# Biochemistry II (BI/CH 422 & BI/CH 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

# Biological Membrane Transport

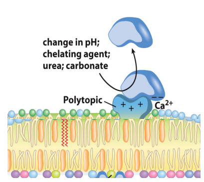
"Crossing the Berlin Wall of the cell - Membrane Transport"

# Physical Properties of Membranes

- Not permeable to large polar solutes and ions
- Permeable to nonpolar compounds and some small polar compounds (e.g., water)
- Permeability can be artificially increased by chemical treatment.
  - when we want to get DNA into the cell
- Very stable yet dynamic and flexible structures
- Can exist in various phases and undergo phase transitions
- Can fuse
- Functionality provided by embedded proteins

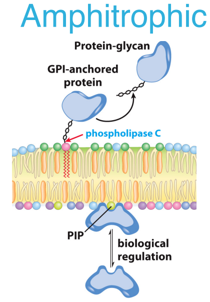
# Three Types of Membrane Proteins

## Peripheral



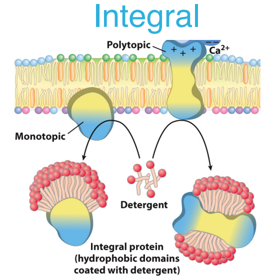

- Associate with the polar head groups of membranes
- Relatively loosely associated with membrane
  - through ionic interactions with the lipids or aqueous domains of integral membrane proteins
- Removed by disrupting ionic interactions either with high salt or change in pH
- Purified peripheral membrane proteins are no longer associated with any lipids.

## Amphitrophic




- Amphitrophic proteins can be conditionally attached to the membrane by covalent interaction with lipids or carbohydrates attached to lipids.
- Biological regulation results in attachment to, or cleavage from, lipids.

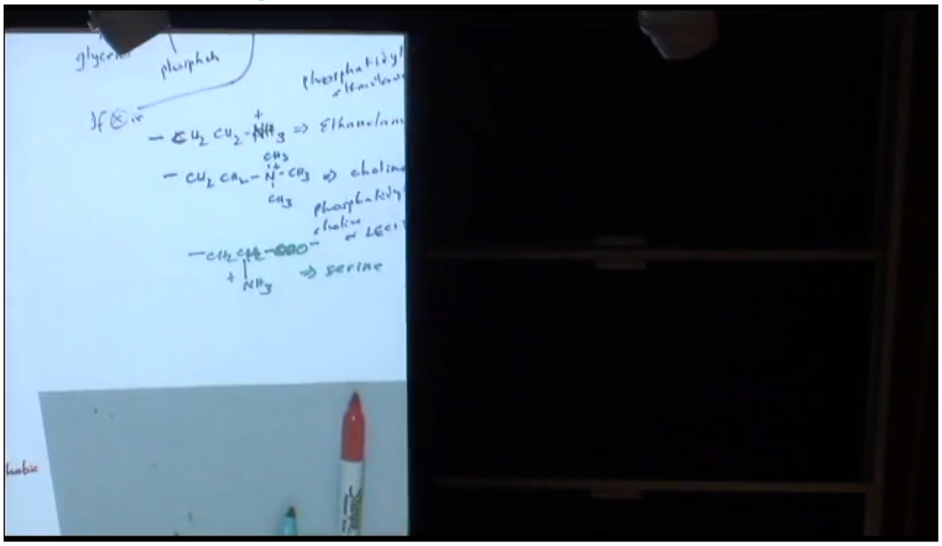
## Integral




- Span the entire membrane
- Have asymmetry like the membrane
  - different domains in different compartments
- Tightly associated with membrane
  - Hydrophobic stretches in the protein interact with the hydrophobic regions of the membrane.
- Removed by detergents that disrupt the membrane
- Purified integral membrane proteins still have phospholipids associated with them.

