# *Review:* Analyzing Structure and Function of Genes

- If you wanted to *permanently* change the protein expression of a target gene, which of the following approaches would work, and why?
  - Design a siRNA that selectively targets the mRNA for the gene of interest, leading to complete degradation of the mRNA and therefore preventing translation.
  - Using a transgenic mouse that has LoxP sites around the gene of interest and activating Cre recombinase in this mouse.
  - Using CRISPR/Cas9 to delete the promoter of the target gene without affecting any of the nucleotide sequence that would be transcribed into RNA.

# *Review:* Analyzing Structure and Function of Genes

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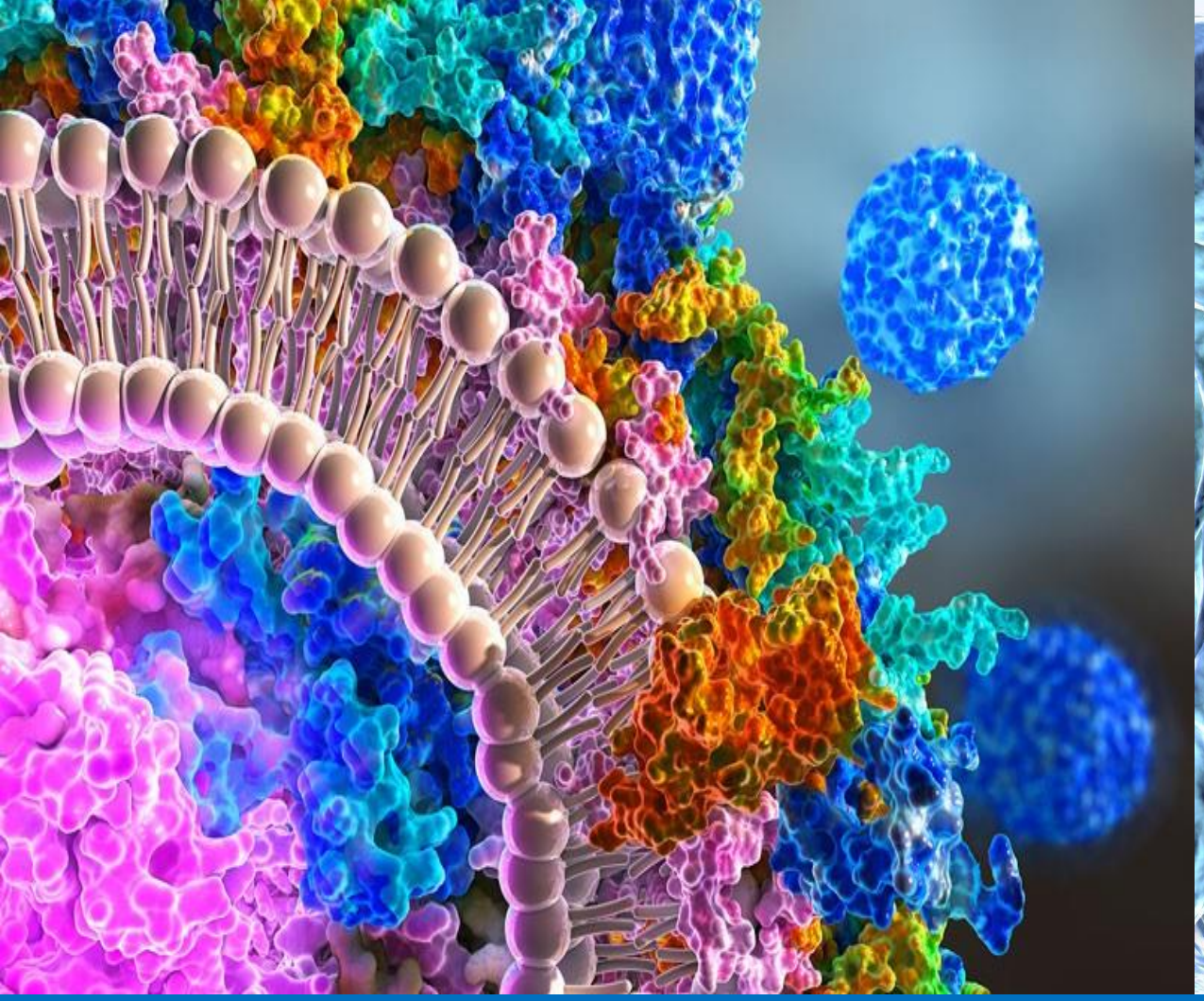
  - Design a siRNA that selectively targets the mRNA for the gene of interest, leading to complete degradation of the mRNA and therefore preventing translation.
    - No, this would serve to knockdown the gene, reducing the amount of mRNA translated to protein, but eventually the siRNA would be degraded while transcription of the mRNA is not being affected and could then continue uninhibited.
  - Using a transgenic mouse that has LoxP sites around the gene of interest and activating Cre recombinase in this mouse.
    - Yes, using this approach will literally cut out the entire gene of interest similarly to a transposon and remove it from the DNA, thereby no allowing transcription or translation to protein to occur.
  - Using CRISPR/Cas9 to delete the promoter of the target gene without affecting any of the nucleotide sequence that would be transcribed into RNA.
    - Yes, if the promoter DNA has been removed by genetic editing, there would be no ability for transcription to occur, and so no protein would be produced even if the gene itself is unaltered.

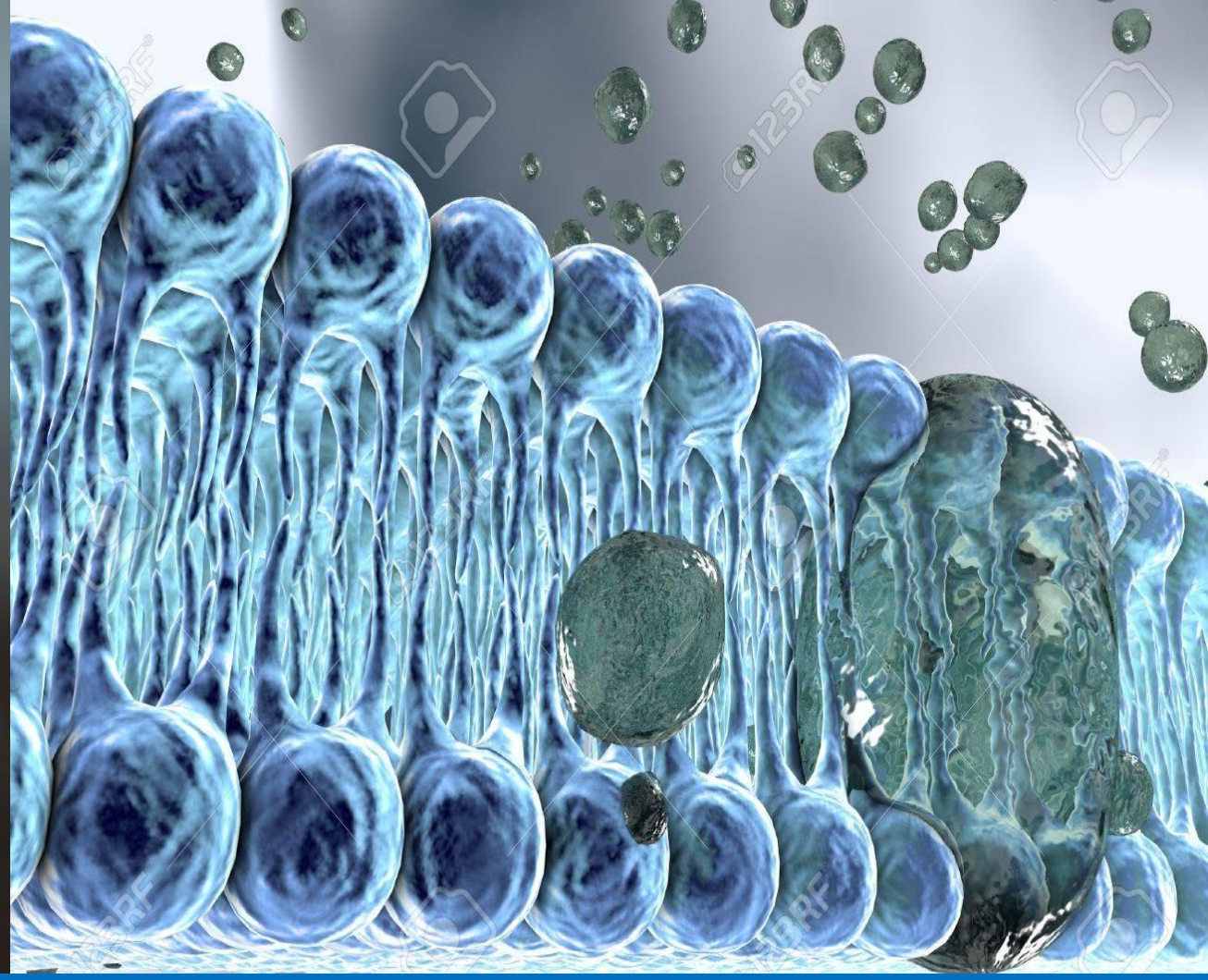
# Medicine Nobel awarded for gene-regulating 'microRNAs'

**Victor Ambros and Gary Ruvkun identified a class of tiny molecules that have a crucial role in controlling gene expression.**

- First discovery in 1993 in nematodes, then expansion to presence in human genome in 2000; now awarded the Nobel Prize in 2024 (last week!)

- Relatively new discovery that we now teach in undergraduate biology due to its impact on gene expression





# Membrane Structure and Transport Across Cell Membranes

Dr. Matthew Ellis

Chapters 11 & 12

Biology 366

# Learning Objectives for Chapters 11 & 12

By the end of this module, you should be able to:

- Describe the functions of the plasma membrane, and how various lipids, proteins, and sugars contribute to these functions.
- Understand the process by which asymmetric distribution of phospholipids within the plasma membrane is established and maintained.

