Fluxes Lows

Diffusion. Permeability

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.

definiamo il flusso per unità di area $J = \frac{dn}{dt}\frac{1}{A}$

Number of moles n flowing in unit time through the surface A

la concentrazione $c = \frac{n}{V}$ per cui $n = cV$

in una sola dimensione $dn = cdV$ oppure $dn = cAdx$

quindi possiamo riscrivere il flusso come

$\frac{dn}{dt} = \frac{Acdx}{dt} = Acv$ (dato $v = \frac{dx}{dt}$ velocità delle particelle di soluto)

quindi $J = cv$

F = force acting on the body
r = resistance
v = speed of the body

per un corpo solido macroscopico in un fluido: $F = \mathrm{r}v$ (STOKES)

$F = \frac{X}{N}$ in cui X è la forza per unita' di mole $X = \frac{forzatotale}{n}$

F e' la forza che agisce sulla singola molecola di soluto

N = numero di Avogadro

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

$f = 6\pi\eta r$ (coeff. frizionale)

r = radius
η = viscosity

ora possiamo riscrivere il flusso

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

definiamo la mobilità $\frac{1}{Nf} = U$

$$J = UcX \qquad \text{(TEORELL)}$$

per più soluti

$Ji = U_i \bullet c_i \bullet X_i$

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

Definiamo il *potenziale chimico* μ che e' la variazione dell'energia libera G per unita' di mole

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

Si tratta della variazione di energia potenziale per unita' di mole

cioe' il lavoro che il sistema puo' compiere per unita' di mole

For ideal solutions the chemical potential of the solute is :

$\mu_i = \mu°_i + RT\ln c_i + V_i P$

$\mu°_i$ = standard chemical potential, the free energy Gibbs value per mole in standard conditions (25°T, 1bar P, 1M concentration)
V_i = partial molar volume of the solute
P = hydrostatic pressure

We are actually interested in the difference of chemical between two states: in this case $\mu°_i$ 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 electricaly charges particles we also have an electrical component contribuiting 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 he 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 $Cmol^{-1}$) multiply for the valence

$$\mu ec_i = \mu°_i + RT\ln c_i + z_iFV$$

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

Riprendiamo TEORELL

Definiamo il *potenziale chimico* μ che e' la variazione dell'energia libera G per unita' di mole

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

Si tratta della variazione di energia potenziale per unita' di mole

cioe' il lavoro che il sistema puo' compiere per unita' di mole

definiamo $X_i = -\frac{d\mu_i}{dx}$ in cui $d\mu =$ energia potenziale (LAVORO)

La forza per unità di mole è la derivata del lavoro, cioè la diminuzione dell'energia potenziale

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

descriviamo il flusso di anelettroliti da TEORELL esplicitando i termini

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

definiamo il coeff. di DIFFUSIONE $\frac{RT}{Nf} = D$

Le dimensioni di D sono cm^2/s

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

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.

Net Diffusion is

- Proportional to the Difference in Particle Concentration (ΔC)

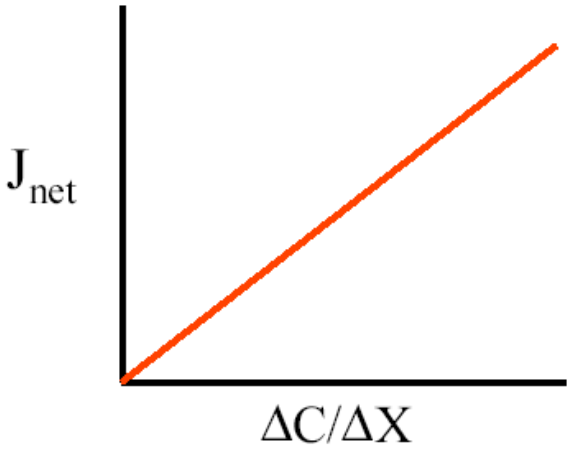
$$J_{net} \propto \frac{\Delta C}{\Delta X}$$

'driving force'

To convert this to an 'equality,' add 'D' (diffusion coefficient)

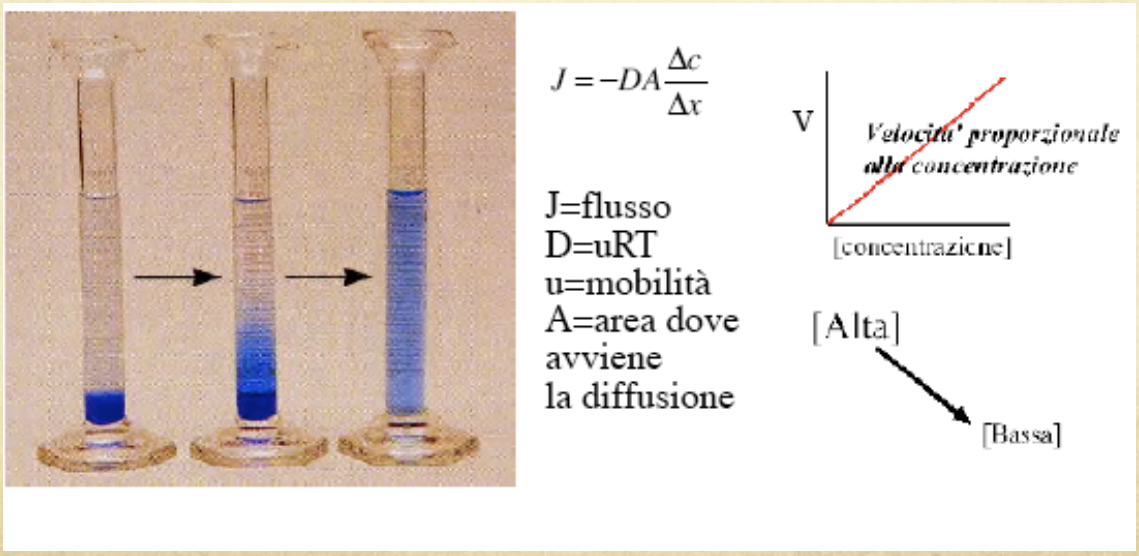
$$J_{net} = D\frac{\Delta C}{\Delta X}$$

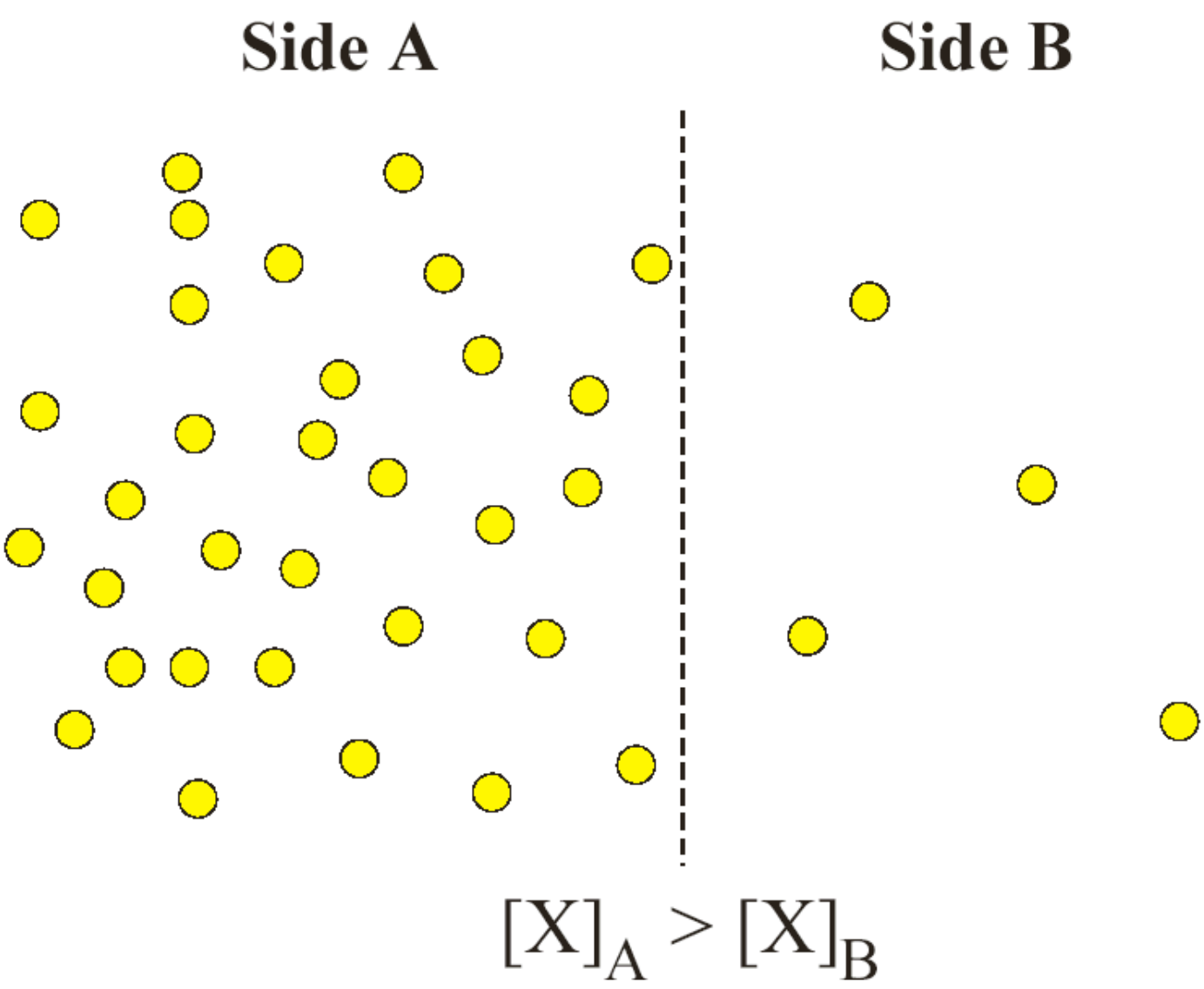
'Fick Equation'



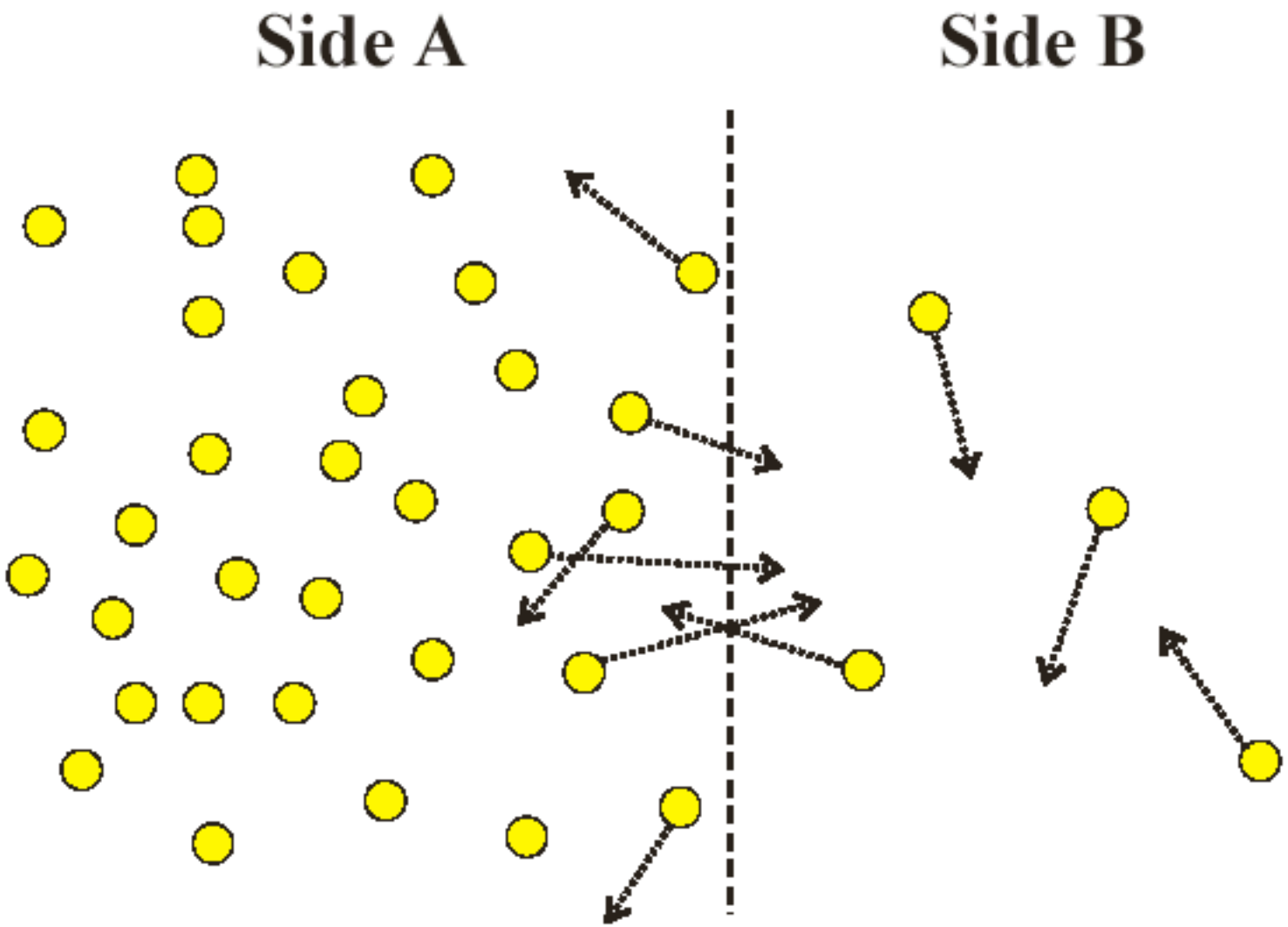
Simple Diffusion

- **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*



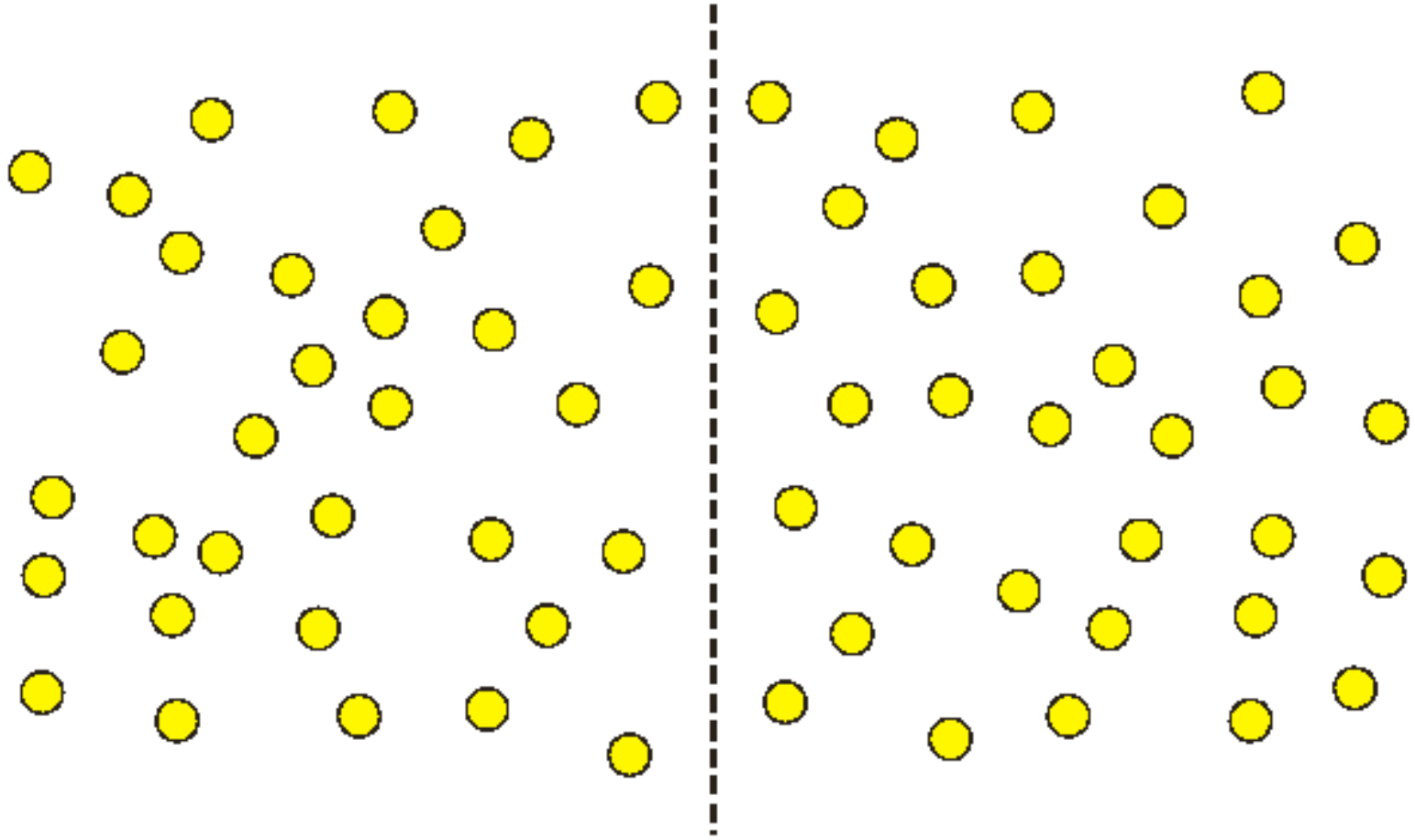


Non-electrolyte



'unidirectional' fluxes are unequal

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



at 'equilibrium'

$$[X]_A = [X]_B$$

$$J_{A\text{-}B} = J_{B\text{-}A}$$

Free diffusion through a membrane

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

FREE DIFFUSION THROUGH A MEMBRANE: IMPLY THE PRESENCE OF TWO DINSTINC PHASES; DISCONTINOUS

IN THIS CASE THE DIFFUSION LOW ARE VALID JUST WITHIN THE MEMBRANE THIKNESS, Δx.

