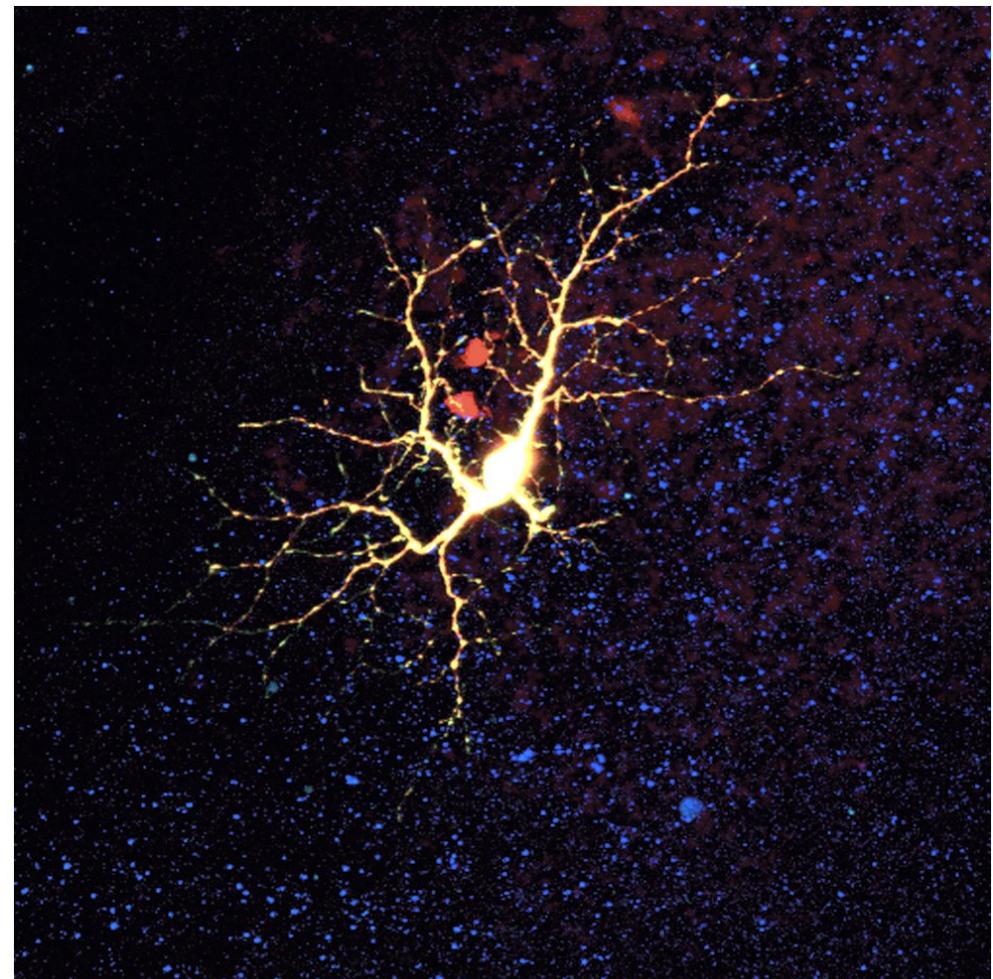


# *Passive and Active Transport*

1. Thermodynamics of transport
2. Passive-mediated transport
3. Active transport



neuron, membrane potential,  
ion transport

# Membranes

- Provide barrier function
  - Extracellular
  - Organelles
- Barrier can be overcome by „transport proteins”
  - To mediate transmembrane movements of ions,  $\text{Na}^+$ ,  $\text{K}^+$
  - Nutrients, glucose, amino acids etc.
  - Water (aquaporins)

# 1) Thermodynamics of Transport

- $A_{\text{out}} \leftrightarrow A_{\text{in}}$  (ressembles a chemical equilibration)
- $G_A - G_A^{\circ} = RT \ln [A]$
- $\Delta G_A = G_{A(\text{in})} - G_{A(\text{out})} = RT \ln ([A]_{\text{in}}/[A]_{\text{out}})$
- $G_A$ : chemical potential of A
- $G_A^{\circ}$ : chemical potential of standard state of A
- If membrane has a potential,  
i.e., plasma membrane: -100mV (inside negative)  
then  $G_A$  is termed the **electrochemical potential** of A

## SAMPLE CALCULATION 10-1

Show that  $\Delta G < 0$  when  $\text{Ca}^{2+}$  ions move from the endoplasmic reticulum (where  $[\text{Ca}^{2+}] = 1 \text{ mM}$ ) to the cytosol (where  $[\text{Ca}^{2+}] = 0.1 \mu\text{M}$ ). Assume  $\Delta\Psi = 0$ .

The cytosol is *in* and the endoplasmic reticulum is *out*.

$$\begin{aligned}\Delta G &= RT \ln \frac{[\text{Ca}^{2+}]_{in}}{[\text{Ca}^{2+}]_{out}} = RT \ln \frac{10^{-7}}{10^{-3}} \\ &= RT(-9.2)\end{aligned}$$

Hence,  $\Delta G$  is negative.

# Two types of transport across a membrane:

- o Nonmediated transport occurs by passive diffusion, i.e.,  $O_2$ ,  $CO_2$   
*driven by chemical potential gradient, i.e. cannot occur against a concentration gradient*
- o Mediated transport occurs by dedicated transport proteins
  1. Passive-mediated transport/facilitated diffusion:  
[high] -> [low]
  2. Active transport: [low] -> [high]  
May require energy in form of ATP or in form of a membrane potential

## 2) Passive-mediated transport

Substances that are too large or too polar to diffuse across the bilayer must be transported by proteins: carriers, permeases, channels and transporters

- A) Ionophores
- B) Porins
- C) Ion Channels
- D) Aquaporins
- E) Transport Proteins

# A) Ionophores

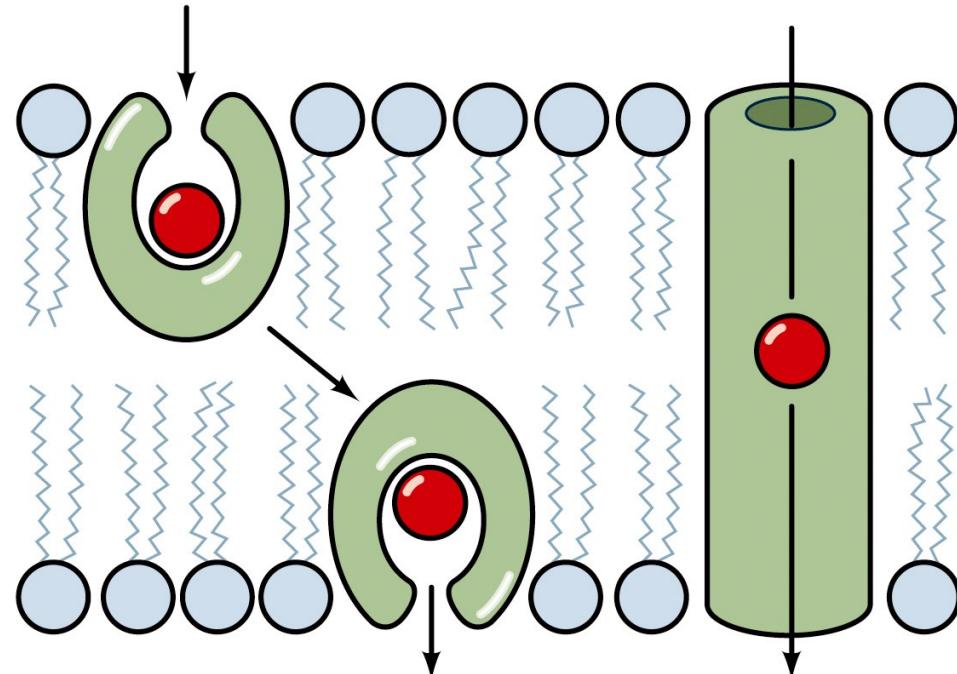
Organic molecules of diverse types, often of bacterial origin

=> Increase the permeability of a target membrane for ions, frequently antibiotic, result in collapse of target membrane potential by ion equilibration

## 1. Carrier Ionophore,

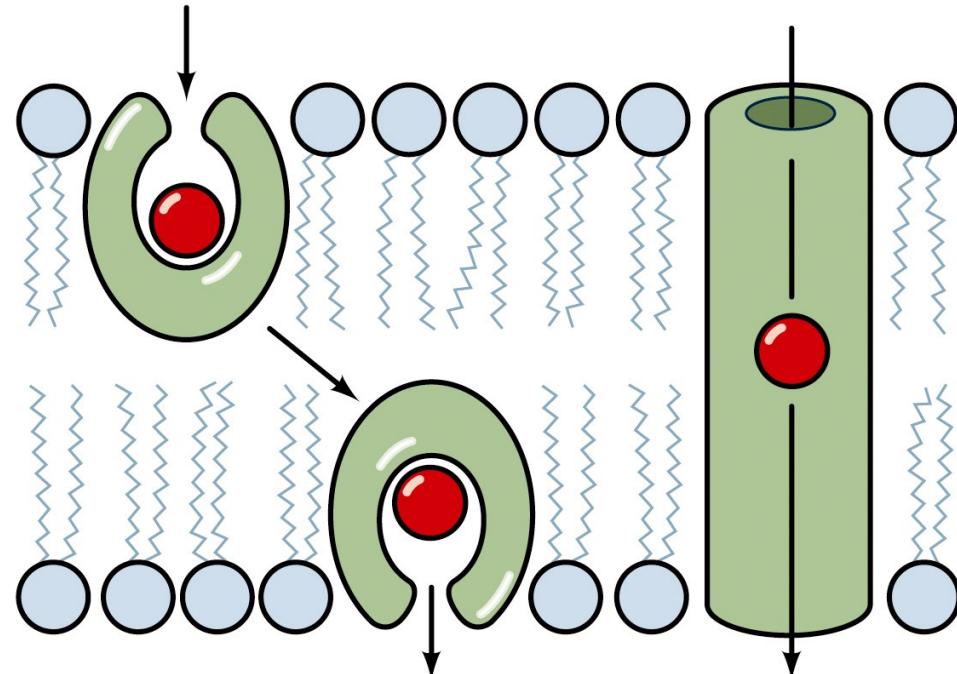
make ion soluble in  
membrane, i.e. valinomycin,  
 $10^4 \text{ K}^+/\text{sec}$

(a) Carrier  
ionophore



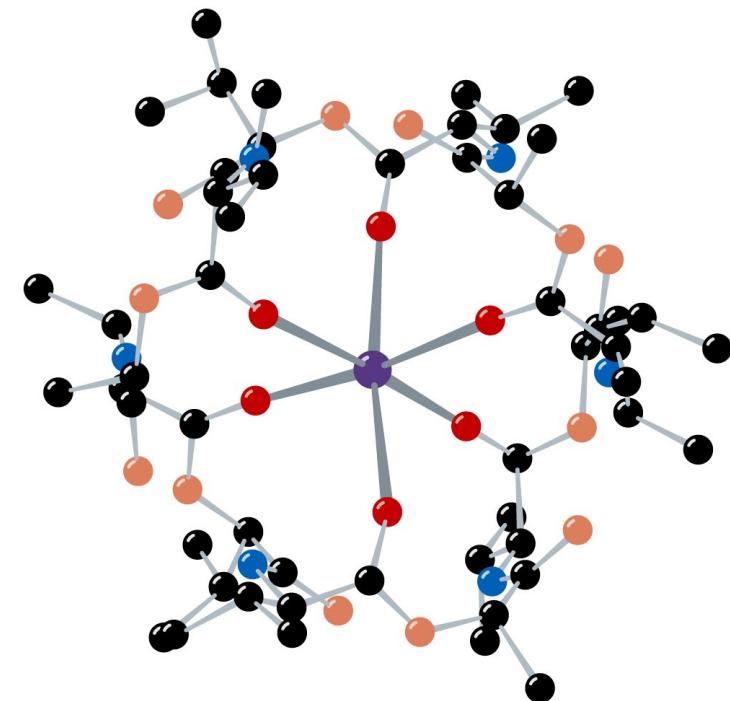
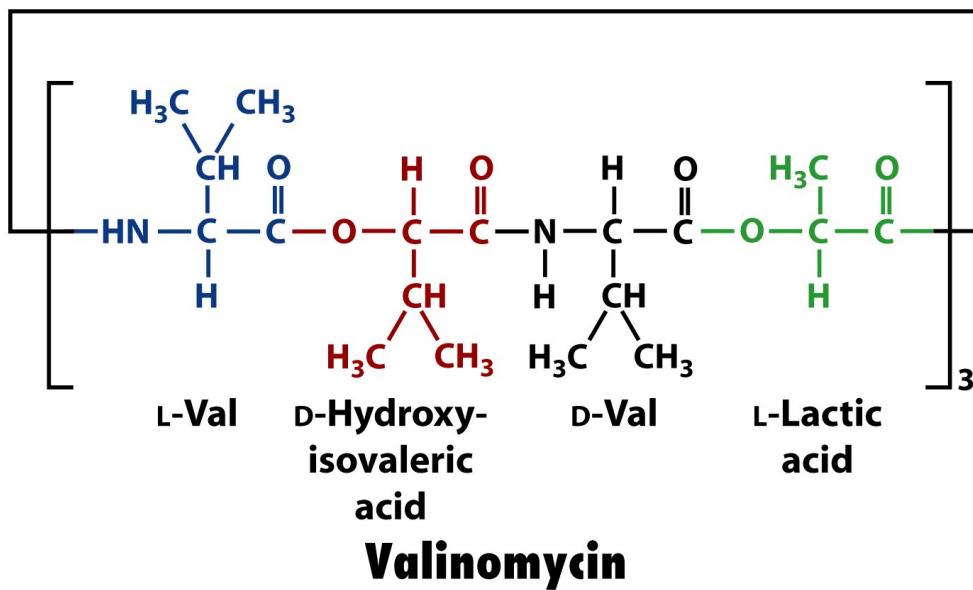
(b) Channel-forming  
ionophore

