Iron Use and Storage in the Body: Ferritin and Molecular Representations

Iron in Biology: Study of the Iron Content in Ferritin, The Iron-Storage Protein.

Authors: Rachel Casiday and Regina Frey
Department of Chemistry, Washington University
St. Louis, MO 63130

For information or comments on this tutorial, please contact R. Frey at gfrey@wuchem.wustl.edu.

Key Concepts:

- Importance of Iron in the Body
- Iron-storage Protein and Control of the Amount of Iron in the Body
- Graphical Molecular Representations
    - Importance of Molecular Visualization
    - Different Types of Representations and What Information They Give
- Structure of Ferritin
    - Amino Acids
    - Peptide Subunits
    - 24-Subunit Structure
- Removal of Fe from Ferritin
    - Crystal-lattice Mineral Structure
    - Channels
    - Polar vs. Nonpolar

Iron in the Body

The Body as a Chemical System: Chemical Elements that Make Up the Body

What are our bodies made of, and how do they work? These questions are fundamental to the study of medicine and to many chemists, biologists, and engineers. We know that our bodies are matter, and thus must be composed of atoms that have been specially arranged to produce the molecules and larger structures that sustain our lives. We know that the properties of an atom (e.g., size, electronegativity, number of valence electrons) determine how that atom will interact with other atoms; furthermore, the properties and reactions of molecules depend on the properties and interactions of the atoms in the molecules. Hence, to study the human body as a complex organization of molecules that undergoes a wide array of interrelated chemical reactions, we should begin by asking one of the most basic questions about any system of molecules: What sort of atoms does the system contain? The complete answer to this question will have two main parts: 1) what elements do the atoms represent, and 2) what interactions are found between the atoms (because the properties of a given atom can be altered by interactions with other atoms).

Hence, our discussion of the human body as a chemical system begins by answering the question, "What type of atoms does the body contain?" Of the more than 100 chemical elements known to scientists today, only a relatively small number of these elements are found in the human body. In fact, only 24 different elements are thought to be essential to the human body. (Other elements, such as mercury, are sometimes found in the body, but do not perform any known essential or beneficial function.) The largest elemental components of the body, by mass, are oxygen (65%), carbon (18%), hydrogen (10%), and nitrogen (3%). The other elements in the body, such as calcium, phosphorus, iron, and copper, are known to physiologists as **mineral elements** and **trace elements**. Although these elements make up a much smaller percentage of the mass of the body than oxygen, carbon, hydrogen, and nitrogen, the mineral and trace elements are vital to the body's proper functioning. These elements must be present in the body in the proper amounts, and they must be available to react with other elements to form critical molecules and participate in important chemical reactions. In this tutorial, we will describe the importance of one essential trace element in the body, **iron**. Although iron comprises only 0.008% of the body's mass (approximately 6 g for a 160-lb (75-kg) adult male), we cannot live without this important element in our bodies.

The Crucial Role of Iron in the Body

You learned from the "Hemoglobin and the Heme Group: Metal Complexes in the Blood" tutorial that iron is necessary for oxygen transport in the blood. Recall that iron is the central atom of the **heme** group, a metal complex that binds molecular oxygen (O_2) in the lungs and carries it to all of the other cells in the body (e.g., the muscles) that need oxygen to perform their activities. Without iron in the heme group, there would be no site for the oxygen to bind, and thus no oxygen would be delivered to the cells (which would result in the cells dying). In addition to **hemoglobin,** other important proteins in the body that contain heme groups (and therefore contain iron) include **myoglobin,** which takes oxygen from hemoglobin and allows the oxygen to diffuse throughout the muscle cells, and the **cytochromes,** which supply the body with its energy currency. (You will learn more about cytochromes in the Chem 152 tutorial, "Energy for the Body: Oxidative Phosphorylation".) Other proteins, such as those needed for DNA synthesis and cell division, also rely on iron. Furthermore, iron is used to help produce the connective tissues in our body, some of the neurotransmitters in our brain, and to maintain the immune system. Hence, iron is necessary for allowing the cells that need oxygen to obtain O_2, for supplying the body with a reliable source of energy, and for maintaining several other important structures and systems in the body.

Iron Disorders

Because iron plays such a crucial role in the body, it is important for us to maintain an adequate supply of iron to form hemoglobin and the other molecules in the body that depend on iron to function properly. Yet, our bodies continually lose iron (in small amounts) through everyday process such as urination, defecation, sweating, and sloughing off skin cells. Bleeding, particularly menstrual bleeding in women, contributes to further loss of iron from the body. To compensate for these losses and to maintain an adequate supply of iron, we should consume approximately 18 mg of iron daily. Certain conditions, including heavy bleeding and pregnancy, further increase the requirement for iron consumption. Good dietary sources of iron include red meat, liver, egg yolk, beans, nuts, and fortified cereals.

When the body's supply of available iron is too low, a condition known as **iron deficiency** results. People with iron deficiency cannot produce an adequate amount of hemoglobin to meet their body's oxygen-transport needs. When the deficiency becomes severe (so that there are too few circulating red blood cells or the hemoglobin content of these cells is very low), the condition is diagnosed as **iron-deficiency anemia**. The most common symptoms of iron-deficiency anemia are tiredness and weakness (due to the inadequate oxygen supply to the body's cells) and paleness in the hands and eyelids (due to the decreased levels of oxygenated hemoglobin, which is red-colored). Iron-deficiency anemia can be treated with iron supplements, and by adopting strategies to improve the body's absorption of the iron in the supplements (e.g., taking iron with vitamin C, which enhances absorption, but not with milk, which limits absorption).

It is also possible to have too much iron deposited in the body tissues. This condition is known as **iron overload.** If the iron overload becomes severe (usually when the total amount of iron in the body exceeds 15 g), the condition is diagnosed as **hemochromatosis.** Hemochromatosis can result in serious damage to the body's tissues, including cirrhosis of the liver, heart failure, diabetes, abdominal pain, and arthritis. A recessive genetic mutation can put some people (e.g., those of Irish or Celtic descent) at a higher risk for developing hemochromatosis. Treatment for hemochromatosis consists of removing blood from the patient to decrease the amount of iron in the body, and treating the symptoms (e.g., liver disease and diabetes).

Ferritin: The Iron-Storage Protein

How does the body regulate the amount of iron? Fortunately, most of us are able to maintain appropriate levels of available iron in the body (enough available iron to ensure an adequate supply of hemoglobin, but not so much as to produce toxic effects), even if our iron consumption does not always exactly match the body's iron loss. **Ferritin** (Figure 1) is the key to this important control of the amount of iron available to the body. **Ferritin is a protein that stores iron and releases it in a controlled fashion.** Hence, the body has a "buffer" against iron deficiency (if the blood has too little iron, ferritin can release more) and, to a lesser extent, iron overload (if the blood and tissues of the body have too much iron, ferritin can help to store the excess iron).

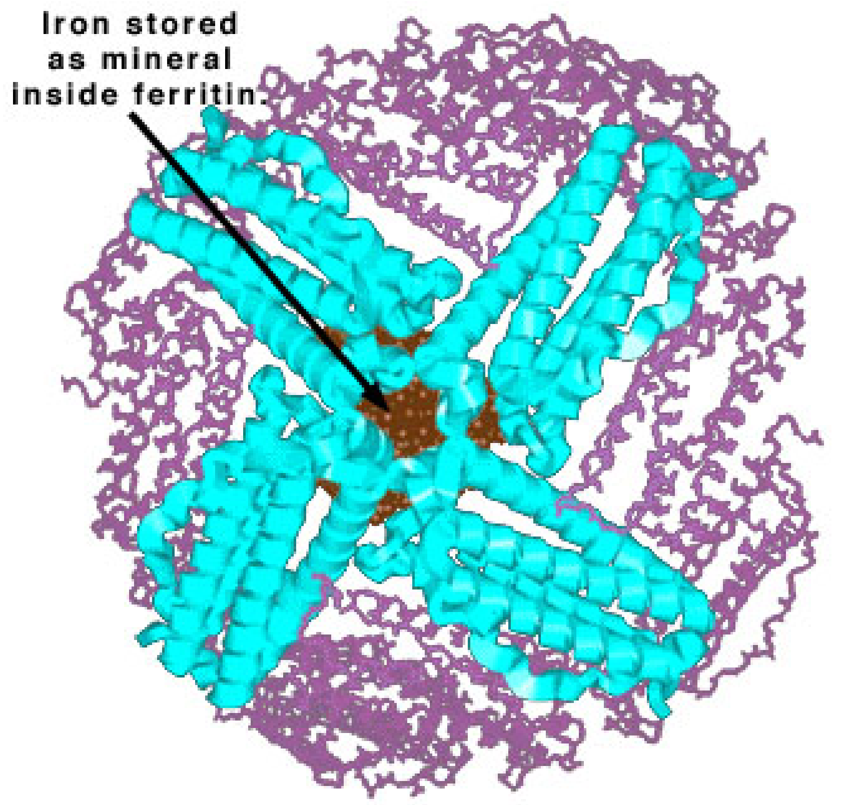


Figure 1

This is a three-dimensional representation showing ferritin, the iron-storage protein in the body. Ferritin has a spherical shape, and iron (brown) is stored as a mineral inside the sphere.