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)

flusso di anelettroliti attraverso una membrana $\Phi = JA$

$\Phi = -AD\frac{\Delta c}{\Delta x}$ in cui Δx è lo spessore della membrana

concentrazione effettiva in membrana $c_m = cr$ in cui r è il coeff. di ripartizione

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

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

definiamo la PERMEABILITA' $P = \frac{Dr}{\Delta x}$

$$J = P_m \Delta c$$

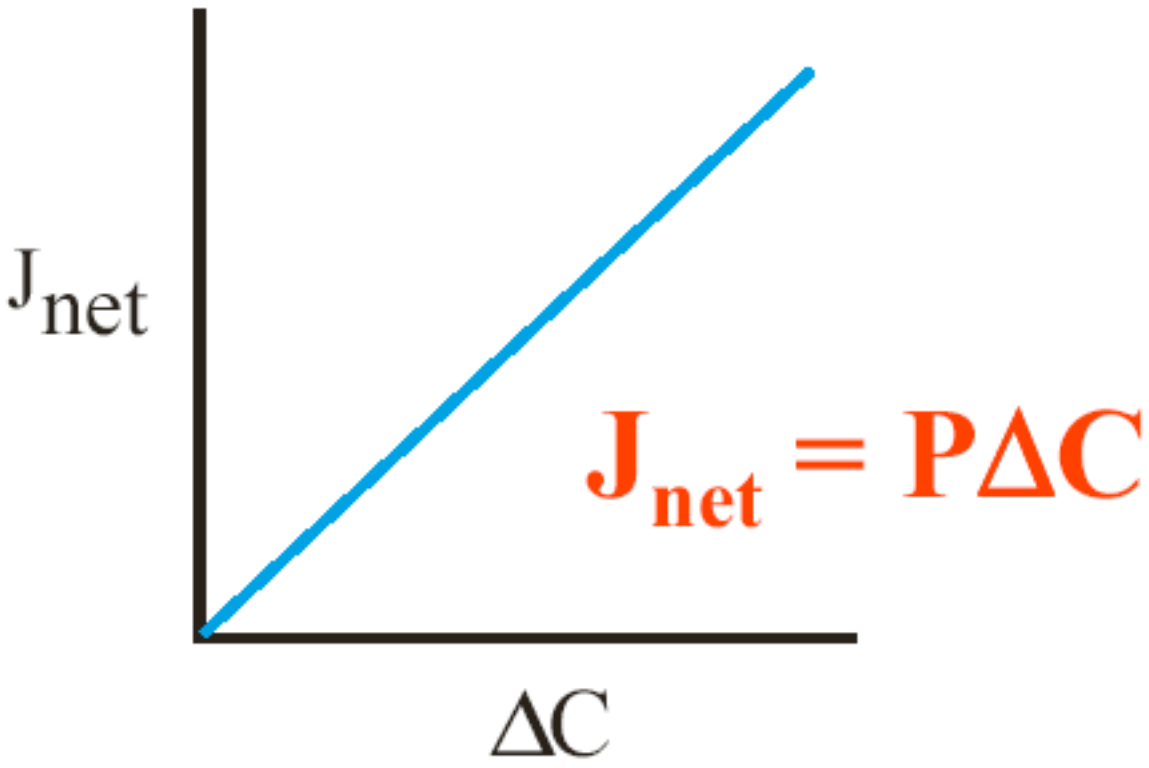
$\frac{J_{1-2}}{J_{2-1}} = \frac{P_m c_1}{P_m c_2} = \frac{c_1}{c_2}$ USSING

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

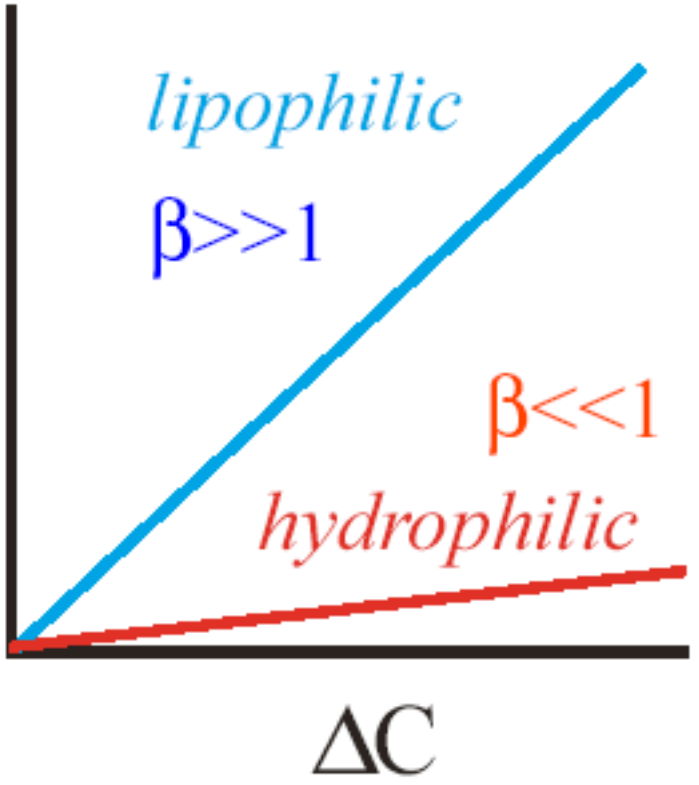
Take Home Issues for 'Permeability'

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

$$J_{net} \propto \Delta C$$



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



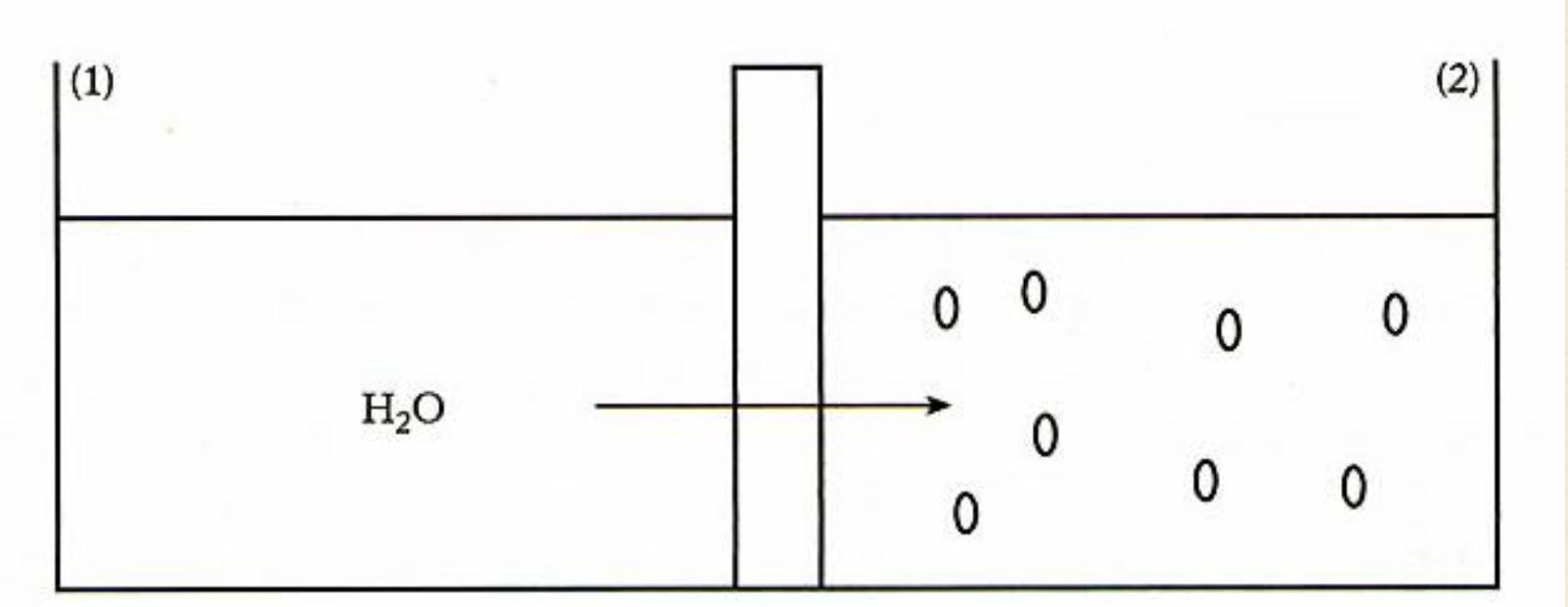
1) A MEMBRANE WHICH IS PERMEABLE ONLY TO SOLVENT

only solvent fluxes. Osmotic phenomenon: volume and pressure changes

2) MEMBRANE PERMEBLE 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 sulur-tes than for solvents)

THE NET FLUXES = 0

Fluxes of charged solutes:

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

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

NERNST-PLANCK (elettrodiffusione)

descriviamo il flusso di elettroliti

$$\mu_{ec} = \mu_c + \mu_e$$

$$J = -Uc\frac{d\mu_c}{dx} \pm Uc\frac{d\mu_e}{dx}$$

$$J = -Uc\frac{RTd\ln c}{dx} \pm Uc\frac{zFd\mathrm{V}}{dx}$$

$$J = -Uc(\frac{RTd\ln c}{dx} \pm \frac{zFdV}{dx})$$

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

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

THIS IS A EQUATION SYSTEM

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

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

$$\mathbf{dV = -RT/z_iF.dc_i/c_i = -RT/z_iF.ln(c_e/c_i)}$$

in condizioni di equilibrio $J = 0$

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

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

$dV = \frac{RT}{zF} d\ln c$ NERNST in forma differenziale

integrando fra V_{int} e V_{est} (ΔV definito come V_{eq} per una singola molecola) e fra C_{int} e C_{est}

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

IF THERE IS A MEMBRANE SEPARETING TWO COMPARTEMENTS (INT and EST), integrating:

Vint – Vest = -RT/ziF.(lnCint-lnCest) =

-RT/ziF.ln Cint/Cest = RT/ziF.ln Cest/Cint

(LEGGE DI NERNST)

Vint-Vest = $\Delta V = V_i$ = EQUILIBRIUM POTENTIALfor the iesim ion

(we can also derive it from $\mu^*_{iINT} = \mu_{iEST}^*$)

Membrane that is permeable to different ions

Se sono soddisfatte le condizioni di

I. Stazionarietà $\sum \mathrm{J} = \text{costante}$

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

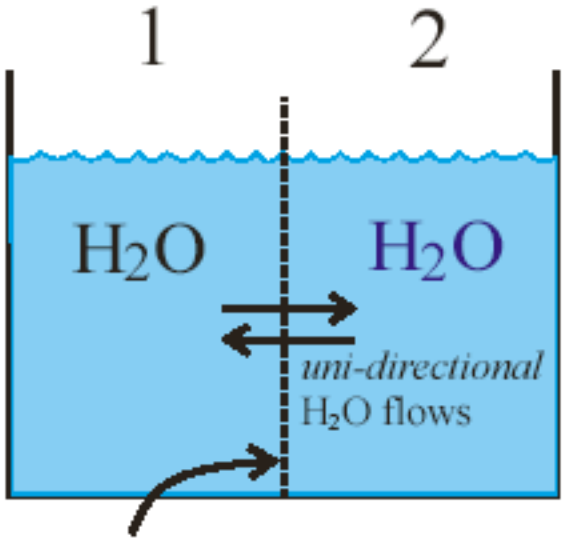
III. Membrana omogenea

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

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

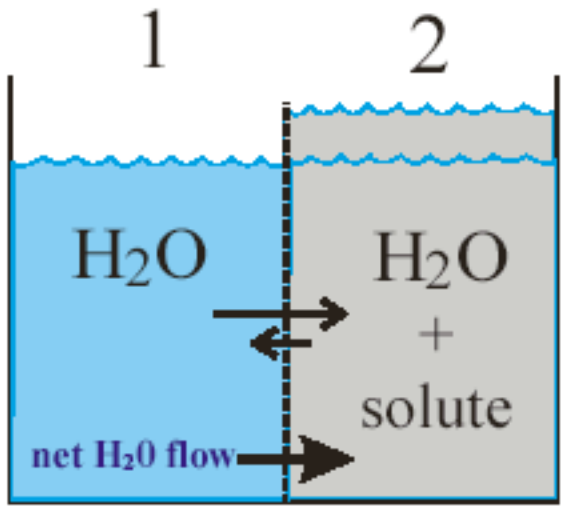
GOLDMAN - HODGKIN - KATZ



semi-permeable membrane *(permeable to H_2O but not to solute)*

pure water on both sides

no NET flux of water

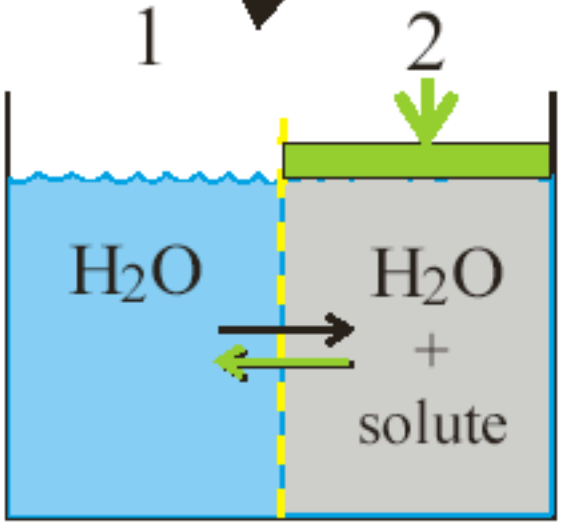


the net flux of water from Side 1 raises the level on Side 2

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 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

Gas Constant [(liter·atm)/(mol·K°)]

$$\Pi = RTC$$

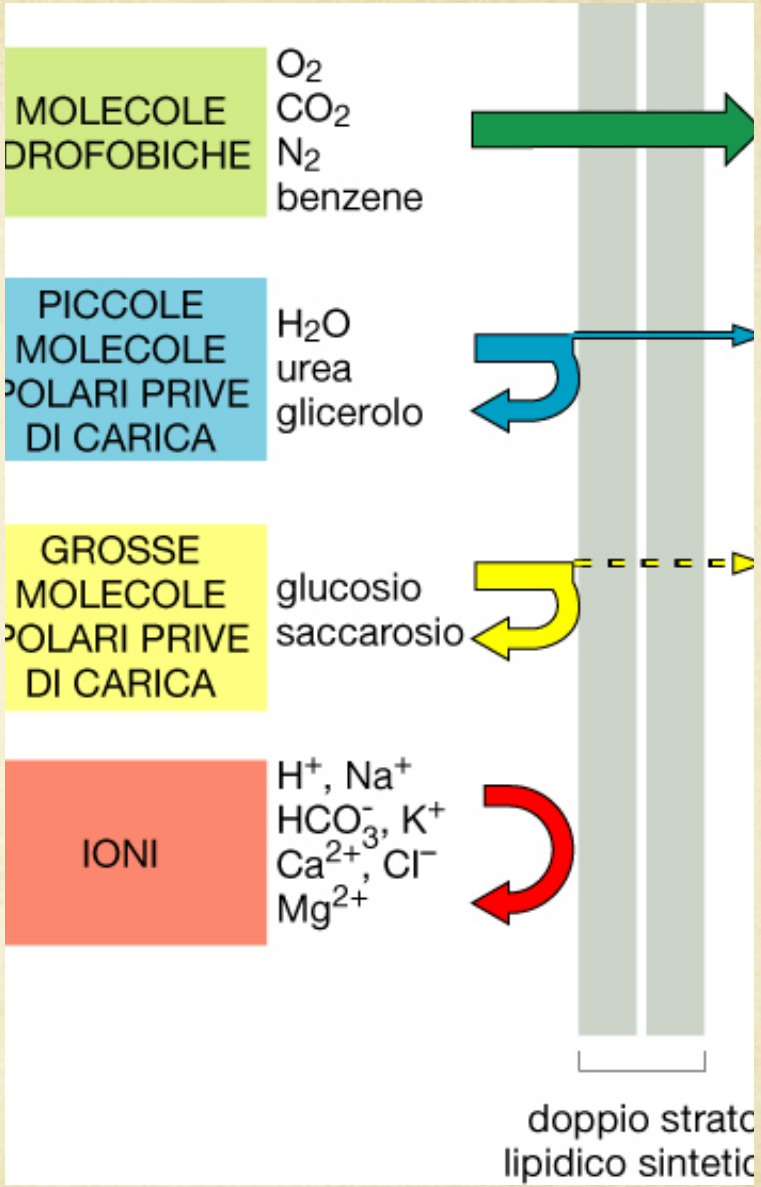
Osmotic pressure

Concentration ('osmotically active' solutes)

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

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

The lipidic component of plasma membrane is **semipermeable**: allows the fluxes of different molecules according to their lipids solubility and dimension

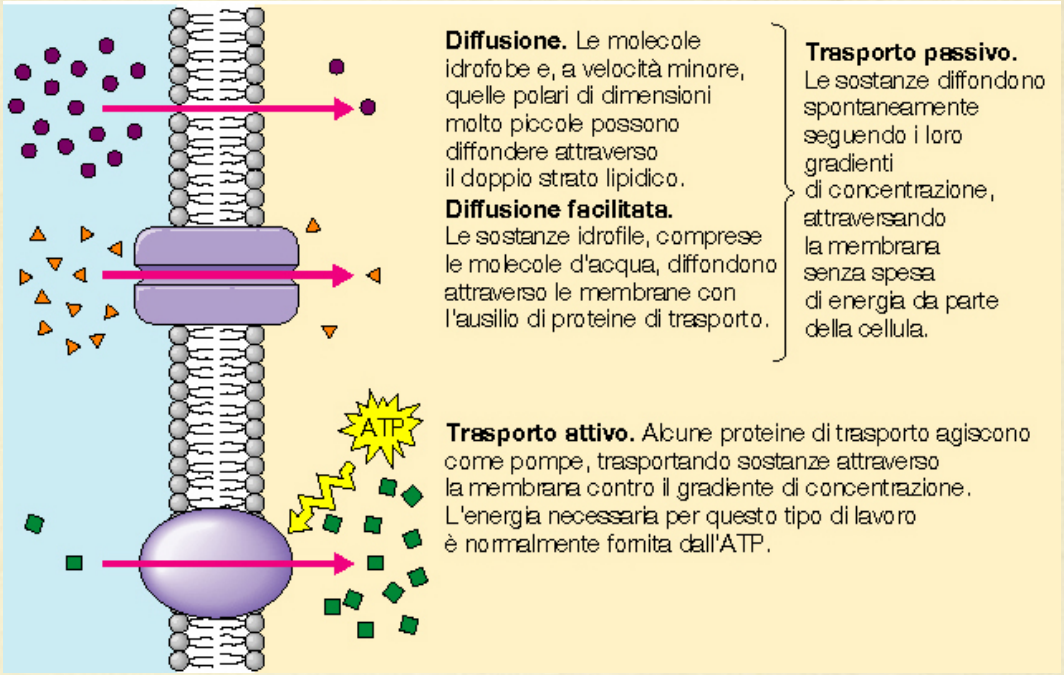


	Extracellulare	Intracellulare
Na^+	145 mM	15 mM
K^+	4 mM	150 mM
Cl^-	110 mM	10 mM
Ca^{2+}	2 mM	10^{-8} M
Mg^{2+}	2 mM	0.5 mM
Fosfato	2 mM	40 mM
H^+	10^{-7} M	10^{-7} M

