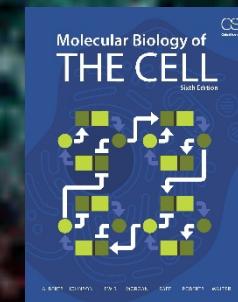


Transport of ions and small molecules across membranes

- Lecture 5 -

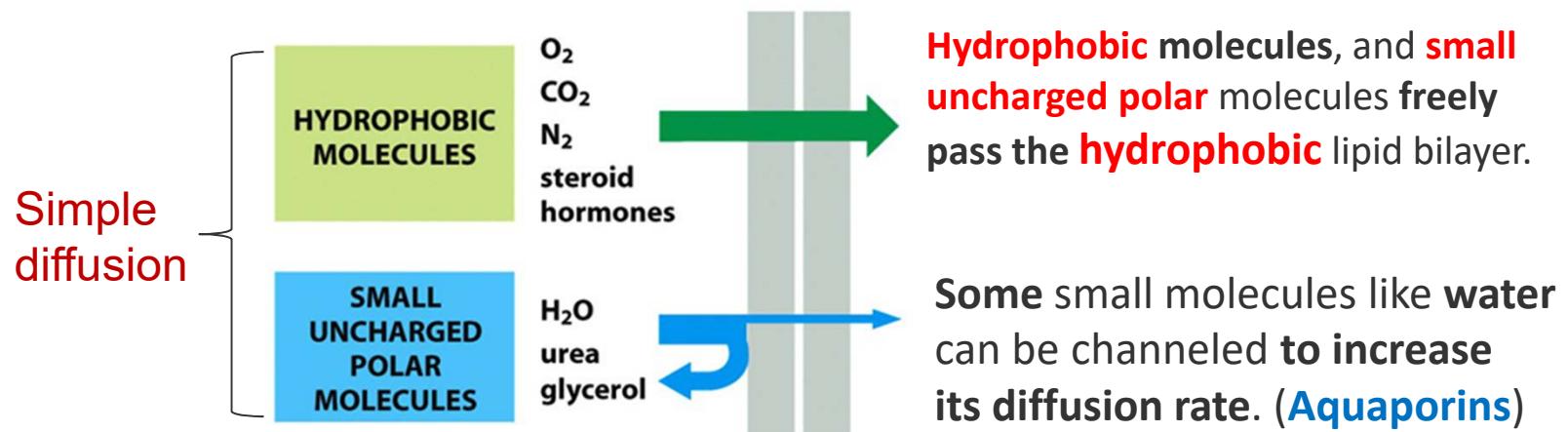
- I. Overview
- II. Channels
- III. Transporters
- IV. ATP-pumps
- V. Membrane potential



Chapter 11

Some molecules can simply cross the lipid bilayer by diffusion...

Diffusion is not transport *strictus sensus*



Hydrophobic molecules, and small uncharged polar molecules freely pass the hydrophobic lipid bilayer.

Some small molecules like water can be channeled to increase its diffusion rate. (Aquaporins)

- Facts:
- Diffusion is **passive** and **not facilitated** by other membrane proteins
- Relatively **slow** process
- Does **not** consume energy
- Movement **only** “down the concentration gradient”
(It is **not possible to concentrate molecules!!!**)
- Applies to **gases & small uncharged molecules.** (O₂, CO₂, N₂, urea, EtOH)
- **Diffusion rate** depends on: **concentration, hydrophobicity & size**
 - The **smaller** the molecule is and the **less strongly it interacts with water**, the **faster** is the permeation!

Permeability of substances

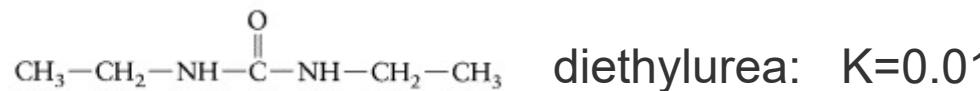
Question:

What will diffuse/permeate membranes more rapidly
diethylurea or urea?

Partition coefficient

(the ratio of concentration of a substance between oil and water)

for:

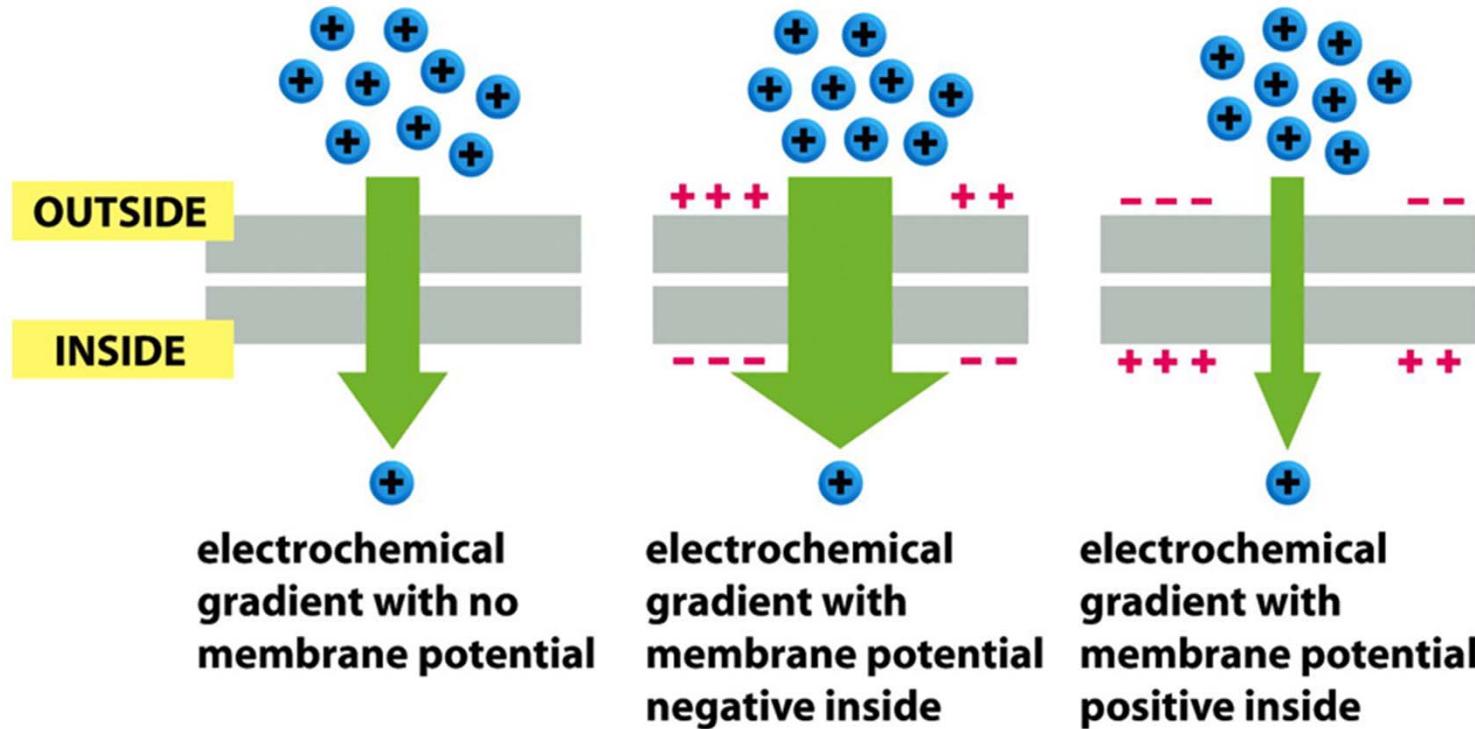


The higher a substance's partition coefficient, the more lipid-soluble it is

Diethylurea, which is 50 times ($0.01 \div 0.0002$) more hydrophobic than urea, will diffuse through phospholipid bilayer membranes about 50 times faster than urea. Diethylurea also enters cells about 50 times faster than urea.

Which of the two substances is more dangerous to handle in the laboratory?

Diffusion is influenced by the electrochemical gradient



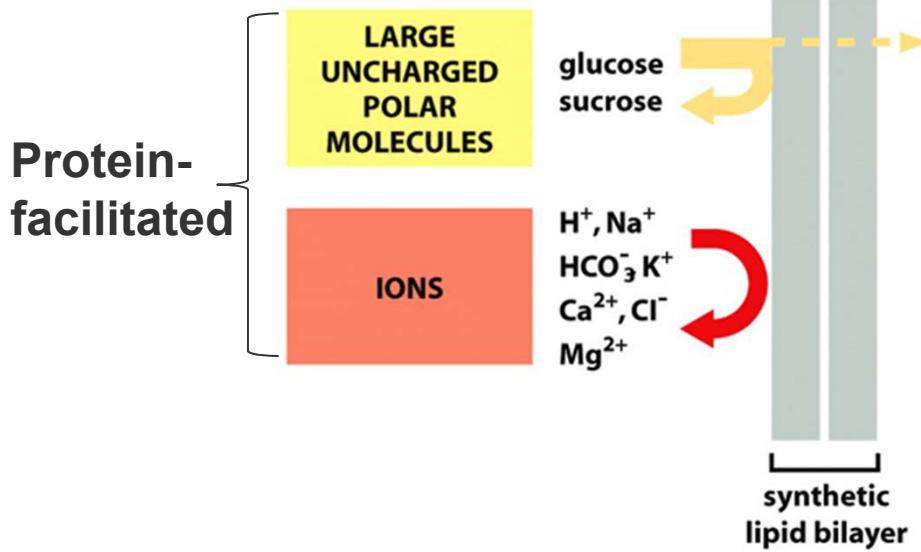
Electrochemical gradient consists of/combines concentration gradient and the electrical gradient

between the inner side and outer side of the membrane.

The electrochemical gradient combines membrane potential and concentration gradient!

... but others cannot cross the lipid bilayer by diffusion!

Ions cannot pass the lipid bilayer



Recorded ion concentrations

	intra-cellular [mM]	extra-cellular [mM]
Cations		
Na ⁺	5-15	145
K ⁺	140	5
Mg ²⁺	0.5	1-2
Ca ²⁺	10 ⁻⁴	1-2
H ⁺	7X10 ⁻⁵	4X10 ⁻⁵
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 (HCO₃⁻, PO₄³⁻, proteins, nucleic acids, metabolites carrying phosphate and carboxyl groups, etc.). The concentrations of Ca²⁺ and Mg²⁺ given are for the free ions. There is a total of about 20 mM Mg²⁺ and 1-2 mM Ca²⁺ in cells, but both are mostly bound to proteins and other substances and, for Ca²⁺, stored within various organelles.

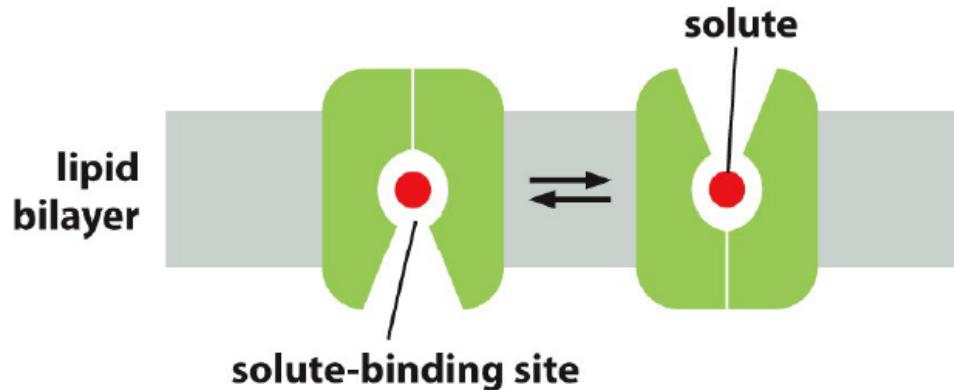
Inside the cells, higher K⁺, but lower Na⁺ and Cl⁻ as compared to outside of a cell!!!

Two major classes of membrane proteins facilitate transport of molecules across membranes: **channels & transporters**

A) Channel



B) Transporter

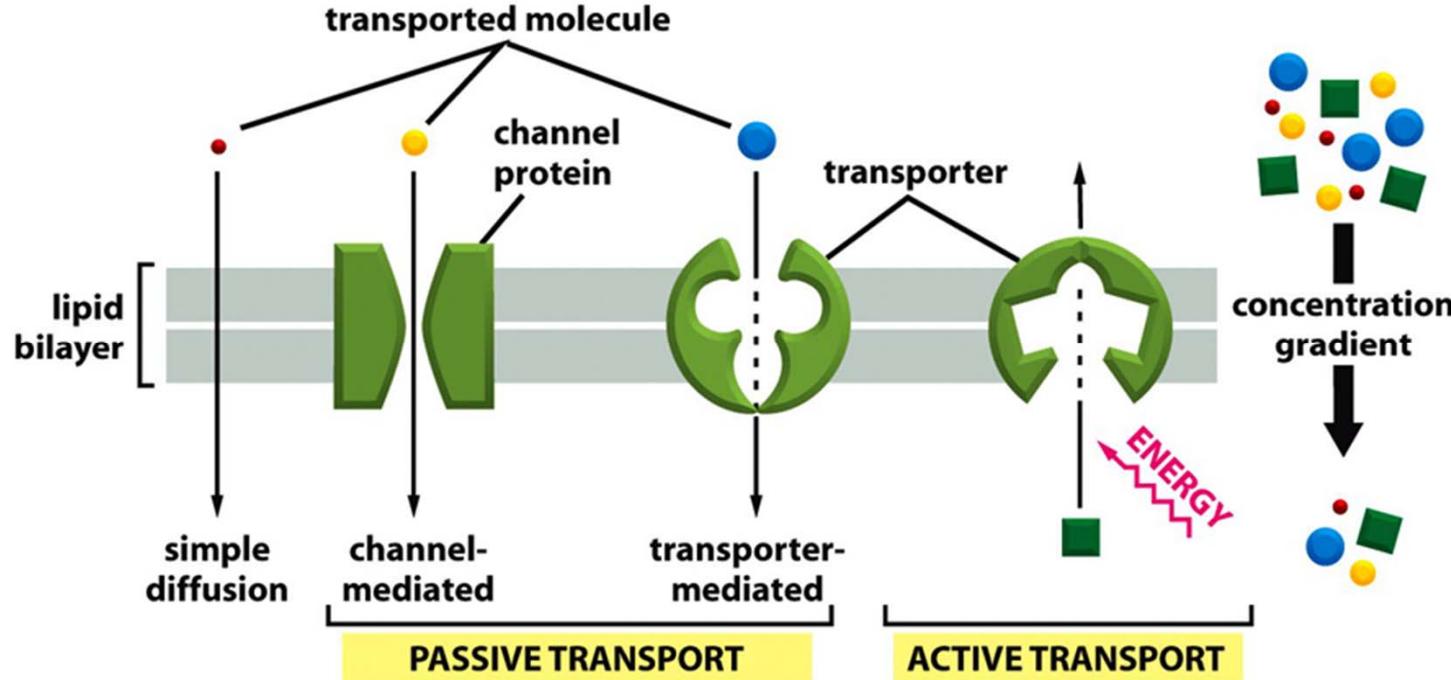


Channels
don't change conformation
during transport

Transporter
change conformation
during transport

Transporters alternate between conformations; channels are water-filled pores!!!

