Molecular Visualization with Chimera

In this session, you will utilize USCF Chimera to view and interrogate two biomolecular structures. This program is freely available for Microsoft Windows, Mac OSX, Linux, and UNIX-based computer operating systems such as IRIX. From home (or your home lab), you may download the Chimera structure viewer from the UCSF website: http://www.cgl.ucsf.edu/chimera/download.html

Chimera menu commands will be designated as: ***Button -> button -> button*** .

Chimera command line commands are indicated after: **command>**

In addition to this handout, you should receive a copy of The Chimera Quick Reference Guide (UCSF).

Section A: Investigating Hemoglobin

1. View the PDB file for human Hemoglobin (1GZX)

In a terminal window, type **chimera**. This is a newer, larger program than e.g. RasMol, and will take much longer to start running. You can drag the bottom right corner of the Chimera window to expand the window size to something comfortable for you.

Now, automatically fetch the structure of hemoglobin from the Protein Data Bank.

1. ***File -> Fetch by ID -> Type 1GZX -> Fetch***
2. Rotate the structure with the left mouse button (button 1)
3. Translate (move) the structure with the middle mouse button (button 2)
4. Zoom in on the structure with the right mouse (button 3)

Here, we start with a PDB code we already know. Usually, we don't know that beforehand, so you would go to rcsb.org and search for your protein of interest. If you haven't done this before, go there and examine the "Molecule of the Month" entry for hemoglobin.

2. Highlight and color individual protein chains

Tools -> Depiction -> Rainbow -> Chain

3. Color the heme cofactors using the Chimera command line

(if the command line is not visible at the bottom of the window, choose ***Favorites -> Command Line***)

1. **command> color purple #0:HEM**
2. **command> color orange #0:HEM@FE**

You'll want to learn the syntax used for specifying selections:

#[model number]:[residue name or number]@[atom name]

Further details can be found under atom specification on this page (it's not a bad idea to keep this page open while working in Chimera):

http://www.cgl.ucsf.edu/chimera/docs/UsersGuide/framecore.html

4. Display heme atoms (and NOT the Fe atoms) as type 'ball & stick'

1. ***Select -> Residue-> HEM***
2. **Shift+Ctrl+click** on Fe atoms to deselect
3. ***Actions -> Atoms/Bonds -> ball and stick***

5. Focus on the heme binding site of hemoglobin Chain B

1. **Ctrl+click** on any atom of the heme to select it (Hover over different hemes to determine which is chain B)
2. **Shift+Ctrl+up-arrow** to expand selection to include entire heme
3. ***Actions-> Focus***

6. Show ONLY the heme binding site for hemoglobin Chain B

1. ***Select -> Zone -> <5.0 angstroms from currently selected atoms***
2. Click button for '***Select all atoms/bonds of any residue in selection zone***'
3. ***Actions -> Atoms/Bonds -> show***
4. ***Select -> Invert (all models)***
5. ***Actions -> Atoms/Bonds -> hide***
6. ***Actions -> Ribbon -> hide***

7. Color the heme and binding site sidechains according to atom type

1. ***Invert the selection again (or re-select the heme and the zone of nearby residues).***
2. ***Actions -> Color -> by heteroatom***

You should now see Nitrogen atoms in blue, Oxygen atoms in red, and Sulfur atoms in yellow.

8. Render the coordinated His residue (H235) as 'ball & stick'

1. **command> select #0:235.B**
2. ***Actions -> Atoms/Bonds -> ball & stick***

9. Color only the H235 side chain

1. ***Actions -> Color -> Atoms/Bonds***
2. ***Actions -> Color -> red***

   Note: You may need to click the "all options" button under the **Action -> Colors** menu to see the Atoms/Bonds option.

10. Color heme binding site side chains yellow (except H235)

1. ***Select -> Structure -> sidechain/base -> without CA/C1'***
2. **command> ~select #0:235.B**
   (That command begins with a "tilde" character meaning de-select.)
3. ***Action -> Color -> yellow***
4. ***Select -> Clear selection***

11. Render all solvent molecules as type "ball"

1. ***Select -> Structure -> solvent <or> Select -> Chain -> water***
2. ***Actions -> Atoms/Bonds -> ball & stick***
3. ***Actions -> Color -> by element***

12. Save the session to resume later

1. ***File -> Save Session -> hg_session.py***
2. ***File -> Close Session***

Section B: Viewing bovine rhodopsin (1L9H)

Exploration of the high resolution X-ray structure for bovine rhodopsin should provide an opportunity for us to combine many of the visualization techniques covered up to this point – plus an opportunity to see a few more. Here, the ligand, 11-cis-retinal (RET), is a covalently bound inverse agonist that locks the protein into an inactive (dark state) conformation. Our annotation of this structure will illustrate in atomic detail the chemical and physical composition of the ligand-binding site of this G-protein coupled receptor, an integral membrane protein that has seven membrane-spanning segments.

1. Basic annotation of the pre-edited PDB file

A pre-edited version of the rhodopsin PDB file exists locally to load into Chimera.

Display the protein backbone trace.

