



StarBiochem

## Hemoglobin & Sickle Cell Anemia Exercise

### Background

Hemoglobin is the protein in red blood cells responsible for carrying oxygen from the lungs to the rest of the body and for returning carbon dioxide from the rest of the body to the lungs. A single amino acid substitution results in sickle cell anemia, which is characterized by the adherence of hemoglobin proteins to one another resulting in long cables that distort red blood cells into sickle-shaped cells. Unlike normal red blood cells that are flexible and can fit through small capillaries, sickle-shaped red blood cells are inflexible and have the tendency to clog blood vessels, which results in organ damage and severe pain in individuals with sickle cell anemia.



Normal red blood cell



Sickle red blood cell

[www.carnegieinstitution.org](http://www.carnegieinstitution.org)

### Learning Objectives

We will explore how a single alteration in the amino acid sequence of the hemoglobin protein results in sickle cell anemia, a genetically-inherited blood disorder.

### Getting started

In this exercise, we will explore the structures of normal and sickled human hemoglobin using a molecular 3-D viewer called StarBiochem.

- To begin using StarBiochem, please navigate to: <http://mit.edu/star/biochem/>.
- Click on the **Start** button for **StarBiochem**.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- In the top menu, click on **Samples** → **Select from Samples**. Within the **Amino Acid/Proteins** → **Protein** tab, select “Deoxyhemoglobin – H. sapiens (1A3N)”. 1A3N is the four-character 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.

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

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).
- Notice that different atoms are slightly different in size.
- Gray = Carbon, Blue = Nitrogen, Red = Oxygen, Yellow = Sulfur, Orange = Iron.

The last page, Reference page, contains useful information that you will refer to during this exercise.

## Exercise

### Hemoglobin's Protein Structure Basics

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

- In the top menu, click **Reset** → **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 code each protein chain.

**Answer**

**2** What is the first and last amino acid of each protein chain in hemoglobin? How many amino acids does each protein chain have?

- Click on the **Primary** tab.
- 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 hemoglobin protein chain. Are any of the protein chains likely identical to the other protein chains within normal hemoglobin? If so, which ones?

**Answer**

**4** Each protein chain in hemoglobin is bound to a chemical group called “heme”, which binds to oxygen. Which chemical elements comprise heme groups?

- In the top menu, click **Reset** → **Reset structure**.
- To visualize only one of the heme groups within the protein, under **View**, click on **View Specific Regions/Set Center of Rotation**.
- Click on the **Non-Peptide** tab. Click on one of the four heme groups ([HEM]) present within this tab. Move the **Show atoms within these Van der Waals radii of selected regions (VDW Radius)** slider completely to the left to make the other heme groups and the rest of the protein disappear.

**Answer**

## Sickle Cell Anemia Questions

We will now compare the structure of hemoglobin (1A3N) with that of sickled 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, click **Reset** → **Reset structure** to reset the structure of hemoglobin (1A3N).
- In the top menu, click on **Samples** → **Select from Samples**. Within the **Amino Acid/Proteins** → **Protein** tab, select “Deoxyhemoglobin S – H. sapiens (2HBS)”. 2HBS is the four-character ID for structure of sickled human hemoglobin.

Unlike the hemoglobin structure that you have examined thus far (1A3N), the sickled hemoglobin structure contains two identical hemoglobin proteins. In the sickled hemoglobin structure (2HBS), protein chain A in the first hemoglobin protein is the same as protein chain E in the second hemoglobin protein, protein chain B in the first protein is the same as protein chain F in the second protein, etc.

**5** Does the single amino acid substitution in sickled hemoglobin (2HBS) influence the overall structure of one hemoglobin protein? Describe and explain in your own words.

**Answer**

**6** We will now explore the specific amino acid substitution in the hemoglobin protein that results in the development and manifestations of sickle cell anemia. Hemoglobin is made up of four protein chains: two identical alpha chains (A and C protein chains) and two identical beta chains (B & D protein chains). An amino acid substitution to valine at position 6 causes sickle cell anemia.

**a)** Which two protein chains (A, B, C, etc.) within the two sickled hemoglobin proteins (2HBS) contain the valine amino acid substitution? Are the amino acid substitutions found within sickled hemoglobins' alpha or beta chains? Remember that the sickled hemoglobin structure you are examining contains two identical sickled hemoglobin proteins.

**Answer**

**b)** What amino acid is present at position 6 within these same two protein chains in normal hemoglobin (1A3N)? How does this amino acid differ from valine? See Reference page.

