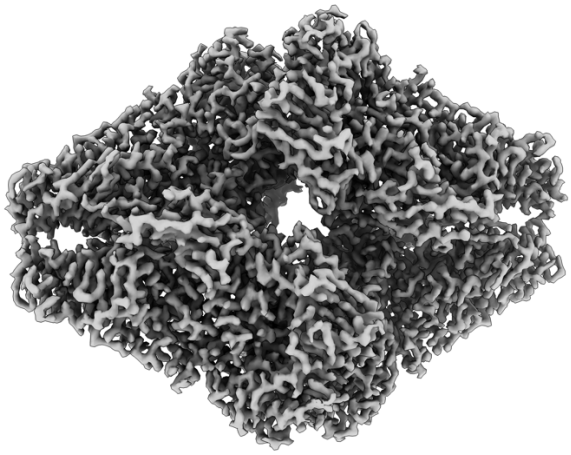
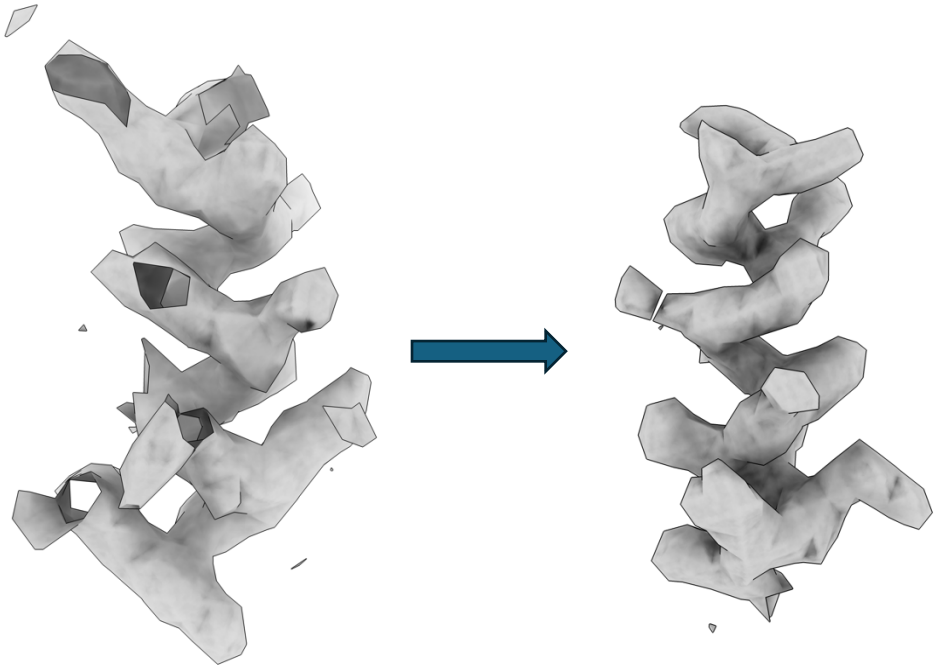
Cryo-EM Data Processing of β-Galactosidase in cryoSPARC 4.4 Tutorial Part II

1- Download the sharp map of b-gal you got during the 3D refinement of cryosparc and open it with chimeraX.
2- Create a working directory somewhere where you will put the map and models.

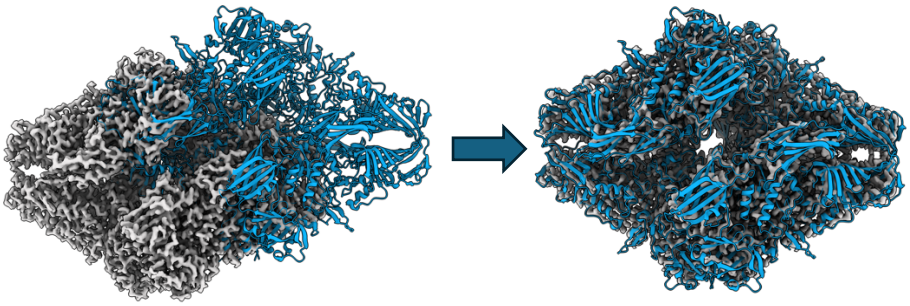


The first step is to check the quality of the map and its orientation. If the map does not appear satisfactory at first glance, there is no need to proceed further; you should continue with the data processing. If you observe secondary features, it's a positive sign and aligns with what we are seeking. Some of you may have an incorrectly oriented map (CryoSPARC does not consider the left-hand orientation as incorrect). If this occurs, you will need to manually flip the orientation.

