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, and 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

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Biological Membrane Transport

"Crossing the Berlin Wall of the cell - Membrane Transport"
– Dr. Kornberg

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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 that provide an alternative diffusion path.
- Such proteins are called transporters or permeases.

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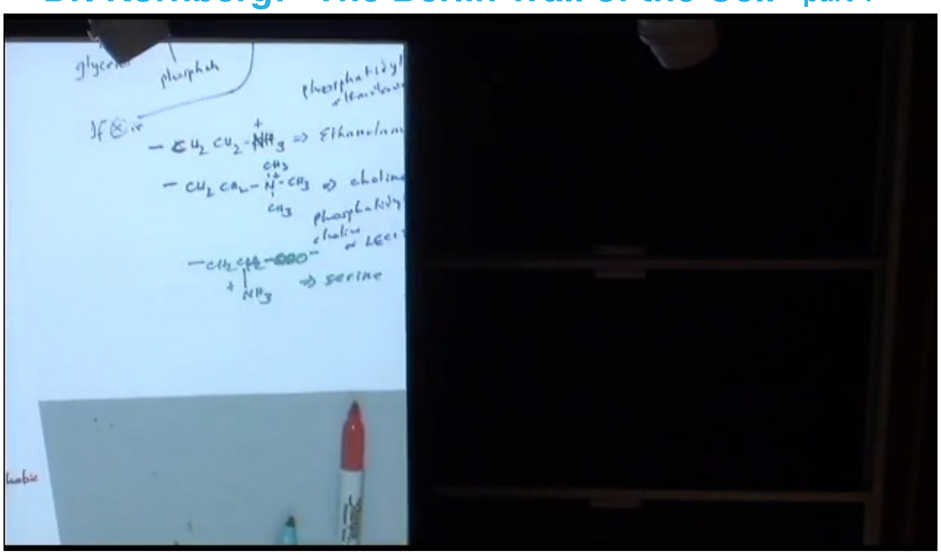
Membrane Transport

Dr. Kornberg: “The Berlin Wall of the Cell”-part 1



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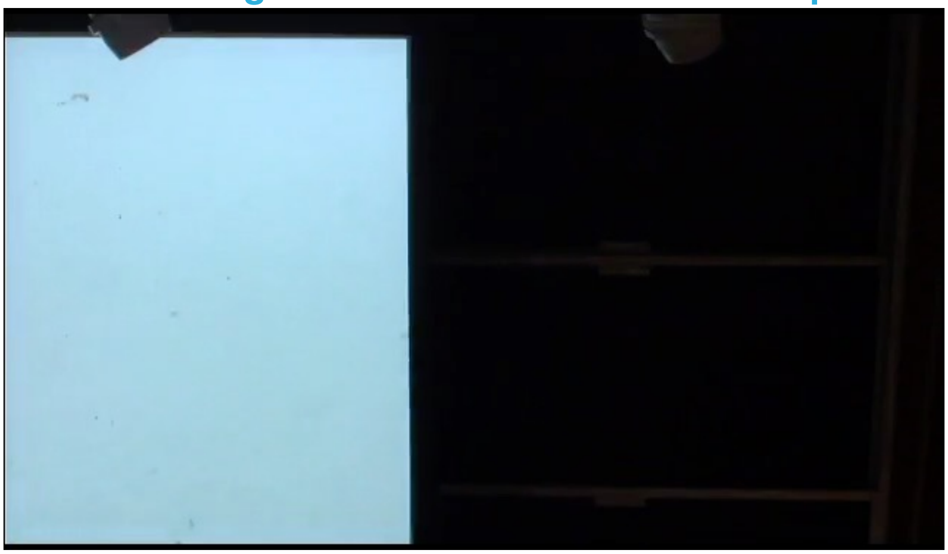
Membrane Transport

