

Two groups of proteins whose functions are binding other molecules: (**Chapter 7**)

Globins

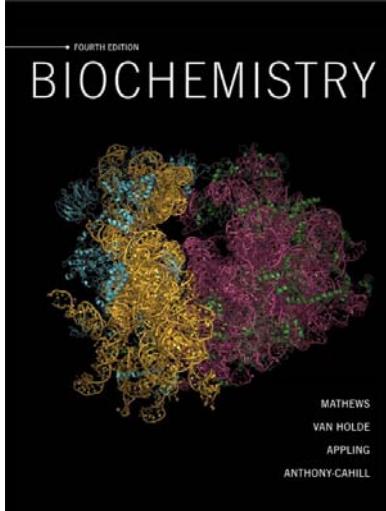
Immunoglobulins

Contractile proteins and molecular motors
(**Chapter 8**)

Structure-function relationships for another diverse and important group of proteins

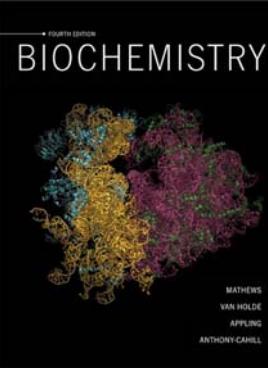
The enzymes (**Chapter 11**)

*Knowing the 3-D structure of a protein is an important part of understanding how the protein functions



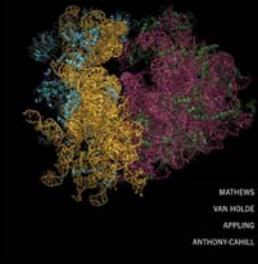
Chapter 7

Protein Function and Evolution

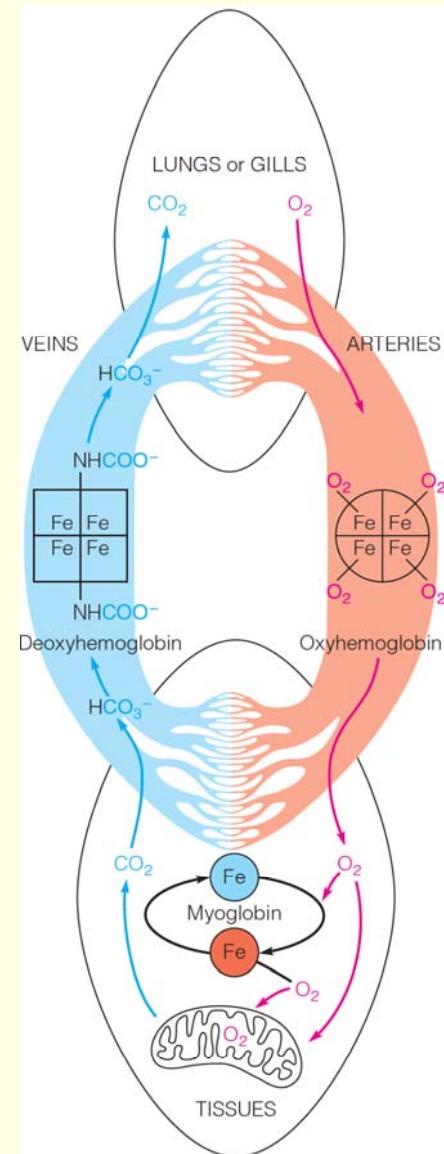


Chapter 7 Outline:

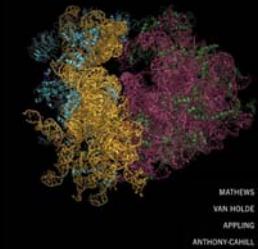
- Oxygen Transport: The Roles of Hemoglobin and Myoglobin
- The Mechanism of Oxygen Binding by Heme Proteins
- Oxygen Transport: Hemoglobin
- Allosteric Effectors of Hemoglobin
- Protein Evolution: Myoglobin and Hemoglobin as Examples
- Hemoglobin Variants: Evolution in Progress
- Immunoglobulins: Variability in Structure Yields Versatility in Binding
- Immunological Methods



Oxygen Transport: The Roles of Hemoglobin and Myoglobin

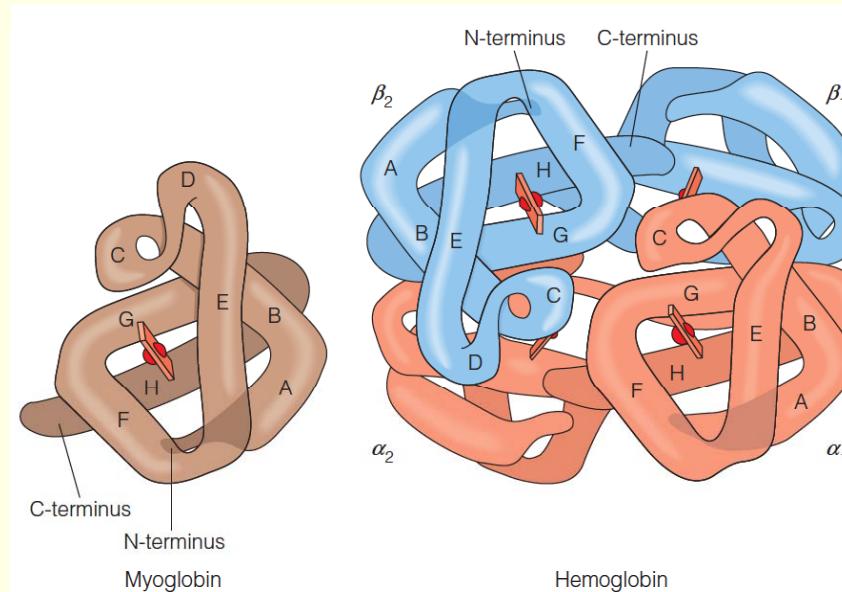


- Vertebrate animals use hemoglobin and myoglobin to provide their tissues with a continuous O_2 supply.
- Hemoglobin transports O_2 from the lungs or gills to the respiring tissues, where it is used for aerobic metabolism in the mitochondria.
- Inside cells, dissolved O_2 diffuses freely or is bound to myoglobin, which aids transport of O_2 to the mitochondria.
- Myoglobin can also store O_2 for later use (as in deep-diving mammals).
- CO_2 produced by oxidative processes in the tissues is carried back to the lungs or gills by hemoglobin and released.

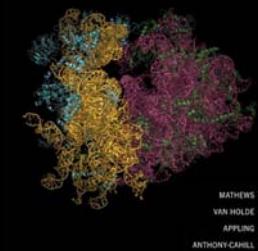


Oxygen Transport: The Roles of Hemoglobin and Myoglobin

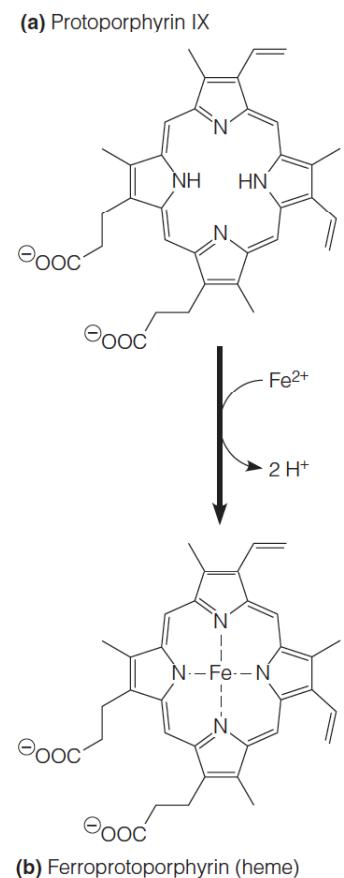
Comparison of myoglobin and hemoglobin:



- Each of the four chains in hemoglobin has a folded structure similar to that of myoglobin, and each carries a heme.
- Hemoglobin contains two identical α chains and two identical β chains. The letters A–H indicate α -helical regions.
- The α and β chains are very similar but have distinct primary structures and folds (note that the α chain does not have a “D” helix).

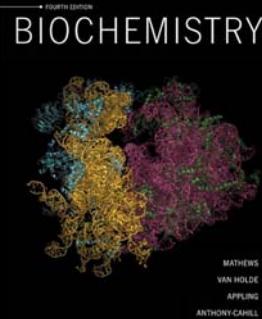


The Mechanism of Oxygen Binding by Heme Proteins



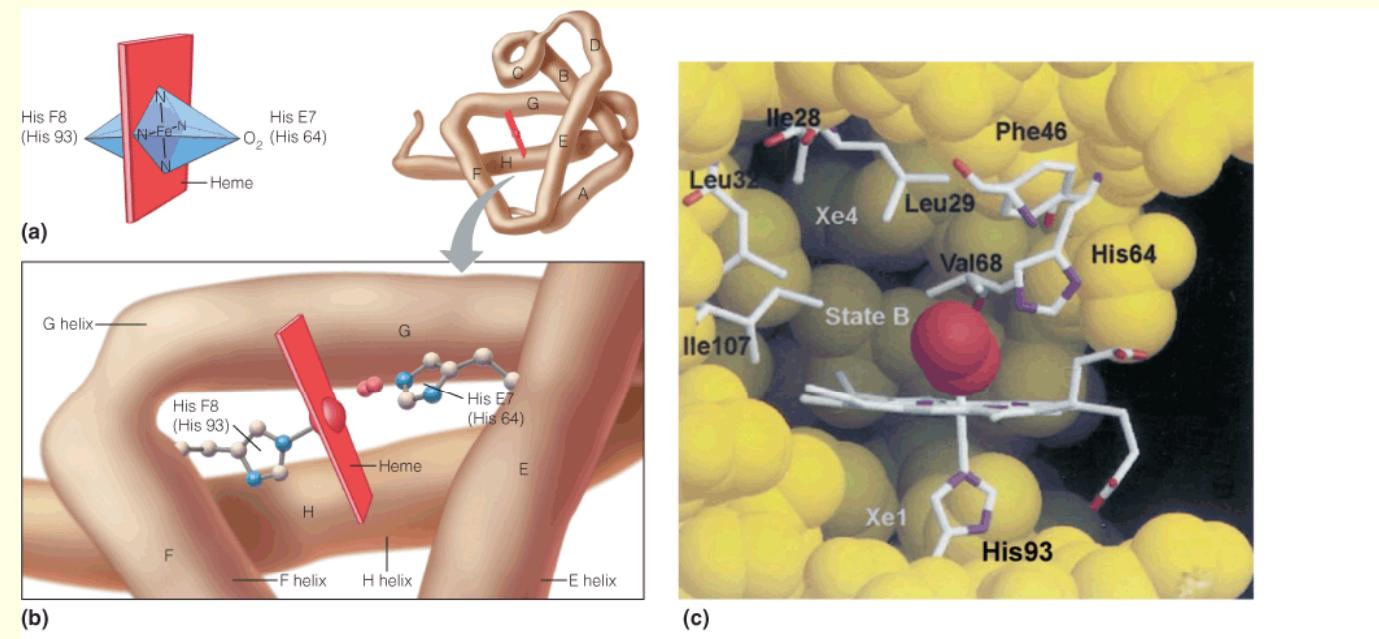
The structures of protoporphyrin IX and heme:

- The **protoporphyrin IX** is the tetrapyrrole portion of the heme molecule.
- **Heme**, which is protoporphyrin IX complexed with $\text{Fe}(\text{II})$, is the **prosthetic group** of hemoglobin and myoglobin.
- Because of resonance delocalization of the electrons in the porphyrin ring, all N–Fe bonds within the heme are equivalent.

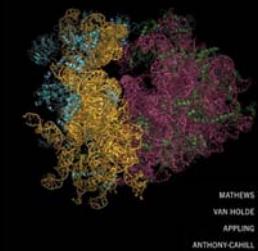


The Mechanism of Oxygen Binding by Heme Proteins

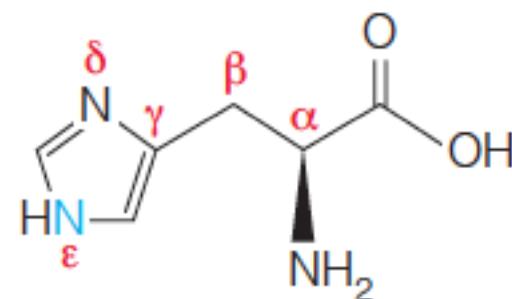
The geometry of iron coordination in oxymyoglobin:



- The octahedral coordination of the iron ion. The iron and the four nitrogens from protoporphyrin IX lie nearly in a plane.
- A histidine (F8, or His 93) occupies one of the axial positions, and O₂ the other.
- Schematic drawing of the heme pocket, showing the proximal (F8; His93) and distal (E7; His64) histidine side chains.

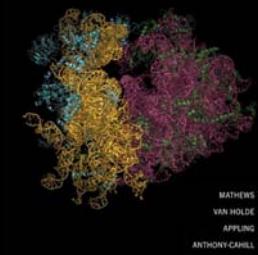


The Mechanism of Oxygen Binding by Heme Proteins

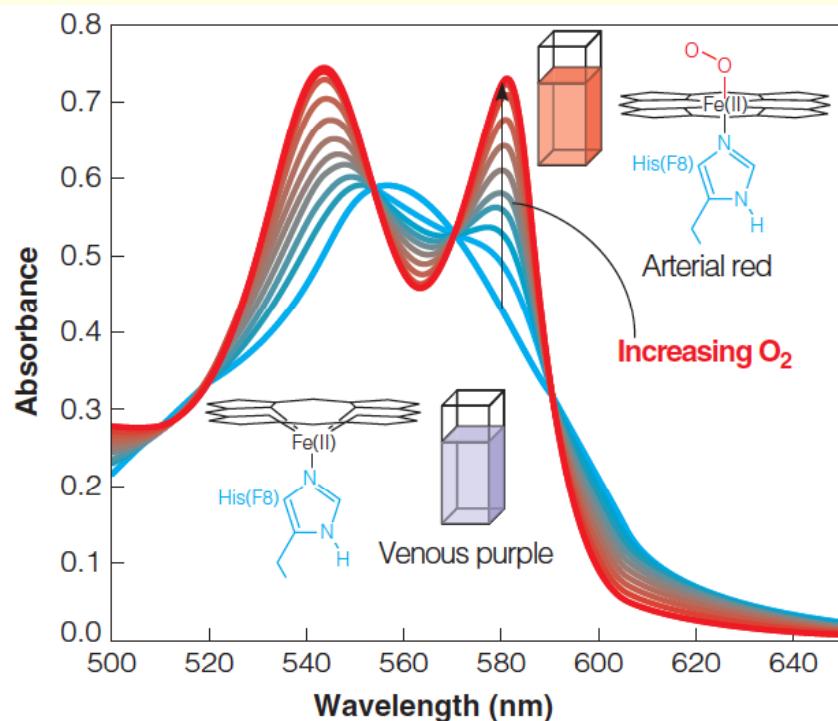


The ϵ -tautomer of histidine

- Coordination of Fe(II) in a porphyrin (heme) within a hydrophobic globin pocket allows reversible O₂ binding without iron oxidation.
- The H-bond between His E7 (the distal histidine) and O₂ selectively increases the affinity of Mb for O₂ vs. CO, which doesn't make a similar bond to His E7.
- Even so, CO binds \sim 200 times more tightly to Mb than does O₂; however, without the E7 H-bond, that ratio would be \sim 6,000:1 in favor of CO.



The Mechanism of Oxygen Binding by Heme Proteins

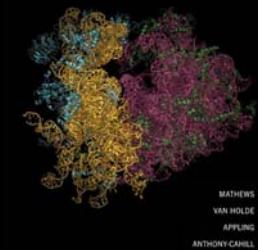


Changes in the visible spectrum of hemoglobin.

Spectra for hemoglobin in the deoxygenated state (blue trace) and the O₂-bound state (red trace) are shown.

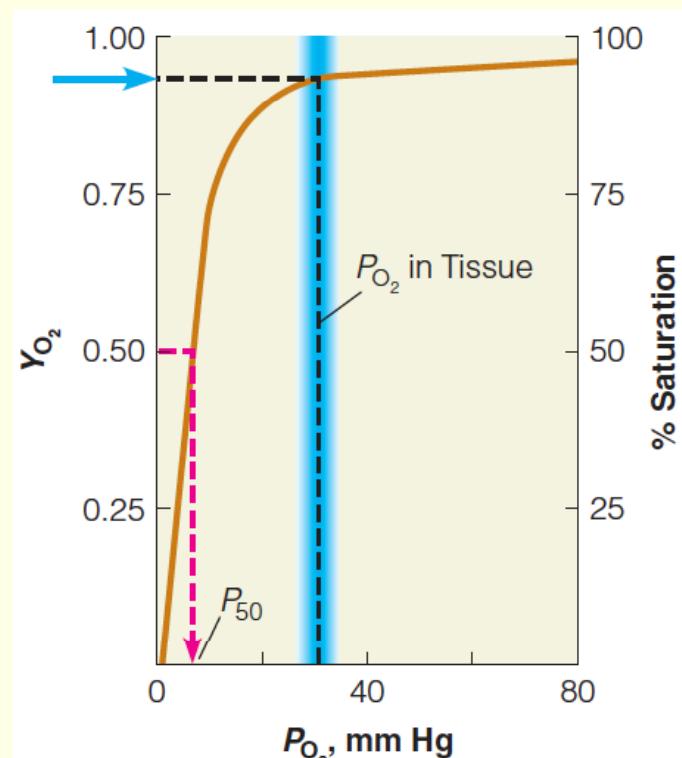
Hemoglobin in the deoxygenated state is a venous purple, whereas completely oxy-Hb is bright red.

As more O₂ binds to Hb, the visible spectrum shifts from the blue to the red trace



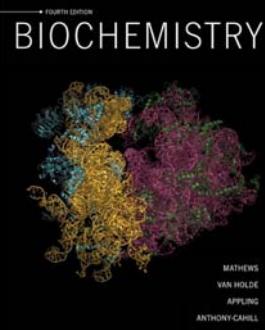
The Mechanism of Oxygen Binding by Heme Proteins

Oxygen-binding curve for myoglobin:



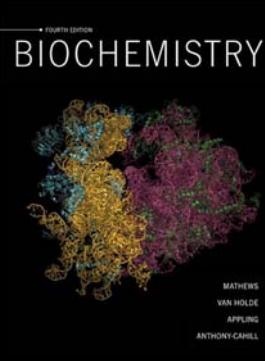
3-4 mm Hg

- The free oxygen concentration is expressed as P_{O_2} , the partial pressure of oxygen.
- The proportion of myoglobin binding sites that are occupied is expressed as a fraction (Y_{O_2} , on the left) or as percent saturation (on the right).
- As P_{O_2} becomes large, 100% saturation is approached asymptotically.
- The value of P_{50} , the partial pressure of oxygen at 50% saturation, is indicated on the graph (red arrow). The dashed blue lines show that at P_{O_2} of 30 mm Hg, Mb would be 90% saturated with O₂.



The Mechanism of Oxygen Binding by Heme Proteins

- The P_{50} is an indicator of the relative binding affinity of a globin for a ligand:
 - For a globin with higher O₂-binding affinity, the value of P_{50} is *lower*.
 - For a globin with lower O₂-binding affinity, the value of P_{50} is *higher*.
- Binding of a ligand (like O₂) to a single site on a protein (like Mb) is described by a **hyperbolic binding curve**.



