Chimera Visualization Tutorials

Presenter: Tom Goddard, Chimera developer

Date: Thursday, July 12, 2012

Time: 9:00 AM - 12:30 PM

**Location:** National University of Singapore
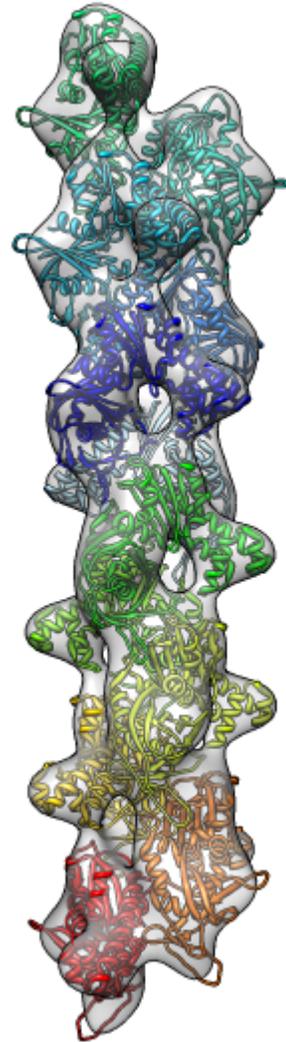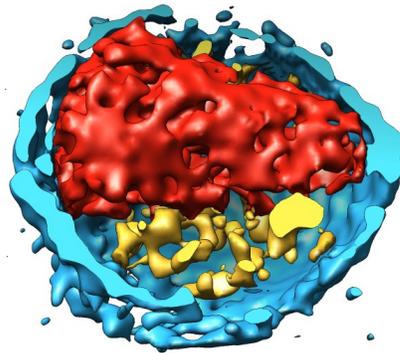
Topics

- Visualization Electron Tomography: HIV virus tutorial (45 minutes)
- Fitting Single-Particle Maps: ParM filaments (45 minutes)
- Making High Quality Images (30 minutes)
- Question and Answer Session. Any Chimera Questions. (30 minutes)

Tutorial Setup

If you will not use a classroom computer, bring your laptop with the following Chimera version and data files.

- Chimera 1.6.1 or newer (32 or 64-bit ok).
- Data files: emviz.zip.

Chimera Tutorial:
Visualizing Electron Tomography

Tom Goddard
July 12, 2012

This tutorial covers basic techniques for viewing, filtering, and segmenting noisy electron tomography maps using Chimera 1.6.

We will look at a map of HIV virus particles from the Fuller lab, EM Databank 1155.
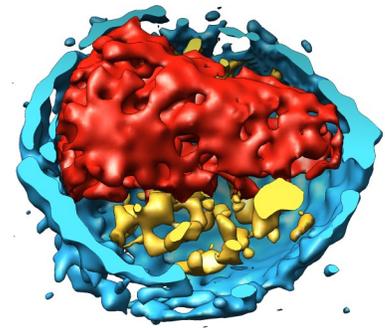
Displaying XY Planes

1. Open map emd_1155.map.
2. Volume dialog, Features / Planes, press One button.
3. Drag markers on histogram brightness yellow curve.
4. Move Plane slider to flip through planes.
5. Change plane axis to z and flip through planes.
6. Show all planes.

Inverting Intensity Values
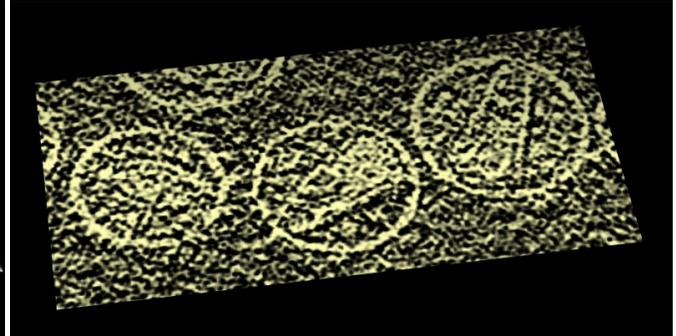
7. Note small map values are high density. Want large map values for high density.
8. Volume dialog, Tools / Volume Filter, type Scale, scale -1, options turn off displayed subregion only, press Filter.
9. Hide original map by clicking "eye" icon above histogram.
10. Switch from surface style to solid, One plane.

