

MacPymol Exercises:

Today we will look at the crystal structure of *Pyrobaculum aerophilum* (*Pae*) SurE α , a homolog of survival protein E (**SurE α**), an acid phosphatase, using the program called PyMol. The structure reveals a dimer in the asymmetric unit of a P3₁21 cell. This is an example taken from Cameron Mura's homepage:

http://mccammon.ucsd.edu/~cmura/PyMOL/pymol_mainFrame.html

1. Get the files we will need and save them onto the desktop.

- Login to one of the CRC computers.
- Open Safari by clicking on the compass icon.
- go to <http://www.chem.umass.edu/~cmartin/Courses/BioStruct/2005/>
- In the "Resources" section click the Download link and a folder called ElectronDensityProject.sit
- ElectronDensityProject.sit contains map2.xplor and ps.pdb.

2. Open MacPymol and load the file we need

- Open MacPymol by clicking on the MacPyMOL icon.
- In Pymol File→Open →ps.pdb
The structure of SurE will appear as lines representing the bonds.
A gear bar labeled PS will also appear.

3. Color and Manipulate the SurE structure. For additional reference, the Users Manual can be found at:
<http://pymol.sourceforge.net/newman/userman.pdf>

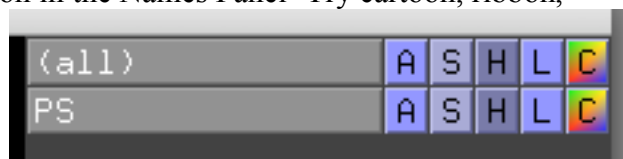
- Moving: The mouse matrix tells you the function of the mouse buttons in the mode you are using. Move the protein around. At any point you can type "zoom" to return to a centered view.



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Mouse Mode 3-Button Viewing
Buttons  L      M      R      Wheel
& Keys   Rota  Move  MovZ  Slab
  Shft   +Box -Box  Clip  MovS
   Ctrl  +/-  PkAt  Pk1   MvSZ
   CtSh   Sele  Orig  Menu  MovZ
SnglClk  +/-  Cent  Menu
  DblClk  Menu   -   PkAt
Selecting Residues
  
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- Draw PS in various ways using the "S" (show) button in the Names Panel- Try cartoon, ribbon, mesh, surface etc.
- To remove a view select "H" (hide) Everything.



- d. Color the molecule in various ways using the “C” (color) button in the Names Panel. (You may want to color it white in between various color schemes to cleanse your pallet.
 - Color by chain as a ribbon diagram
 - What is the oligomeric state of this enzyme?
 - Color by secondary structure (ss) as a cartoon
 - How many strands are there? How many helices?
 - What is the composition of this protein?
 - Color by element in various color combinations as a line drawing or a surface.
 - Color by spectrum→b-factor
 - Which regions are the most mobile?
- e. Change the background to white using Display→Background → White.
 - What does a white background do to your view?
- f. Make a view that looks good to you and save it as a scene as Scene →Store→ F1
- g. Make another view and store as Scene →Store→ F2
- h. Save your work by File → Save Session → yourname.pse
- h. Render your image by pushing the Ray button. (Then don't touch the structure viewing window!)
- i. Save at least one picture you have made using File → Save image→ yourname.png
- j. EXTRA BROWNIE POINTS: Email to me at jhardy@chem.umass.edu

4. View Electron Density Maps:

- a. Show PS as lines only. Color by element using your favorite scheme.
- b. Load the electron Density map (map2.xplor) by File → Load → map2.xplor
- c. Turn the background to black using Display→Background → Black.
- d. Select Wizard → Density. A new bar called w1_map2 will appear in the Names Panel.
- e. PkAt (single left click) at the atom you want to see density for
- f. Use slab and move to get a clear view of the residue you are centered on.
- g. Can you see any clear but unattached spheres of density? What are those?
- h. Find an alpha helix and look at the tube of electron density that is formed by a helix. (Draw a cartoon and color by secondary structure, if necessary, to help you find the helices.)
- i. What do you notice about the density for the loop regions?
- j. OPTIONAL ONLY: An alternative to the Density wizard is to use the command : PYMOL> isomesh msh2,map1,1.0,(chain A),3.0 This command tells Pymol to display an isosurface–mesh contoured at 1.0 Å in a brick about chain A with a border of 3.0 Å.

5. Select regions of the protein to color and work with uniquely.

MANUALLY using the hierarchy of:

/object–name/seg–identifier/chain–identifier/resi–identifier/name–identifier

- a. Color all phenylalanine residues red by typing:
 - PYMOL > color red, ///phe
- b. Draw the electron density around a phe. How unambiguous is that electron density?
- c. Color all tryptophan residues white by typing:
 - PYMOL > color white, ///trp
- d. Draw the electron density around a trp. How unambiguous is that electron density?

e. Color all asparagine residues yellow by typing:

PYMOL > color yellow, ///asn

f. Draw the electron density around an asn. How unambiguous is that electron density? What else might look like that? What other sidechains could fit in that kind of density? How could you know it is not an asp?

USING THE Pk FUNCTIONS

a. PkAt selects an atom and give you a choice of pk levels. You can manipulate the attributes such as color, sphere, etc. at the level of:

Atom (pk1)
molecule (pkmol) can be the same as pkobject
residue (pkresi) the amino acid you selected
chain (pkchain) important if more than one chain
object (pkobject) important for chemicals

(all)	A	S	H	L	C
PS	A	S	H	L	C
map2	A	S	H	L	C
w1_map2	A	S	H	L	C
(pk1)	A	S	H	L	C
(pkmol)	A	S	H	L	C
(pkresi)	A	S	H	L	C
(pkchain)	A	S	H	L	C
(pkobject)	A	S	H	L	C

6. Draw your favorite peptide and chemical.

PEPTIDE

- Hide the protein by clicking the gray bar that says PS.
- Draw an alpha-helix by selecting Build → Residue → Helix
- Add amino acids to your helix by selecting Build → Residue → Phe (or any other amino acid).
Keep going until you have at least 8 amino acids in your helix.
How does your helix look? Are the sidechains in reasonable conformations?
How many turns of helix is this? (Can you tell by drawing it as a ribbon?)
- Note this is a current weakness of PyMOL is that you can draw peptides but not with good geometry.

SMALL MOLECULE

- Hide the peptide by clicking the gray bar that says Phe (or whatever your first amino acid was.)
- Draw a small molecule by selecting Build → Fragment → cyclohexyl (or anyother fragment).
- PkAt (pick the atom) you want to add the next fragment to. Add the next fragment.
- The cool thing about PyMOL is that you can draw chemicals, but you might get strange bond angles so it may be easier to draw your molecule in a program like Chem3D and then make pretty pictures in PyMOL.

7. Homework. Download your favorite protein from the Protein Data Bank (<http://www.rcsb.org/pdb/>). Load the pdb into PyMOL and manipulate it like we did today.