Coot Tutorial

CCP4 School APS 2009

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1 Mousing

First, how do we move around and select things?

Left-mouse Drag	Rotate view
Ctrl Left-Mouse Drag	Translates view
Shift Left-Mouse	Label Atom
Right-Mouse Drag	Zoom in and out
Middle-mouse	Centre on atom
Scroll-wheel Forward	Increase map contour level
Scroll-wheel Backward	Decrease map contour level

2 Introductory Tutorial

In this tutorial, we will learn how to do the following:

1. Start Coot

2. Display coordinates

3. Display a map

4. Zoom in and out

5. Recentre on Different Atoms

6. Change the Clipping (Slab)

7. Recontour the Map

8. Change the Map Colour

9. Display rotamers and refine residue

2.1 Get the files

Before we start, let's get the files on which we will be working:

```
$ wget http://www.ysbl.york.ac.uk/~emsley/coot/tutorial/tutorial-modern.pdb
$ wget http://www.ysbl.york.ac.uk/~emsley/coot/tutorial/rnasa-1.8-all_refmac1.mtz
```
or copy them from
```
/home/emsley/tutorial
```

2.2 Start Coot

To use coot, in a terminal window, type:
```
$ coot
```
When you first start `coot`, it should look something like Figure 1.

2.3 Display Coordinates

So let's read in those coordinates:

- Select "File" from the Coot menu-bar[1]

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[1]Note you can also use "Alt-F" instead of clicking on "File"

Figure 1: Coot at Startup
Not much to see at present... Actually, after this, coot screenshots will be
displayed with a white background, whereas you will see a black one

- Select the "Open Coordinates" menu item

    [Coot displays a Coordinates File Selection window]

- Either

    - Select tutorial-modern.pdb from the "Files" list

    or

    - Type tutorial-modern.pdb in the Selection: entry

- Click "OK" in the Coordinates File Selection window

    [Coot displays the coordinates in the Graphics Window]

2.4 Adjust Virtual Trackball

By default, Coot has a "virtual trackball" to relate the motion of the molecule to
the motion of the mouse. Many people don't like this.

So you might like to try the following. In the Coot main menu-bar:

Edit → Preferences. . .

[Coot displays a Preferences window]

On the left toolbar select General and then on the right top notebook tab HID.
Select "Flat" to change the mouse motion.

(Use the "Spherical Surface" option to turn it back to how it is by default).

What is the difference? "Flat" mode is like the mode used by "O". In both
modes, dragging the mouse near the centre of the screen causes the view to rotate
about the X- or Y- axis. However in spherical mode you can also rotate about the
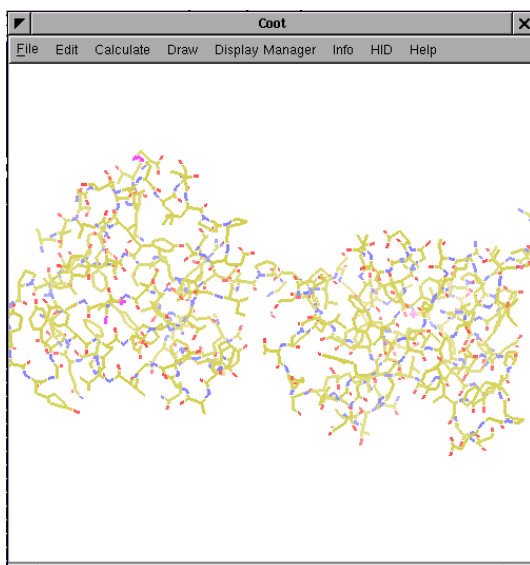z-axis by dragging the mouse along an edge of the window.

Figure 2: Coot After Loading Coordinates

2.5 Display maps

We are at the stage where we are looking at the results of the refinement. The refinement programs stores its data (labelled lists of structure factor amplitudes and phases) in an "MTZ" file. Let's take a look...

