Membrane Biochemistry

Lectures by

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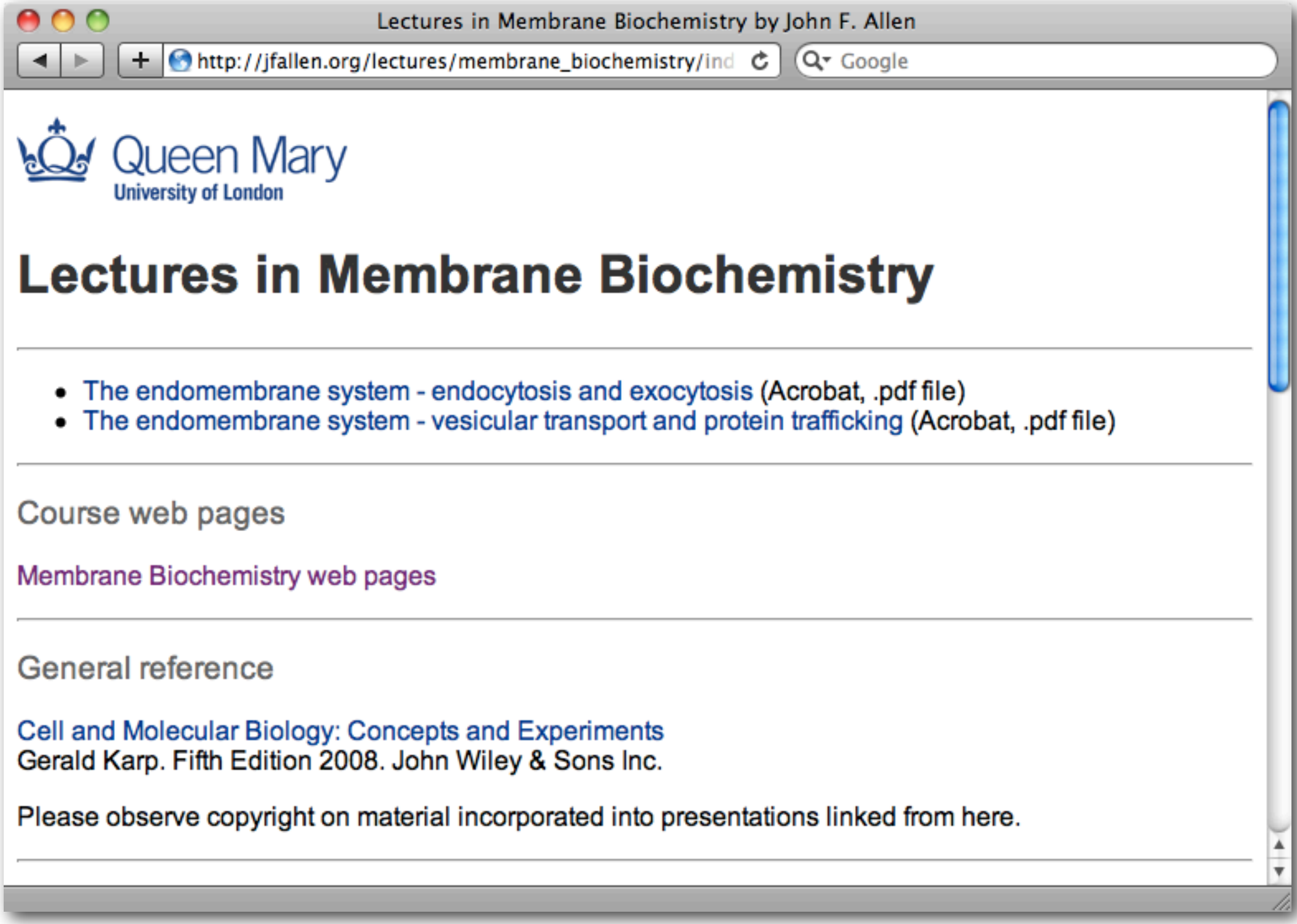
Lectures in Membrane Biochemistry by John F. Allen
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The endomembrane system - endocytosis and exocytosis (Acrobat, .pdf file)
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Course web pages
Membrane Biochemistry web pages
General reference
Cell and Molecular Biology: Concepts and Experiments
Gerald Karp. Fifth Edition 2008. John Wiley & Sons Inc.
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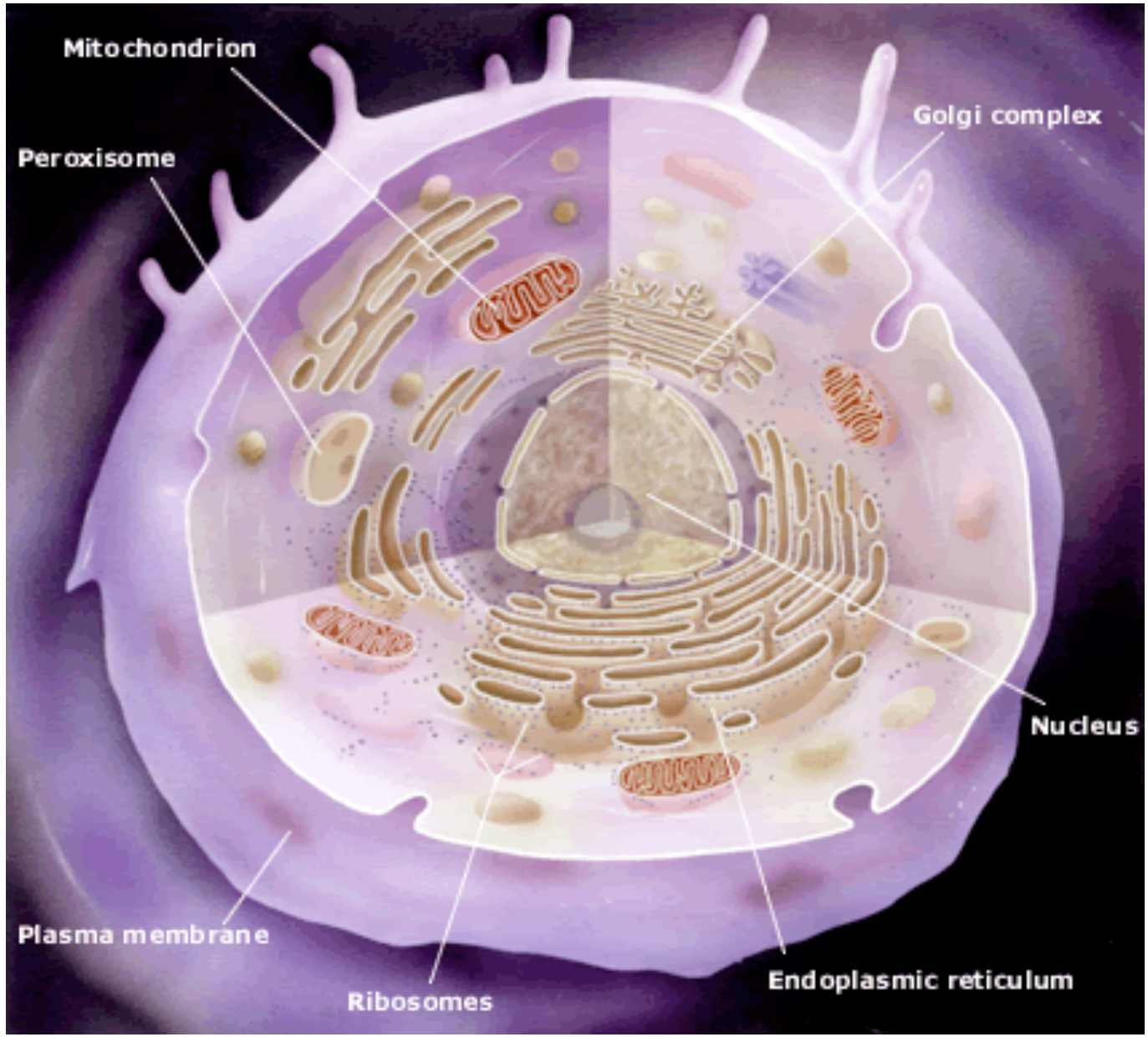


All living organisms are made up of cells. The eukaryotic cell contains a number of different types of organelles each of which is surrounded by a tightly sealed membrane.

