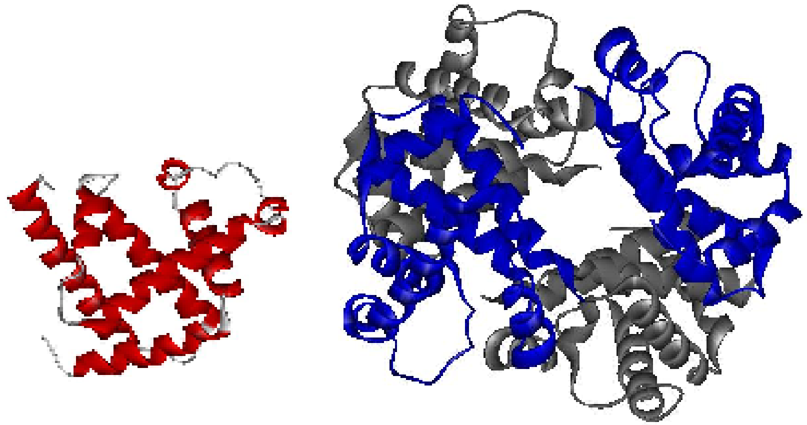


Ch 5 Protein Function

Myoglobin and Hemoglobin

Immune system (Antibody)

Molecular Motors: Actin and Myosin

Oxygen binding proteins

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

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

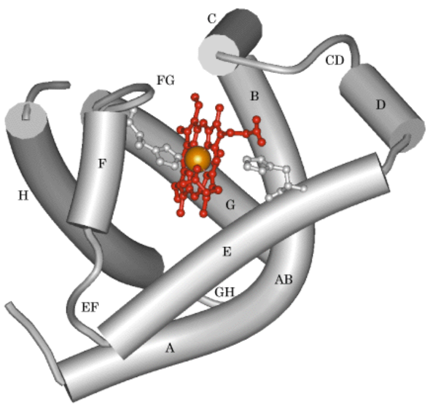


Fig 5-3

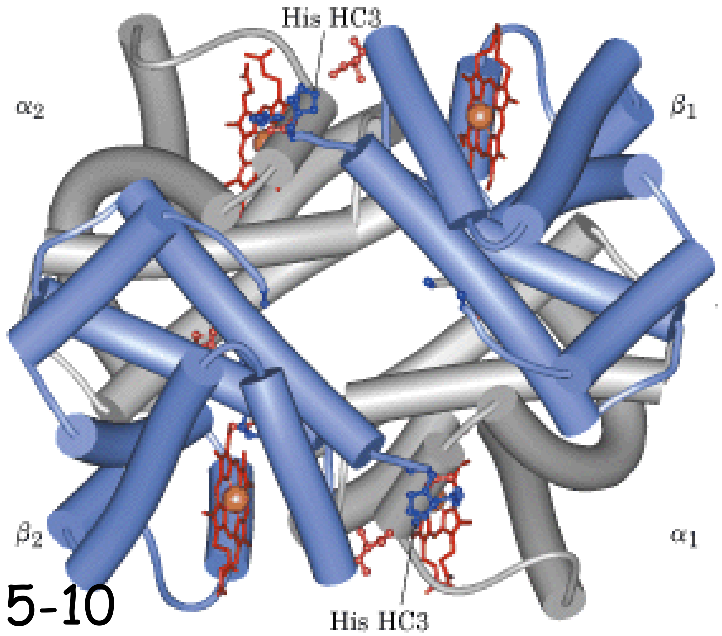


Fig 5-10

O_2 binding to Heme

- Heme group to bind O_2
  - ✓ Heme = organic ring (porphyrin) + Fe^{2+}
  - ✓ Free heme → Fe^{2+} (binds O_2) vs. Fe^{3+}
- O_2 rich blood (bright red) vs. O_2 depleted blood (dark purple)
- CO, NO binds with higher affinity than O_2

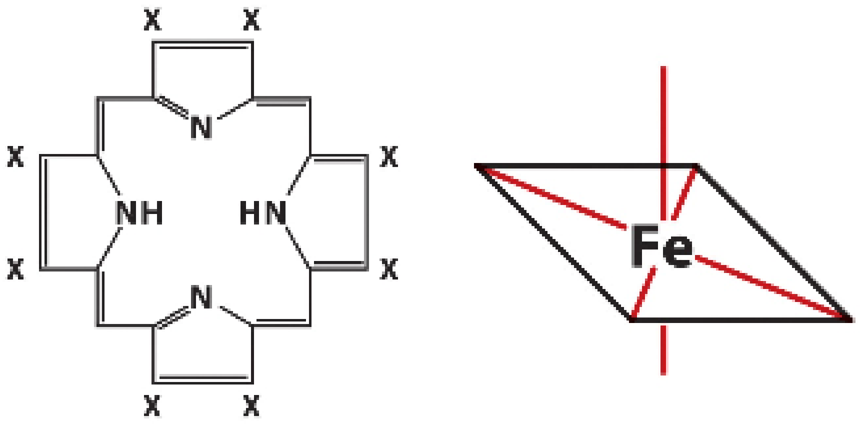


Fig 5-1 a, d, p.154

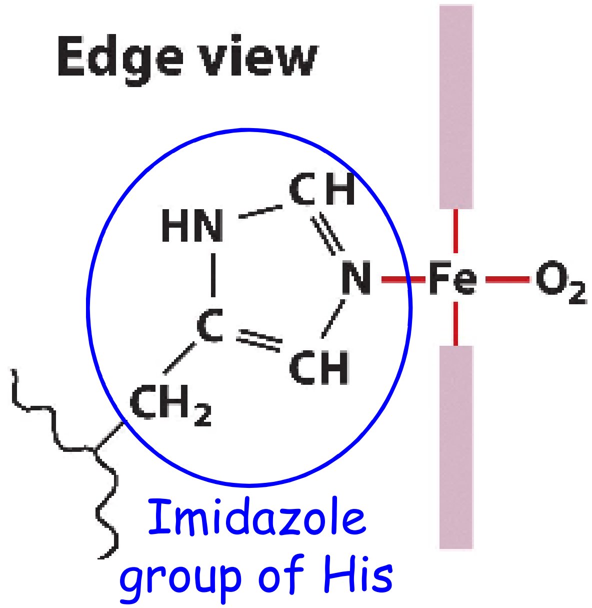


Fig 5-2, p.155

Protein-ligand interaction

p. 155-156

- $P + L \rightleftharpoons PL$

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

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

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

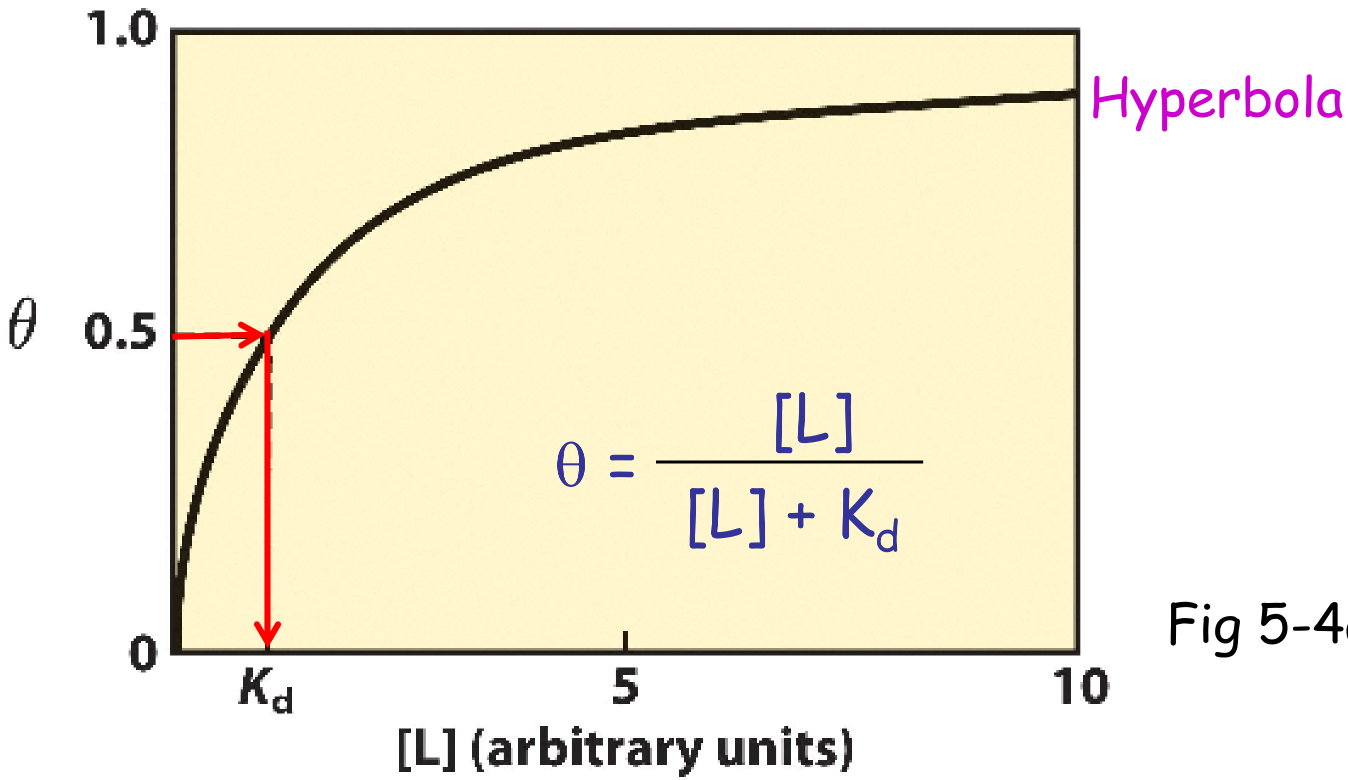
$$\theta = \frac{\text{Binding sites occupied}}{\text{Total binding sites}} = \frac{[PL]}{[PL] + [P]}$$

