



StarBiochem

## Hemoglobin & Sickle Cell Anemia Exercise

### Learning Objectives

In this exercise, you will use StarBiochem, a protein 3D viewer, to explore the structure of the normal hemoglobin protein and the abnormal form of hemoglobin that results in sickle cell anemia, a genetically inherited blood disorder. These structure will be used to understand how an alteration in the amino acid sequence of the hemoglobin protein results in sickle cell anemia.

### Background

Hemoglobin is the protein in red blood cells responsible for carrying oxygen from the lung to the rest of the body and for returning carbon dioxide from the rest of the body to the lung. Hemoglobin is comprised of multiple protein chains. Each of these chains contains a chemical group called “heme”. Each heme group can bind to an oxygen molecule.

Sickle cell anemia is an autosomal recessive genetic disorder. A single amino acid substitution in hemoglobin is responsible for the development of sickle cell anemia. This single amino acid substitution results in the adherence of hemoglobin proteins to each other, forming long cables that can distort normal red blood cells into sickle shaped cells. Unlike normal red blood cells which are flexible and can fit through small capillaries, sickle red blood cells are inflexible and have the tendency to clog blood vessels. This can result in organ damage and severe pain in individuals with sickle cell anemia.



Normal red blood cell



Sickle red blood cell

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### Getting started with StarBiochem

- To begin using StarBiochem, please navigate to: <http://web.mit.edu/star/biochem>.
- Click on the **Start** button for **StarBiochem Ver2**.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- Under **Samples**, select “1A3N” (the four letter ID for the structure of normal human hemoglobin).

Take a moment to look at the structure from various angles by rotating and zooming on the structure.

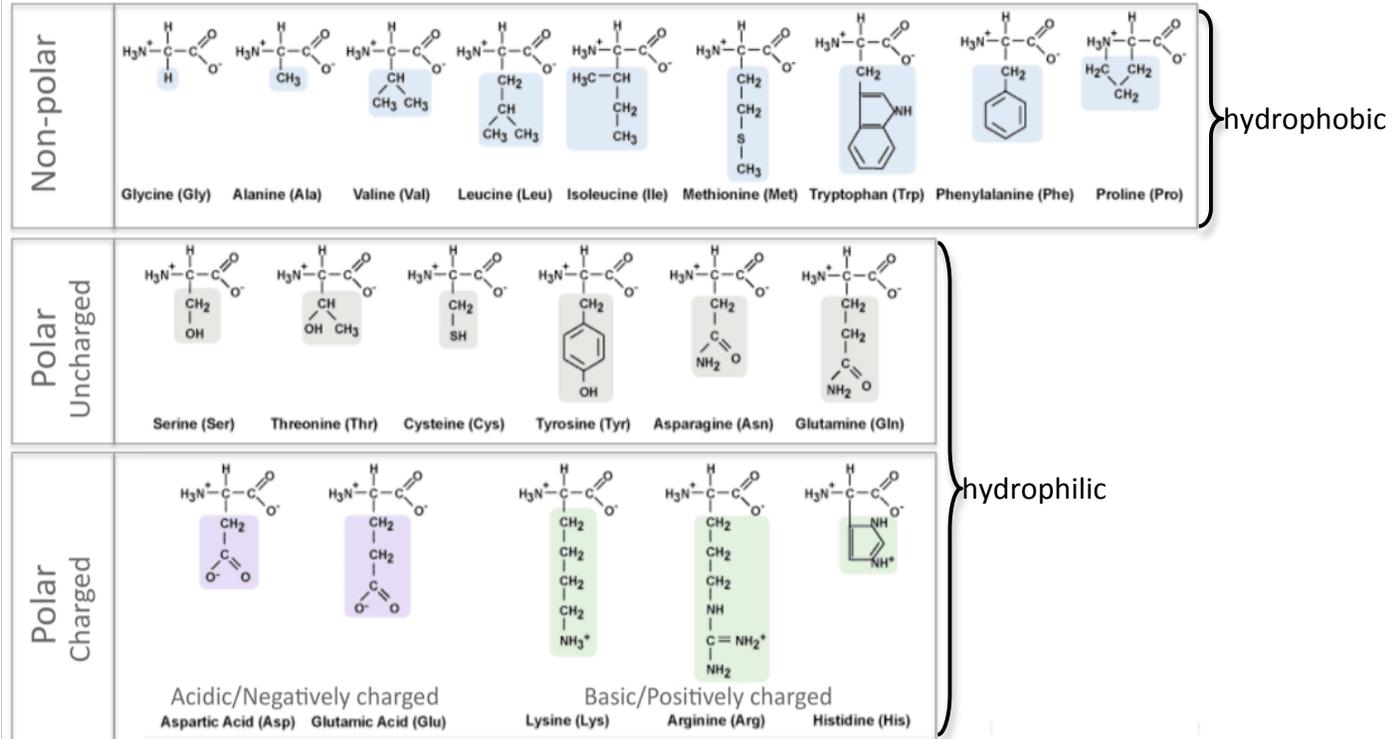
- The following table for changing the view of a structure can be found in the top menu, under **View -> Structure viewing instructions**.

