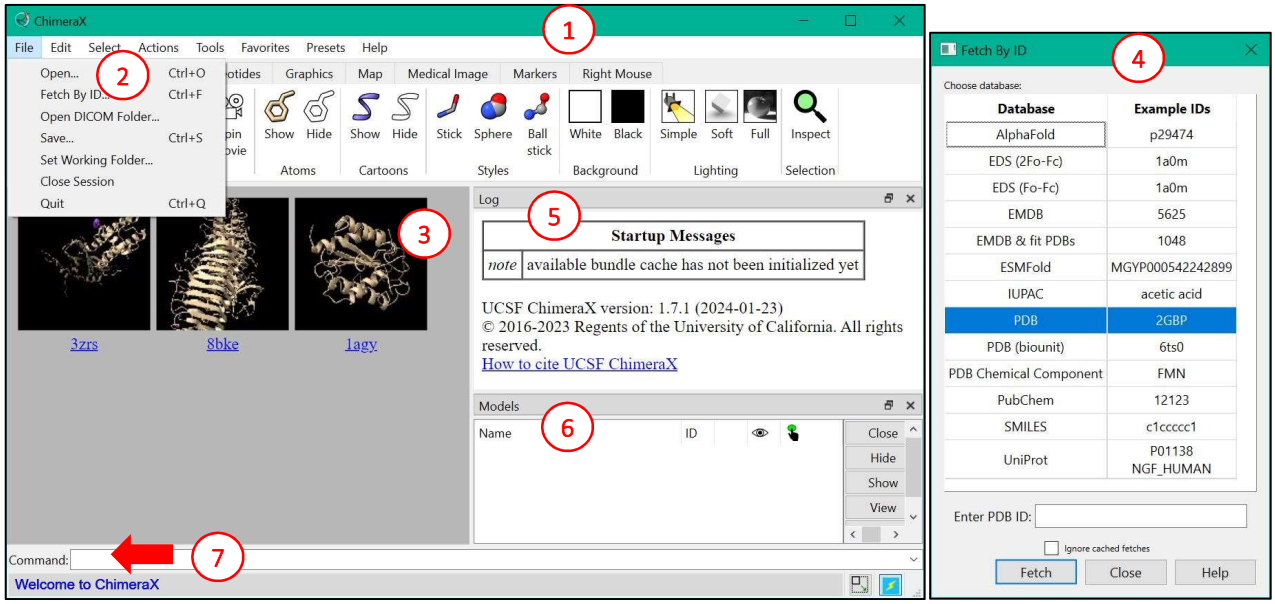
PREPARATION OF IMAGES AND MOVIES OF PROTEIN STRUCTURES USING THE UCSF CHIMERAX SOFTWARE.

The UCSF ChimeraX software [1] is a tool that allows the visualization of protein structures and electron density maps obtained through biocrystallographic or electron microscopy techniques, the comparison of protein conformations, the production of images and small movies of good quality, both of the overall structure and of local details. As input, the software requires a pdb file of a structure, or an electron density map file (usually in ccp4 format, file type .map). Tutorials are available on the software website [2] and on the website of the Brown Lab research group at Virginia Tech University [3]. On the software website, a summary page lists all the available commands and their syntax [4].

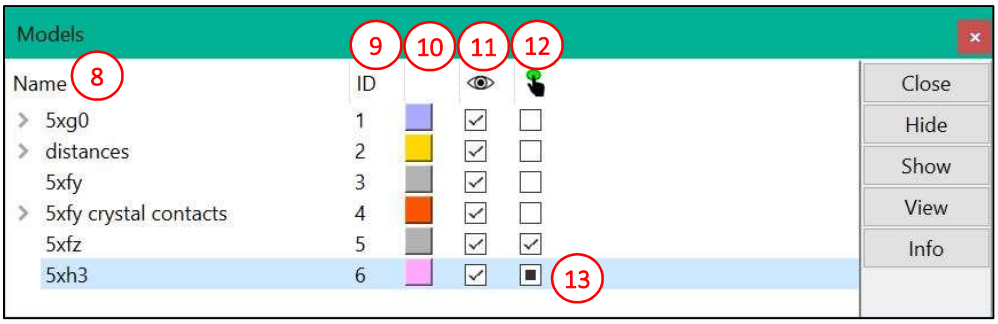
Opening of a pdb file and general options for viewing protein structures

When the program starts, a pdb file can be opened on the graphic window (**1**) by selecting it from the drop-down menu File > Open item (**2**).
Alternatively, if the software has already been previously opened, a list of opened structures appears on the left of the window (**3**). A single click on the name of a structure opens the file.
Furthermore, it is possible to directly obtain a file from PDB or other databases: select the drop-down menu File > Fetch by ID; a dialog window opens; search for the structure in one of the available databases using the specific code (**4**).

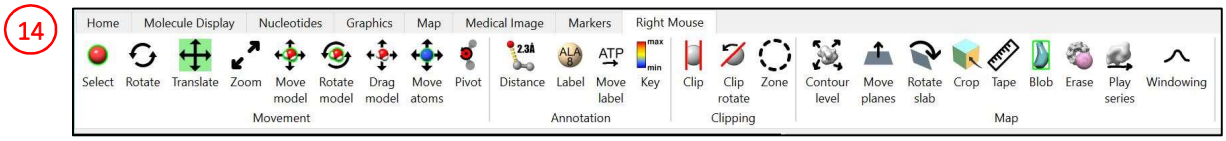


In addition to the display panel (**3**), the main window contains a panel with the list of previous commands (*Log*, **5**) and the list of models displayed on the main window (**6**). From the latter list, it is easy to select what to display and what to remove. At the bottom, a command prompt allows typing the commands (**7**).

