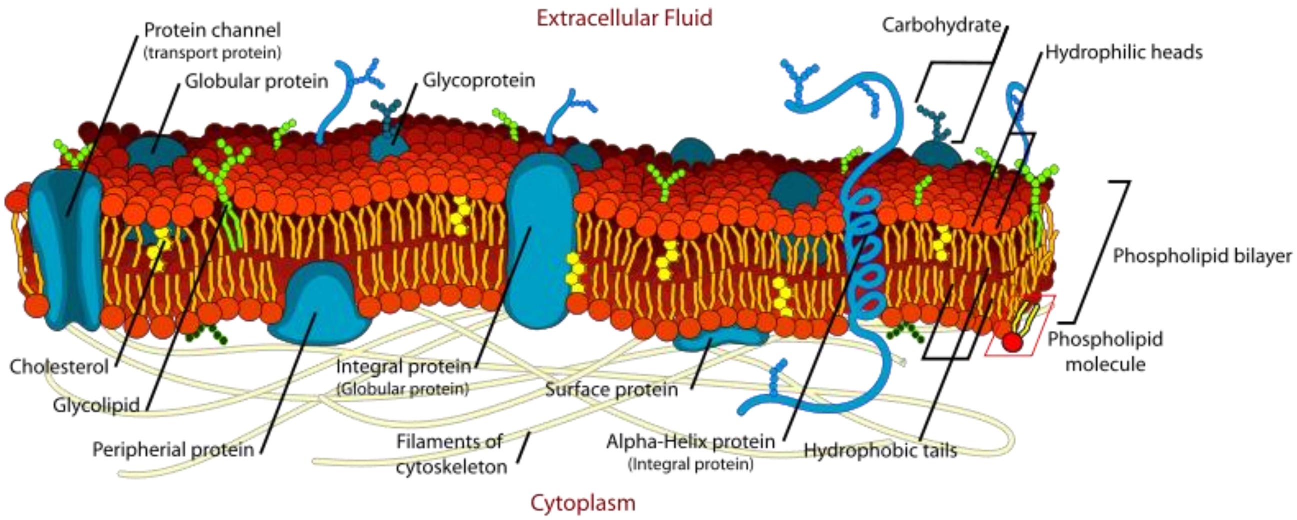
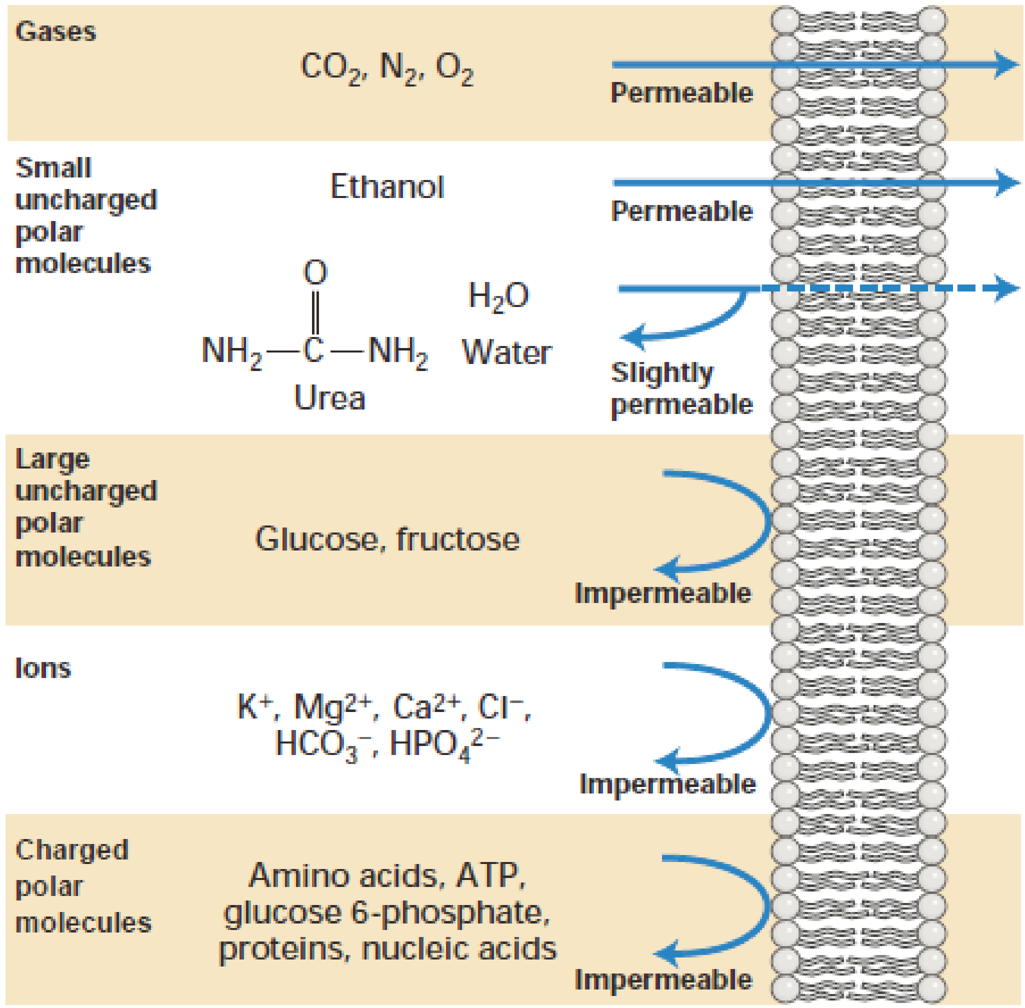
Transport snovi preko celičnih membran

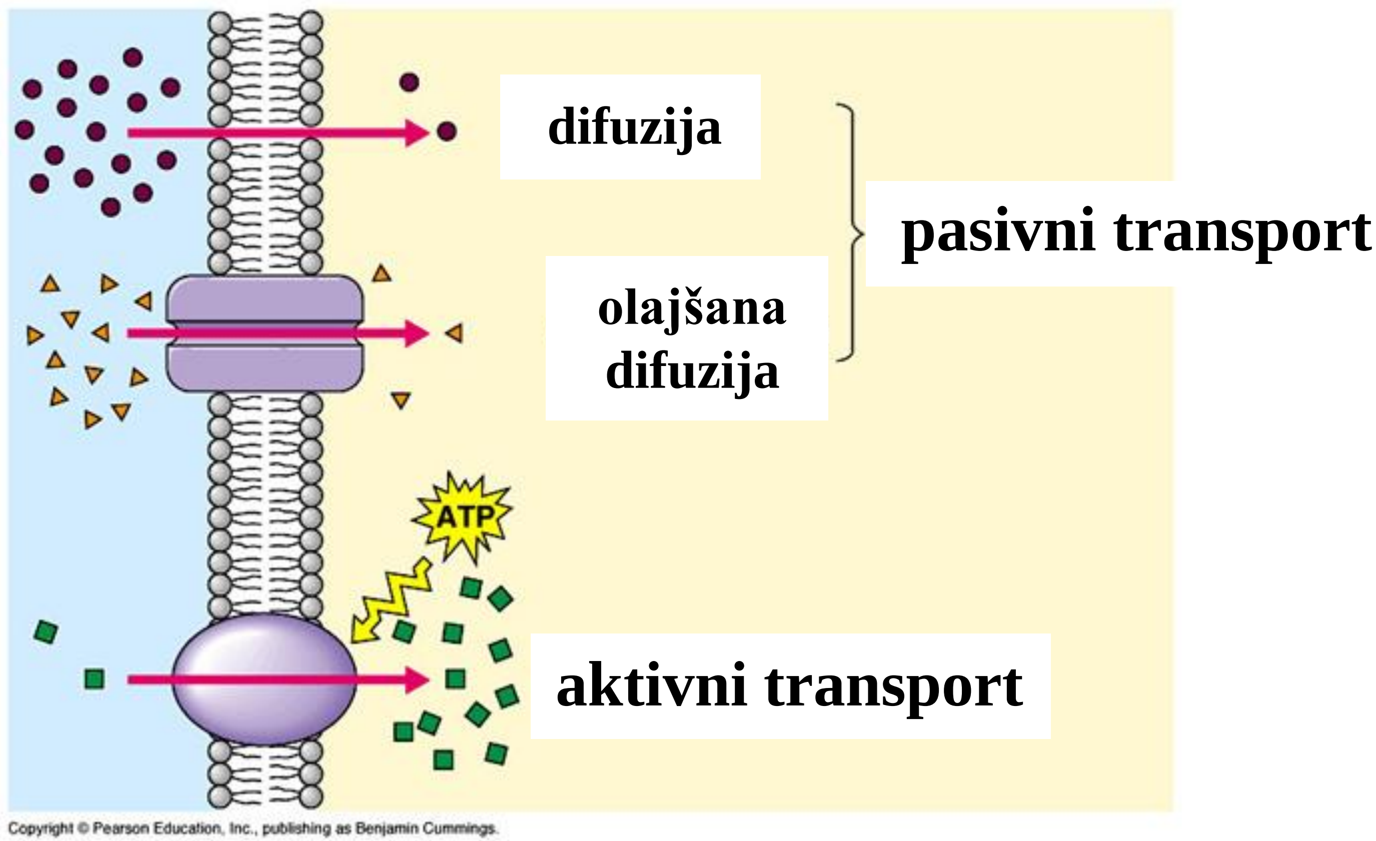


Lodish et al. 4. izdaja, 15. poglavje

Relativna propustnost fosfolipidnega dvosloja za različne molekule



Načini transporta snovi preko celičnih membran



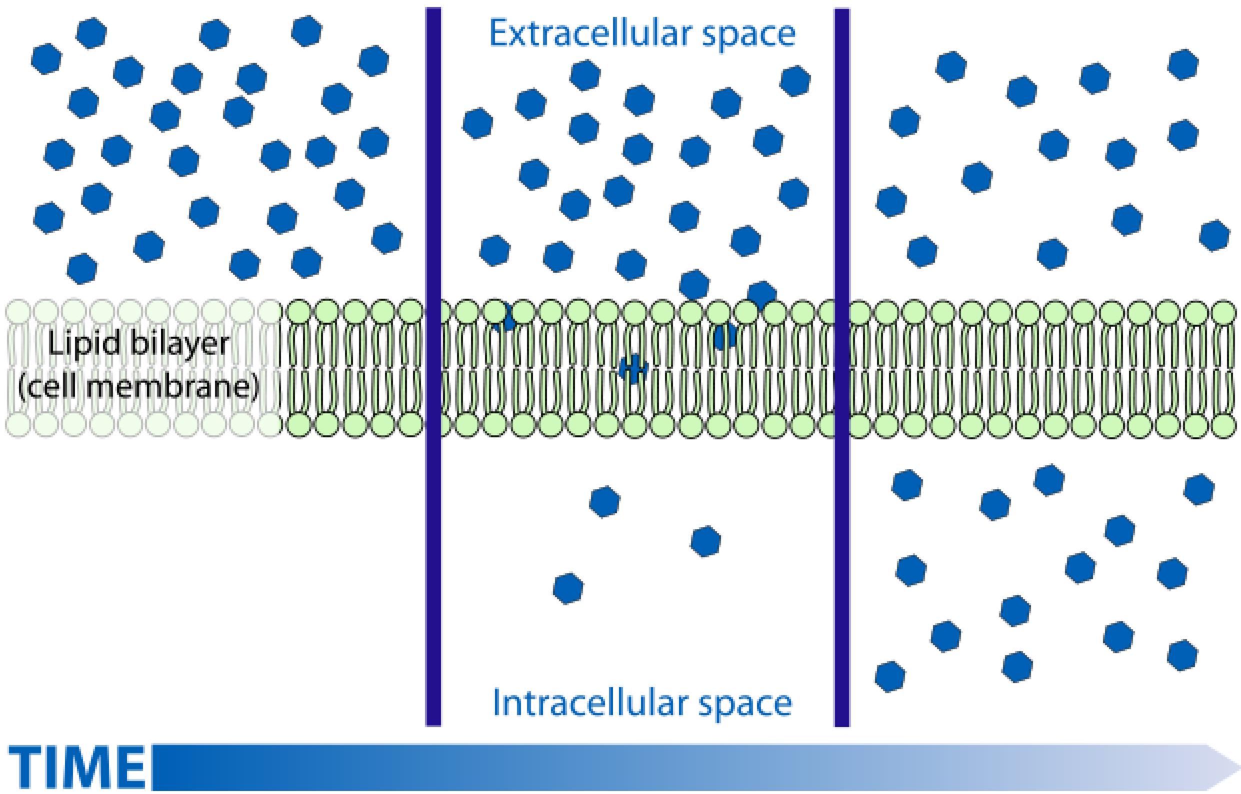
Mehanizmi membranskega transporta

mechanism	molecules	features
direction of molecule movement is **down the concentration gradient**		
passive diffusion	small hydrophobic and polar molecules	direction of flow of molecules determined by concentration of molecules either side of membrane
passive transport / facilitated diffusion	Ions, water	rapid ion transport mediated by channel proteins, different channel proteins move different molecules according to their size and charge – selectivity channels can be open or closed – gated
passive transport / facilitated diffusion	sugars, amino acids, nucleosides	mediated by transmembrane carrier proteins which are selective for the solute they transport, the binding of the correct solute to a carrier protein induces conformational change in the protein and passage of the molecule through the membrane
molecules moving **against the concentration gradient** of transported molecule		
active transport	many different ions, glucose, amino acids, peptides	movement of molecules consumes energy e.g. coupled to ATP hydrolysis

Pasivni transport

- difuzija
- transporterji
- kanalčki

Difuzija



"Difuzija je delovanje snovi, toplote, gibalne količine ali svetlobe, usmerjeno k izničenju koncentracijskega gradienta."

