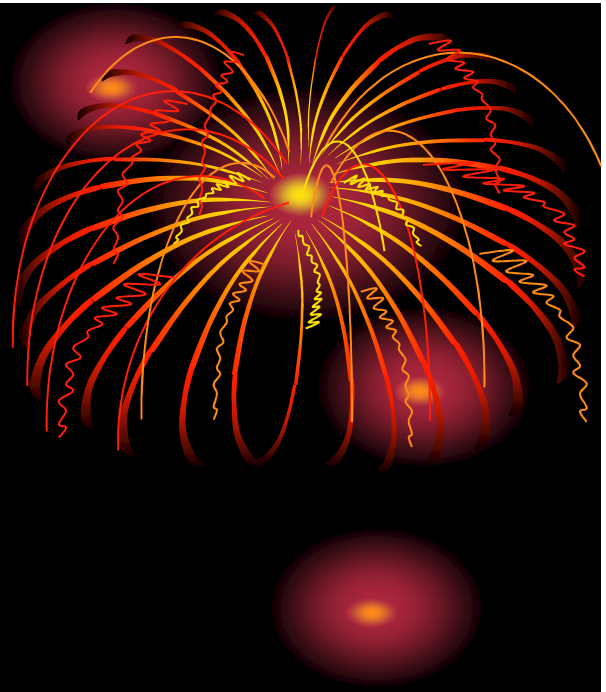
MEDICAL UNIVERSITY – PLEVEN
FACULTY OF PHARMACY

DIVISION OF PHYSICS AND BIOPHYSICS, HIGHER MATHEMATICS AND INFORMATION TECHNOLOGIES



LECTURE No11

ACTIVE TRANSPORT

Primary active transport. Sodium-potassium ATP-ase. Calcium ATP-ase. Basic steps of ion transport processes. Secondary (ion gradient-driven) active transport. Lactose permease requires a proton gradient. Putative mechanism of lactose transport in E. coli.

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Passive-mediated transporters, including ion channels, and proteins such as GLUT1, facilitate the transmembrane movement of substances according to the relative concentrations of the substance on either side of the membrane.

E.g. the glucose concentration in the blood plasma (5 mM) is generally higher than in cells, so GLUT1 allows glucose to enter the erythrocyte to be metabolized.

Many substances, however, are available on one side of a membrane in lower concentrations than are required on the other side of the membrane. Such substances must be actively and selectively transported across the membrane against their concentration gradients.

Def. Active transport is an endergonic process, that in most cases, is coupled to the hydrolysis of ATP.

The elucidation of the mechanism by which the chemical energy released from ATP is used to drive a mechanical process has been a challenging biochemical problem.

The membrane-bound ATPases translocate cations; these proteins carry out **primary active transport.**

In **secondary active transport,** the free energy of the electrochemical gradient generated by another mechanism, such as an ion-pumping ATPase, is used to transport a neutral molecule against its concentration gradient.

(Na–K)–ATPase

One of the most thoroughly studied active transport systems in the plasma membranes of higher eukaryotes.
It was first characterized by Jens Skou (a Danish chemist and Nobel laureate).

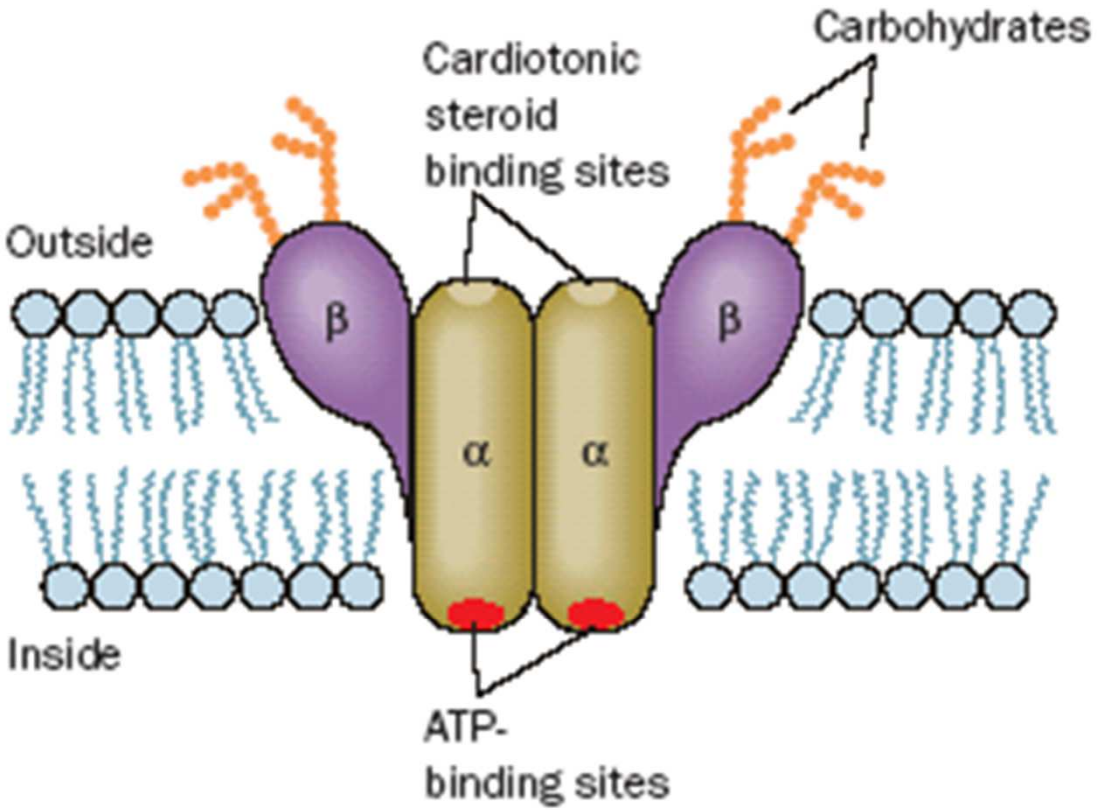
This transmembrane protein consists of two types of subunits:

- ✓ a 110-kD nonglycosylated α subunit that contains the enzyme's catalytic activity and ion-binding sites, and
- ✓ a 55-kD glycoprotein β subunit of unknown function.