Three ways how membrane proteins can facilitate transport:

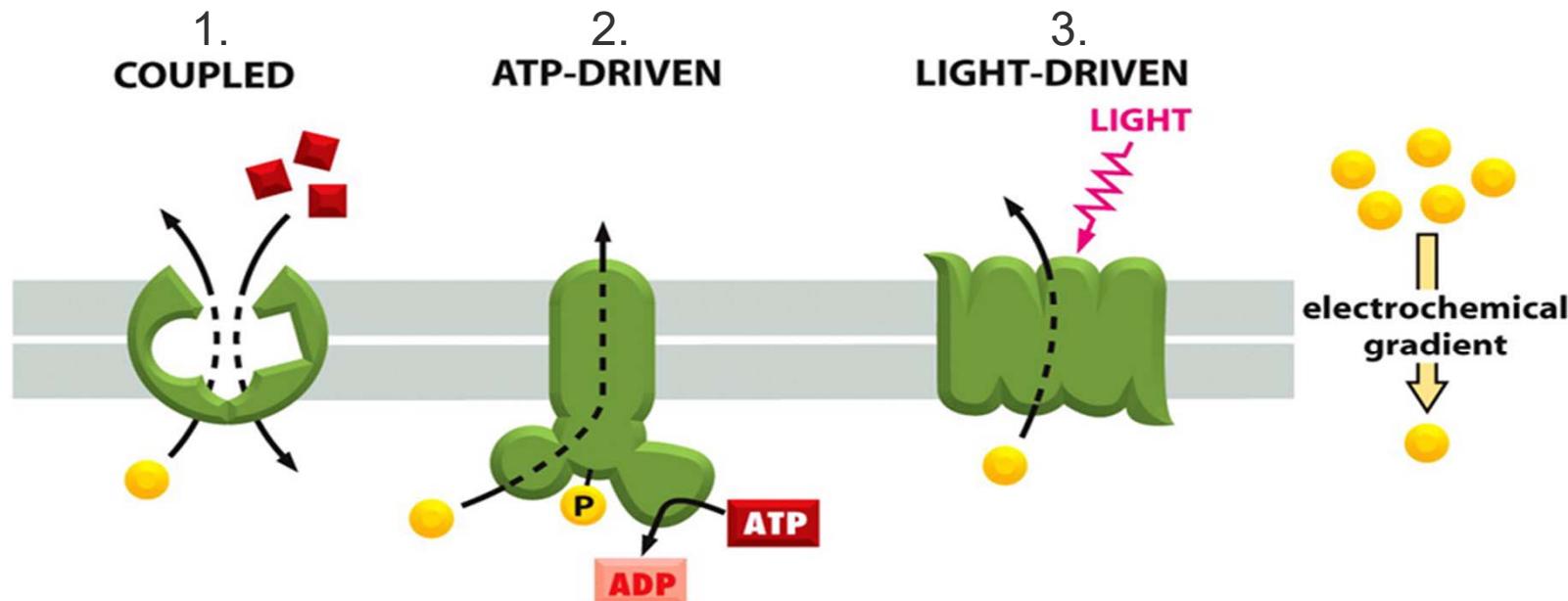


- **Passive transport (channels and transporters):**
 - solutes cross the membrane “**down**” the electrochemical gradient
- **Active transport (transporters only)**
 - solutes cross the membrane **against** the electrochemical gradient
 - needs always some sort of energy

Three different concepts to drive active transport:

Coupled transport, ATP-driven transport & light-driven transport

➤ Always: transport **against** an **electrochemical gradient**



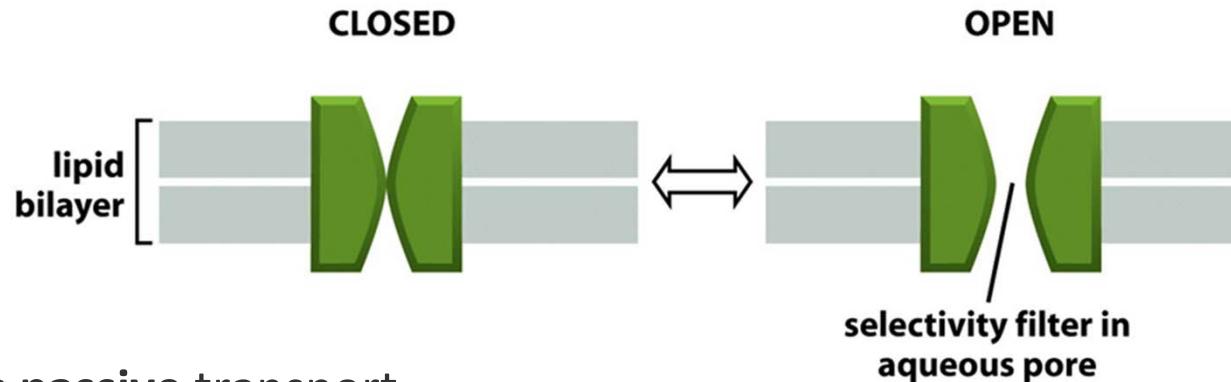
Coupled transporter:

Coupling of an energetically unfavorable transport to an energetically favorable transport to “drive” the transport/reaction

Active transport **always** requires some sort of energy...

Channels: super efficient proteins

Channels:

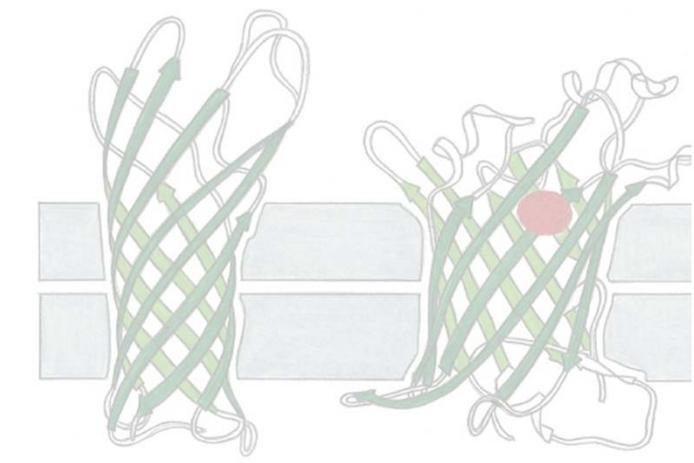


- always **passive** transport
- not coupled to energy consumption
- facilitate molecule movement **only “down”** the gradient
- molecule **selectivity**
- **low** binding affinity
- transport **capacity 10⁷ to 10⁸ ions per second (highly efficient)**

Channels can be regulated to “open” or to “close” (gating):

- **nongated** channels: open most of the time
- **gated** channels: open and close **in response** to signals

Different types of β -barrel proteins...

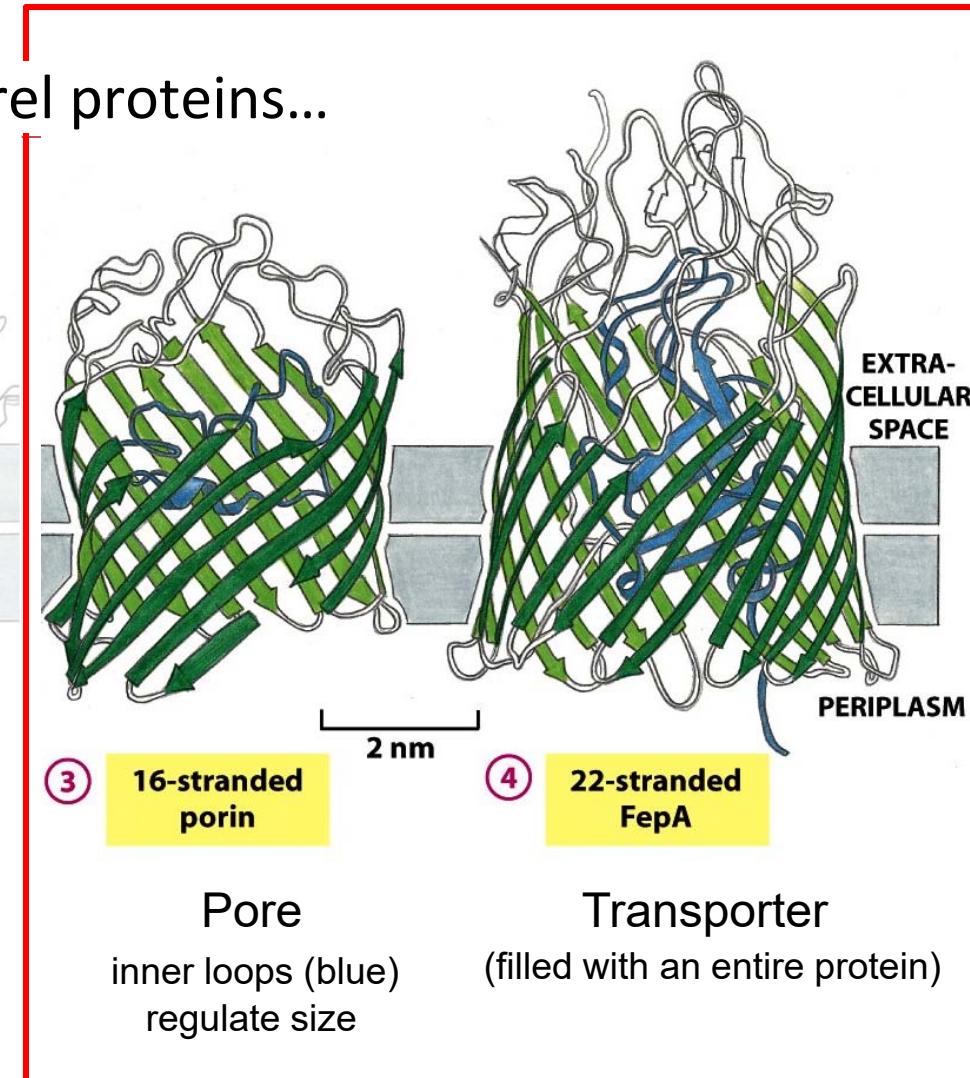


① 8-stranded
OmpA

Receptor

② 12-stranded
OMPLA

Lipase



③ 16-stranded
porin

Pore
inner loops (blue)
regulate size

④ 22-stranded
FepA

Transporter
(filled with an entire protein)

Some β -barrels form large Transmembrane Channels:

Found in: outer membrane of mitochondria and plastids

Number of β -sheets: 8-22

Repetition from lecture 4 !!!

Transmembrane channels: β -barrel proteins

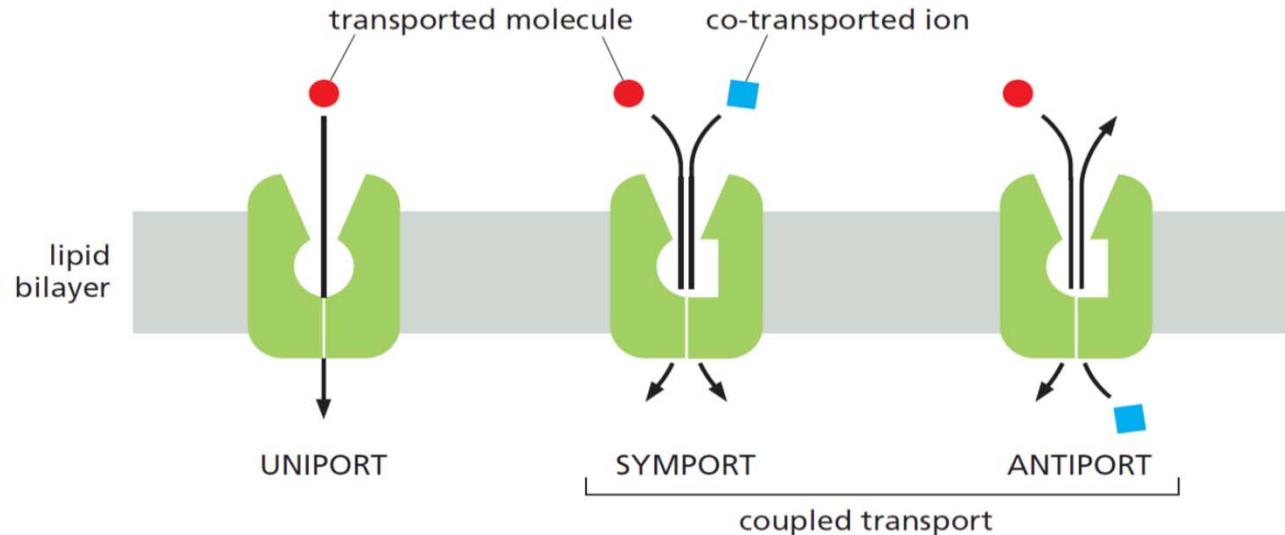
Features:

- Relatively **rigid** structures, **conformational changes** are **less likely** to occur
- Relatively “**easy**” to crystallize for structure determination
- **Abundant** in **outer membranes** of **mitochondria, chloroplast** and **bacteria**.
- **Inside barrel: polar amino acids**
- **Outside barrel: nonpolar amino acids**
- **Loops inside lumen** confer **selectivity**: **only selected molecules can pass**

Repetition from lecture 4 !!!