- Define the roles of passive and active transport across the cell membrane, and how each is used by the cell.
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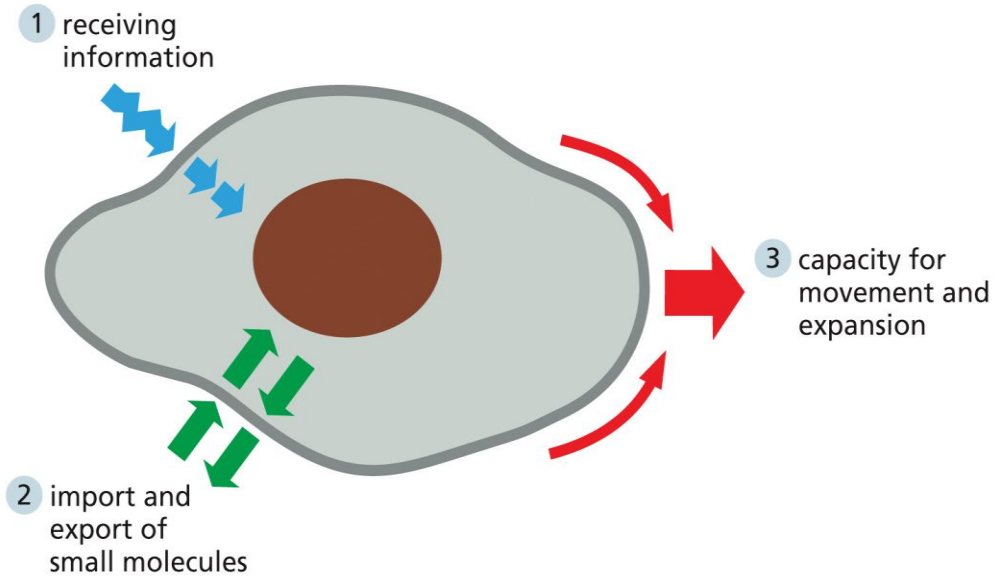
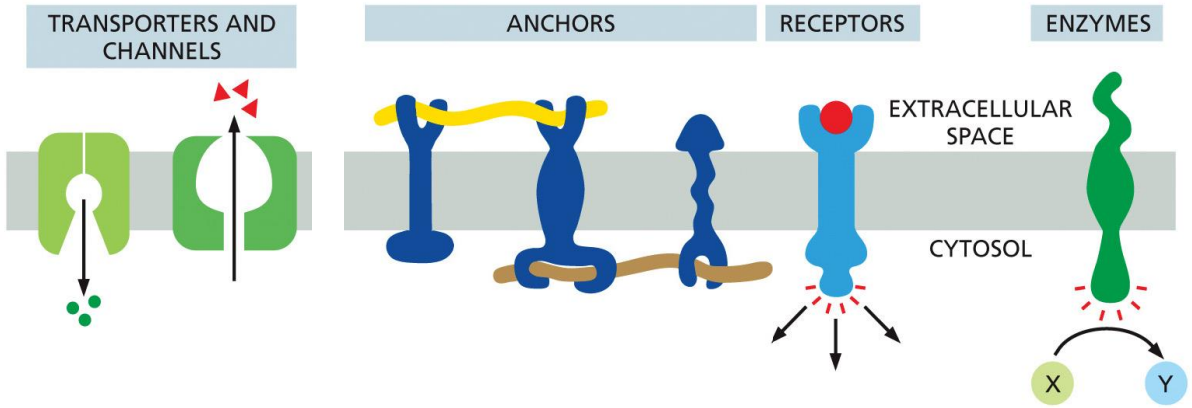
# Cell membranes and membrane proteins play important roles in many cell functions

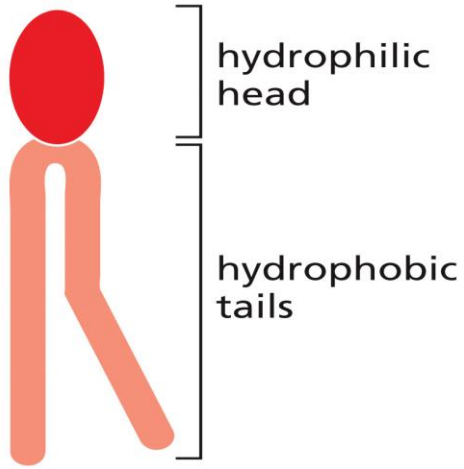
- **Cell communication**
  - Receptor proteins in the plasma membrane enable the cell to receive signals from the environment
- **Import and export of molecules**
  - Channels and transporters in the membrane enable the import and export of small molecules
- **Cell growth and motility**
  - Membrane expansion allows cell growth, changes in cell shape, cell movement (morphological changes)

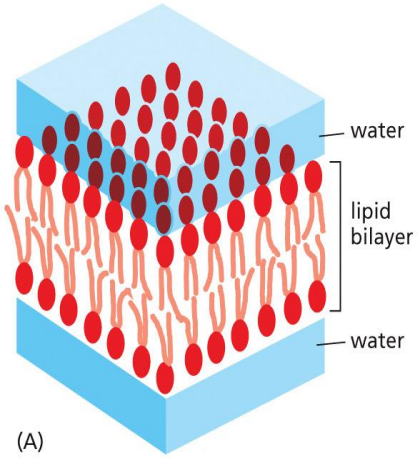


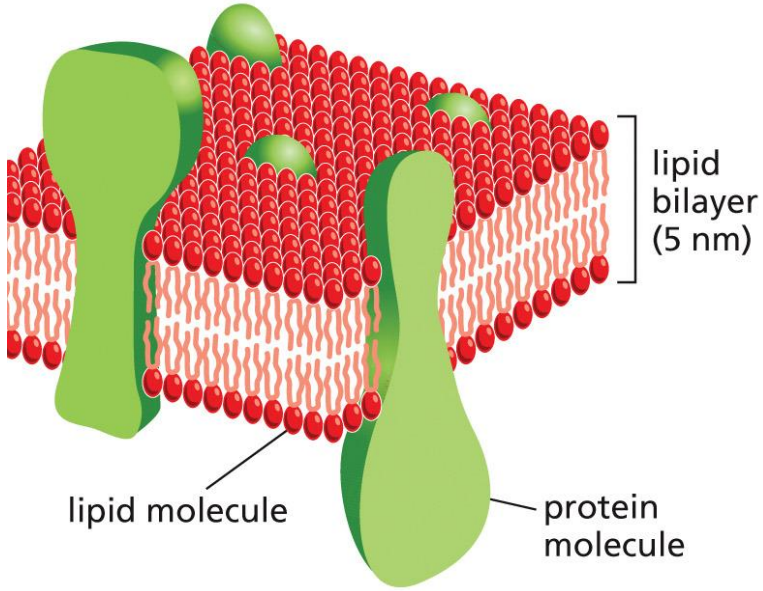



# *Recall:* Cell membranes are packed with phospholipids and embedded with proteins

- The different types of membrane lipids are all **amphipathic:**
  - Hydrophilic head (polar) and hydrophobic tails (fatty acids) lead to the formation of a bilayer in water



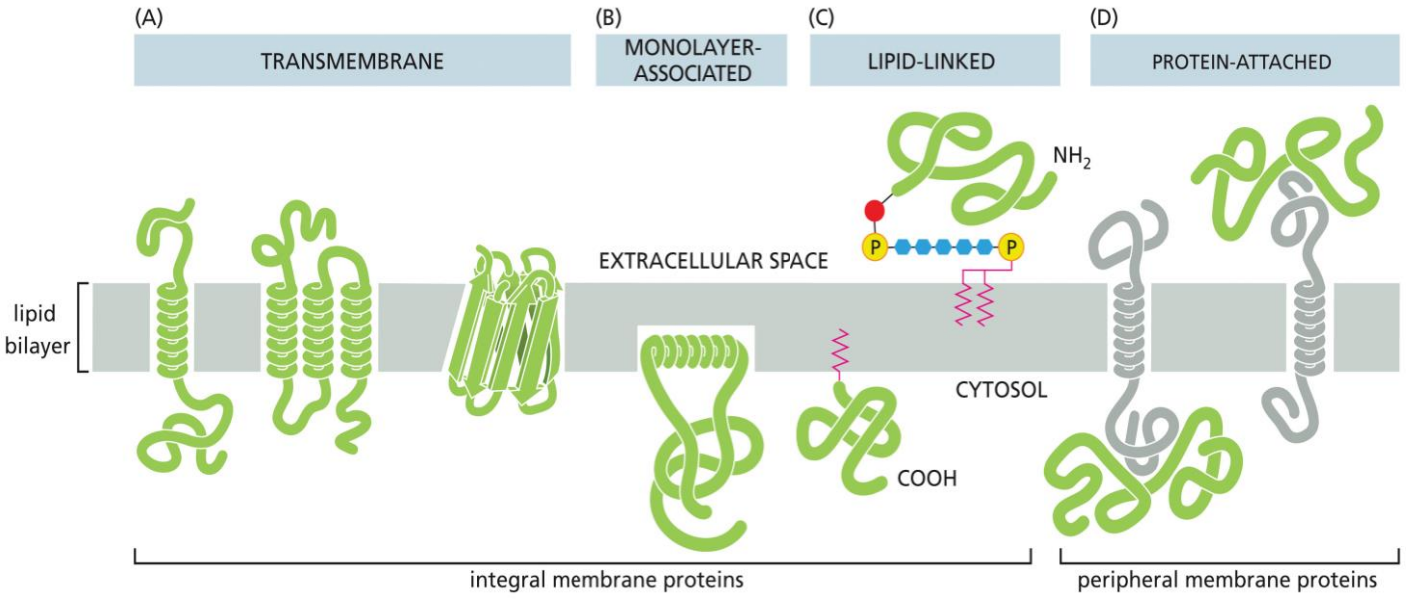



# Membrane proteins associate with the lipid bilayer in multiple ways

**Integral membrane proteins (embedded within the membrane):**

- Transmembrane proteins can extend across the bilayer as a single α-helix, as multiple α-helices, or as a group of rolled-up β-sheets (called a β-barrel)
- Others can be anchored to one half of the lipid bilayer by an amphipathic α-helix
- Another class of proteins can be linked to either side of the bilayer by a covalently attached lipid molecule that is embedded in the membrane




