Simons Electron Microscopy Center

Intro to Model Building & Refinement Tutorial. SEMC winter course 2023.

Please complete this pre-tutorial checklist <u>before</u> the tutorial. Some of these steps may be time consuming.

- ❏ Download tutorial files (models and maps) here:
  https://drive.google.com/drive/folders/17Y4djAv7eFp_Hy42hW3YHs9rK_E93_mb?usp=share_link
- ❏ Install ChimeraX (downloads are here: https://www.cgl.ucsf.edu/chimerax/download.html)
- ❏ Launch ChimeraX and open a model file (Menu: File>Open then choose any model (.pdb or .cif) in the tutorial files you downloaded).
- ❏ Open a map file in ChimeraX (Menu: FIle>Open then choose the map (.map) in the tutorial files)
- ❏ Install Coot. You can use any version of Coot for this tutorial. I recommend version 0.9 or higher. It is significantly updated for lower resolution maps compared to versions 0.8 and below. Note: Coot comes with CCP-EM or CCP4 so if you have installed one of those before, you already have Coot.
  - ❏ For Linux, download from https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/.
  - ❏ For Mac, see http://scottlab.ucsc.edu/xtal/wiki/index.php/Installing_Coot_on_OS_X.
  - ❏ For Windows, download from https://bernhardcl.github.io/coot/wincoot-download.html.
  - ❏ You can use any version of Coot for this tutorial. I recommend version 0.9 or higher. It is significantly updated for lower resolution maps compared to 0.8 versions and below.
- ❏ Launch Coot and open a model file (Menu: File>Open Coordinates then choose any model (.pdb) in the tutorial files)
- ❏ Open a map file in Coot (Menu: File>Open Map then choose the map (.map) in the tutorial files).
- ❏ Install Phenix (downloads are here: https://www.phenix-online.org/)
- ❏ Launch Phenix and create a new project that points to a directory where you will save output files for this tutorial.

If you can check all of these boxes, you're ready to start!

This tutorial will take place over two days. On **day one** we will use **Phenix** and **Coot** to assemble a starting model from other publicly available models.

On **day two** we will use **ChimeraX** to fit our starting model into an experimental cryoEM map and use **Phenix** to perform real space refinements to improve both the fit of the model into the map and the chemical bond geometries of the model. We will use **Coot** to visually assess the output of the refinements and do some minor model adjustments by hand.

The aim of this tutorial is to provide familiarity with available software tools and techniques used for model generation and refinement. We will cover basic features and uses. This will not provide a comprehensive overview or advanced use of any particular software (this would require a much longer course!). We hope this introduction prepares you to work with your own data or attend more advanced tutorials in the future.

Our model system

In this tutorial we will build a model of the TRPV1 ion channel using a published map. TPRV1 is an ion channel, also known as the "capsaicin receptor". It is famous in the single particle cryoEM field as the molecule that ushered in the "resolution revolution". It was also the basis of the work for which the 2021 Nobel Prize in Physiology and Medicine was awarded: https://www.nobelprize.org/prizes/medicine/2021/summary/.
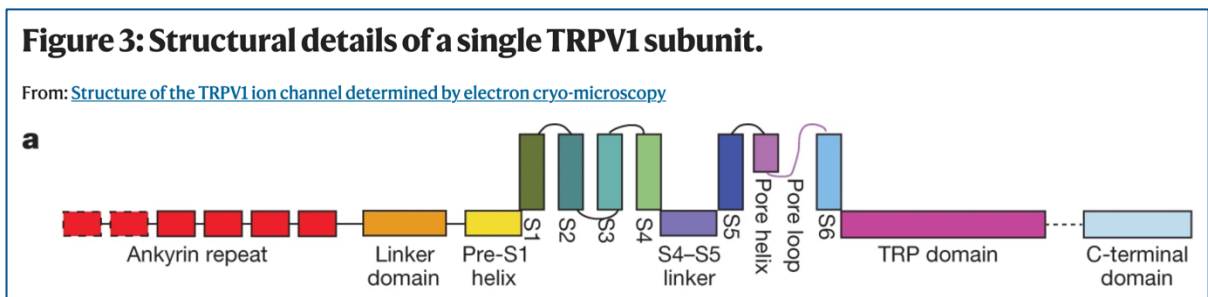
You can also read more about TRPV1 on the PDBs molecule of the month page: https://pdb101.rcsb.org/motm/250

For this tutorial what you need to know is this:
TRPV1 is a homo-tetrameric protein with a transmembrane domain (TMD) and a soluble intracellular domain (see the topology diagram below). The transmembrane domain has 6 transmembrane-spanning helices (S1-S6). The N- and C-termini are intracellular and the N-terminal domain (NTD) has an ankyrin-repeat fold with 6 repeats. The C-terminal domain (CTD) has no predicted fold.

Figure 3: Structural details of a single TRPV1 subunit.
From: Structure of the TRPV1 ion channel determined by electron cryo-microscopy

Figures on this page are from the first published full-length TRPV1 structure. Figure 3 shows the topology from the primary sequence and Figure 2 shows the overall fold of a fully assembled tetramer. [Reference: https://doi.org/10.1038/nature12822]

The map we will work with was obtained for TPRV1 from rat. We will abbreviate it rTRPV1.

Map source:
https://www.emdataresource.org/EMD-8117
Reference:
https://doi.org/10.1038/nature17964 EMDB 8117; PDB 5IRX
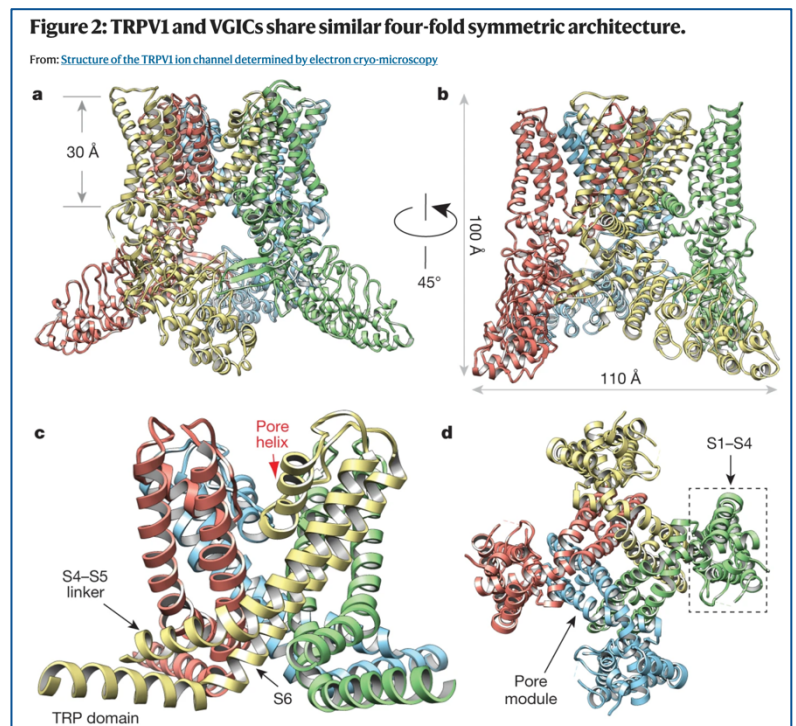
Simulating what you might do if there were not yet a model for this full-length protein available, we will use existing models to build and refine a tetrameric channel into a map.

Figure 2: TRPV1 and VGICs share similar four-fold symmetric architecture.
From: Structure of the TRPV1 ion channel determined by electron cryo-microscopy

Tutorial outline:

Day one:

       Part 1 - Use databases to become familiar with the protein sequence and make a plan.

       Part 2 - Collect model files and manipulate with Phenix and text editors.

       Part 3 - Use Coot to stitch together a starting model from multiple pieces.

Day two:

       Part 4 – Use ChimeraX to dock the starting model into a cryoEM map.

