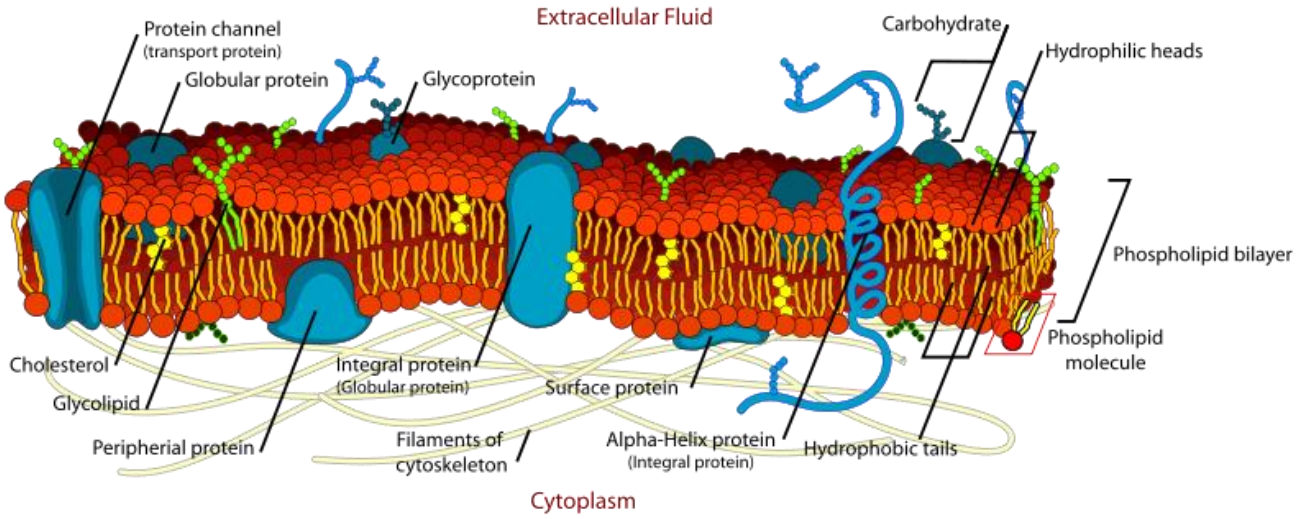
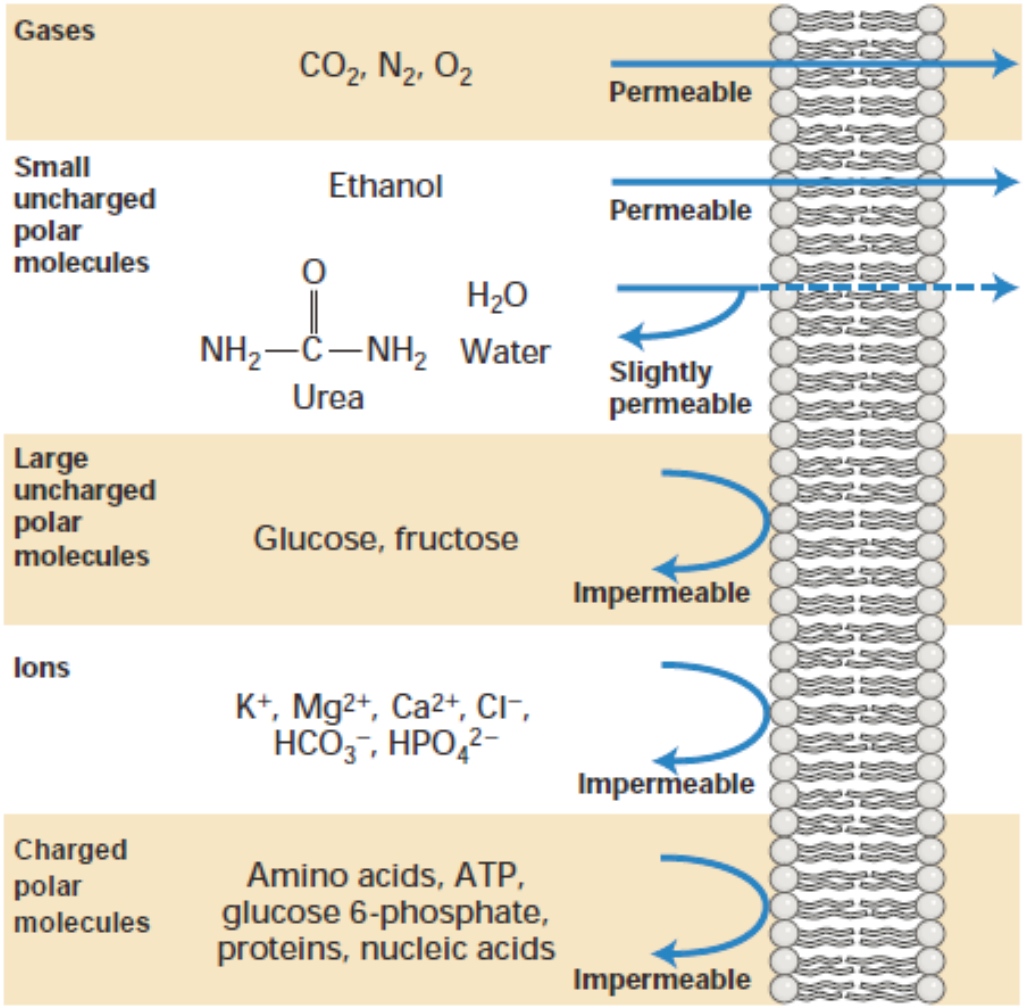
Transport ionov in malih molekul čez lipidne membrane

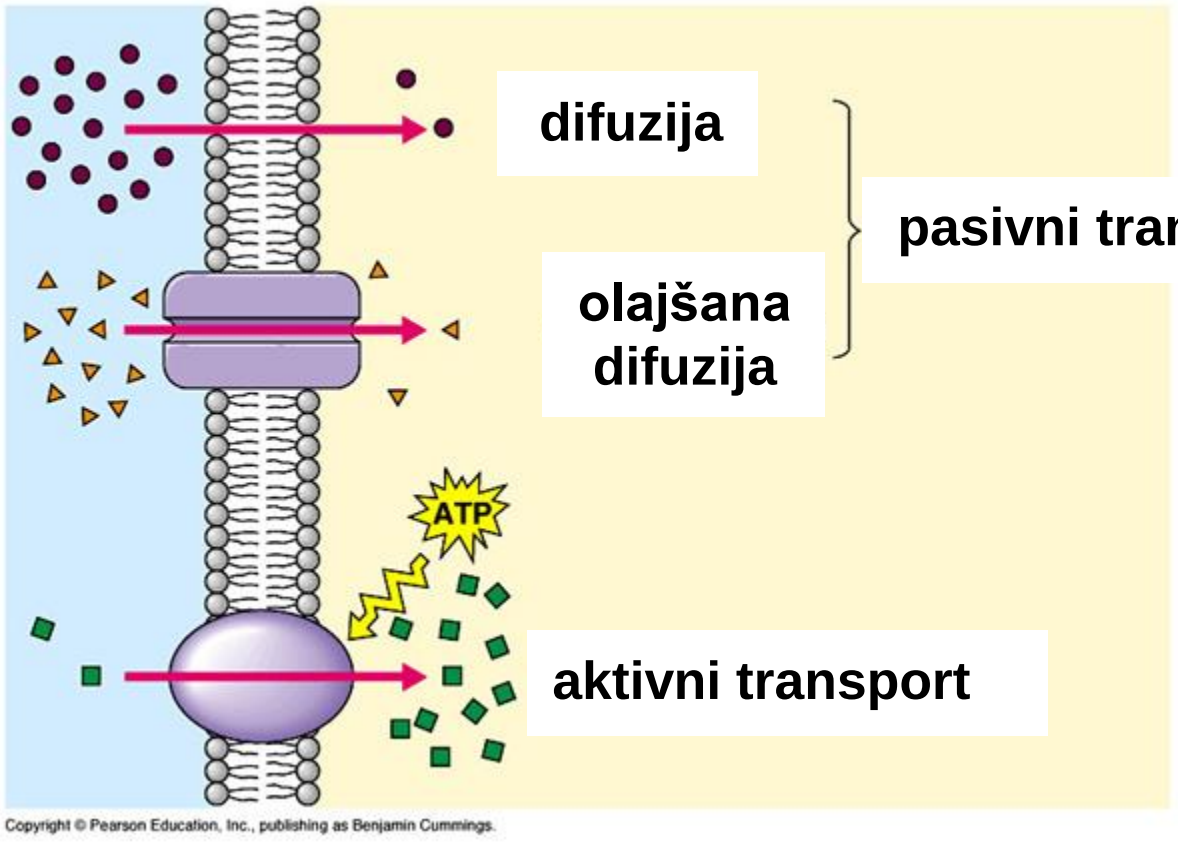


Lodish 8. izdaja, 11. poglavje

Relativna propustnost fosfolipidnega dvosloja za različne molekule



Načini transporta snovi preko celičnih membran



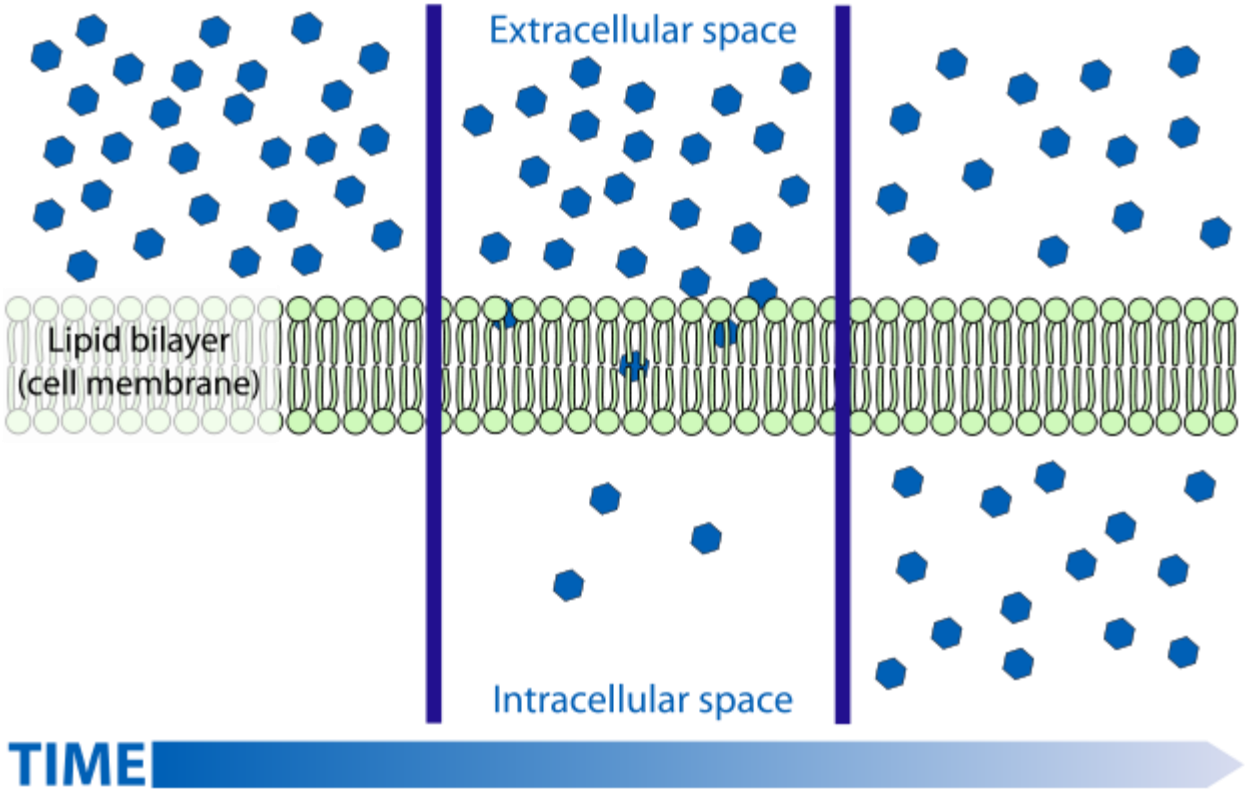
Mehanizmi prenosa snovi čez biološke membrane

mehanizem prenosa	prenašana snov	ključne lastnosti prenosa
gibanje molekul/ionov poteka v smeri **od višje proti nižji koncentraciji**		
pasivni transport z difuzijo	male hidrofobne in polarne molekule	smer toka snovi je določena s koncentracijo molekul na obeh straneh membrane
pasivni transport z olajšano difuzijo	ioni, voda	hiter prenos snovi čez membrano posredujejo proteinski kanalčki; različni proteinski kanalčki prevajajo različne snovi v odvisnosti od njihove velikosti in naboja – selektivni kanalčki so lahko odprti ali zaprti – regulacija
pasivni transport z olajšano difuzijo	sladkorji, aminokisline, nukleozidi	čezmembranski prenos posredujejo prenašalni proteini, ki so selektivni za zvrsti, ki jih prenašajo; vezava specifične molekule na prenašalni protein izzove njegovo konformacijsko spremembo, kar odpre prehod molekuli čez lipidni dvosloj
gibanje molekul/ionov poteka v smeri **od nižje proti višji koncentraciji**		
aktivni transport	različni ioni, glukoza, aminokisline, peptidi	transport molekule/iona čez lipidno membrano zahteva energijo – npr. proces je sklopljen s hidrolizo ATP

