Computer Lab 2 – Molecular Graphics

The analysis and understanding of biomolecular dynamics can be appreciated best by merging in your mind numerical *statistical distributions* with the *static images* apparent from molecular graphics. You will need to use both in this course and you will always want to use both in the future when you consider a molecule.

This lab will give a brief introduction to two molecular graphics applications that we use in the course, PyMOL and VMD. We will use PyMOL to view the results of early Argon and peptide simulations and later to compare molecular density maps. We will use VMD to view the protein simulation trajectories. Both applications are freely available on the Internet and work with most popular computer operating systems. Documentation for both programs is available from the Links page of the course website (pages.jh.edu /pfleming/compbio/links.html).

On a Mac the standard way to launch either PyMOL or VMD is to click on the application icon. But if you are using `tcsh` the applications should be in your path. Enter the word **`alias`** or the following commands in a terminal window to find out if the two applications are in your path,

```
which pymol
which vmd
```

If you get a "`Command not found.`" message, call the instructor.

Answer the questions highlighted in yellow and send the answers to **achin14@jhu.edu** either in the body of an email or as an attached file with your JHEDID in the name (e.g. *JHEDID_lab2.txt* if made with vi or *JHEDID_lab2.docx* if made with MSWord).

A. PyMOL

PyMOL is a molecular graphics program that is one of the best applications for making high quality images and exploring molecular structure of an individual PDB file. The usefulness of **PyMOL** can be extended with python scripts. Documentation for **PyMOL** is available as a link from the course website on the Links page.

1. Image making

Under your home directory on the Mac (or on your flashdrive) make a new subdirectory called *molgraph*, change to that directory. Fetch the files *1RA2.pdb* and *1ra2.xplor* from the *Shared/* directory on kirin to your *molgraph/* directory. (See Lab Guide 1a, page 9 if you forgot how to do this).

Launch **PyMOL** by typing,

```
pymol
```

You will have a new display with three sections, a **command window** at top (takes a while to appear), a black background **main graphics window** and a gray **graphics menu window** (internal GUI) to the right of the main graphics window.

Use the pull down menu **Mouse** from the top of the screen and choose **3 Button Viewing Mode**. (I will usually indicate a multiple-click pull down menu choice with a "pipe" separating the choices: **Mouse | 3 Button Viewing Mode**).

Use the **File | Open** tabs on the screen-top pull down menu to find the file *1RA2.pdb* or in the command window type,

```
PyMOL> load 1RA2.pdb < return >
```

(Note: A quicker way to launch PyMOL with the structure already loaded is to simply type the following at the terminal command line, **`pymol 1RA2.pdb`**).

A wireframe image of the protein will appear in the graphics window.
1. What are all the isolated "plus" signs? (Hint: Click on one and look at the echo in the space above the command window).
Turn them off by clicking on the **H** (**H**ide) next to the **1RA2** label in the internal GUI, slide down to **waters** and unclick to hide the isolated plus signs.

To display the secondary structure of the protein, click on the **H** again and hide everything. Then click on the **S** and **S**how **cartoon**. Then hold down the **C** color button to the right of the **1RA2** label and color by **ss** (secondary structure) using your choice of colors.

Now we will display the prosthetic groups as spheres. In the command window,

```
PyMOL> select folate, resn FOL < return >
```

where **resn** refers to residue name and **FOL** refers to folate name in the PDB file. Now back to the graphics command window,

```
(folate) -> S:Show: spheres
(folate) -> C:Color: magentas : magenta
```

Click on the **(folate)** label in the internal GUI to remove the selection indicators.

For the NADP you can use the sequence viewer.

Display | Sequence on

Scroll over to the NAP residue (164) in the sequence viewer and click on it. In the graphics command window on the right this "residue" is selected and just called (sele)

(sele) -> S:Show: spheres
(sele) -> C:Color: cyans : cyan

Now make the background white,

Display: Background> White

I also prefer the **Display | Orthoscopic** view rather than the default **Perspective** view (your choice).
Now we will make a ray-traced image with highlights and shadows. In the command window (or Viewer) type,

PyMOL> ray < return >

then after the ray traced image finishes redrawing make an image file in PNG format by typing,

PyMOL> png 1ra2.png < return >

And view your **png** format image in Preview or Photoshop.

