Lectures 11-12:
Fibrous & Membrane Proteins

Lecturer:

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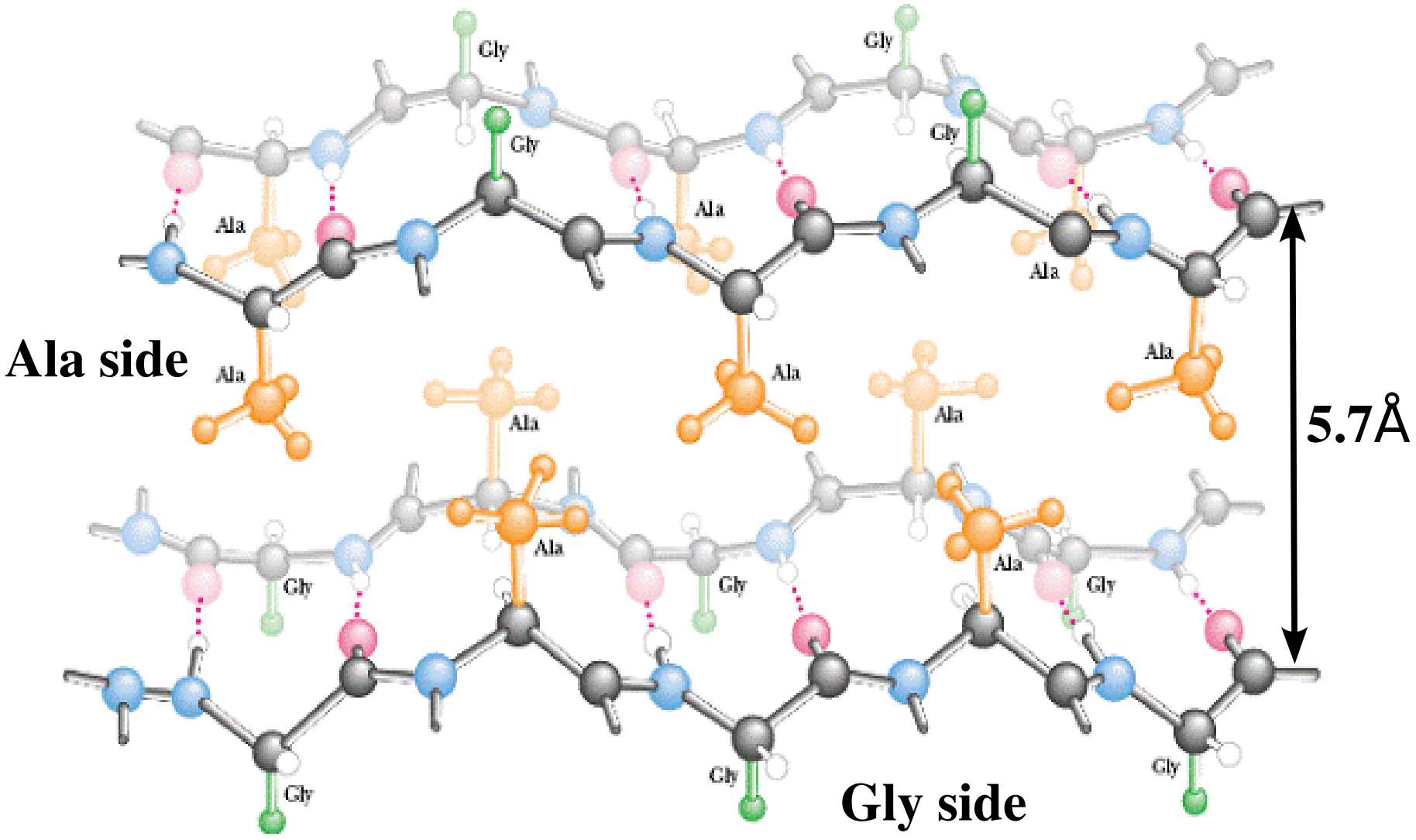
FIBROUS PROTEINS:

- **function: structural**
  - **microfilaments & microtubules**
  - **fibrils, hair, silk**
  - **reinforce membranes**
  - **maintain the structure of cells & tissues**

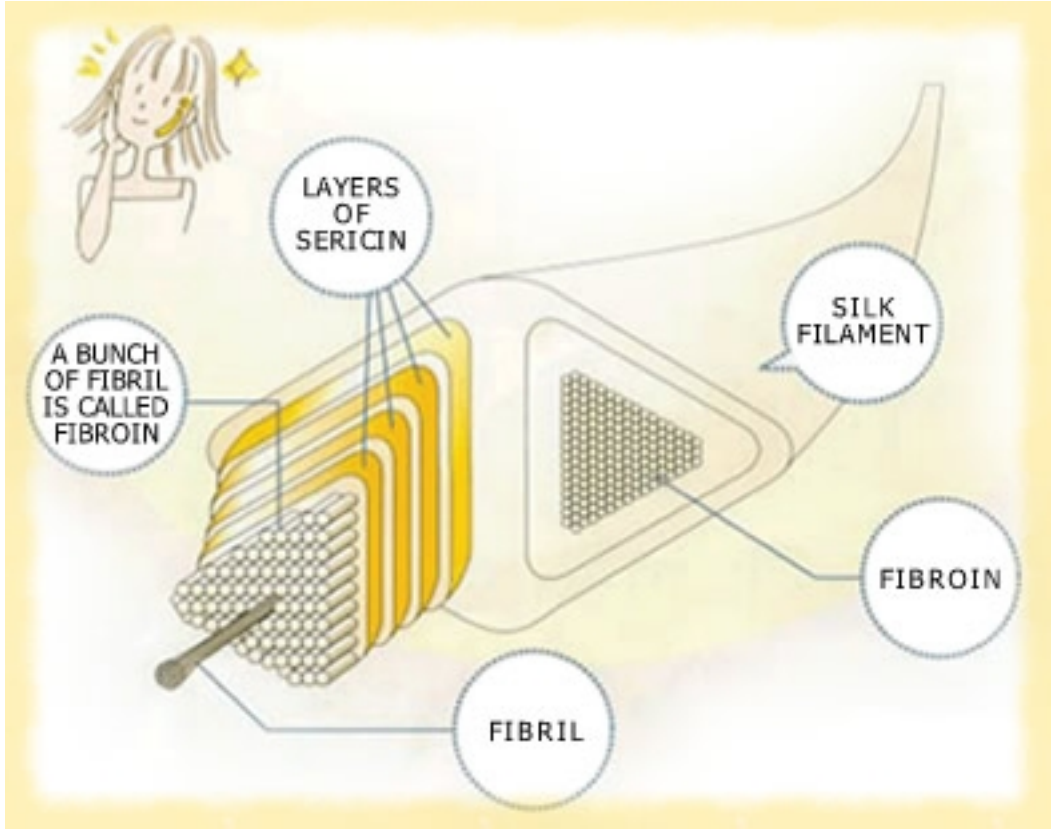
- **large proteins: e.g. titin with ~30,000 amino acids**

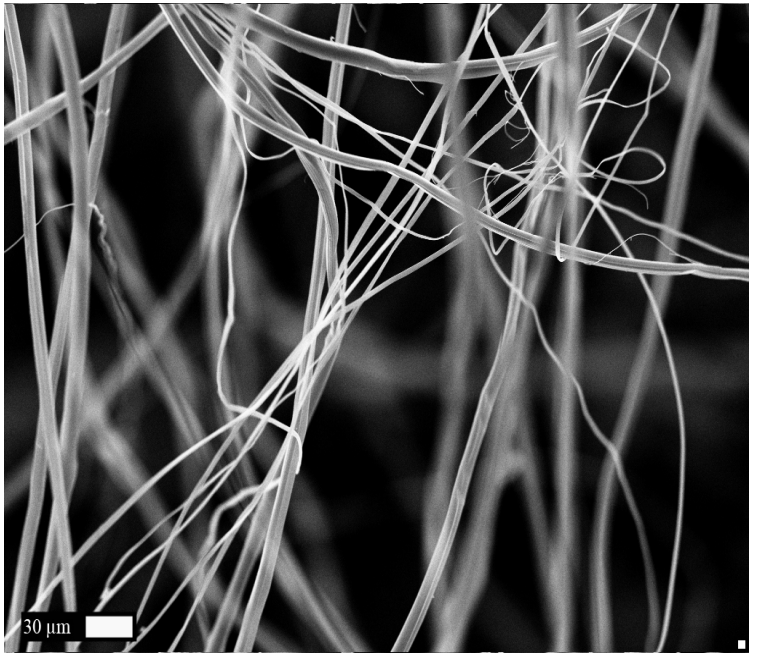
- **highly regular structure:**
  - **huge secondary structure block**
  - **interactions between adjacent chains**
  - **high sequence regularity & periodicity**

A. β-structural fibrous proteins: e.g. silk fibroin



- **the major motif is: … {-Ser-Gly-Ala-Gly-Ala-Gly-}$_8$ …**
- **antiparallel β-sheets: alternating**
  - **face-to-face (5.7Å)**
  - **back-to-back (3.5Å)**