Intra- and extra- ionic composition of typical mammal cell

Transmembrane fluxes

SIMPLE DIFFUSION



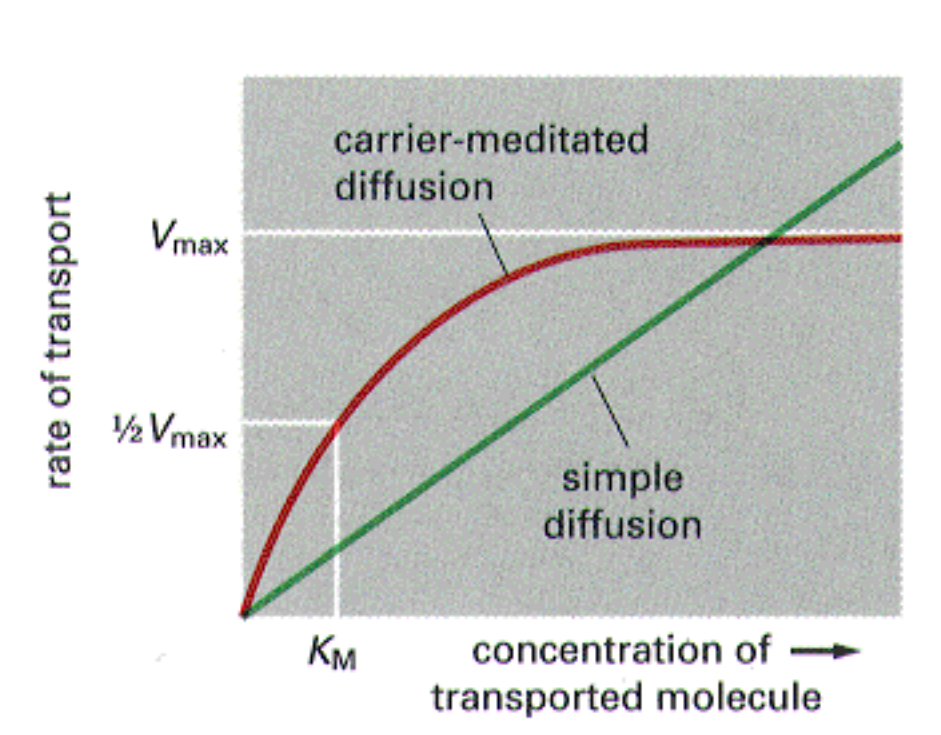
MEDIATED by carriers

- ✓ionc channels
- ✓carriers (transporters)
- ✓pumps

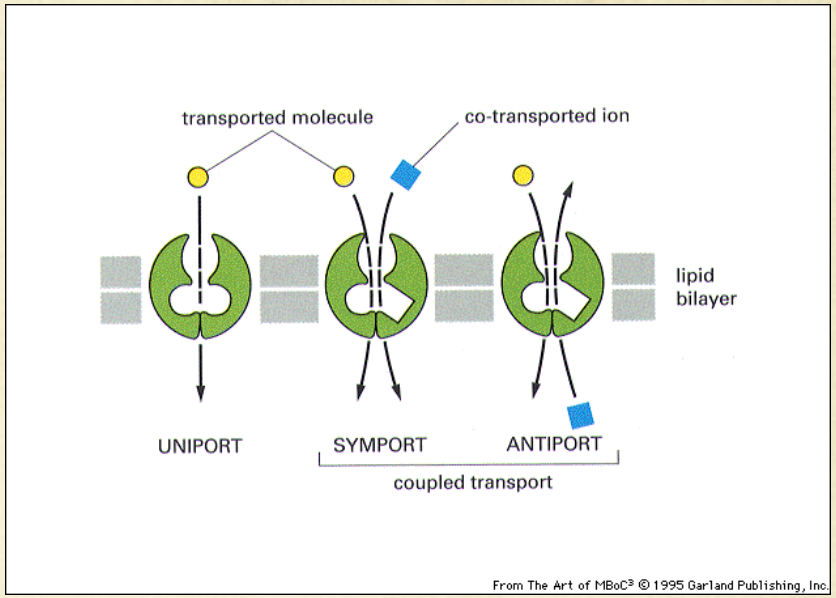
Different transporters

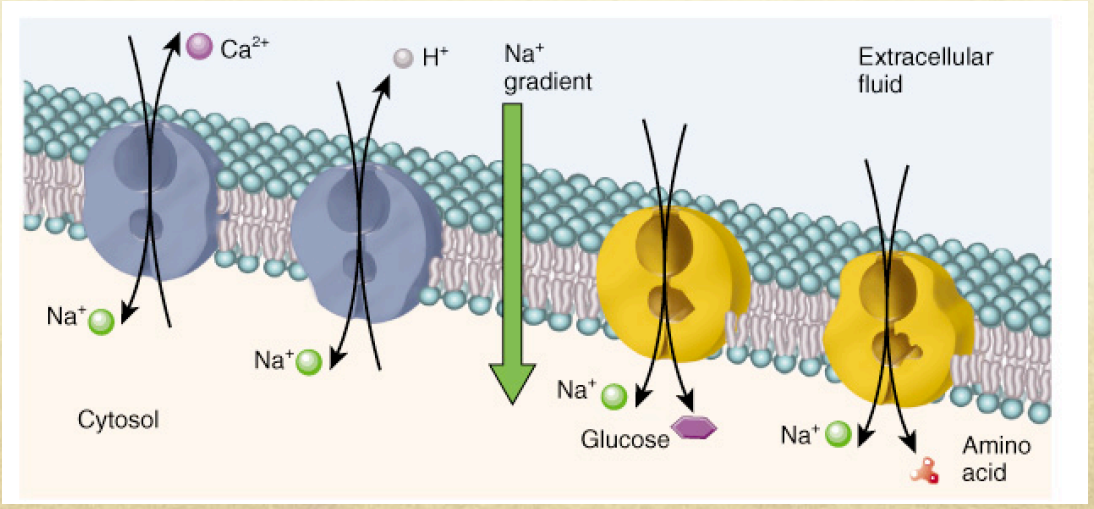
- Proteins that allows fluxes of molecules that can’t cross the lipid bilayer by themselves
- Allow nutrients inflow, osmolality control, detossification
- Fluxes depends form the different forces that act on the molecules transported

Rate of transport is linear and not saturable in Simple diffusion

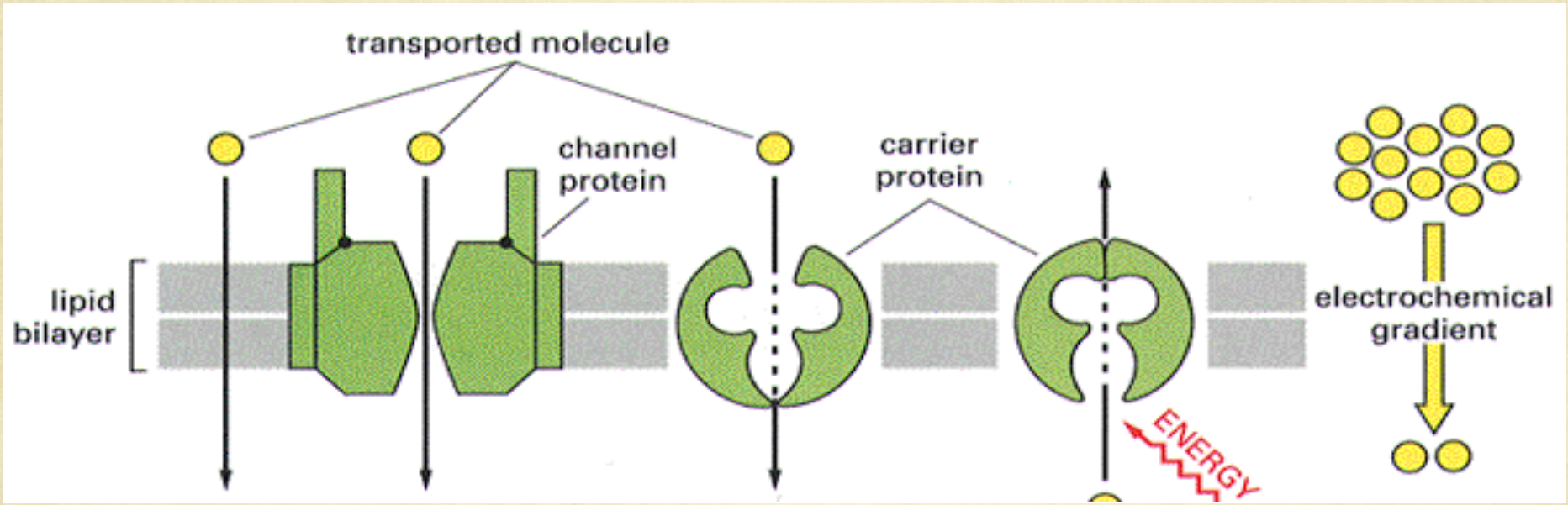


MEDIATED TRANSPORT: CARRIERS





From the energetic point of view the mediated transport can be **PASSIVE** (facilitated diffusion, along chemical or electrochemical gradient) or **ACTIVE** (against gradient)



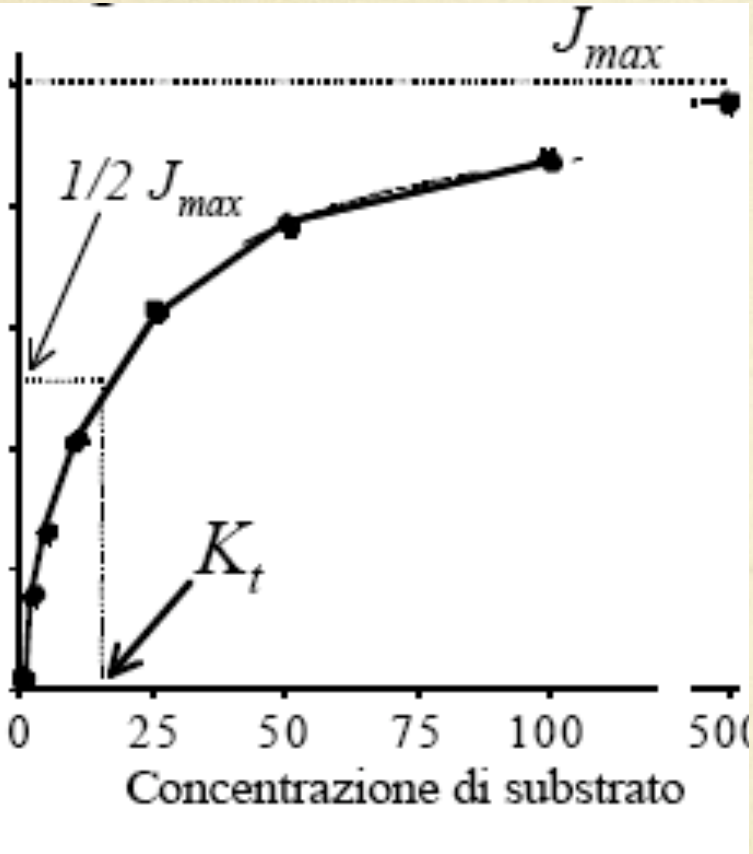
DIFFUSIONE SEMPLICE
trasporto passivo

CANALI IONICI
trasporto passivo

CARRIERS
trasporto passivo o attivo secondario (utilizzo indiretto di ATP)

POMPE IONICHE
trasporto sempre attivo primario (utilizzo diretto di ATP)

MEDIATED TRASPORT "diagnostic characteristics":

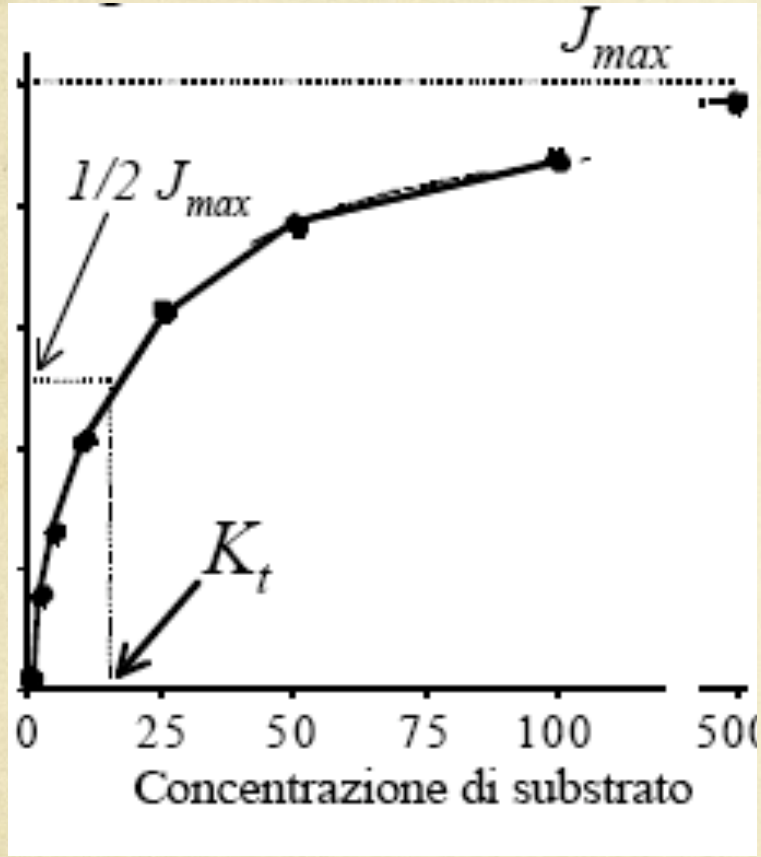


"SATURABILITY"

- Finite numbers of transporters
- Each carrier has its own properties and speed

$$J = \frac{J_{max}[S]}{K_t + [S]}$$

MEDIATED TRASPORT "diagnostic characteristics":



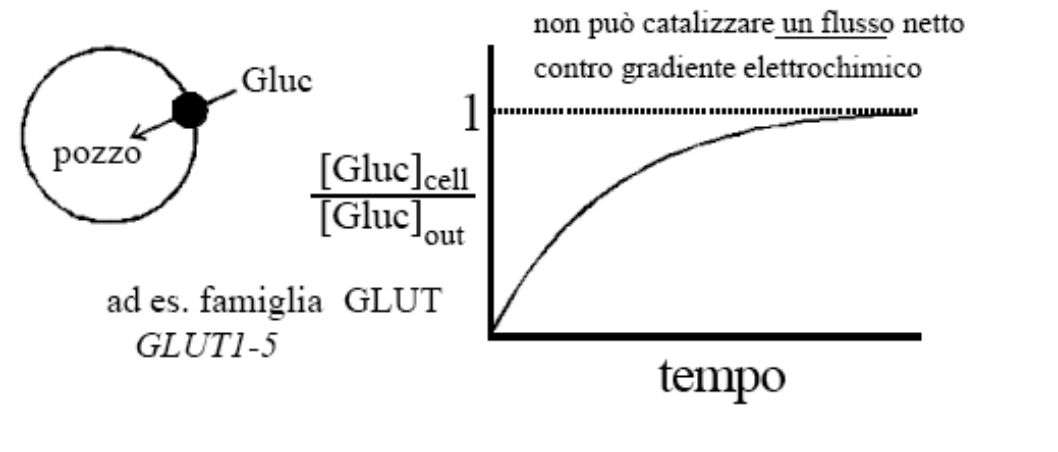
SPECIFICITY

Transporters are quite selective: as an example they are stereoselective (D-Gluc ma non L-Gluc; D-aa ma non L-aa)

In case the Transporter is able to recognize different molecules, the transport present the phenomenon of the **"competitive inhibition"** since different molecules can compete for the same binding site. In this case what changes is the Kt and not the Jmax = to have the same flux it will be necessary a higher concentration of the substrate

If inhibitors bind the transporter blocking its activity, we can talk about ***non competitive inhibitors*** *and they reduce the* Jmax value but not Kt = their effect is to reduce the number of functional transporters

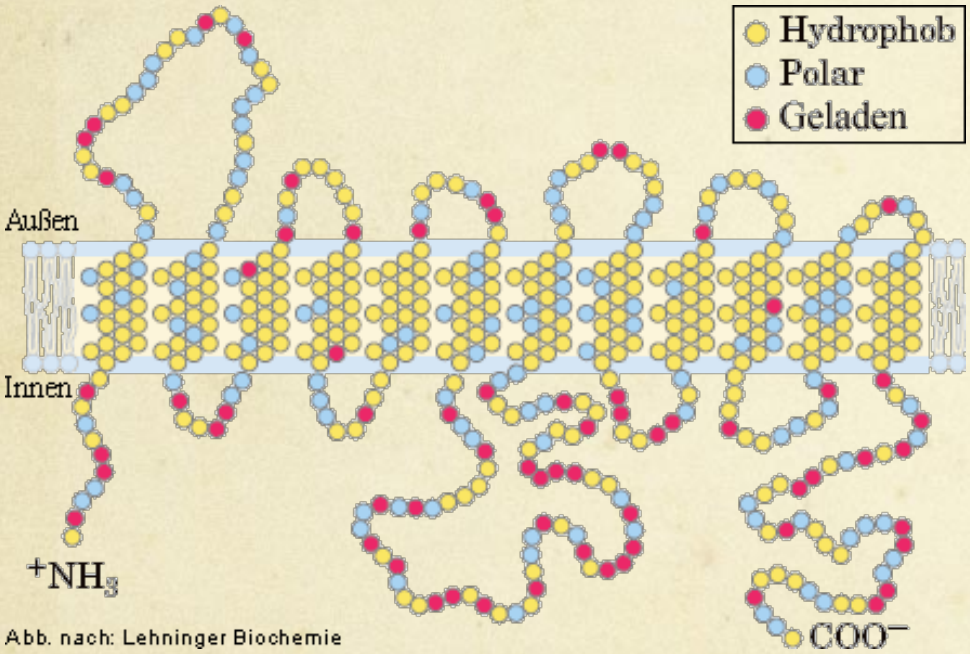
Facilitated Diffusion



The regulation of the transporter can be achieved by:

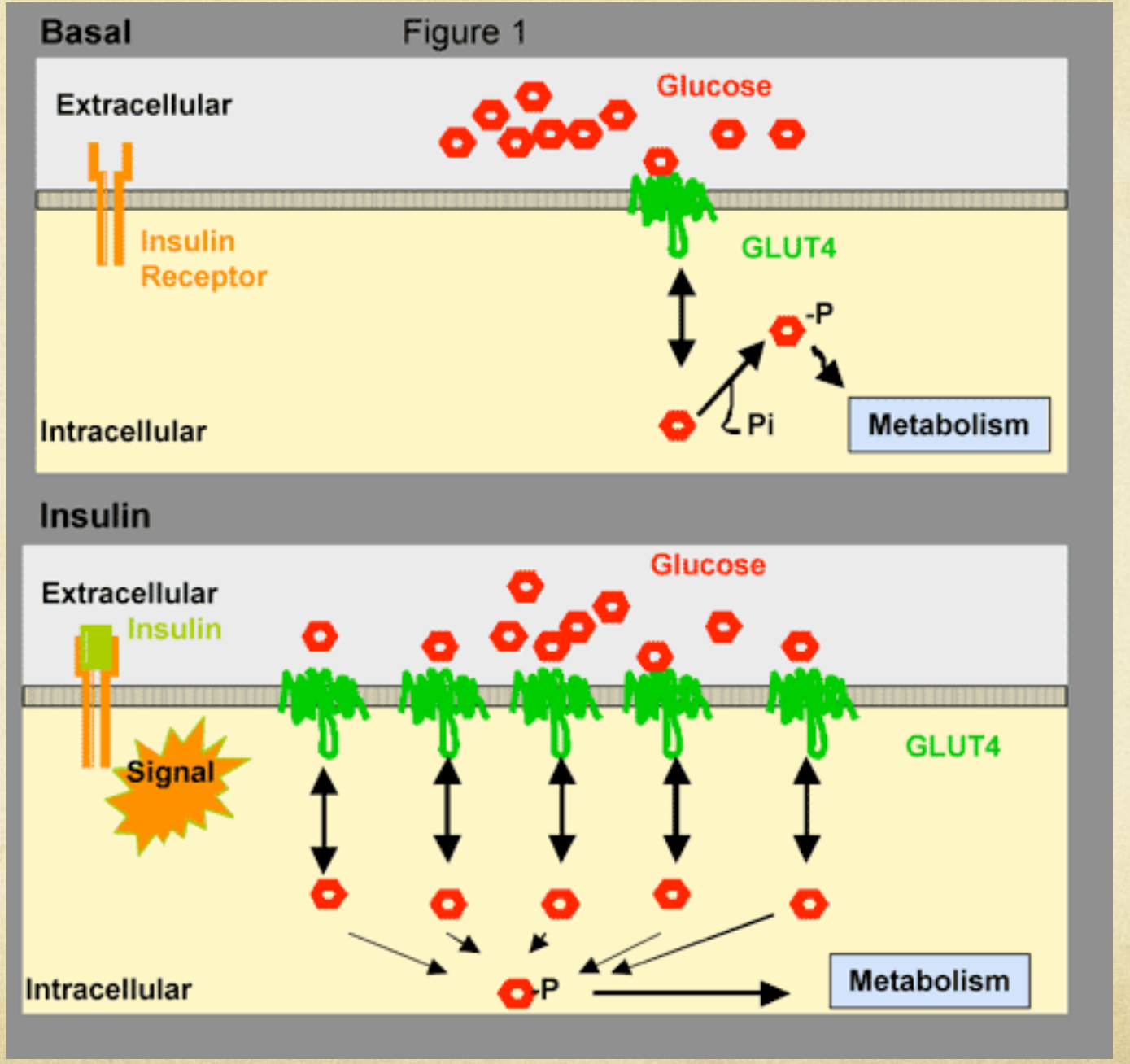
- Changing the speed of the single transporters (as an example by phosphorylating cytoplasmic residues of the transporter)
- Changing the number of expressed transporters in the membrane

Glucose transporter (GLUT)

