

Molecular Graphics Exercises

All of these web sites are best viewed with Mozilla Firefox. IE has too many problems associated with it.

A. Protein Data Base (www.rcsb.org)

- Examine some of the information on the Protein Data Base website
- Call up the 1.5 Å crystal structure of bovine heart cytochrome c determined by Mirkin et. al. (2008) Proteins **70**, 83 : 2B4Z.
 - Take a look at the general layout of a PDB file
 - Distinguish between the atom entries (i.e. protein atoms) and the hetatom (any other atoms for groups other than protein, including H₂O oxygen atoms)
 - *While you are looking at the PDB file for cytochrome c, make note of the atom designations for the two carboxylic acid oxygens of Asp and Glu, the terminal nitrogen of the the Lys side chains, and the nitrogens of the guanadinium group of Arg. Also, find the terminal S atoms of Cys residues, noting their designations. You will need to know these designations in order to complete the initial assignment.*

B. Useful Jmol websites

- Jmol: www.jmol.org
- **The best Jmol command page:**
chemapps.stolaf.edu/jmol/docs/
- Jmol's official interactive scripting:
<http://jmol.sourceforge.net/demo/#Interactive%20applet%20demonstration%20pages>

C. Very useful RasMol websites

- **Select/Restrict:**
<http://www.umass.edu/microbio/rasmol/seleccmd.htm>
- RasMol Manual:
<http://www.umass.edu/microbio/rasmol/distrib/rasman.htm>
- RasMol: <http://www.umass.edu/microbio/rasmol/>

D. Other useful websites for those who want to expand their knowledge of Jmol scripting

- Online Macromolecular Museum: An outstanding collection of artful and informative Jmol routines and scripting tutorial:
http://www.callutheran.edu/Academic_Programs/Departments/BioDev/omm/gallery.htm
- Bioc 462a Jmol routines:
<http://www.biochem.arizona.edu/classes/bioc462/462a/jmol/routines/routines.html>
- Proteopedia (a wiki site for protein structures): www.proteopedia.org

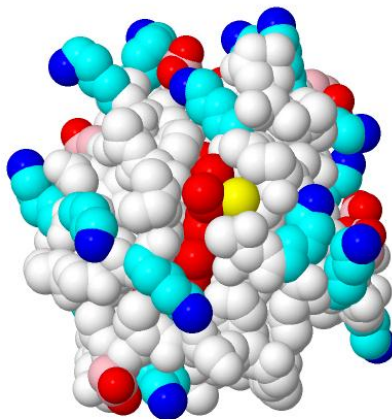
- Center for BioMolecular Modeling: <http://cbm.msoe.edu>
 CBM's Jmol tutorial page: <http://cbm.msoe.edu/teachRes/jmol/index.html>
 A very nicely done influenza virus hemagglutinin webpage:
<http://cbm.msoe.edu/includes/swf/HAAAnimation.swf>

E. Jmol Exercise:

NOTE: If you want to print out any figures, make sure you change the molecular graphics window to WHITE, otherwise you will burn through a print cartridge VERY QUICKLY!

The objective of this assignment is to generate a Jmol image that clearly illustrates why cytochrome c has a greater pI than hemoglobin (which can be downloaded from RCSB as 1G09.pdb, corresponding to carbonmonoxyHb at pH 7.2) and why cytochrome c was bound more tightly to the CMC ion exchange column in last week's chromatography experiment. We want to see a clear representation of the side chains of all the charged amino acids shown in some light shade of color, the atoms which are the sources of the point charge displayed as dark red for negative charges and dark blue as positive charges. Everything else in the protein is to be shown as a uniform color and heme group is to be highlighted, all being shown as CPK structures.

C-type cytochromes get their name from the fact that the heme group is covalently attached to the protein via a thio-ether linkage between two Cys residues and the heme group. In your diagram, also color code the S of the Cys residues as yellow in the CPK representation.