Pasivni transport

- **difuzija**
- **transporterji**
- **kanalčki**

Difuzija



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

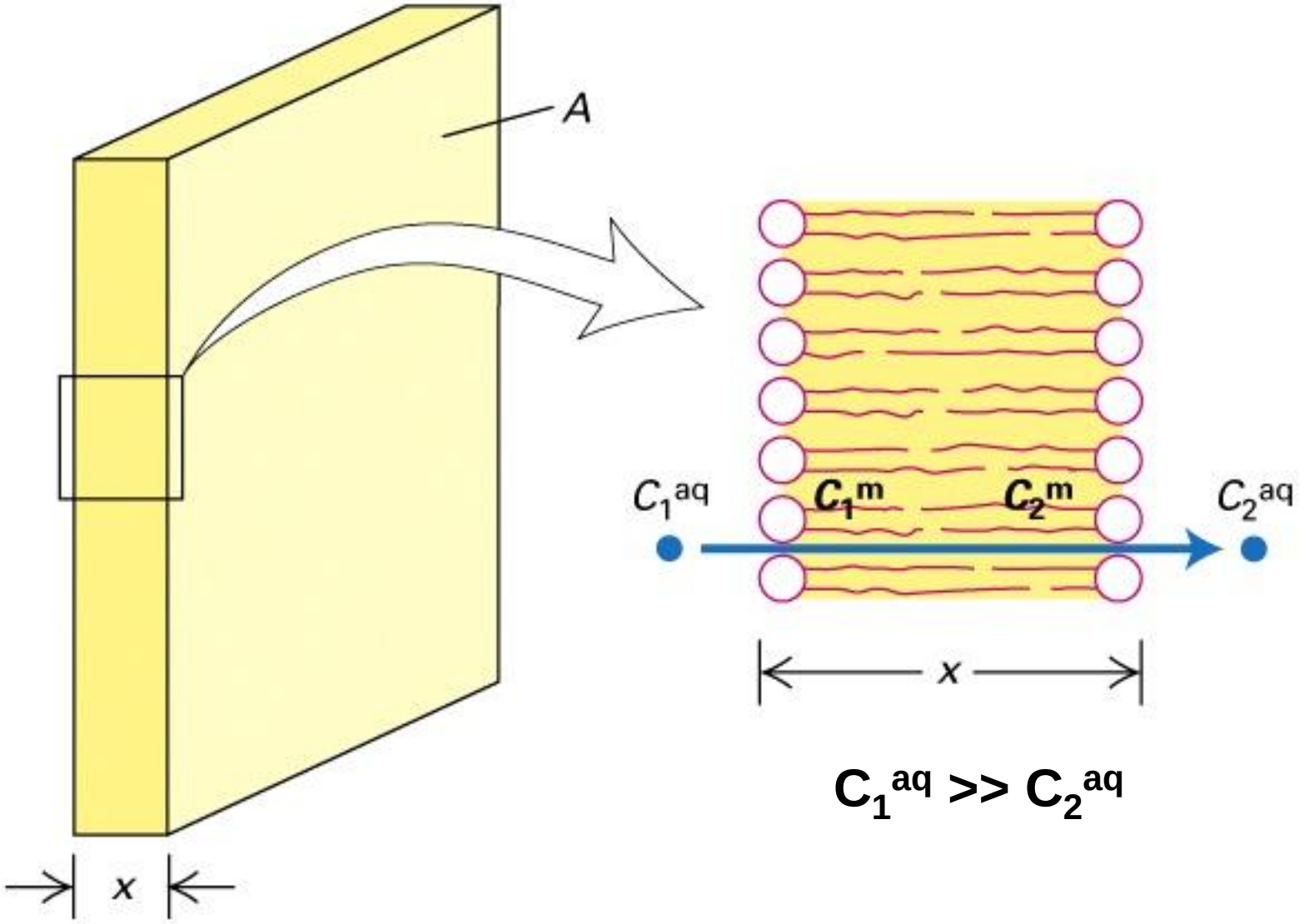
Pasivna difuzija skozi fosfolipidni dvosloj

1. prehod molekule iz vodnega okolja v hidrofobno notranjost dvosloja

2. difuzija skozi hidrofobno sredico
(počasna zaradi 100-1000 krat večje viskoznosti lipidov v primerjavi z vodo)

3. prehod v vodno fazo na drugi strani dvosloja

Difuzija malih molekul skozi membrano



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

Vpliv hidrofobnosti

- Merilo hidrofobnosti snovi je porazdelitveni koeficient K, ravnotežna konstanta porazdelitve snovi med oljno in vodno fazo.

- Ker je notranjost fosfolipidnega dvosloja po sestavi podobna olju, je porazdelitveni koeficient snovi (K) enak razmerju koncentracij snovi v dvoslojni (C^m) in vodni fazi (C^{aq}).

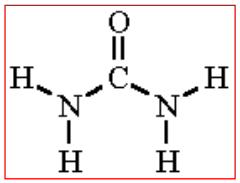
$$K = C^m/C^{aq}$$

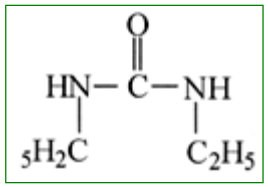
Porazdelitveni koeficient

Porazdelitveni ali particijski koeficient je merilo relativne afinitete snovi za lipid v primerjavi z vodo:

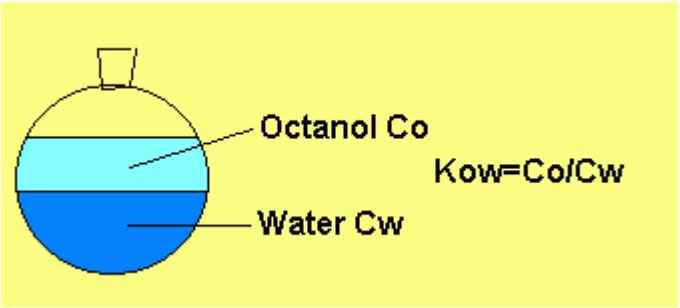
visok K ⇨ boljša topnost snovi v lipidu.

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





urea: K=0,0002; dietilurea: K=0,01 ⇨
dietilurea je 50x bolj hidrofobna kot urea / v PL membrano bo difundirala 50x hitreje kot urea.



Adolf Eugen Fick
Kassel (DE)
03.09.1829*
Blankenberghe (BE)
21.08.1901†

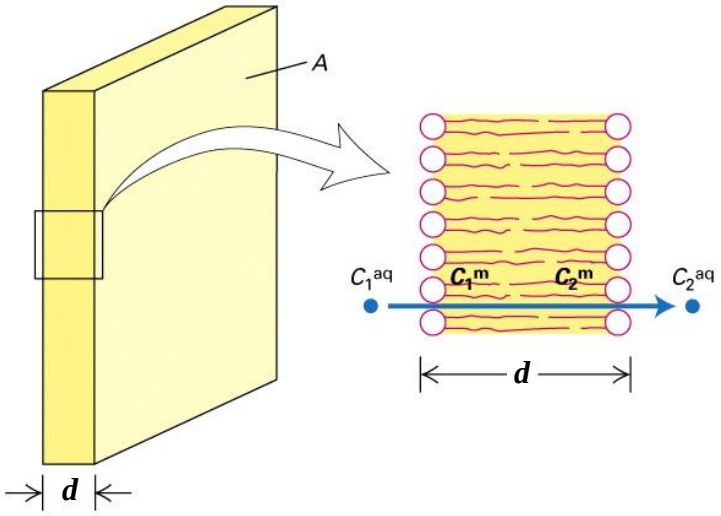
Difuzijski ali 1. Fickov zakon (1855)

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

J – gostota masnega toka
D – difuzijska konstanta
$\delta\phi/\delta x$ – gradient koncentracije

Hitrost difuzije

Hitrost difuzije: dn/dt [mol/s]



J = (dn/dt)/A

dn/dt = J×A

dn/dt = P×A×($C_1^{aq} - C_2^{aq}$)

J … gostota masnega toka

P… prepustnostni koeficient

A… površina membrane

P = K×D / *d*

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

D… difuzijska konstanta snovi v membrani

d … debelina membrane

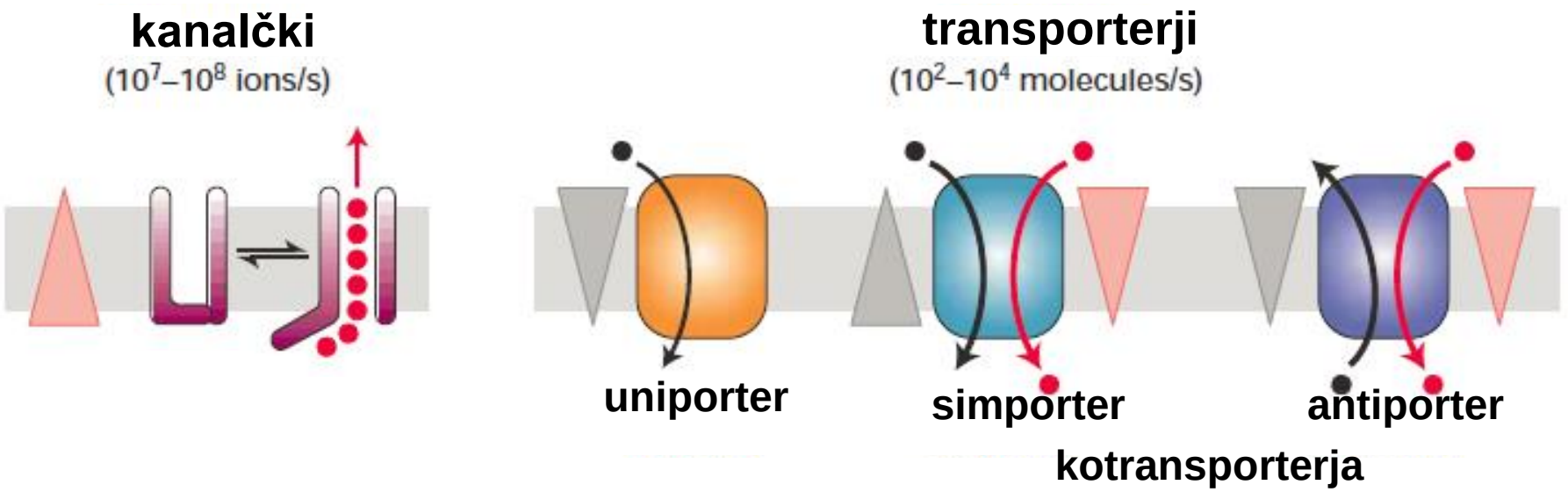
$dn/dt = K \times D \times A \times (C_1^{aq} - C_2^{aq}) / d$

Hitrost difuzije je sorazmerna porazdelitvenemu koeficientu, difuzijski konstanti, prenašalni površini in razliki v koncentracijah snovi na obeh straneh membrane ter obratno sorazmerna z debelino membrane.

Biološke membrane: *d* ≈ 3 nm (≈ konst.); D ≈ konst., A = konst. ⇒

dn/dt ∝ K; K ∝ hidrofobnost snovi → velja za pline in male nenabite molekule ←

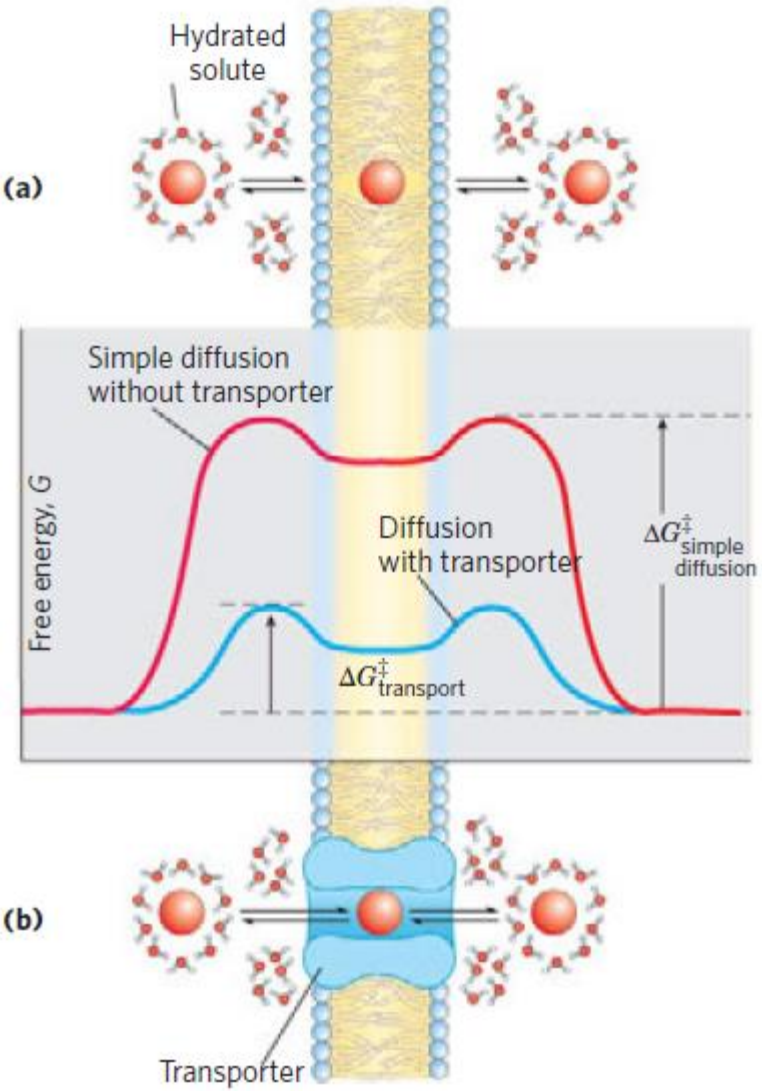
Olajšana difuzija



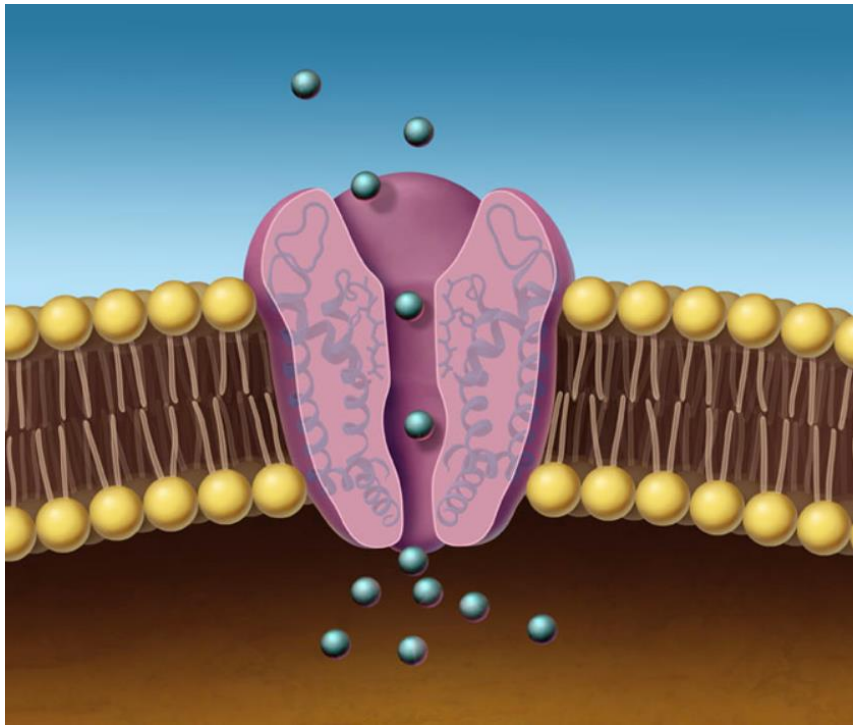
integralni transmembranski proteini /
/ omogočajo hitrejši prehod snovi čez membrano / so specifični

