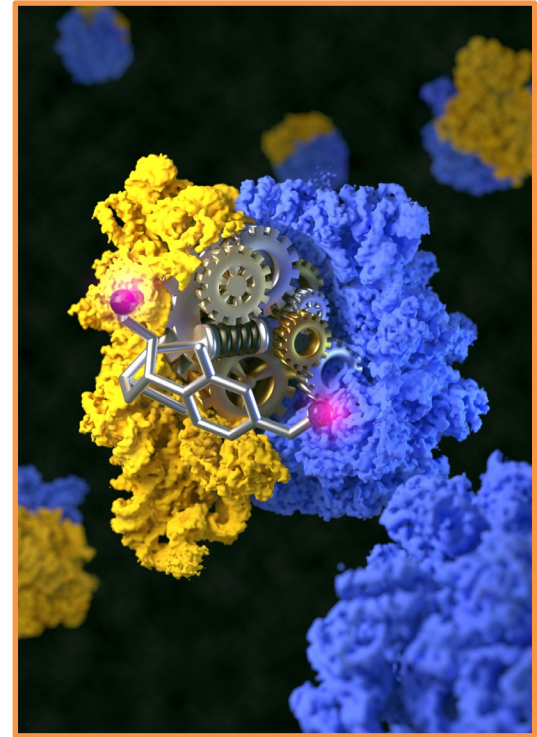
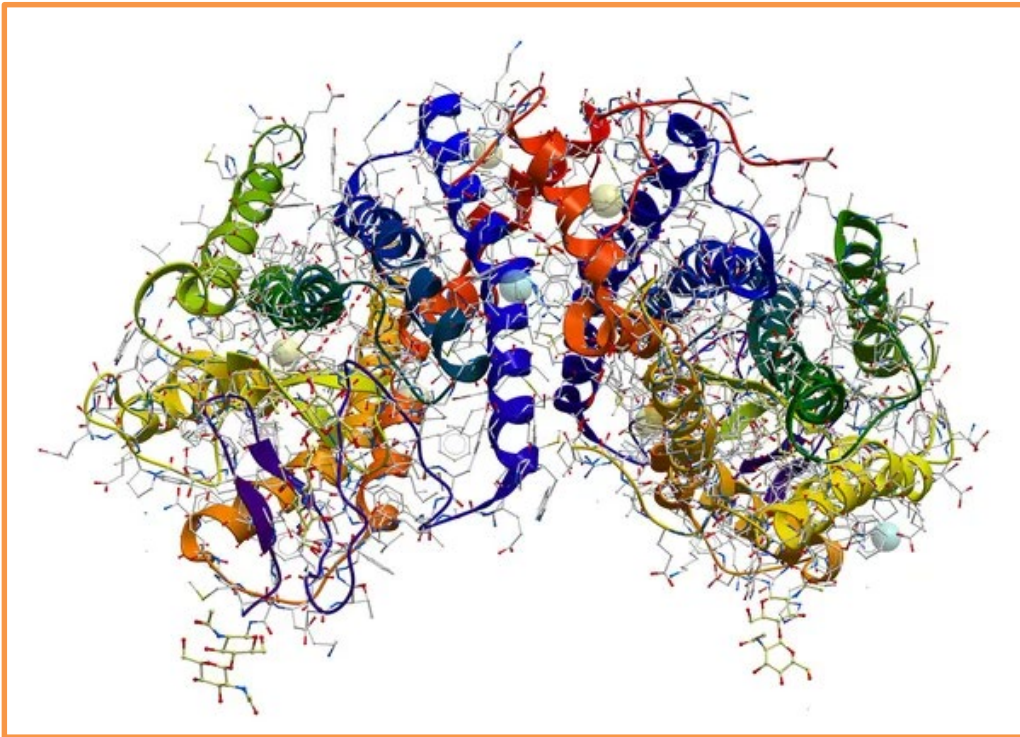


Lecture 5: Structures and Functions of Proteins II



Kwok-On LAI
Department of Neuroscience

Learning Outcomes

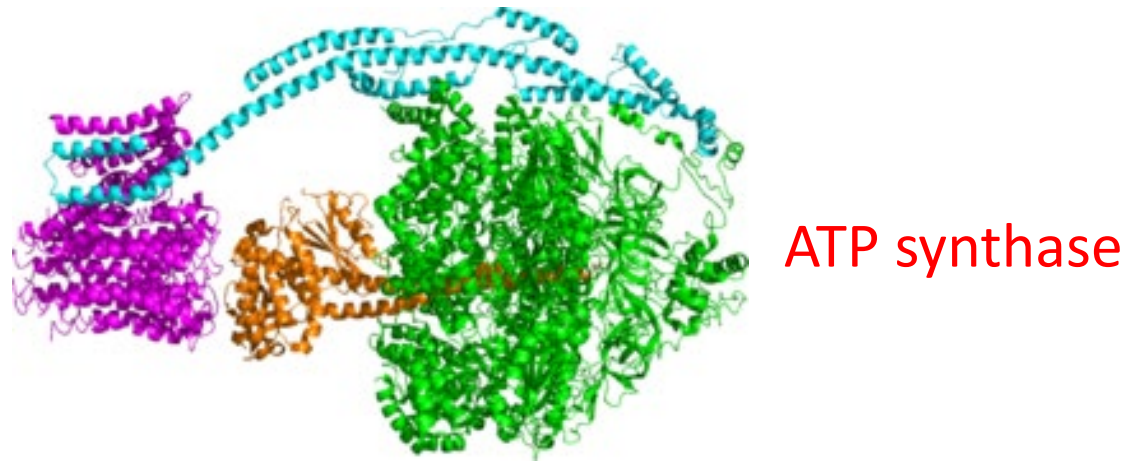
- To define the basic concepts in protein binding
- To describe the structure of heme-binding pocket and reversible binding of hemoglobin to its ligand (oxygen)
- To explain the regulation of binding between oxygen and hemoglobin
- To recognize hemoglobin-related diseases

Basic concepts in protein interactions

- **Ligand**: a molecule bound reversibly by a protein (a ligand can itself be a protein, or another type of molecule); the binding is very specific
- **Binding site**: the site where the ligand binds; it is complementary to the ligand in size, shape, charge and hydrophobic or hydrophilic character
- **Induced fit**: the structure adaptation between protein and ligand upon their binding; involves conformational change of the protein.
- In a multi-subunit protein, a conformational change in one subunit may affect the conformation of other subunits

Basic concepts in protein interactions

What if the protein is an enzyme?



- The ligand is the substrate (what's the difference between a ligand and a substrate?)
- The binding site is the catalytic site