Dr. Kornberg: “The Berlin Wall of the Cell” -part 2



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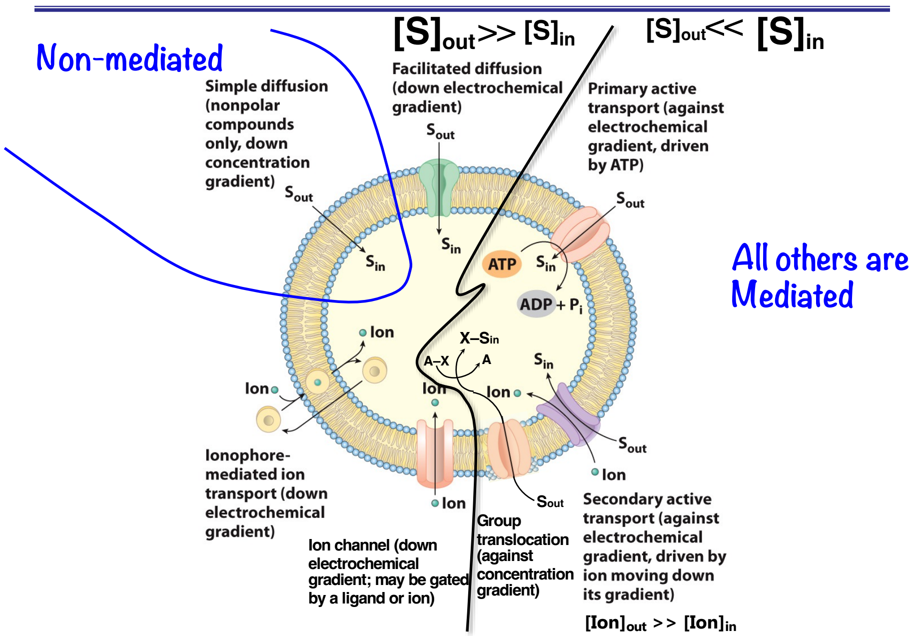
Types of Membrane Transport



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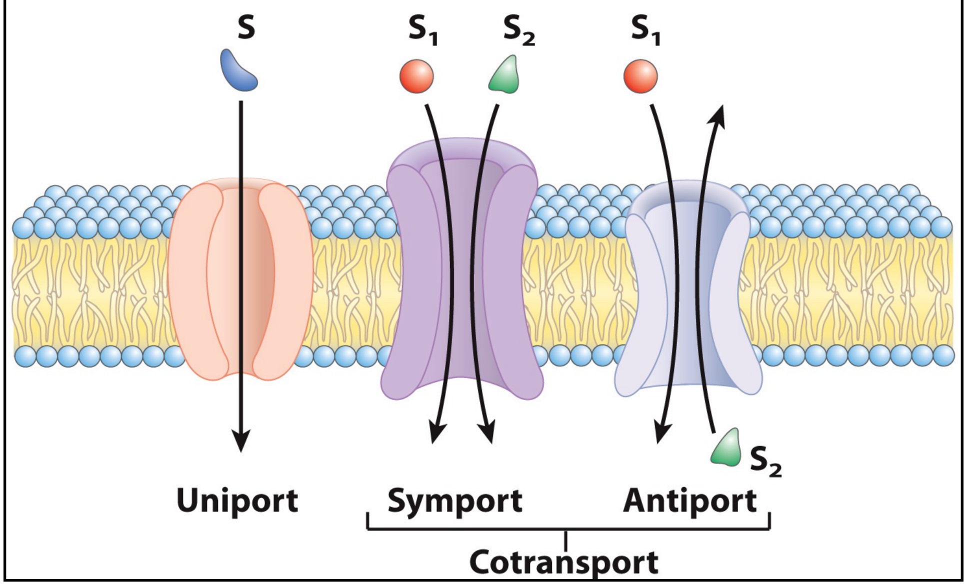
Membrane Transport

Three Classes of Transport Systems



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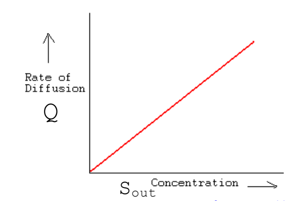
Membrane Transport

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

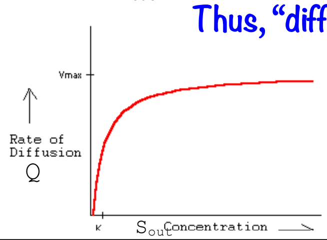
A = area across which diffusion occurs

$[S_{out}]$(P1) and $[S_{in}]$(P2)= 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

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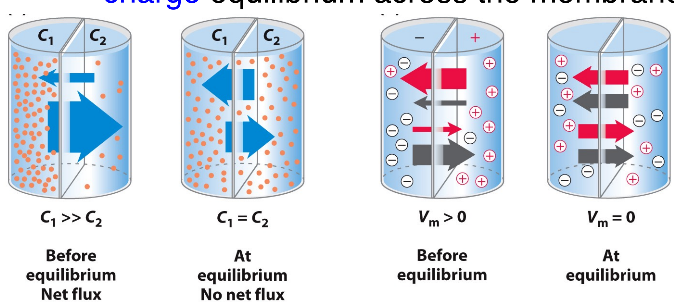
Membrane Transport

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?

