Hemoglobin (Haemoglobin in many varieties of English) is the oxygen binding protein of red blood cells and is a globular protein with quaternary structure. It is a tetramer consisting of four polypeptide subunits; 2 alpha chains and two beta chains.

Each chain is bound to a prosthetic group called heme. A heme consists of an Fe2+ (ferrous) ion contained in the centre of a large heterocyclic organic ring called a porphyrin. The ring is made up of four pyrrolic groups joined together by methine bridges. Porphyrins are quite diffused in nature, (for instance chlorophyll and vitamin B12 contain porphyrin ring) but not all of them contain iron. Hemoproteins are a substantial fraction of porphyrin - containing metalloproteins which have heme as their prosthetic group. Apart from hemoglobin, hemes are also found in a number of other biologically important hemoproteins such as myoglobin, cytochrome, catalase, and endothelial nitric oxide synthase.
As shown in the picture, the heme group is bound to the polypeptide chain by the nitrogen atom in the imidazole ring of Histidine 93 (that is the 93rd amino-acid in the primary chain). Hemes play an essential role in the transport of diatomic gases. The gas binds to the heme ion using a lone pair of their own. Hemoglobin transports oxygen in the blood from the lungs to the rest of the body.

The three-dimensional structure of hemoglobin was solved using X-ray crystallography in 1959 by Max Perutz. The structure of hemoglobin is very similar to the single polypeptide chain in myoglobin despite the fact that their amino acid sequences differ at 83% of the residues. This highlights a relatively common theme in protein structure: that very different primary sequences can specify very similar three-dimensional structures.

Relative Affinity and Efficiency of Hemoglobin and Myoglobin

Hemoglobin does not have an extraordinarily high affinity for oxygen in the first place because it exists in the lungs where oxygen is plentiful and it can easily bind it (at pO2 = 80 torr, 95.8% of binding sites are occupied by oxygen). So when something like carbon dioxide, that has a stronger affinity for oxygen than hemoglobin, exists in the lungs with hemoglobin, it will steal all of the oxygen before hemoglobin can bind it. This will prevent hemoglobin from binding any oxygen and cause the cells to suffocate.
The affinity of hemoglobin for oxygen is less than its structural analog myoglobin. Interestingly enough, however, this does not affect hemoglobin’s usefulness for the body; on the contrary, it allows hemoglobin to be a more efficient oxygen carrier than myoglobin. This is so because hemoglobin can release oxygen more easily than myoglobin can. In facts, while it is important for oxygen to be carried to different areas of the body, it is even more important for the oxygen to be released when needed. The higher the affinity of a given protein for oxygen, the harder it will be for that protein to release oxygen when the time comes. Thus, hemoglobin’s lower affinity for oxygen serves it well because it allows hemoglobin to release oxygen more easily in the cells.

Myoglobin, on the other hand, has a significantly higher affinity for oxygen and will therefore be much less inclined to release it once it is bound. Thus hemoglobin’s lower affinity for oxygen relative to myoglobin allows it to have a higher overall efficiency in binding and then releasing oxygen species. For this reason the body tends to use hemoglobin more often for oxygen-distributing purposes, although myoglobin is used as well, particularly for carrying oxygen to muscle cells. So when hemoglobin travels to the muscles to deliver oxygen, the myoglobin can easily accept the oxygen and the hemoglobin can easily release it.

The following diagram shows that at value of pressure the two proteins have a similar affinity to oxygen. At lower value of pressure (i.e. 15-20 torr) the affinity of myoglobin is much greater.
While Hemoglobin has 4 subunits, Myoglobin has one subunit. It is the protein of oxygen storage within skeletal muscle cells. The reason muscles are red is because they contain large amount of myoglobin. Organisms such as diving mammals have very large amounts of myoglobin so that they can go for an extended period of time without breathing.

**States**

With its sigmoidal shape, the disassociation curve shows how hemoglobin exhibits a cooperative effect related to oxygen. This means that hemoglobin can transmit intramolecular messages to its various functional groups to help it attain a maximum affinity for its ligand oxygen. When a monomer of hemoglobin binds to oxygen, it alerts other nearby hemoglobin monomers to start the binding process as well. This means that the more oxygen is bound by hemoglobin monomers, the more the affinity of hemoglobin will increase. In other words, the affinity of hemoglobin is proportional to the quantity of oxygen bound at a given time.

Hemoglobin exists in two distinct conformational states: the T-state (tense) and the R-state (relaxed). The T state has a less of an affinity for oxygen than the R state. The T-state is the deoxy form of hemoglobin (meaning that it lacks an oxygen species) and is also known as "deoxyhemoglobin." The R-state is the fully oxygenated form: "oxyhemoglobin."

