

(Note: Commands that follow the > symbol should be typed in the terminal window. Commands following O> are typed in the O terminal window.)

1. Login to a mac mini with your LDAP account and password. If you don't have an LDAP account, then I'll log you in on my account.

2. Open the terminal window by clicking on the black TV icon at the bottom of your screen.

- tssh
- mkdir yourname
- cd yourname
- mkdir work
- cd work

3. Insert the CD into your machine. Copy all the files in the folder gar into yourname/work/.

4. PDB is the type of coordinate file that we use for crystallography. Let's go over what each of the columns are. Type:

- more ono.pdb

```
REMARK Written by O version 10.0.1
REMARK Sat Jan 28 12:03:05 2006
CRYST1 73.028 73.028 175.520 90.00 90.00 90.00
ORIGX1 1.000000 0.000000 0.000000 0.000000
ORIGX2 0.000000 1.000000 0.000000 0.000000
ORIGX3 0.000000 0.000000 1.000000 0.000000
SCALE1 0.013693 0.000000 0.000000 0.000000
SCALE2 0.000000 0.013693 0.000000 0.000000
SCALE3 0.000000 0.000000 0.005697 0.000000
```

```
ATOM 1 CB LEU A 1 73.861 49.328 38.575 1.00 39.45 6
ATOM 2 CG LEU A 1 74.191 50.162 37.337 1.00 38.62 6
ATOM 3 CD1 LEU A 1 75.658 49.975 36.975 1.00 37.59 6
ATOM 4 CD2 LEU A 1 73.297 49.743 36.178 1.00 36.01 6
ATOM 5 C LEU A 1 74.380 51.070 40.293 1.00 41.94 6
ATOM 6 O LEU A 1 73.238 51.452 40.532 1.00 42.87 8
```

5. Open the Finder → Applications → O

Drag and drop the file called osx_ono onto the terminal window. This will launch the program O. You will be prompted with a bunch of questions. Push Enter once after each question.

6. What do we have on the screen?

Electron Density Maps (2FOFC_1)
 Crystallographic model (NOW).
 Menus

- Zoom out (pull downward on screen with middle mouse button to zoom out).
- Toggle on and off the model and map in the OBJECT MENU
 - Leave FOFC+_2 and FOFC+_3 off
- Get used to using the mouse and DIAL MENU
- Move around the Menus by clicking the upper RIGHT corner
- Click on Centre_ID then use the middle mouse button to select an atom

- At any point you can save your work by: Controls → Save then click enter in O window.
- To write out the pdb you are working on:

```
O> s_a_o
Sam> Output file name: whatever.pdb
Sam> Coordinate file type assumed from file name is PDB
Sam> What molecule [NOW ]:
Sam> Residue range [all molecule]:
Sam> Define cell constants [ 73.03 73.03 175.52 90.00 90.00 90.00]:
Sam> Write out only selected atoms? [No]:
Sam> Use the B-factor? [Yes]:
Sam> Use the occupancy? [Yes]:
Sam> 3523 atoms written out.
```

Another useful command:

```
O> ce_at a55
@redraw_map
```

7. Manipulate molecule. Toggle off the electron density maps by clicking in the OBJECT MENU.

- Use graph pull down
 - Calculate the Ramachandran Plot
- Rebuild menu, grab, move, flip and refi commands
 - For the grab commands use the middle button to select atom/frag/residue.
- The best way for us to see how to use these commands is to screw things up. So let's move the atoms in a55-a60.
 - Calculate the Ramachandran Plot—Did you screw things up?