Note: This same molecule is shown in another type of representation in Figure 9, below.

Note: The structure of this protein was determined using x-ray crystallography, the structure of the iron core is based on a simplified model compound, and the image was rendered using the Insight II molecular-modeling system from Molecular Simulations, Inc. (see References).

How does ferritin store iron? Ferritin has the shape of a hollow sphere. Inside the sphere, iron is stored in the Fe(III) oxidation state. It is incorporated in the mineral ferrihydrite, $[FeO(OH)]_8[FeO(H_2PO_4)]$, which is attached to the inner wall of the sphere. To release iron when the body needs it, the iron must be changed from the Fe(III) to the Fe(II) oxidation state. Then, the iron leaves through channels in the spherical structure. Thus, the structure of ferritin is extremely important for the protein's ability to store and release iron in a controlled fashion. In order to understand how ferritin helps to maintain the correct amount of available iron in the body, we must study the protein's structure in detail.

Molecular Representations

Proteins (e.g., ferritin) make up a class of very large molecules whose three-dimensional structure allows them to play important roles in biological systems. To understand how ferritin (or any of the many molecules that you will encounter in this course and throughout your experience in the sciences) performs its job, we must be able to visualize the three-dimensional structure of the molecule, and understand the relationship between the structural features and the function of the molecule. Furthermore, we must be able to communicate this image of the three-dimensional structure to others who want to learn about the molecule's structure and function.

There are several strategies that we could use to visualize the ferritin protein's three-dimensional structure, and communicate this image to others. We could make three-dimensional models to depict the structure of ferritin, but these models would be inconvenient for distributing the information widely. The most common formats for distributing information today- in books and on computer screens- necessitate that the image be displayed in two dimensions. Of course, there are many difficulties involved in converting all of the important structural information about a molecule into an easily understandable two-dimensional representation. No two-dimensional representation can show a three-dimensional structure in its entirety. Hence, a variety of molecular representation formats have been developed; each of these representations is designed to show a particular aspect of a molecule's structure. Thus, to illustrate a specific point about a molecule's structure, the type of representation must be chosen carefully. To provide a comprehensive view of a molecule's structure, multiple representations are used. In this tutorial, the 2D-ChemDraw, stick, CPK, and ribbon representations are used to examine the three-dimensional structure of ferritin. These four types of representations are described in the blue box, below.

Types of Representations Used in this Tutorial

Graphical computer modeling has greatly improved our ability to represent three-dimensional structures. One of the goals of graphical computer modeling is to create the computer-generated image such that the image seems three-dimensional. By replicating the effect of light on three-dimensional objects, computers can give the impression of depth to simulate the three-dimensional aspect. The ability of interactive molecular viewing (*e.g.*, using the Chime program) has enhanced our understanding of molecular structure even more, especially in the biochemical area. By interactively rotating the molecules, a clear picture of the three-dimensional structure emerges. In addition, this increases our chemical intuition by looking at two-dimensional images and visualizing the three-dimensional structure in our brains.

This tutorial uses different types of structural representations (Figure 2, Table 1), such as **2D-ChemDraw, stick, CPK, and ribbon,** to illustrate the structure of ferritin. PDB files are also available for viewing the molecules interactively. By using these various representations to study the structure of ferritin, you will become familiar with the different types of information given by each type of molecular representation, as well as the strengths and limitations of each representation.

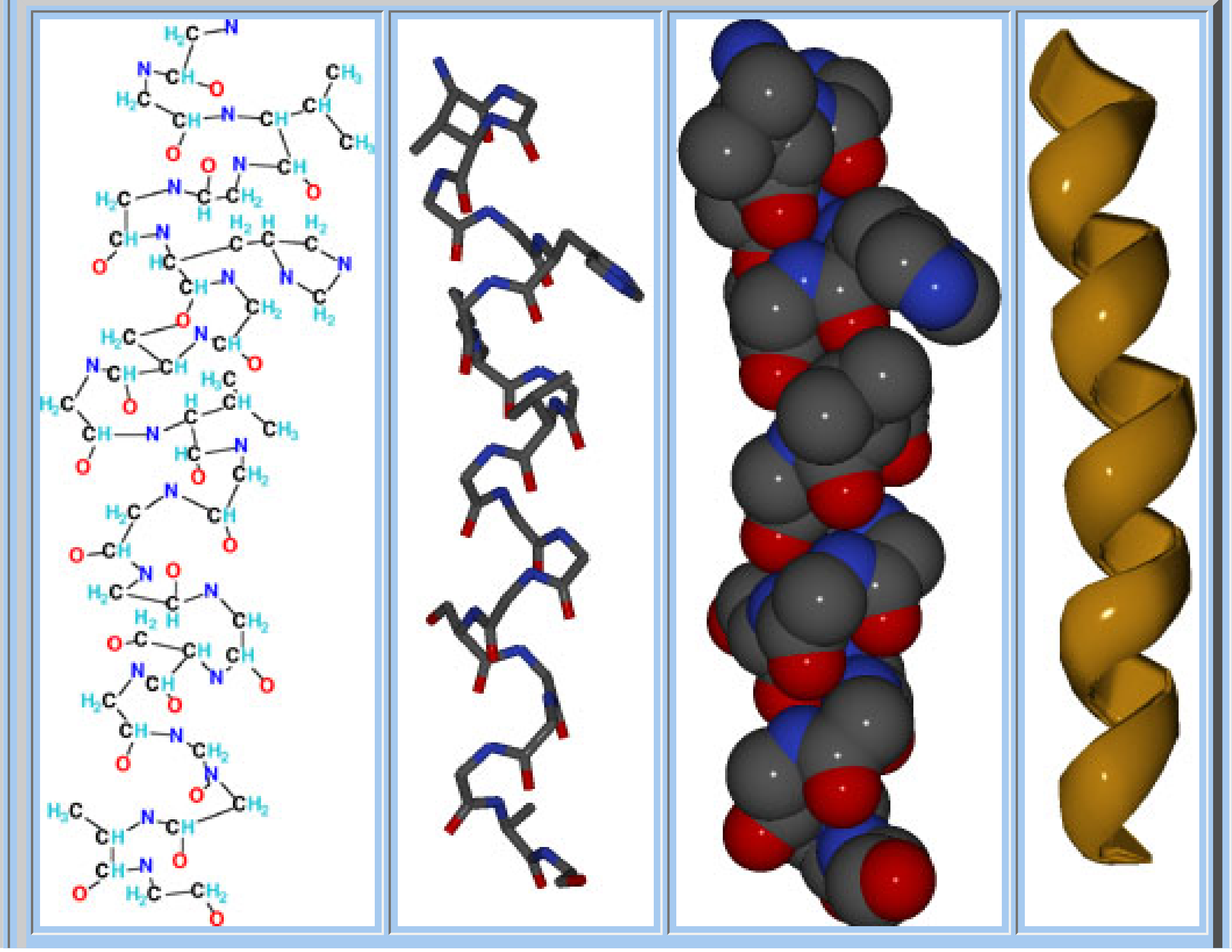


Figure 2

This figure shows an alpha-helix (from the "Hemoglobin and the Heme Group: Metal Complexes in the Blood" tutorial) in four different types of computer-generated molecular representations. The representations are, from left to right, 2D-ChemDraw, stick, CPK, and ribbon. Although all four representations depict the same molecule, they look very different and offer different information about the molecule's structure (see Table 1).

