

Membrane Proteins

Membrane Proteins

- Cells and organelles within them are bounded by membranes, which are extremely thin (4.5 nm) films of lipids and protein molecules.
- The lipids form a bilayered sheet structure that is hydrophilic on its two outer surfaces and hydrophobic in between.
- Protein molecules are embedded in this layer.
- A biological membrane functions basically as a permeability barrier that establishes discrete compartments and prevents the random mixing of the contents of one compartment with those of another.
- However, biological membranes are more than passive containers.

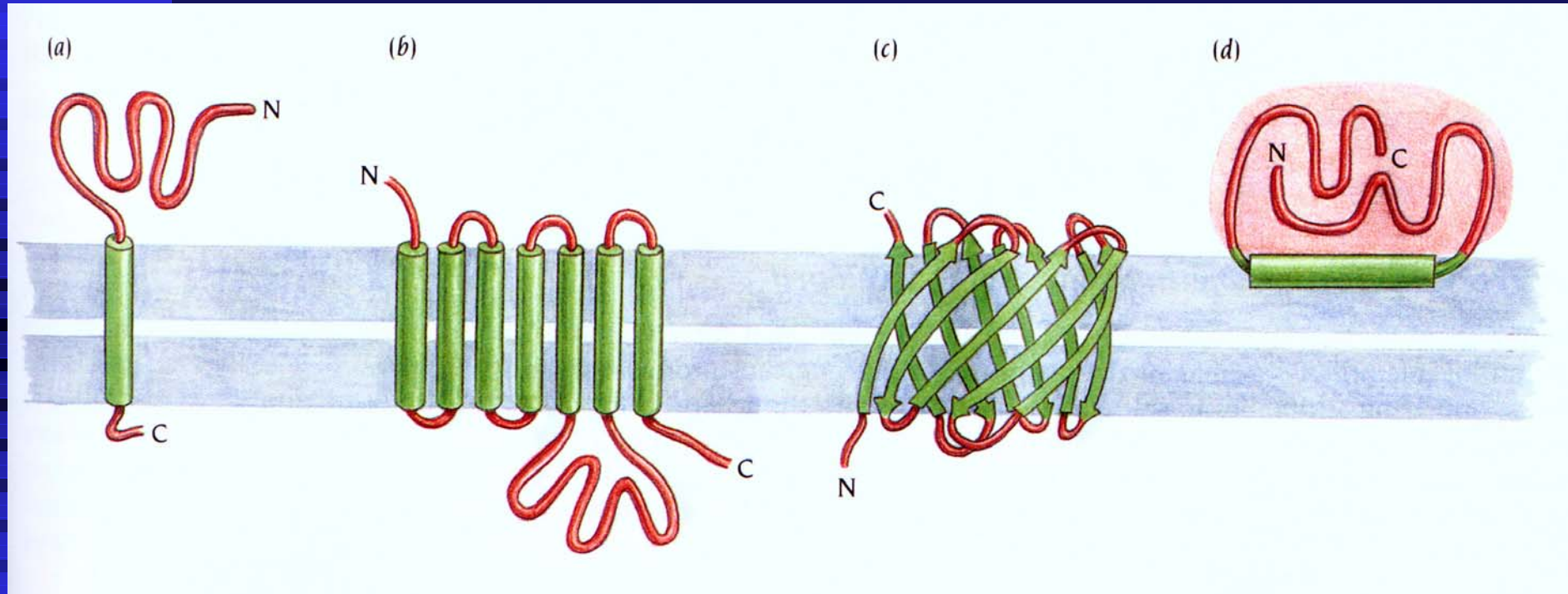
Membrane Proteins

- The embedded proteins serve as highly active mediators between the cell and its environment or the interior of an organelle and the cytosol.
- They catalyze specific transport of metabolites and ions across the membrane barriers.
- They convert the energy of sun light into chemical and electrical energy, and they couple the flow of electrons to synthesis ATP.
- They act as signal receptors and transduce signals across the membrane. The signals can be, for example, neurotransmitters, growth factors, hormones, light or chemical stimuli.
- The transmembrane proteins of the plasma membrane are also involved in cell-cell recognition.

Membrane Proteins

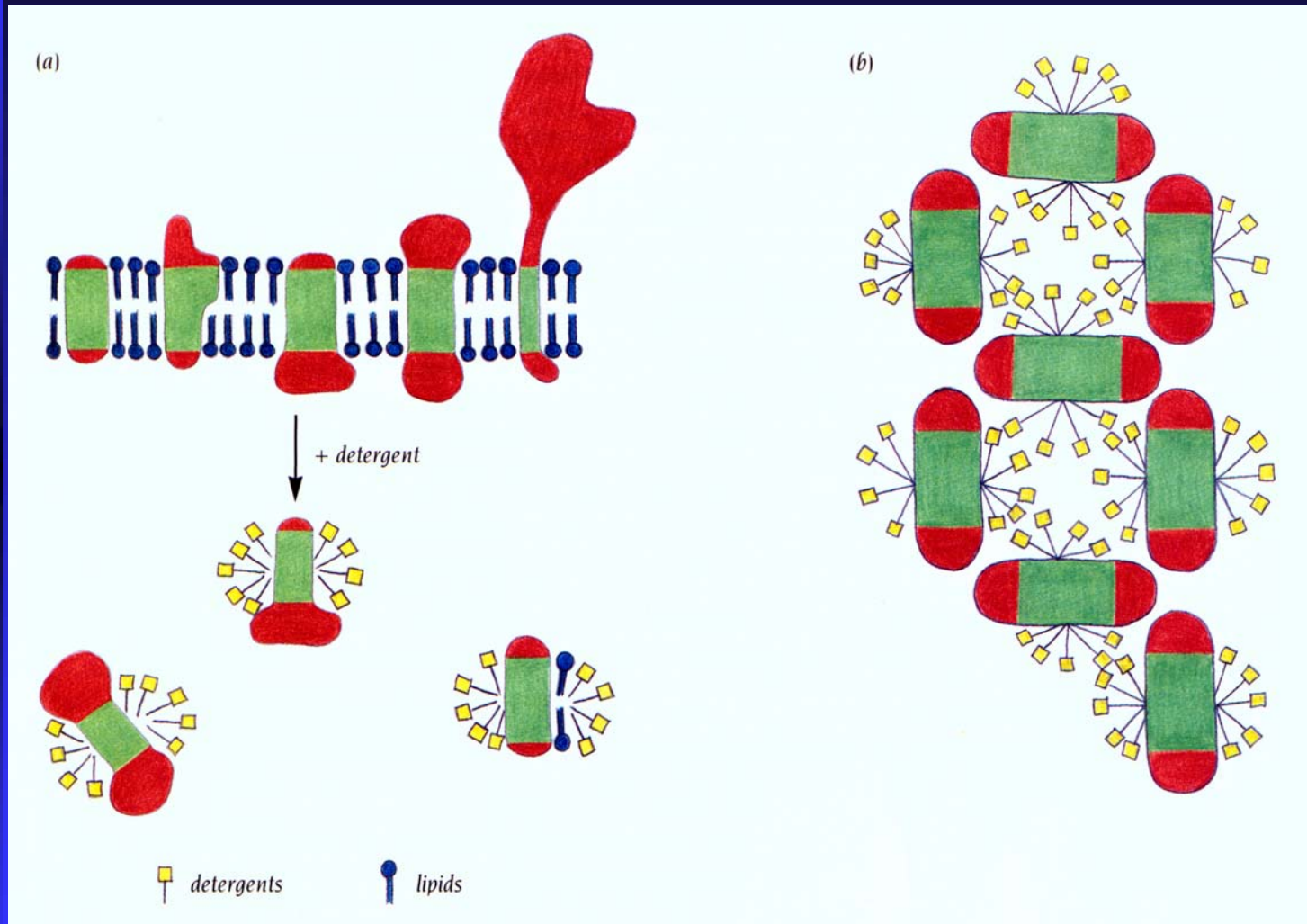
- Here we will discuss some examples of structures of membrane-bound proteins known to high resolution,
- and outline how elucidation of these structures has contributed to understanding the specific function of these proteins,
- as well as some general principles for the construction of membrane-bound proteins.

Membrane proteins



Protein molecules may be bound to a membrane in four different ways. (a) A protein whose polypeptide chain traverses the membrane once as an α helix, (b) a protein that forms several transmembrane α helices connected by hydrophilic loop regions, (c) a protein with several β strands that form a channel through the membrane, and (d) a protein that is anchored to the membrane by one α helix parallel to the plane of the membrane.

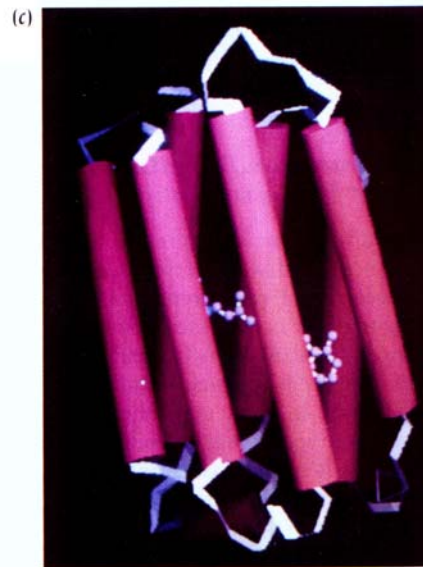
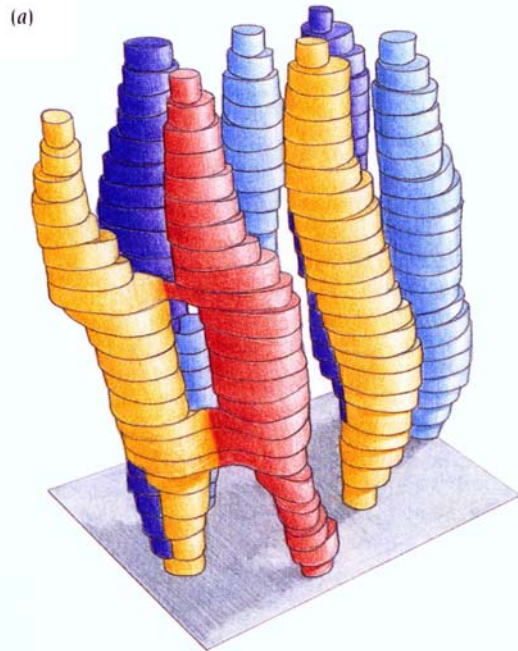
Membrane proteins are difficult to crystallize



(a) Schematic drawing of membrane proteins in a typical membrane and their solubilization by detergents. (b) A membrane protein crystallized with detergents bound to its hydrophobic protein surface.

Bacteriorhodopsin Story

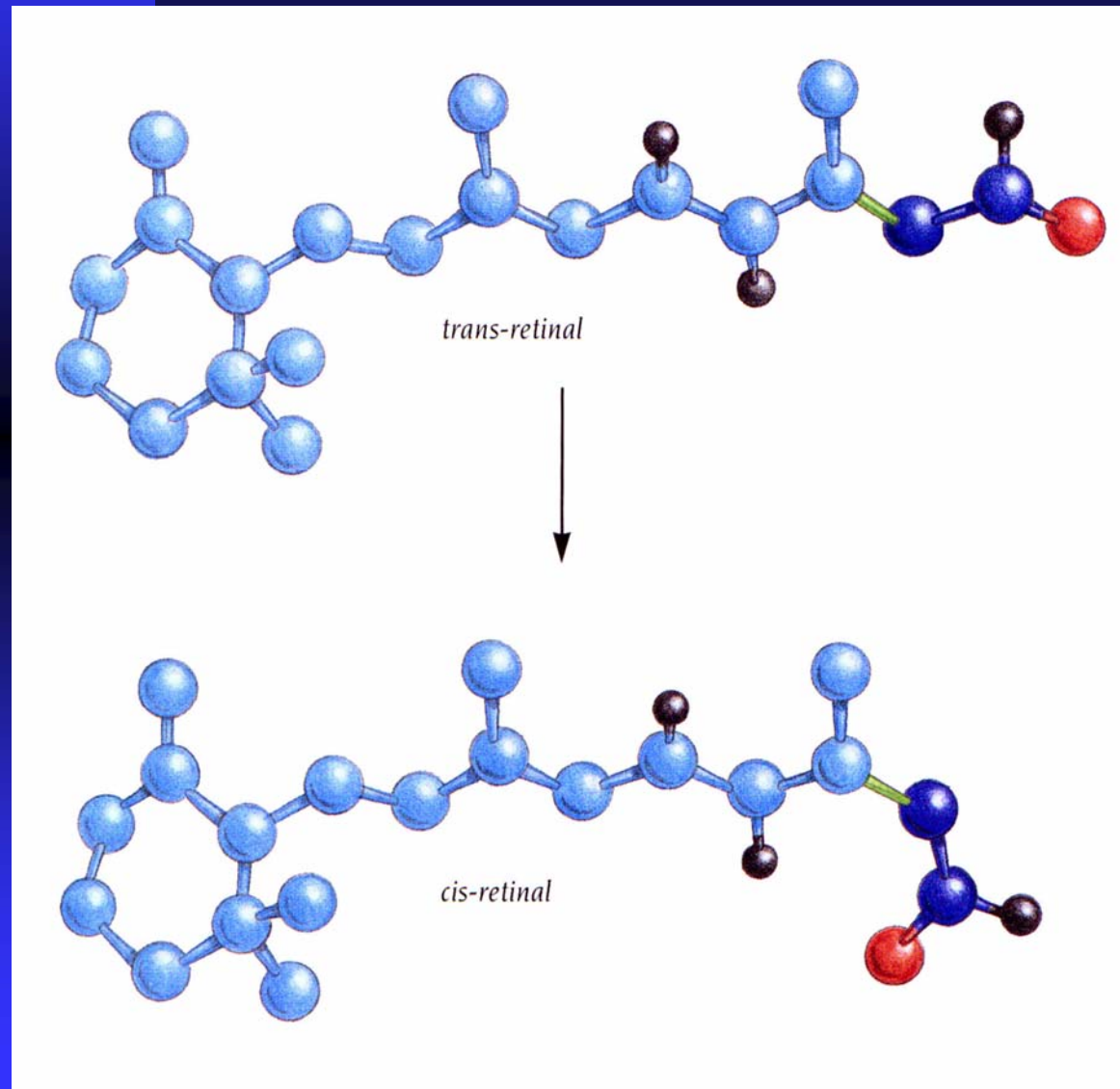
Two-dimensional crystals of membrane proteins can be studied by electron microscopy



A bacteriorhodopsin electron density map solved to 7 Å resolution (a) was obtained and interpreted in terms of seven transmembrane helices (b). In 1990 the resolution was extended to 3 Å, which confirmed the presence of the seven α helices (c). This structure also showed how these helices were connected by loop regions and where the retinal molecule was bound to bacteriorhodopsin.

