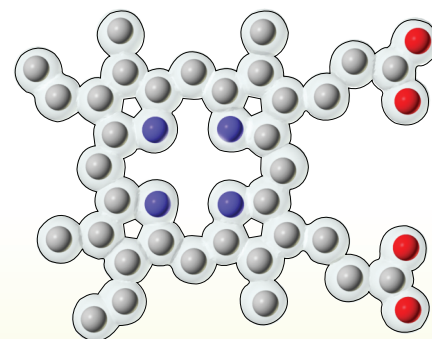




Teacher Notes

Overview of the β -Globin Folding Kit®

Hemoglobin is the classic protein used to introduce quaternary protein structure, both because it is an important protein in the transport of oxygen throughout the body, but also because a single point mutation results in a prominent disease, sickle cell anemia. β -thalassemias are a group of diseases resulting from the underproduction, or total lack of production, of β -globin.

The β -Globin Folding Kit® is best used after introducing your students to protein folding and protein structure with 3D Molecular Designs Amino Acid Starter Kit®. The β -Globin Folding Kit® can also be used as a stand-alone kit. The kit reinforces the following concepts that are covered in the Amino Acid Starter Kit®.

As proteins are synthesized in an aqueous solution, chemical properties of the amino acid side chains determine how proteins fold into 3-dimensional, functional molecular machines.

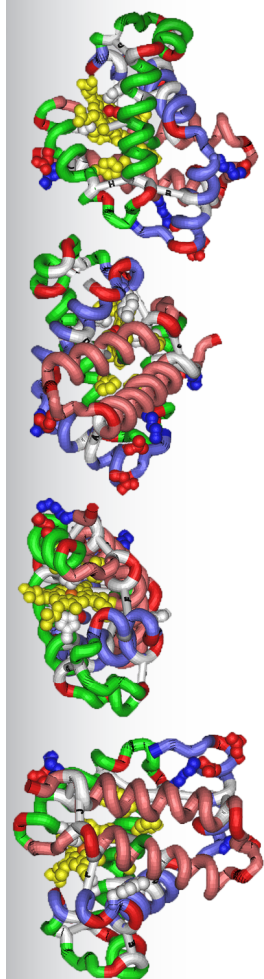
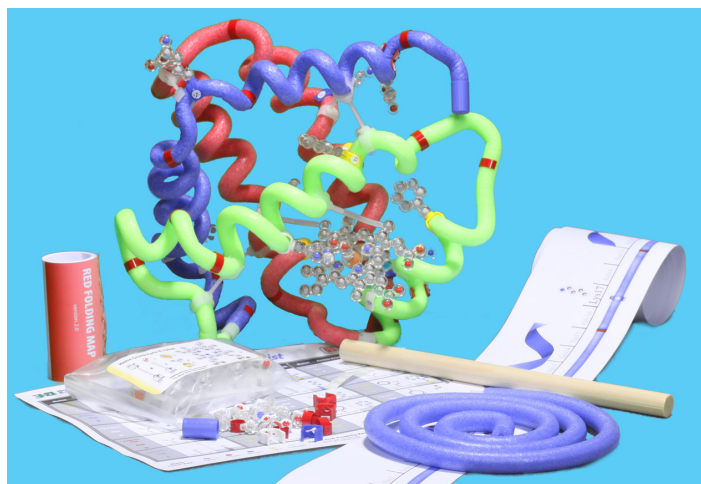
The chemical properties are:

- Hydrophobic amino acids will most often be inside proteins.
- Hydrophilic amino acids will most often be on the surface of proteins.
- Charged amino acids form salt bridges on the surface of proteins. Salt bridges form between oppositely charged amino acids.
- Cysteine residues may form disulfide bonds.

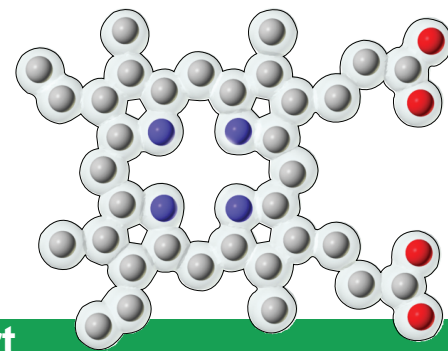
The β -Globin Folding Kit® expands on the Amino Acid Starter Kit® to address additional concepts relating to protein structure and function:

- Some, but not all, proteins exhibit quaternary structure.
- Some proteins contain non-protein cofactors.
- Single bases substitutions in DNA can produce a change in a single amino acid in a protein; sometimes these changes lead to disease.
- Other mutations, such as frameshift mutations or mutations in the promoter region of a gene, can prevent the production of the protein, leading to serious health problems.

See page 1 of the Description, Set-Up & Contents for set-up instructions.



