**Using UCSF Chimera**

We will use a structure of GFP as an introduction to UCSF ChimeraX, a powerful structure viewer for looking into the details of X-ray crystallography and electron microscopy-derived structures. ChimeraX maintains a number of tutorials for specific tools and features of the program that are helpful if you’ll continue to use Chimera. We’ll be looking at PDBID: 1EMA a crystal structure of GFP.

***Opening and Closing Structures***

Open the UCSF ChimeraX application and in the command line type **“open 1EMA”.** Alternatively, you can open local files by selecting File > Open. Both commands can be used to open many structures at the same time. **“close”** will close all structures, or you can specify the model you wish to close by typing **“close #2”** (this will close the second model opened).

**“open 1EMA”** should open a structure of GFP. We’ll now explore representations of structures. The default view is as a ribbon diagram. While we are in the ribbon view, let’s look at the residues that form the chromophore.

***Visualizations and Movement***

**“rainbow”**

This colors the chain from N to C terminus by rainbow color if only a single model is open.

Open the side view controls to make looking at the structure a bit easier.

**Tools > General > Side View**.

Slide the bars to change the perspective and hide some planes.

To rotate the structure, click and move the mouse.

To move a structure, hold **Alt (PC) | Option (Mac)** and drag the structure (or any active model with the mouse) or use the **middle mouse button**.

To select a structure, hold **Ctrl (Both PC and Mac)** and click to select a residue. Press the **up arrow** to select the whole chain. Hold shift to add or subtract to the current selection

To zoom in, click and hold the **right mouse button** or use pinching and expanding motions on a trackpad.

ChimeraX also has ribbons along the top. Select “Right Mouse” and choose “Translate”. This will change the function of the right mouse on a PC, or the two-finger click on a Mac trackpad. Depending on your setup, it may be best to set up the right mouse option (or two-finger click) as the desired action.

***View Residues***

ChimeraX allows input either in through user-interfaces in the dropdown menus or the command line. We’ll now use the command line to look at the residues involved in forming the chromophore:

First select the residues of the chromophore and display them by using the command line:

disp :66

This command tells ChimeraXto display the full amino acid structure, side chains and main chain, for residue (: denotes residue) 66. See [here](https://www.cgl.ucsf.edu/chimerax/docs/user/commands/atomspec.html) for complete description of the ChimeraX target specification.

You can also use the dropdown menus for the same functions: Select residue Thr203 either by clicking as descried above or the command line (sel :203) and show side chain using the drop-down menu:

**Actions > Atoms/Bonds > Show**

**Actions > Color > By Heteroatom**

When Thr203 is mutated to Tyr, GFP becomes Yellow Fluorescent protein (YFP). Measure how close the Threonine side chain is to the chromophore. Open the Structure measurements tools using the dropdown menu:

**Tools> Structure Analysis> Distances**

This will open a dialogue for measuring the distance between two atoms. Measure the distance between the oxygen of Thr203 and the hydroxyl of Tyr66 by selecting both atoms specifically (Control + Click one atom, Shift + Control + Click the other). Then click “Create” in the Distances dialogue.

How many angstroms apart are they?

Do you think that this could be a hydrogen bonding interaction? (It may be helpful to note that many hydrogen bonds occur in the 2.7–3.3 Å distance scale)

Now, let’s look at the space-filling model of GFP to better understand how it interacts with other proteins. Select the ribbon “Molecule Display” and select Surface “Show”

Now let’s look at a mutation A206K that prevents one GFP molecule from interacting with another. Dimer interactions frequently occur through hydrophobic patches. Use the preset (hydrophobicity) to look at the surface of GFP. Is Ala206 in a particularly hydrophobic region of GFP?

Now let’s compare the chromophore of GFP to that of CFP. Close your current session of ChimeraX and start a new session. Open both structures ID: 1EMA and ID: 1OXD. These are structures of GFP and CFP respectively. The two structures will open not perfectly aligned. We can now use ‘matchmaker’ to align the two structures better to compare the change between chromophores using MatchMaker:

**Tools > Structure Analysis > MatchMaker**

Select one reference structure (to stay in the same place) and at least one structure to match. Press OK to align the two sequences in space. You should see that they overlap more perfectly now. If you don’t believe it, use the model panel (Tools > General > Model Panel) to inactivate one of the models and move it farther away from the other structure. Now try MatchMaker again.

Let’s look at how hydrogen bonds have changed with the introduction of the Tyr66Trp mutation Measure the distances between His148 in both structures. Do you think a hydrogen bond is occurring in GFP? What about CFP?