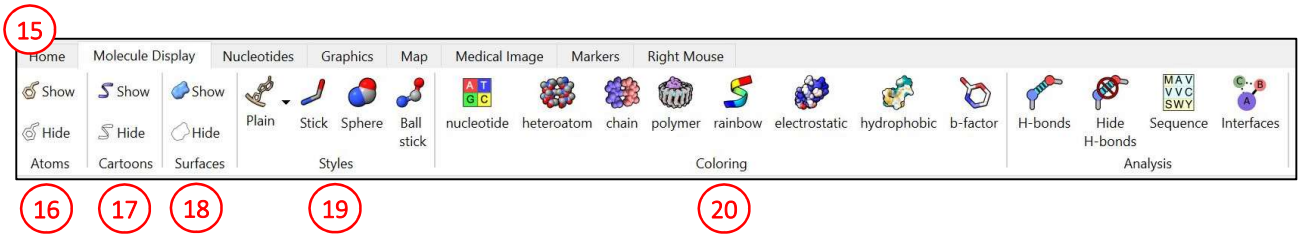
In the *Log* panel (**5**), available information about the structure is displayed when a new pdb file is opened. Sometimes, it is useful to copy the commands provided to be able to reproduce the activity of a previous session; in this case, however, this has to be done manually, as the *Log* panel is not saved automatically.
The *Models* panel (*Models*, **6**) contains the name of the model (**8**) (usually the name of the pdb file), the identification number the software assigns to each model (**9**), and the color of the model (**10**). The identification number must be used for the selection of a specific model (or part of it) preceded by the symbol # (see below). Two columns show which models are on display (**11**) and selected (**12**). The color of a model can be changed from this panel, by clicking on the color in the specific column (**10**). If only part of a model has been selected, a black square symbol (**13**) appears in the selection column.



In the main window of the software, at the top, there are several tabs that open the software menus. Among these, the *Right Mouse* tab (last in the row, **14**) allows you to select the action to be performed by clicking on the right mouse button. This is very useful when you do not have a 3-button mouse or a mouse with a wheel. When the software opens, the mouse controls are as follows: left button for rotation, right button for translation, middle button for zoom. You can change the command linked to the right button by selecting the appropriate icon in this menu. For example, by selecting the *Drag Model* icon you can change the position of just one of the models, when several are on display, and superimpose two models for comparison.



Several display options are available from the second tab (**15**), *Molecule Display*. Furthermore, the same result can be obtained by typing the appropriate commands in the prompt (**7**).

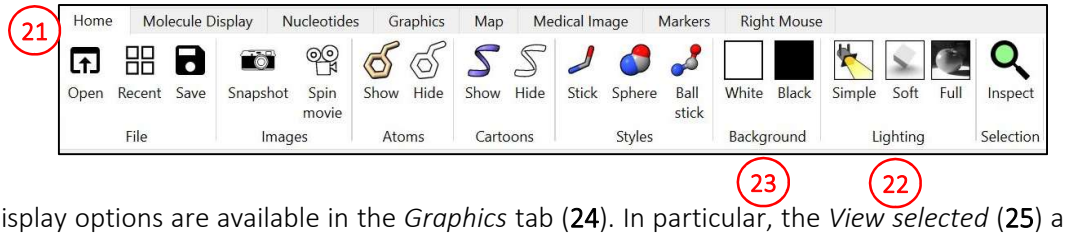


The first sections of this tab (**16-18**) allow activating or deactivating the display of atoms, chains as cartoons and surfaces, respectively. The following sections change the display style of the atoms (**19**) and their color (**20**). After opening the pdb file, you can display all atoms with the *Show* button in the *Atoms* section (**16**). The same result can be obtained by typing in the command line:

> `show atoms`

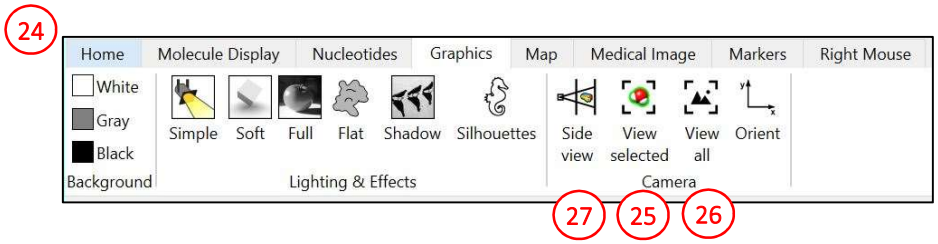
In the *Home* tab (**21**), a series of options allow you to optimize the display of the model, for example improving the lighting (**22**) or changing the background color (**23**). Some of the display options in the *Molecule Display* tab are repeated in this tab. To change the background, type on the command line:

> `set bgColor white`



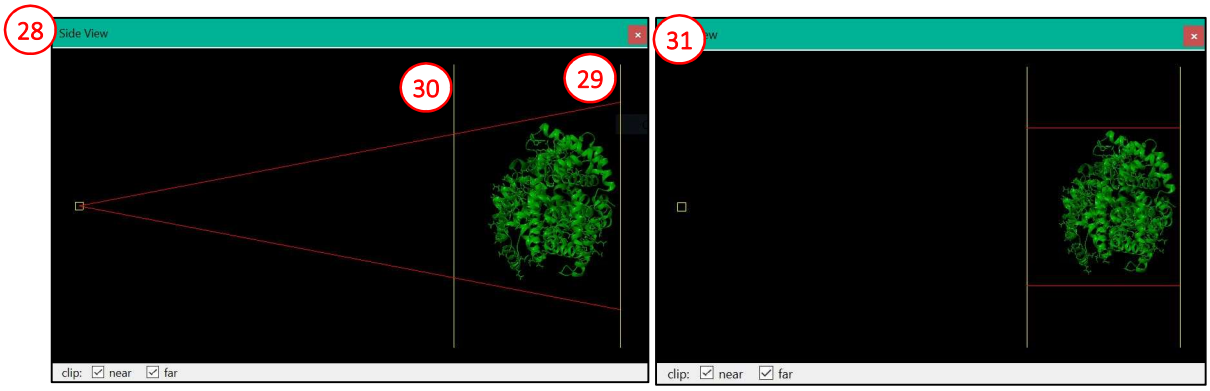
Other display options are available in the *Graphics* tab (**24**). In particular, the *View selected* (**25**) and *View all* (**26**) options allow you to center the display panel on a region of the selected protein (for selection options, see below) or on all the models that are currently loaded and displayed by the software, respectively. Alternatively, type on the command line:

> `view <selection>` [to center the display on a specific portion of the model]
> `view` [to center the display on all currently visualized models]



A useful tool is the lateral view of the display space (**27**, *Side view*), which opens a new window (**28**)that allows the positioning of the frontal (**29**) and posterior (**30**) planes of the view.
The orthoscopic (i.e., without perspective) view (**31**) is generally preferred for protein images, because it does not present deformations due to the position of the focus of the perspective. The orthoscopic view of the model can be activated from the command line by typing:

> `camera ortho`.