"Illustrated Information". Nobelprize.org. 30 Oct 2010
http://nobelprize.org/nobel_prizes/medicine/laureates/1999/illpres/illpres.html

Membrane Transport

Transport across biological membranes

- Transport of proteins
- Transport of ions
- Transport of small molecules

Membrane Transport

Transport across biological membranes

- Transport of ions
- Transport of small molecules

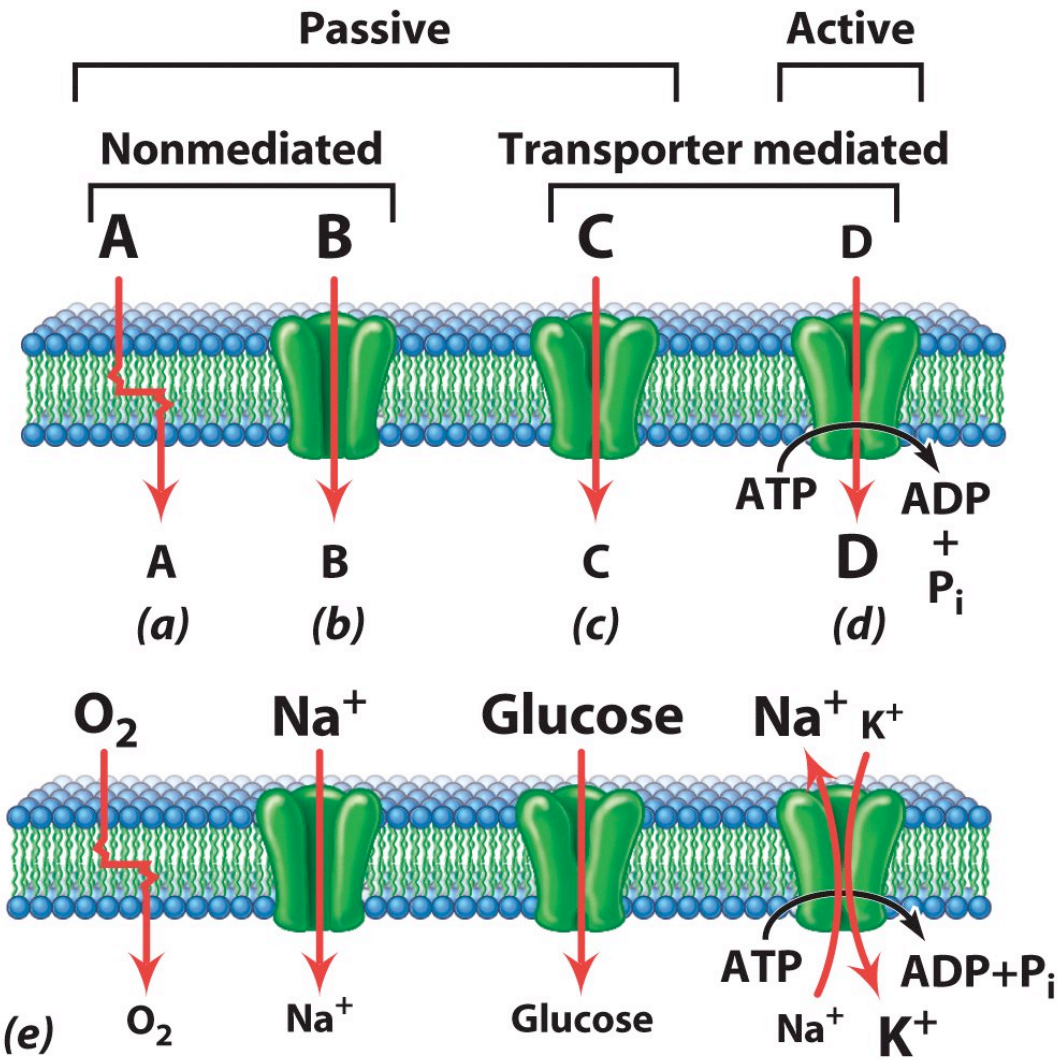


Figure 4-32 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

FIGURE 4.32 **Four basic mechanisms by which solute molecules move across membranes.** The relative sizes of the letters indicate the directions of the concentration gradients. (*a*) Simple diffusion through the bilayer, which always proceeds from high to low concentration. (*b*) Simple diffusion through an aqueous channel formed within an integral membrane protein or a cluster of such proteins. As in *a*, movement is always down a concentration gradient. (*c*) Facilitated diffusion in which solute molecules bind specifically to a membrane protein carrier (a facilitative transporter). As in *a* and *b*, movement is always from high to low concentration. (*d*) Active transport by means of a protein transporter with a specific binding site that undergoes a change in affinity driven with energy released by an exergonic process, such as ATP hydrolysis. Movement occurs against a concentration gradient. (*e*) Examples of each type of mechanism as it occurs in the membrane of an erythrocyte.

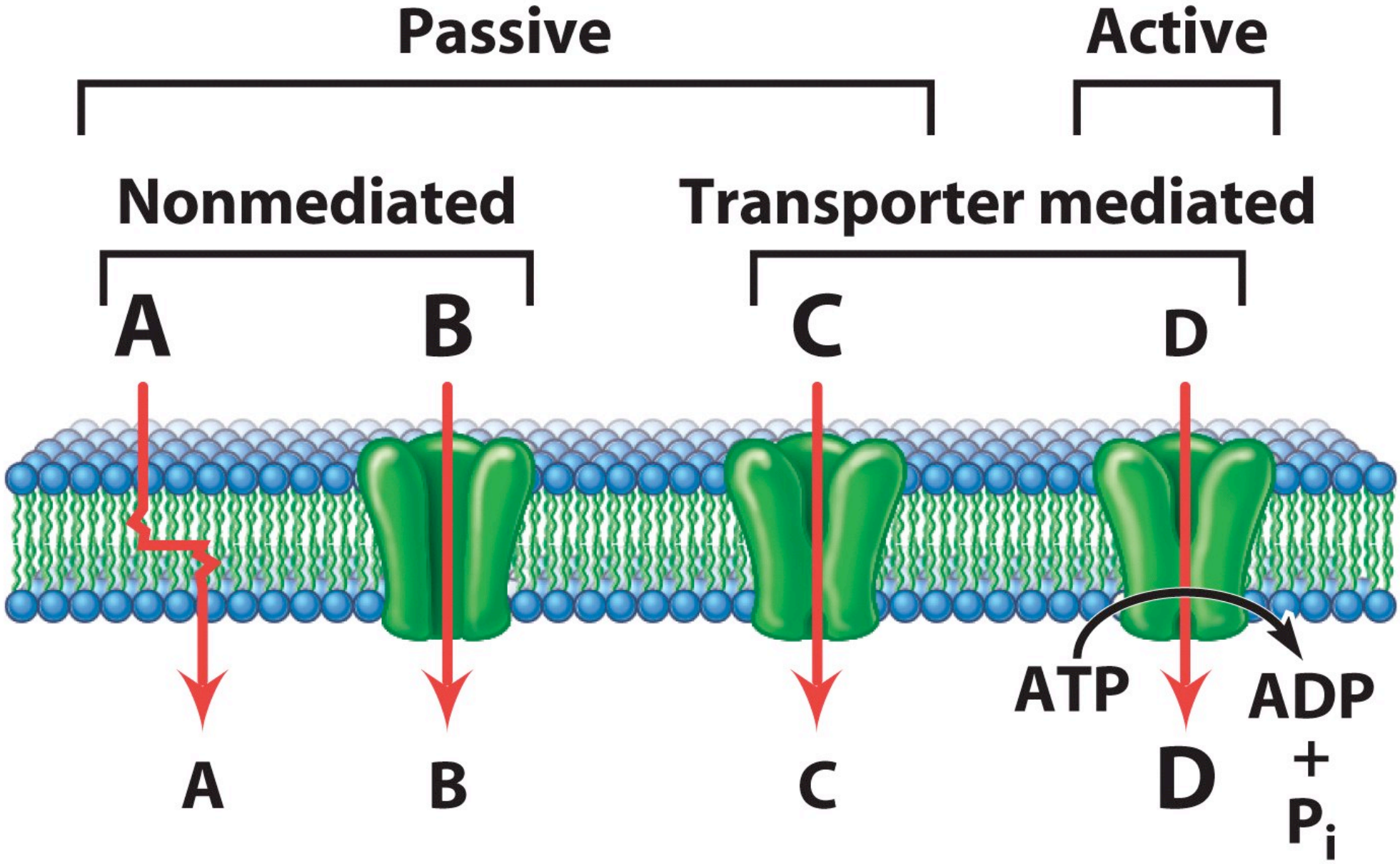


Figure 4-32 part 1 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

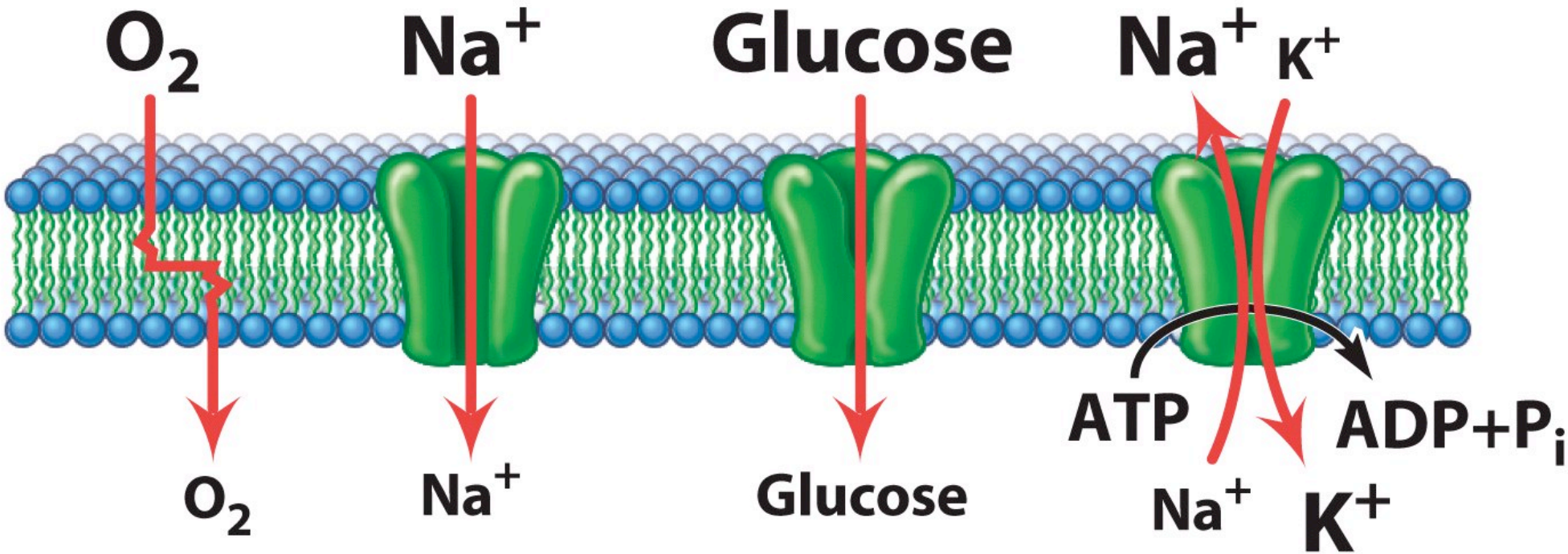


Figure 4-32 part 2 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

Membrane Transport

Transport across biological membranes

Transport of ions and small molecules by means of:

- Free diffusion through the membrane itself
- Free diffusion through channels in the membrane
- Facilitated diffusion
- Active transport

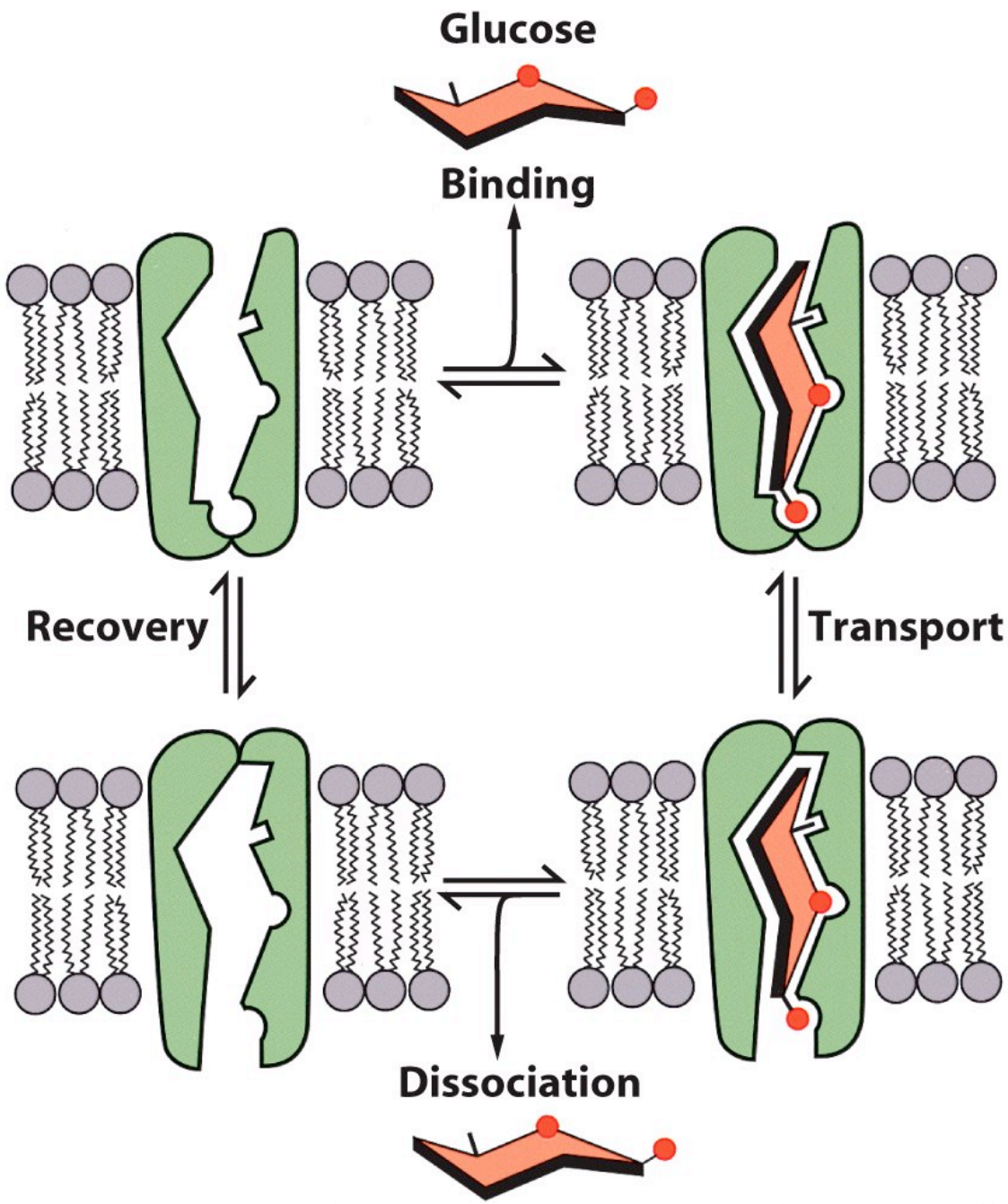


FIGURE 4.43 **Facilitated diffusion.** A schematic model for the facilitated diffusion of glucose depicts the alternating conformation of a carrier that exposes the glucose binding site to either the inside or outside of the membrane. (AFTER S. A. BALDWIN AND G. E. LIENHARD, TRENDS BIOCHEM. SCI. 6:210, 1981.)

Membrane Transport

Transport across biological membranes

Ion channels:

- Simple
- Gated
  - Voltage-gated channels
  - Ligand-gated channels
  - Mechano-gated channels

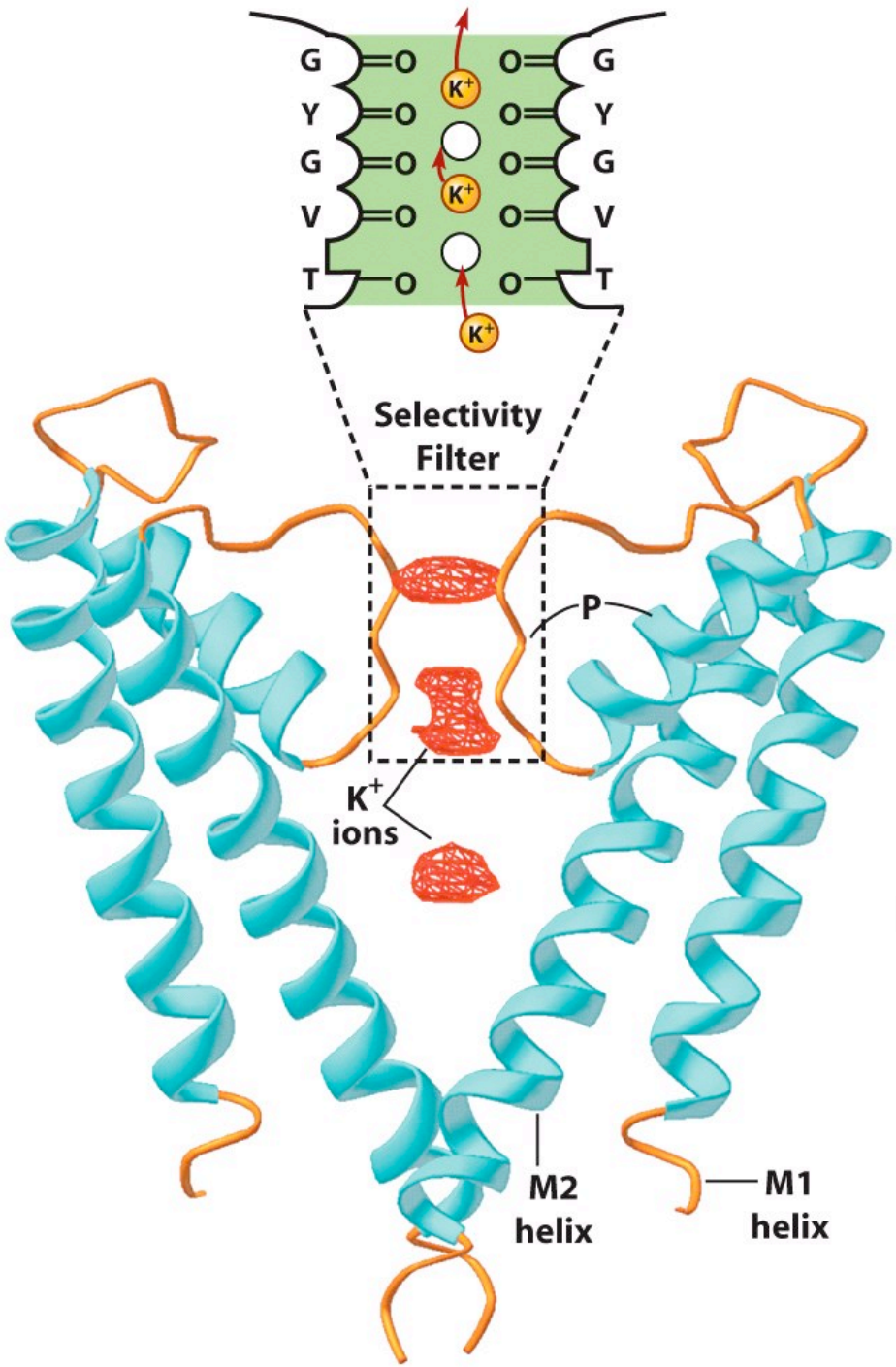
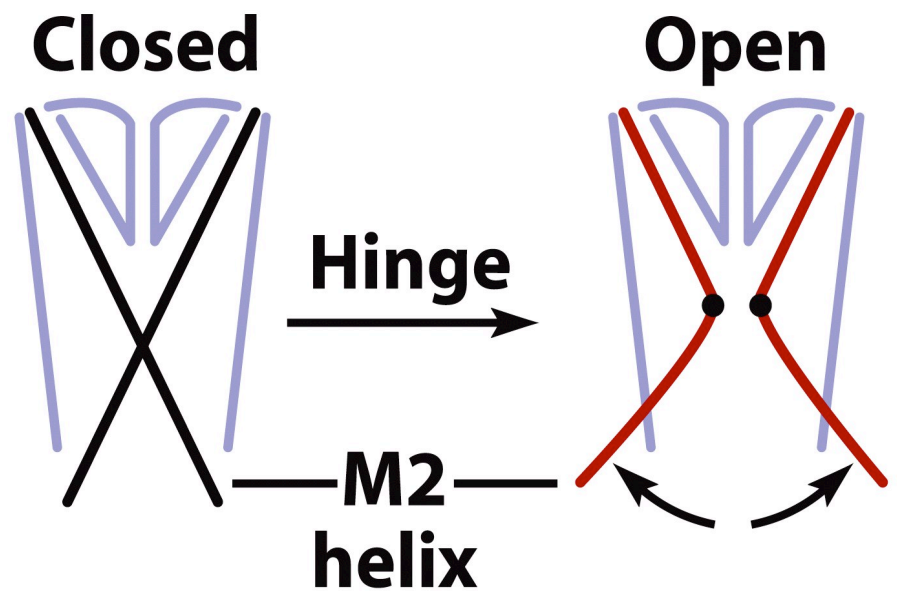


