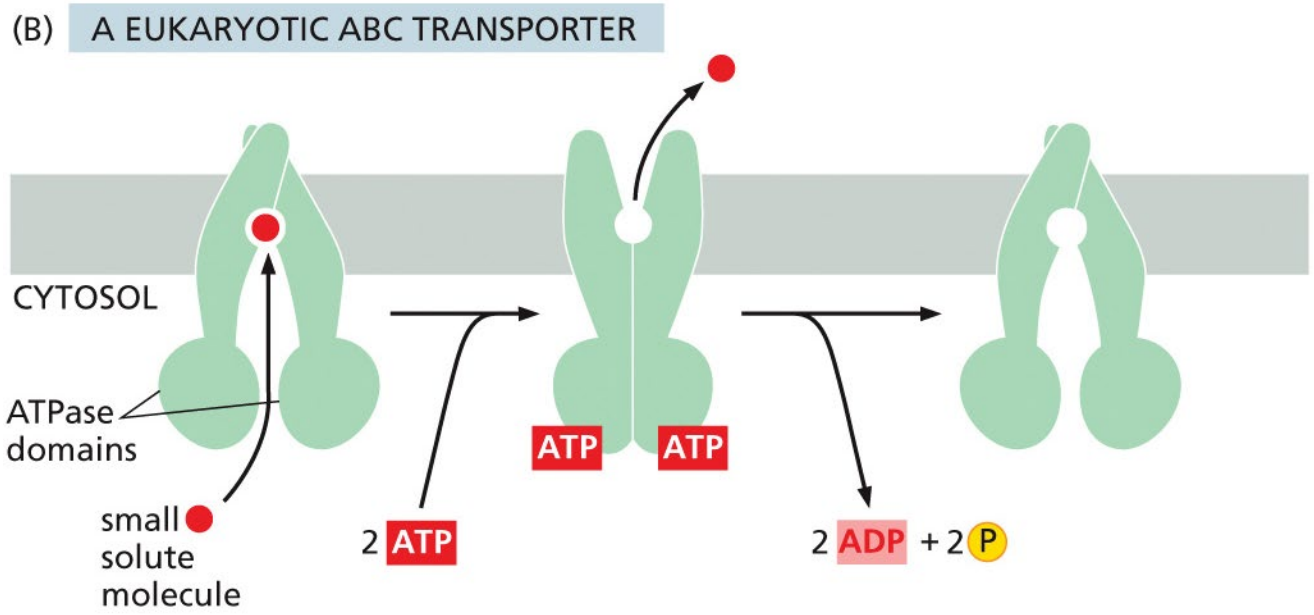
P-TYPE ATPASE PUMPS



- The pumping cycle of the sarcoplasmic reticulum Ca^{2+} pump

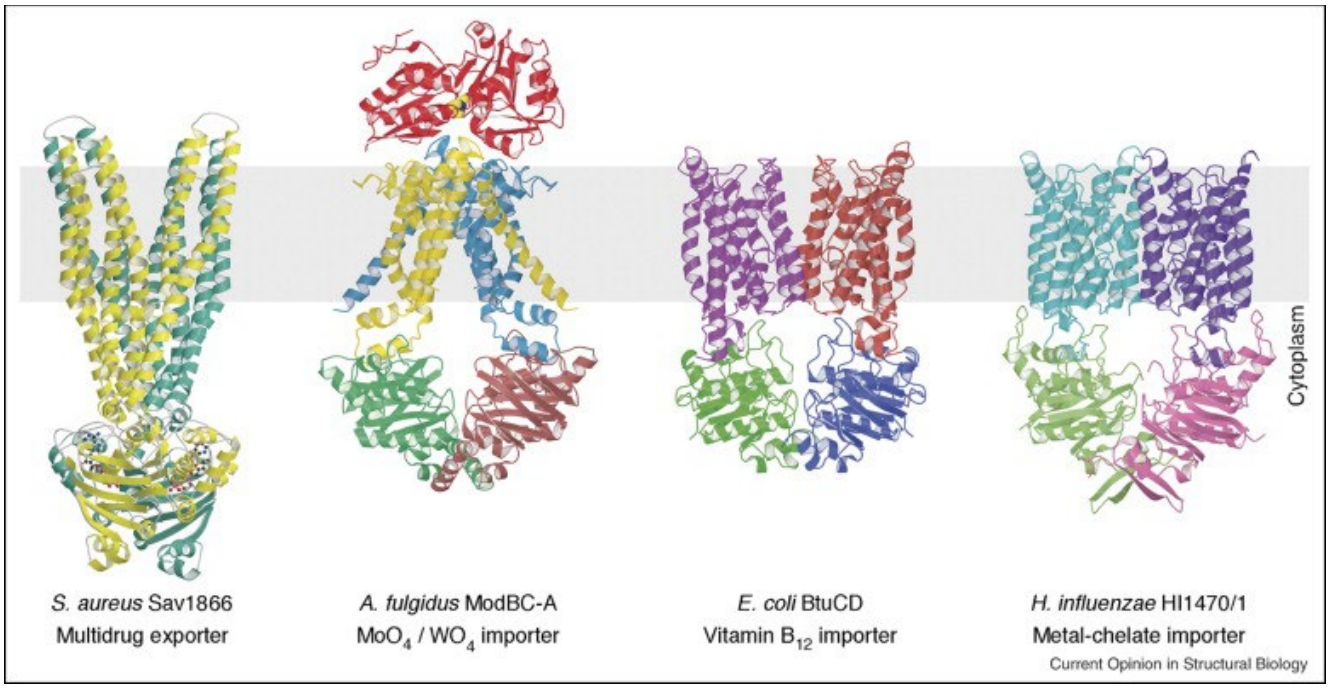
ABC TRANSPORTERS

- Constitute the largest family of membrane transport proteins
- ABC stands for "**A**TP-**b**inding **c**assette"
- Consist of two "cassettes"
- Two ATPase domains protrude into the cytosol.



ABC TRANSPORTERS

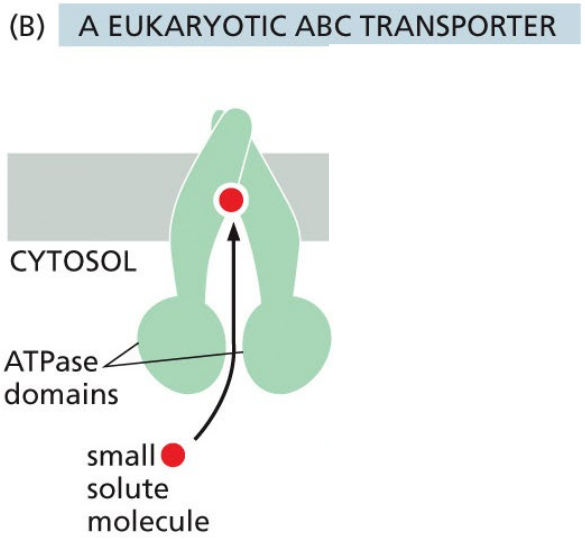
- Typically, two hydrophobic domains, each built of six membrane-spanning α helices, together form the translocation pathway and provide substrate specificity
- In some cases, the two halves of the transporter are formed by a single polypeptide, whereas in other cases they are formed by two or more separate polypeptides that assemble into a similar structure.



Hollenstein, K et al. Current Opinion in Structural Biology, 17, 2007, 412-418, https://doi.org/10.1016/j.sbi.2007.07.003.

