

5 Protein Function

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Learning



Functions of Globular Proteins

- Storage of ions and molecules
 - Storage oxygen Storage iron
 - myoglobin, ferritin
- Transport of ions and molecules
 - hemoglobin, glucose transporter
- Defense against pathogens
 - antibodies, cytokines
- Muscle contraction
 - actin, myosin
- Biological catalysis
 - chymotrypsin, lysozyme

Proteins Function by Interacting Dynamically with Other Molecules

- two types of interactions:
 - protein acting as a reaction catalyst, or **enzyme**, alters the chemical configuration or composition of a bound molecule
 - neither the chemical configuration nor the composition of the bound molecule is changed

5.1 Reversible Binding of a Protein to a Ligand: Oxygen-Binding Proteins

Text

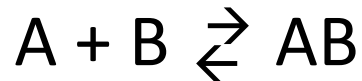


Principle 1

The functions of many proteins involve the reversible binding of other molecules. A molecule bound reversibly by a protein is called a **ligand**. A ligand may be any kind of molecule, including another protein. The **transient** nature of protein-ligand interactions is critical to life, allowing an organism to respond rapidly and reversibly to changing environmental and metabolic **circumstances**.

Interaction with Other Molecules

- Reversible, transient process of chemical equilibrium:



- A molecule that binds to a protein is called a **ligand**
 - Typically a small molecule
 - A region in the protein where the ligand binds is called the **binding site**
 - Ligand binds via same **noncovalent** forces that dictate protein structure (see Chapter 4)
 - Allows the interactions to be transient
- Why reaction must be transient?
- (this is key to life → organism can respond quickly and reversibly to changes)

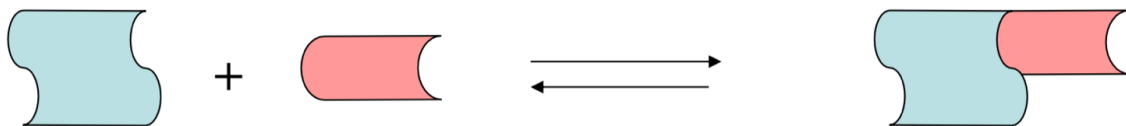
تحدد

Interaction with Other Molecules

- When ligands bind to proteins, some conformational changes (sometimes **quite dramatically**) occur **permitting tighter binding** → this is called **induced fit**
 - In multisubunit proteins, a conformational change of one subunit often affects the others (**cooperativity**)
- Enzymes are special kinds of proteins. They bind and transform other molecules. **Enzyme ligands are called substrates**
- **The binding site is called catalytic site (active site)**

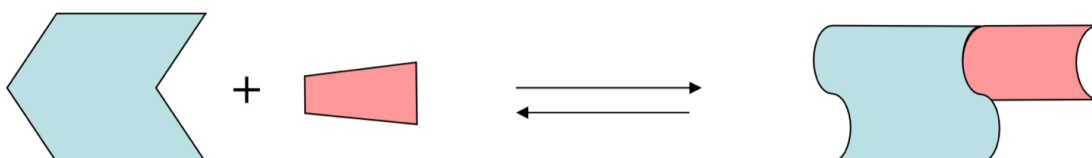
Specificity: Lock-and-Key Model

- Proteins typically have high specificity: only certain ligands bind
- High specificity can be explained by the **complementary** of the binding site and the ligand.
- Complementary in
 - size,
 - shape,
 - charge,
 - or hydrophobic/hydrophilic character
- “Lock and Key” model by Emil Fisher (1894) assumes that complementary surfaces are **preformed**.



Specificity: Induced Fit

- Conformational changes may occur upon ligand binding (Daniel Koshland in 1958)
 - This adaptation is called the **induced fit**
 - Induced fit allows for tighter binding of the ligand
 - **Induced fit allows for high affinity for different ligands**
- Both the ligand and the protein can change their conformations



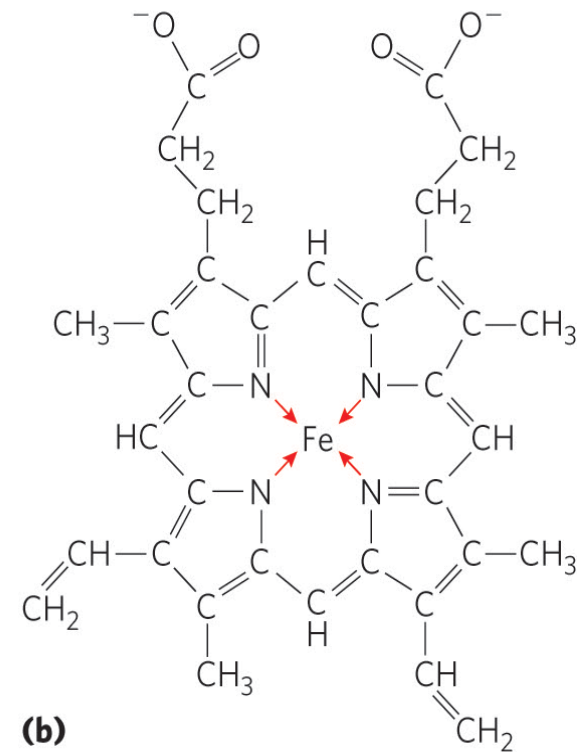
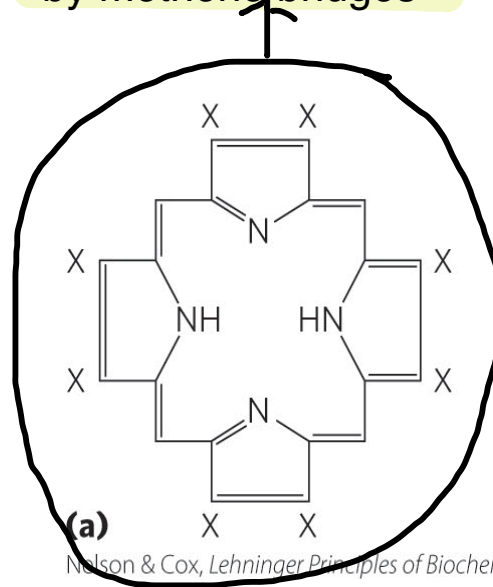
Oxygen Can Bind to a Heme Prosthetic Group

- oxygen:
 - poorly soluble in aqueous solutions
 - diffusion through tissues is ineffective over large distances
 - transition metals have strong tendency to bind (iron, copper)

Heme Prosthetic Group

- * The heme group is a strong chromophore that absorbs both in ultraviolet and visible range
- heme = protein-bound prosthetic group
 - present in myoglobin and **hemoglobin**
 - consists of a complex organic ring structure, **protoporphyrin**, with a bound Fe^{2+} atom

four pyrrole rings linked by methene bridges



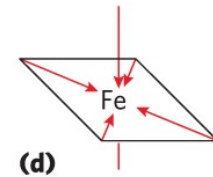
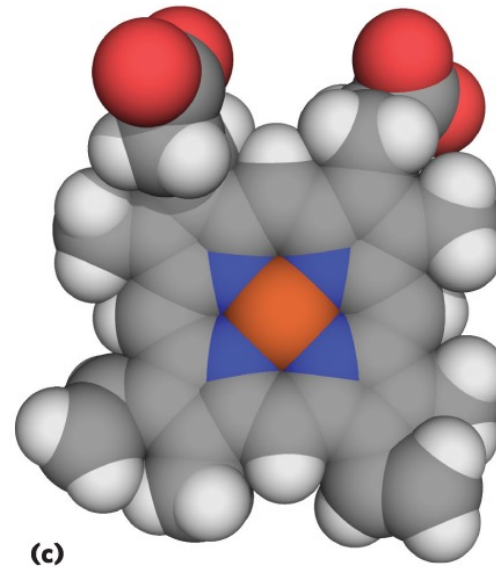
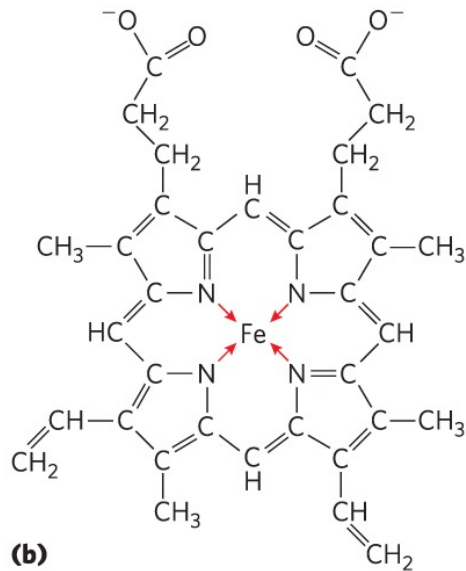
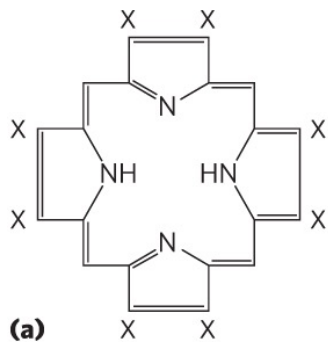
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* Heme is a porphyrin ring complexed with ferrous iron and protoporphyrin

Coordination Bonds of Iron

- six coordination bonds:
 - four to nitrogen atoms in the flat **porphyrin ring**
 - two perpendicular to the porphyrin

One of these two bonds is associated with oxygen and the other is associated with histidine f8



P1

Clicker Question 1

The heme prosthetic group:

- A. consists of protoporphyrin and an iron (II) ion.
- B. is found only in myoglobin and hemoglobin.
- C. contains a single iron atom in its ferrous (Fe^{2+}) state that has a total of 4 coordination bonds.
- D. is only found bound to protein.

Clicker Question 1, Response

The heme prosthetic group:

A. consists of protoporphyrin and an iron (II) ion.

Heme consists of a complex organic ring structure, protoporphyrin, to which is bound a single iron atom in its ferrous (Fe^{2+}) state.

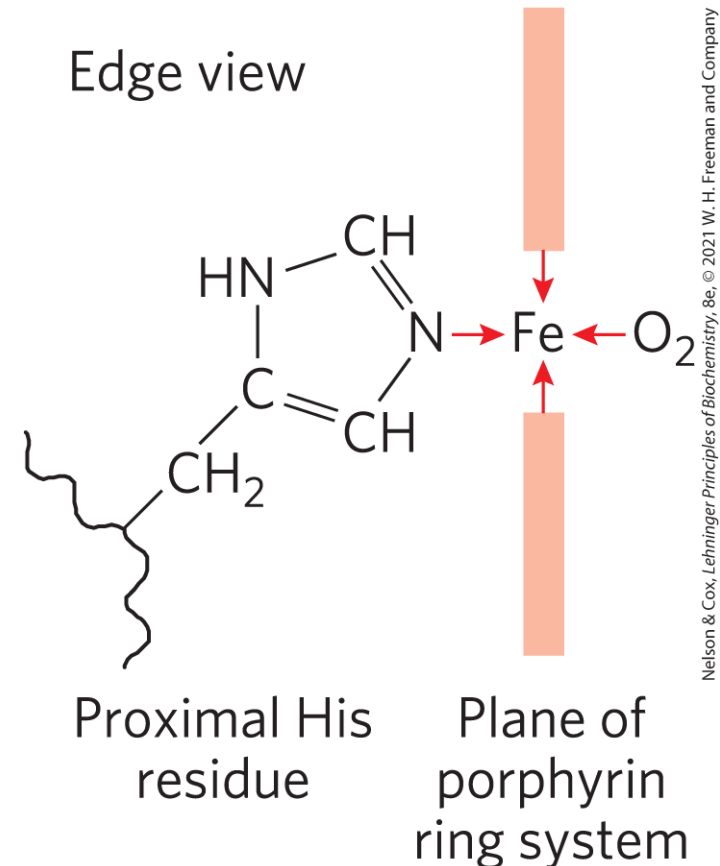


Principle 2

A ligand binds a protein at a binding site that is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character. The interaction is specific: the protein can discriminate among the thousands of different molecules in its environment and selectively bind only one or a few types. A given protein may have separate **binding sites for several different ligands. These specific molecular interactions are crucial in maintaining the high degree of order in a living system.**

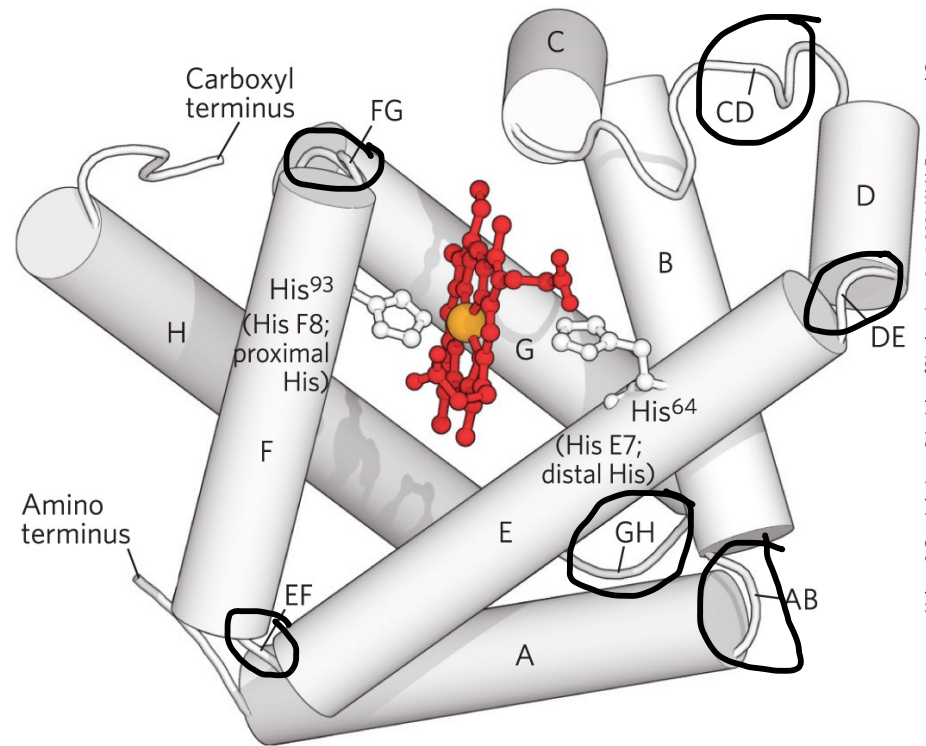
Perpendicular Coordination Bonds

- two perpendicular coordination bonds:
 - one is occupied by a side-chain nitrogen of a highly conserved **proximal His** residue
 - one is the binding site for molecular oxygen (O_2)
 - Fe^{2+} binds O_2 reversibly
 - Fe^{3+} does not bind O_2



Globins Are a Family of Oxygen-Binding Proteins

- **globins** = widespread protein family
 - highly **conserved** محفوظ، مصون tertiary structure: eight α -helical segments connected by **bends (globin fold)**
 - most function in O_2 transport or storage



Types of Globins

- four types in humans and other mammals:
 - **myoglobin** = monomeric, facilitates O₂ diffusion in muscle tissue
 - **hemoglobin** = tetrameric, responsible for O₂ transport in the bloodstream
 - **neuroglobin** = monomeric, expressed largely in neurons to protect the brain from low O₂ or restricted blood supply
 - **cytoglobin** = monomeric, regulates levels of nitric oxide, a localized signal for muscle relaxation

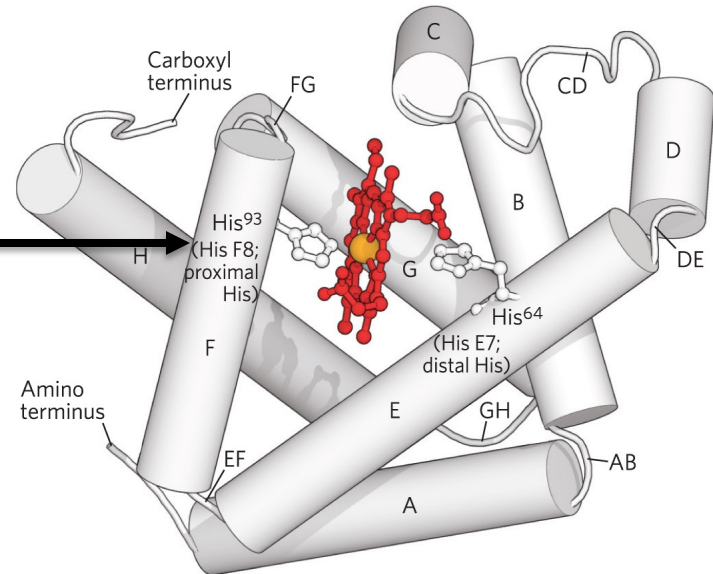
Myoglobin Has a Single Binding Site for Oxygen

- myoglobin:
 - 153 residues + one molecule of heme
 - bends named after the α -helical segments they connect

His⁹³ = ninety-third
reside from the amino
terminal end

His F8 = eighth
residue in α helix F

The
same





Clicker Question 2

Myoglobin:

- A. has quaternary structure.
- B. contains a pocket for binding heme that is made up of largely the C and D helices.
- C. contains multiple binding sites for O₂.
- D. consists mostly of α helices.

