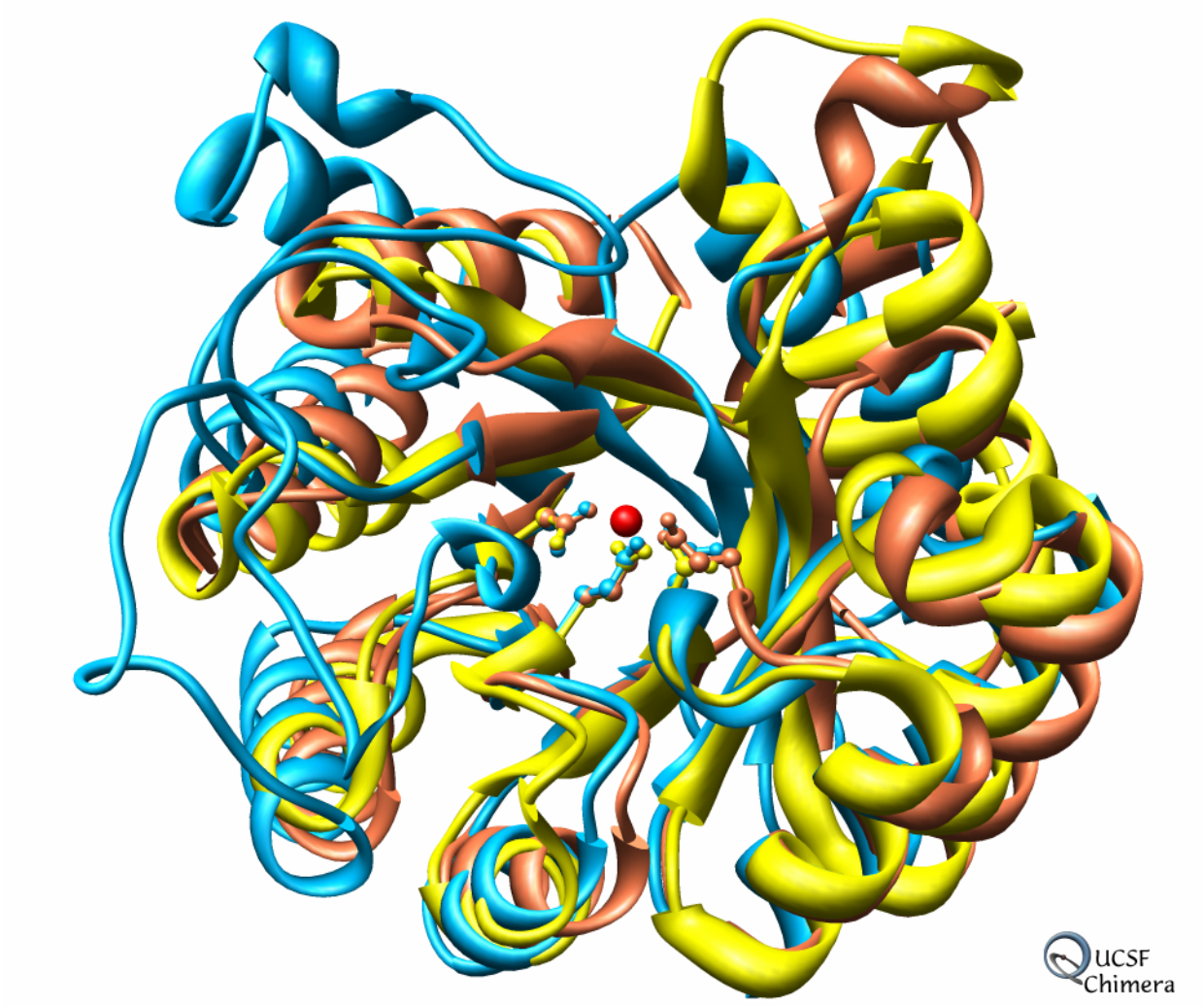
Molecular Visualization

A BBSI Tutorial
http://www.ccbb.pitt.edu/BBSI/index.htm



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Instructions for PDB downloading (from either website)

-Go to:
www.rcsb.org/pdb/

-Type in name of protein (examples at bottom of the page).

- Click on Name icon (first name in purple box).

-On the left side of the screen, click on *Download/Display Structure*

-Under Download the Structure File, right click on the X where the PDB(top) meets with none, under compression (on left) in the table and *save target as*.

- Save under any filename you would like on your hard drive.

- Go to:
www.pdb.bu.edu/oca-bin/pdblite

- Type in name of Protein / Macromolecule (examples at bottom of page).

- Click on *Retrieve Released Data Matching Your Query* icon.

- Highlight any of the molecule name and click on the *View/ Analyze/ Save Macro Molecule* icon.

- Under the Data Retrieval section, click on the *Save ____.pdb* icon.

- A pop up menu will show up, click on the *Express save for Experts* icon

- Save under any filename you would like on your hard drive

Examples for Downloading:

- Any protein/ macromolecule structure you would like to see.

