**Chapter 11C mutagenesis tutorial**

Installation instructions for PyMOL: https://sites.bu.edu/mcneely/it-advice/pymol/
It does require you logging in with your BU user name and password.
Please download PyMOL before you come to your lab section.

It is recommended that you bring a 3-button mouse to lab.

There is no pre-lab and in-lab to complete. The post-lab write-up needs to be completed by the end of your lab day on Gradescope.

The Table below lists the 4 proteins used in this exercise. Your TFs will assign you a PDB file to work on.

| Group number | Protein/enzyme keywords | PDB codes | Align with |
|---|---|---|---|
| 1 | Ketohexokinase-C (human) | 6UL7 | 6P2D |
| 2 | Ketohexokinase-C (mouse) | 6P2D | 6UL7 |
| 3 | Lactate dehydrogenase (plasmodium) | 1LDG | 5YTA |
| 4 | Lactate dehydrogenase (pig) | 5YTA | 1LDG |

1. Load your protein of interest by using the fetch command:

**`fetch`** `<PDB code><chain letter>`

For this tutorial, we are going to load the first monomer (chain **A**) for the protein GAPDH from spiny lobster (PDB code **1IHY**)

**`fetch 1IHYA`**

Alternatively, you can click on File > Get PDB. Type the PDB code in the box provided and any specific chain you want to visualize, and hit "Download."

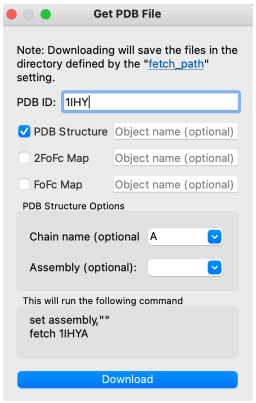



**Figure 1**. Pop up window to load a single chain in PyMOL.

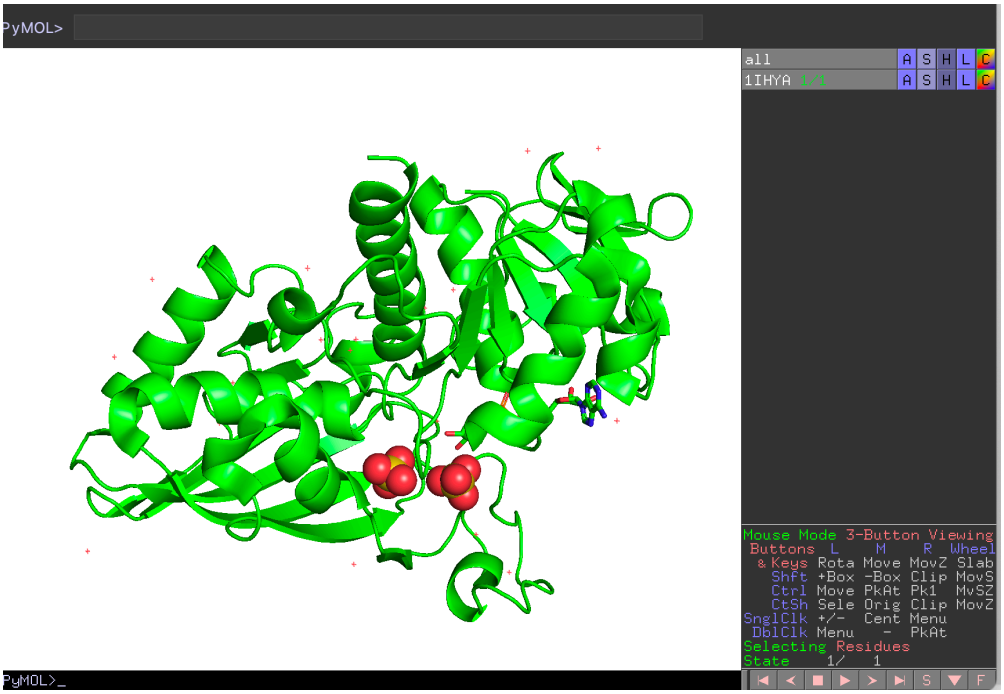



**Figure 2**. Loaded GAPDH monomer in the graphics user interphase window

2. Find your protein ligand by clicking the sequence button at the lower right (Figure 3). The sequence will appear at the top of the window (Figure 3). Scroll to the right until you find your ligand abbreviation. Make sure you find the ligand that sits in the active site cleft (do not choose an inorganic or metal ligand/cofactor).