       Part 5 - Use Phenix to perform rigid body and real space minimization refinements to improve the model.

       Part 6 - Use Coot to evaluate the refinement result and do some manual adjustments.

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1 Use Uniprot to get familiar with the protein

Uniprot will let us quickly familiarize ourselves with the domain architecture and sequence of TRPV1: https://www.uniprot.org/uniprotkb/O35433/entry

For example, we can easily find predicted or experimentally determined domain boundaries:

And we can find models available in public repositories:

We will look at the available models to decide which to use as our starting model. Let's pretend there are no full-length cryoEM structures available. In this case, there is an AlphaFold prediction for the full-length sequence and high-resolution X-ray crystal structures of the N-terminal ARD. We will use these two pieces to assemble a starting model.

Download the PDB files from the sites below to a directory you will use for this tutorial.
AlphaFold2 model: https://alphafold.ebi.ac.uk/entry/O35433
ARD X-ray structure model: https://www.rcsb.org/structure/2PNN

Advanced topics

What if I can't find a structure with an identical sequence? What if the exact pieces don't exist?

Is there an AlphaFold2 model from another species? https://alphafold.ebi.ac.uk/

Running AlphaFold2 on google collabs: https://github.com/sokrypton/ColabFold/

Make a model using RoseTTAFold: https://robetta.bakerlab.org/

Pre-AlphaFold2 suggestions for threading models or homology models:

Modeller: https://salilab.org/modeller/

SWISS-MODEL: https://swissmodel.expasy.org/

I-TASSER: https://zhanglab.ccmb.med.umich.edu/I-TASSER/

Chainsaw (part of CCP4): http://www.ccp4.ac.uk/html/chainsaw.html

If you have a map: De novo model building tools:

ModelAngelo: https://github.com/3dem/model-angelo

DeepTracer: https://deeptracer.uw.edu/home

Buccaneer (part of CCP4): http://www.ccp4.ac.uk/html/cbuccaneer.html

ArpWarp (part of CCP4 - for high resolution maps): https://arpwarp.embl-hamburg.de/

Phenix map_to_model: https://www.phenix-online.org/documentation/reference/map_to_model.html

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2. Use Phenix to modify downloaded files

You can find full documentation for Phenix here: https://www.phenix-online.org/
Phenix tutorial videos are short and helpful: https://www.phenix-online.org/documentation/reference/tutorial_channel.html
Phenix has a suggested workflow for using AlphaFold2 generated models: https://phenix-online.org/version_docs/dev-4380/reference/alphafold.html

2.1 Open Phenix. In the home window make a new project for this tutorial by clicking the "New project" button.
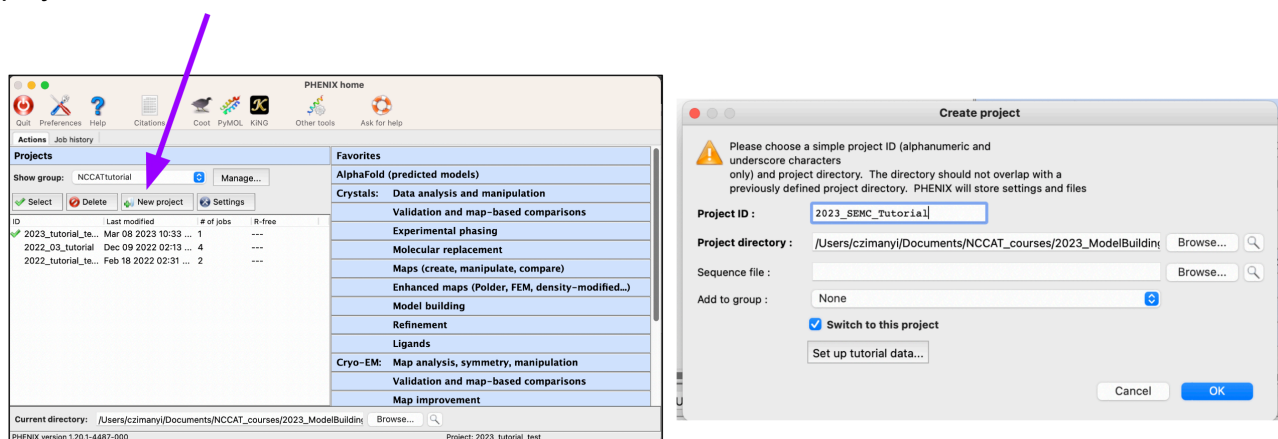

Give your project a sensible ID (I named my project **2023_SEMC_Tutorial),** specify a project directory you would like to work in. Phenix will automatically save outputs in the specified project directory. The sequence file is optional. We'll skip that. Click "OK". Now we can run jobs in that project.

2.2 The first job we will run is "Process Predicted Model" in the AlphaFold tool set to remove parts of the model with a low prediction confidence. These are often "spaghetti" loops hanging off of globular domains and make model building more difficult.

All programs in Phenix have a similar workflow. When you choose the program, it will give you a new window with one or two tabs. Where there are two tabs, one ("Files") is for input/output information and other ("Options") is for run parameters. Details on what input files are required and what the parameter options are can be found in the documentation or you can use the "help" button.

Click on the "Process Predicted Model" job. Give the job a sensible title (this is only for your own reference), find your downloaded AlphaFold2 model using the "Browse…" button, and choose "lddt" for "Contents of B-value field". LDDT is a confidence score determined by AlphaFold2 and ranges from 0-1. LDDT values below 0.7 are considered low. Specify an output file name. I chose "**AF-rTRPV1_pruned**". Check "Remove low-confidence residues". Click the "Processing options" button in the Options. The first option is "Maximum domains". The default is 3 but we don't want to divide the chain into subdomains in this example, so change this to 1 and click "OK". Then click the "Run" button (with the cog icon in the top row) and the job will run, and should fininsh in a few seconds.
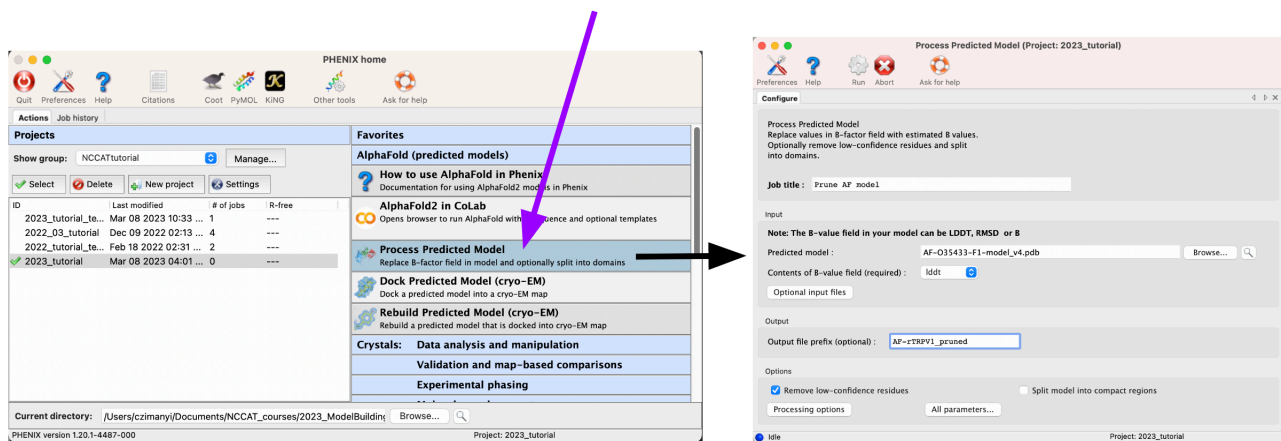
You should now have a new .pdb in a subdirectory of your project directory. We will look at it in a minute.