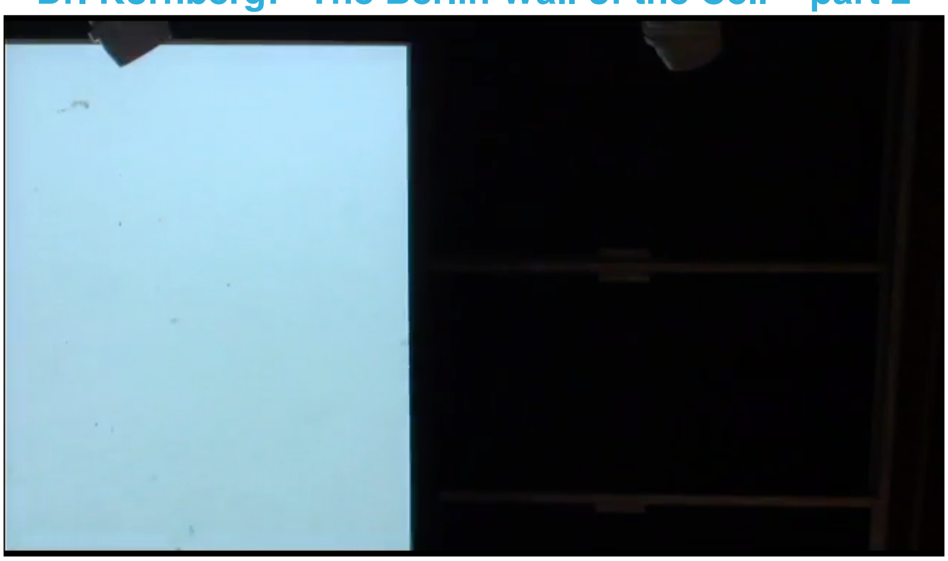
## Membrane Transport

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



## Membrane Transport

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

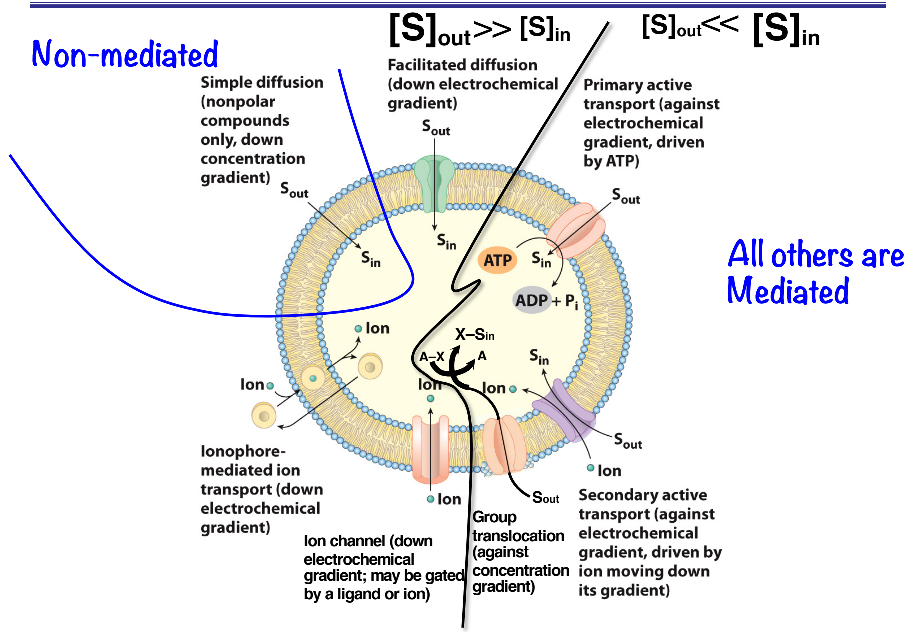


# Membrane Transport

## Dr. Kornberg: “The Berlin Wall of the Cell”

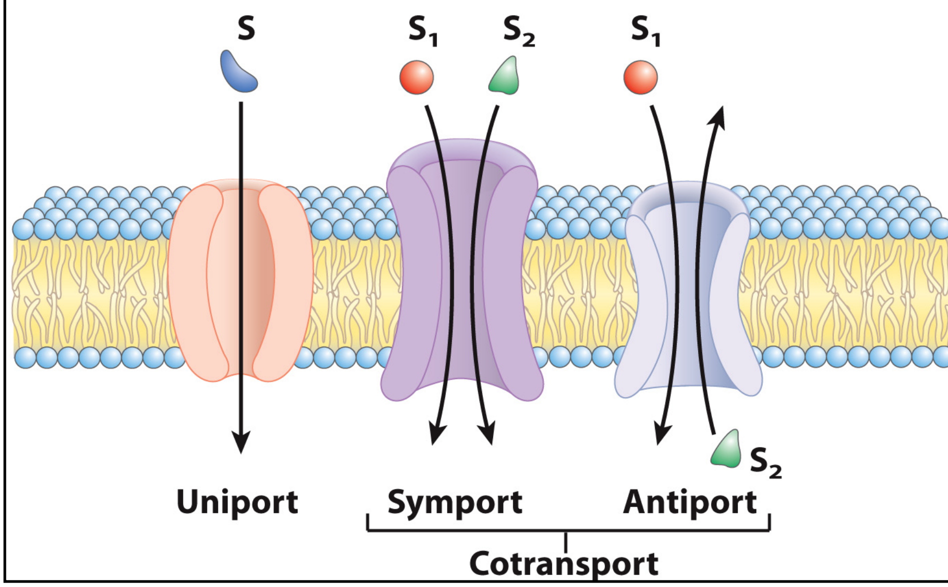
- Cell membranes are permeable to small nonpolar molecules that passively diffuse through the membrane.
- Passive diffusion of polar molecules involves desolvation and thus has a high activation barrier, unless desolvation energy is lowered.
- Transport across the membrane can be facilitated by proteins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

# Types of Membrane Transport



## Membrane Transport

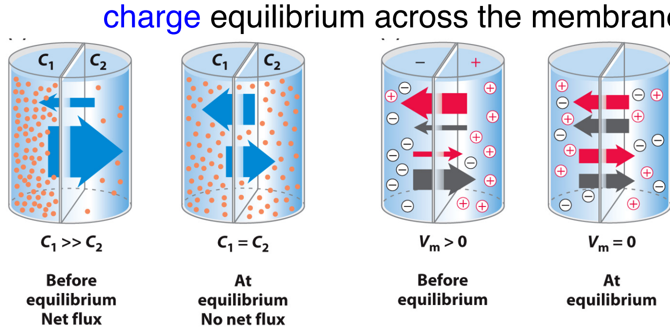
Three Classes of Transport Systems




## 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

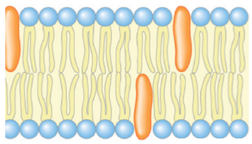
## Energetics

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

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

**$[S_{out}]$**

Outside



Inside

**$[S_{in}]$**

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

But, $\Delta G^{\circ\prime} = 0$ 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, if S is charged, we must account for this:

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

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

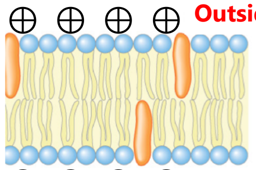
# Membrane Transport

## Energetics

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

**$[S^{\oplus}_{out}]$**

$\oplus$ $\oplus$ $\oplus$ $\oplus$ Outside



$\ominus$ $\ominus$ $\ominus$ $\ominus$ Inside

**$[S^{\oplus}_{in}]$**

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

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

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

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 negative contribution to $\Delta G'$ making it even more favorable.

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

# Membrane Transport

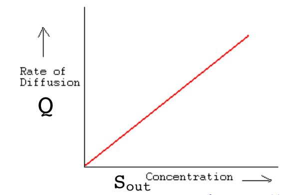
How do you experimentally determine the kind of transport?

