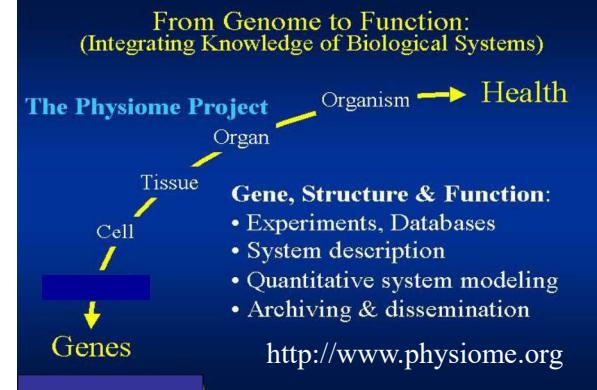


## Cellular Transport

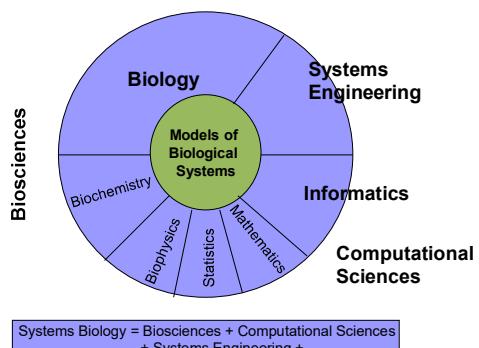
Saikat Chakraborty  
Department of Chemical Engineering  
Indian Institute of Technology -  
Kharagpur



### Systems Biology - an integrative approach

- it seeks to integrate
  1. levels (of structure and scale)
  2. process phases (the many „omics“)
  3. experiment and modeling/computational work
  4. scientific disciplines (multi-disciplinary)
- to achieve quantitative experimental results and
- to build predictive models/simulation environments

### Optimal Formula: Interdisciplinary research



### Bioengineering Research Areas

- Biomechanics
- Bioelectronics, Ion Channels and Organ Function
- Clinical Medicine and Drug Delivery
- Functional Genomics – Microarray Technology, Integrated Systems and Analysis Tools
- Nanotechnology
- Imaging
- Informatics and Computational Applications

### Bioengineering Research Areas

- Medical Implants, Biomembranes & Sensors
- Complex Biological Systems
- Organ Culture Systems and Organogenesis
- Rehabilitation and Prostheses
- Cell and Tissue Engineering and Biomaterials
- Tissue Regeneration
- Integrative Physiology & Organ System Model
- Drug Bioavailability
- Quantification of Human Diseases

## Mechanisms of Transport

- Diffusion
- Convection

**Diffusion:** Transport resulting from random motion of molecules, which occur due to inter-molecular collisions. Collision frequency is of the order of trillion times per second.

### Theory of Brownian Motion (in 2-D):

$$D_{ij} = \frac{\langle x^2 \rangle + \langle y^2 \rangle}{4t} \quad (\text{A. Einstein, 1956})$$

**Problem:** A protein molecule which has a diffusivity of  $1 \times 10^{-7} \text{ cm}^2 \text{ s}^{-1}$  diffuses from the cell membrane to the nucleus located at the cell center. Typical diameter of a cell is 10 microns. Find the time required for it to diffuse.

**Ans: 2.5 seconds**

**Diffusion is a fast process !**

Calculate the time required for the same protein molecule to diffuse across a tissue only 0.2 cm thick.

**Ans: 27.7 hours**

**Diffusion is a slow process?**

Time required for diffusion (characteristic diffusion time) proportional to  $(\text{distance traveled})^2$   
Therefore, diffusion is a preferred carrier mechanism only for short distance travel.

Table 1.1: Range of values of binary diffusion coefficients,  $D_{ij}$ , at  $25^\circ \text{C}$

Diffusing Quantity	$D_{ij}, \text{ cm}^2 \text{ s}^{-1}$
Gases in gases	0.1 to 0.5
Gases in liquids	$1 \times 10^{-7}$ to $7 \times 10^{-5}$
Small molecules in liquids	$1 \times 10^{-5}$
Proteins in liquids	$1 \times 10^{-7}$ to $7 \times 10^{-7}$
Proteins in tissues	$1 \times 10^{-7}$ to $1 \times 10^{-10}$
Lipids in lipid membranes	$1 \times 10^{-9}$
Proteins in lipid membranes	$1 \times 10^{-10}$ to $1 \times 10^{-12}$

## Cellular Transport Processes

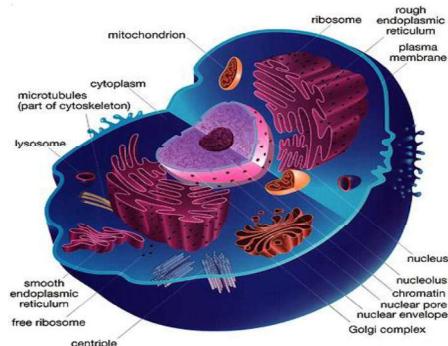
### ■ Simple Diffusion

### ■ Facilitated Diffusion

- Channel Transport  
(Voltage gated, ligand-gated, mechanically gated)
- Passive/Carrier-mediated Transport

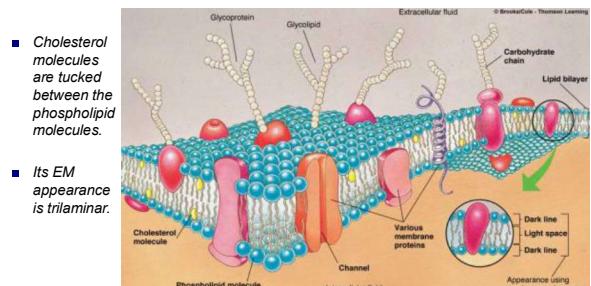
### ■ Active Transport

## Anatomy of a human cell



The plasma membrane is a fluid lipid bilayer embedded with proteins

- Phospholipids form a bilayer. The bilayer has a hydrophobic interior. This interior is sandwiched between hydrophilic inner and outer surfaces.
- Carbohydrates are attached to its outer surface.



## TYPES OF TRANSPORT THROUGH CELL MEMBRANE

### 1. SIMPLE DIFFUSION

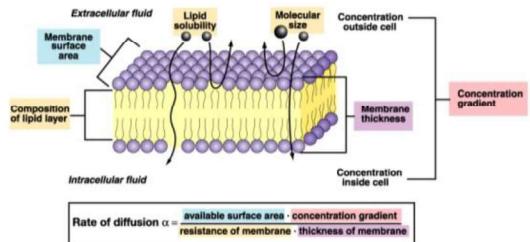
### 2. PASSIVE TRANSPORT (Facilitated Diffusion)

- a) CHANNEL TRANSORT
- b) CARRIER-MEDIATED TRANSPORT

### 3. ACTIVE TRANSPORT (requires energy)

- a) ATP PUMPS (Primary Active Transport)
- b) EXCHANGERS (Secondary Active Transport)

## 1. DIFFUSION



### Diffusion across plasma membrane

Calculate the time required by the protein molecule to diffuse across a plasma membrane of thickness 0.1 micron.

