

## CHEM 4401 Biochemistry Laboratory I

### Protein Structure Analysis

Reading assignment: Lehninger 5<sup>th</sup> Ed. pp. 129, 132-134 (Boxes 4.4, 4.5)

#### Please bring your laptop computer to laboratory

In this week's lab, we will use molecular visualization software available on the internet to study the protein *polyphenoloxidase*. We will use polyphenoloxidase (PPO) as a model protein in the next few experiments to study protein structure, function and enzyme kinetics. The software allows us to view molecular structures in three dimensions. It allows the investigation of protein structure at an amazing level of detail, including: the ability to zoom in on particular structural features, rotate the molecule, identify ligands, amino acid residues and protein secondary structure, examine the contact distances between amino acids and ligands, and a variety of other physical and chemical characteristics

To begin any type of 3D analysis, however, we must have a crystal structure of the molecule. Your text described how X-ray crystallography and nuclear magnetic resonance (NMR) is used to determine the 3D structure of molecules. Data from these types of analyses are manipulated to produce the "protein data bank" (.pdb) files required for use with molecular visualization software.

Today's lab will use an web-based program to interpret the 3D coordinate files obtained from X-ray crystallography of the PPO protein. We will examine the three dimensional structural details in a variety of ways, including rotating and enlarging the structure, identification of secondary structure components ( $\alpha$ -helices,  $\beta$ -strands, etc.), ligands (metal ions, NADH, FMN, etc.), polar and hydrophobic regions, etc. We will even "slice" through the protein structure to view its interior, including the active site. As you will see, this program is quite powerful and enables even a casual user to obtain a great deal of structural and functional information on many proteins.

We will begin by downloading the protein database file (.pdb) for PPO, which contains the necessary X-ray crystallography or NMR coordinates. We will then use the program and different protein "views" to answer a series of questions related to the PPO's structure and function.

#### Procedure

1. To begin, start your internet browser and go to **FirstGlance.Jmol.org**. Your browser must have the Java software installed to work (most browsers already have this software installed).
2. You should arrive at the **FirstGlance in Jmol** page. Enter polyphenoloxidase's Protein Database file ID, **2PHM**, into the **Enter a PDB identification code here** box and return/enter.
3. After several seconds, the structure of polyphenoloxidase should load. There are three components to the webpage: (1) the **molecule visualization window** on the right-hand side of the page (2) A command block which allows you to manipulate views of your structure at the top left of the page, and (3) an information block at the bottom left of the page which allows you to find out additional information

related to your molecule. To increase the speed of protein explorer's ability to respond to commands, toggle the **spin** button to stop molecular rotation. If you see other, unconnected molecules in addition to the protein, toggle the **water** button to remove.

4. One of the first things to learn when viewing 3D structures is how to manipulate them using various mouse or keyboard options. Review the material in the information block for instructions on how to manipulate the structure and what type of information is provided with each view.
5. Look at the different visualization options (views) in the command block (top left portion of your screen) (e.g. secondary structure, Cartoon, etc.). Select these to see how the image changes. Notice how the information block changes with each selection. Use these options in conjunction with the other buttons (Ligands, slab, etc) to answer the questions on your worksheet.

**CHEM4401 Biochemistry Laboratory I**  
**Protein Structure Analysis (10 pts)**

1. Fill in the following table for PPO (4 pt)

No. Chains	No. of amino acid residues?	No. alpha helices?	No. Beta strands? (indicate no. that are parallel and antiparallel)

\*Hint: Using the **PDB** and **OCA** links may prove helpful for some items

2. Does polyphenoloxidase contain any ligands? If so, what are they? (1 pt)
3. In soluble proteins, hydrophobic residues are frequently found buried in the cores of proteins. Large hydrophobic patches on the surface may be sites of protein-protein interaction. How would you describe the core of PPO? Check out the **hydrophobic/polar** view with the **slab** option. Allowing the molecule to **spin** may also be useful (1 pt)
4. Is the surface of PPO largely hydrophilic or hydrophobic? Based on the surface properties, do you think it would be more soluble in an aqueous environment (e.g. cell cytosol), or in a hydrophobic one (e.g. lipid membrane)? (1 pt)
5. How many disulfide bonds does the PPO structure possess. ( 1 pt)
6. The isoelectric point, or pI, is the pH at which a protein has a zero net charge. Calculate the pI for PPO as follows:
- Get the one-letter amino acid sequence for PPO by following the **Key Resources** link to **OCA Browser for 2PHM**. Scroll through this page to find the amino acid sequence for PPO.
  - Highlight and copy the entire amino acid sequence.
  - Open a new window and set the browser to **http:// www3.embl.de/cgi/pi-wrapper.pl** Paste the amino acid sequence into the box and press the **Determine Isoelectric Point** button.
  - What is the isoelectric point (pI) of PPO? Based on the pI, will PPO have a neutral, negative or positive charge at a physiological pH of 7.0? (2 pt)