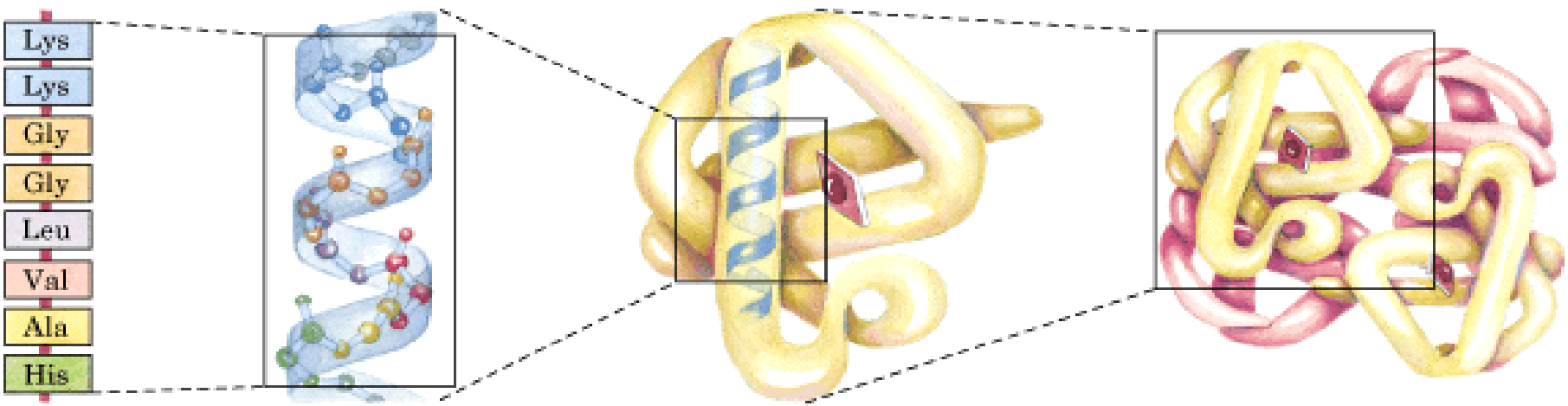
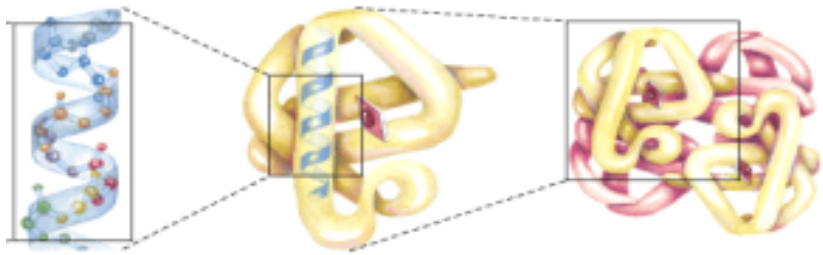
Protein Structures

1. Primary structure
   - Amino acid sequence
   - Edman degradation, MS, deduce from DNA
2. Secondary structure
   - Recurring structural pattern
   - Circular dichroism (CD, 圓二色極化光譜儀)
3. Tertiary structure
   - 3D folding of a polypeptide chain
   - X-ray crystallography, NMR
4. Quaternary structure
   - Subunits arrangement within a protein

Fig 5-16



The 3-D structure of proteins



- Protein conformation in space
- Including *long-range* interactions
- Determined by:
  - ✓ Primary (and secondary) structures
  - ✓ Interactions among R groups
  - ✓ Disulfide bond and weak interactions

Protein stability

Unfolded (denatured)

- High degree of conformational entropy
- H-bond of polypeptide with solvent (H_2O)

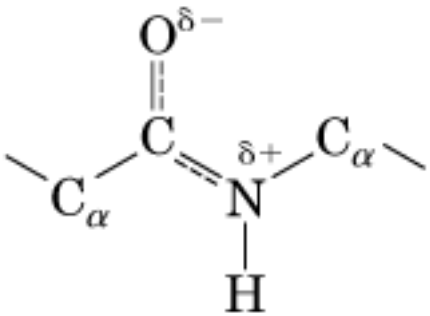
Folded (native)

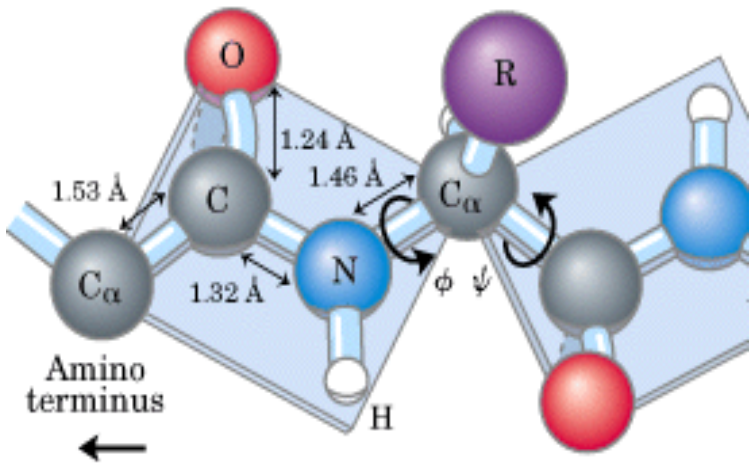
- Lowest free energy
- Stabilized by disulfide bond (covalent) and weak (non-covalent) interactions:
  - ✓ Weak interactions
    - ✓ Van der Waals interaction
    - ✓ H-bond
    - ✓ Hydrophobic
    - ✓ Ionic

In general, the protein conformation with lowest free energy is the one with the max. no. of weak interactions.

Peptide bond

1. OC-NH is shorter
2. Coplanar peptide group
3. Trans configuration (O vs. H)

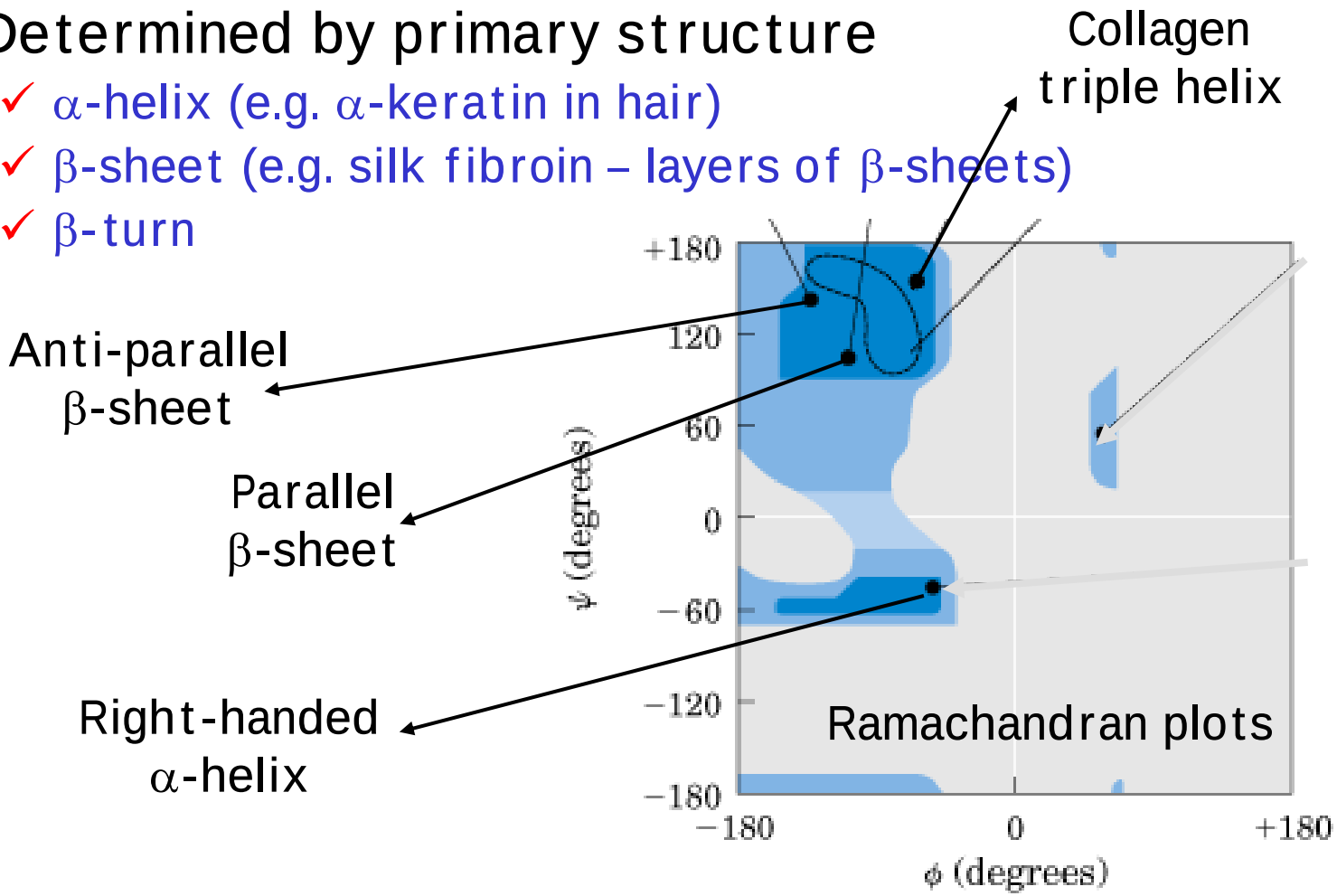




- Electrons resonance (partial sharing) between the carbonyl O and the amide N. (electric dipole)
  - ✓ OC-NH can not rotate
  - ✓ Limited rotation for C_α-C (ψ, psi) and N-C_α (ϕ, phi)

