BioChem 330 **Fall 2011**

Homework Assignment for Protein Structure

Recommended Problems from Chapter 6: 3, 4, 10, 12, 14…..also sample exam is posted and answers

Structural Exercise: (modified with permission from an exercise used by Professor Jaswal) This assignment is designed to help make you familiar with the use the online structure visualization resources Proteopedia and PDB. The assignment will be to turn in a powerpoint file that contains screenshots illustrating a set of requested structure manipulations that give insight into a signaling protein that interests you. Your powerpoint file should contain slides that highlights the important features, relating the images presented to their biochemical relevance if possible. You are free to choose an example that is most interesting to you, but we ask that **you do not choose the same structures as others in the class**. By Tuesday 9/20 at 8 PM we will ask you to declare your structures by creating a powerpoint title slide and have the presentation posted by Friday 5 PM 9/23.

To identify good candidate complex structures, you may you may search in the rcsb.org database or use one of the hormone structures below
PDB ID: 1D2S **Sex Hormone Binding Globulin with testosterone**
PDB ID: 1XF0 **17beta-hydroxysteroid dehydrogenase**
PDB ID: 1A52 **Estrogen receptor hormone-binding domain**
PDB ID: 3ERT **Estrogen receptor and tamoxifen**
PDB ID: 1HGU **Human growth hormone**
PDB ID: 4INS **Insulin**
PDB ID: 2Q1V **Ancient Corticoid Receptor with Cortisone Bound**
PDB ID: 1NHG **Glucocorticoid Receptor Bound to Agonist RU-486**

Contact Prof. O. if you are having problems identifying good candidate structures. In addition to helping highlight protein structures we've just been learning about in class, the larger purpose of this exercise is to empower you to ask your own questions about the relationship of structure to function for any molecular interaction that interests you in the future.

Your Tuesday post should include the following information:

1. Pdb code of complex structure
2. Name of each molecule in the complex
3. Source of information about function and structure (review, lecture notes from date xxx, book or paper citation, website, etc.)
4. Functionally relevant residues and features of the structure you will explore (at least one of the following)
    - hormone or other cofactor binding sites (using ligand explorer)
    - locations of disease-related mutations
    - sites of interactions with other biomolecules

Protein Structure Instructions

THE OVERVIEW:

1. Brief introduction to proteopedia
2. Download template.ppt from the CMS information page and save as yourname.ppt.
3. Become acquainted with the features of the online tools Proteopedia, FirstGlance, OCA, and PDBsum by following the instructions listed below, and replacing the images in slides #2-16 with images you create following the instructions below, for the pdb code corresponding to the signaling protein or complex you have chosen.

HINTS:

• Use control F to search for commands on the page if you don't immediately see where they are!
• If you don't remember how you got to one of the linked resource sites, just use google and remember to type your pdb code in!
• Don't forget to be changing the slide titles and information to reflect your structures instead of the sample template
* asterisks are next to views that you may want to return to as you're putting together your "stories" about your structures

SLIDE #2 (Proteopedia site)

1. Paste the information into slide #2
2. Go to Proteopedia.org
3. In the search box on the left, type in your pdb code and hit enter
4. Below the spinning model of your protein, note the list of "resources"- you will be coming here frequently
5. In the resources list, click on FirstGlance
6. Turn off spin

SLIDE #3 (FirstGlance: Composition, Hide, Charge)

1. Click Composition that will color the components of the complex- beige for protein, purple DNA, red (or is that pink?) RNA
2. If you have more than one chain, click Hide, then leave the button next to "Chain" clicked, and go to the image and click somewhere on the chain you want to hide.
3. Rotate around to get a good sense of the knobbiness of the protein surface- take a **screenshot** with a good view of the crevices
4. Click Charge and click the box next to "Color polar" to show the charged and polar residues. Take a **screenshot** that includes the legend.
   **This is a good way to look at whether the protein interaction surface with a nucleotide, other protein, or bound cofactor has the charge that you expect.*

SLIDE #4 (FirstGlance: Hydrophobic/Polar, Find)

1. Click Hydrophobic/Polar and the box next to slab to view the hydrophobic core of a globular protein. Rotate around and appreciate how hydrophobic the core is, but yet how there are polar parts that can be very buried as well.
2. Click once on the zoom with the big arrow. Find a grey circle next to a purple circle. Hover your cursor above the grey circle, until the residue number, chain, element and atom label appears in a yellow box. **Write the residue, number, and chain letter down on slide #3,** and repeat for the neighboring purple circle- you will need this residue number and chain again later when asked for the core polar residue, so note it.
3. Click Find, and in the new window that shows up, in the box next to the new **Find:** put the number of both residues, separated by a comma, and hit enter. Yellow balls (halos) will appear for each atom of the residue.