- Select "File" from the Coot menu-bar

- Select "Auto Open MTZ" menu item *[Coot displays a Dataset File Selection window]*

- Select the filename `rnasa-1.8-all_refmac1.mtz`

If you choose instead "Open MTZ, cif or phs..." you will see:

- *[Coot displays a Dataset File Selection window]*

- Select the filename `rnasa-1.8-all_refmac1.mtz`

  [Coot displays a Dataset Column Label Selection window]

  Notice that you have a selection of different column labels for the "Amplitudes" and "Phases", however, let's use the defaults: "FWT" and "PHWT".

- Press "OK" in the Column Label Window

  Now open the MTZ file and select column labels "DELFWT" and "PHDELWT".

  So now we have 2 maps (whether auto-opened or not).

2.6 Zoom in and out

To zoom in, click Right-mouse and drag it left-to-right[2]. To zoom out again, move the mouse the opposite way.
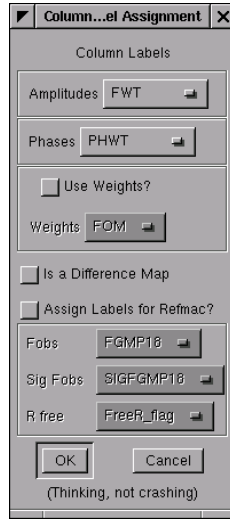
---

[2]or up-to-down, if you prefer that

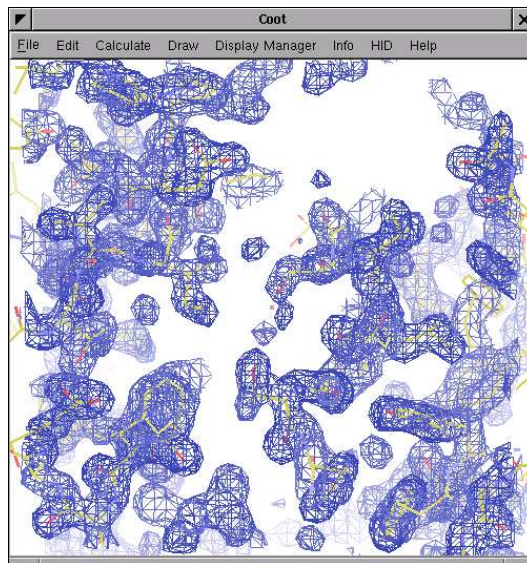Figure 3: Coot MTZ Column Label Selection Window

Figure 4: Coot after reading an MTZ file and zoomed in.

2.7 Recentre on Different Atoms

- Select "Draw" from the Coot menu-bar

- Select "Go To Atom..."

  [Coot displays the Go To Atom window]

- Expand the tree for the "A" chain

- Select 1 ASP in the residue list

- Click "Apply" in the Go To Atom window

- At your leisure, use "Next Residue" and "Previous Residue" (or "Space" and "Shift" "Space"in the graphics window) to move along the chain.

- Click Middle-mouse over an atom in the graphics window

  [Coot recentres on that atom]

- Ctrl Left-mouse & Drag moves the view around. If this is a too slow and jerky:

  – Select Edit → Preferences... from Coot's menu-bar
  – Select the "Maps" toolbutton on the left
  – Select the "Dragged Map" notebook tab.
  – Select "No" in the "Active Map on Dragging" window
  – Click "OK" in the "Preferences" window

  Now the map is recontoured at the end of the drag, not at each step[3].

  Another, additional way to make the movements faster is to change the number of steps for the "Smooth Recentering". Again you find this in the "Preferences" (Preferences → General → Smooth Recentering). Change the "Number of Steps" from the default 80 to something smaller, e.g. 20.

- You can display the contacts too, as you do this:

  – Select "Measures" from the Coot menu-bar
  – Select "Environment Distances..."
  – Click on the "Show Residue Environment?" check-button
    * Also Click "Label Atom?" if you wish the Cα atoms of the residues to be labelled.
  – Click "OK" in the Environment Distances window
  – Click "Apply" in the Go To Atom window
    [You can't change the colour of the Environment distances]

You can turn off the Environment distances if you like.