Note: In the 2D-ChemDraw, stick, and CPK representations, carbon atoms are shown in gray (black), nitrogen atoms are shown in blue, and oxygen atoms are shown in red. In this figure, hydrogen atoms (light blue) are shown in the 2D-ChemDraw representation but hydrogen atoms are not shown in the other representations.

By examining the four representations in Figure 2, you can see that each picture tells us something different about the structure of the molecule. For instance, if we wanted to know how the atoms in an alpha helix are connected to one another, we would use the ChemDraw or stick representation. To see the relative sizes of the atoms in an alpha helix, we would use the CPK representation. Descriptions of the four types of representations, their major strengths, and their drawbacks are given in Table 1, below.

Type of Representation	Description of Representation	Information Depicted Particularly Well by Representation	Drawbacks of Representation
2D-ChemDraw	Shows labeled atoms and bonds connecting atoms in a flat representation.	Connectivities between atoms in small molecules; can also include lone pairs (i.e., Lewis-dot structures).	Difficult to interpret for larger molecules; does not give a good idea of the molecule's three-dimensional structure.

Stick	Shows the bonds between atoms as three-dimensional "sticks", often color-coded to show atom type.	Connectivities between atoms; some idea of the molecule's three-dimensional shape.	Does not depict the size (volume) of the molecule or its constituent atoms, and hence gives a limited view of the molecule's three-dimensional shape.
CPK	Shows atoms as three-dimensional spheres whose radii are scaled to the atoms' van der Waals radii.	Relative volumes of the molecule's components. Usually a good indicator of the molecule's three-dimensional shape and size.	Difficult to view all atoms in the molecule, and to determine how atoms are connected to one another.
Ribbon	Shows molecules with a "backbone" (e.g., polymers, proteins), depicting alpha helices as curled ribbons.	Shows the secondary structure (such as locations of any alpha helices) of a protein.	Used for proteins and other polymers. Does not show individual atoms and other important structural features.

Table 1

This table lists some of the important attributes of the four types of representations pictured in Figure 2 (above).

Questions on Molecular Representations

- Which representation described in the tutorial (see blue box entitled "Types of Representations Used in this Tutorial") would you use to get the most accurate picture of the overall shape and approximate volume of the ferritin protein?

- Which representation described in the tutorial (see blue box entitled "Types of Representations Used in this Tutorial") would you use to see what atoms are connected together in a particular region of the ferritin protein?

Protein Structure

Now we shall use the molecular-modeling tools (representations) described above to zoom in on the ferritin protein and study its structure. We will begin at the smallest level of protein structure, by using 2D-ChemDraw representations to show how atoms are combined to make **amino acids.** Then we will use all four representations to show how amino acids come together to form the protein subunits known as **peptides,** and how the sequence of amino acids determines the shape of the peptide. Finally, we will use the ribbon and CPK representations to show how 24 peptide subunits are combined to make the **hollow-sphere shape** and **channels** in ferritin. The basic structural features of proteins that you will learn about in this tutorial will provide a foundation for understanding the structure and function of any protein. Because proteins are a tremendously important class of biological molecules, you will study many different proteins in these tutorials and in your other coursework and research.

Amino Acids: The Building Blocks of Proteins

All proteins consist of chains of **amino acids.** An amino acid (Figure 3) is a molecule containing a central carbon atom and three special functional groups: a carboxylic acid group (—COOH), an amino group (—NH_2), and variable "side chain" (generically denoted by "R"). (Note: The "—" in "—COOH" and "—NH_2" indicates a bond to another atom in the rest of the molecule.) There are 20 different amino acids that are available to be incorporated into proteins. The side chains of the 20 amino acids have different properties, which in turn give different properties to the amino acids. For instance, side chains may be charged (e.g., glutamate) or electrically neutral (e.g., leucine), bulky (e.g., tryptophan) or consisting only of a hydrogen atom (glycine).

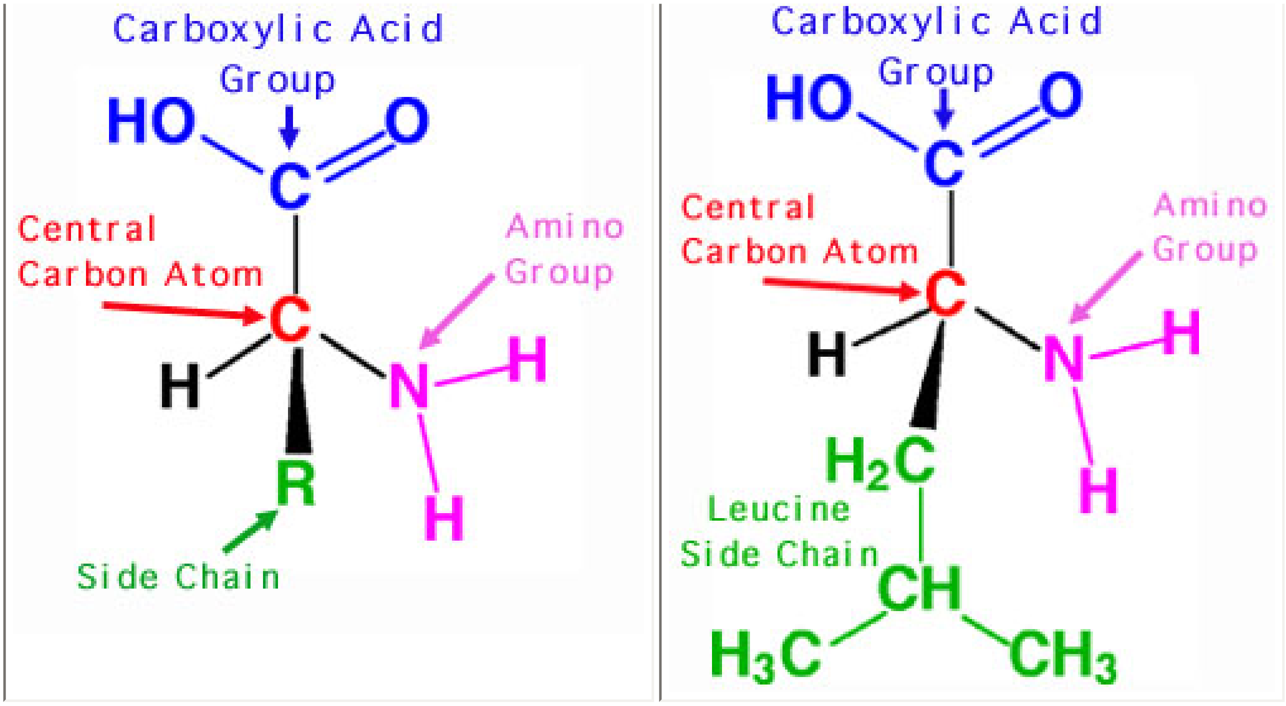


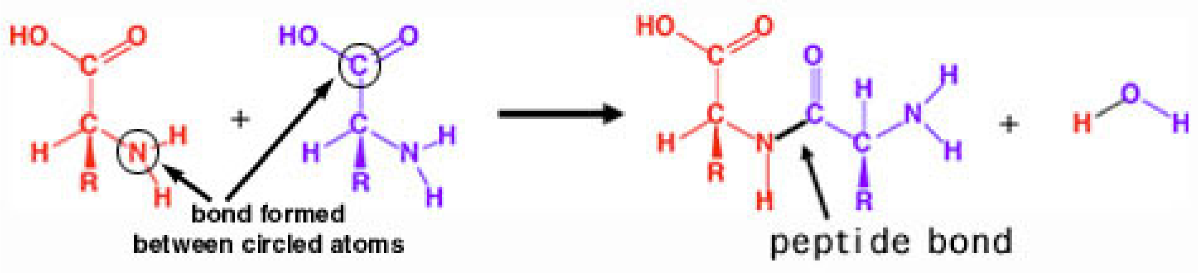
Figure 3

On the left is a two-dimensional ChemDraw model of an amino acid. The carboxylic acid group is shown in blue, the amino group is shown in purple, and the central carbon atom is shown in red. The green "R" represents the side chain, which is different for each amino acid.

On the right is a two-dimensional ChemDraw model of leucine, one of the twenty amino acids available for building proteins. Leucine differs from the other amino acids only in its side chain, shown in green.

