



Department of  
Life Sciences  
and Systems Biology

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# Cellular and Molecular Biophysics

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***Corso di laurea in LM Biotecnologie Industriali- LM Fisica***

# Fluxes Lows

Diffusion. Permeability



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# Solutes and Water Transports across the membranes: FLUXES

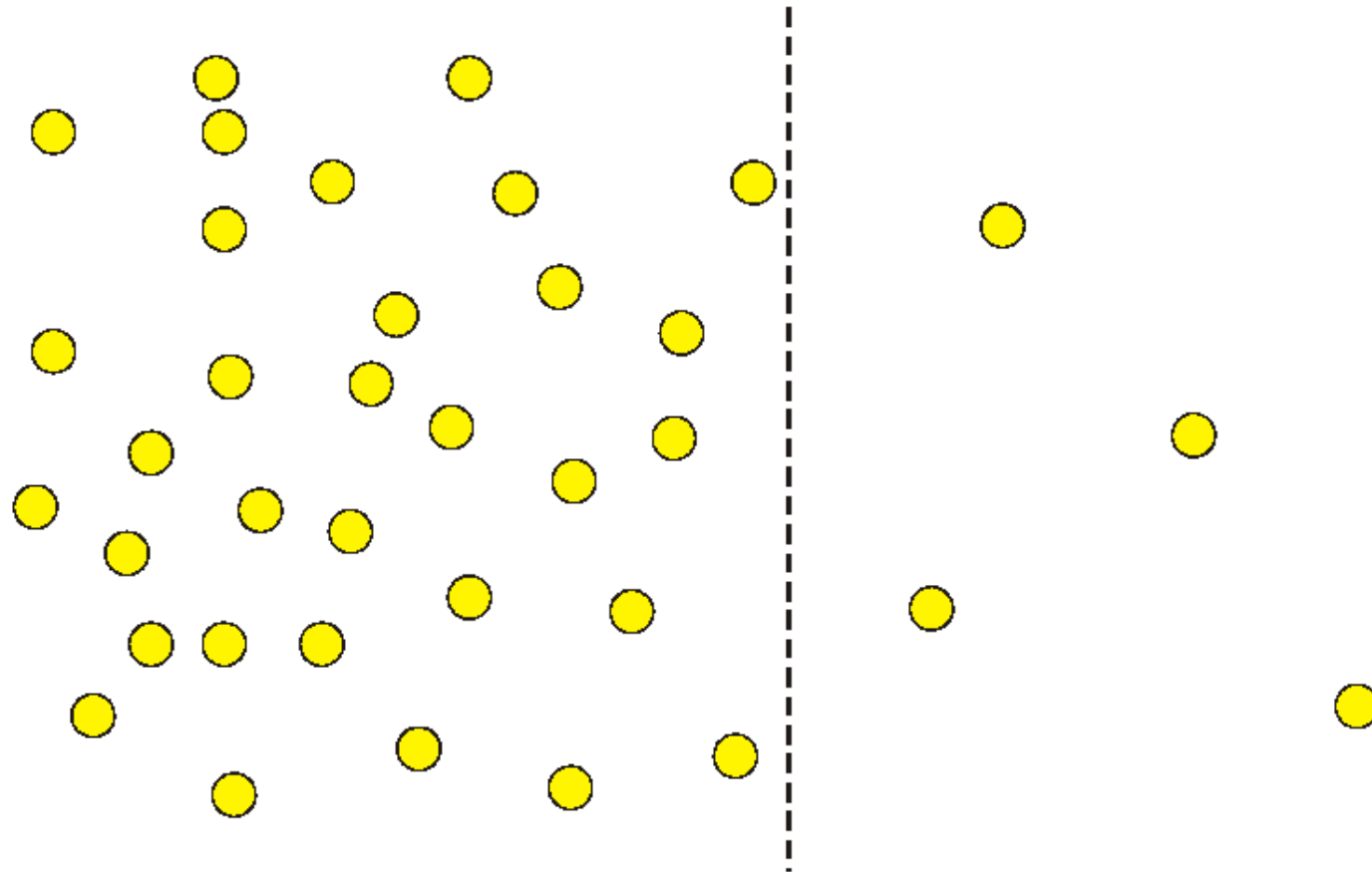
Passive Fluxes (according to the gradient):  
DIFFUSION low

Active Fluxes (against the gradient): on top of the Diffusion low, we need to add the terms regarding the chemical reaction coupled.



Side A

Side B



$$[X]_A > [X]_B$$

*Non-electrolyte*



Flux can be defined as number of moles  $n$  flowing in unit time through the surface  $A$

$$J = \frac{dn}{dt A}$$

The concentration  $c = \frac{n}{V}$  And therefore  $n = cV$   $V = \text{volume}$

We can express in differential form  $dn = c dV$   $dn = c A dx$

Let's define then the flux

$$\frac{dn}{dt} = \frac{c A dx}{dt} = A c v \quad \text{Knowing that } v = \frac{dx}{dt} \text{ Is the speed of the particles in the solute}$$

$$J = cv$$

$F = \text{force acting on the body}$   
 $r = \text{resistance}$   
 $v = \text{speed of the body}$

As for a solid body in a fluid the force that act on it can be defined by STOKES

$$F = rv$$

Also defined as  $F = \frac{X}{N}$   $X = \frac{\text{total force}}{n}$

$F$  is the force acting on the single particle of solute  
 $N = \text{numero di Avogadro}$

$$F = rv$$

$$v = \frac{F}{r} = \frac{X}{rN}$$

$$f = 6\pi\eta r$$

Resistance  $r$  for a particle in a solute can be define as frictional coefficient

$r = \text{radius}$   
 $\eta = \text{viscosity}$



We can therefore rewrite the flux equation

$$J = cv = \frac{1}{Nf} cX$$

Let's define mobility as  $\frac{1}{Nf} = U$

$$J = UcX$$

TEORELL

For more solute

$$J_i = U_i c_i X_i$$



From a thermodynamic point of view the correct expression for the force acting on chemical species is the **chemical potential gradient (potenziale chimico)  $m$**

the **chemical potential  $\mu$**  is the free energy variation in the mole unit

$$\mu_i = \left( \frac{dG}{dn_i} \right)_{T, P, n}$$

It means the free energy variation in the mole unit meaning the work that the system can accomplish per mole unit

For ideal solutions the chemical potential of the solute is :

$$\mu_i = \mu_i^\circ + RT \ln c_i + V_i P$$

$\mu_i^\circ$  = standard chemical potential, the free energy Gibbs value per mole in standard conditions (25°C, 1 bar P, 1 M concentration)

$V_i$  = partial molar volume of the solute

$P$  = hydrostatic pressure



We are actually interested in the difference of chemical potential between two states: in this case  $\mu_i^\circ$  is eliminated in the subtraction and its numerical value is not important anymore.