Sequence analysis suggests that the α-subunit has eight transmembrane α-helical segments and two large cytoplasmic domains. The β subunit has a single transmembrane helix and a large extracellular domain.

The transporter's putative dimeric structure and its orientation in the plasma membrane.

Cardiotonic steroids bind to the external surface of the transporter, thereby inhibiting transport.



The (Na-K) –ATPase is often called the **(Na-K) pump** because it pumps Na^+ out of and K^+ into the cell with the concomitant hydrolysis of intracellular ATP.

The overall stoichiometry of the reaction is:

$$3\ Na^+(in) + 2\ K^+(out) + ATP + H_2O \rightleftharpoons 3\ Na^+(out) + 2\ K^+(in) + ADP + P_i$$

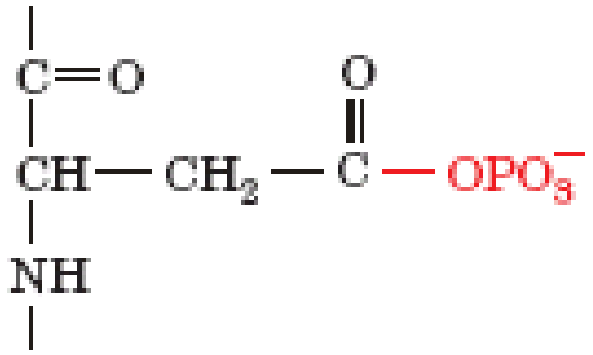
The (Na–K)–ATPase is an antiporter that generates a charge separation across the membrane.

The extrusion of Na^+ enables animal cells to control their water content osmotically; *without functioning (Na-K)–ATPases to maintain a low internal* $[Na]_i$*, water would osmotically rush in to such an extent that animal cells, which lack cell walls, would swell and burst.*

The electrochemical gradient generated by (Na-K)–ATPase is also responsible for the electrical excitability of nerve cells.

The key to the (Na–K)–ATPase is the phosphorylation of a specific Asp residue of the transport protein. ATP phosphorylates the transporter only in the presence of Na, whereas the resulting aspartyl phosphate residue is subjected to hydrolysis only in the presence of K.

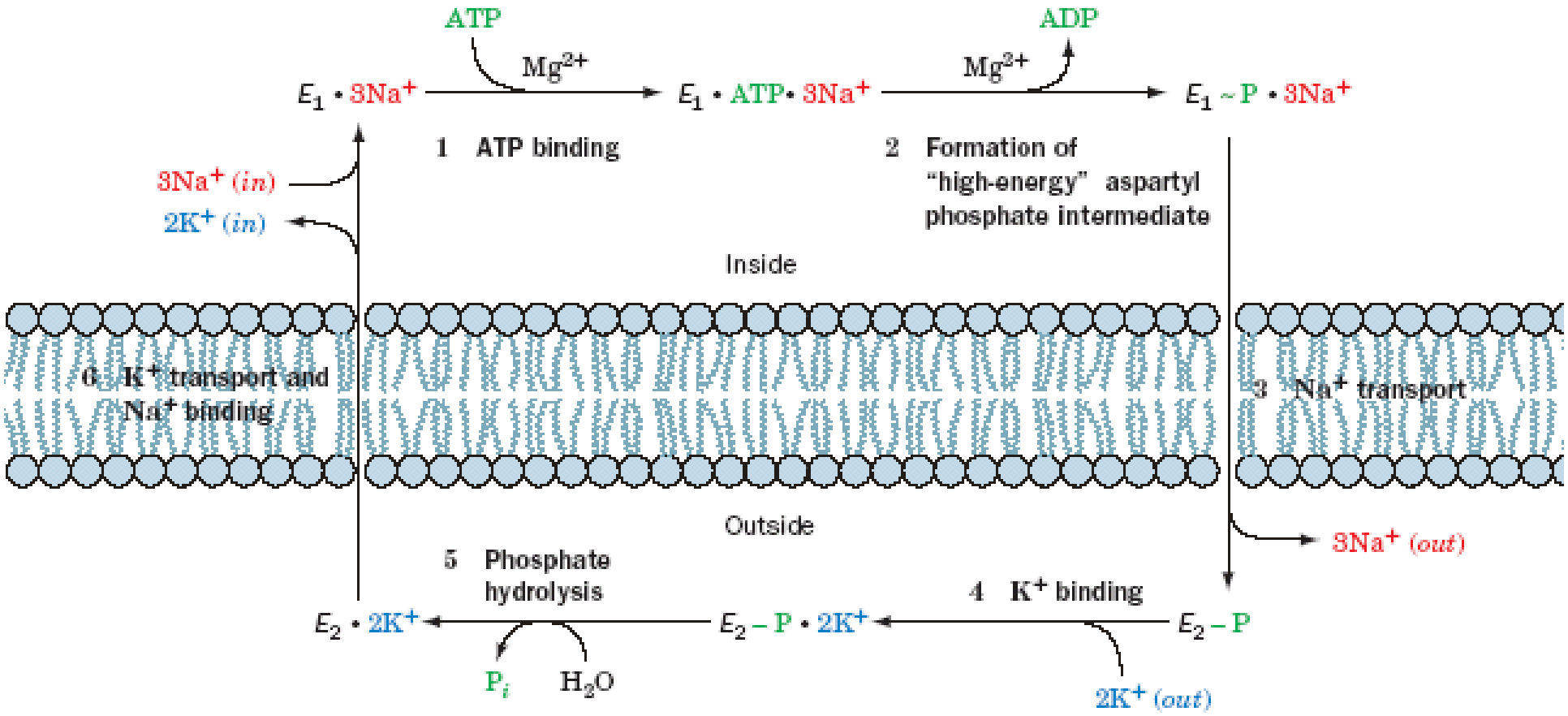
This suggests that the (Na–K)–ATPase has two conformational states (called E_1 and E_2) with different structures, different catalytic activities, and different ligand specificities.