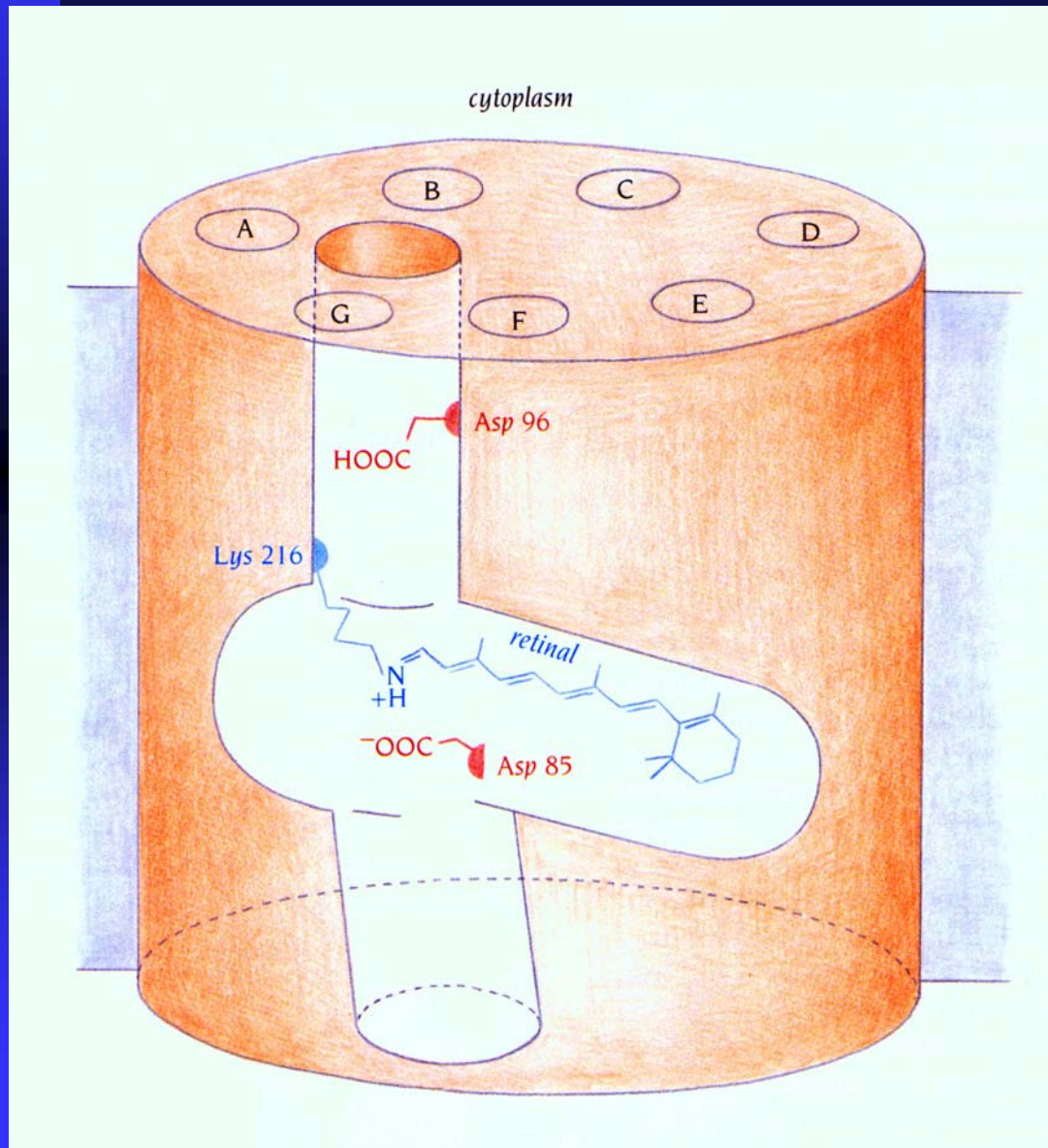
Bacteriorhodopsin is a light-driven proton pump

Bacteriorhodopsin is a light-driven proton pump



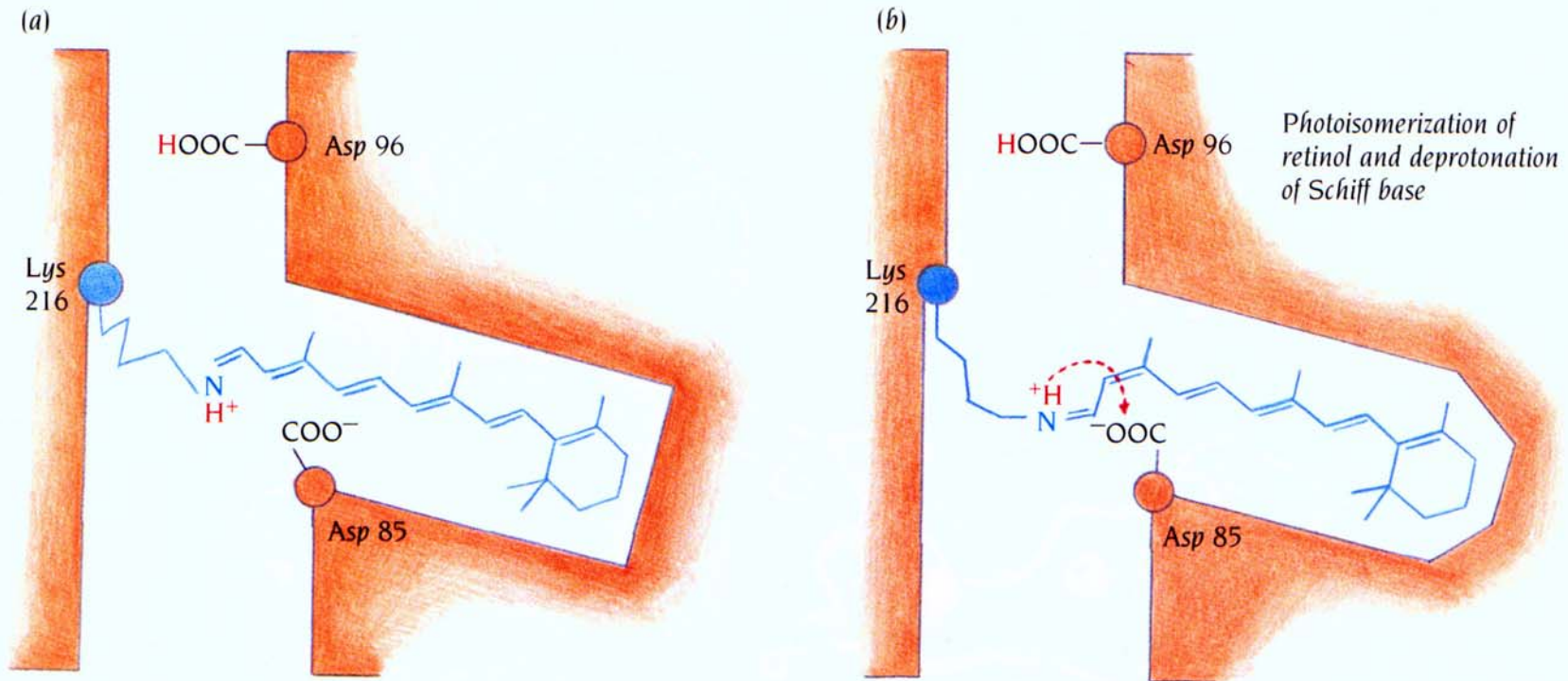
The light-absorbing pigment retinal undergoes a conformational change called isomerization, when it absorbs light. One part of the molecule (dark blue and red) rotates 180° around a double bond between two carbon atoms (green). The geometry of the molecule is changed by this rotation from a *trans* form to a *cis* form. Carbon atoms are blue, hydrogen atoms gray and the oxygen atom red.

Bacteriorhodopsin is a light-driven proton pump



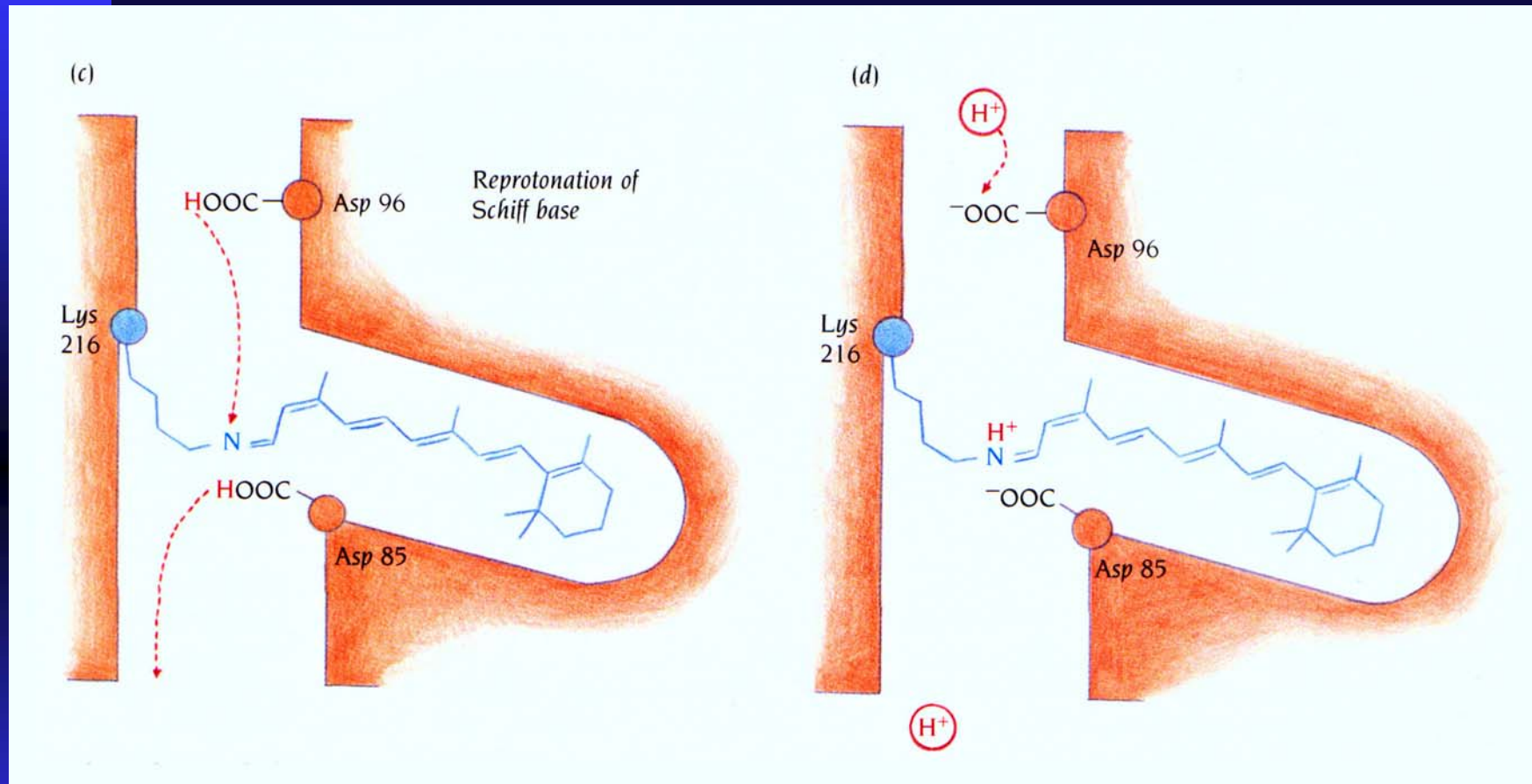
Schematic diagram of the bacteriorhodopsin molecule illustrates the relation between the proton channel and bound retinal in *trans* form. A to E are the seven transmembrane helices. Retinal is covalently bound to a lysine residue. The relative positions of Asp residues, which are important for proton transfer, are also shown.

Bacteriorhodopsin is a light-driven proton pump



The proton movements in the photocycle of bacteriorhodopsin. The protein adopts two main conformational states, tense (T) and relaxed (R). The state T binds *trans*-retinal tightly and the R state binds *cis*-retinal. (a) Structure of bacteriorhodopsin in the T state with *trans*-retinal bound to Lys 216 via a Schiff base. (b) A proton is transferred from the Schiff base to Asp 85 following isomerization of retinal and a conformational change of the protein.

Bacteriorhodopsin is a light-driven proton pump



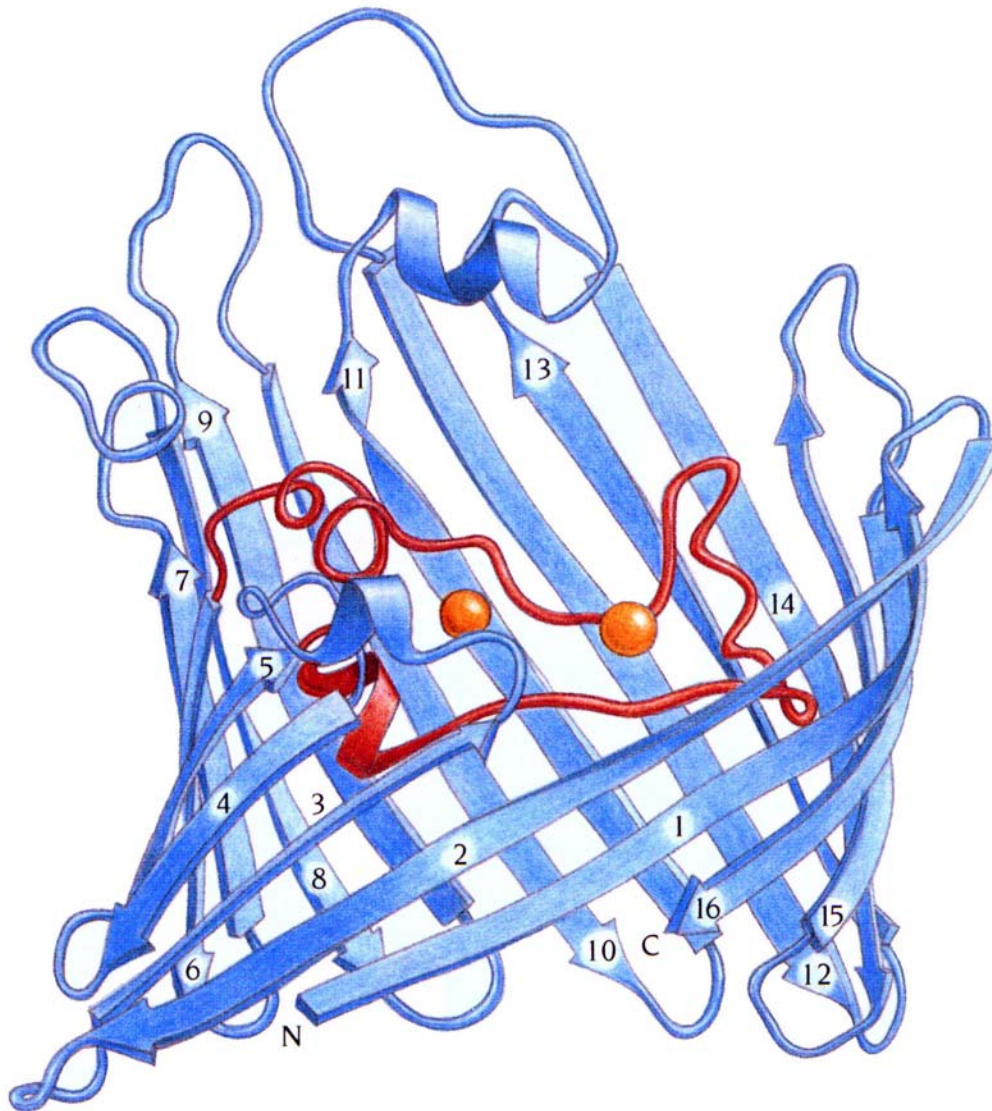
The proton movements in the photocycle of bacteriorhodopsin. The protein adopts two main conformational states, tense (T) and relaxed (R). The state T binds *trans*-retinal tightly and the R state binds *cis*-retinal. (c) Structure of bacteriorhodopsin in the R state with *cis*-retinal bound. A proton is transferred from Asp 96 to the Schiff base and from Asp 85 to the extracellular space. (d) A proton is transferred from the cytoplasm to Asp 96.