Protein secondary structure

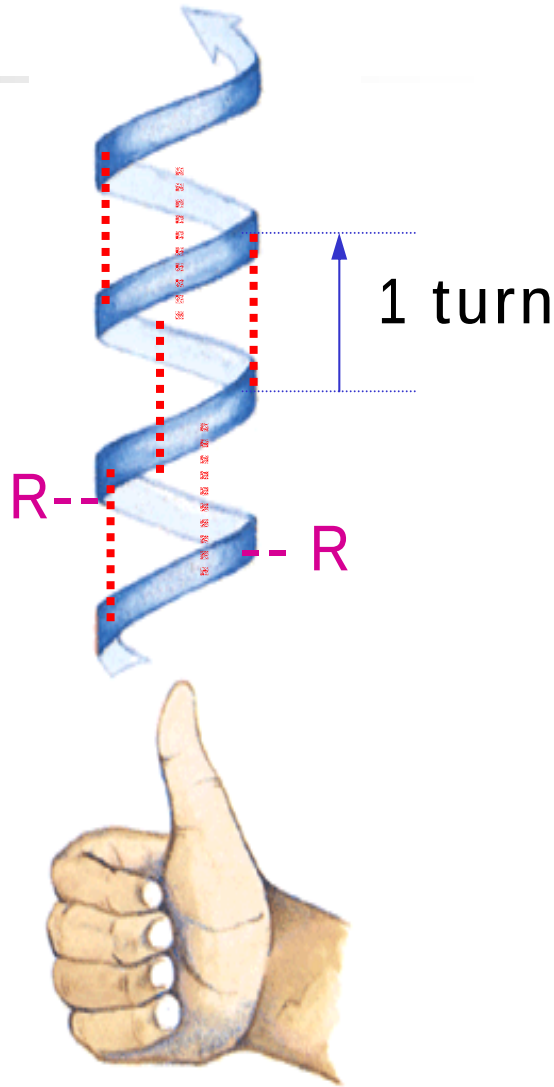
- Local conformation, regular backbone pattern
- Restricted ψ and ϕ in 2º structures
- Determined by primary structure
  - ✓ α-helix (e.g. α-keratin in hair)
  - ✓ β-sheet (e.g. silk fibroin – layers of β-sheets)
  - ✓ β-turn



α-helix

A right-handed α-helix:

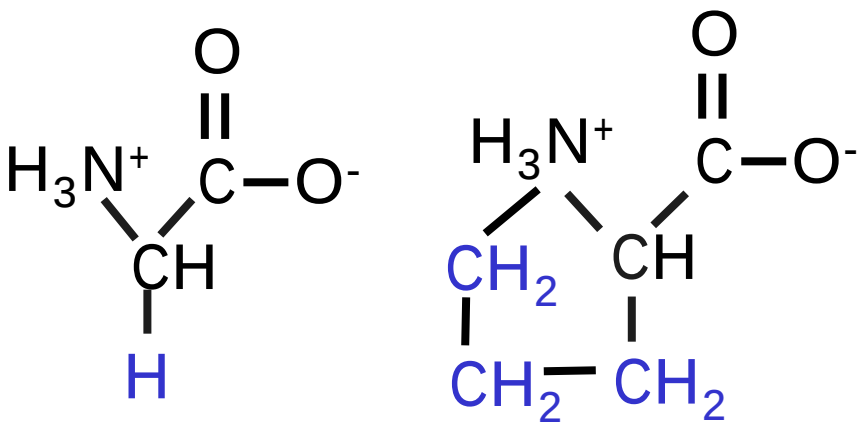
- *3.6 a.a.* per turn
- *5.4 Å* (1 Å = 0.1 nm) per turn
- R groups extended *outward perpendicular* to the helical axis
- *H-bonding* between adjacent turns
  - H-bond between the -CO of residue (i) and the -NH of residue (i+3).
  - 2 H-bonds per residue
  - 3 or 4 H-bonds per turn
  - Provide stability

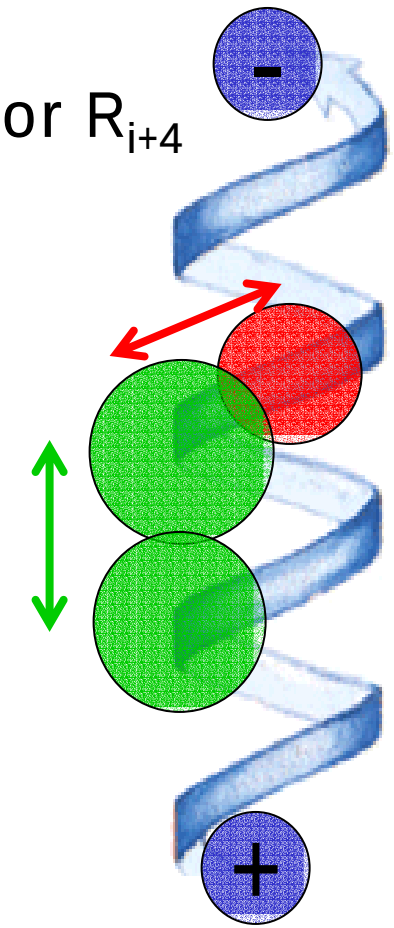


Box 6-1

α-helix constraints

1. Electrostatic interactions of R_i and R_{i+1}
2. Size of the R group
3. Interactions between R_i and R_{i+3} or R_{i+4}
4. Pro and Gly
5. End residues (electric dipole)





Electric dipole of an α-helix

- Peptide bond dipole
- Helix dipole
- End residues and helix stability

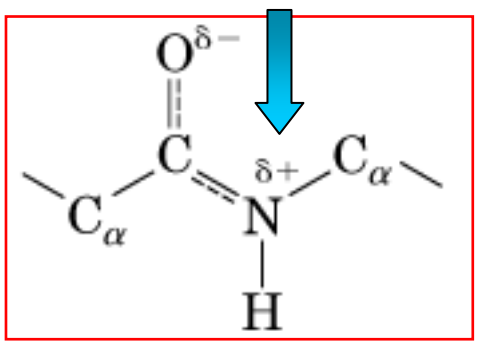


Fig 6-2a

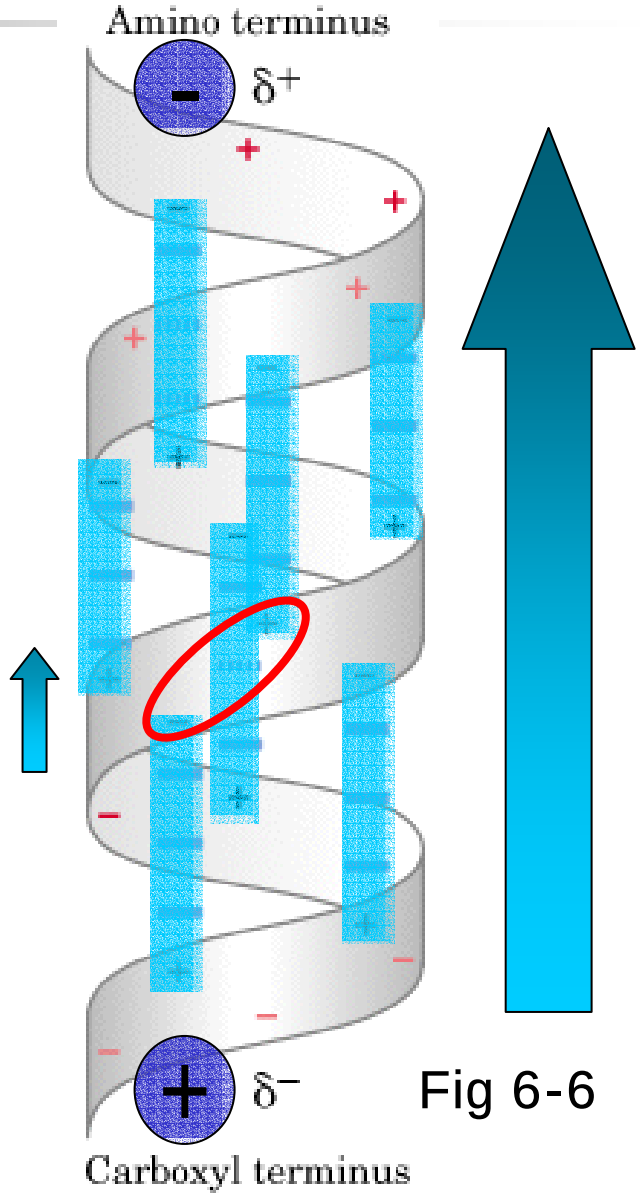
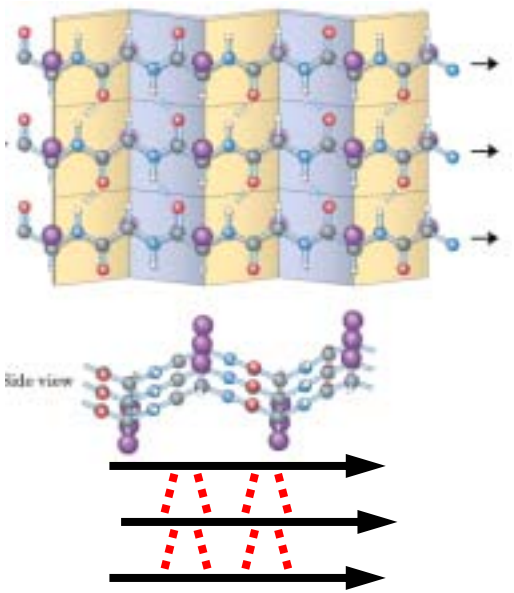


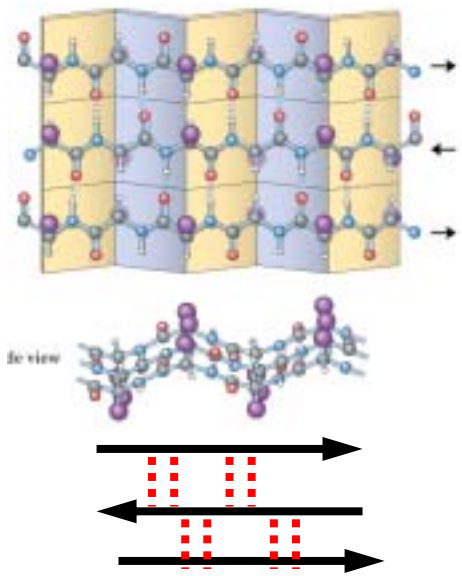
Fig 6-6

β-conformation

- Zigzag, extended protein chain, with the R groups alternating above and below the backbone.
- Side by side β-conformation → β-sheet
  - ✓ H-bonds between adjacent peptide chain (backbone).
  - ✓ Parallel or antiparallel orientations
- Silk fibroin – layers of β-sheets



parallel



antiparallel

β-turn

- A 180° turn involving 4 a.a.
- H-bond between -CO of the 1st a.a. and the -NH of the 4th a.a.
- Common a.a.
  - ✓ Gly (small and flexible, type II β-turn)
  - ✓ Pro (peptide bonds involving the imino N in *cis* configuration)