**Peripheral membrane proteins:**

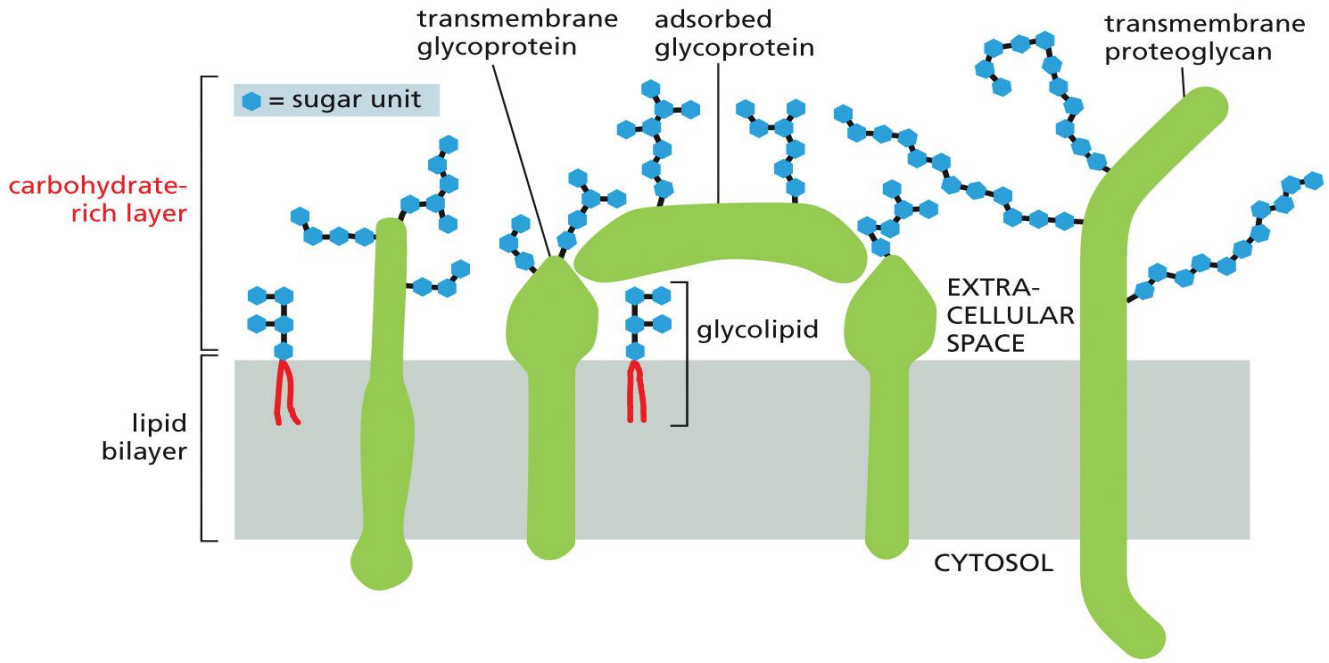
- Attached to the membrane only by relatively weak, noncovalent interactions with other membrane proteins

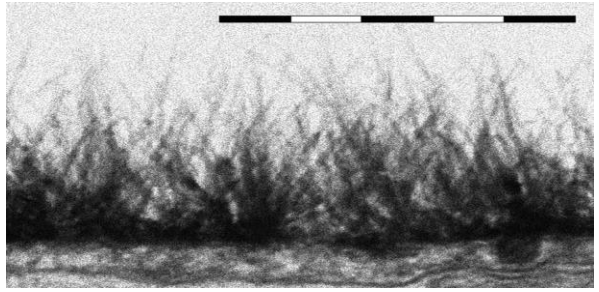
# The cell surface is also coated with sugars

Eukaryotic cells are coated with sugars in a coat called the *glycocalyx* made up of:

- Oligosaccharides (short sugar chains) and polysaccharides (long sugar chains) on membrane proteins
- This meshwork provides cells distinctive patterns important for recognition

Note that all the carbohydrate is on the cell exterior (non-cytosolic) layer of the plasma membrane



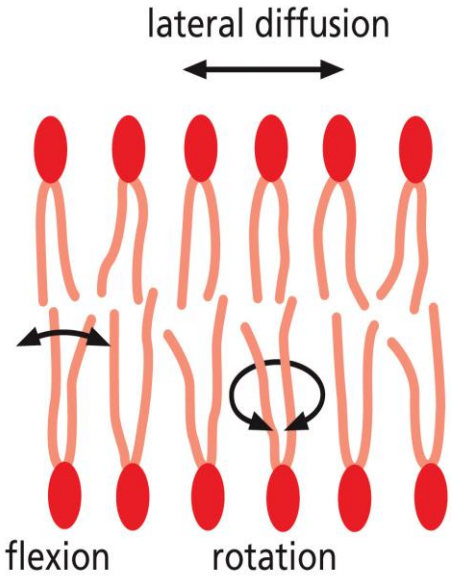



TEM micrograph of endothelial cell glycocalyx

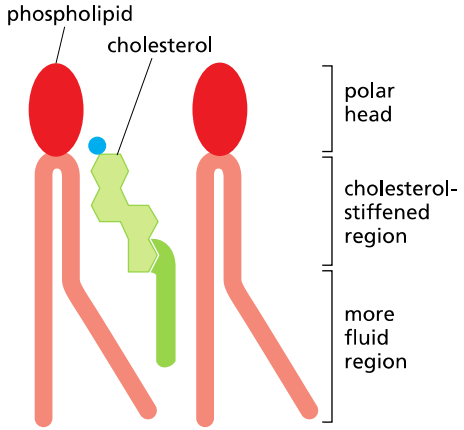
van den Berg B.M. et al. Circulation Research. 2003.

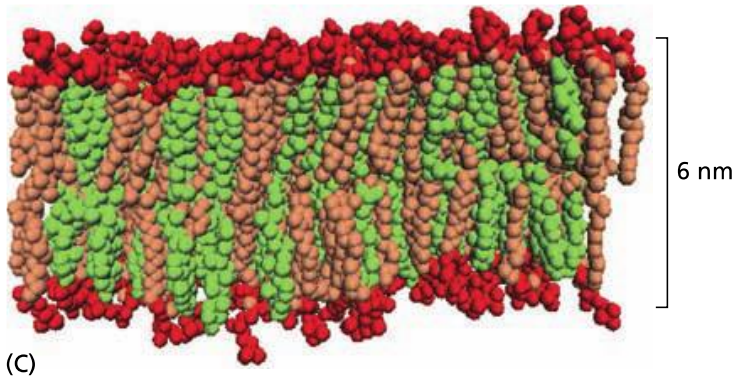
# The lipid composition of a bilayer influences its fluidity

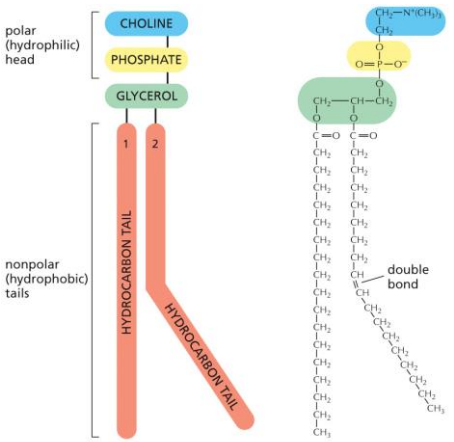
- The hydrocarbon *tail length* and number of *double bonds* affect fluidity
  - Shorter tails increase membrane fluidity (less to move around)
  - Double bonds in the hydrocarbon tails create a kink, due to planar geometry, making it harder to pack tightly for more fluidity (more impactful than tail length)
  - Saturated hydrocarbon tails without double bonds pack more tightly for less fluidity




- Cholesterol (constitutes 20% of membrane lipids) stiffens cell membranes to help maintain optimal membrane fluidity across a range of temperatures
  - Fill in the spaces in the kinks formed by unsaturated hydrocarbon tails



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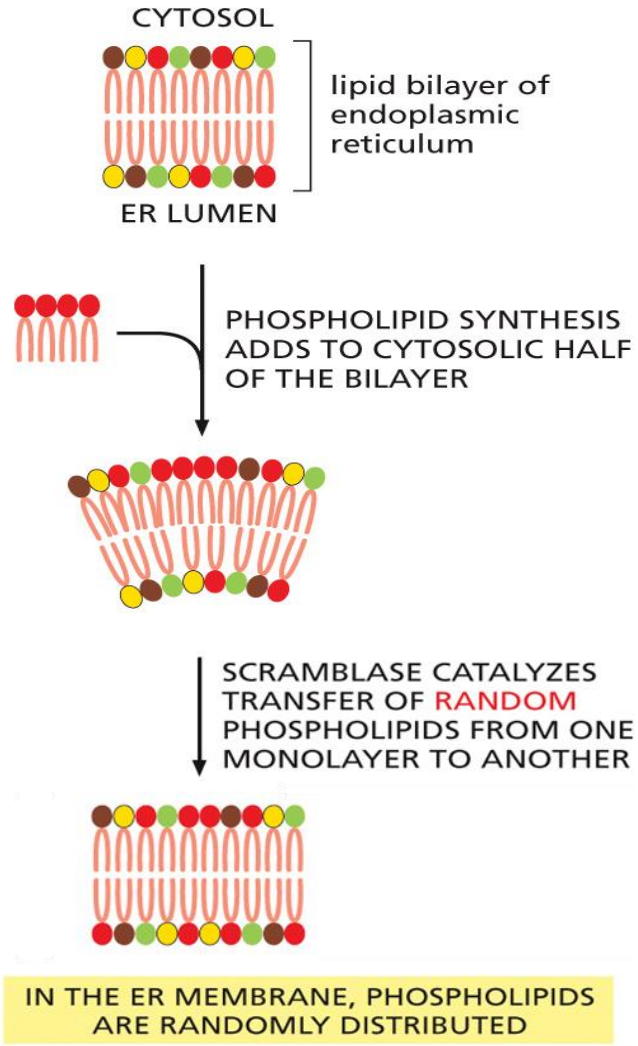
# Membrane assembly and growth begins in the endoplasmic reticulum (ER)