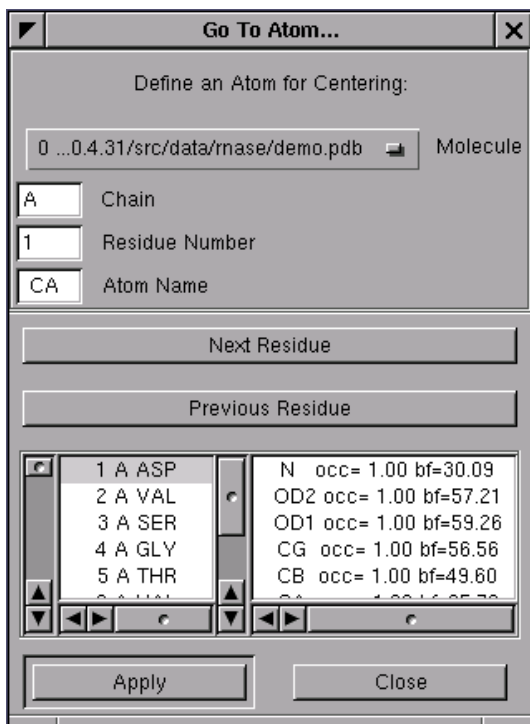[3]which looks less good on faster computers.

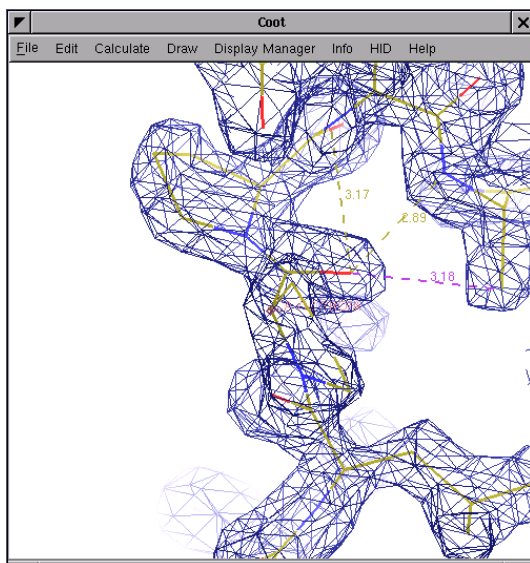Figure 5: Coot's Go To Atom Window.

Figure 6: Coot showing Atom Label and environment distances.

2.8 Change the Clipping (Slab)

- Select "Draw" from the Coot menu-bar

- Select "Clipping..." from the sub-menu
  [Coot displays a Clipping window]

- Adjust the slider to the clipping of your choice

- Click "OK" in the Clipping window

Alternatively, you can use "D" and "F"[4] on the keyboard, or Control Right-mouse up/down (Control Right-mouse left/right does z-translation).

2.9 Recontour the Map

- Scroll your scroll-wheel forwards one click[5]
  [Coot recontours the map using a 0.05electron/$Å^3$ higher contour level]

- Scroll your scroll-wheel forwards and backwards more notches and see the contour level changing.

- If you don't have a wheel on your mouse you can use "+" and "-" on the keyboard.

- Note that the "Scroll" button in the Display Manager allows you to select which map is affected by this[6].

2.10 Change the Map Colour

- Select "Edit" from the Coot menu-bar

- Select "Map Colour" in the sub-menu

- Select "1 xxx FWT PHWT" in the sub-menu
  [Coot displays a Map Colour Selection window]

- Choose a new colour by clicking on the colour widgets
  [Coot changes the map colour to match the selection]

- Click "OK" in the Map Colour Selection window

2.11 Select a Map

Select a map for model building:

Menubar: Calculate → Model/Fit/Refine...
  [Coot displays the Model/Fit/Refine window]
Select "Select Map..." from the Model/Fit/Refine window

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[4]think: <u>D</u>epth of <u>F</u>ield.
[5]don't click it *down*.
[6]by default it is the last map, which is not necessarily the map that you want.

click OK (you want to select the map with "...FWT PHWT")

Alternatively you can use the modelling toolbar icon buttons (here "Map") displayed on the right side of the Coot window. Most options found in the Model/Fit/Refine window are available here too. If you are not sure which icon corresponds to which function look at the displayed tips when over the button with the mouse. Or change the display style of the buttons by clicking on the bottom arrow and select another style to get only or additionally text displayed.