**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

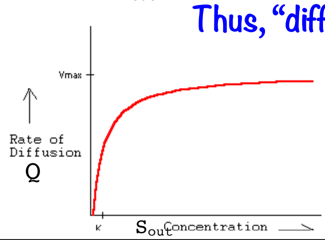
$A$ = area across which diffusion occurs

$[S_{out}]$(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**

# 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)

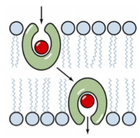
## Membrane Transport

# Facilitative Diffusion

## Membrane Transport

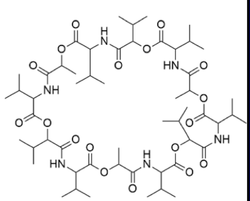
### Examples of Facilitative Diffusion

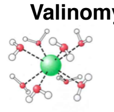
**(including ionophore mediated)**

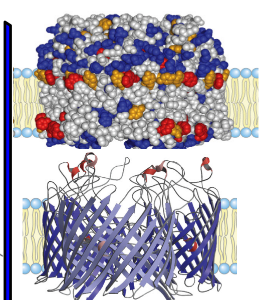


**Ionophore**

**Valinomycin**

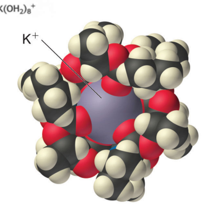




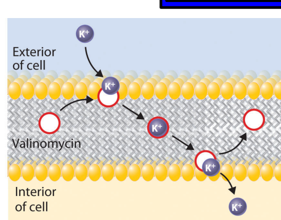



**Maltoporin**

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




**(a) $K^+$–valinomycin complex**




**(b) Transport of $K^+$ across a membrane**

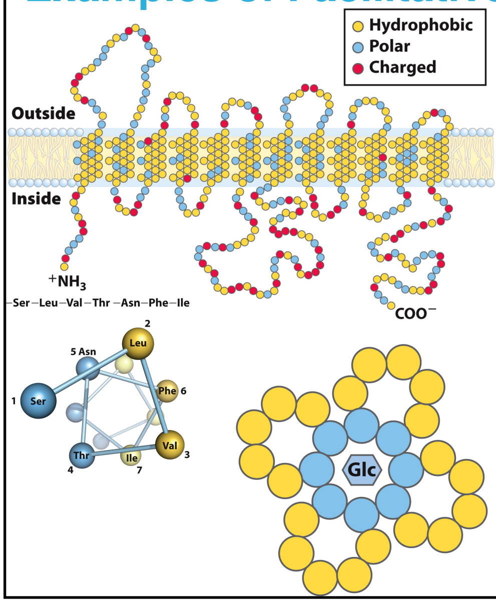
### How it works,

- The six oxygen atoms of the ionophore interract 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!

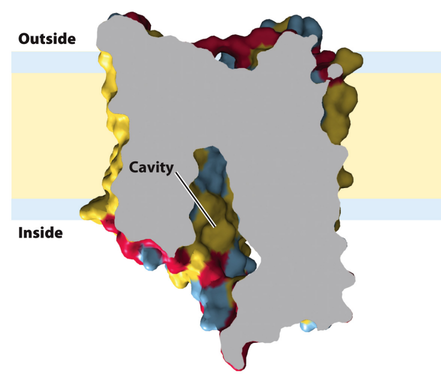
NB. Valinomycin can not carry sodium ions because they are small and there fore can not simultaneously interact with six O-atoms – thus being energetically unfavourable

