Coot Tutorial II: More Advanced Usage

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The idea here is to use more advanced[1] tools of Coot. There will be less description of low-level widget manipulation in this tutorial - we presume that you already have experience with that. You may well trip over issues not discussed here[2].

1 Preamble

When automatic building fails, typically because the resolution limit of your data is too low, then building the molecule "by hand" may be the only way to proceed. Recognizing the shape of main-chain and side-chain densities is valuable and this tutorial aims to introduce these to you. Note that this tutorial map is an easy map to build into, the sidechains are (mostly) clear. If you want a more realistic "bad" map, you can apply a resolution limit to the data read in from the MTZ file[3].

Using just a map and a sequence, we will attempt to generate a model. This model can then be validated and refined with Refmac for several rounds. With some experience you should be able to get an R-factor of less than 20% in less than 30 minutes.

2 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.

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 and you can directly compare it to the real structure afterward[4]. Once you are at residue 2A, use the Display Manager to turn off the `''tutorial-modern.pdb''` and don't look at it again until you have finished building, validating and refining.

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.

So build from the N-terminus to the C (it takes about 15 minutes or so). There are 96 residues to build.

[1] "less commonly-used" might be a better description

[2] Feel free to shout out if you do, several others may have this same problem and we can examine the issue together.

[3] the resolution limit widget will appear when you activate the "Expert Mode" button.

[4] if don't follow this instruction, you could well build a symmetry related molecule, which is perfectly valid, of course, just that the comparison versus the correct structure will be more difficult.

Note that you need at least 6 CA baton points for CA Zone to Mainchain to work[5]

3 Key Bindings

If you look at "Paul's Key Bindings"[6] in the Coot Wiki[7], you will see a page of customizations. One of those customizations can help you in Baton-Building mode - and that is the "quoteleft" key binding.

So, cut the bindings out of the web page, paste them into a file and then use Calculate → Run Script... to evaluate that file[8]. To check that your key-bindings are activated, Use Extensions → Key Bindings....

Now, we can use quoteleft (or "backquote", "`" is how it might appear on the keyboard) to accept the baton position - this is much more convenient than using the "Accept" button[9].

4 At the end of the Chain

At some stage[10] you will come to a point where no progress can be made, the only direction takes us into density we've already built into. OK, so stop: *Dismiss*.

Now we need to turn these CA positions into mainchain. Calculate → Other Modelling Tools → CA Zone to Mainchain. Use the Go To Atom dialog to centre on the first residue of "Baton Atoms", click it, then centre on the last residue of "Baton Atoms" and click on that.

[Coot thinks for a several seconds while building a mainchain]

OK, great, we have a mainchain. Let's tidy it up:

Extensions → Stepped Refine.

Refine the "mainchain" molecule, watch it as it goes. Is it making mistakes?

That refinement may have gone to quickly to make a note of problem areas, so use Validation → Density Fit Analysis on the "mainchain" molecule and find areas that are marked with large spikes.

"There are none" you say? Good[11]. Let's move on.

5 Assign Sequence

Let's tell Coot that we have a sequence associated with this set of CA points. So, Extensions → Dock Sequence → Assign Sequence

Turn on auto-fit of residues

So when the file is assigned "Assign Closest fragment".

[Coot thinks for a several seconds while assigning sidechains, then goes about mutating and fitting the residues]

What's that you say? Coot didn't do that? Well, that's because you mainchain model is too bad for Coot to recognize the sidechain positions. You need to review you mainchain model and make sure sure that the CBs are in density and pointing

[5] otherwise it silently fails - more feedback will be added in later versions.

[6] Use Bernhard's Key-bindings if you are using pythonized or WinCoot

[7] you can find a link to this from the Coot web page

[8] "read it in", you might say

[9] You can do that as well, of course, but *clicky-clicky pressy button* is for Coot noobs, and that's not us, right?

[10] hopefully residue 96

[11] If that's not what you say, you can use the refinement or other tools that we learned about in the first tutorial to improve the fit to density.

in the right direction. When you have improved you model sufficiently well, Coot will apply the sequence to it using the above method.

Change the Chain ID from " " to "A".

6 Cell and Symmetry

Display Symmetry Atoms:

Draw → Cell & Symmetry → Master Switch: Show Symmetry Atoms → Yes and OK.

By zooming out and eyeballing the density, check for unassigned density.

[Coot displays symmetry-related atoms in grey - by default (you may not see many symmetry related atoms, it depends on where in the unit cell you are)]

7 Build another molecule

Now we need to build another molecule (the NCS related copy). So using the map skeleton search around to find a volume of density not already build (and not symmetry related to the model already built).

Here's a hint, find the a helix in the skeleton.

- Using the Other Modelling Tools, place a helix over the skeleton points of the skeleton.
- Improve the fit of the skeleton, taking note that the N and C terminus of the helix are well-fitted.
- Associate the same sequence with the new Helix molecule
- Dock sidechains on the new molecule (it should work if your helix is good)
- Now compare the Helix molecule with the previously built model. Find matching start and end point on the helix and previous model.
- LSQ fit a copy of the previous model on top of the Helix molecule

  [Coot displayes a new molecule that almost fits the so-far unbuilt density.]

  Let's call this new chain, chain "B"
- Now clean up the fit, first do a rigid body refinement of the whole new model…
- then an All Molecule stepped refine should make the fit nice.

8 Merge Molecules

Merge the "B" chain into the "A" chain molecule above:

Calculate → Merge molecules → Append/Insert Molecule(s) *[Choose the most recent mainchain molecule]* into Molecule *[Choose the molecule of the A chain]* → Merge.

9 Ghosts

Unfortunately, there is no slick way to make Coot rebuild ghosts for this composite molecule. We need to write out the pdb file and read it in again - inelegant.

File → Save Coordinates, *[Choose the molecule that does now contains both the A and B chains]* → Select Filename... Pick a filename then use File → Open Coordinates... to read it in again.

Check the console as you do this, Coot will tell you that there are NCS related molecules. If[12] it does this, we're in business.

In the following, you will need to know the first and last residue numbers in the "A" chain. Use the Go To Atom dialog to find them.

If ghosts appear, use:

Extensions → NCS... → Copy NCS Residue Range... using "A"[13] as the Master Chain ID then fill in the first and last residue numbers of the A chain.

[Coot builds the B chain as an NCS copy of the A chain]

10 Rinse, Repeat

Use NCS jumping (the 'O' key) to see NCS differences.

Now unmodelled blobs - like we did before.

Find the ligand (3GP), merge it in.

Refine using Refmac.

Validate.

Rebuild.

11 Make some pictures

- Highlight active site, with ligand. Take a screenshot.
- Use Raster3D to take a screenshot
- Now make a Raster3D image without spheres for atoms, how do you do that?
- Now give the ligand a dotted surface
- Now Use Extension → Mask Map to make a map that has density only around the ligand.
- Now take the residues in the active site, use Copy Fragment and merge molecule to make a single mlecule of them. Display this atom selection as an electrostatic surface

12 Views

Try out the "View" system

- Zoom out to see the whole molecule on the screen
- Recentre and Zoom in to the active site
- Play Views...

[12] When

[13] presumably

13 More Exercises

What does "Another Level" do?

What does "Multi-chicken" do?

Use the skeletonization of a map to find a helix. Use Calculate → Other Modelling Tools to add a helix there.

Try to represent the map with a higher resolution grid (use Edit → Map Parameters). Do you prefer that? Why?

Use the EDS service to download 1H4P. Can you find anything wrong with the main-chain? If so, how can you correct it?