Biochemistry II (BB 422/622)

OUTLINE

Review of 421
Goals of 422
Review of chemical principles
- Thermodynamics
- C/O cycles
- Overview of Metabolism
- ATP cycles

Energy Coupling
Chemical Reactivity
Bioenergetics
Membranes and Transport
- Review of membrane structure, dynamics, & proteins
- **Membrane transport**
  - **Energetics**
  - **Facilitative Diffusion**
  - **Active Transport**
    - Primary
    - Secondary

Examples:
- Facilitative Diffusion
  - Ionophore
  - Maltoporins
  - GLUT1 transporter
  - Aquaporin
  - Selective ion channel for potassium (K-channels)
- Active Transport
  - Primary (1°)
    - Na^+/K^+ ABC
  - Secondary (2°)
    - Na^+/Glc

Biological Membrane Transport

"Crossing the Berlin Wall of the cell - Membrane Transport"

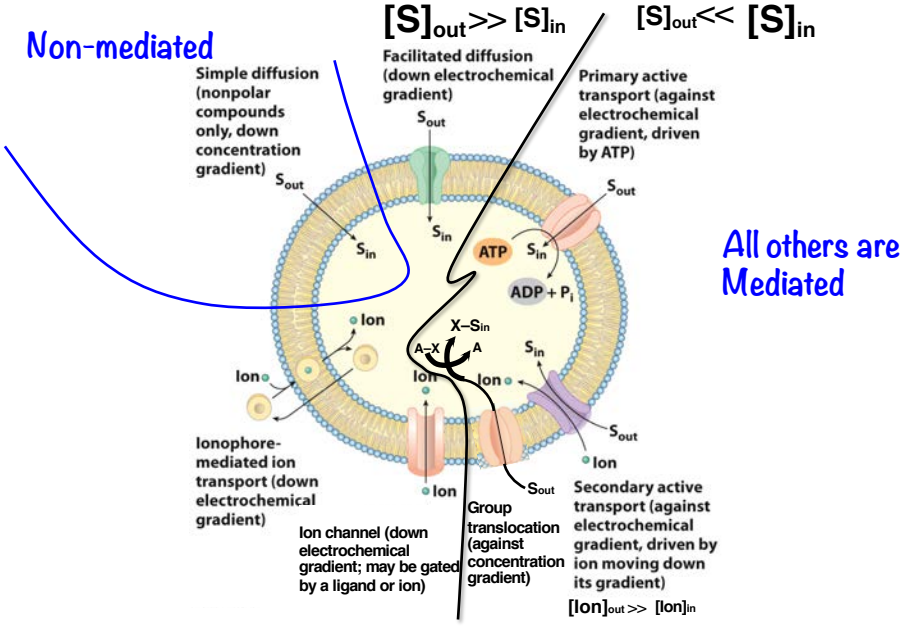
Reminder of membrane structure, membrane proteins, and dynamics
Membrane transport
- **Energetics**
- **Types**

Membrane Transport

Dr. Kornberg: "The Berlin Wall of the Cell"

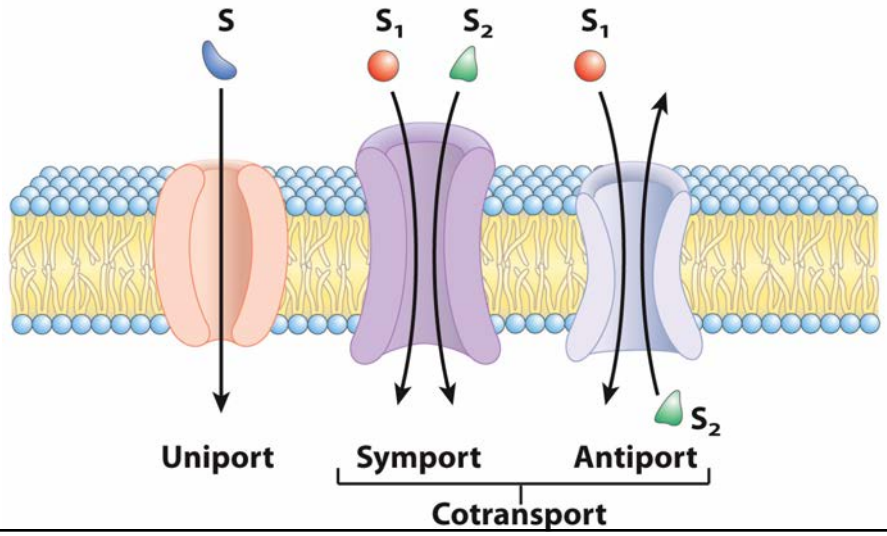
- Cell membranes are permeable to small nonpolar molecules that passively diffuse through the membrane.
- Passive diffusion of polar molecules involves desolvation and thus has a high activation barrier, unless desolvation energy is lowered.
- Transport across the membrane can be facilitated by proteins, which provide an alternative diffusion path.
- Such proteins are called transporters or permeases.

