Building a model into Electron Density : A hands on tutorial

http://aspen.biochem.utah.edu/~heidi/tutorial.htm

This tutorial will take you though from the initial stages of new experimentally phased electron density maps, through modeling building and finally comparing the refined model to the final calculated density. This stage can take a crystallographer anywhere from several weeks/months to a hour depending on the resolution of the data and the quality of the experimental phases.

The tutorial will involve a SGI UNIX workstation. You will first create a directory in which to do the tutorial and then predominately use a program called "O" to observe the electron density and build the new model.

login name = methods (use the computers "vail" or "snowbird")
password = strucm3th

A blue terminal window should be visible on the screen, or is perhaps collapsed and displayed as an icon near the top of the terminal. If a blue terminal window is **not** displayed, then either click on the icon or open a unix shell by clicking the left mouse button on top of the "Desktop" toolbar on the upper left-hand side of the window, drag to the "open unix shell" and release. A blue terminal window should appear.

Throughout this tutorial I shall use the ">" symbol to represent the unix prompt and the commands you need to use are typed afterwards. For additional unix command assistance please refer to the on-line tutorial. Please note that unix is case sensitive. **Please do not be afraid to just hit the carriage return, or click a button to see if the window is responding.**

Place the mouse over the terminal window and type "ls" to list the directory and see the set of directories and file in this area.

```
> ls
```

Change your current location to the users directory, make a directory of your own, list and start Netscape in the background (&). The home page should be the tutorial page.

```
> cd users
> mkdir yourname                    (make a directory)
> cd yourname                       (change directory to a directory called yourname)
> netscape &     (if netscape is already running (icon?) then don't start it a second time.
                  If a window comes up asking you to accept the license agreement then
                  click ACCEPT)
```

You will need a series of starter files to begin work. Copy these files to your directory with the following command:

```
> cp ~methods/data/density-data/files/* .      (note the last item on this line is a "." period)
> ls
```

You will now see a series of files have been written into the directory. You should not have to alter these files in any way. If, for some reason an error occurs and you have to start over you can just clear the directory with “> rm *” and start over again with the copy comannd above.

To start the program called “O” type "o density.o" (lower case) and hit enter (twice) until large black screen appears.

> o density.o

The black window will predominantly cover the blue window. You can “iconize” the graphics window by clicking the [.] button in the top right corner just like a Mac or PC. The [[]] box button will expand the graphics window to full size but this is NOT recommended. You may want to manuver the blue window to the lower left-hand corner of the screen so that you can partially see it. The program will interpret commands typed into either window but this tutorial should minimize the amount of typed commands necessary.

The line of words across the top of the window are already pull down menus and if you draw the mouse across them you can see the different commands available. DO NOT activate a command until you know what you are doing. If a command gets accidentally activated and you want to “cancel” use the CONTROL > CLEAR FLAGS commands from the CONTROL menu on the far left.

The first thing to do is to position a series of virtual menus in the graphics window. Place your mouse on the far right menu area called MENUS. With the left mouse button, drag down until the DIALS menu is showing and then (with the left button still depressed) drag the mouse over to the dials box to a small red box in the left hand corner of this virtual menu. The you can release the mouse and the dials box should remain visible. Now reposition the dials menu using the left mouse button on the right-hand small red box of a menu and drag. A good position for the DIALS menu is the lower right-hand corner of the screen.

Now drag down the "OBJECT" and place it on the left hand side of the screen, about half way down.
Finally, drag down the MENU menu, and leave it positioned above the dials.(These final positions are similar to that seen in the web tutorial)

Click on the @semet option in the MENU menu.

The screen should center on a red cross. Either use the middle mouse button and drag the mouse downwards (to zoom out) or use the dial box. Play with rotation, translation, zoom and slab.

To use the virtual dials you place the mouse adjacent to the requested motion and with the left button depressed move the mouse horizontally. You can also rotate the objects on the screen by depressing the right-hand mouse button anywhere on the screen and moving the mouse.

Before continuing expand the field of view (zoom out) to see the entire unit cell, drawn as a red box.

You will notice two objects have been brought into the OBJECT menu. One is the unit cell and the other the atoms (semet) shown as red crosses. These are the positions of the Selinium atoms which were identified using Patterson methods and used for experimental phasing in a MAD experiment. Please see Chris Hill's lecture notes for more details.

At any time if you wish to remove an object temporarily from the display you can left-click on the object in the OBJECT menu box. The on/off toggle will alternate with the display.

STEREO
IF (and you don't have to do this – it will slow the machine down) you would like to see in stereo you do the following:
1) Turn on the small box which is on top of the SGI monitor. A red light will appear.
2) Find a pair of stereo glasses (might be in the small graphics room)
3) Hit the F1 function key at the top of your keyboard. (Two images should appear on top of each other on the screen . They are very quickly alternating – but your eyes can't detect it without the glasses.
4) Put the glasses on and turn them on – IF there is a small button on the left side then hit that button until you see in stereo. You must be a reasonably close distance to the little box for this to work.
5) His F1 to return the monitor to mono.