3   Model Building

"So what's wrong with this structure?" you might ask.

There are several ways to analyse structural problems and some of them are available in Coot.

Validate → Density Fit Analysis → tutorial-modern.pdb
   [Coot displays a bar graph]

Look at the graph. The bigger and redder the bar the worse the geometry. There are 2 area of outstanding badness in the A chain, around 41A and 89A.

Let's look at 89A first - click on the block for 89A.

   [Coot moves the view so that 89A CA is at the centre of the screen]

3.1   Rotamers

- Examine the situation...*[The sidechain is pointing the wrong way. Let's Fix it...]*

- Menubar: Calculate → Model/Fit/Refine... [7]

   [Coot displays the Model/Fit/Refine window]

- Select "Rotamers" from the Model/Fit/Refine window [8].

- In the graphics window, (left-mouse) click on an atom of residue 89A (the Cγ, say)

   [Coot displays the "Select Rotamer" window]

- Choose the Rotamer that most closely puts the atoms into the side-chain density

- Click "Accept" in the "Select Rotamer" window

   [Coot updates the coordinates to the selected rotamer]

- Click "Real Space Refine Zone" in the Model/Fit/Refine window[9].

- In the graphics window, click on an atom of residue 89A. Click it again.

   [Coot displays the refined coordinates in white in the graphics and a new "Accept Refinement" window]

- Click "Accept" in the "Accept Refinement" window.

   [Coot updates the coordinates to the refined coordinates. 89A now fits the density nicely.]

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[7]or use the modelling toolbar on the right of the Coot window, i.e. you don't need to open the Model/Fit/Refine window

[8]Or click on the "Rotamers" button of the modelling toolbar

[9]...or use the modelling toolbar button

OK. That's good.

Now, how about if we just use Real Space Refinement only?

- Click "Undo" twice

  [Coot puts the sidechain back to the original position]

- Click "Real Space Refine Zone" in the Model/Fit/Refine window[10].

- In the graphics window, click on an atom of residue 89A. Click it again.

  [Coot displays the refined coordinates in white in the graphics and a new "Accept Refinement" window]

- Now using left-mouse, click and drag on the intermediate (white) CZ atom of the PHE (if you mis-click the atom, the view will rotate).

- Can you pull the atom around so that the side-chain fits the density?

  [Yes, you can]
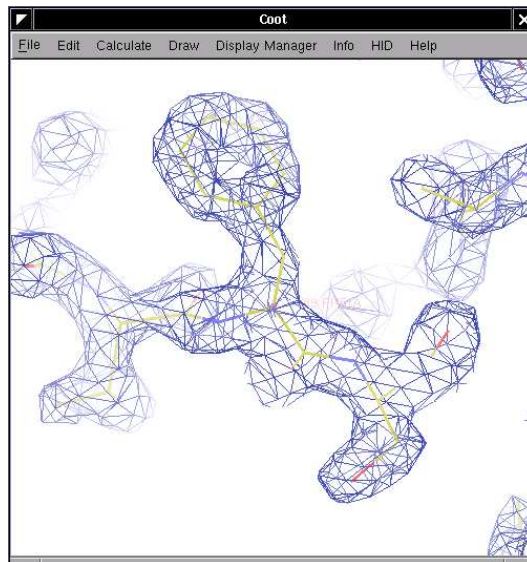
Figure 7: 89A now fits the density nicely.

3.2   More Real Space Refinement

Now let's have a look at the other region of outstanding badness:

- Click on the graph block for 41 A

  [Coot moves the view so that 41A CA is at the centre of the screen]

- Examine the situation. . .

- Residue 41 is in a mess and not fitting to the density. Can you fix it?