Porin Story

Porin story

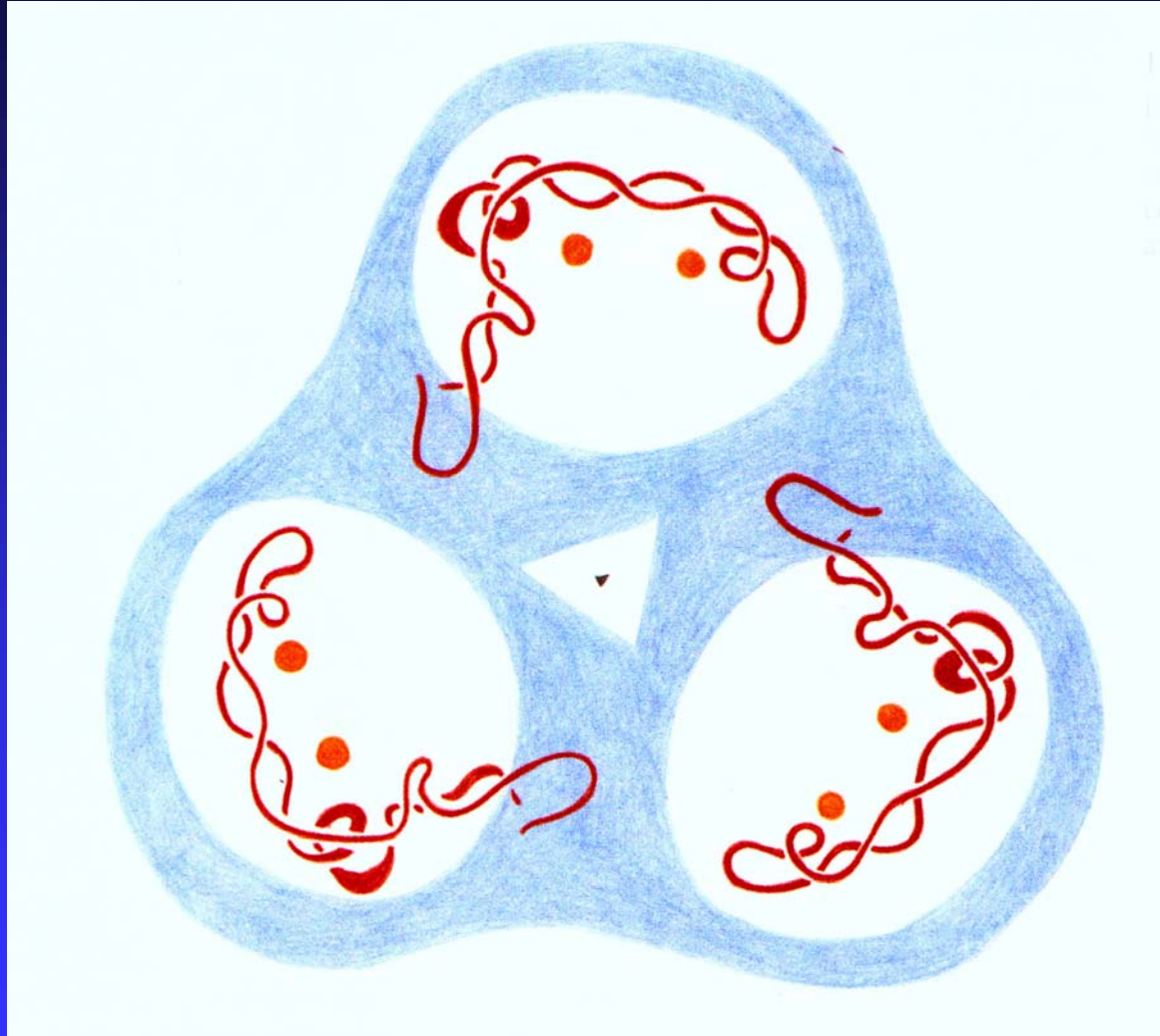
The porins in the outer membrane of Gram-negative bacteria form size-restricted channels for the passive diffusion of molecules in and out of the periplasmic space.

Porin channels are made by up and down β barrels



Ribbon diagram of one subunit of porin from *Rhodobacter capsulatus* viewed from the plane of the membrane. Sixteen β strands form an antiparallel β barrel that traverses the membrane. The loops at the top of the picture are extracellular whereas the short turns at the bottom face the periplasm. The long loop between b strands 5 and 6 (red) constricts the channel of the barrel. Two calcium atoms are shown as orange circles.

Each porin molecule has three channels



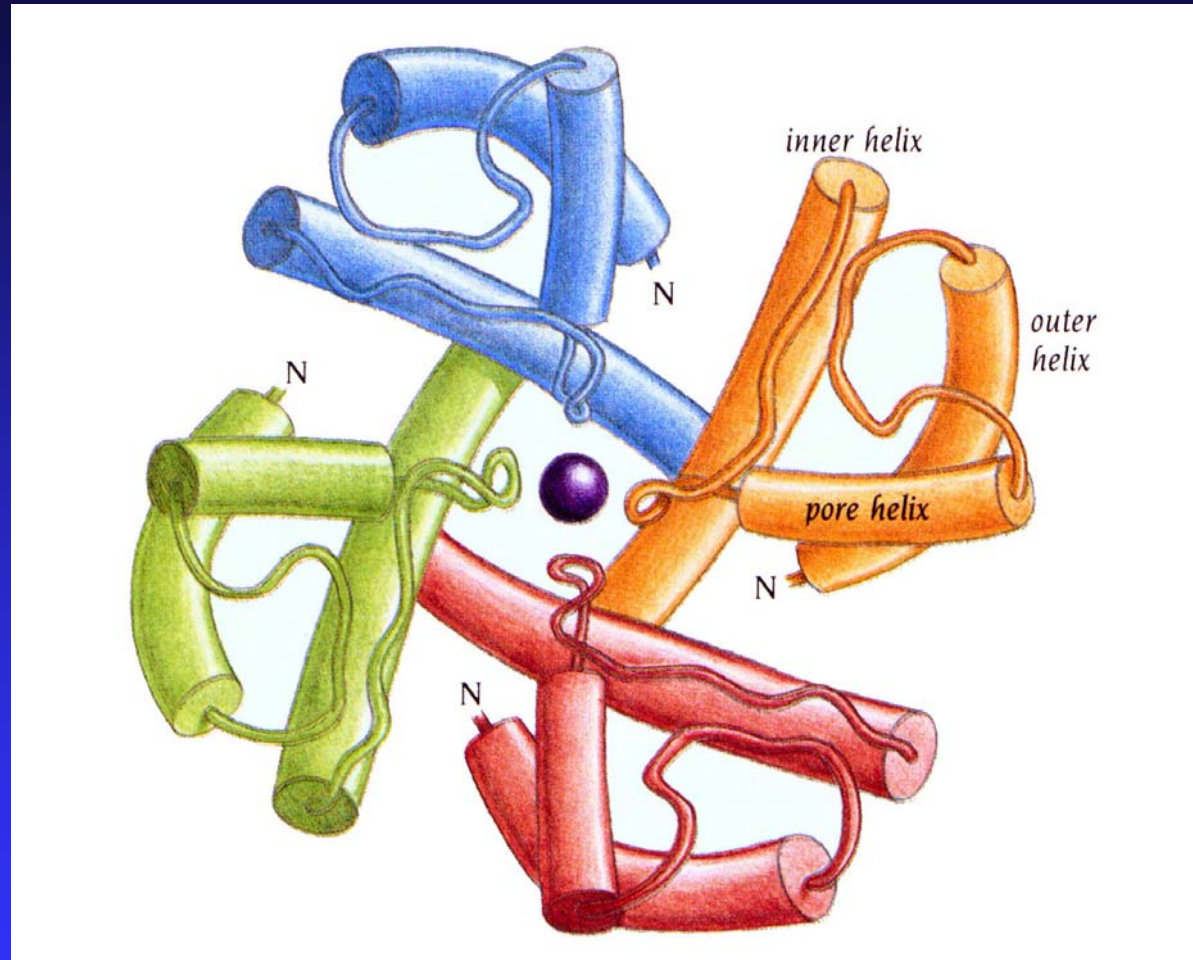
The trimeric porin molecule is viewed from the extracellular space. Blue regions illustrate the walls of the three porin barrels, the loop regions that constrict the channel are red and the calcium atoms are orange.

Ion Channel Story

Ion channels combine ion selectivity with high levels of ion conductance

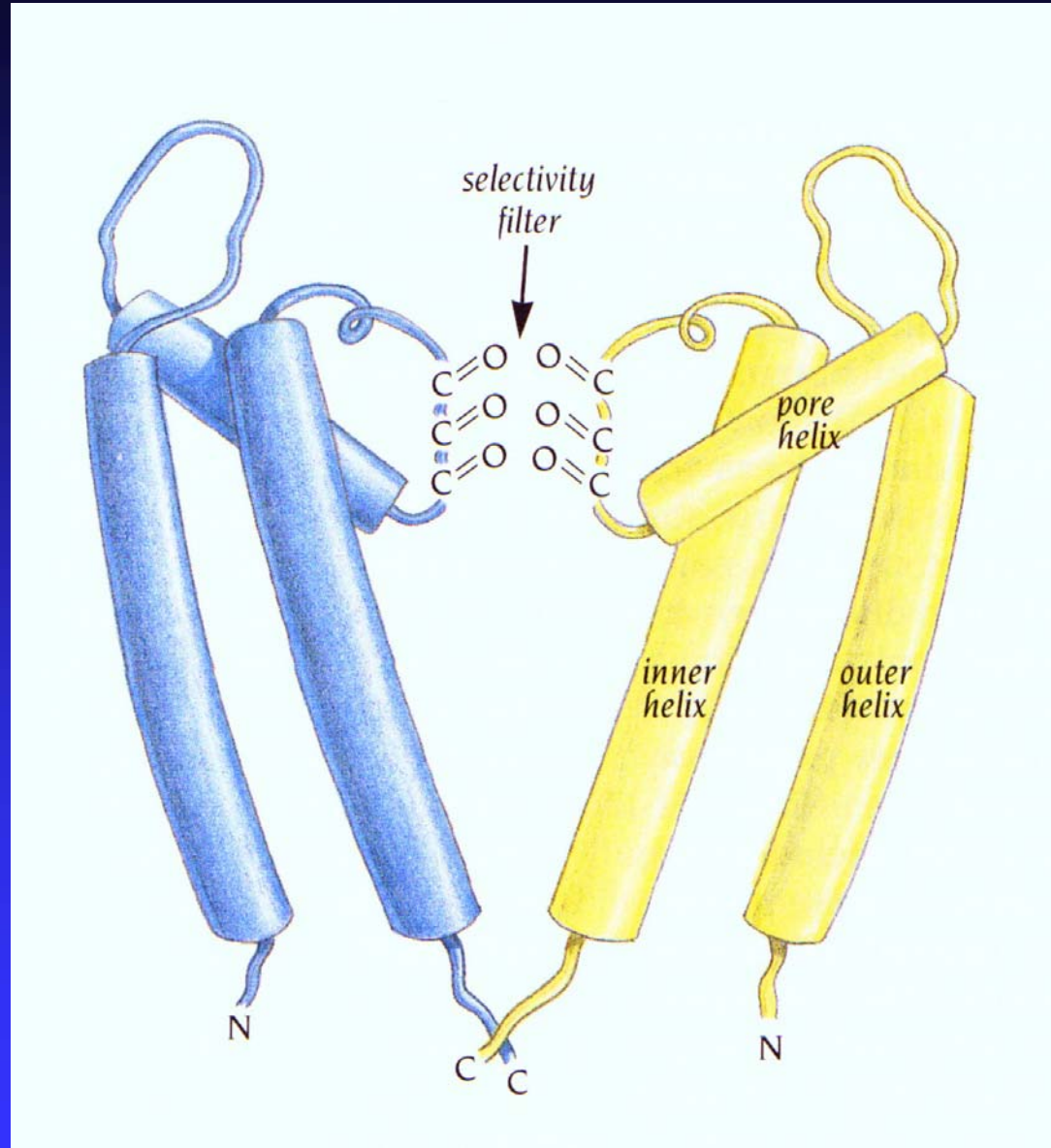
- The bacterial K^+ channel has tilted transmembrane helices, two in each of the subunits of the homotetrameric molecule that has fourfold symmetry.
- These transmembrane helices line the central and inner parts of the channel but do not contribute to the remarkable 10,000-fold selectivity for K^+ ions over Na^+ ions.
- This crucial property is achieved through the narrow selectivity filter that is formed by loop regions from the four subunits and lined by main-chain carbonyl oxygen atoms, to which dehydrated K^+ ions bind.