Transporters: great helpers, but nothing is for free...

Three main types: uniporter, symporter and antiporter



Uniport: transport a **single** type of molecule “**down**” its gradient.

Symport: couple the movement of one type of molecule **against** its gradient in the same direction

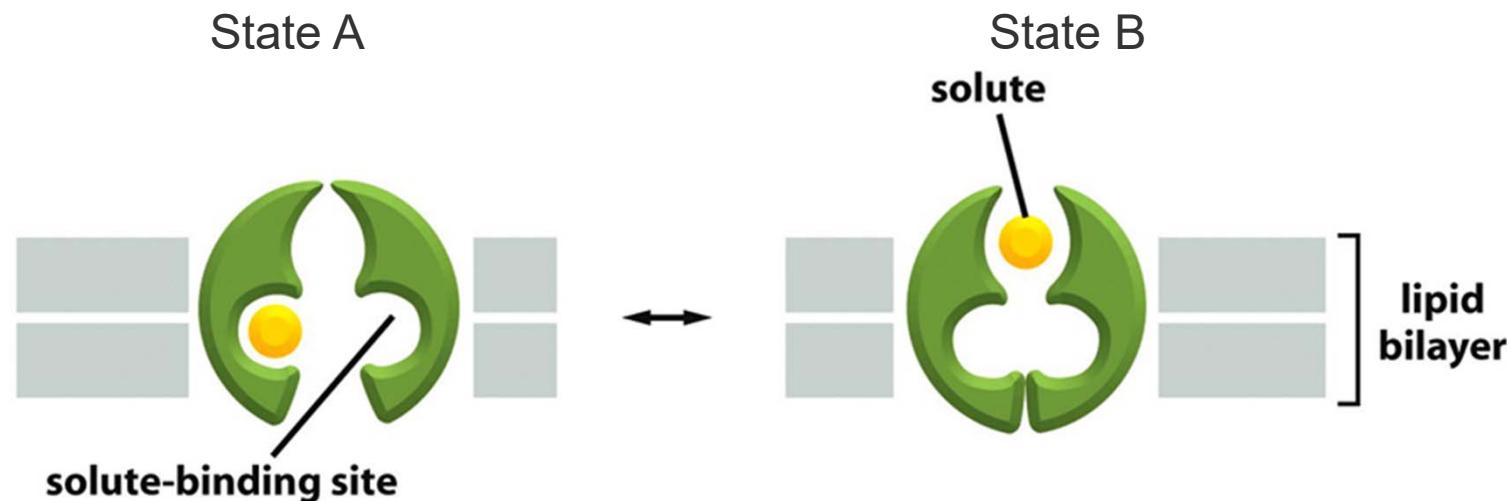
Antiport: couple the movement of one type of molecule **against** its gradient in the opposite direction

- Undergo conformational changes
- Bind molecules more tightly
- Transport molecules at a rate of 100-10,000 molecules per second

Transporters undergo conformational changes

Features:

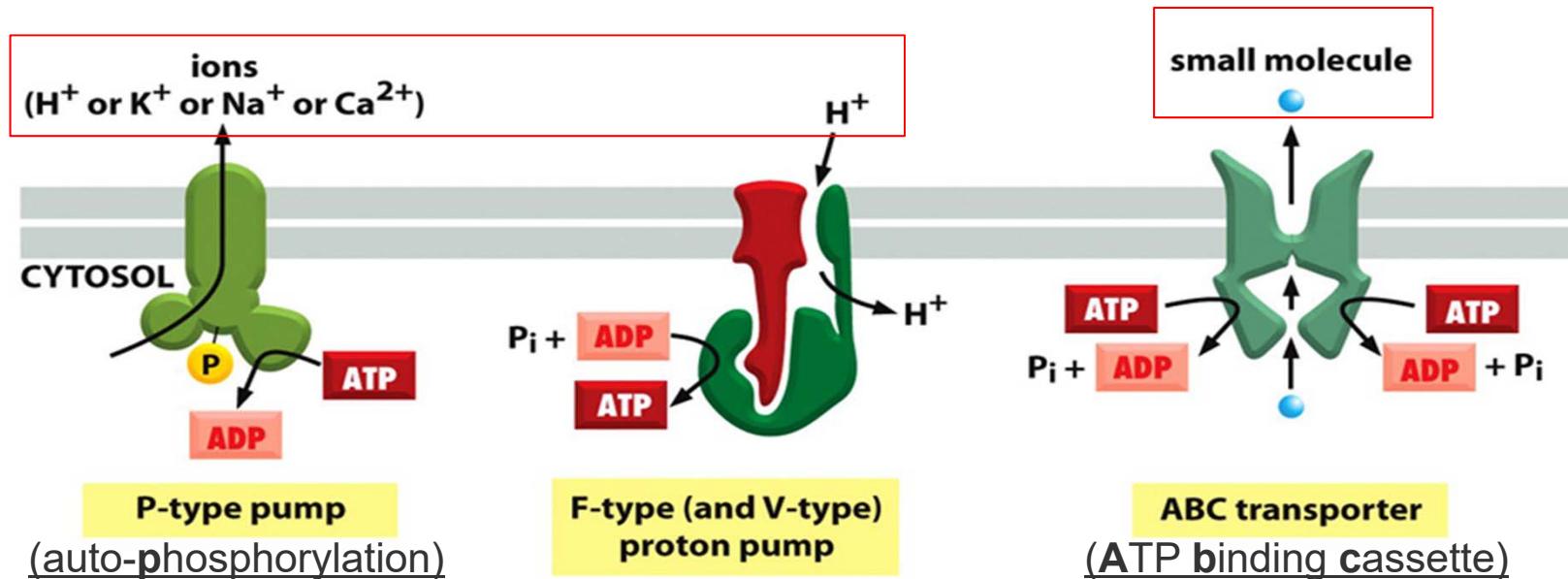
- Undergo conformational changes
- Bind molecules more tightly
- Transport molecules at a rate of 100-10,000 molecules per second

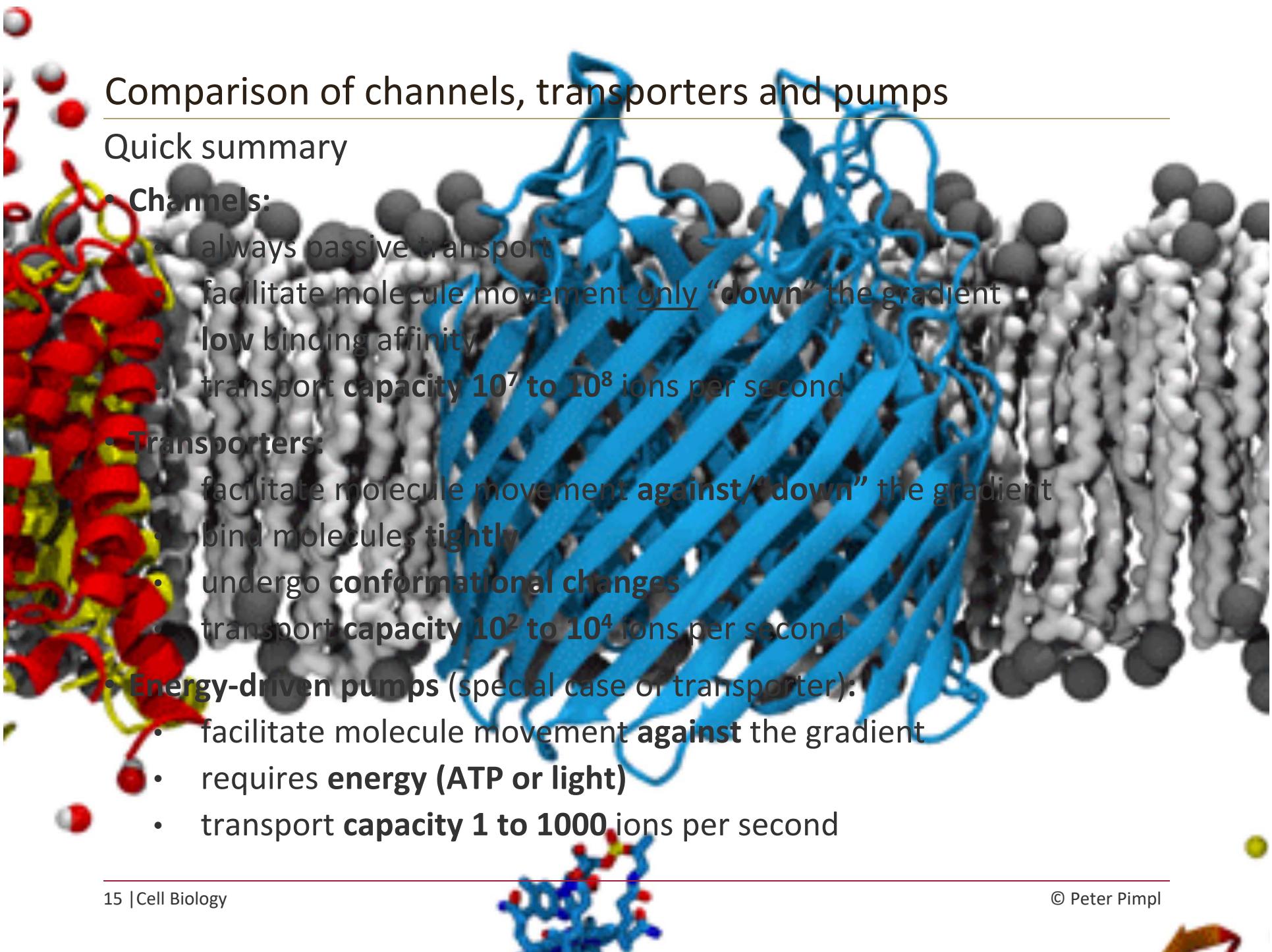


ATP-pumps: molecular machines

Three types of pumps: P-type, F-type/V-type & ABC transporter

- All ATP-pumps are active transporters
- All ATP-pumps have ATPase activity (ATP hydrolysis)
some ATP-pumps have also ATP synthesis activity
- All couple ATP hydrolysis with transport





Comparison of channels, transporters and pumps

Quick summary

- **Channels:**

- always passive transport
- facilitate molecule movement only “down” the gradient
- **low binding affinity**
- transport **capacity 10^7 to 10^8 ions per second**

- **Transporters:**

- facilitate molecule movement **against/“down”** the gradient
- bind molecules **tightly**
- undergo **conformational changes**
- transport **capacity 10^2 to 10^4 ions per second**

- **Energy-driven pumps** (special case of transporter):

- facilitate molecule movement **against** the gradient
- requires **energy (ATP or light)**
- transport **capacity 1 to 1000 ions per second**

II. Channels - functions & examples

Features:

- Unlike transporters, channel proteins form hydrophilic pores across membranes
- Gap junctions (connection between two neighboring cells) are found in virtually all animals
- Ion channels are ion-selective and fluctuate between “open” and “closed” states
- Channels are not be coupled to an energy source
- Two types of channels:
 - A. water channels (aquaporins)
 - B. ion channels (e.g. K⁺ channel)

The biological relevance of transport across membranes:

Cells must maintain appropriate solute concentration to avoid osmotic swelling

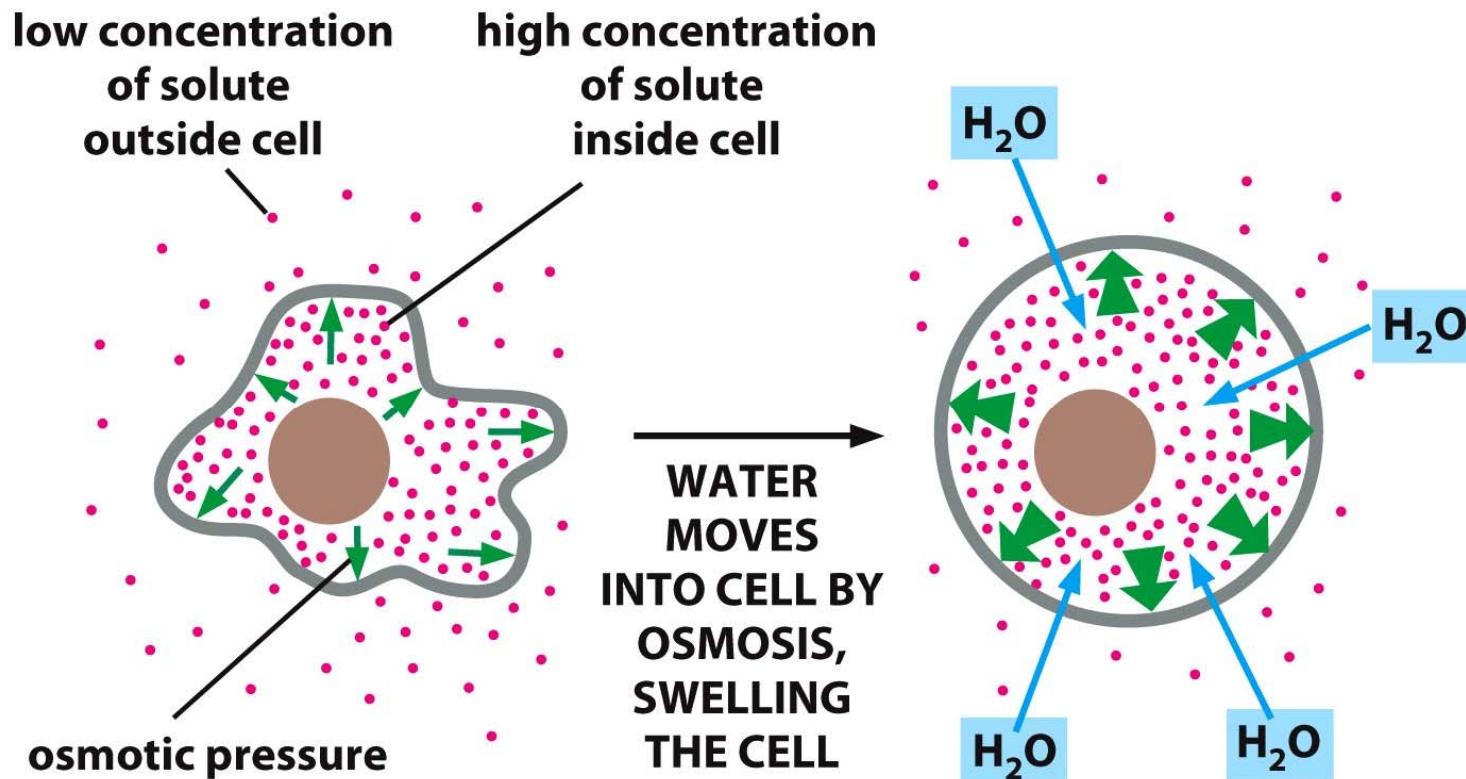
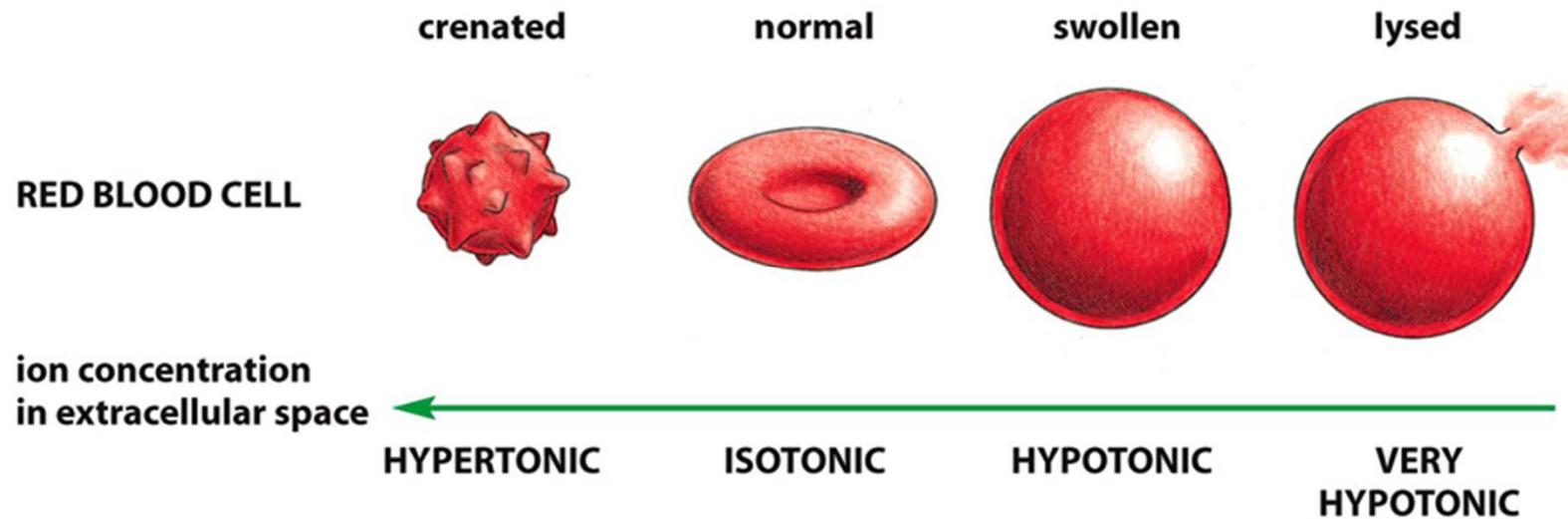


Figure 12-12 Essential Cell Biology 3/e (© Garland Science 2010)

The biological relevance of transport across membranes:

What happens if...



Question: Why don't the cells of fresh-water animals **burst** in water?

Cells have different ways to prevent osmotic swelling

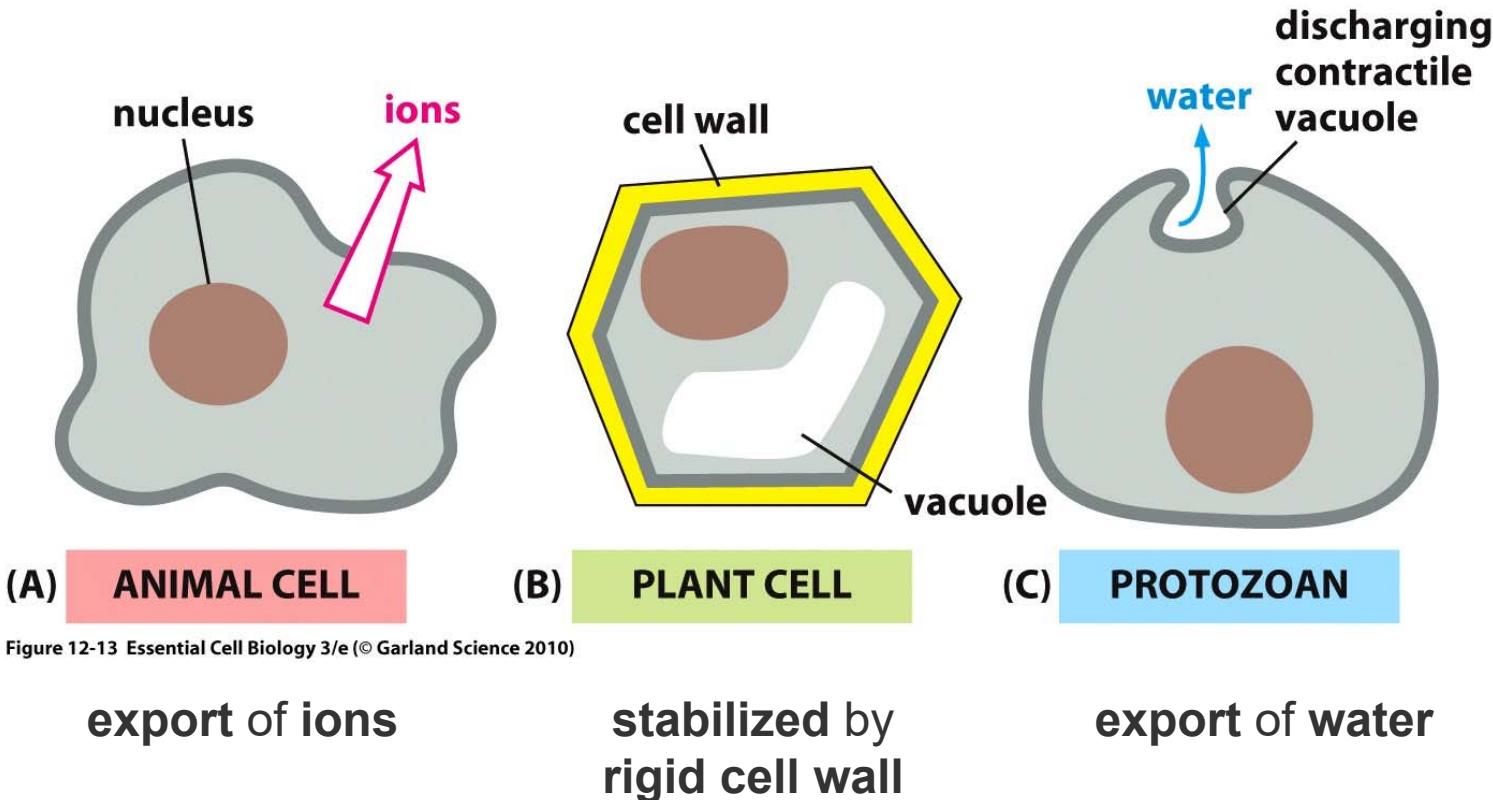


Figure 12-13 Essential Cell Biology 3/e (© Garland Science 2010)

export of ions

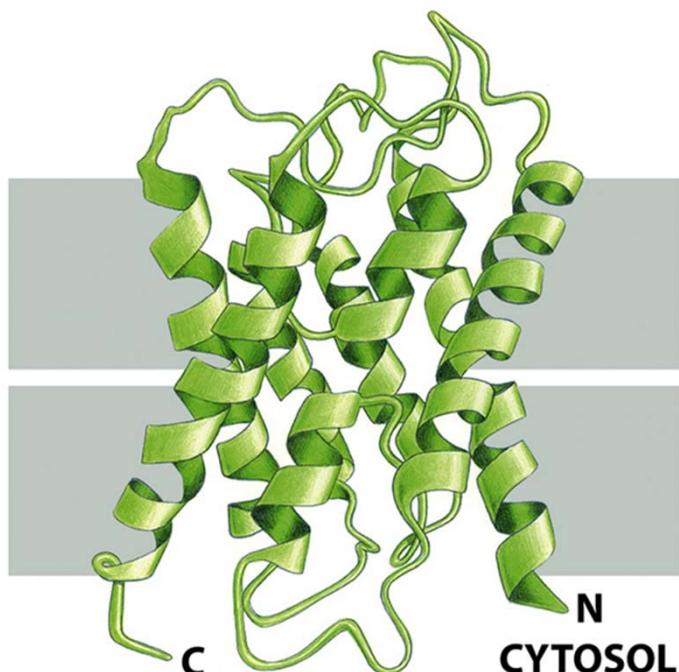
**stabilized by
rigid cell wall**

export of water

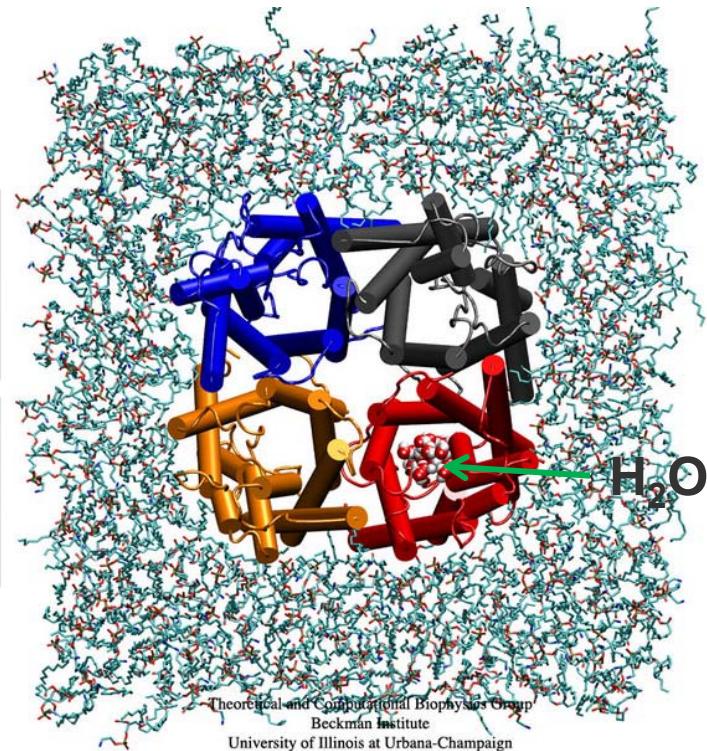
1. Aquaporins increase water permeability of cell membranes

Abundant in cells that transport water quickly, e.g. kidney epithelial cells, and erythrocytes..

Monomer has
6 transmembrane α -helices

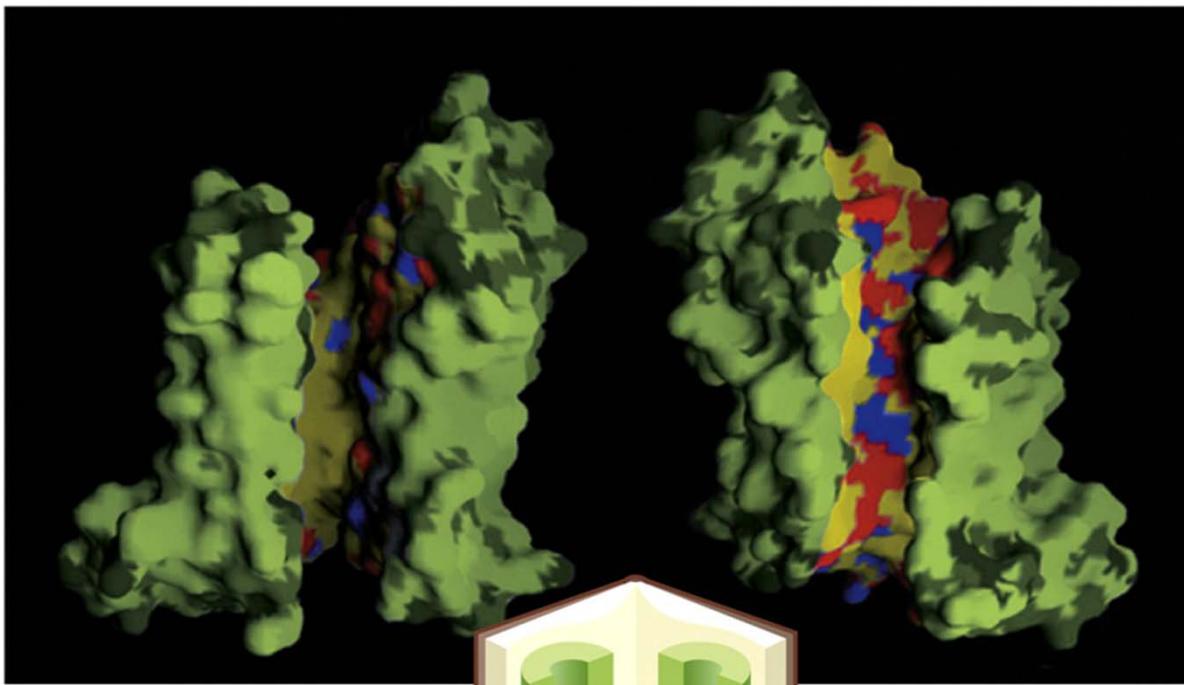


Aquaporin is a
homo tetrameric protein



How does aquaporin transport water?