2. Channel-forming  
ionophores, form  
transmembrane channels,  
gramicidin A,  $10^7 \text{ K}^+/\text{sec}$



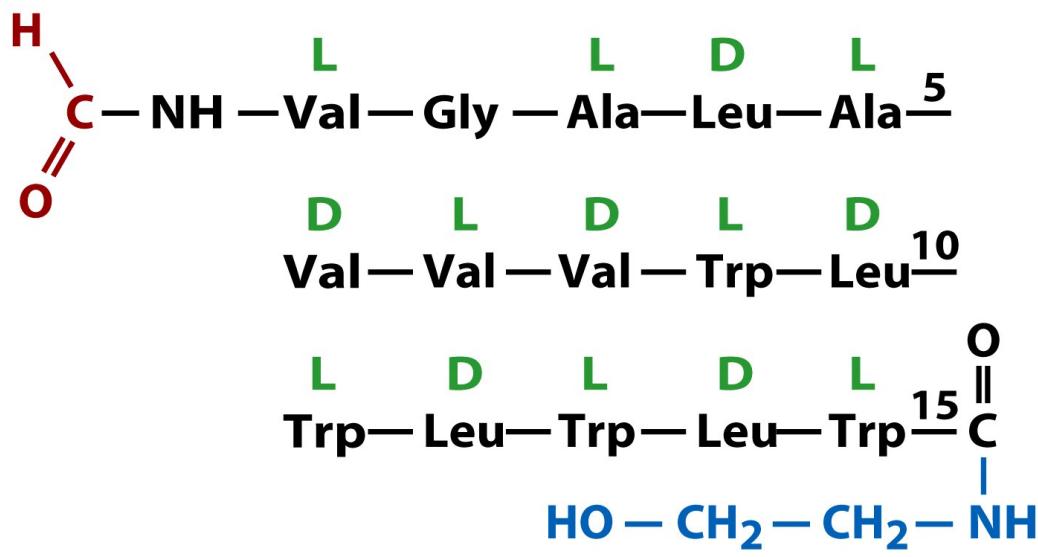
# Valinomycin

- One of the best characterized ionophores, binds K<sup>+</sup> ions
- Cyclic peptide with D- and L-Aa
- Discrimination between Na<sup>+</sup>, K<sup>+</sup>, Li<sup>+</sup> ?  
K<sup>+</sup> (r=1.33 Å), Na<sup>+</sup> (r=0.95 Å)

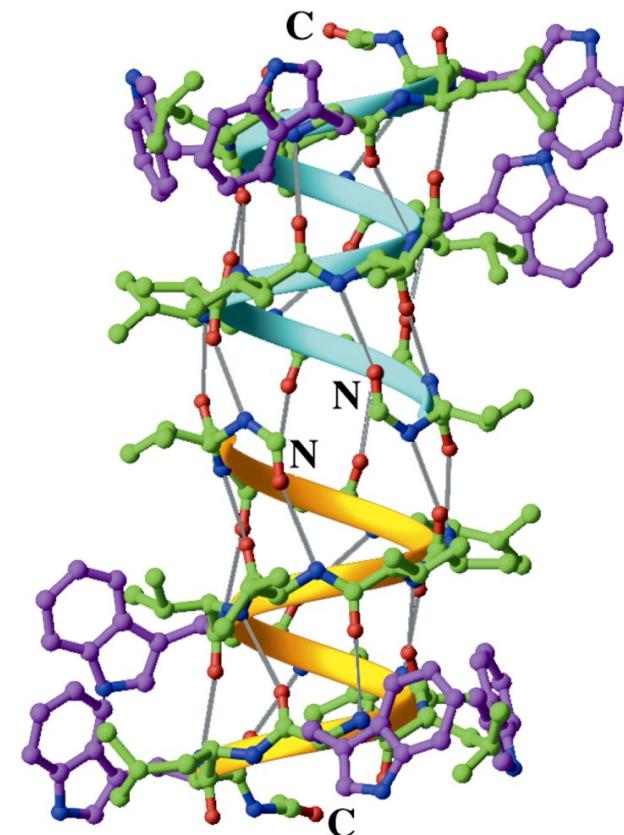


# Gramicidin A

- 15 Aa linear peptide
- Alternating D- and L-Aa, all hydrophobic
- Dimerizes head-to-head to form channel

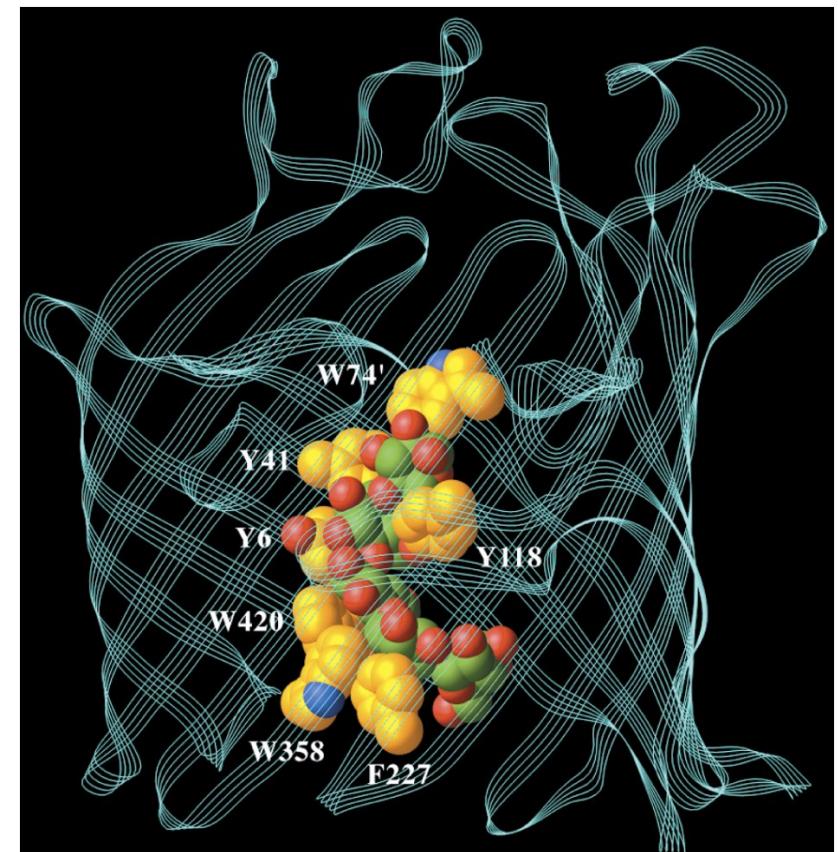


**Gramicidin A**



## B) Porins

- Membrane spanning proteins with  $\beta$ -barrel structure, with central aqueous channel, diameter  $\sim 7 \times 11 \text{ \AA}$   $\sim 600 \text{ D}$ , little substrate selectivity, *E. coli* OmpF
- Maltoporin, substrate selectivity for maltodextrins,  $\alpha(1 \rightarrow 4)$ -linked glucose oligosaccharide degradation products of starch, *greasy slide*



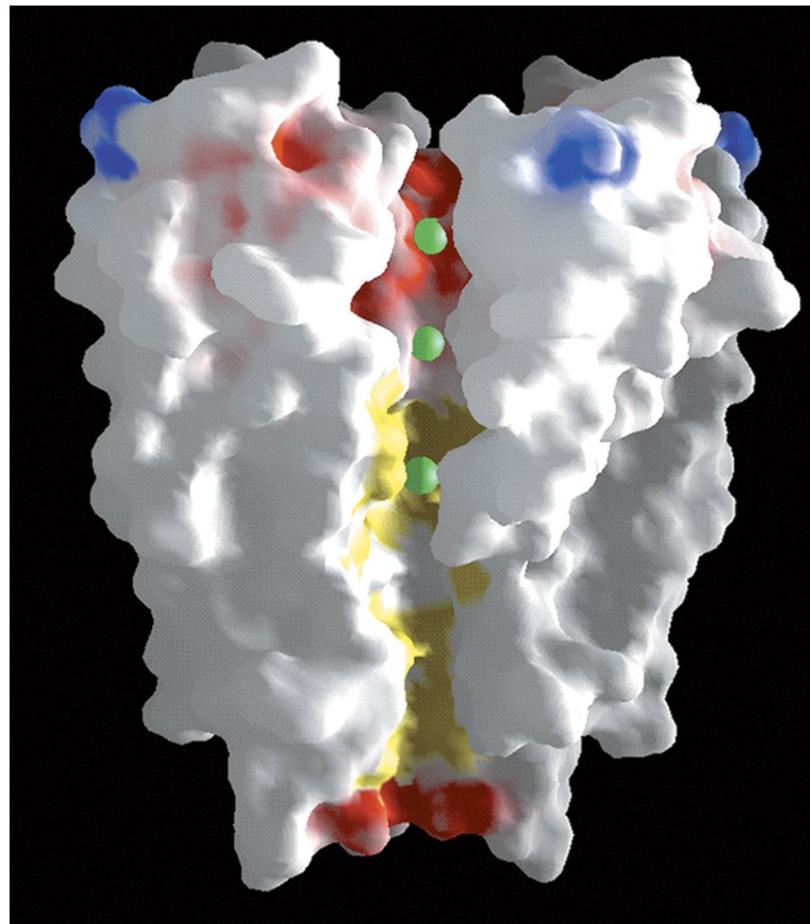
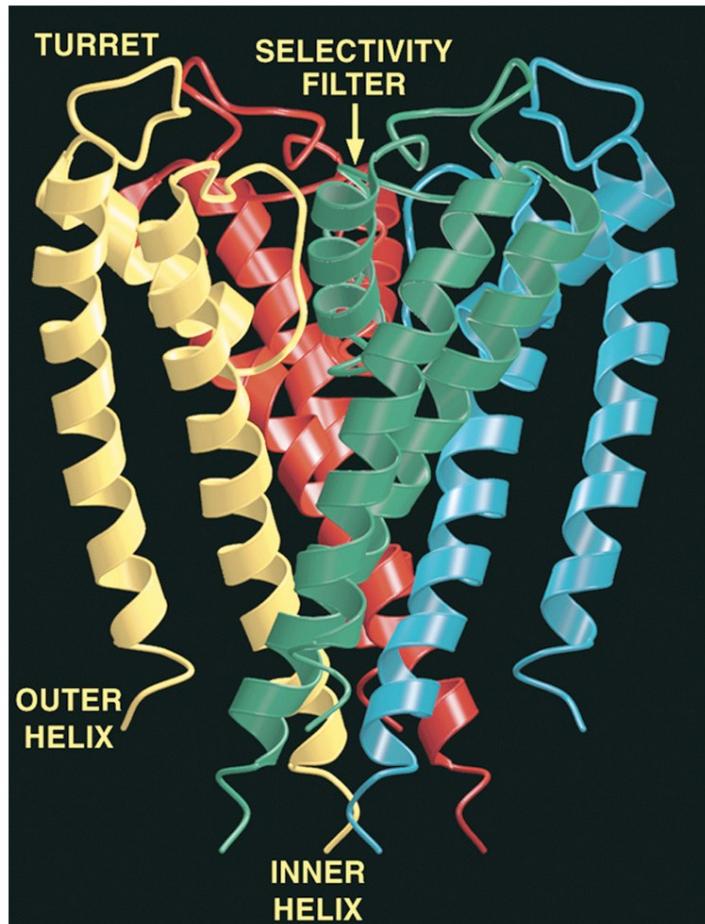
## C) Ion Channels