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

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 ↓



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

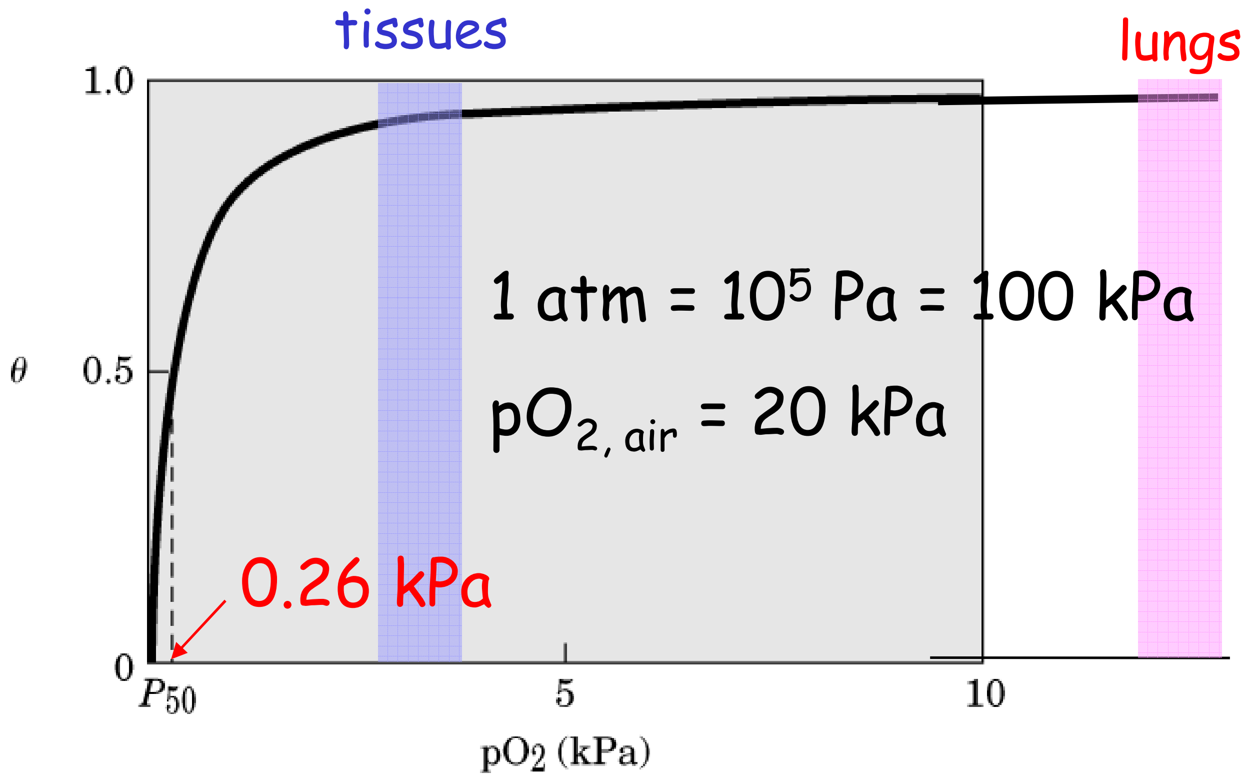
Fig 5-4a, p.156

O_2 binding of Mb

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

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

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



1 atm = 10^5 Pa = 100 kPa

$pO_{2,\,air}$ = 20 kPa

Fig 5-4b, p.156

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

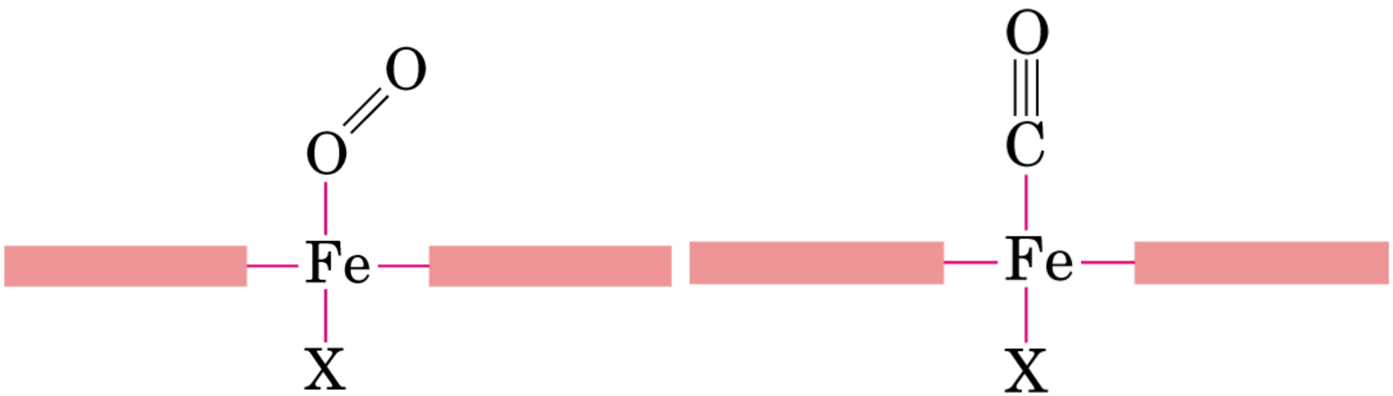


Fig 5-5 a and b, p.158

Mb binding O_2

- Steric hindrance of the distal His (His^{64}) of Mb
  - ✓ H-bond between His^{64} and O_2
- Molecular motion (breathing)
  - ✓ Side chain flexibility allowing O_2 in/out buried cavity

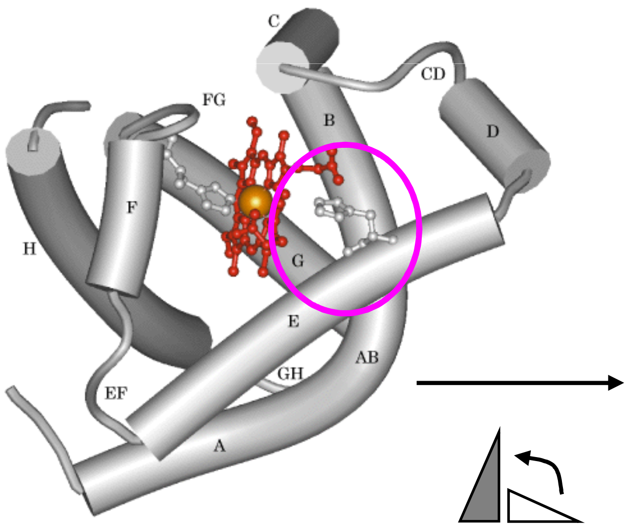
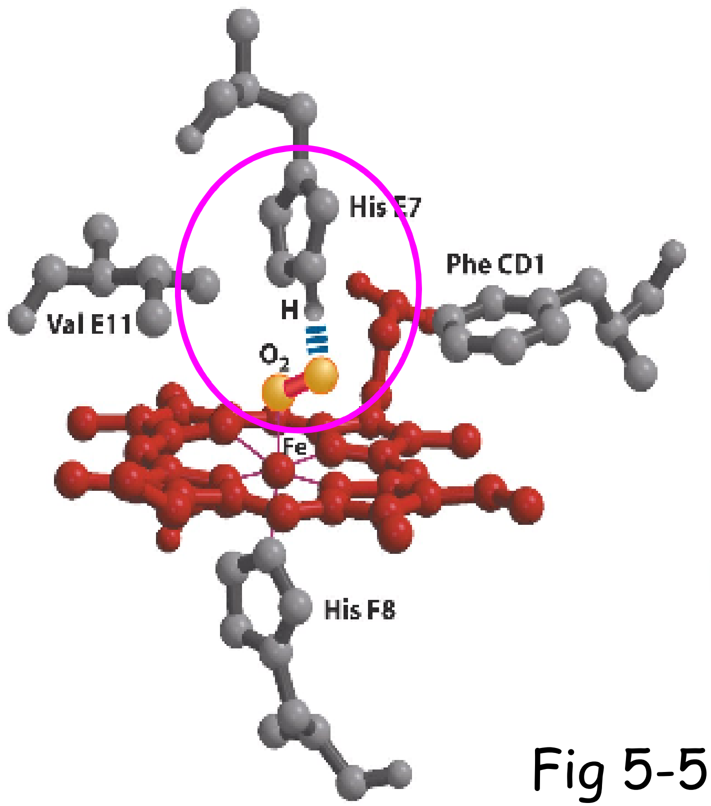