Types of Membrane Transport



Membrane Transport

Aside from any energy concerns: Three Classes of Transport Systems



Membrane Transport

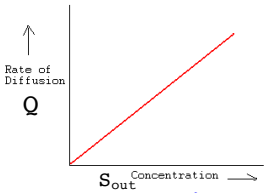
How do you experimentally determine the kind of transport?

Non-mediated Simple diffusion

Mediated Facilitated diffusion, ionophore mediated, active transport

Diffusion is governed by **Fick's law of diffusion**:

$$Q = DA\frac{P_1 - P_2}{L}$$



Q = rate of diffusion.

D = diffusion coefficient

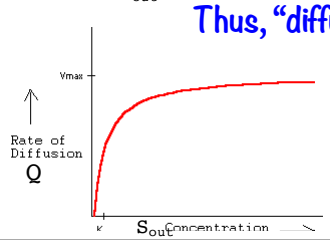
A = area across which diffusion occurs

$[S_{out}]_{(P_1)}$ and $[S_{in}]_{(P_2)}$= concentrations on each side of membrane.

L = thickness of the membrane.

DA/L is the permeability coefficient.

Thus, "diffusion" is Non-mediated



Mediated behaves like saturation kinetics

$$C + S_{out} \rightleftharpoons CS \rightleftharpoons C + S_{in}$$

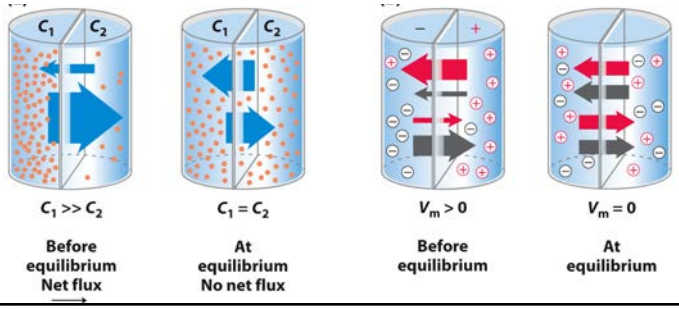
$$Q = \frac{Q_{max}[S_{out}]}{K_d + [S_{out}]}$$

Thus, "diffusion" is Mediated

Membrane Transport

What is this electrochemical gradient?

- Transport across a membrane must be energetically favorable. There are two types of energies at play:
  - Concentration dependence: The solute moves toward chemical equilibrium across the membrane.
  - Electrical dependence: The solute moves toward charge equilibrium across the membrane.



This is why we say "down" or "against" the **electro-chemical** gradient.

How is this gradient quantified?

