BIOLOGICAL FRAMEWORKS FOR ENGINEERS

Laboratory Experience #1 (due in 10/15/12)

MOLECULAR STRUCTURES IN 3D

The purpose of this exercise is to familiarize you with molecular representations of DNA and protein 3D structures. These structures are determined mainly from crystallized preparations using X-ray structure determination techniques. You will use a public database, called The Protein Database to access files for a few structures. (http://www.rcsb.org/pdb/)

I. PROTEIN STRUCTURES

1. Open a web browser and go to the PDB website (http://www.rcsb.org/pdb/). Toward the top of the page is a Search box. Select the "PDB ID or keyword" option and search for **2HHB** (the PDB ID for hemoglobin, the protein in our blood that carries oxygen). A page of information about hemoglobin should appear.

2. Within the menu on the right-hand side, click "SimpleViewer". You may need to launch the installer for this JAVA viewing software. Once the molecule is render in the new window, you can rotate it 3D space by holding the mouse button and moving your mouse in any direction. Holding SHIFT and the moues button together while and moving the mouse up and down = Zoom. Holding CTRL + mouse button + moving the mouse in any direction = Translate position.

This viewer presents each polypeptide chain subunit as a gradient in color from blue to red and allows you to "see" the structure of the hemoglobin molecule. Wherever you place the mouse cursor, the status bar at the bottom of the window identifies the residue number, subunit, secondary structure, and amino acid to which you are pointing

There are also some small, mostly green ball-and-stick structures of different colors – those are not polypeptide chains but heme molecules, non-protein structures that help the protein do its job. When you move the mouse cursor to these structures, the status bar displays the atom you have selected.

Using the mouse cursor and status bar in combination with the summary of information about molecule 2HHB in the previous window from the Protein Data Bank, answer the following questions about hemoglobin.

a) How many polypeptide chains are in hemoglobin?

b) According to the main information page, are all of the chains (also called domains) the same?

c) What is the name of each chain?

d) How many residues are in each chain?

3. Now focus on the heme molecules. Go back and Launch "ProteinWorkshop". You may need to launch the installer for this JAVA viewing software. This software package is similar to SimpleViewer except that individual molecules or amino acids can be rendered at ball-and-stick models. For menu 1), make sure Visibility is selected. For menu 2), select ribbons. In the menu titled "4) Choose items from the tree or 3D view", click on each chain listed to make their ribbon structure disappear. (you can bring it back by clicking on each chain one by one). Note that the atoms shown are color-coded: carbon (C) is green, iron (Fe) is grey, nitrogen (N) is blue, and oxygen (O) is red.

a) How many heme molecules are in the hemoglobin?

b) Do they all appear to be identical?

c) Where are they located?

d) Describe the structure of the binding site for heme.

d) Do you think O_2 can access the heme molecules from the outside of hemoglobin?

4. Now open a second browser window to view the structure for **1GZX.** This is the structure of hemoglobin with O_2 bound. If you compare the two molecules, you will see the site of the new O_2 molecule. Rotate the structure to see it better (look for the 2 new red atoms).

a) Where is the O_2 bound?

b) How many O_2 molecules are bound?

c) Is there a structural difference between the same subunits when O_2 is bound compared to when it was unbound previously? (You will want to own both viewers side-by-side)

5. Let's view protein **2OAU.** This is the file for the structure of a mechanosensitive membrane channel that regulates osmolaric swelling in *e. coli* and may be homologue in mammalian cells to detect mechanical forces at the nanoscale. (Transporters are located in the membrane and move molecules in and out of cells.) Rotate the structure around to observe how it is configured.

a) How many chains are there in the structure?

b) Are they all identical?

c) How might an ion move through this structure?

d) There are two sets of axially aligned alpha-helices at either end of the structure (blue-green vs. yellow-orange). Based upon the residues that form these structures, which ones do you think reside inside the plasma membrane?

II. DNA STRUCTURES & BINDING PROTEINS

1. Now do a search for **140D**. This is the file for the structure of DNA. Within the menu on the right-hand side, click "ProteinWorkshop". You may need to launch the installer for this JAVA viewing software. In the menu titled "4) Choose items from the tree or 3D view", click on Chain A. The nucleotides in the DNA strand are now visible.

a) What is the base pair sequence for each strand?

b) What is matching RNA sequence for each strand?

c) What amino acid sequence arises from the DNA?

2. View file **1TRO.** This is the file for a DNA-binding protein, called the Trp Repressor, bound to a small stretch of DNA. (Repressors block to the process of transcription – they are like switches turn off the transcription machinery) Again, adjust your viewing options so that you can visualize both the protein and DNA structures.

a) How many proteins are bound?

b) Where is the protein bound?

3. View file **1CDW** for the TATA box-binding protein (TBP) which is a transcription factor that binds to DNA and initiates the transcription process.

a) Where is the protein bound?

b) How is the DNA conformation different for TBP than for Trp?

Other interesting structures:

1GOT = trimeric G-protein
1I7X = beta catenin bound to E-cad
1MKX = thrombin active and inactive
2PTC = trypsin
1I6H = RNA Polymerase
1ALM = actin-myosin binding
1BR1 / 2MYS = myosin power stroke
1B7T = myosin
1ATN = actin
1CAG = collagen triple helix
1BKV = collagen
1TUB = alpha-beta tubulin dimer
1YV3 = blebbistatin inhibition of myosin head