The K^+ channel is a tetrameric molecule with one ion pore in the interface between the four subunits



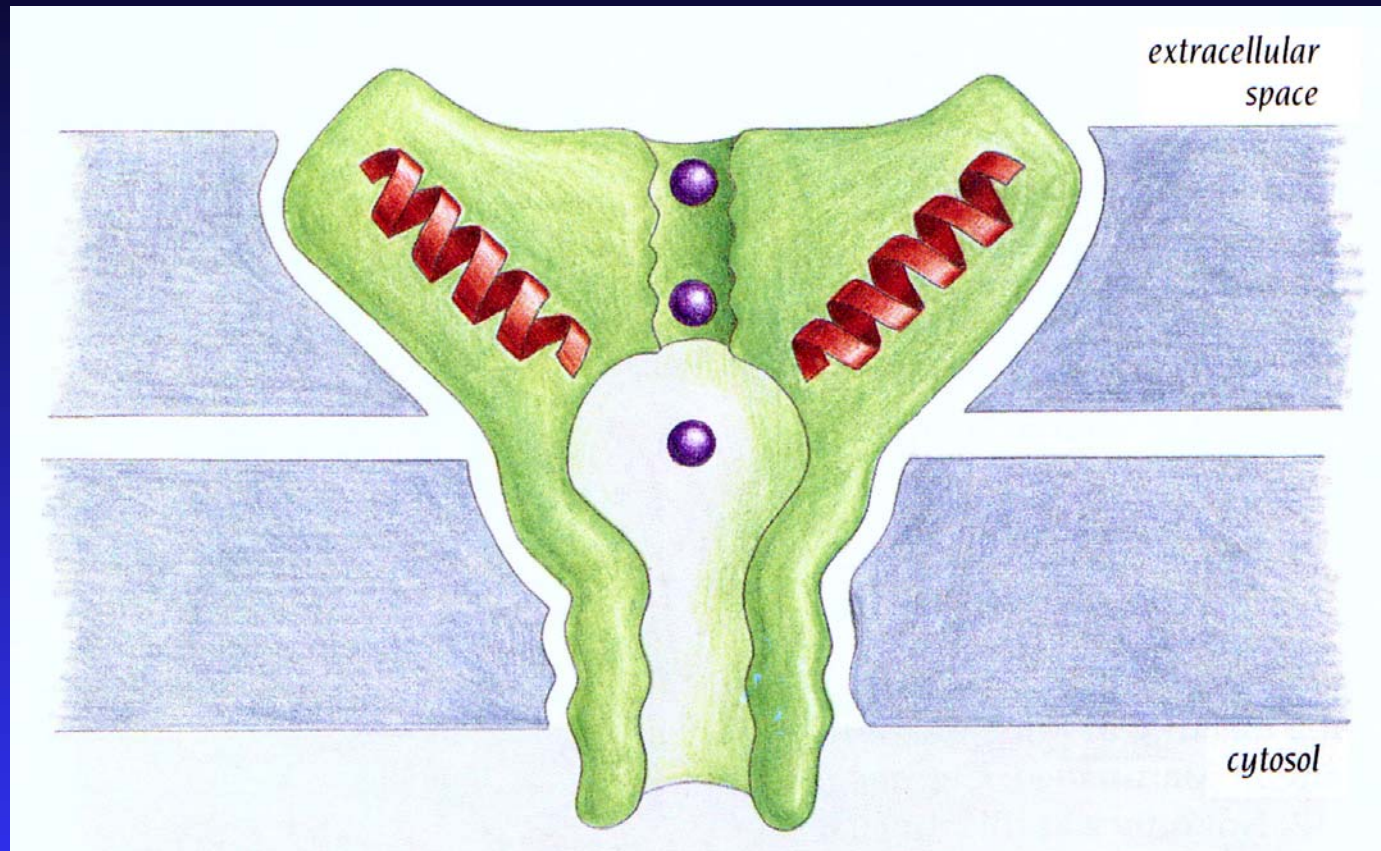
The structure of a potassium channel is viewed perpendicular to the plane of the membrane. The molecule is tetrameric with a hole in the middle that forms the ion pore (purple). Each subunit forms two transmembrane helices, the inner and the outer helix. The pore helix and loop regions build up the ion pore in combination with the inner helix.

The selectivity filter is formed between two subunits of the K⁺ channel



Main-chain atoms line the walls of this narrow passage with carbonyl oxygen atoms pointing into the pore, forming binding sites for K⁺ ions.

The ion pore has a narrow ion selectivity filter

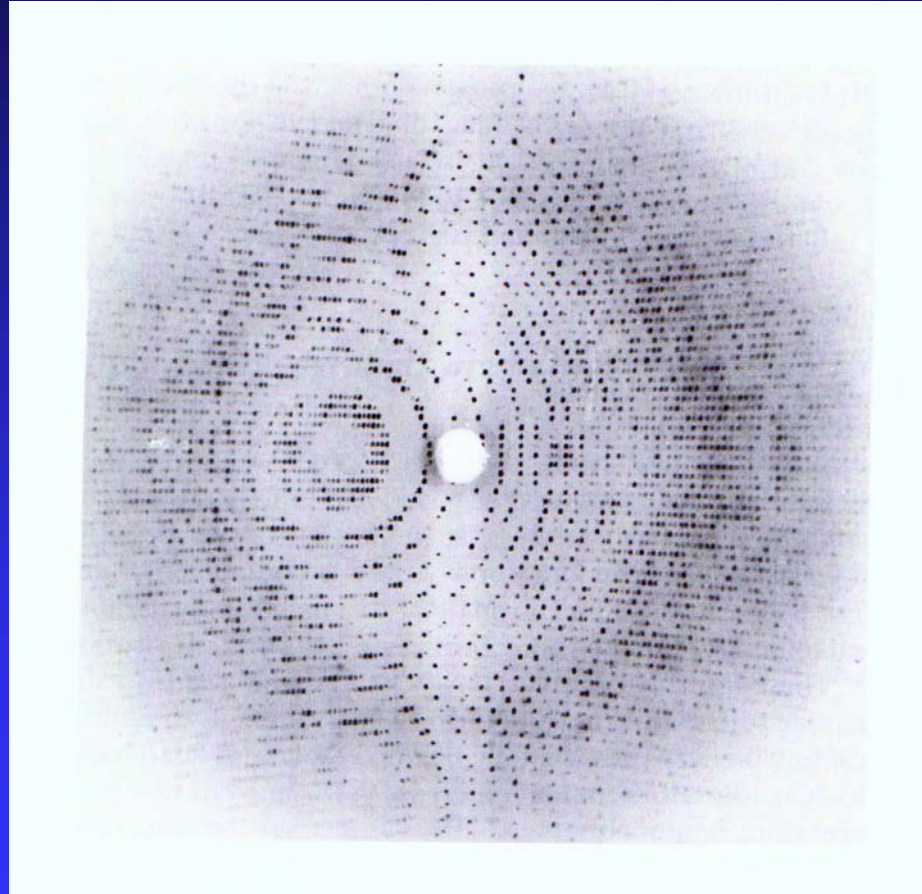


Schematic diagram of the ion pore of the K⁺ channel. From the cytosolic side the pore begins as a water-filled channel that opens up into a water-filled cavity near the middle of the membrane. A narrow passage, the selectivity filter, links this cavity to the external solution. Three potassium ions (purple spheres) bind in the pore. The pore helices (red) are oriented such that their carboxyl end (with a negative dipole moment) is oriented towards the center of the cavity to provide a compensating dipole charge to K⁺ ions.

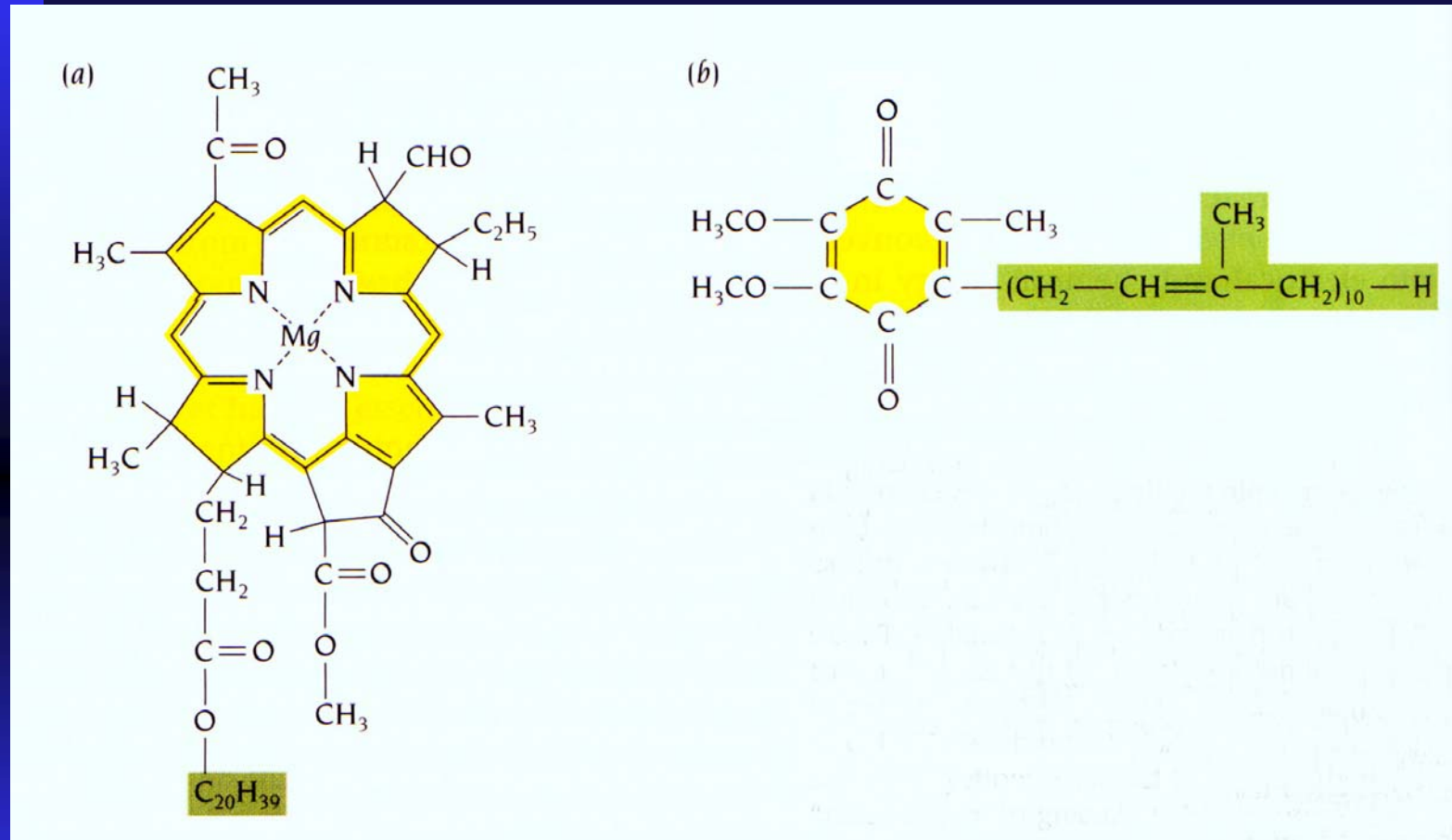
Bacterial Photosynthetic Reaction Center Story

Bacterial photosynthetic reaction center structure

X-ray diffraction pattern from crystals of a membrane-bound protein – the bacterial photosynthetic reaction center