**Ans: 2.5 seconds**

Total time required for the protein to reach the cell-center is 5 seconds.

### Diffusion across plasma membrane...contd.

Assume plasma membrane is composed of a single phase of lipids  
Assume Fick's law to be valid

#### Unsteady-state Model Equation in 1-D:

$$\frac{\partial C_i}{\partial t} = D_g \frac{\partial^2 C_i}{\partial x^2}$$

#### Steady-state Model Equation in 1-D:

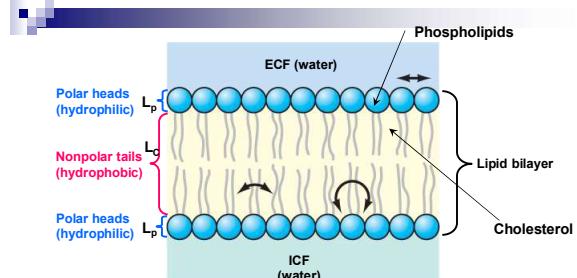
$$0 = D_g \frac{d^2 C_i}{dx^2}$$

B.C.S:  $x=0, C_i = \Phi C_0; x=L, C_i = \Phi C_L$ .

### Solution to 1-D steady state model

$$C_i = \Phi \left[ C_0 - \left( C_0 - C_L \right) \frac{x}{L} \right];$$

$$\text{Steady State flux} = J_{ix} = -D_g \frac{dC_i}{dx} = \frac{D_g \Phi}{L} (C_0 - C_L).$$



$D_p (D_c)$  = Diffusion coefficient of protein in phospholipids (cholesterol)

Calculate the 1-D steady state flux through plasma membrane between ECF and ICF.

B.C.S:  $x=0, C_i = \Phi_p C_0; x=2L_p + L_c, C_i = \Phi_p C_L;$

$\Phi_p$  and  $\Phi_c$  are the partition-coefficients for phospholipds and cholesterol, resp

Other B.C.s (at  $x=L_p$  and at  $x=L_p + L_c$ ):

$$\frac{C_p}{\Phi_p} = \frac{C_c}{\Phi_c} \quad \& \quad N_{px} = N_{cx}.$$

$$\text{Solution: } J_{ix} = \frac{C_0 - C_L}{R_{eff}};$$

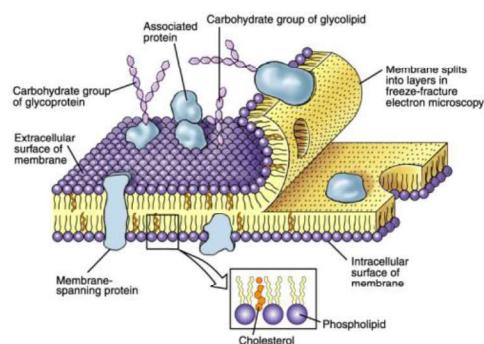
$$\text{where, } R_{eff} = \frac{2L_p}{\Phi_p D_p} + \frac{L_c}{\Phi_c D_c}.$$

For  $n$  layers in series:

$$R_{eff} = \sum_{j=1}^n \frac{L_j}{\Phi_j D_j}.$$

## Ion Concentration Inside and Outside a Mammalian Cell

Ion	Concentration (mM)	
	Intracellular	Extracellular
Na <sup>+</sup>	5-15	145
K <sup>+</sup>	140	5
Mg <sup>++</sup>	.5	1-2
Ca <sup>++</sup>	10 <sup>-7</sup>	1-2
H <sup>+</sup>	7 x 10 <sup>-5</sup> (pH 7.2)	4 x 10 <sup>-5</sup> (pH 7.4)
Cl <sup>-</sup>	5-15	110



## Diffusion Through Plasma Membrane

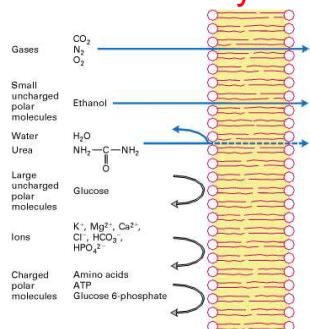
■ Cell membrane is permeable to:

- Non-polar molecules ( $O_2$ ).
- Lipid soluble molecules (steroids).
- Small polar covalent bonds ( $CO_2$ ).
- $H_2O$  (small size, lack charge).

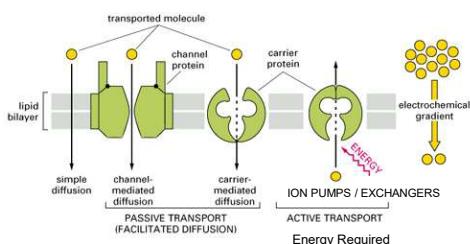
■ Cell membrane impermeable to:

- Large polar molecules (glucose).
- Charged inorganic ions ( $Na^+$ ).

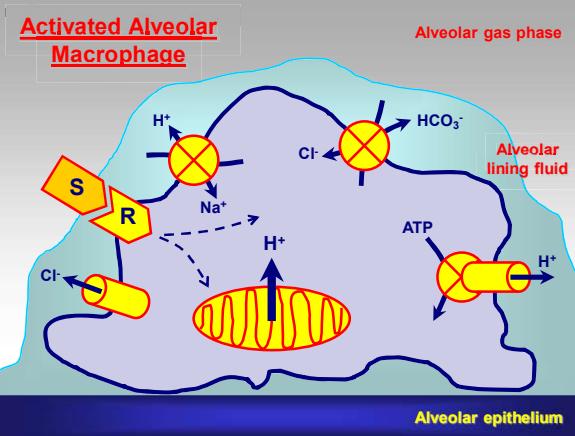
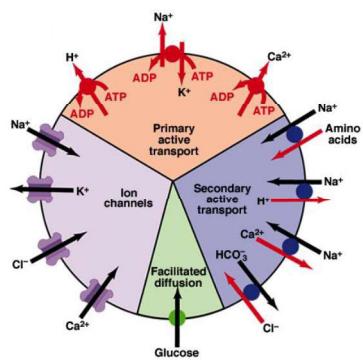
## Membrane Permeability



## Transport Across the Membrane

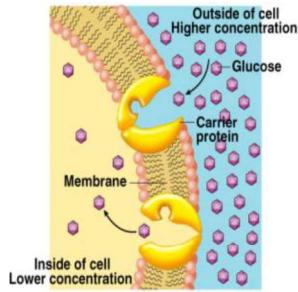


## ION TRANSPORT



## Passive Diffusion

- Passive:
  - ATP not needed.
    - Powered by thermal energy of diffusing molecules.
  - Involves transport of substance through plasma membrane down concentration gradient by carrier proteins.
    - Transport carriers for glucose designated as GLUT.