Peptides: Protein Subunits

Amino acids are linked together to form chains known as **peptides.** These links are formed by covalent bonds known as **peptide bonds** between the carbon atom of the carboxylic acid group (—CO_2H) of one amino acid and the nitrogen atom of the amino group (—NH_2) of an adjacent amino acid (Equation 1).



(1)

The peptide bonds in the —CONH— units (see Figure 4, below) are central to the **backbone** (see Figure 5, below) of the peptide chain. Figures 4 and 5 show the three amino acid residues leucine, alanine, and glutamate (Leu-Ala-Glu) that are bound together and form a part of the peptide subunit found in ferritin.

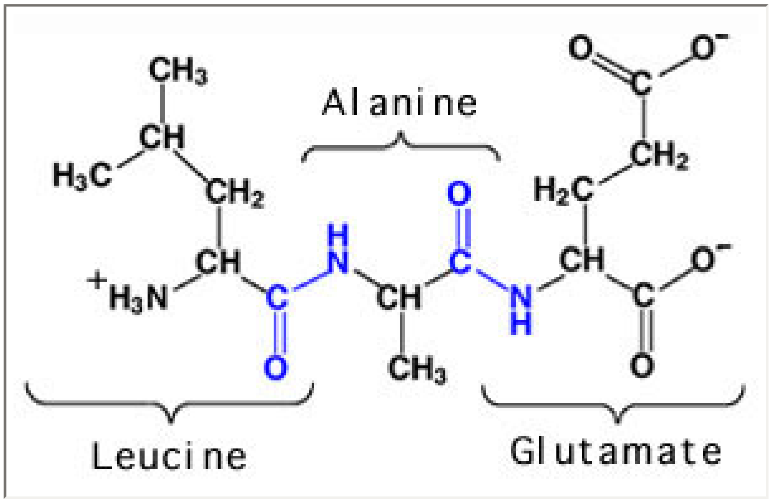


Figure 4

In this figure, the functional groups that form peptide bonds in the amino-acid sequence Leu-Ala-Glu are shown in blue.

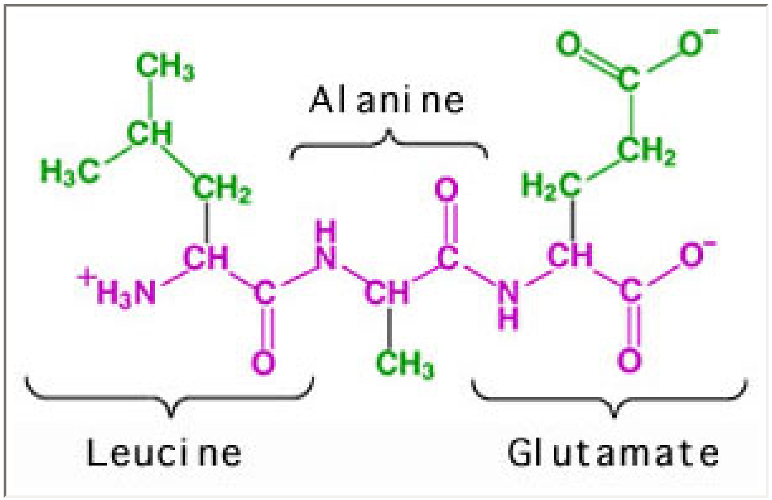


Figure 5

In this figure, the backbone of the amino-acid sequence Leu-Ala-Glu is shown in purple, and the side chains are shown in green.

Peptides may be very long chains of amino acids. There are 184 residues in each peptide subunit in human ferritin. The side chains of amino acids in a peptide can interact with one another, causing the peptide to fold. The shape of the peptide depends on where the peptide is folded, which in turn depends on the sequence of amino acids in the peptide (i.e., the location of side chains whose properties enable them to interact with other side chains). One common example of folding in a peptide is the alpha-helix motif, which is common in many proteins. Recall from the "Hemoglobin and the Heme Group: Metal Complexes in the Blood" tutorial that an alpha helix is formed when there is a regular pattern of side chains that form hydrogen bonds with one another. Figure 6 shows the hydrogen-bonding interactions between amino-acid residues that give rise to the helical structure shown in the ribbon representation.

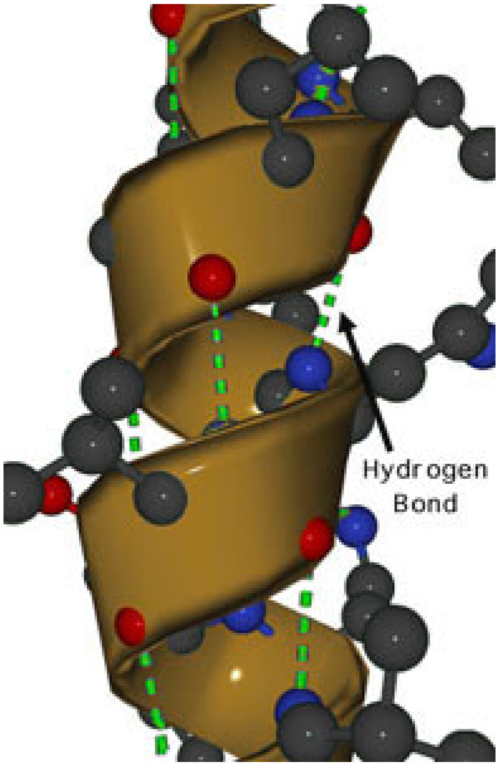


Figure 6

This is a close-up of part of an alpha helix in a peptide chain of ferritin. The helical shape is held by hydrogen bonds (represented by green dotted lines) between the -NH and -CO functional groups in the backbone. In this figure, the ribbon representation (showing only the trace of the backbone) is superimposed on a ball-and-stick representation, in which the non-hydrogen atoms are shown as spheres and the bonds are shown as sticks.

Note: The carbon atoms are gray, the nitrogen atoms are blue, and the oxygen atoms are red in this model. Hydrogen atoms are not shown.

Below are two representations of the peptide subunit in ferritin. The first representation (Figure 7) is a CPK model of the peptide chain. The CPK representation gives an approximate volume of the subunit. Figure 8 shows a ribbon representation of the peptide. The ribbon representation is useful for showing the alpha helices in the peptide.