1. ***File -> Open -> Filename: /home/momokurs/rosetta_workshop/tutorials/day02/sample_files/rho.pdb***
2. ***Actions -> Atoms/Bonds -> backbone only -> chain trace***

Display the protein secondary structure.

1. ***Select -> Structure -> secondary structure-> helix***
2. ***Actions -> Ribbon -> show; Actions -> Ribbon -> edged***
3. ***Actions -> Color -> ribbons; Actions -> Color -> green***
4. ***Select -> Structure -> secondary structure -> strand***
5. ***Actions -> Ribbon -> show; Actions -> Ribbon -> edged***
6. ***Actions -> Color -> orange***

Display and color the conserved disulfide bond in rhodopsin.

1. **command> select #0:110.A,187.A**
2. ***Actions -> Atoms/Bonds -> show***
3. ***Actions -> Focus***
4. ***Actions -> Atoms/Bonds -> stick***
5. ***Actions -> Color -> atoms/bonds; Actions -> Color -> by element***

2. Adjust your viewing parameters to reduce the data displayed

Position the camera looking into the helix bundle from just above the beta strands.

Highlight the ligand, 11-cis retinal (RET)

1. ***Select -> Residue -> RET***
2. ***Actions -> Atoms/Bonds -> stick***
3. ***Actions -> Color -> atoms/bonds***
4. ***Actions -> Color -> medium blue***

Display any water molecules bound near the ligand.

1. ***Select -> Structure -> solvent***
2. ***Actions -> Atoms/Bonds -> ball & stick***
3. ***Actions -> Color -> red***

Try adjusting the clipping planes to hide some detail and focus on the ligand binding site.

1. ***Tools -> Viewing Controls -> Camera -> Side View***

   <or>

2. ***Favorites -> Side View -> drag*** front (left) and back (right) yellow clipping planes to adjust the molecular detail in your view.

Chimera starts in mono viewing mode by default. You can enable stereo viewing mode.

1. ***Tools -> Viewing Controls -> Camera -> camera mode: sequential stereo***
2. Now, put on your 3D-glasses and explore the structure for a while. You can continue in stereo or mono.

3. Highlight the fit into the ligand binding site of the rhodopsin protein.

Focus on the 11-cis retinal ligand-binding site.

1. ***Select -> Residue -> RET***
2. ***Actions -> Focus***

Show amino acid side chains within 5.0 Angstrom of the ligand.

1. ***Select -> Zone -> <= 5.0 angstrom from currently selected atoms***
2. Click '***Select all atoms/bonds of any residue in selection zone'***
3. ***Actions -> Atoms/Bonds -> show***
4. ***Actions -> Atoms/Bonds -> stick***
5. **command>** ~**select #0:RET.A**
   (with a tilde to negate the select command)
6. ***Actions -> Color -> atoms/bonds***
7. ***Actions -> Color -> by element***

Render a semi-transparent surface for the ligand.

1. ***Select -> Residue -> RET***
2. ***Actions -> Surface -> show***
3. ***Actions -> Color -> surfaces***
4. ***Actions -> Color -> grey***
5. ***Actions -> Surface -> transparency -> 50%***

Save the image in a file

1. ***Actions -> Color -> background***
2. ***Actions -> Color -> white***
3. ***File -> Save Image -> adjust size to 1024 pixels***
4. ***Save As -> rhodopsin.tif***

Two additional points. You can see that the repetive use of menus gets tiresome. As you use chimera more, you will get faster if you learn the commands listed on the quick reference sheet, and type them into the commandline.

Secondly, writing and modifying scripts can be very useful for creating figures. The script can also be customized by editing it as a text file. This is a very powerful method for accelerating your visualization work, so an example script has been provided here for you to experiment with and customize.

~/rosetta_workshop/tutorials/day02/sample_files/startup.cmd

Copy it to your home directory. Load a new structure and run the script. View it in a text editor. Try making changes to suit your protein of interest.

Section C: Create a clear visualization of a DNA-ligand complex (1L9H)

Duplicate the DNA-duocarmycin representation you already made with pymol. If you can, try to do it without reading the steps below. Then read through them to find any things you might have missed.

1. ***File -> Fetch by ID -> 1DSM***
2. ***select***
3. ***~select #0.1***
   (That line begins with a "tilde" character)
4. ***delete sel***
5. ***Tools -> Depiction -> Rainbow (by chain, apply)***
6. ***repr stick***
7. select (**Ctrl-click**) the covalent bond between Adenosine 5 and the DSA
8. ***Actions -> Atoms/Bonds -> delete*** (so that the surfacing command can be limited)
9. ***Select -> Residue -> DSA***
10. ***Actions -> Color -> by heteroatom***
11. ***Actions -> Surface -> show***
12. ***Actions -> Surface -> transparency -> 80%***
13. ***Favorites -> Model Panel ->*** double-click on MSMS surface to see attributes, set vertex density=10
14. ***Select -> Chemistry -> Element -> H***
15. ***Actions -> Atoms/Bonds -> hide***
16. ***Tools -> Depictions -> Nucleotides*** (start with defaults. Vary the options according to taste.)
17. Save a **.png** image in your working directory using POV-ray raytracing. Save the session for later.