StarBiochem

Name_________________________

DNA Glycosylase: a case study in protein evolution

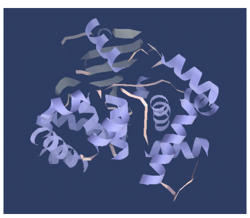
Learning Objectives

- Proteins with similar functions have similar 3D structures
- Proteins with similar function and 3D structures do not need to have identical amino acids sequences.

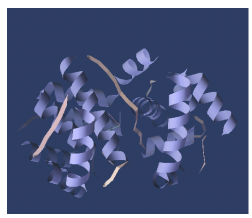
Background

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. DNA repair proteins move along DNA strands, checking for mistakes. DNA glycosylases, a specific type of DNA repair proteins, 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 exploring the structure of one of the human DNA glycosylases called **hOGG1**. We will then explore the structure of a related DNA glycosylase protein found in archaebacteria called ***Pa*-AGOG**.



hOGG1



Pa-AGOG

Getting started with StarBiochem

- To get to 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 "1EBM" (the four letter ID for hOGG1) and click **Open**.

You are now viewing the structure of the human DNA glycosylase hOGG1 (1EBM), with each bond in the protein drawn as a line.

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 hOGG1 (1EBM) from various angles in this "bonds only" view. Before proceeding to answer the questions, review the basic structures and terms on the next page which you can refer to during this lesson.

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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.

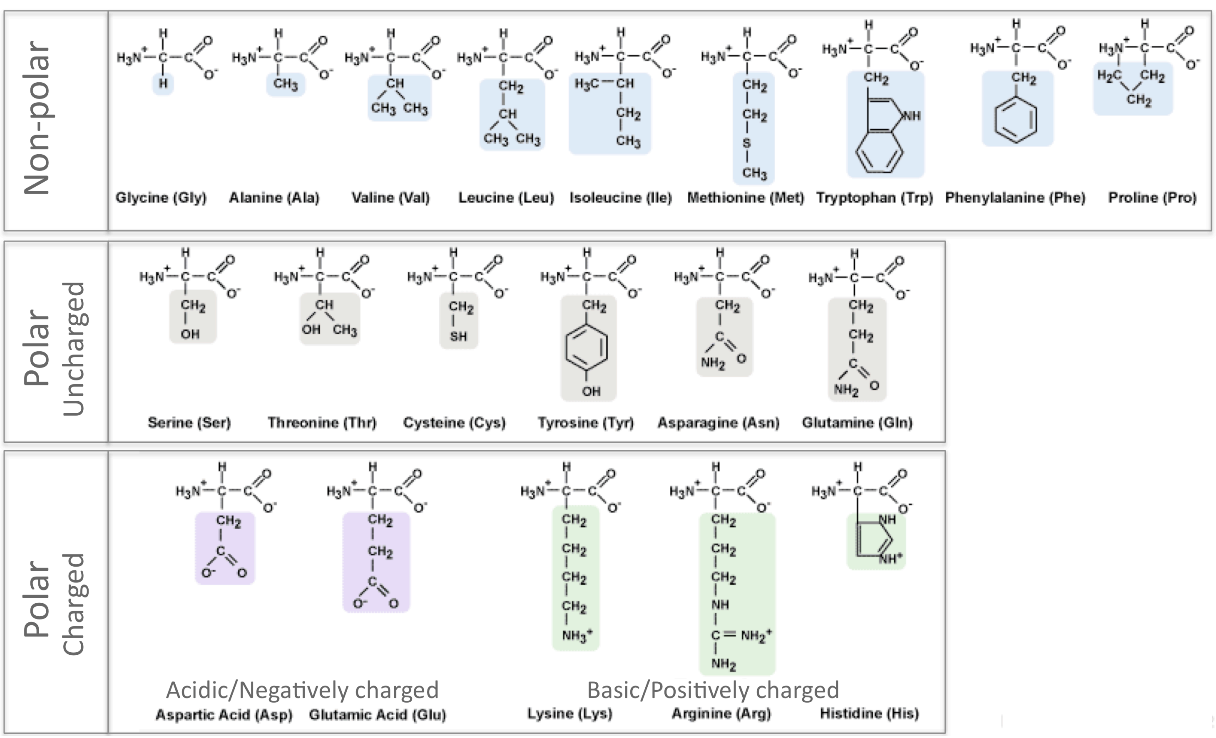
Secondary structure
Describes regions of local folding that form a specific shape, like a helix, a sheet, or a coil.