- Some interesting suggestions:
  1.) Human Hemoglobin
  2.) Chitinase
  3.) HIV Reverse Transcriptase
  4.) Antifreeze Proteins
  5.) Carbonic Anhydrase II

RasMol Tutorial 

For a full on-line manual and tutorial, visit:
http://www.umass.edu/microbio/rasmol/index2.htm
http://info.bio.cmu.edu/Courses/BiochemMols/RasFrames/TOC.HTM

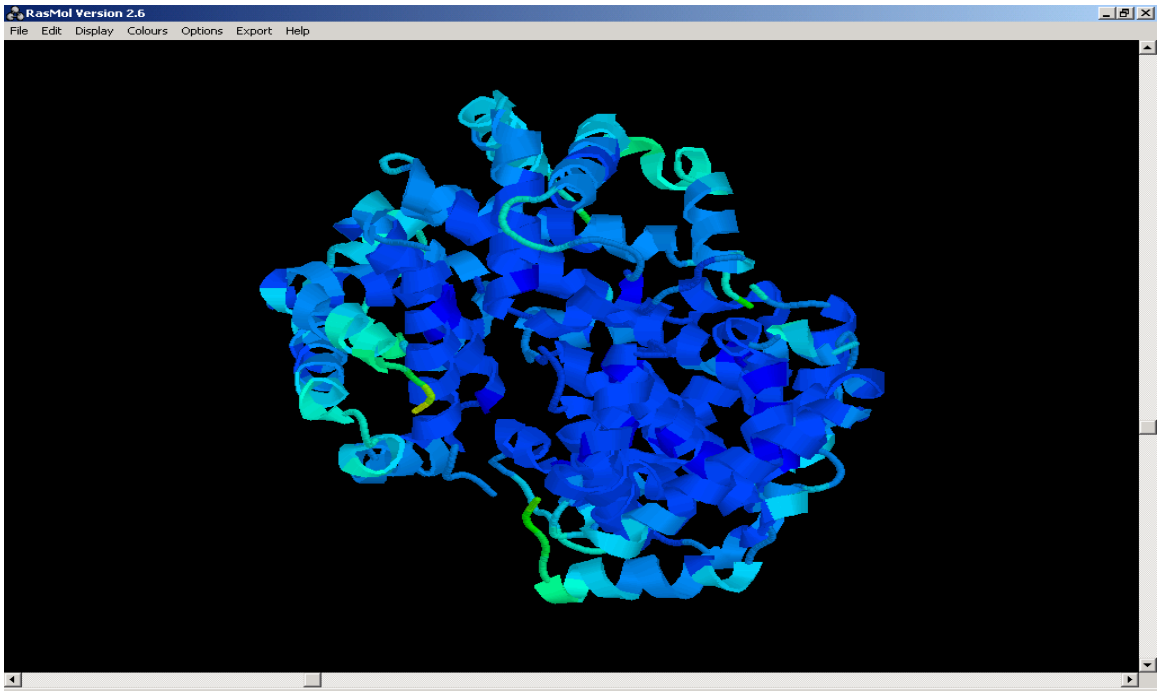
I.) Open a PDB file in RasMol by selecting File and Open.

Use the mouse to rotate, translate, and magnify the molecule. Some useful command include:

- L (left mouse button) will rotate the molecule in the X and Y direction.
- R (right or center mouse button) will translate the molecule in the X and Y direction.
- Shift + L will magnify or shrink the molecule.
- Shift + R will rotate the molecule in the Z direction (axis coming out of the screen)

II.) Explore the options of RasMol by selecting the tabs at the top.

A.) File – For use in opening, closing, printing, and information on files.
B.) Display - Different forms to display the molecule.
C.) Colours – Colors the molecule according to groups, atoms, chains, ect.
D.) Options – Allows user to view the molecule in different ways.
E.) Export – Saves pictures of molecules to be used in other programs.
F.) Help, User manual – Shows the scripts and options available in RasMol.



Try and use the different Display, Color, and viewing Options to view your molecule. Become familiar with the different ways to view the molecule and think about how these different ways to view the molecule would be useful in explaining molecular properties. Also, save the molecule as a picture under the Export tab (I suggest BMP format) so that you can insert this into a MS Word document.

