Protein Analysis Ideas:

Today while you are looking at your favorite protein's crystal structure, you may want to consider the following points listed below. We do not give this list as a comprehensive list. It is just some things to consider. We expect you to analyze your protein in a way that is most appropriate for its structure. In the pymol commands below XXXX is where you would put in your pdb ID:

1. What are the crystal contacts in your structure? (In the command below you must specify a name for the object, the selection is what you want to see the crystal contacts, this should be your protein, and the cutoff is how many angstroms of data you want displayed. Don't pick anything higher than 10 to start with or you might be waiting all day…)
      PYMOL > symexp XXXX = object, (selection), cutoff

2. Does your protein have an active site or binding face? What residues compose that site? What usually binds there? Is it (substrate, for example) present in your structure? What kinds of interactions are made between the protein and substrate or binding partner?

3. What is the ratio of α-helices to β-sheets?

4. How many water molecules are there in the structure? (It specify the number in the pdb header or you can look at the bottom of the pdb files where the non-protein atoms are shown.) Is this reasonable based on the total number of residues in the protein? (The rule of thumb is that at 2Å resolution you can add one water molecule for every two amino acids.)

5. Color your structure by b-factor. What regions are the most mobile? The least? Can you rationalize why based on the function of the protein?

6. Is your protein mono- di- or tri-meric? What does the multi-meric interfaces look like?

7. Are there any significant cavities on your protein surface? What do you think might bind there? You can find them or other structural elements such as Rufness, Electrostatic Potential, Negative Potential, Hydrophobicity, Hydrophilicity, using HotPatch. HotPatch will score your protein based on the selected criteria and report that criteria on a scale of 1 to 10 listed in the b-factor column of the pdb. After analyzing your protein with HotPatch, import the output pdb file into pymol and color by b-factor to see the significant patches. HotPatch can be found at:
      http://nih.mbi.ucla.edu/cgi-bin/pettit/hotpatchweb

8. Are there any unusual regions? Disordered regions? Left-handed helices? Helical kinks---is a proline present?

9. Does your protein have independent domains that can move with respect to one another? What is the functional significance of that?

A few more commands that you may find useful:

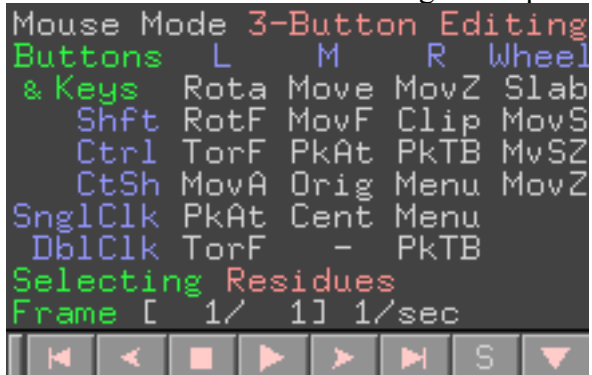1. For a list of commands type:
      PYMOL > help

  For help on one command (eg. symexp) type:
      PYMOL > help symexp

2. The manual is very helpful and can be found at:
   http://pymol.sourceforge.net/html/index.html

3. You can make selections using the sequence of your protein (S box below) tool:

4.To align two proteins use align:
      PYMOL > align XXXX, YYYY

5. To toggle between the help screen and the viewing screen hit esc button.

6. To move one fragment relative to the other.
      MOUSE → 3-button editing mode
      Draw the protein you want to keep stationary as a ribbon. Draw the one you want to move as a ribbon
          with lines also shown.
      Using the mouse use MovF or RotF functions to click on the sidechains of the fragment you want to
          move then move or rotate it.

7. You can always output a pdb of the entire rotated or edited molecule using:
      FILE→ Save molecule.