Name___________________________

StarBiochem

DNA Repair Protein Exercise

Background

In this exercise, you will use StarBiochem, a protein 3-D viewer, to explore the structure of a DNA repair protein found in most species, including bacteria. DNA repair proteins move along DNA strands, checking for mistakes. DNA glycosylases, a specific type of DNA repair protein, recognize DNA bases that have been chemically altered and remove them, leaving a site in the DNA without a base. Other proteins then come along to fill in the missing DNA base.

We will begin this exercise by examining the structure of one of the human DNA glycosylases called human 8-oxoguanine glycosylase or **hOGG1**.

Learning objectives

The structure of hOGG1 will be used to explore the relationship between a protein's structure and its function.

Getting started with StarBiochem

- To begin using StarBiochem, please navigate to: http://mit.edu/star/biochem/version_2.0.
- Click on the Start button and select the version for your operating system.
- Save the file and open or run it as directed depending on what operating system you are using.
- Under Samples, select "1EBM" (the four letter ID for hOGG1).

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.

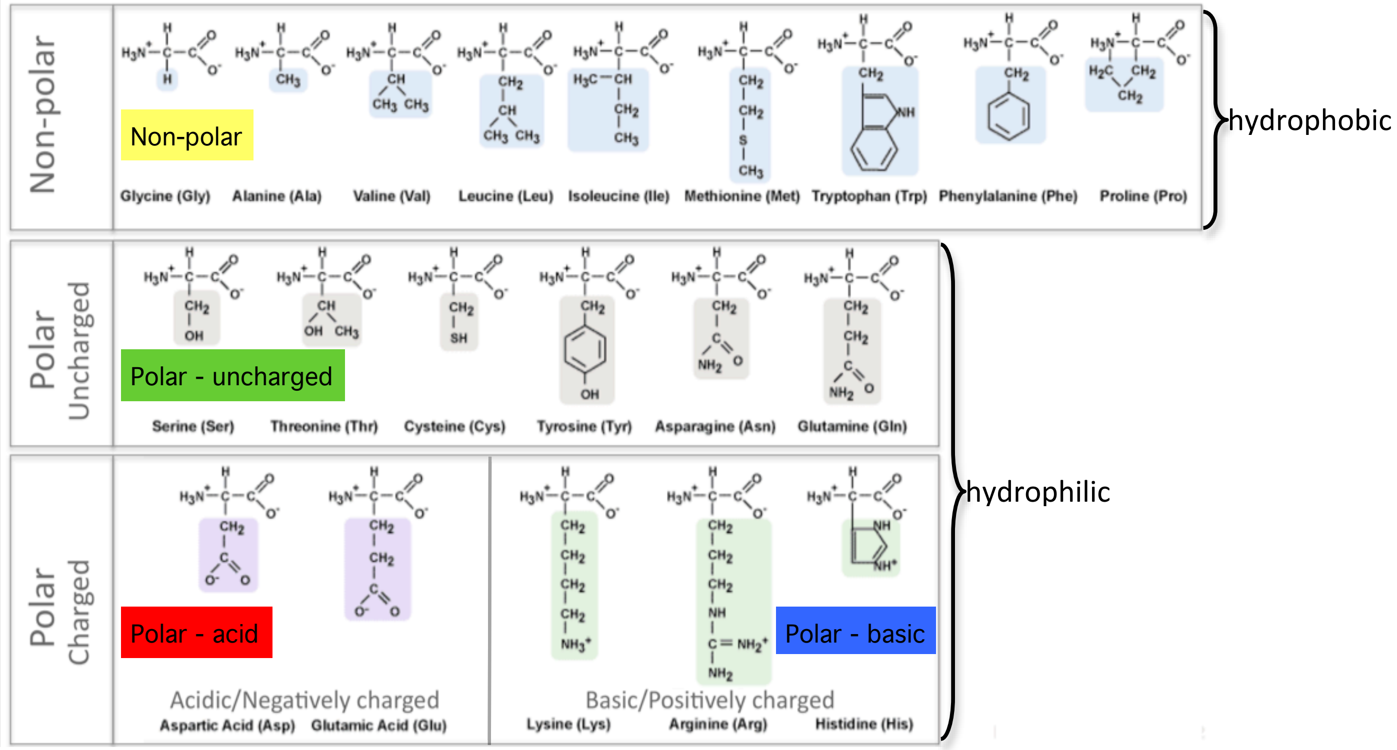
	Windows	Mac	Unix/Linux
Rotate	Click + drag	Click + drag	Click + drag
Zoom	Click + Shift + Vertical drag	Click + Shift + Vertical drag	Click + Shift + Vertical drag
Spin	Click + Shift + Horizontal drag	Click + Shift + Horizontal drag	Click + Shift + Horizontal drag
Move	Click + Ctrl + Alt drag	Click + Ctrl + Option drag	Click + Ctrl + Alt drag