NOTE: *You will not be able to complete this figure by pointing and clicking on the pull down menus within Jmol alone. You will have to eventually resort to writing commands in the Console mode of Jmol. In order to do this, you will first have to learn how to SELECT a specific item (look at the SELECT website given above) and you will have to learn the specific commands (look at the Jmol Command page given above). As far as choosing colors, you can Google "RGB color codes" (or whatever type of color formatting you wish to use) in order to see how they are designated (as a name or a number code) OR you can rummage through the Jmol command documentation to find out how to designate colors.*

Application: In addition to trying to figure out why cytochrome c was bound so tightly to the CMC column, there is a much more important aspect to the charge distribution of this protein that is addressed in the following section.

Notice how the positive charges are distributed on the protein surface relative to the edge of the heme group. Where are the negative charges located relative to the heme edge? Since most of you are in Bioc 462B, where the electron transport chain (ETC) will be discussed, consider the following. Mitochondrial cytochrome c interacts with several different enzymes in the inner membrane space of the mitochondria. It is reduced by the membrane bound cytochrome bc_1 complex (aka Complex III) of the ETC. It is oxidized by cytochrome c oxidase (aka Complex IV), the terminal electron acceptor of the ETC. In yeast, cyt c can also be reduced by flavocytochrome b_2 (aka yeast lactate dehydrogenase), thereby shuttling electrons into the ETC under partially anaerobic conditions. Under these partially anaerobic conditions, it is also possible to get incomplete reduction of O_2 to H_2O by cytochrome c oxidase, resulting in H_2O_2 , a two-electron reduced form of O_2 which is considered a reactive oxygen species leading to peroxidation of membrane fatty acids. Yeast contain a highly efficient enzyme, cytochrome c peroxidase, which very rapidly binds peroxide and carries out a two electron reduction to give 2 H_2O molecules, thereby detoxifying the peroxide. This reaction results in two electron oxidation of the enzyme, requiring subsequent reduction by two cyt c molecules bringing the enzyme back to its active state. In vertebrates, cyt c is reduced by sulfite oxidase, which is responsible for the conversion of sulfite to sulfate during the metabolism of Cys amino acids. The bottom line is that for a fairly simple protein with a very simple function in the cell, cytochrome c interacts with quite a number of different enzymes either embedded in the inner mitochondrial membrane or found in the inner membrane space. *How do you think the charge distribution around the heme edge of cyt c is related to its ability to interact with its partners, in other words how is structure related to function? What type of charges do you think are on the enzymes with which cyt c interacts?*

Now that you know how to do all these things with a relatively simple protein like cytochrome c, repeat the process with bovine hemoglobin which is a tetrameric protein. (The presence of more than one chain introduces a new level of complexity in issuing commands in Jmol or any other molecular graphics program). In this case, simply look at the nature of the charge distribution on the surface of hemoglobin in order to figure out why it eluted off the CMC column at relatively low ionic strength and pH.

F. Repeat above using PyMol

- Go to the PyMol website to download the Educational version, supply the requested information and install the program on your computer
- There is a lot less useful information about PyMol than Jmol available without paying big bucks for the full blown version, but there are some good sources of information:

- On the course homepage are “PyMol1 – PyMol3” pdf files that you can look at. These were modified from Dr. Miyashita’s PyMol exercises in Bioc 462A Honors class.
- An excellent source of information is the “pymol_tutorial_stockwell.pdf” listed on the course homepage; a second less understandable source is “pymol_tutorial_rother.pdf”.
- There is a PyMol Wiki site (Google it), that is fundamentally useless, but you might find something to your liking there.
- As was the case with Jmol, you will have to come to grips with command line syntax and typing in specific commands. Once you get accustomed to the nature of the PyMol commands it is really not that difficult to use, though the learning curve is steeper than for Jmol due to the lesser amount of documentation.
- Two really important features of PyMol are the stunning raster graphics, especially with using a function called RAY. This really adds a nice touch to your figures. The second really nice feature is that you can generate a molecular surface rendition of your molecule where the colors you have chosen for your atoms are displayed on the surface. This cannot be done in Jmol.