Navadna difuzija

Olajšana difuzija

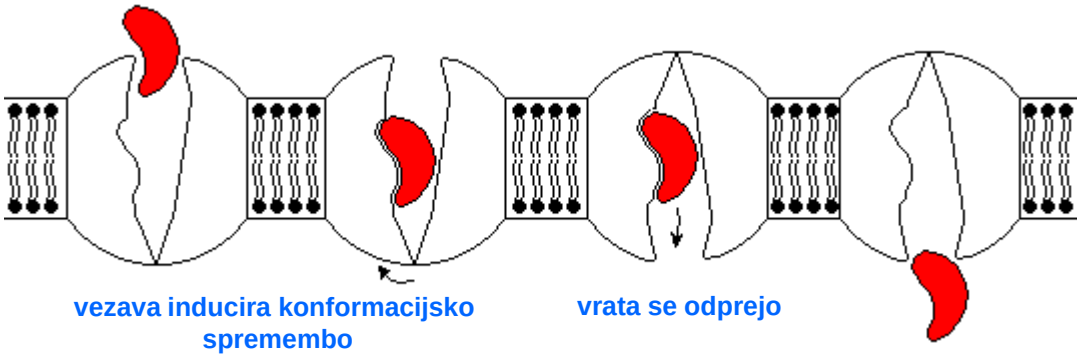


Kanalčki



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

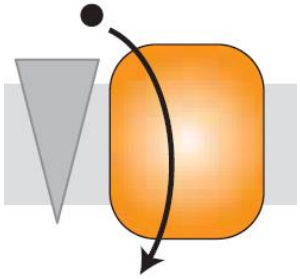
Transporterji



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

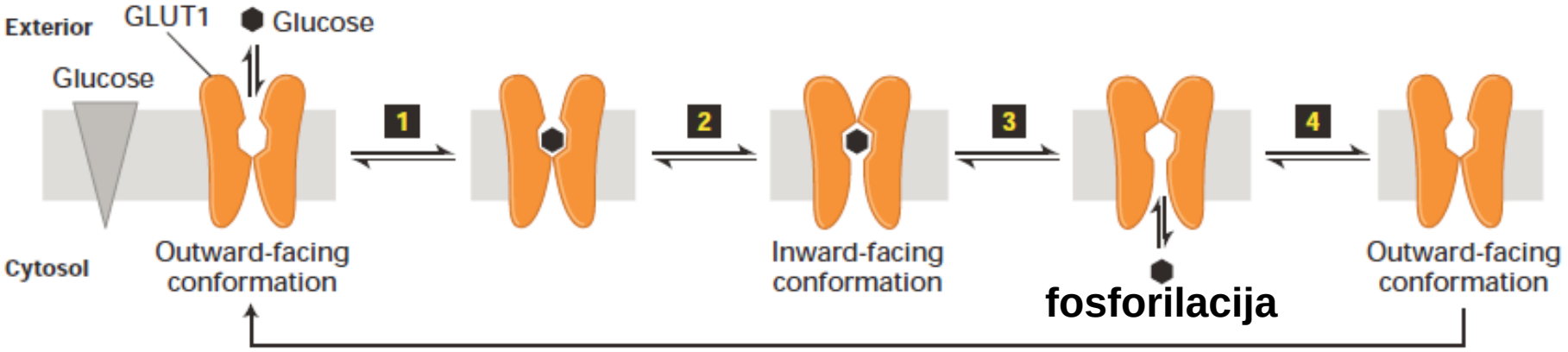
Lastnosti uniporta

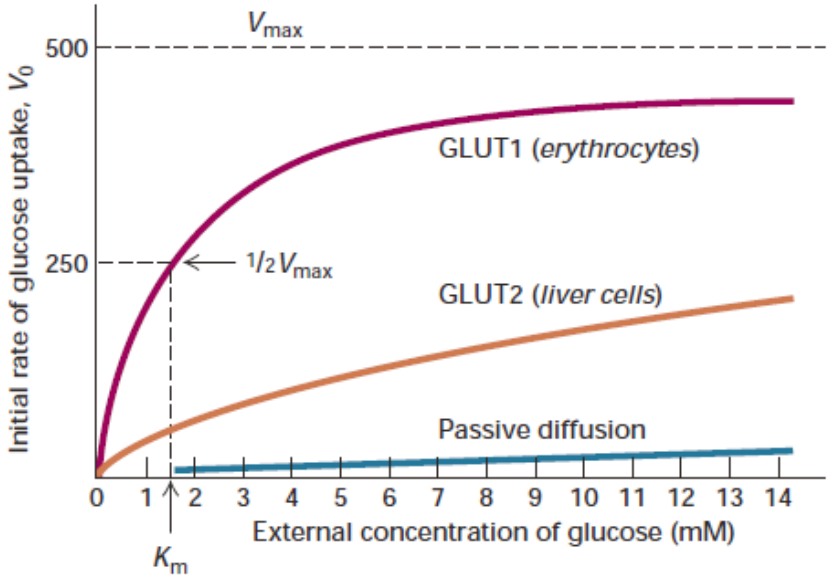
- prenos je **reverzibilen** in poteka v smeri **od višje k nižji koncentraciji** snovi
- kljub gradientu brez proteina do prenosa snovi ne bi prišlo
- prenos snovi je **termodinamsko ugoden** ($\Delta S>0$; $\Delta G<0$)
- gre za **olajšano difuzijo** („facilitated diffusion“), hitrost prenosa je precej višja kot v primeru pasivne difuzije
- proces je podoben encimski reakciji – je **specifičen**, (le substrat se kemijsko ne spremeni) in **saturabilen** (omejen s številom transporterjev v membrani)

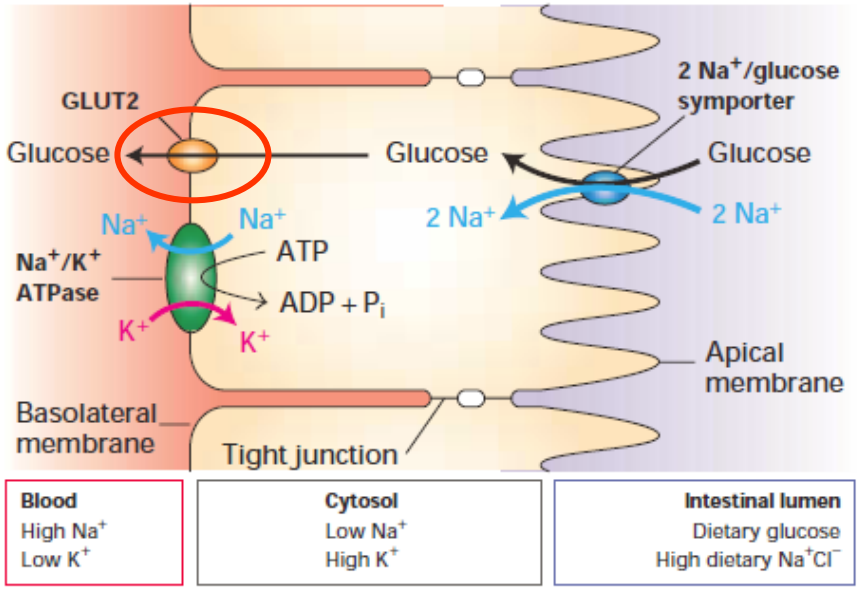


Uniporterji

primera: GLUT1 in GLUT2 (14 GLUT)

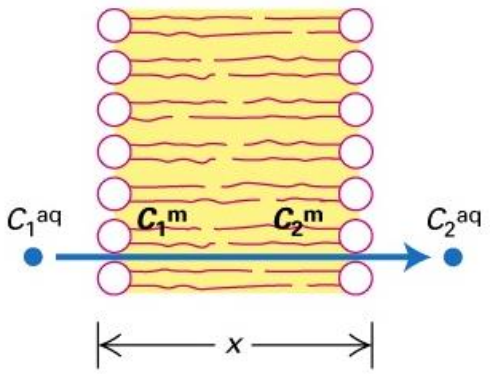






Primerjava uniporta in pasivne difuzije

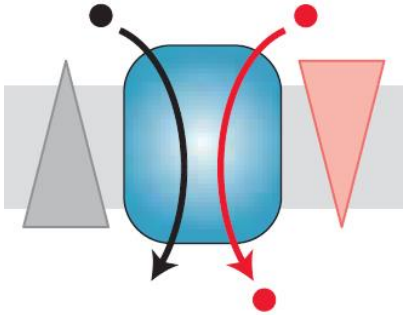
- hitrost prenosa snovi z uniportom je bistveno višja, kot sledi iz Fickovega zakona (molekula ne vstopa v hidrofobni del lipidnega dvosloja, zato K ni potrebno upoštevati)



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

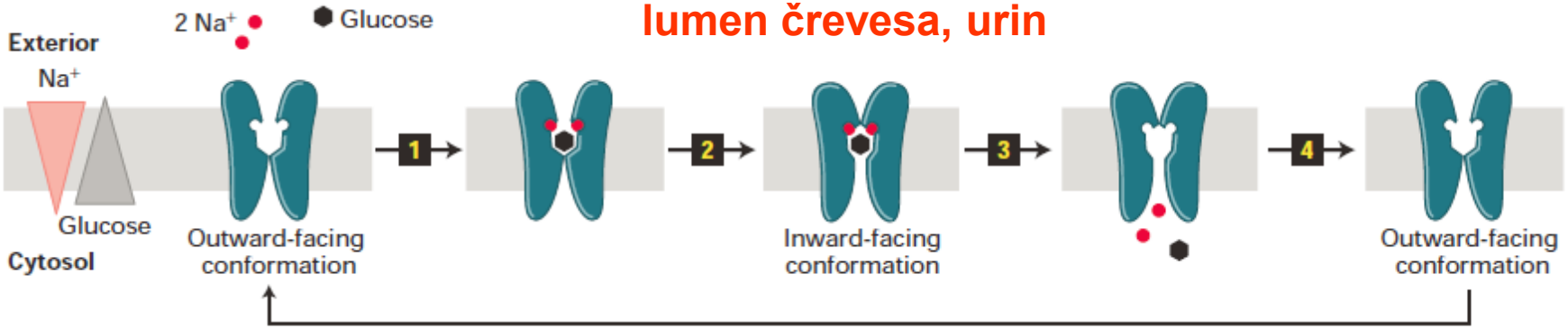
K… porazdelitveni koeficient
D… difuzijska konstanta snovi v membrani
x … debelina membrane
A… površina membrane
(D in *x* sta ≈ konst.)