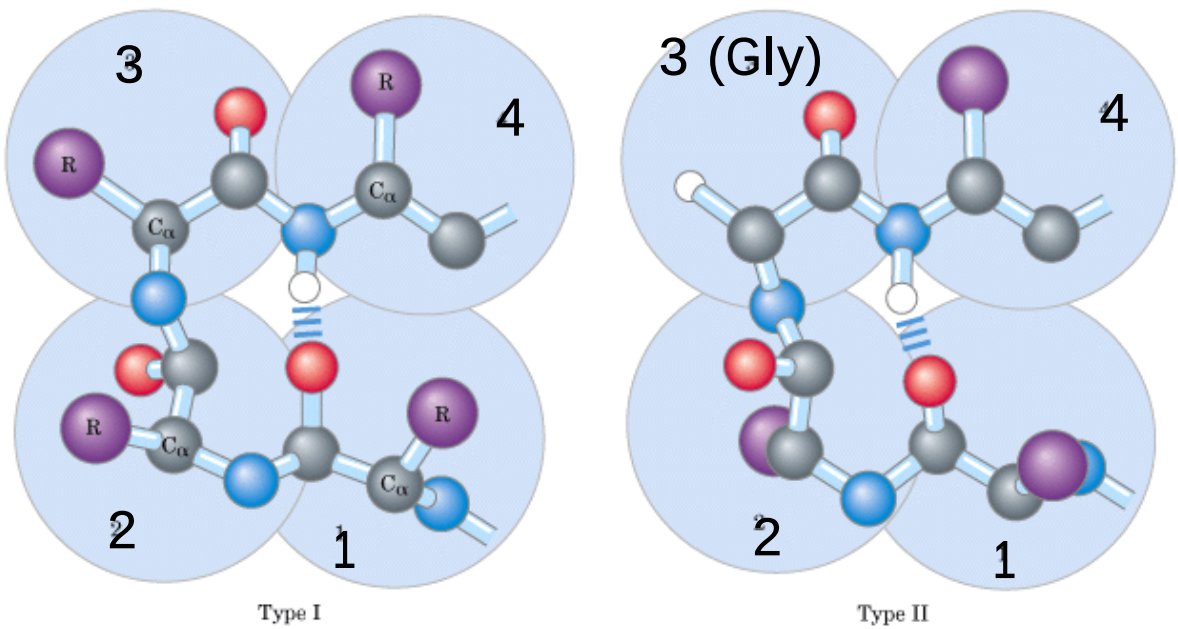


Fig 6-8a

Occurrence in 2º structure

- Relative probability of a.a.

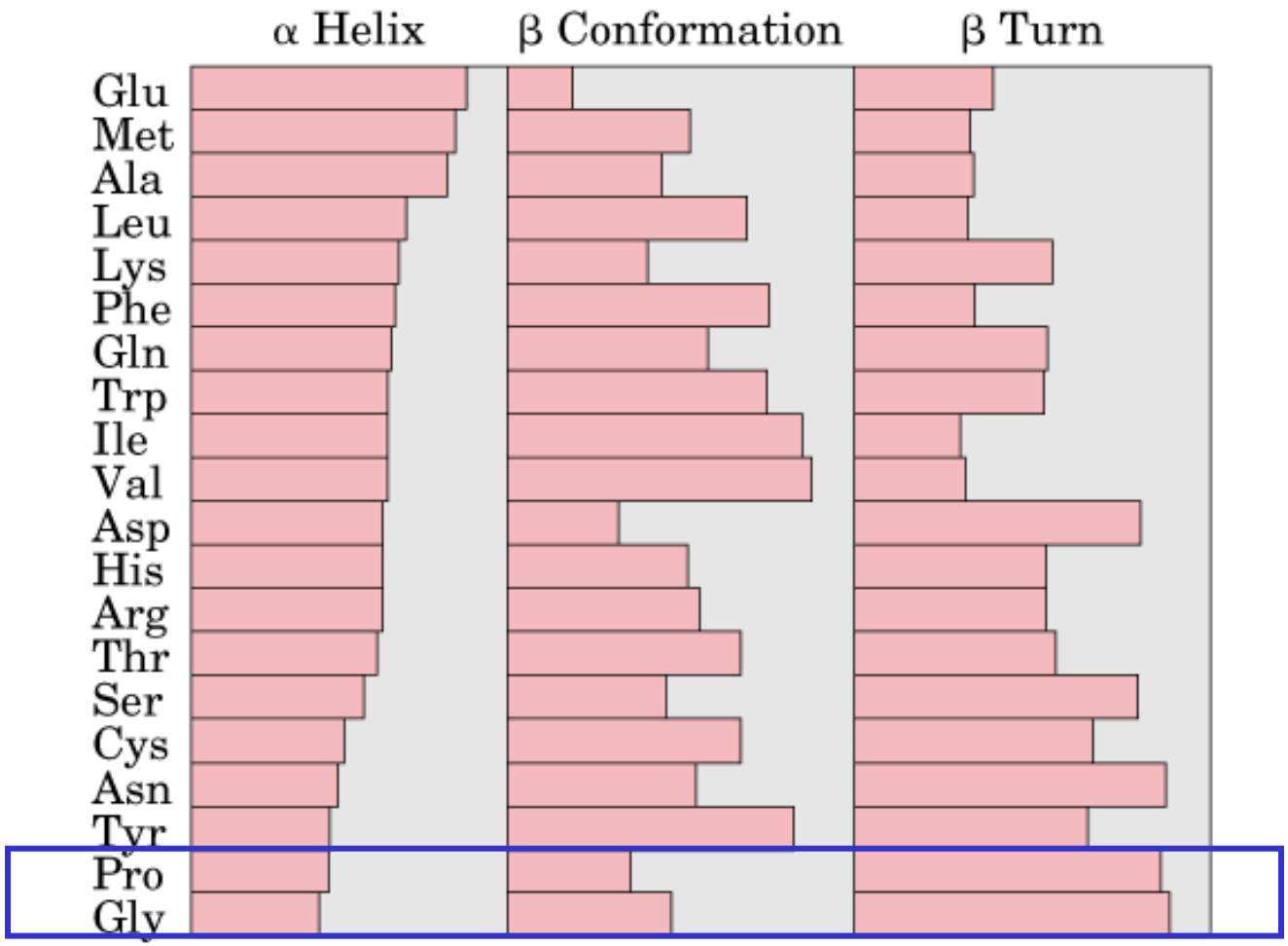
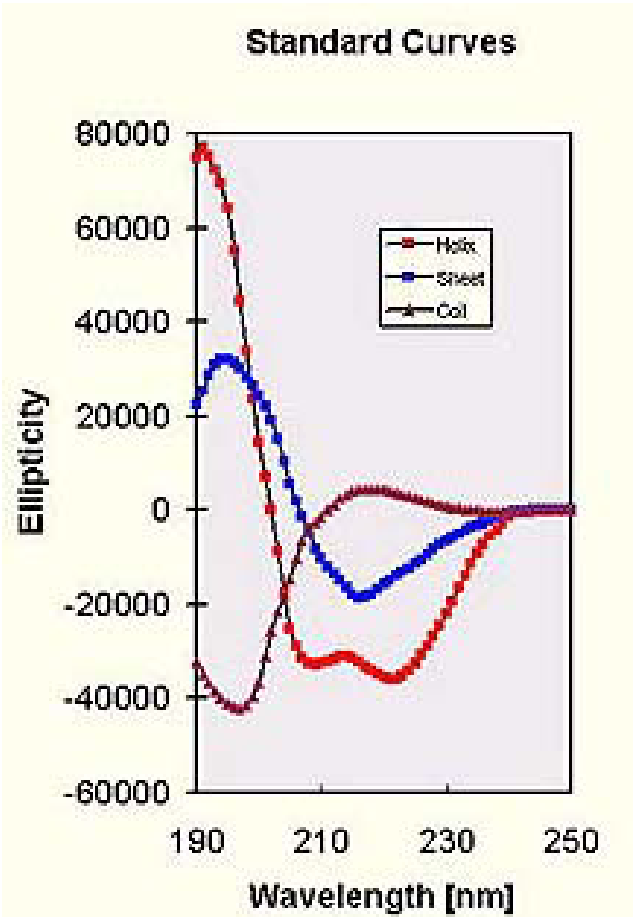
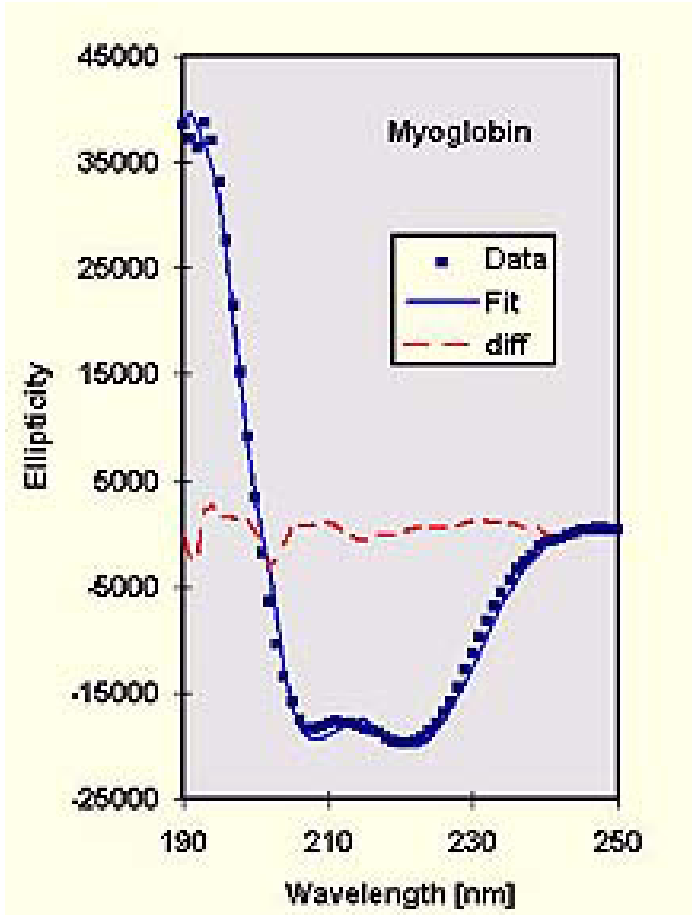