The best way to determine this is by identifying a well-resolved alpha-helix: if you find one, use the following command in the script tab to flip the orientation: vop flip #your_map_ID

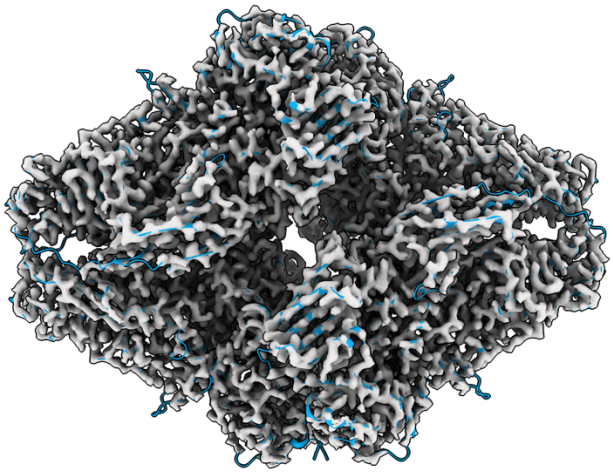


3- Download from the PDB a structure of b-gal (coming from ecoli | **P00722**), I used 8BKG and open the model with chimeraX. The first step is to align the PDB (manually, and roughly) the model with the map.



4- Once you reach this, you can tell chimeraX to perform a map fitting using the command:
fitmap #model_ID inMap #map_ID

It comprises a rigid body optimized for aligning an atomic model with a map. You may need to adjust and realign several times to achieve the perfect fit. While there are more advanced software options for this step, the visual method is typically preferred for its speed and ease of identifying any issues.

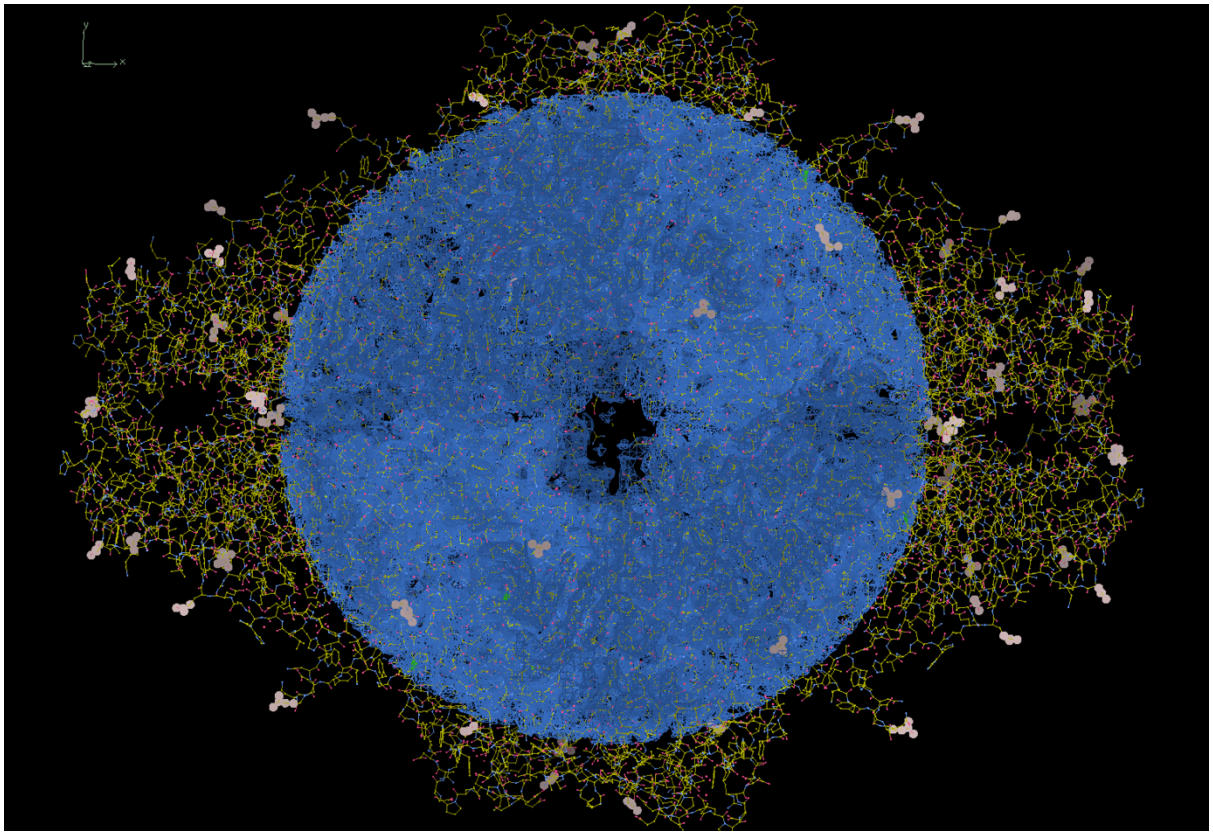


5- You know must save the model (which contains new coordinates after fitting into the map), and the flipped map you generated (if you have one). Using the command:
save /Users/your_choosen_path//fitted-bgal.pdb #model_of_bgal
save /Users/your_choosen_path/z-flip_map.mrc #map_of_bgal

Please pay attention to the extension file: .pdb for the model and .mrc for the EM map.

