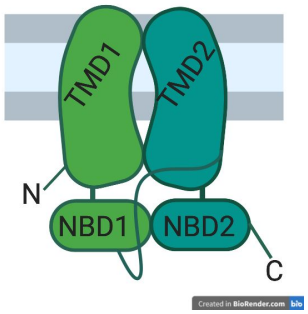
National Center for Cryo-Electron Microscopy Access and Training
Virtual Classroom: Model Building Tutorial. April 2020.

In this tutorial we will build a model of the **C**ystic **F**ibrosis **T**ransmembrane Conductance **R**egulator (CFTR). CFTR belongs to the ATP binding cassette (ABC) transporter superfamily but functions as an anion channel in epithelial cells. Mutations in CFTR that lead to channel dysfunction are causative for cystic fibrosis, making a high resolution structure of this protein highly sought for understanding the molecular basis of the disease and mechanisms of action of small molecule therapeutics.



The ABC transporter family is characterized by two nucleotide binding domains (NBDs) that bind and hydrolyze ATP. The NBDs are connected to transmembrane domains (TMDs). CFTR is a pseudo-dimer consisting of a single polypeptide. An overall topology is shown in the cartoon on the right.

Structures of isolated NBDs have been solved using X-ray crystallography and structures of the full length channel were only more recently solved using cryo-EM (the first published in 2017 by Liu et al. in Cell). We will use a published map, deposited in the EMDB from one of these structures: Molecular structure of the ATP-bound, phosphorylated human CFTR; Zhe Zhang, Fangyu Liu, Jue Chen; *Proc Nat Acad Sci.* Dec 2018, 115(50)12757-12762; DOI: 10.1073/pnas.1815287115

We will start with a model that has most of the TMD already built, but is missing two helices and both NBDs. We will use Chimera to build the NBDs by assembling existing structures and fitting them roughly to the map. We will then use Coot to do manual real-space building and refinement of the NBDs and also build in the two missing TMD helices *de novo.*

Please complete this pre-tutorial checklist prior to the tutorial, some of these steps may be time consuming if you do not already have software installed:

- ❏ Download tutorial materials (models and maps) here: https://drive.google.com/open?id=1Wiwi_ugPOUQoR5uBLpTUHo5T-lwoooT9
- ❏ Install Chimera (downloads are here: https://www.cgl.ucsf.edu/chimera/download.html)
- ❏ Launch Chimera and open a model file (Menu: File>Open then find any model (.pdb) in the downloaded materials)
- ❏ Open a map file in Chimera (Menu: FIle>Open then find any map (.map) in the download materials)
- ❏ Install Coot (for Linux download from https://www2.mrc-lmb.cam.ac.uk/personal/pemsley/coot/. For Mac download from 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) We will be using Coot version 0.8 for this tutorial (version 0.9 is fine too if you already have it).
- ❏ Launch Coot and open a model file (Menu: File>Open Coordinates then find any model (.pdb) in the download materials)
- ❏ Open a map file in Coot (Menu: File>Open Map then find any map (.map) in the download materials).

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

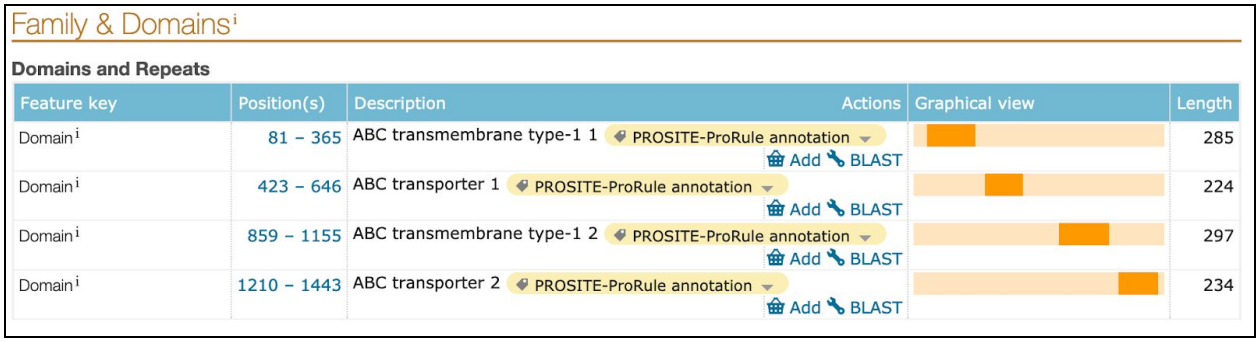
Tutorial outline:

Part 1 - Use Uniprot to become familiar with the sequence and make a plan
Part 2 - Collect model files and fit to a map in Chimera
Part 3 - Refine NBDs into map using Coot
Part 4 - Build missing pieces (protein & ligands) using Coot
Part 5 - Validation

Part 1 - Use Uniprot to become familiar with the sequence and make a plan

We will start by using Uniprot to familiarize ourselves with the domain architecture and sequence of the protein: https://www.uniprot.org/uniprot/P13569

Importantly we can easily find domain boundaries:

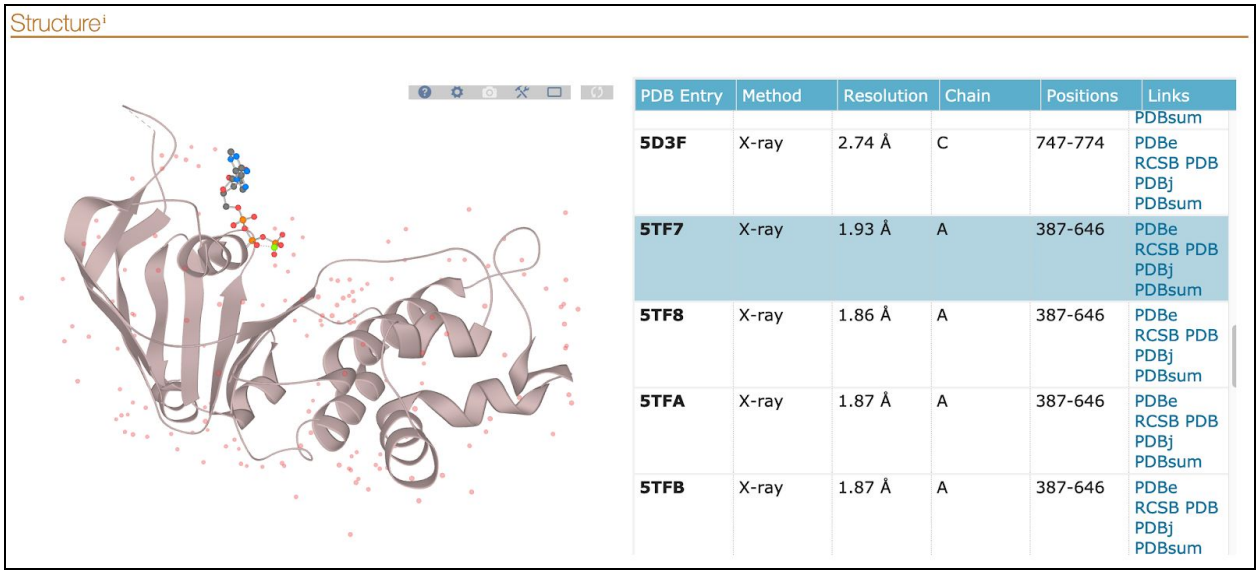


Family & Domains

Domains and Repeats

Feature key	Position(s)	Description	Actions	Graphical view	Length
Domain	81 – 365	ABC transmembrane type-1 1	PROSITE-ProRule annotation Add BLAST		285
Domain	423 – 646	ABC transporter 1	PROSITE-ProRule annotation Add BLAST		224
Domain	859 – 1155	ABC transmembrane type-1 2	PROSITE-ProRule annotation Add BLAST		297
Domain	1210 – 1443	ABC transporter 2	PROSITE-ProRule annotation Add BLAST		234