# Membrane Transport

## Examples of Facilitative Diffusion



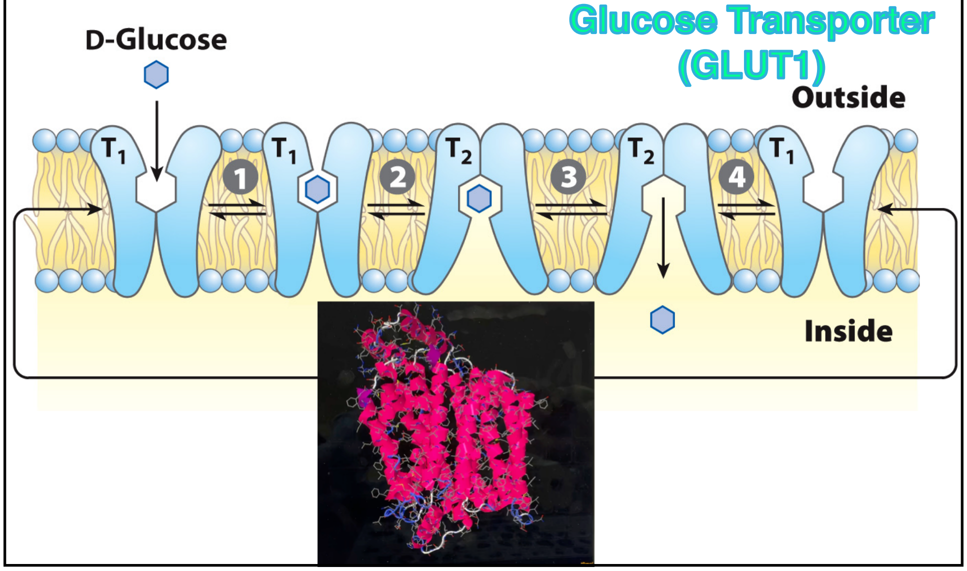

### Glucose Transporter (GLUT1)




# Membrane Transport

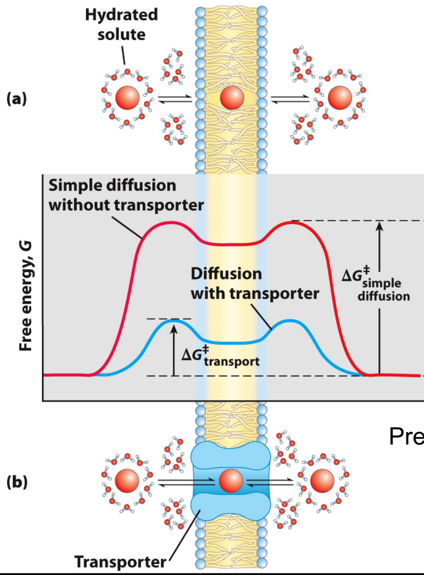
## Examples of Facilitative Diffusion

### Glucose Transporter (GLUT1)

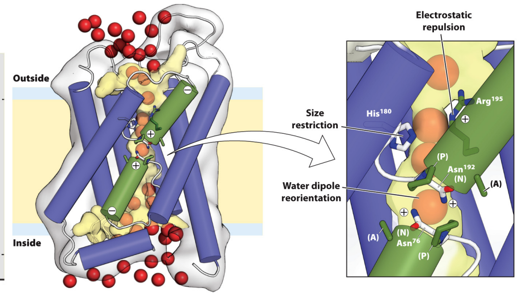


# Membrane Transport

## 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

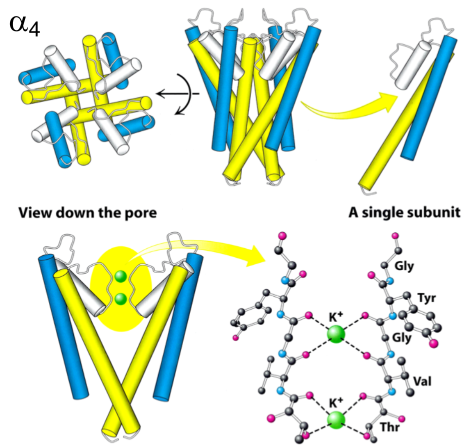
# Membrane Transport

Examples:

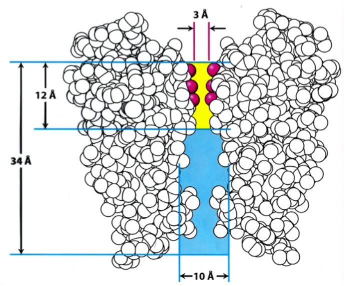
- Facilitative Diffusion
  - Ionophore
  - Maltoporins
  - GLUT1 transprter
  - Aquaporin
  - Selective ion channel for potassium (K-channels)
- Active Transport
  - Primary (1°)
    - Na/K
    - ABC
  - Secondary (2°)
    - Na/Glc
    - Bicarb/Cl
  - Group Translocation
    - Bacterial phosphotransferase system (PTS)