In the sequential mode of cooperativity (Koshland’s hypothesis), the conformation state of the monomer changes as it binds to oxygen. Experimental data obtained from kinetics experiments with hemoglobin reveals that hemoglobin undergoes several conformational changes upon binding with oxygen. In deoxy-hemoglobin the iron ion is bound to the lone pair of nitrogen contained in Histidine side chain (His E8). This bond pulls iron out of the porphyrine plans. When oxygen binds to the iron ion, the new bond pulls back iron on the heme plan. As soon as the iron cation within hemoglobin begins to move, the Histidine residue and the alpha-helix of hemoglobin start moving as well. As a consequence, the carboxyl terminal end of the alpha-helix, which resides at the interface between the two alpha- and beta-dimers, moves too. The positional changes of the carboxyl terminal end create favorable conditions for transitions between the T- and the R-states of hemoglobin. That a new configuration is assumed by the molecule and that’s more favourable to bind oxygen.

![Figure 7.14](biochemistry/seventh-edition/figures/CooperativityModel.png)
Because it can modify its affinity for oxygen, hemoglobin can exhibit a range of different affinities. This makes it quite flexible in terms of how much oxygen it can bind and therefore how much it can release. This is another reason why the body prefers to use hemoglobin, as opposed to myoglobin, for oxygen transport: hemoglobin can modify its own affinity for oxygen to suit the situation (i.e. the oxygen pressure) at hand, making it capable of handling a wider variety of chemical environments and organ systems while still being able to distribute oxygen effectively.
As hemoglobin travels from the lungs to the tissues, the pH value of its surroundings decrease, and the amount of CO₂ that it reacts with increases (we’ll discuss that later). Both these changes cause the hemoglobin to lose its affinity for oxygen, therefore making it drop the oxygen into the tissues. The cooperative effect discussed above makes this loss of affinity more effective. 

The importance of cooperative behavior is that it allows hemoglobin to be more efficient in transporting oxygen. For example, in the lungs, the hemoglobin is at a saturation level of 98%. However, when hemoglobin is present in the tissues and releases oxygen, the saturation level drops to 32%; thus, 66% of the potential oxygen-binding sites are involved in the transportation of oxygen.

This image also shows hemoglobin's oxygen binding affinity compared with myoglobin's affinity. In this graph, on the left, you can see hemoglobin’s sigmoidal curve, how it starts out with a little less affinity than myoglobin, but comparable affinity to oxygen in the lungs. As the pressure drops and the myoglobin and hemoglobin move towards the tissues, myoglobin still attains its high affinity for oxygen, while hemoglobin, because of its cooperativity, suddenly loses its affinity, therefore making it the better transporter of oxygen than myoglobin.

**Allosteric effects of 2,3 biphosphoglycerate.**

**Allosteric regulation** is the process by which the behavior of proteins is controlled by other molecules; the molecules that perform this regulation are known as allosteric regulators. This process involves the binding of an allosteric regulator molecule to the protein in question; the result is a distinct effect on the protein's function. Allosteric regulators that increase or supplement a given protein's function are known as **allosteric activators**. Those that decrease or interrupt a given protein's function are known as **allosteric inhibitors**.

Hemoglobin, like other proteins, has its share of allosteric regulators. Regulation is highly necessary for a protein as important as hemoglobin, since its affinity for oxygen must be just right for the particular organ system that it is currently dealing with. Thus the main concern for most of hemoglobin's allosteric regulators is tweaking its oxygen affinity to match the situation at hand.
Biphosphoglycerate, or BPG, is one of many allosteric regulators for hemoglobin. This molecule binds to the central cavity of the deoxyhemoglobin version of hemoglobin (T-state) and stabilizes it. The increased stability of the T-state results in a decreased affinity for oxygen, since normally it is the intense straining of the T-state that drives deoxyhemoglobin to bind to oxygen; once oxygen is bound, the T-state loses its strain and relaxes into the R-state. Thus, by stabilizing the normally tense T-state, BPG makes hemoglobin less likely to bind oxygen in an attempt to release the strain. This mechanism is necessary, because the T state of hemoglobin is so unstable that the equilibrium lies very strongly in favor of the R state and little to no oxygen is released. In other words, pure hemoglobin binds to oxygen very tightly. 2,3-BPG was thus needed to stabilize the T state. Because BPG decreases hemoglobin’s affinity for oxygen, it is an allosteric inhibitor of hemoglobin. Without 2,3-BPG, hemoglobin would be an extremely inefficient transporter of oxygen from the lungs to the tissues, releasing only about 8% of its oxygen content. However, in the presence of 2,3-BPG, more oxygen-binding sites in the hemoglobin tetramer must be filled in order to obtain the transition from the T to the R state. That means higher concentrations of oxygen must be reached in order for hemoglobin to undergo transition from the lower-affinity T-state to the higher-affinity R state.