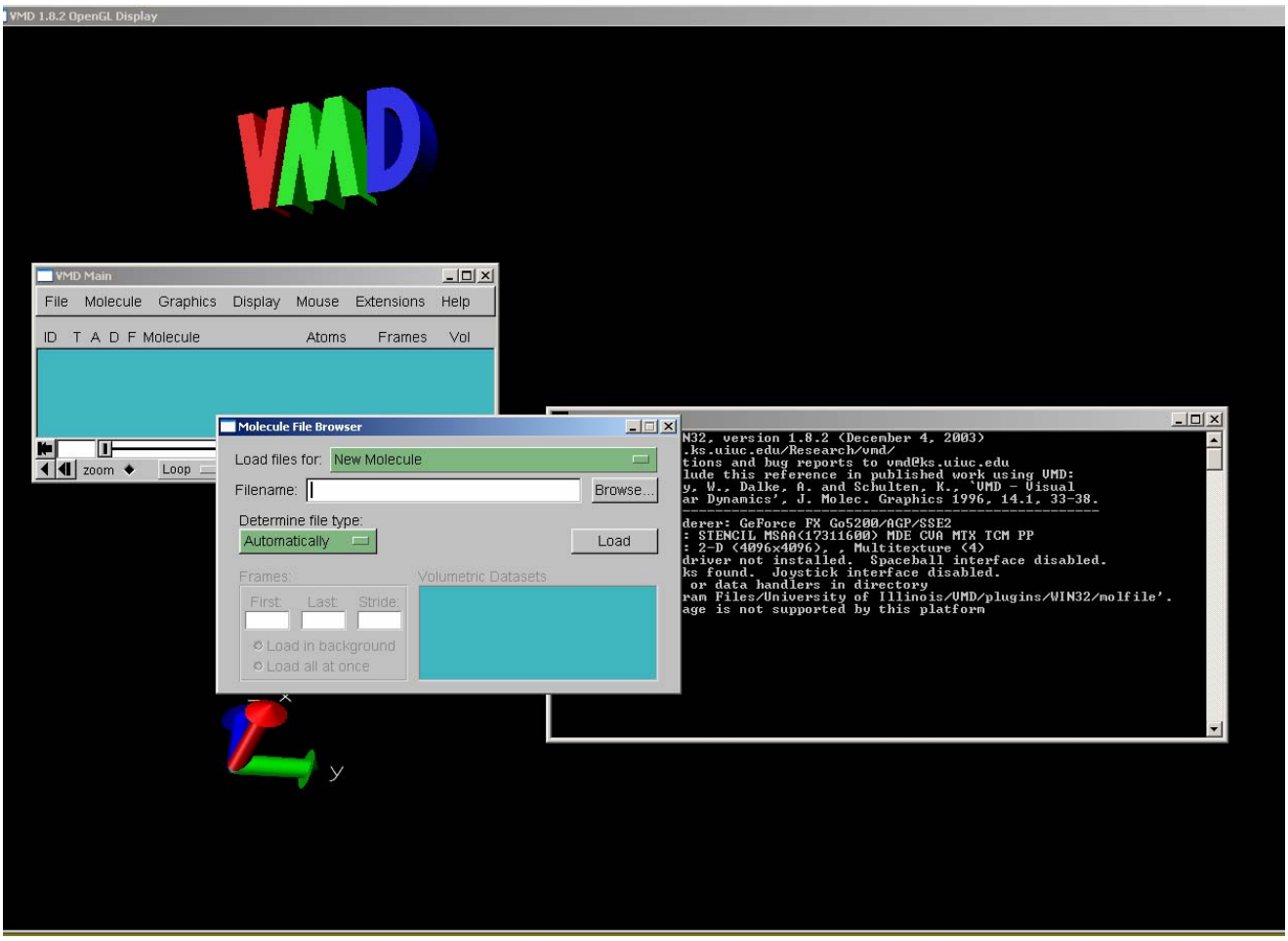
III.) Download a structure of myoglobin from the Protein Data Bank (sperm whale myoglobin). Open the PDB in RasMol. Look at the structure and try to determine where the helices are. Change the color to *colour* by *group* and then *Display* the structure of myoglobin as *Cartoon*. Notice the difference in being able to see the helices of the myoglobin in molecular form and in a cartoon representation; it is much easier to now look at the orientation of the helices with respect to each other. Save a picture of the myoglobin and close the RasMol program.

VMD Tutorial

For more information, visit the VMD website: http://www.ks.uiuc.edu/Research/vmd/

I.) To open a PDB file in VMD, click on *File→New Molecule* in the *main* menu. A pop up window will open and choose the *Browse* option: a new window will open. This will allow you to select the pdb file from your hard drive (the file name will appear in box). Now click on the ***Load*** tab in the middle. An example of the screen is shown below.

When the molecule loads, it will spin (sometimes very annoying). To keep it from doing this just click on it with your mouse. Your mouse will rotate the molecule in VMD (use L to rotate in X/Y directions and C to rotate in Z direction) and zoom by hitting the "s" key first ("r" key returns to rotate) . There are also options to do this under the *Display* tab in the main menu.



II.) Use the tabs in the *main* menu such as Sequence, Color, and Materials under the *Graphics* tab to see the capabilities of VMD. Also, be sure to look at the *Display* and *Mouse* tabs in the main menu.

Use the Graphics menu to change the viewing options of the molecule. Change the *Coloring Method* and *Drawing Method* to view the molecule in different ways (ex. try the Res Type under *Coloring Method* and Bonds under *Drawing Method*).

III.) We will now use some of the advanced functions of VMD to load trajectory and pdb files into VMD to make movies. A trajectory file will have the extension .dcd. The instructors will provide PDB and DCD files for this part of the exercise.

Click on the *File* tab in the main menu as we did before; then click on the *Load Data into Molecule*. Select *a dcd* file fromyour hard drive and then click on *Load* as you did before. (Make sure the pdb file is highlighted in the main window.)

The files will open and you will see lines going across the system. These are the bonds of the oxygens to the hydrogens in water molecules as they move across the periodic boundaries of the system (if you have not discussed periodic boundaries, they will be covered in the near future in the lectures). To make this easier to view we will mask the hydrogens of the water molecules. Go to the *Graphics→Representations* menu and type in "not hydrogen" in the box under Selected atoms, and then click on Apply at the bottom of the menu.

To make the system easier to view, I will suggest a few things to do:

1.) Make the background white-Go to the *Color* menu and select display under Category, then click on background under Names, and click on the White box at the bottom of the screen.
2.) Under *Display*, turn the *Axes*→Off and *Projection*→Orthographic.
3.) Change the protein backbone- Under the *Graphics* menu, click on the *Create New* button at the bottom. In the Selected Atoms box, type "segname SEG1 SEG2" (this selects both of the proteins in this system) and *Apply* at the bottom. In the blue box at the top there will be two selections; highlight the segname one and then choose Under *Drawing Methods* the way you would like to view the backbone (VDW is a nice selection for this protein).

At the bottom of the *main* menu, you will see buttons to control the animation. In this menu you will be able to advance frame by frame through the dcd file, skip frames, play in forward and reverse, and loop (continually run) or stop after one time through the dcd file.