Clicker Question 2, Response

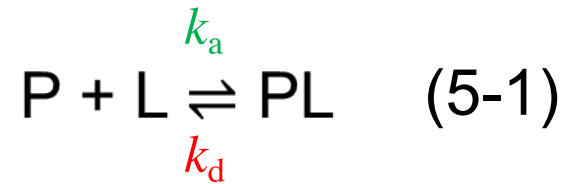
Myoglobin:

D. consists mostly of α helices.

Myoglobin is a single polypeptide of 153 amino acid residues with one molecule of heme. About 78% of the amino acid residues in the protein are found in the eight α helices typical of the globin fold, named A through H.

Protein-Ligand Interactions Can Be Described Quantitatively

- a simple **equilibrium expression** describes the reversible binding of a protein (P) to a ligand (L):



* Forward rate depend on the concentration of L and P \rightarrow rate= $K_a[P][L]$

* Reverse rate depend on the concentration of PL \rightarrow rate= $K_d[PL]$

* At equilibrium, forward rate equal reverse rate

Forward rate= reverse rate

$$K_a[P][L]= K_d[PL]$$

Association Constant

- **association constant (K_a)** = provides a measure of the affinity of the ligand L for the protein
 - higher K_a = higher affinity
 - equivalent to the ratio of the rates of the forward (association) and the reverse (dissociation) reactions that form the PL complex

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d} = \frac{1}{K_d} \quad (5-2)$$

P2 Clicker Question 3

Protein A has a binding site for ligand L with a K_a of 10^5 M^{-1} .
Protein B has a binding site for ligand L with a K_a of 10^8 M^{-1} .
Which protein has the higher affinity for ligand L?

- A. protein A
- B. protein B
- C. It is not possible to determine based only on K_a values.

Clicker Question 3, Response

Protein A has a binding site for ligand L with a K_a of 10^5 M^{-1} .
Protein B has a binding site for ligand L with a K_a of 10^8 M^{-1} .
Which protein has the higher affinity for ligand L?

B. protein B

The association constant provides a measure of the affinity of the ligand L for the protein. A higher value of K_a corresponds to a higher affinity of the ligand for the protein.

[L] Remains Constant

$$K_a = \frac{[PL]}{[P][L]} = \frac{k_a}{k_d} \quad (5-2)$$

$$K_a[L] = \frac{[PL]}{[P]} \quad (5-3)$$

- when $[L] \gg \gg$ [ligand-binding sites], the binding of the ligand by the protein does not **appreciably** change $[L]$

بشكل ملحوظ

Binding Equilibrium

*If Y is small number, we don't have PL

*If Y is equal 1, we don't have p

$$Y = \frac{\text{binding sites occupied}}{\text{total binding sites}} = \frac{[PL]}{[PL] + [P]} \quad (5-4)$$

Fraction of occupied binding sites is the ratio of who many sites are occupied to know the total number of binding sites

substituting $K_a[L][P]$ for $[PL]$:

$$Y = \frac{K_a[L][P]}{K_a[L][P] + [P]} = \frac{K_a[L]}{K_a[L] + 1} = \frac{[L]}{[L] + \frac{1}{K_a}} \quad (5-5)$$



$$1/K_a = K_d$$

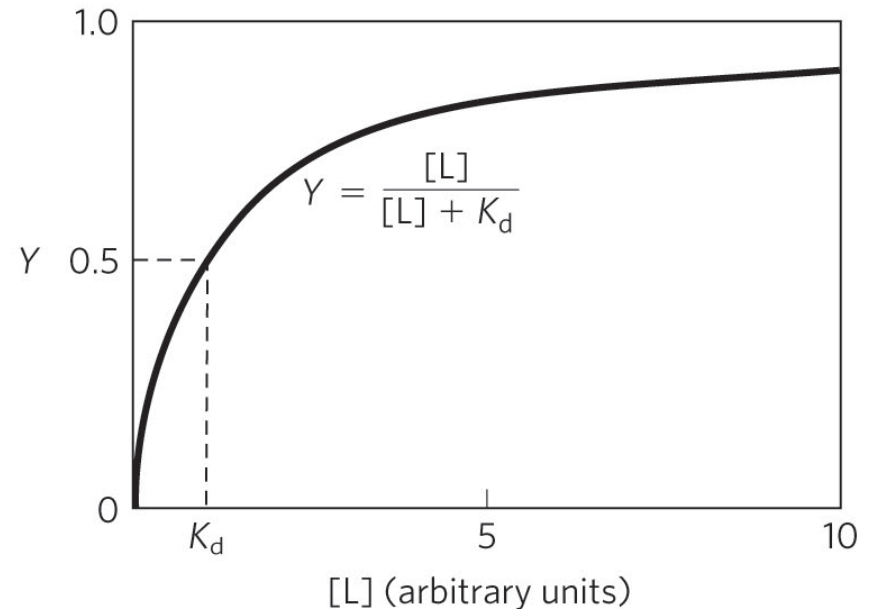
$x = y/(y + z)$ describes
a hyperbola قطع زائد

Graphical Representations of Ligand Binding

$$Y = \frac{[L]}{[L] + \frac{1}{K_a}} \quad (5-5)$$

- [L] at which $\frac{1}{2}$ of the available ligand-binding sites are occupied ($Y = 0.5$) corresponds to $1/K_a$

تکافئ



(a)

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* When concentration of ligand is higher than K_d , we reach saturation (most proteins are bound)

* When K_d increase, affinity of L for P decrease

Dissociation Constant

- **dissociation constant (K_d)** = reciprocal of K_a
 - equilibrium constant for the release of ligand
 - lower K_d = higher affinity

$$K_d = \frac{[P][L]}{[PL]} = \frac{k_d}{k_a} \quad (5-6)$$

$$[PL] = \frac{[P][L]}{K_d} \quad (5-7)$$

$$Y = \frac{[L]}{[L] + K_d} \quad (5-8)$$

- when $[L] = K_d$, $\frac{1}{2}$ of the ligand-binding sites are occupied

- K_d is the molar concentration of ligand at which half of the binding sites are occupied

P2 Clicker Question 4

Protein A has a $K_a = 6.0 \mu\text{M}^{-1}$ for binding ligand L, and the protein B has a $K_d = 4.0 \mu\text{M}$ for binding ligand L. Based on this information, which statement is true?

- A. Protein B binds L with higher affinity.
- B. Protein A is half-saturated with L when $[\text{L}]$ is $6.0 \mu\text{M}^{-1}$.
- C. The K_a of protein B for L is $0.25 \mu\text{M}^{-1}$.
- D. When $[\text{L}] = 1 \mu\text{M}$, $Y = 0.17$ for protein A.

Clicker Question 4, Response

Protein A has a $K_a = 6.0 \mu\text{M}^{-1}$ for binding ligand L, and the protein B has a $K_d = 4.0 \mu\text{M}$ for binding ligand L. Based on this information, which statement is true?

C. The K_a of protein B for L is $0.25 \mu\text{M}^{-1}$.

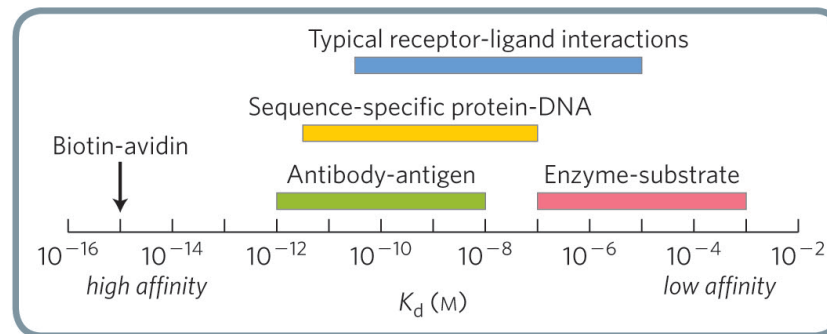
$$K_a = 1/K_d$$

For protein B, $K_a = 1/(4.0 \mu\text{M}) = 0.25 \mu\text{M}^{-1}$.

Representative K_d Values

TABLE 5-1 Protein Dissociation Constants: Some Examples and Range

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



Color bars indicate the range of dissociation constants typical of various classes of interactions in biological systems. A few interactions, such as that between the protein avidin and the enzyme cofactor biotin, fall outside the normal ranges. The avidin-biotin interaction is so tight it may be considered irreversible. Sequence-specific protein-DNA interactions reflect proteins that bind to a particular sequence of nucleotides in DNA, as opposed to general binding to any DNA site.

^aA reported dissociation constant is valid only for the particular solution conditions under which it was measured. K_d values for a protein-ligand interaction can be altered, sometimes by several orders of magnitude, by changes in the solution's salt concentration, pH, or other variables.

^bThis immunoglobulin was isolated as part of an effort to develop a vaccine against HIV. Immunoglobulins (described later in the chapter) are highly variable, and the K_d reported here should not be considered characteristic of all immunoglobulins.

^cCalmodulin has four binding sites for calcium. The values shown reflect the highest- and lowest-affinity binding sites observed in one set of measurements.

Binding of O₂ to Myoglobin

* In this example, myoglobin acts as a protein and oxygen acts as ligand

- substituting the [O₂] for [L]:

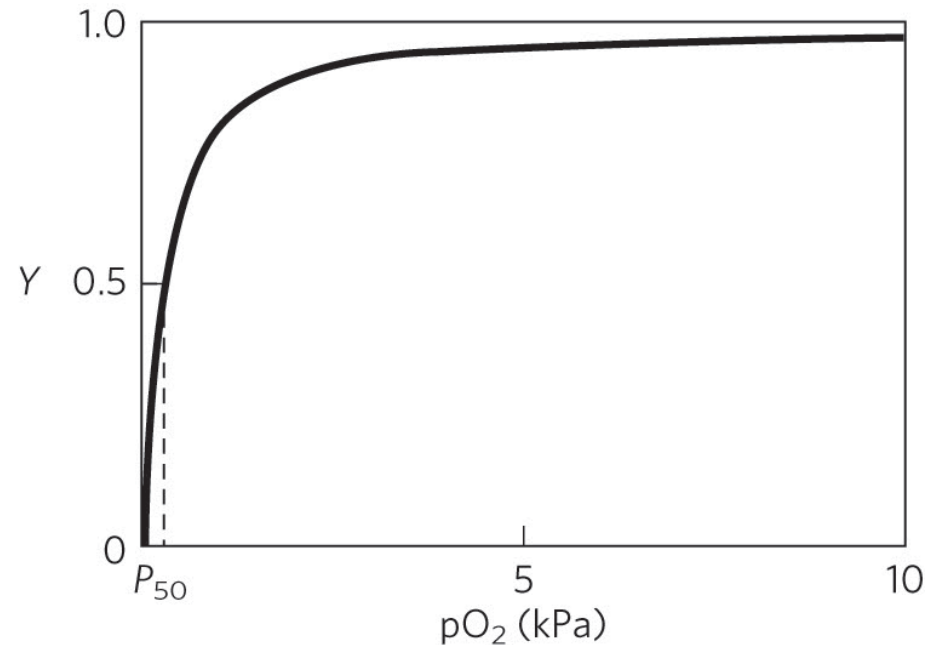
$$Y = \frac{[O_2]}{[O_2] + K_d} \quad (5-9)$$

- K_d equals the [O₂] at which 1/2 of the available ligand-binding sites are occupied, or [O₂]_{0.5}:

$$Y = \frac{[O_2]}{[O_2] + [O_2]_{0.5}} \quad (5-10)$$

Partial Pressure of O₂

- When the ligand is a gas → partial pressure of O₂ (pO₂) is easier to measure than [O₂]
- defining the partial pressure of oxygen at [O₂]_{0.5} as P₅₀:



(b)

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$$Y = \frac{pO_2}{pO_2 + P_{50}} \quad (5-11)$$

* The pressure of oxygen in the lungs equal 13.3 so the value of Y is high is almost 100% of myoglobin will carry oxygen. when this myoglobin reach tissues, the pressure of oxygen in tissues is 4, so myoglobin will not give tissues the oxygen, so myoglobin is a bad transporter and use to storage oxygen

P2 Clicker Question 5

When the partial pressure of oxygen is equal to the P_{50} of myoglobin, what is the value of Y ?

- A. 0.0
- B. 0.25
- C. 0.50
- D. 0.75
- E. 1.0

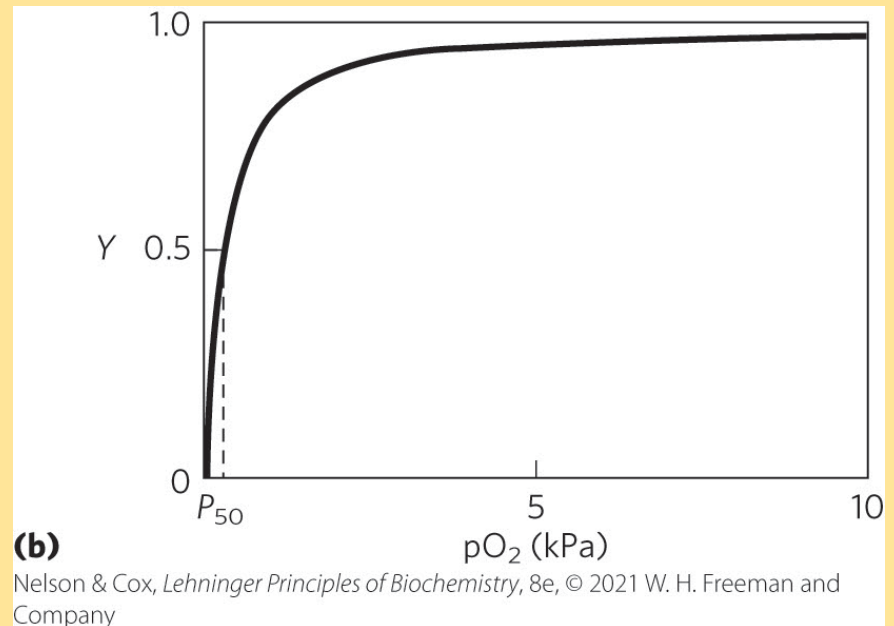
Clicker Question 5, Response

When the partial pressure of oxygen is equal to the P_{50} of myoglobin, what is the value of Y ?

C. 0.50

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

When $pO_2 = P_{50}$, $Y = 0.5$.





Principle 3

Proteins are flexible. Changes in conformation may be subtle, reflecting molecular vibrations and small movements of amino acid residues throughout the protein. Changes in conformation may also be more dramatic, with major segments of the protein structure moving as much as several nanometers. Specific conformational changes are frequently essential to a protein's function.

Example: Oxygen Binding to Myoglobin

* No one of the protinogenic amino acid can bound oxygen

- Free heme molecules not bound in proteins → 2 open coordination bonds
- Reaction of 1 O₂ molecule with two hemes will lead to irreversible conversion of Fe²⁺ to Fe³⁺ which does not bind O₂
- This reaction is prevented in heme-containing proteins because one of the coordination bonds is attached to a His side chain and the other is free to bond O₂
- When O₂ binds, electronic properties of heme changes (color changes from dark purple to bright red)
- CO and NO bind more tightly to heme than O₂ → toxic to aerobic organisms

Binding of Carbon Monoxide

- CO has similar size and shape to O₂; it can fit to the same binding site
- CO binds over 20,000 times better than O₂ because the carbon in CO has a filled lone electron pair that can be donated to vacant d-orbitals on the Fe²⁺
- **Protein pocket** decreases affinity for CO, but it still binds about 250 times better than oxygen
- CO is highly toxic as it competes with oxygen. It blocks the function of myoglobin, hemoglobin, and mitochondrial cytochromes that are involved in oxidative phosphorylation

شاغر

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A cavity on the surface or in the interior of a protein that possesses suitable properties for

binding a ligand

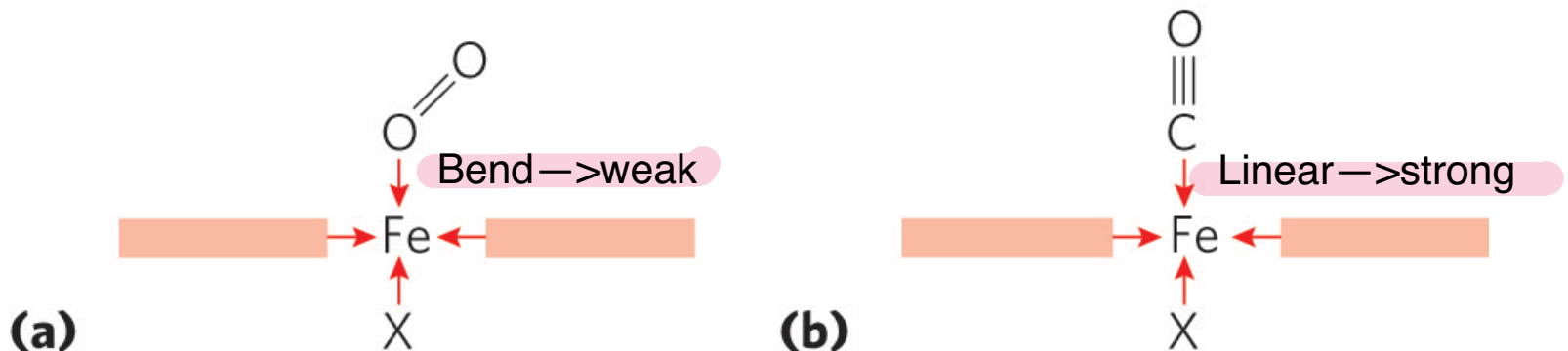
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 Video:

1) Carbon Monoxide Poisoning: Everything You Need to Know

Protein Structure Affects How Ligands Bind

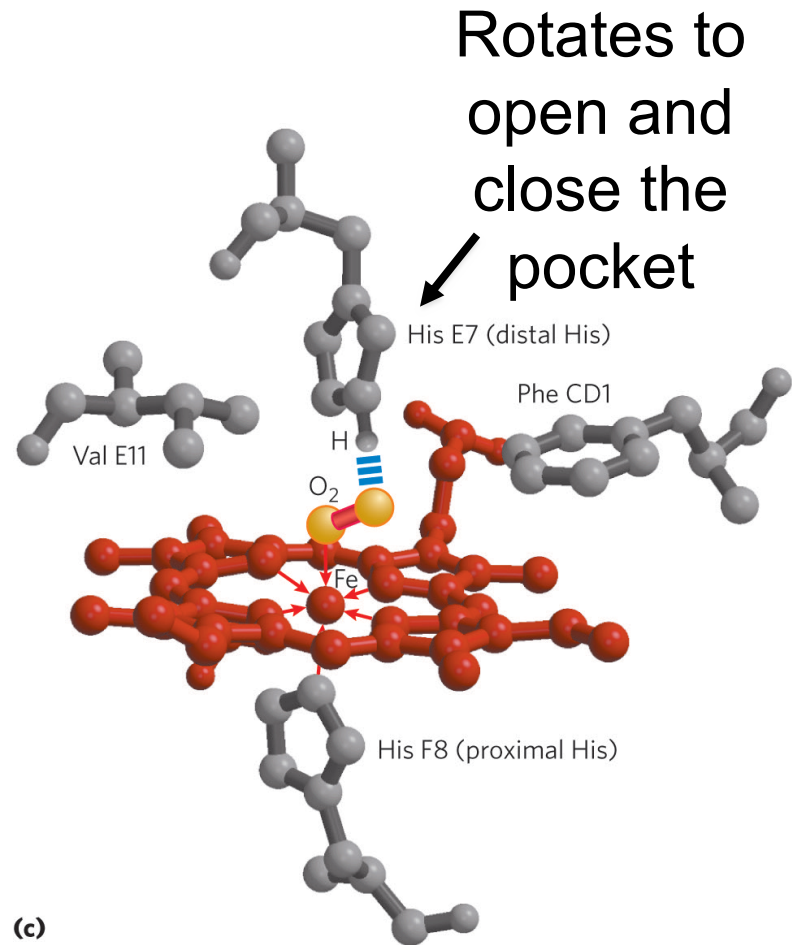
- carbon monoxide (CO) binds free heme more than 20,000 times better than does O₂
 - differences in the orbital structures affect binding geometries



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Myoglobin's Distal His Increases Heme's Affinity for O₂

- hydrogen bond between the imidazole side chain of His E7 and bound O₂ electrostatically stabilizes the Fe-O₂ polar complex
- 20,000-fold stronger binding affinity of free heme for CO compared with O₂ declines to ~40-fold



(c)

Hemoglobin Transports Oxygen in Blood

- erythrocytes (red blood cells) transport O_2
 - formed from **hemocytoblasts** (precursor stem cells)
 - main function is to carry hemoglobin

- arterial blood = ~96% saturated with O_2

- peripheral blood = ~64% saturated with O_2

– Mb is insensitive to small changes in $[O_2]$ —> O_2 - storage protein

– Hb is sensitive to small change —> O_2 -transport protein (multiple subunits)



Clicker Question 6

Oxygen carried by whole blood in animals is bound and transported by hemoglobin in what type of cell?

- A. hemocytoblasts
- B. erythrocytes
- C. white blood cells
- D. stem cells
- E. neurons

Clicker Question 6, Response

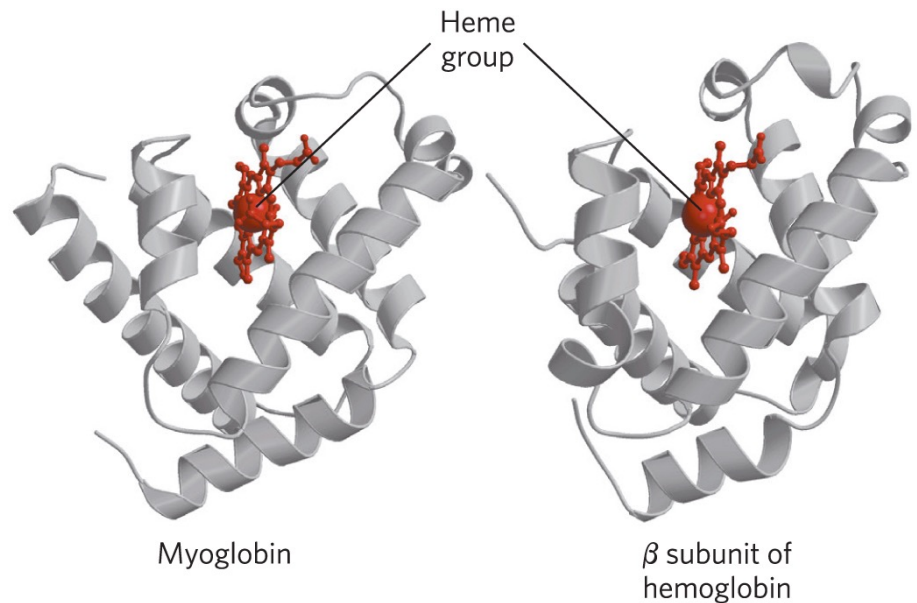
Oxygen carried by whole blood in animals is bound and transported by hemoglobin in what type of cell?

B. erythrocytes

Nearly all the oxygen carried by whole blood in animals is bound and transported by hemoglobin in erythrocytes (red blood cells). They are formed from precursor stem cells called hemocytoblasts.

Hemoglobin Subunits Are Structurally Similar to Myoglobin

- hemoglobin:
 - tetrameric protein with 4 heme groups
 - adult hemoglobin has two globin types: two α chains (141 residues each) and two β chains (146 residues each)



* Hemoglobin is $2\alpha 2\beta$. It is a dimer of dimer

- 3D structure of both a and b is similar
- aa sequences of Mb and a and b Hb are identical in 27 positions

Sequence Similarity between Hemoglobin and Myoglobin

	Mb	Hb α	Hb β
NA1	-- 1V	1V	1V
	---	---	H
	L	L	L
A1	---S	S	T---
	E	P	P
	G	A	E
	E	D	E
	W	K	K
	Q	T	S
	L	N	A
	V	V	V
	L	K	T
	H	A	A
	V	A	L
	W	W	W
	A	G	G
	K	K	K
	V	V	V
A16	---E	G	---
	A	A	---
B1	--20D	20H	N---
	V	A	20V
	A	G	D
	G	E	E
	H	Y	V
	G	G	G
	Q	A	G
	D	E	E
	I	A	A
	L	L	L
	I	E	G
	R	R	R
	L	M	L
	F	F	L
	K	L	V
B16	---S	S	V---
	:	:	:
	:	:	:

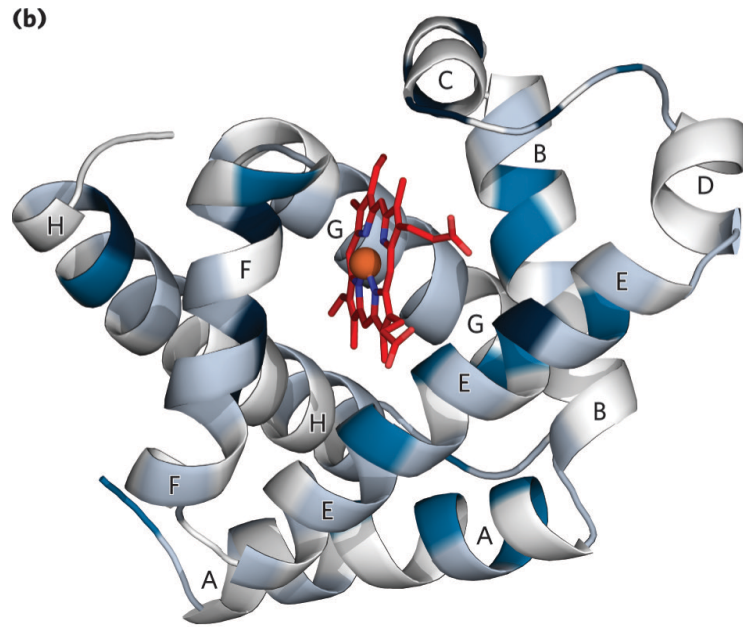
	Mb	Hb α	Hb β
	K	A	A
	K	H	H
	K	V	L
80G	D	D	D
	H	D	80N
	H	M	L
	E	P	K
	A	N	G
	E	A	T
F1	---L	80S	F---
	K	S	A
	P	A	T
	L	L	L
	A	S	S
	Q	D	E
	S	L	L
Proximal His	F8	H	H
	F9	A	C
	T	H	D
	K	K	K
	H	L	L
	K	R	H
	I	V	V
G1	--100P	D	D---
	I	P	100P
	K	V	E
	Y	N	N
	L	F	F
	E	K	R
	F	100L	L
	I	L	L
	S	S	G
	E	H	N
	A	C	V
	I	L	L
	I	L	V
	:	:	:
	:	:	:

	:	:	:
C1	---H	F	Y---
	P	P	P
	E	T	W
	T	T	T
40L	E	40K	Q
	E	T	40R
C7	---K	Y	F---
	F	F	F
	D	P	E
	R	H	S
	F	F	F
	K	---	G
	H	D	D
	L	L	L
	K	S	S
D1	---T	H	T---
	E	---	P
	A	---	D
	E	---	A
	M	---	V
	K	---	M
D7	---A	G	G---
E1	---S	S	N---
	E	A	P
60D	Q	Q	K
	L	V	60V
	K	K	K
	K	G	A
	H	H	H
Distal His	E7	G	G
	V	60K	K
	T	K	K
	V	V	V
	L	A	L
	T	D	G
	A	A	A
	L	L	F
	G	T	S
	A	N	D
	I	A	G

	:	:	:
	H	V	C
	V	T	V
	L	L	L
	H	A	A
	S	A	H
G19	---R	H	H---
	H	L	F
120P	G	A	120K
	D	E	E
	F	F	F
H1	---G	T	T---
	A	P	P
	D	120A	P
	A	V	V
	Q	H	Q
	G	A	A
	A	S	A
	M	L	Y
	N	D	Q
	K	K	K
	A	F	V
	L	L	V
	E	A	A
	L	S	G
	F	V	V
	R	S	A
140K	D	V	140A
	I	L	L
	A	T	A
H21	---A	S	H---
	K	K	K
	Y	140Y	Y
	K	141R	146H
	E		
H26	---L		
	G		
	Y		
	Q		

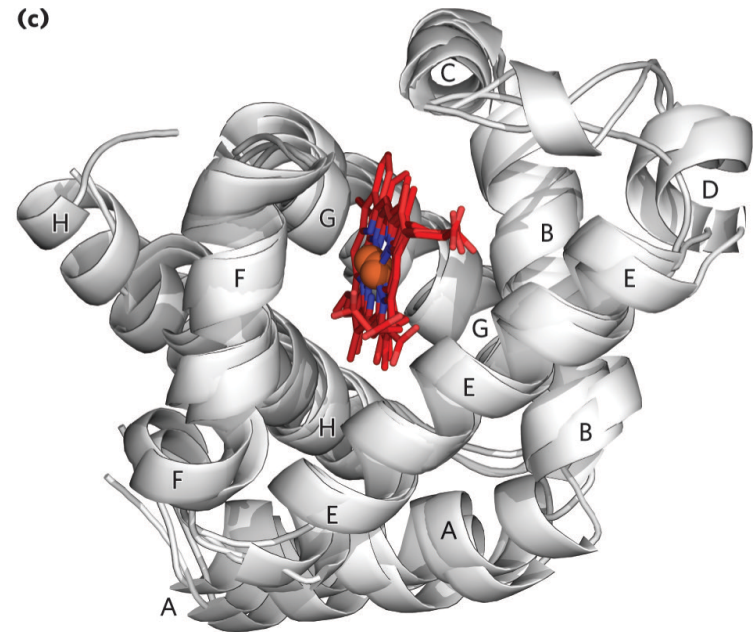
} Hb α
 and
 Hb β
 only

Structural Conservation of Globins



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Low sequence similarity



High structural similarity

The Quaternary Structure of Hemoglobin

- strong interactions between unlike subunits
 - hydrophobic effect
 - hydrogen bonds
 - ion pairs (salt bridges)
- *A dimer of 2 $\alpha\beta$ protomers:*
- $\alpha_1\beta_1$ (and $\alpha_2\beta_2$) interface involves >30 residues → More stable
- $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface involves 19 residues

* Alpha 1 and alpha 2 impossible to intersect (تتقاطع), and same thing with beta 1 and beta 2



Clicker Question 7

The α and β subunits of hemoglobin:

- A. each contain 4 heme prosthetic groups.
- B. contain several stretches of identical amino acids.
- C. are both tetramers.
- D. have high structural similarity to each other.
- E. differ in structure and sequence.

Clicker Question 7, Response

The α and β subunits of hemoglobin:

D. have high structural similarity to each other.

The three-dimensional structures of the two types of subunits are very similar to each other and to myoglobin, reflecting their evolution within the larger globin superfamily.



Principle 4

The binding of a protein and a ligand is often coupled to a conformational change in the protein that makes the binding site more complementary to the ligand, permitting tighter binding. The structural adaptation that occurs between protein and ligand is called **induced fit**.

Hemoglobin Undergoes a Structural Change on Binding Oxygen

- two conformations of hemoglobin:

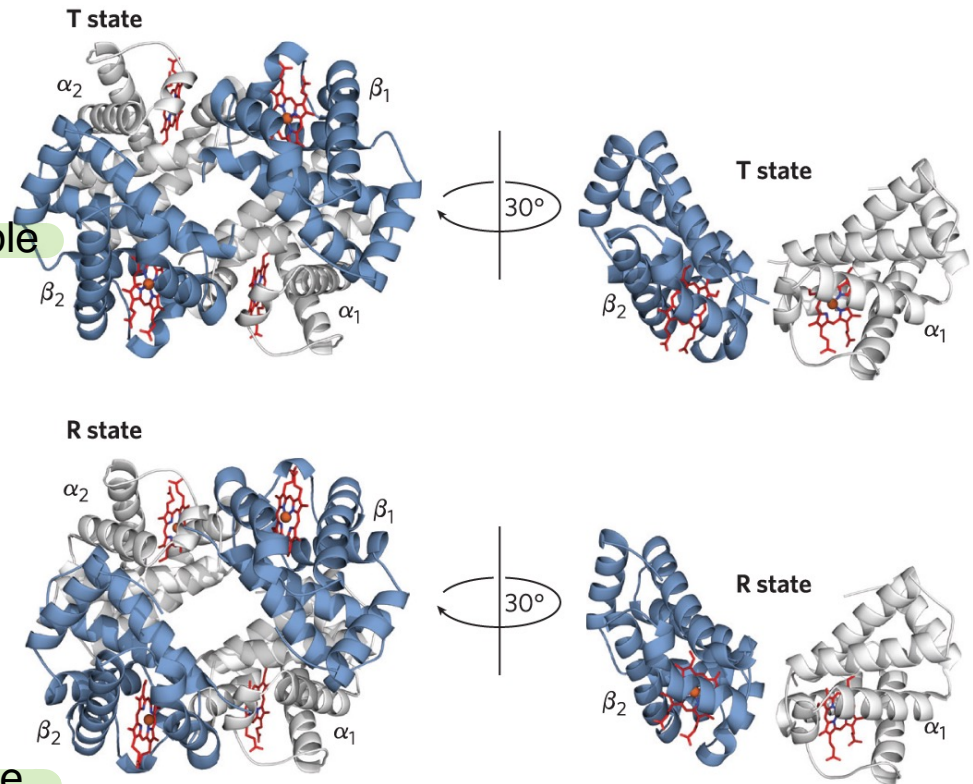
Relax — **R state** = O₂ has a higher affinity for hemoglobin

— Fewer interactions, more flexible

Tense — **T state** = more stable when O₂ is absent, predominant conformation of **deoxyhemoglobin**

