Chimera EM Map Tutorial: RNA Polymerase II

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This tutorial focuses on display of volume data from single particle EM reconstructions. We'll look at maps of two conformations of human RNA polymerase II and a crystal structure of the homologous complex in yeast. This 12 protein complex walks along chromosome DNA making an RNA copy. The EM maps are described in the following article:

Kostek SA, Grob P, De Carlo S, Lipscomb JS, Garczarek F, Nogales E.
Molecular architecture and conformational flexibility of human RNA polymerase II.
Structure. 2006 Nov;14(11):1691-700.

Data sets and the most recent version of this tutorial (html, pdf) are online. The tutorial has been used with Chimera versions 1.2304, 1.2309, and 1.2382 and should work with more recent versions.

Topics

The 6 parts of the tutorial can be done in any order. They are ordered from simple to complex. Time required: ~2 hours.

1. Basic map display options
2. Choosing contour level
3. Coloring parts of a map
4. Morphing between two maps
5. Fitting crystal structure in map
6. Human / yeast structure differences

Part 1: Basic map display options

Use menu entry *File / Open* to open **emd_1283.map** found in the **data** directory of the downloaded tutorial files.

Movement

- Left mouse button - rotate map.
- Middle button - move map in x, y, or z (hold *ctrl* key).
- Right button - zoom in and out.

On a Mac with a one button mouse hold the *option* key down for middle mouse button, and the *apple* key down for right mouse button.

Holding the *shift* key down makes smaller motions for finer control.

Step size

The volume viewer dialog indicates the map size is 120 by 120 by 120. The *step* size of 2 2 2 indicates that every other data plane is being used along the x, y and z axes.

Change the step size to 1 1 1 to show the full resolution data. The surface will appear smoother.

Contour level

The volume dialog shows a histogram of the data values. Move the vertical bar on the histogram to change the contour level.

The displayed surface represents the positions in the map having value equal to the contour level. Higher contour level produces a smaller surface.
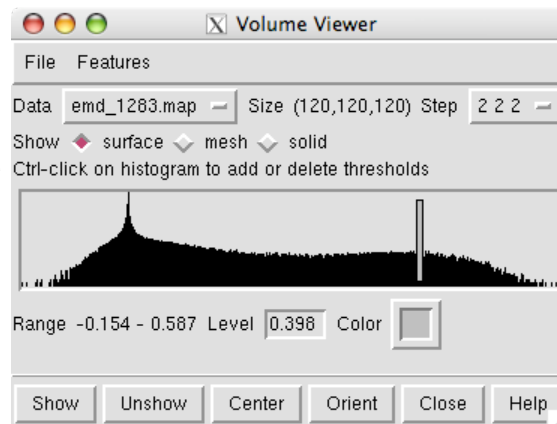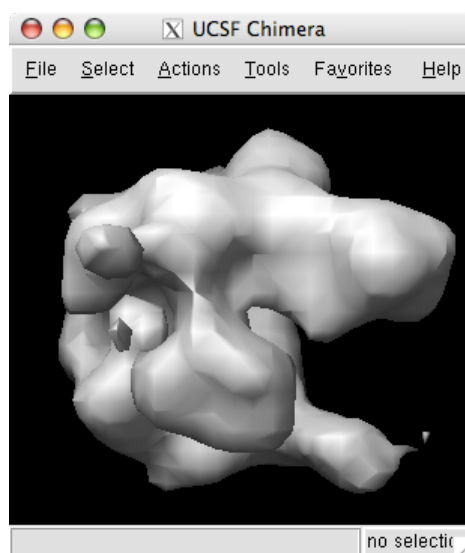
Surface color

Press the square color button in the volume dialog to change the surface color. Move the black boxes on the color bars of the color dialog to adjust red, green and blue components of color.

Press the *Opacity* button on the color dialog and adjust the opacity (bottom color bar labeled with A) to make the surface transparent.

Surface smoothing

Use volume dialog menu entry

Features / Surface and Mesh Options

to display additional options and turn on option

Surface smoothing iterations 2, factor 0.3

This gives the surface a smoother appearance by moving each surface vertex a 0.3 of the way towards the average position of its neighbor vertices 2 times.

Mesh

Click the *Mesh* switch on the second line of the volume dialog.