2.3. Removing waters and ligands from models.

The ARD model from the crystal structure contains water molecules and a bound molecule of ATP. We don't want to include these in our starting model. There is more than one way to remove these atoms (including jobs in Phenix) but here, we will remove them manually using a text editor.

Let's review the anatomy of a .pdb file. It is a human readable text file with the following columns:

Open 2pnn.pdb in your favorite text editor. Non-protein atoms are usually at the end of the file and labeled as HETATM in place of ATOM. Scroll to the bottom of the file and delete the ATP and HOH (water) atoms. Let's save this file as **2pnn_noHETATM.pdb.**

[Tips: When editing .pdf files as text, you can delete anything that isn't atomic coordinates (i.e. REMARK, SEQRES, CRYST1 lines) and still open them in visualization software. Residue numbers should always remain in proper sequence and all HETATM lines should go to the bottom. Remove any TER lines from the middle of contiguous sequences. It doesn't matter if the atom # sequence is sensible – software will usually fix this for you.

We now have the two starting pieces for our model. We will inspect what we did in Coot and then merge them together into a single model.

3. Use Coot to open files, replace the ARD of the AlphaFold model with that from the X-ray crystal structure.

On a Mac or Linux, I like to open Coot from a terminal (i.e. running: /usr/local/bin/coot) in my working directory so that files automatically save in that directory. Also, I can monitor what Coot is doing in that terminal.

3.1 Open Coot and use File>Open Coordinates to open the two initial model files (AF-O35433-F1-model_v4.pdb & 2pnn.pdb) and the two files we've editied (**AF-rTRPV1_pruned.pdb & 2pnn_noHETATM.pdb)**. Spend a few minutes orienting yourself with the navigation, with the table below as a guide.

Action	3-button mouse	Mac trackpad	Windows trackpad
Left click	Left button	click	click
Right click	Right button	Command-click	alt-click
Middle click	Middle button	Control-click	Control-alt-click
Rotate	Left mouse drag	Click drag	Click drag
Translate	Control-left mouse drag or middle mouse drag	Control-click drag	Control-click drag
Label	Shift-left mouse click (or double left mouse click)	Shift click or double click	Shift click or double click
Zoom	Right mouse drag	Command-two finger drag	Alt-drag
Clipping	Control-right mouse drag	Control-two finger drag	Control-alt drag
Rotate around Z	Control shift right mouse drag	Control-shift two finger drag	
Center	Middle mouse click		
Change map contour	Scroll wheel	Two finger scroll	

Disclaimer: This table may not be 100% accurate. Here's a good quick reference:
https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/web/docs/crib-sheet.pdf

A useful feature to use right away is: **Draw>Display manager.**
This gives you a window for managing every object you have open. Try using different display modes to get a feel for how things look.
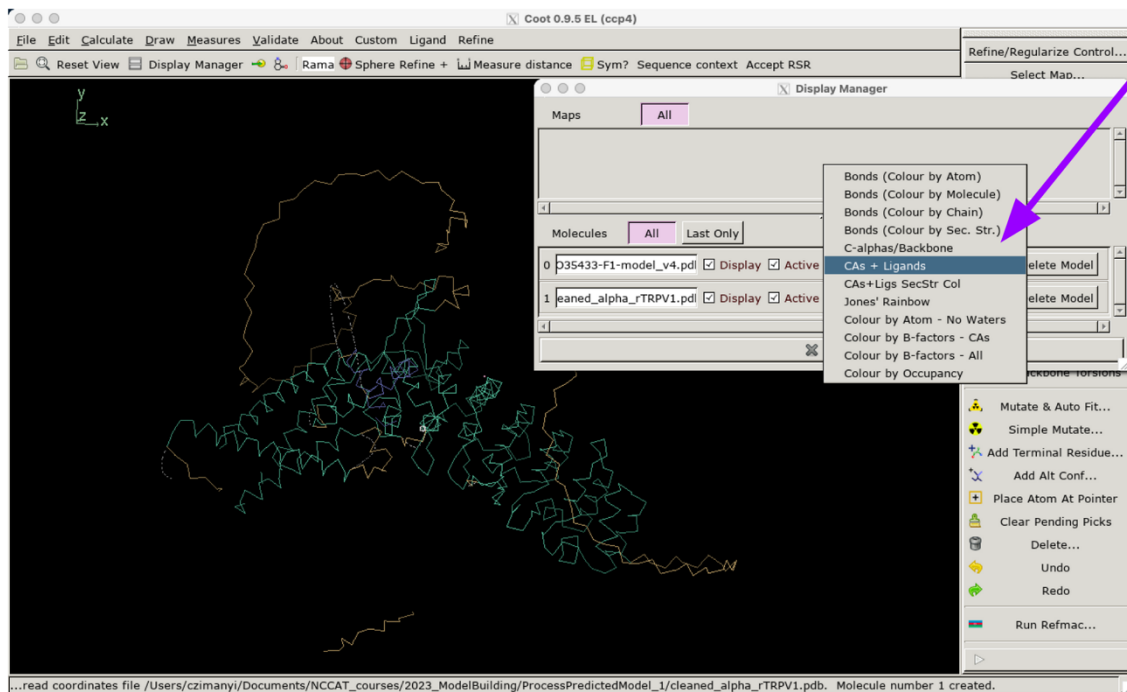
3.2 First, let's look at the AlphaFold Model. You can toggle different models on and off to see what changes. You'll notice the long "spaghetti" loops included in the initial predicted model were removed as intended by the Phenix job because they have low confidence scores.

Now look at the ARD models. You should notice the waters and ATP were removed in your modified model.

3.3. After inspection, you can close the original models using the "Delete Model" button in the Display Manager window.

3.4. Next we want to align the ARD alone model (residues 111-358 of the full sequence) to the full length model because we think the crystal structure will be more accurate. In the Calculate menu, find the LSQ Superpose job. We can use this job because our amino acid sequences are identical. If this wasn't the case, we would choose SSM superpose that aligns based on secondary structure and not sequence. The ARD model only contains residues 111-358, so this will be our alignment range and we will match C-alphas. Click "Fit" to do the alignment.
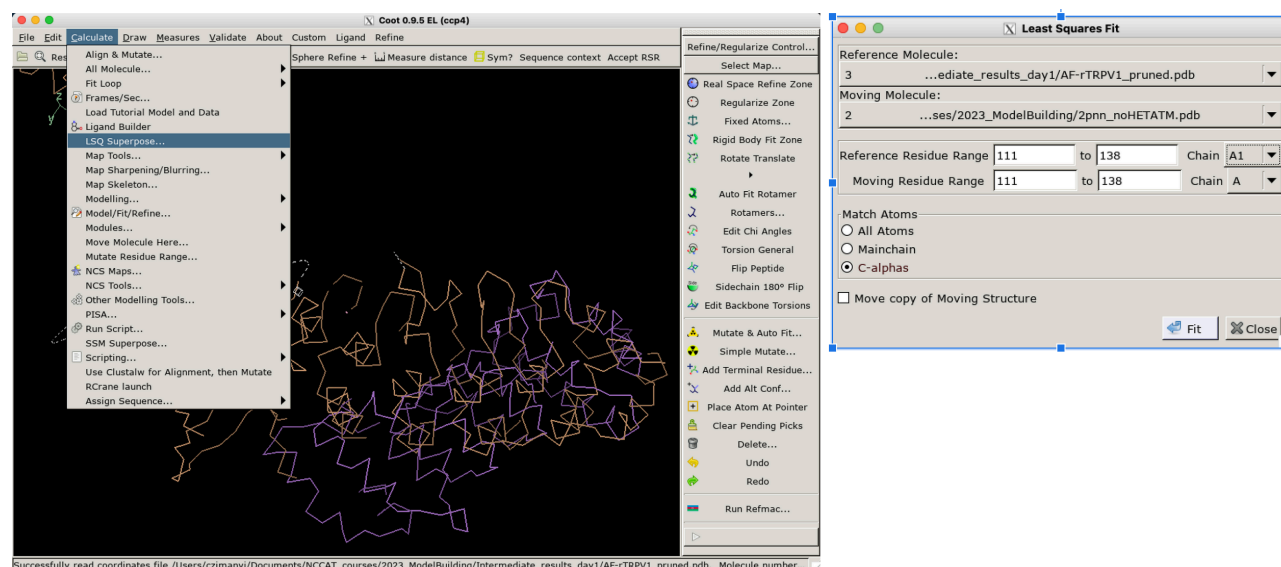
This alignment should work well because the two models are very similar. If needed we could restrict the alignment range to a smaller region that aligns better or in a particular region of interest, but here we don't need to.