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

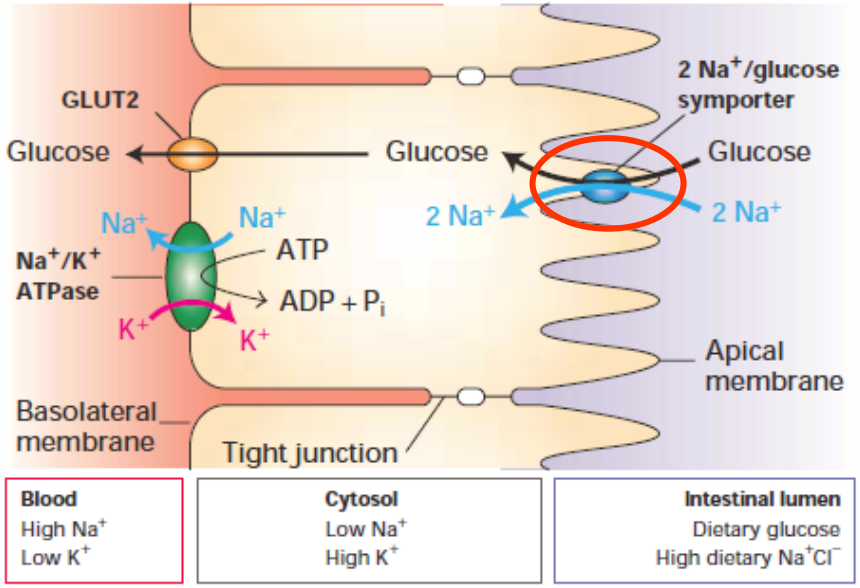


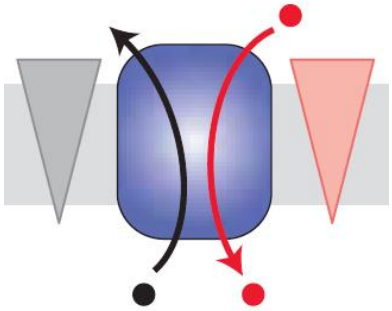
Simporterji

Na^+-simporterji vnašajo Glc in ak v celice – primer: $2Na^+$/Glc simporter



lumen črevesa, urin

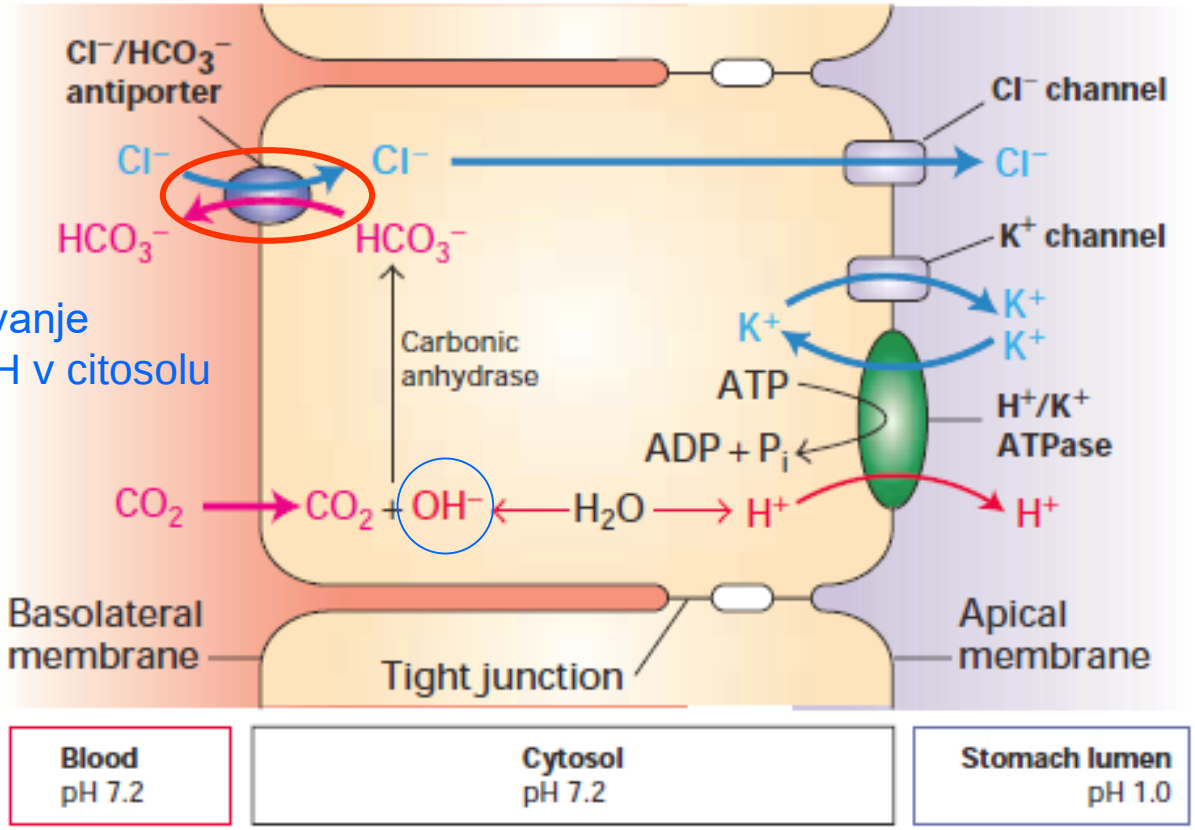




Antiporterji

Primer: Cl^-/HCO_3^- antiporter v procesu kisanja lumna želodca

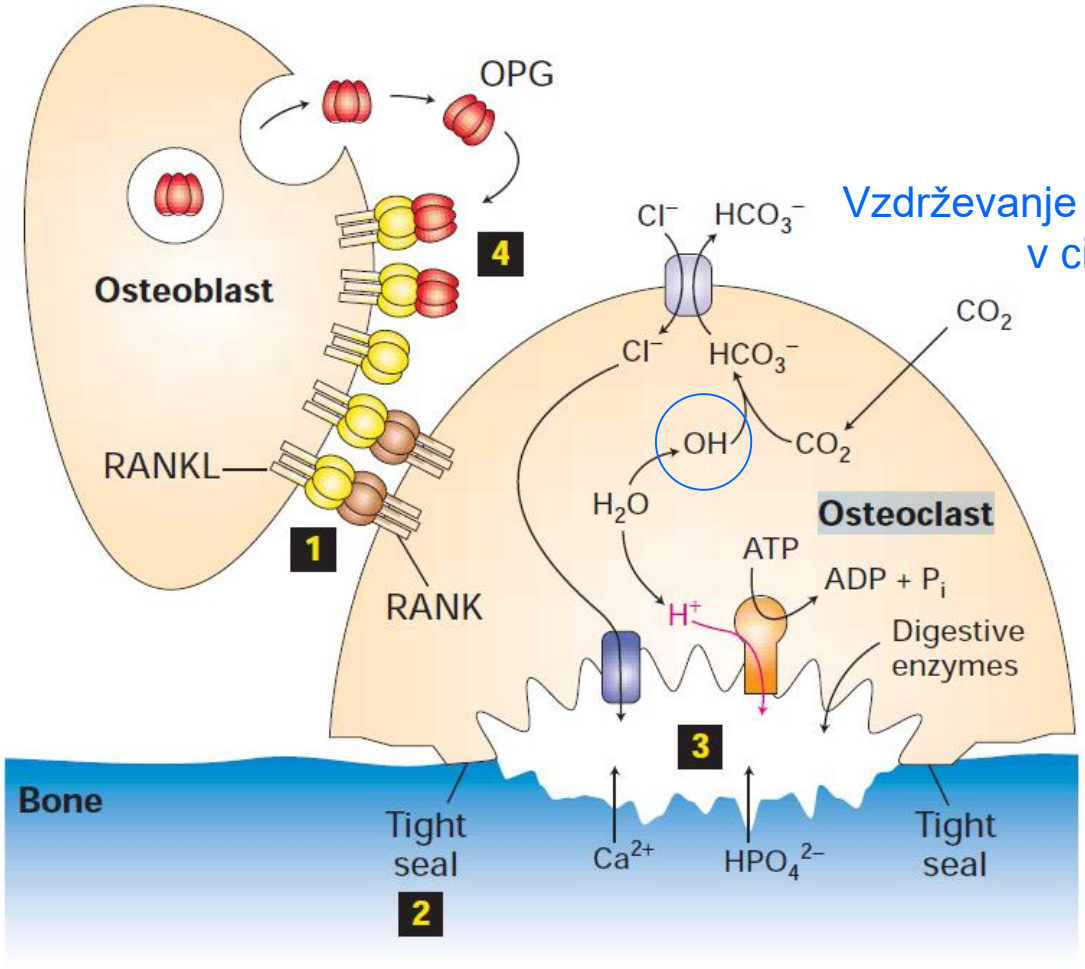
Vzdrževanje nevtralnega pH v citosolu



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

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

Cl^-/HCO_3^- antiporter v procesu raztapljanja kosti



Vzdrževanje nevtralnega pH v citosolu

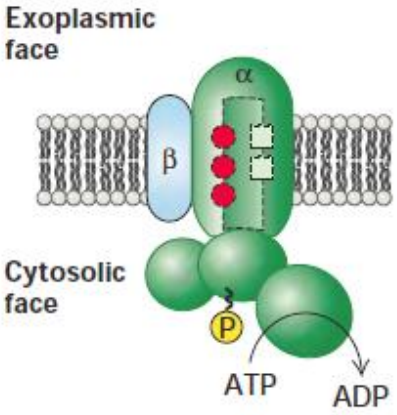
Kotransportni sistemi, ki jih poganja gradient Na^+ ali H^+

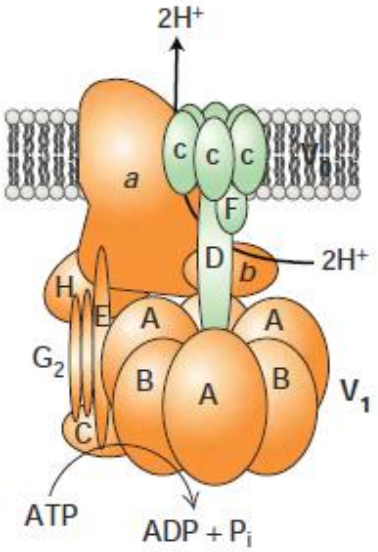
Organism/ tissue/cell type	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
E. coli	Lactose	H^+	Symport
	Proline	H^+	Symport
	Dicarboxylic acids	H^+	Symport
Intestine, kidney (vertebrates)	Glucose	Na^+	Symport
	Amino acids	Na^+	Symport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

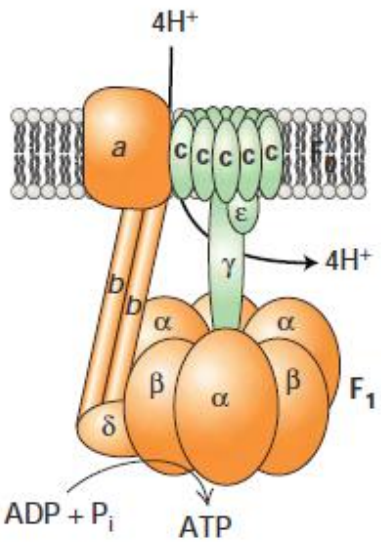
Aktivni transport

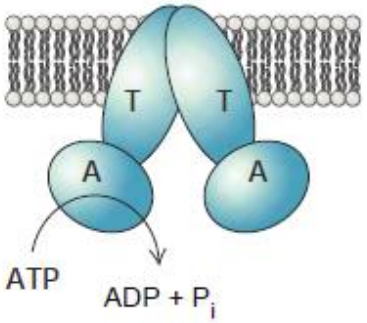
ATP-gnane črpalke

Štiri skupine ATP-gnanih črpalk



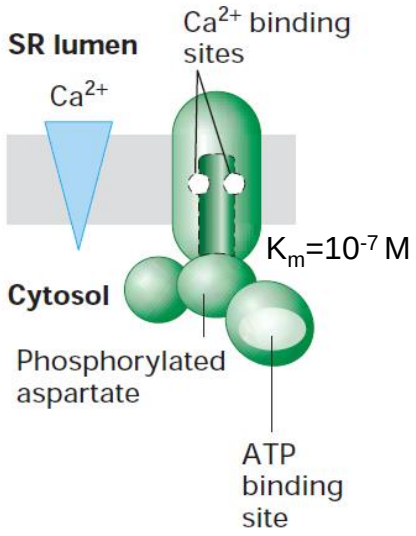


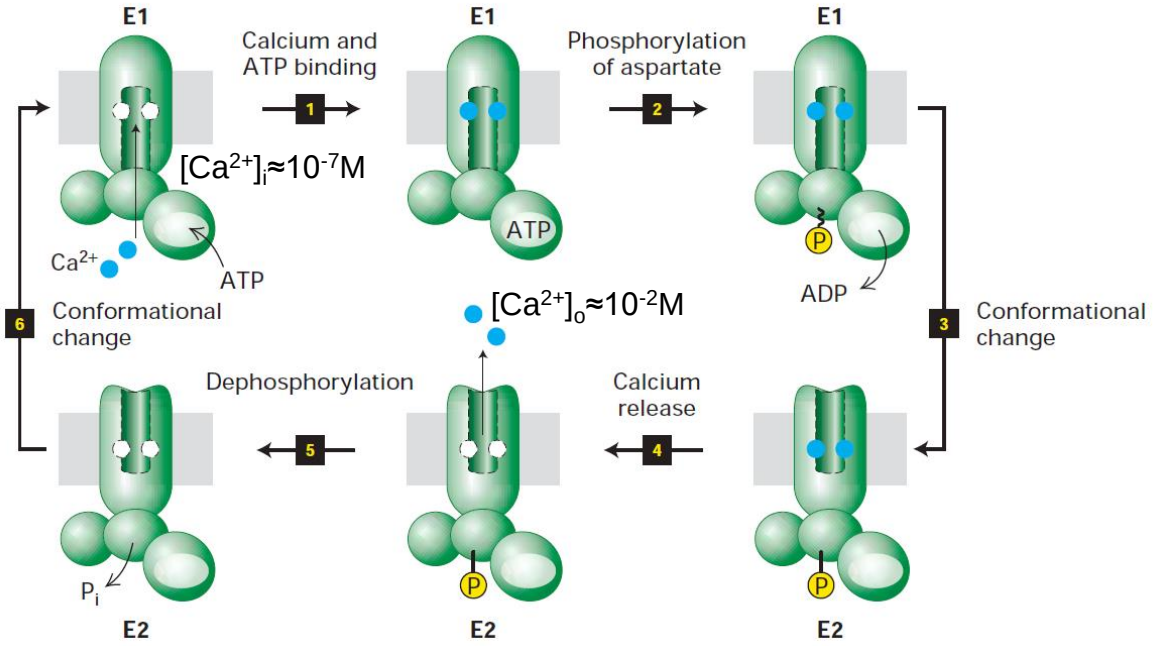


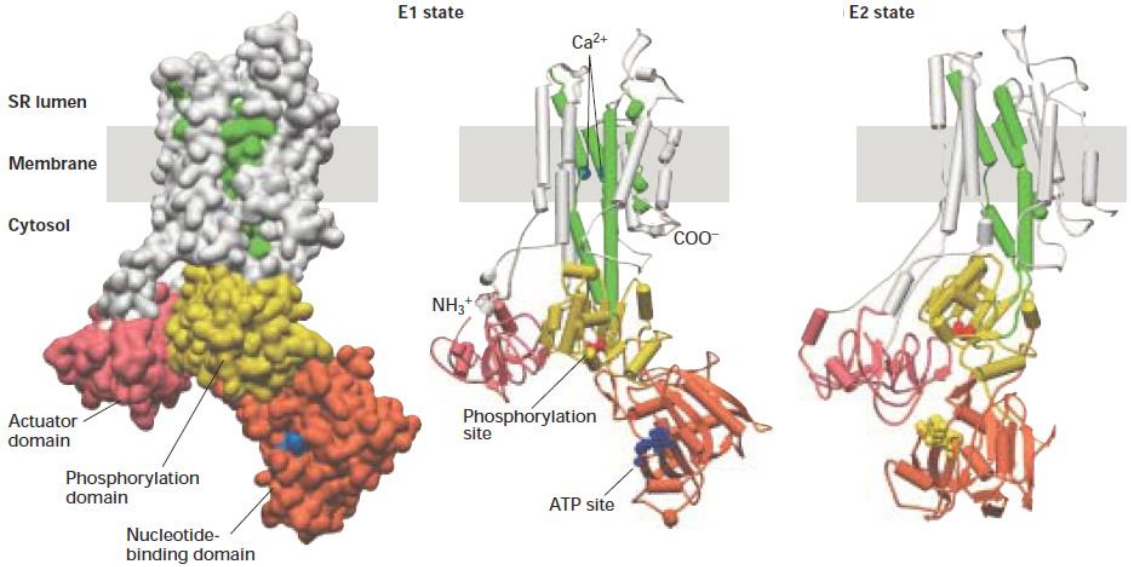


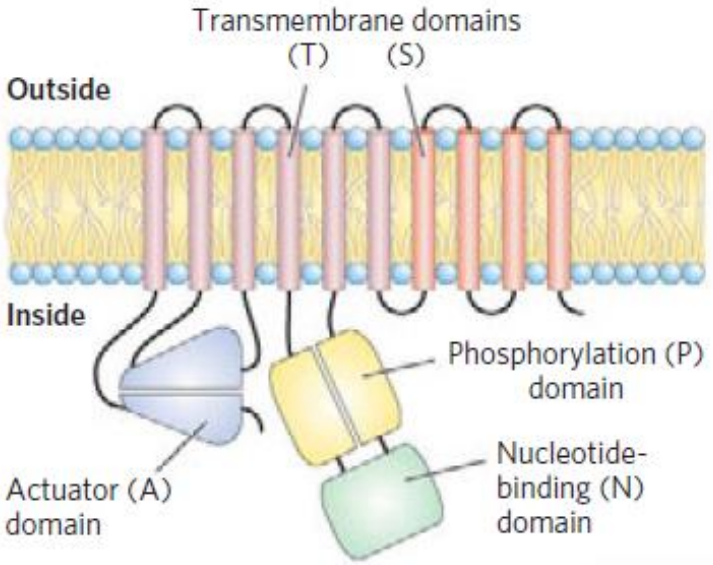
P-tip **V-tip** **F-tip** **ABC transporterji**