A protein can have multiple binding partners

An example: p53

An algorithm
to predict
disordered
structure

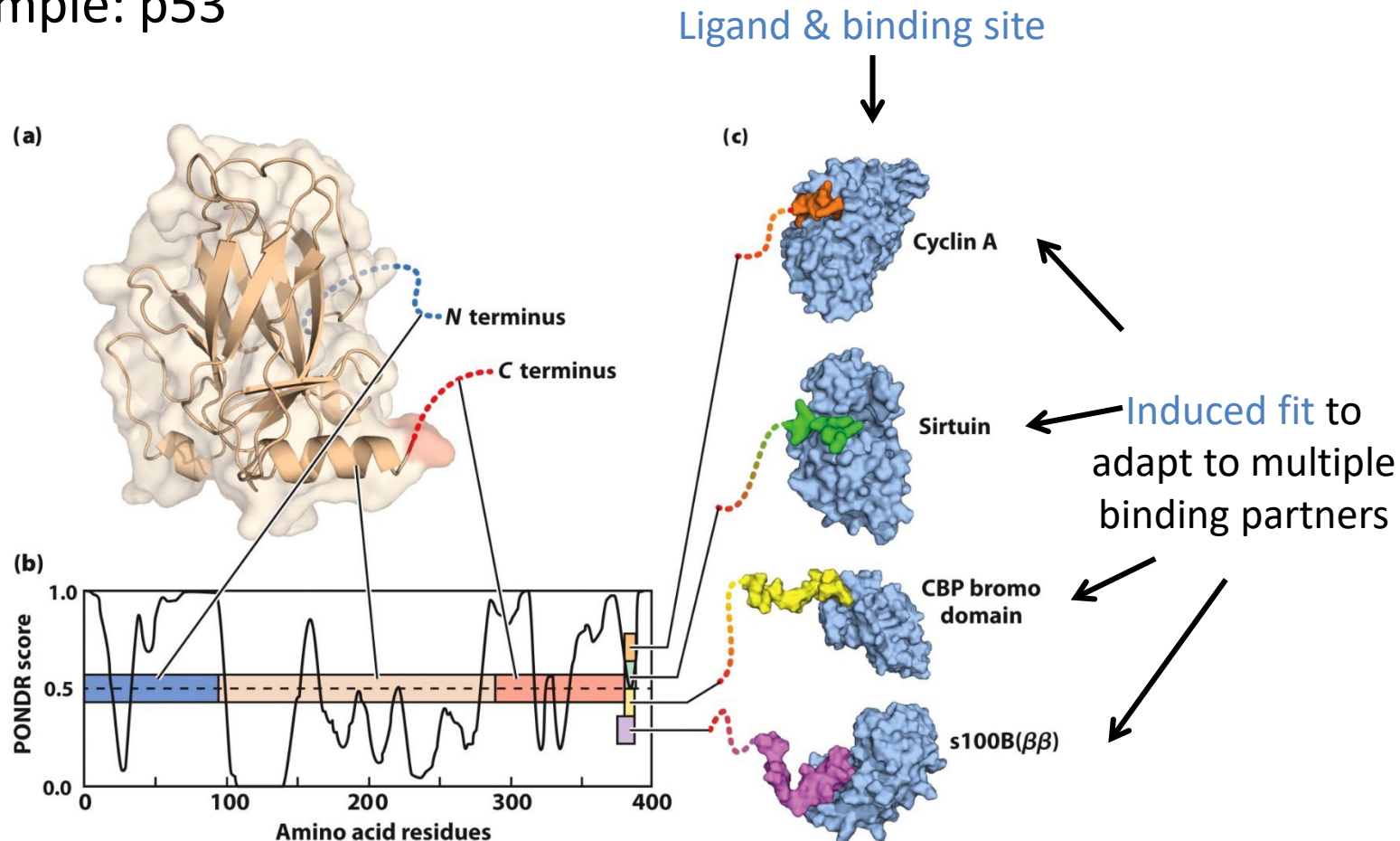
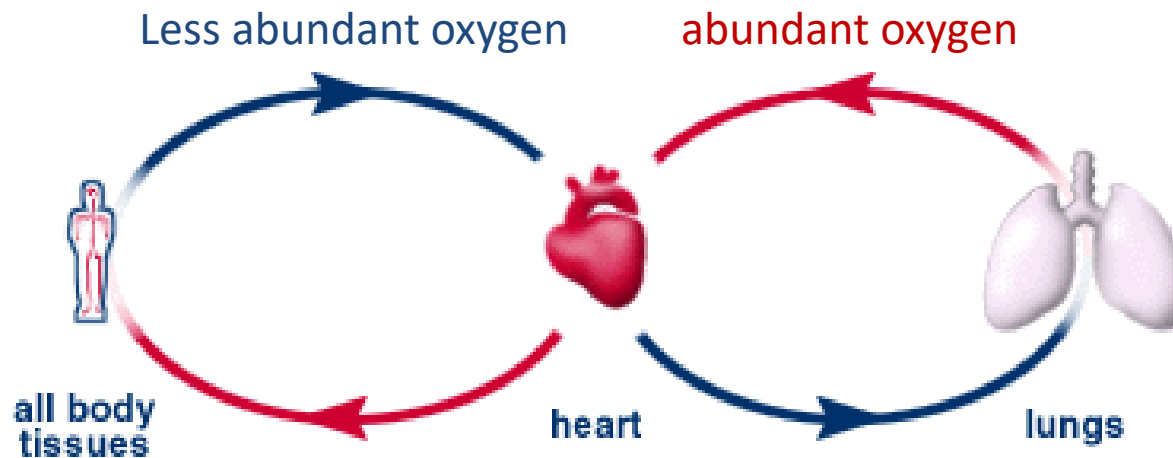


Figure 4-24
Lehninger Principles of Biochemistry, Sixth Edition
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- The C-terminus is structurally disordered and flexible
- Adopt different structures when binds to different ligands

The blood oxygen circulation system



Globin proteins – Carriers of oxygen (*why need a carrier?*)

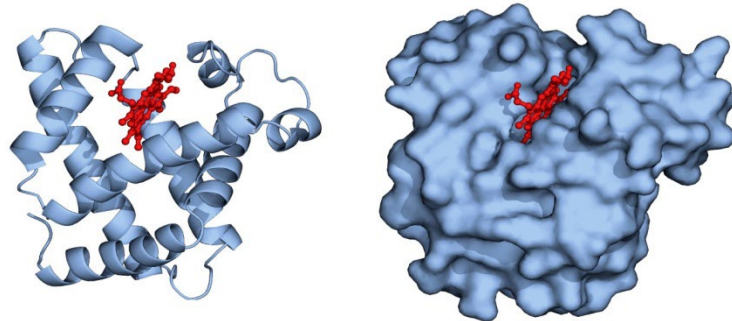
- Myoglobin: found in the muscle tissue in almost all mammals (e.g. Whale); as the storage for oxygen
- Hemoglobin: carried by erythrocytes (red blood cells); as the transporter of oxygen



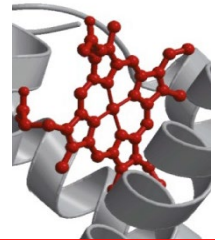
➤ They were the first proteins for which three-dimensional structures were determined

- Myoglobin

- one subunit
- one binding pocket
- 16,700 Da
- consists of 8 α -helices (labelled A-H)

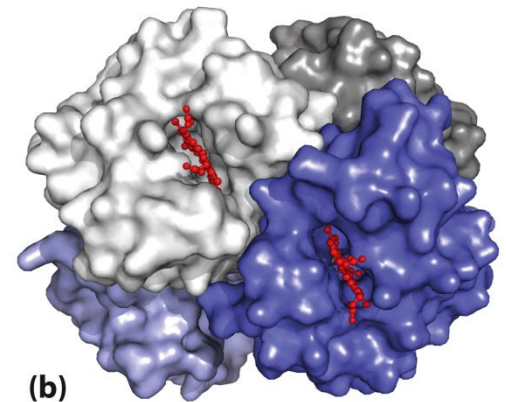
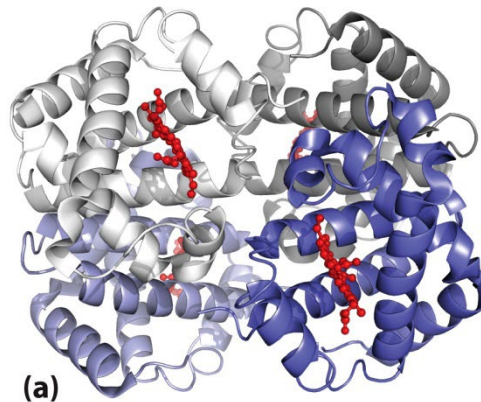