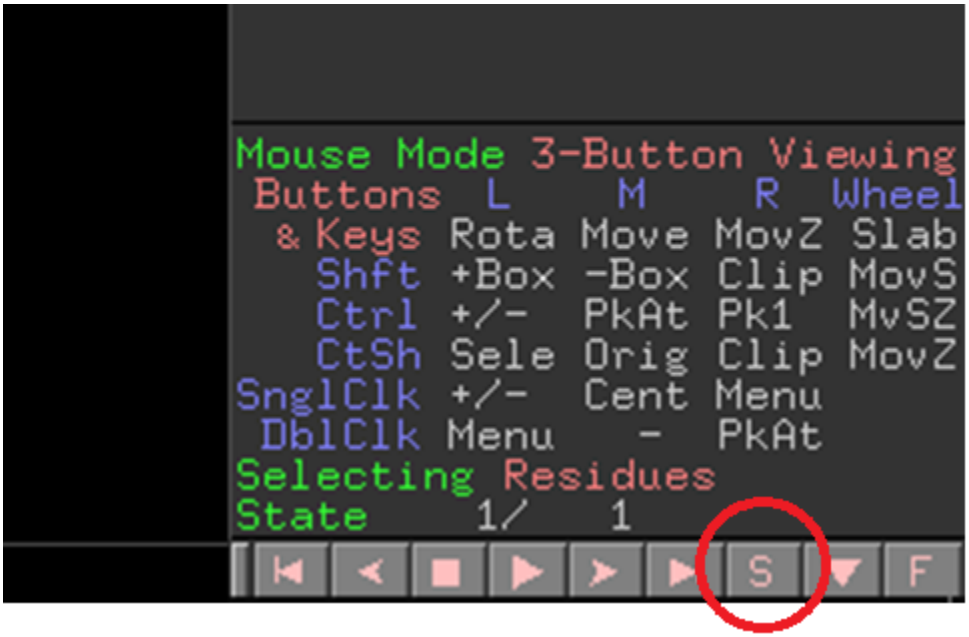



**Figure 3**. The sequence tool button

Click on the ligand. This then should appear as a highlighted selection "(sele)" in the menu at the right.

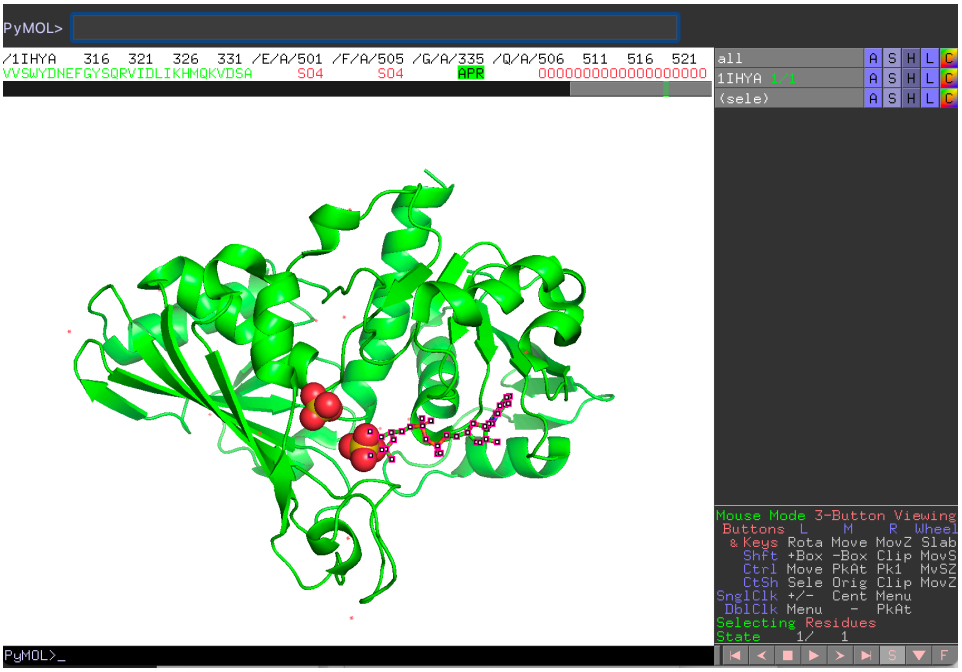



**Figure 4**. The crystalized ligand for GAPDH is 1IHY is APR (adenosine 5'-diphosphoribose).

3. Once you have selected your ligand, use the “color” box to the far right of the (sele) line and color the ligand by element so that it can easily be distinguished from the protein.

