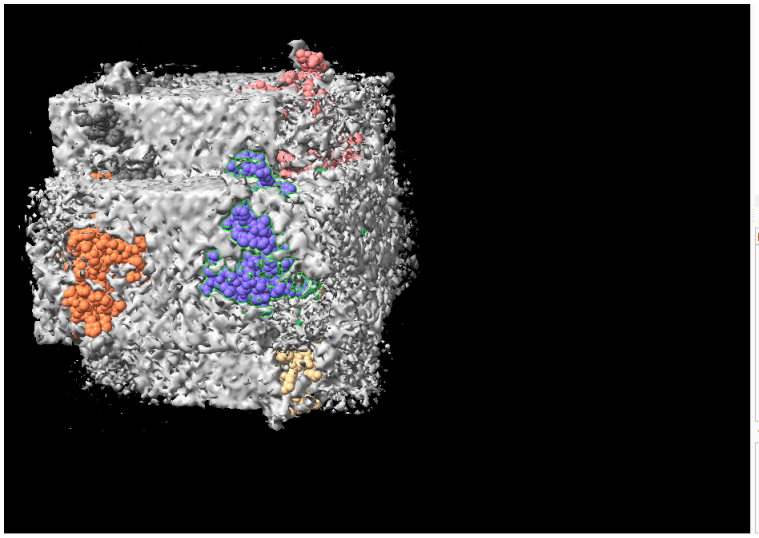
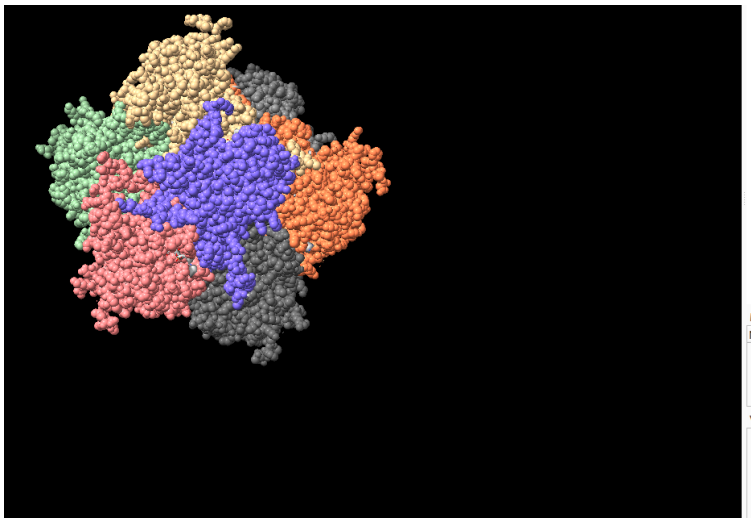
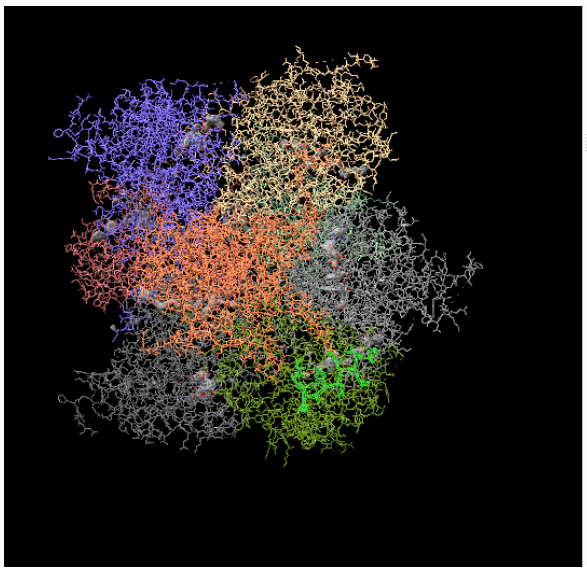
Tutorial