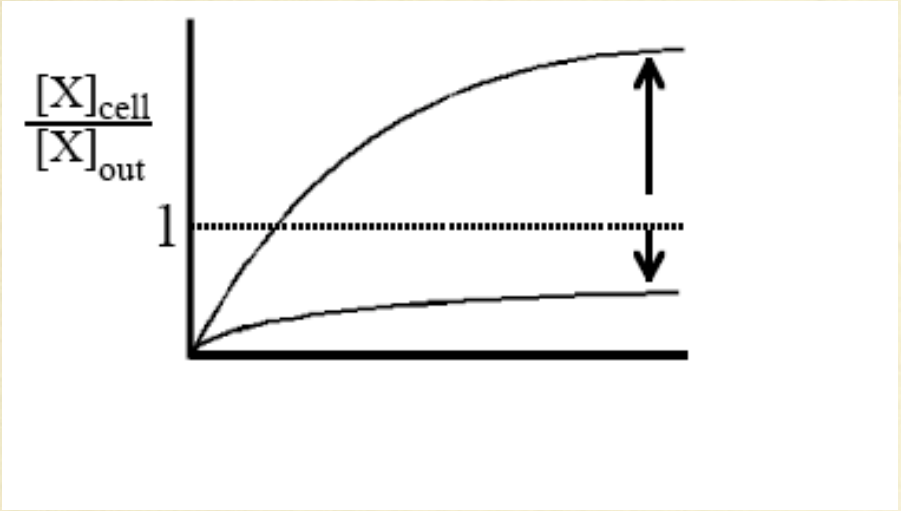


GLUT 1

- **GLUT-1** is the first transporter cloned. It is expressed at different levels depending on the glucose cellular metabolism. Ubiquitary expression
- **GLUT-2** expressed in hepatocytes, neurons and in the basolateral membranes of absorbing epithelia (such as in kidney and intestine)
- **GLUT-3**: neurons
- **GLUT-4**: adipose tissue and muscle. It's regulated by insulin

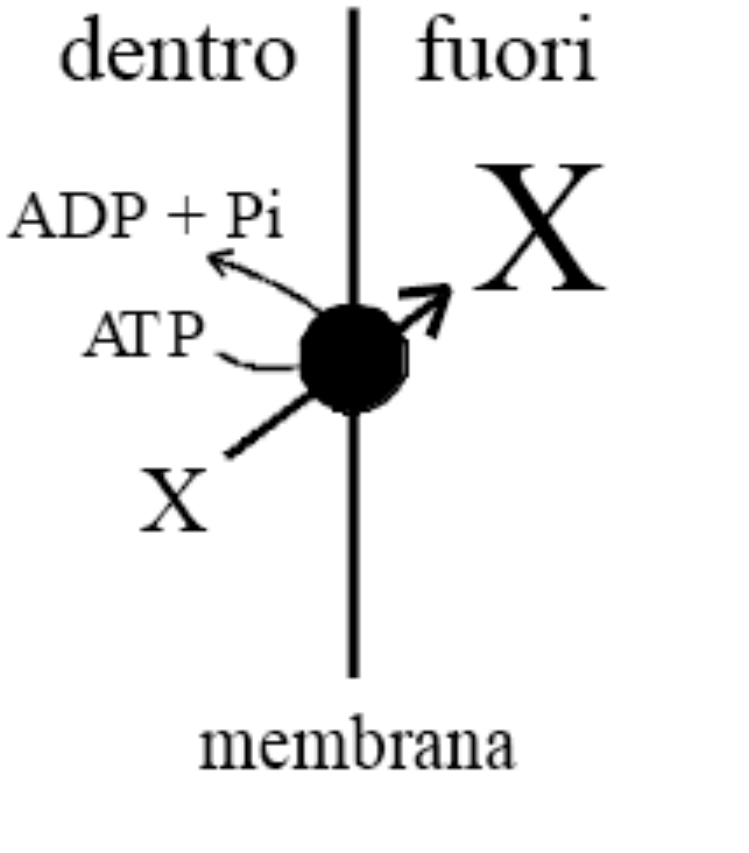

Basal
Figure 1
Extracellular
Glucose
Insulin
Receptor
GLUT4
-P
Pi
Intracellular
Metabolism
Insulin
Extracellular
Glucose
Insulin
GLUT4
Signal
Intracellular
-P
Metabolism

ACTIVE TRANSPORT



- Create and maintain transmembrane gradients of solutes
- Needs energy source
- Common features to facilitated transport:
  - Saturation
  - Inhibition and modulation

PRIMARY ACTIVE TRANSPORT: PUMPS; ATPase

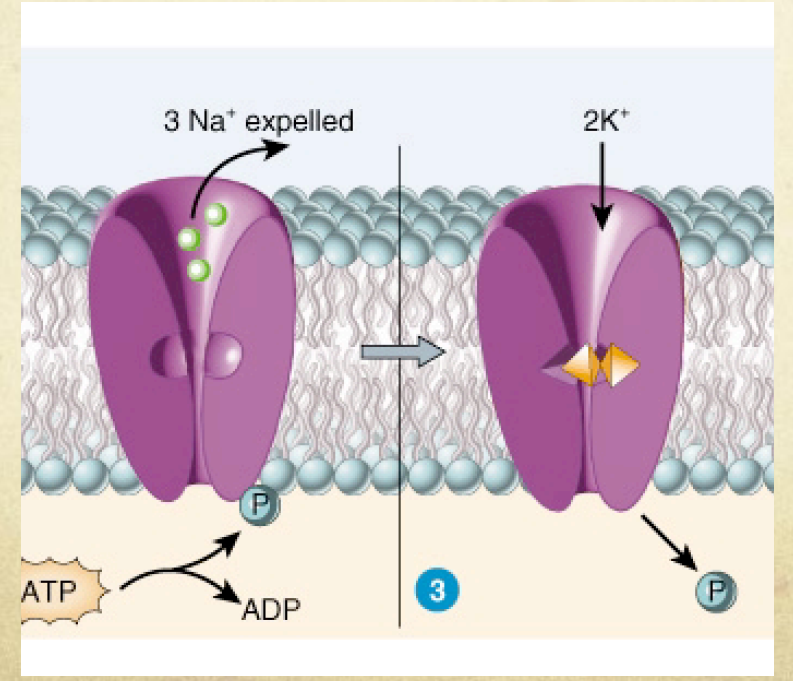


- X is one of the following inorganic cation:
- Na+
- Ca2+
- K+
- H+

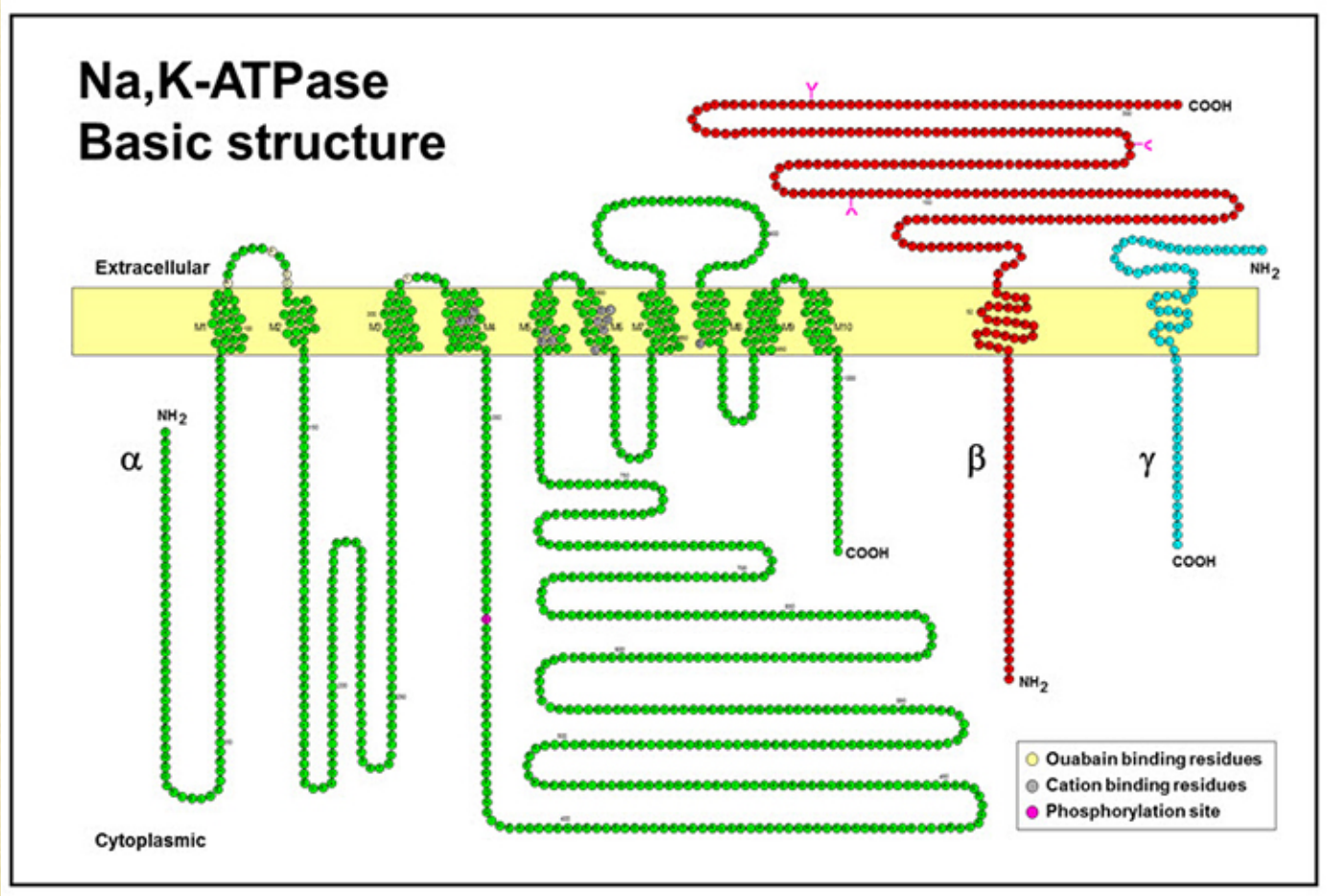
Na+/K+ ATPase

- Use ATP to generate a gradient of Na+ and K+ concentration

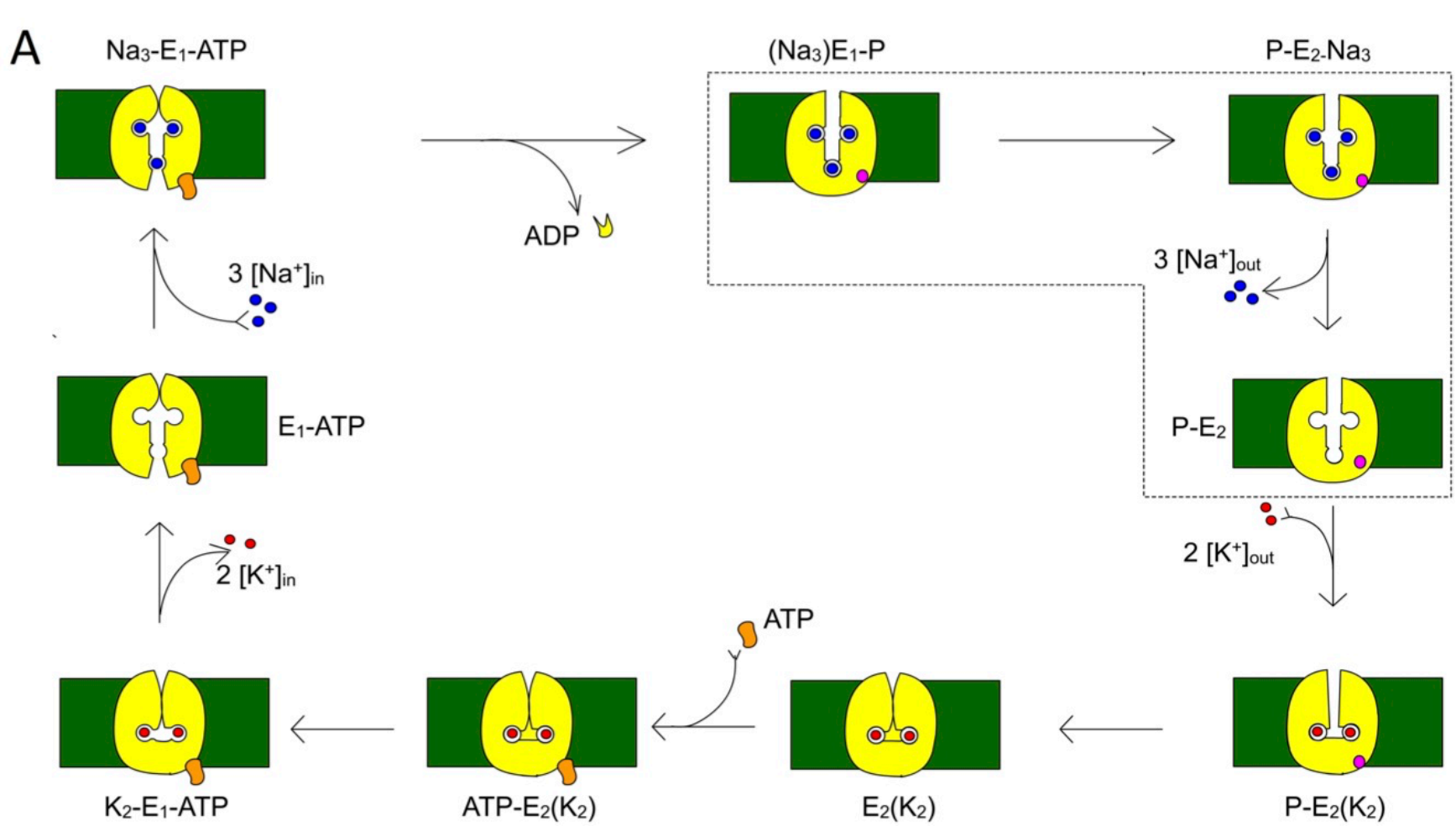
- ELECTROGENIC PUMP :
- Transport proteins that use this mechanism of transport are designated as P types, since ATP cleavage is required and PO3- is covalently transferred to an Asp residue from the ATP.



Na+/K+ ATPasi


Na,K-ATPase
Basic structure
Extracellular
Cytoplasmic
α
β
γ
NH2
COOH
Ouabain binding residues
Cation binding residues
Phosphorylation site

Activity cycle of Na+/K+ ATPase

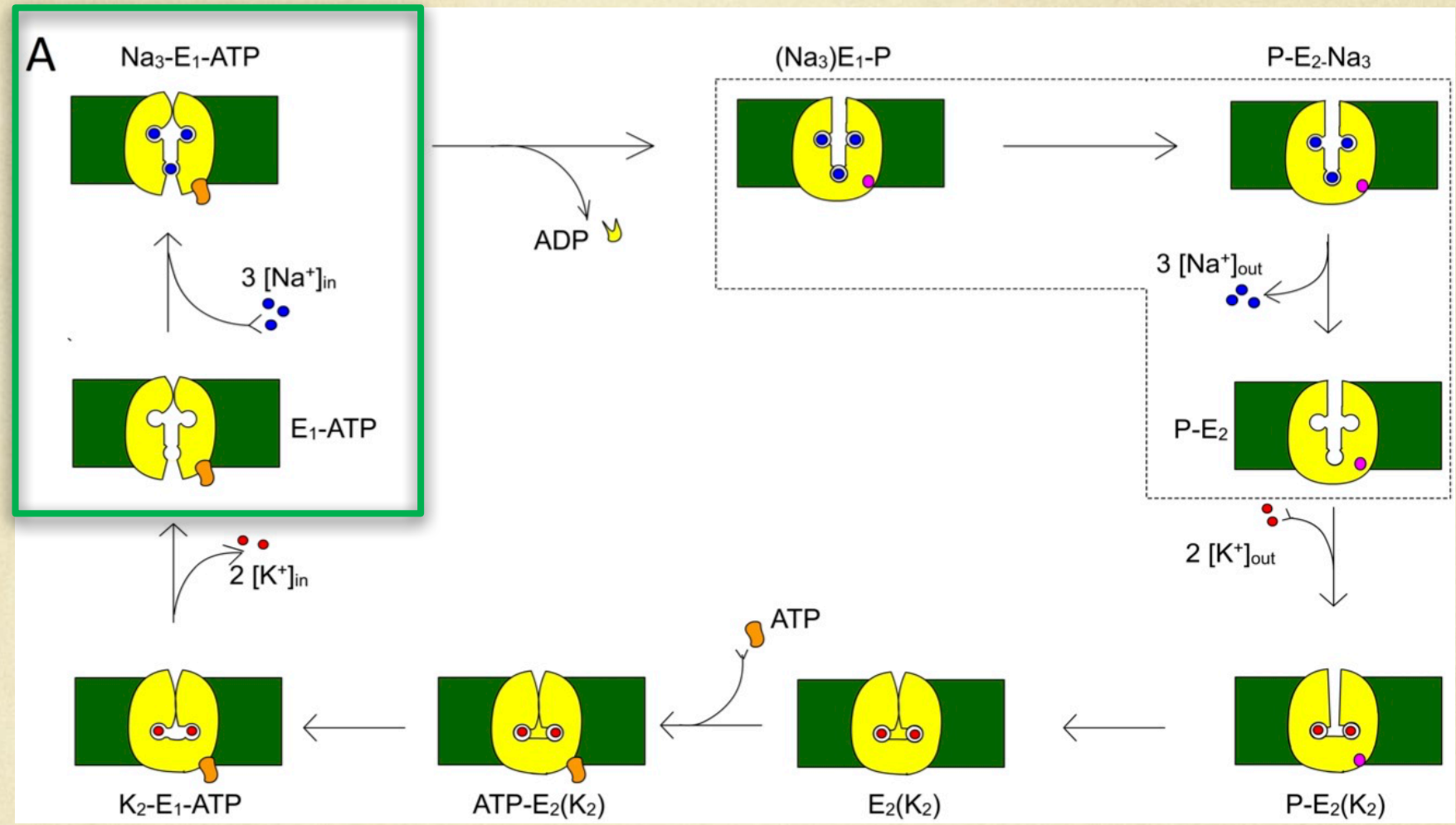


- The protein exists in two essential conformations, E1 and E2, depending on the phosphorylation state of the protein.