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

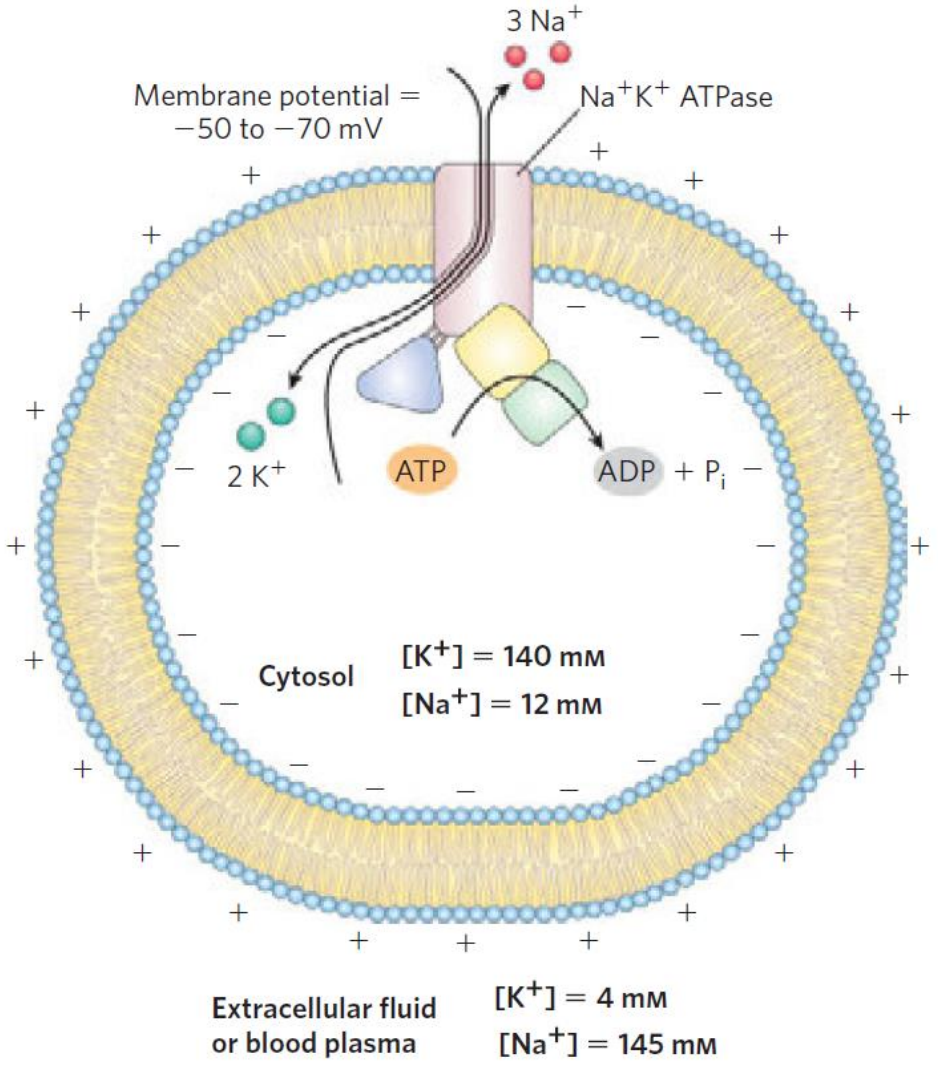




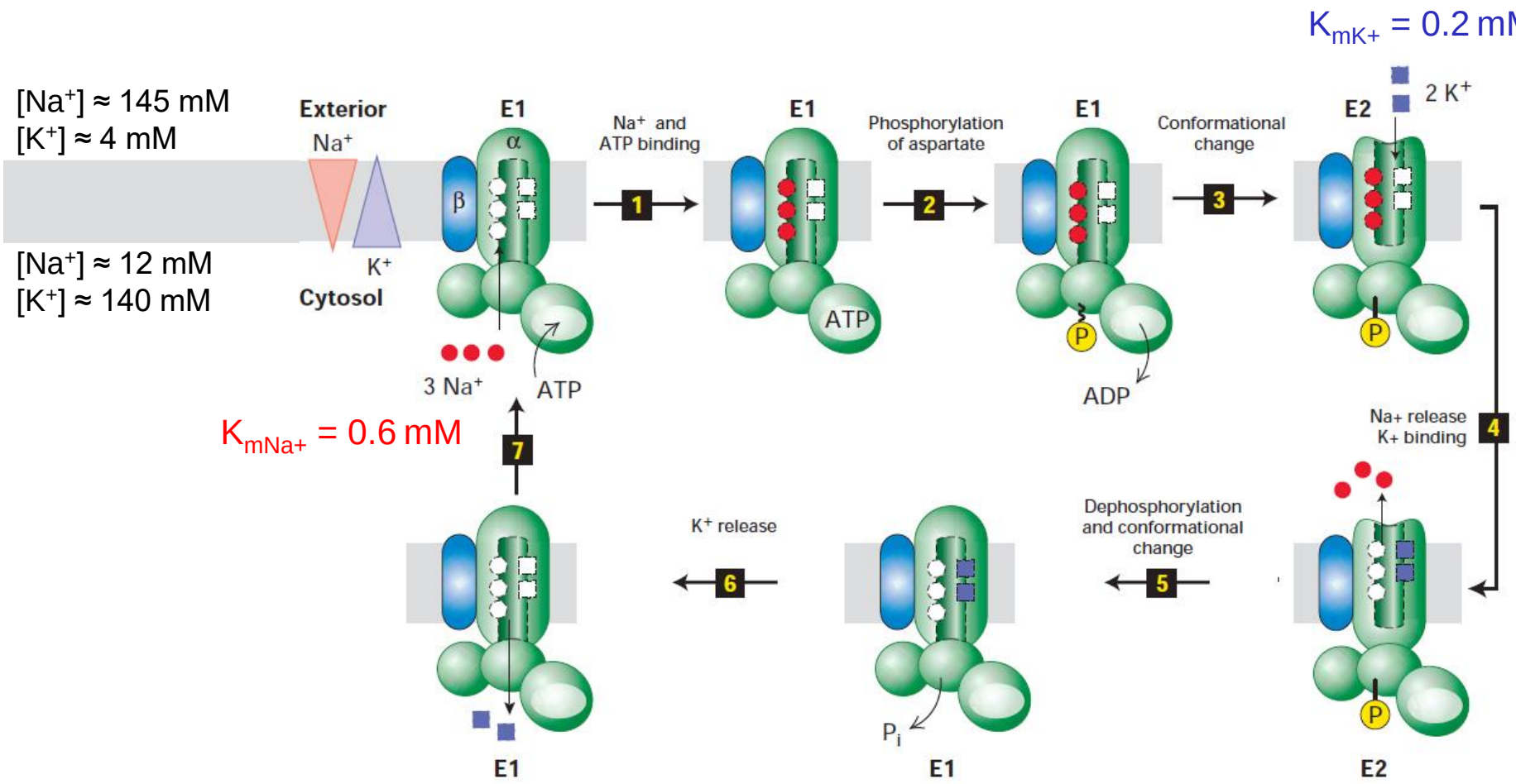




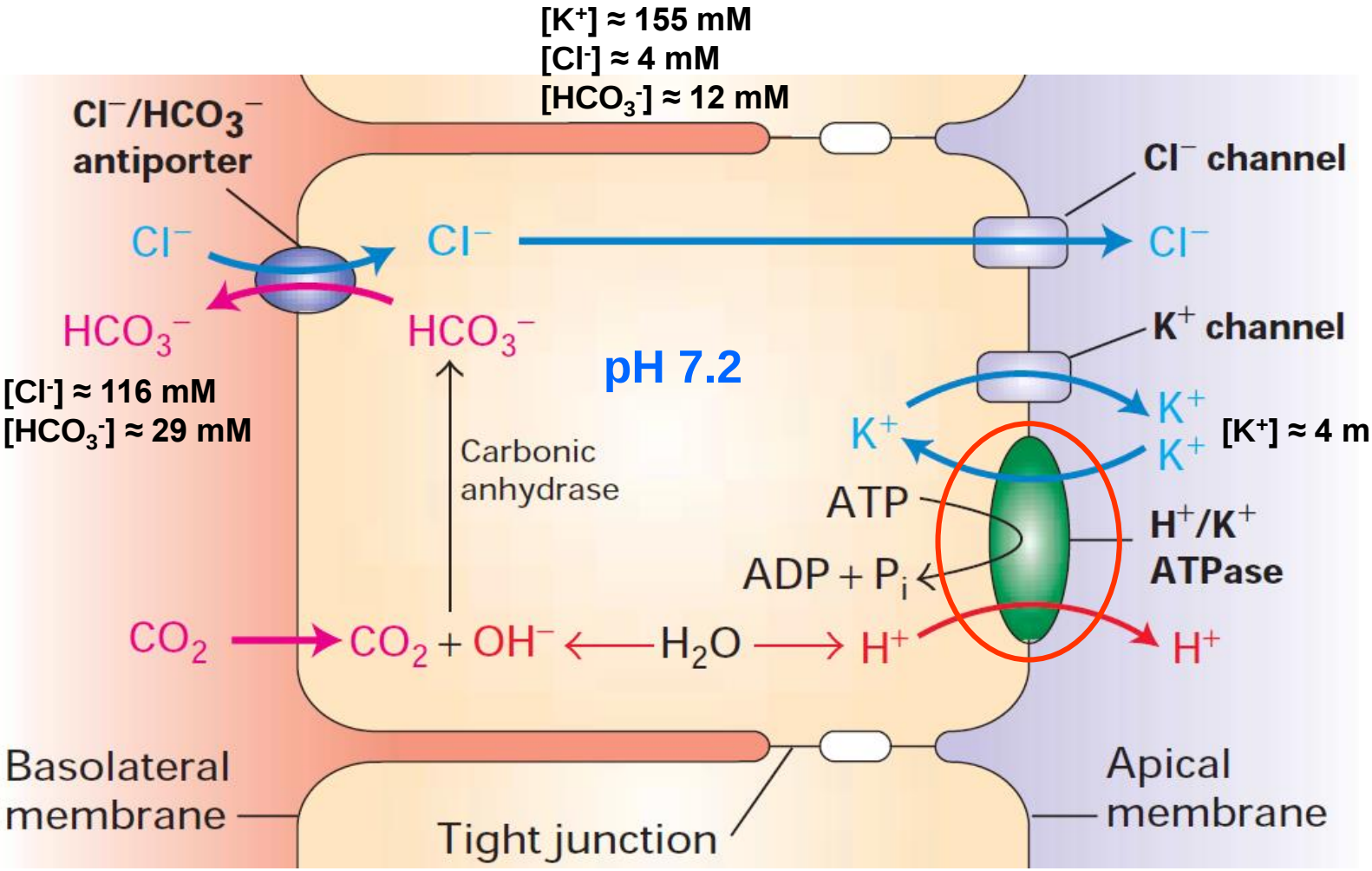
Na^+/K^+ ATPaza je najpomembnejša ionska črpalka za formiranje električnega potenciala na PM živalskih celic



Model mehanizma delovanja Na^+/K^+ ATPaze



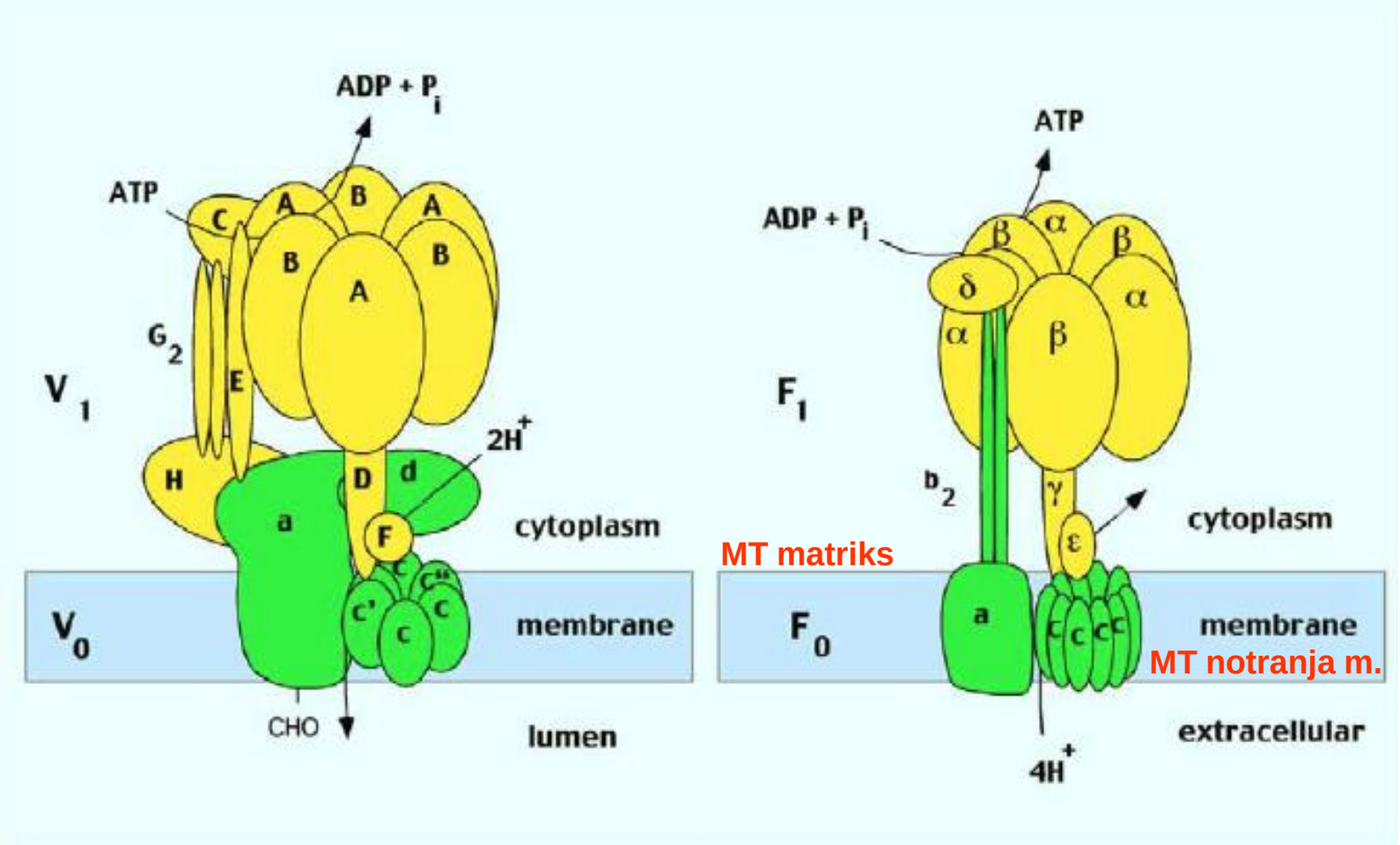
Kisanje lumna želodca z delovanjem P-tip H^+/K^+ ATPaze v apikalni memebrani parietalnih celic



Kri
pH 7.2

Lumen želodca
pH 1.0 (0.1 M HCl)

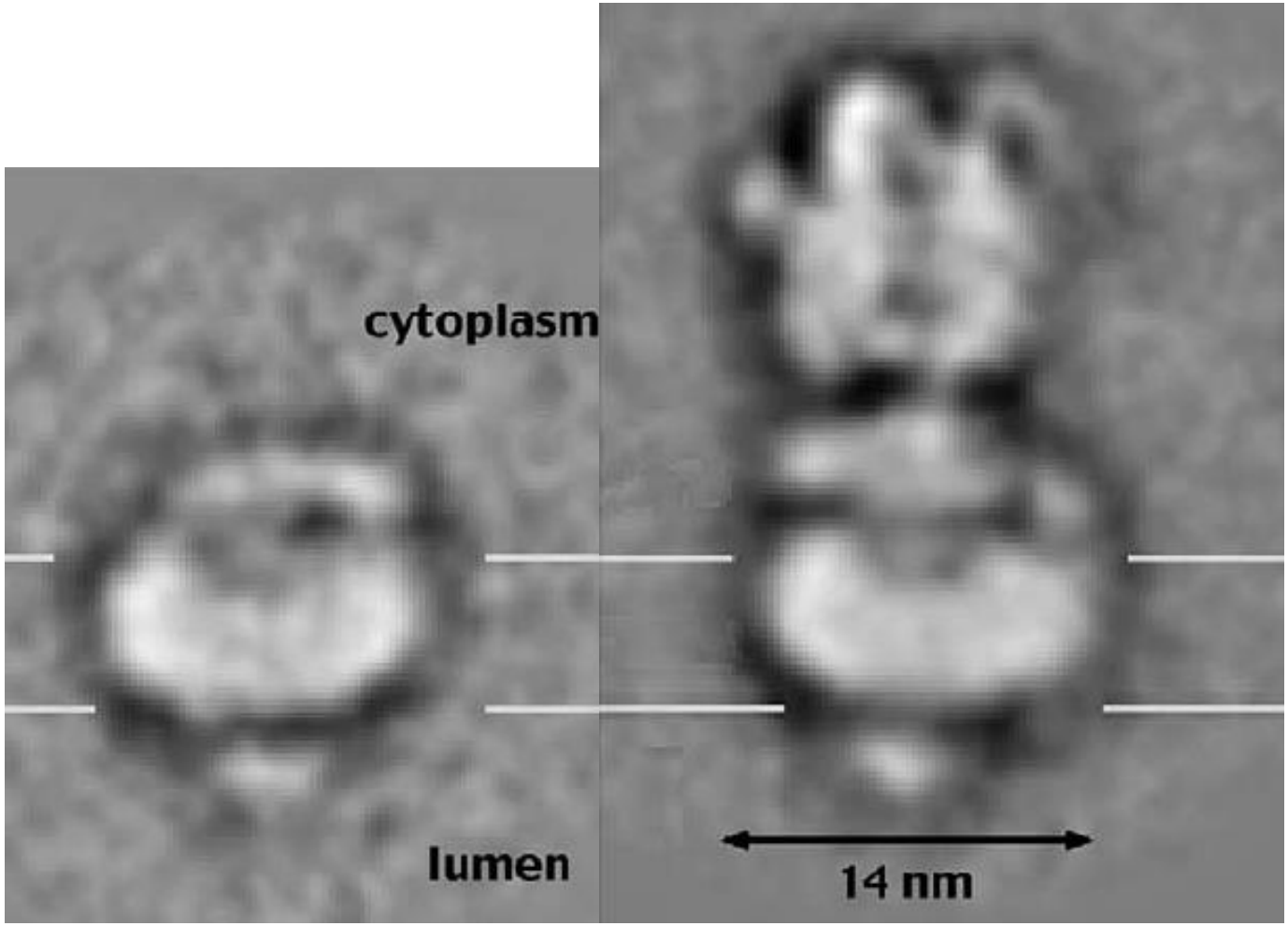
Strukturna primerjava ATPaz tipov V in F