Hide Dust
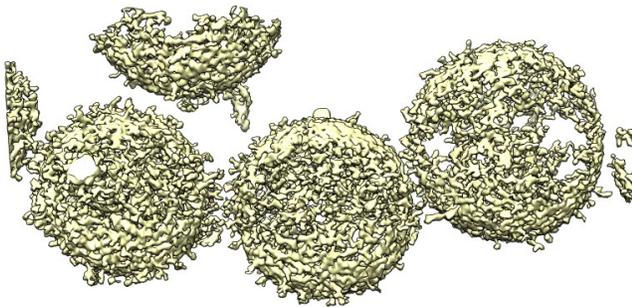
11. Hide small surface blobs to reduce noise.
12. Volume style surface, All planes.
13. Volume dialog, Tools / Hide Dust, Surface emd_1155.map scaled, press Hide.
14. Drag vertical bar on volume dialog histogram to lower contour level.
15. Move size slider in hide dust dialog.
16. Press Unhide.

Gaussian Filtering
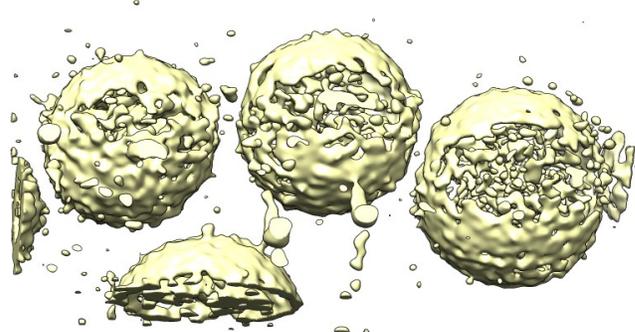
17. Smooth map to reveal large-scale features.
18. Volume dialog, Tools / Volume Filter, type Gaussian, width 30 Angstroms, value type float32, select scaled map in volume dialog, press Filter.
19. Planes One, depth 10, move plane slider.

Extract One Virus
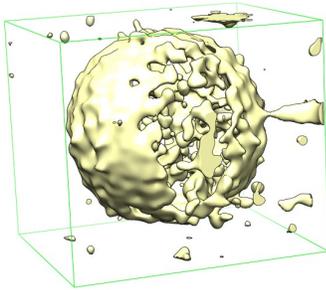

20. Volume dialog, Features / Subregion Selection, enable "Select subregions", drag box around middle virus particle, press Crop.
21. Click on green outline box face (not edge) and drag. Press Crop.
22. Uncheck "Select subregions" to move map using mouse.
23. Can save map to new file with volume dialog, File / Save Map As....

Mask Virus Membrane
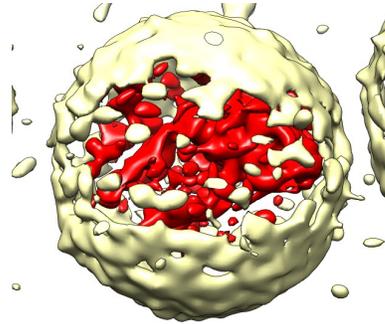
24. Show command line using Favorites / Command Line
25. Enter command "shape sphere radius 550".
26. Click off "Active" sphere model #3 below command-line to stop mouse from moving sphere.
27. Move map to be centered on sphere. Ctrl-middle-mouse moves in z direction.
28. Mesh display style can help see sphere inside map.
29. Create map for inside of sphere, command "mask #2 #3"
30. Hide sphere, Favorites / Model Panel, uncheck shown button for sphere.
31. Create map ouside sphere, command "mask #2 #3 invert true".
32. Color inside map red, command "volume #4 color red".

