

Coot Tutorial

CSHL 2006 Workshop

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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.yesbl.york.ac.uk/~emsley/coot/tutorial/tutorial-  
modern.pdb  
$ wget http://www.yesbl.york.ac.uk/~emsley/coot/tutorial/rnasa-1.8-  
all_refmac1.mtz  
or copyy 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.

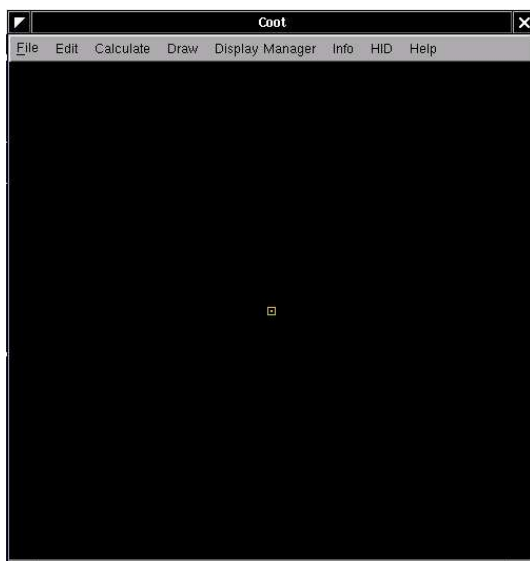


Figure 1: Coot at Startup

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

2.3 Display Coordinates

So let's read in those coordinates:

- Select "File" from the Coot menu-bar¹
- Select the "Open Coordinates" menu item
[Coot displays a Coordinates File Selection window]
- Either
 - Select `tutorial-modern.pdb` from the "Files" listor
 - 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:

HID → Virtual Trackball → Flat

(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.

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

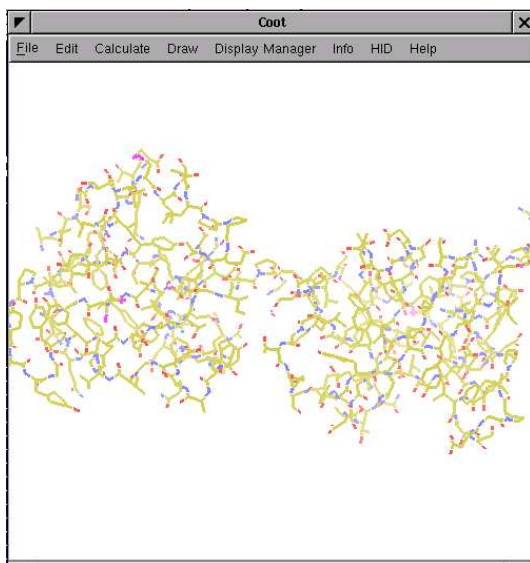


Figure 2: Coot After Loading Coordinates

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-right². To zoom out again, move the mouse the opposite way.

²or up-to-down, if you prefer that

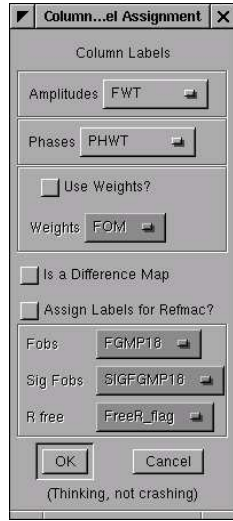


Figure 3: Coot MTZ Column Label Selection Window

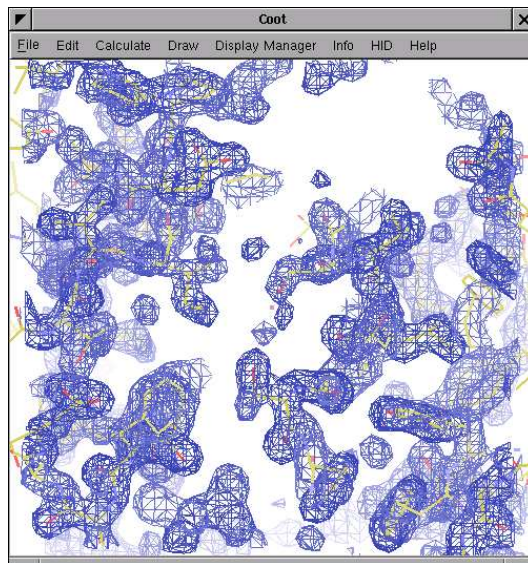


Figure 4: Coot after reading an MTZ file and zoomed in.

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 Draw from Coot’s menu-bar
 - Select the “Dragged Map. . .” menu item
 - Select “No” in the “Active Map on Dragging” window
 - Click “OK” in the “Active Map on Dragging” window

Now the map is recontoured at the end of the drag, not at each step³.

- 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 C α 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.

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).

³which looks less good on faster computers.

⁴think: Depth of Field.

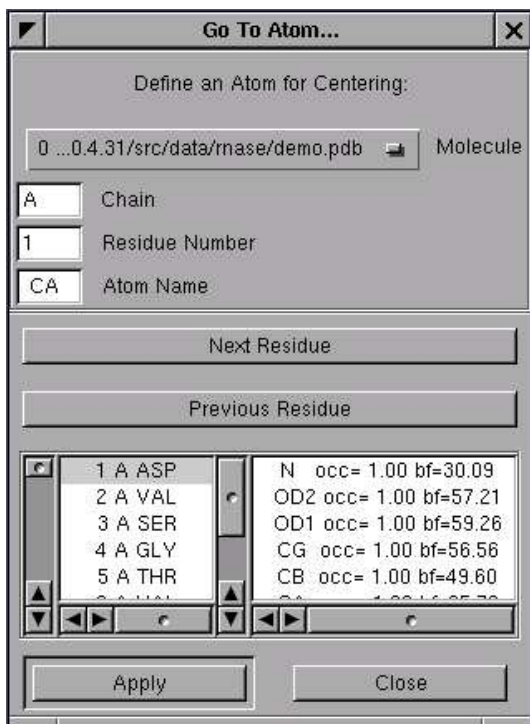


Figure 5: Coot's Go To Atom Window.