Blob validate

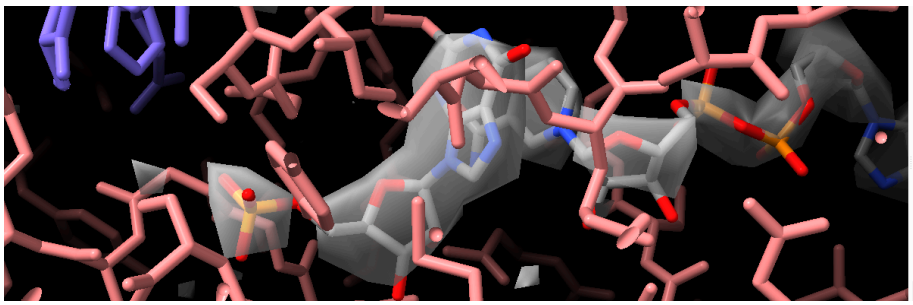
1. Open files
    a. Open .cif and .ccp4 files corresponding to the map and protein.
    b. Run: **open 8FUZ/8FUZ.cif 8FUZ/map_model_difference_1.ccp4**
    c. You will get the corresponding outlook by running the `info` command
    d. 

2. Adjust the view
    a. Run **blob autothreshold** to threshold the density map.It will yield the corresponding view.
    b. 

    c. If you want your view to be better, you can also run **blob autothreshold withstyle True**, which will change display options for atoms and density map as well, making it more readable. The corresponding output looks as follows:

d. 

e. Then, if you want to see what the fuss with this bundle is about, you can run **view /B:601** and see what part of the density map is about to be classified.

f. 

3. Run **blob validate** on the selected ligand:
   a. **blob validate /B:601**
   b. The corresponding output will look as follows:

blob validate /B:601

Attempting to cut ligand (this may take a while)...
23 atoms, 25 bonds, 1 residue, 1 model selected

Ligand Class Predictions
(Click on ligand group name to see the full list of ligands)

Ligand Class	Probability
AMP-like	0.999
ADP-like	0.000
RARE_LIGAND	0.000
5GP-like	0.000
ATP-like	0.000
GDP-like	0.000
AGS	0.000
HEA	0.000
FMN-like	0.000
A3P	0.000

c.

d. You can also manually pass map_id, pdb_id and whether the density map comes from xray crystallography.

e. **blob validate /B:601 #2 #1**

info

4 models
#1, 8FUZ.cif, shown
24008 atoms, 24432 bonds, 3136 residues, 8 chains (A,B,C,D,E,F,G,H)
16 missing structure
#1.1, missing structure, shown, 16 pseudobonds
#2, map_model_difference_1.ccp4, shown size 400,400,400, step 2, voxel size 0.843, level 2.232, value range -50.167 - 83.269, value type float32, 0 symmetry operators
#2.1, surface, shown, 475252 triangles

blob validate /B:601 #2 #1

Attempting to cut ligand (this may take a while)...
23 atoms, 25 bonds, 1 residue, 1 model selected
Ligand Class Predictions
(Click on ligand group name to see the full list of ligands)