- o All organisms have channels for  $\text{Na}^+$ ,  $\text{K}^+$ , and  $\text{Cl}^-$
- o Membrane transport of these ions is important for:
  - o Osmotic balance
  - o Signal transduction
  - o Membrane potential
- o Mammalian cells:
  - extracellular: 150mM  $\text{Na}^+$ , 4mM  $\text{K}^+$
  - intracellular: 12mM  $\text{Na}^+$ , 140mM  $\text{K}^+$

passive diffusion of  $\text{K}^+$  ions through opening of  $\text{K}^+$ -channels from cytosol to extracellular space  
 $\text{K}^+$ -channels have high selectivity of  $\text{K}^+$  over  $\text{Na}^+$   
selectivity  $10^4$

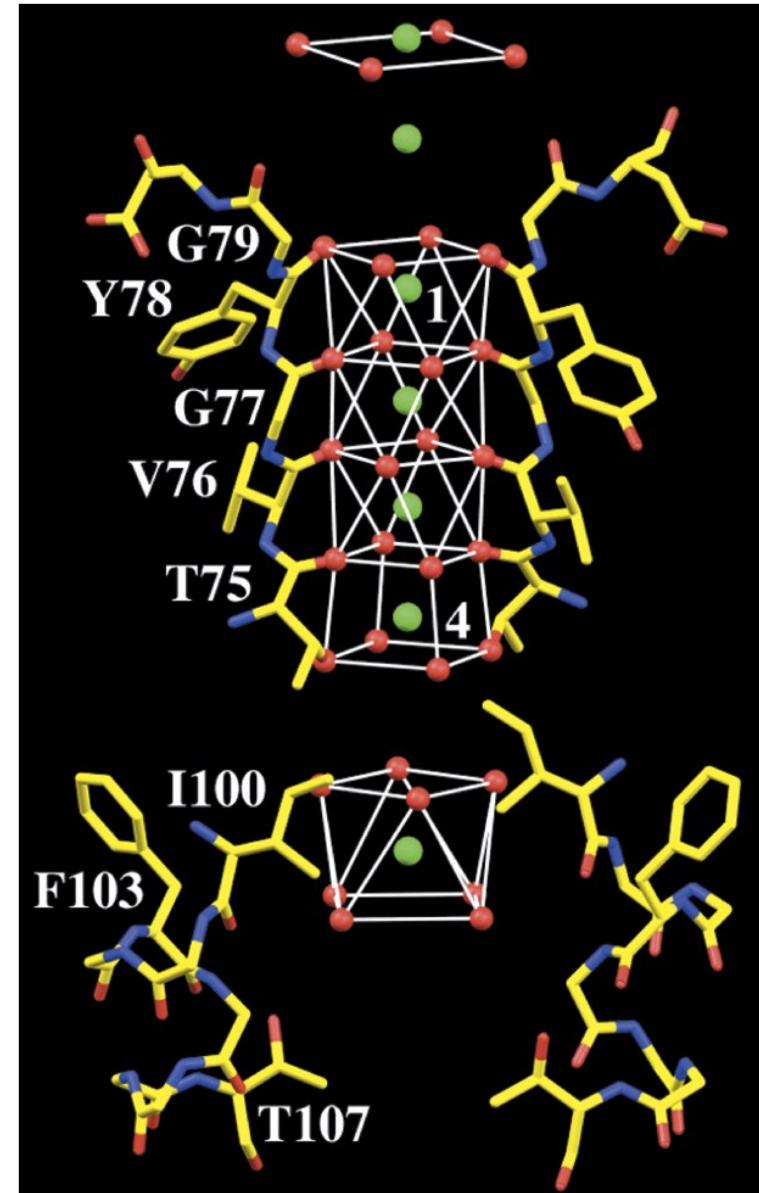
# The K<sup>+</sup>-channel, KcsA

- Streptomyces, Functions as a homotetramer, 158 Aa, 10<sup>8</sup> ions/sec, Roderick MacKinnon
- Selectivity filter allows passage of K<sup>+</sup> but not Na<sup>+</sup>



# The selectivity filter

- The ion needs to be **dehydrated** to pass through the most narrow opening of the channel
- In the dehydration, water is replaced by hydroxyl groups from the channels amino acids
- These hydroxyls will stabilize  $K^+$  but not  $Na^+$ , because  $Na^+$  is much smaller than  $K^+$
- Cavity in the middle of the channel = middle of the membrane !!! Contains water



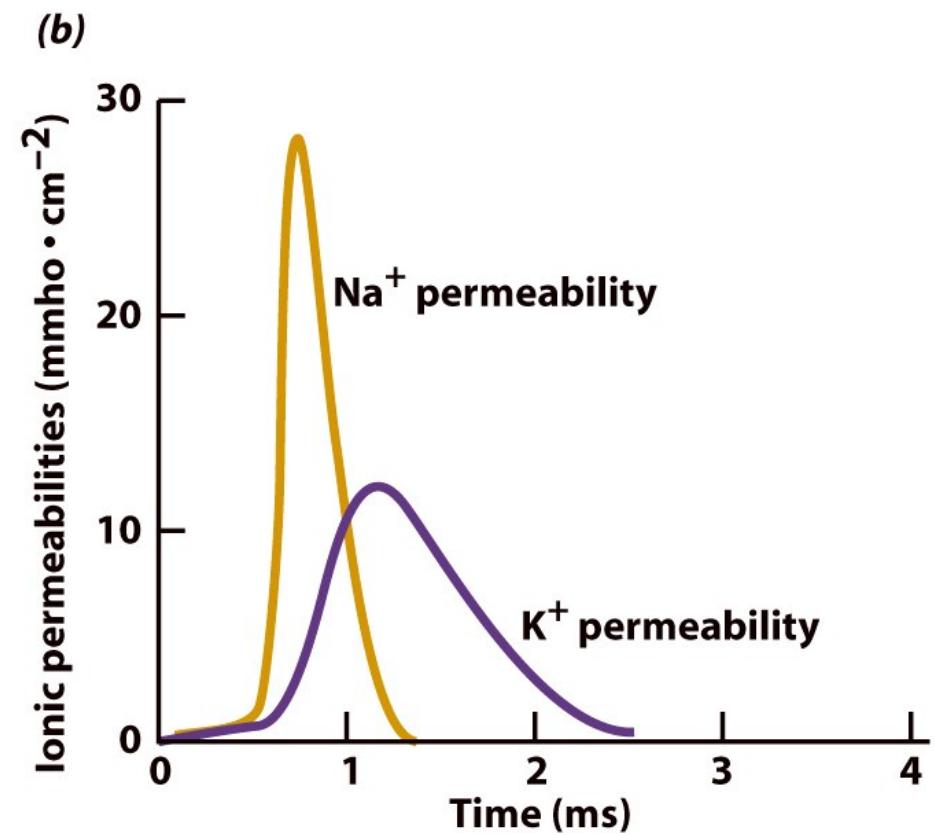
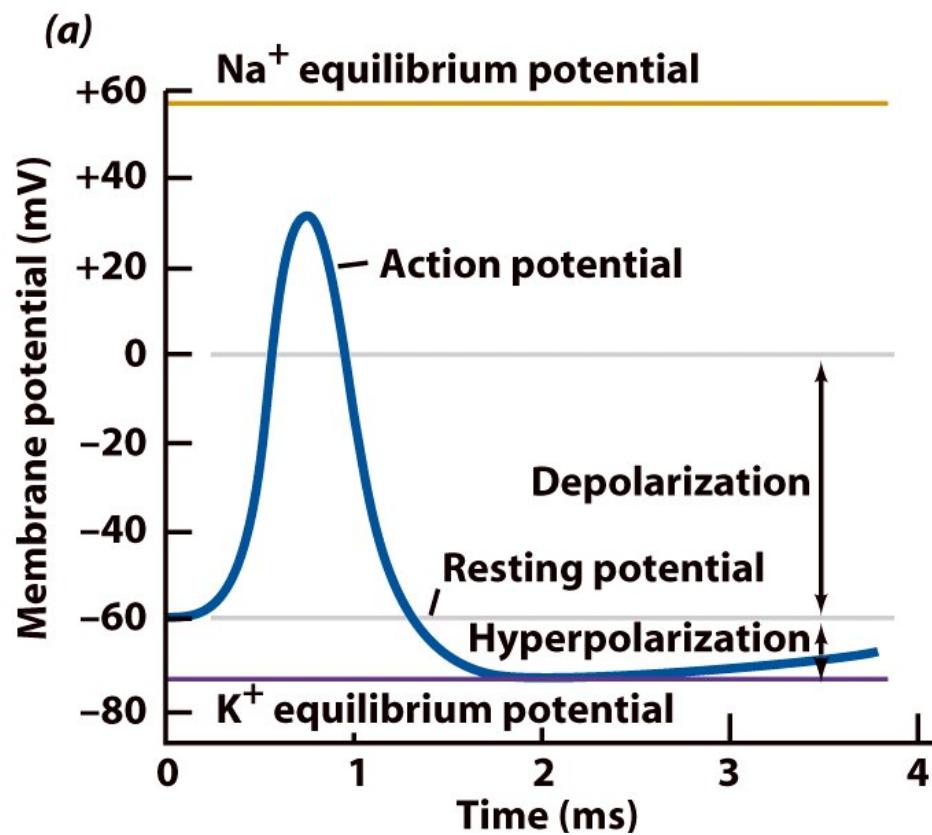
# **Ion channels are gated**

- Channels can be closed and opened upon signal:
  - Mechanosensitive channels open in response to membrane deformation: touch, sound, osmotic pressure
  - Ligand-gated channels open in response to extracellular chemical stimulus: neurotransmission
  - Signal-gated channel, open for example on intracellular binding of  $\text{Ca}^{2+}$
  - Voltage-gated channel: open in response to membrane potential change, transmission of nerve impulses

# Nerve impulses are propagated by action potentials

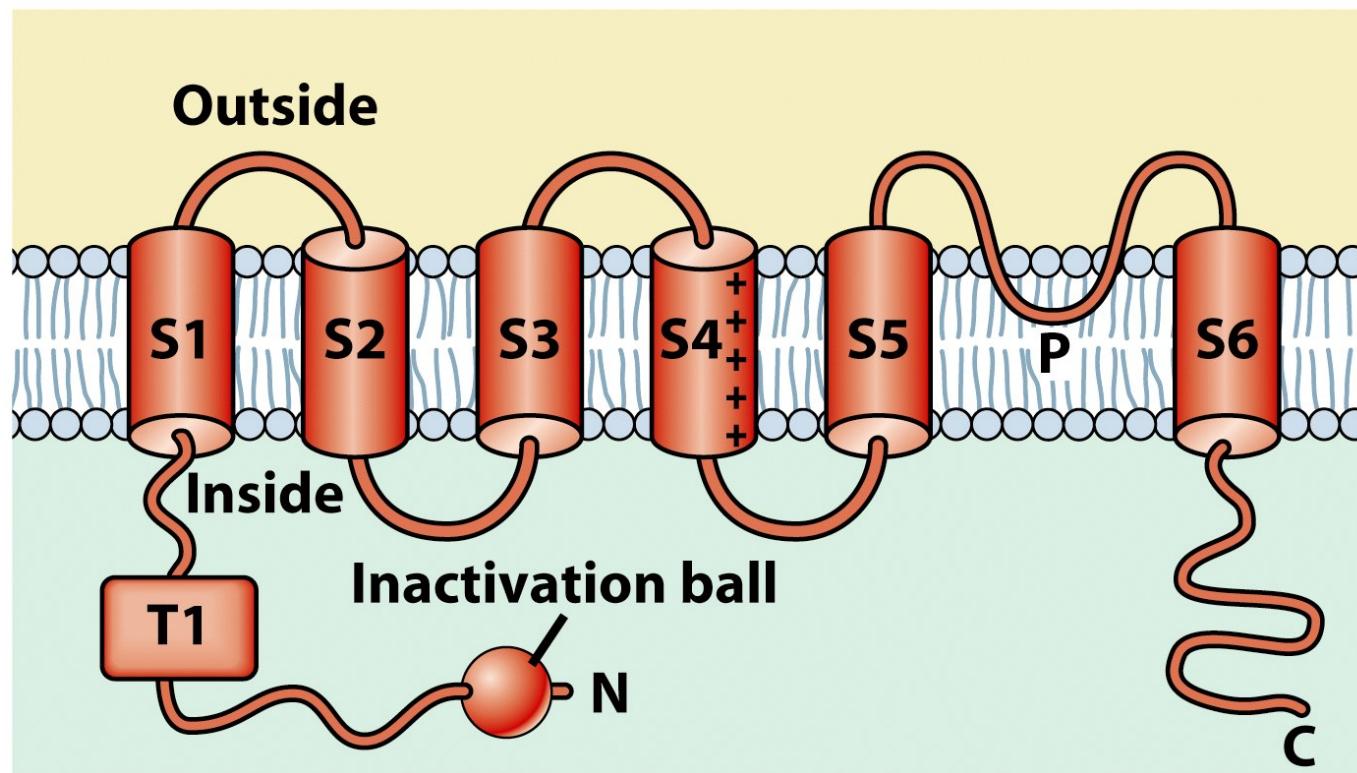
- Stimulus of neuron results in opening of  $\text{Na}^+$  channels -> local depolarization, induces nearby voltage-gated  $\text{K}^+$  to open as well (repolarization)
- Spontaneous closure of channels before  $\text{N}^+ / \text{K}^+$  equilibrium is reached
- Wave of directional transmission to nearby channels -> propagation of signal along the axon = action potential ( $\sim 10\text{m/sec}$ ), no reduction in amplitude ( $\neq$ electrical wire), can be repetitive (ms)