3.5 Now we want to delete the ARD from the AlphaFold model. We could do this in a text editor, but we can also do it in Coot. We will use the "Delete" button in Coot to see how it works.

Before engaging the "Delete" function, I like to label the ends of the sequence I plan to delete by double clicking the terminal atoms. You can navigate quickly to a specific residue using:
> Draw>Go To Atom or
> Draw>Sequence View

Tip: You can increase the font size in Edit>Preferences>Others – Fonts tab.

Label residues 111 and 353 to find them more easily. Then click "Delete" and you will get a new window of delete options.
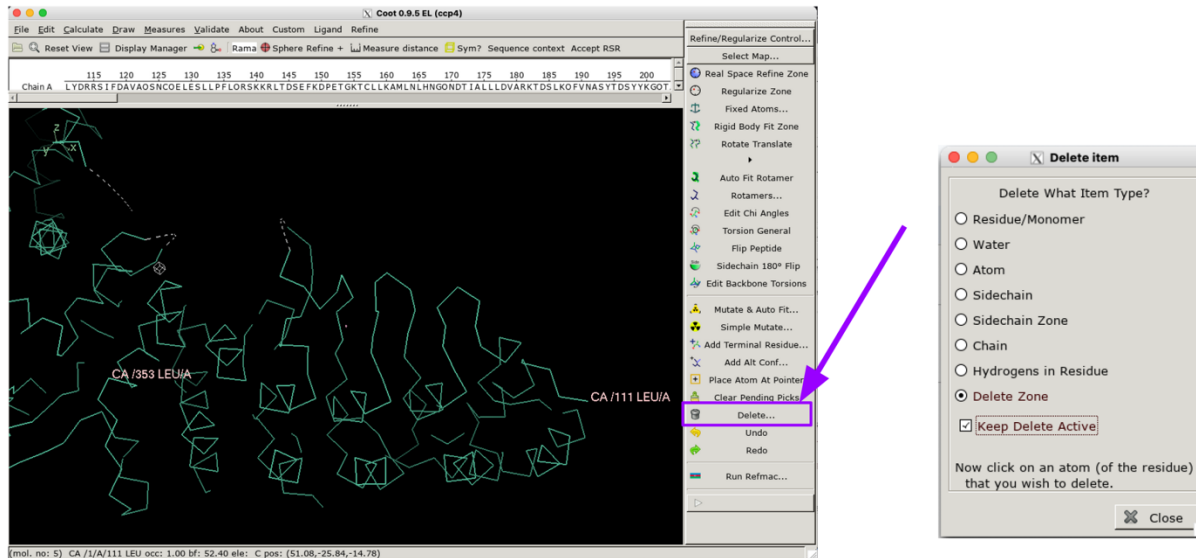
Chose "Delete Zone" and Check "Keep Delete Active" (this is dangerous, but we will use it with care!). Then click on the two ends of the zone you want to click, and they will be deleted. Click carefully! There is an "Undo" button, but it can only be applied to one model. Once you have deleted the region you want to delete click "Close" on the "Delete" window to disengage the deletion cursor.

3.6 Now, save your work! File>Save Coordinates... – make sure you choose the correct model number to save. Rename the file to something sensible (like AF-rTRPV1_pruned_noARD.pdb). Model files are small so you should save your work often.

3.7 We also want to delete resides 353-358 of the ARD model. Do that and save the resulting model (I named mine 2pnn_noHETATM_trimmed.pdb)

3.8 Finally, we will merge both chains into one file using:
Edit>Merge molecules
Pay attention to which file you are merging into.

3.9 Coot gives each chain a unique ID, but we want these to be part of the same chain (chain A). We can change the chain ID using
Edit>Change Chain IDs.
Choose the model you merged into, choose "Chain ID" to "B" in the drop down, chose "Whole Chain" and type "A" into the Chain ID field. Click on "Apply New Chain ID". You will notice it worked if all atoms are now the same color in the display.
[Note: If you have any overlapping residue numbers this will not work. You may need to delete any overlapping residues, renumber residues if they are mis-numbered (also in the Edit menu, or specify a sub-range if needed.]

3.10 Save this file with a new name.
**File>Save coordinates** I called mine **rTRPV1_monomer_chimera.pdb**
That's it for today! Next time we will fit four copies of this model into the cryoEM map and refine it.

Day 2. Today we will use **ChimeraX** to fit our starting model into an experimental cryoEM map (part 4), use **Phenix** to perform refinements (part 5) and use **Coot** to visually assess the refinement output and do some minor model refinements by hand (part 6).

4.1 Open ChimeraX and then open the map file and your **rTRPV1_monomer_chimera.pdb** file from Monday. (File>Open>coordinates.pdb).

Tips: you can open maps or models directly from public databases using the **open** command in the ChimeraX command line: **open 8117 from emdb,** etc.). Web-fetched data are stored locally in **~/Downloads/ChimeraX/** [https://www.cgl.ucsf.edu/chimerax/docs/user/commands/open.htm]

Your map and model will likely not be near each other in space, so we need to move them closer together. But first, let's spend a couple of minutes orienting in ChimeraX. Two useful features of note are the top menus and right column panels. We will use the Models panel and Volume viewer often. Click some buttons and play with the different representations.
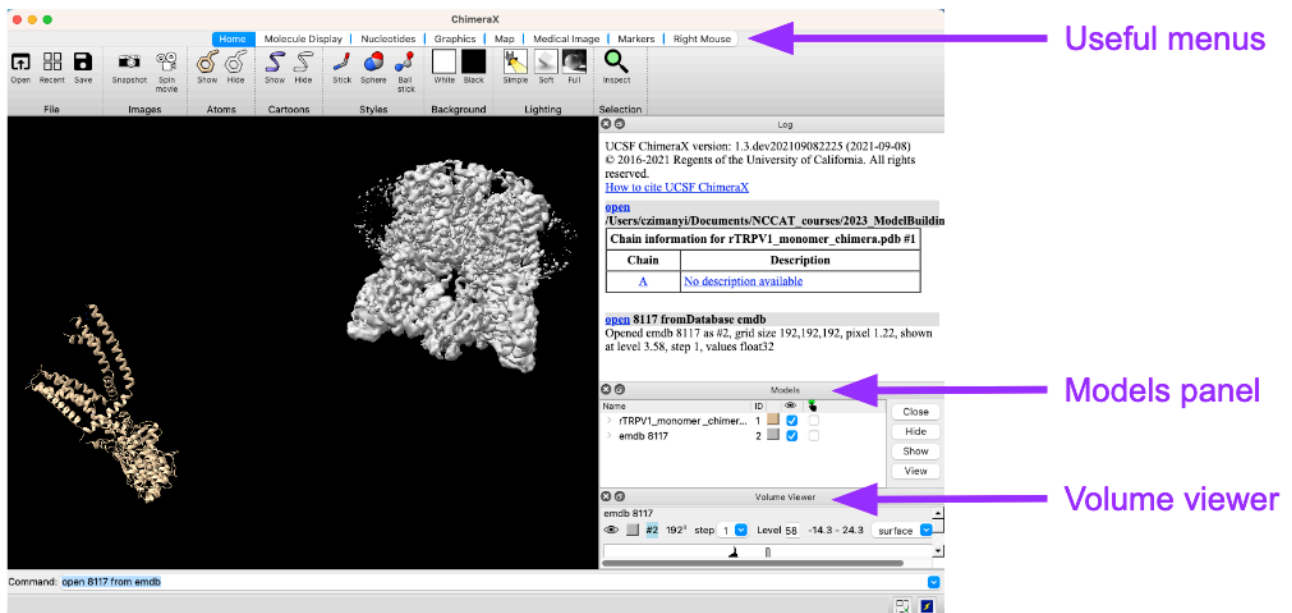