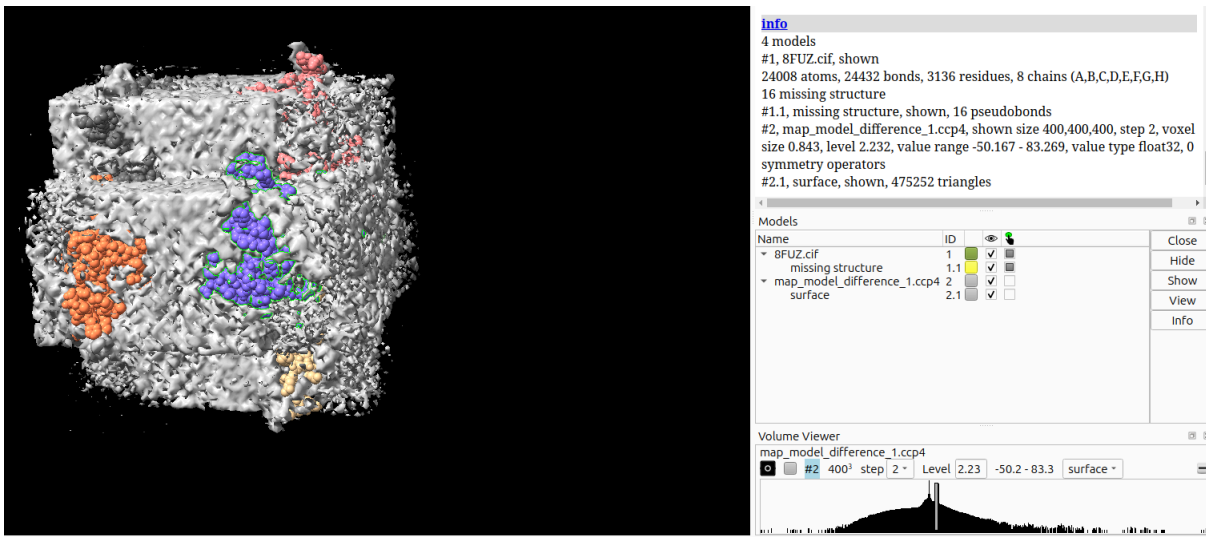
f.