Clip Virus Membrane
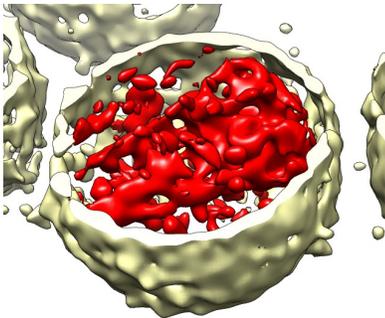
33. Cut virus membrane in half to see inside.
34. Tools / Depiction / Per-Model Clipping, Model #5, check "Enable clipping".
35. Check "Adjust clipping with mouse", move clip plane by dragging with middle mouse button in graphics window.

Chimera Tutorial:
Fitting Molecular Models in Single-Particle EM Maps

Tom Goddard
July 12, 2012

Topics

- Fitting molecules in maps with global search.
- Calculating map symmetry and creating symmetric molecule copies.
- Symmetric fitting to avoid molecular clashes.
- Calculating and displaying difference maps.

What does ParM do?

- The ParM protein forms filaments that segregates DNA plasmids prior to cell division.
- To partition low copy number DNA plasmids in E coli evenly during cell division between the two daughter cells, a plasmid is attached to each end of a growing ParM filament that pushes them to opposite sides of the mother cell.
- ParM filaments look similar to actin filaments.
- Filament growth is driven by ATP and filaments have dynamic instability like microtubles.

Modeling a ParM Filament

- ParM monomers bind ATP and can have the binding cleft open or closed.
- We'll build a model of the open state filament with the ATP binding site empty using x-ray structure 1mwk and cryoEM map EMDB 5129 (19 Angstroms).
- Closed state data is also available: x-ray model 1mwm and map EMDB 5128 (17 Angstroms). Won't have time to look at those.
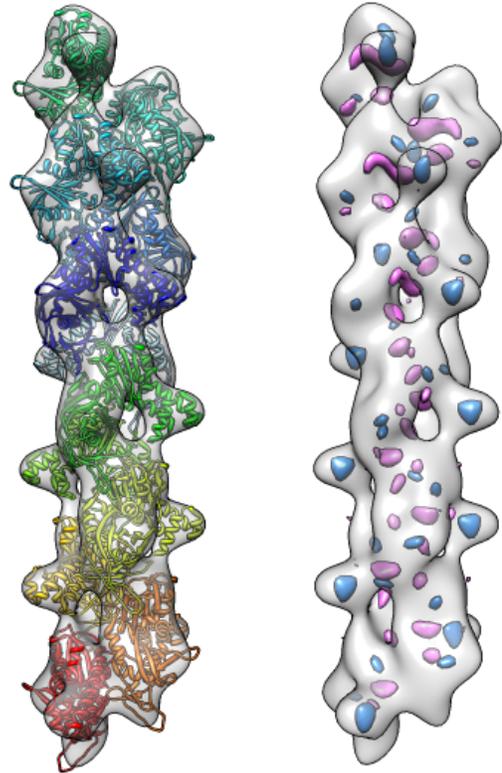
Analysis Steps

Show Molecule and Map
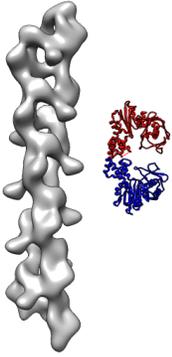
Fit Molecule in Map
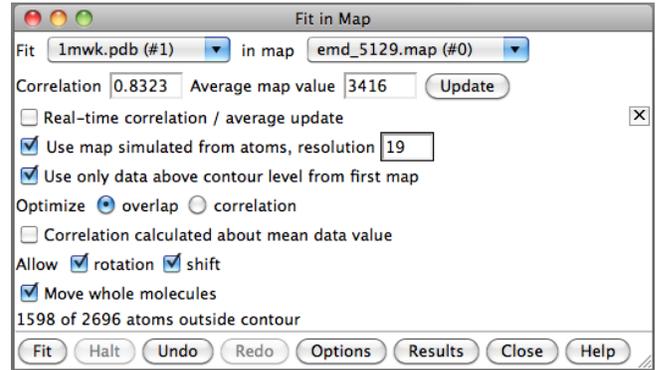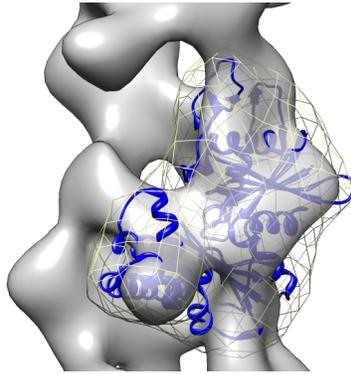
1. Open PDB 1mwk, EMDB 5129.
2. Show Command Line (Favorites menu).
3. Deactivate map (model 0) below command-line to move 1mwk away from map.
4. Command "rainbow chain" to color two ParM monomers.
5. Delete chain B. Use menu Select / Chains / B, then menu Actions / Atoms / Delete.