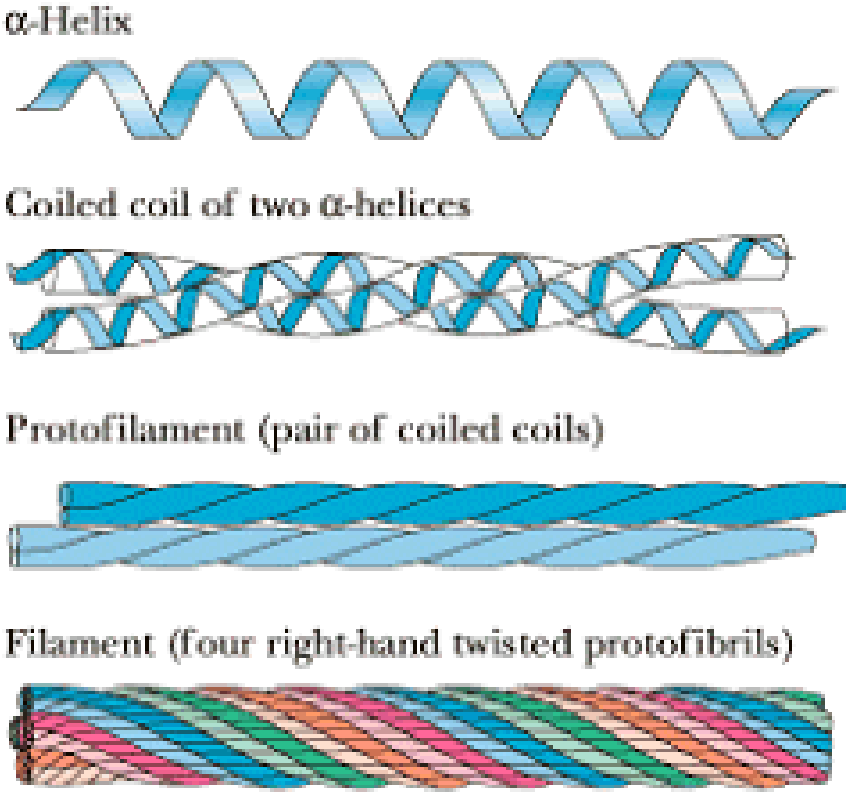
SEM image

B. α-structural fibrous proteins: e.g. α-keratin, tropomyosin

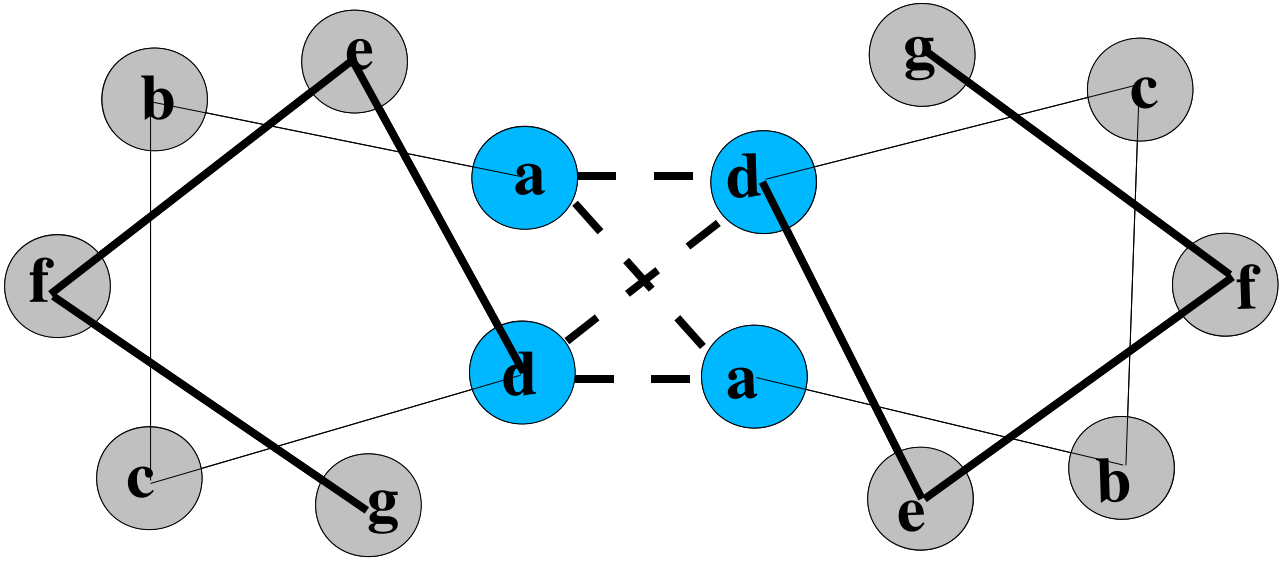
- α-helices → coiled coil helices → protofilament → filament

- 2-3 helices form a coiled coil (supercoil) with 140Å repeat

- 3.5 residues per turn (7 residues involved in one full repeat)



Interactions of two α-helices in a double superhelix



- residues a and d hydrophobic: contacts between helices
- the rest of residues (b, c, e, f, g: hydrophilic)
- increase in hydrophobicity in e & g: triple superhelix
- … -a-b-c-d-e-f-g-a-b-c-d-e-f-g-a-b-c-d-e-f-g- …

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21

α-helical ridges:

➔ **ridges made of side chains**

➔ **"i+4" ridge (left picture)**

contact zone forming parts

d_4-a_8(-e_{12})

(g_7)-d_{11}-a_{15}

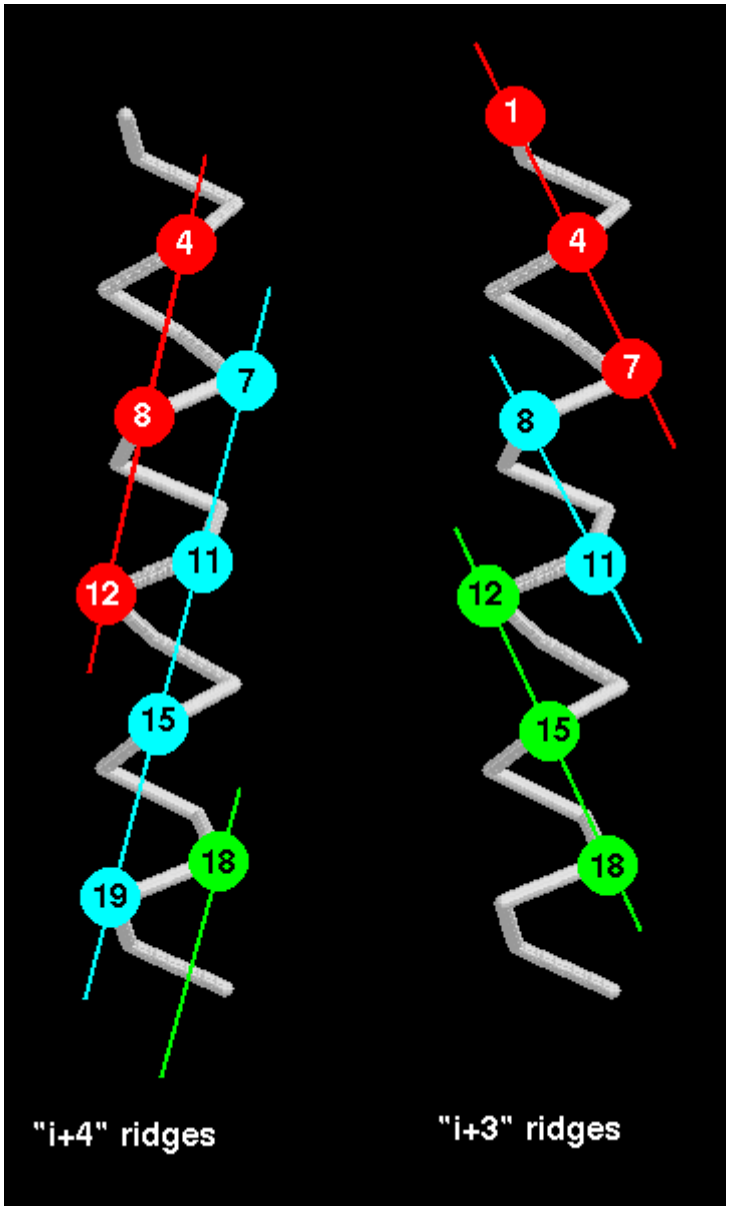
➔ **"i+3" ridge (right picture)**

contact zone forming parts

a_1-d_4(-g_7)