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

The human protein hOGG1 interacts with DNA to help repair damaged DNA bases. In this particular structure, the repair protein is bound to a segment of DNA that has been damaged. We will first focus on the structure of the repair protein and then on how this repair protein interacts with DNA to repair a damaged DNA base.

hOGG1's Protein Structure Basics

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 allow for 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

1 ***Can you identify the DNA and the hOGG1 protein in the structure (1EBM)? Where is the DNA segment?*** Describe in your own words.

Answer

2 hOGG1 contains multiple sulfur atoms. ***Identify the name and sequence number of one of the amino acids in the structure that contains a sulfur atom. Is the sulfur atom in the backbone or the side chain of the amino acid?***

- Reduce the size of the atoms in the structure back to 20%.
- Using your mouse, zoom in and point to a sulfur atom (yellow) in the structure. A small box appears on top of the mouse curser indicating the name of the amino acid and its position in the amino acid sequence (ex: [Ala]223:A CB # 2257 -> amino acid: alanine; position: 223).
- To find if this sulfur atom is in the backbone or the side chain of this amino acid, refer to Reference page 2.
- *Optional:* decrease the size of all the atoms to 0%. In the amino acid sequence window, click on the amino acid you have identified. Unclick the sidechain box and move the Atoms Size slider to 100% to only visualize the atoms that are part of the backbone of that particular amino acid. Zoom out if necessary. Click on the sidechain box to now visualize the atoms that contribute to the side chain.

Answer

3 Next, will explore the primary structure of the hOGG1 protein (Reference page 2). The hOGG1 protein consists of 325 amino acids. *List the 13 amino acids numbered 105 through 117 in order.*

- In the top menu, under View, click on Reset structure.
- Click on the Primary tab. Refer to page 2 for the complete name of each amino acid.

Answer

105 _______ 106 _______ 107 _______ 108 _______
109 _______ 110 _______ 111 _______ 112 _______
113 _______ 114 _______ 115 _______ 116 _______
117 _______

4 Within a protein chain, amino acids form local structures called secondary structures (Reference page 2).

a) Explore the secondary structures found in hOGG1. *Are helices, sheets or coils present in hOGG1 (1EBM)? Describe the color that represents the secondary structures you observe.*

- Click on the Secondary tab.
- To see <u>each</u> of these structures <u>one at a time</u> (ex: helices), unclick the structures you do not want to see at <u>this</u> time (ex: sheets and coils) and move the Structures Size slider to the right to increase the size of the selected structure (ex: helices).
- Visualize another secondary structure by clicking the box next to it (ex: sheets). Repeat this step to visualize the presence of another secondary structure.

Answer

Helices _______ (Yes or No) Sheets _______ (Yes or No) Coils _______ (Yes or No)

b) Amino acids 105 through 117 fold into one of the secondary structures. *Which secondary structure do they fold into?*

- Within Secondary, click on All. Move the Structures Size slider to the left to decrease the size of all secondary structures.
- Within the amino acid sequence window, select amino acids 105-117: click on amino acid 105, hold down Shift and then click on amino acid 117.
- Move the Structures Size slider to the right to increase the size of the secondary structures within amino acids 105-117.