E1= binding sites toward intracellular space

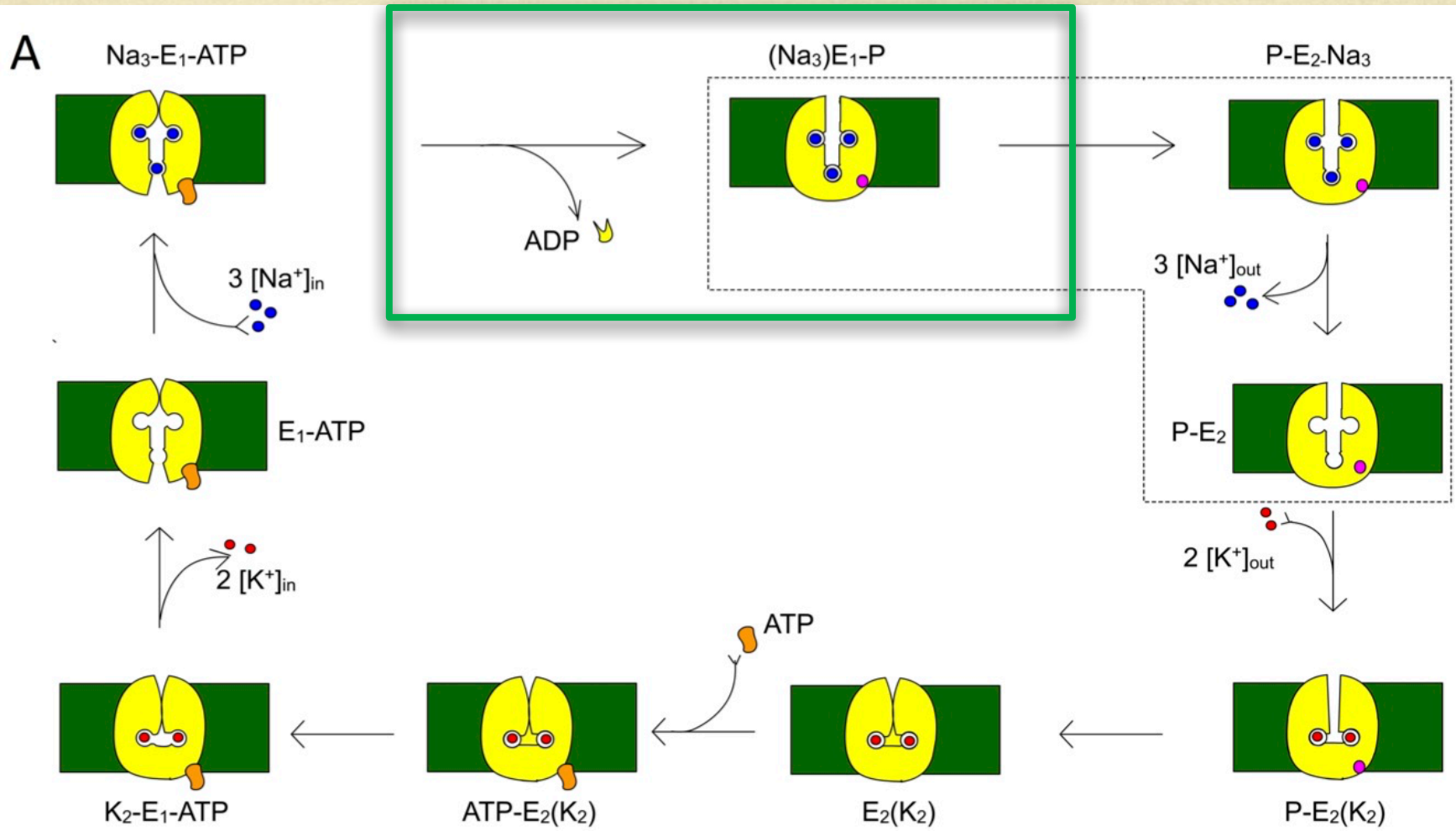
E2= binding sites toward extracellular space

Activity cycle of Na+/K+ ATPase



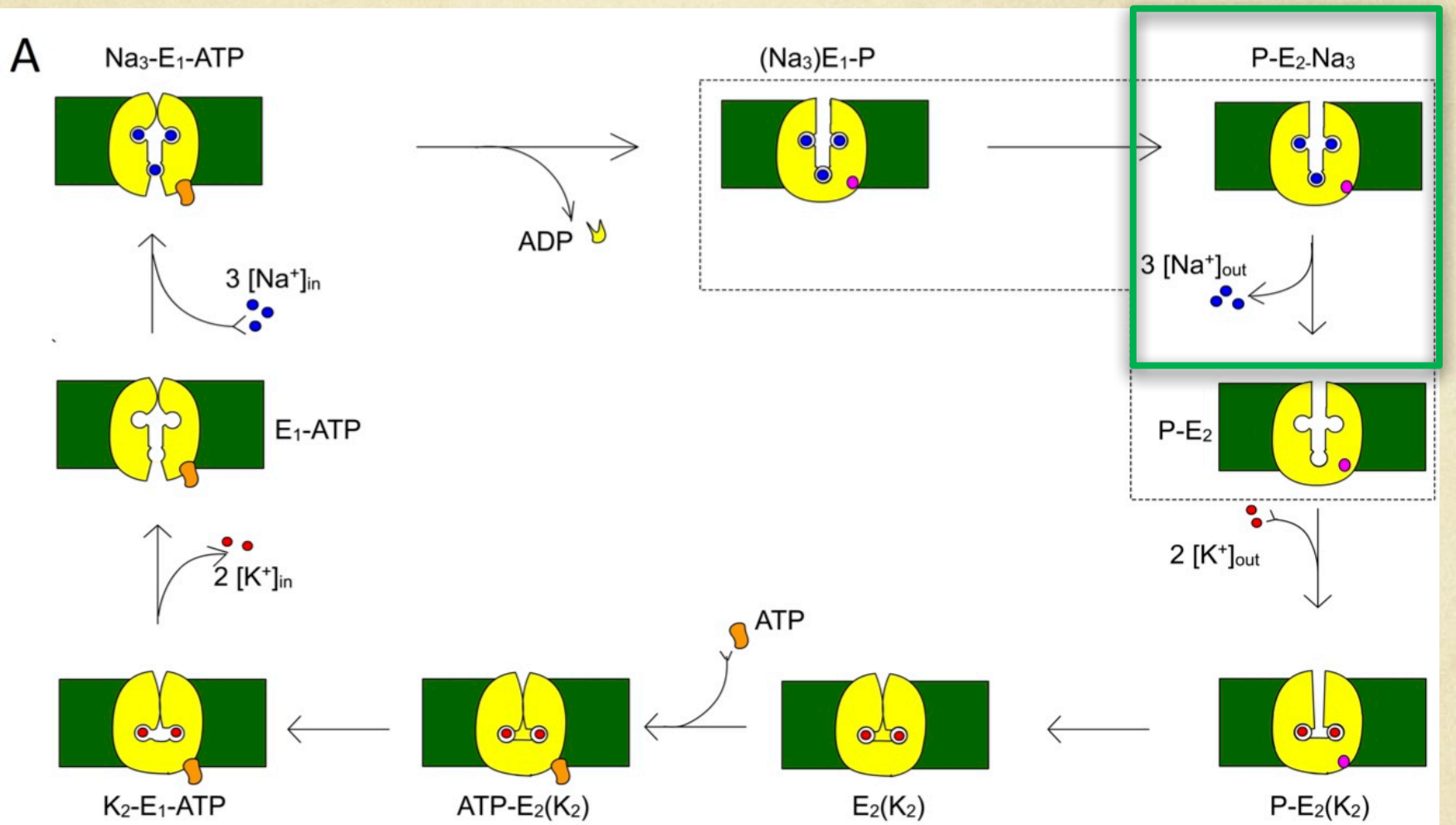
- ATP and 3 Na+ bind to the cytoplasmic domain of the enzyme in the E1 conformation.

Activity cycle of Na+/K+ ATPase



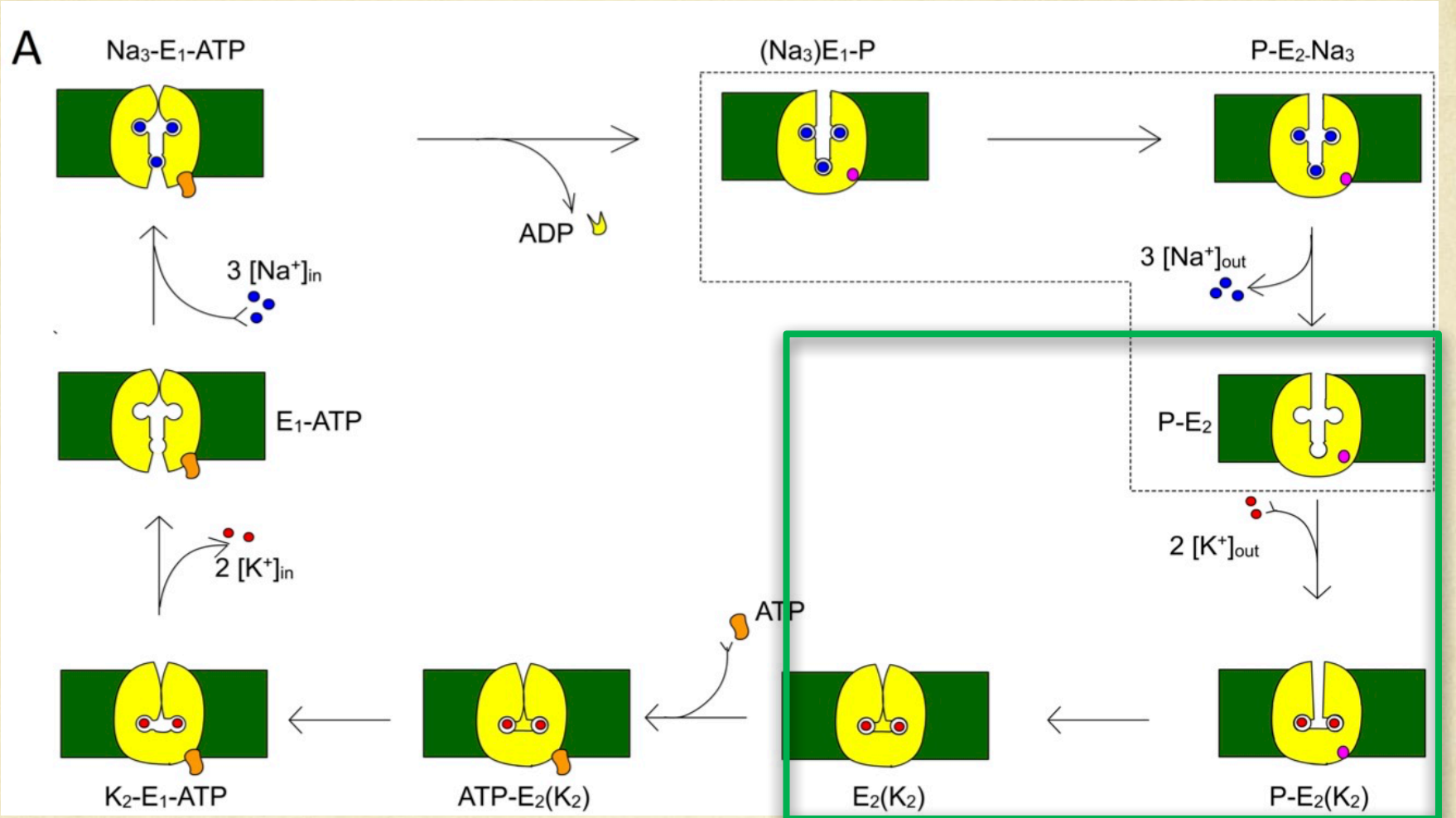
- In the presence of Na+ ions, the bound ATP is cleaved in a nucleophilic attack by an Asp side chain of the protein. (Hence, the protein is a Na+-activated ATPase.

Activity cycle of Na+/K+ ATPase



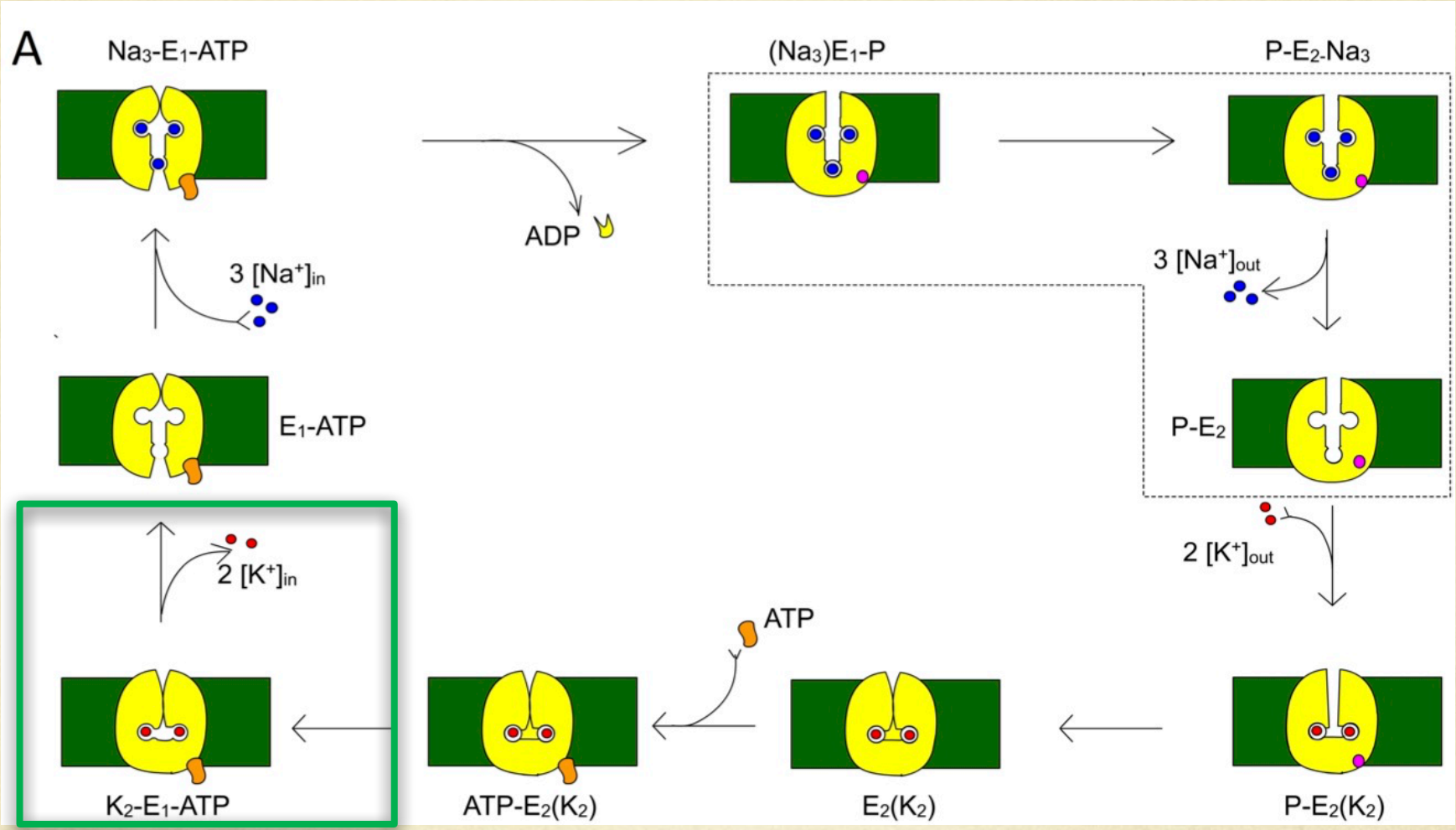
- The phosphorylated enzyme changes conformation to the E2 form in which Na+ ions are now on the outside of the cell membrane, from which they dissociate.

Activity cycle of Na+/K+ ATPase



- The phosphorylated protein in conformation E2 now binds 2 K+ ions on the outside, which activates hydrolysis of the Asp-PO3 mixed anhydride link.

Activity cycle of Na+/K+ ATPase



- The dephosphorylated protein is more stable in the E1 conformation to which it changes as it bring K+ ions into the cell. Transport proteins that use this mechanism of transport are designated as P types, since ATP cleavage is required and PO3- is covalently transferred to an Asp residue from the ATP.

Activity cycle of Na+/K+ ATPase

- The protein exists in two essential conformations, E1 and E2, depending on the phosphorylation state of the protein.
- ATP and 3 Na+ bind to the cytoplasmic domain of the enzyme in the E1 conformation.
- In the presence of Na ions, the bound ATP is cleaved in a nucleophilic attack by an Asp side chain of the protein. (Hence, the protein is a Na+-activated ATPase.
- The phosphorylated enzyme changes conformation to the E2 form in which Na+ ions are now on the outside of the cell membrane, from which they dissociate.
- The phosphorylated protein in conformation E2 now binds 2 K+ ions on the outside, which activates hydrolysis of the Asp-PO3 mixed anhydride link.
- The dephosphorylated protein is more stable in the E1 conformation to which it changes as it bring K+ ions into the cell. This is an example of an electrogenic antiporter. Transport proteins that use this mechanism of transport are designated as P types, since ATP cleavage is required and PO3- is covalently transferred to an Asp residue from the ATP.

Activity cycle of Na+/K+ ATPase

- Na+/K+ ATPase is activated in an asymmetric way from intracellular Na+ and extracellular K+ and NOT vice-versa.
- The 2 cation must be present at the same time in the two compartments to complete the cycle
- HORMONAL REGULATION:

THYROYD hormones induce α e β subunits synthesis therefore modulating the pump expression in tissues

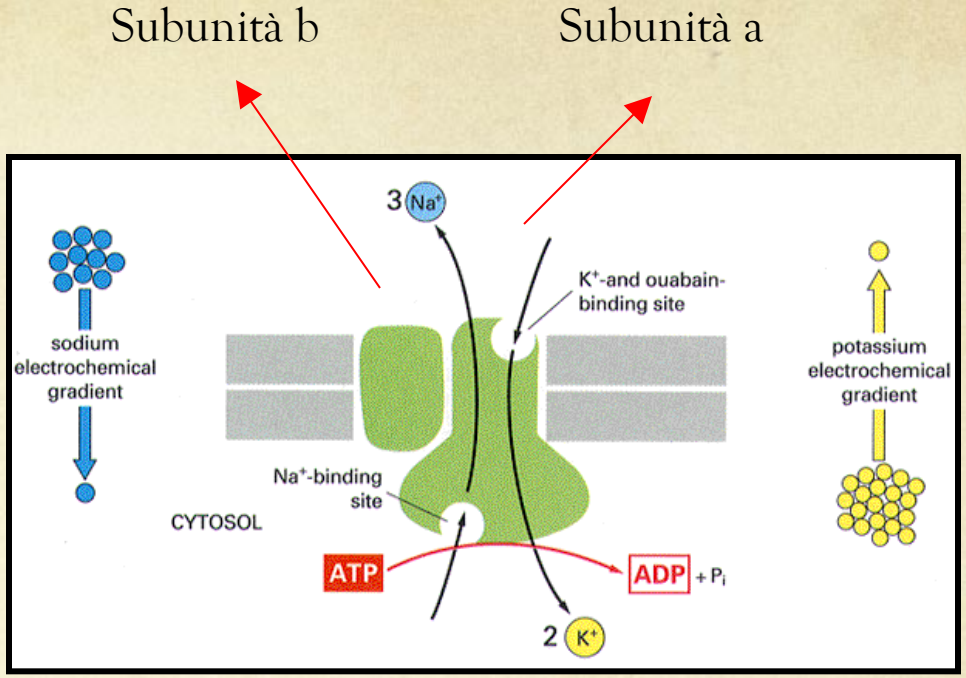
Aldosterone trigger gene transcription of both the subunits

Insulin plays a double role: short term regulation on pump activity and long term regulation on the synthesis

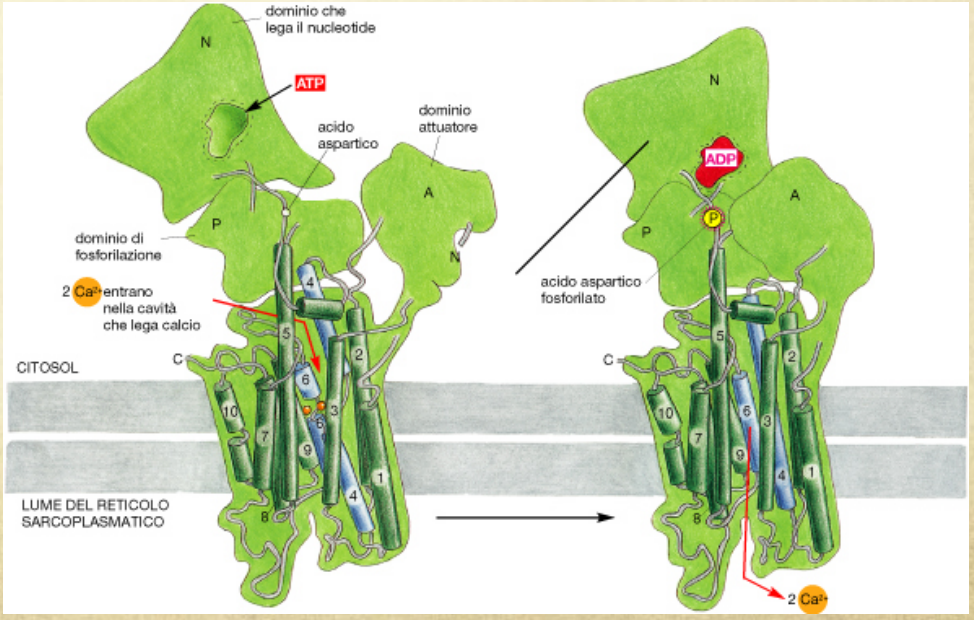
Other ATPase

- Ca2+ ATPase: role in maintaining the low intracellular Ca2+ concentration (10^{-4} M) (PMCA o SERCA)
- H+/K+ ATPase promotes gastric acidification
- V-ATPase pump protons acidifying intracellular organelles such as lysosomes or vacuoles in plant cells
- ABC Transporters (ATP Binding Cassete). An example is MDR (multi drug resistance) which pumps actively cytotoxic drugs out of the cell