Aspartyl phosphate residue

The protein appears to operate in the following manner:

1. The transporter in the E_1 state binds $3Na^+$ inside the cell and then binds ATP to yield an E_1-ATP-3 Na^+ complex.
2. ATP hydrolysis produces ADP and a "high-energy" aspartyl phosphate intermediate E_1~P-3 Na^+ (~ indicates a "high-energy" bond).
3. This "high-energy" intermediate relaxes to its "low-energy" conformation, E_2-P-3 Na^+, and releases its bound Na^+ outside the cell.
4. E_2-P binds $2K^+$ ions from outside the cell to form an E_2-P - 2 K^+ complex.
5. The phosphate group is hydrolyzed, yielding E_2 -2 K^+.
6. E_2-2 K^+ changes conformation, releases its two K^+ ions inside the cell, and replaces them with three Na^+ ions, thereby completing the transport cycle.


ATP
Mg2+
E1 • 3Na+
E1 • ATP• 3Na+
1 ATP binding
ADP
Mg2+
E1 ~ P • 3Na+
2 Formation of "high-energy" aspartyl phosphate intermediate
3Na+ (in)
2K+ (in)
Inside
6 K+ transport and Na+ binding
3 Na+ transport
Outside
3Na+ (out)
5 Phosphate hydrolysis
4 K+ binding
E2 • 2K+
E2 – P • 2K+
E2 – P
Pi
H2O
2K+ (out)

Cardiac glycosides - natural products that increase the intensity of heart muscle contraction.

Digitalis - an extract of purple foxglove leaves, contains a mixture of cardiac glycosides including **digitoxin** (long used to treat congestive heart failure).

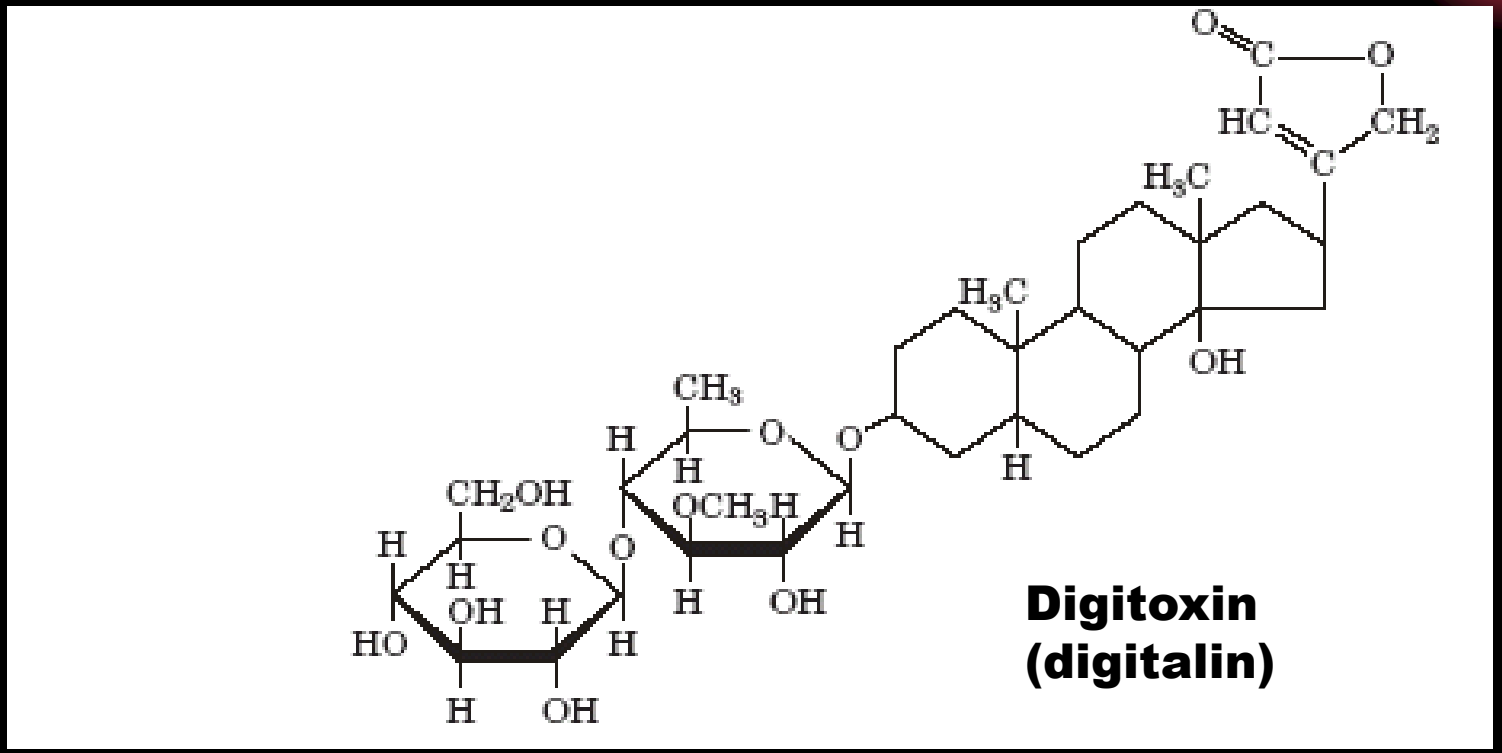
Ouabain - a product of the East African ouabio tree (long used as an arrow poison).