And existing structures that are available in public repositories:



Structure

PDB Entry	Method	Resolution	Chain	Positions	Links
					PDBsum
5D3F	X-ray	2.74 Å	C	747-774	PDBe RCSB PDB PDBj PDBsum
5TF7	X-ray	1.93 Å	A	387-646	PDBe RCSB PDB PDBj PDBsum
5TF8	X-ray	1.86 Å	A	387-646	PDBe RCSB PDB PDBj PDBsum
5TFA	X-ray	1.87 Å	A	387-646	PDBe RCSB PDB PDBj PDBsum
5TFB	X-ray	1.87 Å	A	387-646	PDBe RCSB PDB PDBj PDBsum

Let's identify starting models for the NBDs:

For NBD1 we'll use 5TF7 (residues 387-646) and for NBD2 we'll use 3GD7(1193-1427). We can download these from the PDB or fetch them into Chimera directly. We'll use the PDB search to find an NBD dimer as a useful template (I found 1L2T) and download our map from the EMDataResource (https://www.emdataresource.org/ search ID 9230)

What we've collected so far: A map (emd_9230.map), a model of the TMDs (CFTR_TMDs.pdb), a model of NBD1 (5TF7.pdb), a model of NBD2 (3GD7.pdb) and an ATP bound NDB dimer as a template (1L2T.pdb). Now we assemble:

Part 2 - Collect model files and fit to a map in Chimera

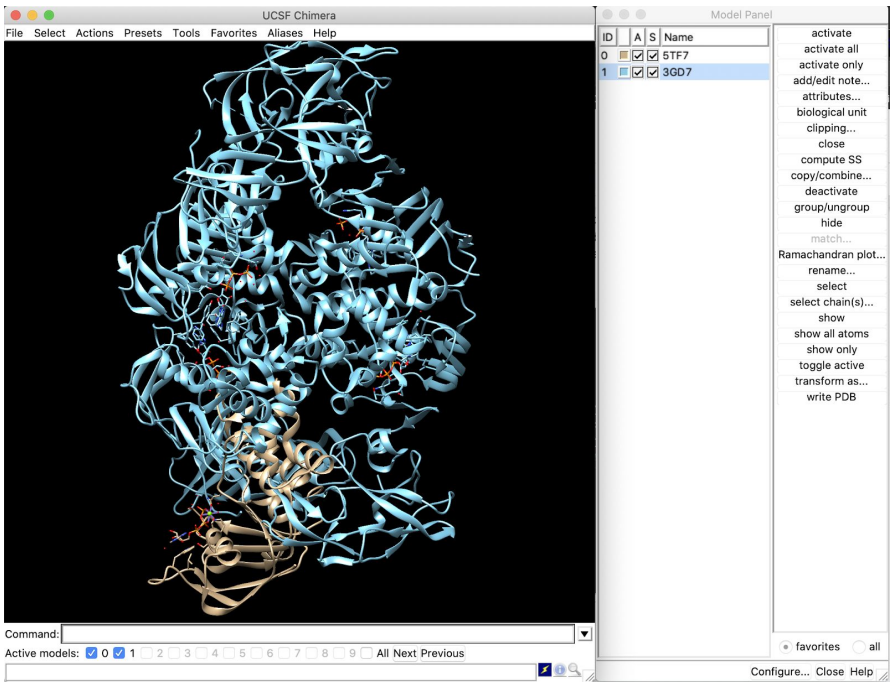
For all of Part 2, we will use Chimera. Chimera has a user guide here: https://www.cgl.ucsf.edu/chimera/docs/UsersGuide/index.html

In this tutorial we will only touch on a few useful functions - there are many we will not cover! We will make use of GUI buttons and the Chimera command line.

2.1 Open the two NBD models, either using the File>Open menu or fetching by ID from the PDB:

5TF7 - This model is a monomer with Mg-ATP and solvent

3GD7 - This model is a fusion protein tetramer. We don't care about the fusion protein or the crystallographic copies, we only want an NBD monomer (residues 1193-1427). We can do this by opening the PDB file in a text editor and manually deleting the unneeded parts of the model, or we can do this using selections in Chimera. We will use command line selections. Open the command line from: Tools>General Controls>Command Line.



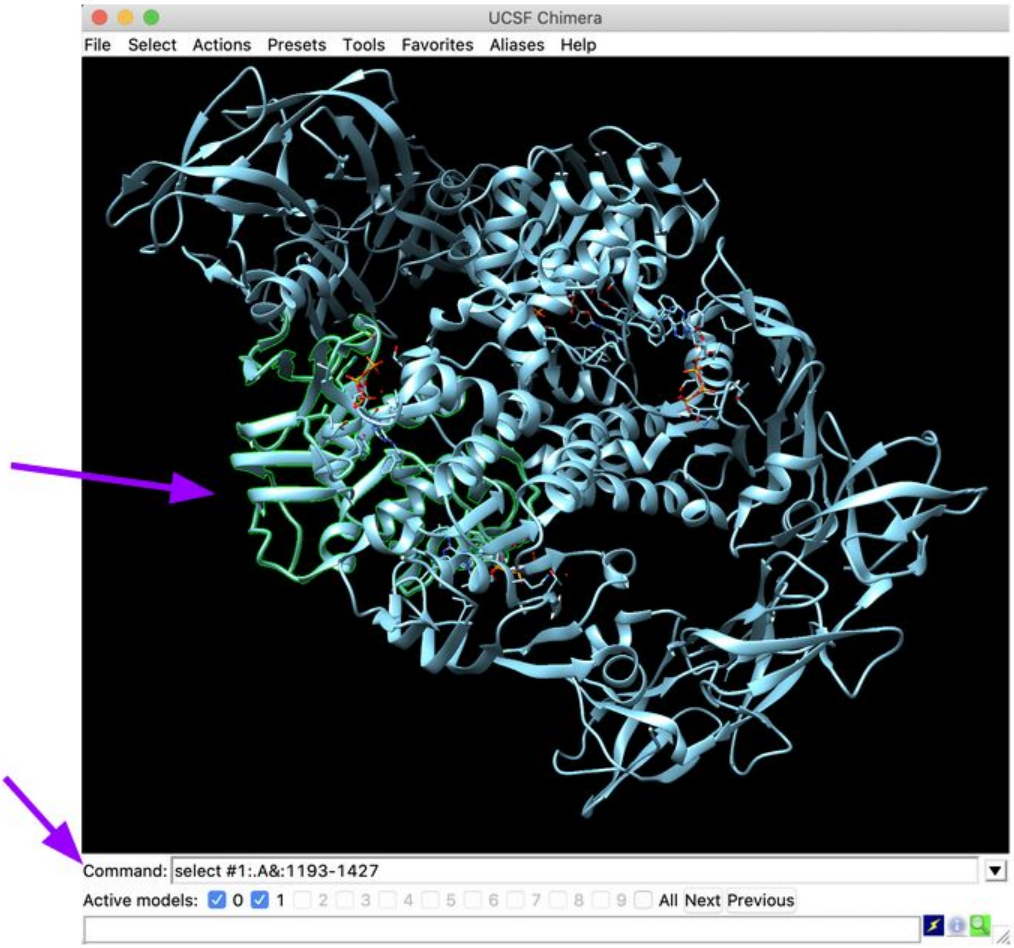
Also, let's open the Model Panel, this will help us keep track of the various objects we have open. Open it from Tools>General Controls>Model Panel. In the model panel you can easily see the ID for each model (here, 0 & 1) and options to **S**how and **A**ctivate each model individually.