6. Fit 1mwk in map.
7. Move 1mwk into map.
8. Press Fit button in Fit in Map dialog (volume dialog Tools menu).
9. Fit using correlation: Fit dialog Options button, enable "Use map simulated from atoms..." resolution 19. Press Fit.
10. Show simulated map (Volume dialog eye icon) as mesh.
11. Correlation depends on domain of calculation. Change simulated map threshold and press Update in Fit dialog.
12. Spend a few minutes trying alternative fit positions.

Global Fit Search
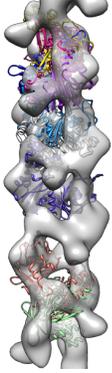
13. Search for best fit using 30 tries with command "fit #1 #0 search 30"
14. Fits appear all along filament, many are equivalent due to symmetry of the filament.

Calculate Map Symmetry

15. Determine map symmetry to eliminate equivalent fits.
16. Command "measure symmetry #0 helix 20,180,opt minimumCorrelation 0.95".
17. The "helix" option gives Chimera a hint about helical parameters.
18. The "minimumCorrelation" option accounts for this unusual map where the helix does not extend to the edges of the volume box.
19. View symmetry copies of molecule with command "sym #1 group #0 surf true".
20. Remove symmetry copies with "~sym #1" (note leading tilde character which means "undo" in Chimera commands).

Fit Asymmetric Unit
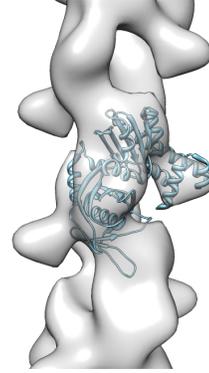
21. Clear fit list.
22. Rerun previous fitting command "fit #1 #0 search 50".
23. Clear fit list.
24. Use correlation optimization "fit #1 #0 search 50 res 19".

Make Filament Model
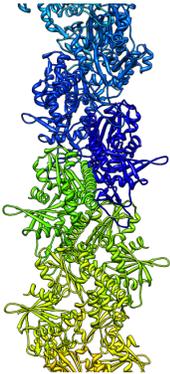
25. Show symmetric molecule copies for best fit. Command "sym #1 group #0 update true".
26. Color molecules distinctly. Command "rainbow model".

Reduce Molecular Clashes
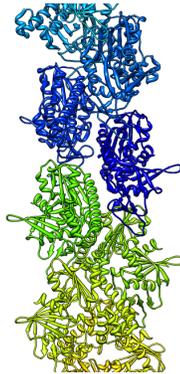
27. Inspect clashes between adjacent ParM molecules.
28. Fit asymmetric unit including all overlapped symmetric molecules. Command "fit #1 #0 sym true res 19"
29. Note increased space between molecules.