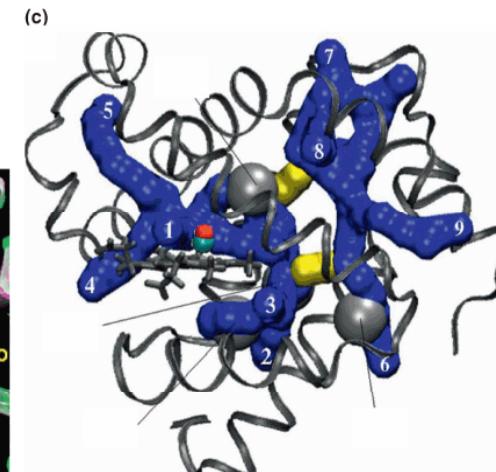
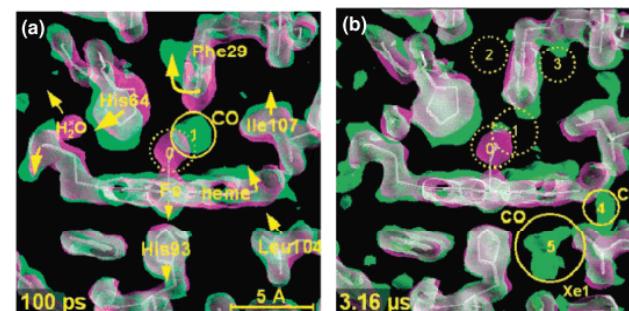
The Mechanism of Oxygen Binding by Heme Proteins

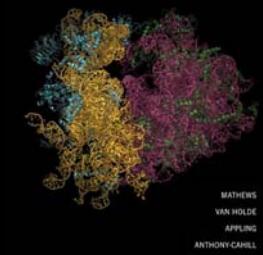
Dynamics of CO release by myoglobin:

(a) and (b) Time-resolved X-ray diffraction data comparing the positions of atoms before (magenta) and 100 ps after (green) photolysis of the L29F mutant of Mb-CO.

(c) Computational modeling of ligand migration pathways in Mb are shown in blue.

(a, b) From Science 300:1944–1947, F. Schotte, M. Lim, T. A. Jackson, A. V. Smirnov, J. Soman, J. S. Olson, G. N. Phillips Jr., M. Wulff, and P. A. Anfinrud, Watching a protein as it functions with 150-ps time-resolved x-ray crystallography. © 2003. Reprinted with permission from AAAS; (c) Reprinted from Proceedings of the National Academy of Sciences of the United States of America 105:9204–9209, J. Z. Ruscio, D. Kumar, M. Shukla, M. G. Prisant, T. M. Murali, A. V. Onufriev, Atomic level computational identification of ligand migration pathways between solvent and binding site in myoglobin. © 2008 National Academy of Sciences, U.S.A.



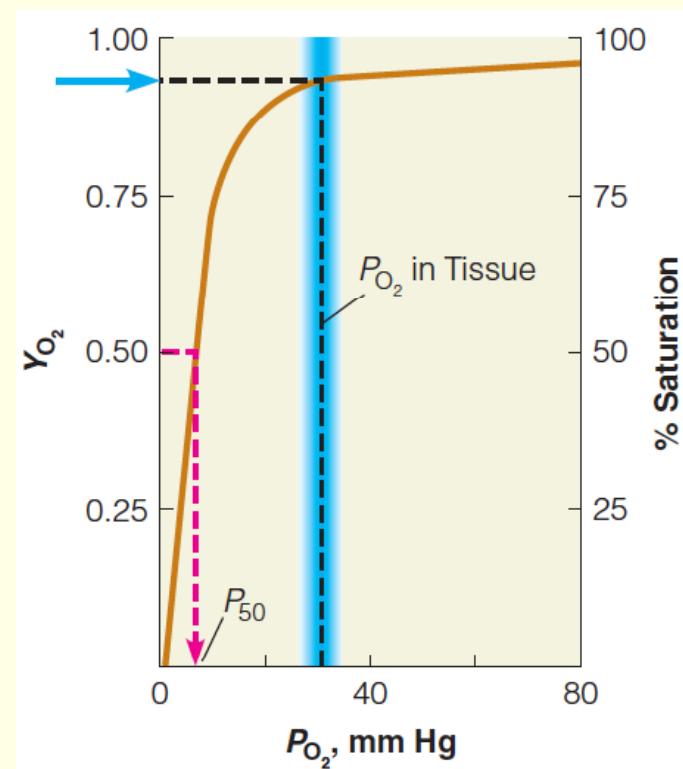


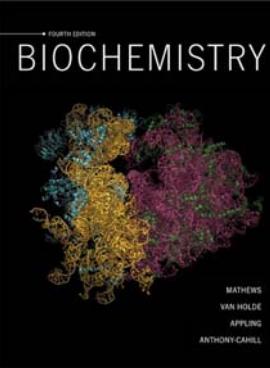
The Mechanism of Oxygen Binding by Heme Proteins

- Dynamic motions of myoglobin facilitate ligand binding and release.
- Myoglobin has evolved to bind and release O₂ under conditions of relatively low oxygen concentration.

PO₂ in the arterial capillaries: ~30 mm Hg

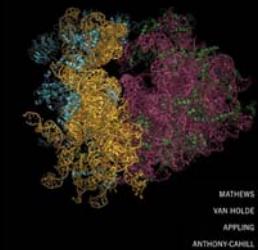
PO₂ in the metabolically active cells: ~3-18 mm Hg





Oxygen Transport: Hemoglobin

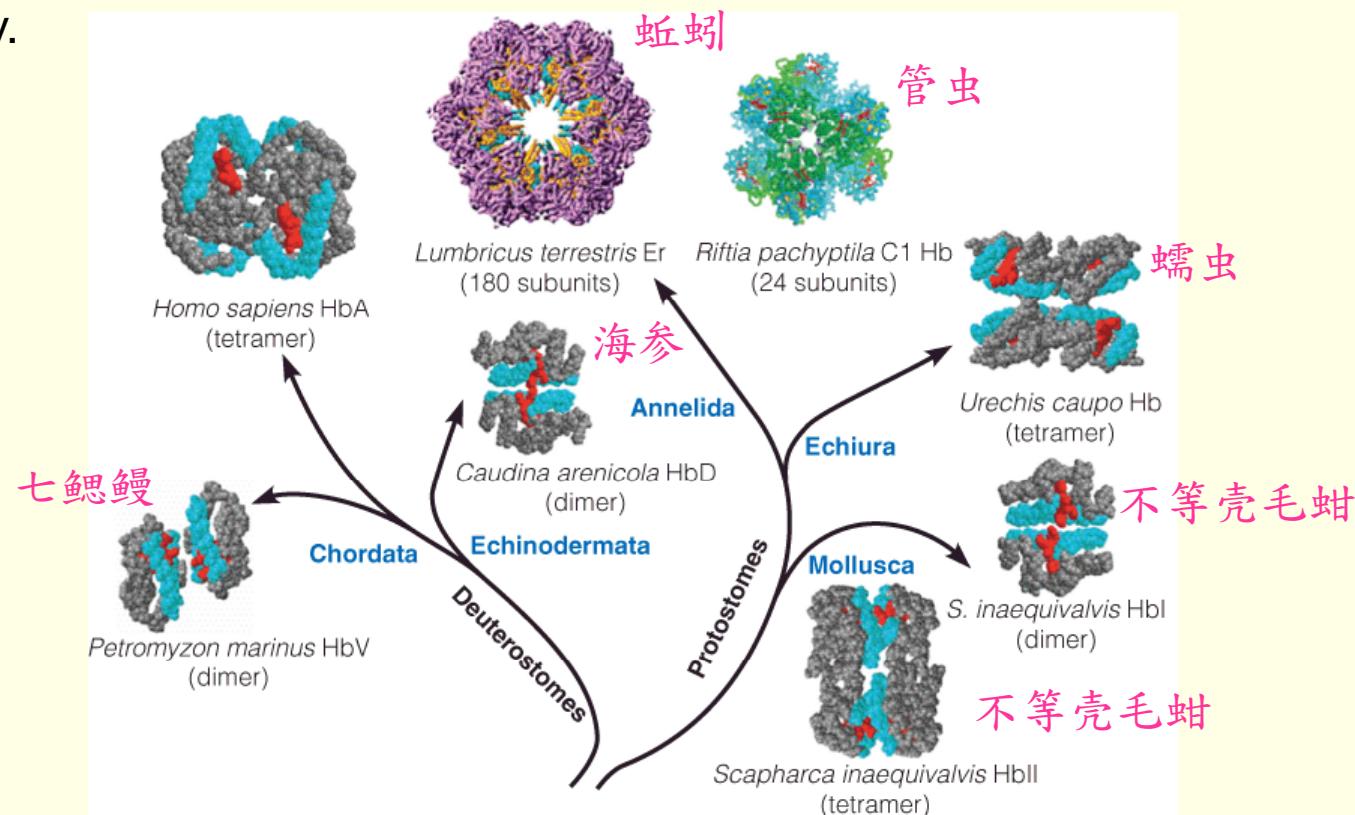
- Higher animals use O₂-binding proteins to transport oxygen from lungs or gills to respiring tissues where it is needed to support metabolism.



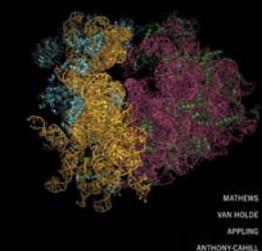
Oxygen Transport: Hemoglobin

Diversity in hemoglobin structures:

Space-filling models of Hb dimers and tetramers are shown with heme groups in red, E and F helices in cyan, and the rest of the main chain in gray.

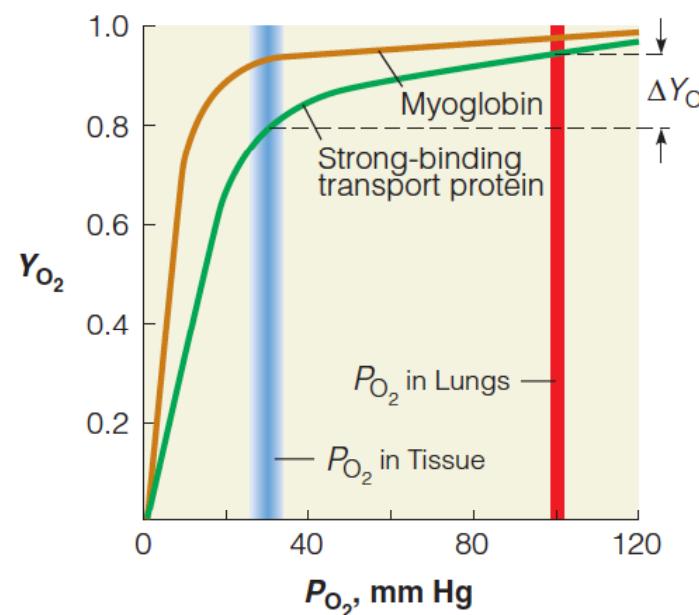


Copyright © 2013 Pearson Canada Inc.

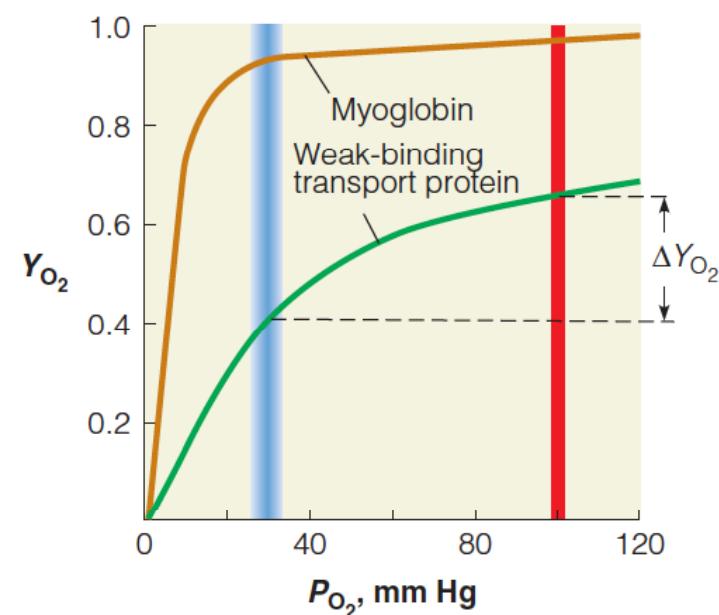


Oxygen Transport: Hemoglobin

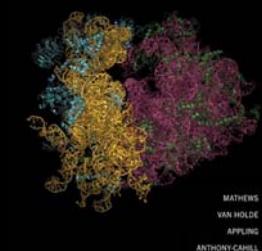
Cooperative vs. noncooperative O₂-binding curves:



- (a) Transport protein efficient in binding but inefficient in unloading (hyperbolic binding curve).

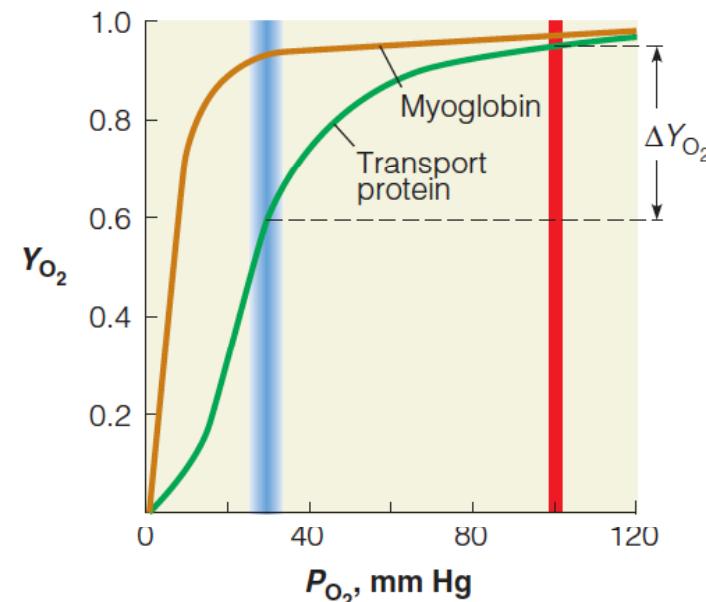


- (b) Transport protein efficient in unloading but inefficient in binding (hyperbolic binding curve).

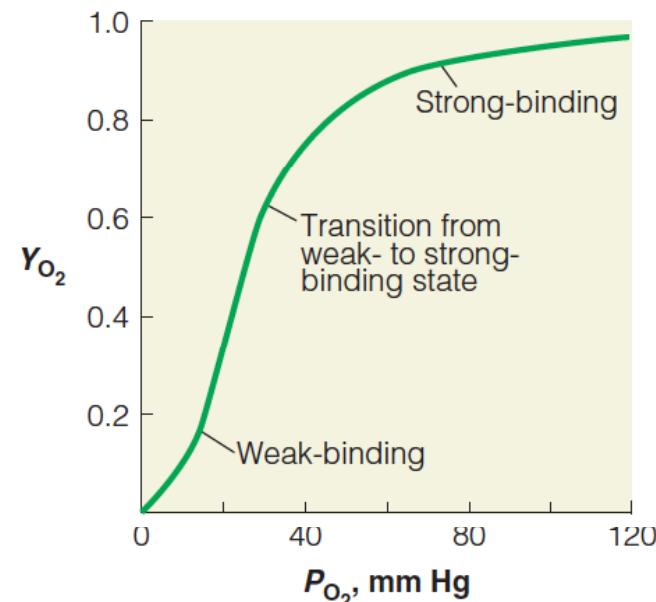


Oxygen Transport: Hemoglobin

Cooperative vs. noncooperative O₂-binding curves:

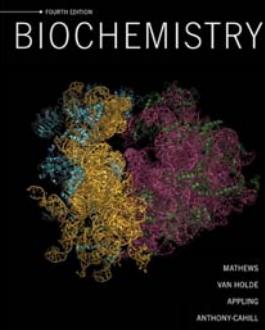


(c) Transport protein efficient in both binding and unloading because it can switch between higher and lower affinity states (sigmoidal binding curve).



(d) Switch from lower to higher affinity states yields the sigmoidal curve.

Efficiency in O₂ transport is achieved by cooperative binding in multisite proteins, described by a *sigmoidal* binding curve.

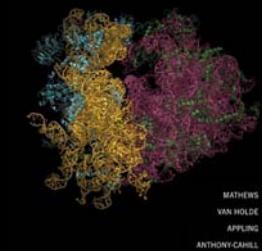


Oxygen Transport: Hemoglobin

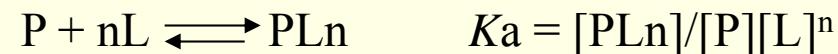
- *Cooperativity* in binding requires communication between binding sites.
- The *Hill equation*

$$\log\left(\frac{Y_{O_2}}{1 - Y_{O_2}}\right) = h \log P_{O_2} - h \log P_{50}$$

- A plot of the Hill equation indicates positive, negative, or no cooperativity.



Cooperative ligand binding can be described quantitatively



$$\text{Dissociation constant, } K_d = [P][L]^n/[PLn]$$

$$\begin{aligned} \theta &= (\text{binding sites occupied})/(\text{total binding sites}) = [PLn]/[PLn] + [P] \\ &= [L]^n/([L]^n + K_d) \end{aligned}$$

$$\theta/(1-\theta) = [L]^n/K_d$$

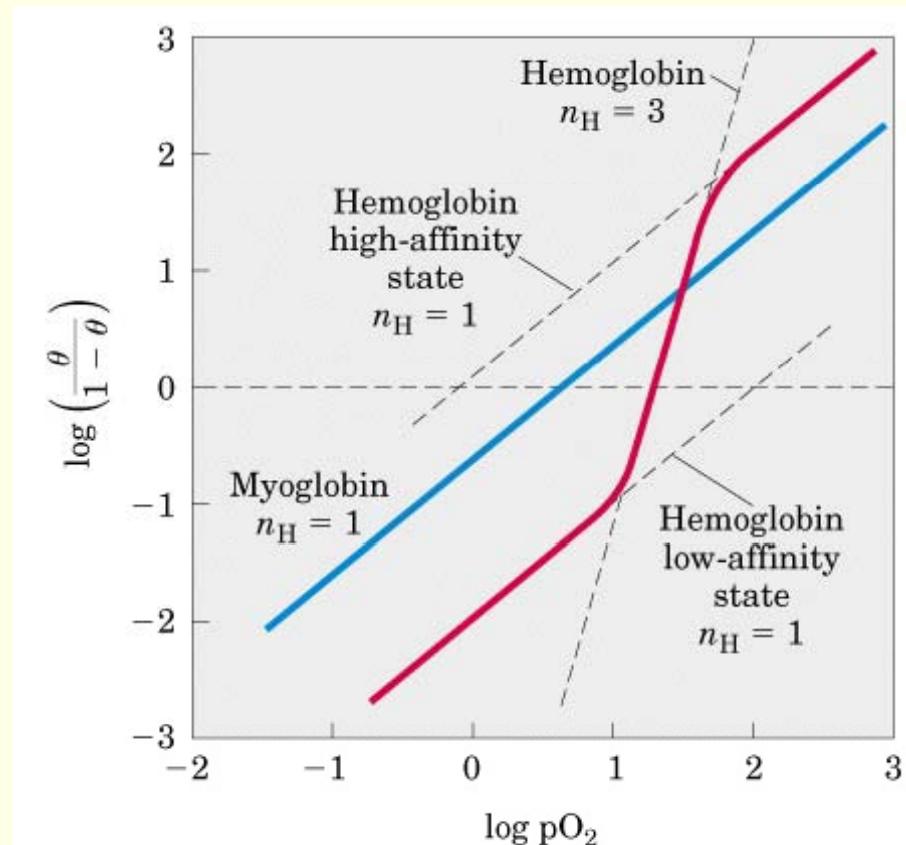
$\log\{\theta/(1-\theta)\} = n \log [L] - \log K_d$
(Hill equation, 1910)

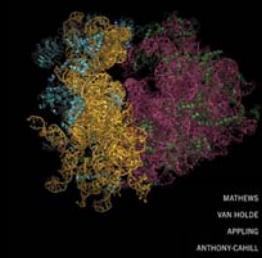
$$\log\{\theta/(1-\theta)\} = n \log pO_2 - \log P_{50}$$

**n_H : the Hill coefficient
(slope of Hill plot)**

When $n_H <, =, > 1$????