Moreover for a solute ( $V_iP$ ) is normally negligible as compared with the other terms and therefore the common expression for chemical potential is

$$d\mu_i = RTd\ln c_i$$

For electrically charged particles we also have an electrical component contributing to the free energy

## ELECTROCHEMICAL POTENTIAL

It is due to the sum of chemical and electrical potential of 1 mole of the substance  $i$ . In general the potential electric energy ( $V$ ) is expressed in Coulomb per charge instead per mole (as the case for the chemical potential). The charge amount (in coulomb) that is transported by 1 mole of a ion with valence  $z_i$  is the one transported by 1 equivalent (Faraday constant =  $96487 \text{ Cmol}^{-1}$ ) multiply for the valence

$$\mu_{ec_i} = \mu_i^\circ + RT\ln c_i + z_i FV$$



$$J = c \cdot v = \frac{1}{Nf} cX$$

Let's go back to TEORELL



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the **chemical potential**  $\mu$  is the free energy variation in the mole unit

$$\mu_i = \left( \frac{dG}{dn_i} \right)_{T, P, n}$$

It means the free energy variation in the mole unit meaning the work that the system can accomplish per mole unit

Let's define  $X = \frac{d\mu_i}{dx}$  Where  $d\mu$  = potential energy (the Work)

The force per mole unit is the derivative of the of the work meaning the reduction of potential energy



$$J = c \cdot v = \frac{1}{Nf} cX$$

Let's describe the flux of anaelectrolites starting from  
TEORELL by explicating the terms

$$J = \frac{1}{Nf} c \frac{d\mu}{dx} = \frac{1}{Nf} c \frac{RT d \ln c}{dx} = \frac{RT}{Nf} c \frac{dc}{c} \frac{1}{dx} = \frac{RT}{Nf} \frac{dc}{dx}$$

$$\frac{RT}{Nf} = D \quad \text{Diffusion coefficient}$$

The dimation of D sono cm<sup>2</sup>/s

$$J = -D \frac{dc}{dx}$$

FICK LOW



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FICK LOW

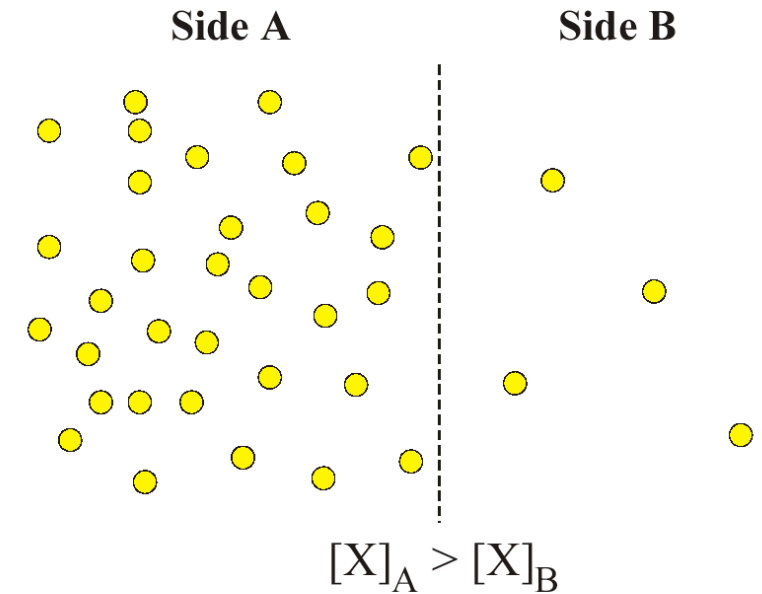
Since:  $dc < 0$  ( $c_B < c_A$ )  
 $dx > 0$  ( $x_B > x_A$ )

