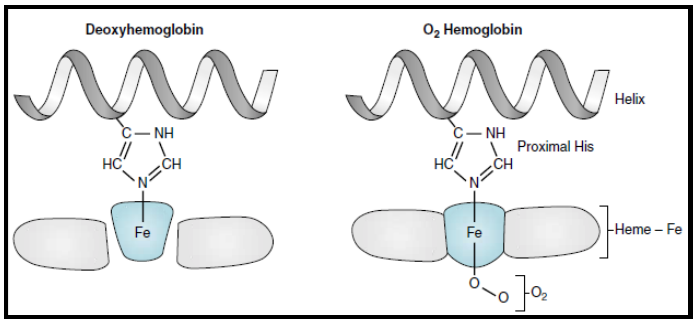
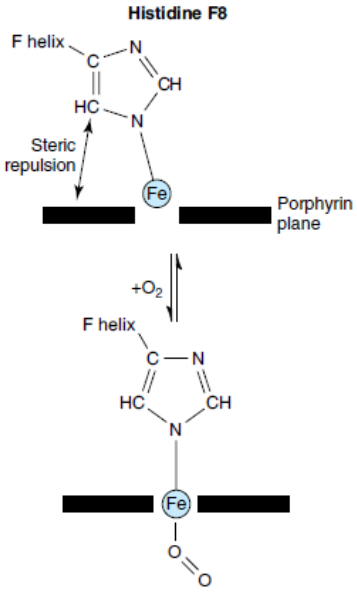
Mechanism of O_2 binding to hemoglobin

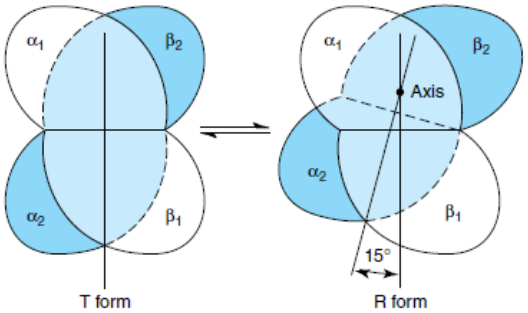
The binding rate for the first oxygen in Hb is very low. Oxygen is accessible only to the heme groups of the α-chains when hemoglobin is in T conformational state. The heme of β-chains in the T state is virtually inaccessible because of steric hindrance by amino acid residues in the helix. This hindrance disappears when the hemoglobin molecule undergoes transition to the R conformational state. Binding of O_2 to the β-chains is thus dependent on the T to R conformational shift, and this shift is triggered by the slight changes that occur when O_2 binds to the α-chain heme groups.

The proximal histidine of myoglobin and hemoglobin is sterically repelled by the heme porphyrin ring this cause the pull of the Fe^{2+} above the plane of the ring. When oxygen binds on the other side of the ring, it induces certain changes in the heme electronic state as a result it pulls the Fe^{2+} back into the plane of the ring. The pull of O_2 binding moves the proximal histidine toward the porphyrin ring, which moves the helix containing the proximal histidine.





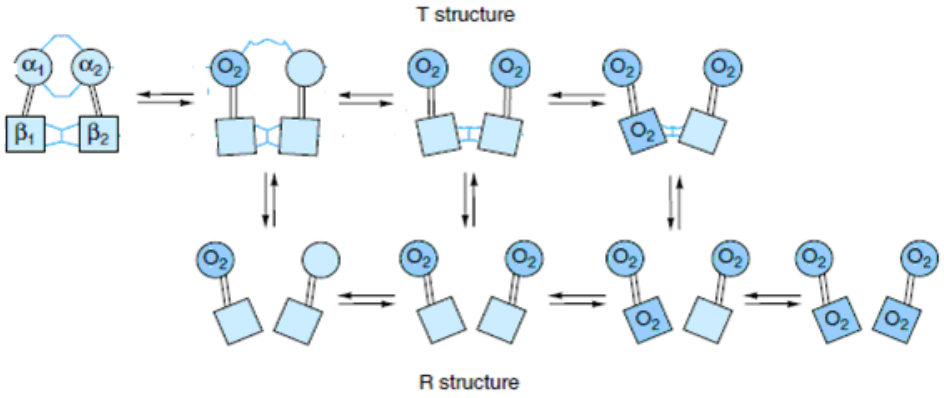
This slight movement is transmitted to adjacent subunits causing one pair of α/β subunits to rotate 15 degrees with respect to the other, this movement cause the break of salt bridges and cause conformational shift from T to R in all other subunits (see figure). These changes significantly increase the affinity of the remaining unoxygenated hemes for O_2. This binding of O_2 to hemoglobin is known as **Cooperativity of O_2 Binding in Hemoglobin**