# Time course of an action potential



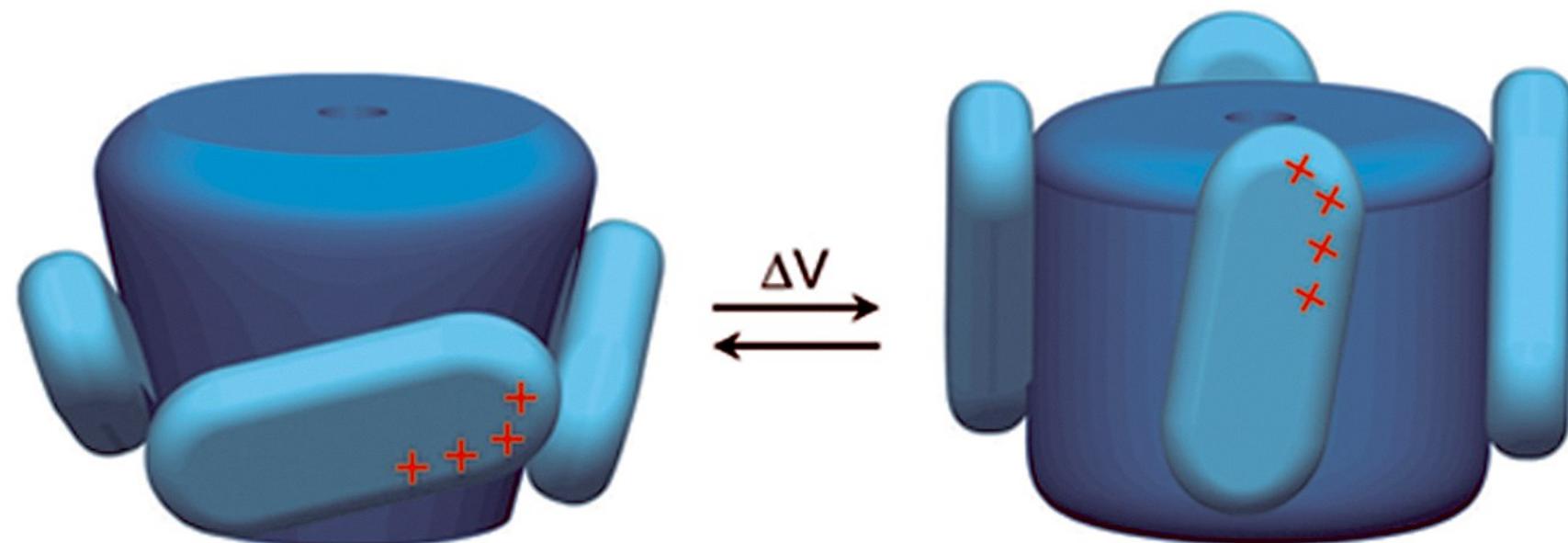
# Voltage gated K<sub>v</sub> channels

- Tetramer, S5,S6 ~KcsA, T1 domain in cytosol
- Gating by the motion of a protein paddle
- S4 helix contains 5 positive charges, spaced by 3 Aa = acts as voltage sensor



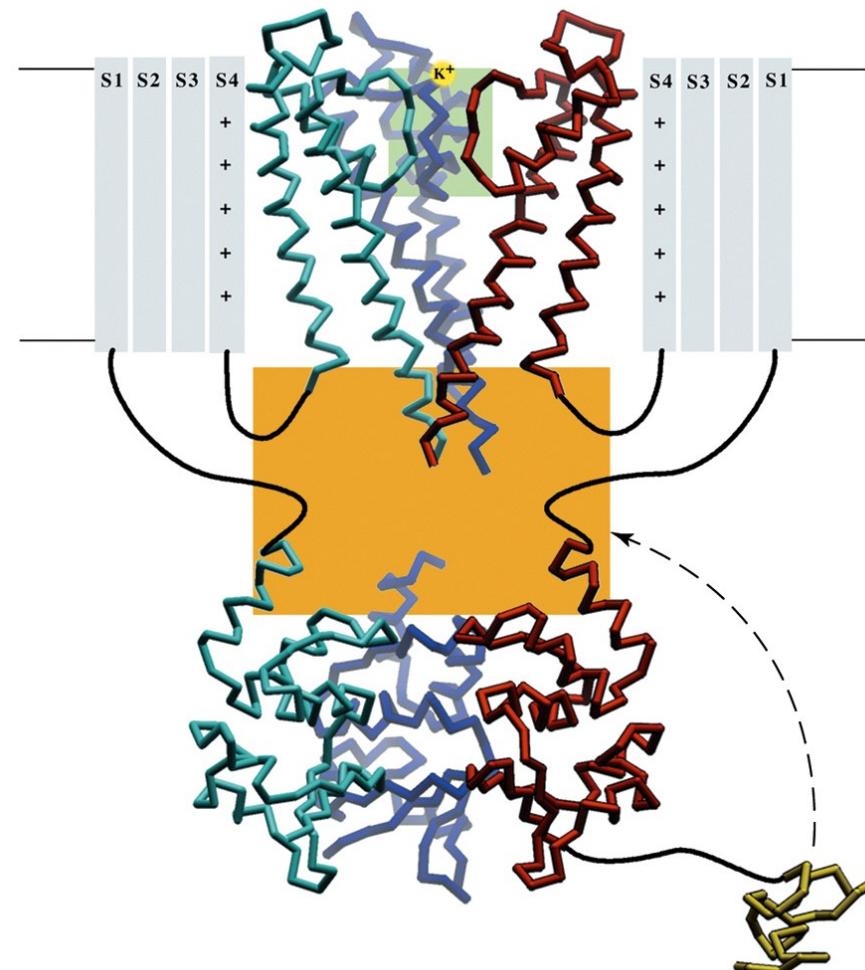
# Model for gating of $K_v$ channels

- Gating by the motion of a protein paddle, S4 helix
- Increase in membrane potential, inside becomes less negative  $\rightarrow$  baddle moves  $\rightarrow$  pore opens



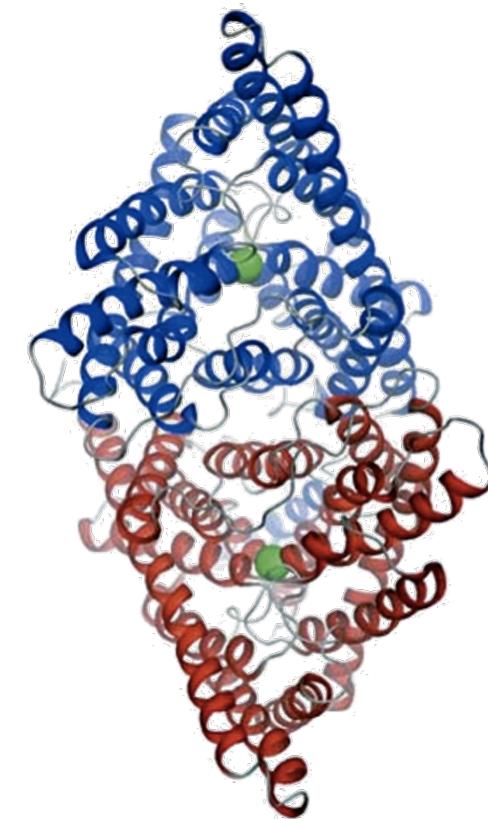
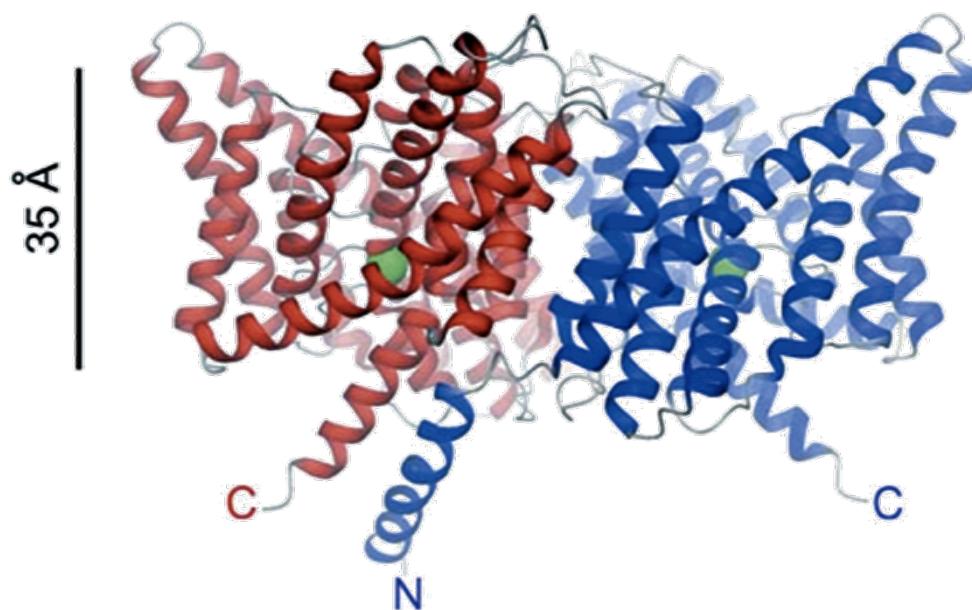
# Ion channels have two gates

- One to open and one to close
- T1 domain contains inactivation peptide that blocks pore entrance a few ms after V-dependent pore opening



