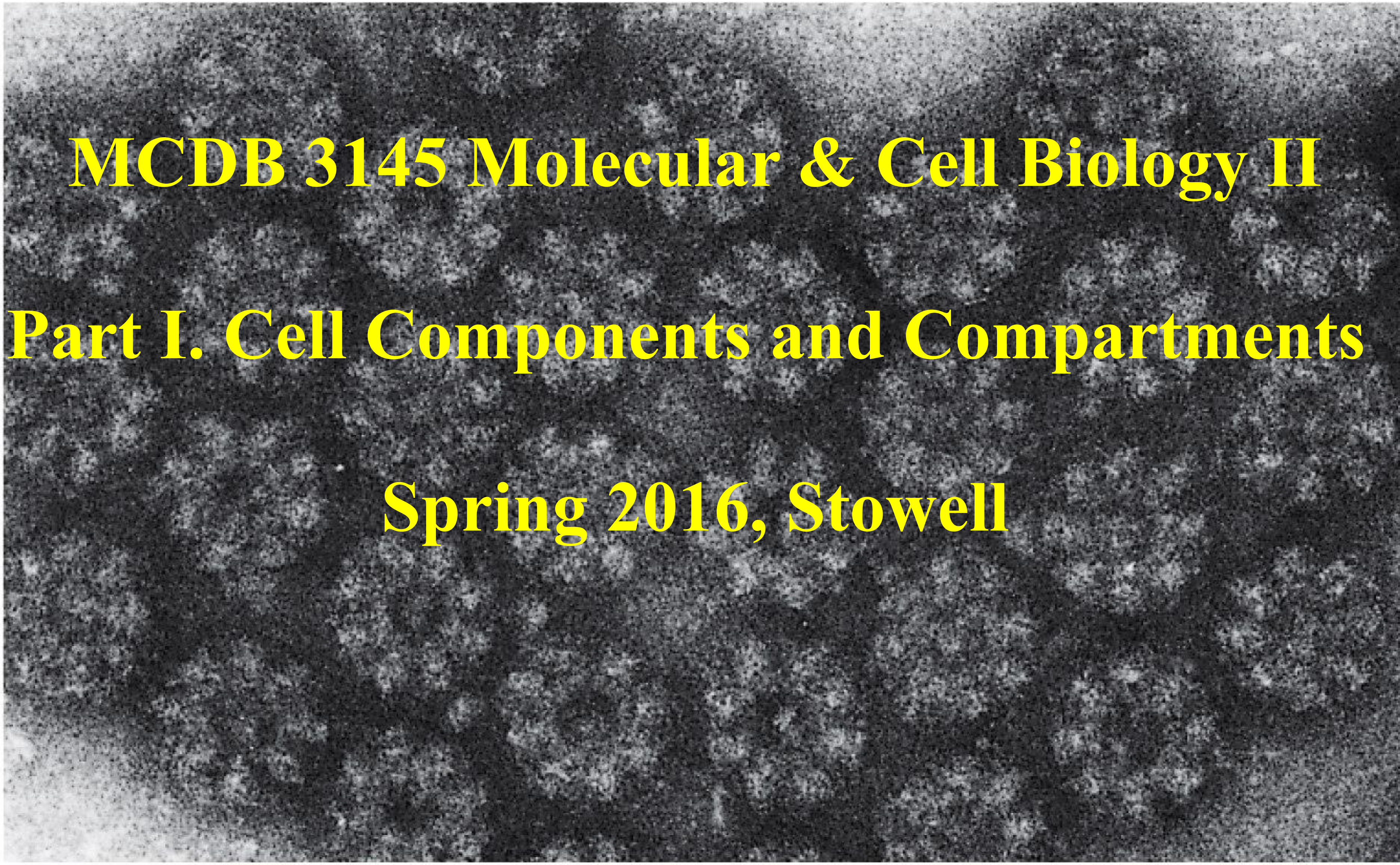
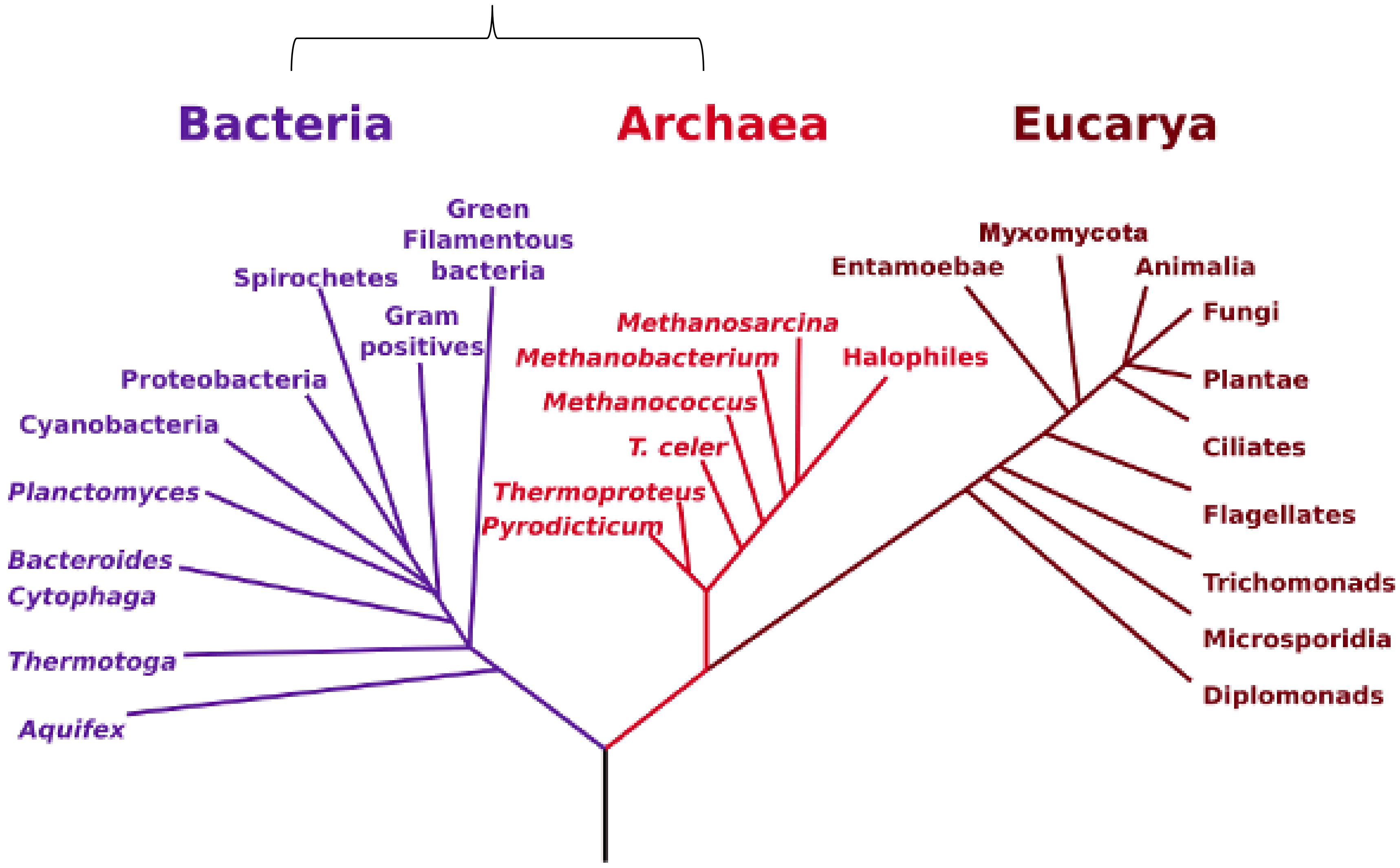


MCDB 3145 Molecular & Cell Biology II

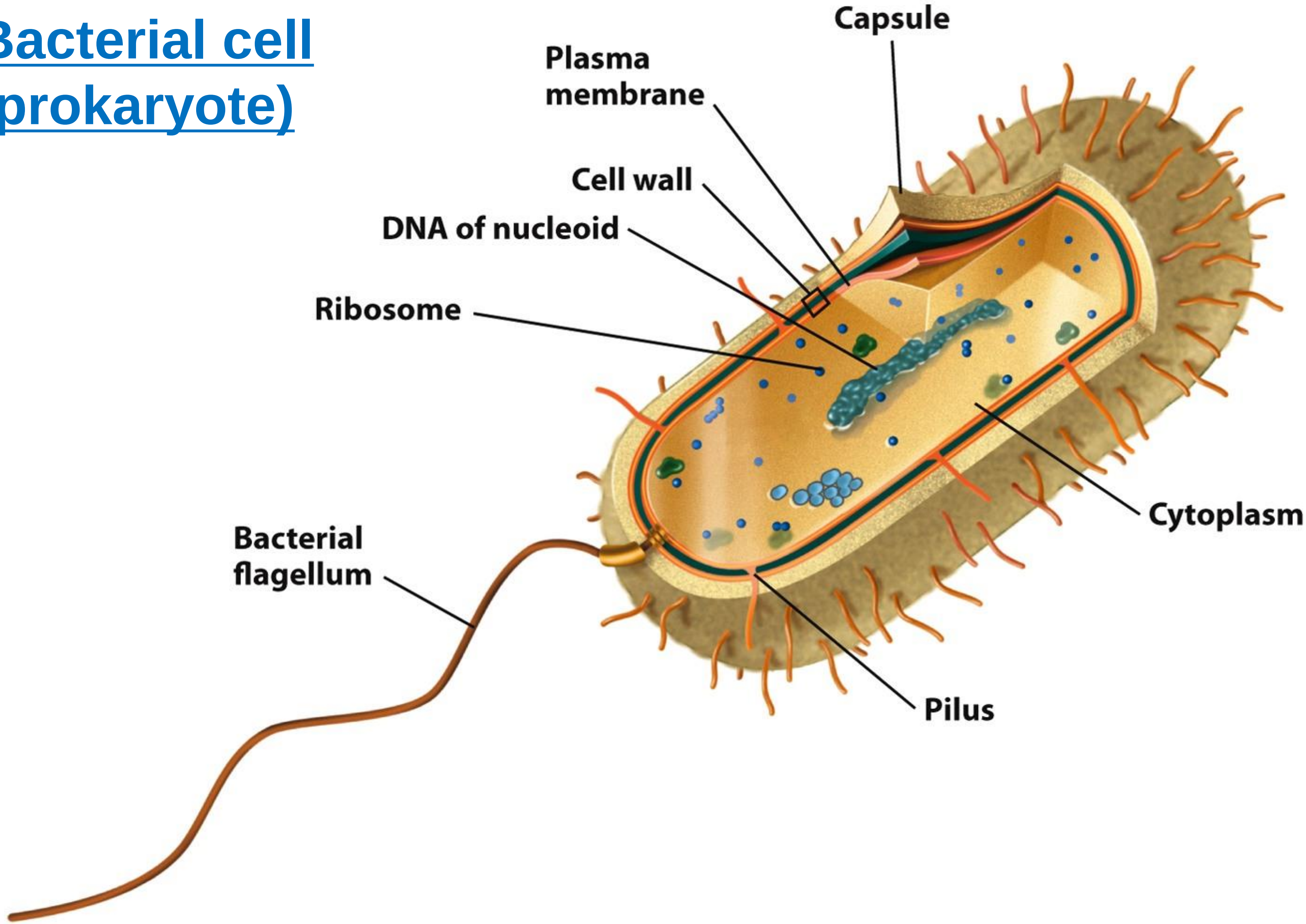
Part I. Cell Components and Compartments

Spring 2016, Stowell

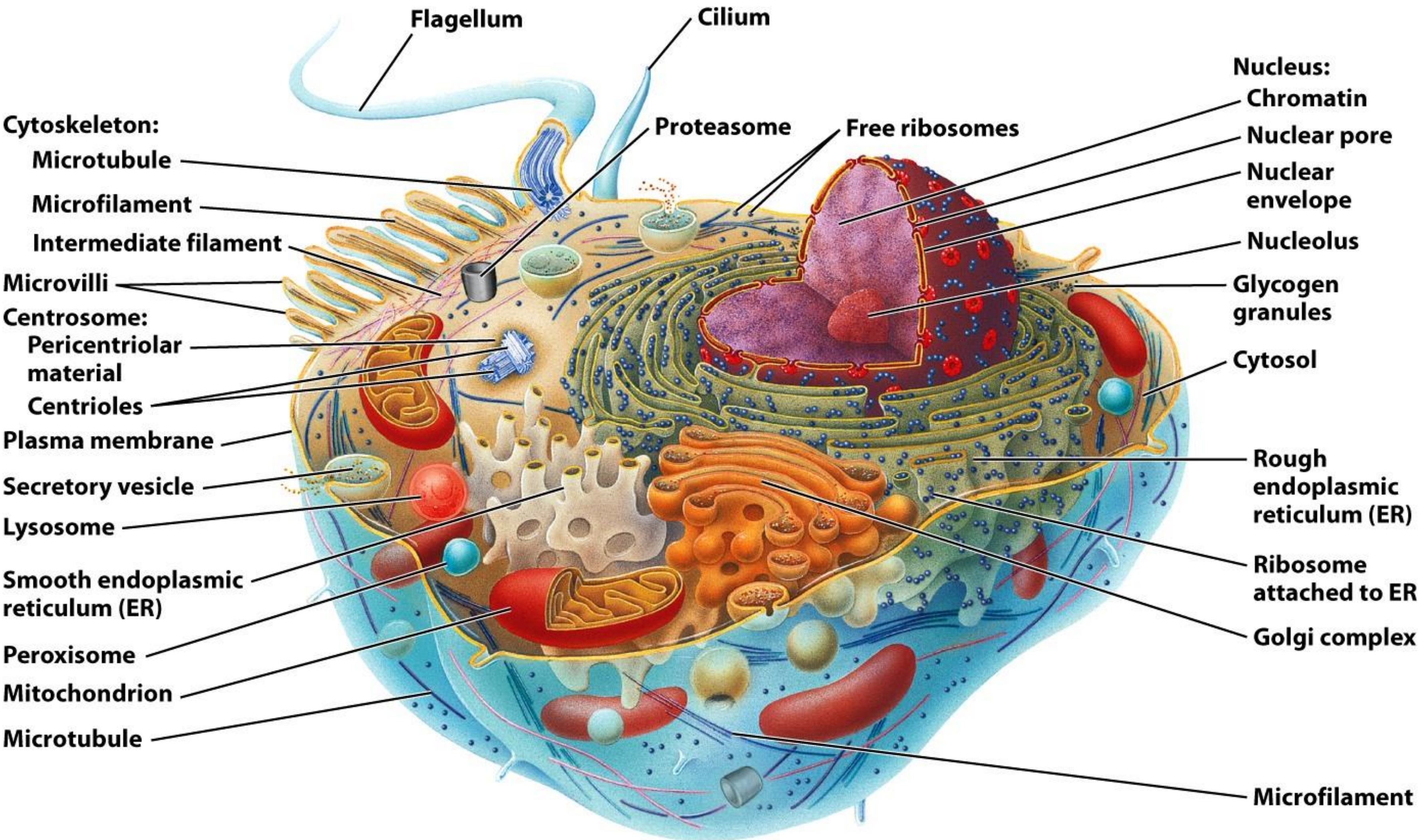
"Prokaryotes"

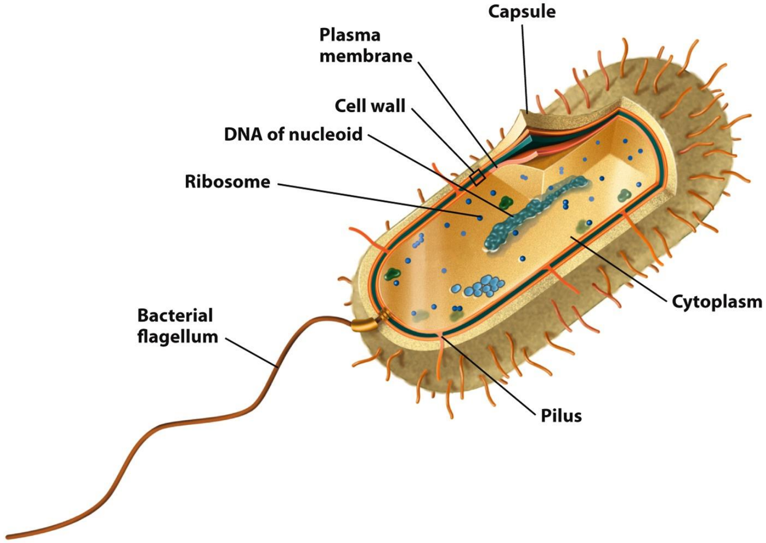


Bacterial cell (prokaryote)



Animal cell (eukaryote)




Capsule
Plasma membrane
Cell wall
DNA of nucleoid
Ribosome
Cytoplasm
Bacterial flagellum
Pilus

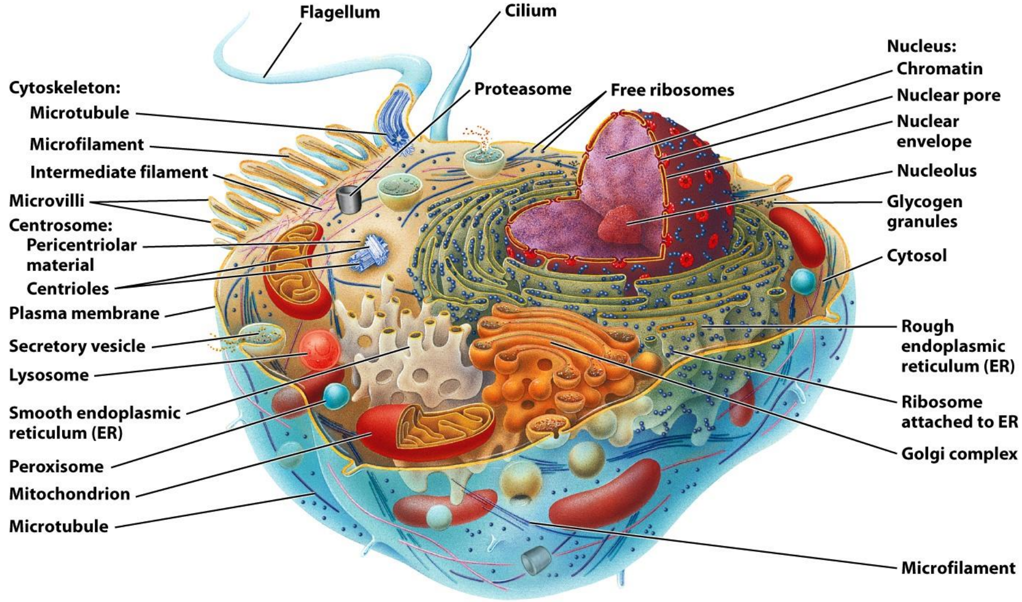

Flagellum
Cilium
Cytoskeleton:
Microtubule
Microfilament
Intermediate filament
Microvilli
Centrosome:
Pericentriolar material
Centrioles
Plasma membrane
Secretory vesicle
Lysosome
Smooth endoplasmic reticulum (ER)
Peroxisome
Mitochondrion
Microtubule
Proteasome
Free ribosomes
Nucleus:
Chromatin
Nuclear pore
Nuclear envelope
Nucleolus
Glycogen granules
Cytosol
Rough endoplasmic reticulum (ER)
Ribosome attached to ER
Golgi complex
Microfilament

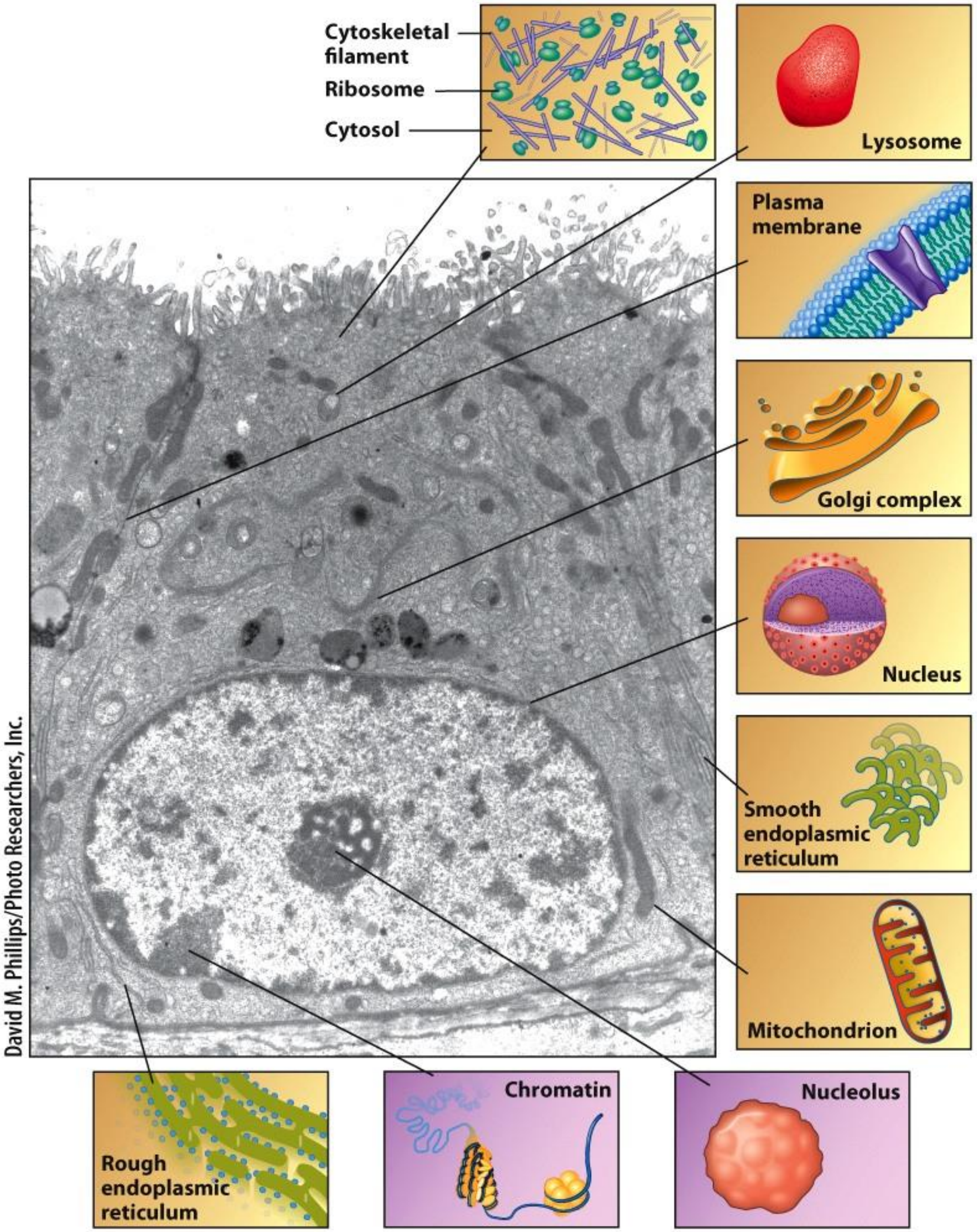
Table 1.1 *A Comparison of Prokaryotic and Eukaryotic Cells*

Features held in common by the two types of cells:

- Plasma membrane of similar construction
- Genetic information encoded in DNA using identical genetic code
- Similar mechanisms for transcription and translation of genetic information, including similar ribosomes
- Shared metabolic pathways (e.g., glycolysis and TCA cycle)
- Similar apparatus for conservation of chemical energy as ATP (located in the plasma membrane of prokaryotes and the mitochondrial membrane of eukaryotes)
- Similar mechanism of photosynthesis (between cyanobacteria and green plants)
- Similar mechanism for synthesizing and inserting membrane proteins
- Proteasomes (protein digesting structures) of similar construction (between archaebacteria and eukaryotes)

Features of eukaryotic cells not found in prokaryotes:

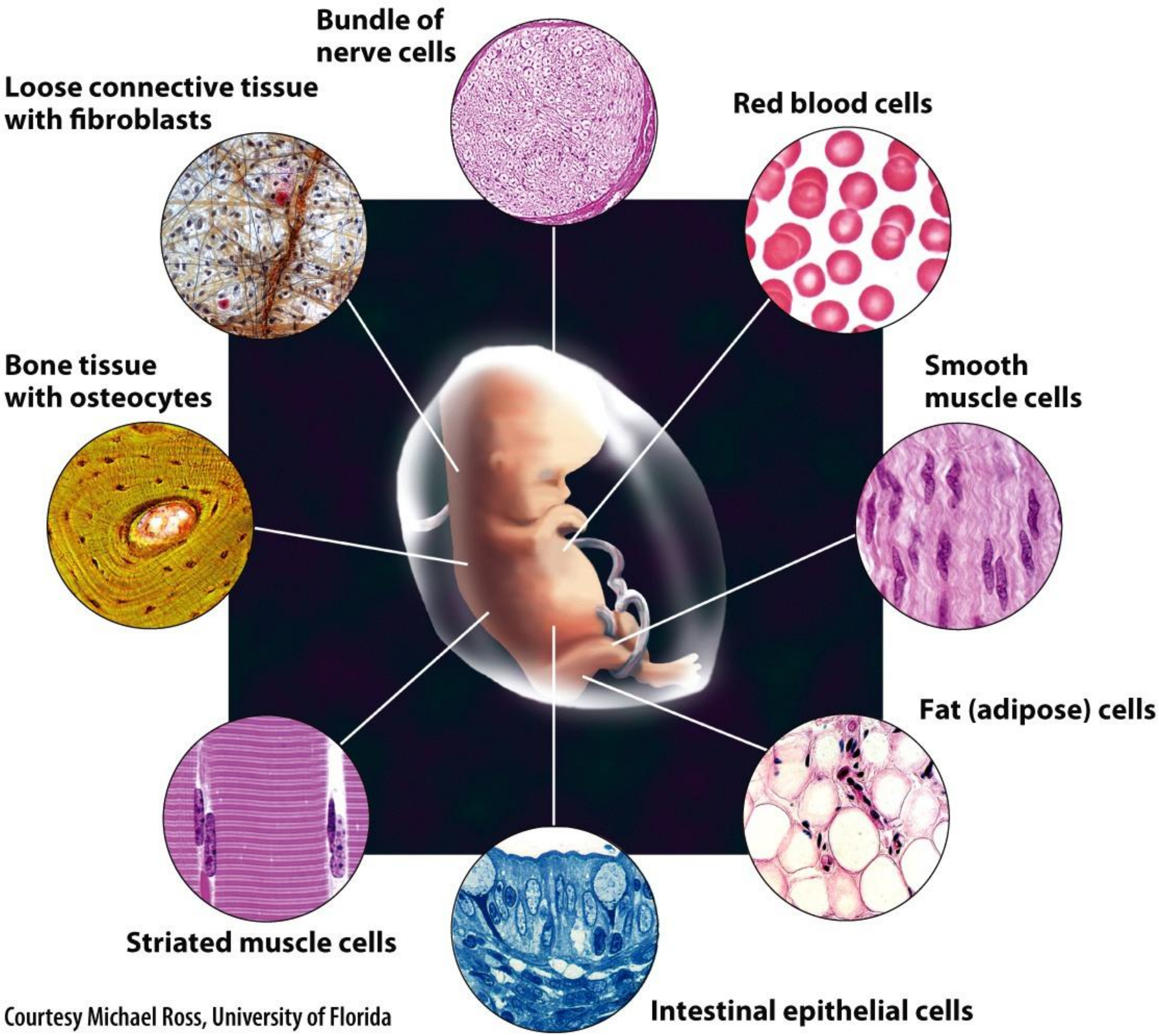
- Division of cells into nucleus and cytoplasm, separated by a nuclear envelope containing complex pore structures
- Complex chromosomes composed of DNA and associated proteins that are capable of compacting into mitotic structures
- Complex membranous cytoplasmic organelles (includes endoplasmic reticulum, Golgi complex, lysosomes, endosomes, peroxisomes, and glyoxisomes)
- Specialized cytoplasmic organelles for aerobic respiration (mitochondria) and photosynthesis (chloroplasts)
- Complex cytoskeletal system (including microfilaments, intermediate filaments, and microtubules) and associated motor proteins
- Complex flagella and cilia
- Ability to ingest particulate material by enclosure within plasma membrane vesicles (phagocytosis)
- Cellulose-containing cell walls (in plants)
- Cell division using a microtubule-containing mitotic spindle that separates chromosomes
- Presence of two copies of genes per cell (diploidy), one from each parent
- Presence of three different RNA synthesizing enzymes (RNA polymerases)
- Sexual reproduction requiring meiosis and fertilization



Basic components of a eukaryotic cell

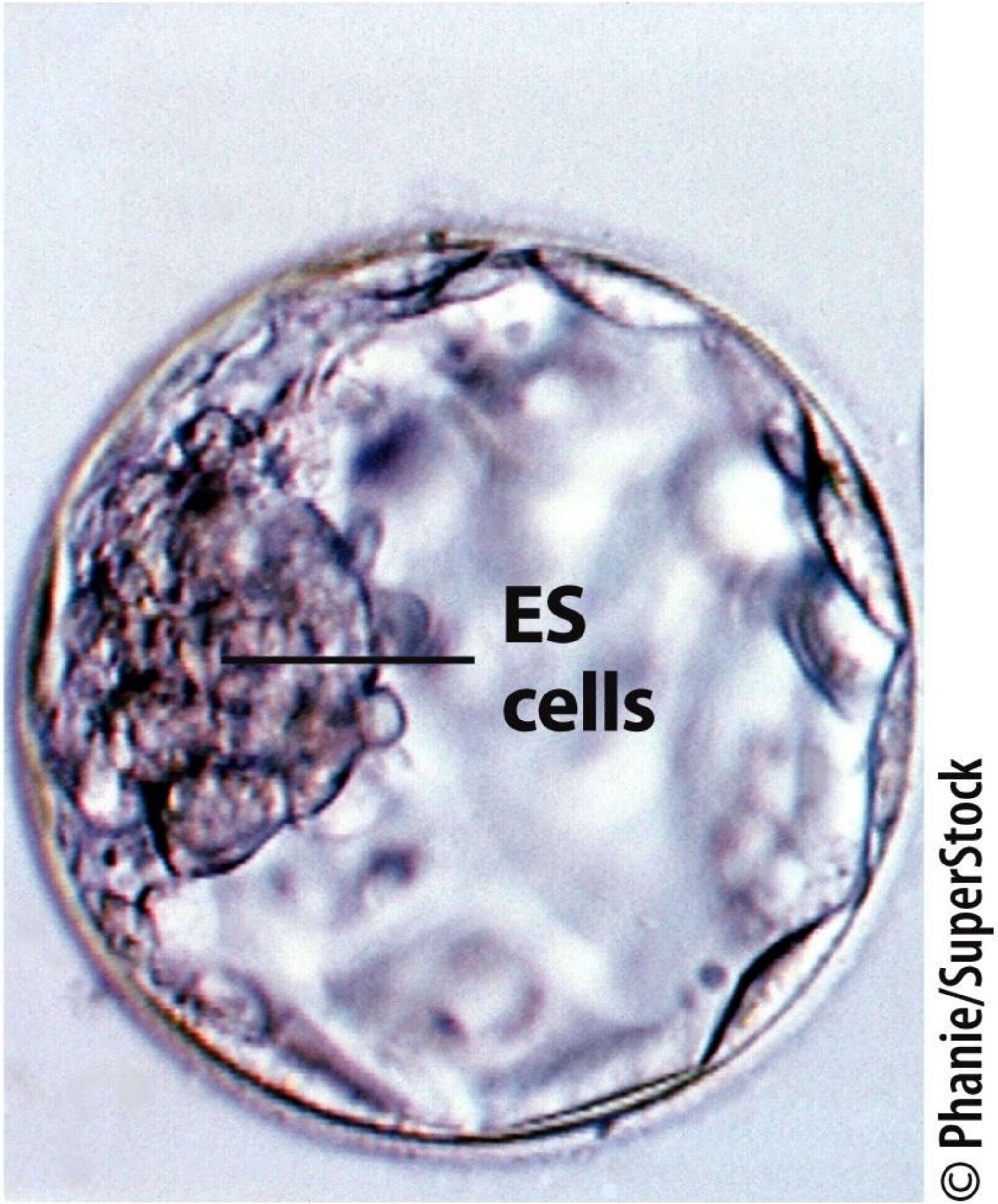
Model organisms commonly used in cell biology research

Many cell types in the human body

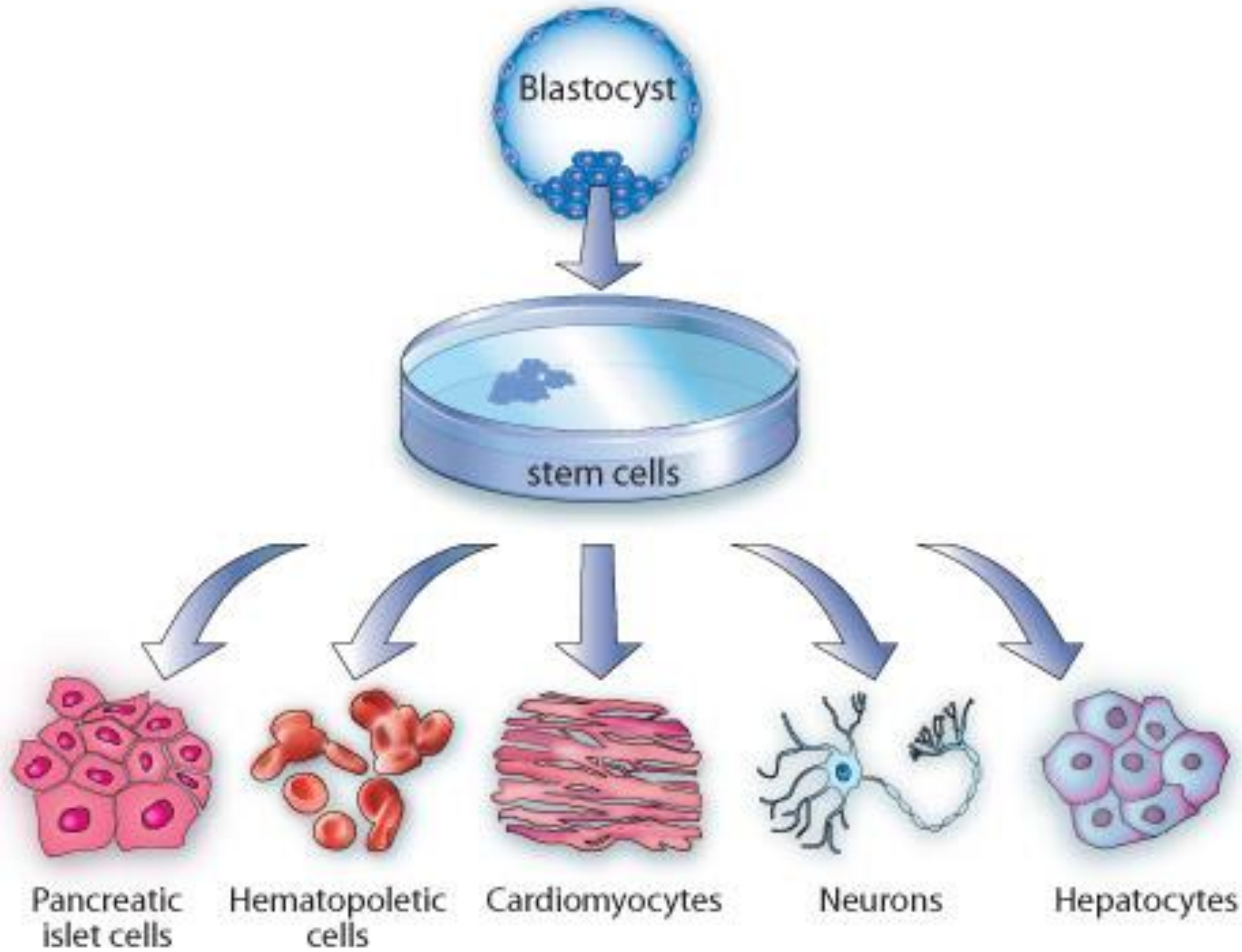


Courtesy Michael Ross, University of Florida

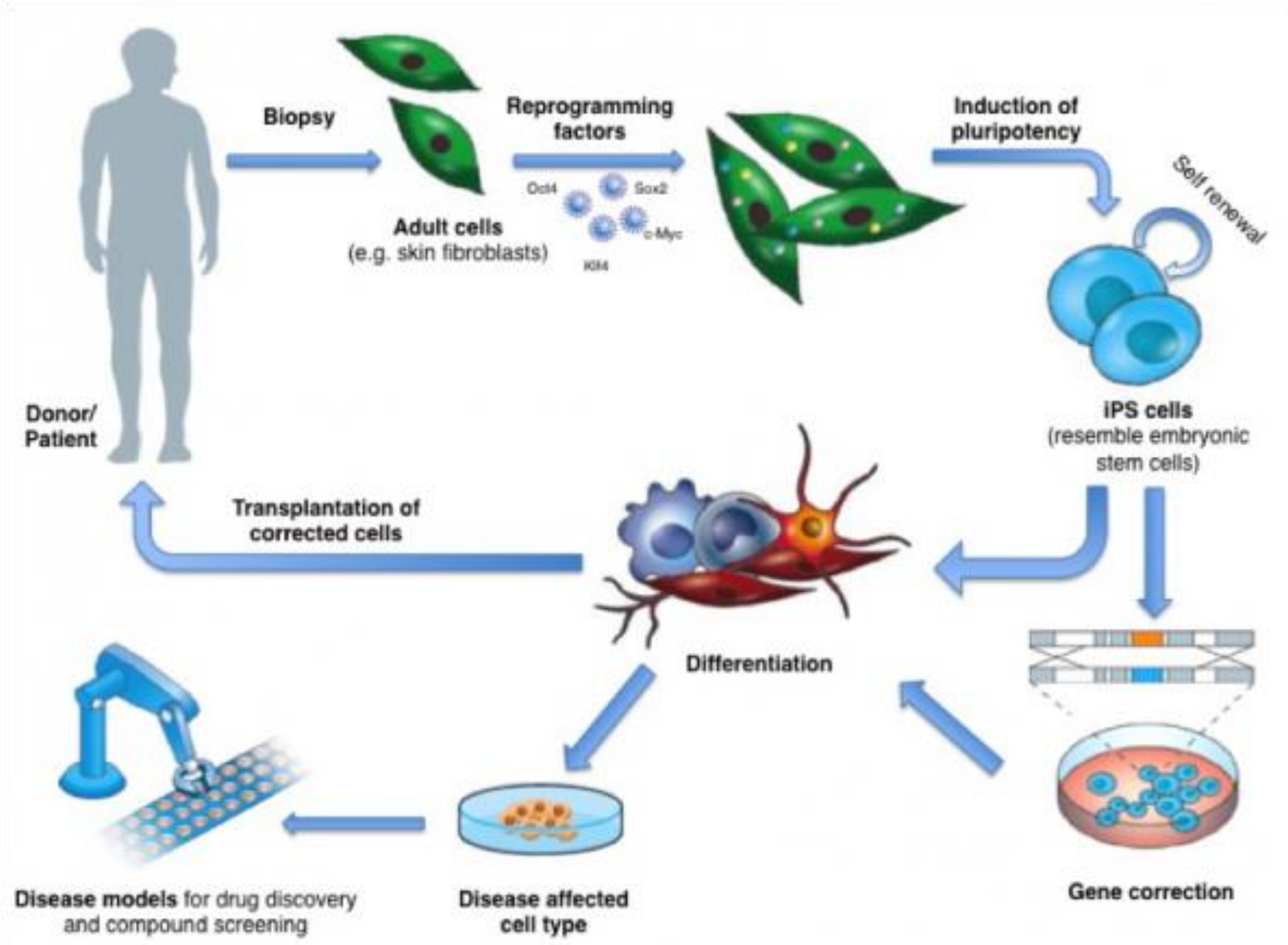
Pluripotent stem cells can be derived from embryos



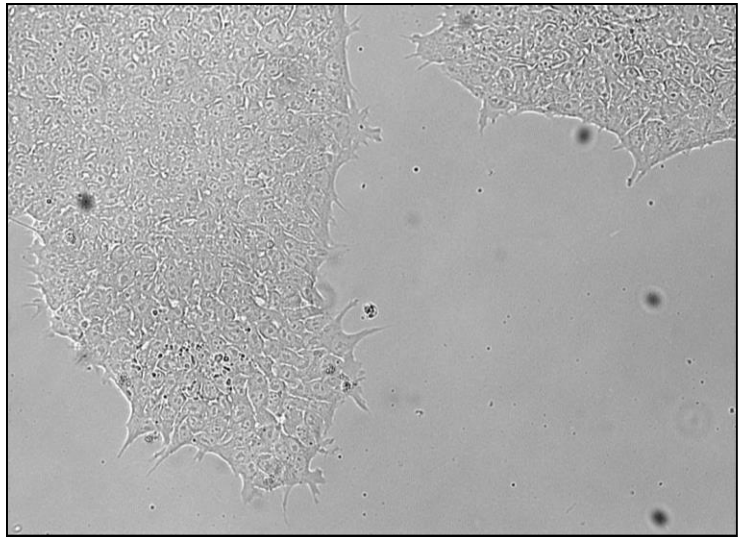
Differentiation of embryonic stem (ES) cells into various cell types



Pluripotent stem cells can be derived from adults (induced pluripotent stem cells, iPS cells)

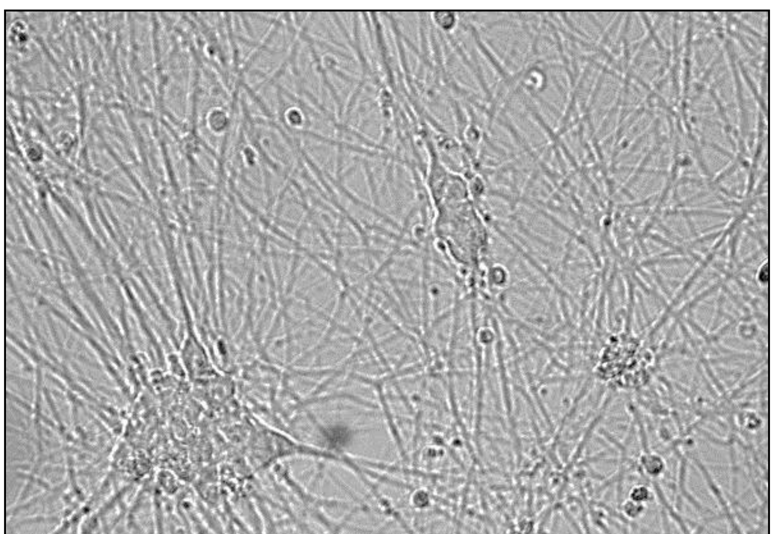


Differentiation of human induced pluripotent stem (iPS) cells

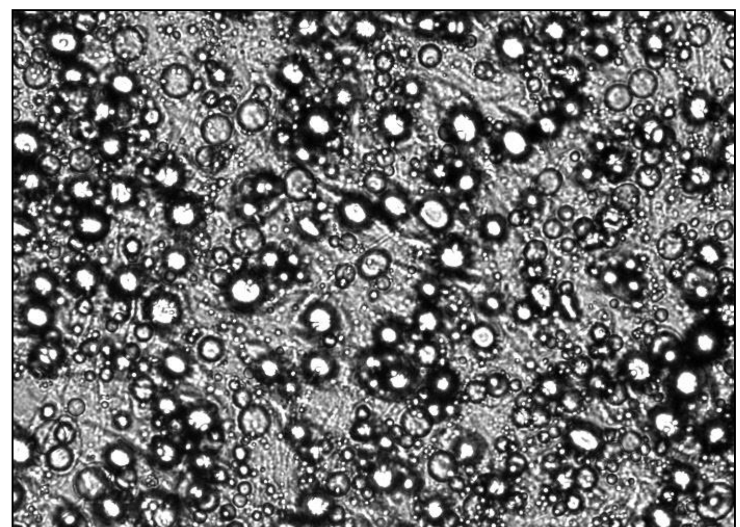


Human iPS cells

<u>Shen lab</u>



Differentiated neurons



Differentiated fat cells

The Nobel Prize in Physiology or Medicine 2012

Photo: U. Montan

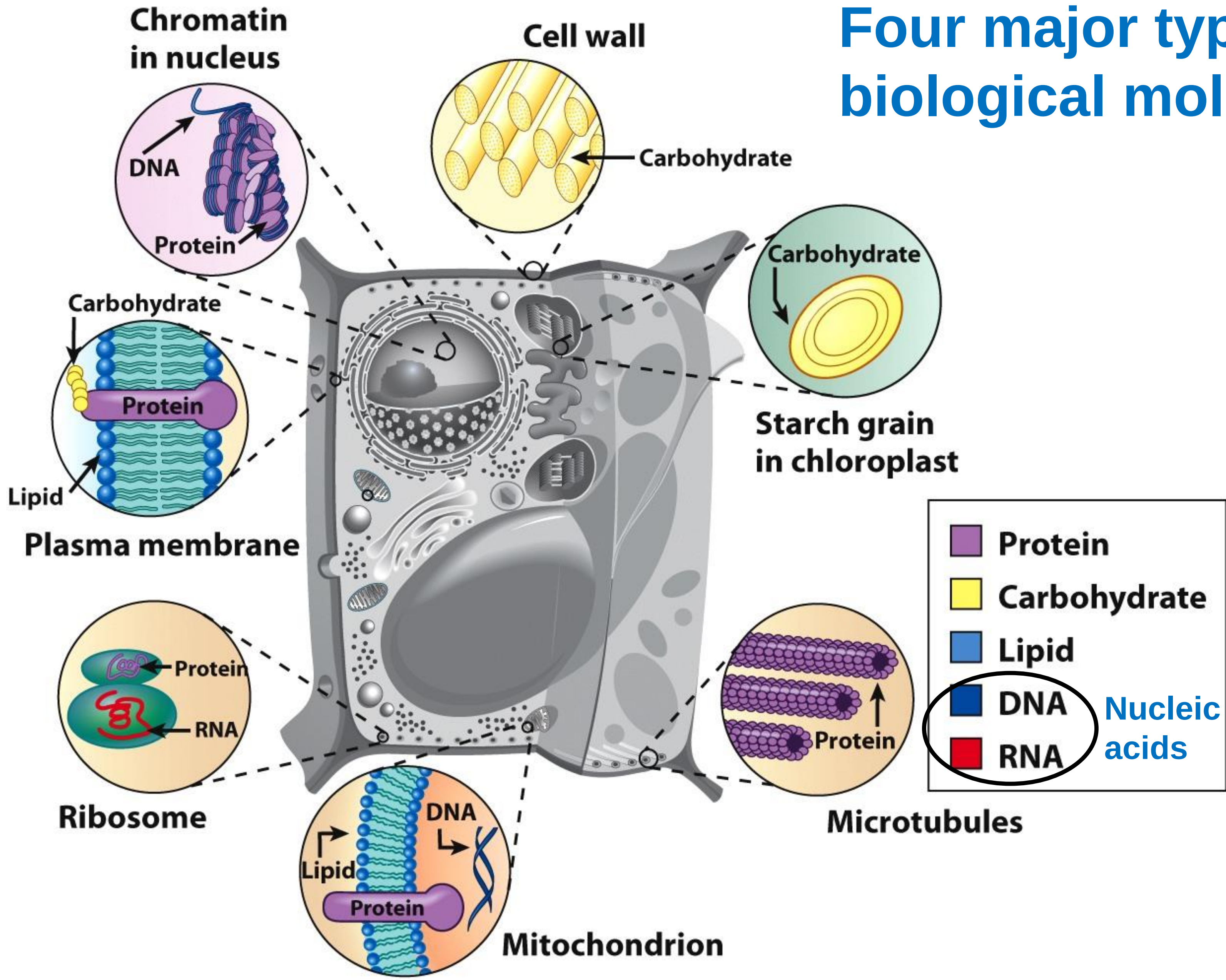
Sir John B. Gurdon

Photo: U. Montan

Shinya Yamanaka

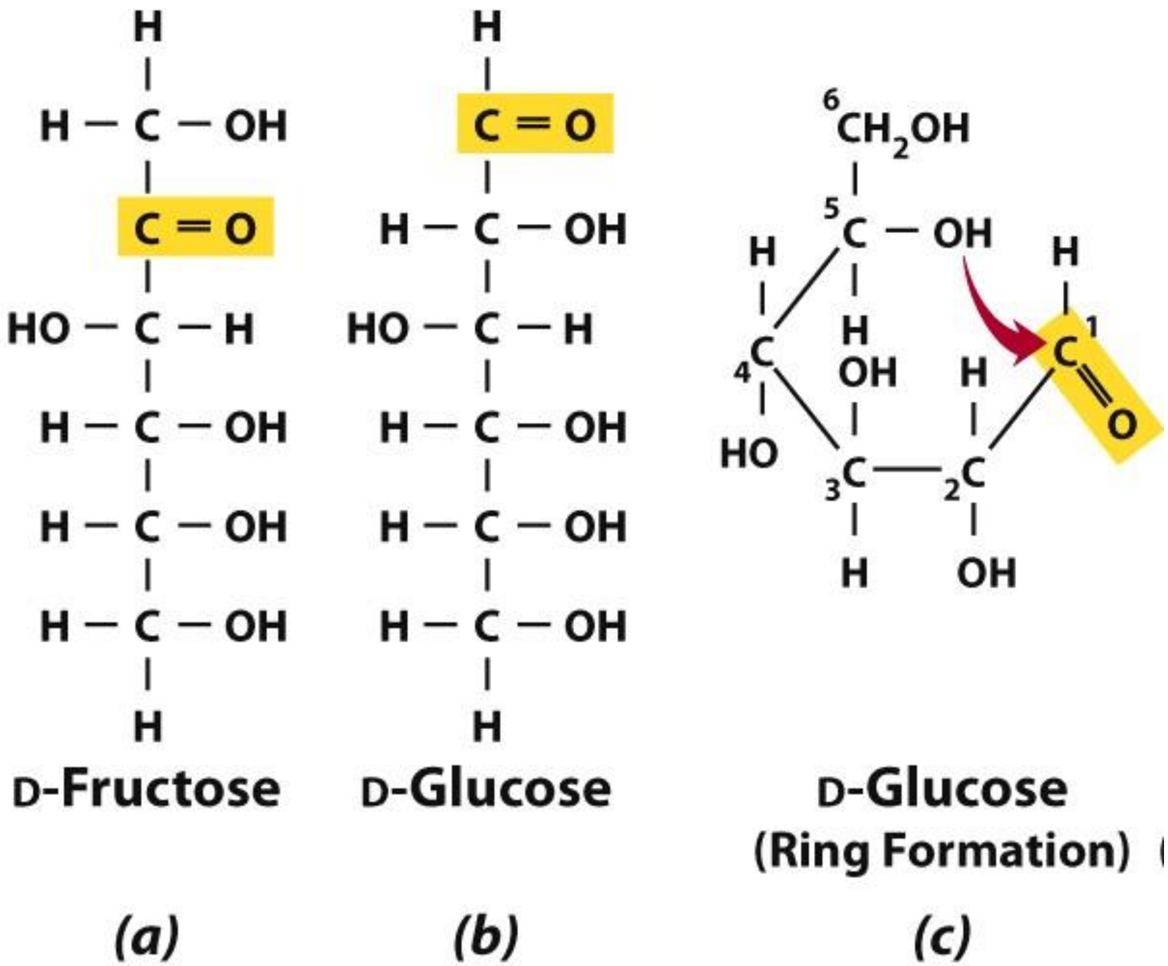
The Nobel Prize in Physiology or Medicine 2012 was awarded jointly to Sir John B. Gurdon and Shinya Yamanaka *"for the discovery that mature cells can be reprogrammed to become pluripotent"*