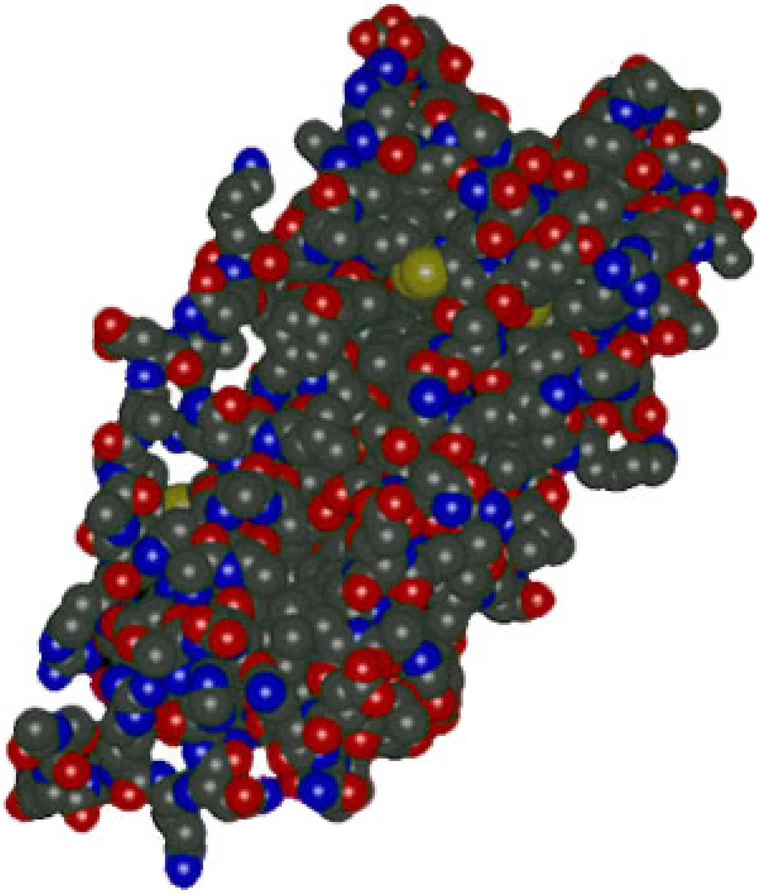


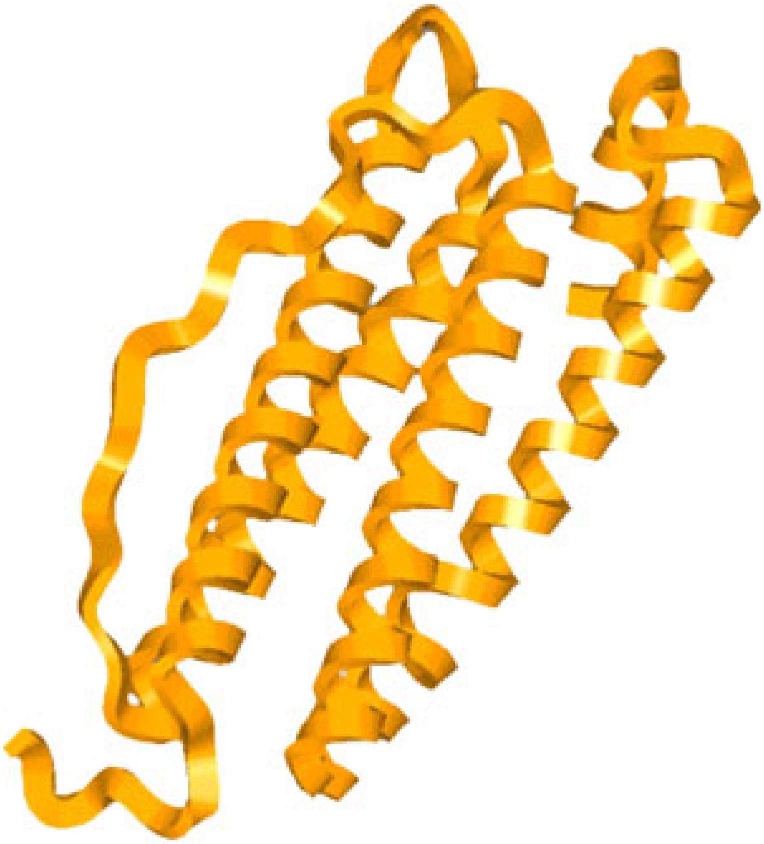
Figure 7

This is a molecular model of a peptide chain in the ferritin protein, shown in the CPK (spacefilled) representation. In this representation all of the heavy (non-hydrogen) atoms are displayed. CPK pictures represent the atoms as spheres, where the radius of the sphere is equal to the van der Waals radius of the atom.

Note: The carbon atoms are gray, the nitrogen atoms are blue, the oxygen atoms are red, and the sulfur atoms are yellow in this CPK model. Hydrogen atoms are not shown in this figure.

Figure 8

Another common representation for proteins and peptides is the ribbon, which traces



the backbone of a protein or peptide. This representation does not include the atoms in the side chains of the residues and is often used to represent the three-dimensional structure. Notice the bundle of helical, or coiled, segments of the backbone.

Ferritin: Assembly of 24 Peptide Subunits

To make the ferritin protein, 24 peptide subunits (Figures 7-8) are assembled into a hollow spherical shell (Figure 9). The sphere that is formed is approximately 80 Angstroms in diameter, and the walls are approximately 10 Angstroms thick. The molecular weight of ferritin (i.e., with all 24 subunits combined) is 474,000 g/mol.

Channels (i.e., small holes through which certain ions or molecules can travel) in the sphere are formed at the intersections of three or four peptide subunits. As we shall see, these channels are critical to ferritin's ability to release iron in a controlled fashion. Two types of channels exist in ferritin. Four-fold channels (such as the one shown in the center of Figure 9) occur at the intersection of four peptide subunits. Three-fold channels (such as those shown on the outskirts of Figure 9) occur at the intersection of three peptide subunits. The two types of channels have different chemical properties, and hence perform different functions, as we shall see later ("Release of Iron" section).

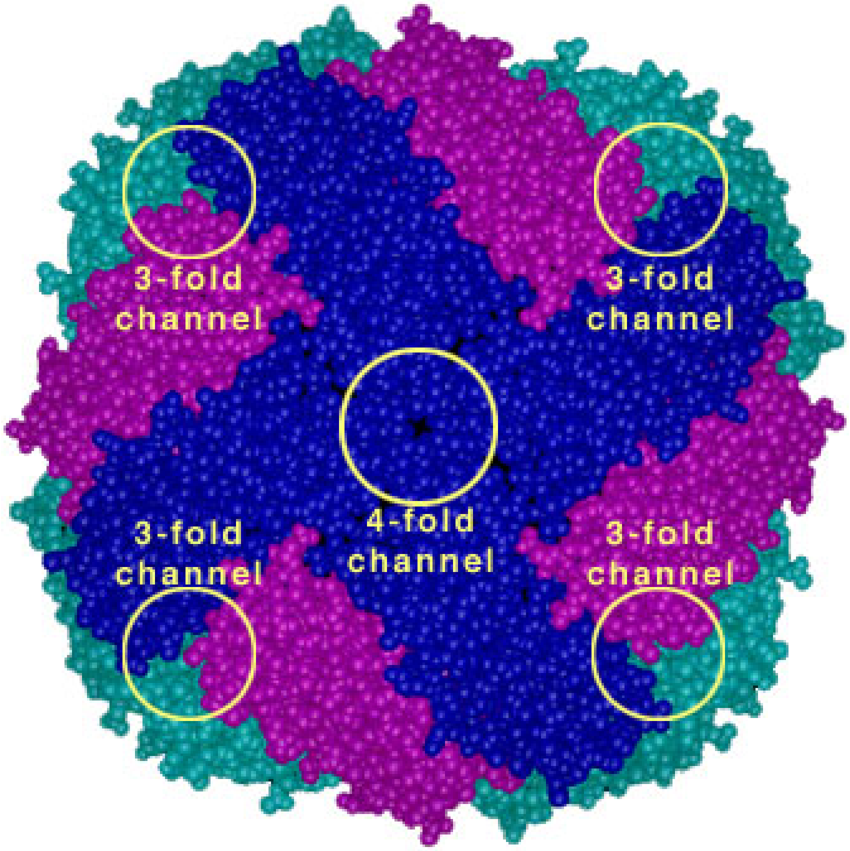


Figure 9

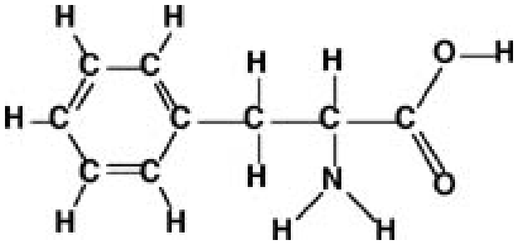
This is a molecular model of ferritin in the CPK representation. CPK pictures represent the atoms as spheres, where the radius of each sphere is equal to the van der Waals radius of the atom. Hence, CPK representations are a good way to show the approximate volume occupied by a molecule.

All of the 24 subunits are identical, but they have been color coded to help illustrate the structure. Dark blue subunits are closest to you, magenta subunits are farther away, and light blue subunits are the farthest away from you. The four (4) subunits colored in dark blue form the walls of a 4-fold channel. The 3-fold channels occur at the intersections of the light blue, dark blue, and magenta-colored subunits. The locations of 3-fold channels are indicated on the figure, but the channels themselves are obscured from this viewing angle.

Note: This Figure shows the same view of Ferritin as Figure 1, but in a different representation. (Figure 1 uses the ribbon representation for the closest peptide subunits, the stick representation for the other subunits, and the CPK representation for the iron core (not shown in Figure 9).) Compare Figures 1 and 9 to see how these representations provide different information about the structure of ferritin.

Questions on Protein Structure

- The two-dimensional (ChemDraw) structure of the amino acid phenylalanine is shown below. Indicate which part of this structure represents the amino acid's **side chain**.



- A segment of a protein is analyzed and found to contain the amino-acid sequence Glu-Leu-Asp. The ChemDraw structures of these three amino acids are in Figures 13 and 15.

  a. Draw a two-dimensional sketch (Lewis structure) of this section, showing the peptide bonds (like Figure 4).
  b. Draw a two-dimensional sketch (Lewis structure) of this section, showing the backbone of the amino-acid sequence (like Figure 5).