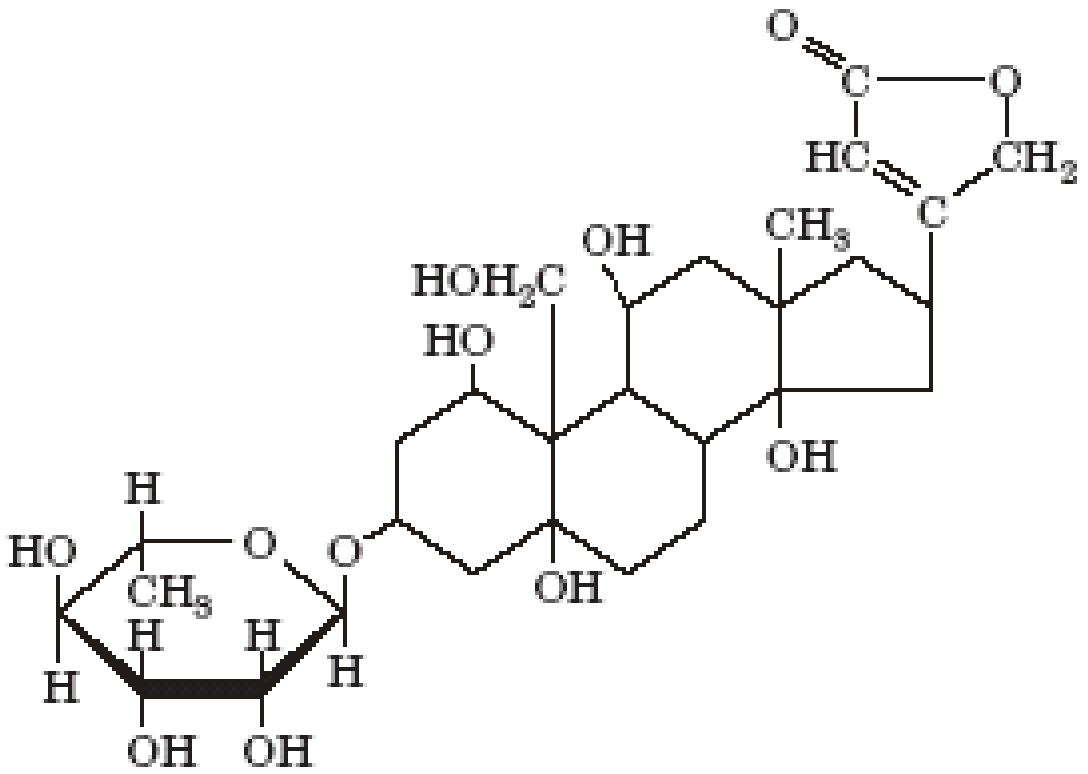
These two steroids, which are still among the most commonly prescribed cardiac drugs, inhibit the (Na–K)– ATPase by binding strongly to an externally exposed portion of the protein so as to block Step 5.

The resultant increase in intracellular $[Na^+]_i$ stimulates the cardiac (Na^+–Ca^{2+}) antiport system, which pumps Na out of and Ca^{2+} into the cell, ultimately boosting the $[Ca^{2+}]$ in the sarcoplasmic reticulum.

The release of Ca^{2+} to trigger muscle contraction produces a larger than normal increase in cytosolic $[Ca^{2+}]_i$, thereby intensifying the force of cardiac muscle contraction.

Ouabain, which was once thought to be produced only by plants, has recently been discovered to be an animal hormone that is secreted by the adrenal cortex and functions to regulate cellular $[Na]_i$ and overall body salt and water balance.





Ouabain

Ca^{2+}–ATPase

Transient increases in cytosolic Ca^{2+} trigger numerous cellular responses: muscle contraction, release of neurotransmitters, glycogen breakdown, oxidative metabolism activation.

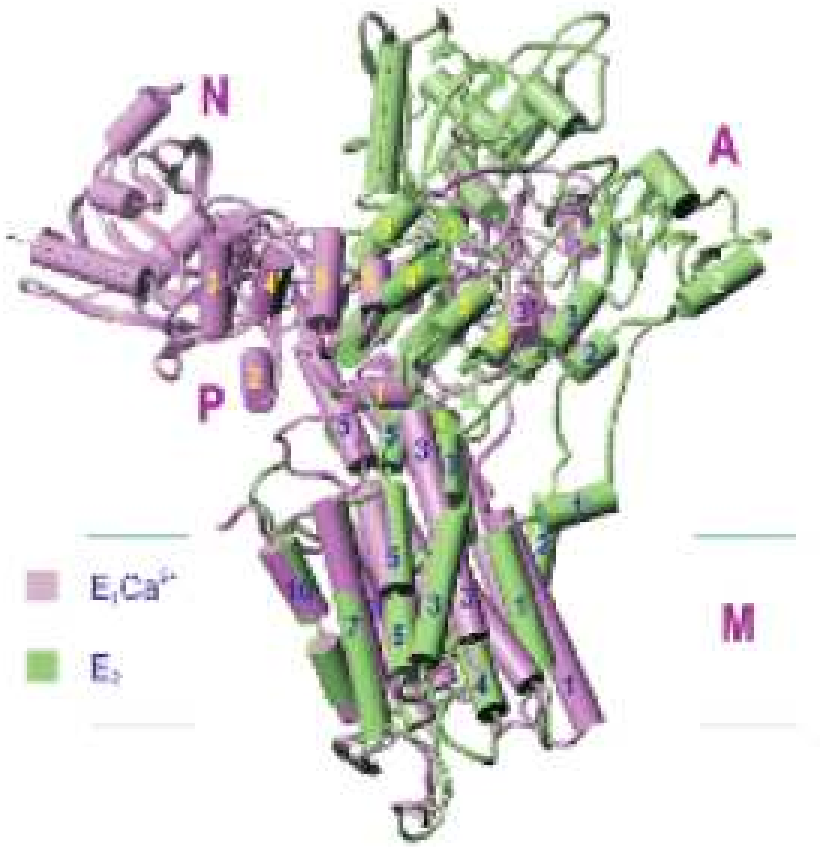
The $[Ca^{2+}]_i$ in the cytosol (0.1 μM) is four orders of magnitude less than it is in the extracellular spaces (1500 μM); Ca_i^{2+} might otherwise combine with phosphate to form $Ca_3(PO_4)_2$, which has a maximum solubility of only 65μM.