Calculate Predicted Map
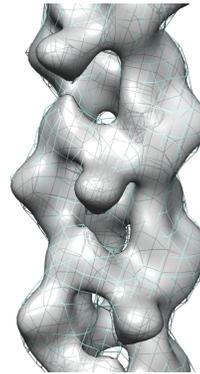
30. Compute difference map between experimental map and predicted map for molecular model.
31. First delete extra ParM molecules outside experimental map. Ctrl-drag mouse to select outside molecules. Press up-arrow key to extend selection to full molecules. Menu Actions / Atoms / Delete to delete.
32. Calculate predicted map. Command "molmap #1,2, 19"

Difference Map

Compare "Open" and "Closed" Filaments.
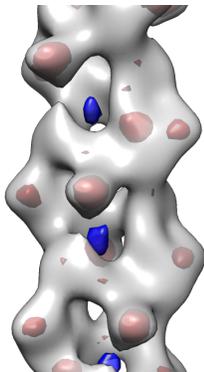
33. Subtract two maps, scaling the second to minimize difference. Command "vop subtract #0 #3 minRMS true"
34. Adjust difference map contour level. Add negative contour with ctrl-click on histogram. Adjust contour colors.
35. Hide molecular models with Model Panel (menu Favorites).

36. Load "closed" filament (GDP bound), EMDB 5128, menu File / Open...
37. Flip closed map 180 degrees and fit to open map.
38. Compute closed map on same grid as open map. Command "vop resample #5 onGrid #0"
39. Use Morph Map (Tools menu of volume dialog) to morph between maps. Movie.

Chimera Tutorial:
# How to Make High Quality Images

Tom Goddard
July 12, 2012

This tutorial shows settings for producing the best looking images in Chimera 1.6.

White Background Color
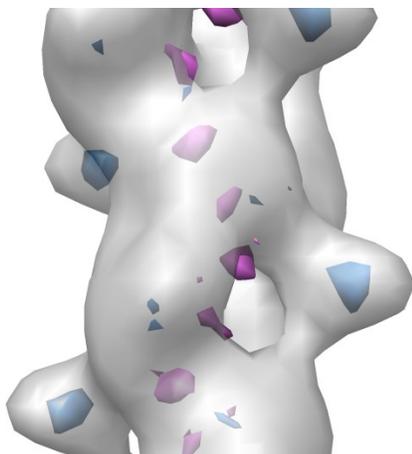
1. Actions / Color / All Options...
2. Click coloring applies to "background".
3. Click color white.
4. Alternatively use Favorites / Command Line, command "set bg_color white".

**Background Color Gradient**
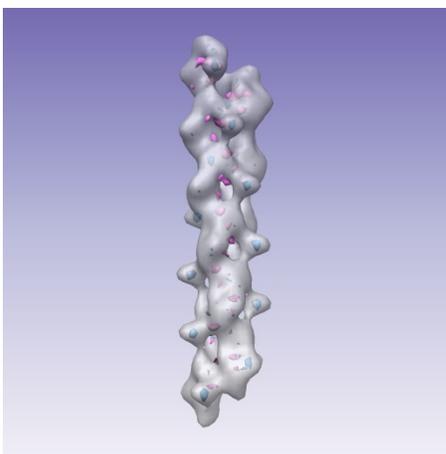
5. Actions / Color / All Options...
6. Click background "More..." button.
7. Change Background Method from "solid" to "gradient".
8. Click blue gradient button to change color scheme.
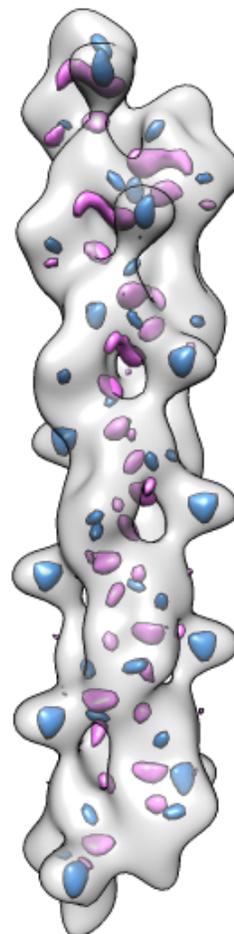
Volume Step Size 1
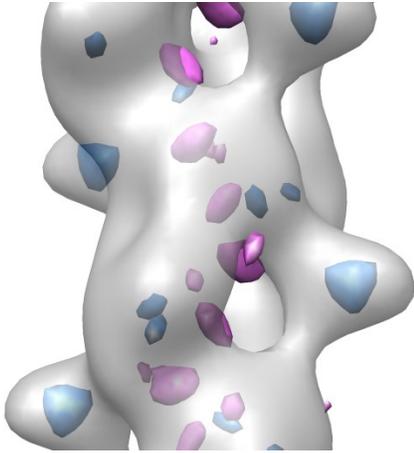
9. Show full-resolution volume data.
10. Change volume dialog "step" to 1 (above histogram).
11. Equivalent command "volume all step 1"