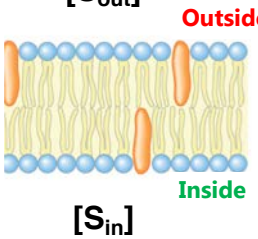
Membrane Transport

Energetics

Transport can be considered like a chemical reaction with regard to mass action of S_{out} to S_{in}

$$S_{out} \rightleftharpoons S_{in}$$

[S_{out}]



[S_{in}]

$$\Delta G' = \Delta \boldsymbol{G}^{o'} + RT\ln \frac{[S_{in}]}{[S_{out}]}$$

But, $\Delta \boldsymbol{G}^{o'} = \mathbf{0}$ because there is no chemical reaction:

$$\Delta G' = RT\ln \frac{[S_{in}]}{[S_{out}]}$$

If $[S_{out}] > [S_{in}]$, $\Delta \boldsymbol{G}'$ is $\ominus$

If $[S_{out}] < [S_{in}]$, $\Delta \boldsymbol{G}'$ is $\oplus$

But, membranes in biology have a difference in charge (inside different from outside).
AND, if S is charged, we must account for this:

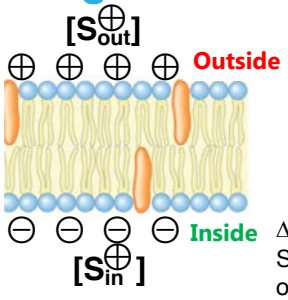
$$\Delta G' = RT\ln \frac{[S_{in}]}{[S_{out}]} + z\mathcal{F}\Delta\psi$$

Where "z" is the charge on S, and $\Delta\psi$ is the membrane electrical potential in volts

Membrane Transport

Energetics

$S_{out} \rightleftharpoons S_{in}$



$$\Delta G' = RT\ln\frac{S_{in}}{S_{out}} + z\mathcal{F}\Delta\psi$$

Where "z" is the charge on S, and $\Delta\psi$ is the membrane electrical potential in volts; simply called the "membrane potential"

$\Delta\psi$ = charge difference "in" versus "out"
So, conventionally the direction of transport is considered out-to-in, as written in the reaction above.

If its more negative in than out, $\Delta\psi$ is ⊖ (as depicted)

And, if its more positive in than out, $\Delta\psi$ is ⊕

Now, if $\Delta\psi$ is negative, and S has a positive charge (z is +1, as depicted), then $z\mathcal{F}\Delta\psi$ makes a contribution to $\Delta G'$ making it even more negative, i.e., favorable.

As a further consequence, if $\Delta\psi$ is maintained, then at equilibrium **$[S_{in}] > [S_{out}]$**.

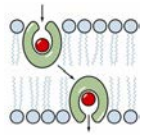
Membrane Transport

Facilitative Diffusion

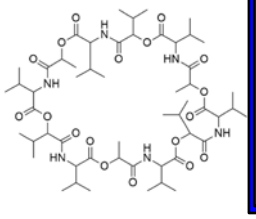
Membrane Transport

Examples of Facilitative Diffusion

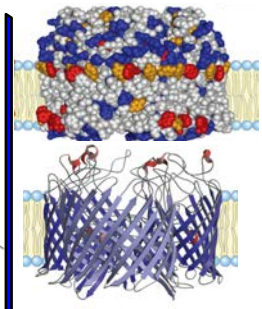
(including ionophore mediated)



Ionophore

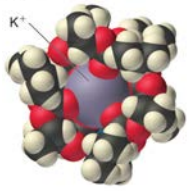


Valinomycin

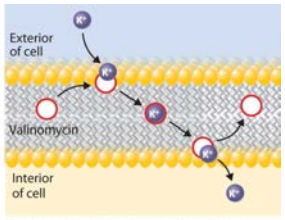


- Proteins of the *E. coli* outer membrane
- Maltoporin (derived from PDB ID 1MAL) is a maltose transporter (a trimer; each monomer consists of 16 β strands).

