How to fit molecular models in EM maps

This practical aims at showing how to dock (rigid body fit) an atomic model into a low-resolution electron microscopy single-particle analysis map using UCSF Chimera 1.12 (https://www.cgl.ucsf.edu/chimera/).

Data

1) Low-pass filtered map of the EMD-5595 beta-galactosidase cryoEM structure: **betagal-30A.mrc** (Bartesaghi *et al*, Proc. Nat. Acad. Sci. USA (2014), 111:11709-11714)
2) Beta-galactosidase monomer from the crystallographic structure PDB 1dp0 : **monomer.pdb** (Juers *et al*, Protein Science (2000), 9:1685-1699)

Information

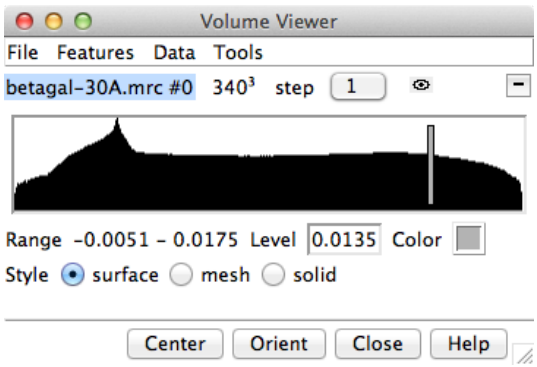
(Biological information, used as constraints, is always helpful to produce a meaningful fitting)

Beta-galactosidase is a 464 kDa homotetramer. Each monomer consists of five domains : domain 1 is a jelly-roll barrel, domain 2 and 4 are fibronectin type III-like barrels, domain 5 is a β-sandwich, and the central domain 3 is a TIM-type barrel. The active site is made up of elements from the third domain of two monomers in the tetramer.

1. Open map

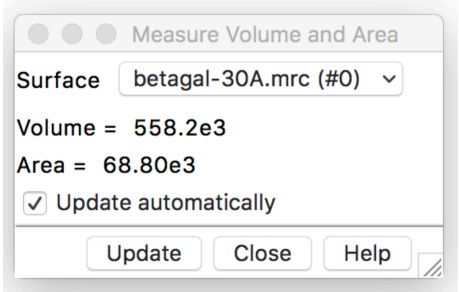
- Use Menu entry *File/Open* and choose betagal-30A.mrc.

- Open the Volume dialog (*Tools/Volume data/Volume viewer*). This gives you map value histogram, contour level, map value range, color, grid size, step size (show effect with mesh).



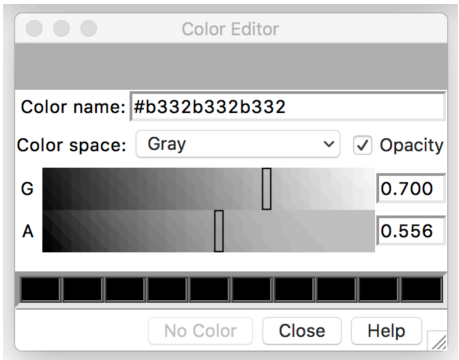
Adjust map surface threshold in volume dialog so that enclosed volume is 5.6 x 10^5 Å^3 i.e the expected volume for the 464 kDa tetramer with 1.21 Å^3 / Da.

- Use *Tools/Measure Volume* and Area and Volume Viewer panel (move vertical bar on histogram).



- Make surface transparent: Press volume viewer panel color button, click opacity and change the value.

- Change background color (*Presets/publication 1*)
- Mouse buttons functions : rotation → left button
  translation → middle button.
  zoom → right button / scroll-wheel
- Show command-line (*Favorites/Command Line*)
- Show model panel (*Favorites/Model Panel*)
- Show side-view (*Favorites/Side View*)



- Inspect the map (symmetry, domains).

- Determine map symmetry using the Command "measure sym #0"

2. Open atomic structure

- Use Menu entry *File/Open* and choose mono.pdb.
Look at the side view panel and zoom out to see that the monomer is far away from the map.

- Center the molecule (left button click on the molecule and drag) and color rainbow (*Tools/Depiction/Rainbow*).
Inspect the molecule (domains, shape).

3. Hand align molecule with map

- Freeze map motion : uncheck the active model 0 button below command-line or in model panel.

- Drag the molecule into the map with mouse.