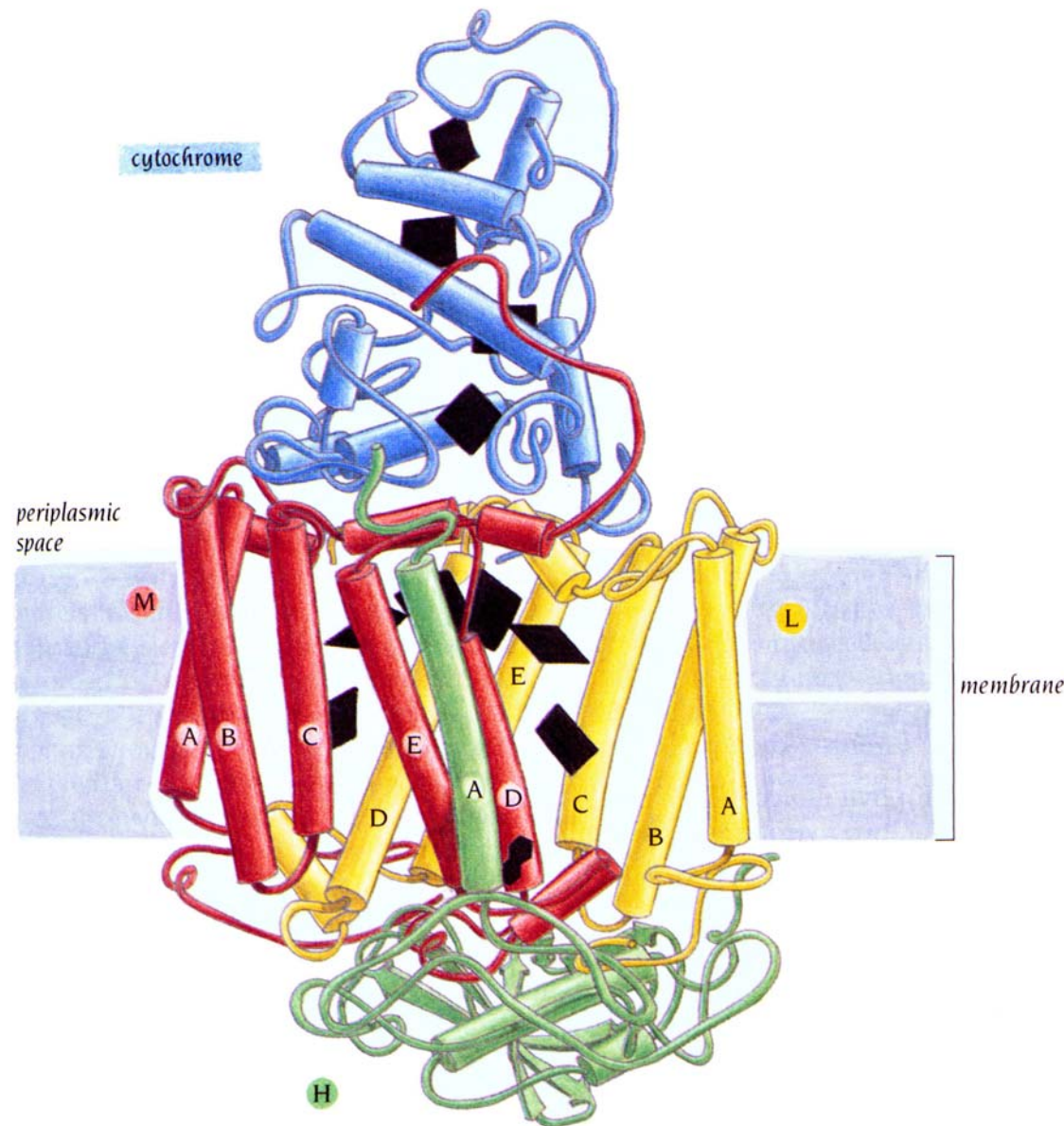


Photosynthetic pigments



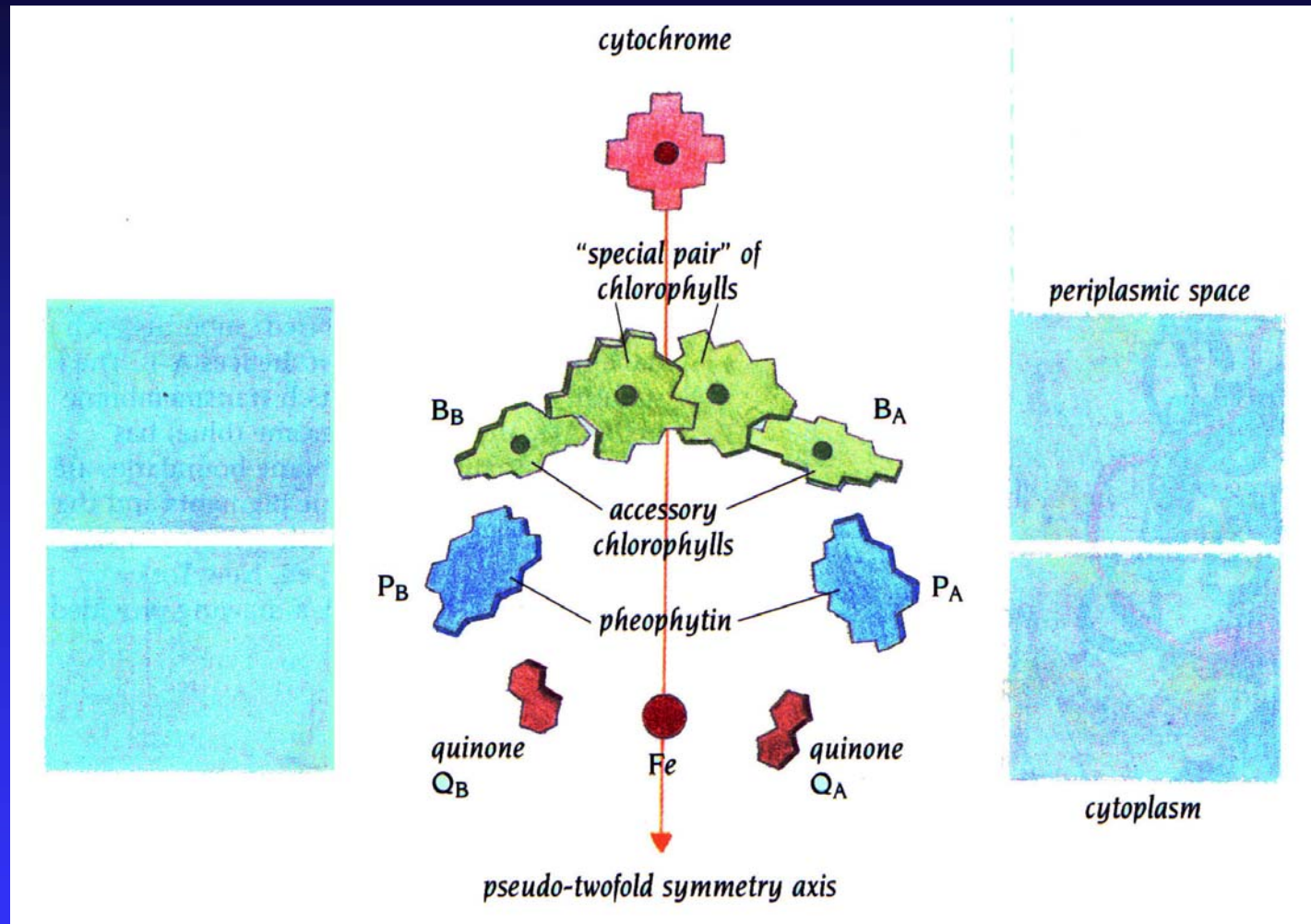
Photosynthetic pigments are used by plants and photosynthetic bacteria to capture photons of light and for electron flow from one side of a membrane to the other side. The diagram shows two such pigments that are present in bacterial reaction centers, **bacteriochlorophyll** (a) and **ubiquinone** (b). The light absorbing parts of the molecules are shown in yellow, attached to hydrocarbon „tails“ shown in green.

The bacterial photosynthetic reaction center is built up from four different polypeptide chains and many pigments



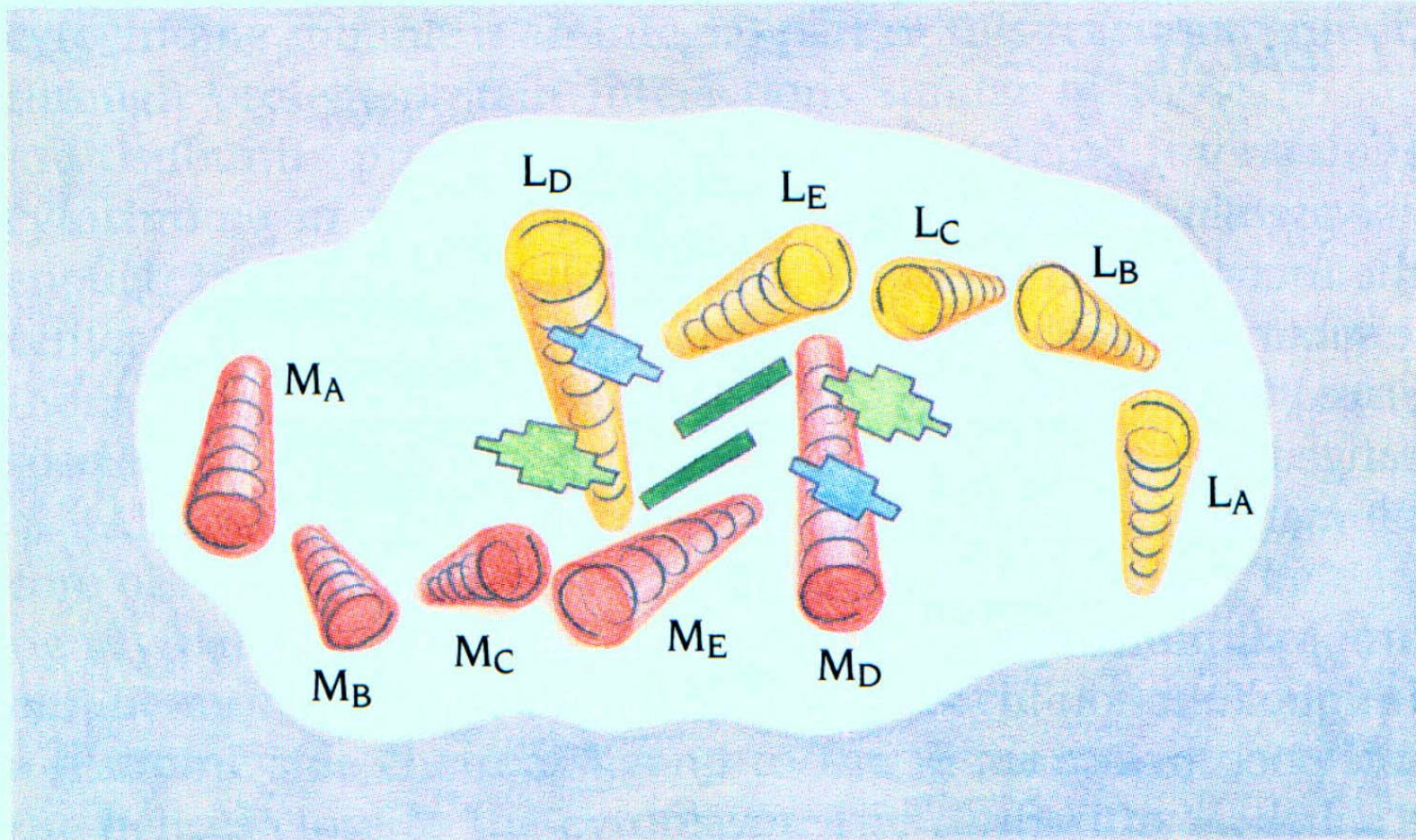
Subunits L and M bind the photosynthetic pigments, and the cytochrome binds four heme groups. The **L** and the **M** subunits each have five transmembrane α helices A-E. The **H** subunit has one such transmembrane helix, AH, and the cytochrome has none. The photosynthetic pigments and the heme groups appear in black.

Arrangement of the photosynthetic pigments in the reaction center of *Rhodospseudomonas viridis*



The twofold symmetry axis that relates the L and the M subunits is aligned vertically in the plane of the paper. Electron transfer proceeds preferentially along the branch to the right. The periplasmic side of the membrane is near the top, and the cytoplasmic side is near the bottom of the structure.

View of the reaction center perpendicular to the membrane



The pigments are bound between the transmembrane helices. The five transmembrane-spanning α helices of the **L** and the **M** subunits are shown as well as the **chlorophyll** and **pheophytin** molecules

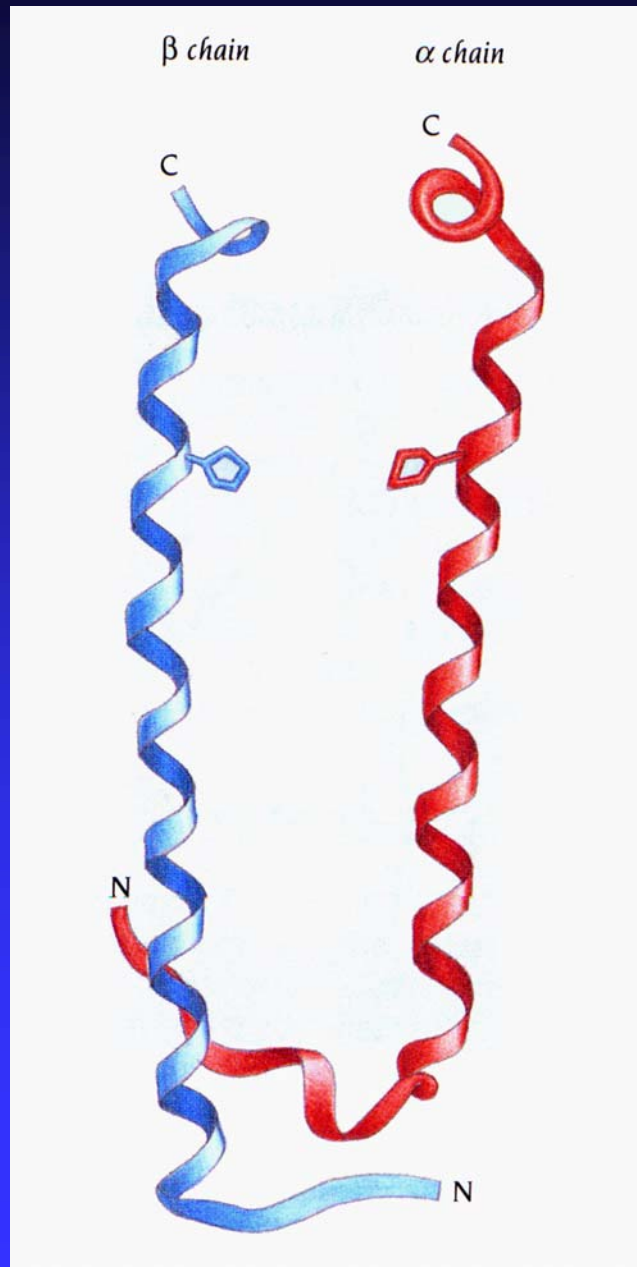
**Antenna pigment proteins assemble into
multimeric light-harvesting particles**

Chlorophyll molecules form circular rings in the light-harvesting complex LH2



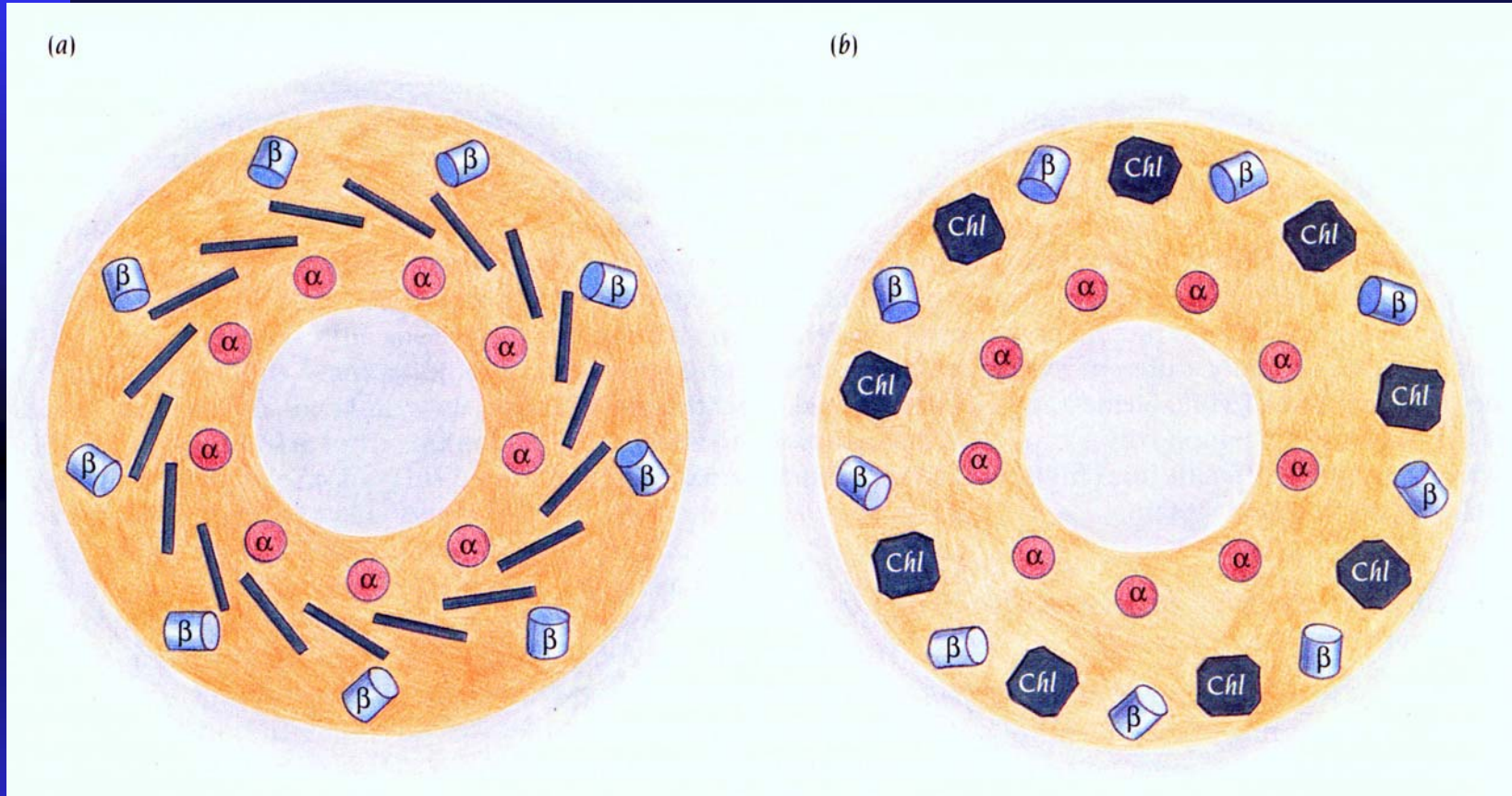
Computer generated diagram of the structure of light-harvesting complex LH2 from *Rhodospseudomonas acidophila*. **Nine α chains** and **nine β chains** form two rings of transmembrane helices between which are bound **9 carotenoids** and **27 bacteriochlorophyll molecules**.