Maltoporin



(a) K⁺–valinomycin complex



(b) Transport of K⁺ across a membrane

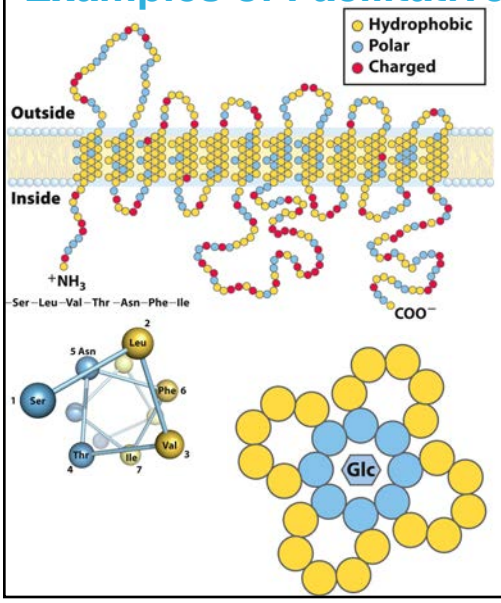
How it works,

- The six oxygen atoms of the ionophore interact with the bound K⁺ ion replacing the O-atoms of water of hydration.
- Each valinomycin molecule is able to carry about 10000 K⁺ ions per second – Very rapid transport rate!

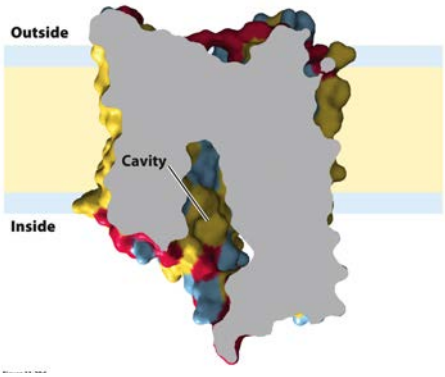
NB. Valinomycin can not carry sodium ions because they are small and there fore can not simultaneously interact with six O-atoms – thus being energetically unfavourable

Membrane Transport

Examples of Facilitative Diffusion



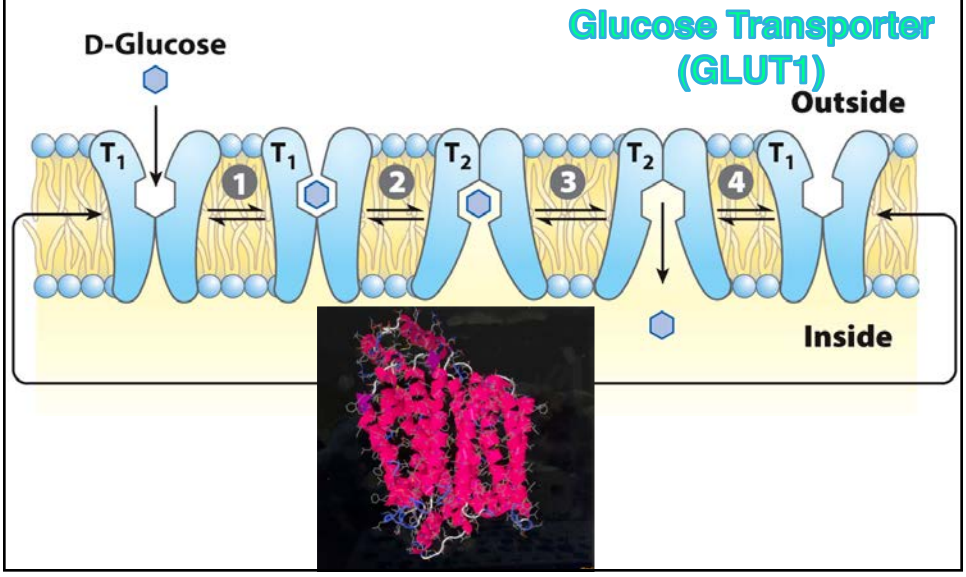
Glucose Transporter (GLUT1)