BPG is really important for O₂ transport in our body. One example is high altitude adaptation. High altitude will induce a rapid increase in the amount of BPG synthesized in erythrocytes. The increased amount of BPG will shift the oxygen binding curve from sea-level position to a lower affinity position (shift to right). This decreases the amount of O₂ binding in the lungs, but, to a greater extent, increases the amount of O₂ released at tissues. So hemoglobin can deliver more O₂ from lungs to tissues.
The binding of 2,3-BPG has further physiological consequences. Fetal hemoglobin has a higher oxygen-binding affinity than that of maternal hemoglobin (see below). Fetal red blood cells have a higher affinity for oxygen than maternal red blood cells because fetal hemoglobin doesn't bind 2,3-BPG as well as maternal hemoglobin does. The result of this difference in oxygen affinity allows oxygen to be transferred effectively from maternal to fetal red blood cells.

**Bohr effect**

Bohr effect is a property of hemoglobin which states that at lower pH (more acidic environment), hemoglobin will bind to oxygen with less affinity. Since carbon dioxide is in direct equilibrium with the concentration of protons in the blood, increasing blood carbon dioxide levels leads to a decrease in pH, which ultimately leads to a decrease in affinity for oxygen by hemoglobin.

The pH, or proton concentration of a given solution, is another allosteric regulator of hemoglobin. Interestingly enough, pH can act as both an allosteric activator and inhibitor, depending on the direction of pH change. As pH decreases, for example, the affinity of hemoglobin for oxygen decreases as well. This is due to the fact that protons help construct salt bridges in the T-state of hemoglobin.

In general, the T-state of hemoglobin is favored by three amino acids that form two salt bridges. One of the salt bridges is formed by the interaction between Beta Histidine 146 (the carboxylate terminal group) and Alpha Lysine 40. This connection will help to orient the histidine residue to also interact in another salt bridge formation with the negatively charged aspartate 94. The second bridge is formed with the aid of an additional proton on the histidine residue. Thus, the higher the proton concentration (or the lower the pH) in the solution, the easier this salt bridge will form. Better salt bridge formation leads to a better and more stable T-state, and as mentioned before, a more stable T-state means decreased oxygen affinity of hemoglobin. Since higher proton concentration corresponds to lower pH, this means that the lower the pH, the more stable the T-state will be. Finally, the more stable the T-state, the lower the affinity for oxygen will be in hemoglobin molecule; thus pH acts as an allosteric inhibitor of hemoglobin when it is decreasing.

Logically, then, the opposite effect would occur when the pH increases. This would signify a lower proton concentration, meaning more difficult salt bridge formation and thus a slower-forming and less stable T-state. A less stable T-state would be much more inclined to bind with oxygen; thus increased pH results in increased oxygen affinity for hemoglobin. The result is that pH (H⁺ actually) acts as an allosteric activator for hemoglobin when it is increasing.
Carbon dioxide, or CO$_2$, is yet another allosteric inhibitor of hemoglobin. There are several reasons for this. The first is that the enzyme carbonic anhydrase can help carbon dioxide react with water to form carbonic acid, which dissociates into bicarbonate and a proton. With enough carbonic anhydrase enzymes present, therefore, carbon dioxide can cause a decrease in the pH of the solution due to all the protons produced from its reaction with water. As mentioned in the previous paragraph, more protons means decreased pH, which in turn means a decreased affinity of hemoglobin for oxygen. Carbon dioxide also neutralizes the positive charge on the amino terminus of hemoglobin (amino groups usually exist in their protonated forms in living systems). This charge neutralization results in production of negatively charged carbamate groups, which form salt bridges that lead to stabilization of the T-state of hemoglobin, which results in a decreased affinity for oxygen. Thus carbon dioxide functions as an effective allosteric inhibitor of hemoglobin.