Release of Iron from Ferritin

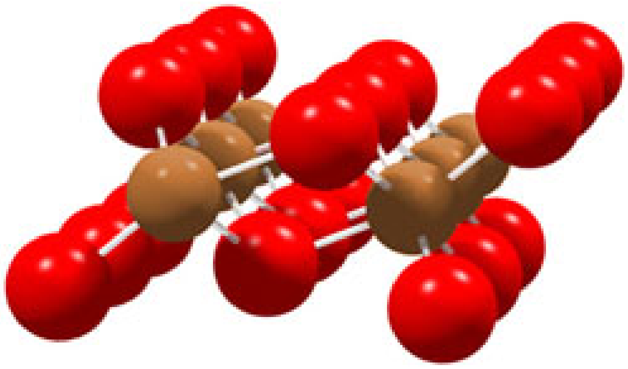
Iron Core

The iron in the ferritin core is stored as Fe(III) in a **crystalline solid** that has the chemical formula $[FeO(OH)]_8[FeO(H_2PO_4)]$. The best model for ferritin's core is the mineral ferrihydrite (Figure 10). A crystalline solid is a three-dimensional structure in which the constituents (i.e., atoms, ions, or molecules) are arranged in a definite repeating pattern. The positions of the constituents' centers are represented by points on a three-dimensional **lattice.** The smallest repeating unit of the lattice is called the **unit cell.** The unit cell for ferrihydrite is shown in Figure 10a. This unit cell is repeated in a specific pattern to form an extended nonmolecular structure (see Figure 10b).

In the mineral ferrihydrite (Figure 10b), every Fe(III) ion is coordinated to six O(II) ions. However, in ferritin, the mineral core has approximately 10% of the Fe(III) ions coordinated to five O(II) ions and 1 phosphate group. Most of the phosphate groups that are coordinated to the iron ions lie on the outside of the crystalline structure, and are used to bind the mineral to the residues on the inside of the ferritin shell (the protein).

As long as a lattice remains intact, the atoms in the lattice are not soluble because they form part of the lattice's continuous structure. (Recall the solvation process for a crystalline solid as described in the "Treating the Public Water Supply: What Is In Your Water, and How Is It Made Safe to Drink?" tutorial.) Thus, in order for iron to be released from ferritin, the mineral lattice must be dissolved (i.e., to allow the iron atoms to break away from the lattice structure). This removal is accomplished by reducing iron from the Fe(III) (ferric) oxidation state to the Fe (II) (ferrous) oxidation state. In the Fe(II) state, iron breaks away from the lattice as the Fe^{2+} ion. The positive charge of the Fe^{2+} ion attracts the electronegative oxygen atoms of water, and so a water "cage" forms around the ion. (In the water cage, six water molecules surround the ion at close range.) Thus, iron becomes soluble as a **hydrated Fe^{2+} ion**, Fe(H$_2$O)$_6^{2+}$, and can be released from the ferritin protein via the channels in the spherical shell.

a.



b.

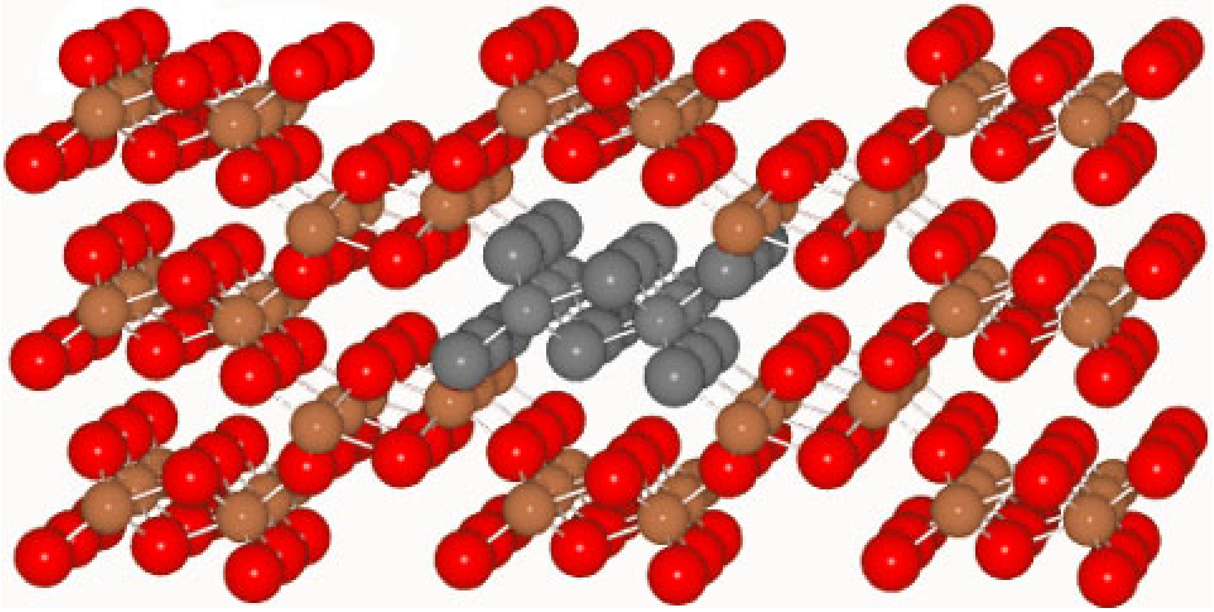


Figure 10

Iron is stored in ferritin as Fe(III) in the mineral $[FeO(OH)]_8[FeO(H_2PO_4)]$. This mineral can be represented by ferrihydrite, FeO(OH) (shown above). Note: the name "ferrihydrite" is used for both $[FeO(OH)]_8[FeO(H_2PO_4)]$ and FeO(OH).

Figure 10a shows the unit cell (the repeating unit) for the ferrihydrite mineral. **Note:** Iron (III) ions are shown in brown, and oxygen (II) ions are shown in red. Hydrogen atoms are not shown in this figure for simplicity.

Figure 10b shows the crystal-lattice structure of ferrihydrite. One unit cell is shown in black and white so that it can be recognized easily.

Note: The crystal structures were drawn using PowderCell for Windows, and the images were rendered using POV-Ray (see References).

Channels in Ferritin

Once the iron is soluble (as $Fe(H_2O)_6^{2+}$), how does it leave the ferritin shell? Recall that ferritin has two types of channels, three-fold and four-fold, in the shell. The soluble Fe^{2+} ion exits through the three-fold channels (Figure 12, below). These channels have a special property, known as **polarity,** that enables the passage of Fe^{2+} ions through these channels.

Polarity refers to significant **differences in electronegativity between adjacent atoms** in a molecule. For instance, the hydroxyl (-OH) functional group consists of an oxygen atom, which is highly electronegative, covalently bound to a hydrogen atom, which is much less electronegative. The highly electronegative oxygen atom draws the (negatively-charged) electrons in the bond to itself more than the less electronegative hydrogen atom does. Hence, the oxygen atom has a slight negative charge relative to the hydrogen atom. The bond between the oxygen and hydrogen atoms is then said to be polar, because it contains a negative pole (the oxygen) and a positive pole (the hydrogen). Because opposite charges attract one another, polar molecules interact well with other polar molecules and charged particles. The negative poles attract positive ions or the positive poles of other polar molecules, while the positive poles attract negative ions or the negative poles of other polar molecules.

Some amino acids have side chains that contain polar groups; these amino acids are known as **polar amino acids**. (Examination of the amino-acid structure in Figure 3 shows that all amino acids have an amino group and a carboxylic-acid group, which are polar. However, these polar groups form part of the backbone and do not contribute to the polarity of an amino-acid residue in a peptide. **Only the side chain determines whether or not the amino acid is considered polar.**) The three-fold channel in ferritin is lined with the polar amino acids aspartate (Asp) and glutamate (Glu) (Figure 13, below). Because it is lined with polar amino-acid side chains, the three-fold channel is also said to be polar. The channel's polarity allows it to interact favorably with the Fe^{2+} ion and with water, because the positive charge of the ion (or the positive pole of water) attracts the negative poles of the side chains (Figure 11). The favorable interaction allows Fe^{2+} to pass through the channel.