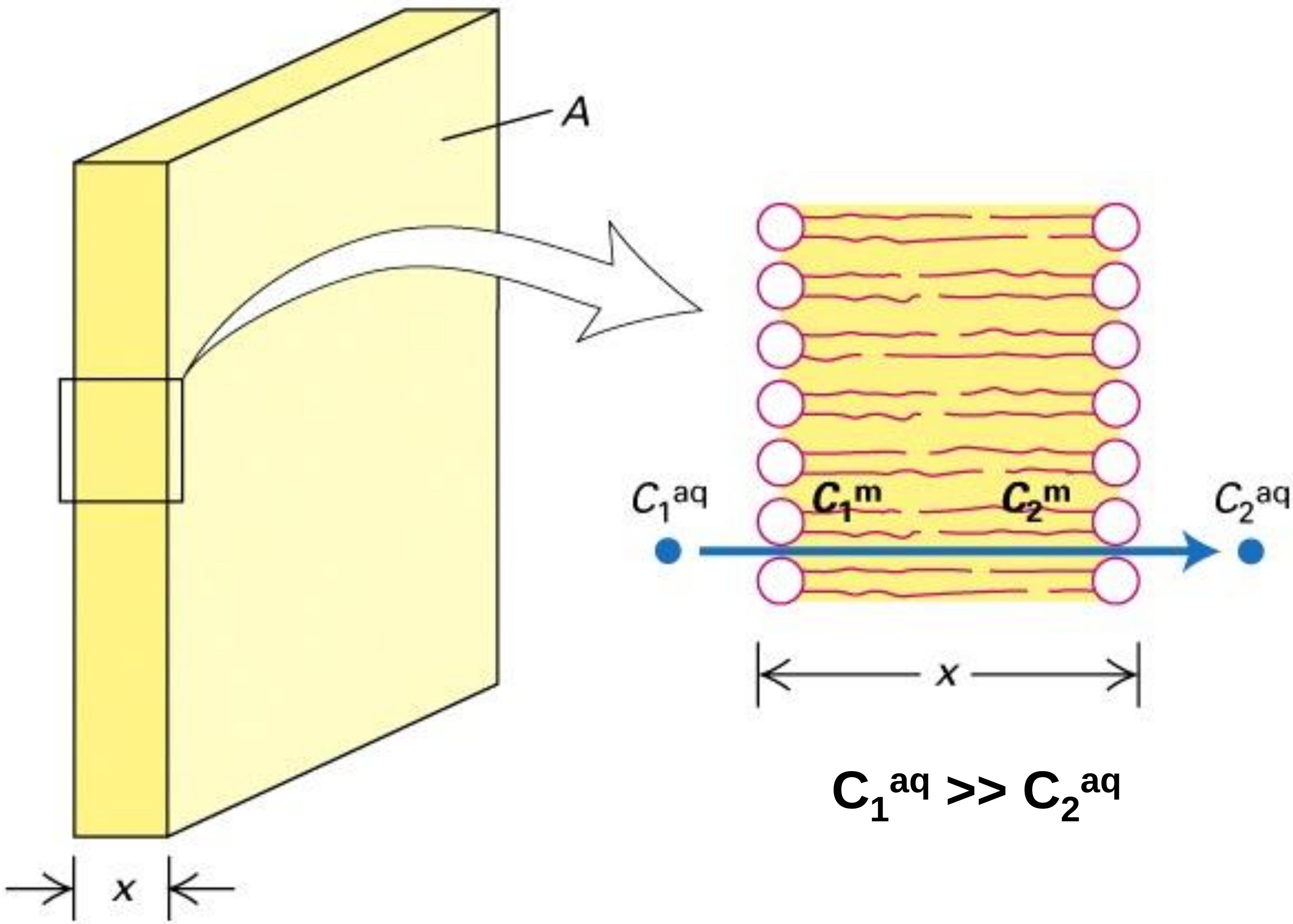
Pasivna difuzija skozi fosfolipidni dvosloj

1. prehod molekule iz vodnega okolja v hidrofobno notranjost dvosloja

2. difuzija skozi hidrofobno sredico
(počasna zaradi večje viskoznosti lipidov v primerjavi z vodo)

3. prehod v vodno fazo na drugi strani

Difuzija malih molekul skozi membrano



$$C_1^{aq} >> C_2^{aq}$$

Vpliv hidrofobnosti

- Merilo hidrofobnosti je porazdelitveni koeficient K (ravnotežna konstanta porazdelitve med vodo in oljem).

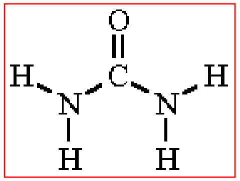
- Ker je notranjost dvosloja po sestavi podobna olju, je porazdelitveni koeficient enak razmerju koncentracij snovi med dvoslojno fazo (C^m) in vodno fazo (C^{aq}).

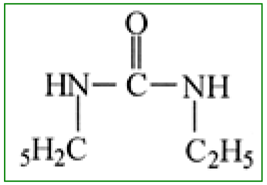
$$K = C^m/C^{aq}$$

Porazdelitveni koeficient

Porazdelitveni ali particijski koeficient je merilo relativne afinitete snovi za lipid v primerjavi z vodo: visok K → boljša topnost v lipidu.

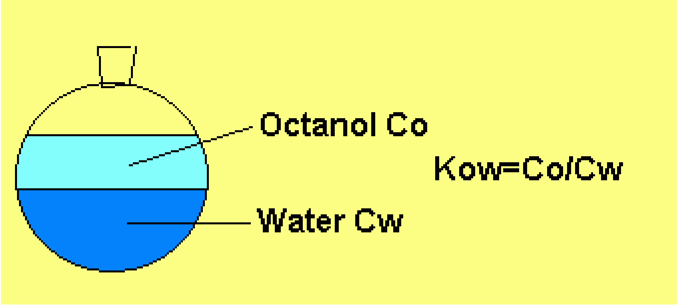
$$K = C^{m}/C^{aq}$$





urea: K=0,0002; dietilurea: K=0,01 ⇨

dietilurea je 50x bolj hidrofobna / difundirala bo 50x hitreje kot urea.



Adolf Eugen Fick
Kassel (DE)
03.09.1829

Blankenberghe (BE)
21.08.1901

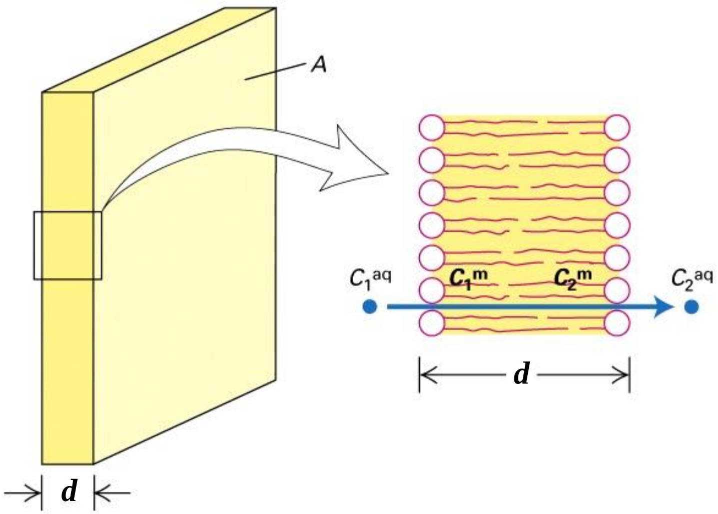
Difuzijski ali 1. Fickov zakon (1855)

$$J = -D\frac{\partial \phi}{\partial x}$$

J – gostota masnega toka
D – difuzijska konstanta
δφ/δx – gradient koncentracije

Hitrost difuzije

Hitrost difuzije: dn/dt [mol/s]



$dn/dt = J \times A$

$dn/dt = P \times A \times (\mathbf{C_1^{aq} – C_2^{aq}})$

J … gostota masnega toka

P… prepustnostna konstanta

A… površina membrane

$P = K \times D / d$

K… porazdelitveni koeficient ← **$K = C^m/C^{aq}$**

D… difuzijska konstanta snovi v membrani

d … debelina membrane

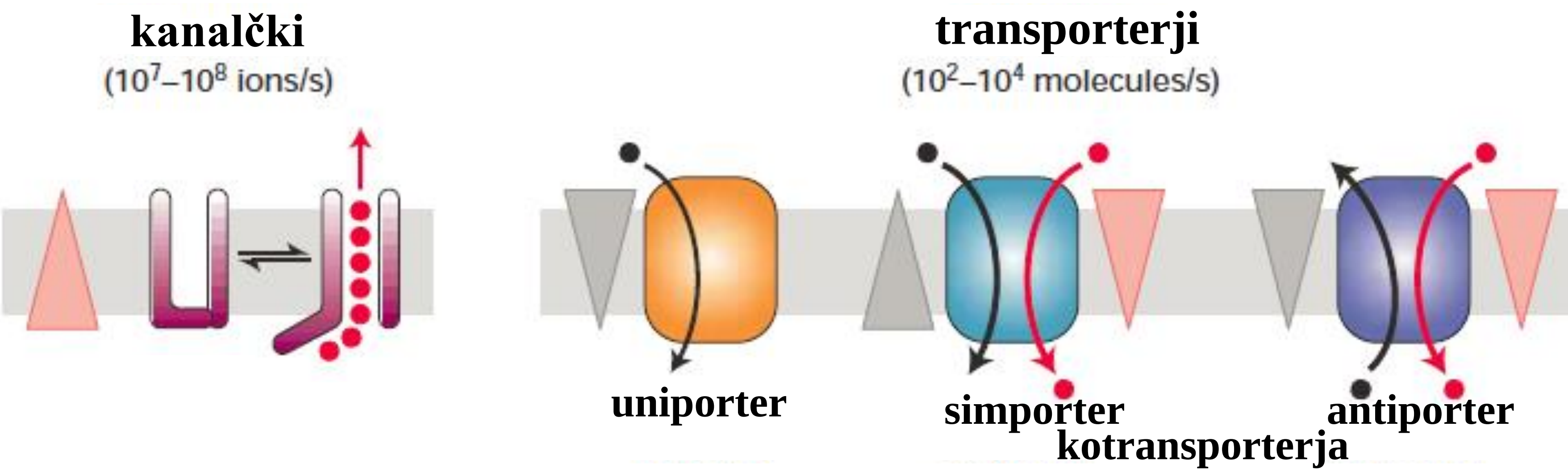
$$\mathbf{dn/dt = K \times D \times A \times (C_1^{aq} – C_2^{aq}) / \mathit{d}}$$

Hitrost difuzije je sorazmerna porazdelitvenemu koeficientu in difuzijski konstanti ter obratno sorazmerna debelini membrane.

Biološke membrane: *d* ≈ 3 nm (≈ konst.); D ≈ konst. ⇒ dn/dt α K; K α hidrofobnost snovi

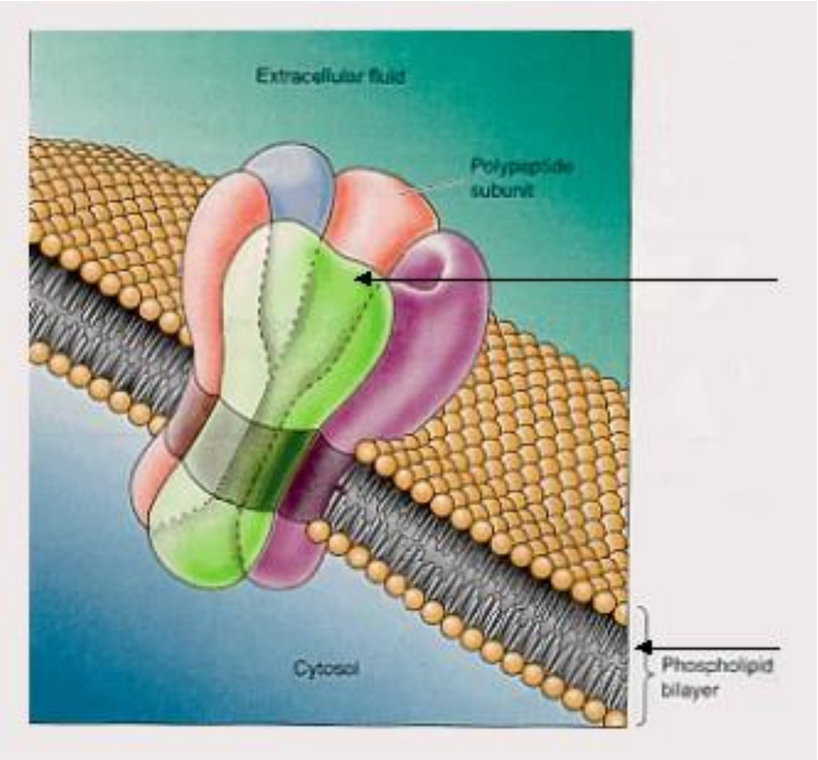
→ velja za pline in male nenabite molekule ←

Olajšana difuzija



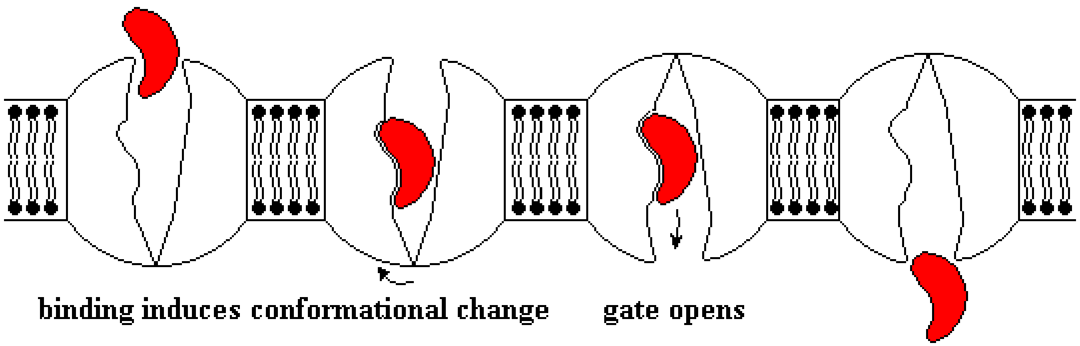
integralni transmembranski proteini /
/ omogočajo hitrejši prehod snovi čez membrano / so specifični

Kanalčki



Kanalčki z veliko hitrostjo prenašajo vodo ali specifične ione v smeri njihovega gradienta (koncentracijskega ali električnega).
Nekateri so ves čas odprti, drugi pa so običajno zaprti in se odprejo le kot odgovor na nek signal.