Click on the

Square mesh

option in the Features / Surface and Mesh Options panel. Turn off surface smoothing. In square mesh mode with no smoothing the displayed mesh corresponds to the intersection of volume xy, yz and xz grid planes with the surface.

Click on

Smooth mesh lines

This enables antialiasing, a method for blurring the jagged pixel jumps on each line segment. Zoom in close to see the effect. With some graphics cards this may not change the appearance.
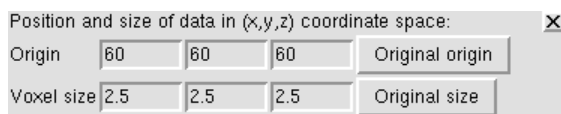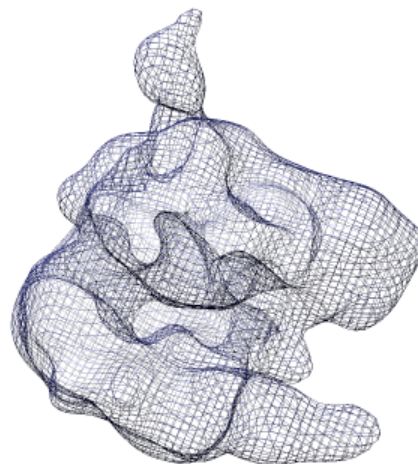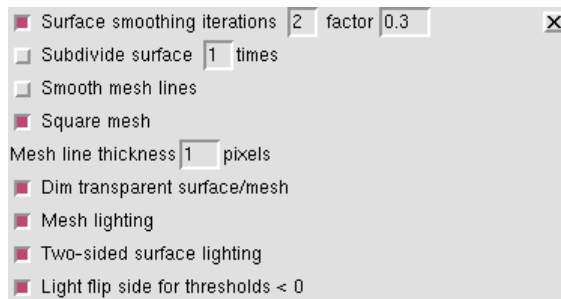
Grid plane spacing

Use volume menu entry

Features / Origin and Scale

The voxel size gives the spacing between grid planes along x, y and z axes. For this map it is 2.5 Angstroms.

Often a map file will not contain the grid plane spacing and in those cases you can enter correct values here. Correct scale is needed for fitting atomic models.

Part 2: Choosing contour level

Open **emd_1283.map** if it is not already opened. We will set the contour level so that the surface encloses the estimated volume of the 12 protein hRNAPII complex of approximately 544,000 Å^3. The estimate uses 1.21 Å^3 per dalton (Harpaz 1994) and molecular weight of 450 kDa.

Use menu entry

> *Tools / Volume Data / Measure Volume and Area*

and press the *Compute Volume and Area* button. Adjust the contour level and recompute the volume to find the contour level giving the desired volume. In Chimera version 1.2382 and newer the enclosed volume will automatically update as you vary the contour level.
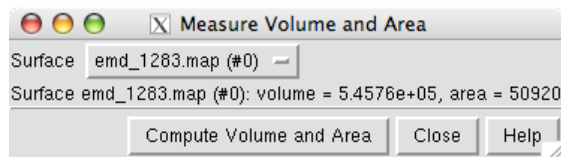
Part 3: Coloring parts of a map

The RNA polymerase complex has features called the "stalk", "clamp" and "jaws". We will color and label these parts of the map. Open **emd_1283.map** if it is not already opened.
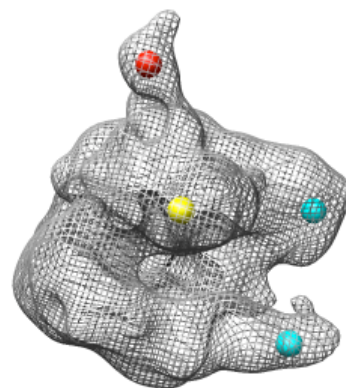
Placing markers

First we will place markers and then color the map near those markers. Open the marker placement dialog with menu entry

> *Tools / Volume Data / Volume Path Tracer*

Click on the map stalk with the middle mouse button to place a marker. Display the map as a mesh so the marker can be seen within the surface.

When a marker is placed its z position (perpendicular to the screen) is the first density maximum along the line of sight through the mouse position.