Ligand Class	Probability
AMP-like	0.999
ADP-like	0.000
RARE_LIGAND	0.000

g. Where #2 is map_id and #1 is pdb_id

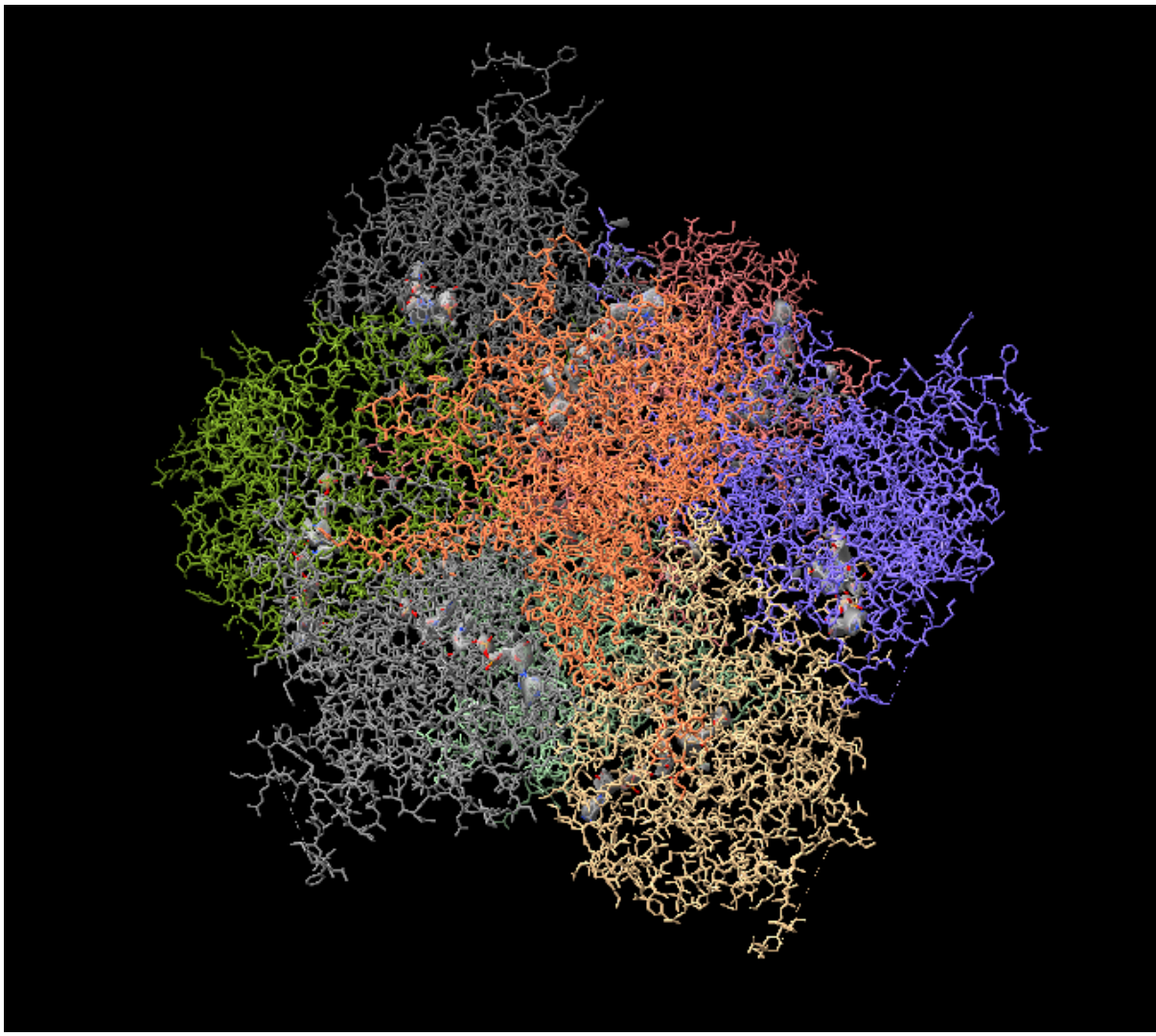
Blob recognize

1. Open files
    a. Open .cif and .ccp4 files corresponding to the map and protein.
    b. Run: open 8FUZ/8FUZ.cif 8FUZ/map_model_difference_1.ccp4
    c. You will get the corresponding outlook by running the `info` command

d. 

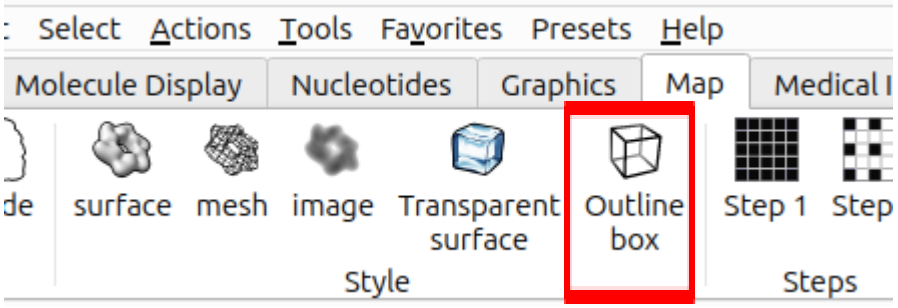
2. Adjust the display

a. Run `blob autothreshold withstyle True` to get better styling:

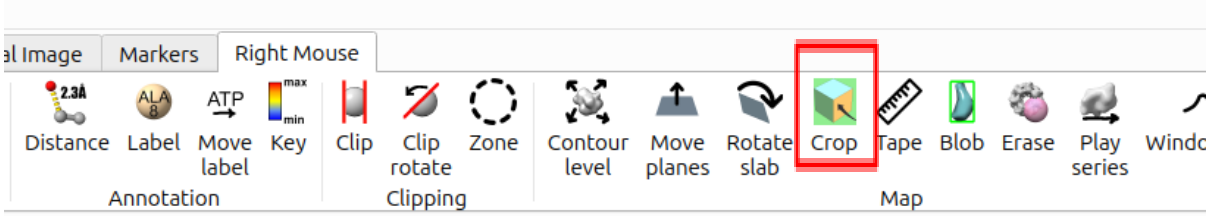
b. 