How to navigate: https://www.cgl.ucsf.edu/chimerax/docs/user/commands/ui.html#mousedefaults

Action	3-button mouse	Mac track pad	Windows track pad
left click	Left button	click	click
Right click	Right button	Command-click	alt-click
Middle click	Middle button	Control-click	Control-alt-click
Rotate	Left mouse drag	Click drag	Click drag
Translate	Middle mouse	alt-click	Control-alt-clic
Zoom	Mouse scroll wheel	Pinch	2 finger drag
Select	Left mouse + ctrl	Control-click	Control-click

4.2 It can be easier to see where a model fits in a map by making a smoother (or filtered) copy of the map. You can do this with the "Smooth" button in the Map menu or with the command
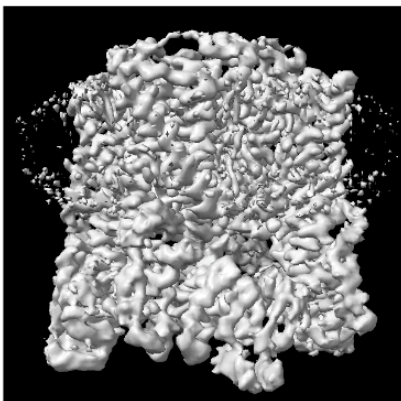
vop gaussian #2 sdev 2

(or whatever standard deviation value gives a map you like). This will make a new map object leaving your original map untouched.
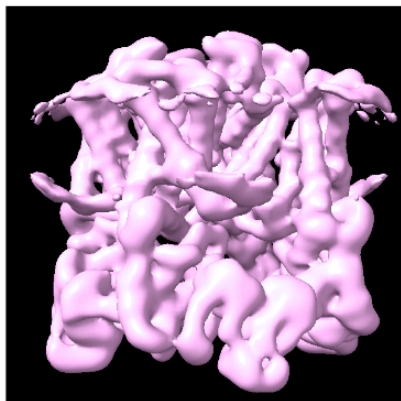
4.3 Now let's fit the model to the map. Select the model in the Models panel by clicking the selection check box. The model can now be selectively modified using your right mouse button as controlled in the "Right Mouse" menu. Use the "Move model" and "Rotate model" options, toggling between right and left mouse movements to roughly orient your model into the map. Click on the color block next to your map to adjust the opacity of the map (and color if you want) to make it easier to see. Get your model roughly in the right place of the map.
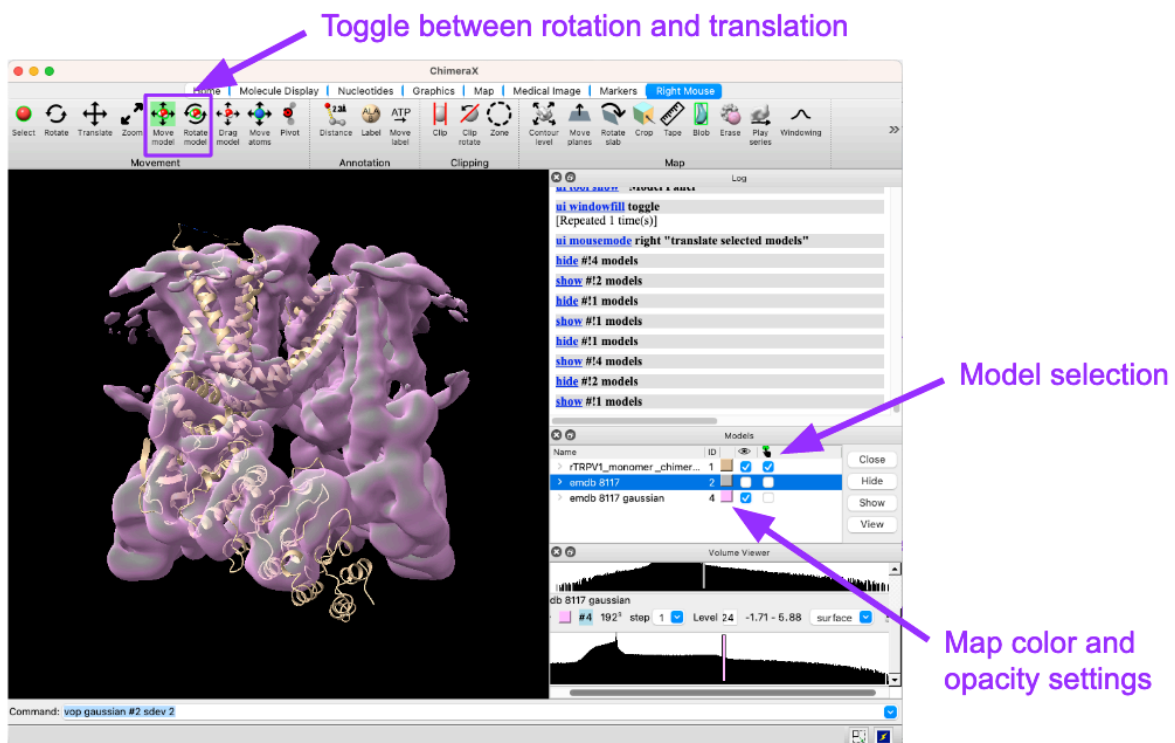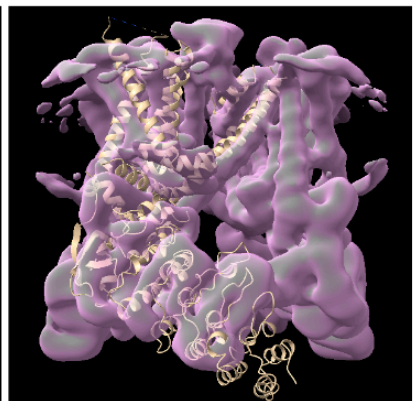

Original map   vop gaussian #selection sdev 2   Transparent map, rough model fit

4.4 We can use the fitmap command (or the Menu Tools>Volume Data>Fit in Map) to optimize the fit once we're close. https://www.rbvi.ucsf.edu/chimerax/docs/user/commands/fitmap.html.

fitmap #1 in #3

or specify the model you want to fit to which map in the Fit in Map panel.

If you're not close enough, the fitmap command won't find a good fit. Try manually placing the model better and run fit map again until you get it right.