Membrane Transport

Examples of Facilitative Diffusion

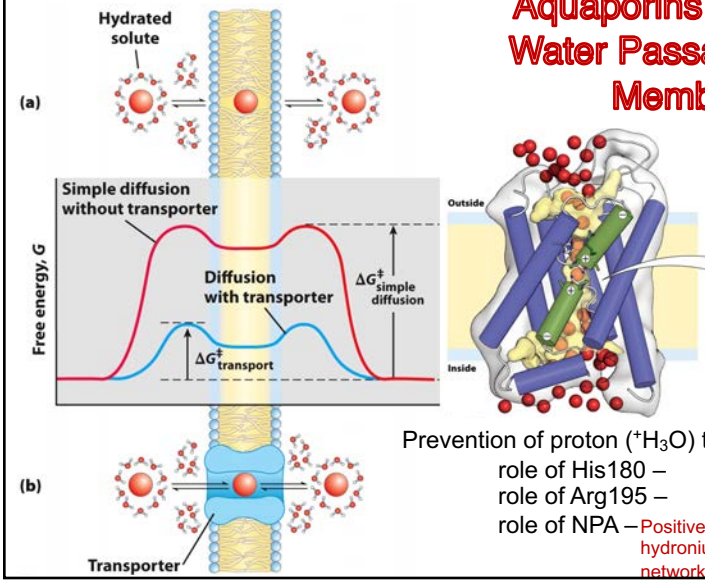
Glucose Transporter (GLUT1)

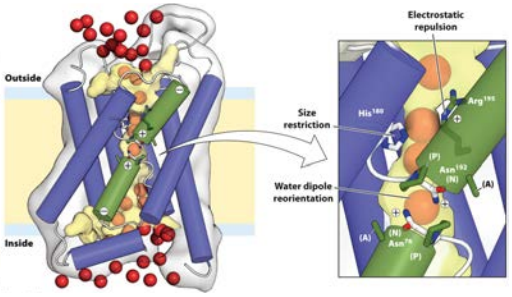


Membrane Transport

Examples of Facilitative Diffusion

Aquaporins Allow Rapid Water Passage Through Membranes





Prevention of proton ($^{+}H_3O$) transport:

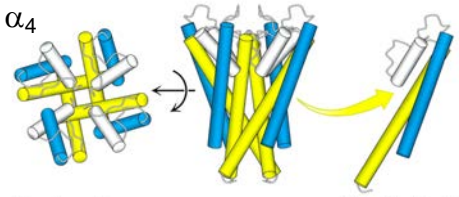
role of His180 – Gates the size of pore at 2.8 Å

role of Arg195 – Repels hydronium ions

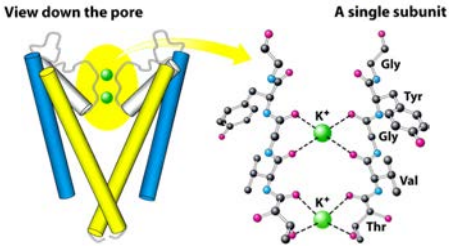
role of NPA – Positive poles at ends of helices repels hydronium ions, but also breaks up network of water molecules

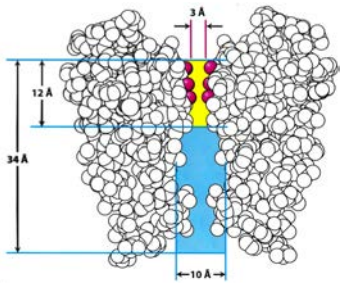
Membrane Transport

Examples of Facilitative Diffusion



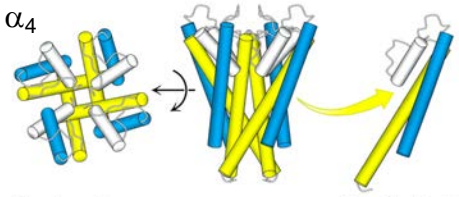
Potassium Channel KcsA K+ allows specific passage of this ion through membranes