Fig 5-3



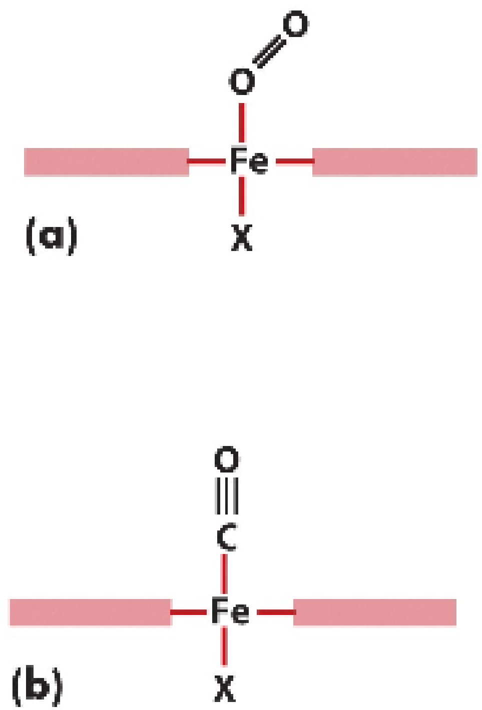


Fig 5-5

Mb vs. Hb

- Sequence homology
- Structure homology

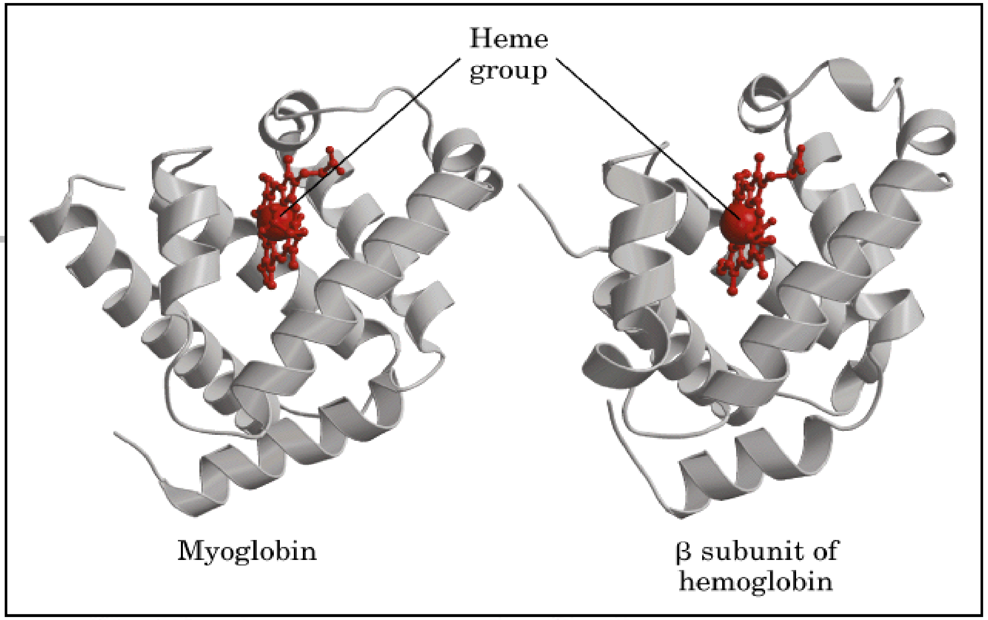
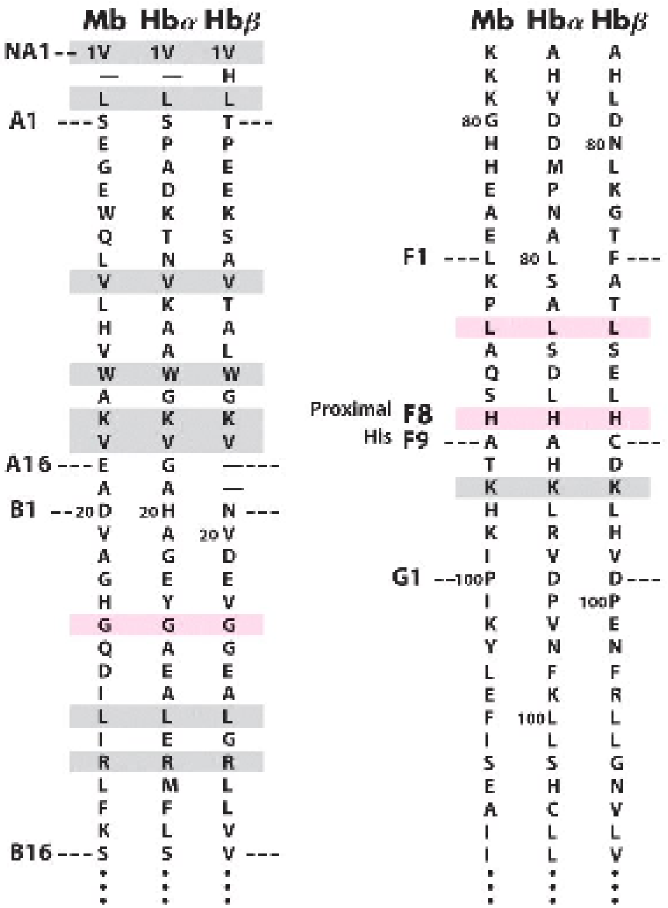