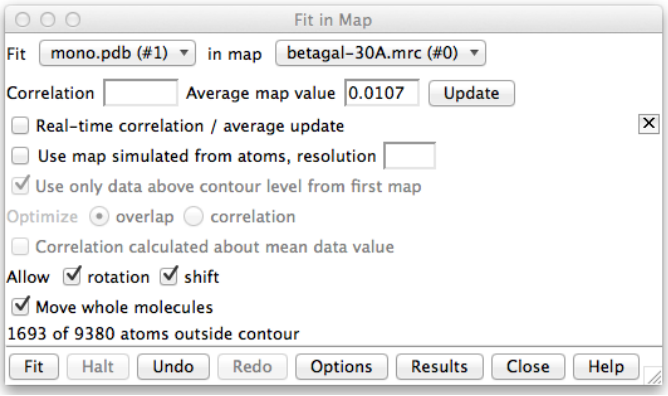
- Check active 0 button (unfreeze map) and rotate to inspect superposition.

- Use repeatedly the active 0 button to hand align molecule in map. Look for similar features in molecule and map to guide docking.

4. Optimize local fit

- Open the *Tools/Volume Data/Fit in map* dialog and Press Fit button.

This fit optimization rotates and shifts atomic model to maximize the average map value at the atom positions.
Be aware that average map values and numbers of atoms outside contour are not useful parameters to report in a paper. They are mainly helpers for the fitting procedure, and only relative values are meaningful.



Press Undo/Redo buttons to see how much it moved. Note average map values and numbers of atoms outside contour before and after optimization.

Press Fit several times. Note slight changes.

Change contour level, press Update in the Fit dialog and look at the number of atoms outside new contour.

- Set contour to the initial value, make surface opaque and zoom in to see where atoms stick out. Mouse over to see their name. Some of them are water and small molecules. Delete these molecules: *Select/Residue/HOH* and *Actions/Atoms/Delete*.
Press fit and note changes.

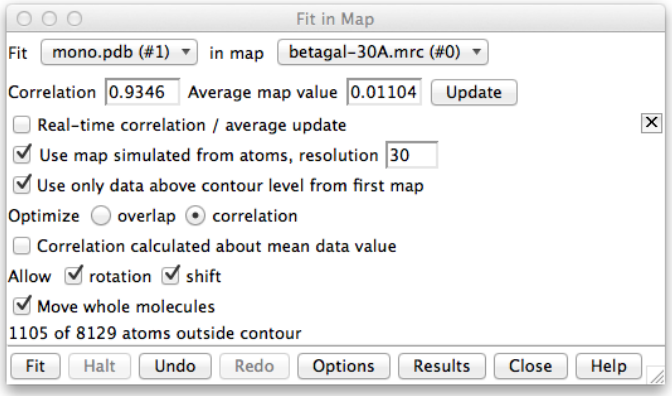
5. Measure correlation coefficient

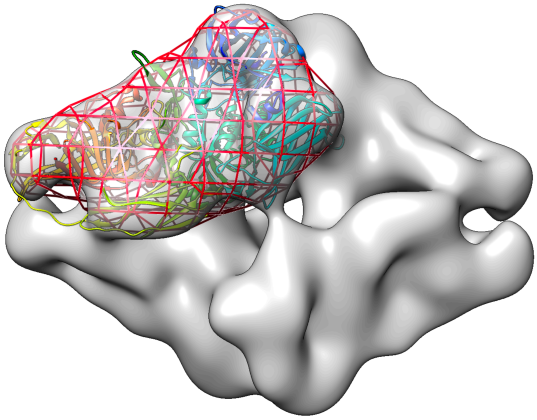
Most commonly reported measure of fit quality is correlation coefficient. Correlation values compare two maps, the experimental map and a simulated map, and range from -1 to 1 (0 indicates no correspondence and 1 identical maps). Correlation coefficient depends on domain - volume within the lowest contour level of simulated map) and hence is an ambiguous value that is not meaningful in itself. Comparison of correlation coefficients is the best way to use them.

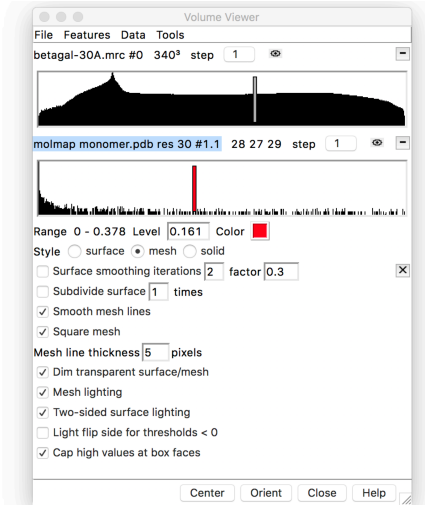
- In the Fit in Map dialog, check Use map simulated from atoms and set resolution to 30 Å. Set optimization mode to correlation and click Fit.