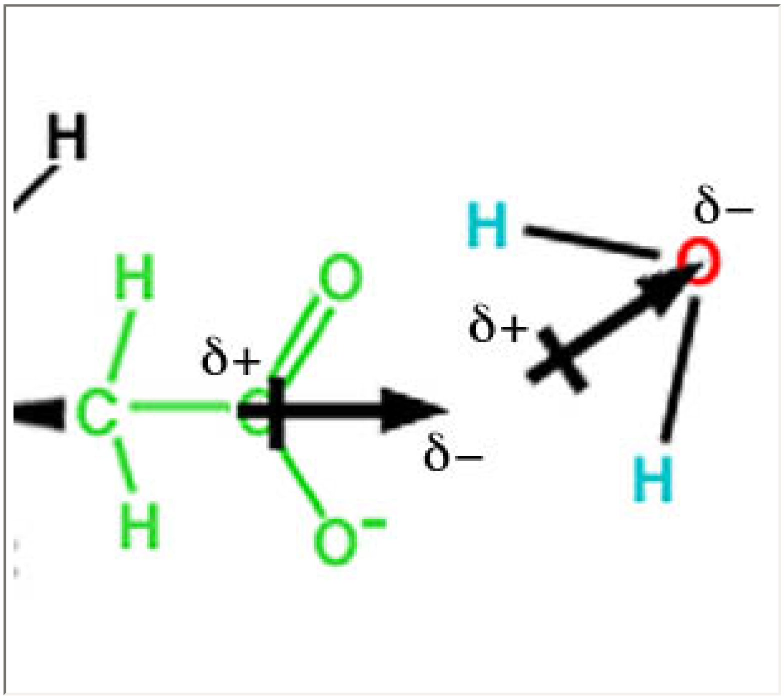


Figure 11

This diagram shows the interaction of a polar water molecule with the polar side chain of aspartate (an amino acid). This figure uses the standard depiction of the direction of polarity: an arrow pointing in the direction of the partial negative charge, with a + sign at the pole with a partial positive charge. In addition, the symbols "δ-" and "δ+" may be used to depict the negative and positive poles, respectively.

Fe(II) is probably not accompanied by all six water molecules of the hydrated complex as it passes through the channel, because this entire complex would be too large to fit through the channel. Most likely, Fe(II) is coordinated to some water molecules and to some of the polar side chains lining the channel as it passes from the inside to the outside of the ferritin shell. Once outside the shell, the Fe(II) then regains the six water molecules and is again solvated as $Fe(H_2O)_6^{2+}$.

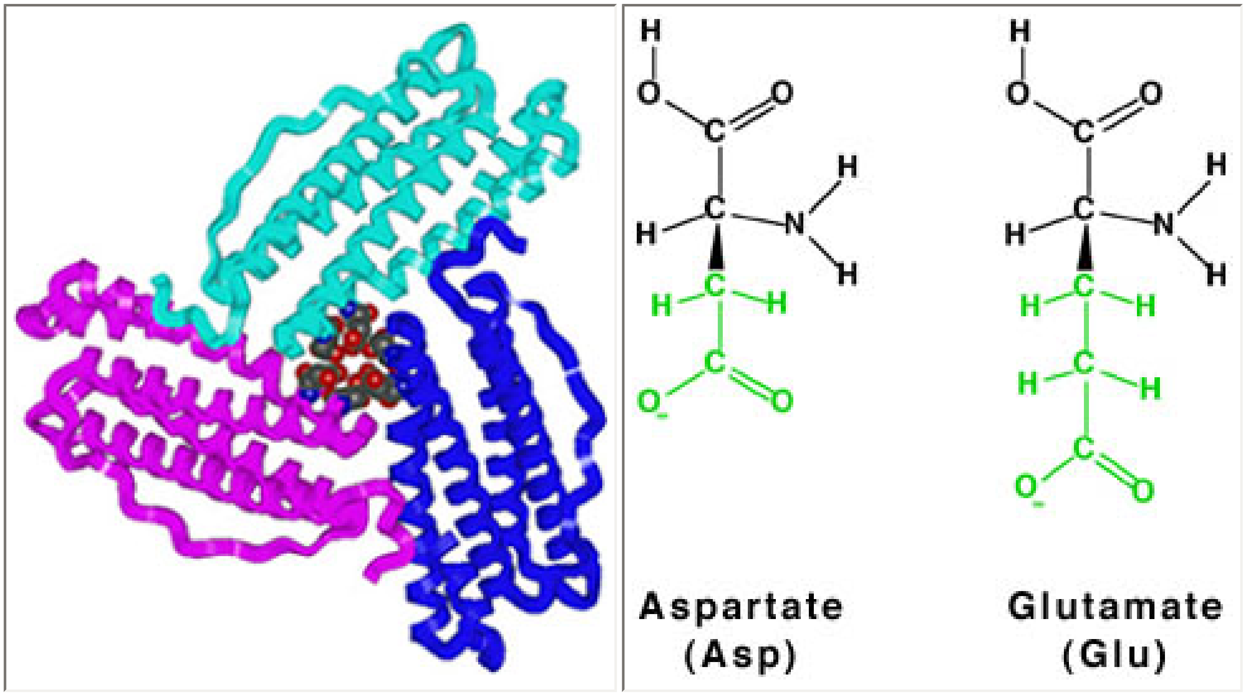


Figure 12

This is a molecular representation of the three-fold (polar) channel in the ferritin protein. Fe(II) can leave the ferritin shell through this channel.

Note: The amino acids that line the channel are shown in the CPK representation, and the remaining portions of the three peptide subunits that form the channel are shown in the ribbon representation.

Figure 13

This is a 2D-ChemDraw representation of aspartate (Asp) and glutamate (Glu), the polar amino acids that line the three-fold channels in ferritin. The side chains are shown in green. Recall that only the side-chain groups contribute to the polarity of the residue in a peptide.

Note: To view these amino acids interactively, please use Chime, and click on the molecule above.

What about the other type of channels in ferritin, the four-fold channels (Figure 14) These channels are lined with the **nonpolar** amino acid leucine (Leu, Figure15). The side chain of leucine contains only carbon and hydrogen atoms, which have similar electronegativities. Hence, the four-fold channel is considered to be nonpolar. Because it is nonpolar, this channel does not interact favorably with the Fe^{2+} ion, and Fe^{2+} does not leave the ferritin shell through these channels. Rather, it is thought that these channels function as the site of electron transfer, whereby the Fe(III) in the mineral lattice is reduced to Fe(II). However, the mechanism of this electron transfer is not well understood.

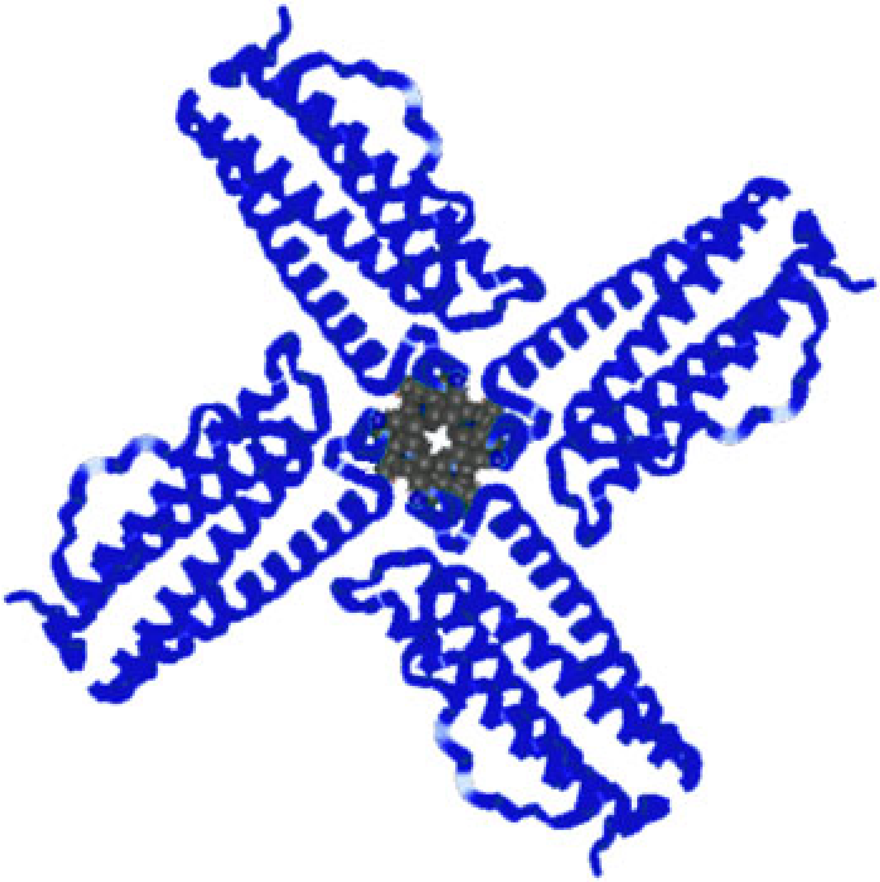


Figure 14

This is a molecular representation of the four-fold (nonpolar) channel in the ferritin protein. Electrons are transferred via this channel to reduce the Fe(III) in the mineral lattice to Fe (II), thereby rendering the iron soluble so that it can be released from ferritin through the three-fold channel shown above (Figure 11).