Fig 5-6



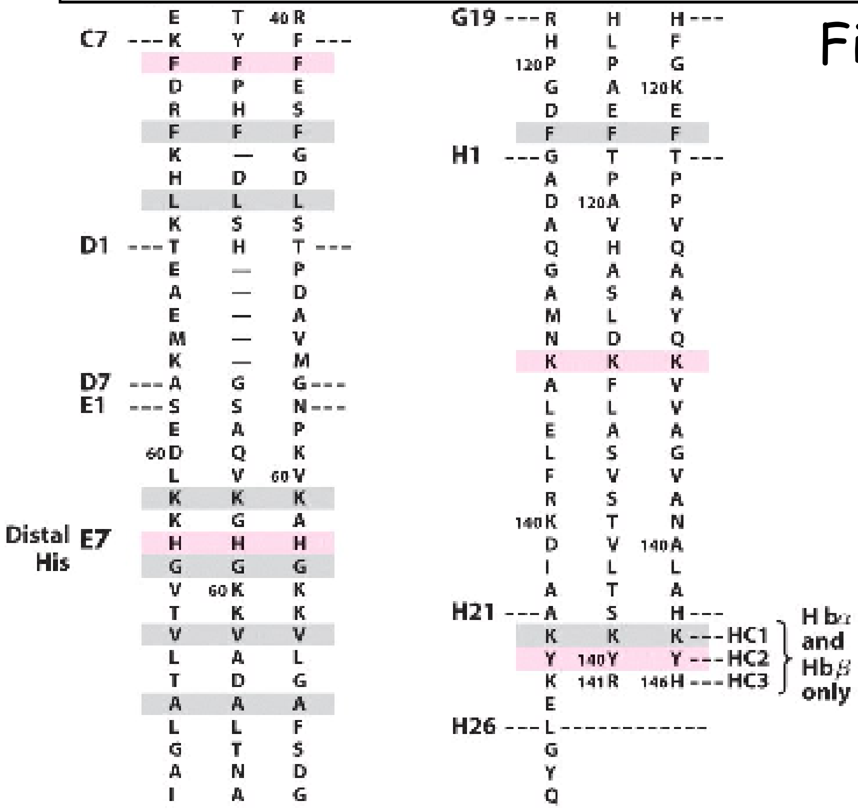


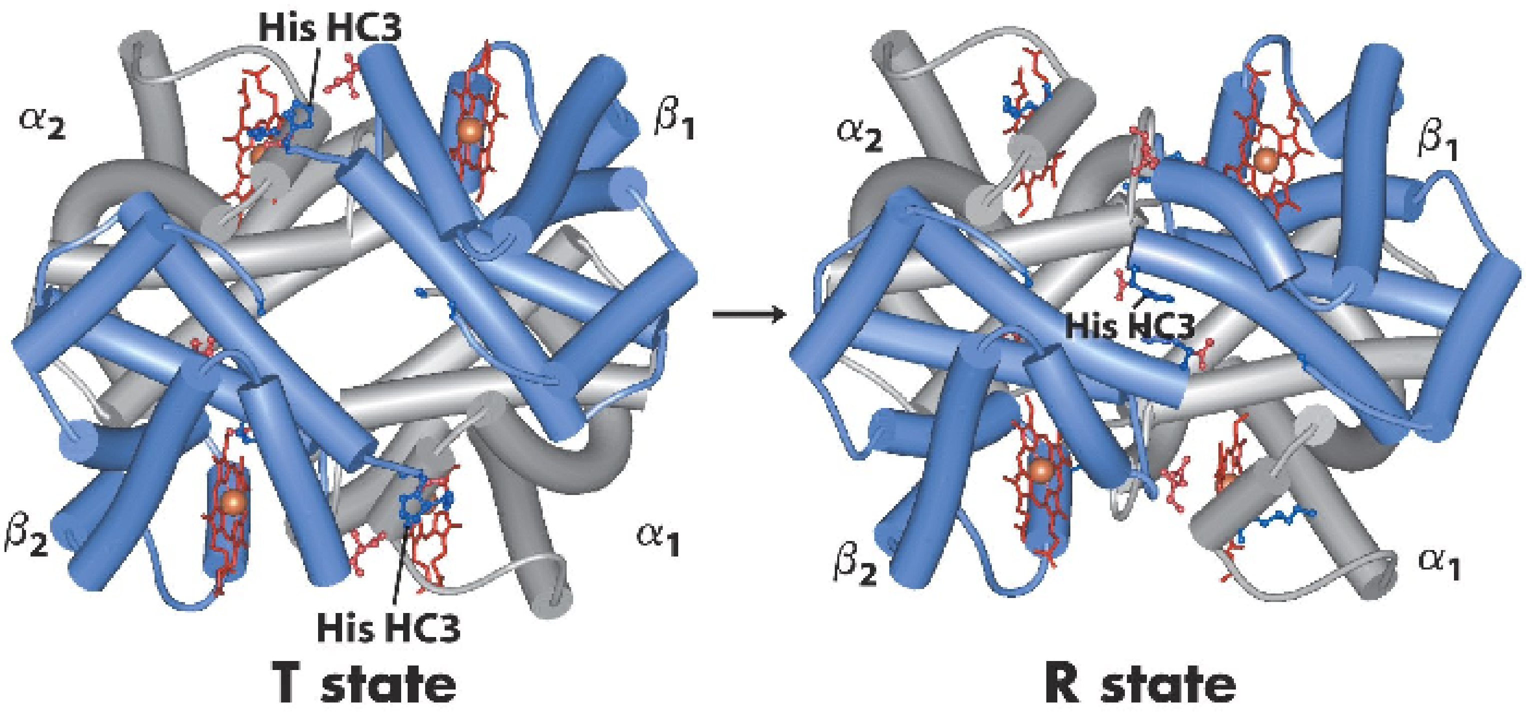
Fig 5-7, p.139

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 5-10, p.161



Hb-O_2 binding curve

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

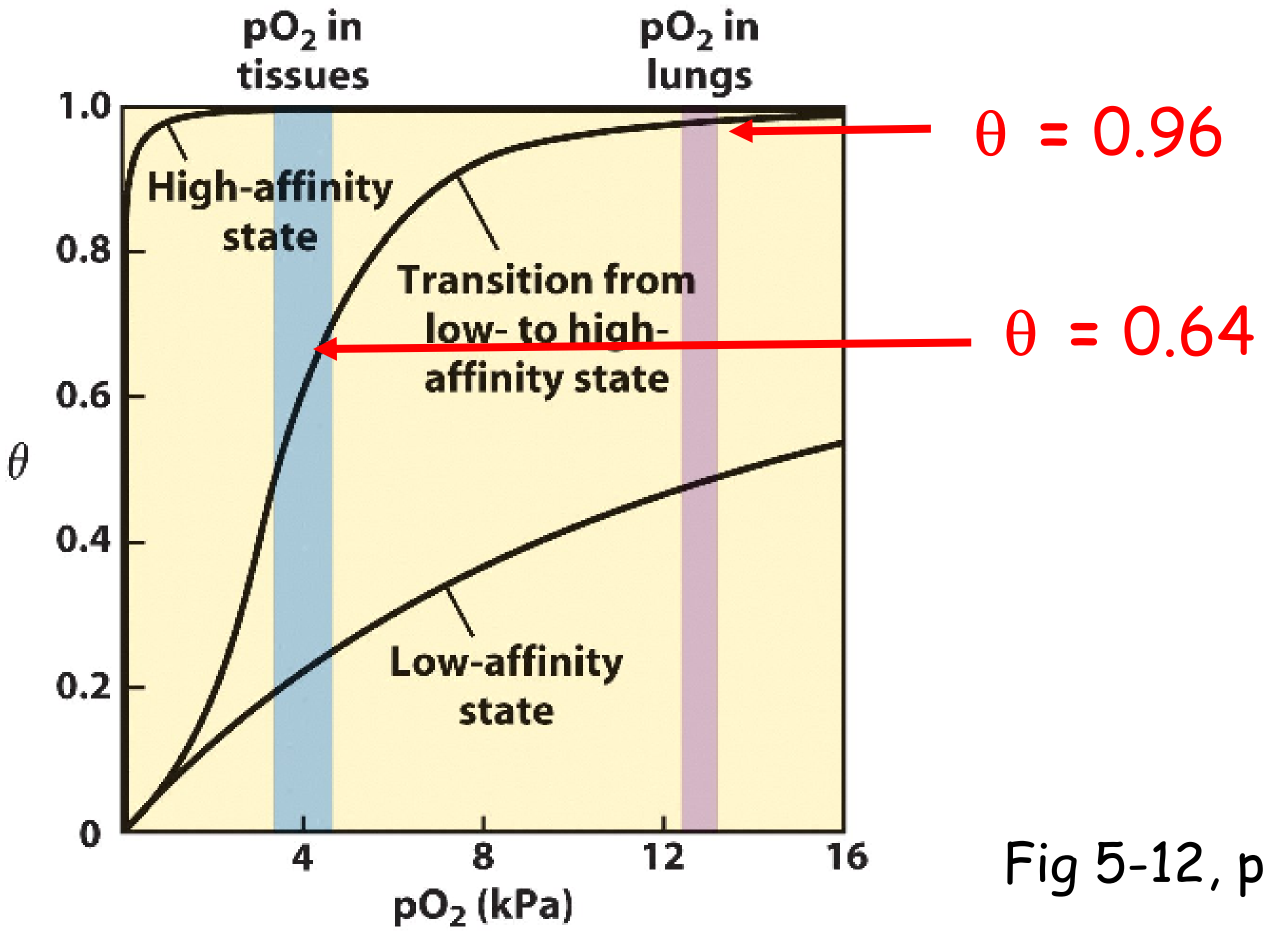


Fig 5-12, p.161

O_2 binding of Mb

- O_2 binds tightly to Mb – good for O_2 storage
- Not sensitive to small changes in pO_2 or [L]