Fig 6-10

Circular Dichroism Spectroscopy

- Determine the content of 2º structure of a protein



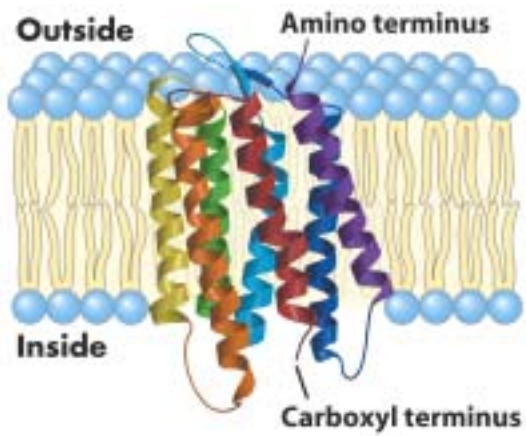


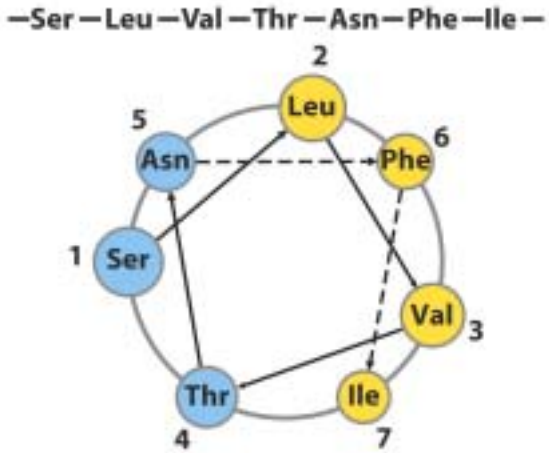
http://www-structure.llnl.gov/cd/cdtutorial.htm

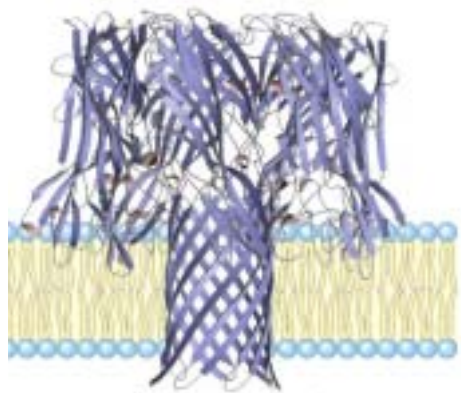
Membrane proteins

Lehninger 4th ed.

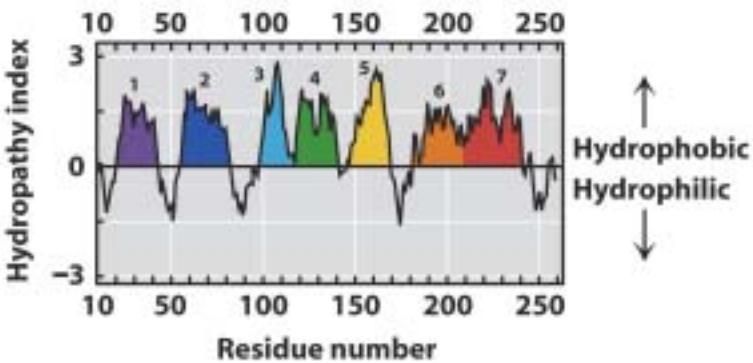
- Membrane spanning protein (hydropathy plot, p. 377)
  - ✓ α helix type channels (helical wheel diagram, p. 393)
  - ✓ β barrel porins (p. 378)

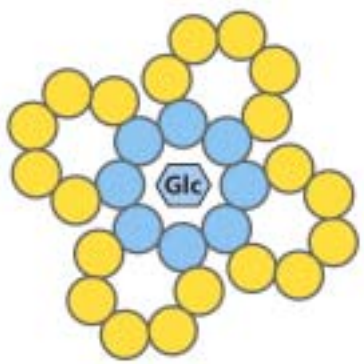


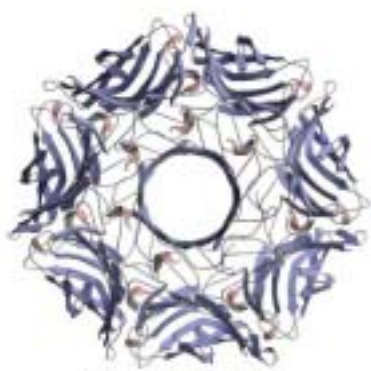




α-Hemolysin toxin







Top view

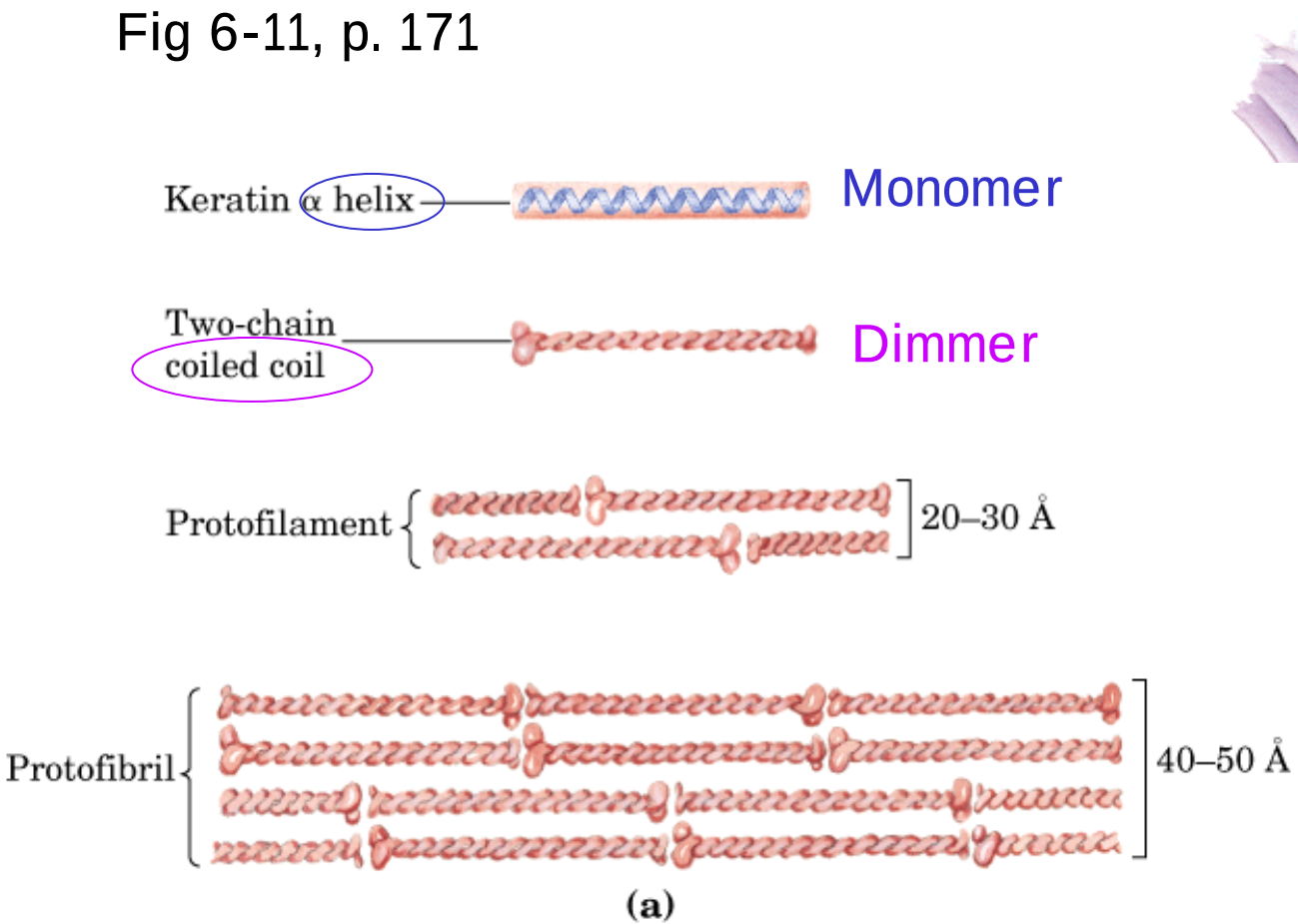
Classification (p. 170)

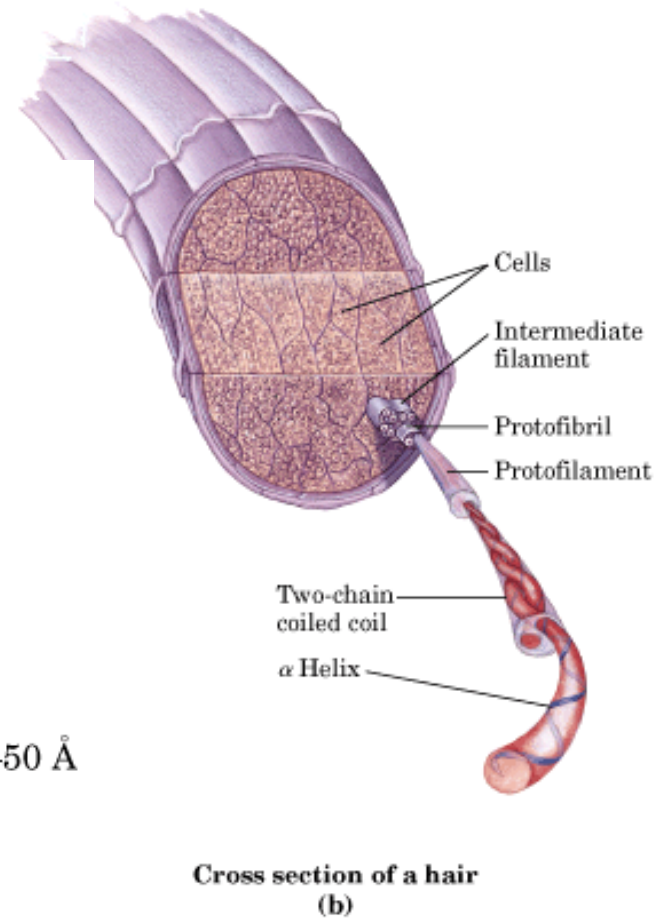
- Fibrous proteins (e.g. Table 6-1)
  - ✓ Long strands or sheets
  - ✓ Consist of a single type of 2° structure
  - ✓ Function in structure, support, protection
  - ✓ α-keratin, collagen
- Globular proteins (e.g. Table 6-2)
  - ✓ Spherical or globular shape
  - ✓ Contain several types of 2° structure
  - ✓ Function in regulation
  - ✓ Myoglobin, hemoglobin

Structure of hair

α-keratin: hair, wool, nails, claws, quills, horns, hooves, and the outer layer of skin