a_8-d_{11}

(e_{12})-a_{15}-d_{18}



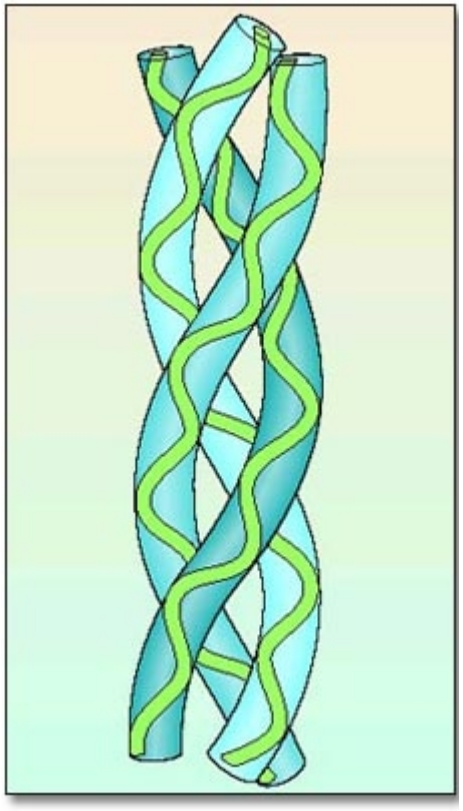
–25° **+45°**

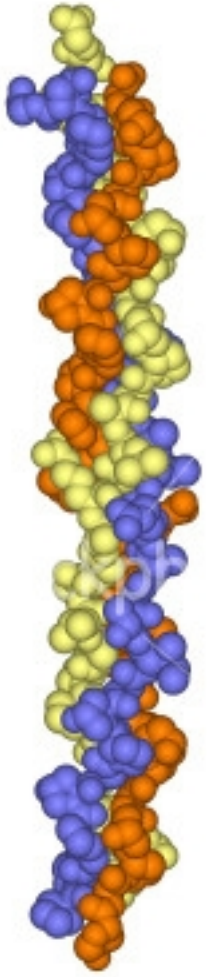
If one of the helices is turned about the vertical axis, superimposed on the other surface, turned about the vertical by +20°, then both types of ridges will be parallel (aligned), maximizing the contacts between the two helices: a groups fit between d groups.

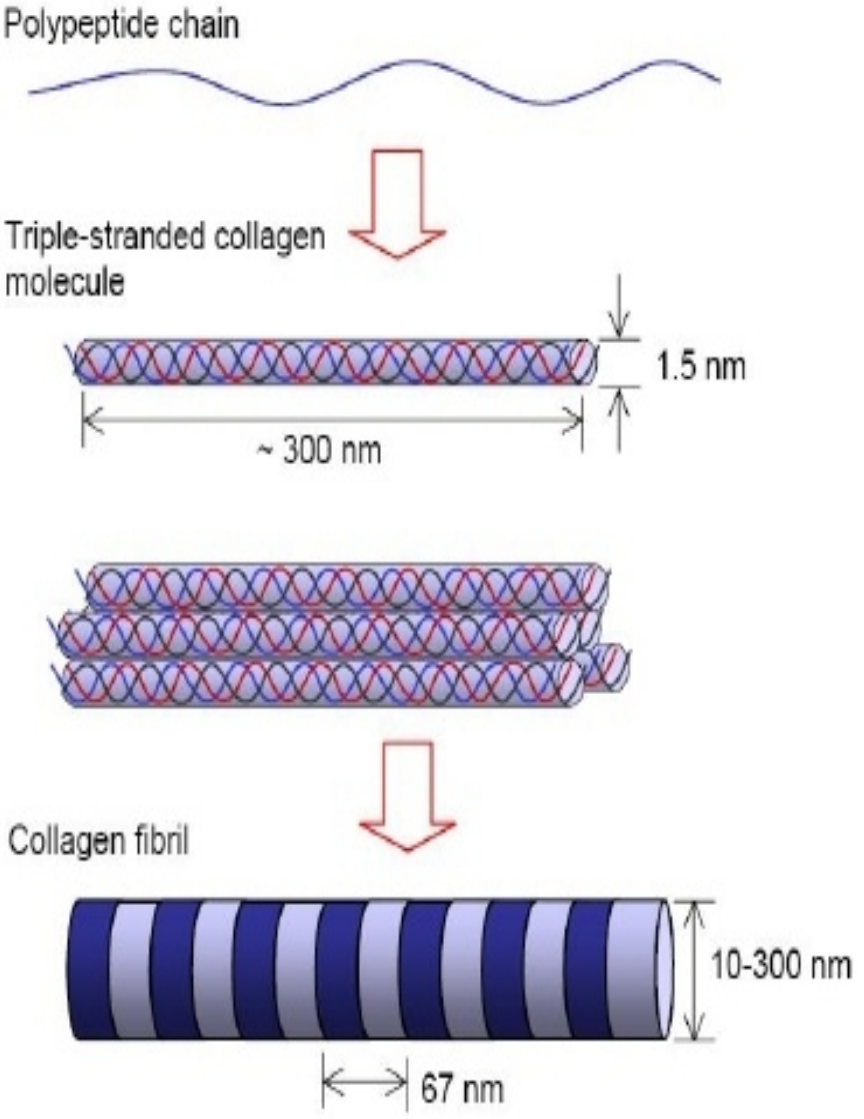
C. Collagen ("glue forming")

- **major structural protein (¼ of the total mass of proteins in vertebrates)**
- **forms strong insoluble fibrils**
- **collagen molecule: special superhelix of 3 polypeptides with only inter-chain hydrogen bonds**
- **each chain in a poly(Pro)II helix conformation**

Collagen Molecular Structure and Assembly





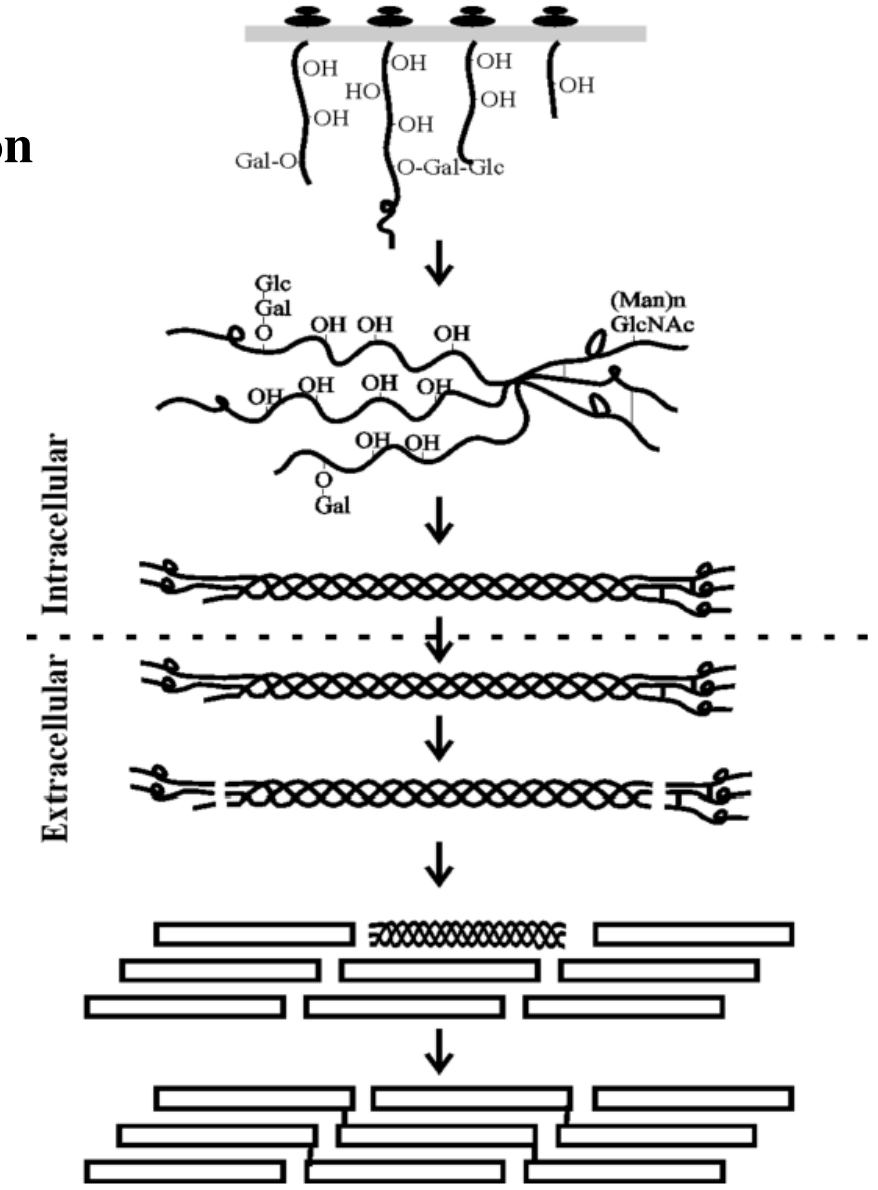


- **each chain is in a left-handed poly(Pro)II helix (3 residue repeat)**
- **sequence repeats: $(\text{-Gly-Pro-Pro-})_n$ or $(\text{-Gly-X-Pro-})_n$**
  - **Gly essential due to small side chain (H)**
  - **Pro (propensity for poly(Pro)II helix)**
- **collagen folding initiated by procollagen (collagen chains + globular heads & tails): heterogeneity & correct register of natural collagen**
- **collagen melts at a temperature only a few degrees higher than the body temperature**
- **mutations in the collagen sequence cause hereditary diseases ("brittle bone" disease):**

Link between protein misfolding and human disease!