Deleting markers

To delete a marker first select it by holding the *ctrl* key and clicking on it with the left mouse button. Then use path tracer dialog menu entry

> *Actions / Delete markers*

You can select more than one marker by dragging a box while holding the *ctrl* key. Or you can hold the *shift* and *ctrl* keys to to add individual markers to the selection.

Connected markers

When you place additional markers they will be connected by cylindrical links. To delete a link first select it (*ctrl* left mouse button) then use path tracer dialog menu entry

> *Actions / Delete links*

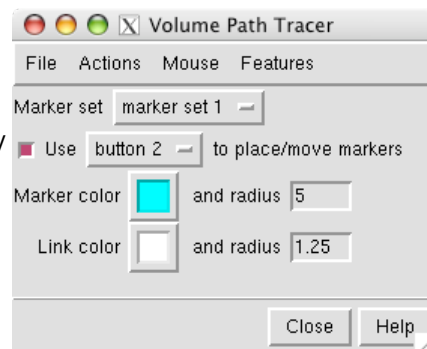To disable automatic connection of markers use menu entry

> *Mouse / Link new marker to selected marker*

Moving markers

To enable moving markers use menu entry

> *Mouse / Move markers with mouse*

Then use the the middle mouse button to drag the markers. To move them perpendicular to the screen hold the *shift* key while dragging.

Marker color

Change the color of the marker by selecting it (*ctrl* left mouse button) and pressing the square *Marker color* button in the path tracer dialog.

Marker radius

Increase the sphere radius of a selected marker to 5 Angstroms by typing 5 in the marker *radius* entry and pressing the Return key.

Coloring map surface

To color the map surface near the markers use main menu entry

> *Tools / Volume Data / Color Zone*

The color zone tool colors the surface within a specified radius of the markers to match the color of the nearest marker.

Select all of the markers by holding the *ctrl* key and dragging a box around all of them with the left mouse button. Press the *Color* button on the color zone dialog and move the radius slider to about 25 Angstroms. Change the map display to *surface* in the volume dialog.
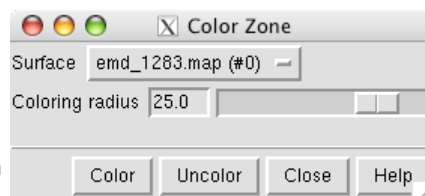
Restoring mouse behaviour

To reenable moving maps with the middle mouse button turn off

> *Use button 2 to place move markers*

in the path tracer dialog.

Text labels

To place text labels on the color regions use main menu entry

> *Tools / Utilities / 2D Labels*

Click in the graphics window and then type to create a text label. Labels can be repositioned with the mouse. They can be deleted using the 2D label dialog. These 2D labels do not move when the map is rotated. They are primarily used for making figures with menu entry

> *File / Save Image*

To reenable rotating maps with the left mouse button close the 2D label dialog.
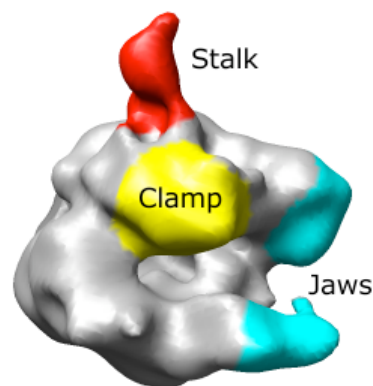
Part 4: Morphing between two maps

The human RNA polymerase II was seen in two conformations. We will now look at the difference between the maps by morphing one map into the other.

Open second map

Open **emd_1283.map** if it is not already opened. Open the second map **emd_1284.map** using menu entry *File / Open*. It is useful to make this map a different color, and display one map in *mesh* style and the other in *surface* style to see both clearly.

Installing morph map tool

You have to tell Chimera where to find the map morphing tool. It is a plug-in that is not distributed with Chimera but is included in the **extensions** directory of the downloaded tutorial files.