- Newly synthesized phospholipids are added to the *cytosolic side* of the ER membrane and then redistributed by transporters that move them from one half of the lipid bilayer to the other side known as the **lumen**, or the internal compartment of a membrane-enclosed organelle

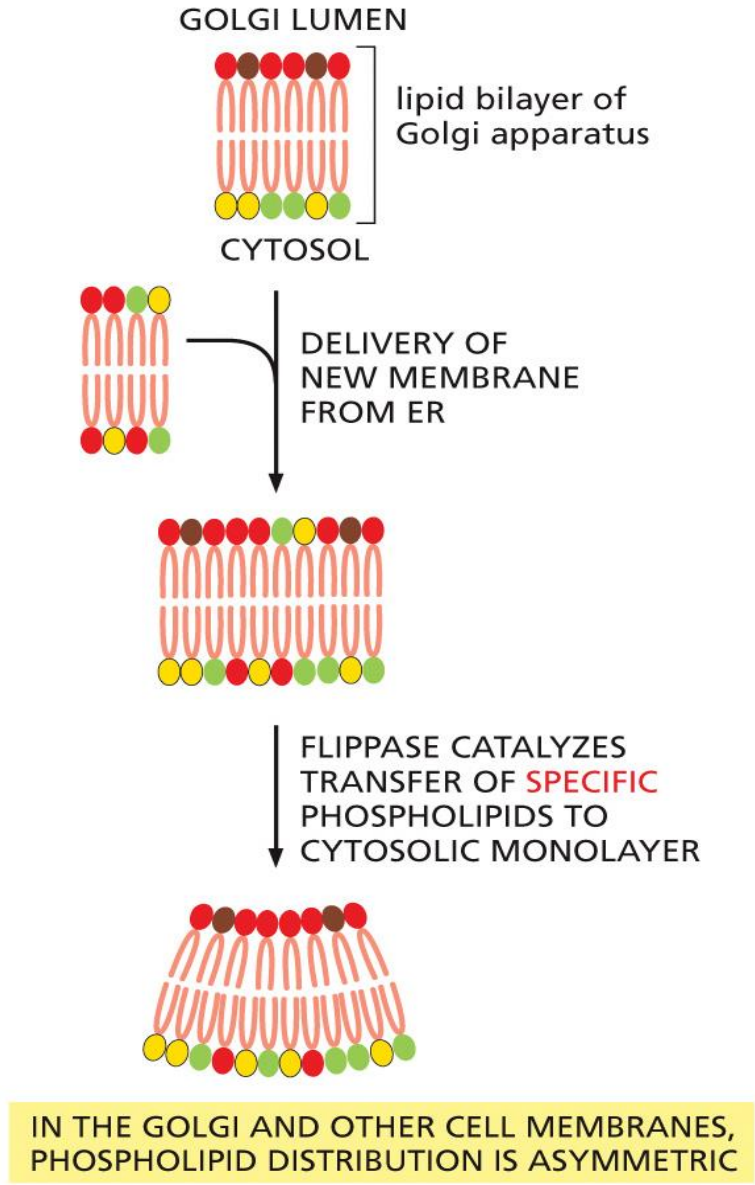
- Transporters called ***scramblases*** then randomly transfer phospholipid molecules from one monolayer to the other



# Selective distribution of phospholipids is achieved in the Golgi

- To move to the next component of the endomembrane system, membranes leave the ER and are incorporated into the Golgi apparatus

- Here transporters called ***flippases*** selectively move specific phospholipids from the *non-cytosolic monolayer* (red and brown) to the *cytosolic monolayer* (green and yellow)
    - This process leaves certain phospholipids concentrated in each layer of the membrane, giving membranes distinct 'inside' and 'outside' faces

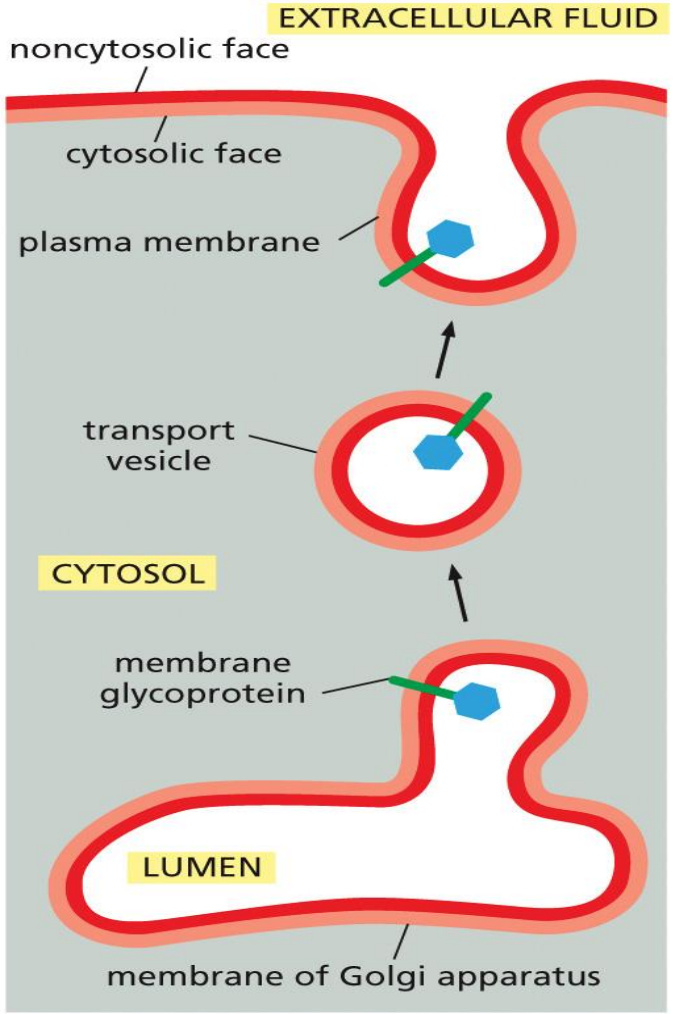


# Membranes retain their cytosolic/non-cytosolic orientation during transfer between cell compartments

- Membranes are transported in the cell by a process of vesicle budding and fusion

- The orientation of membrane lipids and proteins are preserved during this process
  - The original cytosolic surface (pink) of the lipid bilayer always remains facing the cytosol
  - The non-cytosolic surface (red) continues to face away from the cytosol, toward the lumen of Golgi/transport vesicle/extracellular fluid

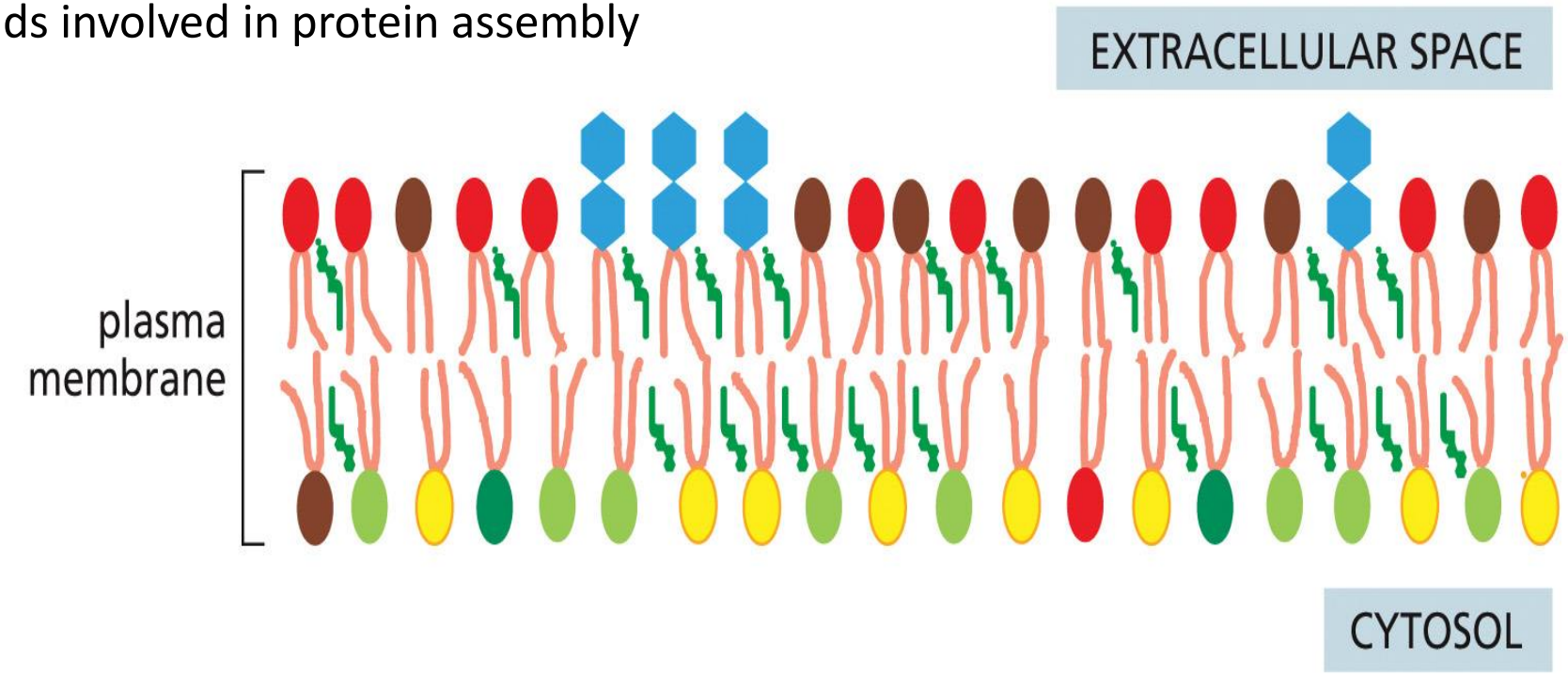
- Sugars are also added by glycosyltransferases within the Golgi lumen to proteins and lipids which will ultimately face the exterior of the cell (glycocalyx)