**Answer**

**c)** Is the amino acid at position 6 that is substituted to valine in sickled hemoglobin (2HBS) located on the inside or the outside of the normal hemoglobin protein (1A3N)?

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

**Answer**

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

- Within the **Protein** tab of the 2HBS structure, click on the **Primary** tab.
- Within the amino acid sequence window, select the desired amino acids by:  
**PC/Linux:** Click on the first amino acid, hold down **Control** and **Alt** and then click on the second amino acid.  
**Mac:** Click on the first amino acid, hold down **Control** and **Apple** and then click on the second amino acid.
- Within the **Primary** tab, 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? Your choices are: 'a covalent bond', 'an ionic bond', 'a hydrogen bond', 'a hydrophobic interaction' or 'a van der Waals interaction? Explain your choice.

**Answer**

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

**Answer**

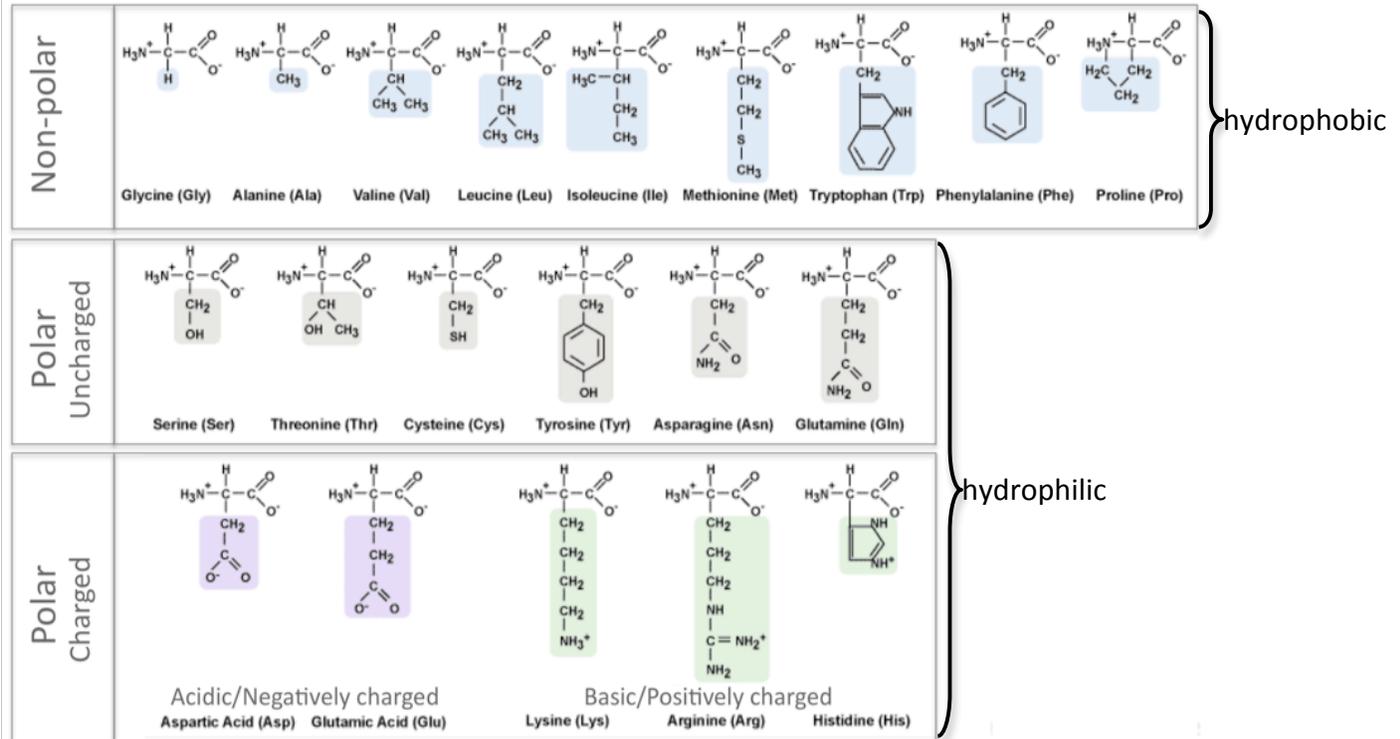
d) The sickled hemoglobin structure (2HBS) reveals a snapshot of how the "sickled hemoglobin cables" may form in patients with this disease. Taking into account what you have learned in this exercise, propose an explanation for how "sickled hemoglobin cables" form. Be specific.

**Answer**

# 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 four 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.