The large concentration gradient is maintained by the active transport of Ca^{2+} across the plasma membrane and the endoplasmic reticulum (the sarcoplasmic reticulum in muscle) by a Ca^{2+} pump.

Ca^{2+}– ATPase actively pumps $2Ca^{2+}$ ions out of the cytosol at the expense of ATP hydrolysis, while countertransporting two or three protons. The mechanism resembles that of the (Na–K)–ATPase.

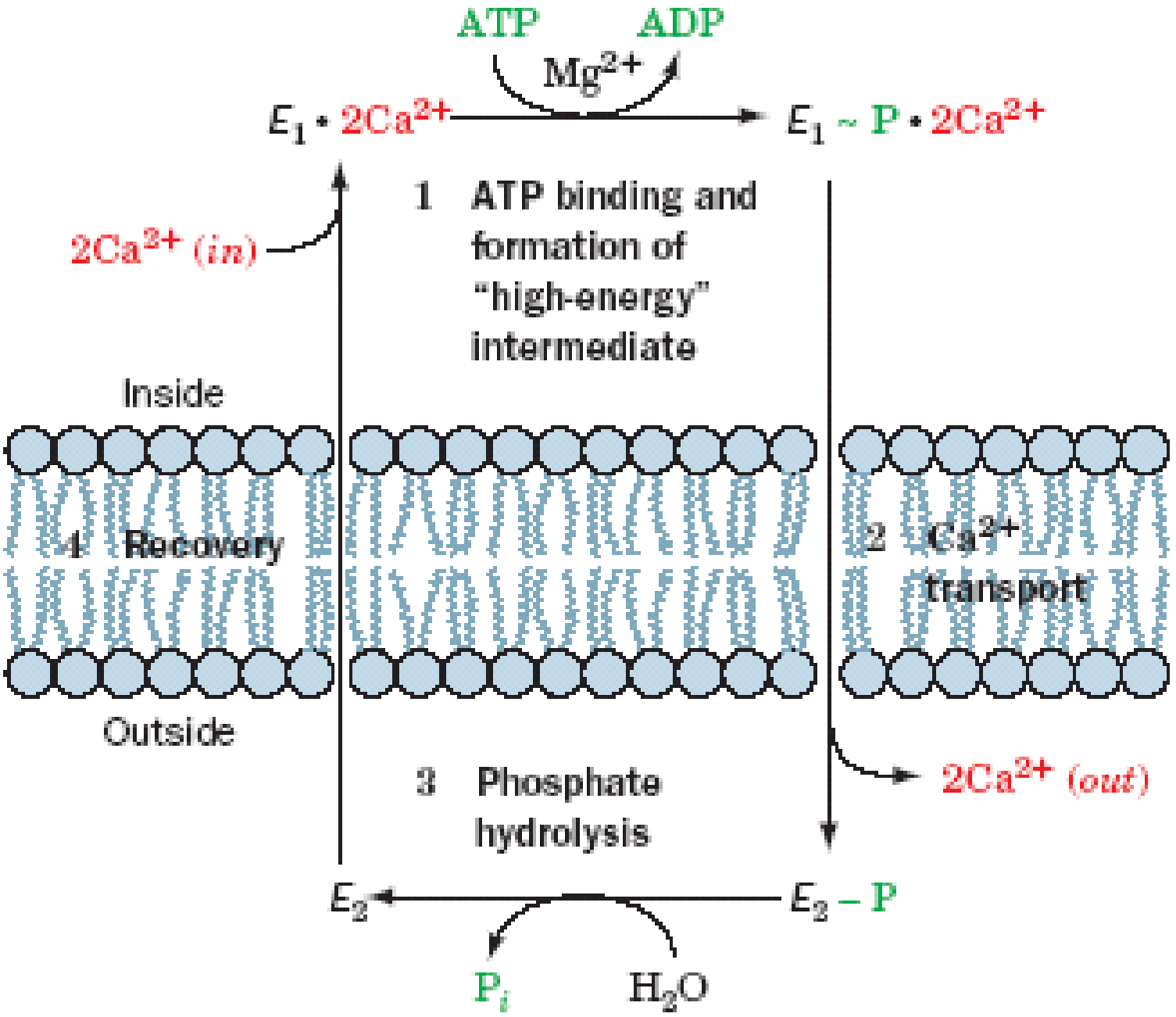
Two Ca^{2+} ions bind within a bundle of 10 trans-membrane helices. Three additional domains form a large structure on the cytoplasmic side of the membrane. The differences between the Ca^{2+}-bound (E_1) and the Ca^{2+}-free (E_2) structures indicate that the transporter undergoes extensive rearrangements during the reaction cycle.

These changes apparently mediate communication between the Ca^{2+} binding sites and the 80-Å-distant site where bound ATP is hydrolyzed.



X-Ray structures of the Ca^{2+}-free (E_2) and Ca^{2+}-bound (E_1) Ca^{2+}–ATPase.

These proteins, which are superimposed on their transmembrane domains, are viewed from within the membrane with the cytosolic side up. Ten transmembrane helices form the M (for membrane) domain, ATP binds to the N (for nucleotide-binding) domain, the Asp residue that is phosphorylated during the reaction cycle is located on the P (for phosphorylation) domain, and the A (for actuator) domain participates in the transmission of major conformational changes. A dashed line highlights the orientation of a helix in the N domain in the two conformations and the horizontal lines delineate the membrane.