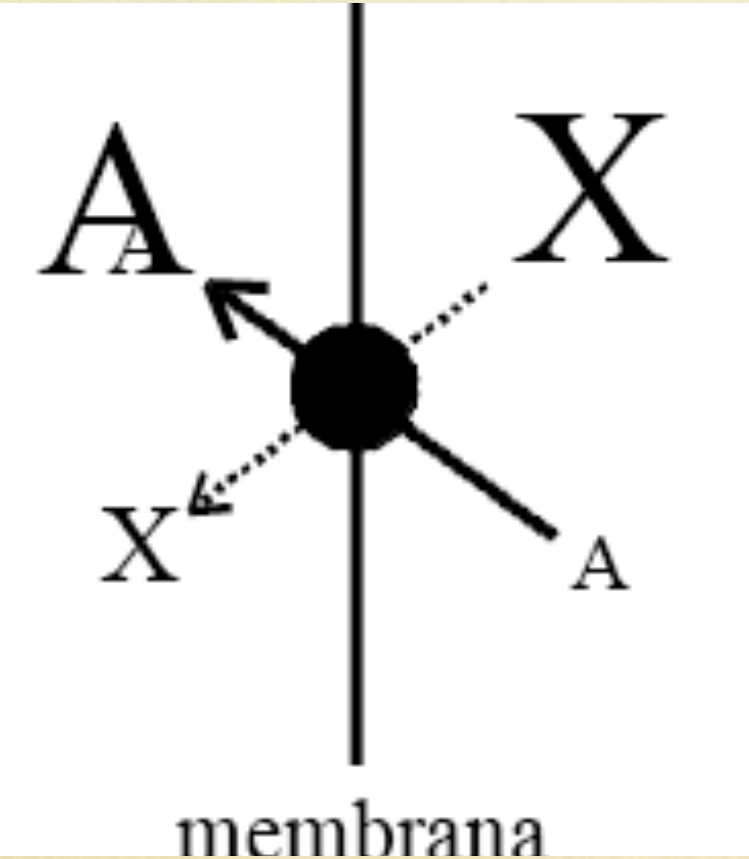
Struttura delle pompe di tipo P



pompa del Ca del reticolo endoplasmatico (SERCA)

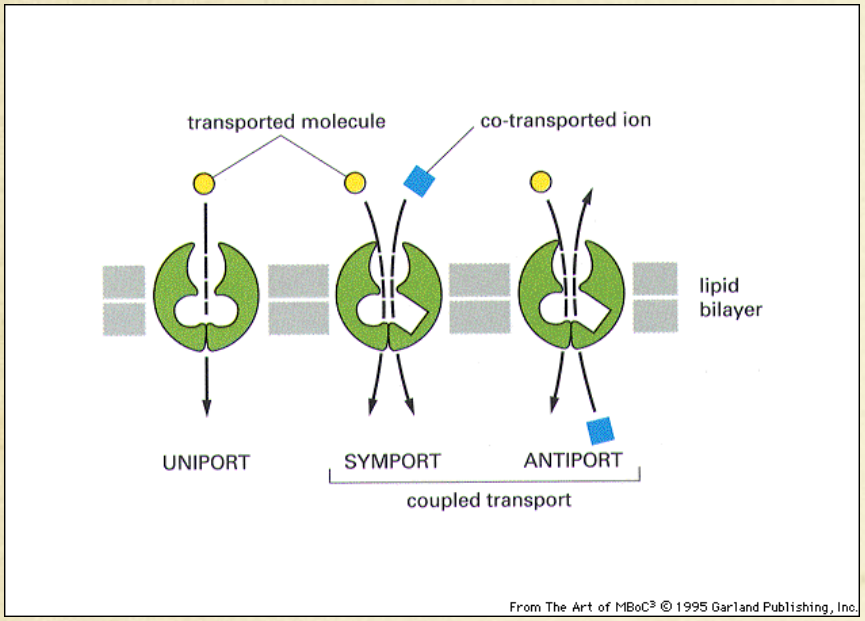


Active Secondary Transports: symport and antiport



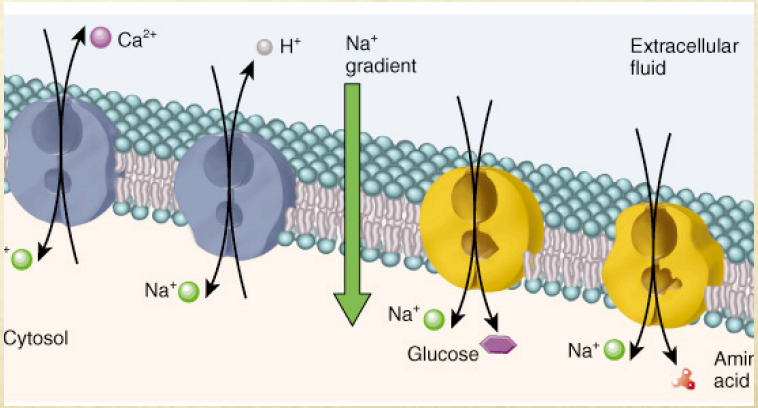
- The use of active secondary transports is a common strategy used to accumulate organics and inorganic nutrients and small molecules
- X is typically Na+ (but also H+). It is not surprising therefore that Na+ and H+ gradients are maintained by active transports
- It has the same characteristics of Facilitated Transports but more complex since there are 3 species playing role: the transporter, the substrate and the driver

Secondary Active Transports: Symport and antiport



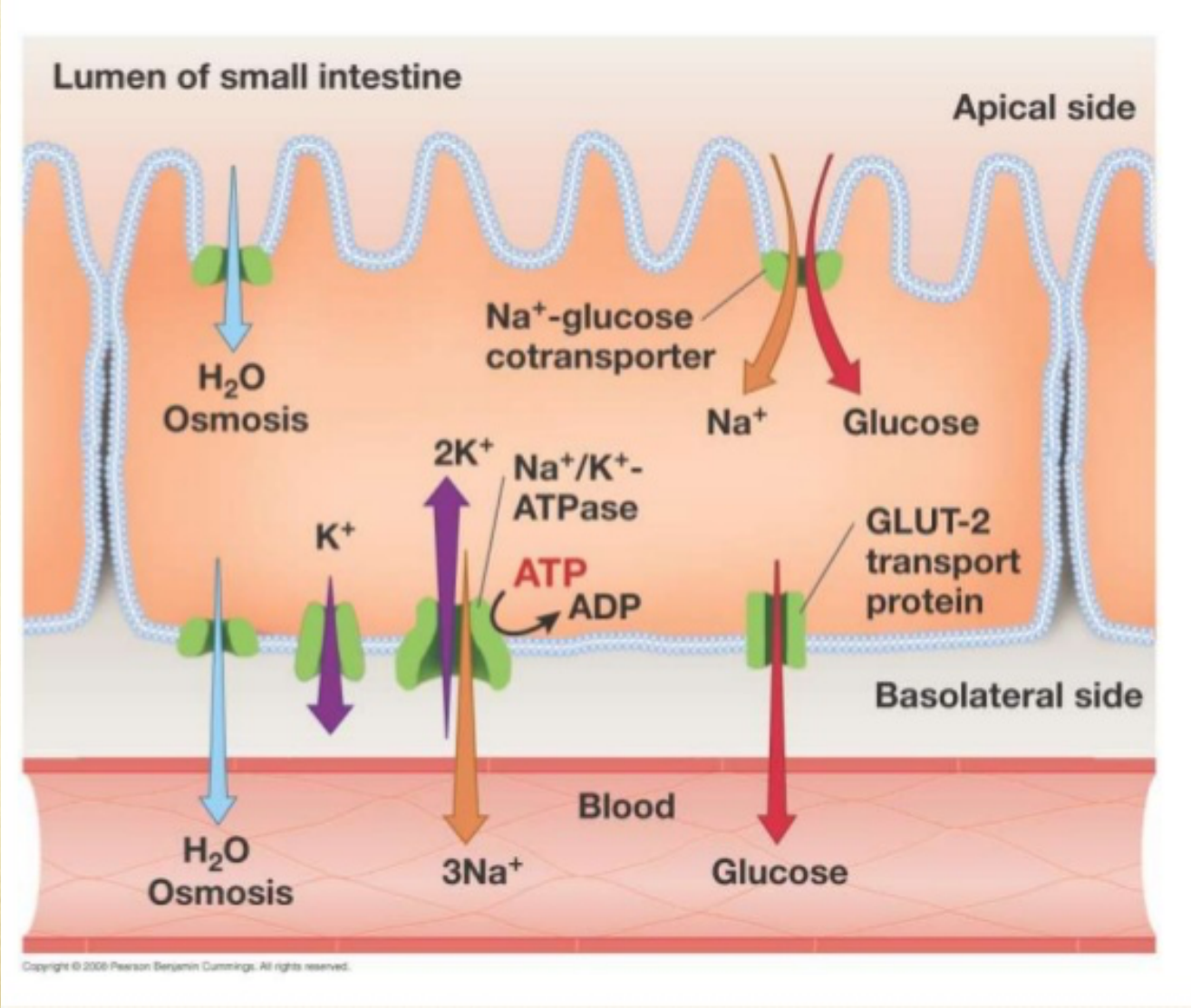
- The electrochemical gradient of Na+ (created by Na+/K+ ATPase) is used to transport molecules such as glucose (SGLT) or aa
- Cotransporters which are involved in neurotransmitter recycle: Na+/Cl- dependent transporters; Excitatory amino acid transporters (EAAT)

- Antiports Na+/H+ and Na+/Ca2+ use Na2+ influx to remove H+ e Ca2+ from the cytosol



SGLT

- SGLT are responsible of glucose reabsorption from intestine and kidney

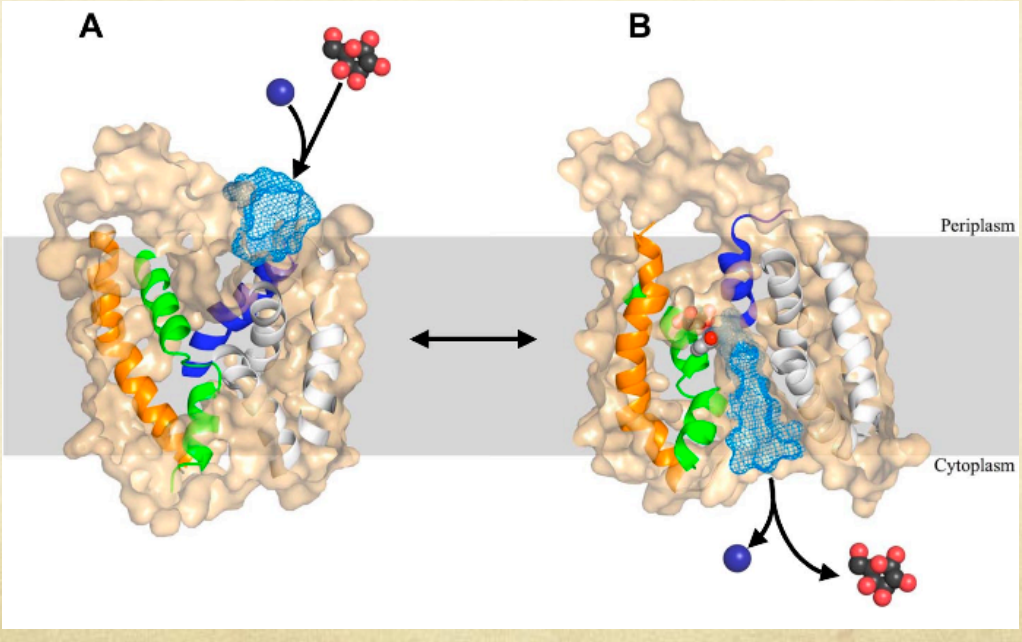


SGLT

- SGLT are responsible of glucose reabsorption from intestine and kidney

Insieme alla biologia molecolare , ha permesso di identificare il significato funzionale di precise sequenze aminoacidiche.

ES: malattia genetica recessiva che impedisce l'assorbimento intestinale del Glu e Gal, e' causata da mutazioni puntiformi del trasportatore (Asp28Asn) causa malassorbimento intestinale



Na+/GLUC transporters (SGLT)

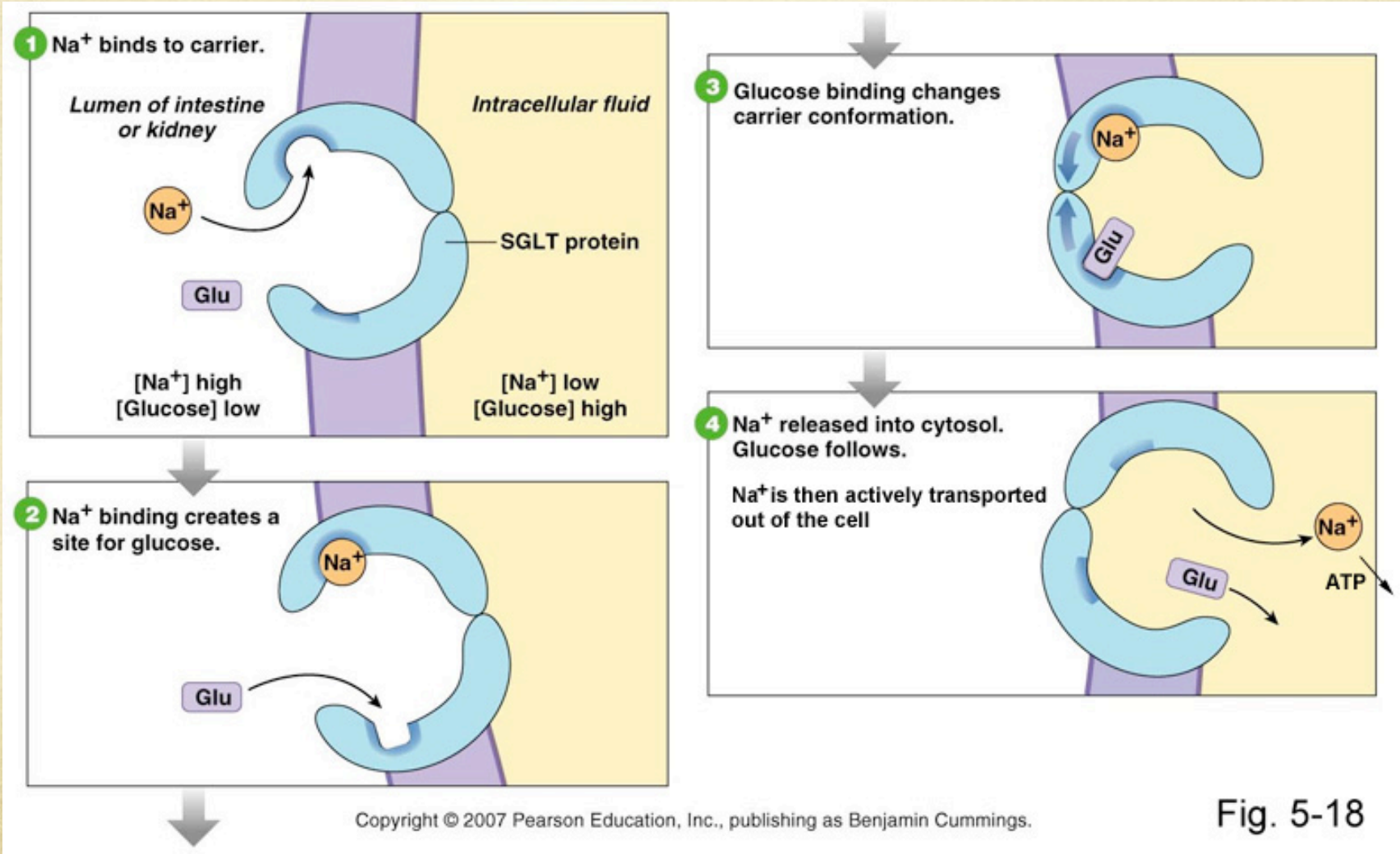


Fig. 5-18

Neurotransmitters co-transporters

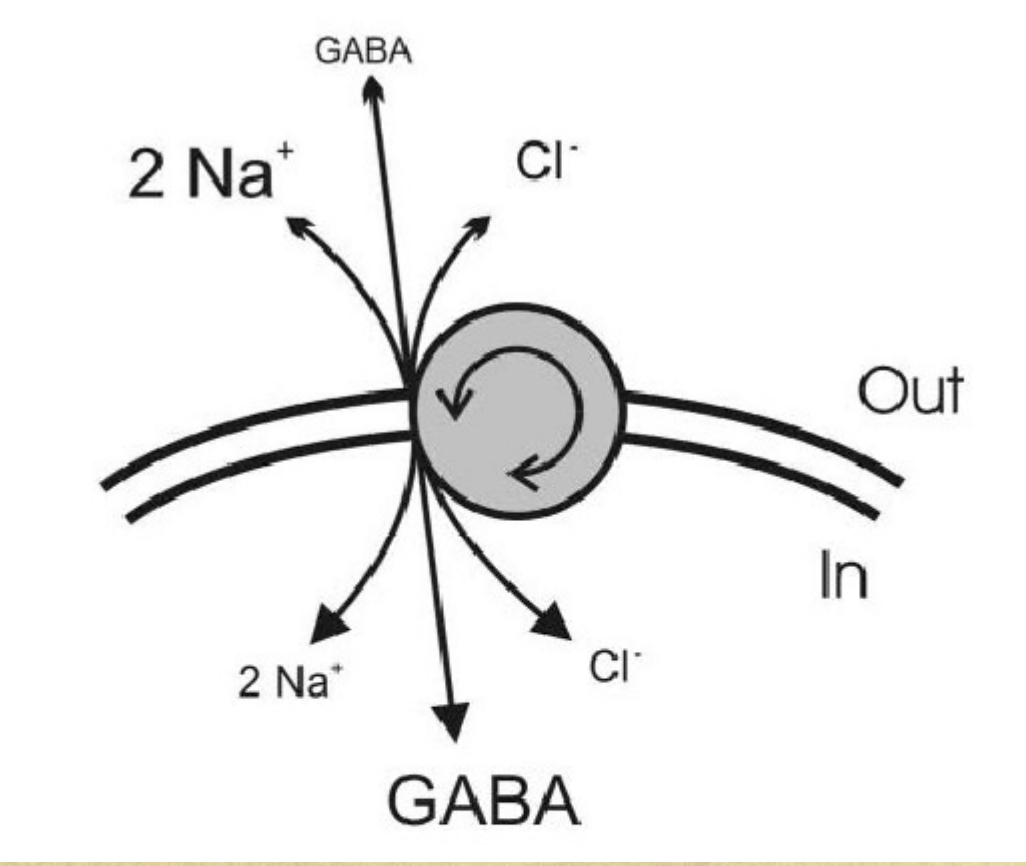
Key role in neurotransmitters removal from post synaptic space.
(This role is also accomplished by specific enzymes).