Tip: Using the Fit in Map window, under options choose "Use map simulated from atoms". This will give you a correlation value that you can use to judge the fit.
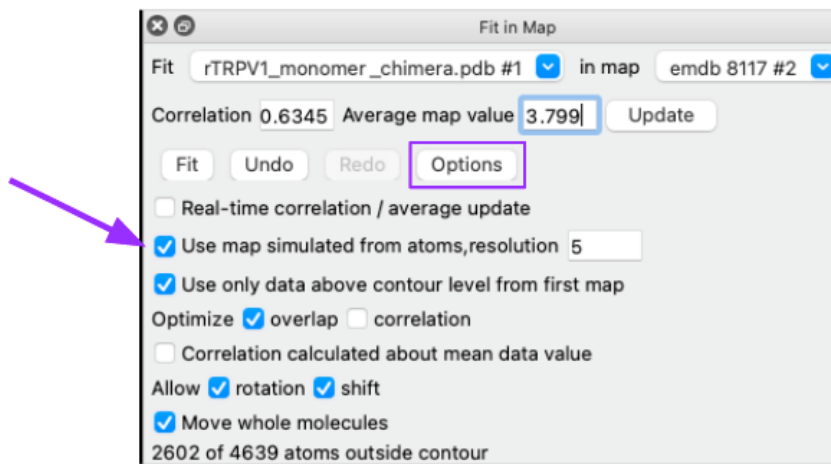
4.5 To make a tetramer, we do this three more times. You can open another copy of the model, it will have a unique model ID. Move it into the density and fit, repeat. To save time today we will use a pre-generated tetramer model. Here is how that was generated:

Combine the four protomers that are separate models into one single model with four chains using the **combine** command. We combine them one by one, each time generating a new model ID. **Warning!** Your model IDs may not be the same as those below!! Use the model ID #'s that make sense for your session.

combine #1 #5 name rTRPV1_dimer
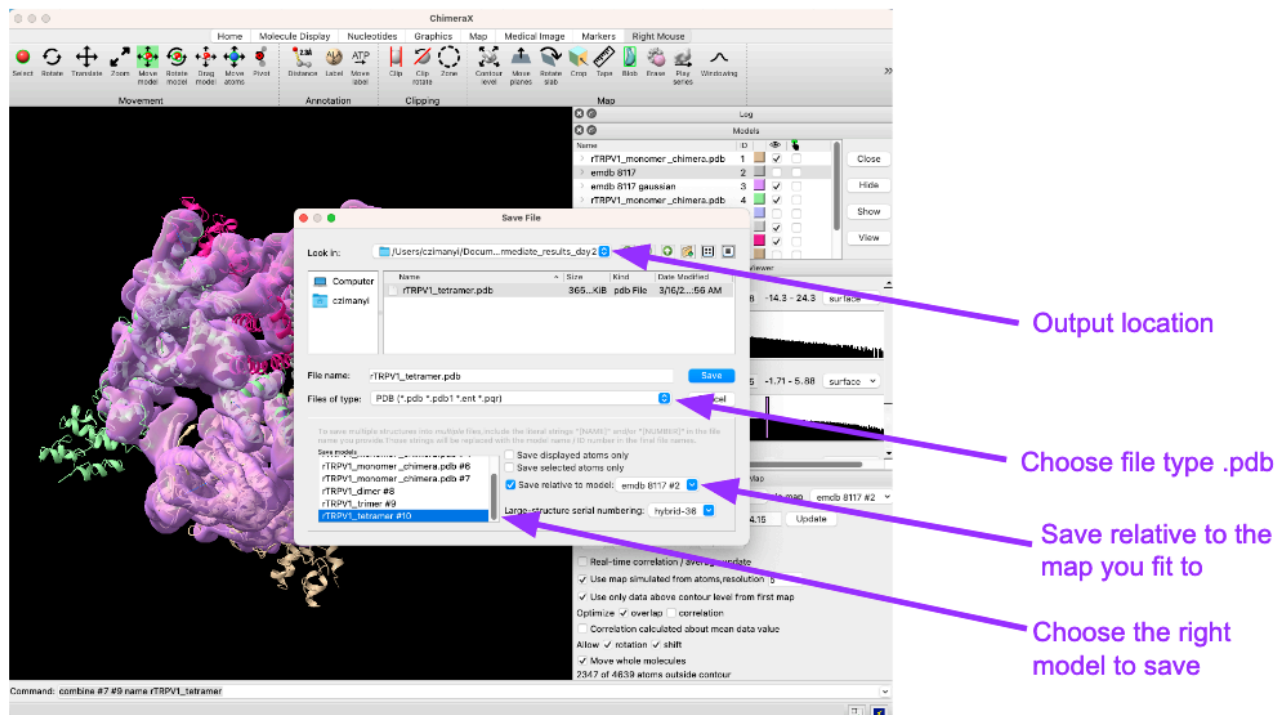   → this combines models 1 and 5 and names the new model (#8) rTRPV1_dimer.
      Iterate this:
combine #6 #8 name rTPRV1_trimer
combine #7 #9 name rTRPV1_tetramer

Then save the final model using **File>Save**

Chose the correct file type (here it's a model so choose PDB) and the correct model. Save it relative to the map you fit it to. Here **rTRPV1_tetramer.pdb**

You can also save from the command line using:
save choose_name.pdb #10 rel #3

Note: the map appears to not be C4 symmetric. Two monomer copies will fit almost perfectly and the other two will have S5 & S6 of the TMD sticking out of density and clashing with other S5/S6 regions in the pore. We can fix this during refinement. Also, the two N-terminal repeats of the ARD do not have density and there is extra density on the extracellular side of the channel that we do not have model for.

4.6 Before we close ChimeraX, lets use it to make a pretty picture. Try different display modes in the **Molecule Display Menu** until you find something you like. Play with the lighting & effects in the **Graphics** menu. The Snapshot button in the Home menu will save a .png to your Desktop (or you can save as an image file format from the Save options).

A few other useful ChimeraX commands

If your map is from a fresh reconstruction of your own data, the hand might be incorrect because handedness cannot be determined from 2D projection images. You can easily flip the hand of a map using:

      volume flip #model
      (default is to flip z, other axes can be flipped by specifying the axis in the command)
      Find all volume commands here:
            https://www.cgl.ucsf.edu/chimerax/docs/user/commands/volume.html

Chimera Selections are not limited to full models. You can select sub-regions using selection specifiers.

Hierarchical Specifiers

Symbol	Reference Level	Definition	Examples
#	model	model number assigned to the data in ChimeraX (hierarchical, with positive integers separated by dots: N, N.N, N.N.N, *etc*.)	**#1** **#1.3**
/	chain	chain identifier (case-insensitive unless both upper- and lowercase chain IDs are present)	**/A**
:	residue	residue number *OR* residue name (case-insensitive)	**:51** **:glu**
@	atom	atom name (case-insensitive)	**@ca**

For example you can select just the N-terminus of chain A using:
select #1/A:111-353

Then save just that selection with the command:
save name.pdb #1 selectedOnly true

We could have done this to quickly make an ARD only model yesterday. There are many ways to do most of the operations we are doing and if you do a lot of model building you'll find your favorites.

See more on saving from the command line here
https://www.cgl.ucsf.edu/chimerax/docs/user/commands/save.html

5.1 Open Phenix and launch a Real Space Refinement window from the Cryo-EM menu.

In the Input/Output tab, add the map and model. Give the resolution (2.95 Å – reported in EMDB) and check "Ignore symmetry conflicts". Give the job a sensible title and output file name (I called mine **rTRPV1_tetramer_rigidbody01)**.

In the parameters tab, for Run options, choose only rigid body (uncheck all others). Set Macro cycles to 1, and Nproc to 4 (or fewer if you have fewer cores to process on).