3. Crop the map – version 1 (not recommended)

a. Go to Map and toggle “Outline Box” style

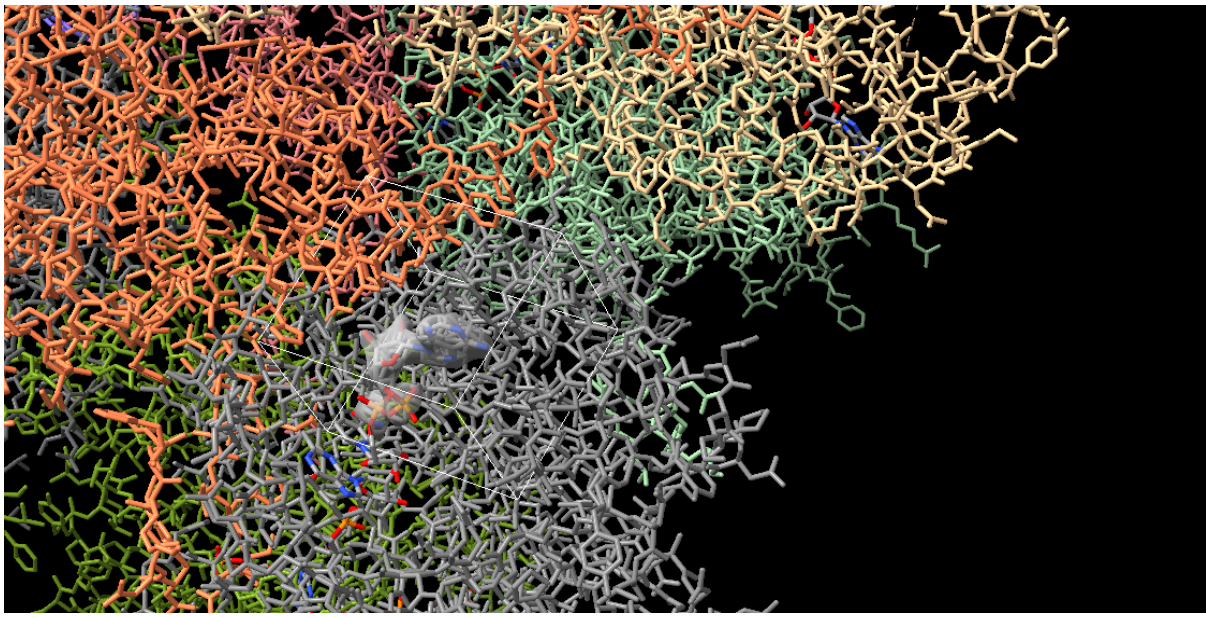
b. 

c. Go to Right Mous and toggle the “Crop” option under Map

d. 

e. Using the right mouse button, limit the outline box to span a part of the density map you want to recognize

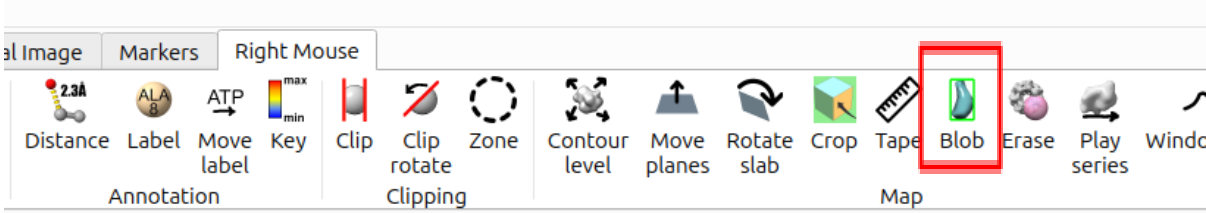
f. The result could look as follows:

g. 

h. With ligand to be recognized inside the Outline Box

i. Optionally, you can hide the protein display to work on density map only

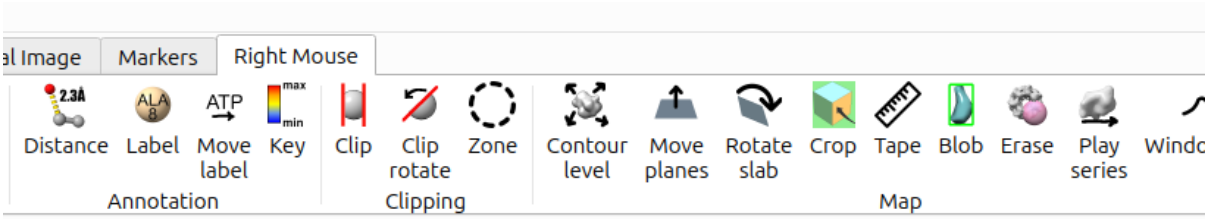
j. You can also use the “Blob” option under Map in Right Mouse settings

k. 