Selection syntax and display/coloring commands

Often, it is useful to select and display only some residues of the protein to analyze their structure in detail, for example when we are interested in the catalytic site residues of an enzyme. In this case, the portions of interest can be selected using the *Select* menu or from the command line.
In the *Select* menu several items are available, including:

- *Chains*, which allows the selection of the polypeptide chains present in the pdb file;
- *Chemistry*, with several sub-items that allow the selection an element type or functional group, or all the atoms of the protein, or if present the nucleic acids;
- *Residues* that allows the selection of a specific residue type (e.g., all the histidine residues present in the structure);
- *Structure* that allows you to alternatively select the atoms of the main chain, the side chains, the ligands, ions, or portions of the protein with a defined secondary structure;
- *Sequence* that allows you to select a specific sequence.

From the *Select* menu we can chose a mode for the next selection: usually, the second selection replaces the first (*Replace*), which is the default option, but you can also add a second selection to the first (*Add*), or identify the intersection between the second and the first (*Intersect*), or subtract the second selection from the first (*Subtract*).
The selection can be cancelled with: *Select* > *Clear*.
For example, aiming to select all the acidic residues of the protein, including both the aspartate and glutamate residues:

- click on *Select* > *Residues* > *Asp*,

- modify the selection method with *Select > Menu Mode > Add* (when this mode is activated, a *+* symbol appears on the menu, *Select+*),
- finally, select glutamate residues by clicking on *Select > Residues > Glu*.

Alternatively, you can select the portions of interest from the command line, using the `select` command. In short, the basic syntax of this command is:

> `select #1` [selects the whole model #1; the identification number of each model is displayes in column *ID* (**9**) of the *Models* panel (**6**)].
> `select /A` [selects chain A in all the available models].
> `select :1-84` [selects residues from 1 to 84 in all chains of all available models].
> `select :Asp` [selects all aspartate residues in all available models].
> `select @C*` [selects all carbon atoms in the available models. Using `@CA` as a specifier, one can select all carbon α atoms of the available models].

These selection options can be combined using the logical operators and (&), or (|) and not (&~). Furthermore, it is possible to combine the selection options even without using the logical operators, but always respecting the hierarchy: #model > /chain > :residue > @atom.
For example, if you want to select all the acidic residues (aspartate and glutamate) of the B chain of the protein identified as model #3, you will write on the command line:

> `select #3/B:Asp|#3/B:Glu`.

To delete the previous selection:

> `select clear`.

You can assign a name to a defined portion of the structure, to be able to recall it later:

> `name <name of the selection> <identifiers of the selected portion>`.

In the previous example:

> `name acidic_res_B #3/B:Asp|#3/B:Glu`.

Once the portion of interest has been selected, it can be displayed with one of the display options offered by the program from the *Molecule Display* tab (see above, **15**). Alternatively, you can type the commands `show`, `hide`, and `color` on the command line:

> `show acidic_res_B atoms` [shows atoms of the selected residues as *sticks*]
> `hide /B cartoon` [hides the *cartoon* visualization of chain B]
> `color :127-234 red` [colors in red residues 127-234 of all protein chains].

Other visualization options can be used with the commands `show/hide`, among these: `atoms/cartoons/ribbons/surfaces`.
For a complete list of color names recognized by ChimeraX, see reference [5].

Visualization of symmetry-related molecules

When a crystallographic structure is loaded, the program displays only the contents of the pdb file, which correspond to the asymmetric unit. However, it is possible (and in many cases useful) to display also symmetry-related molecules. This is the case, for example, of a protein with a quaternary structure in which a crystallographic symmetry element coincides with the symmetry of the quaternary structure. The functional protein in this case can only be obtained by applying the crystallographic symmetry to the asymmetric unit. To display symmetry-related molecules, you need the information about unit cell and symmetry reported in the CRYST1 line of the pdb file, which is automatically read by the software when you open the pdb file.

To display symmetric molecules, select: *Tools > Higher-Order Structure > Crystal Contacts*. At this point, a window allows you to define the contact distance of the symmetric units that you want to be displayed: the software will display all symmetry-related molecules that are closer than the defined threshold. For each symmetry-related copy, the *Log* panel (**5**) reports the symmetry operator and the translations applied.

Furthermore, in the *Models* panel (**6**) the symmetry-related molecules are reported as a new object. In this panel you can select which molecules you want to display or hide, by ticking the appropriate boxes in the column with the eye symbol, **11**).

Alternatively, you can achieve the same result from the command line by typing:

> `crystalcontacts #<model> distance <in Å>`

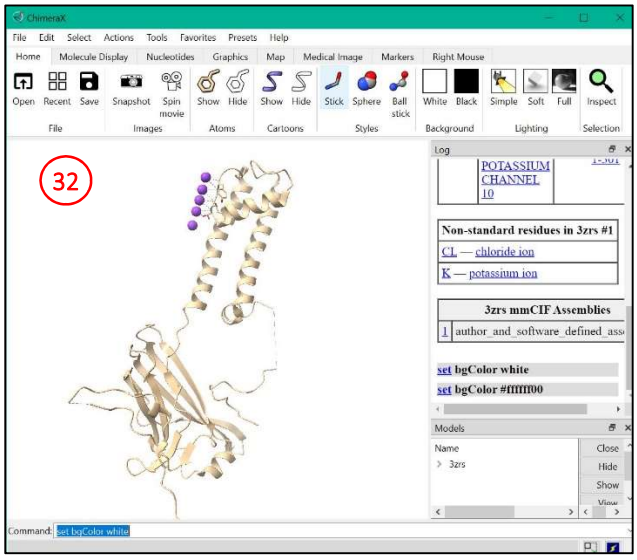
and then, if necessary, hiding some of the symmetry-related molecules with the command:

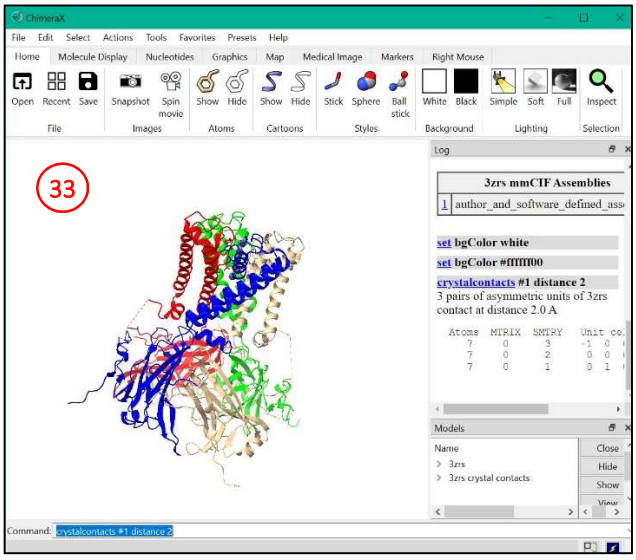
> `hide #<model>.<symmetry-related molecule> cartoons`

(the numerical code of each symmetry-related molecule is reported in the *Models* panel, **6**).