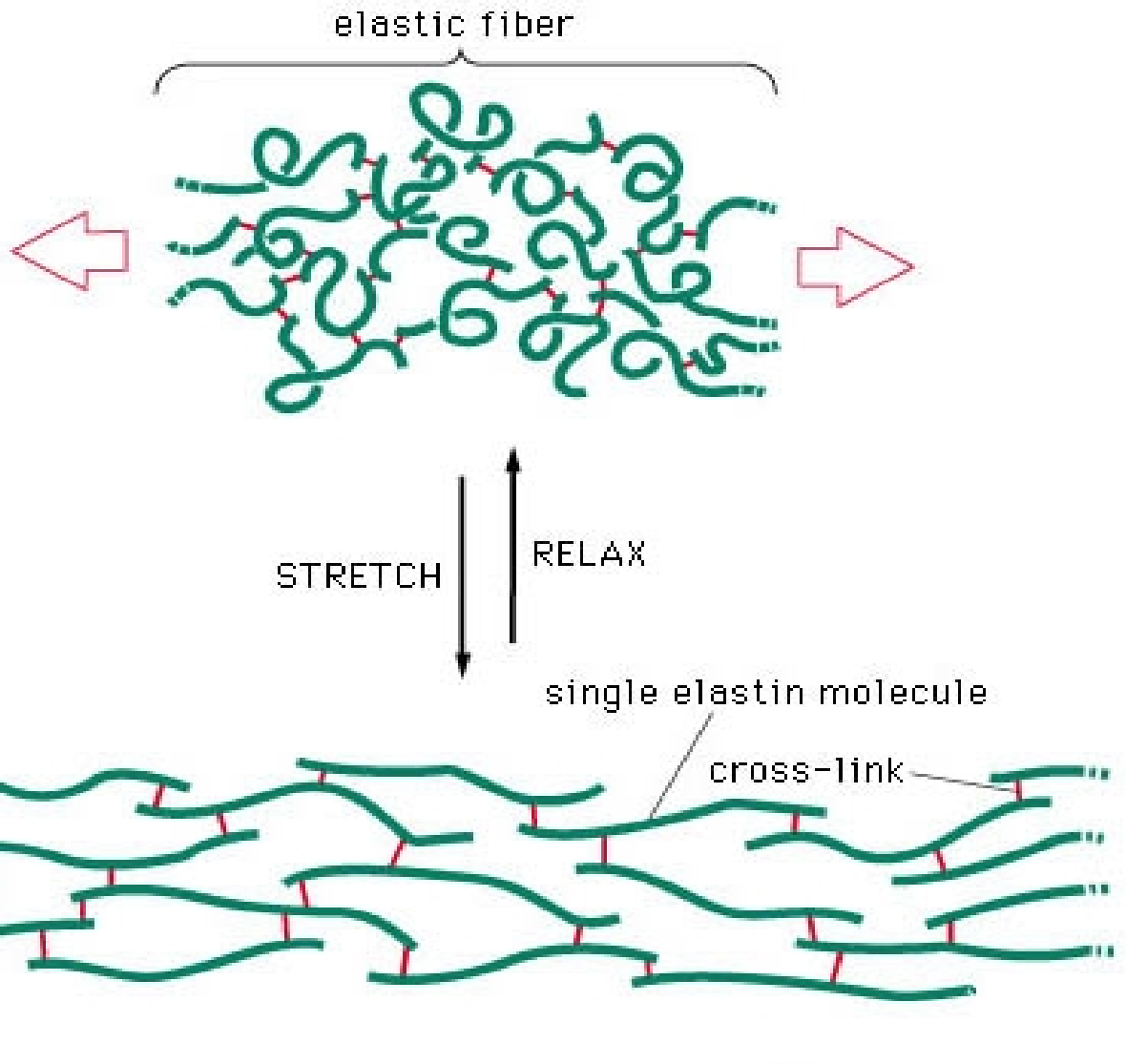
COLLAGEN BIOSYNTHESIS

- **procollagen polypeptide chains synthesized on the ribosomes inside a cell**
- **chain secreted into the lumen, modified by hydroxylation of certain prolines and lysines & glycosylation**
- **chains associate into a triple helix**
- **procollagen molecules are then secreted into the extracellular space**
- **N and C propeptides are cleaved by specific proteases**
- **collagen molecules then assemble into fibrils stabilized by the covalent crosslinks**

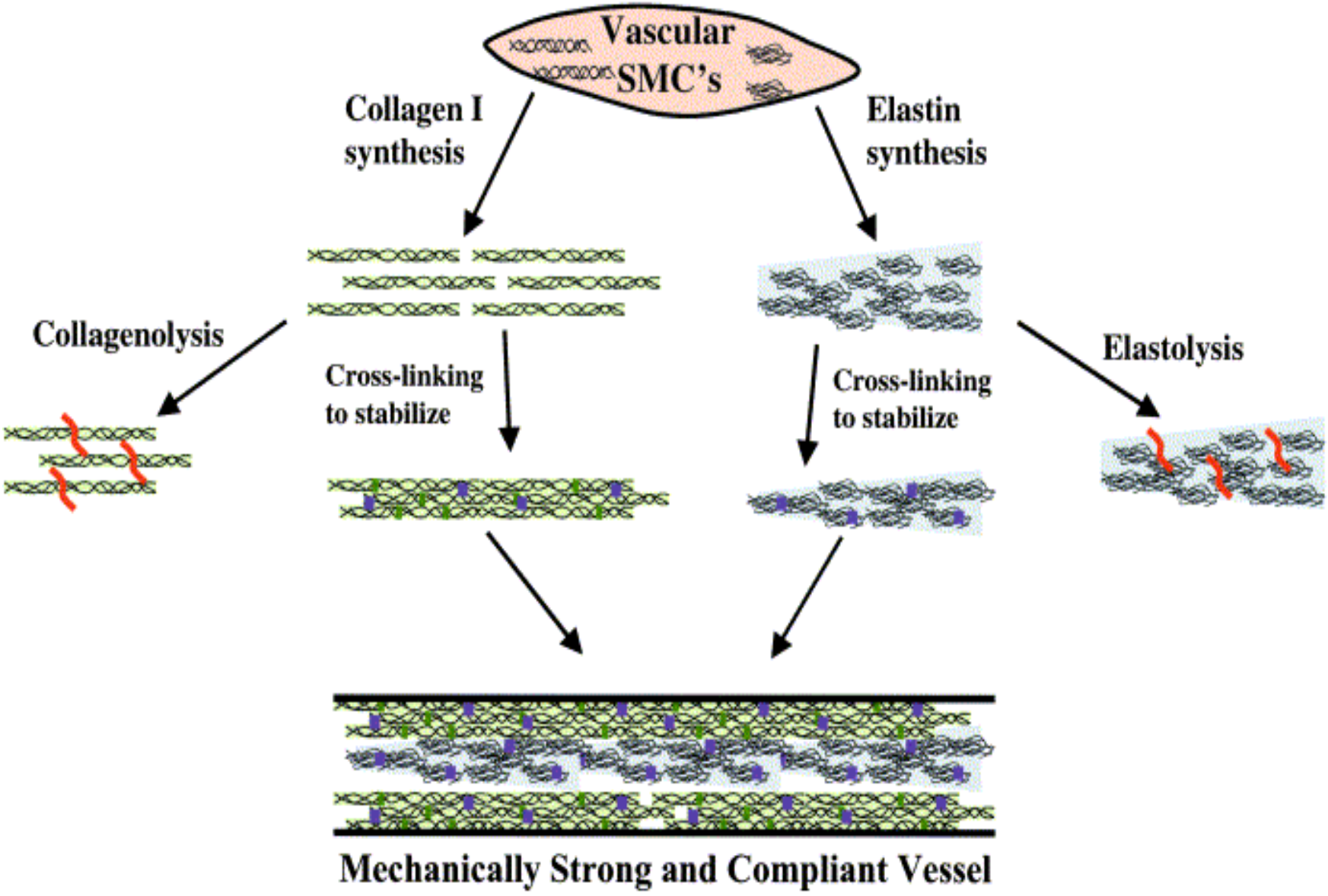


Elastin: a matrix protein (skin, hair, lungs, vessels)

- **links formed by enzyme-modified lysines (4 per knot)**
- **when enzymes malfunction: loss Of elasticity**

