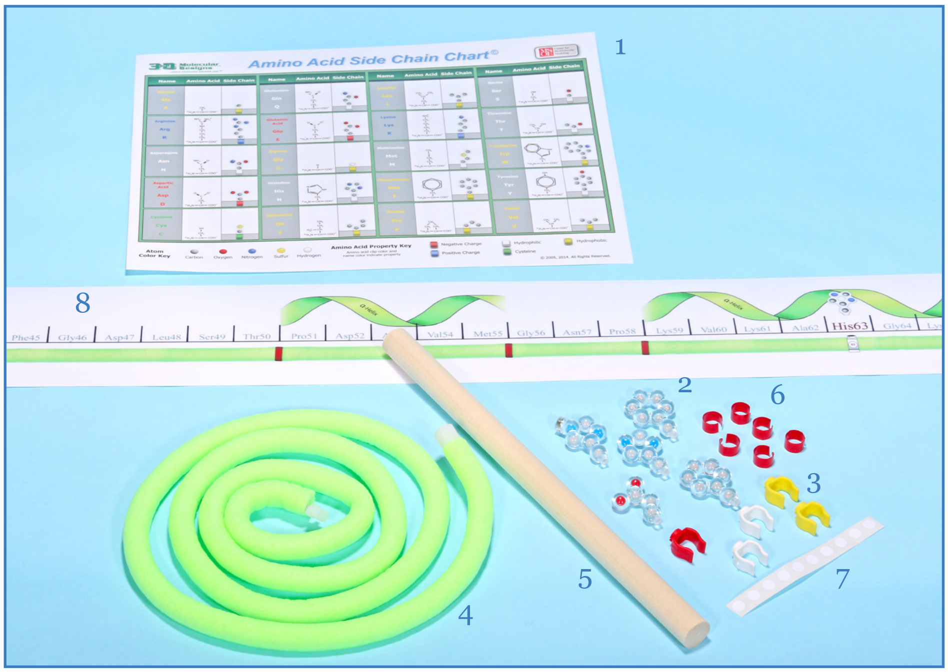
β-Globin Folding Kit©

Green Segment Contents

Parts Reference Guide — Green Segment



1. **Amino Acid Side Chain Chart©** shows the properties and atomic structure of side chains.
2. **Amino Acid Side Chains** affect protein folding, provide stability to protein structure and impact function.
3. **Plastic Clips** mark the location of amino acid side chains. The colors indicate the properties of side chains on models.
4. **Mini Toober** represents the alpha carbon backbone of β-globin.
5. **Dowel** helps form alpha helices.
6. **Red Bands** indicate the beginning or end of each right-handed alpha helix.
7. **White Dots** indicate the amino acid number on plastic clip.
8. **Green Mini Toober β-Globin Folding Map** illustrates how to mark the mini toober for folding and assembly.

Gather all of the parts you need to assemble the green segment. Make sure that the mini toober, parts bag and map are for the green segment of the protein. Use the illustrations on the parts bag and the folding map, and the color keys (page 4) to become familiar with the parts.

3-D Molecular Designs
...where molecules become real ™

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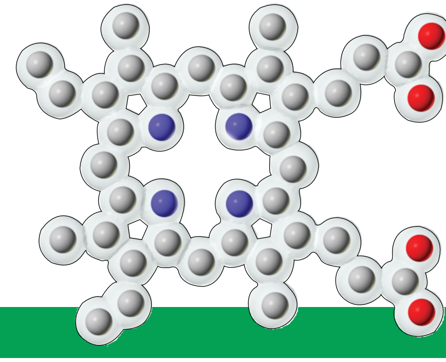
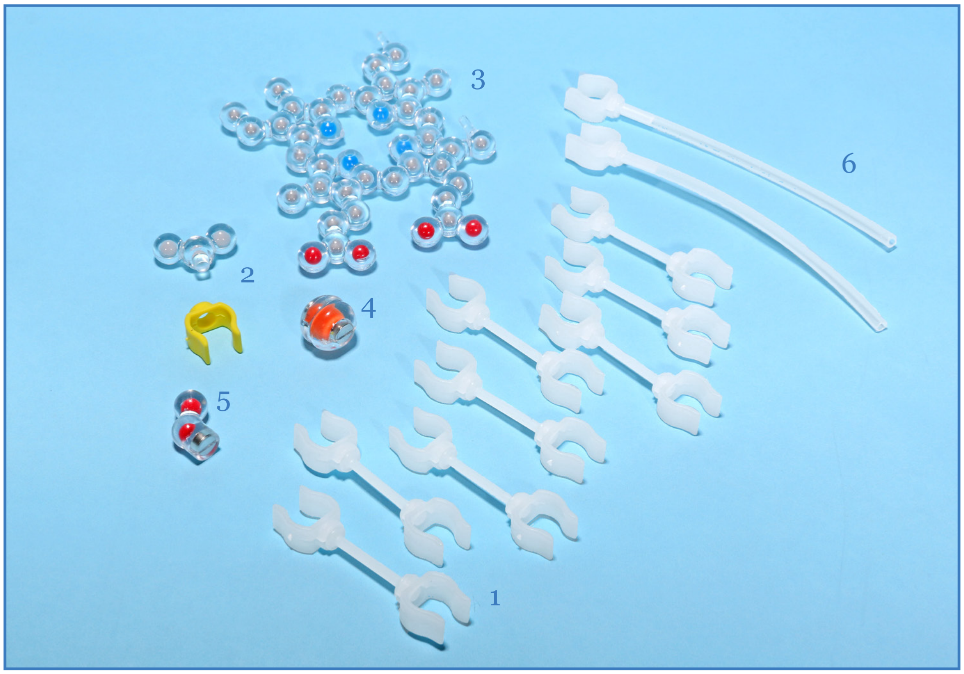
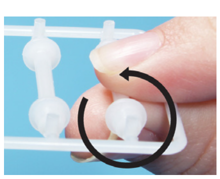


Heme Group



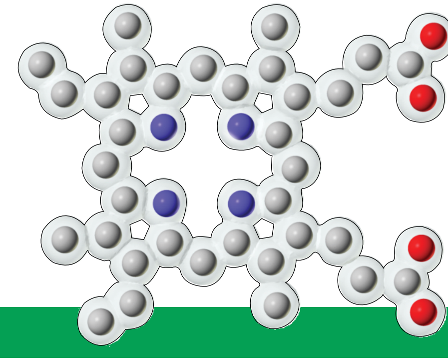
1. **Support Posts** provide support for the β-globin model as the three segments are joined together and correctly positioned in 3-dimensional space.
2. **Valine Side Chain and Clip** is used — once the model is assembled – to replace a glutamic acid to demonstrate the sickle cell mutation.
3. **Heme Group** consists of carbon, nitrogen and oxygen atoms that assume a circular shape to hold an iron atom.
4. **Iron Atom** is contained in the middle of the heme group.
5. **An Oxygen Molecule (O_2)** binds to the iron atom.
6. **Heme Connectors** hold the heme group in place, once the three segments are joined together.

Assembling Support Posts



Twist center post from frame

Insert center post into clip



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Proteins and Amino Acids

Big Idea — Relationship Between Structure and Function

Proteins are more than an important part of your diet. Proteins are complex molecular machines that are involved in nearly all of your cellular functions. Each protein has a specific shape (structure) that enables it to carry out its specific job (function).