Membrane Transport

Examples of Facilitative Diffusion



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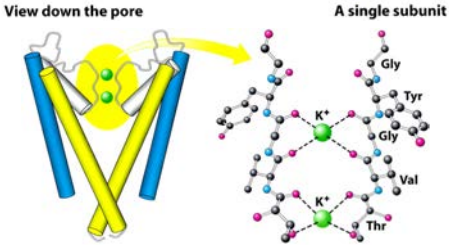
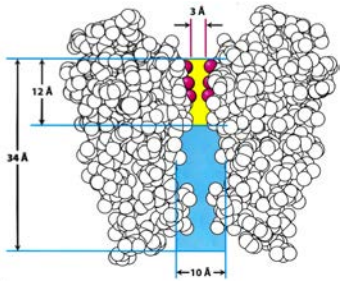
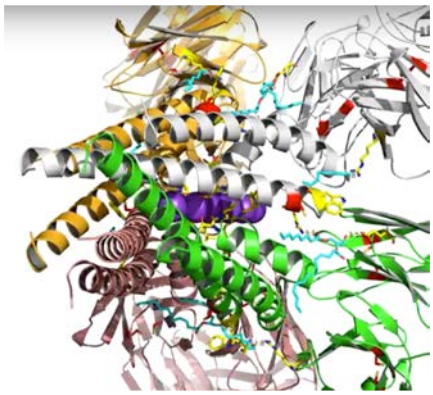




Membrane Transport



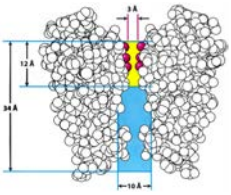
K channel

https://www.youtube.com/watch?v=IXPmgprE8rg&feature=youtu.be

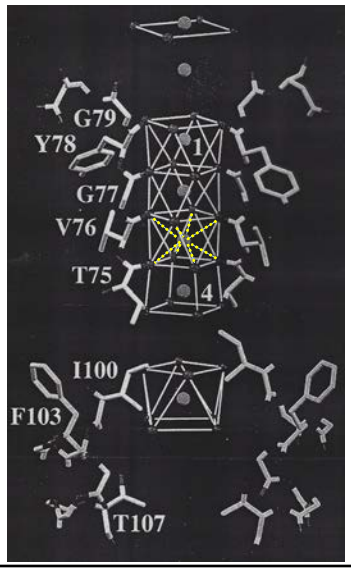
KcsA K^+ channel in complex with a monoclonal Fab antibody fragment

Membrane Transport

Examples of Facilitative Diffusion



This picture is not quite correct as K^+ makes eight coordination bonds; this was revealed by the high resolution structure

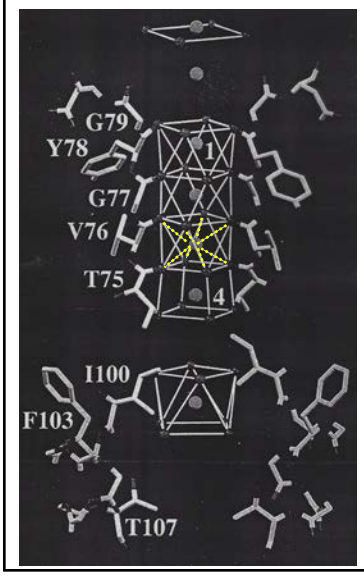


How is the >100X specificity for K^+ achieved?

Ion	Radius (Å)
Li^+	0.6
Na^+	1.0
K^+	1.3
Rb^+	1.5
Cs^+	1.7

Membrane Transport

Examples of Facilitative Diffusion



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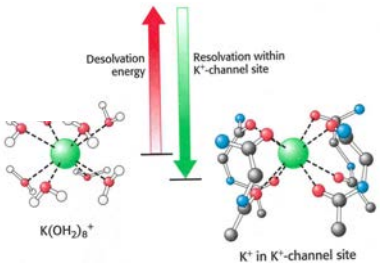
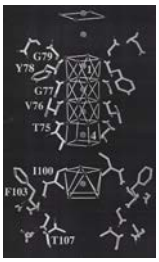


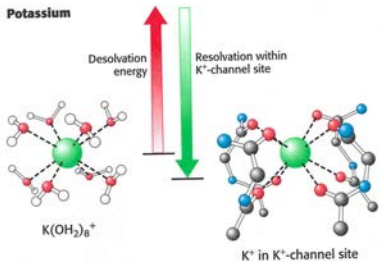
Membrane Transport

Examples of Facilitative Diffusion

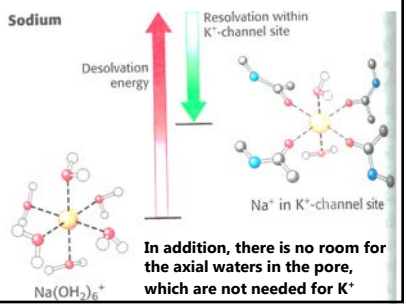


Ion	Radius (Å)	Energy of dehydration (kcal/mole)
Li^+	0.6	– 98
Na^+	1.0	– 72
K^+	1.3	– 55
Rb^+	1.5	– 51
Cs^+	1.7	– 47