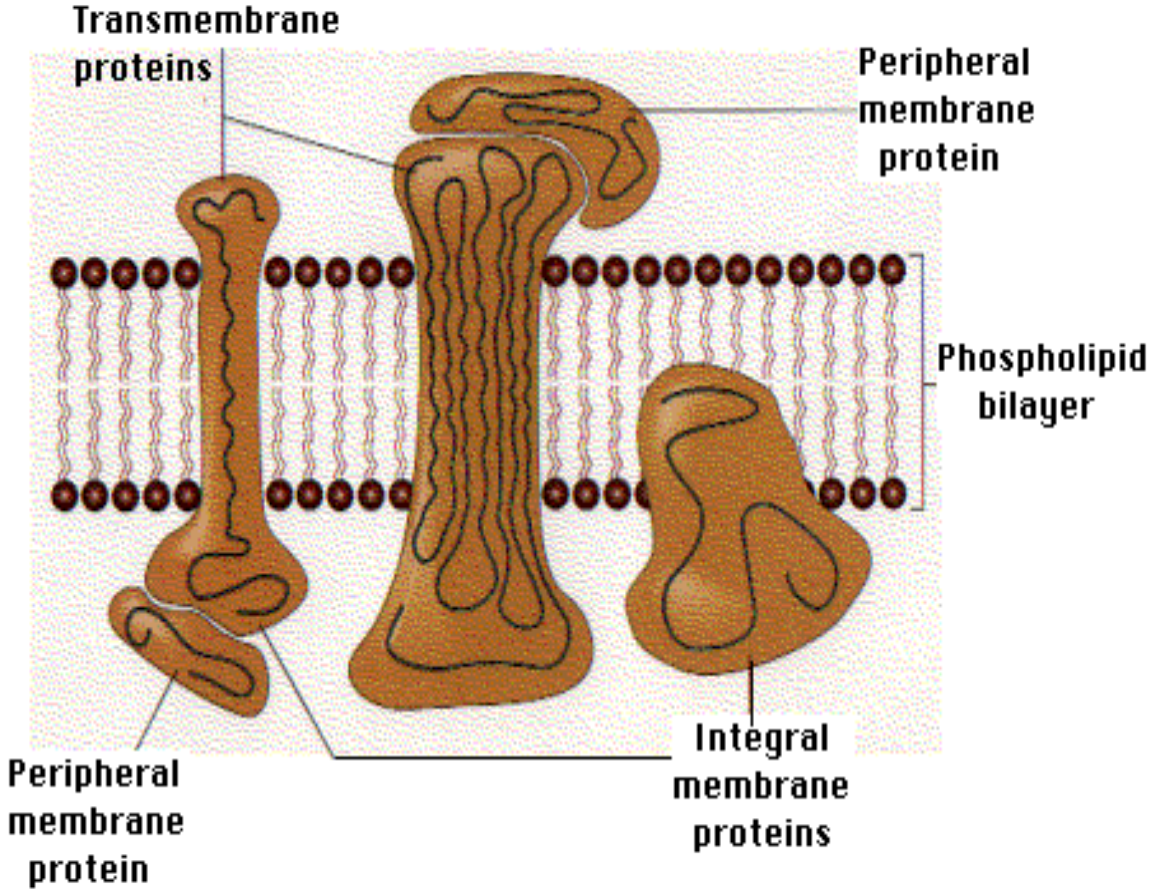


Elastin: Building block of artery & lung walls

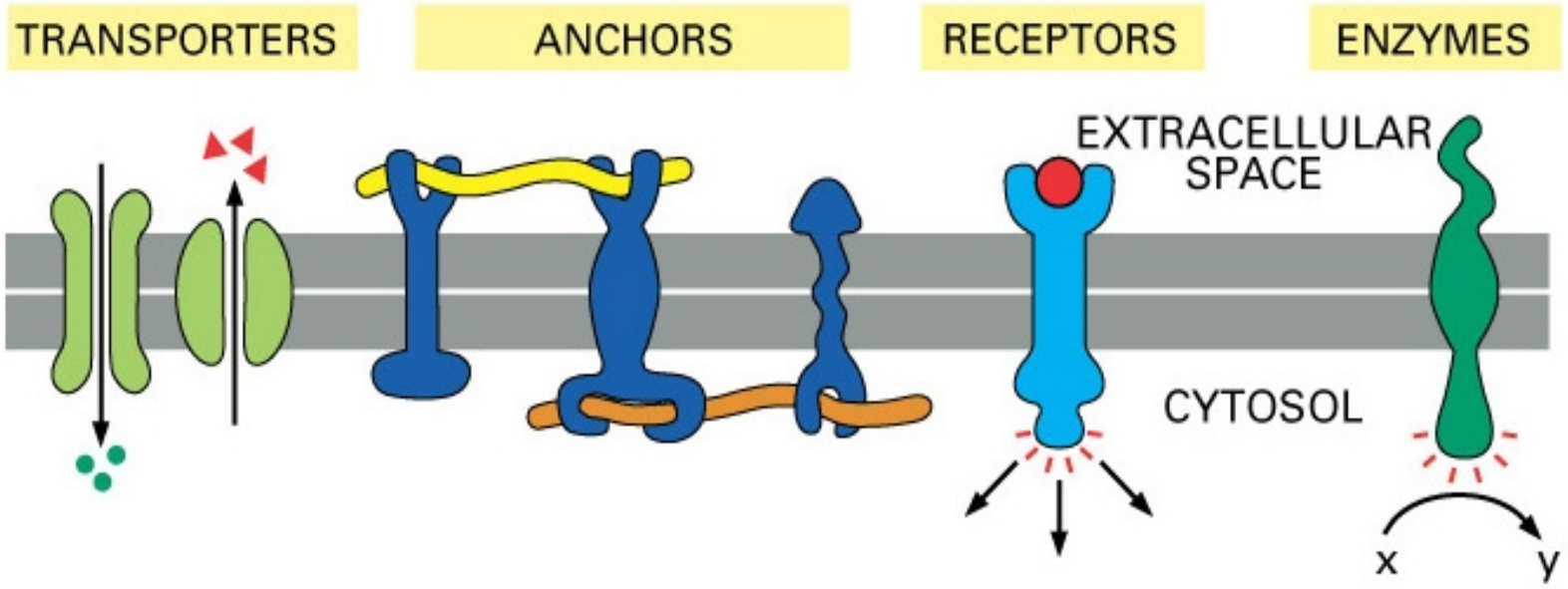


MEMBRANE PROTEINS:

- **membranes: films of lipids & proteins (~40Å thick)**
- **envelop cells and intracellular compartments**
- **membrane proteins contribute to ½ of the weight of the membrane**



What is the function of membrane proteins?

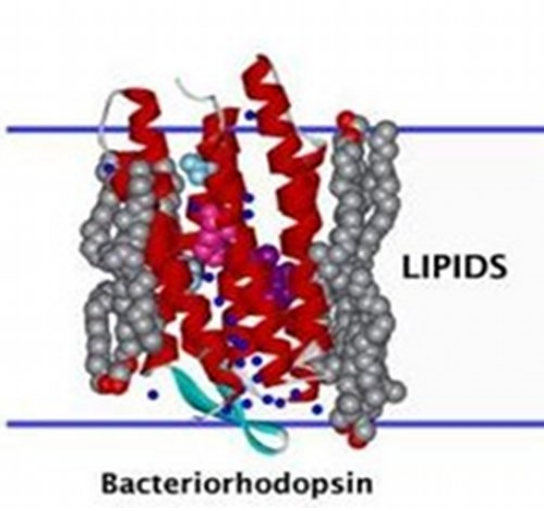


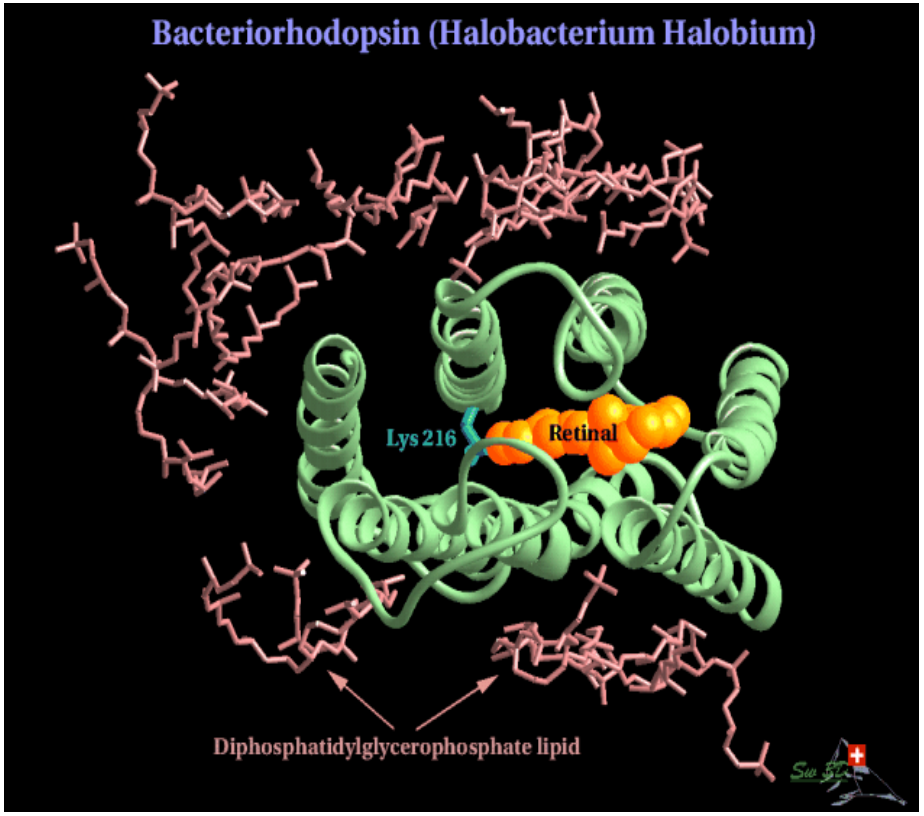
- membrane as an "insulator" & proteins as "conductors"