The α and the β chains of the light-harvesting complex LH2



Each chain forms one transmembrane α helix, which contains a histidine residue that binds to the Mg atom of one bacteriochlorophyll molecule

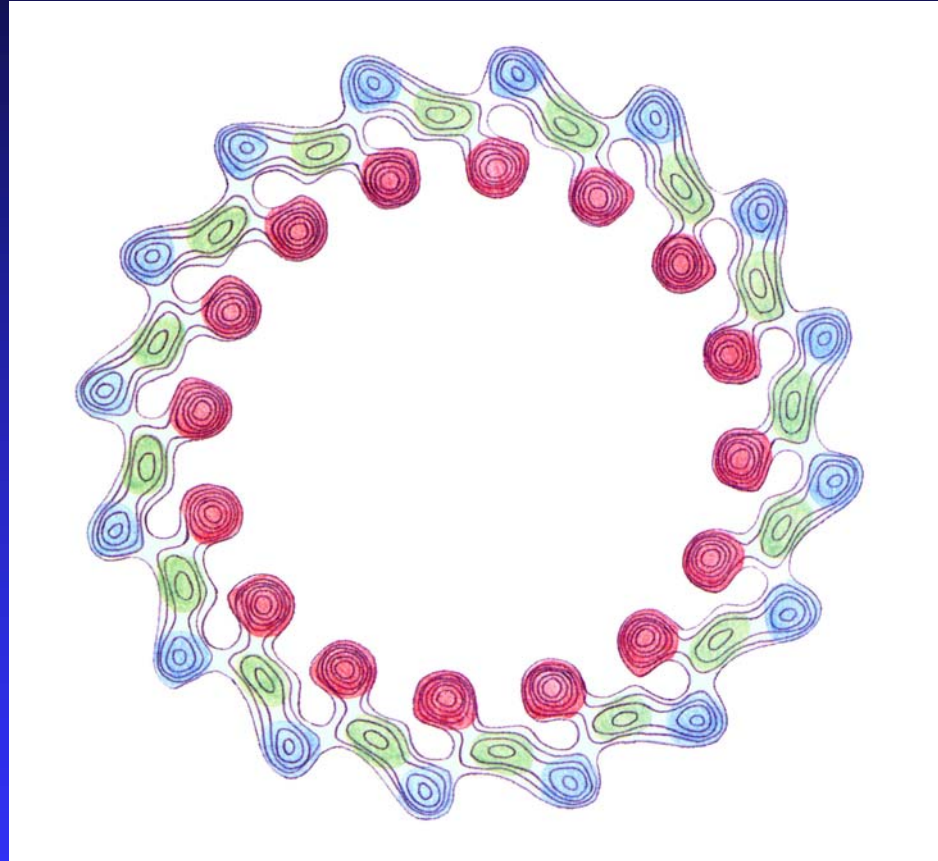
The arrangement of bacteriochlorophyll molecules in the light-harvesting complex LH2



(a) Eighteen bacteriochlorophyll molecules are bound between the two rings of α and β chains. The planes of these molecules are oriented perpendicular to the plane of the membrane and the molecules are bound close to the periplasmic space. (b) Nine bacteriochlorophyll molecules are bound between the β chains with their planes oriented parallel to the plane of the membrane. These molecules are bound in the middle of the membrane.

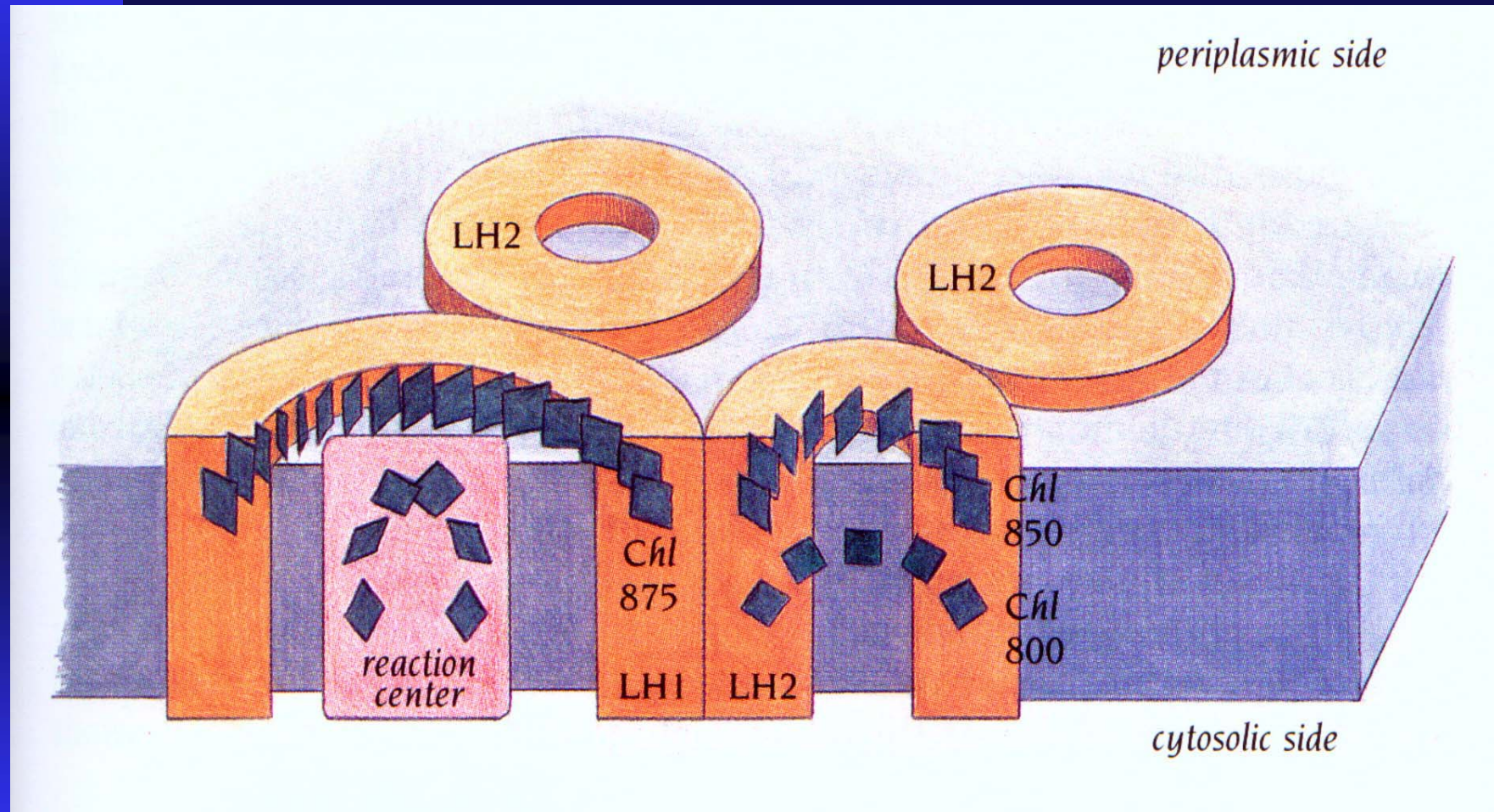
The reaction center is surrounded by a ring of 16 antenna proteins of the light-harvesting complex LH1

Electron density projection of the light-harvesting complex LH1 from *Rhodospirillum rubrum* determined by electron crystallography



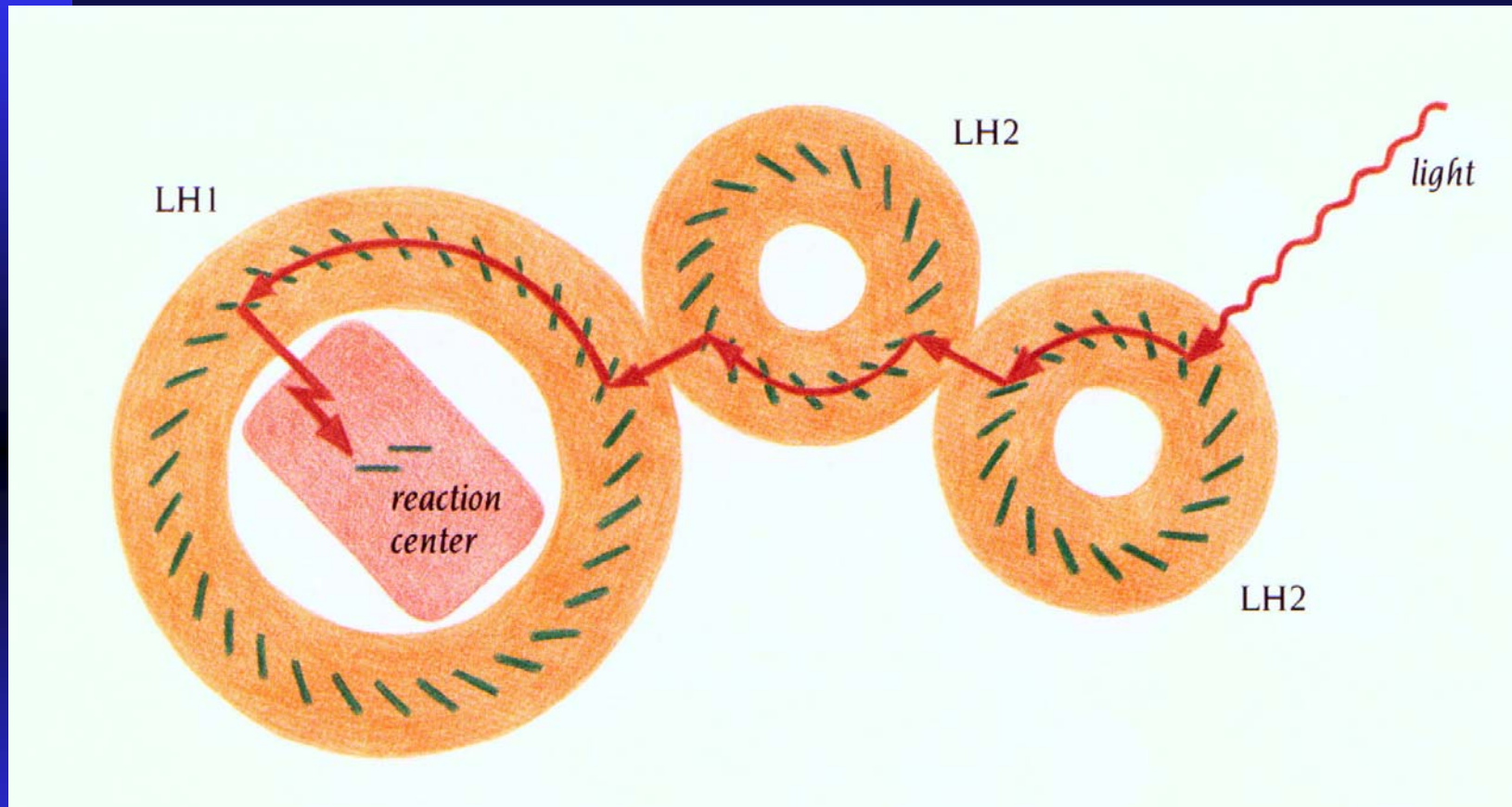
On the basis of comparison with the LH2 complex the red regions can be interpreted as corresponding to the α chains, the blue regions to the β chains and each green region to two bacteriochlorophyll molecules bound between the α and the β chains. The ring of 16 units has a hole in the middle that is large enough to accommodate a complete reaction center molecule.

The relative positions of bacteriochlorophylls in the photosynthetic membrane complexes LH1, LH2, and the reaction center



The special pair of bacteriochlorophyll molecules in the reaction center is located at the same level within the membrane as the periplasmic bacteriochlorophyll molecules Chl 875 in LH1 and the Chl 850 in LH2.

The flow of excitation energy in the bacterial photosynthetic apparatus



The energy of a photon absorbed by LH2 spreads rapidly through the periplasmic ring of **bacteriophyll molecules**. Where two complexes touch in the membrane, the energy can be transmitted to an adjacent LH2 ring. From there it passes by the same mechanism to LH1 and is finally transmitted to the special chlorophyll pair in the reaction center.

Membrane Proteins

What about the general principles for the construction of membrane-bound proteins?

Transmembrane α helices can be predicted from amino acid sequences

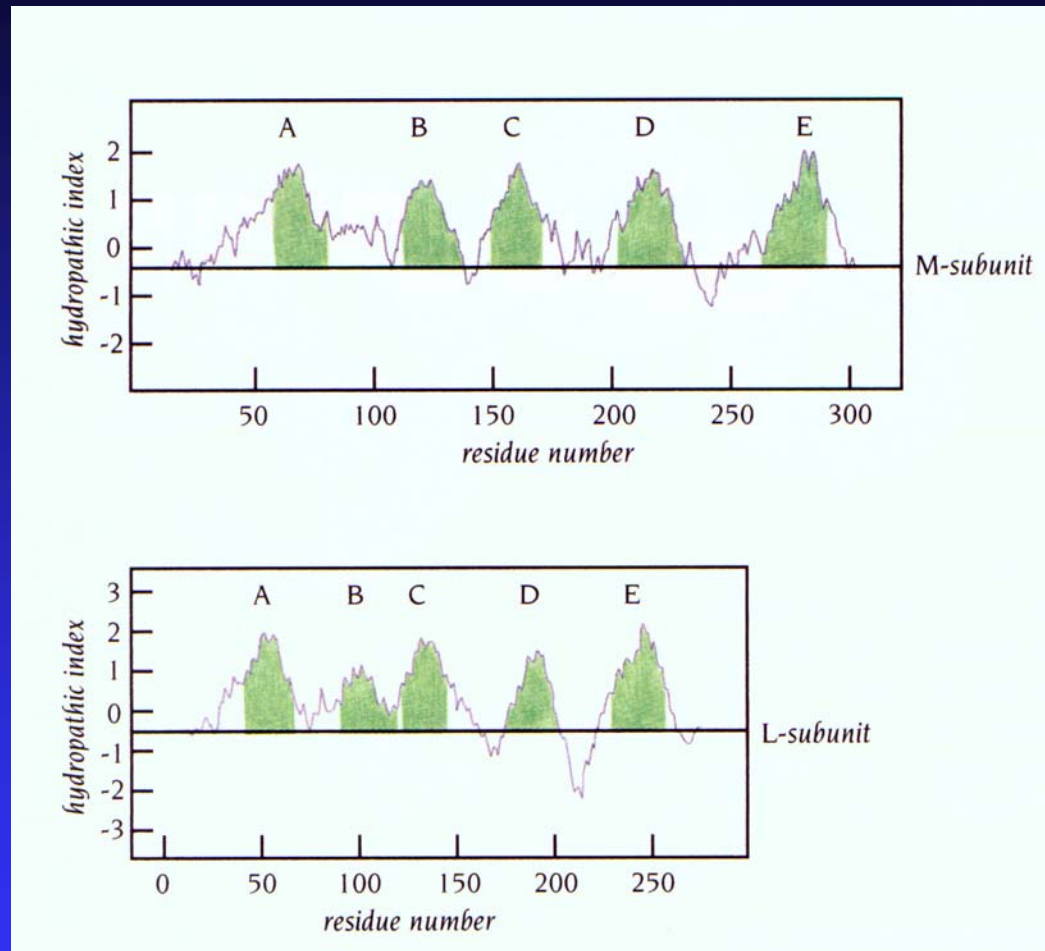
Hydrophobicity scales measure the degree of hydrophobicity of different amino acid side chains

Table 12.1 Hydrophobicity scales

Amino acid	Phe	Met	Ile	Leu	Val	Cys	Trp	Ala	Thr	Gly	Ser	Pro	Tyr	His	Gln	Asn	Glu	Lys	Asp	Arg
A	2.8	1.9	4.5	3.8	4.2	2.5	-0.9	1.8	-0.7	-0.4	-0.8	-1.6	-1.3	-3.2	-3.5	-3.5	-3.5	-3.9	-3.5	-4.5
B	3.7	3.4	3.1	2.8	2.6	2.0	1.9	1.6	1.2	1.0	0.6	-0.2	-0.7	-3.0	-4.1	-4.8	-8.2	-8.8	-9.2	-12.3

Row A is from J. Kyte and R.F. Doolittle; row B, from D.A. Engelman, T.A. Steitz, and A. Goldman.