8. Now we want to fix a region like we would do in Molecular Replacement. We want to rebuild residues 278-289 using the commands we just learned.

```
O> ce_at a289
```

9. Build a region from scratch.

```
O> ce_at a331
-look at the electron density with no model. What kind of secondary structure does it look like?
- What residues are present?
```

9a. In protein crystallography we always know the identity of the residue to be inserted next, so we will insert the residues that are missing. (All these command are typed in the O window or will appear in the O window. You have to type the underlined parts):

```
mutate insert
Mut> Mutate a molecule by inserting residues.
Mut> Molecule ([NOW ]) : (Press enter)
Mut> After which residue: a331
Mut> New residue name and type (<cr> to end) : a332 pro
Mut> New residue name and type (<cr> to end) : a333 phe
Mut> New residue name and type (<cr> to end) : a334 arg
```

```
Mut> New residue name and type (<cr> to end) : a335 tyr
Mut> New residue name and type (<cr> to end) : a336 thr
Mut> New residue name and type (<cr> to end) : a337 thr
Mut> New residue name and type (<cr> to end) : a338 ser
Mut> New residue name and type (<cr> to end) : a339 leu
Mut> New residue name and type (<cr> to end) :
```

9a. The baton is a 3.8Å baton that measures the distance from one Ca to the next. Use baton mode to build the backbone starting from residue a331. (all these command are typed in the O window or will appear in the O window. You have to type the underlined parts):

```
mol di
ca
Mol> Database compressed.
Mol> Created connectivity Db for DI
Mol> Ca zone [all molecule]: (Press enter)
end
mol now
baton build
Aub> What molecule [NOW ]?
Aub> Start residue : a332
Aub> Backwards or Forwards (B/[F])? f
```

Use the FragRotX (etc) commands at the bottom of the Dial Menu box to move the baton. Do not use grab frag or move frag commands. When you have the fragment in the right position press YES in the USER MENU. The baton will jump to the end of the baton you have just placed without drawing the fragment you just placed until you follow 9b.

9b. To visualize the backbone you've drawn (all these command are typed in the O window or will appear in the O window. You have to type the underlined parts):

```
mol
Mol> Molecule code name [NOW ]: (Press enter)
obj
Mol> Name of the new object [NOW ]: ca
ca
Mol> Ca zone [all molecule]: (Press enter)
end
```

Repeat this process after each round if you want to see the backbone the baton has built.

9c. You can draw the molecule Now with the amino acids you have inserted and colored by atom type. You can do this as often as you like. Then use the tools you have learned to reorient the amino acids to the right positions. Also try the commands lego_CA and lego_auto_sc.

```
@pt_by_atom
mol
Mol> Molecule code name [NOW ]: (Press enter)
obj
Mol> Name of the new object [NOW ]: (Press enter)
zone
```

```
Mol> zone [all molecule]: (Press enter)  
end
```

9d. We now want to connect any bonds we need to using the pulldown menu Bones → Make Bond and clicking sequentially between any atoms we want to connect. They whole area can be regularized by `refi_zone`.

9e. (optional)

```
O> ce_at a84
```

-look at the electron density with no model. What kind of secondary structure does it look like?
You can insert the appropriate sequence: 85-GIKALADYVHA-96

10. Measure your success (you will have to type the underlined commands at the appropriate times):

```
O> s_a_i  
Sam> Name of input file: ono2.pdb  
Sam> O associated molecule name: real  
Sam> File type is PDB  
Sam> Database compressed.  
Sam> Space for 6403380 atoms  
Sam> Space for 10000 residues  
Sam> Molecule REAL contained 806 residues and 3637 atoms  
O> mol  
Mol> Molecule code name [ONO ]: real  
O> obj  
Mol> Name of the new object [REAL ]:  
O> zone  
Mol> Zone [all molecule]: (Press enter)  
O> end
```

11. The protocol you used above is the template for drawing more molecular objects. For more details on drawing pictures you can consult section 2.3 on page 27 of:

http://www.doe-mpi.ucla.edu/People/Software/o_for_morons.pdf

other useful sites for your reference:

http://xray.bmc.uu.se/~alwyn/Essential_O/essentials_frameset.html

<http://www.richmond.edu/~jbell2/sb3-xray-protocols-2003.pdf>

http://www.doe-mpi.ucla.edu/People/Software/o_for_morons.pdf

http://sip.clarku.edu/tutorials/intro_emacs.html

<http://www-structmed.cimr.cam.ac.uk/Course/Fitting/fittingtalk.html>