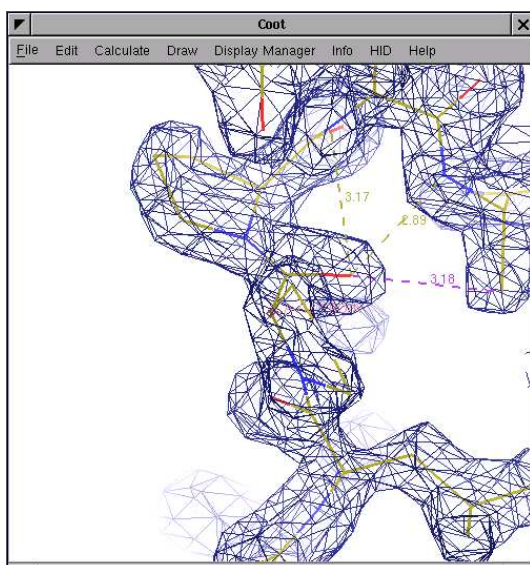


Figure 6: Coot showing Atom Label and environment distances.

2.9 Recontour the Map

- Scroll your scroll-wheel forwards one click⁵
[Coot recontours the map using a 0.05electron/Å³ 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 this⁶.
[This button is not yet available in WinCoot]

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

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]

⁵don't click it *down*.

⁶by default it is the last map, which is not necessarily the map that you want.

3.1 Rotamers

- Examine the situation... [*The sidechain is pointing the wrong way. Let's Fix it...*]
- Menubar: Calculate → Model/Fit/Refine...
[*Coot displays the Model/Fit/Refine window*]
- Select "Rotamers" from the Model/Fit/Refine window.
- In the graphics window, (left-mouse) click on an atom of residue 89A (the C γ , 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.
- 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.*]

OK. That's good.

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

- Click "Undo" twice
[*Coot puts the sicechain back to the original position*]
- Click "Real Space Refine Zone" in the Model/Fit/Refine window.
- 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*]

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...

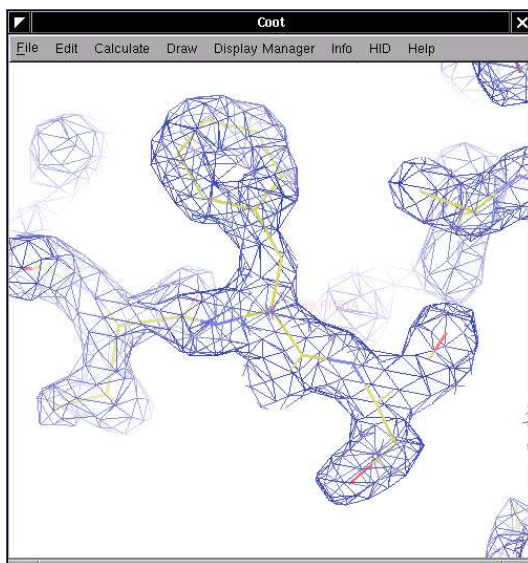


Figure 7: 89A now fits the density nicely.

- 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...
- 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 select and move (just) that 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 unexplained 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
[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 SO₄ twice. Accept)
[The SO₄ fits better now].

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

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]
Note: In WinCoot "File → Get Monomer..." won't work. Instead open the file `monomer-3GP.pdb`.
It is generally easier to work without hydrogens, so let's delete them
- "Delete..." from the Model/Fit/Refine window
- "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

- 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).
- 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 as far as I expect you to get.

4.2 Make a (Pretty?) Picture

(The pretty picture doesn't work with WinCoot yet):

- Arrange a nice view
- Press F8
- Wait a few seconds. . .

5 Extra Fun (if you have time)

5.1 Waters

Fit waters to the structure

- “Find Waters...” in the Model/Fit/Refine window, 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 sliding⁷ you can turn it off:
 - Click “Draw” in the main menubar
 - Click “Smooth Recentering...”
 - Click “No”

5.2 Add Terminal Residue

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

- 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]

⁷it’s not very good for water checking

- 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. . . ⁸)
- 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.
- 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.]

5.3 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]

⁸Actually Jane Richardson may disagree with that statement.

- Symmetry Radius 30Å
- Colour Merge → 0.1
- Show Unit Cell? [on]
- OK
- Zoom out and have a look at how the molecules pack together.

5.4 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 dialog⁹).

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) 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.

5.5 Refine with Refmac

Note: This option does not work on WinCoot.

Refmac is the program that does Maximum Likelihood refinement of the model and the interface to it can be found on the Model/Fit/Refine dialog. First you need to read in an MTZ file (as you did previously) using “File” → “Open MTZ, CIF or pbs. . .” but this time assign the Refmac column labels¹⁰

Now click on “Run Refmac. . .” in 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.

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]

⁹this is quite advanced)

¹⁰Which are the default labels, in this case.

Colophon

This document is written using XEmacs 21.5 in L^AT_EX using AUC_TE_X and is distributed with the Coot source code.