IV.) If you want to save the movie, there are several ways to do this (you can check on the VMD website) In one way to save movies, you will need to download VideoMach from the website and install it on your computer: http://www.gromada.com/VideoMach.html.

You will also need to install the plug in into VMD (you might not have enough user privledges in this lab, but you could do this on your personal computer. This can find this at:
http://www.ks.uiuc.edu/Research/vmd/script_library/scripts/vmdmovie/

You will download the vmdmovie1.0.tar.gz and use WinZip to extract it to a directory on your hard drive. You will then nee to go to the command prompt screen in VMD and type "source c:/temp/vmdmovie.tcl", where c:/temp can be any path name to where you extract the WinZip files to. In the VMD amin menu you will see the *Extensions* tab, under which you will find the vmdmovie button. Click on this and set your output directory, and then just click on the Make Movie button at the bottom. This will save the movie and open VideoMach. Save the movie (avi format works well) in VideoMach You can now play this in Windows Media Player or put it into MS Powerpoint as a movie.

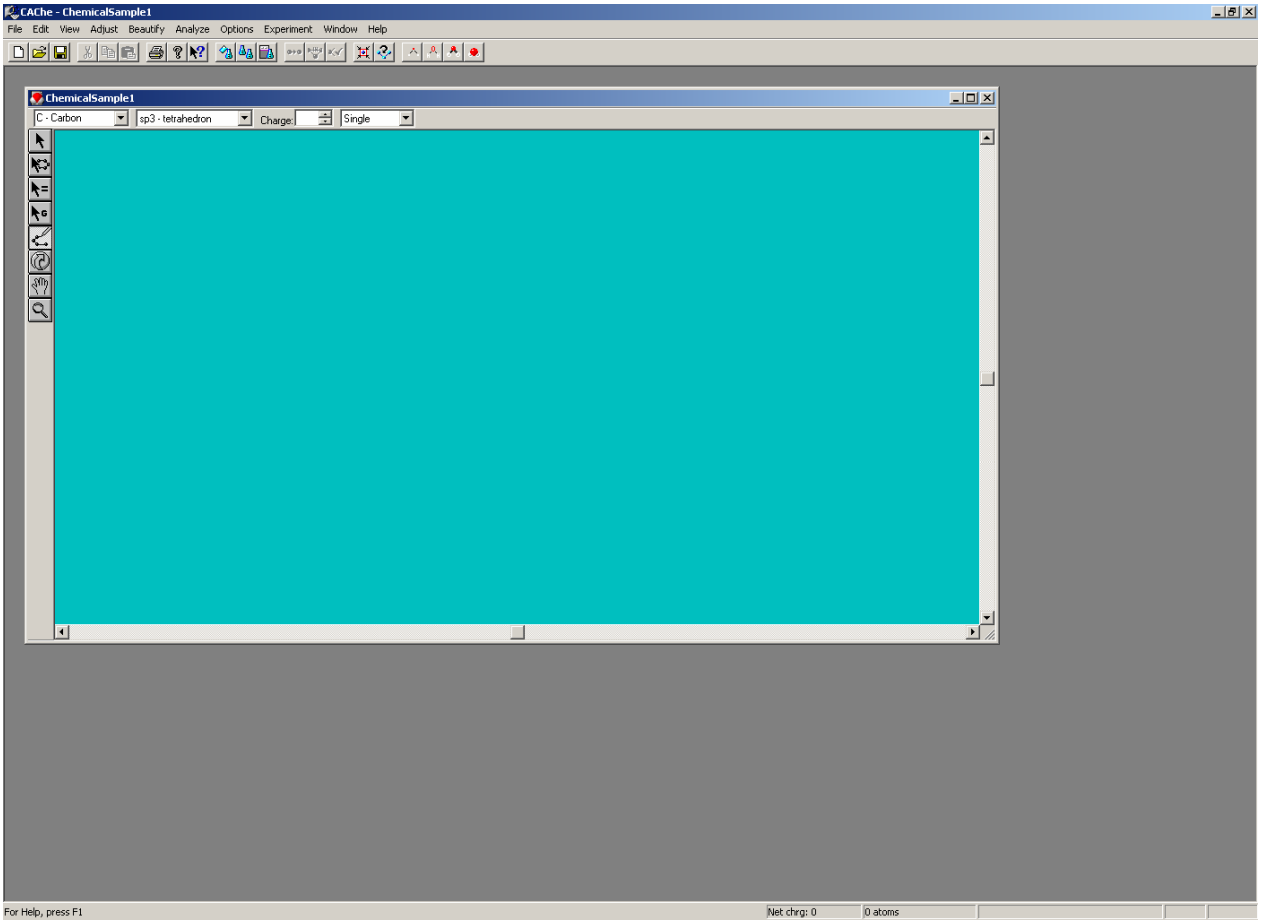
V.) For this example, we will visualize the myoglobin molecule to see important molecular features; we will display the heme group as ball and stick and the backbone as a ribbon. Open the *Myoglobin* pdb file (from the RasMol tutorial) and open it like before. Similar to part3 in step III (above), we will open the *Graphics* menu and click on *Create New* button at the bottom. In the select atom box, type, "resname HEM" and Apply. Click on this selection in the blue box and select the Coloring Method to be *Name* and the Drawing Method to be *CPK*. The click on the other selection in the blue box (the all selection) and select the Coloring Method to be *Chain* and the Drawing Method to be *Ribbon*. Notice how the heme group binds the oxygen and how it would have been very difficult to view if the whole molecule were shown in the same type of drawing method.