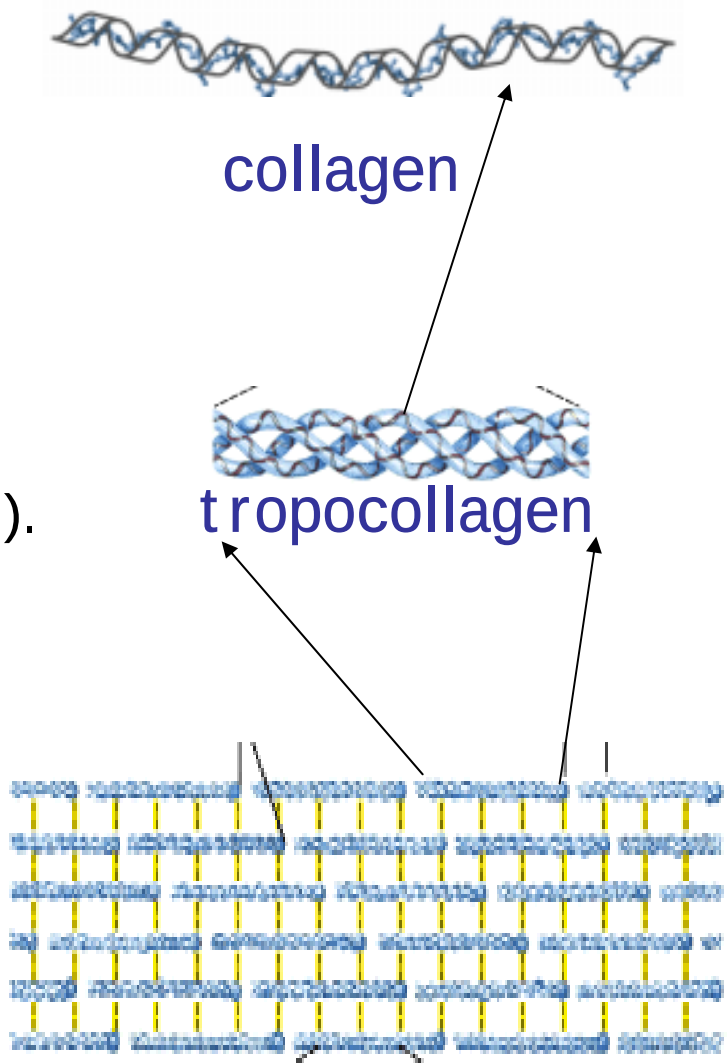
Fig 6-11, p. 171





Collagen

- Tendons, bone, cartilage, skin, and cornea
- Primary sequence:
  - ✓ Gly-X-Pro (HyPro)
  - ✓ Repeating tripeptide unit
- Structure
  - ✓ Monomer (α chain)
    - ✓ Left-handed helix, 3 a.a. per turn
  - ✓ Trimer: coiled-coil (tensile strength).
    - ✓ Stabilized by H-bond
    - ✓ Crosslink between triple helixes
- Genetic defect:
  - ✓ Osteogenesis imperfecta
    - ✓ Abnormal bone formation in babies
  - ✓ Ehlers-Danlos syndrome
    - ✓ Loose joint

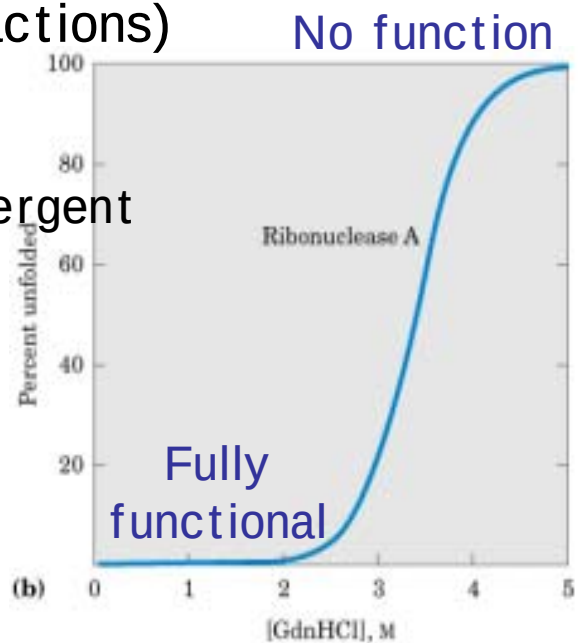


More on Collagen ... Harper's 26th, p. 38-39.

- Procollagen (a larger precursor polypeptide)
  - ✓ Post-translational modification
    - ✓ Pro, Lys → Hydroxyl Pro, Lys (cofactor = ascorbic acid)
    - ✓ Provide H-bond that stablizes the mature protein
    - ✓ Scurvy: a dietary deficiency of Vit C
  - ✓ Central portion → triple helix (procollagen → collagen)
    - ✓ The N-, and C-terminal portions are removed
  - ✓ Certain Lys are modified by lysyl oxidase (a copper-containing protein)
    - ✓ Crosslink between polypeptides → increased strength and rigidity.
    - ✓ Menke's syndrome: a dietary deficiency of the copper

Denature and unfolding

- Loss of function due the structural disruption
  - ✓ Cooperative process
  - ✓ Denatured conformation: random but partially folded
  - ❑ No covalent bonds in the polypeptide are broken !!
- Denaturing agent
  - ✓ Heat (H-bond)
  - ✓ Extreme pH (change ionic interaction)
  - ✓ Miscible organic solvent (hydrophobic interactions)
    - ✓ Alcohol, acetone
  - ✓ Certain solutes (hydrophobic interactions)
    - ✓ Urea, guanidino hydrochloride (Gdn HCl), detergent

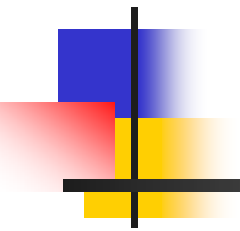


The prion disease

- Spongiform encephalopathies
- Disease caused by a protein (prion)
- Proteinaceous infectious particle
- Related diseases:
  - ✓ Mad cow disease
  - ✓ Kuru
  - ✓ Creutzfeldt-Jakob disease (human)
  - ✓ Scrapie (sheep)
- Misfolded prion

PrP^{C}
(normal)

PrP^{SC}
(infectious)

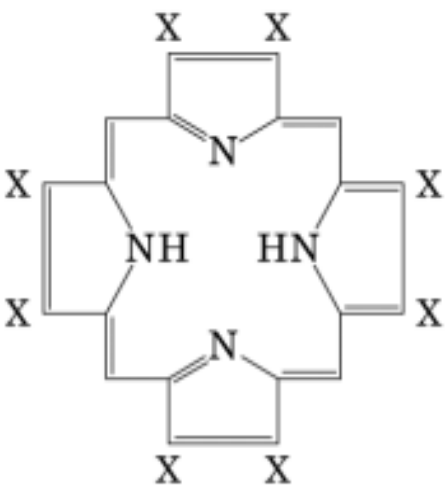


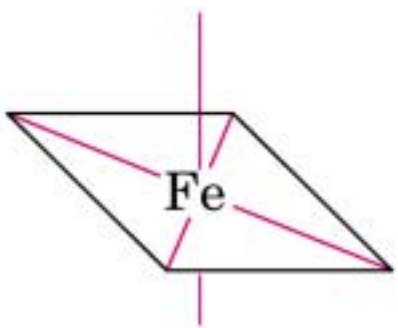
Protein Function

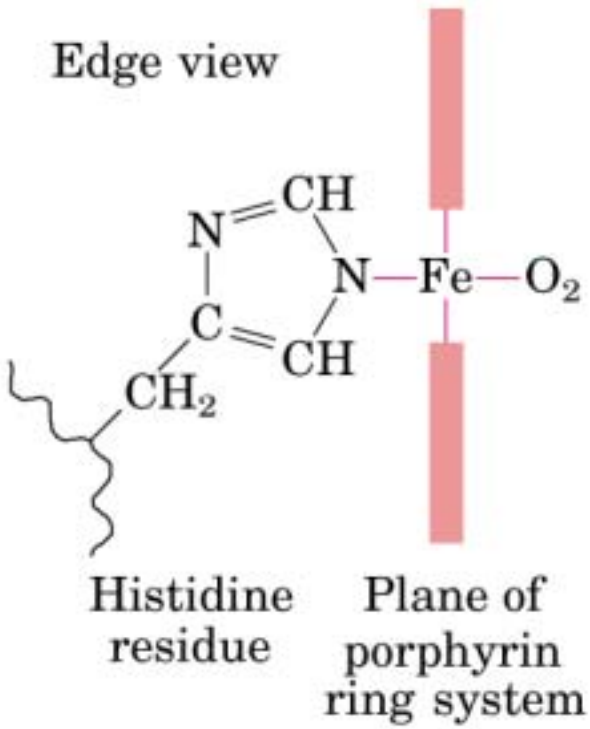
Myoglobin and Hemoglobin

O_2 binding to Heme

- Heme = organic ring (porphyrin) + Fe^{2+}
- Free heme → Fe^{2+} (binds O_2) vs. Fe^{3+} (does not bind)
- O_2 rich blood (bright red) vs. O_2 depleted blood (dark purple)
- CO, NO binds with higher affinity than O_2







Protein-ligand interaction

p. 207

- $P + L \rightleftharpoons PL$

$$K_a = \frac{[PL]}{[P]\,[L]}$$

K_a: association constant (M^{-1})

$$K_a\,[L] = \frac{[PL]}{[P]}$$

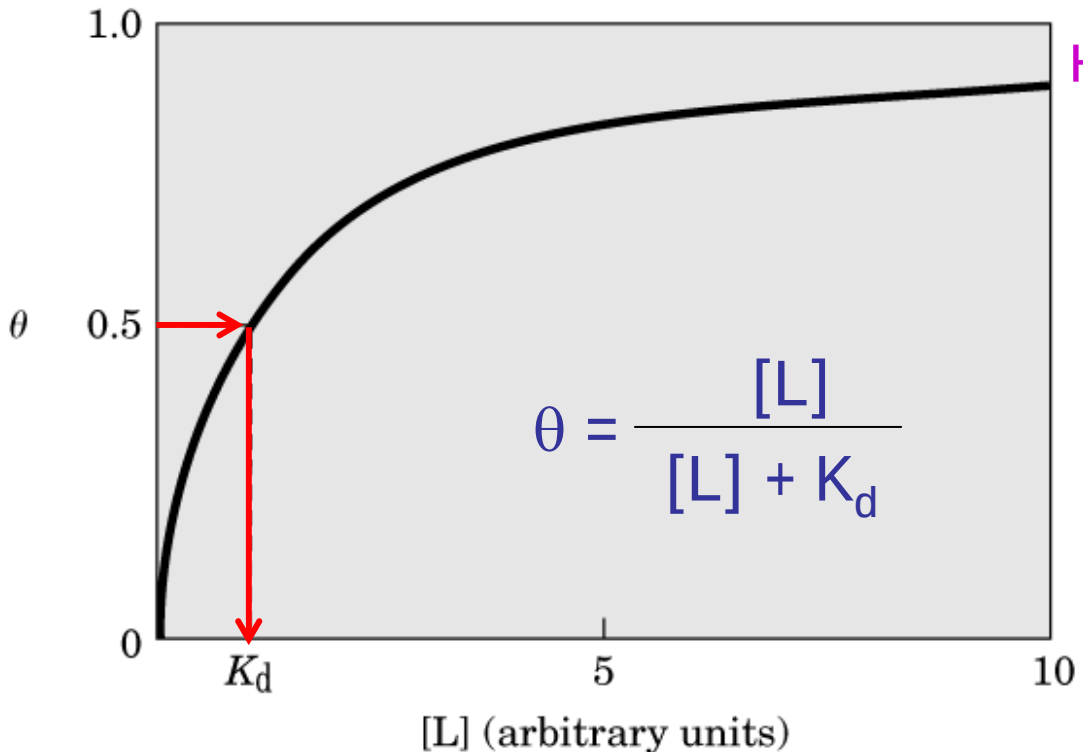
$$\theta = \frac{\text{Binding sites occupied}}{\text{Total binding sites}} = \frac{[PL]}{[PL] + [P]}$$

$$\theta = \frac{[L]}{[L] + 1/K_a} = \frac{[L]}{[L] + K_d}$$

K_d: dissociation constant (M)