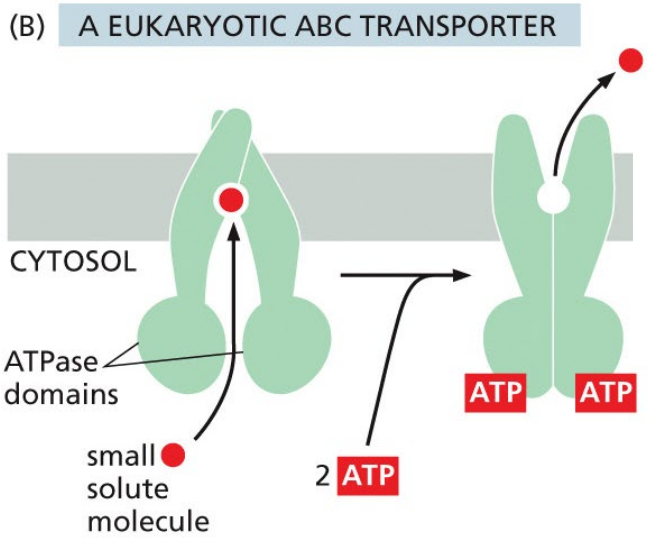
ABC TRANSPORTERS

- Without ATP bound, the transporter exposes a substrate-binding site on one side of the membrane.



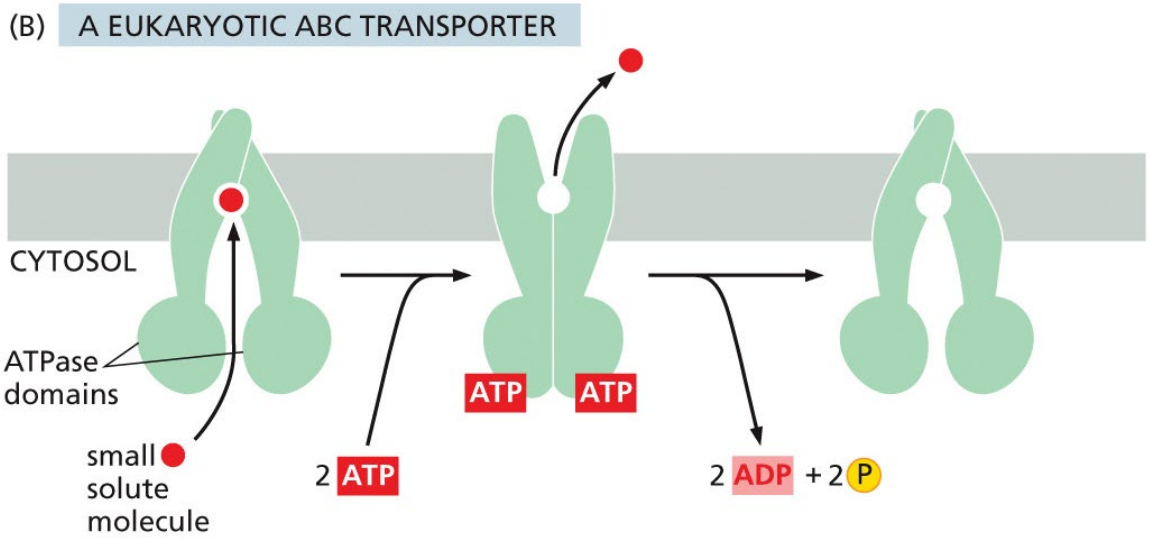
ABC TRANSPORTERS

- Without ATP bound, the transporter exposes a substrate-binding site on one side of the membrane.
- ATP binding induces a conformational change that exposes the substrate-binding site on the opposite side;