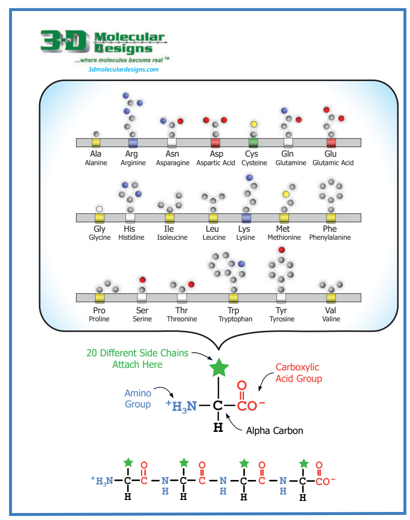
A core idea in the life sciences is that there is a fundamental relationship between a biological structure and the function it must perform. At the macro level, Darwin recognized that the structure of a finch's beak was related to the food it ate. This fundamental structure-function relationship is also true at all levels below the macro level, including proteins and other structures at the molecular level.

This core idea extends across all science and engineering disciplines and will help you whether studying life science, earth and space science, physical science or engineering.

In this activity, you will explore the structure and function of the β-globin protein.

Amino Acid Structure

There are 20 amino acids found in proteins. Each amino acid consists of two parts — a backbone and a side chain. The backbone is the same in all 20 amino acids and the side chain is different in each one.

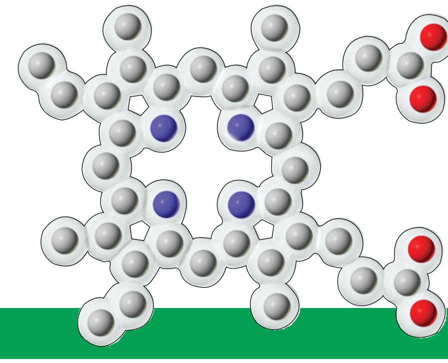


1. Using the Amino Acid Side Chain Chart©, as shown on the right, review the illustration at the bottom of the page, you can see that all of the amino acids have the same **backbone** structure.

   The blue, green and red mini toobers in the kit represent the backbone of the amino acids.

2. Now look at the 20 side chain images, shown on the upper section of the chart. Each side chain consists of a unique combination of atoms.

$$^{+}H_3N-\underset{\displaystyle H}{\overset{\displaystyle CH_3}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}O^{-} + {}^{+}H_3N-\underset{\displaystyle H}{\overset{\displaystyle CH_3}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}O^{-} + {}^{+}H_3N-\underset{\displaystyle H}{\overset{\displaystyle CH_3}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}O^{-} + {}^{+}H_3N-\underset{\displaystyle H}{\overset{\displaystyle CH_3}{C}}-\overset{\displaystyle O}{\overset{\|}{C}}O^{-}$$



Proteins and Amino Acids Continued

Dual Coloring Scheme

The side chains models in this kit feature a dual coloring scheme. The colored balls embedded in clear plastic represent the **atoms** in the side chains. (*See the Atom Color Key below*.)

The specific combination of atoms in each amino acid determines its shape and **properties**. The properties indicate how side chains respond to the watery environment in the cell and how they respond to each other. A side chain could be hydrophobic, hydrophilic, acidic (negatively charged), or basic (positively charged). (*See the Property Key below*.)

The colors of the clips at the base of the side chain represents the properties of the side chain.

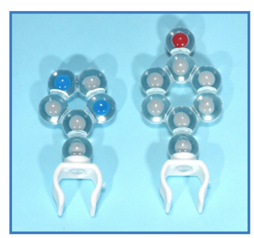
Atom Color Key

- Carbon (C)
- Nitrogen (N)
- Oxygen (O)
- Sulfur (S)
- Hydrogen (H)

Property Key

- Hydrophobic (Non-Polar)
- Hydrophilic (Polar)
- Acidic (Negative Charge)
- Basic (Positive Charge)

Hydrophobic and Hydrophilic Properties



a. Look at the Property Key on page 4. What do you think hydrophobic means?

____

____

____

____

____

b. Can you guess what hydrophilic means?

____

____

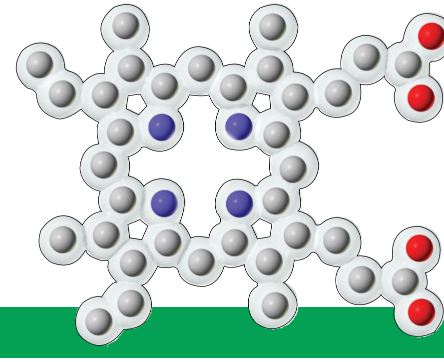
____

c. Look at the hydrophobic (yellow) and hydrophilic (white) side chains in the photos above.

Compare their atomic compositions. From the differences you see, can you explain why hydrophilic side chains like water?

____

____



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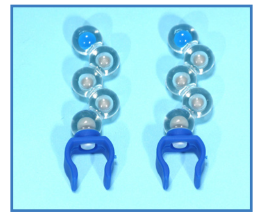
Proteins and Amino Acids Continued

Acidic and Basic

You probably remember from other classes or personal experience that some foods and chemicals are acidic and some are basic. Acidic side chains (red) are negatively charged and basic side chains (blue) are positively charged.



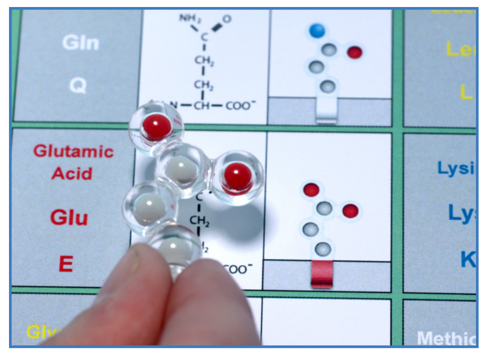
d. Compare the atomic composition of the acidic side chains to the basic side chains in the photos (right). From the differences you see, can you explain why they are acidic or basic?

____

____

____

3. Flip over the Amino Acid Side Chain Chart© and match the amino acid side chain models from your parts bag with the correct images on the chart.

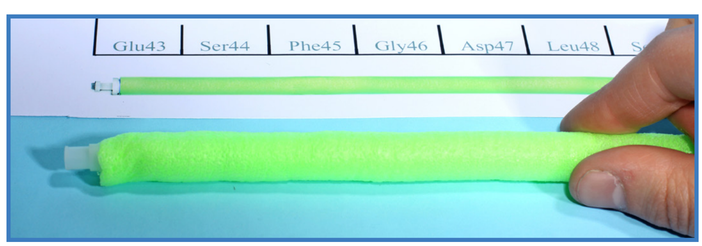


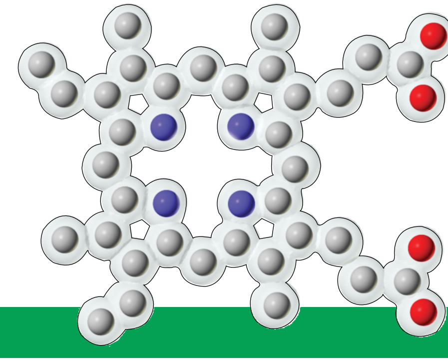
4. Still using the Amino Acid Side Chain Chart©, look at the base of the side chains image for the correct clip color. Insert the side chain into the plastic clip. (*See photo to the right*.)

Preparing Green Segment

5. Using the folding map, line up the mini toober with the illustration on the folding map. The white plastic ends should line up with their identical images on the map.

Examine the ends carefully. They are different and must be aligned correctly with their corresponding images on the map. If they aren't aligned correctly, you won't be able to assemble the model.