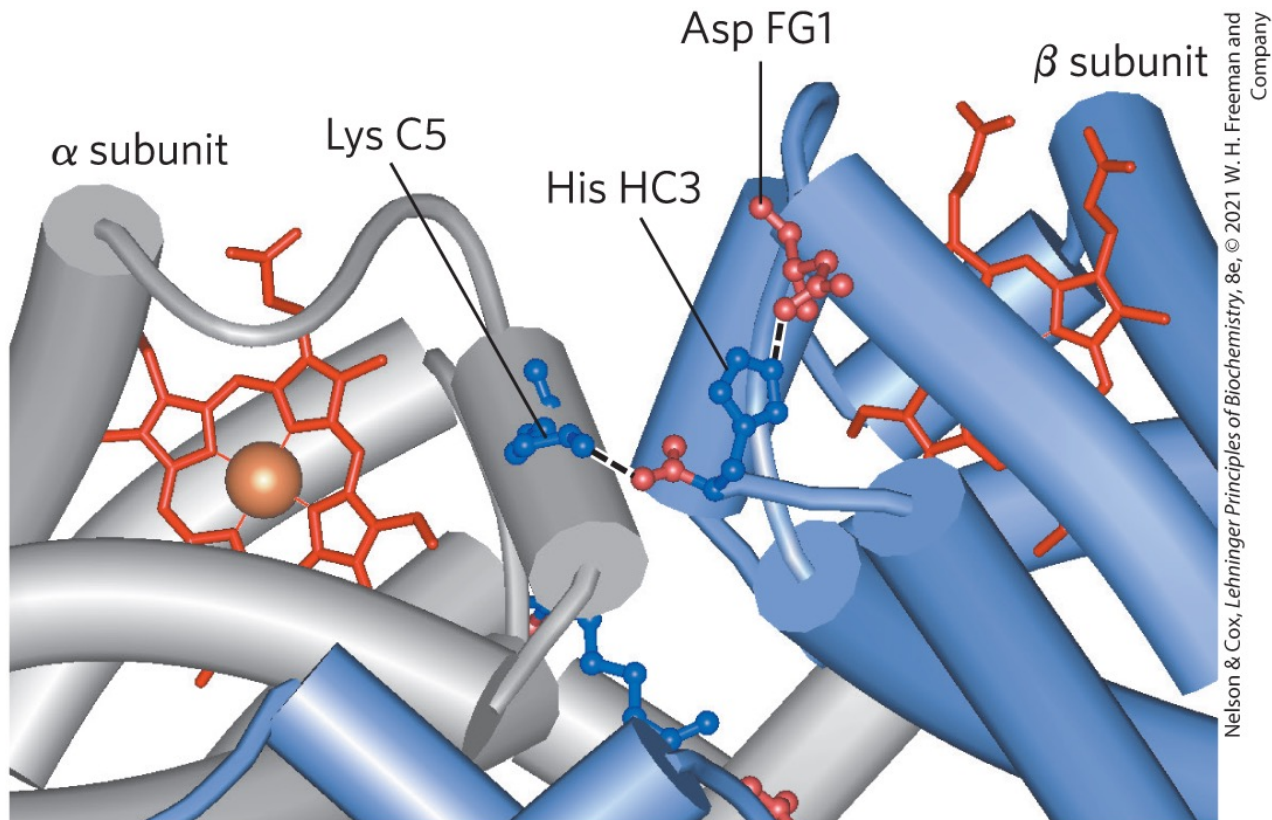
— More interactions, more stable

Greater number of ion pairs



Ion Pairs Stabilize the T State

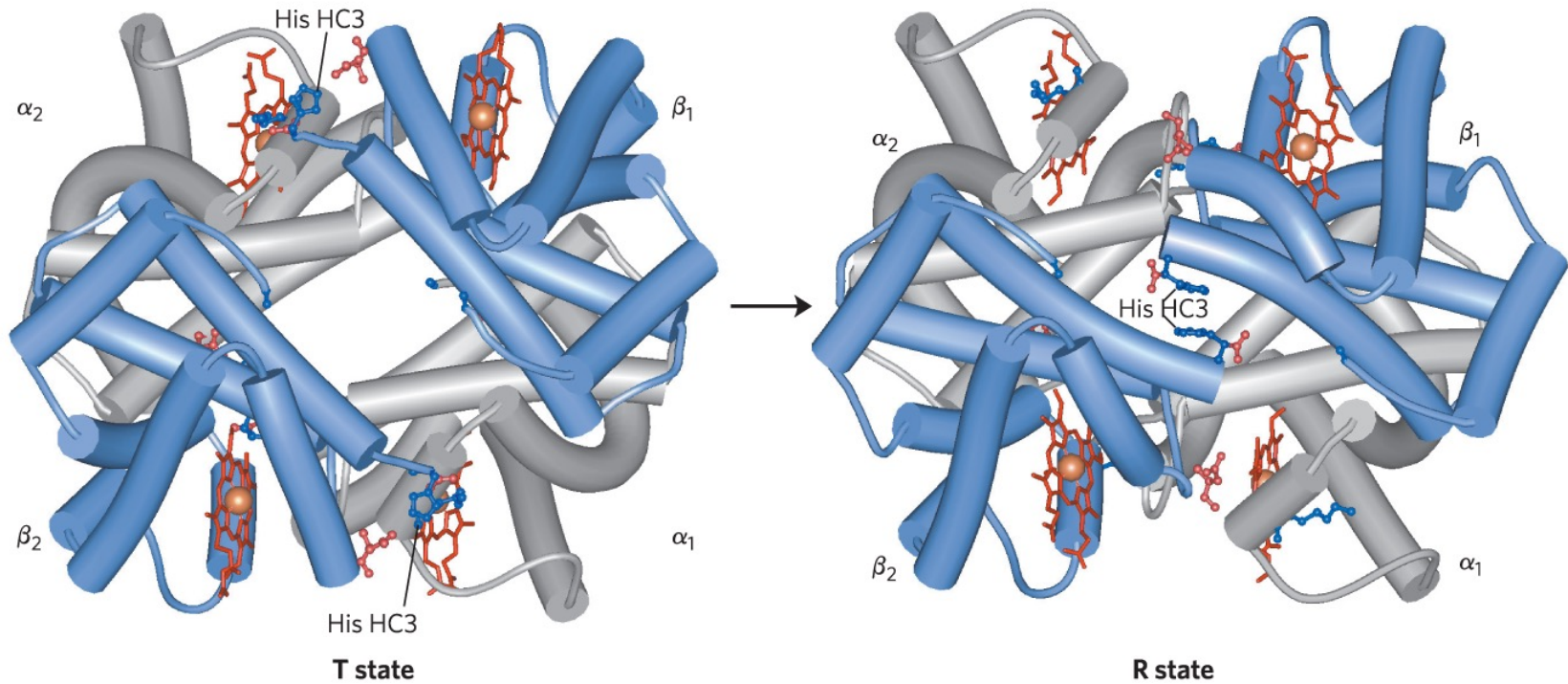
- T state is stabilized by a greater number of ion pairs, many of which lie at the $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface



Conformational Change in Hemoglobin

- O₂ binding to hemoglobin in the T state triggers a conformational change to the R state
 - $\alpha\beta$ subunit pairs slide past each other and rotate
 - the pocket between the β subunits narrow
 - some ion pairs that stabilize the T state break and some new ones form

The T \rightarrow R Transition



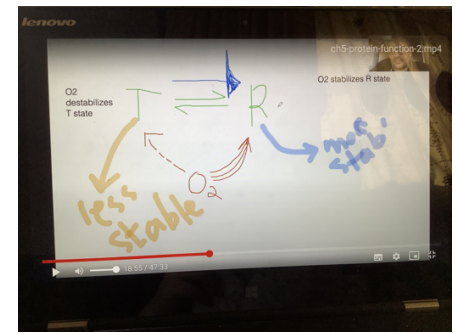
Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2011 W. H. Freeman and Company

 Video:

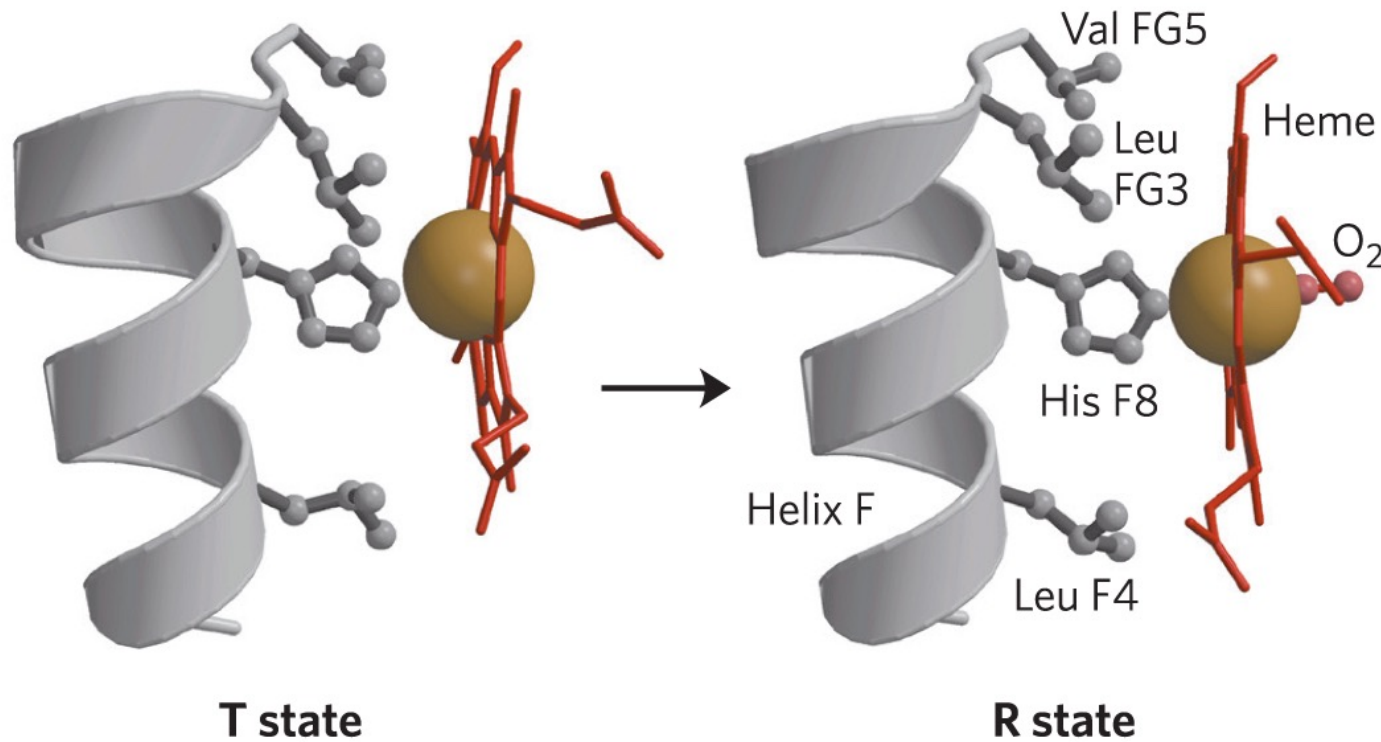
1) HEMOGLOBIN AND MYOGLOBIN BIOCHEMISTRY

Hb Changes Structure after O₂ Binding

- O₂ binding stabilizes R state
- T state is more stable when not bound to O₂ (deoxyhemoglobin)
- *O₂ binding to a Hb subunit at the T state converts the subunit to R state*
- Therefore, O₂ binding triggers a **T** → **R** conformational change
- Conformational change from the T state to the R state involves **breaking ion pairs** between the $\alpha 1$ - $\beta 2$ interface



Changes in Conformation Near Heme



Clicker Question 8

The T \rightarrow R transition is triggered by:

- A. ion pairs forming between residues and O₂ at the $\alpha_1\beta_2$ (and $\alpha_2\beta_1$) interface.
- B. the structures of the individual subunits changing.
- C. O₂ causing the heme to assume a more planar conformation, shifting the position of His F8 and the F helix.
- D. $\alpha\beta$ subunit pairs sliding past each other and rotating, narrowing the pocket between the α subunits.

Clicker Question 8, Response

The T \rightarrow R transition is triggered by:

C. O₂ causing the heme to assume a more planar conformation, shifting the position of His F8 and the F helix.

In the T state, the porphyrin is slightly puckered, causing the heme iron to protrude on the His F8 side. O₂ binding causes the heme to assume a more planar conformation, shifting the position of His F8 and the F helix. This leads to adjustments in the ion pairs at the $\alpha_1\beta_2$ interface.



Principle 5

In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.

Could myoglobin transport O₂?

- pO₂ in lungs is about 13 kPa: it sure binds oxygen well
- pO₂ in tissues is about 4 kPa: it will not release it!

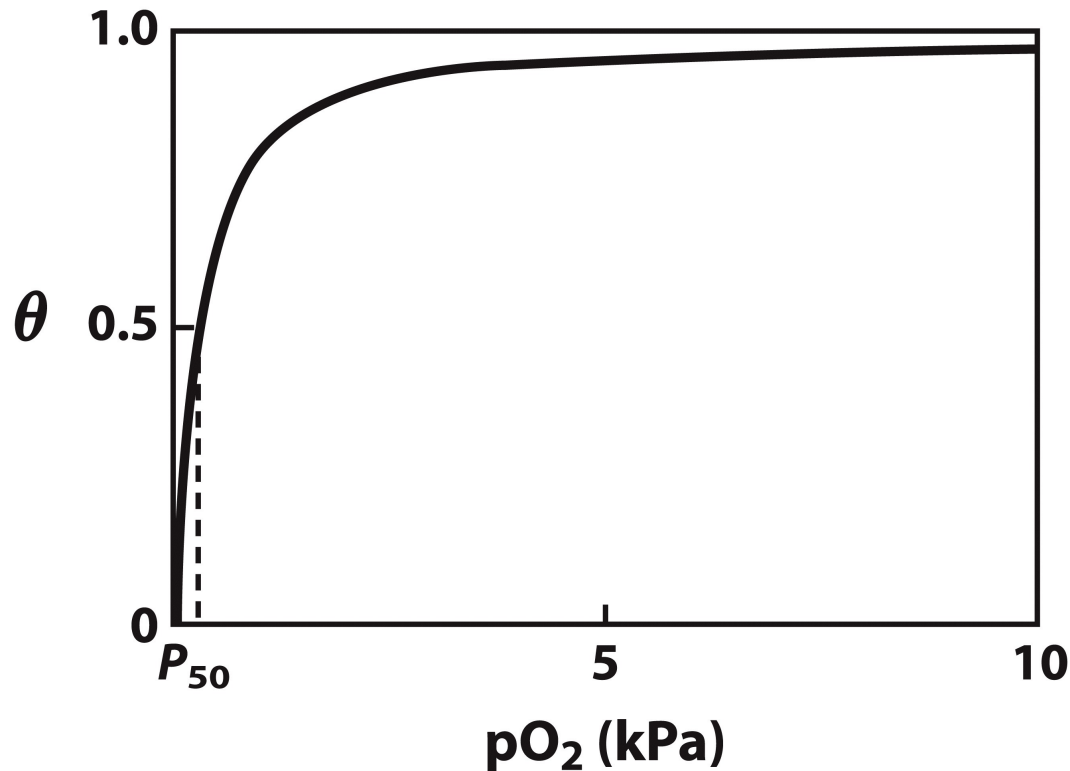
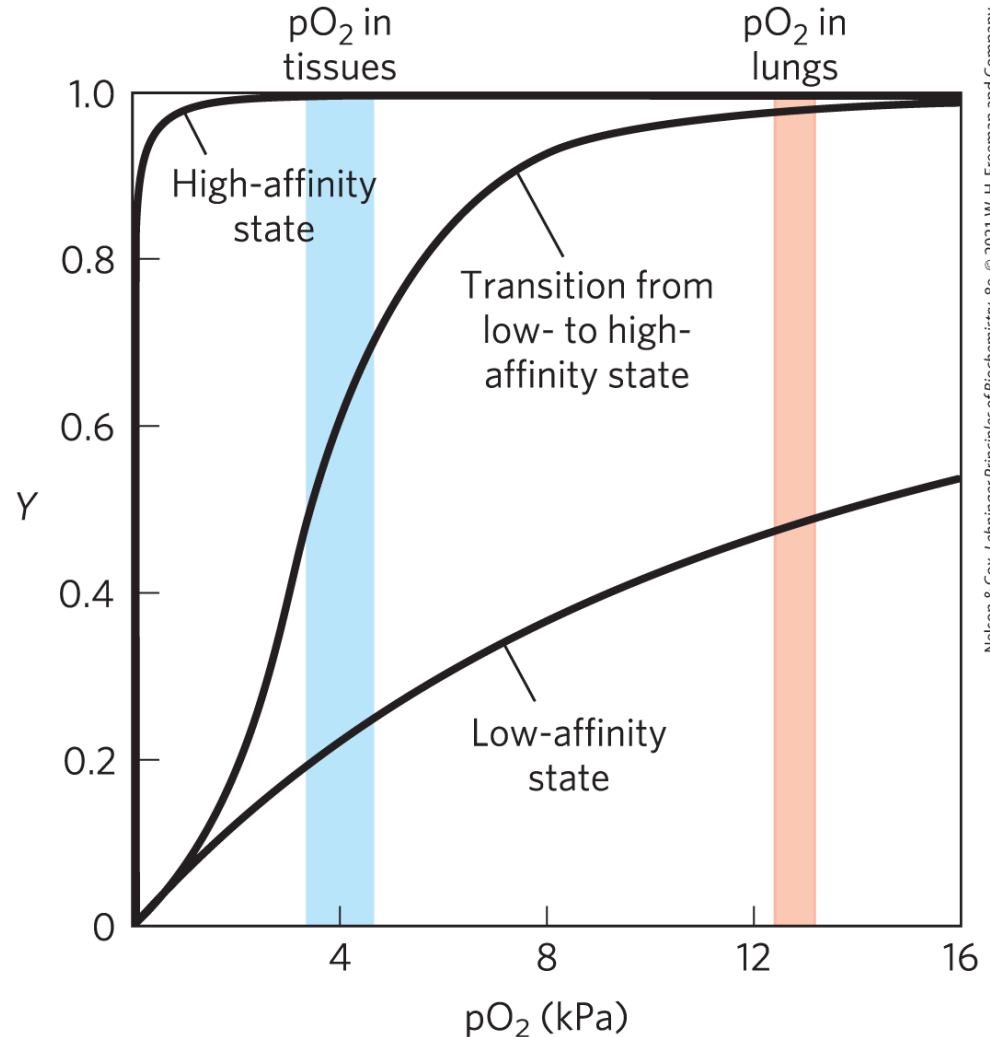


Figure 5-4b
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- Would lowering the affinity (P_{50}) of myoglobin to oxygen help?