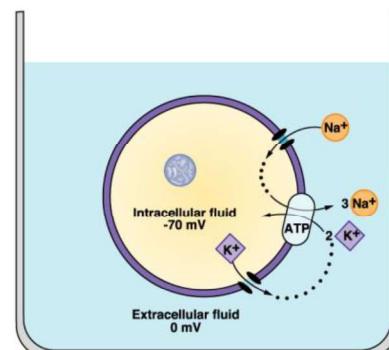
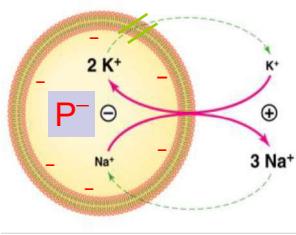


All cells establish a membrane potential. This is a separation of charges across the plasma membrane

- This potential develops due to differences in the concentration and permeability of key ions.
- A cell develops a resting membrane potential when it is not sending electrical signals.

## Membrane Potential ( $E_m < 0$ )

- Membrane more permeable to K<sup>+</sup> than Na<sup>+</sup>. ( $P_K >> P_{Na^+}$ )
  - Concentration gradients for Na<sup>+</sup> and K<sup>+</sup>.
  - K<sup>+</sup> accumulates within cell also due to negatively charged protein molecules that are trapped in cell
  - Na<sup>+</sup>/K<sup>+</sup>ATPase pump 3 Na<sup>+</sup> out for 2 K<sup>+</sup> in.
- Unequal distribution of charges between the inside and outside of the cell, causes each cell to act as a tiny battery.



In the presence of an electric potential gradient (as in the case of facilitated transport),

Total Flux ( $N_i$ ) = Diffusive Flux + Convective Flux + Flux due to electric potential gradient.

Diffusive Flux =  $-D_{ij}\nabla C_i$ , Convective Flux =  $C_i v$

Flux due to electric potential gradient = ?

$$J_i = C_i v_{mi}$$

$$= -\frac{D_{ij}C_i z_i F}{RT} \nabla \psi,$$

where  $z_i$  is the net charge on the molecule (including sign),

$\psi$  is the electric potential (in volts),

F is Faraday's constant =  $e \times$  Avagadro's number  
= 96, 487 coulombs/mol.

$$N_i = -D_{ij}\nabla C_i - \frac{D_{ij}C_i z_i F}{RT} \nabla \psi + C_i v$$

### Nernst-Planck Equation

Two other relations need to characterize ion transport fully:

Electroneutrality: for n ions,  $\sum_{i=1}^n C_i z_i = 0$ .

$$\text{Total Current: } i = F \sum_{i=1}^n N_i z_i$$

### Convection-Diffusion Equation for Electrolytes

$$\frac{\partial C_i}{\partial t} = -\nabla \cdot N_i$$

$$\frac{\partial C_i}{\partial t} + v \nabla C_i = D_{ij} \nabla^2 C_i + \frac{D_{ij} z_i F}{RT} \nabla \cdot (C_i \nabla \psi)$$

$$\text{1-D Models: } \frac{\partial C_i}{\partial t} = -\frac{\partial N_{iz}}{\partial z}$$

$$\frac{\partial C_i}{\partial t} + v \frac{\partial C_i}{\partial z} = D_{ij} \frac{\partial^2 C_i}{\partial z^2} + \frac{D_{ij} z_i F}{RT} \frac{\partial}{\partial z} \left( C_i \frac{\partial \psi}{\partial z} \right)$$

**Problem:** Consider the steady-state 1-D transport of a binary electrolyte across an electrolyte across an uncharged membrane of thickness L. There are no chemical reactions, convection is insignificant, there's no applied electric potential. Derive an expression for the electric potential that arises across the membrane due diffusion of ions.

**Solution:**

$$\text{Steady-state, 1-D Model: } \frac{dN_{iz}}{dz} = 0 \quad (\text{a})$$

$$\text{No net current across the membrane: } 0 = F \sum_{i=1}^n N_i z_i \quad (\text{b})$$

$$\text{Electroneutrality: } \sum_{i=1}^n C_i z_i = 0. \quad (\text{c})$$

**Binary:** n=2

From (b),  $z_+ N_+ = -z_- N_-$

From Nernst-Planck Eqn. (1-D, no convection):

$$N_+ = -D_+ \frac{dC_+}{dz} - \frac{D_+ C_+ z_+ F}{RT} \frac{d\psi}{dz} = \text{constant} \quad [\text{from (a)}]$$

$$N_- = -D_- \frac{dC_-}{dz} - \frac{D_- C_- z_- F}{RT} \frac{d\psi}{dz} = \text{constant} \quad [\text{from (a)}]$$

From (c),  $z_+ C_+ = -z_- C_-$

### Final Solution

Using above expressions,

$$(D_+ - D_-) \frac{dC_+}{dz} + (z_+ D_+ - z_- D_-) \frac{C_+ F}{RT} \frac{d\psi}{dz} = 0.$$

Rearranging,

$$d\psi = - \left( \frac{D_+ - D_-}{z_+ D_+ - z_- D_-} \right) \left( \frac{RT}{F} \right) \frac{dC_+}{C_+}.$$

Integrating over the thickness L of membrane,

$$\psi(L) - \psi(0) = \left( \frac{D_+ - D_-}{z_+ D_+ - z_- D_-} \right) \left( \frac{RT}{F} \right) \ln \left( \frac{C_0}{C_L} \right).$$

### Diffusion Coefficients of Anions and Cations at 25°C

Cation	Charge, $z_+$	$D_+ \times 10^5 \text{ cm}^2 \text{ s}^{-1}$	Anion	Charge, $z_-$	$D_- \times 10^5 \text{ cm}^2 \text{ s}^{-1}$
H <sup>+</sup>	+1	9.312	OH <sup>-</sup>	-1	5.260
Na <sup>+</sup>	+1	1.334	Cl <sup>-</sup>	-1	2.032
K <sup>+</sup>	+1	1.957	NO <sub>3</sub> <sup>-</sup>	-1	1.902
NH <sub>4</sub> <sup>+</sup>	+1	1.954	HCO <sub>3</sub> <sup>-</sup>	-1	1.105
Mg <sup>2+</sup>	+2	0.7063	HCO <sub>3</sub> <sup>-</sup>	-1	1.454
Ca <sup>2+</sup>	+2	0.7920	SO <sub>4</sub> <sup>2-</sup>	-2	1.065
Cu <sup>2+</sup>	+2	0.72	HSO <sub>4</sub> <sup>-</sup>	-1	1.33

N.B.: Hydrogen and hydroxyl ions have higher diffusivity because protons are transferred from solvent to ions via hydrogen ion connections, and proton transfer is important in many biochemical reactions.

### Electrical Potential Gradient in Cell Membranes

For many salts, the diffusion coefficients of the cation and the anion differ slightly in magnitude, e.g. NaCl or KCl. Even if the concentrations differ by a factor of 10, then the diffusion potentials are 12.27 mV for NaCl and 1.11 mV for KCl.

Potential difference across cell membranes ( $\Delta\psi$ ) is small, e.g., 100 mV

Cell membrane thickness ( $\Delta z$ ) is very small, e.g., 0.005 μm

Potential gradient  $\sim \Delta\psi/\Delta z = 2 \times 10^6 \text{ V/cm}$

Therefore, it is often assumed that the potential varies linearly with position within the membrane.