$$D = \text{velocity} = \frac{A}{t} = \frac{\mu m^2}{s}$$

$$dc = \frac{\text{mol}}{\mu m^3}$$

$$\frac{1}{dx} = \frac{1}{\mu m}$$

$$J = \frac{\text{mol}}{\mu m^2 s}$$



Non-electrolyte



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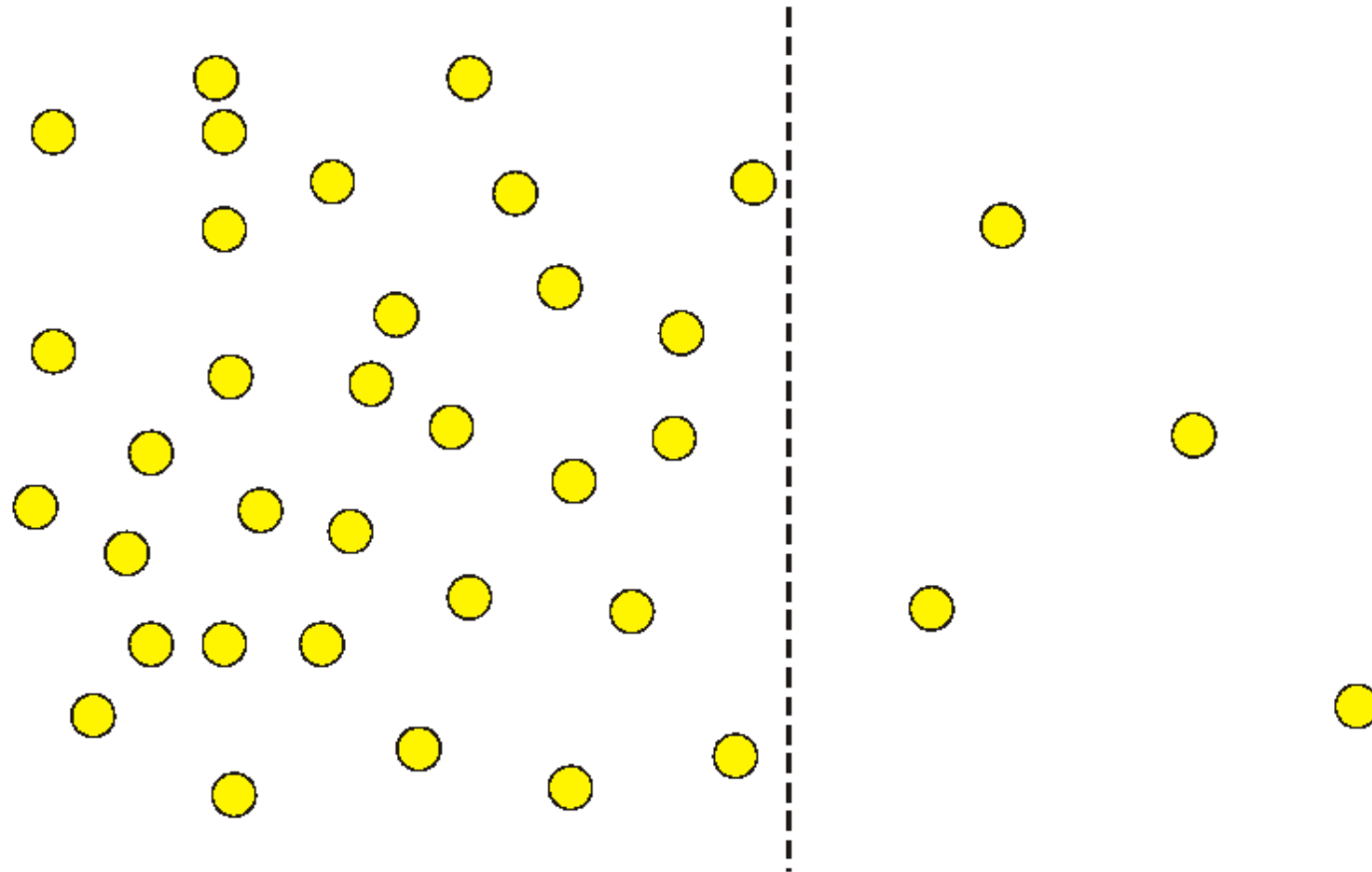
FICK LOW

The Fick low is valid for diffuse fluxes, *passive fluxes*: in a non homogeneous system , the solute (or the solvent) tends to move following its concentration gradient (if it is **nonelectrolyte**; or the electrochemical gradient if it is **electrolyte**), and therefore to dissipate the gradient of potential in the system reaching an **equilibrium state** that can be define as the state in which the **net** flux is 0.