Hemoglobin Binds Oxygen Cooperatively

- For effective transport, affinity *must* vary with pO_2
- Hemoglobin has a hybrid sigmoid binding curve for oxygen



Clicker Question 9

Leghemoglobin is an oxygen-binding protein in root nodules that contain bacteria and fix atmospheric nitrogen. Which statement is true if leghemoglobin is like myoglobin and not hemoglobin?

- A. There are four oxygen-binding sites.
- B. The oxygen-binding curve is hyperbolic.
- C. There is cooperative oxygen binding.
- D. The oxygen-binding curve is sigmoidal.
- E. Oxygen binding changes the heme configuration from T to R.

Clicker Question 9, Response

Leghemoglobin is an oxygen-binding protein in root nodules that contain bacteria and fix atmospheric nitrogen. Which statement is true if leghemoglobin is like myoglobin and not hemoglobin?

B. The oxygen-binding curve is hyperbolic.

For a monomeric protein such as myoglobin, the fraction of binding sites occupied by a ligand is a hyperbolic function of ligand concentration. Thus, if leghemoglobin is like myoglobin, the oxygen-binding curve is hyperbolic.



Principle 6

Interactions between ligands and proteins may be regulated.

Allosteric Proteins

- **allosteric protein** (e.g., hemoglobin) = binding of a ligand to one site affects the binding properties of another site on the same protein
 - **modulators** = ligands that bind to an allosteric protein to induce a conformational change
 - **homotropic** = normal ligand and modulator are identical
 - **heterotropic** = modulator is a molecule other than the normal ligand

* Cooperativity it's a special case of allosteric regulation

P6

Clicker Question 10

Which molecule is a homotropic modulator of oxygen binding to hemoglobin?

- A. oxygen
- B. H^+
- C. carbon dioxide
- D. hemoglobin
- E. carbon monoxide

Clicker Question 10, Response

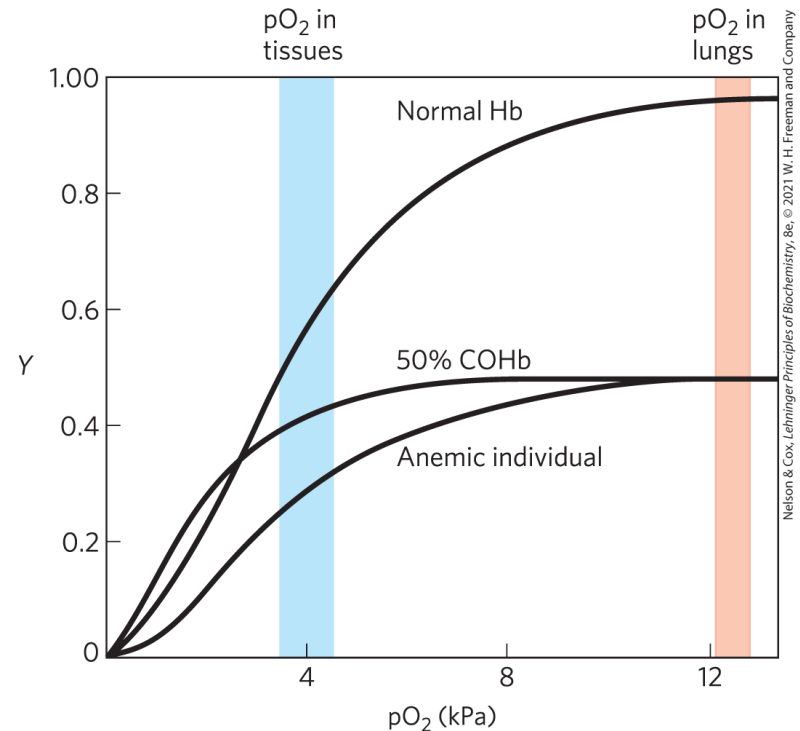
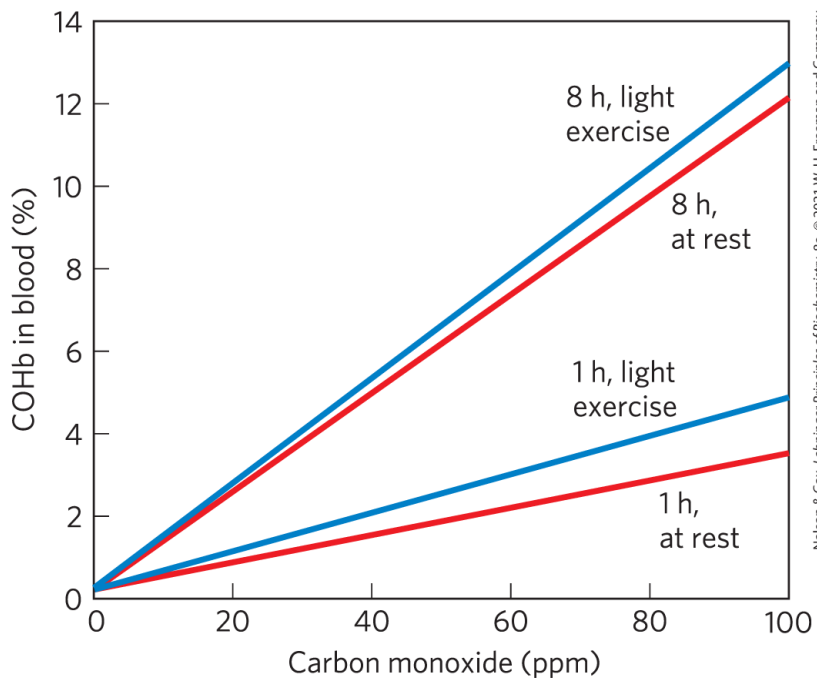
Which molecule is a homotropic modulator of oxygen binding to hemoglobin?

A. oxygen

Cooperative binding of oxygen to hemoglobin, a multimeric protein, is a form of allosteric binding. The binding of one ligand affects the affinities of any remaining unfilled binding sites. Oxygen can be considered as both a ligand and an activating homotropic modulator.

Carbon Monoxide Can Bind to Hemoglobin

- CO has ~250-fold greater affinity for hemoglobin than does O₂



How can affinity to oxygen change?

- Must be a protein with **multiple binding sites**
 - Binding sites must be able to **interact with each other**
 - This phenomenon is called **cooperativity**
 - positive cooperativity
 - first binding event increases affinity at remaining sites
 - **recognized by sigmoidal binding curves**
 - negative cooperativity
 - first binding event reduces affinity at remaining sites
- Curve shows that oxygen binding is cooperative
- At high oxygen pressure, the affinity of protein to oxygen increase
 - At low oxygen pressure, the affinity of protein to oxygen decrease

Cooperative Ligand Binding Can Be Described Quantitatively

- for a protein with n binding sites, the equilibrium becomes:



* Oxygen binding to hemoglobin is a positive cooperativity

The K_a and Y for Cooperative Ligand Binding

- the expression for the association constant becomes:

$$K_a = \frac{[PL_n]}{[P][L]^n} \quad (5-13)$$

- the expression for Y is:

$$Y = \frac{[L]^n}{[L]^n + K_d} \quad (5-14)$$

The Hill Equation

$$Y = \frac{[L]^n}{[L]^n + K_d} \quad (5-14)$$

- rearranging, then taking the log of both sides, yields:

$$\frac{Y}{1 - Y} = \frac{[L]^n}{K_d} \quad (5-15)$$

the **Hill equation**:

$$\log\left(\frac{Y}{1 - Y}\right) = n \log [L] - \log K_d \quad (5-16)$$

Hill Plots and Hill Coefficients

- **Hill plot** = plot of $\log [Y/(1 - Y)]$ versus $\log [L]$
- **Hill coefficient** = n_H = slope of a Hill plot
 - If $n_H = 1$, ligand binding is not cooperative
 - $n_H > 1$ indicates positive cooperativity
 - $n_H < 1$ indicates negative cooperativity

P6

Clicker Question 11

A newly discovered protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site increases the binding affinity of other sites for the ligand. The Hill coefficient (n_H) is:

- A. equal to 1.
- B. greater than 1.
- C. less than 1.
- D. not able to be calculated.

Clicker Question 11, Response

A newly discovered protein has multiple subunits, each with a single ligand-binding site. Binding of ligand to one site increases the binding affinity of other sites for the ligand. The Hill coefficient (n_H) is:

B. greater than 1.

The Hill coefficient (n_H) is the slope of a Hill plot. An n_H of greater than 1 indicates positive cooperativity in ligand binding. This is the situation observed in the newly discovered protein, in which the binding of one molecule of ligand facilitates the binding of others.

Adapting the Hill Equation to the Binding of O₂ to Hemoglobin

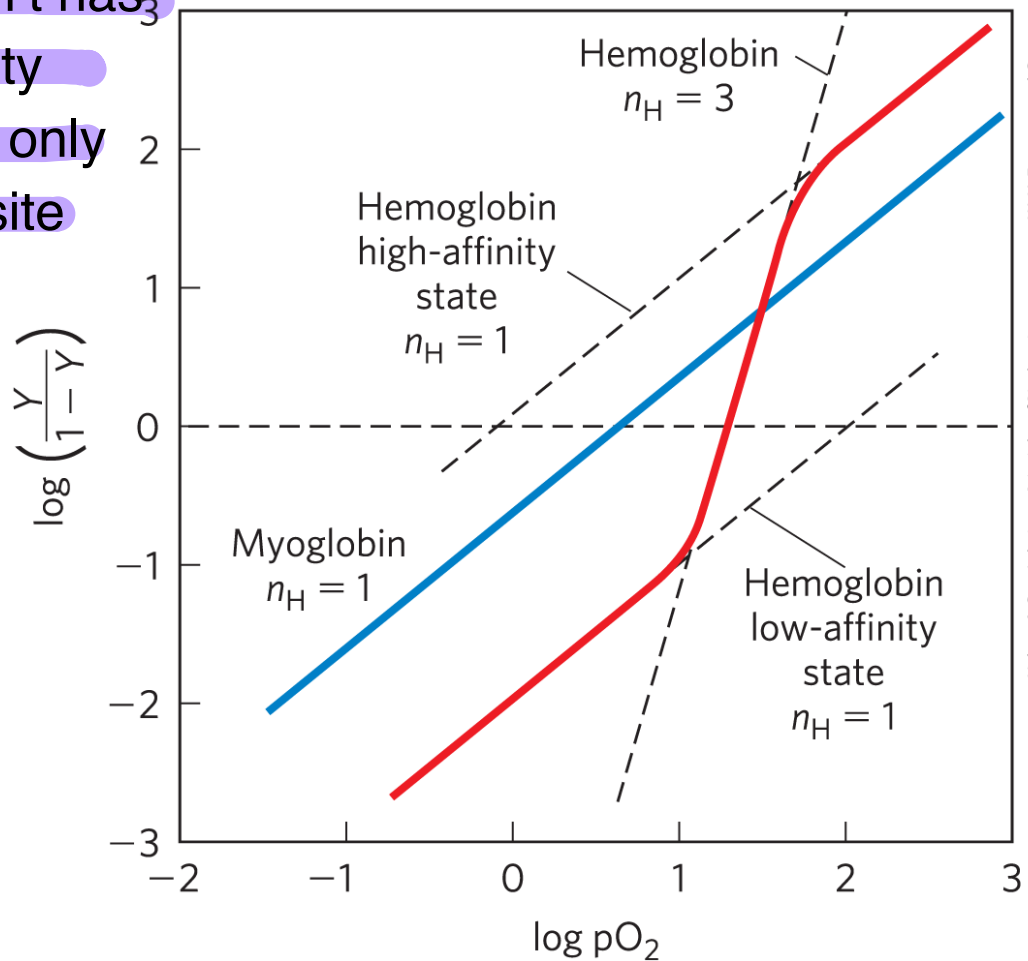
- substituting pO₂ for [L] and P_{50}^n for K_d :

$$\log \left(\frac{Y}{1-Y} \right) = n \log pO_2 - n \log P_{50} \quad (5-17)$$

- **Hill coefficient** = n_H = slope of a Hill plot
 - If $n_H = 1$, ligand binding is not cooperative
 - $n_H > 1$ indicates positive cooperativity
 - $n_H < 1$ indicates negative cooperativity

Hill Plots for Myoglobin and Hemoglobin

* Myoglobin doesn't have cooperativity because it has only one binding site



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P6

Clicker Question 12

There are two binding sites for acetylcholine on its receptor. What would be the shape of a Hill plot if there were cooperativity of binding?

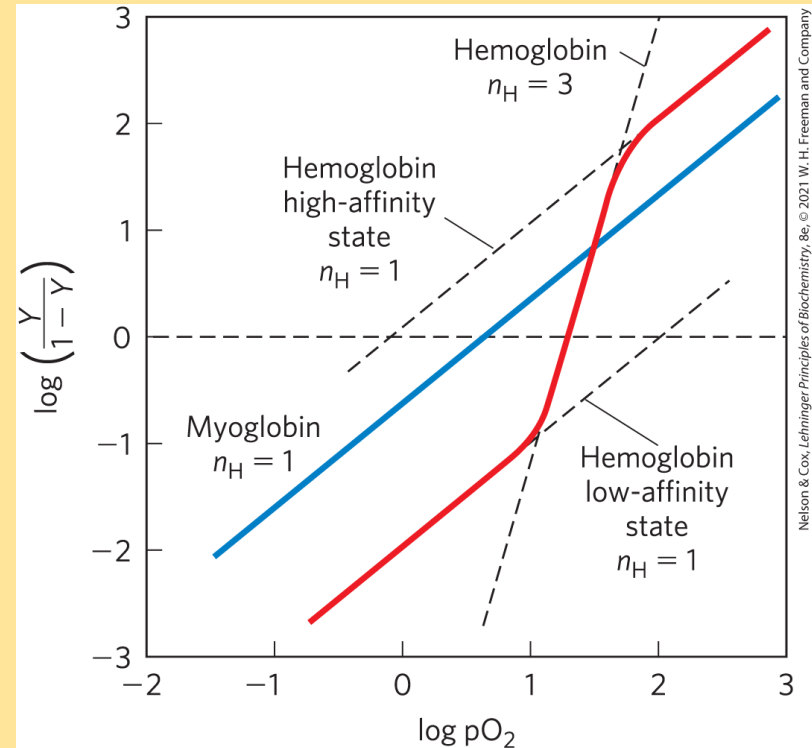
- A. hyperbolic
- B. linear
- C. sigmoidal, with a sharp change in slope
- D. two sigmoidal areas linked together, one for each binding event
- E. two sigmoidal lines, one higher than the other on the graph

Clicker Question 12, Response

There are two binding sites for acetylcholine on its receptor. What would be the shape of a Hill plot if there were cooperativity of binding?

C. sigmoidal, with a sharp change in slope

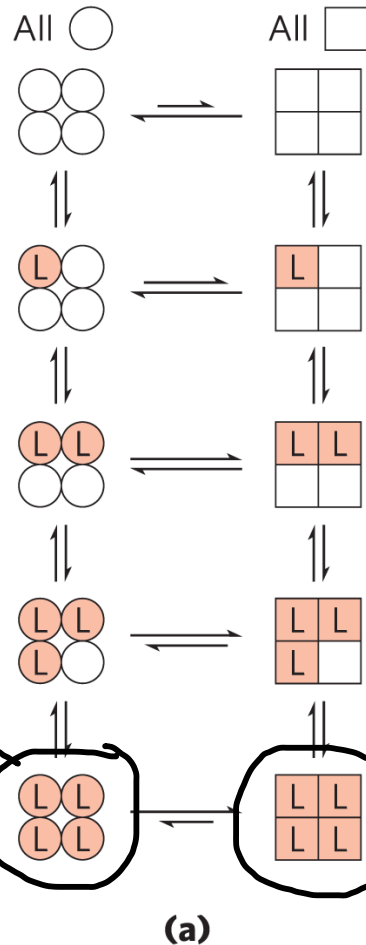
The shape of the Hill plot would resemble that of oxygen binding to hemoglobin.



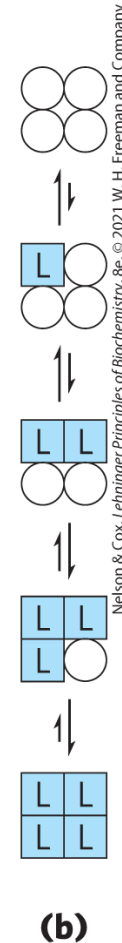
Two Models Suggest Mechanisms for Cooperative Binding

- **MWC model = concerted model**
 - all subunits in the same conformation
 - ligand binds more tightly to the R state
 - **sequential model**
 - each subunit can be in either conformation
 - equilibrium is altered as additional ligands are bound, progressively favoring the R state
- All subunits have high affinity to oxygen or all subunits have low affinity to oxygen

Concerted and Sequential Models



MWC model
(concerted model)



Sequential model

- Low affinity or inactive
- High affinity or active

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P5

Clicker Question 13

Regarding the models of cooperativity:

- A. the concerted model and sequential model only apply to tetramers.
- B. the concerted model and the sequential model are mutually exclusive.
- C. the concerted model is based on the T state and the sequential on the R state.
- D. T state is low affinity and R state is high affinity.