Four major types of biological molecules



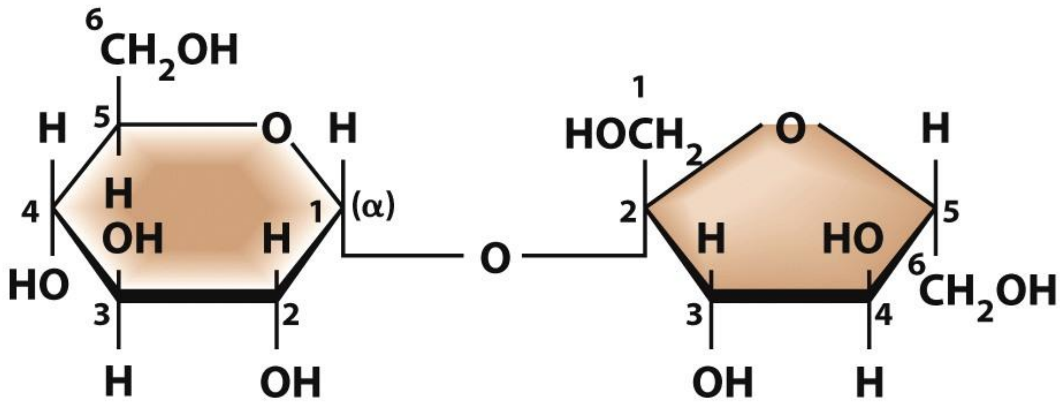
Carbohydrates/ glycans

Simple sugars/ monosaccharides



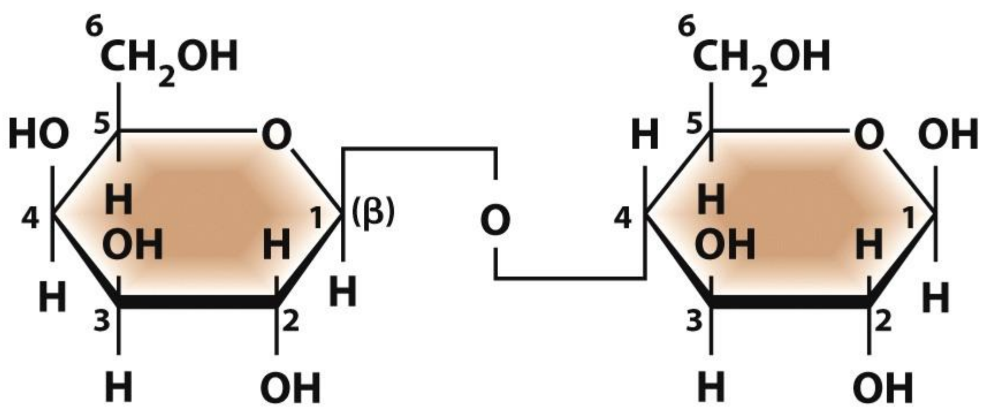
Oligosaccharides

Sucrose



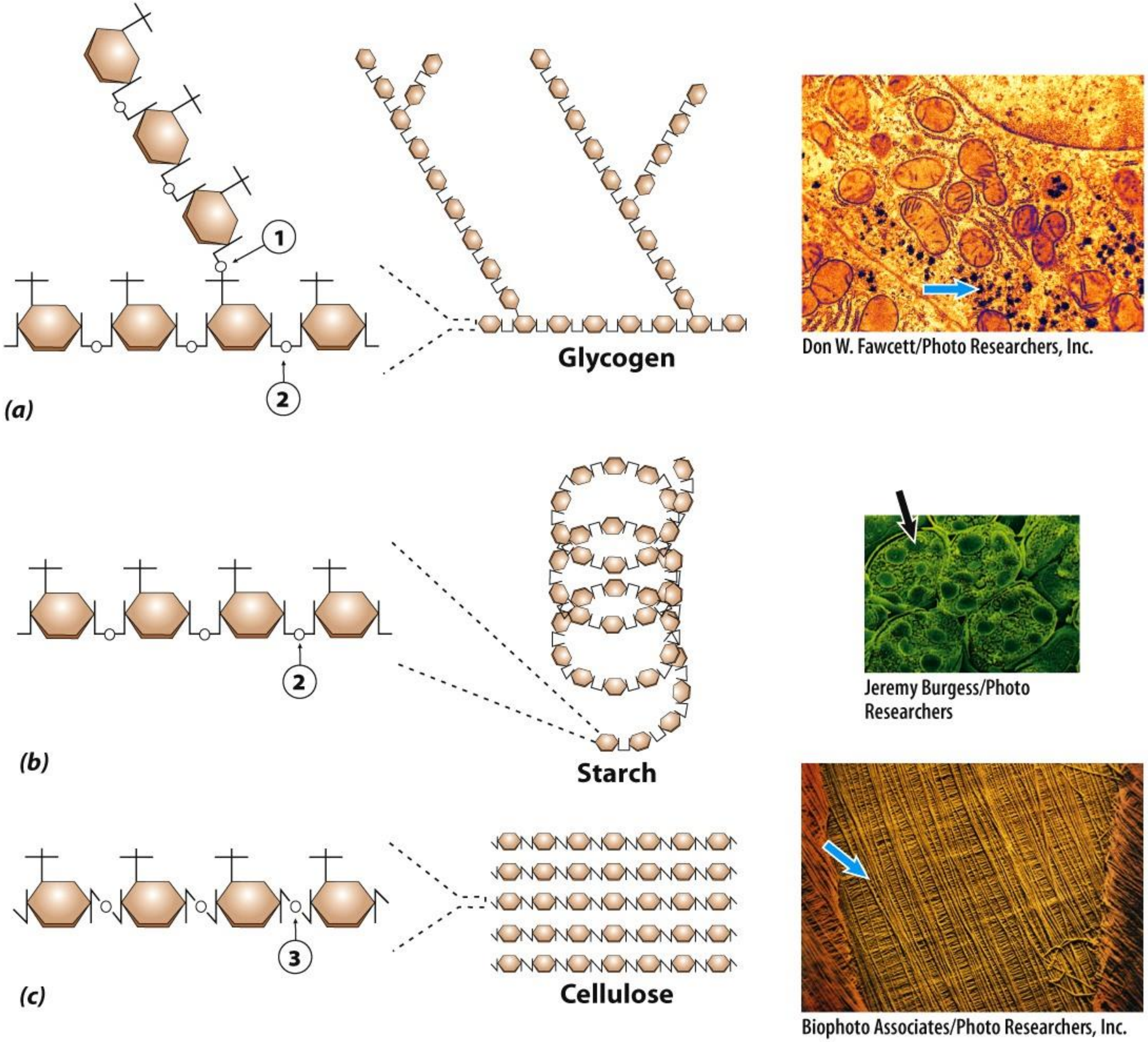
(a)

Lactose



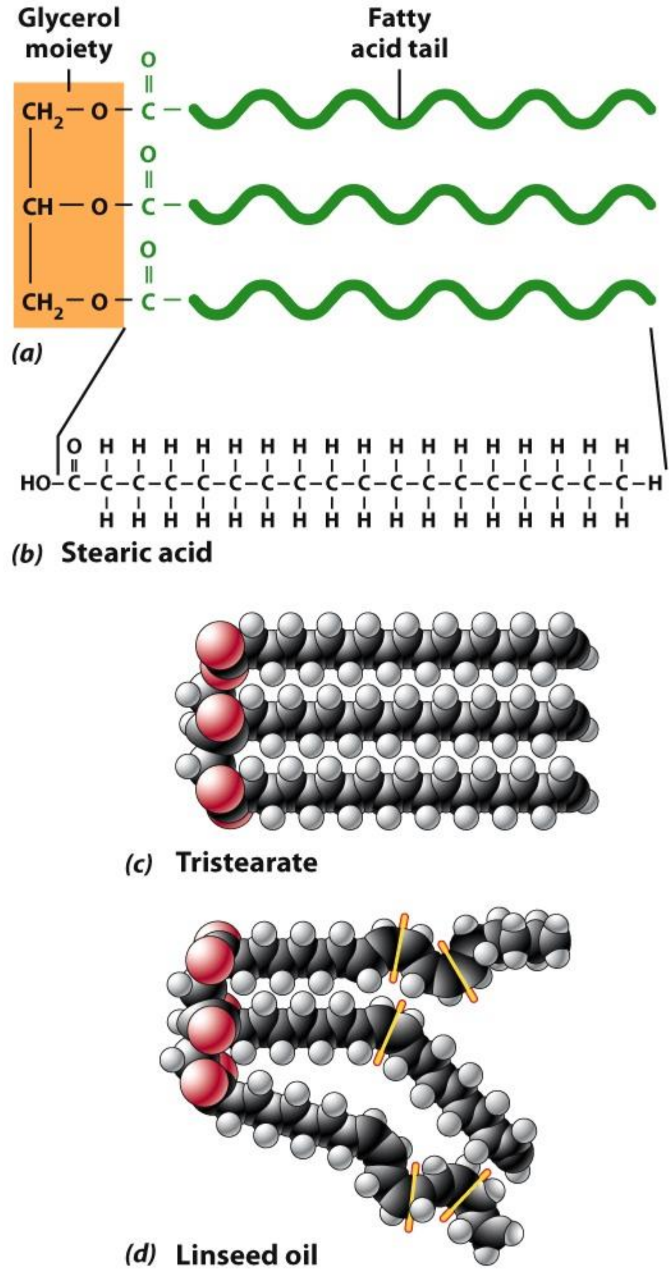
(b)

Polysaccharides



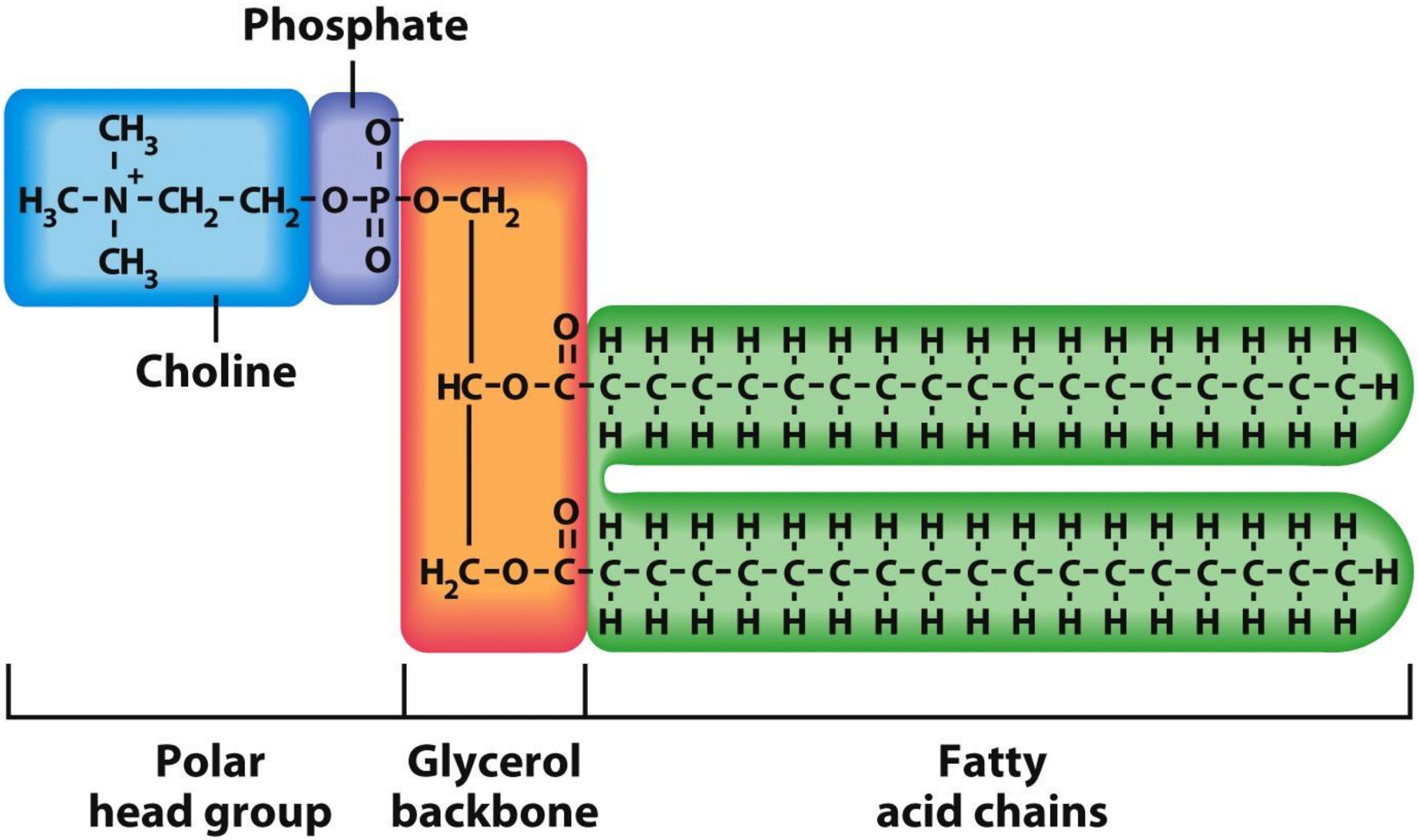
Lipids

Triacylglycerol (triglyceride)

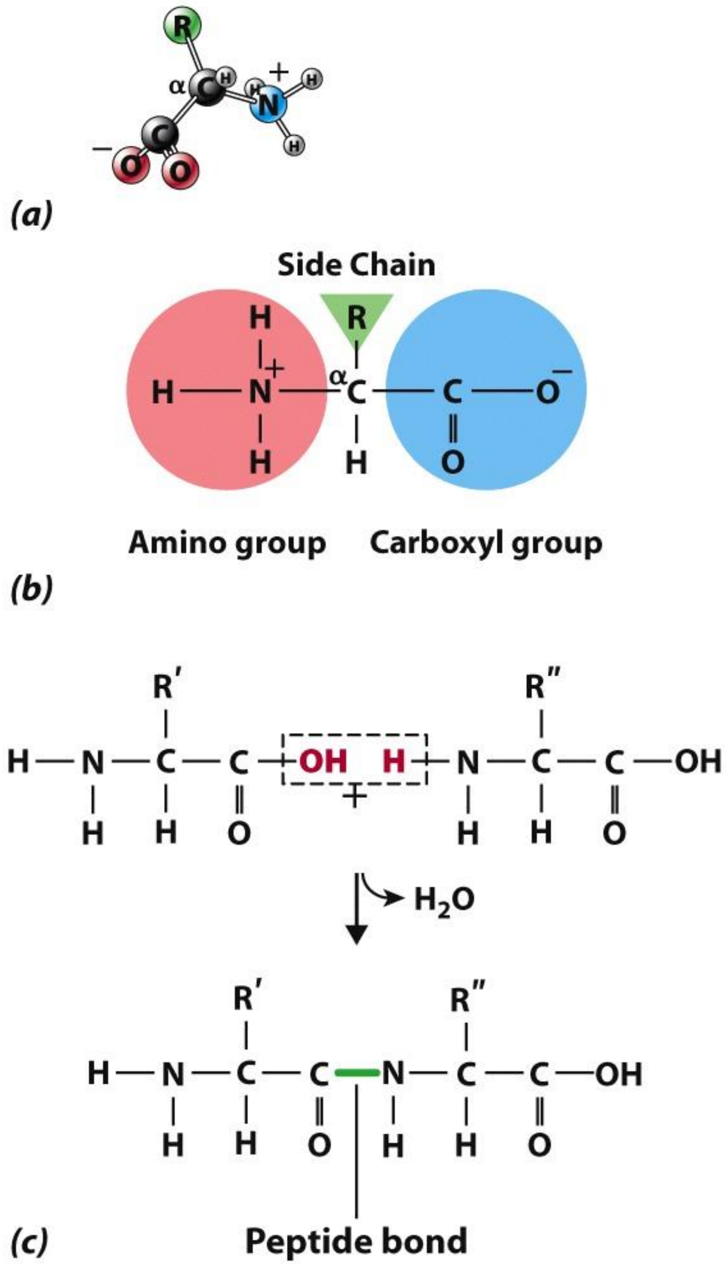


Long-term energy storage in animals

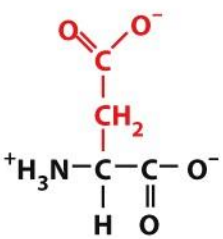
Phospholipid



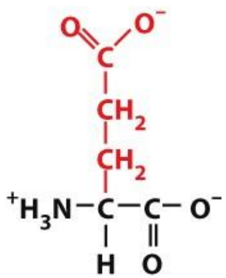
Proteins/polypeptides



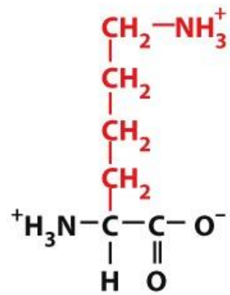
Polar charged



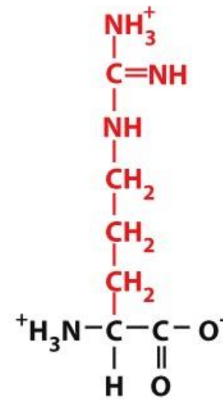
Aspartic acid
(Asp or D)



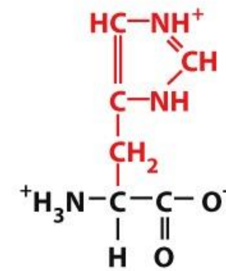
Glutamic acid
(Glu or E)



Lysine
(Lys or K)



Arginine
(Arg or R)

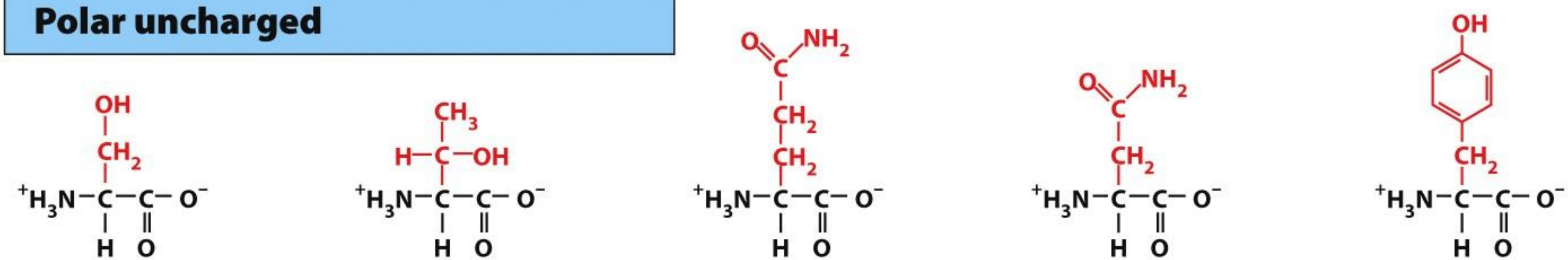


Histidine
(His or H)

Properties of side chains (R groups):

Hydrophilic side chains act as acids or bases which tend to be fully charged (+ or –) under physiologic conditions. Side chains form ionic bonds and are often involved in chemical reactions.

Polar uncharged



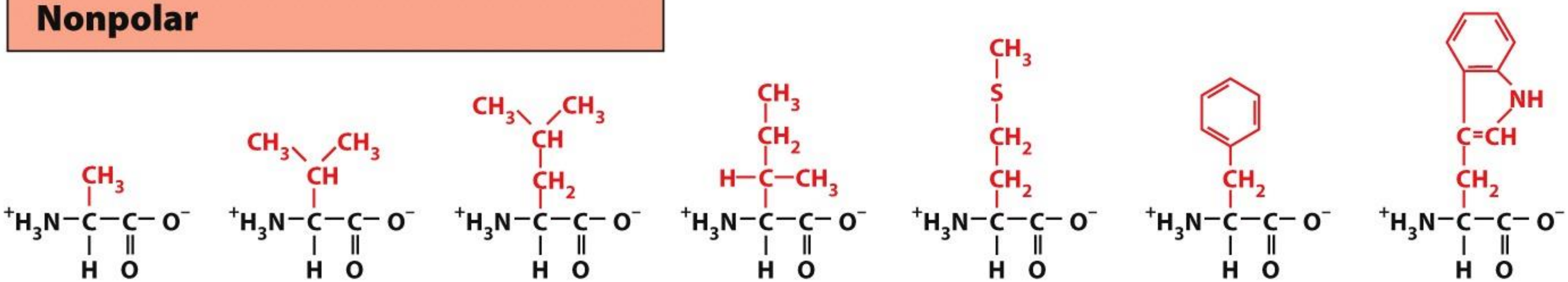
Serine (Ser or S)	**Threonine** (Thr or T)	**Glutamine** (Gln or Q)	**Asparagine** (Asn or N)	**Tyrosine** (Tyr or Y)

Properties of side chains:

Hydrophilic side chains tend to have partial + or – charge allowing them to participate in chemical reactions, form H-bonds, and associate with water.

Potential phosphorylation sites

Nonpolar

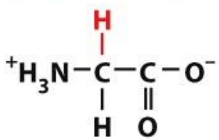


Alanine (Ala or A) | Valine (Val or V) | Leucine (Leu or L) | Isoleucine (Ile or I) | Methionine (Met or M) | Phenylalanine (Phe or F) | Tryptophan (Trp or W)

Properties of side chains:

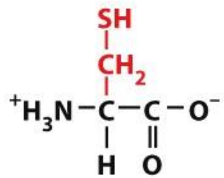
Hydrophobic side chain consists almost entirely of C and H atoms. These amino acids tend to form the inner core of soluble proteins, buried away from the aqueous medium. They play an important role in membranes by associating with the lipid bilayer.

Side chains with unique properties



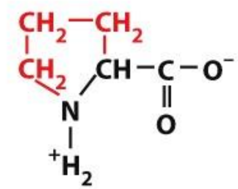
Glycine
(Gly or G)

Side chain consists only of hydrogen atom and can fit into either a hydrophilic or hydrophobic environment.Glycine often resides at sites where two polypeptides come into close contact.



Cysteine
(Cys or C)

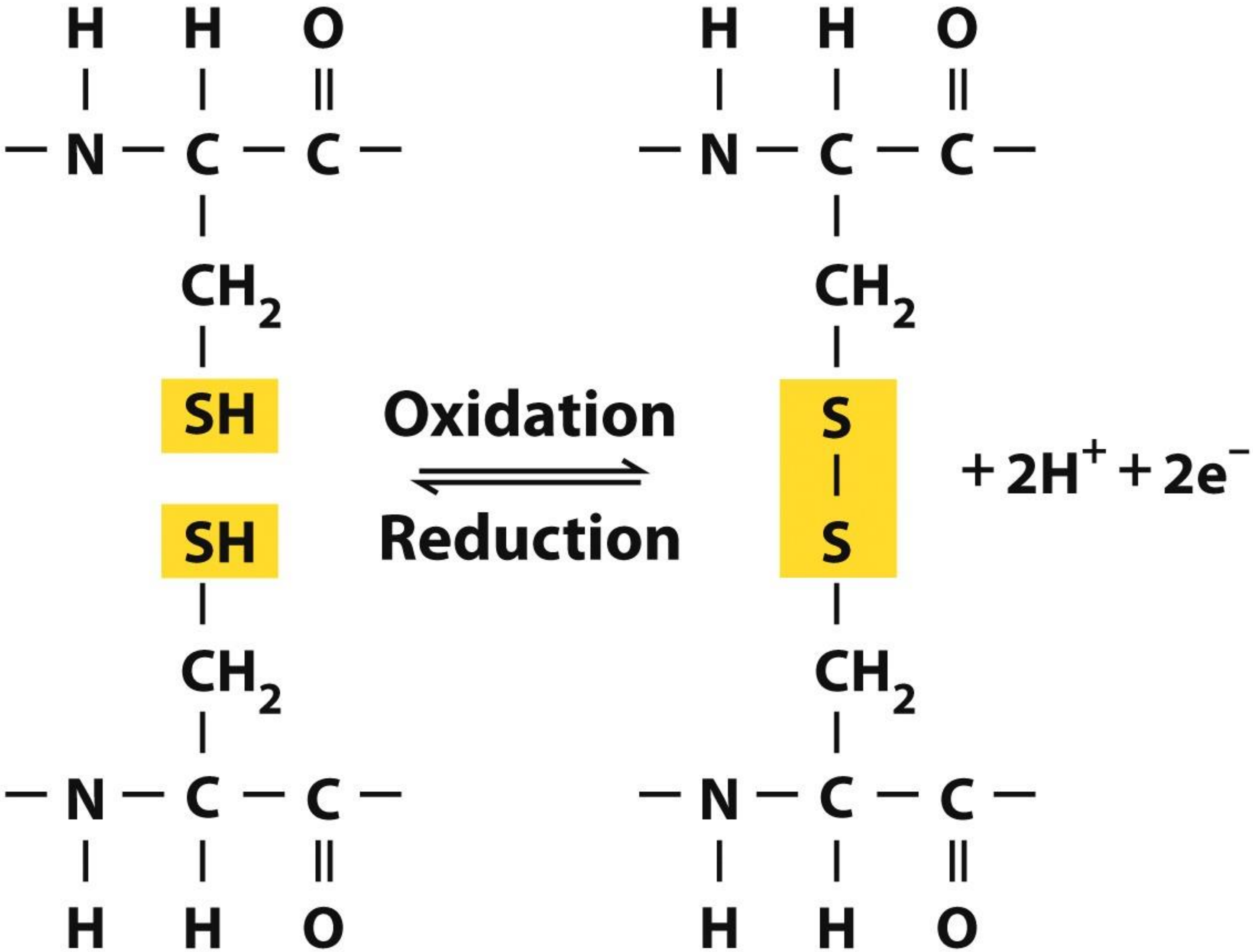
Though side chain has polar, uncharged character, it has the unique property of forming a covalent bond with another cysteine to form a disulfide link.



Proline
(Pro or P)

Though side chain has hydrophobic character, it has the unique property of creating kinks in polypeptide chains and disrupting ordered secondary structure.

Cysteine


H H O
N C C
CH2
SH
SH
CH2
N C C
H H O
Oxidation
Reduction
H H O
N C C
CH2
S
S
CH2
N C C
H H O
+ 2H+ + 2e−

Clicker questions will be uploaded into D2L weekly.

A practice exam will be provided in D2L one week before each actual exam.

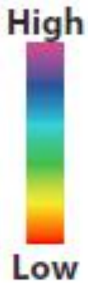
TA Office Hours

TA Name	Time	Location
Kati Buchtel	Tuesday 4 - 5:30	Gold A1B16 (Cell Bio Lab Room)
Christine Crotzer	Tuesday 5:30 - 6:30	Gold A1B16 (Cell Bio Lab Room)
Deanna Langager	Thursday 10 - 11:00	2nd Floor Interaction Room

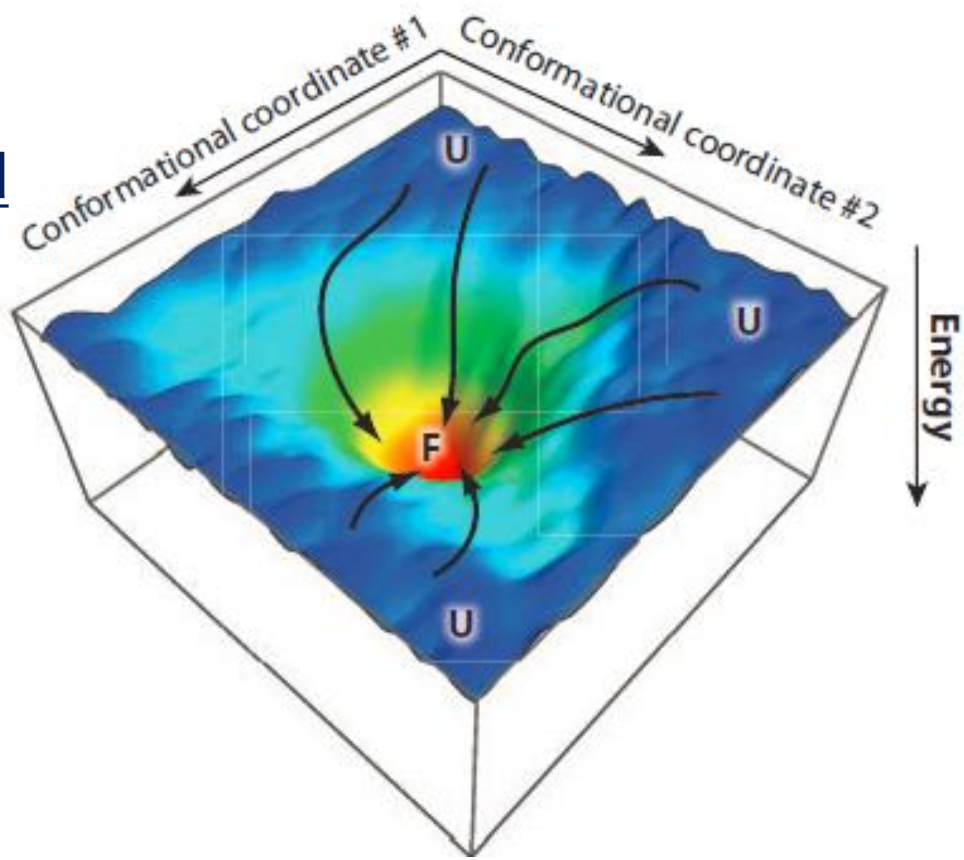
Joy Power's Office is B126D in Porter. Stop by anytime the door is open. If you need to set an appointment, use email: Joy.Power@colorado.edu.

Protein folding – to find the lowest energy state (native conformation)

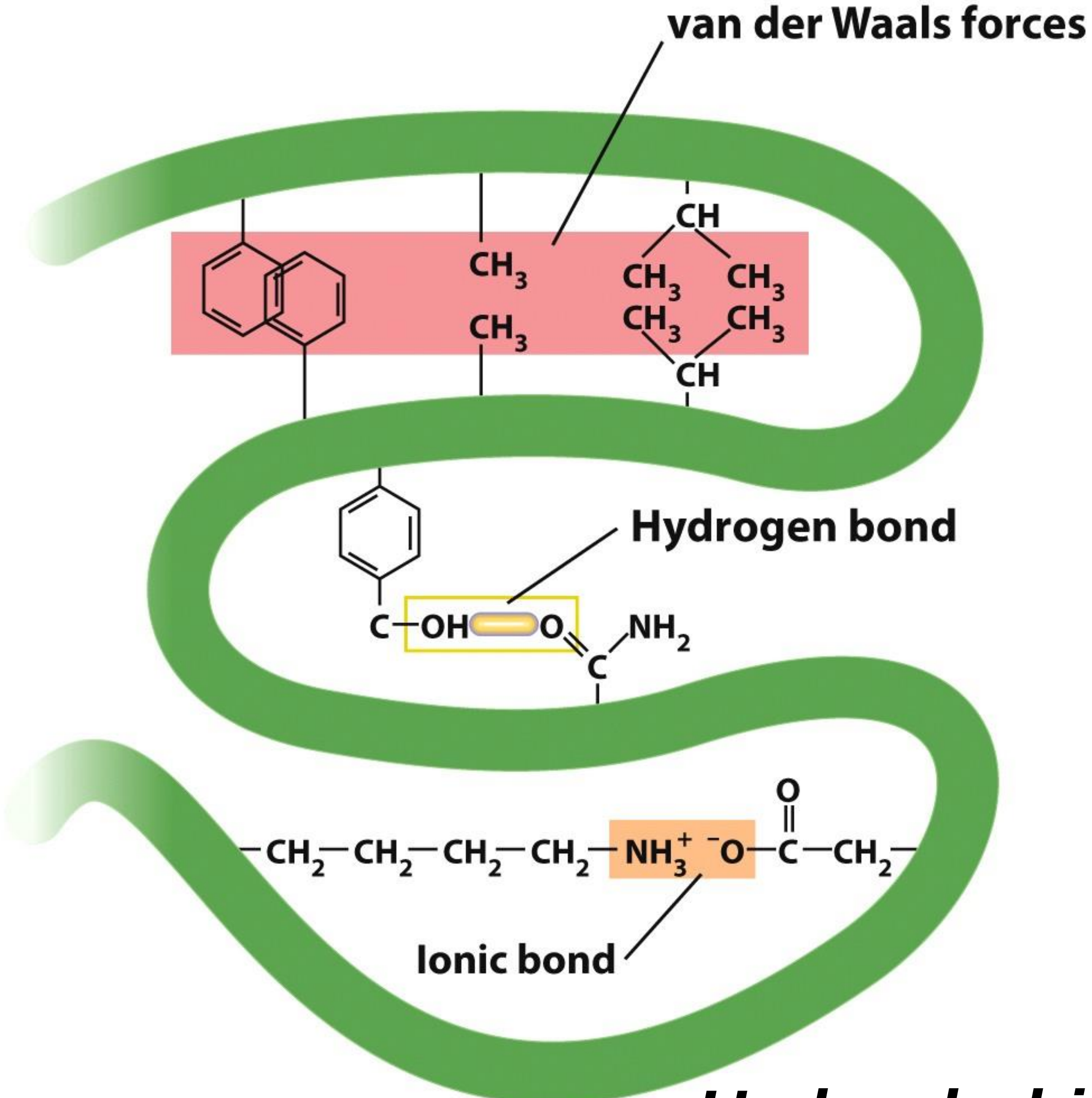
Unfolded



Folded

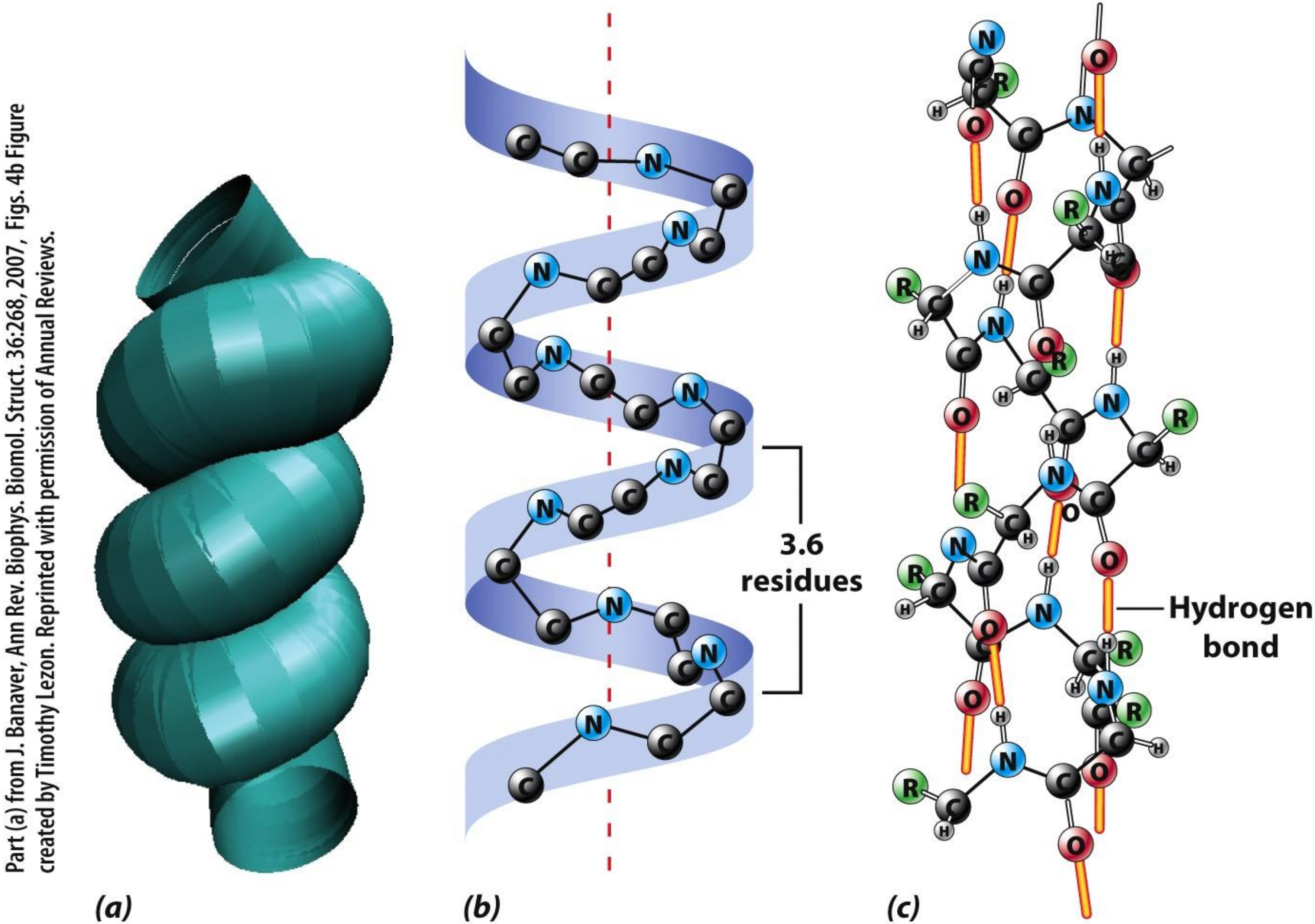


Noncovalent bonds



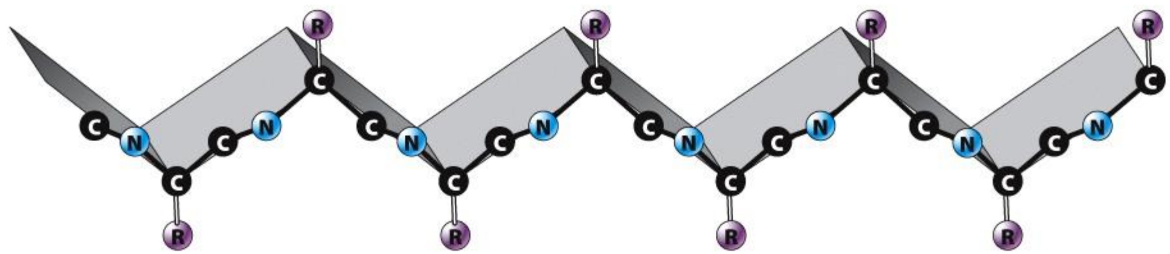
Hydrophobic effect as well

Secondary structures - alpha helix

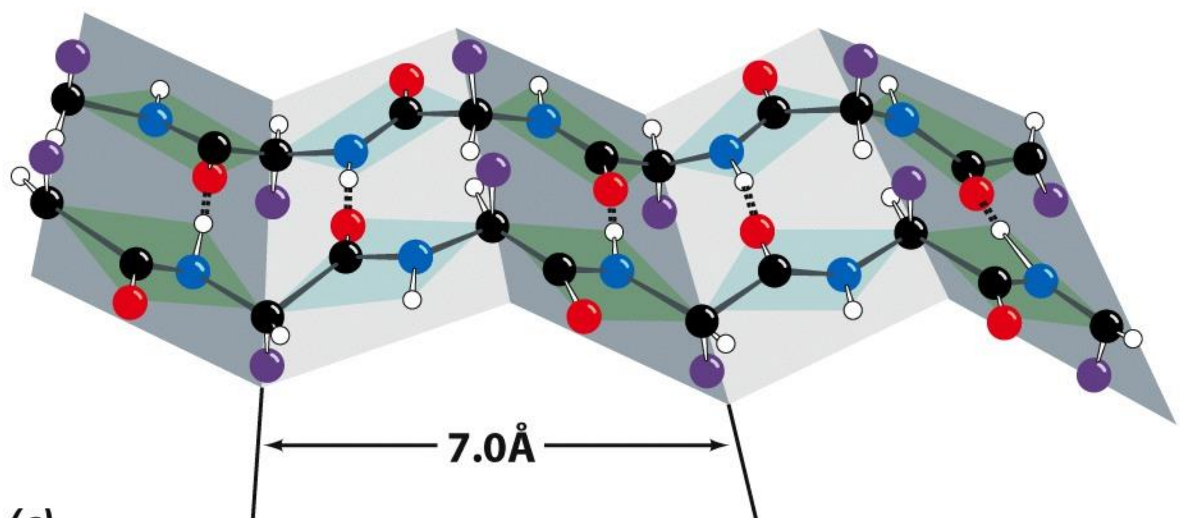


Secondary structures – beta sheet

(a)

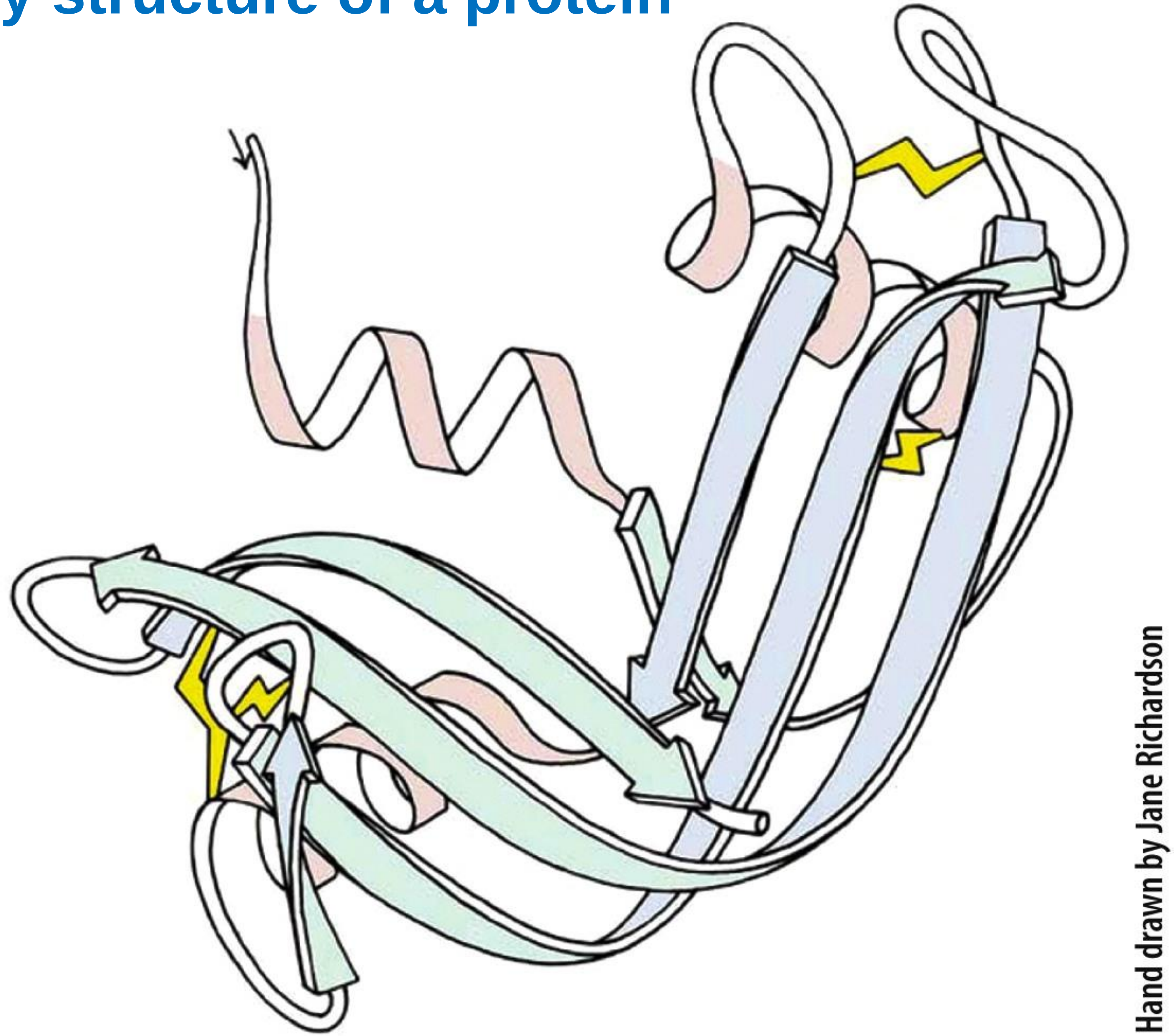


(b)

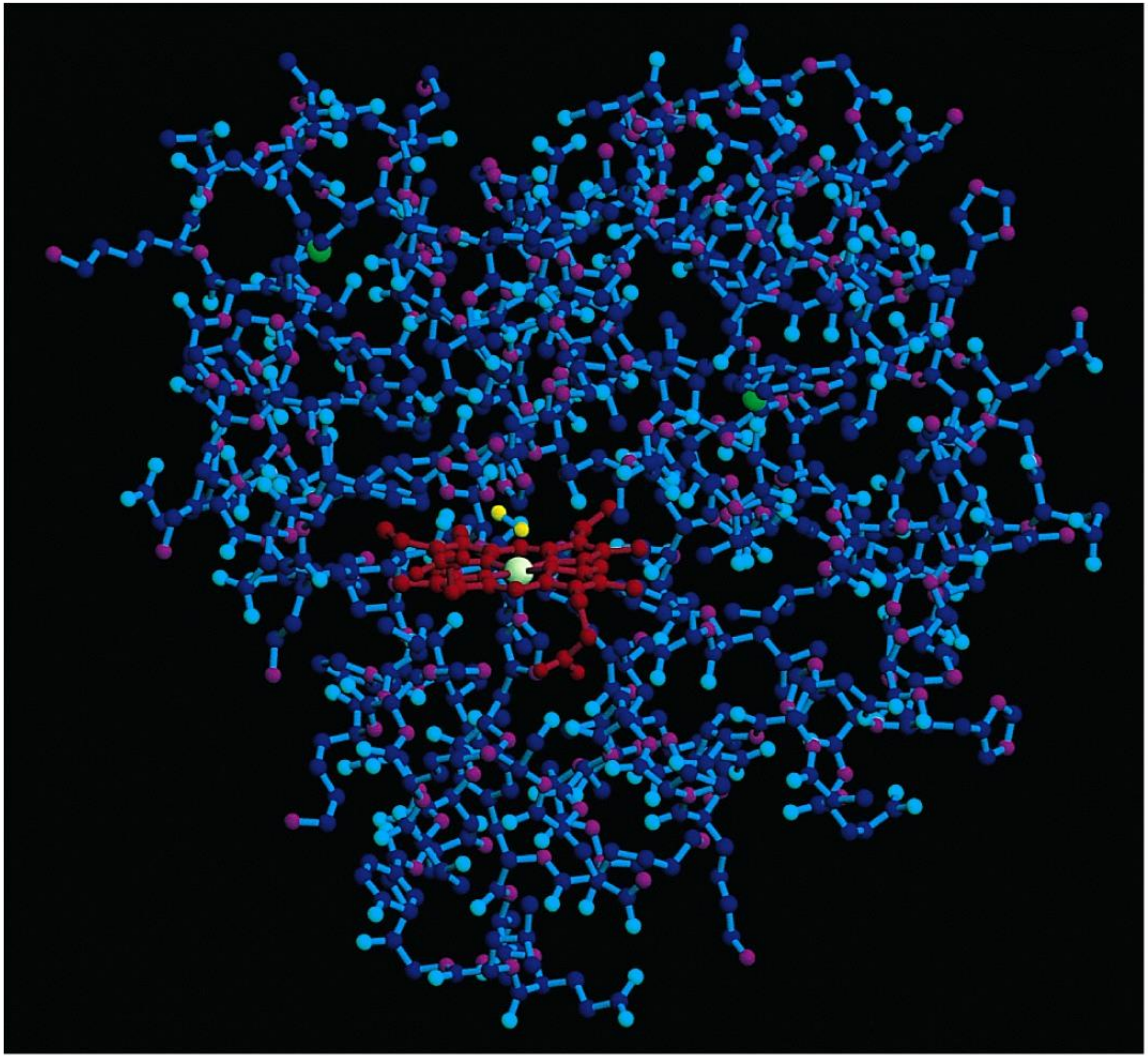


(c)

Tertiary structure of a protein



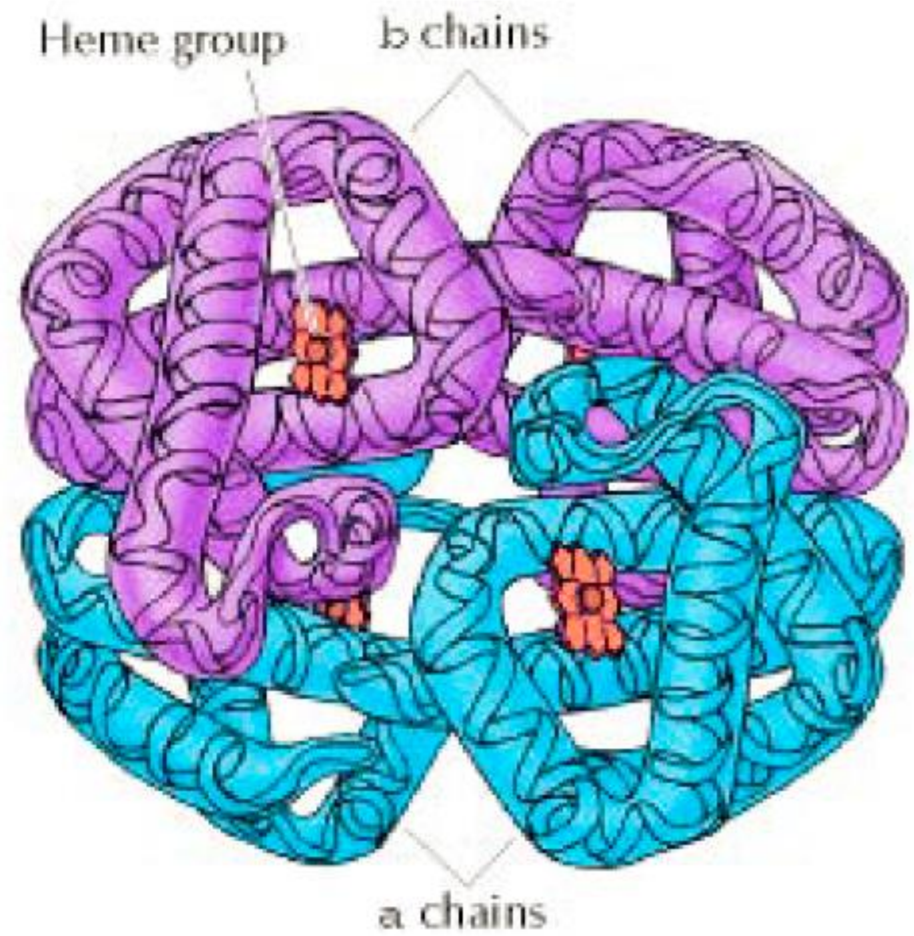
Tertiary structure of a protein



Ken Eward/Photo Researchers

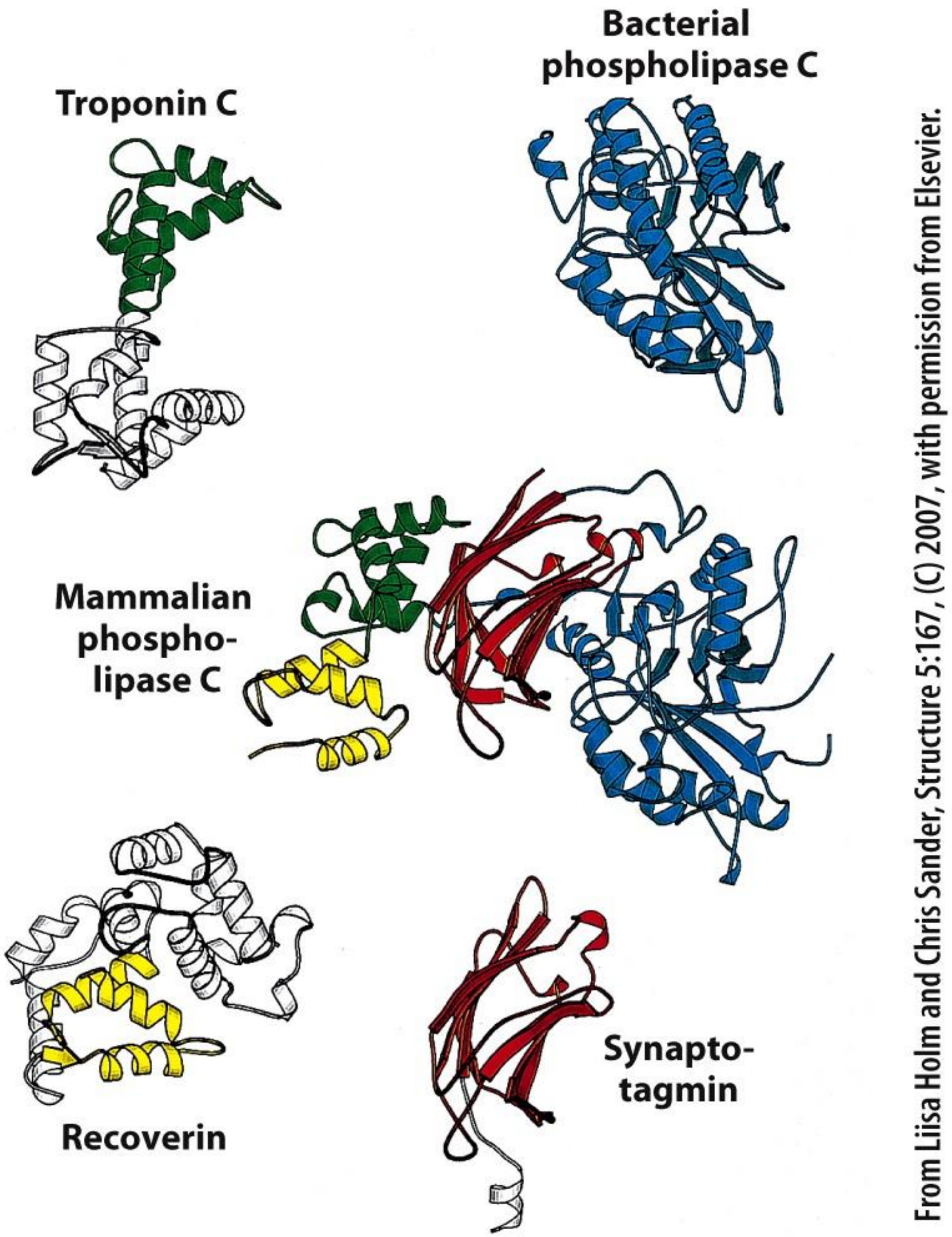
Myoglobin

Quaternary structure of a protein complex

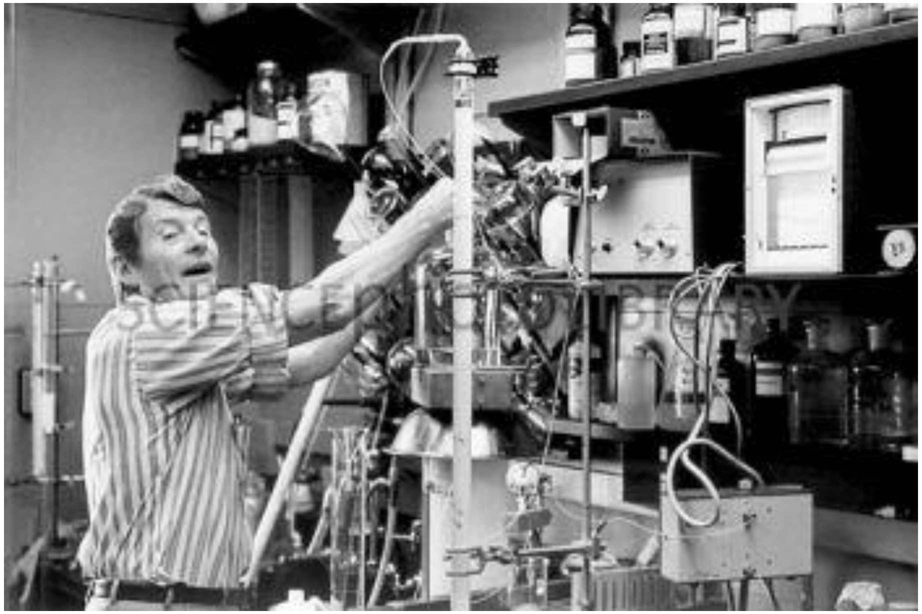


Hemoglobin

Protein domains

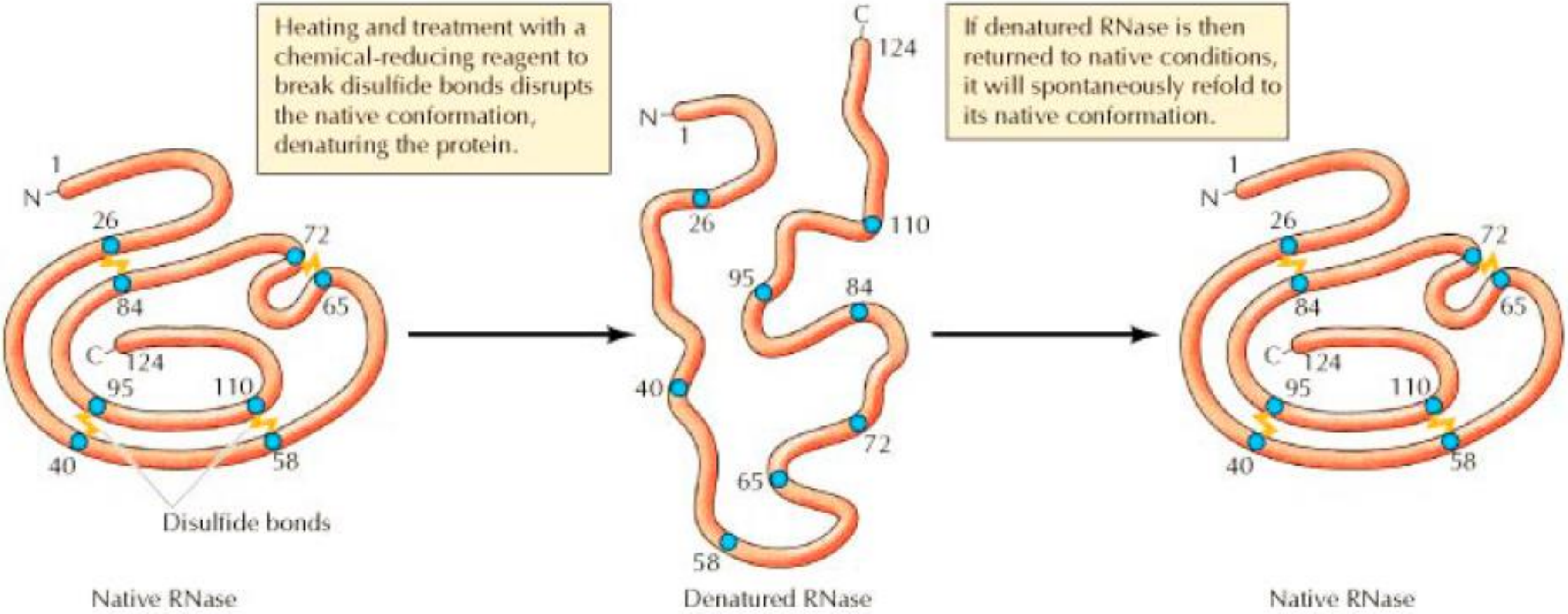


How do proteins fold?