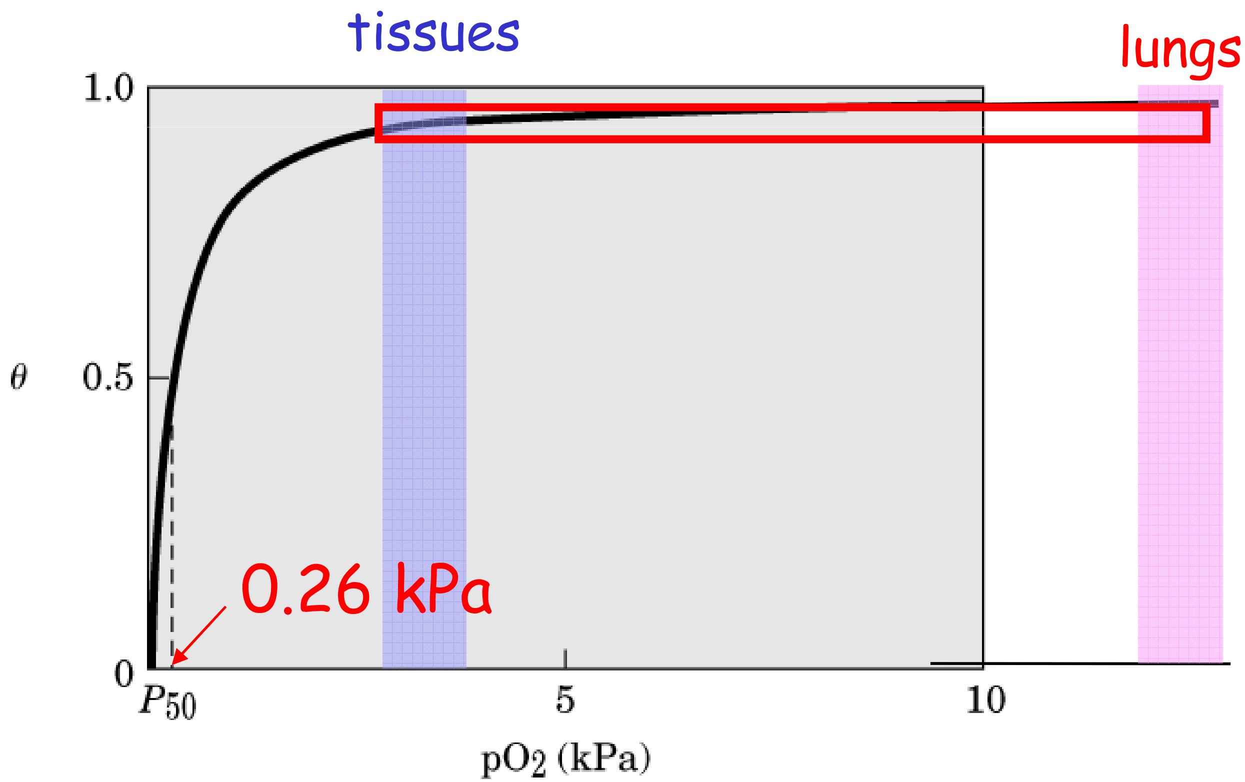


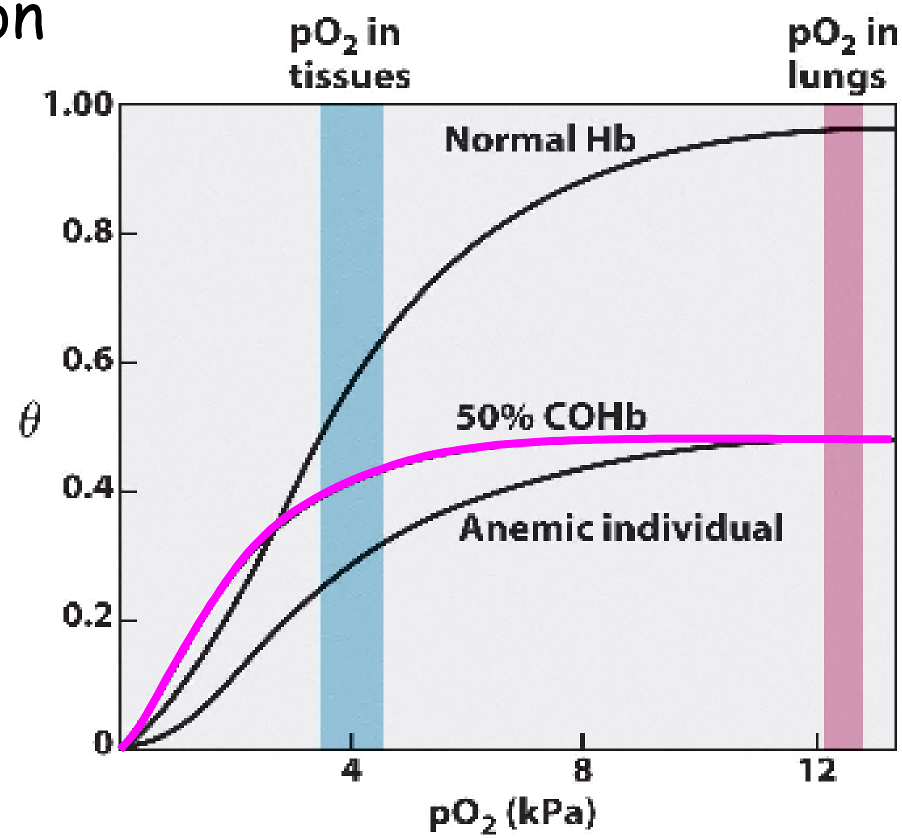
Fig 5-4b, p.156

O_2 binding to Hb

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

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



Quantification

- $P + n\,L \rightleftharpoons PL_n$ p.161~164

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

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

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

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

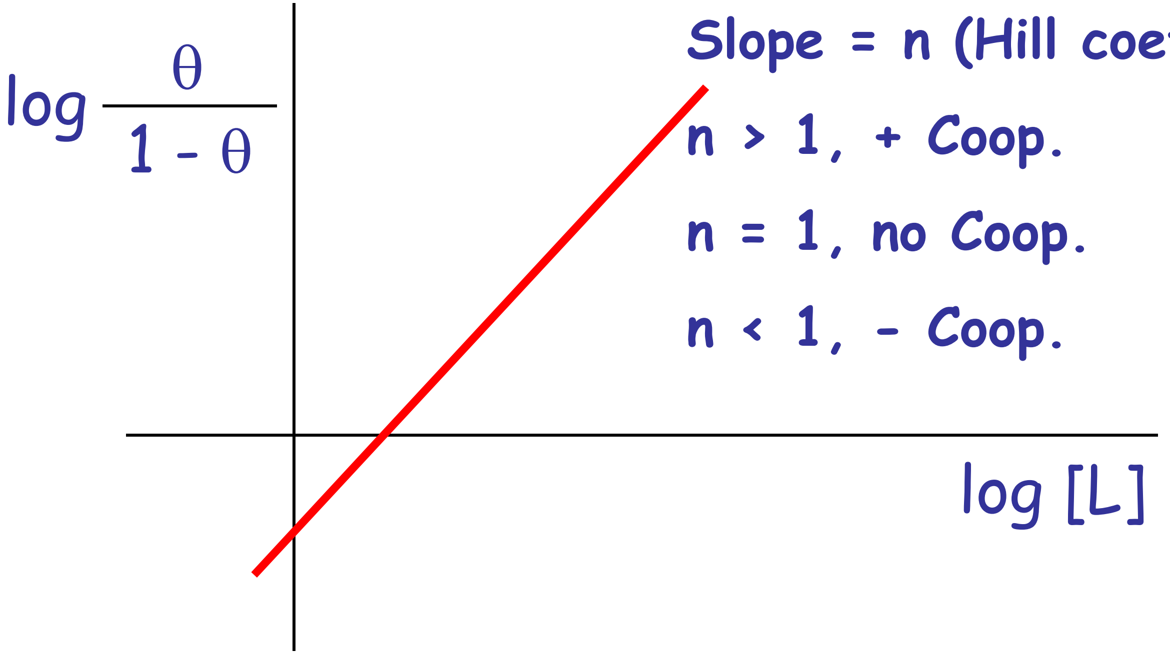
Hill equation

Hill plot

p.161~165

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

$$\log \frac{\theta}{1 - \theta} = n \log [L] - \log K_d \qquad Y = ax - b$$



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

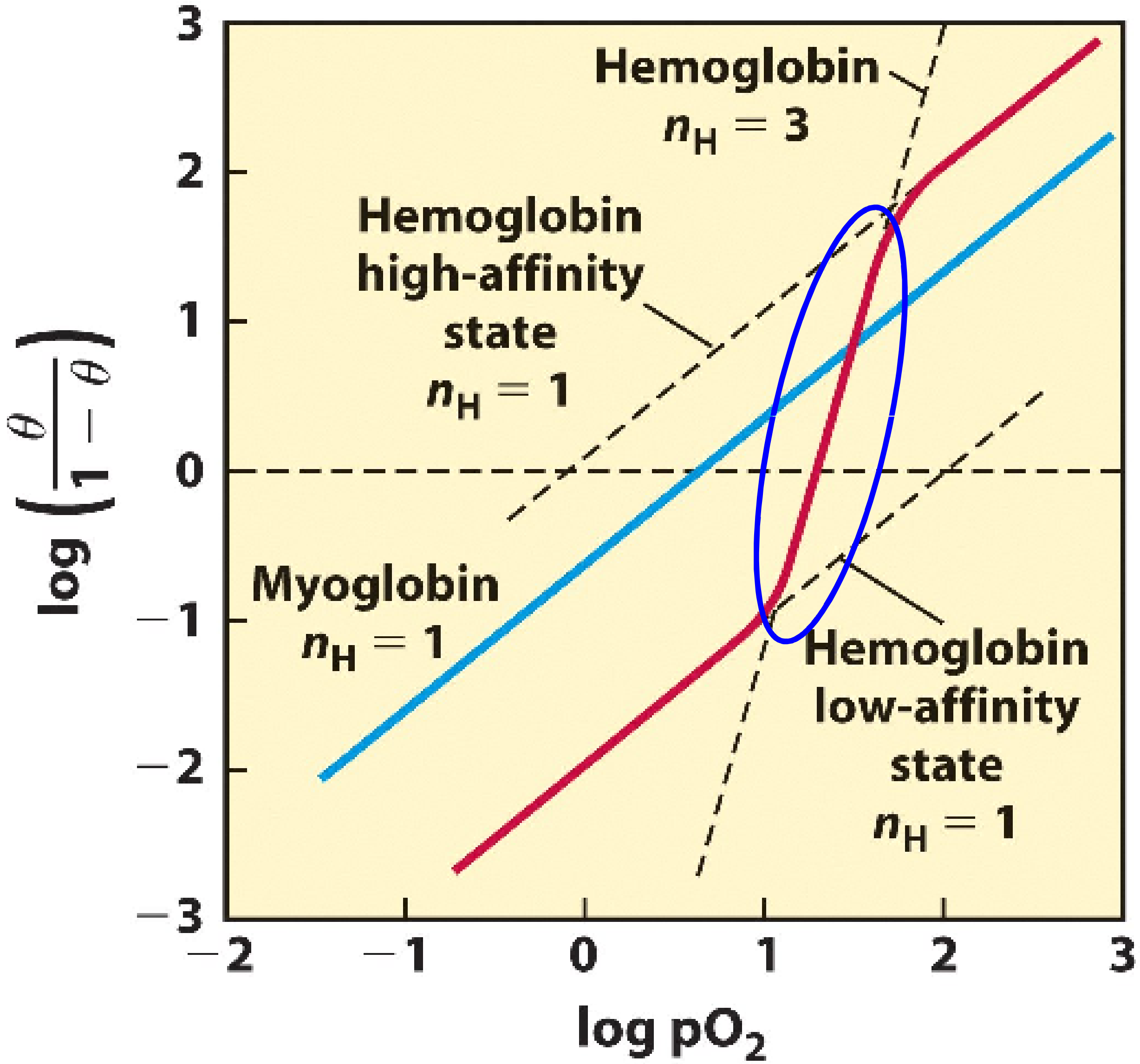


Fig 5-14, p.165

Binding mechanisms (I)