Transporterji

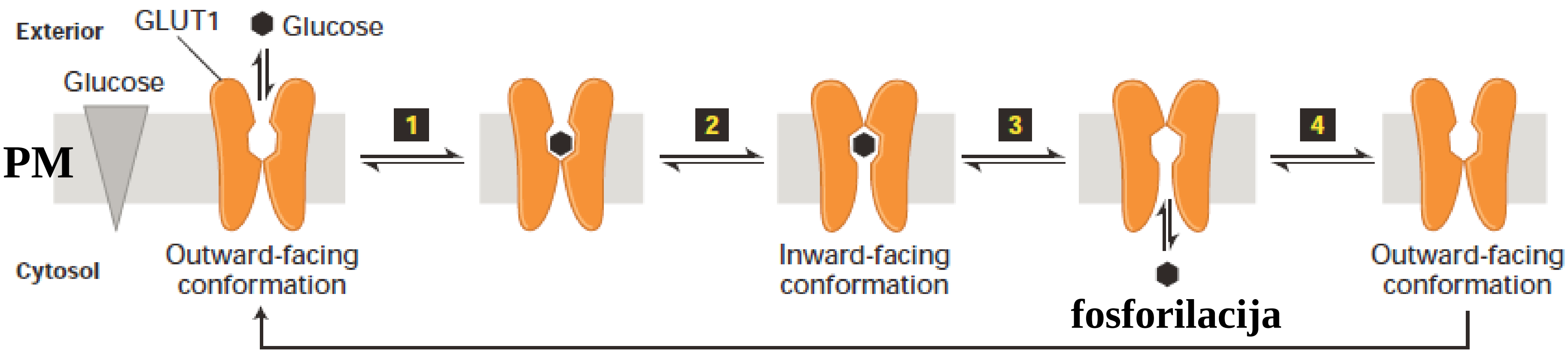


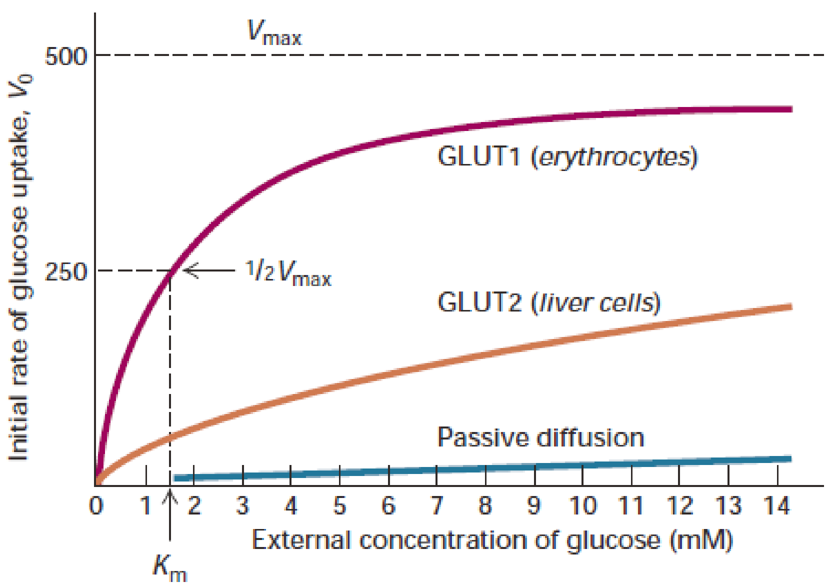
Transporterji običajno prenašajo po 1 molekulo (ion) hkrati skozi membrano. Prenos je povezan s konformacijsko spremembo proteina. Obstojajo 3 različni tipi transporterskih proteinov: **uniporterji**, **simporterji** in **antiporterji**.

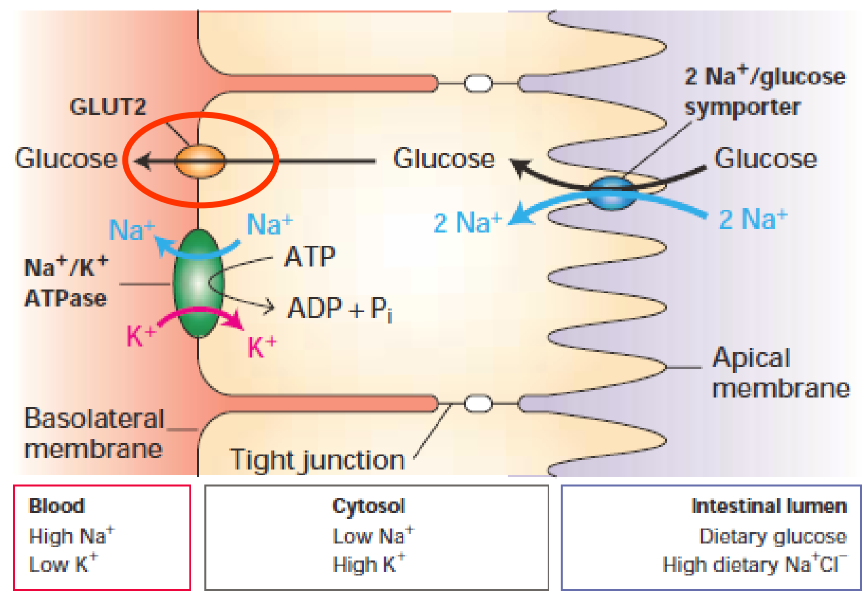
Lastnosti uniporta

- prenos aminokislin, nukleozidov, sladkorjev, …
- prenos v smeri koncentracijskega gradienta
- kljub temu brez proteina do prenosa ne bi prišlo
- termodinamsko ugodna reakcija ($\Delta S>0$; $\Delta G<0$)
- olajšana difuzija (*facilitated diffusion*)
- podobnost z encimsko reakcijo (a substrat se tu kemijsko ne spremeni)

Primera uniporterjev: GLUT1 in GLUT2

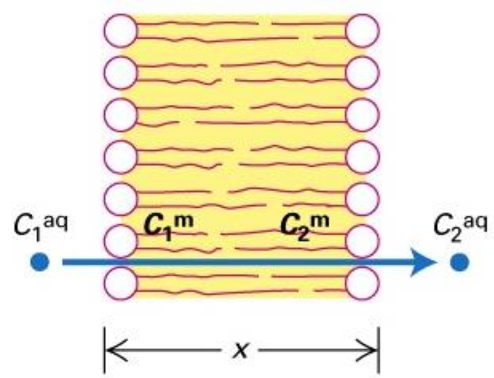






Primerjava uniporta in pasivne difuzije

- pri uniporterjih je hitrost prenosa bistveno večja, kot sledi iz Fickovega zakona, saj K ni treba upoštevati (molekula ne vstopa v hidrofobni del lipidnega dvosloja)



$$dn/dt = A\,K\,D\,(\mathbf{c_1^{aq} - c_2^{aq}})\,/\,x$$

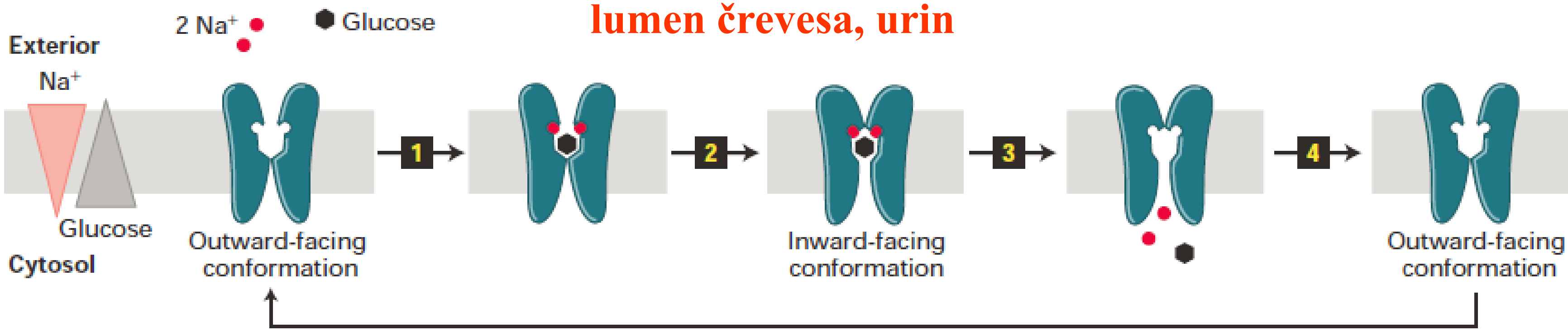
K… porazdelitveni koeficient
D… difuzijska konstanta snovi v membrani
x … debelina membrane
A… površina membrane
(D in *x* sta ≈ konst.)

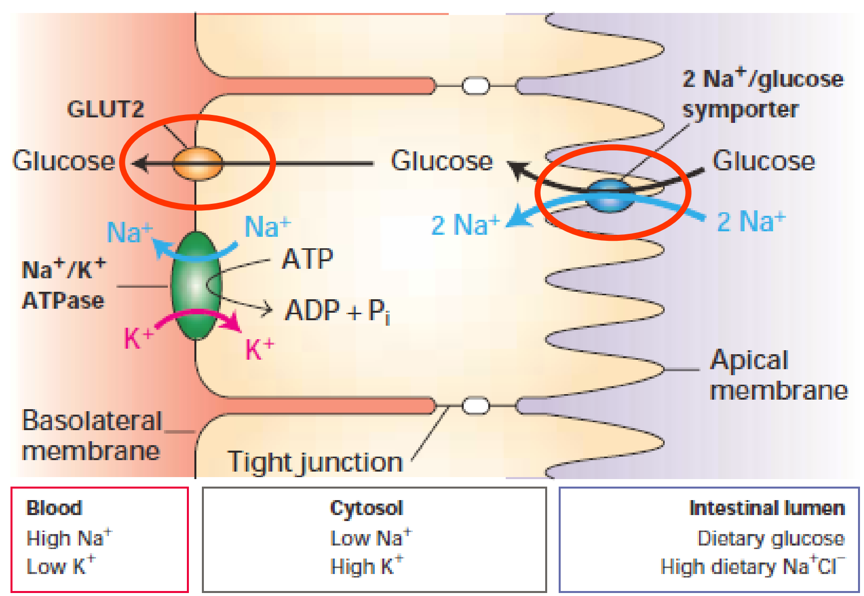
- prenos je specifičen (1 prenašalni protein za 1 tip molekul)
- prenos poteka samo na določenih mestih na membrani, ne pa po vsej površini (nasičenje – v_{max})

Na^+-simporterji vnašajo Glc in ak v celice

2 Na^+/Glc simporter

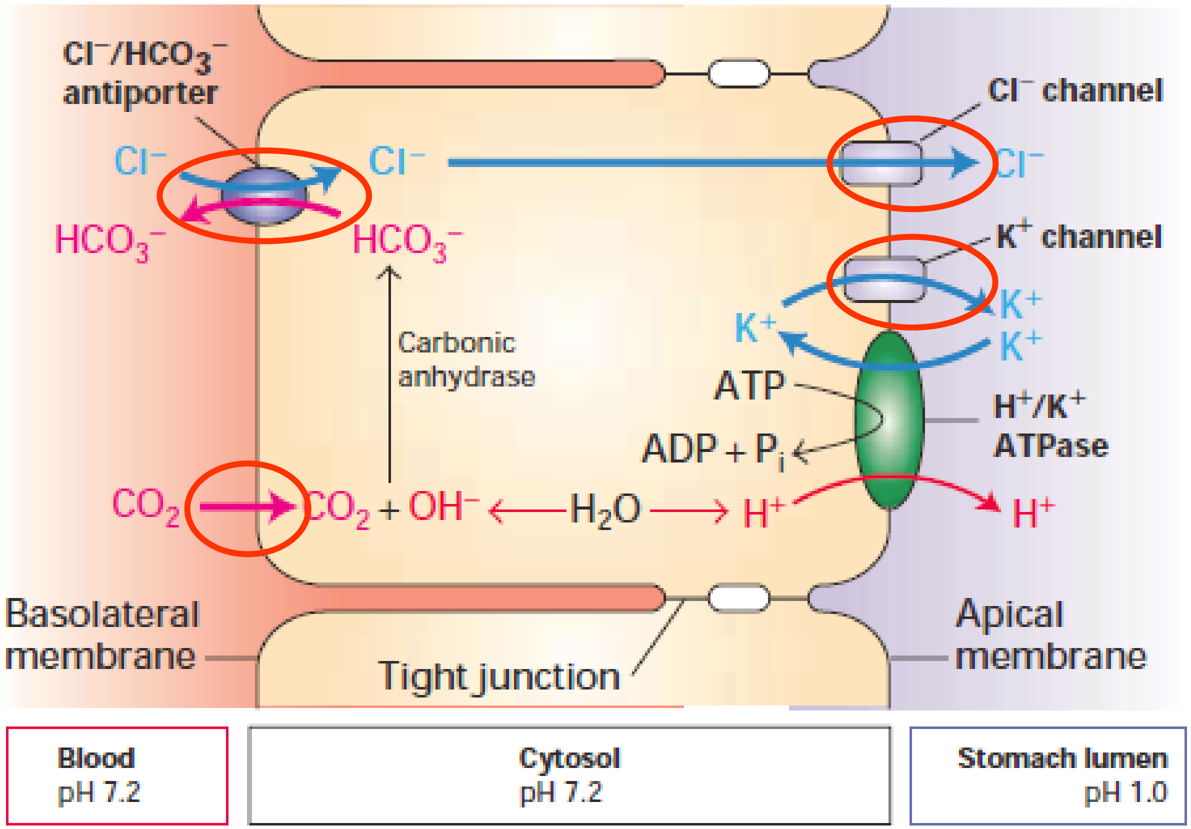
lumen črevesa, urin





Primer antiporterja

Cl^-/HCO_3^- antiporter



$[Cl^-] \approx 116$ mM	**$[Cl^-] \approx 4$ mM**
$[HCO_3^-] \approx 29$ mM	**$[HCO_3^-] \approx 12$ mM**

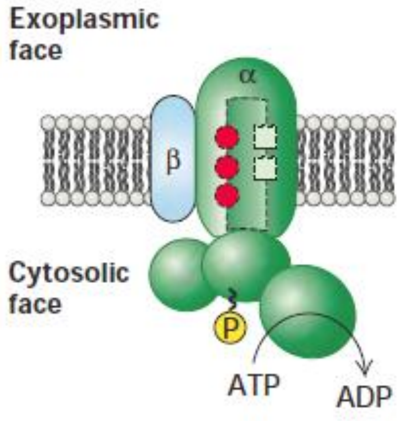
Kotransportni sistemi,
ki jih poganjajo gradienti Na^+ ali H^+