Fig. 5-14

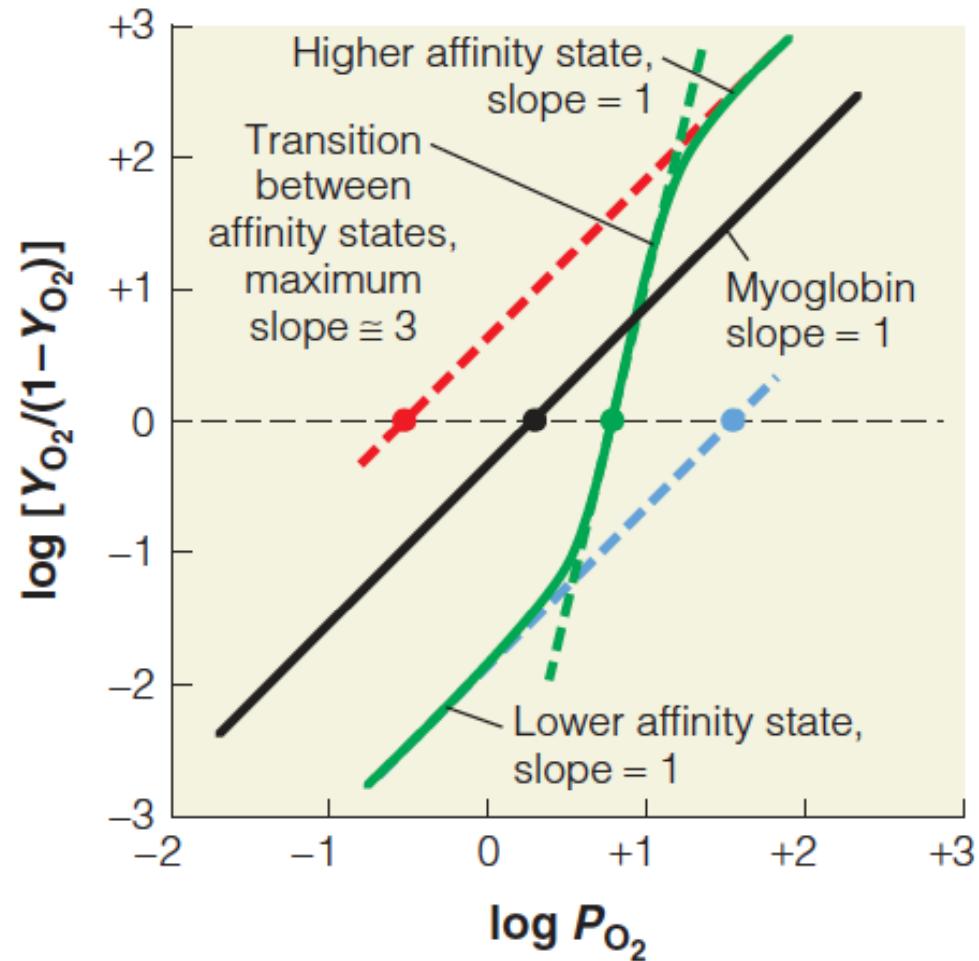


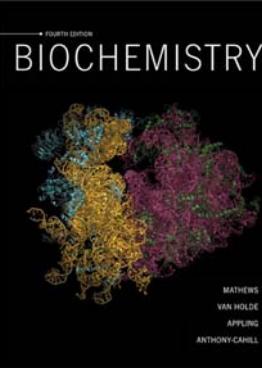


Oxygen Transport: Hemoglobin

Hill plots of oxygen binding for myoglobin and hemoglobin under physiological conditions:

- The plot for myoglobin, which binds oxygen noncooperatively, is a solid black line with a slope of 1.
- The plot for hemoglobin, which binds cooperatively, shows the switch from a lower-affinity state (larger P_{50}) to a higher-affinity state (smaller P_{50}) and has a Hill coefficient of about 3.



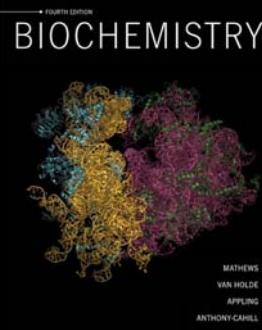


Oxygen Transport: Hemoglobin

For both cooperative and noncooperative systems, the Hill plot gives the value of h as the slope at $\log[Y_{O_2}/(1 - Y_{O_2})] = 0$.

Four cases may be considered for a molecule with n binding sites:

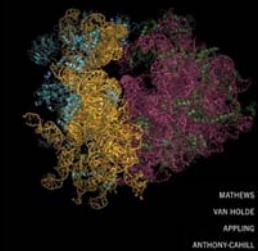
1. $h = 1$: There is no interaction between the sites; thus, the molecule binds ligands noncooperatively (e.g., as for myoglobin).
2. $1 < h < n$: There is interaction between the sites. This situation is the usual one for a protein that binds ligands with so-called “positive” cooperativity, as depicted for hemoglobin.
3. $h = n$: The energy of interaction between sites approaches infinity. In this hypothetical situation the molecule is wholly, or infinitely, cooperative.
4. $h < 1$: In this case, ligand binding at one site reduces binding affinity at other binding sites and is called “negative cooperativity.”



Oxygen Transport: Hemoglobin

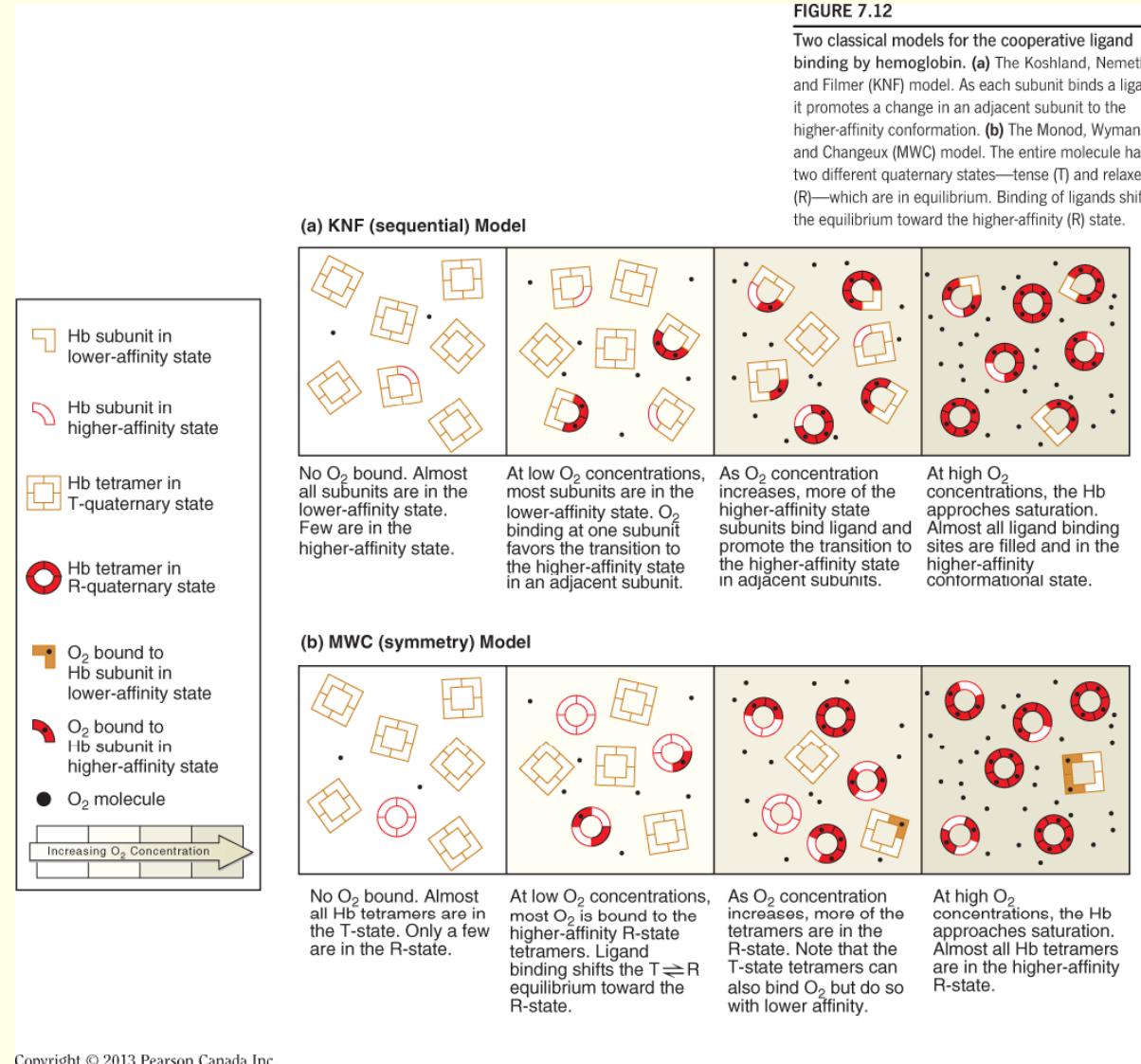
- The cooperative binding of oxygen by hemoglobin is one example of what is referred to as an ***allosteric effect***. (變構效應)
- In allosteric binding, the uptake of one ligand by a protein influences the affinities of remaining unfilled binding sites.
- The ligands may be of the same kind, as in the case of binding to hemoglobin, or they may be different.
- Allostery is also an important mechanism for regulating the activity of enzymes. (變構性)

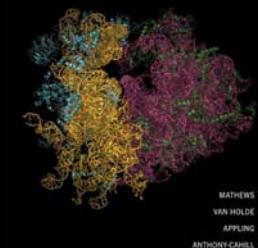
別構調節（Allosteric regulation，源自希臘語*allos*-「其他、*stereos*-「固態（物體）」）是酶活性調節的一種機制，也稱為變構



Oxygen Transport: Hemoglobin

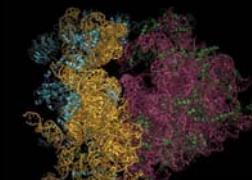
Two classical models for the cooperative ligand binding by hemoglobin:





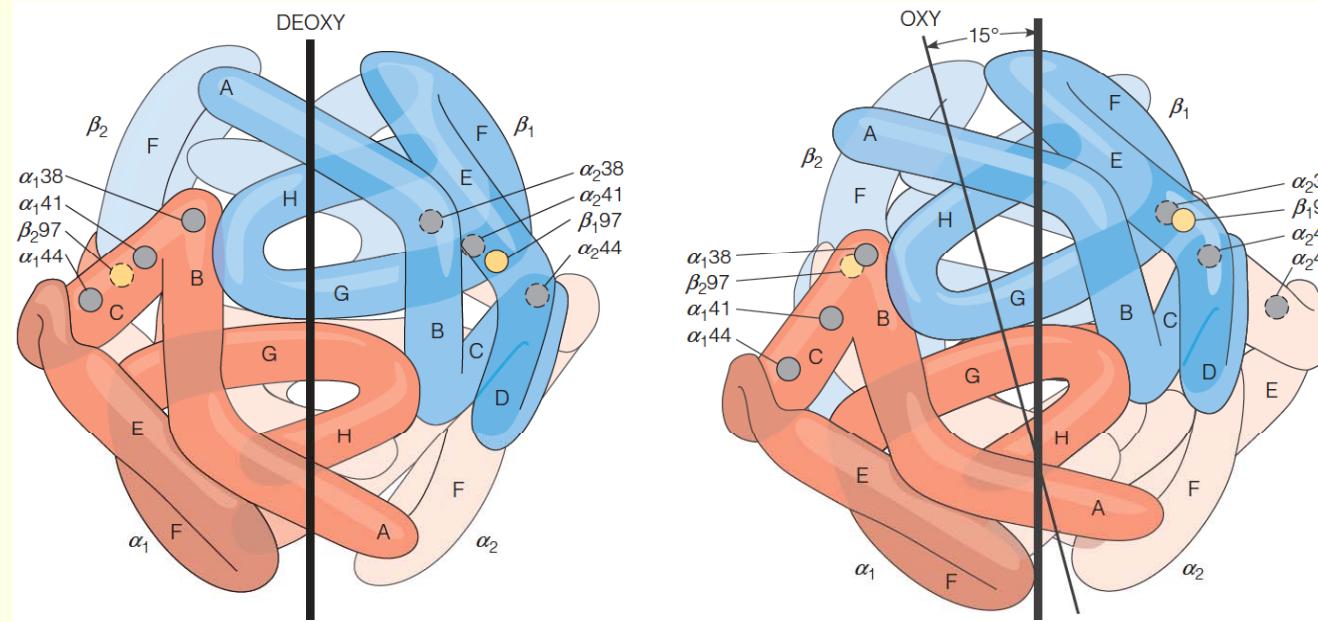
Oxygen Transport: Hemoglobin

- Hemoglobin switches between conformational states with lower and higher O₂-binding affinities.
- In the O₂-rich environment of the lungs or gills the higher affinity state is favored, and oxygen binds to hemoglobin.
- In the O₂-poor environment of respiring tissues the lower affinity state is favored, and oxygen is released from hemoglobin.
 - *R*-state hemoglobin has a higher O₂-binding affinity (lower P_{50}).
 - *T*-state hemoglobin has a lower O₂-binding affinity (higher P_{50}).
- Vertebrate hemoglobins are tetramers ($\alpha_2\beta_2$) made up of two kinds of myoglobin-like chains.
- Oxygenation causes hemoglobin quaternary structure to change:
 - One $\alpha\beta$ dimer rotates and slides with respect to the other.

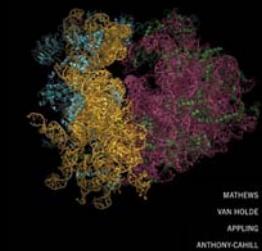
MATHEWS
VAN HOLDE
APPLING
ANTHONY-CAHILL

Oxygen Transport: Hemoglobin

The change in hemoglobin quaternary structure during oxygenation:

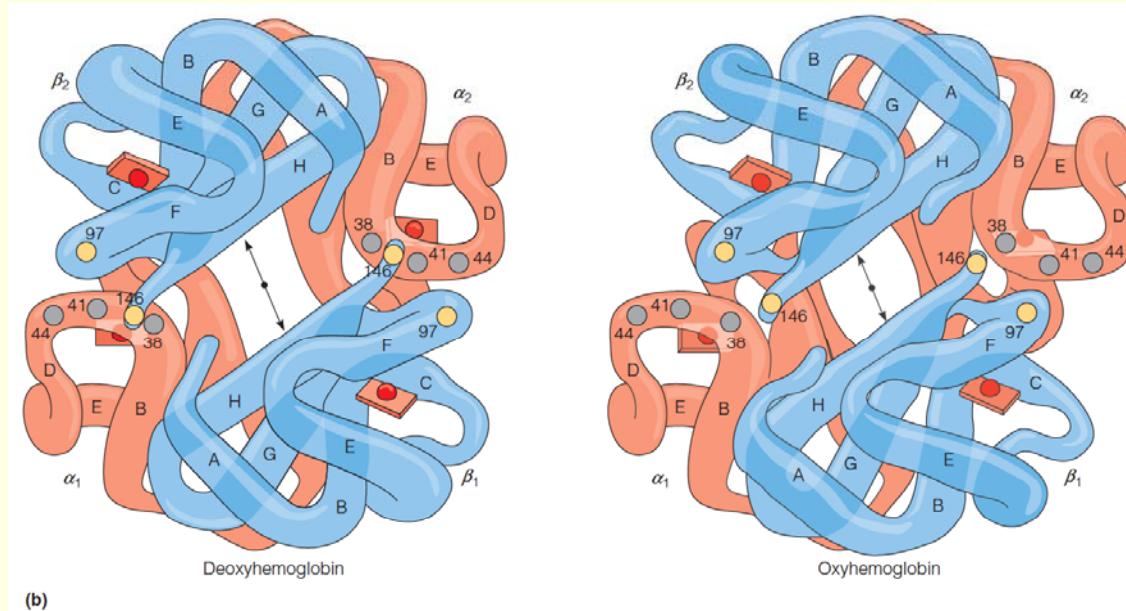


- The transition viewed along an axis perpendicular to the two-fold axis, with the dimer (darker blue and red areas) in front of the dimer.
- Deoxyhemoglobin is shown on the left, and oxyhemoglobin on the right.
- The rotation of about 15° is accompanied by sliding because the center of rotation is not centrally located.

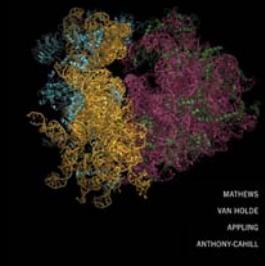


Oxygen Transport: Hemoglobin

The change in hemoglobin quaternary structure during oxygenation:

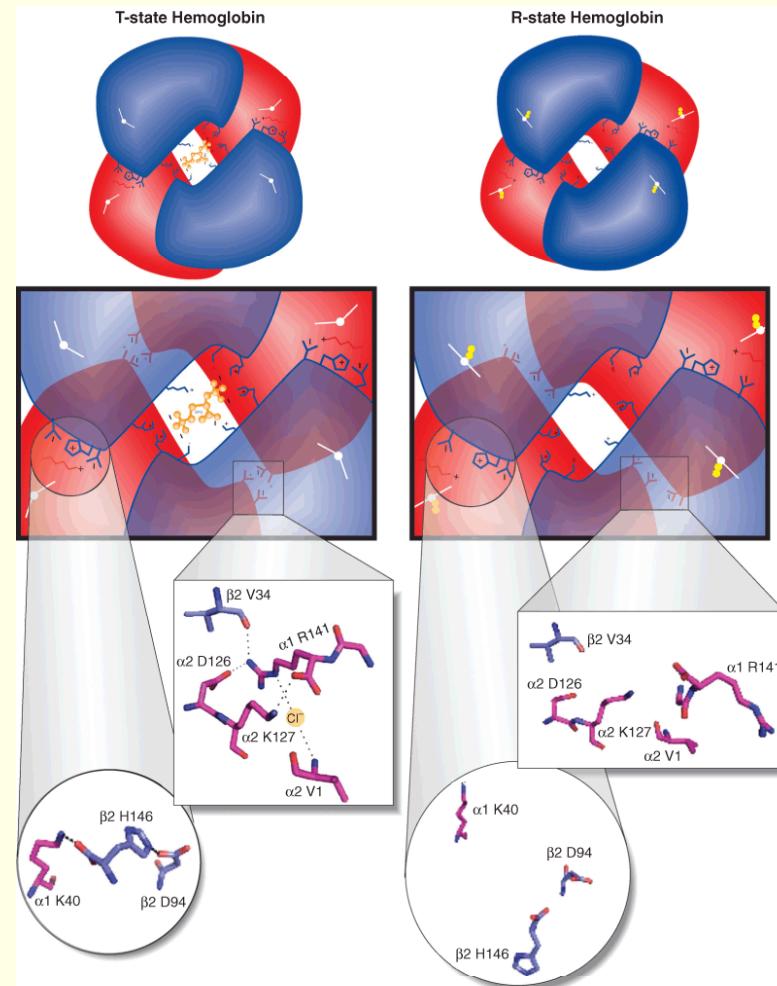


- Top views of hemoglobin, looking down the two-fold axis (dot in center). The two β subunits are in the foreground; α subunits are in the background.
- The shift from the deoxy to the oxy state is evident by the shrinkage of the central cavity and the shift in the contact of residue with the chain.

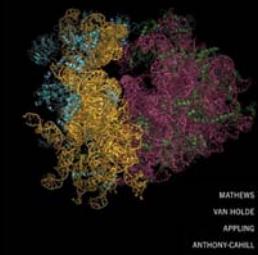


Oxygen Transport: Hemoglobin

Key salt bridge interactions disrupted during the switch between *T* and *R* state hemoglobin quaternary structures.