β-Globin Folding Kit©

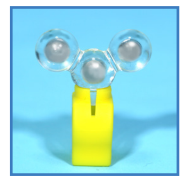
Preparing Green Segment

Primary Structure

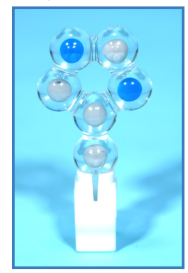
All proteins – including β-globin – consist of a continuous sequence of amino acids. It is the specific sequence that makes each protein unique.

If you put all of the folding maps in the β-Globin Folding Kit© together, from blue to green to red, you will see the sequence of the amino acids that form β-globin. The sequence begins with valine 1 on the blue map and ends with histidine 146 on the red map. This is called the primary structure of the β-globin protein.

While the folding maps show all of the amino acids, you will work with a few key side chains that illustrate concepts that will help you understand how β-globin forms its final structure and performs its function. The blue segment doesn't include any hydrophobic side chains. They are featured in the green and red segments of the β-globin model.



Valine



Histidine

Now you are going to mark the primary structure of the green segment of the β-globin protein on your mini toober.

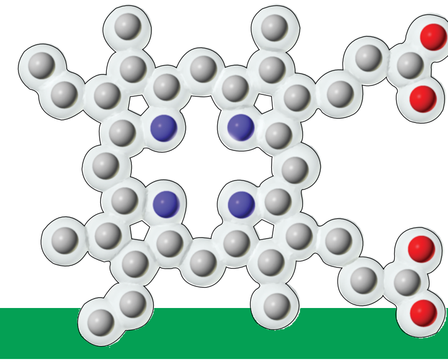
6. Label the white stickers with the numbers of the amino acids shown on the folding map. *(See photo.)*

   The numbers indicate the position of each amino acid.

   The numbering of amino acids begins at the N-terminus (blue endcap) and ends at the C-terminus (red endcap), and corresponds to the order in which the protein is synthesized. The C-terminus is at the end of the red map.

7. Place the white stickers with the numbers on the plastic side chain clips.

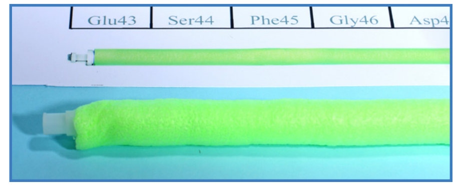
   Remember the clip colors correspond to the chemical properties of the amino acid side chains (page 4).



Preparing Green Segment Continued

√*Checkpoint*

Do you have
2 histidine side chains numbered 63 and 92
2 phenylalanine side chains numbered 71 and 85
1 glutamic acid side chain numbered 90



Is the mini toober aligned with its image on the folding map? (*See top right photo.*)
If the mini toober shifts one direction or the other, your plastic clips (and the side chains) will be in the wrong locations.

8. Place the side chains and plastic clips with stickers on them on the mini toober in the positions indicated on the map.

Remember the amino acids in the β-globin model are represented by the combination of the mini toobers (backbone) and plastic models (side chains).

Now think about what you learned about the properties of side chains on page 4. Predict how the side chains respond in the cell as β-globin spontaneously folds into its 3-dimensional structure. Draw your answer in the diagram below.

a. How will the hydrophilic (white) side chains on this fragment react in the cell?

____

____

b. How will the basic and acidic side chains react in this watery environment?

____

____

c. Predict how the electrostatic nature of charged amino acids might affect the structure of a protein.

____

____

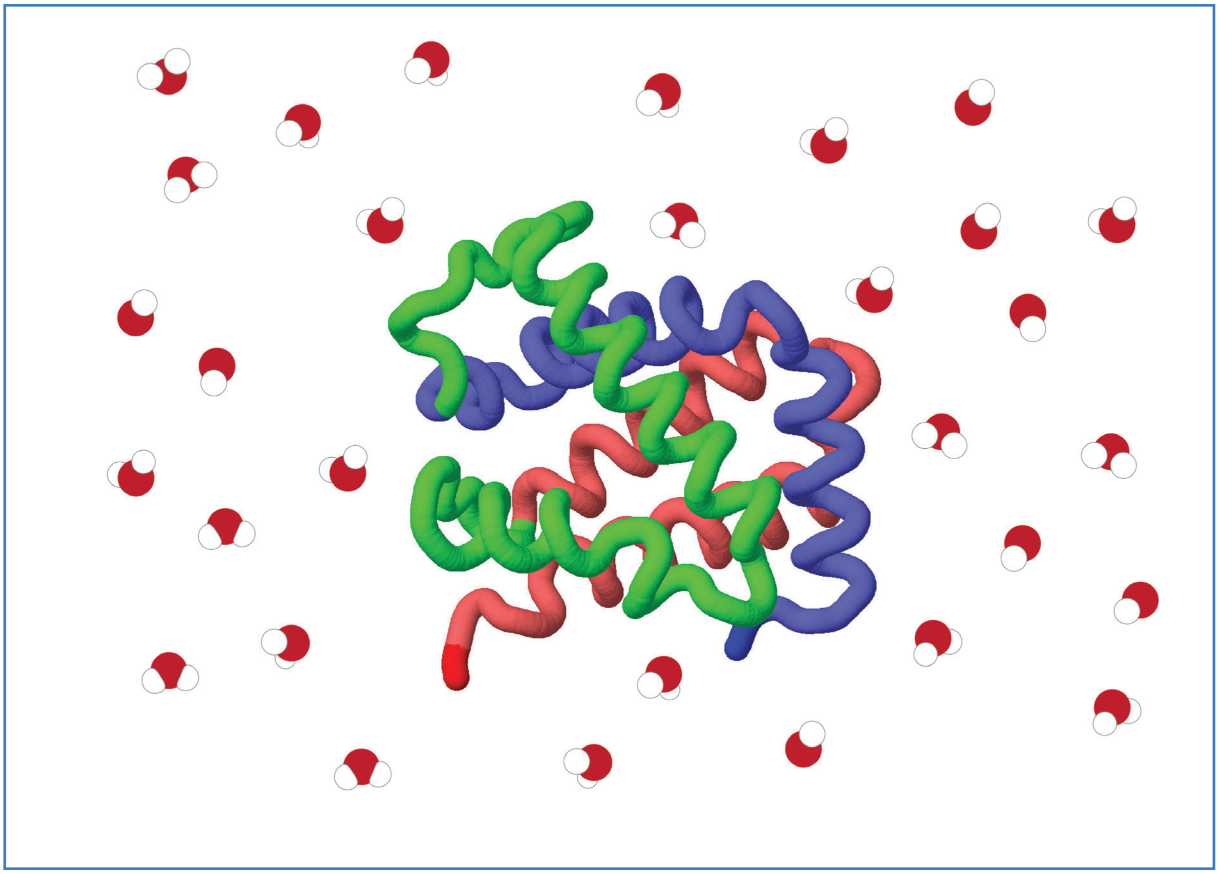
d. If there were hydrophobic (yellow) side chains on this fragment, how would they get away from the water surrounding them?

____

____

Preparing Green Segment Continued

e. On the hypothetical protein below, draw and label **1 hydrophobic side chain, 1 hydrophilic side chain, 1 positive side chain, 1 negative side chain, and 2 side chains forming an electrostatic bond**.



9. Leaving the plastic clips in place, remove the side chains from the alpha carbon backbone (mini toober) and set them aside. The side chains will be put back on the plastic clips once the protein is folded.