Page 2 contains a series of terms and useful information that you will refer to during this exercise. Briefly, review the terms on this page before proceeding.

# Reference

## CHEMICAL STRUCTURES OF THE AMINO ACIDS

The 20 amino acids share a common backbone and are distinguished by different side chains, also called 'R' groups, highlighted by the various colors below.



## PROTEIN STRUCTURE BASICS

All proteins have the following three levels of protein structure:

### Primary structure

Describes the order of the amino acids in the protein chain but does not describe its shape.

### Secondary structure

Describes shapes that form from local folding of regions within the amino acid chain. These smaller structures can be divided into two main types: helices and sheets. Coils are made of amino acids that do not form regular secondary structures (helices and sheets) but play important roles in protein folding.

### Tertiary structure

Describes the entire folded shape of a protein chain.

In addition, some proteins interact with themselves or with other proteins to form larger protein structures. These proteins have an additional level of protein structure:

### Quaternary structure

Describes how multiple protein chains interact and fold to form a larger protein complex.

## Exercise

We will first explore the structure of normal human hemoglobin and then explore the structure of sickle human hemoglobin to reveal how a single amino acid substitution in this protein leads to the development of sickle cell anemia.

### Protein Structure Questions

The current way you are viewing the structure is by seeing each atom and bond in the protein drawn as a ball and a line, respectively. This way of representing a structure is called the **ball-and-stick** model and is the default model in StarBiochem. The **ball-and-stick** model allows you to see how atoms in the structure bond together. However, the space each atom occupies IS NOT accurately represented. To see a more realistic representation of the atoms in the structure you can use the **space-filled** model, where each atom is drawn as a sphere, whose size represents the physical space an atom occupies.

You can switch from the **ball-and-stick** model to the **space-filled** model in StarBiochem by increasing the size of the atoms in the structure:

- Click on the **Primary** tab. The default atom size is 20% (*ball-and-stick* model).
- Move the **Atoms Size** slider to 100% (*space-filled* model).
- Gray = Carbon, Blue = Nitrogen, Red = Oxygen, Orange = Iron

**1** Hemoglobin is composed of multiple protein chains. Therefore, hemoglobin is said to have a quaternary structure (Reference page 2). **How many protein chains does normal hemoglobin have (1A3N)? Given this, which term best describes hemoglobin: monomer, dimer, trimer, tetramer, or pentamer?**

- In the top menu, under **View**, click on **Reset structure**.
- Click on the **Quaternary** tab. To highlight the different protein chains found within this structure, move the **Surfaces Size** slider all the way to the right to increase the size of the atoms found within the different protein chains and to color each subunit differently.

Answer

**2** **How many amino acids does normal hemoglobin (1A3N) have?**

- In the top menu, under **View**, click on **Reset structure**.
- Click on **Primary**.
- The sequence of amino acids that make up the primary structure of hemoglobin - including their identity, position, and respective protein chain - can be found within the amino acid sequence box (ex: “[Val]1:A” -> amino acid: valine; position:1; protein chain: A)

Answer

**3** Briefly look at the amino acid sequence of each protein chain in normal hemoglobin. **Are the various protein chains within normal hemoglobin (1A3N) likely to be identical?** Answer Yes/No and provide a brief explanation for your answer.

Answer

**4** In addition to amino acids, each protein chain in hemoglobin also contains a chemical group called heme, which binds to oxygen. *Which chemical elements comprise heme groups? How many atoms do you find for each chemical element in a heme group?*

- Within the amino acid sequence box, make sure all the amino acids in hemoglobin have been selected (they should all be highlighted in blue). If not, click on the first amino acid, hold down **Shift** and then click on the last amino acid.
- Move the **Atoms Size** slider completely to the left and the **Bonds Translucency** slider to the right (85%) to minimize the appearance of all the amino acids in hemoglobin.
- To increase the size of the atoms found in the heme groups click on the **Hetero Atoms** tab and move the **Atoms Size** slider to the right.

**Answer**

**5** Tertiary and quaternary structure are formed by the bending and folding of protein chains. These two levels of protein structure are stabilized by various covalent and non-covalent interactions between the side-chains of different amino acids. We will now take a closer look at two amino acids involved in the tertiary structure of hemoglobin: amino acids 85 and 88 in the 2<sup>nd</sup> protein chain (protein chain B).