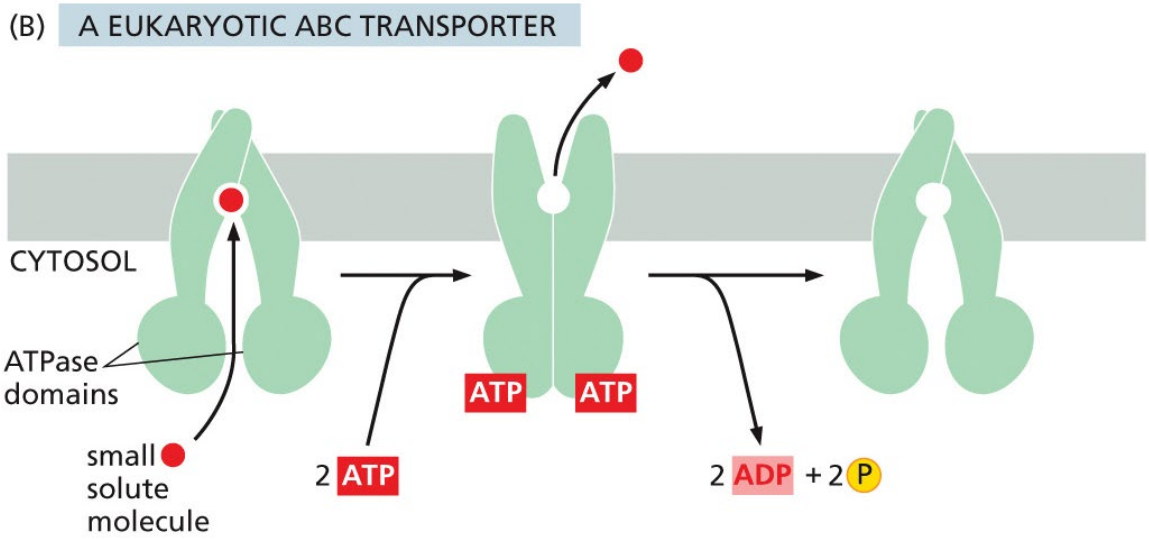
ABC TRANSPORTERS

- Without ATP bound, the transporter exposes a substrate-binding site on one side of the membrane.
- ATP binding induces a conformational change that exposes the substrate-binding site on the opposite side;
- ATP hydrolysis followed by ADP dissociation returns the transporter to its original conformation.



ABC TRANSPORTERS

- Without ATP bound, the transporter exposes a substrate-binding site on one side of the membrane.
- ATP binding induces a conformational change that exposes the substrate-binding site on the opposite side;
- ATP hydrolysis followed by ADP dissociation returns the transporter to its original conformation.
- Most individual ABC transporters are unidirectional.



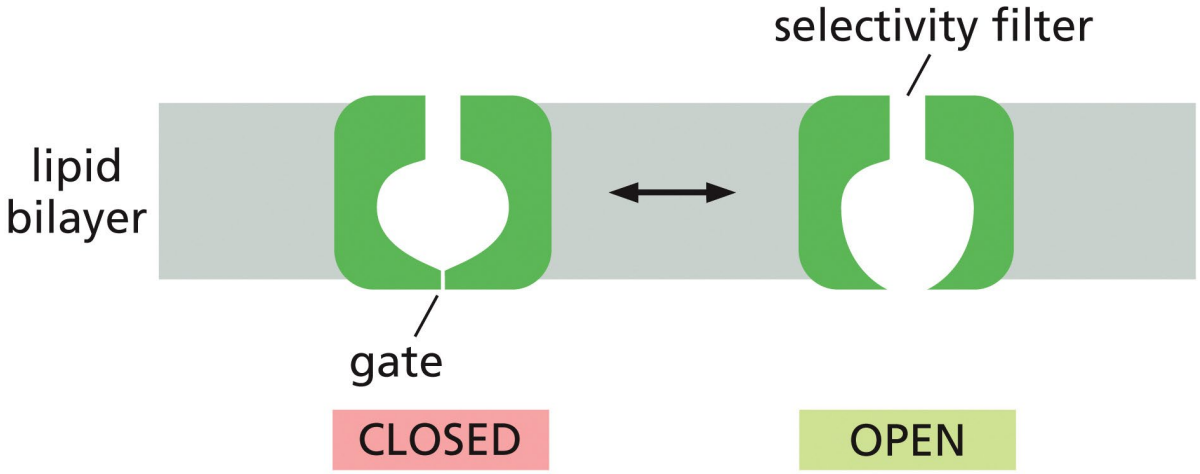
In eukaryotes, most ABC transporters export substances

ABC TRANSPORTERS

- Each transporter is specific to a particular molecule or class of molecules
- Substrates can be ions, amino acids, polysaccharides, peptides, lipids, drugs, even proteins
- Multidrug resistance proteins in cancer cells and pathogens an example