Organism or tissue	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transpor
E. coli	Lactose	H^+	Symport
	Proline	H^+	Symport
	Dicarboxylic acids	H^+	Symport
Intestine, kidney of vertebrates	Glucose	Na^+	Symport
	Amino acids	Na^+	Symport
Vertebrate cells (many types)	Ca^{2+}	Na^+	Antiport
Higher plants	K^+	H^+	Antiport
Fungi (*Neurospora*)	K^+	H^+	Antiport

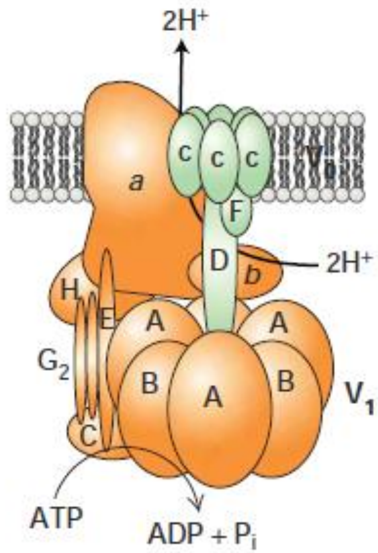
Aktivni transport

ATP-gnane črpalke

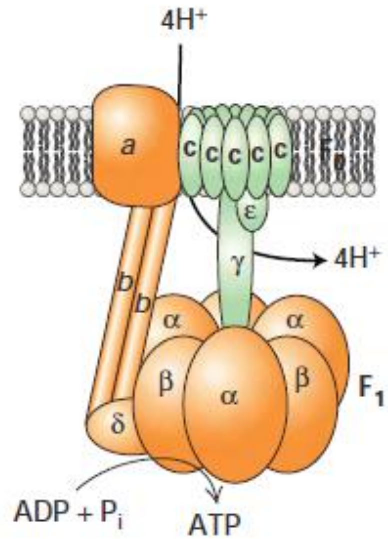
Štiri skupine ATP-gnanih črpalk



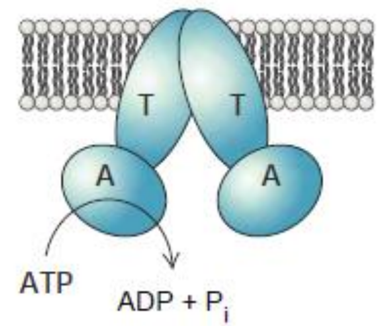
P-tip



V-tip

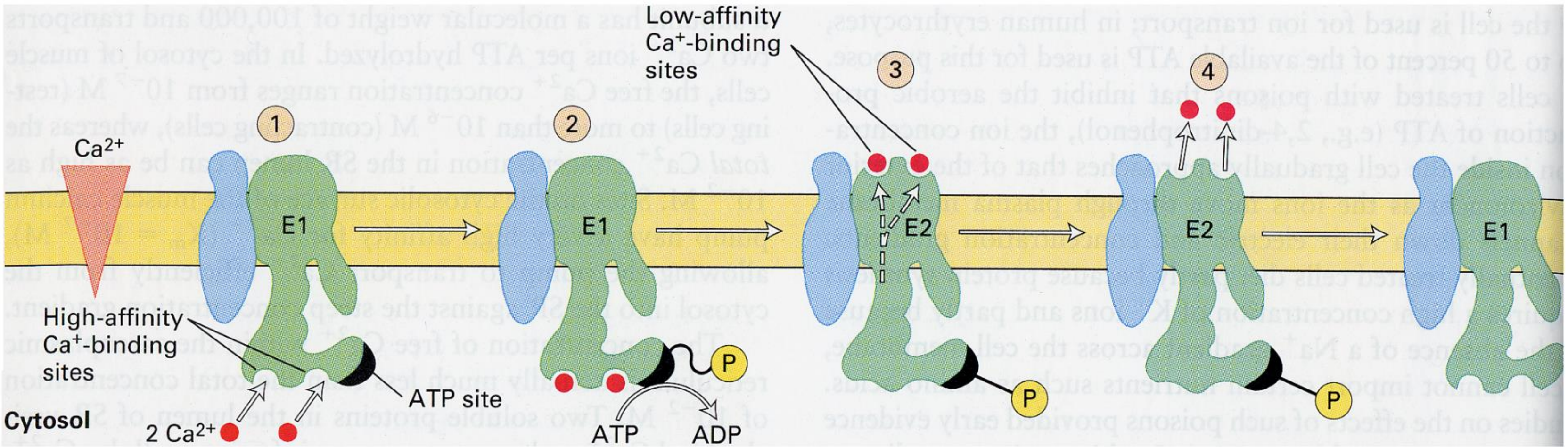


F-tip

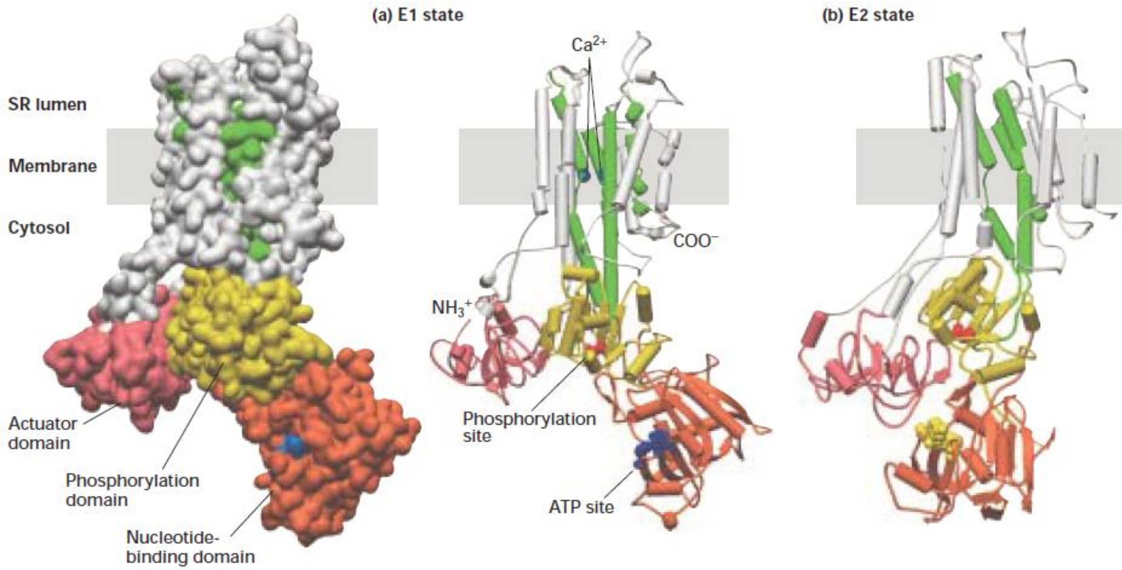


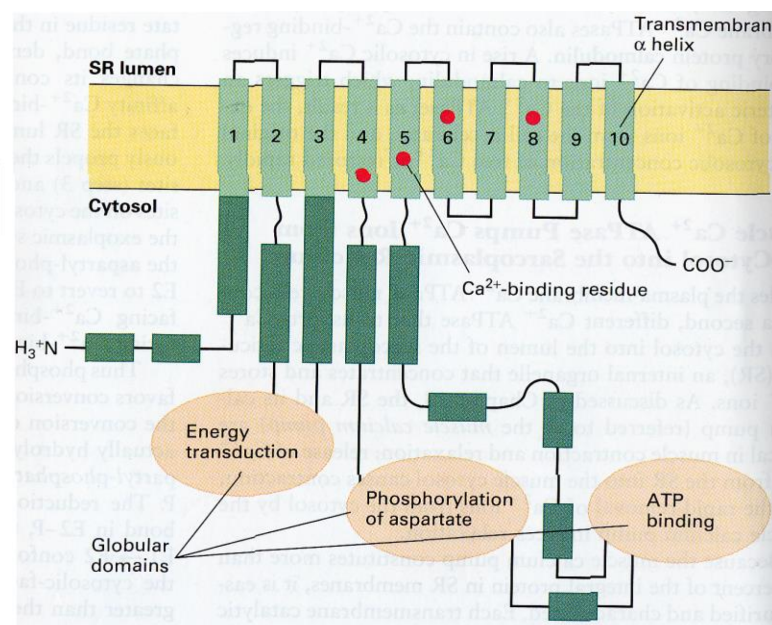
ABC transporterji

Mehanizem delovanja mišičnega tipa Ca^{2+} ATPaze (SERCA)

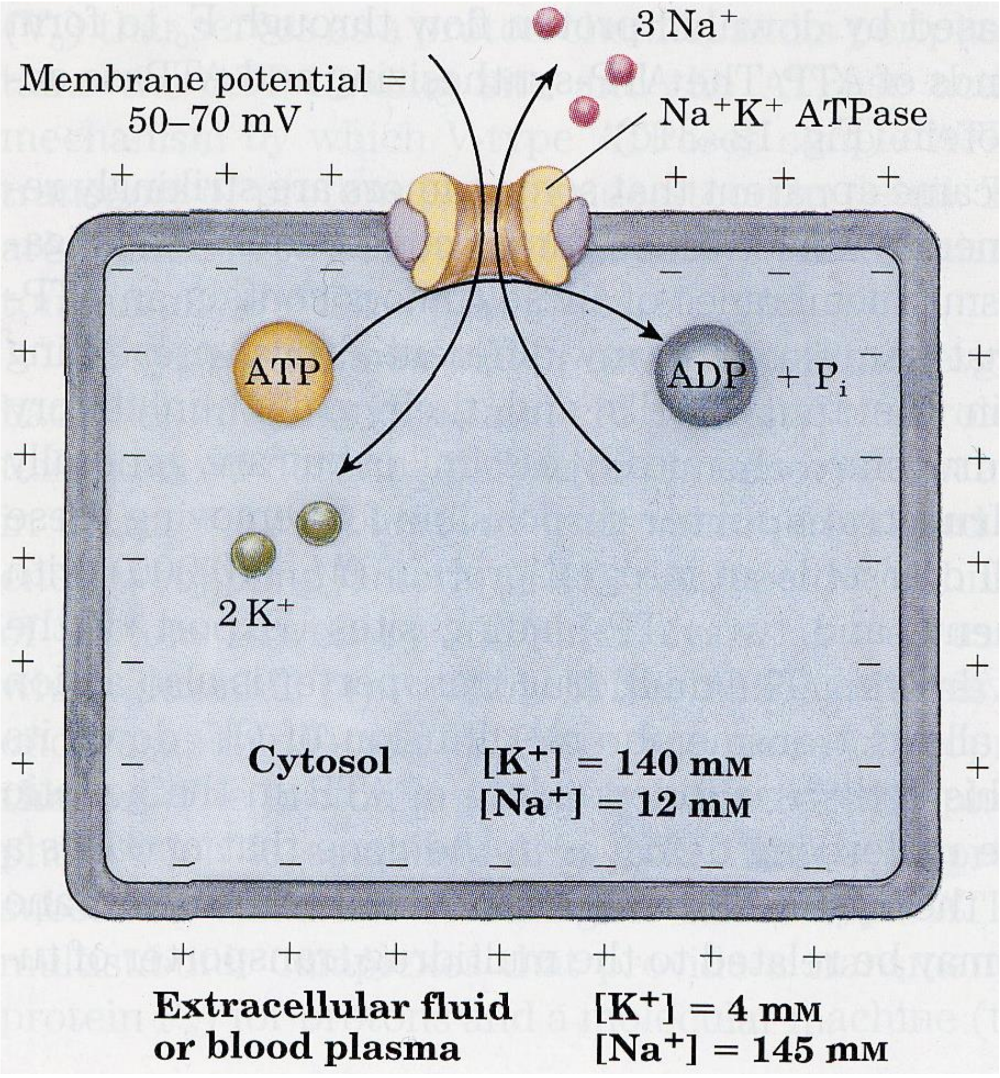


$K_m = 10^{-7}$ M

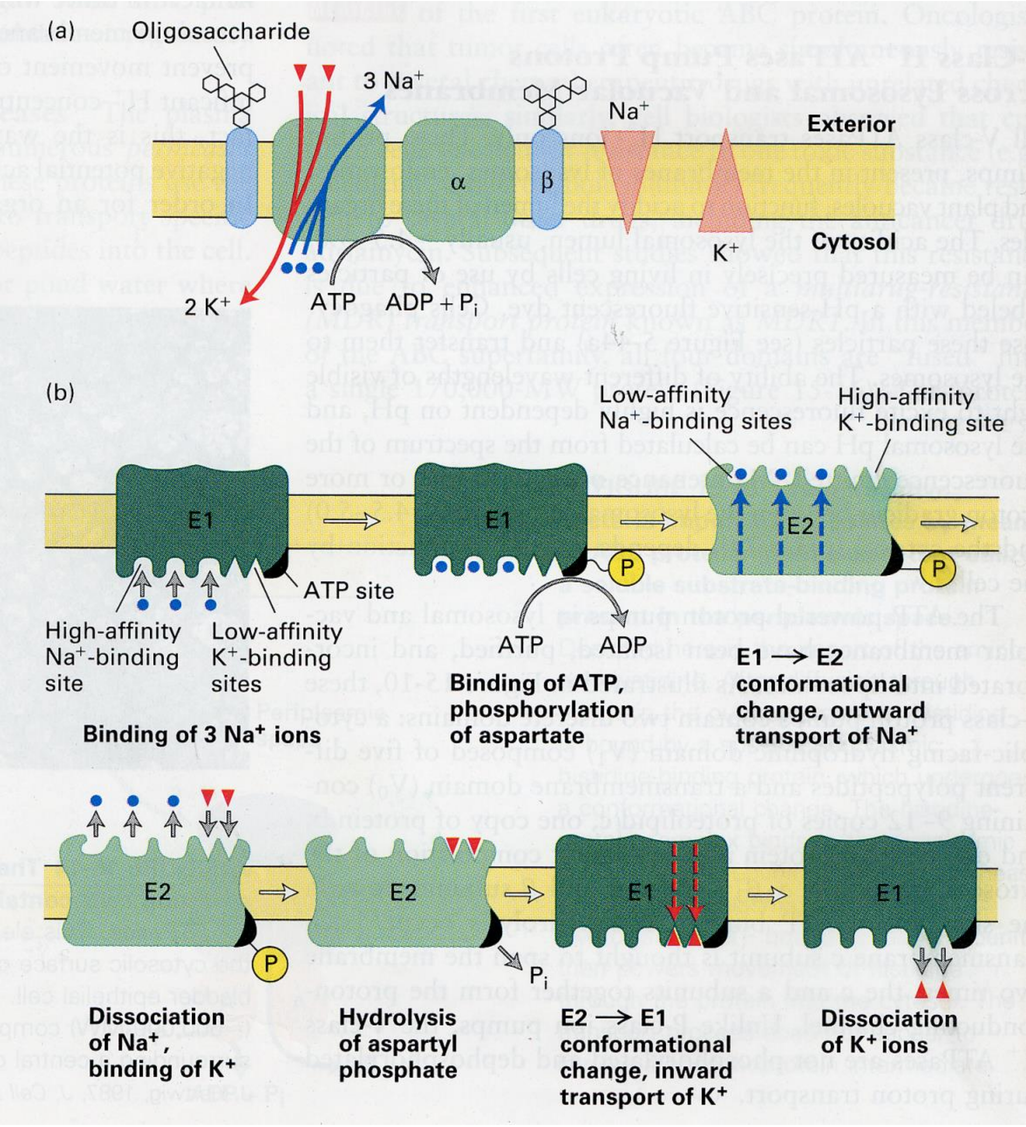




Na^+/K^+ ATPaza je najpomembnejša ionska črpalka za tvorbo električnega potenciala na PM živalskih celic