A view by cutting the monomer in two halves



Blue and red :
hydrophilic residues
lining the pore

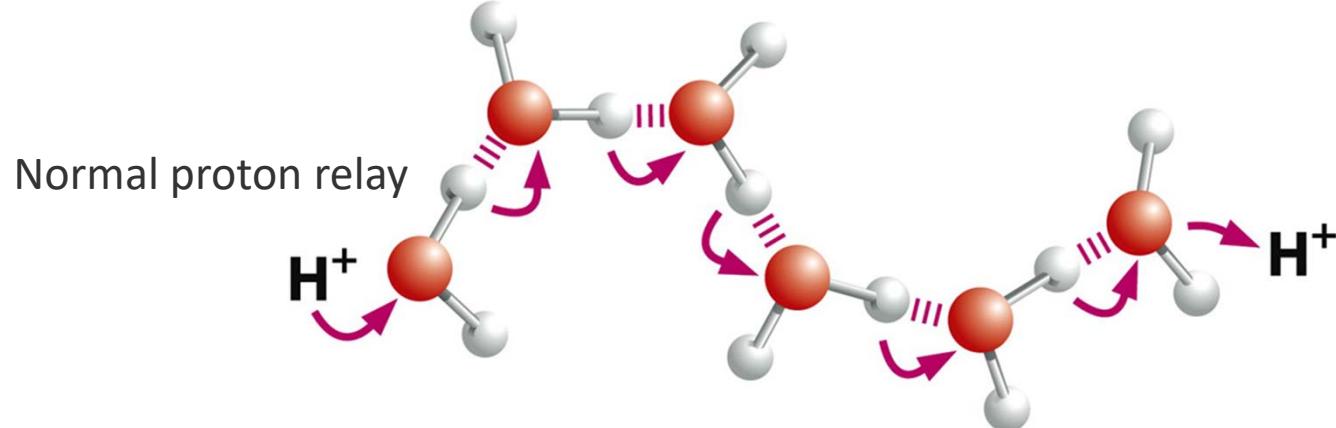
Yellow:
hydrophobic residues
lining the pore

Green:
irrelevant to water
channeling

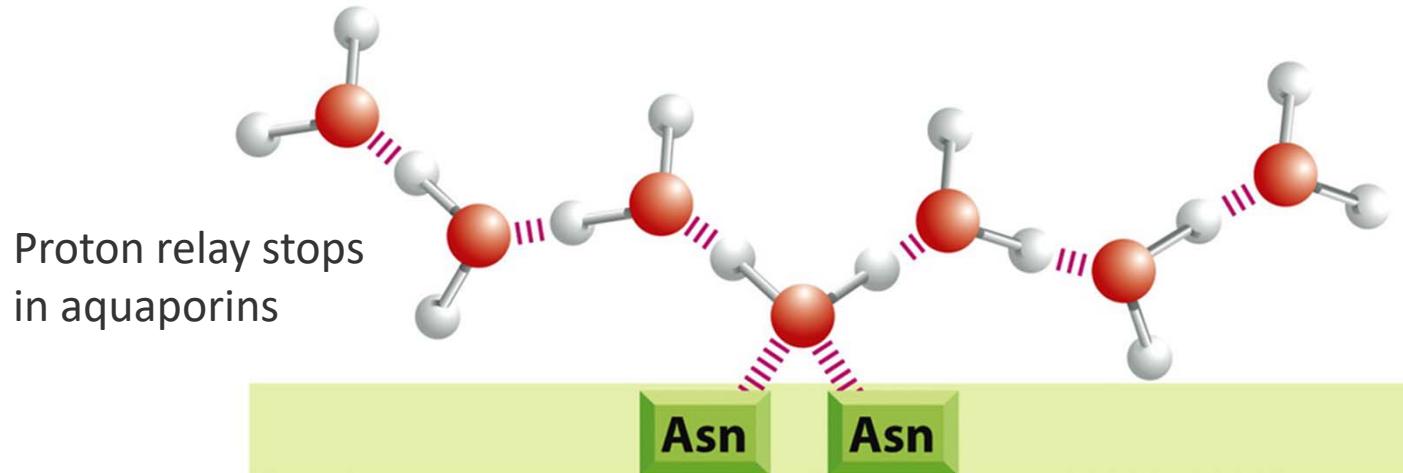
The pore is **lined with hydrophilic amino acids** that provide transient hydrogen bonds to water molecules. These bonds help to line-up water molecules in a **single row and orient them** as they traverse the membrane

How does aquaporin prohibit H⁺ to pass?

In water protons diffuse rapidly by being relayed from one molecule to the next.



In the center of the pore, one water molecule is tethered by two Asparagines. Tethering occupies both valences on it's oxygen, thus preventing the proton relay!

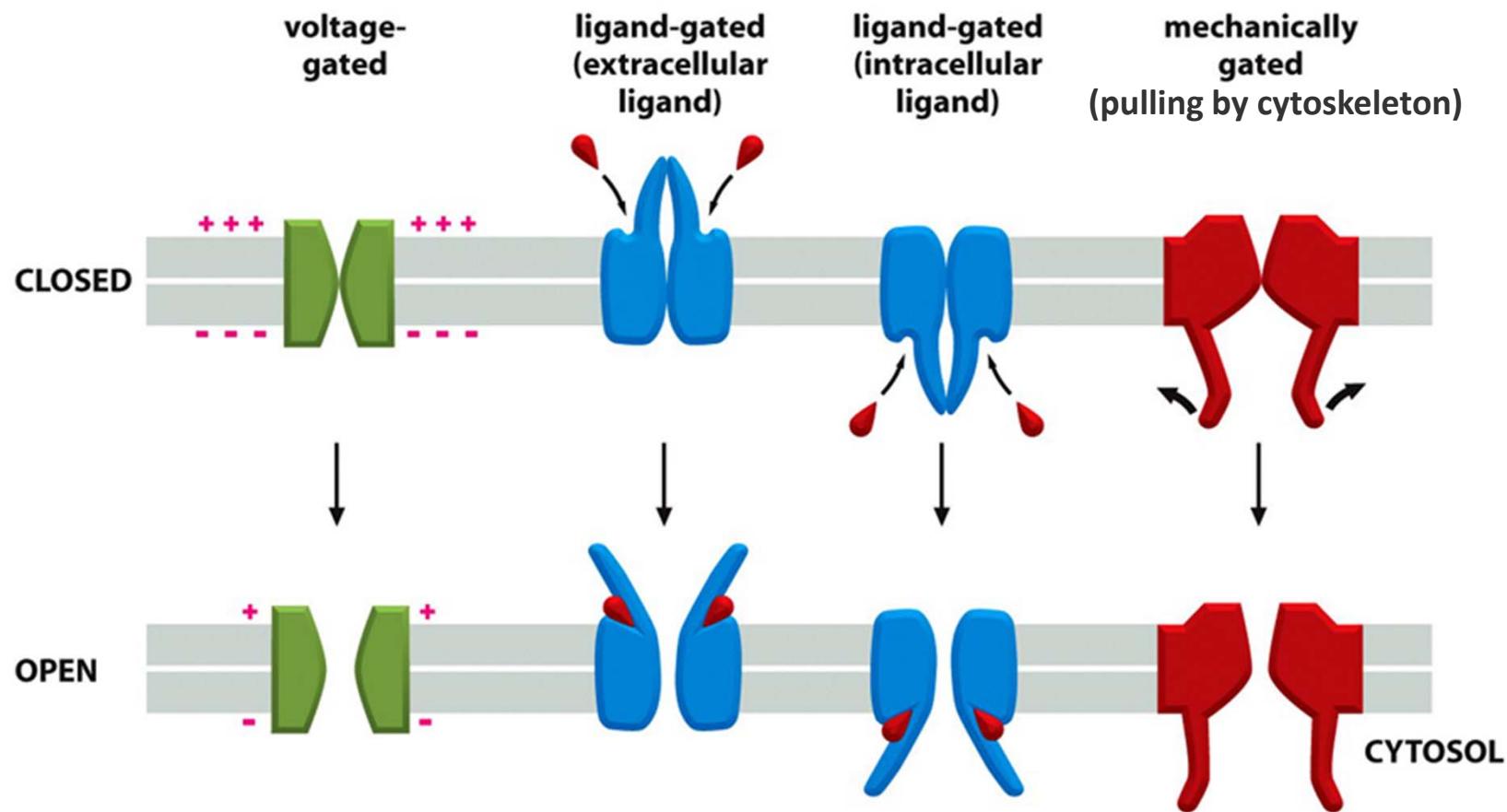


2. Ion channels

Features:

- Highly **selective**, >100 types have been described thus far
- Transport ions **down the gradient**.
- Tight control in its **open and close forms** (Fluctuation!).
- Transport with a **high efficiency: 100 million ions per second** (that is 100,000 times faster than a channel)
- Are important for **muscle and nerve cell function**

The gating of ion channels: four ways to open the “gate”



Mechanically-gated channels in the hair cell of the ear

Organ of Corti, the auditory portion of the inner ear

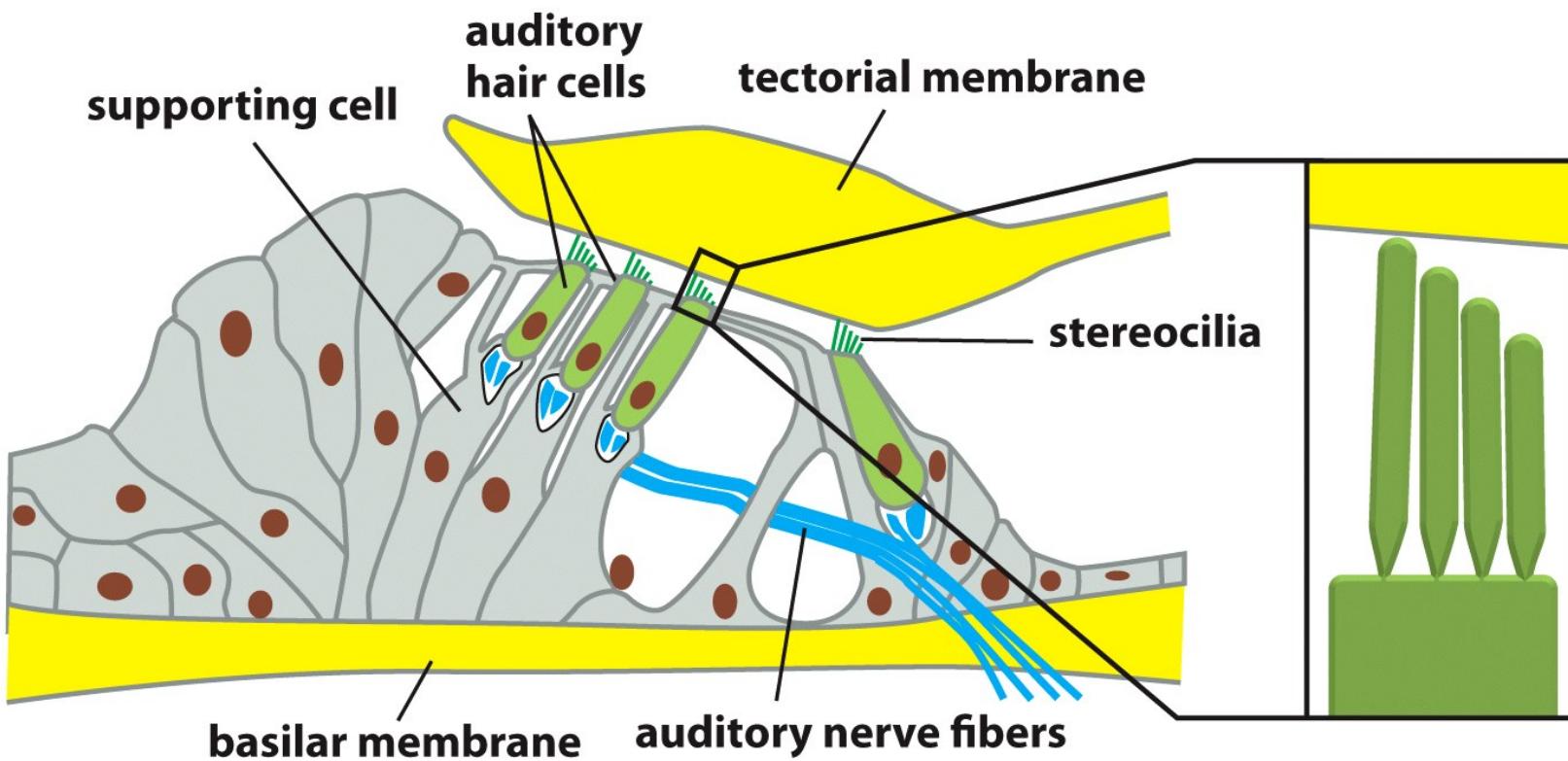


Figure 12-26a Essential Cell Biology 3/e (© Garland Science 2010)