Hydropathy plots identify transmembrane helices



Hydropathy plots for the polypeptide chains L and M of the reaction center of *Rhodobacter sphaeroides*. A window of 19 amino acids was used with the hydrophobicity scales of Kyte and Doolittle. The hydropathy index is plotted against the tenth amino acid of the window. The positions of the transmembrane helices as found by subsequent x-ray analysis are indicated by the green regions.

Amino acid sequences of the transmembrane helices of the photosynthetic reaction center of *Rhodobacter sphaeroides*

Table 12.2 Amino acid sequences of the transmembrane helices of the photosynthetic reaction center in *Rhodobacter sphaeroides*

LA G F F G V A T F F F A A L G I I L I A W S A V L

LB L K R C I E V E R L A W S V F A G T A C I T I I Q W L G G

LC H I P F A F A F A I L A Y L T L V L F R P V M

LD A A S L V L A G H L A L A L A N T F F F S I A I M H A P

LE G T L G I H R L G L L L S L S A V F F S A L C M I I

MA S L G V L S L F S G L M W F F T I G I W F W Y Q A

MB A Q A R L Y T R G W W S W V A V F M F F S A I L W L G G E K L

MC A W A F L S A I W L W M V L G F I R P I L M

MD V A L I T A G H M A F L L A S G Y L F A I S L G H F P

ME M E G I H R W A I W M A V L V T L T G G I G I L L

HA M N E T Q L Y Y I L G A L F I W F S Y I A L S A L

The helices are aligned according to approximate positions within the membrane and with respect to the photosynthetic pigments. LA is the first helix of subunit L, ME is the last helix of subunit M, HA is the only transmembrane helix of subunit H. Charged residues are colored red, polar residues are blue, hydrophobic residues are green, and glycine is yellow. (From T.O. Yeates et al., *Proc. Natl. Acad. Sci. USA* 84: 6438-6442, 1987.)

Signal Transduction

Signal Transduction

- Many signal-transducing receptors are plasma membrane proteins that bind specific extracellular molecules, such as growth factors, hormones, or neurotransmitters, and then transmit to the cell's interior a signal that elicits a specific response.
- These responses are usually cascades of enzymatic reactions giving rise to many different effects within the cell, including changes in gene expression.
- Interference with receptor-signaling systems can therefore have drastic consequences.
- For example, oncogene products that stimulate the function of receptors or their associated signal transmitters cause the loss of cellular growth control.

Signal Transduction

-The DNA sequences of membrane-bound signal-transducing receptor molecules have shown that many are structurally and evolutionary related, and they can be grouped into a few families making up three major classes:

- ion channel linked receptors (homologous to the K⁺ channel molecule)
- G protein-linked receptors
- enzyme-linked receptors

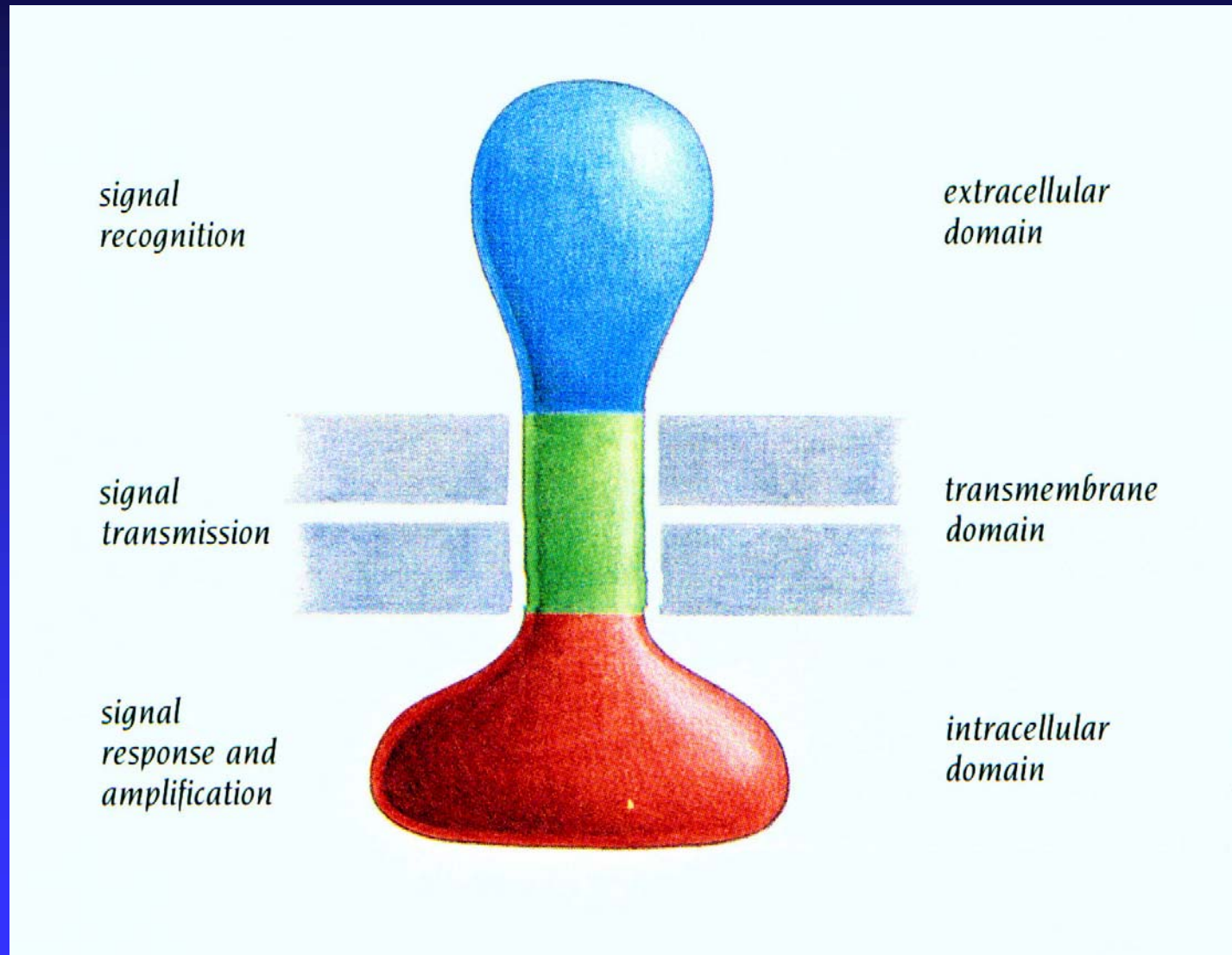
-These last two classes of receptor molecules have

- an extracellular domain that recognizes a specific molecular signal,
- a transmembrane region through which the signal is transmitted,
- and an intracellular domain that produces a response.

Signal Transduction

-These proteins provide yet another example of the now familiar story that there is only a limited number of different types of domains and that protein molecules with different functions have evolved either by accumulation of point mutations or by combining domains in different ways, by gene shuffling.

The basic organization of a membrane receptor molecule



G proteins and G protein-linked receptors

G proteins and G protein-linked receptors

- Several important physiological processes, including **vision, smell, and stress response**, involve large metabolic effects produced from a small number of input signals.
- The receptors that trigger these responses have two things in common:
 - **seven transmembrane helices**
 - the signals transmitted to the intracellular domain of these receptors are amplified by **G proteins**.
- G proteins bind guanine nucleotides and act as molecular switches that are activated by binding GTP and are inactivated when the GTP is hydrolyzed to GDP.
- The hydrolysis of GTP is catalyzed by the G protein itself, but G proteins on their own are very slow GTPases, and switching off the G protein is normally accelerated by regulatory molecules, known as RGS (regulators of GTP hydrolysis).

G proteins and G protein-linked receptors

- RGS bind to the active G protein and increase the rate of GTP hydrolysis.
- When in the active GTP-bound state, the G protein can activate many downstream effectors, greatly amplifying the signal, before RGS binds and the signal is switched off.

G proteins and G protein-linked receptors

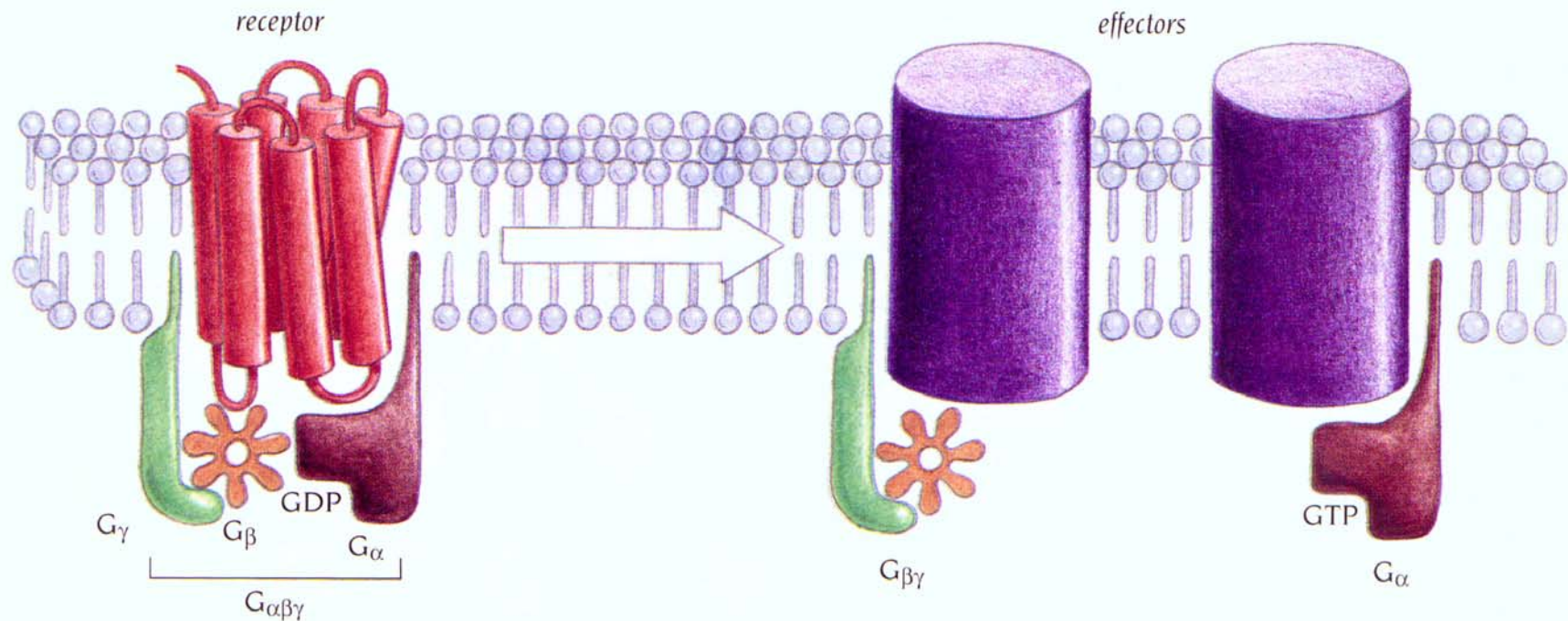
- Most G proteins are heterotrimers consisting of one copy each of
 - α subunit (45 kDa)
 - β subunit (35 kDa)
 - γ subunit (8 kDa).
- The α subunit contains the GTPase activity.
- When G protein is activated by binding GTP, the heterotrimer dissociates into the α subunit and $\beta\gamma$ heterodimer. Both the α subunit and $\beta\gamma$ heterodimer can independently transmit the signal received from the receptor to different effector proteins.
- The human genome is estimated to contain 1000 different genes for these receptors with seven transmembrane helices and, in addition, enough genes for different α , β and γ subunits to allow the formation of hundreds of different G proteins.
- Consequently, there is a very large number of possible combinations of these receptors and G proteins, which provide the potential for a cell to respond to a wide variety of external signals.

Physiological processes mediated by G proteins

Table 13.1 Examples of physiological processes mediated by G proteins

<i>Stimulus</i>	<i>Receptor</i>	<i>Effector</i>	<i>Physiological response</i>
Epinephrine	β -adrenergic receptor	adenylate cyclase	glycogen breakdown
Light	rhodopsin	c-GMP phosphodiesterase	visual excitation
IgE-antigen complexes	mast cell IgE receptor	phospholipase C	histamine secretion in all allergic reactions
Acetylcholine	muscarinic receptor	potassium channel	slowing of pacemaker activity that controls the rate of the heartbeat

G proteins are molecular amplifiers



Activated G protein receptors catalyze the exchange of GTP for GDP on the $G_{\alpha\beta\gamma}$ trimer. The then separated G_α -GTP and $G_{\beta\gamma}$ molecules activate various effector molecules. The receptor is embedded in the membrane, and G_α , $G_{\beta\gamma}$ and $G_{\alpha\beta\gamma}$ are attached to the membrane by lipid anchors, and they all therefore move in two dimensions.

The diagram illustrates the G-protein coupled receptor (GPCR) signaling pathway. A hormone (yellow hexagon) binds to a receptor (blue) on the cell membrane. The receptor is composed of α , β , and γ subunits. The α subunit is bound to GDP. Upon activation, the α subunit releases GDP and binds GTP, becoming an activated G_{α} -GTP. This activated G_{α} -GTP then activates adenylate cyclase (green), which converts ATP to cAMP, the second messenger.

G-protein-mediated activation of adenylate cyclase by hormone binding. Hormone binding on the extracellular side of a receptor such as the β adrenergic receptor activates a G protein on the cytoplasmic side. The activated form of the G protein dissociates from β and γ subunits and activates adenylate cyclase to produce cyclic AMP, which is a second messenger that affects a diverse range of cellular processes.