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

1 The protein hOGG1 (1EBM) interacts with DNA to help repair damaged DNA bases. In this particular structure, the hOGG1 protein is bound to a segment of DNA that has been damaged. ***What color is the protein? What color are the DNA strands?***

- To help distinguish the DNA segment from the hOGG1 protein click on **Structure**.
- Click on the forward arrow until you see **Quaternary**.
- Click on **Quaternary** and then click on **Chain**.

Answer

2 The hOGG1 (1EBM) protein consists of 325 amino acids. ***List the 13 amino acids numbered 105 through 117 in order.***

- Within **Structure**, click on **Primary**.
- Scroll down through the amino acid list if necessary.

Answer

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

3 Within a protein, amino acids sequences form local structures called secondary structures (reference page 2). Secondary structures include helices, sheets and coils.

a) Explore the secondary structures found in hOGG1 (1EBM). ***Are helices, sheets, and/or coils present in hOGG1 (1EBM)?***

- Under **Structure** click on **Secondary**.
- Click on **Helices**, **Sheets** and **Coils** *one at a time* making sure to click each one off before clicking the next one.

Answer

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

b) ***Which secondary structure predominates in the hOGG1 (1EBM) protein?***

Answer

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

- Within **Secondary**, click on **All Ribbons**.
- Click on **Primary**.

- Select all amino acids between 105 through 117 by clicking on amino acids 105 and 117 while holding down **Shift** (Mac/PC).
- Rotate the protein to locate the selected amino acids (white).

Answer

4 DNA is composed of four bases: A, T, C & G. The DNA sequence illustrated in this structure, contains a damaged DNA base

- Under **View**, click on **Reset molecule**.
- Click on **PDB Tree**, **IEBM** and then click on **8OG_25**.
- The highlighted structural element (white), 8OG_25 (8-oxoguanine), is a type of damage for the DNA base guanine (G).

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

- Close **PDB Tree** and click on **Structure**.
- Click on **Secondary** and then on **Track Selection**.
- Within **Helix Selection** click on **Helix 1** and **Helix 16**.
- Close **Structure**.
- To visualize all the atoms within the helices and the damaged based click on **View Controls**.
- Under **Atoms**, click on **Draw**. Under this view, each atom in the protein is shown. [Carbon is grey, Nitrogen is blue, Oxygen is red, and Sulfur is yellow. *Note: hydrogen is not shown.*]
- To help isolate the amino acids within the protein and the damaged DNA base move the **Unselected** transparency slider to the left until you see most of the amino acids that were NOT selected disappear.
- ***Optional***: under **Atoms** click on **Fill space** to have a more realistic view of the space occupied by the selected atoms . This view allows you to see whether or not the damaged DNA is contacting either one of the helices.

Answer

Protein Evolution Questions

The hOGG1 protein is part of large superfamily of DNA glycosylases. Protein families represent groups of proteins from different organisms whose function (for instance, repairing DNA damage) has been conserved throughout evolution. Typically, protein family members not only have conserved **function**, but in many instances their **3D structure** (or parts of their structure) have also been conserved throughout evolution.

We will now explore another member of the DNA glycosylase protein family: *Pa*-AGOG. *Pa*-AGOG is found in a species of archaebacteria, *Pyrobaculum aerophilum*, that live in hot marine water holes. *Pyrobaculum aerophilum* prefers living in extreme environment. It's optimal growing temperature is 100 ˚C (212 ˚F)!

We will explore the differences and similarities between the human DNA glycosylase protein, hOGG and its archaebacteria protein counterpart, *Pa*-AGOG.