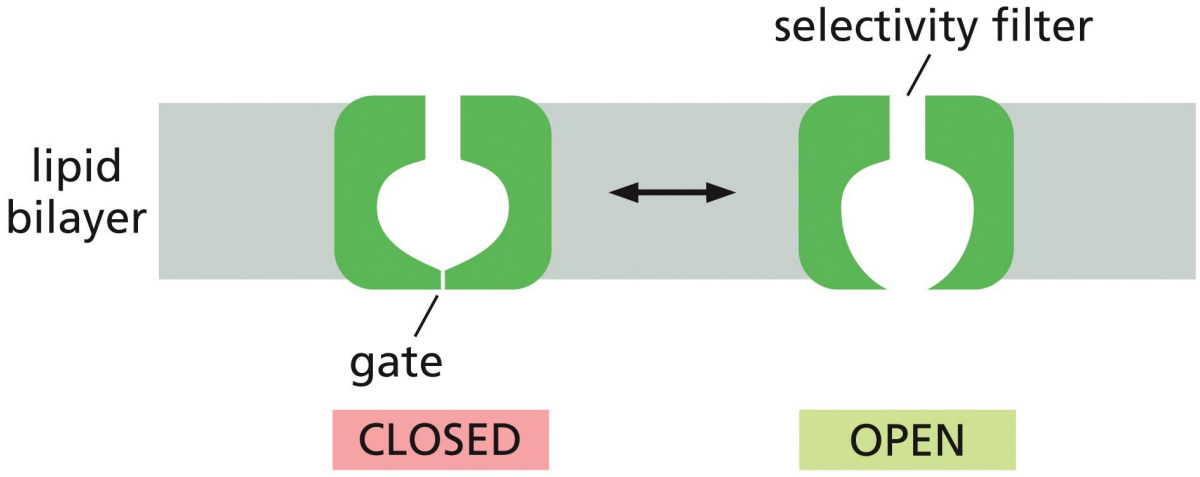
CHANNELS AND THE ELECTRICAL PROPERTIES OF MEMBRANES

- Much **faster** than transporters (10^5 times greater rate)
- Transport always **passive**
- Channels are **selective** and can be turned **on** or **off**



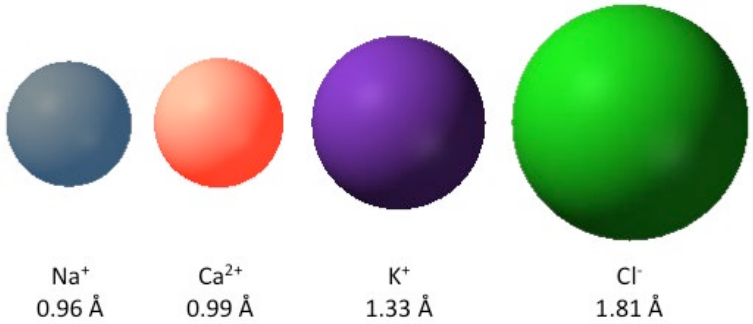
CHANNELS ARE SELECTIVE AND HAVE GATES

- The pore narrows to atomic dimensions in one region (*the selectivity filter*), where **the ion selectivity** of the channel is largely determined
- Another region of the channel forms the **gate**
- The ion channel shown here forms a pore across the lipid bilayer only in the “open” conformational state