Growth hormone receptors

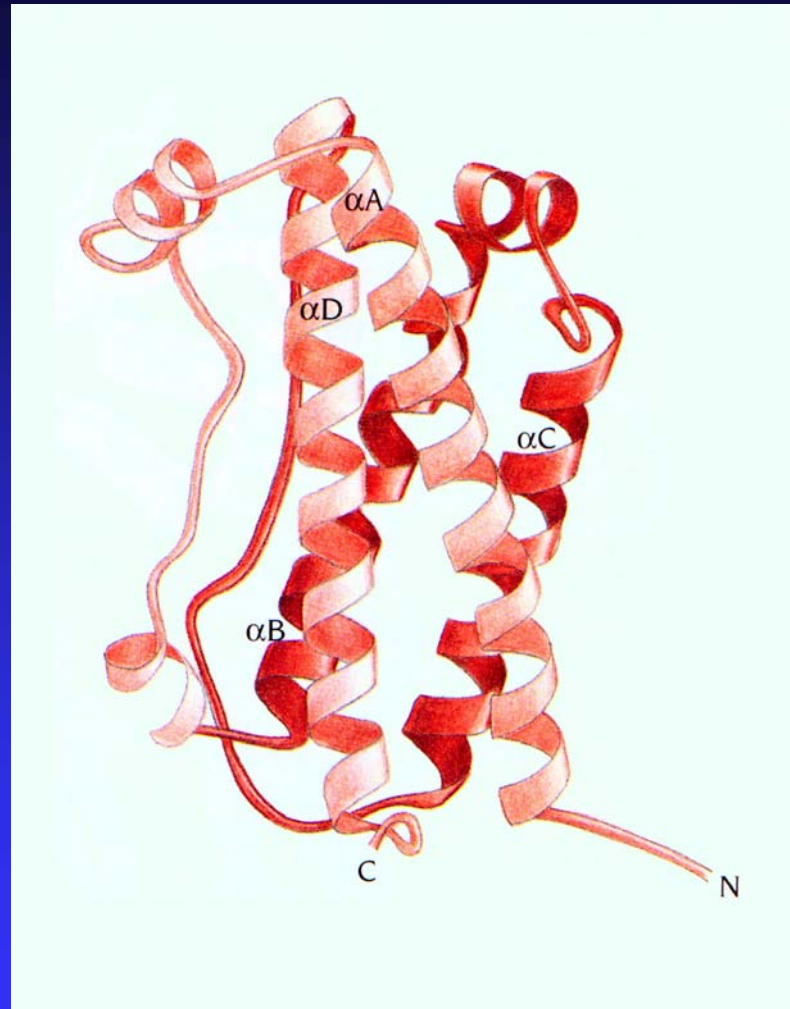
Growth hormones and growth hormone receptors

- Peptide hormone receptors are sensory machines that direct cells to proliferate, differentiate, or even die.
- The growth hormone receptor belongs to one class of the cytokine superfamily of hormone receptors that mediate signals from more than 20 known cytokines such as interleukin, erythropoietin and prolactin, as well as growth factors.
- Members of this receptor superfamily have a three-domain organization comprising an extracellular ligand-binding domain, a single transmembrane segment, and an intracellular domain that is quite diverse in sequence among the family members.
- The intracellular domain is structurally not well characterized, but it is known to associate reversibly with different cytoplasmic tyrosine kinases, which transmit the hormone-induced signal to activate a specific family of transcription factors.

Growth hormones and growth hormone receptors

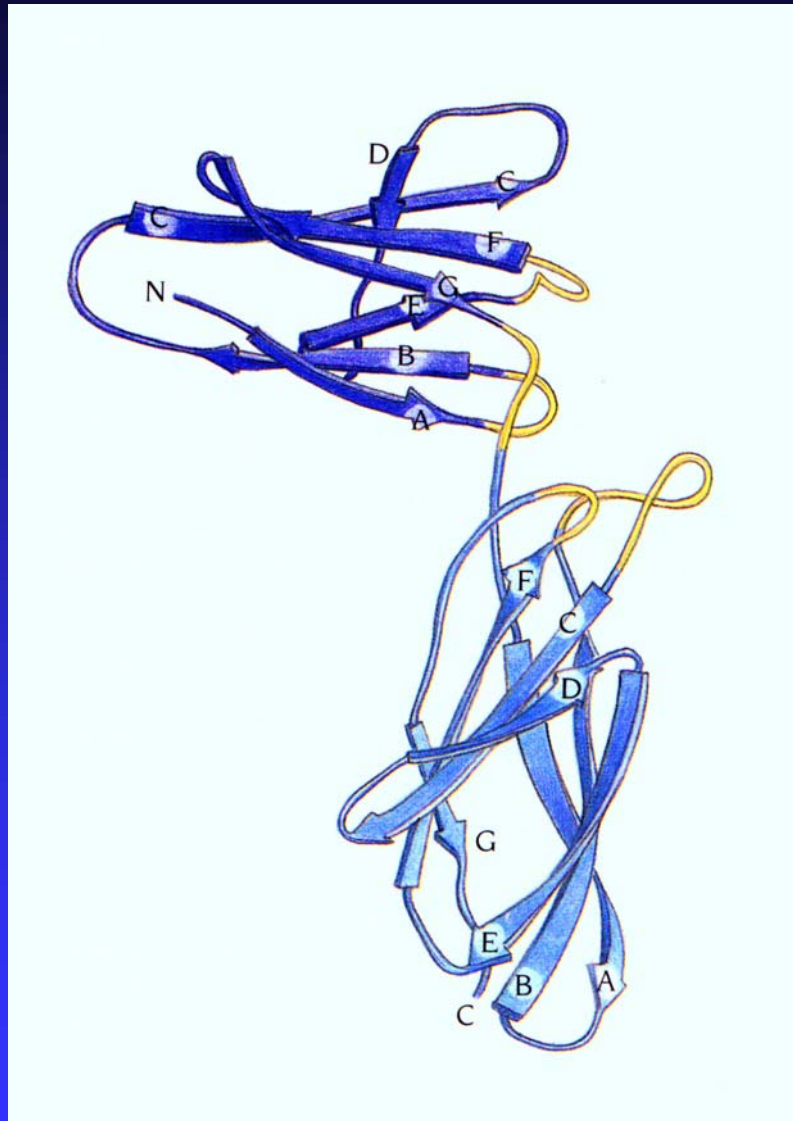
- Crystal structure of the complex between the human growth hormone, GH, and the extracellular domain of specific receptor, GHR, will be discussed
- This structure will be compared with that of growth hormone complexed with the prolactin receptor, PLR.

Human growth hormone



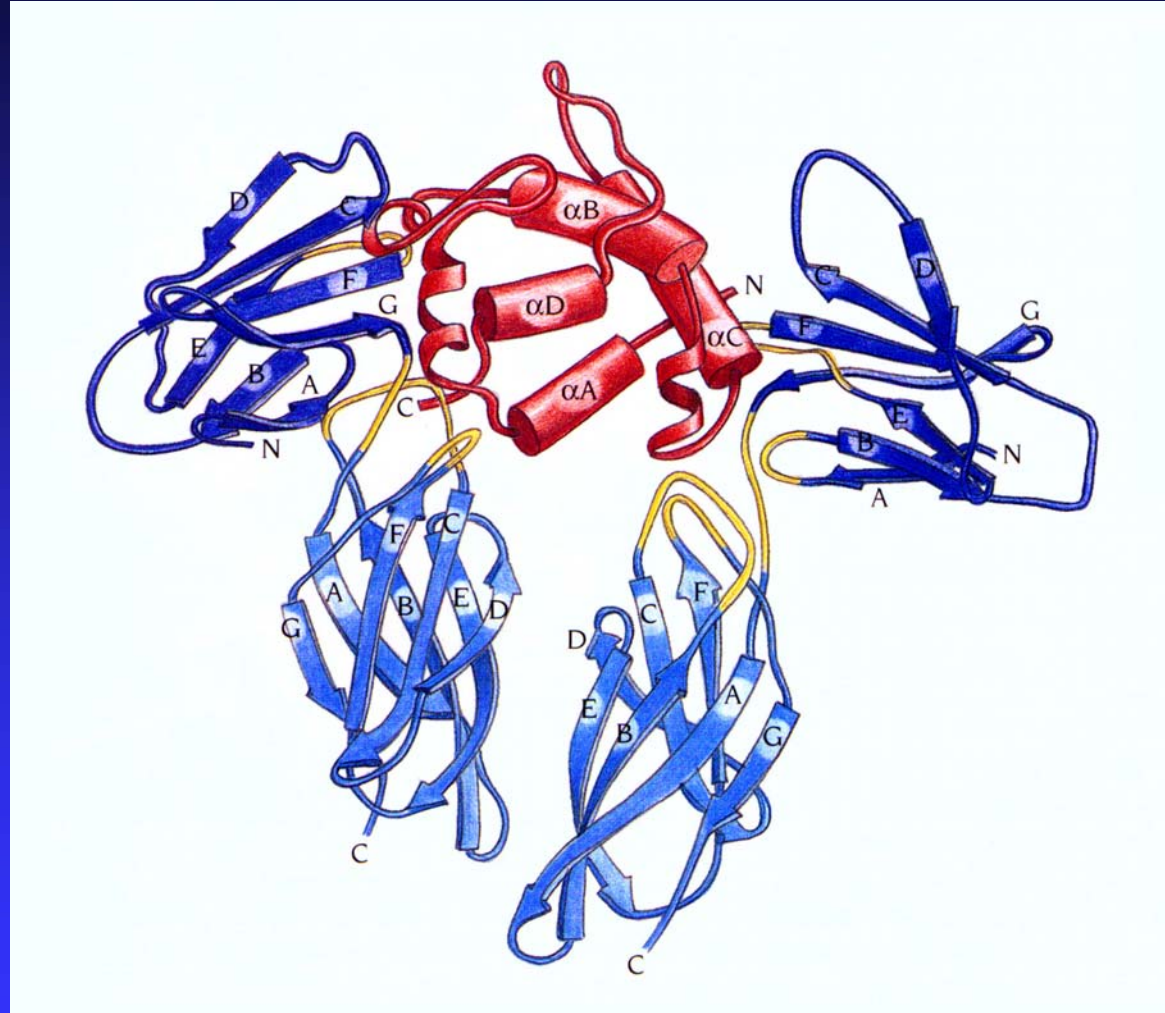
The fold is a four-helix bundle with up-up-down-down topology, and consequently there are two long cross-connections between helices A and B as well as between helices C and D.

Extracellular domain of the human growth hormone receptor



The hormone-binding region is formed by loops (yellow) at the hinge region between two fibronectin type III domains

Complex between the human growth hormone and the extracellular domains of two receptor molecules



The two receptor molecules (blue) bind the hormone (red) with essentially the same loop regions (yellow). In contrast, the hormone molecule uses totally different surface regions to bind the two receptor molecules.

Dimerization of the growth hormone receptor is a sequential process

- The receptor has a single ligand-binding site that can interact with either of two different sites on the hormone.
- Both receptor molecules in the complex use their A-B, C-D and E-F loops of the N-terminal domain as well as linker and loops B-C and F-G of the C-terminal domain to form the binding site. Not only are essentially the same amino acid residues on both receptors used for binding, but the three-dimensional structures of these loop regions are also very similar.
- In contrast, the two binding sites on the hormone are very different; one site comprises residues from a helices A and D, and the other site is formed by residues from helices A and C.

Dimerization of the growth hormone receptor is a sequential process

- There is no structural similarity to these two binding sites; the **A-C binding site is flat** whereas the **A-D site is concave**, neither is there any similarity in the nature of the amino acids that form these two different binding sites. **Nevertheless they bind to the same site on the receptor.**
- However, the **binding strengths of these two sites on the hormone are different.**
- At high concentrations of the hormone a 1:1 complex is formed with receptor instead of the normal 1:2 complex.**
- Experiments with mutants of the hormone show that **this complex is formed by interactions to the A-D site on the hormone.** Mutants in which binding to the A-C site is blocked still form the 1:1 complex whereas mutants that block binding to the A-D site do not form such complexes.

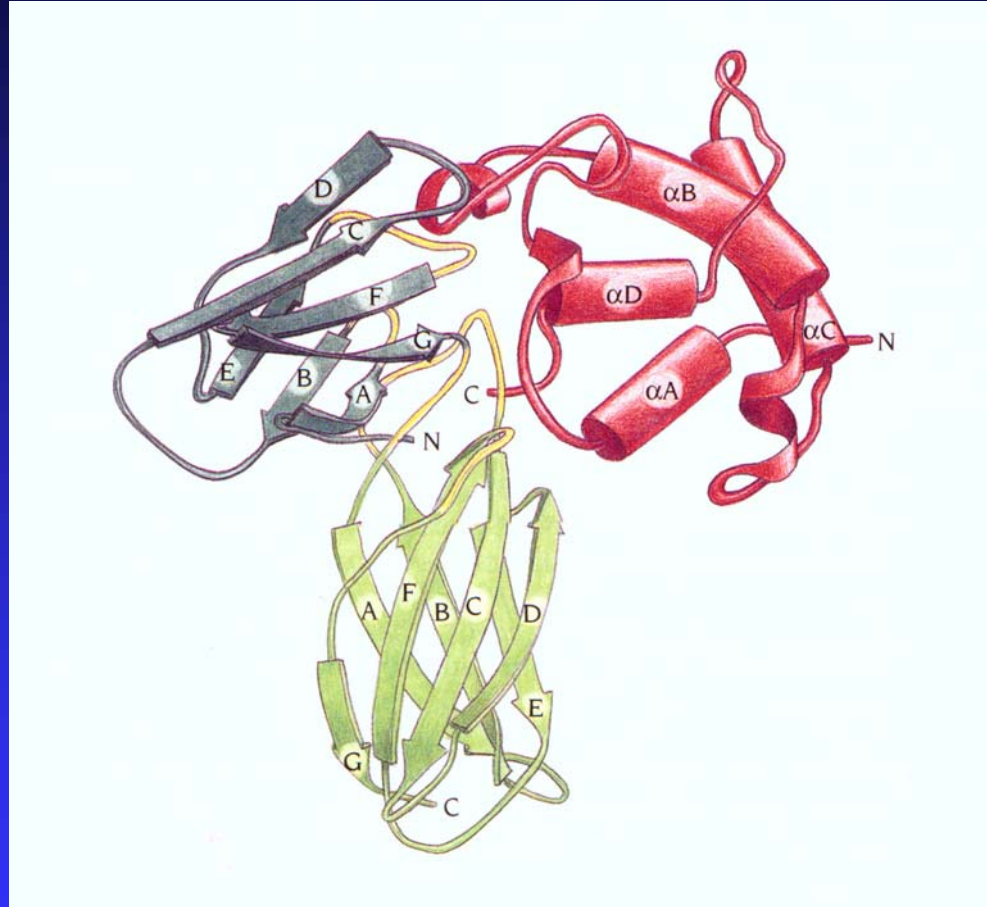
Dimerization of the growth hormone receptor is a sequential process

- These results nicely correlate to the structure of the complexes. The **A-D binding site** involves **31 residues** and the receptor covers an area of **1300 Å²** of the hormone surface. **The A-C site** on the other hand only involves **24 residues** and the binding area is **850 Å²**.
- Even though binding strength depends on the specific interactions involved there is in general direct correlation between the size of the binding area and the strength of binding when proteins interact.
- These results suggest that activation of the receptor by dimerization through binding the hormone is a sequential process.
- A **1:1 complex** is first formed by hormone binding firmly to one receptor molecule through its **A-D site**. Once this complex is formed the binding of a second receptor to the **A-C site** of the hormone is facilitated by the interactions between the C-terminal domains of the two receptor molecules.

The growth hormone also binds to the prolactin receptor

- The prolactin receptor, PLR, which regulates milk production in mammals, belongs to the same receptor class as the growth hormone receptor.
- PLR also binds and is activated by growth hormone.
- The extracellular domain of PLR forms a very stable 1:1 complex with growth hormone in solution: this complex has been crystallized and its structure determined.

Structure of a 1:1 complex between the human growth hormone and the extracellular domain of the prolactin receptor



The hormone binds to the prolactin receptor using the same binding area as in the strong binding site to the growth hormone receptor. The two domains of the prolactin receptor have similar structures to the corresponding domains of the growth hormone receptor, but the orientation between the domains is different, causing differences in the details of the binding area.

Receptor tyrosine kinases