Ligand binding and K_d

- When [L] = K_d, 50% ligand-binding sites are occupied
- K_d: dissociation constant
- K_d = [L] at half-saturation
- Affinity ↑, K_d ↓



Hyperbola

Fig 7-4a

O_2 binding of Mb

- O_2 binds tightly to Mb
- Good for O_2 storage
- Not good for O_2 transport

$$\theta = \frac{[L]}{[L] + K_d}$$

$$\theta = \frac{pO_2}{pO_2 + P_{50}}$$

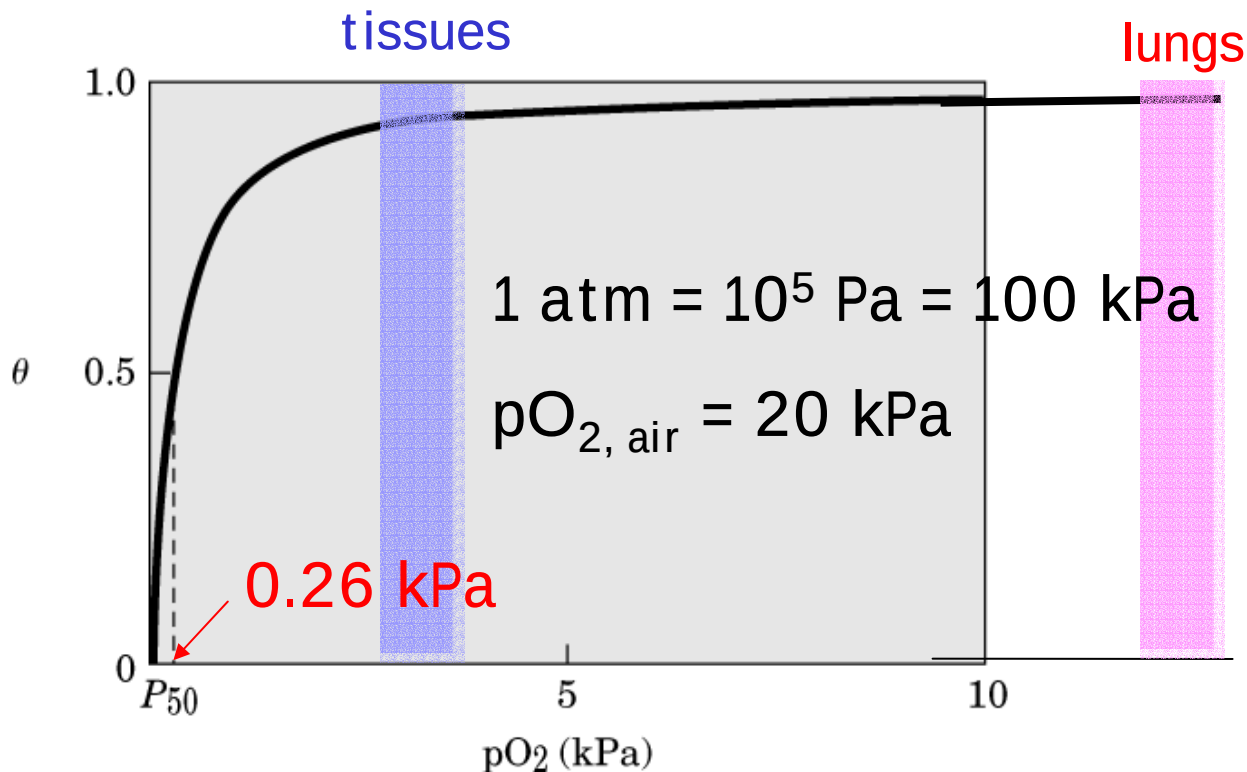
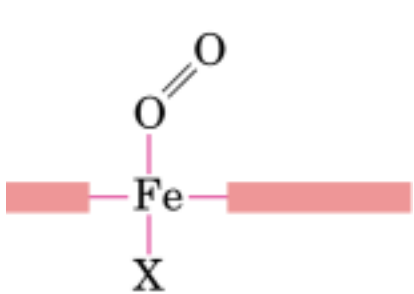
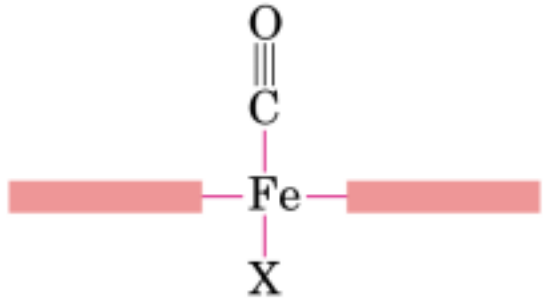


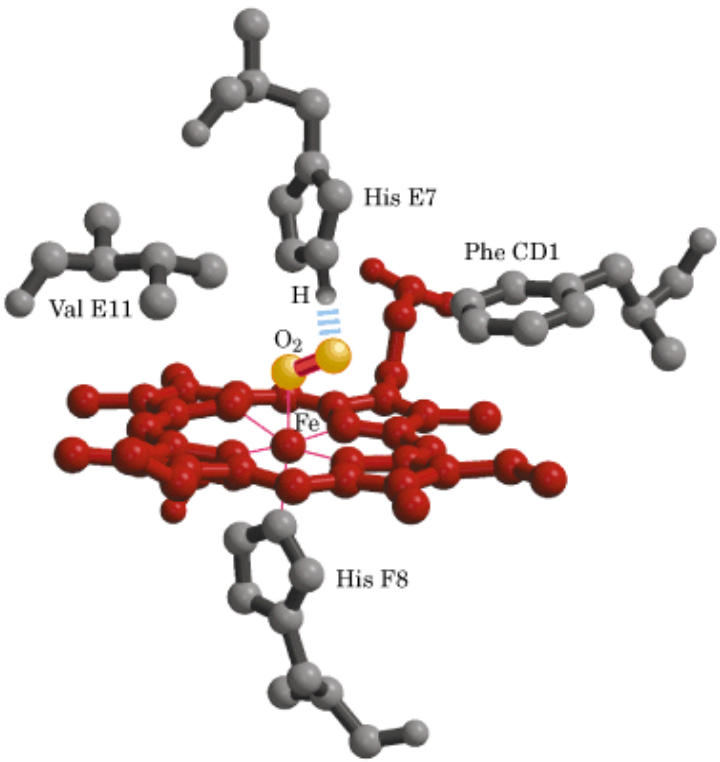
Fig 7-4b

Structure affects K_d

	K_d for O_2	K_d for CO
Free heme	1x	1/20,000x
Heme in Mb	1x	1/200x







- Steric hindrance
  - ✓ Distal His, (His^{64} of Mb)
- Molecular motion (breathing)
  - ✓ O_2 in/out buried cavity

Mb vs. Hb

- O_2 storage
- In muscle tissue
- Mb = monomer
  - ✓ 1 polypeptide chain (153 a.a.) + 1 heme
- Mb m.w. = 16.7 kDa

- O_2 transport
- Found in erythrocyte
- Hb = tetramer
  - ✓ 4 x (polypeptide chain + heme)
- Hb m.w. = 64.5 KDa
- Interactions between subunits (tetramer)

Sequence vs. structure homology

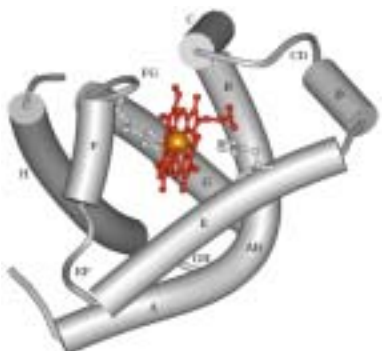


Fig 7-3

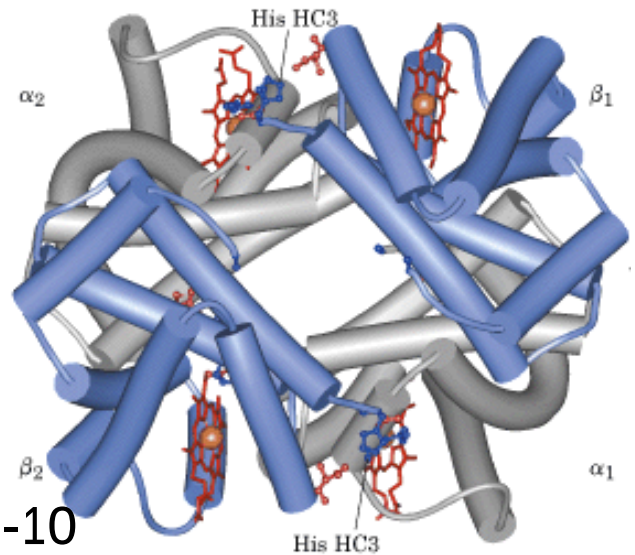


Fig 7-10

Hb has 2 conformations

	T state	R state
$-O_2$	structure stable	unstable
$+O_2$	unstable	stable
K_d (O_2)	large	small