Note: The amino acids that line the channel are shown in the CPK representation, and the remaining portions of the four peptide subunits that form the channel are shown in the ribbon representation.

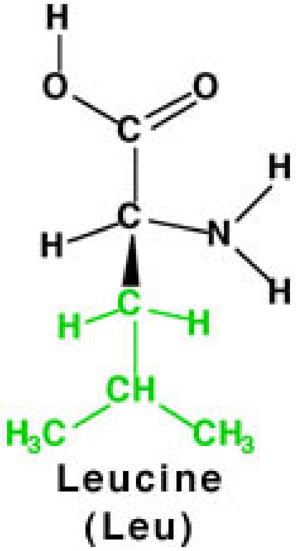


Figure 15

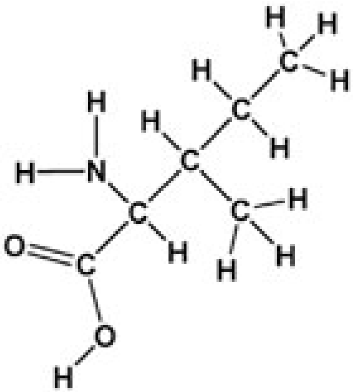
This is a 2D-ChemDraw representation of leucine (Leu), the nonpolar amino acid that lines the four-fold channels in ferritin. The side chain is shown in green. Recall that only the side-chain groups contribute to the polarity of the residue in a peptide.

Note: To view this amino acid interactively, please use Chime, and click on the molecule above.

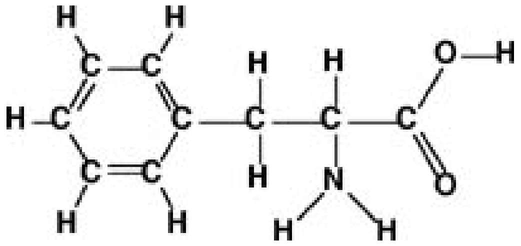
Questions on Release of Iron from Ferritin

- The two-dimensional (ChemDraw) structures of three amino acids are shown below. For each amino acid, tell whether it is polar or nonpolar. **HINT:** Remember that the polarity of an amino acid is considered to depend only on its side chain, because the other groups of amino acids become incorporated into peptide bonds forming the backbone of a peptide. The amino acids are not all shown in the same orientation, so you will need to determine which part of each amino acid is the side chain.

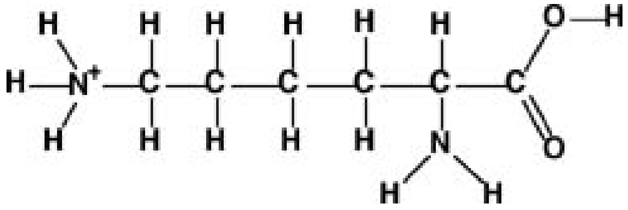
    a. Isoleucine (Ile)

    b. Phenylalanine (Phe)

    c. Lysine (Lys)

- If these three residues formed part of a channel, would the channel permit, Fe^{2+} to pass through it? Explain your reasoning.

Conclusion

Iron is an essential trace element that is used to form molecules in the body, such as hemoglobin. Ferritin is the protein within the body that stores iron and releases it through channels in a controlled fashion. The unique structure of ferritin forms a spherical shell in which the iron is "stored" as Fe(III) in a crystalline mineral. Ferritin consists of 24 peptide subunits that form two types of channels where these subunits intersect; the 3-fold channel is polar and the 4-fold channel is nonpolar. (The residues that line the channels determine the polarity of the channel.) When the Fe(III) in the crystalline mineral is reduced to Fe(II), the iron becomes solvated and ferritin releases the solvated iron, $Fe(H_2O)_6^{2+}$, through the 3-fold polar channel. Hence, ferritin can control the amount of available iron in the body, preventing iron disorders like anemia and iron overload.

The three-dimensional structure of ferritin is crucial to its function within the body. (In fact, the three-dimensional stucture of any molecule is critical in determining a molecule's properties and function.) Hence, to better understand ferritin's role in the body, we used different types of molecular representations to study ferritin's three-dimensional stucture. Each representation used in this tutorial gives important information about ferritin. However, none of the representations by themselves can tell us everything we need to know about ferritin. Only by recognizing the value and limitations of each type of representation, and using these representations in conjunction with one another and with other information about the molecule, can we begin to understand the complex relationship between the protein's structure and its function.

Additional Links:

- The American Hemochromatosis Society provides a wealth of information about iron overload and hemochromatosis, including a new diagnostic test that uses the same procedure for analyzing the iron content in ferritin that you performed in the Experiment!
- The Iron Disorders Institute is an organization dedicated to helping people understand the relationship between iron and disease.
-

References:

Berkow, R., ed. "Iron Overload/Hemochromatosis," *The Merck Manual*, 16th ed., 1992. Reprinted by Thomas, S.; Snyder, D., web authors, The American Hemochromatosis Society, Inc.

Frey, R.; Donlin, M.; Bashkin, J. "Ferritin Molecular-Graphics Tutorial," Washington University: St. Louis, MO, 1995. URL: *LabTutorials/Ferritin/FerritinTutorial.html*.

Griffith, H. W. "Anemia, Iron-Deficiency," *The Complete Guide to Symptoms, Illness & Surgery*, The Putnam Berkley Group, Inc., 1995; electronic rights by Medical Data Exchange.

Harrison, P.M., *et al.* In *Iron Transport and Storage*; Ponka, P.; Schulman, H.M.; Woodworth, R.C., Eds.; CRC: Boca Raton, FL, 1990; pp 81-101.

Insight II graphical program; Molecular Simulations, Inc. URL: *http://www.msi.com*.

Lawson, D.M., *et al.* "Solving the Structure of Human H Ferritin by Genetically Engineering Crystal Contacts," *Nature* **1991**, 349, 541-544. (Ferritin PDB coordinates, Brookhaven Protein Data Bank.)

Modern Life Guide- Body Elements, 1996. URL: *http://www.modlife.com/MLG/body.html*.

K.L. Taft, *et al.* "A Mixed-Valent Polyiron Oxo Complex that Models the Biomineralization of the Ferritin Core," *Science*, **1993**, 259, 1302.

Persistence of Vision Ray Tracer (POV-Ray). URL: *http://www.povray.org*.

Theil, E.C. "Ferritin: Structure, Gene Regulation, and Cellular Function in Animals, Plants, and Microorganisms," *Annu. Rev. Biochem.* **1987**, 56, 289-316.

Stryer, L. In *Biochemistry*, 4th. ed., W.H. Freeman and Co.: New York, 1995, pp. 18-24.

Vander, A.J.; Sherman, J.H.; Luciano, D.S. In *Human Physiology: The Mechanisms of Body Function*, 6th ed., Mc-Graw-Hill, Inc.: New York, 1994, p. 398.

Acknowledgements:

The authors thank Bill Buhro for obtaining the structural information for the iron-mineral core, Greg Noelken for creating the chime script files, and Dewey Holten, Michelle Gilbertson, Jody Proctor and Carolyn Herman for many helpful suggestions in the writing of this tutorial.

The development of this tutorial was supported by a grant from the Howard Hughes Medical Institute, through the Undergraduate Biological Sciences Education program, Grant HHMI# 71199-502008 to Washington University.