β-Globin Folding Kit®



Role of Hemoglobin in Oxygen Transport

Oxygen is not very soluble in water; hemoglobin is a protein that is highly specialized to transport oxygen. Hemoglobin consists of two α-globin and two β-globin subunits. Each of the four subunits contains a non-protein cofactor called a heme group. The heme group consists of a porphyrin ring containing a single iron atom. Each of the four iron atoms in a hemoglobin molecule binds a single oxygen molecule. Hemoglobin is slow to pick up the first oxygen molecule, but once the initial oxygen molecule binds to hemoglobin, there is a conformational change in the protein. This change allows each of the remaining heme groups to take up additional oxygen molecules quickly. Similarly, hemoglobin binds the oxygen tightly unless oxygen levels are low in surrounding tissues. Once one oxygen molecule is released, the resulting conformational change in hemoglobin causes the remaining three oxygen molecules to be released in rapid succession. The dissociation curve for oxygen bound to hemoglobin (Figure 1) shows this cooperativity among the hemoglobin subunits. At the oxygen concentration within the lungs, hemoglobin becomes fully loaded with oxygen. At the lower oxygen concentration in the tissues, oxygen is released from hemoglobin.

Oxyhemoglobin secretion (%)

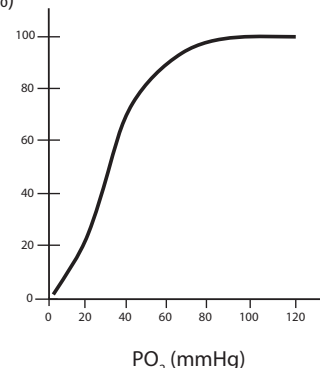
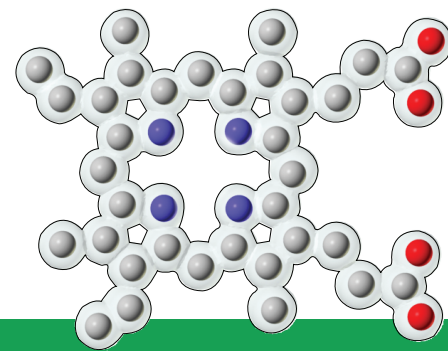


Figure 1: oxygen dissociation curve for hemoglobin. The PO₂ in the lungs is around 100 mm, and the PO₂ in the tissues is around 40 mm.

Role of Hemoglobin in Carbon Dioxide Transport

Once cells utilize oxygen in cellular respiration, carbon dioxide is produced. Only a small amount of carbon dioxide (about 7%) dissolves in blood plasma. Another 23% binds to various amino acids in hemoglobin. The remaining 70% of carbon dioxide combines with water to form carbonic acid (H₂CO₃), which then dissociates into hydrogen ions (H⁺) and bicarbonate ions (HCO₃⁻). The hydrogen ions bind to hemoglobin, which serves as a buffer. The bicarbonate ions can thus be transported through the blood without impacting the pH of the blood.

When hydrogen ions bind to hemoglobin, there is a conformational change in the protein. This leads to a shift in the oxygen dissociation curve to the right, lowering the percent of hemoglobin that is saturated with oxygen at a given concentration of oxygen. This is known as the Bohr effect. Thus, in rapidly metabolizing tissues, the carbon dioxide produced causes a localized drop in pH. This results in hemoglobin releasing more oxygen to these tissues.



β-Globin Folding Kit®

Sickle Cell Anemia - A Balancing Act

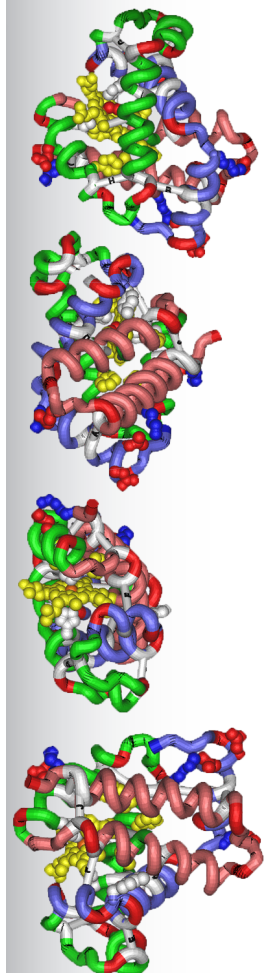
Sickle cell anemia results from a point mutation in codon 6 of hemoglobin. This mutation codes for valine, a hydrophobic residue. The normal amino acid at this position is a glutamic acid, a negatively charged amino acid located on the surface of the protein. In both normal (HbB) and sickle cell (HbS) hemoglobin, there is an uncharged patch of amino acids that is exposed to the surface in deoxyhemoglobin. The valine in HbS is attracted to this hydrophobic patch, leading to the aggregation of hemoglobin in low oxygen concentrations found in the tissues. When the red blood cells return to the lungs, the high oxygen concentration allows hemoglobin to bind oxygen and change its conformation so the hydrophobic patches are again shielded. Thus, in the lungs, the sickle cell hemoglobin is not clumped.