- O_2 binding to T triggers a conformational change to R

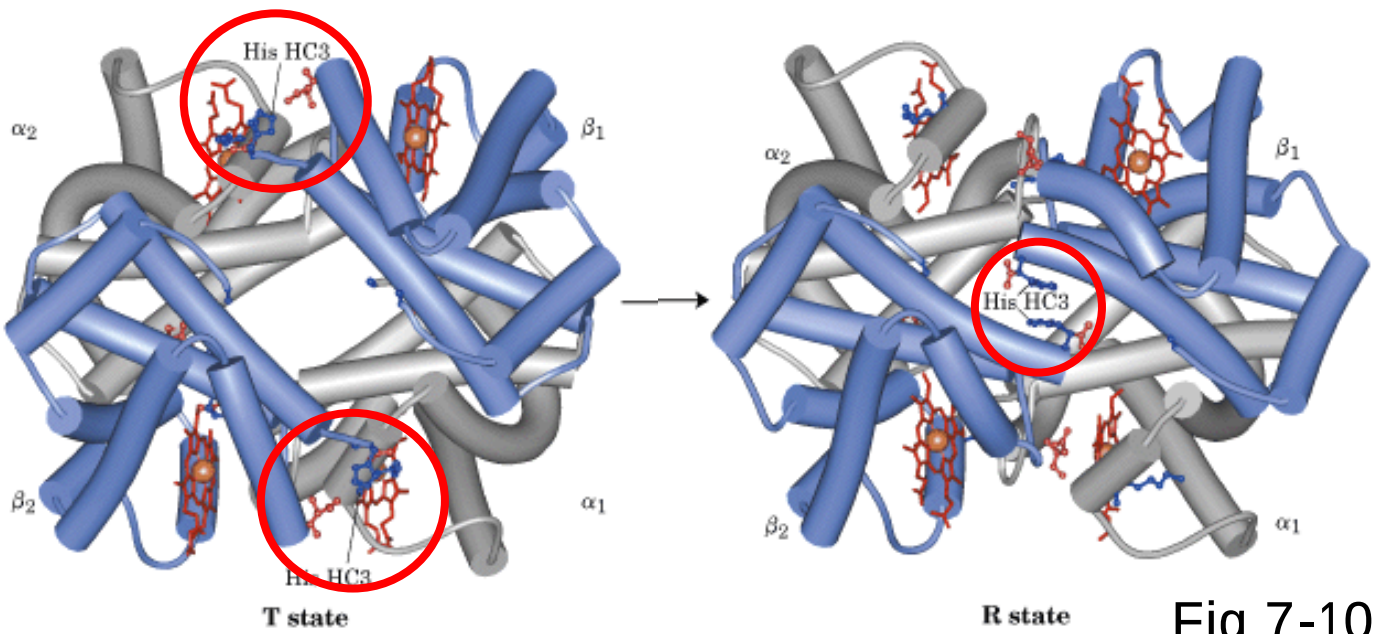


Fig 7-10

Hb–O_2 binding curve

- A sigmoid (S-shape) binding curve
- Permit highly sensitive response to small change in pO_2 or [L]

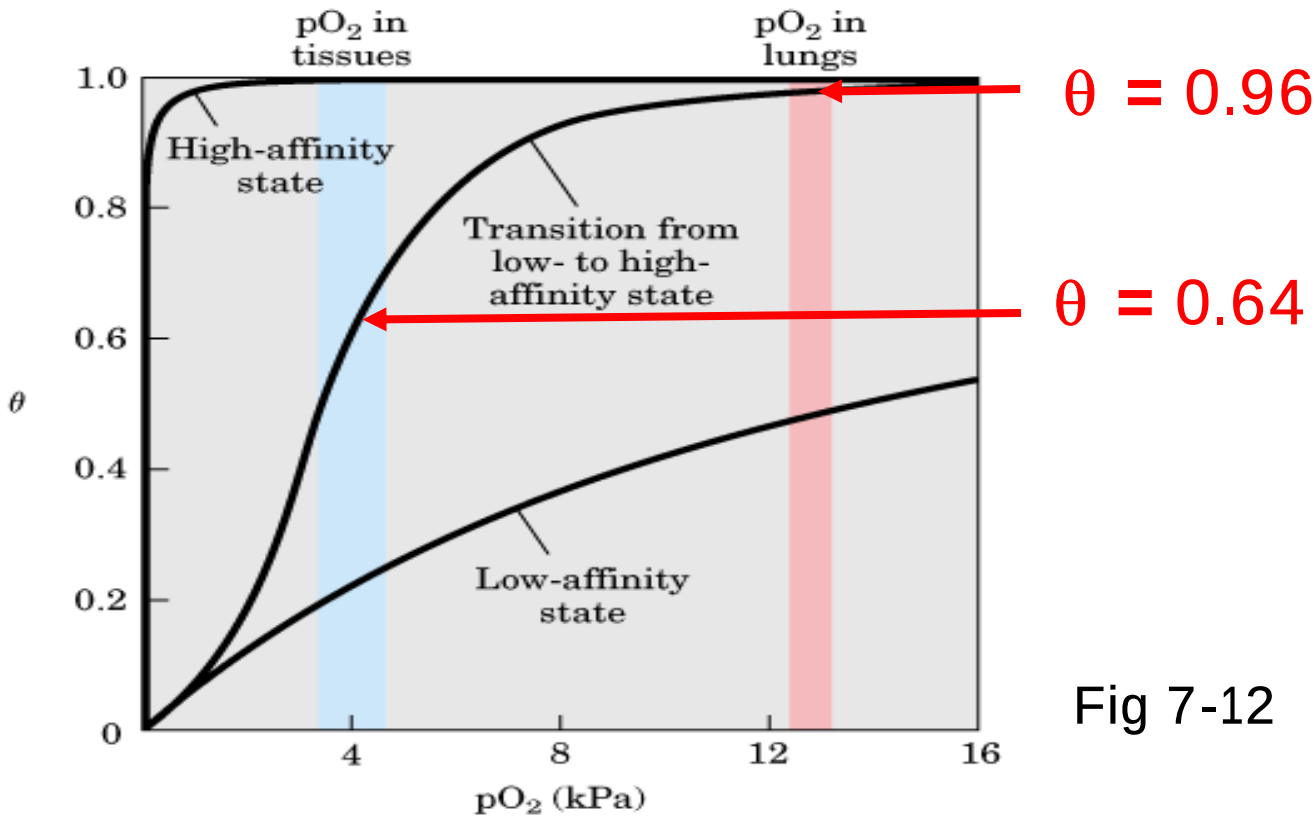


Fig 7-12

O_2 binding to Hb

- Cooperativity
  - ✓ One subunit binding of O_2 affects K_d of the adjacent subunits
  - ✓ 4 x (subunit + O_2)
    - ✓ 1st O_2 binds Hb (**T**) weakly, initiate T → R
    - ✓ 2nd O_2 binds Hb (**T**→R) with higher affinity
    - ✓ 3rd O_2 binds Hb (T→**R**) with even higher affinity
    - ✓ 4th O_2 binds Hb (**R**) with highest affinity
  - ✓ S-shaped (sigmoid) binding curve – multimer only
- Allosteric protein
  - ✓ Homotropic: modulator = ligand (substrate)
    - ✓ e.g. O_2, CO
  - ✓ Heterotropic: modulator ≠ ligand (substrate)
    - ✓ e.g. H^+, CO_2, BPG

Quantification

- $P + n\,L \rightleftharpoons PL_n$

$$K_a = \frac{[PL_n]}{[P]\,[L]^n}$$

$$\theta = \frac{\text{Binding sites occupied}}{\text{Total binding sites}} = \frac{[L]^n}{[L]^n + K_d}$$

$$\frac{\theta}{1 - \theta} = \frac{[L]^n}{K_d}$$

$$\log \frac{\theta}{1 - \theta} = n \log [L] - \log K_d$$

$$Y = ax - b$$

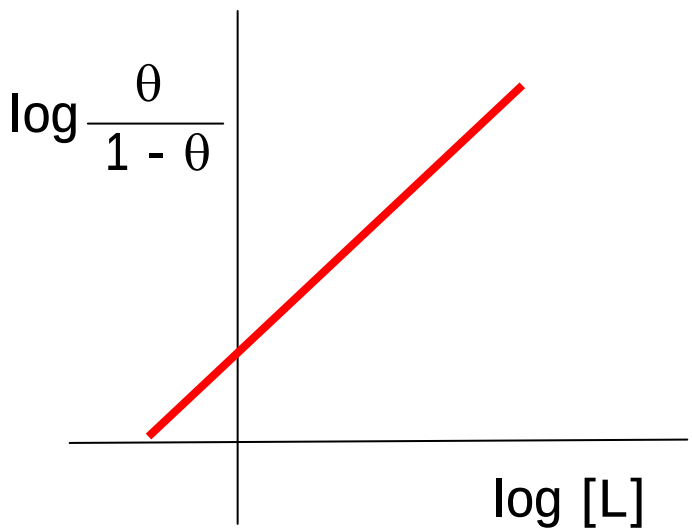
Hill equation

Slope = n (Hill coefficient)

n > 1, + Coop.

n = 1, no Coop.

n < 1, - Coop.