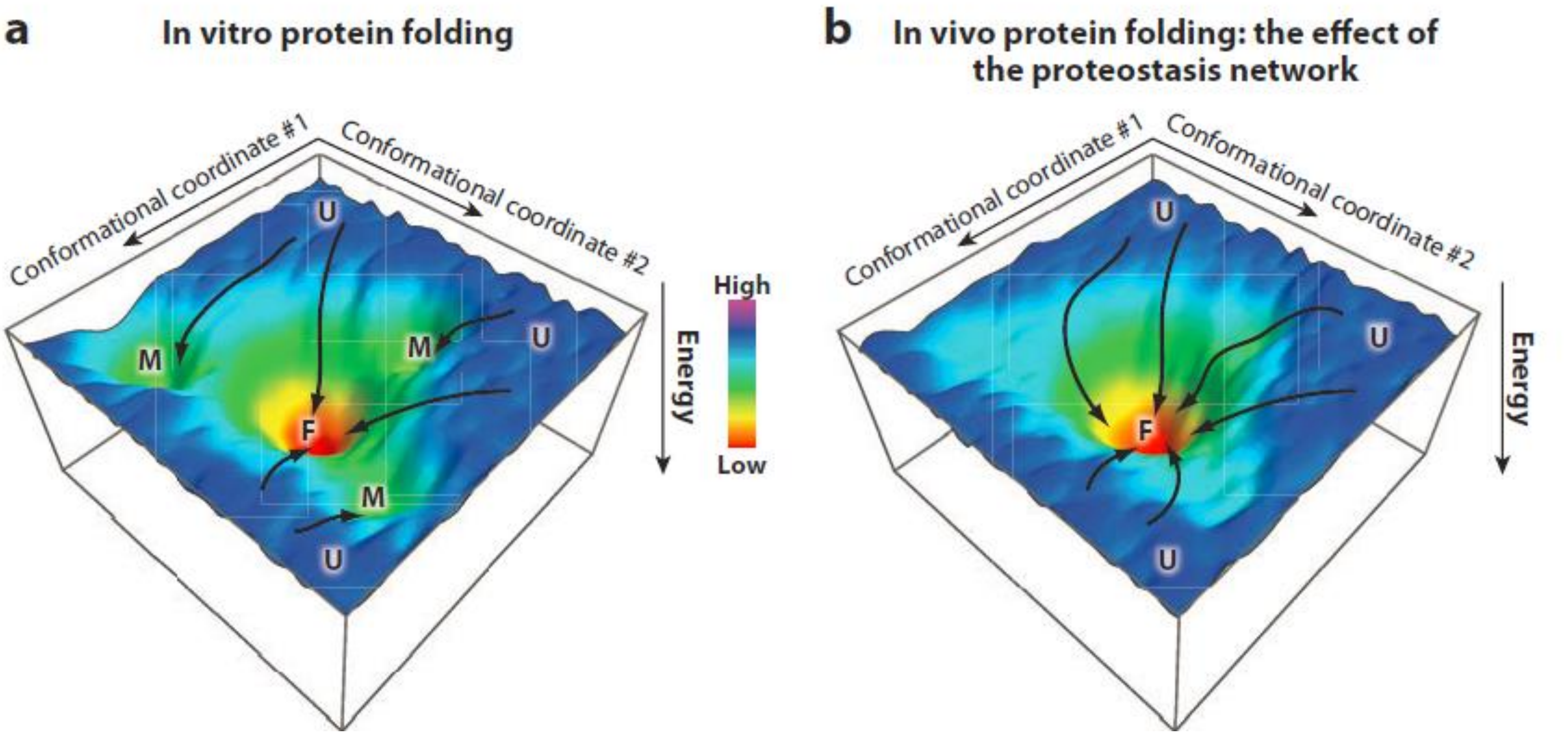


Christian Anfinsen
1972 Nobel Prize in Chemistry

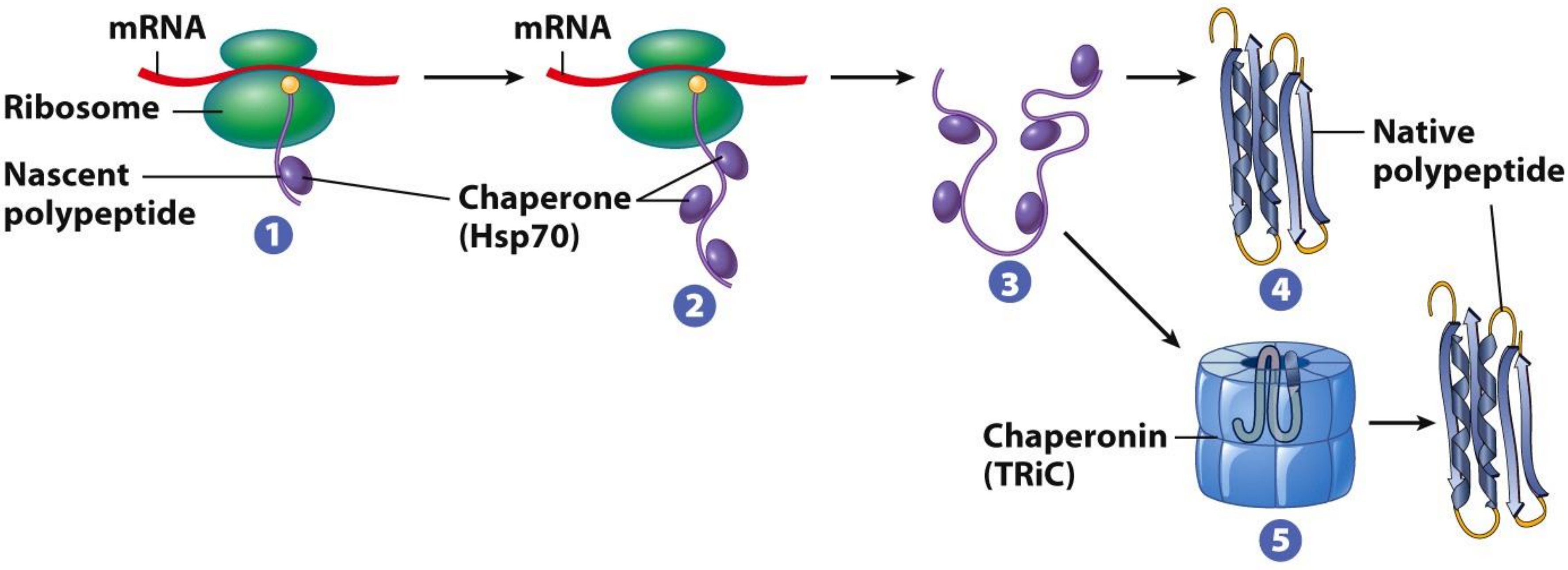
Denatured RNase spontaneously folds into its native conformation



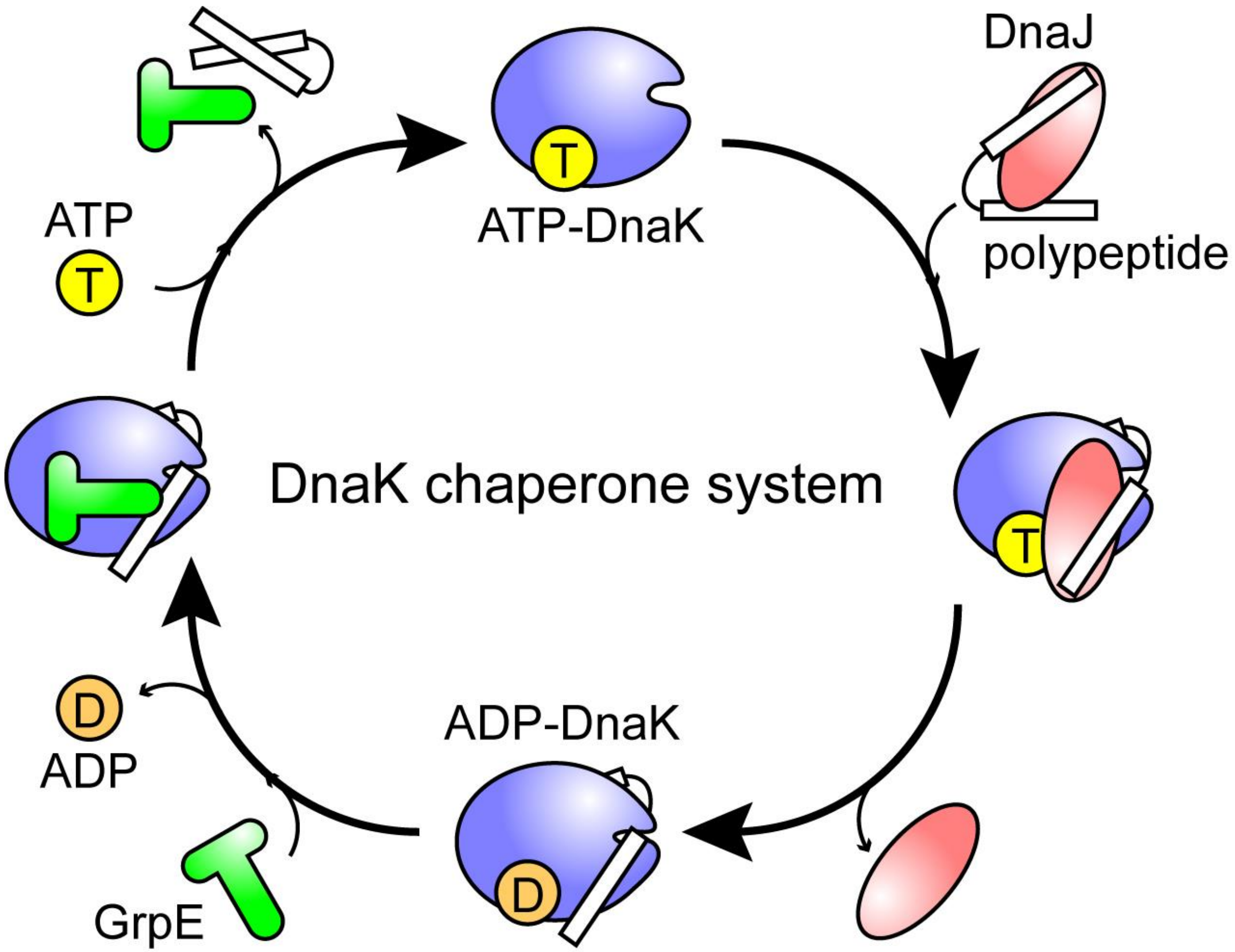
Protein folding – to find the lowest energy state (native conformation)



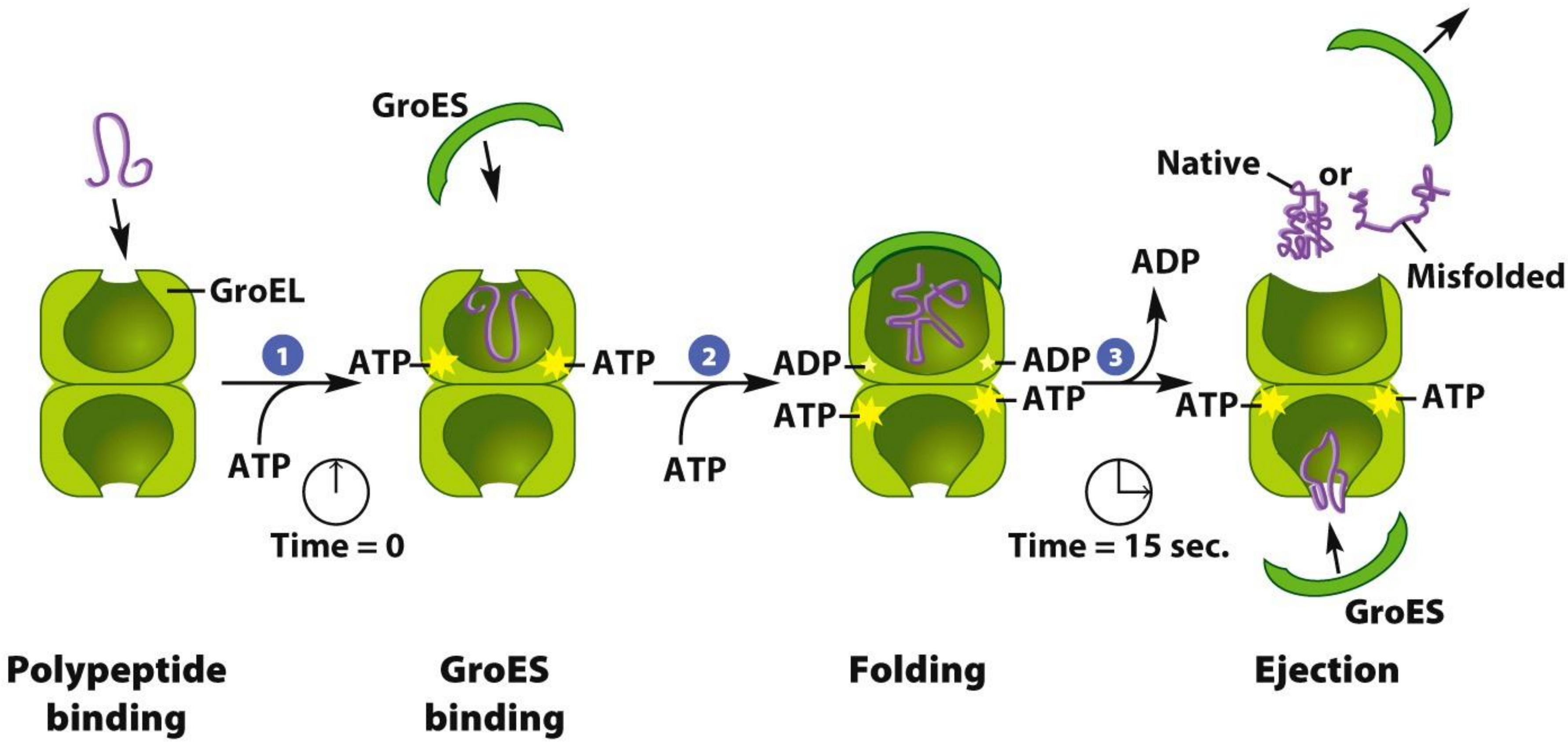
Chaperones assist protein folding in the cell



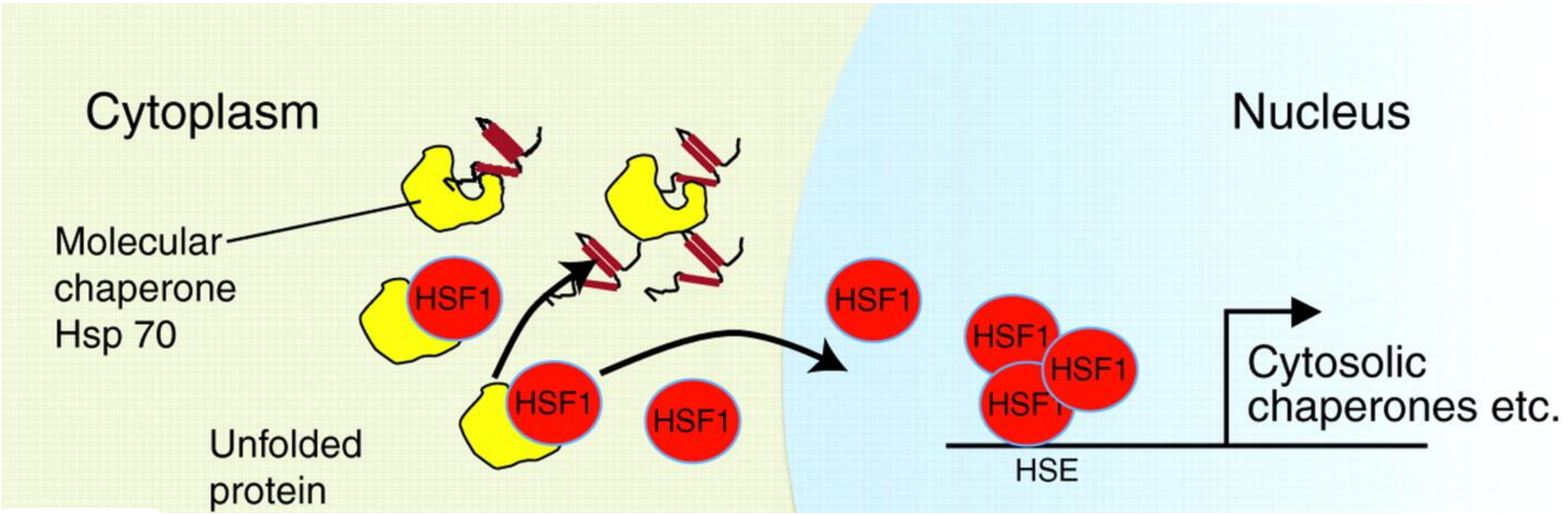
DnaK/Hsp70 chaperones promote protein folding



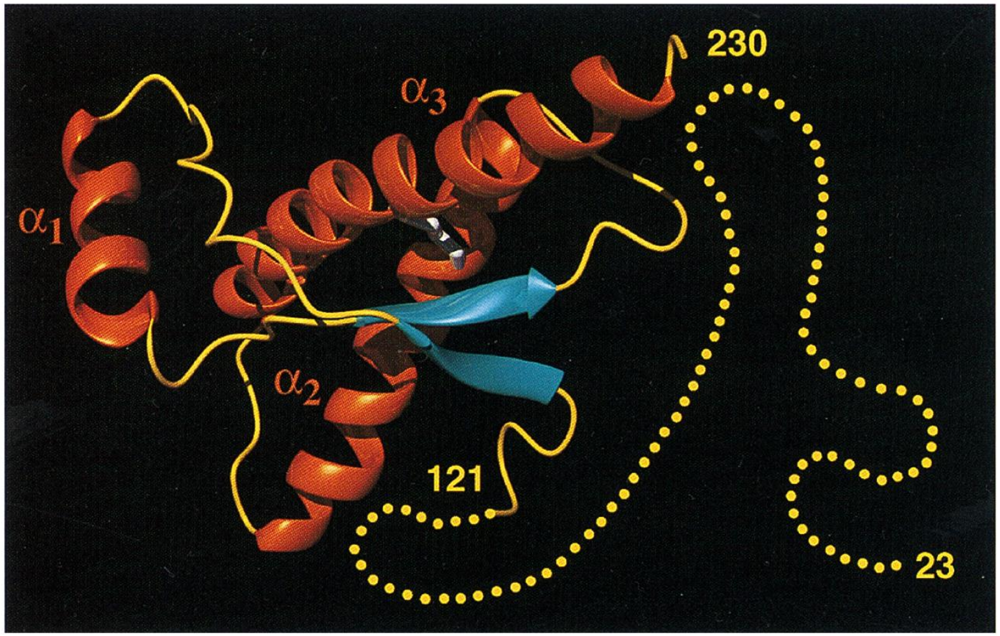
The chaperonin system



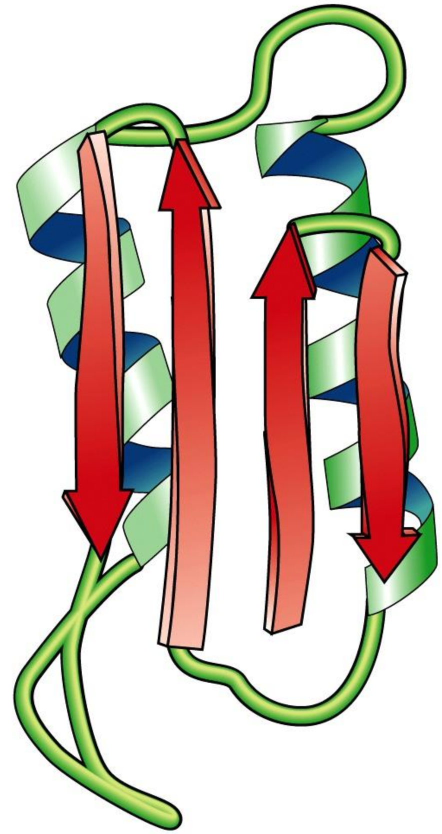
Heat shock response



Prion protein (PrP)

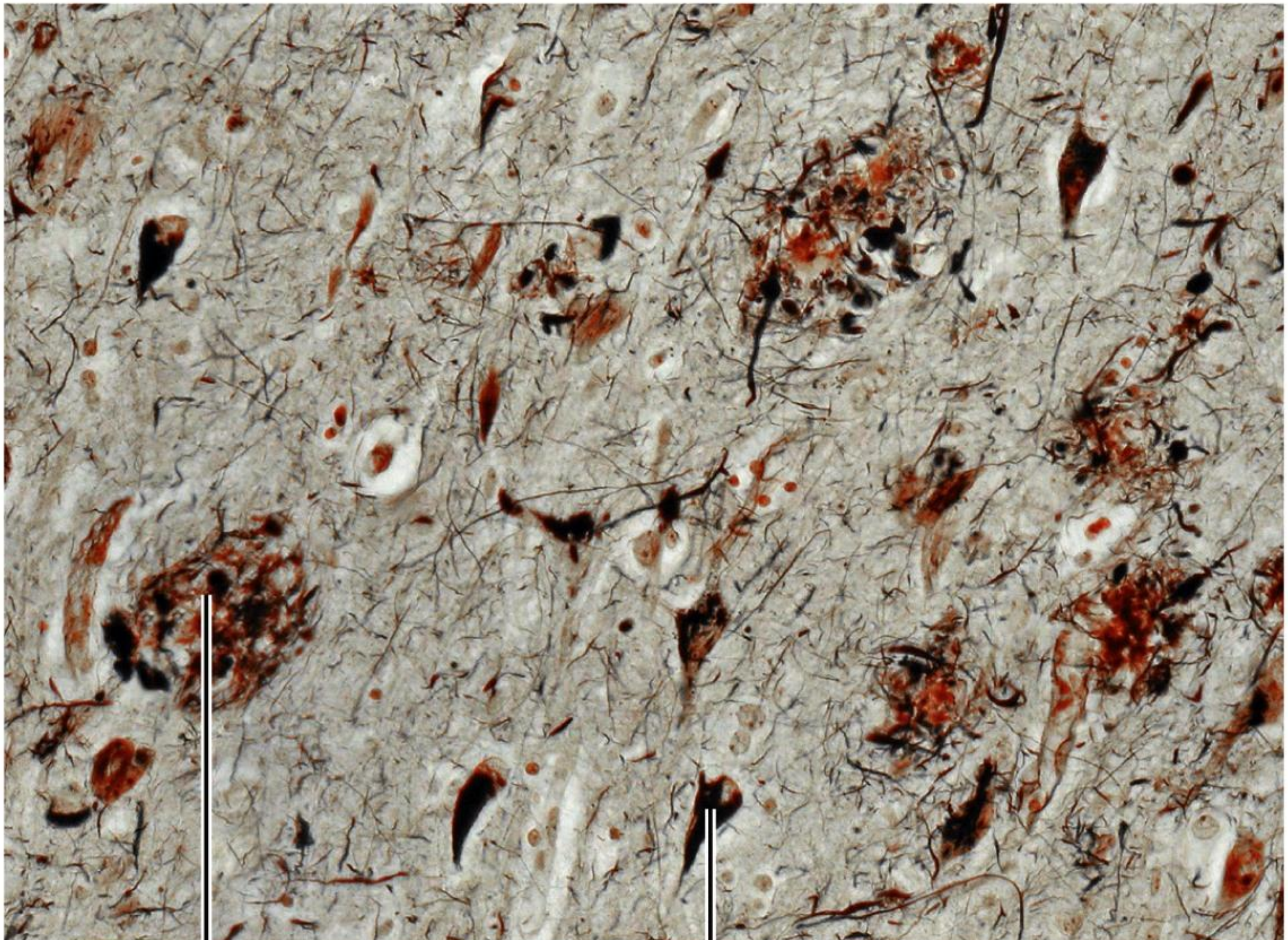


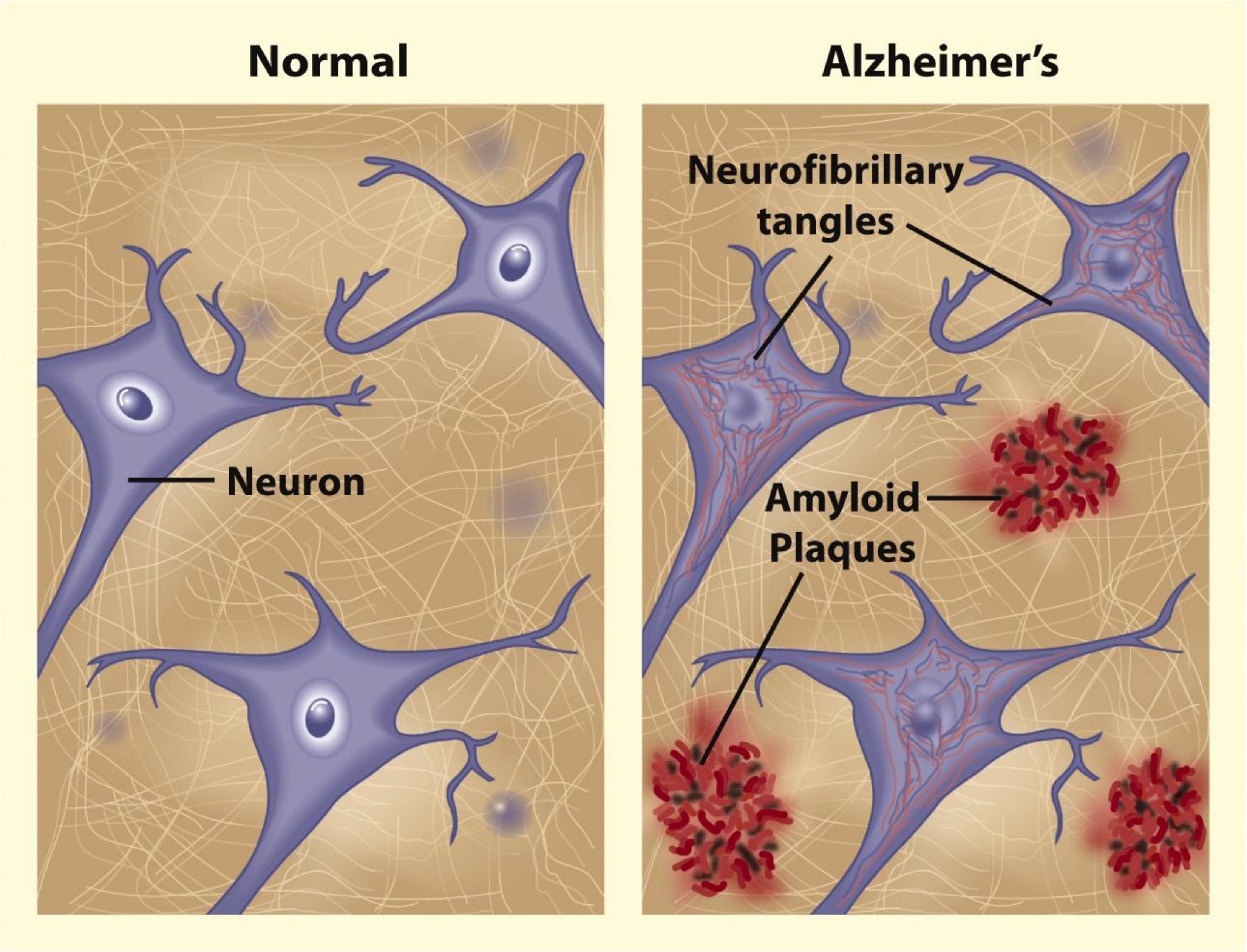
PrP^{C}

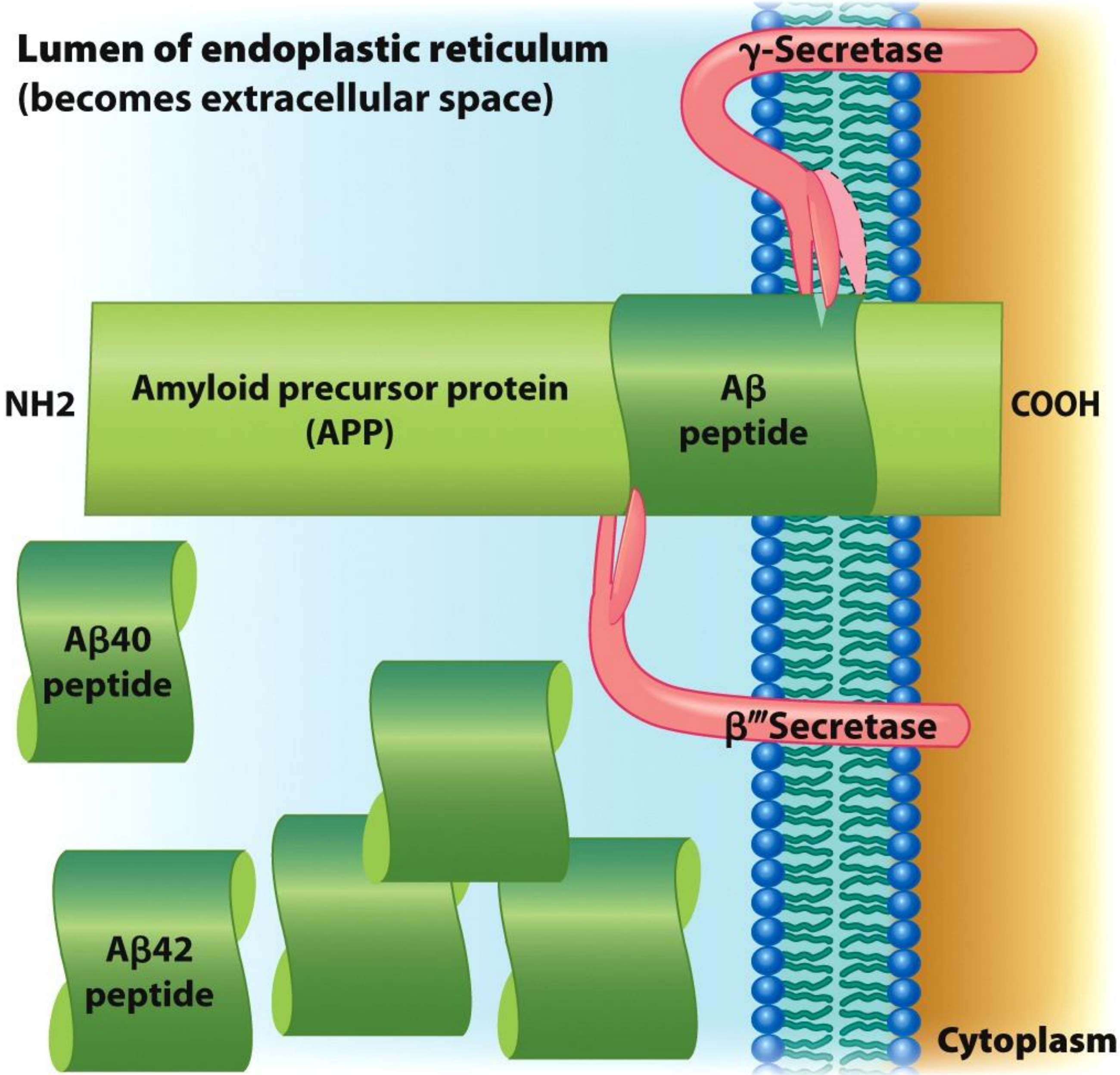


PrP^{Sc}

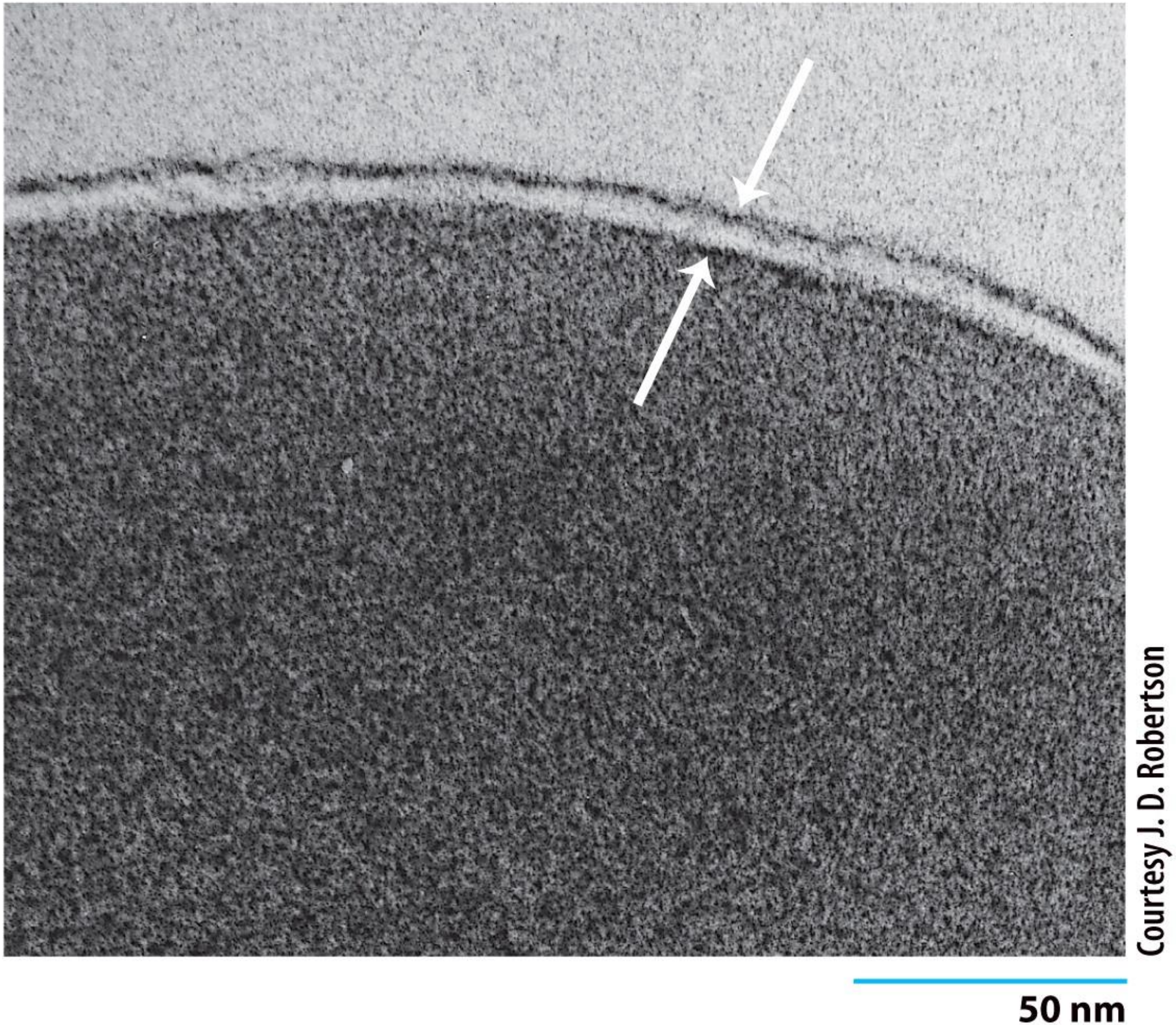
Alzheimer's Disease

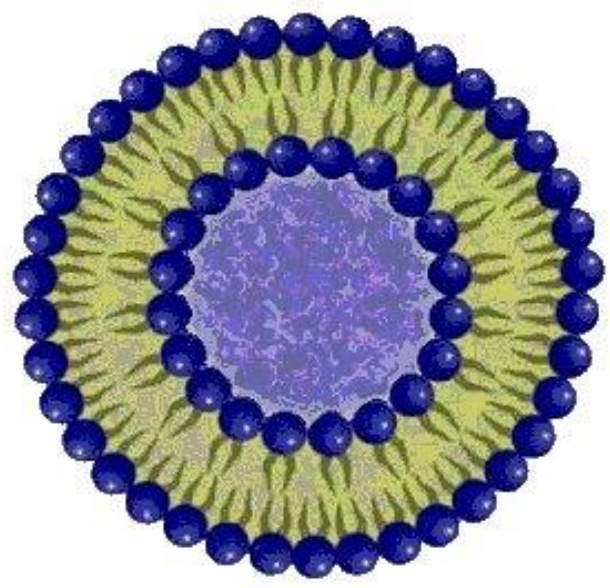



Normal
Alzheimer's
Neuron
Neurofibrillary tangles
Amyloid Plaques


Lumen of endoplastic reticulum
(becomes extracellular space)
γ-Secretase
NH2
Amyloid precursor protein
(APP)
Aβ
peptide
COOH
Aβ40
peptide
β‴Secretase
Aβ42
peptide
Cytoplasm

The plasma membrane

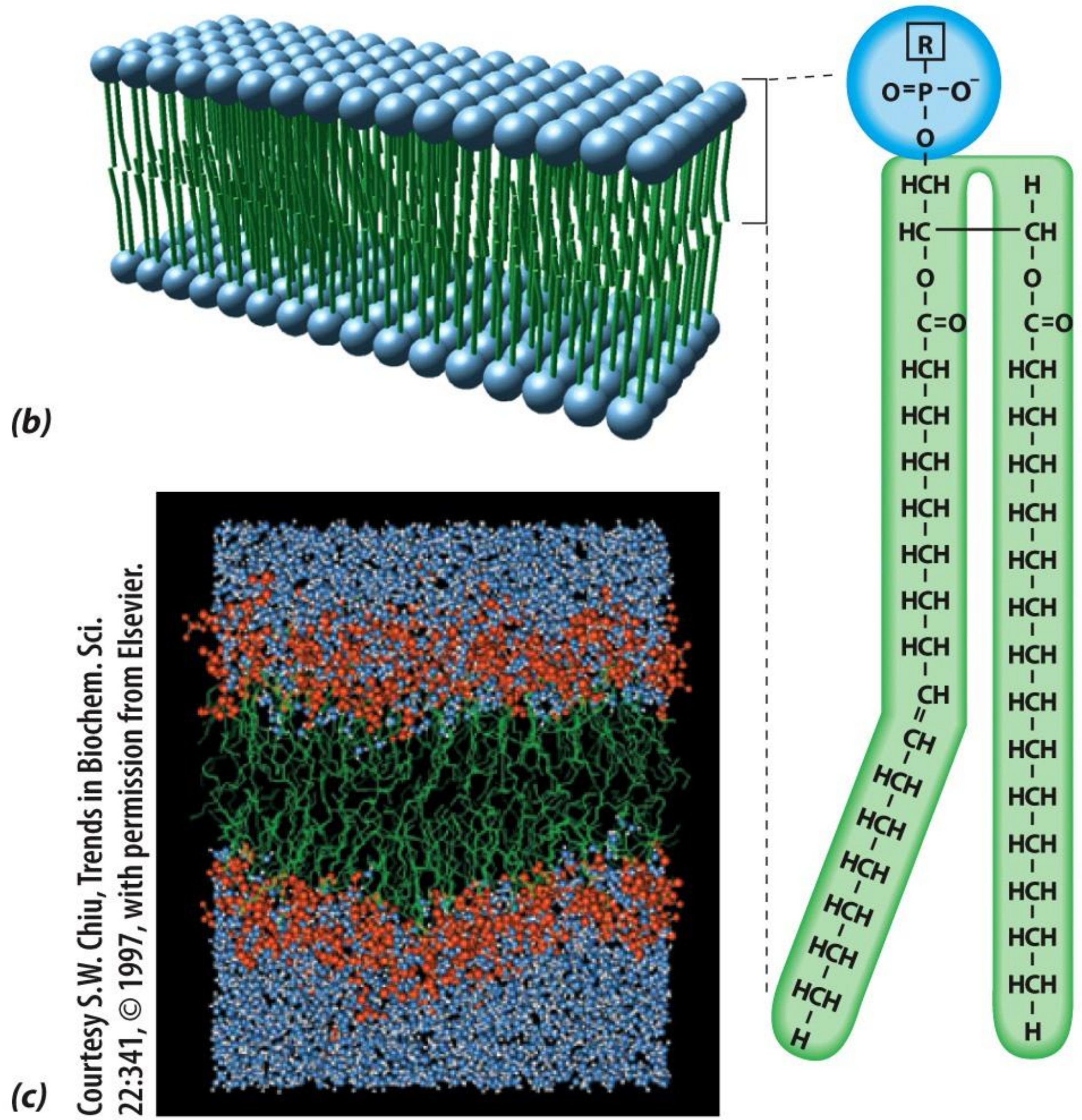




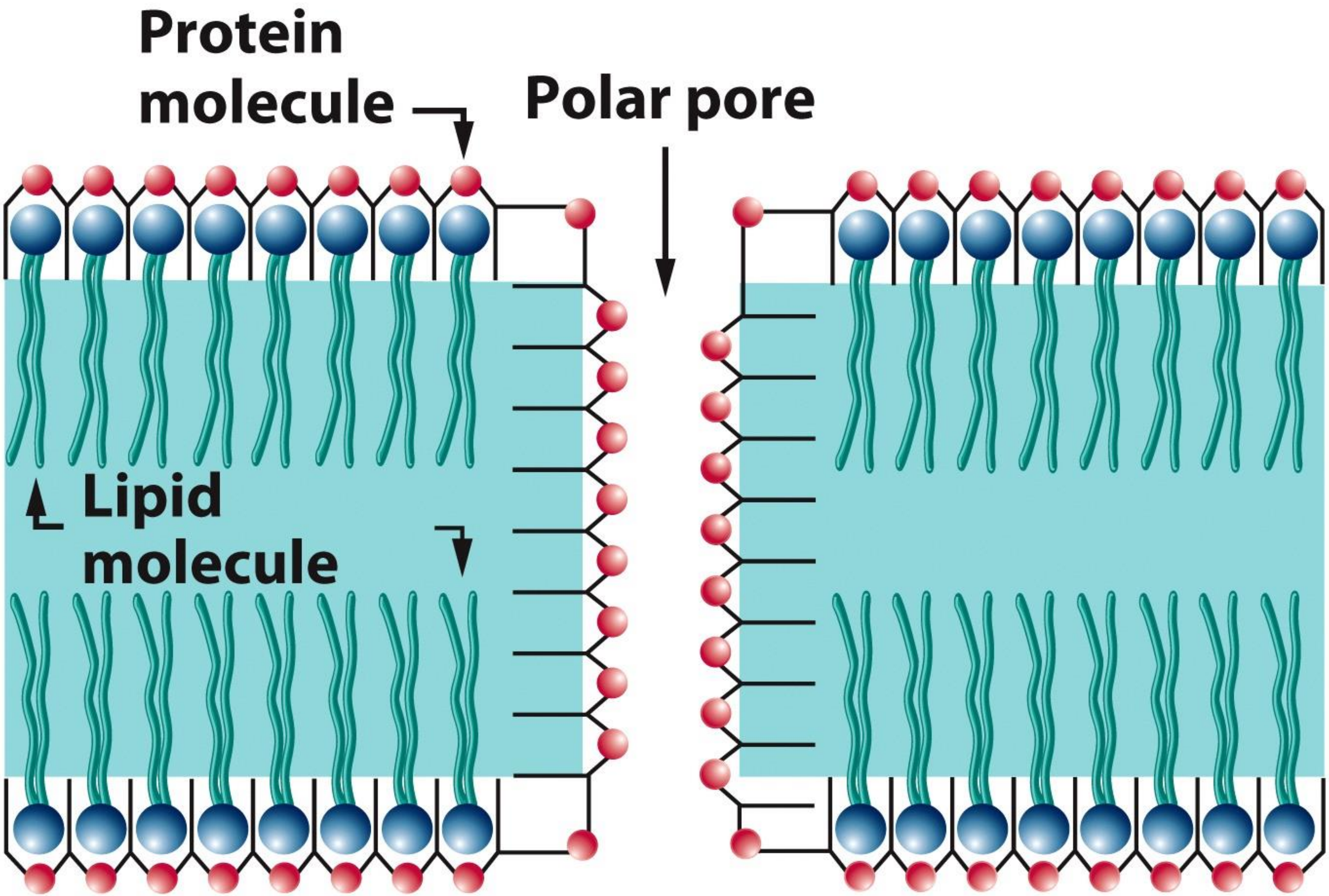
Membrane - lipid bilayer

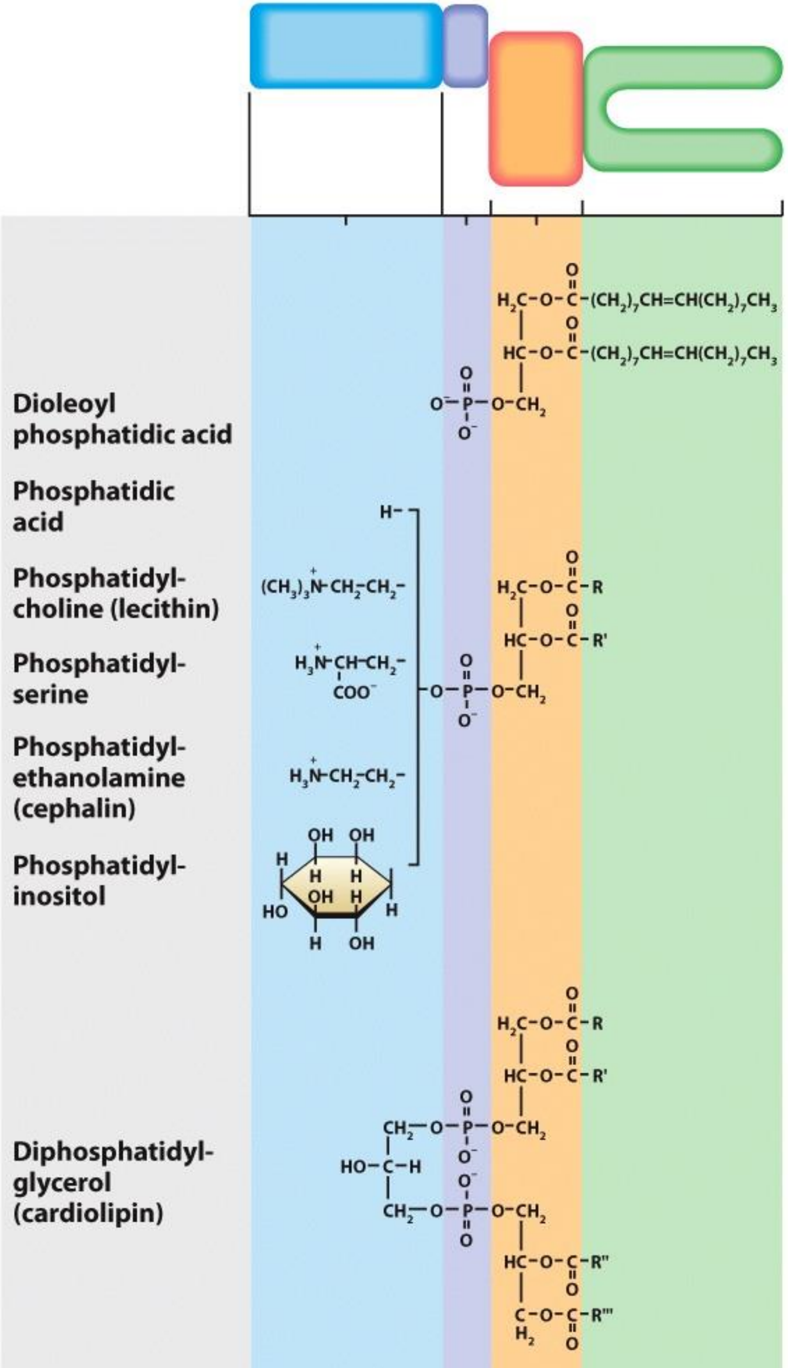
(Formed by mixing phospholipids and water)