2.2 For making selections in Chimera, the basic syntax is here:
https://www.cgl.ucsf.edu/chimera/docs/UsersGuide/midas/frameatom_spec.html

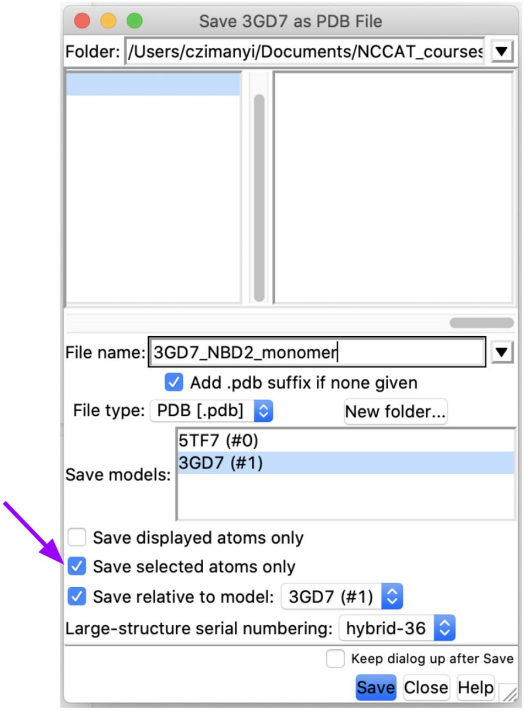
Most important for now, we can select a **#**(model), **:.**(ChainID), or **:**(residue number).

In this case to choose residues 1193-1427 of chain A in 3GD7 we type:
select #1:.A&:1193-1427
(make sure 3GD7 is model #1 in your session using the Model Panel. If it's not, choose the correct model number for your session).

Selected residues are highlighted with a green outline. We can then save just those residues to a new file.



Go to Actions>Write PDB. Make sure to save selected atoms only. Give it a useful name and save it in a useful place. Now we can close the original model and open our newly saved monomer. You'll notice we have lost the Mg-ATP that was in the original model because we didn't select it. No worries, we'll add it back later by hand in Coot.

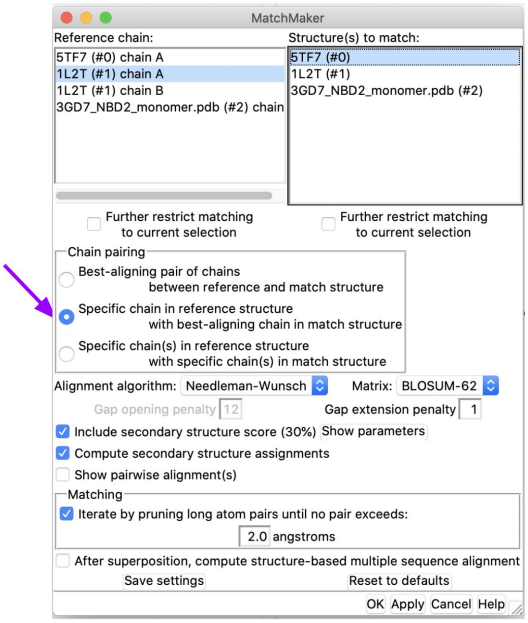


Chimera tip: For interactive selections you can also use: Control + left mouse to click on what you want to select or you can use the Select menu options.

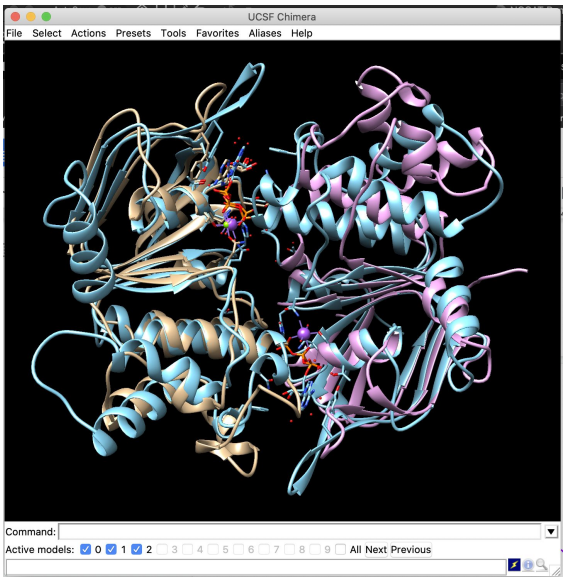
2.3 Our two NBDs are in random positions based on our starting models. It will be easier to fit them to a map as a dimer first, so lets orient them together by aligning to a known ATP bound dimer structure. Open 1L2T. This is a heterodimer with two chains, A & B. We will align NDB1 (5TF7) to chain A, and NBD2 (3GD7) to chain B.

We will use the MatchMaker in Chimera to do the alignment. Choose Tools>Structure Comparison>MatchMaker

Choose to pair a "specific chain in reference structure" so that you can choose chain A as the reference and choose 5TF7 as the structure to match. All other defaults should be fine. Then in a second run of MatchMaker, match 3GD7 monomer model to chain B of 1L2T.