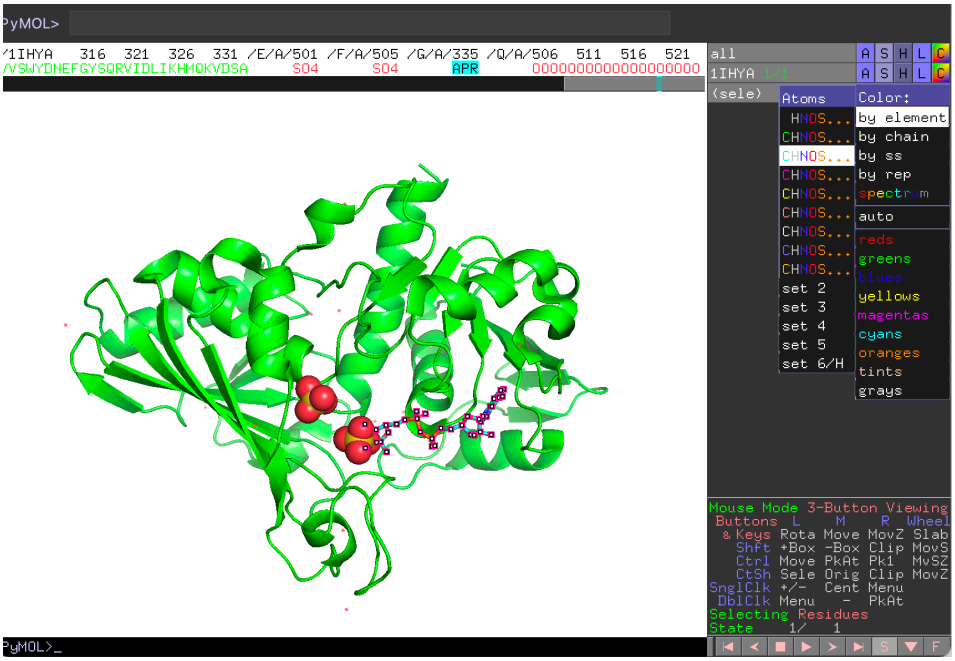



**Figure 5**. The ligand APR is colored by element.

Under the “**S**” box (for “**S**how”), select the “licorice: sticks” command.

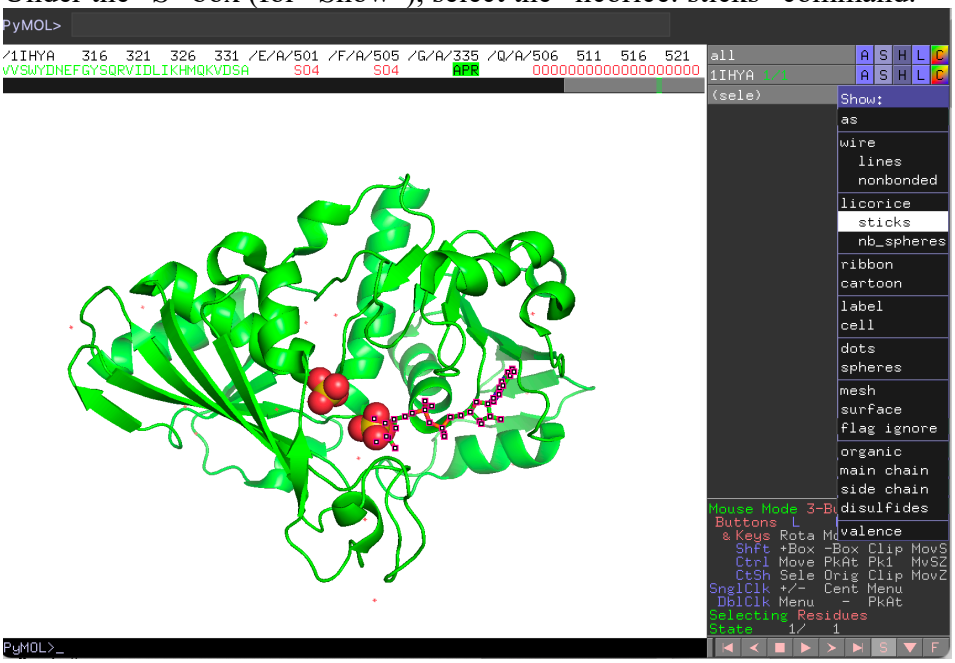



**Figure 6**. The ligand APR is shown in sticks.

You should now see the ligand and protein completely with the oxygen atoms in red and nitrogen atoms in blue, but carbons in the chosen colors with ligand different than protein.