2. Electron Density Maps
Let's delete the separate prosthetic group selections and go back to a basic stick figure image.

(sele) -> A:Actions: delete selection
(folate) -> A:Actions: delete selection
1RA2 -> H:Hide: everything
1RA2 -> S:Show: sticks
1RA2 -> C:Color: greens : green
Display: Background> Black

Now load and display the electron density map *1ra2.xplor*. First in the command window enter the following command,

PyMOL> ls < return >

This should list the contents of the directory from which you launched PyMOL (If you used the command line to start PyMOL; if you clicked the icon this will list the contents of your home directory.) If you don't see the file *1ra2.xplor* you will have to use **cd** to change to the *molgraph/* directory where this file should exist.

Note that the above means that the PyMOL> window understands (some) UNIX commands!

PyMOL> load 1ra2.xplor, map < return >
PyMOL> isomesh msh, map, 1.0 < return>

Make the image small (right button, drag up). Rotate the image to get a sense of the 3D shape of the electron density map.

Restrict the electron density map to within 3.0 Å of the protein object,

```
PyMOL> isomesh msh, map, 1.0,(1RA2),carve=3.0
```

Now enlarge the image so the green protein just fills the Viewer (right button, drag down) and compare the position of the model with the electron density contours. (I like to color the **msh** object magenta.)

2. Is there electron density information for all residues of the protein model?

Contour the densities at a lower electron density (you can use the up arrow to retrieve previous commands),

```
PyMOL> isomesh msh, map, 0.5,(1RA2),carve=3.0
```

3. Now is there electron density information for all atoms of all residues of the protein model?

4. What are defining physical characteristics of the residues that do not have electron density? E.g. polar, non-polar, large, small, aromatic, etc. (Double click on the naked atoms to see which residue type they are part of.)

5. How were the conformations of these side chains obtained for the model if there is no density to indicate its conformation?

Later in the course you will calculate probability density maps from your protein simulations and use **PyMOL** to help interpret the probabilities of different protein conformations. Quit **PyMOL**.

B. VMD (Visual Molecular Dynamics)

VMD is a molecular graphics program especially designed to visualize molecular dynamics trajectories including making movies of the trajectories. VMD is very powerful and that means it can be very complicated. This lab is an introduction but we will only touch on a few commands that will be useful in this course. **VMD** can be extended with the TCL script language and we will use scripts later in the course. There are a large number of TCL scripts for analysis; some are built-in and others are available on-line. Documentation for **VMD** is available as a link from the course website on the Links page (pages.jh.edu/pfleming/compbio/links.html). Some of the steps below were taken from a VMD tutorial that is also available on the course website from the Links page (it is a PDF). The tutorial has much more than the following introduction but it uses files that you have to download from the VMD site.

1. Visualizing simulation trajectories.
In the first half of this course your simulation trajectories will be large PDB files that contain multiple structures. These are readable by humans (they are ASCII format) and we will use PyMOL to visualize them. However, most research grade trajectories are so large that, instead of being saved in ASCII format, they are in a binary format (*.dcd* extension) that is much more compact but is not readable by humans. In order to make sense of binary files VMD needs to also have what is called a structure file (*.psf* extension) that describes the atoms names of the structure. Use **sftp** to download two files from the *compbio2/Shared/* directory on kirin*: ionized.ps*f and *production.dcd*. Make sure the two files, *ionized.psf* and *production.dcd* are in your *molgraph/* directory. Launch **vmd**,

```
vmd
```

In the **VMD main** window, choose,

File | New Molecule...

Another window, the **Molecule File Browser** window will appear. Use the **Browse...** button to find the *ionized.psf* file. Then click the Load button.