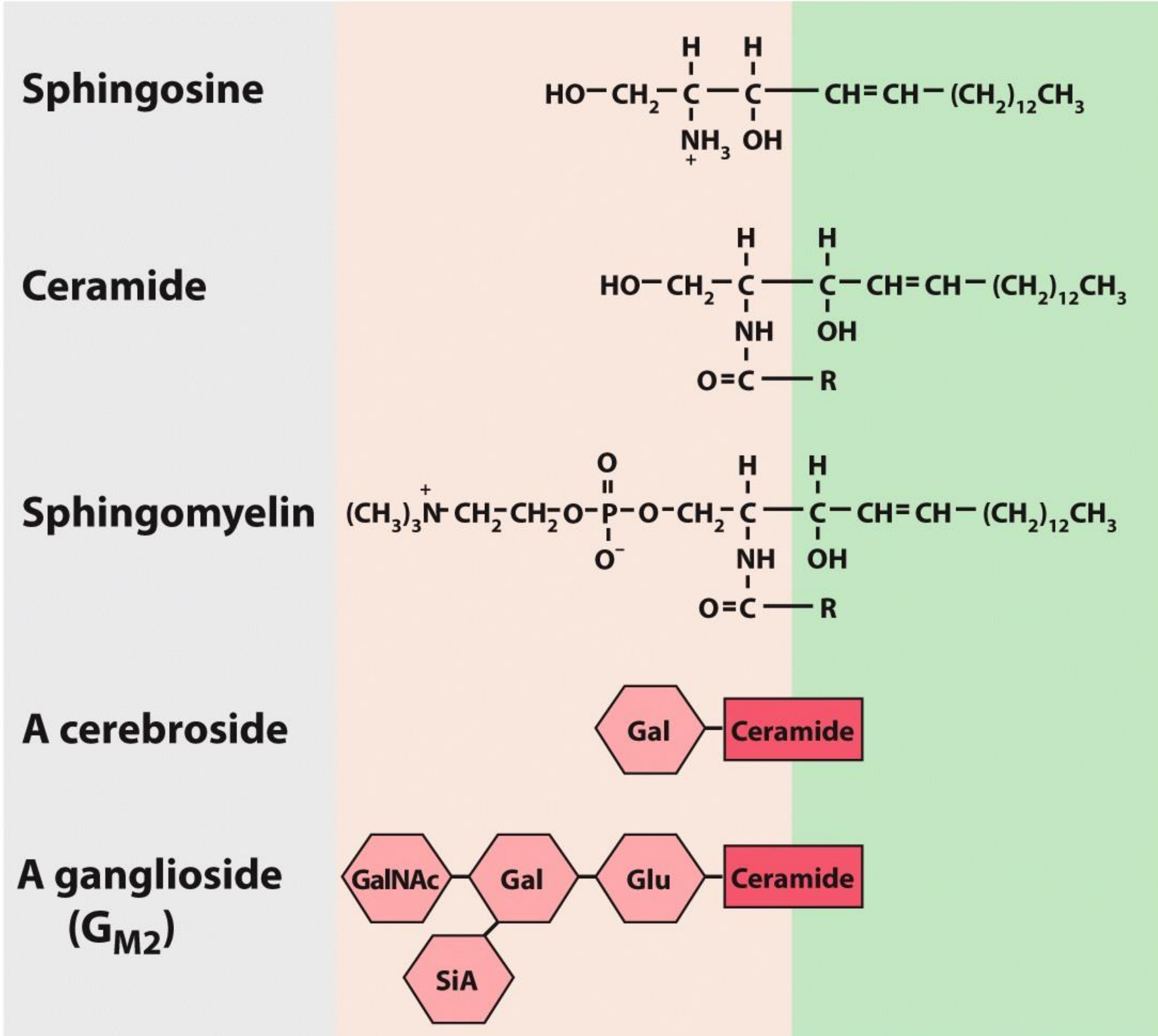
Phospholipids: building blocks of membranes



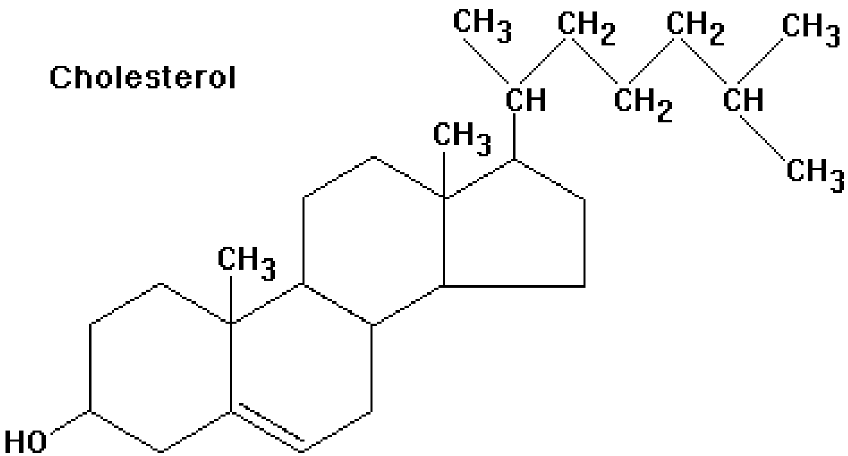
Phospholipids: building blocks of membranes

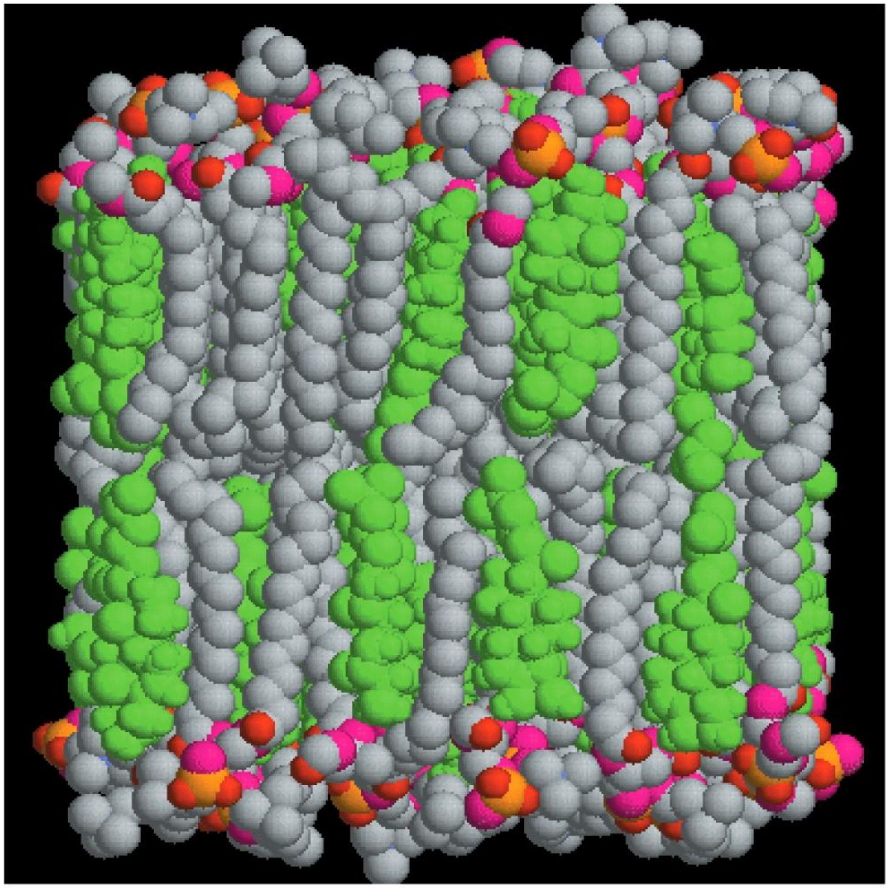



Dioleoyl phosphatidic acid
Phosphatidic acid
Phosphatidyl-choline (lecithin)
Phosphatidyl-serine
Phosphatidyl-ethanolamine (cephalin)
Phosphatidyl-inositol
Diphosphatidyl-glycerol (cardiolipin)


Sphingosine
Ceramide
Sphingomyelin
A cerebroside
Gal
Ceramide
A ganglioside (G_{M2})
GalNAc
Gal
Glu
Ceramide
SiA

Cholesterol





Lipid asymmetry

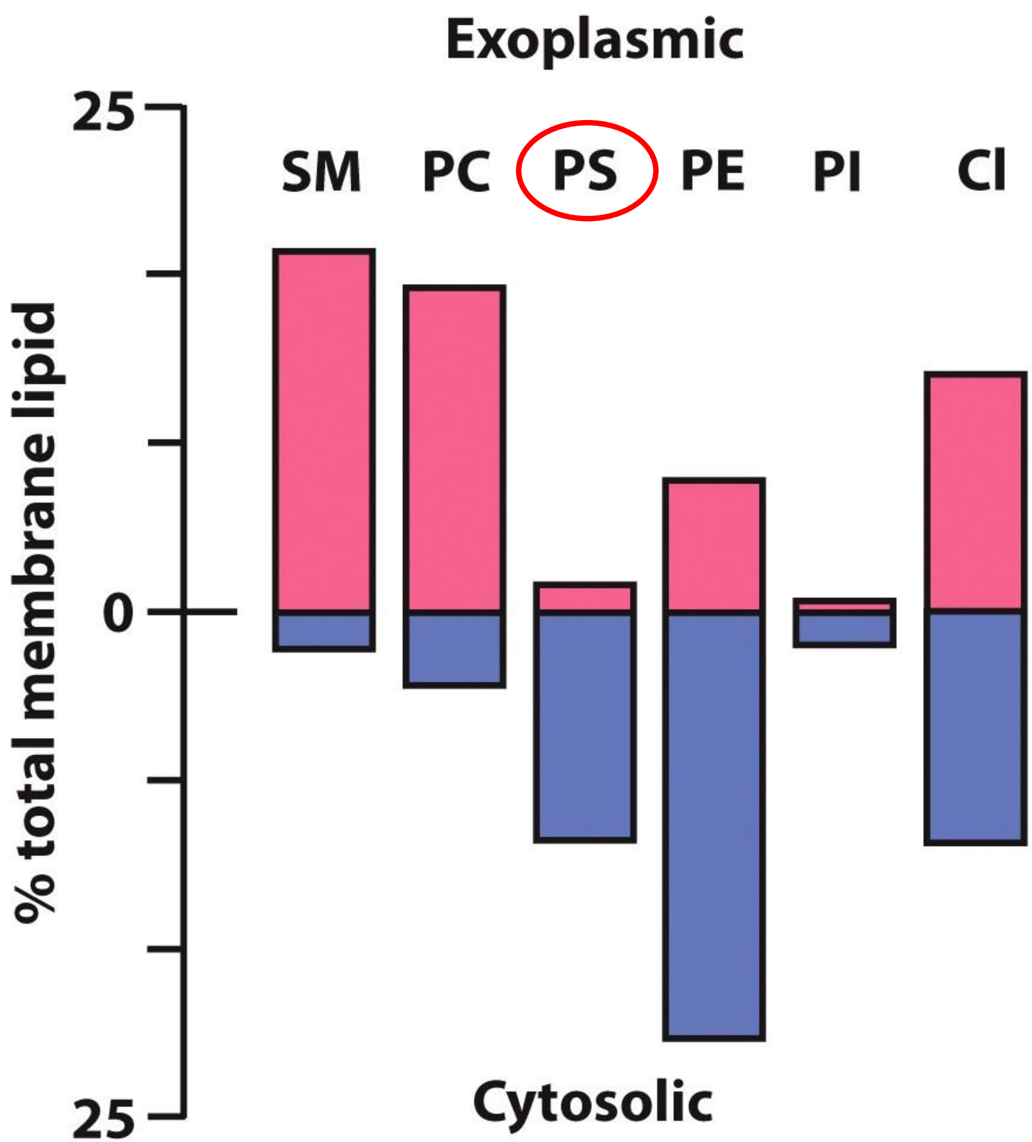
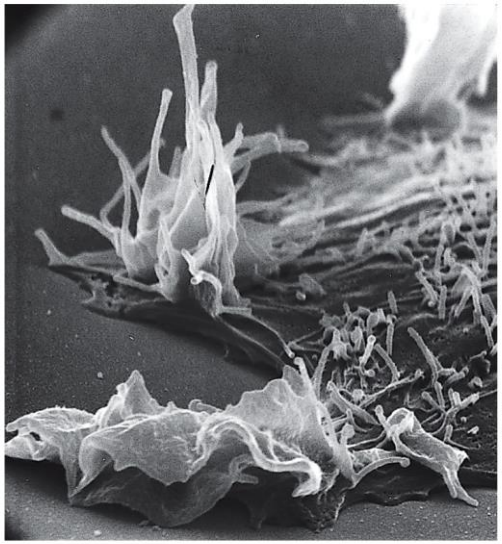


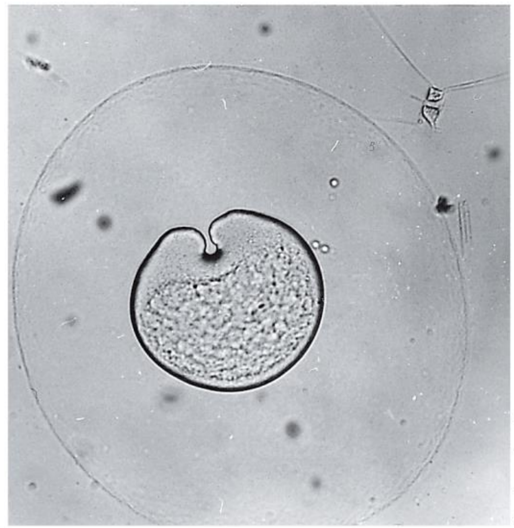
Table 4.1 Lipid Compositions of Some Biological Membranes*

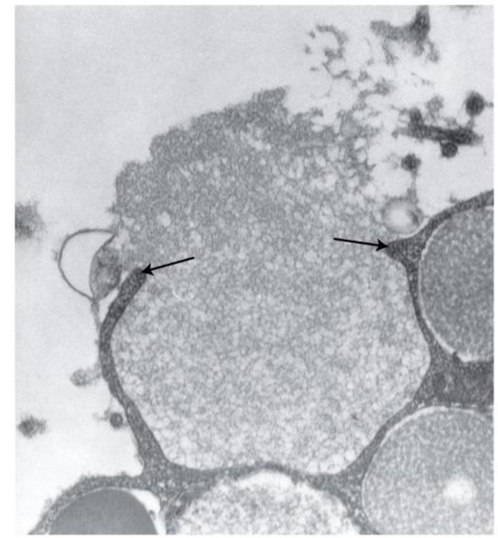
Lipid	Human erythrocyte	Human myelin	Beef heart mitochondria	*E. coli*
Phosphatidic acid	1.5	0.5	0	0
Phosphatidylcholine	19	10	39	0
Phosphatidyl-ethanolamine	18	20	27	65
Phosphatidylgycerol	0	0	0	18
Phosphatidylserine	8.5	8.5	0.5	0
Cardiolipin	0	0	22.5	12
Sphingomyelin	17.5	8.5	0	0
Glycolipids	10	26	0	0
Cholesterol	25	26	3	0

*The values given are weight percent of total lipid.
Source: C. Tanford, *The Hydrophobic Effect*, p. 109, copyright 1980, John Wiley & Sons, Inc. Reprinted by permission of John Wiley & Sons, Inc.

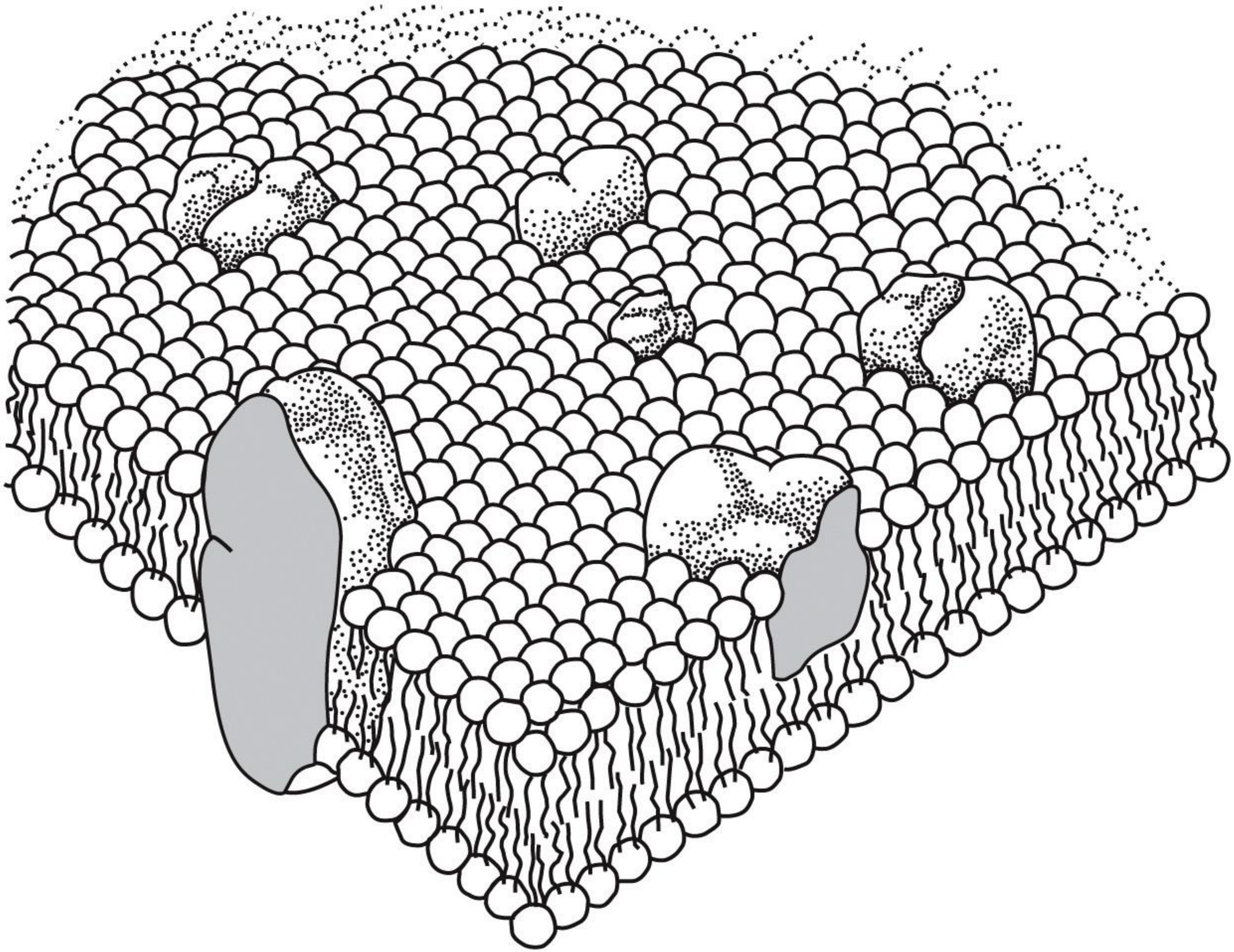
Dynamic plasma membrane


Courtesy Jean Paul Revel

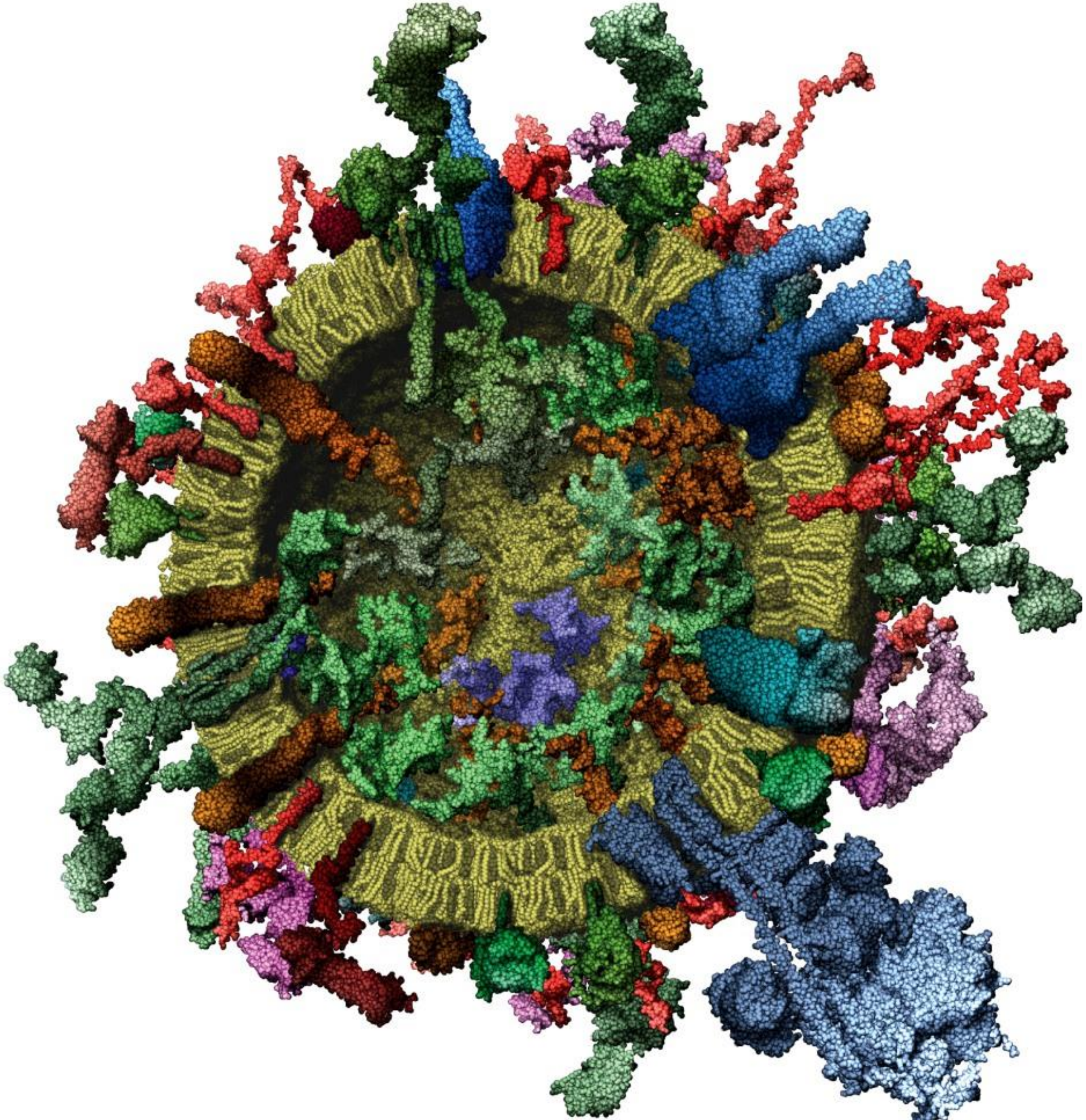

Courtesy Gary Freeman


Courtesy Susan Jo Burwen

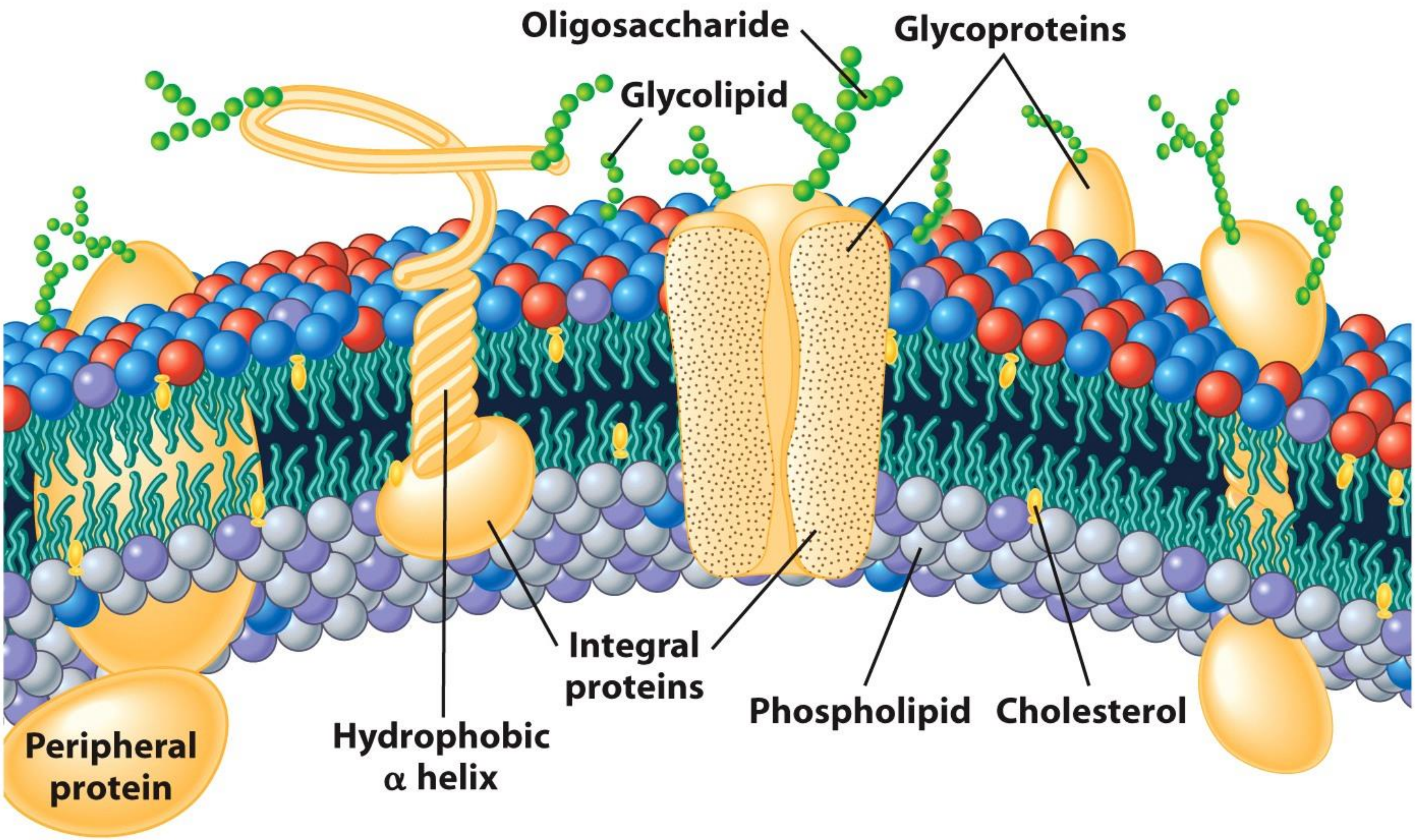
Fluid-mosaic model



Synaptic vesicle model

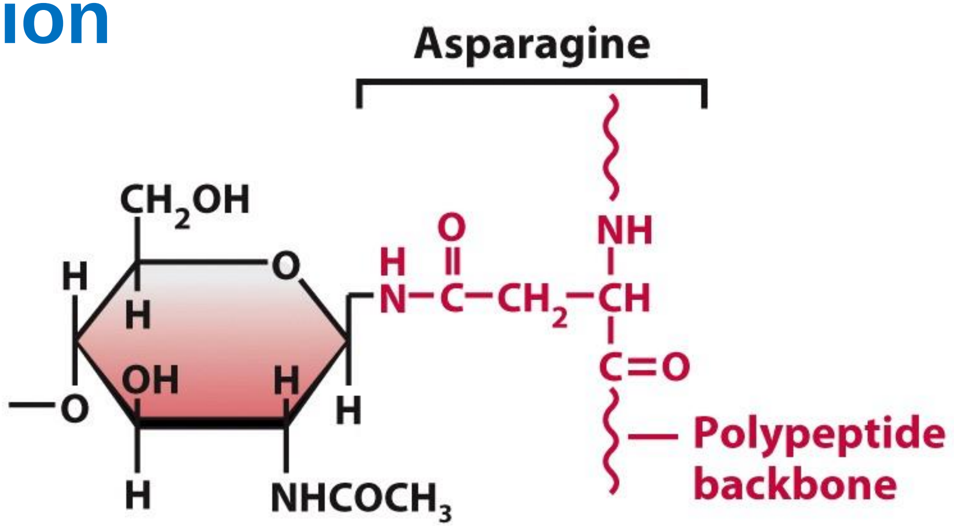


Fluid-mosaic model



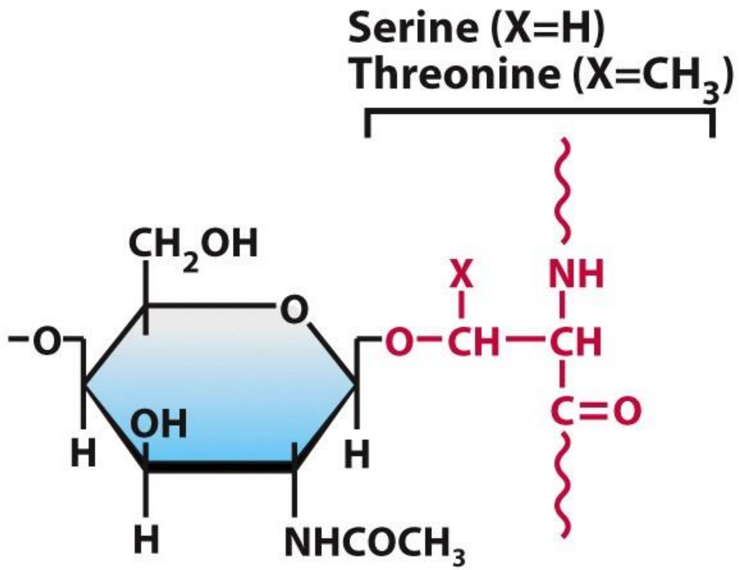
Protein glycosylation

N-linked glycosylation



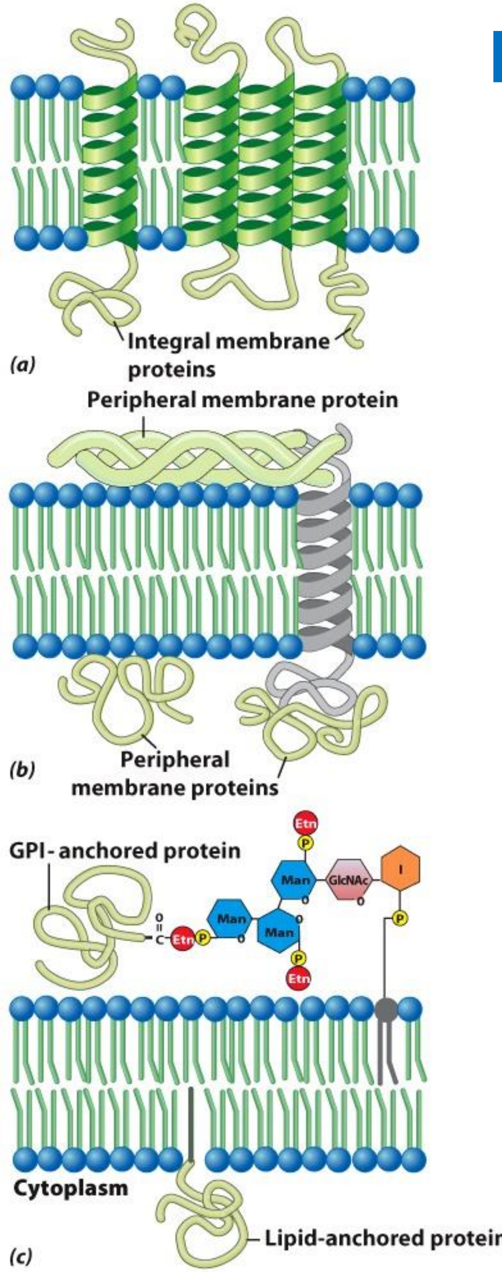
N-Acetylglucosamine

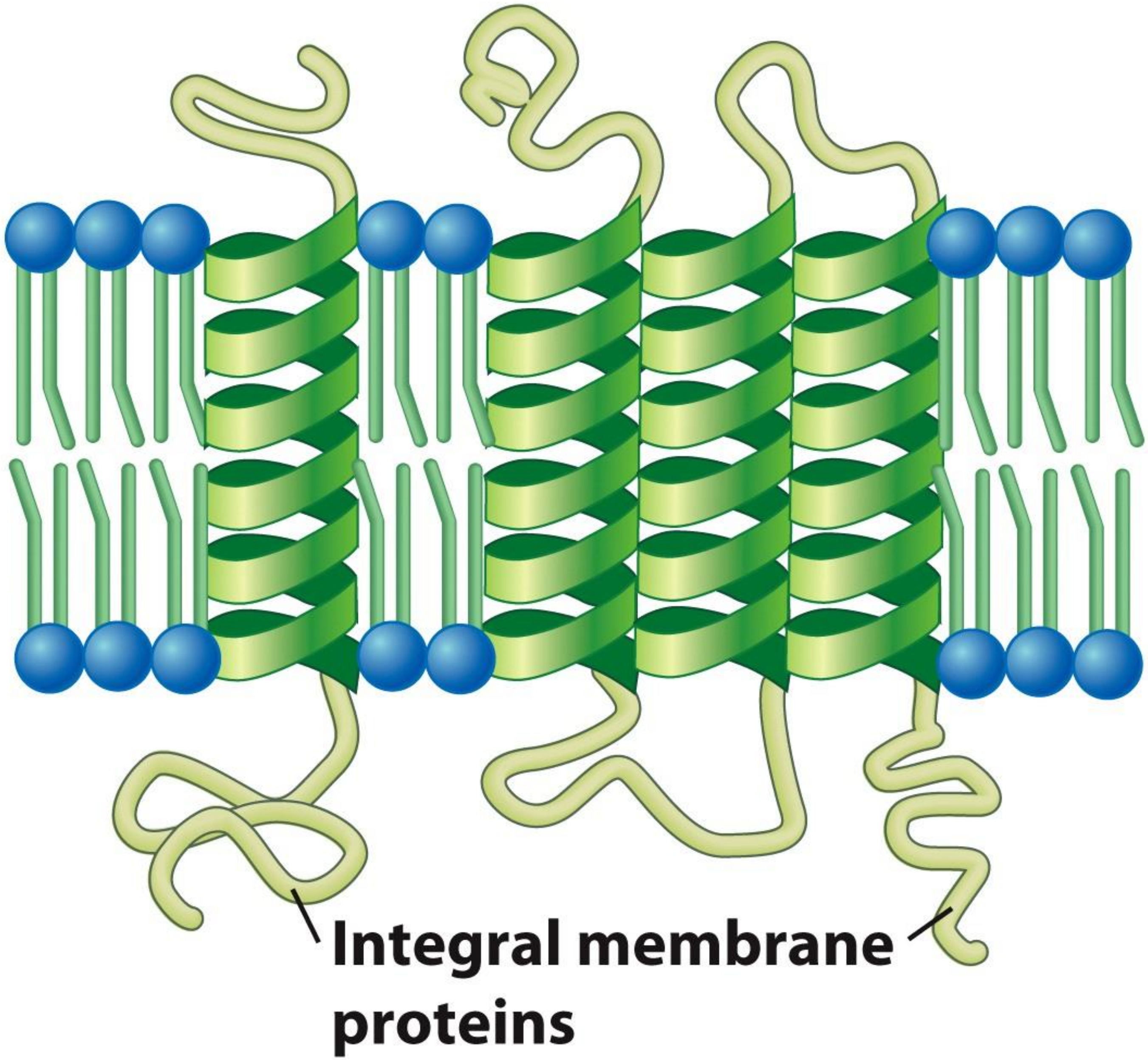
O-linked glycosylation



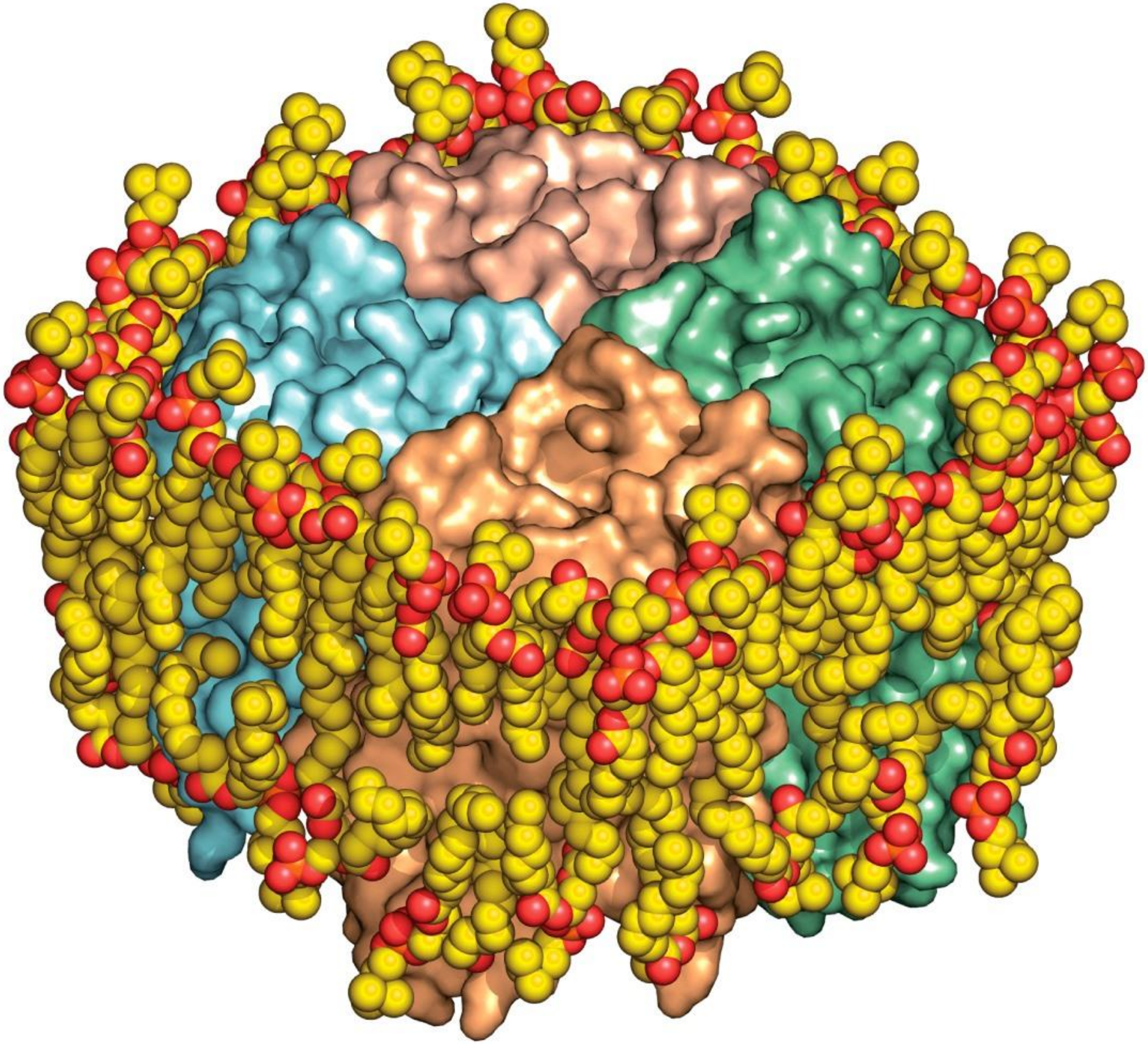
N-Acetylgalactosamine

Membrane proteins



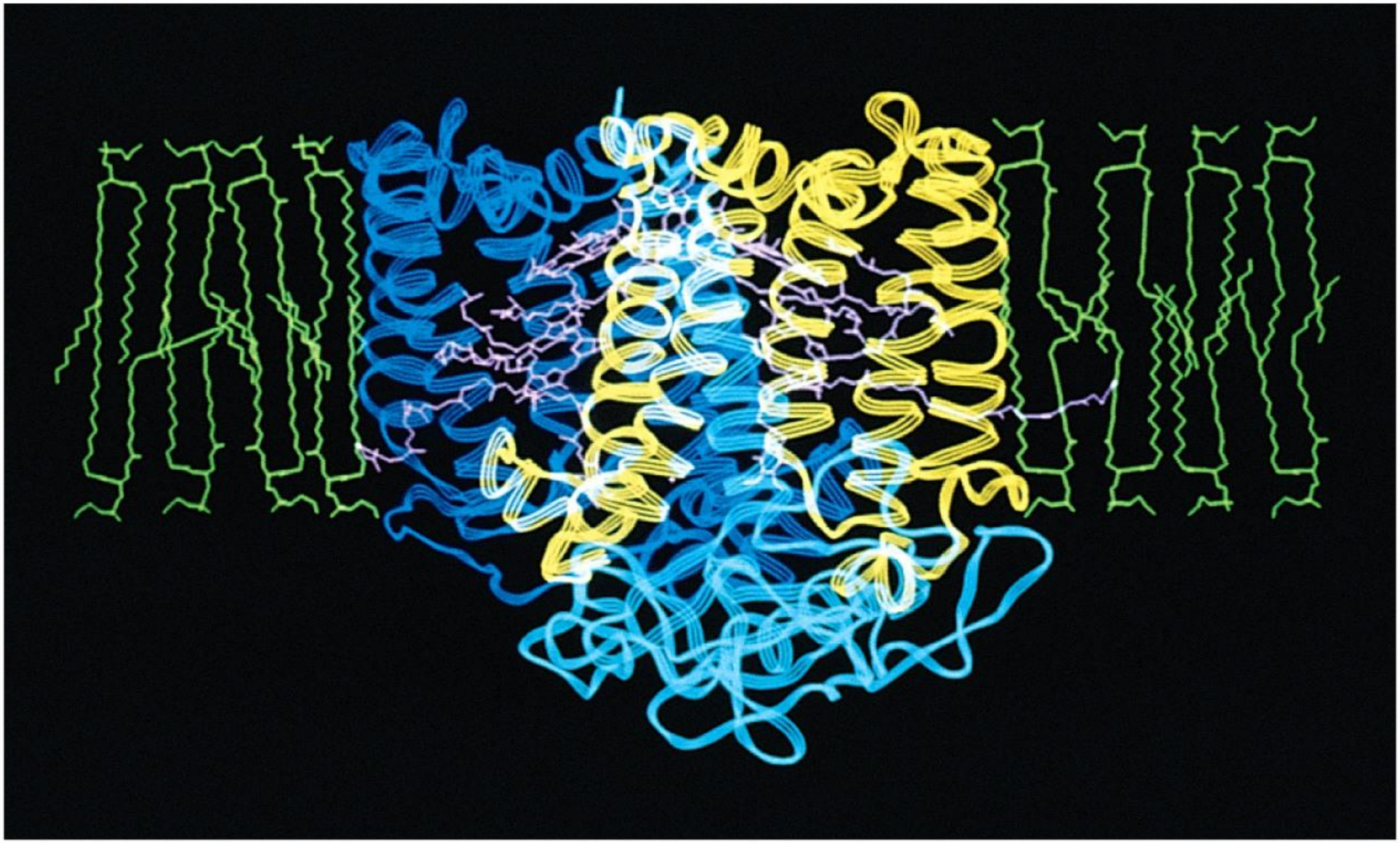

Integral membrane
proteins

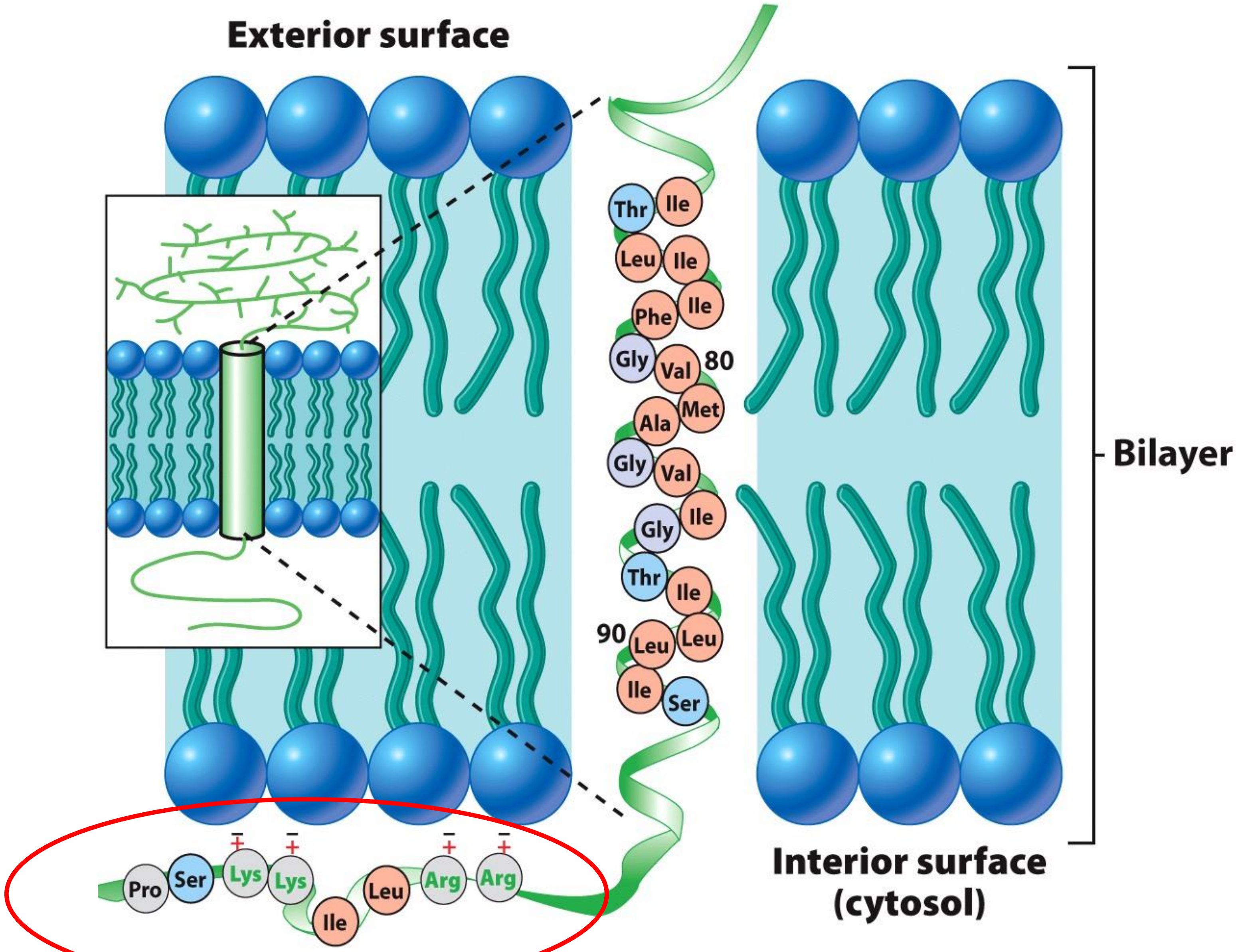
Protein-membrane interactions



From Carola Hunte and Sebastian Richers, Curr. Opin. Struct. Biol. 18: 407, 2008, © 2008, with permission of Elsevier Science

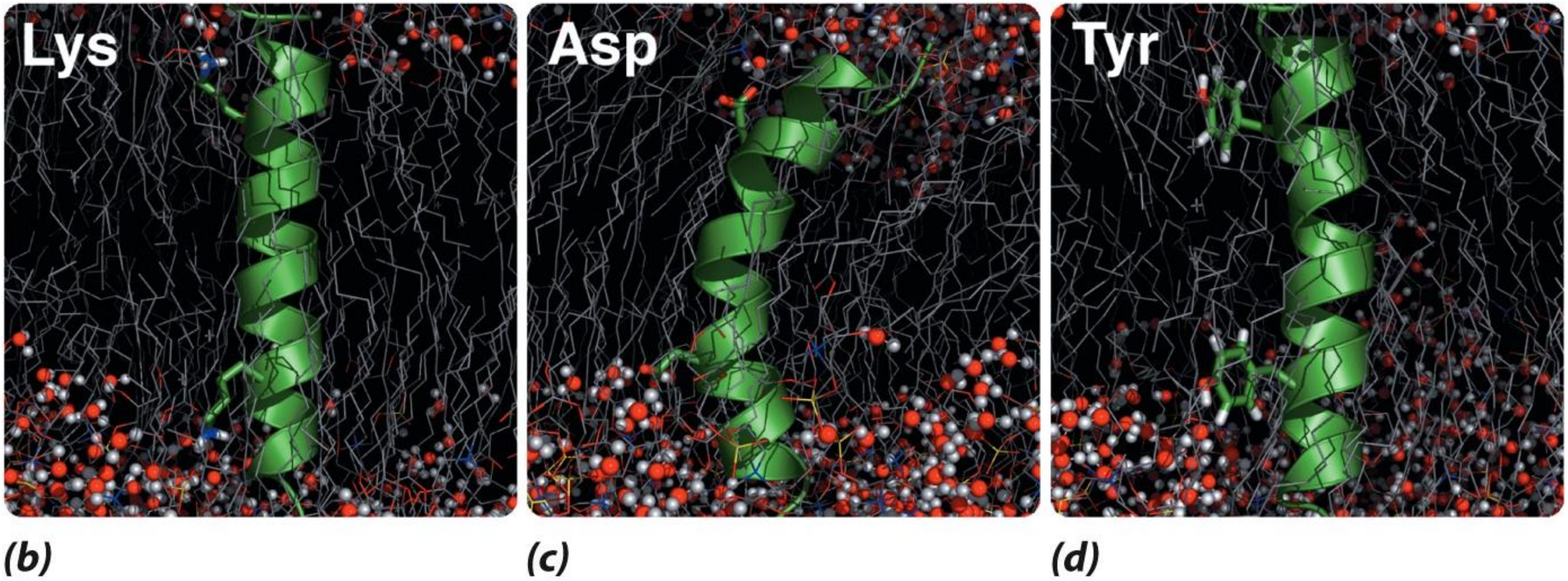
Structure of an integral membrane protein

