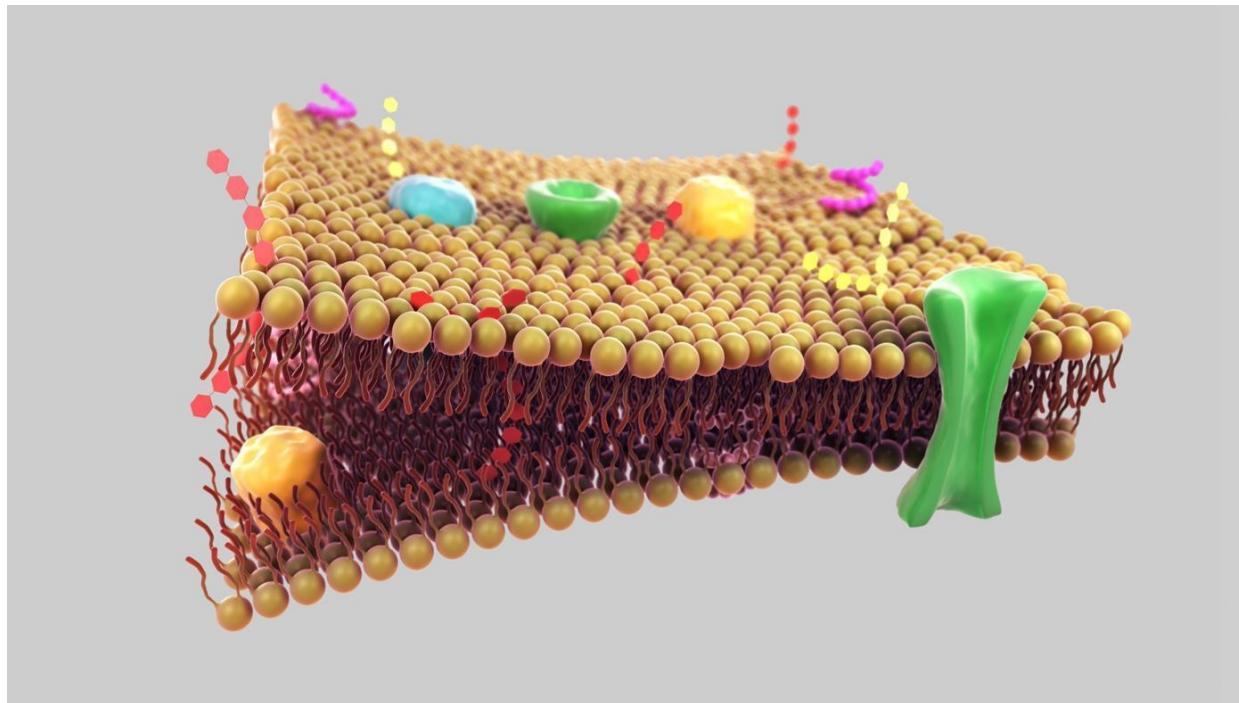


# Introduction to Molecular and Cellular Biology

## LECTURES 14-16:

### Cell membrane



# LECTURES 14-16: CELL MEMBRANE

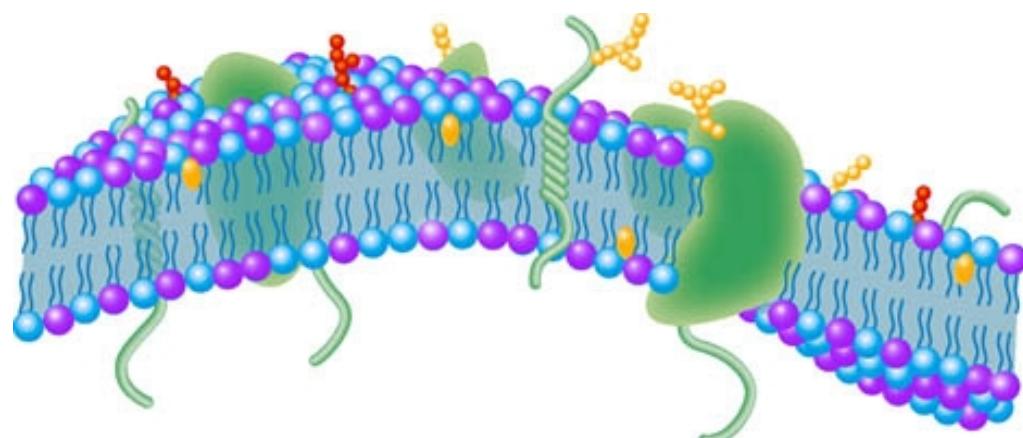
➤ **Membrane components:**

- lipids
- membrane proteins
- glycoproteins
- carbohydrates

➤ **Membrane recognition and penetration by toxins**

➤ **Transport through membrane:**

- principles
- transporters
- ion channels



# INTRODUCTION

Biological membrane separating cell interior from outside environment

➤ 5 nm ~ 50 atoms thick

➤ Functions:

- mechanical barrier structure

- selective permeability

- active transport

- vesicular transport

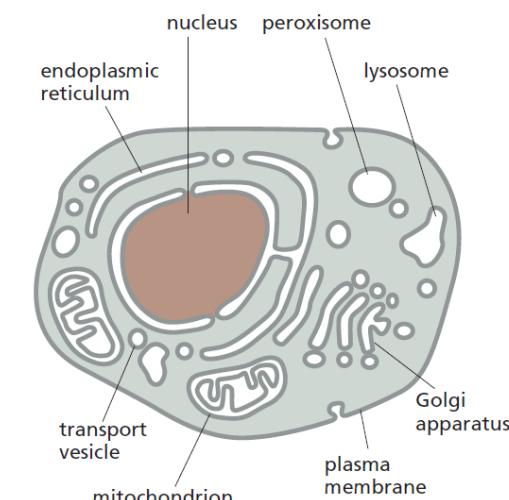
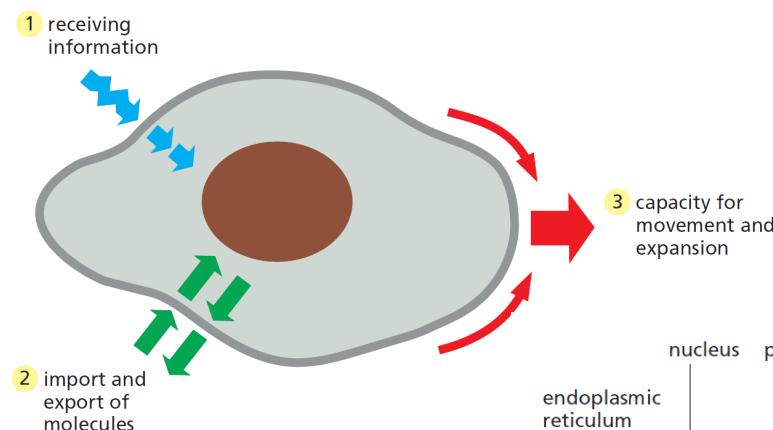
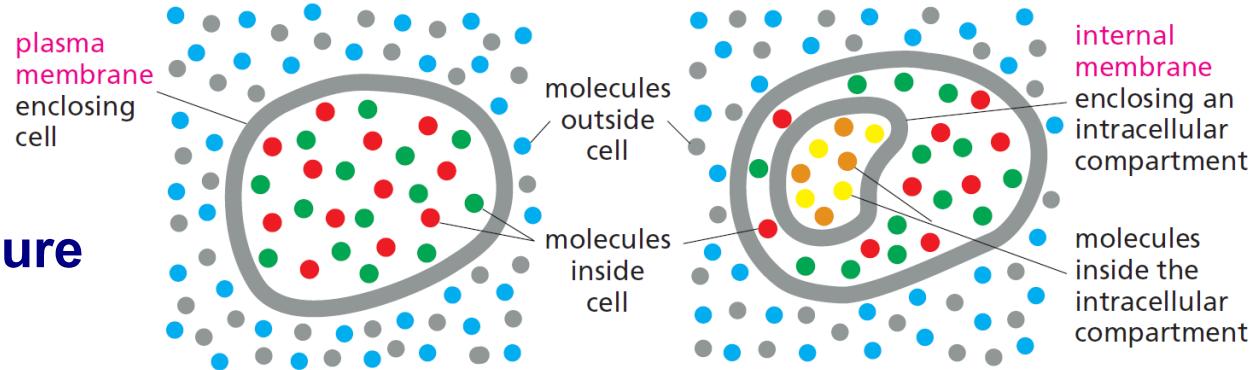
- cell communication

- metabolic activity

➤ Eukaryotes: many membranes

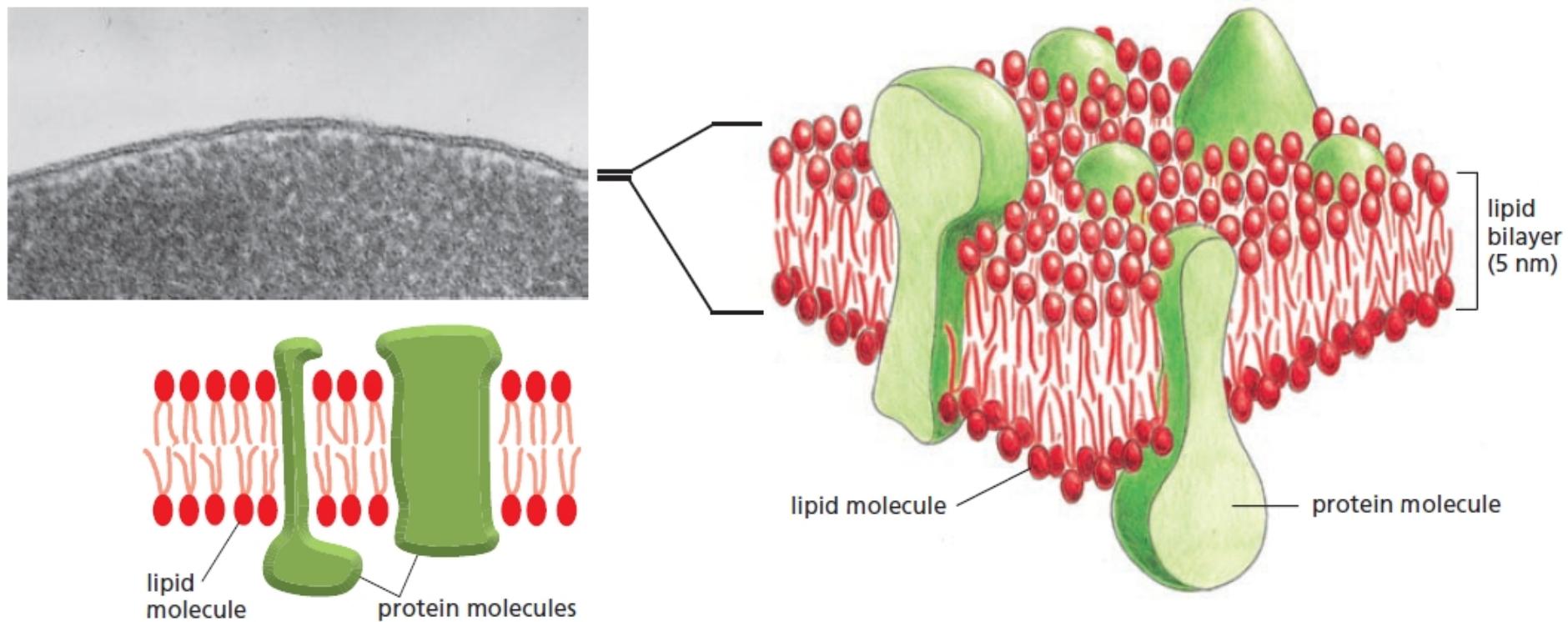
➤ Prokaryotes: one membrane

➤ Membrane adapts to the size of the cell



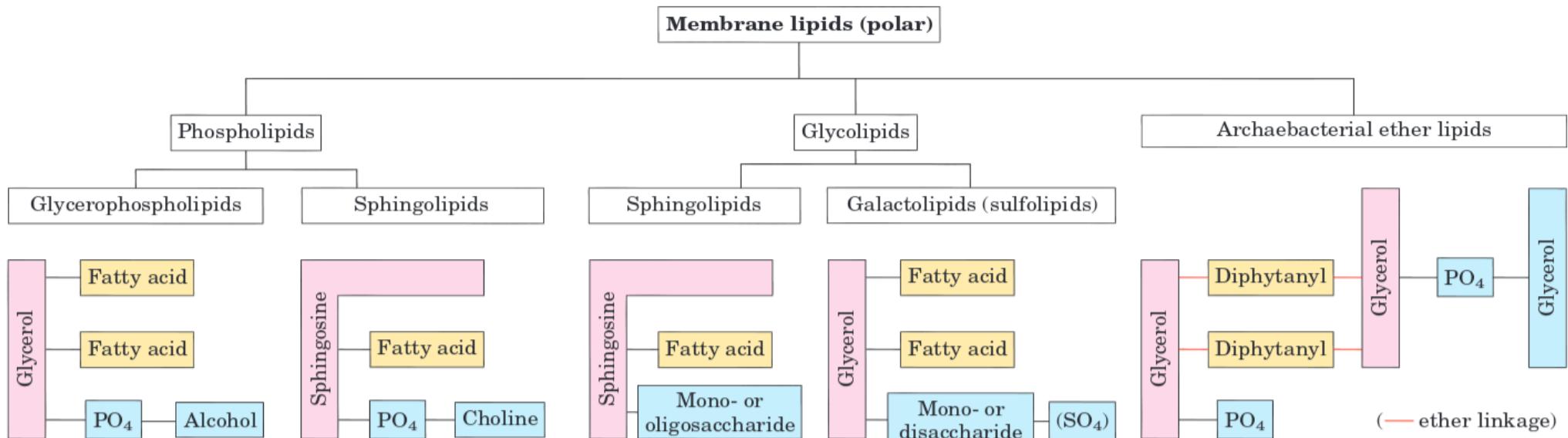
# LIPID BILAYER AND MEMBRANE COMPOSITION

**Lipid bilayer: thin polar membrane made of two layers of lipid molecules**



- **Lipids (phospholipids, glycolipids, sterols)**
- **Proteins**
- **Glycoproteins**
- **Carbohydrates**

# MEMBRANE LIPIDS

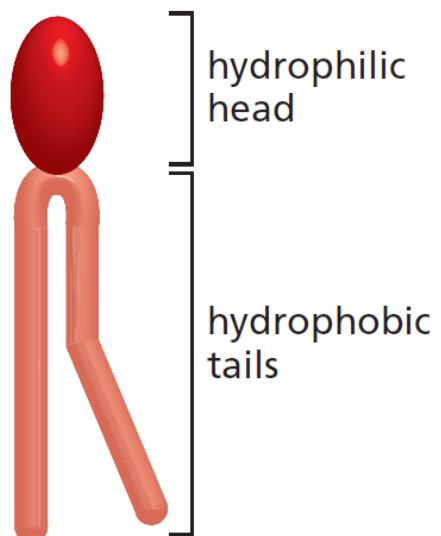


## ➤ Tails:

- Fatty acids
- P<sub>i</sub>, alcohol, cholin, oligosaccharides, glycerol

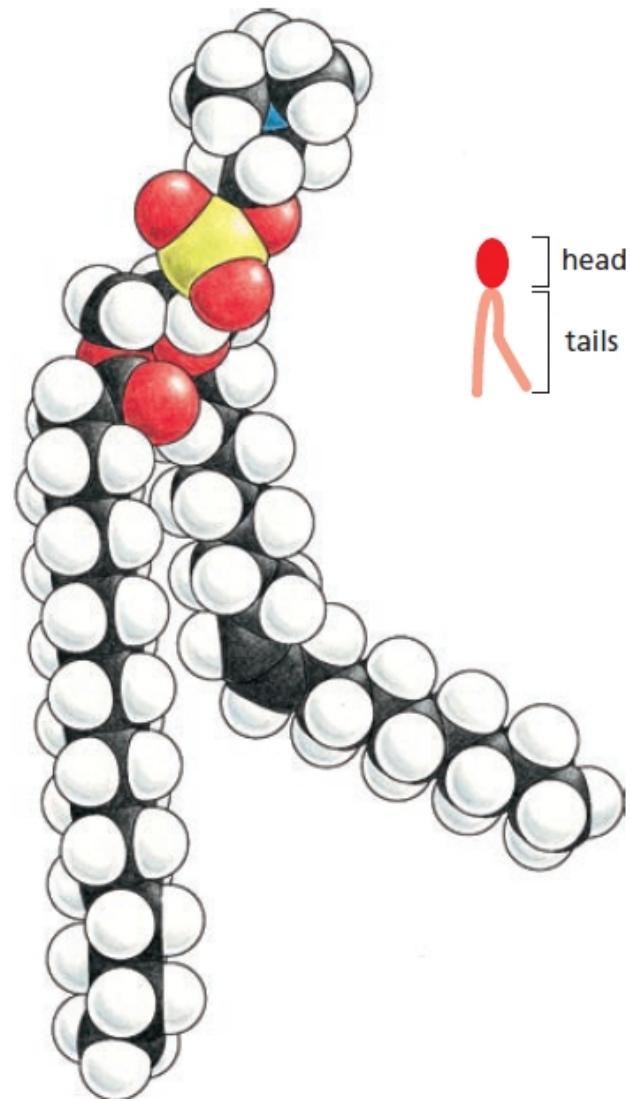
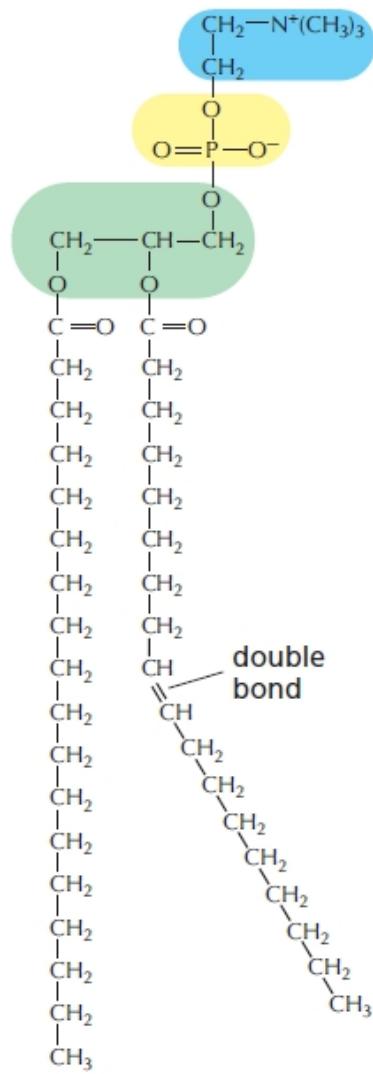
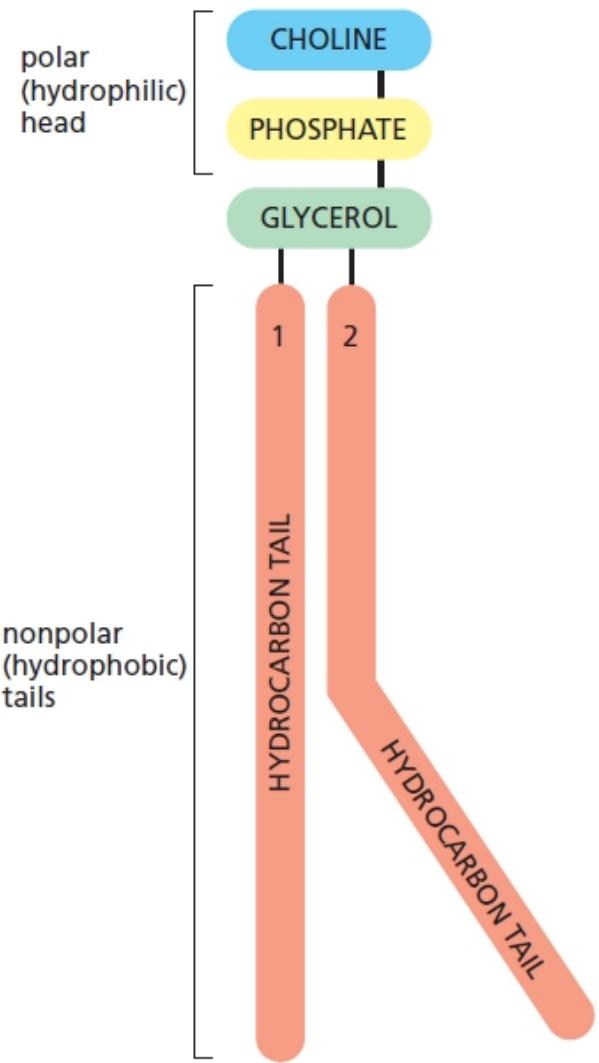
## ➤ Heads

- Glycerol, sphingosine



# PHOSPHOTIDYLCHOLINE

## Most common lipid in the cell membrane

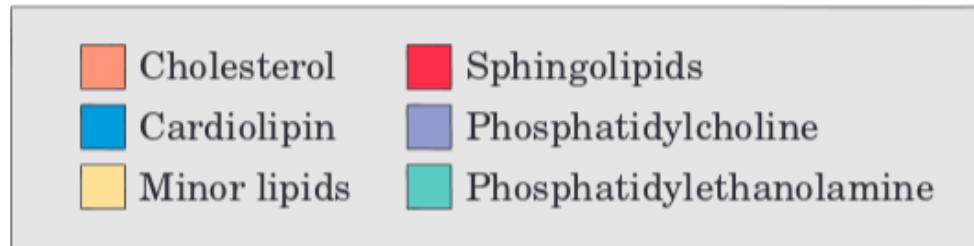
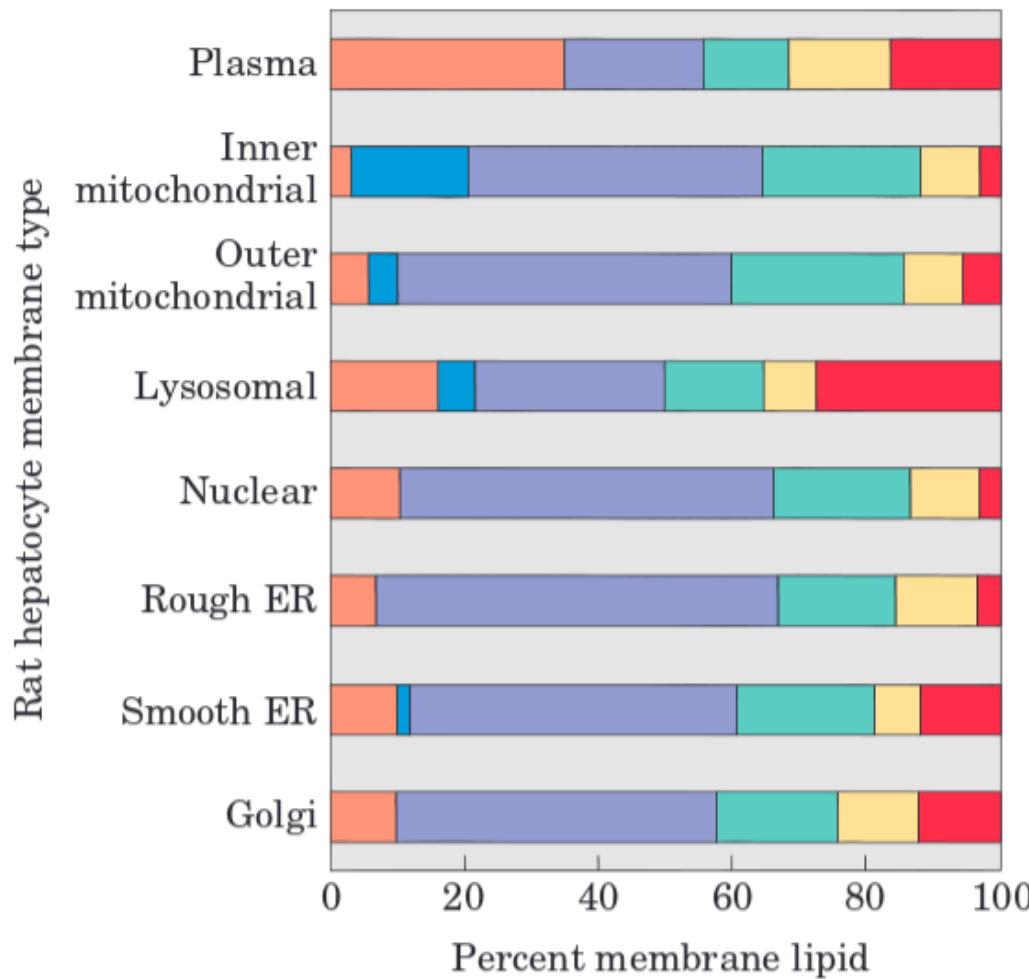


# DIVERSITY OF MEMBRANES

Different membranes in the cell are very diverse in their composition

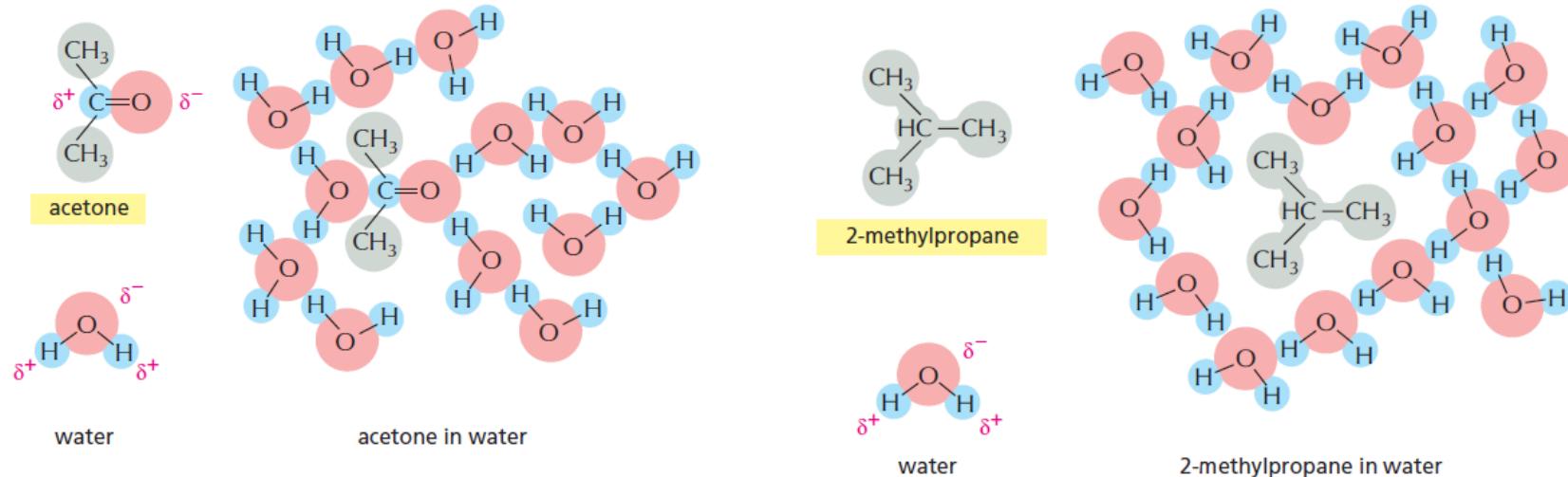
LIPID	PERCENTAGE OF TOTAL LIPID BY WEIGHT					
	LIVER CELL PLASMA MEMBRANE	RED BLOOD CELL PLASMA MEMBRANE	MYELIN	MITOCHONDRION (INNER AND OUTER MEMBRANES)	ENDOPLASMIC RETICULUM	E. COLI BACTERIUM
Cholesterol	17	23	22	3	6	0
Phosphatidylethanolamine	7	18	15	25	17	70
Phosphatidylserine	4	7	9	2	5	trace
Phosphatidylcholine	24	17	10	39	40	0
Sphingomyelin	19	18	8	0	5	0
Glycolipids	7	3	28	trace	trace	0
Others	22	13	8	21	27	30

# DIVERSITY OF MEMBRANES

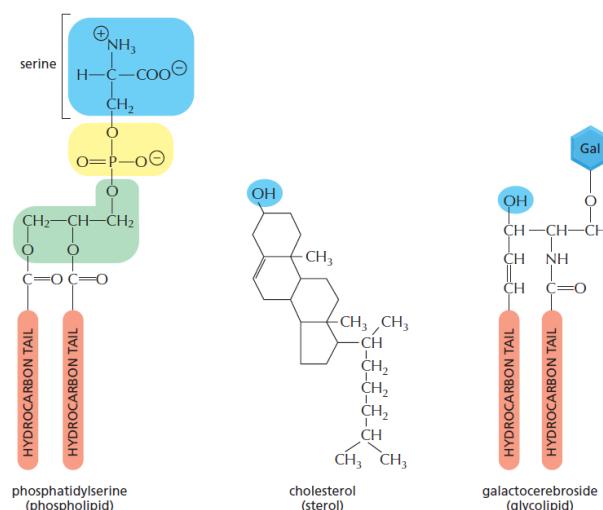


# AMPHIPATHICITY OF MEMBRANE LIPIDS

Combination of hydrophobic and hydrophilic properties  
in the same molecules

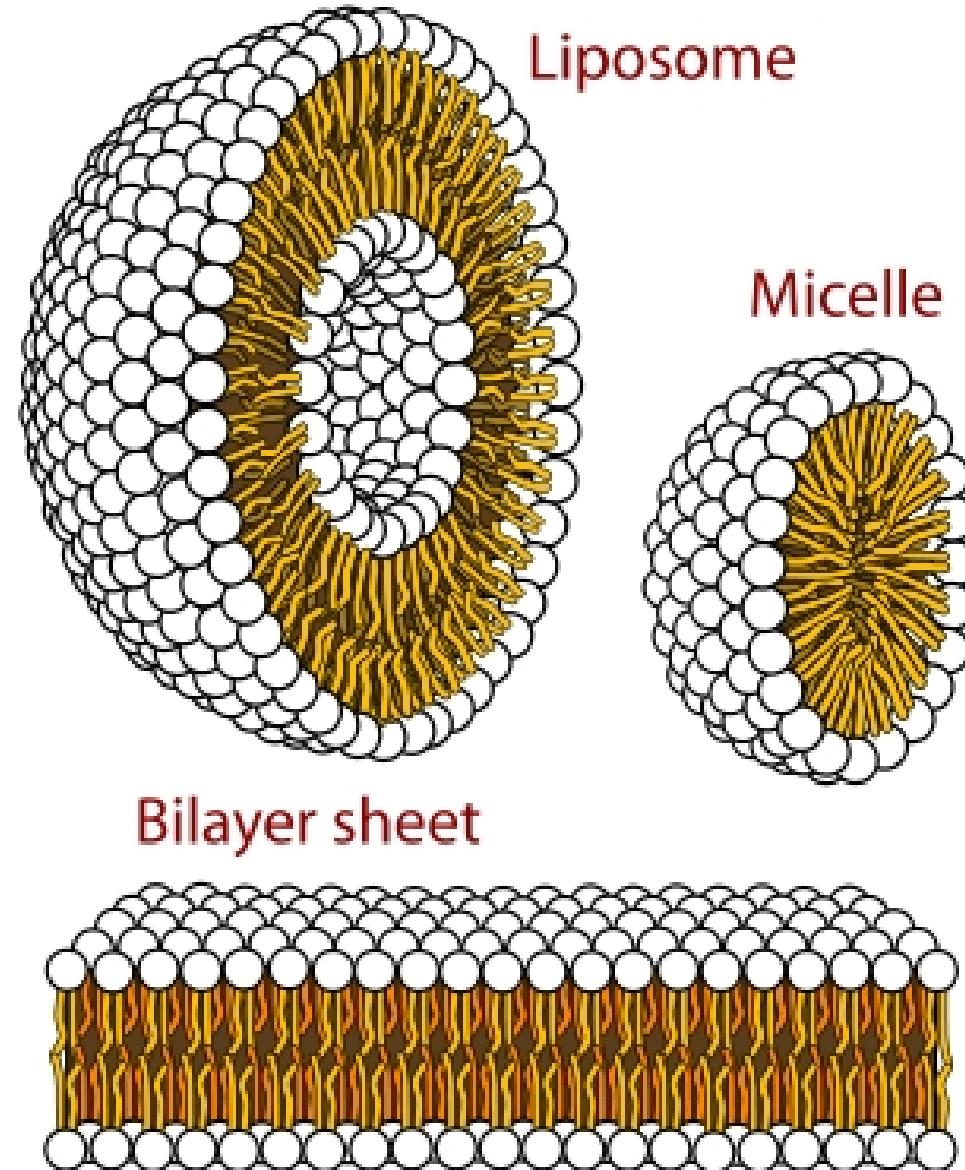


Membrane lipids are amphipathic

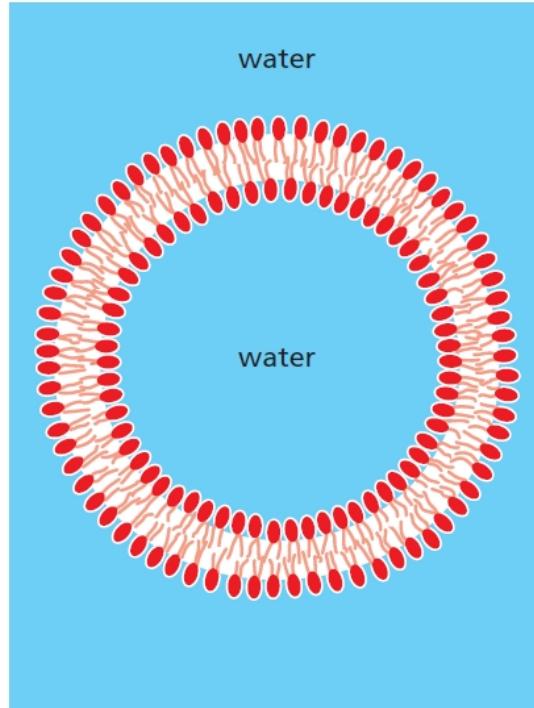
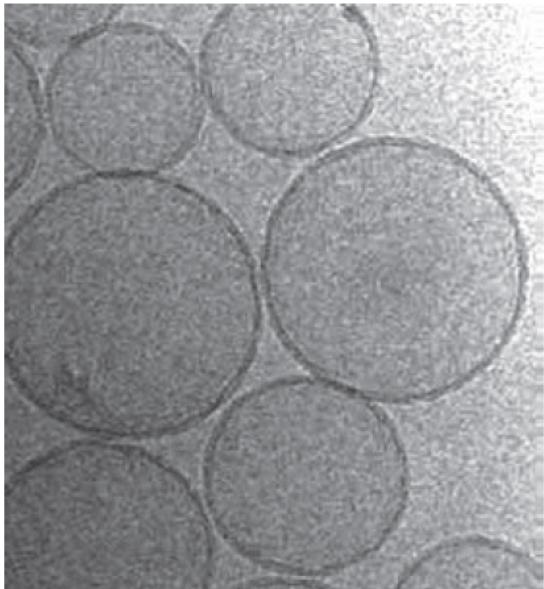


# STRUCTURES OF AMPHIPATHIC MOLECULES

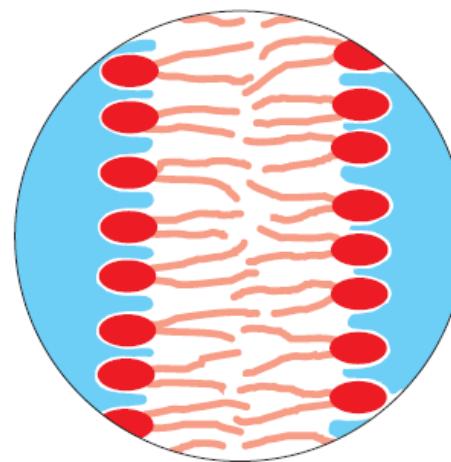
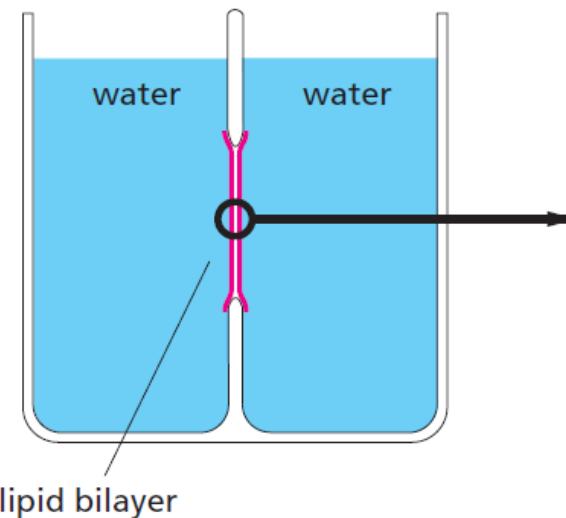
- Micelles
- Bilayers:
  - Liposomes 25 nm-1mm
  - Flat bilayers



# STRUCTURES OF AMPHIPATHIC MOLECULES



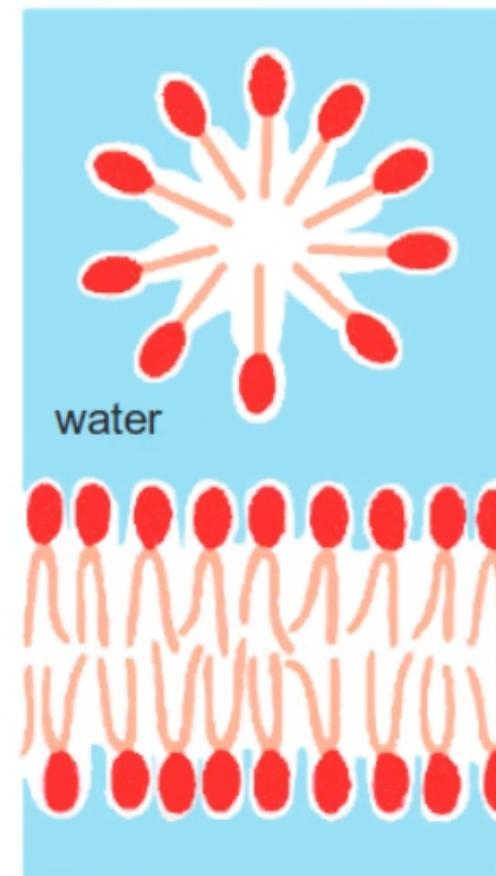
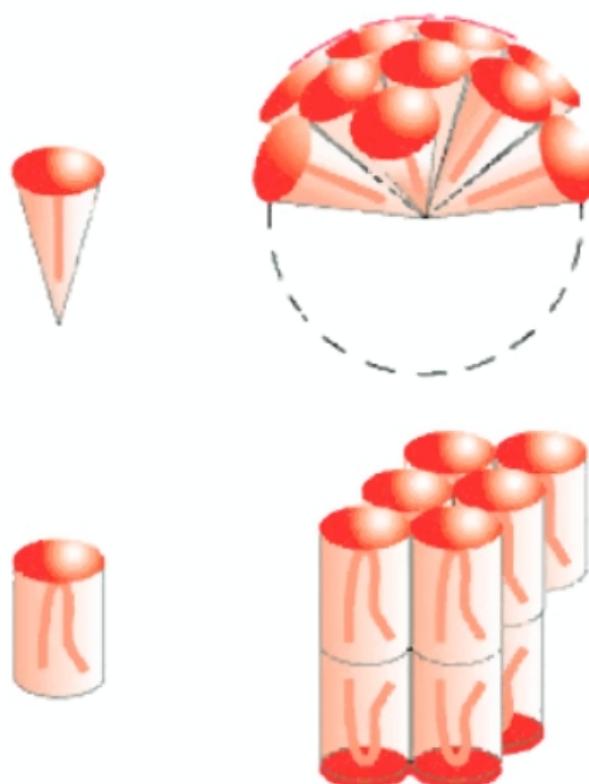
Liposomes



Flat lipid bilayer

# STRUCTURES OF AMPHIPATHIC MOLECULES: LIPIDS GEOMETRY AND PACKING

Molecular shape      Molecular packing



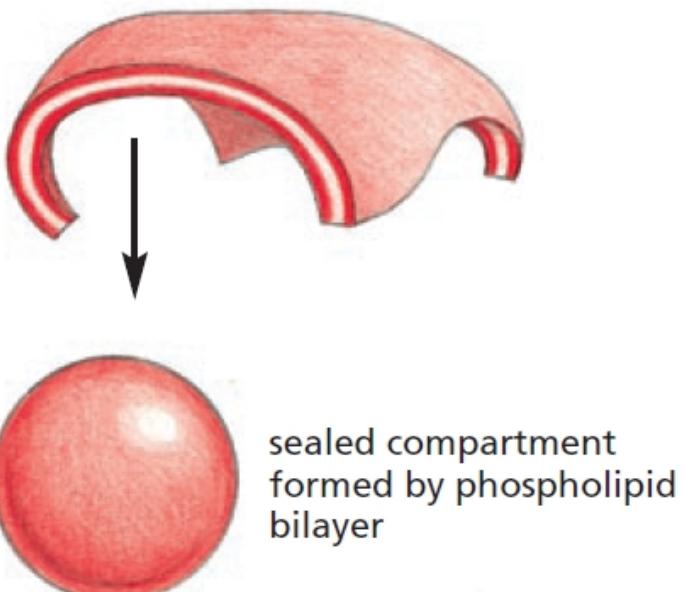
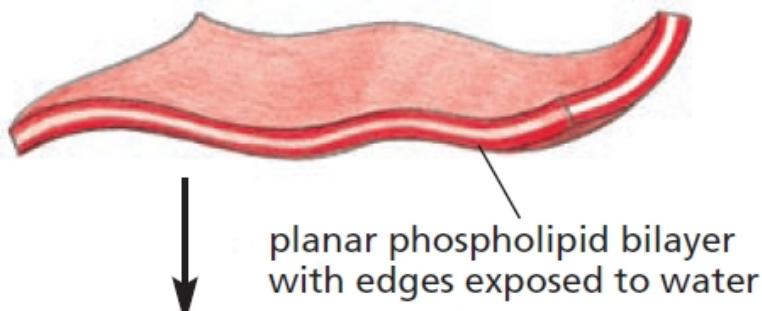
lipid  
micelle

lipid  
bilayer

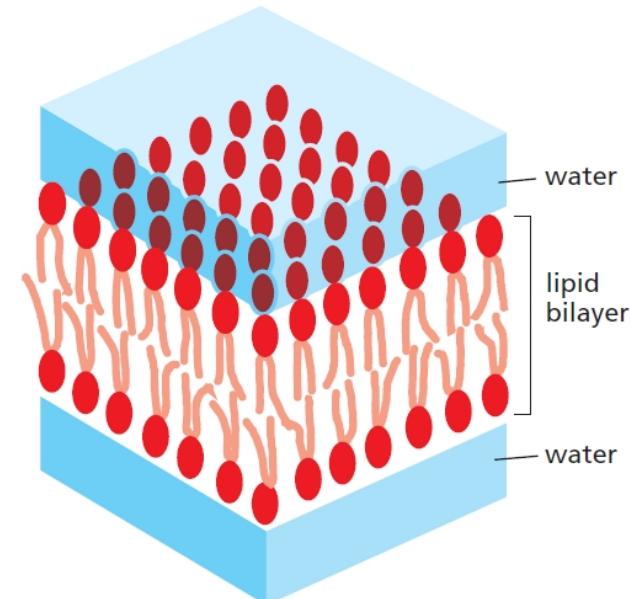
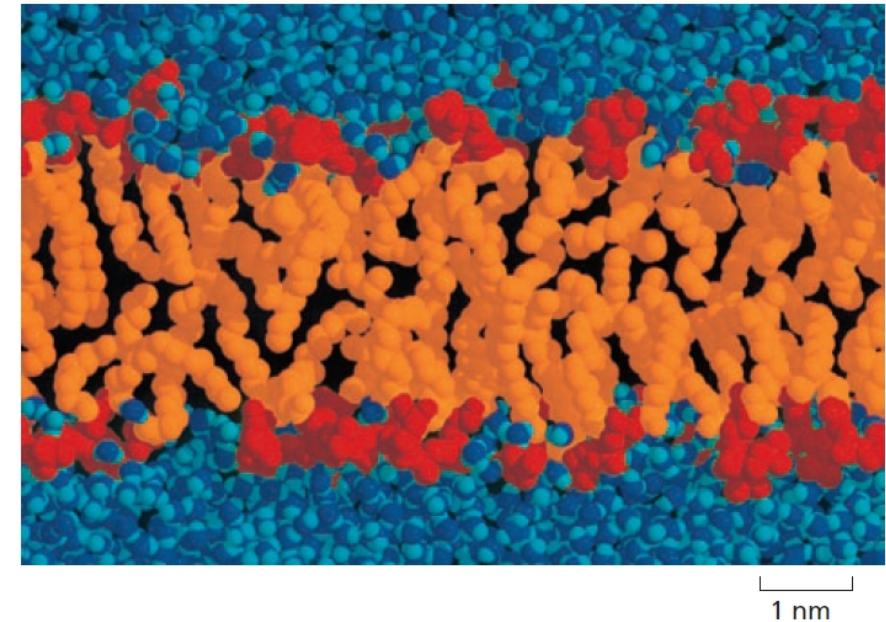
# BILAYERS ARE FORMED SPONTANEOUSLY

Exposure of the edges is energetically unfavourable

ENERGETICALLY UNFAVORABLE



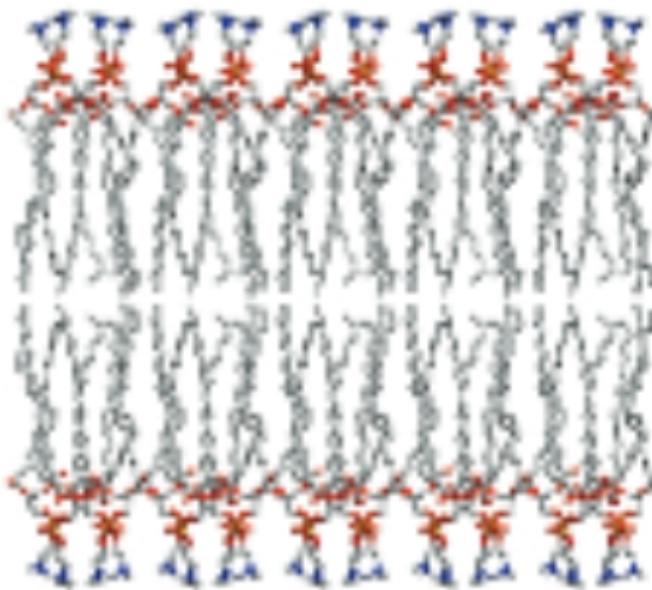
ENERGETICALLY FAVORABLE



# LIPIDS PHASES IN THE BILAYER

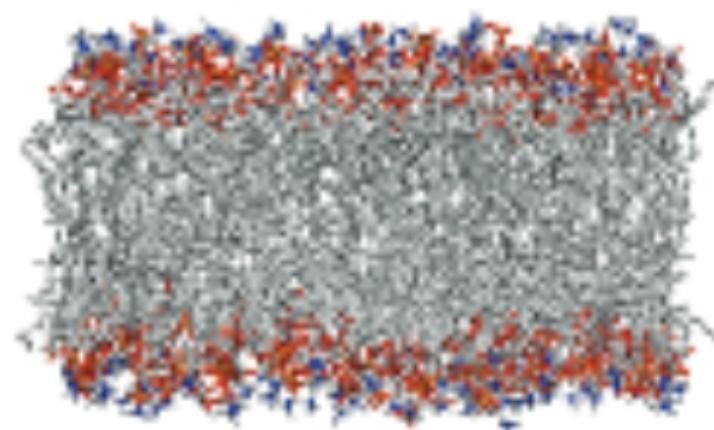
Paracrystalline state (gel)

- Gel phase
- Liquid disordered phase
- Liquid ordered phase



Heat produces thermal motion of side chains  
(gel → fluid transition)