...where molecules become real ™

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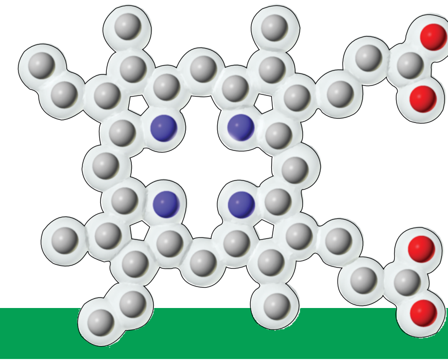
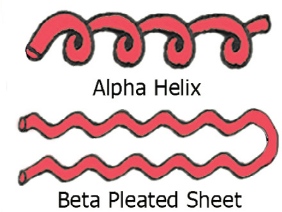


Preparing Green Segment Continued

From Amino Acids to Protein

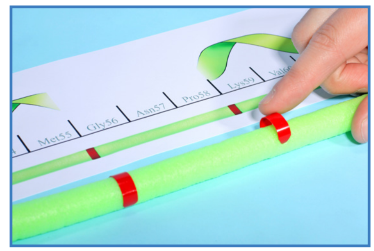
As a protein is synthesized in the ribosome and emerges into the watery environment of the cell, the protein sequence changes from its linear sequence (primary structure) and spontaneously folds into a 3-dimensional structure based the properties of its amino acids.



Secondary Structure

Secondary Structure

Next you will mark the secondary structure of the protein. Secondary structure **consists of the alpha helices and beta pleated sheets that form as a protein spontaneously folds into it's final shape.**



10. Using the folding map as a guide, add the red bands to mark the beginning and end of the alpha helices.

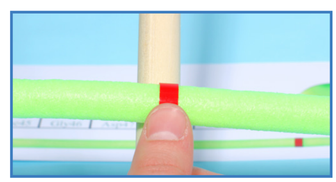
√*Checkpoint*

Do you have three alpha helices marked on the mini toober with red bands?

The first one begins at ____________________ and ends at _______________________.

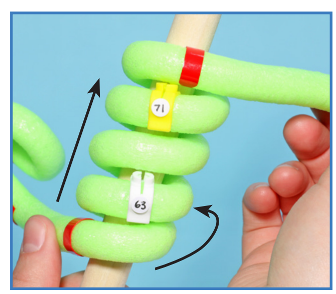
The second one begins at __________________ and ends at _______________________.

The third one begins at __________________ and ends at _______________________.



11. Using the wooden dowel, form a *right-handed* alpha helix as indicated between the red bands.

    Hold the dowel in your left hand. Place the mini toober on top of and perpendicular to the bottom of the dowel, positioning the first red band on top of the dowel. (*See photo.*)



12. Wrap the mini toober around the dowel in a counterclockwise direction, moving up the dowel as you wind the toober around it. Stop wrapping at the next red band. (*See photo.*)

√*Checkpoint*

To be sure that you have formed a right-handed helix, imagine the coiled mini toober is a spiral staircase, and you are walking **up** the staircase with your hand on the **outside rail** of the staircase. Which hand is on the rail? If it is your right hand, you have a right-handed alpha helix.

If your left hand is on the rail, you'll need to unwind the helix and wind it in the *opposite direction*.

13. Repeat for each section marked as an alpha helix.

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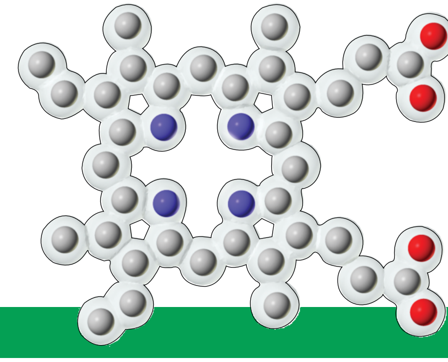
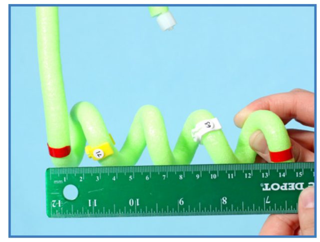
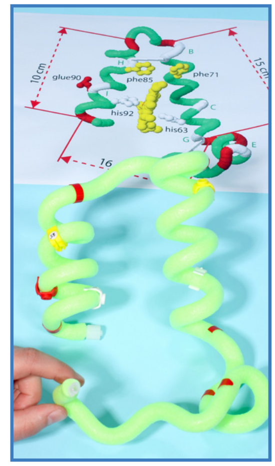


Preparing Green Segment Continued

14. Expand or compress the alpha helixes to match the indicated length(s) on the printed illustrations or Jmol at *3dmoleculardesigns.com/Teacher-Resources/B-Globin-Folding-Kit/Jmols-and-Tutorials.htm*. Don't worry about positioning the alpha helixes in relation to each other at this point. That will be completed in the tertiary structure section.





15. Add the side chains to the mini toober, using the numbers and the folding map to place the side chains in the correct locations. You may rotate the clip to see the dot with the number.

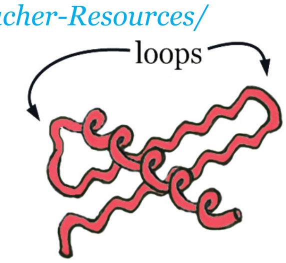
    The side chains should point away from the inside of the helices.

Tertiary Structure

Tertiary structure is the final 3-D shape of a protein. The final shape is determined by the properties of the amino acid.

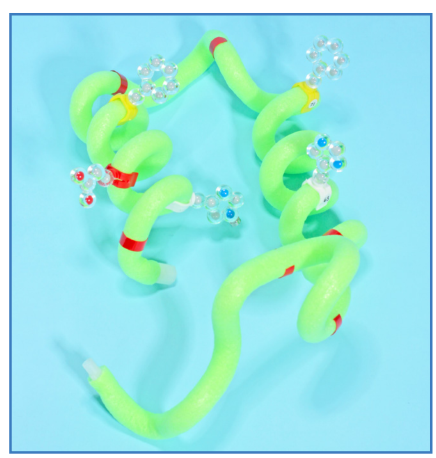
16. Use the green segment Jmol images at *3dmoleculardesigns.com/Teacher-Resources/B-Globin-Folding-Kit/Jmols-and-Tutorials.htm* and/or the printed illustrations as guides. Fold the toober so that the alpha helices are aligned correctly in three dimensional space. You may slide side chains ½" up or down the mini toober as necessary or adjust the alpha helices by slightly twisting or untwisting them.

    Add short bends to the non-helical regions (these are called loops) so that they equal the lengths indicated in the illustrations. The two long helices should lie somewhat flat. They should be at less than a 90° angle. The short helix should point up from this plane and angle toward the nearest long helix.



Tertiary Structure

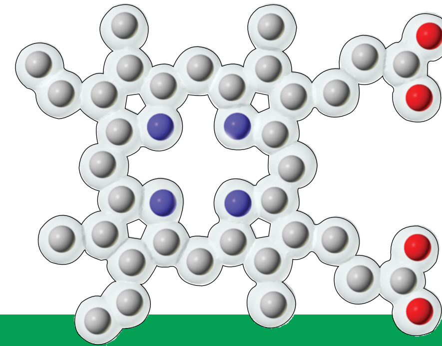
Position the connecting end as shown in the photo.



√*Checkpoint*

Does your finished green segment look similar to the photo to the left?

When the blue and red segments are folded you and your classmates will put the segments together to complete the model.



