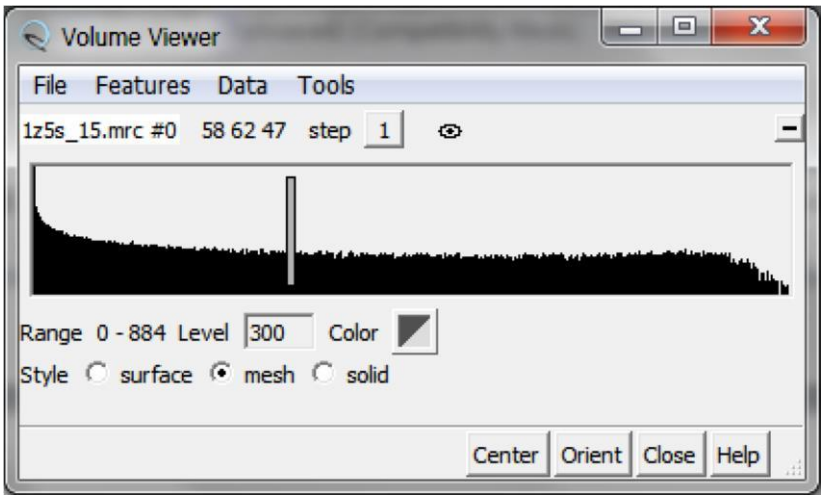
Hands-on Practice

MutiFit

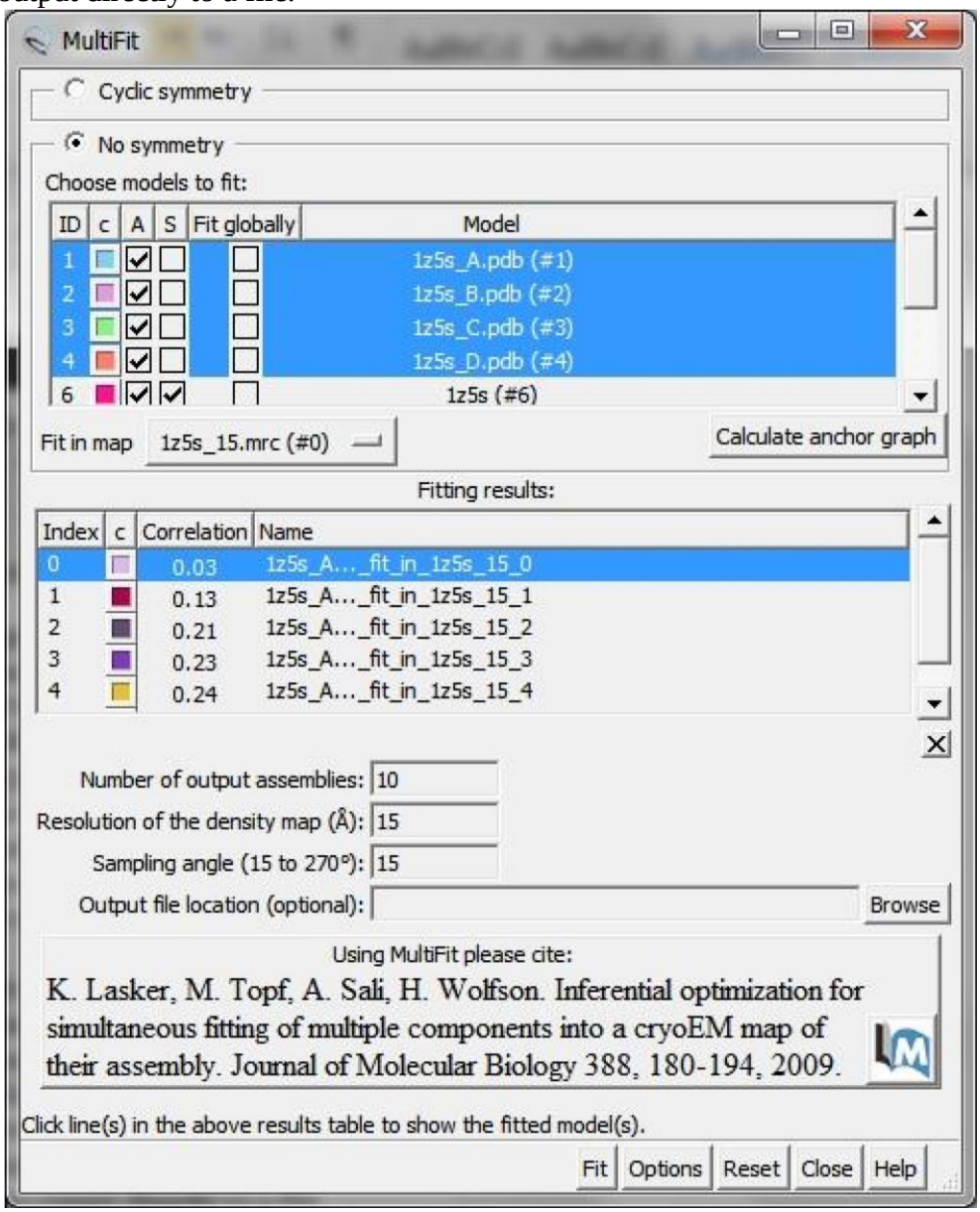
Complex between UBC9, SUMO-1, RANGAP1 and NUP358/RANBP2

This complex involves in regulating pathways of differentiation, apoptosis, the cell cycle and responses to stress. We will try to find the correct orientation of the complex with MultiFit.
We would like to use lower resolution data such as the one received from Cryo-EM experiments. The data that we get from Cryo-EM experiments is a 3D grid with a given resolution and for each point we get a density value. Points with high density represent the molecule under test and points with density lower than a threshold represent "noise" or parts outside the molecule.
In the Exercise file you will find 1z5s_15.mrc. This is the Cryo EM map of our complex. Load it to Chimera.
We need to decide the threshold we would like to use in order to get the real molecular surface. That threshold is a unitless value, with a specific scale. Each CryoEM file has its own level values. The threshold for this map is 300. Select mesh Style, it will come in handy when you want to show crystal structure embedded in the volume data.

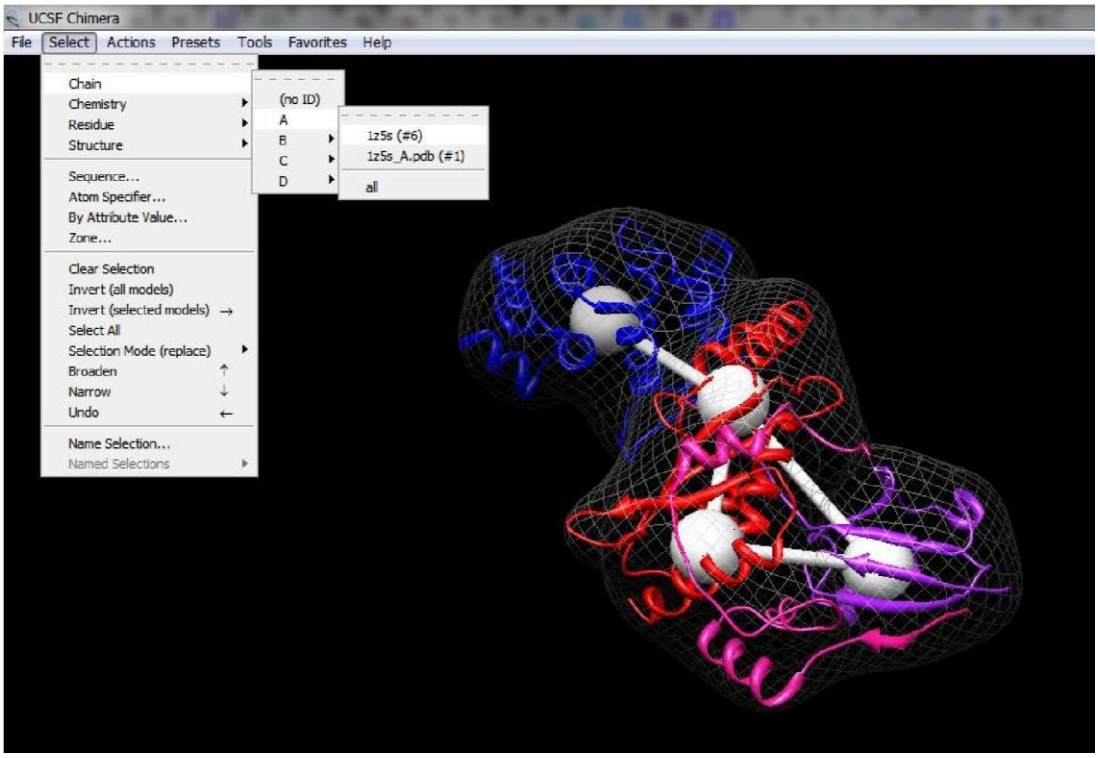


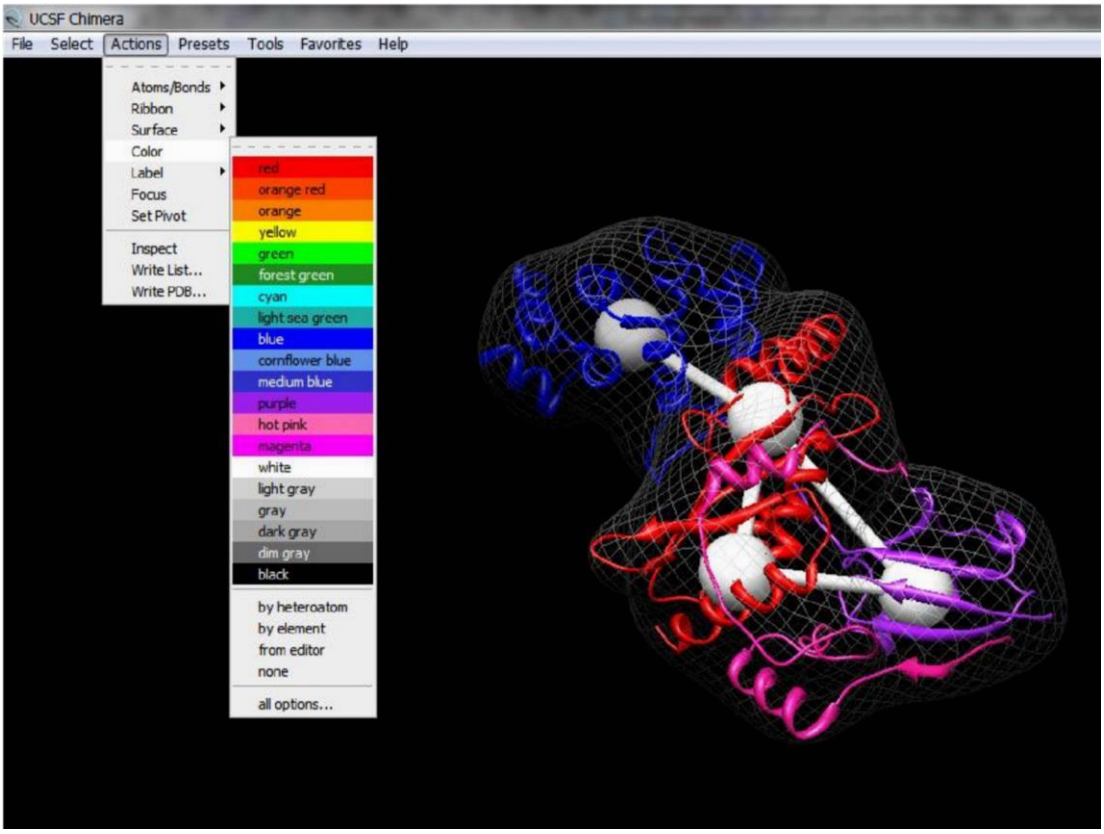
Load all the complex subunits from the Exercise file (4 subunits). Using the model panel, activate each time only one subunit, rotate and translate it. Now we will use MultiFit in order to fix the subunits in their correct orientation in the Cryo EM map.
Go to Tools->Volume data->Multifit
We can choose cyclic symmetry fitting or No symmetry to be used for the fitting. Let's choose "No symmetry". Select all the monomers to be fit into the map and turn all of the models to be active so they can be moved by the algorithm.
Before we do the actual multifit prediction, we would like to view the anchor graph calculated by the algorithm. Let's click the "calculate anchor graph" and wait for the result.
While the anchor graph is being calculated, let's check out the advanced options:
- We can decide how many output complexes we would like to get.

- We can provide the density map resolution. Our Cryo EM resolution is 10A.
- Sampling size for the rotation angles can be decided. Set it to 15°. - We can write output directly to a file.



Press fit and you can see that the data is sent to the MultiFit web service and we now have to wait for it to be processed.
You can see the progress information on the lower part of the screen.
This might take some time. Meanwhile, let's play with the result anchor graph and see that it does give an accurate estimation.
To do so we need the correct complex structure. Fetch it from the pdb: 1z5s.
For better visualization, color each chain in a different color: Select ->
Chain -> A -> 1z5s.
And then: Action -> Color

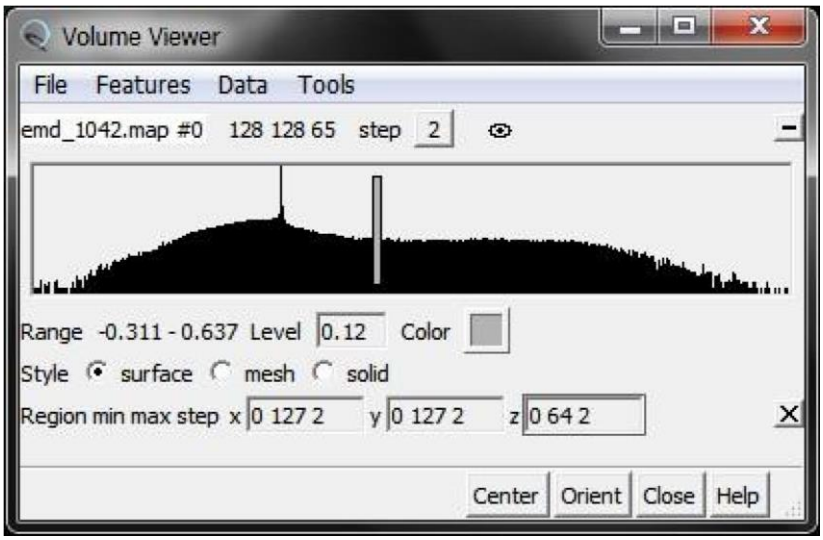




When the results are ready, go over the results.

GroEL

Now we will try the symmetric version of Multifit but using one of the most favorable cryo-EM map in the field - the GroEL. This is an E.coli version of the GroEL. The GroEL is a Chaperonin found in many types of bacteria and is very important for the proper folding of proteins. The GroEL has a 7-fold circular symmetry so we will be predicting its structure using Sym-multifit.
Let's start by fetching the GroEL emdb file - emdb:1042 . Again we need to set the correct contour level. The recommended level is 0.12.
Now that we have loaded the structure we can see that it has in fact dihedral symmetry - you have a C7 rotational symmetry but also a reflection symmetry creating the double ring formation. As multifit is limited to rotational symmetry we would like to cut the structure in two. We can do that by using Tools->Volume data>Volume Viewer->Features->Region bounds. This allows us to limit the part of the map that we are reading. Let's limit it on the Z axis to only half of the map (we assume that the ring is centered around the center of the map).



Now that we have the map ready, we would like to get the GroEL monomer. At first, we will cut it out of the full GroEL atomic. Load the PDB structure for the entire GroEL , pdb 1oel.
Split the structure:

1. Tools-> Command line
2. Type: "split #1" in the Command Line
3. Remove 6 out of 7 chains

Select Tools->Volume data->Multifit, choose the symmetric version, select the monomer and volume data and press fit.
Load again the correct pdb and compare the results.