Mechanical gated channel is found in the hair cell of the ear

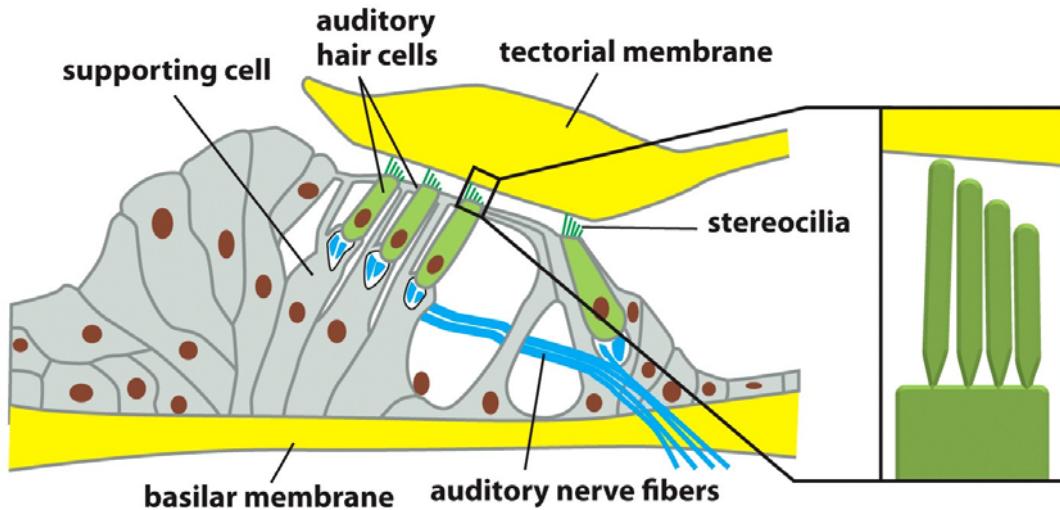
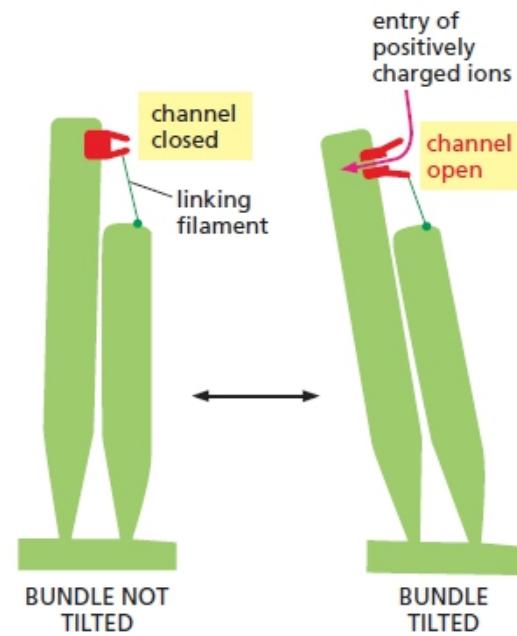


Figure 12-26a Essential Cell Biology 3/e (© Garland Science 2010)

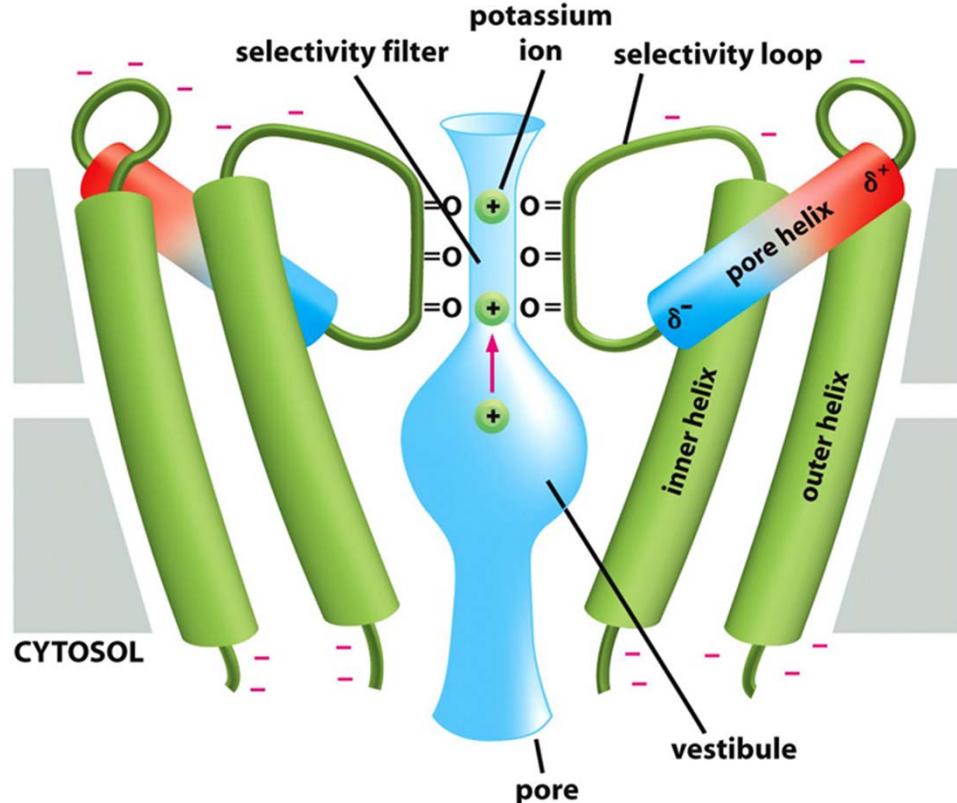
- **Sound vibrations** cause **tilting** of stereocilia. Each stereocilium in the staggered array is **attached** to the next shorter stereocilium **by a fine filament**.
- Tilting stretches the filaments, which **pulls open** **mechanically-gated ion channels** in the stereocilium plasma membrane, allowing **entry of positively charged ions** from the surrounding fluid.

The faintest sounds we can hear **stretch the filaments** about **0.04 nm**, which is **less** than the diameter of a hydrogen ion



Ion channels are highly selective: the bacterial K⁺ channel

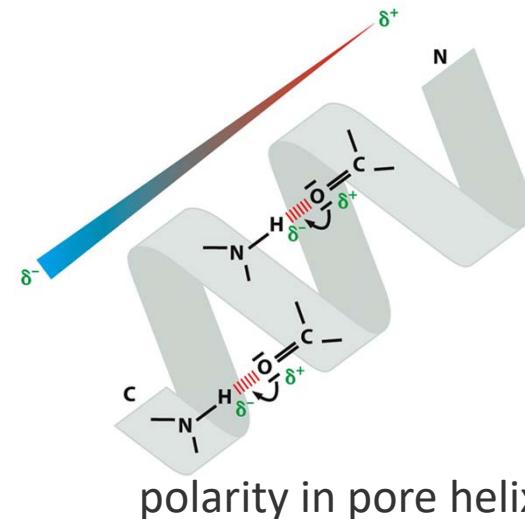
Na⁺ is smaller than K⁺, how can one be too small to sneak through the pore?



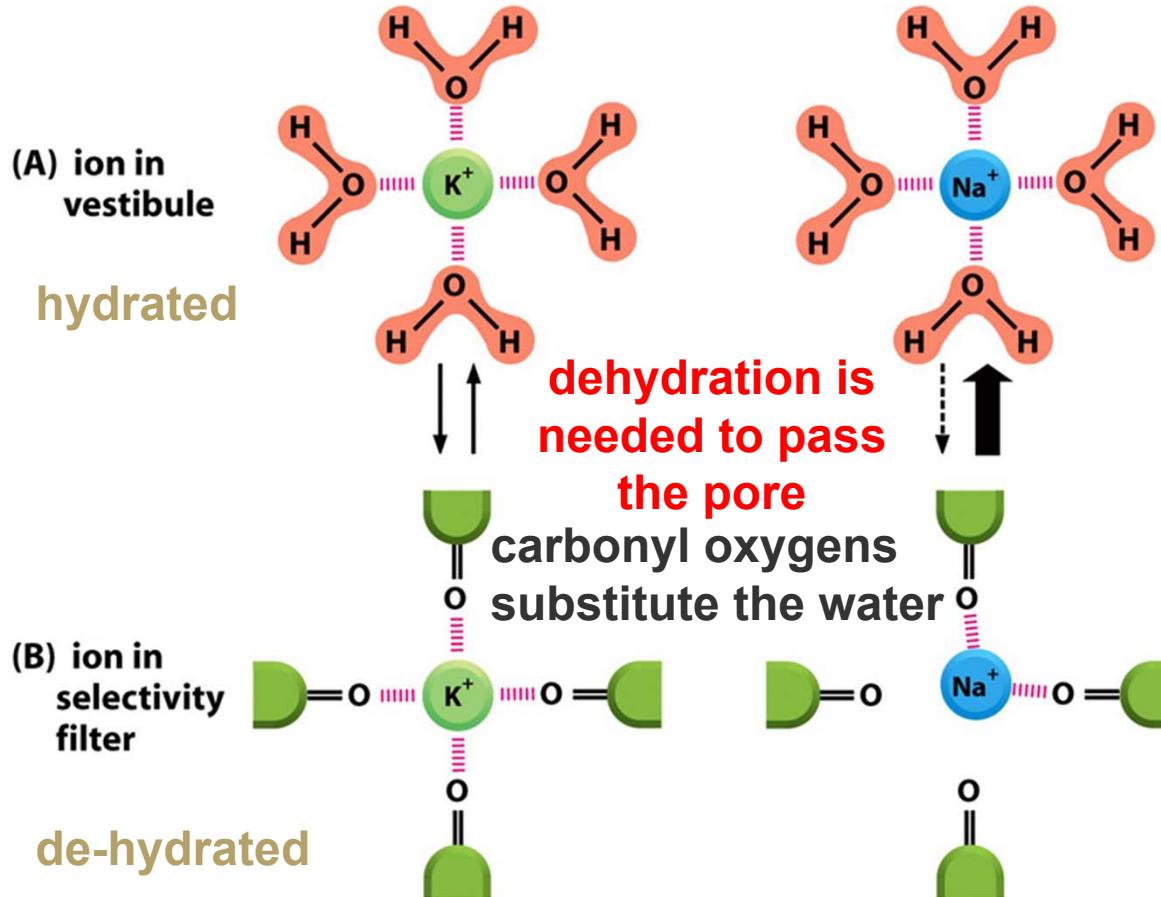
Only TWO out of the FOUR subunits with two TMDs each are shown. They possess negatively charged amino acids at entrance. That attracts cations and repels anions (selective for cations)

The K⁺ channel conducts K⁺ 10,000 fold better than Na⁺; how is that possible?

Selectivity:
Carbonyl oxygen lines the wall of the **selectivity filter** and form binding sites for dehydrated K⁺ ions