- Make betagal-30A.mrc map surface transparent and show simulated map as mesh (Volume dialog eye icon).

- Change simulated map threshold and press Update in Fit dialog to see how correlation varies with domain of calculation.



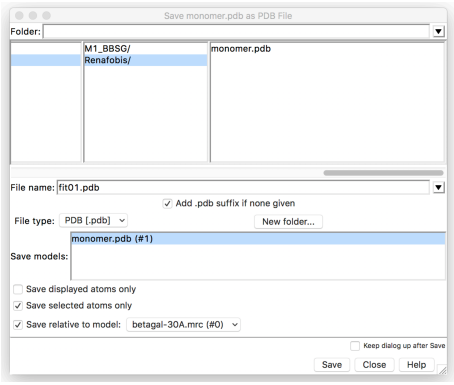




- Save this molecule position relative to the map : in Model panel select mono.pdb and click on Write PDB, check save relative to model betagal-30A.mrc and save as fit01.pdb.

- Search for alternative fits (30 trials) with the command "fit #1 #0 search 30" and inspect them.

- Close all pdb files and open your fit01.pdb molecule.

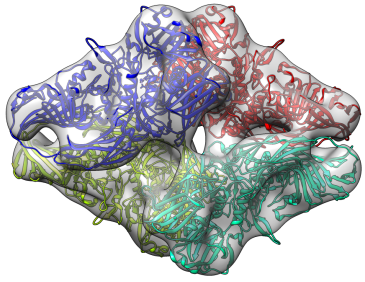


6. Make tetramer model

- View symmetry regions with command "sym #2 group #0 surf true".

- Show symmetric molecule copies for fit01.pdb. Command "sym #2 group #0 update true".

- Color molecules distinctly. Select the four chains and in *Tools/Depiction/Rainbow* check Model.

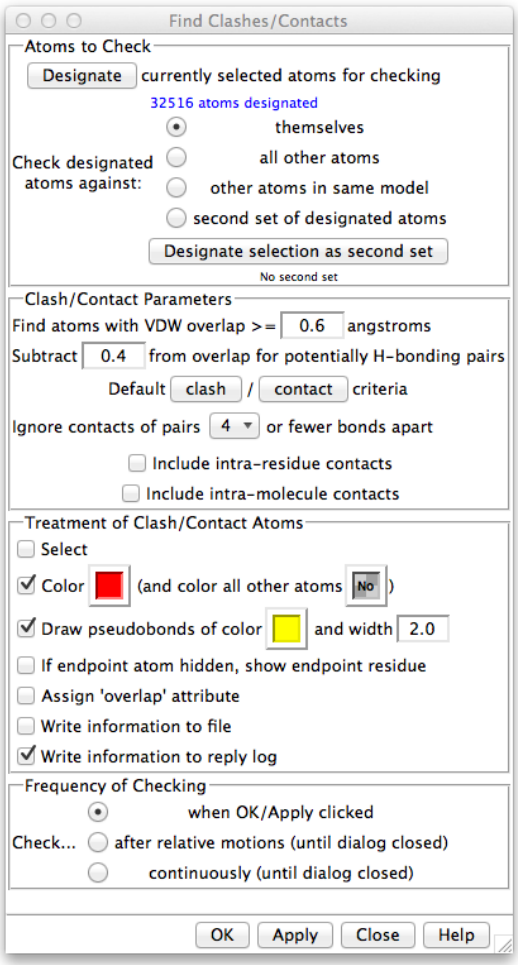


- Inspect clashes between molecules. Hide the map and select all chains by pressing ctrl and drawing a square around the molecules.

Open *Tools/Structure analysis/Find clashes/contacts* and fill in the dialog as shown here.
Click Apply. Note number of clashes.

- Fit asymmetric unit and all symmetric molecules with the command "fit #2 #0 sym true res 30".

- Re-inspect clashes.



7. Save session & model

- Use *File/Save session as* to save you fitting session.

- Select the four molecules on the display and in the Model panel dialog, click “Write pdb” and save in a single file relative to the map.