Adjust Transparency
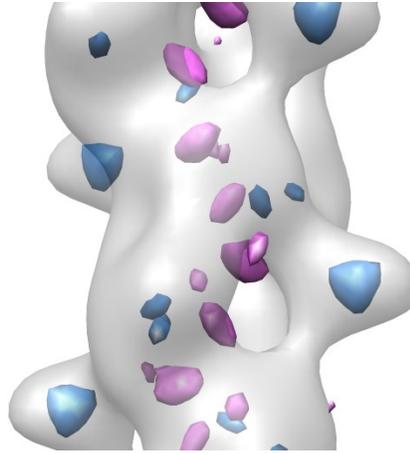
12. Adjust surface transparency
13. Click top histogram in volume dialog to choose emd_5129.map.
14. Click volume dialog color button (square to right of "Color").
15. Slide "A" slider to control opacity, 0.5 good.

Glossy Lighting
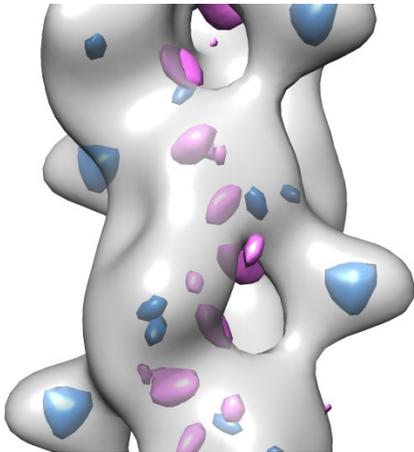
16. Enable better quality "glossy" lighting.
17. Tools / Viewing Controls / Lighting, Quality "glossy".

Silhouette Edges
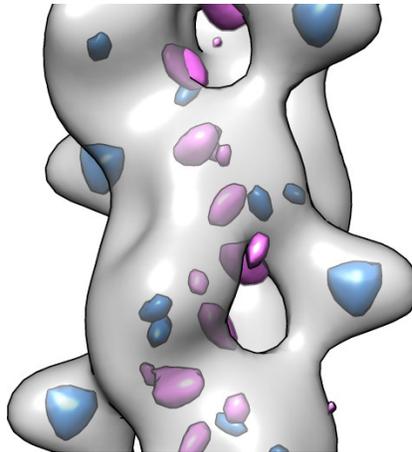

18. Add black edges to surfaces.
19. Tools / Viewing Controls / Effects, turn on "Silhouettes"
20. Try silhouette width 3, press enter. Width is in pixels.