Fluid state



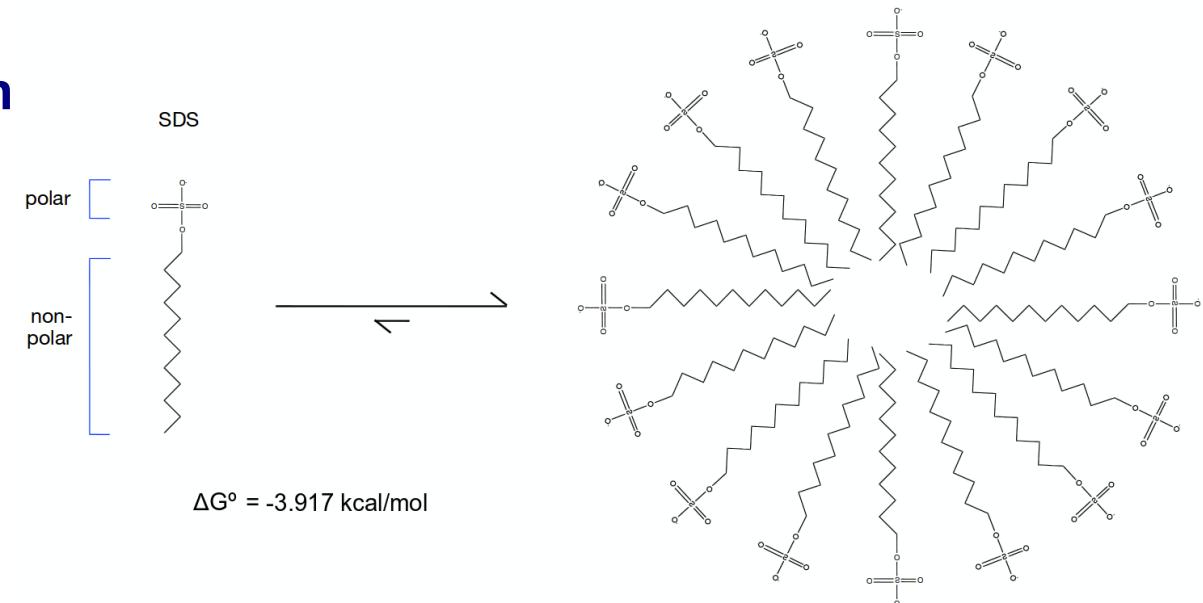
# DETERGENT

Detergent/surfactant: lowers the surface tension

➤ **Critical micelle concentration**

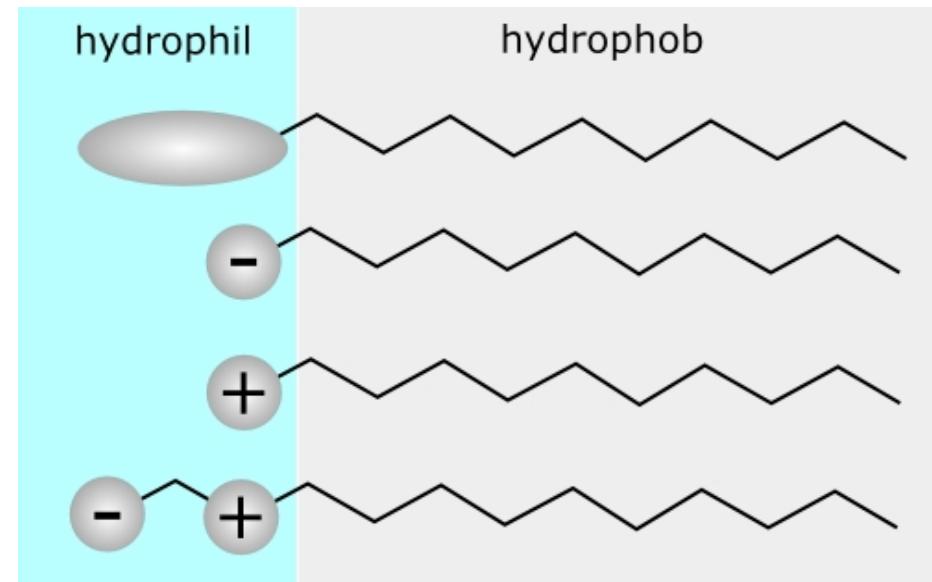
➤ **Action:**

- disassembly of bilayers
- unfolding of proteins
- extraction of TM-proteins

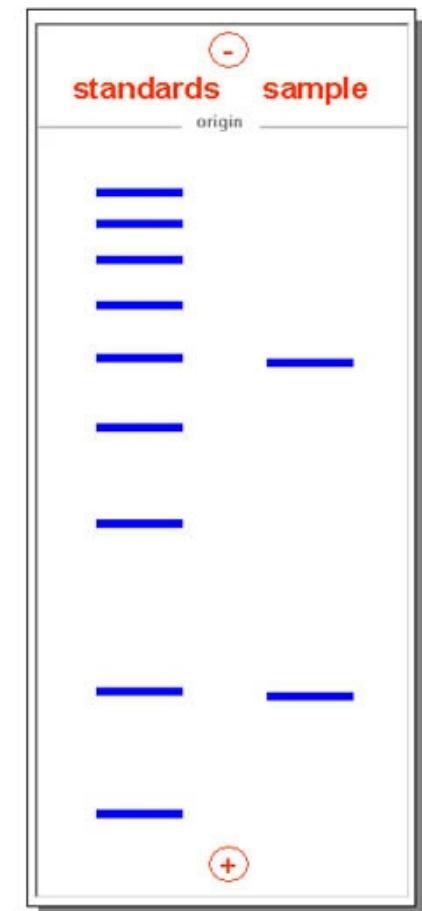
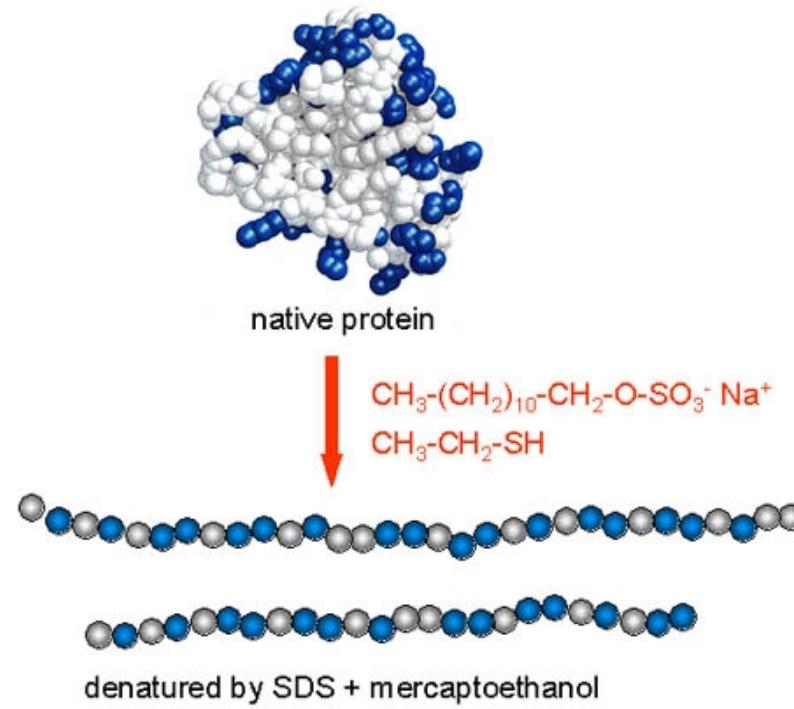
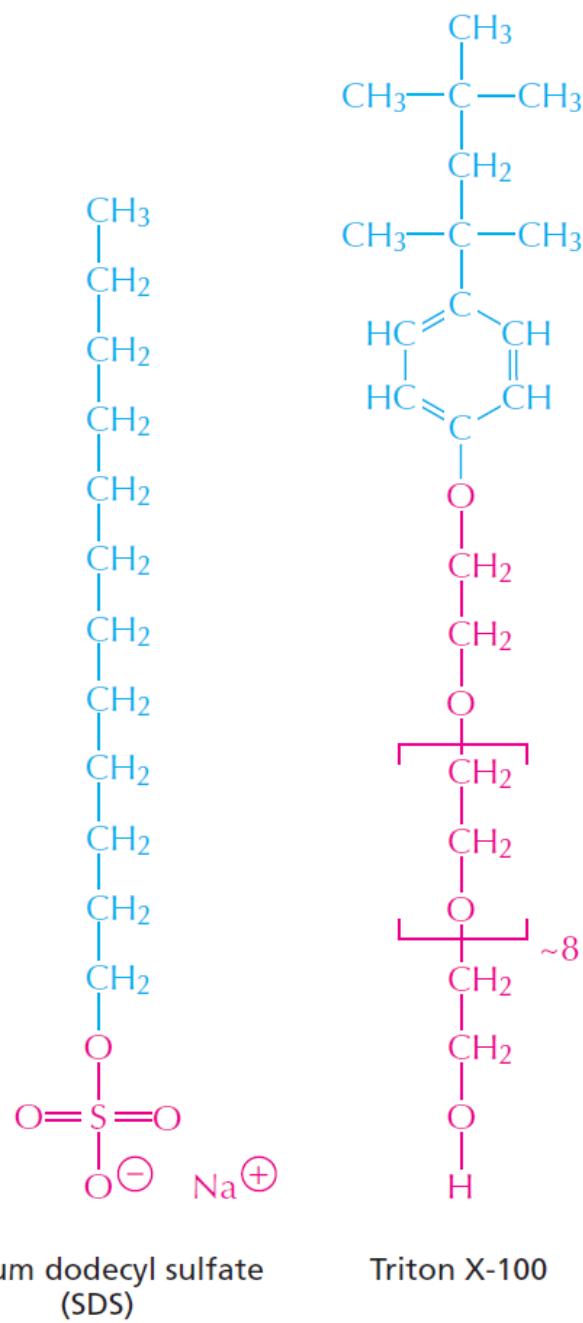


➤ **Classes:**

- non-ionic
- anionic
- cationic
- amphoteric



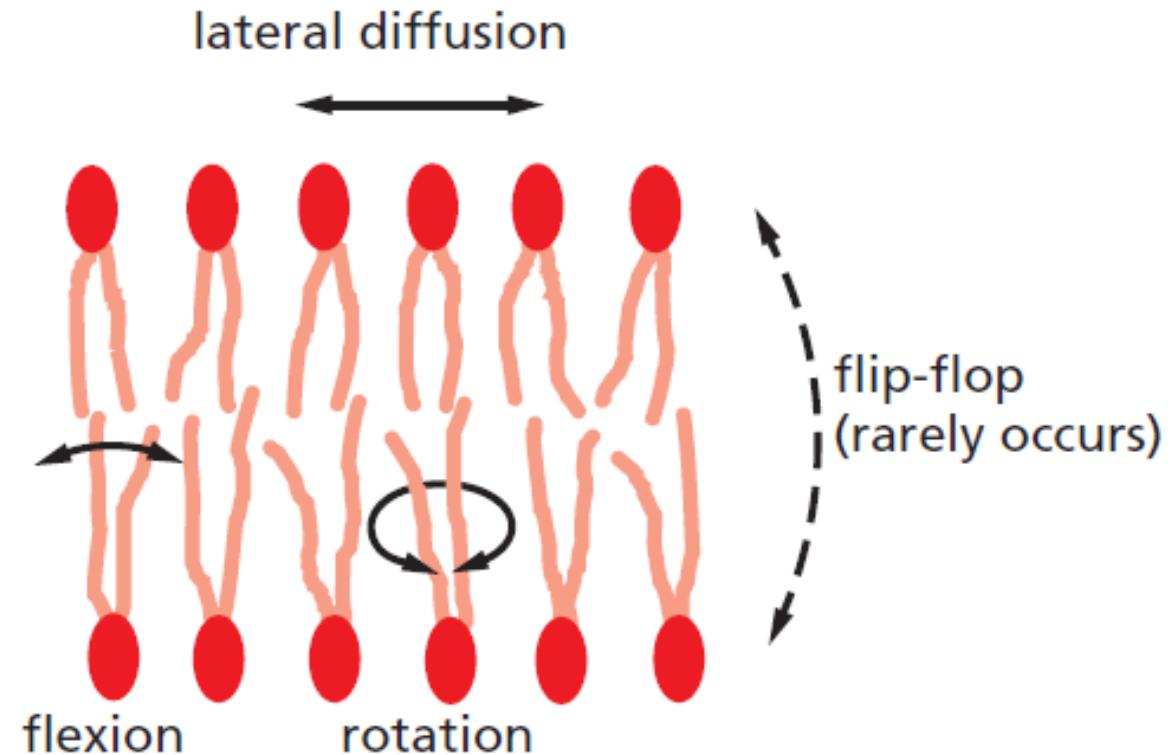
# DETERGENTS



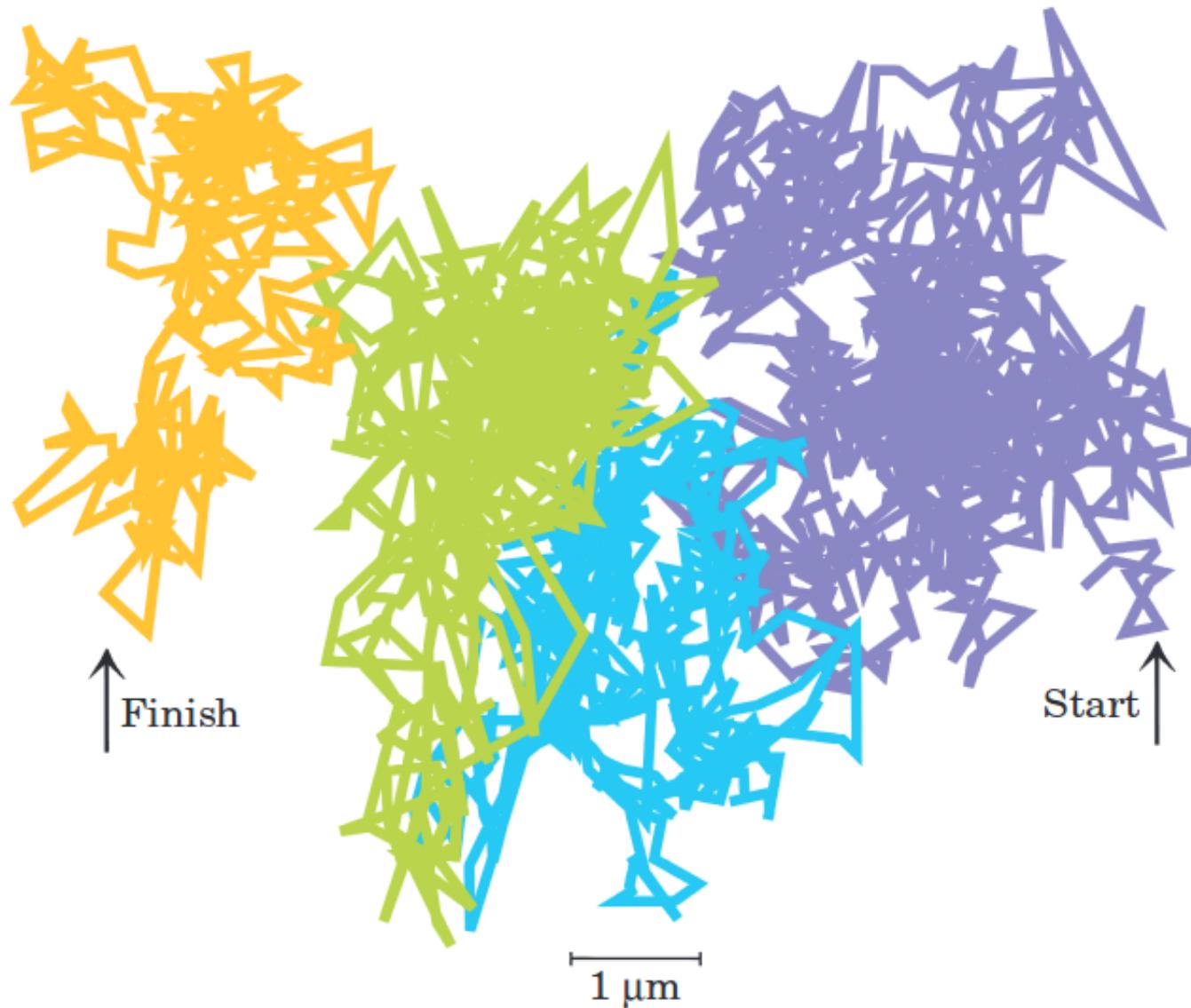
## SDS-electrophoresis

# MOVEMENTS IN THE BILAYER

- Flexibility (ability to bend)
- Fluidity (diffusion, f.i. 2 µm/s)
- Rotation (~ $10^4$  per minute)
- Flexion
- Flip-flops



# MOVEMENTS IN THE BILAYER

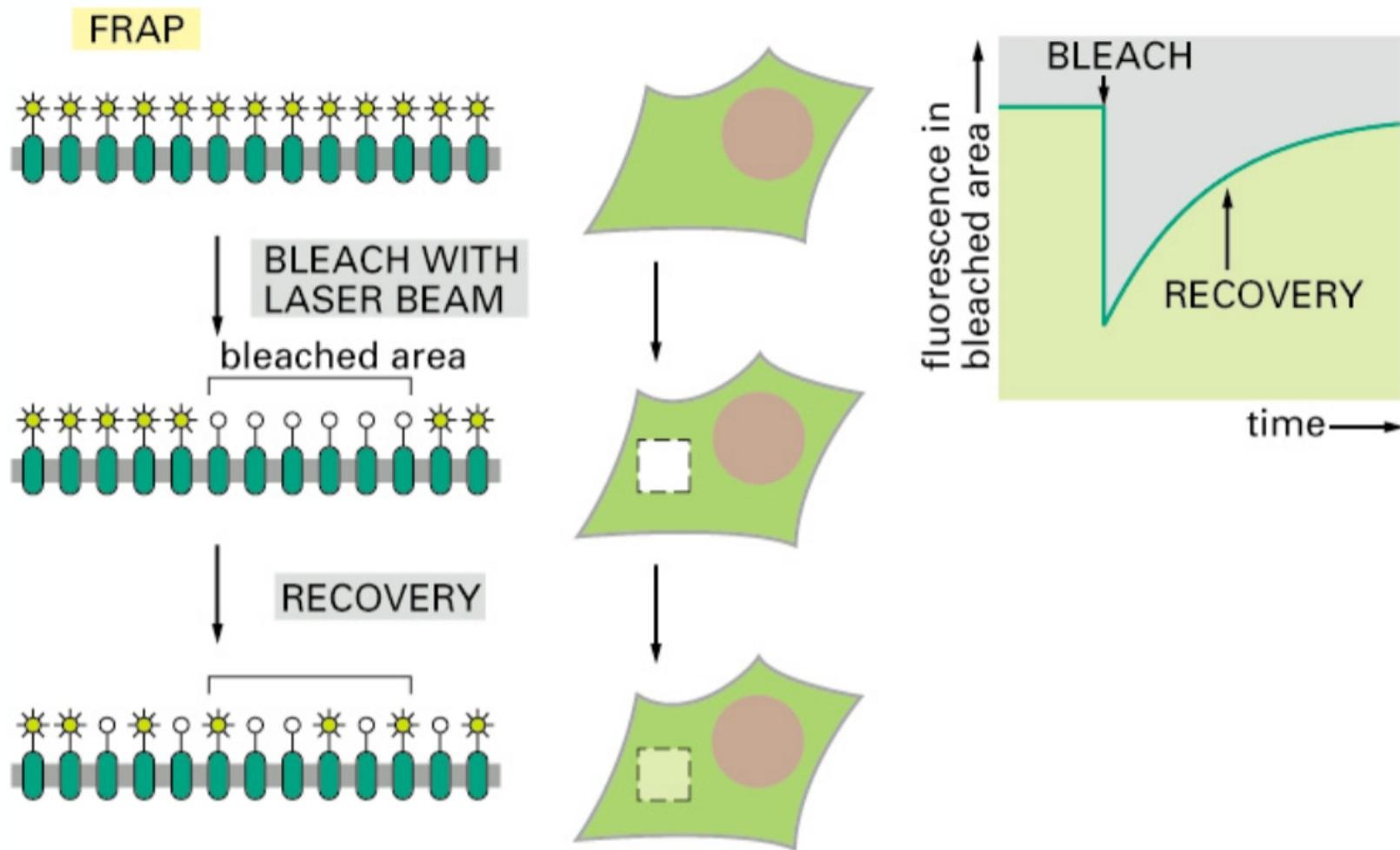


$t = 56 \text{ ms}$

# MOVEMENTS IN THE BILAYER: DESCRIBING FLUIDITY PROPERTIES

FRAP: fluorescence recovery after photobleaching

Diffusion coefficient:  $D = r^2/4t_D$



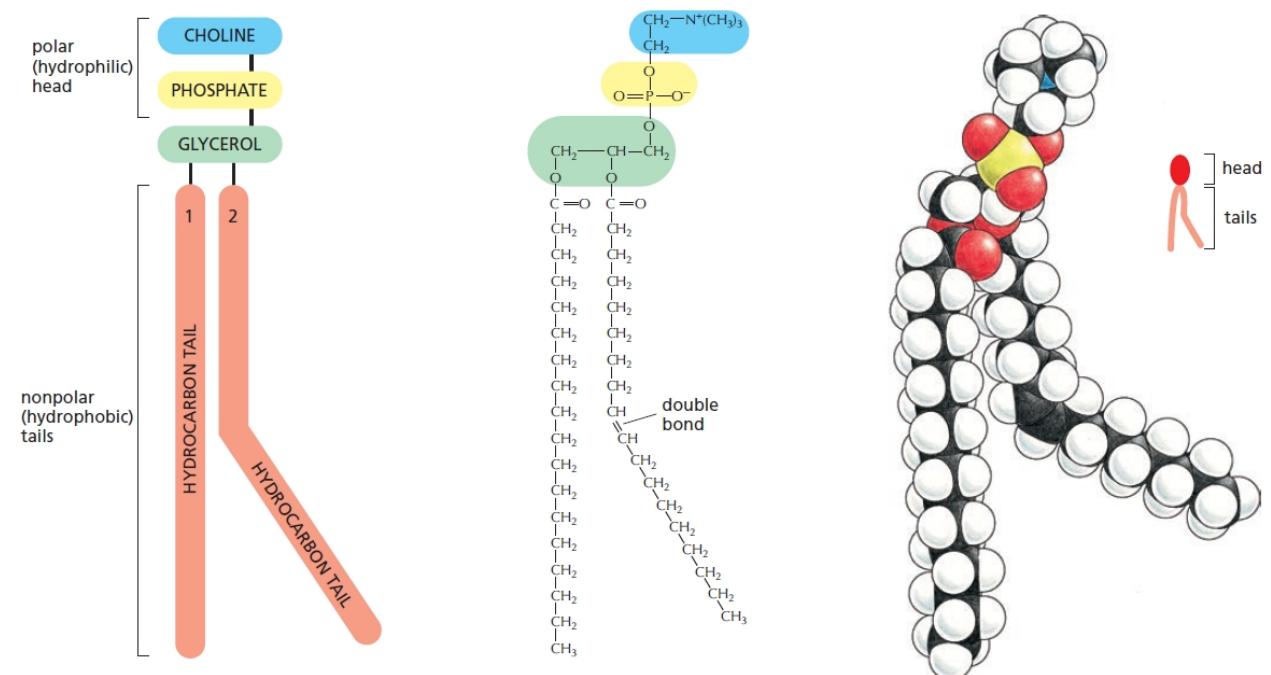
# MOVEMENTS IN THE BILAYER: LIPIDS COMPOSITION

## ➤ Packing of lipids:

- length of hydrophobic tails (14-24 C atoms, most 18-20)
- number of double bonds (saturated/unsaturated)

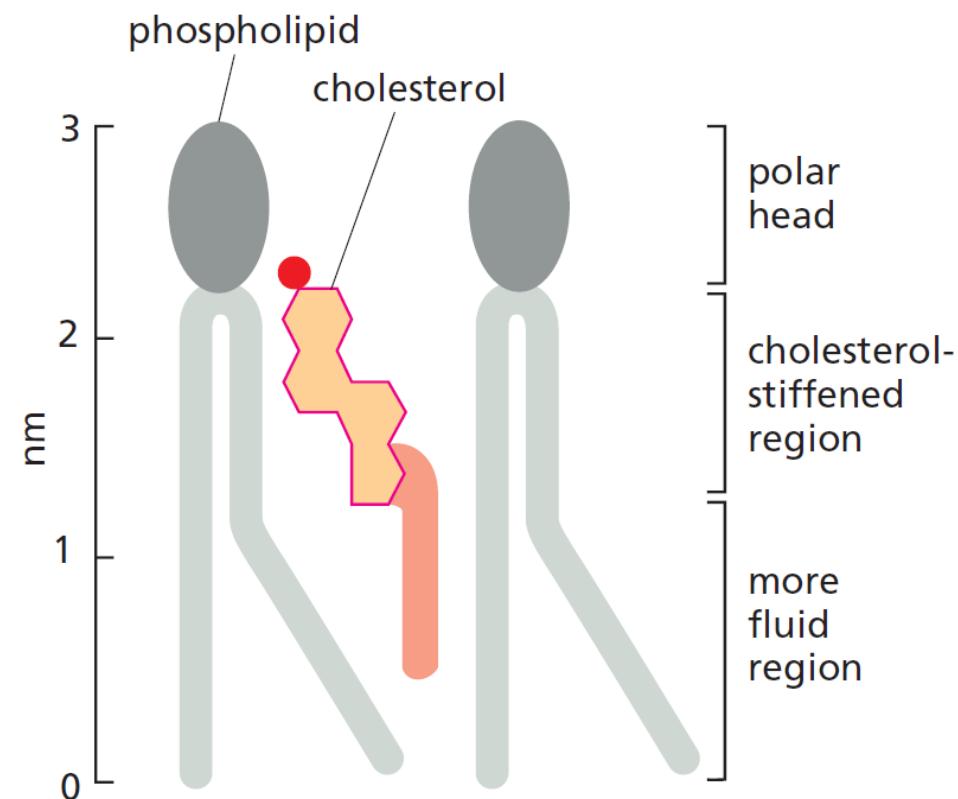
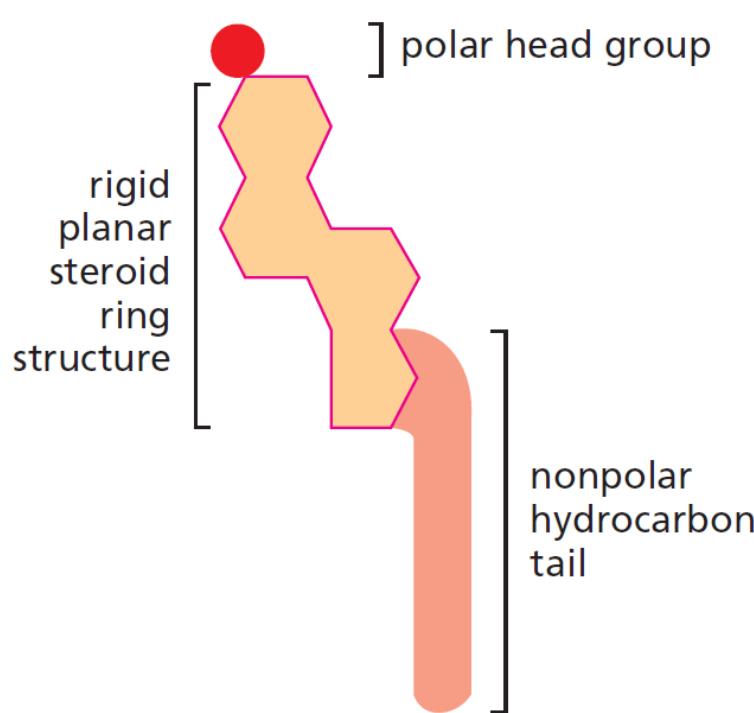
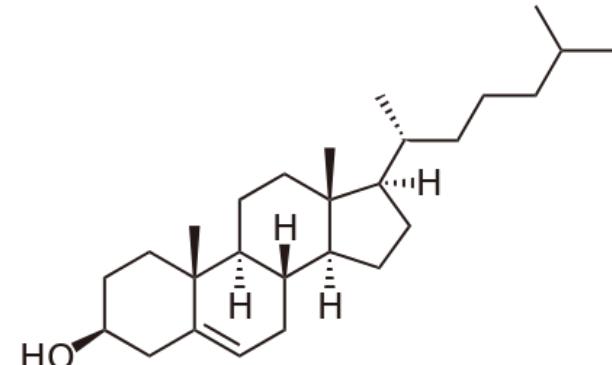
## ➤ Adaptation to the temperature

## ➤ Cholesterol (~20% of total membranes)



# CHOLESTEROL IN THE LIPID BILAYER

- Fluidity
- Reduced permeability
- Steroid hormones/bile acids
- Antioxidation
- Cell signalling, nerve conduction, intracellular transport



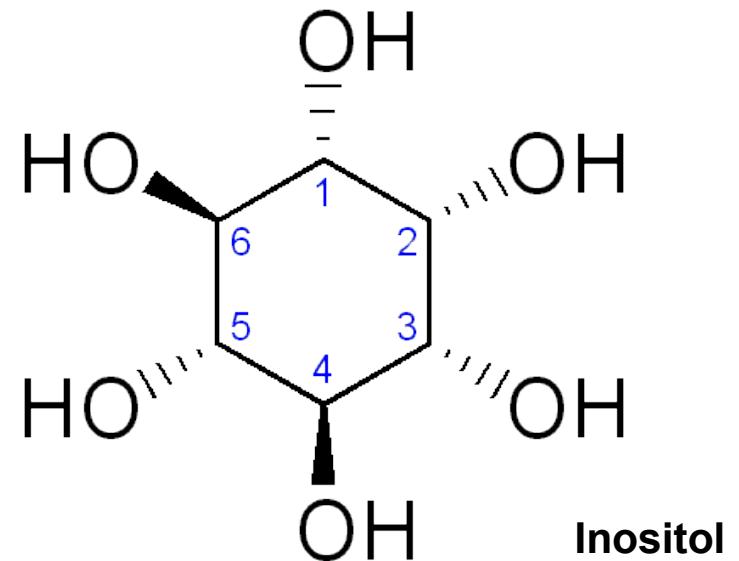
# ASSYMETRY IN THE LIPID BILAYER

➤ Different composition of:

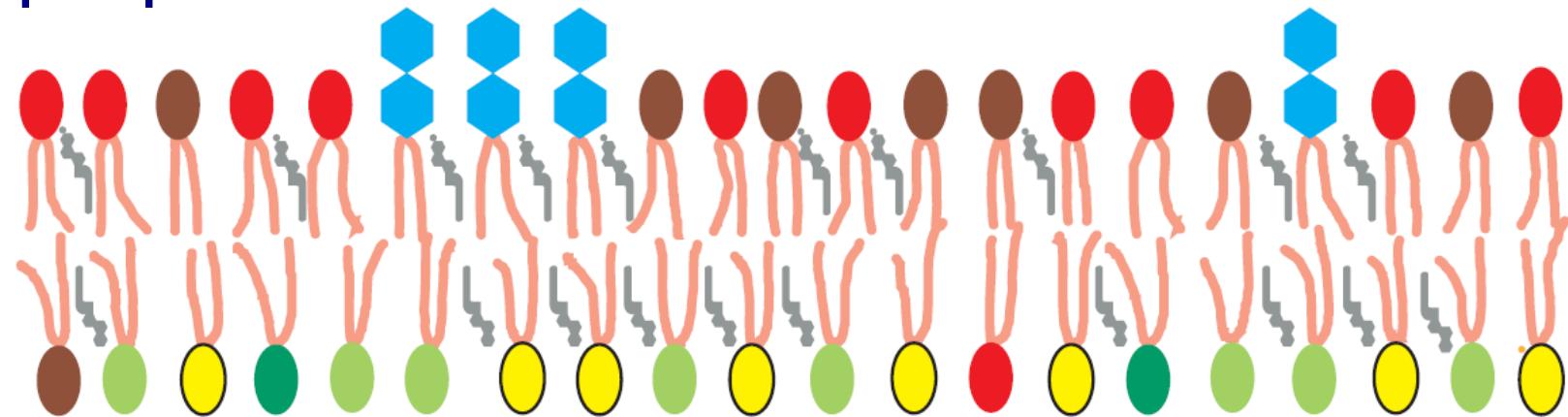
- lipids
- glycolipids
- saccharides
- proteins

➤ Affects:

- mechanical properties/function
- Inositol phospholipids

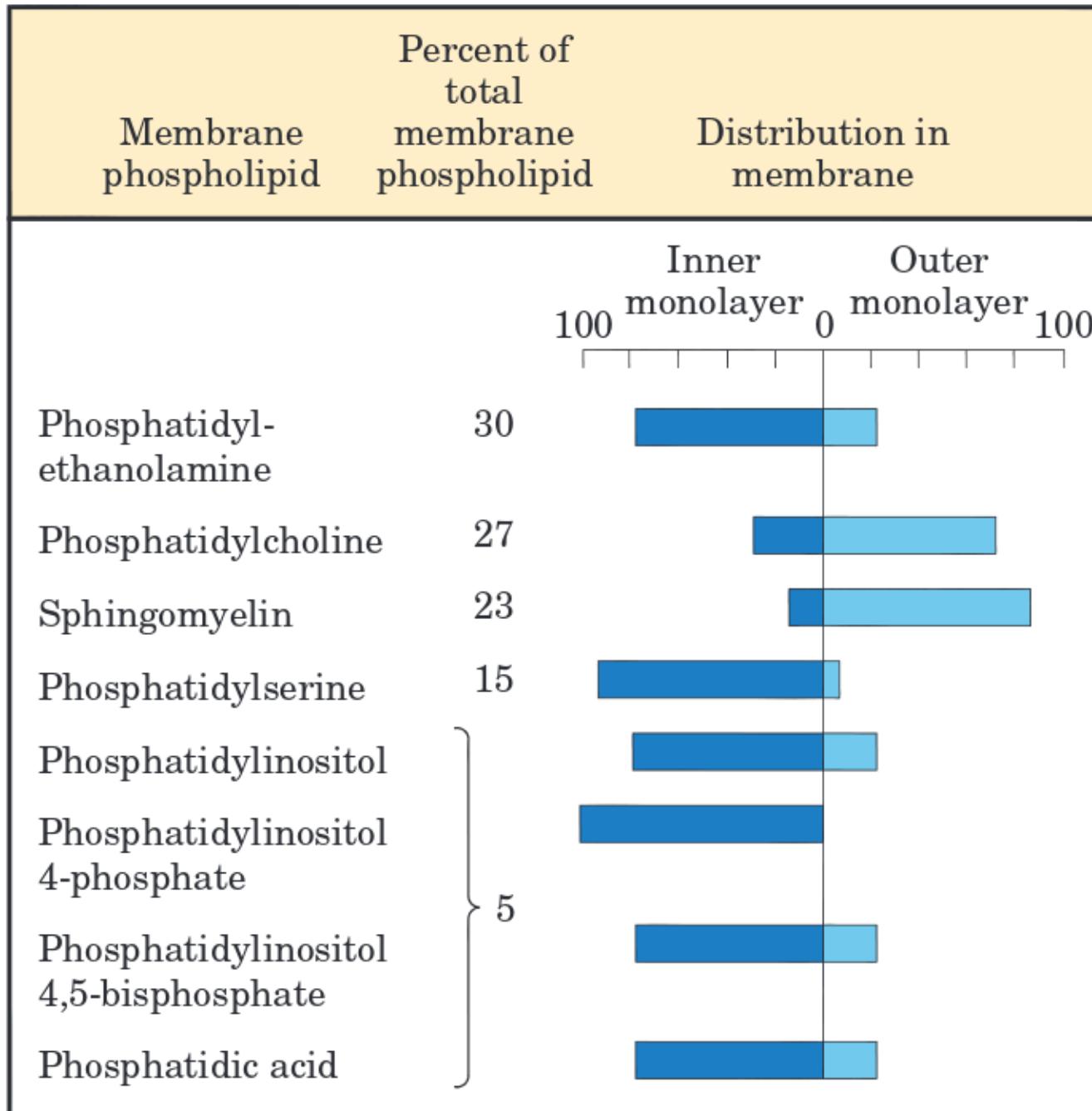


Inositol



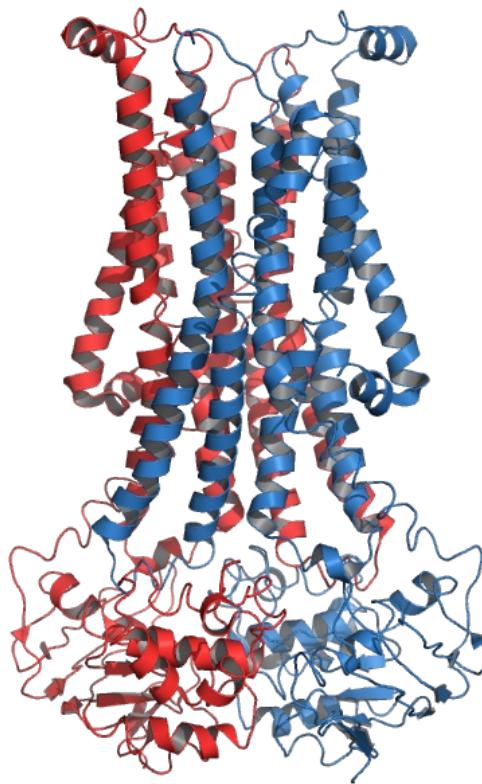
CYTOSOL

# ASSYMETRY IN THE LIPID BILAYER

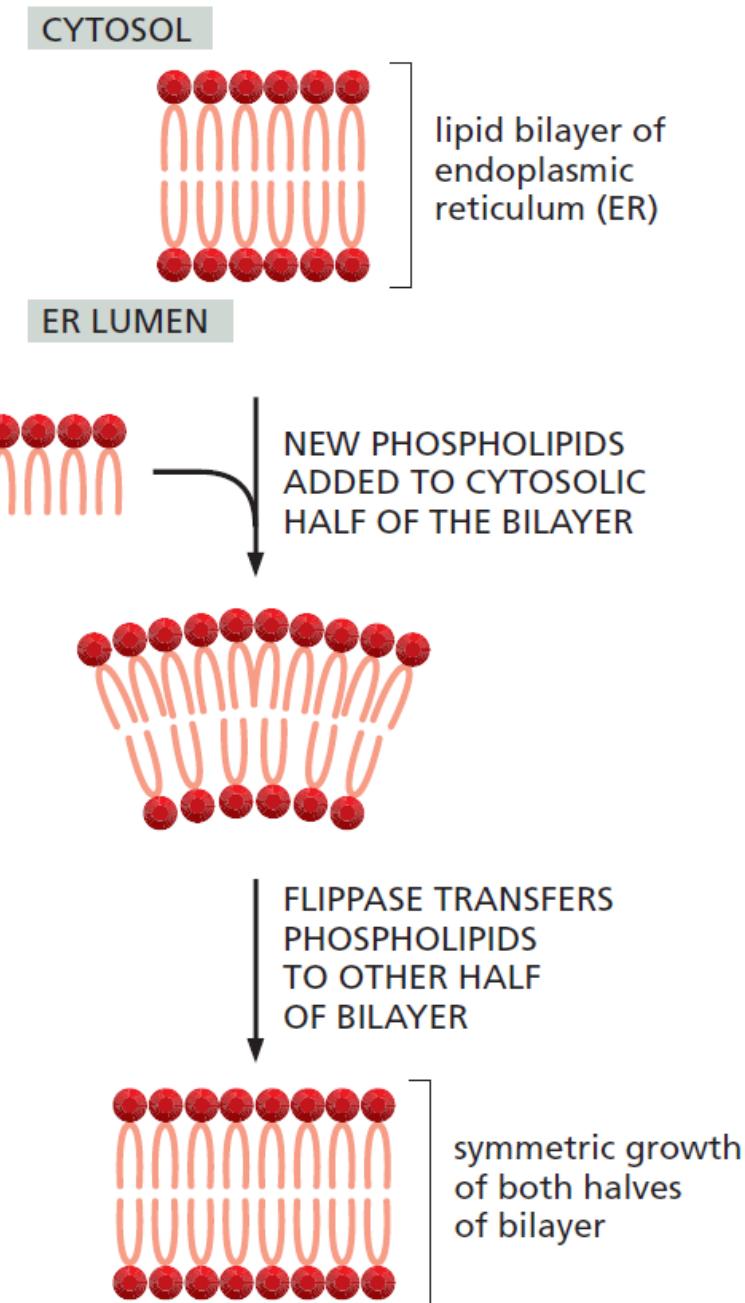


# FLIPPASES/FLOPPASES

- Flip-flop transfers
- ATP/Ca<sup>2+</sup>-dependence/independence
- ER localization
- Co-synthetical mechanism



PDB ID: 5C73



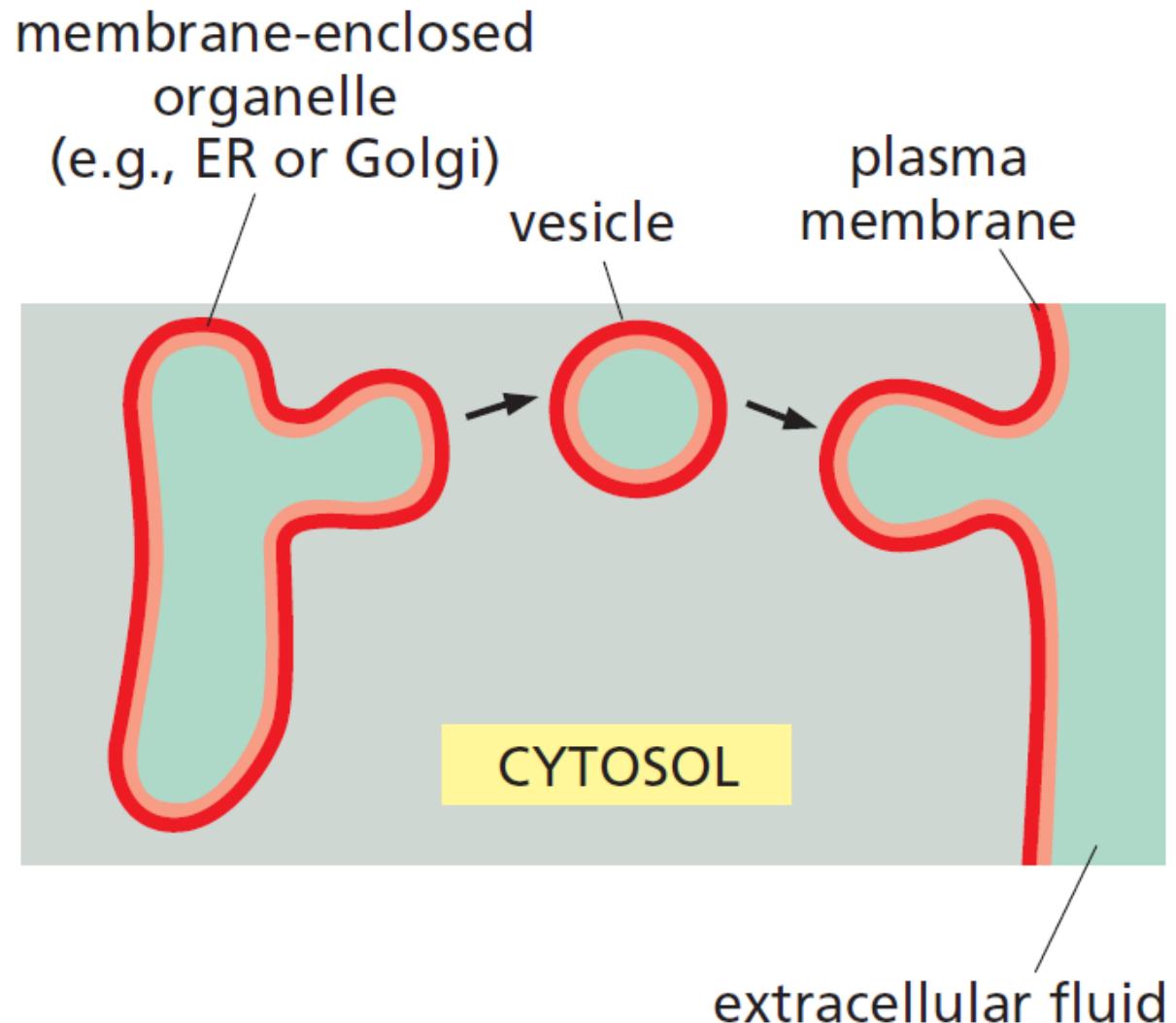
# ASSYMETRY IN THE LIPID BILAYER: MEMBRANE TRANSPORT

## ➤ Preservation of two faces:

- cytosolic
- noncytosolic

## ➤ Glycolipids:

- AG synthesis
- only noncytosolic face



# GLYCOLIPIDS

➤ Tendency to self-association

➤ ~ 5% of cell membranes

➤ Many types:

- sphingoglycolipids

- glyceroglycolipids

- gangliosides

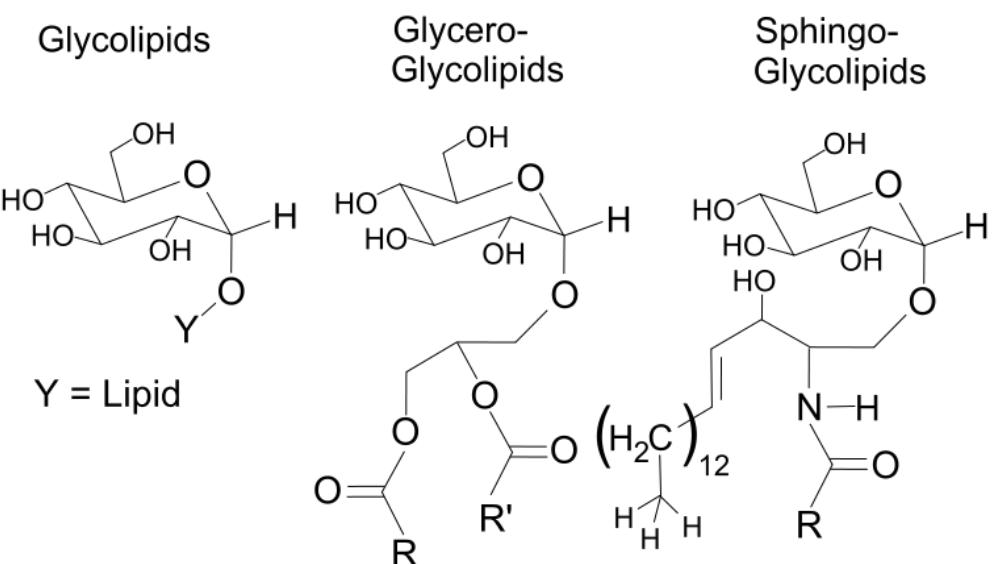
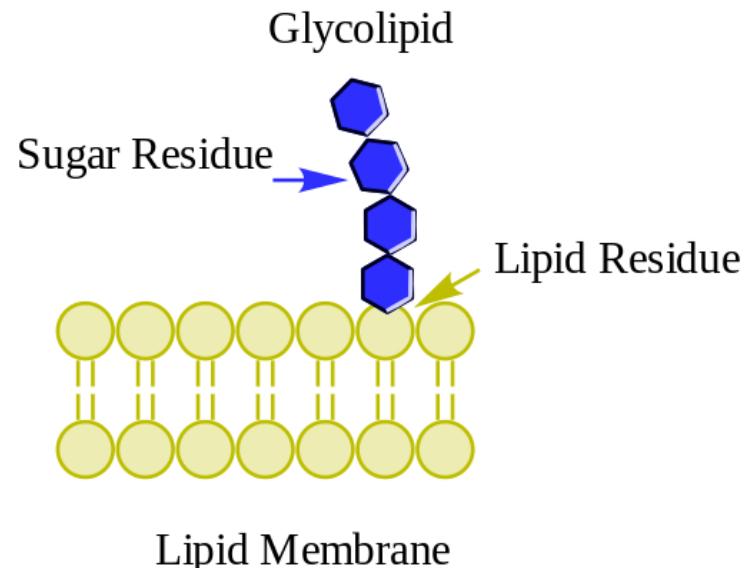
- ...