Then click the Select Atoms button to open the Selection Editor. In the Selection Editor, Choose the "Rigid Body" tab and use Add Selection to add 7 selections.
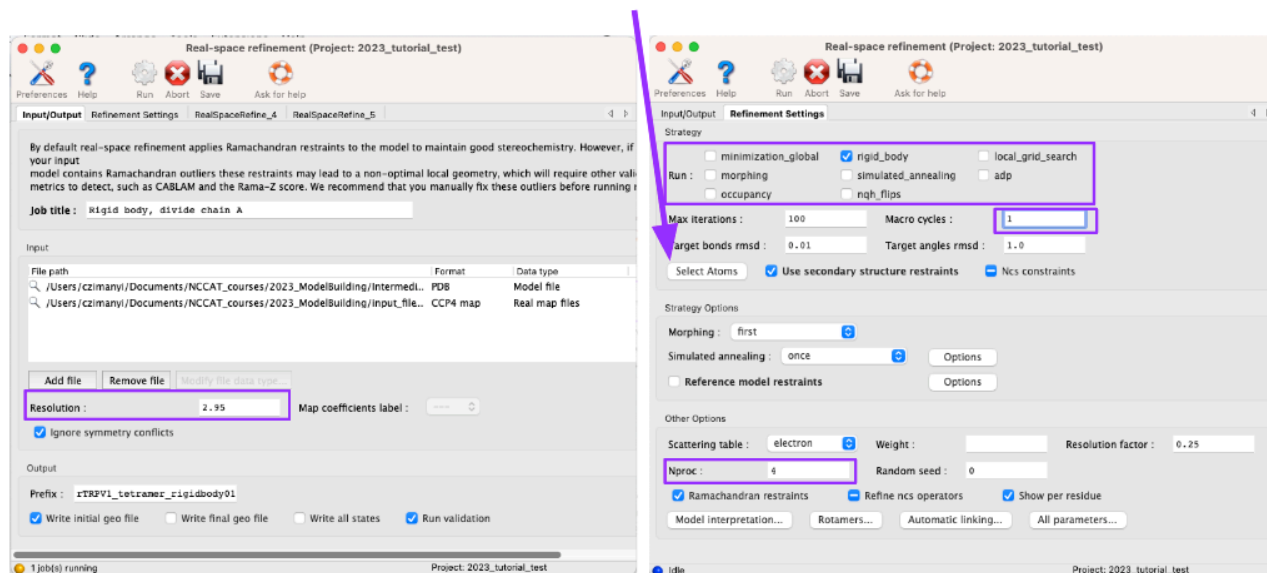

We will use the following selections:
For one chain, we will break the structure down to four regions that we treat as separate rigid bodies:

1) Chain A and resid 111:430 (N-terminal ARD to start of TMD)
2) Chain A and resid 431:588 (S1-S4)
3) Chain A and resid 560:689 (S5-S6)
4) Chain A and resid 699:748 (CTD)

To compare how that differs from leaving the full chain as a rigid body, we will treat the other three chains as single rigid bodies. The selection editor should look as in the figure:
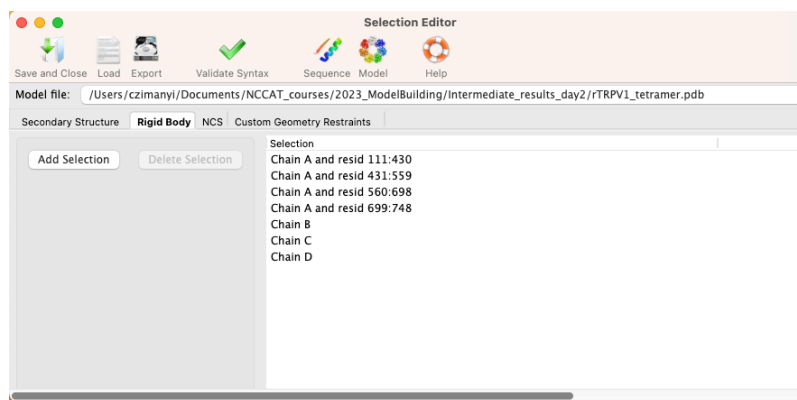

For full Phenix selection syntax see:
https://www.phenix-online.org/documentation/reference/atom_selections.html

You can add as many selections as you'd like and check that they make sense using the "Validate syntax" button. Click "Save and Close" to finish.

Click run. This took ~4 minutes to run on my MacBook Pro.

5.2 When it's finished, you'll get new tabs showing you the location of the output files and in the validation tab, multiple types of validation results for the refined model:

Dynamic Link to Coot   Geometry Validation   Fit-to-map validation
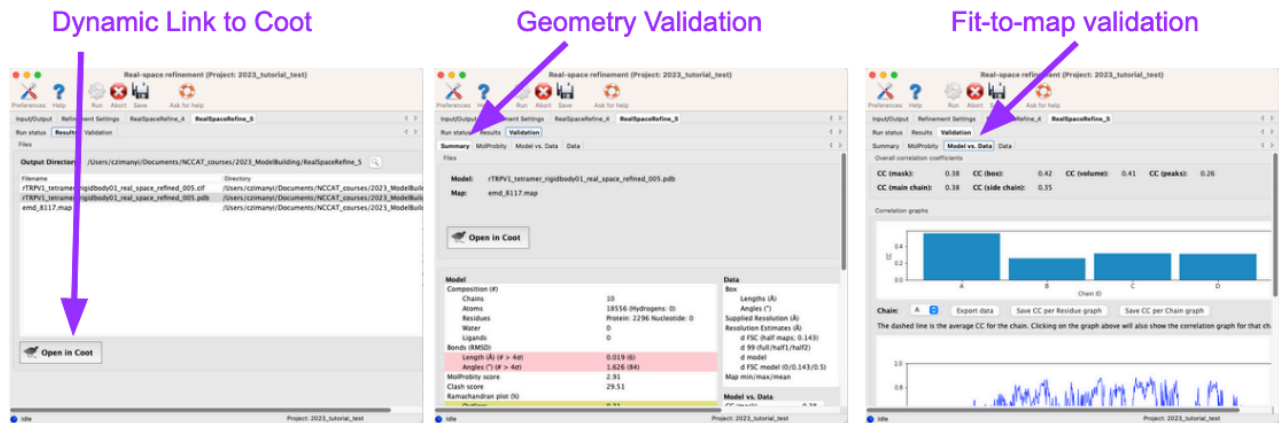
Notice the fit-to-map correlation for Chain A is highest – probably because we used more rigid bodies allowing a better fit.

6.1 Let's inspect in Coot using the dynamic link (otherwise, open a new Coot session and load in the files). After Coot opens with your refined model, also open the input model so we can see what happened. Try hiding the map (using **Display Manager**) to just see what happened to the model during refinement. The rigid body movements should be clear.

Note: If the clashes displayed when opening Coot from Phenix are too much too look at, turn them off using:
Draw>Generic Display Objects turn off clashes. (If closing that window crashes your Coot, just leave it open in the background).

Take some time to explore in Coot. You can threshold the map using your middle mouse, change the model display in the Display Manager, etc.
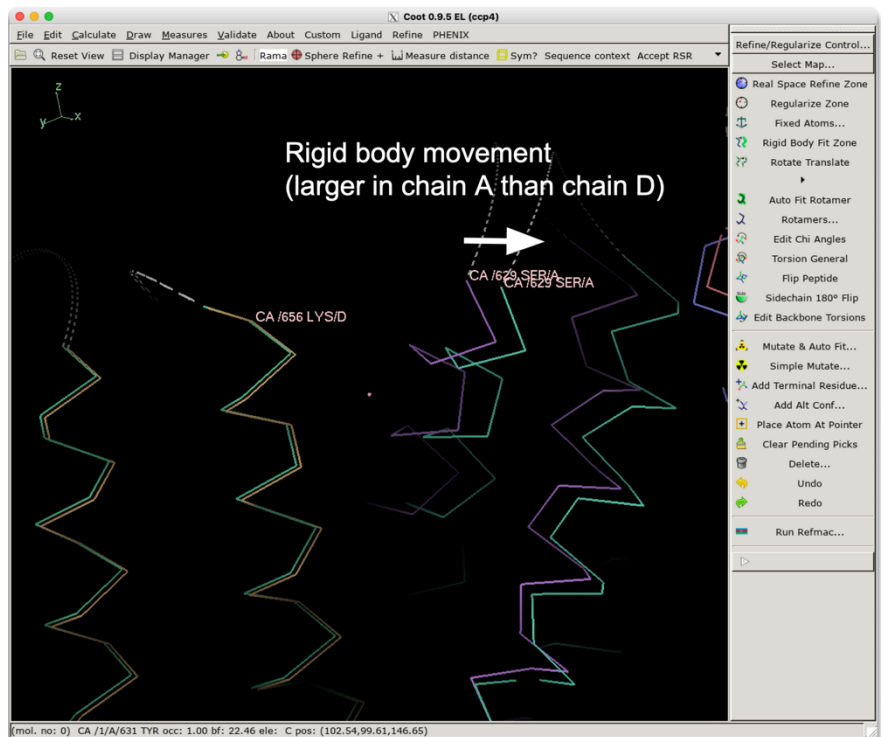
6.2 Different refinement modes in Coot are useful for different tasks. The two major modes are rigid body and dynamic refinement. After clicking a refinement button, two mouse clicks let you choose a range of residues to model.