Use menu entry

Favorites / Preferences
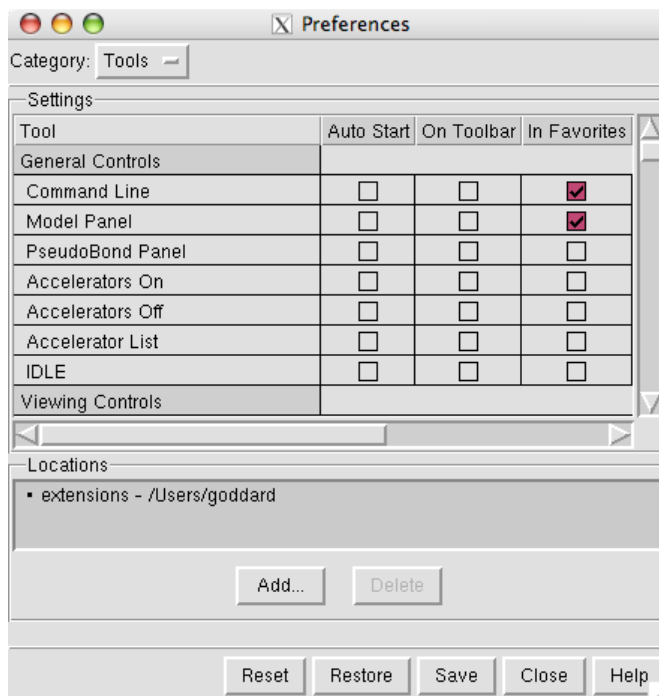
select

Category *Tools*

and press the button

Add...

near the bottom of the preferences dialog and choose the directory called

extensions

that contains a directory called **MorphMap**. You add the **extensions** directory and Chimera finds all plug-ins in that directory.

If you do not have the morph map tool you can obtain it online from the Chimera experimental features web page.

Morphing between maps

To display the morph map dialog use main menu entry

Tools / Volume Data / Morph Map

Choose **emd_1283.map** as the first map and **emd_1284.map** as the second map, and press

Play

at the bottom of the dialog.

Normalizing maps

The two maps are not identically normalized, they do not enclose the same volume at the same contour level. A contour level of 0.339 gives volume 540,000 $Å^3$ for map emd_1283 but a lower contour level of 0.326 is needed for map emd_1284. To morph between equal volume end-points turn on the option

Multiplier for second map 1.04

using 1.04 (= 0.339 / 0.326) as the multiplier for the second map.

Saving an animation

The morph animation can be saved in several formats (Quicktime, MPEG-2, ...). Press the *Record* button to save the animation.

You may wish to decrease the *step* value to create a smoother animation. A step of 0.1 creates 20 frames in going from an interpolation parameter of 0 (first map shown) to 1 (second map shown) and back to 0. A step of 0.02 would produce an animation of 100 frames.
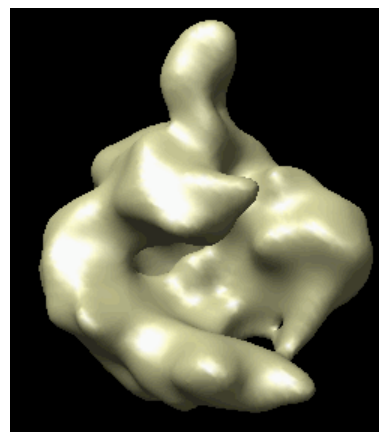
Part 5: Fitting crystal structure in map

We will fit a crystal structure of yeast RNA polymerase II into the EM map of the human complex. Currently no crystal structure of the human complex is available.

Close your current Chimera models using menu entry

> *File / Close Session*

and open **1y1v.pdb** and **emd_1283.map**.

Put model and map in view

The PDB model and map are far apart. To see both use menu entry

> *Favorites / Side View*

The side view window shows the models from the side (perpendicular to the view in the main window). Move the vertical yellow lines which represent clip planes so both the map and model lie between them. The yellow square on the left in the side view represents your eye. Move it left or right to zoom out or in.
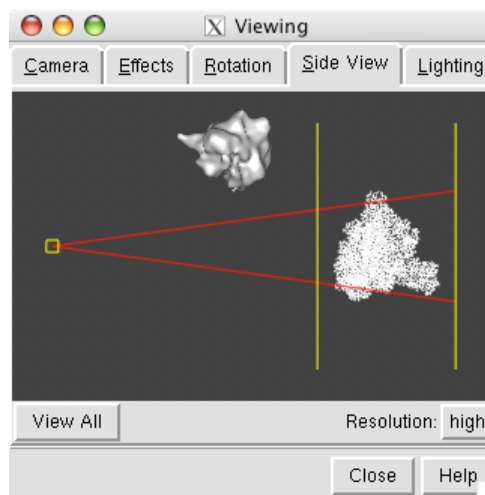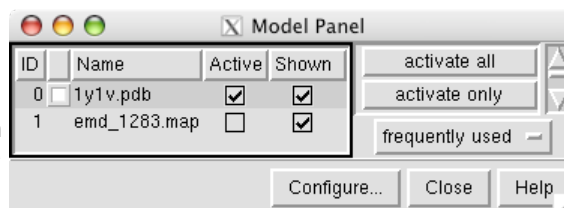
Move model and map close to each other