Figure 4-38 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

FIGURE 4.38 **Three dimensional structure of the bacterial KcsA channel and the selection of K^+ ions.** This K^+ ion channel consists of four subunits, two of which are shown here. Each subunit is comprised of M1 and M2 helices joined by a P (pore) segment consisting of a short helix and a nonhelical portion that lines the channel through which the ions pass. A portion of each P segment contains a conserved pentapeptide (GYGVT) whose residues line the selectivity filter that screens for K^+ ions. The oxygen atoms of the carbonyl groups of these residues project into the channel where they can interact selectively with K^+ ions (indicated by the red mesh objects) within the filter. As indicated in the top inset, the selectivity filter contains four rings of carbonyl O atoms and one ring of threonyl O atoms; each of these five rings contains four O atoms, one donated by each subunit. The diameter of the rings is just large enough so that eight O atoms can coordinate a single K^+ ion, replacing its normal water of hydration. Although four K^+ binding sites are shown, only two are occupied at one time. (FROM RODERICK MACKINNON, REPRINTED WITH PERMISSION FROM NATURE MED. 5:1108, 1999; COPYRIGHT 1999, MACMILLAN MAGAZINES LIMITED.)



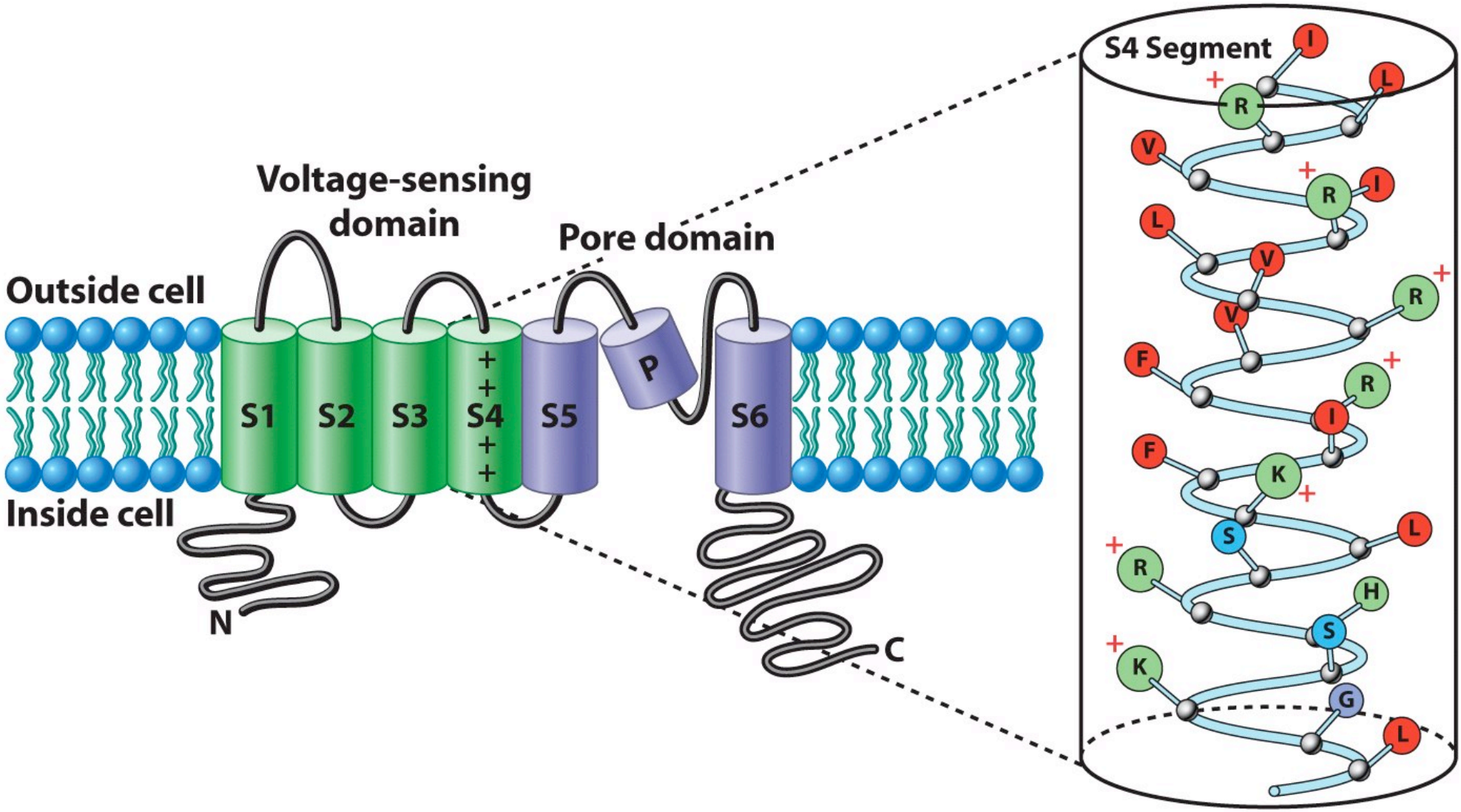


Figure 4-40 Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

FIGURE 4.40 The structure of a eukaryotic, voltage-gated K^+ channel. A two-dimensional portrait of a K^+ channel subunit showing its six transmembrane helices and a portion of the polypeptide (called the pore helix or P) that dips into the protein to form part of the channel's wall. The inset shows the sequence of amino acids of the positively charged S4 helix of the *Drosophila* K^+ *Shaker* ion channel, which serves as a voltage sensor. The positively charged side chains are situated at every third residue along the otherwise hydrophobic helix. This member of the Kv family is called a *Shaker* channel because flies with certain mutations in the protein shake vigorously when anesthesized with ether. The *Shaker* channel was the first K^+ channel to be identified and cloned in 1987.

