**ChimeraX tutorial**

Note: this is just to give you the basic idea and uses. Many actions are illustrated with command line instructions relevant to the ChimeraX session Bxb1\_Tail\_Tip\_Models.cxs. For anything not covered here, there is a great User Guide accessible either in the program or online. You can also check out the Brown Lab’s video tutorials on YouTube!

**Important areas of the software**

* Top toolbar - important areas:
	+ Select: here you can select by chain name, residue type, sequence, secondary structure etc.
	+ Actions: most important here is the color command
	+ Tools: here you can access the sequence of each chain, perform structural analysis, and predict new structural models
* Top Ribbon:
	+ Home: make a spin movie; change background color; change the lighting
	+ Molecule Display: show/hide atoms, cartoons, or surfaces; surface coloring options; view sequences of all displayed models; view model interface map
	+ Graphics: display effects like silhouettes; view orient; side view
	+ Right mouse: select different actions for the right mouse
* Right side windows (can be collapsed into one with tabs for easier viewing; drag and drop):
	+ Log: reports all actions and displays any error messages
	+ Models: details the Name, ID, and color of each model and gives you control over which models are visible (eyeball icon) and/or selected (green button with pressing finger) via checkboxes; lets you close models completely (close button)
* Command line: bottom text box that gives you more control over various commands and can be used as a shortcut

**Basic use of the mouse**

* Rotate: left click and drag.
	+ Two different modes depending on location of your initial click:
		- Click near center of page: rotate about x and y (horizontal and vertical) axes
		- Click near edge of page: rotate about z (in and out of page) axis
* Translate: right click and drag
* Zoom: scroll

**Basic viewing and depiction instructions**

* To reset your viewing angle: in the command line type “view orient”
* Viewing one model at a time: check/uncheck models in the Models window, right panel
	+ Try showing just Model #8 (ChainP\_gp29\_TailSpike.pdb)
* Showing atoms, cartoons, surfaces: use the options in the Molecule Display ribbon toolbar
	+ This works on either all displayed models or just on the selection (if there is one)
	+ If you’re showing just a single chain and click one of these buttons, the other chains in the model will also be shown
* Coloring the model by chain or by rainbow: use coloring options in Molecule Display toolbar
	+ This works on either all displayed models or just on the selection (if there is one)
* Surface mapping:
	+ Electrostatics
	+ Lipophilicity
	+ Heteroatom
	+ If you’re showing just a single chain and click one of these buttons, the other chains in the model will also be shown
* To close a model: in the right window for “Models” click on the name of the model you want to delete (let’s close #1) and click “Close” at the upper right

**Advanced use of the mouse using the Right Mouse ribbon toolbar**

* Select: right click on residues or atoms to select them
	+ Clearing a selection: type “select clear” into the command line or right click on empty space while using the Select right mouse tool
* Move model: translate a selected model
* Rotate model: rotate a selected model relative to everything else
* Distance: right click two atoms in a row to display the distance between them (must be displaying atoms for this to work) – we will come back to this

**Advanced viewing and depiction instructions**

* Viewing one chain at a time (You can see a particular chain’s name by hovering over it):
	+ For example, to view only chain Pa of model #8:
		- In the command line type: select #8/Pb/Pc
		- Hide atoms/cartoons/surfaces in the Molecule Display ribbon toolbar
	+ Alternatively (useful for models with many chains):
		- select #8/Pa
		- Toolbar/Select/Invert/Invert Selection
		- Hide atoms/cartoons/surfaces
* Viewing the sequence of a single chain; two options:
	+ Use the command line to select the chain, then click the Sequence button in the Molecule Display ribbon toolbar
	+ Tools/Sequence/Show Sequence Viewer and select the chain you’re interested in
* Selecting residues
	+ By eye: show atoms and click on them with the right mouse Select option
	+ By sequence: click and drag on a particular residue or region in the sequence viewer
* Using the side viewer to focus in on a region:
	+ Graphics in the Ribbon toolbar / Side view
	+ Click and drag the vertical yellow lines to narrow the field of view

**Basic editing of a model**

* Making a copy of a model (useful if you want to delete fragments while keeping the original around):
	+ In the command line type: combine #8 name NewModel
	+ (or whatever name makes sense)
	+ The new model will get the first available model # (1 in this case)
* Deleting chains or atoms:
	+ select #1/Pb/Pc
	+ Top toolbar/Actions/Atoms and Bonds/Delete
* Try it: make three copies of model #8 and delete 2 chains from each so you end up with three models each with a single (distinct) chain

**Structure analysis**

* Aligning models/chains with matchmaker
	+ Example: align the three chains of the tail spike protein created in “Basic editing of a model” above
	+ Method 1 using buttons:
		- Top toolbar/Tools/Structure Analysis/Matchmaker
		- Reference structure: model #1 (chain Pa)
		- Structure(s) to match: models #13 and 14
	+ Method 2 using command line:
		- matchmaker #13 #14 to #1

OK we are done with those, so close the copied models in the right panel and click to display model #5 (ChainM\_gp23\_TailTipCage.pdb) and model #7 (ChainO\_TailTipCore.pdb) for the next steps. Command line: view orient

* H-bonds: upper toolbar/Tools/Structure Analysis/H-Bonds
	+ Lots of options here; let’s just look at the H-bonds between the tail tip cage and the baseplate hub. Here’s how:
	+ Click the “select” checkbox for each displayed model in the right “Models” window
	+ Adjust these options in your H-bonds dialog box:
		- Pseudobond display color – choose something other than red or yellow
		- Limit results:
			* Limit by selection with both ends selected
			* Include intermodal
			* Nothing else checked
		- Treatment of results:
			* Select atoms
			* Reveal atoms of H-bonding residues
		- Click “OK” or “Apply” – it will take a couple minutes to complete
		- Without de-selecting anything, color the residues involved in H-bonds and show the atoms as sticks by upper toolbar/Actions/Color
		- Now in the command line: select clear
		- Ribbon toolbar Molecule Display/Stick
		- Zoom around and translate so you can see things; use your right mouse tool and the side view tool to make a clear view of bonded atoms
* Close contacts: upper toolbar/Tools/Structure Analysis/Contacts
	+ Similar in spirit to the H-bond tool
	+ This is useful if you don’t have hydrogen bonds but still want to show a close contact between chains or residues

**Sharing your work**

* Saving a high-res image:
	+ Upper toolbar/File/Save
	+ File of type: TIFF image (\*.tif \*.tiff)
		- Don’t be deceived by ImageJ TIFF map (\*.tif \*.tiff)
	+ Can make the background transparent if you like (helpful for presentations)
	+ Supersample: None
	+ If you want super high-res, increase the size of the image with the text boxes
* Making a spin movie
	+ Ribbon toolbar / Home / Spin Movie
	+ Check the log to see where the file got saved to – probably Desktop

**Structure prediction with Colab-fold (Alphafold lite)**

* Upper toolbar/Tools/Structure Prediction/Alphafold
	+ Brings up a new window on the right side
	+ Sequence dropdown box: select one of the chains or Paste a new sequence
	+ Click “Predict” and sign in with your google account to run it
	+ By default the predicted models are saved to a random spot on your computer – downloads for me. The protein names are generally not included in the file name.
* General notes:
	+ I’d recommend using the Alphafold Server first, since it’s faster and easier to use
	+ However, sometimes Alphafold3 is very bad and the earlier version (under the hood here) works better