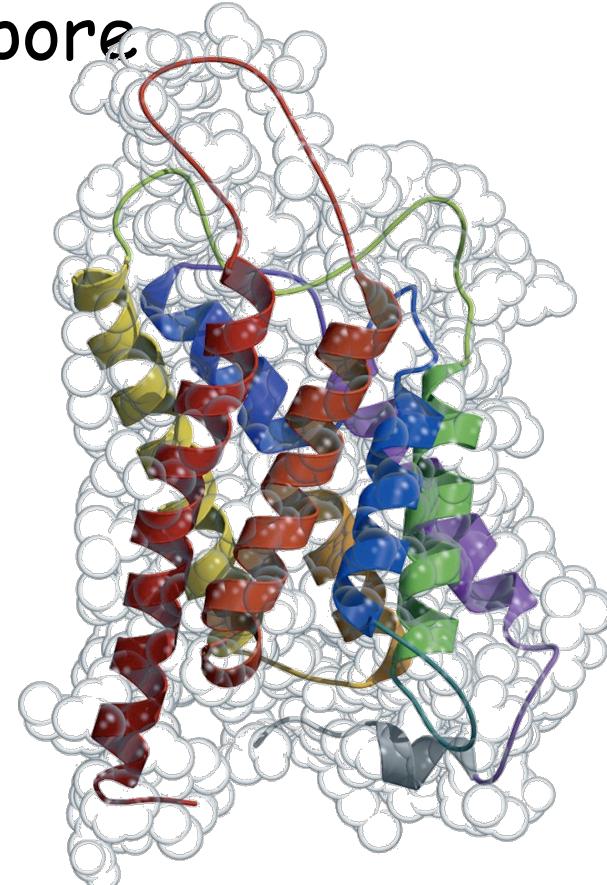
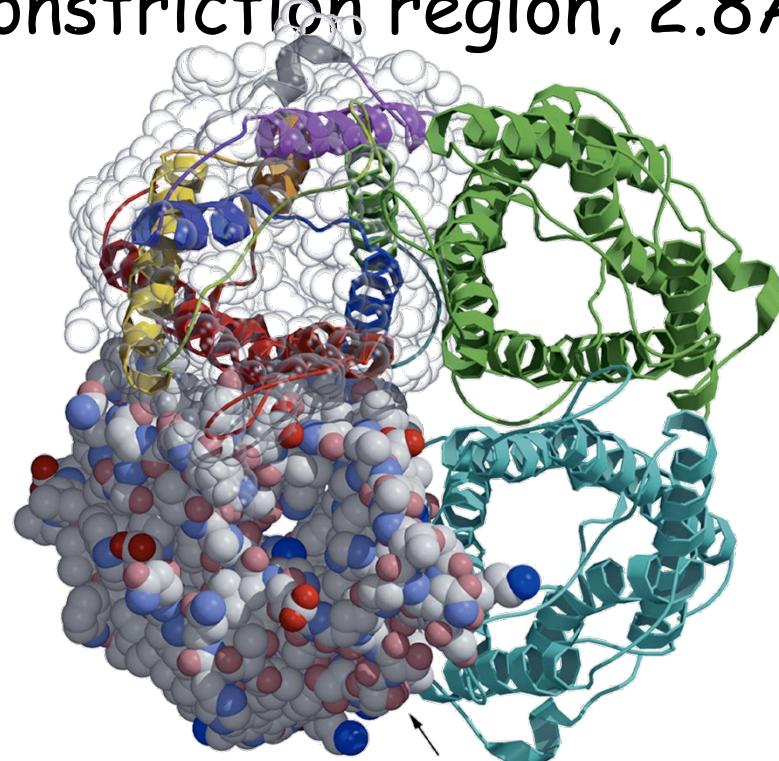
# $\text{Cl}^-$ channel differ from cation channels

- Present in all cell types. Permit transmembrane movement of chloride ions along concentration gradient:  $[\text{Cl}^-]$  extracellular: 120mM; intracellular 4mM
- Homodimer with each 18TMDs



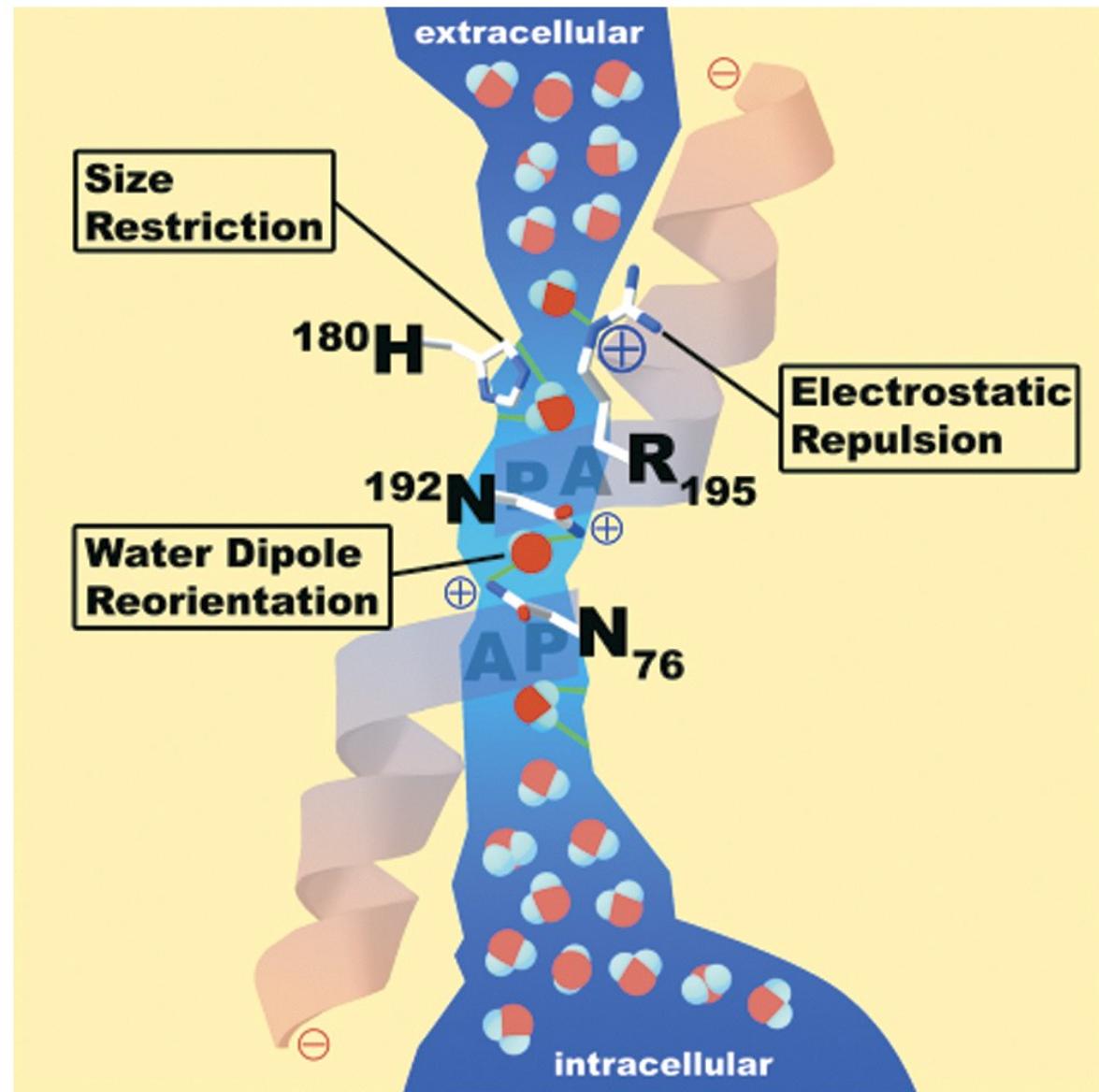
## D) Aquaporins

- Permit rapid rates of water transport across the membrane (kidney,  $\text{Hg}^{2+}$ ), ( $3 \times 10^9/\text{sec}$ ), Peter Agre (1992); 11 genes in human
- AQP1, homotetrameric glycoprotein, 6 TMDs, elongated hourglass with central pore.
- Constriction region,  $2.8\text{\AA}$  narrow



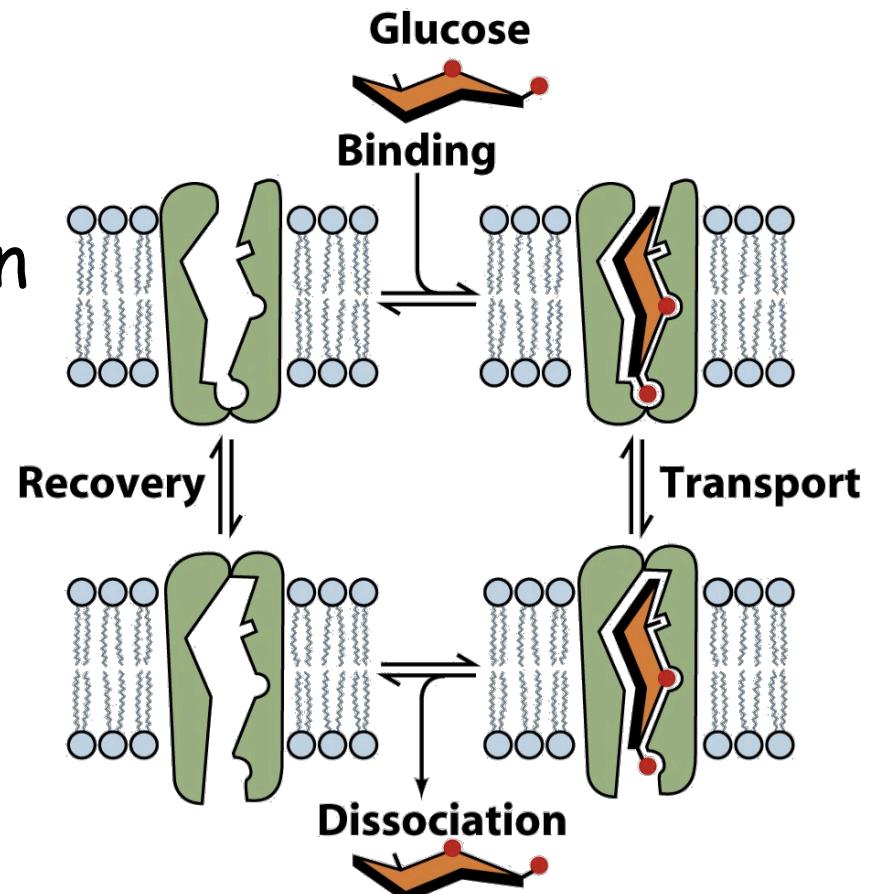
# Aquaporins (2)

- Only dehydrated water can pass
- Only 1 at the time, reorientation of dipol
- Selectivity filter similar to KscA
- No proton conducting wire allowed

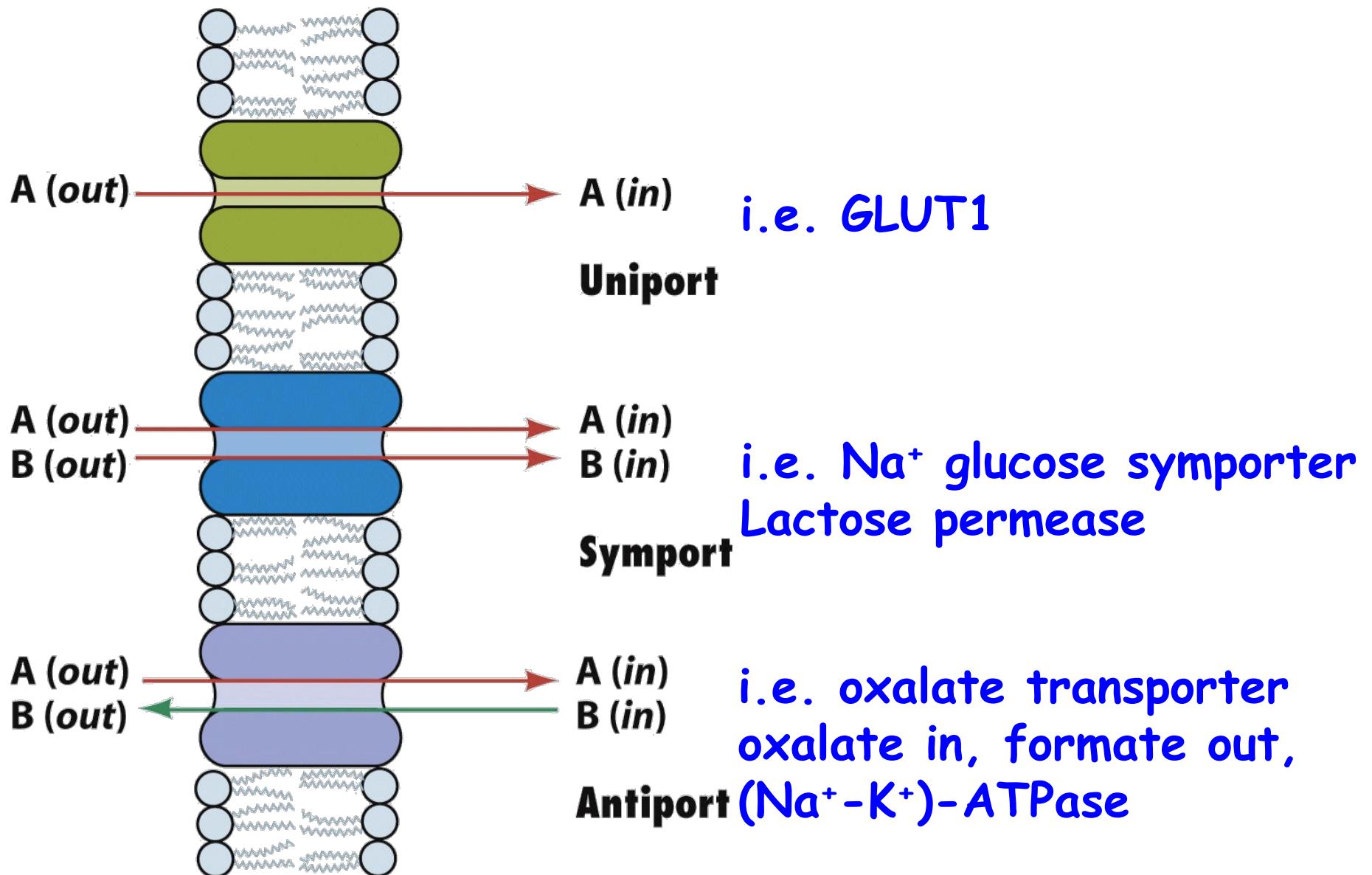


## E) Transport proteins

- Transport proteins alternate between two conformations
- Erythrocyte glucose transporter, GLUT1
- Has glucose binding sites on both sides of membrane (propyl on C1 prevents bdg to outer surface, propyl on C6 prevents bdg on inner site)
- Assymetric bdg sites
- 12 TMDs forms tetramer