*not unlike chemical reactions where there are two energies at play, concentration and chemical reactivity

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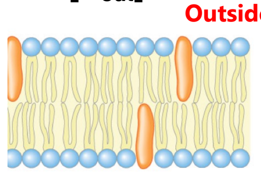
Membrane Transport

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

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

$[S_{out}]$

Outside



Inside

$[S_{in}]$

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

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

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

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

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

But, membranes in biology have a difference in charge (inside is 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

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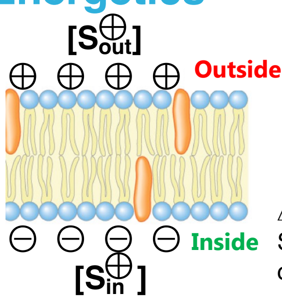
Membrane Transport

Energetics

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



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

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 as 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 $z\mathscr{F}\Delta\psi$ makes a contribution to $\Delta G'$ making it even more negative, i.e., more favorable.

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

*For ΔG to be favorable, with z as a positive molecule, the potential ($\Delta\psi$) needs to have a negative sign.

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Membrane Transport

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
  - Bicarb/Cl
- Group Translocation
  - Bacterial phosphotransferase system (PTS)

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Membrane Transport

Facilitative Diffusion

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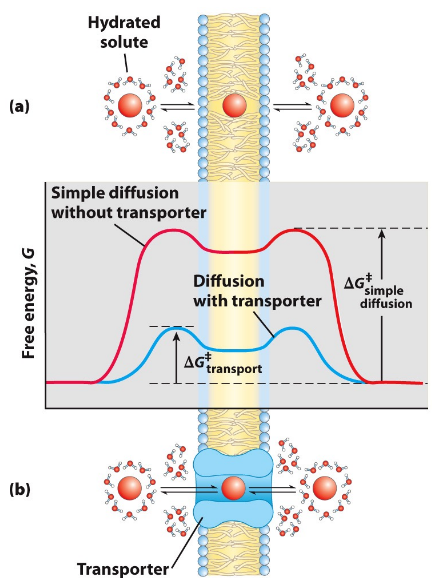
Membrane Transport

Examples of Facilitative Diffusion



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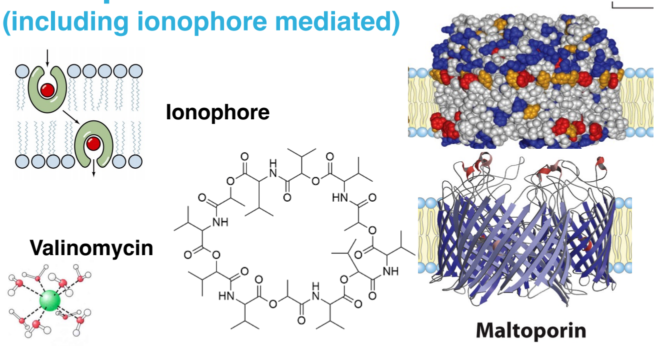
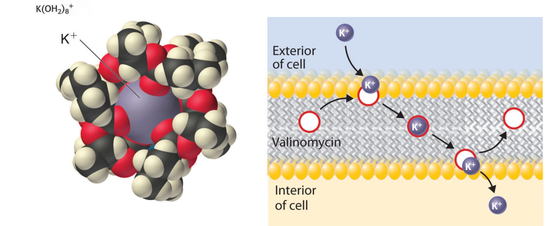
Membrane Transport

Examples of Facilitative Diffusion

(including ionophore mediated)



- 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).



