Nyholm-Strino-Koppisetty 2009-03-03

Practical Exercises on Drug Design 1

1) Fetch the structure of protein tyrosine phosphatase 1B, pdb entry 1PTY, from the PDB and display the complex in Chimera. Try to localize the ligand that is in the active site. Also try to read the article about this enzyme.

2) Fetch the structure of neuraminidase with inhibitor, pdb-entry 1F8E, and display the complex.

3) Fetch the structure of PPAR-delta, i.e. 3GWX, and try to locate the position of the ligand. Note the deep pocket.

4) Search in the PDB for "saquinavir" and fetch an entry which contains the structure of HIV-1 protease with this compound. Toggle the display of the heteroatoms on/off and note the position of the inhibitor in a cavity in the enzyme.

5) Fetch the pdb-file 1STC and display the structure which is protein kinase C with the inhibitor staurosporine. Locate the inhibitor.

Guided solution to exercise 1 – A short Chimera tutorial

To most of the tasks in this exercise a *short description of how to accomplish the task in Chimera is given in italics*. **Key tasks are highlighted in bold**. If you want more extensive information about Chimera, there is a good online documentation and some tutorials on the Chimera website http://www.cgl.ucsf.edu/chimera/. Chimera is free for noncommercial use.

A – Getting familiar with your structure

Tyrosine phosphatase 1B (PTP1B) is an important tyrosine phosphatase enzyme that has been indicated as key target for type II diabetes. More information can be found on the article by Doman et al. *(J. Med Chem*, 2002, 45, 2213-2221). A crystal structure of this enzyme with the natural ligand tyrosine phosphate can be found on the Protein Data Bank, entry 1PTY.

1. Start Chimera by typing "chimera" on a shell window.

2. Fetch the structure file of the complex from the Protein Data bank
2.1. File -> Fetch by ID…
2.2. Select the radio button PDB ID
2.3. Type 1PTY in the text field
2.4. Press the button fetch

The protein structure is displayed to you in wire representation. It can be **rotated by pressing the left mouse button** while moving the mouse, **translated by pressing the middle button** while moving the mouse and **scaled by pressing the right** mouse button while moving the mouse (up and left to zoom out, down and right to zoom in).

3. As you might see there are small dots surrounding the protein. These are **water molecules** which we will **remove**.
3.1. Before deleting the water we first need to select it.
3.1.1. Select -> Chain -> Water
3.1.2. The water molecules are highlighted in green.
3.2. Delete all selected atoms, i.e. the water molecules.
3.2.1. Actions -> Atoms/Bonds -> Delete

4. Now that the water is removed, are there any other molecules included in the crystal structure apart from the protein itself? In that case what do they look like and what are they? **Highlight them all by changing their display style from the wire representation to the ball and stick representation and color them according to their atom type**.
4.1 Select all non-protein atoms
4.1.1 Select -> Chain -> het
4.2 Change the display style of all selected atoms.
4.2.1 Actions -> Atom/Bonds -> ball & stick
4.3 Color atoms according to atom type
4.3.1 Actions -> Color -> Check the atom/bonds option
4.3.2 Actions -> Color -> by element
4.4 Now that the changes of the display style of the non-protein atoms are finished unselect them.
4.4.1 Select -> Clear selection
4.4.2 No atoms should be highlighted in green.
Notice that there are two tyrosine phosphate molecules and a molecule of Magnesium ion (shown in cyan or in green in newer versions of Chimera). As the latter is not interesting, it will be removed.

5. It is possible to **select one atom or one bond by left-clicking on it while pressing the Ctrl key**. Multiple selections can be obtained by left-clicking while pressing Ctrl and shift keys. If no atom or bond is pointed by the cursor, the current selection is cleared. A group of atoms or bonds can be selected by

dragging the mouse while keeping the Ctrl key pressed. **Once an atom (or a bond) is selected it is possible to select the atoms (or the bonds) of his residue by pressing the upper arrow key once**. The complete chain can be selected by pressing the upper arrow key again. Finally, all atoms can be selected by pressing the upper arrow key again. The current selection can be then reduced to the chain, to the residue, and to the atom by pressing the down key arrow.
5.1. Select the Mg atom by left clicking on it while keeping the Ctrl key pressed.
5.1.1 The atom will now be highlighted in green.
5.2. Actions -> Atoms/Bonds -> Delete

B - Identifying secondary structure arrangement

1. Show and hide ribbons
1.1 Actions -> Ribbon -> show
1.2 Tools -> Graphics -> Color Secondary Structure
Note: In recent versions of Chimera this dialog is under Tool->Depiction-> Color Secondary Structure
1.2.1 Make sure the "Color ribbons" checkbox is checked and then press OK.
1.3. Hide the protein atoms.
1.3.1. Select -> Chain -> A
1.3.2. Action -> Atoms/Bonds -> hide.
1.3.3 Just the ribbon representation and the two ligands should be seen now.
1.4 Change the ribbon visualization mode.
1.4.1. Action -> Ribbon -> round.
1.5 Observe the secondary structure motifs of the protein.
1.6 Hide the ribbon representation.
1.6.1 Action -> Ribbon -> hide.
1.6.2 Only the two tyrosine phosphate molecules should now be visible.