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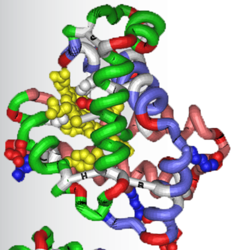
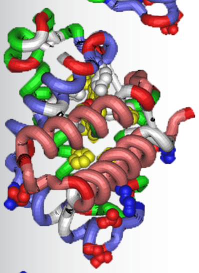
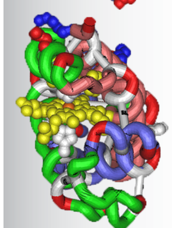
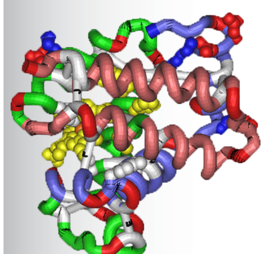




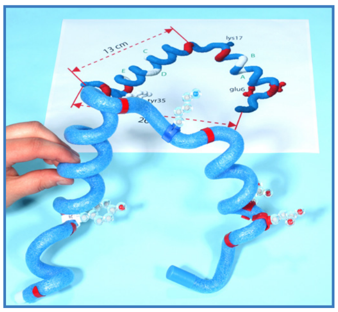


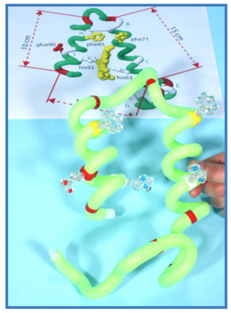


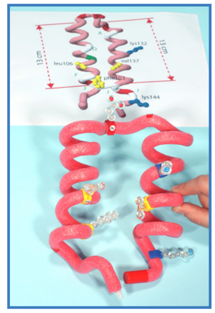
Assembling the β-Globin Model

Examine the Segments

Check each segment using the printed illustrations and/or Jmol visualizations on your computer monitor to verify that each one is aligned correctly in 3-D space. Make sure all of the side chains are also positioned as indicated.





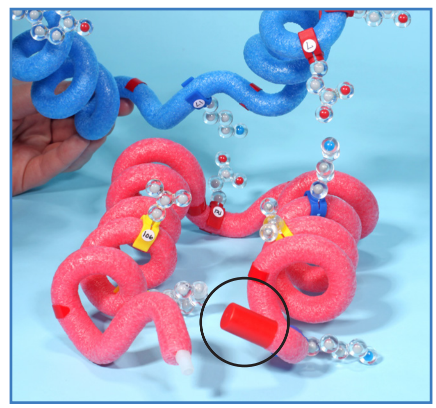


√Checkpoint

As you assemble the model you may need to make some minor adjustments to each segment to properly align the segments and side chains.

Form Salt Bridges

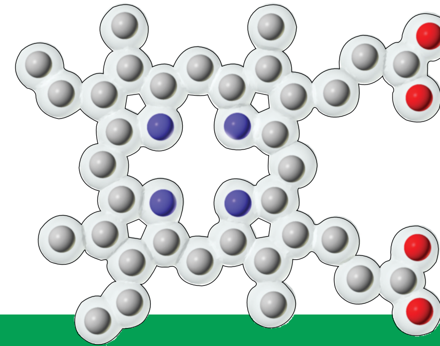
Salt bridges stabilize proteins and occur between amino acid side chains with opposite positive or negative charges.

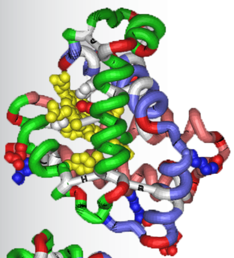


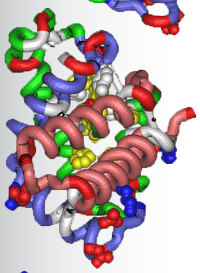
17. Form two salt bridges between the red and blue fragments. Start with the red segment as positioned in the photo. The red endcap should be on the right.

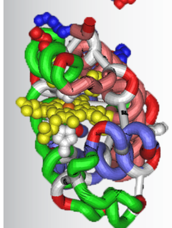
    Hold the blue segment parallel to the red, with the blue endcap on the right.

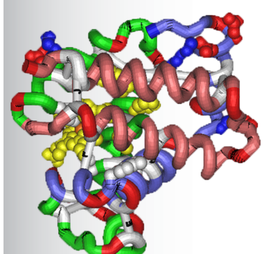
    Connect two salt bridges with support posts.
    Glutamic acid 7 will connect with lysine 132.
    Glutamic acid 121 will connect with lysine 17.



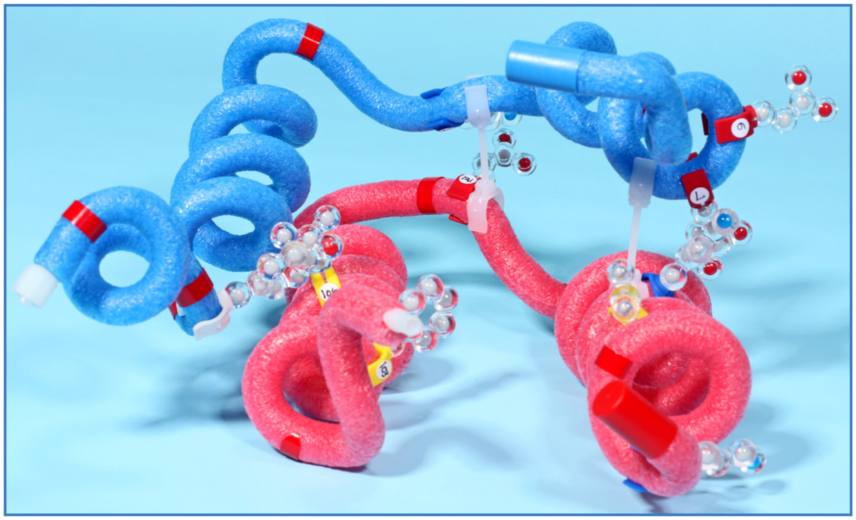




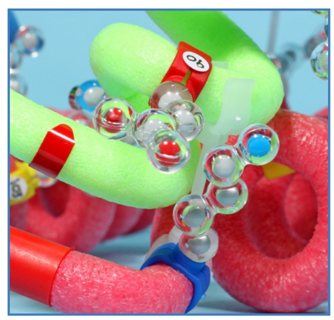




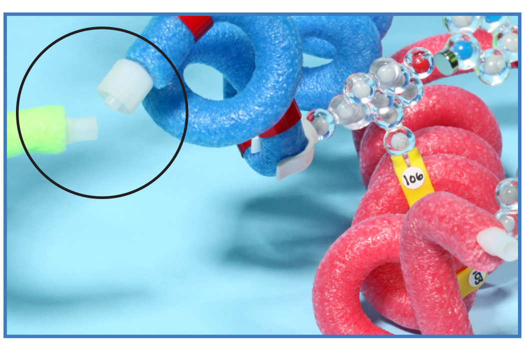
Assembling the β-Globin Model Cont.

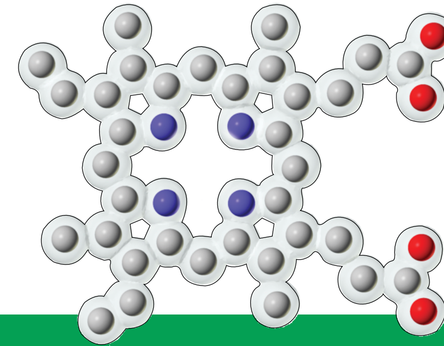


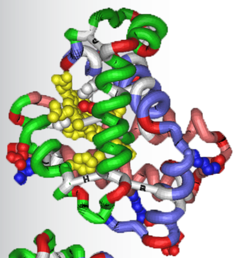
18. Form a third salt bridge between the red and green fragments: glutamic acid 90 with lysine 144.