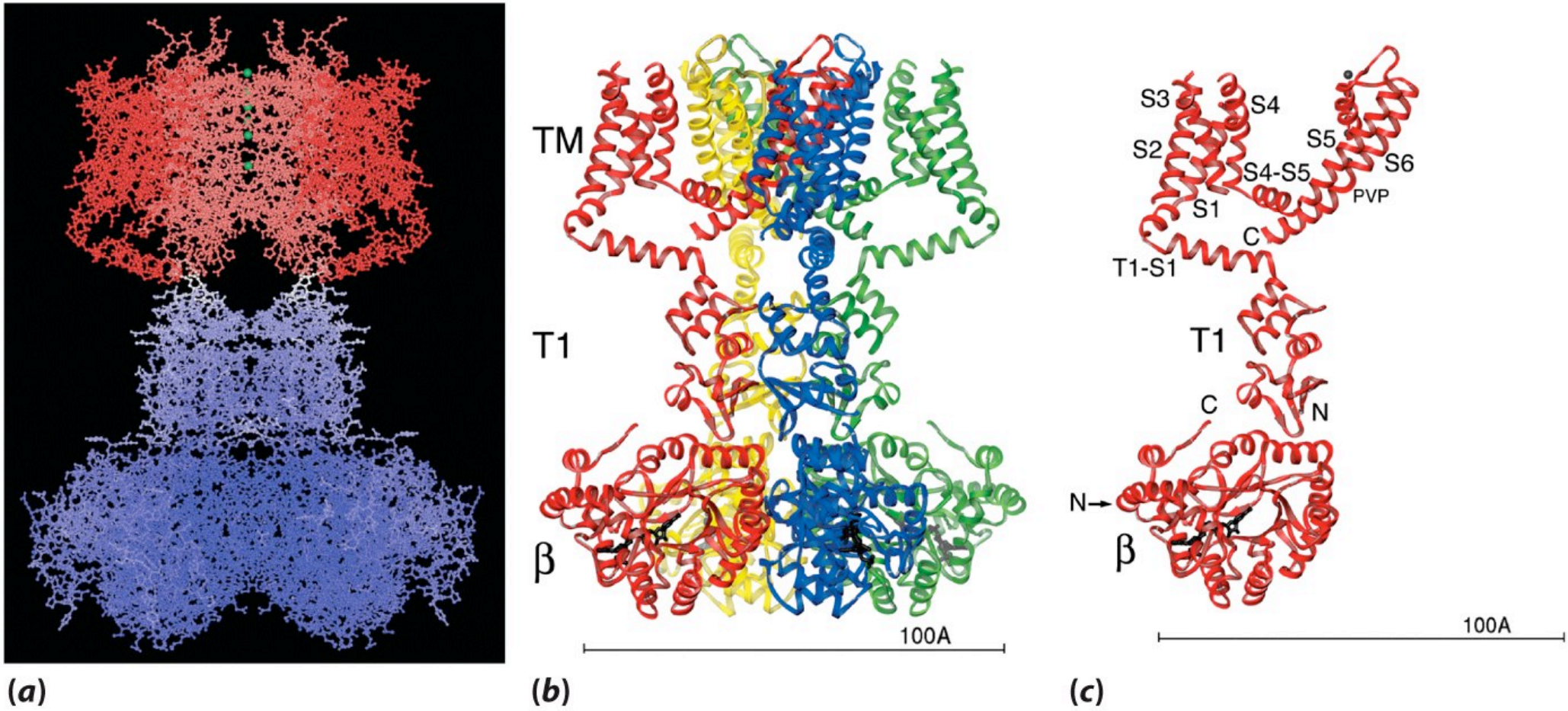
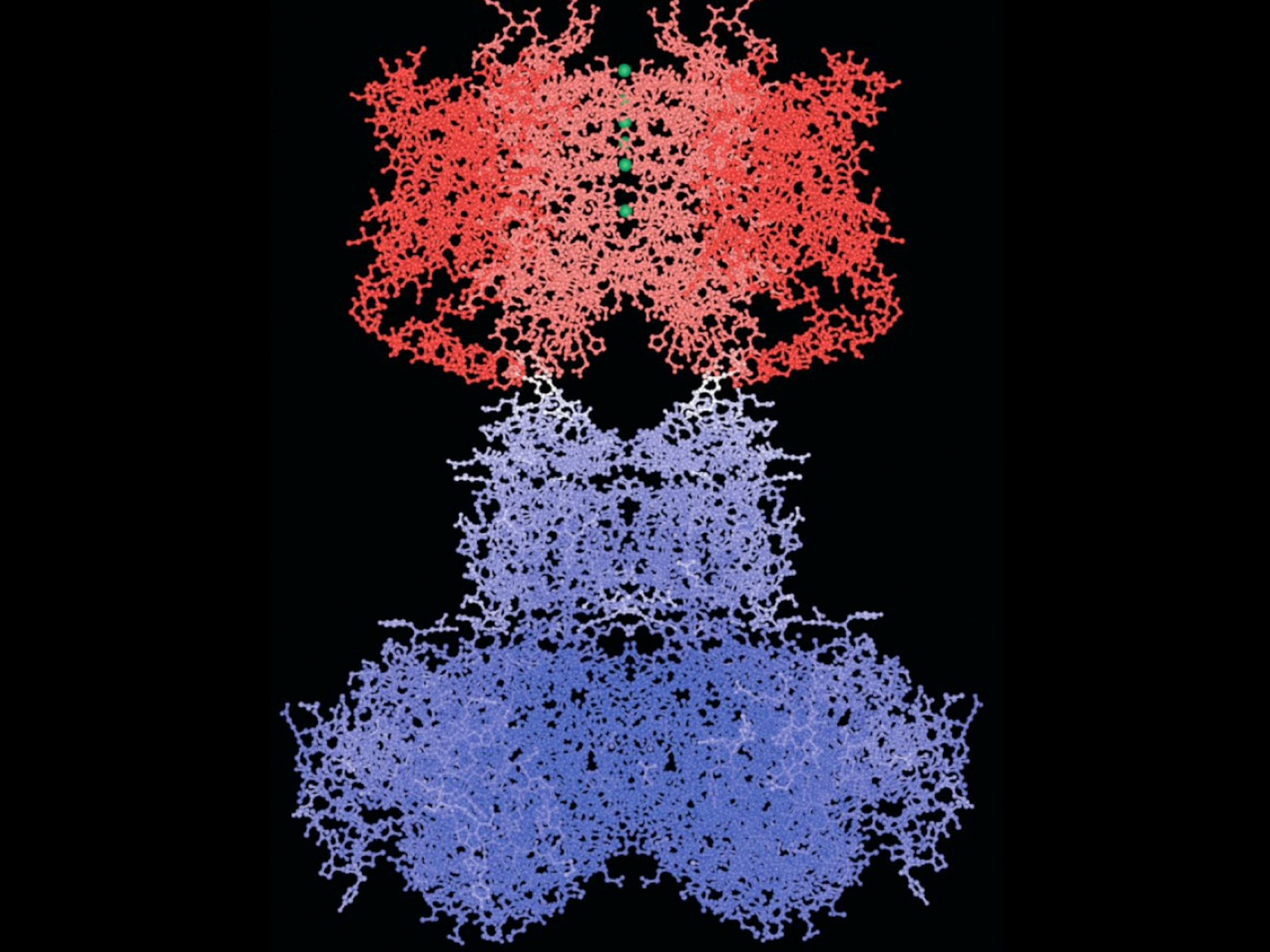
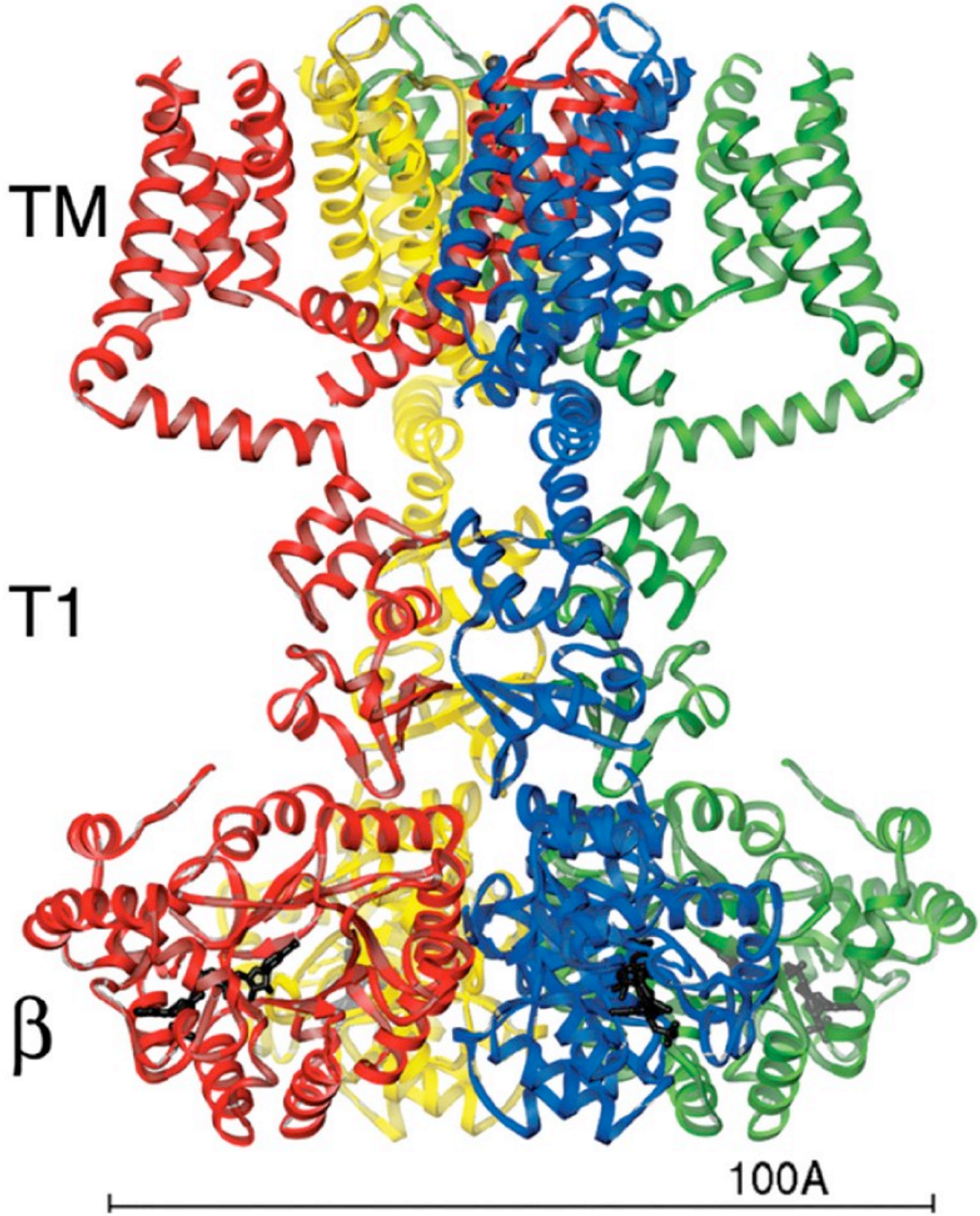
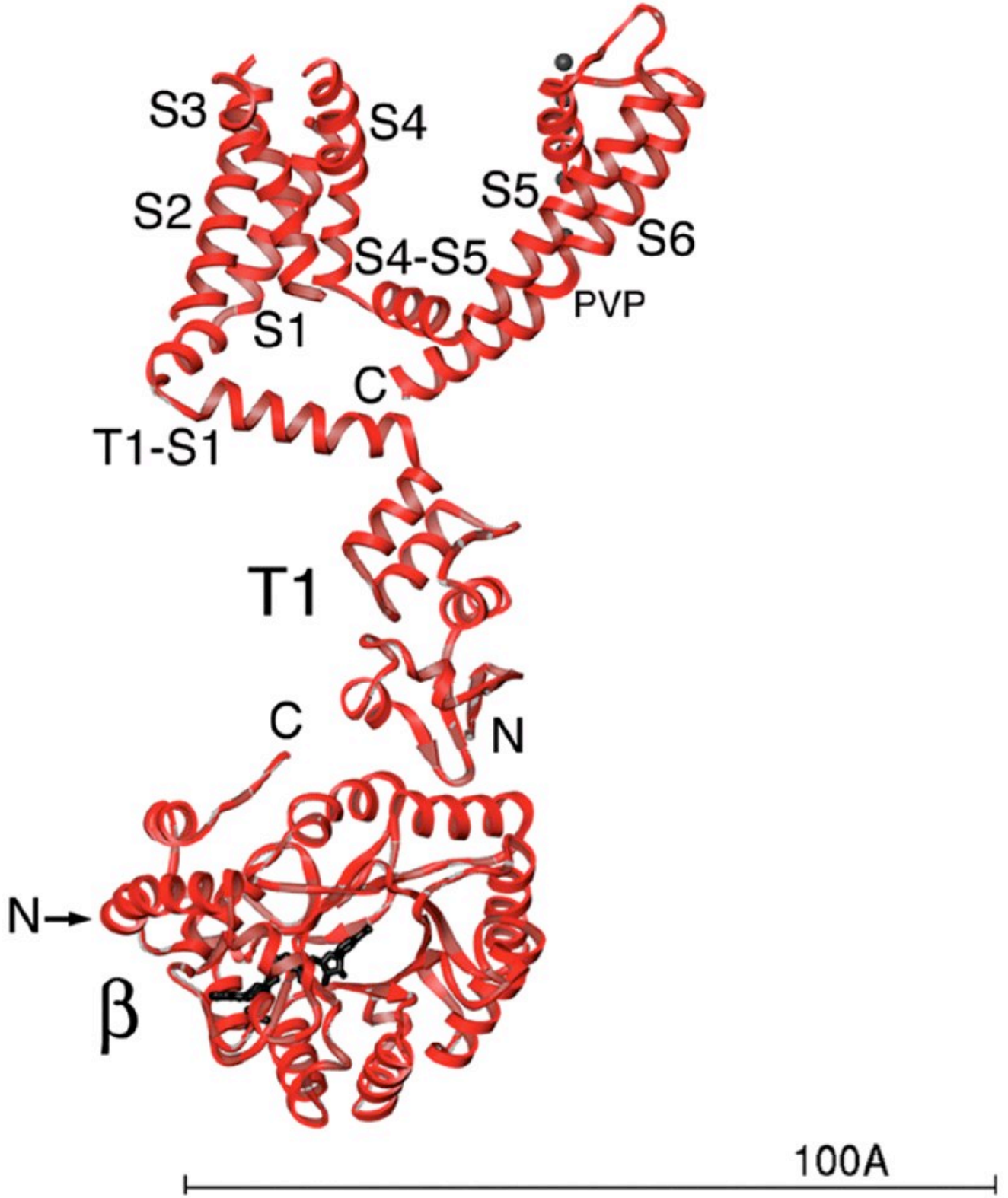