Side A

Side B

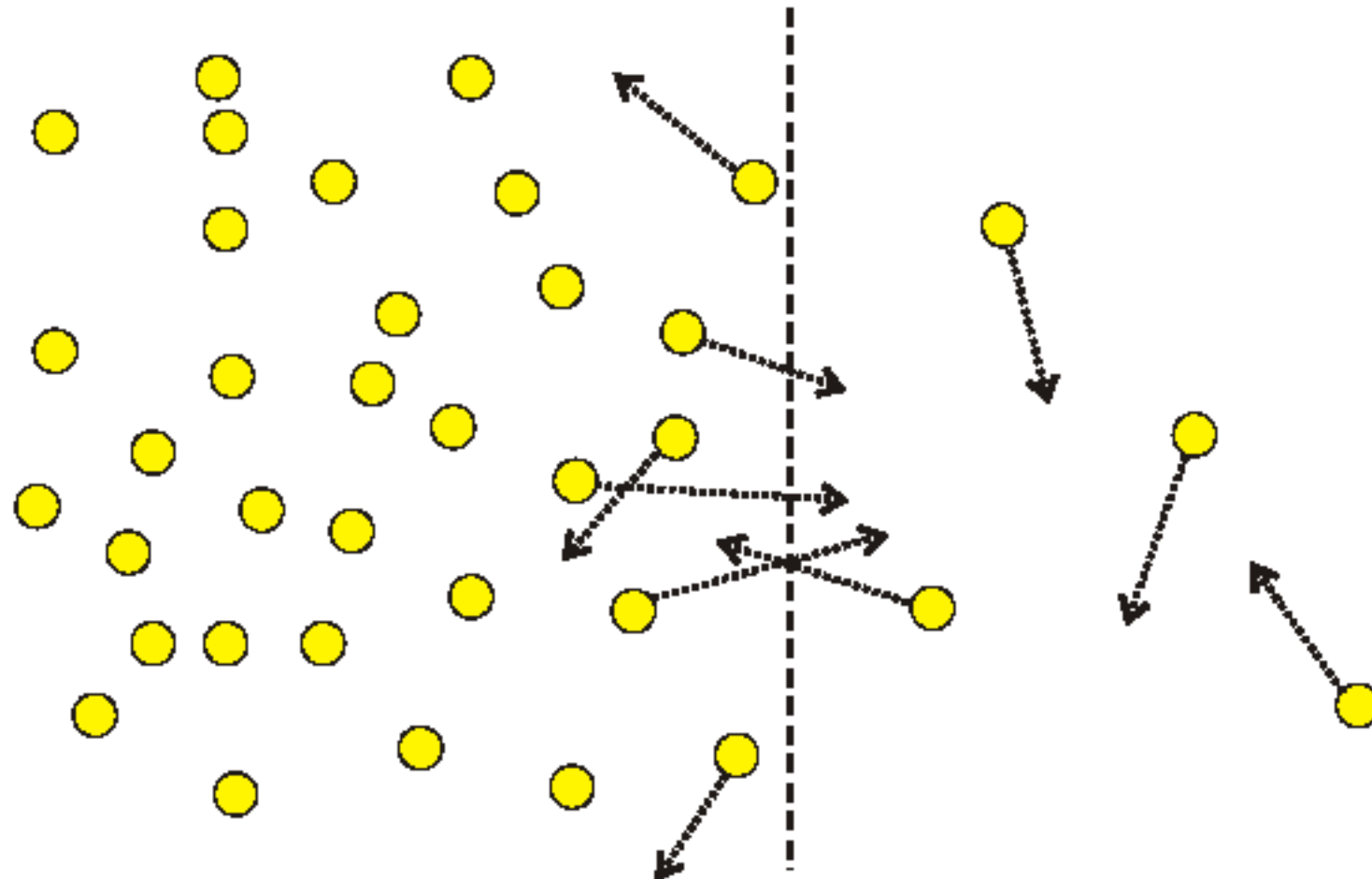


$$[X]_A > [X]_B$$

*Non-electrolyte*

Side A

Side B

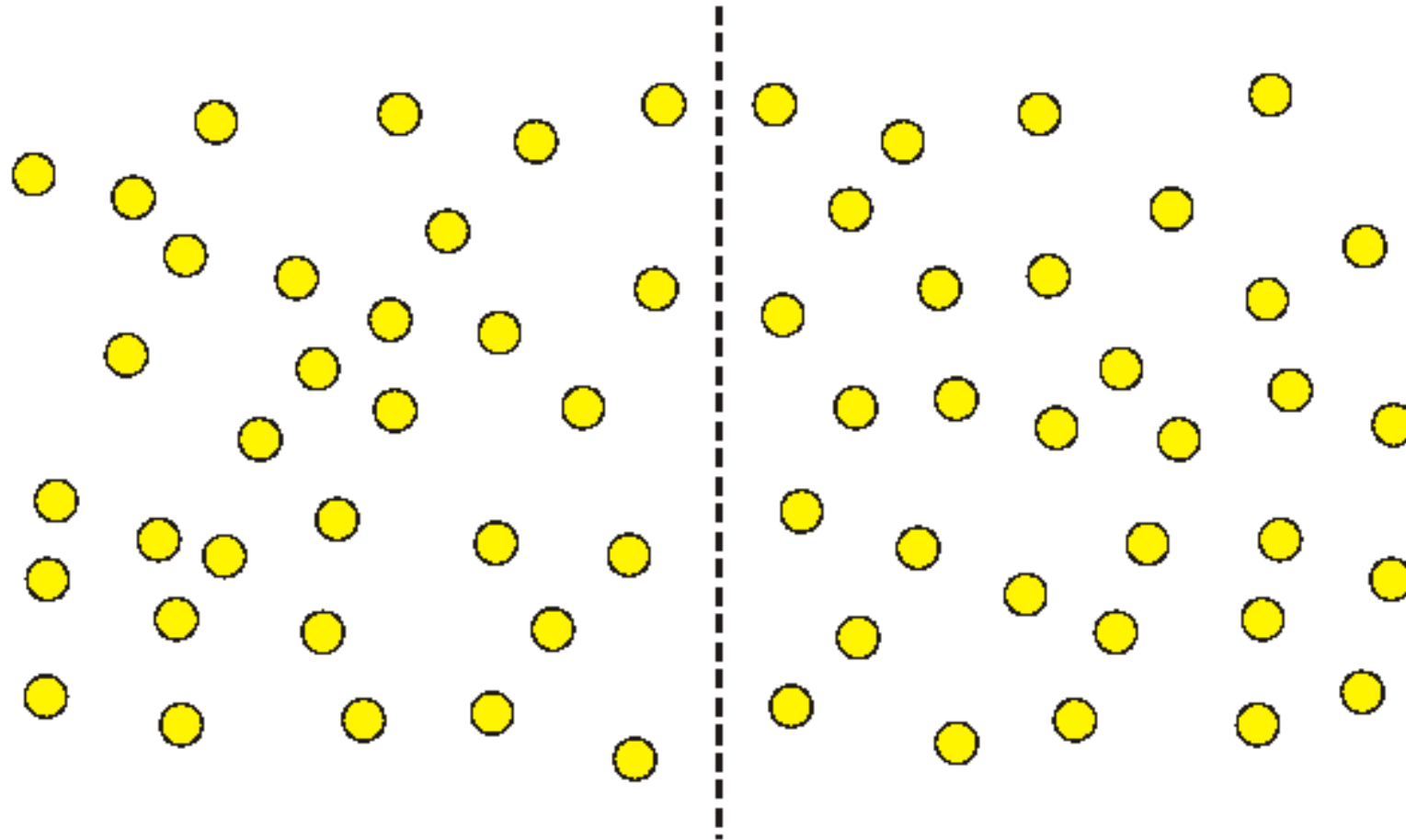


*'unidirectional' fluxes  
are unequal*

$$J_{A-B} > J_{B-A}$$

Side A

Side B



*at 'equilibrium'*

$$[X]_A = [X]_B$$
$$J_{A-B} = J_{B-A}$$

# Net Diffusion is

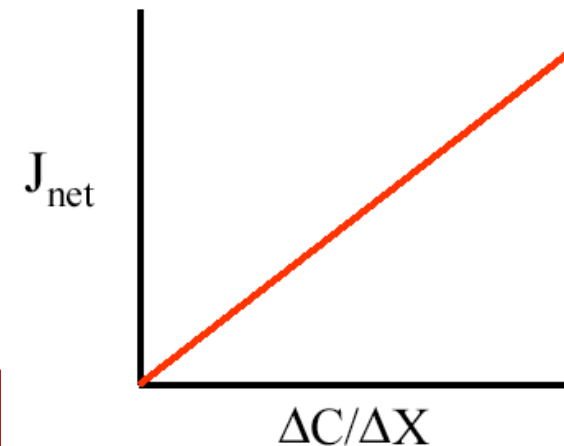


Proportional to the Difference in  
Particle Concentration ( $\Delta C$ )

$$J_{\text{net}} \propto \frac{\Delta C}{\Delta X} \quad \leftarrow \text{‘driving force’}$$

To convert this to an ‘equality,’  
add ‘D’ (diffusion coefficient)  $J_{\text{net}} = D \frac{\Delta C}{\Delta X}$

‘Fick Equation’