➤ Function:

- protection

- membrane electric polarization

- cell-recognition



# GANGLEOSIDES

- 5-10% of lipids in neurons
- NANA (N-acetylneuraminic acid) blocks

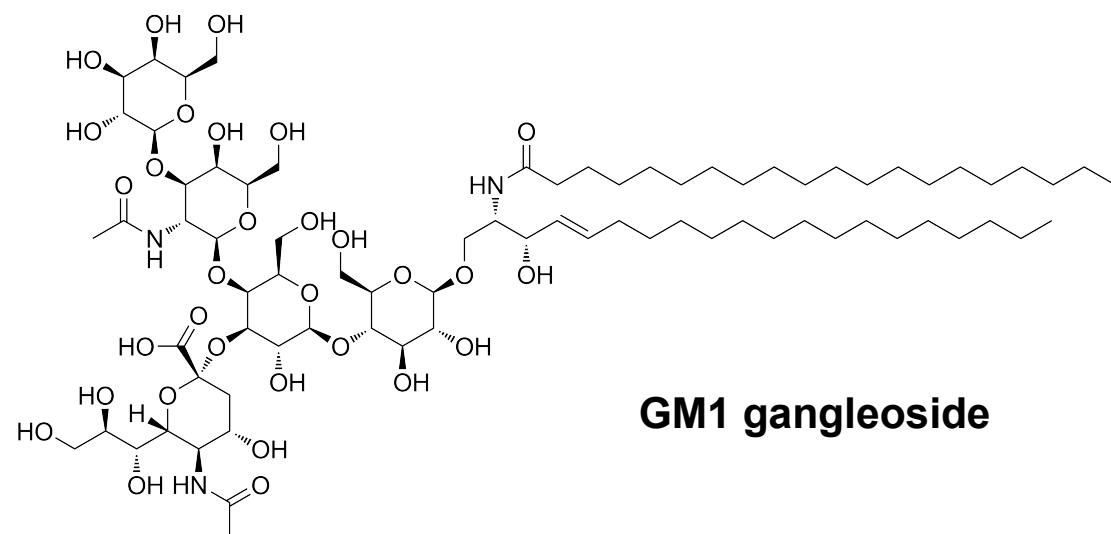
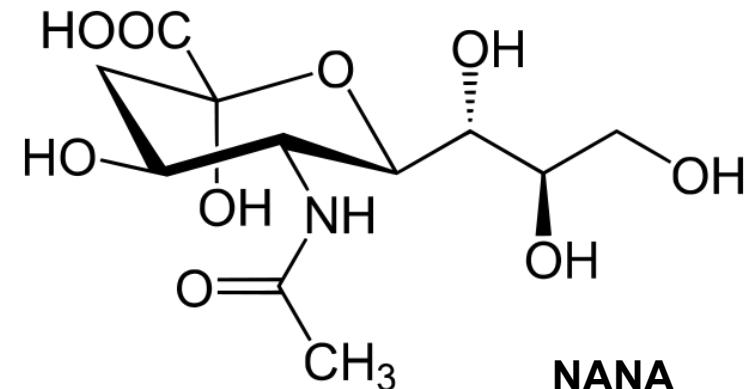
➤ ~60 types

➤ Function:

- cell communication
- cell signalling
- lipid rafts

➤ Associated diseases:

- influenza
- tetanus
- cholera
- botulism
- leprosy

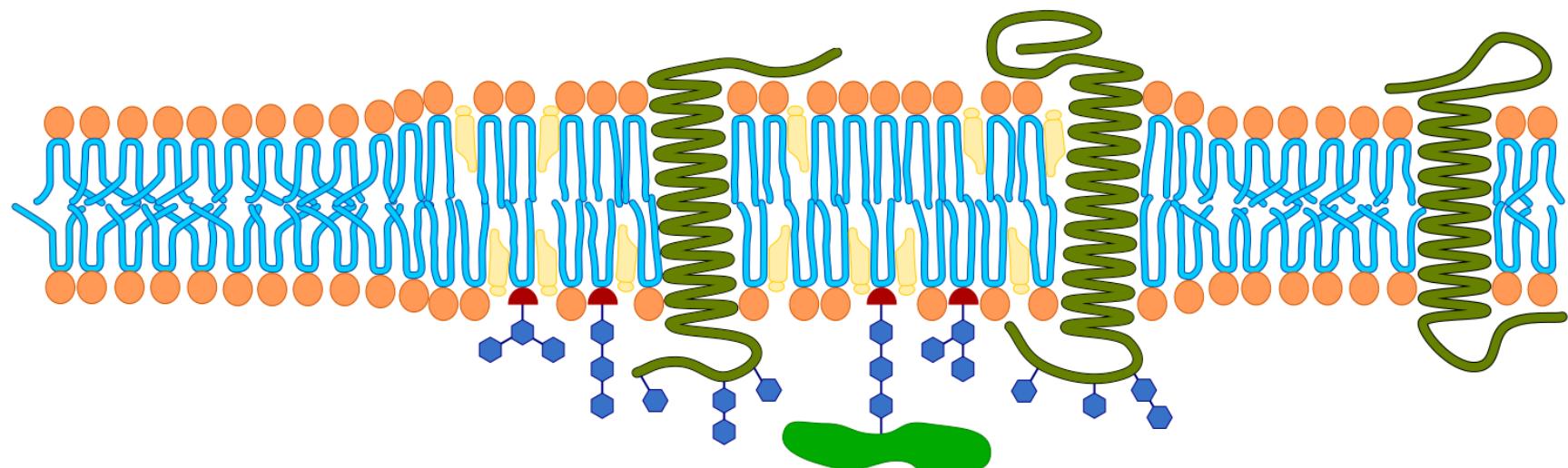
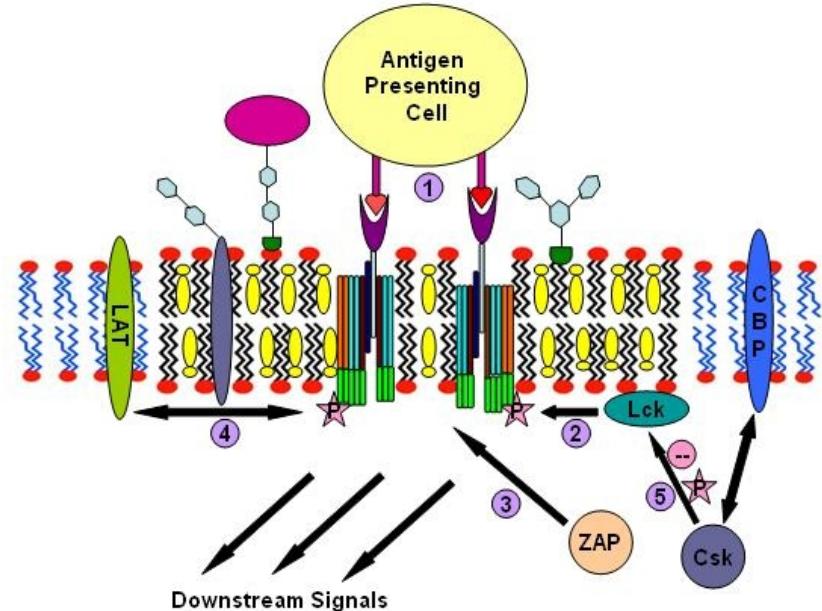


GM1 ganglioside

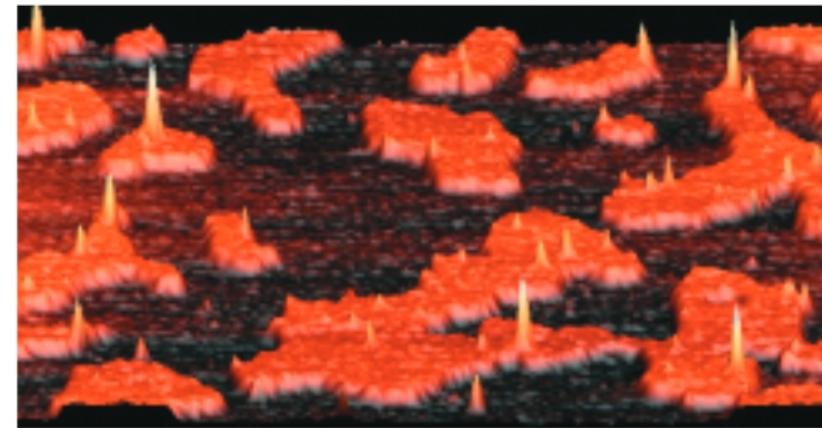
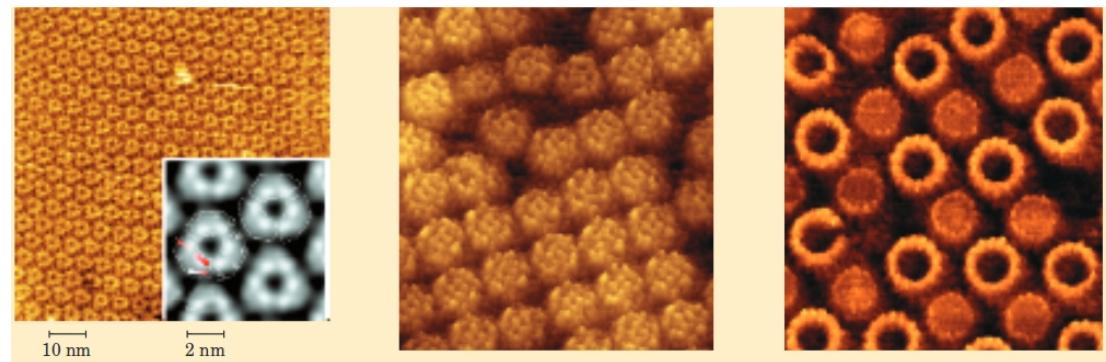
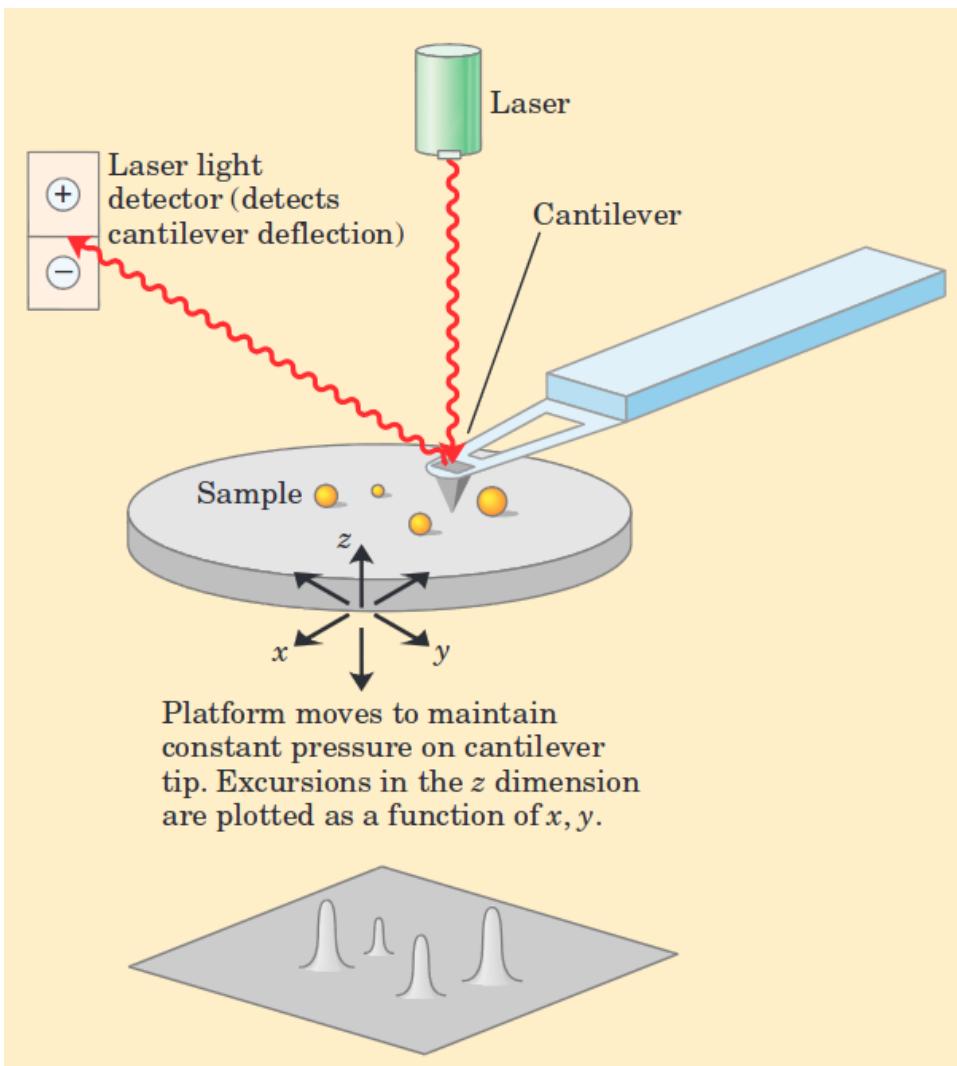
# LIPID RAFTS

Glycophospholipids + cholesterol + proteins

- Thicker membranes
- Higher concentration of proteins
- Communication through lipid tails
- Different diffusion properties
- Functions:
  - components concentration
  - cell signalling



# LIPID RAFTS IN ATOMIC FORCE SPECTROSCOPY

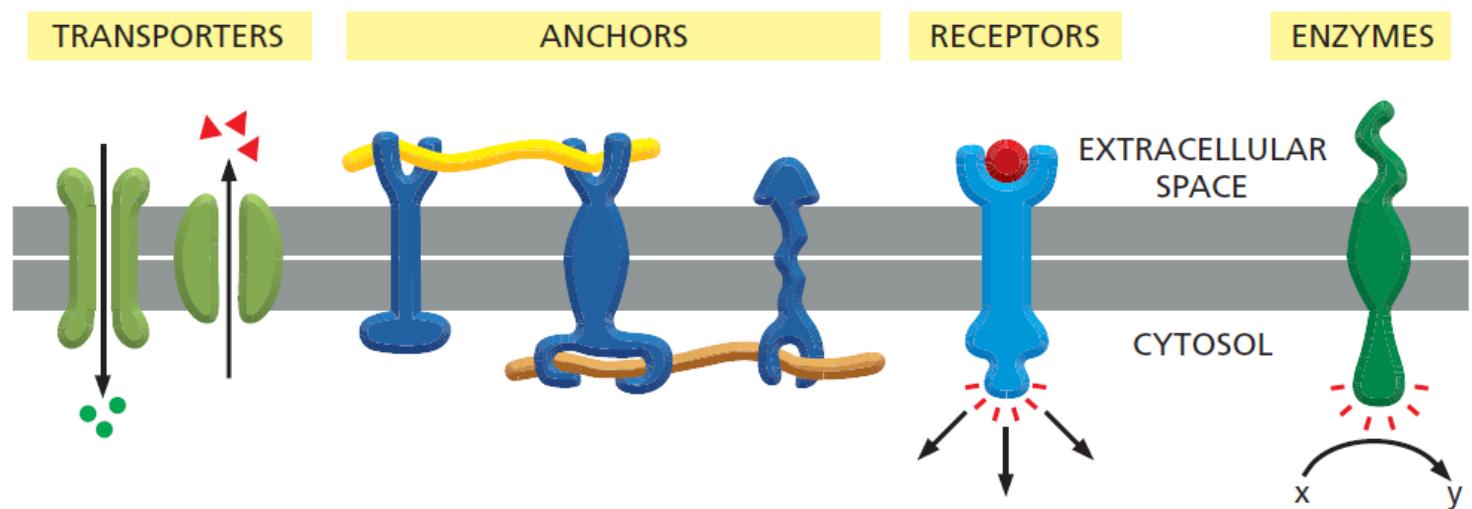


Lipid rafts visualized with AFM

# MEMBRANE PROTEINS

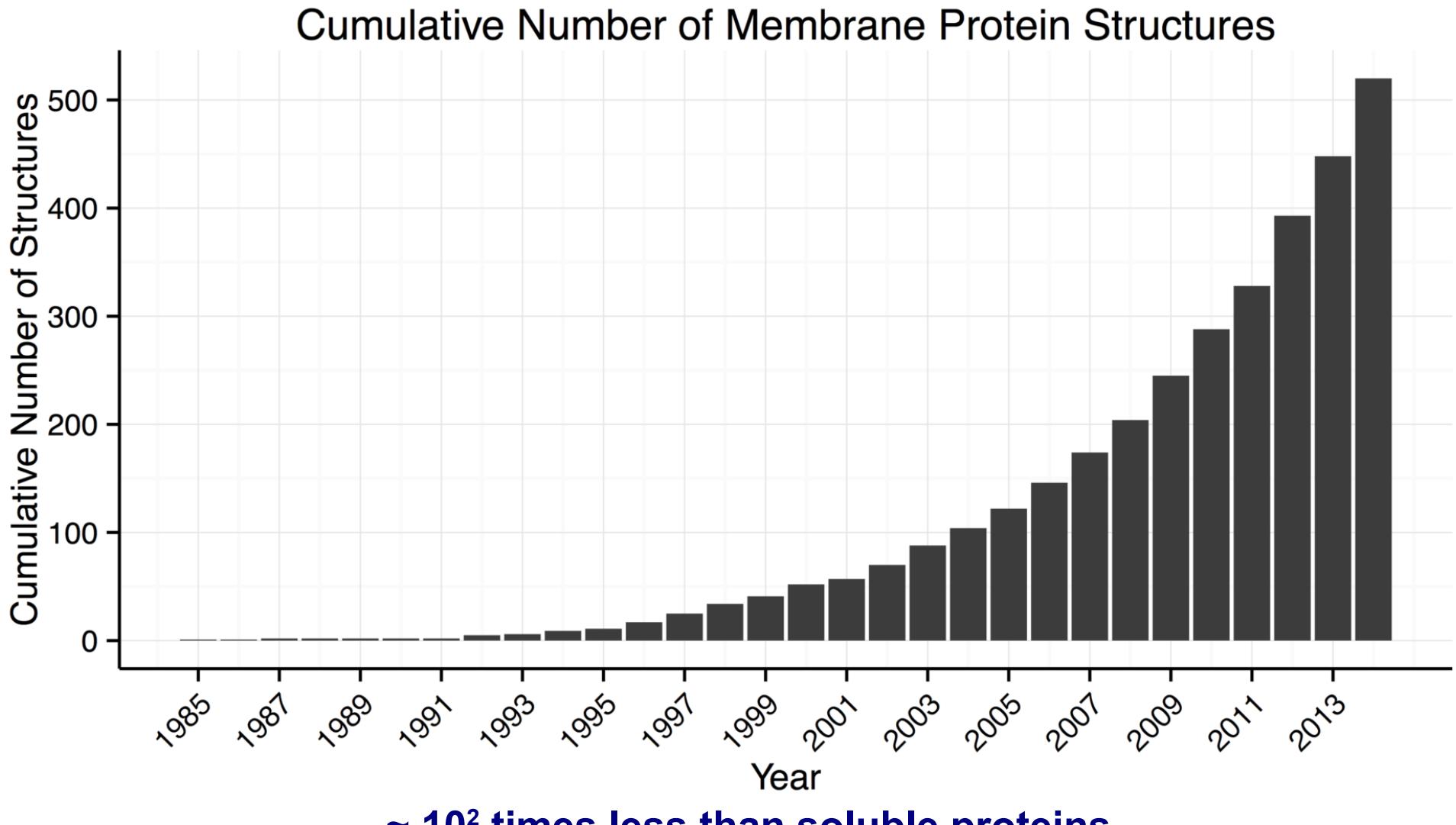
- ~50% of the mass
- Protein/lipid ~ 1/50
- Classes based on functions:

- transporters
- anchors
- enzymes
- receptors



FUNCTIONAL CLASS	PROTEIN EXAMPLE	SPECIFIC FUNCTION
Transporters	Na <sup>+</sup> pump	actively pumps Na <sup>+</sup> out of cells and K <sup>+</sup> in
Anchors	integrins	link intracellular actin filaments to extracellular matrix proteins
Receptors	platelet-derived growth factor (PDGF) receptor	binds extracellular PDGF and, as a consequence, generates intracellular signals that cause the cell to grow and divide
Enzymes	adenylyl cyclase	catalyzes the production of intracellular signaling molecule cyclic AMP in response to extracellular signals

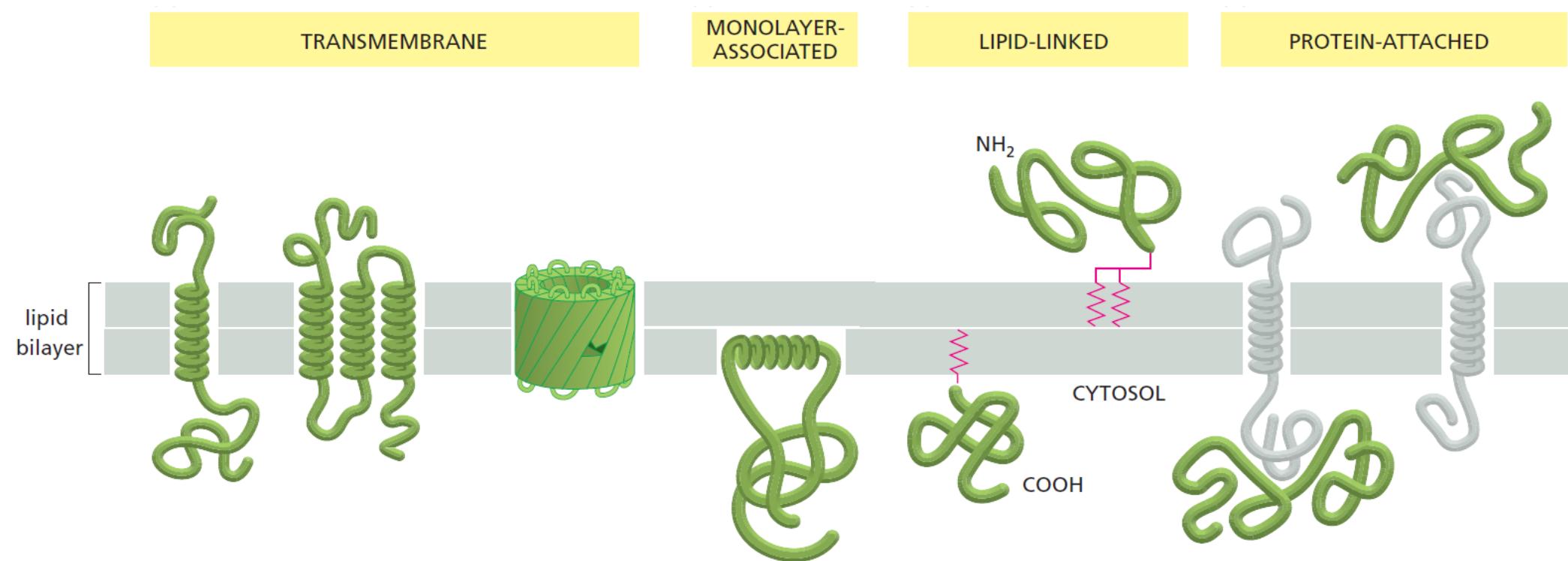
# MEMBRANE PROTEINS: STRUCTURES



➤ Challenges:

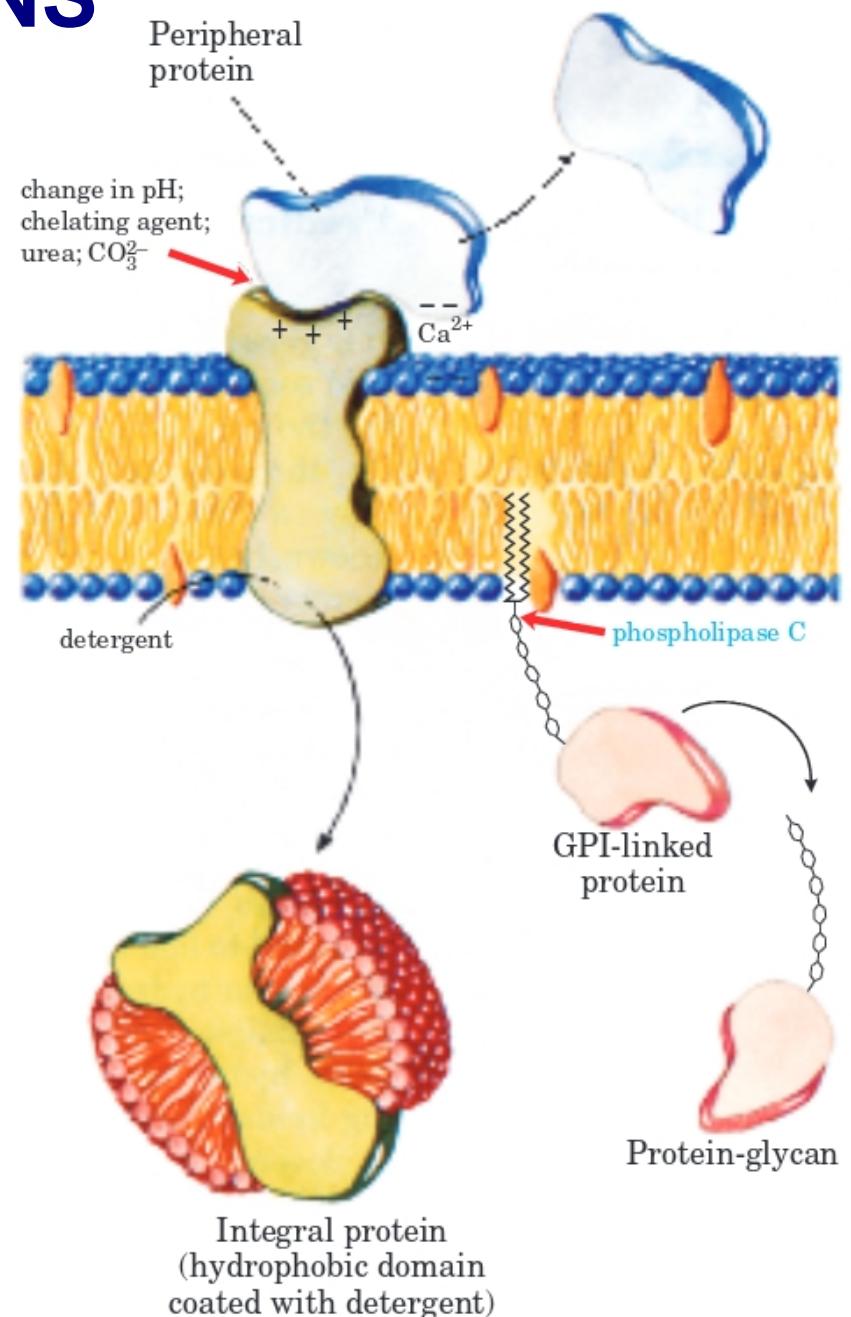
- extraction
- crystallization

# MEMBRANE PROTEINS: TYPES OF VARIOUS ASSOCIATIONS WITH LIPID BILAYER

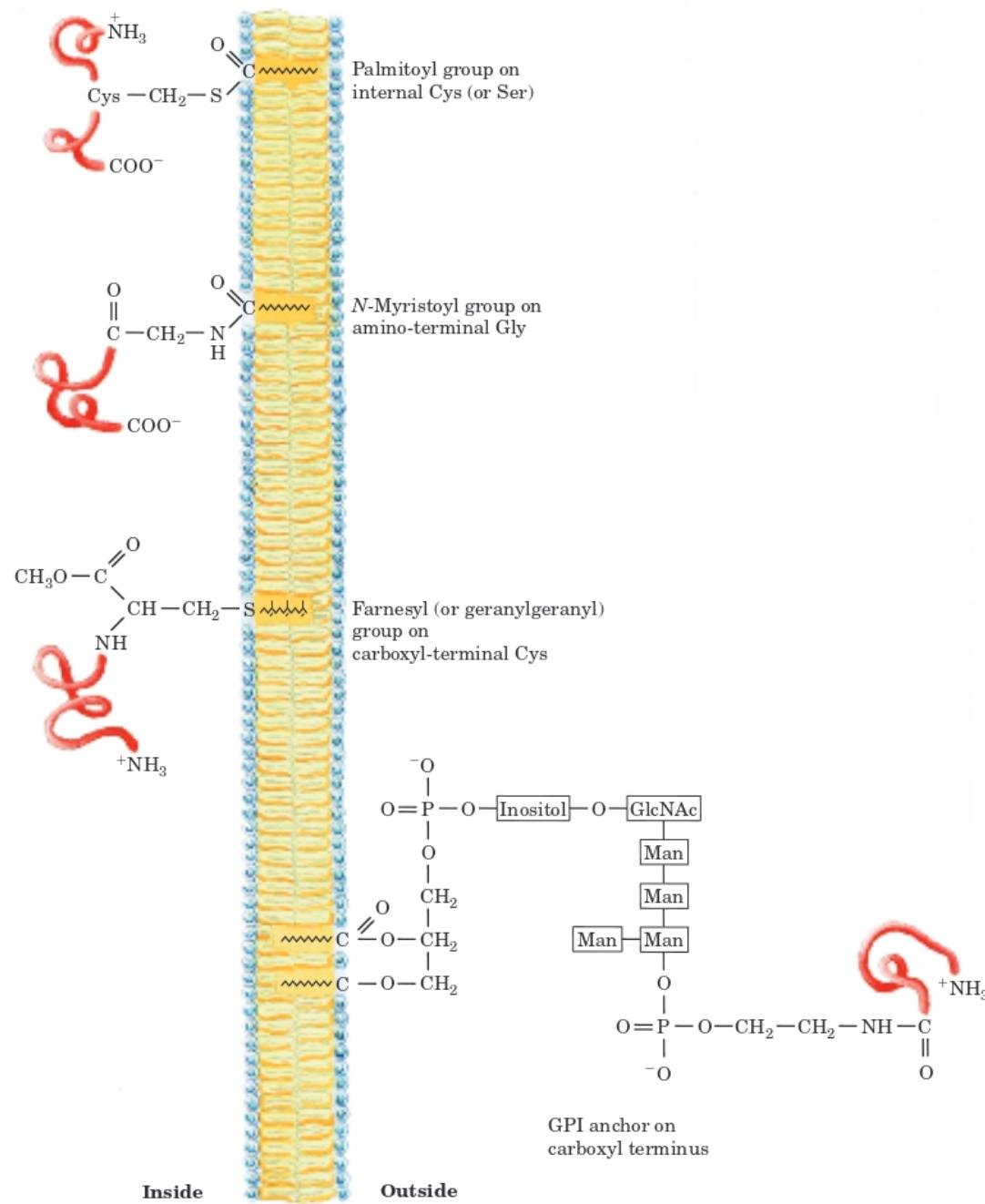


# INTERGRAL AND PERIPHERAL MEMBRANE PROTEINS

- Integral: can be extracted by detergent
- Peripheral: can be extracted leaving the bilayer intact

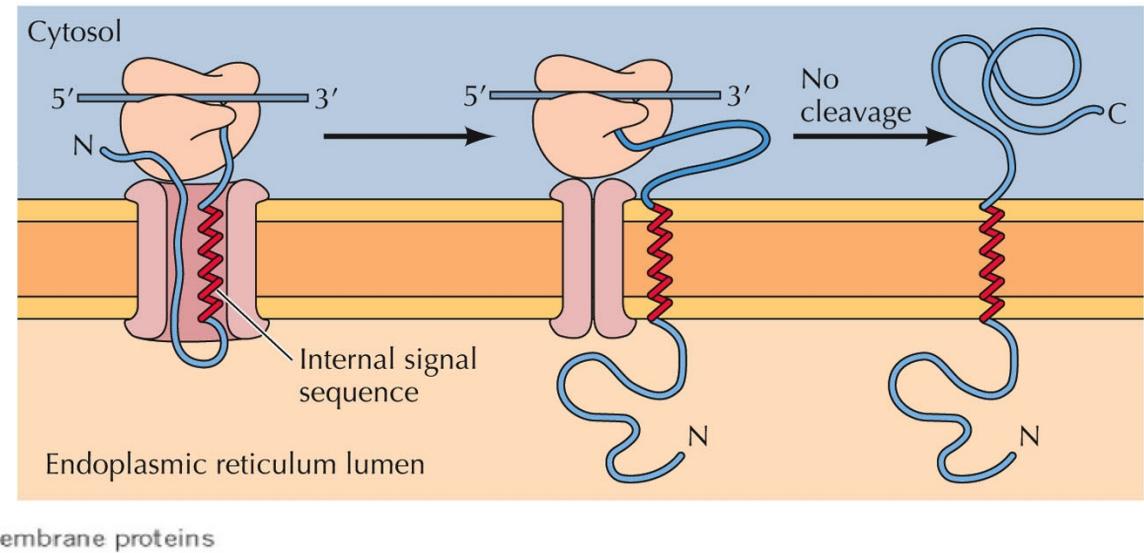


# LIPID-LINKED PROTEINS

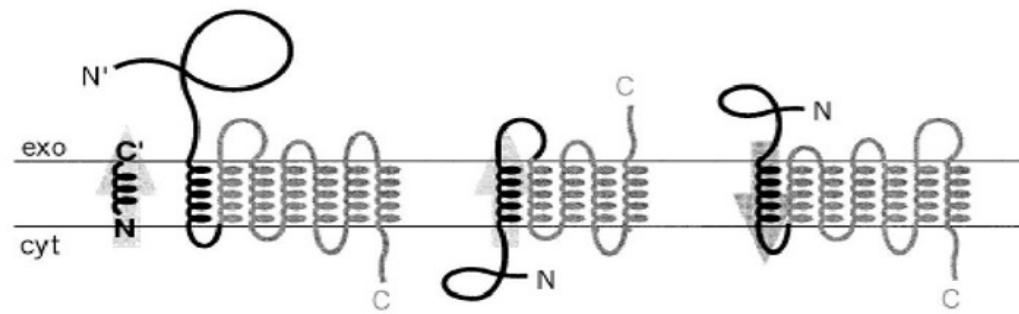
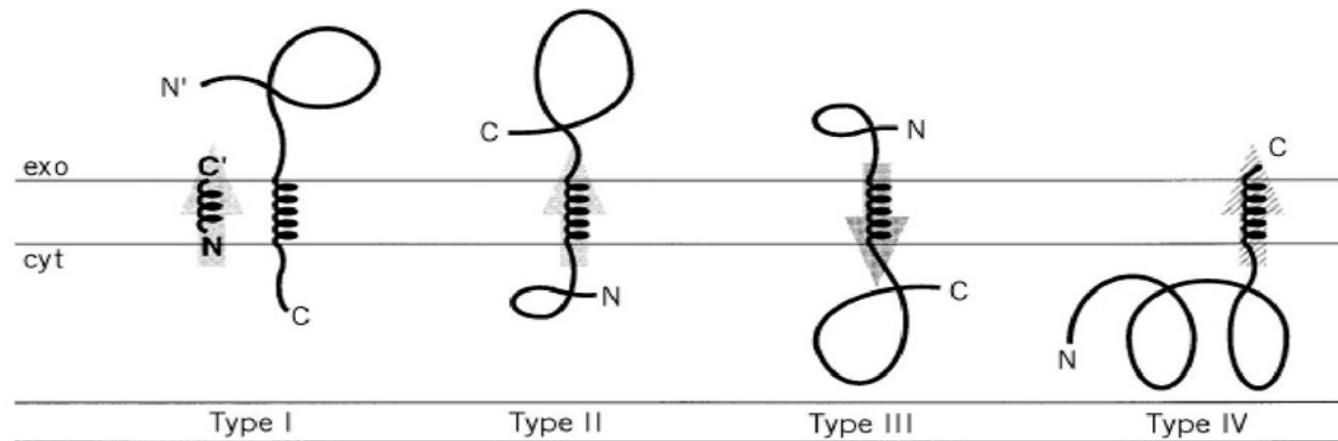


# TRANSMEMBRANE PROTEINS: TOPOLOGIES

- Single/multiple spanning
- Orientation of N/C-termini:
  - signals (cleaved, direct/reverse anchor)



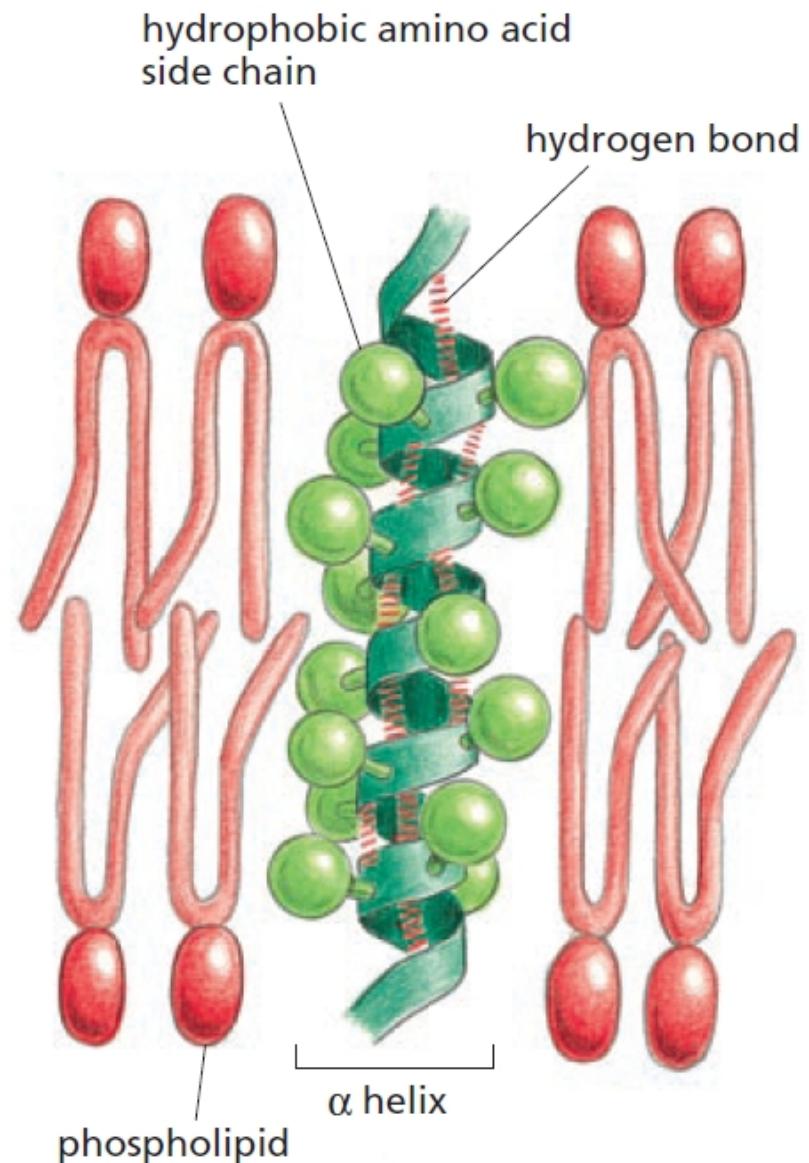
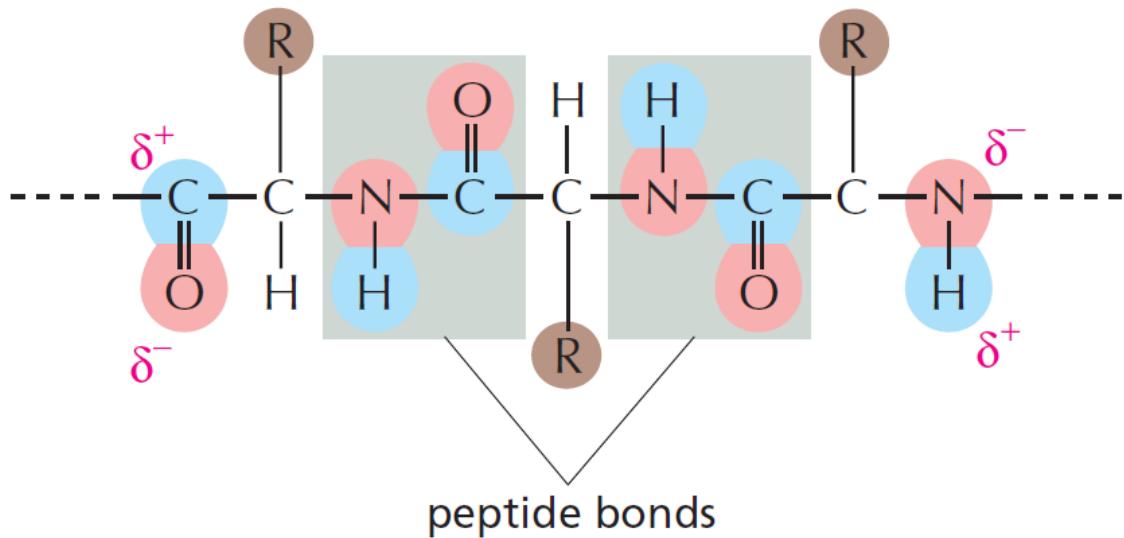
Single-spanning membrane proteins



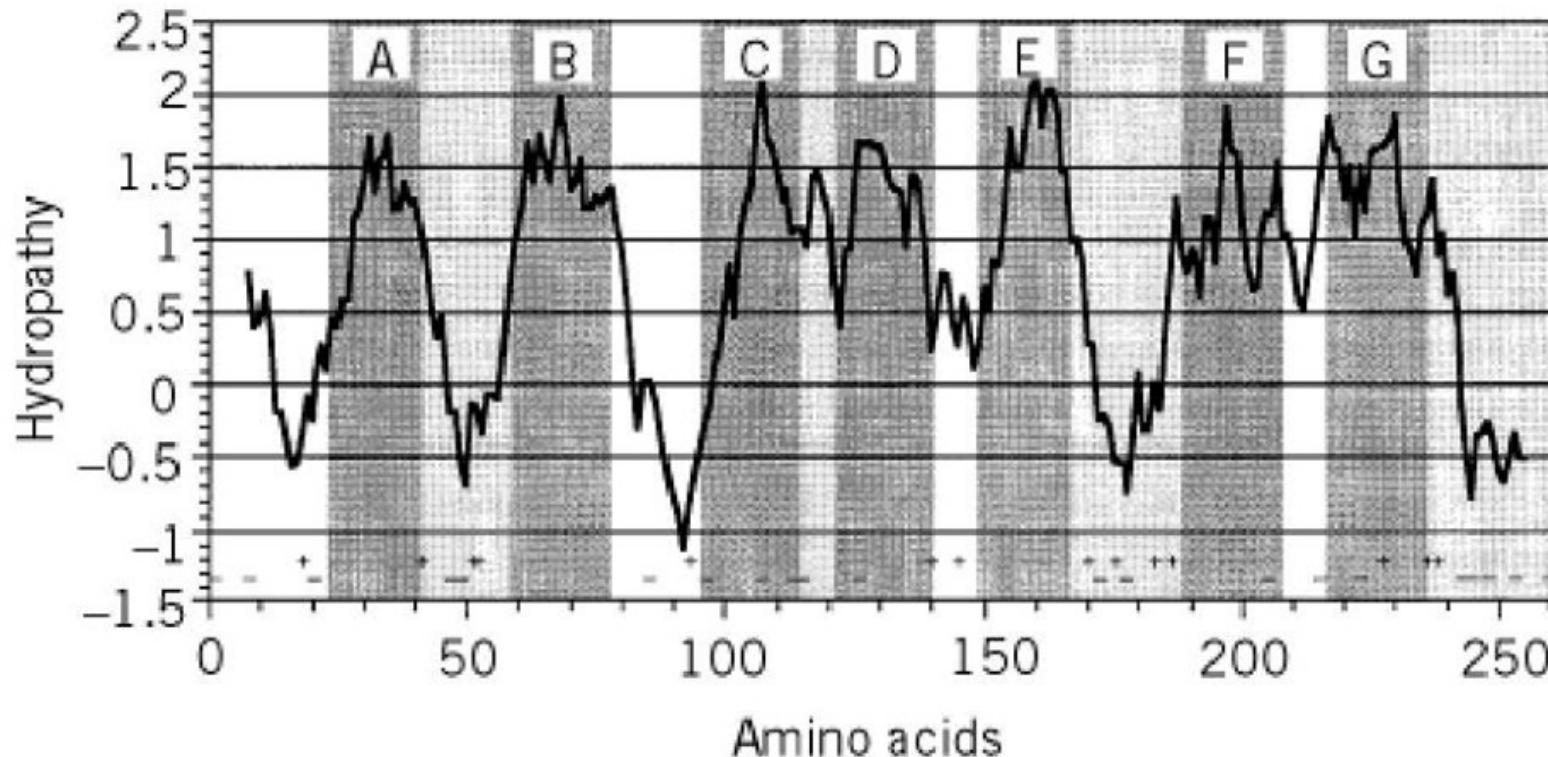
Multi-spanning membrane proteins

# TRANSMEMBRANE PROTEINS: INTERACTION WITH THE LIPID BILAYER

- TM-segment is hydrophobic
- Often  $\alpha$ -helix (~20 residues, 5.5 turns)



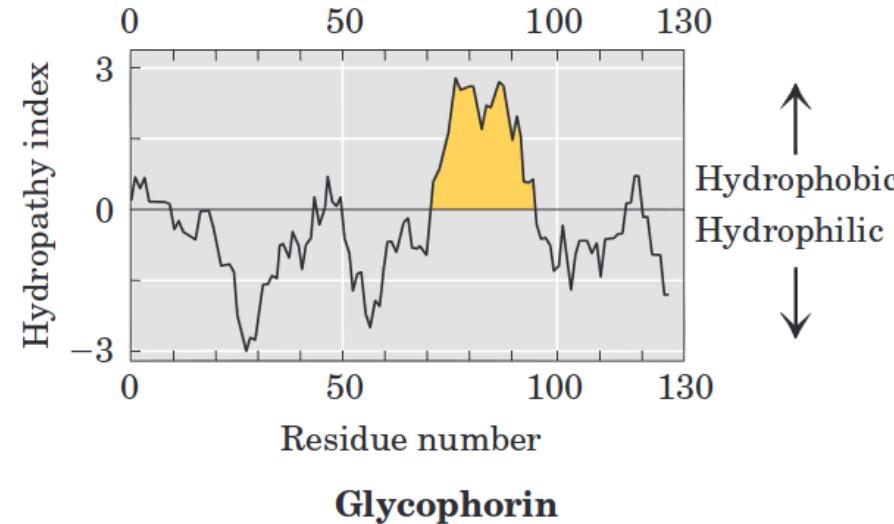
# TRANSMEMBRANE PROTEINS: PREDICTIONS BASED ON SEQUENCE



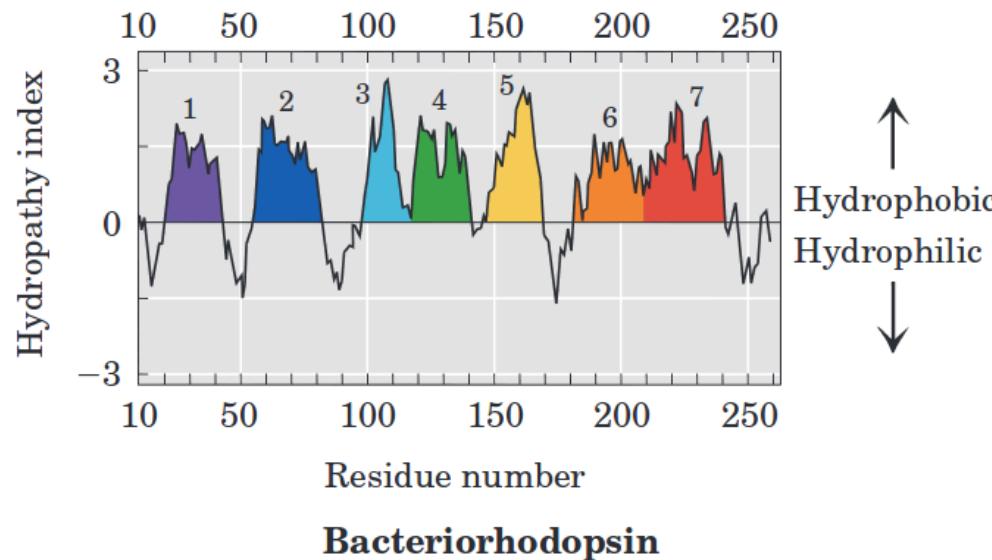
- Hydropathy index
- Length of segments

# TRANSMEMBRANE PROTEINS: PREDICTIONS BASED ON SEQUENCE

Single TMD



Multiple TMD



# TRANSMEMBRANE PROTEINS: PREDICTIONS

## Transmembrane Prediction Sites

- [DAS](#) (Stockholm University, Sweden)  
Prediction of transmembrane regions in prokaryotes using the Dense Alignment Surface method
  - input format: sequence only
  - output: html page with gif image

- [HMMTOP](#) (Hungarian Academy of Sciences, Budapest)  
Prediction of transmembrane helices and topology of proteins using Hidden Markov Model
  - input format: fasta, pir/nbrf, swissprot id or accession number
  - has options to select
  - output: html page

- [MEMSAT](#) (old original version of David Jones's software)  
Prediction Tool using Dynamic Programming
  - input format: fasta sequence
  - output format: html page with TOPO2 image

- [PredictProtein](#)  
general predictprotein page
  - input format: sequence only
  - register for free account, limited to 3 unique queries a month
  - number of hoops to get the account
  - output: email message

- [PRED-TMR](#) (University of Athens, Greece)  
Transmembrane segment prediction in proteins based on a statistical analysis of the SwissProt database

- [PRED-TMR2](#)(University of Athens, Greece)  
Prediction of Transmembrane regions in proteins

- [PSIPRED V2.3](#) (University College London)
  - input format: sequence only
  - enter server, select MEMSAT3 from list of prediction methods
  - output format: email message containing prediction

- [SOSUI](#)(Tokyo University of Agriculture & Technology)  
Prediction of transmembrane regions using Hydrophobicity Analysis for Topology and Probe Helix Method for Tertiol Structure  
**Requires JAVA enabled browser**
  - input format: sequence only
  - output format: html page with hydrophaty plot and helical wheel diagrams of predicted helices

- [Split](#) (University of Split, Croatia - Membrane Protein Transmembrane Secondary Structure Prediction Server)  
Prediction Tool using Preference Functions Method

- [TMHMM](#)(Center for Biological Sequence Analysis, Technical University of Denmark)  
Prediction of transmembrane helices in proteins using Hidden Markov Model
  - input format: fasta
  - output: html page with image of predicted locations

- [TMPred](#)(European Molecular Biology Network, Swiss node)  
Prediction of transmembrane regions and protein orientation
  - input format: sequence, swissprot id or accession number, Genbank gi
  - output: html or ascii

## Prediction of Transmembrane Regions and Orientation

The TMpred program makes a prediction of membrane-spanning regions and their orientation. The algorithm is based on the statistical analysis of TMbase, a database of naturally occurring transmembrane proteins. The prediction is made using a combination of several weight-matrices for scoring.

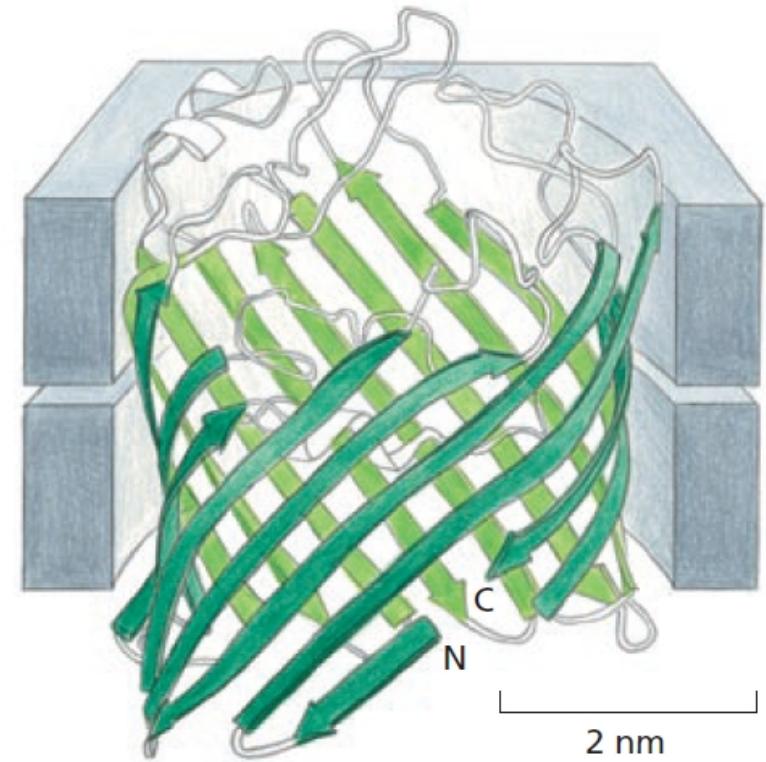
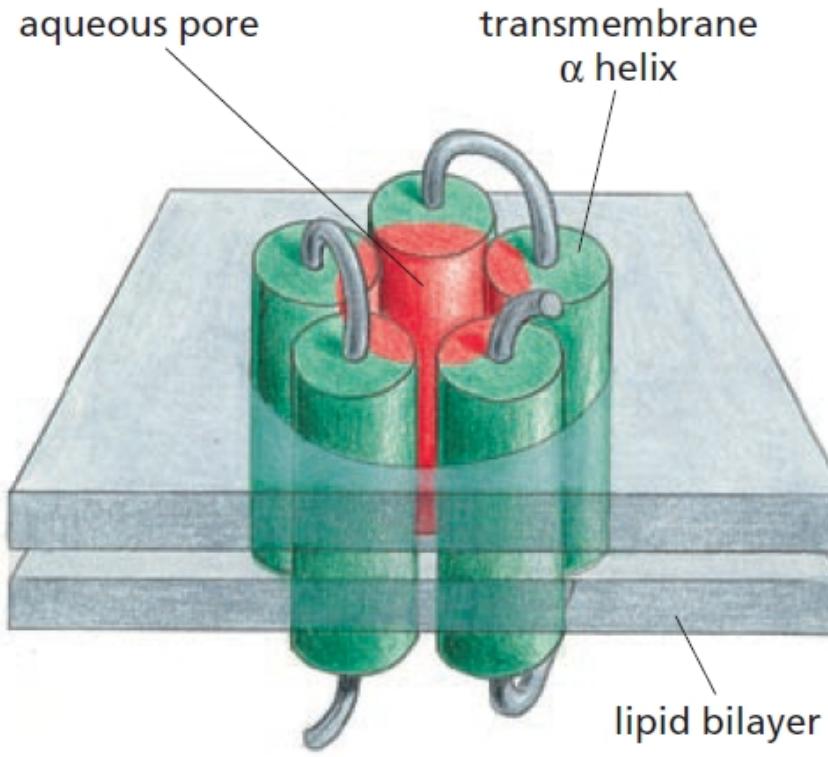
- **K. Hofmann & W. Stoffel (1993)**  
**TMbase - A database of membrane spanning proteins segments**  
**Biol. Chem. Hoppe-Seyler 374,166**

For further information see the [TMbase documentation](#).