FIGURE 4.41 **Three-dimensional structure of a voltage-gated mammalian K^+ channel.** (*a*) The crystal structure of the entire tetrameric Kv1.2 channel, a member of the *Shaker* family of K^+ ion channels found in nerve cells of the brain. The transmembrane portion is shown in red, and the cytoplasmic portion in blue. The potassium ion binding sites are indicated in green. (*b*) Ribbon drawing of the same channel shown in *a*, with the four subunits that make up the channel shown in different colors. If you focus on the red subunit, you can see (1) the spatial separation between the voltage-sensing and pore domains of the subunit and (2) the manner in which the voltage-sensing domains from each subunit are present on the outer edge of the pore domain of a neighboring subunit. The cytoplasmic portion of this particular channel consists of a T1 domain, which is part of the channel polypeptide itself, and a separate β polypeptide. (*c*) Ribbon drawing of a single subunit showing the spatial orientation of the six membrane-spanning helices (S1–S6) and also the presence of the S4–S5 linker helix, which connects the voltage-sensing and pore domains. This linker transmits the signal from the S4 voltage sensor that opens the channel. The inner surface of the channel below the pore domain is lined by the S6 helix (roughly similar to the M2 helix of the bacterial channel shown in Figure 4.38). The channel shown here is present in the open configuration with the S6 helices curved outward (compare to Figure 4.39) at the site marked PVP (standing for Pro-Val-Pro, which is likely the amino acid sequence of the "hinge"). (REPRINTED WITH PERMISSION FROM STEPHEN B. LONG, ET AL., SCIENCE 309:867, 899, 2005, COURTESY OF RODERICK MACKINNON; COPYRIGHT 2005, AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE.)




TM
T1
β
100A


S3
S4
S2
S5
S6
S4-S5
PVP
S1
C
T1-S1
T1
C
N
N
β
100A

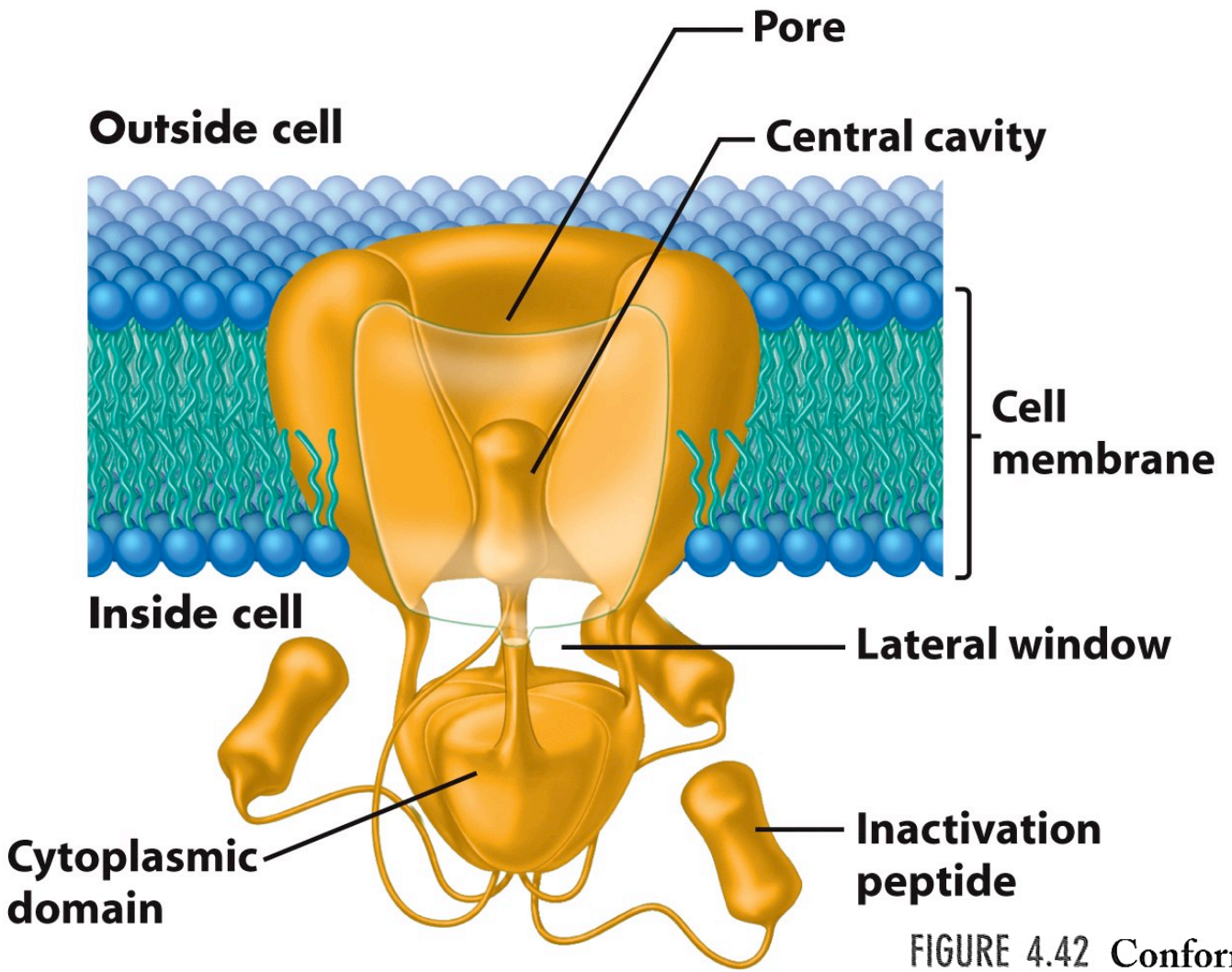


Figure 4-42a Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

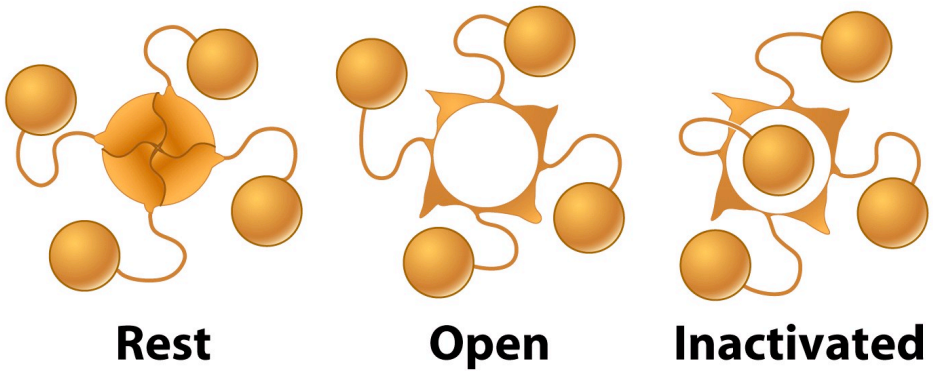


Figure 4-42b Cell and Molecular Biology, 5/e (© 2008 John Wiley & Sons)

FIGURE 4.42 **Conformational states of a voltage-gated K^+ ion channel.** (*a*) Three-dimensional model of a eukaryotic K^+ ion channel. Inactivation of channel activity occurs as the inactivation peptide, which dangles from the cytoplasmic portion of the complex, fits into the cytoplasmic opening of the channel. (*b*) Schematic representation of a view into a K^+ ion channel, perpendicular to the membrane from the cytoplasmic side, showing the channel in the closed (resting), open, and inactivated state. (B: REPRINTED FROM NEURON, VOL. 20, C. M. ARMSTRONG AND B. HILLE, VOLTAGE-GATED ION CHANNELS AND ELECTRICAL EXCITABILITY, PAGE 377; COPYRIGHT 1998, WITH PERMISSION FROM ELSEVIER SCIENCE.)