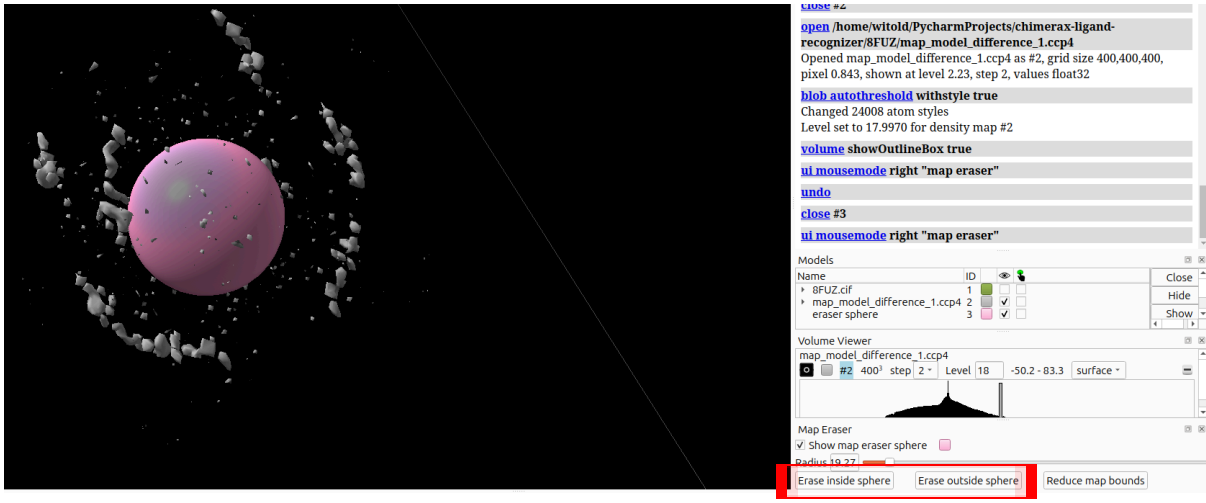
l. You can outline many blobs; however, the final command must be run using only one blob (one surface). To separate the blobs into distinct surfaces, you should run **surface splitbycolor #2**, where **#2** is the ID of the density map for which the blobs were outlined.

4. Crop the map – version 2 (recommended)

a. Go to the Right Mouse menu, and choose “Erase” under Map settings:

b. 

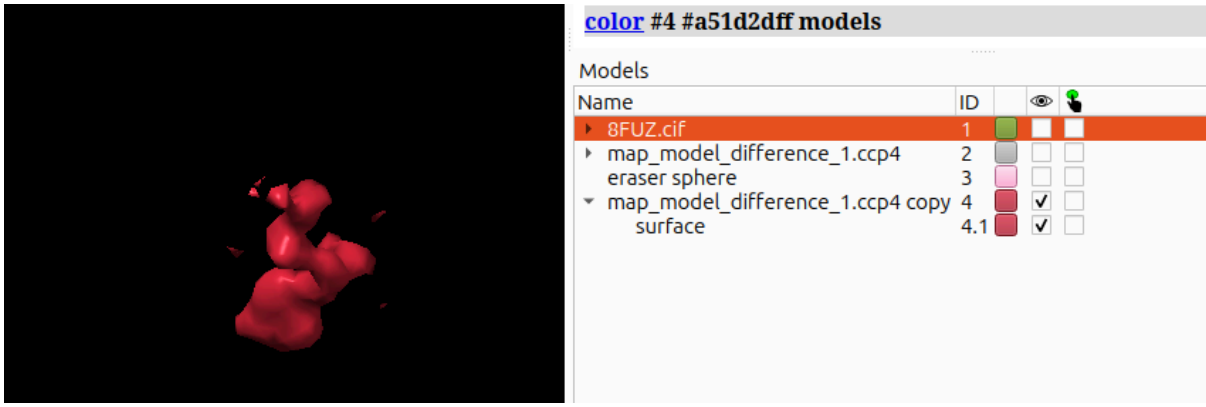
c. Upon right-clicking on the UI, an eraser sphere will appear

