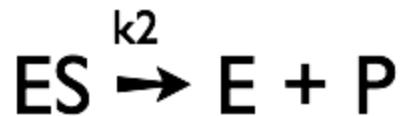
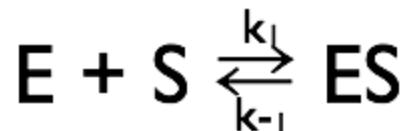


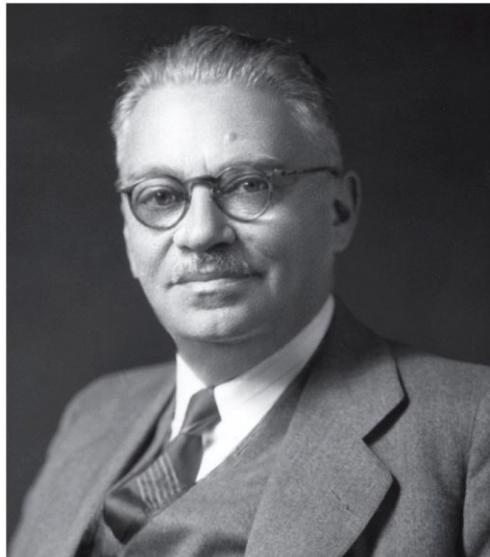
Enzyme Kinetics

General Observations

- Enzymes are able to exert their influence at very low concentrations $\sim [\text{enzyme}] = \text{nM}$
- The initial velocity (rate) is linear with [enzyme].
- The initial velocity increases with [substrate] at low [substrate].
- Saturation: The initial velocity reaches a maximum at high [substrate] and does not increase with increasing [substrate].



Michaelis and Menten developed a formalism for studying enzyme catalyzed reaction kinetics.



Leonor Michaelis, 1875–1949

Unnumbered 6 p199a

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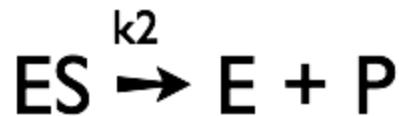
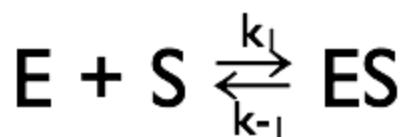


Maud Menten, 1879–1960

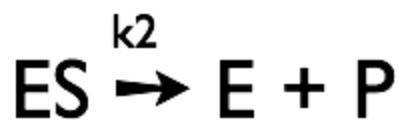
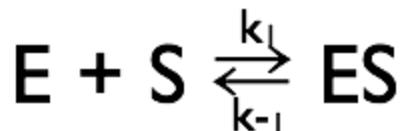
Unnumbered 6 p199b

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Michaelis-Menten Kinetics



Simplest enzyme mechanism

- One reactant (S)
- One intermediate (ES)
- One product (P)

How to make kinetic measurements

Experiment:

1. Mix enzyme + substrate.
2. Record rate of substrate disappearance and/or product formation as a function of time (the velocity of reaction).
3. Plot initial velocity versus substrate concentration.
4. Change substrate concentration and repeat.

The initial reaction velocity $[(d[P]/dt)_{ini}]$ increases with $[S]$ at low $[S]$.

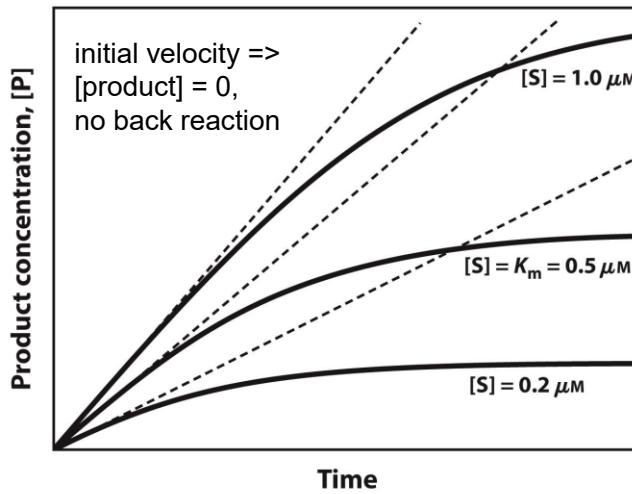
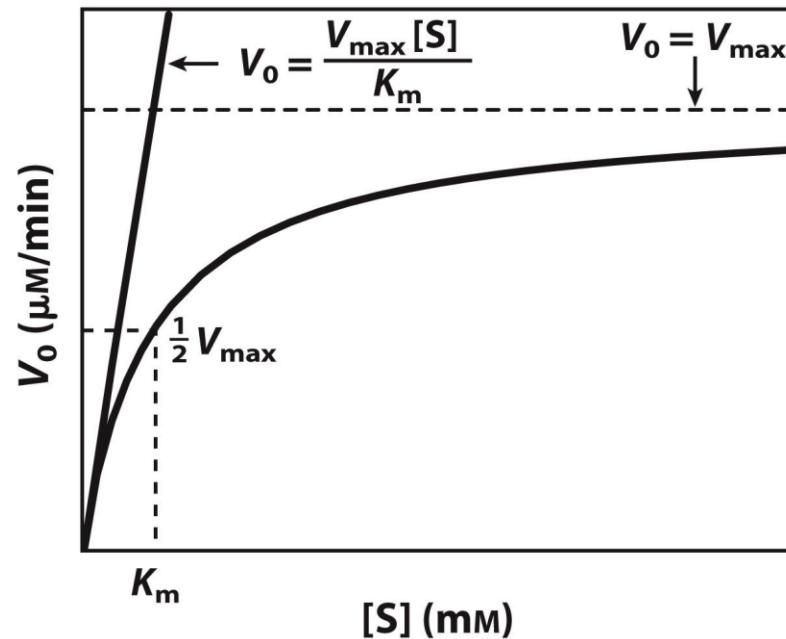


Figure 6-10
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Saturation Kinetics:

At low [S], Velocity is proportional to [S]
At high [S], Velocity is independent of [S]



Always measure initial velocity

[velocity = $d[P]/dt$, P=product]

Figure 6-12
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Michaelis-Menten Kinetics

$$v = \frac{v_{\max}[S]}{K_M + [S]} \quad \text{Michaelis-Menten Equation}$$

When $[S] = K_M$ then,

$$v = \frac{v_{\max}[S]}{[S]+[S]} = \frac{v_{\max}}{2}$$

This is saying that when $K_M = [S]$, the reaction runs at half maximum velocity.

Michaelis-Menten Kinetics

K_m is the substrate concentration required to reach half-maximal velocity ($v_{max}/2$).

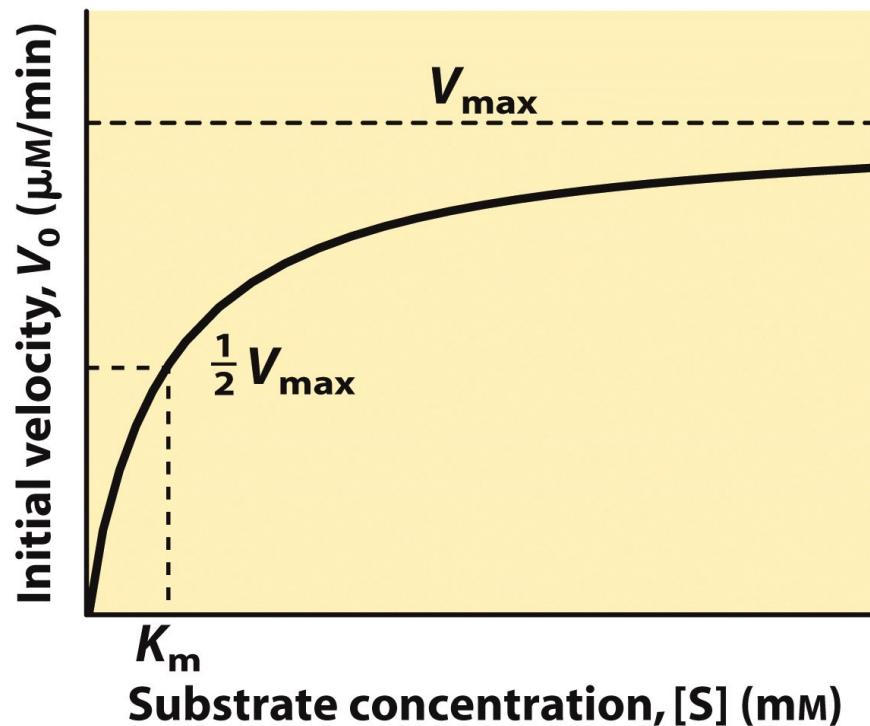


Figure 6-11
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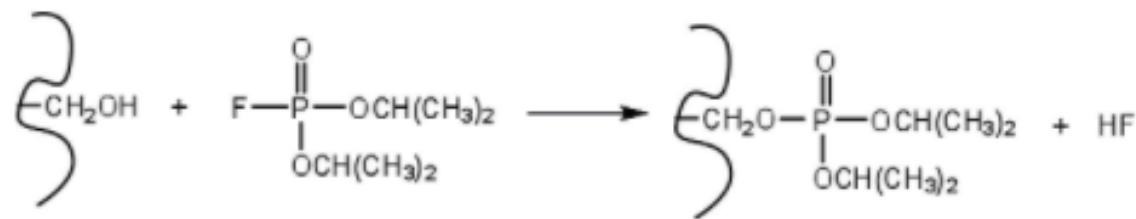
Enzyme Inhibition

An enzyme inhibitor decreases enzymatic activity.