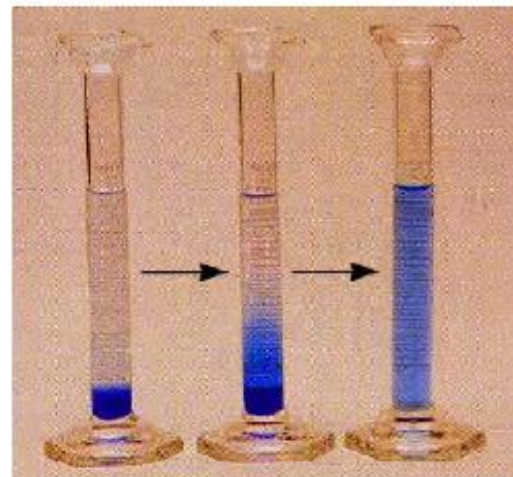




# Simple Diffusion

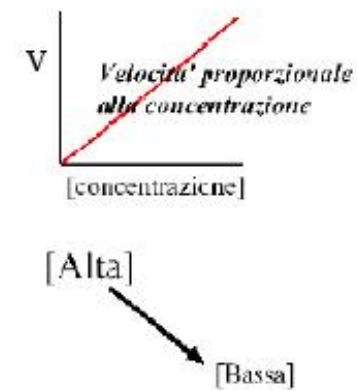
Simple free diffusion is due to thermal agitation: it's a probabilistic process

1. The net diffusion is directly proportional to the concentration gradient.
2. Diffusion is fast for short distances (cellular) but slow for long distances ('organ' level)
3. Diffusion depends on the diffusion coefficient of the solute

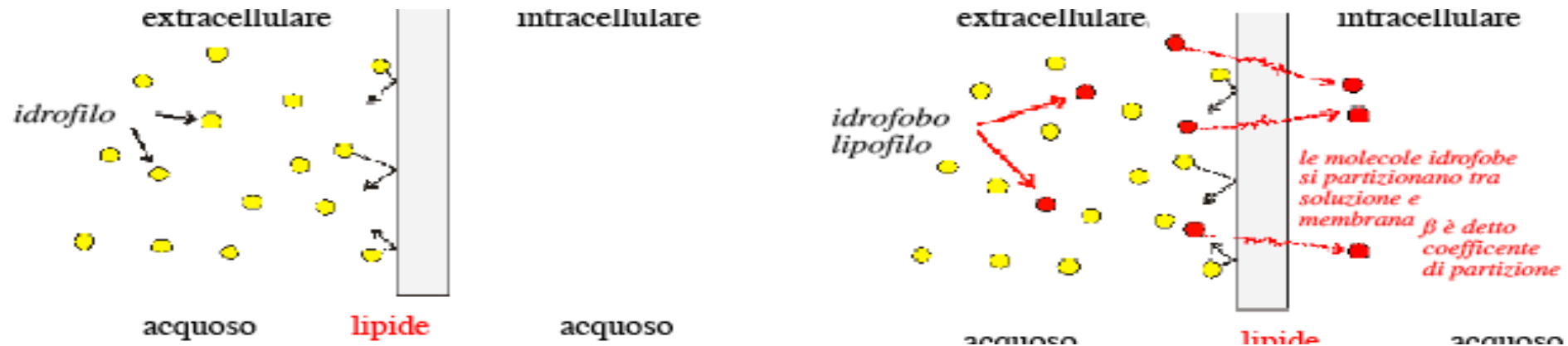


$$J = -DA \frac{\Delta c}{\Delta x}$$

J=flusso  
D=uRT  
u=mobilità  
A=area dove avviene la diffusione



# Free diffusion through a membrane

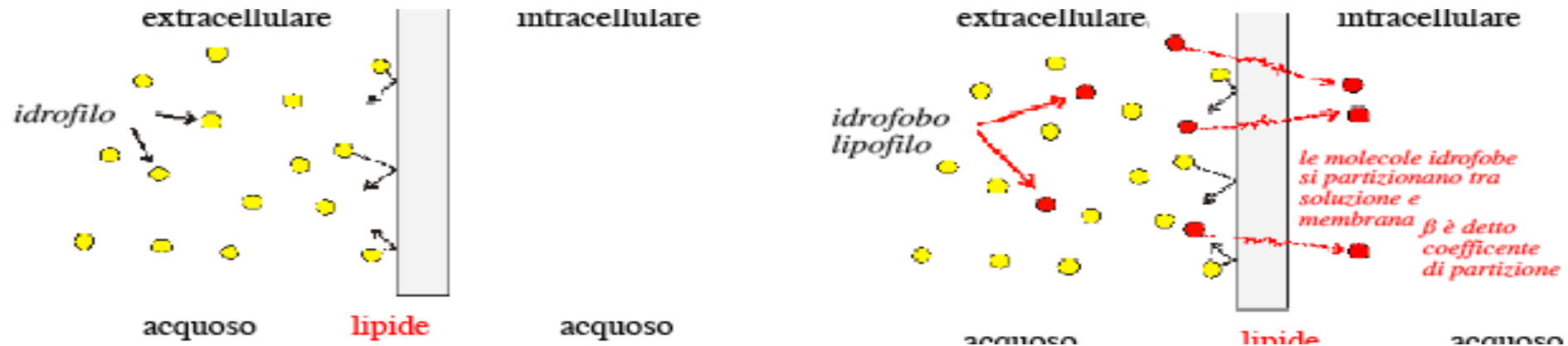


FREE DIFFUSION IN THE ABSENCE OF BARRIERS: EX. DROP OF SOLUTE IN A CILINDER  
(CONTINUOUS SYSTEM)

FREE DIFFUSION THROUGH A **MEMBRANE**: IMPLY THE PRESENCE OF **TWO DISTINCT PHASES**; DISCONTINUOUS

IN THIS CASE THE DIFFUSION LAWS ARE VALID **JUST WITHIN THE MEMBRANE THICKNESS**,  $\Delta x$ .