Two main classes of cotransporters for neurotransmitters:

- NA+/CL- DEPENDENT TRANSPORTERS
- EXCITATORY AMINOACID TRANSPORTERS (EAAT)

• NA+/CL- DEPENDENT TRANSPORTERS

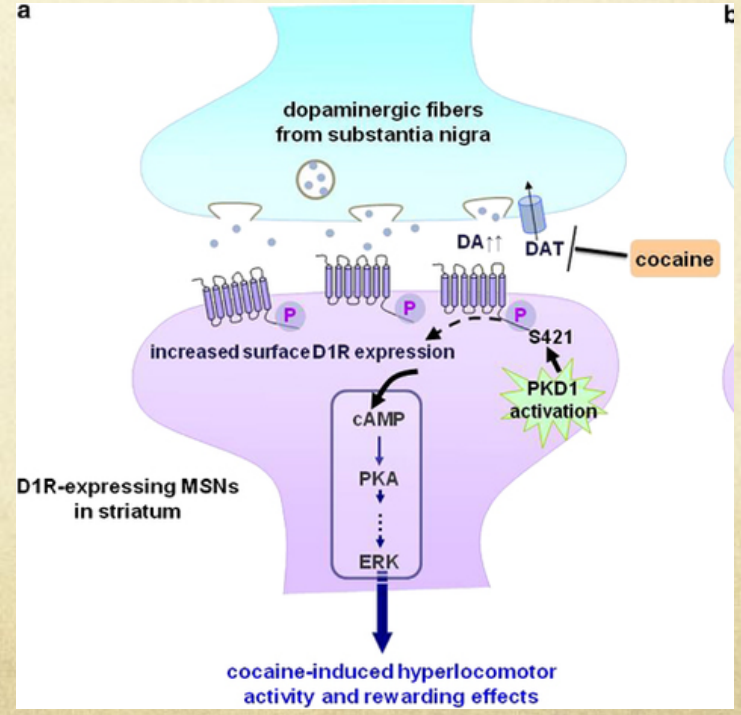
Classical example
GABA transporters GAT: remove GABA (the principal inhibitory neurotransmitter in the CNS).



The uptake of GABA is dependent upon the coupled translocation of 2Na+ and 1Cl- and thus the net uptake of one positive charge.

• NA+/CL- DEPENDENT TRANSPORTERS

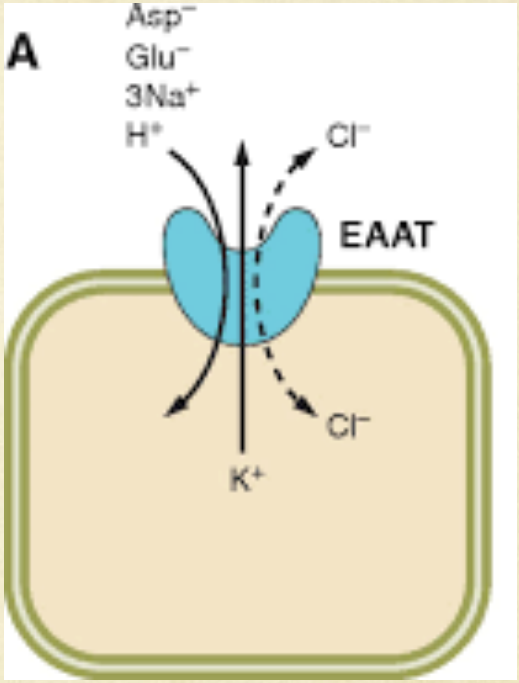
This family of transporters also comprehends:
Proline (PROT) and Glycine (GLYT) transporters
Dopamine (DAT) transporters
Serotonin (SERT)
Noradrenalin (NET)
All these transporters are targets for narcotic drugs and psychotropic substances



EXCITATORY AMINOACID TRANSPORTERS (EAAT)

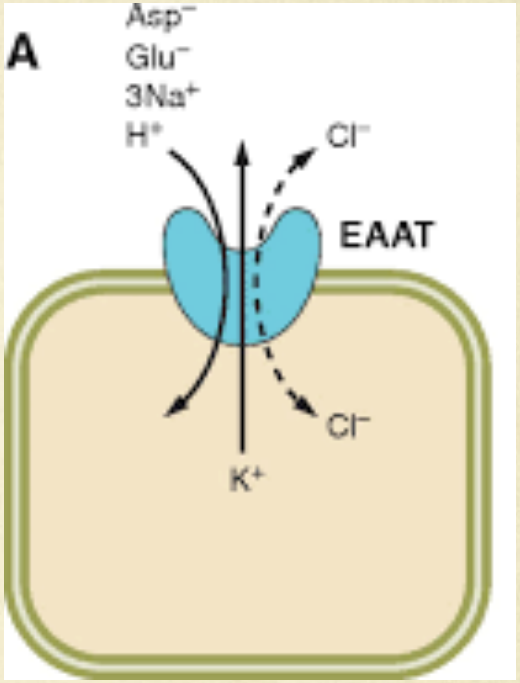
Glutamate transport by EAAT1, 2, and 3 is coupled to the cotransport of 3 Na+ and 1 H+ followed by the countertransport of 1 K+.

At equilibrium under standard physiological conditions, this coupling ratio is able to support a **106 fold gradient** of glutamate across the cell membrane. Theoretically, this coupling ratio should ensure that the resting extracellular glutamate concentration should be in the low nanomolar range.



EXCITATORY AMINOACID TRANSPORTERS (EAAT)

In addition to the coupled glutamate ion fluxes, substrate binding to the EAATs generates a chloride (Cl-) flux through the transporter. The extent of channel activity varies between transporter subtypes. The neuronal transporters EAAT4 and EAAT5 behave predominantly as Cl- channels, while for EAAT1, EAAT2, and EAAT3 the channel activity represents a much smaller proportion of the ion fluxes associated with transporter function.



AMINOACID TRANSPORTERS

Amino acids fundamental for cell metabolism cross the plasma membrane by means of UNIPORTERS, crossing the membrane along their gradient, or CONTRANSPORTERS, crossing the membrane against their gradient.

The main criteria for the classification of the Amino acid Transporters is based on aa types. We therefore can divide the transporters in 3 main categories:

- Neutral amino acid Transporters
- Positive charged amino acid Transporters
- Negatively charged amino acid Transporters

The ubiquitary expressed aa Transporters are generally poorly selective: broad selectivity for different a-amino acids.
On the other hand the neurotransmitters transporters expressed at synaptic level are highly selectivity for their specific substrate (as shown earlier)

AMINOACID TRANSPORTERS

Another criteria for the classification of the Amino acid Transporters is based on molecular structure.

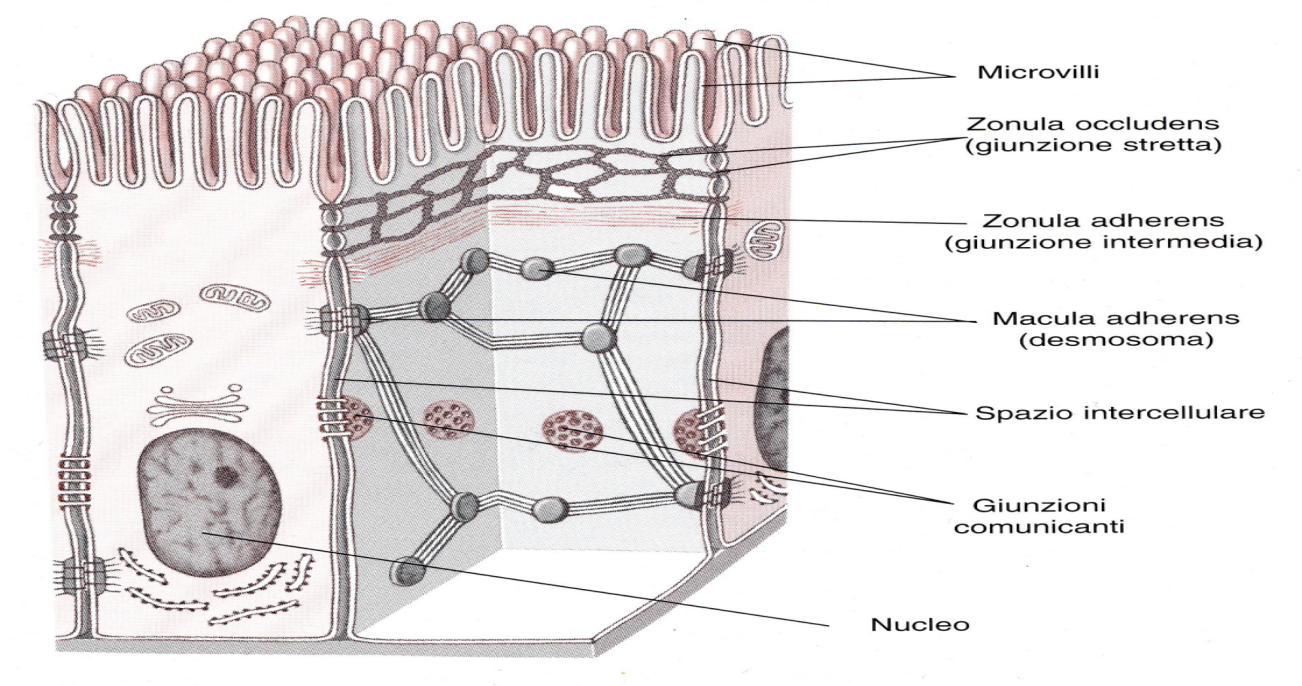
Using this classification criteria we can enumerate:

- EAAT Transporters (Excitatory aa transporters) = Na+/Cl
- SNAT (Na+-coupled neutral aa transporters)

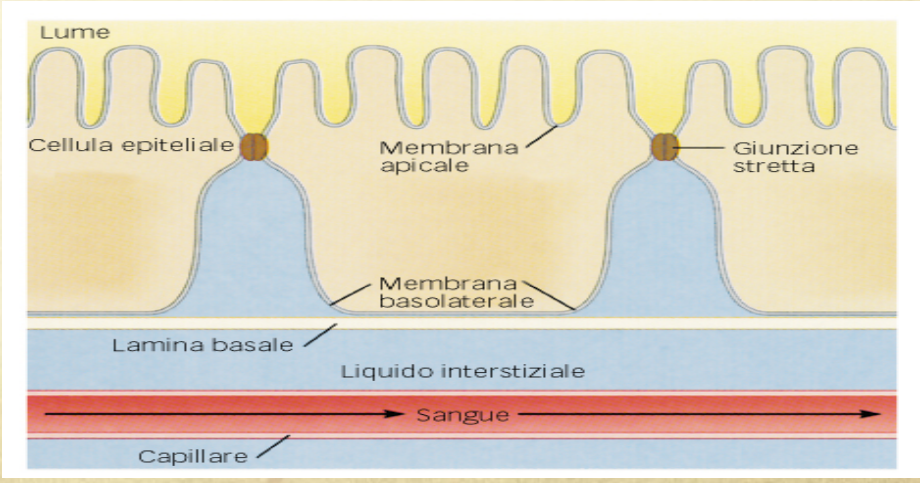
This family accounts for both neutral aa and small polar aa such as Asparagine (Asn) or Glutamine (Gln). Important role in several processes such as Gln recycling for GABA synthesis, NH3 detossification, liver gluconeogenesis, acidosis response in kidney

- CAT (cationic-Na+ aa transporters) mediate transmembrane fluxes of aa such as Arginine (Arg), Lysine (Lys), Histidine (His). Important role in NO synthesis
- HAT (heteromeric aa transporters) are formed by 2 different subunits. They trasnport neutral or basic aa

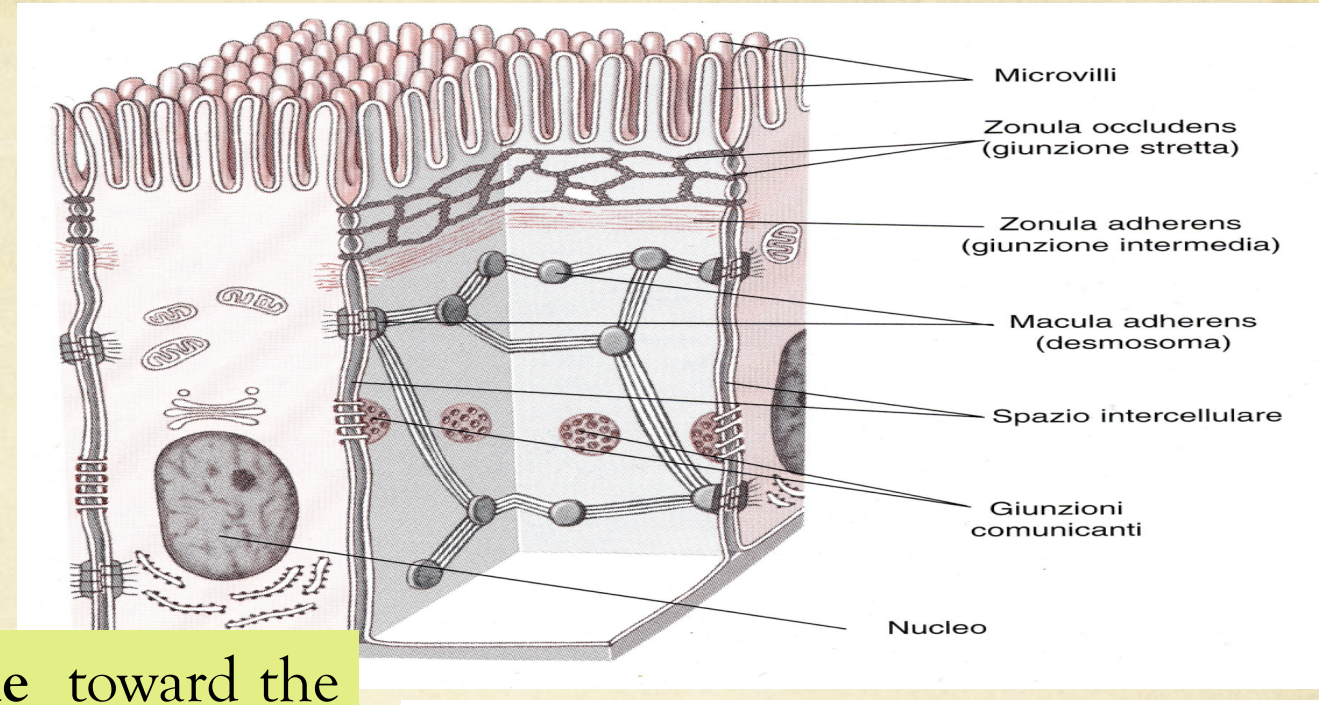
Absorbent Epithelial structure



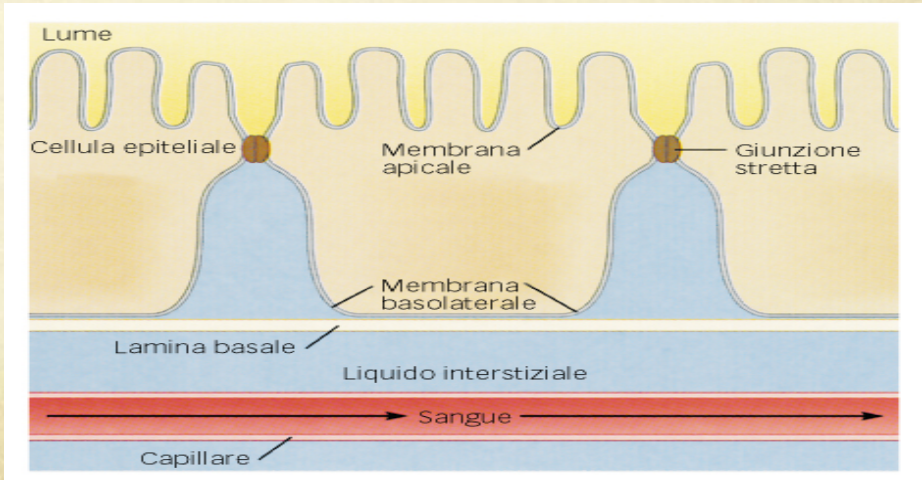
- Cell-cell contact is mainly due to tight junctions which limit the flow of material therefore delimiting the two epithelial faces.



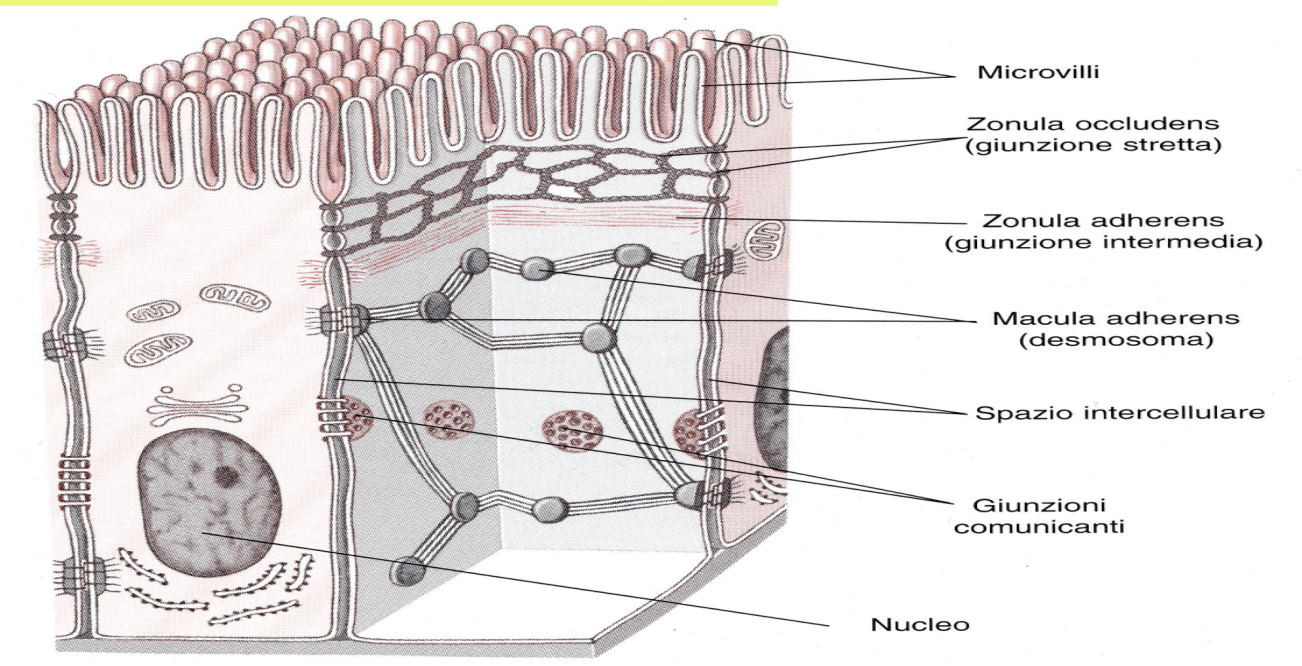
Absorbent Epithelial structure



• **Apical membrane** toward the internal lumen. Often presenting microvilla that amplify the exchange surface. In this domain there is a large number of enzymes, uniporters and contransporters

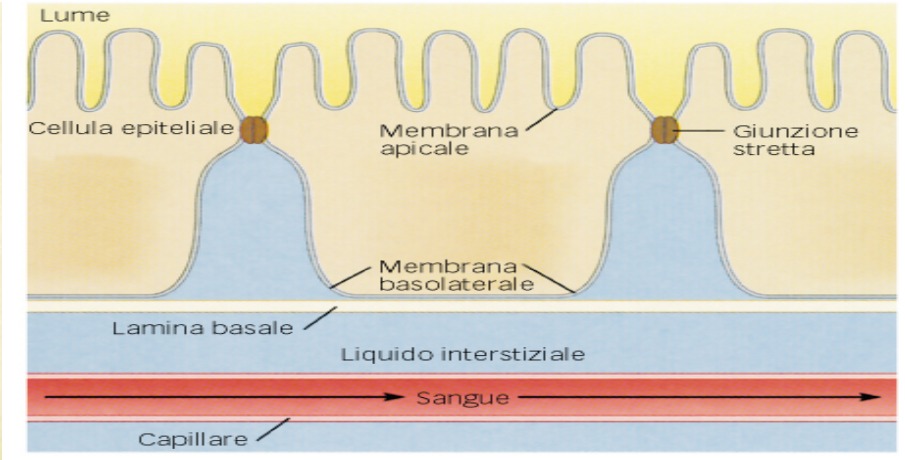


Absorbent Epithelial structure: Polarized epithelia



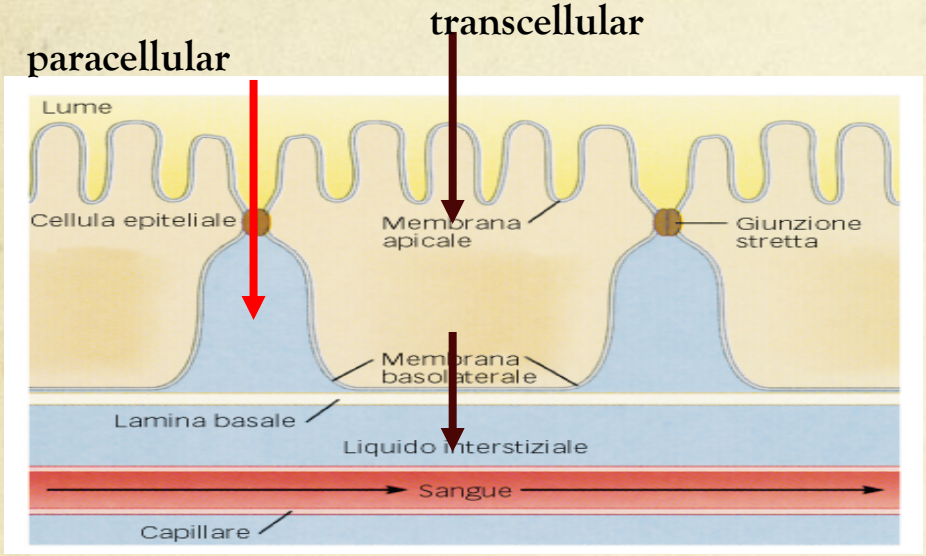
- **Basolateral Membrane toward the internal part of the body** is in contact with the interstitial liquid exchanging substances with blood circulation.