Answer

5 Now we will explore the relationship between DNA glycosylase's structure and one of the several types of amino acids that contribute to hOGG1's overall shape, its tertiary structure (Reference page 2).

a) Negatively charged amino acids are hydrophilic (Reference page 2). *Are the negatively charged amino acids located on the <u>inside</u> (buried) or <u>outside</u> (exposed) of this protein? What does that suggest about the cellular environment surrounding this protein, is it hydrophobic or hydrophilic?* Explain your answer.

- Reset the structure.
- Click on the **Primary** tab. Move the **Atoms Size** slider to the left to decrease atom size for all the atoms in the protein.
- Click on the **Tertiary** tab.
- Click on the **– charged/acidic** button. Move the **Atoms Size** slider to the right to increase the atom size for the negatively charged/acidic amino acids.

Answer

hOGG1's Interaction with DNA

6 Now let's explore how DNA glycosylase interacts with DNA to recognize the damaged DNA base within its sequence. DNA is composed of four bases: Adenine (A), Thymine (T), Cytosine (C) and Guanine (G). In this particular structure (1EBM), the hOGG1 protein is bound to a segment of DNA that contains an oxidized guanine. First, let's make the DNA segment more visible.

- Reset the structure.
- Click on the **Nucleic Acids** tab. Move the **Atoms Size** slider to the right (35%) to increase the size of the DNA segment.
- Within the **Protein** tab, click on the **Primary** tab. 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 the hOGG1 protein.
- Click on the **Water** tab. Move the **Atoms Size** slider completely to the left to hide the water atoms present in this structure.

Second, let's take a closer look at how the DNA bases are oriented within the double helix. Each base is attached to a sugar and phosphate backbone to form a complete nucleotide. Within the double helix, a base within one strand pairs with another base from the opposite strand by hydrogen bonding forming a base pair: Adenine (A) base pairs with Thymine (T) and Cytosine (C) base pairs with Guanine (G).

a) *How many DNA base pairs can you count within this double helix?*

- Click on the **Nucleic Acids** tab.
- Unclick **phosphates** and **sugars**. Move the **Atoms Size** slider to the left to decrease the size of the bases while leaving the size of the phosphates and sugar nucleotide components intact. This will allow you to count the base pairs more easily.

Answer

b) *How many DNA bases are unpaired (not paired to its partner on the other strand)?*

Answer

c) *Is the oxidized guanine base paired or unpaired? Describe the position of the oxidized guanine with respect to the hOGG1 protein and the double helix. What does this suggest about the mechanism that hOGG1 uses to identify damaged DNA bases?*

- Click on the **Hetero Atoms** tab. Move the **Atoms Size** slider to the right (100%) to increase the size of the atoms in the oxidized guanine ([8OG]25).

Answer

7 *(Optional)* Certain amino acids within hOGG1 form contacts with the DNA and are able to recognize if a guanine base has been damaged by oxidation. ***Where are you more likely to find the amino acids that recognize damaged guanine bases within hOGG1, in Helix 1 or Helix 16?*** Explain why.

- Follow the first set of instructions in question 6 for making the DNA segment more visible.
- Click on the **Hetero Atoms** tab. Move the **Atoms Size** slider to the right (100%) to increase the size of the atoms in the oxidized guanine ([8OG]25).
- Within the **Protein** tab, click on the **Secondary** tab.
- Within the amino acid sequence box, select the amino acids within Helix 1. Move the **Structure Size** slider to the right to increase the size of Helix 1. Select the amino acids within Helix 16.
- *Optional:* These set of steps will allow you to see if any of the side chains within Helix 1 or Helix 16 contact the oxidized guanine ([8OG]25). Click on the **Primary** tab. Select the amino acids within Helix 1. Unclick **backbone**. Move the **Atoms Size** slider to the right to visualize the side chains of the amino acids within Helix 1. Select the amino acids within Helix 16.

Answer