# Free diffusion through a membrane



- In order to diffuse through the cell membrane, the solutes need to enter in the lipid bilayer
- Gas particles can diffuse by free diffusion
- Polar solutes (hydrophilic) can't
- Hydrophobic solutes can diffuse (depending on the nature and dimensions)

Analectrolite flux across a membrane

$$\Phi = JA$$

$$\Phi = -AD \frac{\Delta c}{\Delta x} \quad \Delta x = \text{the membrane thickness}$$

We have to consider the real concentration within the membrane  $c_m = cr$   $r =$  ripartition coefficient

$$\Phi = -ADr \frac{\Delta c}{\Delta x}$$

$$J = -Dr \frac{\Delta c}{\Delta x}$$

We define the PERMEABILITY as  $P = \frac{Dr}{\Delta x}$

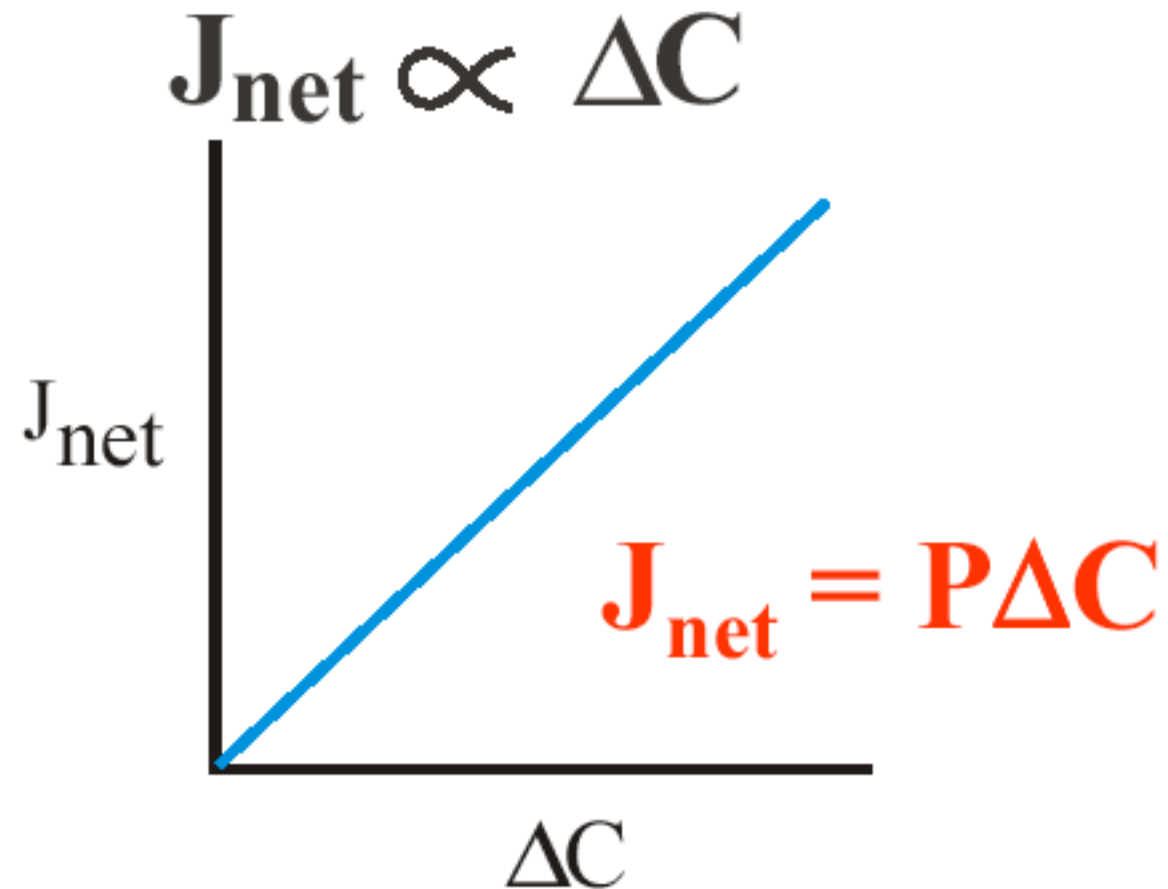
$$J = -P\Delta c$$

$$\frac{J_{1-2}}{J_{2-1}} = \frac{P_m c_1}{P_m c_2} = \frac{c_1}{c_2} \quad \text{USSING}$$

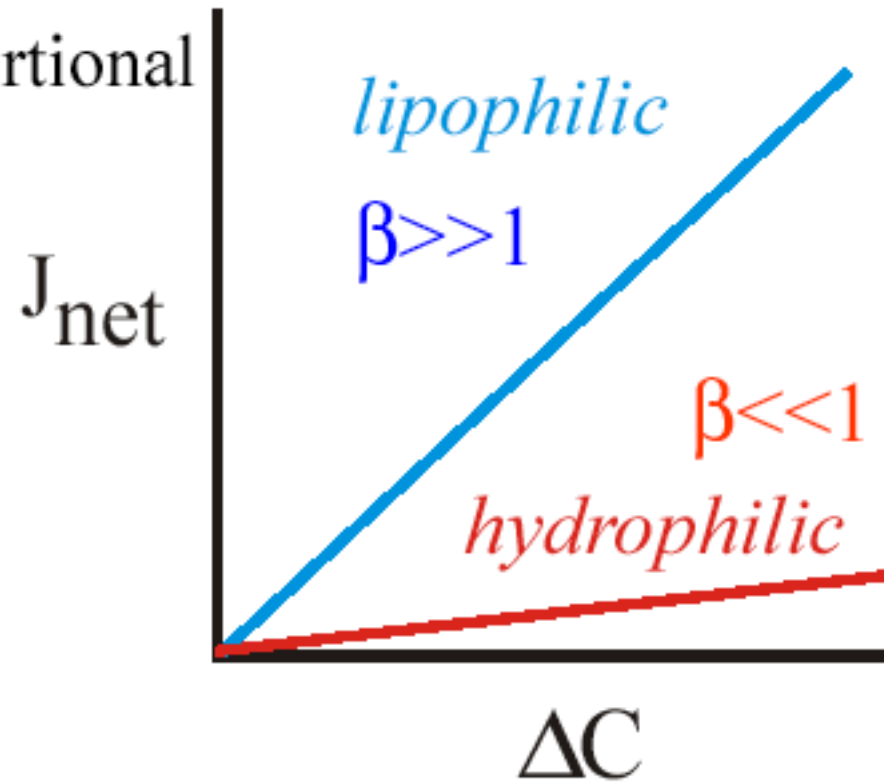
D is dependent from both solute and solvent while P is also depending from the membrane properties