To move the model closer to the map use menu entry

> *Favorites / Model Panel*

Lock the map position by clicking off the checkbutton in the *Active* column next to emd_1283.map. Then move the PDB model so that it overlaps the map. Then turn back on the *Active* checkbutton so the map and model can both be moved.
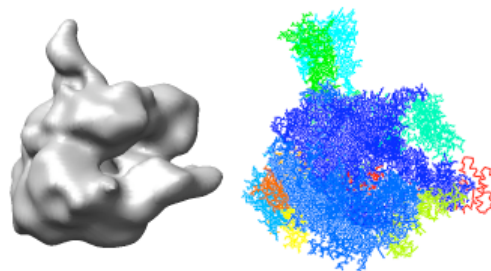
Color the PDB model

To color the distinct proteins of the PDB model first display the Chimera command line using menu entry

> *Favorites / Command Line*

then type the command

    rainbow chain

in the entry field at the bottom of the Chimera graphics window.

Hand align model and map

Orient the map so its stalk is pointing up and you look into the cleft. Then lock the map position using the model panel dialog and rotate the PDB model so it is similarly oriented and superimposed on the map.
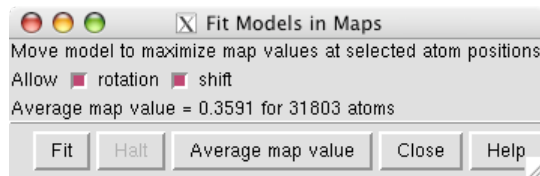
Note that *ctrl* middle mouse button moves the model perpendicular to the screen.

Optimize the fit

Do a computational optimization of the fit of the model in the map using menu entry

> *Tools / Volume Data / Fit Model in Map*

Select all atoms by dragging a box around them using the *ctrl* key and

the left mouse button. Then press the *Fit* button. After a few seconds of steepest descent optimization the PDB model is moved to its optimized position. Sometimes pressing *Fit* again will improve the fit further.

Unselect all atoms by clicking with *ctrl* left mouse button on the black background in the Chimera graphics window.

Atoms as spheres

If you have fast graphics you can display atoms as spheres using menu entry

>   *Actions / Atoms+Bonds / sphere*

If rotating the map and model is too slow return to the default display style with

>   *Actions / Atoms+Bonds / wire*

Alternate fitting method

An alternate approach to fitting is to create a theoretical density for the PDB model at the experimental map resolution (22 Å) using the *pdb2mrc* program in the EMAN package. Then fit that map into the experimental map. Chimera version 1.2318 and newer versions can optimize the fit of one density map in another map using

>   *Tools / Volume Data / Fit Map in Map*

and report the correlation.
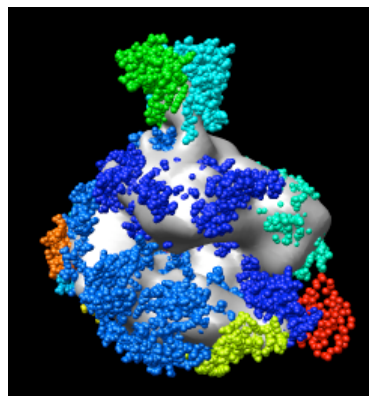
Part 6: Human / yeast structure differences

Chain S of 1y1v shown in red (when colored with command *rainbow chain*) is an elongation factor that was not present in the EM sample. To delete it, hold the *ctrl* key and click on a single red atom to select it. Then press the *up arrow* key to extend that selection to all of chain S. Then use menu entry

>   *Actions / Atoms+Bonds / delete*

Human stalk crystal structure

A crystal structure is available for the human RNA polymerase II stalk that we will compare to the yeast stalk. Open **2c35.pdb**, color its chains with Chimera command

>   rainbow chain

and delete all chains except A and B with command

>   delete #2:.C-V

(delete model number 2 chains C through V). The asymmetric unit of this crystal structure contained four copies of the stalk dimer, three of which you just deleted (chains C,D,E,F,G,H), plus 4 chains (S,T,U,V) containing water molecules.