  [Yes, you can]

- The trick is Real Space Refine that zone. So. . .

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[10]. . . or modelling toolbar button

- You can either Real Space Refine a few residues (40, 41 and 42) or just 41. Take your pick.

- Click on "Real Space Refine Zone" in the Model/Fit/Refine window

- Select a range by clicking on atoms in the graphics window (either atoms in 40 then 42 or an atom in 41 twice)

  [Coot displays intermediate (white) atoms]

- Click and drag on some atoms until the atoms fit nicely in the density.

  If you want to move a *single atom* then *Ctrl Left-mouse* to pick and move (just) that intermediate atom.

  [Note: Selecting and moving just the Carbonyl Oxygen is a good idea - use Ctrl Left-mouse to move just one atom.]

4 Blobology

4.1 Find Blobs

To be found under Validate (called "Unmodelled Blobs").

You can use the defaults in the subsequent pop-up. Press "Find Blobs" and wait a short while.

You will get a new window that tell you that it has found unexplaned blobs. Time to find out what they are.

4.1.1 Blob 3

Let's start from Blob 3 (the blobs are ordered biggest to smallest - Blobs 3 and 4 (if you have it) are the smallest).

- Click on "Blob 3"

  [Coot centres the screen on a blob]

- Examine the situation. . .

  [We need something tetrahedral there. . .]

- "Place Atom At Pointer" on the Model/Fit/Refine window[11]

  [Coot shows a Pointer Atom Type window]

- "SO4" in the new window. . .

- In the "Pointer Atom Added to Molecule:" frame, change "New Molecule" to "tutorial-modern.pdb"

- Click OK.

- Examine the situation. . .

- the orientation is not quite right.

- Let's Real Space Refine it (you should know what to do by now. . .)

- ("Real Space Refine Zone" then click an atom in the SO_4 twice. Accept)

  [The SO_4 fits better now].

  Blob 4 is like Blob 3 (isn't it?)

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[11]also available in the modelling toolbar

4.1.2 Blob 2

Click on the button "Blob 2" and examine the density. Something is missing from the model. What?

This protein and has been co-crystallized with its ligand substrate. That's what missing: 3' GMP. So let's add it...

- File → Get Monomer...

- Type 3GP in the box (Use Upper Case). Press return...

  [Coot pauses for a few seconds while LIBCHECK and REFMAC run]

  [3GP appears]

  It is generally easier to work without hydrogens, so let's delete them

- "Delete..." from the Model/Fit/Refine window [12]

- "Hydrogens in Residue"

- click on an atom in 3GP

  [Hydrogens disappear]

  The 3GP is displaced from where we want it to be.

- "Rotate/Translate Zone" from the Model/Fit/Refine window[13]

- click on an atom in 3GP twice

  [Intermediate (white) atoms appear]

- Drag it or use the sliders to move the intermediate fragment to the approximately the right place.

- Click "OK" in the slider window

- Now optimize the fit using "Real Space Refine Zone" like we did before.

  Now merge this ligand into the protein:

- Calculate → Merge Molecules...

- Click (activate) "monomer-3GP.pdb"

- into Molecule:

  tutorial-modern.pdb

- Merge

4.1.3 Blob 1

- Click on "Blob 1"

- Examine the situation... What is this density?

  [We need to add residues to the C-terminus of the A chain.]

  The missing residues are GLU, THR, CYS (QTC).

---

[12] Found in the modelling toolbar? Of course!

[13] Found it in the modelling toolbar?

- Calculate → Fit Loop. . .

  [Coot pops-ups a Fit Loop dialog]

  Add residue number 94 to 96 in the A chain.

  The single letter codes of the extra residues are QTC

- Make sure that you can see the unmodelled density well, then click "Fit Loop"

- Watch. . . (fun eh?)

- Examine the density fit.

  [It should be fine, except at the C-terminus. We need to add an OXT atom to the residue]

- Calculate → Other Modelling Tools. . .

- Add OXT to Residue. . .

- Add it

This is a far as I expect you to get.