During transition of the T form to the R form of hemoglobin, one pair of subunits rotates through 15 degrees relative to the other pair. In the diagram, the unshaded pair is shown fixed while the colored pair both rotates.

The affinity of hemoglobin for the last oxygen bound is approximately 300 times greater than its affinity for the first oxygen bound.

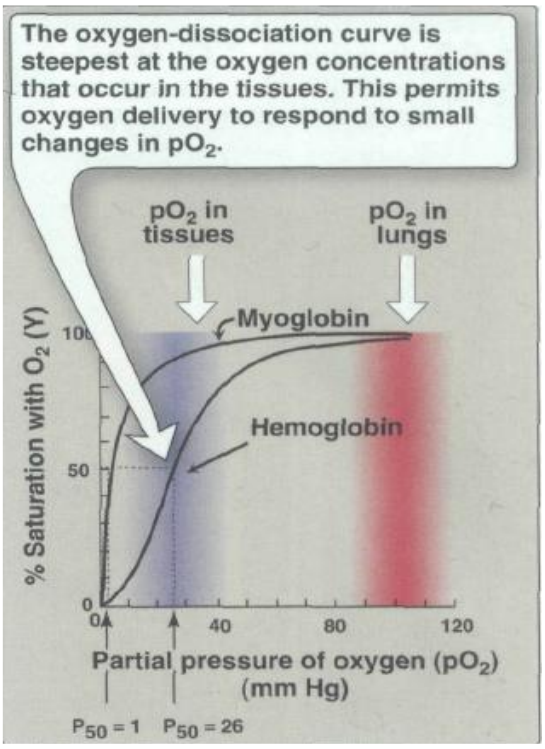
This phenomenon, known as positive cooperativity, is responsible for the sigmoidal oxygen saturation curve of hemoglobin.



Transition from the T structure to the R structure. In this model, salt bridges (thin lines) linking the subunits in the T structure break progressively as oxygen is added, and even those salt bridges that have not yet ruptured are progressively weakened (wavy lines). The transition from T to R does not take place after a fixed number of oxygen molecules have been bound but becomes more probable as each successive oxygen binds. The transition between the two structures is influenced by protons, carbon dioxide, chloride, and BPG; the higher their concentration, the more oxygen must be bound to trigger the transition.

Oxygen dissociation curve

The oxyhemoglobin dissociation curve is important for understanding how our blood carries and releases oxygen by relating oxygen saturation (SO_2) and partial pressure of oxygen in the blood (PO_2). It describes the relation between the partial pressure of oxygen (x axis) and the oxygen saturation (y axis). The oxygen saturation is the ratio of the amount of oxygen bound to the hemoglobin, to the oxygen carrying capacity of the hemoglobin. Each haemoglobin molecule has the capacity to carry four oxygen molecules. How much of that capacity is filled by oxygen at any time is called the oxygen saturation. The amount of oxygen bound to the hemoglobin is related to the O_2 pressure in the alveolar-capillary where the partial pressure of oxygen is about 100 torr and therefore the oxygen binds readily to hemoglobin that is present.