For a membrane of thickness L, the potential distribution is approximated by

$$\frac{d\psi}{dz} \approx \frac{\psi_m - \psi_L}{L} = \frac{V_m}{L},$$

where  $V_m$  represents the difference between extra- and intra-cellular potential.

Constant Field Assumption

### Convection-Diffusion Eqn. for Electrolytes (Revisited)

$$\frac{\partial C_i}{\partial t} + v \frac{\partial C_i}{\partial z} = D_{ij} \frac{\partial^2 C_i}{\partial z^2} + \frac{D_{ij} z_i F}{RT} \frac{\partial}{\partial z} \left( C_i \frac{\partial \psi}{\partial z} \right)$$

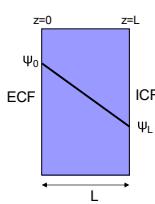
Steady-state, No Convection, Constant Field Assumption

$$0 = D_{ij} \frac{d^2 C_i}{dz^2} + \frac{D_{ij} z_i F}{RT} \left( \frac{V_m}{L} \right) \frac{dC_i}{dz}$$

Boundary Conditions: at  $z=0$ ,  $C_i = \Phi_i C_{i,0}$ ;

at  $z=L$ ,  $C_i = \Phi_i C_{i,L}$ ,

where  $\Phi_i$  is the partition coefficient of ion  $i$ .



### Solution:

$$C_i = - \frac{\left[ \Phi_i (C_{i,L} - C_{i,0}) \left[ \exp\left(-\frac{V_m z_i F z}{RT L}\right) - 1 \right] \right]}{1 - \exp\left(-\frac{V_m z_i F}{RT}\right)} + \Phi_i C_0, \quad (\text{= Function of } z)$$

$$N_i = - \left( \frac{\Phi_i D_{ij}}{L} \right) \left( \frac{V_m z_i F}{RT} \right) \left( \frac{C_{i,0} \exp(V_m z_i F / RT) - C_{i,L}}{\exp(V_m z_i F / RT) - 1} \right), \quad (\text{= Constant})$$

$$= -P_i^j \left( \frac{V_m z_i F}{RT} \right) \left( \frac{C_{i,0} - C_{i,L} \exp(-V_m z_i F / RT)}{1 - \exp(-V_m z_i F / RT)} \right),$$

where  $P_i^j$  is the Goldman Permeability Coefficient of Ions.

Goldman-Hodgkin-Katz Equation

### Evaluation of Membrane Potential by Application of Goldman-Hodgkin-Katz Equation

The principal ions transported across channels are K<sup>+</sup>, Na<sup>+</sup> and Cl<sup>-</sup>

Note that net current across the membrane is zero.

$$0 = N_{K^+,z} + N_{Na^+,z} - N_{Cl^-,z} \quad \left( \sum_{i=1}^n N_i z_i = 0 \right)$$

Applying G-H-K:

$$\sum_{i=1}^n P_i^j z_i \left( \frac{C_{i,0} - C_{i,L} \exp(-V_m z_i F / RT)}{1 - \exp(-V_m z_i F / RT)} \right) = 0;$$

On simplification:

$$V_m = \frac{RT}{F} \ln \left[ \frac{P_{K^+} C_{K^+,L} + P_{Na^+} C_{Na^+,L} + P_{Cl^-} C_{Cl^-,L}}{P_{K^+} C_{K^+,0} + P_{Na^+} C_{Na^+,0} + P_{Cl^-} C_{Cl^-,0}} \right].$$

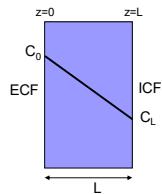
In the absence of other ions, for any single ion i, the previous equation becomes

$$V_m = \frac{RT}{z_i F} \ln \left[ \frac{P_i C_{i,L}}{P_i C_{i,0}} \right].$$

Nernst's Equation

Problem 1: Determine the flux of Na<sup>+</sup> across an epithelial membrane via Na<sup>+</sup> channels between concentrations of C<sub>0</sub> = 9.5 mM and C<sub>L</sub> = 28 mM.

Given: P<sub>Na<sup>+</sup></sub> = 1.19 × 10<sup>-6</sup> cm s<sup>-1</sup>, T=310 K, R=8.314 × 10<sup>-2</sup> J mmol<sup>-1</sup> K<sup>-1</sup>, F=96.48 C mmol<sup>-1</sup>.



**Problem 2:** For the membrane shown here, obtain the steady-state flux and the concentration profile in the absence of any potential gradient (applied or developed; i.e. no ions are present on either side of the membrane) but the in the presence of convection.

**Solution:**

$$C_i = C_0 - (C_0 - C_L) \left( \frac{1 - \exp(-Pe z/L)}{1 - \exp(-Pe)} \right),$$

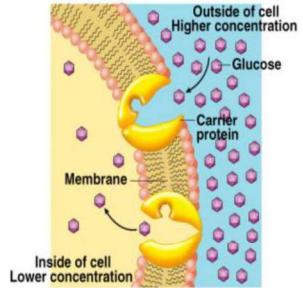
where  $Pe = vL / D_{ij}$ .

$$N_{i,z} = \frac{D_{ij}}{L} Pe \left[ C_0 - \frac{C_0 - C_L}{1 - \exp(-Pe)} \right].$$

## Carrier-mediated Transport: Passive Diffusion

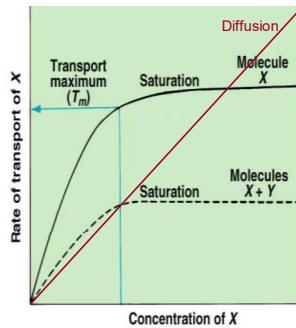
### ■ Passive:

- ATP not needed.
  - Powered by thermal energy of diffusing molecules.
- Involves transport of substance through plasma membrane down concentration gradient by carrier proteins.
- Transport carriers for glucose designated as GLUT.



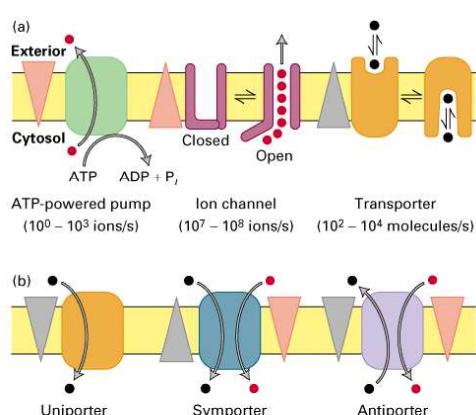
## Carrier-Mediated Transport

- Molecules that are too large (and polar) to diffuse are transported across plasma membrane by protein carriers.
- Characteristics of protein carriers:
  - Specificity:
    - Interact with specific molecule only.
  - Competition:
    - Molecules with similar chemical structures compete for carrier site.
  - Saturation:
    - $T_m$  (transport maximum):
      - Carrier sites have become saturated.



## Other kinds of assisted transport

- Vesicular transport - Materials move in or out of the cell wrapped in a membrane.
- Examples of vesicular transport as endocytosis and exocytosis.
- By endocytosis substances move into the cell.
- Exocytosis is the reverse process.