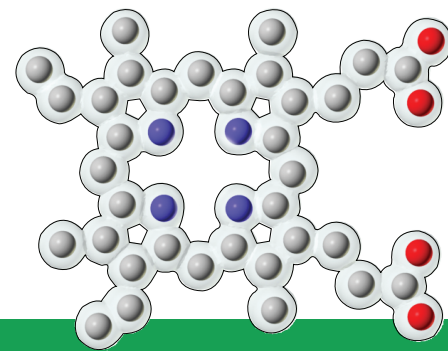
Clumping of hemoglobin in persons with sickle cell disease leads to distorted red blood cells, some of which are sickle shaped (hence the name of the disease). These distorted cells have difficulty traversing the narrow passages of the capillaries and tend to break easily and block the blood vessels. The decline in the number of red blood cells results in anemia, and the clogging of capillaries leads to excruciating pain and poor circulation. Persons who are carriers (with one normal and one mutated beta globin gene) make enough normal hemoglobin that they don't have any symptoms, but they can pass the trait on to their offspring. Heterozygotes are said to have sickle cell trait.

The sickle cell mutation is found at a very high frequency in certain regions of Africa, the Mediterranean, the Middle East and India. In some tribes in Africa, the frequency of homozygotes is as high as 4%, and heterozygotes comprise 32% of the population. The reason for this high frequency of such a deleterious allele is that it confers a selective advantage for carriers in malaria-prone regions. People with sickle cell trait are more resistant to malaria, though the mechanism by which the mutation confers resistance is not clearly understood.

In the United States, sickle cell trait is found predominantly in people of African descent. Within the African American population, only one in 500 is homozygous for sickle cell (0.2%), and about 8% are carriers for the trait. Since the mosquito that transmits malaria is not present in the U.S., there is no selective advantage for sickle cell trait.

The sickle cell mutation results from changing an A to a T in codon 6 of the β -globin gene. This mutation disrupts a restriction enzyme recognition site. Screening newborns for the presence of the mutation simply requires PCR amplification of the region of the gene containing codon 6. Following digestion with the restriction enzyme, samples are run on gel electrophoresis. Mutants cannot be cleaved at codon 6, so have a longer fragment, rather than the two shorter fragments of normal genes. Samples containing both the long fragment and the two shorter fragments are from persons who are heterozygous for the mutation.





β-Globin Folding Kit®

Beta Thalassemias

A number of mutations in the β-globin gene result in little or no production of β-globin, and the associated disease is beta thalassemia. There are two forms of thalassemia, depending on the level of β-globin produced. The most serious is beta zero thalassemia, in which no β-globin is produced. Other mutations lead to a low level of β-globin production; these are classified as beta plus thalassemias. Mutations leading to thalassemia range from frameshift and chain termination mutations to mutations in promoter and polyadenylation sites. Traditional treatment consists of monthly blood transfusions, though recent focus has been on stimulating the production of fetal hemoglobin, which is usually turned off shortly after birth.

Teaching Points

The β-Globin Folding Kit® allows three teams of students to each fold a portion of the protein, then assemble the three sections to form the beta globin gene. Students use a folding map to correctly position key amino acids (primary structure) and alpha helices (secondary structure). They then fold the protein into its tertiary structure, adding connectors to stabilize the structure.

Key amino acids include:

- Histidines involved in coordinating the heme group
- Three salt bridges formed on the surface of the protein
- Hydrophobic amino acids on the interior of the protein
- Glutamic acid 6, the amino acid that changes to valine in sickle cell anemia

You may wish to note the following in presenting this material:

- The secondary structure of hemoglobin consists of only alpha helices.
- Hemoglobin has quaternary structure, as it consists of two α-globin subunits and two β-globin subunits. Note that the use of α and β here does not refer to the secondary structure. Researchers typically name the subunits of a protein using Greek letters

Note: although the β-globin gene consists of three sections (exons), separated by introns, the toober sections do NOT correspond with the exons of the gene. Instead, the protein is divided roughly into three pieces for ease of folding. The kit is designed so that an entire alpha helix remains in one segment of the folding kit, so the three toobers vary in length.

The support posts used in this modeling kit are provided for structural stability and do not represent hydrogen bonds. Students who have used the Amino Acid Starter Kit® should be alerted to this alternate representation for a component used in both kits.

Reference

Nelson, Nathan. "Sickle Cell Anemia and Malarial Resistance." Department of Physics, The Ohio State University, Columbus, OH 43210. Web.
www.physics.ohio-state.edu/~wilkins/writing/Samples/shortmed/nelson/sickle.html.