CAChe Tutuorial 

For more information, visit the Help menu at the top of the page. It contains a full user manual in pdf format.

1.) Start with opening the CAChe workspace. It will be a blue window inside of a gray window, which can be seen below.

Become familiar with the controls of the CAChe style bar located at the top of the light blue window. The four scroll down buttons at the top will show you the atom type, hybridization, charge, and bond type.



Going down the right side of the blue screen will be a series of tabs. The default highlighted tab is the Drawing tab. Below this you will see the Rotate, Translate, and Magnify tabs, respectively and above the drawing tab is the Select Group, Select Similar, Select Molecule, and Select Tabs, again respectively.

Also investigate the tabs in the main CAChe window; the *View* tab offers a variety of ways to draw atoms and molecules. Use the select tools discussed above to highlight certain areas of your drawn structure, and change the atoms/ bond types of your molecule; also, be sure to vary the color of your molecule.

2.) Use the drawing tool to build several different molecules of your choice. Some that you may want to try are:

Pentane
Octane
Cyclohexane
Benzene

In the main CAChe window, you will see the *Beautify* tab. This tab will give you the opportunity to clean up your structure. You have the choice of doing this individually to the rings, hybridization, ect., or you can do this all at once with the Comprehensive choice at the top.

3.) Downloaded the PDB file, GP-39 from the PDB website and open it in the CaChe workspace. Use the curved arrow in the CAChe window to rotate the molecule; look for any distinctive feature on the surface of the molecule.

Use the *View* tab in the main window to adjust the backbone and the molecule types for viewing. You should be able to see the overall structure of the molecule by showing the backbone and/or viewing the individual atoms. Rotate the molecule; notice how the backbone aids in seeing the 3-dimensional features of the macro-molecule. Can you identify the active site of the molecule (Hint: function follows form)?

MOE Tutorial

For a more complete tutorial, see the *Help* tab at the top of the MOE window or see the CCG website at http://www.chemcomp.com/fdept/back.htm.

I.) Open a PDB file of your choice in MOE by using *File→ Open.* Select the PDB file and either click on *OK* (at the bottom of the screen) or on *Load PDB* (to the right). Under the Load Options, select Center Molecule and under the Read Options, select Load All Models and Auto-connect Atoms. It may take a couple of seconds for the file to load.

II.) Explore the abilities of MOE by rotating the molecule with C (center mouse button) or the dials at the bottom of the screen and translating the molecule with Shift + C or the dials at the bottom of the screen. Use Ctrl + C and the mouse to zoom in or out of the molecule.

III.) Use the L (right mouse button) to select single atoms or group of atoms; use the Ctrl and Shift keys with L to select residues and multiple atoms respectively. Selected atoms will appear in pink. Use the *Selection* menu at the top of the screen to select atoms using advanced techniques such as geometry and elements and atom selector.

Now, use the invert key to select all of the other atoms that were not selected before and use extend to select the rest of the residue, chain, ect. Be sure to look at some of the available functions of the *Atom Selector* such as the Connectivity and Extend functions in this menu; the proximity function can be set to any distance (in Å) and can be very powerful for the determine how close certain atoms are to each other. The screen for the atom selector is shown below in *Figure 1*.

IV.) Use some of the main menu features (on the right side of the main MOE window). The Distances, Angles, and Dihedrals tabs can be useful in determining exact structural properties that could be useful to you; Remove allows you to take these back off of the screen. Atoms and Sequence can show you the exact order of the molecule giving you the atom names/types and the sequence of the amino acids. The Builder tab allows you to change the molecule or build your own unique molecule (this would need to be minimized, but is not covered in this tutorial).

These are some of the advanced features of MOE that make it unique from the other programs discussed. After understanding these selecting functions, we can implement them in using more powerful functions of MOE to show VDW contacts, hydrogen bonding, and backbone manipulation on part or all of eh structure.