All contain Heme-Fe-O₂ binding pocket



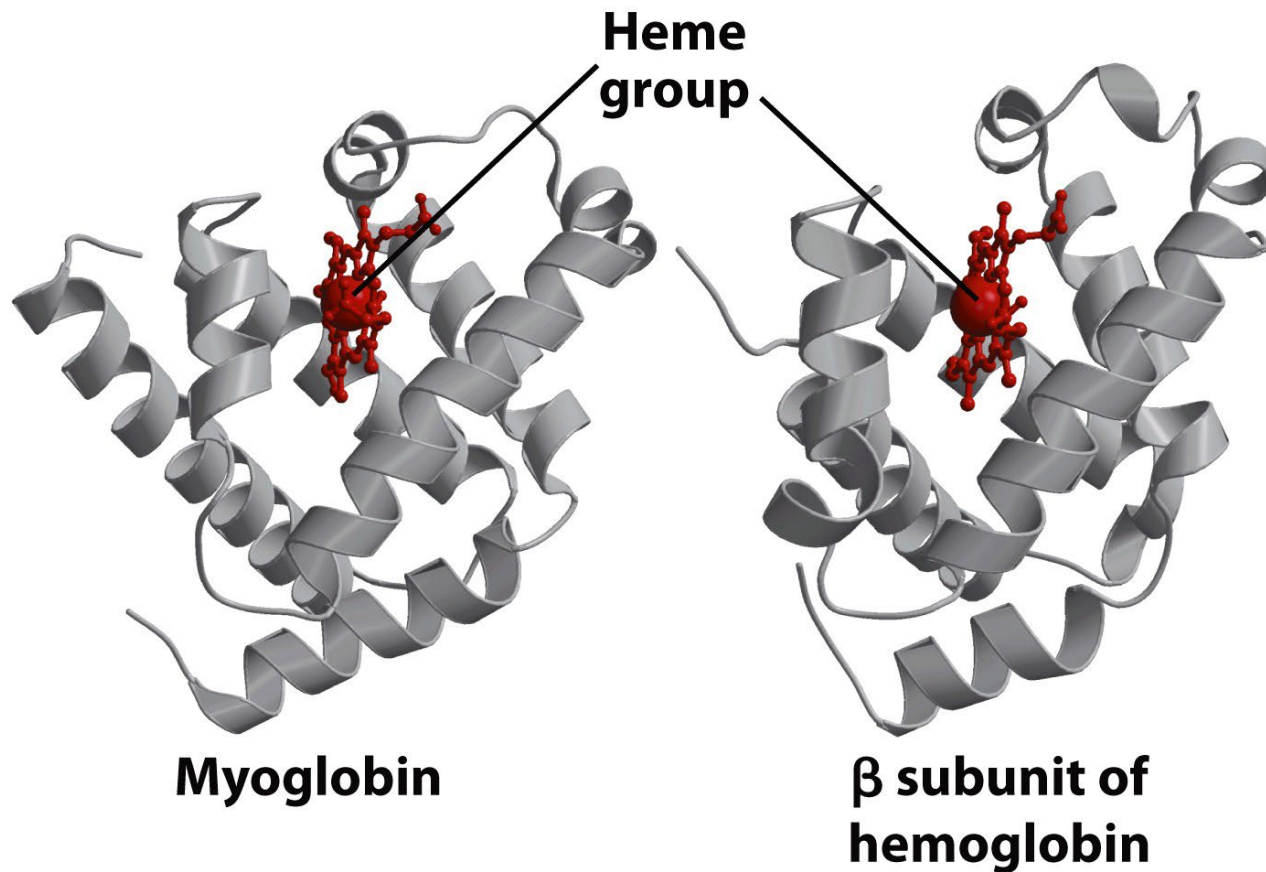
- Hemoglobin

- tetramer (four subunits)
- two α subunits (α 1, α 2) and two β subunits (β 1, β 2)
- four binding pockets
- total 64,500 Da



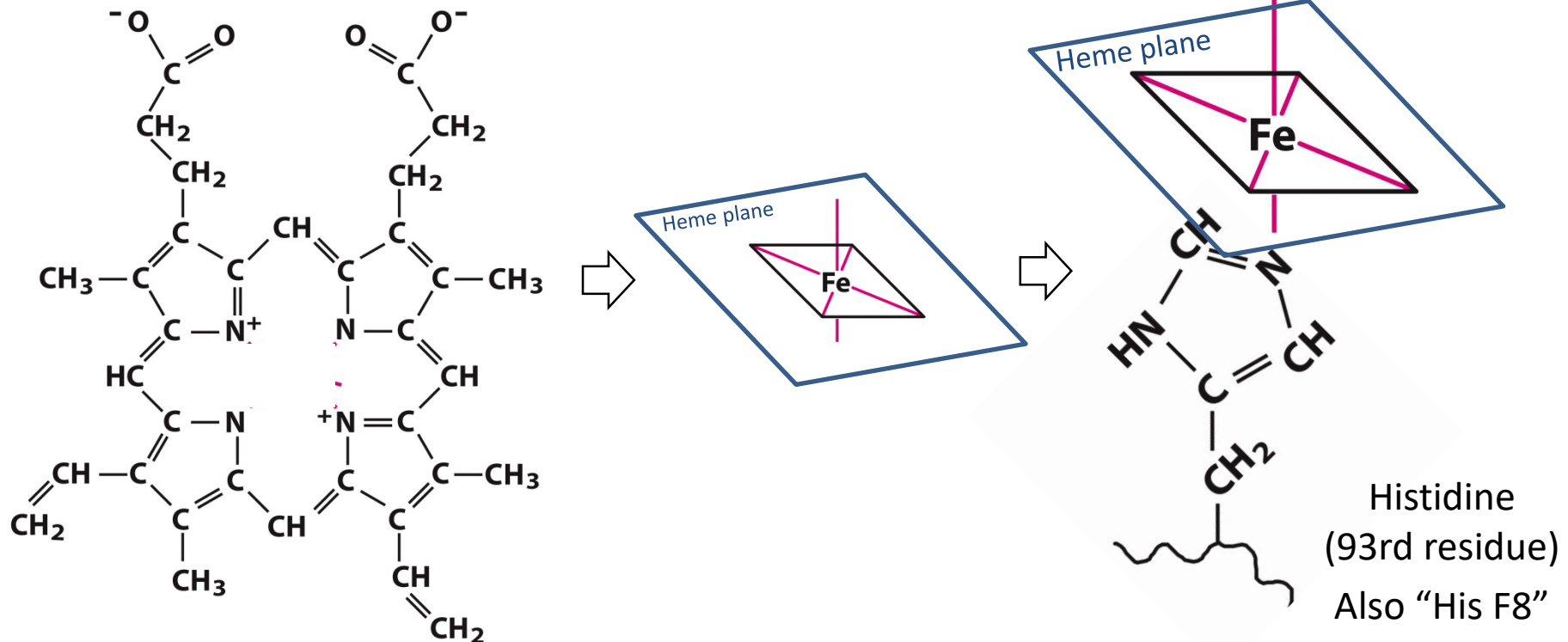
Similar structures between myoglobin and the subunit of hemoglobin

The primary sequence is less similar (only 30%-50% identity)



