

**Texas A&M University-Corpus Christi**  
**CHEM4402 Biochemistry II Laboratory**  
**Laboratory 10: Molecular Graphics & Visualization**

Before we begin the expression and purification of the Green Fluorescent protein from the recombinant plasmid, let's take the opportunity to view the three-dimensional structure of the protein. Three-dimensional structure allows us to see the relationship between the structure of a protein and its function. The relationship becomes especially important in situations where a protein is dysfunctional, or when researchers attempt to design a compound (drug) that will interact with the protein to alter its function or activity. This approach is known as rational drug design. Much of the work involved in rational drug design comes from obtaining the three dimensional structure of the protein. Three dimensional protein structures are determined either by analyzing the spin of protein atomic nuclei (Nuclear Magnetic Resonance (NMR)) or by crystallizing a very pure protein preparation and analyzing the crystal using a technique known as X-ray diffraction (figure 1). X-ray diffraction determines the three dimensional position of every single atom of the protein through the analysis of X-ray diffraction patterns. These patterns reflect regions of electron density, which can be mathematically analyzed to reveal the positions of atoms. Many of the techniques we have used so far are also used by protein crystallographers to make enough protein to form a crystal. Today, many hundreds of proteins have been crystallized and their three-dimensional structure determined. Most of these structure determinations have been placed in publicly available databanks, such as those found at the National Center for Biotechnology Information web site.

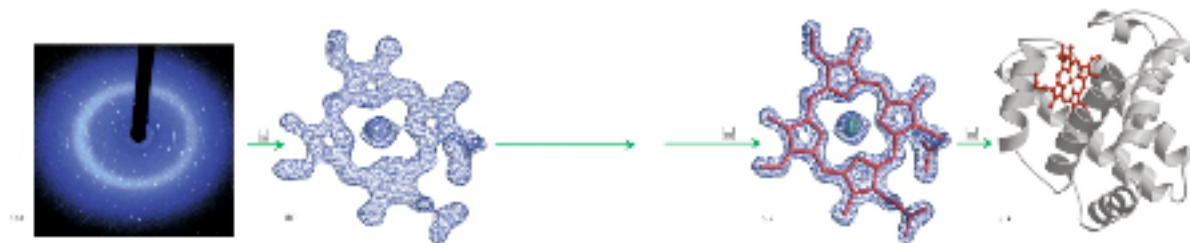


Figure 1. X-ray crystallographic protein structure determination (Lehninger 2000).

Fortunately, the three dimensional structure of the green fluorescent protein has been determined. We will examine the structure of this protein by using the *Protein Explorer* web site. This site contains a molecular graphics program that is capable of interpreting the 3D coordinate files obtained from the X-ray diffraction patterns. *Protein explorer* allows the examination of structural details in a variety of ways, including rotation or enlarging of the structure, identification of regional secondary structure ( $\alpha$ -helices,  $\beta$ -strands, etc.), ligands, polar or hydrophobic regions, etc. The program even allows you to “slice through” a structure to view its interior. It also allows you to identify individual amino acids, hydrogen or disulfide bonds. In sum, the program is quite powerful, and enables even a casual observer to obtain a great deal of structural and functional information about a macromolecule.

We will begin today by downloading the protein database file (.pdb) which contains the X-ray crystallography coordinates for green fluorescent protein. We will then explore the features of the protein to answer a series of questions related to it's structure and function.

## Procedure

1. To begin, start your internet browser and go to **www.proteinexplorer.org**. Select the **FirstGlance in Jmol** hyperlink. Your browser must have the Java software installed to work.
2. You should arrive at the **FirstGlance in Jmol** page. Before we can view the structure of GFP we need to load its database (.pdb) file into the website. Enter GFP's file ID, **1EMB**, into the **Enter PDB identification code here** box and return/enter.
3. After several seconds, the structure of GFP should load in Protein Explorer. 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/keyboard button combinations. You can rotate your structure by holding down the mouse button (left PC's) and dragging the structure left/right or up/down. You can zoom in or out of your structure by holding down the **shift** and **mouse buttons** (left PC's) while dragging the mouse back and forth.
5. Look at the different visualization options in the top left portion of your screen (secondary structure, Cartoon, etc.). Press these to see how the image changes. Notice how the information in the bottom left of your screen changes to provide information on the selected view. Use these options in conjunction with the information presented to answer the questions on your worksheet. Play with it a bit. It will take a little practice so please be patient, this is an opportunity to learn how to use an import computer tool.

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**Molecular Graphics Laboratory (10 pt)**

1. Describe what type of information the following views provide (1 pt each).
  - a. Secondary structure
  - b. Cartoon
  - c. Composition
  - d. Hydrophobic/Polar
  - e. Charge
  
2. How many protein chains are there in GFP? (1 pt)
  
3. What is the predominant secondary structure (2°) of green fluorescent protein? (1 pt)
  
4. Is the structure of GFP primarily what we would consider parallel or anti-parallel? (1 pt)
  
5. Is the surface of the GFP protein largely hydrophilic or hydrophobic? Based on these surface characteristics, do you think the protein would be found primarily in the cytosol or in a membrane? Why? (1 pt)
  
6. The chromophore in GFP is especially interesting. It relies upon the oxidation of part of its *own structure* and consists entirely of only *three amino acids*. This is significantly different from other biological reactions which give off light, as found in fireflies and deep sea animals. These other types of fluorescent reactions rely upon the conversion of a substrate to another product, giving off light in the process. Such reactions are often energetically expensive for the organism, requiring large amounts of ATP. Where is the chromophore in GFP located? (Hint - use a molecular view allows you to see through the molecule and select the **Ligands** button) (1 pt)