---

5   Extra Fun (if you have time)

5.1   Waters

Fit waters to the structure

- "Find Waters. . . " in the Other Modelling Tools dialog [14], then OK (using the defaults)

  [Waters appear]

To check the waters:

- Click on "Measures" in the main menubar.

- Click on Environment Distances

- Click on the "Show Residue Environment?" check-button

- Change the distances as you like

- Use the Go To Atom widget to go to the first water (probably towards the end of the list of residues in Chain "W")

- Use the Spacebar to navigate to the next residue (and Shift Spacebar to go backwards)

- If you don't like the fast sliding[15] you can turn it off (if you haven't done so already):

  - Click Edit → Preferences. . . in the main menubar
  - Click General → "Smooth Recentering..."
  - Click "No"

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[14]In older versions of Coot, the Find Waters button can be found in the Model/Fit/Refine dialog or the water drop in the modelling toolbar

[15]it's not very good for water checking

5.2 Working with NCS

The tutorial data has two-fold NCS. With good data it is interesting to investigate the differences between the NCS copies. This may be done by comparing the NCS related electron density, comparing the NCS related chains, or comparing the Ramachandran plots for related chains:

- Comparing the NCS related electron density.

  Go to Calculate/NCS maps and select the first map and model. Now, if you go to anywhere in the A chain, you will see two sets of electron density - the original density for the A chain, and the density for the B chain transformed to overlap the A chain density. This gives you a visual comparison of any differences.

  Look at the density in the B molecule. Why does the density not agree here? (Hint: the NCS is not a 2-fold rotation in this case, it is improper).

- Comparing the NCS-related chains.

  Go to Draw/NCS ghost control and select Draw non-crystallographic ghosts. You will now see a copy of the B chain superimposed on the A chain. The B chain is draw using thin bonds.

  Look for regions where the models differ. Using the NCS maps tool, check that the differences in the models are supported by the density.

- Compare the NCS-related chains using "NCS jumping".

  This is an alternative way of examining NCS similarities and differences. Use the Display Manager to undisplay the NCS maps you recently created above.

  Use the "Goto Atom" dialog and press the "Update from Current Position" button.

  [Coot shifts the centre of the screen to the closest displayed CA atom]

  Identify the chain that you are looking at (for example, by double-clicking on an atom)

  Now press the "o" key on the keyboard.

  [Coot shifts the centre of the screen to a NCS-related chain]

  Identify the chain that you are looking at now.

  Press "o" again.

  What is happening?

  (Note: "o" stands for "Other NCS-related chain".)

- Comparing the Ramachandran plots for related chains.

  Go to Validate/Kleywegt plot. Check the Use specific chain options and select chains A and B. You will get a Ramachandran plot with equivalent residues from the A and B chains linked by arrows. Long arrows indicate significant differences. Click on a dot to view the residue concerned.

Another tool for identifying NCS differences is the Validate/NCS differences graph. This gives a clickable histogram of differences.

5.3 Add Terminal Residue

[To be found on the Model/Fit/Refine dialog[16]]

- Use the Go To Atom window to go to residue 72 A.

- What do you see?

  [You see missing atoms (that is, you don't see them). This residue is supposed to be a CYS! Bad things have happened to it. Let's get rid of this residue.]

  [There may also be waters in the side-chain positions too (they have to be removed too).]

- Delete. . .

- (Make sure "Residue/Monomer" is active)

- Click on an atom of residue 72 A.

  [Residue 72 A disappears]

- Lets' put back an ALA.

- Add Terminal Residue. . .

  [Coot adds intermediate white atoms and pops up a dialog]

- Accept the new atoms

  OK, we now have a ALA. We want a CYS:

- Mutate & Auto Fit. . .

- Click on an atom in the new ALA

  [Coot pops up a residue type chooser]

- Choose residue type CYS

  [Coot mutates and fits a CYS]

- Examine the situation.

  [There is an extra blob of density on that CYS. What is it?] It is an alternative conformation.

  Let's model it

- Add Alt Conf. . .