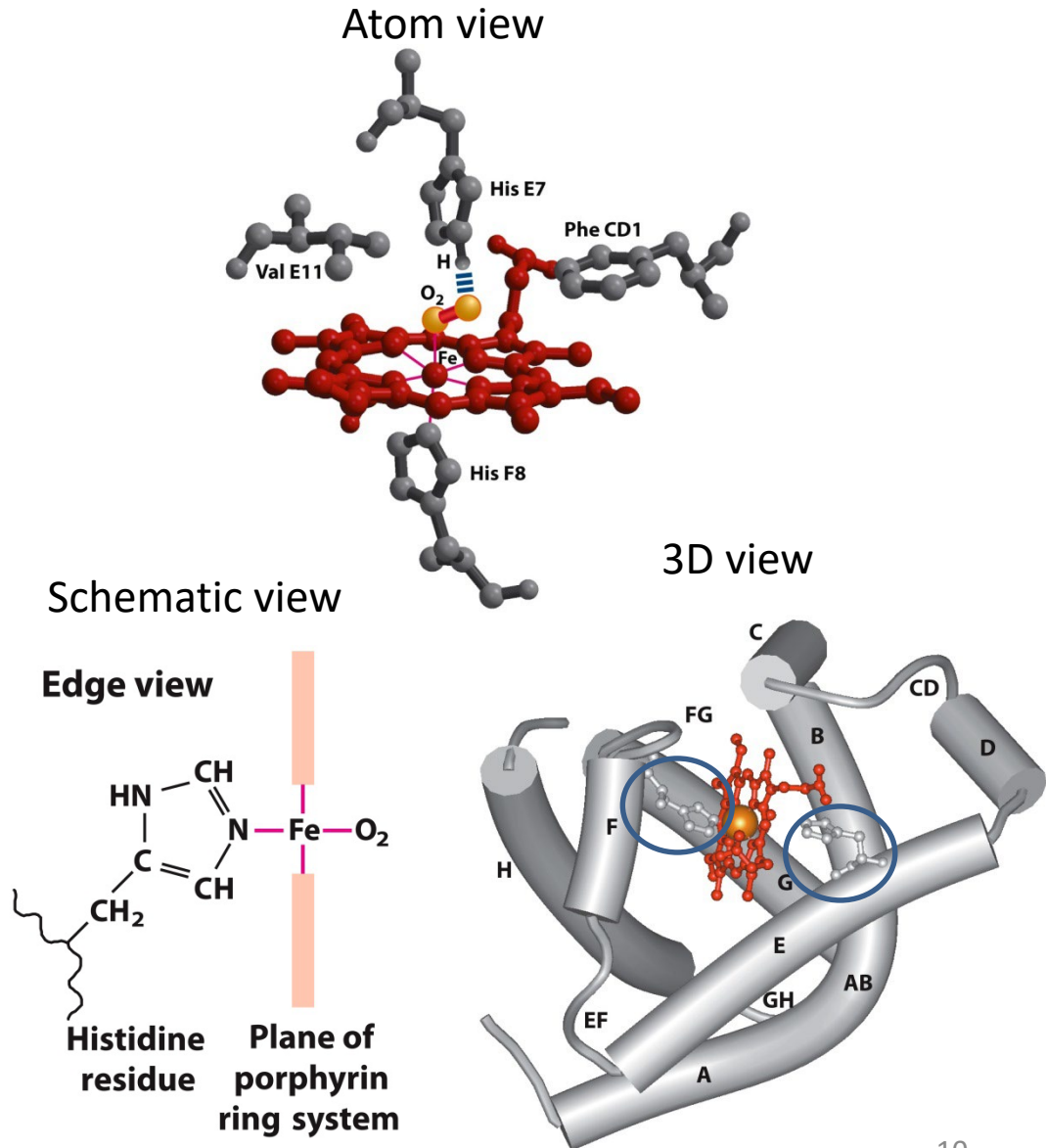
A simple model for ligand binding: Myoglobin-Heme-Fe-O₂

- Heme: protoporphyrin ring (forms a plane) binds to iron atom in its ferrous (Fe²⁺) state
- Four nitrogen atoms take four coordination bonds of Fe to maintain the Fe²⁺ state



Binding pocket for Heme-Fe-O₂

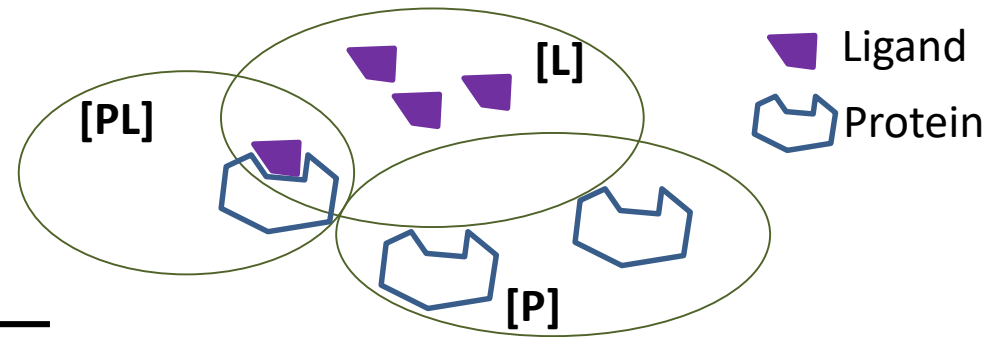
- O₂ could have a reversible coordination bond with Fe²⁺ in myoglobin
- Binding of O₂ to Fe²⁺ and Heme is stabilized by His F8 (by a coordination bond of Fe) and His E7 (by a hydrogen bond) of myoglobin



Quantitative description of protein-ligand binding

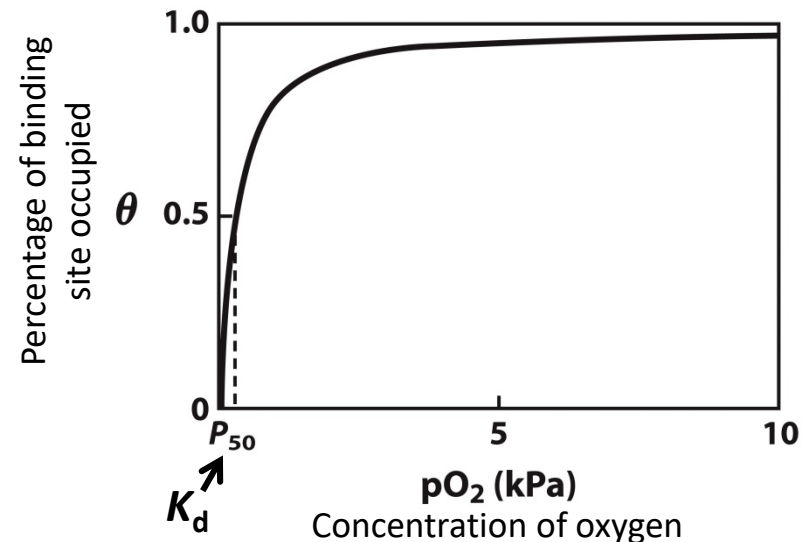
- θ : Percentage of binding site occupied

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

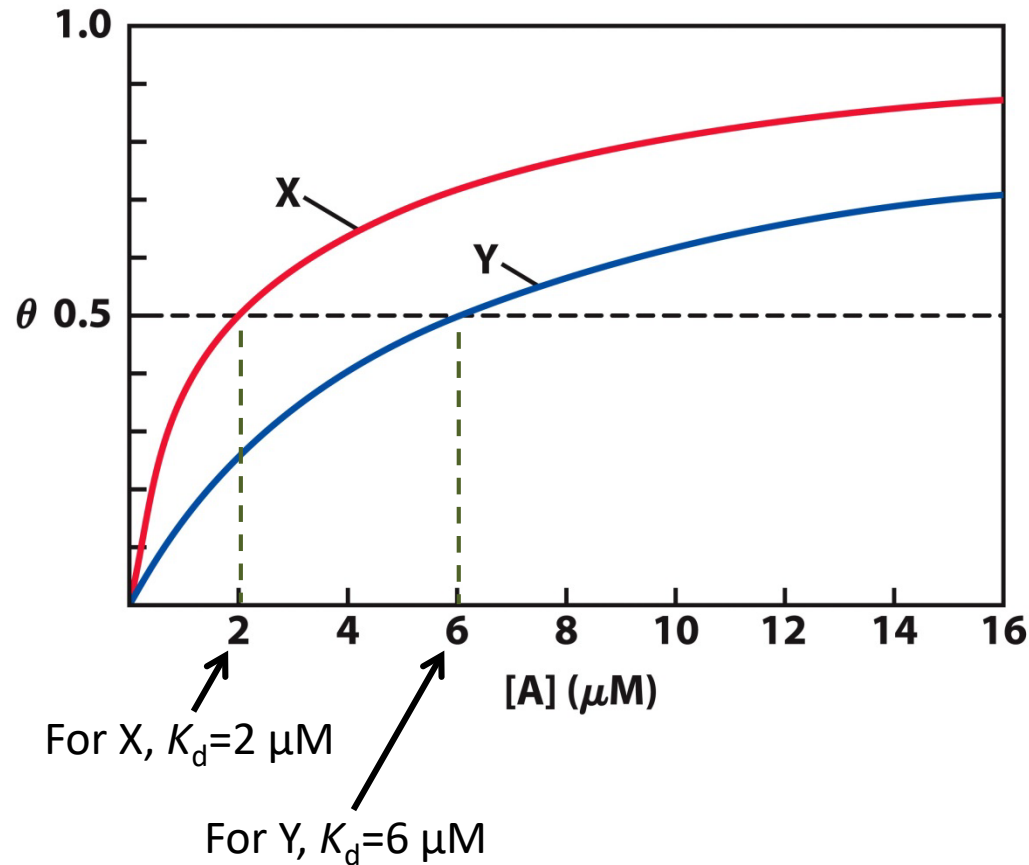


- K_d dissociation constant
- K_d equals to the concentration (in molar) of the ligand, at which half of the binding sites are occupied by the ligand ($\theta = 0.5$)

Binding curve of oxygen to myoglobin



What are the K_d of these two ligands?