Save Large Image

21. Resize window to desired aspect ratio and border width.
22. File / Save Image...
23. Change width or height to larger size, 2000 pixels high, press Enter.
24. Press Save As.
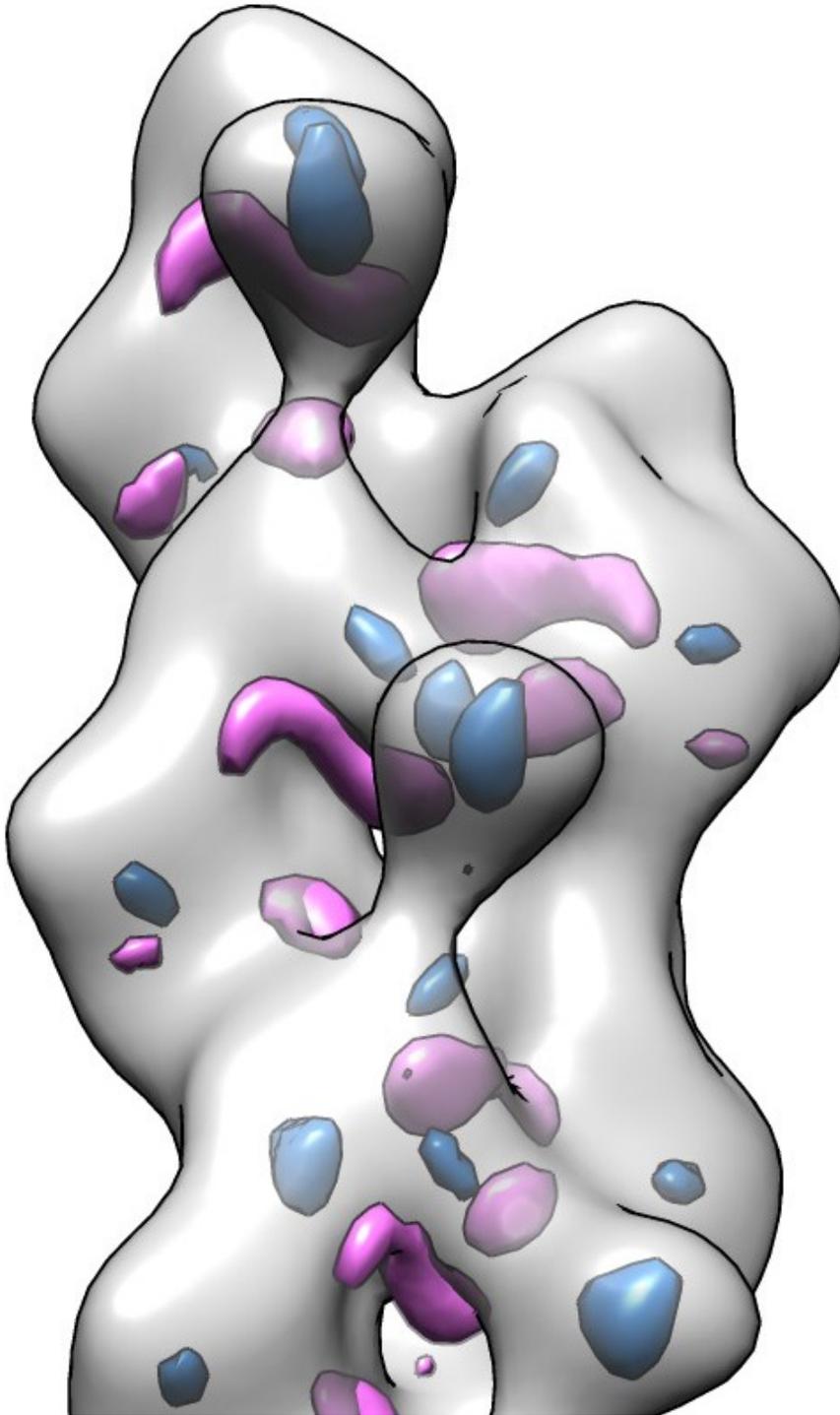
Shadows and Raytracing
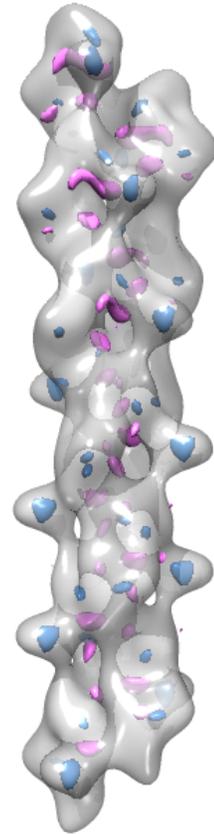
25. File / Save Image... has "Raytracing" option which produces shadows.
26. Raytracing disadvantages:
    - Darkness and transparency will look different.
    - Trial and error required.
    - Slow to save image.
    - Multiple transparent surface layers always shown.
    - No silhouette edges.
27. Interactive shadows. Tools / Viewing Controls / Effects.
    - Not available with some graphics card. Not on Mac.
    - Shadows may have rough edges.
    - May not work with glossy lighting.