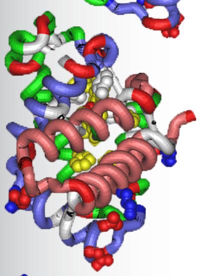


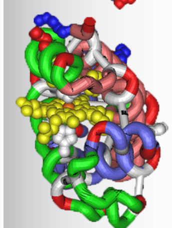
19. Connect the ends of the blue and green fragments by inserting the peg and twisting the collar. Then connect the end of the green and red segments.

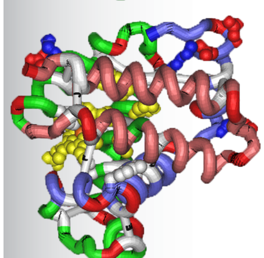












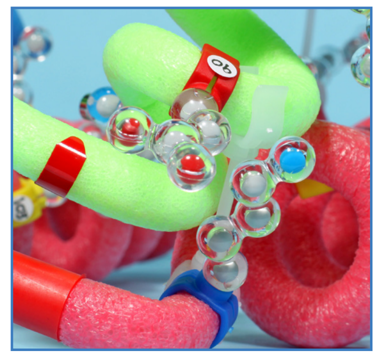
β-Globin Folding Kit©

Assembling the β-Globin Model Cont.

20. Adjust the location of the side chains so that they follow the basic principles of chemistry that affect protein folding:

Are the ***hydrophobic*** side chains facing to the *inside* of the protein, forming a hydrophobic pocket?

Do the ***negatively charged*** and ***positively charged*** side chains face each other on the *outside* of the protein? Interactions between negatively charged and positively charged side chains on the outside of protein are called ***salt bridges.*** *(See right photos.)*

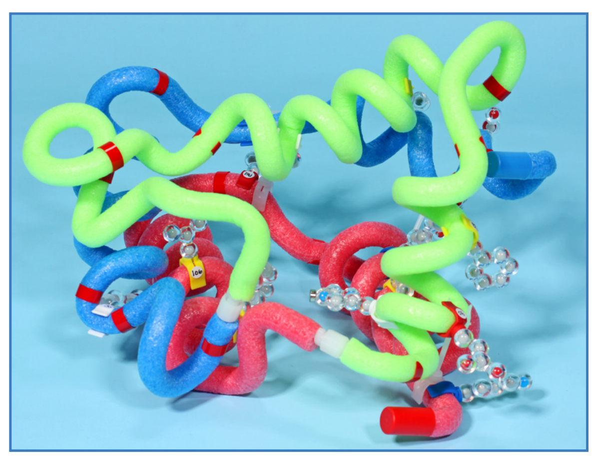


Are the ***hydrophilic*** side chains on the *outside* of the protein, except for the two histidines?

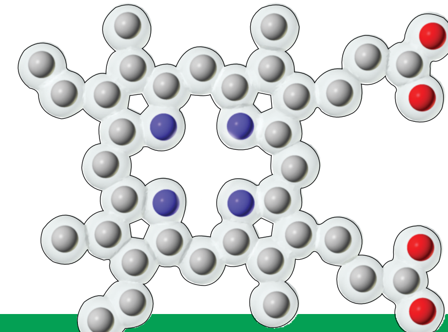
Are the two histidines pointed toward each other in the open space between the two green helices? *(See photo.)*

√***Checkpoint***

Does your model look similar to the model in the photo on the right?



Compare your model to the Jmol image of β-globin. Rotate the β-globin on your monitor as you turn your β-globin model. View your model from different perspectives. Does it look as though you folded and assembled the model correctly?



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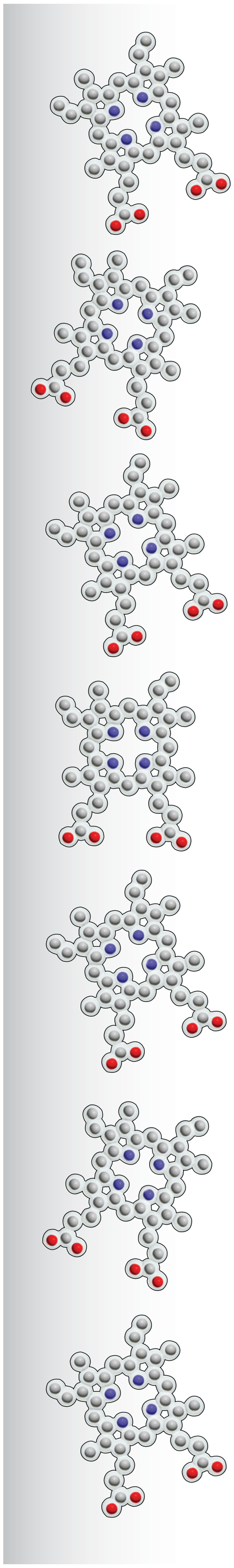


Adding the Heme Group

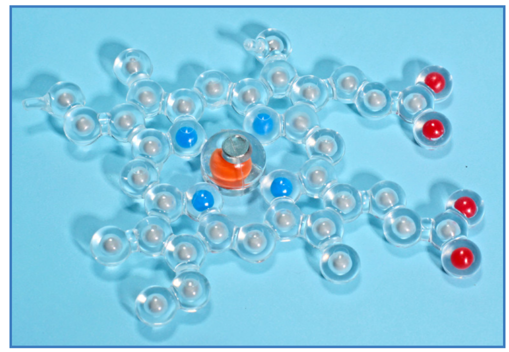
The heme group is an iron-binding complex found in β-globin and α-globin proteins. The iron atom binds to oxygen and transports it to cells where it is used for energy and growth. The iron is coordinated by the four nitrogen atoms in the heme complex. (*See photo below.*)

21. Take the parts out of the heme group bag and examine the heme group.

    Gray represents ______________________________

    Blue represents ______________________________

    Red represents ______________________________

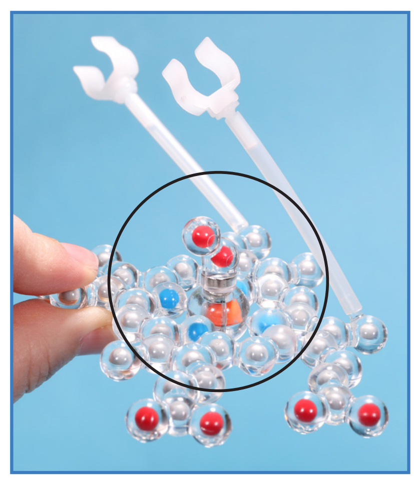


22. Insert the orange iron atom model into the center of the heme group. It will snap into place between the four blue nitrogen atoms, fitting into the groove on the iron atom. See the photo to correctly orient the magnets on the iron atom model.

23. Add the heme connectors to the posts on the heme group.

24. Add the red oxygen molecule to the magnet on the orange iron atom that is on the same side as the heme connectors. (*See right photo.*)

    Now return to your mini toober model.



√*Checkpoint*

The histidine side chains in the β-globin model should point toward each other in the interior of your β-globin model.