This CO$_2$ action is called the Bohr effect and it facilitates oxygen transport. Hemoglobin binds to oxygen in the lungs and releases it in the tissues predominately to those tissues in most need of oxygen. When a tissue's metabolic rate increases, (e.g. under exercise) its carbon dioxide production increases. Carbon dioxide forms bicarbonate through the following reaction:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons H^+ + HCO_3^-$$

This reaction usually progresses very slowly. With the help of the enzyme carbonic anhydrase, the formation of bicarbonate and protons in the red blood cells is accelerated. This causes the pH of
the tissue to decrease and promote the dissociation of oxygen from hemoglobin to the tissue, allowing the tissue to obtain enough oxygen to meet its demands.

Conversely, in the lungs, oxygen concentration is high. The binding of oxygen causes hemoglobin to release protons, which combine with bicarbonate to drive off carbon dioxide in exhalation. Since these two reactions are closely matched, there is little change in blood pH.

Sickle Cell Anemia

A disease that affects many individuals's hemoglobin functionalities is sickle cell anemia, which cause by substitution of Valine instead of Glutamate at position 6 in amino acid sequence. Symptoms occur when an individual is several months old. Sickle cell anemia is characterized by decreased breath intake, delayed growth and development, fever, jaundice, rapid heart rate, and many other ailments.
The problem is that hemoglobin in these individuals are mutated. This mutated form of hemoglobin is called hemoglobin S and is less soluble than regular hemoglobin forms. Examination of the structure of hemoglobin S reveals that a new valine residue lies on the surface of the T-state molecule. As a result of this change deoxyhemoglobin has a hydrophobic patch on its surface. The hydrophobic patch interacts with other hydrophobic patches causing the molecule to aggregate into strands that align into insoluble fibers. Because this mutated form cannot move freely when they accumulate in the blood stream they end up rupturing or distorting the shape of the red blood cells when delivering oxygen. The red blood cells end up becoming a sickle or crescent shape. These inflicted cells are much less efficient in deliver oxygen through the body’s circulation. They can clog fairly easily in smaller areas of blood flow causing them to disrupt blood flow. Sickle cell anemia should not be mistaken with hemophilia which is a disease in which an individual’s body cannot form blood clots. If gone without proper treatment, people with this disease usually die from organ failure from ages 20 to 40. Better technology and data on this disease has led to treatments that involve folic acid supplements that activate the product of new healthy red blood cells. Treatment must be ongoing and is meant to limit the number of pains and emergencies of the disease. Overall immune system also suffers from this disease so often people take antibiotics and vaccines to prevent themselves from getting sick.

Sickle cell anemia is passed down through families and a child can only receive sickle cell anemia if both parents also have the disease. About 1 in 12 African Americans have this trait. There is a significant correlation between regions with high frequency of sickle cell anemia and regions with high prevalence of malaria. People with the sickle cell trait are resistant to malaria because the parasite that carries the disease needs to live within a red blood cell at some point in its life and is unable to survive in a sickle cell. Therefore, due to natural selection overtime the number of people with sickle cell anemia grew because before there was a cure for malaria the majority of the people who got malaria would die.

It is now possible to diagnose sickle cell anemia during pregnancy. Patients with the disease are encouraged to drink enough fluids, get enough oxygen, responding quickly to infections. Strenuous physical activity should be avoided, smoking should be avoided, and too much sun exposure should also be avoided. Extreme consequences of sickle cell anemia no doubt includes death but others include blindness, spleen malfunction, tissue death, strokes, and acute chest pains. Below are pictures comparing healthy red blood cells to blood cells inflicted by sickle cell anemia.
**Thalassemia**

Just as sickle cell anemia is a difference in one amino acid, thalassemia is also an inherited disease where there is a reduction or loss of a hemoglobin chain. This leads to lower levels of hemoglobin and those with the disease experience anemia, fatigue, pale skin as well as spleen and liver malfunctions. Thalassemia branches into two different types: α-thalessemia and β-thalessemia. In α-thalessemia, the α-helix of hemoglobin is in low supply. This makes hemoglobin with high affinity for oxygen and no cooperativity therefore, making the release of oxygen in tissue poor. This is caused by a disruption in 4 alleles on chromosome 16 and is more rare.

In β-thalessemia, the β-chain is in low supply. the extra α-helixes will aggregate and precipitate inside red blood cells which can result in anemia. β-thalessemia is caused by disruption on two alleles on chromosome 1.

**Carbon Monoxide Poisoning**

Carbon Monoxide (CO) is a dangerous gas because it is odorless and colorless. Sources of carbon monoxide include running automobiles and gas-powered appliances. When inhaled, it binds at the same sites as oxygen and can negatively impact the body's ability to absorb oxygen. Carbon monoxide binds to hemoglobin 200 times more tightly than oxygen does. Even at low partial pressures, carbon monoxide will prevent hemoglobin from delivering oxygen to the body. Once carbon monoxide binds to one site of hemoglobin, hemoglobin turns into the R-state which increases oxygen affinity and prevents oxygen dissociation in tissues.