(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

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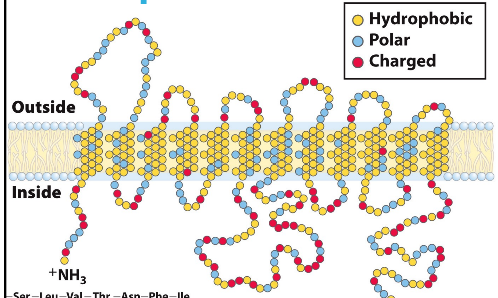
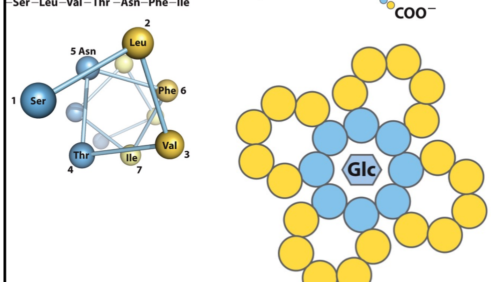
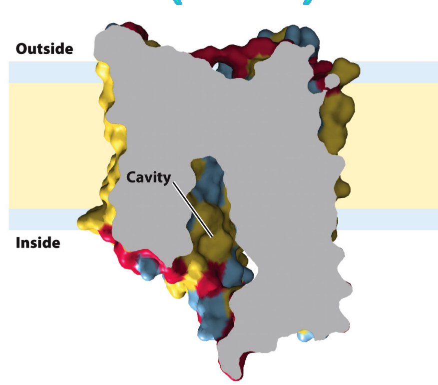
Membrane Transport

Examples of Facilitative Diffusion

Glucose Transporter (GLUT1)







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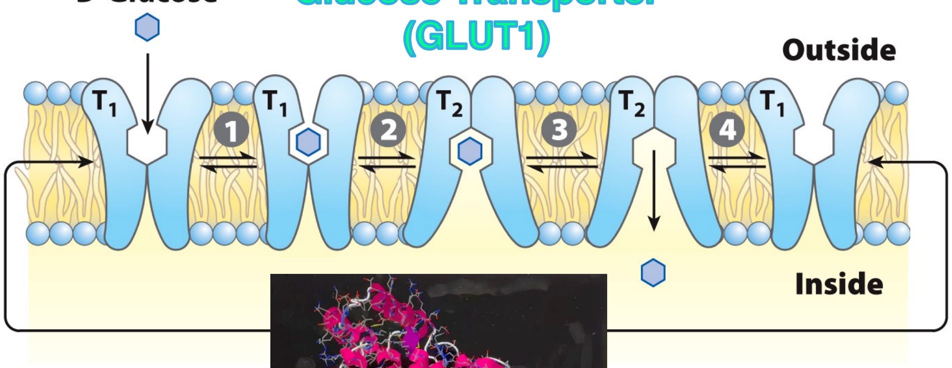
Membrane Transport

Examples of Facilitative Diffusion

Glucose Transporter (GLUT1)



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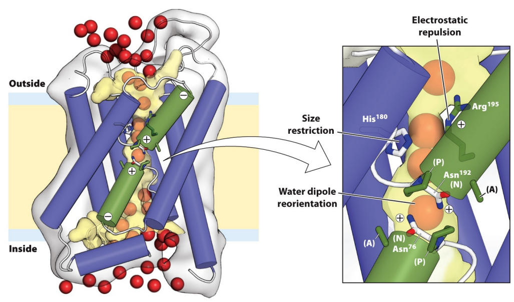
Membrane Transport

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

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Membrane Transport

Examples:

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  - Group Translocation
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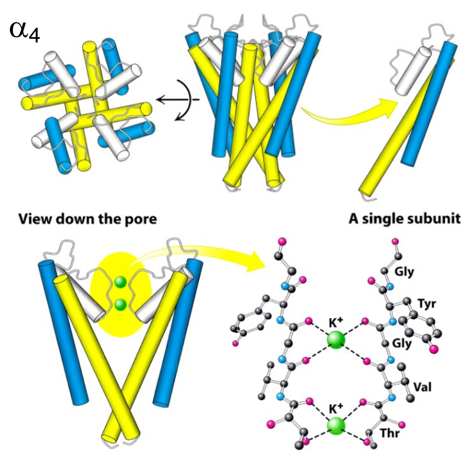
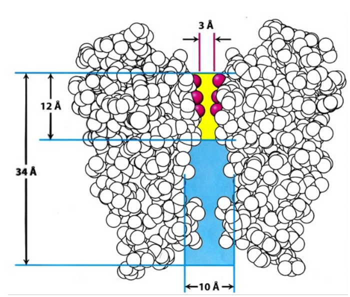
Membrane Transport