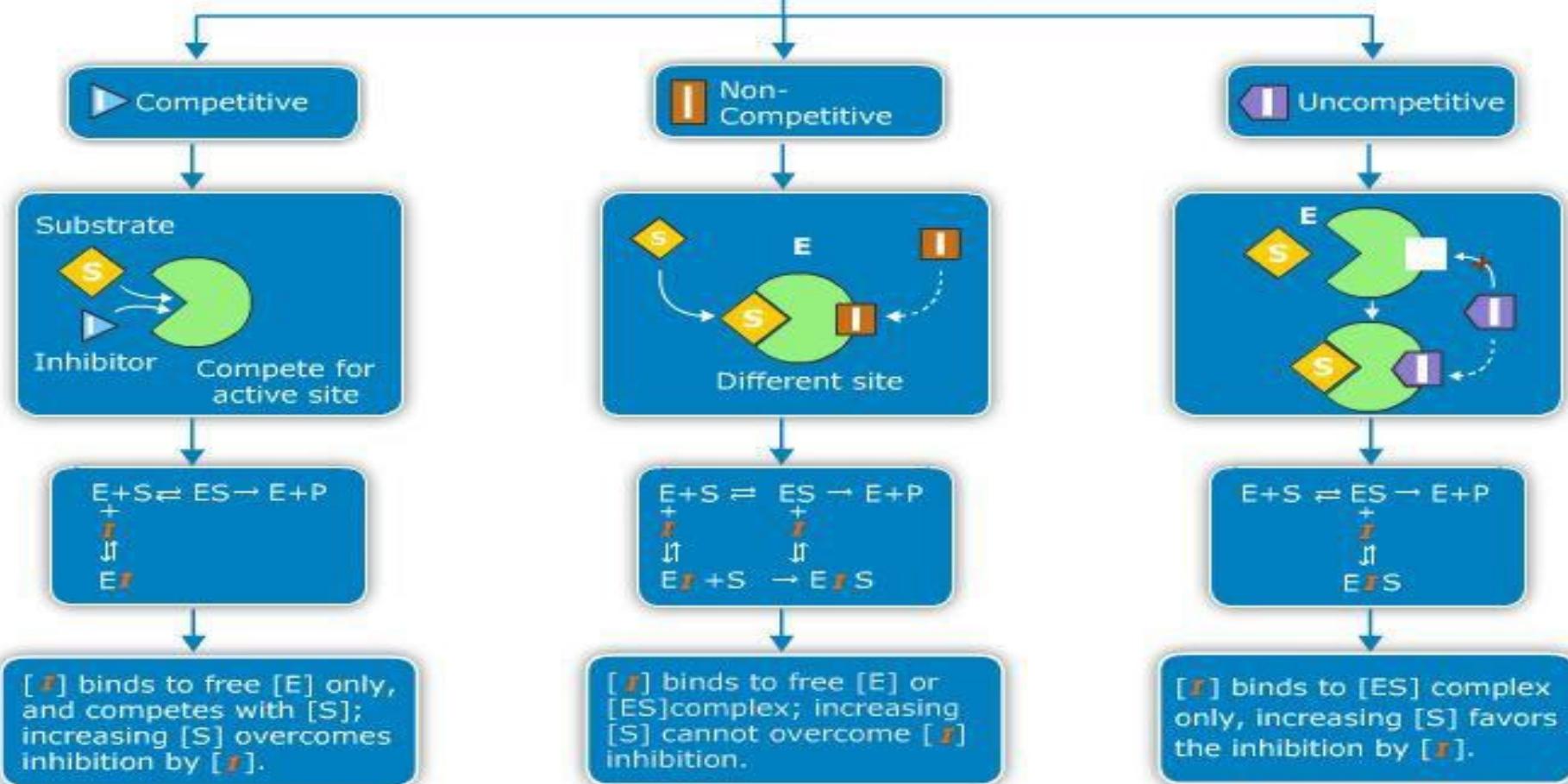
- Irreversible inhibitors (inactivators) react covalently with the enzyme.
 - One inhibitor molecule can permanently shut off one enzyme molecule.
 - are often **powerful toxins** but also may be used as drugs.
- Reversible inhibitors bind by non-covalent interactions and can associate and dissociate from the enzyme.
 - are often structural analogs of substrates or products.
 - are often **used as drugs** to slow down a specific enzyme.
 - can bind to the free enzyme and prevent the binding of the substrate.
 - can bind to the enzyme-substrate complex and prevent the reaction.

Irreversible Inhibition

Poison	Formula	Example of Enzyme Inhibited	Action
arsenate	AsO_4^{3-}	glyceraldehyde 3-phosphate dehydrogenase	substitutes for phosphate
iodoacetate	ICH_2COO^-	triose phosphate dehydrogenase	binds to cysteine SH group
diisopropylfluoro-phosphate (DIFP; a nerve poison)	$\begin{array}{c} \text{F} \\ \\ \text{O}=\text{P}-\text{OCH}(\text{CH}_3)_2 \\ \\ \text{OCH}(\text{CH}_3)_2 \end{array}$	acetylcholinesterase	binds to serine OH group



Enzyme Inhibition (Mechanism)



Summary of enzyme inhibition kinetics analysis

Table 12-2 Effects of Inhibitors on Michaelis–Menten Reactions^a

Type of Inhibition	Michaelis–Menten Equation	Lineweaver–Burk Equation	Effect of Inhibitor
None	$v_o = \frac{V_{max}[S]}{K_M + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$	None
Competitive	$v_o = \frac{V_{max}[S]}{\alpha K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}} \frac{1}{[S]} + \frac{1}{V_{max}}$	Increases K_M^{app}
Uncompetitive	$v_o = \frac{V_{max}[S]}{K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{K_M/\alpha' + [S]}$	$\frac{1}{v_o} = \frac{K_M}{V_{max}} \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases K_M^{app} and V_{max}^{app}
Mixed (noncompetitive)	$v_o = \frac{V_{max}[S]}{\alpha K_M + \alpha'[S]} = \frac{(V_{max}/\alpha')[S]}{(\alpha/\alpha')K_M + [S]}$	$\frac{1}{v_o} = \frac{\alpha K_M}{V_{max}} \frac{1}{[S]} + \frac{\alpha'}{V_{max}}$	Decreases V_{max}^{app} ; may increase or decrease K_M^{app}

$$^a \alpha = 1 + \frac{[I]}{K_1} \quad \text{and} \quad \alpha' = 1 + \frac{[I]}{K'_1}$$

Control of enzyme activity

- 1. **control of enzyme availability:** through change of the rate of synthesis and the rate of degradation of the enzyme in the cell.
- 2. **control of enzyme activity:** through structural alteration that influence the enzyme's substrate-binding affinity or turnover number.
 - A. "allosteric regulation". an enzyme's substrate-binding affinity may likewise vary with the binding of small molecules, called **allosteric effectors**.
 - B. covalent modification through phosphorylation or dephosphorylation of specific residues.

Drug design

- Structure-based drug design, also known as rational drug design
- Combinatorial chemistry and high-throughput screening, back to "make many compounds and see what they do" approach.
- Many drugs target to inhibit enzymes or signaling proteins (receptors).

Role of metal ions in biology

Element	% weight of	
	Earth crust (non living matter)	Human body (living matter)
Hydrogen	0.14	0.5
Carbon	0.03	18.5
Oxygen	46.6	65.0
Nitrogen	Very little	3.3
Sulphur	0.03	0.3
Sodium	2.8	0.2
Calcium	3.6	1.5
Magnesium	2.1	0.1
Silicon	27.7	Negligible

- Elements most abundant on earth are also those used primarily by life??
- Two main factors to consider: **bioavailability** and **chemical properties**.
- While the chemical property fit is the key factor for the theoretical applicability of an element for a desired process, the bioavailability of this element determines its actual use by the respective organism.
- Accordingly, the correlation between the geospheric abundance of elements and their relative distribution in, for example, the human body is low.
- Bioinorganic chemistry is the understanding of the role of inorganic metals in biology.

Biochemist's periodic table

Bulk elements

Trace elements

Lanthanides
Actinides

1 H																	2 He
3 Li	4 Be																
11 Na	12 Mg																
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba		72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra																

- Four most abundant elements in living organisms: H, O, N, C, 99% of the mass of most cells.
- The trace elements are essential.

Cofactors

Essential
ions

Coenzymes
(loosely bound)

Prosthetic
groups
(tightly bound)

Activator metal
ions
(loosely bound)

Metal ions of
metalloenzymes
(tightly bound)

Metalloproteins

Proteins contain metal ions as a cofactor

More than half of the known protein are metalloprotein

TABLE 6–1

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^+	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

Biochemist's periodic table

Bulk elements

Trace elements

Lanthanides
Actinides

1 H															2 He		
3 Li	4 Be																
11 Na	12 Mg																
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba		72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra																

- Four most abundant elements in living organisms: H, O, N, C, 99% of the mass of most cells.
- The trace elements are essential.

Coordination chemistry studies the formation, structure, and reactivity of coordination compounds, where metal ions (Lewis acids) bind to ligands (Lewis bases) via dative bonds.

The Hard-Soft Acid-Base (HSAB) principle, proposed by Ralph G. Pearson in 1963, provides a powerful framework for predicting these interactions by classifying acids and bases as "hard" or "soft" based on their polarizability, charge density, and electron-donating/accepting tendencies.

Hard species have low polarizability (e.g., small, highly charged ions with tightly held electrons), favoring ionic bonding, while soft species have high polarizability (e.g., larger ions with diffuse electrons), favoring covalent bonding.

The core HSAB rule—"like binds with like"—directly explains stability in coordination complexes: Hard metal ions prefer hard donor atoms in ligands for stable, ionic-like bonds, while soft metals prefer soft donors for covalent-like interactions. This connection is fundamental to understanding selectivity, thermodynamics, and applications in catalysis, bioinorganic chemistry, and materials science.

Biochemist's periodic table

Bulk elements

Trace elements

Lanthanides
Actinides

1 H																2 He	
3 Li	4 Be																
11 Na	12 Mg																
19 K	20 Ca	21 Sc	22 Ti	23 V	24 Cr	25 Mn	26 Fe	27 Co	28 Ni	29 Cu	30 Zn	31 Ga	32 Ge	33 As	34 Se	35 Br	36 Kr
37 Rb	38 Sr	39 Y	40 Zr	41 Nb	42 Mo	43 Tc	44 Ru	45 Rh	46 Pd	47 Ag	48 Cd	49 In	50 Sn	51 Sb	52 Te	53 I	54 Xe
55 Cs	56 Ba		72 Hf	73 Ta	74 W	75 Re	76 Os	77 Ir	78 Pt	79 Au	80 Hg	81 Tl	82 Pb	83 Bi	84 Po	85 At	86 Rn
87 Fr	88 Ra																

- Four most abundant elements in living organisms: H, O, N, C, 99% of the mass of most cells.
- The trace elements are essential.

Category	Metal Ion Acids (Examples)	Ligand Bases (Donor Atoms/Examples)
Hard	H ⁺ , alkali metals (Li ⁺ , Na ⁺), early transition metals (Ti ⁴⁺ , Cr ³⁺ , Fe ³⁺)	O-donors (H ₂ O, OH ⁻ , carboxylates like EDTA's O); N-donors (NH ₃ , amines)
Soft	Late transition metals (Cu ⁺ , Ag ⁺ , Au ⁺ , Pd ²⁺ , Pt ²⁺), p-block (Hg ²⁺ , I ₂)	S-donors (thiols RS ⁻ , thioethers); P-donors (PR ₃ like PPh ₃); C-donors (CO, CN ⁻)
Borderline	Mid-transition metals (Fe ²⁺ , Co ²⁺ , Ni ²⁺ , Cu ²⁺ , Zn ²⁺)	Halogens (Cl ⁻ , Br ⁻); N-heterocycles (pyridine)

In general 'hard' acids prefer 'hard' ligands whereas 'intermediate' and 'soft' acids form more stable complexes with 'soft' bases.

Hard-hard interactions will be primarily ionic in nature whereas soft-soft interactions will be governed by 'orbital' interactions.

Non-biological metal ions, which are of importance in medicine or as environmental pollutants, can also use the same ligands. Thus, Al³⁺ and Ga³⁺ fall into the 'hard' category, while Cd²⁺, Pt²⁺, Pt⁴⁺, Hg²⁺ and Pb²⁺ are classified as 'soft'.

Biological Ligands for Metal Ions

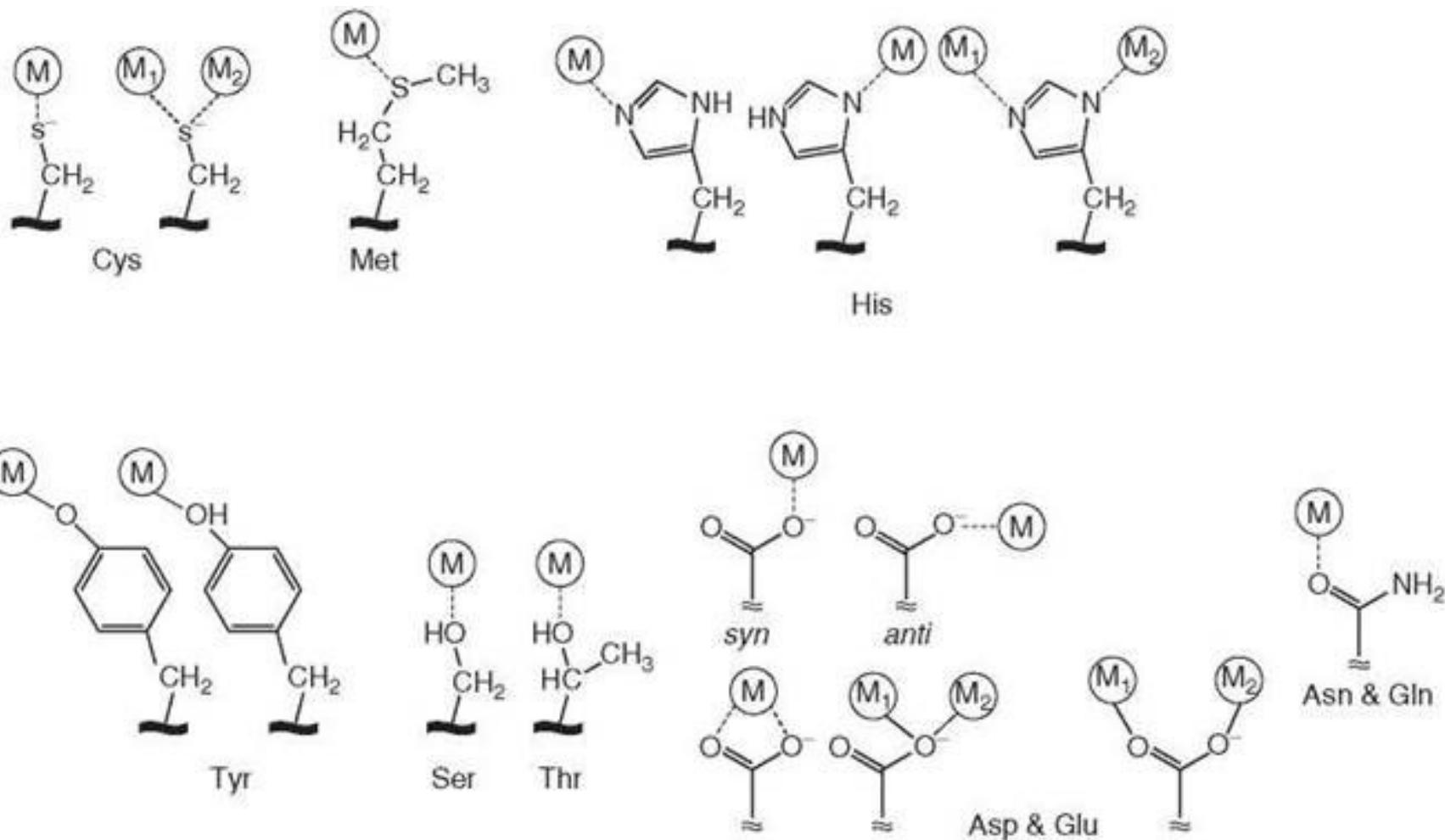
Naturally occurring amino acids in the protein itself

amino acids that have been chemically modified in order to bind specific metal ions

low-molecular weight inorganic ligands, such as carbonate, cyanide and carbon monoxide

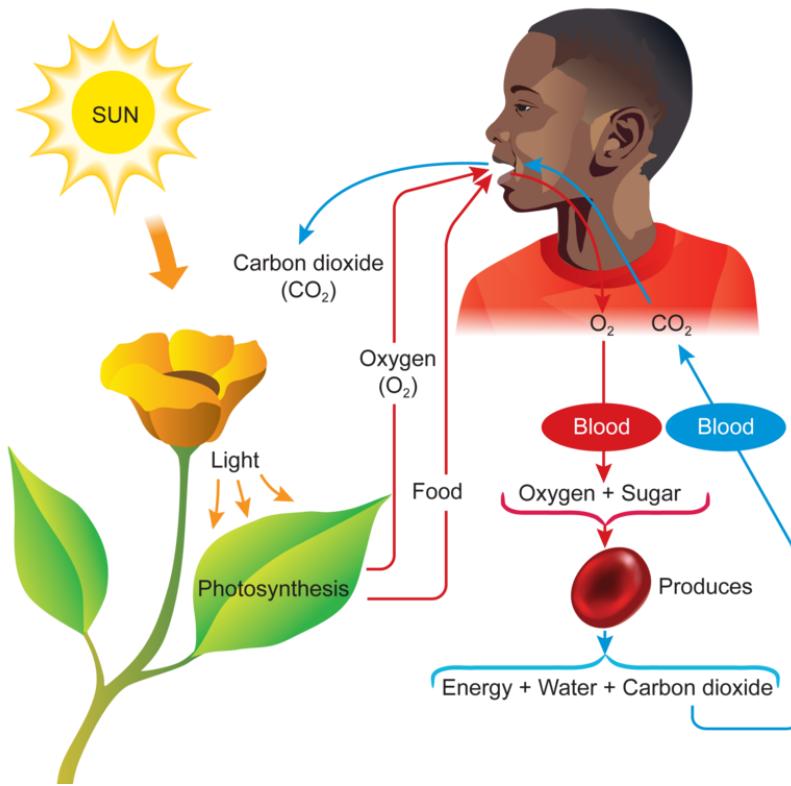
metal-binding organic cofactors that have been introduced into the protein (such as porphyrins, corrins and iron-sulfur (Fe-S) clusters, the molybdenum cofactor, MoCo, the CuZ centre of nitrous oxide reductase and the FeMoCo and P-clusters of nitrogenase)

metal-binding molecules excreted from the cell and then taken up as the metal chelate (siderophores)



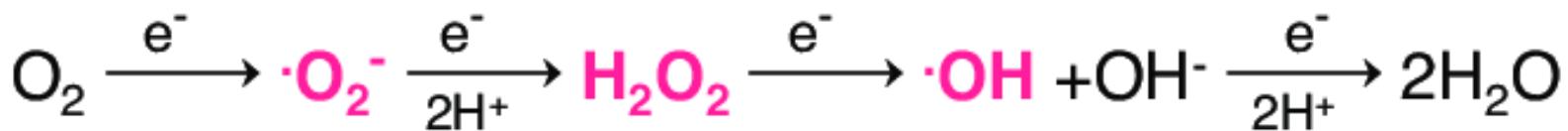
Oxygen Transport Proteins

Oxygen is essential for life



Source: www.ck12.org

Complete O₂ Reduction



*Superoxide
anion*

*Hydrogen
peroxide*

*Hydroxyl
radical*

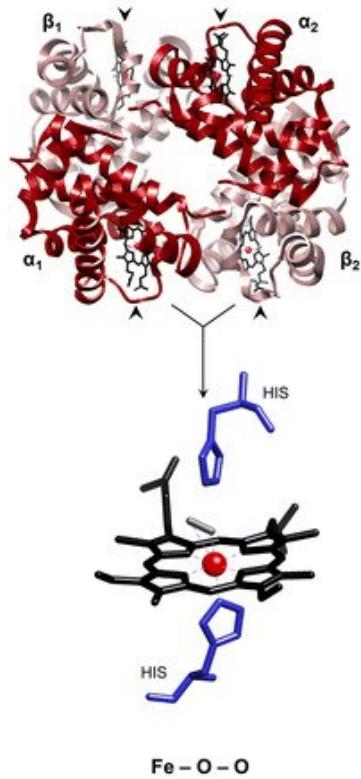
Incomplete O₂ Reduction

Comparison of different O_2 transport protein

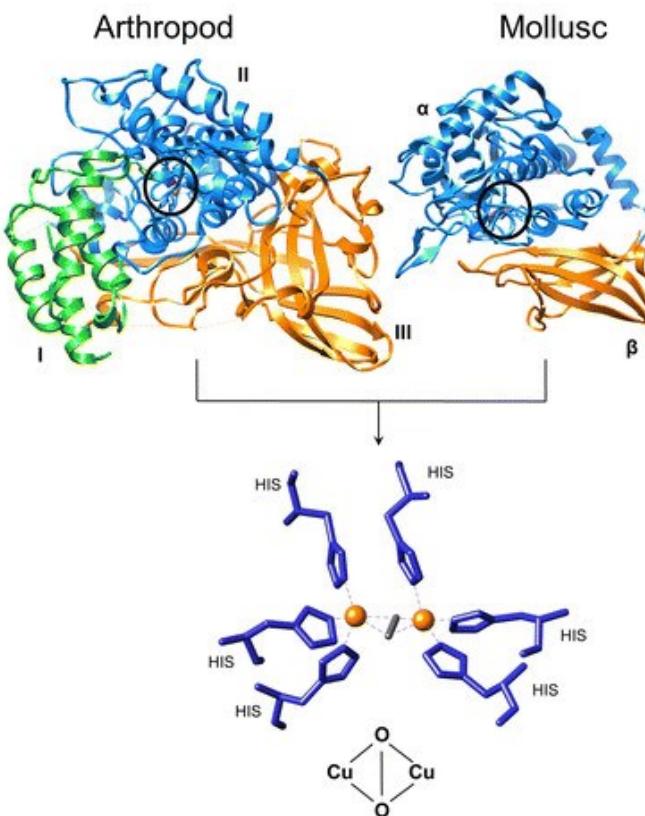
Property	Hemoglobin	Hemerythrin	Hemocyanin
Metal (M)	Fe	Fe	Cu
DeoxyM(O^{xn})	II	II	I
Metal: O_2	1Fe:1 O_2	2Fe:1 O_2	2Cu:1 O_2
Colour (Oxy)	Red	Violet-pink	Blue
Colour (Deoxy)	Red-purple	Colourless	Colourless
Metal Coordination	Porphyrin	Protein Side Chains	Protein Side Chain
Molecular Weight	65,000	108,000	400,000 – 20,000, 000
Number of subunits	4	8	Many
Amino acids/subunit	153	113	628

Lippard (1994; p 293)

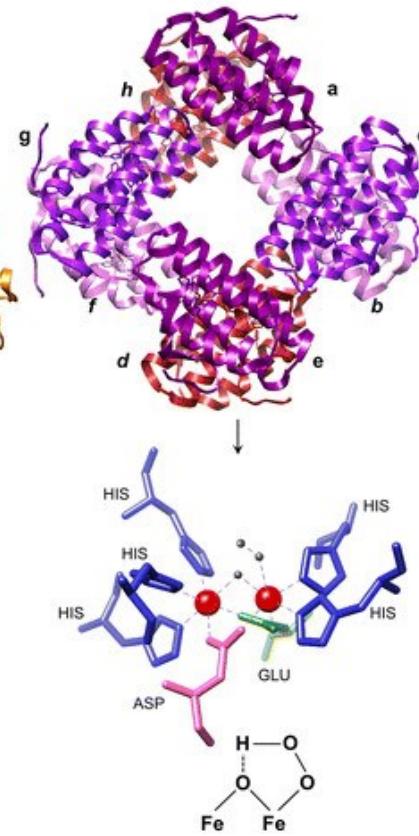
Hemoglobin



Hemocyanins

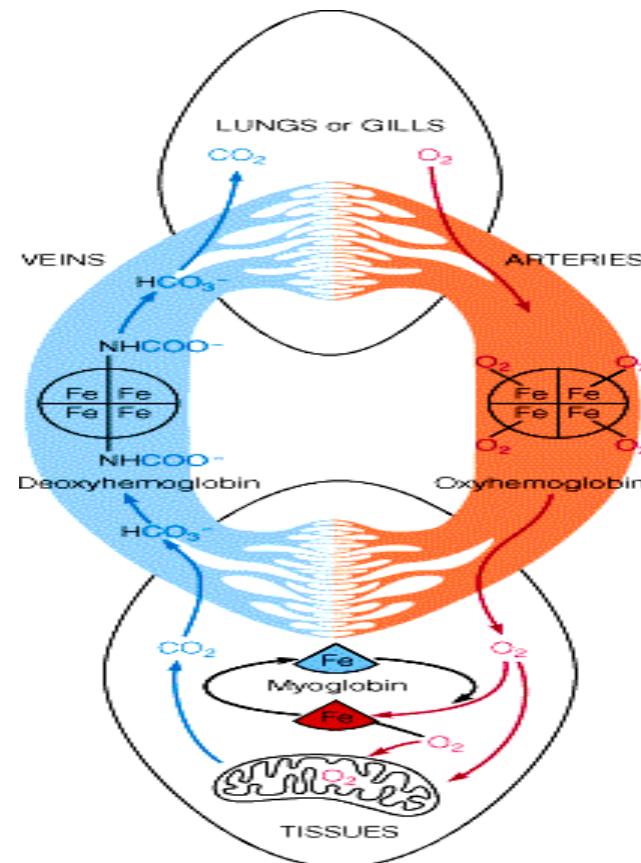


Hemerythrin

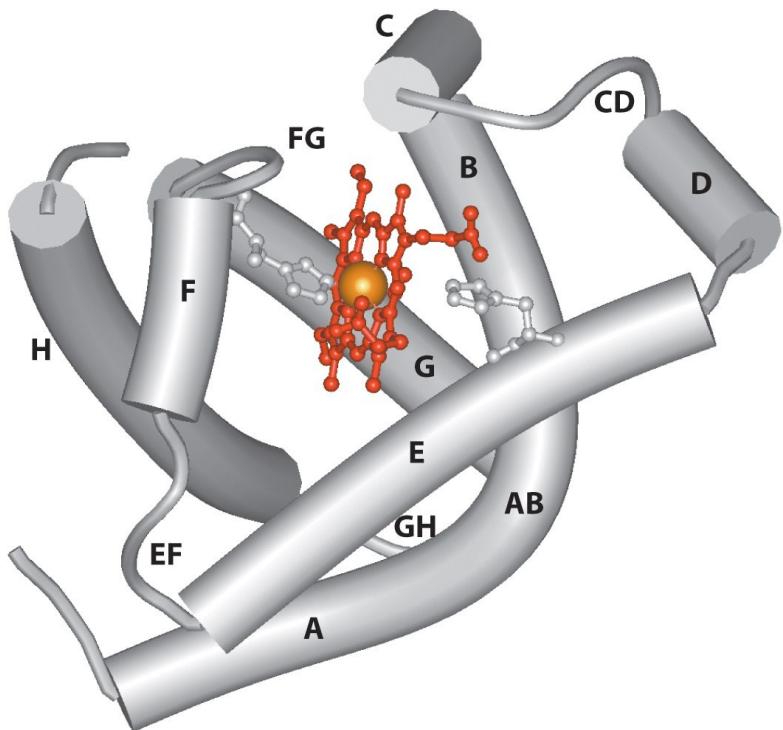


Hemoglobin and Myoglobin: Paradigms of Protein Structure and Function

Oxygen Transport and Storage

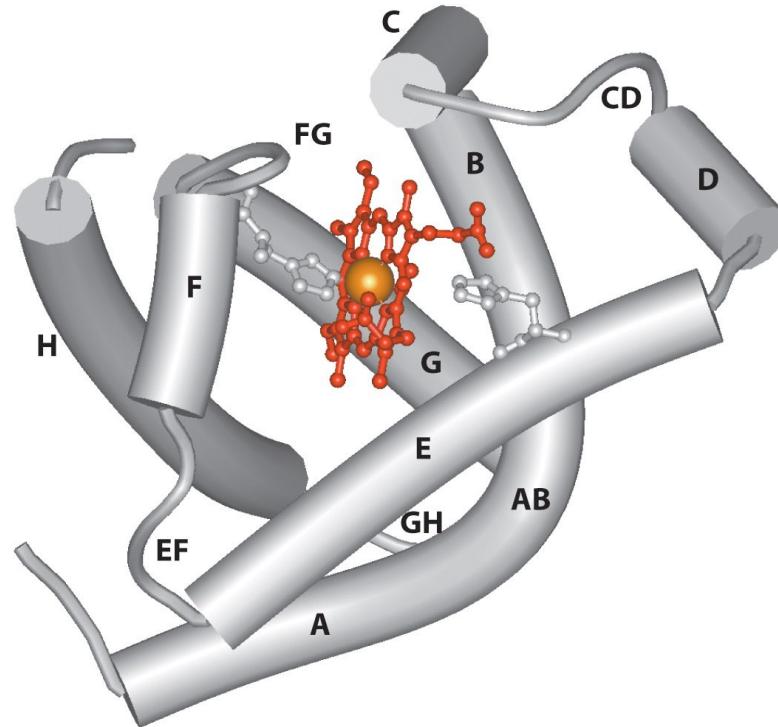


Myoglobin: a monomeric protein



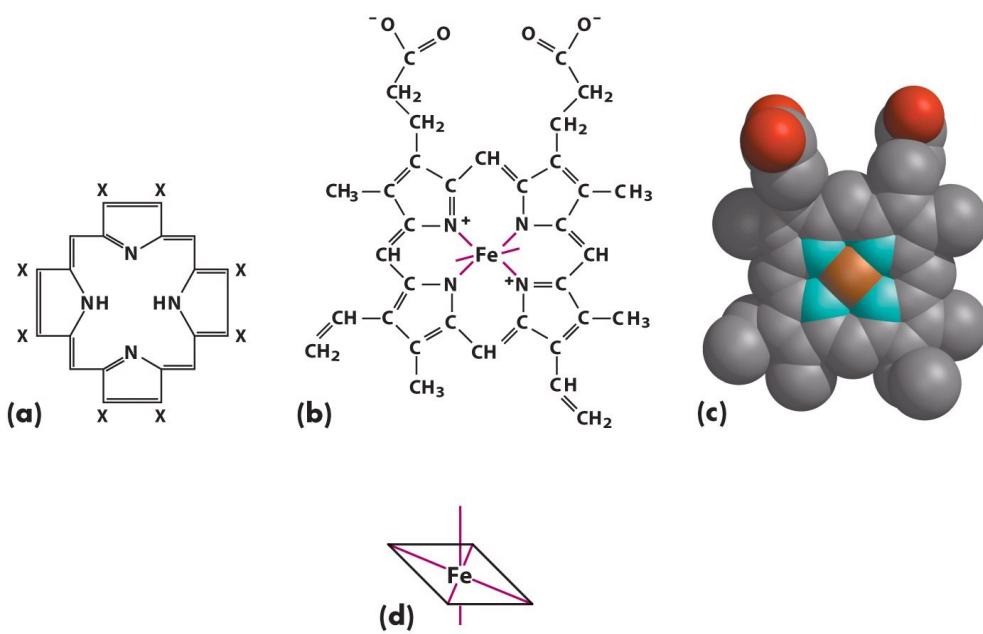
Myoglobin serves as an oxygen storage protein primarily in muscle tissue. It contains one binding site for one O_2 molecule. This binding site is a heme group.

Structure of sperm whale myoglobin: 153 residues, 8 α -helices.



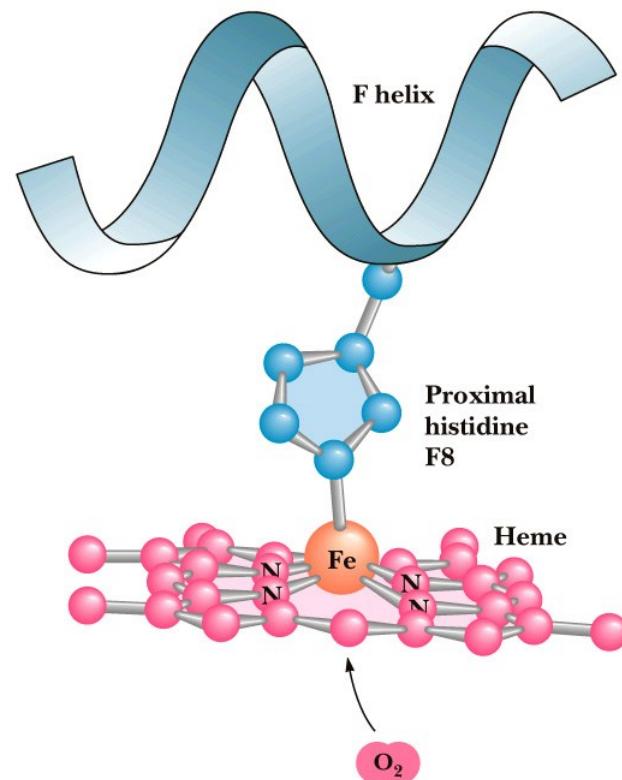
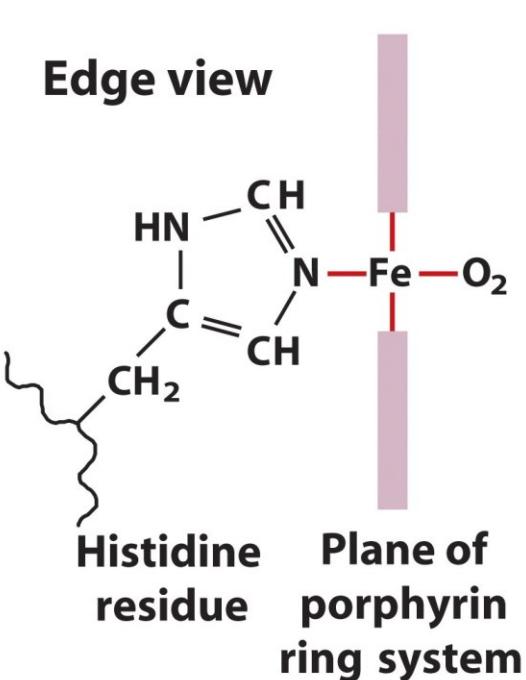
Myoglobin facilitates O_2 diffusion in muscle (O_2 has low water solubility).

What is a heme group?



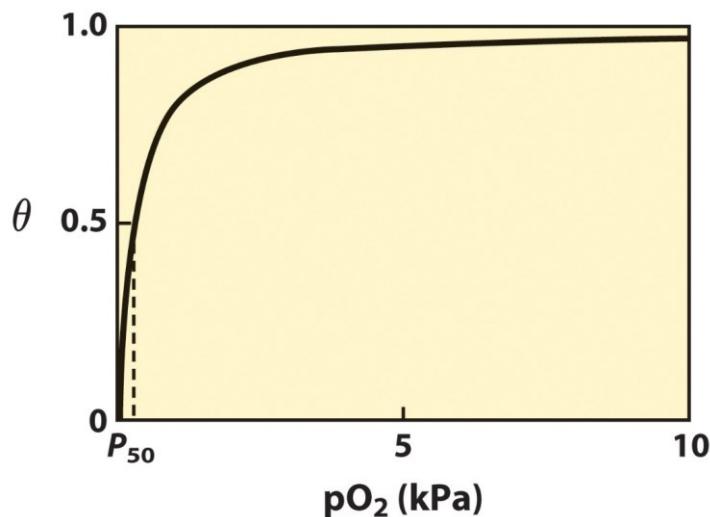
Heme consists of a complex organic ring structure, protoporphyrin, to which is bound a single iron atom in ferrous (Fe^{2+}) state. The iron atom has six coordination bonds, four to nitrogen atoms that are part of the semi-flat porphyrin ring and two perpendicular to the porphyrin.

Oxygen Binding to the Heme Group of Myoglobin



Oxygen Binding Curve for Myoglobin

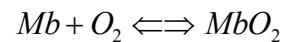
Myoglobin's oxygen-binding curve is hyperbolic



If we define the partial pressure of oxygen at $[O_2]_{0.5}$ as P_{50} , then $\theta = \frac{pO_2}{pO_2 + p_{50}}$

The above plot was obtained experimentally by plotting pO_2 (oxygen pressure can be measured) against θ (bound state of myoglobin can be measured spectrometer)

Why hyperbolic? Being adaptive to the environment (tissue has lower oxygen pressure compared to blood, so small pO_2 change can cause efficient storage and release of O_2 .)



$$K_d = \frac{[Mb][O_2]}{[MbO_2]}$$

$$\text{define } \theta = \frac{\text{occupied} \cdot \text{binding} \cdot \text{sites}}{\text{total} \cdot \text{binding} \cdot \text{sites}}$$

$$\begin{aligned} &= \frac{[Mb][O_2]}{[MbO_2] + [Mb]} = \frac{K_d}{\frac{[Mb][O_2]}{[MbO_2] + [Mb]} + K_d} \\ &= \frac{[O_2]}{[O_2] + K_d} = \frac{pO_2}{pO_2 + K} \end{aligned}$$

Structure of Myoglobin

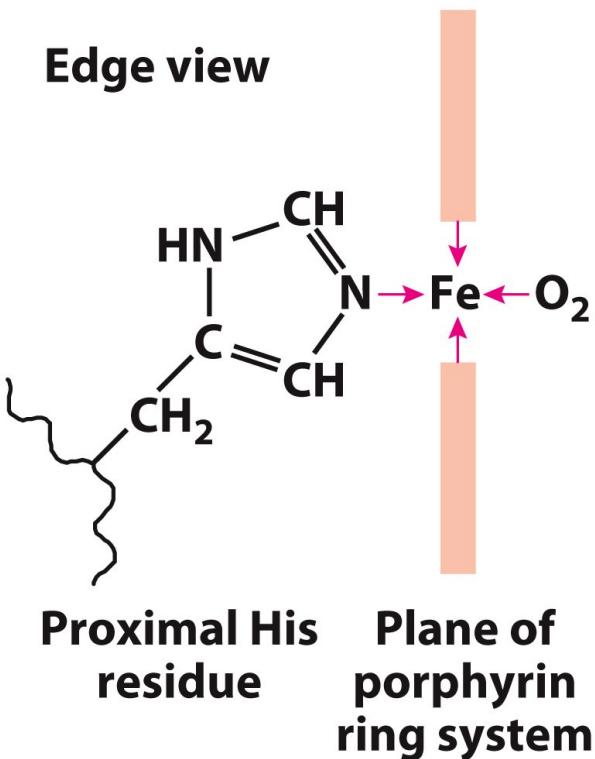


Figure 5-2
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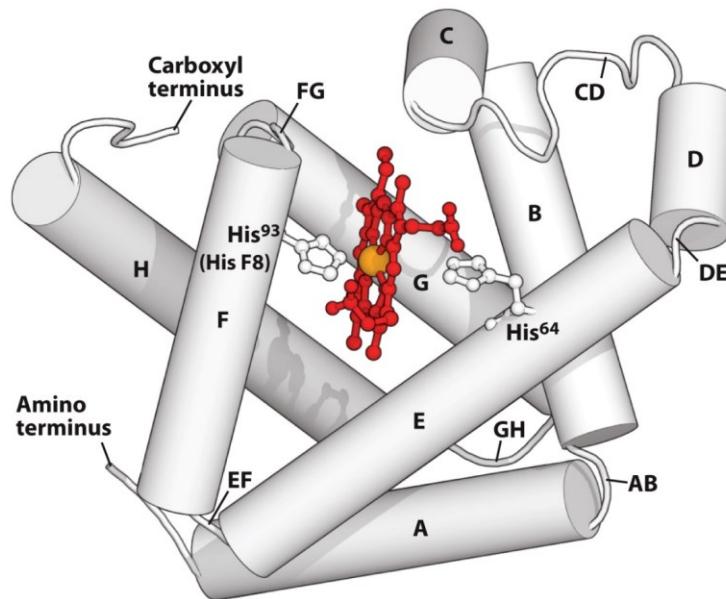


Figure 5-3
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Carbon Monoxide Binding

- CO has similar size and shape to O_2 ; it can fit to the same binding site.
- CO binds heme over 20,000 times better than O_2 because the carbon in CO has a filled lone electron pair that can be donated to vacant d-orbitals on the Fe^{2+} (and because of p back bonding)
- The protein pocket decreases affinity for CO, but it still binds about 250 times better than oxygen.
- CO is toxic, as it competes with oxygen. It blocks the function of myoglobin, hemoglobin, and mitochondrial cytochromes that are involved in oxidative phosphorylation. COHb has a blood half-life of 4 to 6 hours.

CO vs. O_2 Binding to Free Heme

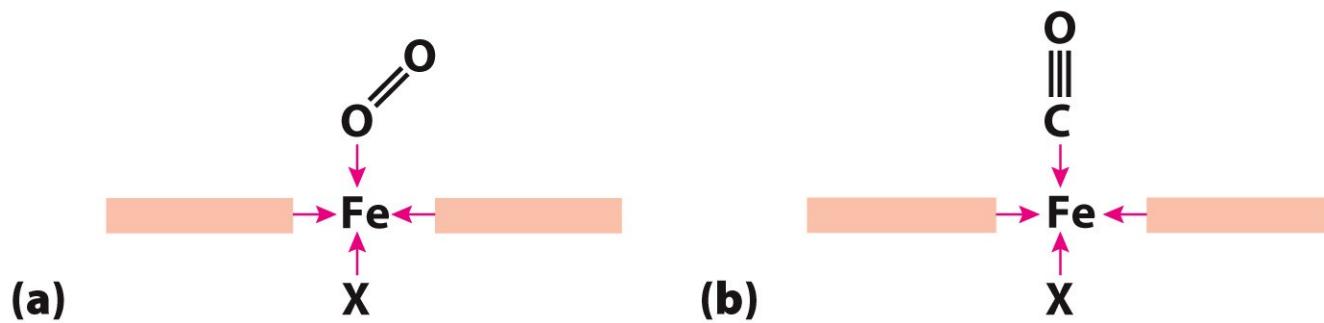


Figure 5-5ab

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Heme Binding to Protein Affects CO vs. O₂ Binding

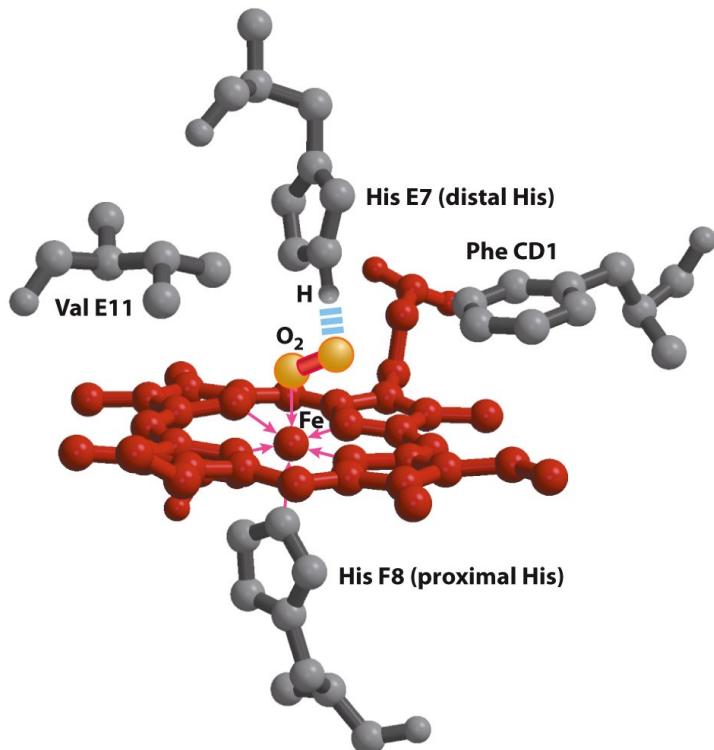


Figure 5-5c
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Could Myoglobin Transport O₂?

- pO₂ in lungs is about 13 kPa (100 torr): Myb binds oxygen well.
- pO₂ in tissues is about 4 kPa (30 torr): it will not release O₂

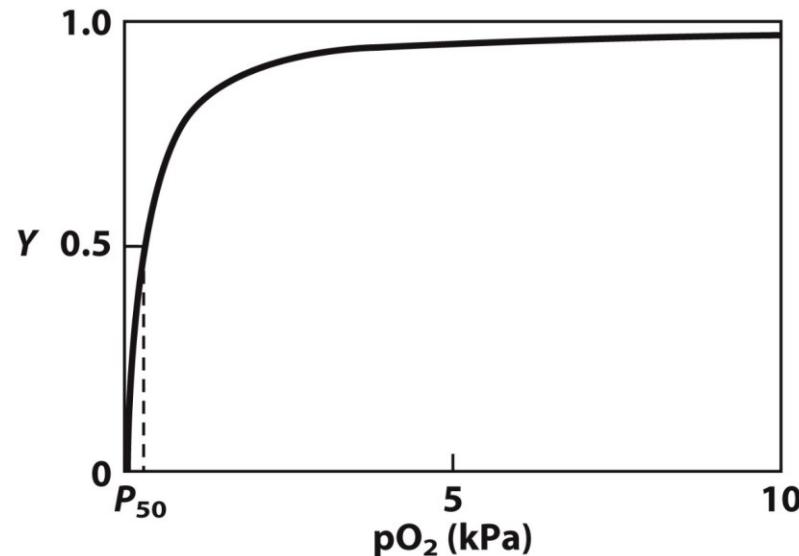
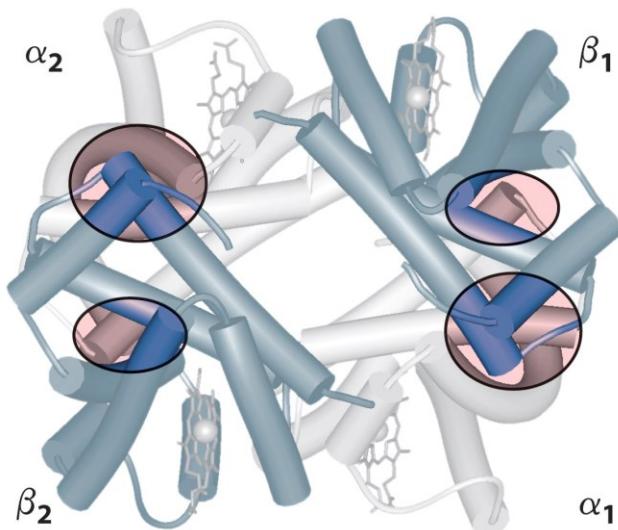


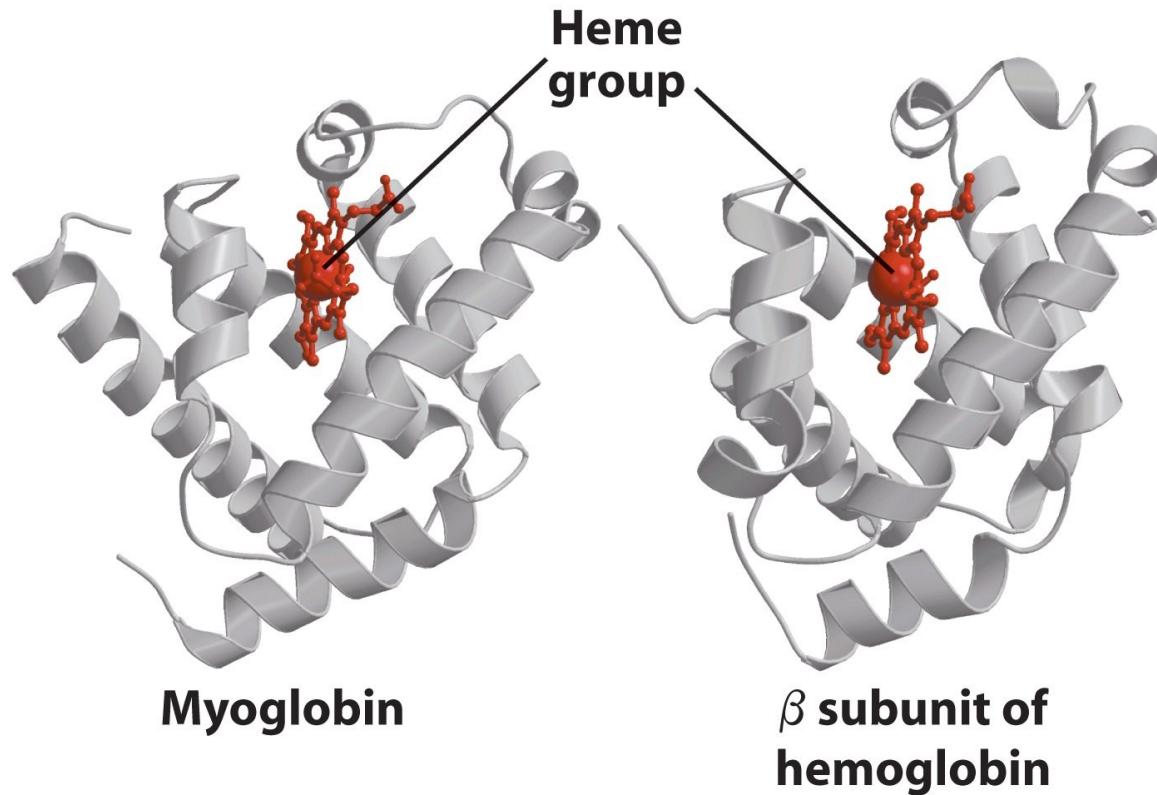
Figure 5-4b
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Hemoglobin: a tetrameric protein

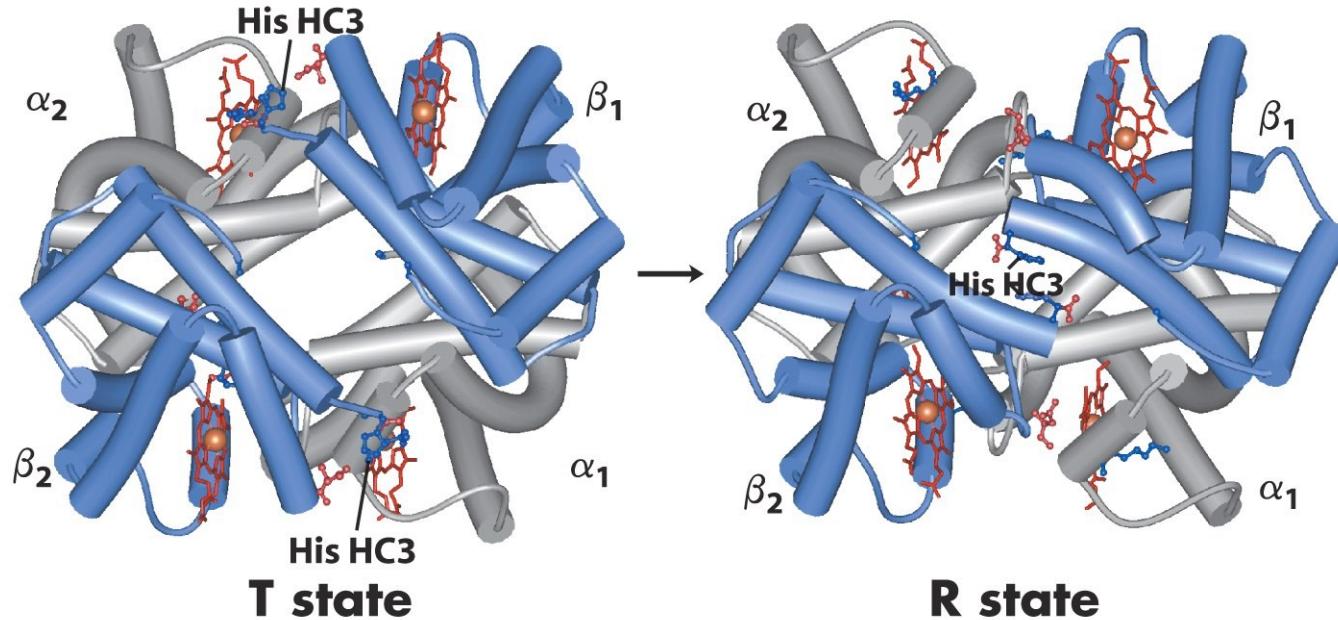


Hemoglobin serves as an oxygen storage protein primarily in blood. It is a tetrameric protein (two α subunits and two β subunits) contains four binding site (heme group too)for four O_2 molecule.

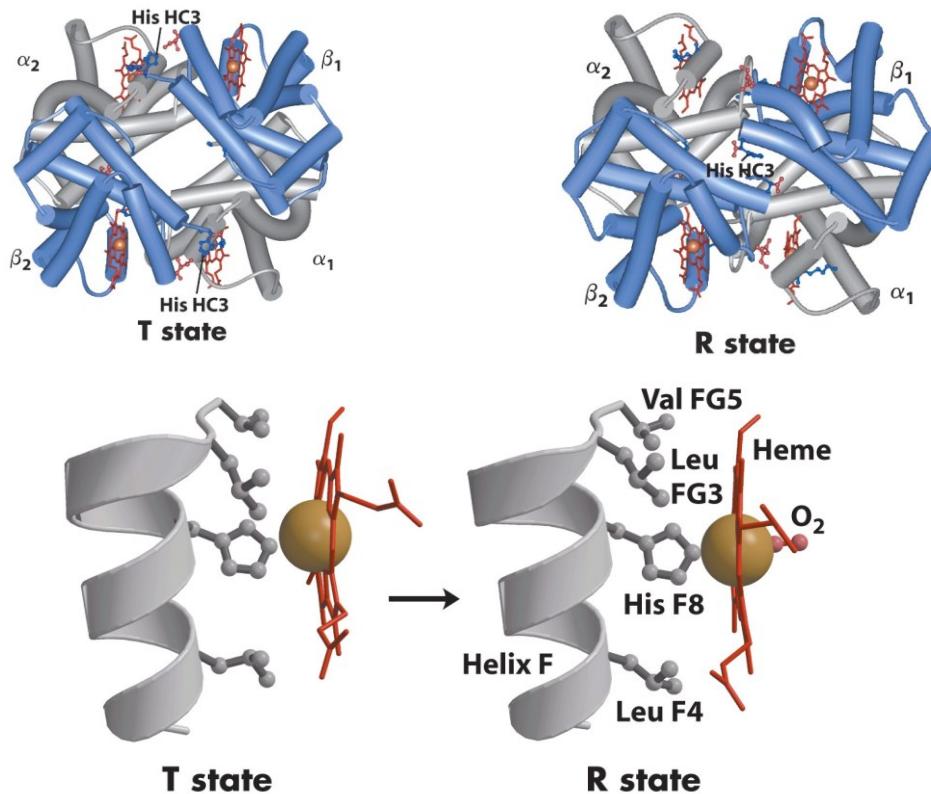
Hemoglobin subunits are structurally similar to Myoglobin



Hb undergoes a structural change on binding oxygen



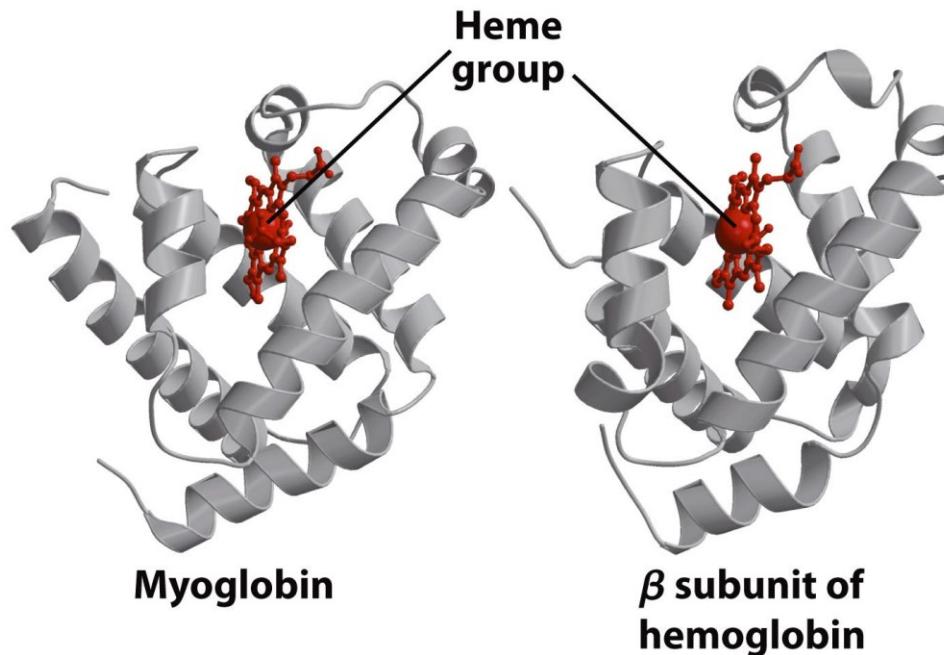
Although oxygen binds to hemoglobin in either state, it has higher affinity for Hb in the R (relaxed) state. When oxygen is absent, the T (tense) state is dominant.



The binding of O₂ causes the heme to assume a more planar conformation, shifting the position of the proximal His and the attached F helix

Hemoglobin Binds Oxygen Cooperatively

- Hemoglobin (Hb) is a tetramer of two subunits ($\alpha_2\beta_2$).
- Each subunit is similar to myoglobin.



Subunit Interactions in Hemoglobin

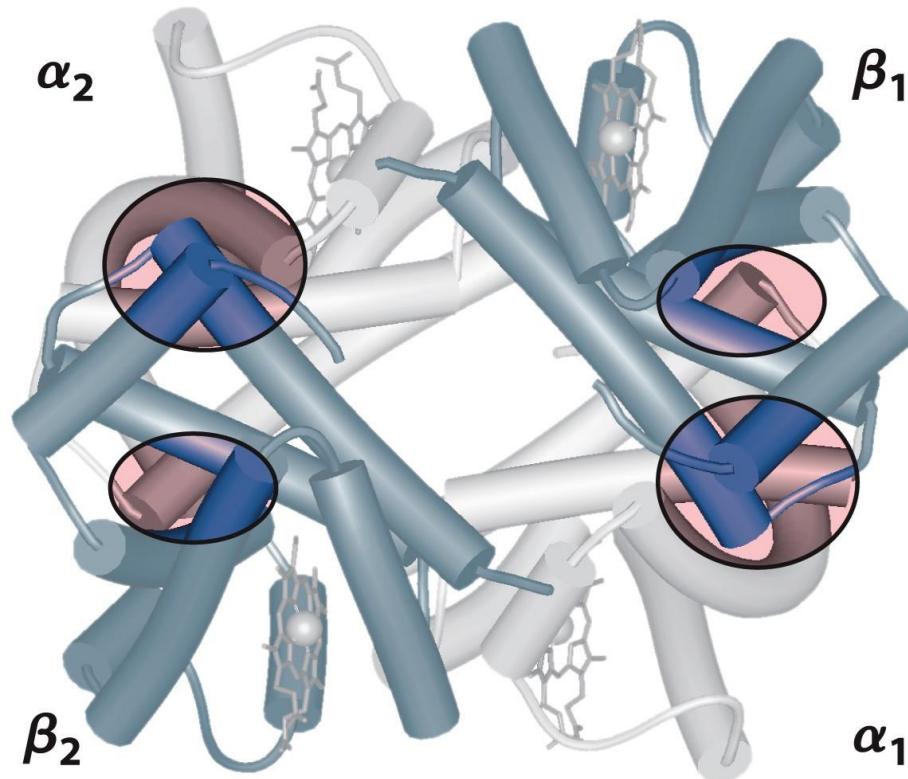


Figure 5-8
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Subunit Interactions: Details

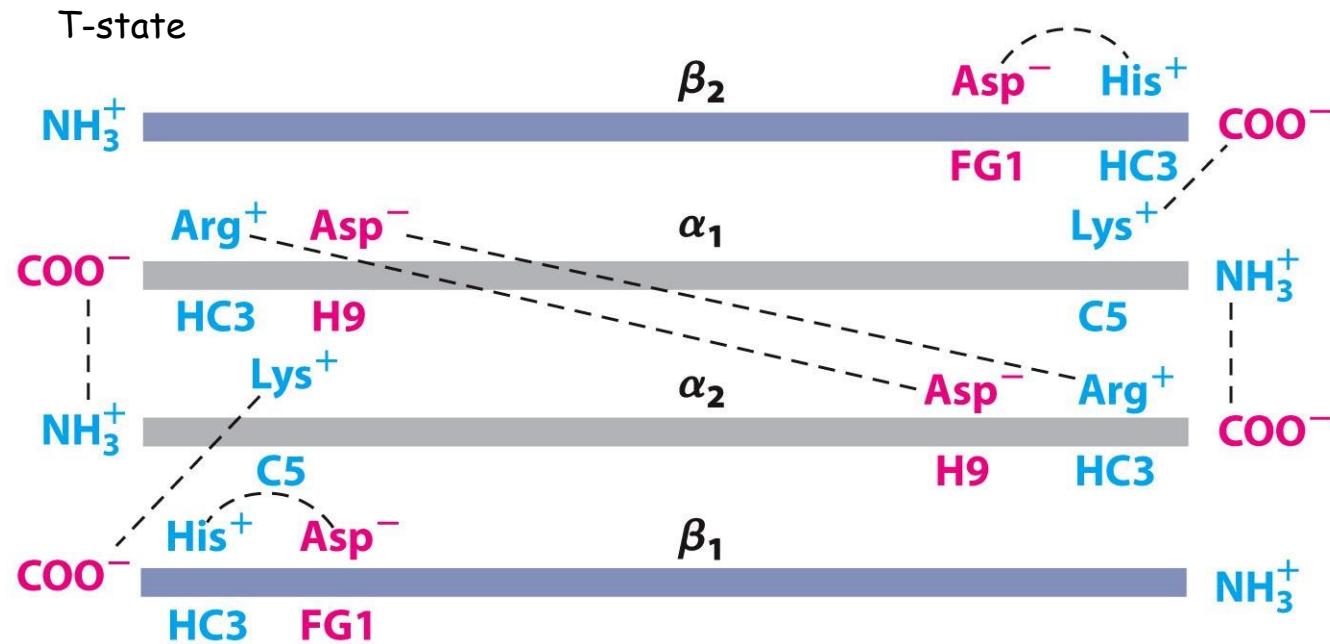


Figure 5-9b

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Subunit Interactions: Details

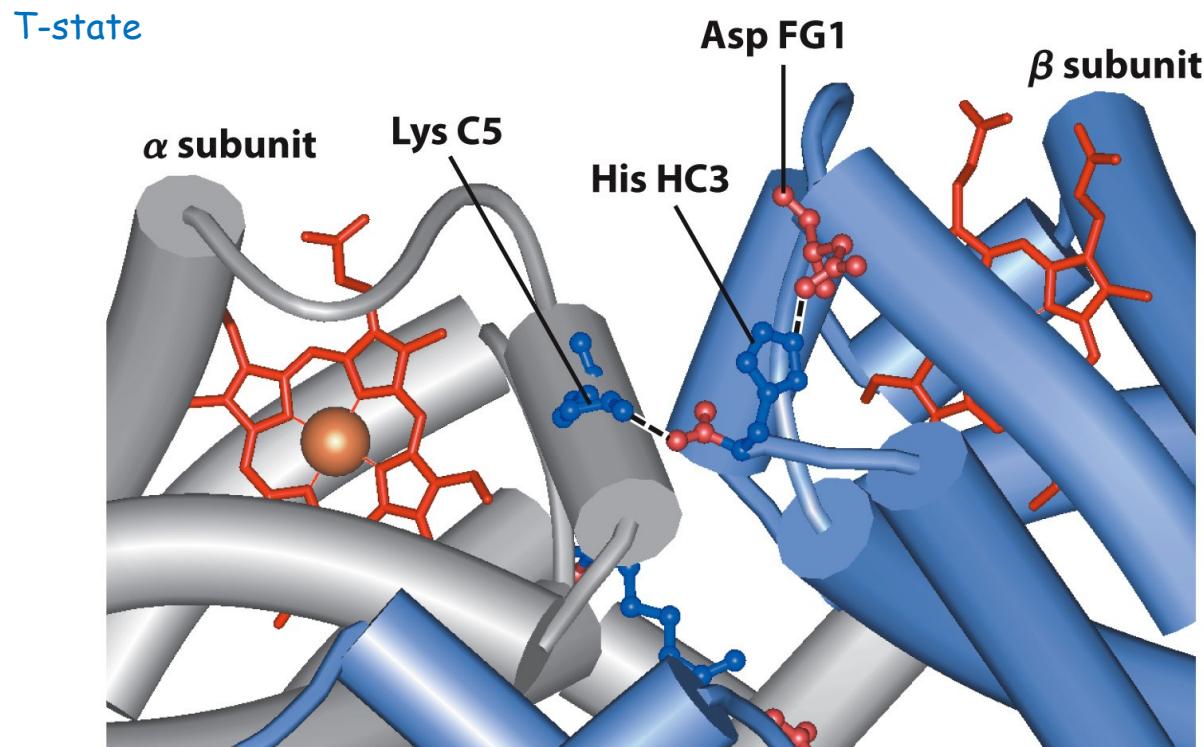


Figure 5-9a
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Conformational Change Is Triggered by Oxygen Binding

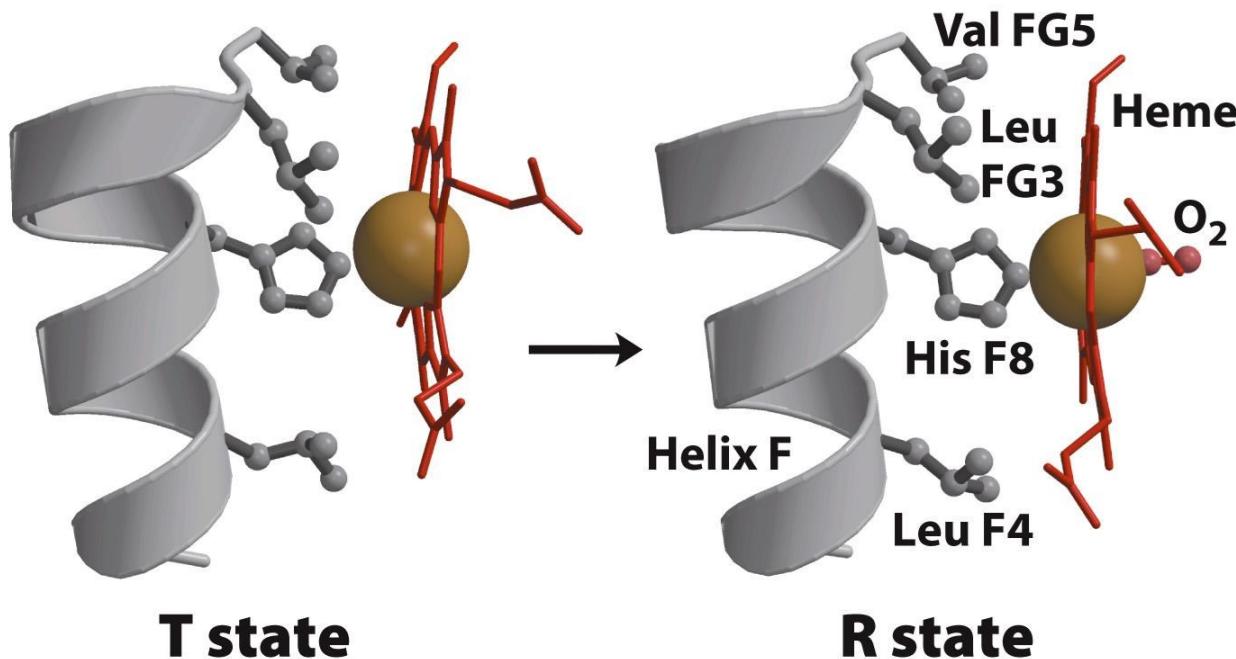
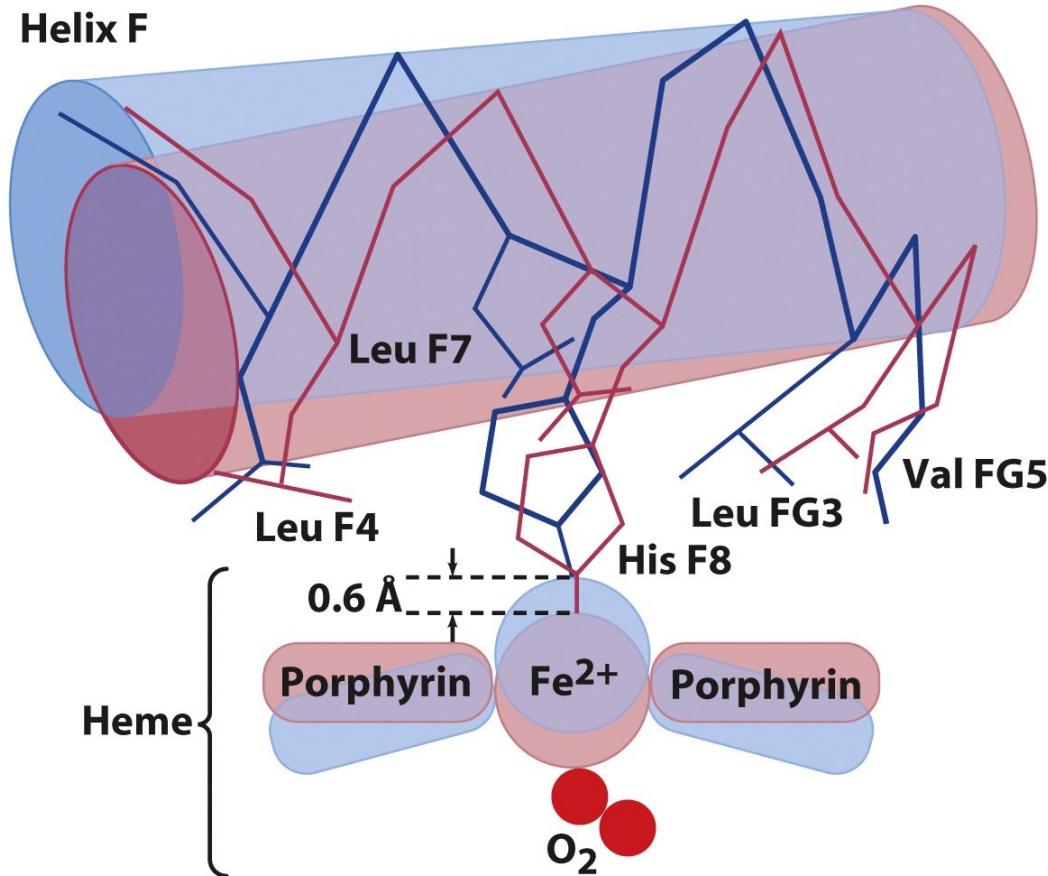


Figure 5-11
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Movement of the heme and F helix during the T \rightarrow R transition.



1. Fe(II) moves into heme plane.
1. Fe(II) pulls on His F8, which causes helix F to tilt and translate.
1. Movement of $\alpha_1-\beta_2$ and $\alpha_2-\beta_1$ interface.
1. Change in interactions at C-terminal residues of each subunit.

Figure 7-8
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R and T States of Hemoglobin

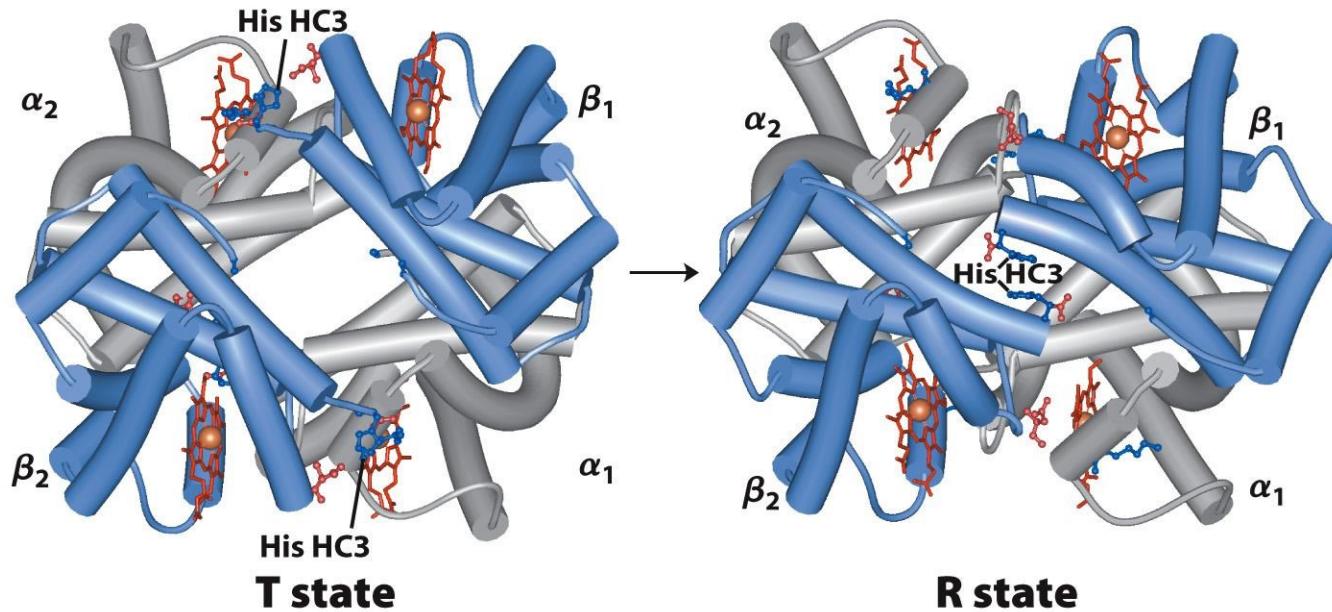


Figure 5-10
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Cooperative Effect

O₂ binds
to one
subunit

Fe²⁺
contracts
and move
into plane
of
porphyrin
ring

Moves His
Attached
to it

Triggers
conformat
ional
change in
the globin
change

Translated
through
the
network

Enhances
the ability
of another
three
units to
bind O₂

Oxygen binding curve of myoglobin and hemoglobin

Dashed line: If hemoglobin (4 binding sites) exhibited hyperbolic (non-cooperative) with same O_2 binding constant as actual hemoglobin.

Myoglobin and non-cooperative hemoglobin (not real) are not suitable for O_2 delivery.

pO_2 in tissues is about 4 kPa (30 torr)

pO_2 in lungs is about 13 kPa (100 torr)