Now load the trajectory that corresponds to that structure file. Use the Browse... button to find the *production.dcd* file. Then click the Load button again. Close the Molecule File Browser window.

(Note: A quicker way to launch VMD with the structure and trajectory already loaded is to simply type the following at the terminal command window, **`vmd ionized.psf production.dcd`**).

Click the following from the **VMD Main** window: **Display | Orthographic**. Before learning the mouse conventions let's un-display the water. Click the **Graphics | Representations...** menu items from the VMD Main window. In the **Selected Atoms** box enter the word, **`protein`** and hit the return key. Notice that the water disappears. Now use the **Drawing Method** menu to choose **VDW**. You should now have the protein displayed as space-filled atomic spheres. Move the molecule around with the left and right buttons of the mouse. Scroll the middle button to zoom the display in and out. Below is a table of "hot keys" to change the function of the mouse left button.

Hot Key	Purpose
r	enter rotate mode
t	enter translate mode
s	enter scaling mode
0	query atom (Info appears in terminal window when you click the atom.)
c	assign rotation center

2	display distance (two atoms)
3	display dihedral (three atoms)
=	reset to original view

View the trajectory by clicking on the ▶ button (lower right in the **VMD Main** window). Slow the movie down using the **speed** slider. Every frame of the movie is 2 ps of simulation time.

Stop the movie by clicking the ▶ button again.

2. Selecting components for display

Selecting atoms, residues and fragments is the key to making molecular graphics programs useful. In this section you will learn a few things about selection in VMD using the GUI. Note that one can also use the command line to select things, but at first, the VMD selection syntax maybe particularly unintuitive (see below) so we will use the GUI windows if possible.

Click the **Graphics | Representations...** menu items from the **VMD Main** window if you previously closed it. The idea here is to make multiple "representations" of the structure and display specific components of each representation. Click the **Create Rep** button. In the **Selected Atoms** box enter the word, **`water`**. Now the water is so dense that you can't see the protein. Change the water **Drawing Method** to **Dynamic Bonds**. Start the movie again.

Notice that the protein drifts toward the edge of the water box and even partially exits it. Let's center everything on the protein and "wrap" the water box around it. (This uses periodic boundary conditions that you learn about in lecture.) Stop the movie and enter the following command in the VMD active terminal window,

```
pbc wrap -center com -centersel protein -compound fragment -all
```

This means **wrap** molecular fragments (i.e. the water) around the center of mass (**com**) of the selected center component (**protein**) and do it for **all** frames in the trajectory.

Start the movie again. The water box now moves around to keep the protein solvated. Let's look only at the protein again. In the **Graphical Representations** window double click the **DynamicBonds Name water** line to turn off the water display.

The protein molecule drifts during the trajectory and this makes it difficult to concentrate on one residue. Let's center the protein for each frame. To do this we will align the protein in all frames to the protein in the first frame. Stop the movie and in the **VMD Main** window use the slider to display frame number zero. Then click the following,

Extensions | Analysis | RMSD Trajectory Tool
Selection Modifiers = Trace, Reference mol = Top, and click **ALIGN**
Close the **RMSD Trajectory Tool**

Start the movie and find a hydrogen bond.
6. Stop the movie, query the identities of the two hydrogen bonded atoms and enter the Info) records as the answer to this question.

Now let's look at the water in more detail. In the **Graphical Representations** window change the **Selected Atoms** from **`water`** to **`water and within 3 of protein`**, enter return and click this representation to display it. Play the movie. Notice that in the movie the waters drift more than 3 Å away from the protein. To fix this go to the **Trajectory** tab and click the button labeled **Update Selection Every Frame**. Play the movie again.

7. In general, do the individual water-protein hydrogens bonds persist for a longer or shorter time than the protein-protein hydrogen bonds?

Click the **DynamicBonds Name water** line to turn off the water display again and display the protein in other **Drawing Methods** to become familiar with what's available.

Quit the VMD program by clicking on **File | Quit** or typing **`quit`** in the VMD-active terminal window.