Model mehanizma delovanja Na^+/K^+ ATPaze



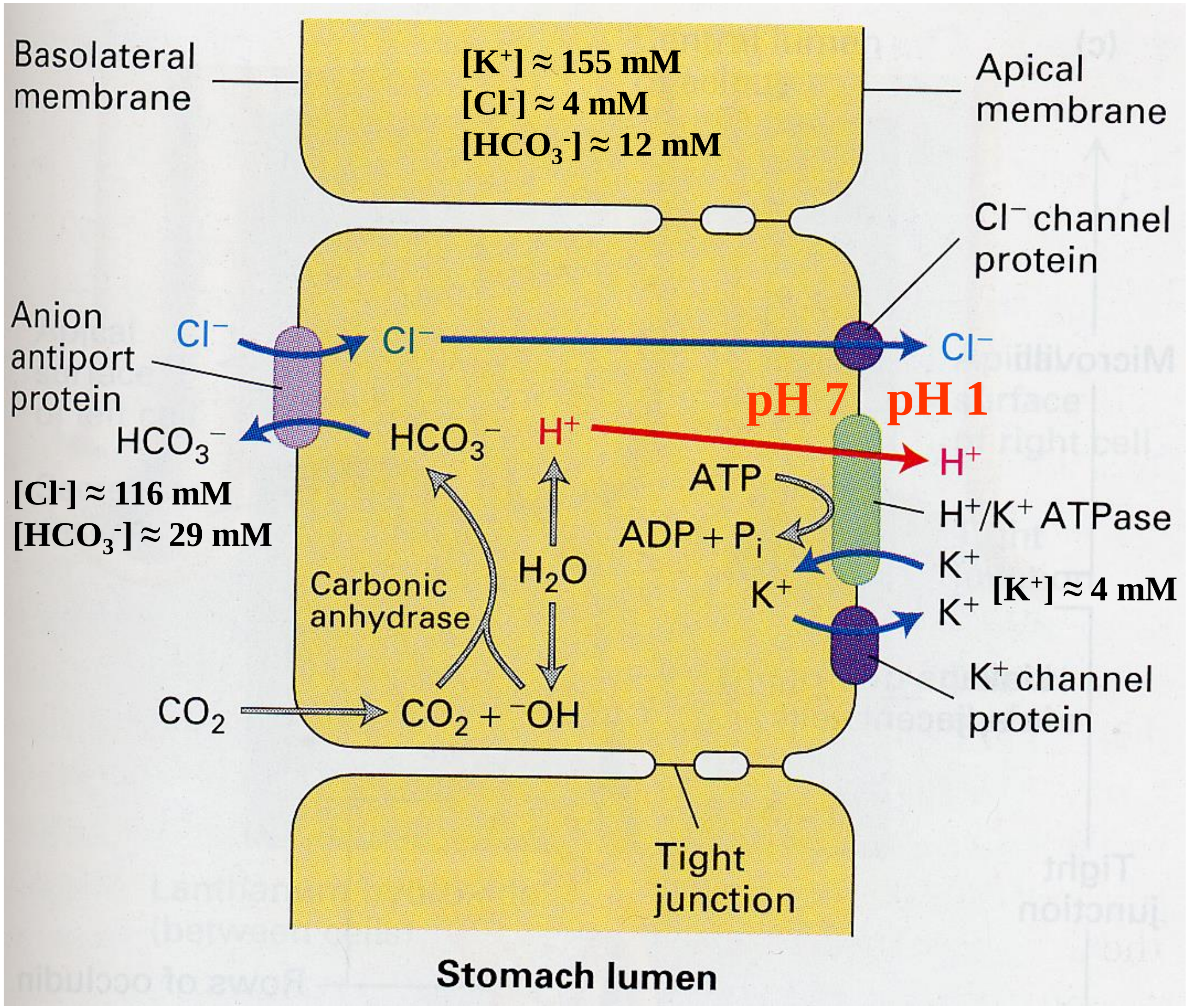
Zunajcelično:
$[Na^+] \approx 142$ mM
$[K^+] \approx 4$ mM

$K_{mK^+} = 0.2$ mM

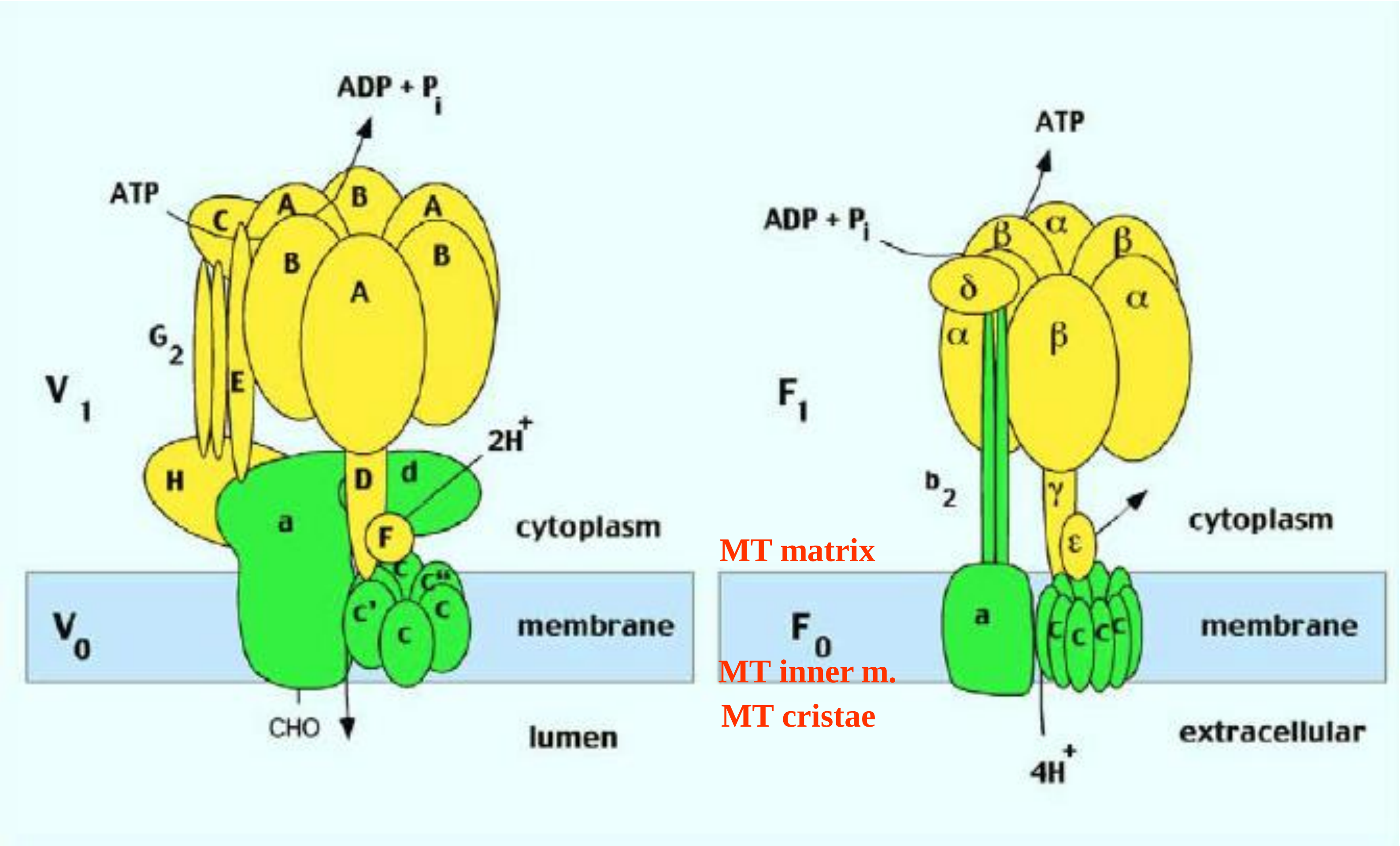
$K_{mNa^+} = 0.6$ mM

Znotrajcelično:
$[Na^+] \approx 10$ mM
$[K^+] \approx 155$ mM

Kisanje lumna želodca z delovanjem P-tip H^+/K^+ ATPaze v apikalni m. parietalnih celic



Strukturna primerjava V- in F-tipa ATPaz



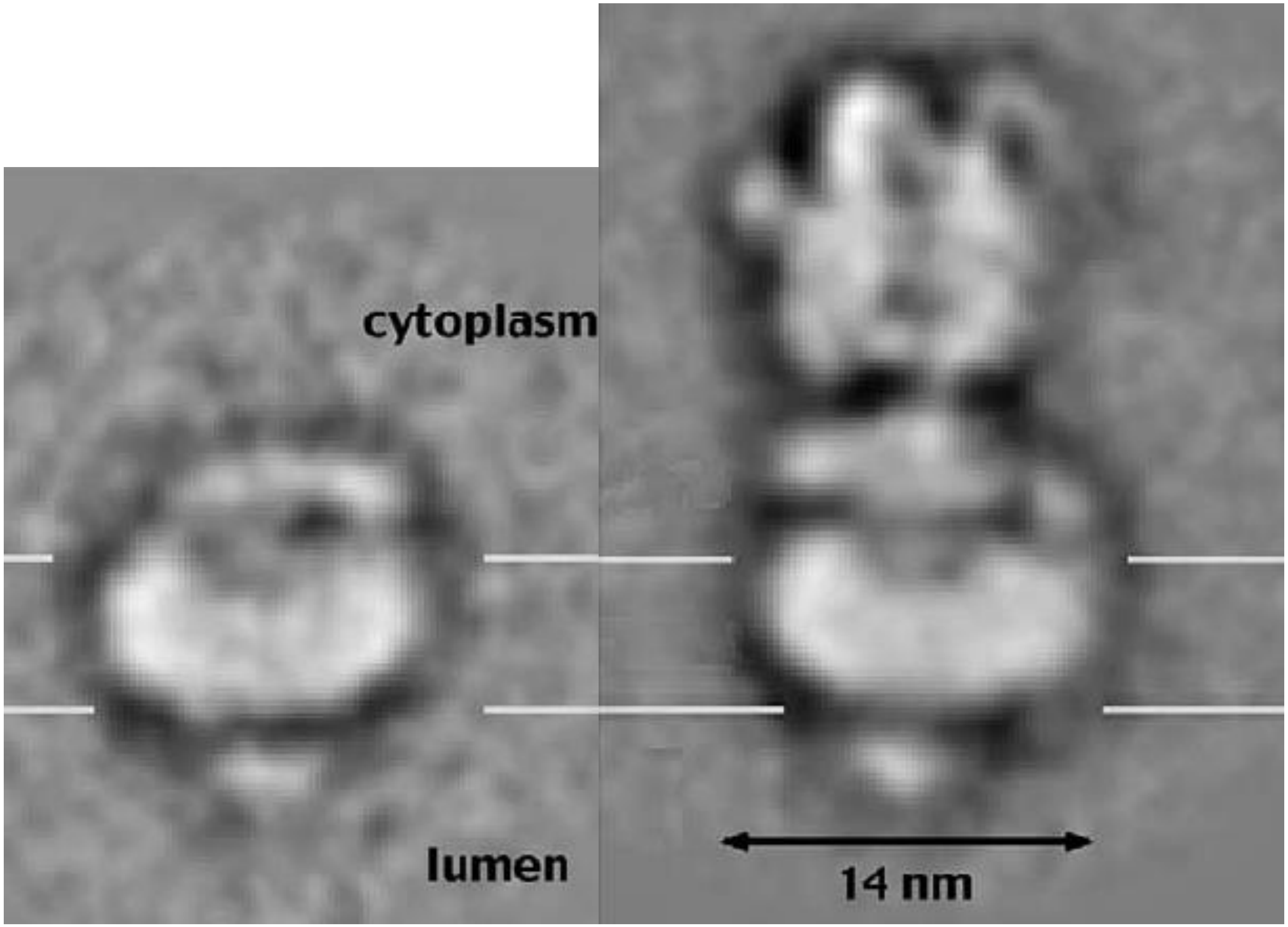
Kawasaki-Nishi, S. *et al.* (2003) FEBS Lett. 545, 76-85

Lastnosti podenot V-ATPaze

Subunit	Molecular mass (kDa)	Yeast gene	Mammalian isoforms* (tissue/cell)	Subunit function
V_1 domain				
A	70	*VMA1*		ATP hydrolytic site, regulation via non-homologous domain, stator subunit
B	60	*VMA2*	B1(renal, epididymis); B2 (ubiquitous)	Non-catalytic ATP site, binds actin and aldolase, stator subunit
C	40	*VMA5*	C1(ubiquitous); C2a,b (lung, renal, epididymis)	Regulatory, stator subunit, binds actin
D	34	*VMA8*		Rotary subunit
E	33	*VMA4*	E1(testis); E2 (ubiquitous)	Stator subunit, binds RAVE and aldolase
F	14	*VMA7*		Rotary subunit
G	13	*VMA10*	G1 (ubiquitous); G2 (neural); G3 (renal, epididymis)	Stator subunit, binds RAVE
H	50	*VMA13*	Two alternatively spliced variants	Regulatory, stator subunit, binds NEF
V_0 domain				
a	100	*VPH1* (vacuole); *STV1* (Golgi)	a1 (neural); a2 (endothelial); a3 (osteoclasts); a4 (renal, epididymis)	H^+ transport, targeting, binds aldolase, stator subunit
d	38	*VMA6*	d1 (ubiquitous); d2 (renal, epididymis)	Coupling, rotary subunit
e	9	*VMA9*		Unknown
c	17	*VMA3*		H^+ transport, rotary subunit
c′	17	*VMA11*	No mammalian gene	H^+ transport, binds Vma21 assembly factor, rotary subunit
c″	21	*VMA16*		H^+ transport, rotary subunit
Ac45	45	No yeast gene		Unknown