Clicker Question 13, Response

Regarding the models of cooperativity:

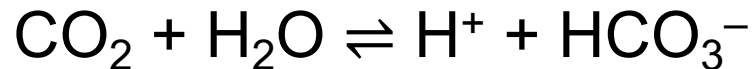
D. T state is low affinity and R state is high affinity.

In both the concerted model and sequential models of cooperativity, the T state is the low affinity state and the R state is the high affinity state.

Hemoglobin Also Transports H⁺ and CO₂

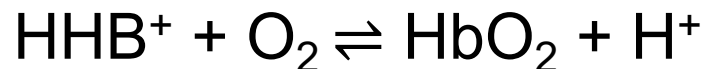
* Actively metabolizing tissues generate H⁺, lowering the pH of the blood near the tissues relative to the lungs

- hemoglobin carries two end products of cellular respiration: H⁺ and CO₂
- **carbonic anhydrase** catalyzes the hydration of CO₂ to bicarbonate:



The Bohr Effect

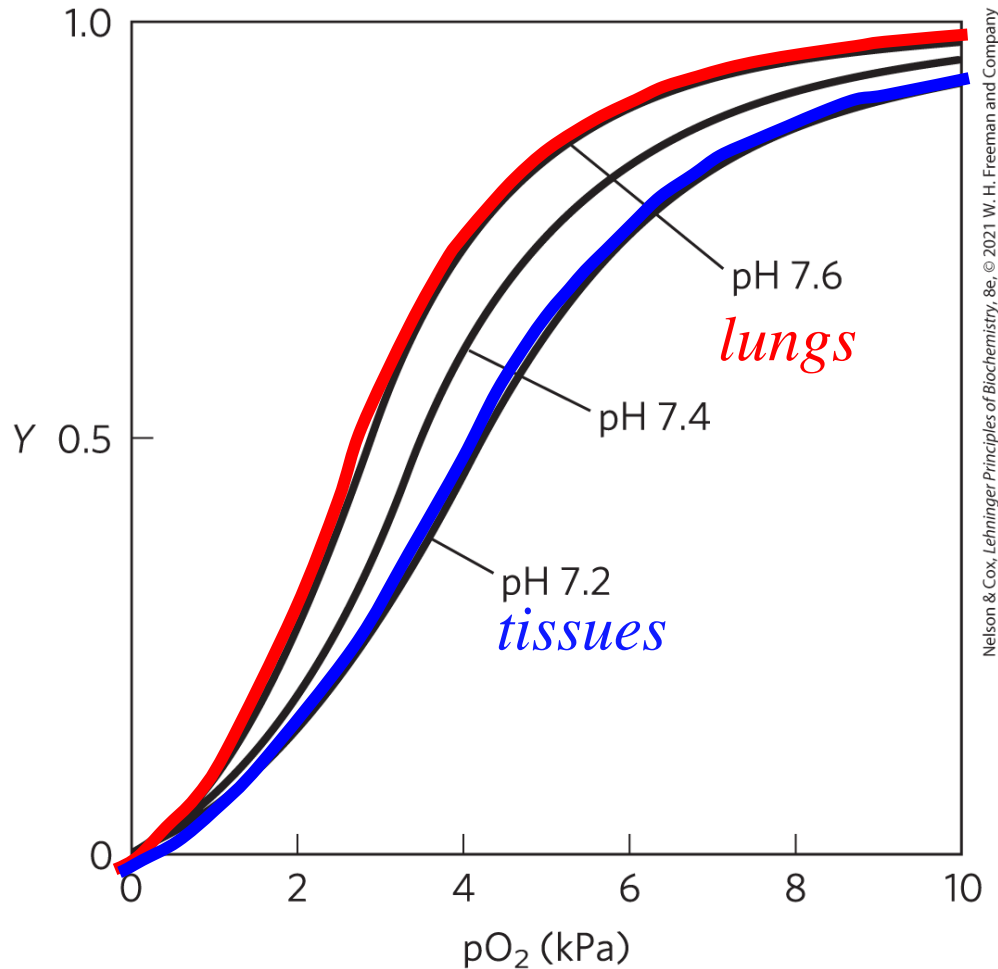
- the structural effects of H⁺ and CO₂ binding to hemoglobin favor the T state
 - the binding of H⁺ and CO₂ is inversely related to the binding of O₂
- **Bohr effect** = describes the effect of pH and [CO₂] on the binding and release of O₂ by hemoglobin



* The pH difference between lungs and metabolic tissues increases efficiency of the O₂ transport.

The Effect of pH on O₂ Binding to Hemoglobin

Negative hetrotropic regulation

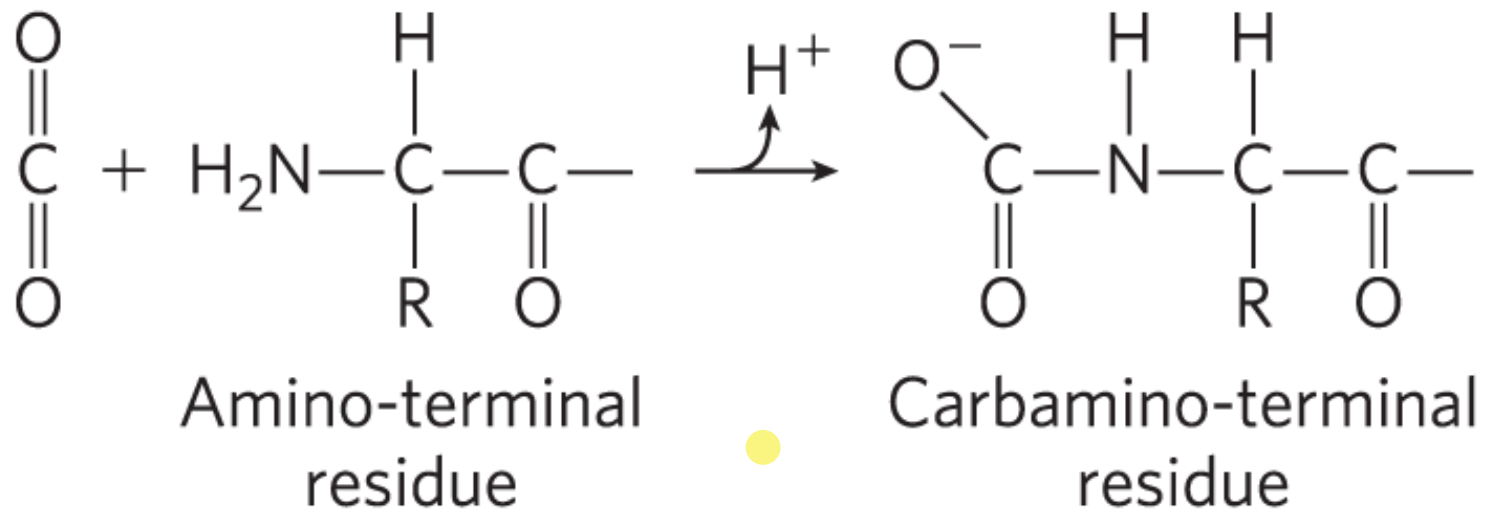


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- when [O₂] is high, hemoglobin binds O₂ and releases H⁺
- when [O₂] is low, hemoglobin releases O₂ and binds H⁺

Hemoglobin Binds CO₂

- CO₂ binding to hemoglobin is inversely related to binding of O₂
 - contributes to the Bohr effect by producing H⁺



- 15–20% of CO₂ is exported in the form of a carbamate on the amino terminal residues of each of the polypeptide subunits.
 - the formation of a carbamate yields a proton which can contribute to the Bohr Effect
 - the carbamate forms additional salt bridges stabilizing the T state

P6

Clicker Question 14

In peripheral tissues:

- A. the affinity of hemoglobin for oxygen increases and the protein binds more O_2 .
- B. CO_2 is excreted.
- C. the structural effects of H^+ and CO_2 binding to hemoglobin favor the R state.
- D. O_2 is released.

Clicker Question 14, Response

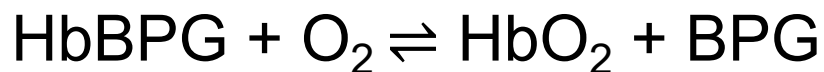
In peripheral tissues:

D. O₂ is released.

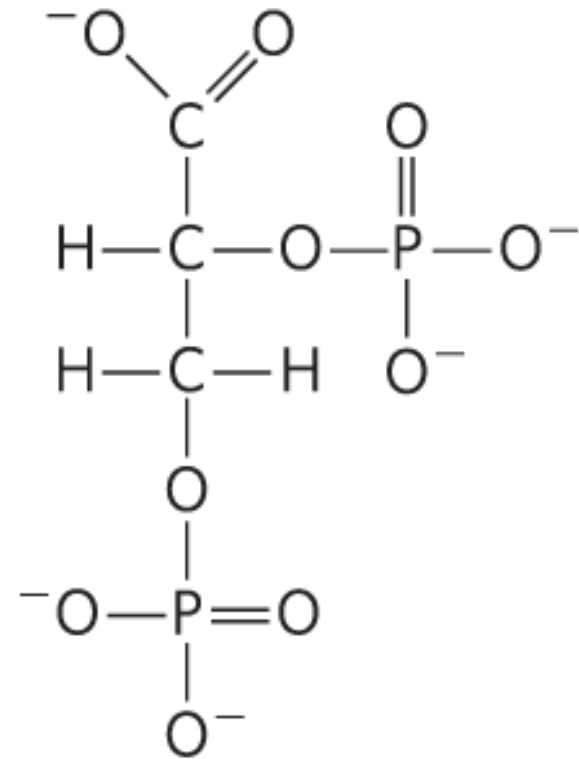
At the relatively low pH and high CO₂ concentration of peripheral tissues, the affinity of hemoglobin for oxygen decreases as H⁺ and CO₂ are bound, and O₂ is released to the tissues.

Oxygen Binding to Hemoglobin Is Regulated by 2,3-Bisphosphoglycerate

- **2,3-bisphosphoglycerate (BPG):**
 - example of heterotropic allosteric modulation
 - binds to a site distant from O₂-binding site
 - greatly reduces the affinity of hemoglobin for oxygen (**stabilizes the T state**)

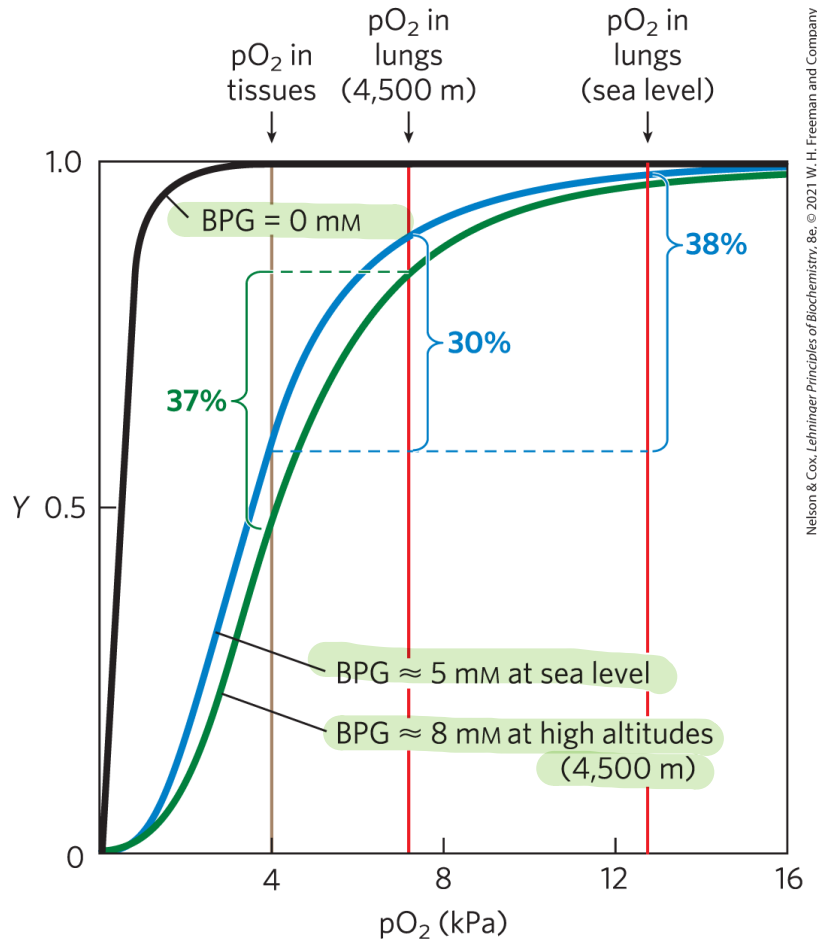


Small negatively charged molecule, binds to the positively charged central cavity of Hb



2,3-Bisphosphoglycerate

Effect of BPG on O₂ Binding to Hemoglobin



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- BPG increases at high altitudes
- **hypoxia** = lowered oxygenation of peripheral tissues
 - causes BPG increases

*At $BPG = 0$, hemoglobin binding curve resemble myoglobin binding curve. There are no cooperativity, so oxygen binding will be very fast

*At sea level, Hb is nearly saturated with O_2 in the lungs

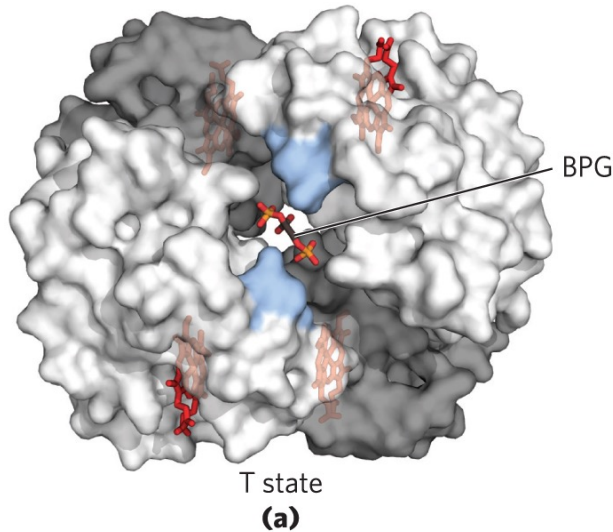
-Hb is just over 60% saturated in the tissues

-The amount of O_2 released in the tissues is about 38% of the maximum that can be carried in the blood

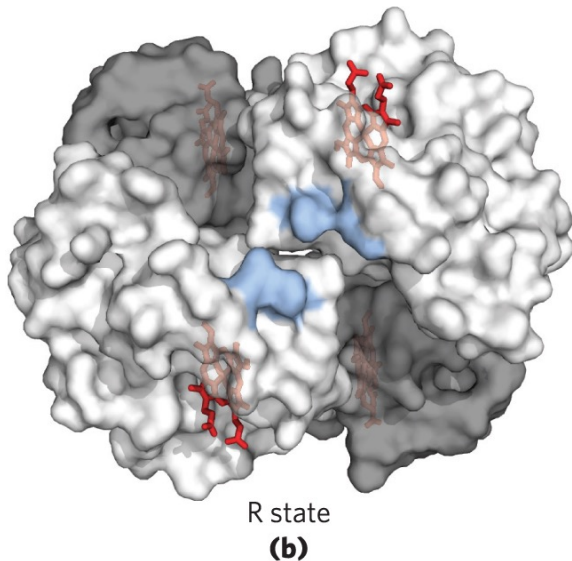
*At high altitudes, O_2 delivery declines to 30% of maximum

-An increase in $[BPG]$ decreases the affinity of Hb for O_2 , so ~ 37% of what can be carried is again delivered to the tissues

Binding of BPG to Deoxyhemoglobin



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- BPG binds to the cavity between the β subunits in the T state
 - cavity is lined with positively charged residues
 - BPG stabilizes the T state

P6

Clicker Question 15

What is the actual, significant effect of 2,3-bisphosphoglycerate on oxygen binding by hemoglobin?

- A. There is an increase in K_d for oxygen in peripheral tissues.
- B. A higher concentration of oxygen can bind at high altitudes compared to low altitudes.
- C. The affinity of hemoglobin for oxygen in peripheral tissues increases at high altitudes.
- D. Heme further decreases affinity for both CO_2 and H^+ in lungs, allowing more O_2 to bind.
- E. It has no effect on O_2 affinity, but it forces heme into the R state.

Clicker Question 15, Response

What is the actual, significant effect of 2,3-bisphosphoglycerate on oxygen binding by hemoglobin?

A. There is an increase in K_d for oxygen in peripheral tissues.

2,3-Bisphosphoglycerate greatly reduces the affinity of hemoglobin for oxygen. A higher value of K_d corresponds to a lower affinity of ligand for the protein. Thus, 2,3-bisphosphoglycerate increases the K_d for oxygen binding by hemoglobin.