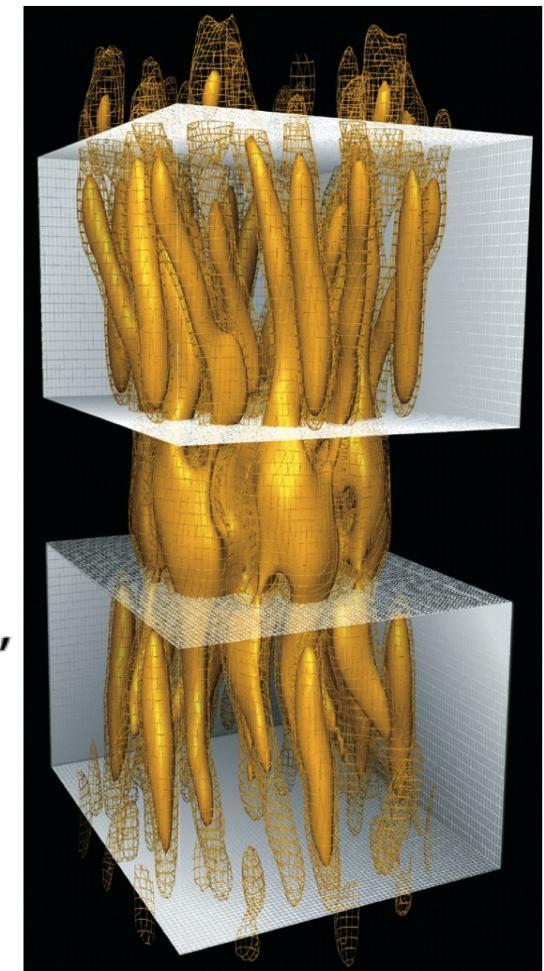
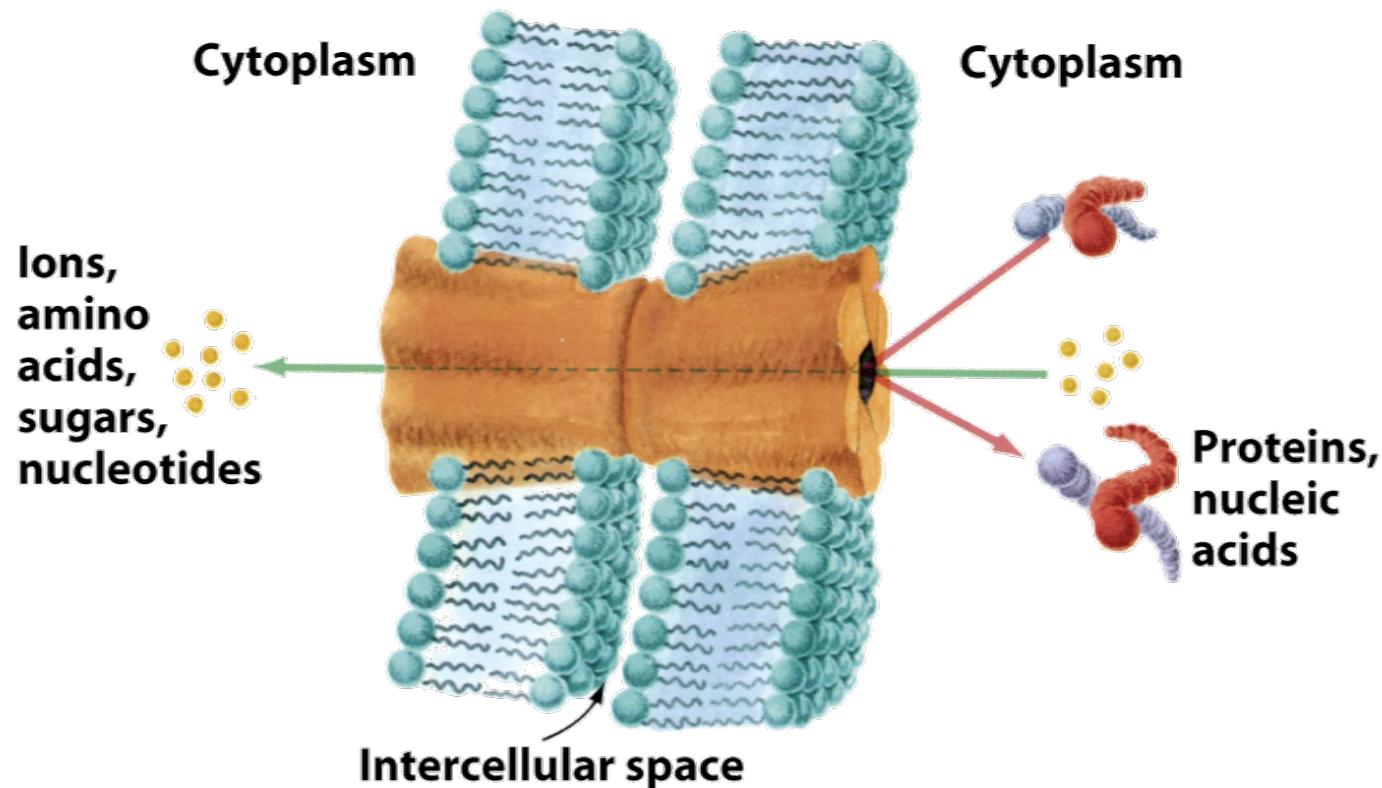


# Transport proteins (2)



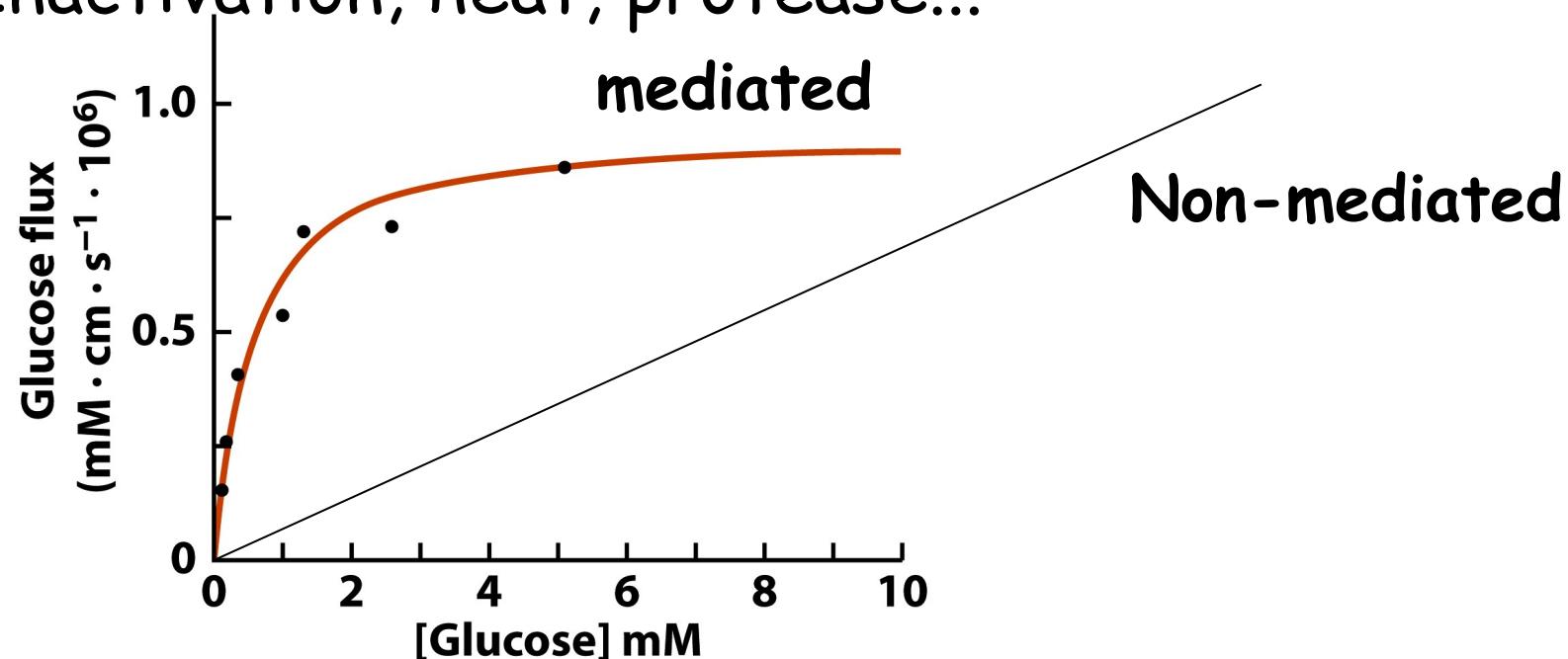
# Gap Junctions

- Form Cell-Cell connections, Connexins form gap junctions
- Cells within an organ are in metabolic and physical contact with neighboring cells through gap junctions, 16-20 Å pore, 1000 D
- Two apposed plasma membrane complexes
- Closed by  $\text{Ca}^{2+}$



# Differentiation between mediated and nonmediated transport

1. Speed and specificity: Mannitol  $\leftrightarrow$  glucose, structurally similar but uptake of glucose is much faster  $\Rightarrow$  must be mediated
2. Saturation
3. Competition, with similar substrates
4. Inactivation, heat, protease...



### 3) Active Transport

Transport against a concentration gradient,  
endergonic process, frequently requires ATP  
or other energy sources (i.e., trans-  
membrane potential = secondary active  
transport !)

Example: Glucose concentration in blood is around 5mM Glut1 cannot increase the intracellular glucose concentration in the erythrocyte above this 5mM this would require an energy consuming transporter

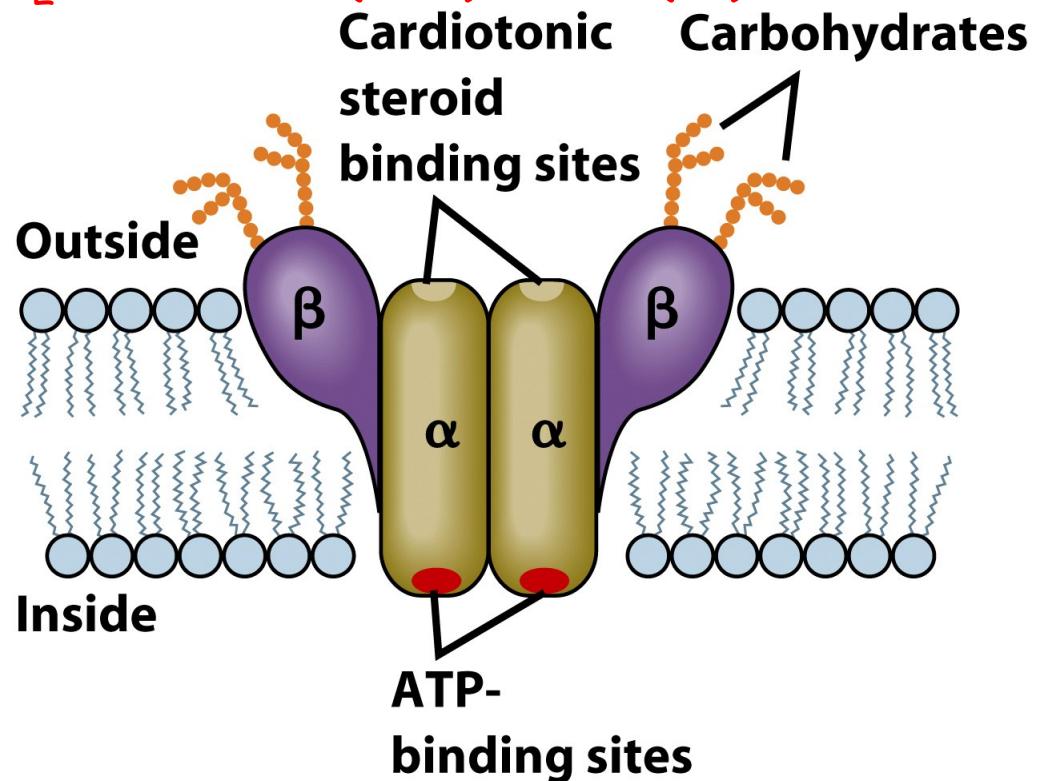
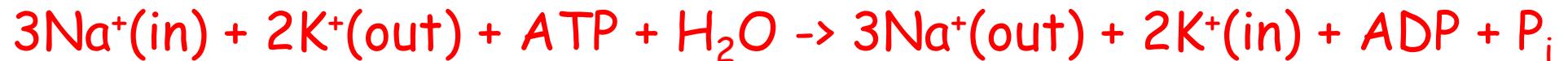
# Classes of ATPases