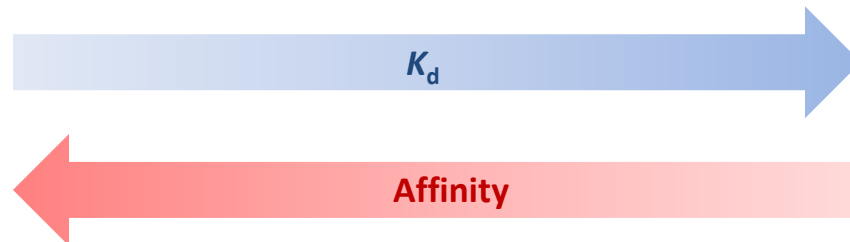
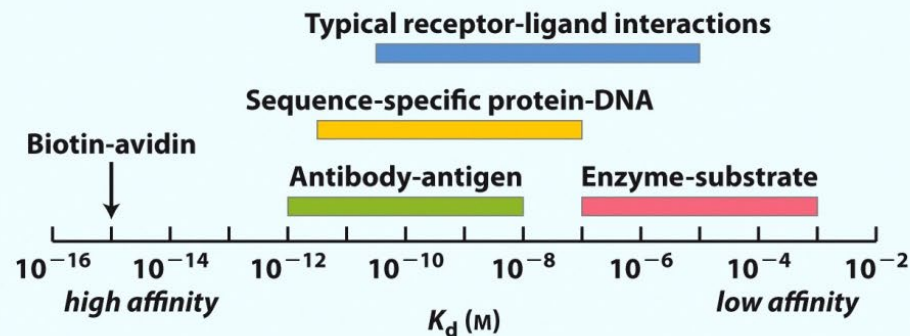


Which ligand has higher affinity?

K_d and the binding affinity

TABLE 5-1 Some Protein Dissociation Constants

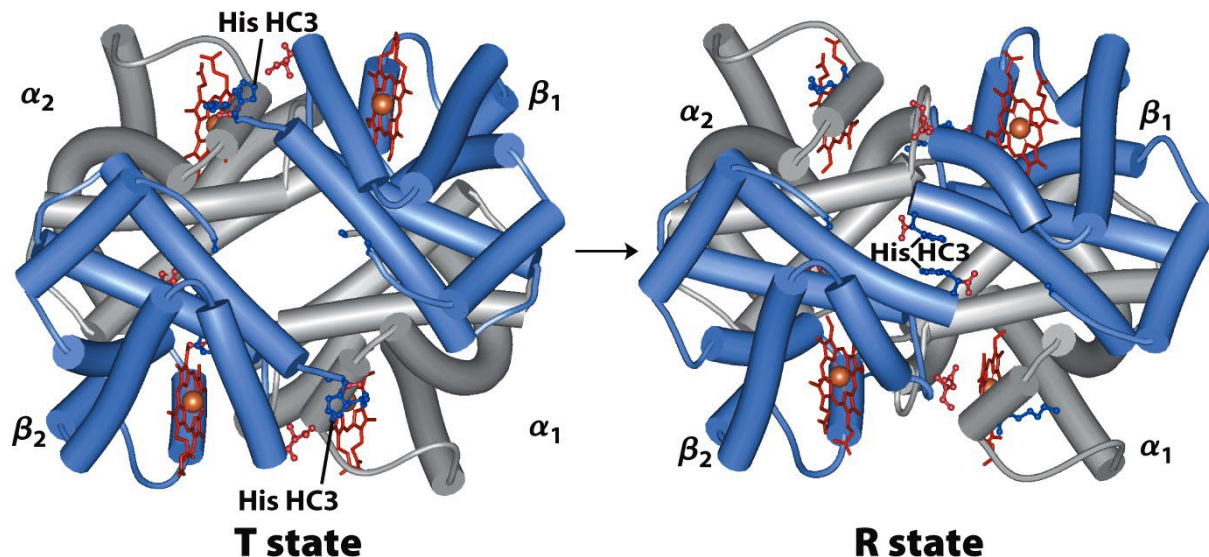
Protein	Ligand	K_d (M)*
Avidin (egg white)	Biotin	1×10^{-15}
Insulin receptor (human)	Insulin	1×10^{-10}
Anti-HIV immunoglobulin (human) [†]	gp41 (HIV-1 surface protein)	4×10^{-10}
Nickel-binding protein (<i>E. coli</i>)	Ni^{2+}	1×10^{-7}
Calmodulin (rat) [‡]	Ca^{2+}	3×10^{-6}
		2×10^{-5}



A more complex model for ligand binding

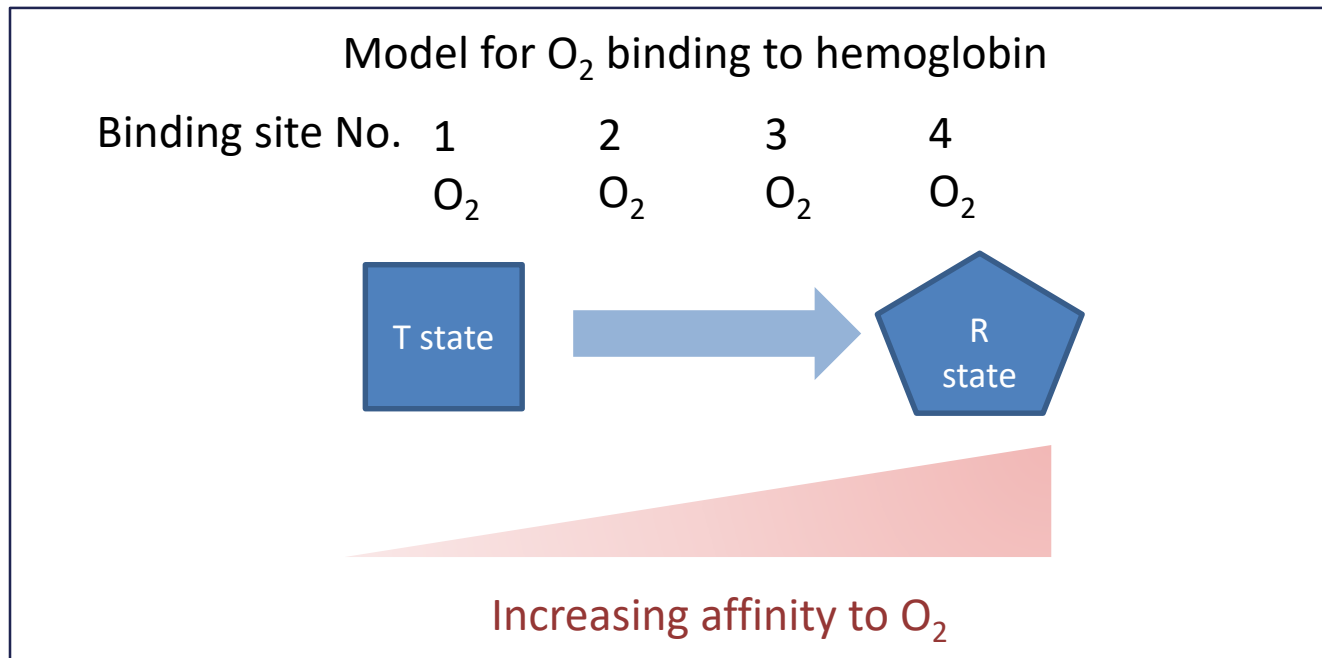
- A hemoglobin has four subunits, each binds to one Heme and O₂
- Two crystal structures of hemoglobin are present, the **T state** and **R state**, both can bind to O₂, but **T state** usually has less O₂
- O₂ has significant higher affinity for the **R state** of hemoglobin and stabilizes the **R state**