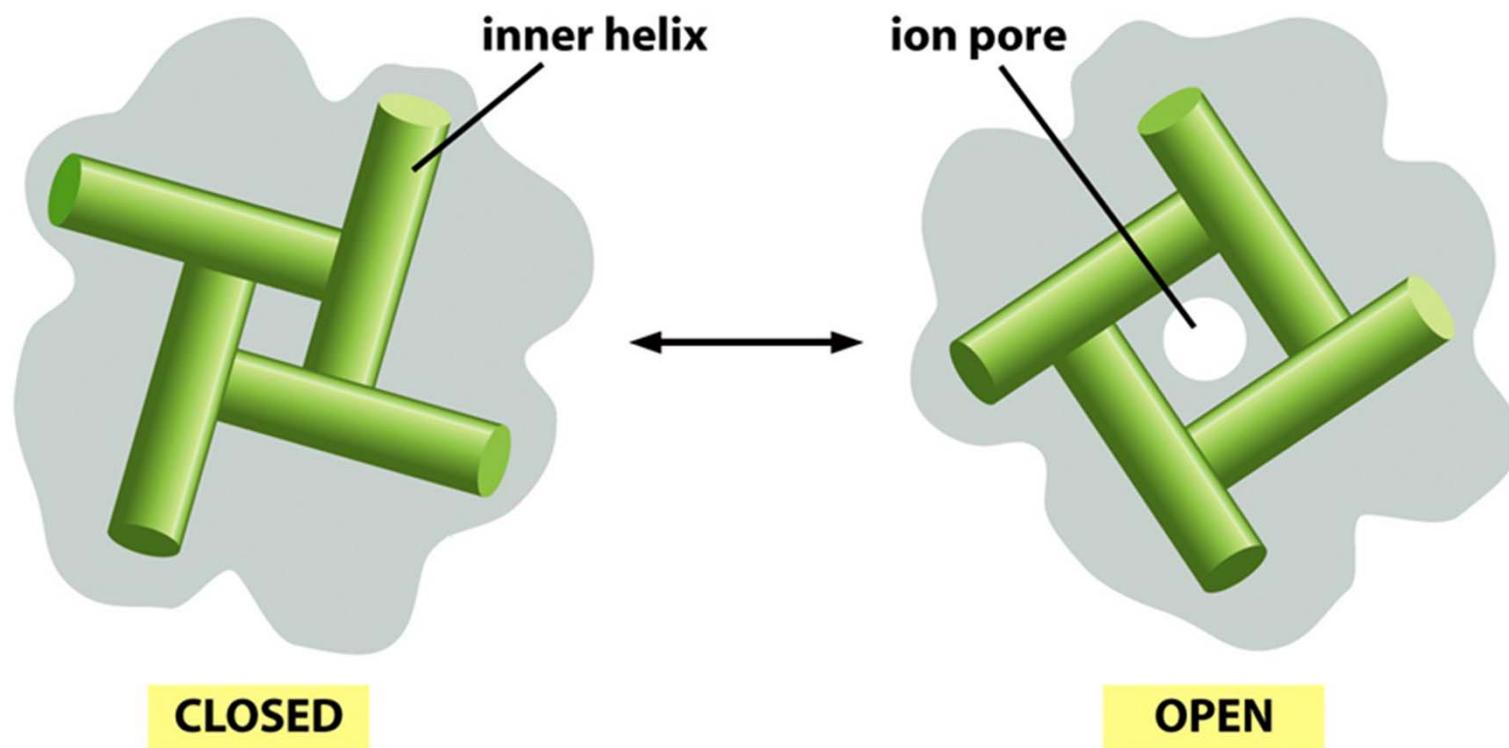


Specificity for K⁺ due to the selectivity filter



Sodium ion is too small to interact with **all four** oxygens

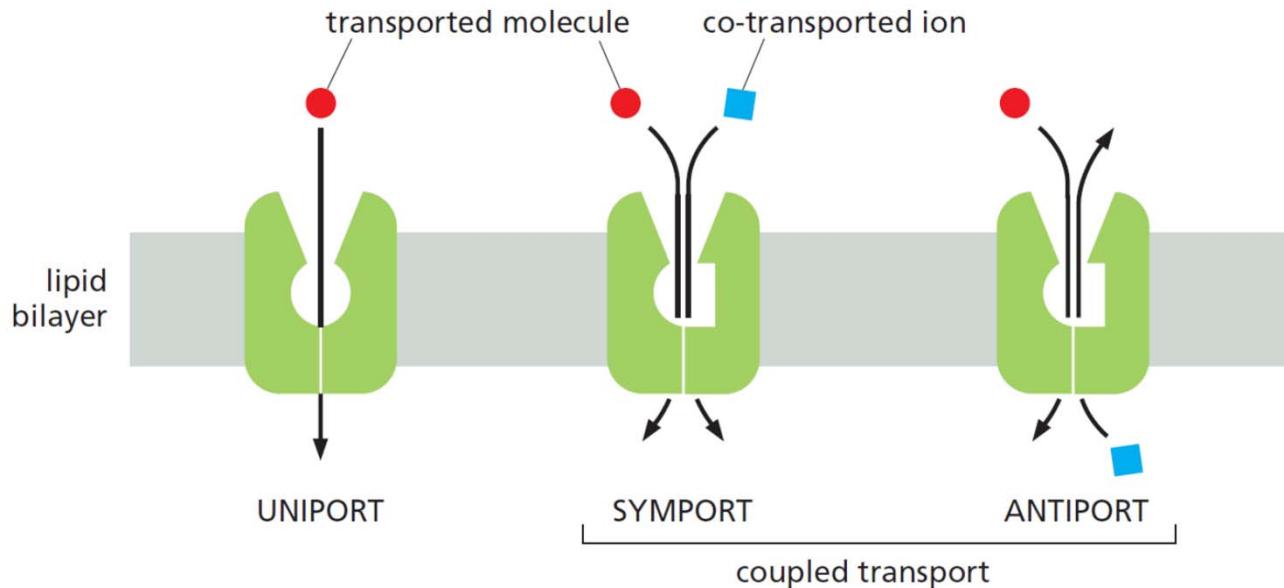
Model for gating of a bacterial K⁺ channel



Closing/opening occurs by tilting of the four inner helices

III. Transporters

Three main types: uniporter, symporter and antiporter



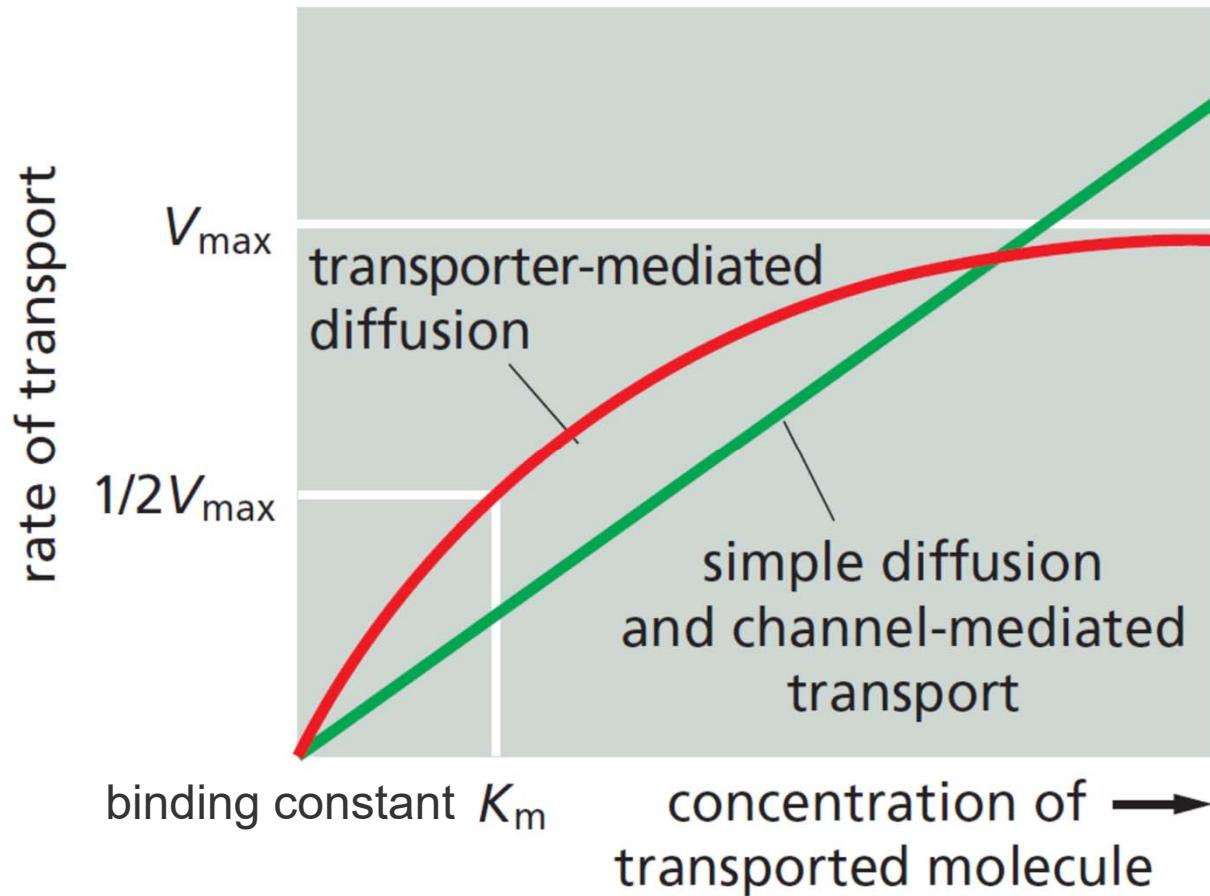
1. Uniporter: **glucose transporter 1 (GLUT1)**
2. Symporter: **lactose permease**
3. Antiporter :
 Na^+/H^+ exchanger
 Na^+ -driven $\text{Cl}^-/\text{HCO}_3^-$ exchanger
 Na^+ -independent $\text{Cl}^-/\text{HCO}_3^-$ exchanger
4. Transcellular transport via asymmetrically-distributed transporters

Transporters

Features:

1. Each transporter has individual biochemical properties:
 - one or more specific binding sites for its solute
 - a characteristic V_{\max}
 - a characteristic affinity, K_m
2. Binding and transport is reversible.
3. Binding can be competitively or non-competitively inhibited.
4. Transport is selective for only one single or a group of closely related molecules.
8. Transport rate is much higher than simple diffusion
9. Hydrophobicity and size of solutes does not matter.

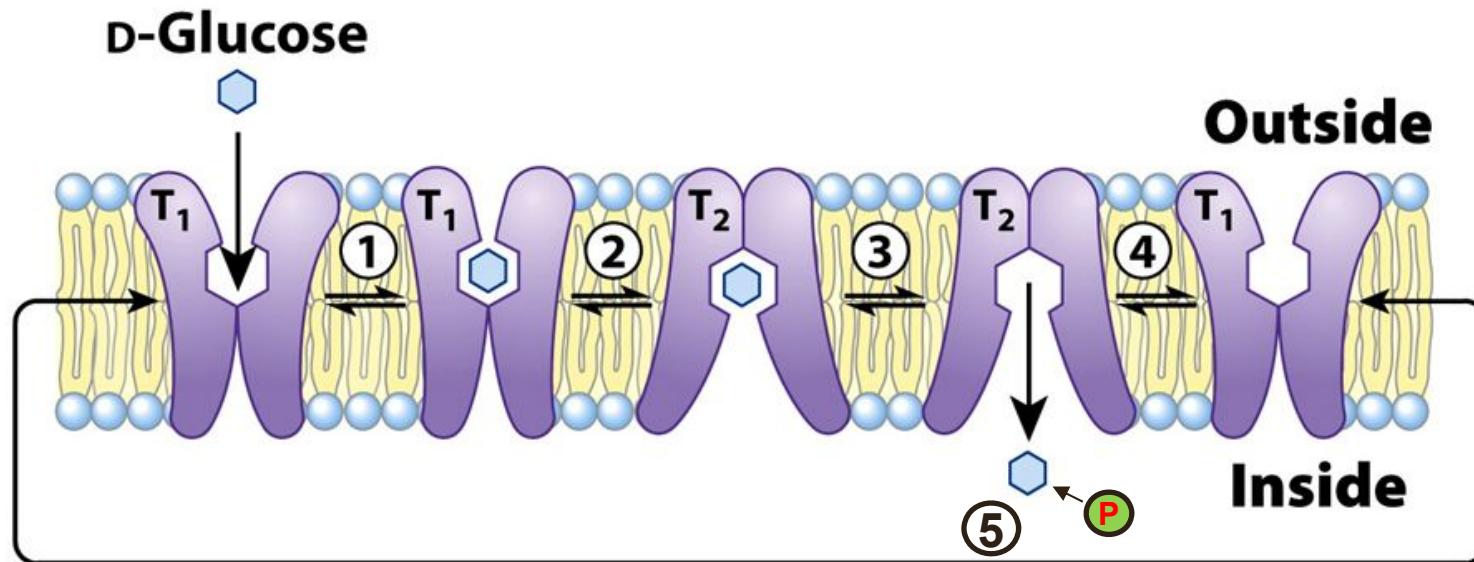
Binding and transporting resembles enzyme kinetics



Diffusion is proportional to the solute concentration,
transporter-mediated diffusion is saturable (V_{max})

The uniporter glucose transporter 1 (GLUT1)

GLUT1 transports glucose from the extracellular space into the cytosol
(transports glucose across the blood-brain barrier)



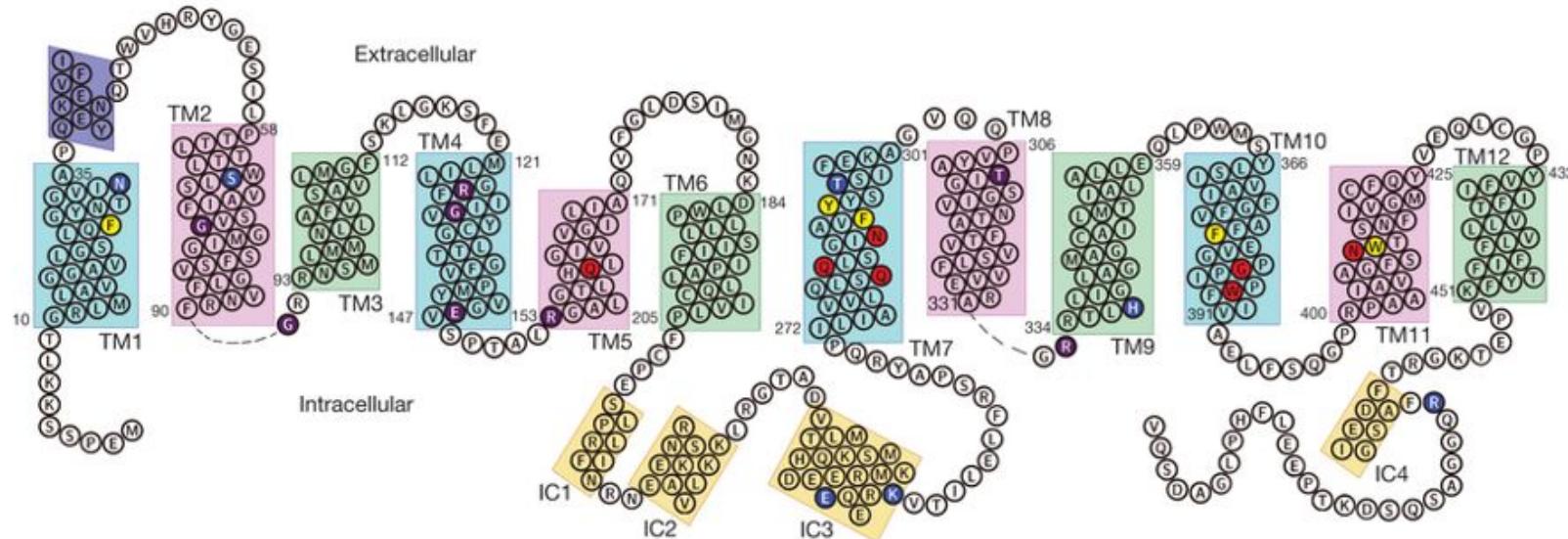
Transport requires conformational change:

- 1) binding pocket is exposed outside and glucose binds
- 2) binding triggers conformational change (transport)
- 3) release triggers conformational change, to
- 4) expose the binding pocket on the outside again
- 5) phosphorylation of glucose to glucose 6-phosphate