- Usage: Paste your sequence in one of the supported **formats** into the sequence field below and press the "Run TMpred" button.  
**Make sure that the format button (next to the sequence field) shows the correct format**  
Choose the minimal and maximal length of the hydrophobic part of the transmembrane helix

Output format	<input type="button" value="html"/> minimum 17 maximum 33
Query title (optional)	<input type="text"/>
Input sequence format	<input type="button" value="Plain Text"/>
Query sequence: or ID or AC or GI (see above for valid formats)	<input type="text"/>
<input type="button" value="Run TMpred"/> <input type="button" value="Clear Input"/>	

# MULTITRANSMEMBRANE PROTEINS



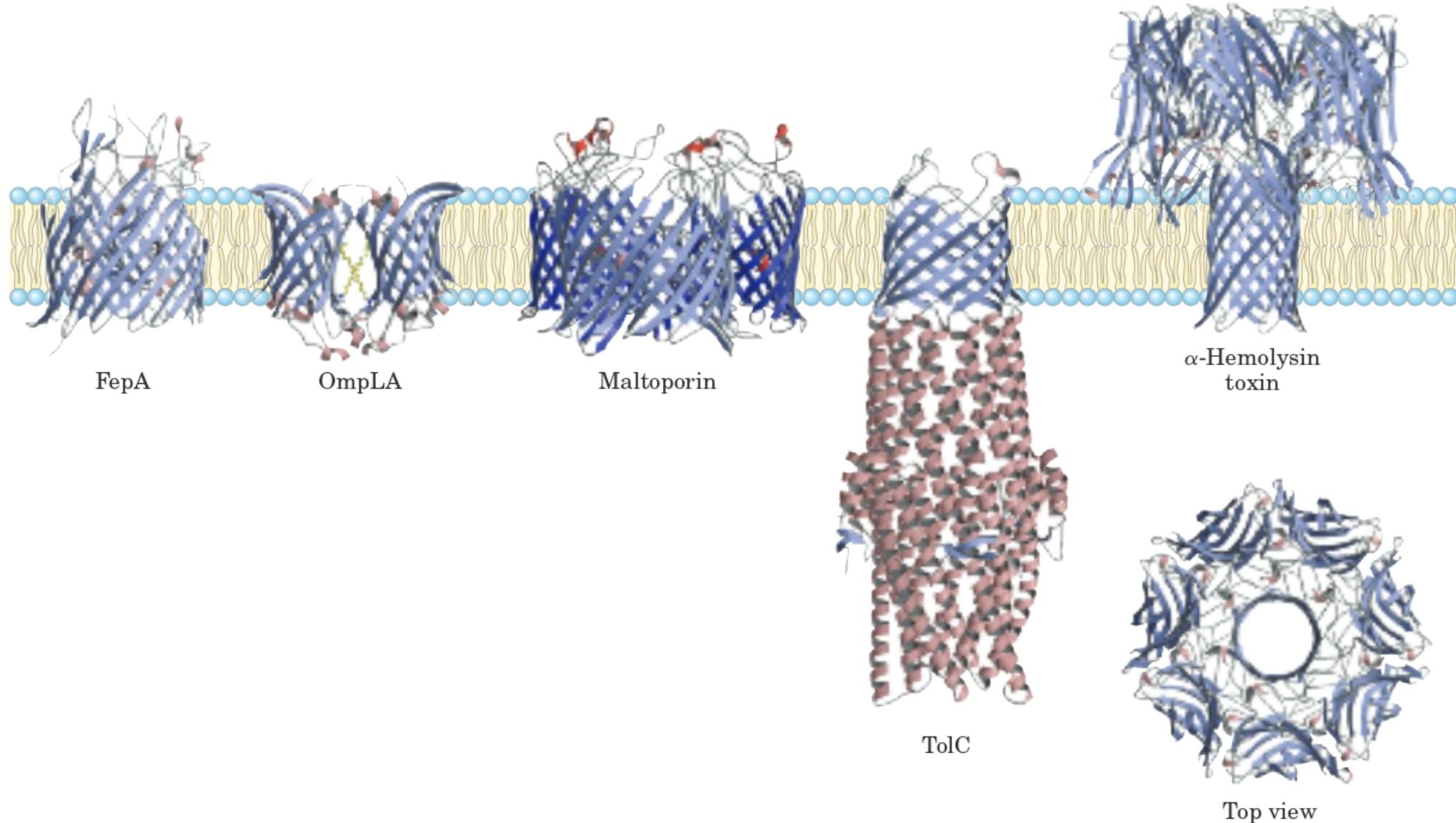
Hydrophilic pore formed by  $\alpha$ -helices

Porin:  $\beta$ -barrel

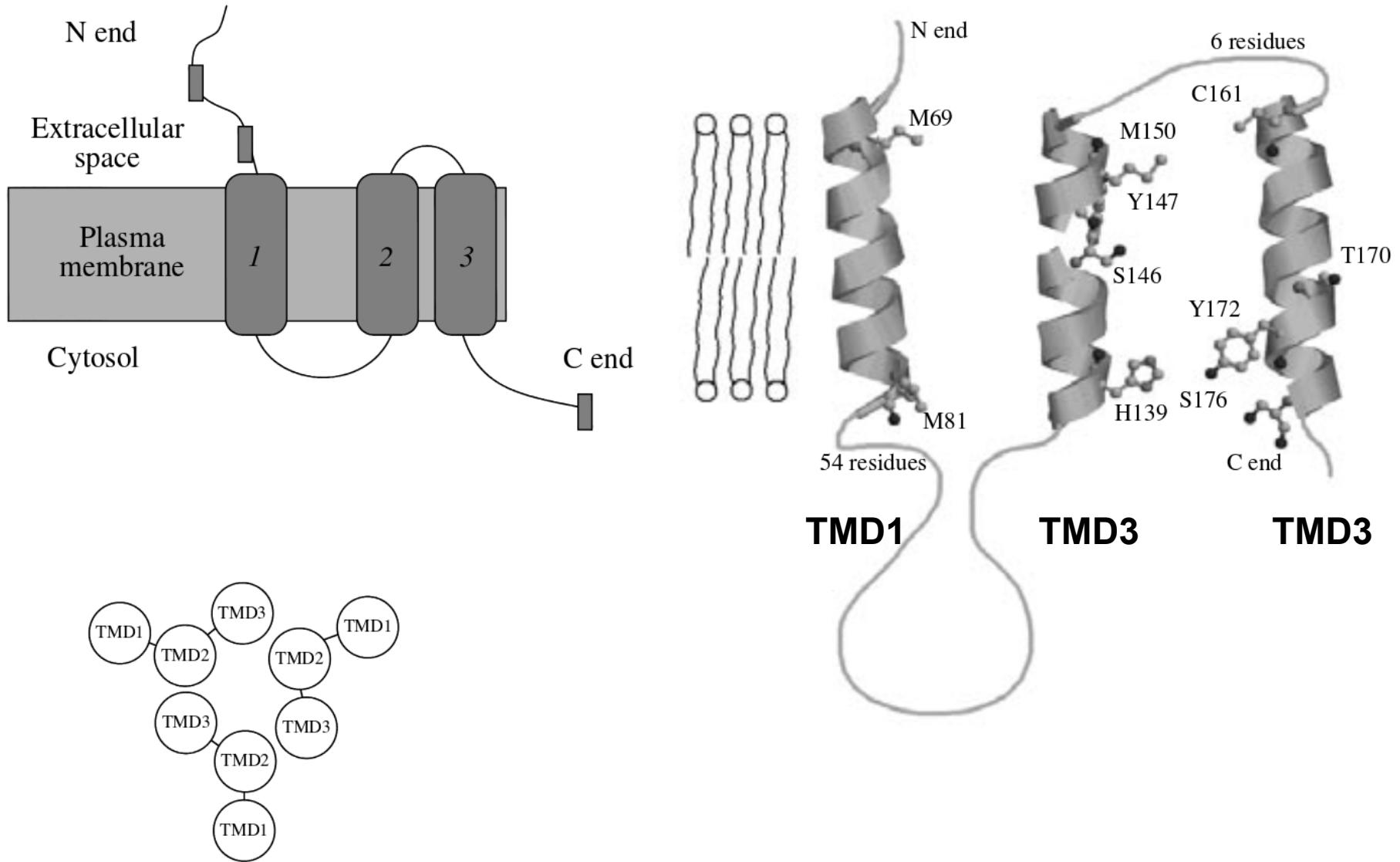
(16 antiparallel  $\beta$ -sheets)

Difference in rigidity

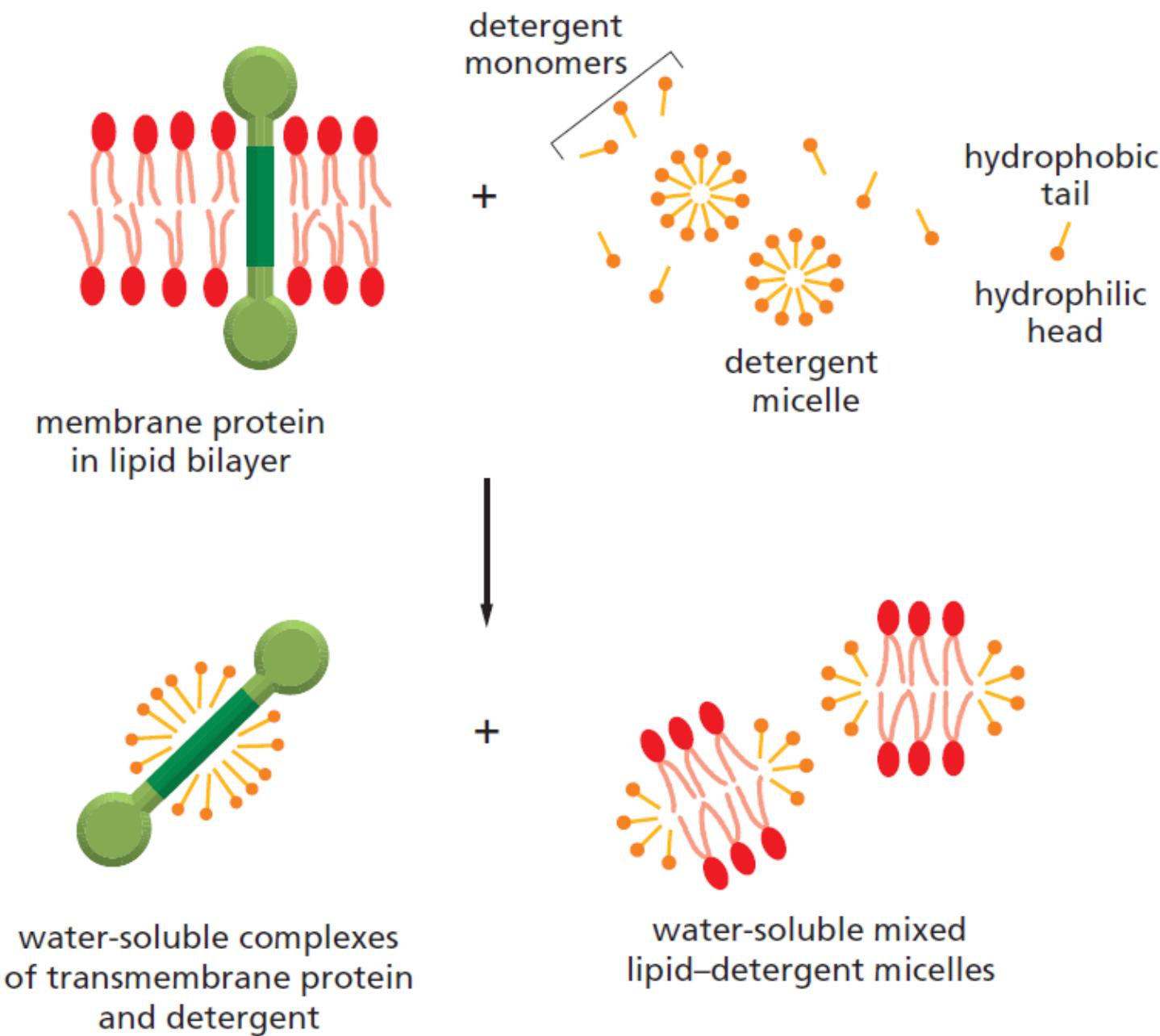
# BETA-BARRELS PROTEINS



# EXAMPLE OF PREDICTION: COPPER TRANSPORTER-1

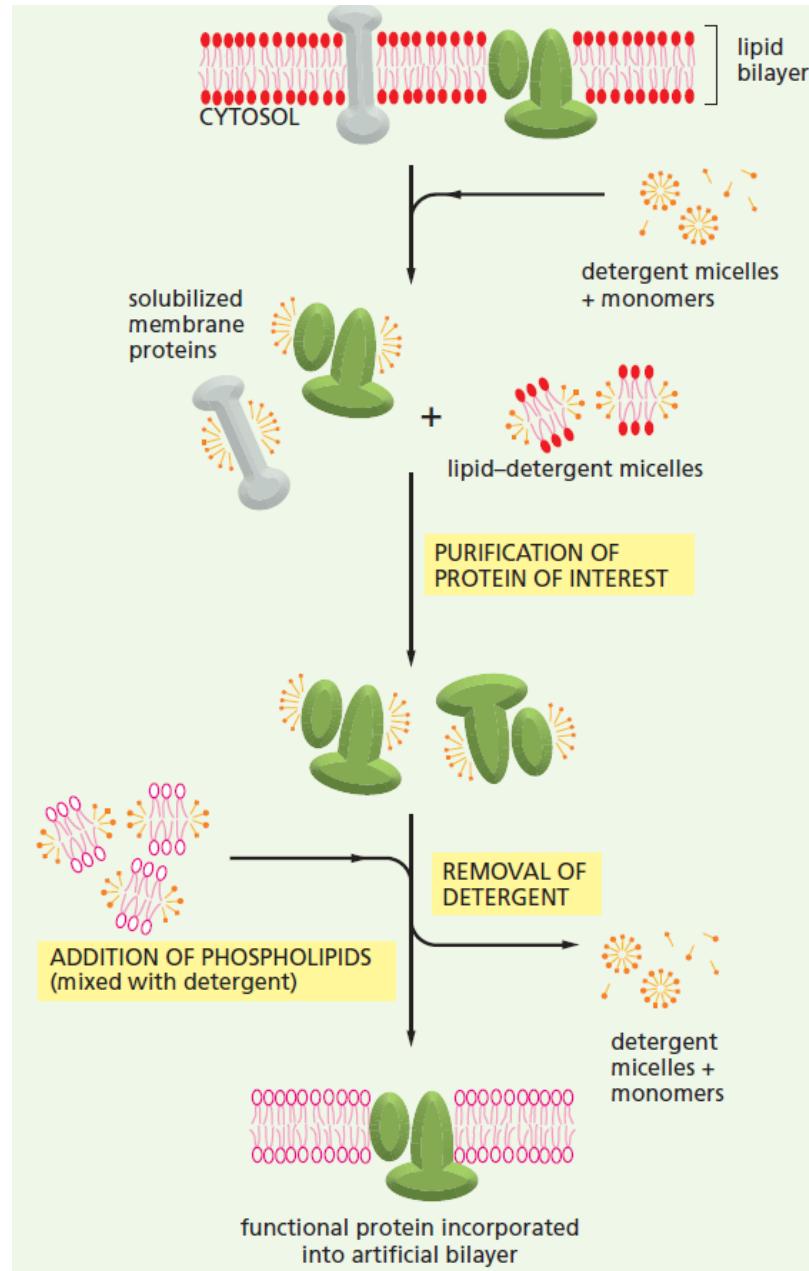


# EXTRACTION OF TRANSMEMBRANE PROTEINS

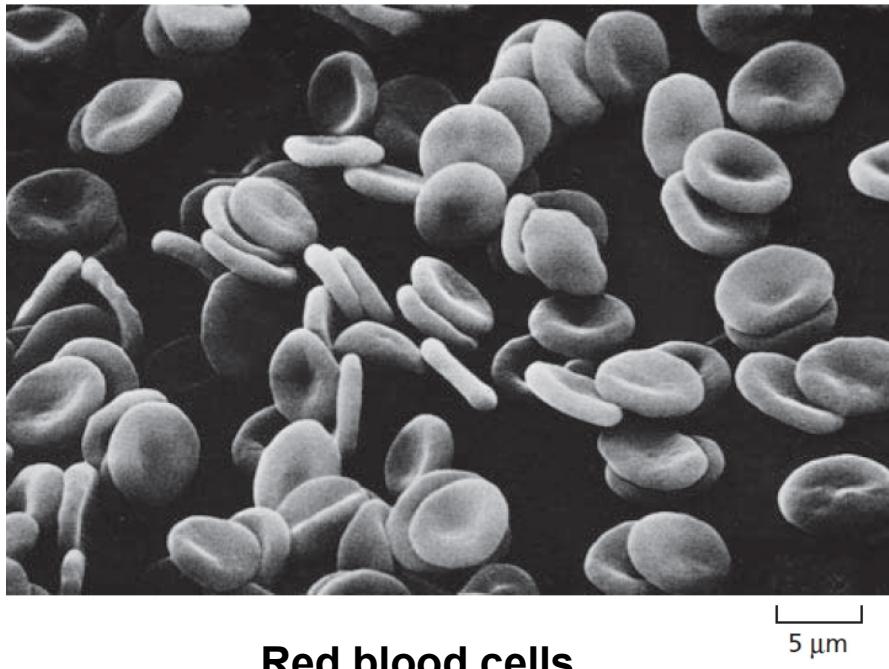


# TRANSMEMBRANE PROTEINS: RECONSTITUTION

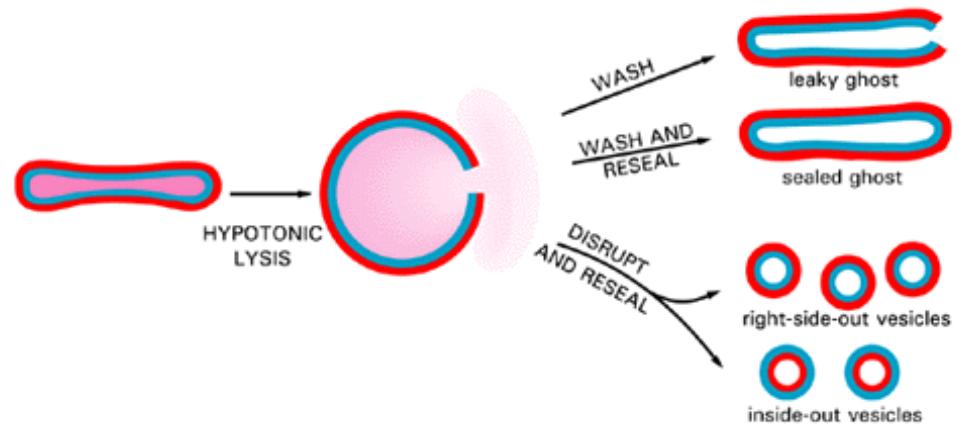
To isolate and study TM-protein, it can be extracted and reconstituted



# TRANSMEMBRANE PROTEINS: RECONSTITUTION



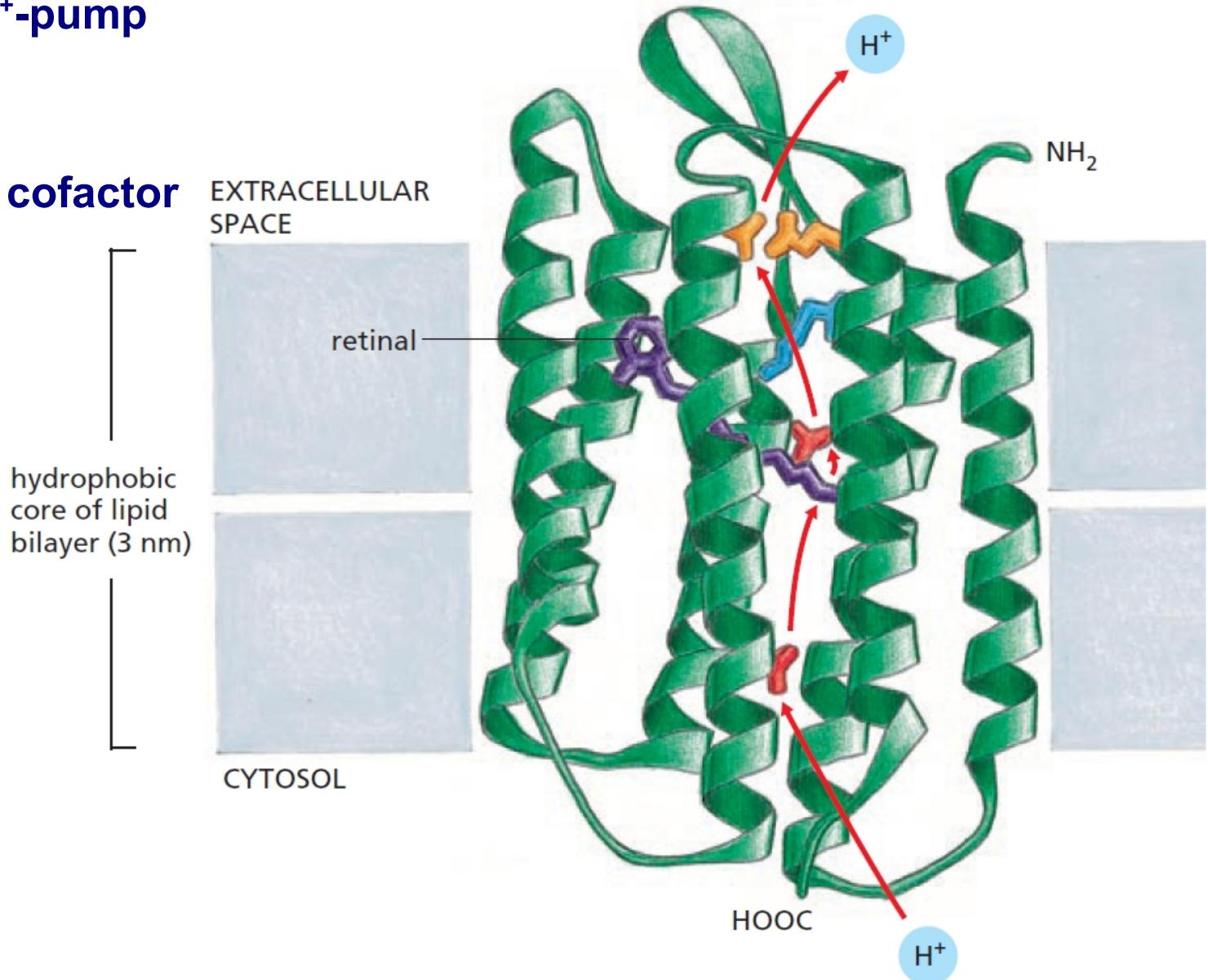
Red blood cells



- Identification of TM-protein localization:
  - vectorial labelling (radioactive + SDS gel)
  - enzymatic reactions
  - antibodies

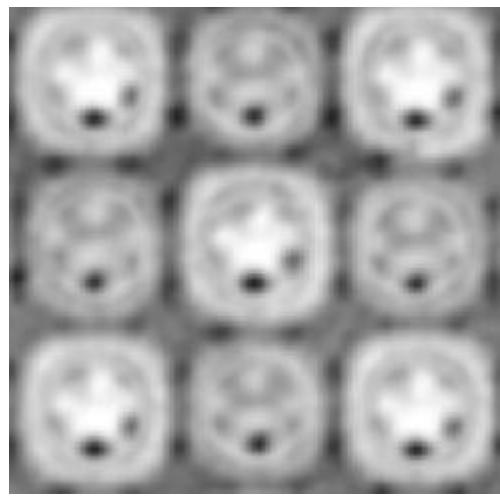
# EXAMPLE: BACTERIORHODOPSIN

- ~ 250aa
- Transporter: H<sup>+</sup>-pump
- 7 TM-domains
- Light-sensitive cofactor

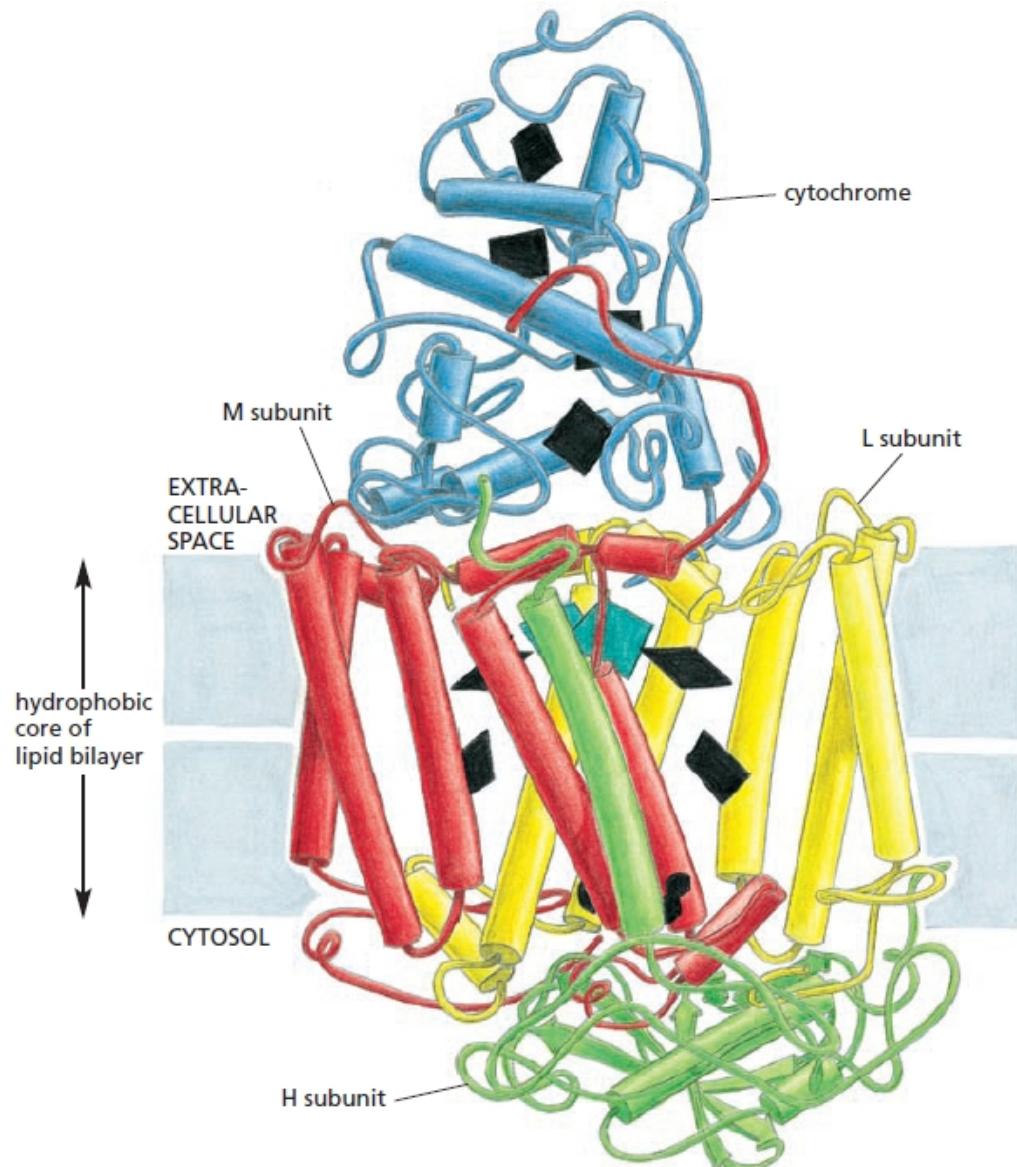


# EXAMPLE: PHOTOSYNTHETIC REACTION CENTER

- Production of high-energy electrons
- Part of photosystems
- Light-sensitive cofactors



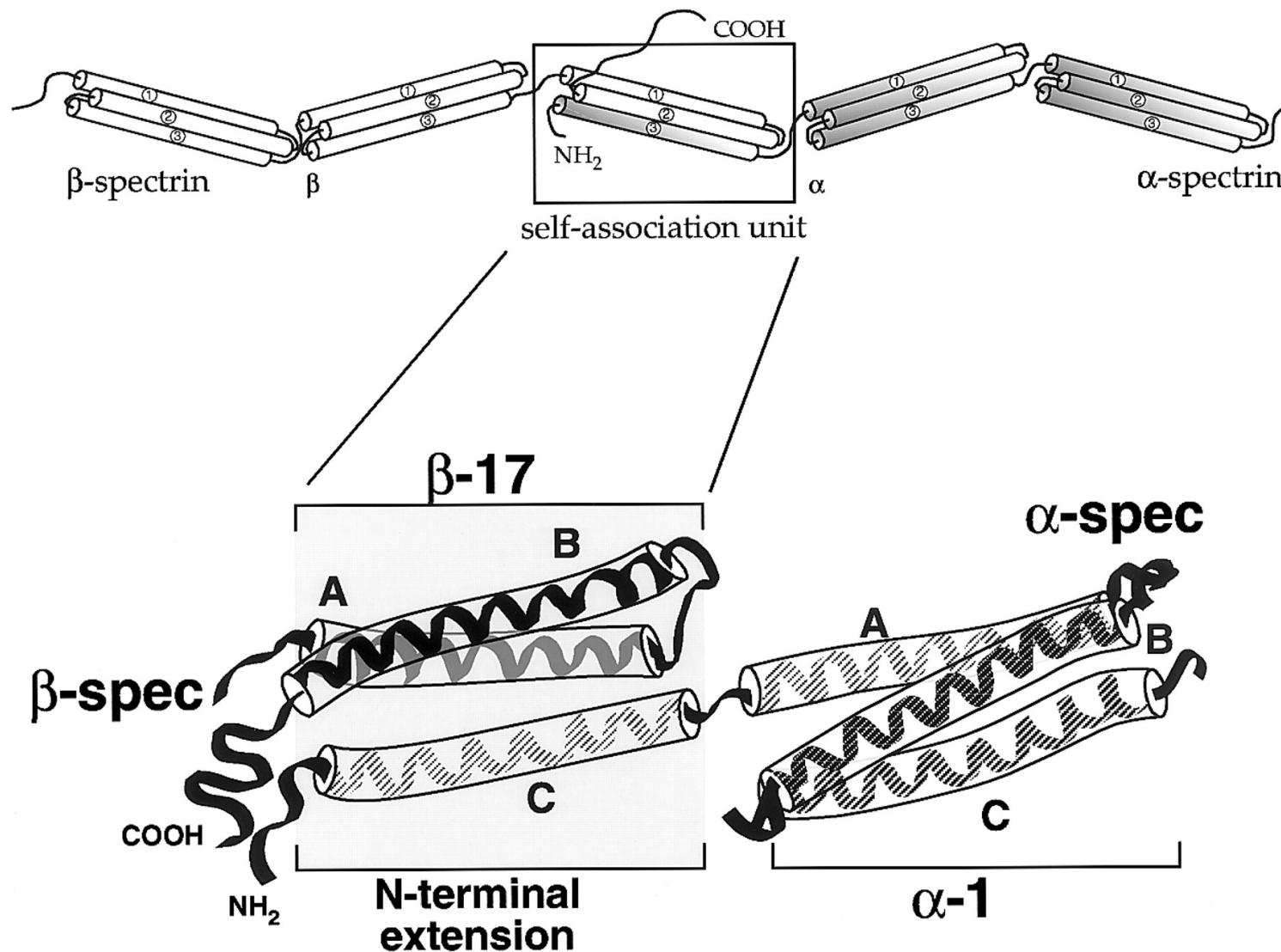
2D-crystals



# EXAMPLE: SPECTRIN

➤ Two long chains => heterodimer

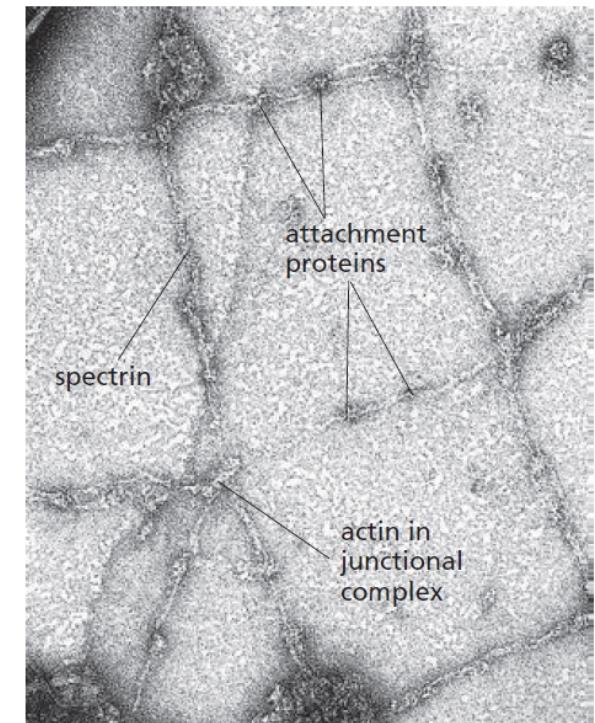
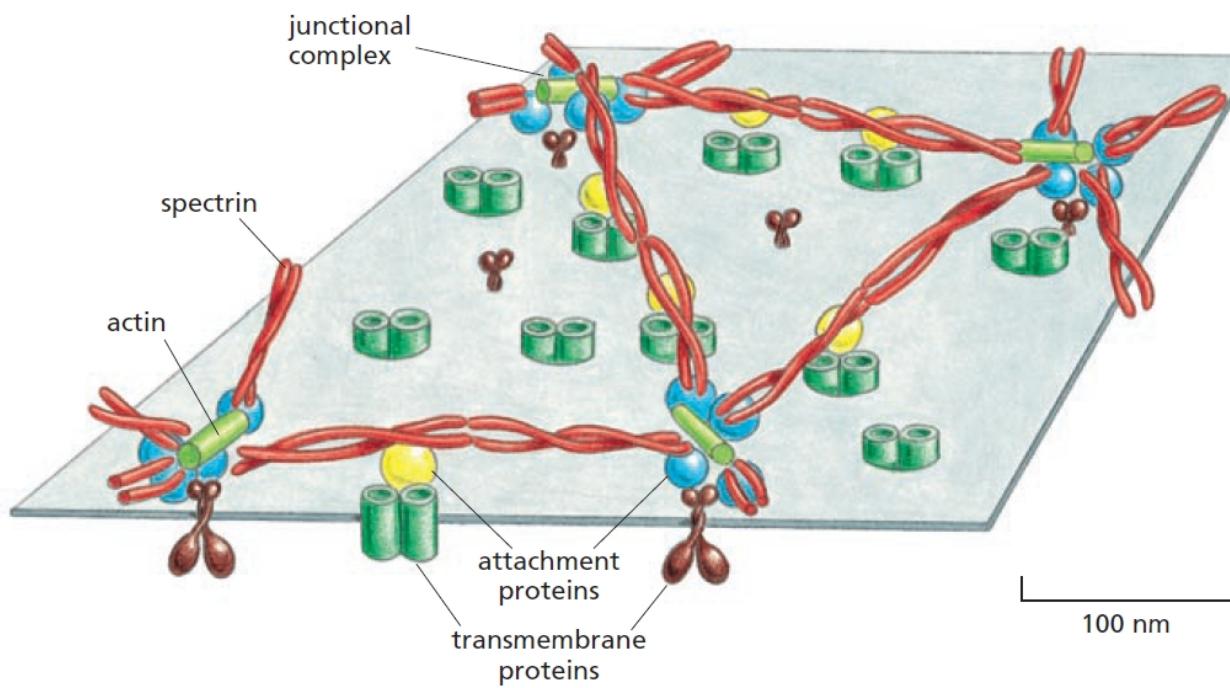
➤ 2 heterodimers => tetramer



# EXAMPLE: SPECTRIN

## ➤ Function:

- shape of red blood cells
- association with cytoskeleton



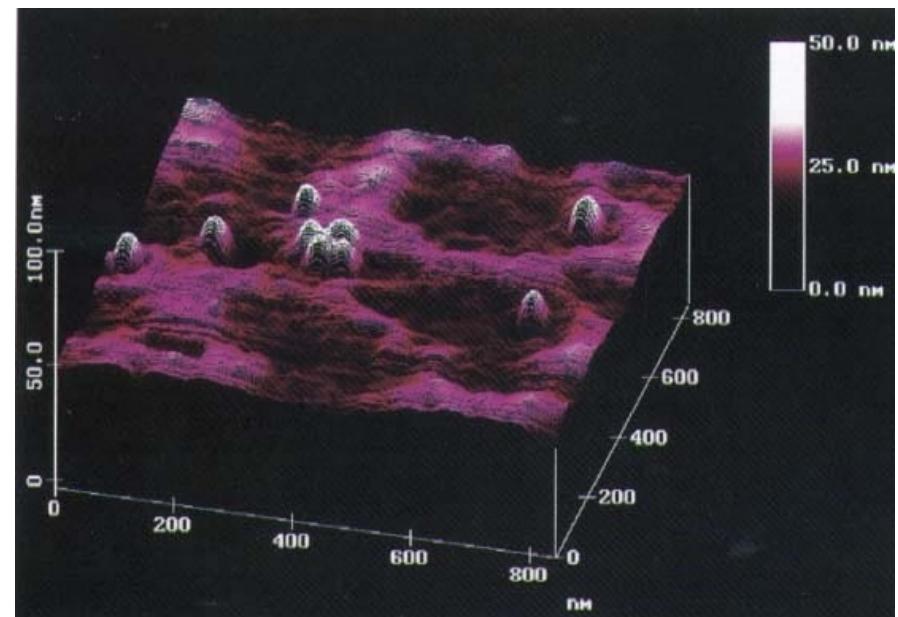
# EXAMPLE: GLYCOPORIN/BAND 3

## ➤ Glycoporin:

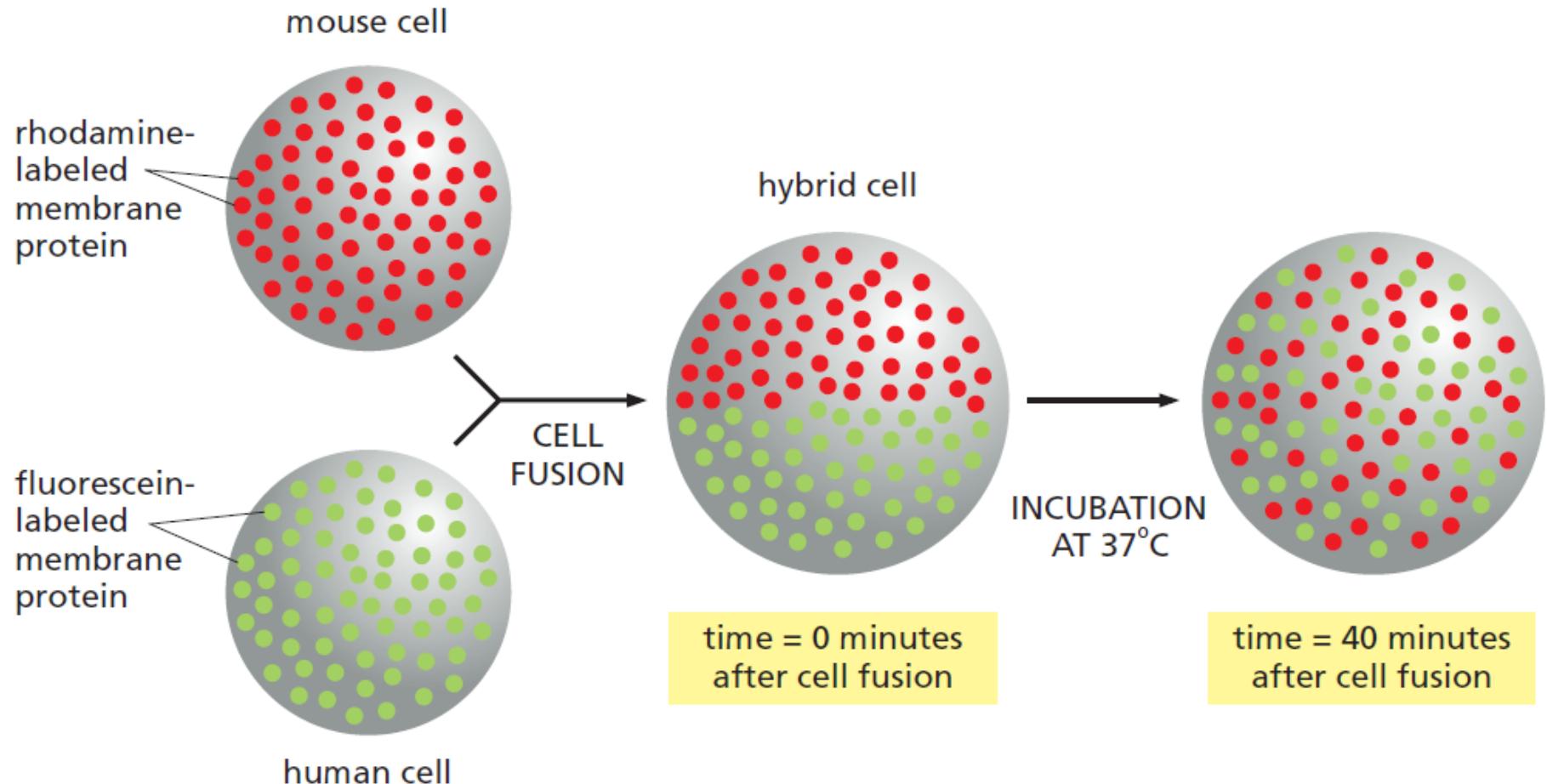
- major component of red blood cells
- 131 aa
- 1 TMD (23 aa)
- dimer
- sialised N-terminus
- fixes spectrin

## ➤ Band 3:

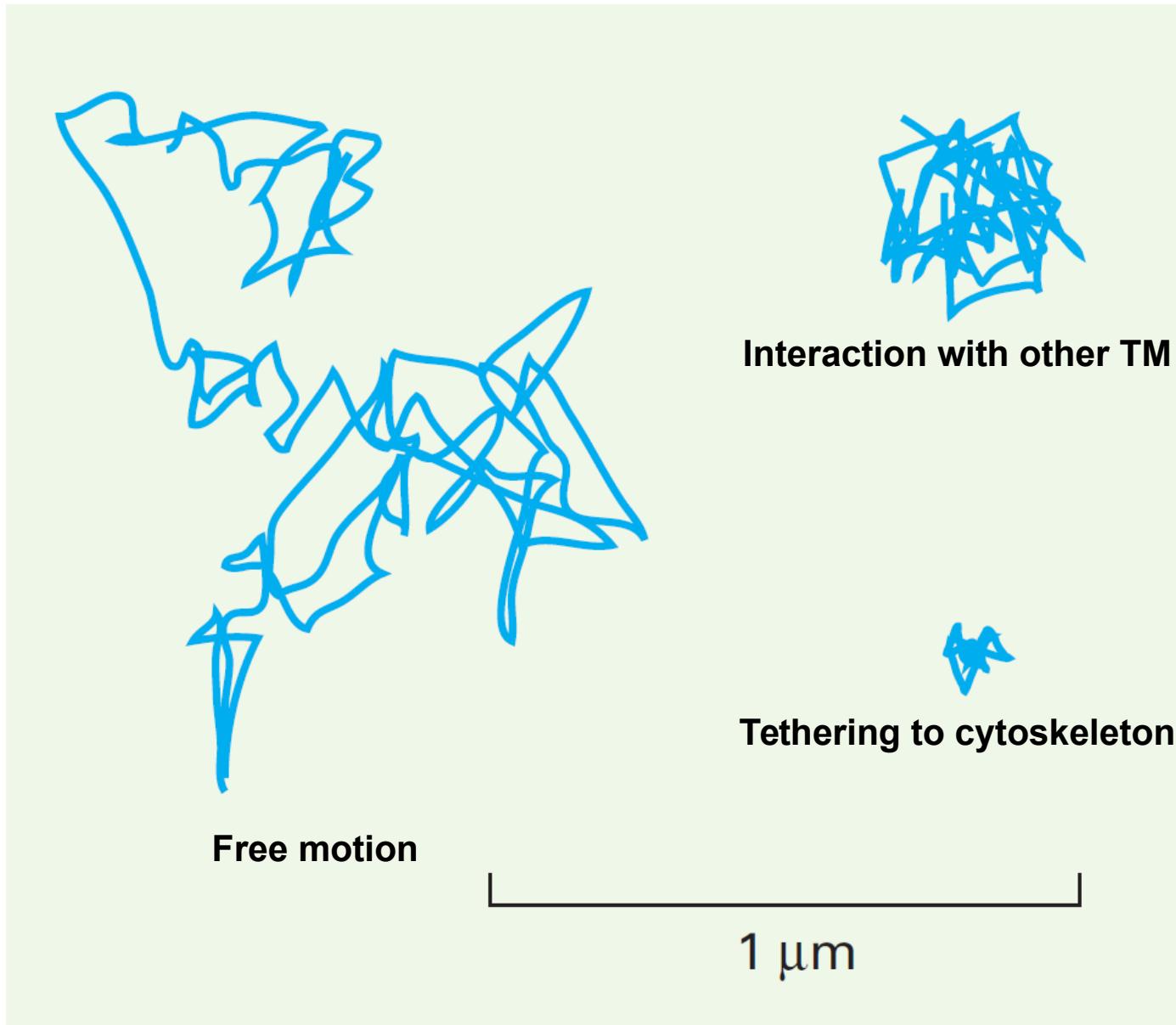
- major component of red blood cells
- 930 aa
- 12 TMD
- $\text{HCO}_3^-/\text{Cl}^-$  exchange transporter
- association with cytoskeleton



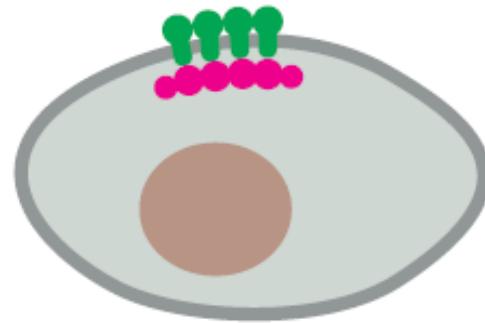
# DIFFUSION OF MEMBRANE PROTEINS



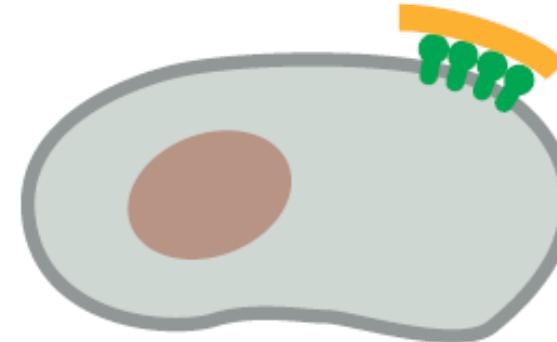
# DIFFUSION OF MEMBRANE PROTEINS



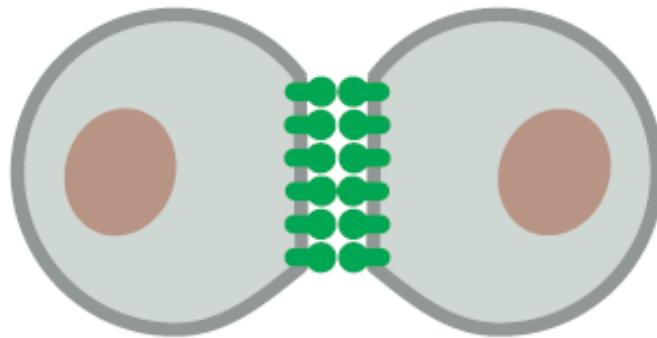
# RESTRICTION OF MEMBRANE PROTEINS DIFFUSION



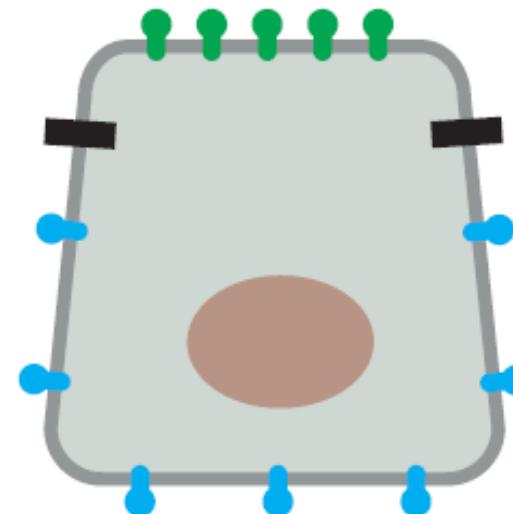
Tethering to the cell cortex



Tethering to the EM

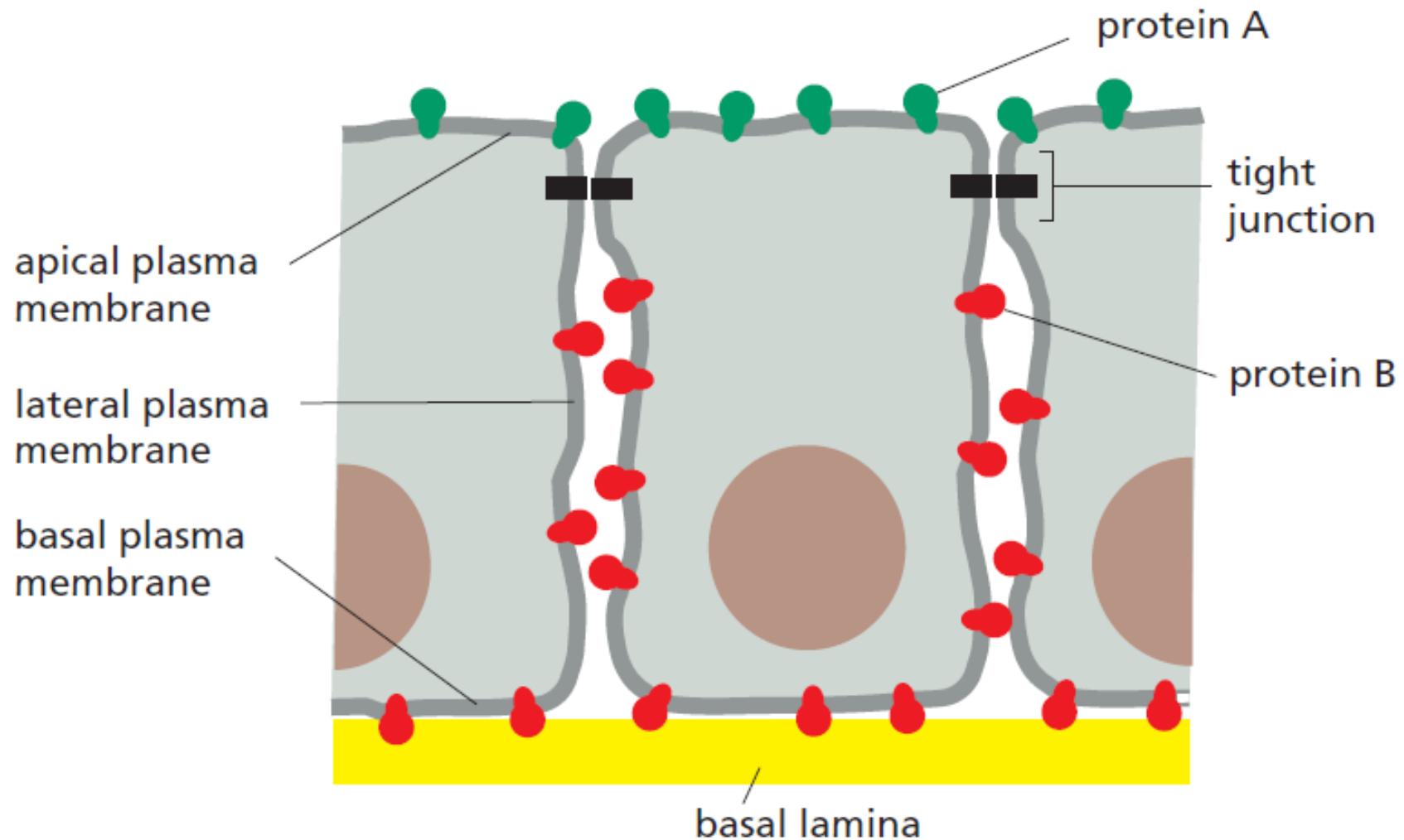


Tethering to the proteins of  
another cell

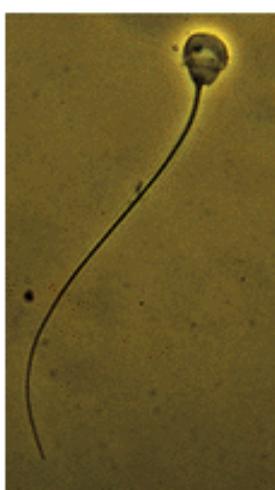
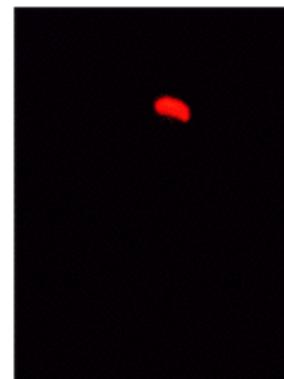
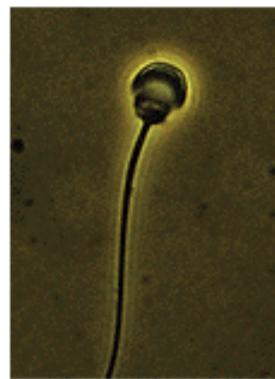
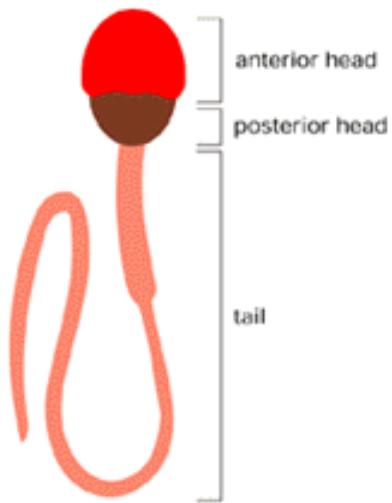