For example, when opening the structure with PDB code 3zrs (bacterial KIR-type potassium channel), the software only shows a monomer (**32**), which represents the asymmetric unit of the structure, but not its functional unit (the protein is homotetrameric). With the following command we can display the symmetry-related molecules, obtaining the visualization of the tetramer (**33**):

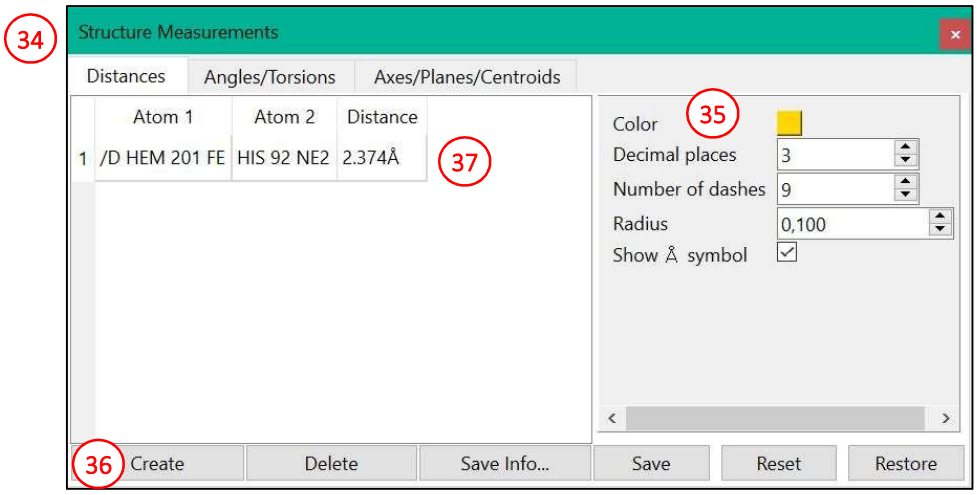
> `crystalcontacts #1 distance 2`.





How to measure distances, angles and torsion angles

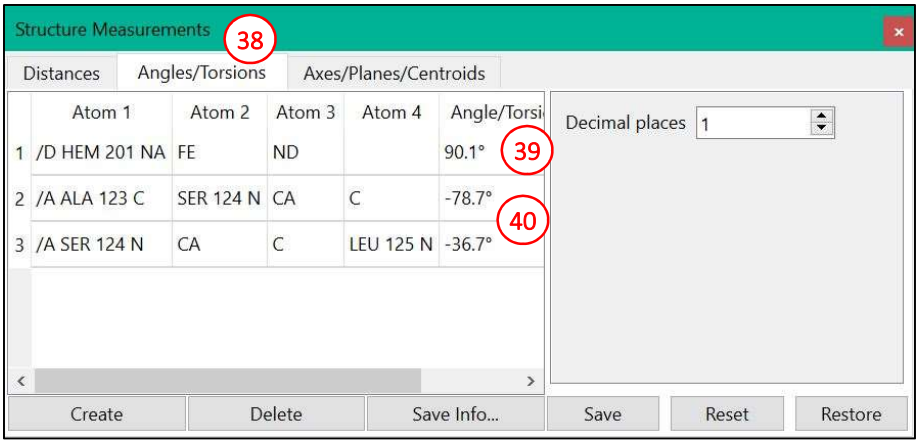
To measure the distance between two atoms, first select the atoms using the left mouse button, together with the *Ctrl* and *Shift* keys. At this point, select *Tools* > *Structure Analysis* > *Distance*. This command opens a dialog box (**34**) to measure the distance and select the measurement display options (**35**). Press the *Create* button (**36**) in the window to measure the distance. At this point, the distance appears in the dialog box (**37**), while the display panel shows a dashed line between the selected atoms.



You can obtain the same result by typing the `distance` command with the references of the selected atoms. For example:

> `distance #1/A:127@O #1/A:124@N` [measures the distance between the carbonyl oxygen atom of residue 127 of chain A and the amide nitrogen atom of residue 124 of the same polypeptide chain].

To measure the angles and torsion angles, you can use the second tab of the same window (**38**), selecting 3 or 4 atoms, respectively, and pressing the Create button. In the figure, an example of measurement of the angle between a nitrogen atom of the heme group, the iron atom and another nitrogen atom (hemoglobin structure, PDB code 6r2o) (**39**). In the second and third rows, examples of measurement of the torsion angles Φ and Ψ for a single residue of the structure (**40**).



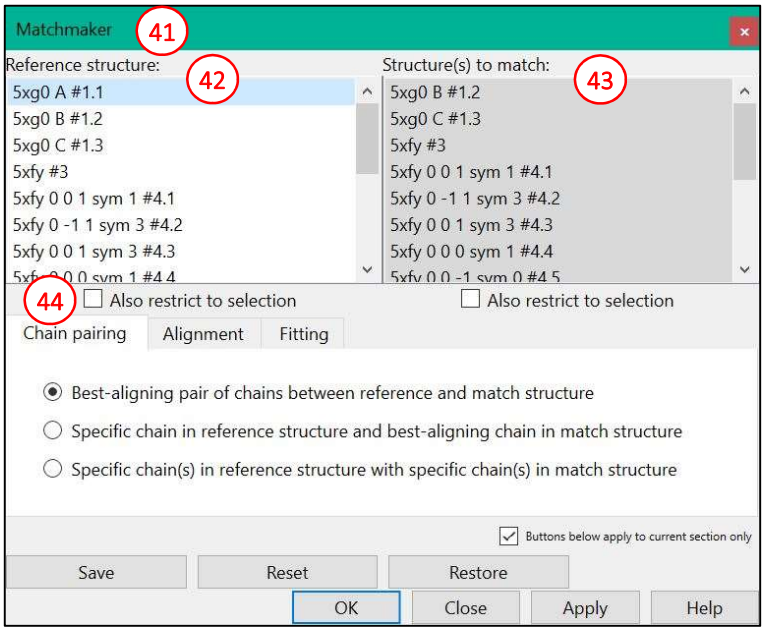
From the command line, you can display in the Log panel (**5**) the same angle or torsion angles, respectively, by typing the following commands:

> `angle /D:201@NA /D:201@FE /D:201@ND`
> `torsion /A:123@C /A:124@N /A:124@CA /A:124@C` [for the Φ torsion angle]
> `torsion /A:124@N /A:124@CA /A:124@C /A:125@N` [for the Ψ torsion angle].