Membrane Transport

Transport across biological membranes

Active Transport

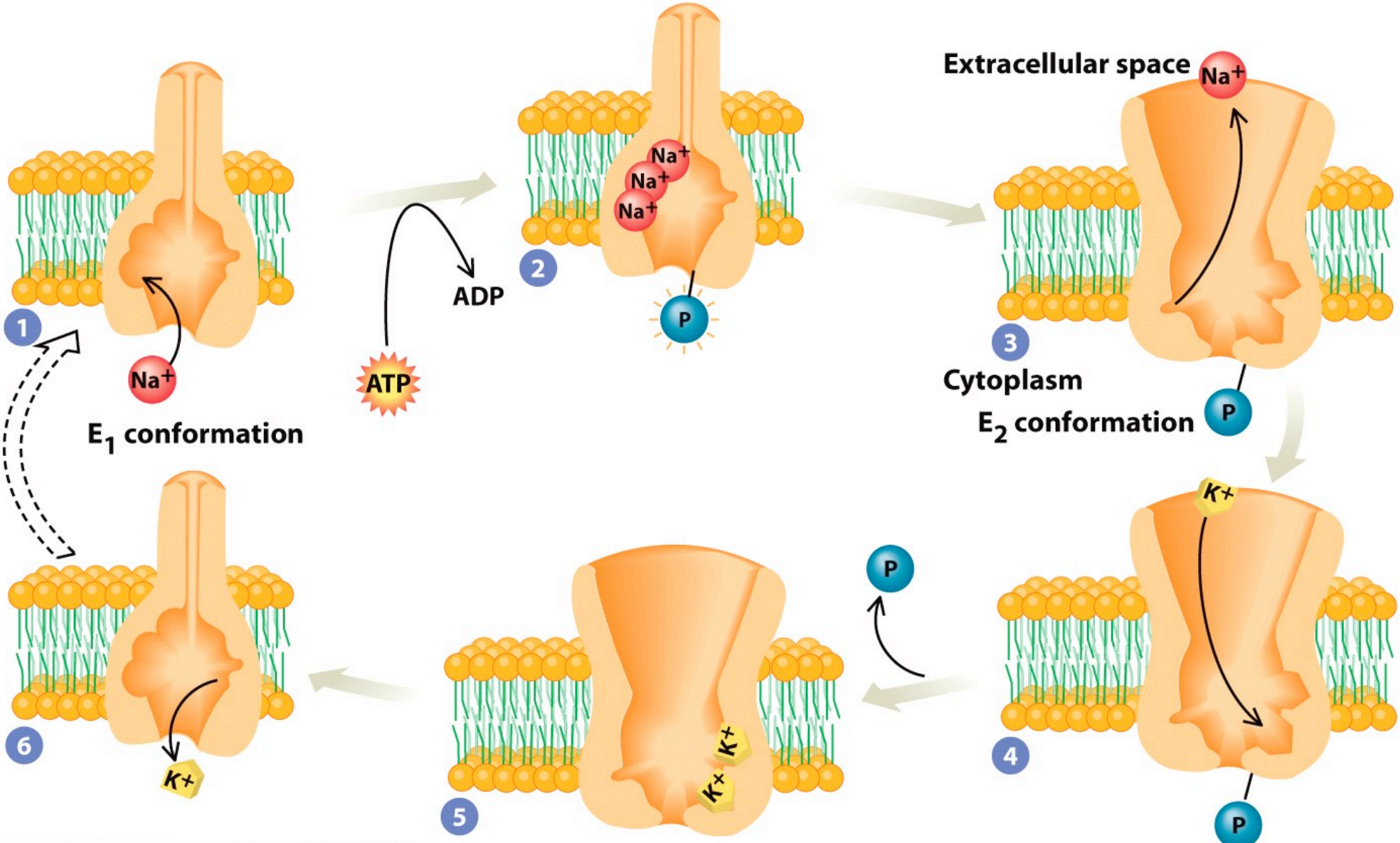
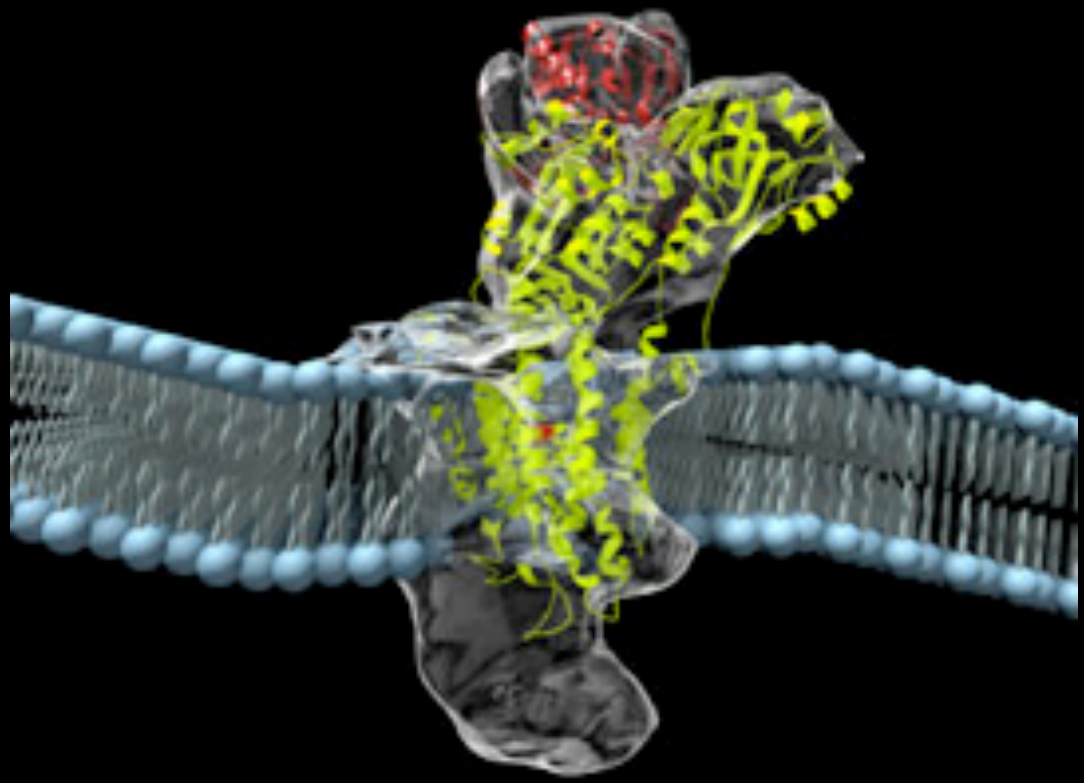


FIGURE 4.45 **Simplified schematic model of the Na^+/K^+-ATPase transport cycle.** Sodium ions (1) bind to the protein on the inside of the membrane. ATP is hydrolyzed, and the phosphate is transferred to the protein (2), changing its conformation (3) and allowing sodium ions to be expelled to the external space. Potassium ions then bind to the protein (4), and the phosphate group is subsequently lost (5), which causes the protein to snap back to its original conformation, allowing the potassium ions to diffuse into the cell (6). Note that the actual Na^+/K^+-ATPase is composed of two different membrane-spanning subunits: a larger α subunit, which carries out the transport activity, and a smaller β subunit, which functions primarily in the maturation and assembly of the pump within the membrane. The cation binding sites are located in the transmembrane domain, which consists of ten membrane-spanning helices. (Steps where ATP binds to the protein prior to hydrolysis are not included.) An animation of the pump cycle can be found at www.mark-hilge.com/nak/nak-atpase_ f.htm