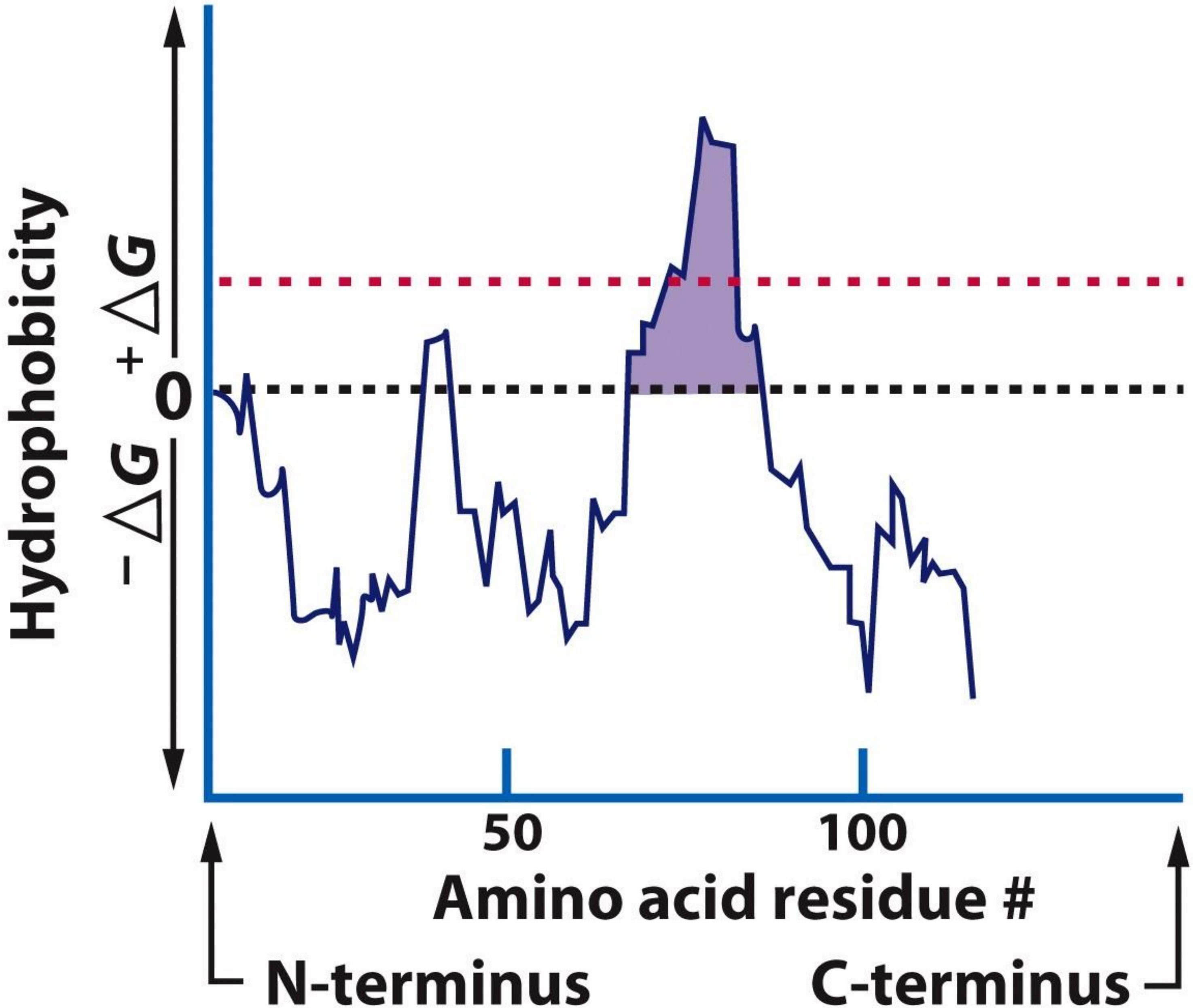




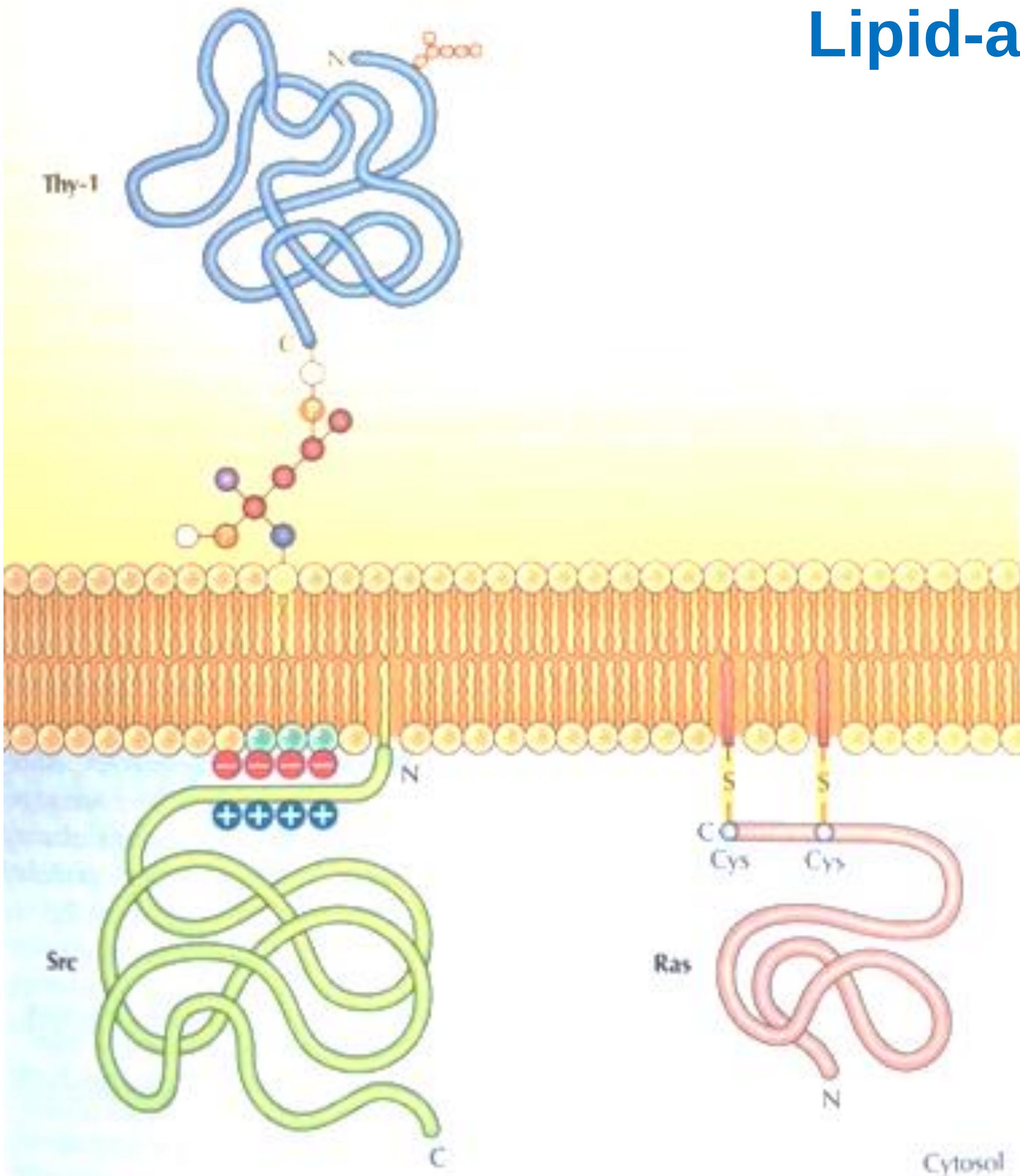
Exterior surface
Thr
Ile
Leu
Ile
Phe
Ile
Gly
Val
80
Ala
Met
Gly
Val
Gly
Ile
Thr
Ile
90
Leu
Leu
Ile
Ser
Bilayer
Pro
Ser
Lys
Lys
Ile
Leu
Arg
Arg
Interior surface
(cytosol)

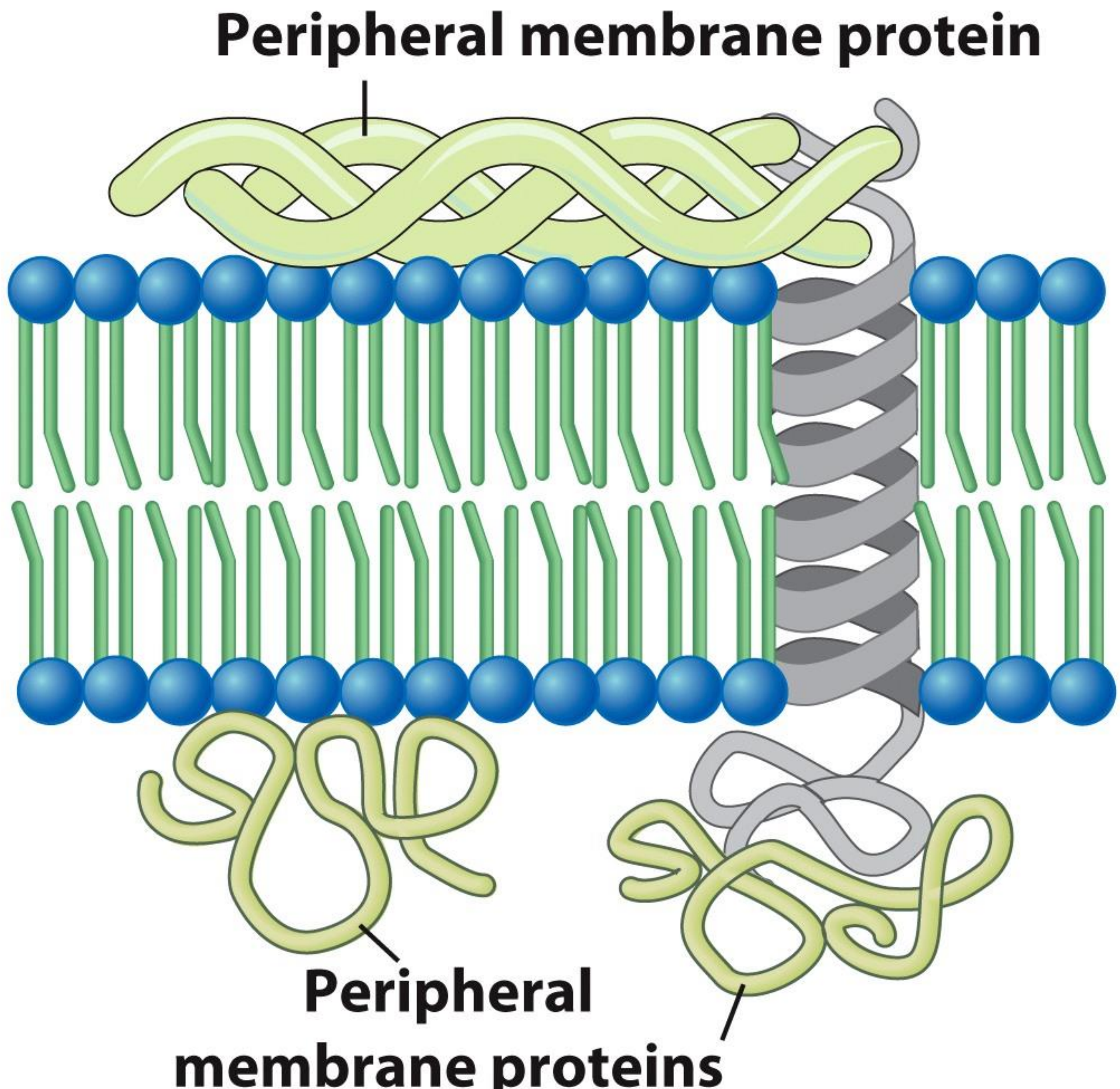
Orientation of residues in the membrane



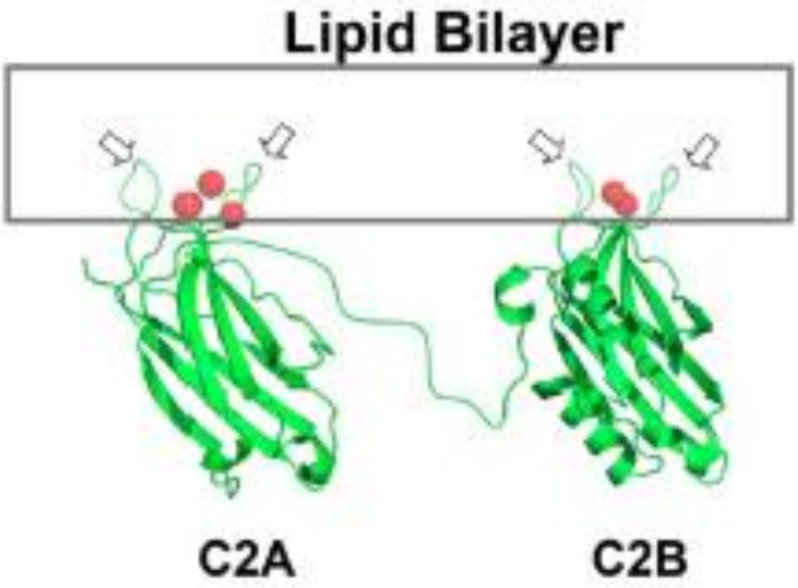

Hydrophobicity
$-\Delta G$
0
$+\Delta G$
50
100
Amino acid residue #
N-terminus
C-terminus

Lipid-anchored proteins

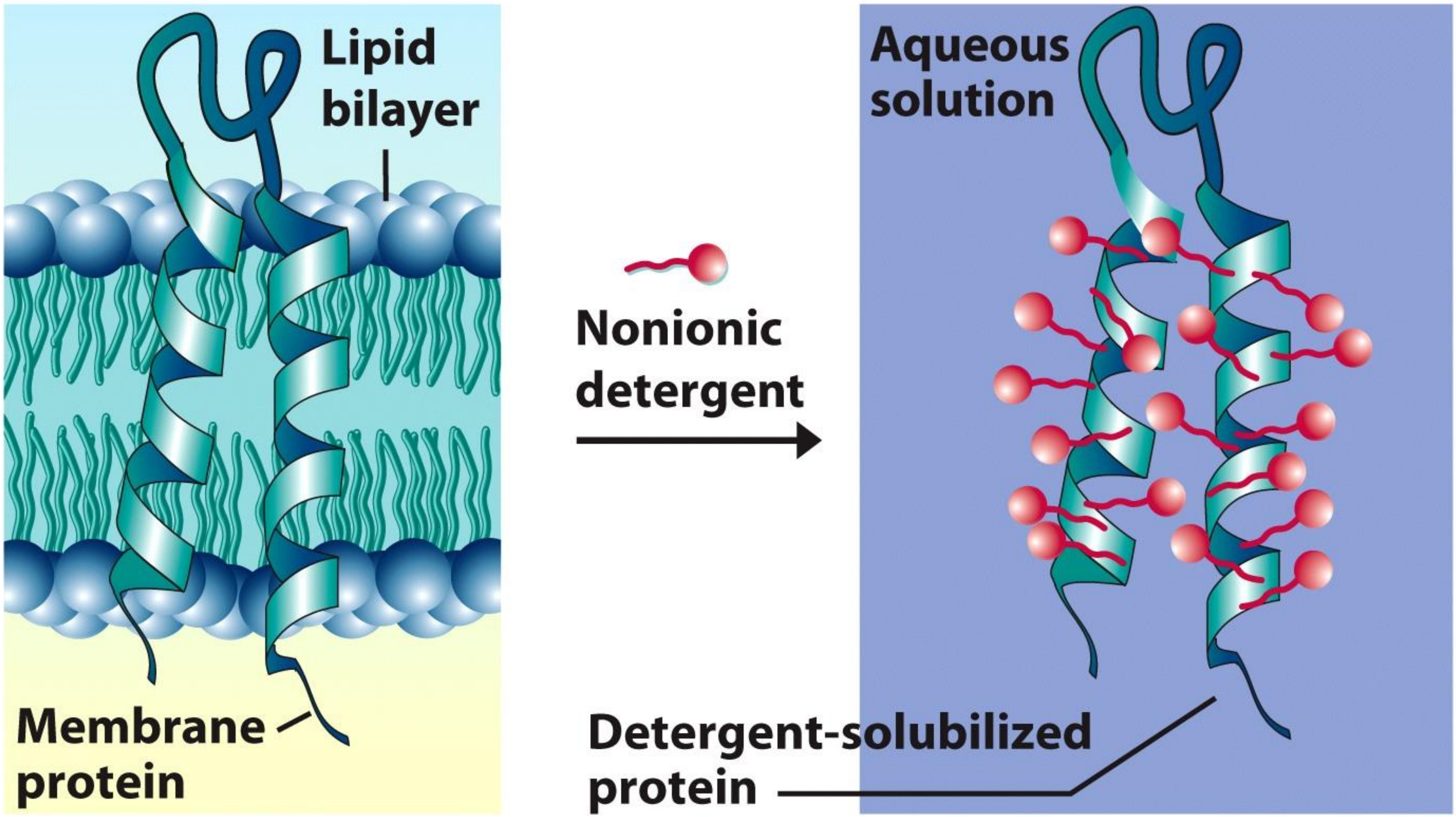



Peripheral membrane protein
Peripheral
membrane proteins

Doc2b: a calcium-regulated peripheral membrane protein



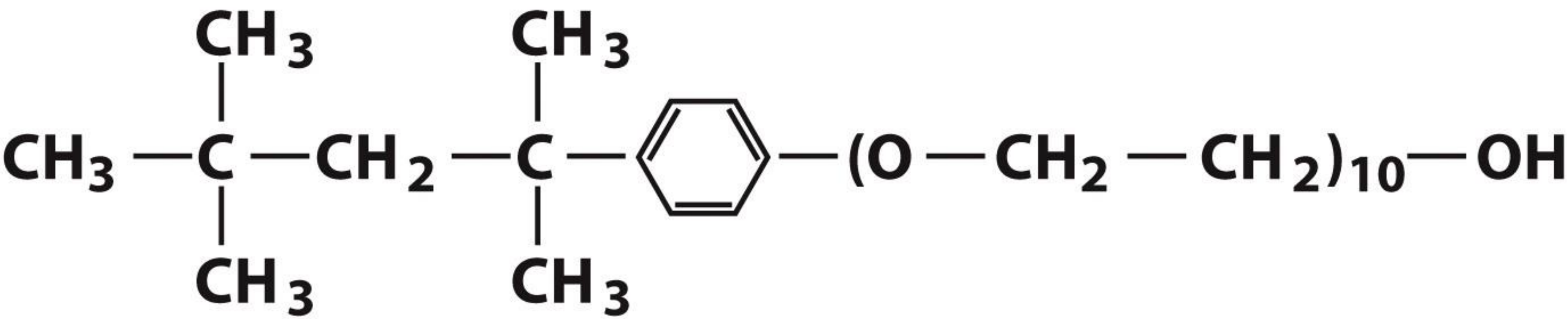
Solubilization of membrane proteins



Detergents

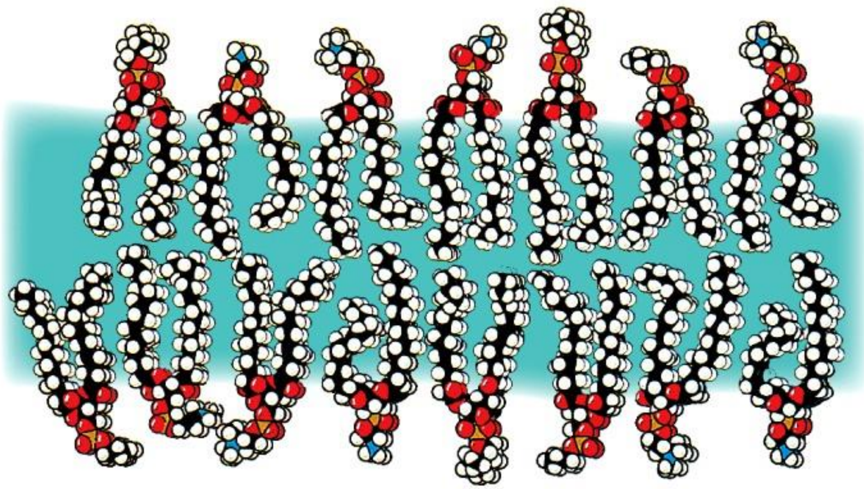
$$CH_3{-}(CH_2)_{11}{-}OSO_3^{-}\,Na^{+}$$

Sodium dodecyl sulfate (SDS)

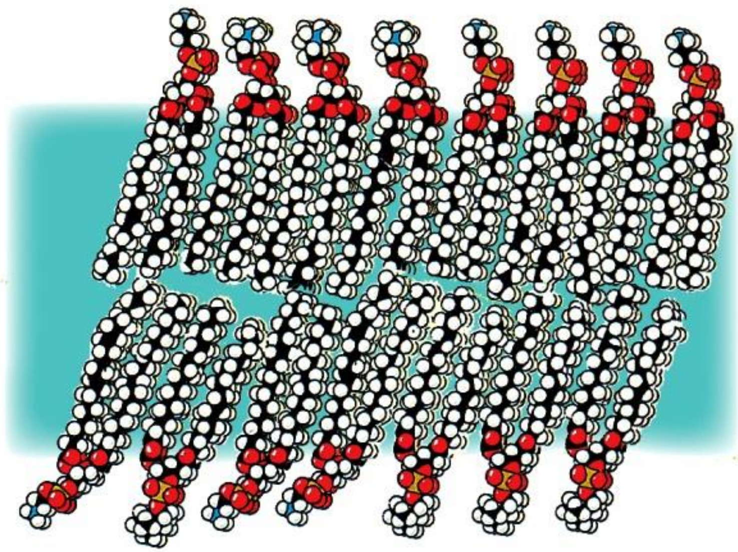


Triton X-100

Temperature and membrane structure



(a)



(b)

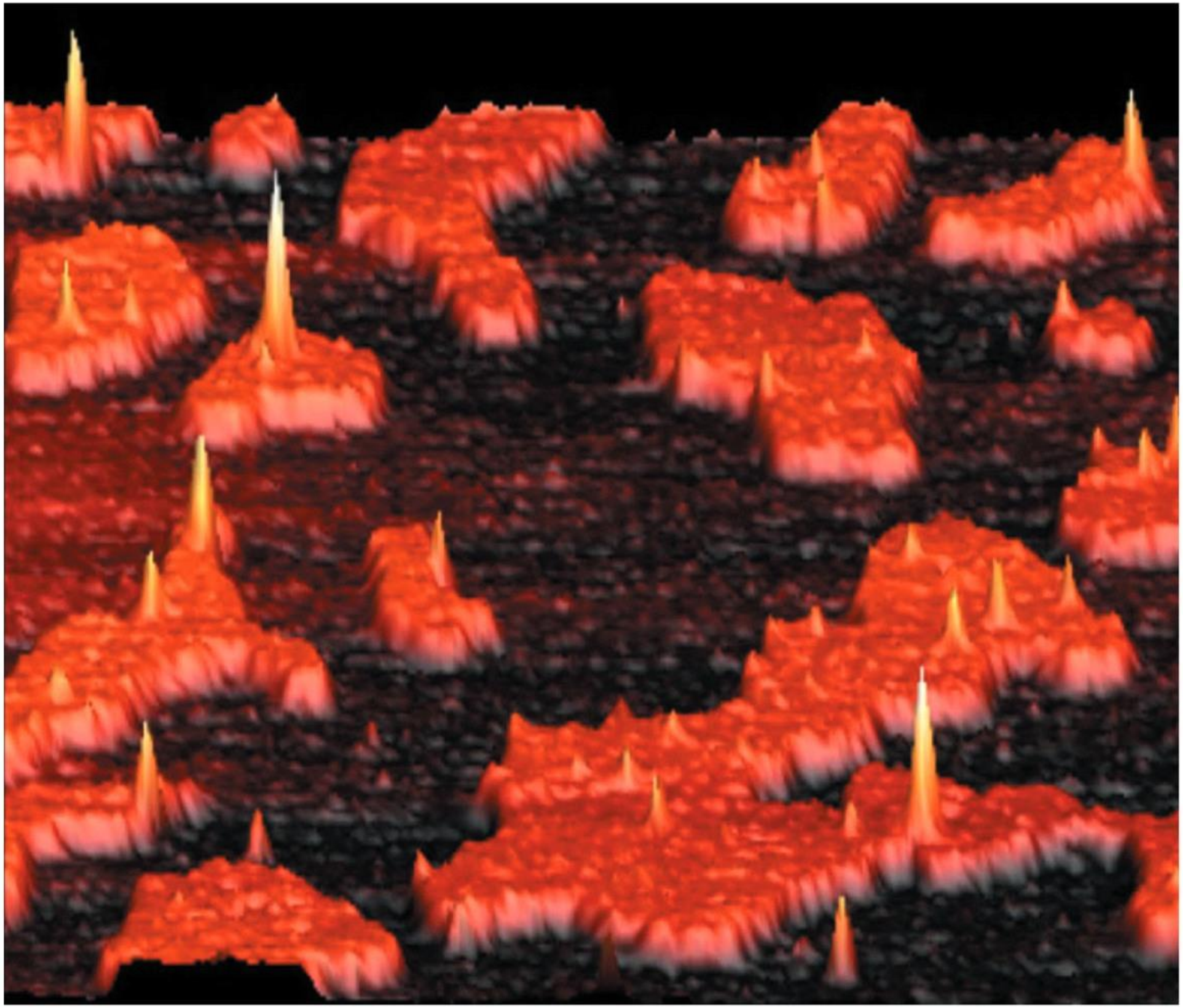
Fluid **Crystalline gel**

Table 4.2 *Melting Points of the Common 18-Carbon Fatty Acids*

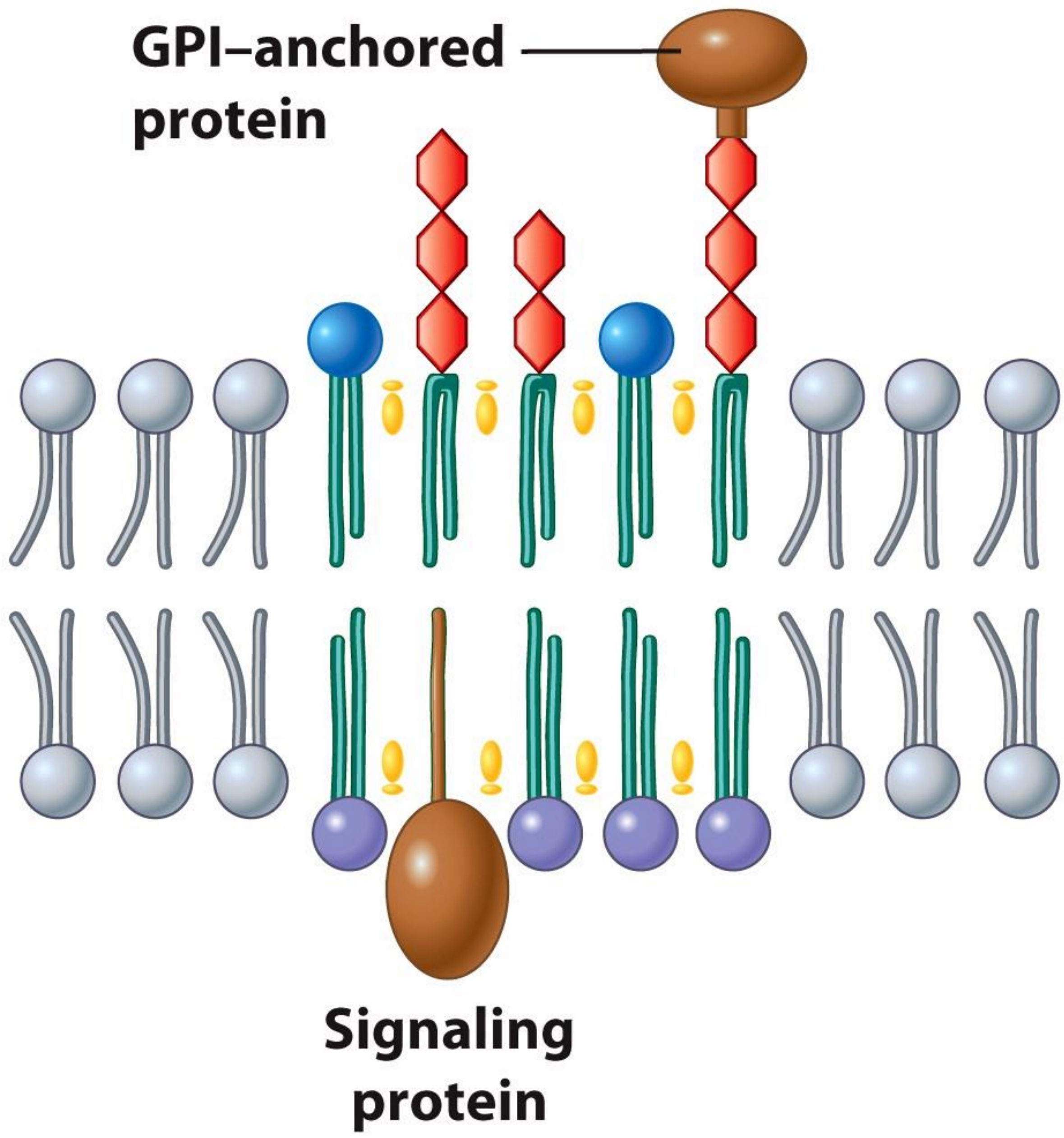
Fatty acid	*cis* Double bonds	M.p.(°C)
Stearic acid	0	70
Oleic acid	1	13
Linoleic acid	2	−9
Linolenic acid	3	−17
Eicosapentanoic acid (EPA)*	5	−54

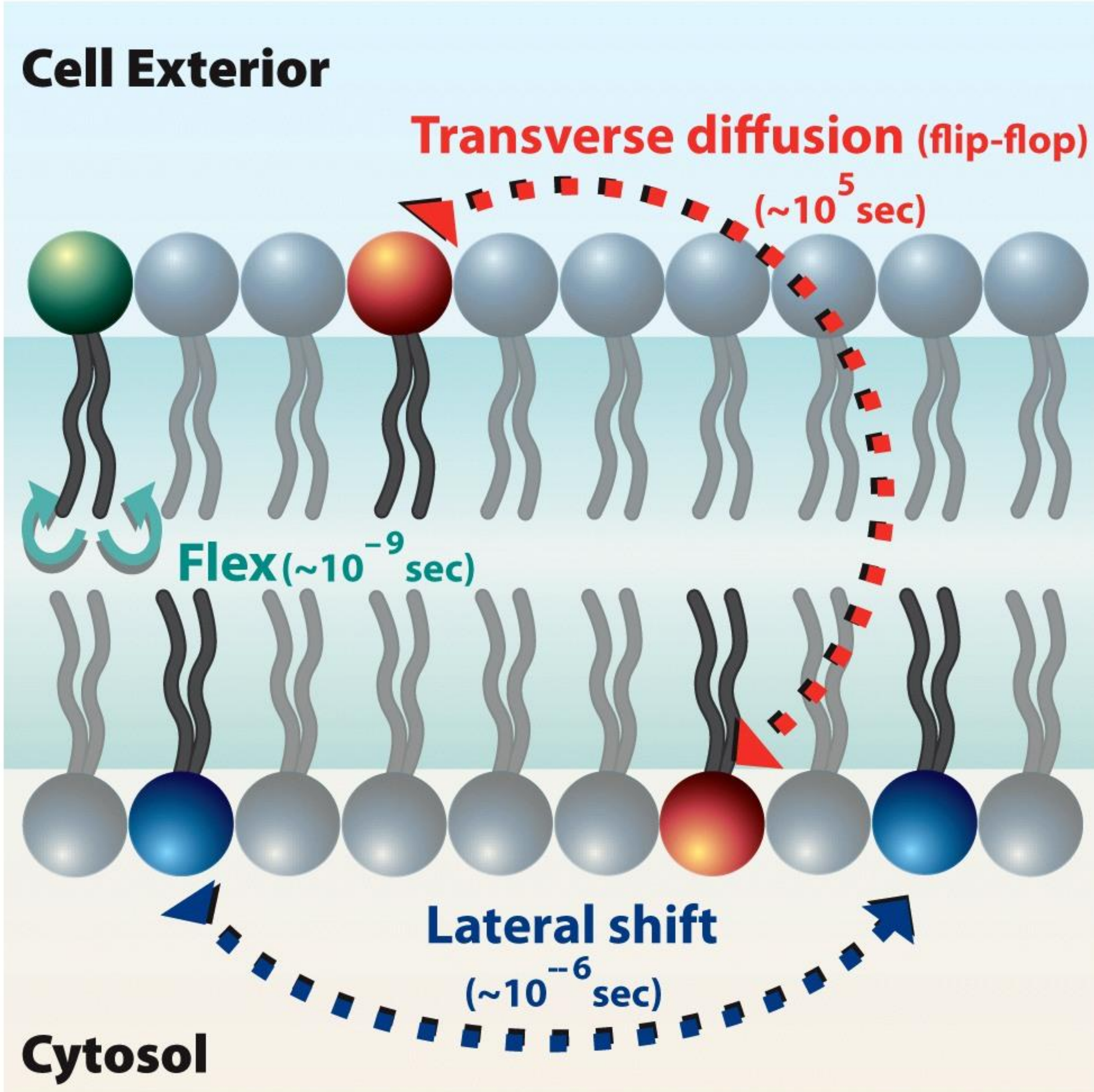
*EPA has 20 carbons.

Lipid rafts

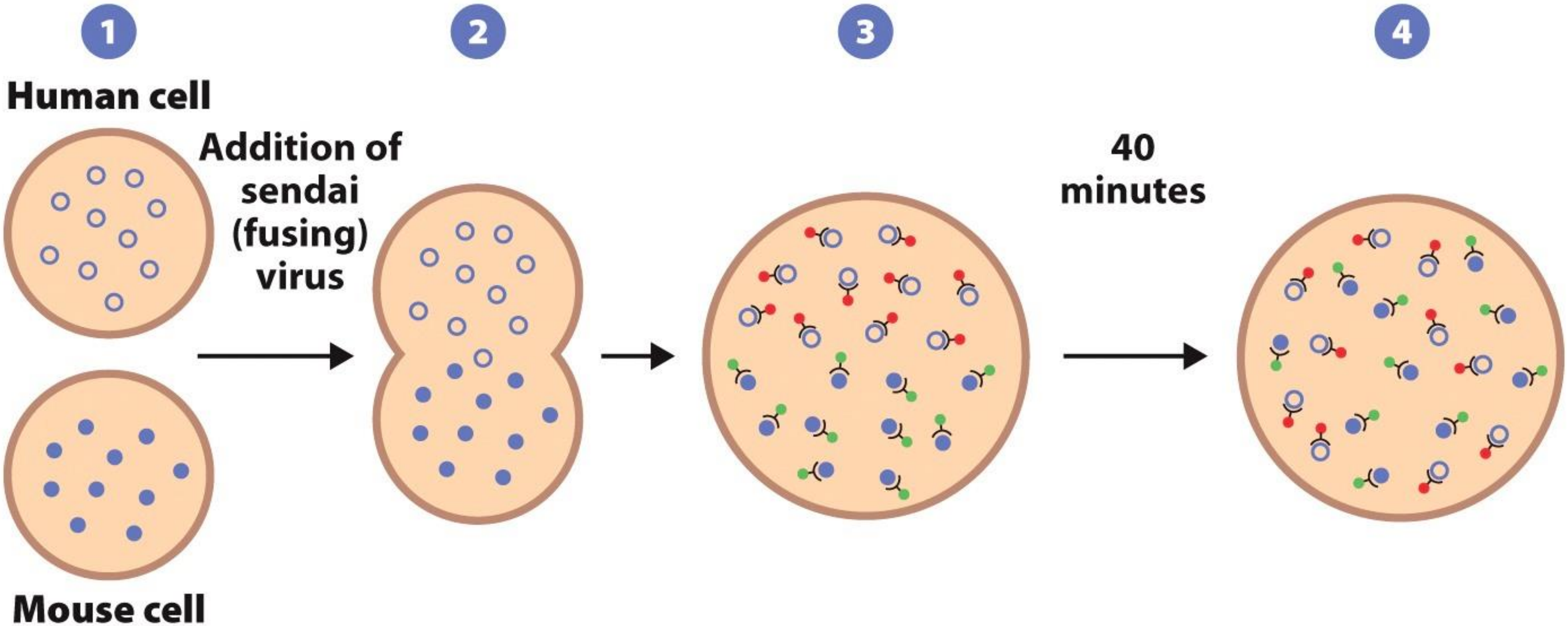


From D.E. Saslowsky, et al, J. Biol. Chem. 277, 26966.26970, July 26, © 2002

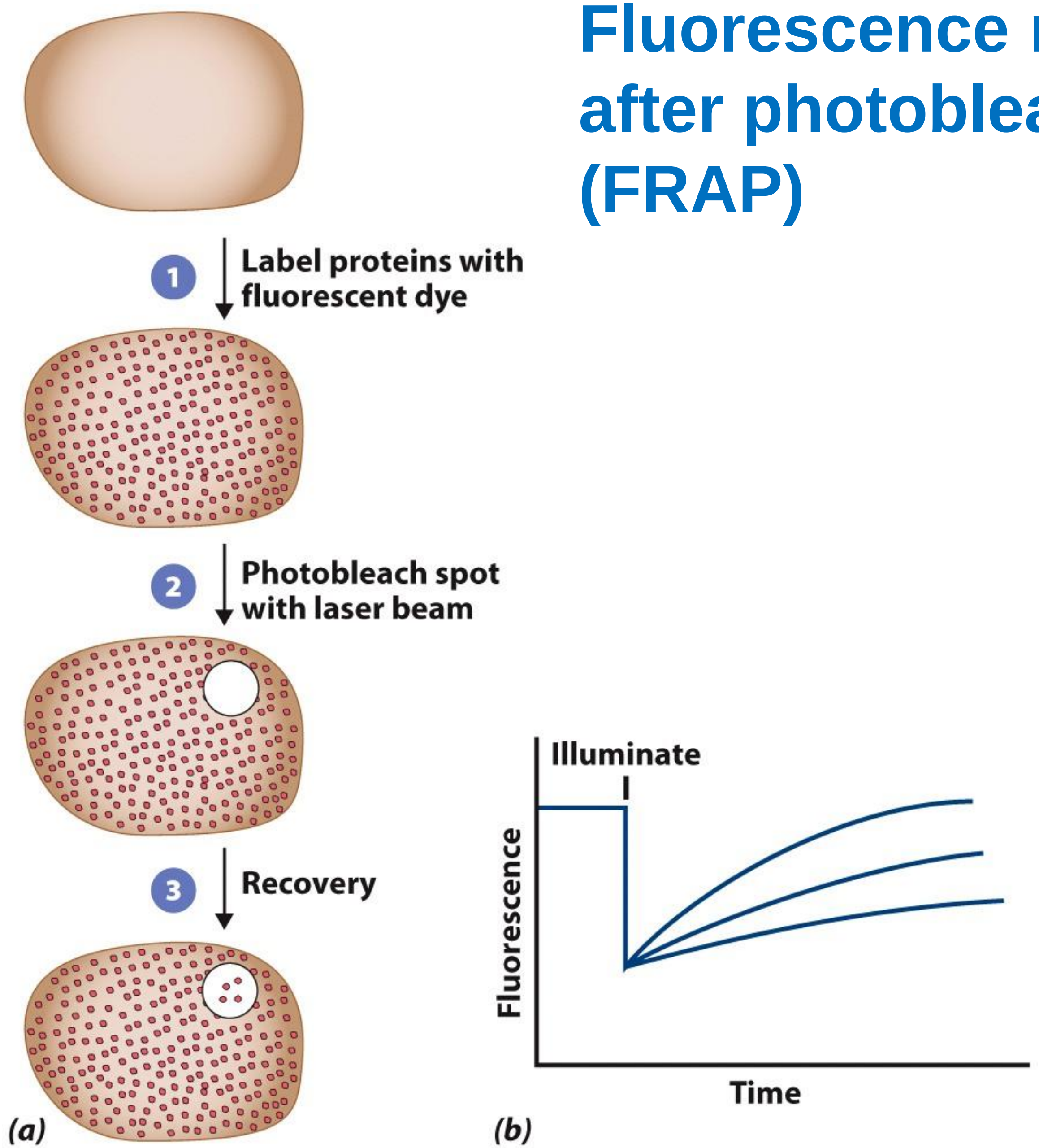

GPI–anchored
protein
Signaling
protein


Cell Exterior
Transverse diffusion (flip-flop)
(~10^5 sec)
Flex (~10^−9 sec)
Lateral shift
(~10^--6 sec)
Cytosol

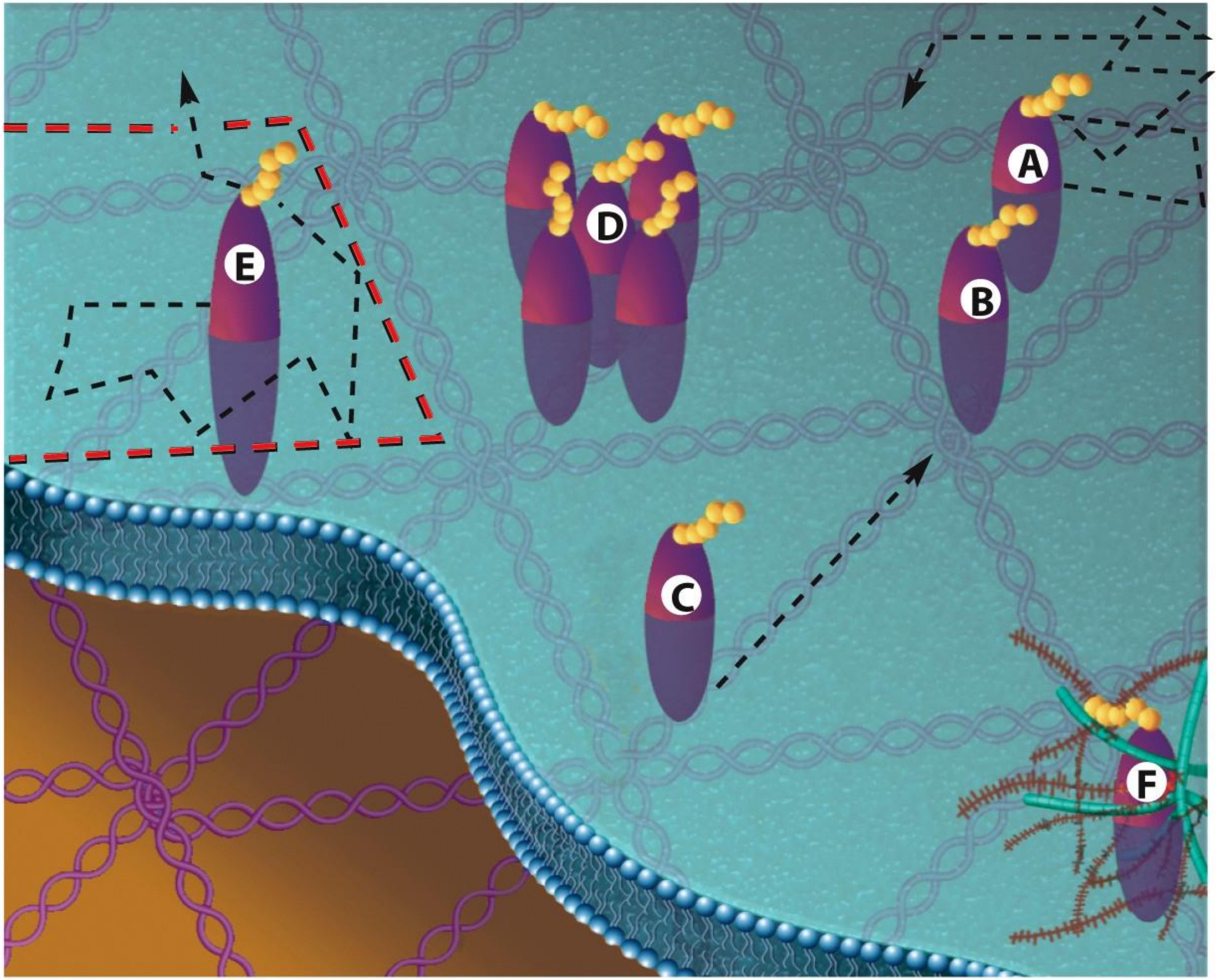
Membrane protein dynamics



Fluorescence recovery after photobleaching (FRAP)



Membrane protein mobility

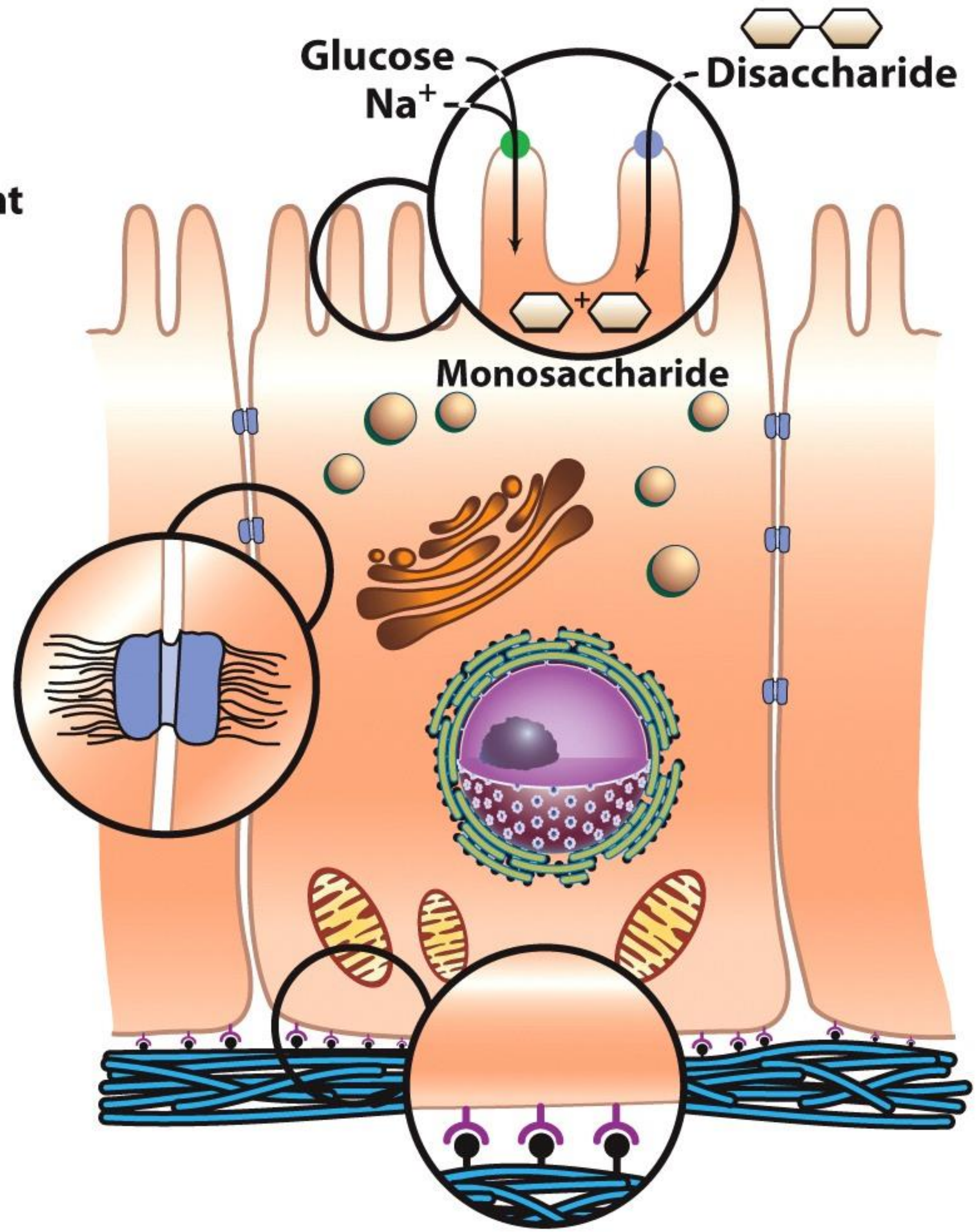


Polarized cell

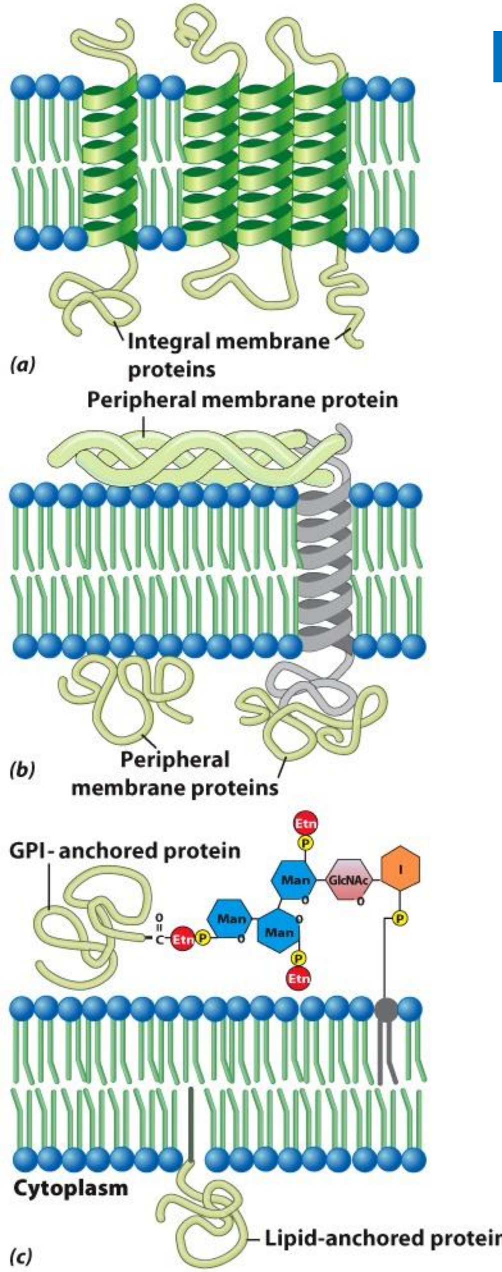
Apical plasma membrane
- regulation of nutrient and water intake
- regulated secretion
- protection