All consume intracellular ATP for the transport process:

1. P-type ATPases undergo internal phosphorylation during transport cycle, i.e.  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$
2. F-type ATPase, proton transports in mitochondria, synthesize ATP
3. V-type ATPase, acidify a cellular compartment: vacuole, lysosome
4. A-type ATPase transport anions across membranes
5. ABC transporters, ATP-binding cassette, transport wide variety os substances for example ions, small metabolites, lipids, drugs

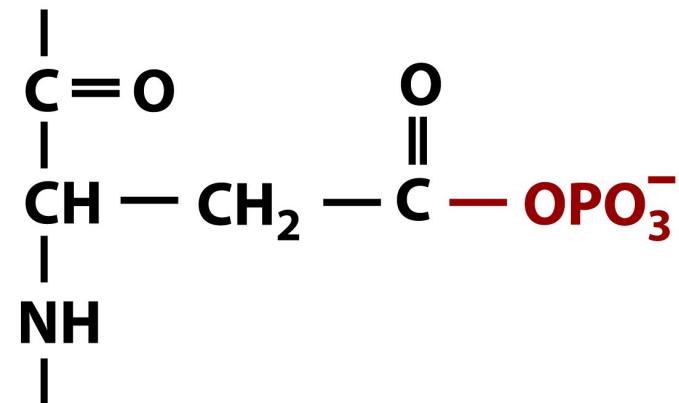
# A) $(\text{Na}^+ - \text{K}^+)$ -ATPase

- o Plasma membrane,  $(\alpha\beta)_2$  tetramer
- o **Antiporter** that generates charge separation across membrane  $\rightarrow$  osmoregulation and nerve excitability



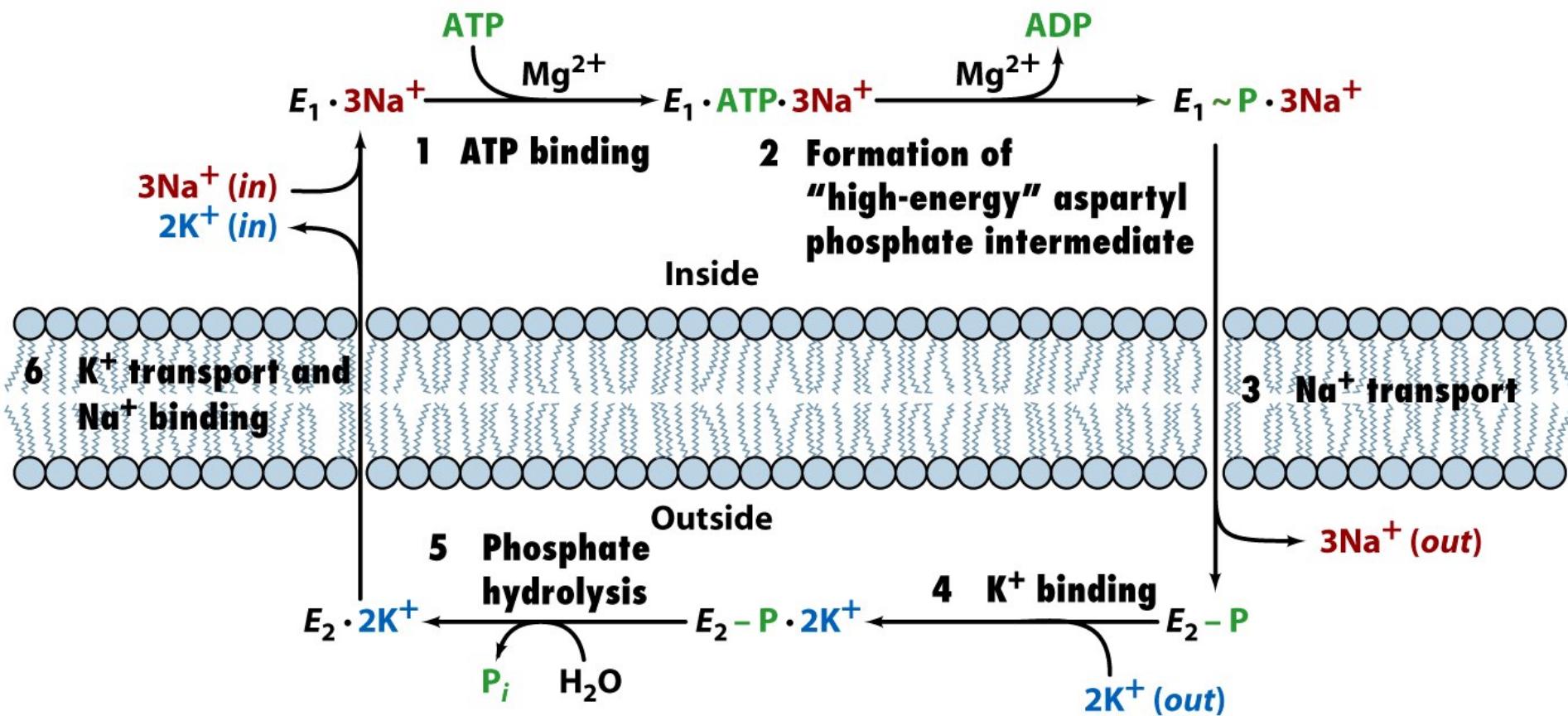
## (Na<sup>+</sup>-K<sup>+</sup>)-ATPase (2)

- o ATP transiently phosphorylates Asp to form a high energy intermediate = All P-type ATPases
- o While every single reaction step is reversible, the entire transport cycle is not



**Aspartyl phosphate residue**

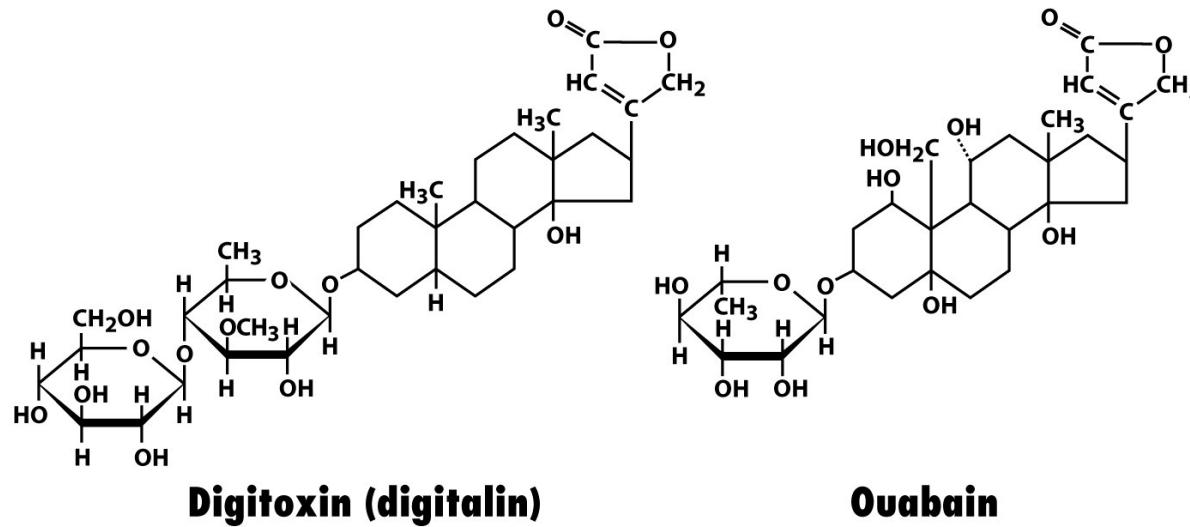
# $(\text{Na}^+ - \text{K}^+)$ -ATPase (3)



Step 5 is inhibited by cardiac glycosides  $\rightarrow$  increase in  $\text{Na}^+$

# Cardiac Glycosides

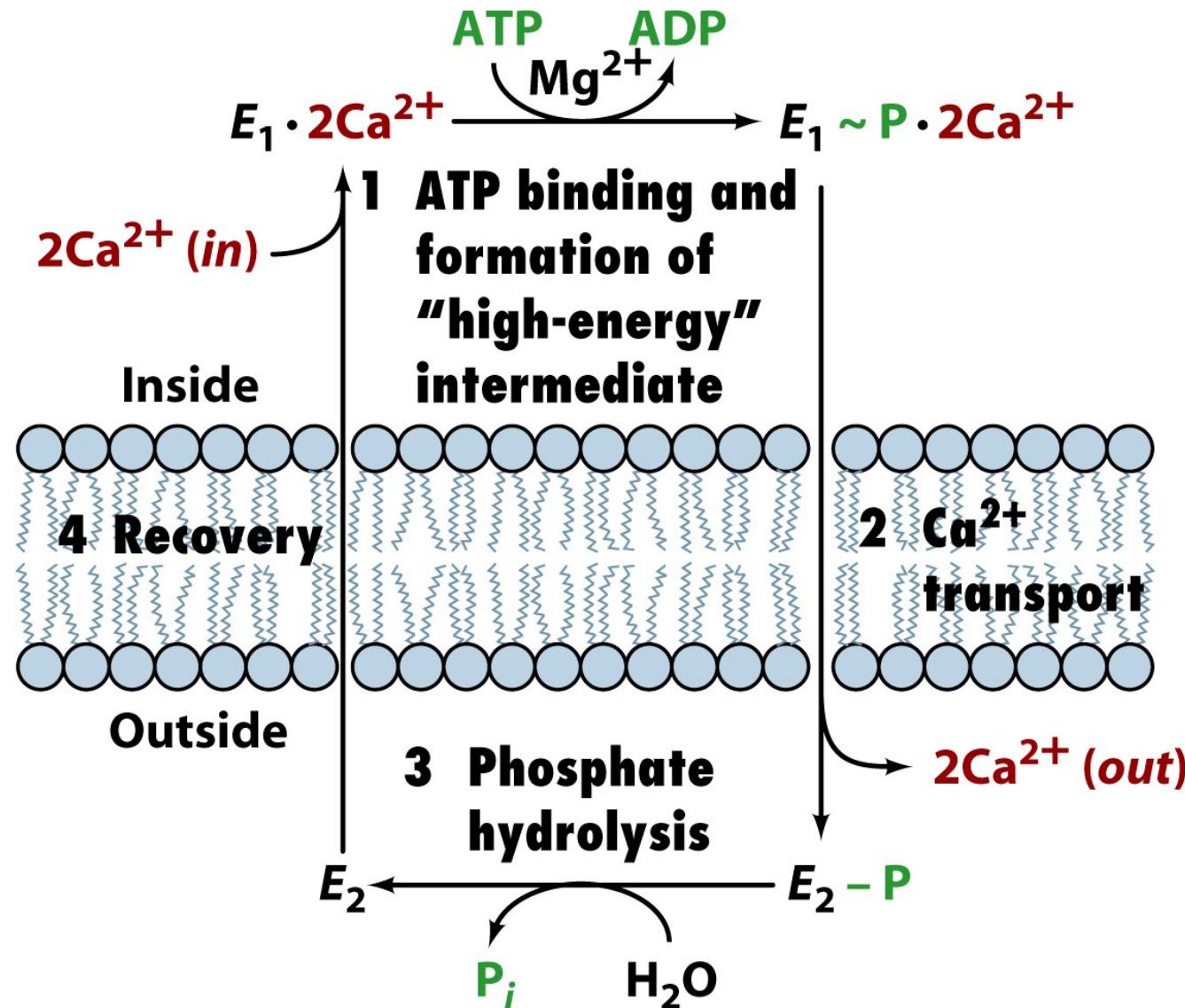
- Increase intensity of heart muscle contraction  
Digitalis contains digitoxin  
Oubain (wabane), arrow poison (East African Ouabio tree)
- Belong to the steroids, inhibit  $(\text{Na}^+ - \text{K}^+)$ -ATPase  
Block step 5 → increase intracellular  $[\text{Na}^+]$ 
  - stimulate  $(\text{Na}^+ - \text{Ca}^{2+})$  antiporter
  - increases intracellular  $\text{Ca}^{2+}$  and ER stores
  - reinforces muscle contraction (higher  $\text{Ca}^{2+}$  peaks)