# End result: Phospholipids and glycolipids are distributed asymmetrically in the lipid bilayer of the plasma membrane

**Non-cytosolic monolayer**
Cell recognition markers and lipids involved in protein assembly

**Both monolayers**
Cholesterol distributed relatively equally




**Cytosolic monolayer**
Lipids and proteins with important roles in cell signaling

# Squarecap #1-2

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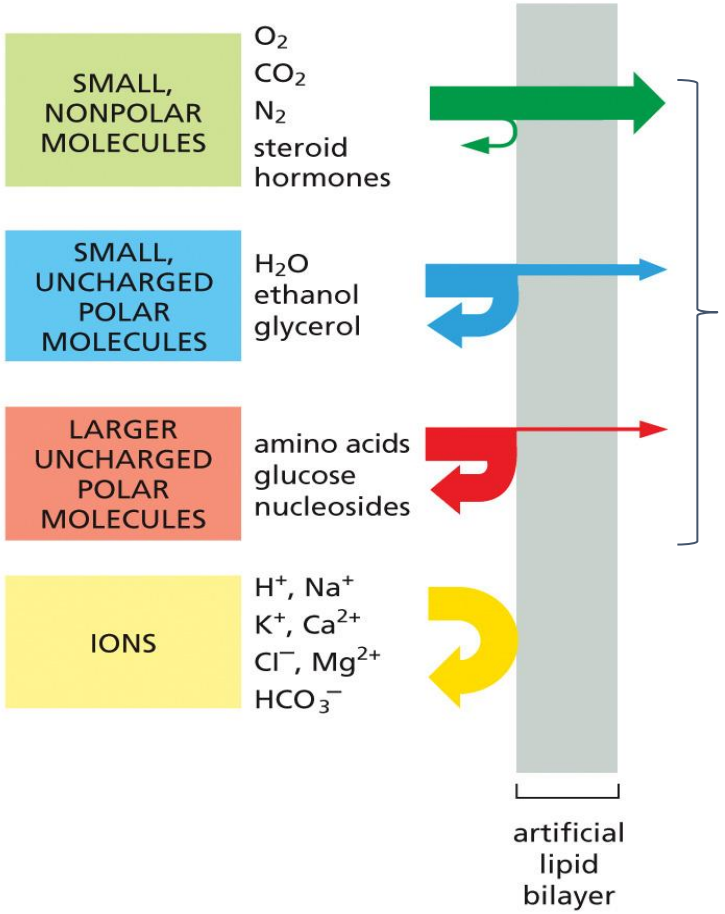
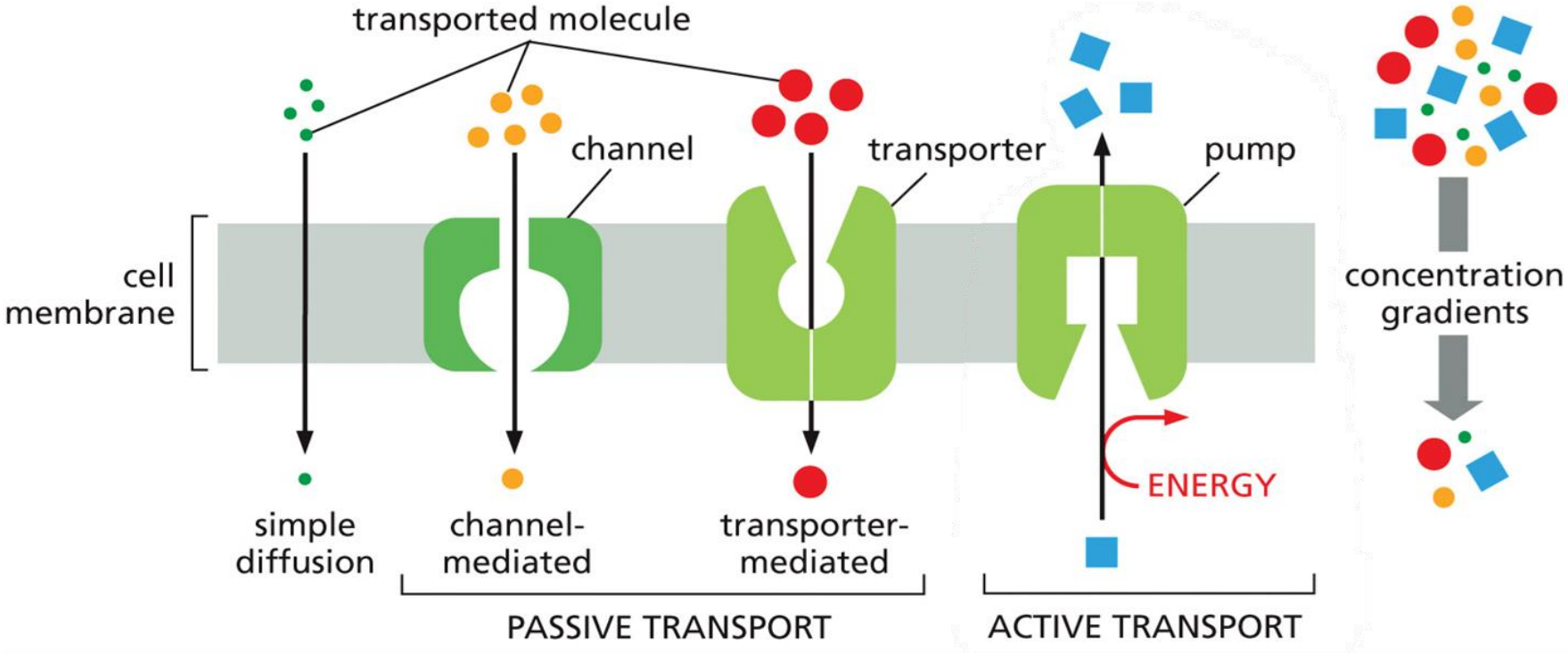
# The permeability barrier to transporting solutes across membranes




**Simple diffusion:** molecules cross the membranes without the help of proteins. The smaller and more hydrophobic the molecule, the more rapidly it will diffuse across bilayer

Lipid bilayers are impermeable to ions, and *most* uncharged polar molecules

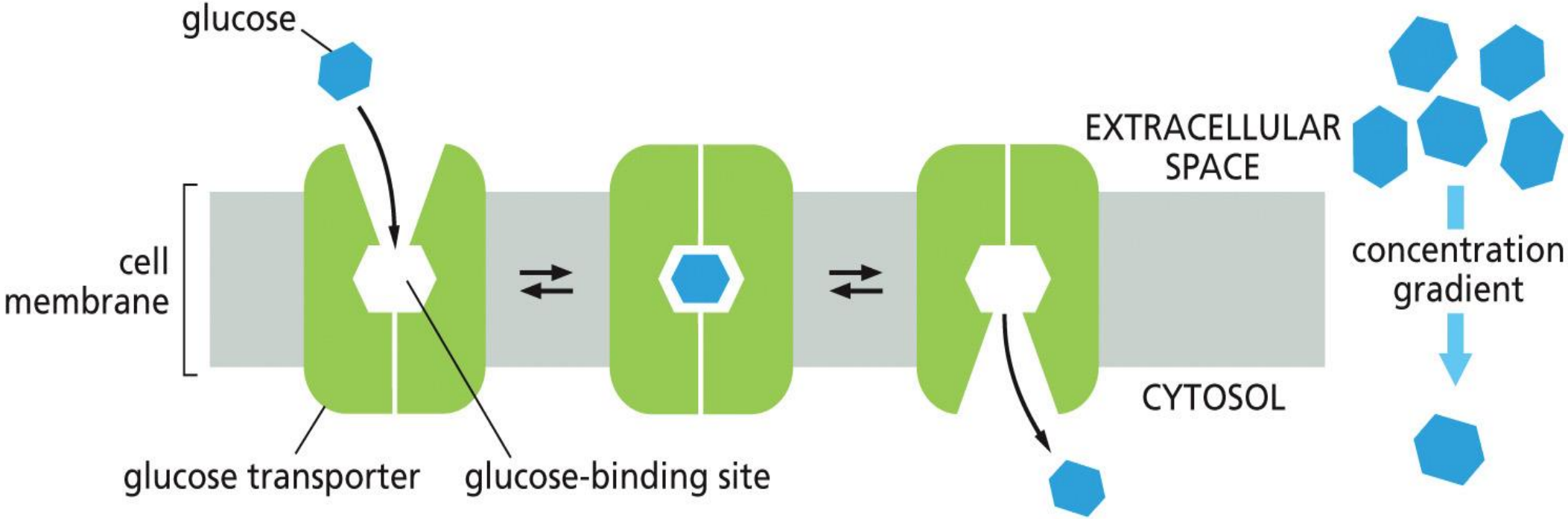
# Solutes cross the cell membrane by *passive* or *active* transport



