StarBiochem

Name_____

Hemoglobin & Sickle Cell Anemia Exercise

Learning Objectives

In this exercise, you will use StarBiochem, a protein 3D viewer, to explore:

- the structure of the hemoglobin (Hb) protein
- the structure of the abnormal form of hemoglobin (HbS) that results in sickle cell anemia, a genetically inherited blood disorder
- the specific amino acid substitution in HbS that causes sickle cell anemia

Background

Hemoglobin (Hb) is a protein that functions by binding to the oxygen molecules (O_2) in the O_2-rich environment of the lungs, traveling to the rest of the body within red blood cells in the circulatory system, and then releasing O_2 rapidly in the relatively O_2-poor environment of various body tissues. Hemoglobin has the capacity to bind between 1 to 4 O_2 molecules. The binding of each O_2 molecule to hemoglobin increases its affinity for the next O_2 molecule.

The Hb protein is comprised of polypeptide chains called "globin "chains. Each of these globin chains is attached to the iron containing "heme" group.

A single amino acid substitution in a specific globin chain of Hb results in the HbS form of the protein. The HbS molecules may adhere to each other, forming large complexes that can distort normal red blood cells (RBC) into sickle shaped cells. The sickled RBCs have a reduced life span. Additionally, the sickled RBCs can clog blood vessels, which can lead to organ damage and pain in individuals with sickle cell anemia.

Normal red blood cell

Sickle red blood cell

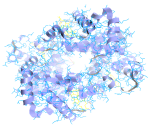
www.carnegieinstitution.org

Sickle cell anemia is a genetic disorder that shows an autosomal recessive mode of inheritance. The prevalance of this disorder in United States is approximately 1 in 5000 individuals, and it mostly affects African Americans, South Asians and Hispanics.

Getting started with StarBiochem

- To being using StarBiochem, please navigate to: **http://web.mit.edu/star/biochem**.
- Click on the **Start** button to launch the application.
- Click **Trust** when a prompt appears asking if you trust the certificate.
- Under **File**, click on **Open/Import** and select "1A3N" and click **Open**.

You are now viewing the structure of human hemoglobin (1A3N), with each bond in the protein drawn as a line ("bonds only" view).



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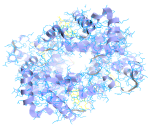
Practice changing the viewpoint of this protein in the view window:

	Mac	PC
TO ROTATE	**click** and **drag** the mouse	**left-click** and **drag** the mouse
TO MOVE UP/DOWN RIGHT/LEFT	**apple-click** and **drag** the mouse	**right-click** and **drag** the mouse
TO ZOOM	**option-click** and **drag** the mouse	**Alt-left-click** and **drag** the mouse

Take a moment to look at the structure of human hemoglobin (1A3N) from various angles in this "bonds only" view. Before proceeding to answer the questions, you should review the basic structures and terms on the next page which you may refer to during this exercise.



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PROTEIN STRUCTURE BASICS

Each protein has the following three levels of protein structure:

Primary structure
Lists the amino acids that make up a protein's sequence, but does not describe its shape.