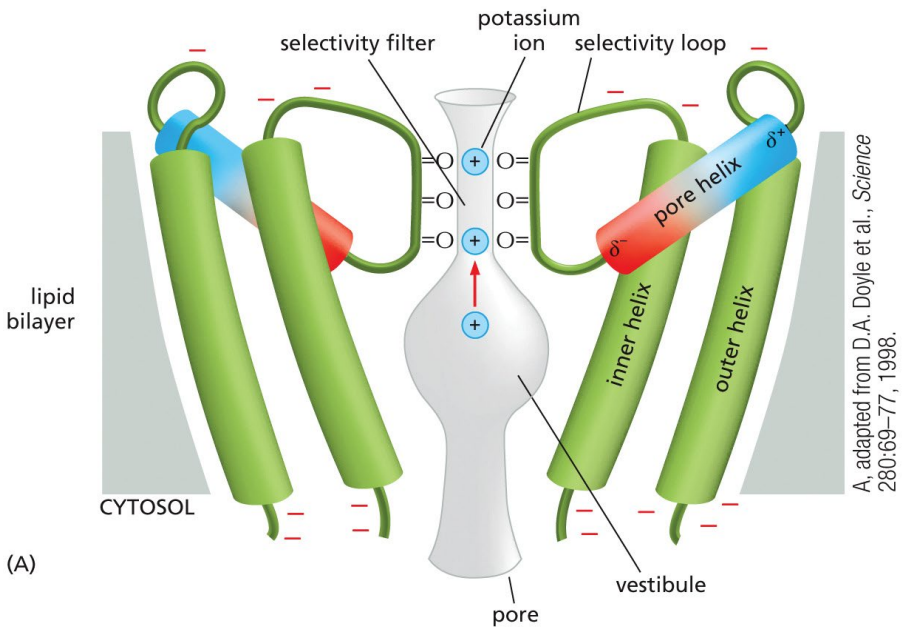
BACTERIAL K^+ CHANNEL AS AN EXAMPLE OF THE SPECIFICITY OF AN ION CHANNEL

- Selective to K+
- Larger ions and ions with different charge can be excluded
- How to select only K+ and not Na+? (10,000-fold faster rate for K+)



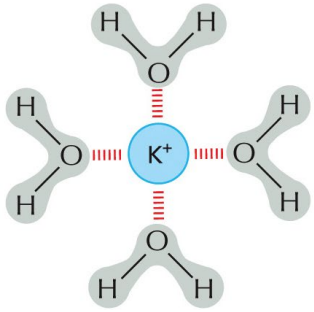
BACTERIAL K^+ CHANNEL AS AN EXAMPLE OF THE SPECIFICITY OF AN ION CHANNEL

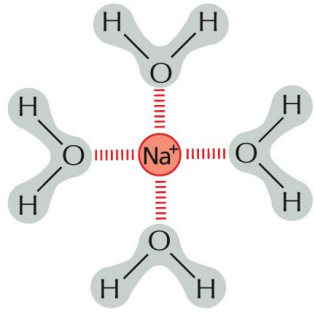
- 4 identical transmembrane subunits form a pore
- Pore contains a vestibule and then narrows into a selectivity filter



- Both K+ and Na+ can enter the **vestibule** in hydrated state

ion in vestibule





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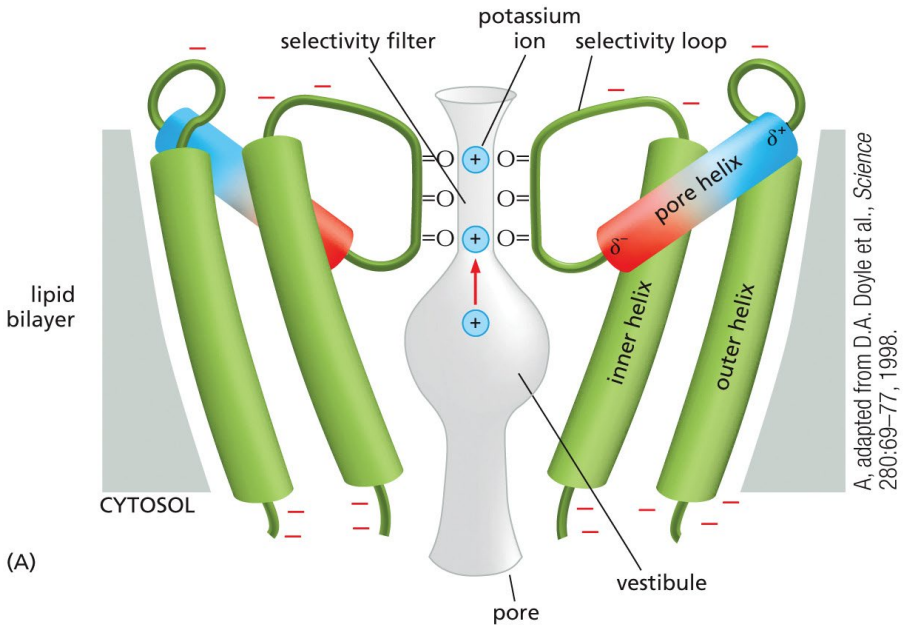
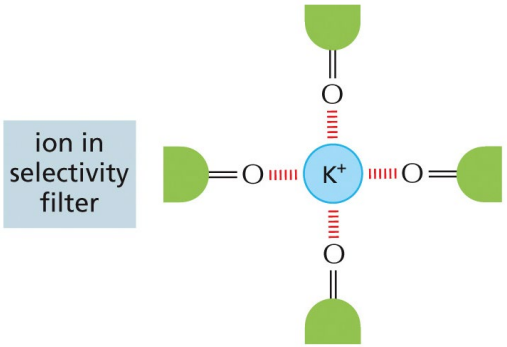