ATP
ADP
Mg^{2+}
$E_1 \cdot 2Ca^{2+}$
$E_1 \sim P \cdot 2Ca^{2+}$
$2Ca^{2+}$ (*in*)
1 ATP binding and formation of "high-energy" intermediate
Inside
4 Recovery
2 Ca^{2+} transport
Outside
3 Phosphate hydrolysis
$2Ca^{2+}$ (*out*)
E_2
$E_2 - P$
P_i
H_2O

Ion Gradient–Driven Active Transport

Systems such as the (Na–K)–ATPase generates electrochemical gradients across membranes. The free energy stored in an electrochemical gradient can be harnessed to power various endergonic physiological processes.

For example, cells of the intestinal epithelium take up dietary glucose by Na-dependent symport. The immediate energy source for this "uphill" transport process is the Na gradient.

This process is an example of secondary active transport because the Na gradient in these cells is maintained by the (Na–K)–ATPase. The Na–glucose transport system concentrates glucose inside the cell. Glucose is then transported into the capillaries through a passive-mediated glucose uniport (which resembles GLUT1).

- Since glucose enhances Na resorption, which in turn enhances water resorption, glucose, in addition to salt and water, should be fed to individuals suffering from salt and water losses due to diarrhea.
- The brushlike villi lining the small intestine greatly increase its surface area (a), thereby facilitating the absorption of nutrients.

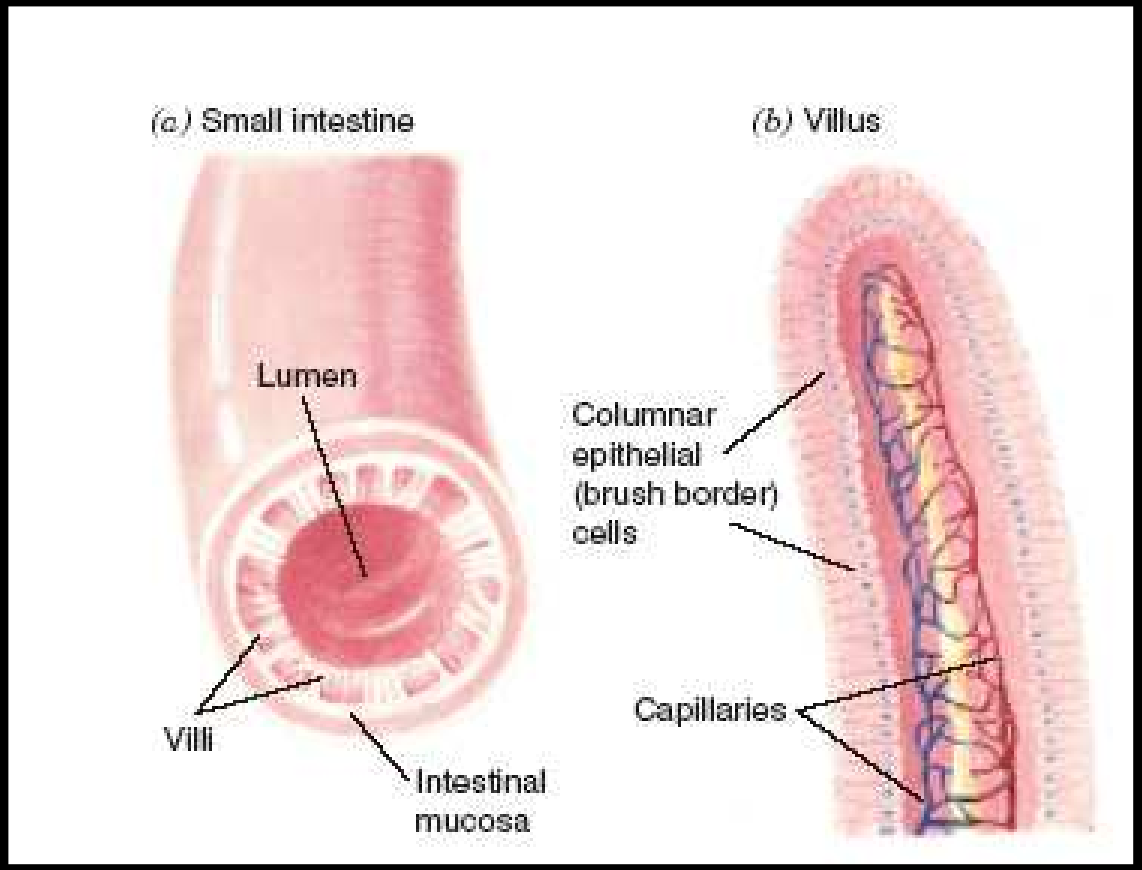
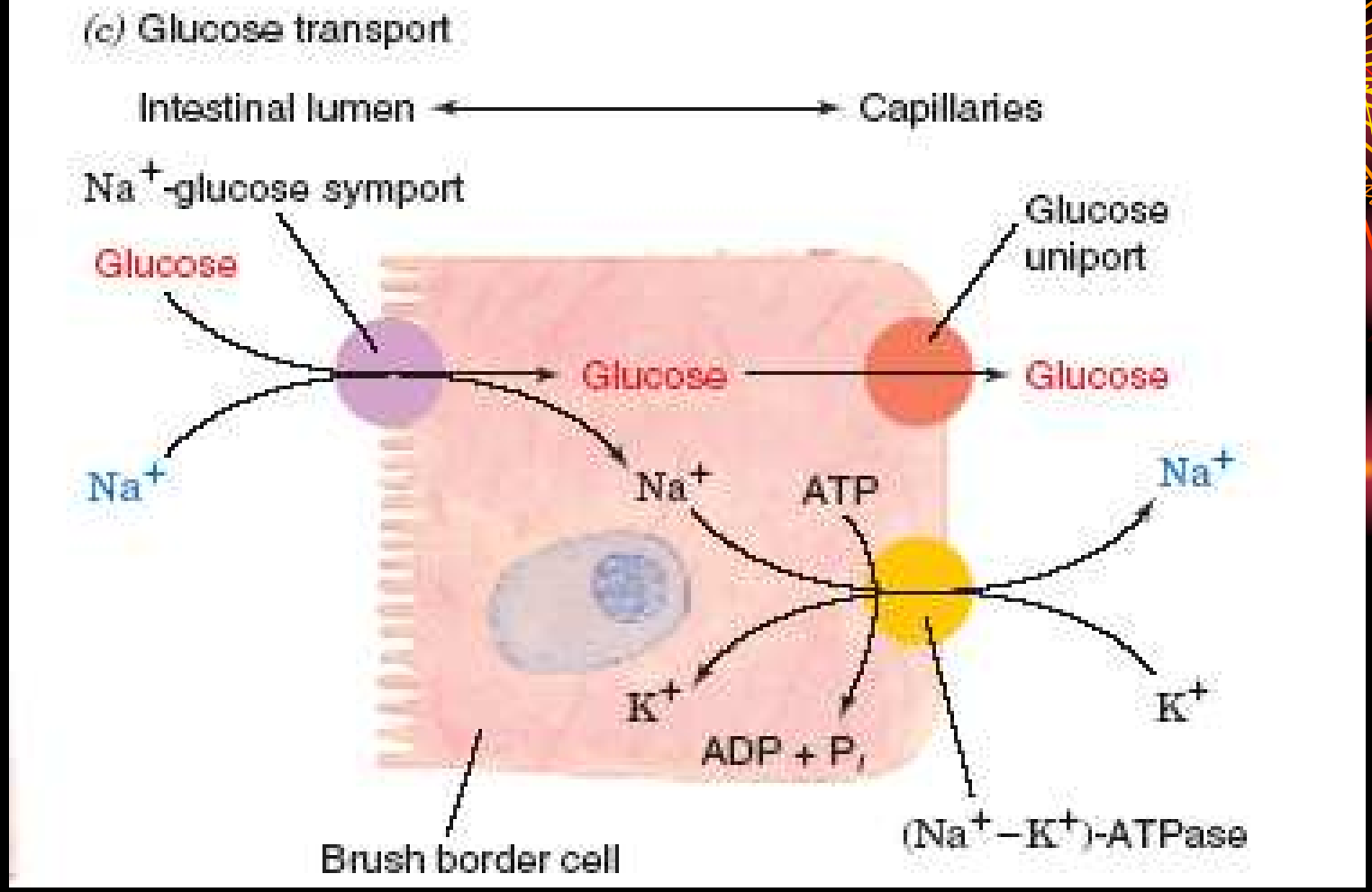