Diffusion barriers

# TIGHT FUNCTION IN GUT CELLS



# MEMBRANE DOMAINS IN A SPERM CELL



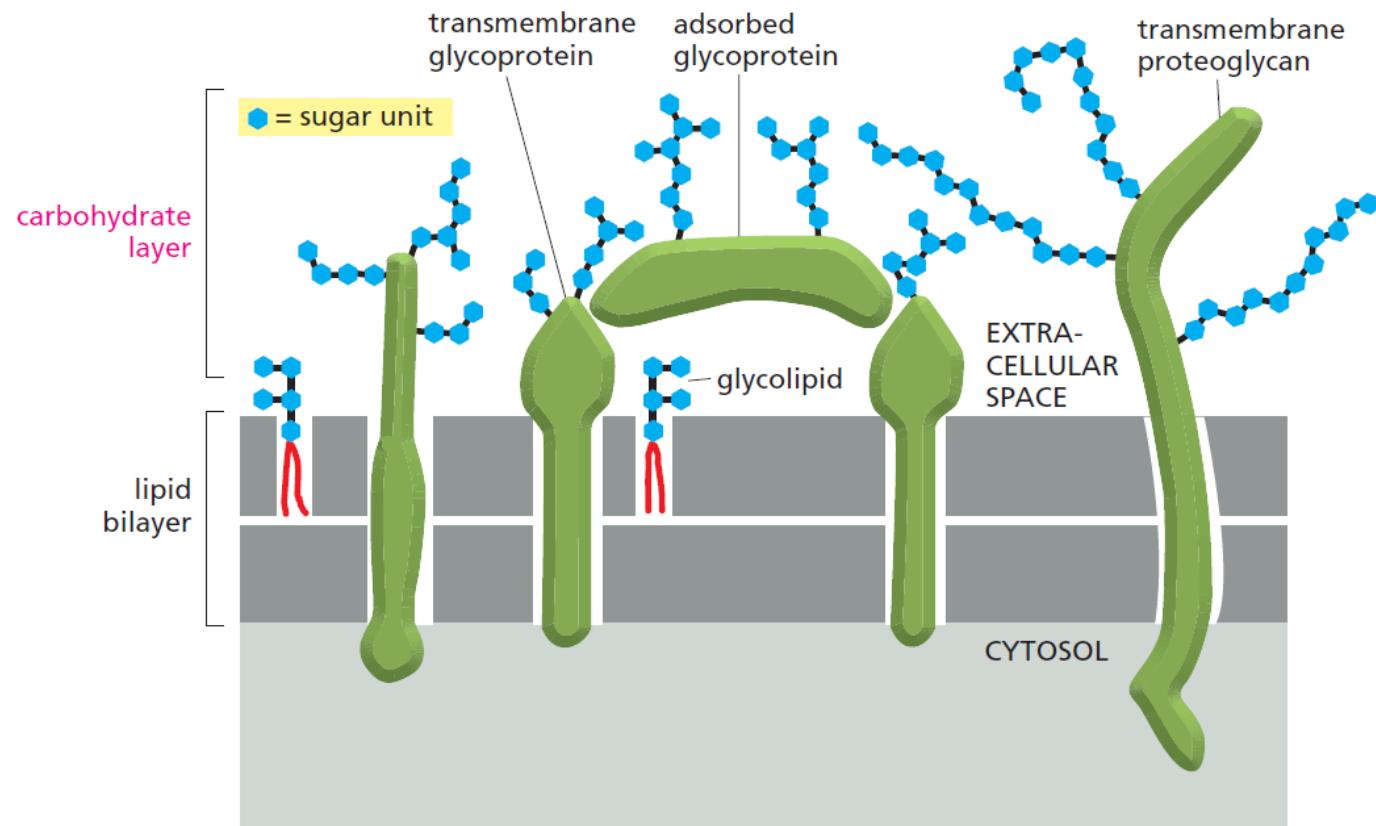
# CARBOHYDRATE LAYER OF THE MEMBRANE

## ➤ Classes:

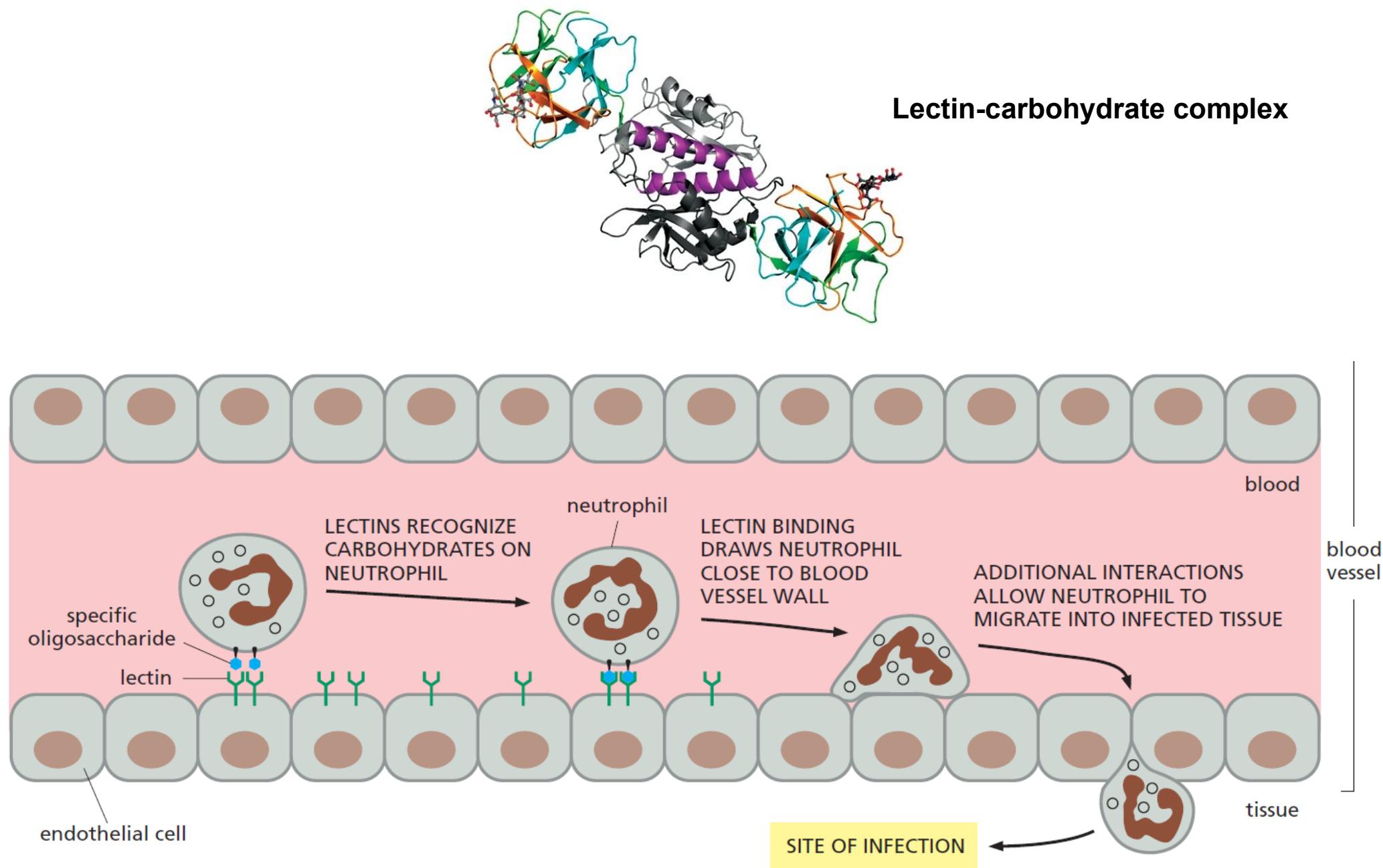
- glycolipids
- glycoproteins
- proteoglycans

## ➤ Function:

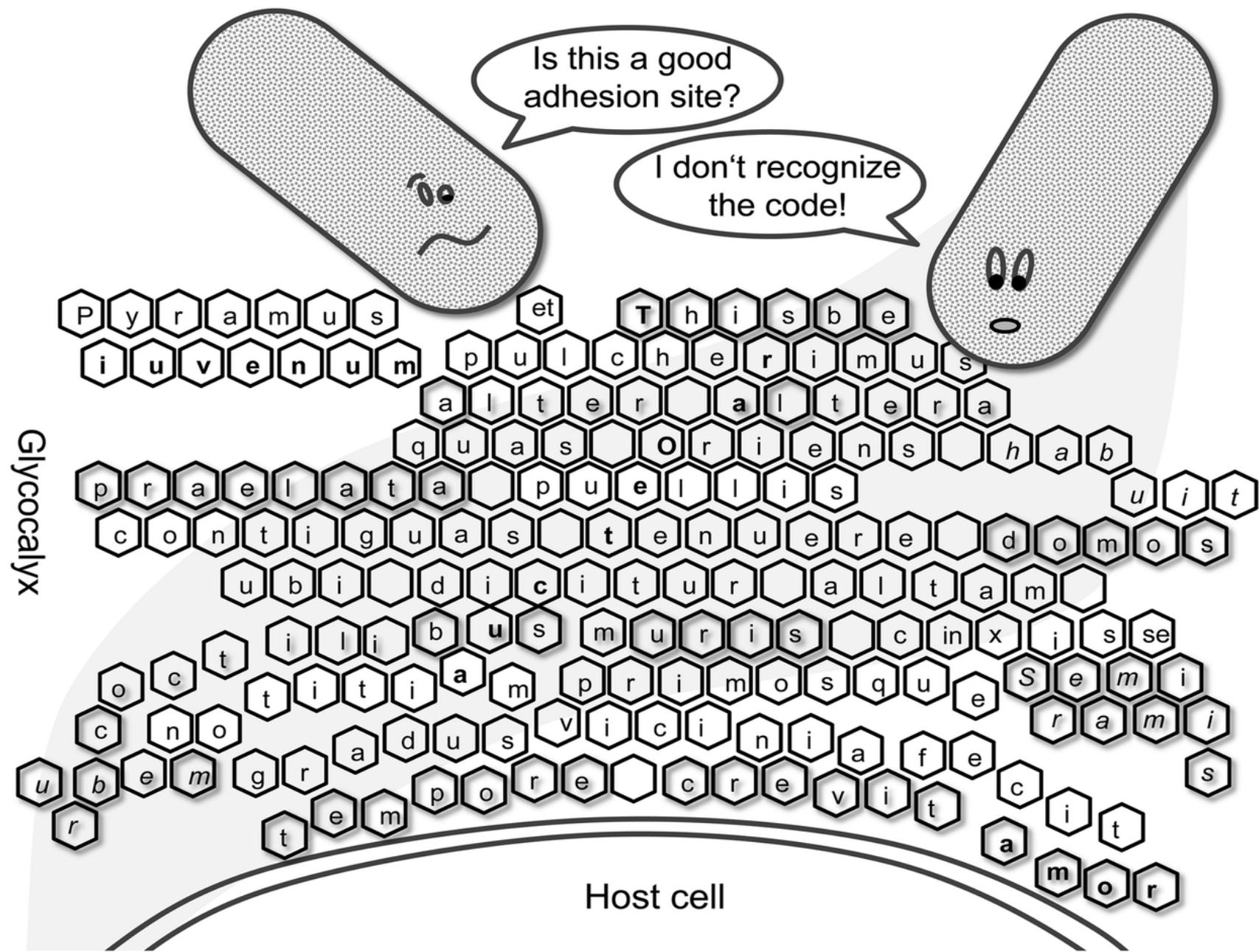
- protection
- maintenance of proper hydration
- cell recognition (lectins)



# CARBOHYDRATE LAYER OF THE MEMBRANE: LECTINS-NEUTROPHIL ACTION

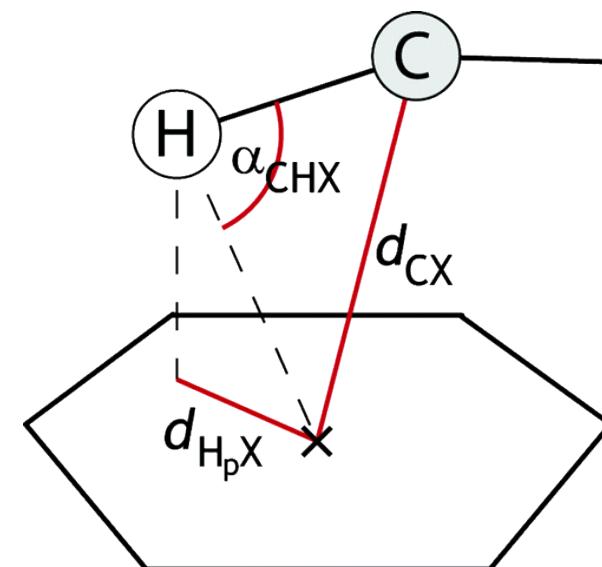


# CARBOHYDRATE CODE



# SPECIFIC FEATURES OF PROTEIN-CARBOHYDRATE INTERACTIONS

- Relatively low affinities (up to  $\sim\mu\text{M}$ )
- Importance of polyvalence
- Contributions to free energy:
  - Hydrophobic effect: classical and non-classical,  $\sim 25\text{-}100\%$  of enthalpy
  - CH/ $\pi$  interactions
  - Hydrogen bonds (also water-mediated)
  - Electrostatics+vdW
  - Solvation/Desolvation
- Not biased to cavities binding



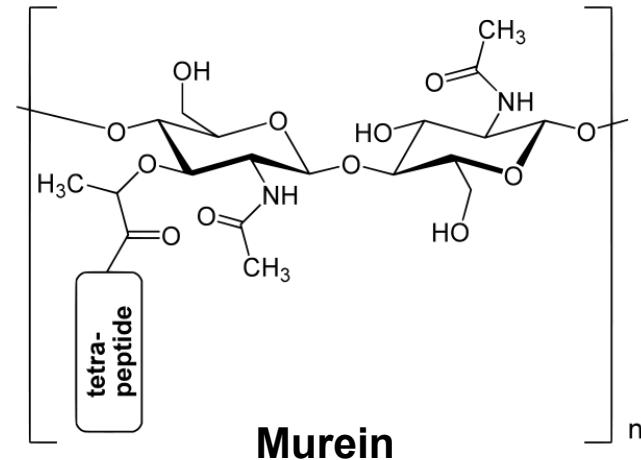
# MEMBRANE PENETRATION BY TOXINS

Toxin: poisonous substance

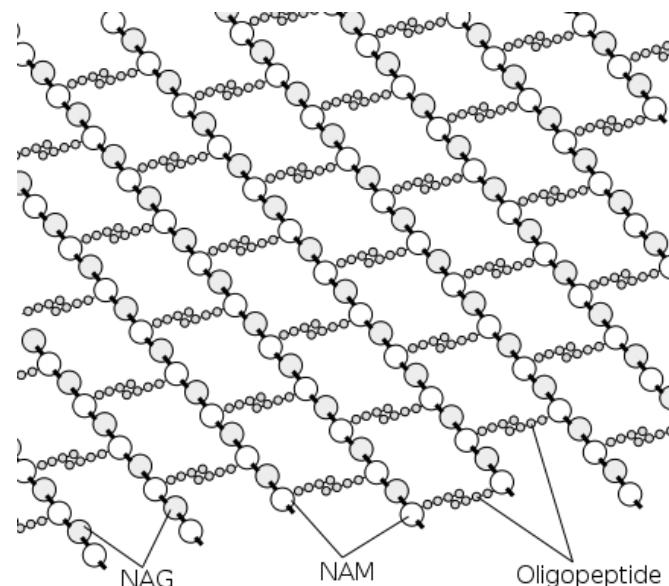
Bacteriocin: proteinic toxin inhibiting growth of bacteria

Colicin:

- Mechanism of action:
  - DNAase activity
  - RNAase acitivity
  - ribosome inactivation
  - inhibition of murein ( $\text{NAG-NAM}_n$ ) synthesis
- Structure:
  - N-terminal translocation domain
  - central domain (receptor binding)
  - C-terminal cytotoxic domain



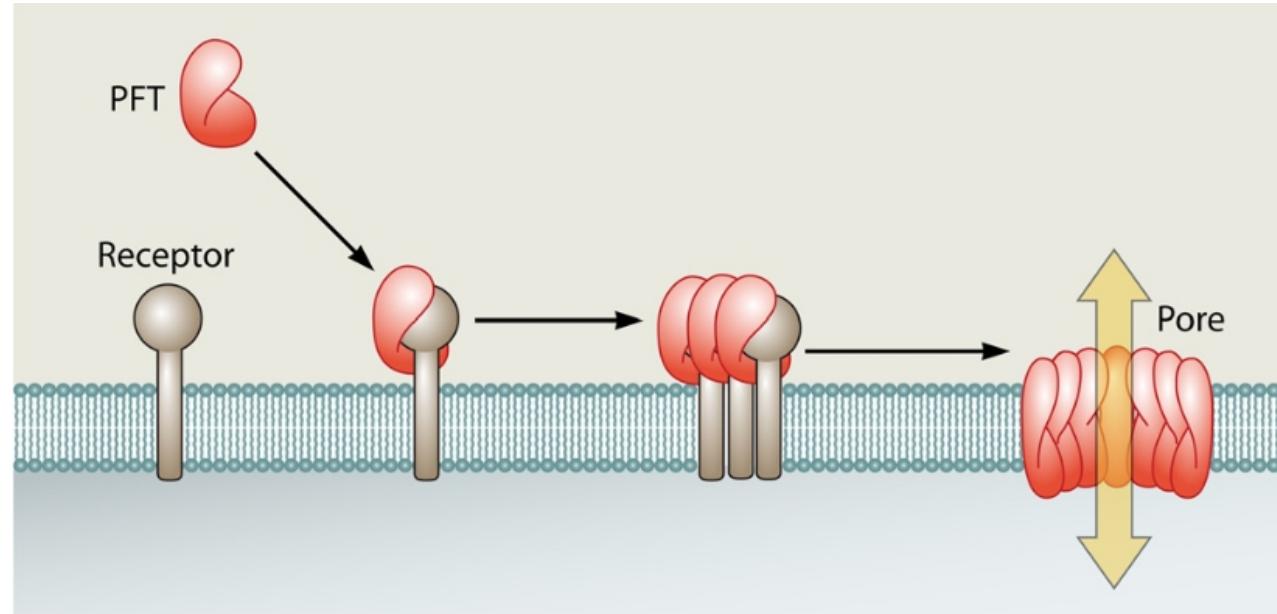
Murein



# BACTERIAL PORE FORMING TOXINS (PTFS)

## Common bacterial cytotoxic proteins

- Steps:
  - Receptor recognition
  - Membrane association
  - Conformational change
  - Pore formation



- Structural diversity

- Size: 0.5-100 nm

- Pathology:

- pneumoniae

- tuberculosis

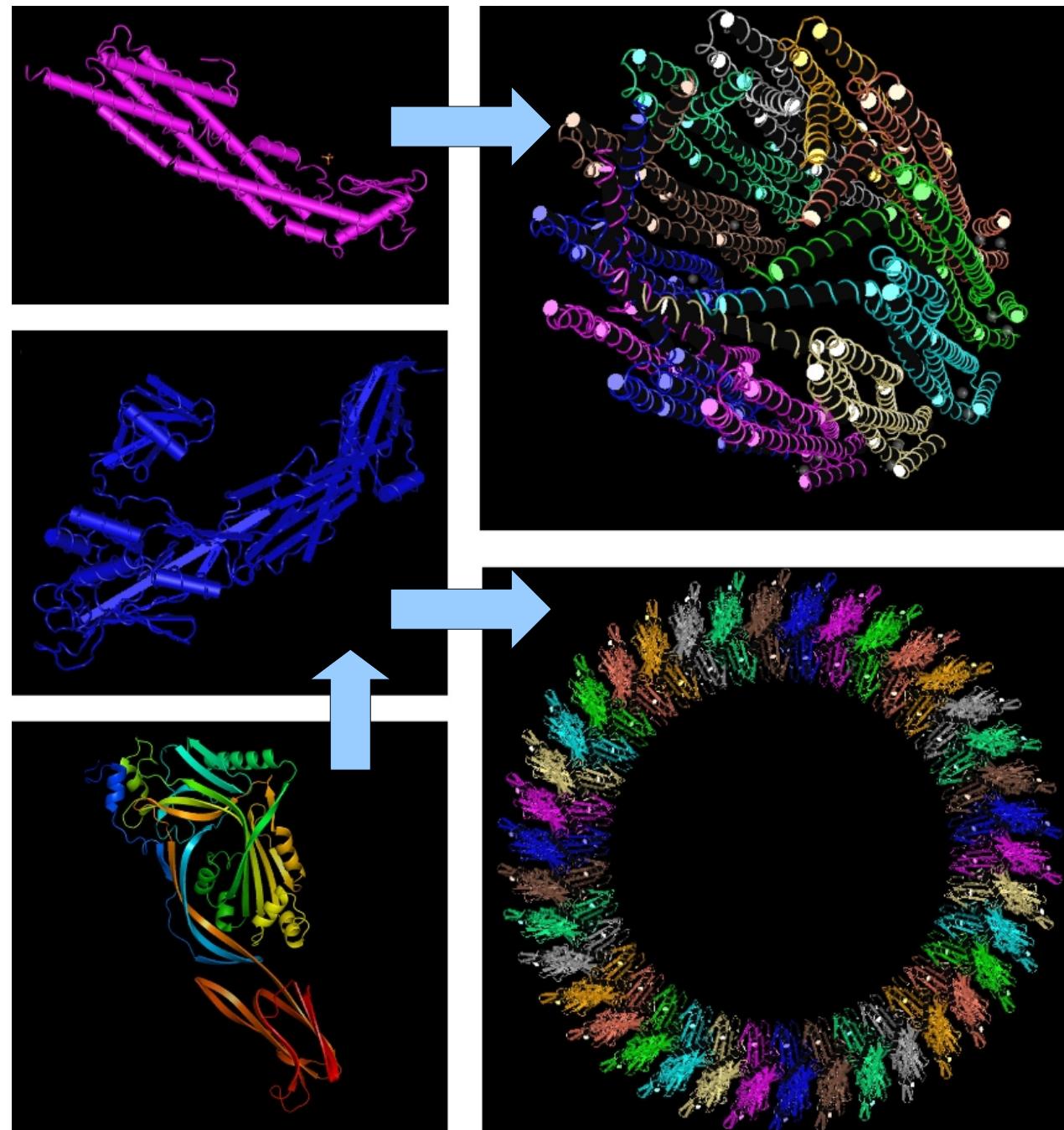
- Some are highly antibiotic-resistant

Soluble

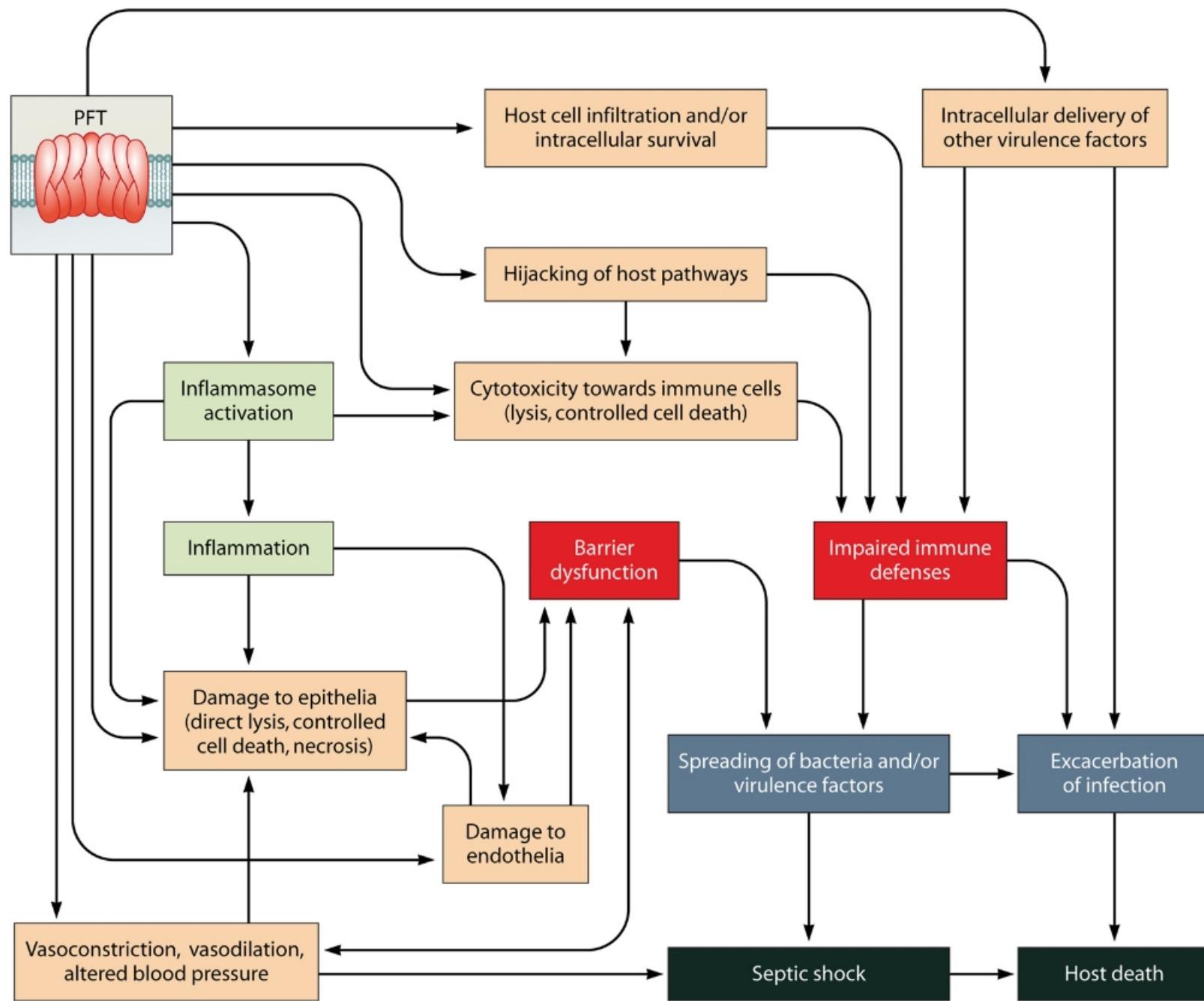


TM

# PFTS STRUCTURAL DIVERSITY



# PFTS: ACTION



# PFTS: SCENARIOS

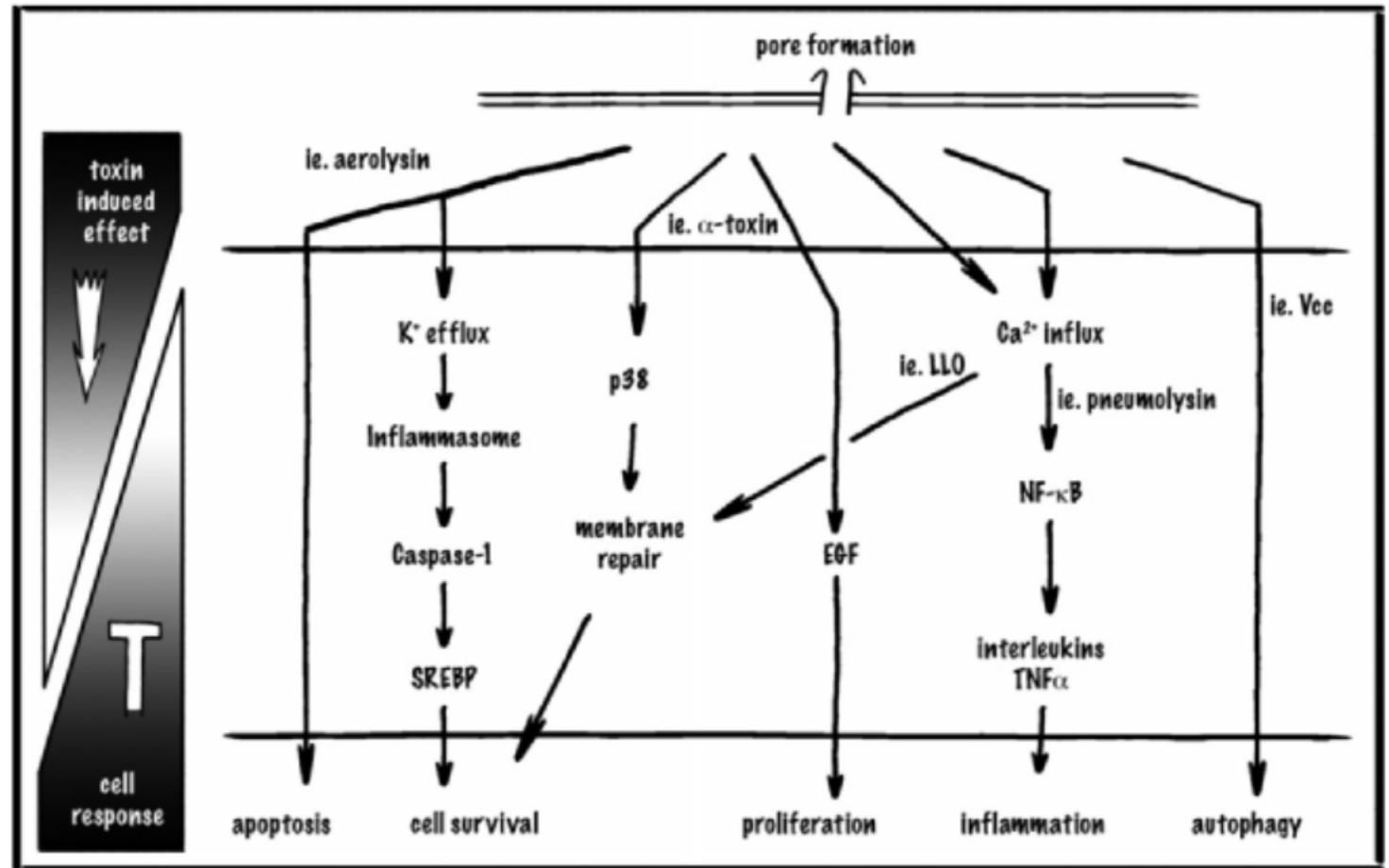
➤ Apoptosis

➤ Cell survival

➤ Proliferation

➤ Inflammation

➤ Autophagy

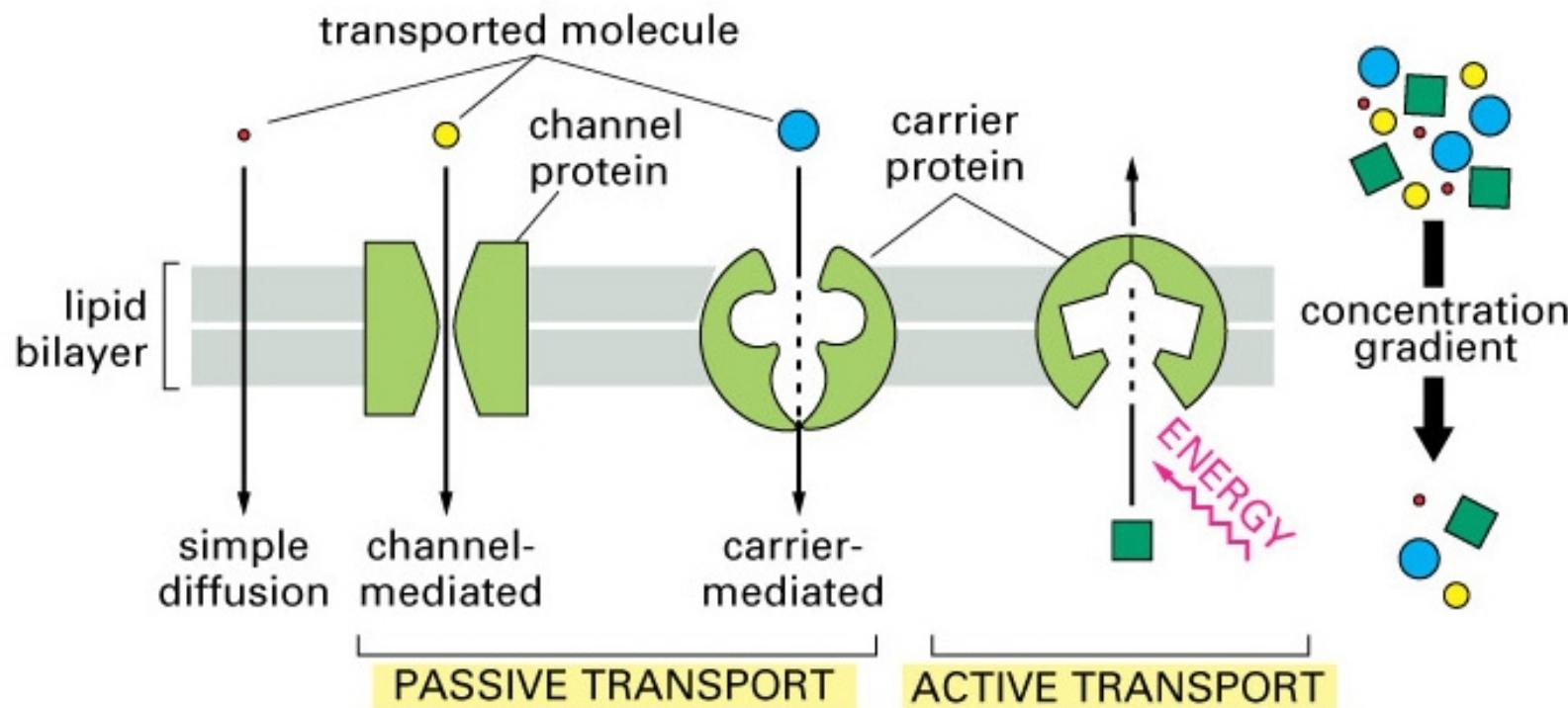
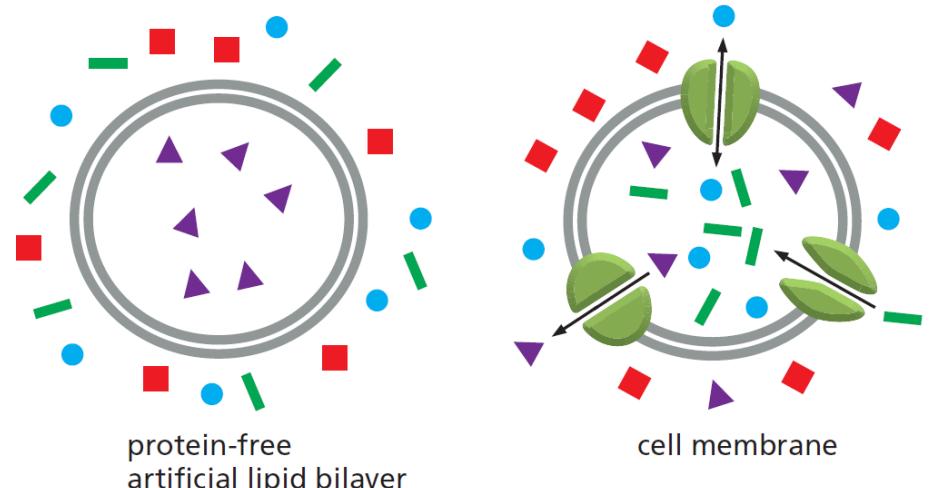


# MEMBRANE TRANSPORT: TYPES

Mechanisms regulating the passage of solutes through membranes

➤ Classifications:

- passive/active
- diffusion/protein-mediated
- specialized/non-specialized



# IONS INSIDE AND OUTSIDE THE CELL

➤ **Transport affects:**

- difference in concentrations of different ions: specificity
- charge balancing

➤ **Controls:**

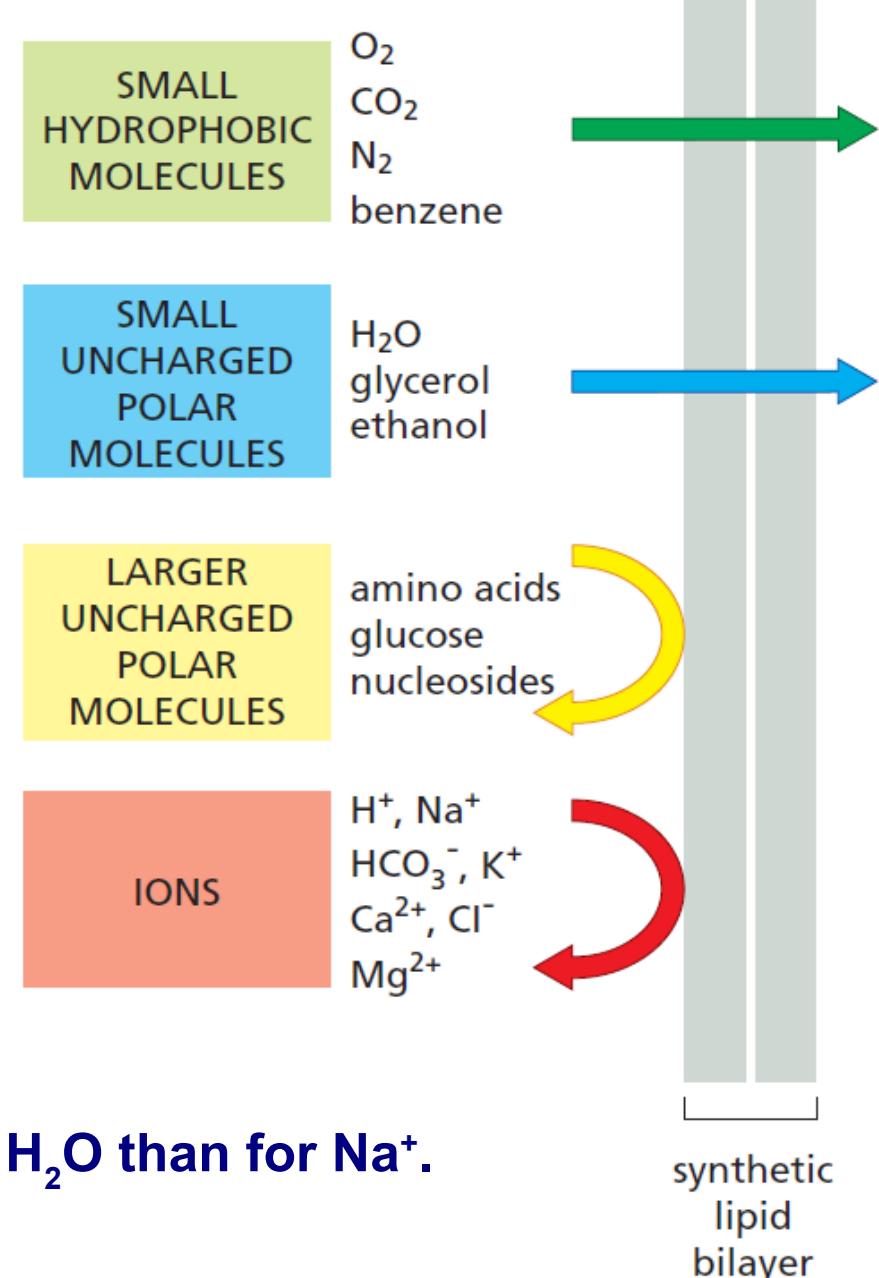
- permeability
- transporters

COMPONENT	INTRACELLULAR CONCENTRATION (mM)	EXTRACELLULAR CONCENTRATION (mM)
Cations		
Na <sup>+</sup>	5–15	145
K <sup>+</sup>	140	5
Mg <sup>2+</sup>	0.5	1–2
Ca <sup>2+</sup>	10 <sup>-4</sup>	1–2
H <sup>+</sup>	$7 \times 10^{-5}$ ( $10^{-7.2}$ M or pH 7.2)	$4 \times 10^{-5}$ ( $10^{-7.4}$ M or pH 7.4)
Anions*		
Cl <sup>-</sup>	5–15	110

# MEMBRANE PERMEABILITY

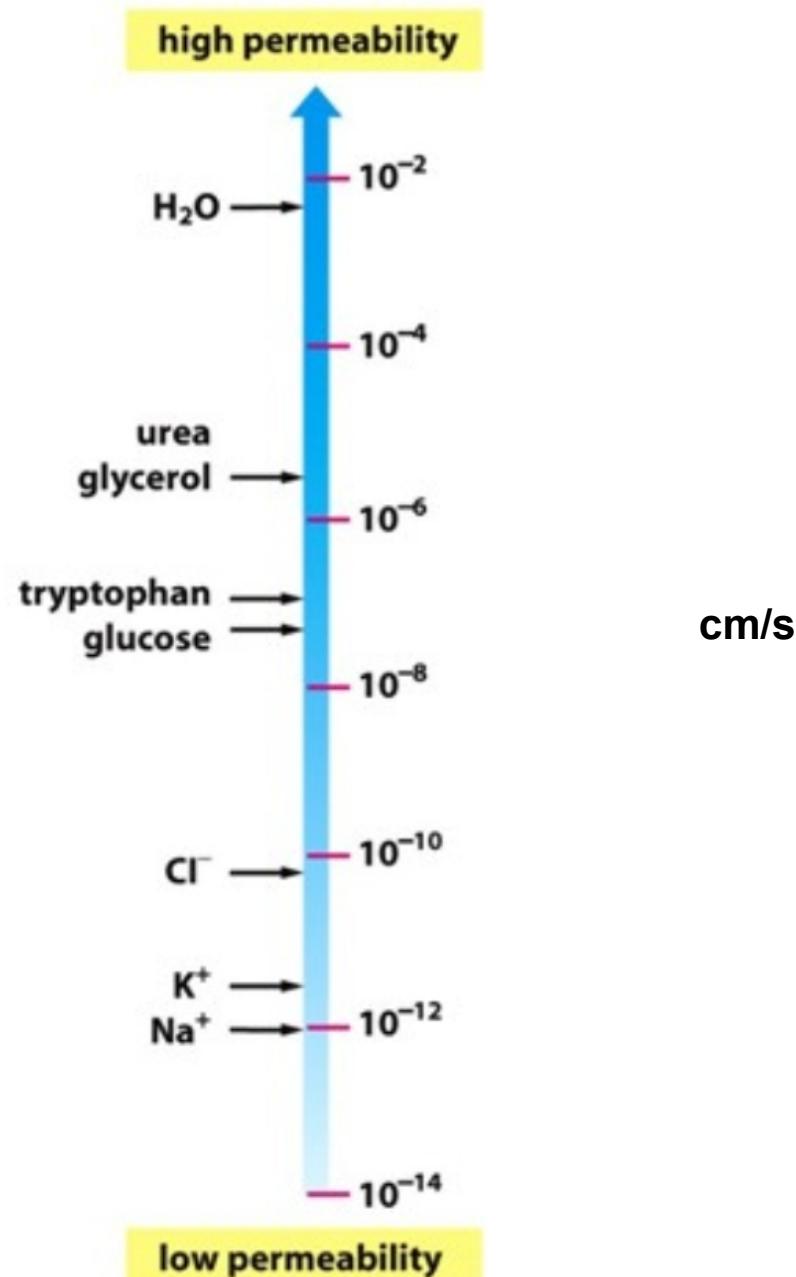
ALL molecules can diffuse through the membrane BUT with different rates.