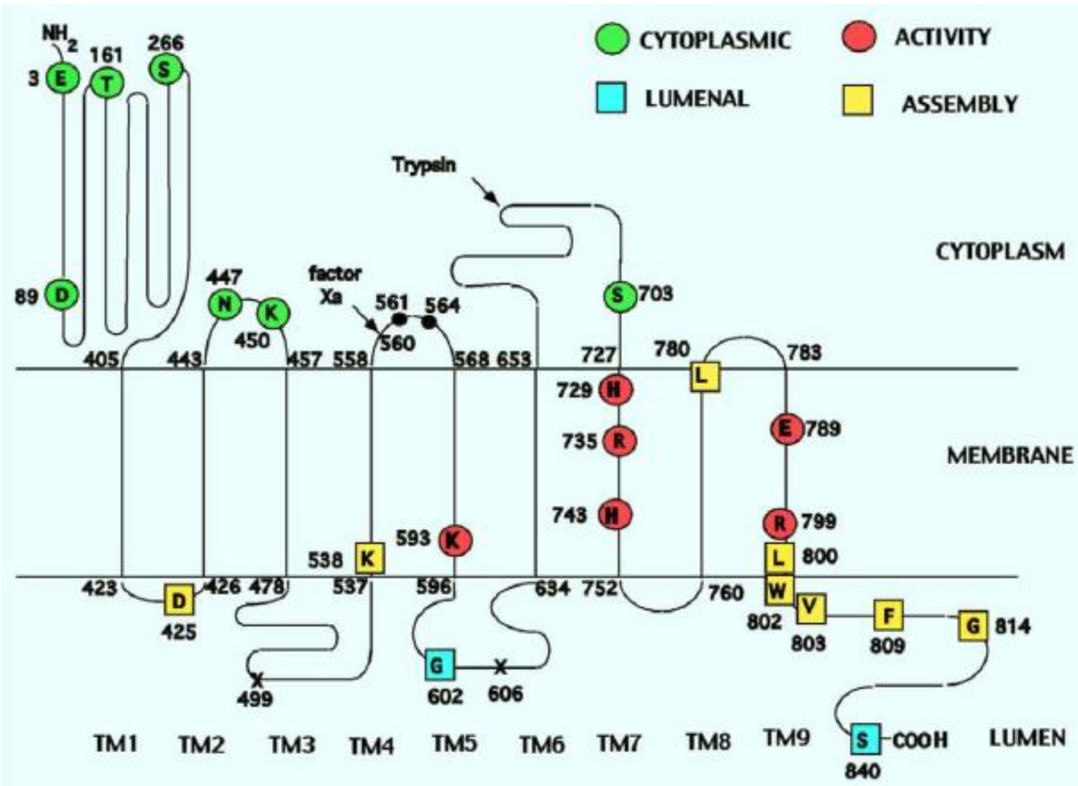
Forgac, M. (2007) Nature Rev. MCB 8, 917-929

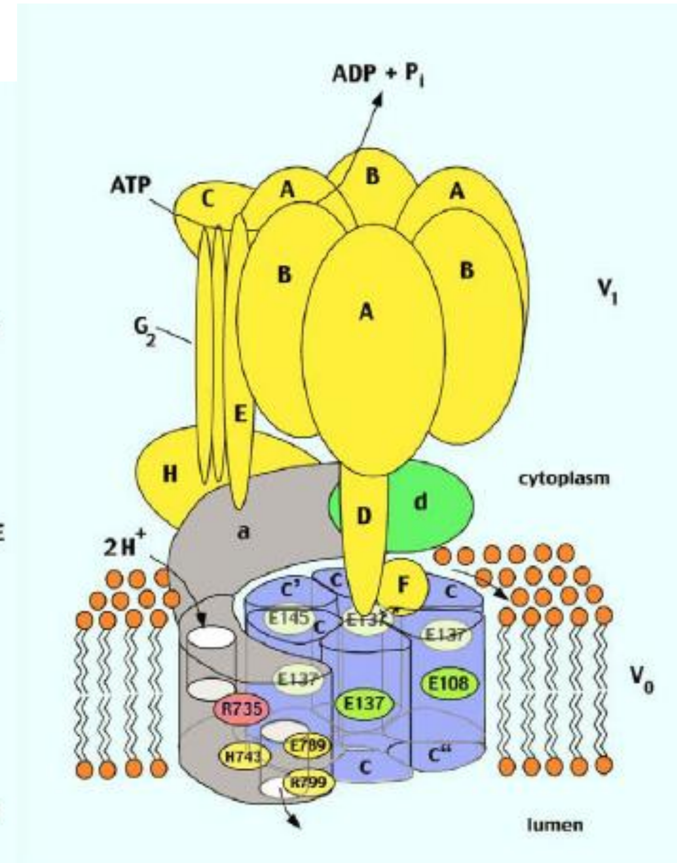
V_0 sektor in V_1V_0 kompleks goveje možganske V-tip ATP-aze pod elektronskim mikroskopom



Kawasaki-Nishi, S. *et al.* (2003) FEBS Lett. 545, 76-85

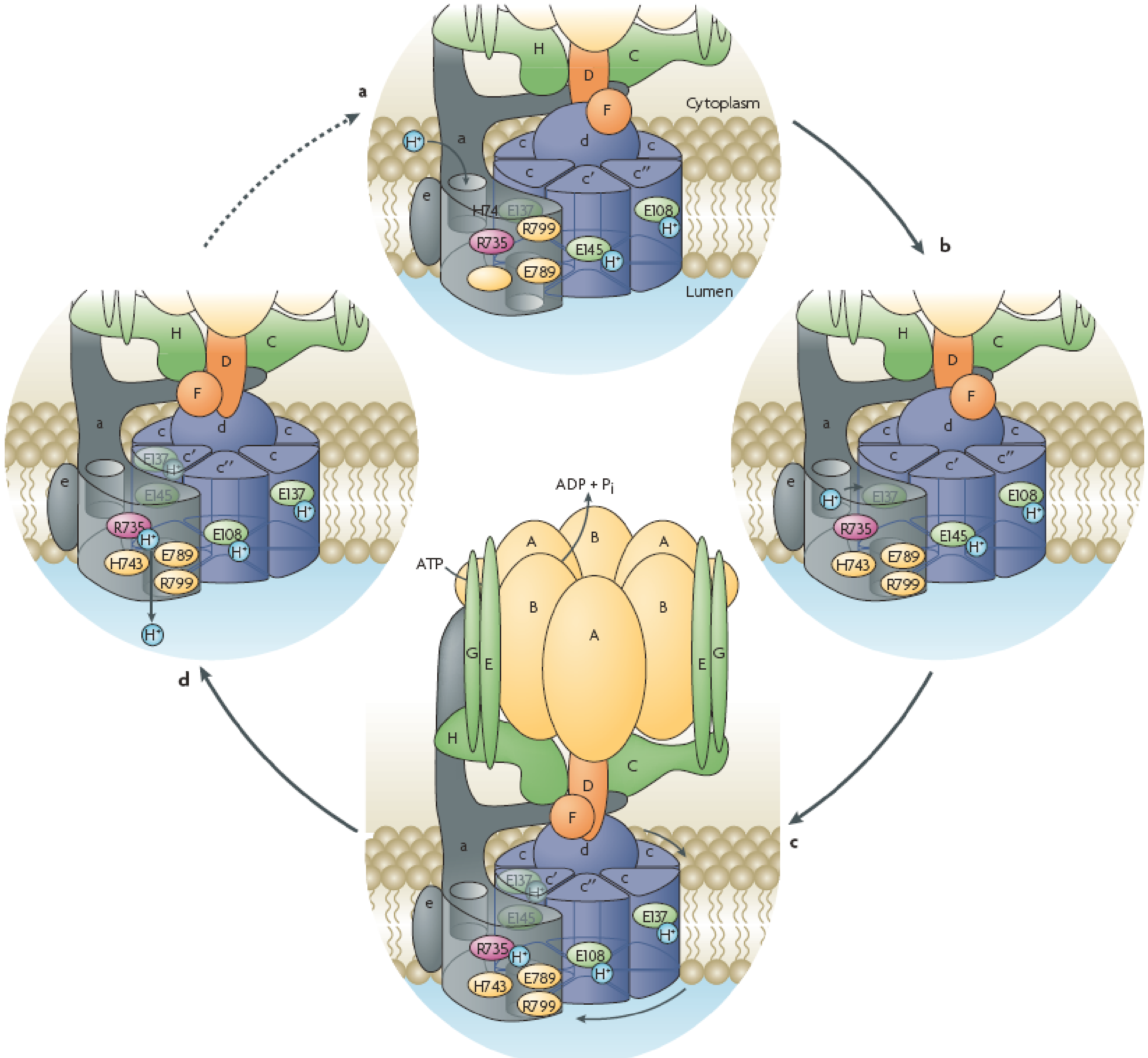
Struktura in funkcija V_o domene





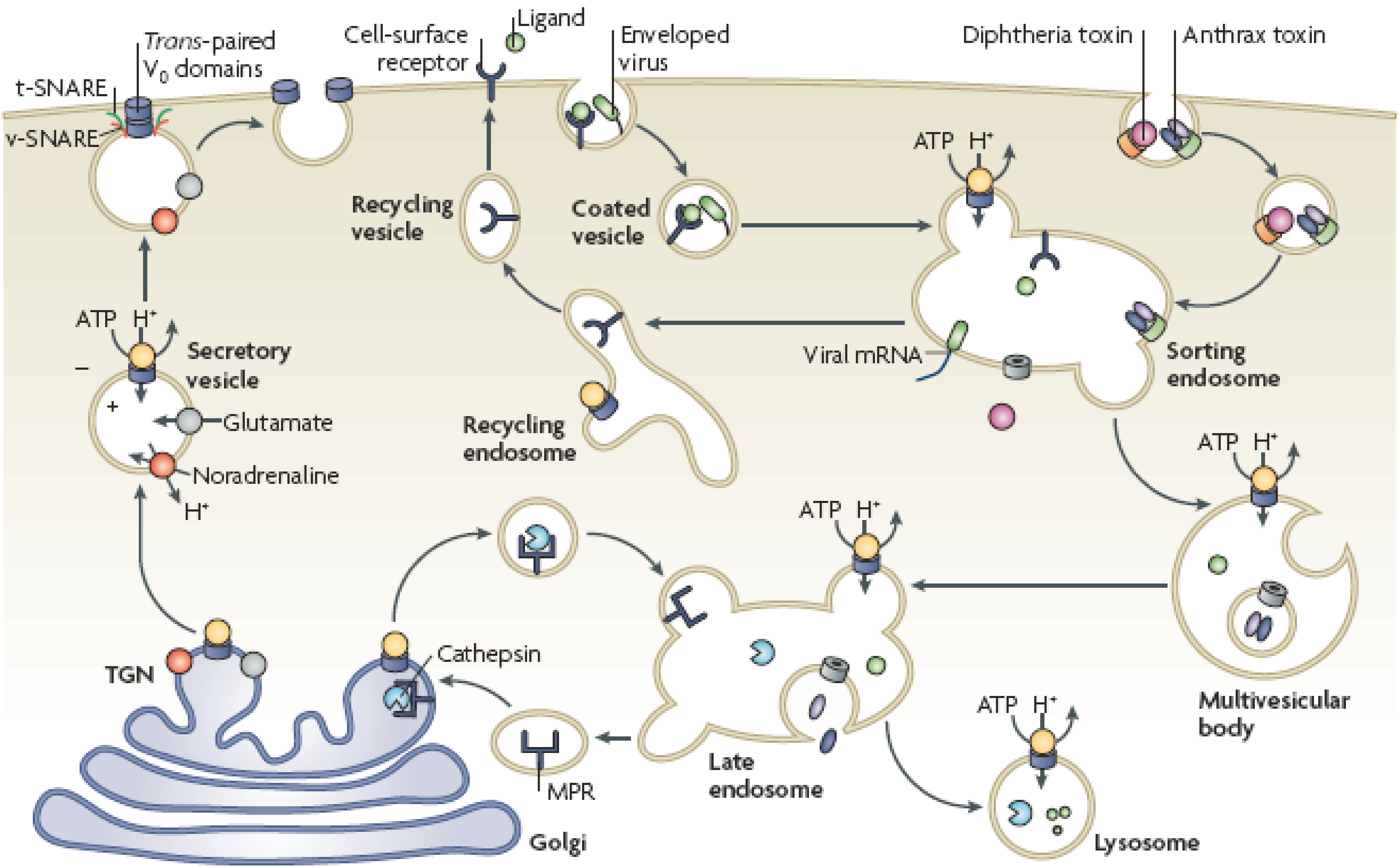
Kawasaki-Nishi, S. *et al.* (2003) FEBS Lett. 545, 76-85