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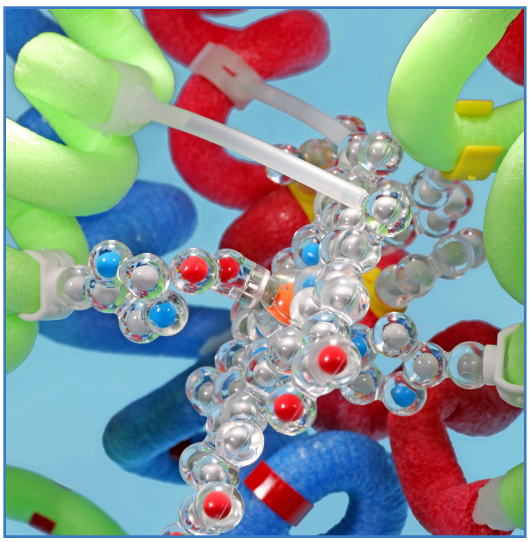
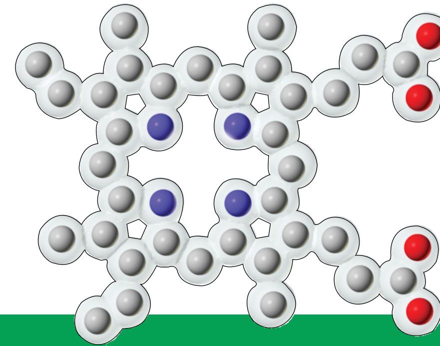
Adding the Heme Group Continued

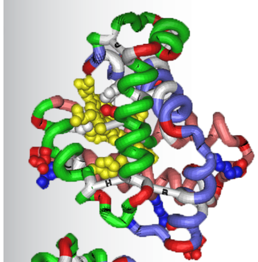


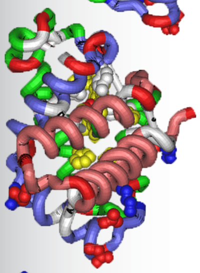
25. Add the heme group to the mini toober model by attaching the clips to the mini toober so that the orange iron atom can bond to the histidine 92 with the magnet.

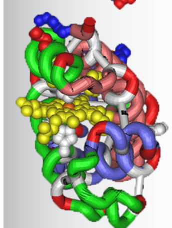
26. Adjust the histidine with the magnet (His92) so the magnet attaches to the iron atom in the heme group. (*See left photos.*)

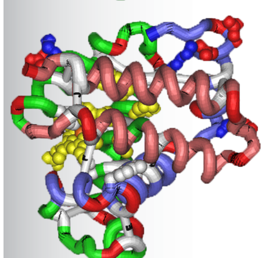


β-Globin Folding Kit©

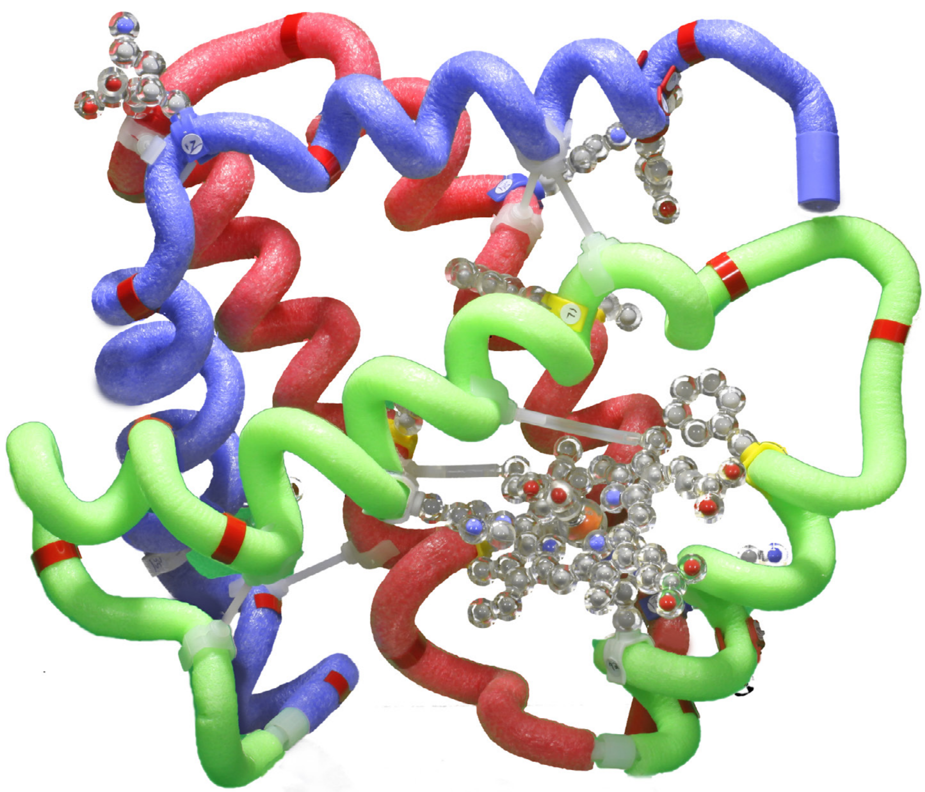








Completed β-Globin Model



√*Checkpoint*

Does your model approximately look like the one in the photo above and/or the Jmol image?

Do you see three alpha helices on the blue segment, three on the green segment and two on the red segment?

Do you see a hydrophobic pocket inside the β-globin structure? Are the yellow side chains clustered there?

Is the heme group connected correctly?

Do you see three salt bridges (negatively charged glutamic acid interacting with positively charged lysine) on your model?

Are the salt bridges on the outside surface of your model?

Is there also a hydrophilic tyrosine on the outside surface of your model?

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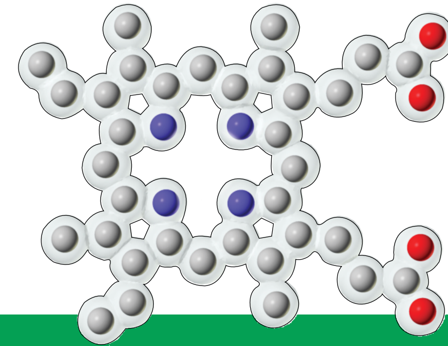
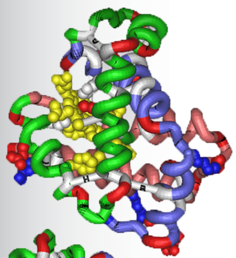
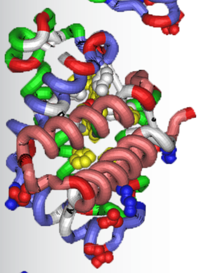
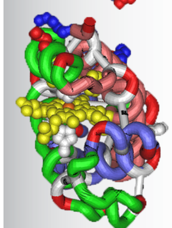
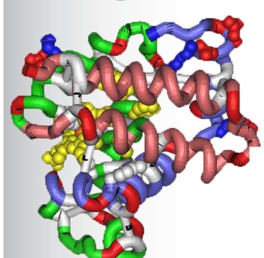


From Structure to Function









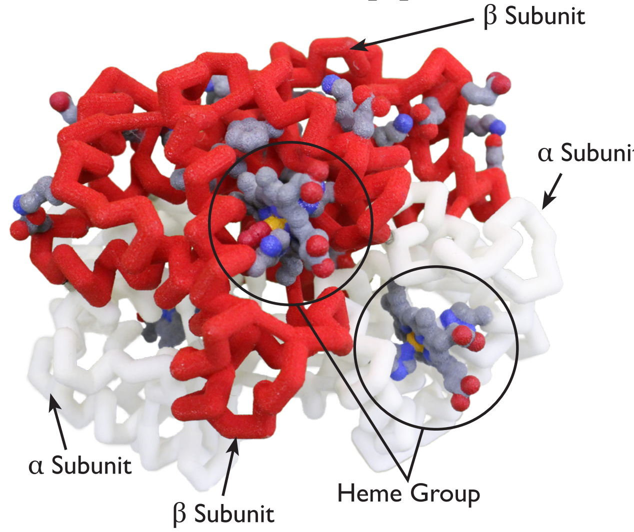
Quaternary Structure

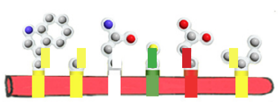
The β–globin protein is a subunit of hemoglobin. Each hemoglobin consists of two α–globin and two β–globin. When two or more proteins form a multi-unit structure the assembled protein complex exhibits a quaternary structure.