Real Space Refine Zone is dynamic refinement guided by map restraints. We want to set a good refinement weight using the **Refine/Regularize Control** parameters. This value controls the weight on geometry vs. fit to map and is calculated for the particular map you have loaded. You can adjust it later if needed – higher numbers give more weight to the map.

Let's do three types of manual adjustments.

1) Go to residue S693.
   You can navigate quickly using:
      Draw>Go To Atom or
      Draw>Sequence View
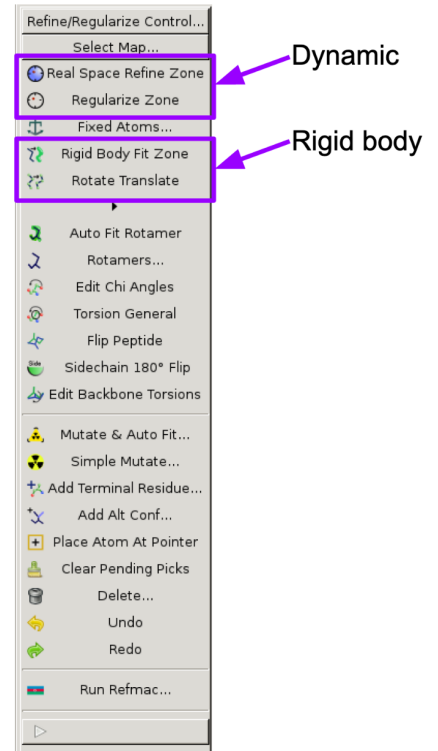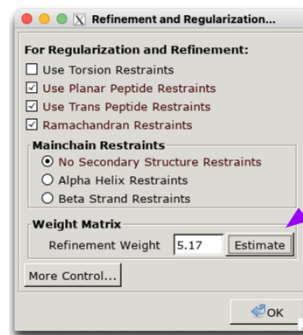
There is an incorrectly modeled helix around S693. We can try to fit it better using Real Space Refine. Click on the range from ~687-701 for this refinement. The dynamic region will become moveable, and a validation box will pop up to help you decide to Accept or Reject a change. Tug on the bonds with your mouse to see how they respond. If you do anything terrible, you can always reject the change and start over. It's ok if every residue isn't perfect. You can fix residues within smaller refinement ranges later.
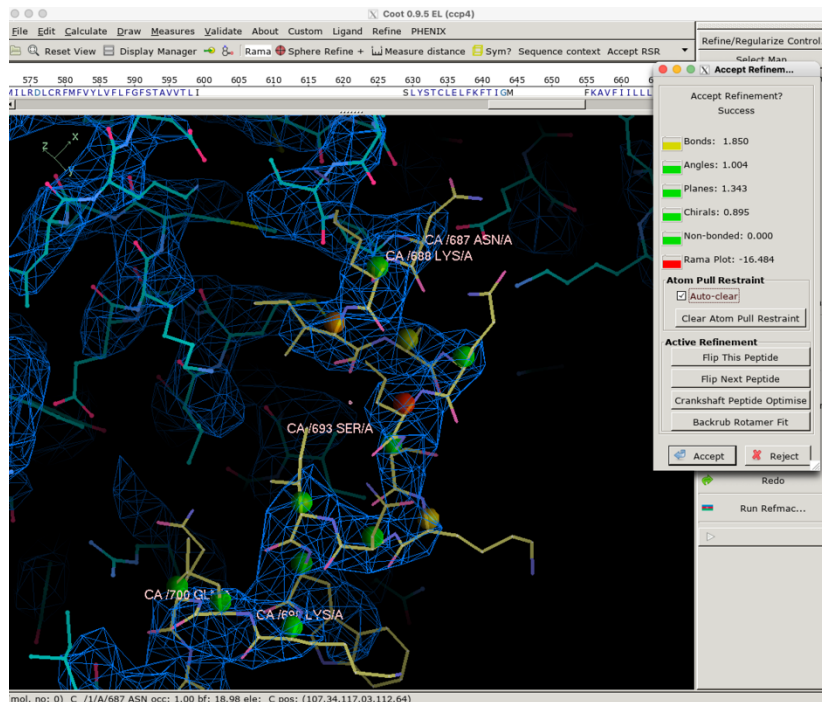
2) Go to residue N467. You can see that there is density for unmodeled amino acids. We can add additional residues with the **Add Terminal Residue** button. Add one. It will be an alanine by default. Then we have to add the proper side chain. Residue 466 is a lysine. You can use **Simple Mutate** or **Mutate and Autofit**. Here the density isn't great, so choose Simple Mutate and make this a Lys.

3) Go to Y511. The backbone looks ok, but the side chain in pointing in the wrong direction. Click on **Rotamers**. You'll get a new window that lets you toggles the most commonly observed side chain rotamers. None of them are perfect, but #2 fits better than the current position. Chose #2 and then try using real space refinement to fit it even better.

Now Save your work! File>Save coordinates. I like to save often and give the files new file names so I can come back to intermediate steps if needed.

Useful tip: If you have symmetry, you can propagate changes easily to symmetric chains. From the menu, choose Calculate>NCS tools>Copy NCS Residue Range... Chose the chain you modified as the Master, click "OK". In the next pop-up window, input the residue range you want to copy onto the other chains and apply.

Here are a few ideas to find additional places that need work:
- Validate>Density Fit analysis
- Validate>Geometry analysis
- Use the validation tables in the Phenix window to navigate to side chains with outlier rotamer geometry.

Bonus Round:

7.1 In Phenix, start another real-space refinement job. Add the file you saved from Coot as your input for the next round of refinement (and remove the old one if it's still there). Give your job a useful title (ie "first round of minimization - 3 cycles") and change the file name prefix for the output file if you want. In the refinement settings, check minimization_global. Make sure macro cycles is set to 3 (this is just to make it faster today – 5 is better).

Click run.

7.2 If you want to try other options, you can run multiple refinements simultaneously. Let's also try "morphing" and see if it helps. This will take longer to run (about ~30 min. longer on my MacBook Pro) but it shouldn't slow down your other run. Check both minimization_global & morphing, give it a useful job tile and output file name prefix and run it.

This is as far as we will work for this tutorial. There is certainly much more building and refinement one can do!

Advanced topics: Other model building/real space refinement tools
        ISOLDE (integrated into ChimeraX): **https://isolde.cimr.cam.ac.uk/**

Advanced topics: Ligands
Remember that ATP we deleted way back when? What if we want to add it back? In Coot you can use File>Get Monomer to get common molecules from a standard library (there are over 2000). The three letter code for ATP for example is ATP. The default is to open ligands as a new model so don't forget to merge them into your working model before saving your pdb.
Sources of ligand coordinates and restraint files:
        PDBe: https://www.ebi.ac.uk/pdbe-srv/pdbechem/
        Cambridge Crystallographic Data Center: https://www.ccdc.cam.ac.uk/
        Crystallography Open Database: http://www.crystallography.net/cod/
        Acedrg program in CCP4: https://www2.mrc-lmb.cam.ac.uk/groups/murshudov/content/acedrg/acedrg.html
        Generate yourself using eLBOW in Phenix.