- Small nonpolar molecules
- Uncharged polar molecules
- Ions and charged molecules



# MEMBRANE PERMEABILITY COEFFICIENT

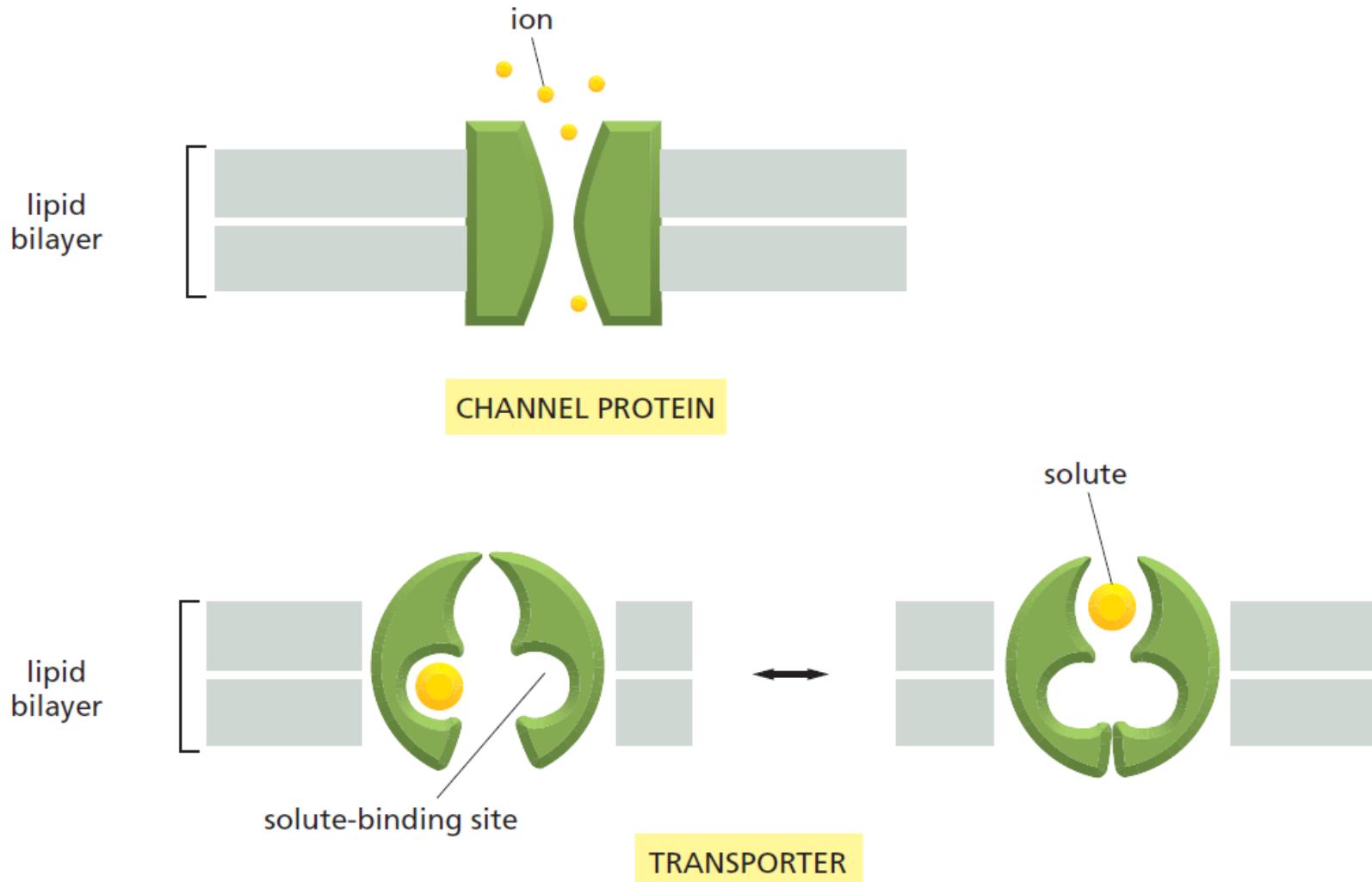
- Size
- Charge



# PROTEIN-MEDIATED MEMBRANE TRANSPORT

➤ Channels: size and electric charge

➤ Transporters: binding site



# PASSIVE AND ACTIVE TRANSPORT

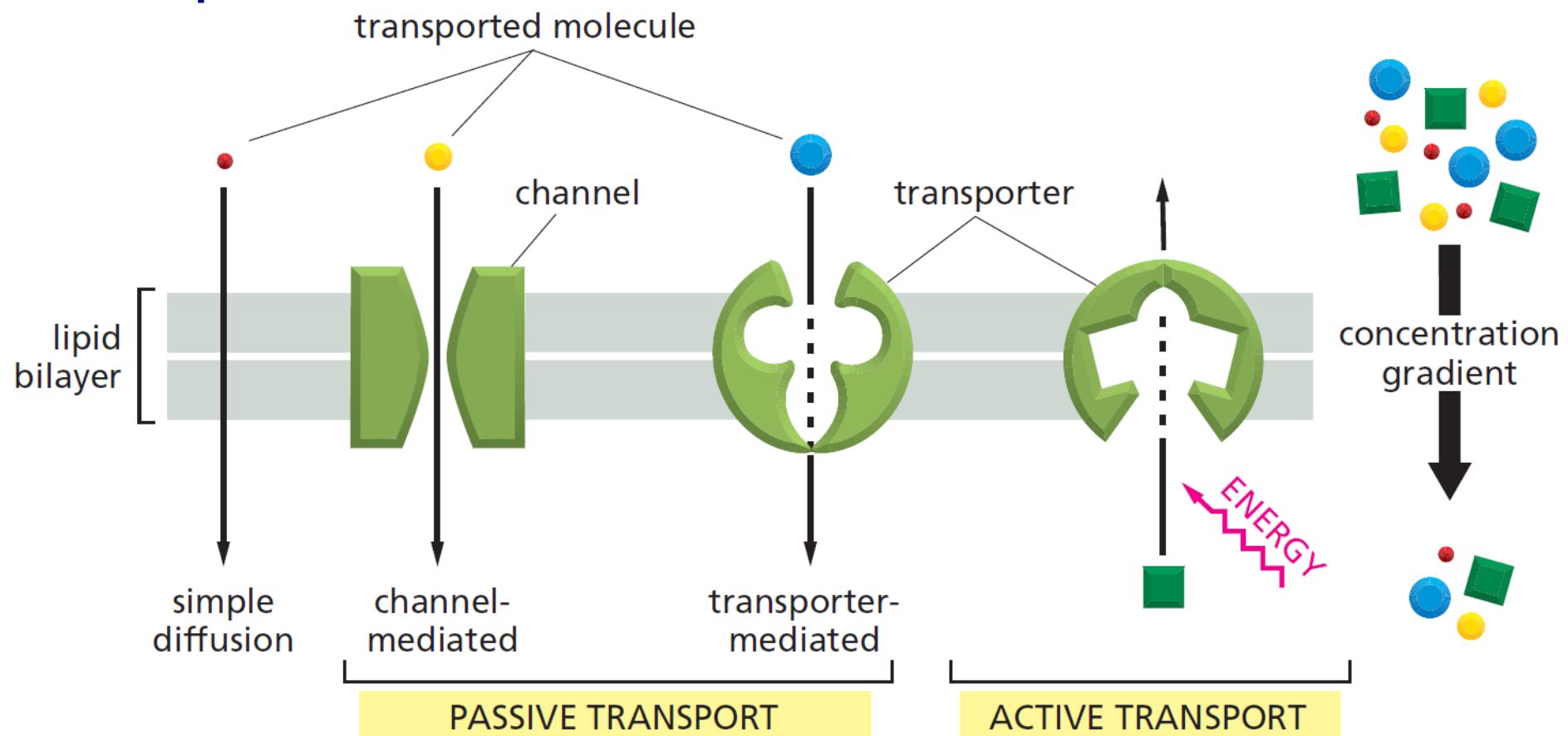
➤ Passive: concentration-dependent

- all channels

- some transporters

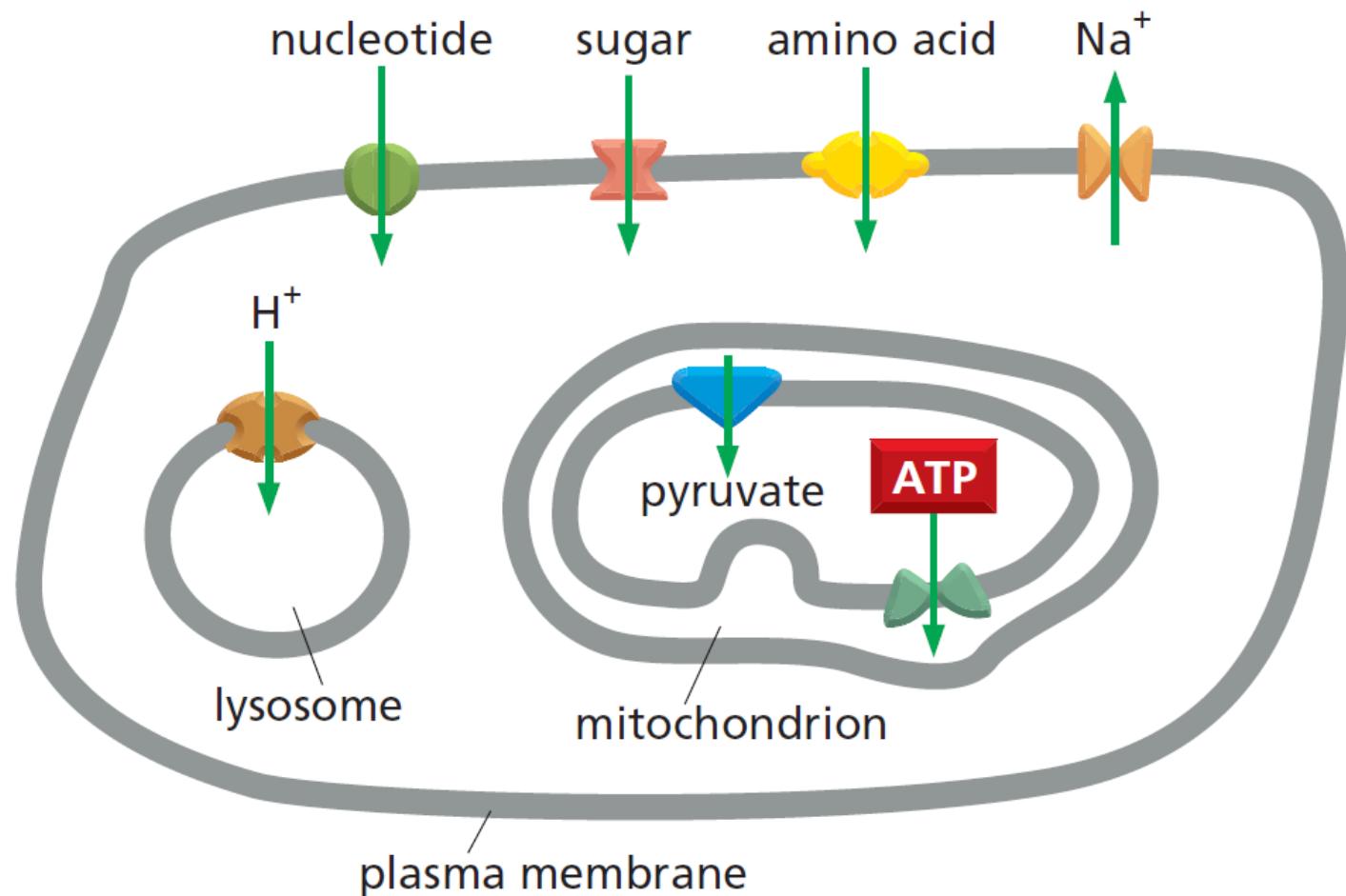
➤ Active: against the gradient of concentration

- transporters



# TRANSPORTERS

- Most of organic molecules (exception: fat soluble, small uncharged)
- High specificity:
  - transported molecules
  - organelle



# PASSIVE TRANSPORT

➤ Electrochemical gradient:

- concentration

- voltage

➤ Glucose transporter:

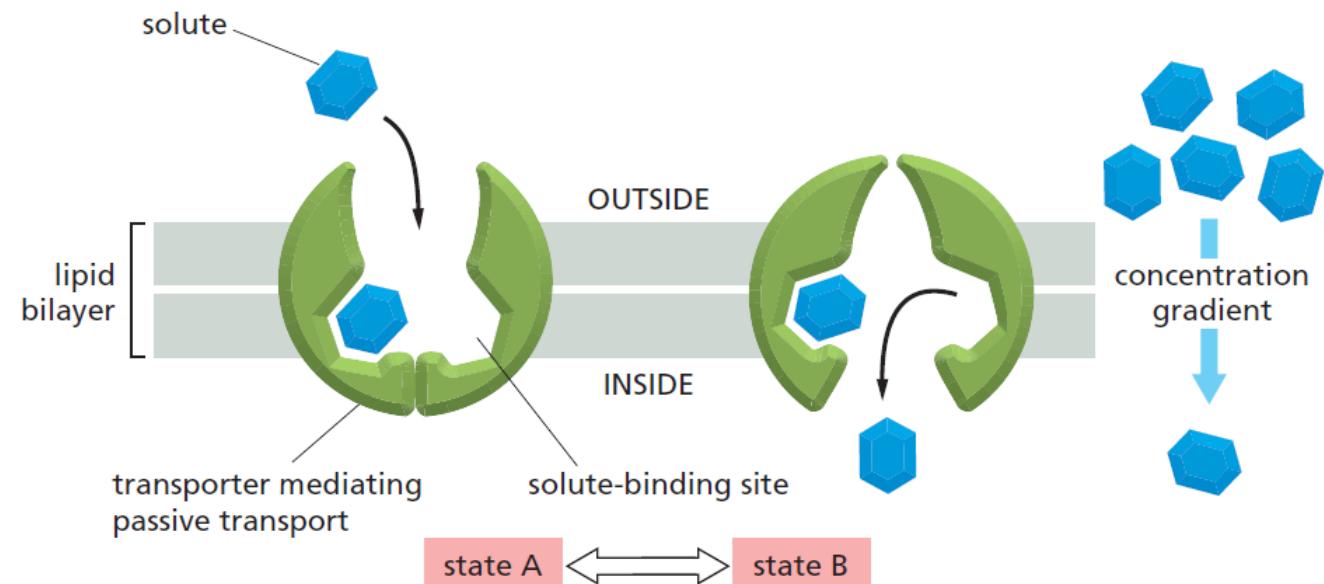
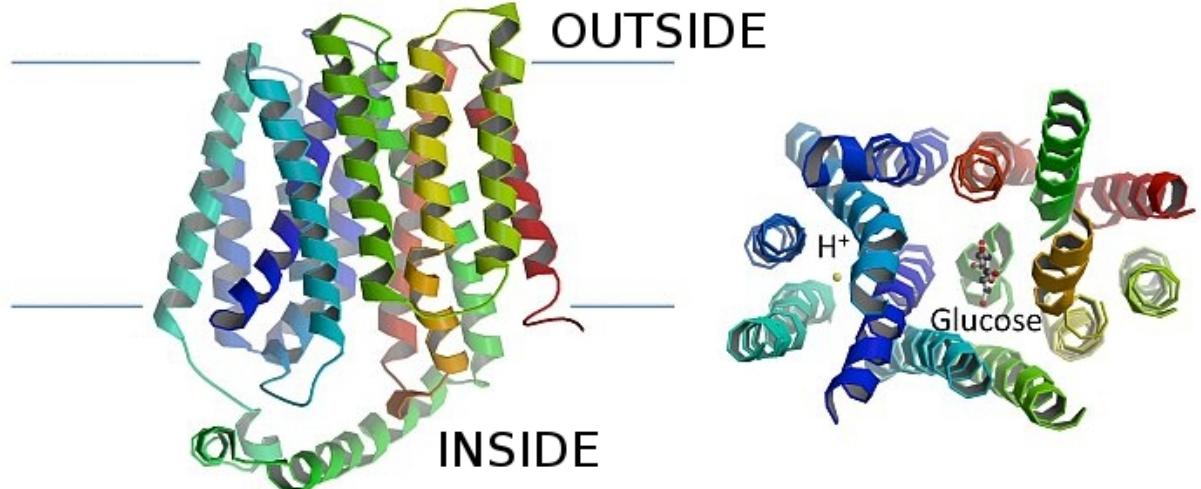
- 12 TMD

- 2 conformations

- random switch

- either directions

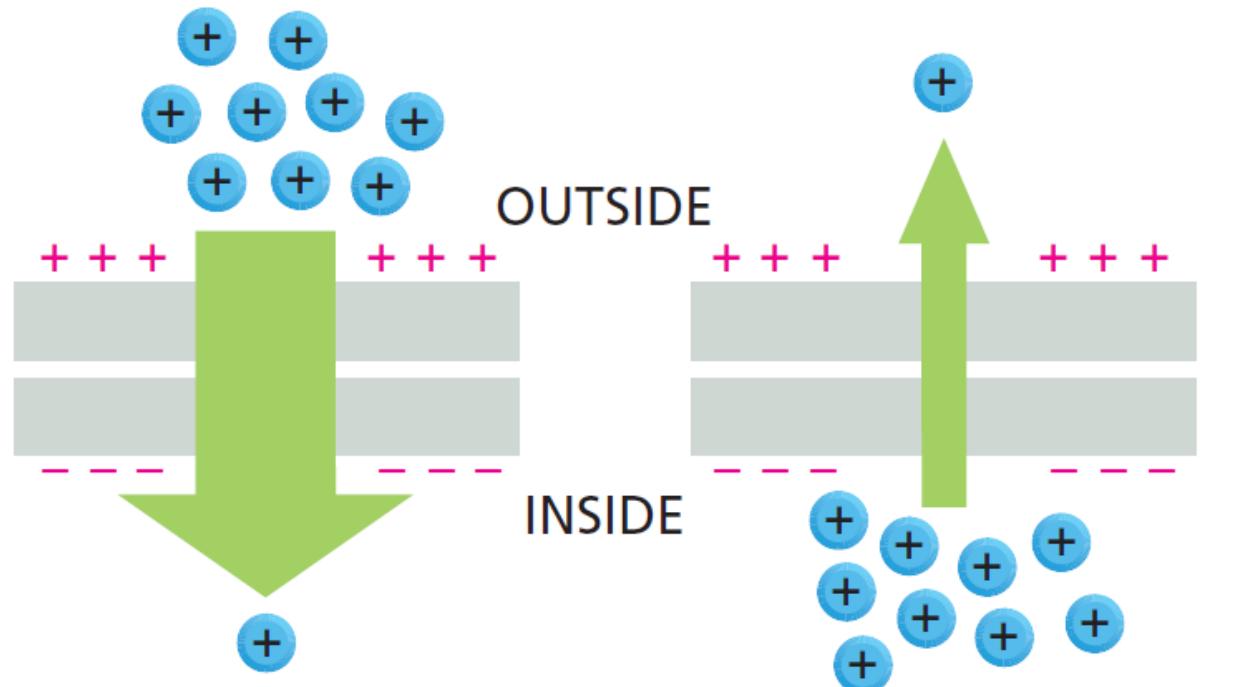
- physiological control



# PASSIVE TRANSPORT

## ➤ Electrochemical gradient:

- voltage and concentration: same direction ( $\text{Na}^+$ )
- voltage and concentration: different direction ( $\text{K}^+$ )



electrochemical  
gradient when voltage  
and concentration  
gradients work in  
the same direction

electrochemical  
gradient when voltage  
and concentration  
gradients work in  
opposite directions

# ACTIVE TRANSPORT

➤ Against electrochemical gradient

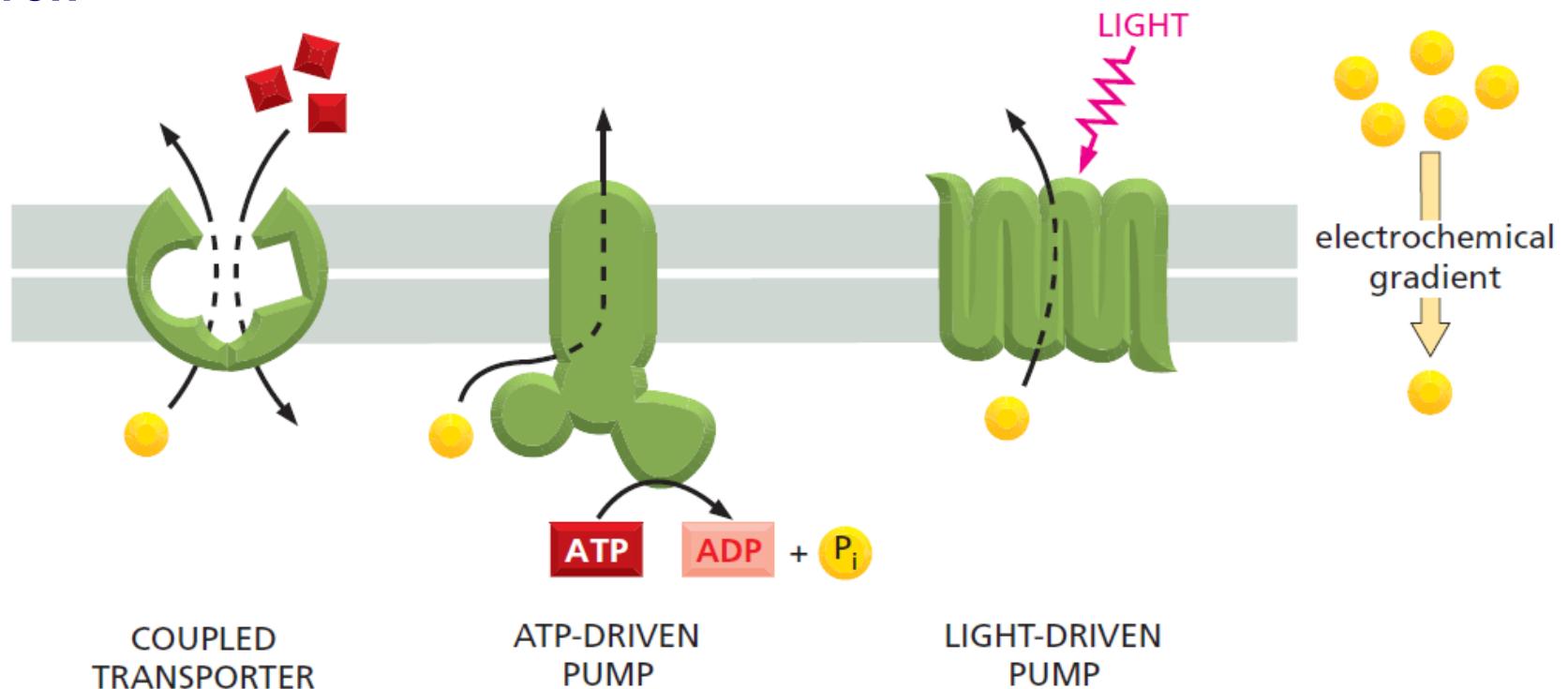
➤ Requires energy

➤ Classes:

- coupled

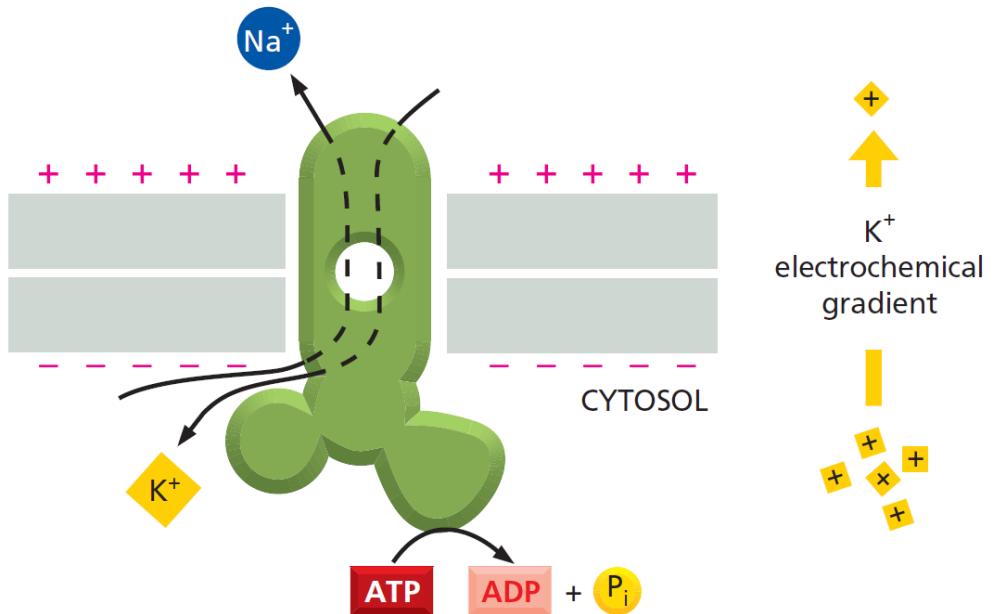
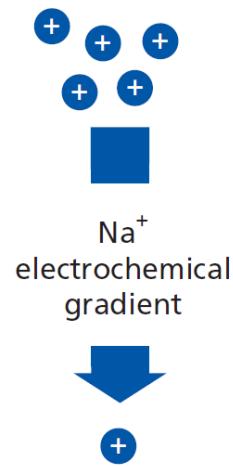
- ATP-driven

- light-driven

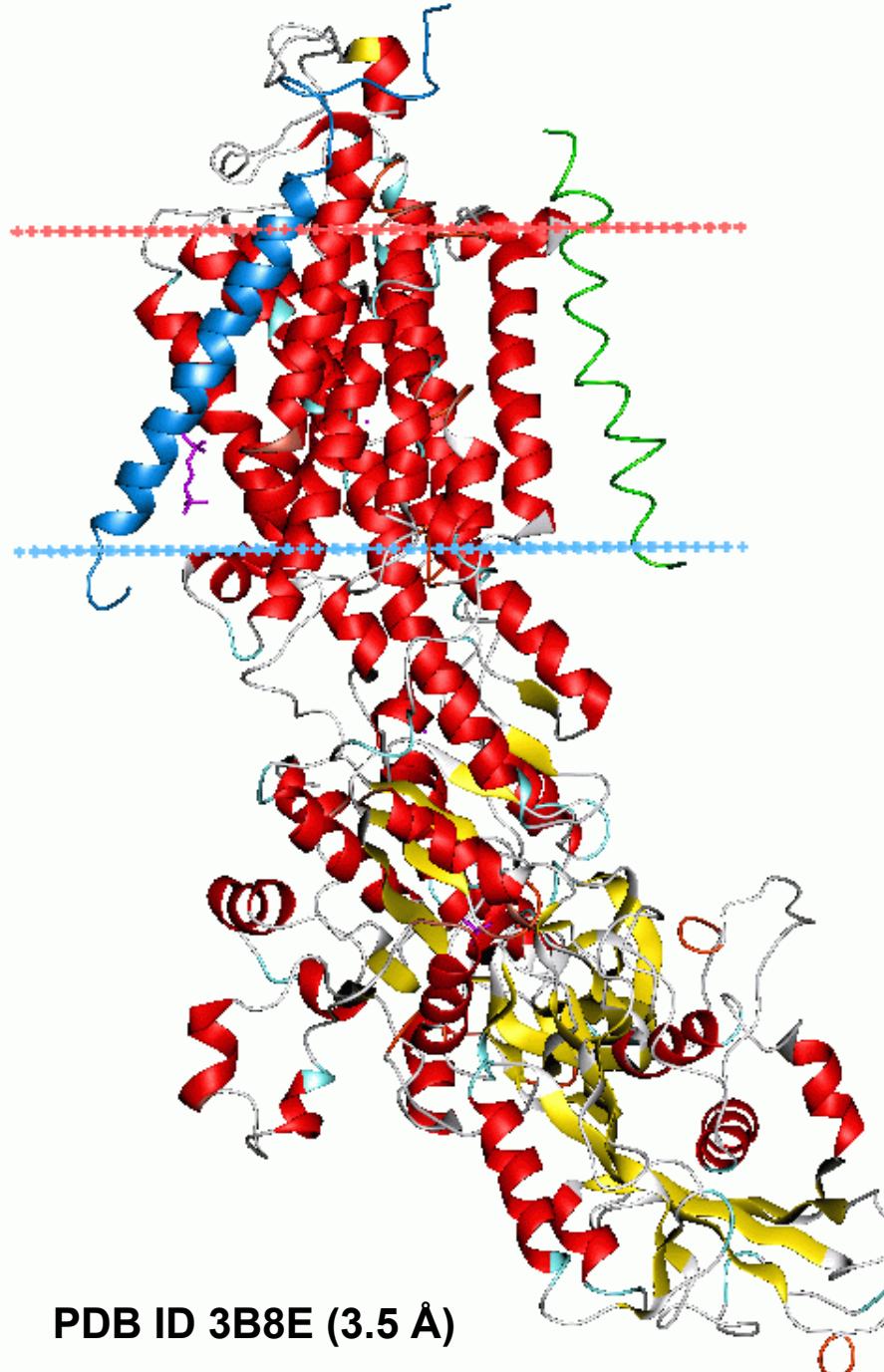


# $\text{Na}^+/\text{K}^+$ ATPASE

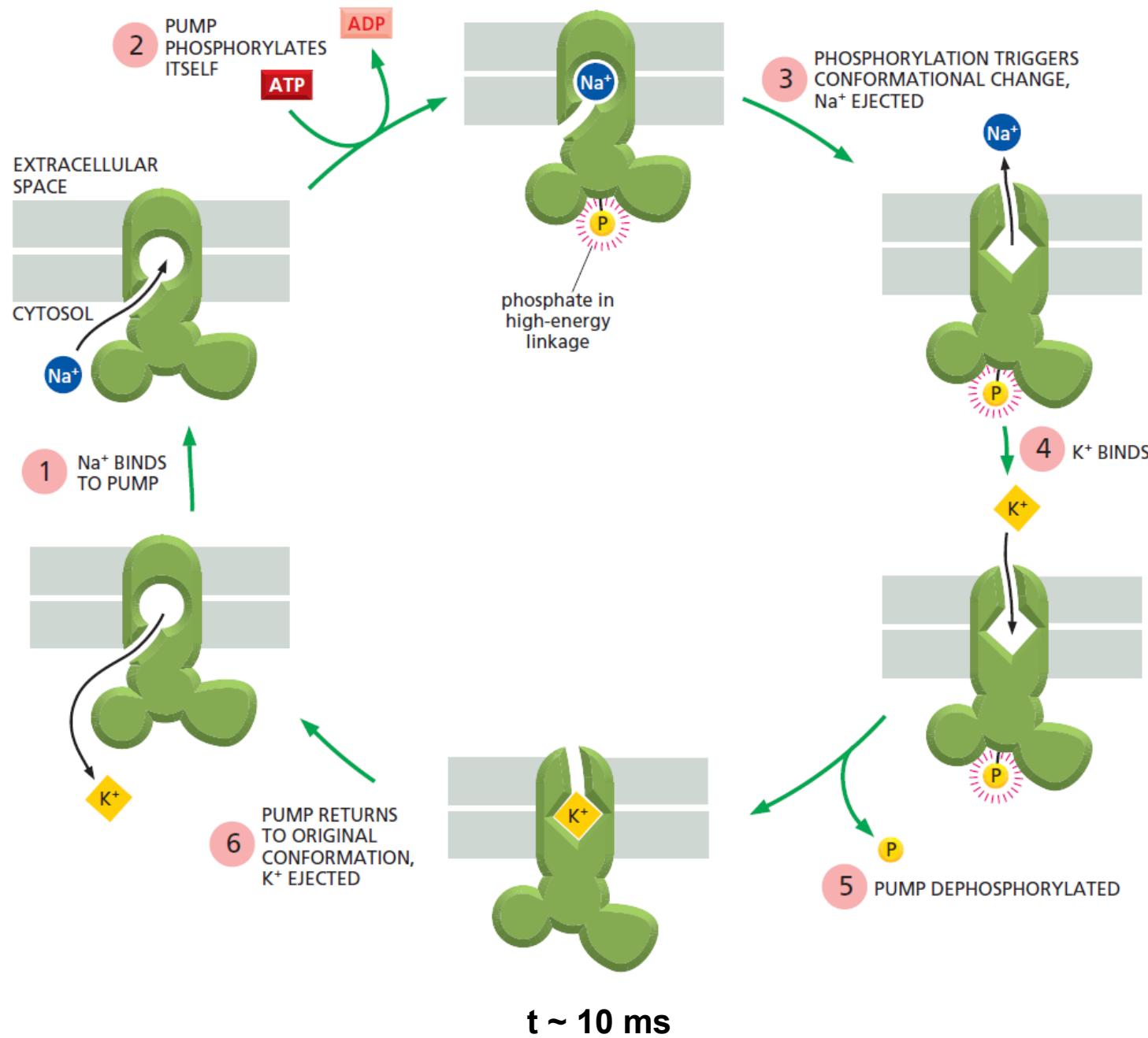
- $[\text{Na}^+]_{\text{out}} \sim 10-30 [\text{Na}^+]_{\text{in}}$
- $[\text{K}^+]_{\text{out}} \sim 0.01-0.03 [\text{K}^+]_{\text{in}}$
- $q(\text{membrane})_{\text{in}} < 0$
- 30% ATP production



# NA<sup>+</sup>/K<sup>+</sup> ATPASE STRUCTURE



# $\text{Na}^+/\text{K}^+$ ATPASE MECHANISM



# $\text{Na}^+/\text{K}^+$ ATPASE

## ➤ P-type ATPase:

- cations/ATPase

- 10 TMD

- vanadate inhibition

## ➤ Balance:

- $\text{ATP} + \text{H}_2\text{O} \Rightarrow \text{ADP} + \text{P}_i$

- $3\text{Na}^+ \Rightarrow \text{out}$

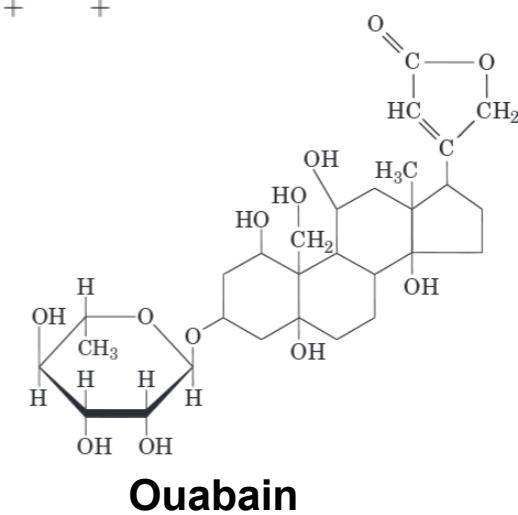
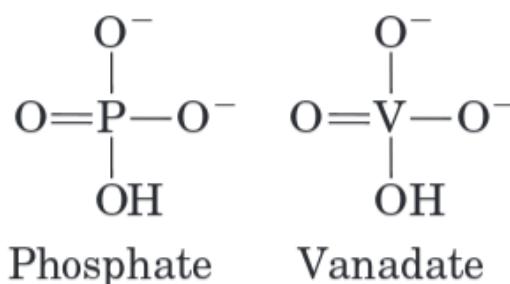
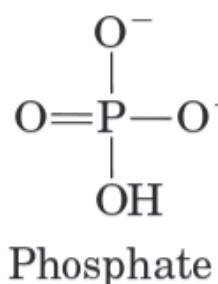
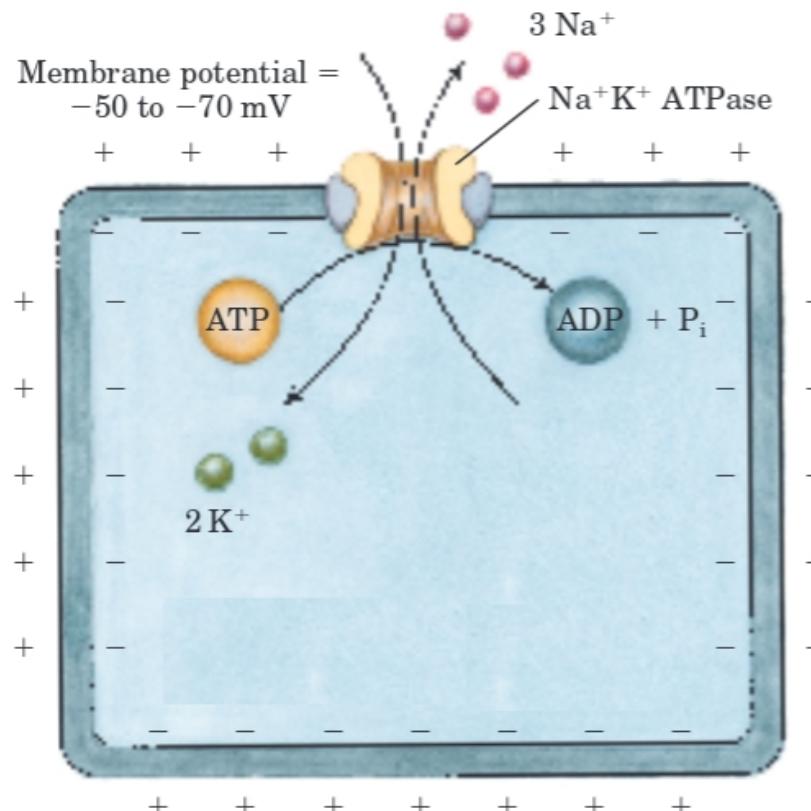
- $2\text{K}^+ \Rightarrow \text{in}$

## ➤ Inhibitors:

- ouabain: locks  $2\text{Na}^+$

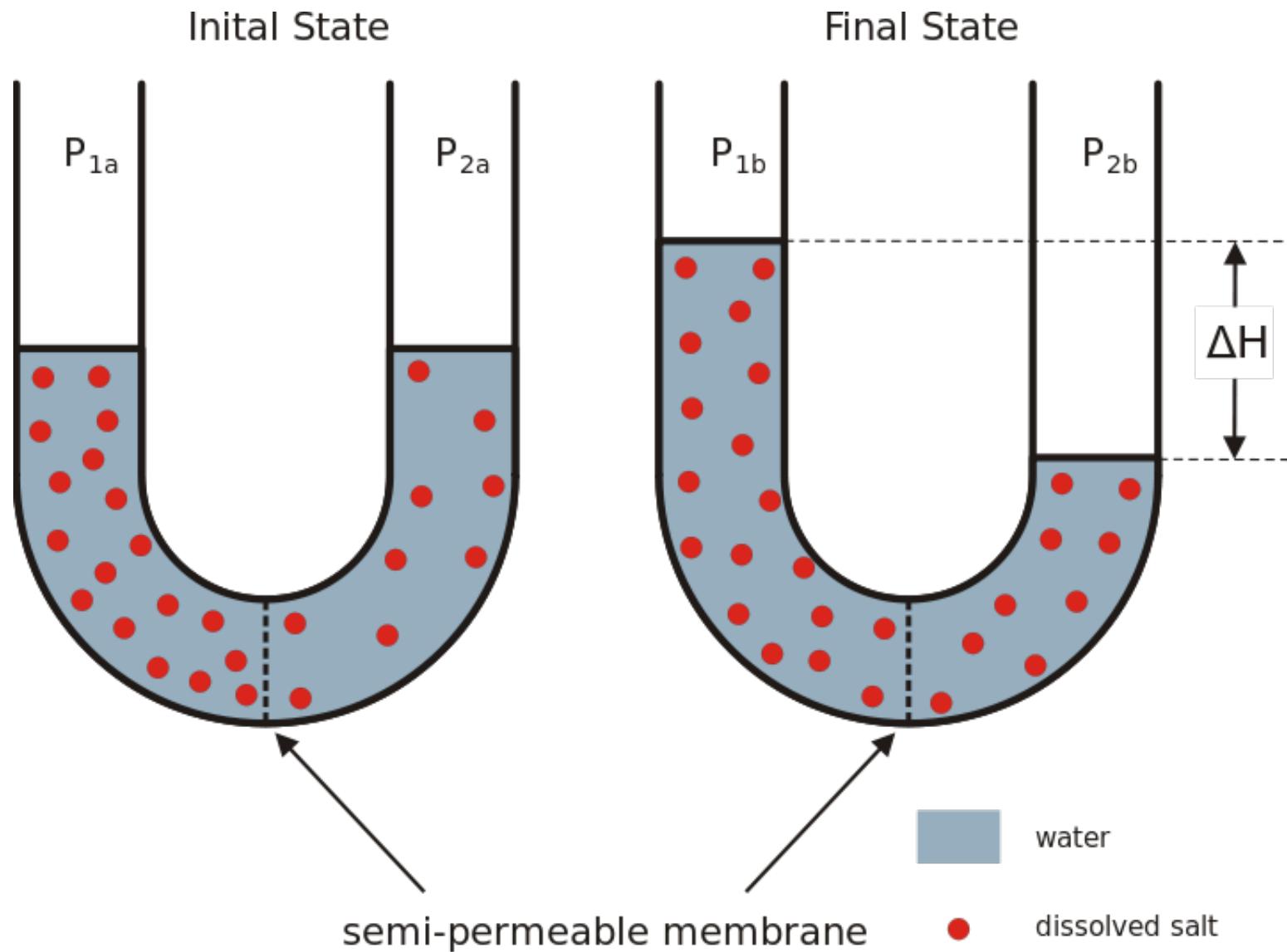
- palytoxin: non-specific channel

- digitalis  $\Rightarrow \text{Na}^+/\text{Ca}^{2+}$

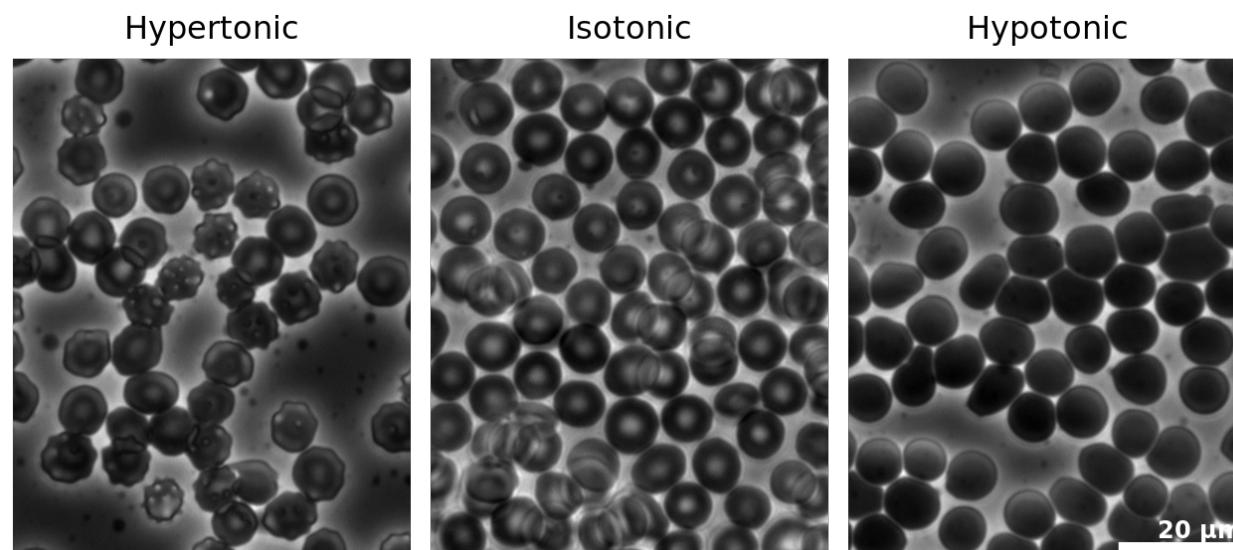
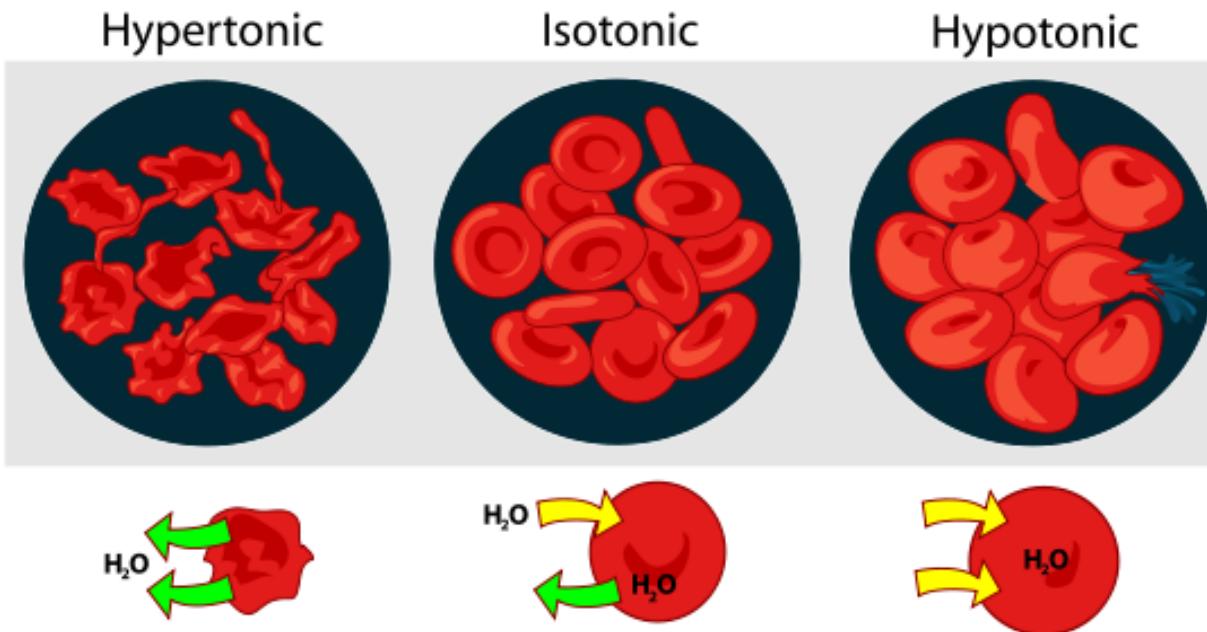


# OSMOSIS

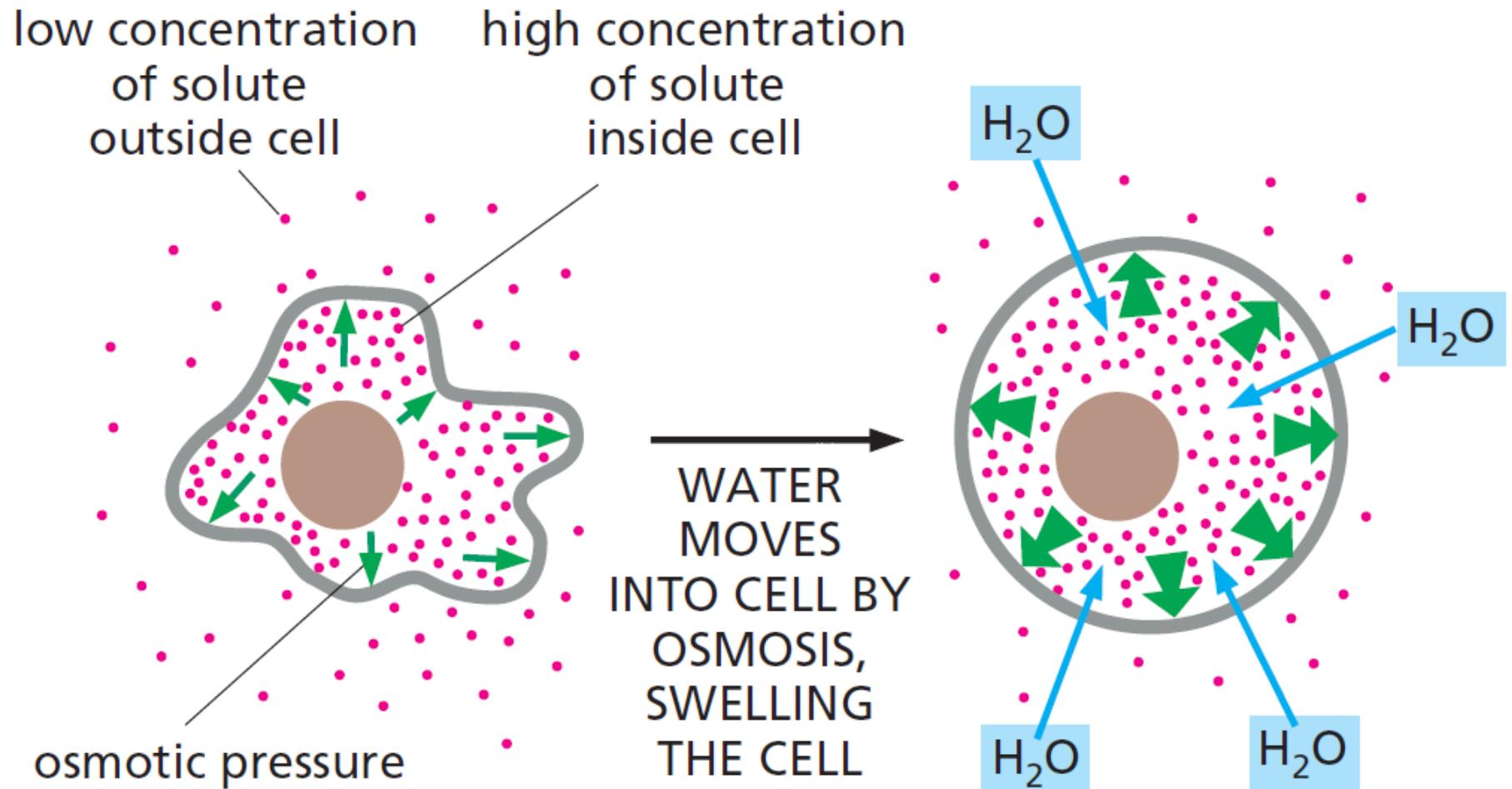
Osmosis: the movement of solvent from region of low [solute] to high [solute]



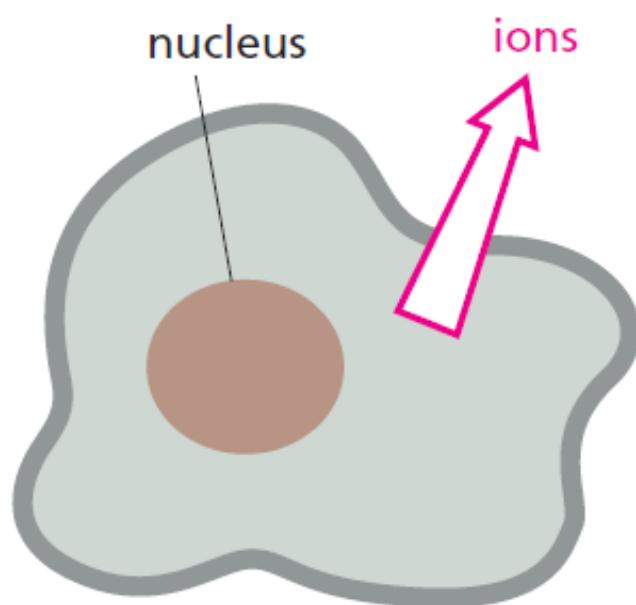
# OSMOSIS AND RED BLOOD CELLS



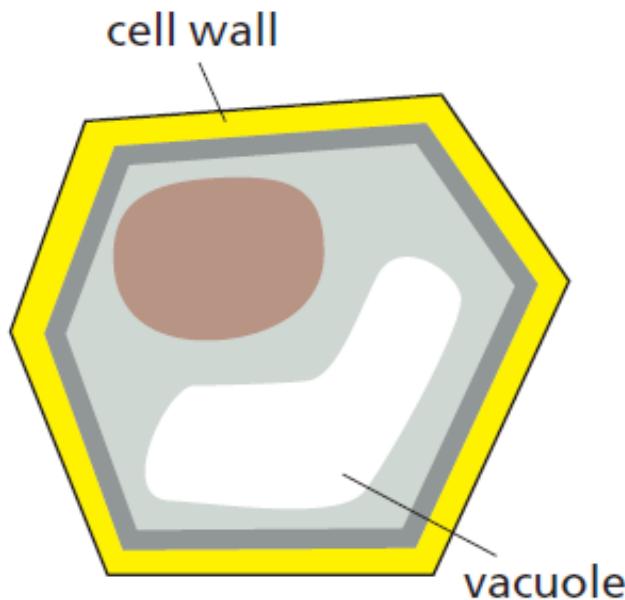
# OSMOSIS: $\text{Na}^+/\text{Cl}^-$ CONCENTRATIONS



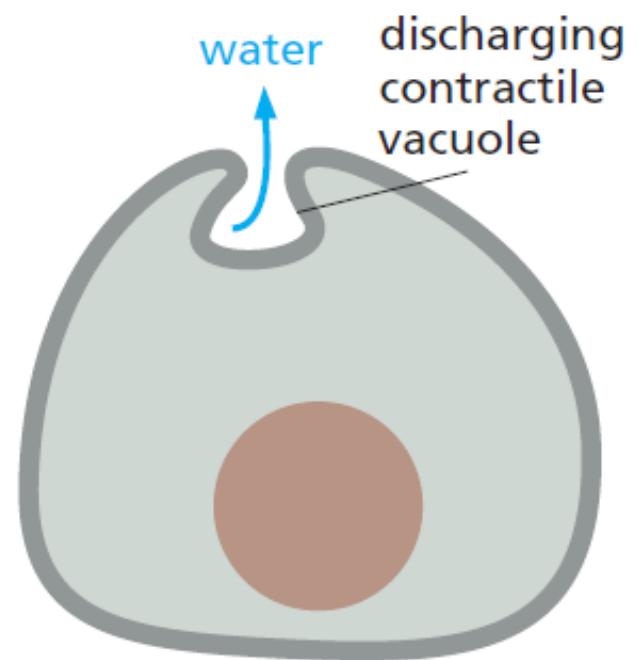
# OSMOSIS IN DIFFERENT CELLS



ANIMAL CELL



PLANT CELL



PROTOZOAN

**Na<sup>+</sup>/K<sup>+</sup> ATPase**

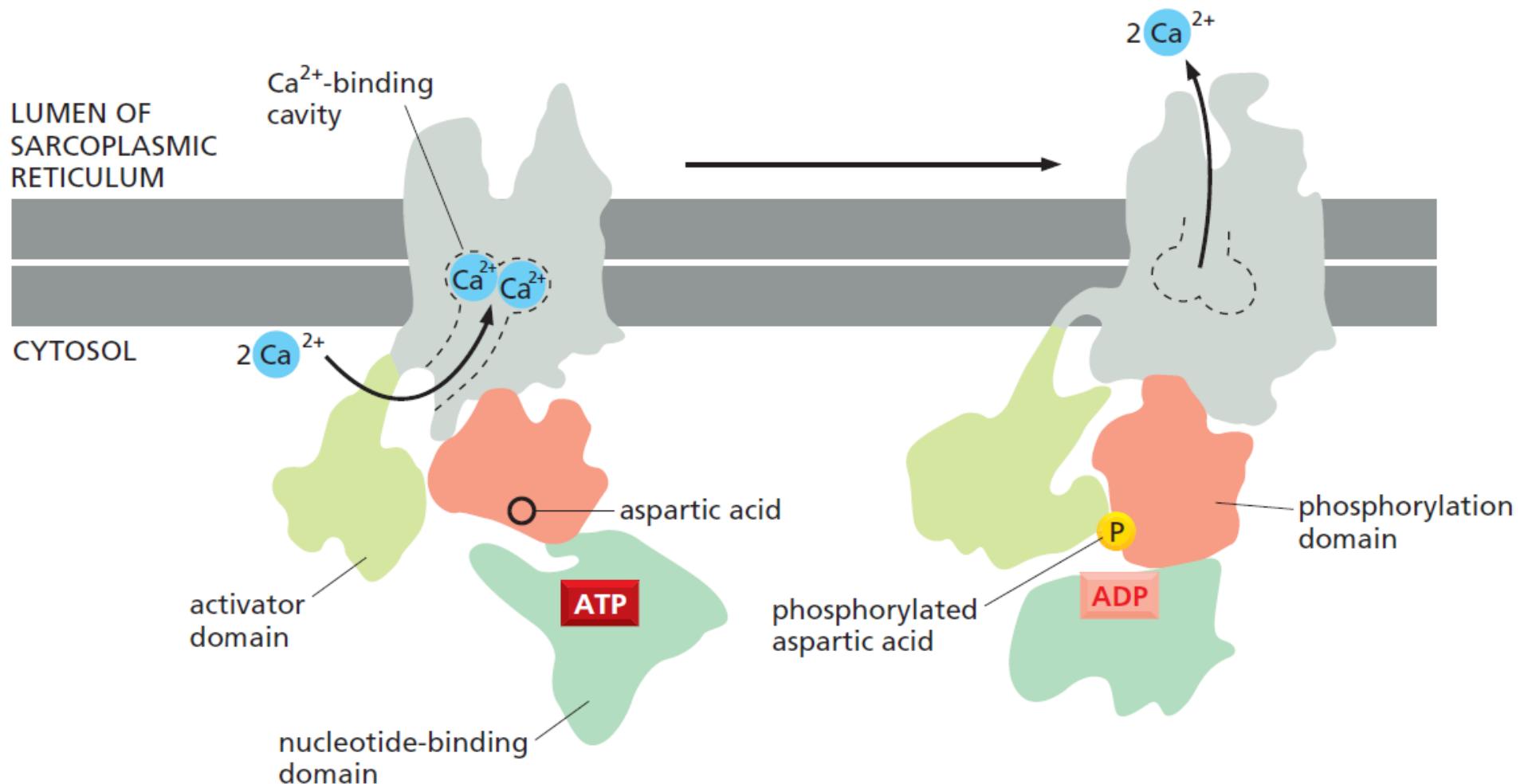
**Vacuole and cell wall:  
turgor pressure**