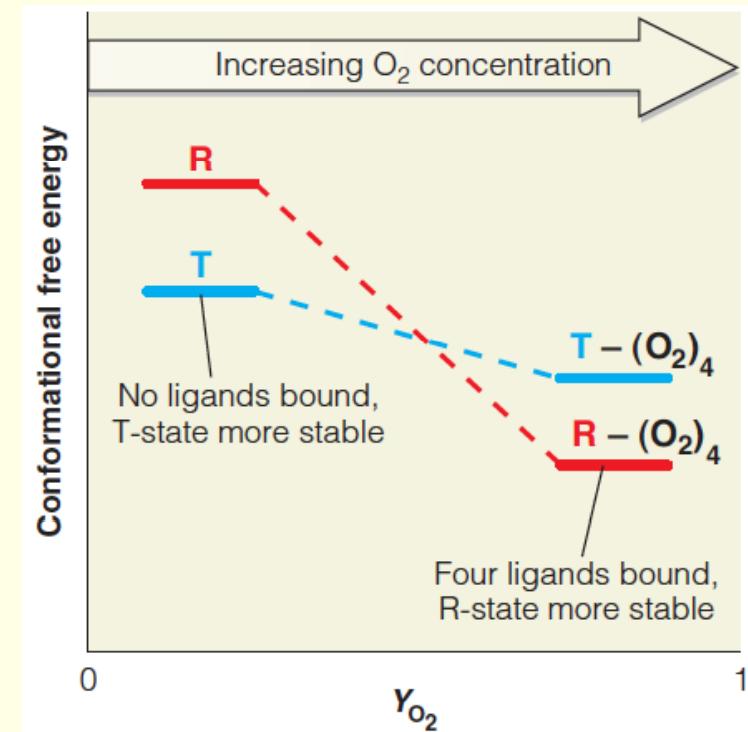
Copyright © 2013 Pearson Canada Inc.

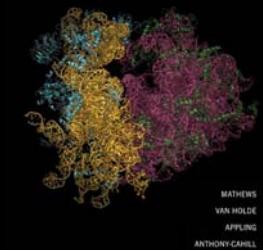


Oxygen Transport: Hemoglobin

A simplified view of ligand binding and conformation energies in hemoglobin:

- The deoxy (*T*) conformation is favored when no ligands are bound, due to the increased number of noncovalent interactions in the *T* state.
- As Y_{O_2} increases (i.e., more ligands are bound) the energy provided by formation of the Fe-O₂ bond stabilizes the *R* conformation relative to the *T* conformation.
- The energetic cost of breaking stabilizing interactions in the deoxy state is paid by the formation of Fe-O₂ bonds in the oxy state.

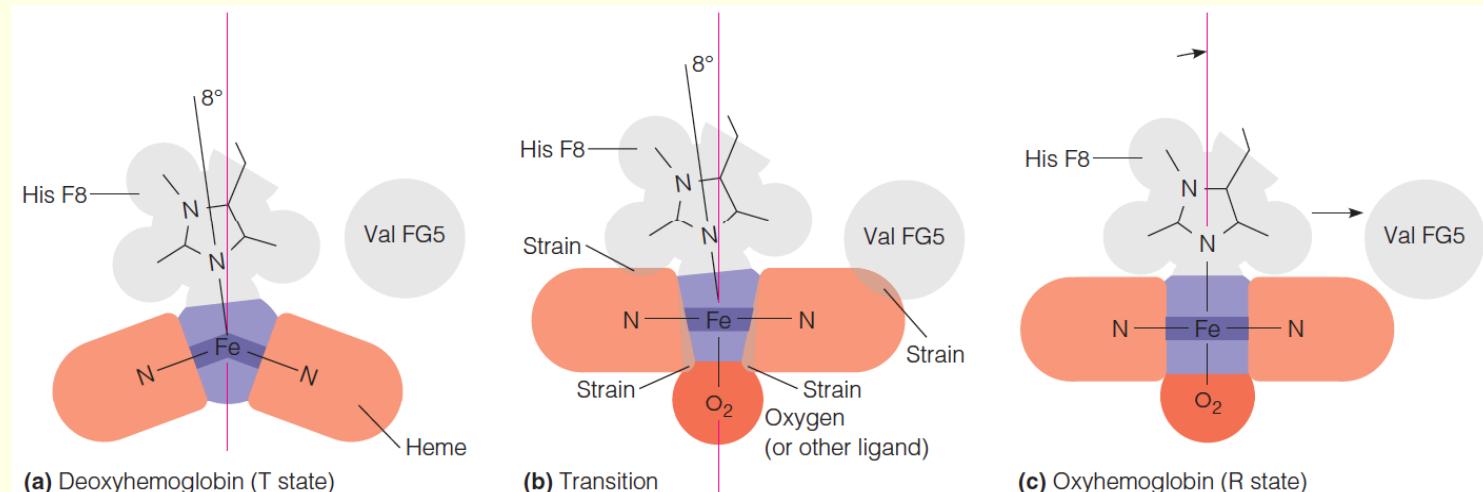


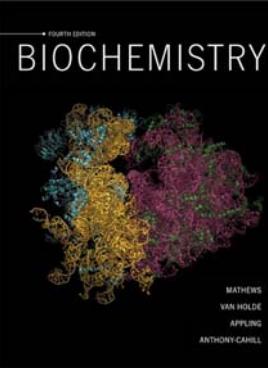


Oxygen Transport: Hemoglobin

The essential features of the “Perutz mechanism” of the T-R transition in hemoglobin:

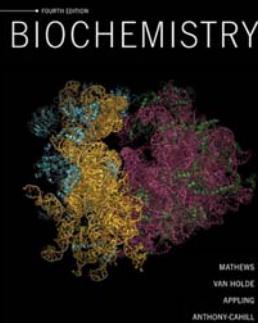
- The binding of oxygen to deoxyhemoglobin causes conformational changes in the heme.
- In the deoxy state, heme has a dome shape.
- Binding of the O₂ ligand pulls the iron into the heme plane, flattening the heme and causing strain.
- A shift in the orientation of His F8 relieves the strain, partly because Val FG5 is pushed to the right. In this way, the tertiary change in heme is communicated to the FG corner.





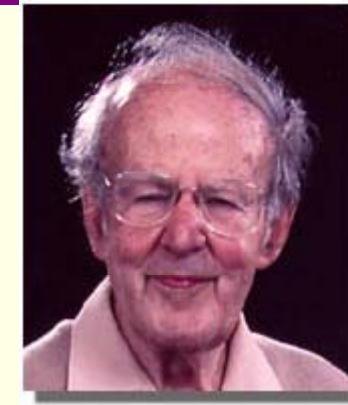
Oxygen Transport: Hemoglobin

- In the **Perutz mechanism**, a small movement of the heme iron upon O₂-binding is translated into a larger movement of the F helix by the covalent connection between the F helix and the proximal histidine.

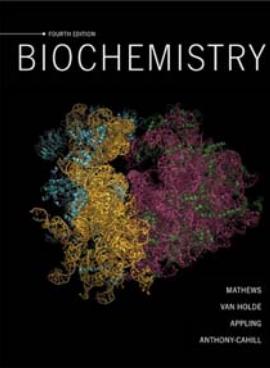


Max Perutz 1914 - 2002

Born 19 May 1914 in Vienna, Austria



Early on Wednesday, 6th February, Max Perutz died of cancer after a long and productive life. Starting a Ph.D. in 1936 under J.D. Bernal at the Cavendish Laboratory, he applied X-ray crystallography to proteins and in 1953 developed the method of isomorphous replacement using heavy atoms to solve the phase problem. **This led to the solution of the first protein structures, those of myoglobin by his colleague John Kendrew and his collaborators, and of haemoglobin by Perutz and his collaborators. For this, Perutz and Kendrew were awarded the 1962 Nobel Prize for Chemistry.** With a great deal more work during the 1960's, Perutz and his colleagues went on to solve the atomic structures of both oxy- and deoxy-haemoglobin which allowed him to propose a stereochemical mechanism for the cooperative binding of oxygen to haemoglobin. Max was the first author of a recent review (Ann. Rev. Biophysics and Biomolecular Structure 1998) of cooperativity in haemoglobin in which he noted that his mechanism still appeared to be correct.



Biochemistry, 4th Edition

LIST OF PUBLICATIONS OF M.F. PERUTZ, OM, CH, CBE, PhD, FRS

Hemoglobin Papers

M.F. Perutz. Umkristallisieren von kleinen Mengen.
Z. Kristallogr. (A) 96, 328-329, 1937.

J.D. Bernal, I. Fankuchen & M.F. Perutz. An X-ray study of chymotrypsin and haemoglobin.
Nature, 141, 523-524, 1938.

M.F. Perutz. Absorption spectra of single crystals of haemoglobin in polarized light.
Nature, 143, 731-734, 1939.

M.F. Perutz. X-ray analysis of haemoglobin. Nature, 149, 491-496, 1942.

M.F. Perutz. Crystal structure of oxyhaemoglobin. Nature, 150, 324-329, 1942

J. Boyes-Watson & M.F. Perutz. X-ray analysis of haemoglobin. Nature, 151, 714-720, 1943.

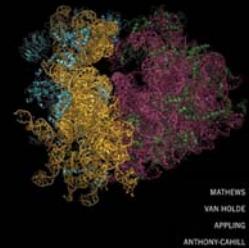
M.F. Perutz. The composition and swelling properties of haemoglobin crystals. Trans. Faraday Soc. Vol.XLII B, 187-195, 1946.

M.F. Perutz & G.L. Rogers. A vacuum tank for use with a single crystal X-ray goniometer.
J. Sci. Instruments, 23, 1946.

J. Boyes-Watson, E. Davidson & M.F. Perutz. An X-ray study of horse methaemoglobin. I.
Proc. Roy. Soc. A. 191, 83-132, 1947.

M.F. Perutz. An X-ray study of horse methaemoglobin. II. Proc. Roy. Soc. A 195, 474-499,
1949.

M.F. Perutz & O. Weisz. Crystal structure of human carboxyhaemoglobin. Nature, 160, 786-
788, 1947.



Biochemistry, 4th Edition

"for their studies of the structures of globular proteins"



The Nobel Prize in Chemistry 1962



Max Ferdinand Perutz

1/2 of the prize

United Kingdom

MRC Laboratory of Molecular Biology
Cambridge, United Kingdom

b. 1914
(in Vienna, Austria)
d. 2002



John Cowdery Kendrew

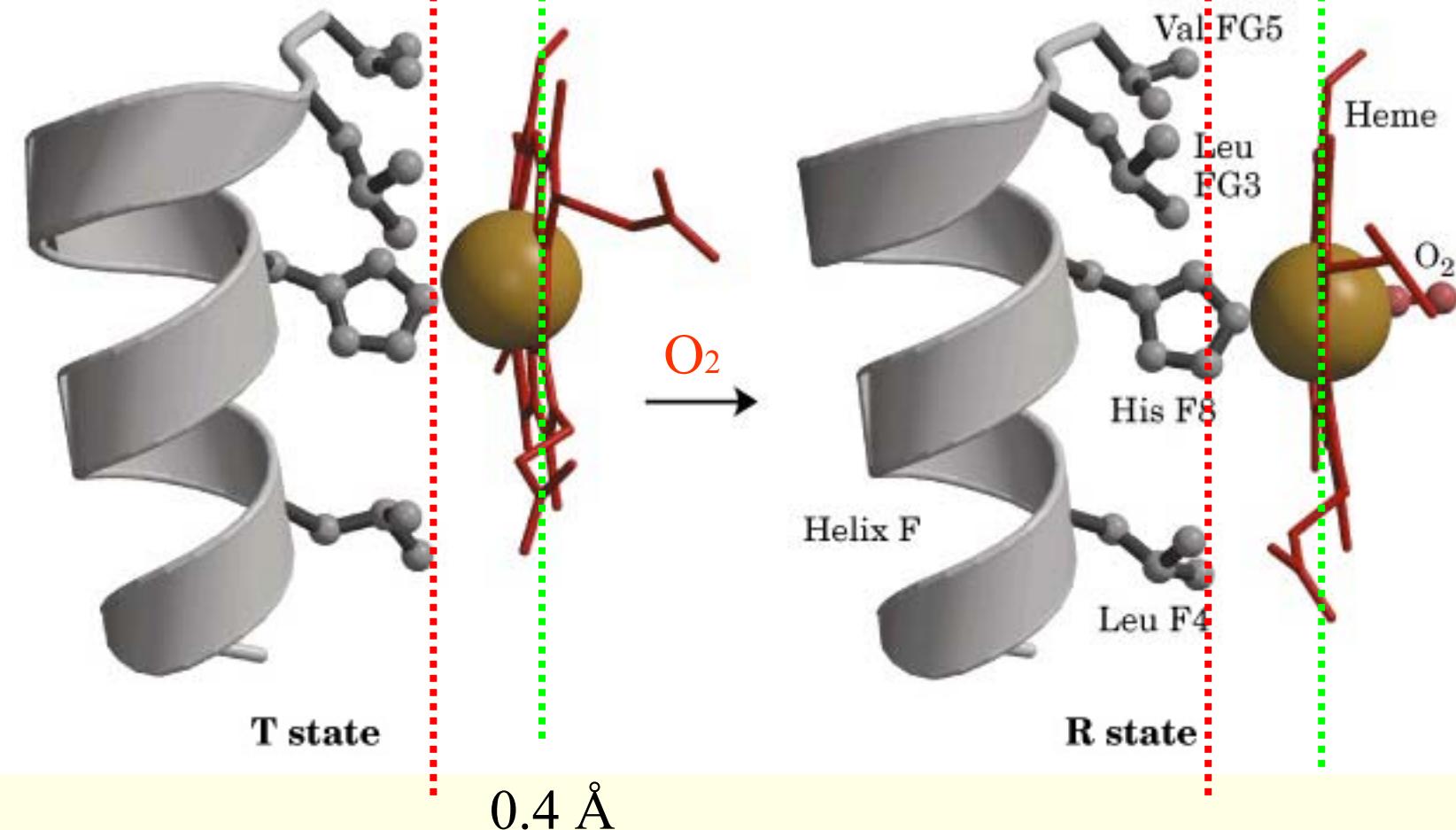
1/2 of the prize

United Kingdom

MRC Laboratory of Molecular Biology
Cambridge, United Kingdom

b. 1917
d. 1997

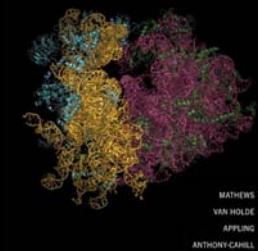
Changes in conformation near heme on O₂ binding



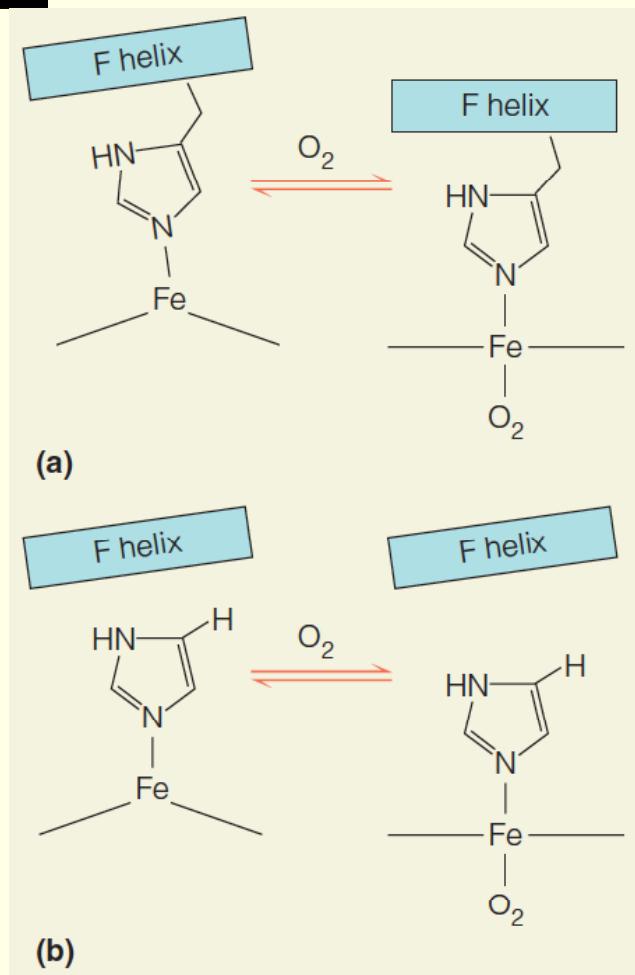
0.4 \AA

Angstrom ($1 \text{ \AA} = 10^{-10} \text{ m}$)

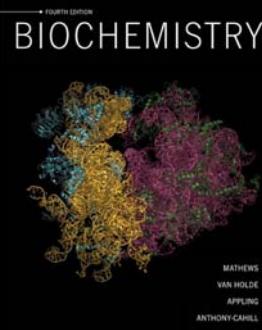
? $^{\circ} \text{A}$



Allosteric Effectors of Hemoglobin



- The effect of replacing the proximal **histidine** in hemoglobin with a **glycine** residue and adding a noncovalently bonded imidazole.
 - The effect of O_2 binding according to the Perutz model: the F helix is drawn toward the heme.
 - Now lacking a connection to the heme, the F helix is not disturbed by O_2 binding and there is significantly reduced cooperativity.

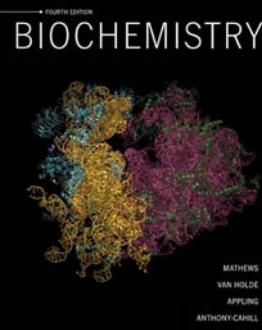


Allosteric Effectors of Hemoglobin

- Cooperative binding and transport of oxygen are only part of the allosteric behavior of hemoglobin.
- As oxygen is utilized in tissues, carbon dioxide is produced and must be transported back to the lungs or gills.
- Accumulation of CO₂ also lowers the pH in erythrocytes through the bicarbonate reaction catalyzed carbonic anhydrase:

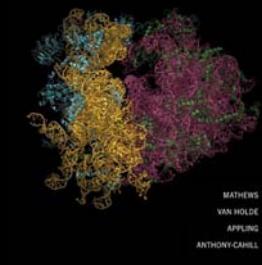


- At the same time, the high demand for oxygen, especially in muscle involved in vigorous activity, can result in oxygen deficit, or hypoxia, which lowers the pH by the production of lactic acid.
- The falling pH in tissue and venous blood signals a demand for more oxygen delivery. Hemoglobin does so through its allosteric transition between high-affinity oxy (*R*) and low-affinity deoxy (*T*) states.

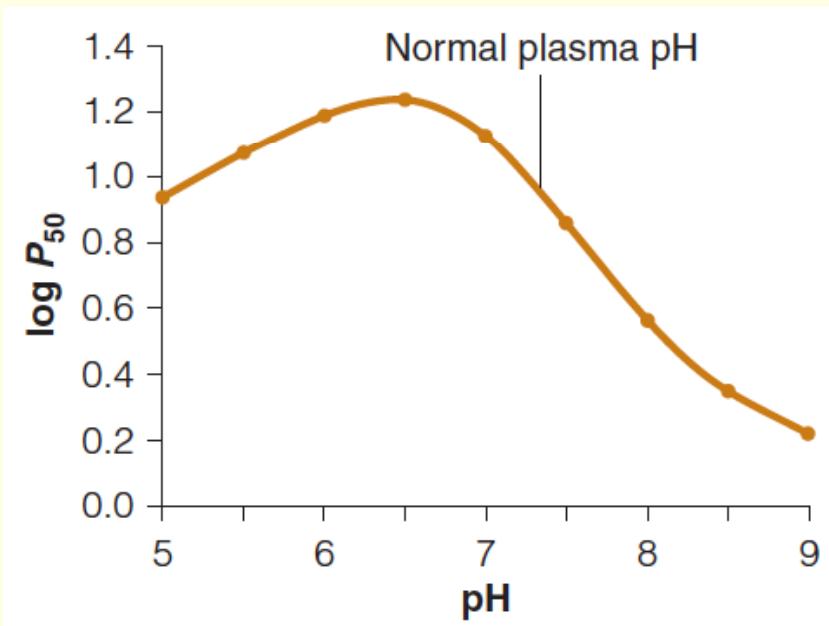


Allosteric Effectors of Hemoglobin

- The **active site** of a protein is where the protein must bind one or more substrate molecules to carry out its primary function.
- The heme pocket is the “active site” of hemoglobin because that is where the ligand is bound.
- In addition to the active site, there may be other sites, called **regulatory sites**, which bind specifically to molecules that regulate the function of the protein.
- The molecules that regulate protein function in this way are called **effectors**, and they typically exert their effects through an **allosteric** mechanism.
 - Those that increase protein activity are **positive effectors**.
 - Those that decrease activity are **negative effectors**.
 - A **homotropic effector** like O₂ in Hb binds at the **active site**.
 - A **heterotropic effector** binds at the **regulatory sites**.