Comparison of the structures of homologous molecules

To compare the 3D structure of proteins, graphical tools are indispensable. ChimeraX can perform this comparison overlaying homologous structures in order to minimize the distance between corresponding residues. This operation can be performed either through the menus or from the command line.

Selecting from the top menu *Tools* > *StructureAnalysis* > *Matchmaker*, a new window opens (**41**) where you can select the structures you want to overlay. In the left column (**42**), select the structure that will be used as a reference (i.e. the structure that in the overlay remains in the initial position); in the right column (**43**) select the structure to overlay. The overlay can also be performed only on a previously selected part of the structure, by ticking the box (**44**) under the list of structures. More sophisticated tools include the alignment of protein sequences (*Alignment*) or the optimization of the overlay through iterative cycles (*Fitting*).



Preparing images of the protein structure

Before saving an image, it is advisable to optimize the position of the model that you want to display. You can use the mouse to manually perform rotations and movements of the model, and the *Side View* panel (**28**), to move the foreground and background planes of the display window. Thus, only the selected part of the model will appear in the image.

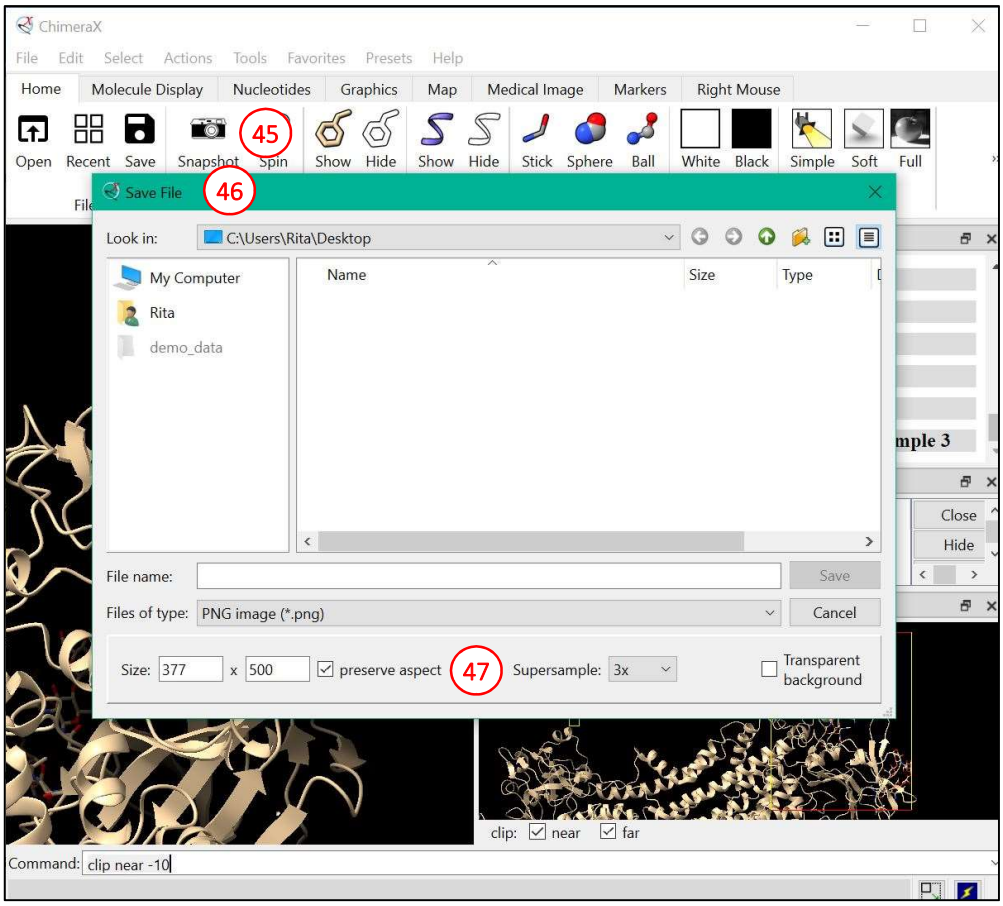
The same result can be obtained with the `clip` command and option `far` and `near`:

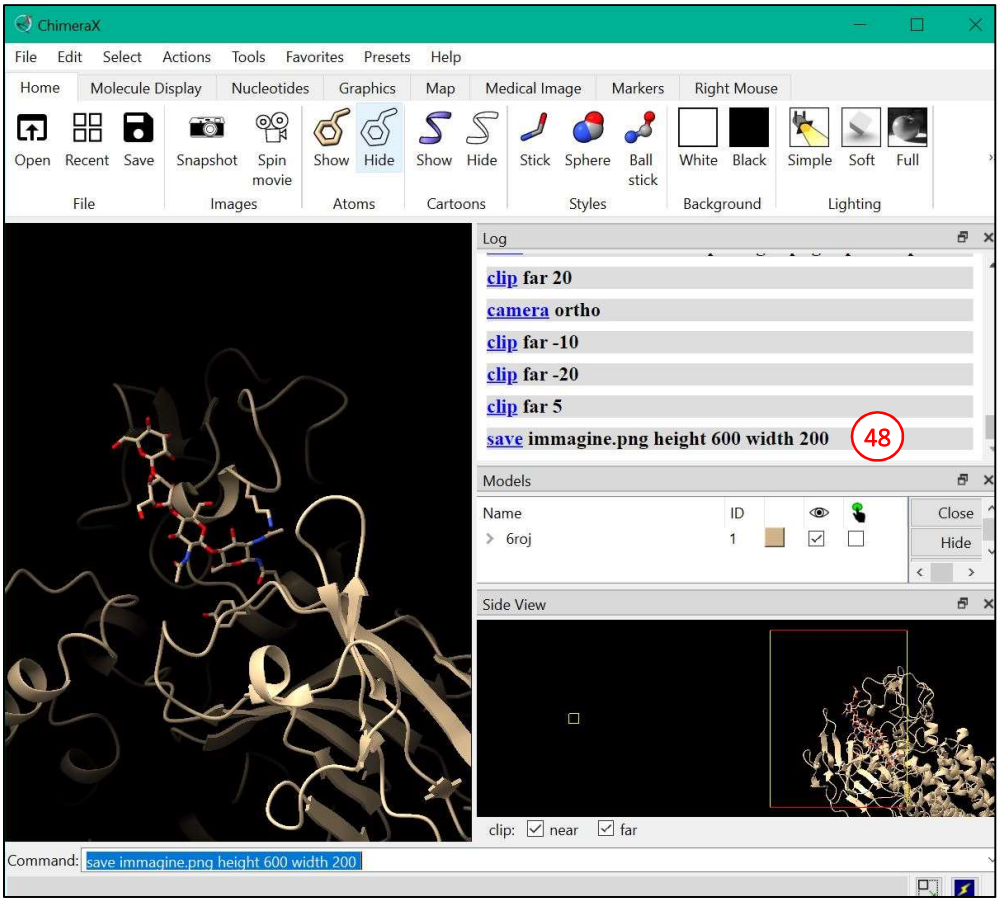
> `clip near 20` [moves the foreground plane 20 Å closer to the observer]
> `clip near -10` [moves the foreground plane 10 Å further from the observer]
> `clip far 5` [moves the background plane 5 Å further from the observer]
> `clip far -2` [moves the background plane 2 Å closer to the observer].