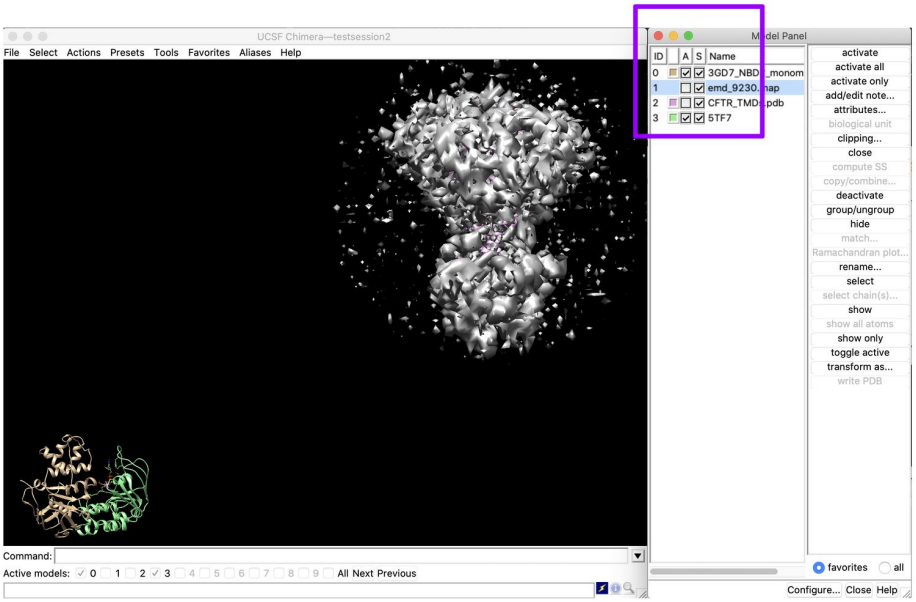
Now I have an approximate dimer overlaid on my template that looks like this:



We can close the template from the Model Panel, (we're done with it) and next we can fit this approximate dimer into a map. First let's save our progress. You can save a Chimera session from the File menu.

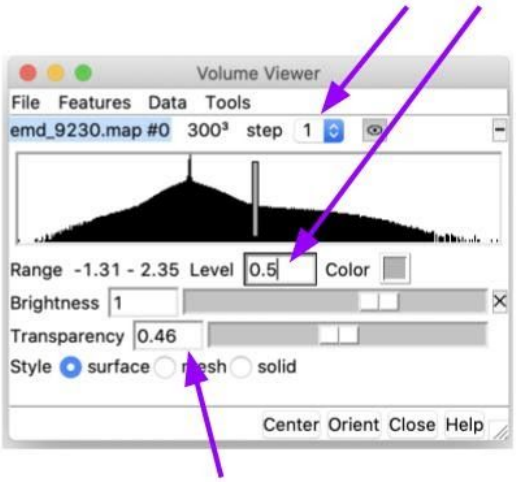
2.4 Open the map, either using the menu: File>Open or File>Fetch by ID and choose 9230 from the EMDB. Let's open the TMD model now as well (CFTR_TMDs.pdb). This should already be well aligned in the map.

You'll notice the map and TMD model is far away in real space from the NBD models, so let's move the NBDs towards the map with the help of the Model Panel.



Uncheck the A column for the map and we can now move the models using middle mouse (or option + left mouse) and drag them towards the map. You may need a few rounds of (de)activing the map to move in all three dimensions. Once you've got them in the right neighborhood let's make the map easier to use.

We can change the representation: Use the Volume viewer (Tools>Volume Data>Volume viewer) to change the contour to the **level** recommended by the depositors (here, 0.5). We can also use the Features>Brightness and **Transparency**: to adjust the transparency. Also, set the **Step** to 1.



This is a good place to start, but there are many other useful manipulations of volume representation that may be useful for you. See some here:

https://www.cgl.ucsf.edu/chimera/data/tutorials/maps08/volume-basics.html

Now we will fit the NBDs into the map. Chimera does a good job of calculating a fit of a model into a map, but it needs to be fairly well positioned to start, so we will put the NBDs into the map as best as we can by hand, by translating and rotating our approximate dimer. Once it's about right, we can fit each NBD on it's own because the dimer isn't exactly right.

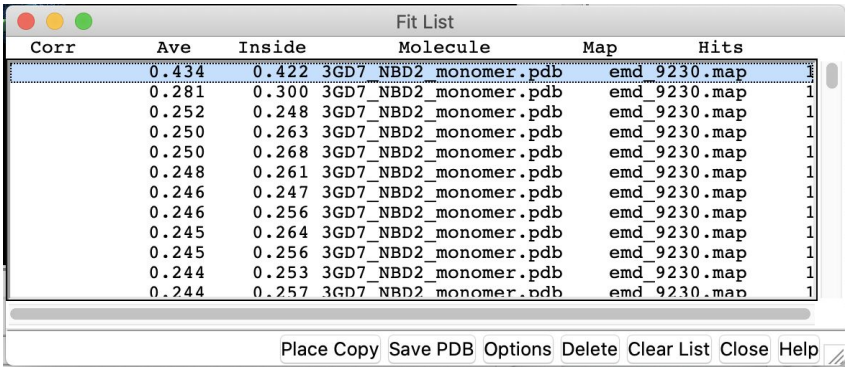
Once we're close we can use the fitmap command:

fitmap #model #map search 100 radius 5

For my session this looks like:

For NBD2: fitmap #0 #1 search 100 radius 5
For NBD1: fitmap #3 #1 search 100 radius 5

The fitting job will output a table of the 100 search results we asked for with statistics.



Choose the best fit from the list (and see how it looks). In this case the fit isn't amazing but it's close. We will need to make some adjustments that aren't rigid body movements of the full chain, but we'll do these in Coot.

Once you have both NBD's positioned, we want to save them so that they are in the right place relative to the map. Merging is easier in Coot than in Chimera so we'll take the three separate model files and open them in Coot.
(A quick note - to cheat and/or save time, you can always check your progress against the full published model: 6MSM.pdb).

Part 3 - Refine NBDs into map using Coot

If you're opening Coot from the command line (in Linux or MacOS) open it from the directory containing the files you want to work with (this isn't strictly necessary but makes life easier).

3.1 Open the three model files that were aligned to the map in Chimera (File>Open Coordinates).

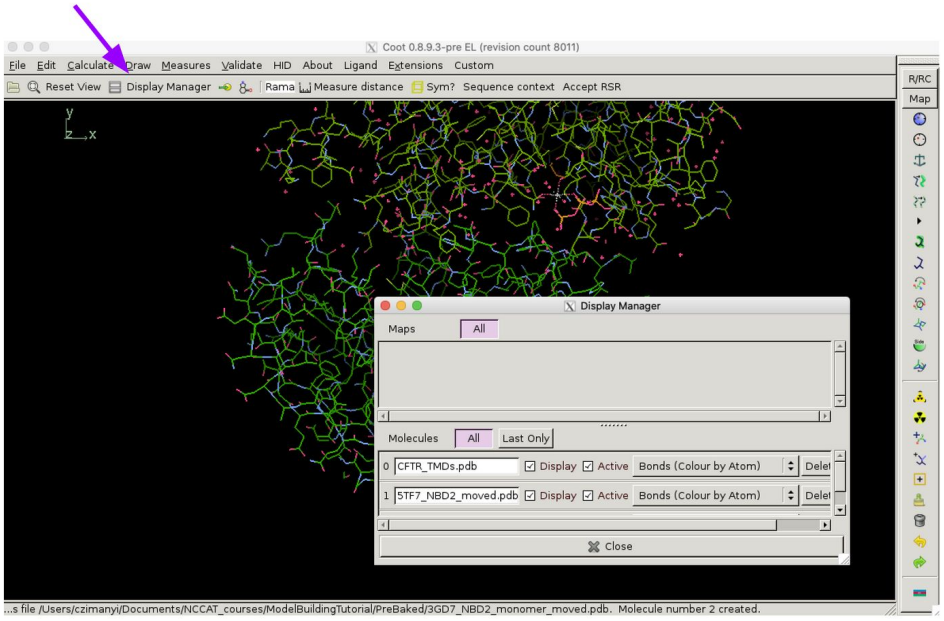
Here are some useful navigation tips for Coot:

Manipulate:

- Ctrl-click-drag (or middle mouse) to translate view
- Click-drag to rotate view
- Right-click drag (or right mouse) to zoom in/out
- Middle-click on atom to center
- Shift-click (or double click) to label atom/residue

View:

- Ctrl-right-click-drag up/down to translate slab in/out of plane
- Ctrl-right-click-drag left/right to change thickness of slab



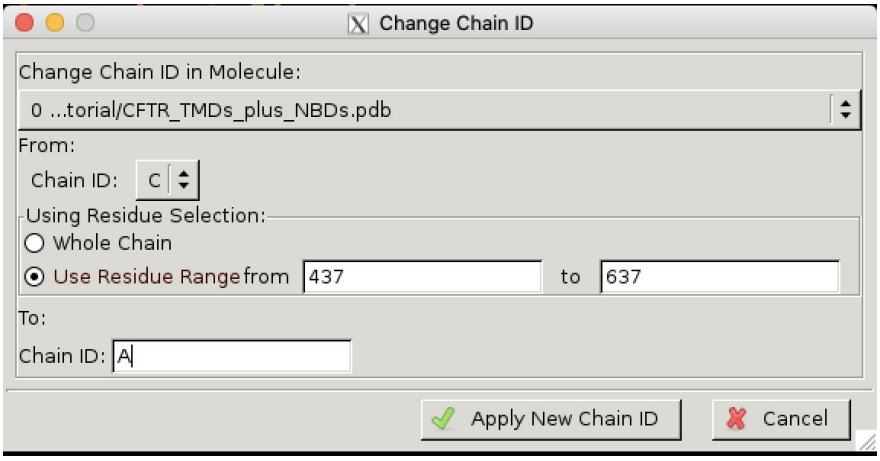
Similar to the model panel in Chimera, Coot has a Display Manager. You can find the Display Manager in Draw>Display Manager or the button on the top of the display.

3.2 Merge the models using Edit>Merge Molecules.

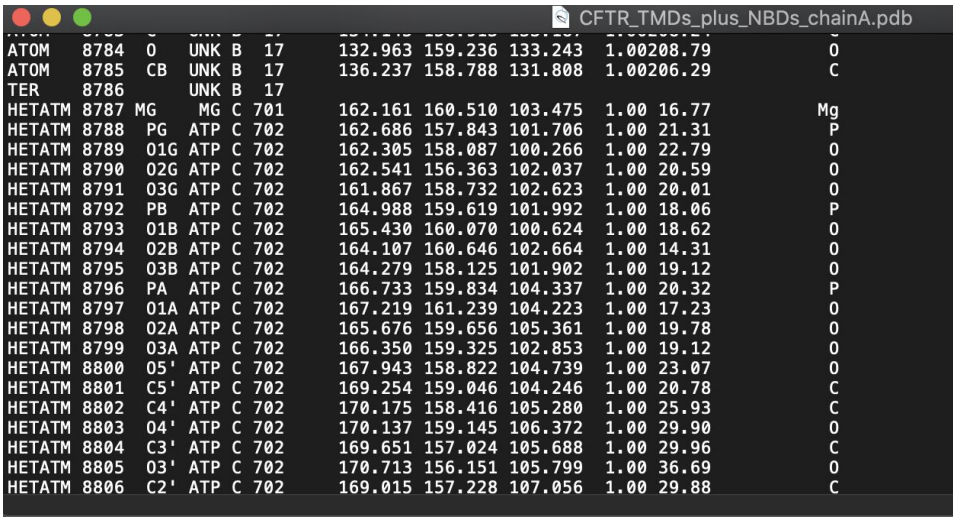
Choose all three and merge into any of the existing models. Save the file you merged with a new and meaningful name. Then we can delete the unmerged models.

You'll notice when you click on the atoms in the different domains that the merged chains were given unique ChainIDs. You can also visualize this using the sequence viewer in Draw>Sequence View.

3.3 We want everything to be part of Chain A so we will change the Chain IDs using Edit>Change Chain ID. Because we want to change Chains C & D to a name that already exists, we have to specify the exact range of residues. If you're forgotten those ranges, the sequence viewer can help with this. [NBD1 is 390-637 and NBD2 is 1202-1427].