Is charachterrized by the **presence of Na+/K+ ATPase that is absent in the apical membrane**



Transports Across Epithelia

- 2 types of transports



- **Paracellular fluxes: Passive diffusion of ions** (Na^+, Cl^-, K^+, Ca^{2+}, Mg^{2+}) **and** e H_2O.

In absorbent epithelia, this way is limited by the presence of tight juntion

- **Transcellular fluxes: fluxes of ions and small molecules mediated by transporters**

Composed by:

- Passive or secondary active transporters on apical membranes
- Active Transports (Na^+/K^+-ATPasi) and other passive or active transports on basolateral membranes

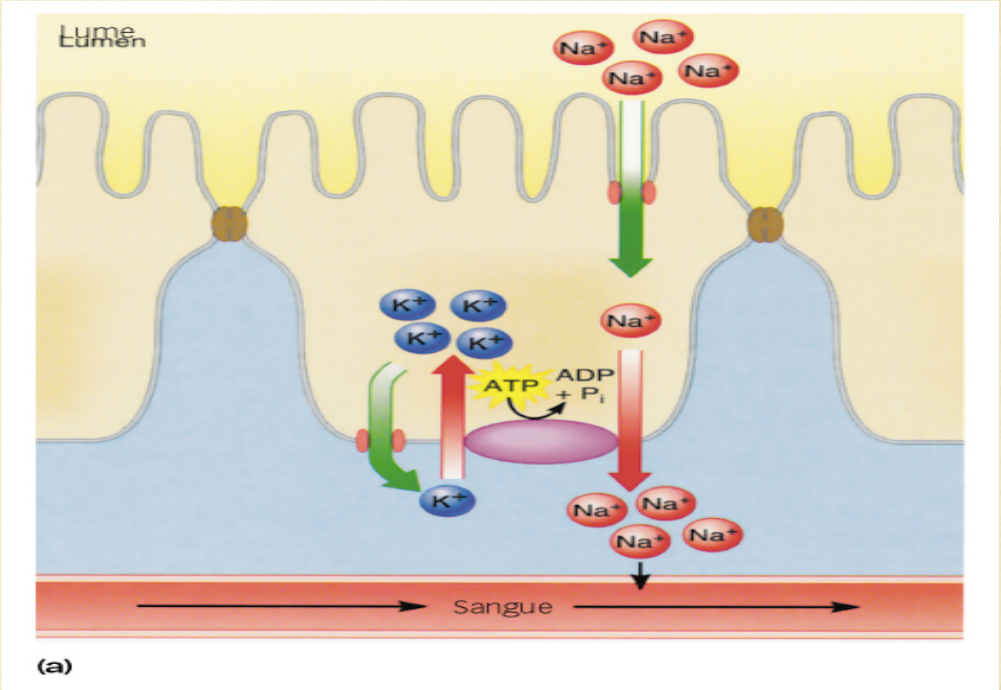
Intestinal Na^+ Absortion

Apical membrane:

- Passive transport of Na^+ (Na^+/H^+ antiport; Na+/aa or Na+/K+/2Cl-cotransports)

Basolateral membrane:

- Active Transport Na^+ e K^+ (Na^+/K^+-ATPase)
- Passive transport K^+ and Cl- (ion channels)



Net absorption of NaCl

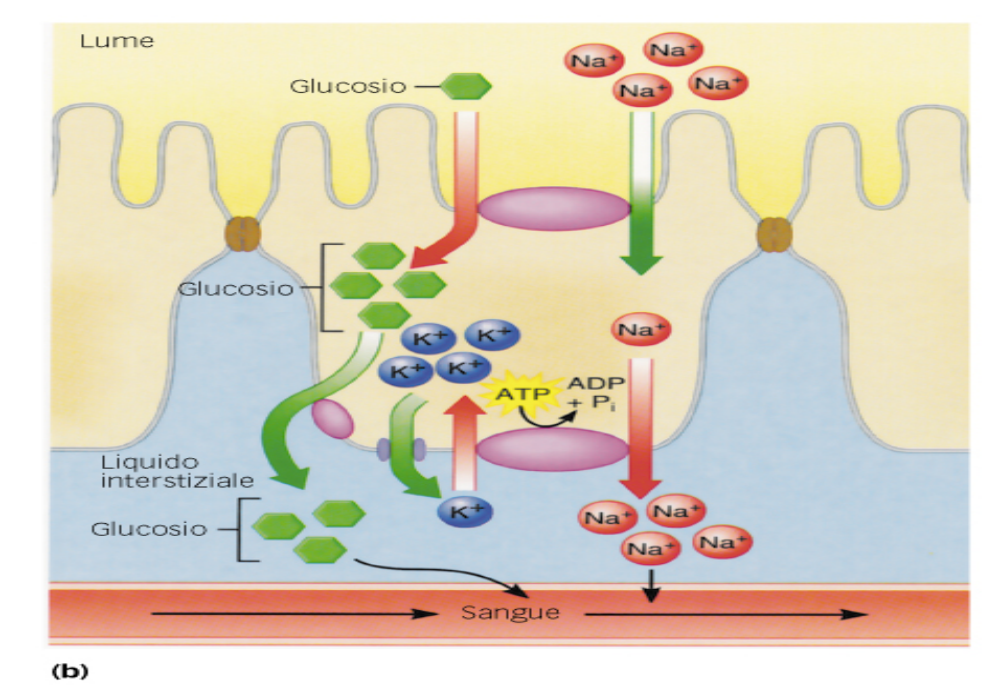
Intestinal Na^+ and Glucose Absortion

Apical Membrane

- Secondary active Transport of Na^+ and glucose (SGLT)

Basolateral Membrane

- Active Transport Na^+ and K^+ sulla m. basolaterale (Na^+/K^+-ATPase)
- Passive Transport for glucose (GLUT) and K^+ (ion channels)



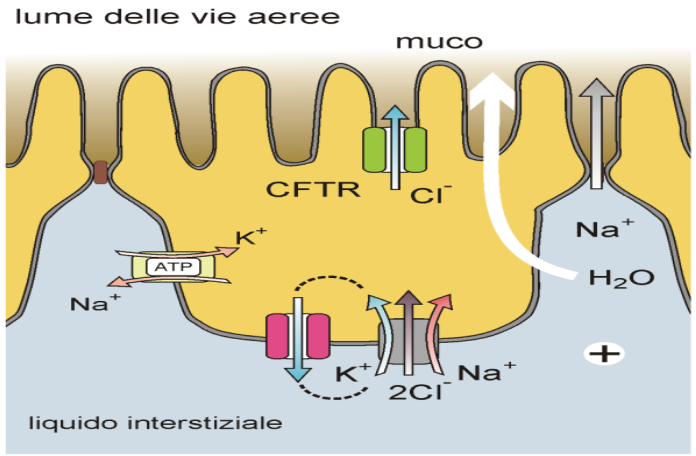
Role of coupled fluxes across the epithelia

- Fluxes depends on coupled forces and simmetric crossed coefficients
- H2O fluxes brings solutes
- Solutes fluxed influence H2O movements
- The net fluxes are dependent on energy sources : most of the times Na+ gradient which is created by 3Na/2K ATPase

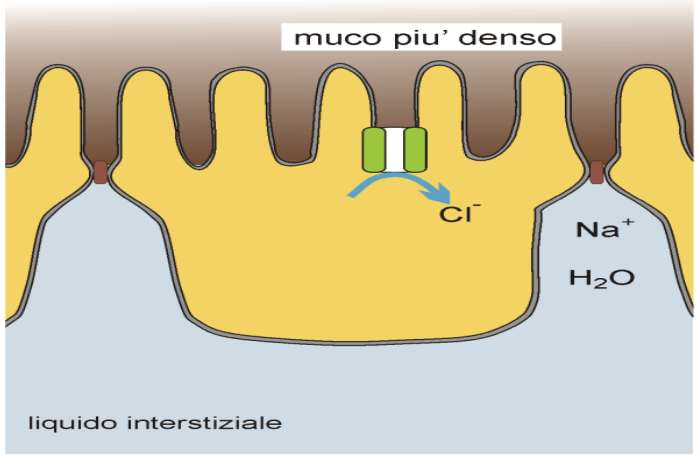
Patologie del trasporto epiteliale: la fibrosi cistica

In alcune condizioni patologiche il trasporto epiteliale di H_2O è eccessivo o insufficiente:

- Nella ***fibrosi cistica*** l'epitelio che riveste le vie respiratorie non trasporta abbastanza soluti (Na^+ e Cl^-) per produrre liquidi.
- Un difetto del ***canale del Cl^- (CFTR)*** interferisce con il trasporto del Na^+, viene meno il gradiente osmotico e quindi la secrezione di acqua.
- I polmoni si intasano di muco rendendo difficile la respirazione e portando gravi infezioni batteriche.

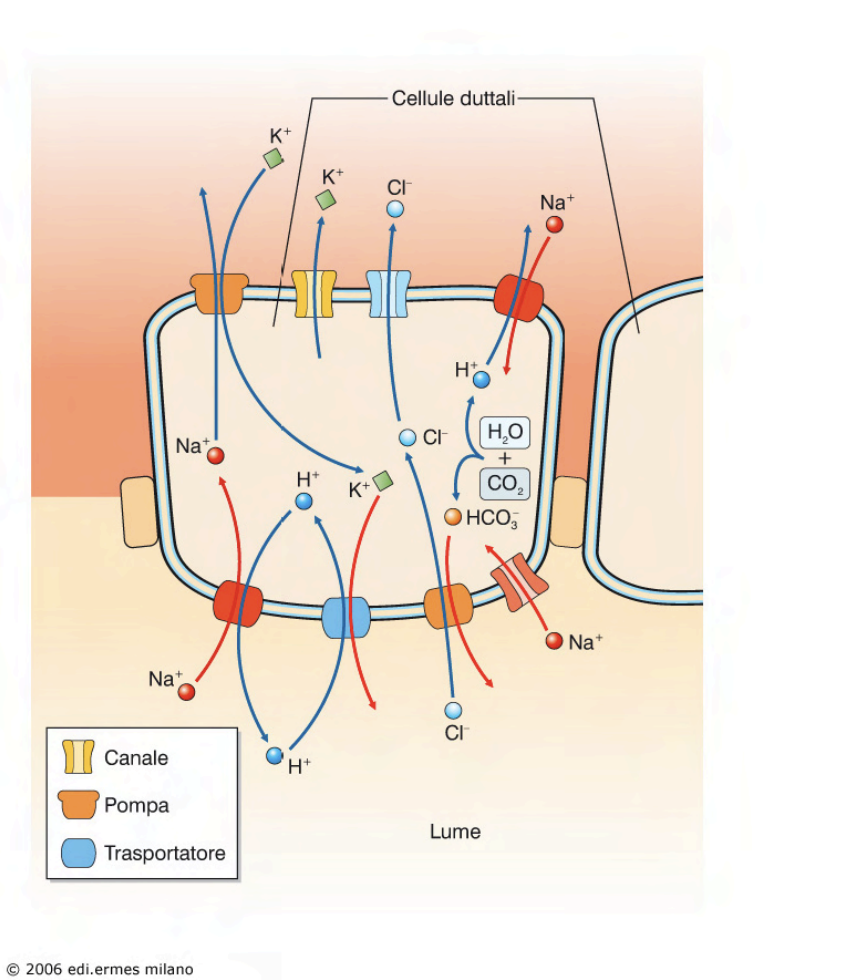


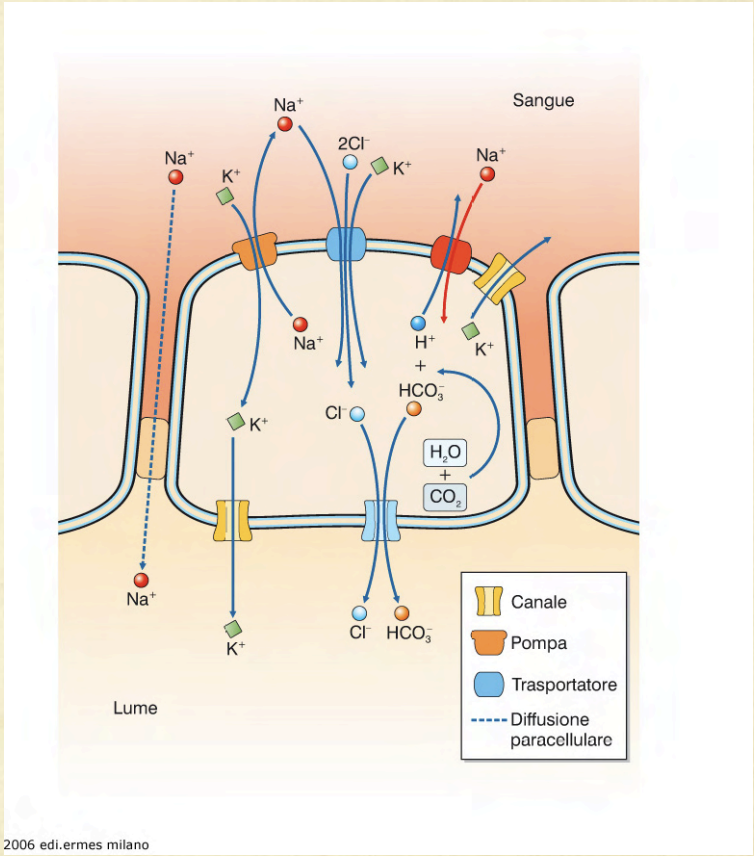
Trasporto di Cl^-, Na^+ e H_2O per la normale produzione di muco



Trasporto di Cl^-, Na^+ e H_2O compromesso nella fibrosi cistica (CFTR non funzionante) con conseguente aumentata densita' di muco nelle vie aeree

ACCOPPIAMENTO DEI FLUSSI



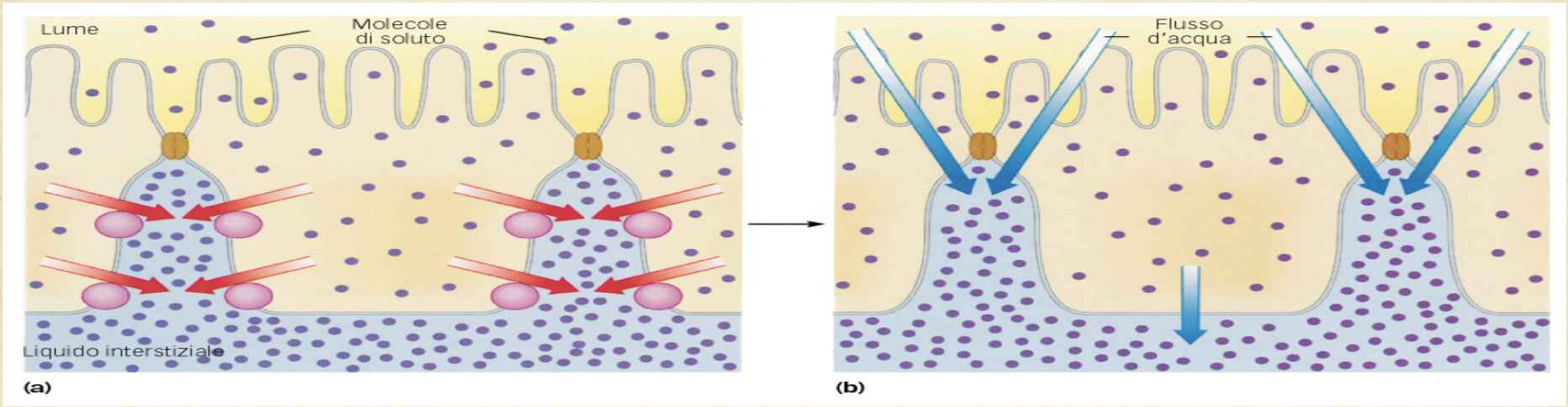


Assorbimento di soluti e H_2O

- Gli epiteli assorbono (o secernono) H_2O utilizzando il trasporto attivo di soluti.

- Si crea prima un ***gradiente osmotico*** tra le soluzioni poste ai due lati dell'epitelio

- L'H_2O quindi fluisce passivamente attraverso l'epitelio per ***osmosi***



Il trasporto attivo sulla membrana basolaterale crea una ***pressione osmotica*** attraverso l'epitelio che genera un flusso di acqua dal lume al liquido interstiziale (***assorbimento***). Può accadere il contrario se l'H_2O è ***secreta***.

Adesioni cellula-cellula

- durante lo sviluppo e la crescita le membrane di cellule adiacenti formano connessioni (giunzioni) che possono rimanere permanenti
- si distinguono tre tipi di giunzioni: ***serrate***, ***comunicanti*** e ***aderenti*** (***desmosomi***)

Giunzioni serrate (tight junctions)

- sono presenti nei tessuti epiteliali degli organi cavi che assorbono e rilasciano ioni e molecole (rene e tratto gastrointestinale)
- formano una barriera impermeabile alle sostanze polari tra il lato luminale e il lato sierosale
- ***trasporto transepiteliale*** (attraverso le cellule)
- ***trasporto paracellulare*** (tra le cellule)