To center the ligand on your window, right-click on the ligand and select “orient” or “center” (Figure 7).

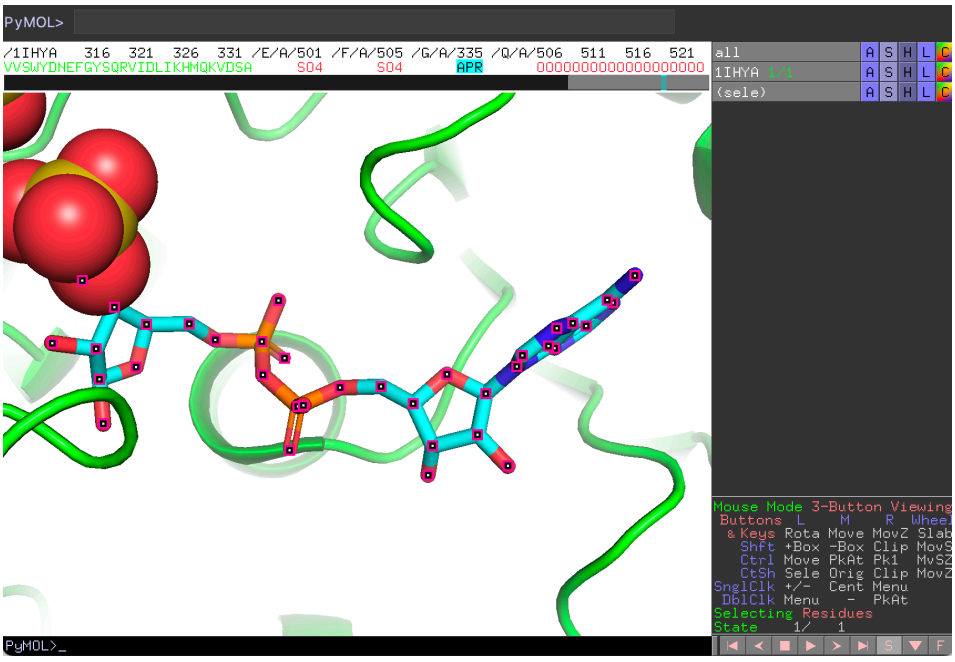



**Figure 7**. The ligand APR is centered on the window.

4. Finding an electrostatic interaction between ligand and protein.

Click on the ligand so that all the atoms are selected.

Go the wizard menu and drop down to “Measurement” A new set of commands should appear in the menu to the right above those commands for Mouse Mode called “Measurement”.
Click on the top line under “Measurement” and slide down menu to highlight “Polar Neighbors” and select “in all objects”. Careful now because the next click needs to be on an atom in your ligand.

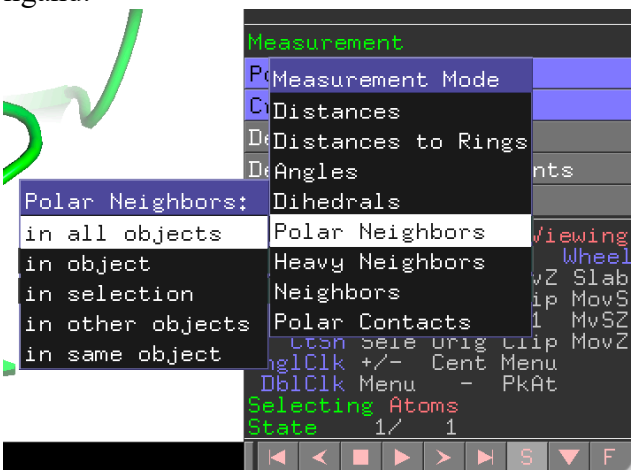



**Figure 8**. Going into “measurement” mode and selecting “polar neighbors” and “in all objects”.

Click on a polar atom in the ligand.  All neighbors will appear along with the distances.

Click on another polar atom, and then another until all the polar atoms have been selected.  This causes each measurement from each polar atom of the ligand to appear in the menu at the right as a "measure##" (Figure 9).
Click "Done" at the bottom of the Measurement menu.