Fig. Glucose transport in the intestinal epithelium.

The brush border cells from which the villi are formed (b) concentrate glucose from the intestinal lumen in symport with Na (c),



The process is driven by the (Na–K)–ATPase, which is located on the capillary side of the cell and functions to maintain a low internal $[Na]_i$. The glucose is exported to the bloodstream via a separate passive-mediated uniport system similar to GLUT1.

Lactose Permease Requires a Proton Gradient

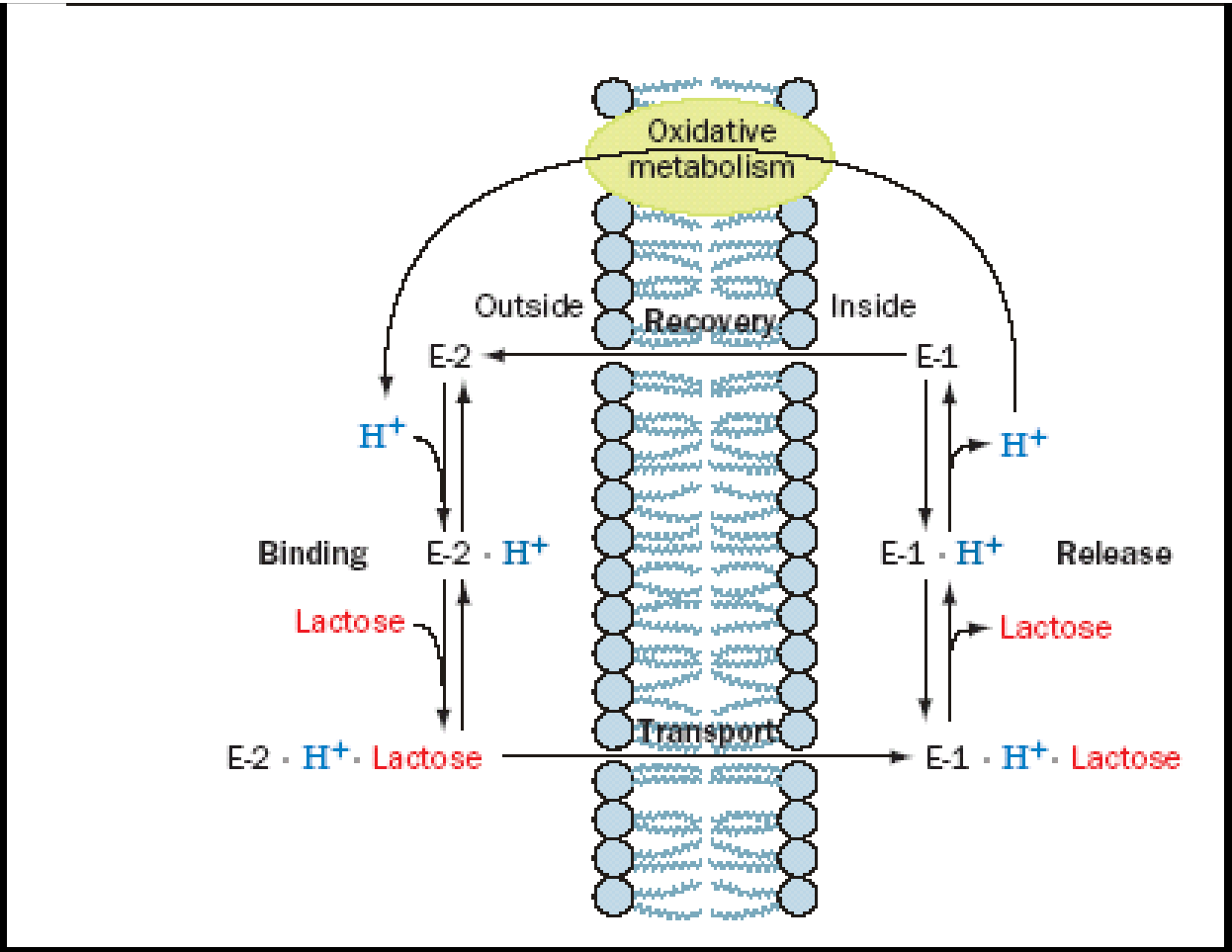
Gram-negative bacteria such as E. coli contain several active transport systems for concentrating sugars. One extensively studied system, lactose permease (a.k.a galactoside permease), utilizes the proton gradient across the bacterial cell membrane to cotransport H^+ and lactose.

