**Chapter 5 Protein Function**

* It will be explored how proteins interact with other molecules.
* The functions of many proteins involve the reversible binding of other molecules called **ligand**.
* A ligand may be any kind of molecule, including another protein.
* A ligand binds at a site on the protein called the **binding site**.
* is complementary to the ligand in size, shape, charge, and hydrophobic or hydrophilic character.
* Interaction between protein and ligand is specific.
* The binding is coupled to a conformational change in the protein since proteins are flexible.
* The structural adaptation occurring between protein and ligand is called **induced fit**.
* In a multisubunit protein, a conformational change in one subunit often affects the conformation of other subunits.
* Enzymes bind and chemically transform other molecules called reaction **substrates** rather than ligands.
* The ligand-binding site is called **catalytic site** or **active site**.

**5.1 Reversible Binding of a Protein to a Ligand: Oxygen-Binding Proteins**

* Myoglobin and hemoglobin may be the most-studied and best-understood proteins.
* They are examples for the reversible binding of a ligand to a protein.

**Oxygen Can Bind to a Heme Prosthetic Group**

* Oxygen is poorly soluble in aqueous solutions and cannot be carried to tissues in sufficient quantity if it is simply dissolved in blood serum.
* Diffusion of oxygen through tissues is also ineffective.
* Some proteins could transport and store oxygen.
* However, none of the amino acid side chains in proteins are suited for the reversible binding of oxygen molecules.
* Multicellular organisms exploit the properties of metals, most commonly iron, for oxygen transport.
* Iron is often incorporated into a protein-bound prosthetic group called **heme**.
* Heme consists of a complex organic ring structure, **protoporphyrin**, to which is bound a single iron atom in its ferrous (Fe2+) state **(Fig. 5-1)**.





* The iron atom has six coordination bonds, four to nitrogen atoms that are part of the flat **porphyrin ring** system and two perpendicular to the porphyrin.
* Iron in the Fe2+ state binds oxygen reversibly; in the Fe3+ state it does not bind oxygen.
* One of these two coordination bonds is occupied by a side chain nitrogen of a His residue.
* The other is the binding site for molecular oxygen (O2) **(Fig. 5–2)**.





* Heme is found in many oxygen-transporting proteins.
* When oxygen binds, the electronic properties of heme iron change; this accounts for the change in color from the dark purple of oxygen-depleted venous blood to the bright red of oxygen-rich arterial blood.
* Some small molecules, such as carbon monoxide (CO) and nitric oxide (NO), coordinate to heme iron with greater affinity than does O2. When a molecule of CO is bound to heme, O2 is excluded, which is why CO is highly toxic to aerobic organisms.

**Myoglobin Has a Single Binding Site for Oxygen**

* It is a simple oxygen-binding protein found in almost all mammals, primarily in muscle tissue.
* As a transport protein, it facilitates oxygen diffusion in muscle.
* It is a single polypeptide of 153 amino acid residues with one molecule of heme.
* About 78 % of the amino acid residues in the protein are found in  helices.
* The polypeptide is made up of eight -helical segments connected by bends **(Fig. 5-3)**.





**Protein-Ligand Interactions Can Be Described Quantitatively**

* The function of myoglobin depends on the protein’s ability not only to bind oxygen but also to release it when and where it is needed.
* Function in biochemistry revolves around a reversible protein-ligand interaction of this type.
* A quantitative description of this interaction is a central part of many biochemical investigations.
* The reversible binding of a protein (P) to a ligand (L) can be described by a simple equilibrium expression:

P + L PL

* The reaction is characterized by an equilibrium constant, *K*a,

*K*a = [PL] / [P][L]

* ***K*a** is an **association constant**.
* *K*a provides a measure of the affinity of the ligand for the protein.
* A higher value of *K*a corresponds to a higher affinity of the ligand for the protein.
* It is more common to consider the **dissociation constant**, ***K*d**,

*K*d = 1 / *K*a

* *K*d is the equilibrium constant for the release of ligand.
* In practice, *K*d is used much more often than *K*a to express the affinity of a protein for a ligand.
* A lower value of *K*d corresponds to a higher affinity of ligand for the protein.

**Protein Structure Affects How Ligands Bind**

* The specificity of heme is different for its various ligands.
* CO binds about 40 times better than O2.

**Hemoglobin Transports Oxygen in Blood**

* Oxygen is bound and transported by hemoglobin in erythrocytes (red blood cells).
* Erythrocytes are destined to survive for only about 120 days.