# Take Home Issues for 'Permeability'

1. Rate of diffusion across a membrane is proportional to the concentration gradient



2. Rate of diffusion across membranes is proportional to the lipophilicity of the particle





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## 1) A MEMBRANE WHICH IS PERMEABLE ONLY TO SOLVENT

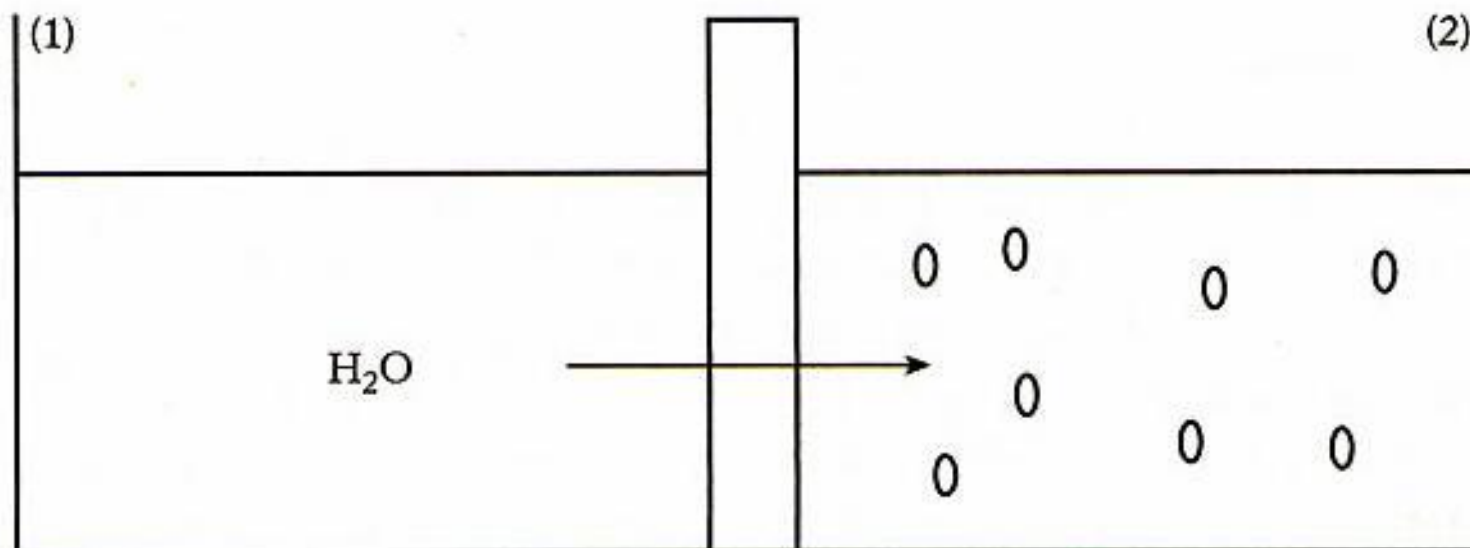
only solvent fluxes. Osmotic phenomenon  
volume and pressure changes

## 2) MEMBRANE PERMEABLE TO BOTH SOLUTE AND SOLVENT

Both solute and solvent can diffuse: in general  
volume changes are momentary; the system  
tend to reach and EQUILIBRIUM state



## A MEMBRANE WHICH IS PERMEABLE ONLY TO SOLVENT







## EQUILIBRIUM:

$\mu_1 = \mu_2$  this means that, by definitions of  
chemical potential,  $c_1 = c_2$ .

(this is valid both for chemical potential – and  
concentration – of solutes than for solvents)

**THE NET FLUXES = 0**

# Fluxes of charged solutes:

It is possible to derive the Fick law valid for ionic solutes: ELECTRODIFFUSION LAW

$$J = -D \left( \frac{dc}{dx} + c z \frac{F}{RT} \frac{dV(x)}{dx} \right)$$

NERNST-PLANCK  
(elettrodifusione)

Let's describe the electrolytes fluxes



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$$m_{ec} = m_c + m_e$$

$$J = -Uc \frac{dm_c}{dx} \pm Uc \frac{dm_e}{dx}$$

$$J = -Uc \frac{RT d \ln c}{dx} \pm Uc \frac{zF dV}{dx}$$

$$J = -Uc \left( \frac{RT d \ln c}{dx} \pm \frac{zF dV}{dx} \right)$$

$$J = - \frac{RT}{Nf} c \left( \frac{dc}{cdx} \pm \frac{zF}{RT} \frac{dV}{dx} \right)$$

$$J = -D \left( \frac{dc}{dx} + c \frac{zF}{RT} \frac{dV}{dx} \right) \quad \text{NERNST-PLANCK}$$

THIS IS A EQUATION SYSTEM

(n ions = 2n +1 incognita = 2n + 1 equations)



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TO SIMPLIFY THE SOLUTION OF THE  
SYSTEMS OF EQUATIONS:

1) LET'S CONSIDER THE EQUILIBRIUM  
STATE ( $J_i$  TOTAL= 0)

IN THIS CASE WE CAN DERIVE THE  
FOLLOWING SOLUTION

$$V_{eq} = \frac{RT}{zf} \ln \frac{C_{out}}{C_{in}}$$



At the equilibrium  $J = 0$

$$\frac{dc}{dx} = c \frac{zF}{RT} \frac{dV}{dx}$$

$$\frac{dV}{dx} = \frac{RT}{zF} \frac{dc}{c} \frac{1}{dx} = \frac{RT}{zF} \frac{d \ln c}{dx}$$

$$dV = \frac{RT}{zF} d \ln c \quad \text{NERNST in the differential form}$$

Integrating between  $V_{\text{int}}$  and  $V_{\text{est}}$  ( $\Delta V$  defined as  $V_{\text{eq}}$  for a single molecule) and between  $C_{\text{int}}$  and  $C_{\text{est}}$

$$V_{\text{eq}} = \frac{RT}{zF} \log \frac{C_{\text{out}}}{C_{\text{in}}} \quad \text{NERNST (forma finita)}$$



Calculation of equilibrium potentials for the most relevant inorganic ions  
in biological solutions at 298 K (25°C)

$$V_{\text{Na}} = 58 \log_{54} V = +45 \text{mV} \quad (z = +1) \quad V_{\text{K}} = 58 \log_{10} V = -91 \text{mV} \quad (z = +1)$$

$$V_{\text{Cl}} = 58 \log_{10} V = -86 \text{mV} \quad (z = -1) \quad V_{\text{Ca}} = 29 \log_{10} V = +96 \text{mV} \quad (z = +2)$$

This means that when a biological membrane is only permeable for one ionic species, its voltage will approach to the correspondent  $V_{\text{eq}}$ .