The proton gradient is metabolically generated through oxidative metabolism in a manner similar to that in mitochondria. The electrochemical potential gradient created by both these systems is used mainly to drive the synthesis of ATP.

As does (Na–K)–ATPase, lactose permease has two major conformational states (Fig.):

1. E-1, which has a low-affinity lactose-binding site facing the interior of the cell.
2. E-2, which has a high-affinity lactose-binding site facing the exterior of the cell.

Ronald Kaback established that E-1 and E-2 can interconvert only when their H^+ and lactose binding sites are either both filled or both empty.This prevents dissipation of the H gradient without cotransport of lactose into the cell. It also prevents transport of lactose out of the cell since this would require cotransport of H against its concentration gradient.

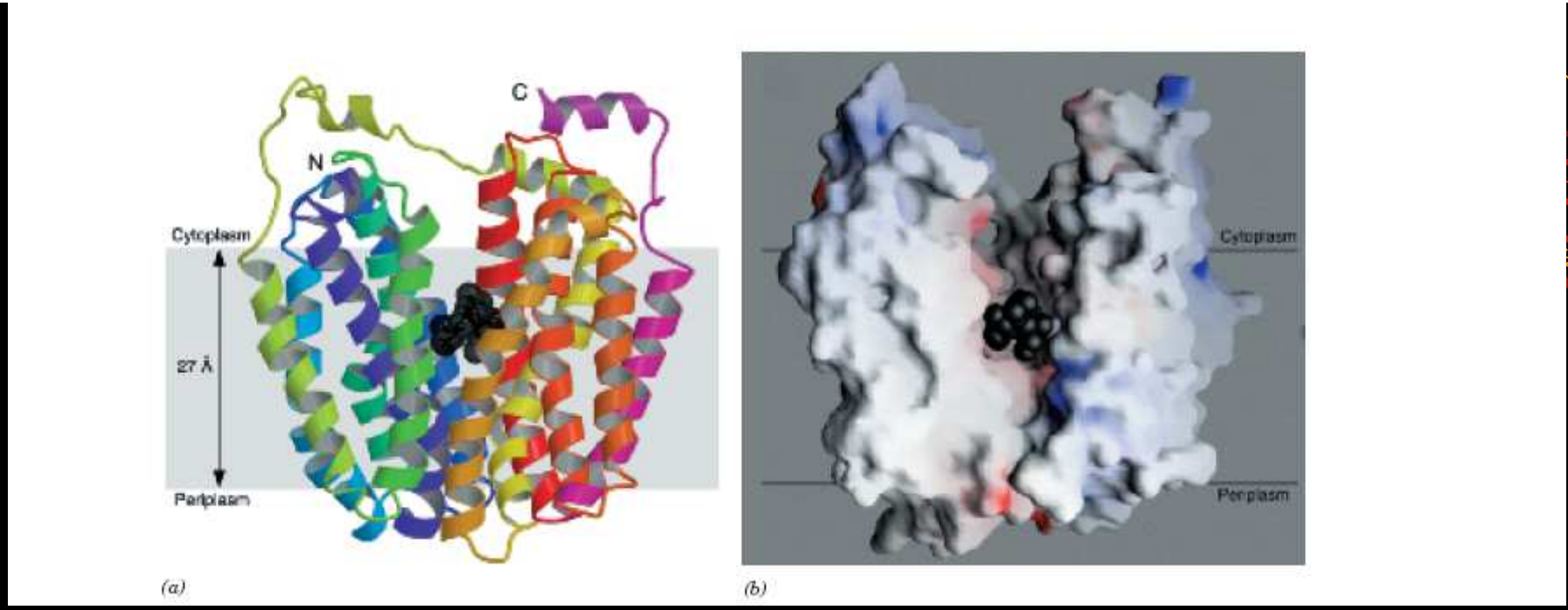


Scheme for the cotransport of H and lactose by lactose permease in E. coli.

H^+ binds first to E-2 outside the cell, followed by lactose. They are sequentially released from E-1 inside the cell. E-2 must bind to lactose and H^+ in order to change conformation to E-1, thereby cotransporting these substances into the cell. E-1 changes conformation to E-2 when neither lactose nor H^+ is bound, thus completing the transport cycle.

The X-ray structure of lactose permease in complex with a tight-binding lactose analog, reveals that this protein consists of two structurally similar and twofold symmetrically positioned domains containing six transmembrane helices each.

A large internal hydrophilic cavity is open to the cytoplasmic side of the membrane so that the structure represents the E-1 state of the protein.The lactose analog is bound in the cavity at a position that is approximately equidistant from both sides of the membrane, consistent with the model that the lactose binding site is alternately accessible from each side of the membrane.



X-Ray structure of lactose permease from E. coli. (a) Ribbon diagram as viewed from the membrane with the cytoplasmic side up. The protein's 12 transmembrane helices are colored in rainbow order with the N-terminus purple and the C-terminus pink. The bound lactose analog is represented by black spheres. (b) Surface model with the two helices closest to the viewer in Part a removed to reveal the lactosebinding cavity. The surface is colored according to its electrostatic potential with positively charged areas blue, negatively charged areas red, and neutral areas white.