**Hemoglobin Subunits Are Structurally Similar to Myoglobin**

* It is a tetrameric protein containing 4 heme prosthetic groups (4 O2-binding sites)
* one associated with each polypeptide chain **(Fig. 5-10)**.





* It contains two  chains (141 residues each) and two  chains (146 residues each).
* The three-dimensional structures of the two types of subunits are very similar.
* Their structures are very similar to myoglobin.

**Hemoglobin Undergoes a Structural Change on Binding Oxygen**

* Interactions among the subunits cause conformational changes
* alter the affinity of the protein for oxygen.
* Hemoglobin has 2 major conformations: **R state** and **T state** (relaxed and tense) **(Fig. 5-10)**.
* Although oxygen binds to hemoglobin in either state, it has a significantly higher affinity in the R state.
* oxygen binding stabilizes the R state.
* When oxygen is absent, the T state is more stable **(deoxyhemoglobin)**.
* The binding of O2 to a subunit in the T state triggers a change in conformation to the R state.

**Hemoglobin Binds Oxygen Cooperatively**

* Hemoglobin must bind oxygen in the lungs and release oxygen in the tissues.
* O2 binding to individual subunits of hemoglobin can alter the affinity for O2 in adjacent subunits.
* The first molecule of O2 binds to a subunit in the T state.
* Its binding leads to conformational changes that are communicated to adjacent subunits, making it easier for additional molecules of O2 to bind.
* T R transition occurs in the second subunit once O2 is bound to the first subunit.
* The last (fourth) O2 molecule binds to a heme in a subunit that is in the R state. It binds with much higher affinity than the first molecule.
* Binding of a ligand to one site affects the binding properties of another site on the same protein.
* Cooperative binding of a ligand to a multimeric protein is a form of allosteric binding.
* Hemoglobin is called an **allosteric protein**.

**Hemoglobin Also Transports H+ and CO2**

* In addition to carrying all the O2 required by cells from the lungs to the tissues
* hemoglobin carries two end products of cellular respiration H+ and CO2 from the tissues to the lungs and the kidneys, where they are excreted.
* The CO2, produced by oxidation of organic fuels in mitochondria, is hydrated to form bicarbonate:

CO2 + H2O H+ + HCO3-

* This reaction is catalyzed by carbonic anhydrase, an enzyme particularly abundant in erythrocytes.
* The hydration of CO2 results in an increase in the H+ concentration (a decrease in pH) in the tissues.
* The binding of O2 by hemoglobin is influenced by pH and CO2 concentration.
* Hemoglobin transports about 40% of the total H+ and 15% to 20% of the CO2 formed in the tissues to the lungs and kidneys.
* The binding of H+ and CO2 is inversely related to the binding of oxygen.
* At the relatively low pH and high CO2 concentration of peripheral tissues,
* the affinity of hemoglobin for oxygen decreases as H+ and CO2 are bound, and O2 is released to the tissues.
* Conversely, in the capillaries of the lung, as CO2 is excreted and the blood pH consequently rises,
* the affinity of hemoglobin for oxygen increases and the protein binds more O2 for transport to the peripheral tissues.
* This effect of pH and CO2 concentration on the binding and release of oxygen by hemoglobin is called the **Bohr effect**.
* When the O2 concentration is high, as in the lungs,
* hemoglobin binds O2 and releases protons.
* When the O2 concentration is low, as in the peripheral tissues,
* H+ is bound and O2 is released.
* O2 and H+ are not bound at the same sites in hemoglobin.
* O2 binds to the iron atoms of the hemes, whereas H+ binds to any of several amino acid residues in the protein.
* Hemoglobin also binds CO2, inversely related to the binding of oxygen.
* CO2 binds as a carbamate group to the -amino group at the amino-terminal end of each globin chain, forming carbaminohemoglobin.



* This reaction produces H+, contributing to the Bohr effect.

**Oxygen Binding to Hemoglobin Is Regulated by 2,3-Bisphosphoglycerate**

* 2,3-Bisphosphoglycerate (BPG) is present in relatively high concentrations in erythrocytes.



* BPG reduces the affinity of hemoglobin for O2
* there is an inverse relationship between the binding of O2 and the binding of BPG.
* BPG binds at a site distant from the O2-binding site and regulates the O2-binding affinity of hemoglobin.

**Sickle-Cell Anemia Is a Molecular Disease of Hemoglobin**

* Almost 500 genetic variants of hemoglobin are known to occur in the human population.
* Most variations consist of differences in a single amino acid residue.