DENSITY
Click on @resolve from the MENU menu. (the menu item will remain lit red while it's working)

This displays two maps. The maps are at 2.8A resolution. This is a medium resolution structure but the phases are of high quality and the solvent flattened map is easily interpretable.

If you left click on "resmap_1" in the OBJECT menu then the blue map should go away and leave you with the green map. The green map is the original MAD phased map. You should be able to rotate the map (right mouse) and see a regions of electron density which vary in their coverage. The dense areas are where the protein is and the less dense areas are the solvent channels between proteins in the unit cell. (You may want to "slab down" on the region so that you don't have to look through all the density at the same time (dials – drag the mouse to the left on the slab area))

Now click off the "solmap_1" and click back on the "resmap_1" by left-clicking on each name in the OBJECT menu.

The blue map is the solvent flattened map. These crystals contain three molecules in the asymmetric unit in addition to 60% solvent. If you click the maps on and off in the OBJECT window you can see that the less dense areas of the original (solmap) correspond to the empty solvent channels of the solvent flattened map (resmap). The names correspond to the programs which created the maps, solve and resolve.

Can you identify any regions of secondary structure in the density – a spiral helix or series of consecutive straight beta-strands in a beta-sheet? If you have not translated the screen much in your

dial trails then you should still be centered on a Se atom. Zoom in on the atom and see that it lies in side chain density just like the methionine side chain it represents.

Click on @remove_sol in the MENU menu. This macro will delete the original map for space considerations – if you click on the OBJECT menu title bar the "solmap_1"will go away..

BONES
In this section we will draw a "skeleton." The skeleton is basically a line drawn through the center of the electron density. Long sections of connected density are draw blue and short side segments are colored red. In a good map these are almost equivalent to the main chain and side chain regions of the final structure.

Using the original built-in menus along the top of the O window drag the following command:
BONES > DRAW ALL (20)
The blue/red skeleton should appear on the screen. Zoom in on the skeleton and rotate the screen. Try again to identify any regions of secondary structure in the molecule. Turn the skeleton on and off in the OBJECT window (MSCSK6) and test weather you could have identified the helix in the electron density without the skeleton. You can also toggle the map on and off to view the skeleton more clearly. A skeleton doesn't tell you anything new with respect to the electron density, but it does clarify the connections and simplify the screen view.

You can move around the map and look at other regions of the molecule now. Since I have the program only showing you a 20A sphere of electron density and skeleton To recenter the screen to a new location use the pull down menu in the top left corner. CONTROLS > CENTER_ID > click on a connection in the skeleton where you want to center. Now redraw the skeleton and the map with the BONES >DRAW ALL (20) and @MAPUPDATE commands like above or from the MENU menu.

Occasionally, when you try and left-click on an atom the atoms are too close together and they don't get selected or labeled. An error message will come up in the upper left hand corner with this message. To get around this problem you may need to zoom in further or rotate the screen to get a better view of the atom you are trying to select.

BUILD
For the purpose of this tutorial I will start you by building an alpha-helix. You can then go onto build other secondary structure if you would like. To start this section
Click on @build

This macro recenters the screen sothat we all start building in the same place. If you can not see electron density, or it is not centered on the screen, then rebuild the map with @mapupdate and the bones with BONES > DRAW ALL (20).

You are now centered on an alpha-helix. There are more than nine residues in this helix but we will build nine residues initially. Use the DENSITY pull down menu along the top of the screen for the following series of commands.

DENSITY > 2ry UNIT ALPHA9 > alpha 9 residues
(It turns out that this is the default, but you could choose other types of secondary structure from this menu)
DENSITY > TEMPALTE > FIT alpha9

The program is now waiting for you to click on a section of this helix. Try and click on a C-alpha atom of an amino acid residue. This would be the location of a branch point between the main chain atoms which spiral up the helix and the red skeleton of the side chain branches. Also try to click on the center of the final nine residue helix for best results. It turns out that this is the center of the screen.

The program will use a series of real space refinement algorithms to determine the placement of the helix and try and decide the directionality of the helix (N to C-terminus). This takes a few moments, when the computer is done a poly-alanine model of a nine residue helix should be visible in atom colors (mostly yellow for carbon).

(I had a problem with the tutorial here. If you don't see a yellow helix show up within 60 seconds then start these series over again from the @build command.)

Do the carbon-beta atoms of the alanine side chains point in the right direction for the corresponding electron density? Remember the "Christmas tree" effect of the side chains of any alpha helix – they point towards the N-terminus.

If you agree with the position where the program has built the helix then answer YES by dragging from the CONTROLS menu in the upper left hand corner.
CONTROL > YES

At this point you can feel free to build other regions of secondary structure using a combination of commands: (Always accept YES before starting a new section of secondary structure)

If a helix is not inserted in the incorrect orientation you can reverse the direction of the helix by dragging the following command:
DENSITY > FIT > REVERSE
Now the helix would be in the opposite orientation and you would accept the result with CONTROLS > YES.