This energetic difference is due to **Coulomb's** law; as the distance of water to the positively charged nucleus is shorter for sodium than potassium.



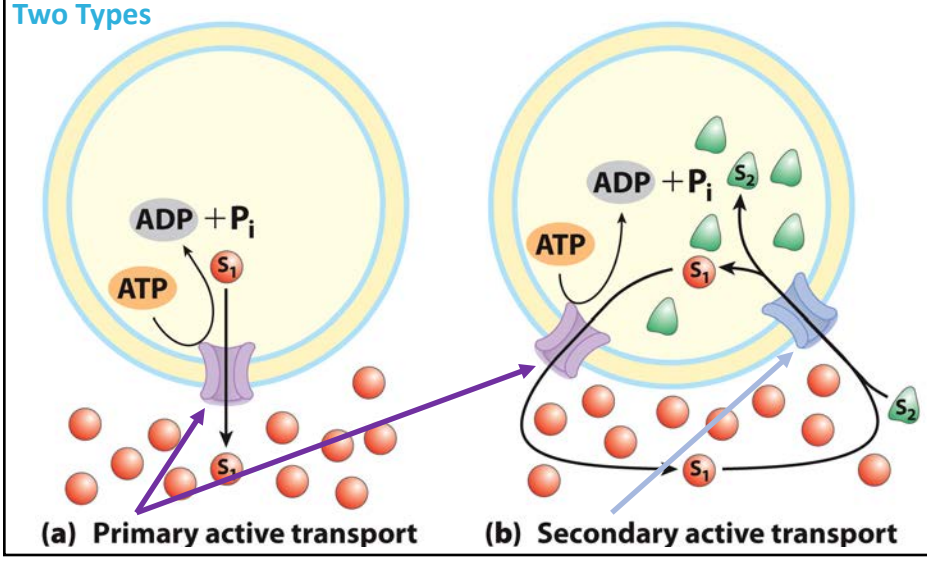
While specificity for Na^+ over K^+ can be achieved by the size of the binding site, that won't work the other way around. To get specificity of K^+ over Na^+, the protein takes advantage of the higher (30%) energy of dehydration. It takes more energy to dehydrate Na^+ than K^+ and the protein doesn't make as good bonds to Na^+ ion as does water shell (see off-set oxygens in polygon).



Active Transport

Examples of Active Transport

Two Types



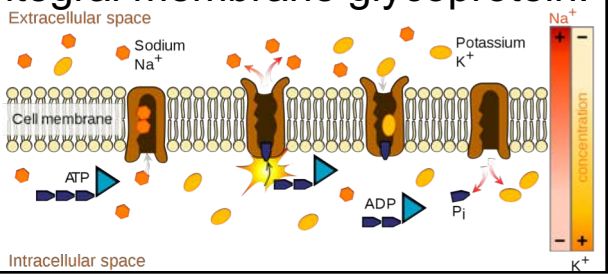
Membrane Transport

One of the best and most important examples of PRIMARY Active Transport:

The **sodium–potassium (Na^+–K^+) pump** is a primary active anti-porter.

Found in all animal cells.

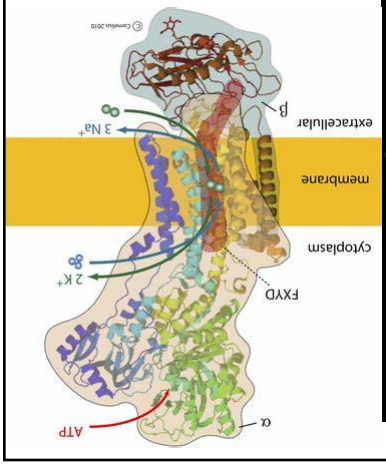
The pump is an integral membrane glycoprotein.