Structure transition from **T state** to **R state** upon the binding of O₂



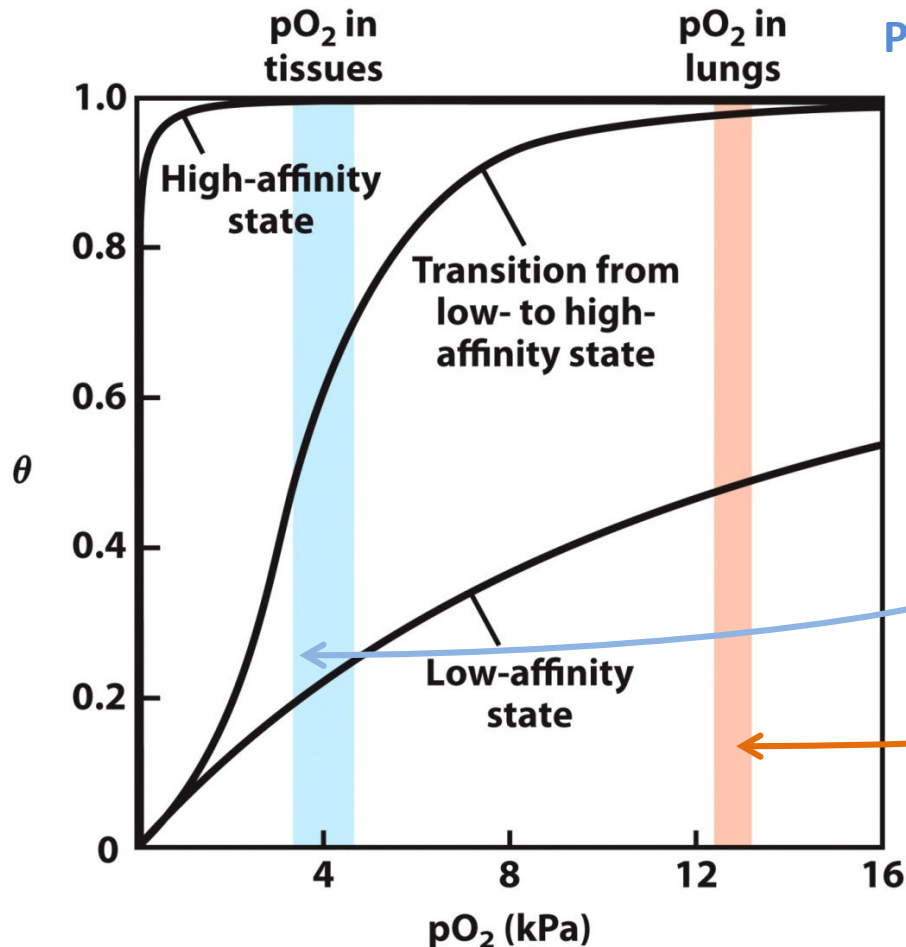
The cooperative binding model

- Binding of O_2 stimulates the conformational change of hemoglobin towards the R state and promote the affinity of O_2 at the other binding sites (**cooperative binding**)
- **Allosteric protein**: binding of one site affects the binding of the other sites. Hemoglobin is one example



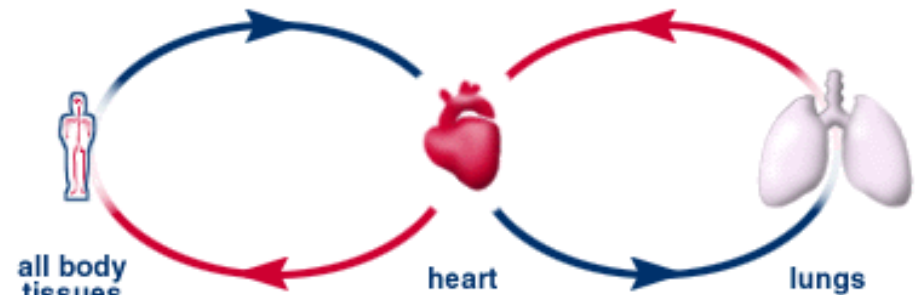
Cooperative binding is an adaption to different levels of O_2 in different tissues

A sigmoid binding curve (the middle curve) of O_2 binding to hemoglobin



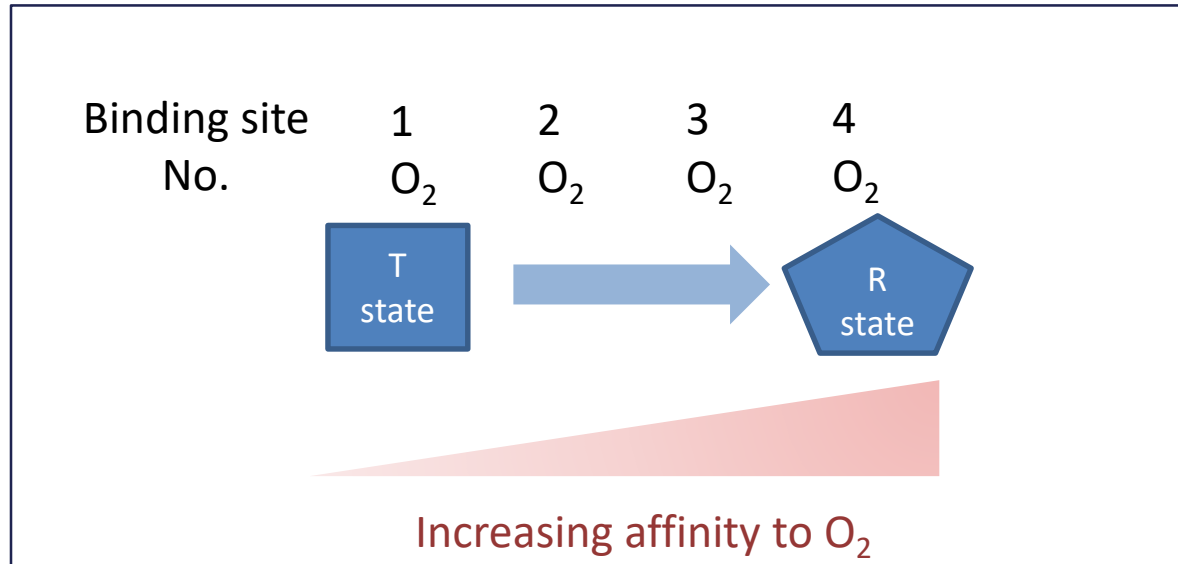
More in T state
Promote releasing of O_2
(release about 30%)

More in R state
Promote binding of O_2
(bind about 100%)



Modulation of hemoglobin binding to O₂

- Hemoglobin is an **allosteric protein**: binding of oxygen to one site affects oxygen binding to the other sites.



- There are other molecules besides oxygen that affect hemoglobin-O₂ interaction

CO₂, H⁺, BPG and CO also bind to hemoglobin

More layers of complexity:

Binding of H^+ and CO_2 by Hemoglobin

- Hemoglobin can also bind to H^+ and CO_2 ; their binding is inversely related to the binding of O_2 “Bohr effect”

- In tissues**

- CO_2 is high (oxidation in mitochondria)

- pH decreases because:



- Decrease binding affinity of O_2

- Release O_2 and bind to CO_2 and H^+

- In lung**

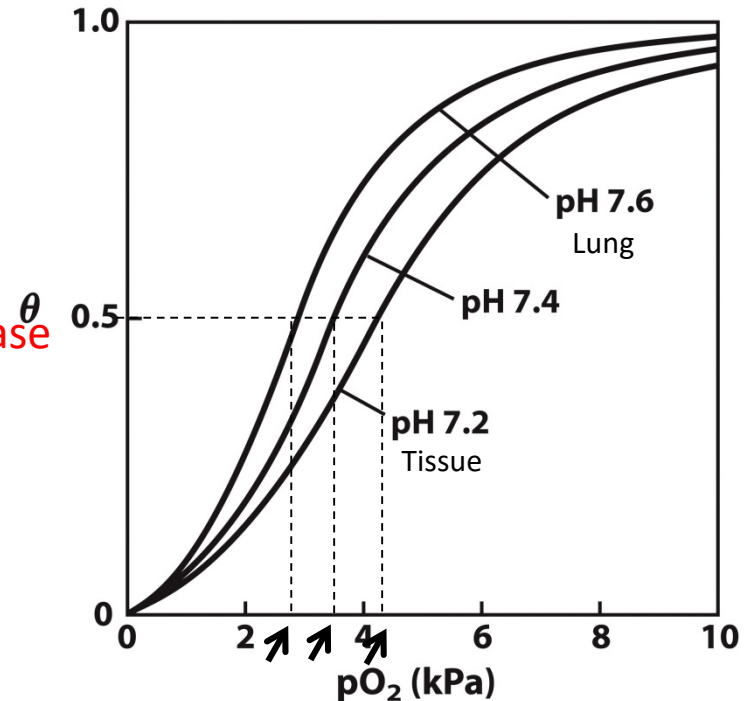
- CO_2 is low (excreted)