The ChimeraX software allows you to prepare good quality images of the structure of interest. The images can be prepared by selecting the *Snapshot* button from the *Home* menu at the top (**45**), or by selecting *File* > *Save*. In the latter case, the software opens a window (**46**) from which you can select the type of file you want to save (in this case png). From this window you can define the size and quality of the image to be saved (**47**).

The image can be saved also using the `save` command and options to define the pixel size (`pixelsize`), the height (`height`) e width (`width`) of the image (**48**):

> `save immagine.png height 600 width 200` [saves a 600 x 200 pixel png image].


ChimeraX
File Edit Select Actions Tools Favorites Presets Help
Home Molecule Display Nucleotides Graphics Map Medical Image Markers Right Mouse
Open Recent Save Snapshot Spin Show Hide Show Hide Stick Sphere Ball White Black Simple Soft Full
45
Save File
46
Look in: C:\Users\Rita\Desktop
My Computer
Rita
demo_data
Name Size Type
File name:
Save
Files of type: PNG image (*.png)
Cancel
Size: 377 x 500 preserve aspect
47
Supersample: 3x
Transparent background
Close
Hide
clip: near far
Command: clip near -10


ChimeraX
File Edit Select Actions Tools Favorites Presets Help
Home Molecule Display Nucleotides Graphics Map Medical Image Markers Right Mouse
Open Recent Save Snapshot Spin movie Show Hide Show Hide Stick Sphere Ball stick White Black Simple Soft Full
File Images Atoms Cartoons Styles Background Lighting
Log
clip far 20
camera ortho
clip far -10
clip far -20
clip far 5
save immagine.png height 600 width 200
48
Models
Name ID
6roj 1
Close
Hide
Side View
clip: near far
Command: save immagine.png height 600 width 200

Preparing Movies – Commands for Positioning and Moving the Displayed Models

The simplest type of movies that can be made with ChimeraX are those in which the model in question is rotated along the vertical axis of the figure (y-axis). This type of movies can be immediately saved using the *Spin Movie* button in the *Images* section of the *Home* tab (**21**). The software saves a movie of the model rotating along the y-axis at a speed of 60°/second. The file name and location are shown in the *Log* panel (**5**) after the file has been saved.

To prepare more sophisticated movies, you need to play with commands that move the display to a specific position of the model without using the mouse, thus increasing the precision and reproducibility of the display movement. The movement/rotation commands are applied to all selected models of the session, which can be identified by the check mark in the relevant column (**13**) of the *Models* panel (**6**).

Useful commands to move/rotate the models include:

- > `turn <axis> <rotation angle>` [allows the rotation of the model by a defined angle around a specific axis (x, y or z)]
  - e.g.: `turn x 90` [rotates the model by 90 degrees around the x-axis, which is the horizontal axis of the displayed image]
- > `move <axis> <movement in Å>` [moves the model along the defined axis, by a specified translation]
  - e.g.: `move y -9` [9 Å movement of the model along the –y direction, i.e. downwards]
- > `zoom <value>` [zooms in on the structure, if the assigned value is greater than 1, or zooms out, if the assigned value is less than 1]
- > `roll` [This command without further specifications continuously rotates the model around the y-axis, at a speed of 25°/second, producing one frame per degree.]
- > `roll <axis> <rotation angle per image> <number of frames>` [the model is continuously rotated around the defined axis]
  - e.g.: `roll x 2 180` [continuous rotation of the model by 2 degrees per frame, for 180 frames]
- > `stop` [This command stops the previous action, which can be, for example, the continuous rotation around an axis.]
- > `wait <number of frames>` [Pauses the action for a defined number of frames.]

When separated by a semicolon, multiple commands can be executed simultaneously. On the contrary, when commands have to be executed one after the other, you can separate them with a semicolon followed by the command `wait`.

When working on the movie of the model of interest, it is useful to save different orientations with the view name command:

- > `view name <orientation name>`.

To recall a specific orientation, use the command `view` (see above). Pay attention that the `view name` command only saves the view orientation but not the displayed contents: when a view orientation is recalled with the `view` command, models are visualized/hidden as they were before reorienting them, with the same colors and styles previously set, and NOT with the settings on display when the view orientation had been saved.

To start recording a movie with a specific sequence of actions, type the command:

- > `movie record`

At this point you can type movement commands, such as the `roll` command, or the commands:

- > `fly <1st orientation> <2nd orientation>` [moves the camera from the first view orientation to the second while saving 100 frames]

> `fly <view1> <frame number> <view2>` [as above, but with a defined number of frames between the two view orientations]

> `crossfade <#frames>; hide /C` [this is an assembled command: the *crossfade* command morphs between two images along the defined number of frames; at the same time, the *hide* command blurs the specified portion, i.e. chain C].