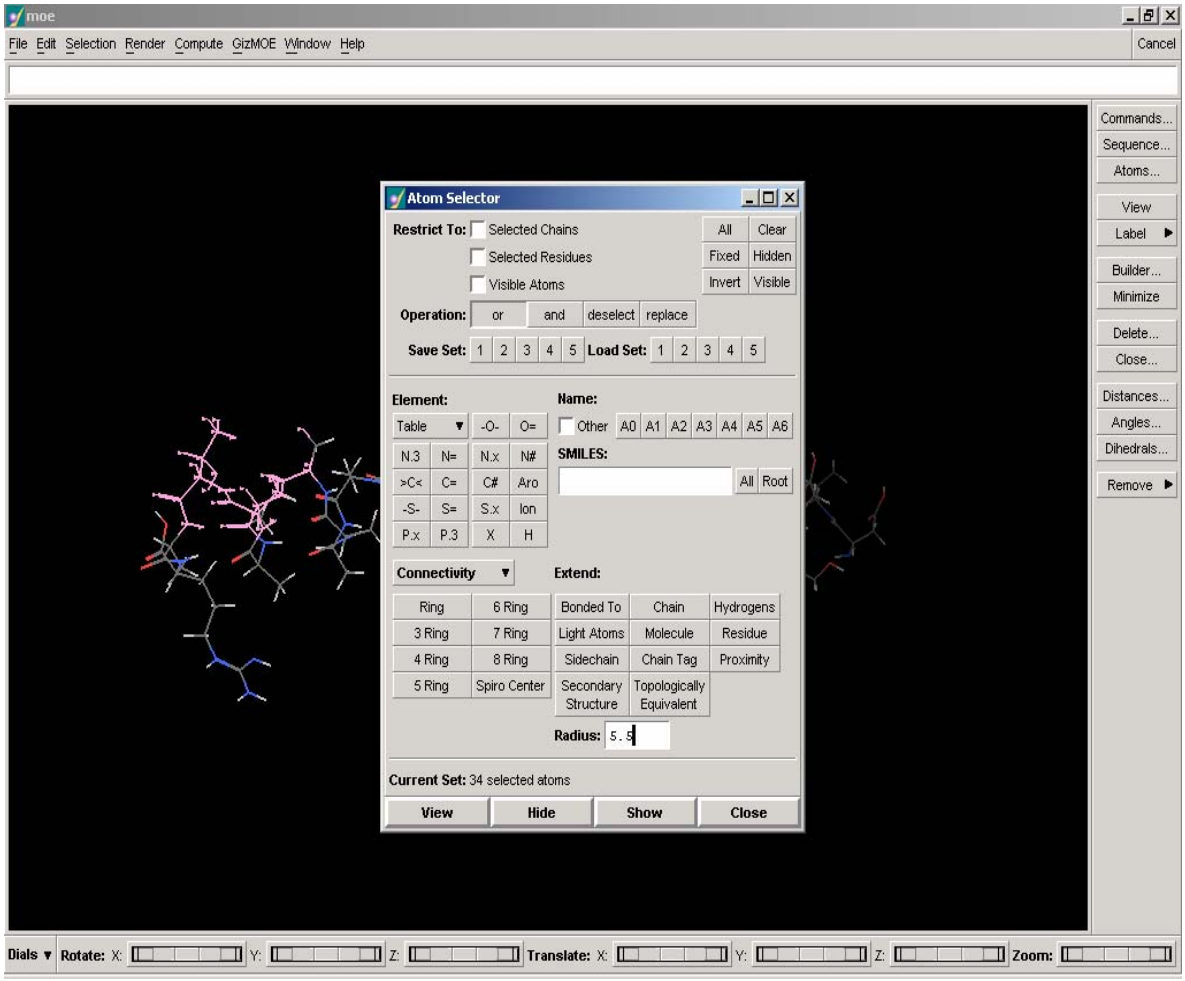


Figure 1: The Atom Selector can be used to extend the selection to the chain, the atoms that your selection is bonded to, secondary structure, proximity (in Å), ect.

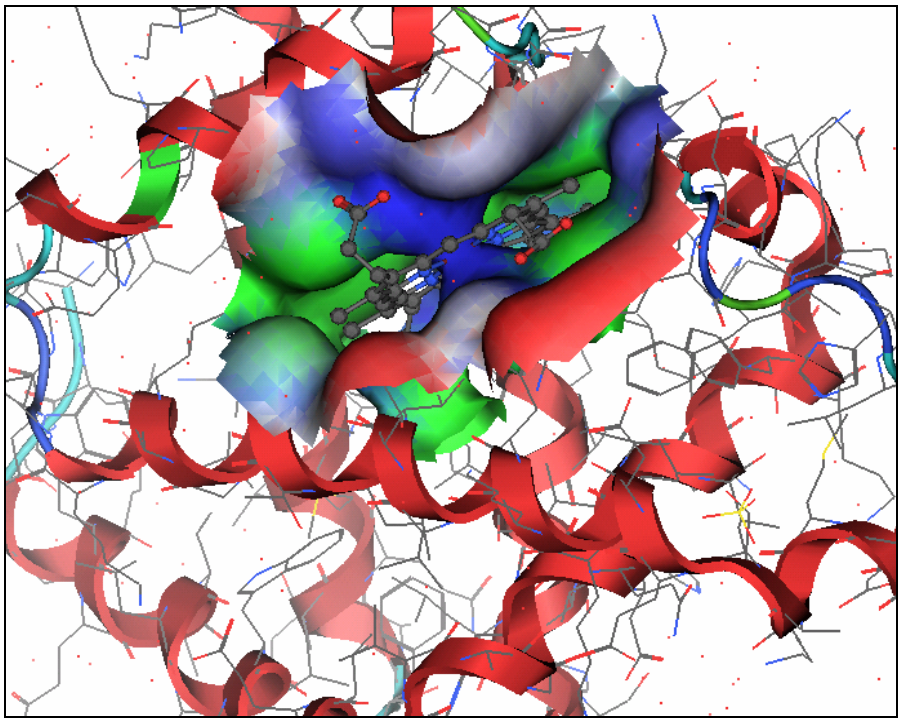
V.) Under the *Render* tab, use the functions under the *Draw* function, such as Hydrogen bonds and VDW contacts to show the contacts of the molecule chosen.

Use the *Backbone* function to change the prominent structure to a more easily viewed structure; you can show the backbone as cartoon, tube, ect. and you have the ability to change the color of the backbone as well.

You can change the way to view individual atoms and bonds with the Line, Stick, Ball and Line, Ball and Stick, and Space Filling functions. The Hide and Show Functions allow you to mask or view only certain features of the molecule. The Color, Nucleus, and Bond functions of this menu allow you to change the way that the molecule/atoms and bonds are colored or viewed; the color function will allow you to color based on many distinguishing features of the molecule. The boxes will allow you to visually see the molecule in different ways. Under *Setup* you can change the color of any of the viewing

functions in MOE: the range goes from 0 (R,G,B zero's will be viewed as black) to 255 (R,G,B 255's will be white). You can change the background to white for easier viewing or for saving a picture.