**Contractile vacuole**

**Na<sup>+</sup>/K<sup>+</sup> ATPase is involved in osmosis regulation in the animal cell.**

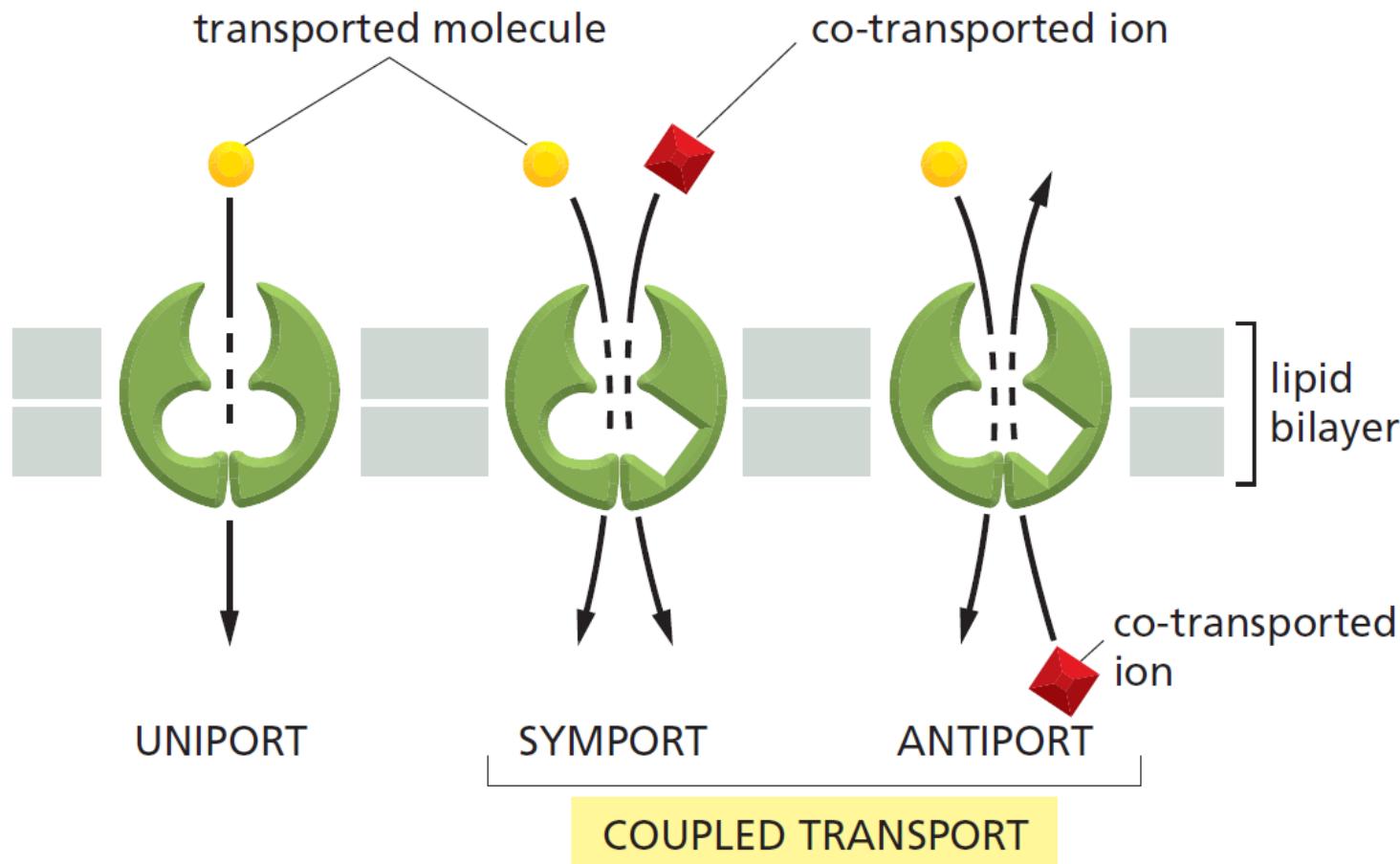
# CA<sup>2+</sup> ATPASE

- $[Ca^{2+}]_{out} \sim 10000 [Ca^{2+}]_{in}$
- $[Ca^{2+}]$ : signalling, contraction etc.
- P-type ATPase (35% identity and 65% similarity with Na<sup>+</sup>/K<sup>+</sup> ATPase)

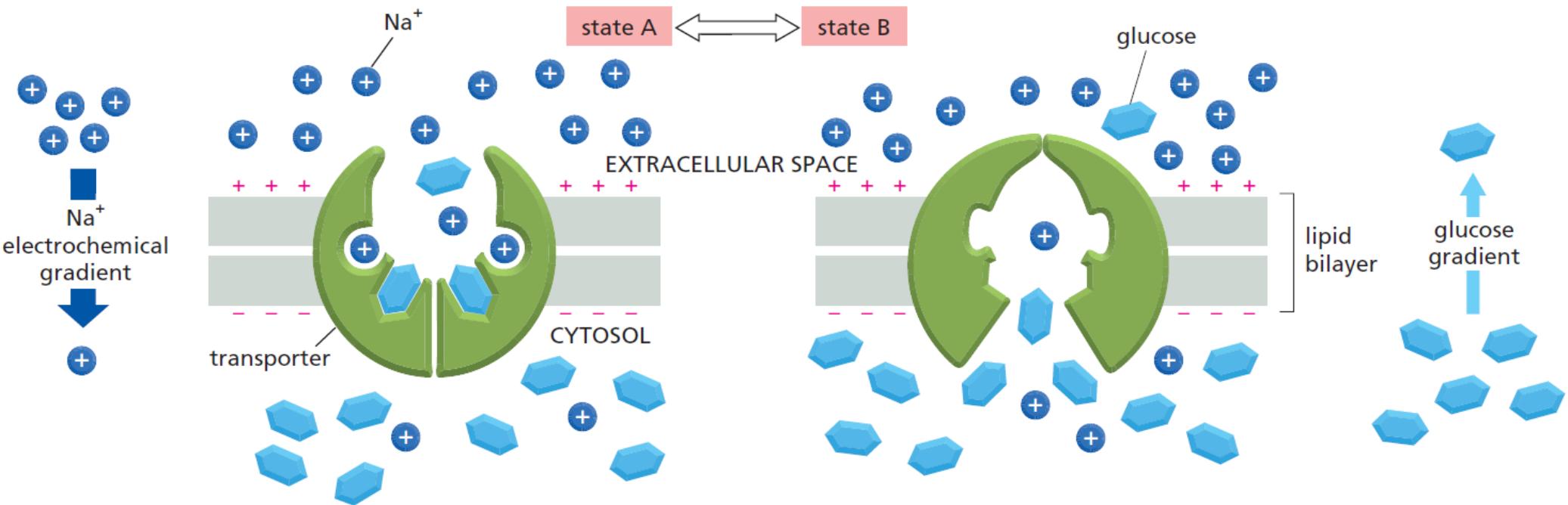


# COUPLED TRANSPORTERS

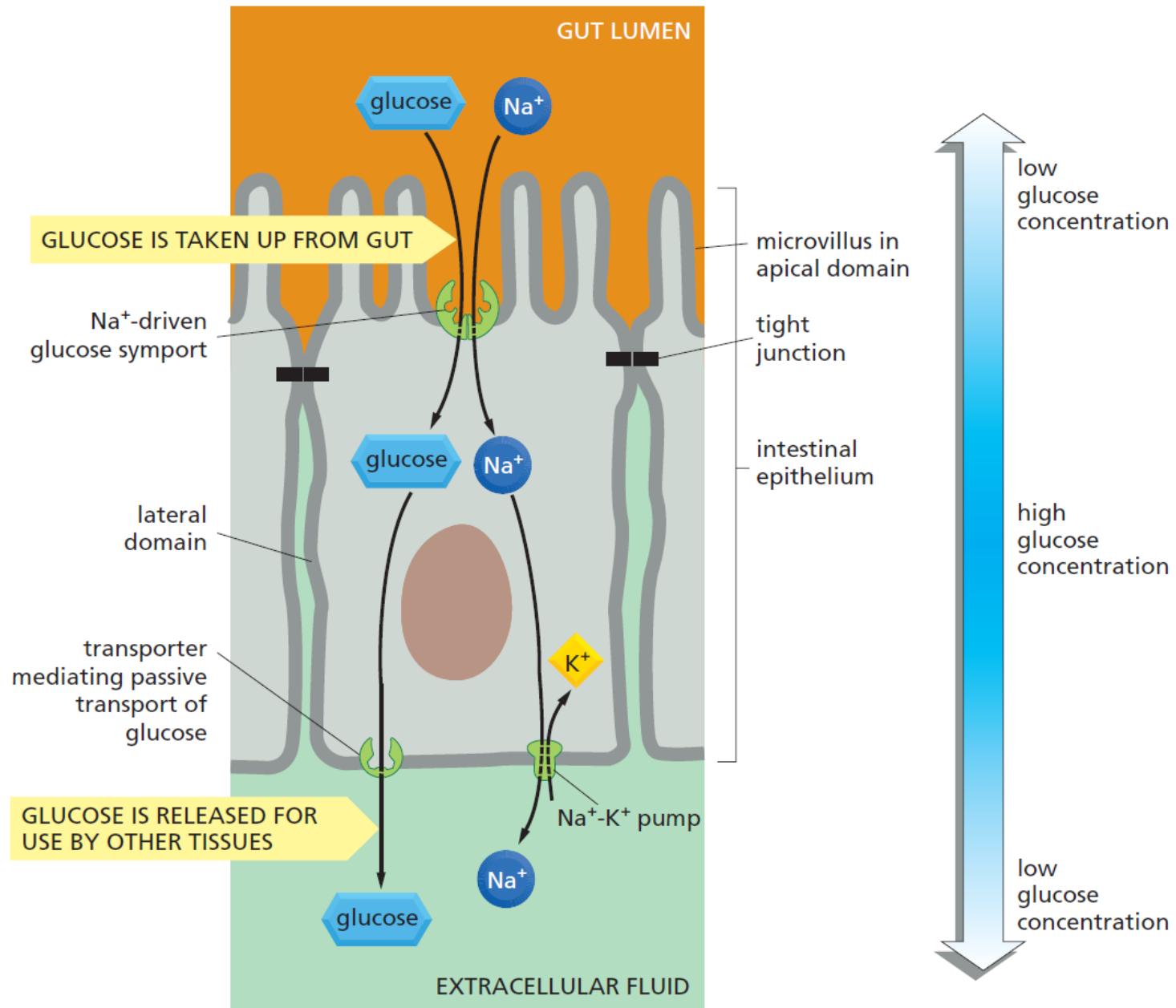
- Coupling: pumping one molecule induces transport of a second one
- Symports: different molecules => same direction
- Antiport: different molecules => opposite direction
- Uniport: only one molecule



# COUPLED TRANSPORTERS: ENERGY-INDEPENDENT GLUCOSE/NA<sup>+</sup> SYMPORT



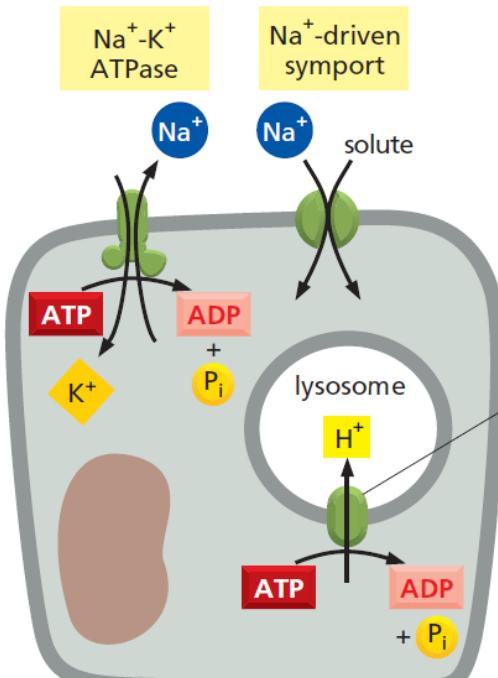
# TWO TYPES OF GLUCOSE TRANSPORTERS IN THE GUT CELL



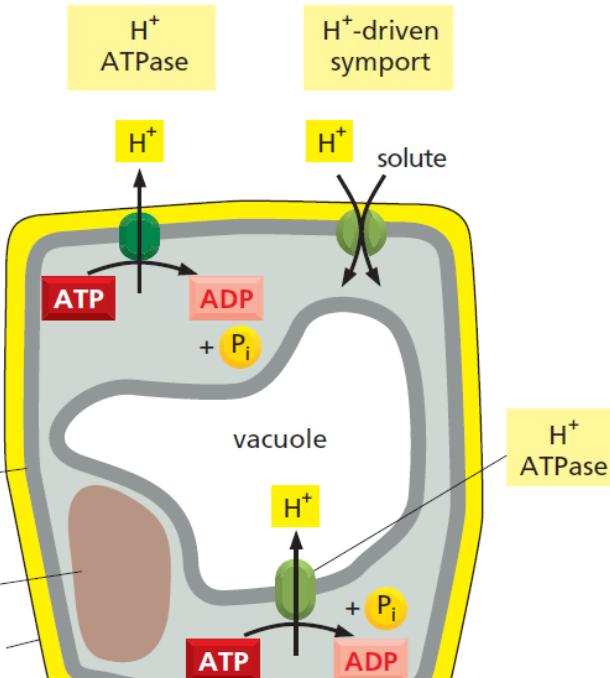
# H<sup>+</sup> ATPASES

➤ Plants, fungi, bacteria: H<sup>+</sup> instead of Na<sup>+</sup>

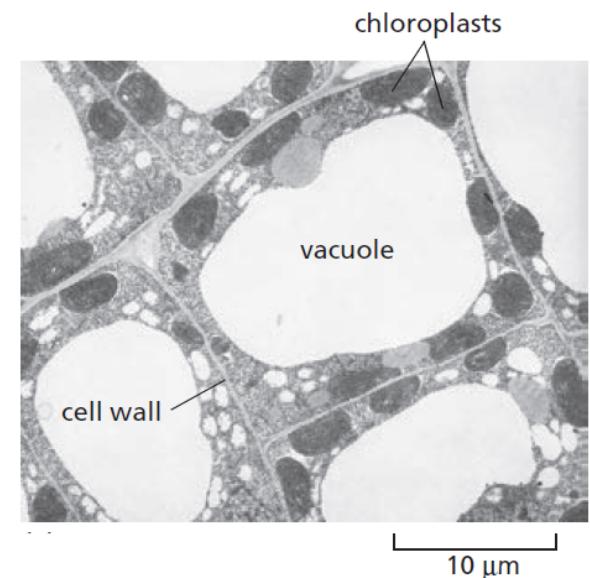
➤ pH<sub>cytosole</sub> regulation



ANIMAL CELL



PLANT CELL



# $H^+$ ATPASES

## ➤ F-type

- mitochondria, chloroplasts

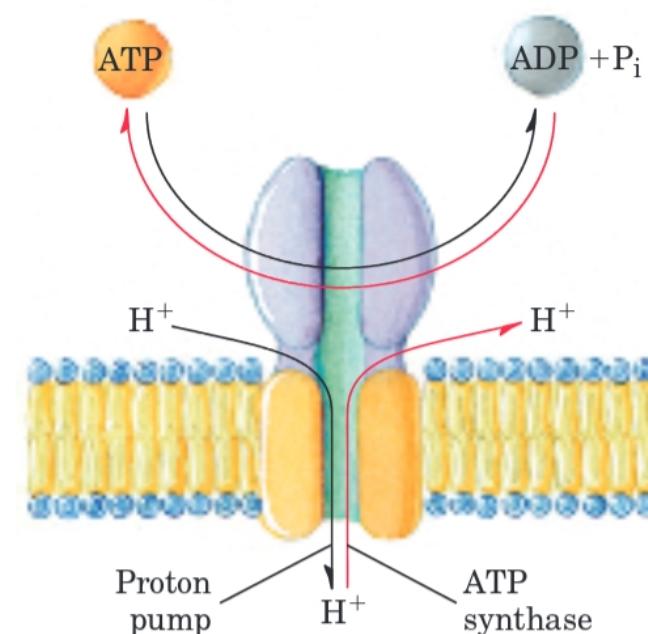
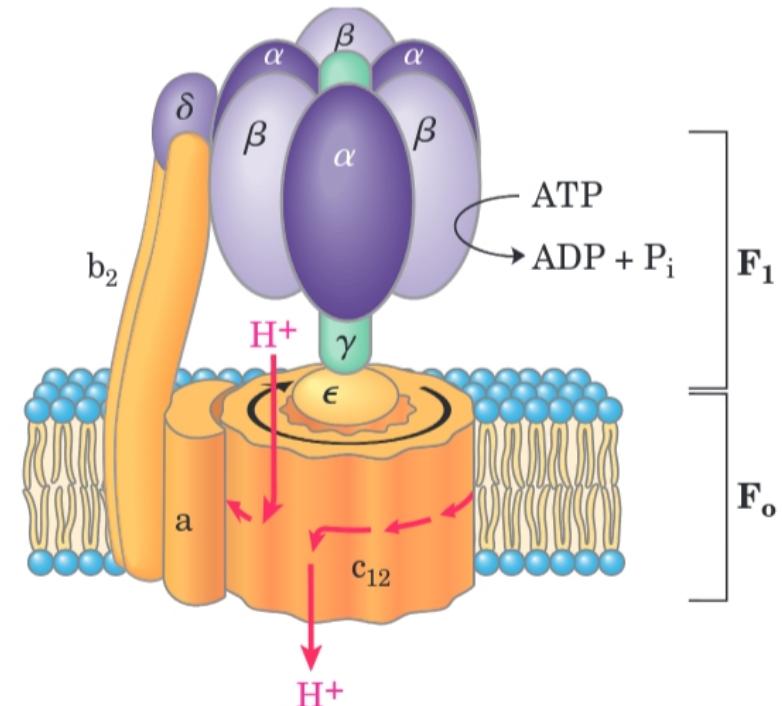
- $F_0$  and  $F_1$  units

- reversible

## ➤ V-type:

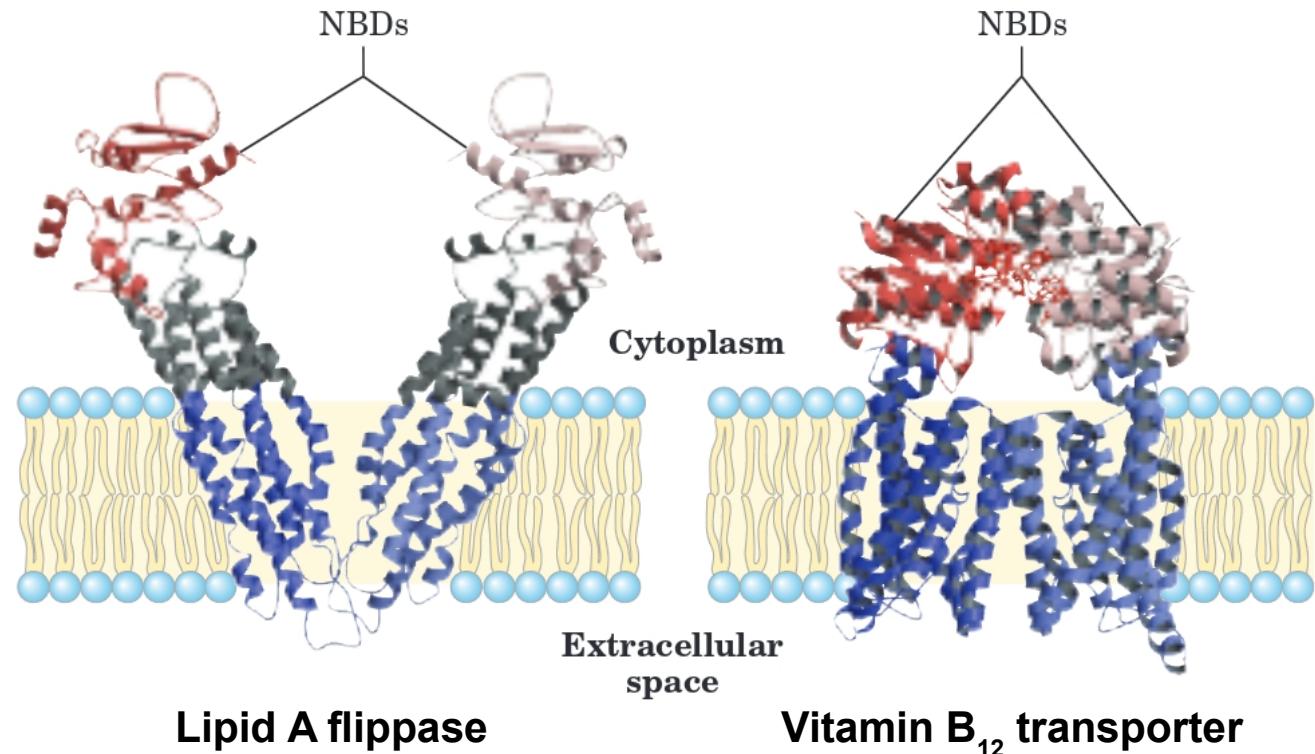
- vacuoles

- very similar to F-type



# ABC-TRANSPORTERS

- ATP-dependent
- Amino acids, peptides, proteins, lipids, ions, bile salts, drugs etc.
- 2 NBDs: nucleotide binding site
- 12 TMD
- Example: multidrug transporter (MDR1)
- Specific/non-specific
- Variety:
  - Human ~ 50 genes
  - *E.coli* ~80 genes (2%)



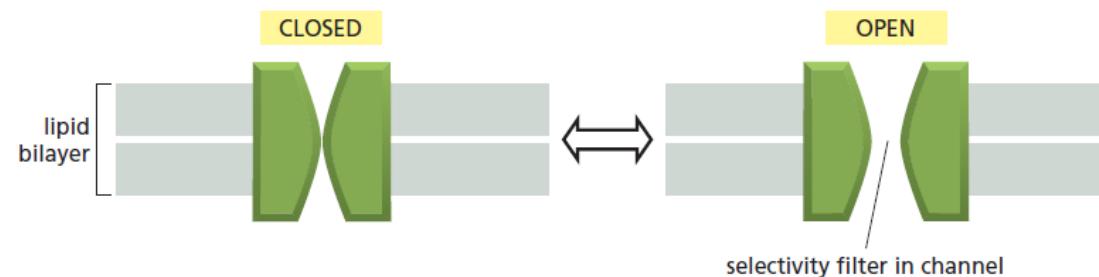
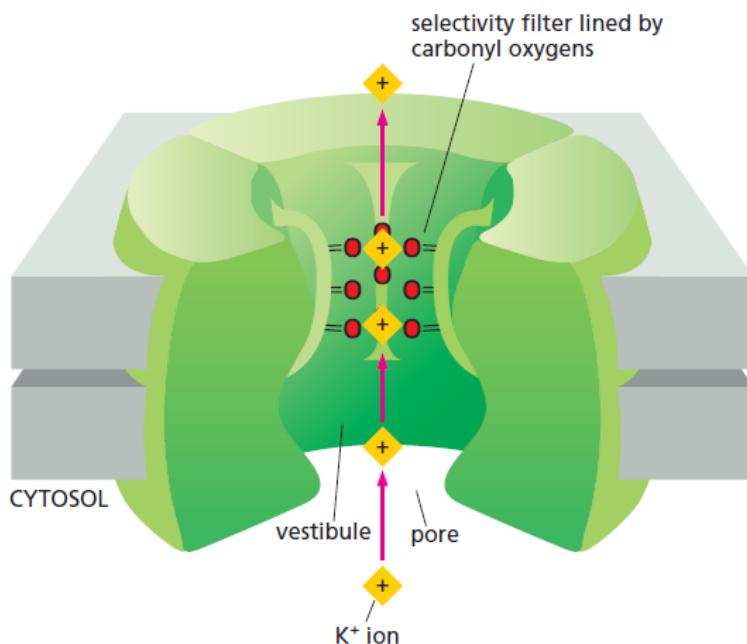
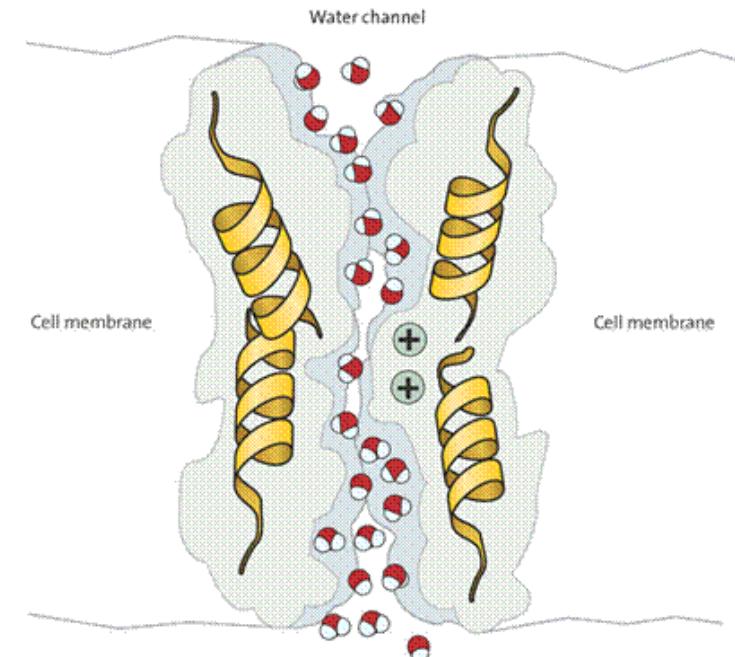
# EXAMPLES OF TRANSPORTERS

TRANSPORTER	LOCATION	ENERGY SOURCE	FUNCTION
Glucose transporter	plasma membrane of most animal cells	none	passive import of glucose
Na <sup>+</sup> -driven glucose pump	apical plasma membrane of kidney and intestinal cells	Na <sup>+</sup> gradient	active import of glucose
Na <sup>+</sup> -H <sup>+</sup> exchanger	plasma membrane of animal cells	Na <sup>+</sup> gradient	active export of H <sup>+</sup> ions, pH regulation
Na <sup>+</sup> -K <sup>+</sup> pump (Na <sup>+</sup> -K <sup>+</sup> ATPase)	plasma membrane of most animal cells	ATP hydrolysis	active export of Na <sup>+</sup> and import of K <sup>+</sup>
Ca <sup>2+</sup> pump (Ca <sup>2+</sup> ATPase)	plasma membrane of eucaryotic cells	ATP hydrolysis	active export of Ca <sup>2+</sup>
H <sup>+</sup> pump (H <sup>+</sup> ATPase)	plasma membrane of plant cells, fungi, and some bacteria	ATP hydrolysis	active export of H <sup>+</sup>
H <sup>+</sup> pump (H <sup>+</sup> ATPase)	membranes of lysosomes in animal cells and of vacuoles in plant and fungal cells	ATP hydrolysis	active export of H <sup>+</sup> from cytosol into vacuole
Bacteriorhodopsin	plasma membrane of some bacteria	light	active export of H <sup>+</sup>

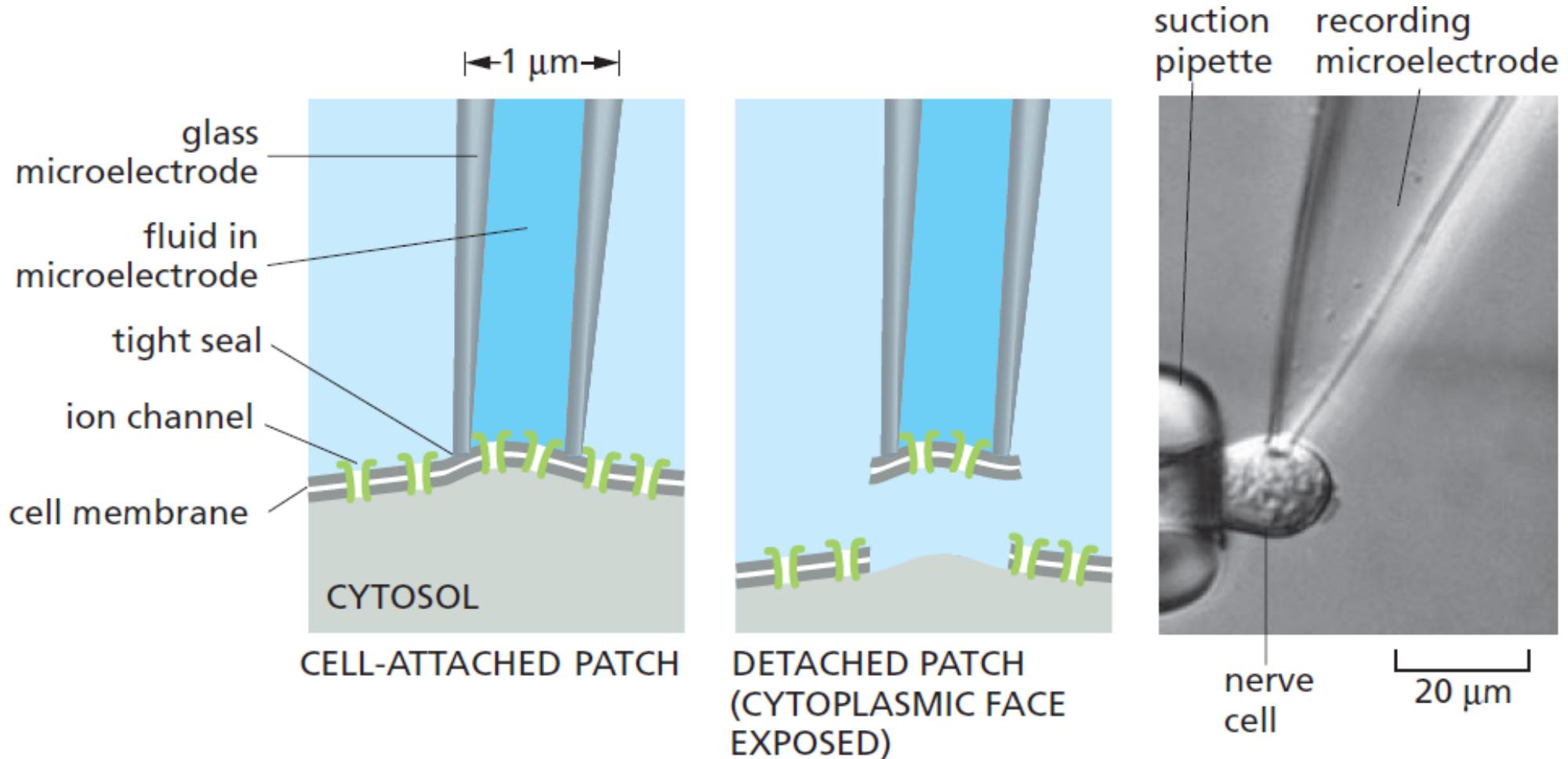
# CHANNELS

Hydrophilic pores across membrane

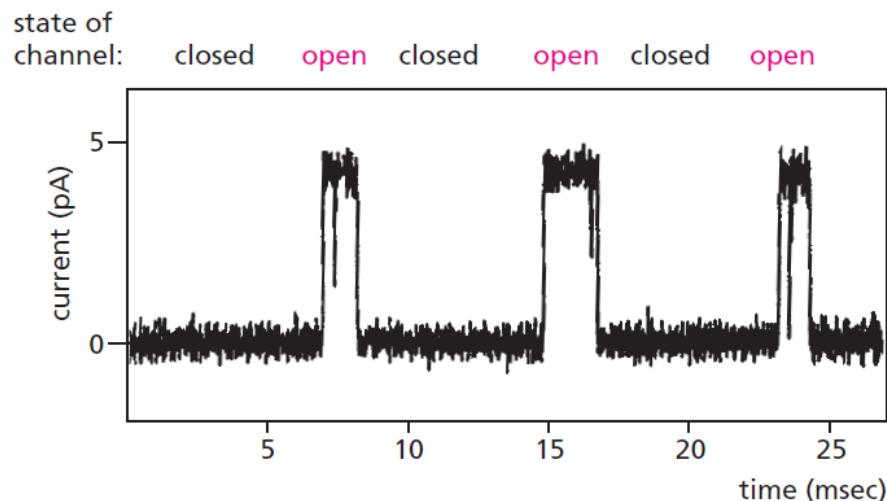
- Aquaporin
- Ion channels:  $K^+$ ,  $Na^+$ ,  $Ca^{2+}$  etc.
  - ion-selective
  - gated
  - $\sim 10^3$  faster than transporters
  - electrochemical effects



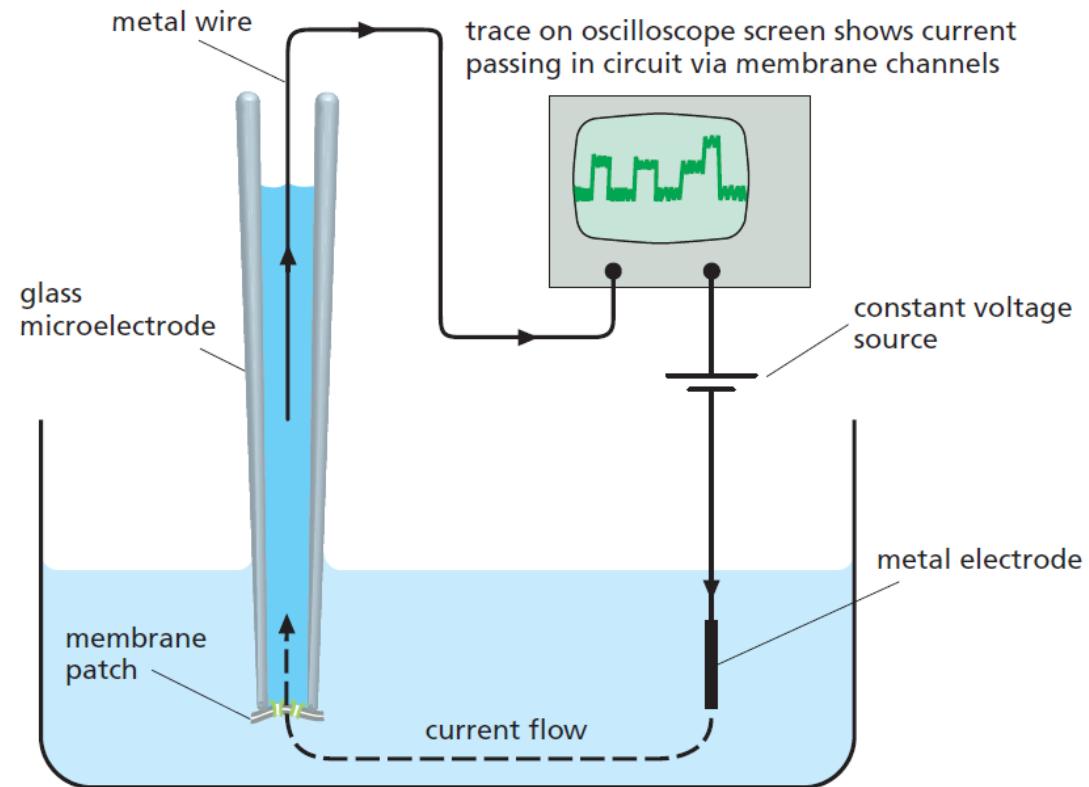
# ION CHANNELS: PATCH-CLAMP TECHNIQUE



# ION CHANNELS: PATCH-CLAMP TECHNIQUE



**Channels are in equilibration  
between open and closed states.**



# ION CHANNELS: TYPES OF GATING

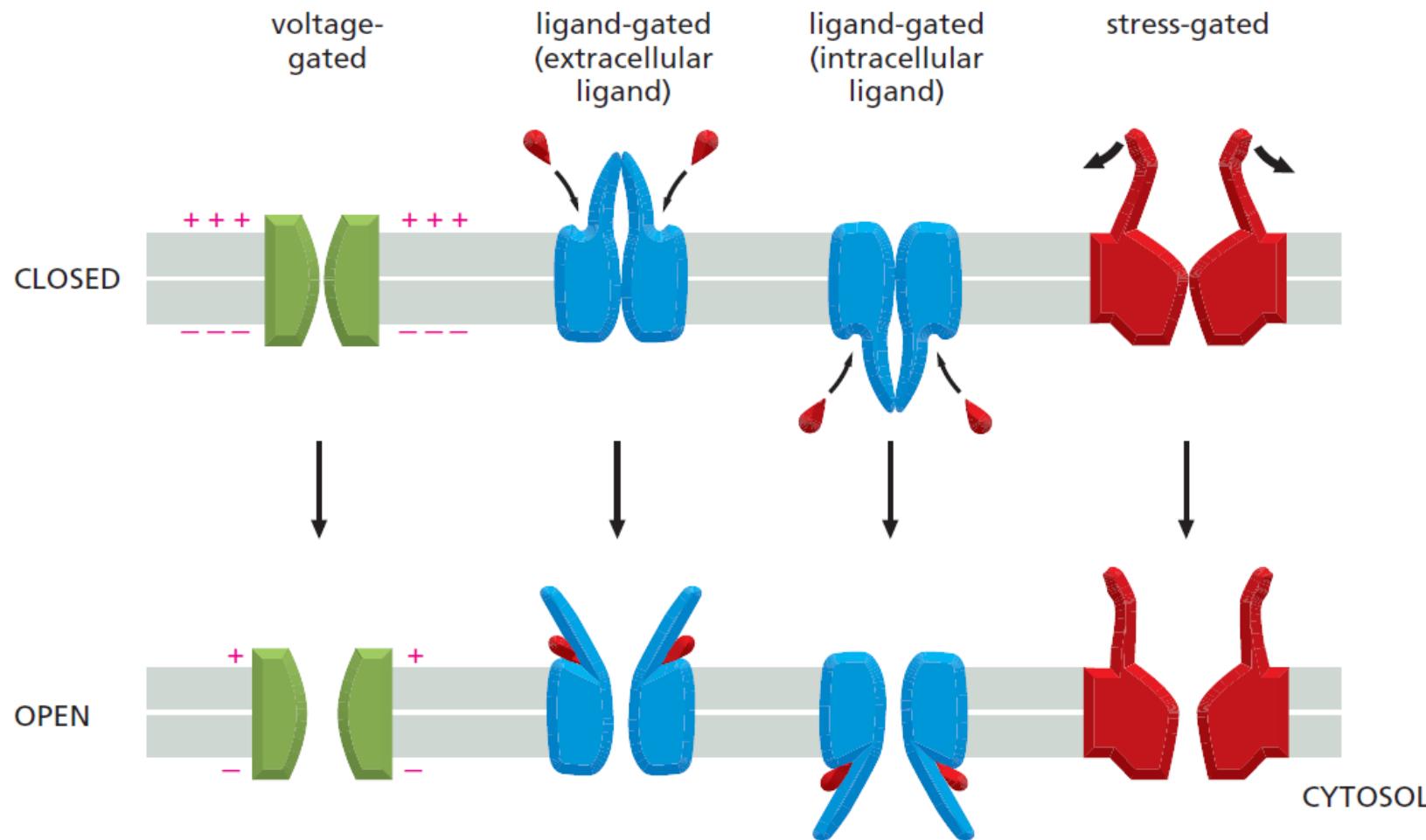
➤ **Voltage**

➤ **Ligand:**

- intracellular

- extracellular

➤ **Stress**

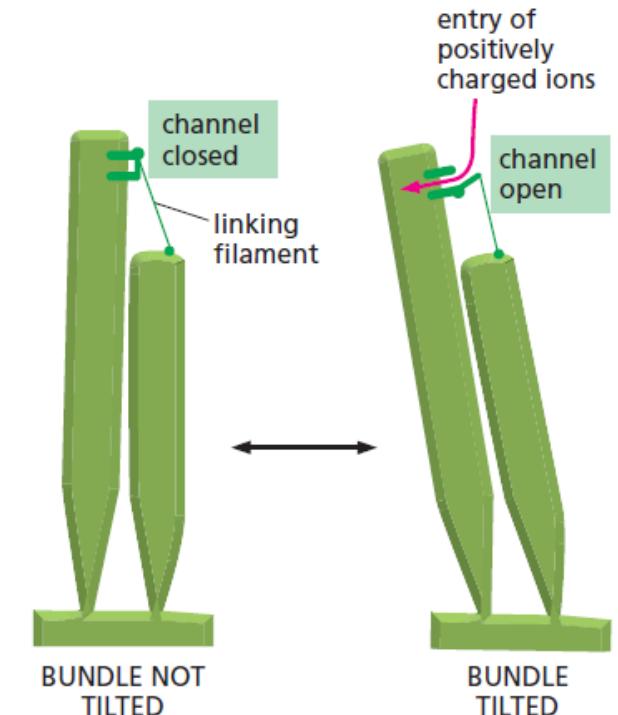
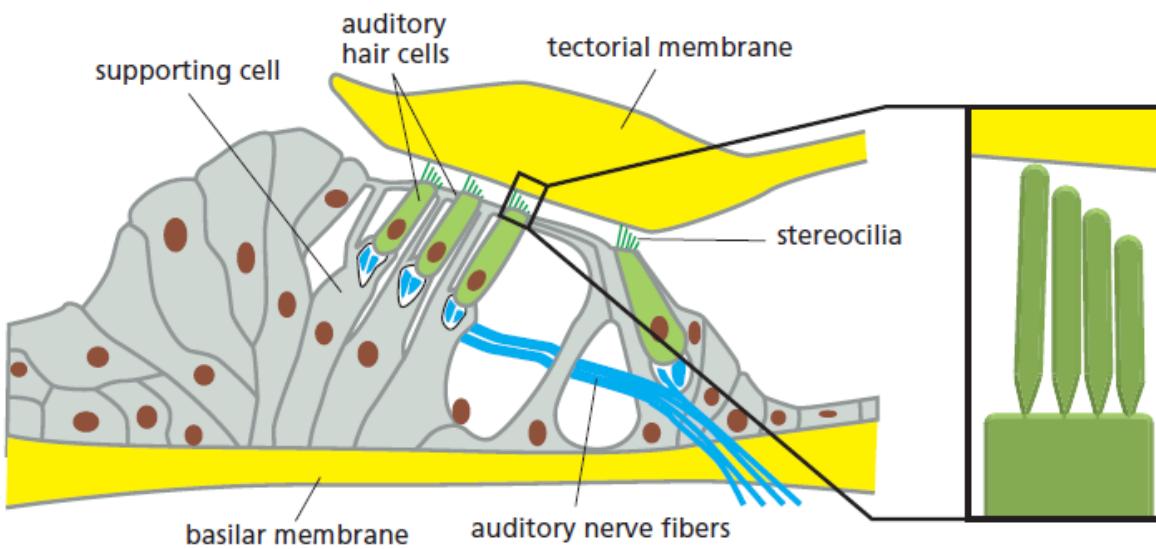


Gating affects probability of open state.

Ion channels

Membrane potential

# ION CHANNELS EXAMPLES



Stress gating: ability to hear



Voltage gating: touching mimosa leaves

# MEMBRANE POTENTIAL

➤ K<sup>+</sup> plays a key role

➤ K<sup>+</sup> transport:

- Na<sup>+</sup>/K<sup>+</sup> ATPase

- K<sup>+</sup> leak channels

➤ Nernst equation:

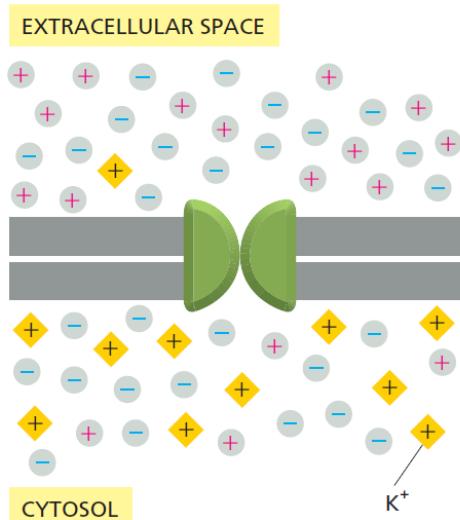
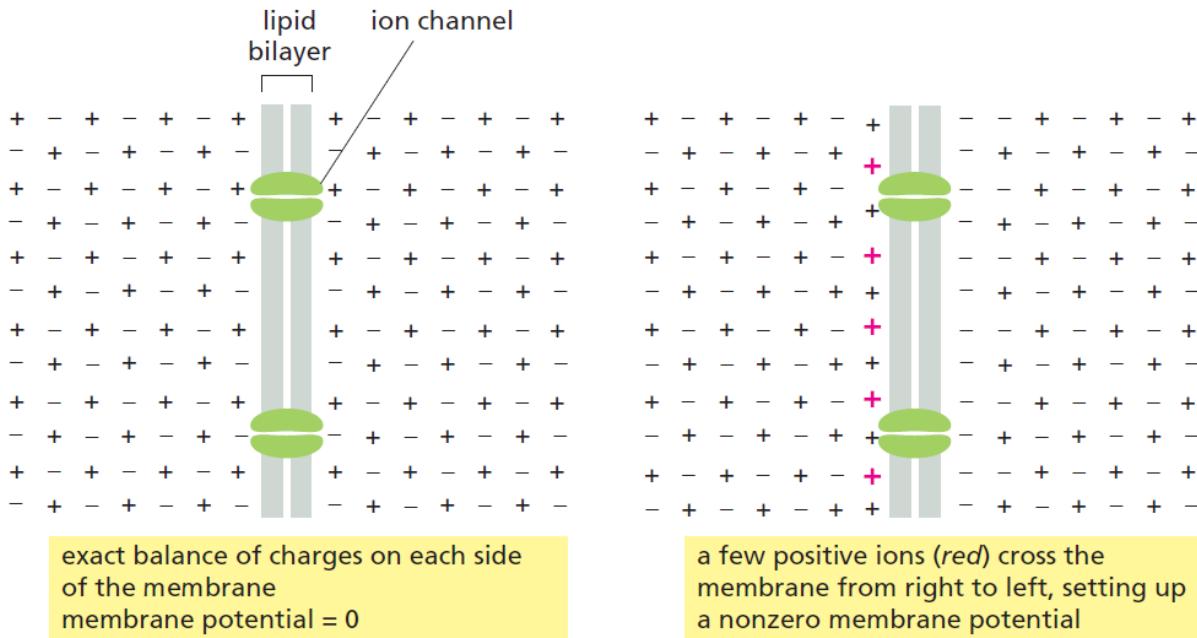
$$V = 62 \lg ([\text{Ion+}]_{\text{outside}} / [\text{Ion+}]_{\text{inside}})$$

➤ Normally: [-20; -200] mV

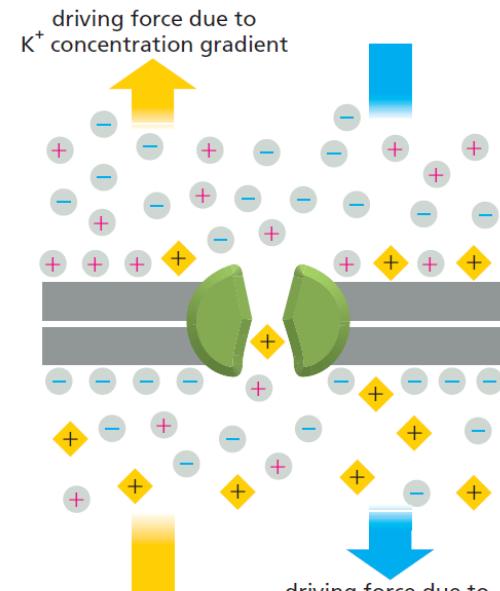
➤ V depends on:

- concentrations

- ion channels state



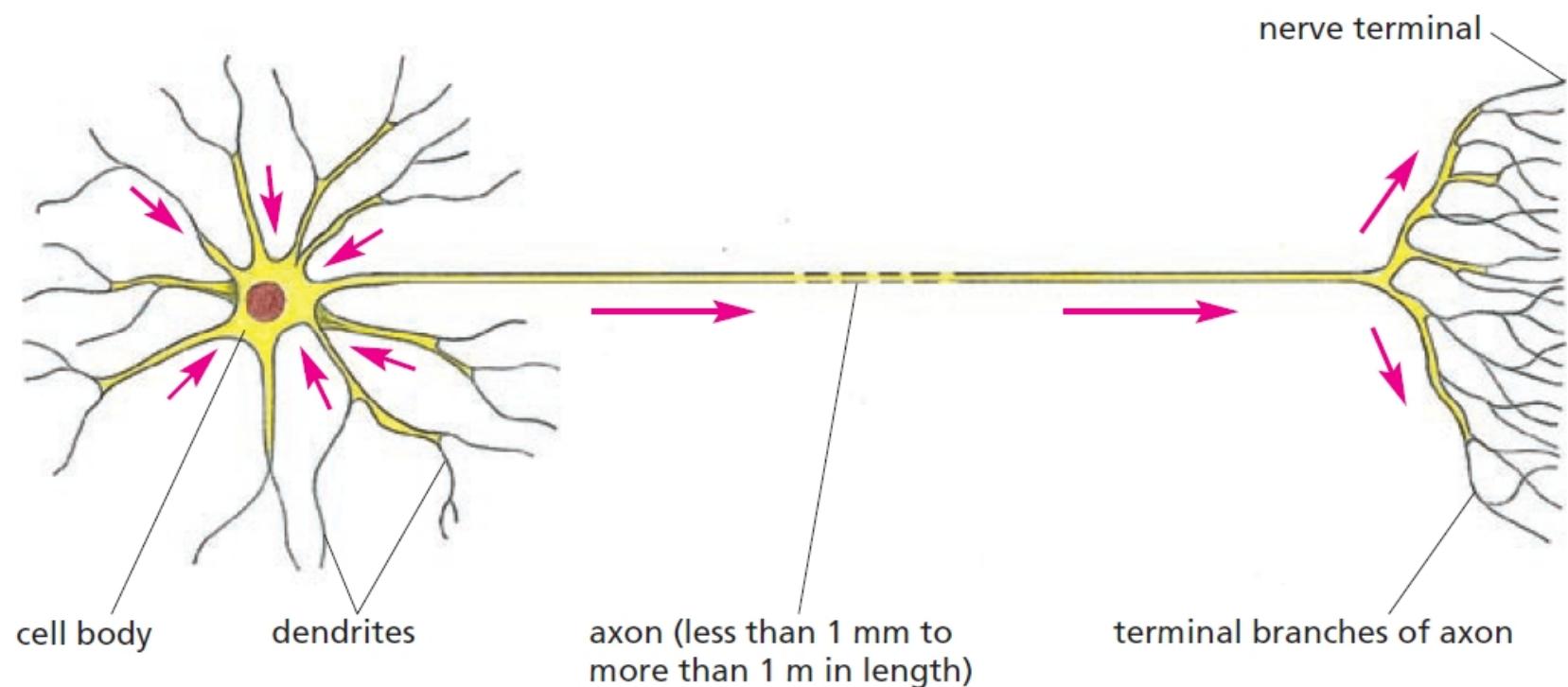
K<sup>+</sup> channel closed, membrane potential = 0; more K<sup>+</sup> inside the cell than outside, but zero net charge on each side (positive and negative charges balanced exactly)