When all actions that you want to represent in the movie have been typed in, the movie has to be saved as a file with the command `movie encode`:

> `movie encode C:/<path>/<name>.mp4`

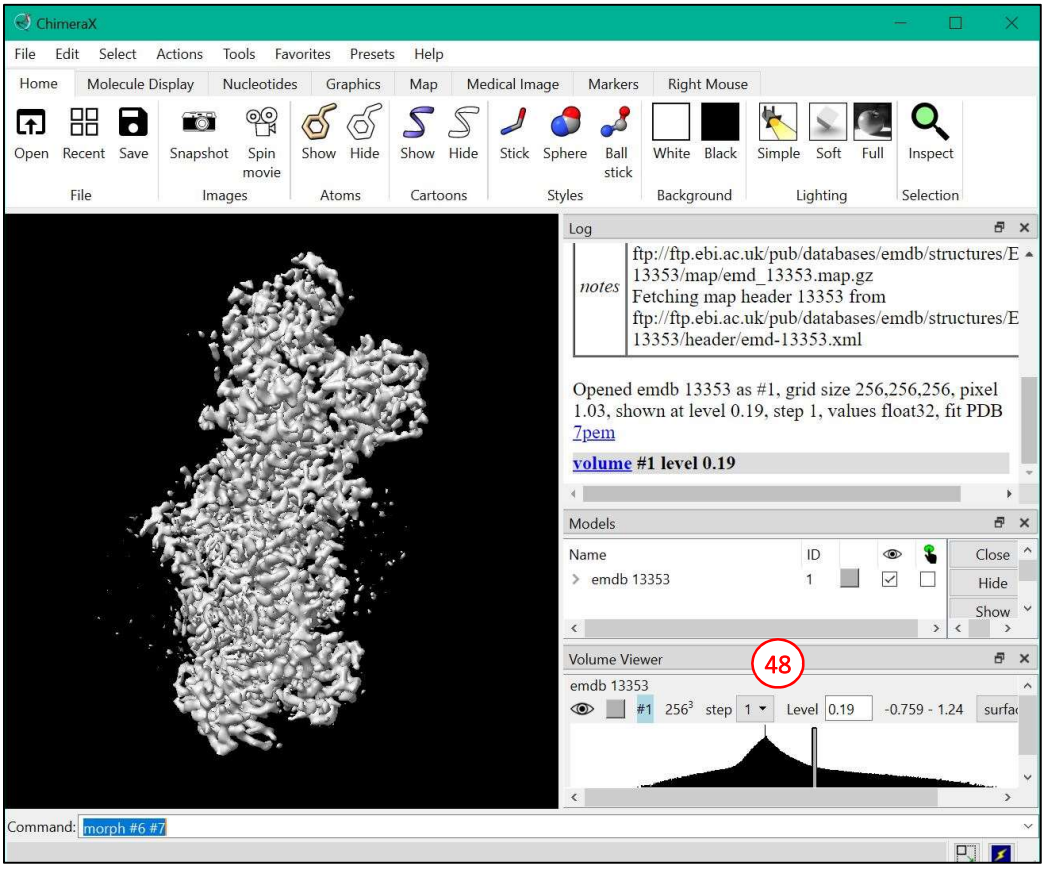
Viewing maps obtained from electron microscopy data

The ChimeraX software allows the visualization of electron density maps (or electrostatic potential maps) obtained from both diffraction and electron microscopy data. The typical format for these maps is the CCP4 format encoded in .map files. As with atomic models, to open a map use the command:

> `open #<number> fromDatabase EMDB` [which opens a map from the EM database]

or click on the menu *File* > *Open* (**2**) (selecting an already downloaded file) or *File* > *Fetch By ID* (**4**).
When opening the map, the software automatically opens the *Volume Viewer* panel (**49**) where the contour line value of the displayed map can be selected by changing the position of the bar. The same panel can also be opened from the menu *Tools* > *Volume Data* > *Volume Viewer*. Alternatively, from the command line you can type the command:

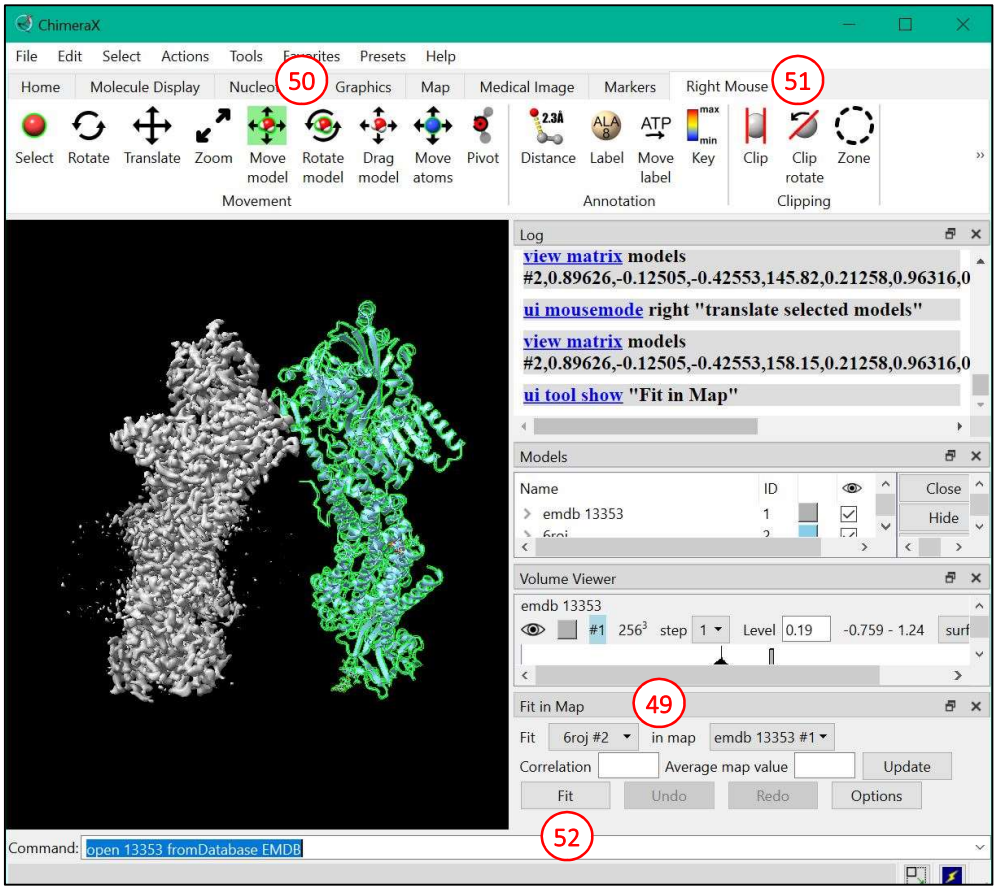
> `volume #<map ID> level #<contour level value>`



We often need to insert an existing model in the map (for example, when a previously determined crystallographic structure is available for the protein of interest and we want to interpret EM data). To this aim, load the file with the coordinates of the structural model. Then, the software fits the model within the

map using the *Tools > Volume Data > Fit* in the *Map* menu. After selecting this option, the software opens a panel in the main window (**49**) from which you can select the model to be fit and the electron density map to be used.