VI.) We will now look at how to display a molecular surface in MOE.



First, open the *Myoglobin* pdb file and display the backone as cartoon. Next, display the heme group as ball and stick. Go to *Sequence Editor* (right side of main screen) and right click on the heme (HEM) group in the sequence; choose *Render→Ball and Stick*. In the main window find the heme group and click on one of the atoms using Ctrl + L to select the whole residue (this will highlight the heme in pink). Go to *Compute→Molecular Surface;* click on the *Restrict to* block (the default distance value of 4.5 Å should suit for this case) and click on Apply at the bottom. Rotate the molecule and see how the pocket around the heme group is shaped.

Chimera Tutorial 

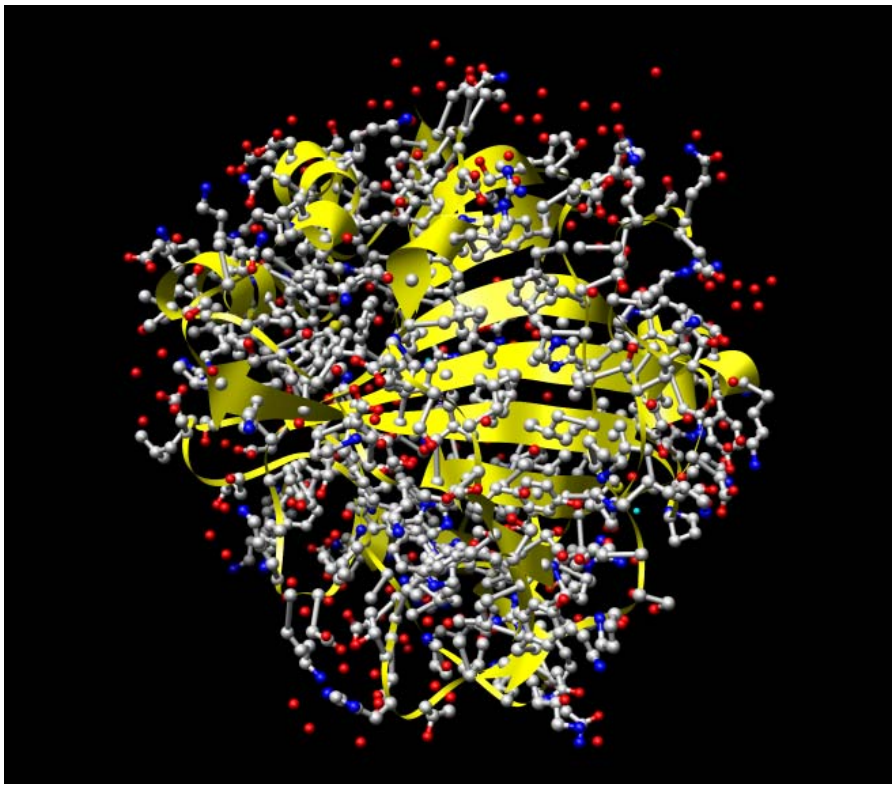
For a complete tutorial visit the UCSF website at http://www.cgl.ucsf.edu/chimera/ or click on the help tab for a list of topics on a variety of features.

I.) Open a PDB structure of your choice in CHIMERA using *File → Open.* A navigation window will be displayed. Click through the directories in order to find your PDB file. Once your PDB file is available, click on it and click *Open*. Your structure should be displayed in the main window.

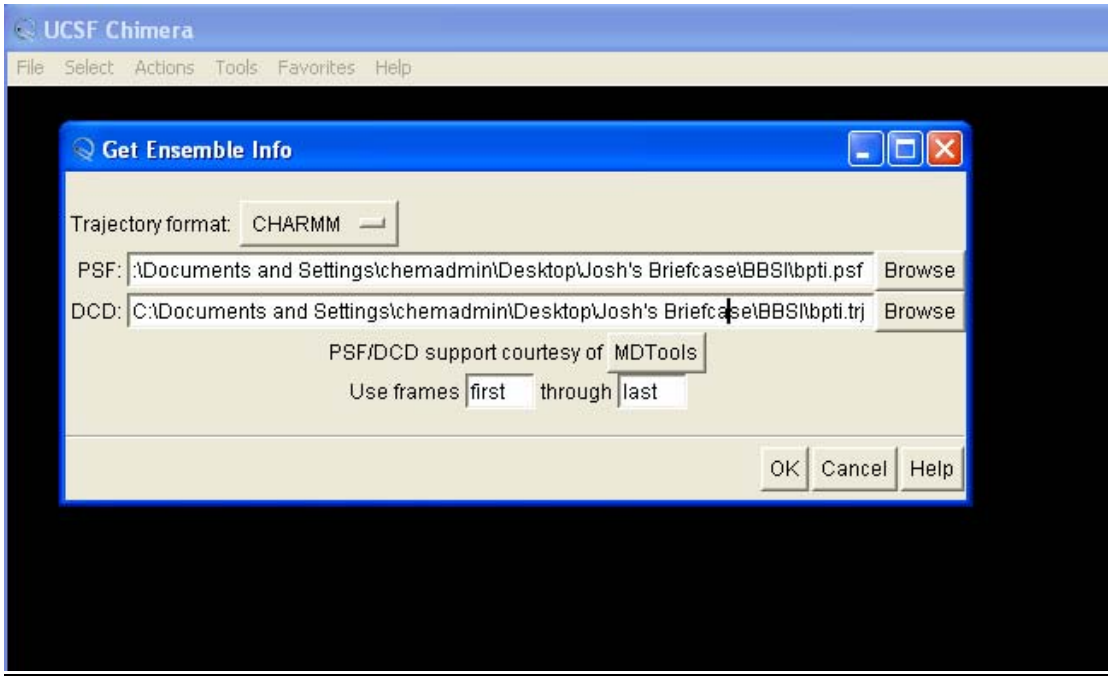
II.) Chimera is a useful program, with a plethora of features. A useful way to learn any visualization program is to just explore the commands, through the drop down menus and determine what they do. This tutorial is just a way to allow you to become familiar with the program in order to explore the variety of the commands at your own leisure.