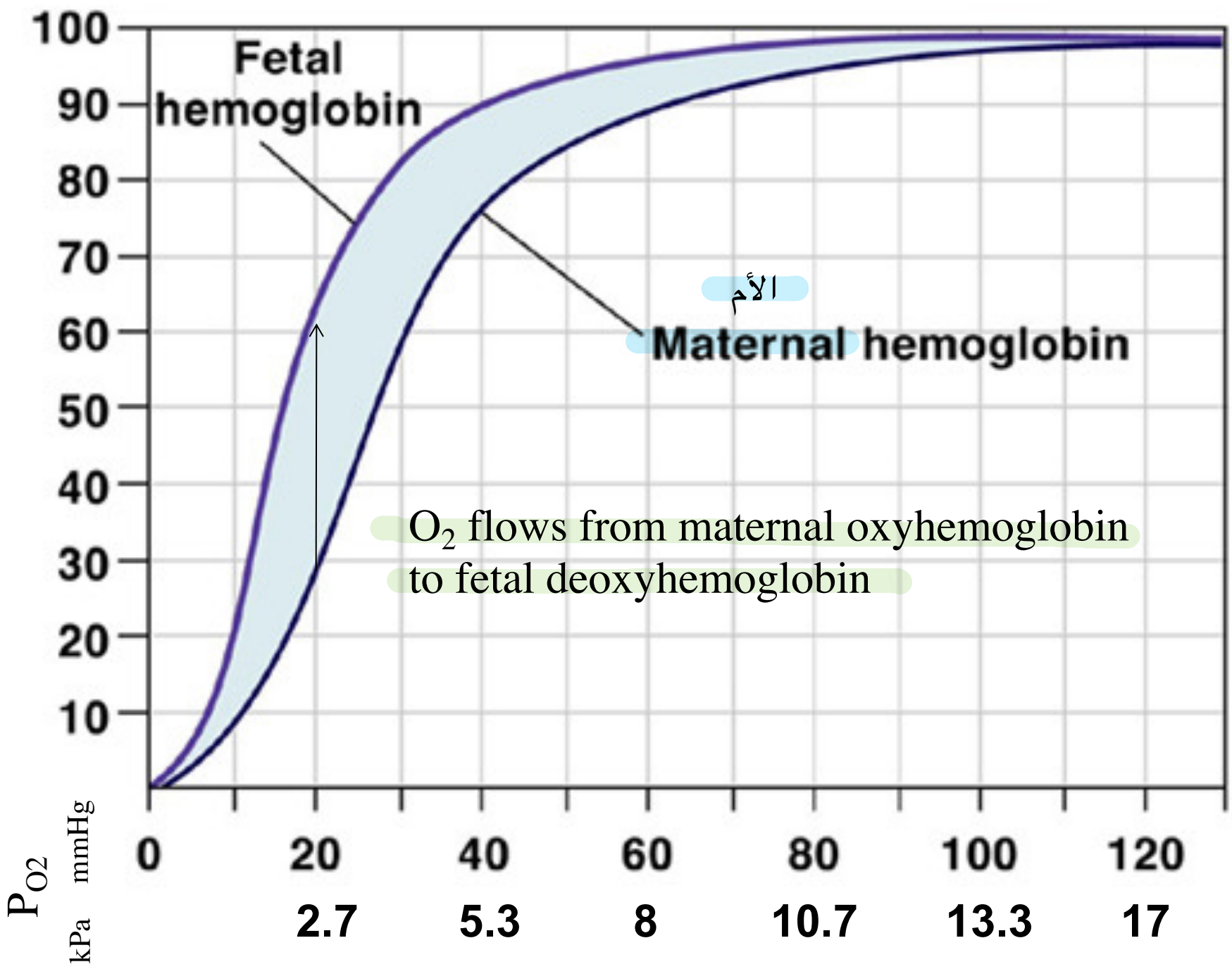
Fetal Hemoglobin (HbF)

- fetus synthesizes $\alpha_2\gamma_2$ hemoglobin
 - Fewer positive charges than the adult hemoglobin β subunit; *2,3-BPG binds less*)
 - Higher affinity for O_2 than normal adult hemoglobin
 - fetus can *يستخرج، يستخلص* extract O_2 from his/her mother bloodstream easily
- The affinity of HbF for oxygen > that of HbA (P₅₀ HbF ~ 2.5 kPa; P₅₀ HbA ~ 3.7 kPa)
- The oxygen saturation curve is shifted to the left for HbF
- HbF does not interact with 2,3-BPG (which decreases the affinity of HbA for oxygen) → **HbF binds O₂ tighter than HbA**

A → adult

F → fetus

Percent O₂ saturation of hemoglobin



Fetal hemoglobin

الأم
Maternal hemoglobin

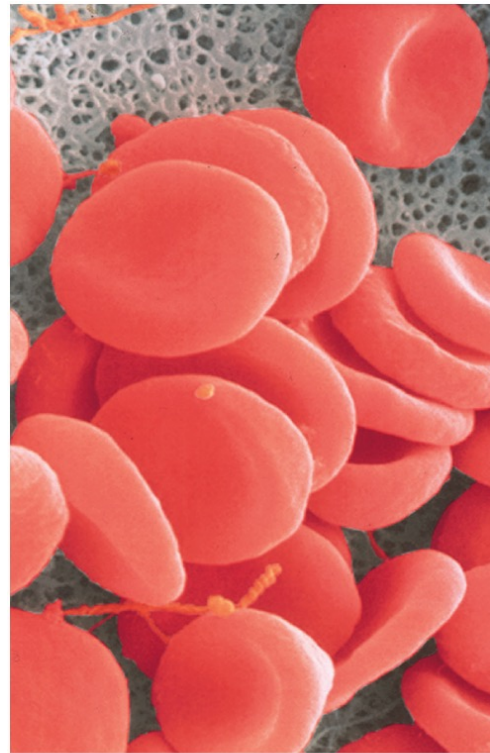
O₂ flows from maternal oxyhemoglobin to fetal deoxyhemoglobin

P_{O2}
kPa

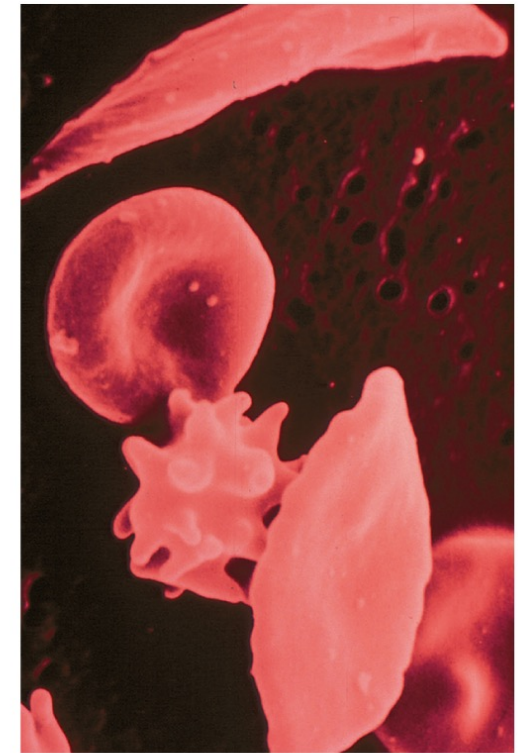
0 20 40 60 80 100 120
2.7 5.3 8 10.7 13.3 17

Sickle Cell Anemia Is a Molecular Disease of Hemoglobin

- sickle cell anemia:
 - homozygous condition
 - single amino acid substitution (Glu⁶ to Val⁶) β chains produces a hydrophobic patch



(a) 2 μ m



(b)

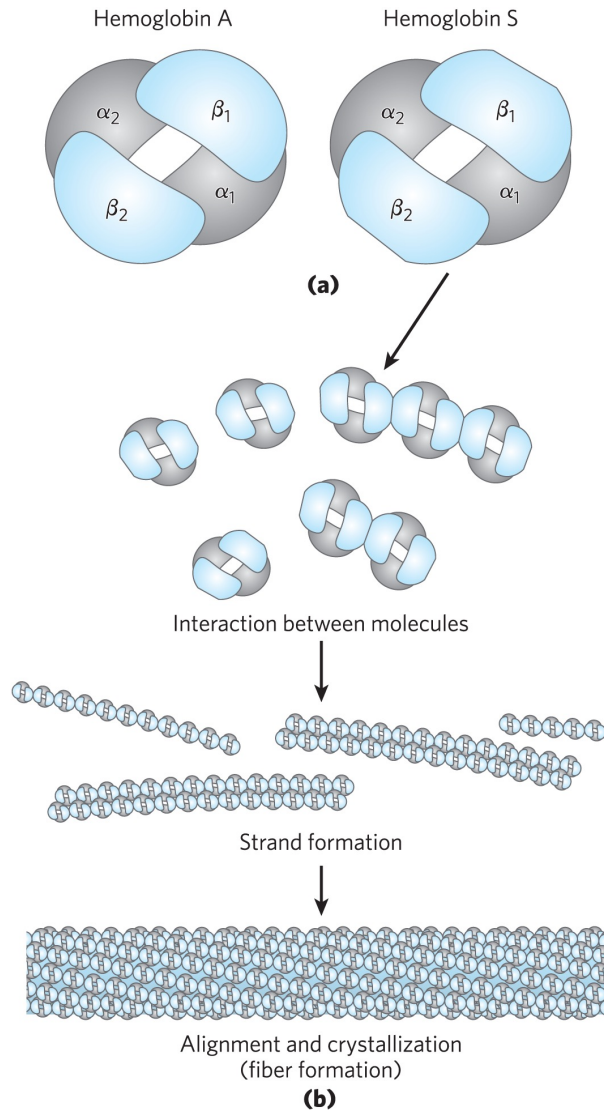
When Hb from a sick patient is deoxygenated (Hb S) it aggregates and precipitates (normal Hb, Hb A does not precipitate upon deoxygenation)

(a) A. Syred/Science; (b) Jackie Lewin, Royal Free Hospital/Science Source.

 Video:

1) Sickle Cell Disease, Animation

Normal and Sickle Cell Hemoglobin



- deoxygenated hemoglobin becomes insoluble and forms polymers that aggregate
- normal hemoglobin remains soluble upon deoxygenation

Clicker Question 16

Which statement is false?

- A. Hemoglobin can be considered a dimer of $\alpha\beta$ dimers.
- B. O_2 binding to myoglobin is allosteric but not cooperative.
- C. 2,3-bisphosphoglycerate decreases the affinity of hemoglobin for O_2 .
- D. Fetal hemoglobin has a lower affinity for BPG than normal adult hemoglobin.
- E. The Glu⁶ to Val⁶ that causes sickle cell anemia produces a hydrophobic patch on hemoglobin's surface.

Clicker Question 16, Response

Which statement is false?

B. O₂ binding to myoglobin is allosteric but not cooperative.

Cooperative binding of a ligand to a multimeric protein, such as the binding of O₂ to hemoglobin, is a form of allosteric binding. As such, O₂ binding to myoglobin is both allosteric and cooperative.

5.2 Complementary Interactions between Proteins and Ligands: The Immune System and Immunoglobulins

Immune Responses

منسق

- **immune response** = coordinated set of interactions among many classes of proteins, molecules, and cell types
 - distinguishes molecular “self” from “nonself” and destroys “nonself”
 - eliminates viruses, bacteria, and other pathogens and molecules

* Autoimmune diseases occur when immune system attack the body cells because the ability of immune system to distinguish between self or non-self become weak

The Immune Response Includes a Specialized Array of Cells and Proteins

- **leukocytes** = white blood cells, including **macrophages** and **lymphocytes**
- The immune response consists of two complementary systems:
 - **humoral immune system** = directed at bacterial infections and extracellular viruses
 - **cellular immune system** = destroys infected host cells, parasites, and foreign tissues

 Videos:

- 1) The Immune Response HD Animation
- 2) The Adaptive Immune System

The Humoral Immune Response

* 20% of blood is antibodies

- **antibodies = immunoglobulins (Ig)** = bind bacteria, viruses, or large molecules identified as foreign and target them for destruction
 - produced by **B lymphocytes or B cells**

* B cells produce in bones marrow and mature in bone marrow

* T cells produce in bone marrow and mature in thymus gland



The Cellular Immune Response

- **T lymphocytes = cytotoxic T cells (T_C cells)**
 - recognition of infected cells or parasites involves **T-cell receptors** on the surface of T_C cells
- **helper T cells (T_H cells)** = produce soluble signaling proteins called cytokines
 - interact with macrophages توالد
 - stimulate the selective **proliferation** of T_C and B cells that can bind to a particular antigen (**clonal selection**)
- **memory cells** = permit a rapid response to pathogens previously encountered

Vaccines

- often consists of weakened or killed virus or isolated proteins from a viral or bacterial protein coat
- “teaches” the immune system what the viral particles look like, stimulating the production of memory cells

P2

Clicker Question 17

Which statement is false?

- A. T lymphocytes produce immunoglobulins.
- B. Immunoglobulins target foreign bacteria and viruses for destruction.
- C. Memory cells allow the body to quickly respond to viruses it has encountered before.
- D. Clonal selection increases the number of immune system cells that can respond to a particular pathogen.
- E. Vaccines stimulate the production of memory cells.

Clicker Question 17, Response

Which statement is false?

A. T lymphocytes produce immunoglobulins.

T lymphocytes produce T-cell receptors on the surface of T cells. Recognition of infected cells or parasites involves these T-cell receptor proteins.

Antigens and Haptens

* Antigen is a protein or macromolecule

إثارة

- **antigen** = molecule or pathogen capable of eliciting an immune response
 - can be a virus, a bacterial cell wall, or an individual protein or other macromolecule
 - antibodies or T-cell receptors bind to an **antigenic determinant** or **epitope** within the antigen
- The part of an antigen molecule to which an antibody attaches itself
- **haptens** = small molecules that can elicit an immune response when covalently attached to large proteins

Antibodies Have Two Identical Antigen-Binding Sites

- **immunoglobulin G (IgG)** = major class of antibodies
 - one of the most abundant blood serum proteins
 - 4 polypeptide chains: 2 heavy chains and 2 light chains
 - cleavage with protease papain releases the basal fragment **Fc** and two **Fab** branches (each with a single antigen-binding site)
 - constant domains contain the **immunoglobulin fold** structural motif
- * Variable domains give the specificity for the IgG



Clicker Question 18

The molecular structure for which an individual antibody or T-cell receptor is specific is known as:

- A. an epitope.
- B. its target.
- C. an immune response.
- D. an immunoglobulin fold.

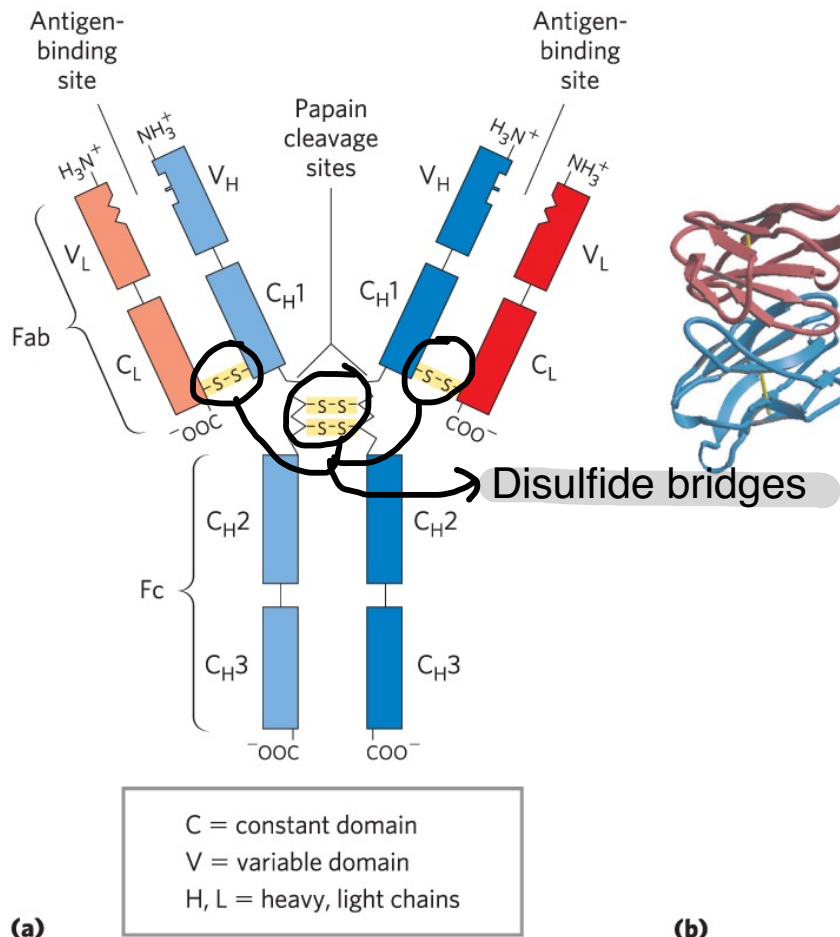
Clicker Question 18, Response

The molecular structure for which an individual antibody or T-cell receptor is specific is known as:

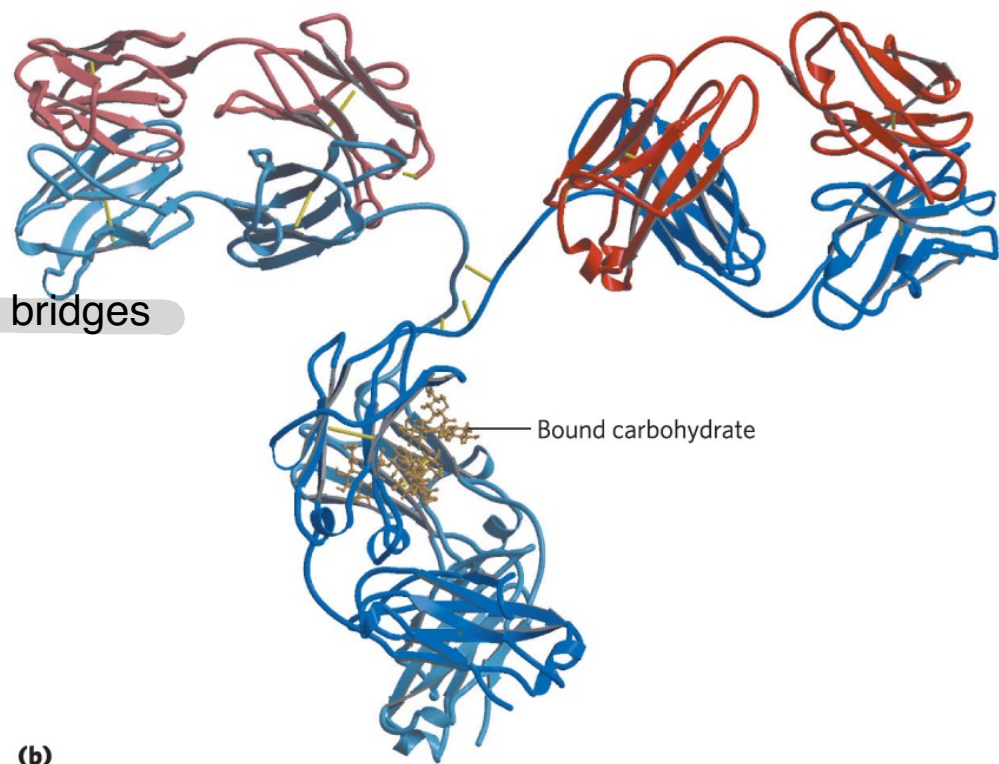
A. an epitope.

