Cryo-EM map-based structure modeling

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Structure Modeling Workshop

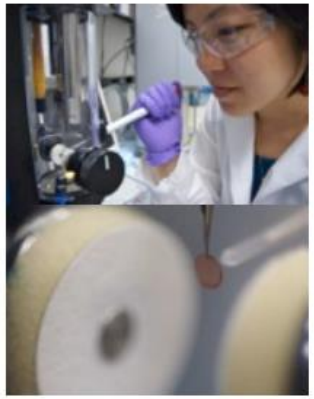
Michigan State University

May 2015

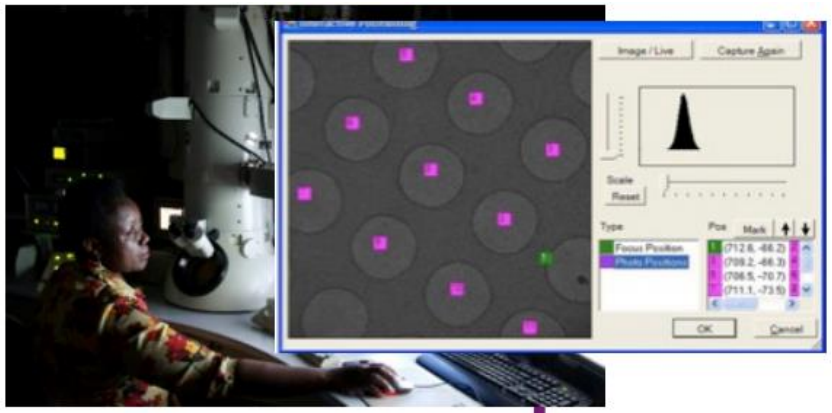
Pipeline in Single Particle Cryo-EM

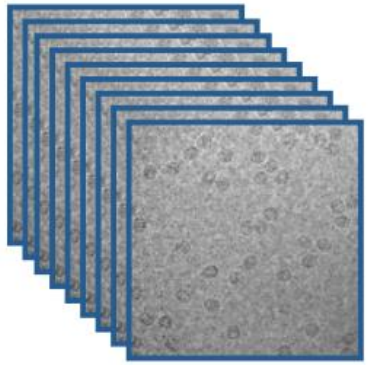
Biochemical Preparation

Cryo-EM Sample Preparation

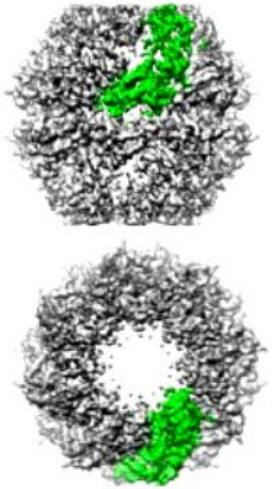


High Resolution Automated Data Collection



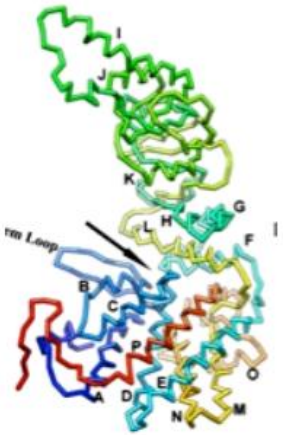


Data Archiving & Processing



3D Reconstruction

EMAN



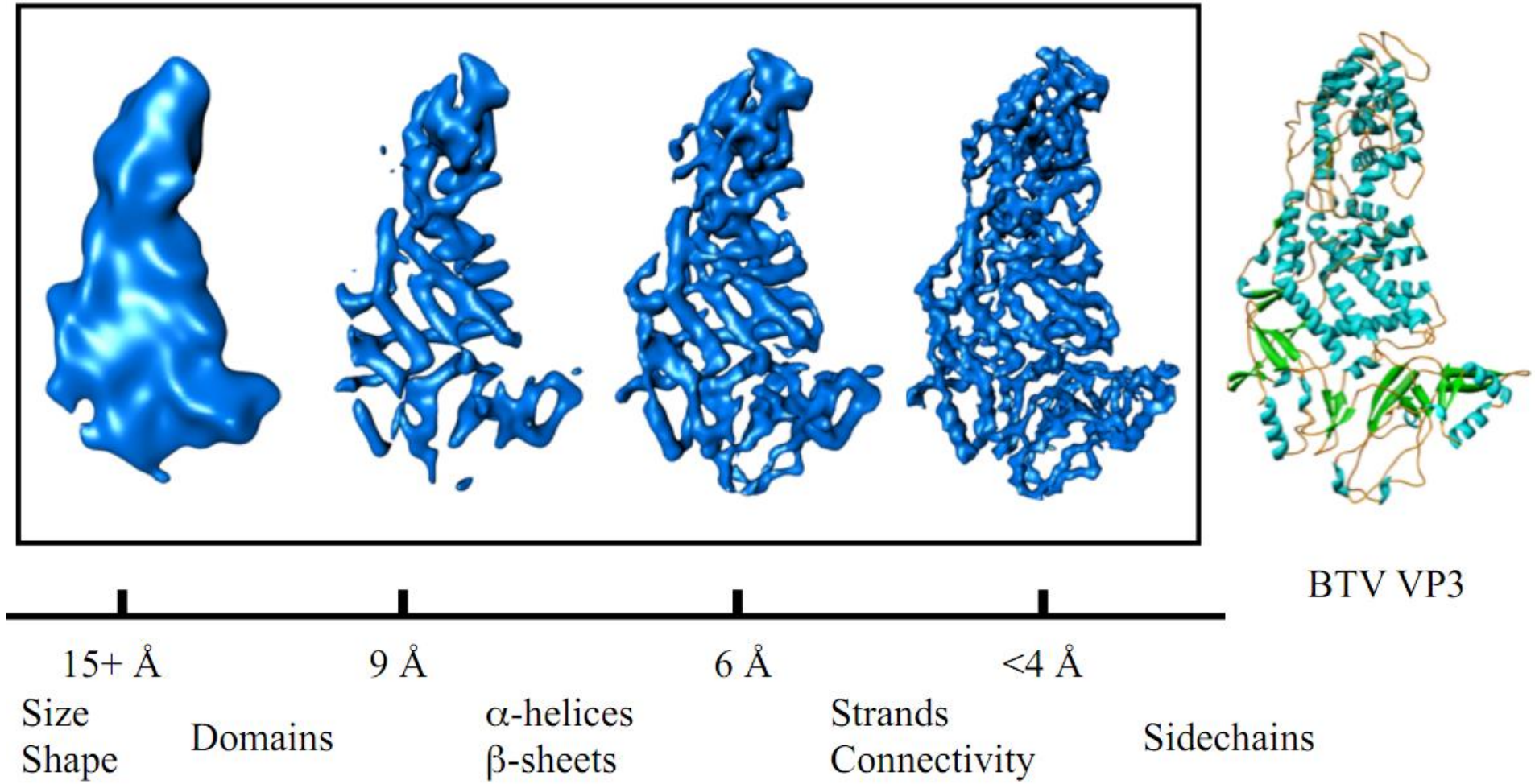
Model Building

Chimera, Situs, MDFF…..

WORLDWIDE PDB PROTEIN DATA BANK

Structure Deposition

Structural Features at Different Resolutions



EMDatabank

1

2015-05-13 : 2934 EMDB map entries, 832 PDB coordinate entries PDBe | RCSB

EMDataBank
Unified Data Resource for 3DEM

One-stop shop for 3DEM deposition and retrieval

Home | About | Deposit | Search | Tools | Events | News | Links | Help

Unified Data Resource for 3-Dimensional Electron Microscopy

EMDataBank is a unified global portal for deposition and retrieval of 3DEM density maps, atomic models, and associated metadata, as well as a resource for news, events, software tools, data standards, validation methods for the 3DEM community.

Recently released entries — All recent entries

EMD-2902

PDBe | RCSB

Feb. 19, 2015 RELEASED ON May 13, 2015 singleParticle 7.4Å **NEW**

Cryo electron microscopy of actively translating human polysomes (POST-i2 state).

Behrmann E, Loerke J, Budkevich TV, Yamamoto K, Schmidt A, Penczek PA, Vos MR, Burger J, Mielke T, Scheerer P, Spahn CMT

News — All news

EMPIAR: Raw Data Image Archive

EMPIAR (Electron Microscopy Pilot Image ARchive; http://pdbe.org/empiar) is a public archive for raw image data (micrographs, particles, and tilt-series) related to EMDB entries. Important uses of EMPIAR data include validation and as test-data for methods development and teaching.

Read more...

Tilt Validation Server

The PDBe tilt-pair validation web-server is now available from: http://www.pdbe.org/tiltpair. You can use this service to perform a tilt-pair analysis on your own data.

Read more...

RSS

Supported by
National Institutes of Health
National Institute of General Medical Sciences

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http://emdatabank.org/

2

Search EMDB

Browse/search:

- Simple search (RCSB): http://emsearch.rutgers.edu
- Advanced search (PDBe): http://pdbe.org/emsearch

Resources:

- Searching, Visualizing and Analysing EMDB Data--PDBe Webinar Recording (July 8, 2013)

Four numbers ID

3

ID code:	EMDB
Author Last Name:	
Title:	
Sample Name:	
Citation Abstract:	
Method Type:	Any Type
Specimen State:	Any Type
Resolution:	low: high:
Date:	Release From To

Start search

Try:1940

4

Click on "Start search" to browse all the entries

Home — **EMD-1940**

Title: Negative stain EM density of green-type rubisco activase (R294V) from tobacco
Authors: Stotz M, Mueller-Cajar O, Ciniawsky S, Wendler P, Hartl FU, Bracher A, Hayer-Hartl M
Sample: Nicotiana tabacum Rubisco Activase (R294V)
Method: Single particle reconstruction (20 angstroms resolution)
Latest update: 2011-12-09

- Summary
- Experimental details
- Visualization
- Map information
- Downloads

Summary

Status: Released
Deposition date: 2011-07-28
Deposition site: PDBe
Processing site: PDBe
Header release date: 2011-08-26
Map release date: 2011-11-11
Primary citation: Structure of green-type Rubisco activase from tobacco.
Stotz M, Mueller-Cajar O, Ciniawsky S, Wendler P, Hartl FU, Bracher A, Hayer-Hartl M
NAT.STRUCT.MOL.BIOL. (2011) **18**, pp. 1366-1370 [PubMed 22056769] [DOI]
Sample: Nicotiana tabacum Rubisco Activase (R294V)
Resolution: 20 Å (determined by FSC 0.5)
Fitted PDB:

PDB	Authors
3zw6	Stotz, M., Mueller-Cajar, O., Ciniawsky, S., Wendler, P., Hartl, F.U., Bracher, A., Hayer-Hartl, M.

Details on Cryo-EM data

Home | EMD-1940 | Mirrors

Title: Negative stain EM density of green-type rubisco activase (R294V) from tobacco
Authors: Stotz M, Mueller-Cajar O, Ciniawsky S, Wendler P, Hartl FU, Bracher A, Hayer-Hartl M
Sample: Nicotiana tabacum Rubisco Activase (R294V)
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Map information

3d grid box information

Map: emd_1940.map.gz (compressed: 152KB; uncompressed: 8.0MB)
Map data type: Image stored as Reals (mode=2)

Map size

Map axis order:	X	Y	Z
Dimensions (voxels):	128	128	128
Voxel spacing:	3.31 Å	3.31 Å	3.31 Å
Map extent:	423.4 Å	423.4 Å	423.4 Å
Origin (voxels):	0	0	0

Grid size

Isovalue range

Map statistics:	Minimum	Maximum	Average	Standard deviation
	-0.17	0.32	0.00	0.01

Recommended contour level: 0.02 (source: emdb)

- Summary
- Experimental details
- Visualization
- Map information
- Downloads

View clearly

Visualization

Negative stain EM density of green-type rubisco activase from tobacco. Hexameric model based on p97 D2 ring is fitted into the structure.

Suggested contour level: **0.02**

Volume viewer
Map visual analysis (PDBe)

Atlas images are either supplied by the depositor or generated by EMDataBank using UCSF Chimera.

- Summary
- Experimental details
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Resolution: 20 Å (determined by FSC 0.5)

Fitted final complex

Fitted PDB:	PDB	Authors
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Sample

Sample name: Nicotiana tabacum Rubisco Activase (R294V)
Oligomeric state: Hexamer
Theoretical molecular weight of the sample: 0.3

Components:

ID	Type	Name	Exp. MW (MDa)	Oligomeric details	Recombinant expression	Organism	InterPro identifier	Details
1	protein	Ribulose bisphosphate carboxylase activase 1, chloroplastic	0.3	Hexamer	true	Nicotiana tabacum	IPR003959	Hexamer assembles upon addition of 1 mM ATPgammaS at 25 deg C

Biological experiment details

Experiment

Specimen state: Particle

Specimen preparation:

pH	Specimen conc.	Details	Staining	Specimen support details
8.0	0.044 mg/mL	20mM Tris-HCl, 50mM NaCl, 2mM MgCl2, 1mM ATPgammaS	Grids were stained twice with 2% w/v uranyl acetate	Plain carbon

Vitrification:

Cryogen name	Instr.
NONE	NONE

Imaging:

Microscope	Voltage	Illumination mode	Imaging mode	Cs	Defocus min.	Defocus max.	Electron source	Detector	Astigmatism
FEI TECNAI 12	120 kV	FLOOD BEAM	BRIGHT FIELD	2.0 mm	470 nm	950 nm	LAB6	FEI EAGLE (2k x 2k)	Objective lens astigmatism was corrected for at 110k magnification

Specimen holder	Holder model	Electron dose	Date
Single tilt	SIDE ENTRY, EUCENTRIC	20 e/Å^2	22-Jun-2011

Processing

Protocol: Angular reconstitution
Software: MRC, IMAGIC, SPIDER
CTF correction: Phase flipping, each particle
Number of particles: 599
Imposed symmetry: C6
Resolution by author: 20 Å
Resolution method: FSC 0.5

Cryo-EM Data reconstruction

Atomic structure fitting into Cryo-EM map

Fitting:

PDB	Protocol	Target crit.	Software	Fitting space	Details
3T15	rigid body	Local minimisation, fit in map	Chimera	REAL	Protocol: Rigid body. A module of alpha helical domain and alpha-beta domain of neighbouring subunit was overlaid with p97 D2 (3CF3) structure and the hexamer was fitted into the EM map

Fitting atomic structure

Fitting protocol and software

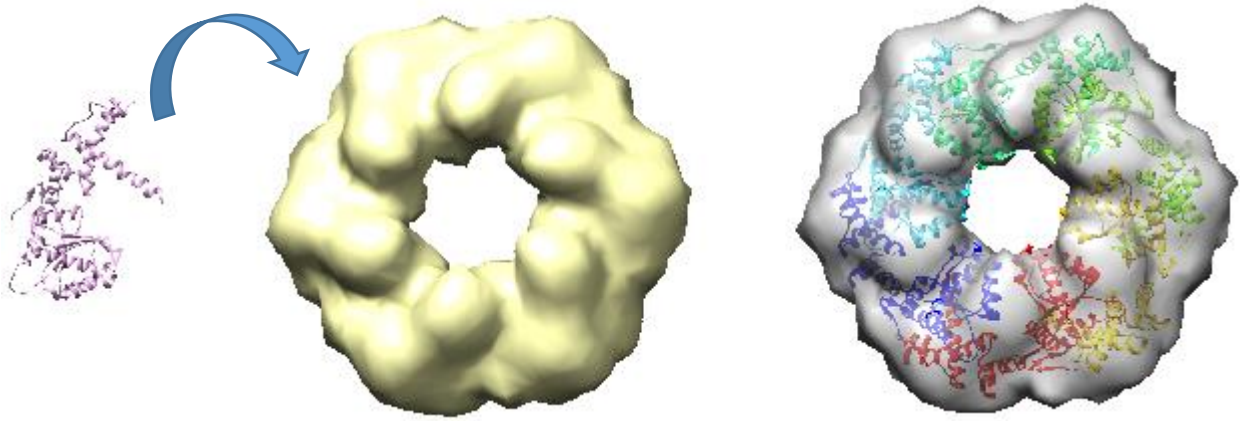
Fitting details

Atomic Structure for Cryo-EM vs X-Ray

Experimental Details Hide

Method: X-RAY DIFFRACTION
Exp. Data:
Structure Factors
EMDataBank: emd-1940
EDS
Resolution[Å]: 2.95
R-Value: 0.229 (obs.)
R-Free: 0.296
Space Group: P 6_5
Unit Cell:

Length [Å]	Angles [°]
a = 103.64	α = 90.00
b = 103.64	β = 90.00
c = 56.67	γ = 120.00



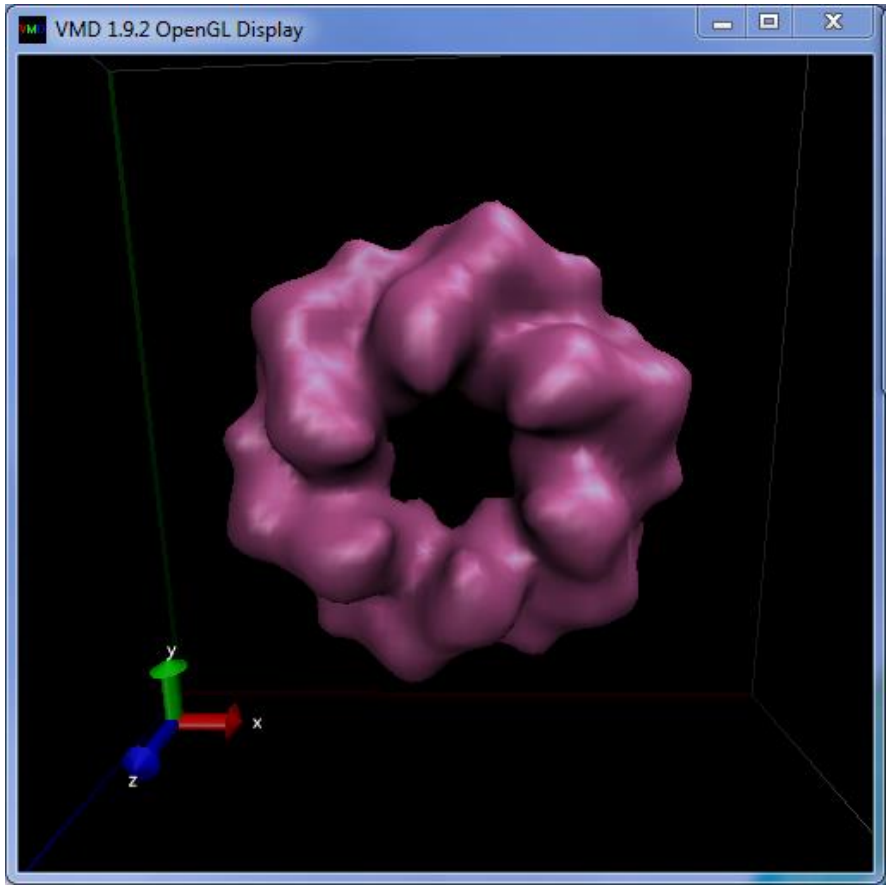
Experimental Details Hide

Method: ELECTRON MICROSCOPY
Exp. Data:
EMDataBank: 1940
EM Resolution [Å]: 20.0
Aggregation State: PARTICLE
Symmetry Type: MIXED SYMMETRY
Reconstruction Method: SINGLE PARTICLE
Specimen Type: NEGATIVE STAIN

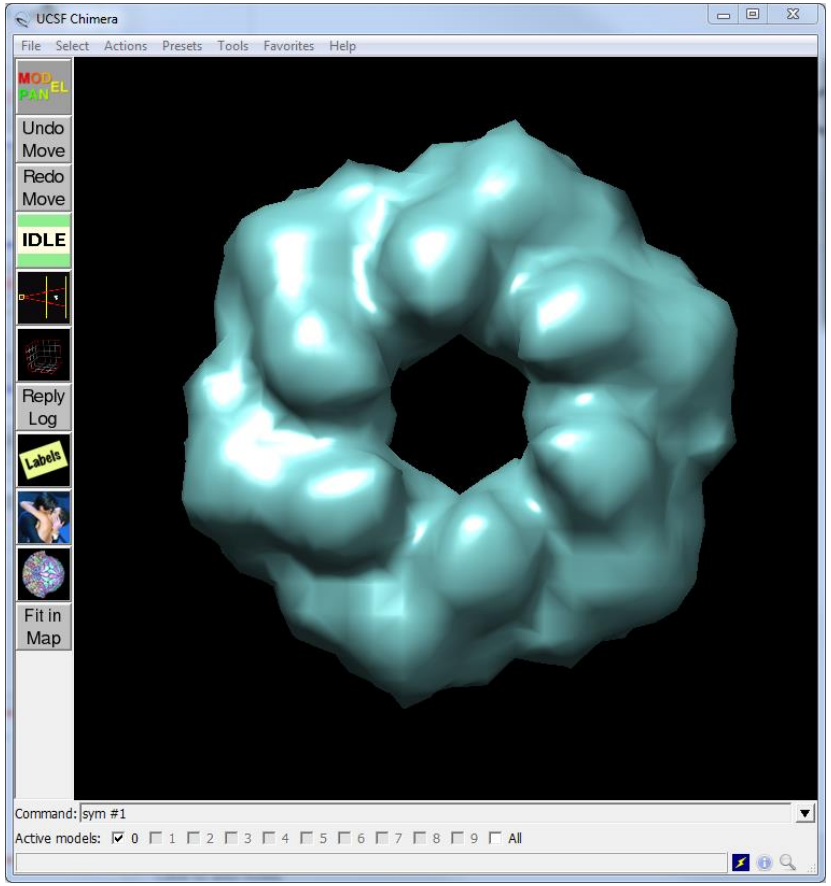
Fitting model, not a real experimental atomic structure

The unit for fitting could also be obtained by homology modeling.

Visualization of Cryo-EM data



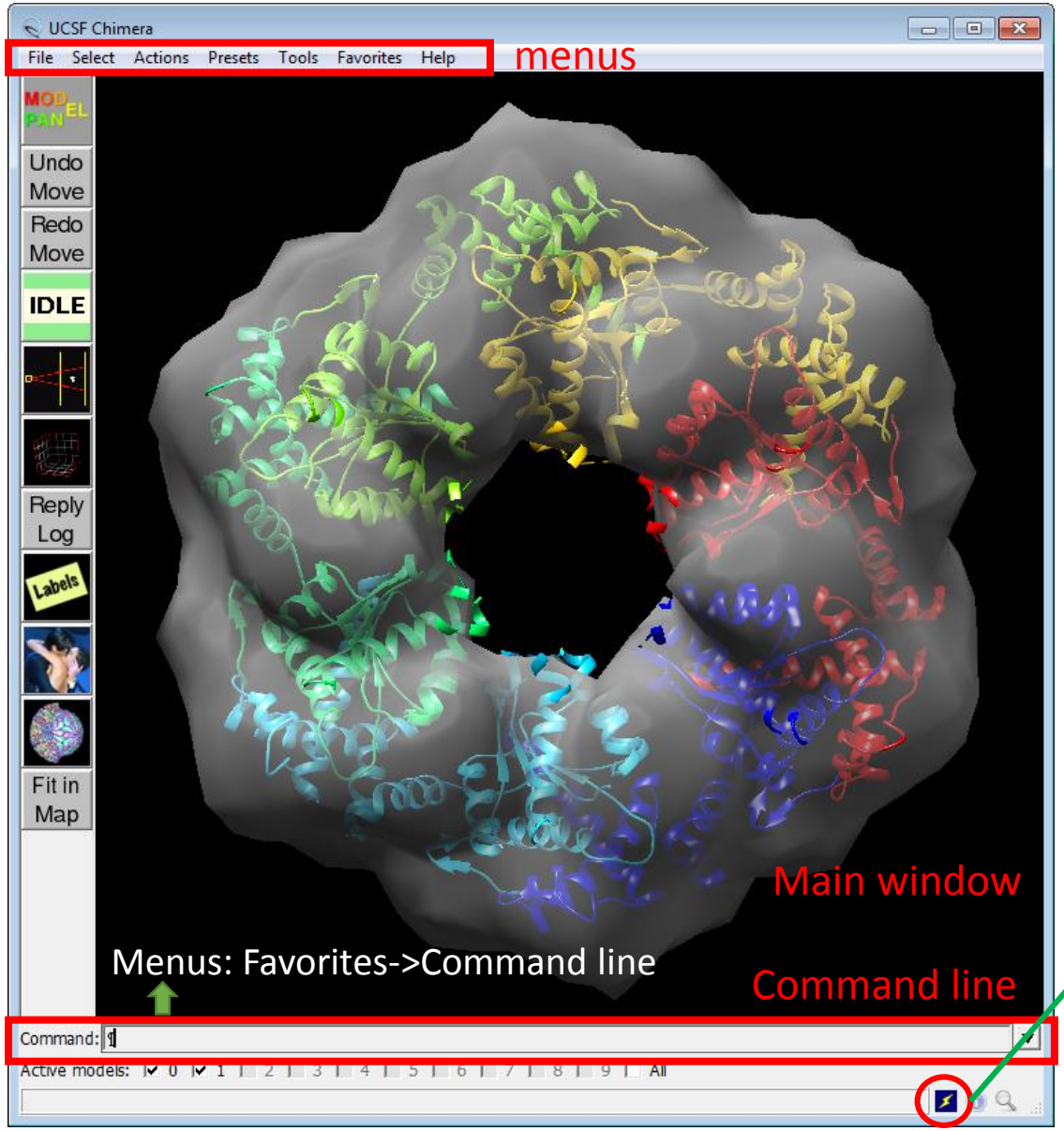
VMD
http://www.ks.uiuc.edu/Research/vmd/



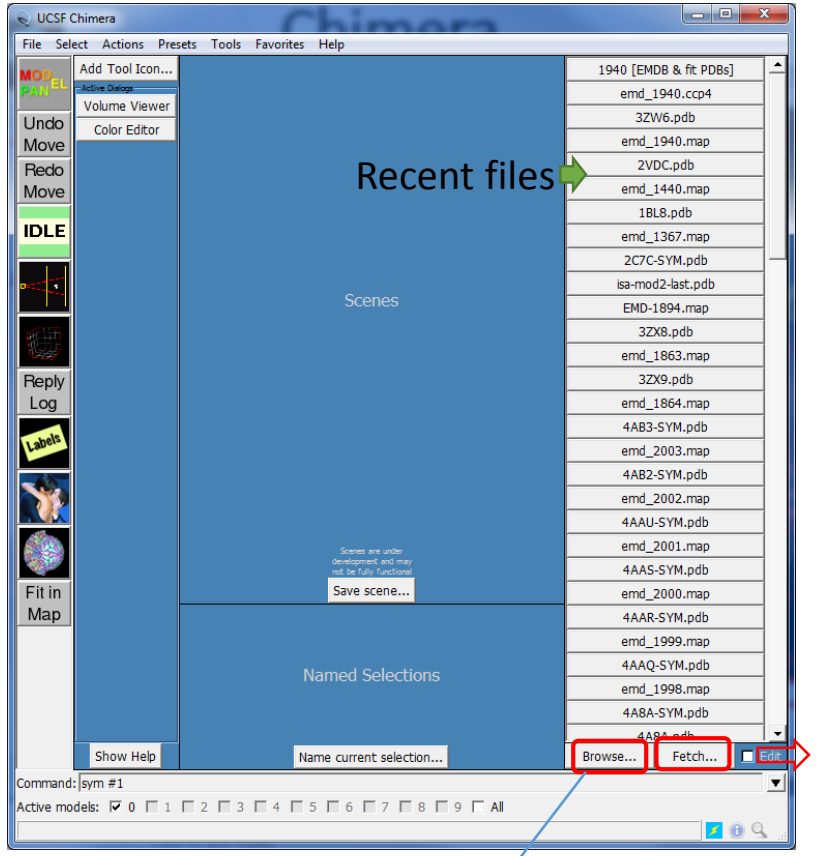
Chimera
http://www.cgl.ucsf.edu/chimera/

- VMD could help to perform MDFF. Use the latest version(1.9.2)
- Chimera contains more tools for cryo-EM data processing.

Chimera

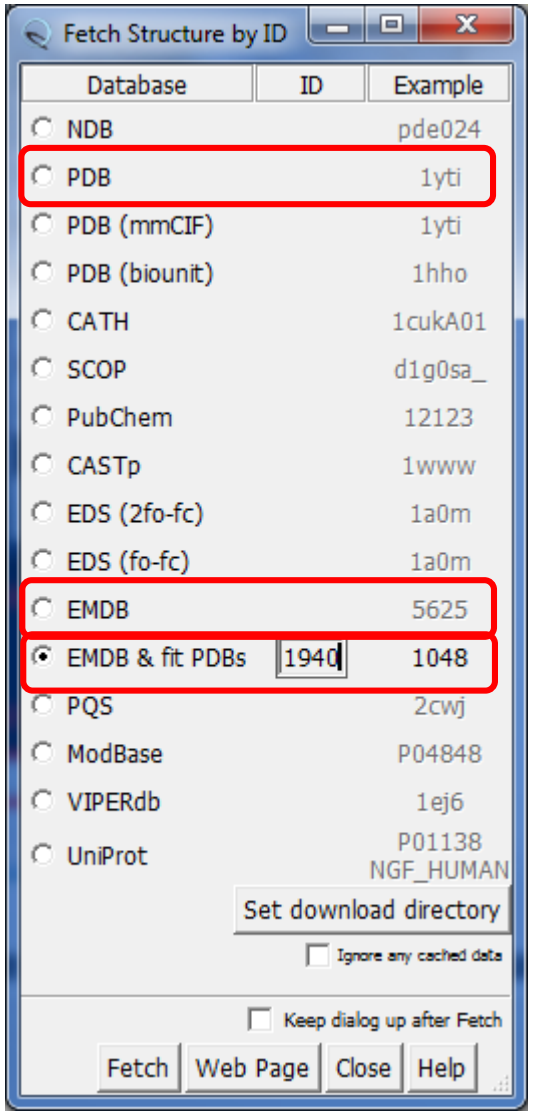


Open file window



Choose & open a selected file

Get file online



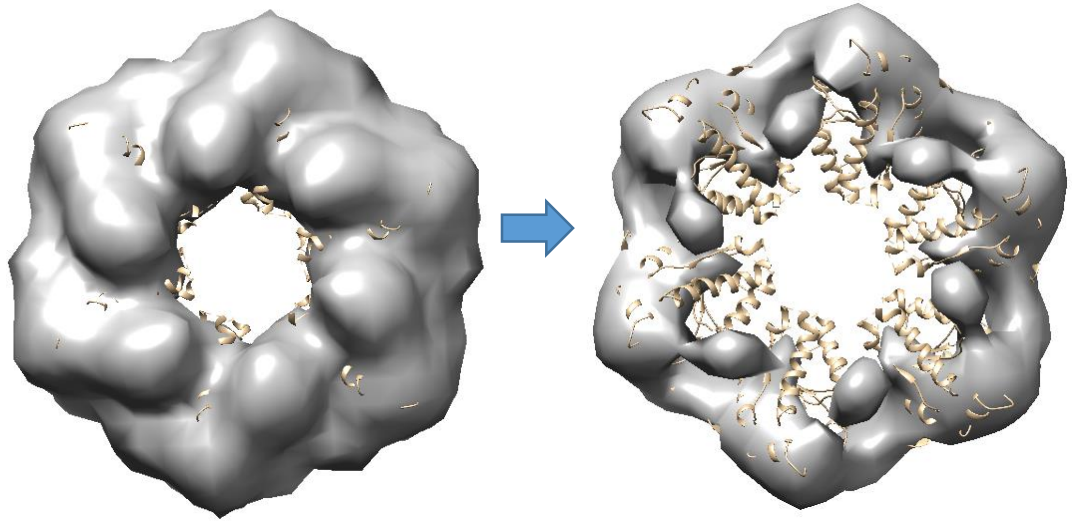
Menus: Files->Fetch by ID

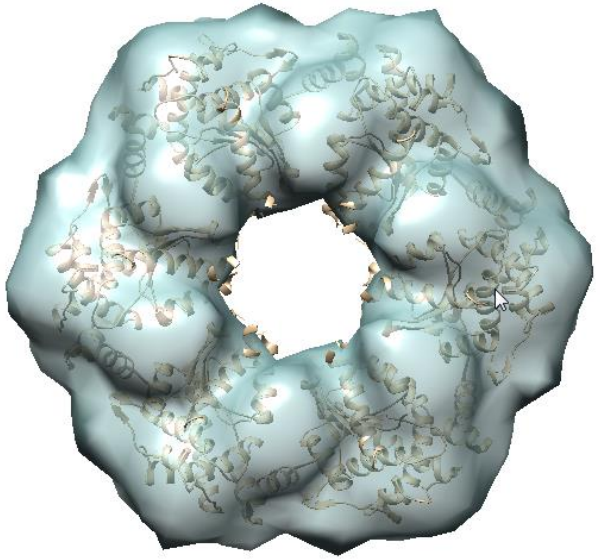
Left click and move: **Rotation**

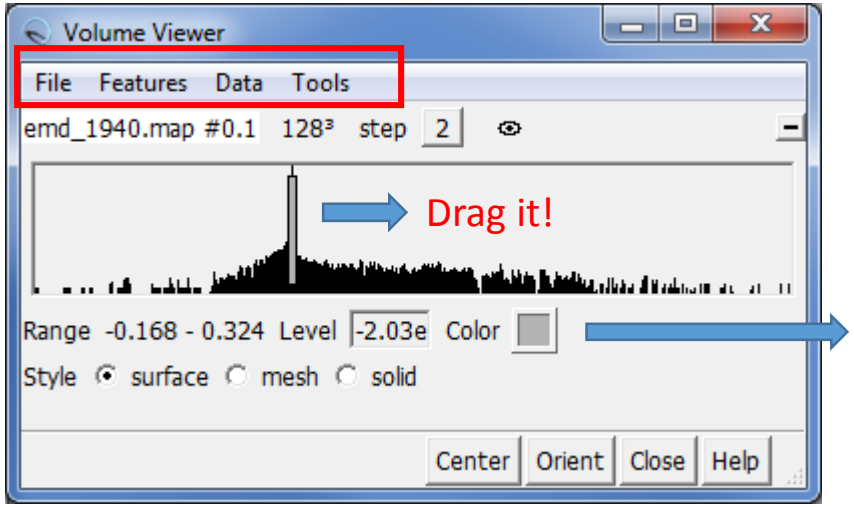
Middle click and move: **Translation**

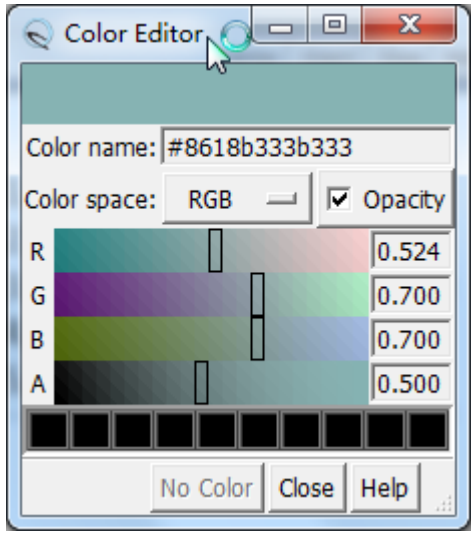
Right click and move: **Scaling**

Volume Viewer

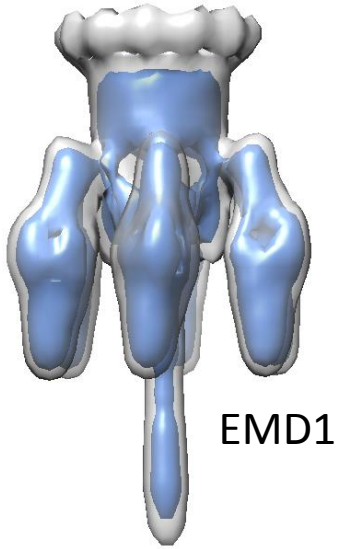








1. **Control isovalue surface**
2. **Show/Hide/Delete map**
3. **Surface/Mesh**
4. **Color/Transparent**
5. **Tools/Save map**



EMD1119

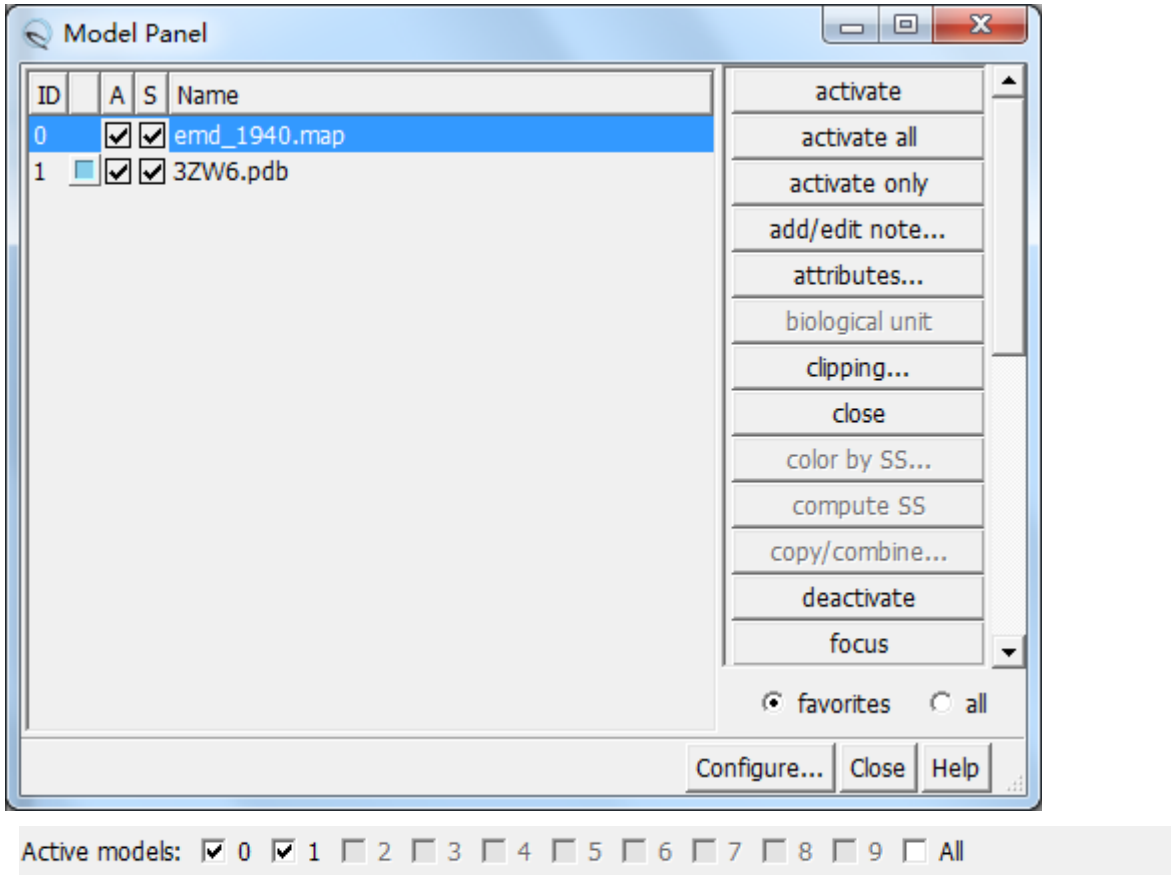
Multiple contour levels

To generate a map for atomic structure:
Commands: molmap #0.2 20

Control & Select model

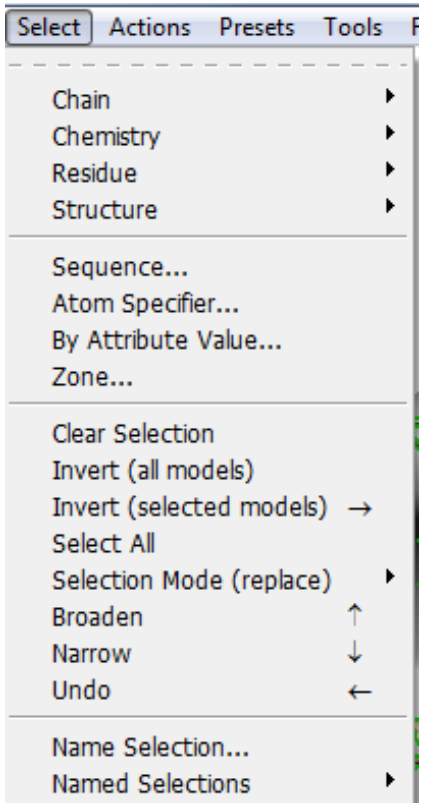
Reopen emd:1940 and pdb:3ZW6

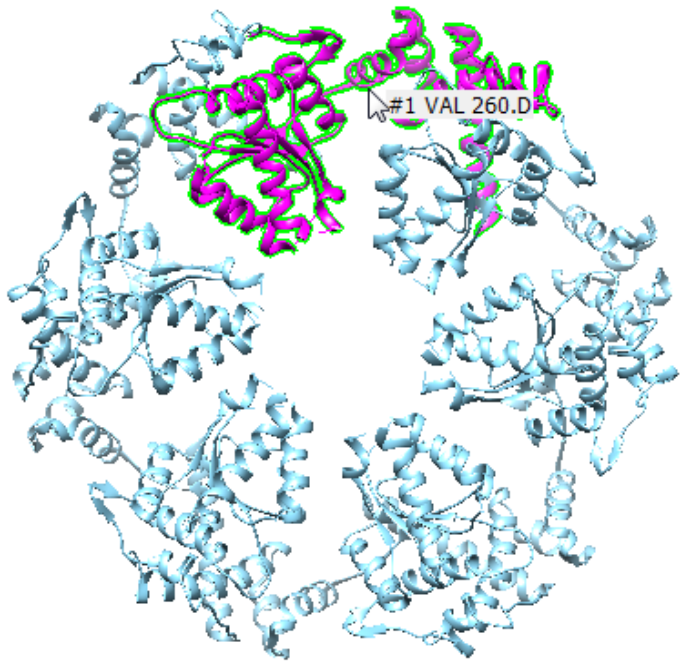
Active & Shown



Only active model will move!

Use selection menus to help!





1. **Ctrl**+left click to select
2. **Up** and **down** to broaden and narrow the selection
3. -> to invert selection
4. <- to undo the last selection

Atom Specifier

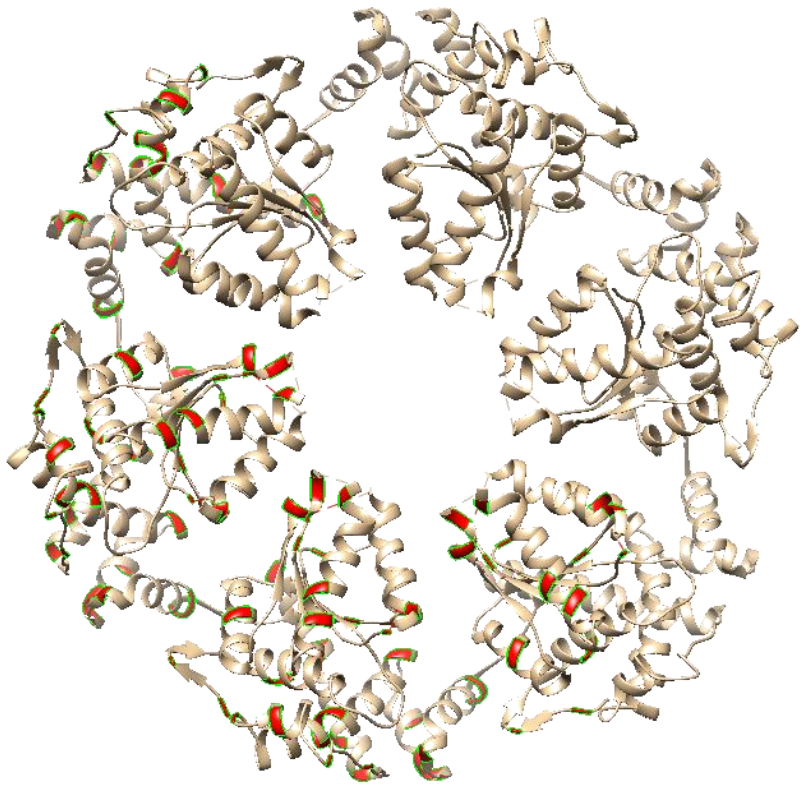
- #model or #model.submodel
- :residue or :residue.chain
- @atom
- Special: **all** **sel**

select atom-spec

Try:
Select chain E
Select all CA
Select CA,C,N in residue PHE and PRO of chain D

color colorname atom-spec
~*color* sel to un-color.
~*command* perform the reverse function.

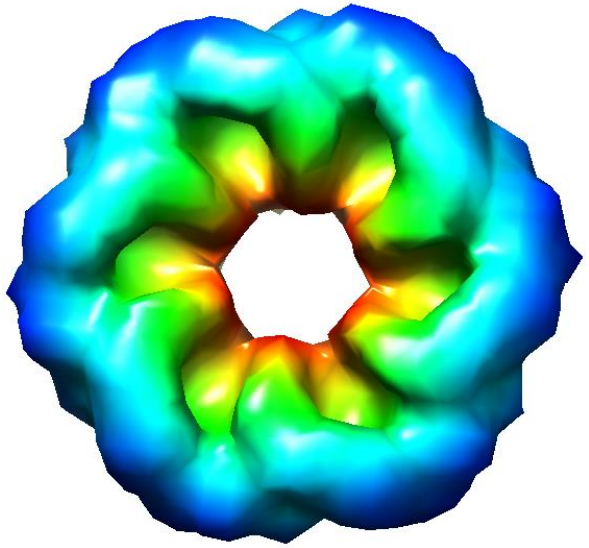
Select acidic residues in chain A to C



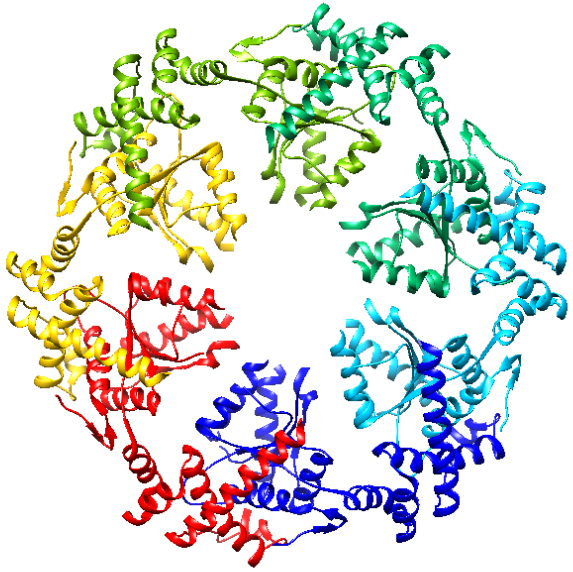
select :GLU.A-C,ASP.A-C
Color red sel

More on atom specifier: https://www.cgl.ucsf.edu/chimera/docs/UsersGuide/midas/atom_spec.html

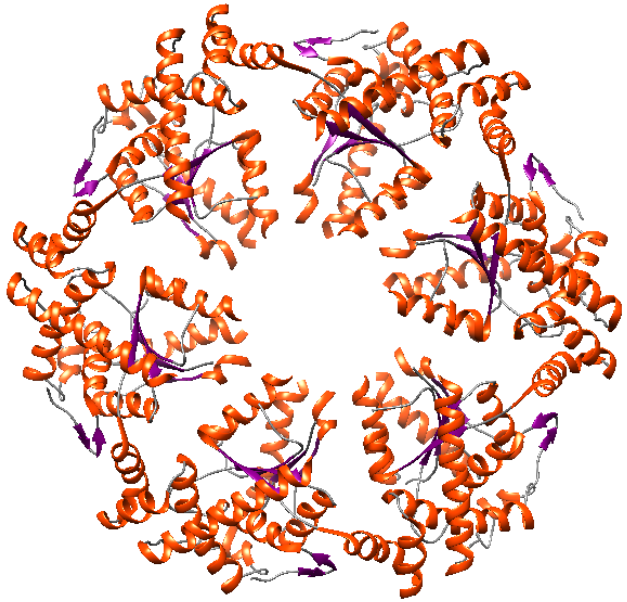
Coloring



Volume Data->Surface color
Colored by radius



Tools->Depiction->Rainbow
Rainbow chain #1

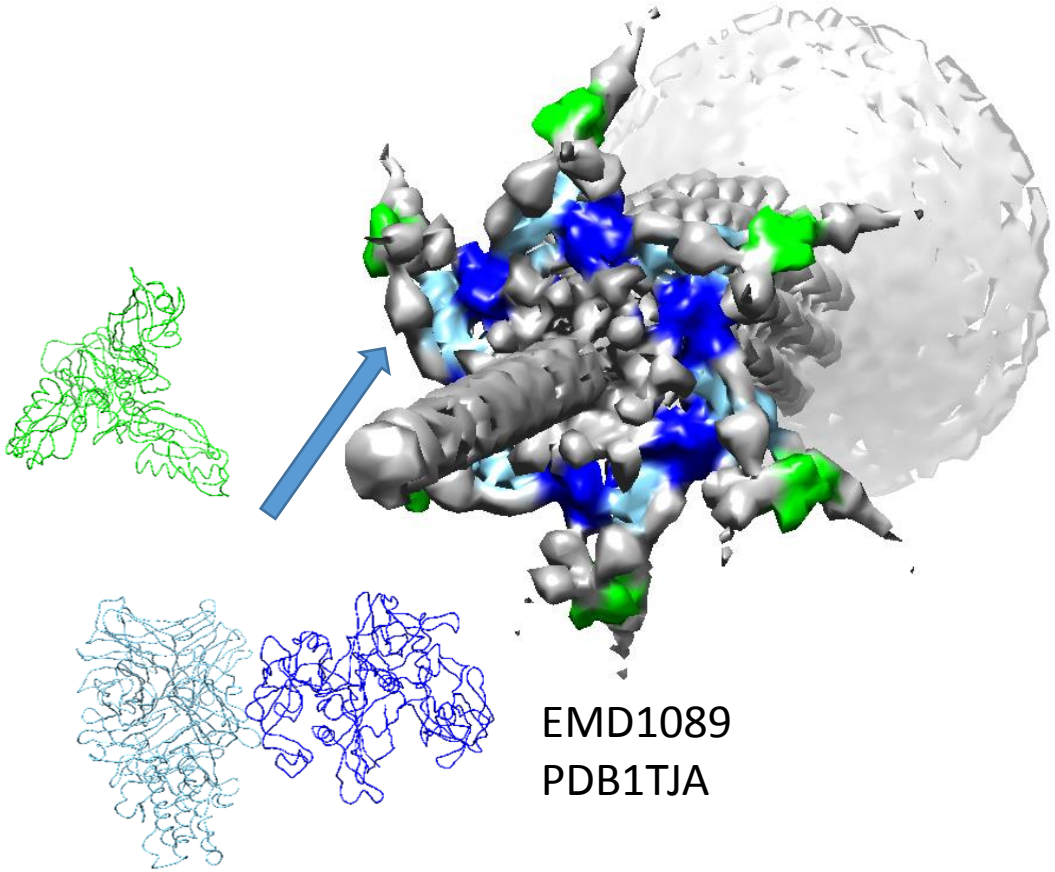


Tools->Depiction->Color Sec. Stru.

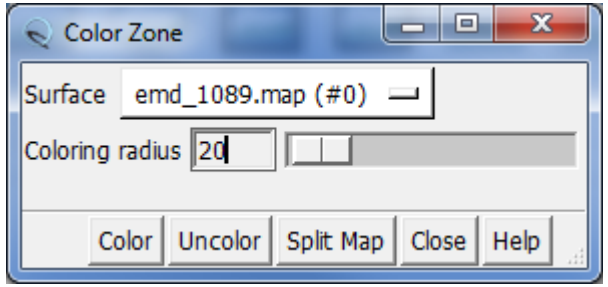
Color by commands: color red *#1:.A*

Color zone

Patches of a surface near selected atoms or markers can be colored to match the atom or marker colors.



EMD1089
PDB1TJA



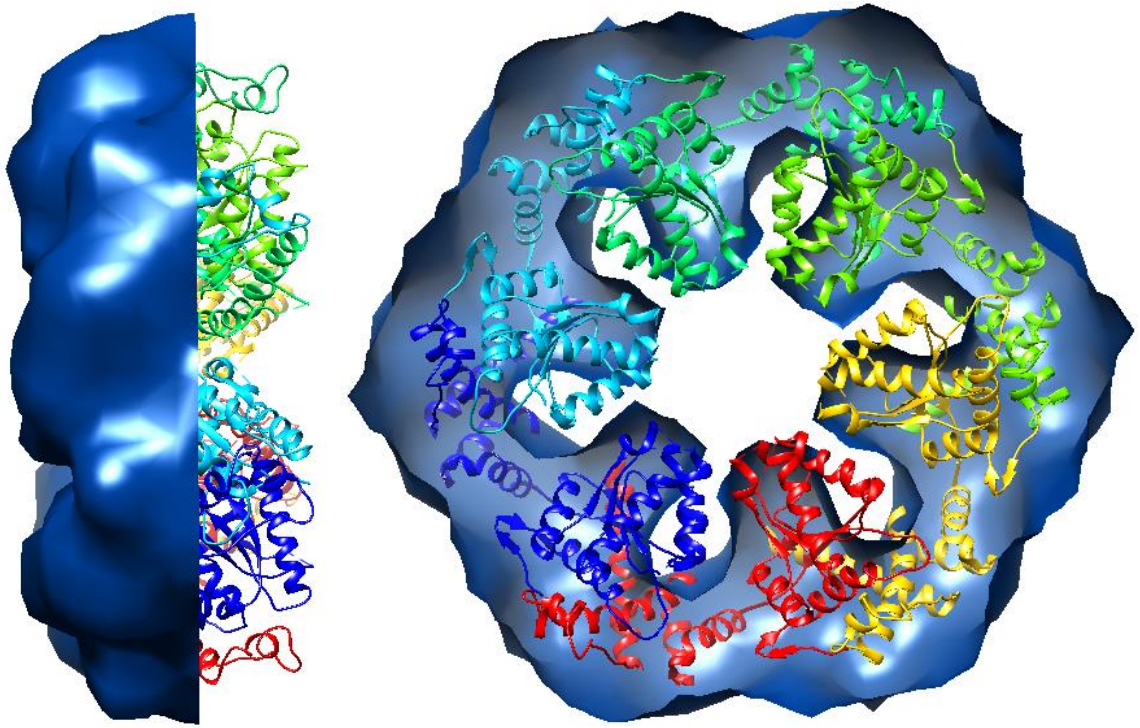
Tools->Volume Data->Color Zone

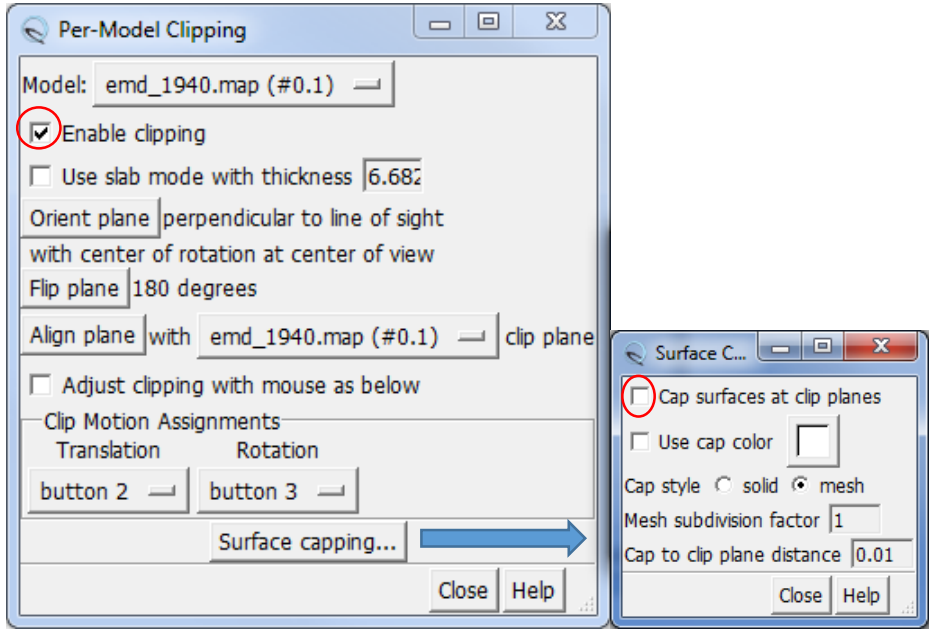
1. Color selected atoms
2. Select atoms and patch color to map

Color blue :.A,.B
Color green :.F-H
Select #1 #2

Slice clipping

Help you view the details of fitting.

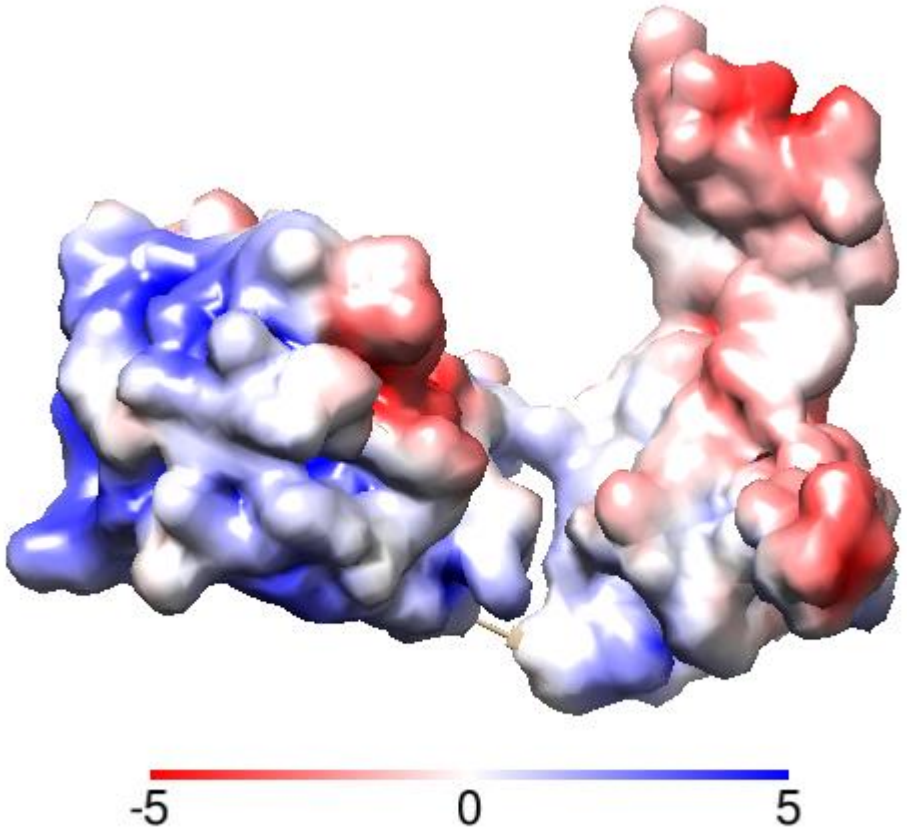




Menus: Tools->Depictions->Per-Model Clipping

*Adjust the vision of map and click orient plane to slice the map surface.

Electrostatic potential surface

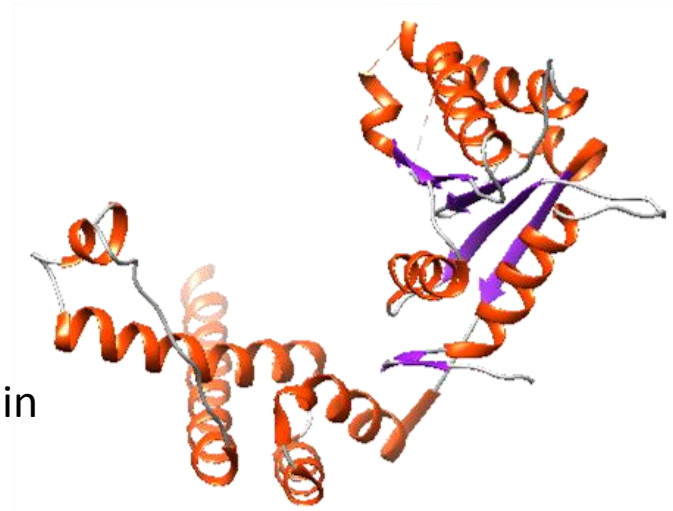


Step1: Add charges for atoms

Step2: Calculate electrostatic potential

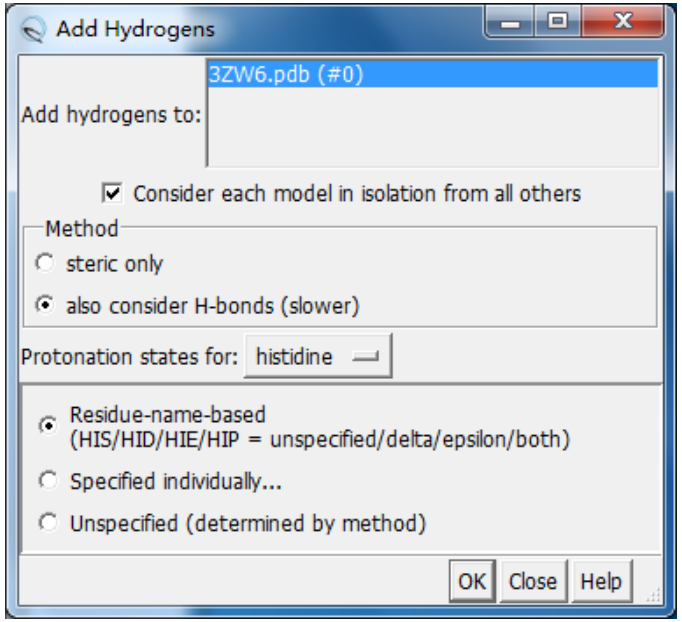
Step3: Color by electrostatic potential

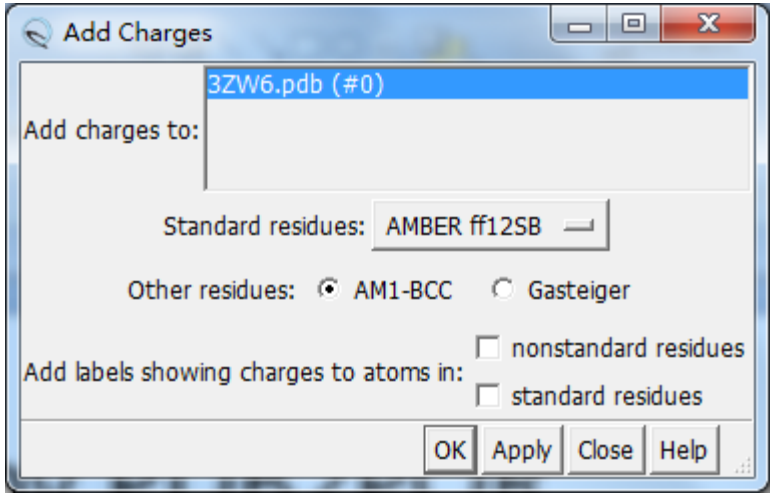
- Close session
- Open pdb 3ZW6
- Only retain the first chain



Add charges for atoms

1



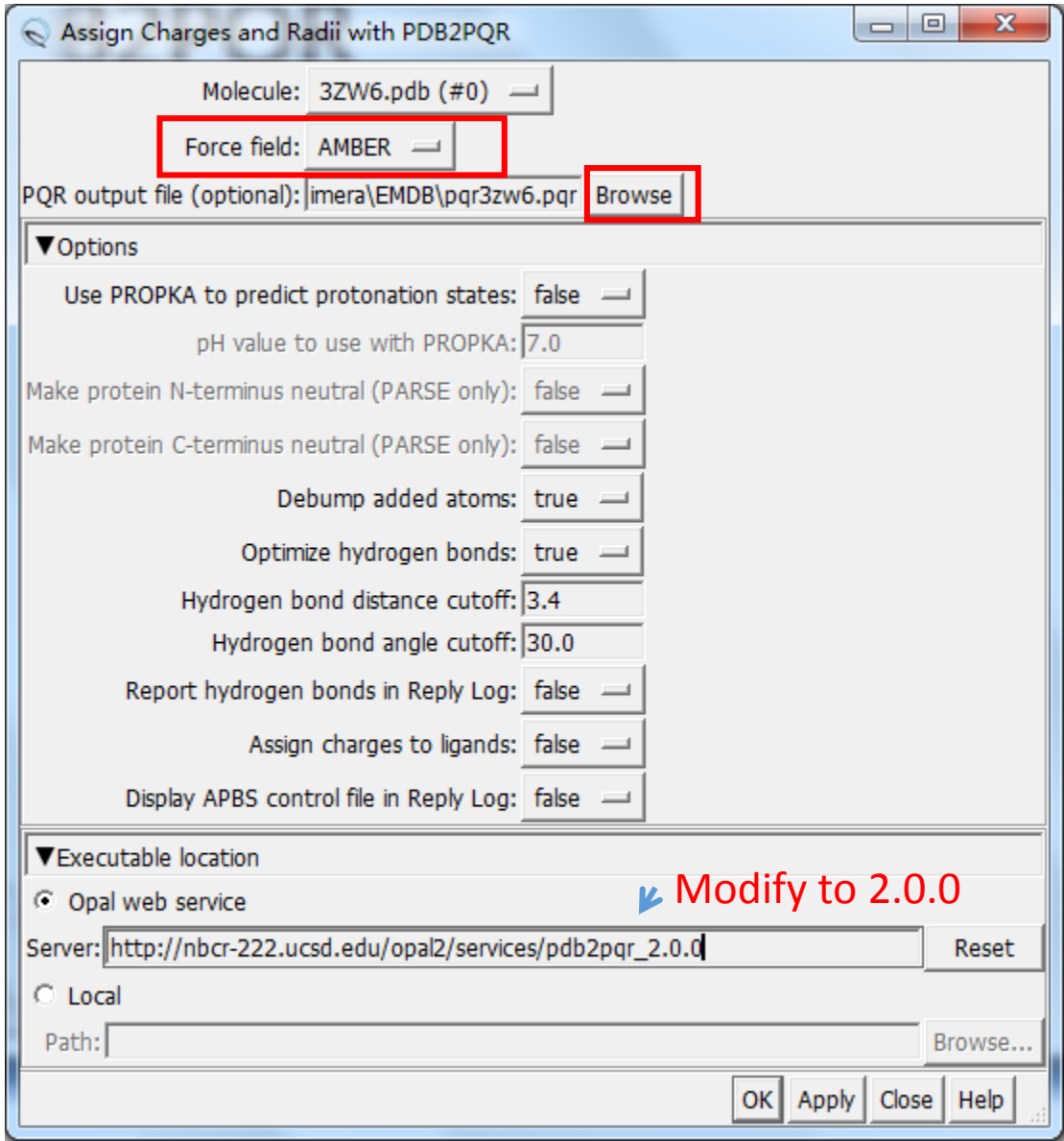


Total charge for #0: -1.000

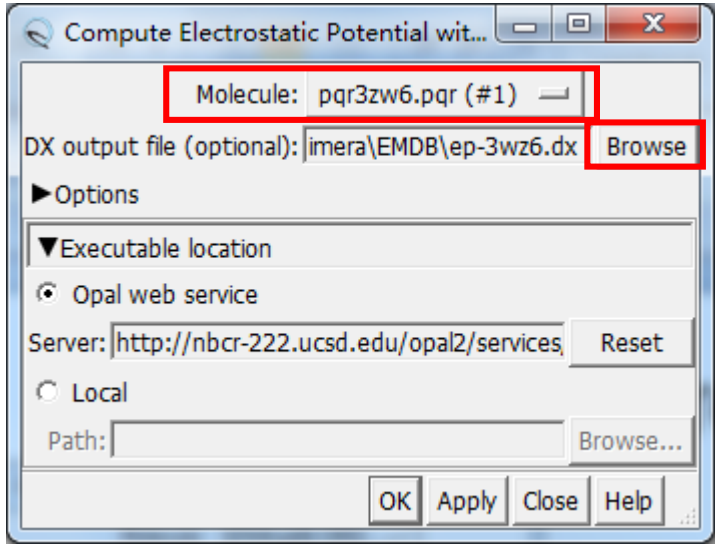
Tools->Structure Editing->
AddH
Add Charge
PDB2PQR

(pdb2pqr will automatically add H)

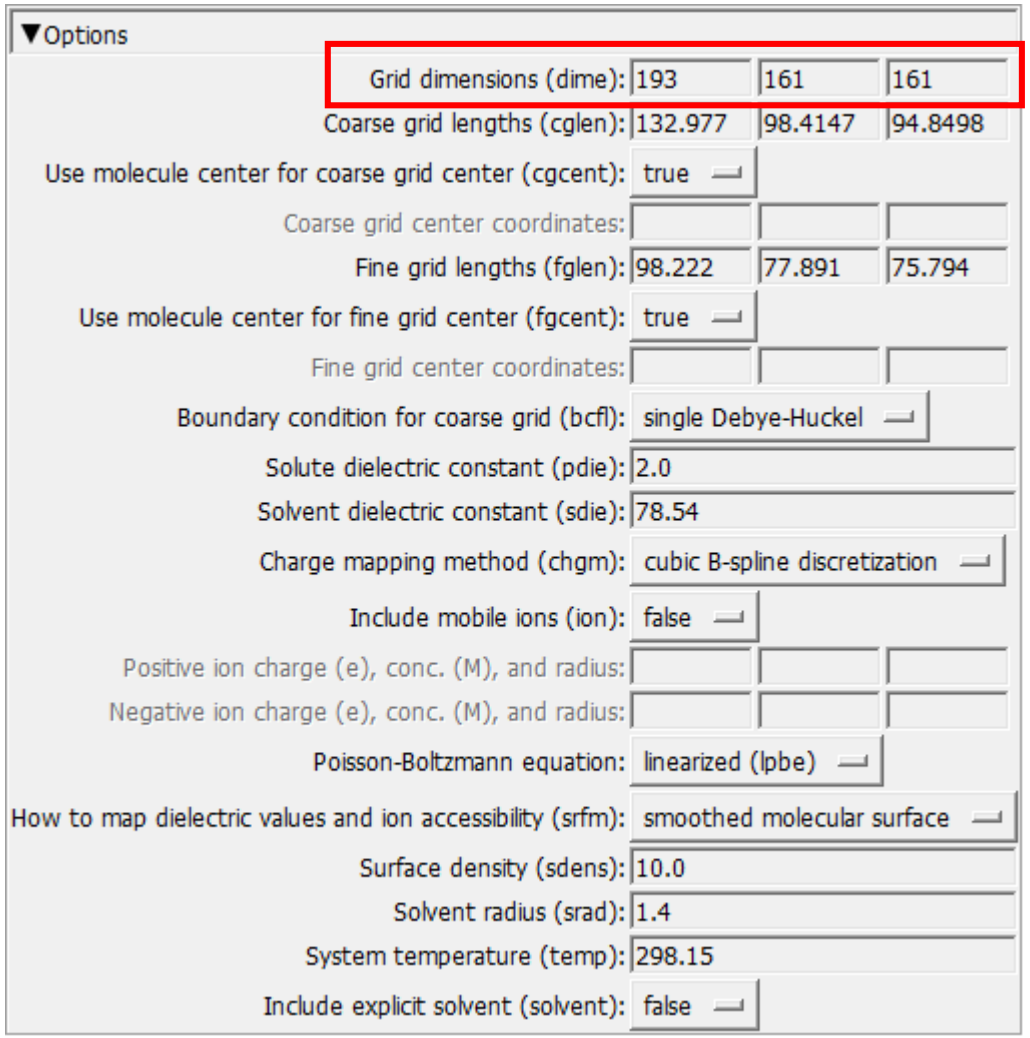
2



Calculate electrostatic potential by APBS

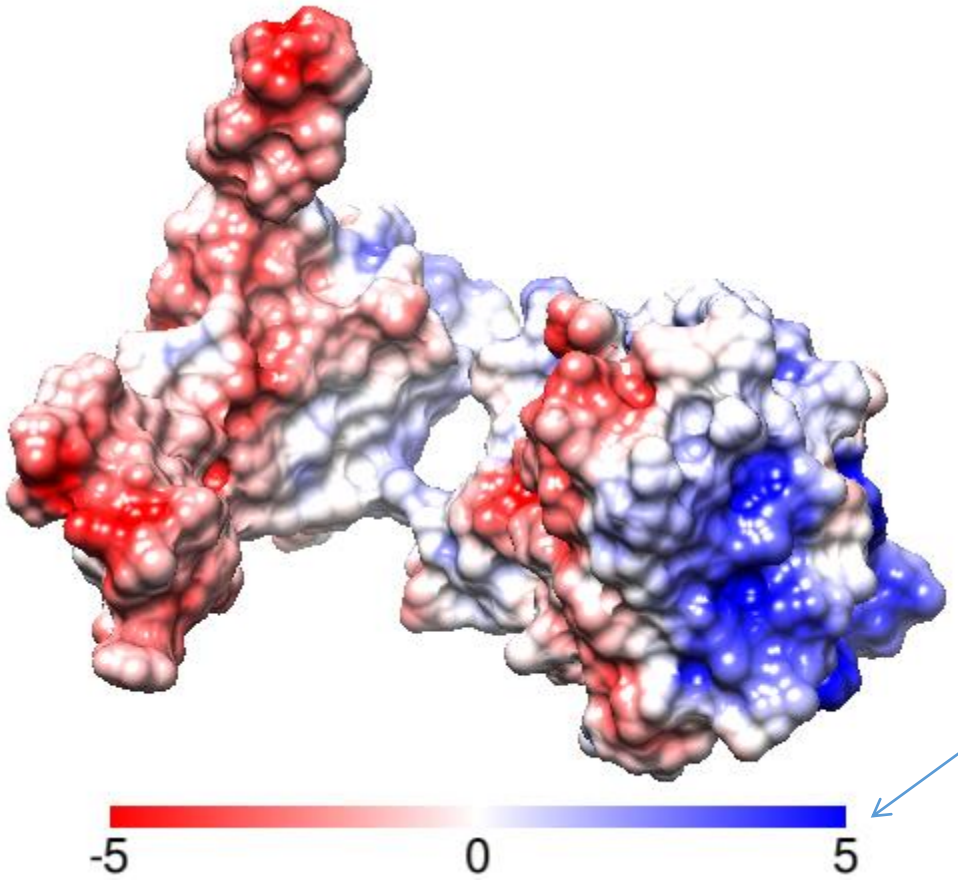


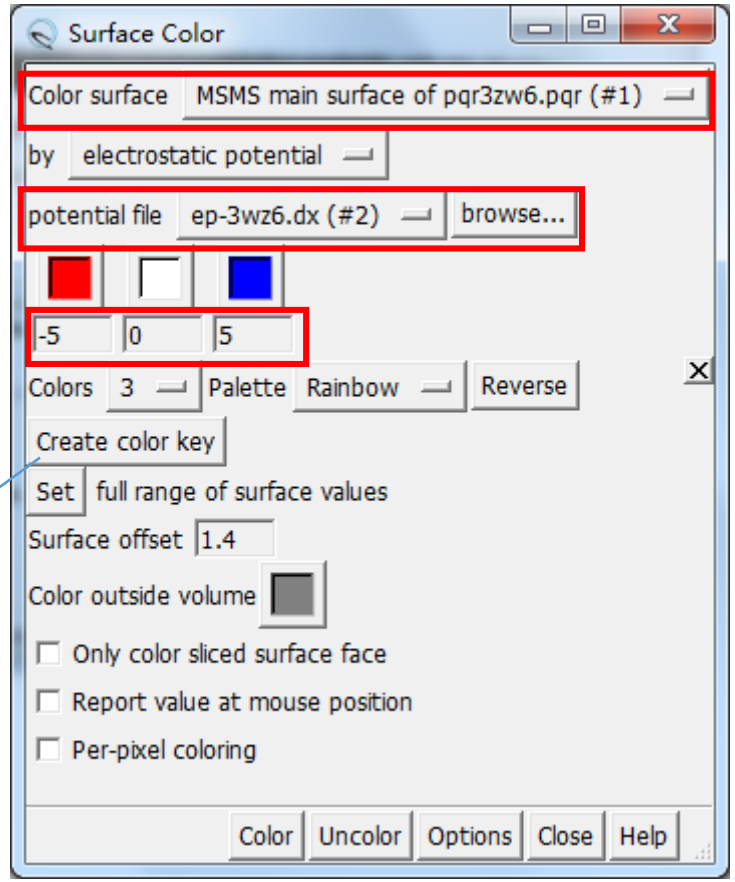
Tools->Surface/Binding analysis->APBS



Change to a smaller map size can accelerate the calculation

Color by electrostatic potential



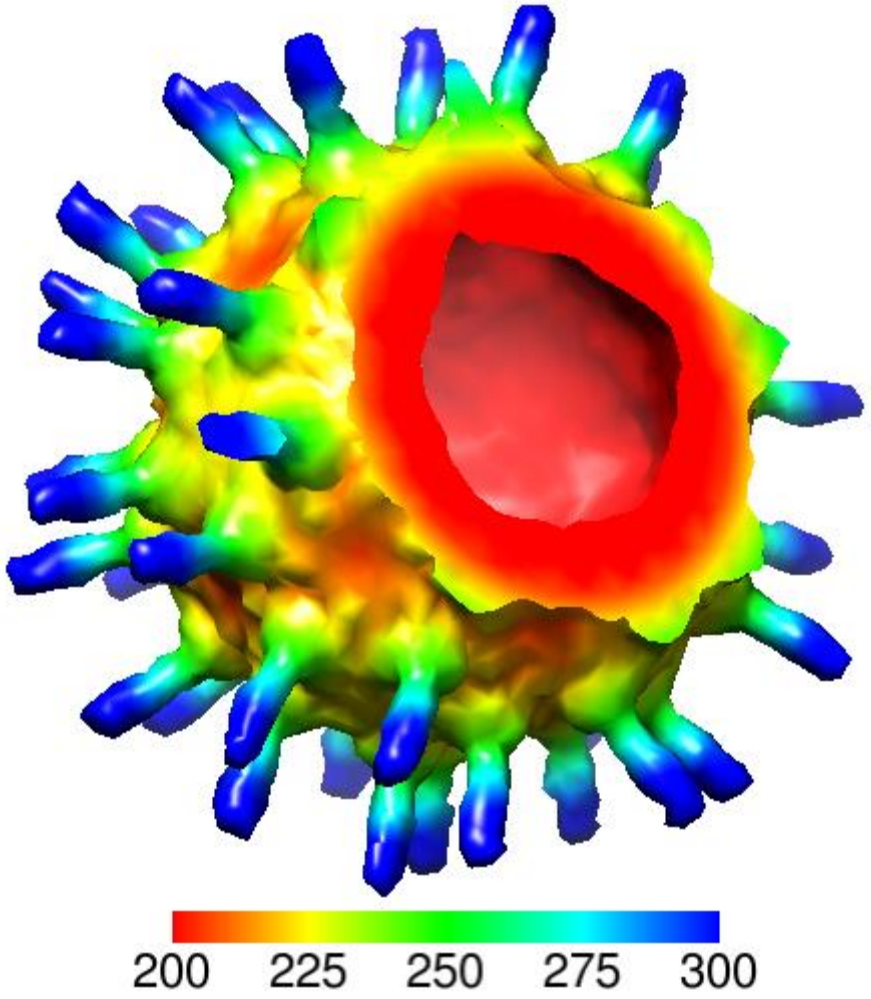


Create a **MSMS** molecular surface: Actions->Surface->Show

Create a volume map for atomic structure: Molmap #1 5

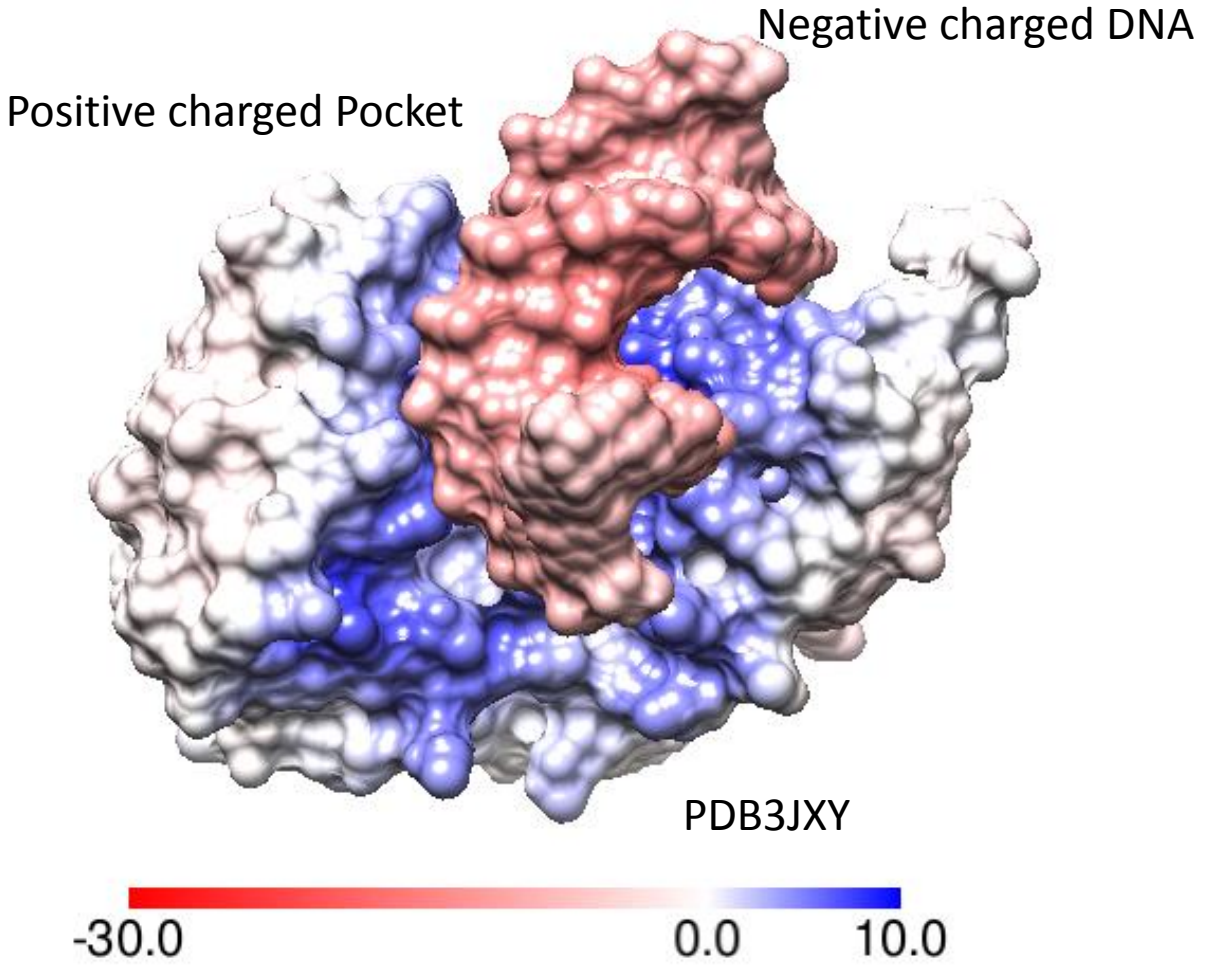
Tools->Surface/Binding analysis->
Electrostatic surface coloring

Practice



EMD1116

Hints:
Tools->Depiction->Per-Model Clipping
Tools->Volume Data->Surface Color



Hints:
Separate protein and DNA
Split #0 atoms :.A atoms :.B,.C
Surface #1

Adjustment of Cryo-EM data

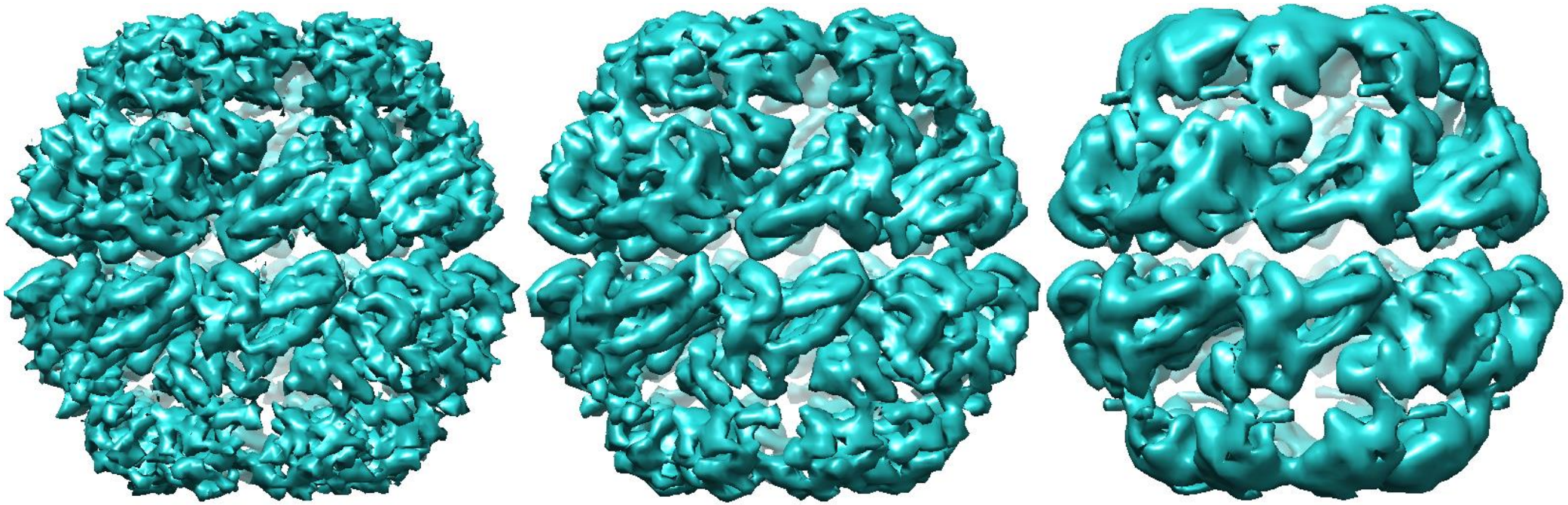
- Smooth the map
- Morph map
- Erase region
- Vop command

- Segmentation

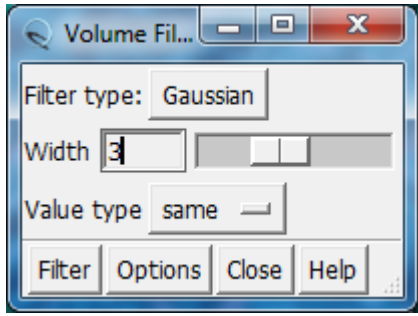
Try: smooth by Gaussian at width at 1.5, scale to 2-fold value and 100*100*100 cells, then save it.

Smooth cryo-EM map

Fetch-EMD:5248
Tools->Volume Data->Volume Filter

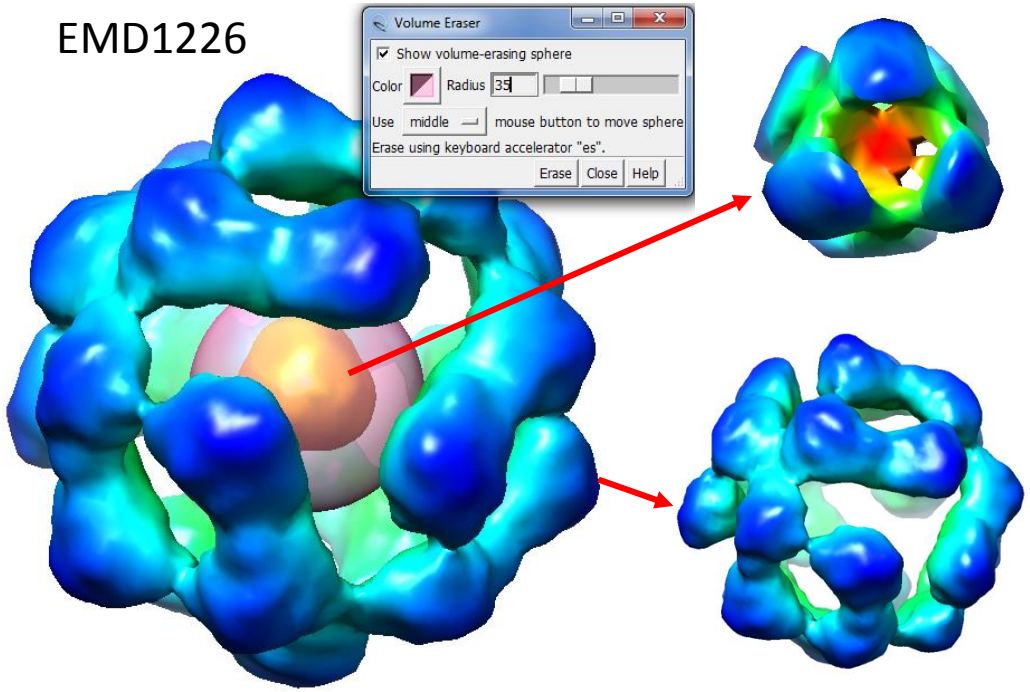


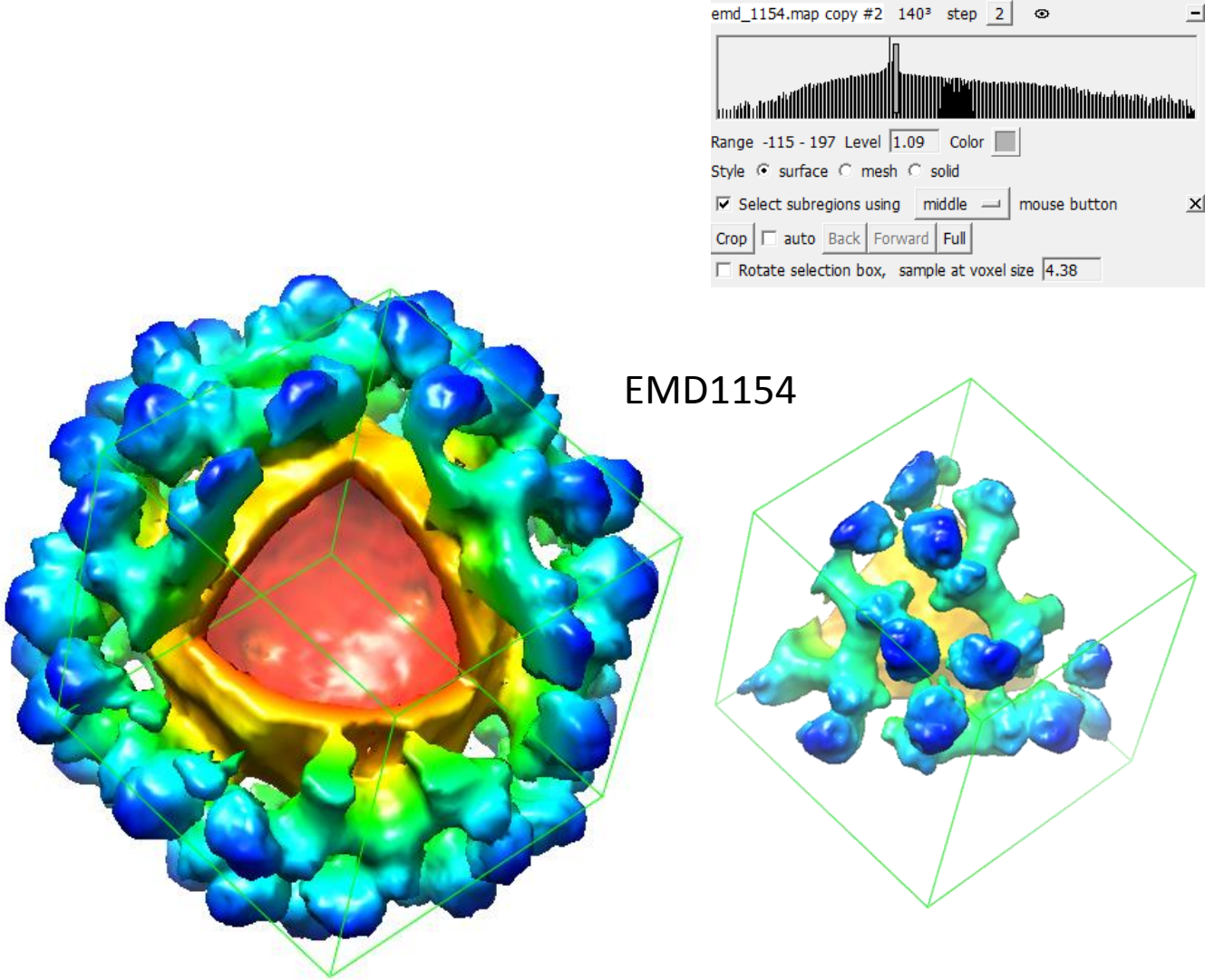
Origin Map | Gaussian Filter at width 1.5 | Gaussian Filter at width 3



- **Volume Filter** includes several options for smoothing or transforming volume data.
- **Gaussian filtering** is the most used for smoothing.
- User can also scale the data values by "**Scale**" or change the map size by "**Bin**" methods.
- The changed map can be saved via "Volume viewer menus->File->Save map as.."

Erase Region





Tools->Volume Data->Volume Eraser

Use **middle** button to select the pink ball and move it.

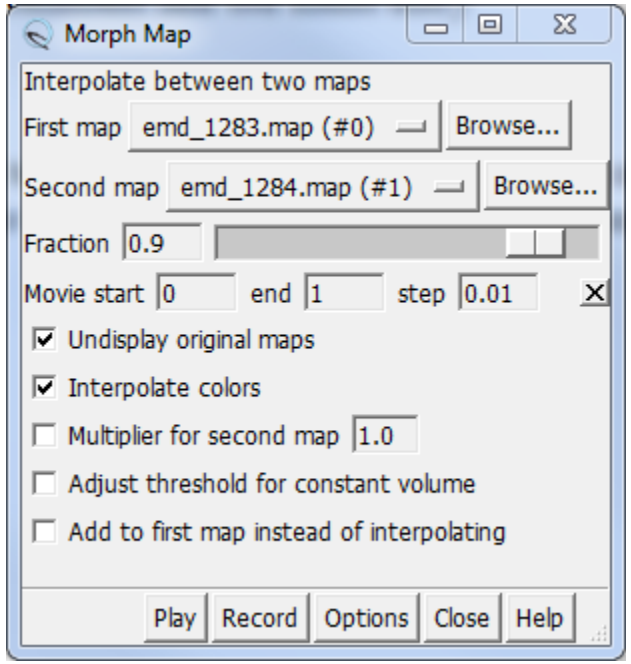
Activate keyboard shortcuts:
Tools->General Controls->Accelerators On
Try press "**eo**" in the window to erase outside

Volume Viewer->Features->Subregion Selection
Use **middle** button to move the **face** to build a subregion.
"Crop" button to retain the subregion
Shortcuts "**eb**" to delete the subregion

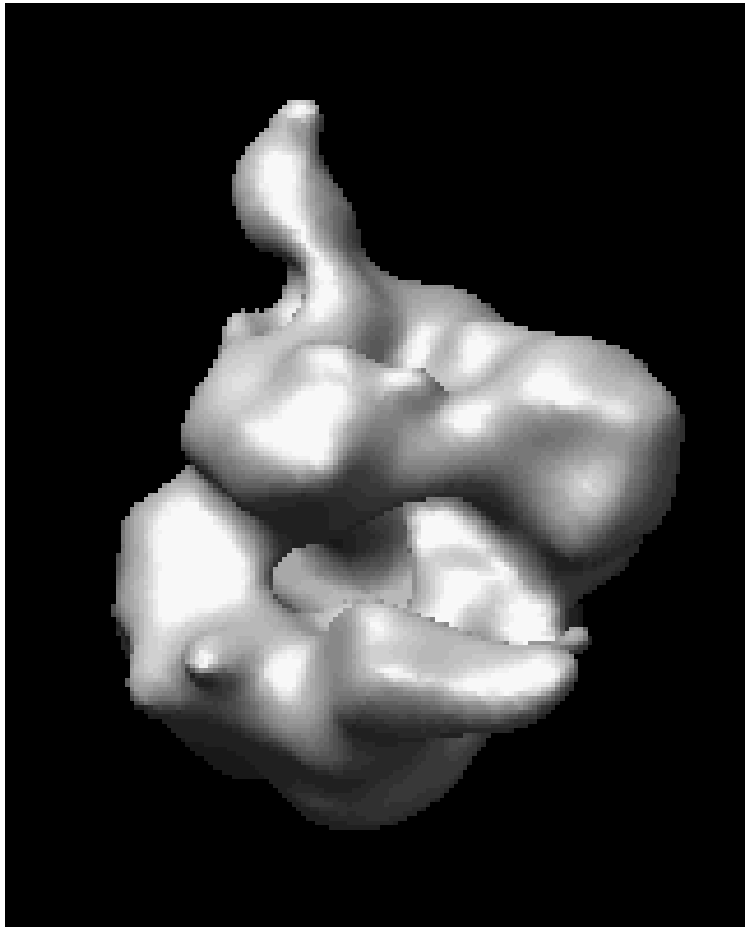
PS: remember to close the subwindow to renew the middle button

Morph map

Morph Map morphs between two related sets of volume data (maps). It can help you to get the possible intermediate map based on linear interpolation.



Tools->Volume data->Morph Map



Command: vop

- Add two map:
  vop add #0,#1
- Map 3 – Map 2
  vop add #2,#1 scaleFactors 1,-1
- Set each value to the minimum at that point in two or more input maps.
  vop minimum #0,#1
- Replace all values below *min(0.1)* value with *newmin(0)*.
  vop replace #0 minimum 0.1 set 0
- More functions:
  http://www.cgl.ucsf.edu/chimera/current/docs/UsersGuide/midas/vop.html

Handle cryo-EM map data by Matlab

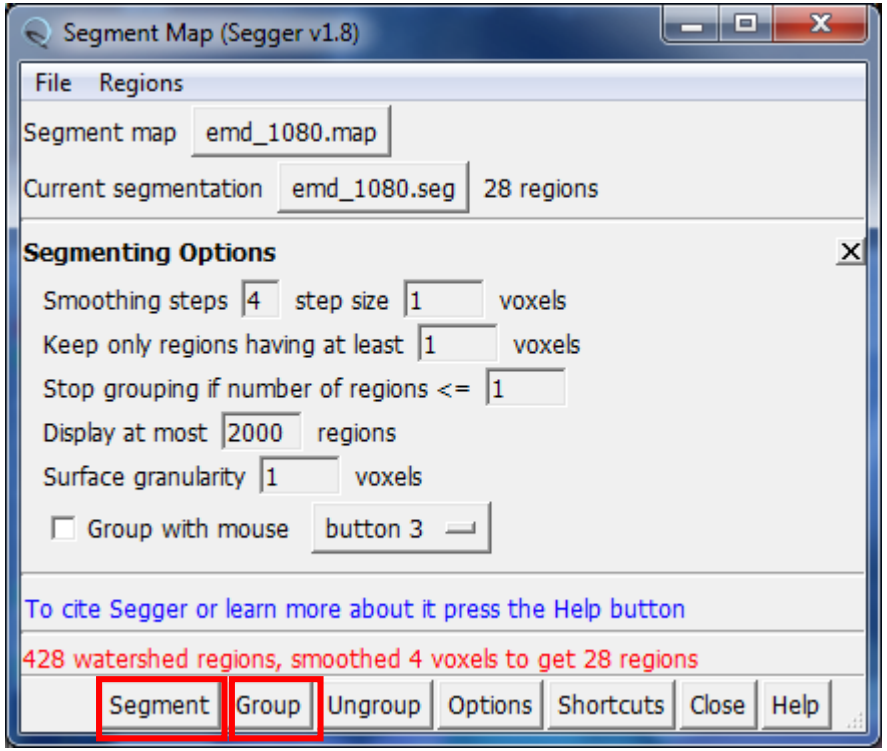
```
function a= readMRCfile(fname)
% readMRCfile readMRCfile (fname)
[fid,message]=fopen(fname,'r');
if fid == -1
   error('can''t open file');
   a= -1;
   return;
end
nx=fread(fid,1,'long');
ny=fread(fid,1,'long');
nz=fread(fid,1,'long');
type= fread(fid,1,'long');
fprintf(1,'nx= %d ny= %d nz= %d type= %d', nx, ny,nz,type);
% Seek to start of data
status=fseek(fid,1024,-1);
% Shorts
if type== 1
   a=fread(fid,nx*ny*nz,'int16');
end
%floats
if type == 2
   a=fread(fid,nx*ny*nz,'float32');
end
fclose( fid);
a= reshape(a, [nx ny nz]);
if nz == 1
   a= reshape(a, [nx ny]);
end
```

Most map file for cryo-EM is the **MRC** format file. It's a **binary** file and has a 1024 bytes head. 1~3 long word for the map size (nx, ny , nz) and the forth one is the data type for the isovalue stored in the map.

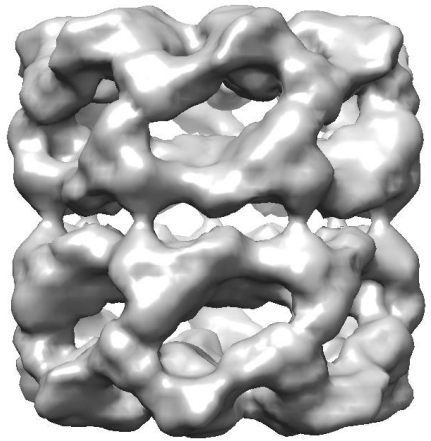
http://ami.scripps.edu/software/mrctools/mrc_specification.php

Segmentation

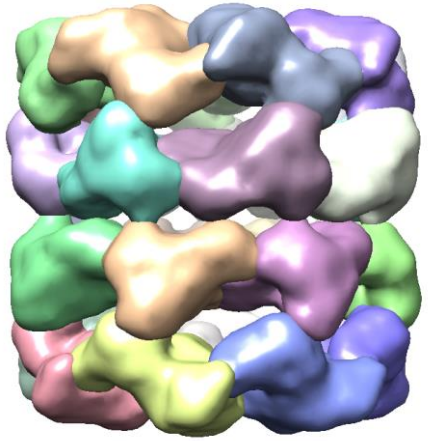
Tools->Volume Data->Segment Map



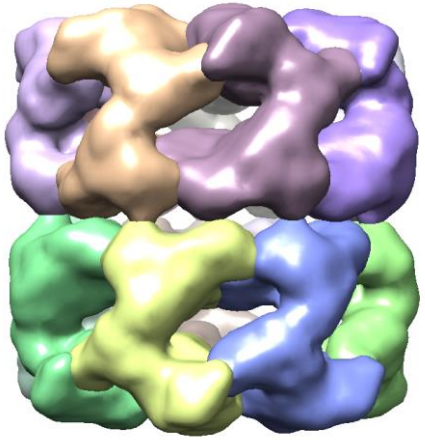
Try steps 1 and 'segment', then 'group'. Notice the voxels and regions.



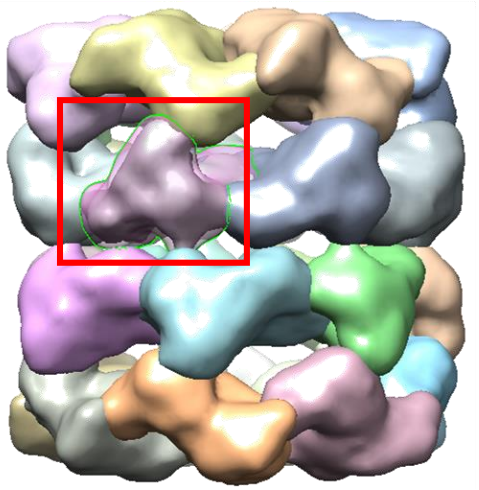
EMD1080. Level1.12



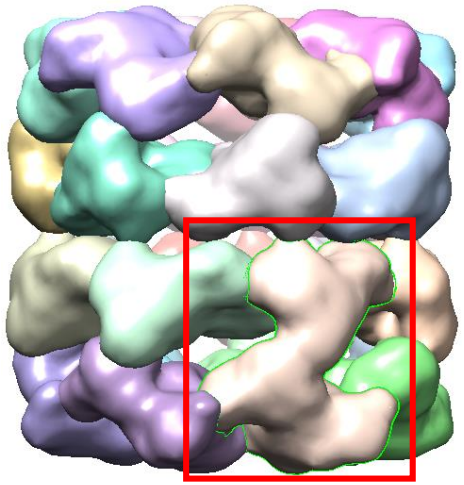
Segmentation



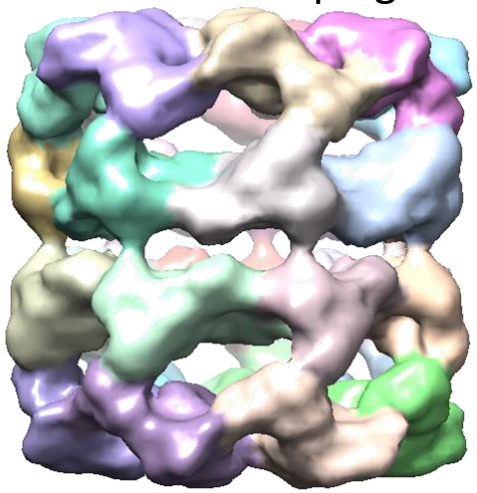
Further Grouping



Select one and transparent



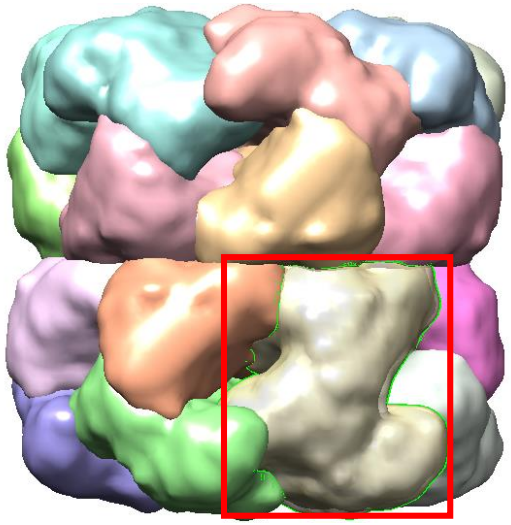
Group selected



Color map based on segments

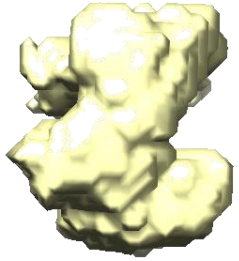
The Segmentation uses the Segger program with the watershed algorithm.

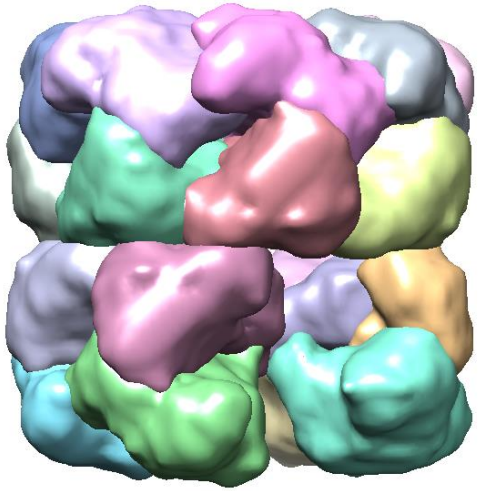
Segmentation



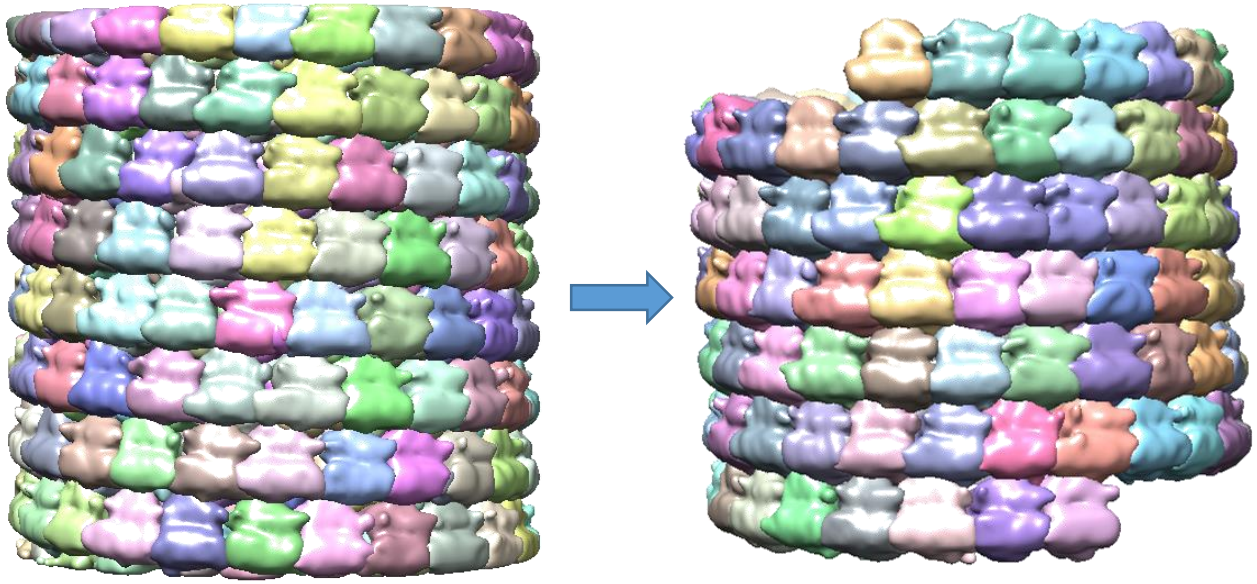
EMD1080,level0.2
Select two regions

Save select region as map

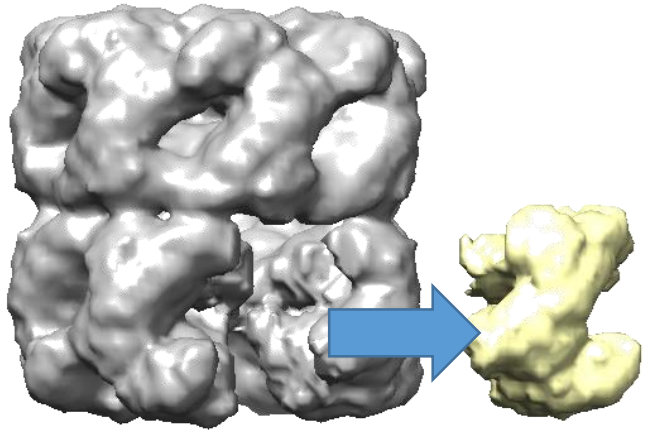




Subtract selected from map



Try to remove incomplete segment from EMD1129



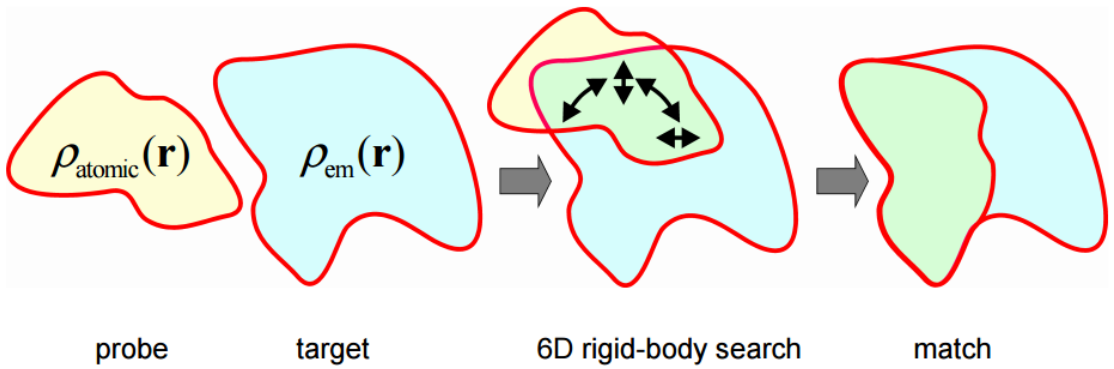
Vop add #0,#1

Fitting atomic structure into Cryo-EM map

- Rigid Fitting by Chimera

- Flexible Fitting by MDFF

Rigid Fitting



- Atoms-in-map

The average map value at fit atom positions is maximized. For each atom within the bounds of the reference map, the map value is found by trilinear interpolation from the eight corners of the enclosing data grid cell.

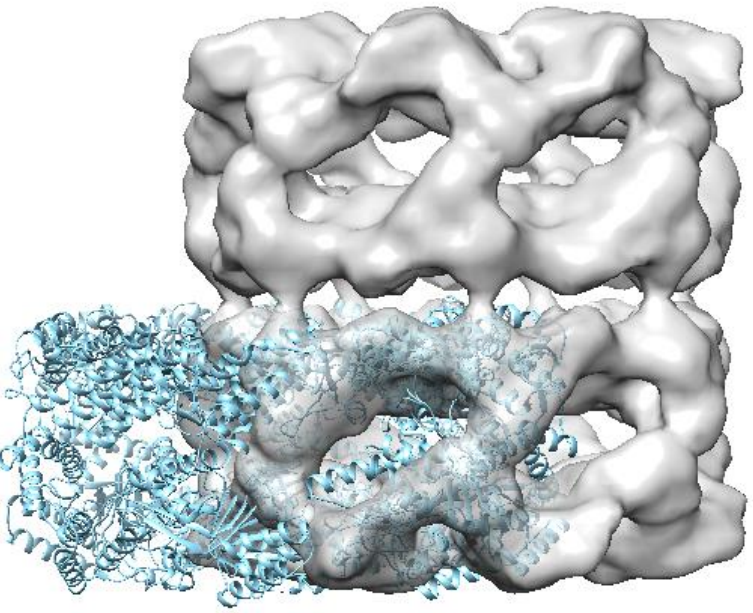
- Map-in-map

For atomic structure, the volumetric data as a probe map at a certain resolution has to be built in advance. Usually, the Gaussian kernel is used to reconstruct the map for atomic structure. The correlation value is maximized. There are many forms for the correlation.
In Chimera, the ***overlap*** is the sum over fit map grid points of the product of the fit map value and the reference map value at that point, determined by trilinear interpolation.

$$overlap = \langle \mathbf{u},\mathbf{v} \rangle \qquad correlation = \frac{\langle \mathbf{u},\mathbf{v} \rangle}{|\mathbf{u}||\mathbf{v}|} \qquad CCC = \frac{\langle (S - \langle S \rangle)(E - \langle E \rangle) \rangle}{\sigma_S \sigma_E}$$

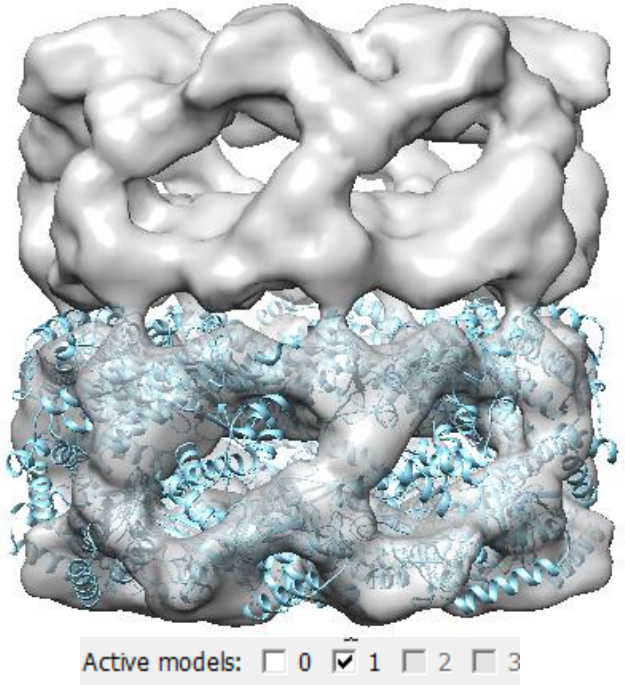
The most used tools for rigid fitting is **Chimera** and Situs.

Rigid Fitting: Atom in Map

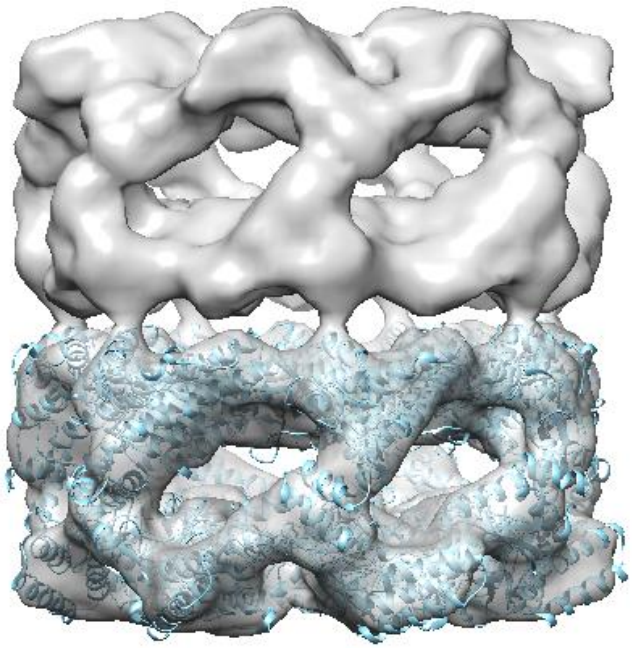


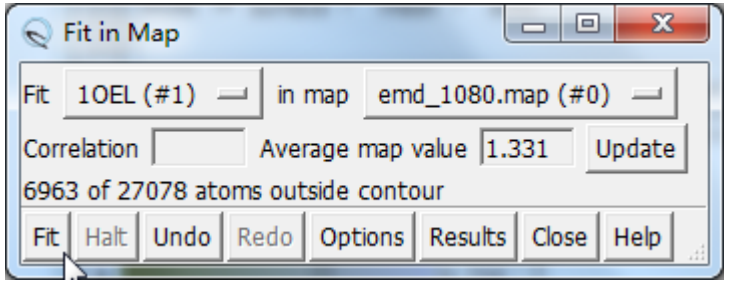
EMD1080 & PDB1OEL

Set transparent for EMD map



Fix map and move PDB into it



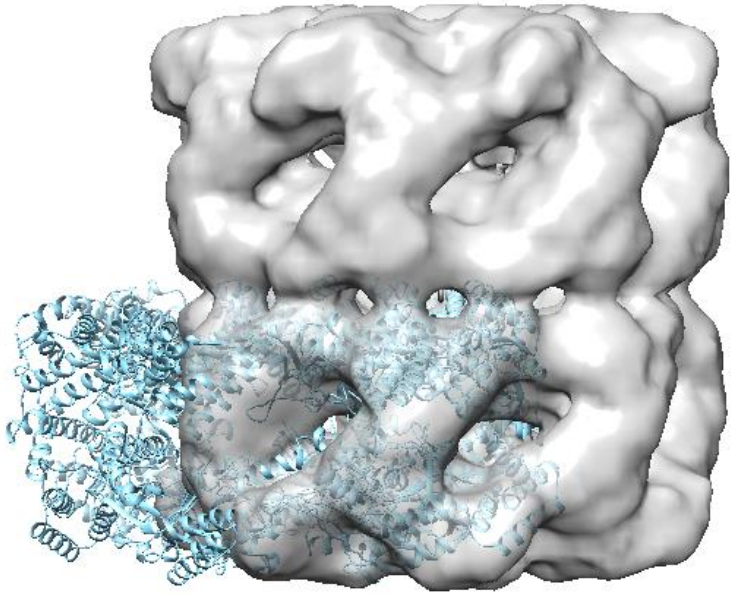


Volume Data->Fit in Map

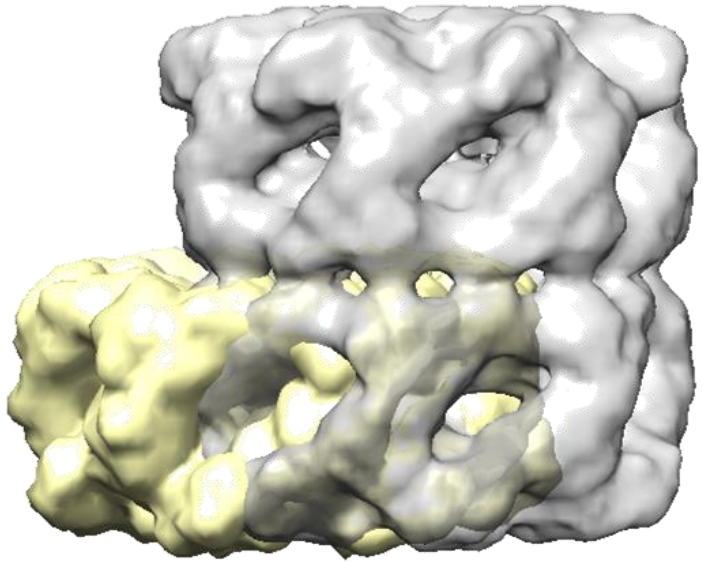
Commands: **Fitmap** #1 #0

Try move PDB a little and press ‘Update’

Rigid Fitting: Map in Map

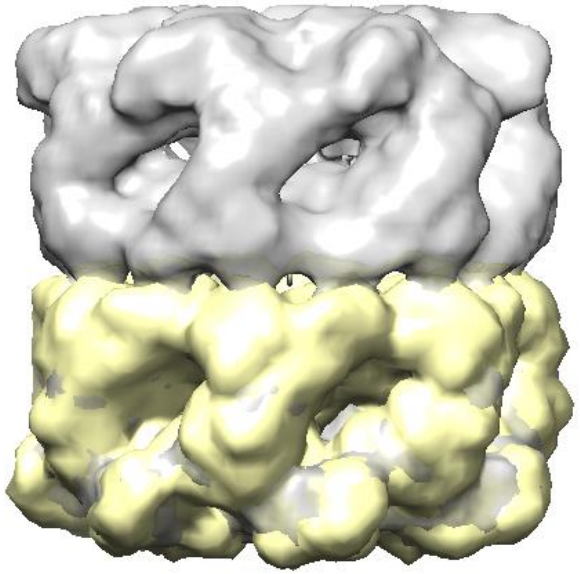


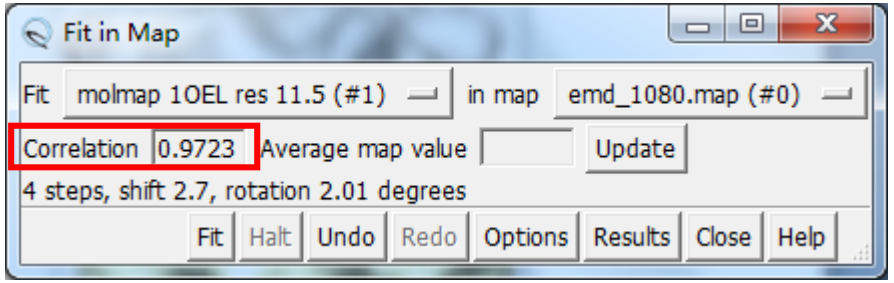
Reopen EMD1080 & PDB1OEL
Here map level I used at 0.5



Generate a reconstructed map for PDB
Molmap #1 11.5

Resolution at 11.5 A

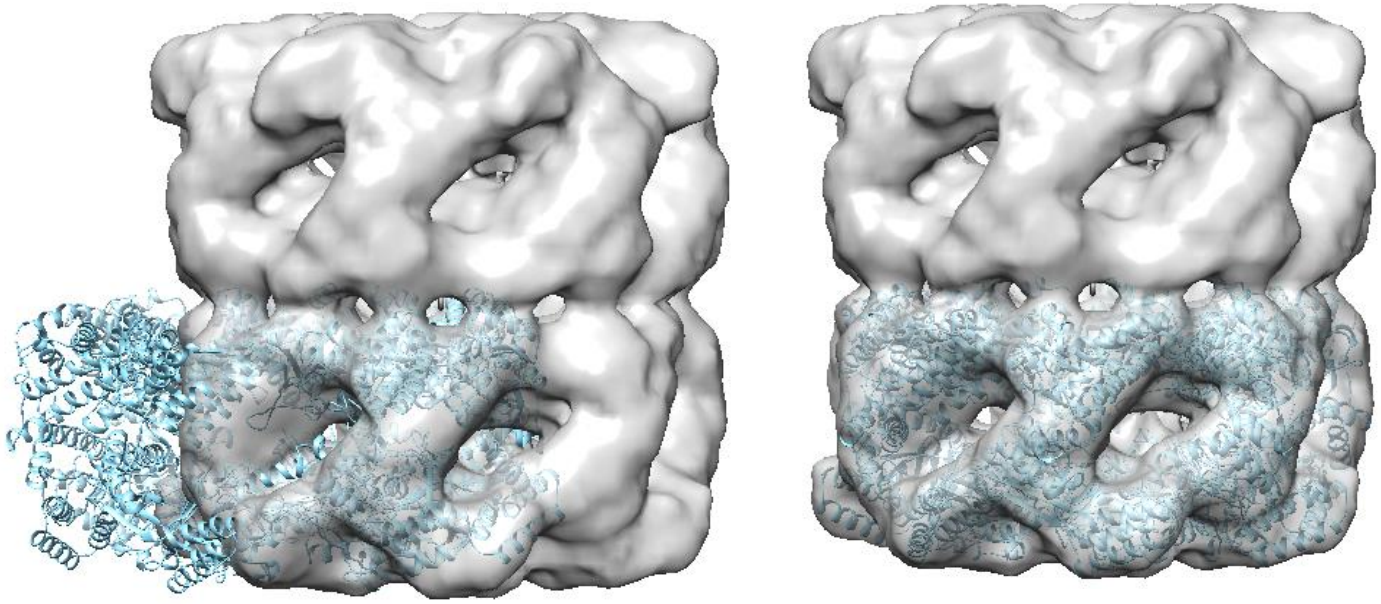




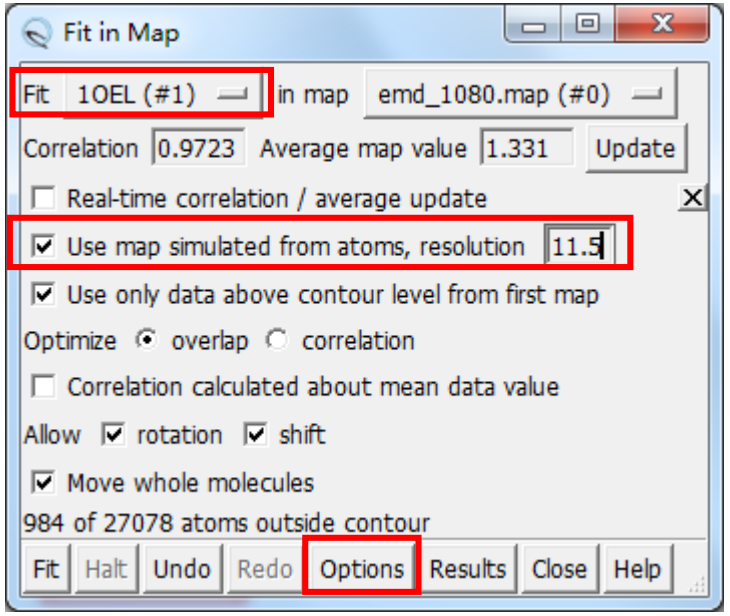
Volume Data->Fit in Map
Choose the reconstructed map for fitting

Try move PDB a little and press ‘Update’

Rigid Fitting: Map in Map



Reopen EMD1080 & PDB1OEL
Here map level I used at 0.5

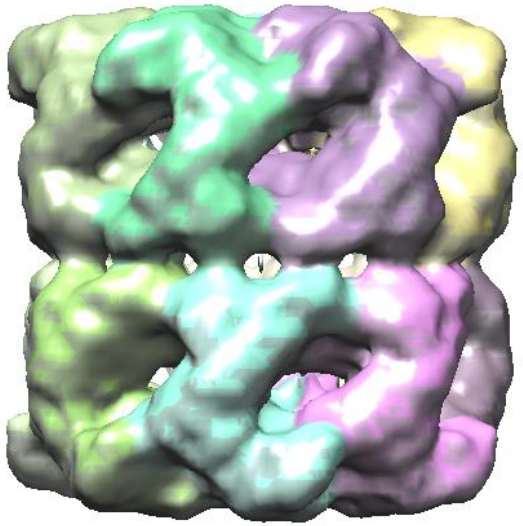


Volume Data->Fit in Map
Option and select the use map from atoms
Resolution at 11.5

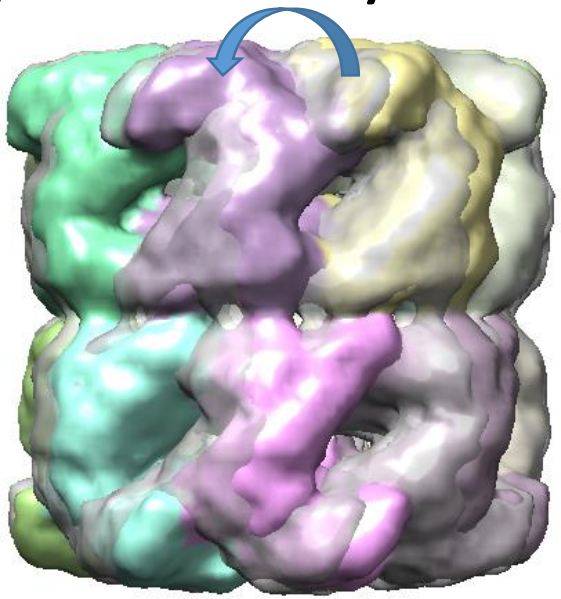
It's the same as the previous map-in-map method.
It'd better to move the PDB structure to its possible position before fitting! Specially when the map it's complicated.

Commands: **Fitmap** #1 #0 **res** 11.5

Measure Symmetry

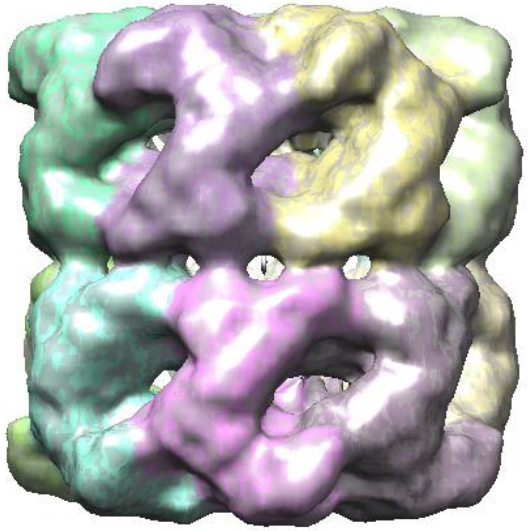


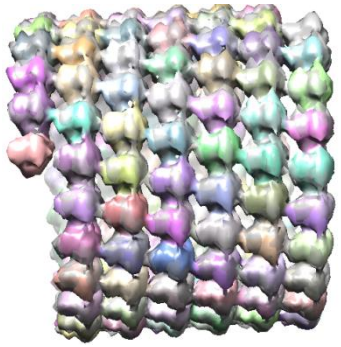
Same maps fitting as before



Rotate to next fitting pose

Fitting





Try 1129-remove

Favorites->Reply Log

Fit map emd_1080.map in map emd_1080.map using 65406 points
correlation = 0.9998, correlation about mean = 0.9984, overlap = 1.08e+05
steps = 60, shift = 0.00746, angle = 8.43 degrees
Position of emd_1080.map (#1) relative to emd_1080.map (#0) coordinates:
Matrix rotation and translation
0.62345646 0.78185807 -0.00001558 -0.00122013
-0.78185807 0.62345646 0.00008531 0.00001018
0.00007641 -0.00004100 1.00000000 0.02749075
Axis -0.00008078 -0.00005883 -1.00000000
Axis point -0.00060228 0.00127333 0.00000000
Rotation angle (degrees) 51.43101493
Shift along axis -0.02749065

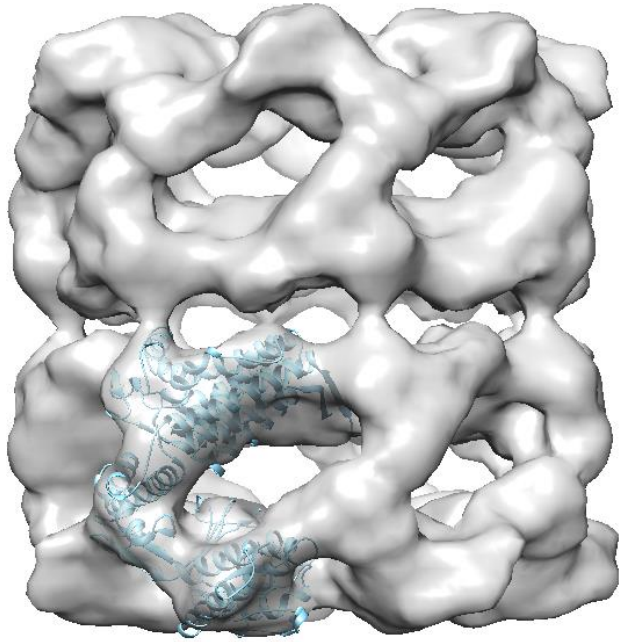
Obtain symmetric information, such as rotation 51.4 degree for C7 symmetry.

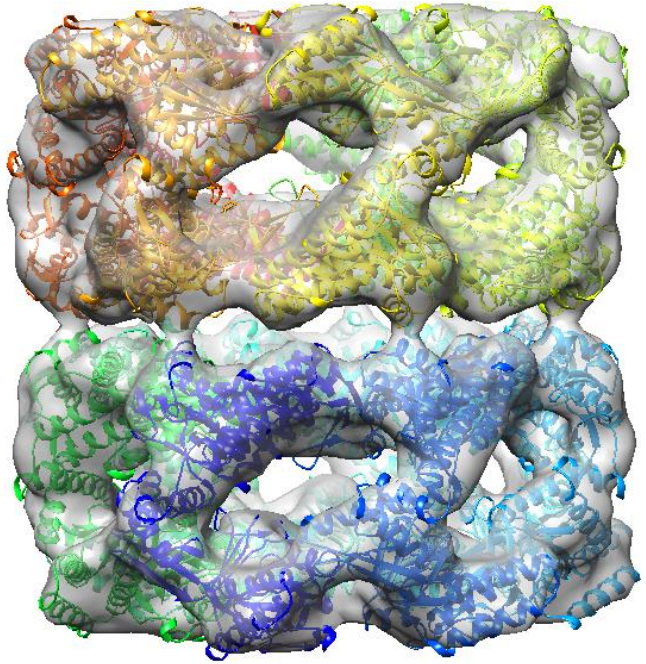
Use command to measure and save symmetry into map:
Measure sym #0

```
Symmetry emd_1080.map: D7, center 50 50 50
```

Assign symmetry to map: **Volume** #0 **sym** sym-type

Symmetrize





sym [molmodel] [*options*]

Group *model*: use symmetry from given model

Group *symmetry*: use given symmetry

update *true*: update complex based on unit position

Biomtset true: write symmetric information to structure and PDB

More options please refer to the 'help-command index'.

Measure sym #0

sym #1 group #0

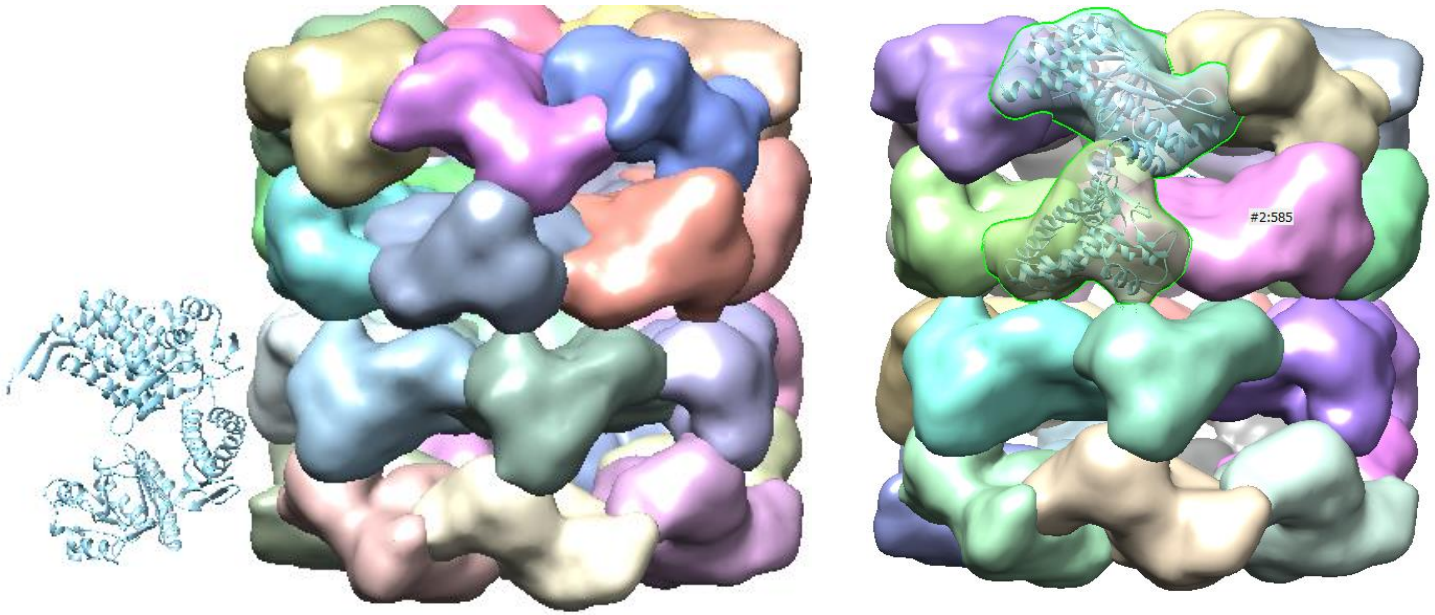
Rainbow model #1,#2

~sym

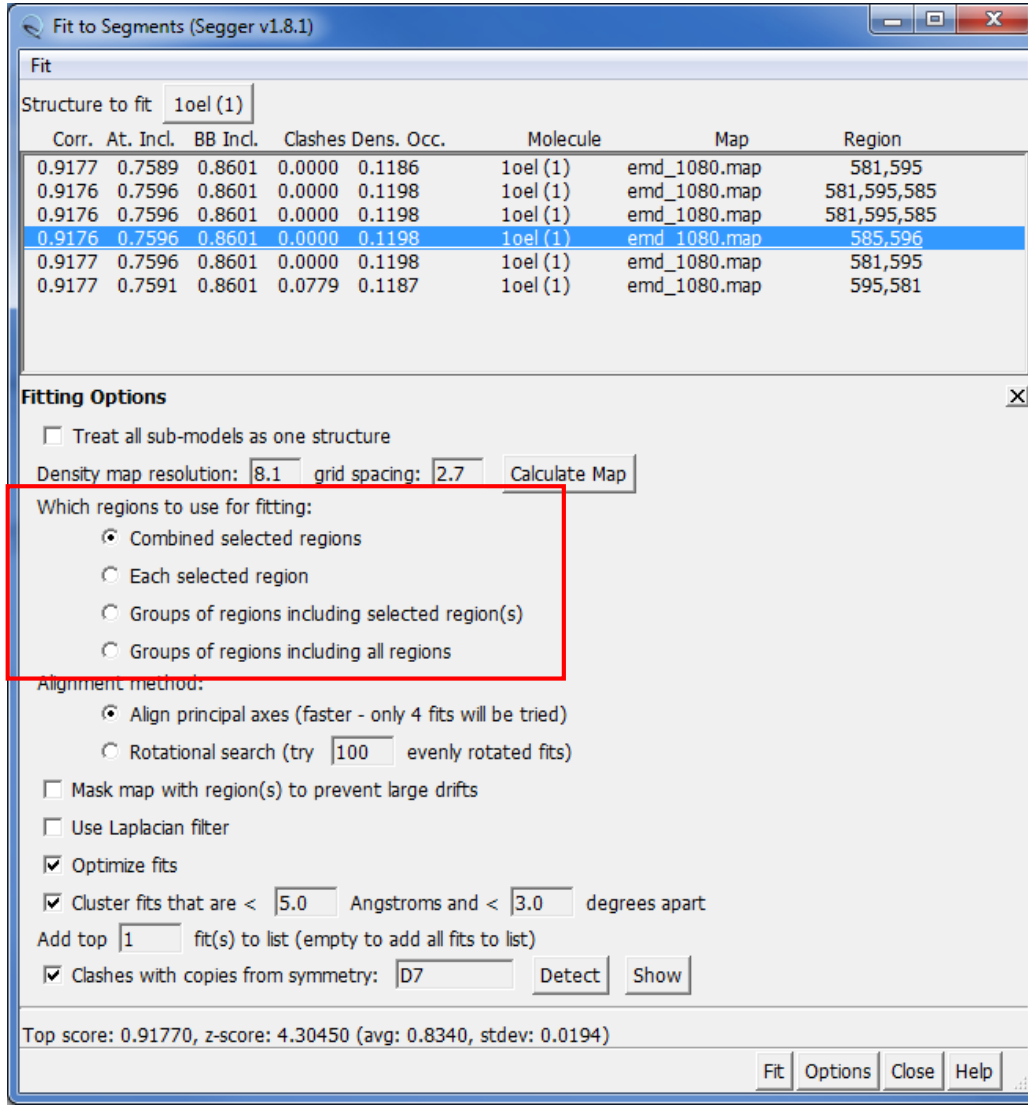
Sym #1 group #0 update true

Try to move the unit monomer.

Fit into Segment



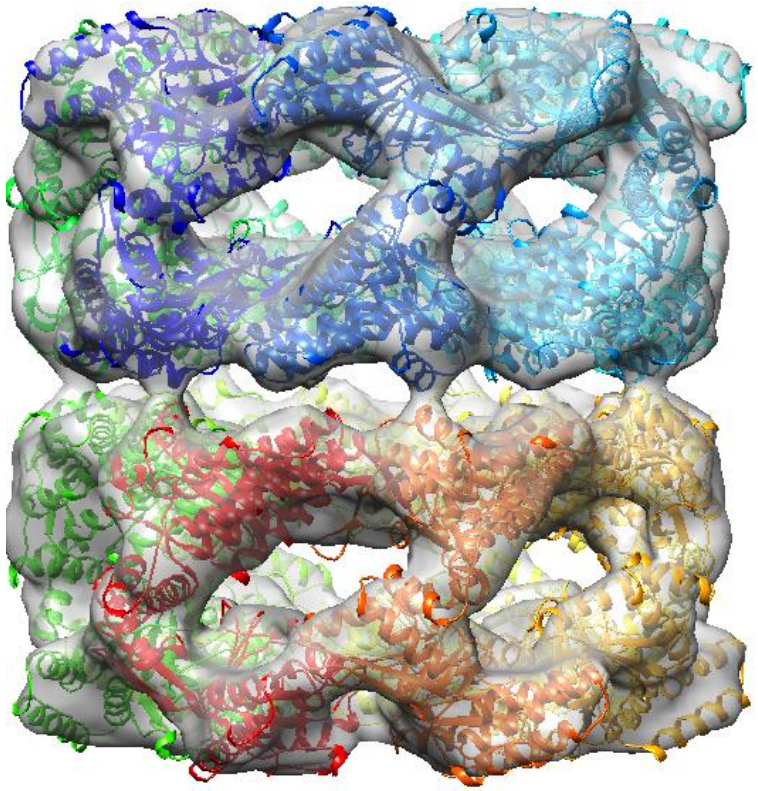
EMD:1080,PDB:1OEL

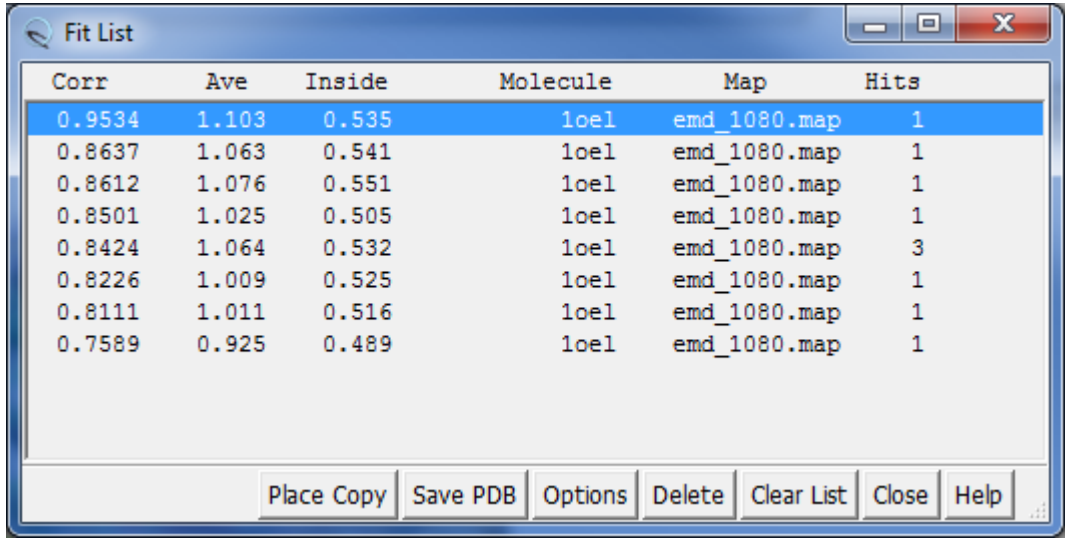


Only use selected regions as the map for fitting.
It depends on the segmentation result and segments you selected.

G.D. Pintilie et al. J. Struct. Biol. 2010, 427–438

Rigid Fitting: Global Searching





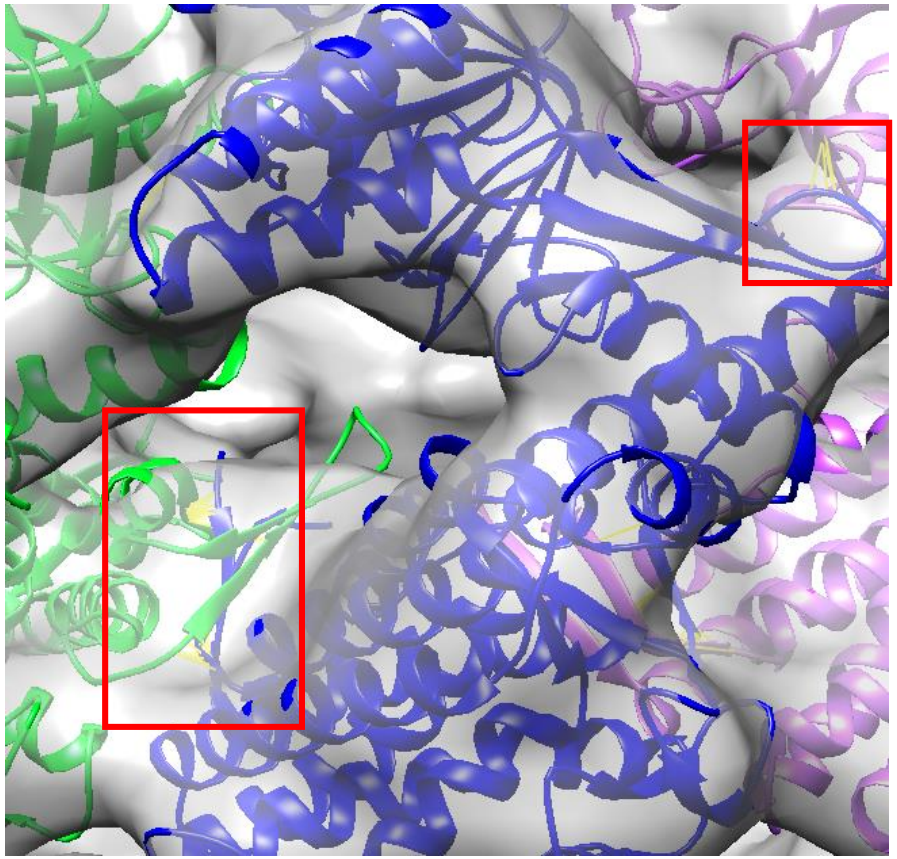
Corr	Ave	Inside	Molecule	Map	Hits
0.9534	1.103	0.535	1oel	emd_1080.map	1
0.8637	1.063	0.541	1oel	emd_1080.map	1
0.8612	1.076	0.551	1oel	emd_1080.map	1
0.8501	1.025	0.505	1oel	emd_1080.map	1
0.8424	1.064	0.532	1oel	emd_1080.map	3
0.8226	1.009	0.525	1oel	emd_1080.map	1
0.8111	1.011	0.516	1oel	emd_1080.map	1
0.7589	0.925	0.489	1oel	emd_1080.map	1

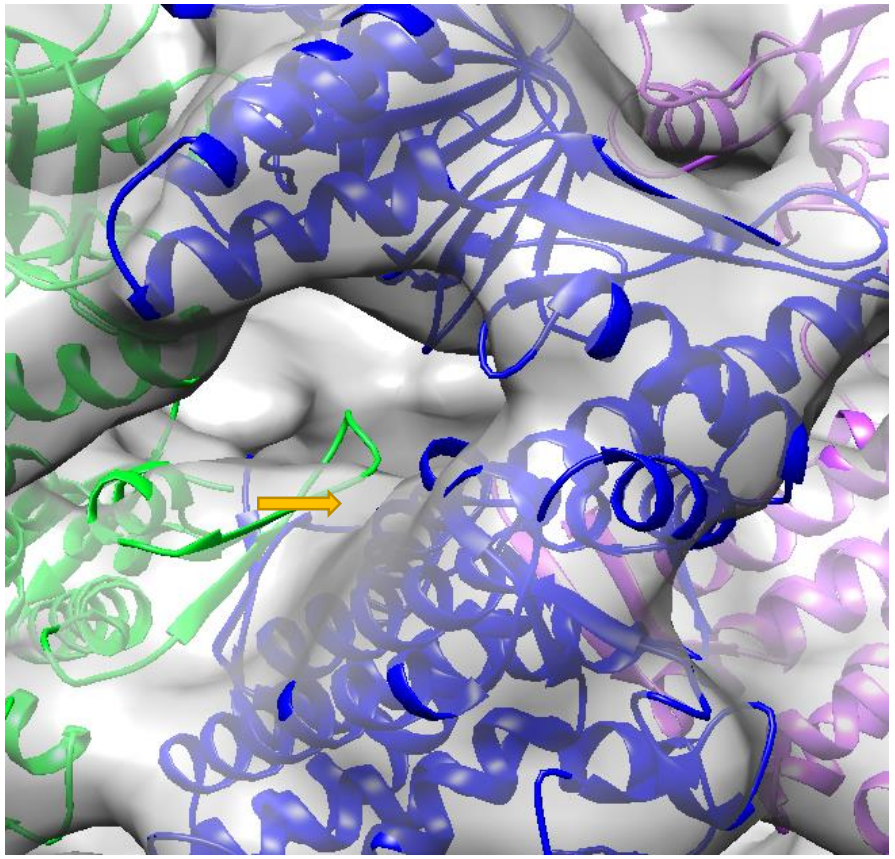
Fitmap #1 #0 res 11.5 **search** 10 asym true
Search N: number of initial placement for further fitting. Default: 0
Asym true/false: whether to only list asymmetric results. Default: True

measure sym #0
Sym #1 group #0 update true
Fit #1 #0 res 11.5 search 10

- Global searching could help to find many possible fitting results automatically. It doesn't depend on the original position.
- Global searching can only be used in command line.

Rigid Fitting: using Symmetry



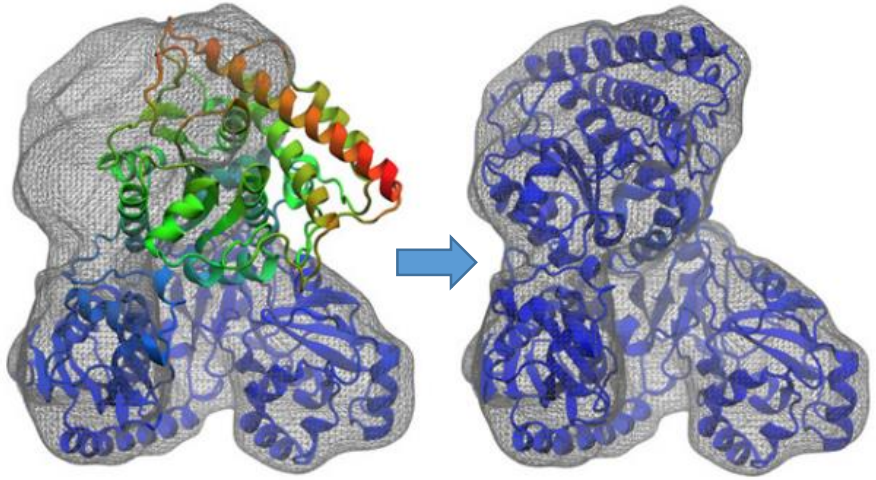


- findclash #1 intersubmodel true
- fitmap #1 #0 res 11.5 **sym true**

Yellow lines mean the clash between models.

- There may be many clashes between the units after symmetrization because the fitting don't consider the symmetric units. We can use "sym" option when doing fitting. It's often used after global searching.
- "Sym" option could only be used for map-in-map fitting and so "res" option is always used. "sym" option could not be used in global searching or sequential fitting.

Flexible fitting by MDFF



RMSD to target structure: 0.0 7.3 14.6 21.9 29.2 36.5

- Flexible fitting of carbon monoxide dehydrogenase

An external potential U_{EM} derived from the EM map is introduced into an MD simulation to steer the atoms into high-density regions. $\Phi(\mathbf{r})$ ovalue in EM map and is thre Φ_{thr} to disregard data.

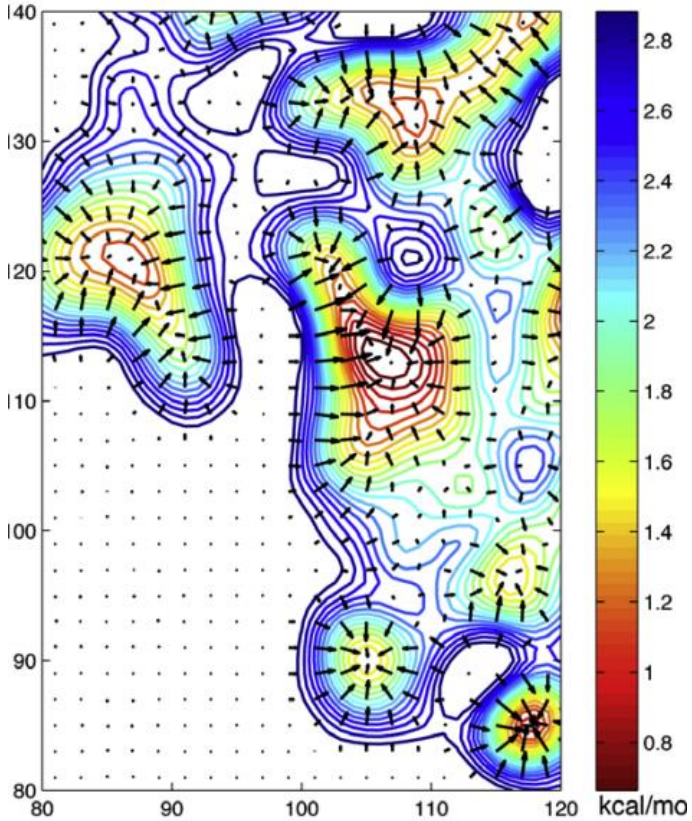
The stereochemical quality of the structure is preserved by the MD force field and also through harmonic restraints (U_{SS}) applied to enforce the integrity of secondary structure.

$$U_{total} = U_{MD} + U_{EM} + U_{SS}$$

$$U_{EM}(\mathbf{R}) = \sum_i w_j V_{EM}(\mathbf{r}_j)$$

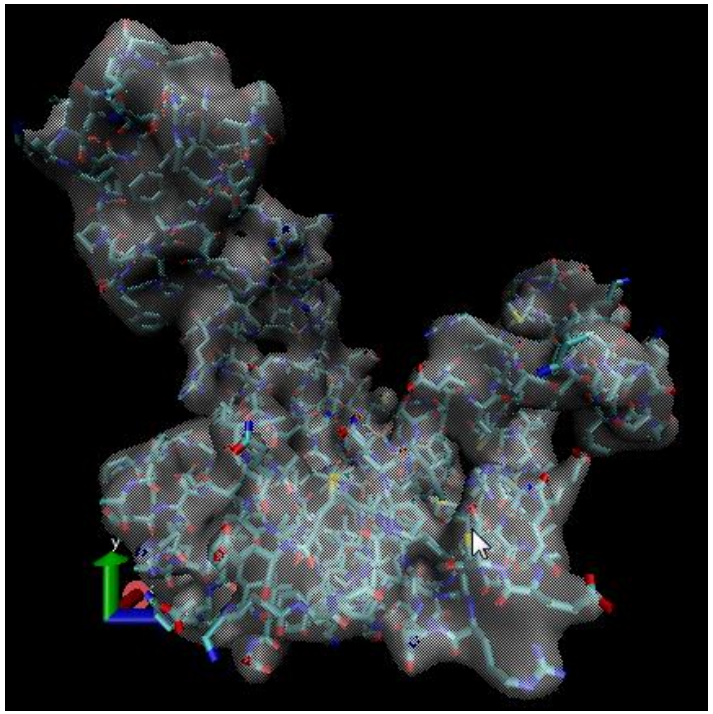
$$V_{EM}(\mathbf{r}) = \begin{cases} \xi\left[1 - \frac{\Phi(\mathbf{r})-\Phi_{thr}}{\Phi_{max}-\Phi_{thr}}\right] & \text{if } \Phi(\mathbf{r}) \geq \Phi_{thr}, \\ \xi & \text{if } \Phi(\mathbf{r}) < \Phi_{thr}. \end{cases}$$

$$\mathbf{f}_i^{EM} = -\frac{\partial}{\partial \mathbf{r}_i} U_{EM}(\mathbf{R}) = -w_i \frac{\partial}{\partial \mathbf{r}_i} V_{EM}(\mathbf{r}_i)$$

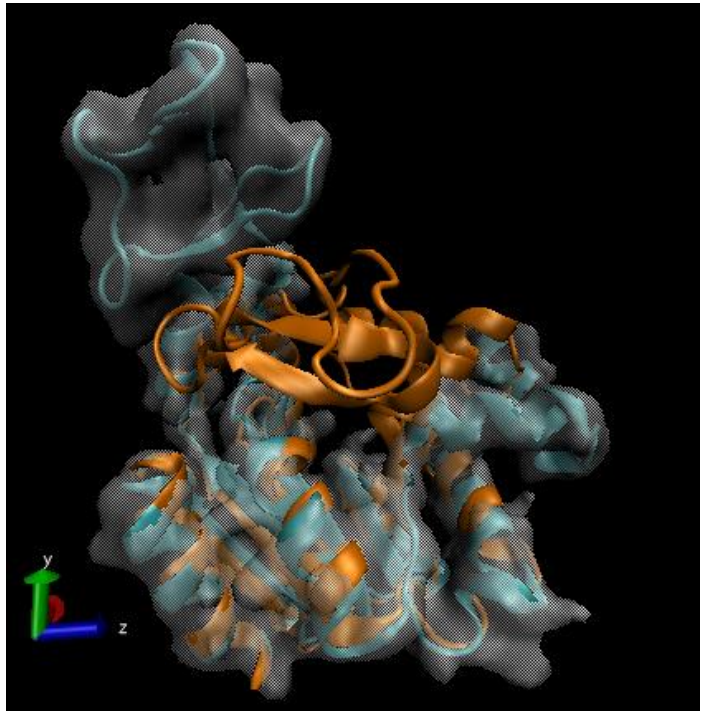


Potential and Force in a 2D slice

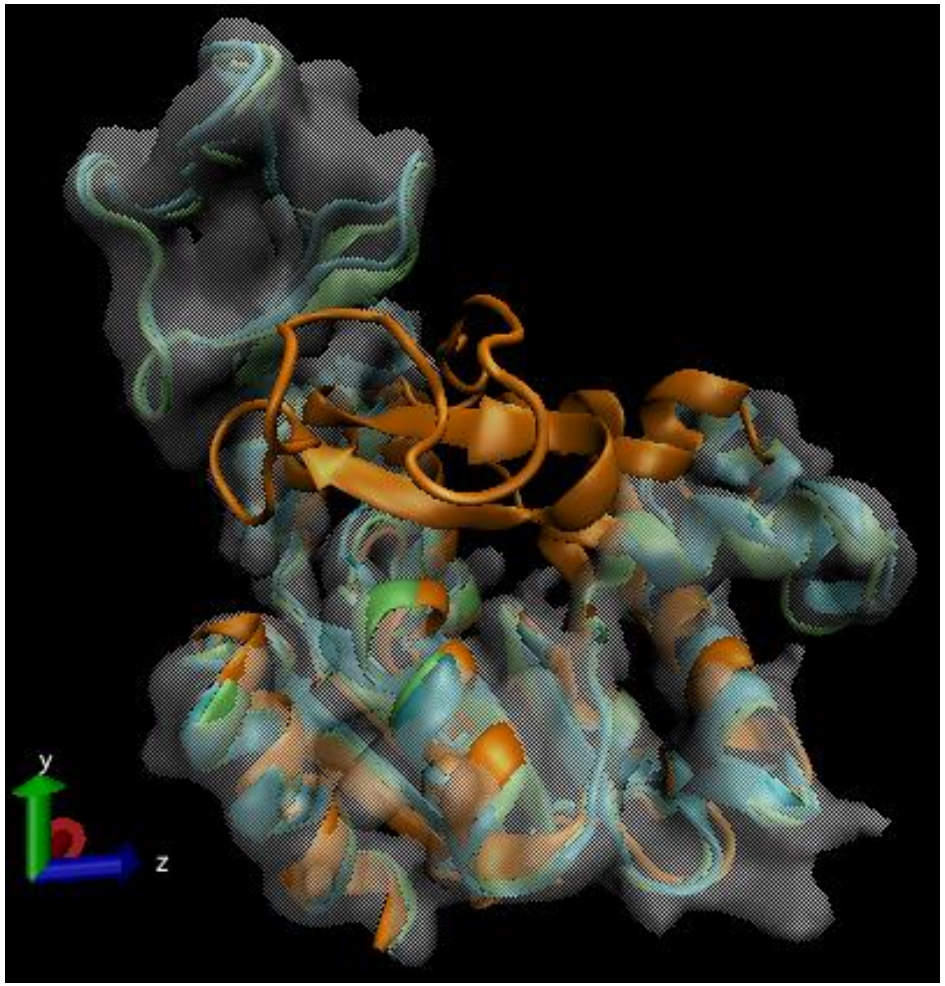
MDFF-test1:
different conformations



Generate simulated Map for target
source test1-1.tcl



Dock initial structure into Map
AutoPSF for parameters
MDFF parameters
source test1-2/3/4.tcl



Green one is the result

MDFF-steps

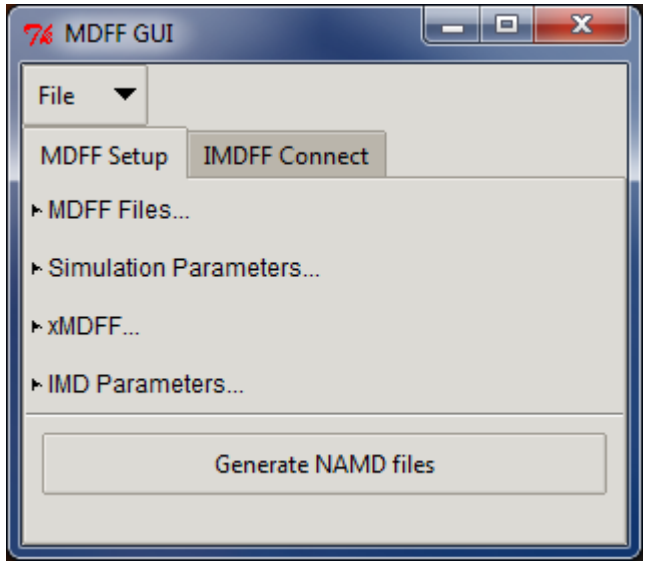
$$U_{EM}(\mathbf{R}) = \sum_i w_j V_{EM}(\mathbf{r}_j)$$

$$V_{EM}(\mathbf{r}) = \begin{cases} \xi\left[1 - \frac{\Phi(\mathbf{r})-\Phi_{thr}}{\Phi_{max}-\Phi_{thr}}\right] & \text{if } \Phi(\mathbf{r}) \geq \Phi_{thr}, \\ \xi & \text{if } \Phi(\mathbf{r}) < \Phi_{thr}. \end{cases}$$

$$\mathbf{f}_i^{EM} = -\frac{\partial}{\partial \mathbf{r}_i} U_{EM}(\mathbf{R}) = -w_i \frac{\partial}{\partial \mathbf{r}_i} V_{EM}(\mathbf{r}_i)$$

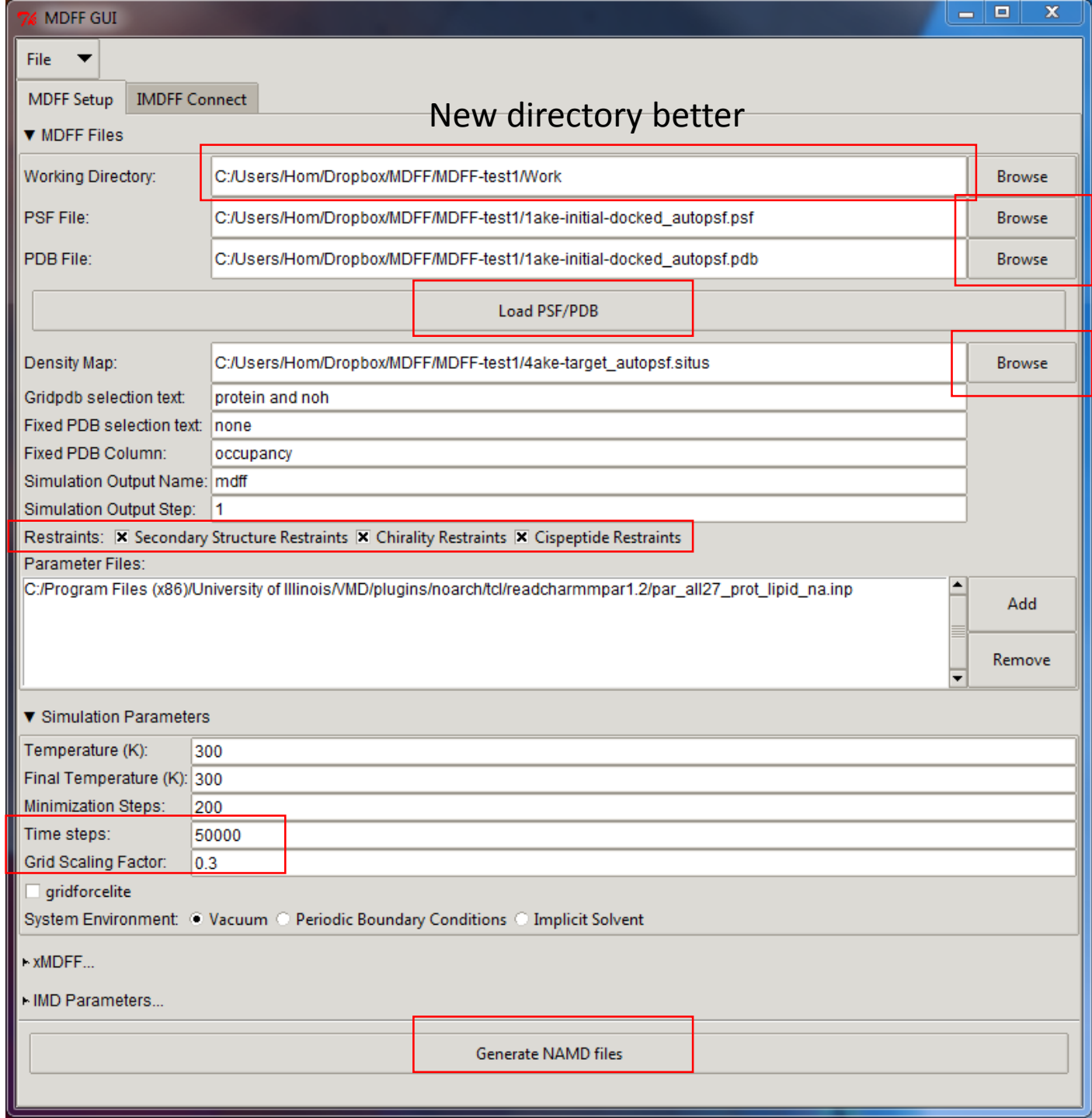
- Prepare a volumetric map and a dx file containing potential information V_m/ξ derived from a map (test1-1/2.tcl)
- Prepare the rigid fitting result as initial structure
- AutoPSF to generate NAMD topological files with parameters
- Generate a PDB file containing the per-atom scaling factors w_j (test-3.tcl).
- Prepare the structural restraint files (test-3.tcl)
- Prepare the MDFF MD-running parameters (test-4.tcl)
- Exit VMD and Run NAMD2

MDFF plugins

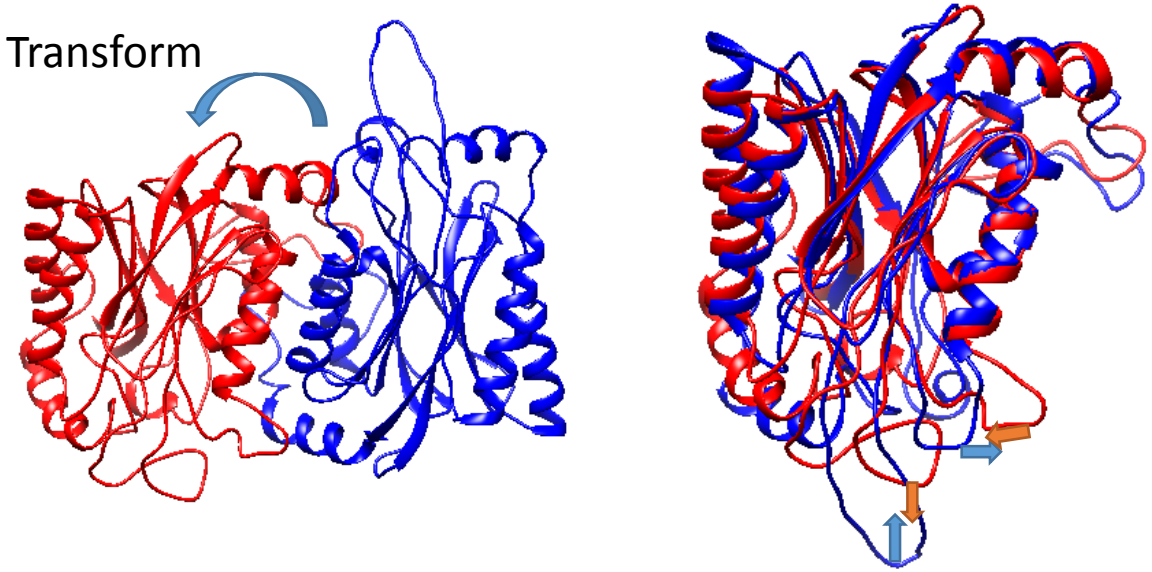


Extentions->Modeling->MDFF
Only exist >= version 1.9.2

It can help you to generate input files easily.
But it still need AutoPSF to generate PSF files for docked structure.

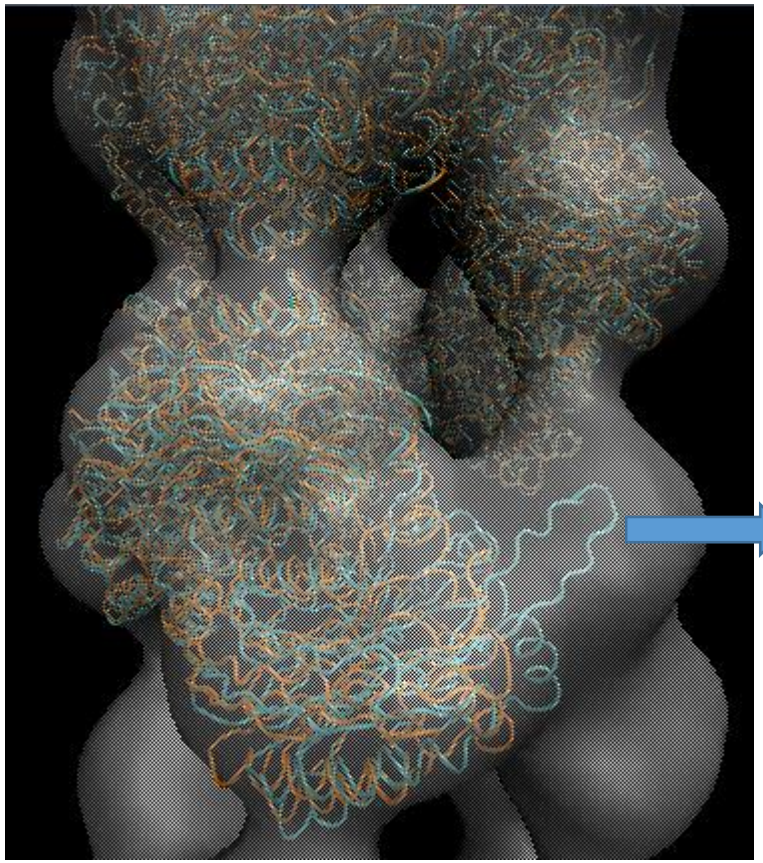


MDFF with symmetric restraint



- Transform all the units to the first unit based on symmetric matrices.
- Obtain an averaged structure
- Add force based on difference between each unit and averaged structure

- Symmetric restraint can help to reduce the differences from each unit to keep the complex symmetry during MD simulation.



Unreasonable movement

Orange: with symmetric restraint
Blue: without symmetric restraint

MDFF with symmetric restraint

- Compared to the normal MDFF, there are three more steps:
- Mark the restraint atoms (often the alpha-C) on occupancy column in PDB and distinguish each asymmetric unit on b-factor column in PDB (set_symmetry.tcl, you have to modify it as you need)
- Measure the transformation matrixes of all the asymmetric units compared to the first unit. (vmd command: measure fit)
- Write the symmetric restraint information into the “namd” input files

Practice: Refitting structure for HSP16.3

- Rigid fitting the atomic model from homology modeling (ita_align.pdb) to cryo-EM map (emd_1149.ccp4)
- Symmetrize
- Setup the MDFF files
- Use symmetric restraint (sym-matrices.txt)
- Try to run 50000 steps