  [Coot pops up a residue splitter dialog]

- Choose how you want to split your residue (the Right Way is an unresolved philosophical issue. . .[17])

- Click on an atom in 72 A

  [Coot adds intermediate white atoms and pops up a Rotamer Chooser]

- Choose the correct rotamer - it shouldn't be difficult to tell which is the closest.

- Accept when you are happy with you choice.

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[16]and in the modelling toolbar
[17]Actually Jane Richardson may disagree with that statement.

- Now optimize the fit of this new residue with Real Space Refinement.
  The refinement of each alternate conformations is independent.

- Real Space Refine Zone

- click on (say) SG,B of 72A

- Press "A" (on the keyboard)
  [Coot moves the atoms a bit]

- Accept

- Now do the same with the other conformation.
  [Ahhh! Much better.]

5.4 Display Symmetry Atoms

To be found on "Draw" → "Cell & Symmetry" menu item in the main menu.
Try:

- Show Symmetry Atoms? → Yes

- Symmetry as Calphas? [on]

- Colour symmetry by molecule [on]

- Symmetry Radius 30Å

- Colour Merge → 0.1

- Show Unit Cell? [on]

- OK

- Zoom out and have a look at how the molecules pack together.

5.5 Skeletonization and Baton Building

You can calculate the map skeleton in Coot directly:
   Calculate → Map Skeleton... → On.
   This can be used to "baton build" a map. You can turn off the coordinates and try it if you like (the Baton Building window can be found by clicking "Ca Baton Mode..." in the Other Modelling Tools dialog[18].
   If you want to do this, I suggest you use Go To Atom and start residue 2 A (this allows you to build the complete A chain in the correct direction (it takes about 15 minutes or so) and you can compare it to the real structure afterwards.
   Remember, when you start, you are placing a CA at the baton **tip** and at the start you are placing atom CA 1. This might seem that you are "double-backing" on yourself - which can be confusing the first time.

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[18]this is quite advanced)

5.6 Refine with Refmac

Refmac is a program that does Maximum Likelihood refinement of the model, and the interface to it can be found on the Model/Fit/Refine dialog. (By default there is no icon on the toolbar for this action - open the window from the calculate menu[19]). Now click on "Run Refmac. . . " (at the bottom of the Model/Fit/Refine window).

The defaults should be fine...

"Run Refmac" in the new window. . .

. . . Wait. . .

[The cycle continues. . .]

- Use "Validate → Difference Map Peaks" to find interesting features in the newly created map and model.

- Python enabled version only: a dialog with geometric outliers and other interesting things (if they exist) detected by Refmac shows up [20].

6 Use the EDS

Let's validate a structure deposited in the PDB.

Use a web browser and go to `http://eds.bmc.uu.se/eds/`. Use the keyword search to find the structure of a particular protein/ligand/author.

File → Get PDB and Map Using EDS

Get the accession code and paste it into the entry box that pops up.

Use the validation tools in Coot to examine how good a PDB file this is...

[the Difference Map Peaks can be interesting]

6.1 Make a (Pretty?) Picture

(Depends on the appropriate supporting software being available):

- Arrange a nice view

- Press F8

- Wait a few seconds. . .

Colophon

This document is written using XEmacs 21.5 in LATEX using AUCTEX and is distributed with the Coot source code.

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[19]or add your own "Run Refmac" button to the toolbar or the main/top toolbar. To add the button to the modelling/side toolbar, open the "Preference" window. Now go to Preferences → General → Refinement Toolbar. In the right hand window scroll down and tick the box besides "Run Refmac. . . ". A corresponding button will appear on the modelling toolbar. Alternatively you can add your own "Run Refmac. . . " button to the top toolbar of the Coot window (only in Python versions). Click Right-mouse on the toolbar (besides the "Display Manager" button) and you will see a pop-up menu. Select "Manage Buttons", tick the box besides "Add Alt Conf" and click "Apply". A new button named "Add Alt Conf" will appear on the toolbar.

[20]This is an analysis from the Refmac log file and can alternatively be opened from Extensions... → Refine... → Read REFMAC log