Align human and yeast stalks

To align the human stalk (2c35) with the yeast stalk (1y1v) use menu entry

>   *Tools / Structure Comparison / MatchMaker*

select reference structure 1y1v and structure to match 2c35 and press *Apply*. This will do a sequence-based structure alignment, move the human stalk model to align it with the yeast model, and display a sequence alignment of the best matching pair of chains.
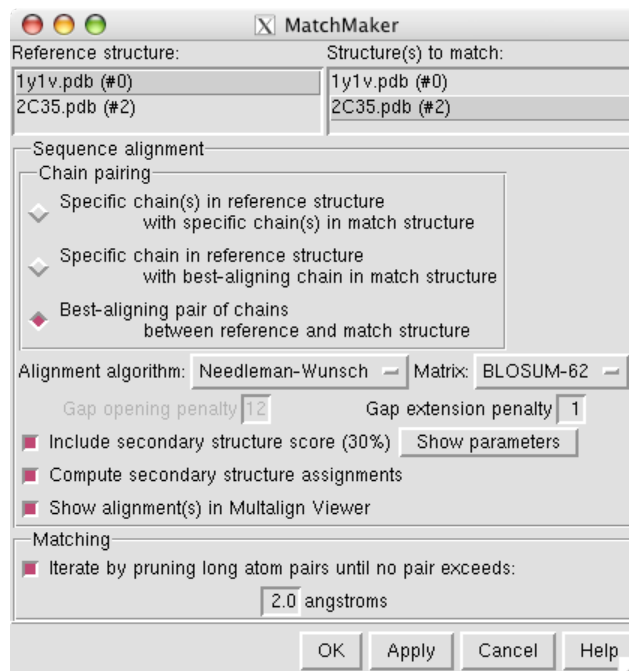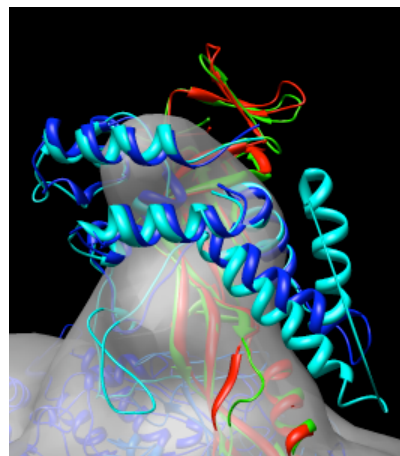
Show the models with ribbons using the two menu entries

> *Actions / Atoms+Bonds / hide*
> *Actions / Ribbon / show*

and for better looking ribbons

> *Actions / Ribbon / round*

While the structures are very similar notice that one alpha helix of the yeast stalk (cyan) that is far out of the density is not part of the human structure (dark blue).

Sequence alignment

The sequence alignment shown by the match maker tool is for the human and yeast chains that matched best (B and G). To look at a sequence alignment of the other pair of chains where yeast has an extra helix use the match maker chain pairing option

> *Specific chain in reference structure with best-aligning chain in match structure*

choose 1y1v chain D as the match maker reference chain and press *Ok*.

Dragging a box around the yeast sequence insertion (positions 75 - 91) in the MultAlignViewer sequence window will highlight the corresponding residues (the extra helix) in the graphics windows.

For a closer view of this helix, select it and use menu entry

> *Tools / Focus*

to zoom in, set the near and far clip planes, and set the center of rotation about those atoms.

Data Sets

The data sets for this tutorial are included in the **data** directory of the tutorial files (chimera-tutorial.zip), or can be obtained from public databases:

- EM Databank 1283 and 1284
- Protein Databank 1Y1V and 2C35

The map files will have to be uncompressed using command-line program **gunzip** on Linux or **Stuffit Expander** on Mac OS X. On Windows XP you have to install decompression software such as Win-GZ.

More Chimera volume features

For more information about Chimera volume display capabilities look at the

> Guide to Volume Data Display

Also see additional tutorials, experimental features, image gallery, animations, examples, user's guide, and the Chimera home page http://www.cgl.ucsf.edu/chimera.