The four levels of protein structure are described and illustrated below:

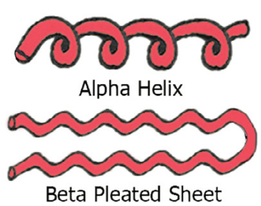
- Primary – the sequence of amino acids in the protein chain
- Secondary – initial folding into right-handed alpha helices or beta pleated sheets
- Tertiary – final folding of the protein in three-dimensional space
- Quaternary structure – assembly of two or more subunits to make a functional protein

Hemoglobin $\alpha_2\beta_2$

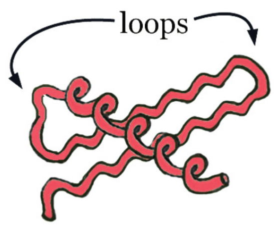




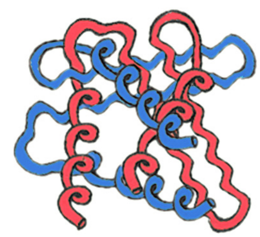
Primary Structure



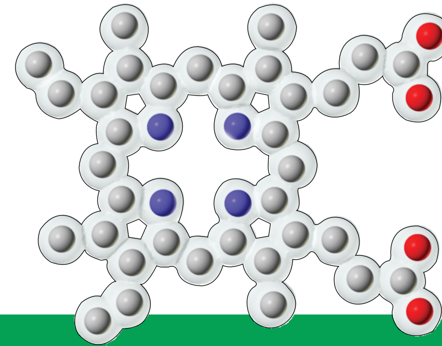
Secondary Structure



Tertiary Structure



Quaternary Structure



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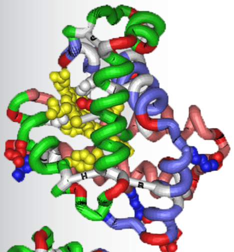
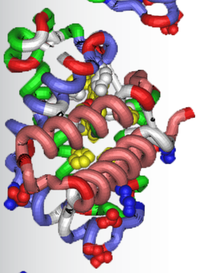
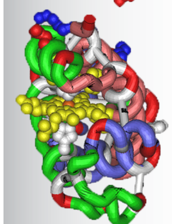
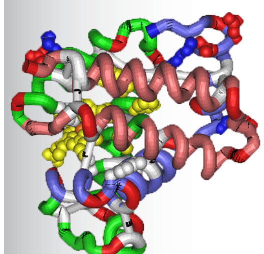

From Structure to Function

Oxygen Transport

The function of the the β-globin protein — as part of hemoglobin — is to transport oxygen. Take a deep breath. Oxygen-rich air is sweeping into your lungs, entering passages with many branches that lead to delicate air sacs, called alveoli. Many tiny vessels, called capillaries, supply your alveoli with blood. The alveoli and capillaries come into contact with each other over a very large surface area. In this ideal environment, oxygen diffuses into your blood where it binds to hemoglobin proteins in your red blood cells. In each red blood cell you have **300 million hemoglobin proteins** ready to bind oxygen. Hemoglobin has four subunits: two α–globin and two β–globin. You folded one of the β–globin.

27. Now go back to your model and remove the O_2 from iron in the heme group.

    a. What would happen if the β-globin in your hemoglobin couldn't bind oxygen?

Sickle Cell Anemia

One single mutation in the gene for the β subunit – located at amino acid six – changes a glutamic acid (a charged amino acid) to valine (a hydrophobic amino acid).

28. Replace **glutamic acid 6** with the valine from the heme group parts bag. Change in the sequence causes sickle cell anemia.

This mutation results in sickle cell anemia. The hydrophobic valine in the β-globin subunits of hemoglobin causes proteins to clump together, forming long chains in the cell. As a result, the normal doughnut shape of the red blood cell is distorted into a crescent shape, similar to the shape of an old fashioned sickle for cutting hay. The sickle-shaped cells tend to clog capillaries and rupture easily.

People with this mutation in both copies of their β-globin gene have fewer red blood cells, resulting in anemia. Symptoms include fatigue (from the anemia and blood clots) and episodes of severe pain (called sickle cell crisis). Some people with the mutation experience a sickle cell crisis infrequently (perhaps only once a year), while others experience crises more frequently.

People with the sickle cell mutation in only one copy of their two β-globin genes do not exhibit the disease, since half of their hemoglobin is normal. They are, however, resistant to malaria. As a result, people in malaria-ridden parts of the world (particularly Africa and India) are at an advantage if they carry one copy of the sickle cell β-globin gene. This is why the sickle cell gene is found more commonly in people who originated from these regions of the world.

3-D Molecular Designs
...where molecules become real ™

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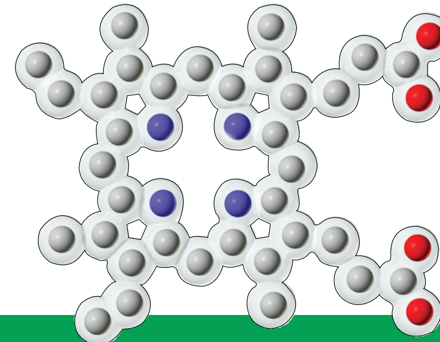

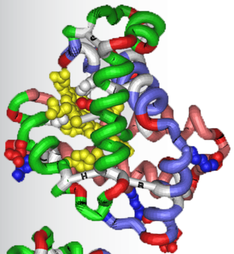

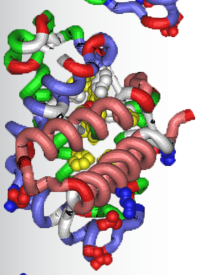

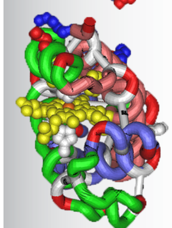

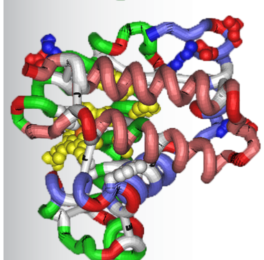

Optional Activities

Optional Activities and Other Resources

1. To learn more about the structure of β-globin, see Jmols at *3dmoleculardesigns.com/Teacher-Resources/-Globin-Folding-Kit/Jmols-and-Tutorials.htm* and follow the directions.

2. Use the 3D Molecular Designs Map of the Human β-Globin Gene© to learn how the DNA sequence for the β-globin gene is translated so it can be synthesized as a protein.

3. Use the 3D Molecular Designs Map of the Human β-Globin Gene© to learn about other mutations of the gene.

4. Use the Internet to discover how fetal β-globin is different from adult β-globin.

5. Use the Internet to research the process hemoglobin undergoes to bind O_2.

6. Use the Internet to research how carbon monoxide poisons the hemoglobin, which then prevents the protein from binding oxygen and transporting oxygen to cells.

What questions do you have about β-globin and/or hemoglobin?