Treatment of carbon monoxide poisoning includes the administration of 100% oxygen at higher partial pressures. Because of the higher pressure, this will displace most of the carbon monoxide from hemoglobin.

Breathing of 100% O2 helps reduce the half-life of COHb, Carboxyhemoglobin, a stable complex of CO and hemoglobin formed in red blood cells with the presence of CO. Measurement of COHb level in red blood cells is used to confirm exposure to CO and assess the severity of poisoning. Elevated level of COHb is determined more than 2% for nonsmokers and more than 9% for smokers.

By replacing oxygen in hemoglobin, CO cuts off the supply of oxygen to tissues and cells, which can result in neurological problems in adults, learning disabilities and developmental issues in children, and miscarriage in women during pregnancy.

CO poisoning symptoms are not obvious, including headache, dizziness, nausea, fatigue and weakness. They can be mistaken as food poisoning, influenza, migraine headache, or substance abuse.

2 main types of CO poisoning: acute, caused by exposure to high level of CO in a short period of time, and chronic or subacute, caused by exposure to low level of CO in a long period of time.
Fetal Hemoglobin

During the entire fetal formation period, three different types of hemoglobin are produced, with the succeeding hemoglobin deactivating its predecessor. All three types have the same heme molecules and iron atom, but differ slightly in structure.

In the first eight weeks, majority of the hemoglobin is a type called embryonic hemoglobin. After that period the fetal hemoglobin (Hemoglobin F) is produced. It is the predominant form of hemoglobin expressed in the fetus development. The Hemoglobin F is produced until a few months after birth.

Around the thirty-fifth week, the adult hemoglobin (Hemoglobin A) starts to be produced. Eventually, the blood cells will only contain Hemoglobin A, which is the only one produced for the duration of the human life.

There are some structural differences between the adult hemoglobin and the fetal hemoglobin. As we know, the adult hemoglobin is composed of 4 heme groups, 2 alpha chains and 2 beta chains. The fetal hemoglobin is also composed of 4 heme groups, 2 alpha chains and 2 gamma chains. The fetal hemoglobin and adult hemoglobin are found to be different near the 2,3 BPG binding site. The 2,3 BPG binds less tightly with the deoxy form of fetal hemoglobin as compared to the deoxy form of adult hemoglobin.

Another form of haemoglobin, termed haemoglobin A2, and comprising of two alpha and two delta chains; is produced in small quantities throughout childhood and adulthood. Haemoglobin A2 accounts for around 2-3% of total haemoglobin levels.
A fetus obtains its source of oxygen from the mother’s lungs. The oxygen in the mother’s bloodstream attaches to hemoglobin molecules in the red blood cells and diffuses to the fetal bloodstream at the placenta. By the time the blood reaches the fetus, the pressure is much lower, which is not enough for a normal adult. The fetal hemoglobin has a noticeably higher affinity for oxygen than does maternal hemoglobin. This is of crucial importance during pregnancy in human females (and presumably in other pregnant mammalian females) because it allows the fetus to obtain much-needed oxygen during development. Basically, the hemoglobin present in the fetus is able to strip oxygen species from the maternal hemoglobin when the mother's blood comes into contact with fetal material. The portion of the mother’s blood that does not touch the fetus transfers oxygen as normal to the mother's organ systems.

**Blood Doping**

There is a hormone that can induce the increase of red blood cell production. Erythropoietin is a glycoprotein hormone that controls erythropoiesis, or otherwise known as red blood cell production. It is a protein signaling molecule (cytokine) for erythocyte (red blood cell) precursors in the bone marrow. This hormone is produced in the interstitial fibroblasts in the kidney and in the perisinusoidal cells in the liver.

**Superoxide**

Oxygen binding to iron in the heme group pulls part of the electron density from the ferrous ion to the oxygen molecule. It is important to leave hemoglobin (and myoglobin) in the dioxygen form rather than superoxide form ($O_2^-$, a free radical, with an unpaired electron) when the oxygen is released.
Superoxide and superoxide-derived oxygen species (ROS, radical oxygen species) are so reactive compared to the stable O2 molecule that they could have a destructive effect both within the cell and in its environment.

![Superoxide and Superoxide-Derived Oxygen Species](image)

Figure 7.4  
*Chemistry, Seventh Edition*  
© 2012 WH Freeman and Company