The pump cycle of Na,K-ATPase



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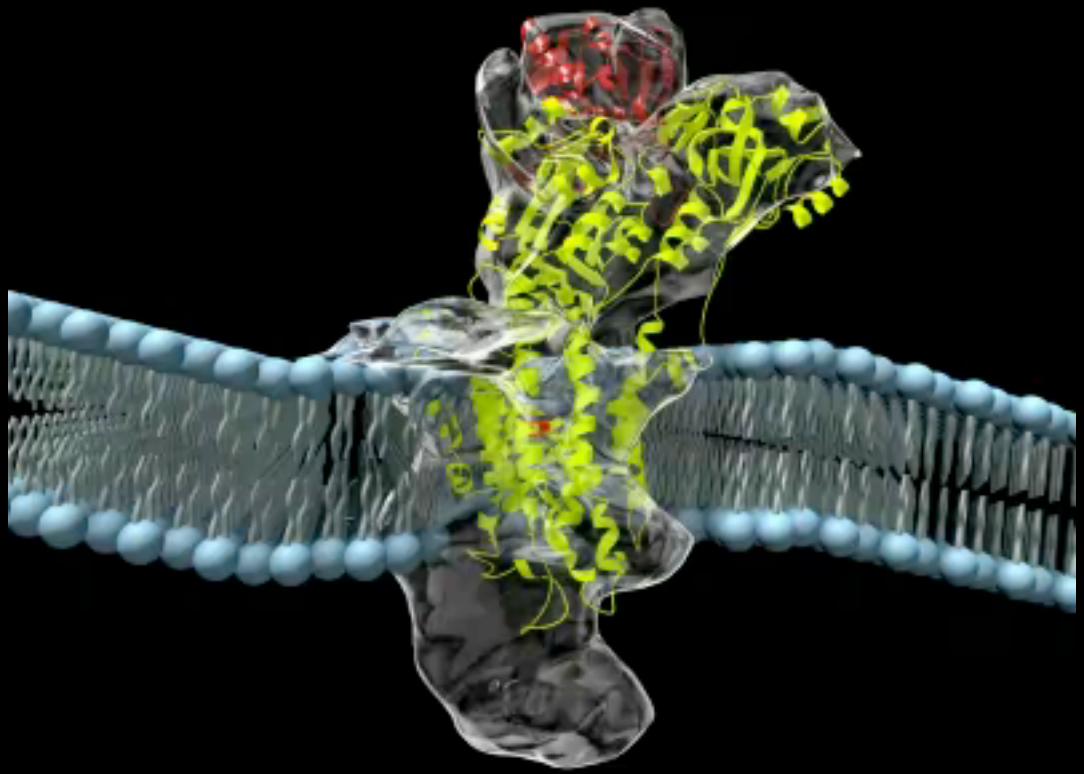


Na,K-ATPase

Membrane Transport

Transport across biological membranes

Active transport by coupling of ion transport with electron transfer

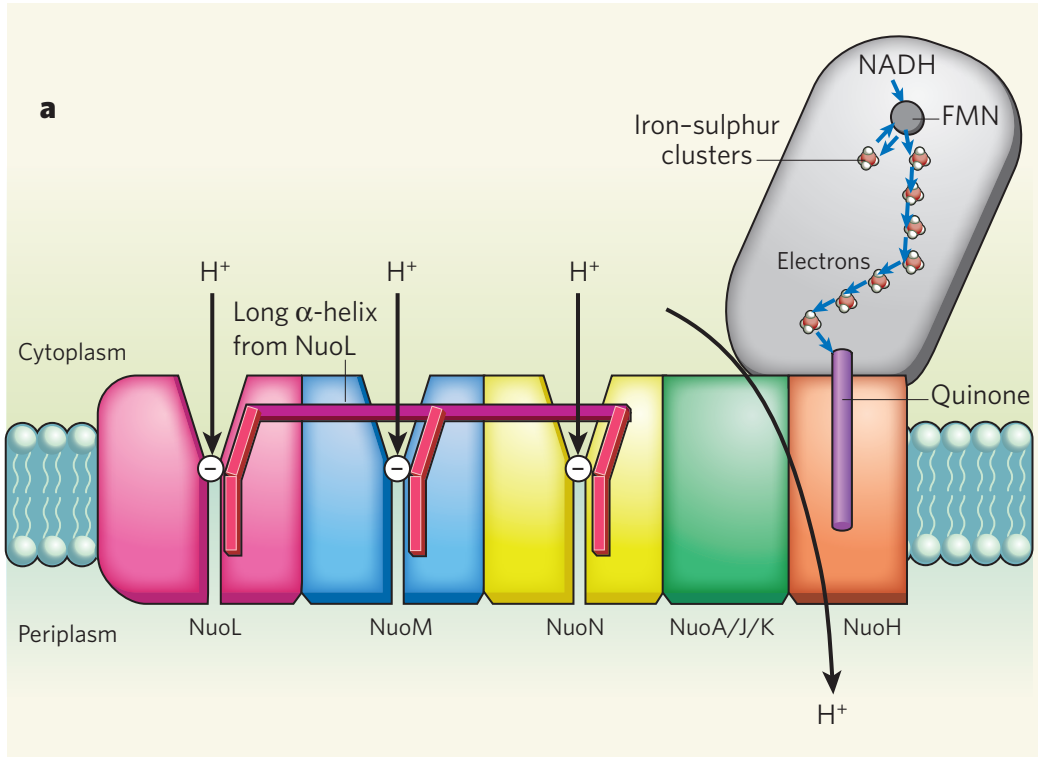
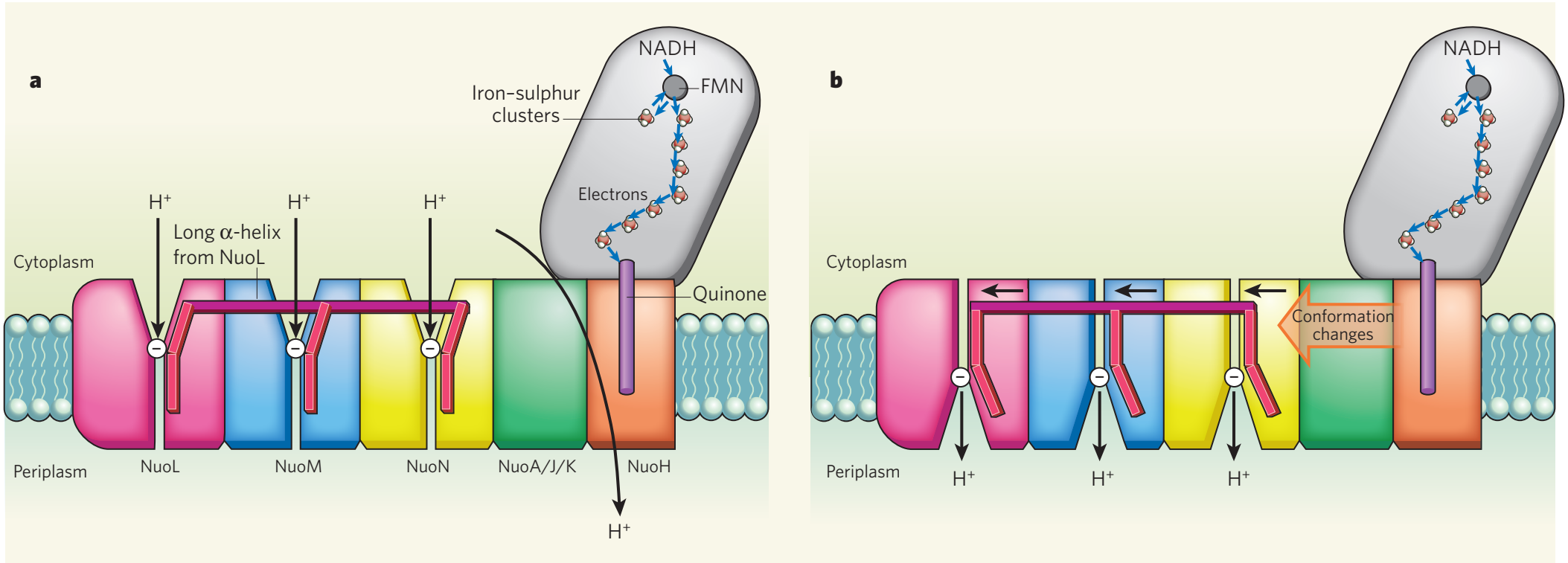


Figure 1 | Indirect coupling of electron transfer to proton pumping in complex I. Complex I, an enzyme found in mitochondrial and bacterial membranes, converts energy by coupling electron transfer to proton pumping. Sazanov and colleagues' crystal structures[1] of bacterial complex I reveal that the transmembrane NuoL subunit of the enzyme projects a long α-helix through the adjacent NuoM and NuoN subunits. They suggest the following mechanism to explain how electron transfer drives proton pumping. **a**, Pairs of electrons from the metabolic intermediate NADH are transferred to a cofactor (flavin mononucleotide, FMN) and then passed along a chain of iron–sulphur clusters in the extramembrane region of complex I, eventually reaching a quinone cofactor; blue arrows indicate the electron-transfer pathway. This allows a proton (H^+) to pass through complex I at the interface of the extra- and intramembrane regions. Protons can also enter channels in NuoL, NuoM and NuoN from the cytoplasm, but cannot pass through. White circles with minus signs represent negatively charged amino acids, which are key to proton transport. **b**, Conformational changes in the NuoA/J/K/H subunits push the long α-helix towards the other transmembrane subunits. This tilts three other helices in NuoL, NuoM and NuoN, causing the reorientation of certain residues in the subunits' channels. These local conformational changes allow protons in the channels to pass through the channels and enter the periplasm (the space between the inner and outer bacterial membranes).

Ohnishi, T. (2010) Structural Biology. Piston drives and proton pump. Nature 465, 428-429



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Ohnishi, T. (2010) Structural Biology. Piston drives and proton pump. Nature 465, 428-429

Membrane Transport

Summary:
Transport across biological membranes

- Transport of proteins
- Transport of ions
- Transport of small molecules

Membrane Biochemistry

Next Lecture...

Mitochondria and chloroplasts - analysis of compartments

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