Allosteric Effectors of Hemoglobin

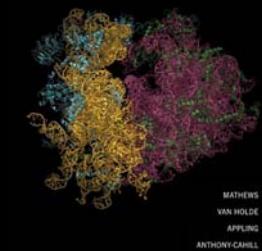


Oxygen affinity of hemoglobin as a function of pH.

A decrease in blood pH results in stabilization of the deoxy state and thereby favors greater O₂ released from hemoglobin.

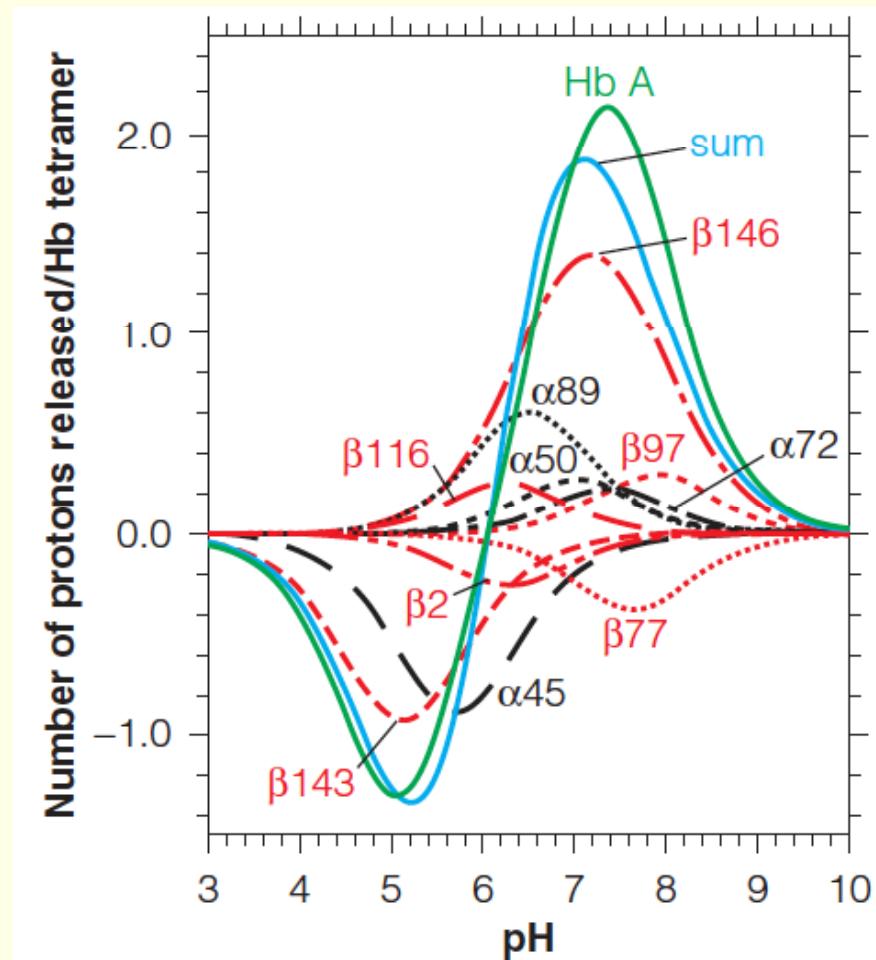
This response of hemoglobin to pH change is called the **Bohr effect**.

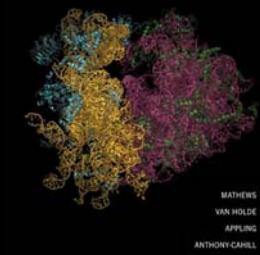




Allosteric Effectors of Hemoglobin

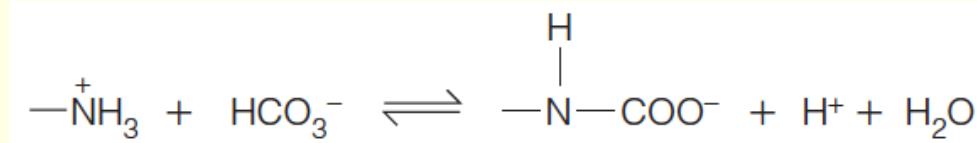
Contributions of various His residues to the Bohr effect in hemoglobin:



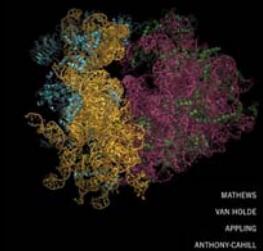


Allosteric Effectors of Hemoglobin

- A small portion of the CO_2 (estimated to be 5–13%) reacts directly with hemoglobin, binding to the *N-terminal amino groups* of the chains to form carbamates:

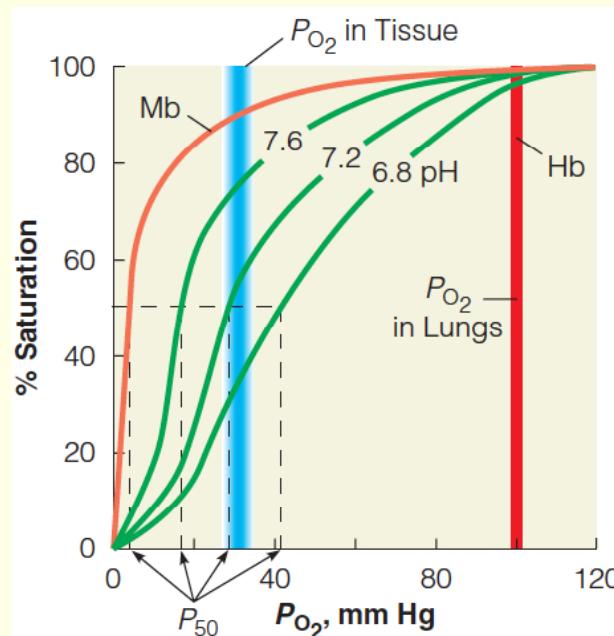


- This *carbamation reaction* allows hemoglobin to aid in the transport of CO_2 from tissues to lungs or gills, and *the protons released on carbamate formation contribute to the Bohr effect.*

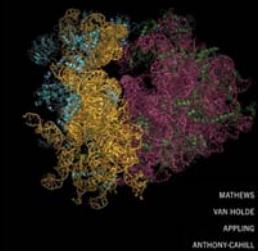


Allosteric Effectors of Hemoglobin

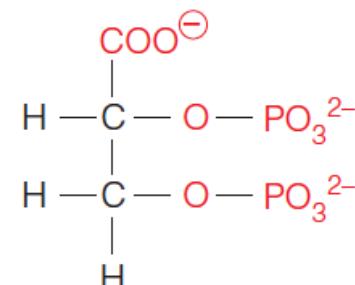
The Bohr effect in hemoglobin:



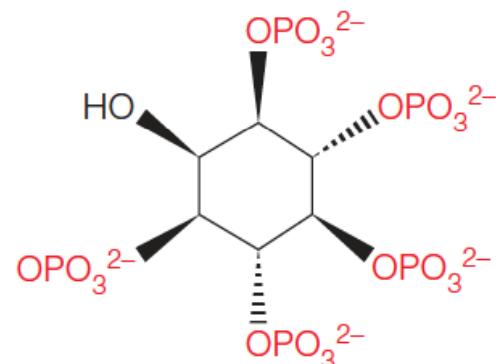
- O_2 -binding curves for hemoglobin are shown for pH 7.6, 7.2, and 6.8.
- Note that the efficiency of O_2 unloading, as measured by the differences in the curves at a P_{O_2} of 30 mm Hg, increases greatly as the pH drops.
- As the hemoglobin circulates from lungs to tissues, the lower pH favors the lower-affinity conformation.
- Myoglobin displays little Bohr effect, so its O_2 -binding curve is approximately the same at all three pH values.



Allosteric Effectors of Hemoglobin



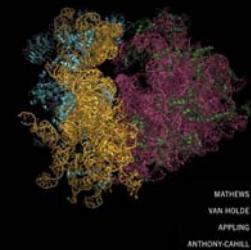
(a) 2,3-Bisphosphoglycerate



(b) myo-Inositol-1,3,4,5,6-pentaphosphate

Two anionic compounds that bind to deoxyhemoglobin:

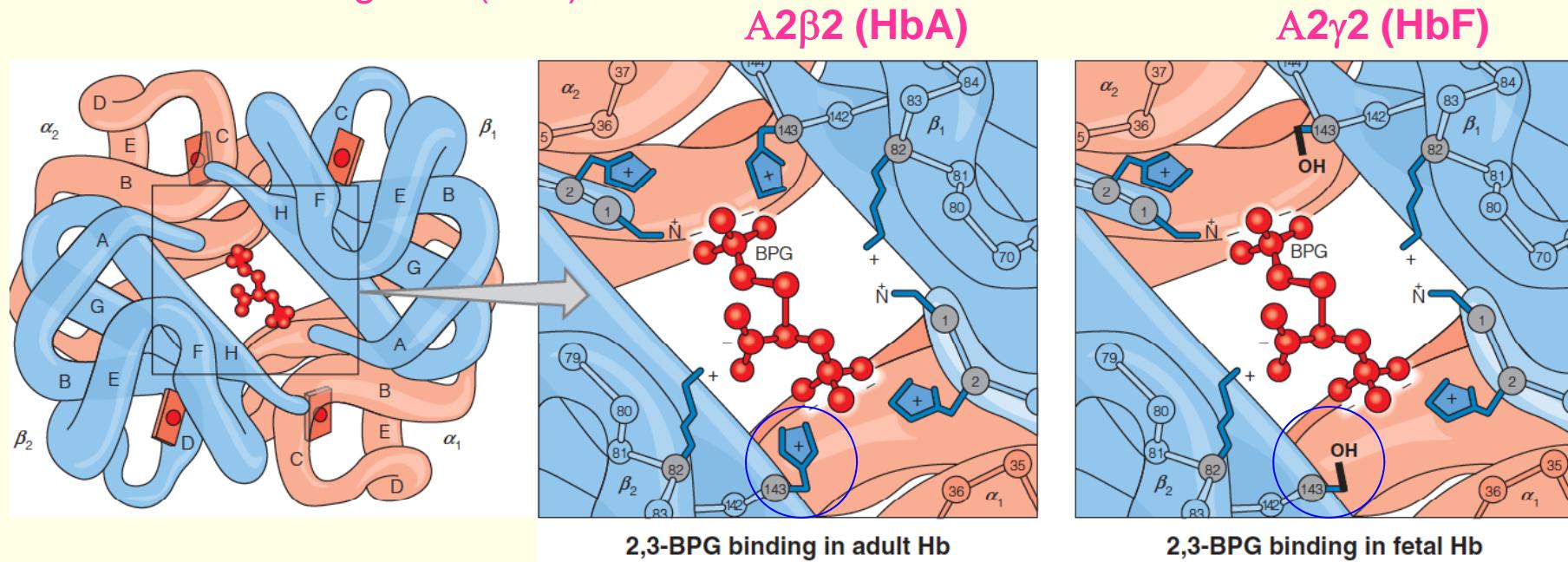
- 2,3-Bisphosphoglycerate (2,3-BPG), found in mammals.
- Myo-Inositol-1,3,4,5,6-pentaphosphate (IPP), found in birds.
- 2,3-BPG is found inside red blood cells and is a potent allosteric effector that lowers the affinity of hemoglobin.

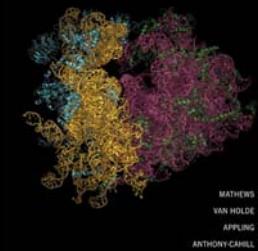


Allosteric Effectors of Hemoglobin

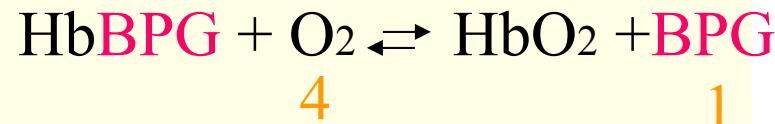
Binding of 2,3-bisphosphoglycerate to deoxyhemoglobin:

- The binding site, in the central cavity of the HbA tetramer is lined with eight positively charged groups that help bind the negatively charged 2,3-BPG molecule.
- Note the His residues ($\beta 143$) that are replaced by Ser in fetal hemoglobin (HbF).

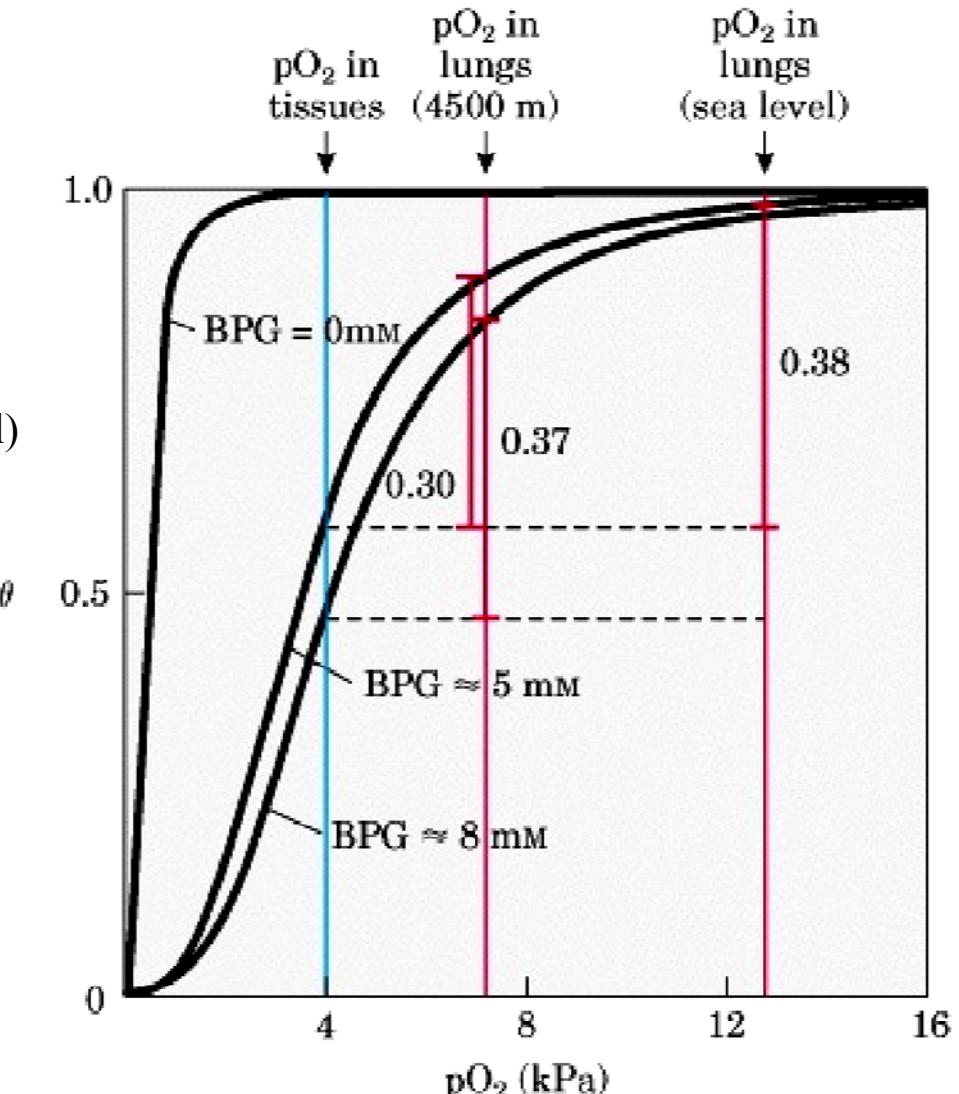
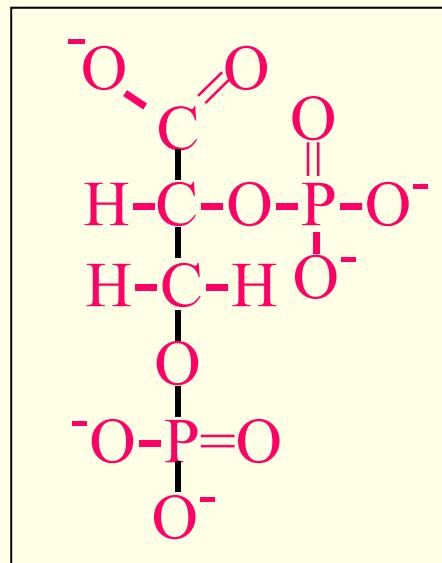


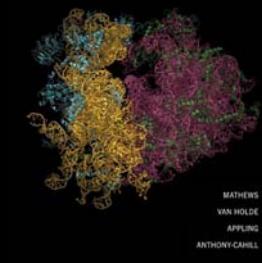


O₂ binding to Hb is regulated by 2,3-bisphosphoglycerate (BPG) (heterotropic allosteric modulation)



[BPG] ↑ during hypoxia
(highly abundant in erythrocytes)
(8 mM at high altitudes, 5 mM at sea level)

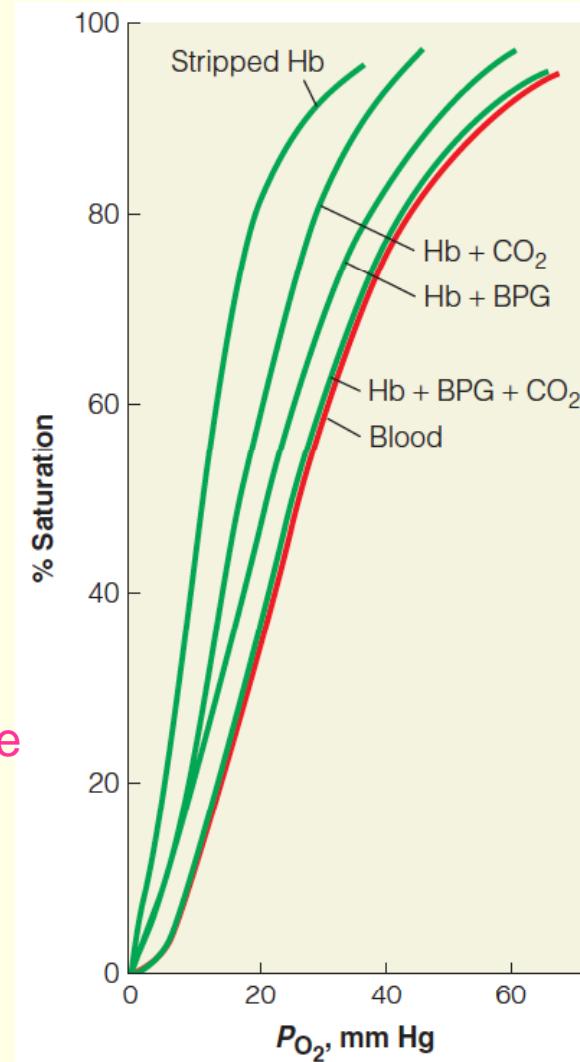


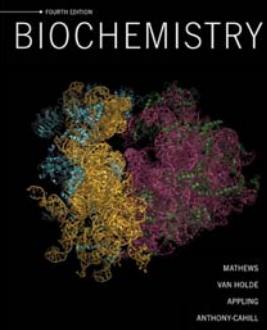


Allosteric Effectors of Hemoglobin

Combined effects of CO₂ and BPG on oxygen binding by hemoglobin:

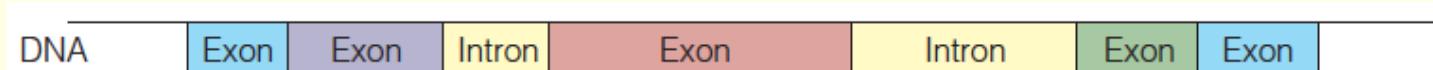
- Hemoglobin that has been stripped of both CO₂ and BPG has a high oxygen affinity.
- When both substances are added to hemoglobin at the levels found in blood emerging from the capillaries, the hemoglobin displays **almost exactly the same binding curve** as observed for whole blood.