An example:

$\text{K}^+$  currents are the prevalent

$$V_m \sim V_k \sim -90 \text{mV}$$

ionic fluxes across resting membranes

## Membrane that is permeable to different ions



Se sono soddisfatte le condizioni di

I. Stazionarietà  $\dot{a}J = \text{costante}$

II. Elettroneutralità puntuale  $\frac{dV}{dx} = E = \text{costante}$  e cioè il potenziale varia linearmente

III. Membrana omogenea

IV. Indipendenza dei flussi (ogni specie ionica fluisce seguendo il suo gradiente elettrochimico, indipendentemente dal movimento delle altre)

il potenziale di membrana a riposo è descritto dall'equazione

$$V_m = \frac{RT}{F} \ln \frac{P_K [K^+]_e + P_{Na} [Na^+]_e + P_{Cl} [Cl^-]_i}{P_K [K^+]_i + P_{Na} [Na^+]_i + P_{Cl} [Cl^-]_e} \quad \text{GOLDMAN - HODGKIN - KATZ}$$

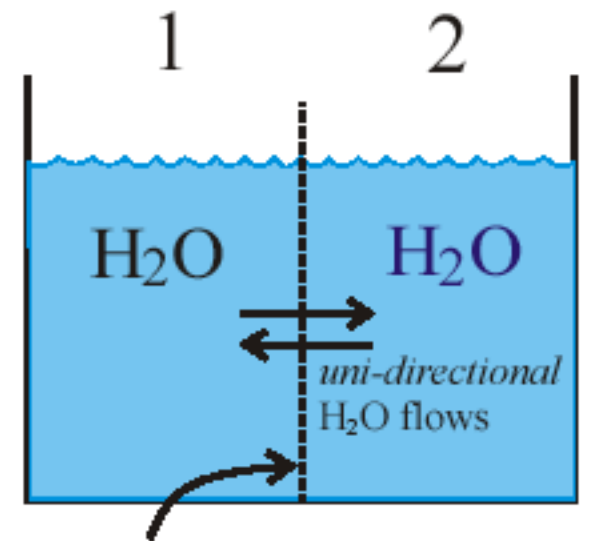
## Membrane Permeability (P) for a given species

P depends on 3 factors:

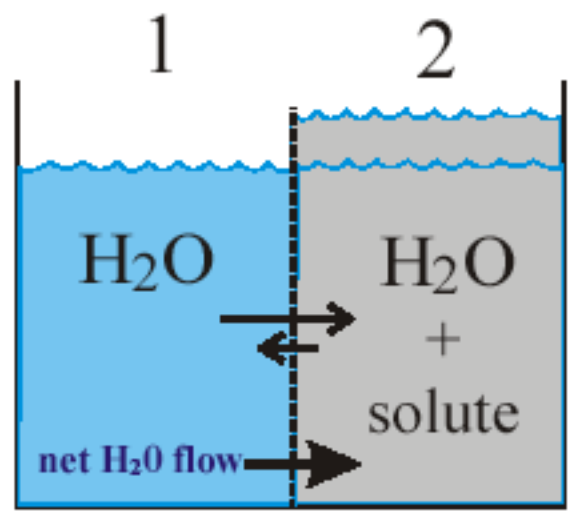
- n= number of channels expressed in the cell
- p= open probability for the channels
- $\gamma$ = channel **conductance**, the current flowing through the open channel over time

$$P = f(n p \gamma)$$



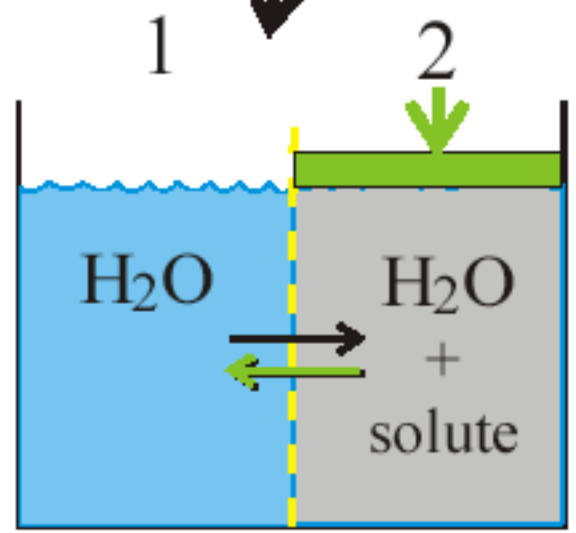


semi-permeable (permeable to  $H_2O$  membrane but not to solute)  
pure water on both sides  
**no NET flux of water**



presence of solute in Side 2 reduces water flux from Side 2  
**now there is a NET flux of water from Side 1 to Side 2**  
the net flux of water from Side 1 raises the level on Side 2

The hydrostatic pressure that eliminates the NET flux of water from Side 1 (pure water) to Side 2 (solution) is equal to the **OSMOTIC PRESSURE** of the solution on side 2



hydrostatic pressure pushing down (e.g., with a piston) can increase the flux of water from Side 2 to 1

# Osmotic Pressure (quantitative basis)

Van't Hoff Relationship



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$$\Pi = RTC$$

*Gas Constant* [(liter·atm)/(mol·K°)]

*Osmotic pressure*

*Concentration*  
(*'osmotically active' solutes*)

The DIFFERENCE ('delta';  $\Delta$ ) in osmotic concentration between two compartments (e.g., inside/outside of cell)

$$\Delta\Pi = RT\Delta C$$

Osmotic pressure is measured in **Osmolarity** = Osmoles osm / L

**Osm** = defines the number of moles of solute that contribute to the osmotic pressure of a solution. A milliosmole (mOsm) is 1/1,000 of an osmole. A microsmole ( $\mu$ Osm) (also spelled micro-osmole) is 1/1,000,000 of an osmole.

1 mol/L NaCl solution is a 2 osmol/L NaCl



# Key questions Diffusion

- What parameters affect the diffusion of uncharged and charged particles?
- What forces act on the particles?
- What parameters of diffusion laws are changed in the presence of biological membranes?
- What is the biological significance of the Nernst equation?
- What parameters define the membrane potential?



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**Thank you**