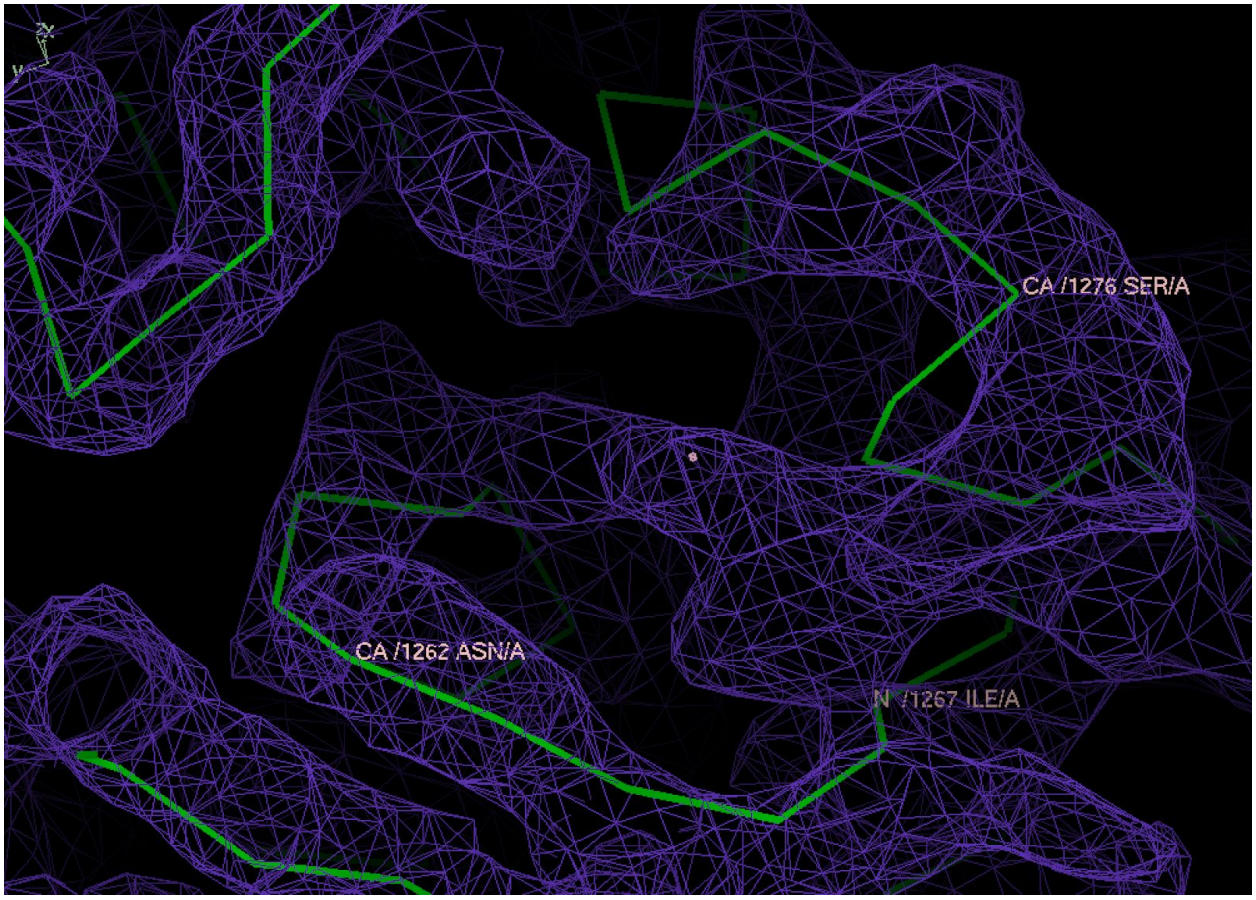


Close and reopen the sequence viewer to make sure this worked as intended. You'll notice chain C still exists with a number of heteroatoms. 701 & 702 are Mg-ATP. We want to keep this for now. Atoms 801-968 are waters. We don't want to include these in our model, so lets remove them. This time, let's do it manually in a text editor. Save your current file with a useful name (I called mine CFTR_TMDs_plus_NBDs_chainA.pdb). Then open the .pdb file with your favorite text editor. Waters have the three letter code HOH and are labeled as HETATMs. Find them, delete them and save the file with a new name and the extention .pdb (I called mine CFTR_TMDs_plus_NBDs_noHOH.pdb).



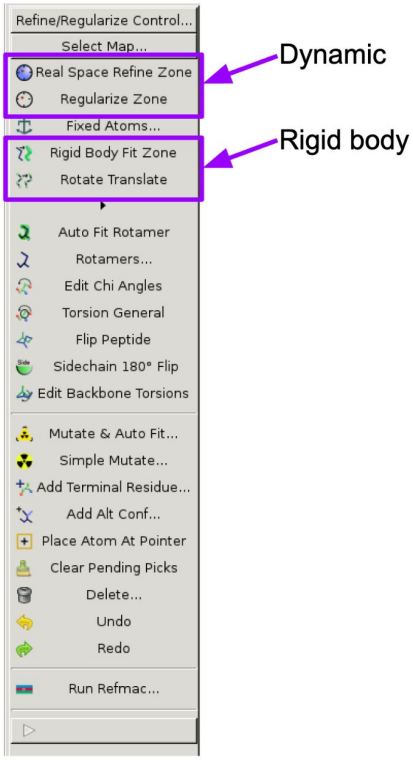
3.4 Open this file in Coot. If it looks good, we can delete the model with waters. Then open the map (I find this to be somewhat slow on my MacBook). You can change the map display using Edit>Map parameters and also the properties button in the Display Manager. Set the level to the suggested level of 0.5 in the properties window.

3.5 Now we can use the modeling tools. It can help to toggle between representation types in the Display Manager to get a sense of what will need work. For example I can see that the beta-strand containing Asn1262 is placed in the density fairly well, but Ser1276 needs to be repositioned substantially.

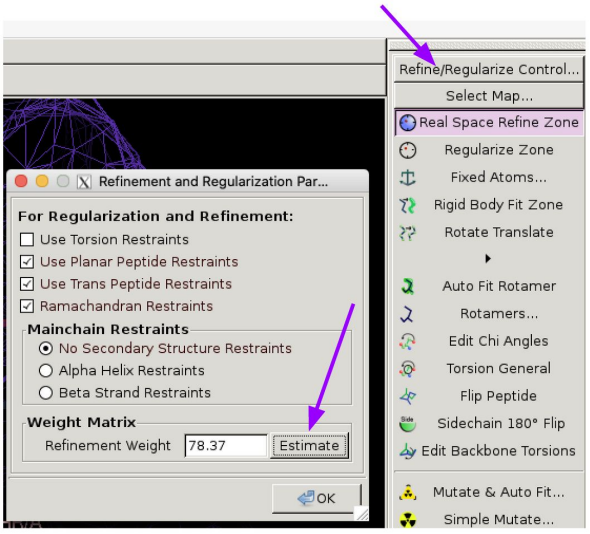


Your model may differ slightly based on your fit from Chimera, so I won't outline detailed steps, we will just work through our own models using the different refinement modes as needed.

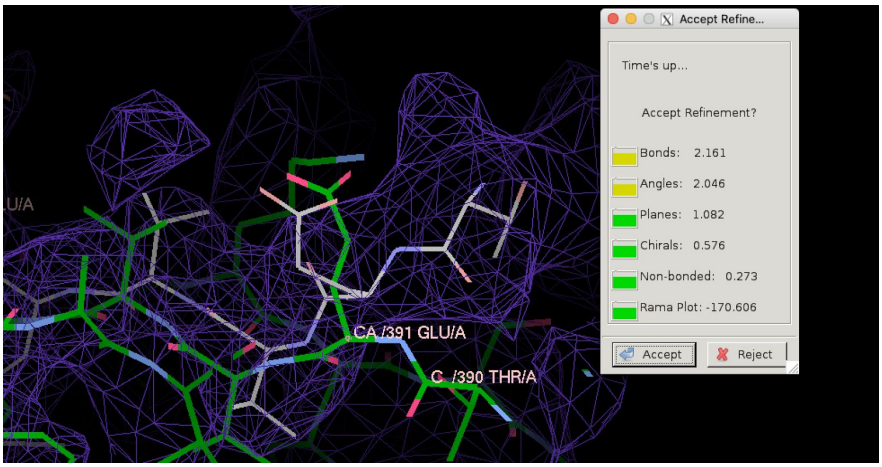
Different refinement modes are useful for different tasks.The two major modes are rigid body and dynamic refinement.



For dynamic refinement guided by map restraints (Real Space Refine Zone), we want to set the refinement weight using the Refine/Regularize control parameters. Click “Estimate” to give a good starting refinement weight. This usually works well.



I suggest using Real Space Refine Zone with larger selections of atoms initially for things that are close but not exactly in the density. This will be a little slow but works. Then you can use shorter (1-4 residue) selections to spot clean. Dynamic refinement will be guided by color coded validation parameters. It isn’t vital for these to be perfect at every step, especially at early stages of building but they are a helpful guide.



For bigger issues and pieces far from fitting into the density, the rigid body options will be more useful. You can translate and rotate any selected range of atoms. In some cases where there is a large rearrangement that can't be adjusted with rigid body motion, it can be useful to just delete residues and build them back with the Add Terminal Residue button.