- Carbonyl oxygens in the selectivity filter provide transient binding sites for K+, to replace the interactions with water



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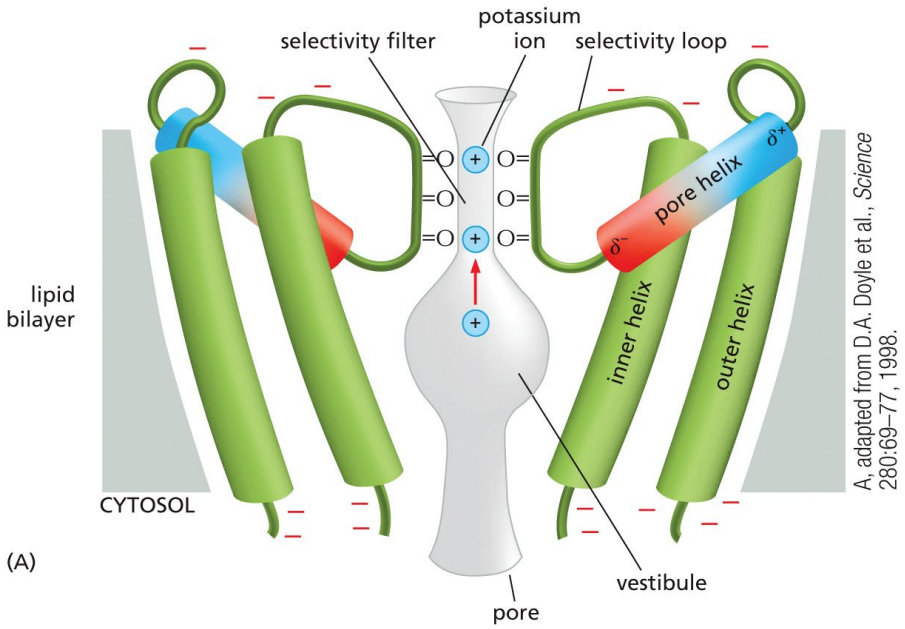
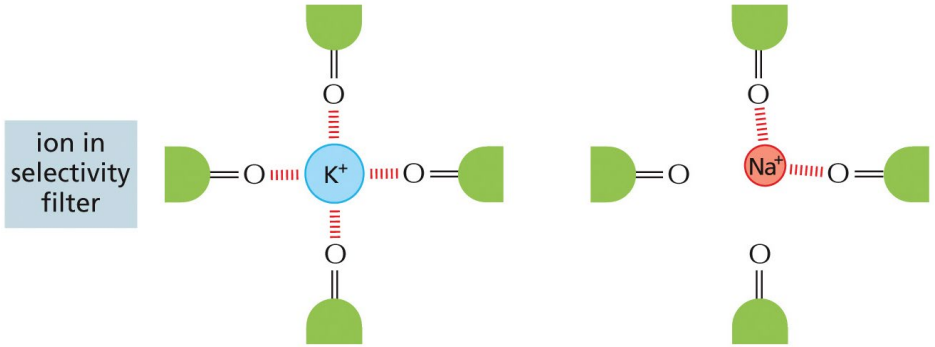