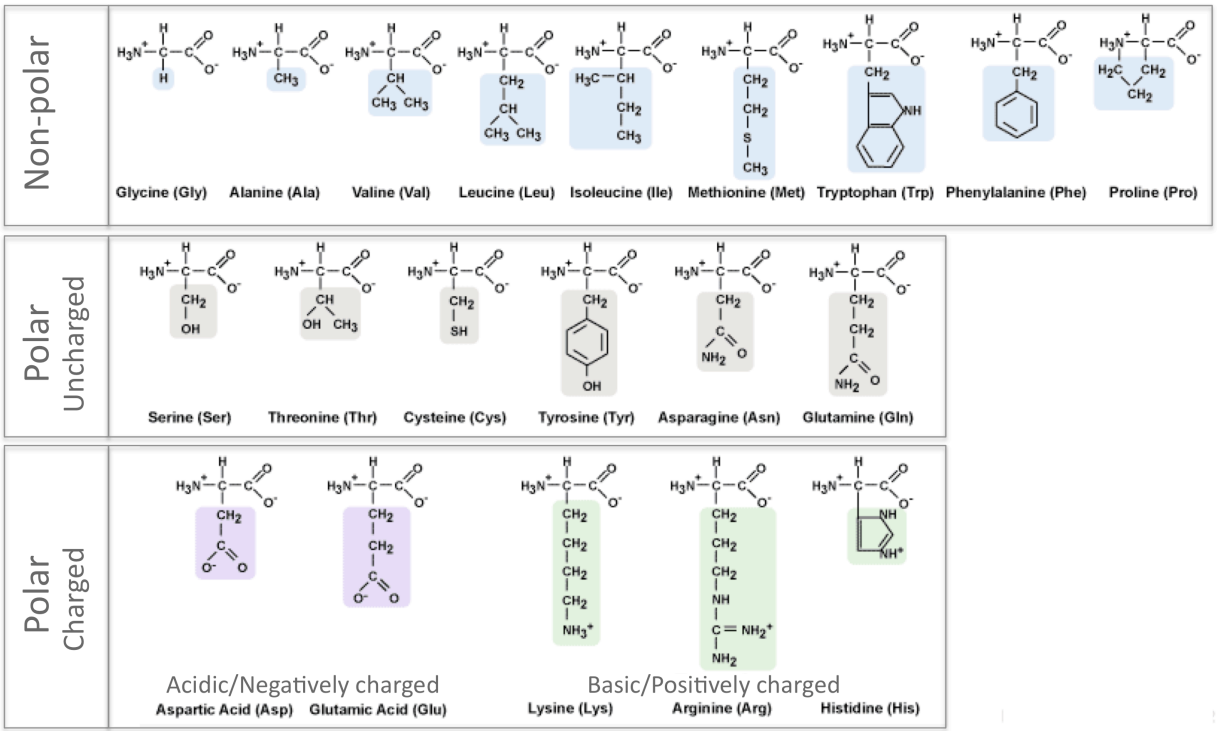
Secondary structure
Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

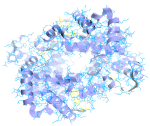
Tertiary structure
Describes the entire folded shape of a whole protein chain.

In addition, some proteins interact with themselves or with other proteins to form larger protein structures. How these proteins interact and fold to form a larger protein complex is termed **Quaternary structure**.

CHEMICAL STRUCTURES OF THE AMINO ACIDS

The 20 amino acids share a common backbone and are distinguished by different 'R' groups, highlighted in various colors below.





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Protein Structure Questions - Level 1

1 ***How many amino acids comprise the primary structure of hemoglobin (1A3N)?***

- Click on **Structure**.
- Click on **Primary** which shows the amino acids that are sequentially joined through peptide bonds to make the amino/polypeptide chain. The amino acids of each chain are highlighted by a specific color and can be distinguished from those of other chains.

Answer

2 ***How many monomer globin chain(s) do you see in the current view of hemoglobin (1A3N)? Given this, which term best describes the structure of hemoglobin: a monomer, dimer, trimer, tetramer, or pentamer?***

- To distinguish between the different monomers that make up 1A3N, under **Structure** click on **Quaternary**.
- Click on **Chain**.

Answer

3 Briefly look at the primary sequence of each monomer/protein chain. ***Are the protein chains within hemoglobin (1A3N) likely to be identical or different?*** Answer Yes/No and provide a brief explanation for your choice.

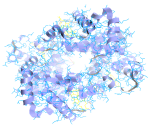
- Within **Structure**, click on **Primary**.

Answer

4 In addition to containing amino acids, hemoglobin also contains four chemical groups called hemes, which bind to the oxygen in our blood stream. ***Which elements comprise the structure of heme groups? How many atoms of each of these elements are present per heme group?***

- Click on **View** and choose **Reset Molecule**.
- Click on **PDB Tree** and then click on the file labeled **"1A3N"**.
- Click on all Heme groups while holding down **shift** to select them at the same time.
- In **View Controls**, set the **Unselected** transparency slider to **"0.2"**.
- Within the **Atoms** box, click on **Draw** to see what atoms are present. Each atom is color-coded: Carbon is grey, Nitrogen is blue, Oxygen is red and, in this structure, Iron is orange.

Answer



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Protein Structure Questions - Level 2

5 Follow the instructions provided below to answer the next set of questions.

- Under **Selection Controls**, click on **Residues**.
- Go to **Measurement Tools** and click on **Enable Radius**.
- From the pull down menu choose **Residues**.
- Slide the **Within Radius** slider until it reads "**7.61"** and click on **Select Within Radius.**
- Go back to **Structure** and under **Primary** look at the amino acid residues that are being highlighted.
- Click on **View Controls** and bring the **Unselected** slider to "**0"** while keeping the **Selected** slider at **"1"**. You may zoom in the selected amino acid for a better view.

a) ***Identify the globin chain(s) (1, 2, 3 and/or 4) that contain the highlighted amino acids.***

Answer

b) ***In the 1st globin chain, name the amino acid that is closest to the N-terminus end.*** Explain why you selected this amino acid.