- MWC model (concerted)
  - ✓ Equilibrium
  - ✓ Bind ligand with different affinity
  - ✓ All subunits change at the same time

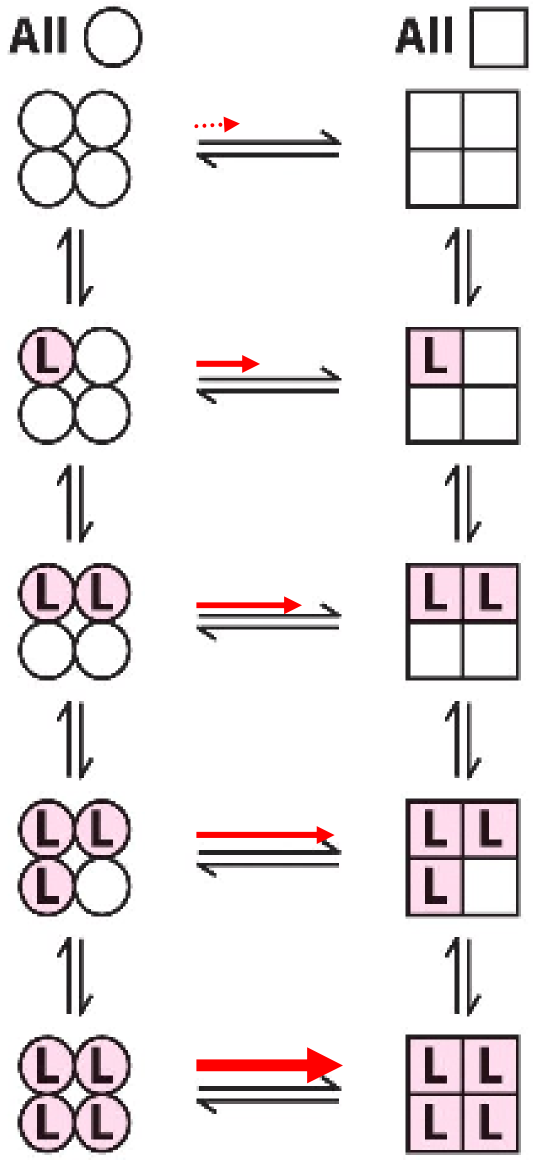


Fig 5-15a, p.165

Binding mechanisms (II)

- Sequential model
  - ✓ Subunits change conformation individually
  - ✓ More intermediate states

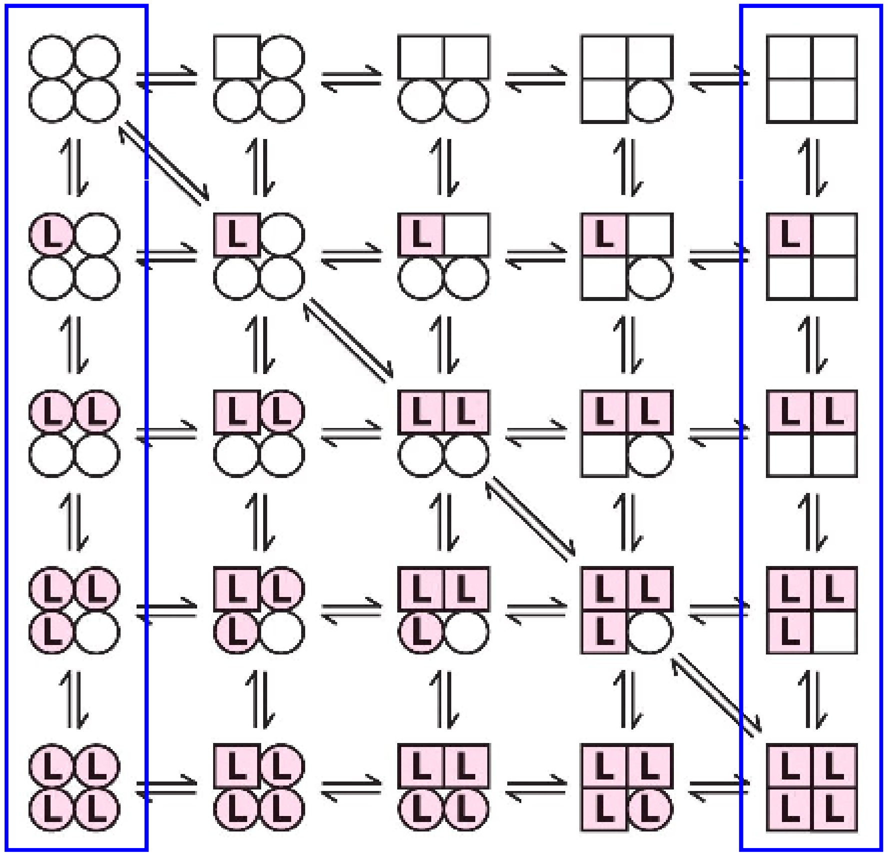


Fig 5-15b, p.165

Hb also transports H^+ and CO_2

$$CO_2 + H_2O \rightleftharpoons H^+ + HCO_3^-$$

Carbonic anhydrase
rich in erythrocytes

- Bohr effect
- pH and CO_2 modulate the affinity of Hb for O_2
  - ✓ 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

p.165

Hb binds H^+ and CO_2

- Hb binds O_2 and (H^+ or CO_2) with inverse affinity
- Hb binds O_2, H^+, and CO_2 at different sites

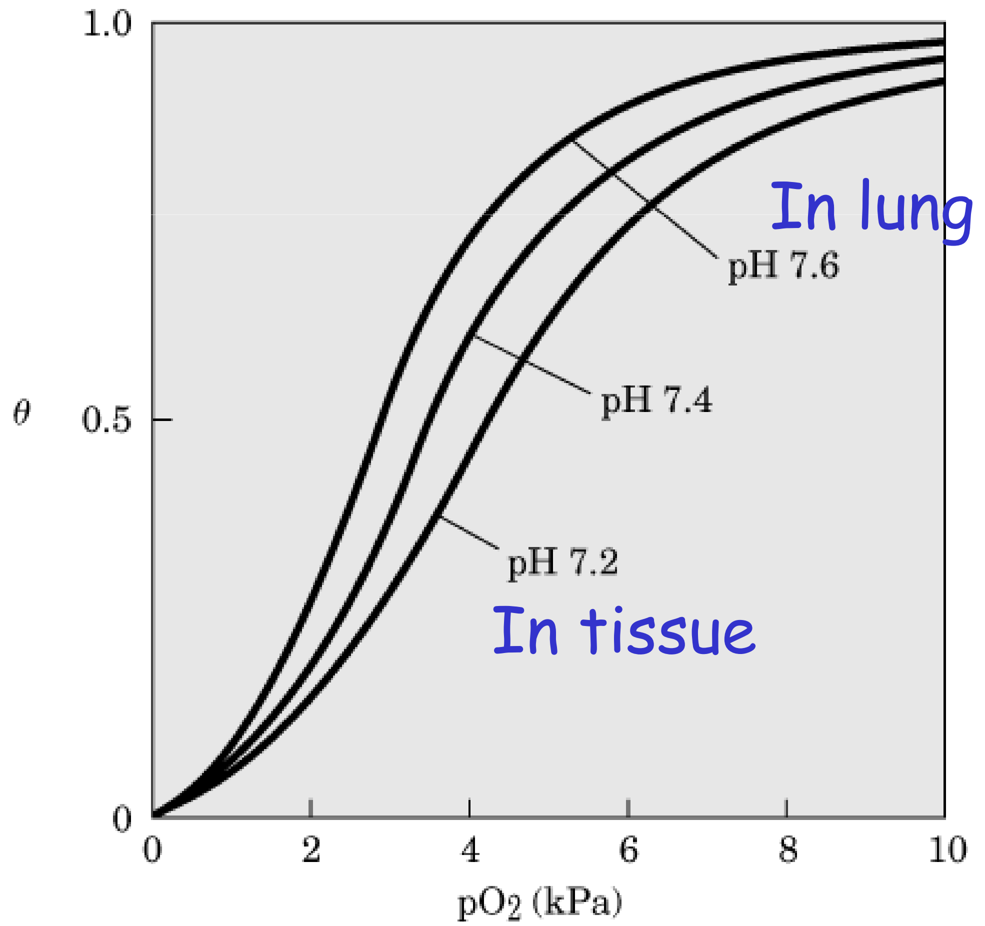
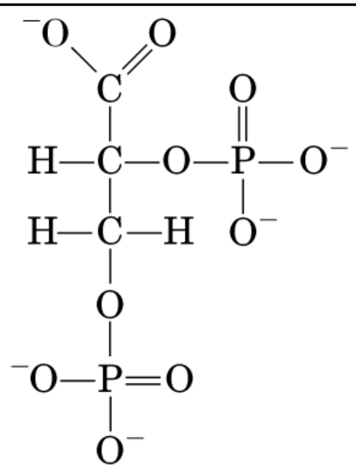