Glucose transporter GLUT1

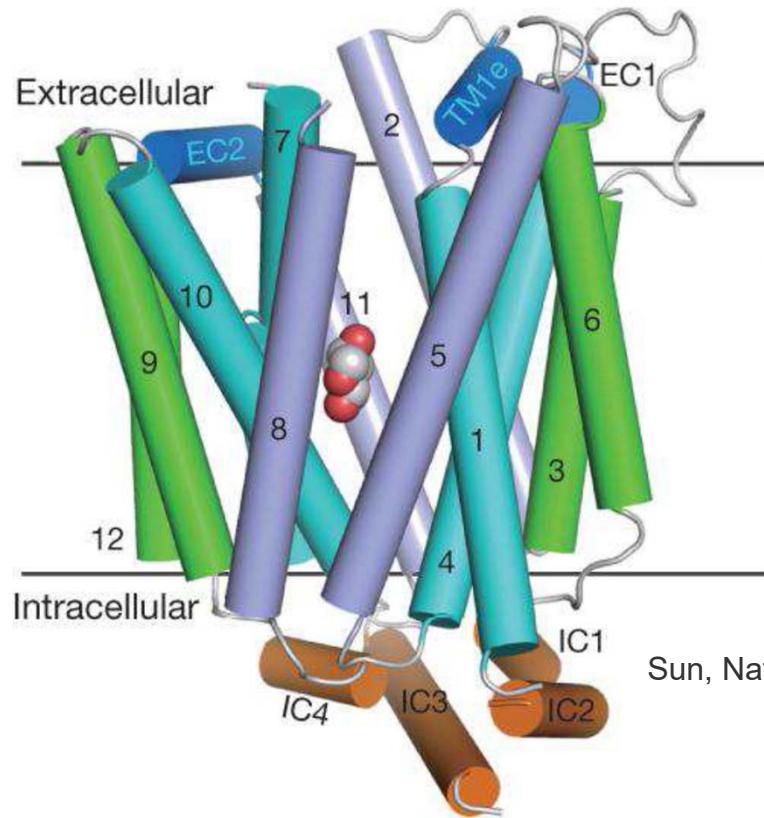
Features:

- The human genome encodes 14 homologs, differential tissue expression
- Abundant in erythrocytes
- Is more specific for D-glucose (K_m 1.5 mM) than for D-mannose (K_m 20 mM) or for D-galactose (K_m 30 mM)
- Immediately after import, glucose is phosphorylated to Glu-6-P:
 - Maintaining gradient for further rapid glucose import

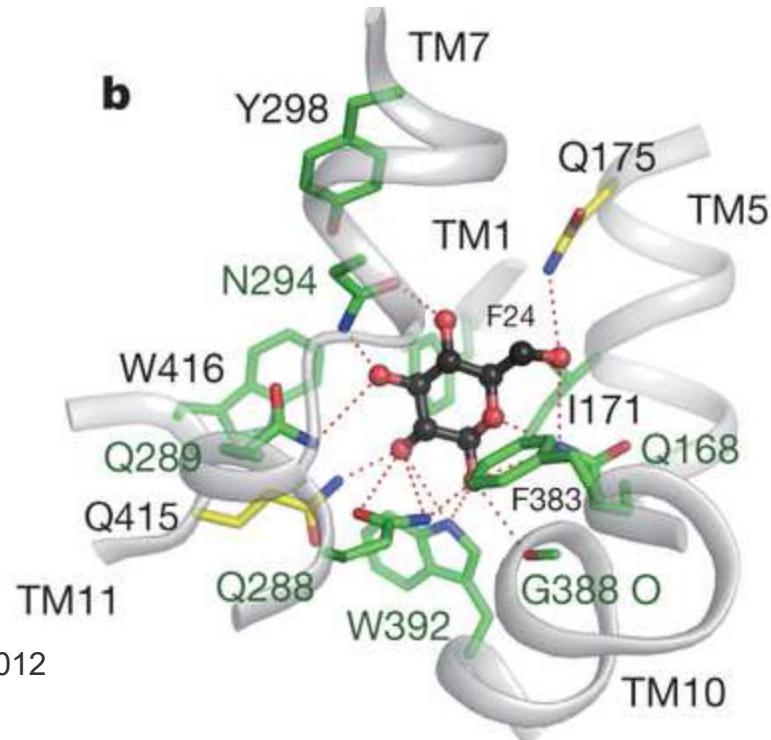


Nice and colorful, but how does GLUT1 work?

GLUT1: from structure to function



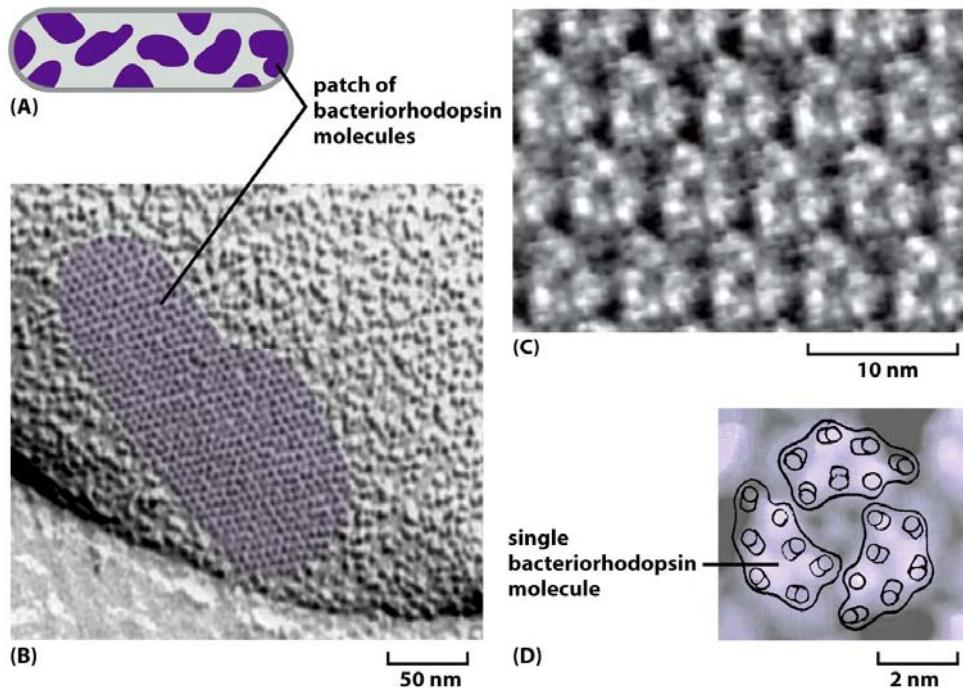
Sun, Nature, 2012



- 12 transmembrane segments and a unique intracellular four-helix domain.
- Captured in an outward-facing, partly occluded conformation.
- Most of the important amino acids responsible for recognition of D-xylose or D-glucose are invariant in GLUT1-4

Now , guess why glucose is phosphorylated after transport....

Bacteriorhodopsin: light-driven proton pump

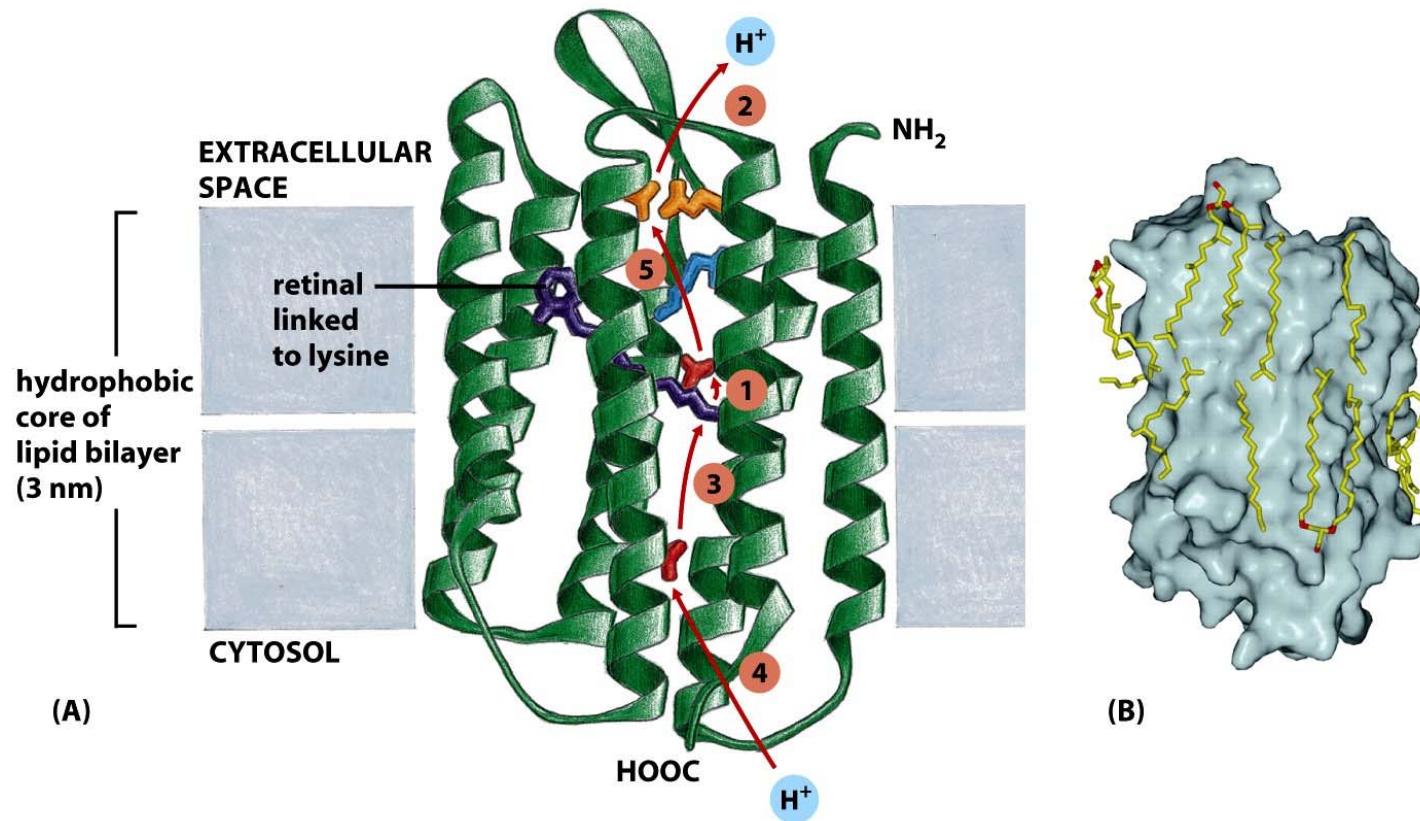


Exist in the plasma membrane of archaean *Halobacterium salinarum* who lives in sea water

Pump protons in the presence of sunlight and set up proton gradients across the membrane.

Use the proton gradients to harvest ATP or other energy requiring activities.

Bacteriorhodopsin: light-driven proton pump



Retinal: **chromophore** (light absorbing group) is **linked to a lysine**

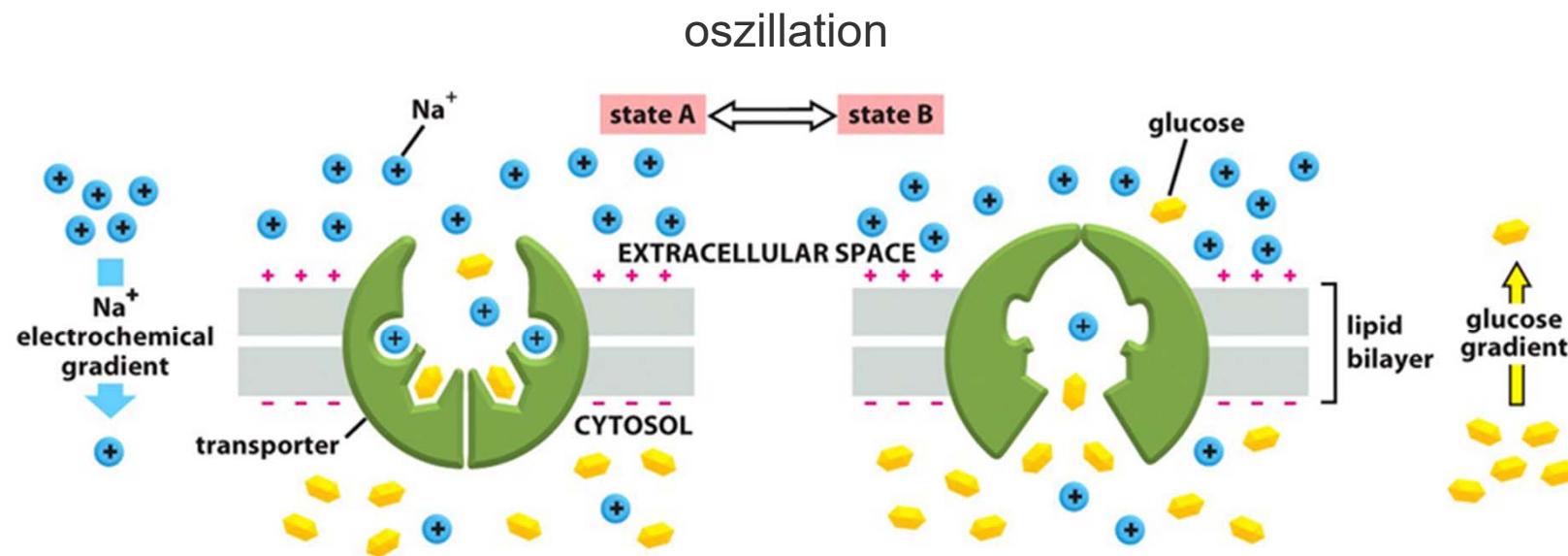
- (1) transfer of a proton from the chromophore to **aspartic acid 85**
- (2) transfer of the proton to **glutamic acid** and release
- (3) conformational change and (4) “refilling” with a new proton...

Symporter: ... you never walk alone!!!

- **Energy** is stored in electrochemical gradients
- Symporter **couple** the Na^+ or H^+ gradient with the transport of other molecules.
- Transport can only occur if “**both**” molecules are present
- **Eukaryotic** cells mainly use Na^+ as a primary cotransporting ion
- **Bacteria** and **yeast** or **subcellular organelles** in **animal** cells mainly use H^+ as a primary cotransporting ion

The sodium-dependent glucose transporter 1 (SGLT1)

SGLT1 is Symporter: Glucose transport is driven by a Na^+ gradient



- Binding of **either** Glucose or Na^+ increases binding affinity of the transporter for the respective other molecule.
- Transport requires binding of **both molecules**, binding of only one of the two will not trigger the conformational change/transport