## Passive Carriers vs. Ion Channels

■ Transport by carriers can be saturated easily due to limited number of carriers in the membrane. The saturation phenomenon is a more common for characteristic for carriers than for channels.

■ Carrier proteins are specific to solutes. Although the degree of specificity varies, it's usually higher than that of channel proteins. This is because the carrier specificity is determined both by the geometrical and physical complementarities between solutes and carriers rather than by molecular sieving.

■ Carriers facilitate the transport of large molecules such as glucose and amino acids across the cell membrane, whereas channels allow only small molecules to pass through.

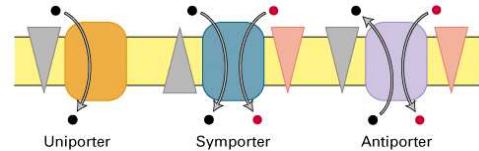
■ Transport via carriers is typically several orders of magnitude slower than transport via channels, since it depends on carrier proteins rather than solute movement in the system. Rate of transport through ion channels:  $10^7\text{-}10^8$  ions/s. Rate of carrier-mediated transport:  $10^2\text{-}10^4$  molecules/s.

## Passive Carriers vs. Ion Channels...contd.

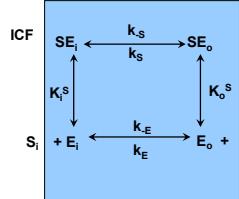
- Carrier-mediated transport is more sensitive to changes in membrane structure than is transport through channels.
- Carrier-mediated transport is often asymmetric, due to an asymmetric distribution of carrier proteins in the membrane and the difference in free energy of these proteins among distinct conformation. Thus, solute transport from one side to another of the membrane may involve less change in free energy than transport in opposite direction.
- Carrier-mediated transport may be inhibited by
  - (a) Non-competitive inhibition, in which inhibition is caused by binding of inhibitors to either free carriers or carrier-solute complexes.
  - (b) Competitive inhibition, in which the inhibitors compete with solutes for the same binding sites in free carriers.

## Types of Carrier-mediated transport

- Unipporter e.g. Transport of glucose across plasma membrane of red blood cells.
- Symporter (or Cotransporter) e.g.  $\text{Na}^+/\text{Cl}^-$  symporter across epithelial cell membrane in human kidney
- Antiporter (or Exchanger) e.g.  $\text{Cl}^-/\text{HCO}_3^-$  exchanger for  $\text{CO}_2$  transport in human red blood cells.



## Mathematical Modeling of Uniporter Transport



### Assumptions:

- Rate limiting step is the translocation of the substrate between ICF and ECF through the membrane.
- Thus, binding between substrate and uniporter is assumed to have reached equilibrium.
- Total number of uniports (on both sides of the membrane, together) is constant ( $=E_T$ )
- Total number of uniports is time independent, i.e., total flux of uniports is zero.

Due to assumption b)

$$[\text{SE}_i] = [\text{S}_i][\text{E}_i]/K_i^S,$$

$$[\text{SE}_o] = [\text{S}_o][\text{E}_o]/K_o^S,$$

where  $K$ 's are equilibrium constants and  $i$  and  $o$  indicate extracellular and intracellular, respectively.

Due to assumption c)

$$[\text{E}_i] + [\text{SE}_i] + [\text{E}_o] + [\text{SE}_o] = E_T.$$

Due to assumption d)

$$k_{-S}[\text{SE}_i] - k_S[\text{SE}_o] + k_{-E}[\text{E}_i] - k_E[\text{E}_o] = 0.$$

### Solving above four equations:

$$[\text{E}_o] = \frac{E_T \left( k_{-E} + k_{-S} \frac{[\text{S}_i]}{K_i^S} \right)}{\Phi},$$

$$\Phi = \left( 1 + \frac{[\text{S}_i]}{K_i^S} \right) \left( k_{-E} + k_{-S} \frac{[\text{S}_i]}{K_i^S} \right) + \left( 1 + \frac{[\text{S}_o]}{K_o^S} \right) \left( k_E + k_S \frac{[\text{S}_o]}{K_o^S} \right)$$

Net flux of S into the cell is

$$J_S = k_S [\text{SE}_o] - k_{-S} [\text{SE}_i] = \frac{E_T}{\Phi} \left( k_S k_{-E} \frac{[\text{S}_o]}{K_o^S} - k_{-S} k_E \frac{[\text{S}_i]}{K_i^S} \right).$$

For  $[\text{S}_i] = [\text{S}_o]$ ,  $J_S = 0$ ;

$\therefore K_i^S k_S k_{-E} = K_o^S k_{-S} k_E$ ,  $\Rightarrow$  constants are not independent.

**Problem:** Consider the uniporter transport of glucose into human red blood cells. The concentration of glucose in the plasma is  $[S_o]$  and that inside the red blood cell is negligible. Determine the net flux of glucose into the red blood cell.

Solution:

$$[\text{S}_i] = 0, \Phi = (k_{-E} + k_E) + (k_{-S} + k_S) \frac{[\text{S}_o]}{K_o^S},$$

$$J_S = \frac{E_T}{\Phi} k_S k_{-E} \frac{[\text{S}_o]}{K_o^S}.$$

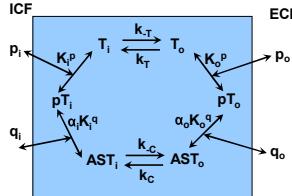
Define:

$$K_m = K_o^S \frac{k_{-E} + k_E}{k_{-E} + k_S} \quad \& \quad V_{\max} = E_T \frac{k_{-E} k_S}{k_{-E} + k_S}.$$

Michaelis-Menten Kinetics

$K_m$  and  $V_{\max}$  measured by fitting experimental data of  $J_S$  to  $[S_o]$ .  
At 37°C,  $K_m = 4 - 10 \text{ mM}$ ,  $V_{\max} = 600 \text{ mM min}^{-1}$

### Schematic of Symporter Transport: Random Model



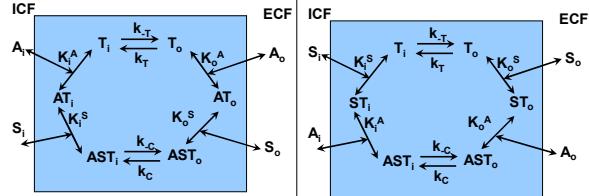
#### Random Model

A and S are substrates  
 $p = A$  or  $S$ ,  
 $q = A$  or  $S$   
 $T$  = symporter  
 $\alpha$  = substrate cooperativity in binding to T  
 $K$  = equilibrium constants,  
 $k$  = dislocation (across membrane) constants

- Assumptions:**
- Rate limiting step is the translocation of the substrate between ICF and ECF through the membrane.
  - Thus, binding between substrates and symporter is assumed to have reached equilibrium.

- Total number of symporters (on both sides of the membrane, together) is constant ( $= E_t$ )
- Total number of symporters is time independent, i.e., total flux of symporters is zero.