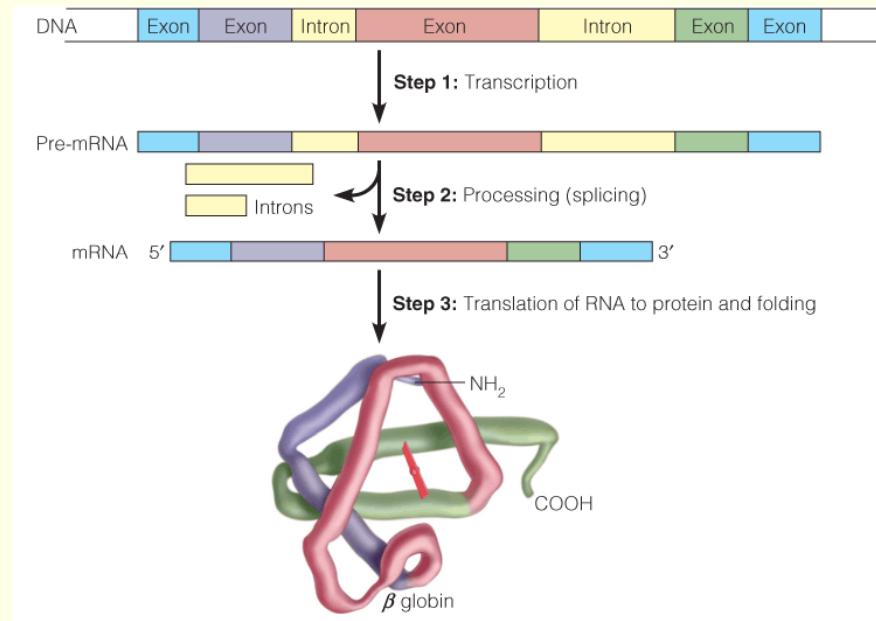
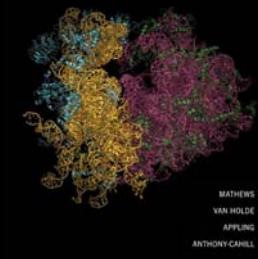




Protein Evolution: Myoglobin and Hemoglobin as Examples

- Eukaryotic genes are discontinuous, containing regulatory and protein-encoding sequences (**exons**) and intervening sequences (**introns**).
- The gene for the human hemoglobin chain has exons, alternating with introns.

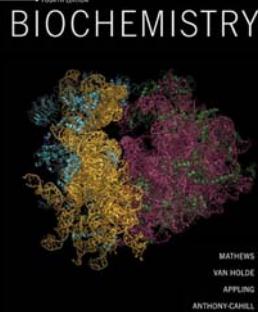




Step 1, transcription: A primary transcript (premRNA) containing complementary copies of the exons and introns is produced from the gene.

Step 2, splicing: The intron sequences are removed and the exons spliced together to yield the final mRNA.

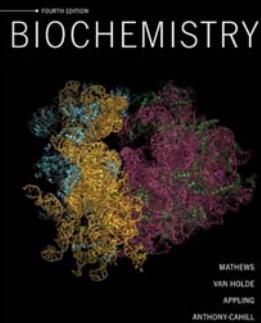
Step 3, translation: The coding regions of the spliced mRNA produce a chain, which adopts its favored three-dimensional structure and incorporates a heme group. Note that the **entire heme-binding region (red) is coded for by one exon (the central exon).**



Protein Evolution: Myoglobin and Hemoglobin as Examples

Residue number	1	2	3	4	5	6	7	8	9	10	
Normal β gene	...A T G	G T G Val	C A c His	C T G Leu	A C T Thr	C C T Pro	G A G Glu	G A G Glu	A A G Lys	T C T Ser	G C C Ala
(a) Silent, or synonymous mutation	...A T G	G T G Val	C A T His	C T G Leu	A C T Thr	C C T Pro	G A G Glu	G A G Glu	A A G Lys	T C T Ser	G C C Ala
(b) Missense, or non synonymous mutation	...A T G	G T G Val	C A C His	C T G Leu	A C T Thr	C C T Pro	G T G Val	G A G Glu	A A G Lys	T C T Ser	G C C Ala
(c) Nonsense mutation	...A T G	G T G Val	C A C His	C T G Leu	A C T Thr	C C T Pro	G A G Glu	G A G Glu	T A G Stop	T C T G C C	...
(d) Frameshift mutation by deletion	...A T G	G T G Val	C A C His	C T G Leu	A C □ Thr	C C T G Leu	A G G Arg	A G A Arg	A G T Ser	C T G Leu	G C C ...

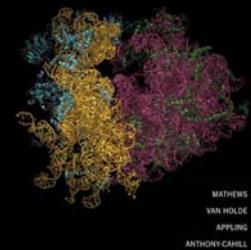
- a) A silent, or synonymous, mutation has occurred in the codon for residue 2 (CAC to CAT).
- b) A missense, or nonsynonymous, mutation has occurred in the codon for residue 6 (GAG to GTG). This is the sickle-cell mutation.
- c) A nonsense mutation has introduced a stop signal after the codon for residue 7 (AAG to TAG), terminating the chain prematurely.
- d) A frameshift mutation has occurred by deletion of a single T residue. The rest of the chain, with a completely altered sequence, will continue to be produced until a stop signal is encountered in the new frame. Both (c) and (d) would result in β -thalassemia.



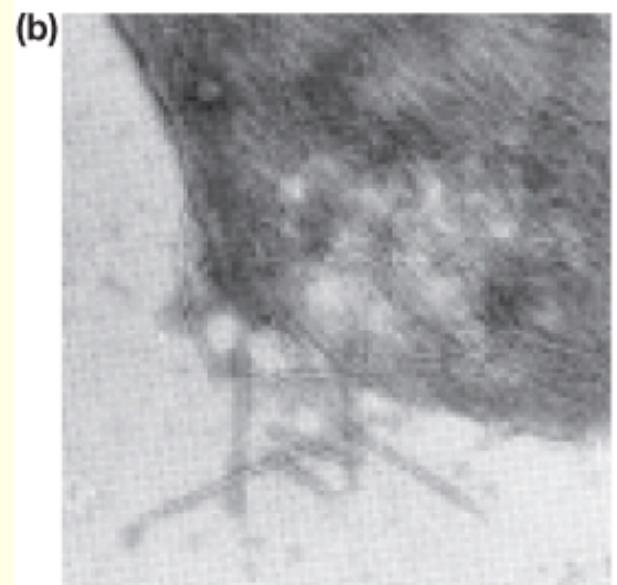
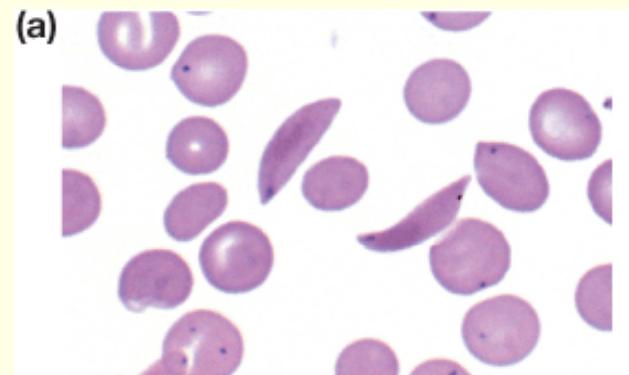
Hemoglobin Variants: Evolution in Progress

TABLE 7.1 Selected list of missense mutations in human hemoglobins

Effect	Residue Changed	Change	Name	Consequences of Mutation	Explanation
Sickling	β 6 (A3)	Glu \longrightarrow Val	S	Sickling	Val fits into EF pocket in chain of another hemoglobin molecule.
	β 6 (A3)	Glu \longrightarrow Ala	G Makassar	Not significant	Ala probably does not fit the pocket as well.
	β 121 (GH4)	Glu \longrightarrow Lys	O Arab, Egypt	Enhances sickling in S/O heterozygote	β 121 lies close to residue β 6; Lys increases interaction between molecules.
Change in O ₂ affinity	α 87 (F8)	His \longrightarrow Tyr	M Iwate	Forms methemoglobin, decreased O ₂ affinity	The His normally ligated to Fe has been replaced by Tyr.
	α 141 (HC3)	Arg \longrightarrow His	Suresnes	Increases O ₂ affinity by favoring R state	Replacement eliminates bond between Arg 141 and Asn 126 in deoxy state.
	β 74 (E18)	Gly \longrightarrow Asp	Shepherds Bush	Increases O ₂ affinity by decrease in BPG binding	The negative charge at this point decreases BPG binding.
	β 146 (HC3)	His \longrightarrow Asp	Hiroshima	Increases O ₂ affinity, reduced Bohr effect	Disrupts salt bridge in deoxy state and removes a His that binds a Bohr-effect proton.
	β 92 (F8)	His \longrightarrow Gln	St. Etienne	Loss of heme	The normal bond from F8 to Fe is lost, and the polar glutamine tends to open the heme pocket.
Heme loss	β 42 (CD1)	Phe \longrightarrow Ser	Hammersmith	Unstable, loses heme	Replacement of hydrophobic Phe with Ser attracts water into heme pocket.
Dissociation of tetramer	α 95 (G2)	Pro \longrightarrow Arg	St. Lukes	Dissociation	Chain geometry is altered in subunit contact region.
	α 136 (H19)	Leu \longrightarrow Pro	Bibba	Dissociation	Pro interrupts helix H.



Hemoglobin Variants: Evolution in Progress



Erythrocytes in sickle-cell disease:

- Typical sickled cells, together with some normal, rounded red blood cells.
- Scanning electron micrograph of a sickled cell that has ruptured, with **hemoglobin fibers** spilling out.

Hemoglobin Variants: Evolution in Progress

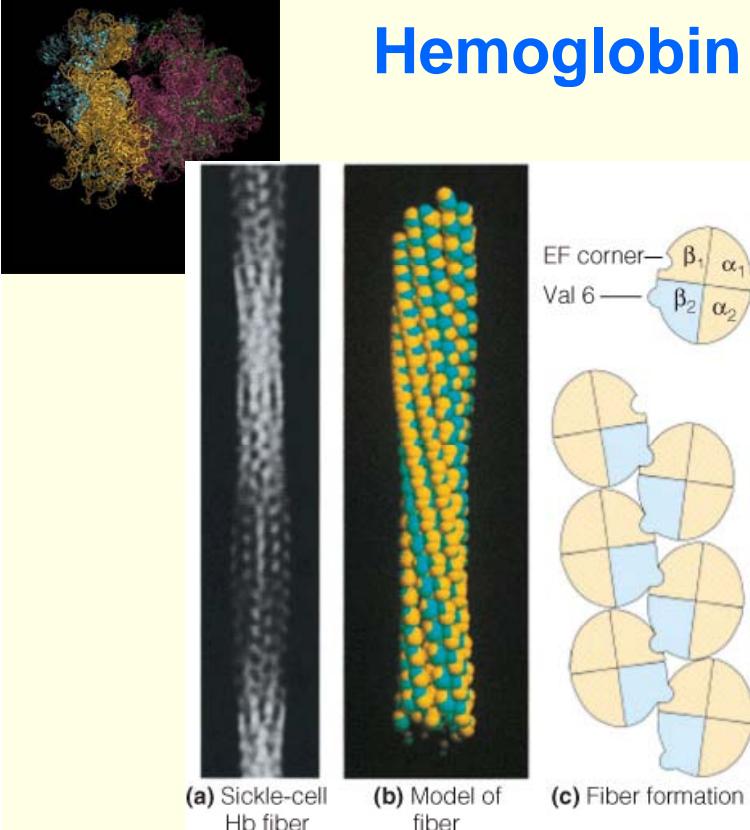


FIGURE 7.35

Sickle-cell hemoglobin. Molecules of sickle-cell hemoglobin tend to aggregate, forming long fibers. (a) An electron micrograph of one sickle-cell fiber. (b) A computer-graphic depiction of one fiber. (c) A schematic model of fiber formation. Deoxyhemoglobin S molecules lock together to form a two-stranded cluster because Val 6 in the β chain of one hemoglobin molecule fits into a pocket in an adjacent molecule. Interaction of these two-stranded structures with one another produces the multistrand fibers shown in (a) and (b).

Courtesy of B. Carragher, D. Bluemke, M. Potell, and R. Josephs.

Sickle-cell hemoglobin:

Molecules of sickle-cell Hb tend to aggregate, forming long fibers.

- An electron micrograph of one sickle-cell fiber.
- A computer-graphic depiction of one fiber.
- A schematic model of fiber formation.

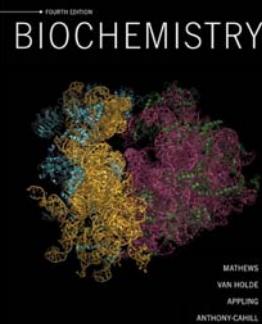
DeoxyHb S molecules lock together to form a two-stranded cluster because Val 6 in the β chain of one Hb molecule fits into a pocket in an adjacent molecule.

Interaction of these two-stranded structures with one another produces the multistrand fibers shown in (a) & (b).

Sickle-cell disease is confined in malaria-infected regions.

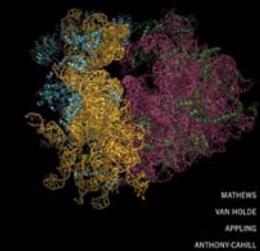
1998, USA FDA approved hydroxyurea for treatment.

($\alpha_2\gamma_2$ induction)



Immunoglobulins: Variability in Structure Yields Versatility in Binding

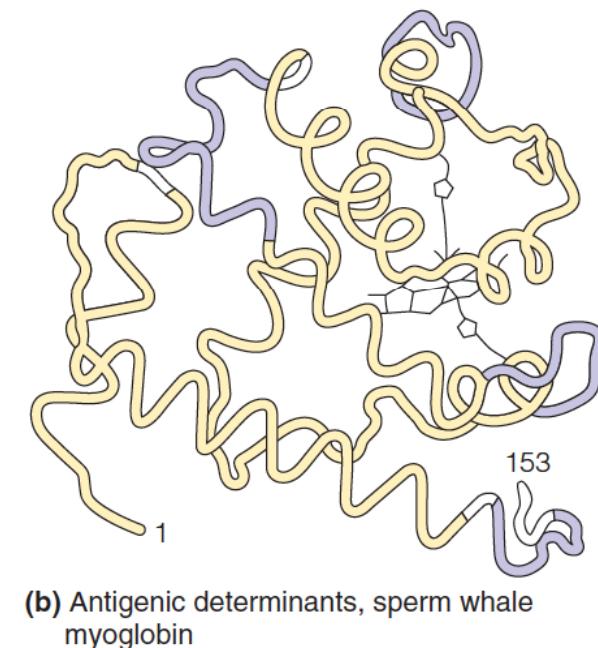
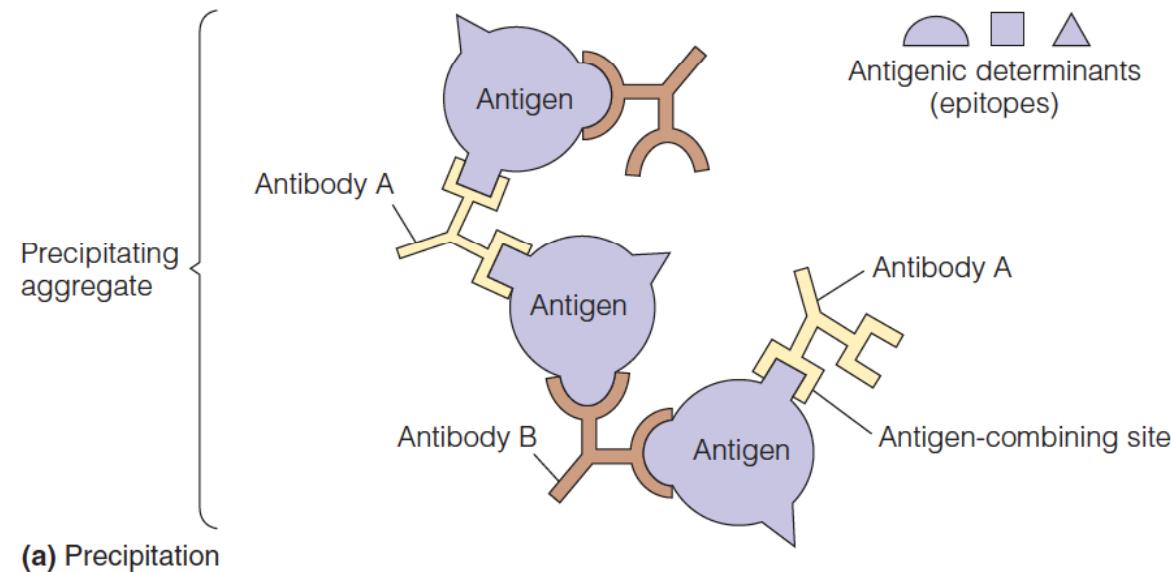
- The immune response involves the defense of the body against foreign substances or pathogens and operates via many different cellular mechanisms.
- In the ***humoral*** immune response, ***B lymphocytes*** secrete ***antibodies (immunoglobulins)*** that react with specific antigens.
- These ***immunoglobulin proteins***, whose primary function is the specific, and essentially irreversible, binding of substances that appear to be of nonself origin, such as bacterial or viral pathogens.

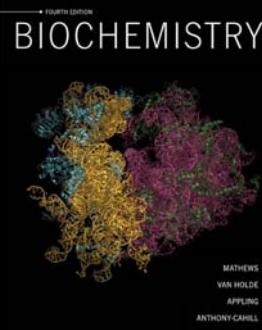


Immunoglobulins: Variability in Structure Yields Versatility in Binding

Antigenic determinants (*epitopes*):

Some antigenic determinants involve portions of chain that are far apart in the primary sequence but close together in the tertiary structure, a so-called **discontinuous epitope**.



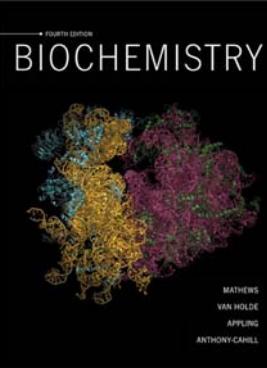


Immunoglobulins: Variability in Structure Yields Versatility in Binding

In the ***clonal selection theory***, the body has an inherent ability to produce an immense diversity of antibodies with different amino acid sequences that are able to bind an enormous range of antigens.