Remember that to move around the unit cell you need to recenter and refresh the map and bones.
CONTROL > CENTER_ID >click on an atom of the skeleton (bones)
DENSITY > DRAW (all atoms)
@mapupdate
and then use the DENSITY pull down menu to build structure. If you don't like what you've built you can always CONTROLS > NO

Decide what type of secondary structure to build and how long it should be. Place it in the electron density and remember to click on an C-alpha atom. This is what it feels like to build a structure

from scratch. Imagine being the first person to see what a molecule looks like. Zoom back to see how a-helices might pack together or how several beta-strands might form a beta-sheet.

SIDE CHAINS

Once you've had your fill of building secondary structure, start the next section where you will start to identify side chains

Click on @poly-ala

This macros brings in a poly-alanine trace of most of the asymmetric unit. Zoom back if you want to see the dimer, but then zoom down to look at the particular amino acid side chains in this region. Display the electron density for this region.

How well does your poly-alanine trace conform to the refined poly-alanine model? Think about what kind of residue this might be. There are 20 amino acids, small, large, hydrophobic, hydrophilic. You can probably rule out a glycine residue in this positional already. Try and guess what residue it might be.

Luckily we have tools to help us compare the electron density to the different sizes of amino acids. One other complicating feature is that many amino acids can adopt alternate side chain conformations. You'll need to check them all.

Click on @center (this just puts everyone at the same position)
Click on @side_chain

Now the computer is waiting for you to click on a residue you wish to investigate. Go ahead and select the residue in the center of the screen. Residue A87. Then, if you click in the middle of the title bar of the DIALS menu you will see two new menu items appear. Residue and Rotomer. You can use the left mouse button, dragging left and right, to scroll though the different residues. They start small and get bigger. The residues will appear on the screen in green but they may not be completely visible or at a funny orientation. The name of the residue is displayed in the upper left hand corner of the screen on the second line of text. You can also stop scrolling at any time and rotate the screen (right mouse button) to get a better view.

If you reach an amino acid which might be about the right size, but is not in the electron density then you want to stop scrolling with the RESIDUE dial and move to the ROTOMER dial. Scrolling here takes you through the top three or four most common rotomer conformations.

This particular piece of density could be a variety of side chains. You should also look at the surrounding density. The residue is inside a beta-strand which are typically in the center of proteins and hydrophobic – but this residue points away from the core of the structure towards an open area of solvent. While a likely residue for a beta-strand is a leucine, this residue is not a leucine. In general you want to choose the largest residue which fits in the density. HINT: it turns out that there are two correct answers for this residue which cannot be distinguished by the electron density. When you have made your best guess cancel the side chain command by dragging down from the CONTROL menu.

CONTROL > NO

Now using the REBUILD menu in the center of the screen drag down to the bottom line (MUTATE) and continue dragging till you get to the three letter amino acid code for the amino acid you think fits in the electron density you were just looking at. When you release the left mouse button on an amino acid the computer will then be waiting for you to click on an atom in that residue. Don't worry if you make a mistake. You can always mutate back to ALAnine.

The mutate command causes the molecule on the screen to be redrawn and the new molecule (sph) will contain your mutated amino acid in a purple color. To obtain the entire molecule again please hit the @redraw command from the MENU menu.

The most common side chain rotomer will have been used to place the side chain. This may not be in the electron density. Use the REBUILD pull down menu again to fit it.
REBUILD > DATABASE > ROTOMER TO BUILD

The ROTOMER option will appear again in the DIALS menu box again and you can use the left mouse and scroll through the side chain conformations until you get to the one you want. When you are ready, hit CONTROL > YES, or if you don't like the change CONTROL >NO. You can always mutate it again or review all the amino acids with the @sidechain command.

Now try and determine what the two amino acids are on either side of this residue (residues A86 and A89). I'll give you a hint and say that they are both the same residue. (recenter by CONTROLS > CENTER BY ATOM >click on the CA atom and then start over from @sidechain.

Follow the above procedure and mutate the molecule to contain the new residues at both positions. You may also do this for any other region of space you would like. Just keep updating the map with @mapupdate and the model with @redraw

CHECK YOUR WORK
Once you are satisfied with a few of your amino acid choices then check you model against the final structure. (note that crystallographers don't usually get this option)
Click @check in the MENU menu.

Now the entire structure is visible with all the atoms and their corresponding CA-trace. This can make for a busy screen. Click on and off objects by left-clicking on the OBJECT menu.

How did you do? Did you pick the correct amino acids? When a crystallographer builds a structure from scratch they have the amino acid sequence in hand and once they identify a stretch of residues in the structure, and in the sequence, they can mutate residues N- and C-terminal to that location to build all the other residues.

Click on @final_dens to display the final refined electron density in sky_blue color. This is called a 2Fo-Fc map which means that it's map using two times the observed (o) structure factors minus one times the calculated (c) structure factors and the calculated phases. The calculated portion comes directly from the refined model.

If you turn off all the maps and zoom out you will see the full atom model of the homodimer in the asymmetric unit. A final schematic picture of this structure is presented in the web pages which accompanied this tutorial.