- membrane proteins: transmembrane transport of molecules & signals

Example 1: Bacteriorhodopsin

- pumps protons across the membrane
- structure determined by EM
- 7 regular α-helices, β-hairpin & irregular segments outside the membrane
- hydrophobic groups of α-helices turned toward the lipid molecules (no water → max #Hbs)
- cofactor retinal key to proton flow (photon induced)



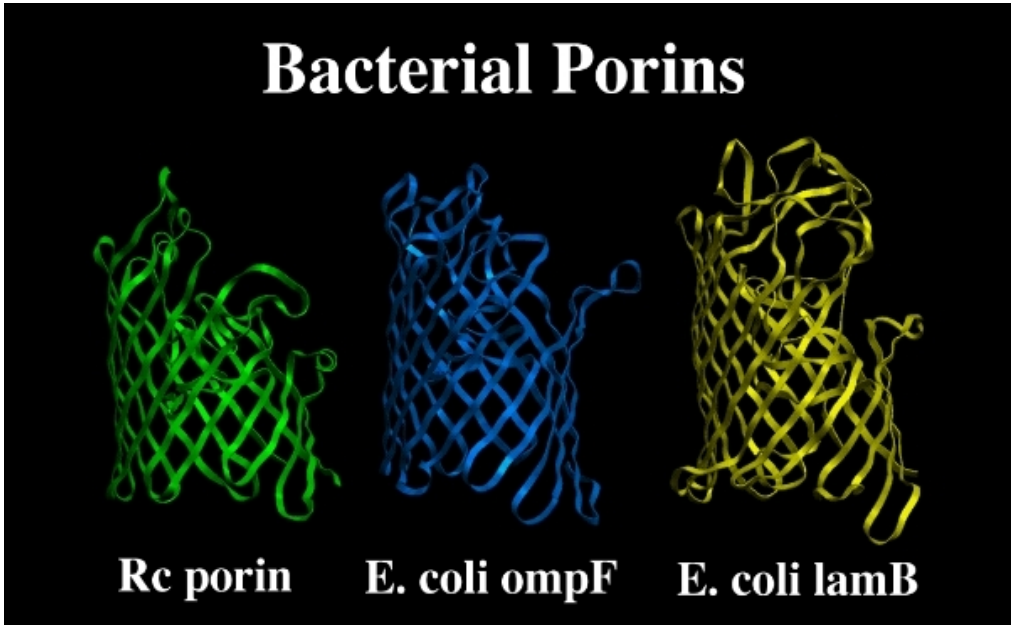


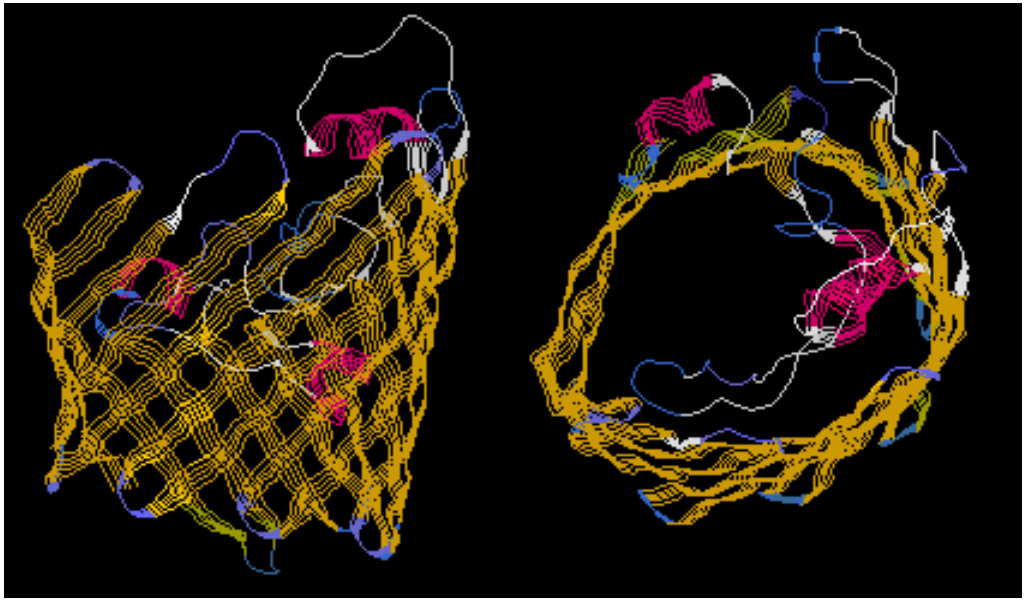
Example 2: Porin

- wide cylinder build up from 16 β-segments: closed β-cylinder (no edges with free HB donors/acceptors) - diameter 15Å

- non-selective transport of polar molecules

- ion transport?





- the free energy of a charge q in a medium with permittivity ε:

$$F = q^2/(8\pi\varepsilon_0\varepsilon r)$$

r – van der Waals radius

- free energy in water:

$F(\varepsilon_w = 80$, q – unit charge, r=1.5Å$) \approx$ 1.5 kcal/mol

- free energy in membrane:

$F(\varepsilon_m = 3$, q – unit charge, r=1.5Å$) \approx$ 37 kcal/mol

$\Rightarrow$

- free energy change upon q transfer (water → membrane)

$$\Delta F \approx 35 \text{ kcal/mol}$$

- probability: $\exp(-\Delta F/k_B T) \approx \exp(-35/0.6) \approx 10^{-25}$
- In only 1 of 10^{25} attacks on membrane, q can penetrate it
- If each attack takes 10^{-13} s, the total time needed: 10^{12} s

$\approx$ 10,000 years

- **transport of charges across the membrane is key to correct cell functions**
- **in a membrane protein with a broad water-filled channel:**

$$F = q^2/[4\pi\varepsilon_0(\varepsilon_w\varepsilon_m)^{1/2}R]$$

R – radius of the ion channel

$$(\varepsilon_w\varepsilon_m)^{1/2} = 15.5$$

- **for R=1.5Å → a fraction of 1 s**
- **for R=3Å → a fraction of 1 ms**
- **the size of the ion channel strongly affects the ion transport**
- **position of the charged amino acids at the entrance of the channel regulates ion transport**