Mehanizem transporta protonov



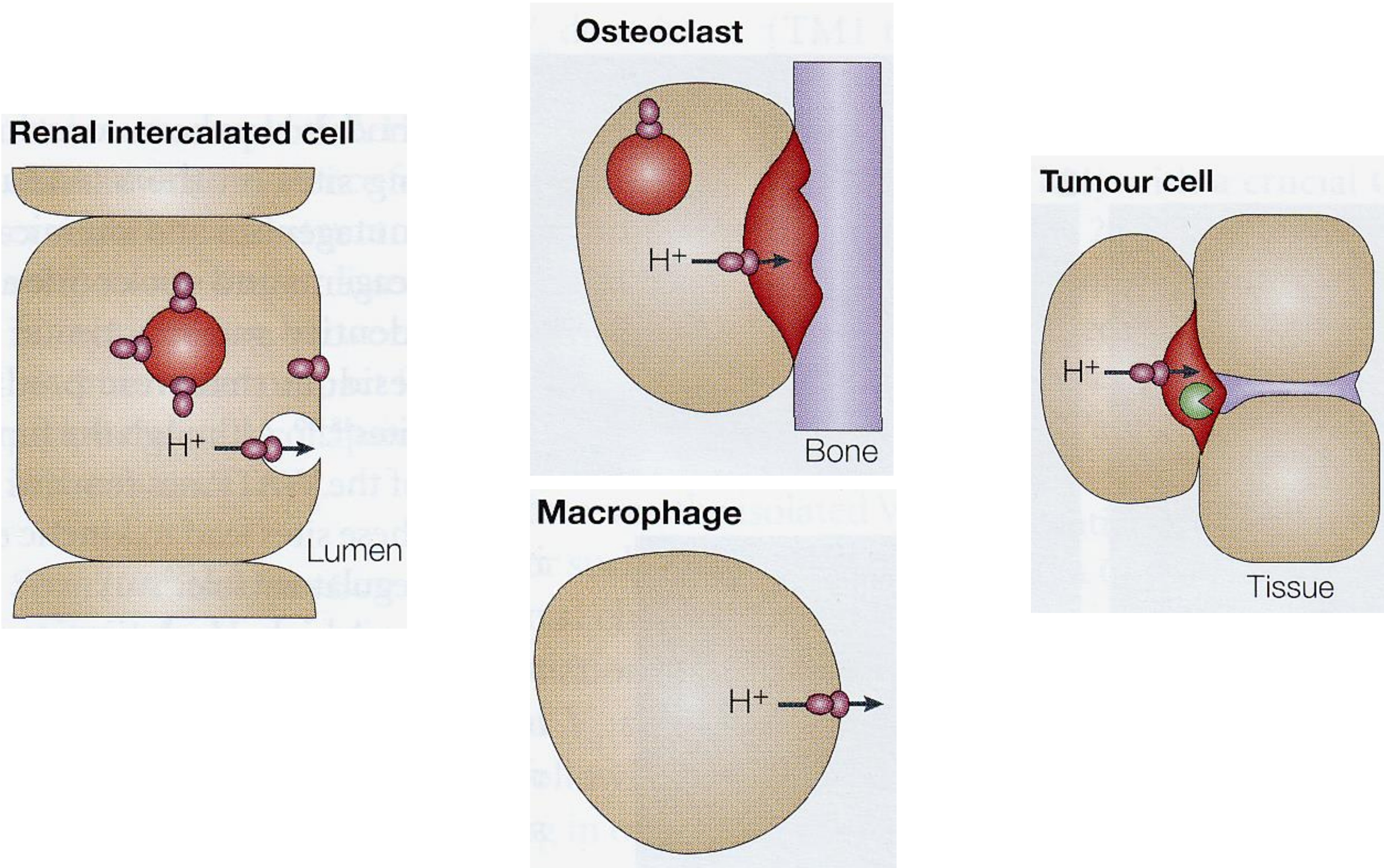
Forgac, M. (2007) Nature Rev. MCB 8, 917-929

Funkcije V-ATPaze v ekso/endocitozi in pri znotrajceličnem transportu



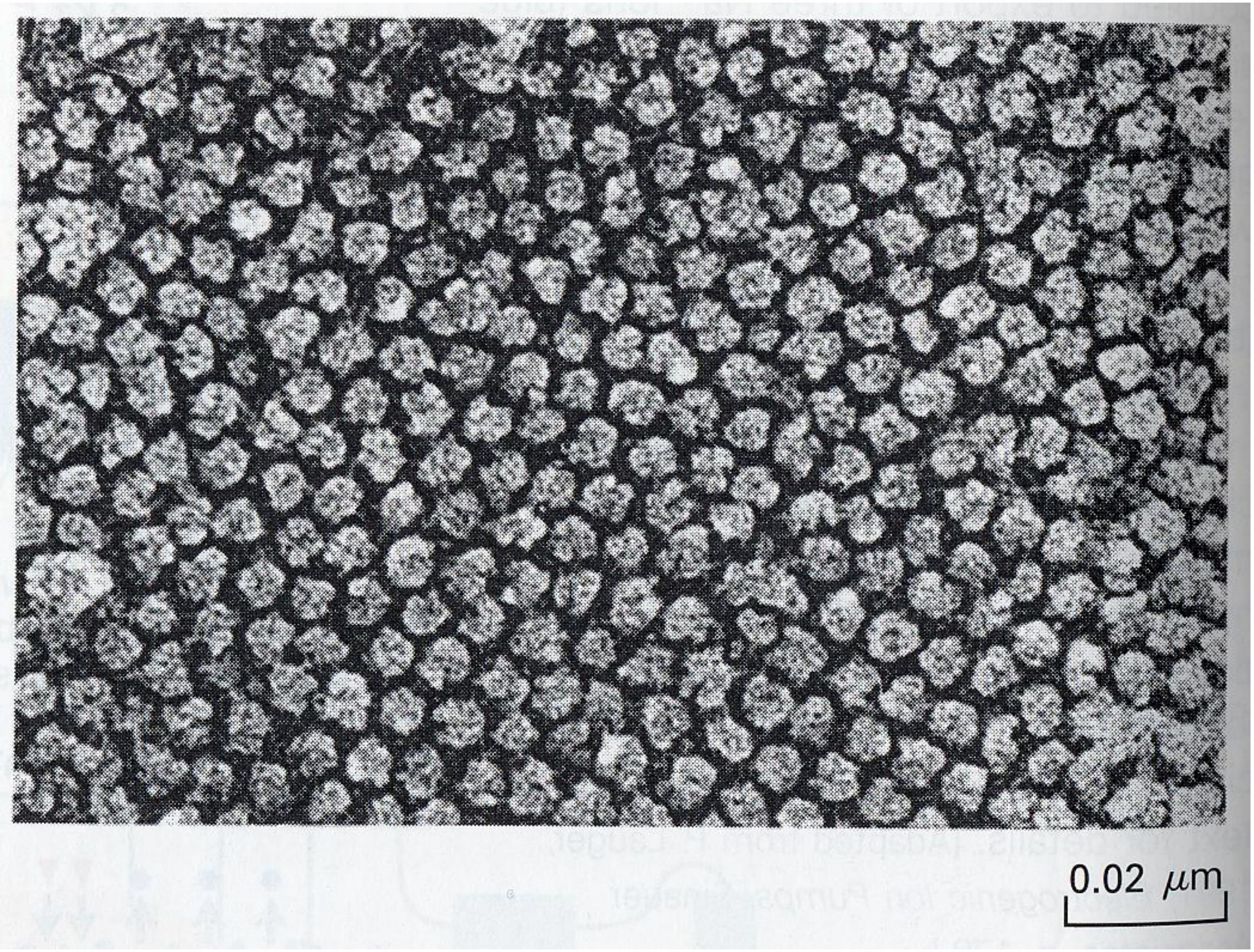
Forgac, M. (2007) Nature Rev. MCB 8, 917-929

Funkcije V-ATPaze v plazemski membrani

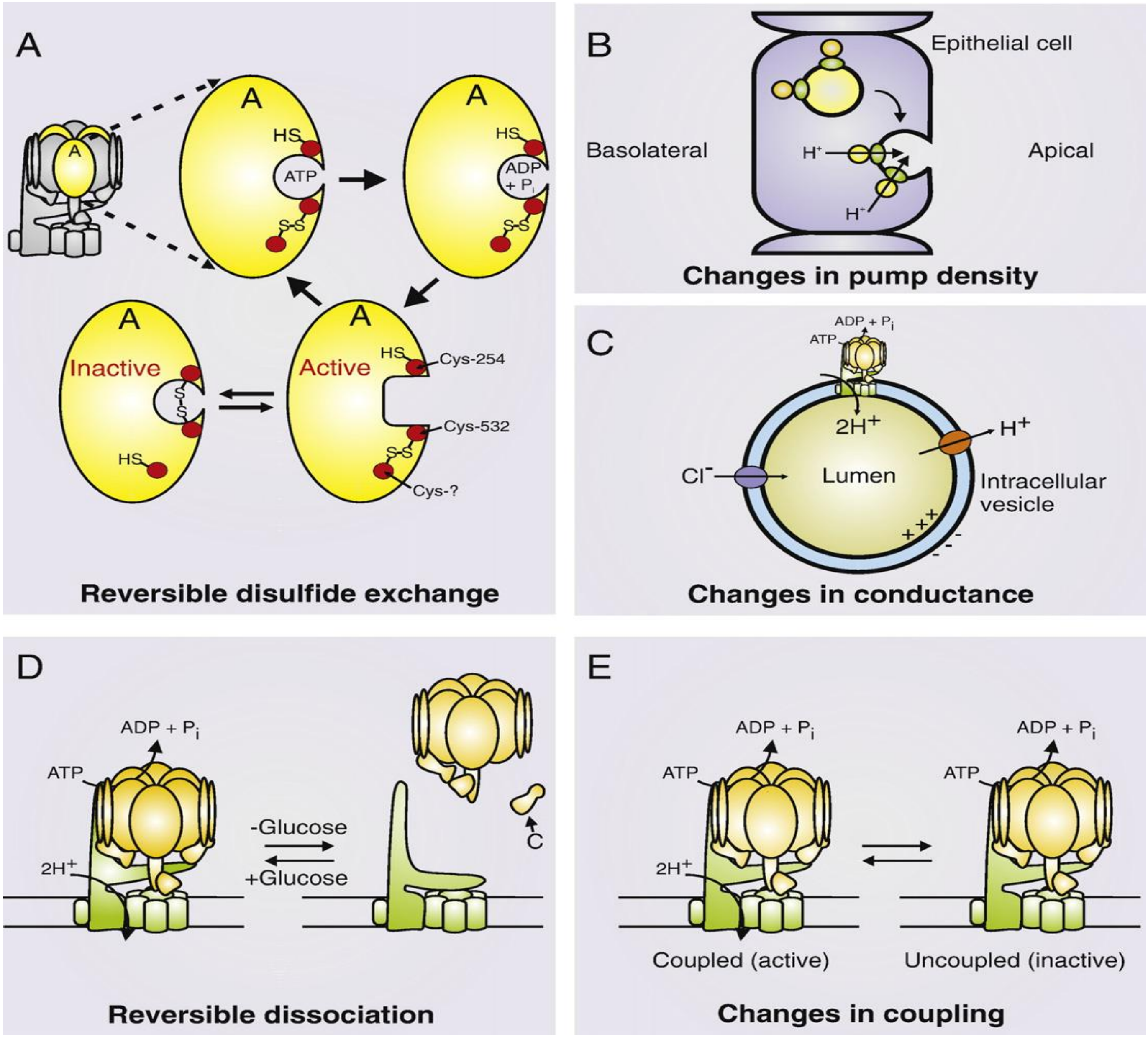


Nishi, T. and Forgac, M. (2002) Nat. Rev. Mol. Cell Biol. 3, 94-103

V-class ATPases in toad bladder epithelial cells



Regulacija aktivnosti V-tip ATPaze



Cipriano, D.J. et al. (2008) BBA 1777, 599-604

Kotransportni sistemi,
ki jih poganjajo gradienti Na^{+} ali H^{+}

Organism or tissue	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transpor
E. coli	Lactose	H^{+}	Symport
	Proline	H^{+}	Symport
	Dicarboxylic acids	H^{+}	Symport
Intestine, kidney of vertebrates	Glucose	Na^{+}	Symport
	Amino acids	Na^{+}	Symport
Vertebrate cells (many types)	Ca^{2+}	Na^{+}	Antiport
Higher plants	K^{+}	H^{+}	Antiport
Fungi (*Neurospora*)	K^{+}	H^{+}	Antiport