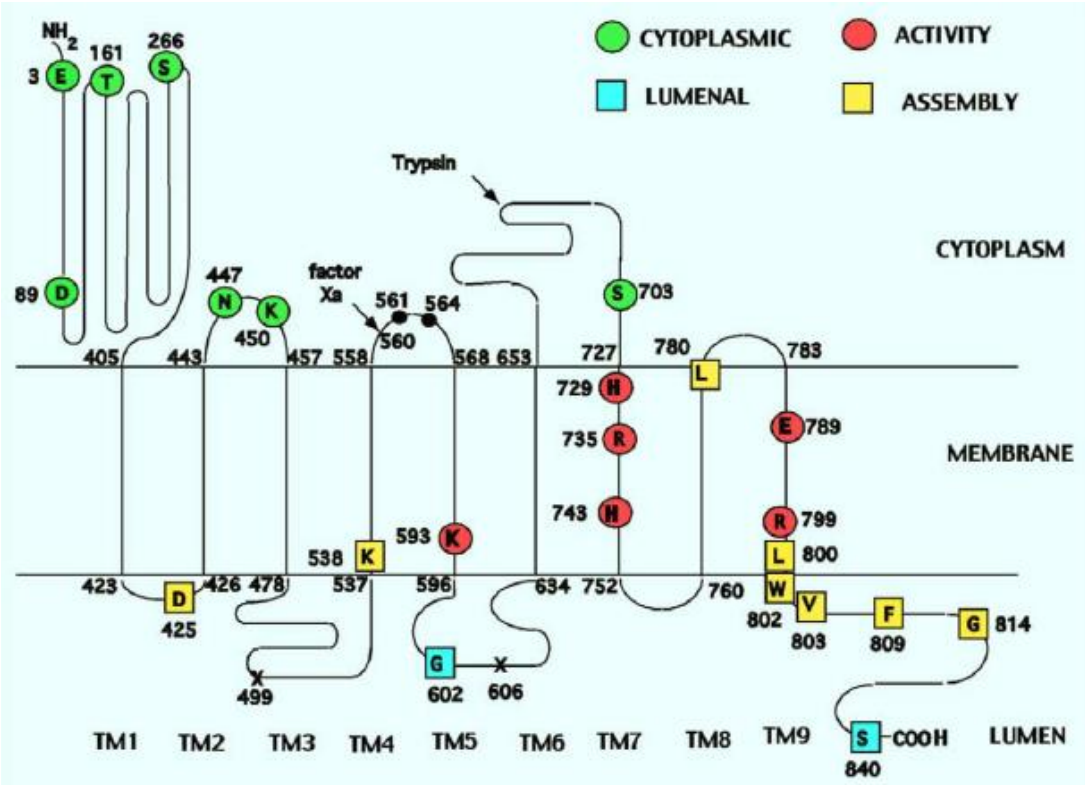
Lastnosti podenot V-tip ATPaze

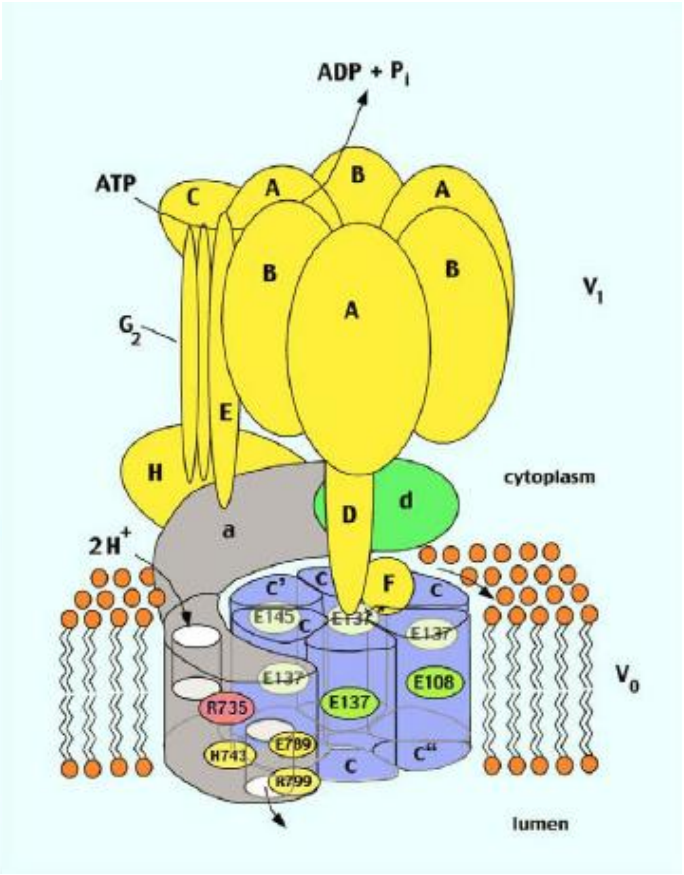
Subunit	Molecular mass (kDa)	Yeast gene	Mammalian isoforms* (tissue/cell)	Subunit function
V_1 domain				
A	70	*VMA1*		ATP hydrolytic site, regulation via non-homologous domain, stator subunit
B	60	*VMA2*	B1(renal, epididymis); B2 (ubiquitous)	Non-catalytic ATP site, binds actin and aldolase, stator 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