### Schematic of Symporter Transport – Ordered Models



$p = A$   
 $q = S$   
 Binding of A and S to T occurs in a sequential manner  
 A binds to the symporter first on both sides of the membrane  
 $ST$  does not exist in the membrane  
 $AT$  does not exist in the membrane

$p = S$   
 $q = A$   
 Binding of S and A to T occurs in a sequential manner  
 S binds to the symporter first on both sides of the membrane  
 $AT$  does not exist in the membrane

### Mathematical Model for Symporter Transport – Random Model

Due to assumption b)

$$[AT_o] = [A_o][T_o]/K_o^A,$$

$$[ST_o] = [S_o][T_o]/K_o^S,$$

$$[AST_o] = \frac{[AT_o][S_o]}{\left(\alpha_o K_o^S\right)} = \frac{[A][T_o][S_o]}{\left(\alpha_o K_o^S K_o^A\right)},$$

$$\text{or, } [AST_o] = \frac{[AT_o][S_o]}{\left(\alpha_o K_o^A\right)} = \frac{[A][T_o][S_o]}{\left(\alpha_o K_o^S K_o^A\right)}.$$

where K's are equilibrium constants  
 and o indicates extracellular.

### Mathematical Model for Symporter Transport – Random Model

Due to assumption c)

$$[T_o] + [AT_o] + [ST_o] + [AST_o] + [T_i] + [AT_i] + [ST_i] + [AST_i] = T_t,$$

where  $T_t$  is the total conc. of symporters in the membrane.

Due to assumption d)

$$k_T [T_o] - k_{-T} [T_i] + k_C [AST_o] - k_{-C} [AST_i] = 0,$$

where k's are the rate constant for translocation across the membrane.

### Solving above 8 equations

$$[T_o] = \frac{T_t \left( k_{-T} + k_{-C} \frac{[A_i][S_i]}{\alpha_i K_i^A K_i^S} \right)}{X}, \quad [T_i] = \frac{T_t \left( k_T + k_C \frac{[A_o][S_o]}{\alpha_o K_o^A K_o^S} \right)}{X},$$

where

$$X = \left( 1 + \frac{[A_o]}{K_o^A} + \frac{[S_o]}{K_o^S} + \frac{[A_o][S_o]}{\alpha_o K_o^A K_o^S} \right) \left( k_{-T} + k_{-C} \frac{[A_i][S_i]}{\alpha_i K_i^A K_i^S} \right) \\ + \left( 1 + \frac{[A_i]}{K_i^A} + \frac{[S_i]}{K_i^S} + \frac{[A_i][S_i]}{\alpha_i K_i^A K_i^S} \right) \left( k_T + k_C \frac{[A_o][S_o]}{\alpha_o K_o^A K_o^S} \right)$$

### Net Fluxes of A and S into the cell are

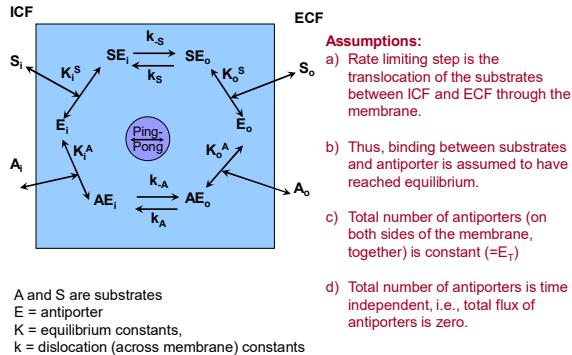
$$J_A = J_S = k_C [AST_o] - k_{-C} [AST_i] \\ = \frac{T_t}{X} \left( k_C k_{-T} \frac{[A_o][S_o]}{\alpha_o K_o^A K_o^S} - k_{-C} k_T \frac{[A_i][S_i]}{\alpha_i K_i^A K_i^S} \right).$$

Fluxes should be zero when  $[A_i] = [A_o]$  and  $[S_i] = [S_o]$ .  
 $\therefore$  the following relationship must be satisfied:

$$\frac{k_C k_{-T}}{\alpha_o K_o^A K_o^S} = \frac{k_{-C} k_T}{\alpha_i K_i^A K_i^S}.$$

This means, only 9 of the 10 constants could be specified independently.

### Schematic of Antiporter Transport



### Mathematical Model for Antiporter Transport

Due to assumption b)

$$[AE_o] = [A_o][E_o]/K_o^A,$$

$$[SE_o] = [S_o][E_o]/K_o^S,$$

$$[AE_i] = [A_i][E_i]/K_i^A;$$

$$[SE_i] = [S_i][E_i]/K_i^S;$$

where K's are equilibrium constants and o and i indicate extracellular & intracellular, respectively.

### Mathematical Model for Antiporter Transport

Due to assumption c)

$$[E_o] + [AE_o] + [SE_o] + [E_i] + [AE_i] + [SE_i] = E_T,$$

where  $E_T$  is the total conc. of symporters in the membrane.

Due to assumption d)

$$k_A [AE_o] - k_{-A} [AE_i] + k_S [SE_o] - k_{-S} [SE_i] = 0,$$

where k's are the rate constant for translocation across the membrane.

### Solving above 6 equations

$$[E_o] = \frac{E_T \left( k_{-A} \frac{[A_i]}{K_i^A} + k_{-S} \frac{[S_i]}{K_i^S} \right)}{Y}; [E_i] = \frac{E_T \left( k_A \frac{[A_o]}{K_o^A} + k_S \frac{[S_o]}{K_o^S} \right)}{Y},$$

where

$$Y = \left( 1 + \frac{[A_o]}{K_o^A} + \frac{[S_o]}{K_o^S} \right) \left( k_{-S} \frac{[S_i]}{K_i^S} + k_{-A} \frac{[A_i]}{K_i^A} \right) + \left( 1 + \frac{[A_i]}{K_i^A} + \frac{[S_i]}{K_i^S} \right) \left( k_S \frac{[S_o]}{K_o^S} + k_A \frac{[A_o]}{K_o^A} \right)$$

Fluxes should be zero when  $[A_i] = [A_o]$  and  $[S_i] = [S_o]$ .

∴ the following constraint relationship must be satisfied:

$$\frac{k_A k_{-S}}{K_o^A K_i^S} = \frac{k_{-A} k_S}{K_i^A K_o^S}.$$

This means only 7 out of 8 constants are independent. Also, if  $J_A = 0$ ,  $k_A [AE_o] = k_{-A} [AE_i]$ .

Using the above eqn. and the two equilibrium relations for A, the constraint eqn. could be rearranged as

$$\frac{K_A^A k_{-A}}{K_o^A K_i^A} = \frac{k_{-S} K_o^S}{K_i^A k_S} = \frac{[E_o]}{[E_i]} = \lambda \quad (\text{asymmetry ratio of unbound carriers between intra- and extra-cellular space}).$$

$\lambda$  is independent of substrate and intrinsic property of the antiporter. For anion-exchangers in the red blood cells,  $\lambda=0.1$ .