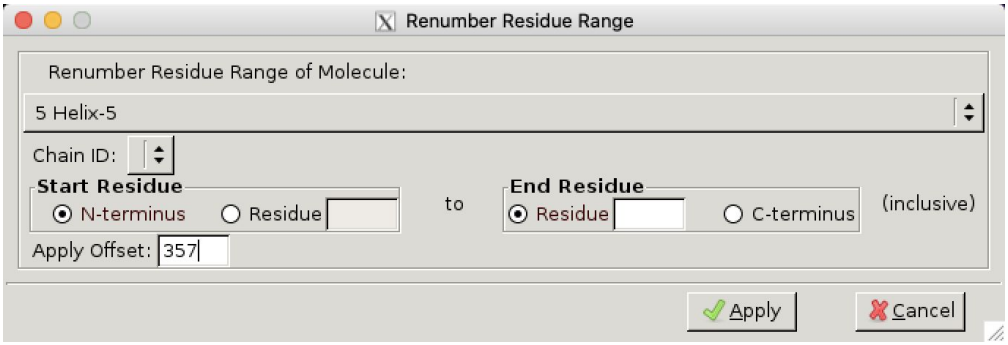
Spend some time playing with the refinement modes to get used to them.

Note - Coot does not autosave work, so save your progress often!

Part 4 - Build missing pieces (protein & ligands) using Coot

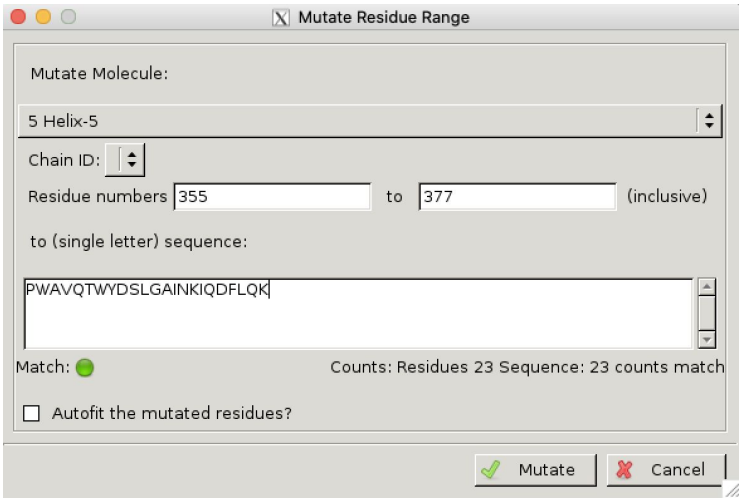
Not all starting models will have all of the residues you need. Here we are missing two long helices in the TMD.

4.1 Find the density with the missing helices by navigating around the map (and if you can't find them, they are adjacent to residues 354 and 1144). Find the unmodelled density following residue 354. Navigate so that the center pointer is near the middle of the missing helix. Choose Calculate>Other Modelling Tools from the menu and click on "Place Helix Here". Coot will auto generate a helix into the density. This is a new molecule (which you can see if you look in the Display Manager). We can attach it properly by deleting any overlapping residues, and changing the residue numbering (Edit>Renumber residues). We know the first new residue should be 355 so apply the appropriate offset.



You can then merge the helix into the same molecule and change chain ID's as you did in the earlier steps to combine this into a single model file. Now if you Real Space Refine across the junction, it should automatically form a bond.

4.2 Coot made a poly-alanine helix so now we need to change the amino acids to have the correct side chains. For this we need to know our sequence. You can find it in the hCFTRsequence.fasta file. [As a cheat sheet, the missing residues are highlighted in the sequence below]. You can change them one at a time using the mutate buttons in the menu, or use the Calculate>Mutate Residue Range and paste the missing range.



Once you're satisfied with this helix, you can try again by building, merging, attaching and mutating a helix extending from residue 1144.

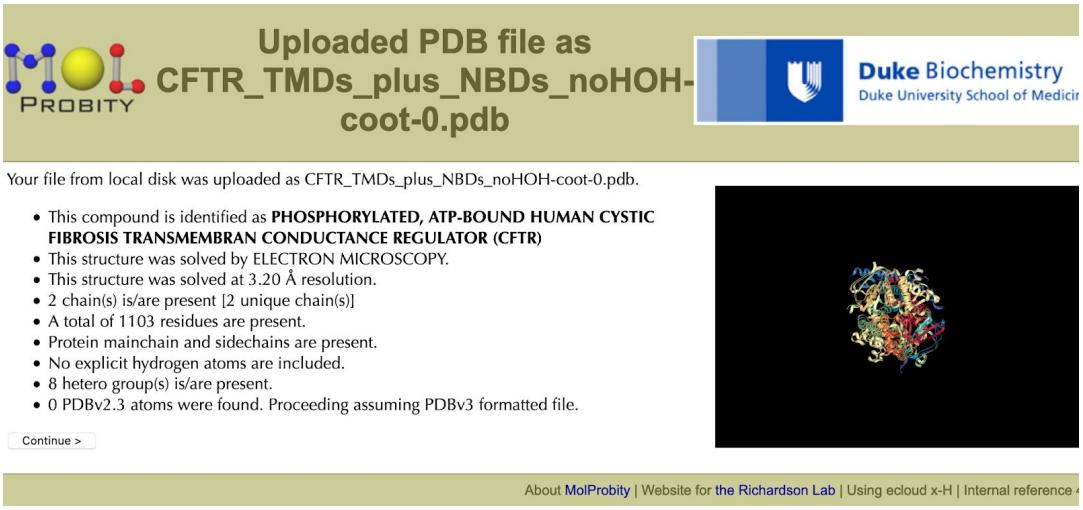
4.3 Our final step is to add back the ATP molecule we lost from NBD2. You should find empty density for it near Tyr1219 (it may be difficult to recognize - you can use the initial model 3GD7 or the final published model 6MSM if you need help seeing it). You can add common ligands in Coot using File>Get Monomer. Coot uses the Refmac monomer library that contains common ligands (http://www.ccp4.ac.uk/html/refmac5/dictionary/list-of-ligands.html).

Because ATP is common, you can just type in the three letter code; ATP. It will be placed at the cursor center and then you can refine it into the density just as you did for protein atoms. It will have hydrogen atoms, but you can delete these easily using the delete button. By default, ligands added this way are placed into a separate model, so you'll have to merge it with the protein if you want to have them in the same file (but by now, you're a pro at this!).

Part 5 - Validation

How do I know when my model is done? I should satisfy some cutoff of validation metrics and decide that I have done my best, for myself and future users of my model. Validation encompasses many metrics. Two major aspects are model quality & fit to map. We will only look at model quality for now.

Ideally, you would visually check your model residue by residue. There are some quick ways to check for big issues within Coot first, using the Validate menu. (Ramachandran, Geometry analysis, rotamer analysis are useful). Once you're done in Coot you can use validation servers to check your work. Molprobity is great one: http://molprobity.biochem.duke.edu/



MOLPROBITY

Uploaded PDB file as CFTR_TMDs_plus_NBDs_noHOH-coot-0.pdb

Duke Biochemistry
Duke University School of Medicir

Your file from local disk was uploaded as CFTR_TMDs_plus_NBDs_noHOH-coot-0.pdb.

- This compound is identified as **PHOSPHORYLATED, ATP-BOUND HUMAN CYSTIC FIBROSIS TRANSMEMBRAN CONDUCTANCE REGULATOR (CFTR)**
- This structure was solved by ELECTRON MICROSCOPY.
- This structure was solved at 3.20 Å resolution.
- 2 chain(s) is/are present [2 unique chain(s)]
- A total of 1103 residues are present.
- Protein mainchain and sidechains are present.
- No explicit hydrogen atoms are included.
- 8 hetero group(s) is/are present.
- 0 PDBv2.3 atoms were found. Proceeding assuming PDBv3 formatted file.