C – Creating a molecular surface around the protein

1. **Create a molecular surface over the entire protein and color that surface in green**.
1.1 Select the protein.
1.1.1 Select -> Structure -> main
1.2 Actions -> Surface -> show
1.3 Actions -> Color -> check the surfaces option
1.4 Actions -> Color -> green

2. Color the surface according to different properties of the amino acids such as hydrophobicity by selecting those amino acid residues having the desired properties.
2.1.1 Select -> Residue -> amino acid category -> hydrophobic
2.1.2 Color the surface of the selected residues in forest green
2.2 Repeat using another property such as polarity.
2.3 If you want to return to the all green surface select all and then color the surface green.
2.3.1 Select -> Select all
2.3.2 Actions -> Color -> check the surfaces option
2.3.3 Actions -> Color -> green

D - Studying the protein-ligand interaction

Observe the two tyrosine phosphates. Which one looks more interesting? Where is the main active site probably located? From now on, we will focus on the ligand inside the deep pocket. However, a similar investigation can be done for the other ligand or for both at the same time. Note: The interesting molecule is labeled PTR 1; you can see this by keeping the cursor over one atom of the molecule for more then a second (ex. The label "#0 PTR 1.het C" indicates a carbon atom of the most interesting ligand).
The ligand PTR 2 can now be deleted (see A5).

1. Hide the surface
1.2.1 Select -> Select all
1.2.2 Actions -> Surface -> hide
1.2.3. Select -> Clear Selection

2. Show the residues within 5.0Å from the tyrosine in the deep pocket.
2.1. Select the molecule (see A5).
2.2. Select -> Zone
2.2.1. Check that the radio button next to "<=" is checked and write 5.0 in the text field.
2.2.2. Check "Select all atoms/bonds of any residue in the selection zone".
2.2.3. Press "OK"
2.3. Show the atoms in the selection
2.3.1. Action -> Atoms/Bonds -> show

3. Identify the residues involved in the binding
3.1. Select the protein residues. (Ex. Select -> Clear, Selection Select -> Chain -> A
3.2. It might be useful to visualize the residues as sticks.
3.2.1. Action-> Atom/ Bonds -> stick
3.3. Label the residue names and types
3.3.1. Action-> Label -> residue -> name + specifier
3.3.2. Action-> Color -> check the "atom labels" option
Note: In recent versions of Chimera the option "residue labels" should be selected
3.3.3. Action-> Color -> Dim Gray

4. Show the hydrogen bonds
4.1 Select the tyrosine phosphate in the binding pocket (see A5).
4.2 Tools -> Utilities -> FindHbond
Note: In recent versions of Chimera this dialog is under Tool->Structure Analysis-> FindHbond
4.2.1 Select the radio button "Only find H bonds with" and change the option into "exactly one end".
4.2.2. Press "OK"
4.3 **Which residues interact strongly with the tyrosine phosphate? Which residues are involved with the phosphate recognition function of the enzyme?** Check your answer with the article (Fig 2A and first paragraph of page 2215). Note: The cysteine 215 residue in Fig 2A has been substituted with a serine, as explained in the caption of figure 2.
4.3.1. It might be helpful to color some the important residues with different colors.
4.3.1.1. Select one or more residues (see A5)
4.3.1.2. Action-> Color -> check the "atoms/bonds" option
4.3.2. Choose a color, ex Blue
4.3.2.1. Action-> Color -> blue
4.3.3. Repeat the procedure for few other interesting residues (4.3.1.2 can be skipped).

5. Another look at the surface
5.1 Show the surface again.
5.1.1 Select -> Clear Selection.
5.1.2 Action -> surface -> show.
5.2 **Make the surface transparent** above all atoms that are within 6Å from the ligand
5.2.1. Select the tyrosine phosphate *(see A5).*
5.2.2. Select all atoms within a 6Å radius from the selected ligand
5.2.2.1. Select -> Zone -> all atoms <= 6Å from the currently selected atom
5.2.3. Actions -> Surface -> transparency -> 50%
5.3. Observe again the ligand in the binding pocket and its interactions with the enzyme.

6. Change the clipping plane. This option allows you to visualize only a part of the complex and is particularly useful to examine deep binding pockets, like the PPAR-delta in exercise 3.
6.1. Favorites -> Side view
6.1.1. Drag the yellow lines in order to change the clipping planes.