d. 

e. Now, move the sphere using the right mouse button and use it to erase part of a map that shouldn’t be recognized. Notably, you can modify its radius using a bar in the bottom-right part of the screen

f. You can use either option “Erase inside/outside sphere” (marked red in the picture above). The only difference is where you will put your sphere. If you put a ligand to be recognized inside it, then use “Erase outside sphere”. Otherwise, you can reposition the sphere to span non-relevant parts of a map and use “Erase inside sphere”

g. IMPORTANT! Conversely to blob/crop from Version 1, this will create a copy of a volume with a surface limited to the blob we positioned inside the sphere. This allows us to reposition the sphere and recognize further blobs, <u>without</u> altering the original map. The created copy is under id #4 in the Models section.

h. 

i. Notably, the original volume (#2) remains unchanged.

j. Now, we can run `blob recognize`, which requires the map_id of the original volume (#2) and surface_id of the blob we want to classify (#4.1 in this case). We cannot use #4, since it refers to a volume, not surface.

k.

blob recognize #2 #4.1

Opened map_model_difference_1.ccp4 copy masked as #5, grid size 400,400,400, pixel 0.843, shown at step 1, values float32

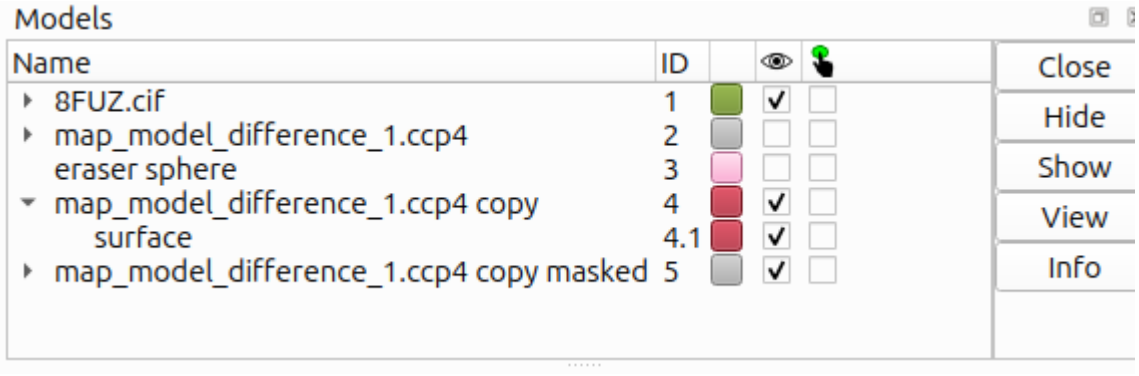
Ligand Class Predictions

(Click on ligand group name to see the full list of ligands)

Ligand Class	Probability
ADP-like	0.633
ATP-like	0.249
SAM	0.048
AGS	0.023
RARE_LIGAND	0.022
NAD-like	0.013
AMP-like	0.008
SAH-like	0.003
ACO	0.001
TYR-like	0.001

l. Running `blob recognize` opens another object, namely a masked copy of a volume (#5 in the Models section):

m.



n. While it is necessary to open it to perform computations, it is redundant for any analysis and can be closed using the “Close” button to the right.

o. Afterwards, the sphere can be repositioned and the process can begin anew. Make sure to toggle on/off the visibility of the entire map and/or protein.