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

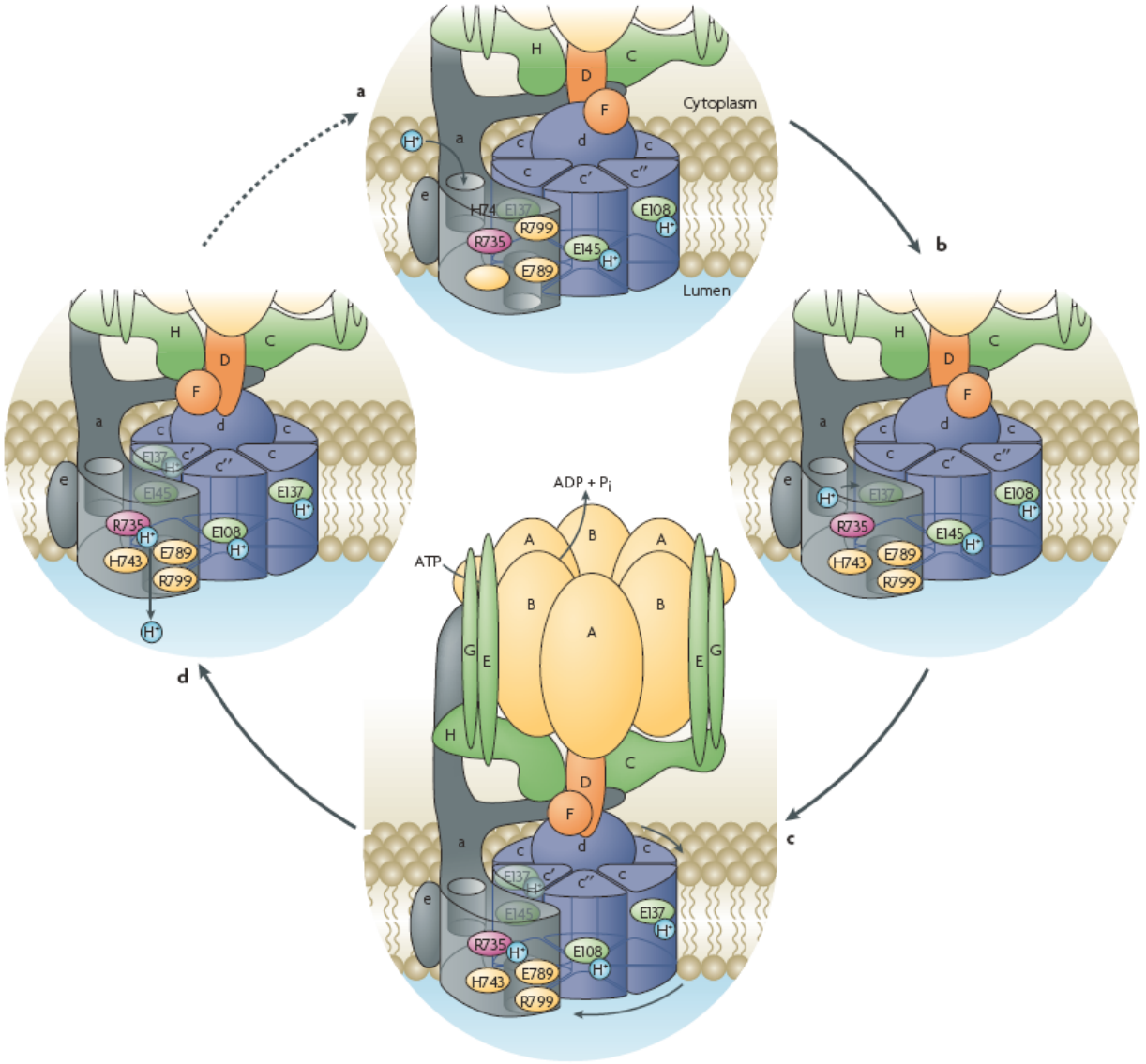


Struktura in funkcija V_0 sektorja V-ATPaze



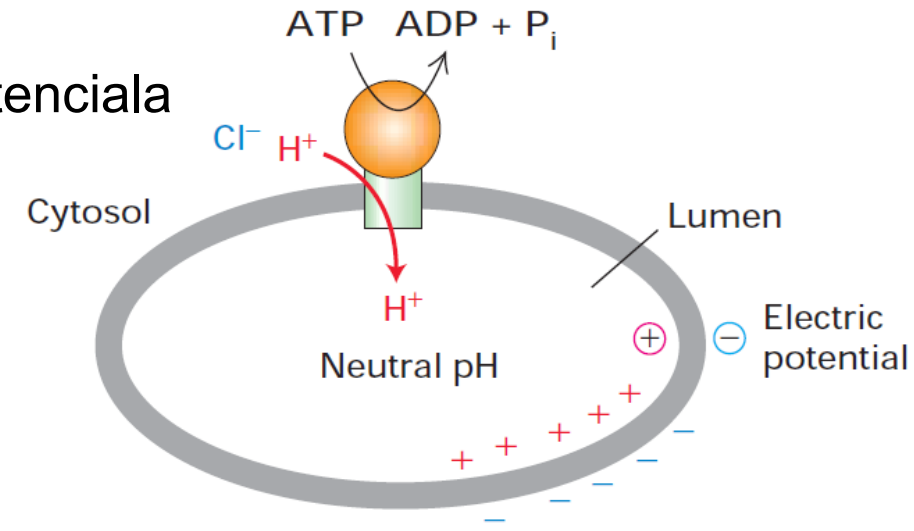


Mehanizem transporta protonov z V-tip ATPazo

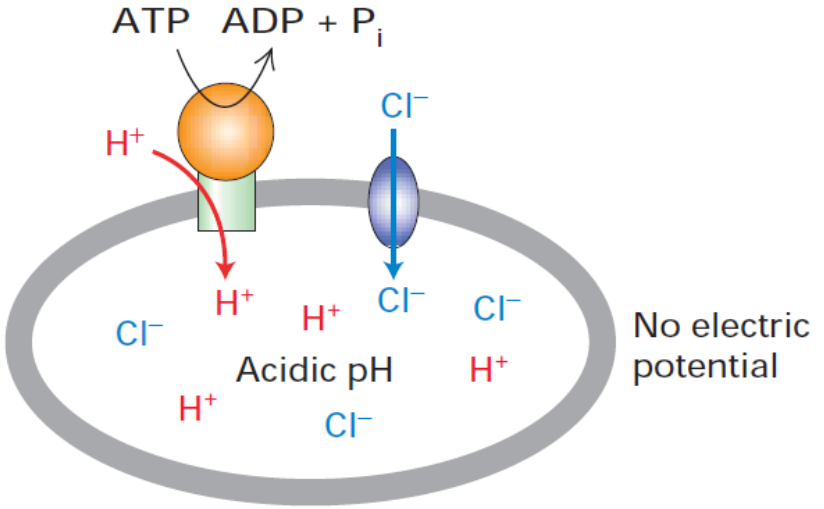


Temeljna učinka delovanja V-tip ATPaze

Vzpostavitev električnega potenciala

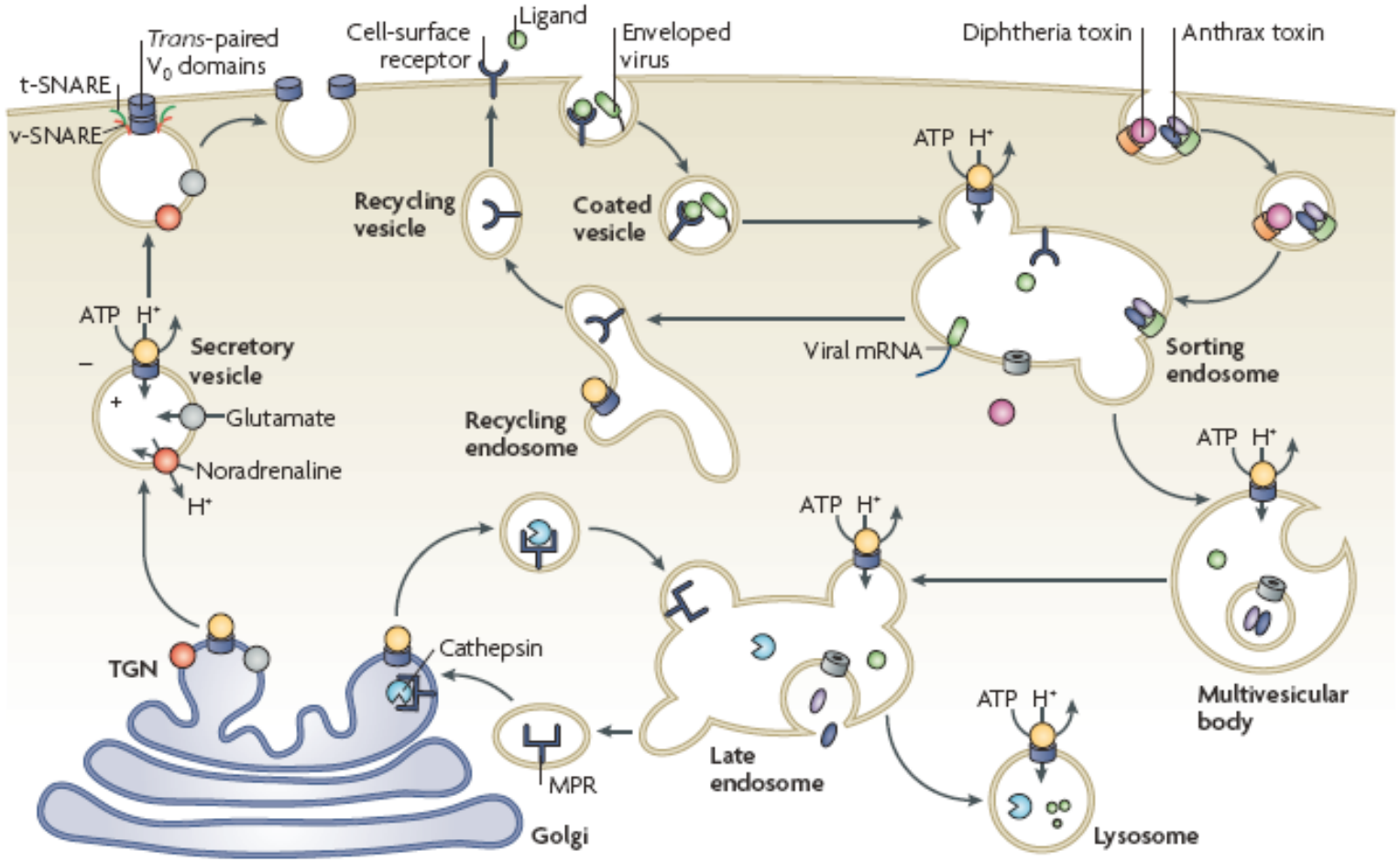


Nakisanje lumna organela/celice

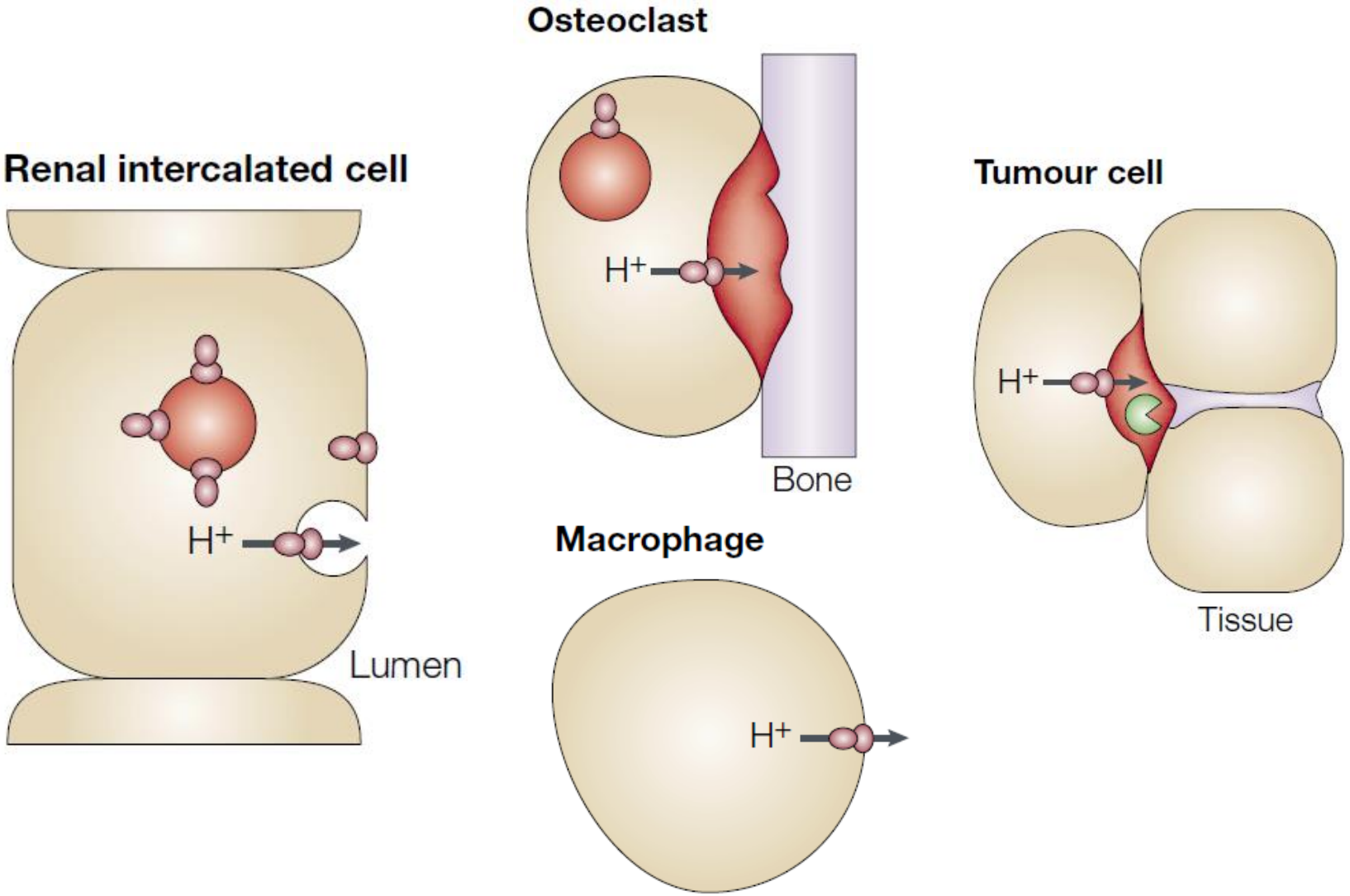


Pomembne znotrajcelične funkcije V-tip ATPaze:

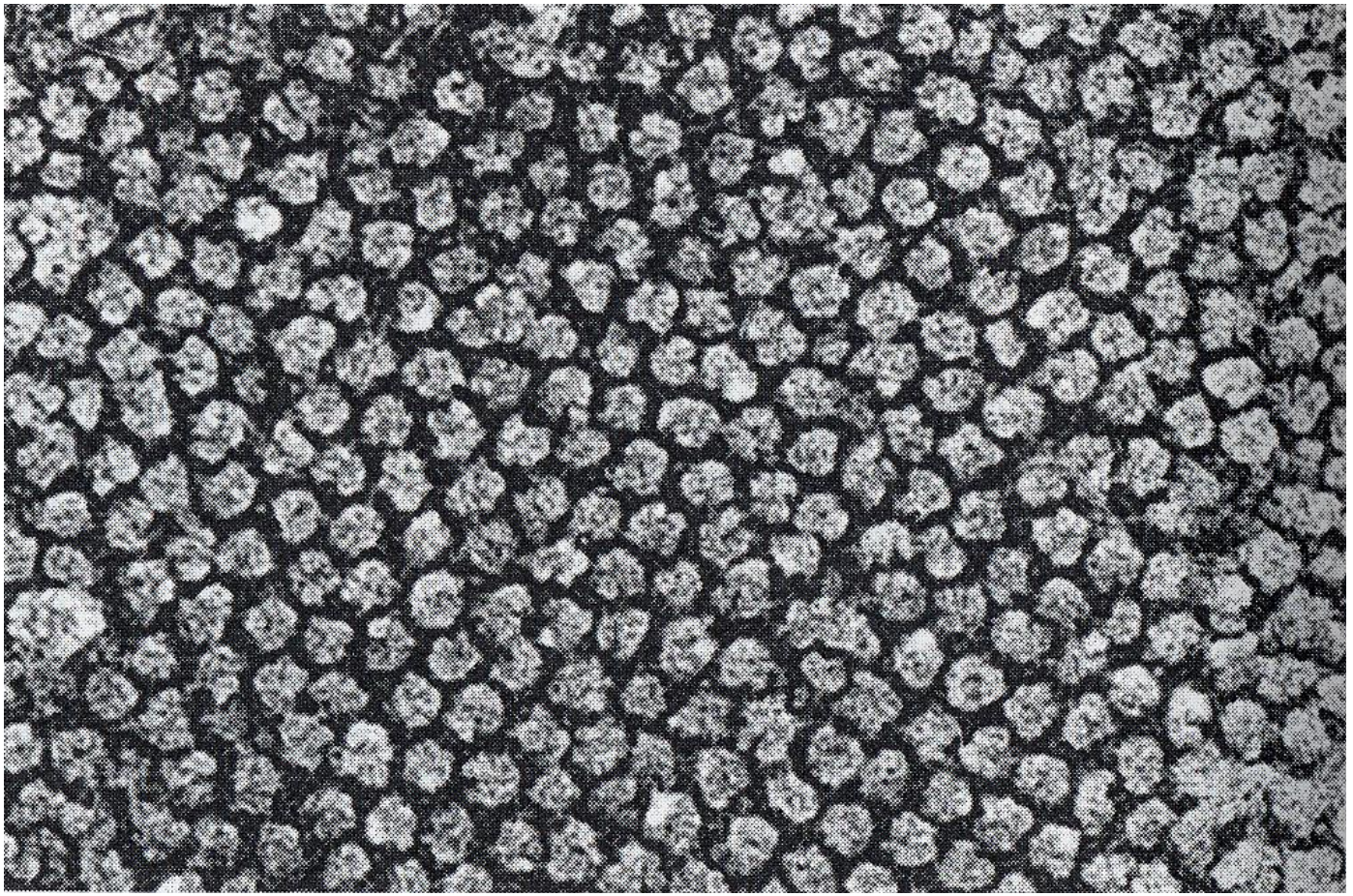
transport in razgradnja snovi,
zlivanje membran – eksocitoza, celična signalizacija …



Nekatere funkcije V-tip ATPaze v plazemski membrani



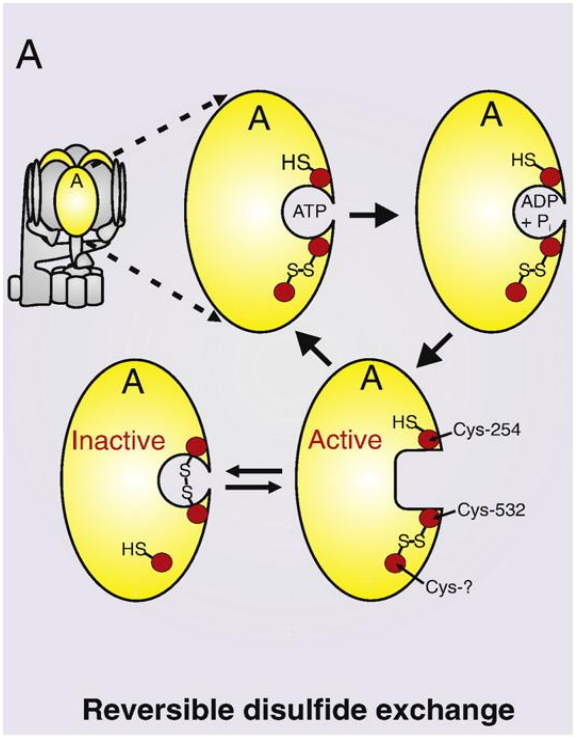
V-tip ATPaze v PM epitelijske celice mehurja krastače



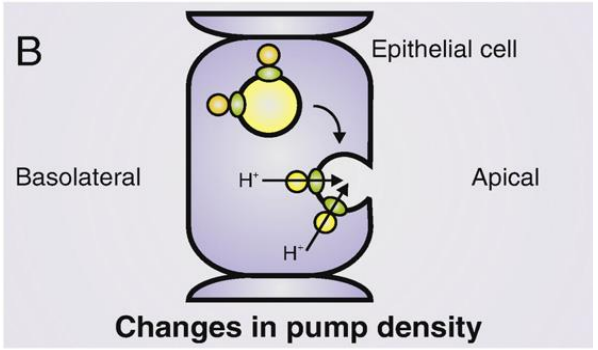
Poganjanje transportnih sistemov (kotransporterjev)

Organism/ tissue/cell type	Transported solute (moving against its gradient)	Cotransported solute (moving down its gradient)	Type of transport
E. coli	Lactose	H^+	Symport
	Proline	H^+	Symport
	Dicarboxylic acids	H^+	Symport
Higher plants	K^+	H^+	Antiport
Fungi (*Neurospora*)	K^+	H^+	Antiport