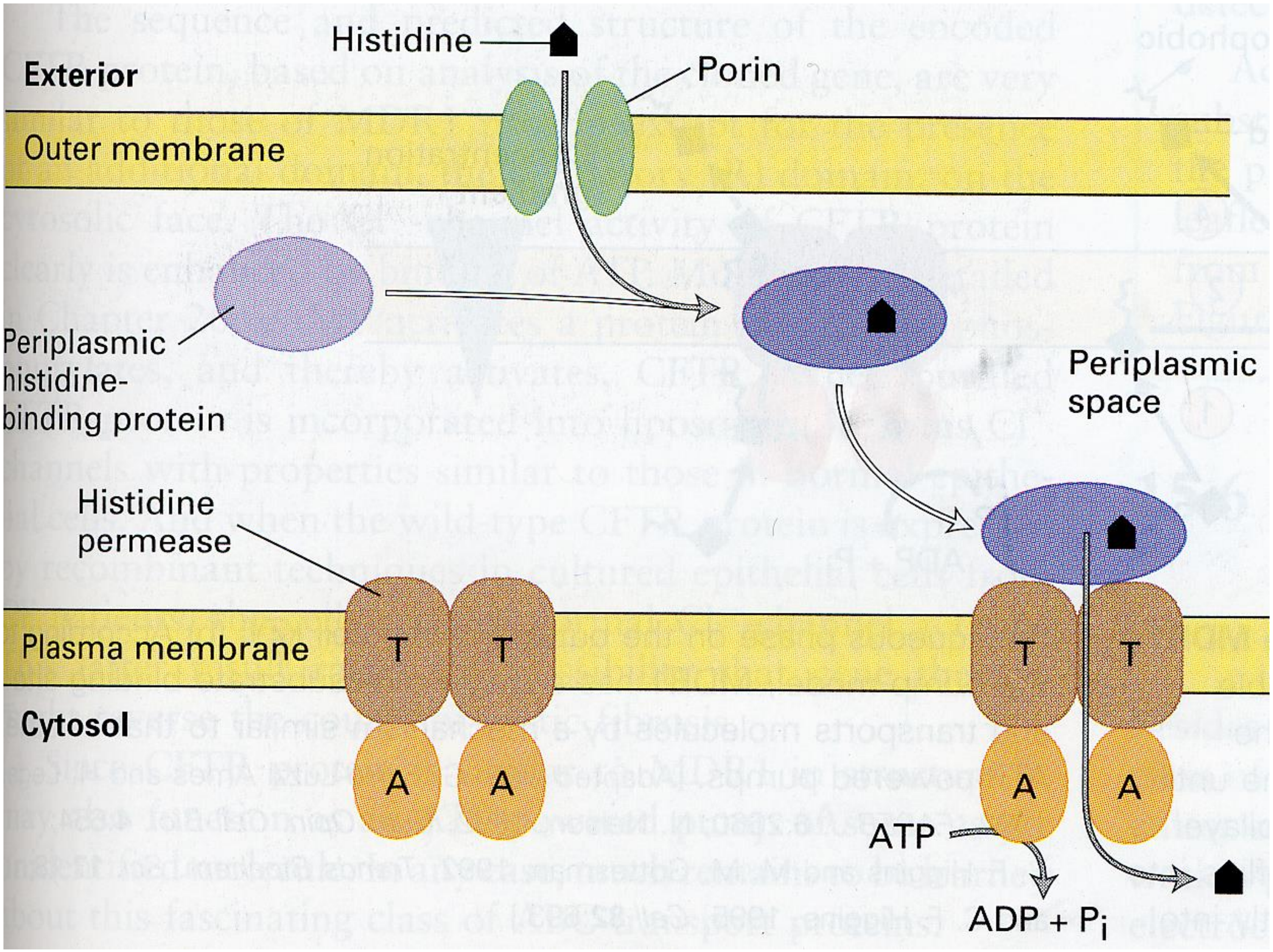
ABC transporterji

- Permeaze v plazemski membrani bakterij

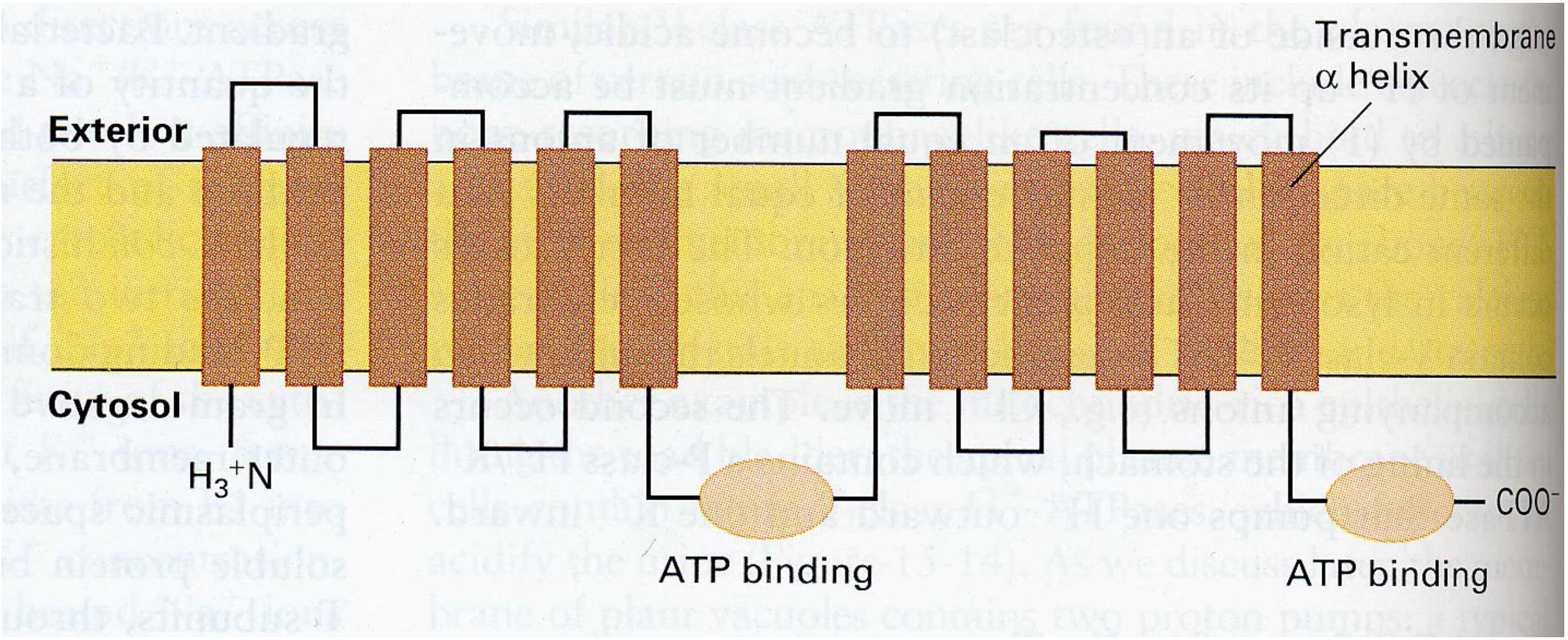
- Multidrug-resistance proteini (MDR1, MDR2)

- Cystic fibrosis transmembrane regulator (CFTR) protein

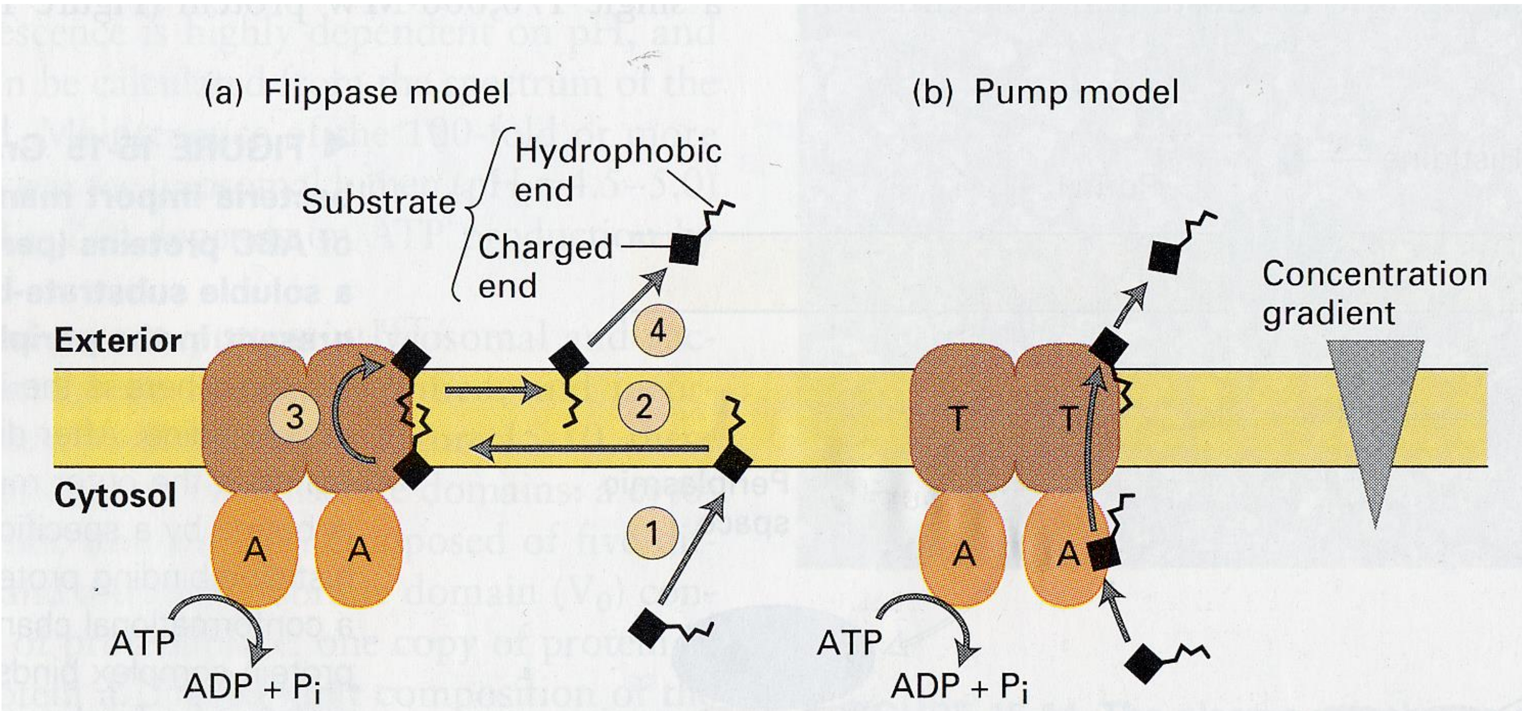
PM številnih bakterij vsebuje različne ABC transporteje - permeaze



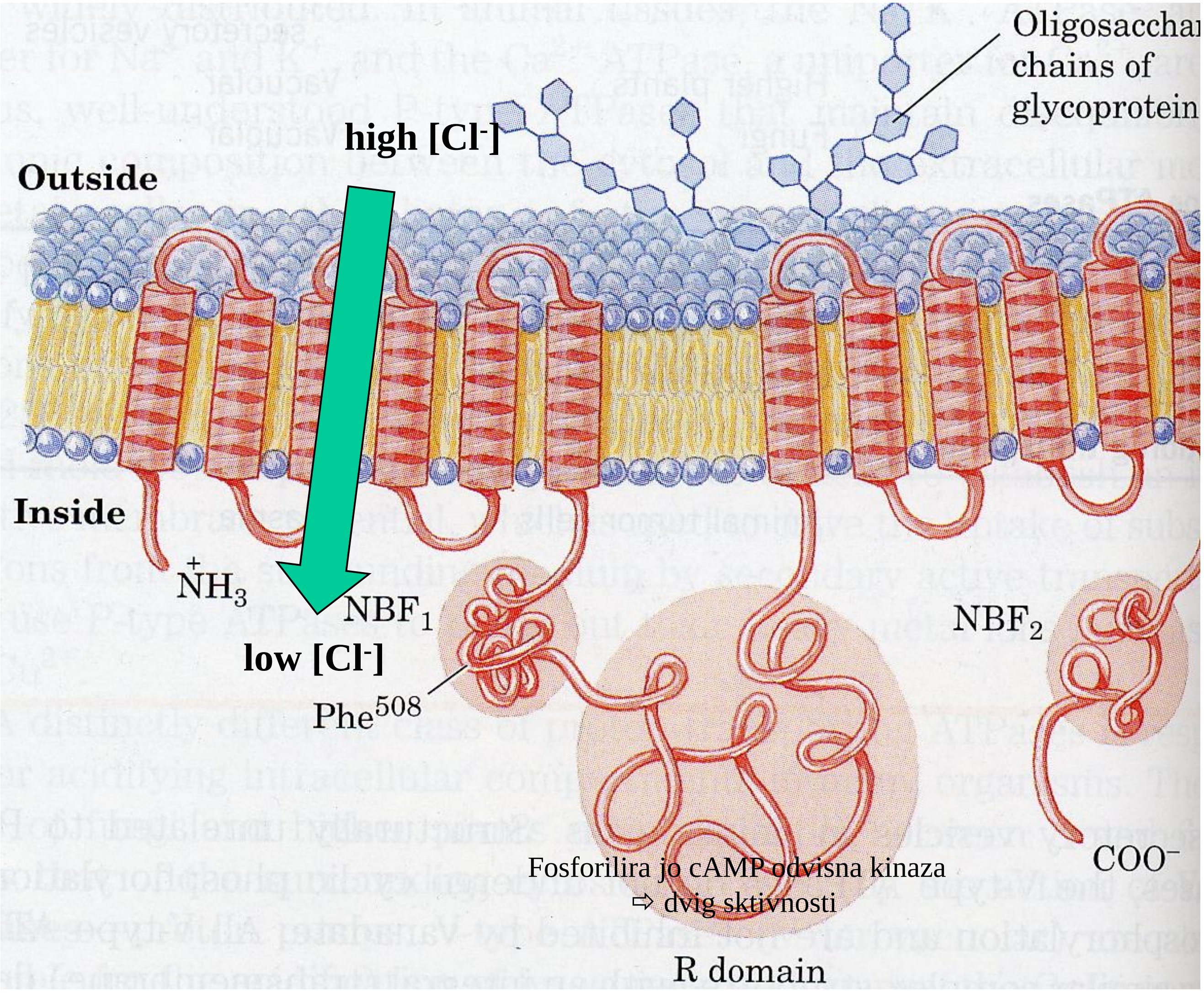
Shematski strukturni model sesalskega multidrug-resistance (MDR) transporterja - - splošni strukturni model ABC transporterjev



Alternativni možnosti delovanja MDR transporterjev



Cystic fibrosis transmembrane-conductance regulator (CFTR)
Protein je Cl^- kanalček s strukturo ABC transporterja



Povzetek – aktivni transport

	Organism or tissue	Type of membrane	Role of ATPase
P-type ATPases			
Na^+K^+	Animal tissues	Plasma	Maintains low $[Na^+]$, high $[K^+]$ inside cell; creates transmembrane electrical potential
H^+K^+	Acid-secreting (parietal) cells of mammals	Plasma	Acidifies contents of stomach
H^+	Fungi *(Neurospora)*	Plasma	Create H^+ gradient to drive secondary transport of extracellular solutes into cell
H^+	Higher plants	Plasma	
Ca^{2+}	Animal tissues	Plasma	Maintains low $[Ca^{2+}]$ in cytosol
Ca^{2+}	Myocytes of animals	Sarcoplasmic reticulum (endoplasmic reticulum)	Sequesters intracellular Ca^{2+}, keeping cytosolic $[Ca^{2+}]$ low
Cd^{2+}, Hg^{2+}, Cu^{2+}	Bacteria	Plasma	Pumps heavy metal ions out of cell
V-type ATPases			
H^+	Animals	Lysosomal, endosomal, secretory vesicles	Create low pH in compartment, activating proteases and other hydrolytic enzymes
H^+	Higher plants	Vacuolar	
H^+	Fungi	Vacuolar	
F-type ATPases			
H^+	Eukaryotes	Inner mitochondrial	Catalyze formation of ATP from ADP + P_i
H^+	Higher plants	Thylakoid	
H^+	Prokaryotes	Plasma	
Multidrug transporter			
	Animal tumor cells	Plasma	Removes a wide variety of hydrophobic natural products and synthetic drugs from cytosol, including vinblastine, doxorubicin, actinomycin D, mitomycin, taxol, colchicine, and puromycin

Povzetek – transport čez BM

TABLE 7-1 Mechanisms for Transporting Ions and Small Molecules Across Cell Membranes

	Transport Mechanism			
Property	**Passive Diffusion**	**Facilitated Diffusion**	**Active Transport**	**Cotransport***
Requires specific protein	–	+	+	+
Solute transported against its gradient	–	–	+	+
Coupled to ATP hydrolysis	–	–	+	–
Driven by movement of a cotransported ion down its gradient	–	–	–	+
Examples of molecules transported	O_2, CO_2, steroid hormones, many drugs	Glucose and amino acids (uniporters); ions and water (channels)	Ions, small hydrophilic molecules, lipids (ATP-powered pumps)	Glucose and amino acids (symporters); various ions and sucrose (antiporters)

*Also called *secondary active transport.*