Lateral plasma membrane
- cell contact and adhesion
- cell communication

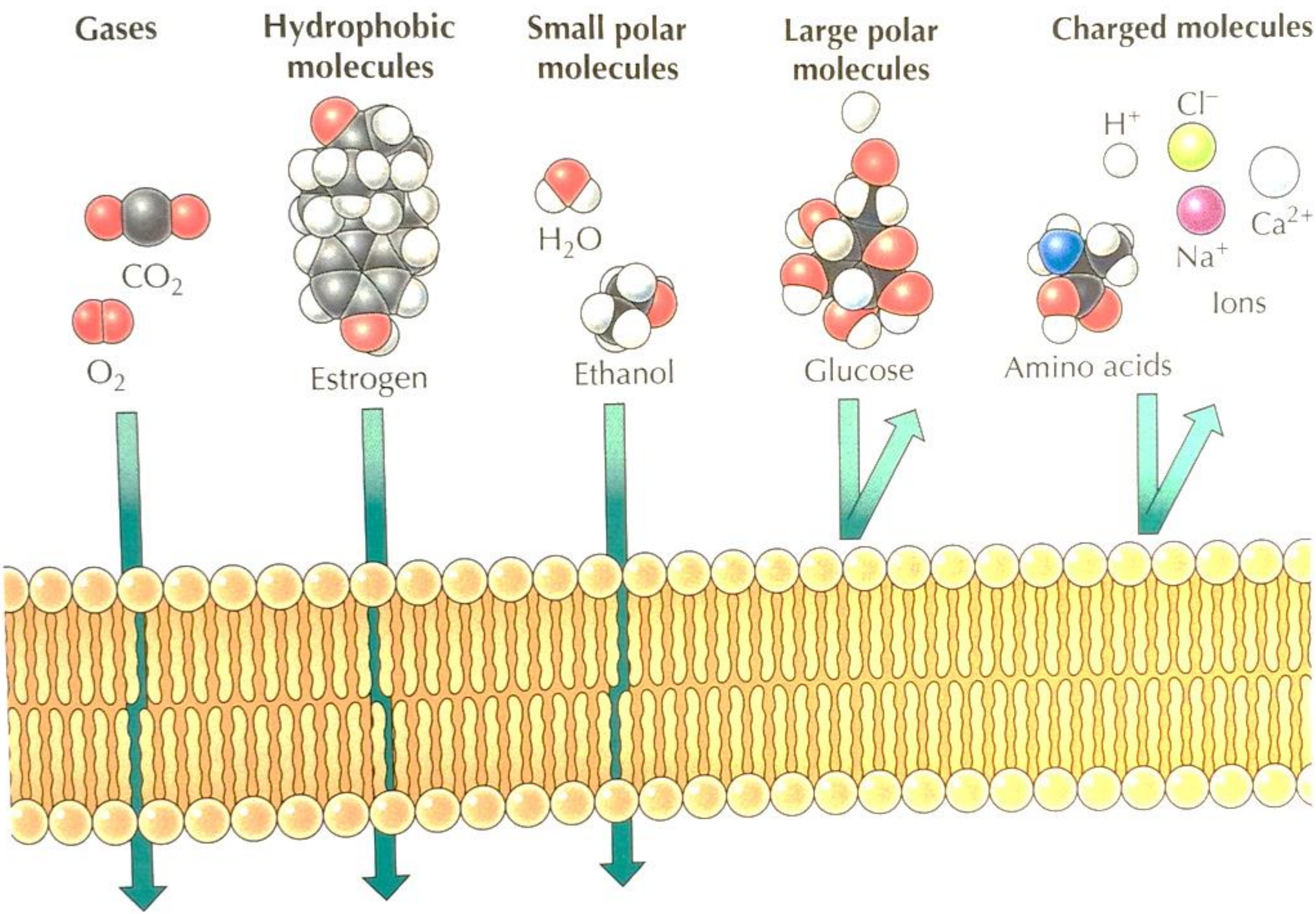
Basal membrane
- cell-substratum contact
- generation of ion gradients

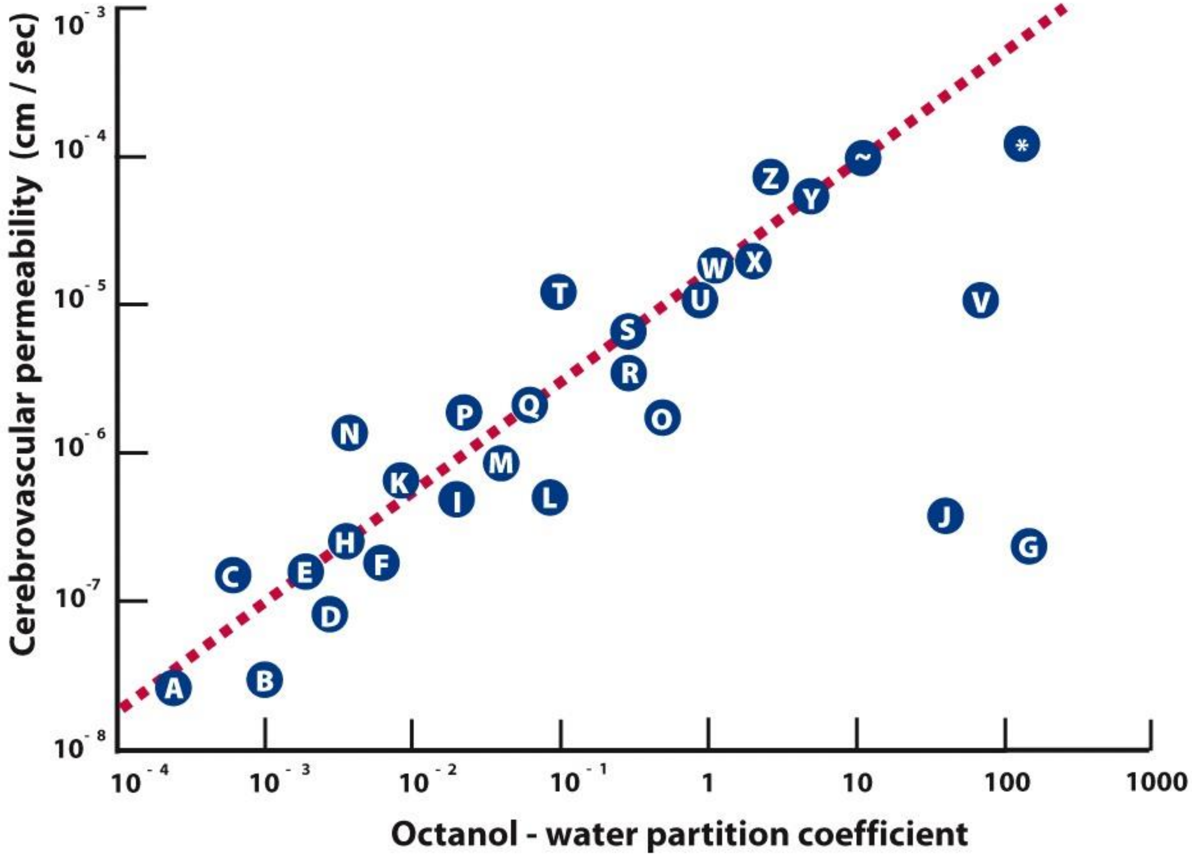


Membrane proteins



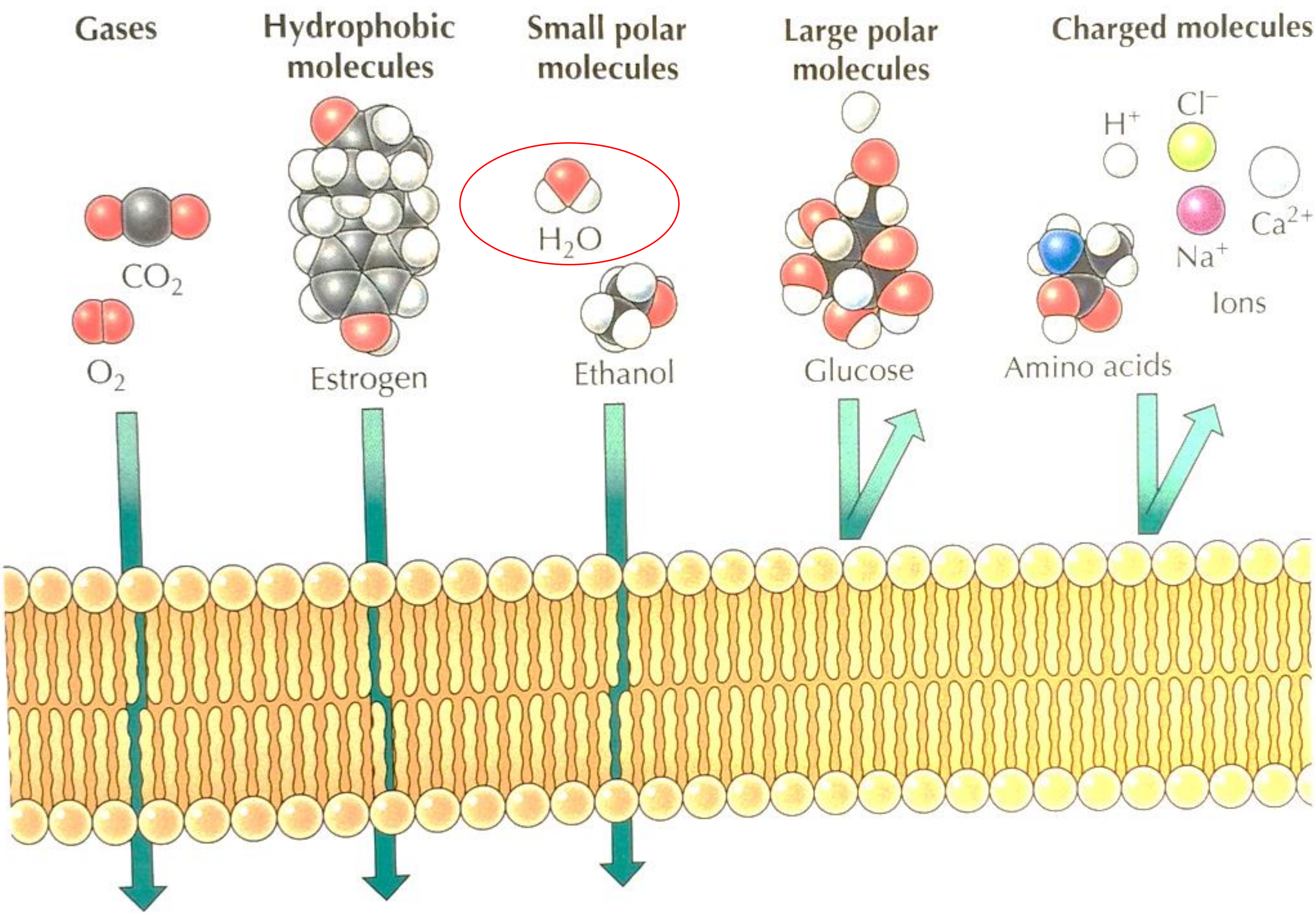
Molecular transport across membranes

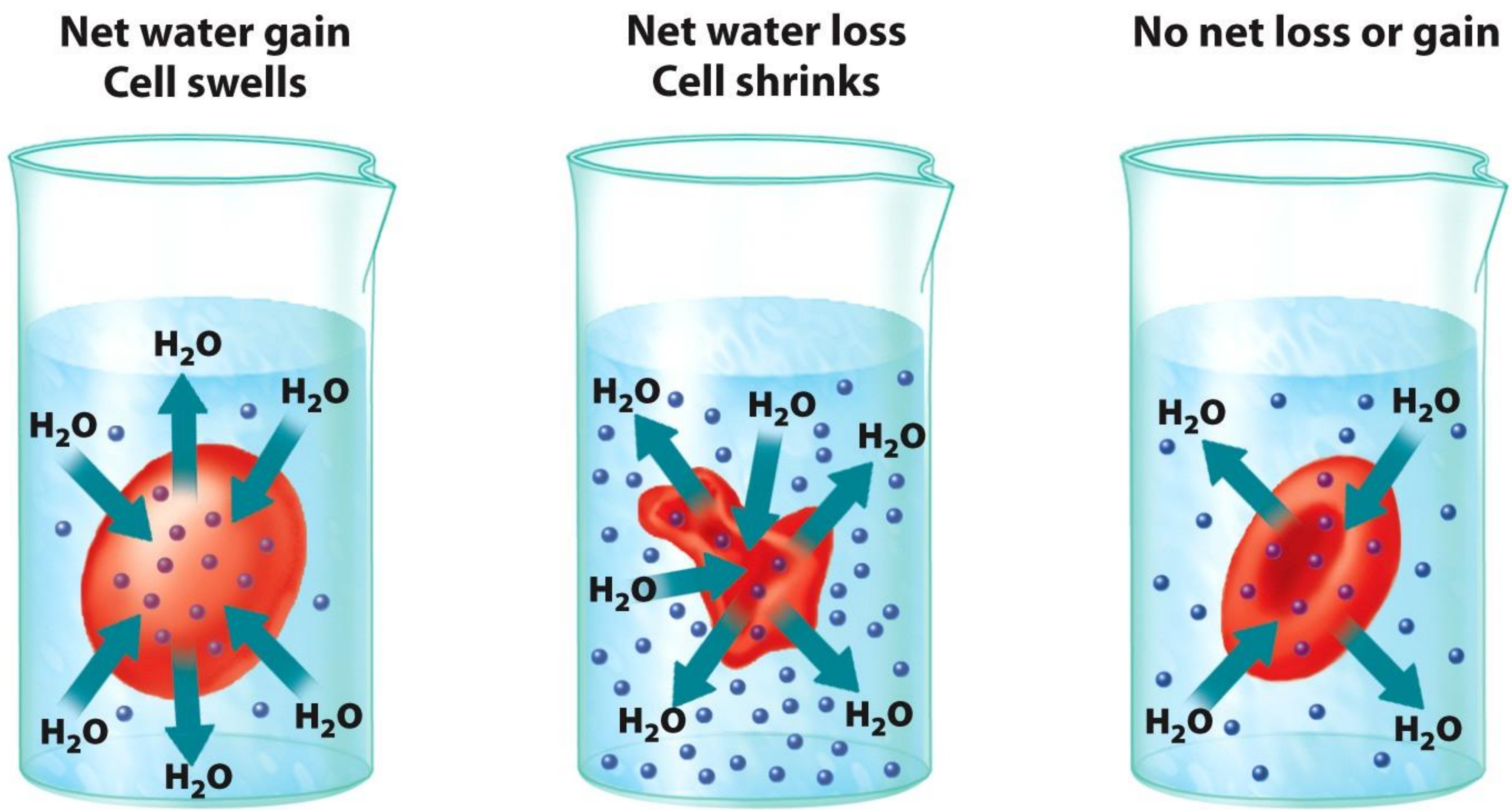




A. Sucrose
B. Epipodophyllotoxin
C. Mannitol
D. Arabinose
E. *N*-methyl nicotinamide
F. Methotrexate
G. Vincristine
H. Urea
I. Formamide
J. Vinblastine
K. Curare
L. Thiourea
M. Dianhydrogalacticol
N. Glycerol
O. 5-FU
P. Ethylene glycol
Q. Acetamide
R. Ftorafur
S. Misonidazole
T. Propylene glycol
U. Metronidazole
V. Spirohydantoin mustard
W. Procarbazine
X. PCNU
Y. Antipyrine
Z. Caffeine
~. BCNU
***.** CCNU

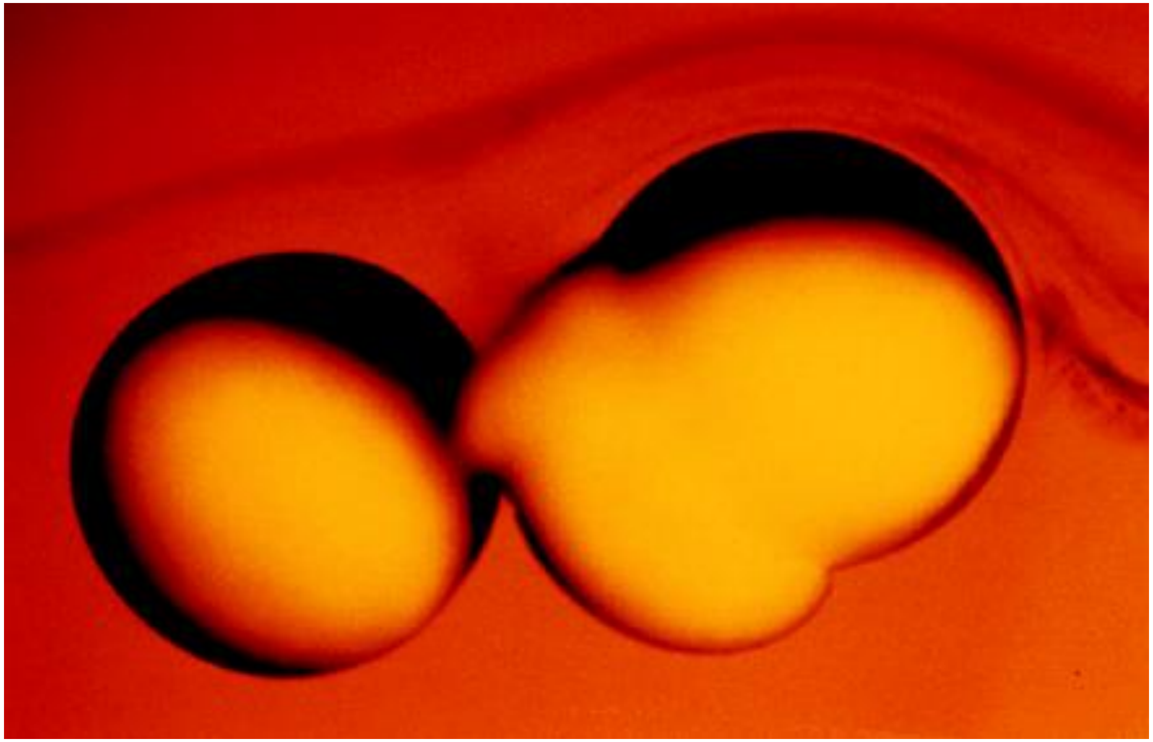
Molecular transport across membranes



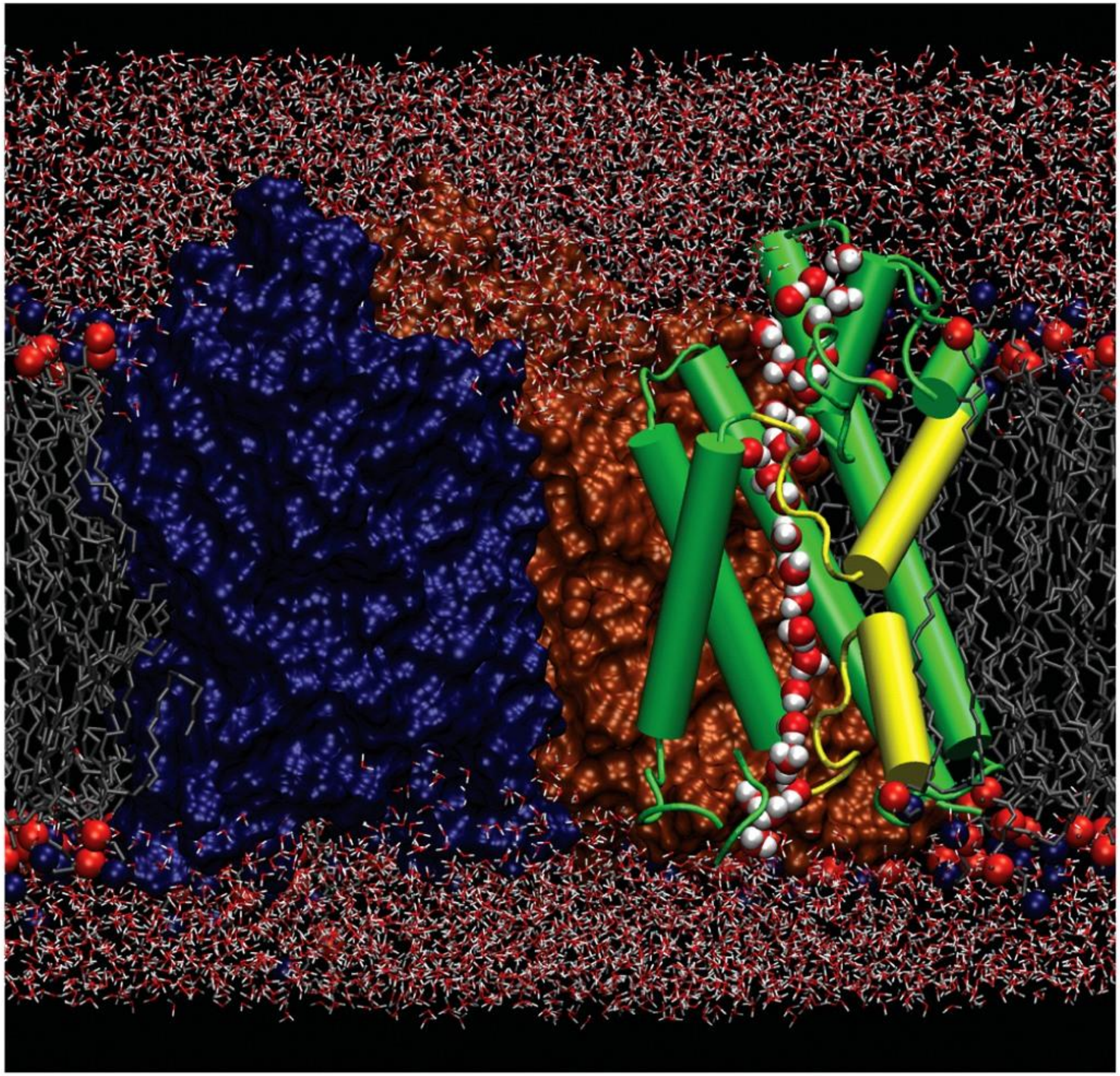


(a) **Hypotonic solution** *(b)* **Hypertonic solution** *(c)* **Isotonic solution**

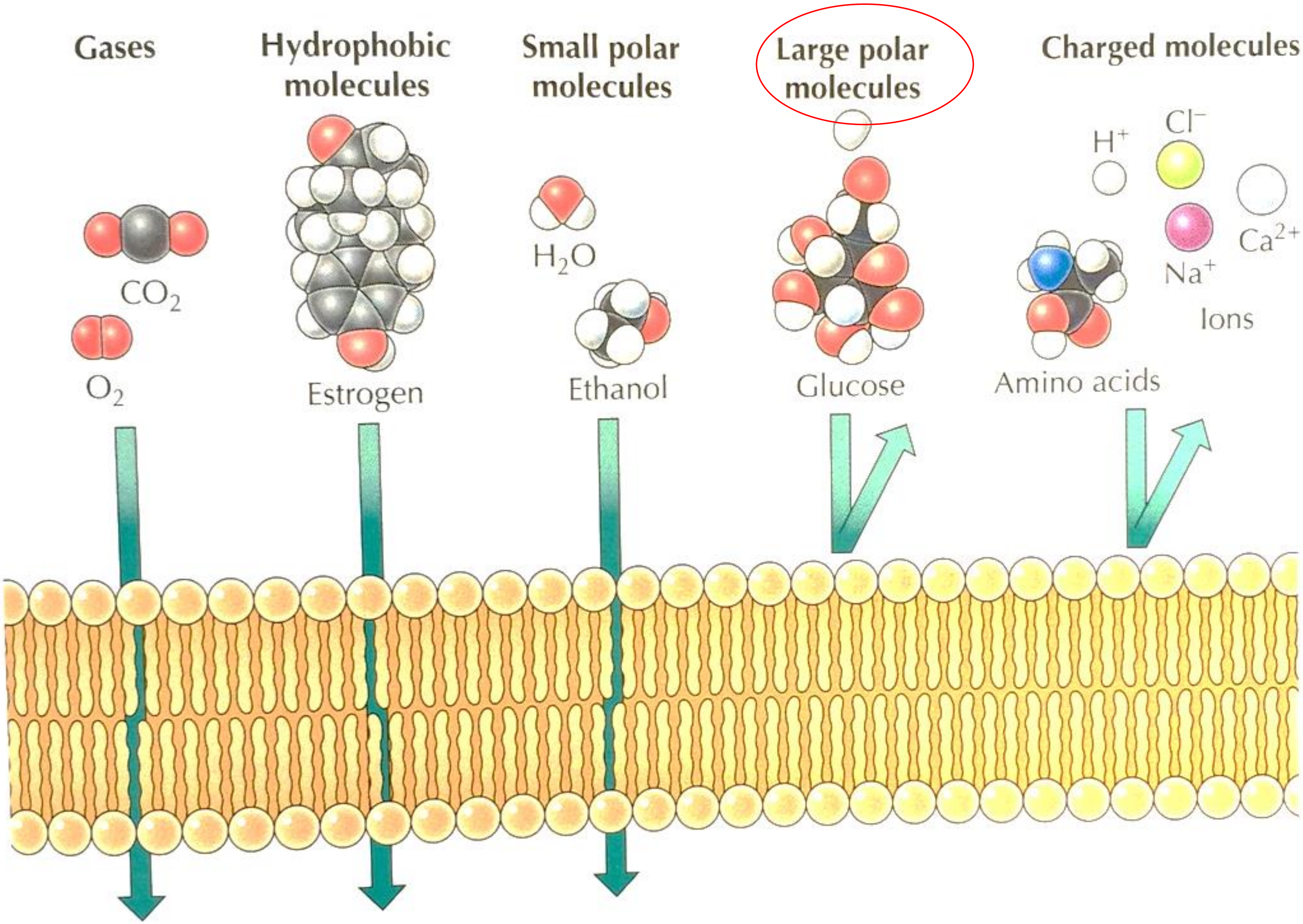
Aquaporin – water channel



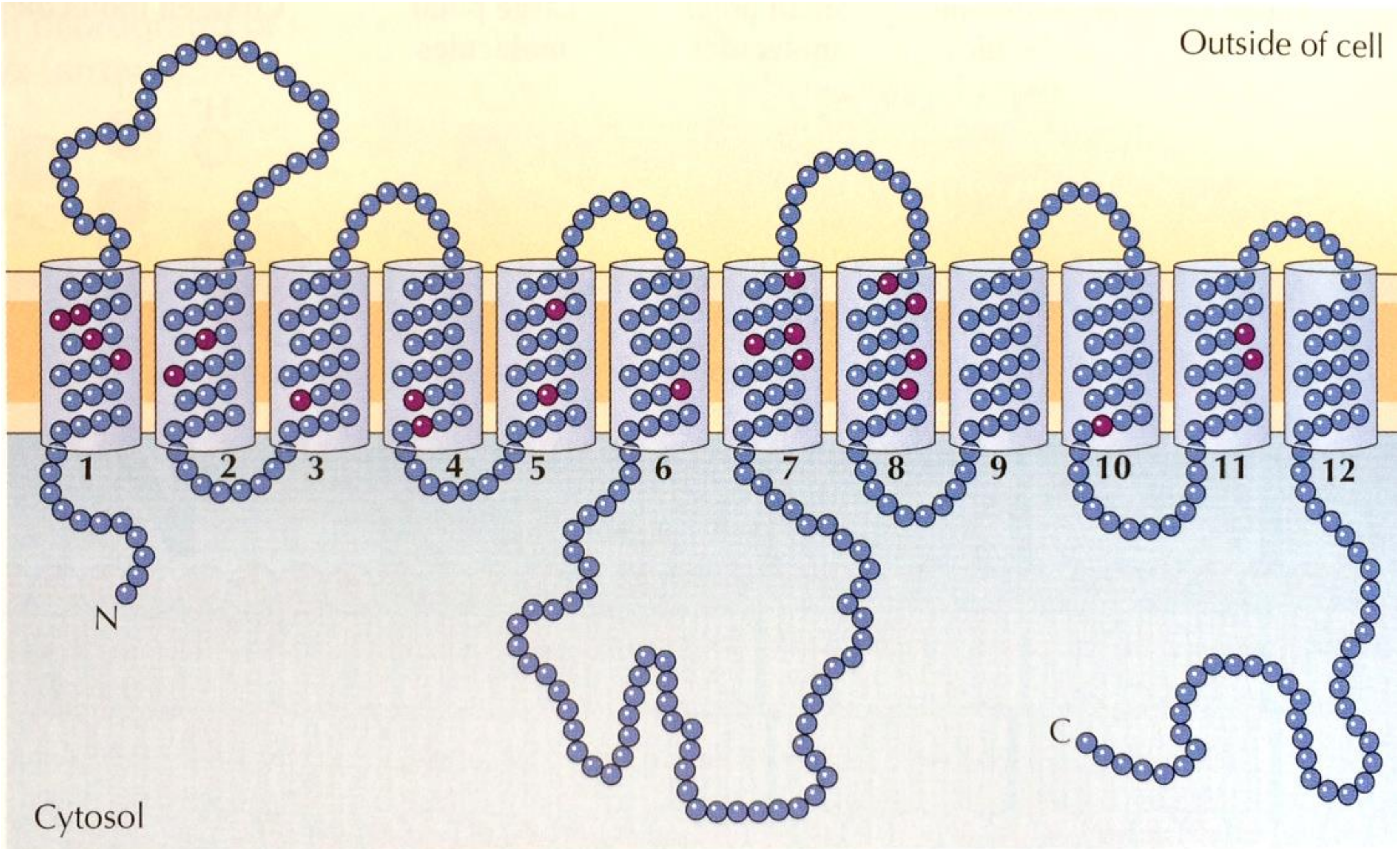
Aquaporin – water channel



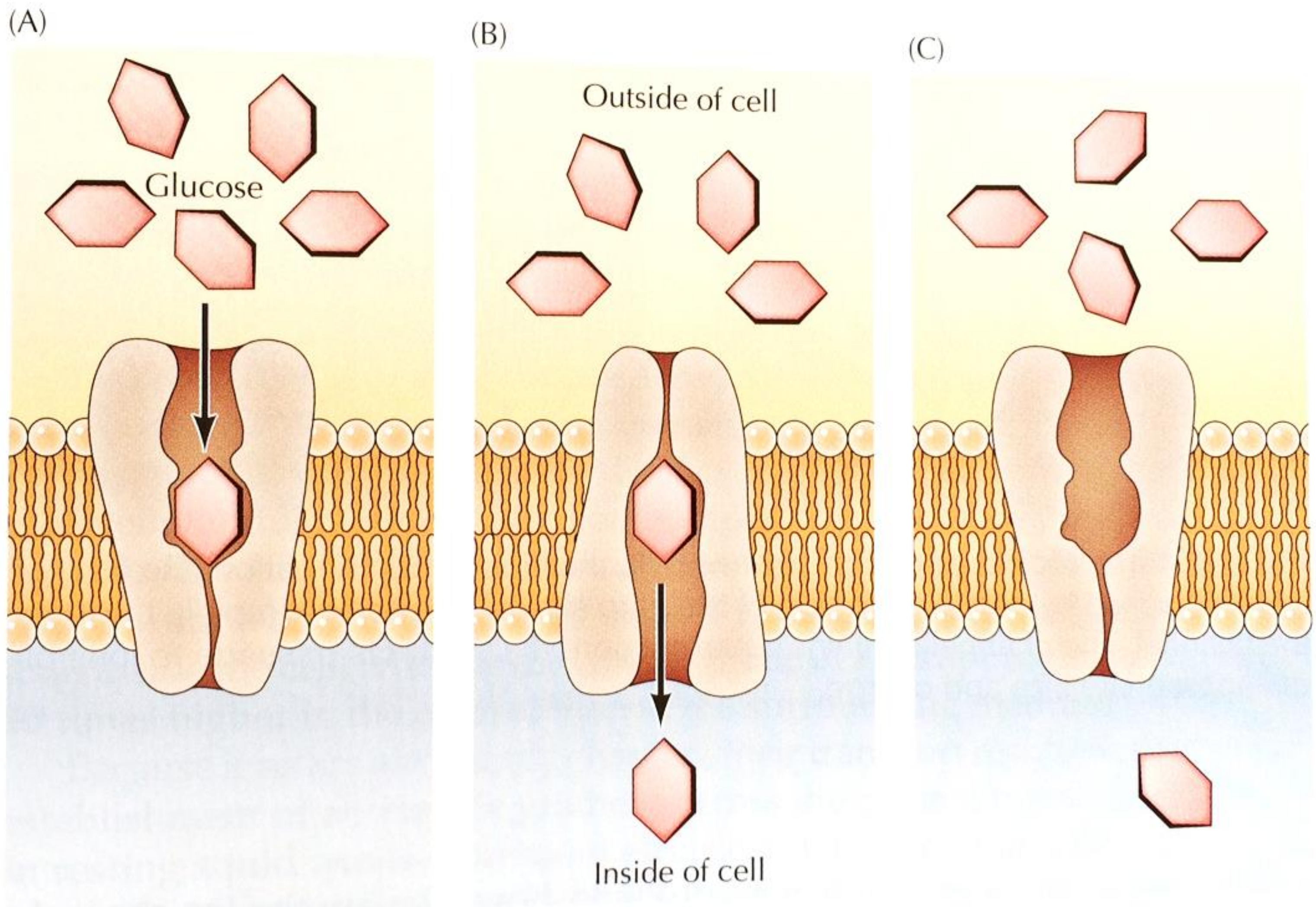
Molecular transport across membranes



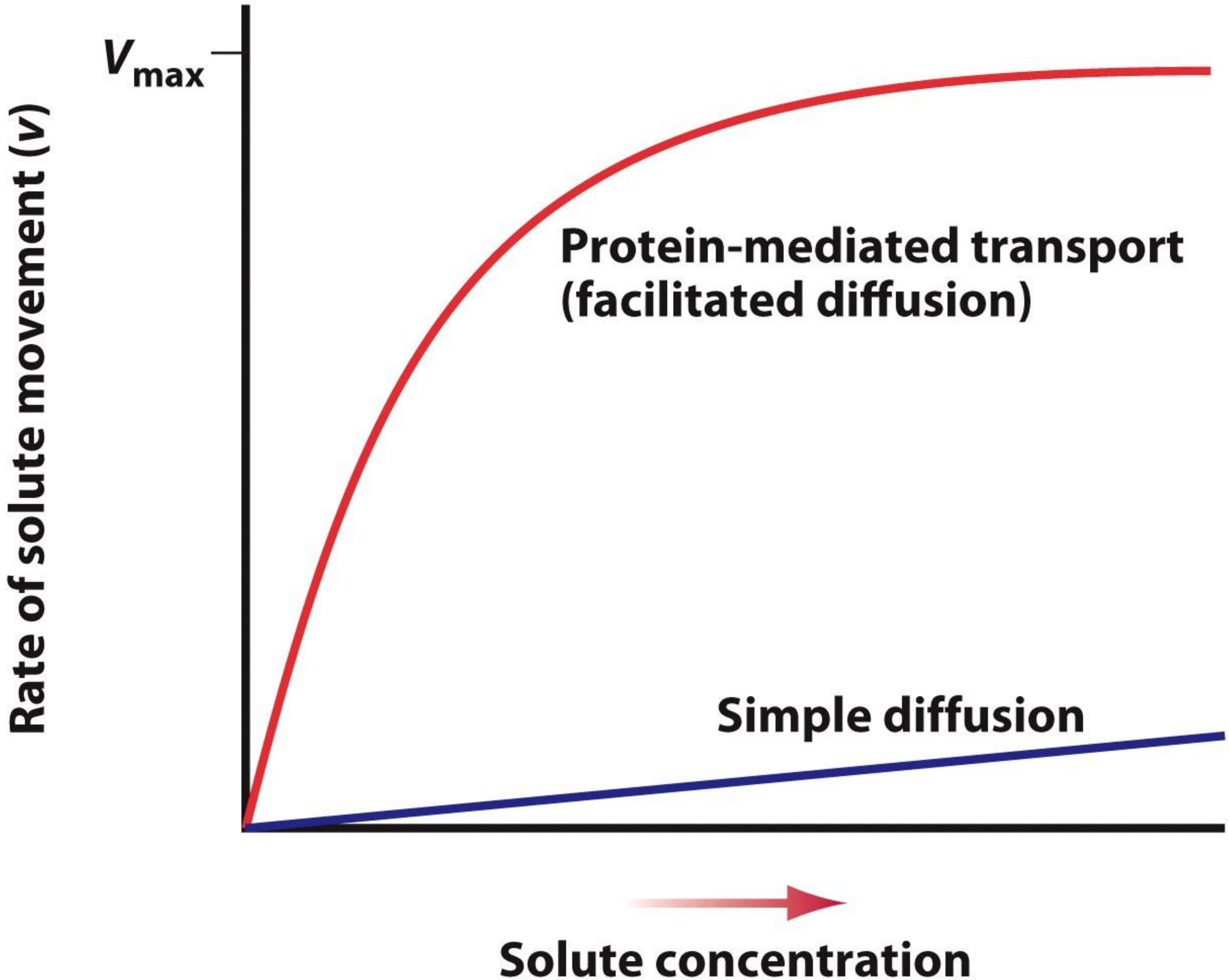
Facilitative glucose transporter



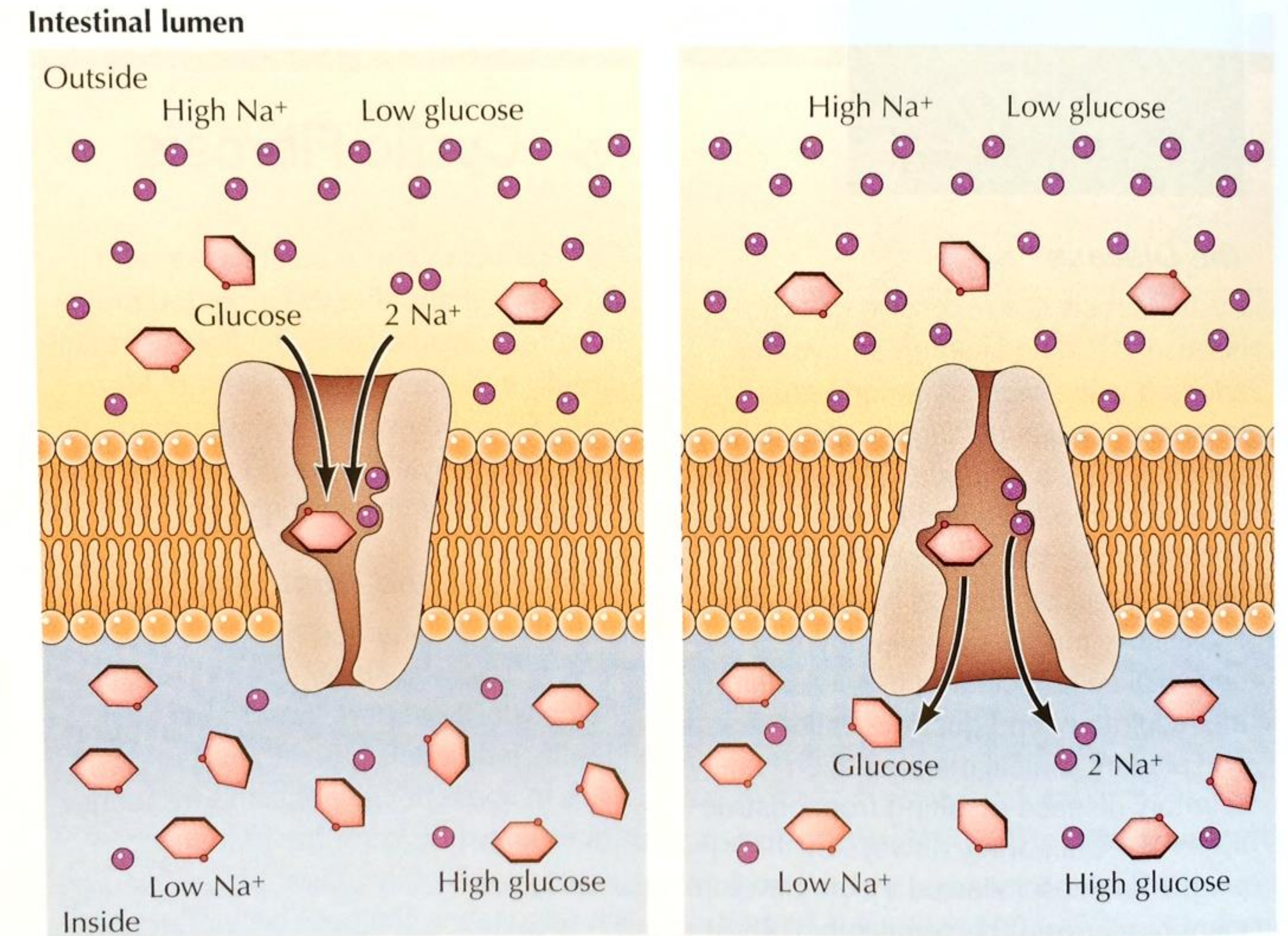
Facilitative glucose transporter



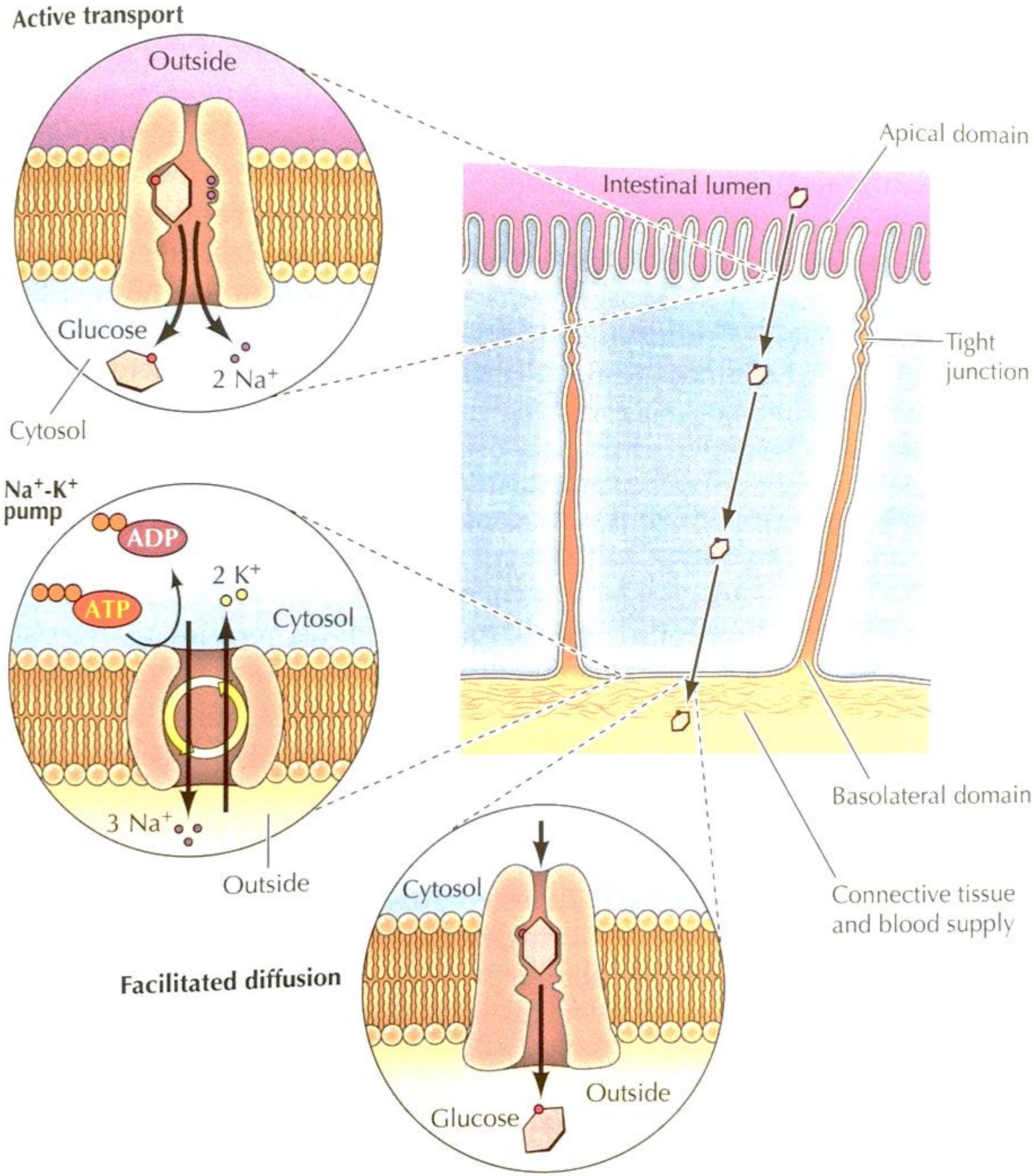
Transporters can work in both directions


V_{max}
Rate of solute movement (v)
Protein-mediated transport (facilitated diffusion)
Simple diffusion
Solute concentration

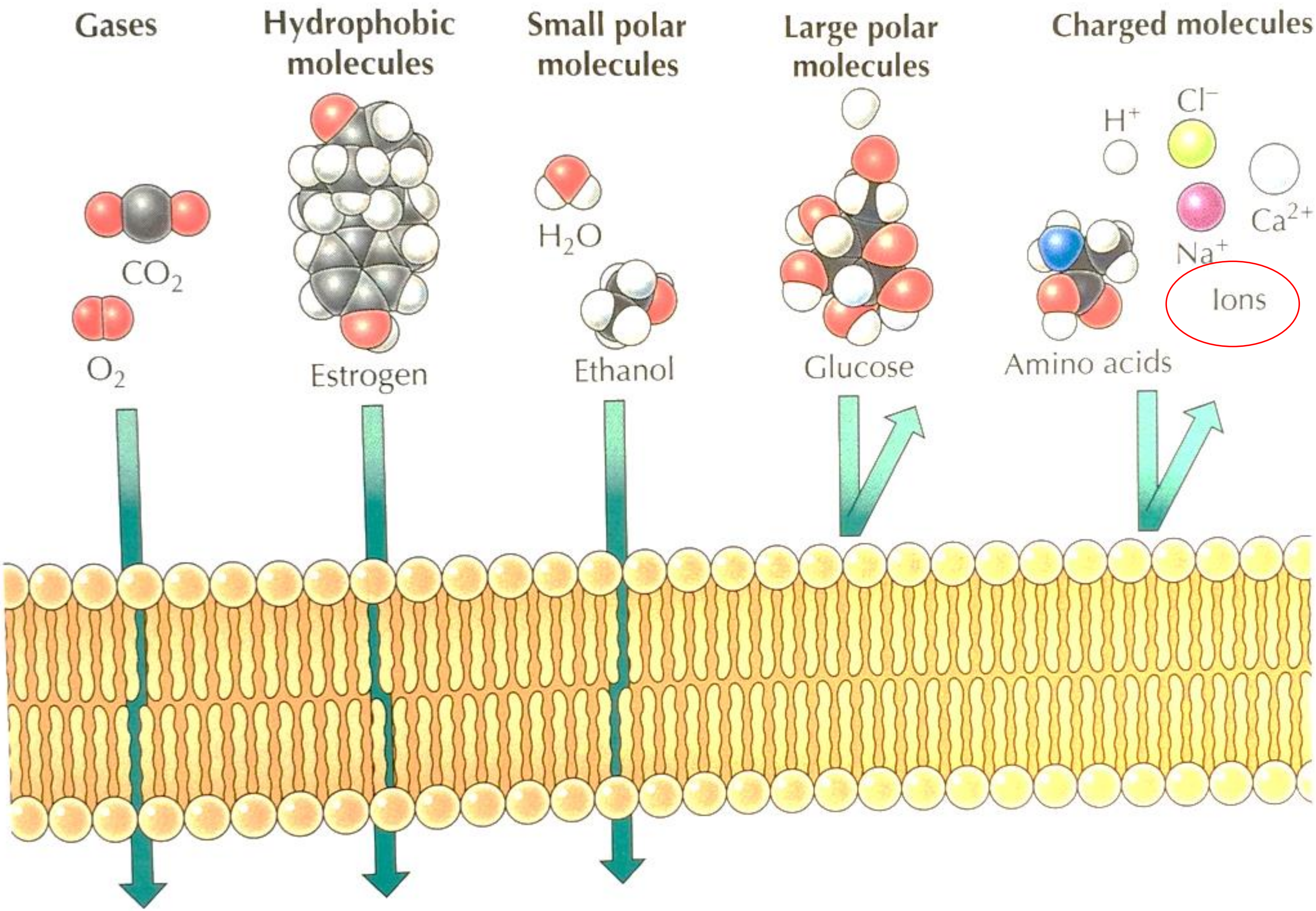
Active glucose transport
(Na^+/glucose cotransporter)