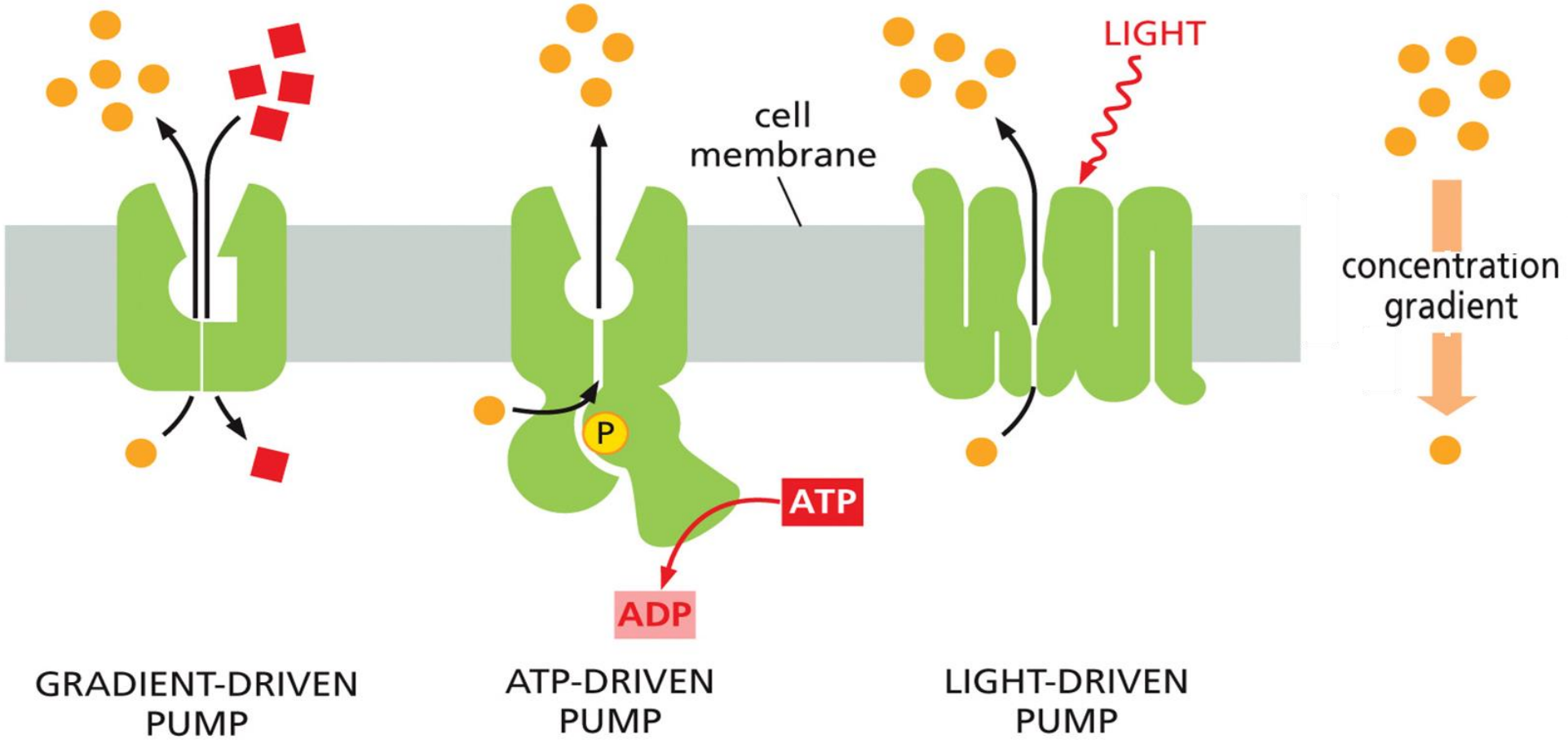

- **Passive transport** uses the *concentration gradient* to drive the passive flow of solutes from the side with the highest concentration to the side with the lowest concentration

- **Active transport** requires energy input to power a pump to move a solute *against* its concentration gradient

# Passive transporters move solutes along their concentration gradient

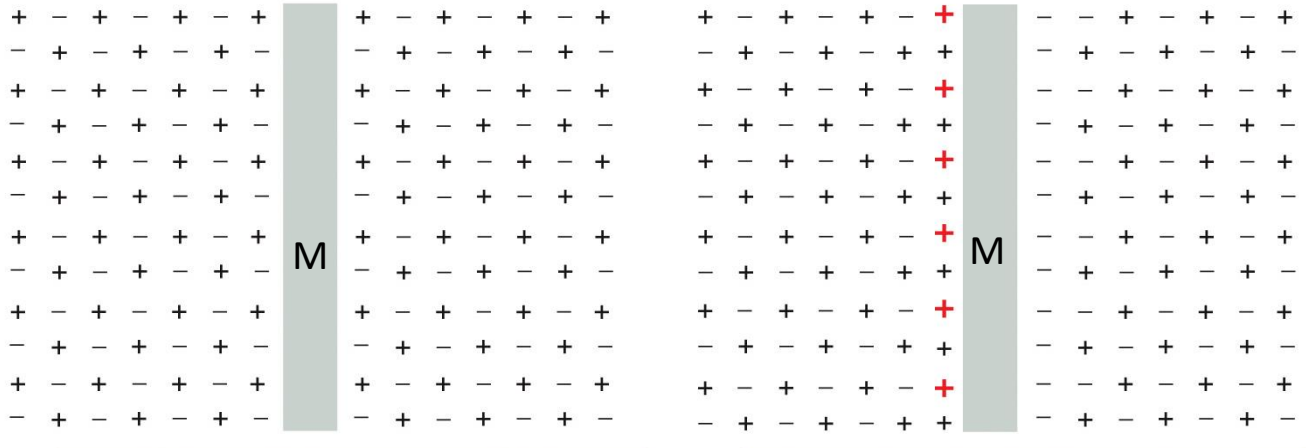


# Pumps actively transport solutes against their concentration gradient using energy



# The *membrane potential* also influences passive transport of charged solutes

**M** = Membrane




(A) exact balance of charges on each side of the membrane: membrane potential = 0

(B) a few positive ions (*red*) cross the membrane from right to left, setting up a nonzero membrane potential

charge balanced = no membrane potential

charge not balanced = membrane potential

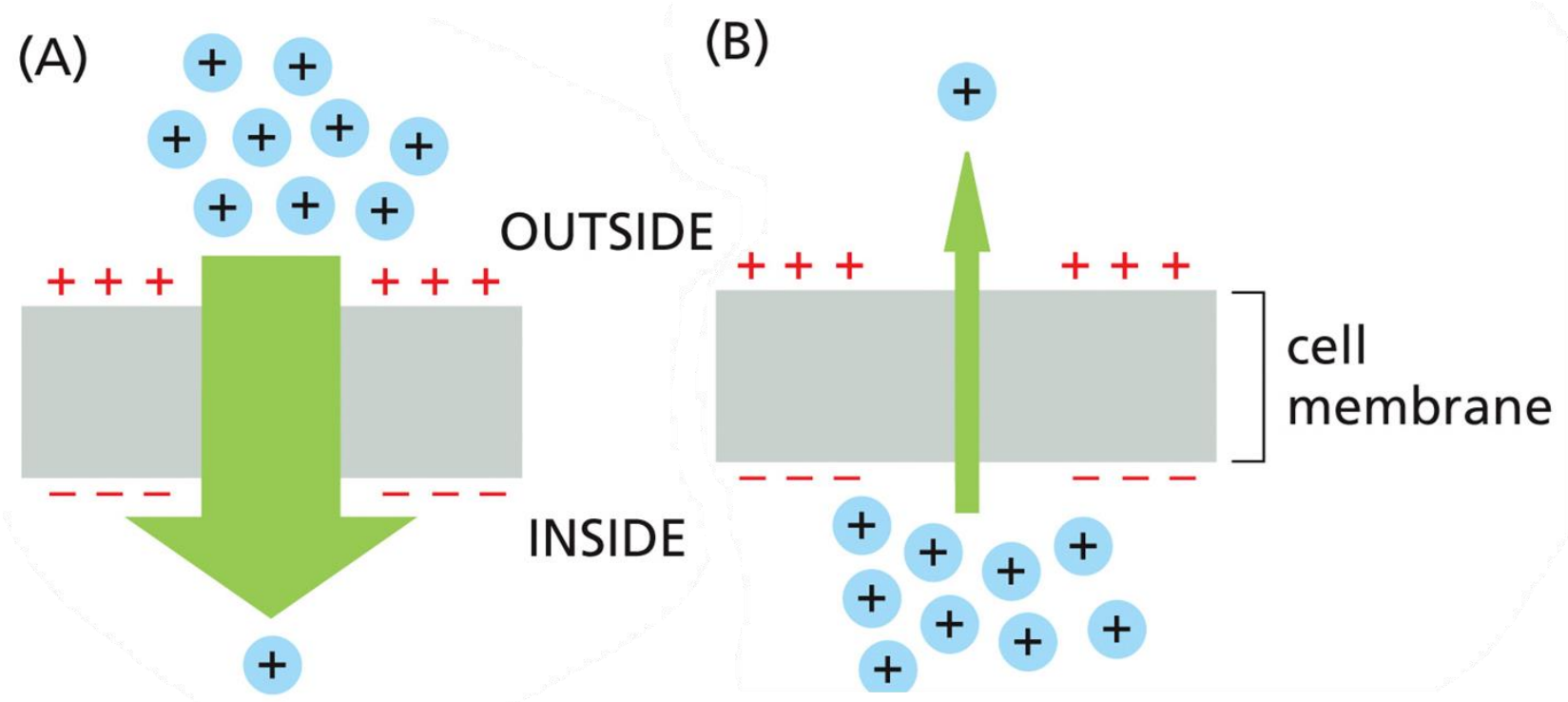
- Unequal distribution of charged ions on either side of a cell membrane gives rise to the "*membrane potential*," or the difference in voltage on one side of the membrane versus the other
- In general, the outside of the cell is positive (high $Na^+$) and the inside of the cell is negative (high $Cl^-$) so cells tend to draw in positively charged and expel negatively charged molecules
  - The overall membrane potential of a cell at rest is negative

# The *electrochemical gradient* for a solute is the *net sum* of the *concentration gradient* and the *membrane potential*