*Based on the nature of their side-chains, what type of interaction do amino acids 85 and 88 in the 2nd protein chain form with each other's side chains?* Your choices are 'a covalent bond', 'an ionic bond', 'a hydrogen bond', 'a hydrophobic interaction' or 'a van der Waals interaction'?

Explain your choice.

- *Optional:* This set of steps are not necessary for answering this question but will illustrate how you can use StarBiochem to visualize the interaction between these two amino acids.
- In the top menu, under **View**, click on **Reset structure**.
- Within the **Protein** tab, click on the **Tertiary** tab.
- Within the amino acid sequence window, select amino acids 85 and 88 only: click on amino acid 85, hold down **Control** and **Alt** [PC/Linux]/**Control** and **Apple** [Apple] and then click on amino acid 88. Move the **Atoms Size** slider to the right to increase the size of the atoms found in these two amino acids.
- Within the **Tertiary** tab, click on the different amino acid type choices to explore the categories that these two amino acids belong to.

**Answer**

## Structure -> Function -> Disease Questions

**6** We will now compare the structure of normal hemoglobin (1A3N) with that of sickle hemoglobin (2HBS) to understand how a single amino acid change in hemoglobin leads to sickle cell anemia. We will first begin by exploring overall differences between the two structures.

- In the top menu, under **View**, click on **Reset structure**.
- Under **Samples**, select "2HBS" (the four letter ID for the structure of sickle human hemoglobin). A new tab will appear showing the 2HBS structure right next to the 1A3N structure tab.

a) How does the overall structure of normal hemoglobin (1A3N) differ from that of sickle hemoglobin (2HBS)? Describe and explain in your own words.

Answer

b) Circle the correct statement from the options below.

The single amino acid substitution in sickle hemoglobin:

Answer

...influences the overall structure of an individual hemoglobin protein

...does not influence the overall structure of an individual hemoglobin protein

7 We will now explore the specific alteration in the hemoglobin protein that results in the development and manifestations of sickle cell anemia. A single amino acid substitution at position 6 in two of hemoglobin's protein chains to the amino acid valine results in sickle cell anemia.

a) Identify the protein chains (A, B, C, etc) in sickle hemoglobin (2HBS) with this specific amino acid substitution.

Answer

b) Name the amino acid present in normal hemoglobin (1A3N) that is substituted to valine at position 6 in sickle hemoglobin (2HBS). How does this amino acid differ from valine?

Answer

c) Where is the amino acid at position 6 that is substituted to valine in sickle cell anemia located: on the inside or the outside of the normal hemoglobin protein(1A3N)?

- In the **Protein** tab of the 1A3N structure, click on the **Primary** tab. Within the amino acid sequence window, click on the specific amino acid(s). Move the **Atoms Size** slider to the right to increase the size of the selected amino acid(s).

Answer

8 In the sickle hemoglobin structure (2HBS) valine 6 in one sickle hemoglobin protein interacts with phenylalanine 85 and leucine 88 in another sickle hemoglobin protein.

a) Identify which valine 6 and which phenylalanine 85 and leucine 88 interact in this structure (2HBS) by stating the protein chains (A, B, C, etc) in which each of them are found.

- In the **Protein** tab of the 2HBS structure, click on the **Primary** tab. Within the amino acid sequence window, select the desired amino acids one at a time by individually clicking on each and simultaneously holding down **Control** and **Alt** [PC/Linux]/**Control** and **Apple** [Apple]. Move the **Atoms Size** slider to the right to increase the size of the atoms found in the selected amino acids.

**Answer**

**b) What is the most likely type of interaction between valine 6, phenylalanine 85 and leucine 88?** Explain your answer.

**Answer**

**c) In unaffected, normal patients, the amino acid located at position 6 in either of the two protein chains of normal hemoglobin does not interact with phenylalanine 85 and leucine 88 in another normal hemoglobin protein. Why?**

**Answer**

**d) The sickle hemoglobin structure (2HBS) reveals a snapshot of how the “sickle hemoglobin cables” may form in patients with this disease. Taking into account what you have learned in this exercise, propose an explanation for how “sickle hemoglobin cables” form. Be specific in your answer.**

**Answer**

## Keywords

Sickle cell anemia, and autosomal recessive genetic disorder.

## Extra Credit Questions

**1** Sickle cell anemia patients are often asked to avoid dehydration by increasing their fluid intake significantly. Explain how this medical recommendation may help these patients.

**2** Abnormalities in the hemoglobin protein account for a variety of genetically inherited disorders such as sickle cell anemia and thalassemia. The genetic mutations responsible for these diseases are much more common in certain regions of the world, i.e. Africa, Eastern Europe and South East Asia. Propose how certain mutations in the hemoglobin gene could have been selected for and favored in certain regions of the world.