- Na+ is smaller and only part of the interactions can be formed at the time



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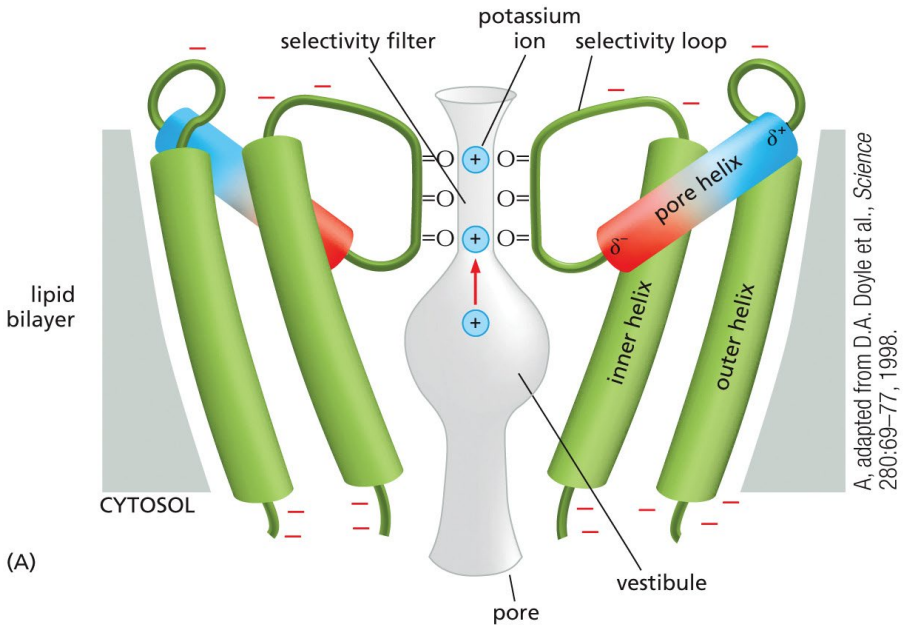

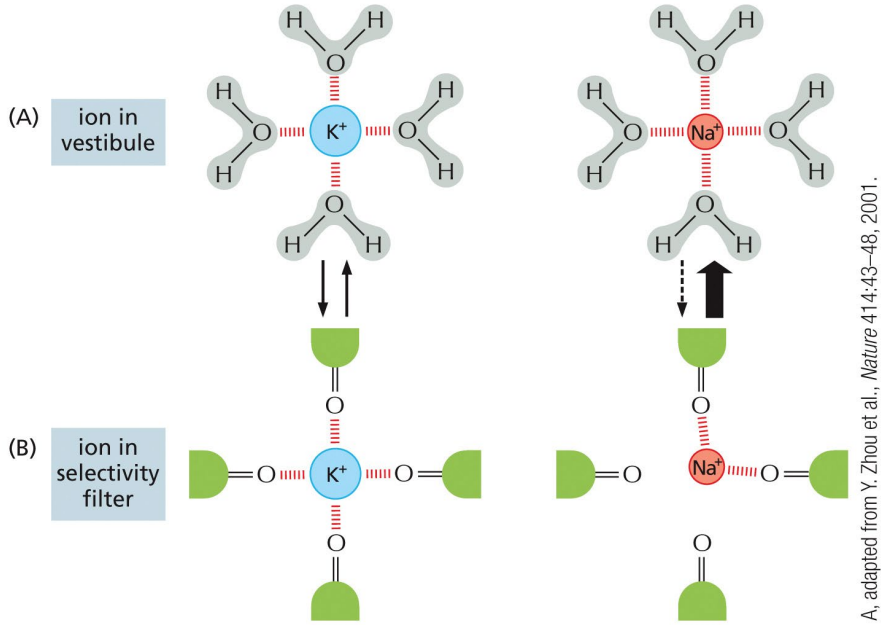

- Na+ is excluded by the selectivity filter

SUMMARY

- **Transporters** are *actively* transporting molecules, whereas **channels** allow them to move *passively*
- Both are highly *selective* and their activities are *controlled*
- Transporters use energy by cotransporting *cosolutes* or by *hydrolyzing ATP*