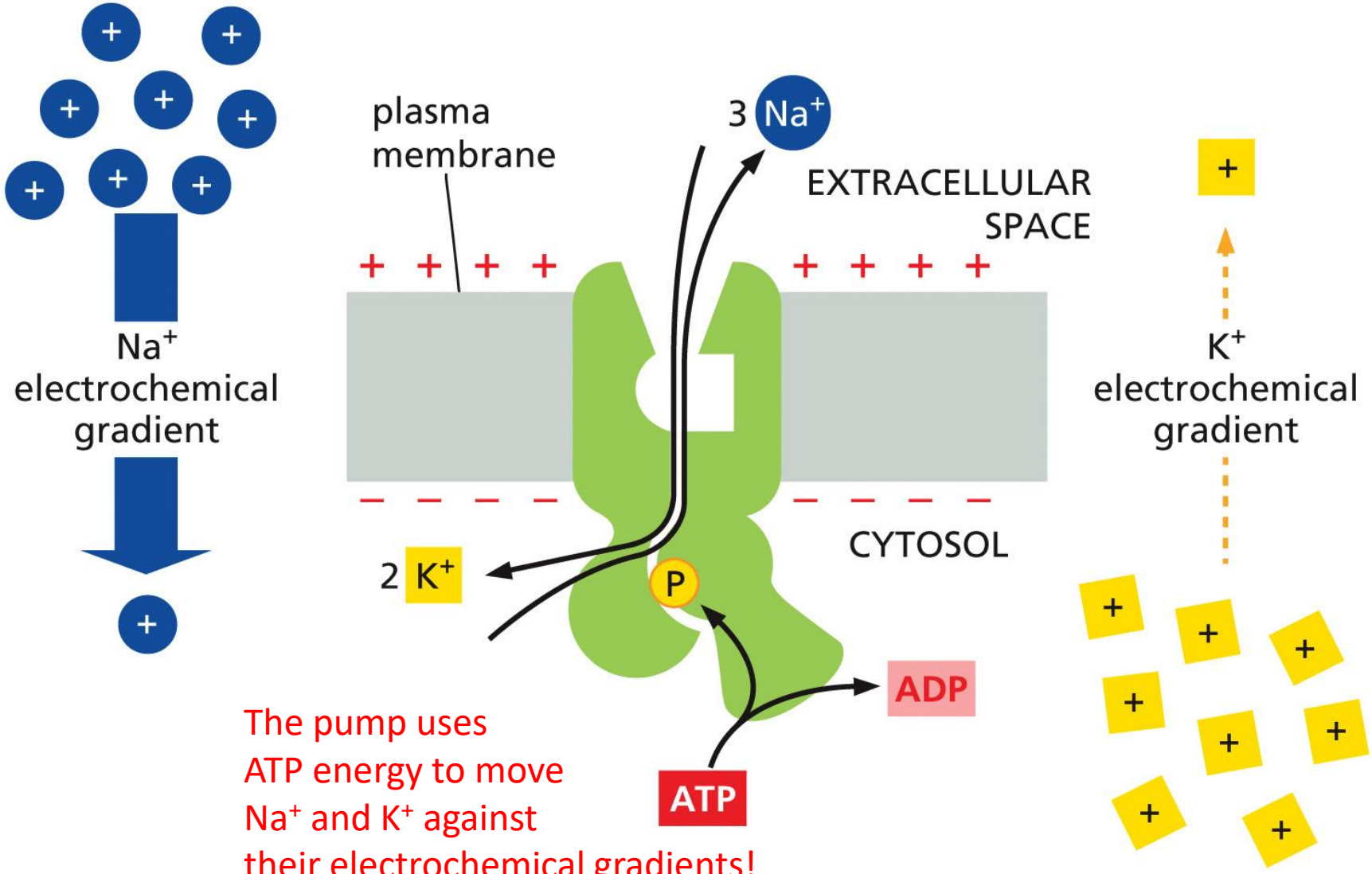
**The concentration gradient** & the **membrane potential** can work in the **SAME** direction

**The concentration gradient** & the **membrane potential** can also work in the **OPPOSITE**

→ = direction and magnitude (width of arrow) of the electrochemical gradient



# Example: The $Na^+/K^+$ pump uses ATP to power $3Na^+$ out of the cell and $2K^+$ into the cell




The pump uses ATP energy to move $Na^+$ and $K^+$ against their electrochemical gradients!

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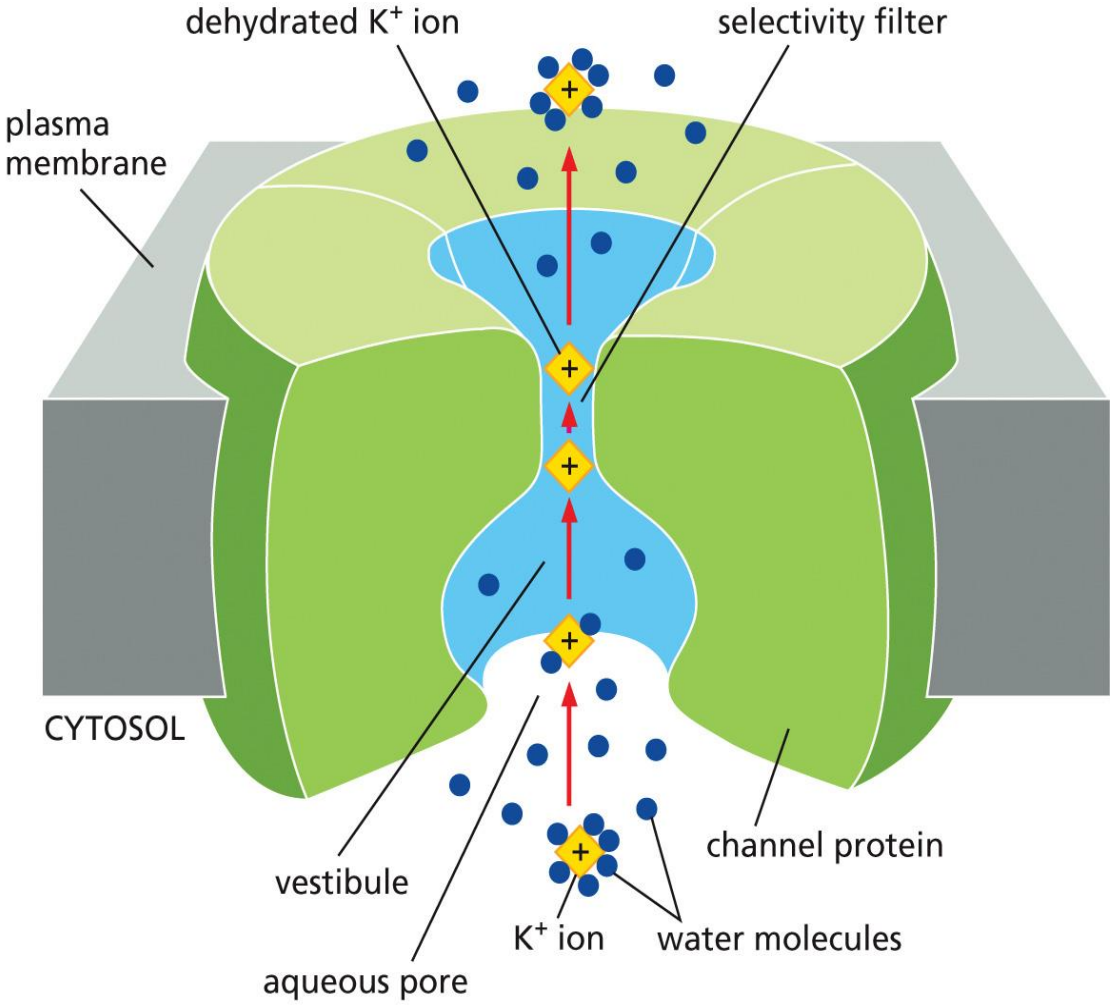
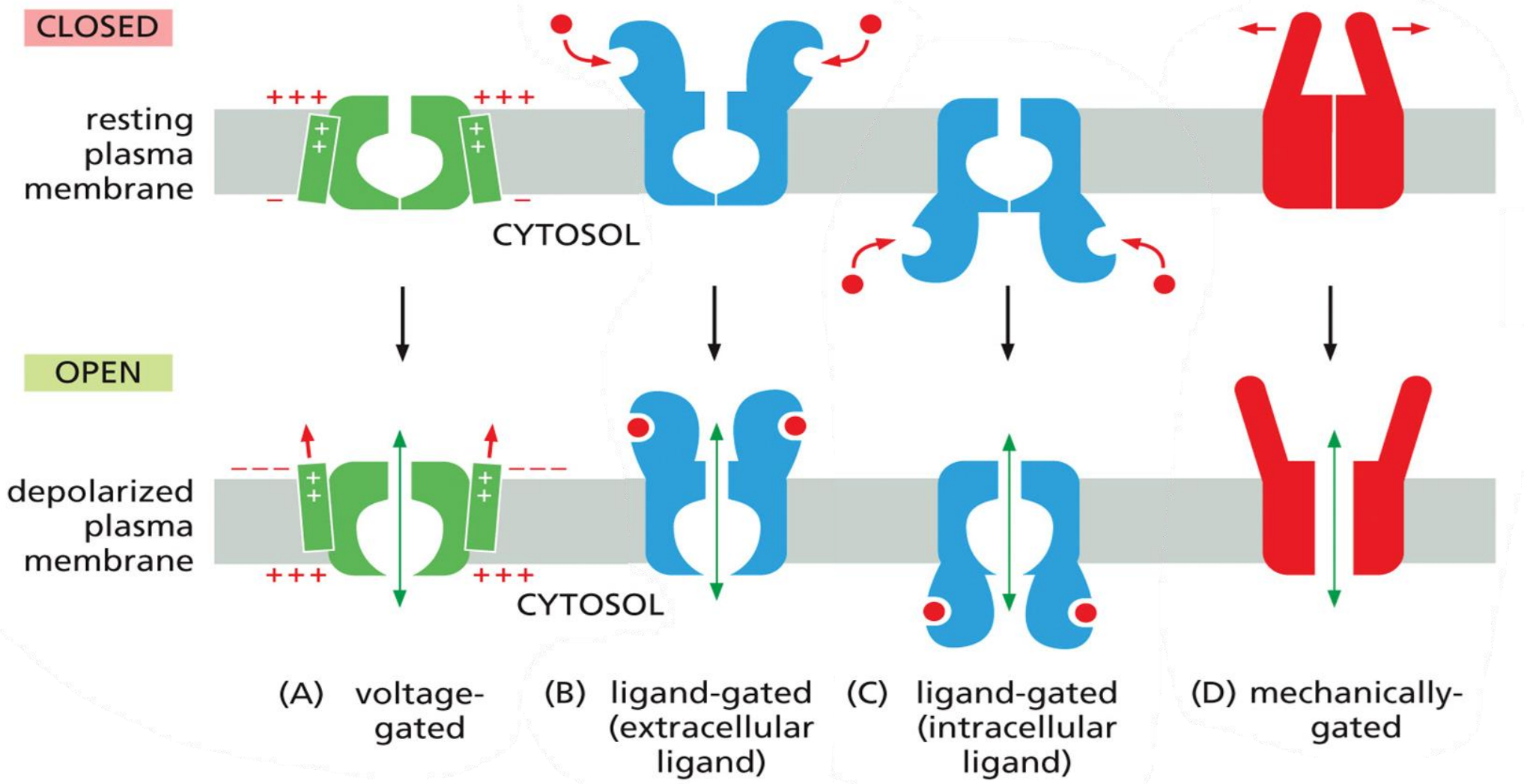
# Ion channels are highly selective, narrow pores for allowing passage of one type of charged ion through the membrane while excluding others