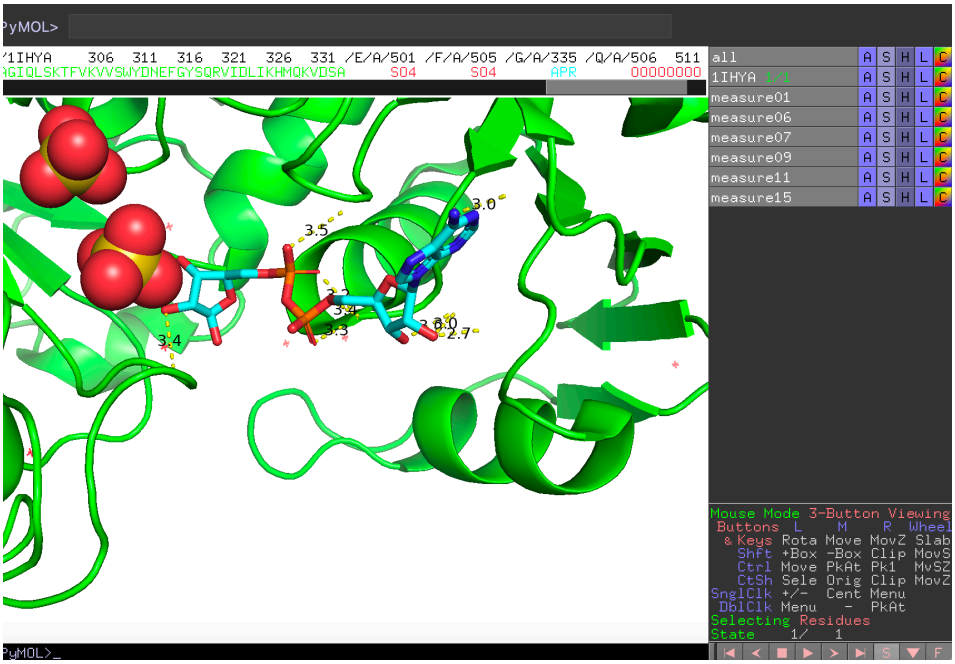



**Figure 9**. Distances between polar atoms in APR ligand and neighbors.

Under the "**S**" box (for "**S**how") for your protein, select the "licorice: sticks" command. This shows all the residues in your protein as sticks.
To visualize the polar residues interacting between your ligand and the protein better, you can adjust the clipping plane by rolling the scroll wheel on your mouse.

Take a screen shot of the ligand interacting with residues on the protein with all the measurements made (Figure 10).  Upload on Gradescope.

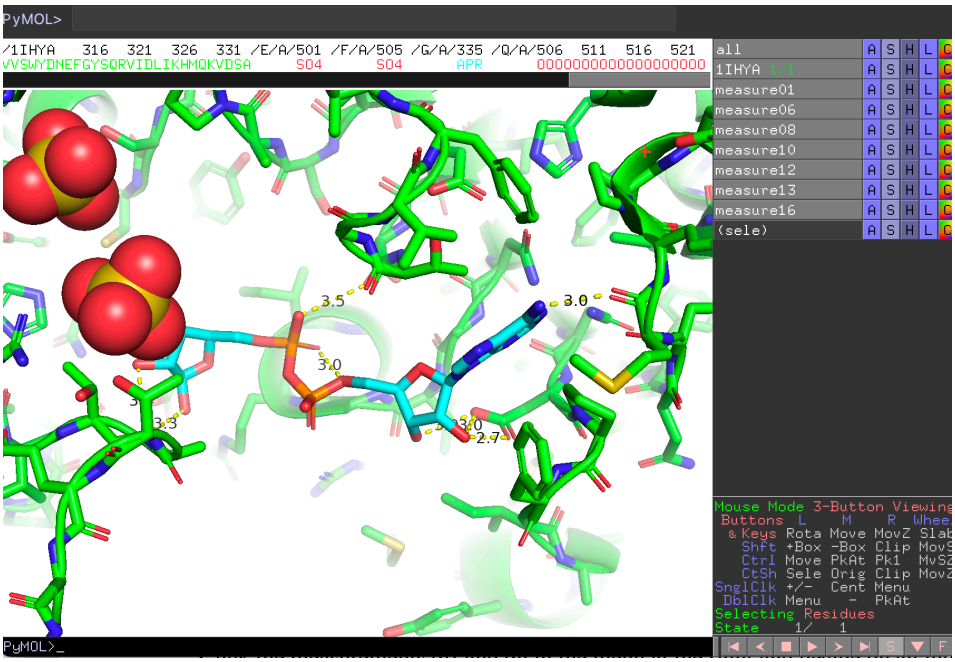



**Figure 10**. Polar residues on APR ligand interacting with residues on the protein with distances shown.

5. Identifying the residue in the protein that you would like to mutate.

Click the measurements off and on in the menu at the right and decide on an interaction between an atom on the ligand and a residue that makes a good H-bond or salt bridge to the ligand. Click all the "measure##" off except the one you have chosen (Figure 11). Good starting distances for hydrogen bonds are between 2.5 to 4.0 angstroms