# Membrane Transport

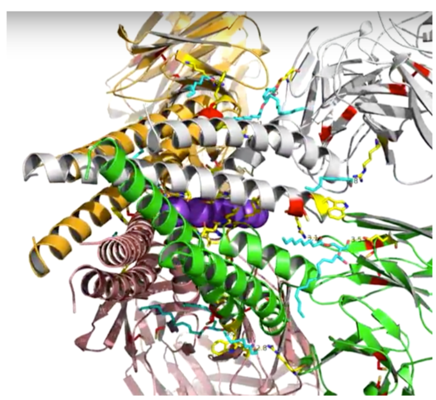
## Examples of Facilitative Diffusion




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




# Membrane Transport



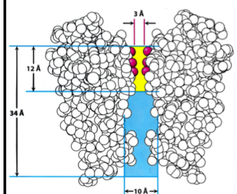
K channel

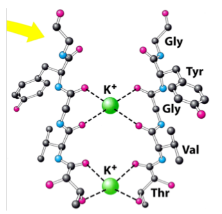
https://www.youtube.com/watch?v=IXPmgprE8rg&feature=youtu.be

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

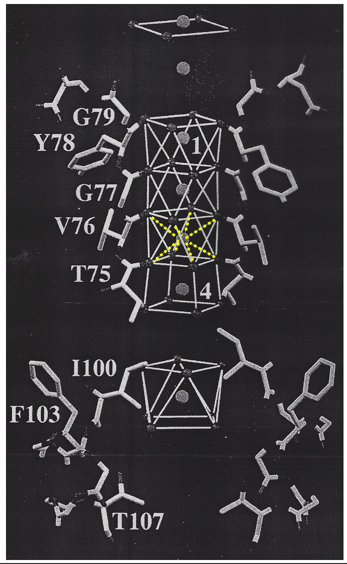
# Membrane Transport

## Examples of Facilitative Diffusion




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

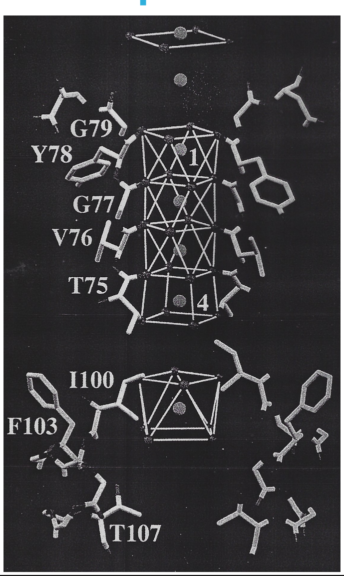



How is the >100X specificity for $K^+$ achieved?

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

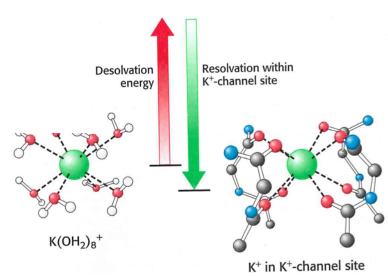

# Membrane Transport

## Examples of Facilitative Diffusion

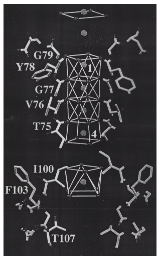

| Ion | Radius (Å) |
|---|---|
| $Li^+$ | 0.6 |
| $Na^+$ | 1.0 |
| $K^+$ | 1.3 |
| $Rb^+$ | 1.5 |
| $Cs^+$ | 1.7 |

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

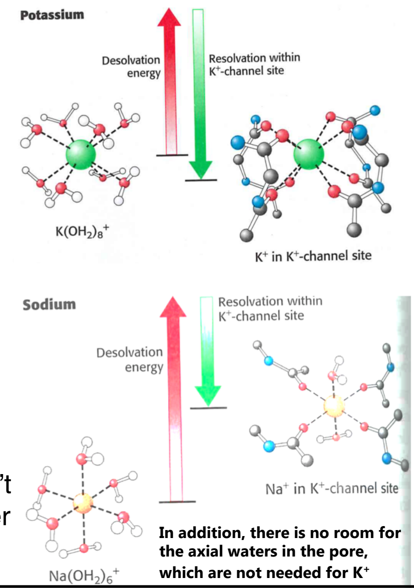


# Membrane Transport

## 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 |




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).