Fig 5-16, p.165

BPG (2,3-bisphosphoglycerate)

- BPG binds Hb and reduce the Hb affinity for O_2
- Blood [BPG] ↑ at high altitude
- Sea level vs. high altitude in O_2 – saturation curve



2,3-Bisphosphoglycerate

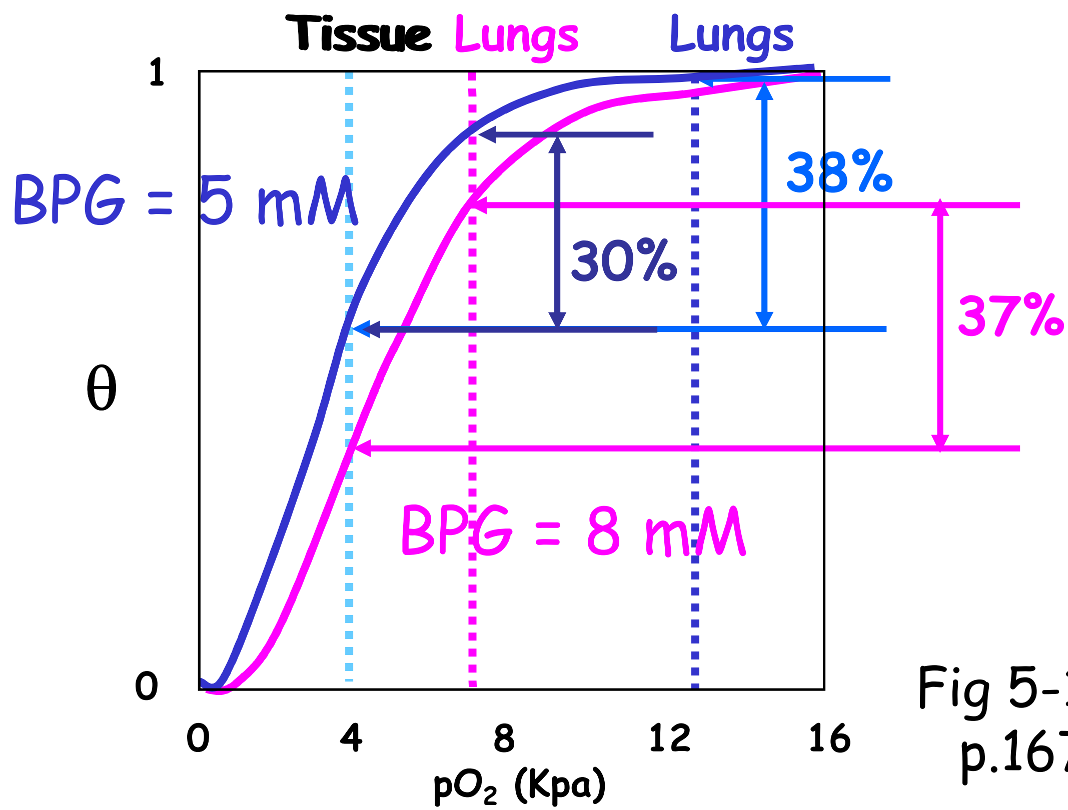


Fig 5-17, p.167

BPG in fetal development

- BPG binds to ⊕ a.a. in the cavity between β subunits in Hb (T state)
- BPG stabilize T state ⇒ O_2 affinity ↓
- Fetal Hb – needs to have a higher O_2 affinity than mother's Hb
- Fetal Hb : $\alpha_2\gamma_2$

T, deoxy-Hb R, oxy-Hb

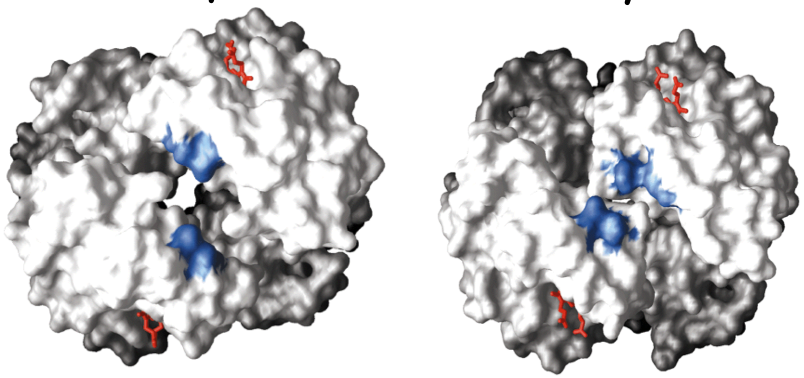


Fig 5-18 b and c, ⊕ group in blue

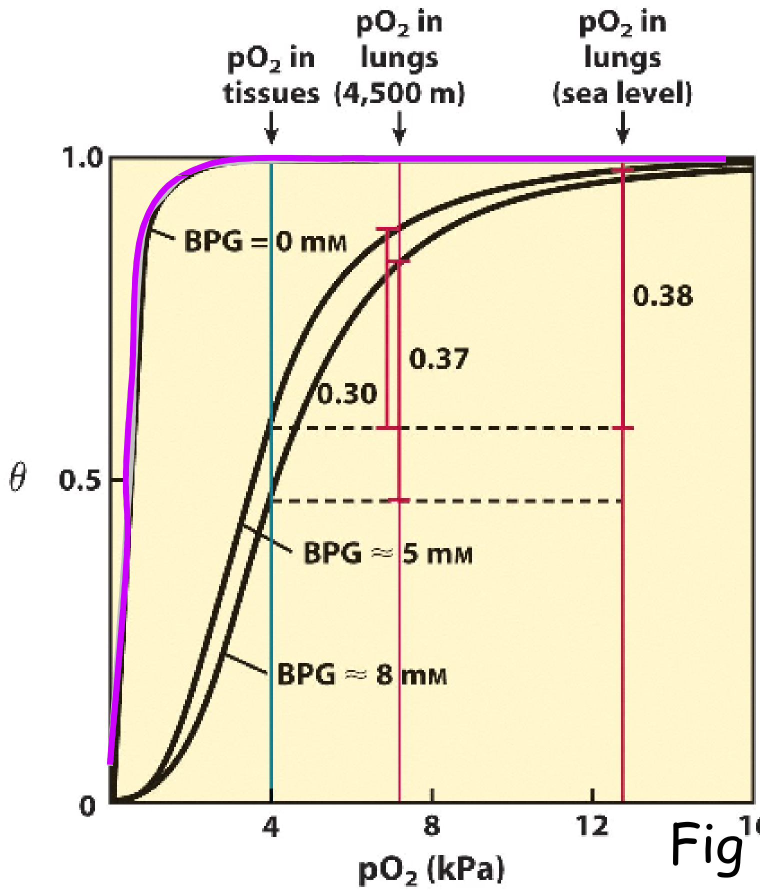


Fig 5-17

Sickle-cell anemia

- Homozygous allele for the β subunit gene
- Fewer and abnormal erythrocytes: sickle blade
- Due to one a.a. in β chains