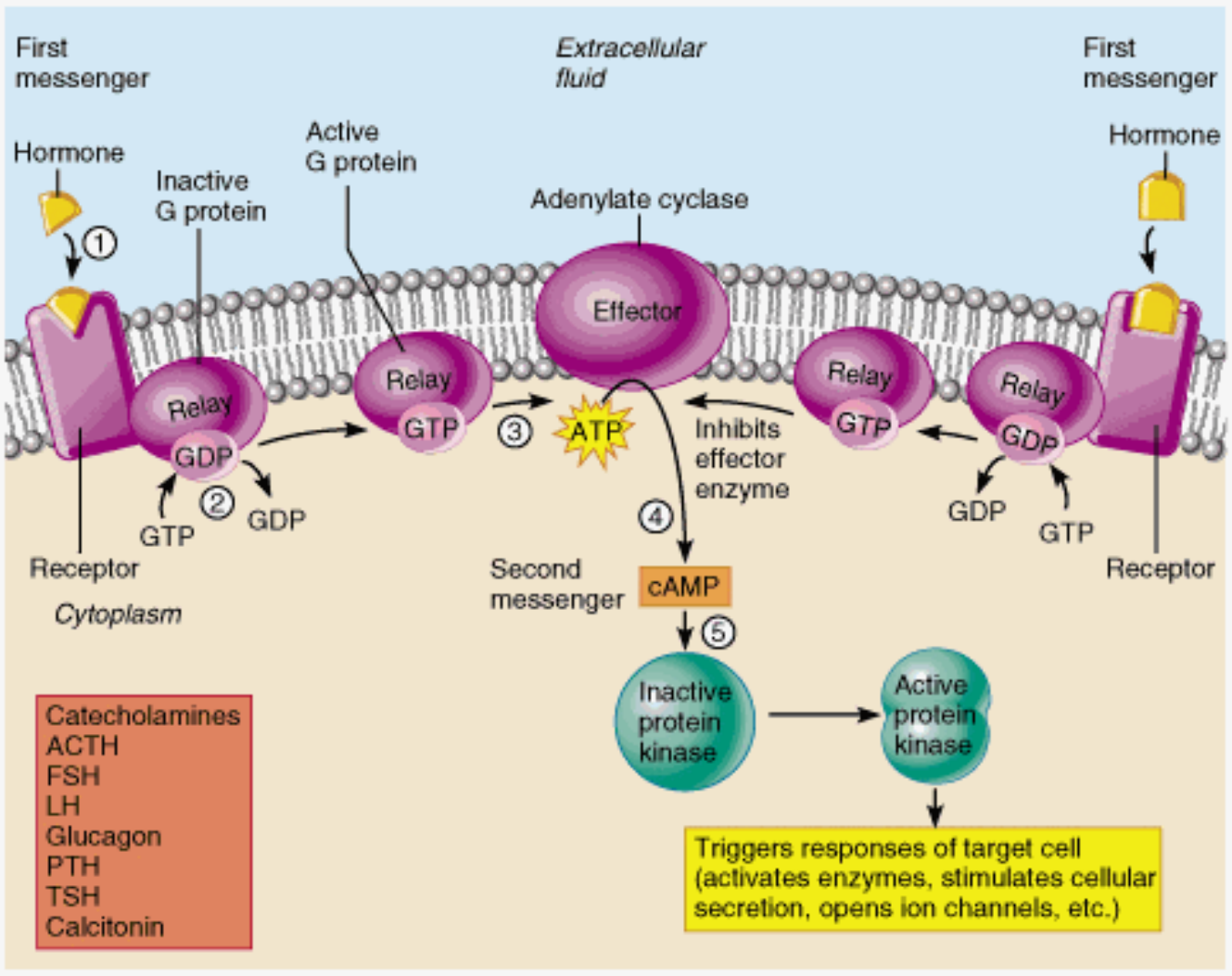
Example 3: Hormone Receptor

(1) hormone binds to the receptor → changes its Structure

(2) G-protein releases GDP and takes up GTP

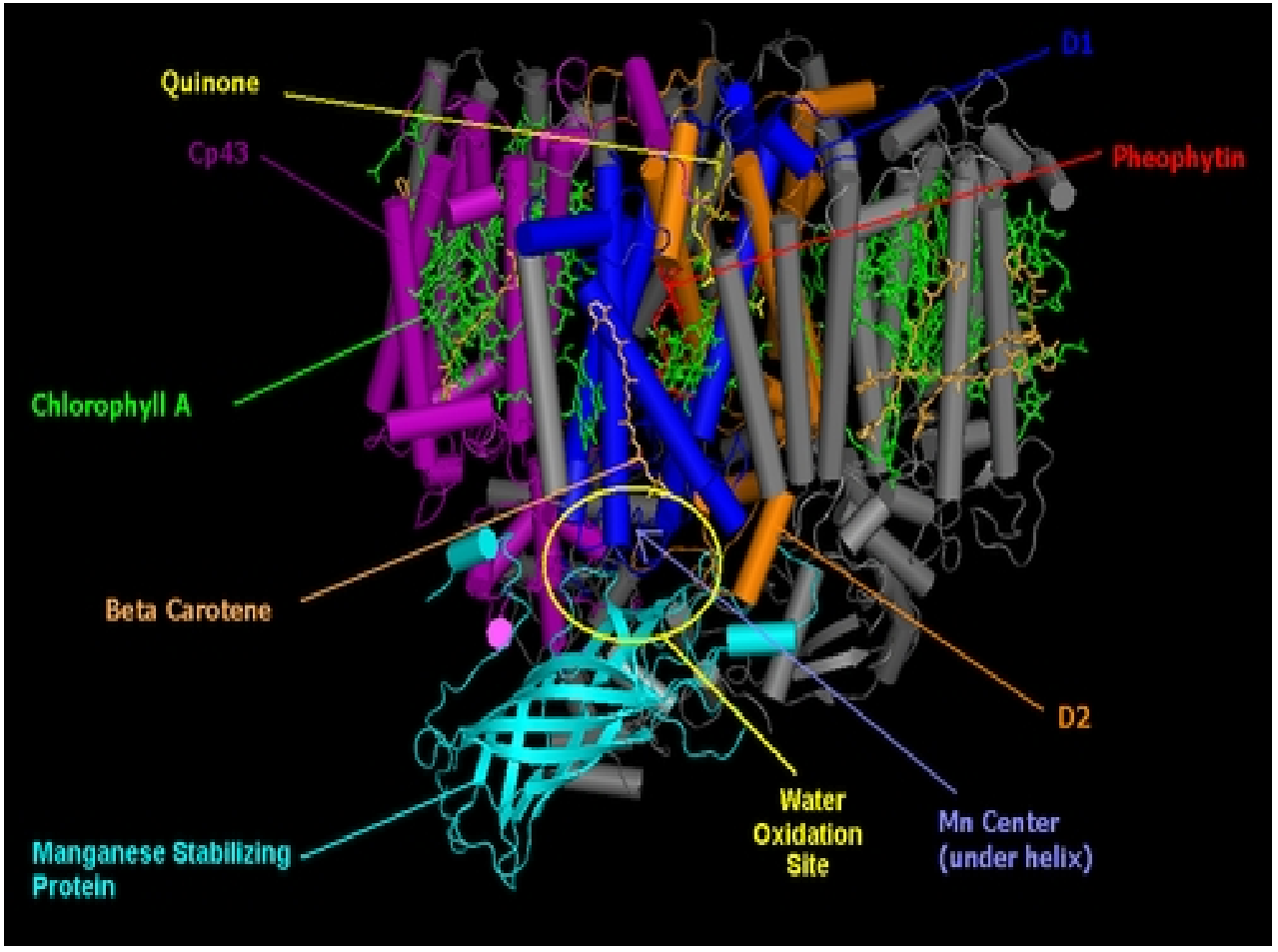
(3) G-protein with GTP leaves the receptor to bind adenylate cyclase

(4) physiological reaction (ATP→cAMP)



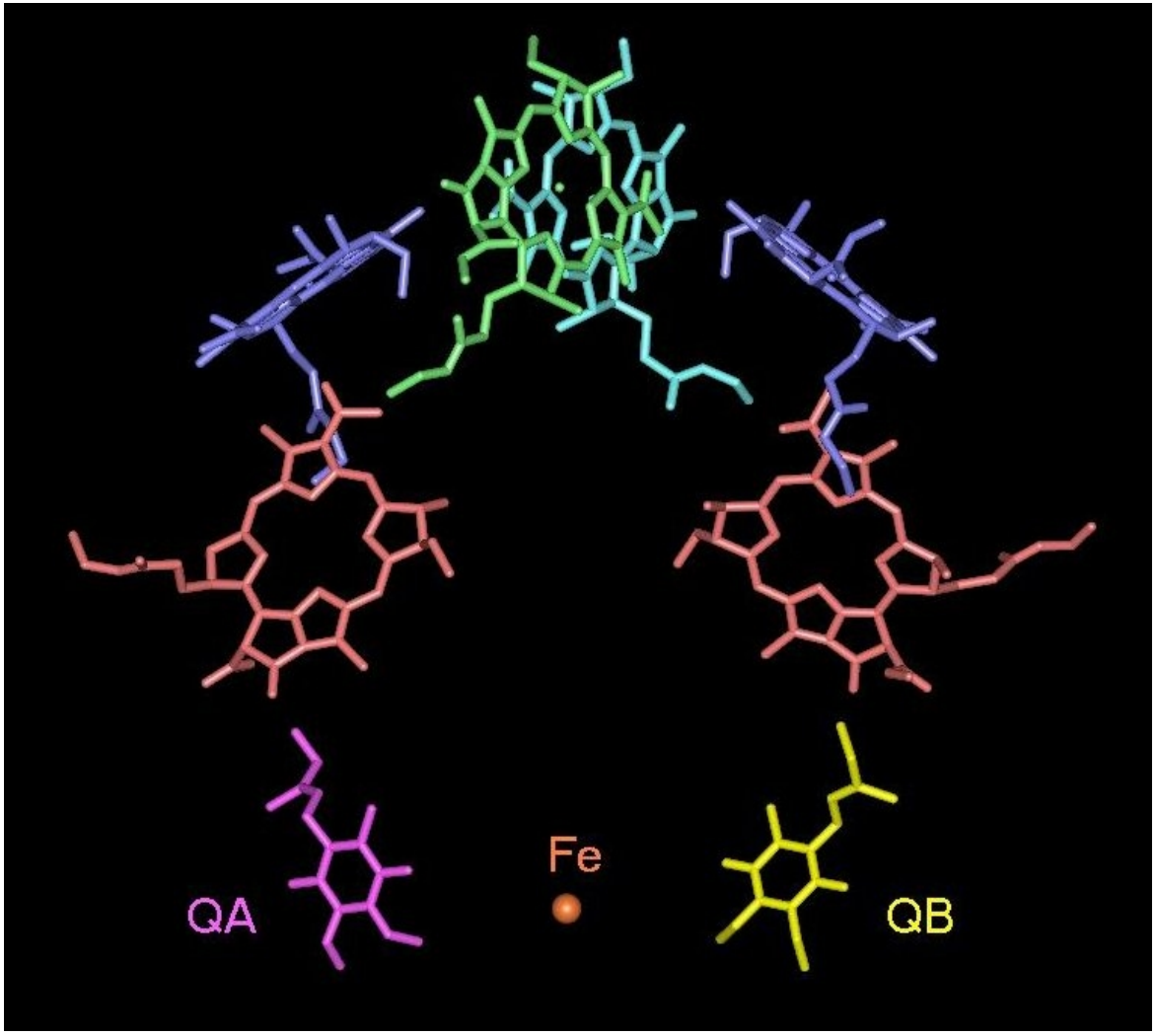
Example 4: Photosynthetic reaction center complex