First, position your cursor over your structure and press the left mouse button down. With the left mouse button pressed down, move your cursor in order to rotate your molecule in the corresponding direction. To zoom in and out, keep the right mouse button pressed down and move the cursor. In order to translate your molecule, keep the center mouse button pressed and move your cursor in the desired direction.

III.) Chimera allows a user to manipulate the structure into a variety of formats such as wire, stick, ball and stick, etc. To explore these features click *Actions→ Atoms/Bonds.* Press ball and stick to display atoms as balls and bonds as sticks or lines. As you will notice all the bonds and atoms are white. To make it clear as to what sphere is what atom we can color the molecule to designate each atom with its own corresponding color. Click *Actions → Color → Atoms/Bonds.* Then press *Actions → Color → by element.* Secondary structure ribbons for peptides, proteins, and nucleic acids can be displayed using the command *Actions → Ribbons → show.* The color of the ribbon can also be changed by clicking *Actions → Color → ribbons* then click *Actions → Color →* (*color of your choice).* If you followed the above instructions on the bpti structure you will develop the following pretty picture.



IV.) Now let's take a look at creating a movie as we did with VMD. Creating a movie in Chimera is more straight forward than VMD. Two files are necessary for creating a movie utilizing Chimera, the .psf file and the .trj file. These files will be given to you by the instructor. Click on *Tools* → *MD/Ensemble Analysis* → *MD movie.* A window will pop up with the heading "Get ensemble Information", as shown below. Make sure the trajectory format is CHARMM. Other formats are available. However, for this exercise CHARMM will be utilized. Load the .psf and .trj files. Once that is complete click OK, and the "MD Movie" window will pop up. The play back speed can be adjusted accordingly. Keep in mind the slower the playback speed the longer it takes to record. To record the movie click *File* → *Record Movie.* You will be prompted to save the movie as whatever you like. Click Record. After recording is complete go to where you saved the movie and open the movie file. Grab some popcorn and enjoy the fantastic movie clip.


UCSF Chimera
File Select Actions Tools Favorites Help
Get Ensemble Info
Trajectory format: CHARMM
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Browse
DCD: C:\Documents and Settings\chemadmin\Desktop\Josh's Briefcase\BBSI\bpti.trj
Browse
PSF/DCD support courtesy of MDTools
Use frames first through last
OK
Cancel
Help

Exercises

THESE ARE TO BE WRITTEN UP AND TURNED IN AT THE END OF CLASS!!
Don't worry, it just an exercise and there is no grade.

RasMol and CAChe

I.) Download and open up the 3CA2 (Carbonic Anhydrase II) PDB file and open it up in both the RasMol and CAChe programs. Open the molecule and render them in a style of your choosing. Write a brief description of the 3-dimensional structure and render a picture.

VMD

II.) Open up the AVM1 and AMD1 PDB files (Carbonic Anhydrase II). Hide the water molecules like you did in the RasMol exercise. Display the protein structure the same way that was done in RasMol with the helices in cartoon formand the active site in ball and stick form (CPK). Notice the differences in the displayed format and the ease of the VMD program. As before, also save an image of the structure.

MOE

III.) Download the GP-39 and Chitinase (you may have already done this) and open them in MOE. Do the routine as the RasMol and VMD exercises and display the molecules in the exact same fashion. Note, this should be a lot easier than the previous two exercises. Save an image.

To overlay several protein structures, open up multiple pdb files in MOE (Carbonic Anhydrase II with different bound ligands is a good choice). Use the *Sequence Editor* and hide all of the water molecules. Click on chain numbers of the protein (holding Ctrl) to highlight them all in the *Sequence Editor* and use the *Homology→Align* selection to align the proteins and use *Homology→Superimpose* to put the proteins on top of each other. Using the selection features shown previously, highlight the bound ligands and show the molecular surface around the active site. Explain the active site structure and can you see hydrogen bonding? What other types of interactions might be occurring here?

Chimera

IV.) Open a PDB structure of your choice and apply colors and structure formats as you desire. Write a caption for your figure as if you were going to publish a journal article about your molecule. Do not be wordy but be clear and concise.

Bonus: Then next logical step in molecular visualization would be to observe how the ligand is bound in the active site, i.e. what is the orientation and how might this change with mutations. We will use Carbonic Anhydrase again for this example. Go to the pdb website and find 3-5 Carbonic Anhydrase (II) mutated structures with the same bound ligand. Use the steps shown above to align and superimpose the structures. Highlight the molecular surface around he active site. Use the selection techniques to highlight the bound ligands from each of the

downloaded structures. See if the orientation is the same as the other ligands. If the orientation changes, what kinds of interactions with the ligand were made/destroyed from mutating the protein? How might these interactions change the orientation of the ligand in the active site?