### Net Fluxes of A entering (or S exiting) the cell are

$$J_A = -J_S = k_A [AE_o] - k_{-A} [AE_i] \\ = \frac{E_T}{Y} \left( k_A k_{-S} \frac{[A_o][S_i]}{K_o^A K_i^S} - k_{-A} k_S \frac{[A_i][S_o]}{K_i^A K_o^S} \right).$$

Problem: Determine the fluxes of  $\text{Na}^+$  and  $\text{Cl}^-$  through a  $\text{Na}^+/\text{Cl}^-$  symporter in the epithelial cells of the distal tubule of the human kidney, as a function of the extracellular  $\text{Na}^+$  concentration  $[Na_o^{+}]$ .  $\alpha_o = \alpha_i = 1$ ,  $K_o^{\text{Na}} = K_i^{\text{Na}} = 51.1\text{mM}$ ,  $K_o^{\text{Cl}} = K_i^{\text{Cl}} = 19.2\text{mM}$ ,  $k_T = k_{-T} = k_C = k_{-C}$ , and  $J_{\max} = k_f T_f = 1.04 \times 10^{-4} \text{ mmol cm}^2 \text{s}^{-1} [Na_i^{+}] = 9.5\text{mM}$ ,  $[Cl_i^{-}] = 35.7\text{mM}$ ,  $[Cl_o^{-}] = [Na_o^{+}]$ .

Evaluate the net flux of  $\text{Na}^+$  for  $[Na_o^{+}] = 20, 40, 60\text{ mM}$ .

$$\text{Solution: } J_{Na} = \frac{1.06 \times 10^{-7} [Na_o^{+}]^2 - 0.36 \times 10^{-4}}{4.75 + 0.097 [Na_o^{+}] + 4.87 \times 10^{-3} [Na_o^{+}]^2}.$$

$[Na_o]$ , mM	$J_{Na} \times 10^{-4} \text{ mmol/cm}^2/\text{s}$
20	0.01
40	0.08
60	0.12

## Active Transport

### Coupled transporters

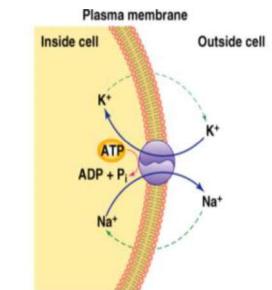
- uphill transport of one solute across the membrane to the downhill transport of another
  - $\text{Na}^+ - \text{K}^+$  pump - 30% of the total ATP consumption

### ATP driven pumps

- couple uphill transport with energy from ATP

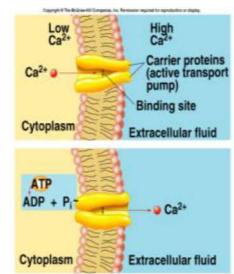
## Na<sup>+</sup>/K<sup>+</sup> Pump

- Carrier protein is also an ATP enzyme that converts ATP to ADP and  $P_i$ .
  - Actively extrudes 3  $\text{Na}^+$  and transports 2  $\text{K}^+$  inward against concentration gradient.
- Steep gradient serves 4 functions:
  - Provides energy for "coupled transport" of other molecules.
  - Regulates resting calorie expenditure and BMR.
  - Involvement in electrochemical impulses.
  - Promotes osmotic flow.



## Primary Active Transport

- Hydrolysis of ATP directly required for the function of the carriers.
- Molecule or ion binds to "recognition site" on one side of carrier protein.
- Binding stimulates phosphorylation (breakdown of ATP) of carrier protein.
- Carrier protein undergoes conformational change.
  - Hinge-like motion releases transported molecules to opposite side of membrane.

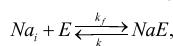


## Mathematical Model for Na<sup>+</sup>/K<sup>+</sup> ATPase

Na<sup>+</sup>/K<sup>+</sup> ATPase is an antiporter and therefore, the antiporter model described above can be used. However, here we present an alternate model. The active transport of  $\text{Na}^+$  via Na<sup>+</sup>/K<sup>+</sup> ATPase involves

- The binding of  $\text{Na}^+$  to the ATPase
- The transmembrane translocation of  $\text{Na}^+$  due to conformational change of the ATPase.

The binding process could be approximated by the reaction:



where,  $Na_i$  is the intracellular  $\text{Na}^+$ ,  $E$  is the ATPase, and  $NaE$  is the complex of  $\text{Na}^+$  and  $E$ .

$$\text{The kinetic equation is } \frac{dC_{NaE}}{dt} = k_f C_{Na,i} C_E - k_r C_{NaE}$$

In the steady state,

$$k_f C_{Na,i} C_E = k_r C_{NaE}.$$

Number of free ATPase molecules is equal to total number of ATPase molecules minus those which have bound to  $\text{Na}^+$ ; i.e.,

$$C_E = \langle C_E \rangle_t - C_{NaE},$$

Where  $\langle C_E \rangle_t$  is the total concentration of ATPase in the membrane. Substituting the above eqn. into the one above that

$$C_{NaE} = \frac{k_f \langle C_E \rangle_t C_{Na,i}}{K'_{Na} + C_{Na,i}},$$

Where  $K'_{Na} = k_r/k_f$  is the apparent dissociation constant for  $\text{Na}^+$ .

The last equation shows that the probability of forming a complex of  $\text{Na}^+$  and E is proportional to  $C_{\text{Na},i}/(K'_{\text{Na}} + C_{\text{Na},i})$ .

Similarly it can be shown that the probability of forming a complex of  $\text{K}^+$  and E is proportional to  $C_{\text{K},o}/(K'_{\text{K}} + C_{\text{K},o})$ .

Therefore the probability of forming a complex of one E, two  $\text{K}^+$  and three  $\text{Na}^+$  is proportional to

$$P_s = \left[ \frac{C_{\text{Na},i}}{K'_{\text{Na}} + C_{\text{Na},i}} \right]^3 \left[ \frac{C_{\text{K},o}}{K'_{\text{K}} + C_{\text{K},o}} \right]^2,$$

if there is no co-operativity in ion/ATPase binding.

Assuming that active transport of  $\text{Na}^+$  or  $\text{K}^+$  always involves a simultaneous binding of three  $\text{Na}^+$  and two  $\text{K}^+$  ions to the  $\text{Na}^+/\text{K}^+$ -ATPase, and that there is no binding cooperativity, the efflux of  $\text{Na}^+$  via the  $\text{Na}^+/\text{K}^+$ -ATPase,  $J_{\text{Na}}$  should be proportional to  $p_s$ , and is given by

$$J_{\text{Na}} = J_{\text{Na,max}} p_s \\ = J_{\text{Na,max}} \left[ \frac{C_{\text{Na},i}}{K'_{\text{Na}} + C_{\text{Na},i}} \right]^3 \left[ \frac{C_{\text{K},o}}{K'_{\text{K}} + C_{\text{K},o}} \right]^2,$$

where  $J_{\text{Na}} = J_{\text{Na,max}}$  when  $p_s = 1$ .