- **Example:** $K^+$ channels have polar amino acid side-chains that create a selectivity filter for dehydrated $K^+$ ions based on size and charge

- When channels are open, ions flow rapidly through, down their electrochemical gradients



# Most ion channels are gated so that different stimuli regulate their opening

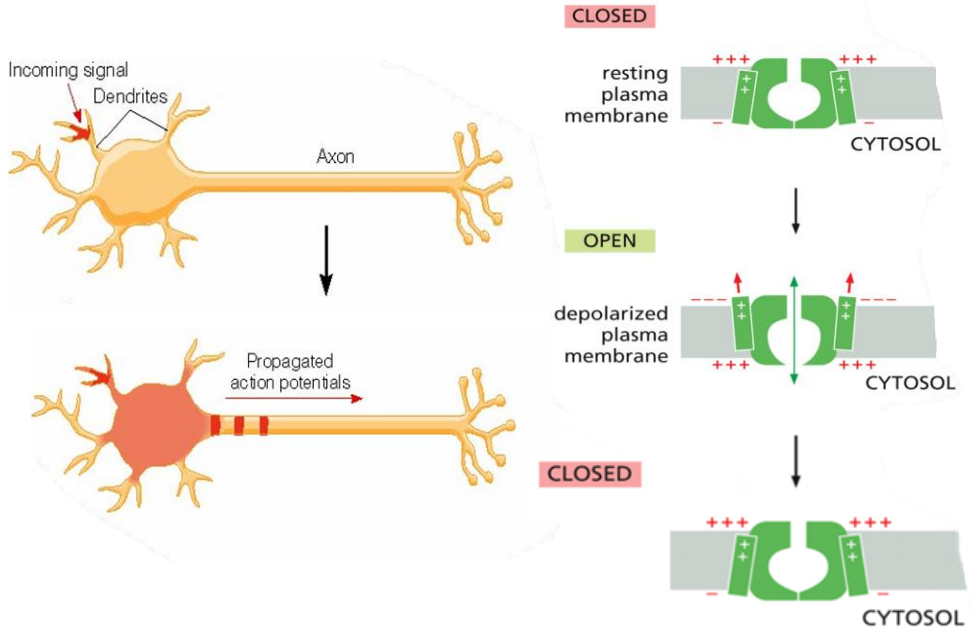


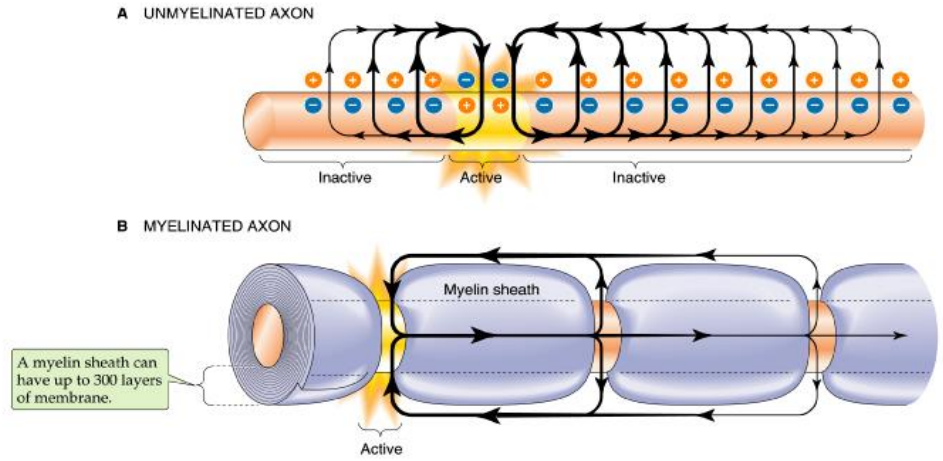
# Action potentials are mediated by voltage-gated ion channels

- **Example:** Axon signal propagation in neurons

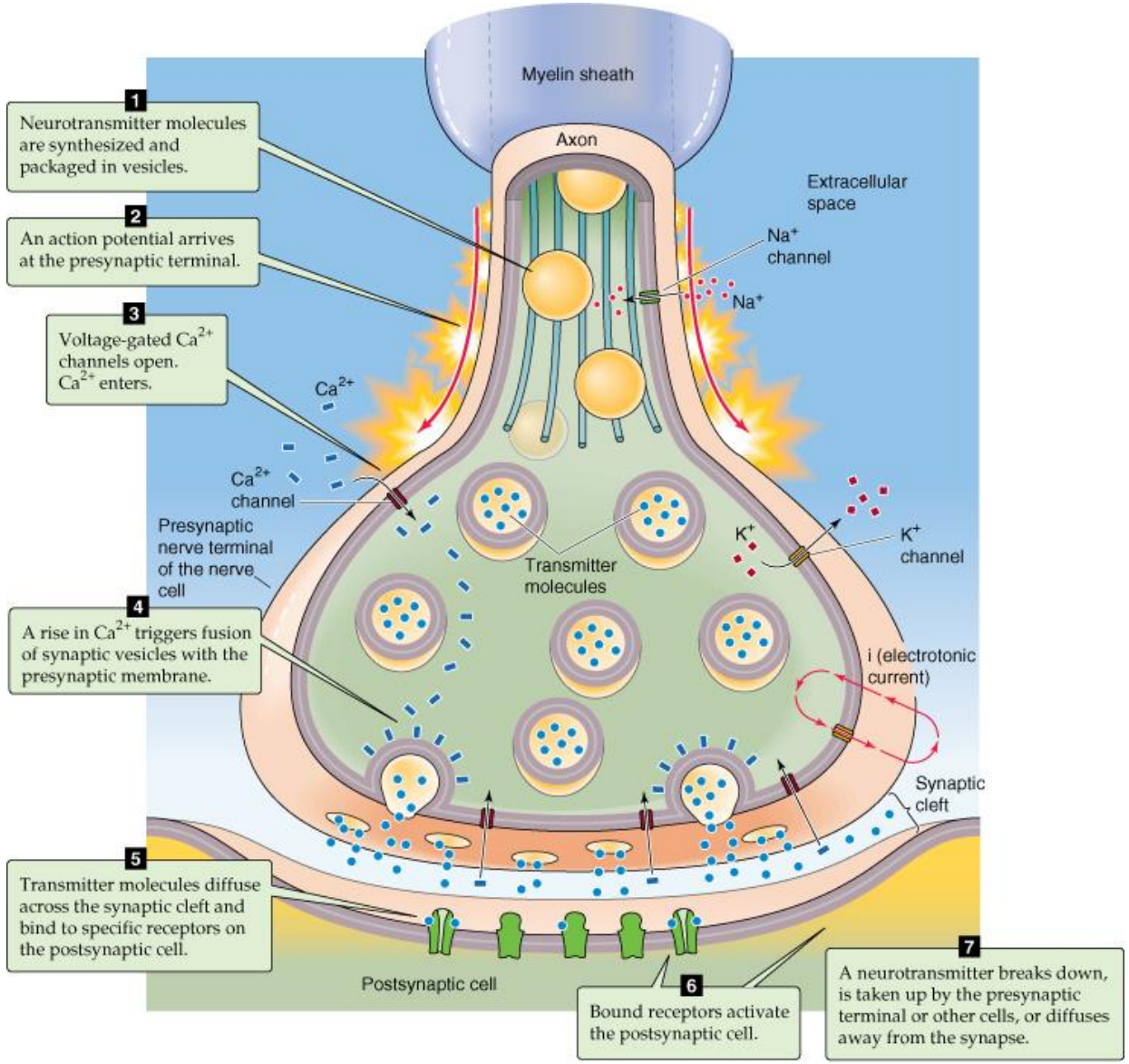
- A stimulus *depolarizes* (raises the membrane potential) the axon's plasma membrane and voltage sensors twist and alter conformation
- Voltage-gated $Na^+$ channels open and $Na^+$ enters the cell down its steep electrochemical gradient
  - This triggers what is known as an *action potential* that further depolarizes the axon's plasma membrane
- Within milliseconds, voltage-gated $Na^+$ channels inactivate/close, and they will not open again during a *refractory period*, allowing for unidirectional signal propagation

- Neurons are insulated in a myelin sheath (many layers of cell membrane) that isolates the leakage of ions to specific sites to continue signal propagation without lowering intensity



# At the synapse (axon terminal) the arrival of the action potential triggers release of neurotransmitters to signal to a neighboring cell





Neurotransmitters are chemical signals in synaptic vesicles that are released from the axon terminal to bind to a receptor on a neighboring neuron

Electrical signals are converted to chemical signals at the axon terminal of neurons and back into electrical signal in postsynaptic cell (basis for neural networks, neuromuscular junctions)

# Squarecap #3-5

# Thank You!

- This was my first time teaching a large lecture course, so I will always remember you as my first group of students. I hope you enjoyed the course and found my instruction valuable.

- It would be a great help to me if you would take the time to fill out a detailed feedback survey next week on my instruction:
  - I will send out an announcement on Canvas next week with the link to the survey.
  - If you complete the survey within one week, I will award you **10 points of extra credit** in the exam category (the one with the most weight) as a thank you.
  - Please be honest in your feedback, I will separately be sent the names of those who completed the survey for assigning extra credit and the anonymized feedback from the survey.
  - If you feel inclined to create a Rate My Professor entry for me, this would also be helpful for me as I apply for faculty positions and would be appreciated.

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# Reflection/Feedback

https://forms.gle/vbdZXee89Dh9diY78