K<sup>+</sup> channel open; K<sup>+</sup> moves out, leaving negative ions behind, and this charge distribution creates a membrane potential that balances the tendency of K<sup>+</sup> to move out

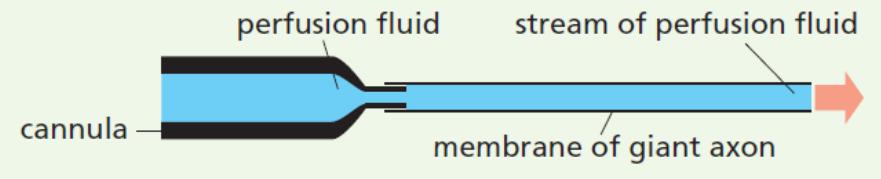
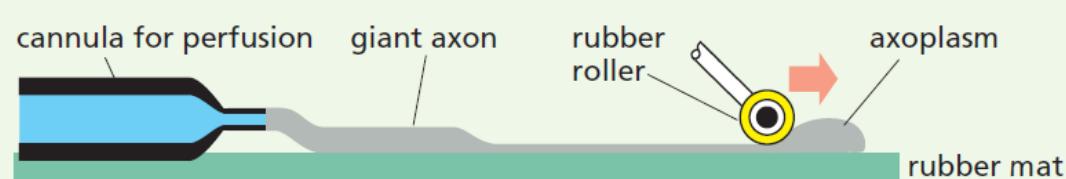
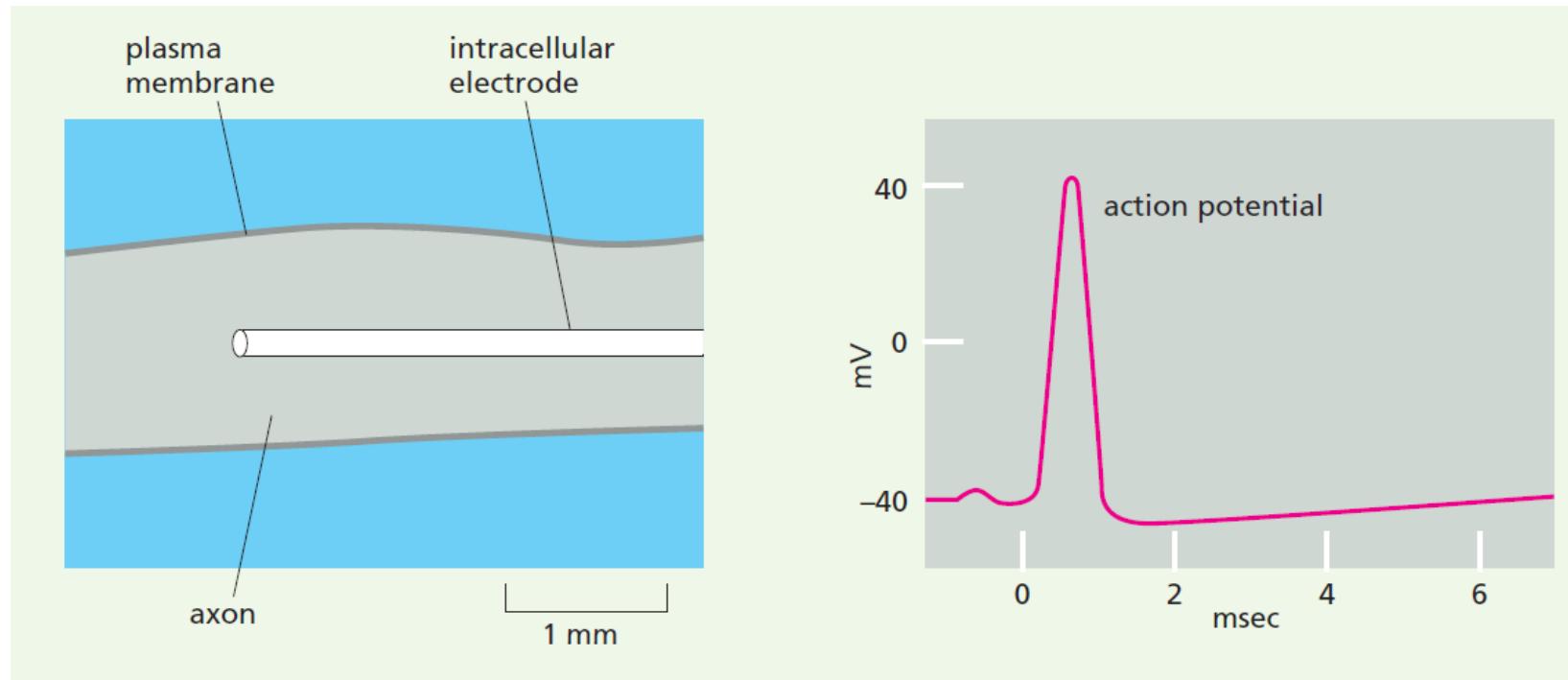
# ION CHANNELS IN SIGNALLING IN NERVE CELLS

- Neurons need to transport the signal
- Signal should weakens with the time
- Action potential speed ~ 100 m/s



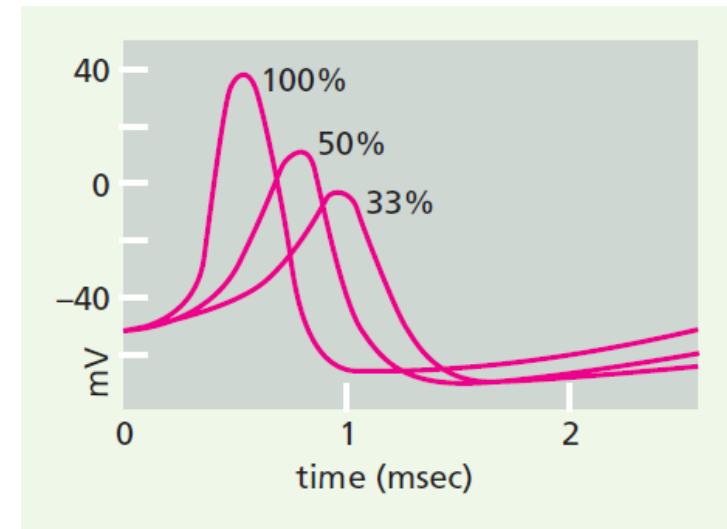
# ACTION POTENTIAL

- Axon: ~10cm long, ~1cm thick
- Action potential was measured:
- Perfusion showed that  $\text{Na}^+$ ,  $\text{K}^+$  are important

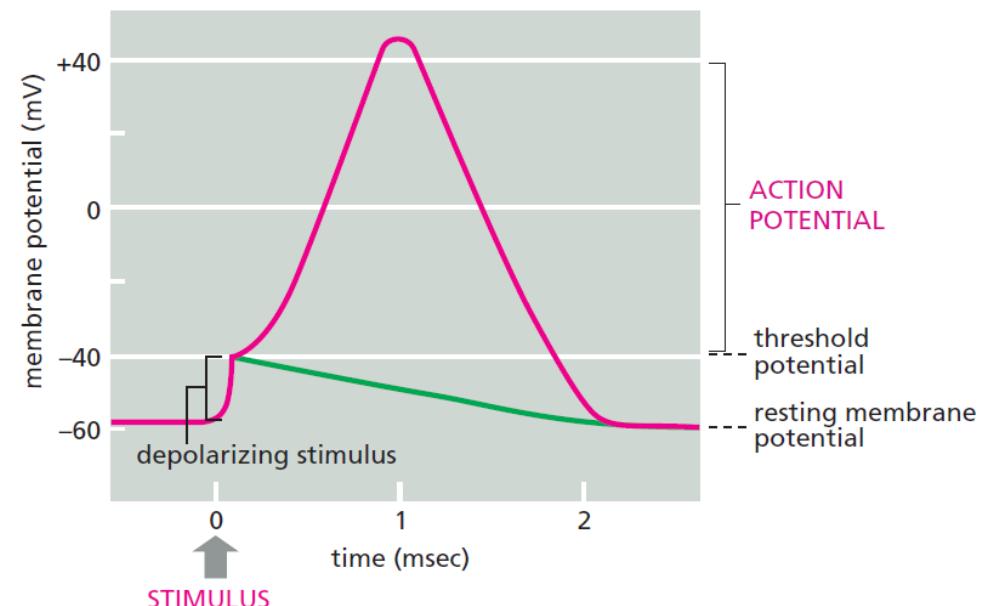


# ACTION POTENTIAL

- Resting potential =  $f ([K^+])$
- Resting potential  $\neq f ([Na^+])$
- Height of action potential =  $f ([Na^+]_{\text{outside}})$
- Action potential mechanism:
  - $Na^+$ -channels open (voltage-gated)
  - $[Na^+]$  increases inside
  - membrane depolarizes ( $-60mV \Rightarrow 40mV$ )
  - $Na^+$ -channels close
  - $K^+$ -channels open (voltage-gated)
  - Potential recovers by  $Na^+/K^+$  ATPase



Dependence on  $[Na^+]_{\text{outside}}$



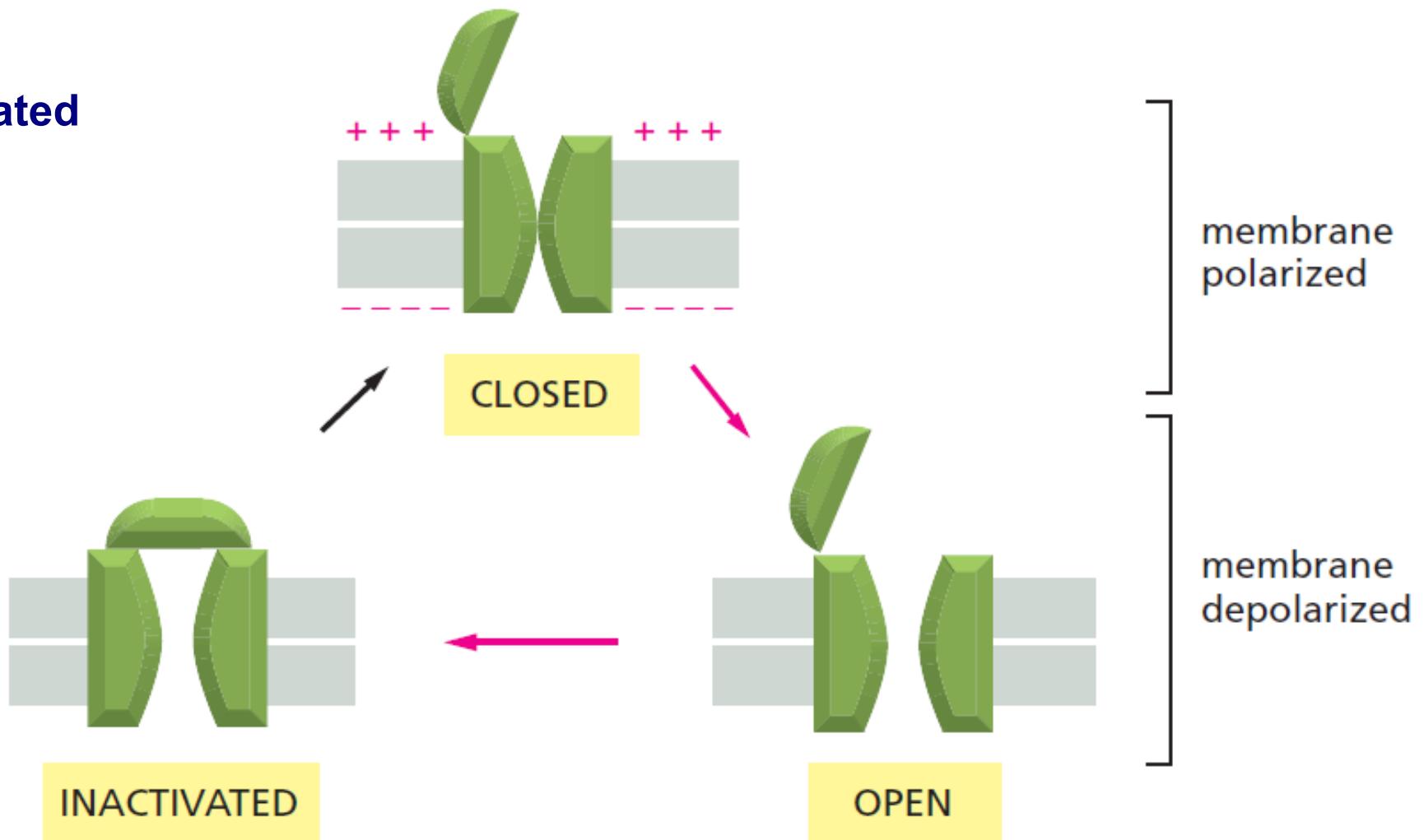
# ACTION POTENTIAL

## ➤ Na<sup>+</sup>-channel states:

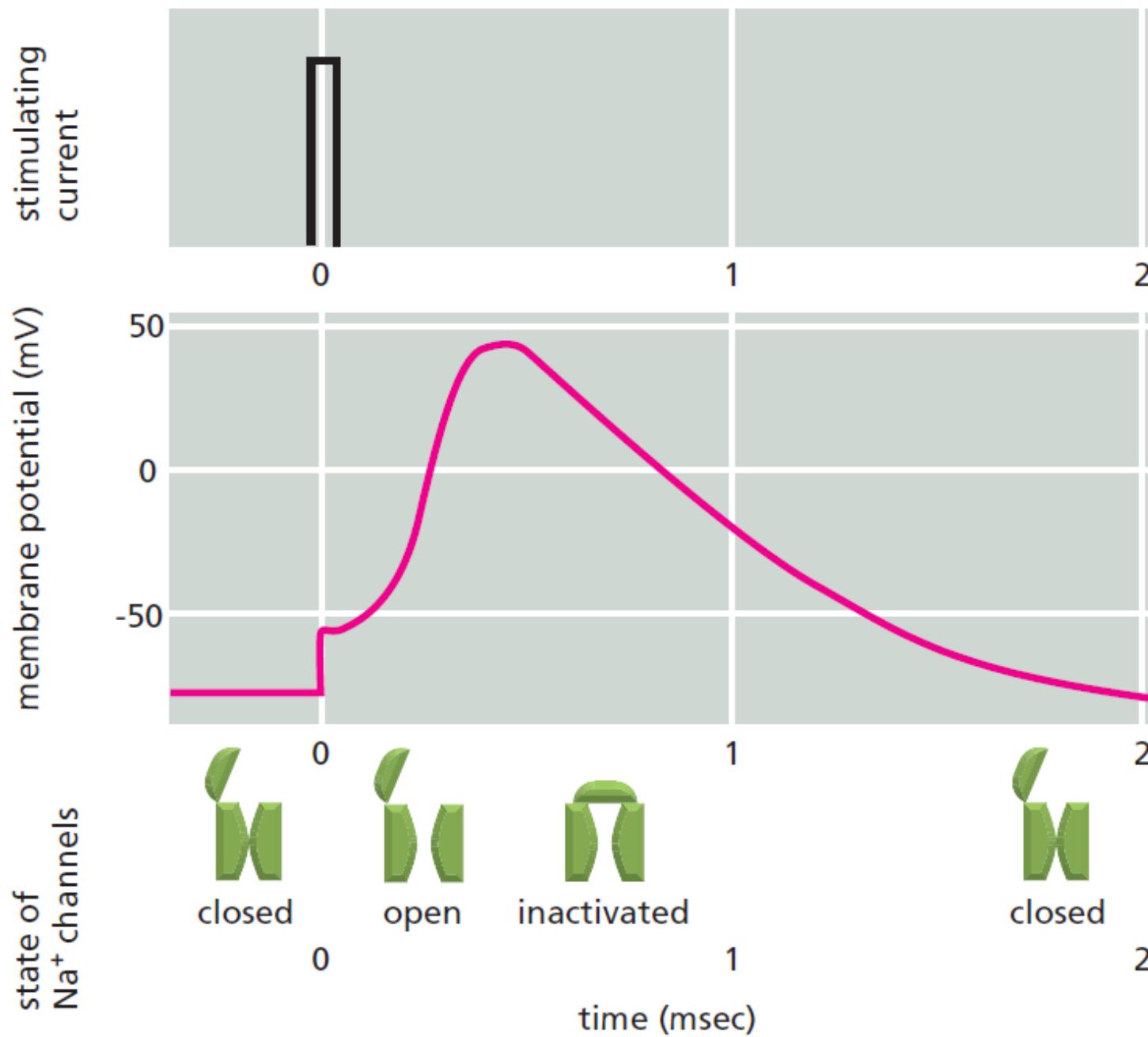
- closed

- open

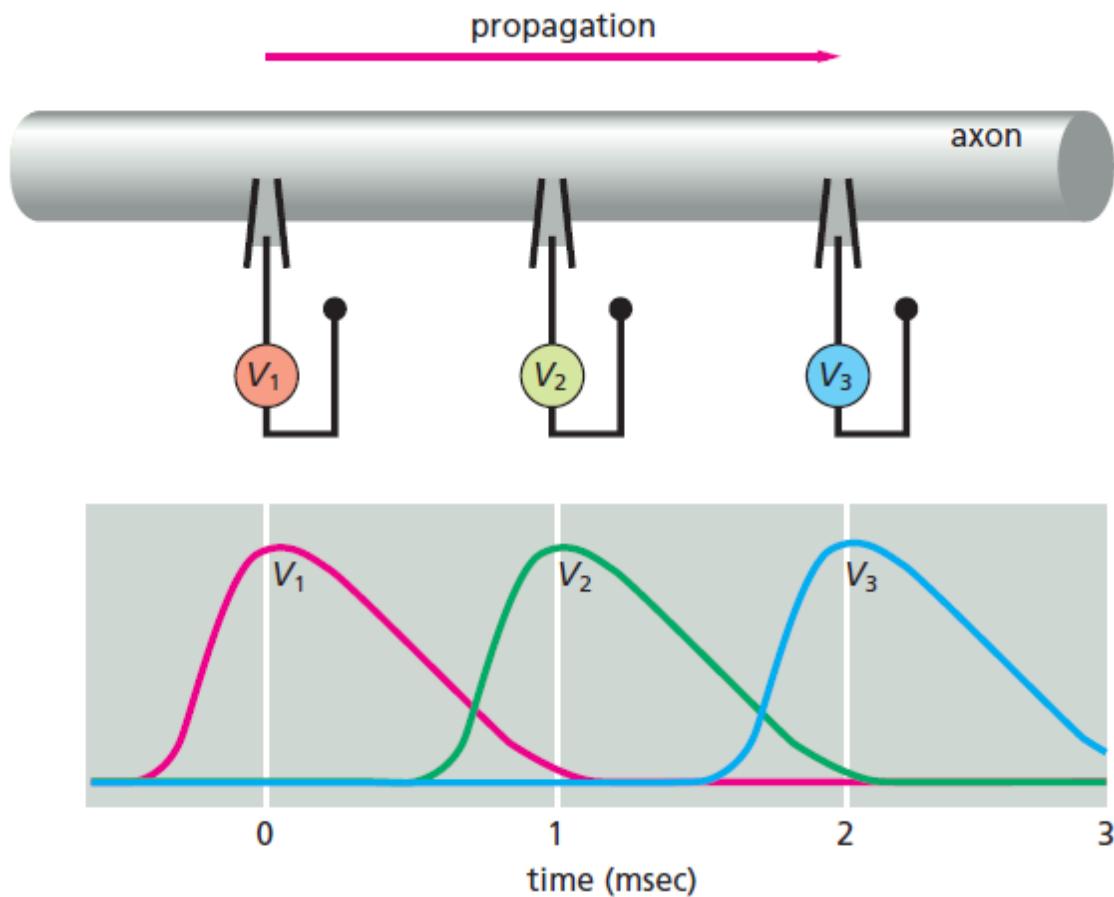
- inactivated



# ACTION POTENTIAL



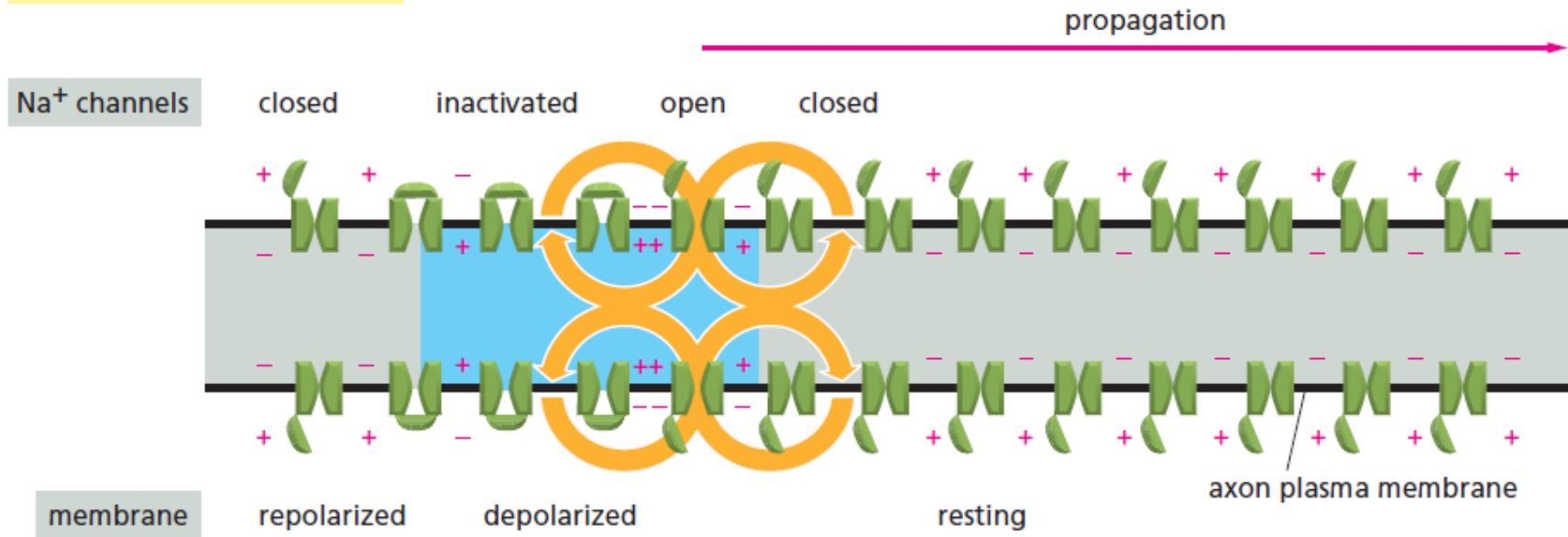
# ACTION POTENTIAL



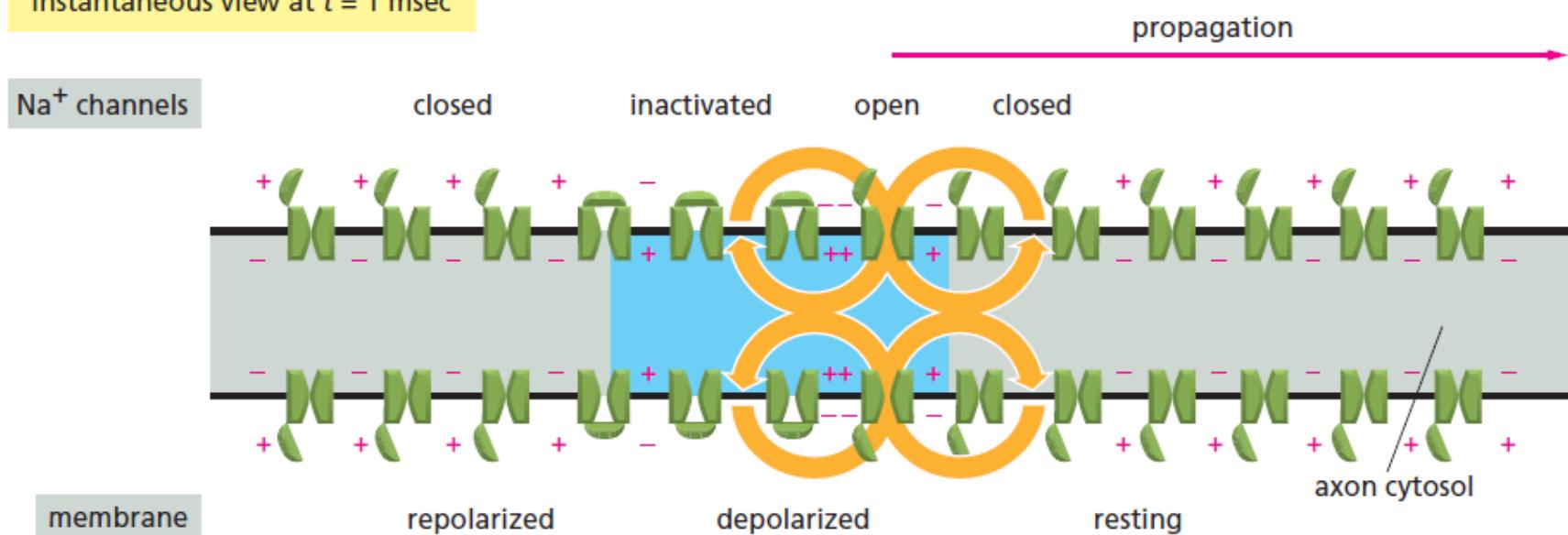
Action potential can be measured by inserting  
electrode in different points of the axon.

# ACTION POTENTIAL

instantaneous view at  $t = 0$

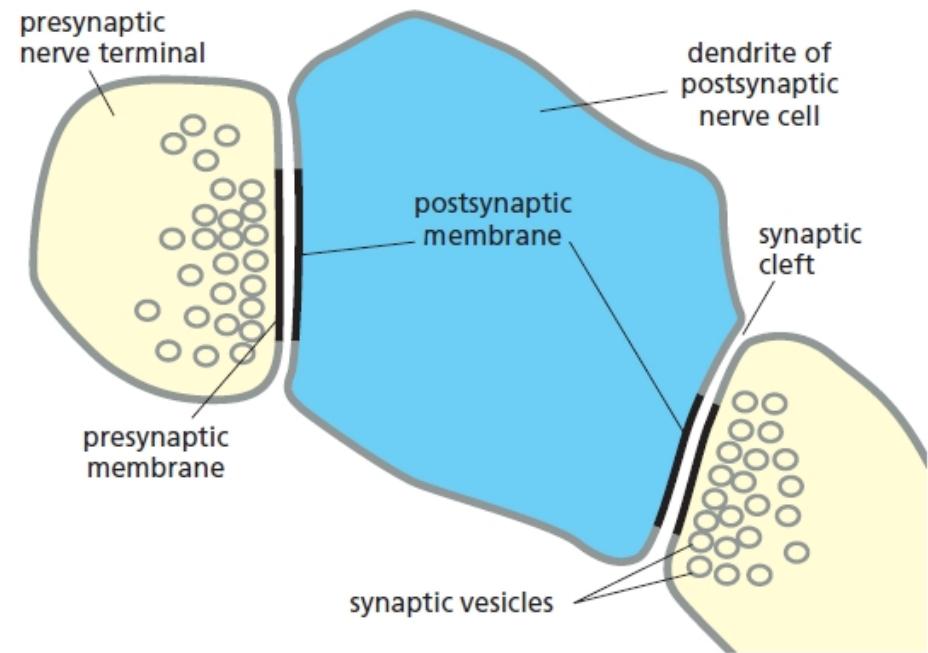


instantaneous view at  $t = 1 \text{ msec}$

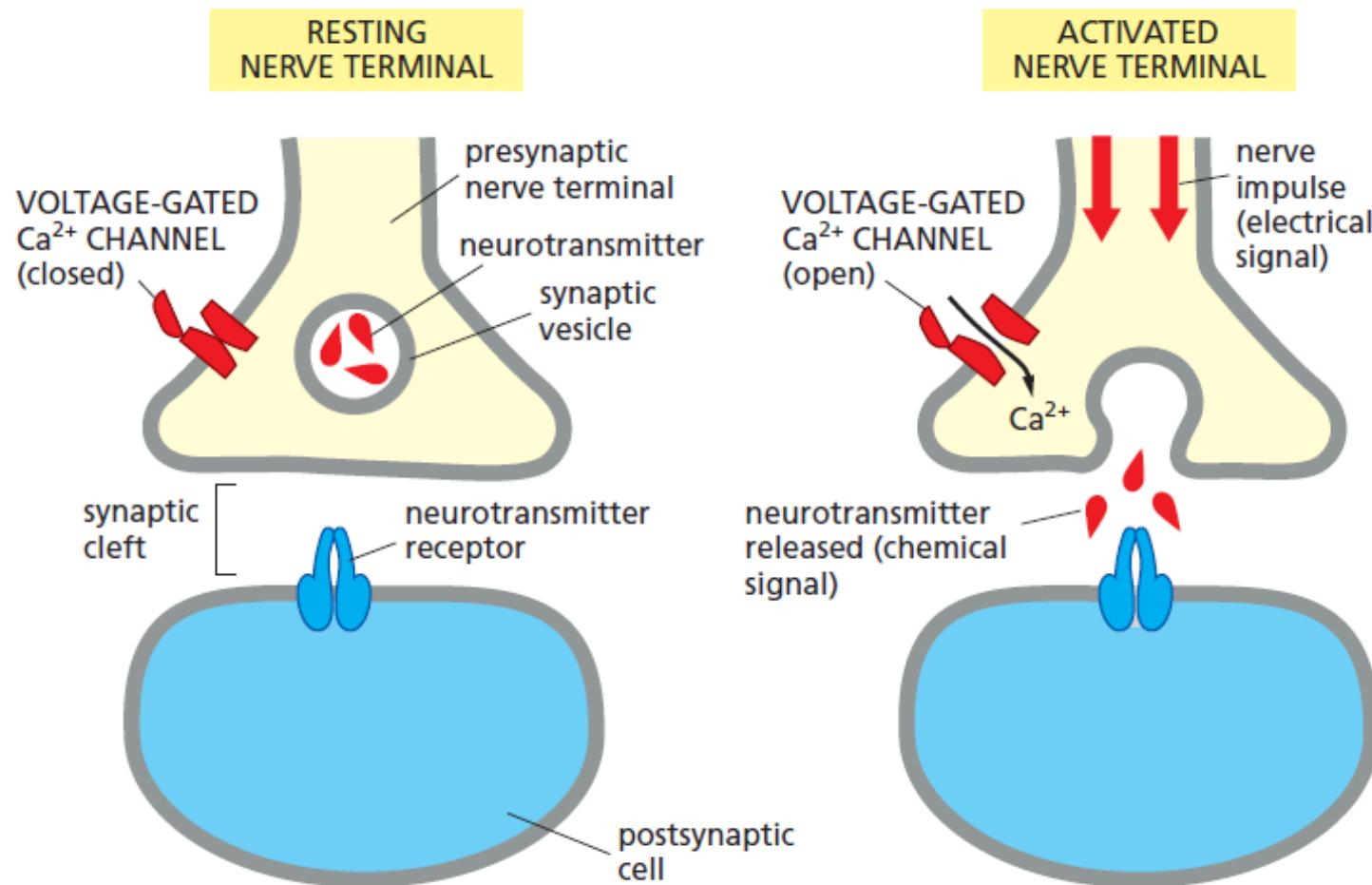


# CONVERSION OF ELECTRIC SIGNALS INTO CHEMICAL ONES

- Action potential reaches synapse: target cell junction.
- Voltage-gated  $\text{Ca}^{2+}$ -channels open.
- $[\text{Ca}^{2+}]_{\text{inside}}$  increases.
- Synaptic vesicles with neurotransmitters are released.

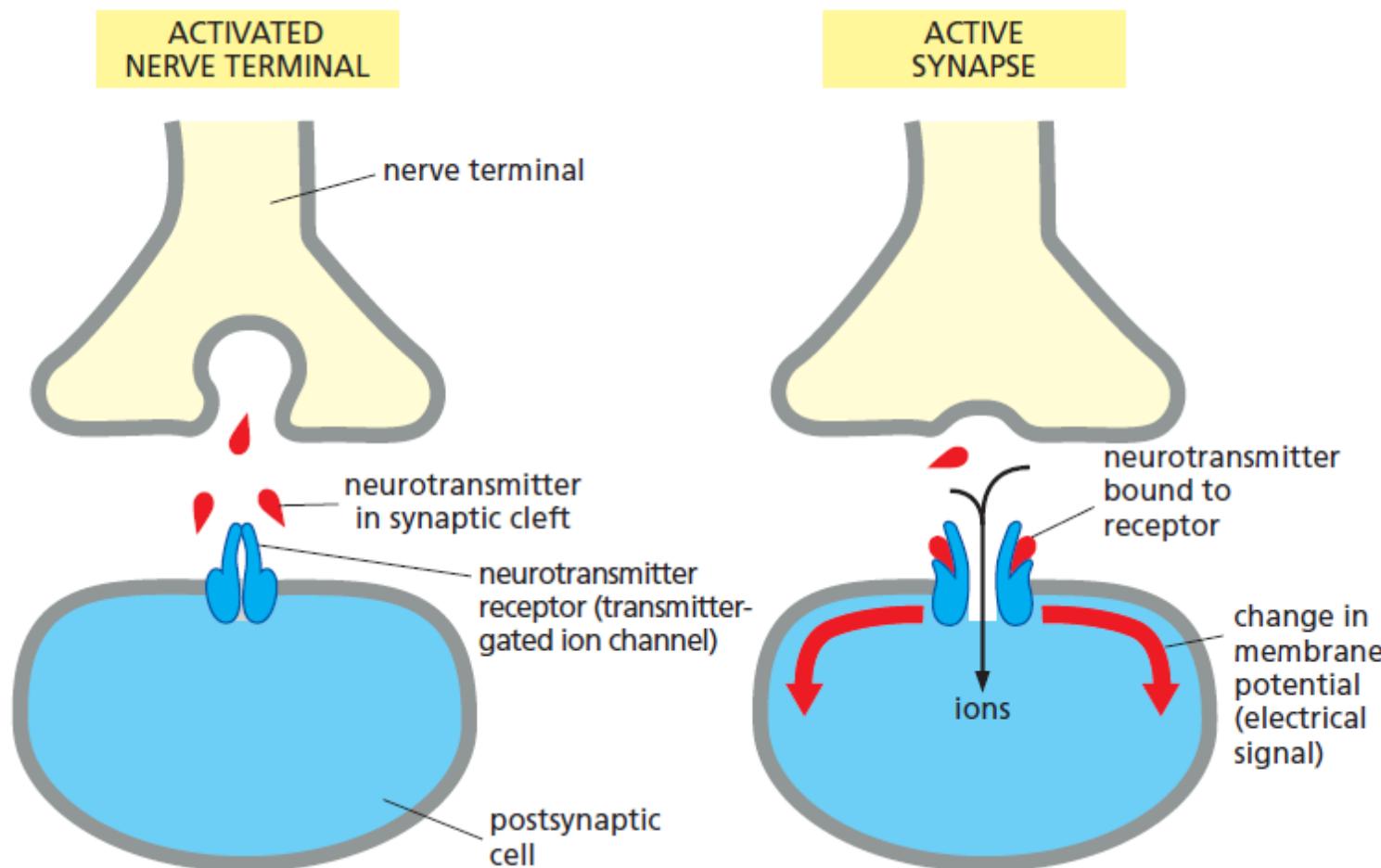


# CONVERSION OF ELECTRIC SIGNALS INTO CHEMICAL ONES

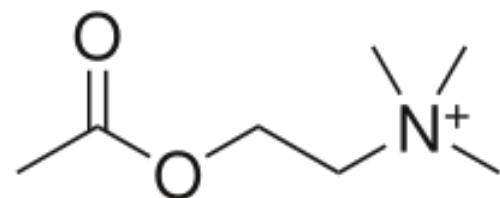


# CONVERSION OF CHEMICAL SIGNALS INTO ELECTRIC ONES

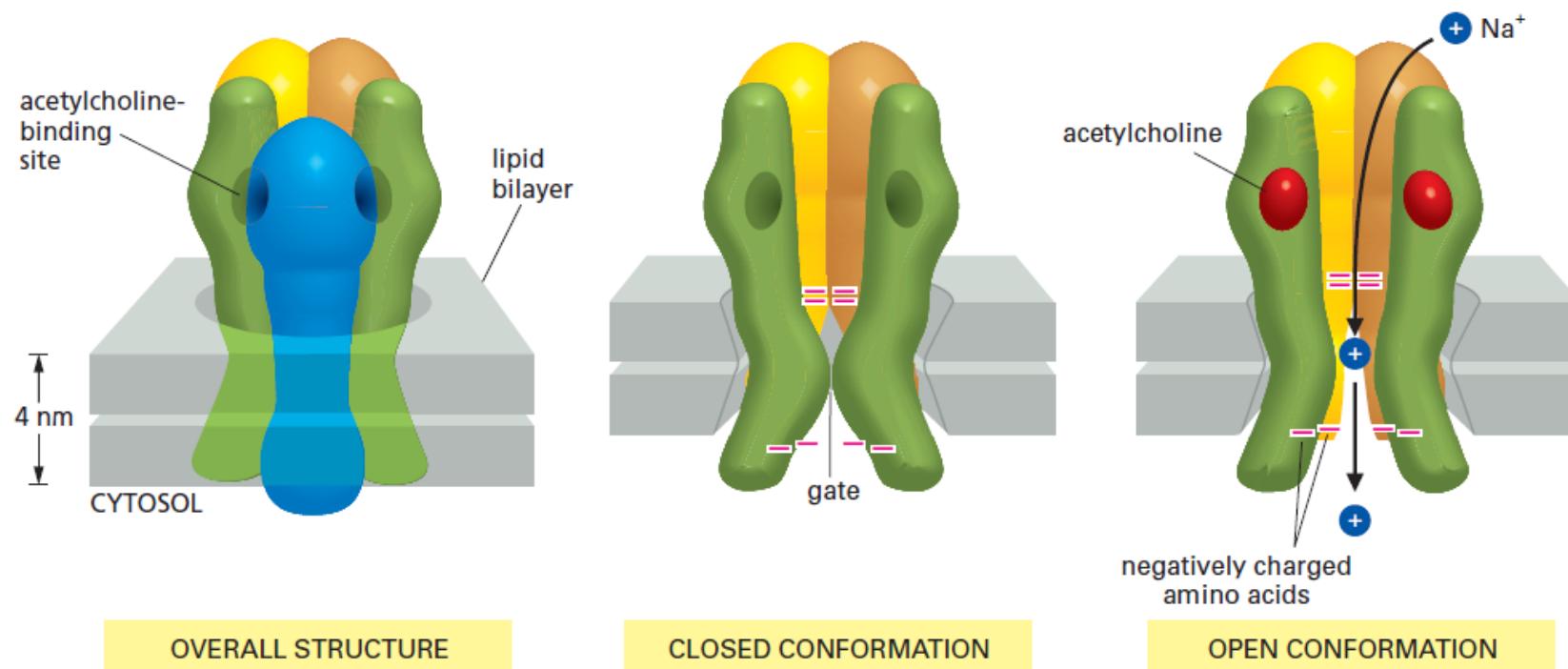
- Neurotransmitters bind their receptors.
- This activates the action potential.
- Neurotransmitters are removed/destroyed.



# EXAMPLE: ACETYLCHOLINE



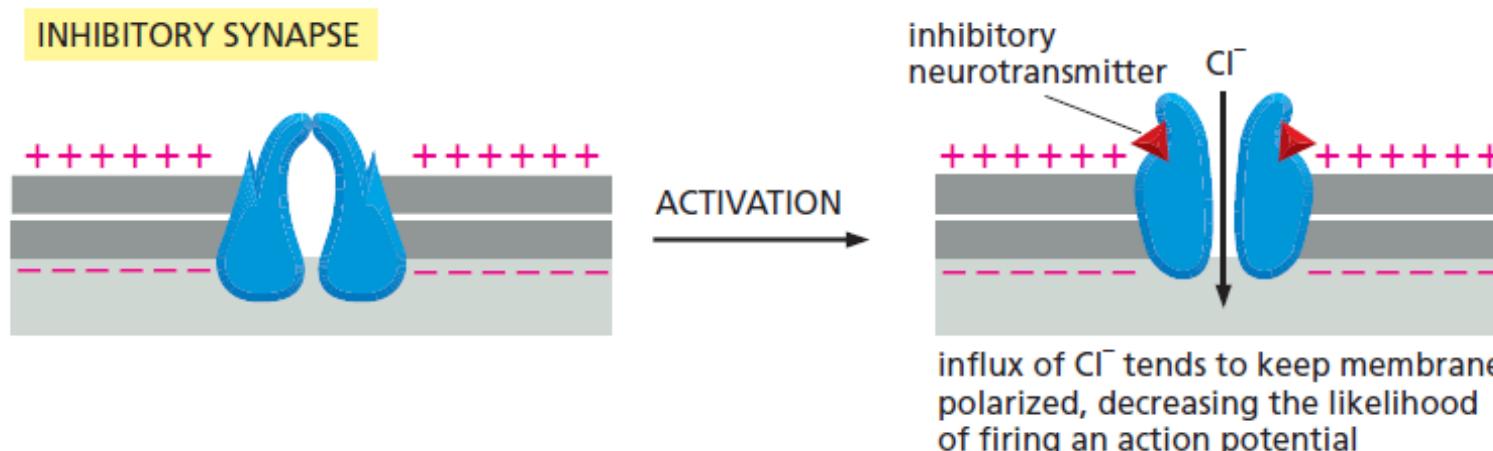
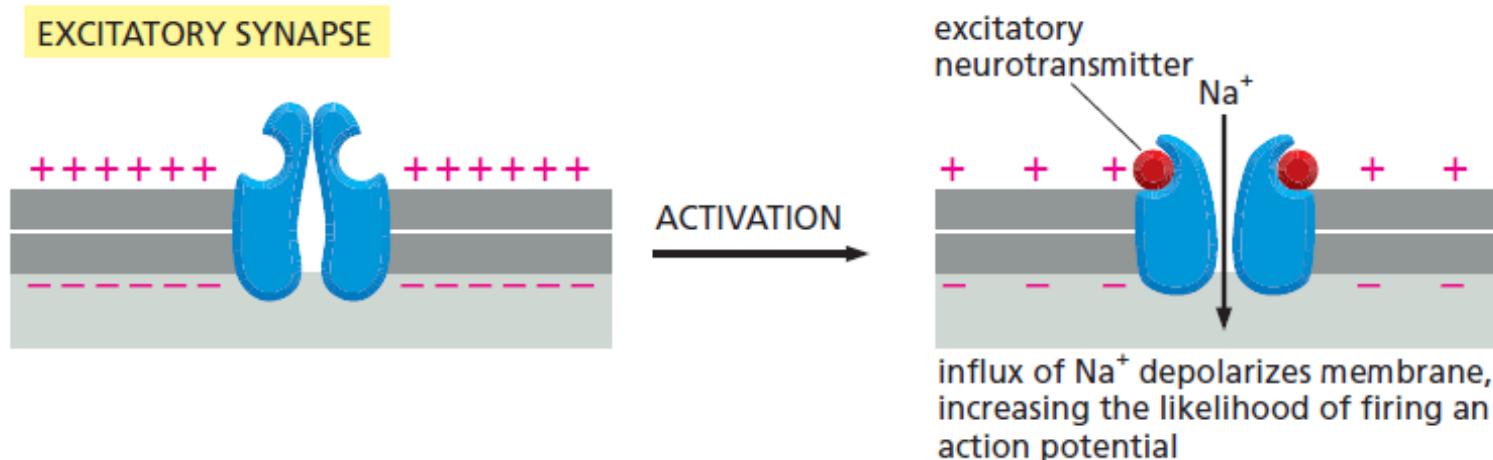
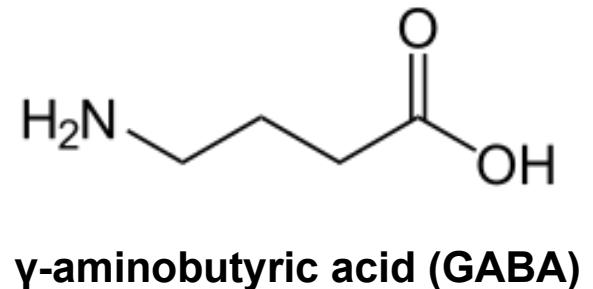
Acetylcholine



Acetylcholine receptor

# NEUROTRANSMITTERS

- Excitatory (acetylcholine, Glu):  $\text{Na}^+$
- Inhibitory (GABA, Gly):  $\text{Cl}^-$
- Drugs interact with the receptors:
  - barbiturates (GABA-gated  $\text{Cl}^-$  channels)
  - Prozac blocks serotonin uptake

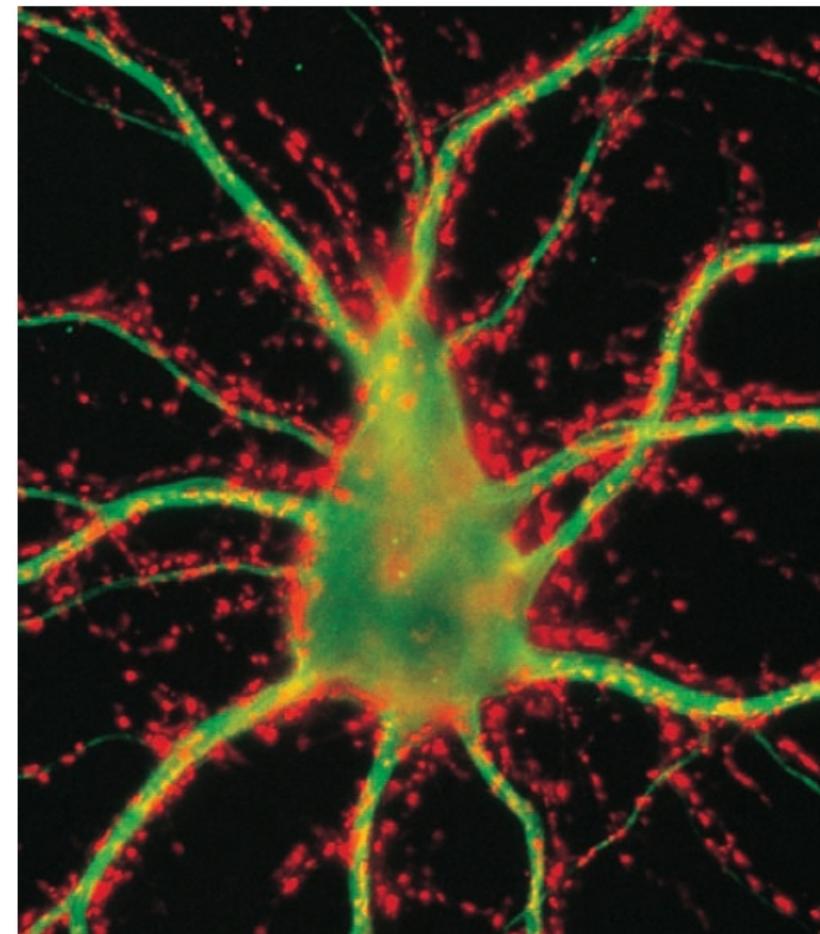
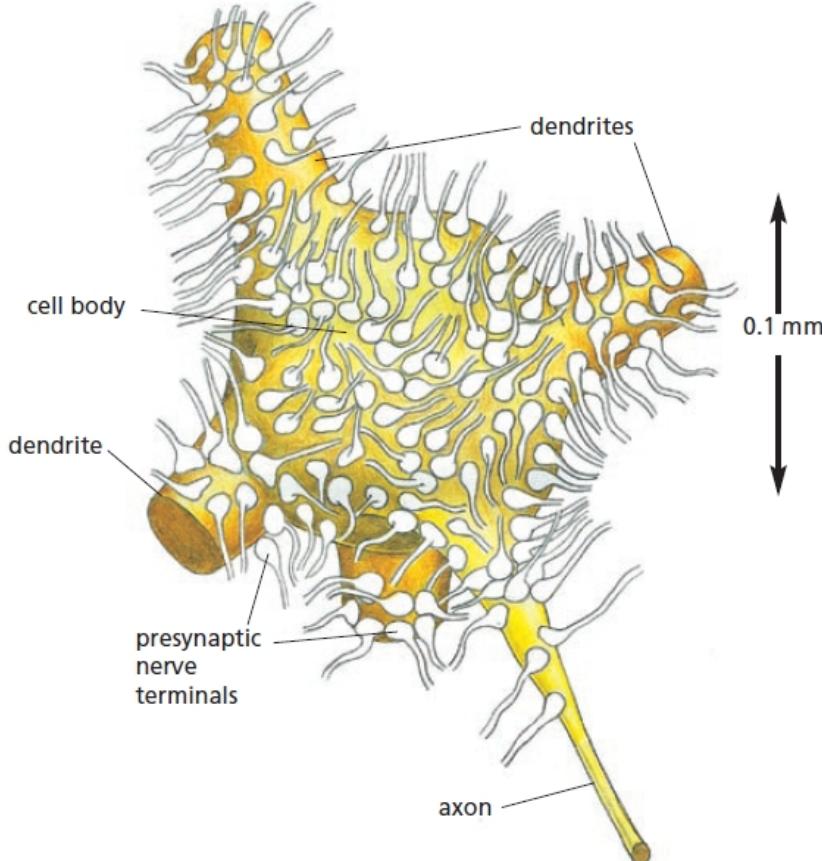


# SYNAPTIC SIGNALS

➤ Combination of electric and chemical signals:

- specificity

- tuned control mechanisms



# EXAMPLES OF ION CHANNELS

ION CHANNEL	TYPICAL LOCATION	FUNCTION
K <sup>+</sup> leak channel	plasma membrane of most animal cells	maintenance of resting membrane potential
Voltage-gated Na <sup>+</sup> channel	plasma membrane of nerve cell axon	generation of action potentials
Voltage-gated K <sup>+</sup> channel	plasma membrane of nerve cell axon	return of membrane to resting potential after initiation of an action potential
Voltage-gated Ca <sup>2+</sup> channel	plasma membrane of nerve terminal	stimulation of neurotransmitter release
Acetylcholine receptor (acetylcholine-gated Na <sup>+</sup> and Ca <sup>2+</sup> channel)	plasma membrane of muscle cell (at neuromuscular junction)	excitatory synaptic signaling
Glutamate receptors (glutamate-gated Na <sup>+</sup> and Ca <sup>2+</sup> channels)	plasma membrane of many neurons (at synapses)	excitatory synaptic signaling
GABA receptor (GABA-gated Cl <sup>-</sup> channel)	plasma membrane of many neurons (at synapses)	inhibitory synaptic signaling
Glycine receptor (glycine-gated Cl <sup>-</sup> channel)	plasma membrane of many neurons (at synapses)	inhibitory synaptic signaling
Stress-activated cation channel	auditory hair cell in inner ear	detection of sound vibrations

# LECTURES 14-16: CELL MEMBRANE

➤ **Membrane components:**

- lipids
- membrane proteins
- glycoproteins
- carbohydrates

➤ **Membrane recognition and penetration by toxins**

➤ **Transport through membrane:**

- principles
- transporters
- ion channels