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MOLPROBITY

Viewing CFTR_TMDs_plus_NBDs_noHOH-coot-0FH-multi.table

Duke Biochemistry
Duke University School of Medicine

When finished, you should close this window .

Hint: Use File | Save As... to save a copy of this page.

All-Atom Contacts	Clashscore, all atoms:	19.54		95th percentile* (N=41, 2.95Å - 9999Å)
	Clashscore is the number of serious steric overlaps (> 0.4 Å) per 1000 atoms.			
Protein Geometry	Poor rotamers	32	3.43%	Goal: <0.3%
	Favored rotamers	847	90.78%	Goal: >98%
	Ramachandran outliers	0	0.00%	Goal: <0.05%
	Ramachandran favored	1037	96.55%	Goal: >98%
	MolProbity score^	2.42		97th percentile* (N=1544, 3.20Å ± 0.25Å)
	Cβ deviations >0.25Å	19	1.84%	Goal: 0
	Bad bonds:	42 / 8788	0.48%	Goal: 0%
	Bad angles:	54 / 11886	0.45%	Goal: <0.1%
Peptide Omegas	Cis Prolines:	2 / 28	7.14%	Expected: ≤1 per chain, or ≤5%
Low-resolution Criteria	CaBLAM outliers	4	1.8%	Goal: <1.0%
	CA Geometry outliers	0	0.00%	Goal: <0.5%
Additional validations	Tetrahedral geometry outliers	2		
	Waters with clashes	0/0	0.00%	See UnDowser table for details

In the two column results, the left column gives the raw count, right column gives the percentage.
* 100th percentile is the best among structures of comparable resolution; 0th percentile is the worst. For clashscore the comparative set of structures was selected in 2004, for MolProbity score in 2006.
^ MolProbity score combines the clashscore, rotamer, and Ramachandran evaluations into a single score, normalized to be on the same scale as X-ray resolution.
Key to table colors and cutoffs here:

Use the feedback from Molprobity to go back and fix up your model in Coot.

Homework:

Finish refinement of the NBDs into the map, adding as many of the unmodeled residues as you can. Finish rebuilding the TMD helices and connect them to the NBDs if possible. Use validation to judge your progress. When you're "done" report the following (MolProbity will provide most of these):

1) How many residues are modelled (out of how many total):
2) What percent of residues are Ramachandran favored/Ramachandran outliers:
3) What percent of residues have favored rotomers/poor rotomers:
4) What is your clash score:

Advanced resources:

This was just a taste of the basics of model building. There are many other tools at your disposal (this list is not exhaustive):

Making 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

Automated building:
- Buccaneer (part of CCP4): http://www.ccp4.ac.uk/html/cbuccaneer.html
- ArpWarp (part of CCP4 - stated to work for high resolution maps): https://arpwarp.embl-hamburg.de/
- Phenix map_to_model: https://www.phenix-online.org/documentation/reference/map_to_model.html

Refinement
- Isolde: https://isolde.cimr.cam.ac.uk/
- Rosetta: https://www.rosettacommons.org/software (also available in Phenix)
- Phenix refine: https://www.phenix-online.org/documentation/reference/refine_gui.html

Chimera/Coot advanced use:
- See the new version of Coot (optimized for lower resolution maps) in action: https://www.youtube.com/watch?v=hhB8qUBBnJQ
- Oli Clarkes customization scripts: https://github.com/olibclarke?tab=repositories

Appendix

Human CFTR amino acid sequence. Residues missing in our initial model are highlighted in yellow.

MQRSPLEKASVVSKLFFSWTRPILRKGYRQRLELSDIYQIPSVDSADNLSEKLEREWDRELASK
KNPKLINALRRCFFWRFMFYGIFLYLGEVTKAVQPLLLGRIIASYDPDNKEERSIAIYLGIGLCLLFI
VRTLLLHPAIFGLHHIGMQMRIAMFSLIYKKTLKLSSRVLDKISIGQLVSLLSNNLNKFDEGLALAH
FVWIAPLQVALLMGLIWELLQASAFCGLGFLIVLALFQAGLGRMMMKYRDQRAGKISERLVITSE
MIENIQSVKAYCWEEAMEKMIENLRQTELKLTRKAAYVRYFNSSAFFFSGFFVVFLSVLPYALIK
GIILRKIFTTISFCIVLRMAVTRQFPWAVQTWYDSLGAINKIQDFLQKQEYKTLEYNLTTTEVVME
NVTAFWEEGFGELFEKAKQNNNNRKTSNGDDSLFFSNFSLLGTPVLKDINFKIERGQLLAVAGS
TGAGKTSLLMVIMGELEPSEGKIKHSGRISFCSQFSWIMPGTIKENIIFGVSYDEYRYRSVIKACQ
LEEDISKFAEKDNIVLGEGGITLSGGQRARISLARAVYKDADLYLLDSPFGYLDVLTEKEIFESCV
CKLMANKTRILVTSKMEHLKKADKILILHEGSSYFYGTFSELQNLQPDFSSKLMGCDSFDQFSA
ERRNSILTETLHRFSLEGDAPVSWTETKKQSFKQTGEFGEKRKNSILNPINSIRKFSIVQKTPLQ
MNGIEEDSDEPLERRLSLVPDSEQGEAILPRISVISTGPTLQARRRQSVLNLMTHSVNQGQNIH
RKTTASTRKVSLAPQANLTELDIYSRRLSQETGLEISEEINEEDLKECFFDDMESIPAVTTWNTYL
RYITVHKSLIFVLIWCLVIFLAEVAASLVVLWLLGNTPLQDKGNSTHSRNNSYAVIITSTSSYYVFY
IYVGVADTLLAMGFFRGLPLVHTLITVSKILHHKMLHSVLQAPMSTLNTLKAGGILNRFSKDIAILD
DLLPLTIFDFIQLLLIVIGAIAVVAVLQPYIFVATVPVIVAFIMLRAYFLQTSQQLKQLESEGRSPIFT
HLVTSLKGLWTLRAFGRQPYFETLFHKALNLHTANWFLYLSTLRWFQMRIEMIFVIFFIAVTFISIL
TTGEGEGRVGIILTLAMNIMSTLQWAVNSSIDVDSLMRSVSRVFKFIDMPTEGKPTKSTKPYKN
GQLSKVMIIENSHVKKDDIWPSGGQMTVKDLTAKYTEGGNAILENISFSISPGQRVGLLGRTGS
GKSTLLSAFLRLLNTEGEIQIDGVSWDSITLQQWRKAFGVIPQKVFIFSGTFRKNLDPYEQWSD
QEIWKVADEVGLRSVIEQFPGKLDFVLVDGGCVLSHGHKQLMCLARSVLSKAKILLLDEPSAHL
DPVTYQIIRRTLKQAFADCTVILCEHRIEAMLECQQFLVIEENKVRQYDSIQKLLNERSLFRQAIS
PSDRVKLFPHRNSSKCKSKPQIAALKEETEEEVQDTRL