Hill plot of Mb vs. Hb

- Mb: $n_H = 1$
- Hb: $n_H = 3$

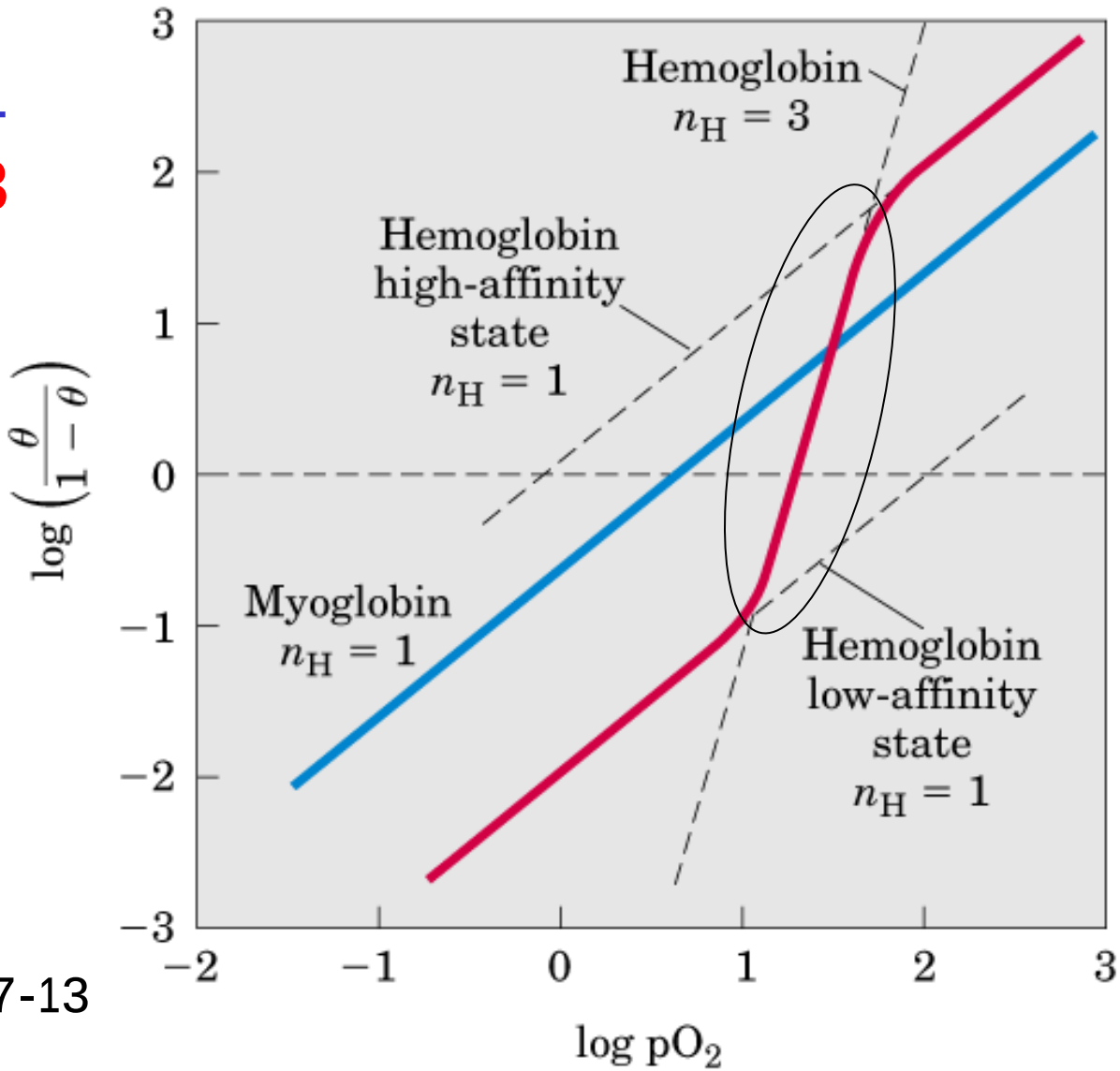
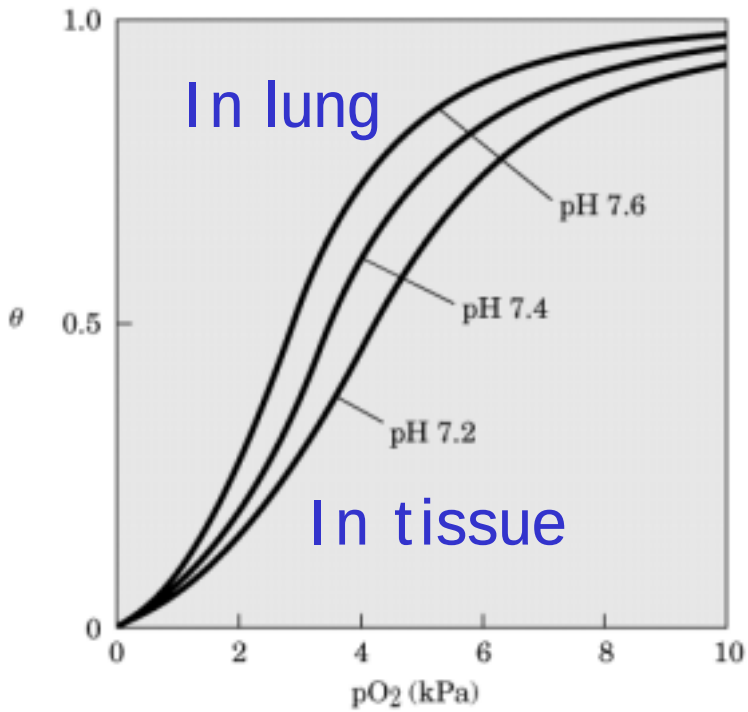


Fig 7-13

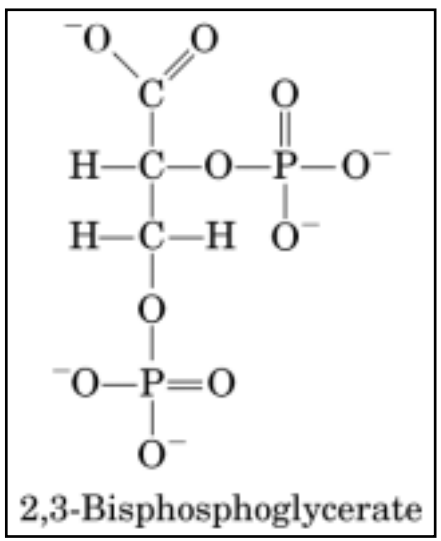
Hb also transports H^+ and CO_2

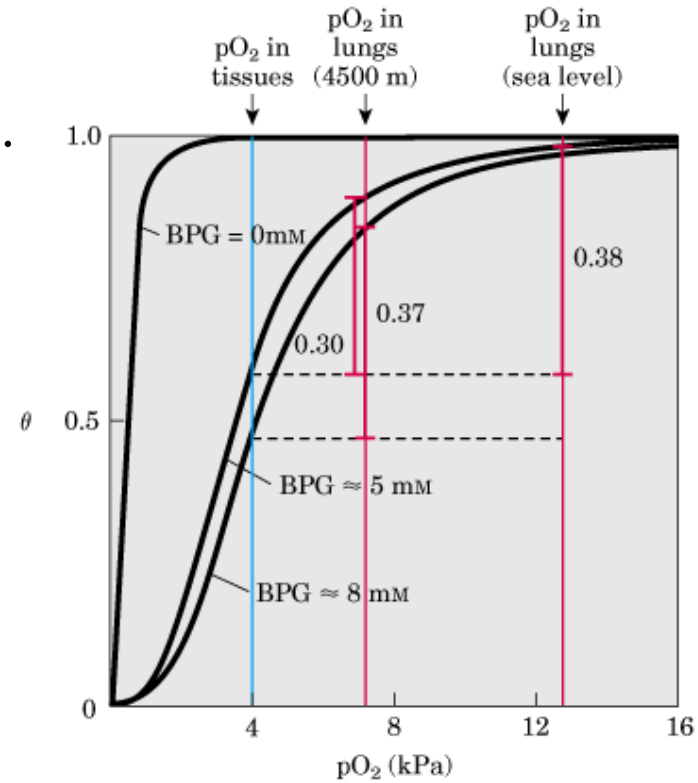
- Bohr effect
- pH and CO_2 modulate the affinity of Hb for O_2
  - ✓ Hb binds O_2 and (H^+ or CO_2) with inverse affinity
  - ✓ Hb binds O_2, H^+, and CO_2 at different sites
    - ✓ Tissues: pH ↓ and CO_2↑, O_2 affinity ↓, Hb release O_2
    - ✓ Lungs: pH ↑ and CO_2 ↓, O_2 affinity ↑, Hb binds more O_2



BPG (2,3-bisphosphoglycerate)

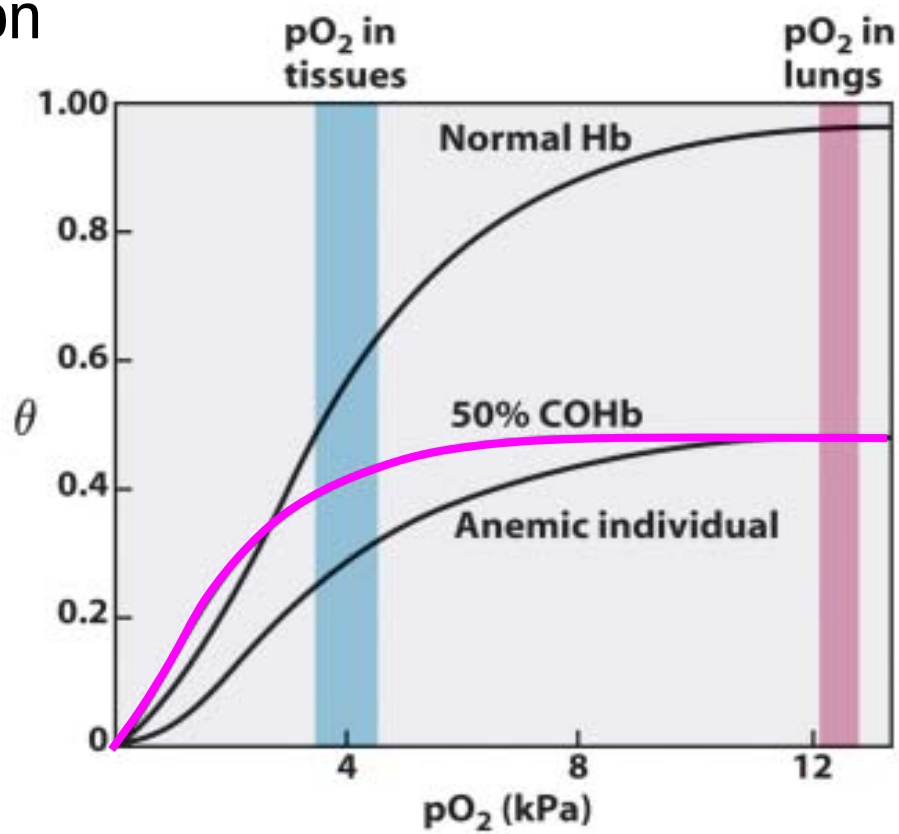
- BPG binds to ⊕ a.a. in the cavity between β subunits in Hb (T state)
  - ✓ BPG stabilize T state ⇒ O_2 affinity ↓
- [BPG] at sea level vs. high altitude
- Fetal Hb – needs to have a higher O_2 affinity than mother's Hb
  - ✓ Fetal Hb : $\alpha_2\gamma_2$
- [BPG] ↓, after storage, transfusion…
- People suffering from hypoxia, [BPG] ↑ …





CO intoxication (Box 5-1)

- CO has a higher affinity for Hb
  - ✓ Smoker has higher level of COHb (3~15%) vs. < 1%
  - ✓ Binding of CO to Hb increase the O_2 affinity of Hb
    - ✓ O_2 transport become less efficient (Fig 2)
- Suspected CO intoxication
  - ✓ Rapid evacuation
  - ✓ Administer 100% O_2



Lehninger 4th ed.

Sickle-cell anemia

- Homozygous allele for the β subunit gene
  - ✓ Hb A (Glu^6) vs. Hb S (Val^6) on β subunits surface
  - ✓ "Sticky" hydrophobic contacts
  - ✓ deoxyHb S: insoluble and form aggregates
- Heterozygous: malaria resistance
- Anemia or Malaria ?