- 3 integral proteins
- 1 peripheral protein
- cofactors:
  - -heme
  - -chlorophyll
  - -quinone



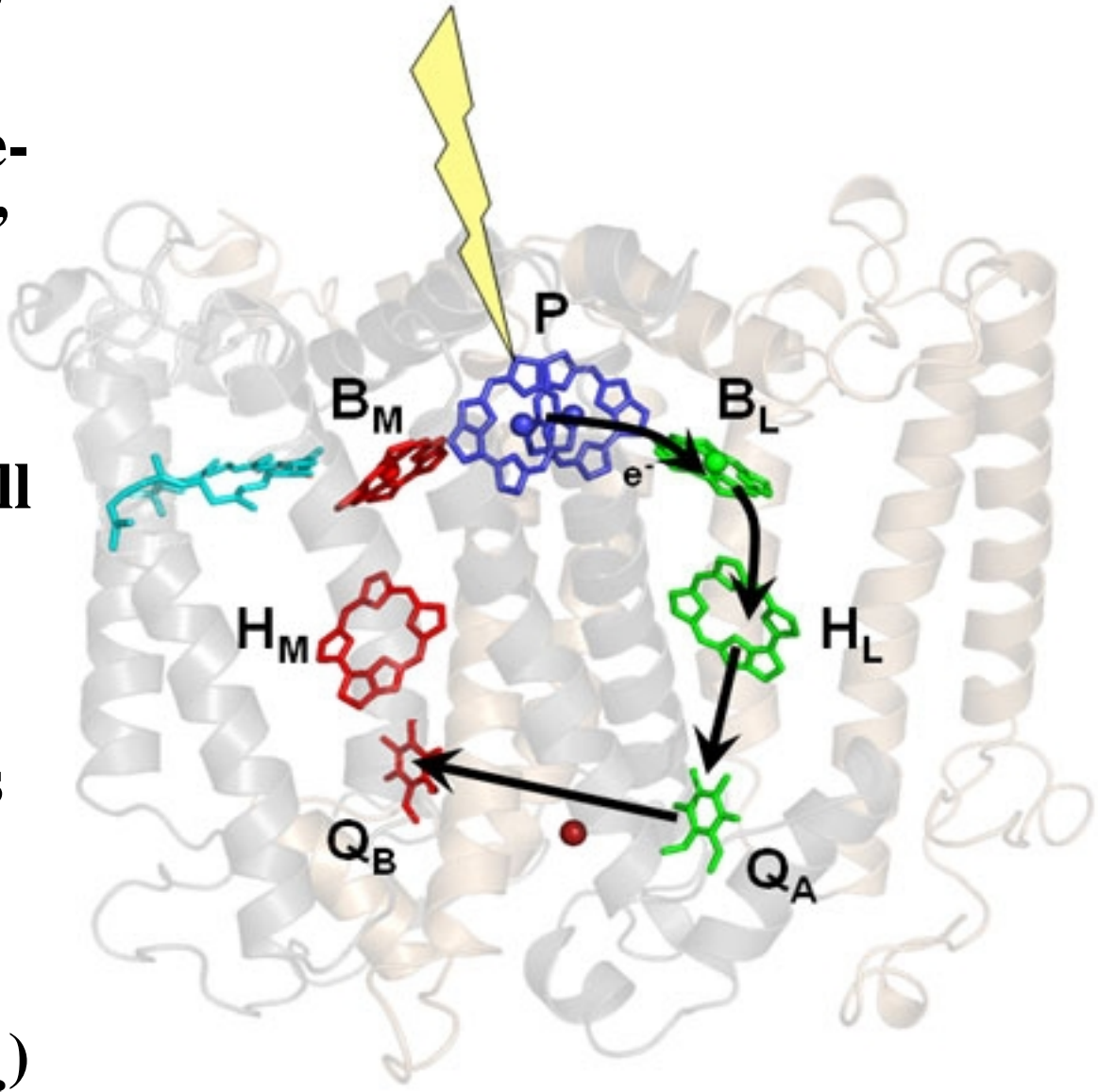
Photosynthetic pigments within the reaction center

- **pigment: a small cyclic molecule**
- **pigments act like small conductors in the protein complex which serves as a shaping insulator**



Electron transfer through the reaction center:

- a photon displaces an e- from the "special pair" of chlorophylls (P)
- e- passes through the "accessory" chlorophyll (B_L) in ~10^{-12} s onto the pheophytin (H_L)
- in ~10^{-10} s, the e- jumps to the quinone A (Q_A)
- in ~10^{-4}s, the e- arrives onto the quinone B (Q_B)



- another half-cycle brings the second e- to $Q_B \rightarrow Q_B^{2-}$
- Q_B^{2-} leaves the membrane easily to participate further in photosynthesis
- e- transport from the lower to the bottom compartment efficiently (50% of photons converted into e- transport)
- ONLY the right pathway used (despite the symmetry)
- all pigments contain partial double bonds:

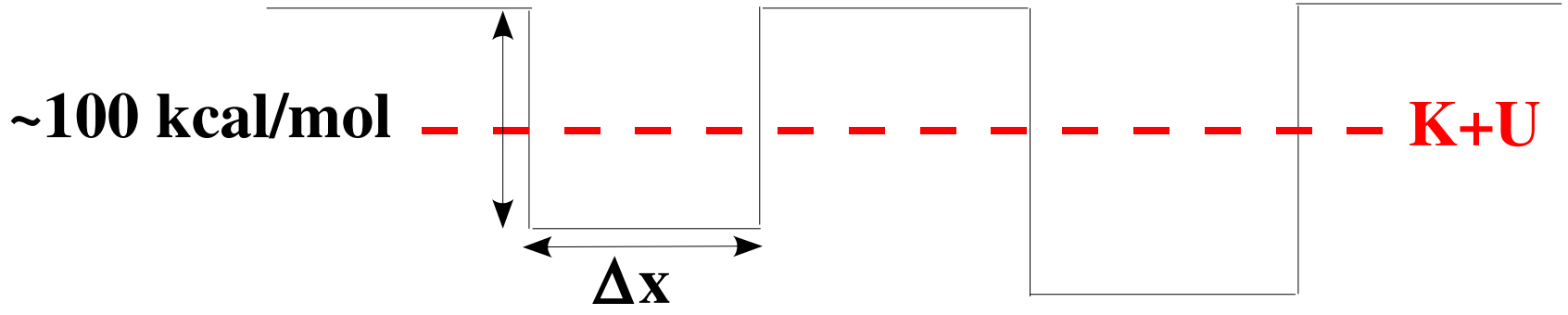
$$\ldots -C=C- C= \ldots$$

$$\Rightarrow$$

- e- are delocalized on distances greater than atom diameter (delocalized e- excited by visible light ⇒ pigment color)
- e- transfer from pigment to pigment: quantum tunneling (no direct contact between pigment molecules)

Quantum Tunneling:

- total energy of e-, K+U (kinetic+potential), smaller than the energy barrier:
  - each pigment molecule (chlorophyll, pheophytin, etc.) represents a potential well
  - in-between pigments molecules: a potential barrier



- Heisenberg uncertainty principle: $\Delta x\, \Delta p \sim \hbar$
- $K \propto \Delta p^2 \propto 1/\Delta x^2$ & $U \propto 1/\Delta x \rightarrow \Delta x > 0$ for e-

- **a typical distance over which the e- probability decreases by 10-fold is ~1Å (atom radius)**

- **10^3 – times lower e- probability at ~3Å**
- **when in the well, e- vibrates with f=10^{15} s^{-1} (visible light)**
- **if the next pigment molecule ~3Å away, time needed to tunnel across is 10^{-15}s × 10^3 = 10^{-12} s**
- **if ~5Å away, then 10^{-15}s × 10^5 = 10^{-10} s**
- **if ~10Å away, then 10^{-15}s × 10^{10} = 10^{-5} s**

…

⇒

- **40Å– thick membrane: e- jumps across in one step**

10^{-15}s × 10^{40} = 10^{25}s = 10^{17} years!

Why does the e- not jump backwards on the pathway?

- **each next well is deeper (lower potential energy) to ensure reversibility**
- **e- does not loose any energy due to tunneling**
- **e- changes the conformational state of the pigment molecule (deformed pigment conformation)**
- **pigment molecule (during conformational change) Dissipates energy to the surroundings**
- **e- looses some of its energy as it jumps from well to well**