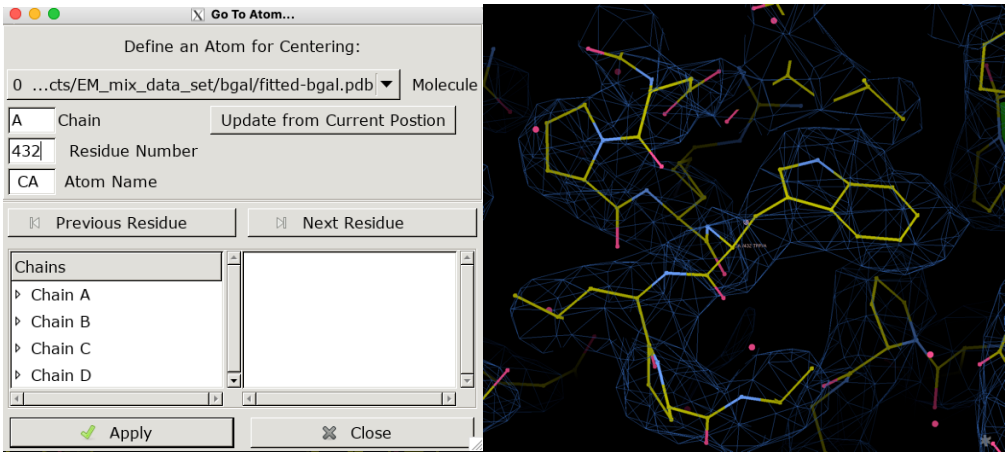
6- We can look a bit closer into the map and the quality of fitting. Of course that will depend on your final map quality. For this, we will use Coot (same as what you did during the X-ray training).

Open coot
Select the model go to File > open coordinates > fitted-bgal.pdb
Select the map go to File > open map > z-flip_map.mrc



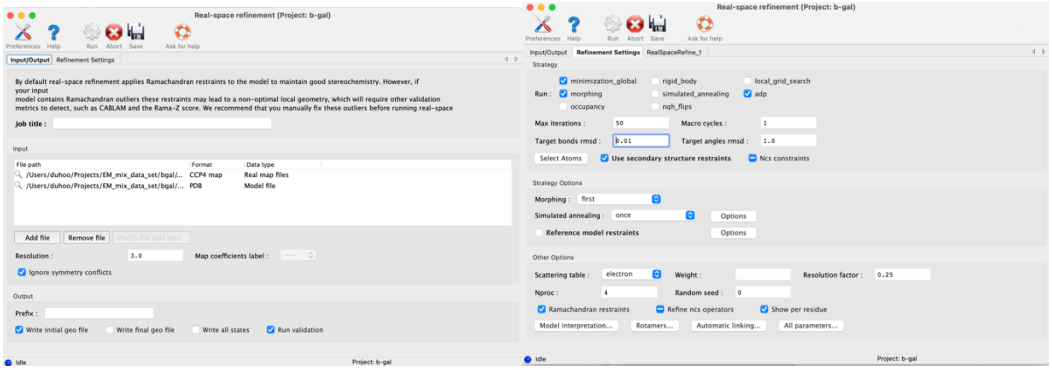
The cryoEM map is usually very large, and most laptops cannot handle the cache demanding to show everything. To avoid slow motion and crash you can quickly go to Edit > Map parameters > Map Radius EM: 15A and press Apply.

Let's have a look around a nicely resolved (in most models) alpha-helix: 432-447. Go to atom a seek for this one and have a look around. Usually in cryoEM visual inspection (already from ChimeraX) is important.



If your final resolution is good enough, you should be able to see the TRP432 fitted into the cryoEM density. You can play with the level to see more or less the contrast.
If the fitting is not correct, you might want to go back to the previous steps and redo it again.

7- Once you are happy with the fitting, we will perform a Real Space Refinement of the model into the cryoEM map.
For this, you will open Phenix, create a new project (where you saved your previous data) and select the job real-space refinement.
Select the EM_map and the PDB model.
Select the resolution you reached (and ignore symmetry conflicts).
Go to the Refinement Settings tab and select the same settings than the images.
We do not go into much detail here for a matter of time.
Press Run



8- Once you are done with the RSR, you can look at the validation report and start fixing the problem until your model matches the obtained data. We will not do this today.