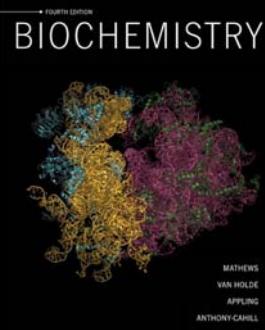
The basic postulates of the clonal selection theory are as follows:

1. B stem cells in the bone marrow differentiate to become B lymphocytes, each producing a single type of immunoglobulin molecule, each type with a binding site that will recognize a specific molecular shape.
 - These immunoglobulins, or antibodies, are attached to the cell membrane and exposed on the outer surfaces of the B lymphocytes.



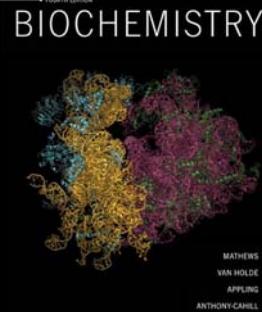
Immunoglobulins: Variability in Structure Yields Versatility in Binding

2. Binding of an antigen to one of these antibodies stimulates the cell carrying it to replicate, generating a clone (a collection of cells with identical genetic information).
 - This primary response is aided by a special class of T cells called helper T cells.
 - If a helper T cell recognizes a bound antigen, it binds to the appropriate B lymphocyte and transmits to it a signal protein (interleukin-2) that stimulates B-cell reproduction.
 - Thus, only those clones of B cells that recognize antigens are stimulated to continued cell division.



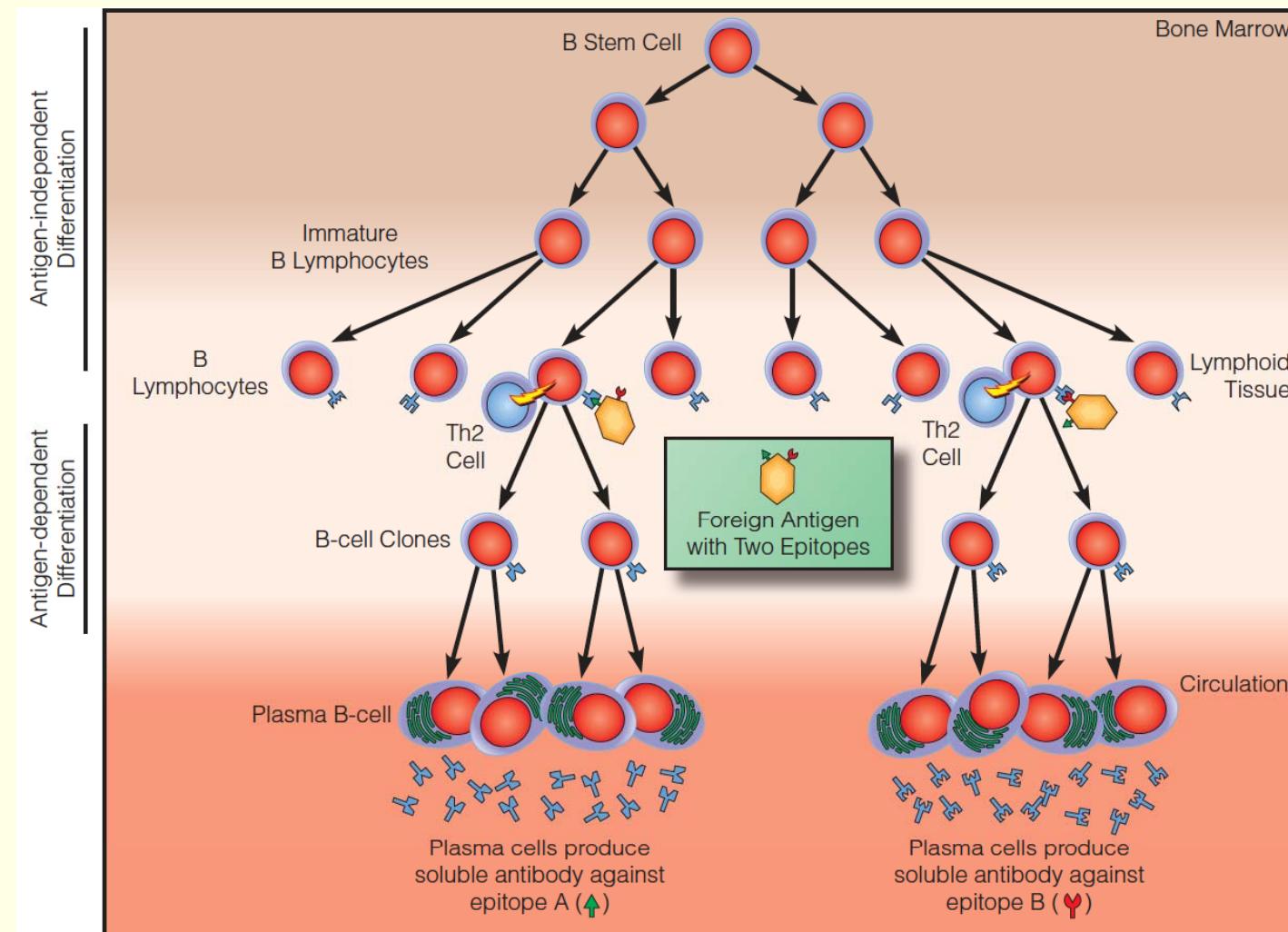
Immunoglobulins: Variability in Structure Yields Versatility in Binding

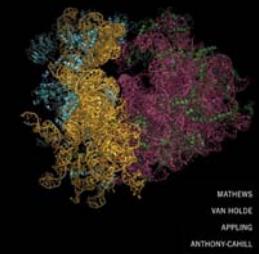
3. Two classes of cloned B cells are produced, effector B cells, or plasma cells, now produce soluble antibodies, which are secreted into the circulatory system.
 - These antibodies have the same antigen binding sites as the surface antibodies of the B lymphocyte from which the effector cells arose, but they lack the hydrophobic tail that bound the surface antibodies to the lymphocyte membrane. The other class of cells in the clone—memory cells—will persist for some time, even after antigen is no longer present.
 - This persistence constitutes the immune memory: It allows a rapid secondary response to a second stimulation by the same antigen



Immunoglobulins: Variability in Structure Yields Versatility in Binding

The clonal selection theory of the adaptive immune response:

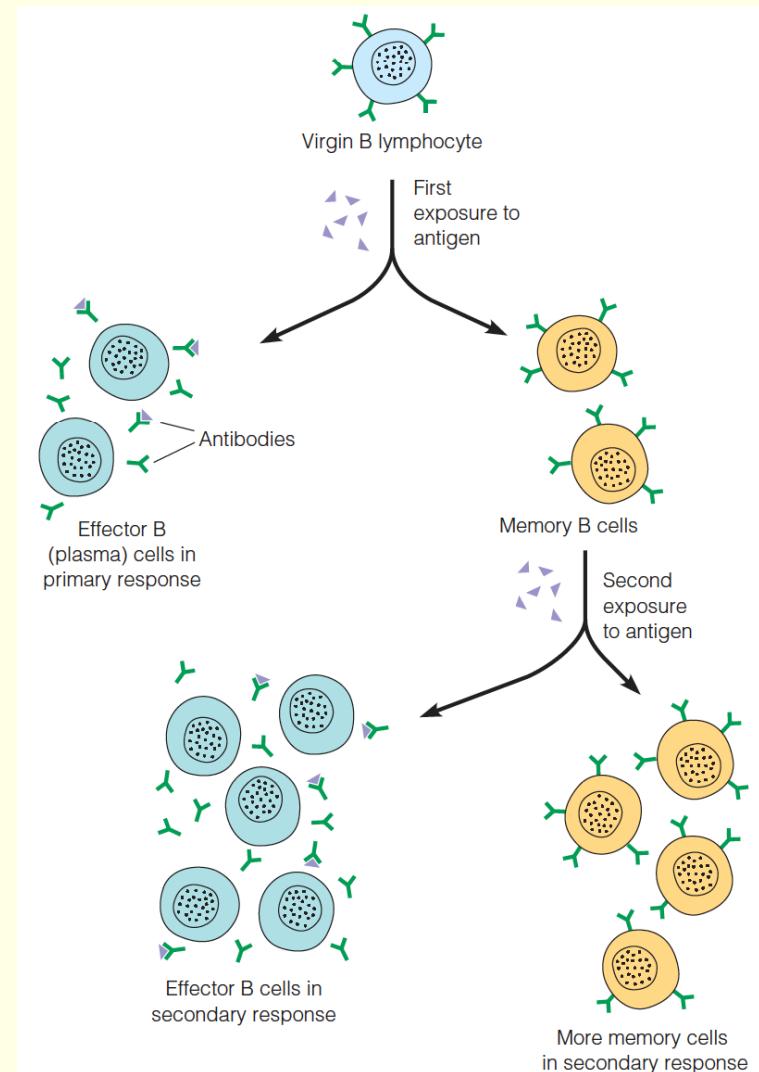


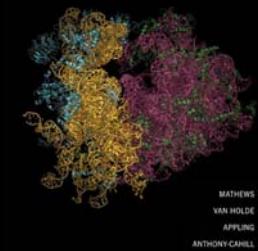


Immunoglobulins: Variability in Structure Yields Versatility in Binding

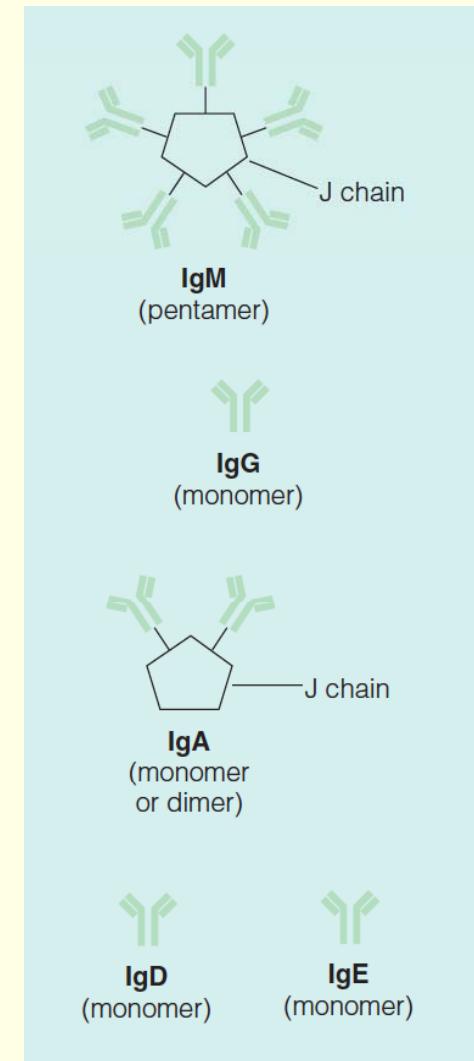
Two developmental paths for stimulated B lymphocytes:

- Exposure to antigen causes two kinds of cells to develop from B lymphocytes.
 - Cells of one type (effector B cells, or plasma cells) synthesize soluble antibody.
 - Cells of the second class (memory cells) carry membrane-bound antibody to allow a rapid and enhanced response to a second exposure of the same antigen.

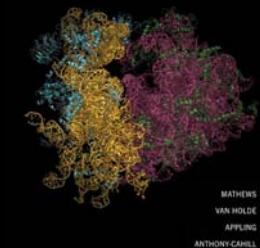




Immunoglobulins: Variability in Structure Yields Versatility in Binding



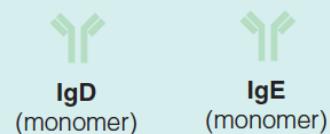
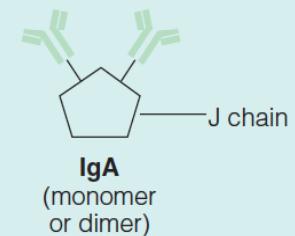
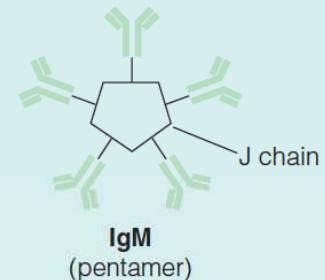
- Each immunoglobulin monomer (e.g., IgG) consists of four chains:
 - Two identical heavy chains ($M = 53,000$ Da each)
 - Two identical light chains ($M = 23,000$ Da each)
 - Held together by disulfide bonds.
- Immunoglobulin molecules contain both **constant** and **variable** regions.
- The **variable regions** are the **antigen binding sites**.



Immunoglobulins: Variability in Structure Yields Versatility in Binding

TABLE 7.2 The five classes of immunoglobulins

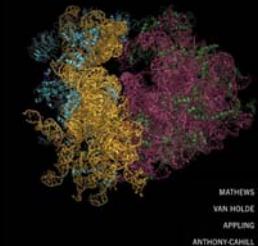
IgM is produced during the early response to an invading microorganism. It is the largest immunoglobulin, containing five Y-shaped units of two light and two heavy chains each. The units are held together by a component called a J chain. The relatively large size of IgM restricts it to the bloodstream. It is also effective in triggering an important mechanism for foreign cell destruction, called the complement system.



IgG molecules, also known as γ -globulin, are the most abundant of circulating antibodies. A variant is attached to B-cell surfaces. IgG molecules consist of a single Y-shaped unit and can traverse blood vessel walls rather readily; they also cross the placenta to carry some of the mother's immune protection to the developing fetus. Specific receptors allow such passage. IgG also triggers the complement system.

IgA is found in body secretions, including saliva, sweat, and tears, and along the walls of the intestines. It is the major antibody of colostrum, the initial secretion from a mother's breasts after birth, and of milk. IgA occurs as a monomer or as double-unit aggregates of the Y-shaped protein molecule. IgA molecules tend to be arranged along the surface of body cells and to combine there with antigens, such as those on a bacterium, thus preventing the foreign substance from directly attaching to the body cell. The invading substance can then be swept out of the body together with the IgA molecule.

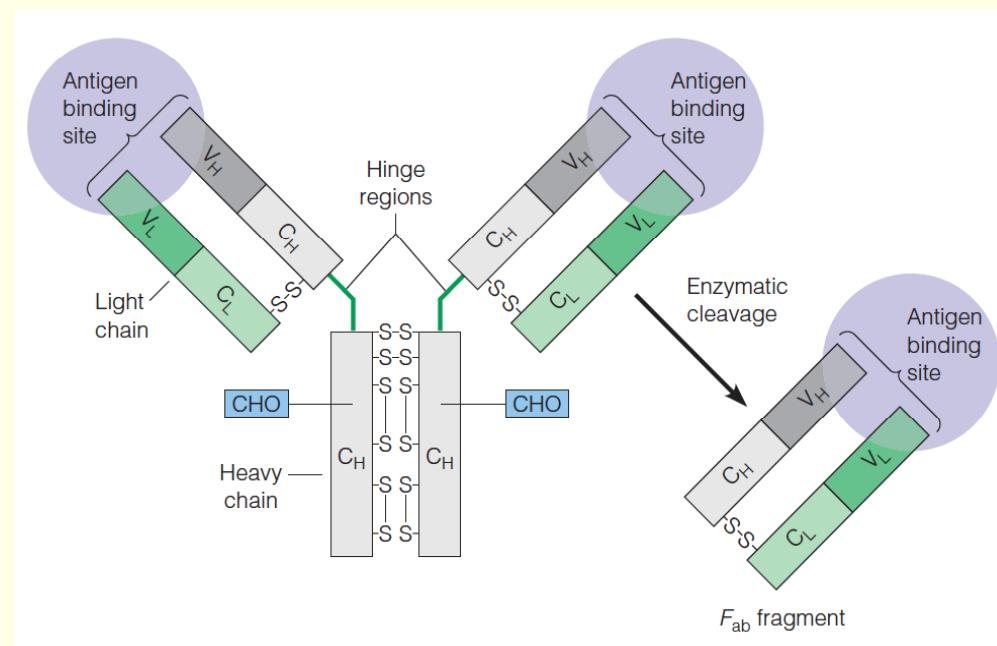
Less is known about the IgD and IgE immunoglobulins. IgD molecules are found on the surface of B cells, though little is known about their function. IgE is associated with some of the body's allergic responses, and its levels are elevated in individuals who have allergies. The constant regions of IgE molecules can bind tightly to mast cells, a type of epithelial and connective tissue cell that releases histamines as part of the allergic response. Both IgD and IgE consist of single Y-shaped units.



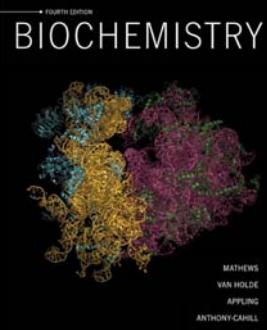
Immunoglobulins: Variability in Structure Yields Versatility in Binding

Schematic models of an IgG antibody molecule and an F_{ab} fragment:

- The IgG is made from two identical heavy chains and two identical light chains, all held together by disulfide bonds. Each chain contains both constant domains (C) and variable domains (V).

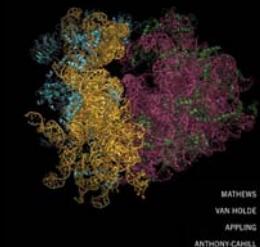


- Constant domains are the same in all antibody molecules of a given class, whereas variable domains confer specificity to a given antigenic determinant.
- Cleavage by certain proteolytic enzymes such as papain at the hinge and one Fc fragment regions allows production of two identical monovalent F_{ab} fragments and one F_c fragment.



Immunoglobulins: Variability in Structure Yields Versatility in Binding

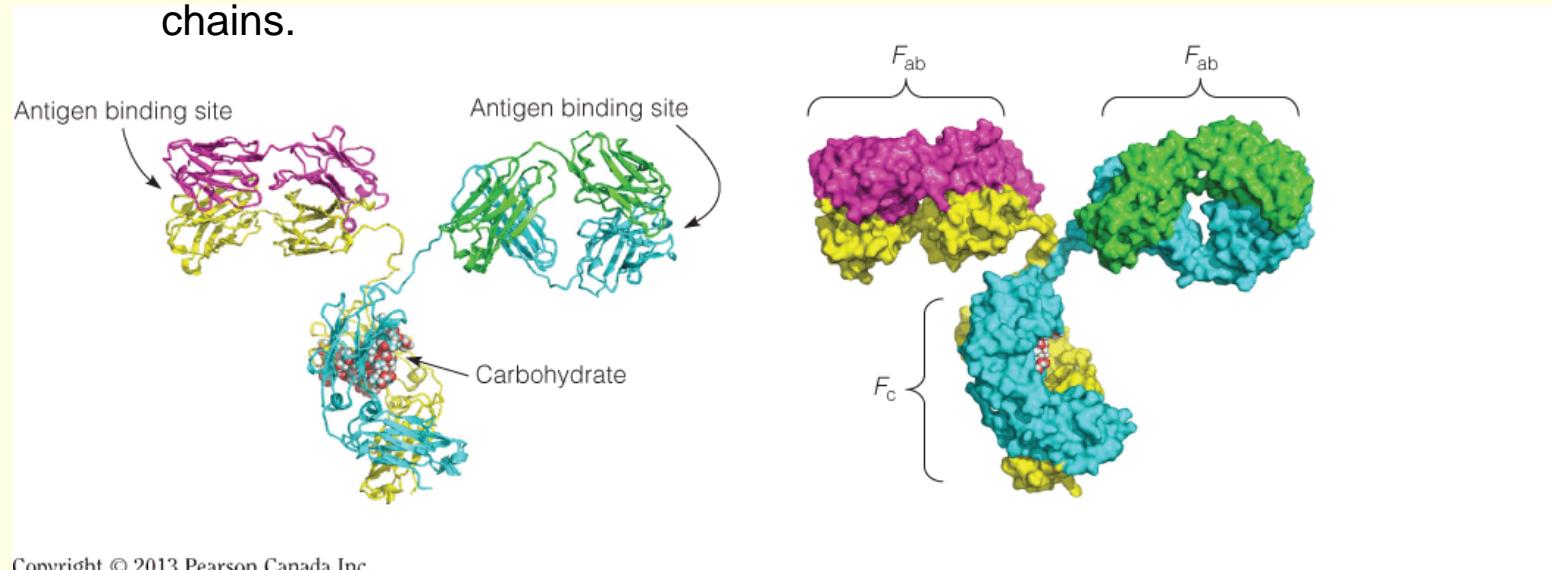
- The diversity as well as the exquisite specificity of antigen binding sites is determined by the ***hypervariable complementarity determining regions (CDR)*** from both the light and the heavy chains.