$$J_K = -\frac{2}{3} J_{\text{Na}}.$$

In general,  $J_{\text{Na,Max}}$  increases with the intracellular concentration  $C_{\text{K},i}$  of  $\text{K}^+$  and reaches a plateau when  $C_{\text{K},i} > 30 \text{ mM}$ . Under normal conditions,  $C_{\text{K},i}$  is of the order of 150 mM. Therefore,  $J_{\text{Na,Max}}$  is a constant with a value ranging from  $0.15 \times 10^{-6}$  to  $2.69 \times 10^{-6} \text{ mmol/cm}^2/\text{s}$  in the kidney. However,  $K'_{\text{Na}}$  and  $K'_{\text{K}}$  are linear functions of concentrations of  $\text{Na}^+$  and  $\text{K}^+$  and given by

$$K'_{\text{Na}} = a_{\text{Na}} + b_{\text{Na}} C_{\text{K},i},$$

$$K'_{\text{K}} = a_K + b_K C_{\text{Na},o},$$

Approximate values:

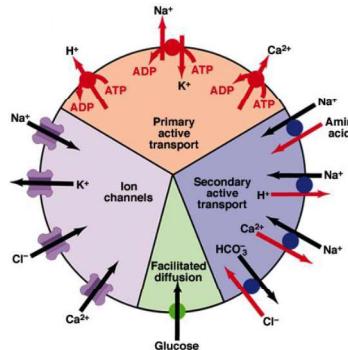
$$a_{\text{Na}} = 0.2 \text{ mM}, a_K = 0.1 \text{ mM},$$

$$b_{\text{Na}} = 2.4 \times 10^{-2}, b_K = 5.4 \times 10^{-3}.$$

The dependence of  $K'_{\text{Na}}$  on  $C_{\text{K},i}$  is due to the competitive inhibition of the binding of  $\text{Na}^+$  to the  $\text{Na}^+/\text{K}^+$ -ATPase by the  $\text{K}^+$  in the cytosol of cells.

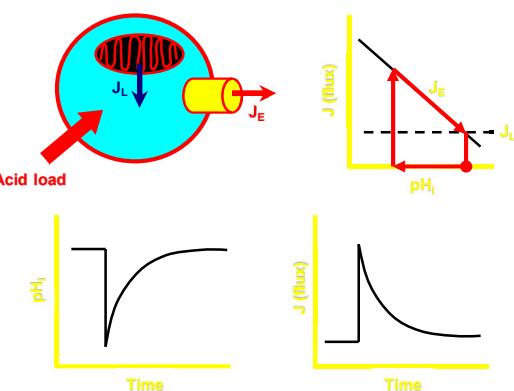
Similarly, the dependence of  $K'_{\text{K}}$  on  $C_{\text{Na},o}$  is due to competitive inhibition of the binding of  $\text{K}^+$  to the  $\text{Na}^+/\text{K}^+$ -ATPase by  $\text{Na}^+$  in the extracellular medium.

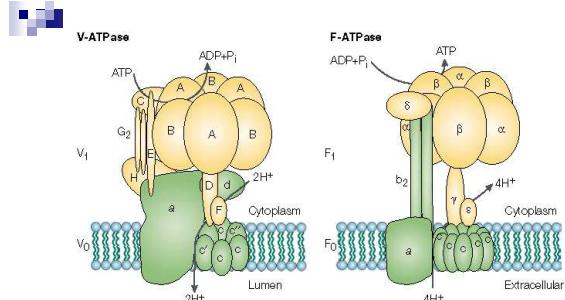
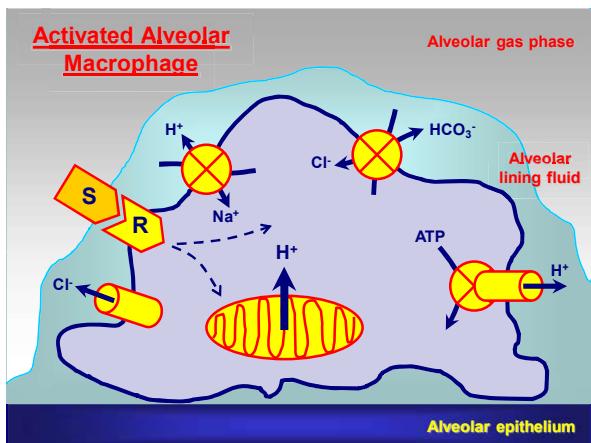
## ION TRANSPORT



**Problem:** Determine the efflux of  $\text{Na}^+$  across the epithelial membrane via the  $\text{Na}^+/\text{K}^+$ -ATPase if  $C_{\text{Na},i} = 10 \text{ mM}$ ,  $C_{\text{K},i} = 140 \text{ mM}$ ,  $C_{\text{Na},o} = 145 \text{ mM}$ , and  $C_{\text{K},o} = 5 \text{ mM}$ .  $J_{\text{Na,Max}}$  ranges from  $0.15 - 2.69 \times 10^{-6} \text{ mmol/cm}^2/\text{sec}$ .

Ans: Flux of  $\text{Na}^+$  varies from  $0.43 - 7.79 \times 10^{-7} \text{ mmol/cm}^2/\text{sec}$ .





**Figure 1 | Structural comparison of the V- and F-ATPases.** For the V-ATPases, ATP hydrolysis by the peripheral  $V_1$  domain (shown in yellow) drives proton transport through the integral  $V_0$  domain (shown in green) from the cytoplasm to the lumen. By contrast, the F-ATPases (or ATP synthases) normally function in the opposite direction (that is, in ATP synthesis), coupling the downhill movement of protons through  $F_0$  to the synthesis of ATP in  $F_1$ . Both enzymes are thought to operate through a rotary mechanism (see text).

### Relative Importance of Convection and Diffusion

$$\text{Pecllet Number } Pe = \frac{\text{Diffusion Time}}{\text{Convection Time}} = \frac{L^2 / D_g}{v / L} = \frac{vL}{D_g}$$

#### Relevant Length Scales in Biological Systems

Quantity	Length scale, m
Proteins & Nucleic Acids	$10^{-8}$
Organelles	$10^{-7}$
Cells	$10^{-5}$ to $10^{-6}$
Capillary Spacing	$10^{-4}$
Organs	$10^{-1}$
Whole Body	$10^0$

If  $Pe \ll 1$ , Diffusion Time  $\ll$  Convection Time  
Therefore, **Convection Limited**.

If  $Pe \gg 1$ , Diffusion Time  $\gg$  Convection Time  
Therefore, **Diffusion Limited**.

If  $Pe \sim O(1)$ , Diffusion Time  $\sim$  Convection Time  
Both Diffusion and Convection are equally important.

### Relative Importance of Convection and Diffusion

Molecule	MW, g mole <sup>-1</sup>	$D_{ij}$ (cm <sup>2</sup> /s)	Diffusion Time, $L^2/D_{ij}$ (s)	$Pe = Lv/D_{ij}$
Oxygen	32	$2 \times 10^{-5}$	5	0.05
Glucose	180	$2 \times 10^{-6}$	50	0.5
Insulin	6000	$1 \times 10^{-6}$	100	1
Antibody	150,000	$6 \times 10^{-7}$	167	1.67
Particles	Diameter			
Virus	0.1 $\mu\text{m}$	$5 \times 10^{-8}$	2000	20
Bacterium	1 $\mu\text{m}$	$5 \times 10^{-9}$	20000	200
Cell	10 $\mu\text{m}$	$5 \times 10^{-10}$	200000	2000

$L=100$  microns,  $v=1$  micron/s