## B) $\text{Ca}^{2+}$ -ATPase

- Transient  $[\text{Ca}^{2+}]$  increase triggers many processes, such as:
  - Muscle contraction
  - Neurotransmitter release
  - Glycogen breakdown
- Cytosolic  $[\text{Ca}^{2+}] \sim 0.1 \mu\text{M}$ , extracellular  $1500 \mu\text{M}$
- Concentration gradient (1000x) is maintained by active transport across the plasma membrane and the ER by  $\text{Ca}^{2+}$ -ATPases, antiport of protons

# $\text{Ca}^{2+}$ -ATPase (2)



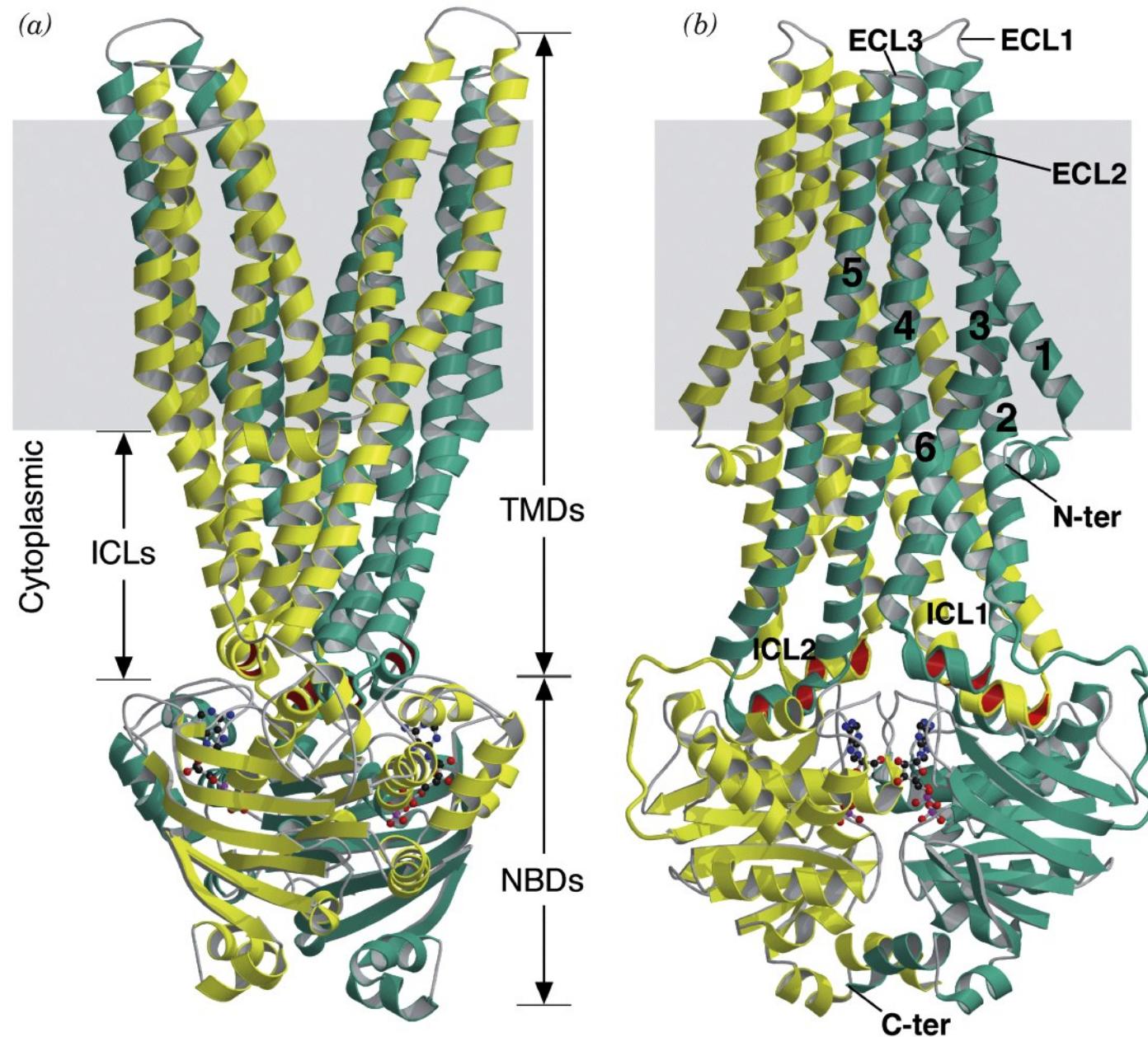
## C) ABC transporters are responsible for drug resistance

- o If anti-cancer drugs do not show any positive effect, this is frequently due to overexpression of the P-glycoprotein, a member of the ABC transporter superfamily or **multidrug resistance (MDR) transporters**
- o Built from 4 modules: 2x cytoplasmic nucleotide binding sites, 2x TMDs with 6 helices each,
- o In bacteria these 4 domains can be coded by 2 or 4 single proteins; in eukaryotes all 4 domains on a single protein
- o In bacteria, ABC transporters can act as importers or exporters, in eukaryotes only as exporters (?)

# Structure of an ABC transporter

- Sav1866 from Staphylococcus

- Homodimer, intertwined subunits

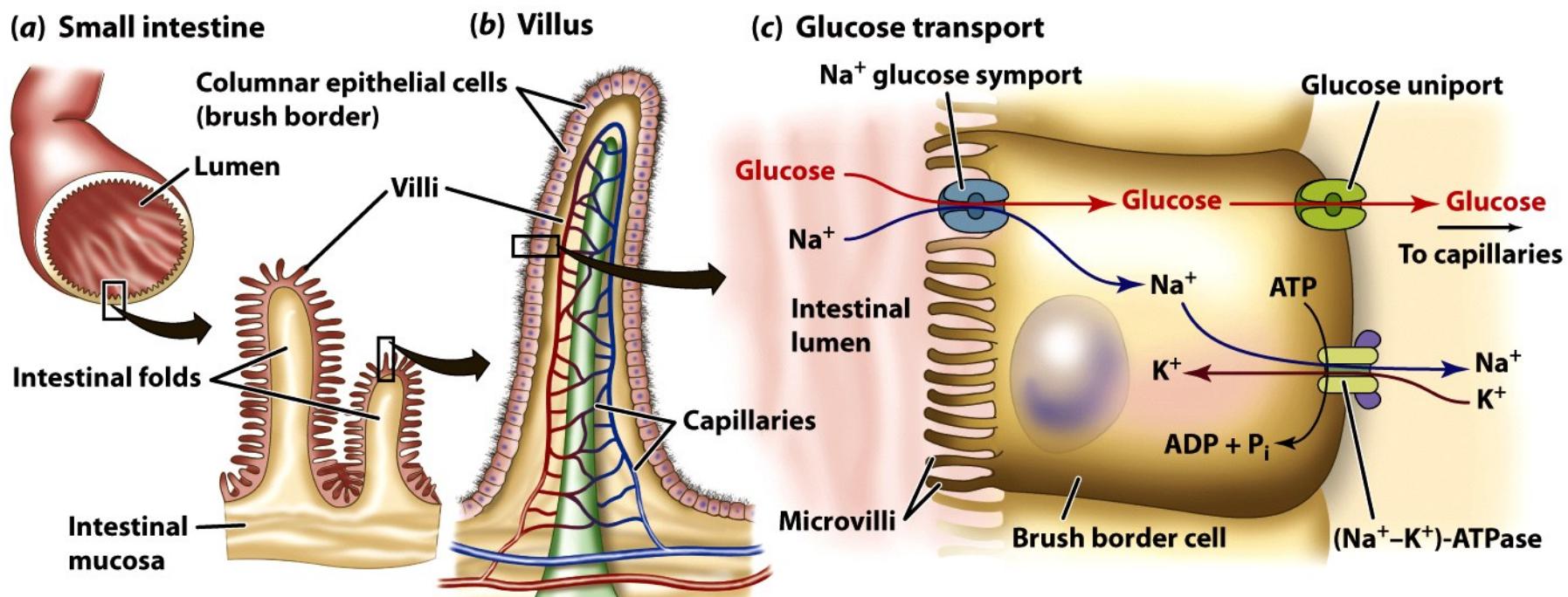


# CFT is an ABC transporter

- o CFTR, cystic fibrosis transmembrane conducting channel, is the only ion transporting of the ca 100 known ABC transporters (see Box 3-1)
- o Allows Cl<sup>-</sup> ions to flow out of the cell, by ATP hydrolysis
- o More than 1000 mutations known, most frequent is Phe508 deletion, protein is functional, but improperly folded and degradet in the ER before transport to PM
- o Homozygous have lung problems, thick mucus in the airways -> suffer from chronic lung infections, early death

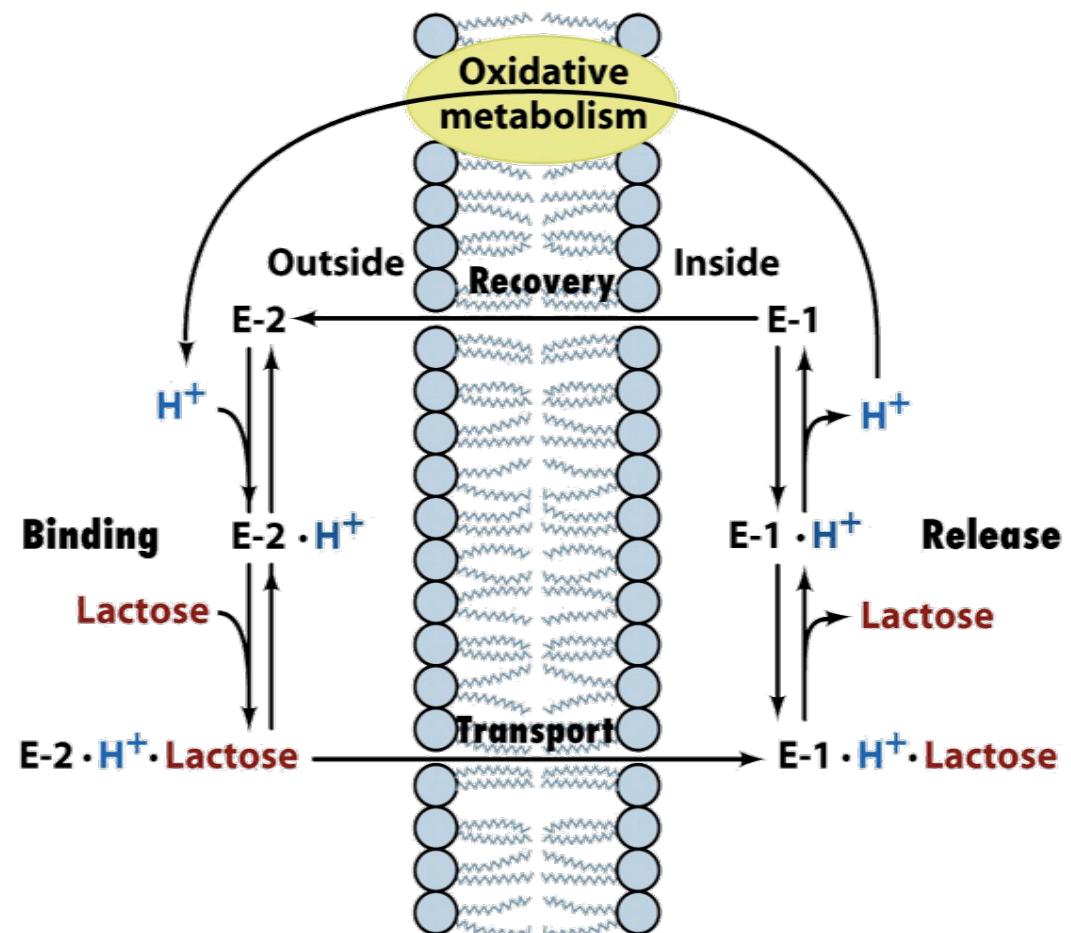
## D) Ion Gradient-Driven Active Transport

- Free energy of the electrochemical gradient can be utilized to power active transport for example uptake of glucose by symport with  $\text{Na}^+$  (which is transported down the gradient)



# Lactose permease requires a proton gradient

- *E. coli* lactose permease (galactoside permease), utilizes proton gradient to symport lactose and H<sup>+</sup>



# The lactose permease

- High affinity Bdg site extracellular
- Low affinity intracellular
- Binds sugar only in combination with proton