Answer

c) ***From the choices provided below, select the level of protein structure that is represented by the highlighted amino acids*****.** Your choices are 'primary', 'secondary', 'tertiary' and 'quaternary'. Select all that apply and explain why you selected a specific option.

Answer

d) ***Which of these highlighted amino acids can pair together to ….***

- ***form hydrogen bonds?***
- ***exhibit hydrophobic interactions?***

Answer

6 Tertiary and quaternary structure are formed by the bending and folding of peptide chains. These two levels of structure are stabilized by various covalent and non-covalent interactions between the side-chains of different amino acid residues. We will now take a deeper look at the amino acids involved in the tertiary structure of hemoglobin: amino acids #85 & #88 in the 2nd globin chain. ***Based on the nature of their side-chains, how would you characterize these amino acids?*** Your choices are 'ionic', 'hydrogen bonding, 'van der Waals forces', 'hydrophobic' or 'covalent'.

- Under **Structure**, click on **Primary**.
- Select the amino acids by individually clicking on them and simultaneously pressing **Control** and **Apple** key(Mac)/**right-click** (PC).
- Go to **Tertiary** and within the **Color by Residue** window click on each option one at a time.

Answer

Structure -> Function -> Disease Questions

7 We will now take a look at the structure of sickle hemoglobin, HbS (2HBS), and compare its structure to that of normal (wild type) hemoglobin, Hb (1A3N), to understand how a single amino acid change in hemoglobin leads to sickle cell anemia.

- Click on **View** and choose **Reset Molecule**.
- Open a new window of StarBiochem while keeping the structure of Hb (1A3N) open.
- In the top menu under **File** click on **Open/Import**.
- Click on "2HBS" and click **Open**.

a) ***Comparing the crystal structures of the two PDB files, 2HbS and 1A3N, how does the overall structure of normal (wild type) hemoglobin differ from that of sickle hemoglobin?***

Answer

b) Carefully look at the PDB structure of the two molecules within 2HBS and the molecule within 1A3N. ***Circle the correct statement(s) from the options below.*** The single amino acid substitution in sickle hemoglobin**:**

Answer

- influences the overall structure of individual Hb molecules.
- does not influence the overall structure of individual Hb molecules.
- creates sticky regions between two individual Hb molecules.

8 The single amino acid substitution of valine at position #6 in a specific globin chain of hemoglobin results in sickle cell anemia.

a) ***Identify the globin chain(s) in HbS (2HBS) where you observe this amino acid substitution.***

Answer

b) ***Name the amino acid present in normal hemoglobin, Hb (1A3N), that is being substituted by valine 6 in sickle hemoglobin, HbS (2HBS).***

Answer

9 In HbS (2HBS), valine 6 in a specific globin chain interacts with phenylalanine 85 and leucine 88 located in the globin chain of another HbS molecule.

a) ***In the HbS structure (2HBS), identify the globin chains (i.e. 1, 2, 3, 4) that contain these three amino acids in a configuration that allows them to interact with each other.***

- Under **Structure** click on **Primary**.
- Select more than one amino acid residue by by individually clicking on them and simultaneously pressing **Control** and **Apple** key(Mac)/**right-click** (PC).
- The amino acids you select get highlighted in the structure (white). For a better view you can go to **View Controls** and move the **Unselected** transparency slider to **"0"**.

Answer

b) ***What is the most likely interaction between valine 6 and phenylalanine 85 and leucine 88?*** Please explain.

Answer

c) In question 8 (b) of this exercise you have identified the amino acid located at position #6 in normal hemoglobin, Hb (1A3N). This amino acid, unlike valine 6 in sickle hemoglobin (2HBS), does not interact with phenylalanine 85 and leucine 88. ***Propose an explanation for this observation.***

Answer

d) ***Based on what you have learned from this exercise, explain why an amino acid substitution to valine at position 6 results in sickle cell anemia.***

Answer

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Keywords:

Sickle cell anemia, essential amino acids, oxyhemoglobin or saturated hemoglobin, deoxyhemoglobin or desaturated hemoglobin, and autosomal recessive genetic disorder.

Thought Questions

1 Sickle cell patients are very often asked to avoid dehydration by significantly increasing their fluid intake. Explain how this 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 nature could have selected for the mutant copy of the hemoglobin gene in certain regions of the world.

3 World class tennis player, Peter Sampras, and football star Zinedine Zidane are thalassemia carriers. These players perform much better in short versus long lasting matches. Based on what you have learned about hemoglobin from this exercise, explain why this may be so.