**This is a good way to locate specific residues that are important. You can also type in just an amino acid name, ie "Trp" and view all of the tryptophans in the protein. (when you want to view different residues in the future, don't forget to clear halos, but don't do it now…)*

4. Click Center Atom, click back on one of your haloed residues, then take a screenshot.

SLIDE #5 (FirstGlance: Zoom, Distance, Salt Bridges, Cation Pi)

1. Zoom in on the two residues and click Vines
2. Measure the distance between the alpha-carbons of the two residues: double click on the first atom. Now, as you touch other atoms, the distances will be shown on a red line connecting the atoms (except in Mac Firefox where you may have to click multiple times). **Double click** on a second atom to fix the distance report, note the distance.
   How well did you do at identifying neighbors- how far apart are they?
   **This will be useful to make arguments about how close in proximity two things are in your structures*
3. Turn off slab, under More Views, click Show in the line Show all protein Cation-Pi interactions and salt bridges, then click the circle next to "Show Protein Salt Bridges" and write down the partners for two salt bridges.
4. Click the circle next to "Show protein cation-pi interactions", write down two cation-pi interactions
5. For one of these views, take a screenshot that captures the interactions, and the distance you measured.

SLIDE #6

1. Go back to Find, clear halos, and enter the number of only the polar core residue you selected .
2. Click Contacts, in the window at the bottom left that appears, click the circle next to cartoon, and the square next to "atoms with halos", then Show Atoms Contacting Target

3. Under the four small snapshots, click the square that says “More views”, the click the box next to “Label Contacts” and select target and contacts as balls and sticks
4. Then click uncheck, and click the box next to show putatively hydrogen bonded non-water, use zoom and center atom as necessary to get the right screenshot to show the residues hydrogen bonding to your selected atom.
5. Uncheck putatively hydrogen bonded non-water and check show hydrophobic vanderwalas interactions, and adjust as before to get a schreenshot.

**This will be very useful for looking at important residues, in particular mutations, to see what interactions might be disrupted the most by a particular mutation.*

Now we’re going to explore other resources that proteopedia links to that give you general information about your protein and its structure.

SLIDE #7 (OCA site)

1. Go back to the tab with proteopedia that has your structure pulled up. Under the rotating model, next to resources, click OCA
2. Note what organism the protein is from (organism_scientific), what expression_system organism was used, and what the resolution of the structure is.
3. Above the green gene ontology row is a row with “related structures” listed, if you have any- note those.

SLIDE #8 (OCA site: Contacts of Structural Units)

1. Under the Data retrieval heading, click Contacts of Structural Units
2. Scroll down to the big #2, and type in the number only of the core polar residue, and the chain letter, and hit select.
3. How well did you do at picking a core residue (expect 0% solvent accessibility). Look at table I- how accessible is the surface of this residue in the protein relative to what it would be if it were free? Copy the table and paste into the ppt.
4. Look at table II: this is a very helpful list of all of the specific interactions with the target residue, specifying type of interaction, distance between residues, and surface of that residue interacting with the target!
5. Look at table III: copy the list of putative hydrogen bonds- how does do the H bonds predicted by CSU here compare with the H bonds identified graphically in FirstGlance?

SLIDE #9 (Proteopedia, ConSurfDB)

1. Go back to the proteopedia tab that has your structure pulled up, click [show] next to evolutionary conservation.
2. Under further information, click "Complete results at ConSurfDB", and under "View 3D structure colored by conservation…" click a protein chain.
3. Check out what's conserved and what's not- are you surprised? What features might you expect to be conserved? Copy a screenshot.

**This is very useful for looking at conservation, showing that a mutation or site is or isn't conserved can be a part of your story…*

SLIDE #10 (Proteopedia-> pdbsum-> protein)

1. Go back to the proteopedia tab that has your structure pulled up, click pdbsum
2. Scroll down and for an enzyme you may find the enzyme reaction diagrammed, and below that the 1st page of the paper that reported the structure, and below that references that cite this paper

**could be a good place to look for newer information and/or biochemical studies that have built off of the findings based on this structure*

3. Click the Protein link near the top of the page, scroll down - any residues in a yellow box outlined with red are those catalytic residues, and ligand and DNA/RNA interacting residues are also noted
4. Under motifs, click the residue conservation link, and take a screenshot.

**Great way to find features to profile in your structure story*

SLIDE #11 (pdbsum: toppage)

1. Go back to the "top page" tab of pdbsum, and on the right side, click the procheck button and copy the ramachandran plot.
   **This can be part of a story, particularly for a protein that's all alpha, or all beta…*

SLIDE #12 and #13 if you need another (why interesting slide)

1. This one or two is up to you, you can use ligand explorer from pdb site if the protein has a ligand bounds, or discuss mutations if this is a mutant protein