Examples of Facilitative Diffusion



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



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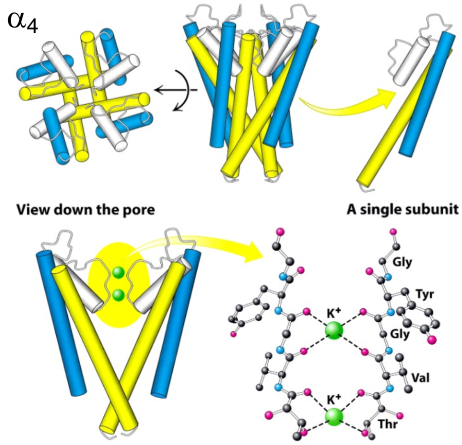
Membrane Transport

Examples of Facilitative Diffusion



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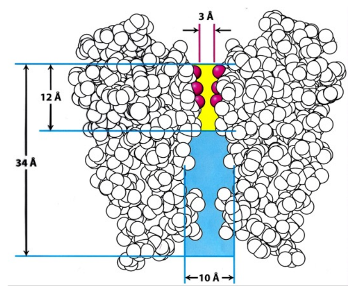

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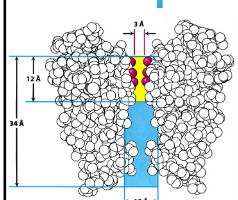
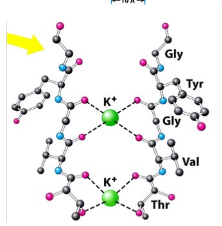
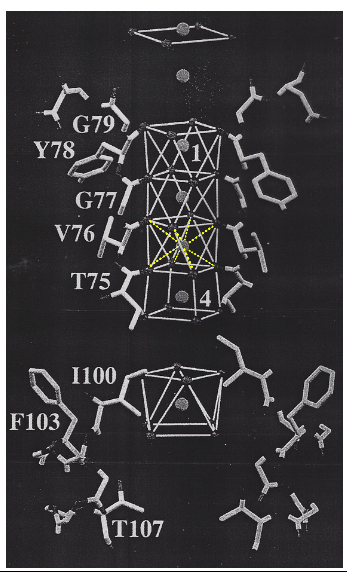
Membrane Transport

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

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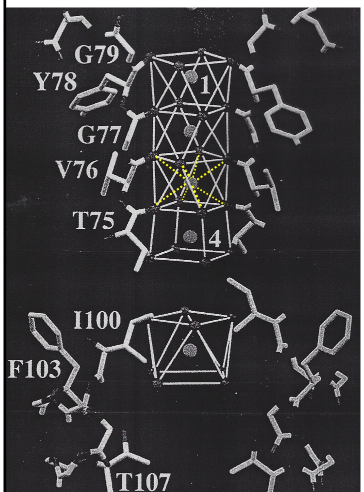
Membrane Transport

Examples of Facilitative Diffusion



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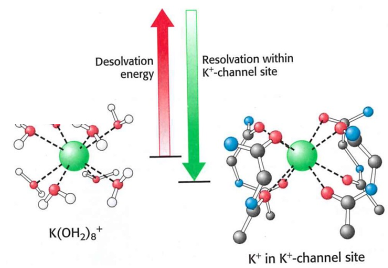
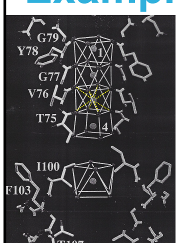


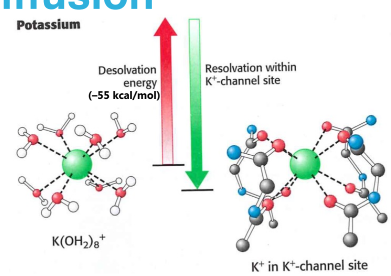
Membrane Transport

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).