The initial slope of the oxygen binding curve is low which represent the first O_2, however, hemoglobin affinity for oxygen increases as more molecules of oxygen bind. More molecules bind as the oxygen partial pressure increases. This continues until the maximum amount that can be bound is reached. At this point, very little additional binding occurs and the curve levels out as the hemoglobin becomes saturated with oxygen for this reason the curve has a sigmoidal or S-shape (The sigmoid shape of the oxygen dissociation curve is a result of the cooperative binding of oxygen to the four polypeptide chains). The sharp rise of the oxygen-dissociation curve over the range of oxygen concentrations that occur between the lungs and

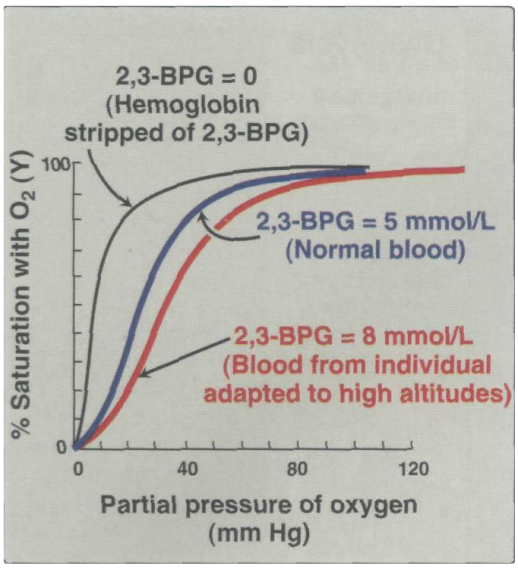
the tissues permits hemoglobin to carry and deliver oxygen efficiently from sites of high to sites of low pO_2.
At pressures above about 60 mmHg, the standard dissociation curve is relatively flat, which means that the oxygen content of the blood does not change significantly even with large increases in the oxygen partial pressure.
As the blood circulates to body tissues in which the partial pressure of oxygen is about 40 torr, the hemoglobin releases the oxygen into the tissue because the hemoglobin cannot maintain its full bound capacity of oxygen in the presence of lower oxygen partial pressures.

Atmospheric pO_2 is 160 mmHg at sea level. The partial pressure of oxygen in the blood at which the haemoglobin is 50% saturated, typically about 26.6 mmHg for a healthy person, is known as the P50. The P50 is a conventional measure of haemoglobin affinity for oxygen. In the presence of disease or other conditions that change the haemoglobin's oxygen affinity and, consequently, shift the curve to the right or left, the P50 changes accordingly. An increased P_{50} indicates a rightward shift of the standard curve, which means that a larger partial pressure is necessary to maintain 50% oxygen saturation. This indicates a decreased affinity. On the other hand, a lower P_{50} indicates a leftward shift and a higher affinity.

pO2 (torr)	**% saturation of Hb**
100 in alveoli	98
40 in resting muscle	75, thus it deliver 23% of its O_2 to resting muscle and leaving the rest of the oxygen in the blood as a reserve example for exercising and to maintain life for four to five minutes if breathing is interrupted.
20 in working muscle	20
10 in vigorous exercising muscle	10

1 Torr is approximately equal to 1 mmHg (millimeter of mercury)

Myoglobin: Myoglobin is designed to bind oxygen released by hemoglobin at the low pO_2 found in muscle. Myoglobin, in turn, releases oxygen within the muscle cell in response to oxygen demand. When strenuous exercise lowers the PO_2 of muscle tissue to about 5 mm Hg, myoglobin releases O_2 for mitochondrial synthesis of ATP, permitting continued muscular activity.
The oxygen dissociation curve for myoglobin has a **hyperbolic** shape. This reflects the fact that myoglobin reversibly binds a single molecule of oxygen.



Agents that affect oxygen binding

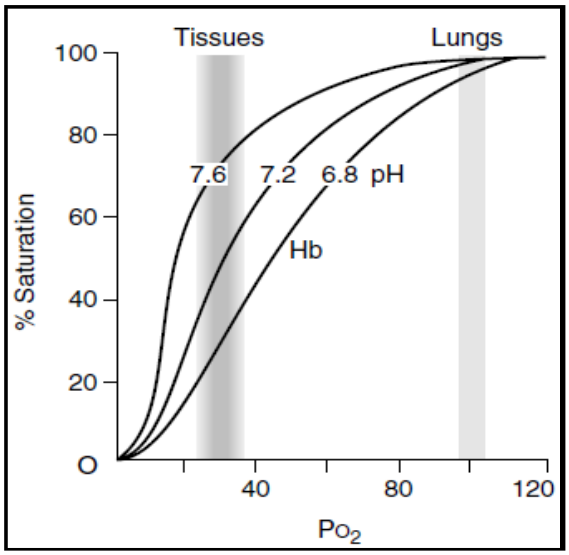
The major agents that affect oxygen binding to hemoglobin including BPG, Bohr effect, and binding to CO_2:

1- The 2,3-bisphosphoglycerate (BPG) also known as 2,3-diphosphoglycerate (DPG)
Hemoglobin, as the oxygen carrier, becomes saturated with O_2 in the lungs, where the partial pressure of O_2 (pO_2) is about 100 mm Hg. In the capillaries of tissues, pO_2 is typically 40 mm Hg, and oxygen is released from Hb.

BPG is an important regulator of the binding of oxygen to hemoglobin. The binding of BPG to Hb promotes the release of O_2. Hemoglobin stripped of BPG is virtually saturated with O_2 at low pO_2 of only 20 mm Hg, and it cannot release its oxygen within tissues, where the pO_2 is typically 40 mm Hg.
Hemoglobin from which 2,3-BPG has been removed has a high affinity for oxygen. However, the presence of 2,3-BPG significantly stabilise the deoxy structure and thus reduces the affinity of hemoglobin for oxygen, shifting the oxygen-dissociation curve to the right. This reduced affinity enables hemoglobin to release oxygen efficiently at the partial pressures found in the tissues.

High levels of 2,3-BPG shift the curve to the right, while low levels of 2,3-BPG cause a leftward shift.
The concentration of 2,3-BPG in the red blood cell increases in response to chronic hypoxia, such as that observed in obstructive pulmonary emphysema, or at high altitudes, where circulating hemoglobin may have difficulty receiving sufficient oxygen. Intracellular levels of 2,3-BPG are also elevated in chronic anemia, in which fewer than normal red blood cells are available to supply the body's oxygen needs. Elevated 2,3-BPG levels lower the oxygen affinity of hemoglobin, permitting greater unloading of oxygen in the capillaries of the tissues (Reduced Hb-O2 affinity------- shift the curve to right; <u>while</u>
Increase Hb-O2 affinity -------shift the curve to the left).

<u>2- Bohr effect</u>:
The binding of protons to hemoglobin lowers its affinity for oxygen (see Fig) therefore, a shift to the right in the oxygen dissociation curve.
Conversely, raising the pH or lowering the concentration of CO_2 results in a greater affinity for oxygen, and a shift to the left in the oxygen dissociation curve. This phenomenon is known as the Bohr Effect. The pH of the blood decreases as it enters the tissues (and the proton concentration rises) because the CO_2 produced by metabolism is converted to carbonic acid (as we discussed in the first lecture). Dissociation of carbonic acid produces protons that react with several amino acid residues in hemoglobin (such specific histidine side chains), causing conformational changes that promote the release of oxygen.



In the lungs, this process is reversed. Oxygen binds to hemoglobin, causing breakage of salt bridges involving β-chain residue in His 146 and release of protons, which combine with bicarbonate to form carbonic acid. This decrease of protons causes the pH of the blood to rise. Carbonic anhydrase cleaves the carbonic acid to H_2O and CO_2, and the CO_2 is exhaled.
Thus, in tissues in where the pH of the blood is low because of the CO_2 produced by metabolism, oxygen is released from hemoglobin. In the lungs, where the pH of the blood is higher because CO_2 is being exhaled, oxygen binds to hemoglobin.

3- Binding of CO_2:
The rise in CO_2 induces decrease in pH which cause decrease affinity to O2 and lose of O_2 from oxyhemoglobin.
Most of the carbon dioxide produced in metabolism dissolve in water and transported as bicarbonate ion. However, some CO_2 is carried as **carbamino (carbamate)** bound to the uncharged α-amino groups of hemoglobin.

The binding of CO_2 stabilizes the T (taut) or deoxyhemoglobin, resulting in a decrease in its affinity for oxygen. In the lungs, CO_2 disassociates from the hemoglobin, and is released in the breath.
An increase in pCO2 and decrease in pH are both characteristic of actively metabolizing cell these cells promote the release of O2 from oxyhemoglobin thus a right shift to curve meaning additional O2 can supply the tissue.