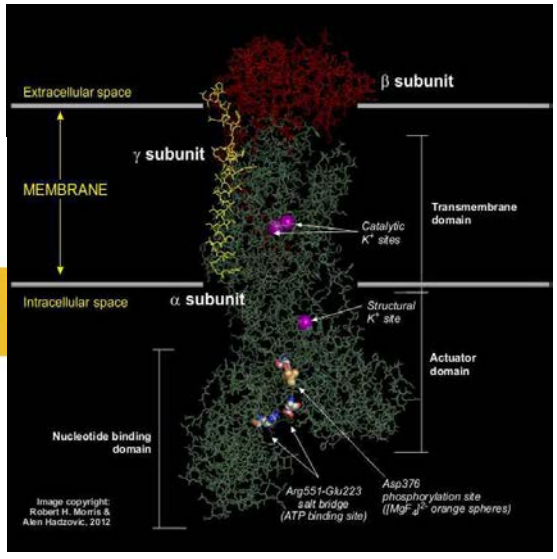


Membrane Transport

Sodium–potassium (Na^+–K^+) pump

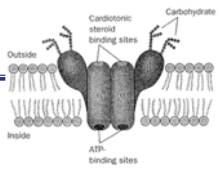
$\alpha_2\beta_2\gamma$ pentamer

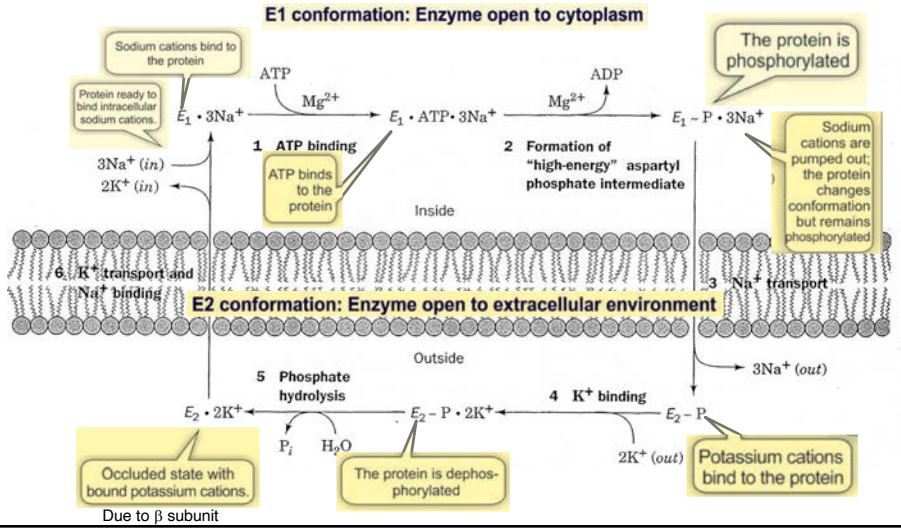




Membrane Transport

Sodium–potassium (Na^+–K^+) pump

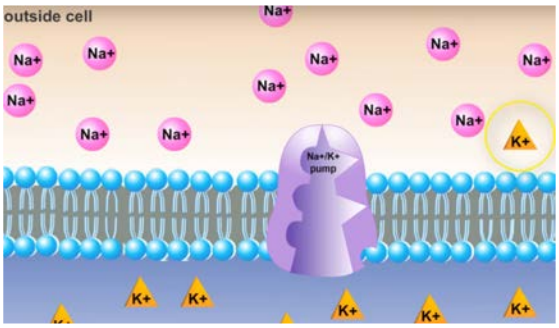




Due to β subunit

Membrane Transport

Sodium–potassium (Na^+–K^+) pump



Potassium binding causes change to E1, which then causes P_i hydrolysis

https://www.youtube.com/watch?v=_bPFKDdWlCg

Start at 0:30 – 2:00