Immunoglobulins: Variability in Structure Yields Versatility in Binding

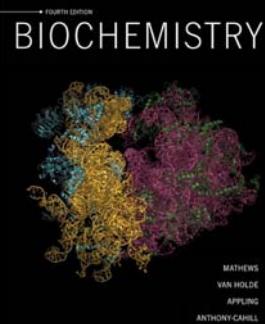
The crystal structure of an IgG molecule from mouse:

- The identical heavy chains are colored yellow and cyan; the identical light chains are magenta and green.
- A cartoon model, illustrating the high degree of secondary structure, is shown on the left.
- On the right is a surface rendering showing the intimate contact between the chains.



Copyright © 2013 Pearson Canada Inc.

Copyright © 2013 Pearson Canada Inc.

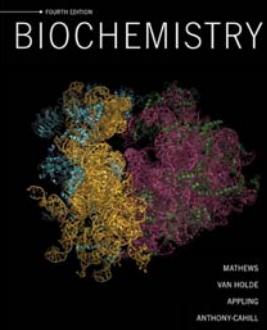


Immunoglobulins: Variability in Structure Yields Versatility in Binding



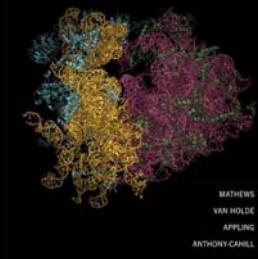
The immunoglobulin fold:

- A common structure in domains of many proteins in the immunoglobulin superfamily.
- Two antiparallel sheets (cyan and orange) are stacked face to face and covalently bonded by a disulfide bond (not shown).
- This folding motif is found 12 times in the IgG molecule and 4 times within an F_{ab} .

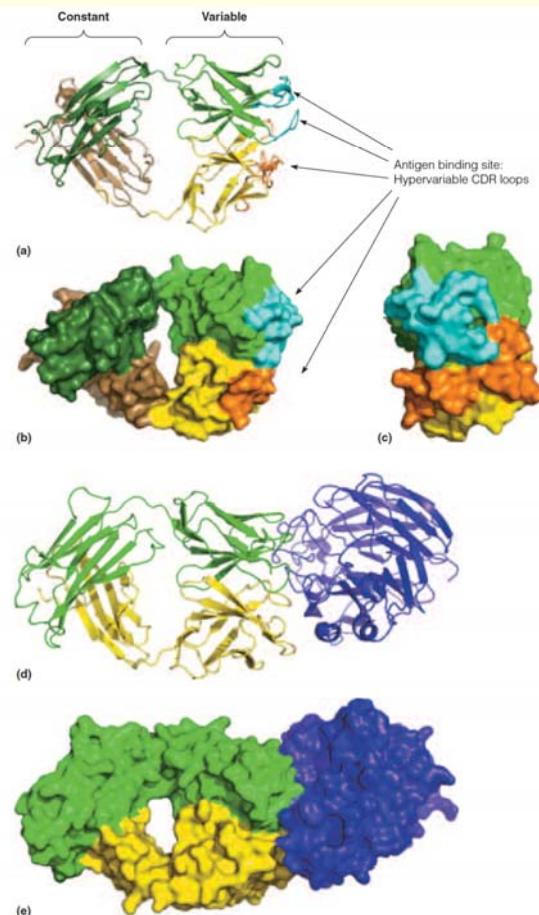


Immunoglobulins: Variability in Structure Yields Versatility in Binding

- Through **somatic recombination and rapid mutation**, a human can generate over **10 billion different antibodies**.
- The cellular immune response uses killer T cells to destroy foreign or infected cells.

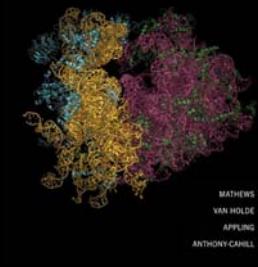


Immunoglobulins: Variability in Structure Yields Versatility in Binding

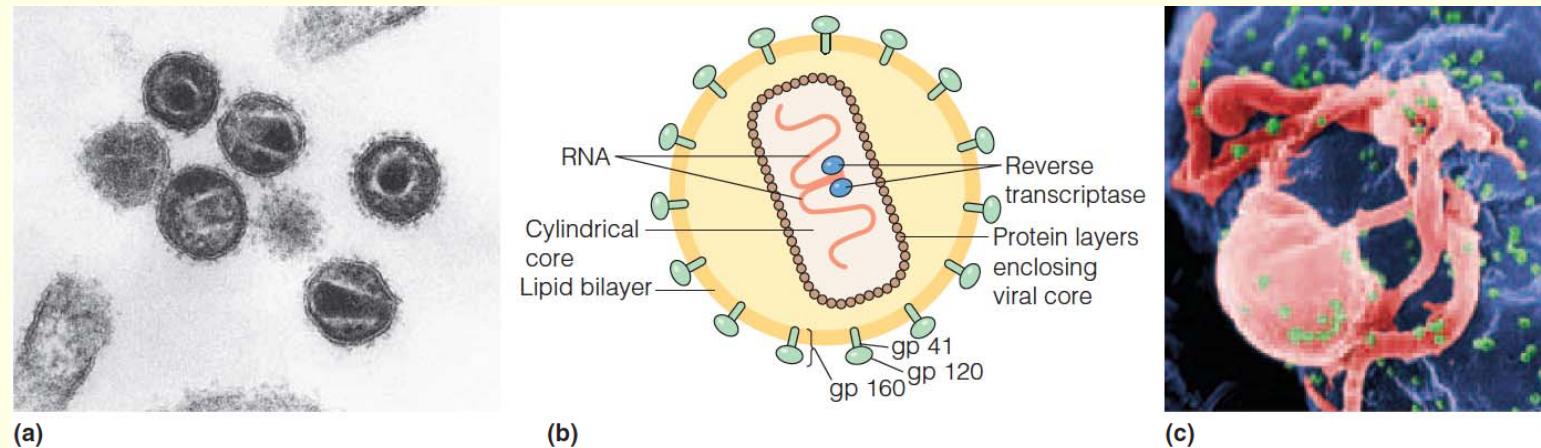


Antigen binding by an F_{ab} fragment:

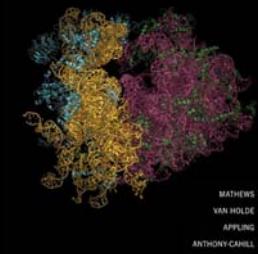
- Backbone structure of an F_{ab} fragment.
- Surface rendering of F_{ab} fragment.
- Same rendering as in (b), but rotated 90 degrees to view the surface of the antigen binding site formed by the CDR loops.
- The close contact that occurs between antigen and antibody surfaces is shown in the backbone and surface renderings of a murine F_{ab} fragment bound to the viral protein **neuraminidase**.



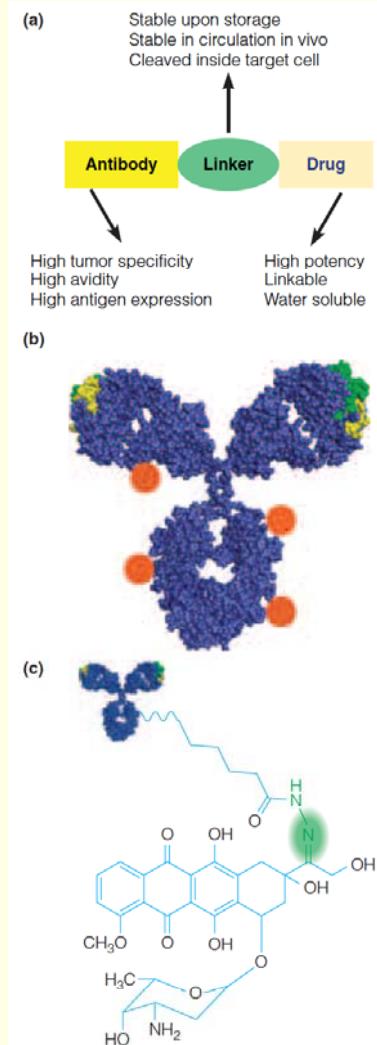
Immunoglobulins: Variability in Structure Yields Versatility in Binding



- Electron micrograph of the human immunodeficiency virus (HIV) that is responsible for AIDS.
- A schematic model of HIV. The surface protein gp160 is composed of two fragments, gp41 and gp120. The RNA genome is transcribed into DNA by an error-prone reverse transcriptase. This DNA integrates into the host cell genome, and is then retranscribed to produce new viral RNA.
- False-color scanning electron micrograph of budding HIV-1 virus particles (green spheres) on the surface of a human lymphocyte (red).



Immunoglobulins: Variability in Structure Yields Versatility in Binding

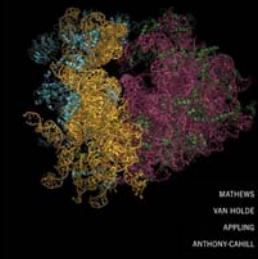


Immunoconjugate drugs for targeted chemotherapy:

a) The desirable features for each component of the immunoconjugate: targeting antibody, linker, and cytotoxic drug.

b) Common sites of attachment of drugs to the antibody constant regions are shown as orange spheres. The tumor-specific antigen binding sites are shown in green and yellow.

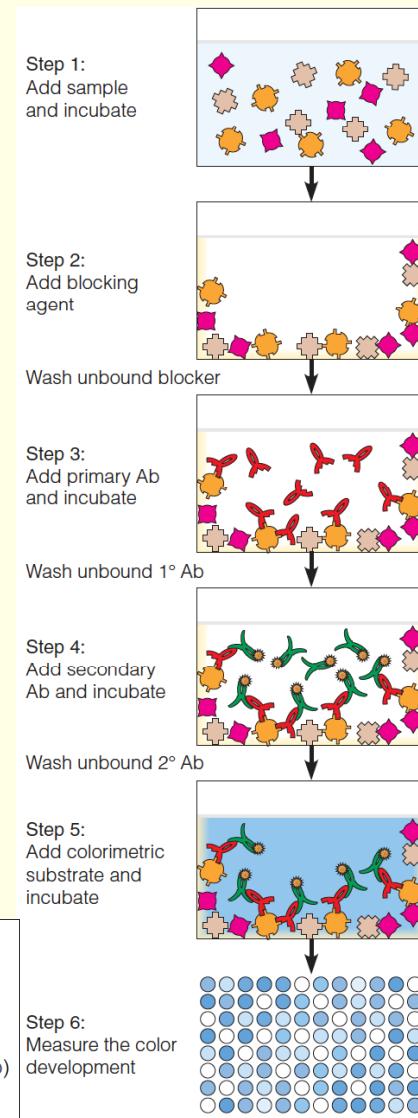
c) A schematic of the immunoconjugate. The acid-labile hydrazone linker is highlighted in green. The linker is stable in circulation in blood (pH 7.4), but is cleaved in the acidic environment of the endosome following endocytosis into the tumor cell.

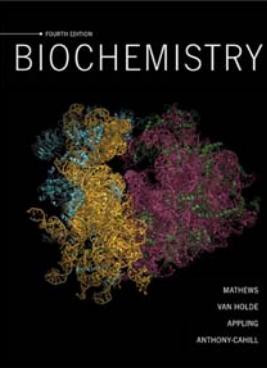


Immunological Methods

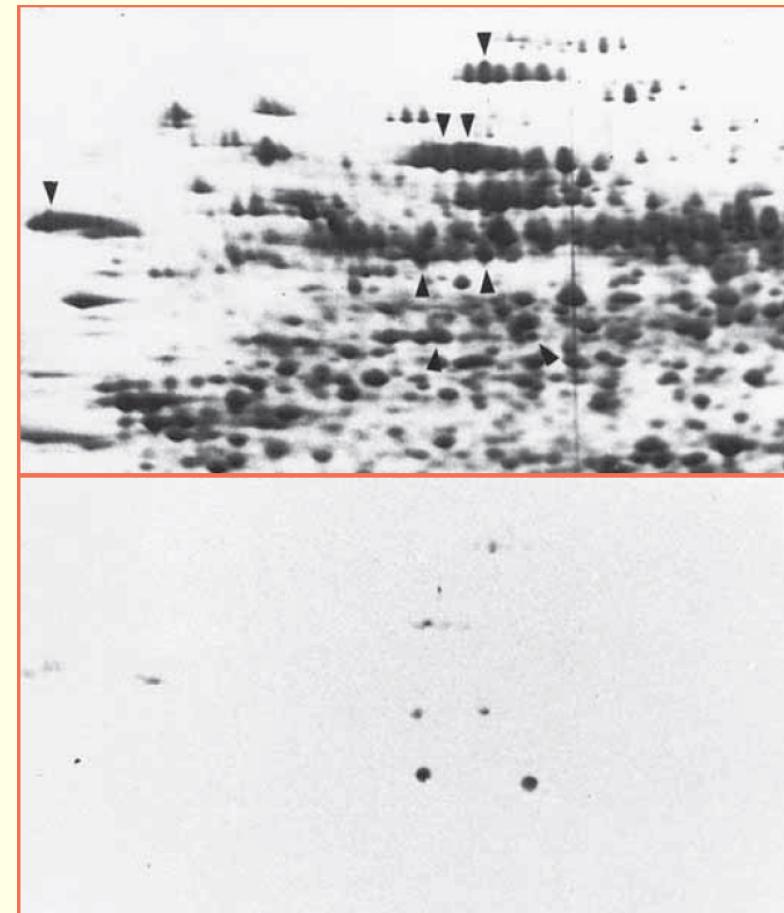
The indirect ELISA assay:

- Detection of one protein in a complex mixture is shown.
- Steps 1–5 show a close-up view of a single well.
- In step 5 color develops in the well due to the action of the enzyme linked to the 2° antibody.
- The enzyme causes a chromogenic substrate to change color.
- In step 6, the entire 96-well plate is analyzed (each circle represents one of the 96 wells). Those wells with color are presumed to contain the target antigen.



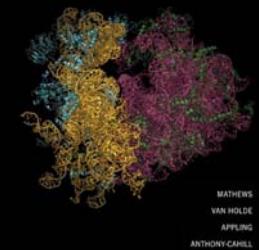


Immunological Methods

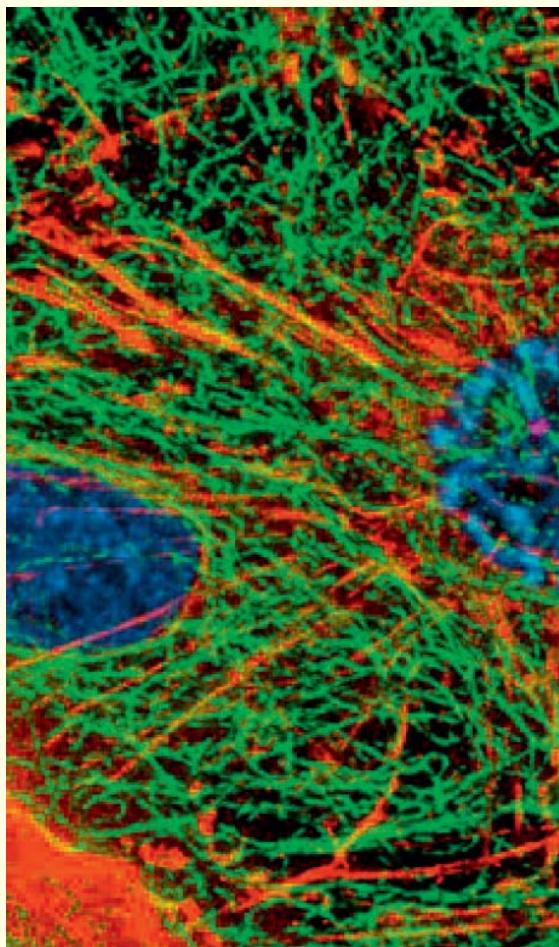


Western blotting:

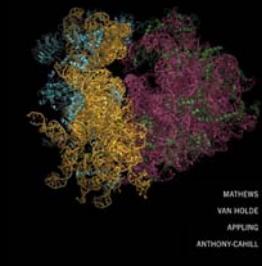
- On the top is a 2-D gel of total protein from tobacco leaf.
- On the bottom is the same gel, blotted with an antibody against proteins containing phosphothreonine residues.



Immunological Methods



- Immunofluorescent light micrograph of a rat kangaroo kidney epithelial cell during mitotic cell division.
- The chromosomes (blue, center) are condensed, after replication.
- The actin microfilaments (red) and tubulin microtubules (green) of the cytoskeleton maintain the structure of the cell.
- Antibodies have been used to attach different fluorescent dyes to the chromatin, actin, and tubulin.

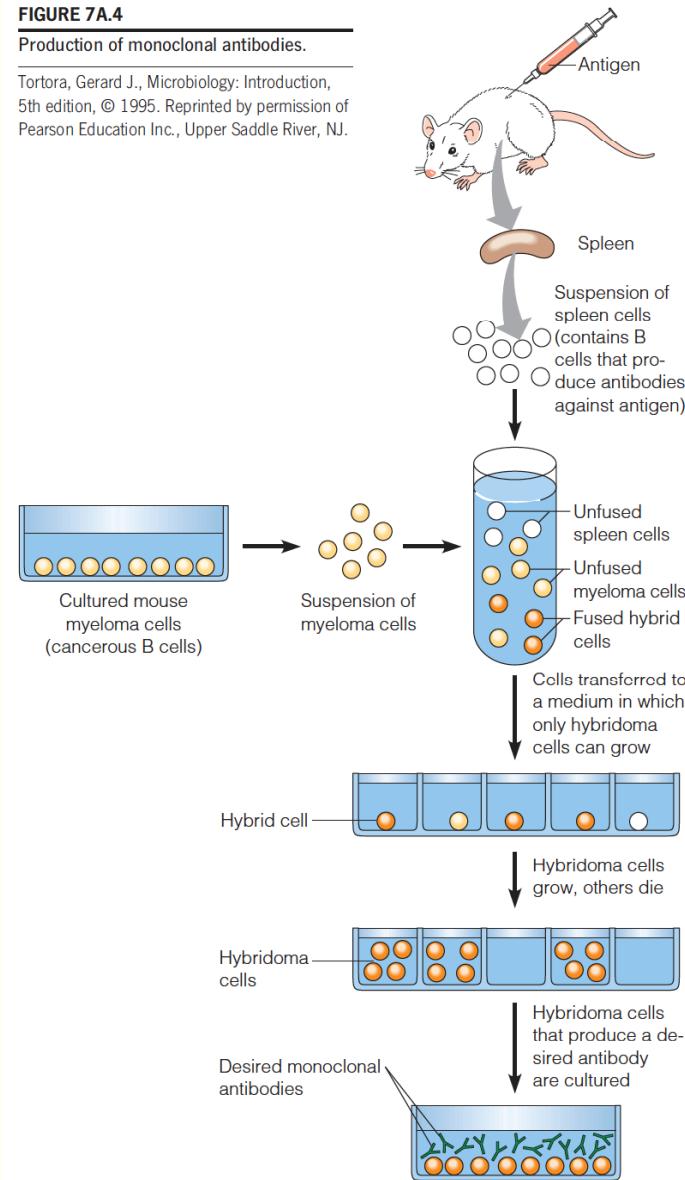


Immunological Methods

FIGURE 7A.4

Production of monoclonal antibodies.

Tortora, Gerard J., *Microbiology: Introduction*, 5th edition, © 1995. Reprinted by permission of Pearson Education Inc., Upper Saddle River, NJ.



Production of monoclonal antibodies: