[redacted] PORTRAIT OF AN ALLOSTERIC PROTEIN [redacted]

The transition from anaerobic to aerobic life was a major step in evolution because it uncovered a rich reservoir of energy.

Vertebrates have evolved two principal mechanisms for supplying their cells with a continuous, and adequate flow of oxygen:

- A circulatory system that actively delivers oxygen to cells
- Use of oxygen-carrying molecules to overcome the limitations imposed by the low solubility of oxygen in water

The oxygen carriers in vertebrates are the proteins hemoglobin and myoglobin

Hemoglobin: serves as the oxygen carrier in blood and also plays a vital role in the transport of carbon dioxide and hydrogen ion (red blood cells)

Myoglobin: provides a reserve supply of oxygen and facilitates the movement of oxygen within muscle (located in muscle)

The structures of hemoglobin and myoglobin are known in atomic detail

The binding of O_2 by hemoglobin is regulated by H^+, CO_2, and organic phosphates (allosteric interactions)

Hemoglobin:

- Best understood allosteric protein
- Discovery of mutant hemoglobins (concept of molecular disease)
- A rich source of insight into the molecular basis of evolution

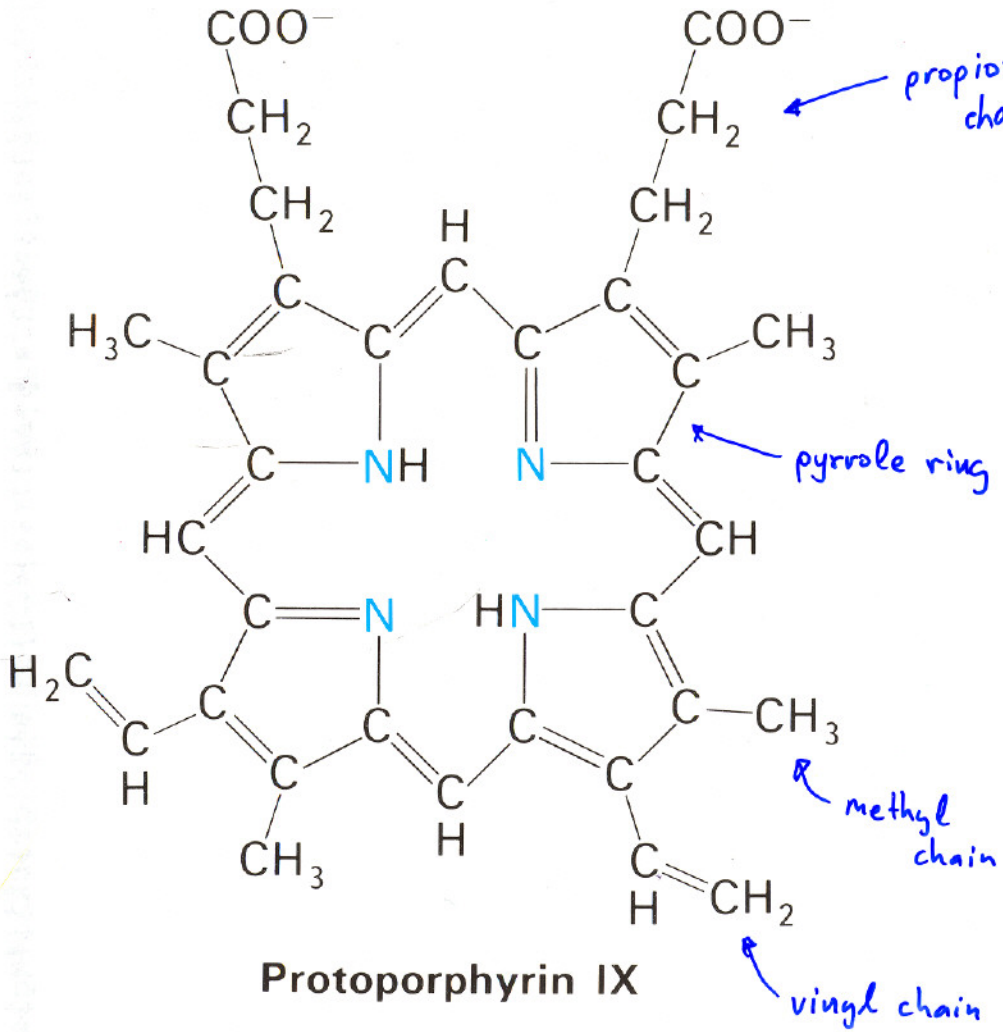
OXYGEN BINDS TO A HEME PROSTHETIC GROUP

The capacity of myoglobin or hemoglobin to bind oxygen depends on the presence of a nonpolypeptide unit, called heme group

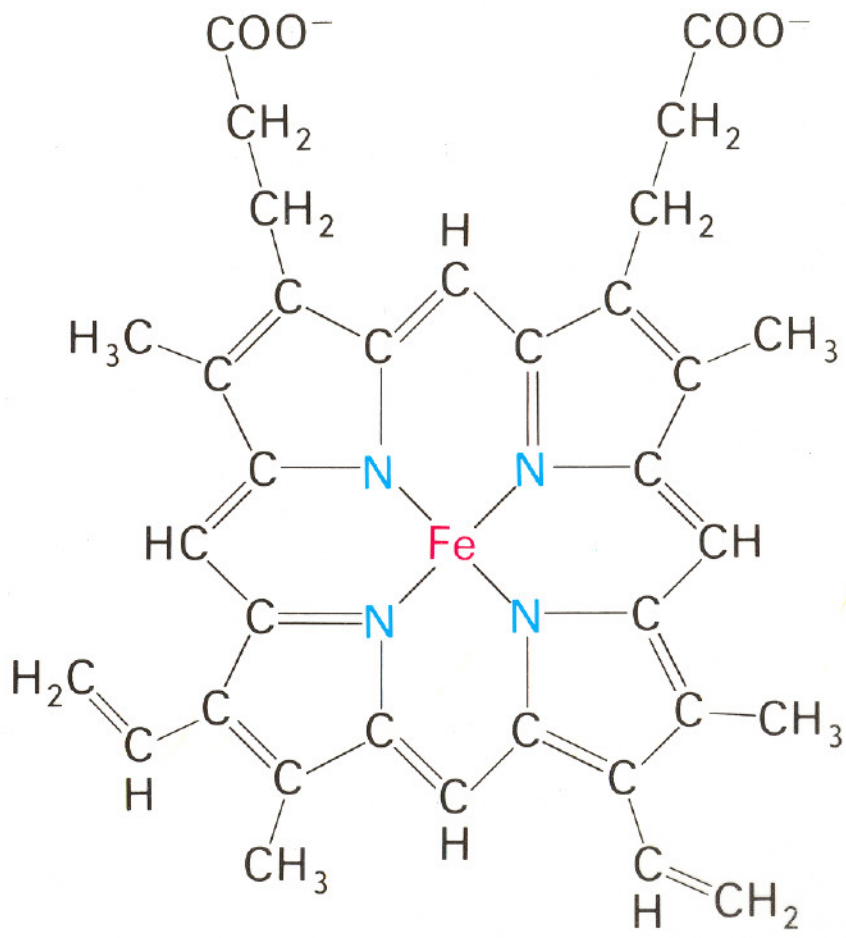
Myoglobin or hemoglobin polypeptide without heme: apoprotein

Heme: prosthetic group

The structure of heme



Protoporphyrin IX



Heme
(Fe–protoporphyrin IX)

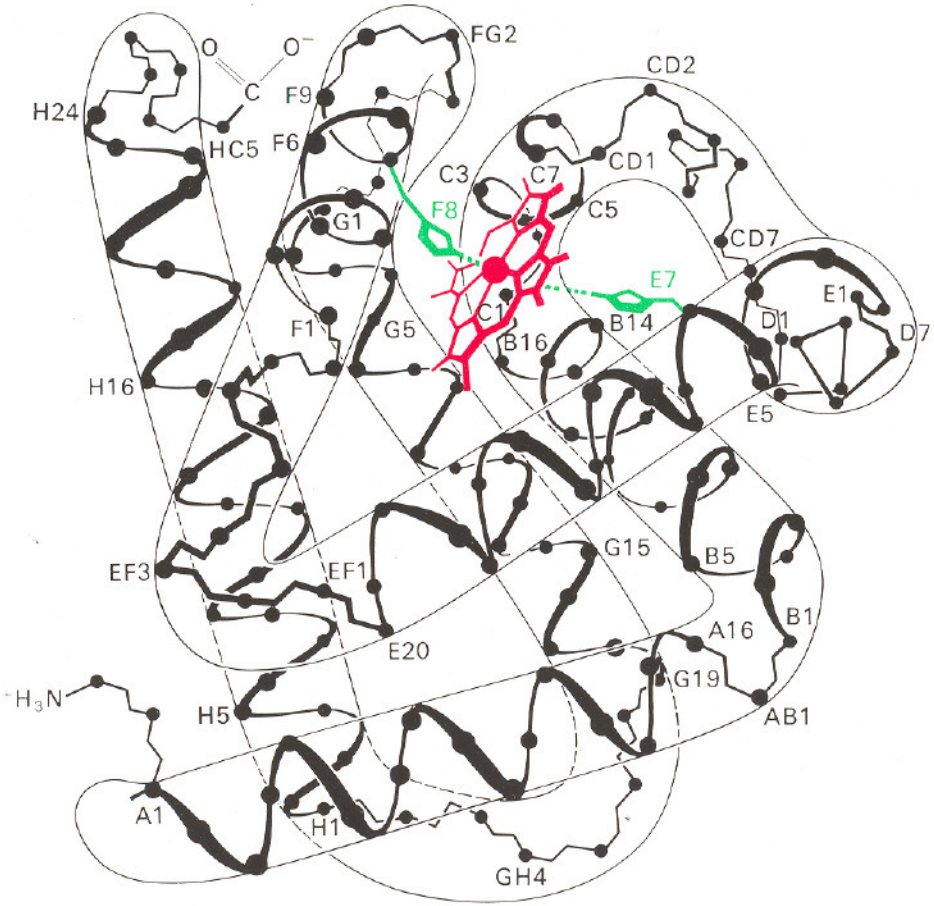
Figures for protoporphyrin IX and heme, page 148

MYOGLOBIN HAS A COMPACT STRUCTURE AND A HIGH CONTENT OF α-HELICES

The 3D structure of myoglobin was determined by John Kendrew and of hemoglobin by Max Perutz (1950s)

Some important features of myoglobin:

- **Extremely compact structure (45 x 35x 25 Å)**
- **About 75% of the main chain is in an α-helical conformation**
- **Four of the helices are terminated by a proline residue**
- **The main- chain peptide groups are planar, and the carbonyl group of each is trans to the NH**
- **The interior consists almost entirely of nonpolar residues, whereas the outside of the protein has both polar and nonpolar residues**



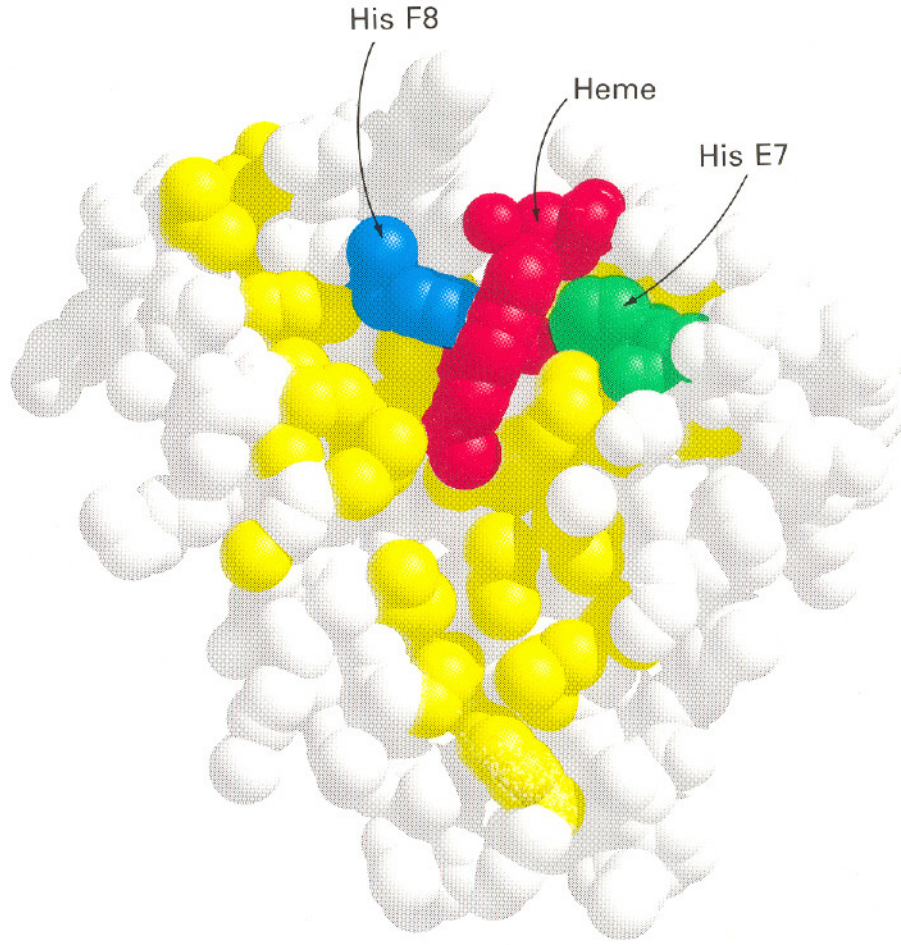


Figure 7-4, page 149; Figure 7-6, page 150

A HINDERED HEME ENVIRONMENT IS ESSENTIAL FOR REVERSIBLE OXYGENATION

The heme group is located in a crevice in the myoglobin molecule

The iron atom of the heme is directly bonded to one histidine residue, which is called the proximal histidine (F8)

The oxygen- binding site is on the other side of the heme plane

A second histidine (E7), termed the distal histidine, is near the heme but not bonded to it

There are three physiologically pertinent forms of myoglobin:

1. Deoxymyoglobin- Empty
2. Oxymyoglobin- Occupied by O_2
3. Ferrimyoglobin- Occupied by water

THE CENTRAL EXON OF MYOGLOBIN ENCODES A FUNCTIONAL HEME- BINDING UNIT

The gene for myoglobin consists of three exons:

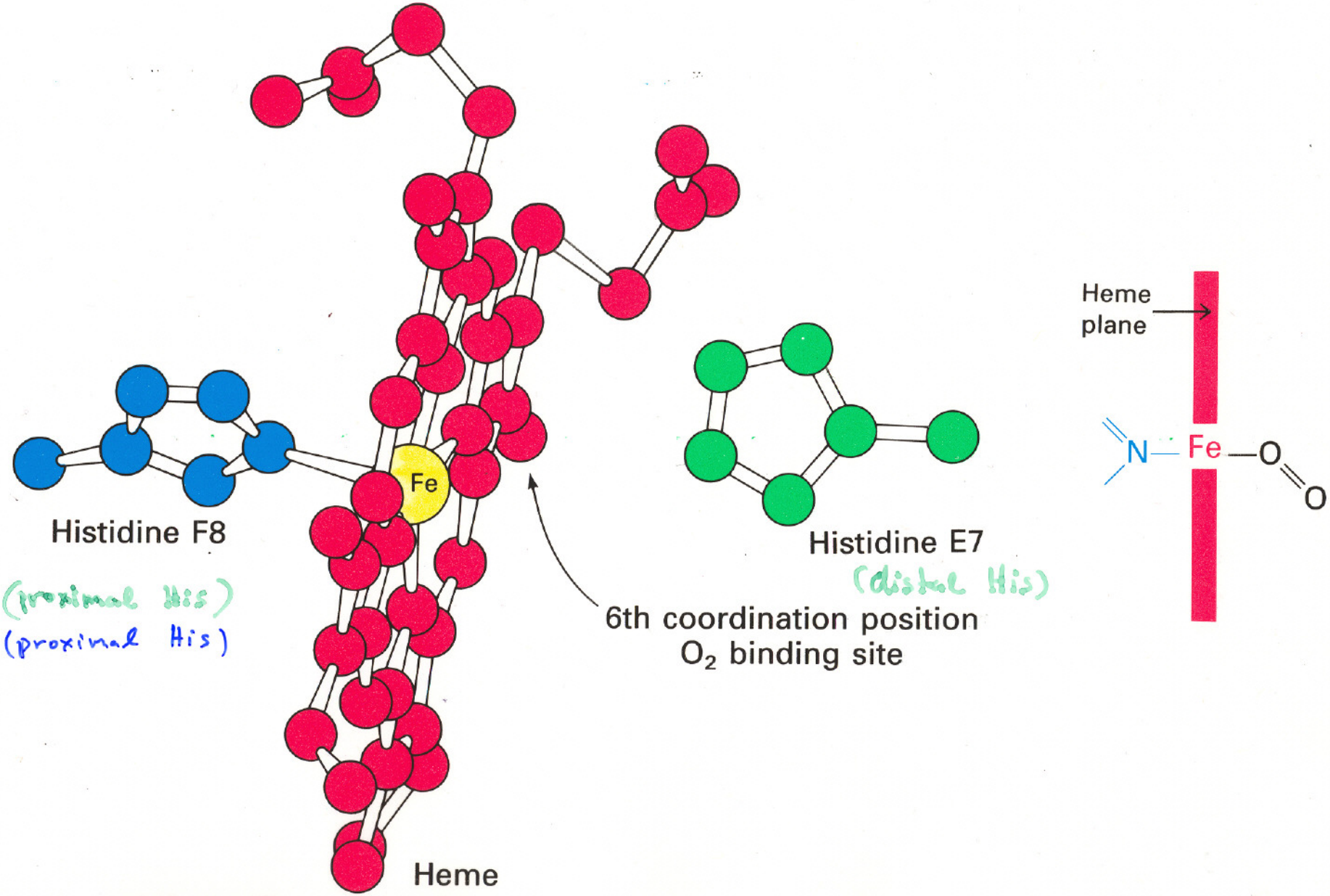
1. An amino- terminal one encoding residues 1 to 30
2. A central one encoding residues 31 to 105
3. A carboxyl- terminal one encoding residues 106 to 153

The entire heme- binding site is specified by the central exon.

What are the functional properties of an isolated polypeptide consisting of residues 31 to 105?

EXPERIMENT:

Digestion of apomyoglobin with clostripain→ generation of a polypeptide containing residues 32 to 139. This polypeptide fragment binds heme (minimyoglobin). Minimyoglobin binds O_2 and CO reversibly with similar association and dissociation rates as the intact protein.



Figures 7-8 and 7-10, page 151

Oxygen is directly bonded only to the iron atom of the heme.

The heme group is much less susceptible to oxidation, because two myoglobin molecules cannot readily associate to form a heme- O_2- heme complex. heme – O – heme

Studies of synthetic model compounds by James Collman, using picket- fence iron porphyrin complexes

CARBON MONOXIDE BINDING IS DIMINISHED BY THE PRESENCE OF THE DISTAL HISTIDINE

Carbon monoxide (CO) is a poison because it combines with ferrimyoglobin and ferrohemoglobin and thereby blocks oxygen transport

The binding affinity of myoglobin and hemoglobin for CO is about 200 times as great as for O_2

The protein forces CO to bind at an angle rather than in line. This bent geometry in the globins weakens the interactions of CO with the heme.

The decreased affinity of myoglobin and hemoglobin for CO is biologically important because:

- Endogenously produced CO would cause massive poisoning if the affinity of these proteins for CO was like that of the isolated iron, porphyrins.
- This challenge was solved by the evolution of heme proteins that discriminate O_2 and CO

The function of a prosthetic group is modulated by its polypeptide environment

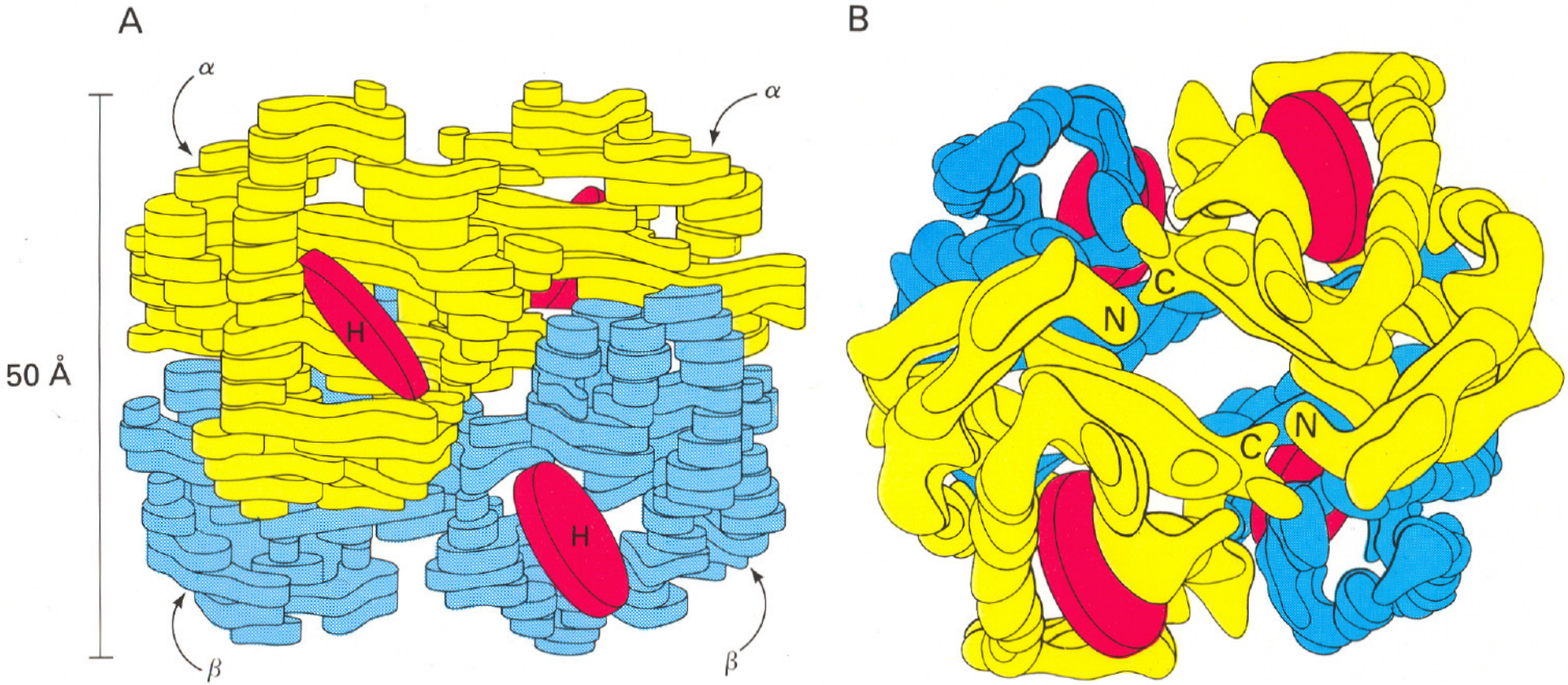


Figure 7-16, page 155

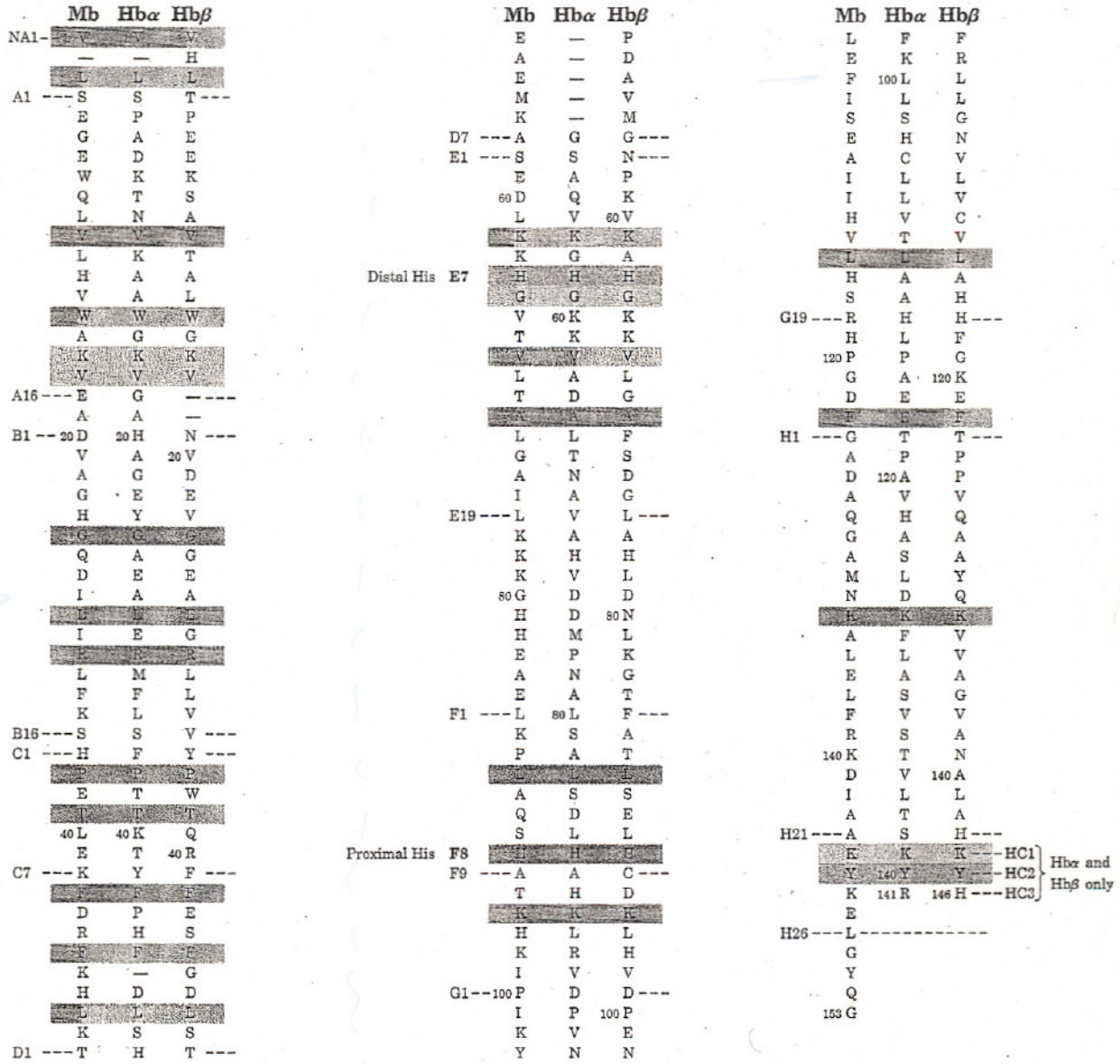
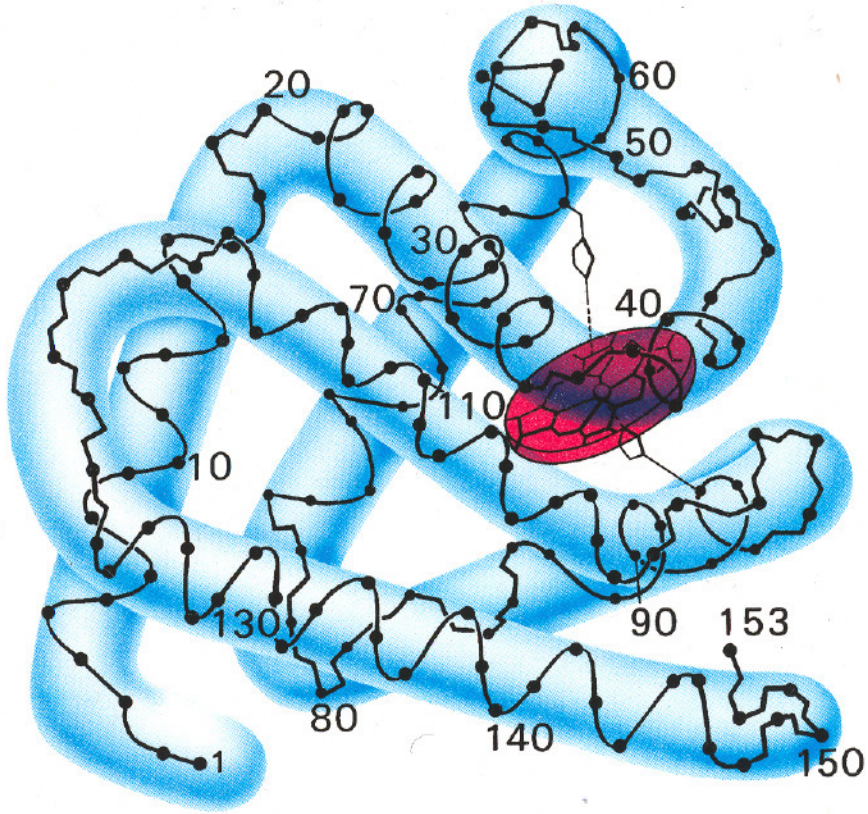
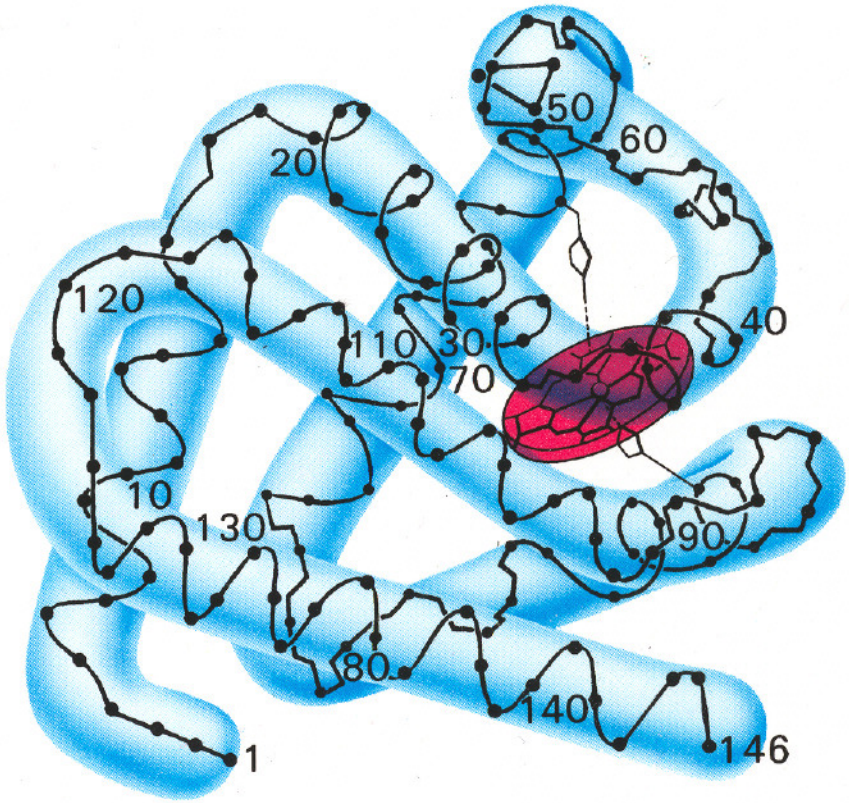


figure 7–7

The amino acid sequences of whale myoglobin and the α and β chains of human hemoglobin. Dashed lines mark helix boundaries. To align the sequences optimally, short breaks must be incorporated into both Hb sequences where a few amino acids are present in the other sequences. With the exception of the missing D helix in Hbα, this alignment permits the use of the helix lettering convention that emphasizes the common positioning of amino acid residues that are identical in all three structures (shaded). Residues shaded in red are conserved in all known globins. Note that a common letter-and-number designation for amino acids in two or three different structures does not necessarily correspond to a common position in the linear sequence of amino acids in the polypeptides. For example, the distal His residue is His E7 in all three structures, but corresponds to His^{64}, His^{58}, and His^{63} in the linear sequences of Mb, Hbα, and Hbβ, respectively. Nonhelical residues at the amino and carboxyl termini, beyond the first (A) and last (H) α-helical segments, are labeled NA and HC, respectively.

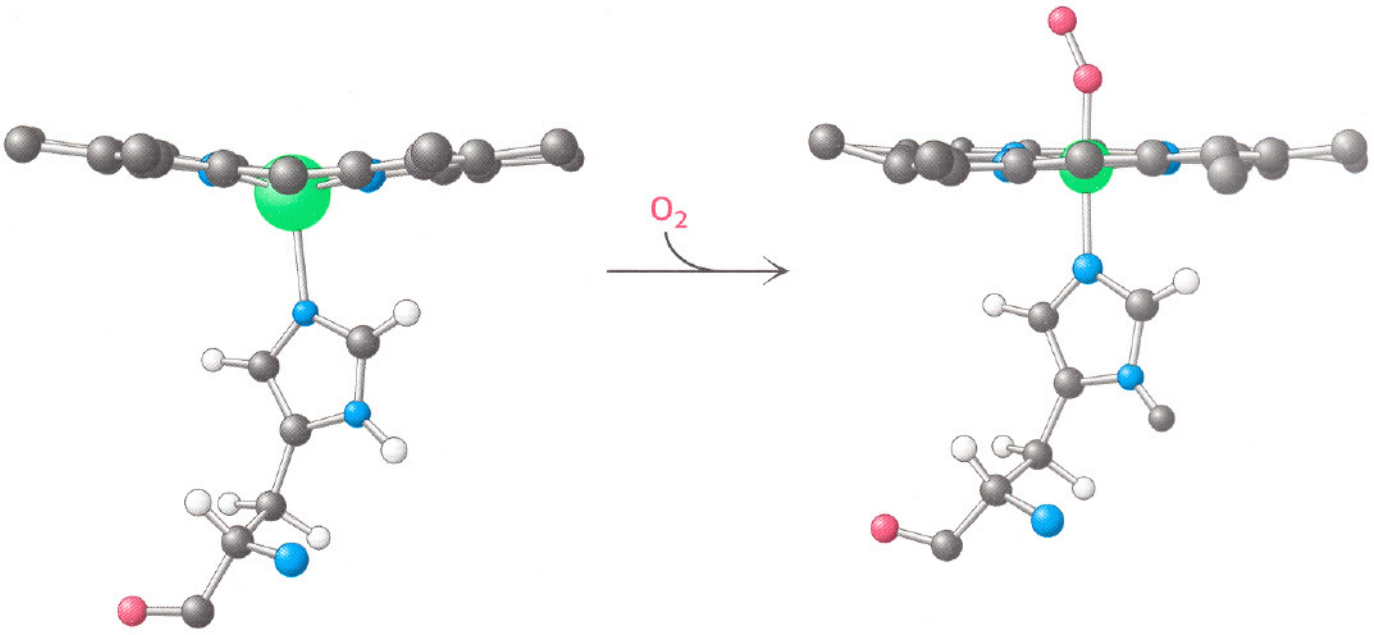


Myoglobin

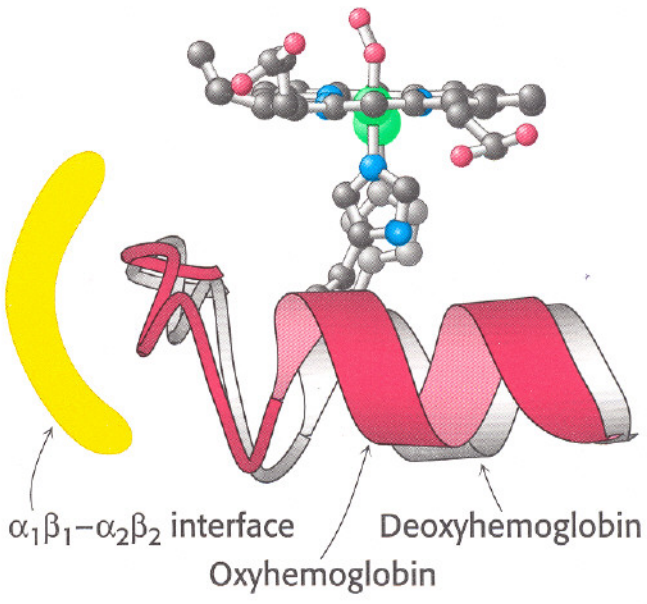


β chain of hemoglobin

Figure 7-17, page 155

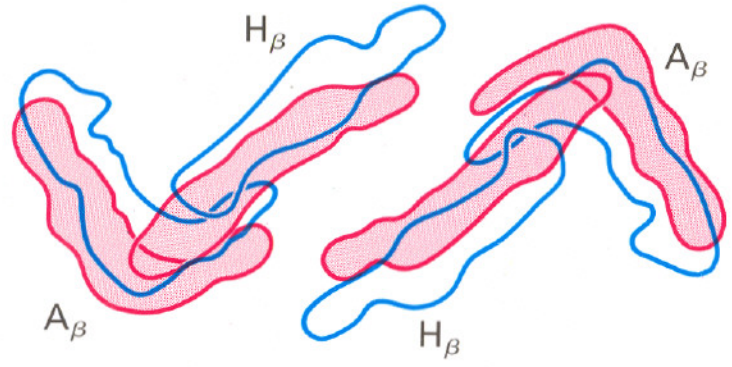


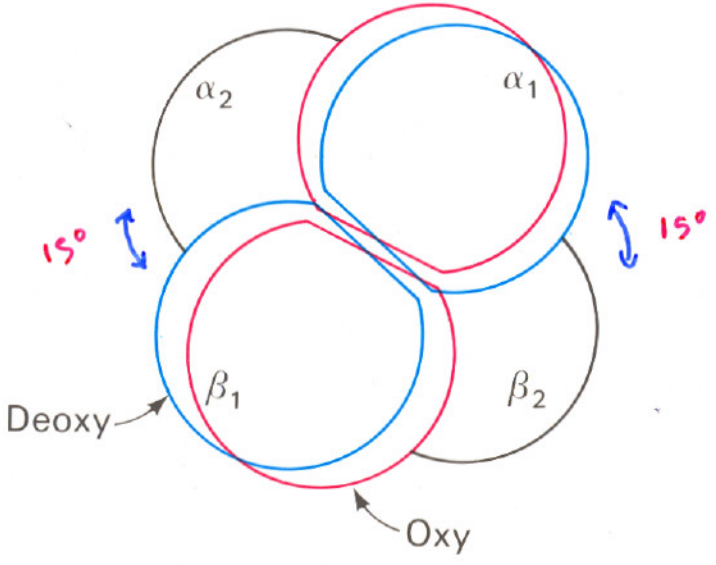
O_2 binding initiates structural changes.

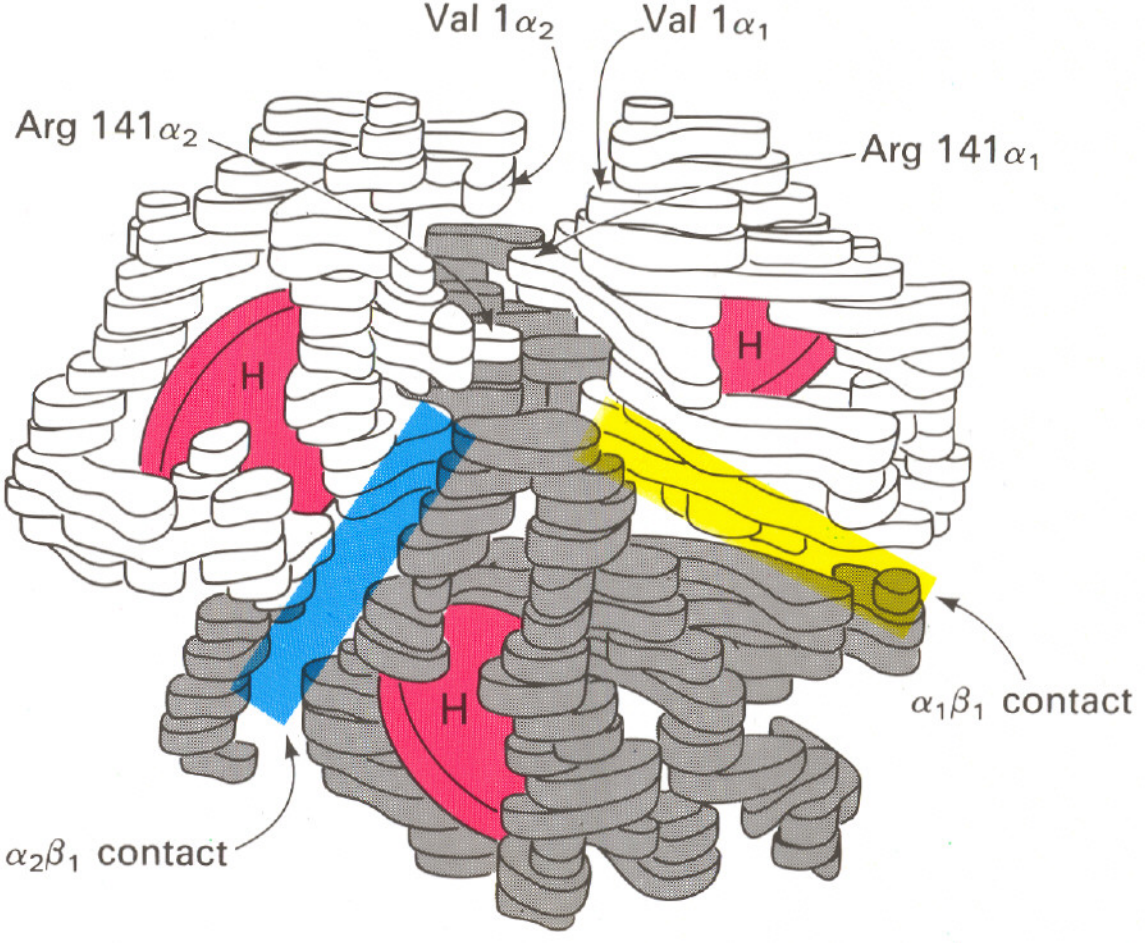


Conformational changes in hemoglobin

Figure 10-19, 10-22
Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.



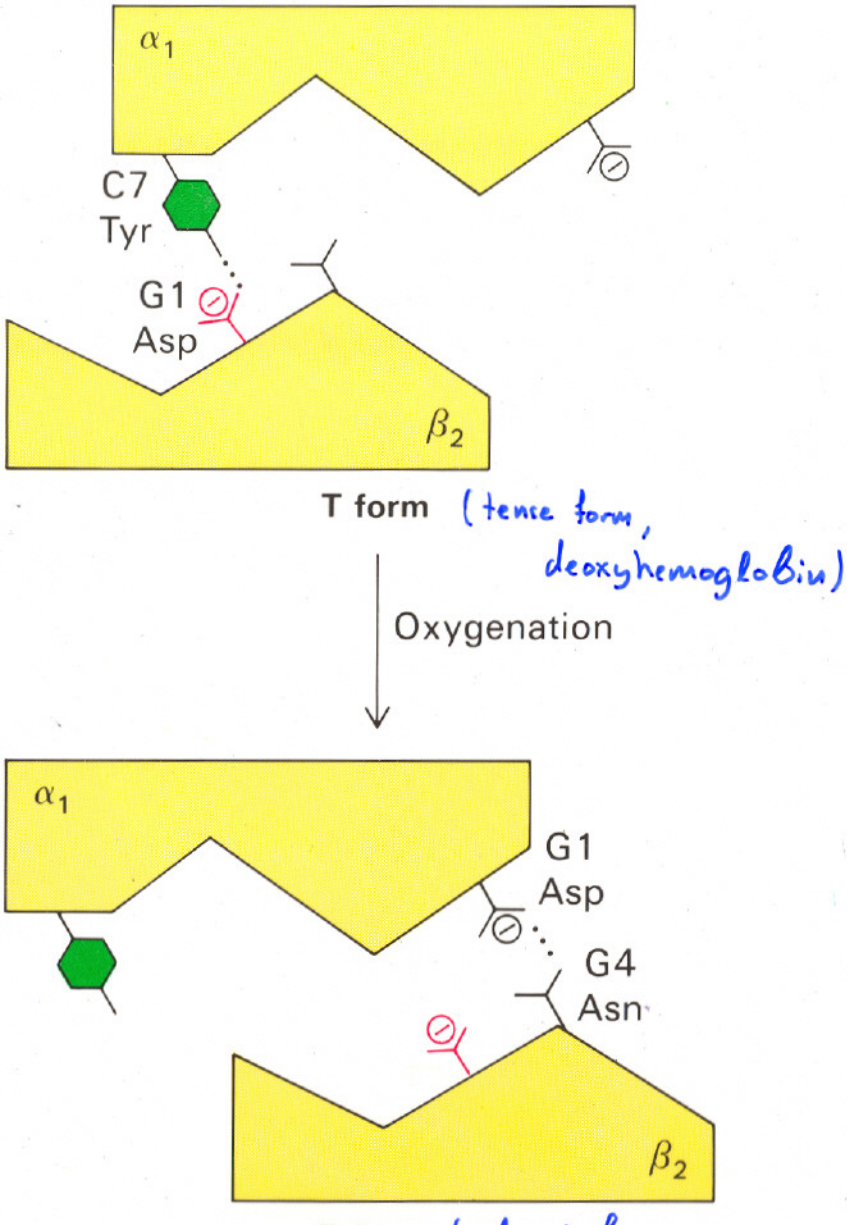




the a_1b_2 and a_2b_1 contact regions act as switches between the alternative subunits