- pH rises

- Increase binding affinity of O_2

- Release CO_2 and H^+ and bind to O_2

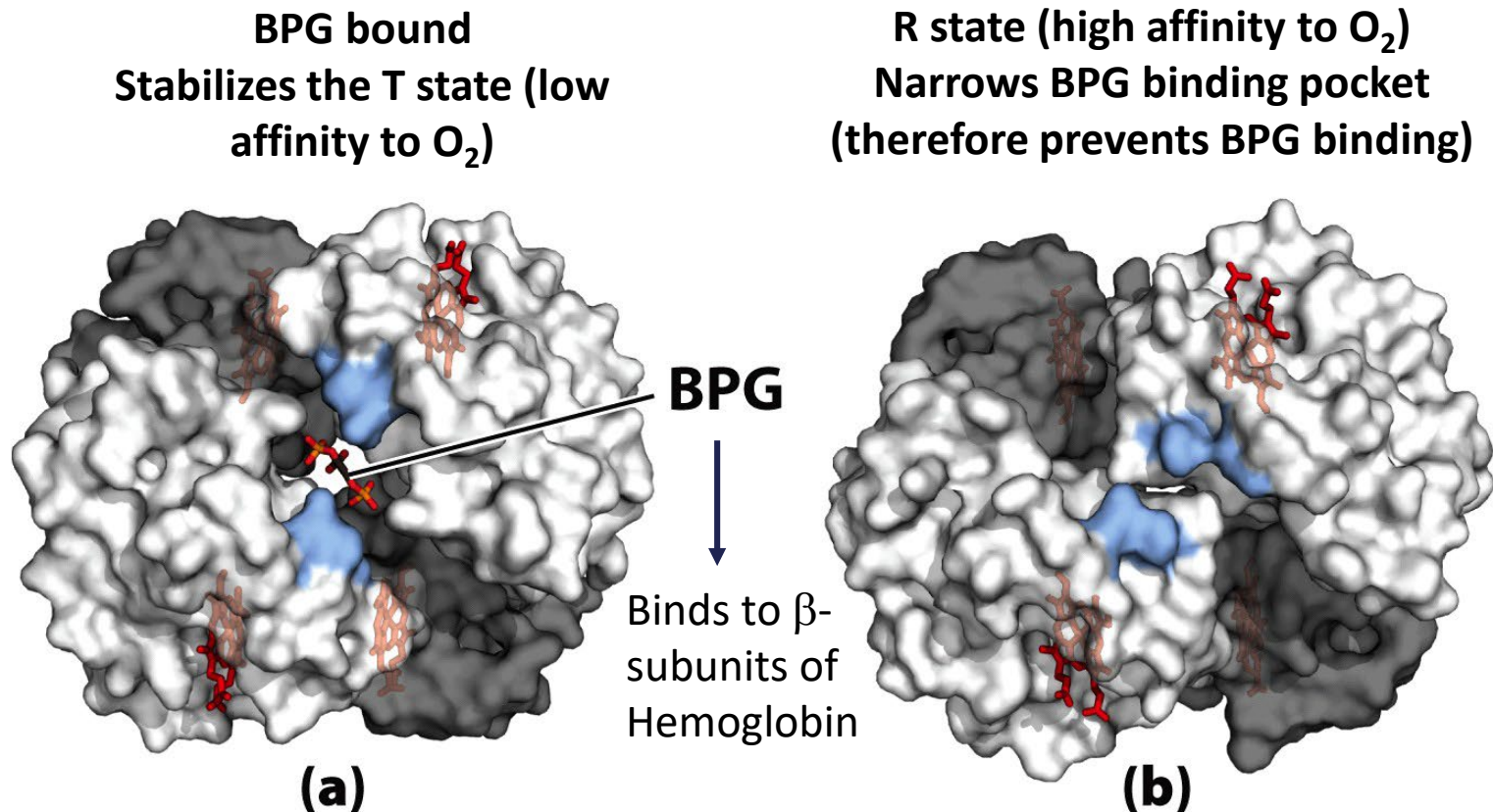
Effect of pH on oxygen binding to hemoglobin



K_d (in lung) < K_d (in tissue)

Regulation of oxygen binding to hemoglobin by 2,3-Bisphosphoglycerate (BPG)

- BPG is present in relatively high concentration in erythrocytes (red blood cells)
- BPG reduces the binding of oxygen to hemoglobin



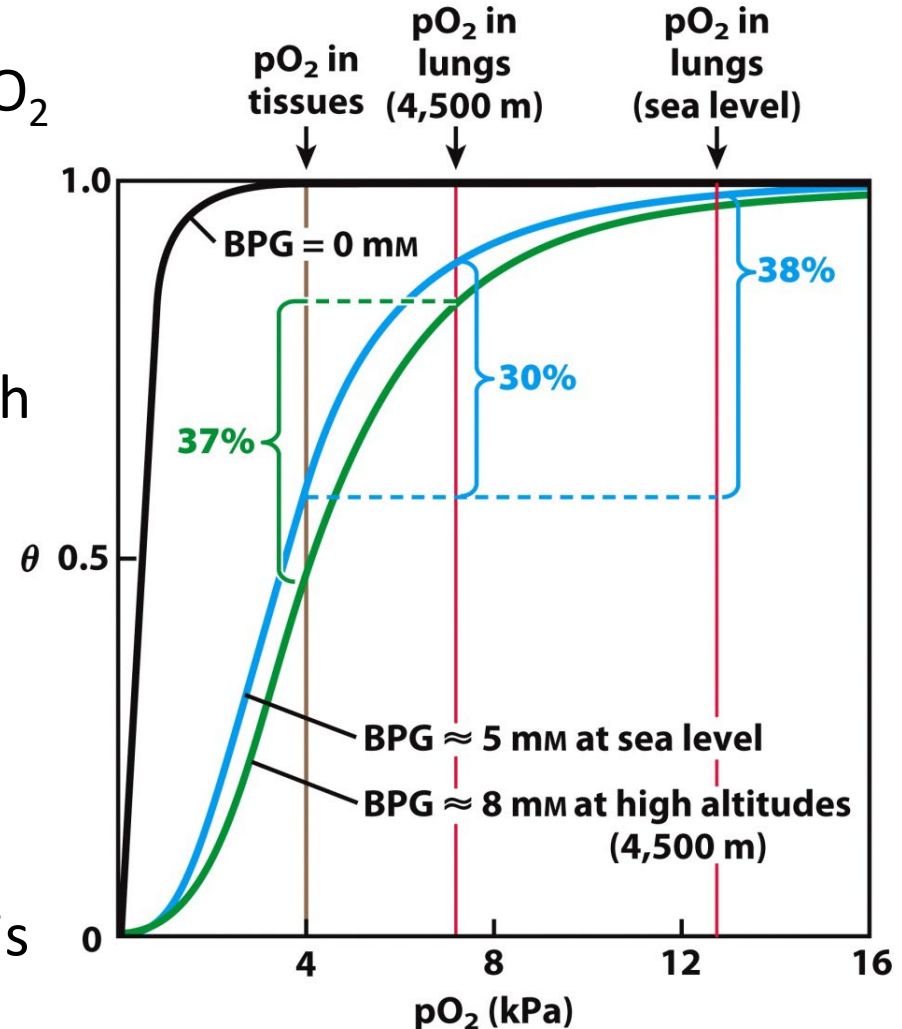
Elevated level of BPG in blood at high altitude promotes the release of oxygen