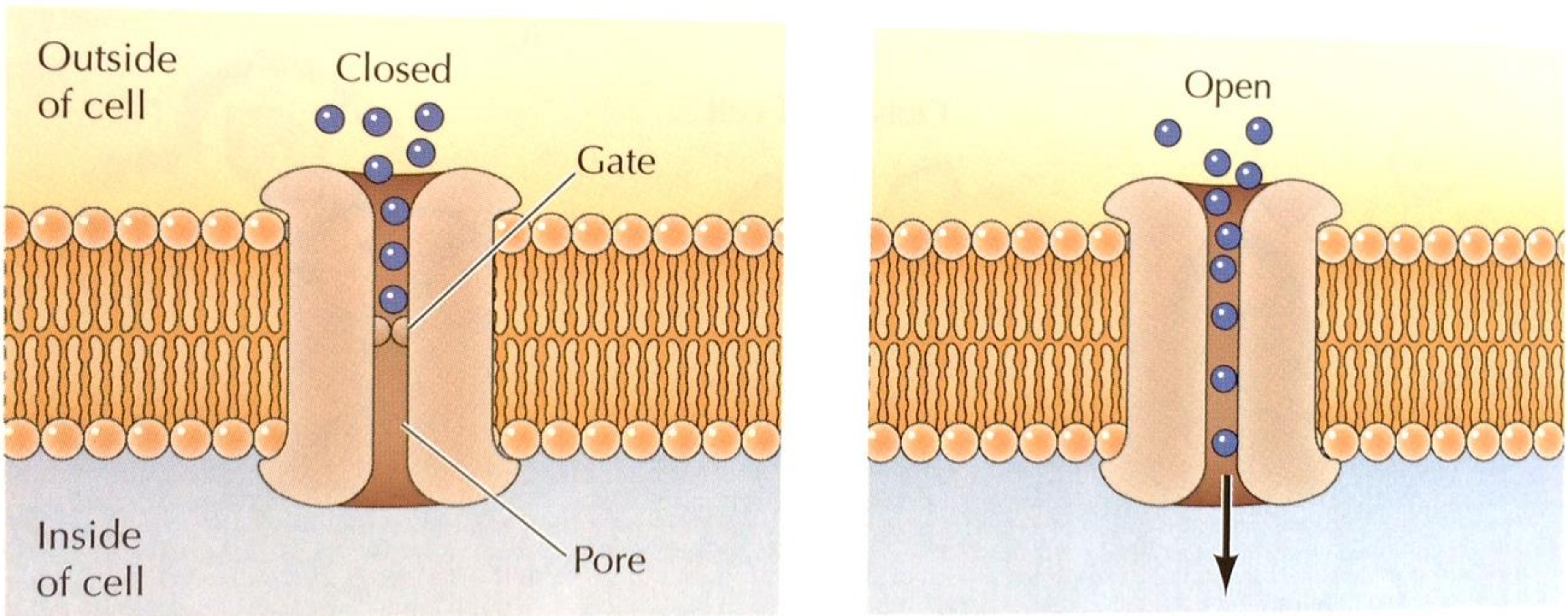
Active glucose transport



Molecular transport across membranes

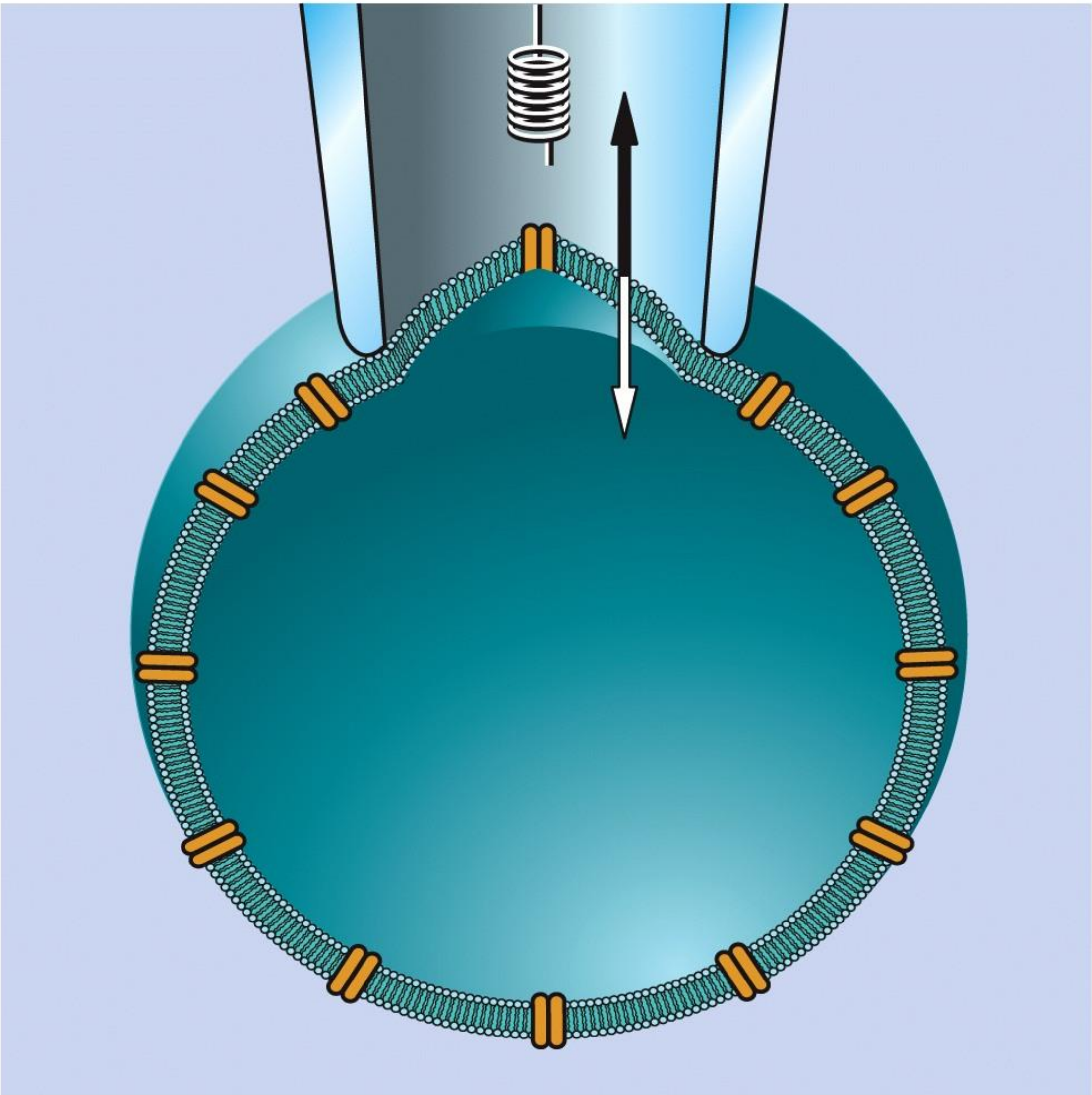


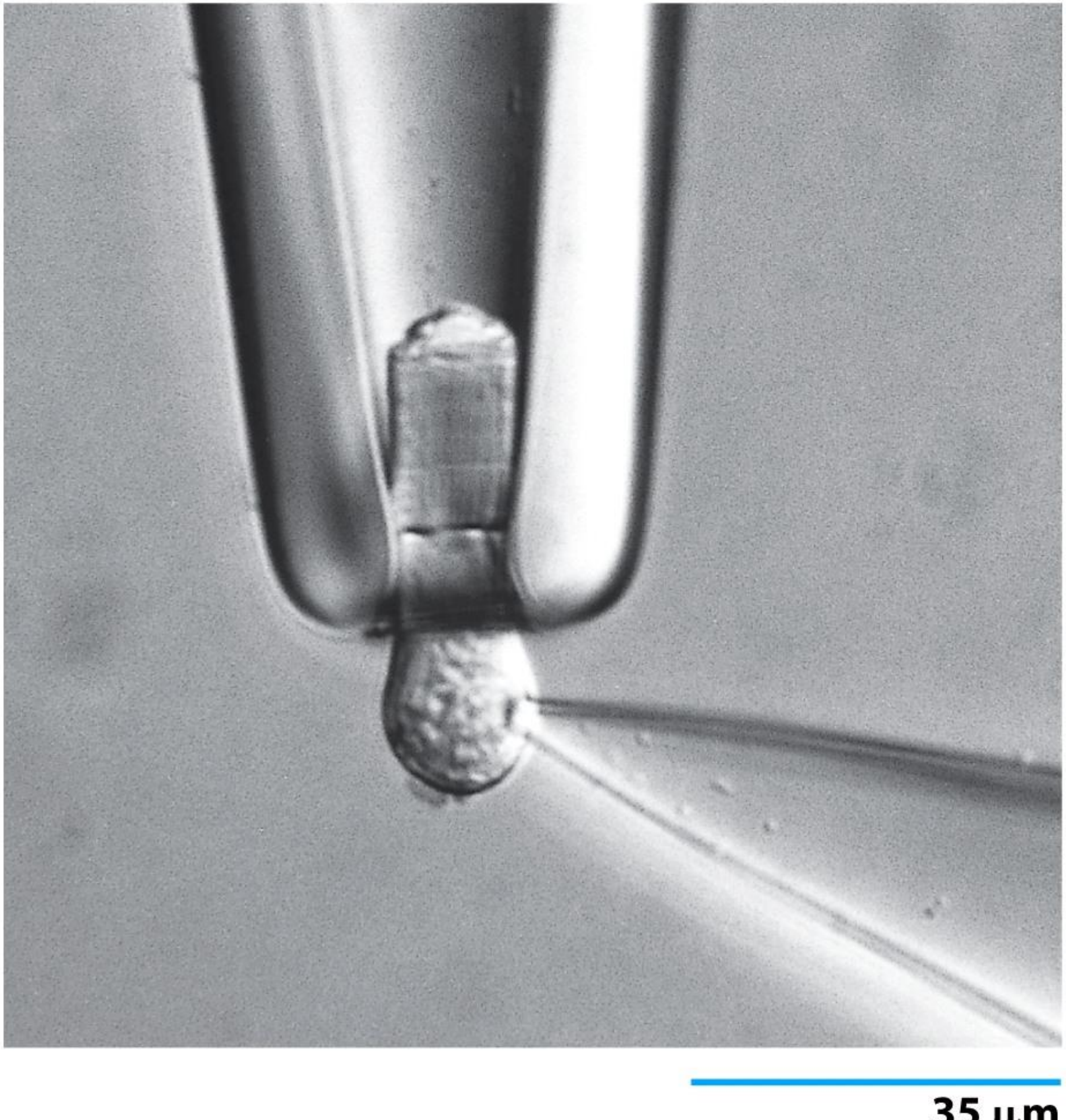
Ion channel



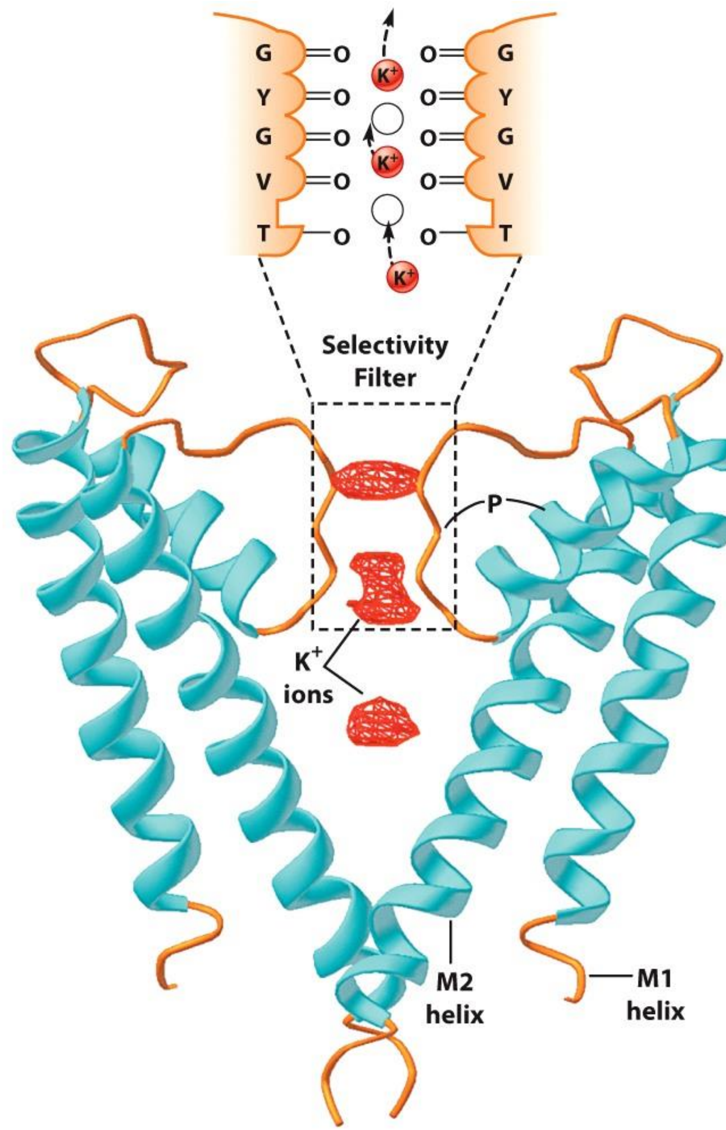
Ion transport is electrogenic

Patch clamp

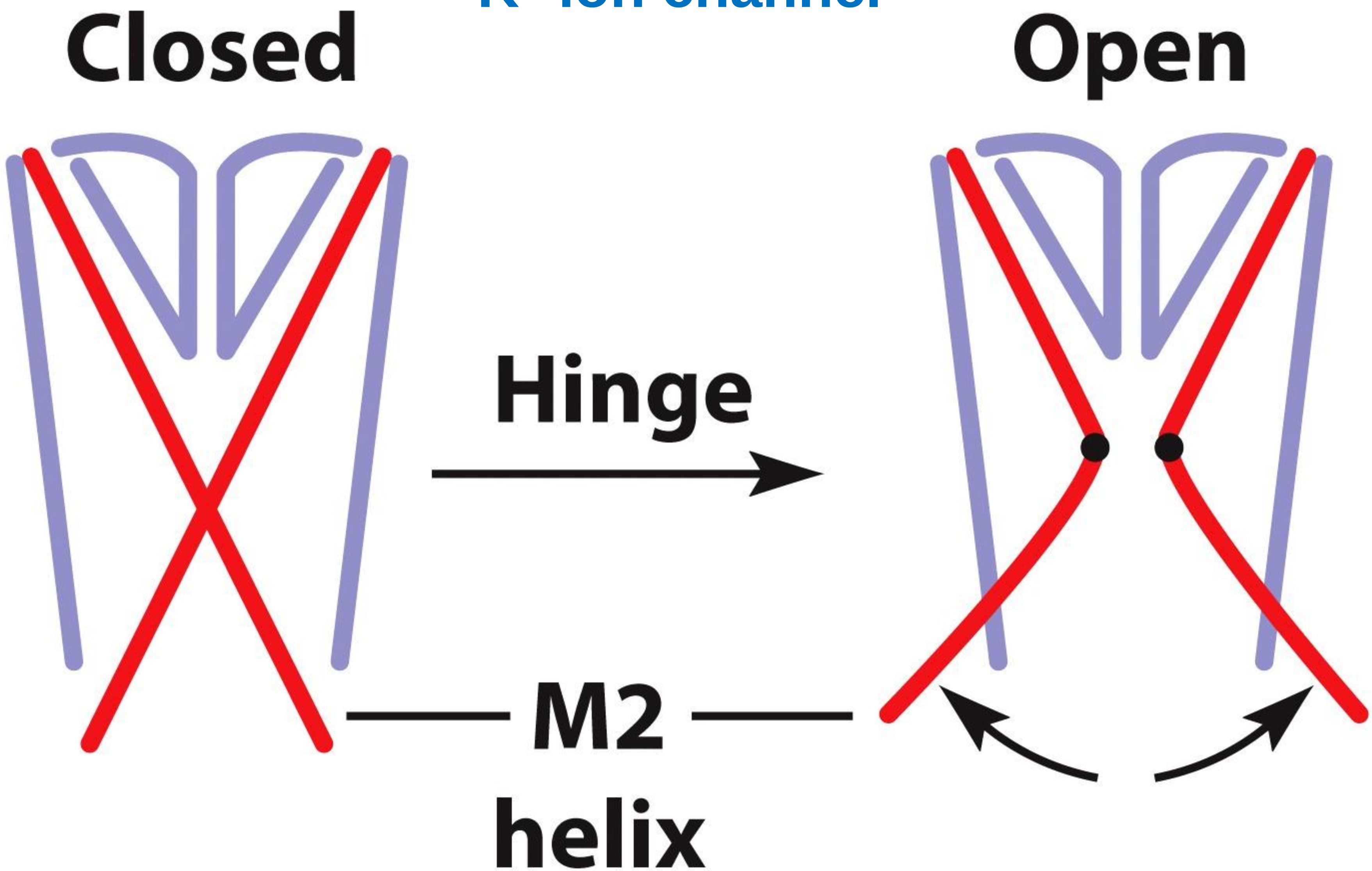



35 μm

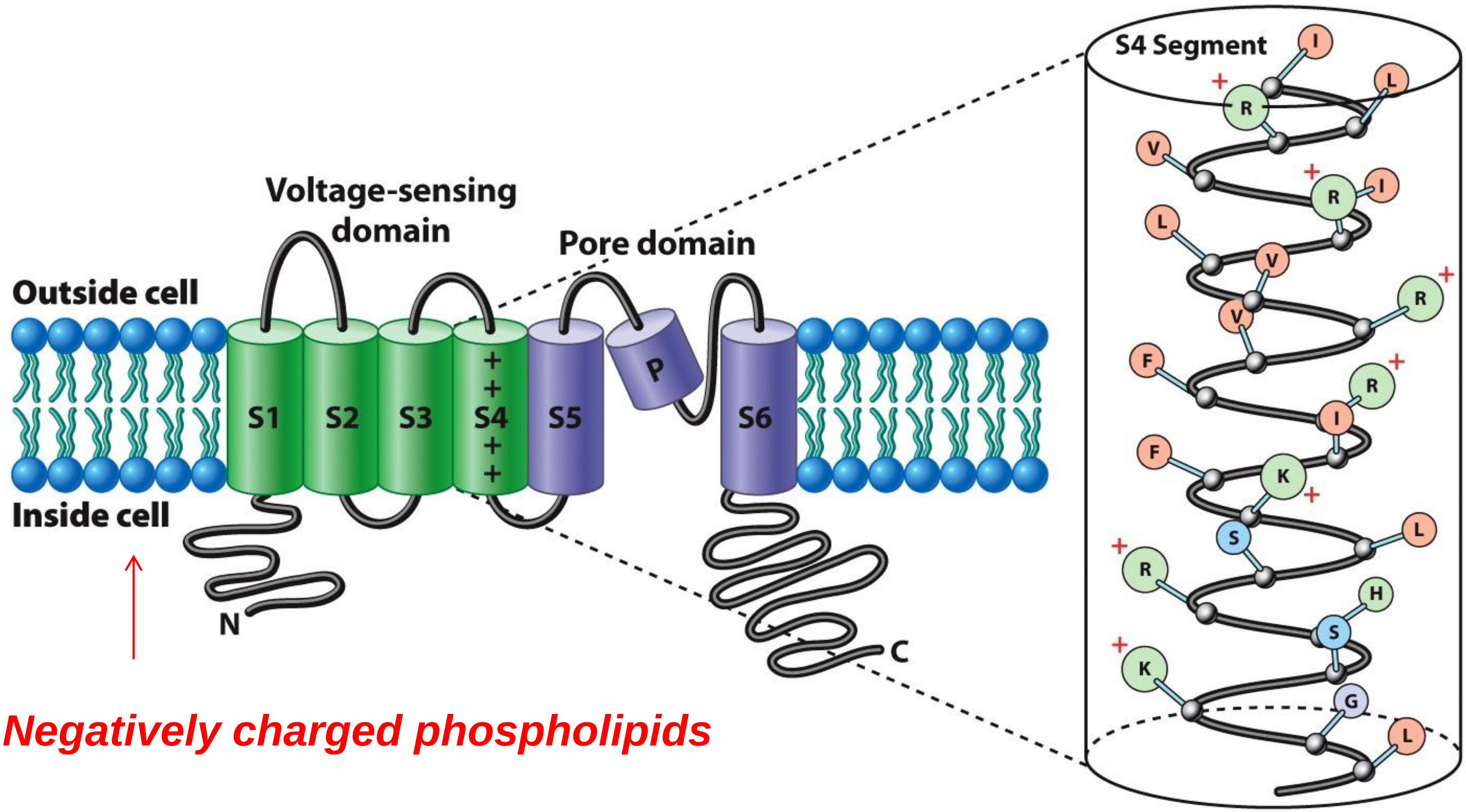
K^+ ion channel



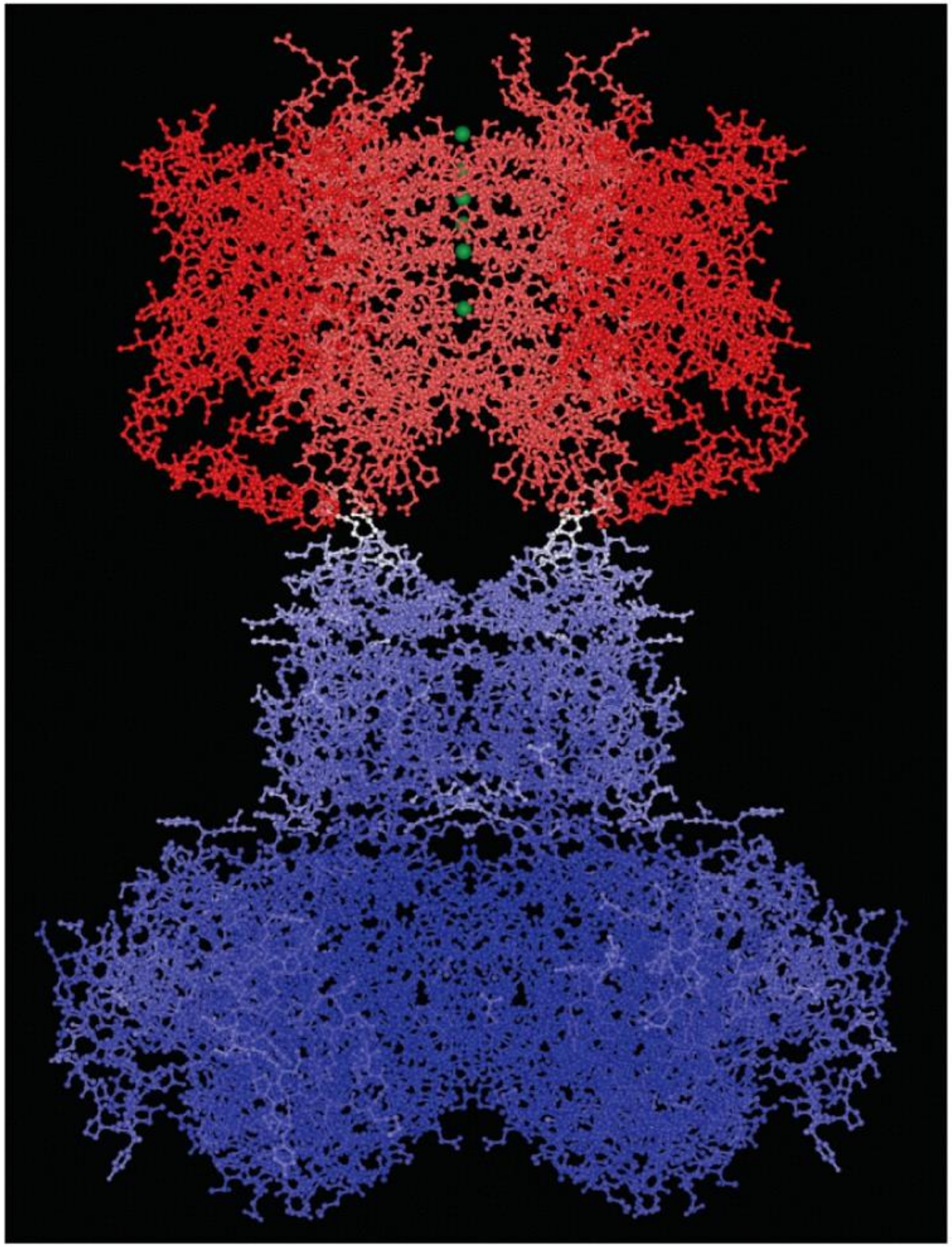
K$^+$ ion channel



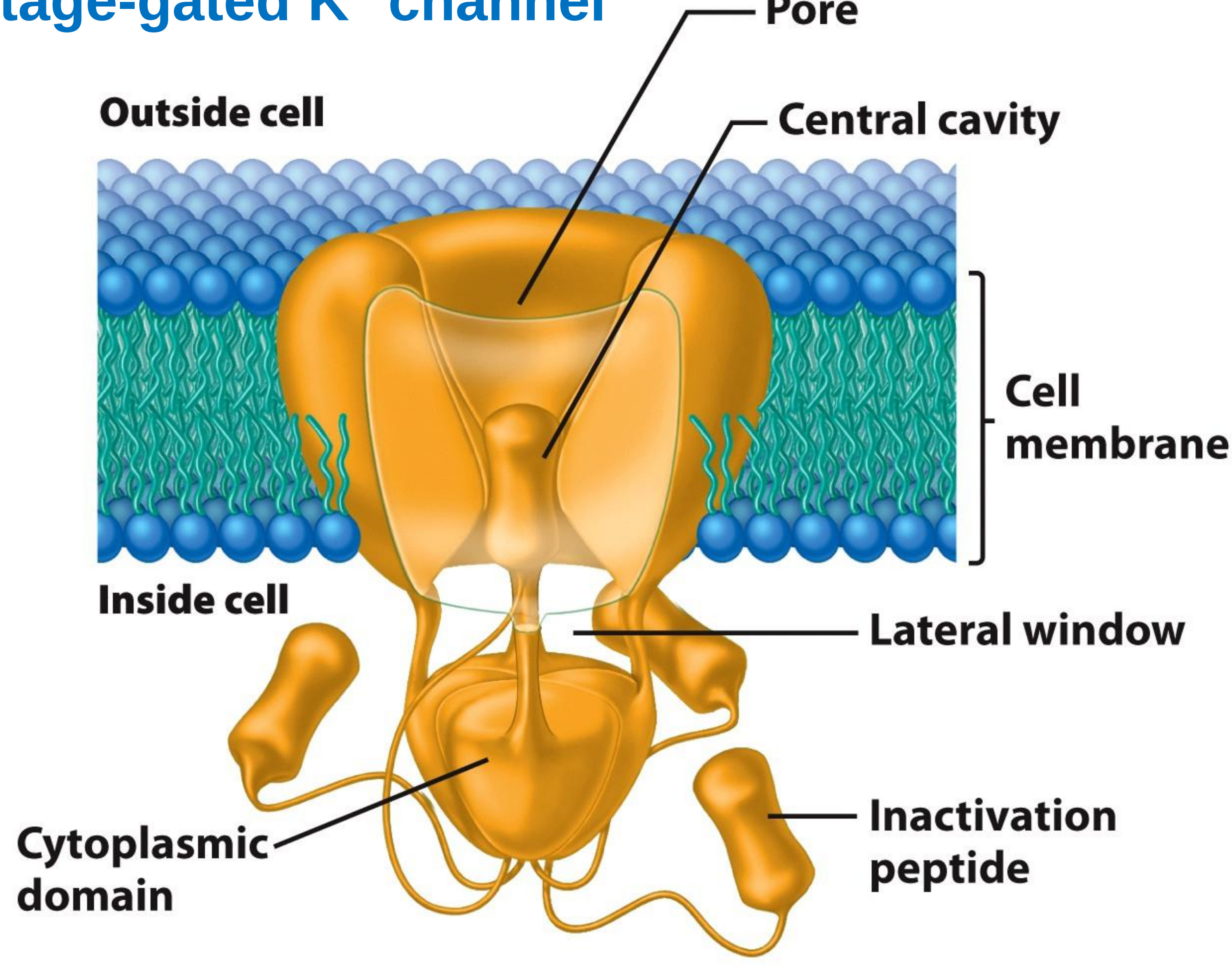
Voltage-gated K^+ channel



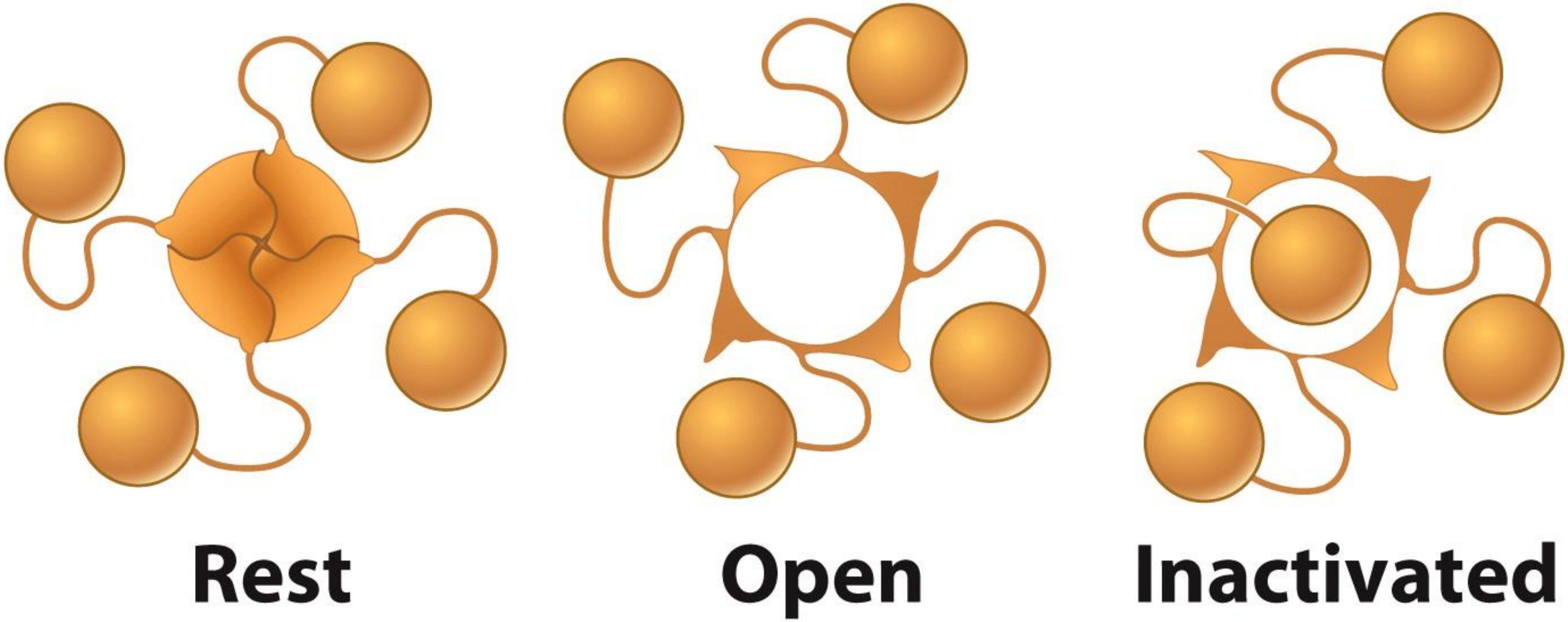
Negatively charged phospholipids



Voltage-gated K^+ channel



Voltage-gated K^+ channel



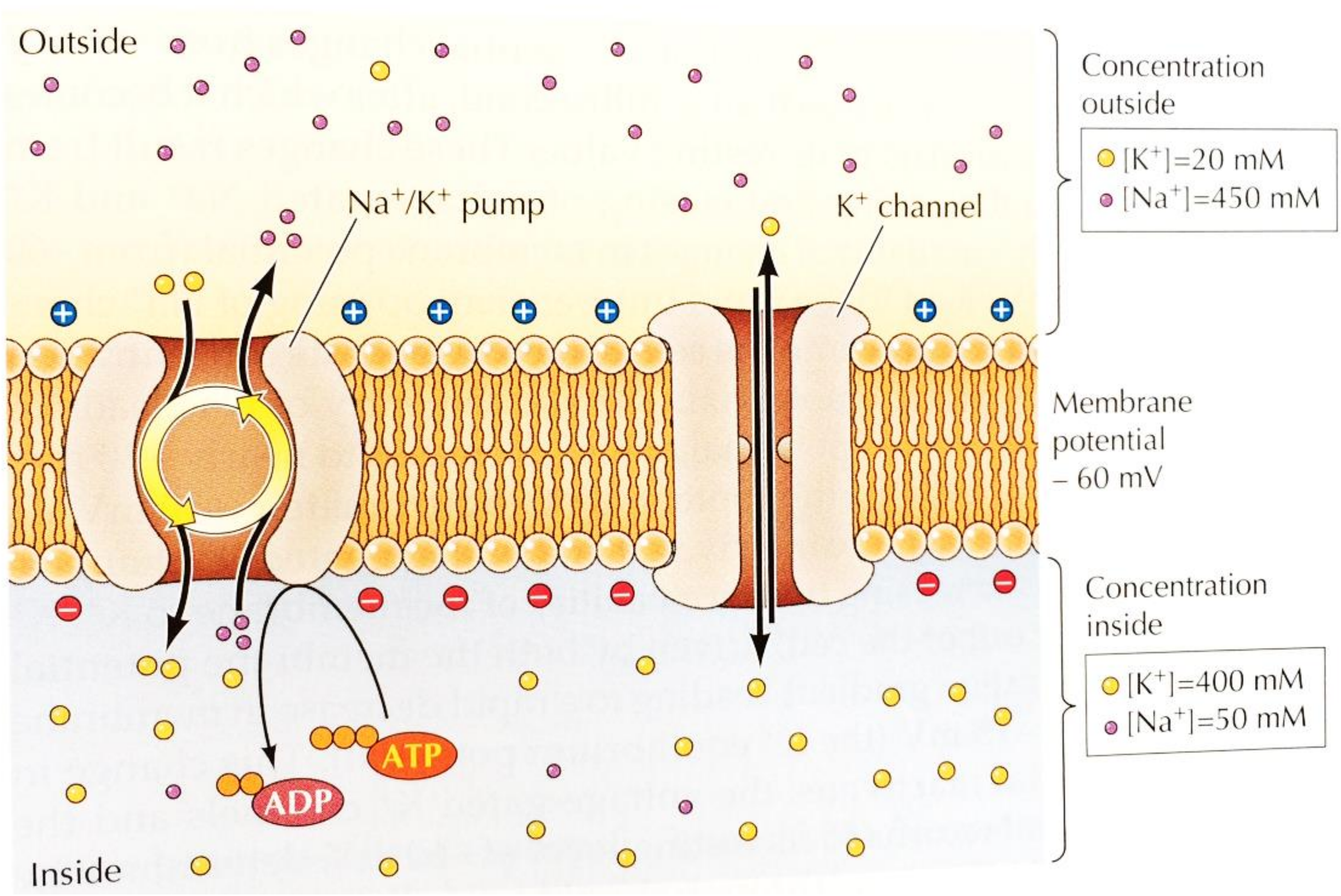
“closed”

Table 4.3 Ion Concentrations Inside and Outside of a Typical Mammalian Cell

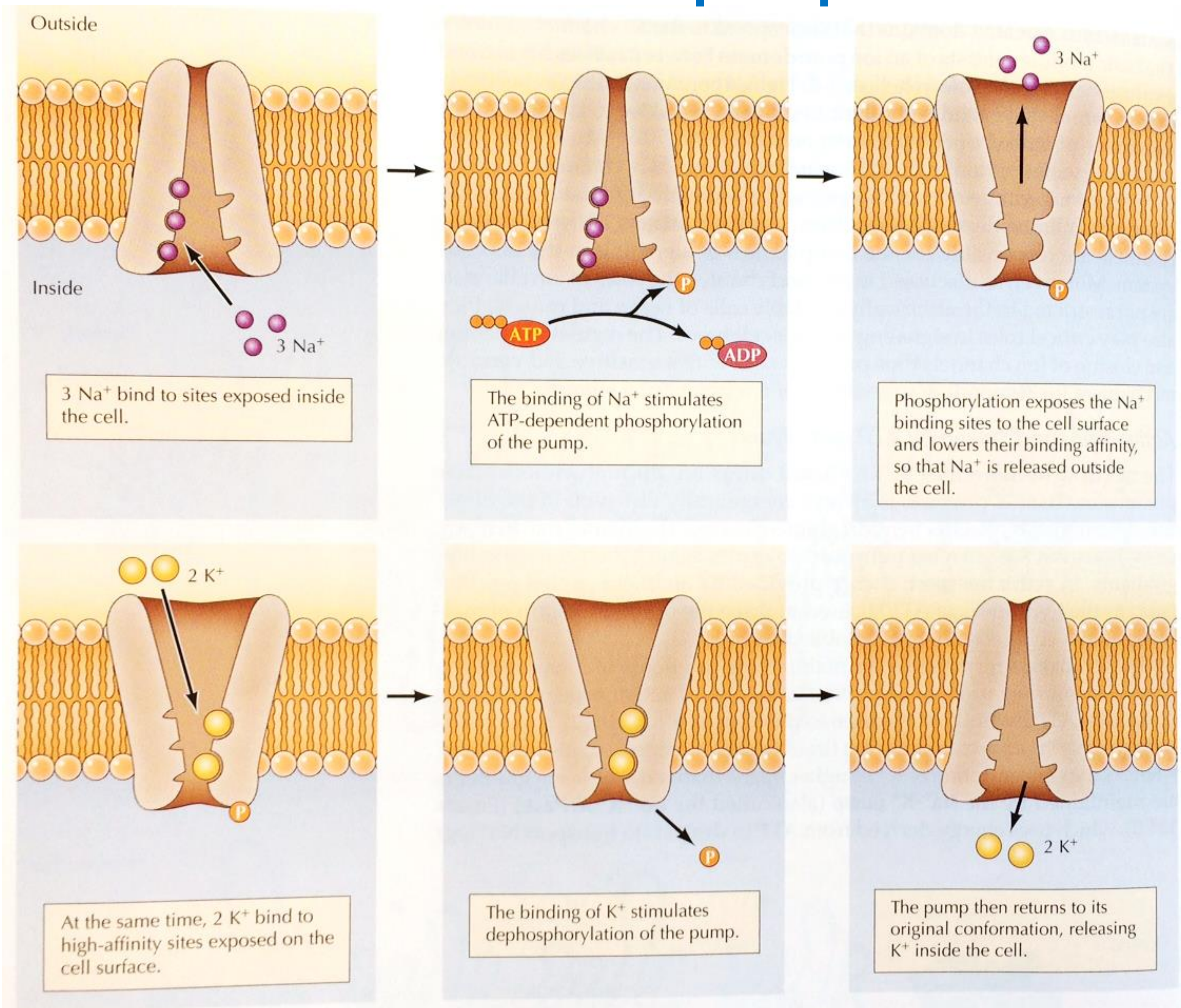
	Extracellular concentration	Intracellular concentration	Ionic gradient
Na^{+}	150 mM	10 mM	15×
K^{+}	5 mM	140 mM	28×
Cl^{-}	120 mM	10 mM	12×
Ca^{2+}	10^{-3} M	10^{-7} M	10,000×
H^{+}	$10^{-7.4}$ M (pH of 7.4)	$10^{-7.2}$ M (pH of 7.2)	Nearly 2×

The ion concentrations for the squid axon are given on page 177.

Na^+ /K^+ pump

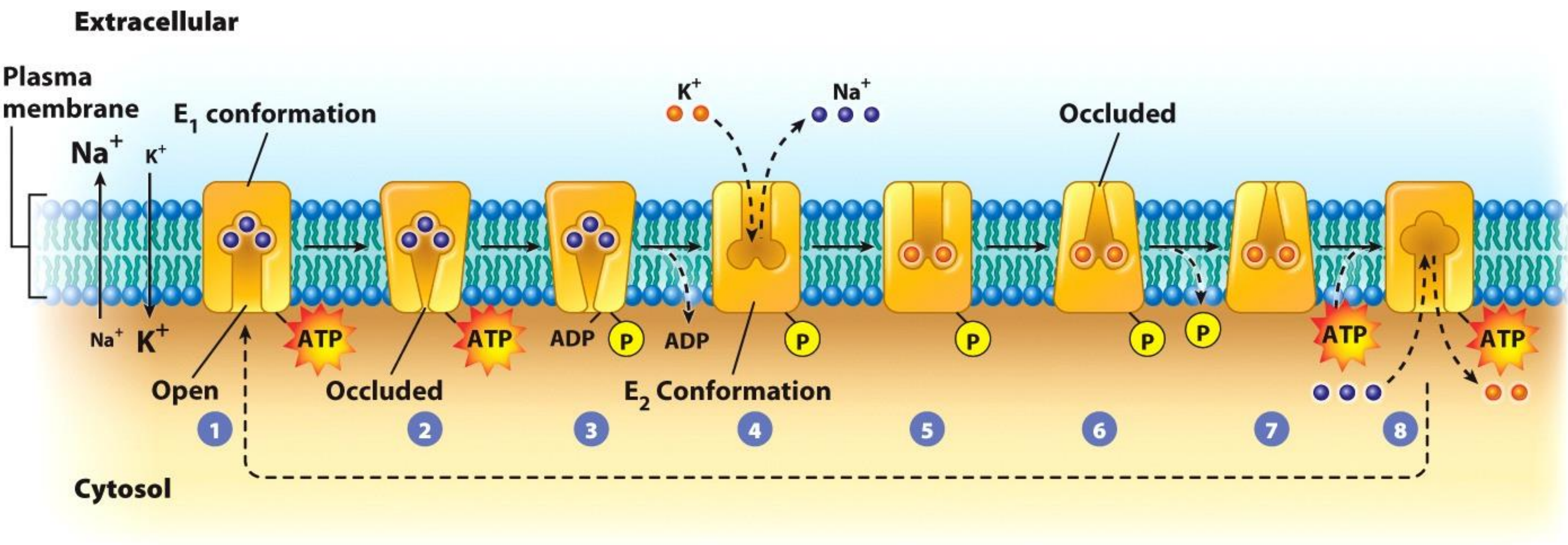

Outside
Na+/K+ pump
K+ channel
Concentration outside
[K+]=20 mM
[Na+]=450 mM
Membrane potential – 60 mV
Concentration inside
[K+]=400 mM
[Na+]=50 mM
ATP
ADP
Inside

Na^+ /K^+ pump



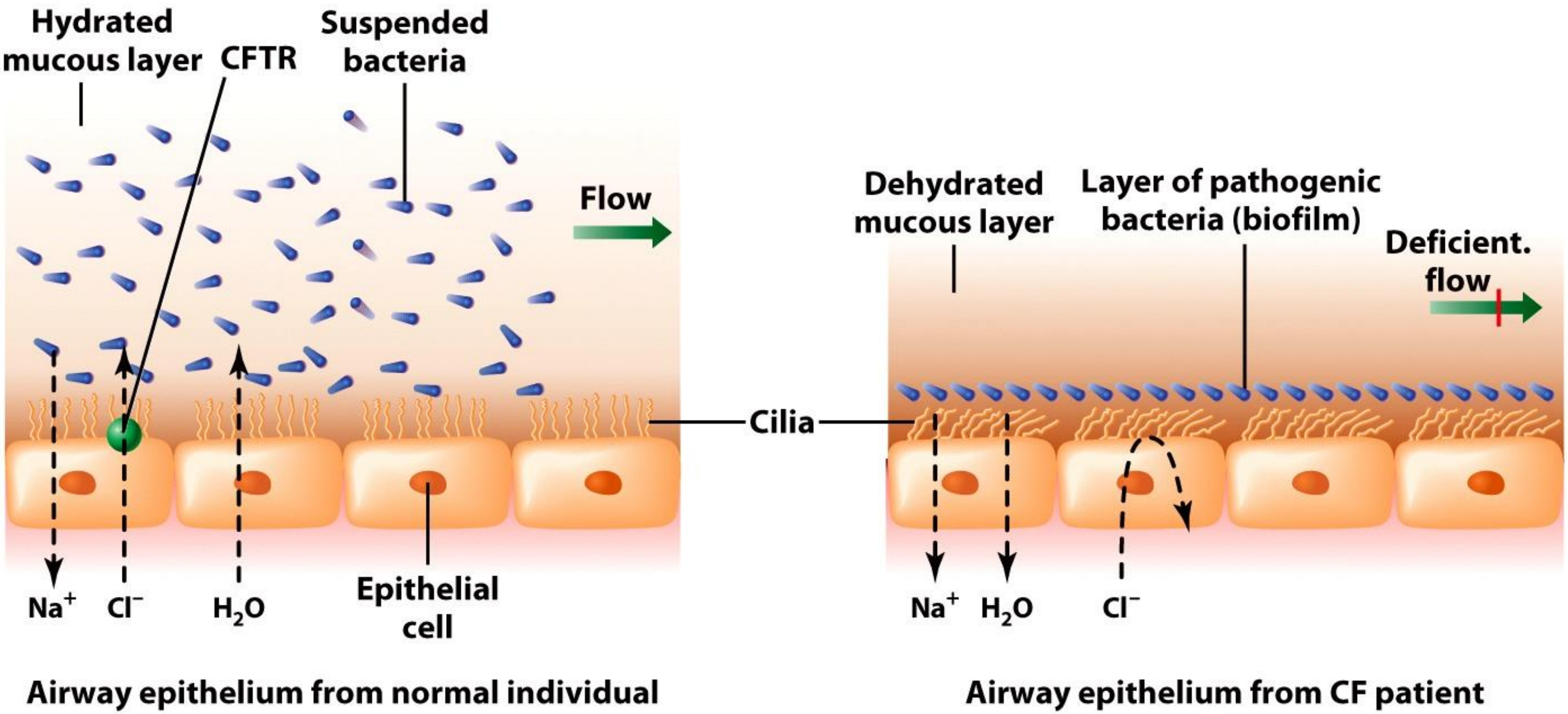
Outside
Inside
3 Na⁺
3 Na⁺ bind to sites exposed inside the cell.
ATP
ADP
P
The binding of Na⁺ stimulates ATP-dependent phosphorylation of the pump.
3 Na⁺
P
Phosphorylation exposes the Na⁺ binding sites to the cell surface and lowers their binding affinity, so that Na⁺ is released outside the cell.
2 K⁺
P
At the same time, 2 K⁺ bind to high-affinity sites exposed on the cell surface.
P
The binding of K⁺ stimulates dephosphorylation of the pump.
2 K⁺
The pump then returns to its original conformation, releasing K⁺ inside the cell.

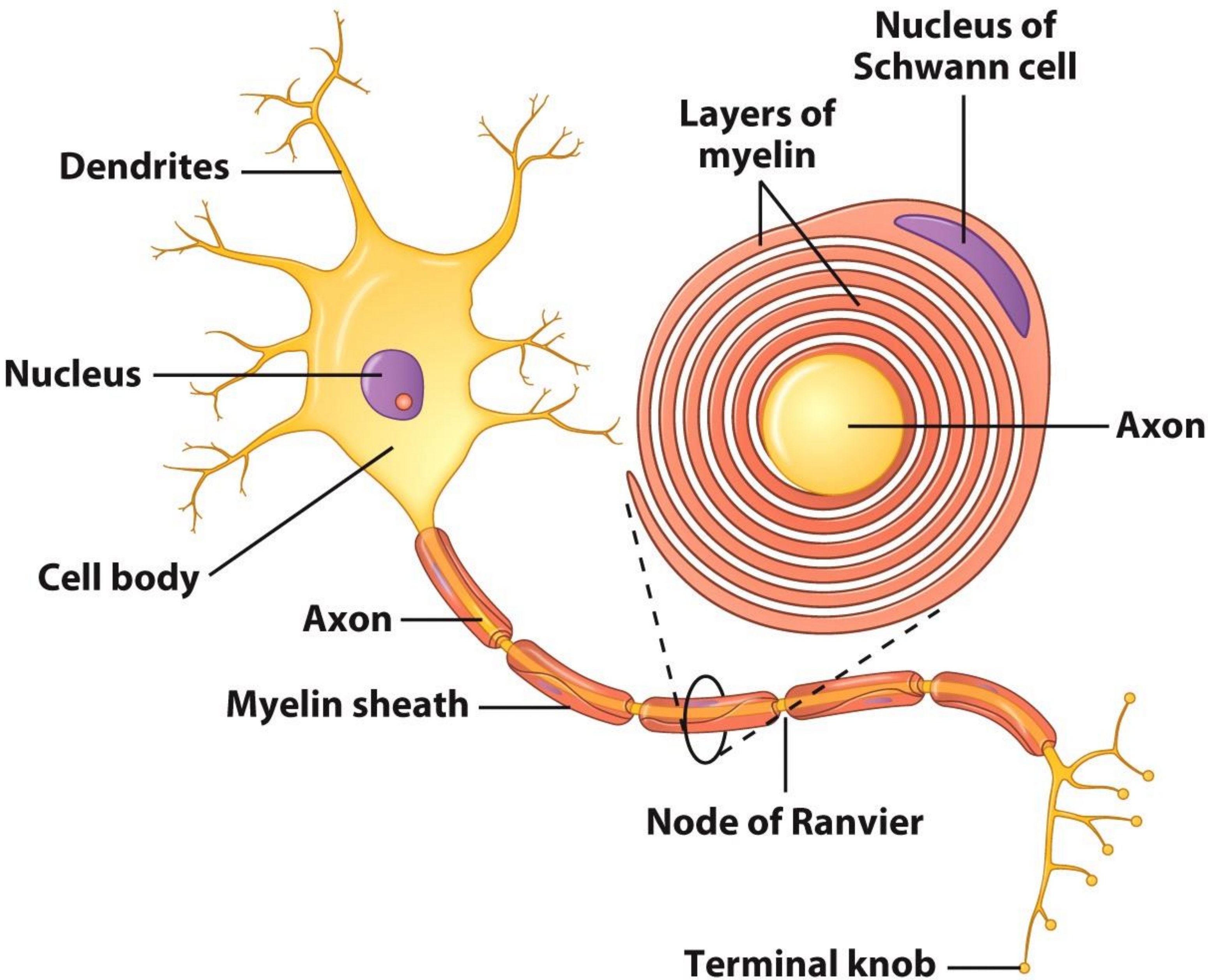
Na^+ /K^+ pump

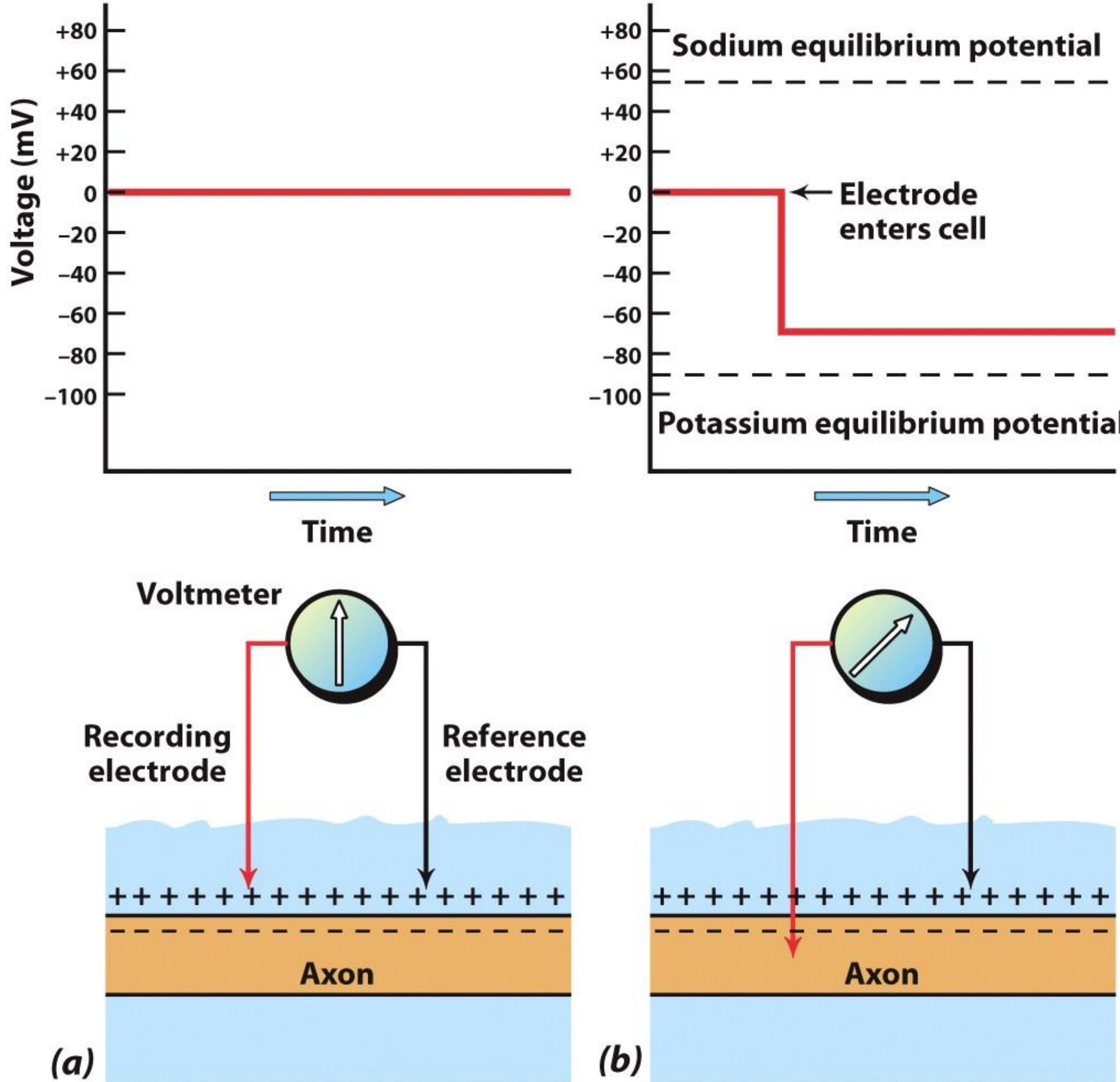


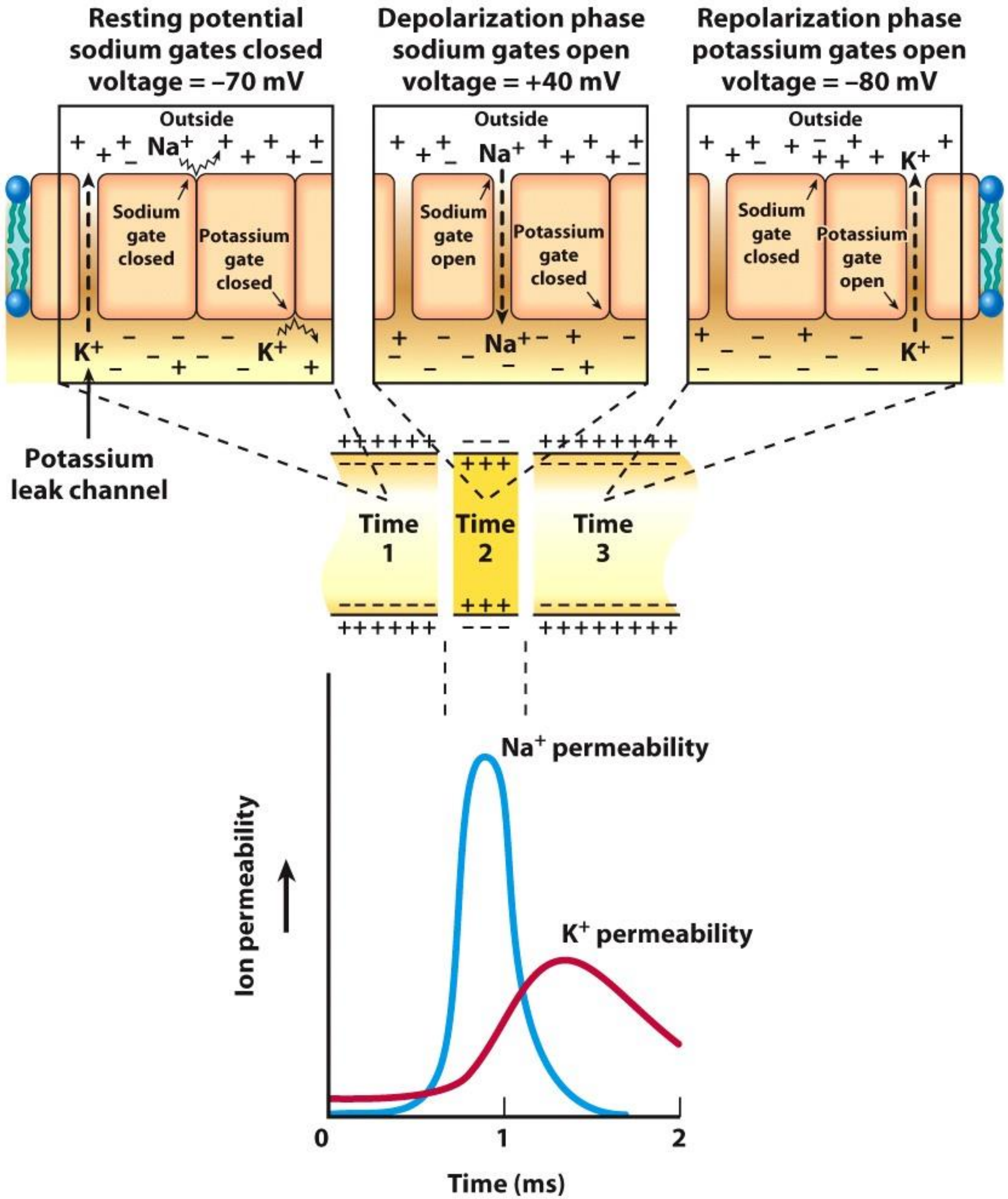
Extracellular
Plasma membrane
E1 conformation
Na+
K+
K+
Na+
Occluded
Na+
K+
ATP
ATP
ADP
P
ADP
P
P
P
P
ATP
ATP
Open
Occluded
E2 Conformation
1
2
3
4
5
6
7
8
Cytosol