Before proceeding with the automatic fitting, it is useful to pre-position the model within the map to obtain a better correspondence between model and map. This can be done by selecting only the atomic model (from the *Models* panel or from the command line, see above) and manually inserting the model in the map with the *Move model* and *Rotate model* actions (**50**) from the *Right Mouse* menu (**51**). This sequence of commands will allow you to move the model but not the electron density.

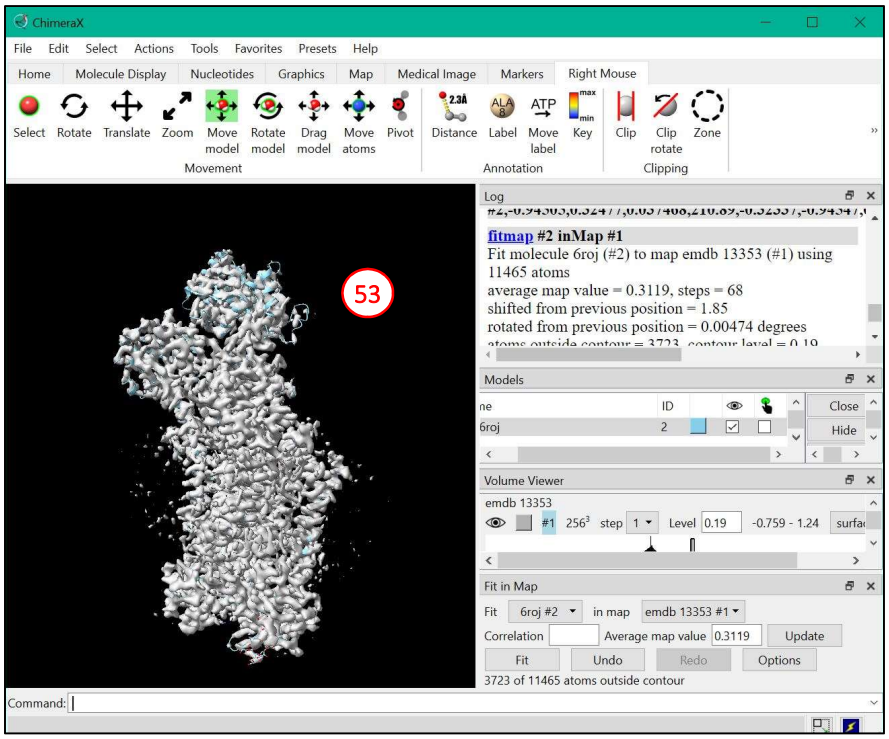


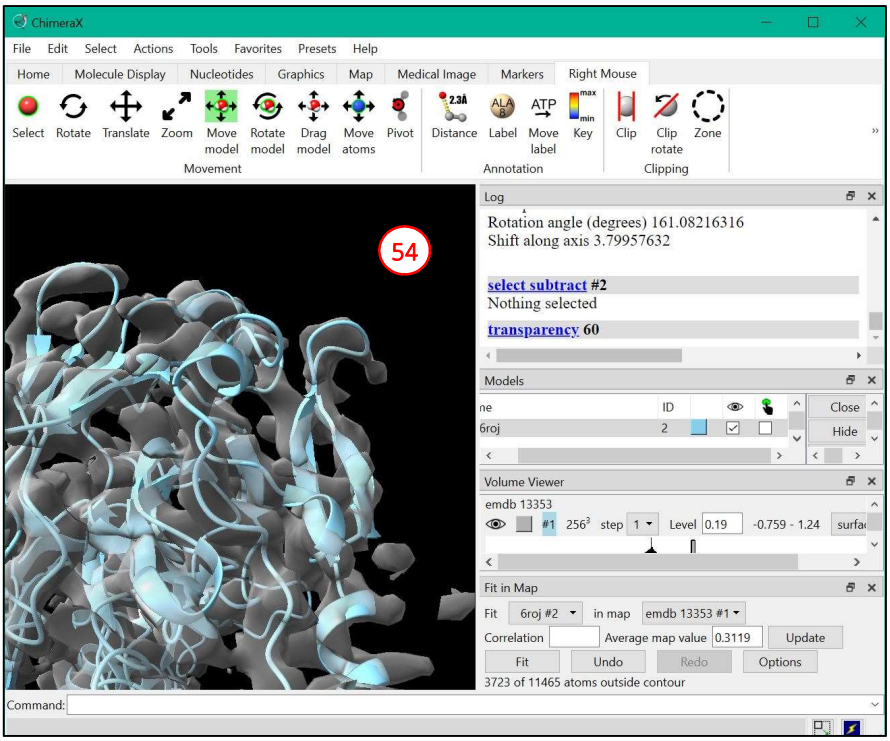
Once the model has been inserted into the electron density, the *Fit* button (**52**) will perform the required translations and rotations of the model in order to optimize its correspondence with the electron density (**53**). The same result can be obtained with the command:

> `fitmap #<model ID> inMap #<map ID>`

Once the model has been inserted into the map, their correspondence can be better visualized by making the map transparent (**54**), selecting the *Actions > Surface > Transparency* menu and the desired transparency value, or with the command:

> `transparency #1 60` [sets a transparency value of 60% for map #1].





References

[1] Goddard TD, Huang CC, Meng EC, Pettersen EF, Couch GS, Morris JH, Ferrin TE. "*UCSF ChimeraX: Meeting modern challenges in visualization and analysis.*" Protein Sci. 2018. 27(1):14-25. doi: 10.1002/pro.3235.

[2] https://www.cgl.ucsf.edu/chimerax/

[3] https://www.youtube.com/watch?v=8XmbMROKNnQ&list=PL4eF1KHNgDfIYSKCS3_S0PTRYtYTV9Myi

[4] https://www.cgl.ucsf.edu/chimerax/docs/user/index.html#commands

[5] https://www.cgl.ucsf.edu/chimerax/docs/user/commands/palettes.html