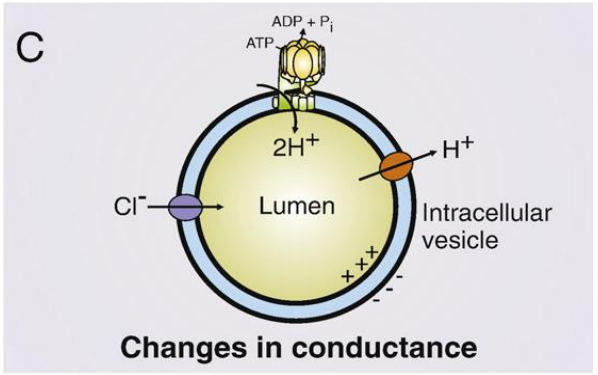
Regulacija aktivnosti V-tip ATPaze



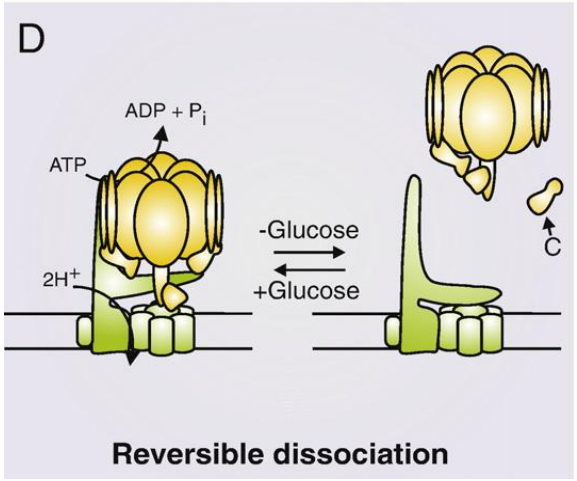
Reversible disulfide exchange



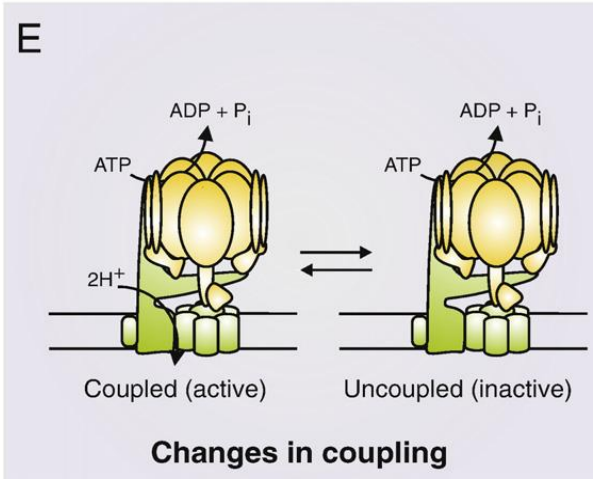
Changes in pump density



Changes in conductance



Reversible dissociation



Changes in coupling

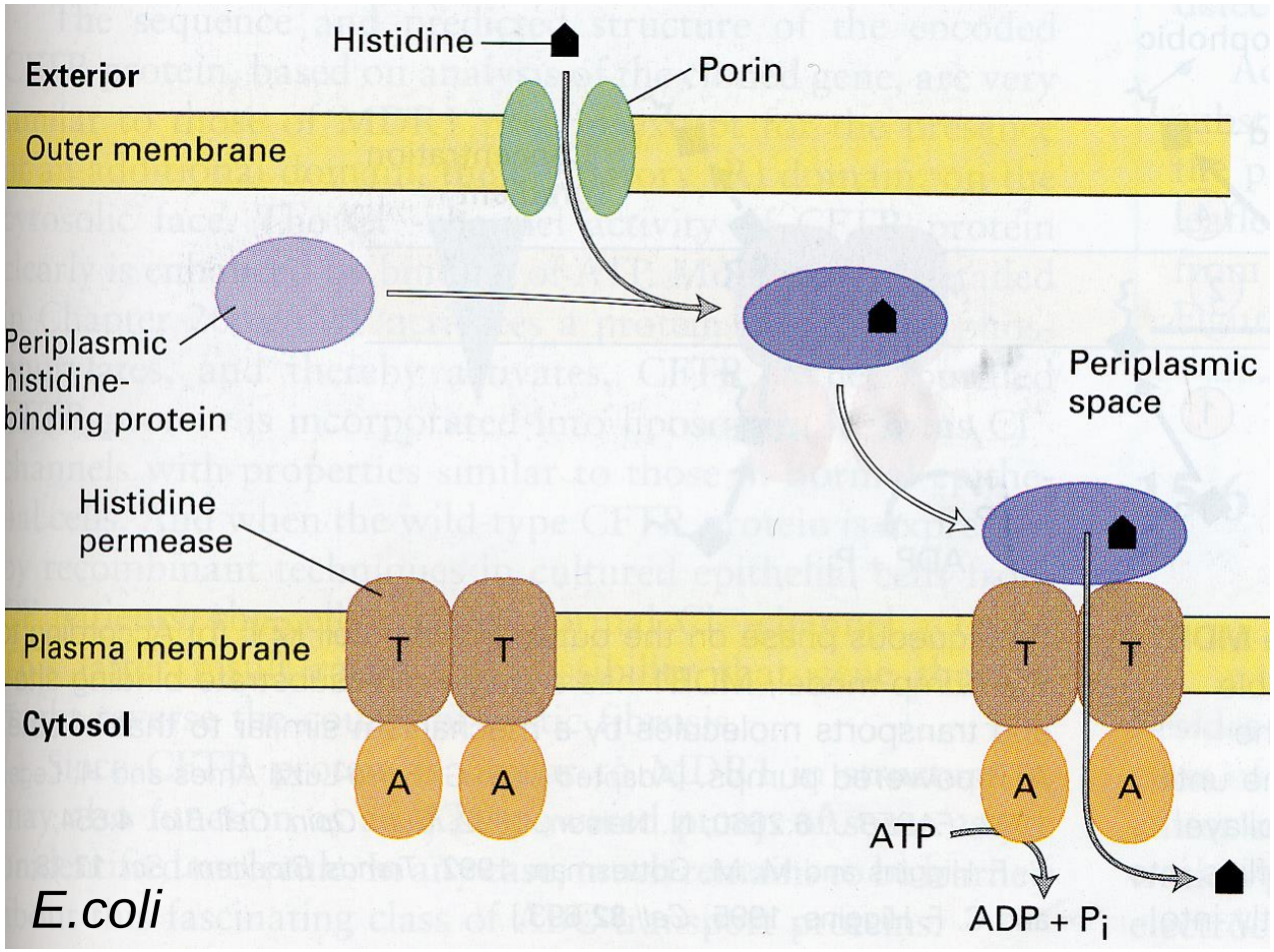
ABC transporterji

- **Permeaze** v PM bakterij

- **MDR proteina 1 in 2** (“MultiDrug-Resistance”)

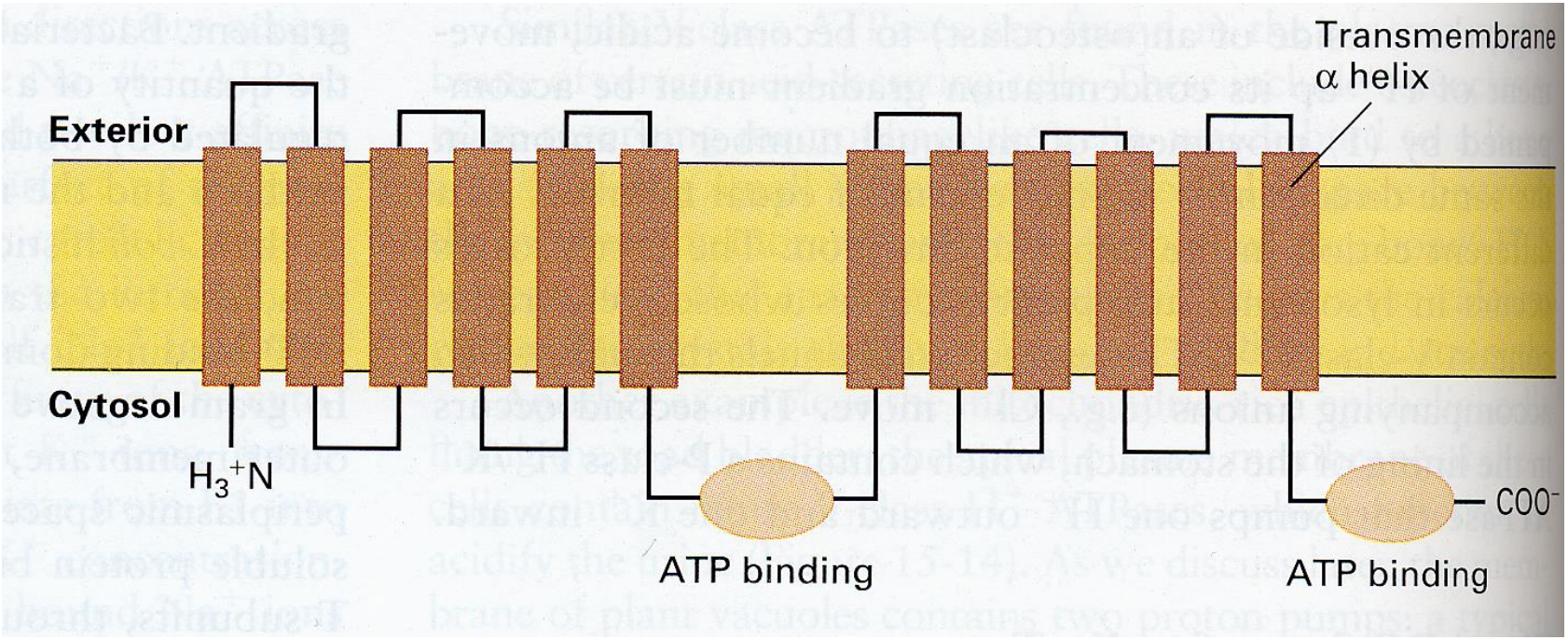
- **CFTR protein** (“Cystic Fibrosis Transmembrane-
-conductance Regulator”)
- **Flipaze**

PM bakterij vsebuje različne ABC transporterje - PERMEAZE



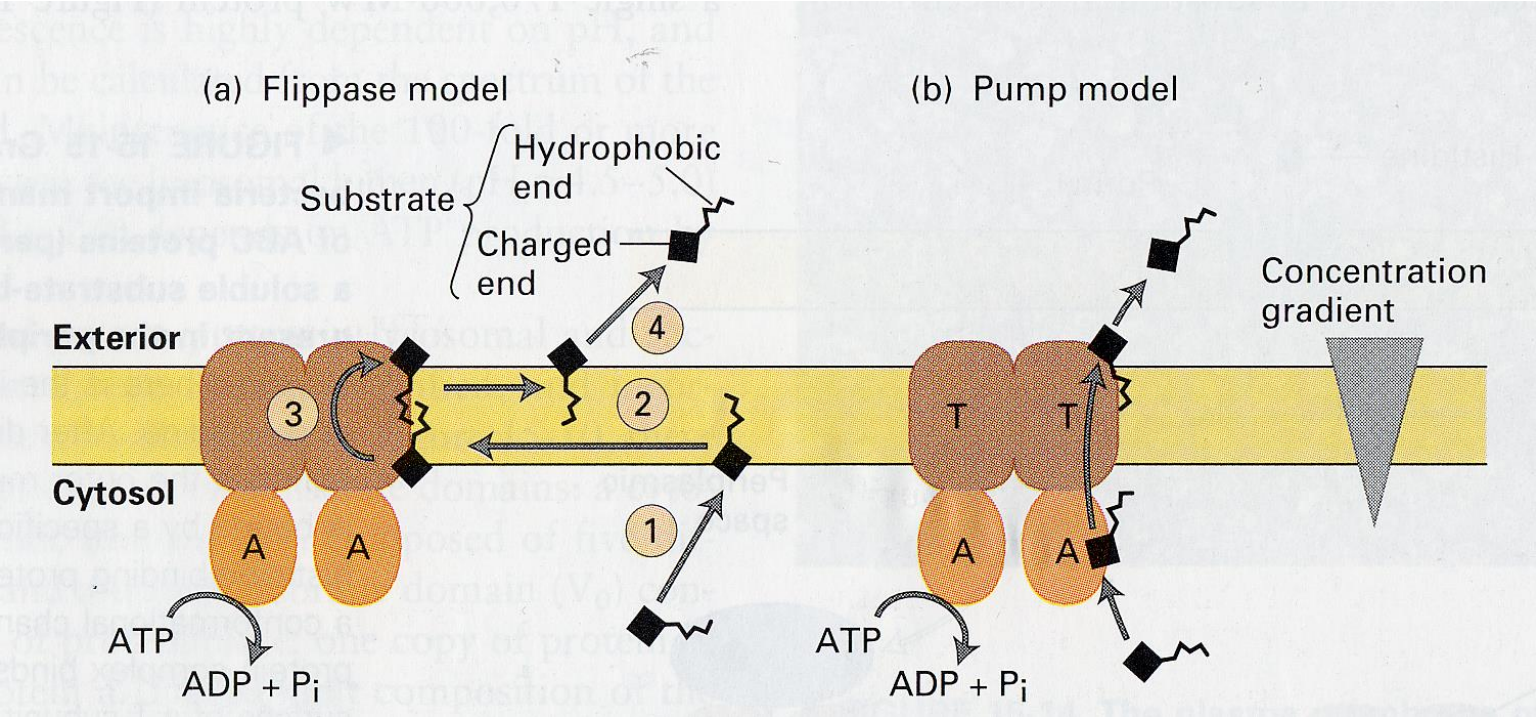
2 transmembranski (T) in 2 citosolni ATP-vezavni (A) podenoti

Shematski strukturni model sesalskih MDR transporterjev: splošni strukturni model ABC transporterjev



M ~ 170.000 kDa – ena veriga, štiri domene

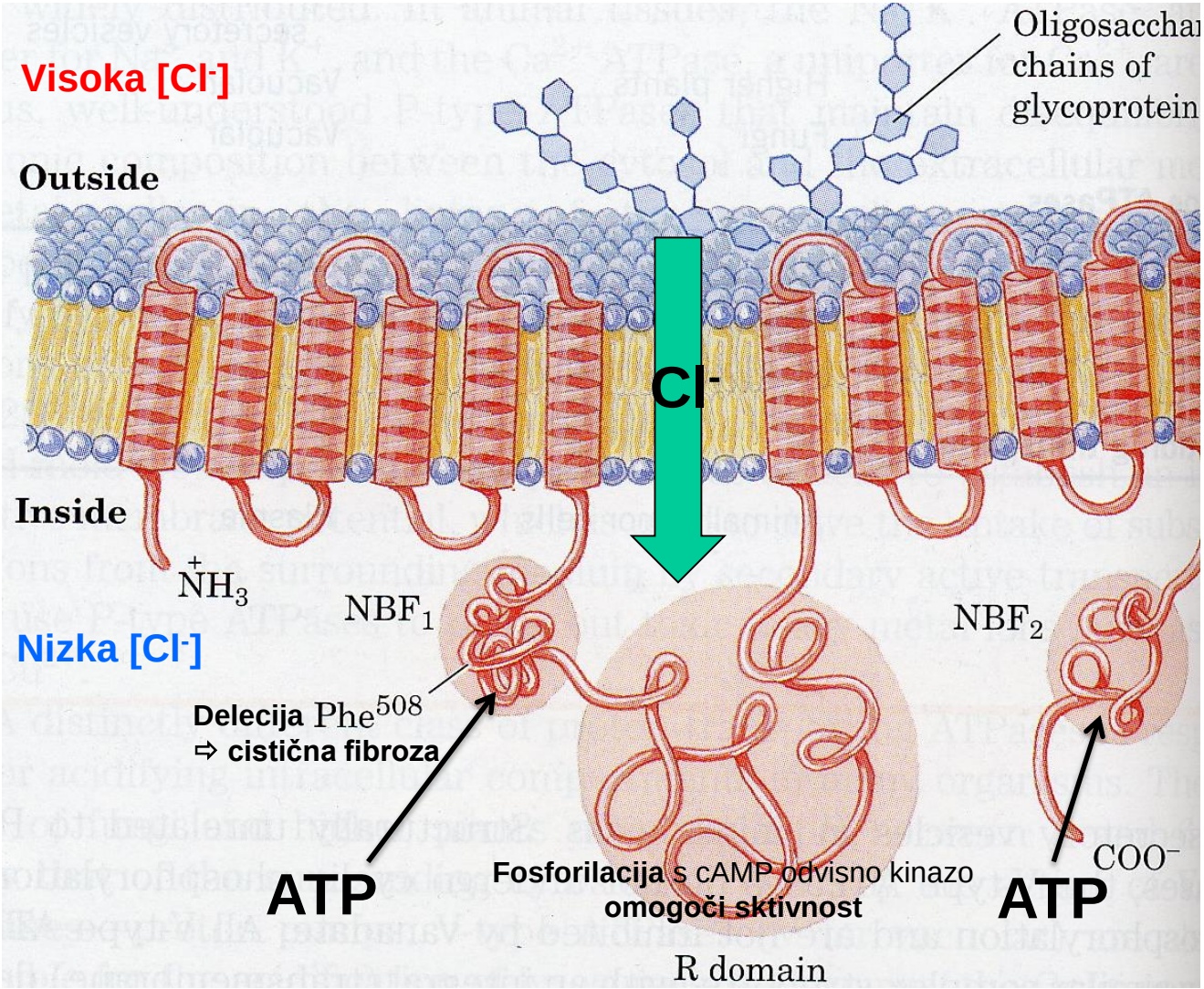
Načini delovanja ABC transporterjev



MDR2 (**ABCB4**) v PM jetrnih celic (prenos PL za tvorbo žolča)

MDR1 (**ABCB1**) v PM jetrnih, črevesnih in ledvičnih celic (izločanje naravnih toksinov in odpadnih produktov presnove); Bakterijske **PERMEAZE**

CFTR protein je ABC transporter – – ionski kanalček



Domenska struktura CFTR: T1-A1-R-T2-A2

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 snovi čez BM

	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*.