Figures 7-27 and 7-28, page 161; Figure 7-29, page 162

Stryer: *Biochemistry*, Fourth Edition



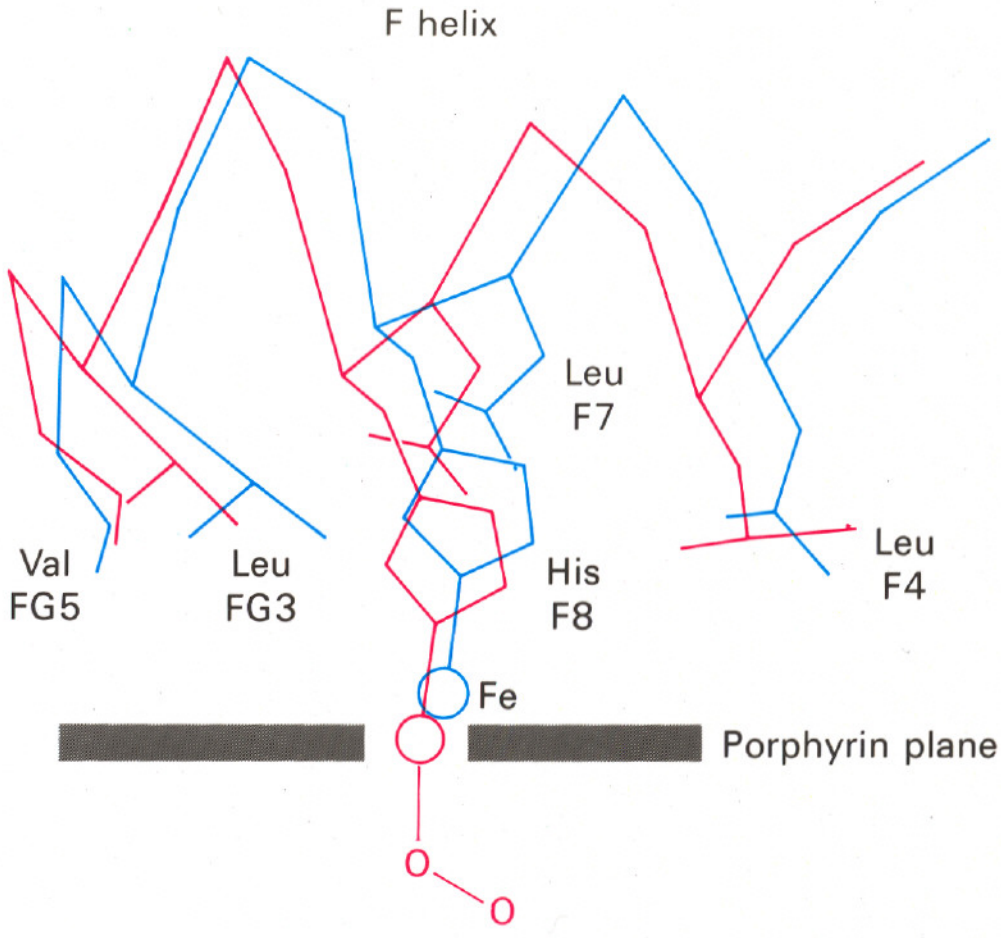


Figure 7-30, page 162; Figure 7-32, page 163

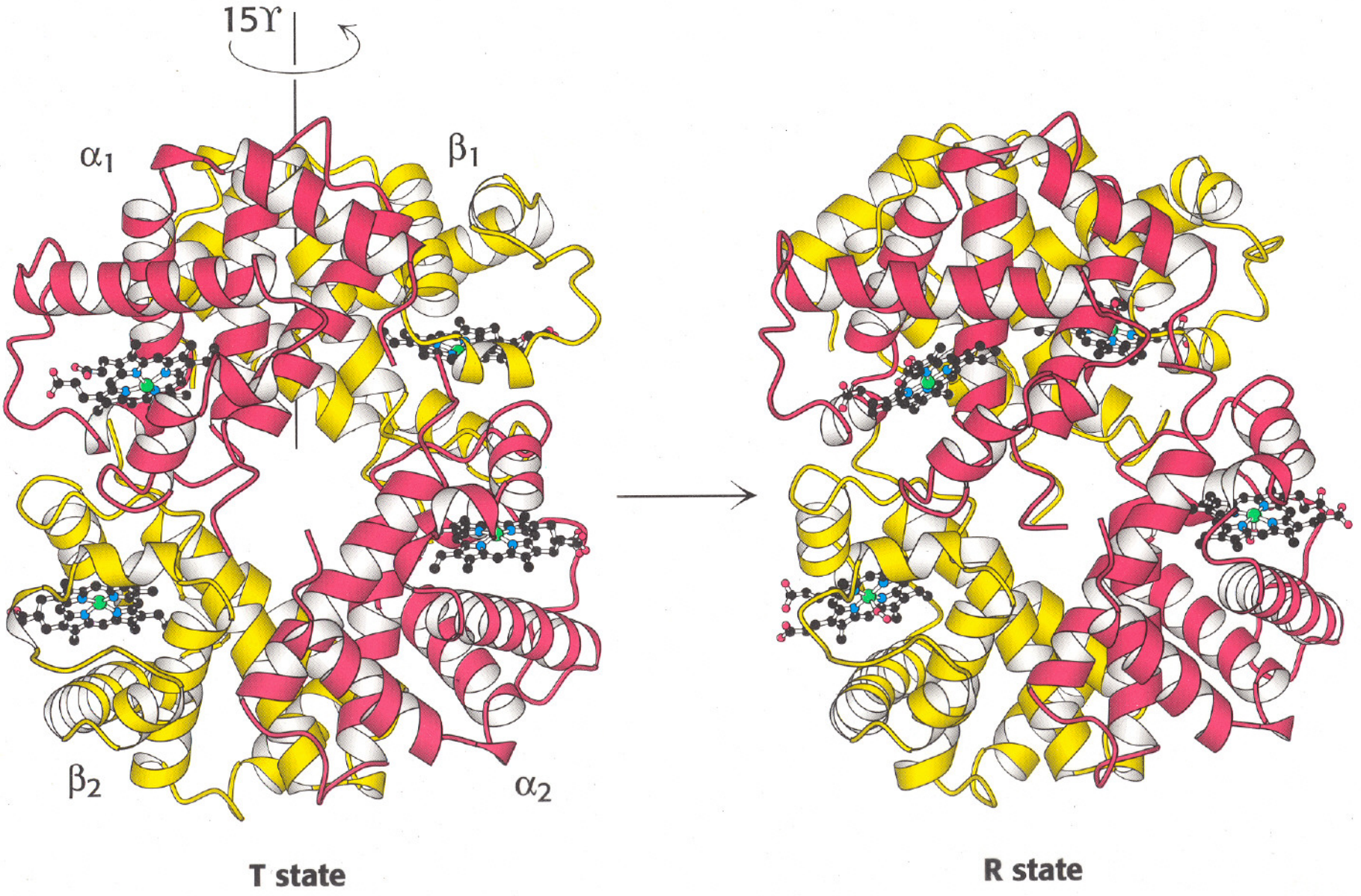


Figure 10-21 Transition from T to R state in hemoglobin

Stryer, Tymoczko, & Berg, BIOCHEMISTRY, Fifth Edition.

Allosteric interactions:

(i) O_2 binds cooperatively to hemoglobin

(ii) Affinity of hemoglobin for oxygen depends on pH, CO_2, H^+, and organic phosphates (BPG)

The saturation Y is defined as the fractional occupancy of all the oxygen binding sites in a solution

Y= 0 (all sites empty) – 1 (all sites filled)

<u>Oxygen dissociation curve</u>: a plot of Y versus pO_2 (the partial pressure of oxygen)

- **myoglogin has a higher affinity for oxygen than does hemoglobin**

P_{50} ..... Y = 0.5

P_{50} myoglobin = 1 Torr

P_{50} hemoglobin = 26 Torr

- **the oxygen dissociation curve of myoglobin is hyperbolic, whereas that of hemoglobin is sigmoidal**

The cooperative binding of oxygen by hemoglobin enables it to deliver 1.83 times as much oxygen under typical physiological conditions as it would be if the sites were independent.