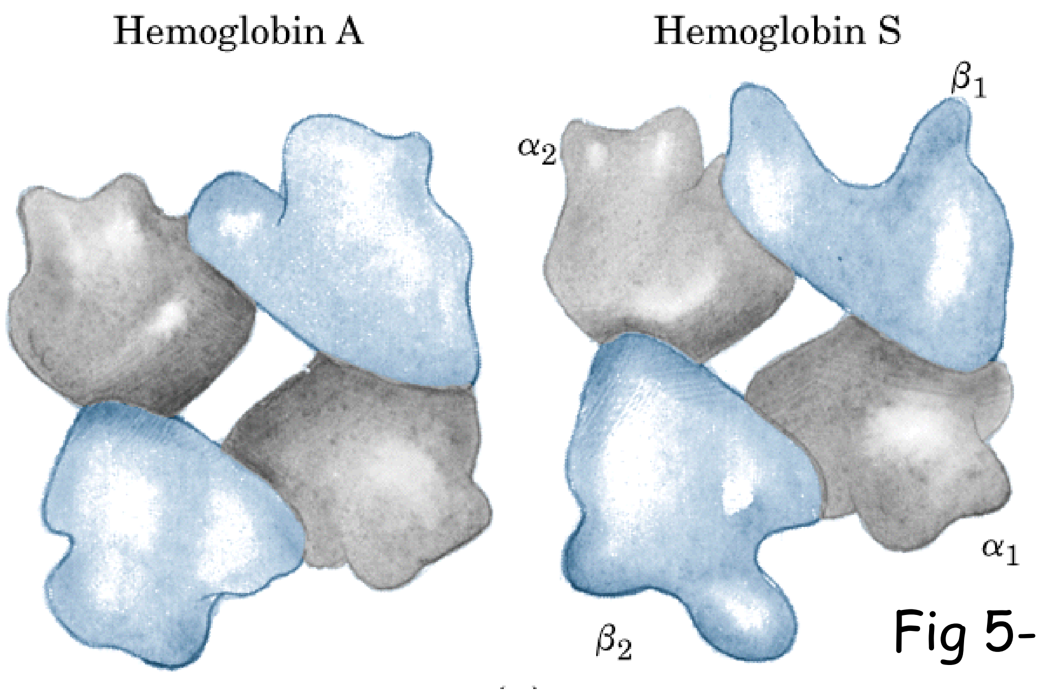


Fig 5-20a, p.169

A single a.a. substitution

- Hb A (Glu^6) vs. Hb S (Val^6) on β subunits surface
- "Sticky" hydrophobic contacts
- deoxyHb S: insoluble and form aggregates

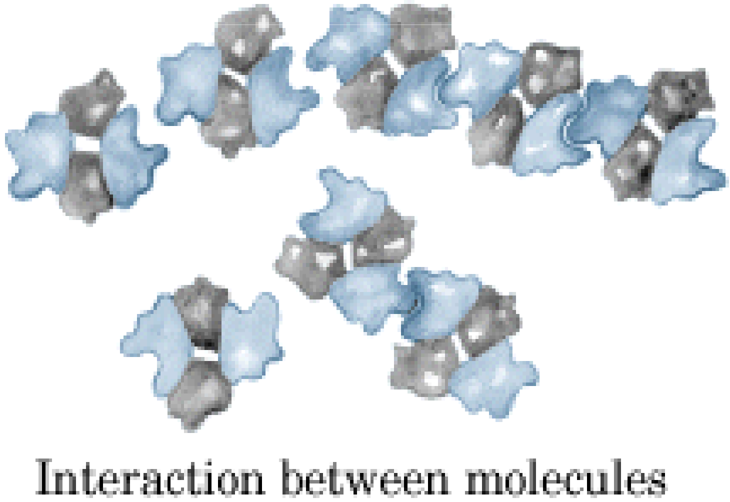
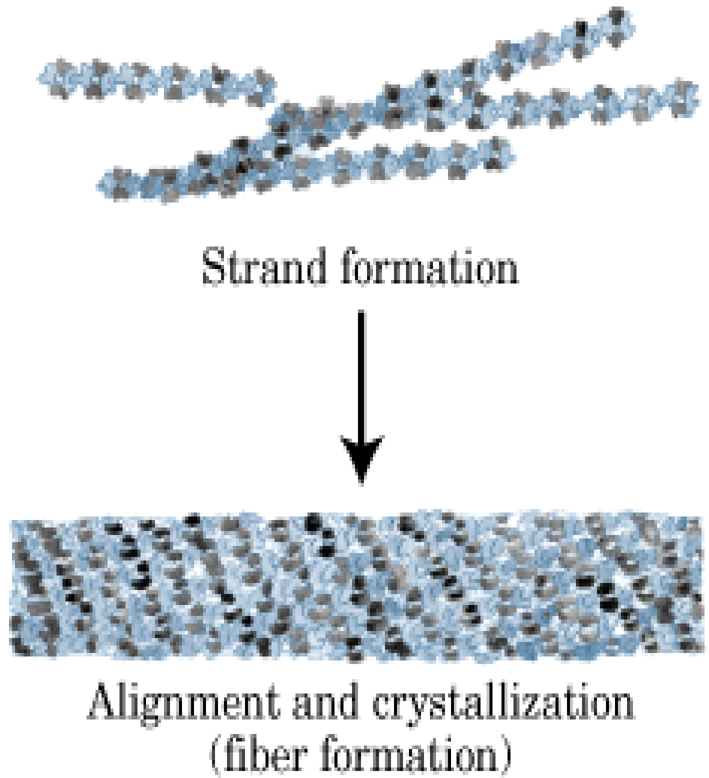


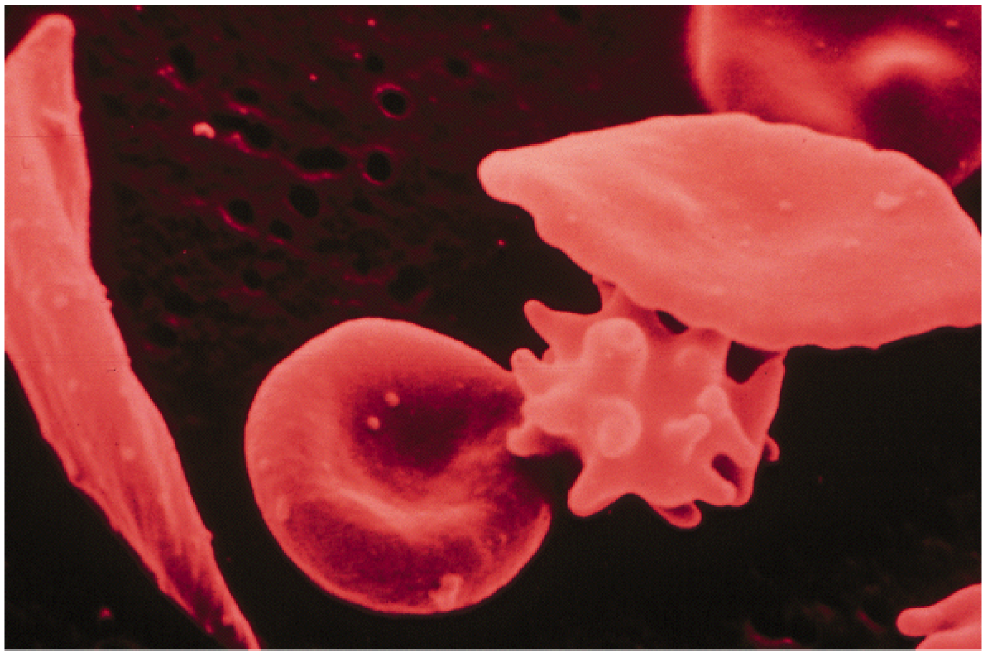
Fig 5-20b, p.169



Natural selection

- Homozygous: anemia, blocked capillaries
- Heterozygous: malaria resistance
- Anemia or Malaria ?

Time ?



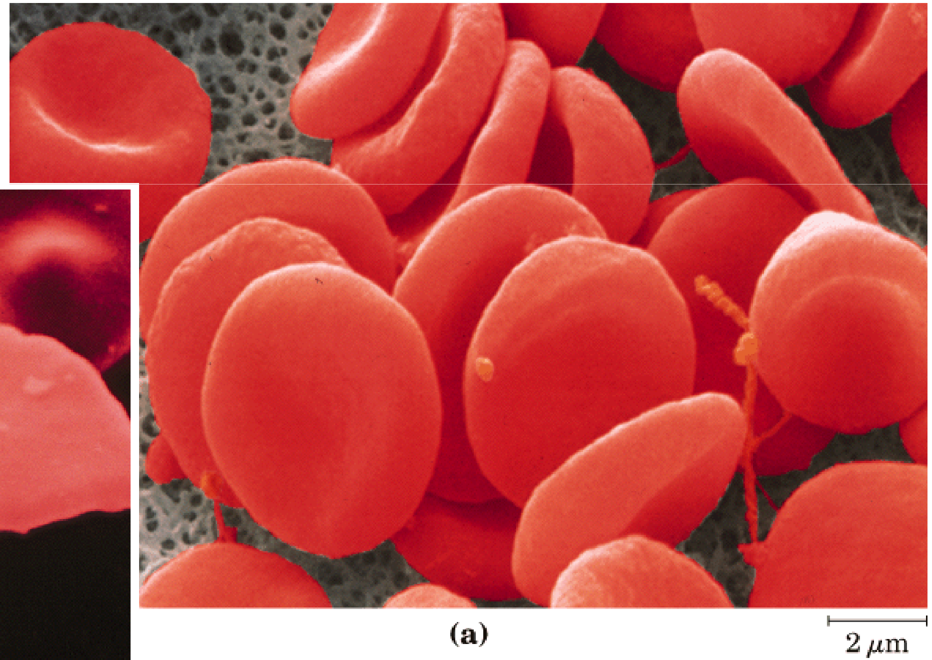


Fig 5-19, p.168

Summary

- O_2 binding protein: Mb and Hb
- Protein-ligand interactions
  - Affinity and K_d
  - Cooperativity, Hill plot
  - Allosteric protein
    - Homotropic and heterotropic modulators
- Problems: 1, 3, 4, 5, 6, 7, 8