- BPG level is important for people adaptation to high altitude where O_2 concentration is lower:

-Concentration of BPG is increased when people are transferred to high altitude

-Affinity of O_2 to hemoglobin is reduced (from blue line to green line)

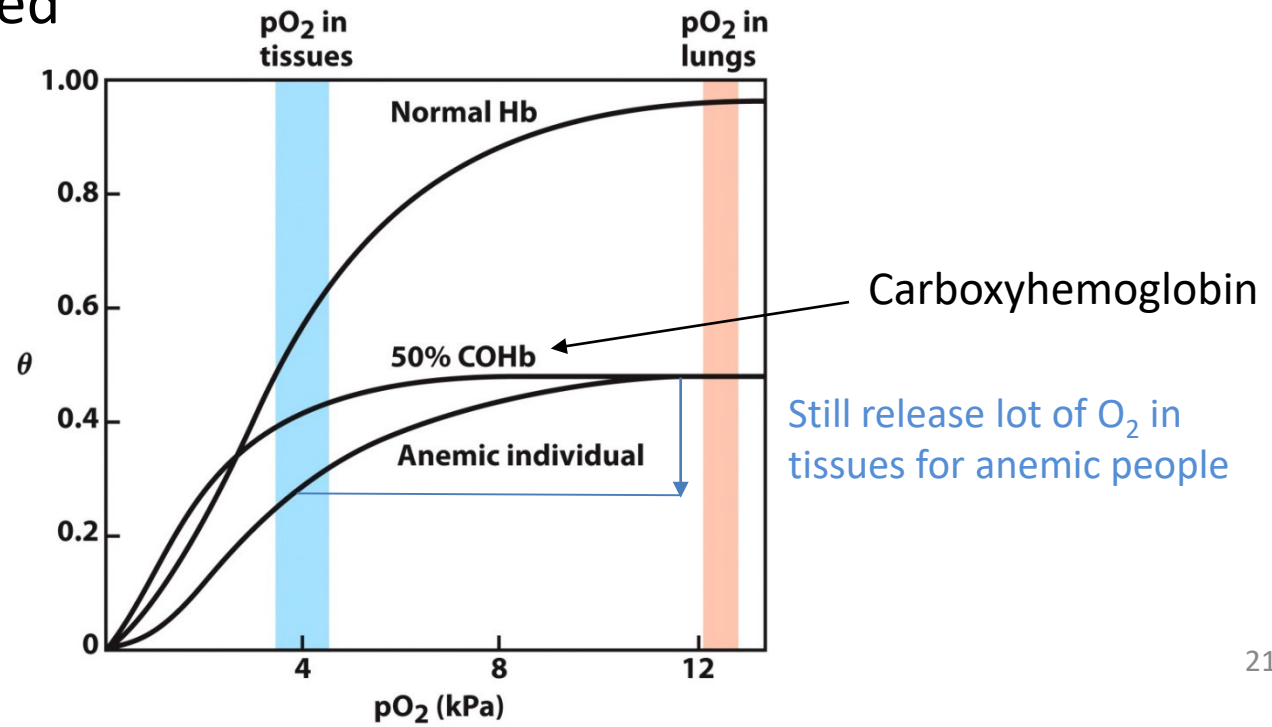
-Percentage of O_2 release in tissue is increased (from 30% back to 37%, which is similar to that in sea level)



Hemoglobin related diseases:

Why can CO kill people?

- Carbon monoxide (CO) binds to hemoglobin with an affinity 250-fold greater than O_2 (competing with O_2 at the same binding pocket)
- CO is a byproduct of incomplete burn of fossil fuels; also present in tobacco smoke
- >60% of CO-Hemoglobin causes death because O_2 uptake and release are blocked



Sickle cell anemia



- A disease caused by genetic mutation in residue 6 of β chain of hemoglobin (Glu \rightarrow Val, acidic \rightarrow hydrophobic) resulting in variable abnormal red blood cells
- Caused by formation of aberrant aggregation of hemoglobin S (the mutated type of hemoglobin in patient) when deoxygenated (hence changing the shape of the red blood cells)
- Homozygous mutation causes serious disease because after physical exertion hemoglobin can become only half of the normal value
- Heterozygous mutation causes 1% of abnormal red cells; live normally if avoid vigorous exercise

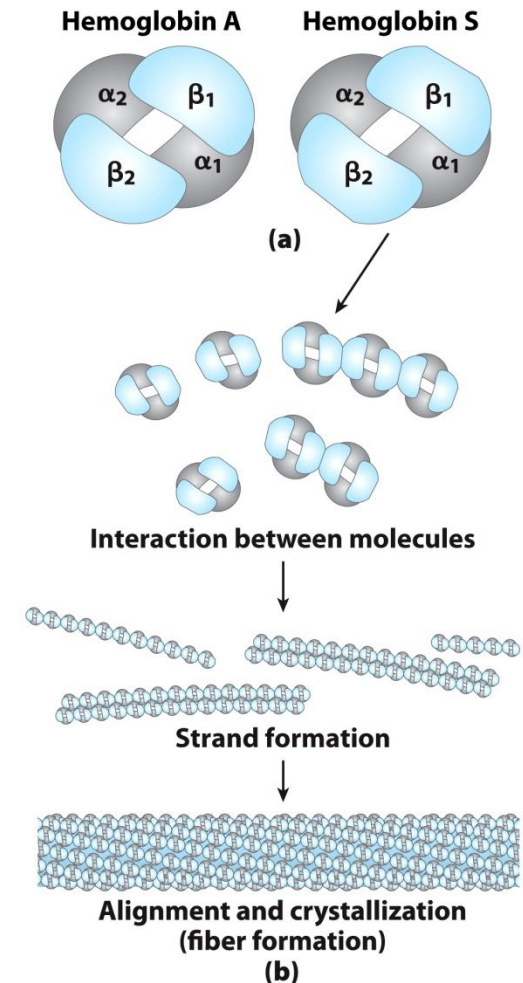
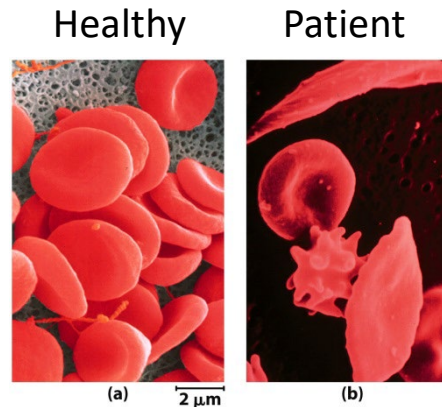


Figure 5-20
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Summary

- **Basic concepts in protein binding**
Ligand, binding site and induced fit
- **Reversible binding of a protein to a ligand**
Myoglobin and hemoglobin
Binding pocket conformation
 K_d
The cooperative binding model
- **Regulation of the binding between oxygen and hemoglobin**
 H^+ and CO_2
BPG
- **Hemoglobin related diseases**
CO poisoning
Sickle cell anemia

Study Question

Three proteins (1, 2, 3) can bind to a membrane protein with different affinities. According to the information below, which of the following statements is CORRECT?

Hormone concentration (nM)	Y		
	Protein 1	Protein 2	Protein 3
0.2	0.048	0.29	0.17
0.5	0.11	0.5	0.33
1	0.2	0.67	0.5

- A. Protein 1 has the highest binding affinity to the membrane protein
- B. Protein 2 has the highest binding affinity to the membrane protein
- C. Protein 3 has the lowest binding affinity to the membrane protein
- D. Not sufficient information to tell which protein has the highest affinity to the membrane protein

Study Question

Which of the following statements about hemoglobin is CORRECT?

- A. Each tetramer of hemoglobin binds to four BPG molecules
- B. CO_2 and O_2 bind to the same site on hemoglobin
- C. BPG in red blood cells helps people living in high altitude by increasing the hemoglobin binding affinity to O_2 in the lung
- D. None of the above