Open another viewer window of StarBiochem. (Do not close the viewer that contains the hOGG1 (1EBM) protein.)

- Launch another window of StarBiochem.
- In the top menu under **File** click on **Open/Import** and select "1XQP", the four letter ID for the *Pa*-AGOG protein, and click **Open**.

You are now viewing the structure of *Pa*-AGOG (1XQP), with each bond in the protein drawn as a line. Note that this structure only contains the protein *Pa*-AGOG (1XQP) bound to a single damaged DNA base (no DNA helices are present). Take a minute to look at the structure of *Pa*-AGOG and then answer the following questions.

5 *Pa*-AGOG (1XQP) and hOGG1 (1EBM) have similar 3D structures. In particular *Pa*-AGOG (1XQP) shares some of the same secondary structures as hOGG1 (1EBM).

a) ***Which secondary structure(s) are present in both Pa-AGOG (1XQP) and hOGG1 (1EBM)?***

- Within the hOGG1 (1EBM) viewer, under **View** click on **Reset molecule**.
- Click on **Structure**.
- Within **Secondary**, click on **All Ribbons**.
- Go to the *Pa*-AGOG (1XQP) viewer.
- Click on **Structure**.
- Within **Secondary**, click on **All Ribbons**.

Answer

b) ***Which secondary structure(s) are missing in Pa-AGOG (1XQP), but present in hOGG1 (1EBM)?***

Answer

In the archeabacteria protein *Pa*-AGOG (1XQP) the phenylalanine amino acid 144, Phe_144, is involved in recognizing damaged DNA bases. First take a look at Phe_144 in the *Pa*-AGOG (1XQP) protein. Note its location within the protein and with respect to the damaged DNA base (yellow).

- In the *Pa*-AGOG (1XQP) viewer, under **View** click on **Reset molecule**.
- Within **Structure** click on **Primary**.
- Click on Phe_144. The selected amino acid is now shown in white.
- Zoom in and rotate the molecule to observe the orientation of Phe_144 with respect to the damaged DNA base (yellow). Note both its angle and the distance from the damaged DNA base.

6 In the human protein hOGG1 (1EBM), a phenylalanine amino acid is **also** involved in recognizing damaged DNA bases. Although hOGG1 (1EBM) contains a phenylalanine at position 144, this one is **NOT** the one involved in the recognition of damaged DNA bases. We will examine the orientation and closeness of Phe_144 with respect to the damaged DNA base in hOGG1 (1EBM).

a) ***What is different about the angle between Phe_144 and the damaged DNA base in Pa-AGOG (1XQP) and in hOGG1 (1EBM)?***

- In the hOGG1 (1EBM) viewer, click on **Reset molecule** under **View** in the main menu.
- Within **Structure** click on **Primary**.
- Click on Phe_144. The selected amino acid is now shown in white.
- Zoom in and rotate the molecule to observe the orientation of Phe_144 with respect to the damaged DNA base (yellow).

Answer

b) ***What else is different in the placement of Phe_144 in Pa-AGOG (1XQP) and in hOGG1 (1EBM), with respect to the damaged DNA base?***

Answer

c) Given that in the human DNA glycosylase, hOGG1 (1EBM), Phe_144 is **NOT** responsible for recognizing damaged DNA bases, find the phenylalanine in hOGG1 (1EBM) that would recognize damaged DNA bases. Use the placement and orientation of Phel_144 with respect to the damaged base in *Pa*-AGOG as a reference.
What is the amino acid number of this phenylalanine in hOGG1 (1EBM)? Why?

- In the hOGG1 (1EBM) viewer, click on **Reset molecule** under **View** in the main menu.
- Zoom in to see amino acids surrounding the damaged DNA based colored in yellow.
- Click on **Selection Controls**.
- Under **Select by mouse click** click on **Residue**. This will allow you to click on a particular amino acid within the actual structure in the viewer and determine which one it is by looking at the highlighted amino acid in **Primary** within the **Structure** window.

Answer