An individual antibody or T-cell receptor binds only a particular molecular structure within the antigen, called its epitope.

The Structure of Immunoglobulin G



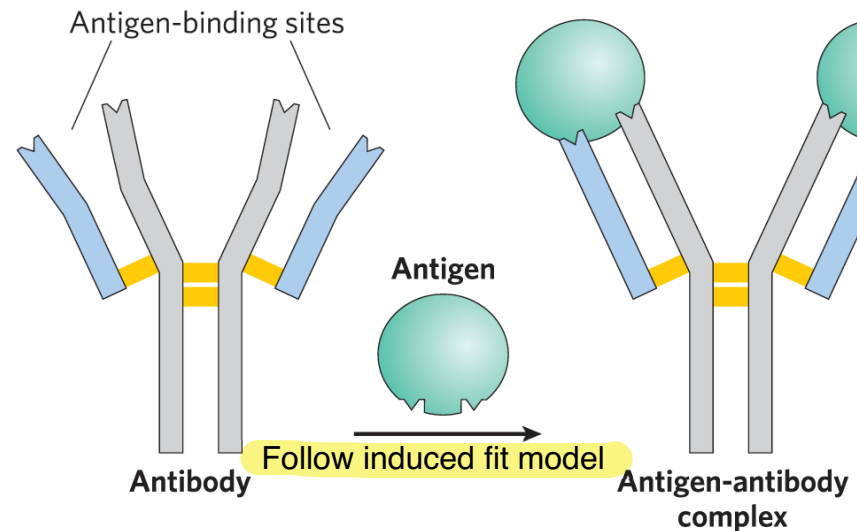
(a)



(b)

The Variable Domain of Immunoglobulin G

- heavy and light chains each have a variable domain
 - variable domains associate to create the antigen-binding site
 - allows formation of an antigen-antibody complex



Classes of Immunoglobulins

- 5 classes in vertebrates:
 - characterized by heavy chain:
 - α for **IgA**
 - δ for **IgD**
 - ϵ for **IgE**
 - γ for **IgG**
 - μ for **IgM**

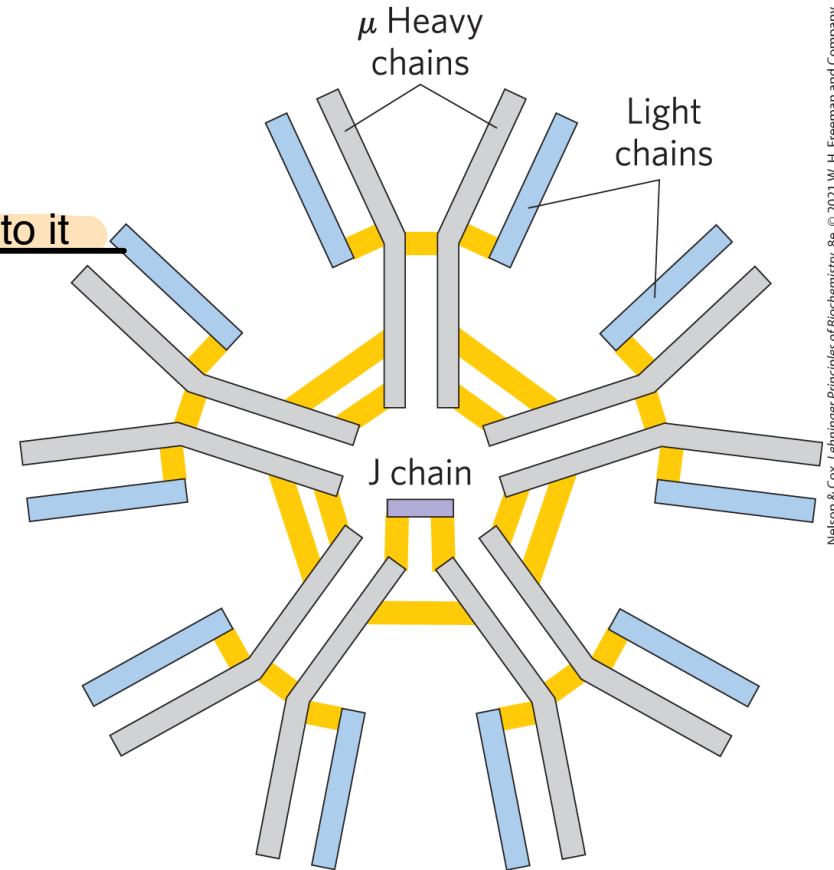
Structure of Immunoglobulins

- **IgD** and **IgE** = similar in structure to IgG

The first one that the body response to it

- **IgM** = monomeric, membrane-bound form or in a secreted form that is a cross-linked pentamer of this basic structure

- **IgA** = monomer, dimer, or trimer



IgM pentamer



Clicker Question 19

Which description is associated with immunoglobulin G?

- A. consists of two Fc fragments and two Fab fragments
- B. consists of one heavy and one light chain
- C. most abundant immunoglobulin
- D. important in the allergic response

Clicker Question 19, Response

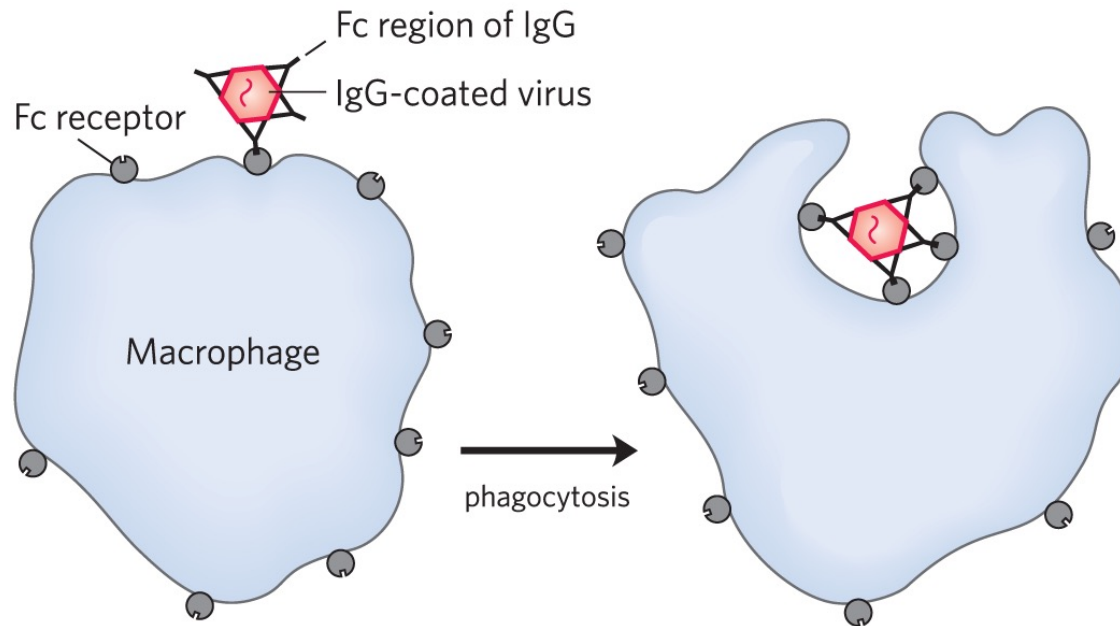
Which description is associated with immunoglobulin G?

C. most abundant immunoglobulin

The IgG described above is the major antibody in secondary immune responses, which are initiated by a class of B cells called memory B cells. IgG is the most abundant immunoglobulin in the blood.

Phagocytosis of Antibody-Bound Viruses by Macrophages

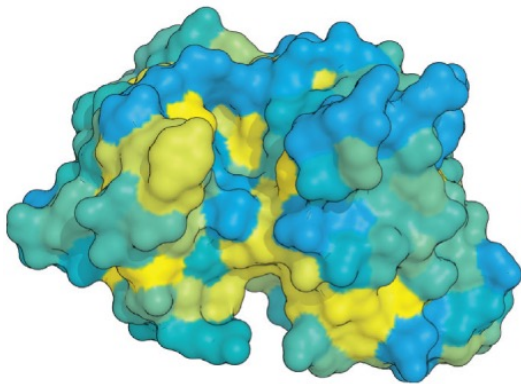
- When Fc receptors bind an antibody pathogen complex, macrophages engulf the complex



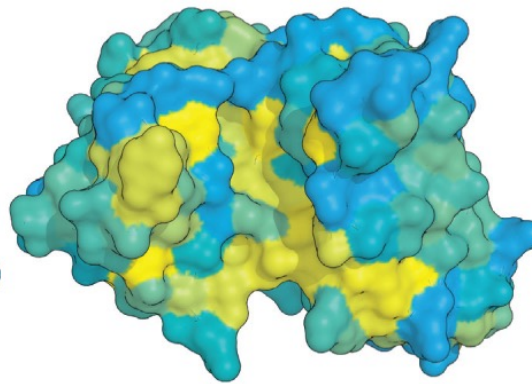
Antibodies Bind Tightly and Specifically to Antigen

IgE is an antibody that bond to the Fc receptors in specific cells like phagocytes and glycoocytes in blood and mast cells that produce histamine. When antibody bind to Fc receptors, then If any thing make allergy enter the body and bind to LgE, the response will occur, this response allow secret of histamine from mast cells, this histamine increase the size and permeability of blood vessels to allow white blood cells to reach the area that suffer inflammation.

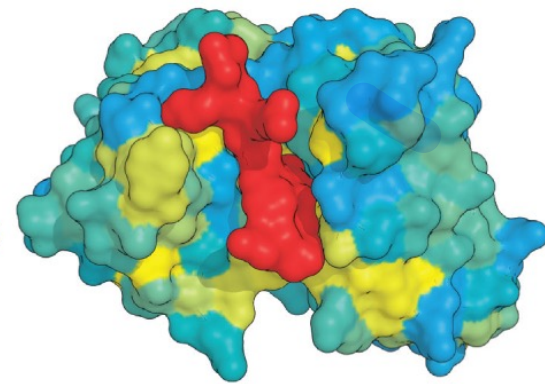
- induced fit = conformational changes in the antibody and/or antigen allow the complementary groups to interact fully
- K_d values as low as 10^{-10} M



(a) Conformation with no antigen bound



(b) Antigen bound (but not shown)



(c) Antigen bound (shown)

Clicker Question 20

Which biochemical principle is NOT true of immunoglobulins as examples of protein structure and function?

- A. Disulfide linkages strengthen the quaternary structure.
- B. Because the antigen-binding domain has high affinity, there is no observed induced fit.
- C. Amino acid chain flexibility and bond rotation permit optimal epitope binding.
- D. There are two specific and identical binding sites with high affinity on the same molecule.
- E. There is 100% sequence homology between the two antigen-binding sites.

Clicker Question 20, Response

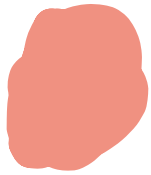
Which biochemical principle is NOT true of immunoglobulins as examples of protein structure and function?

B. Because the antigen-binding domain has high affinity, there is no observed induced fit.

Complementarity between the antigen and its specific binding site is often achieved interactively as the structures of antigen and binding site influence each other. Conformational changes in the antibody and/or the antigen then allow the complementary groups to interact fully. This is an example of induced fit.

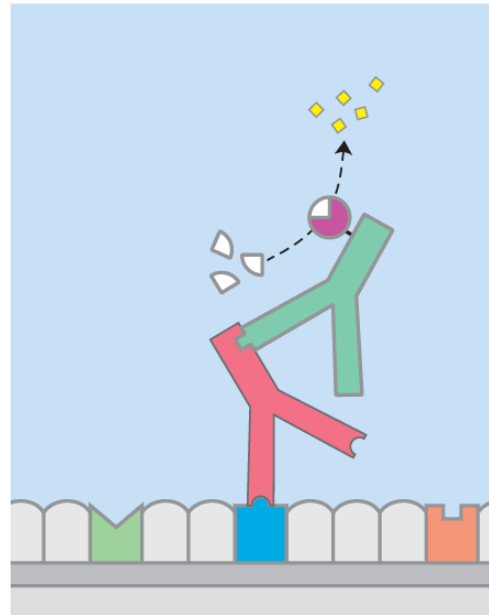
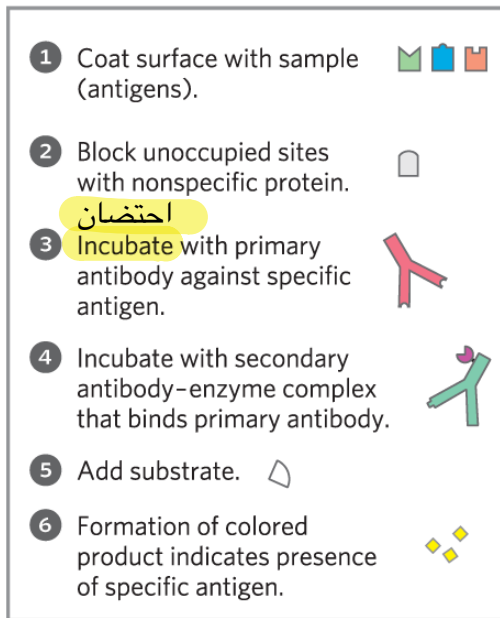
The Antibody-Antigen Interaction Is the Basis for a Variety of Important Analytical Procedures

- two types of antibody preparations are used:
 - **polyclonal antibodies**
 - produced by injecting a protein into an animal
 - contain a mixture of antibodies that recognize different parts of the protein
 - **monoclonal antibodies**
 - Synthesized by a population of identical B cells (a **clone**)
زراعة في المختبر
 - homogeneous, all recognizing the same epitope.

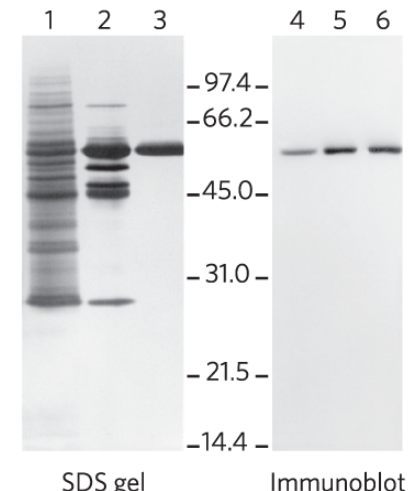


Western Blots

- **immunoblot = Western blot** assay = uses antibodies to detect a protein



(a)



(b)

Nelson & Cox, *Lehninger Principles of Biochemistry*, 8e, © 2013 W. H. Freeman and Company, (b) State of Wisconsin Lab of Hygiene, Madison, WI.



P2

Clicker Question 21

Polyclonal antibodies:

- A. are synthesized by a population of identical B cells.
- B. all recognize the same epitope.
- C. are identical to monoclonal antibodies.
- D. are used as analytic reagents in Western blot assays.

Clicker Question 21, Response

Polyclonal antibodies:

D. are used as analytic reagents in Western blot assays.

Polyclonal antibodies are used in Western blot assays.

The specific reaction of the antibody with its antigen makes it possible to identify and quantify a specific protein in a complex sample.