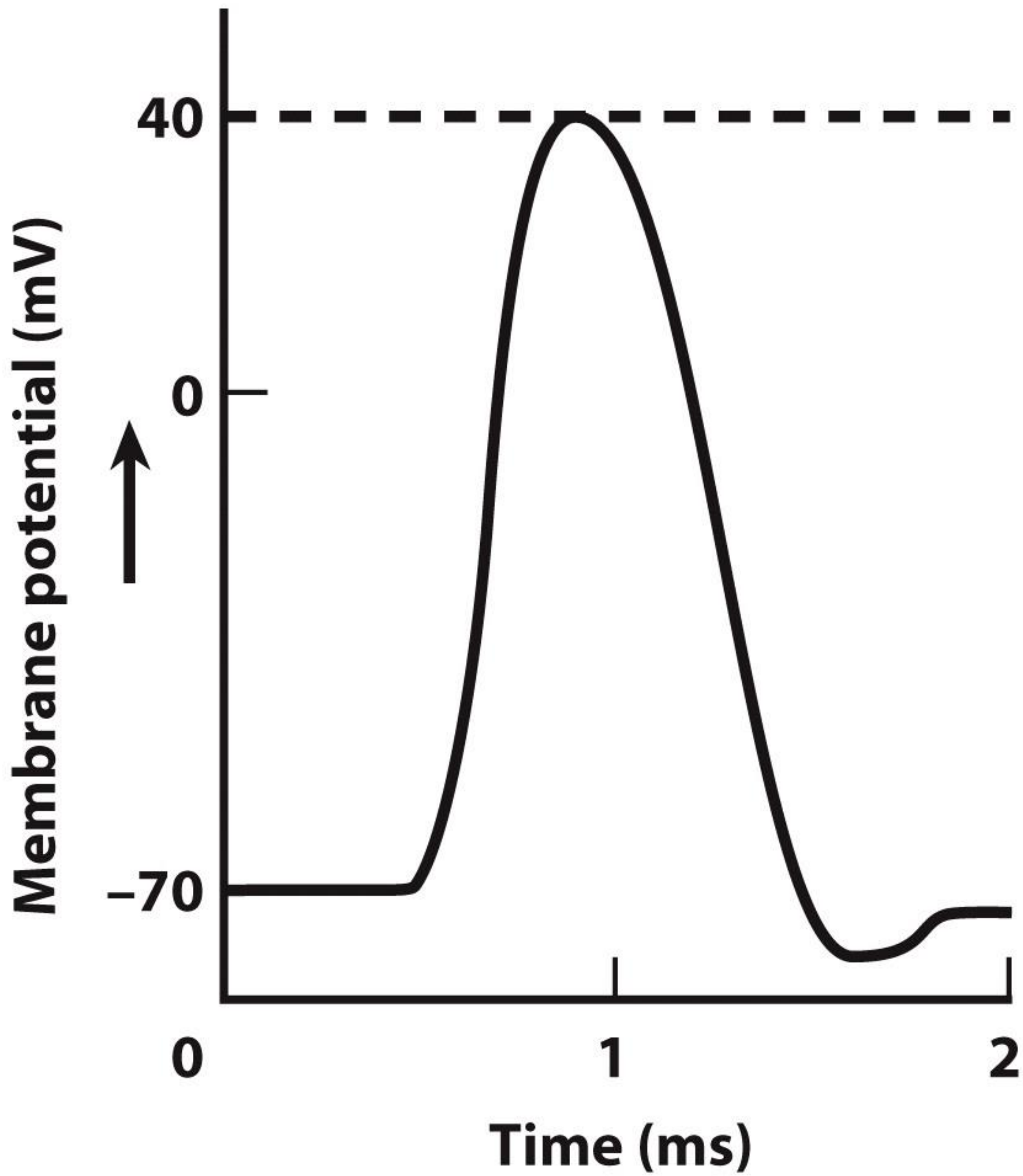
Cystic fibrosis transmembrane conductance regulator (CFTR)

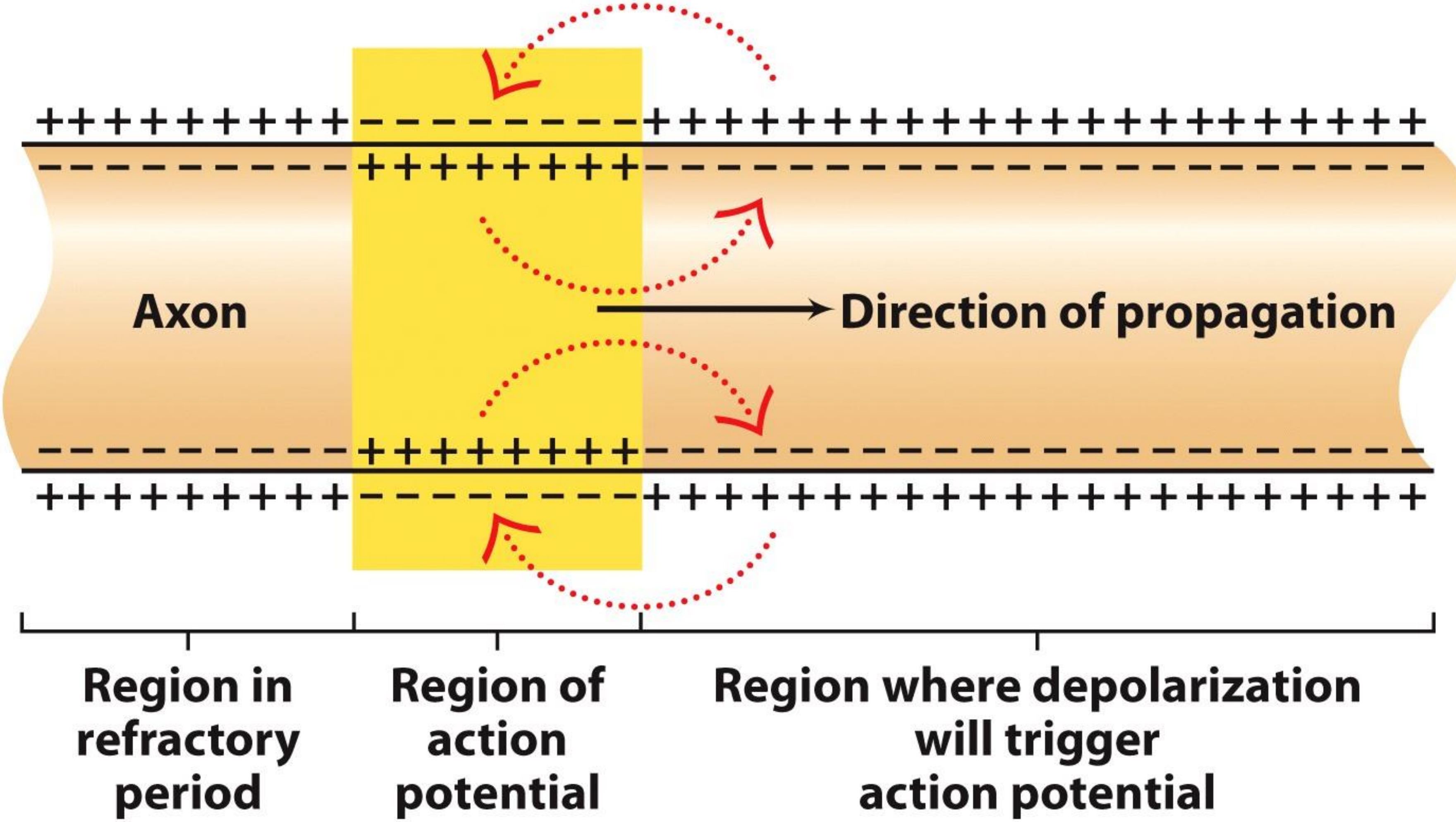


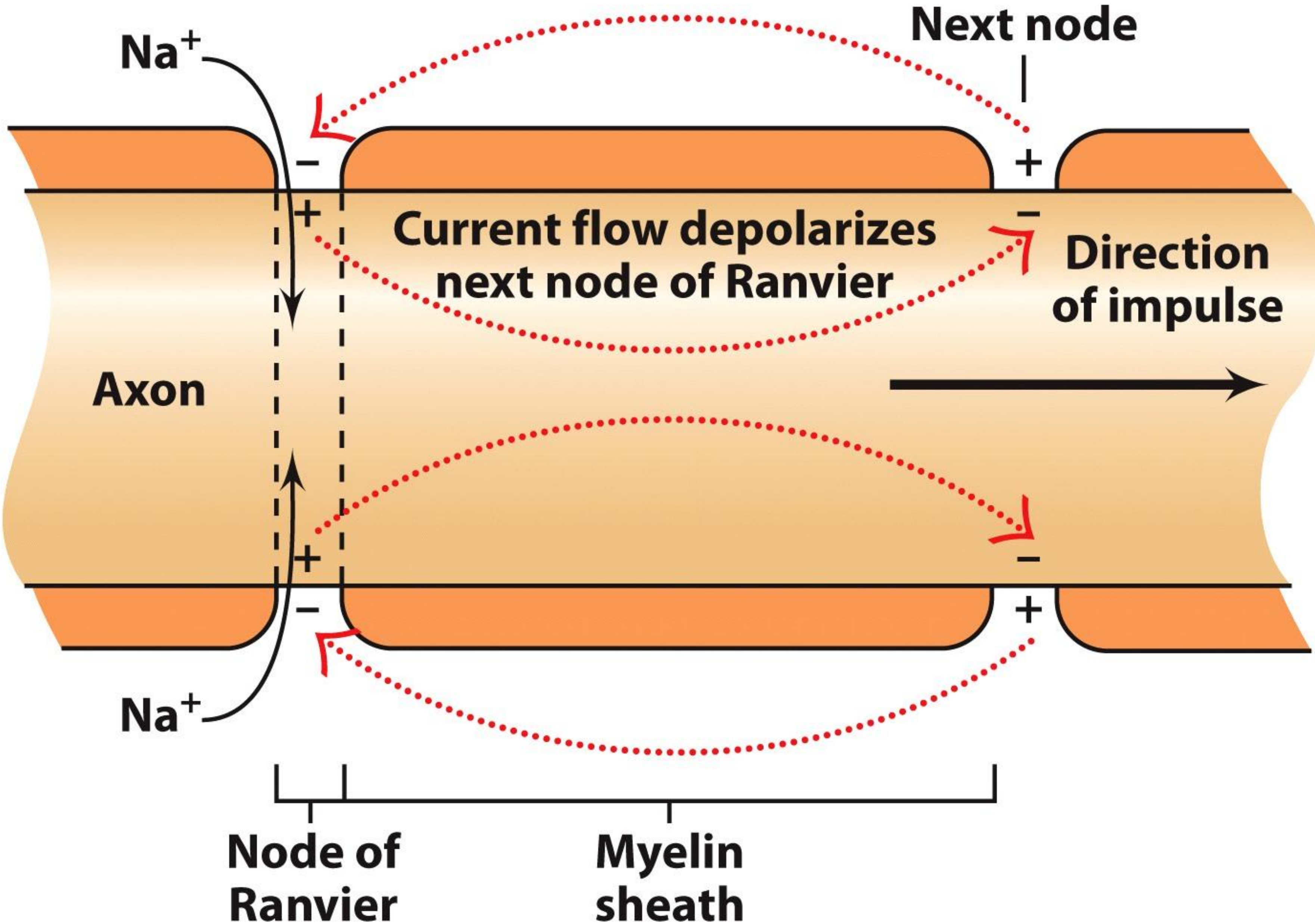

Nucleus of
Schwann cell
Layers of
myelin
Dendrites
Nucleus
Axon
Cell body
Axon
Myelin sheath
Node of Ranvier
Terminal knob


Voltage (mV)
+80
+60
+40
+20
0
−20
−40
−60
−80
−100
Time
Voltmeter
Recording electrode
Reference electrode
Axon
(a)
Sodium equilibrium potential
Electrode enters cell
Potassium equilibrium potential
Time
Axon
(b)

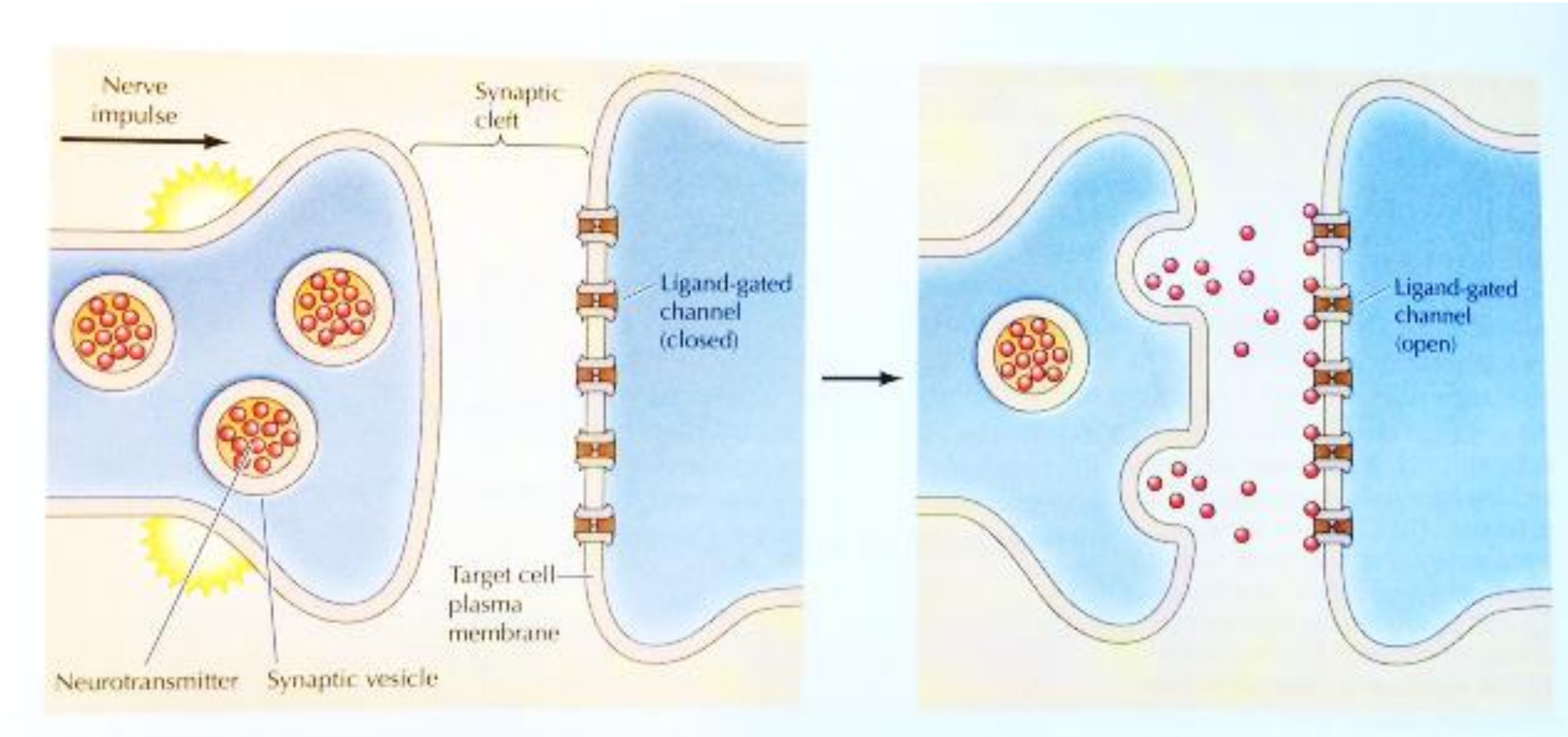

Resting potential
sodium gates closed
voltage = –70 mV
Depolarization phase
sodium gates open
voltage = +40 mV
Repolarization phase
potassium gates open
voltage = –80 mV
Outside
Na+
K+
Sodium gate closed
Potassium gate closed
Sodium gate open
Potassium gate open
Potassium leak channel
Time 1
Time 2
Time 3
Na+ permeability
K+ permeability
Ion permeability
0
1
2
Time (ms)

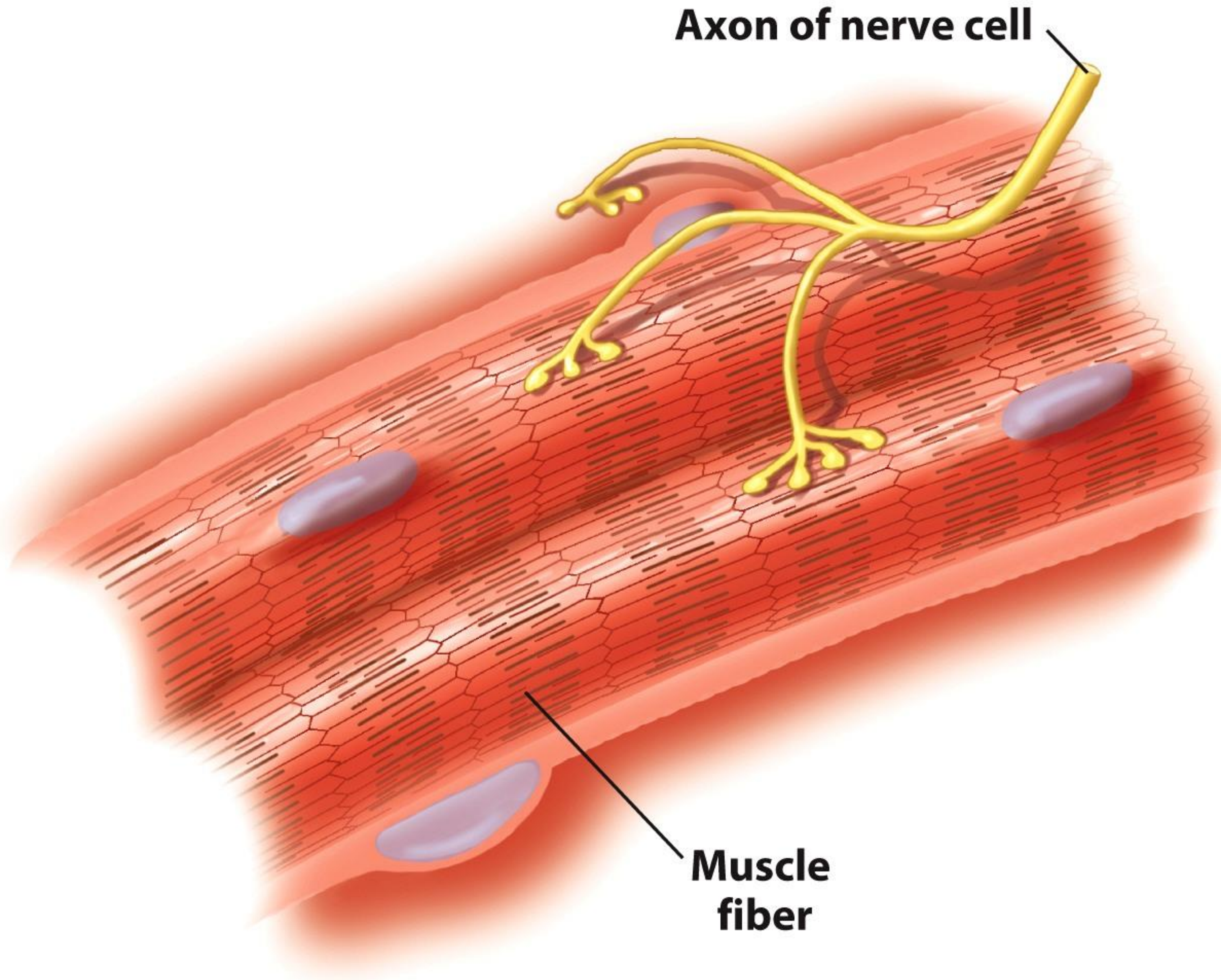

Membrane potential (mV)
40
0
–70
0
1
2
Time (ms)

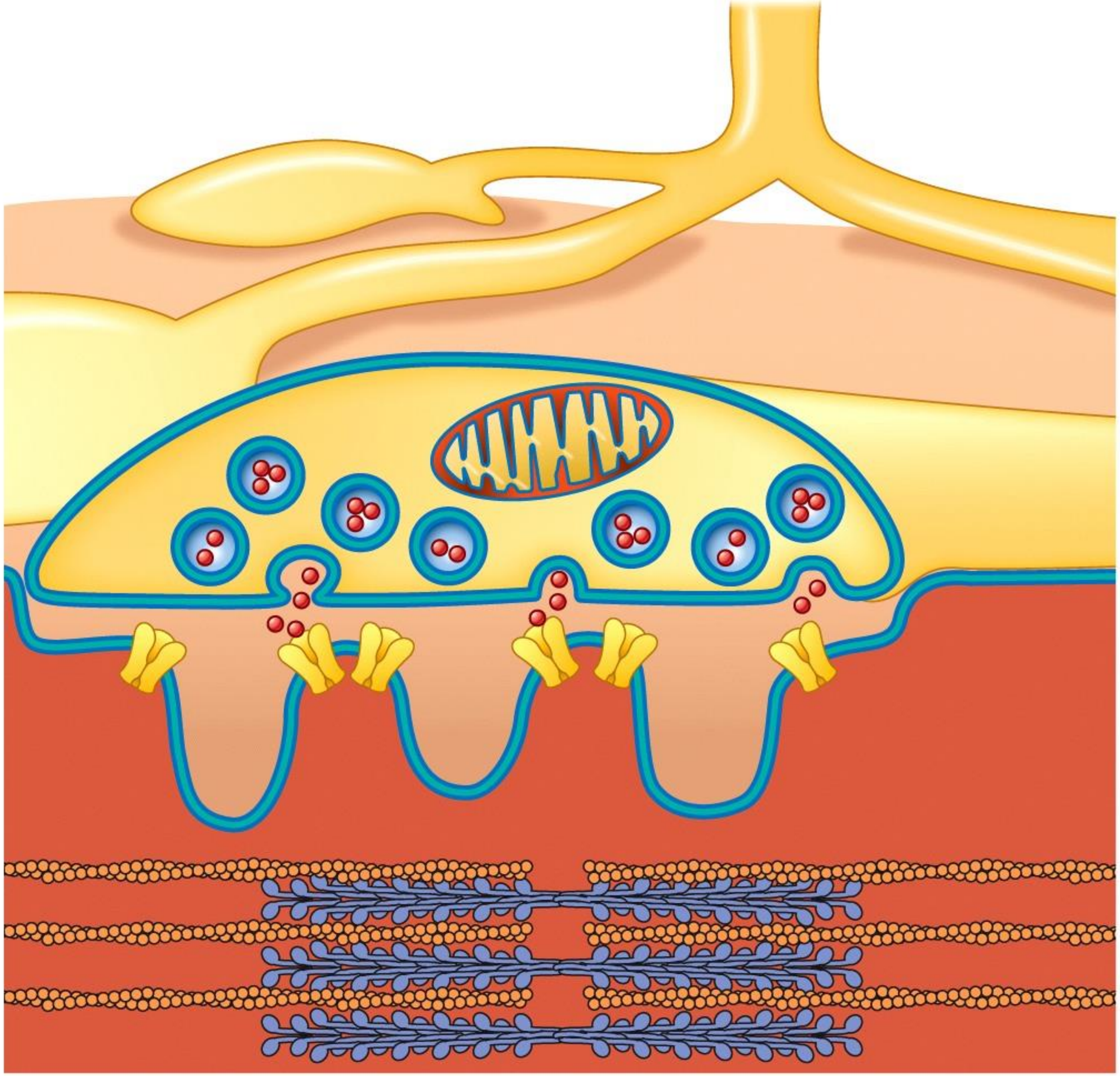

Axon
Direction of propagation
Region in refractory period
Region of action potential
Region where depolarization will trigger action potential

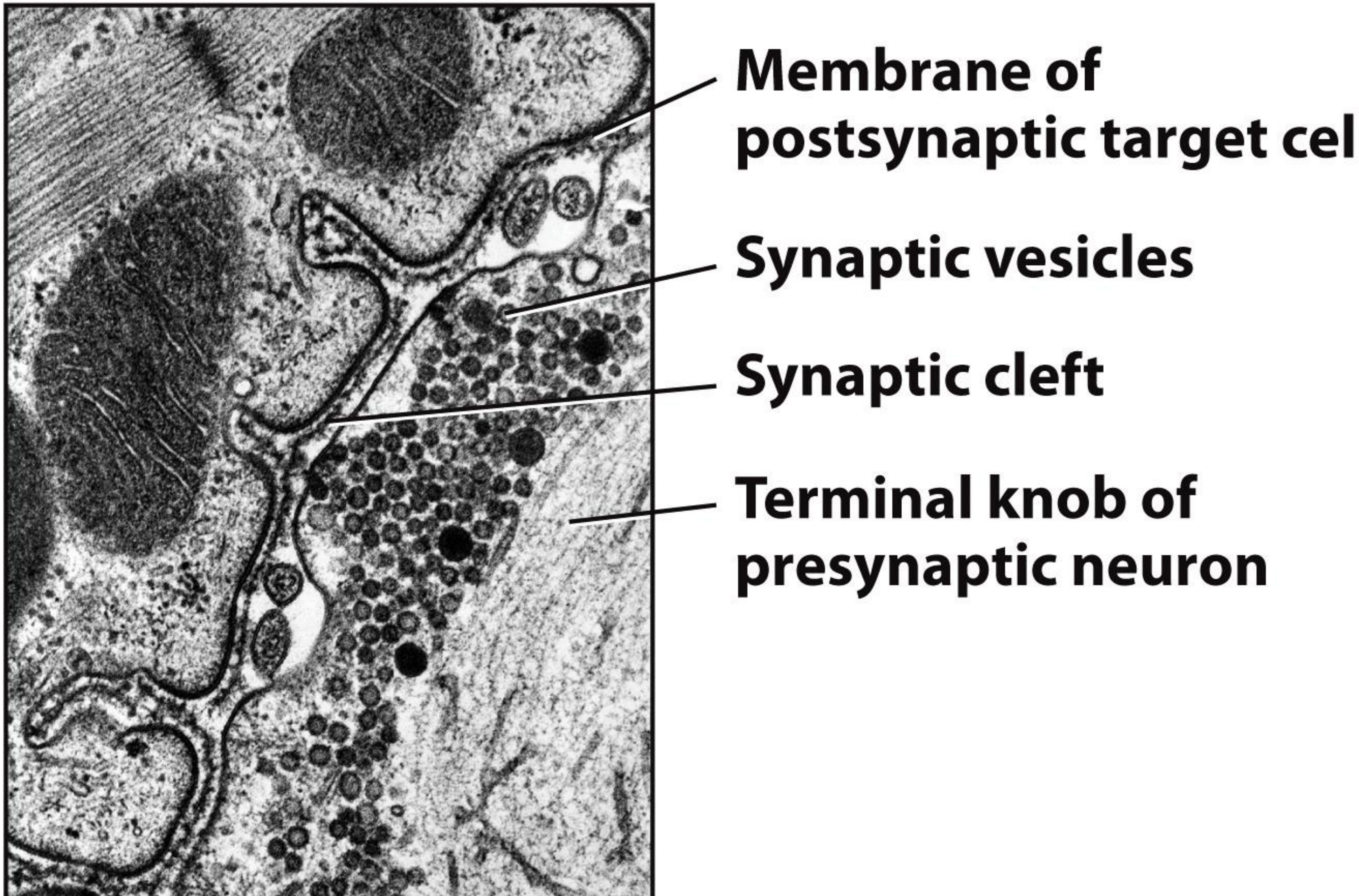

Next node
Na+
Current flow depolarizes next node of Ranvier
Direction of impulse
Axon
Na+
Node of Ranvier
Myelin sheath

Synaptic transmission

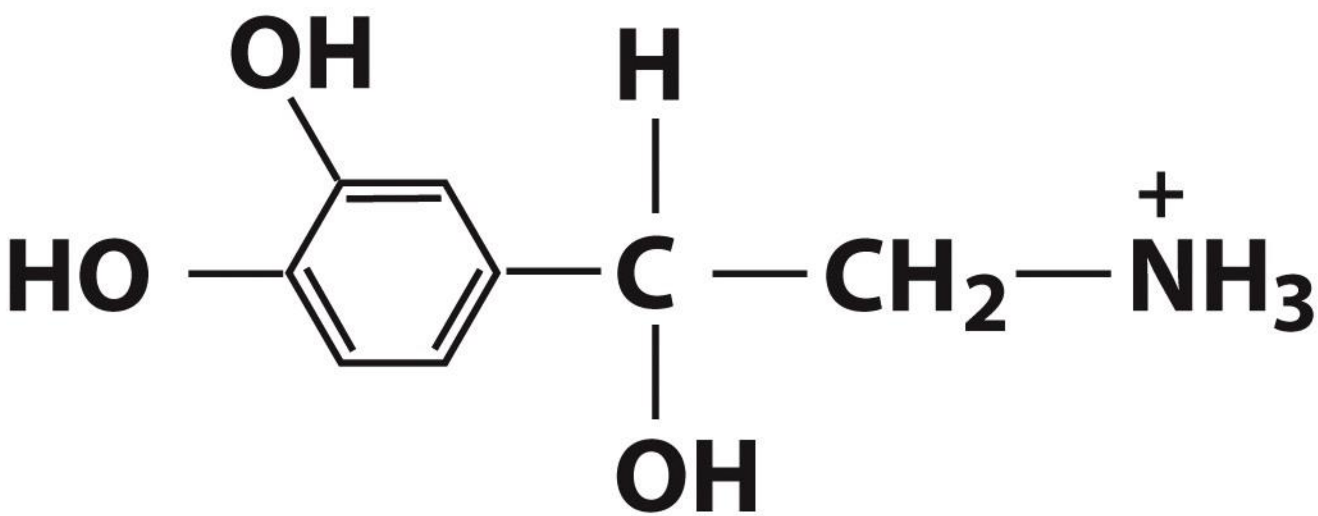



Axon of nerve cell
Muscle fiber



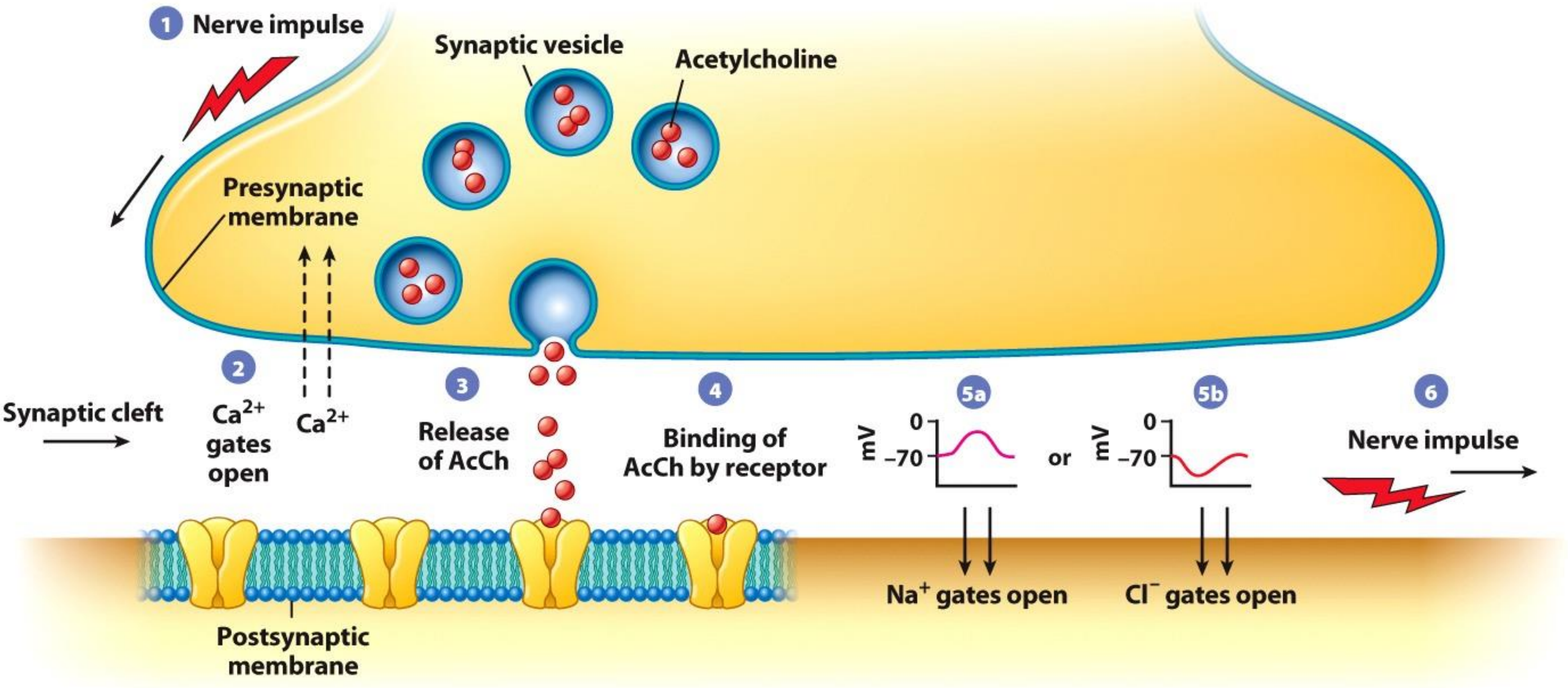

Membrane of postsynaptic target cell
Synaptic vesicles
Synaptic cleft
Terminal knob of presynaptic neuron

Acetylcholine (ACh)

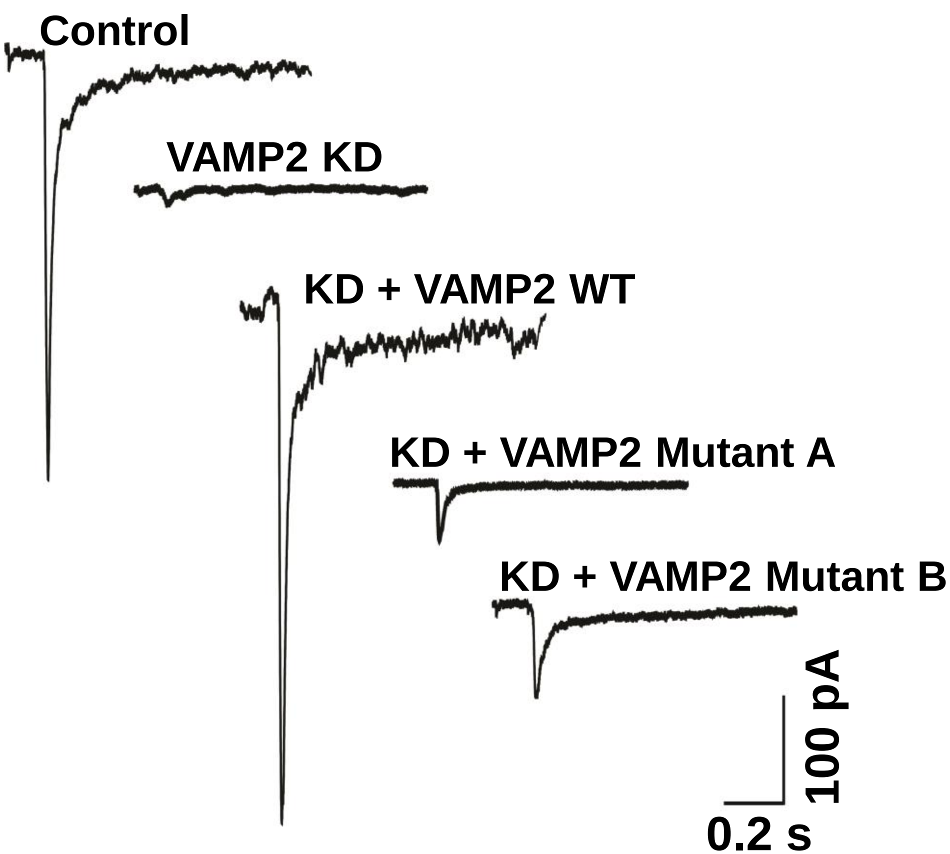


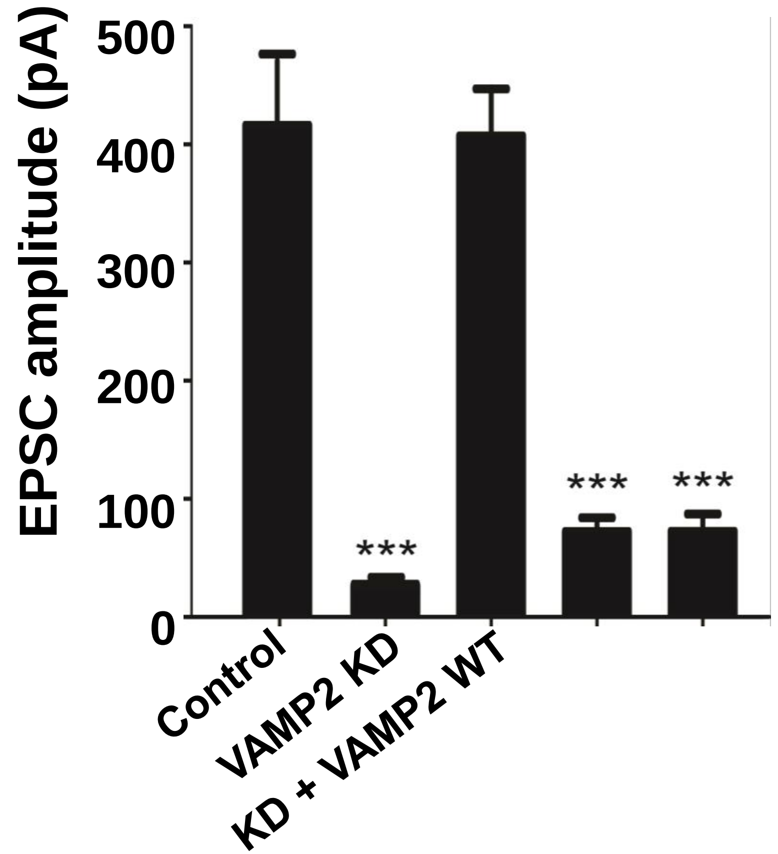
Norepinephrine

Synaptic transmission



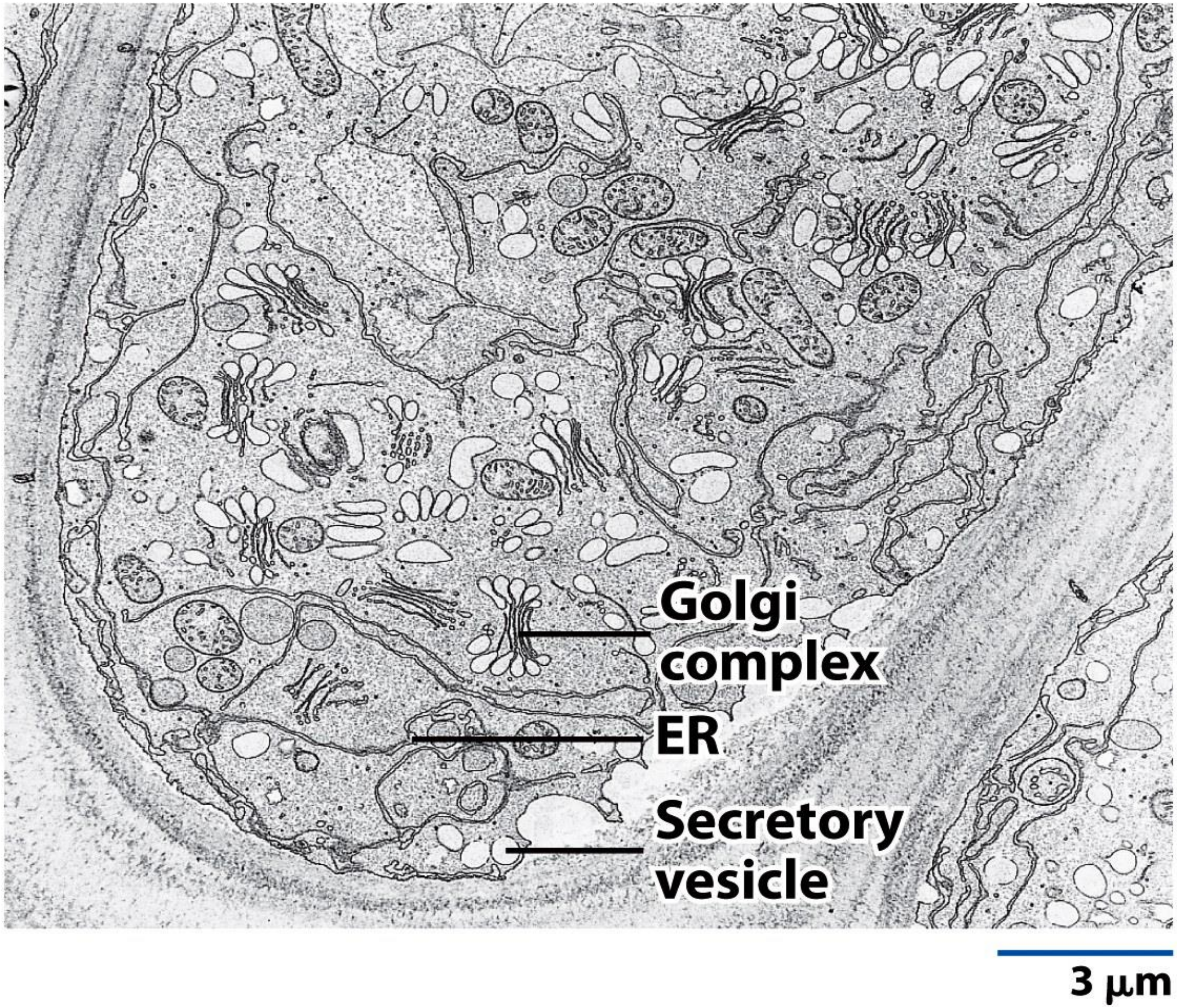
Postsynaptic responses





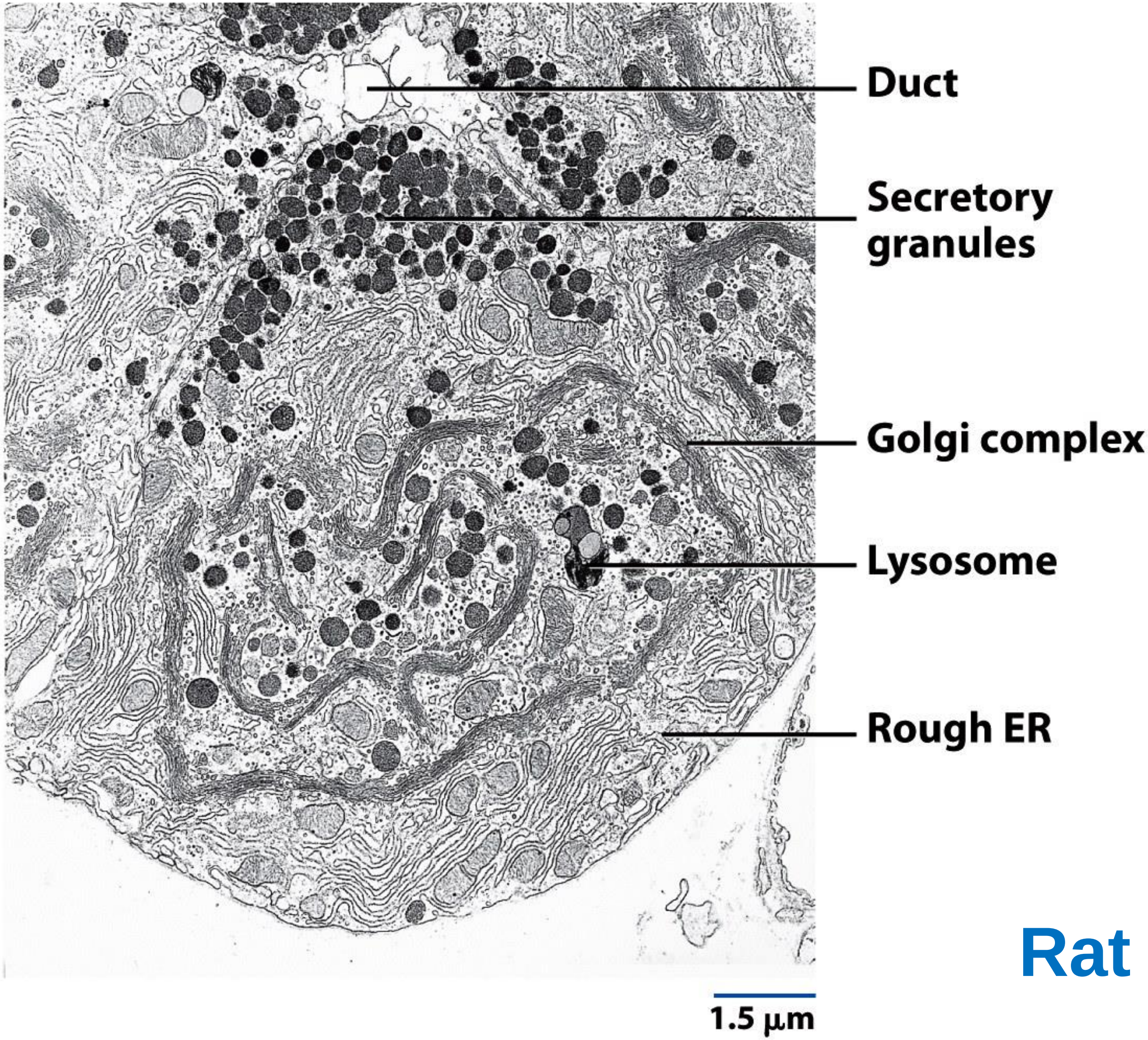
Shen lab

Cytoplasmic organelles



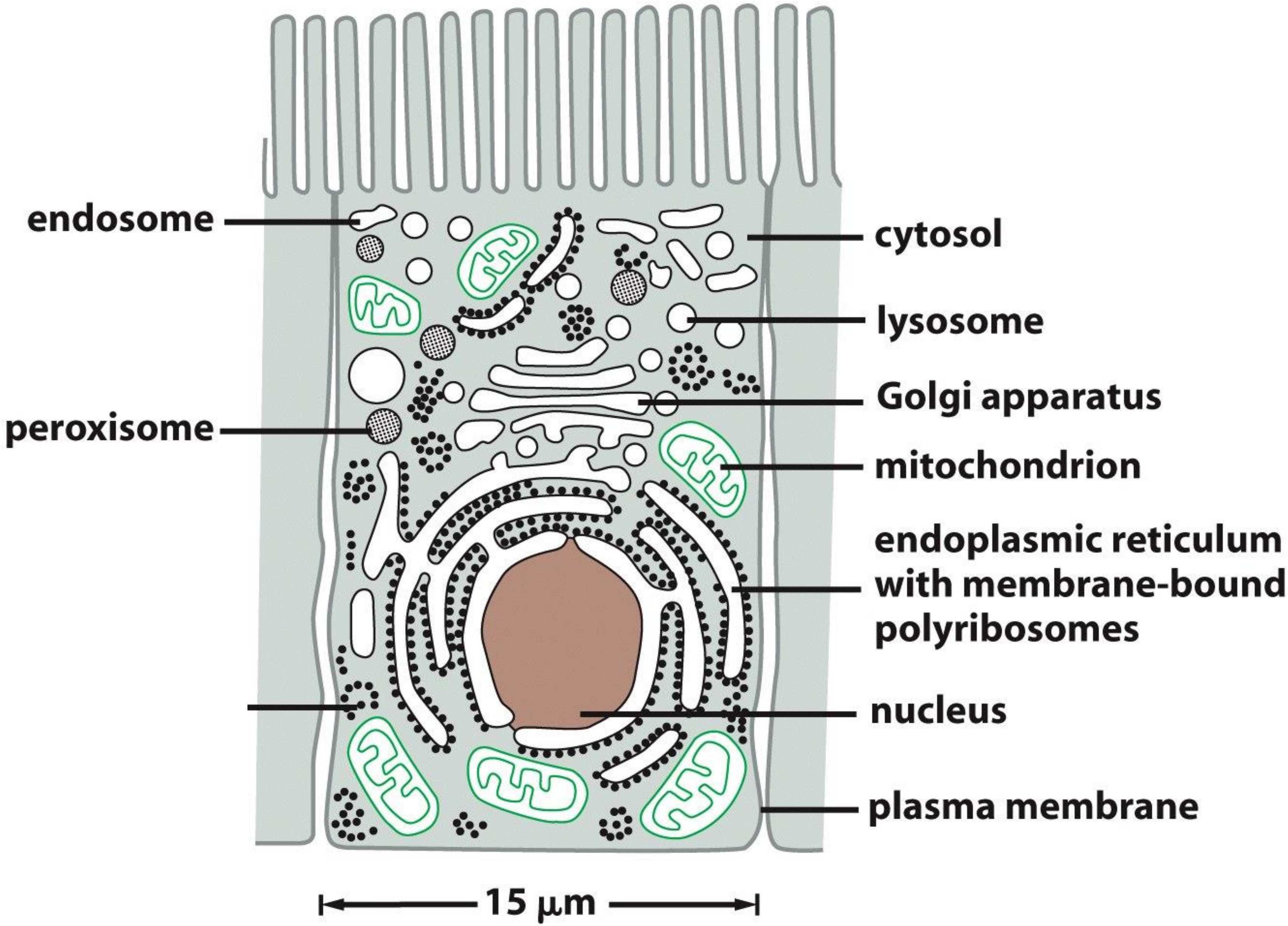
Plant root cell

~50% of the cell volume is in membrane-bound organelles



From Alain Rambourg and Yves Clermont, *Eur. J. Cell Biol.* 51:196, 1990

Rat goblet cell



Why does the cell need such diverse organelles?