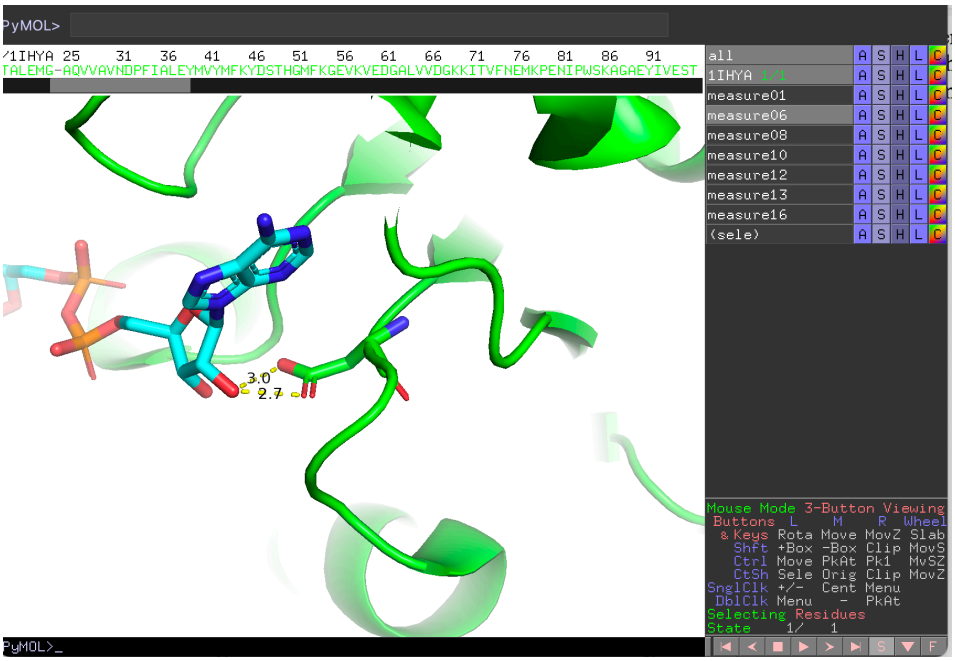
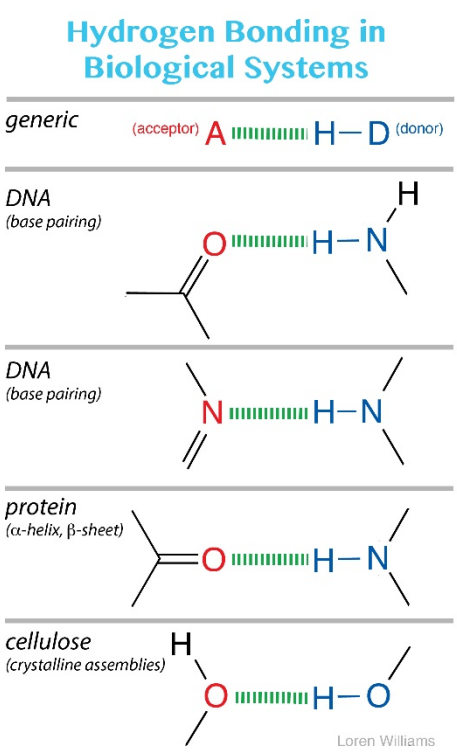



**Figure 11**. Selected H-bond distance between ligand and residue D32 on the GAPDH protein.

Identify the residue on the protein involved in the interaction you have chosen.
Show this residue as sticks and color it by element. Ensure that you hide all the other residues on your protein by clicking on the "**H**" box (for "**H**ide") for your protein, select the "licorice: sticks" command.

Take a screen shot of the interaction between the ligand and the residue on the protein with the distance clearly shown (Figure 11). Upload it on Gradescope.

(Optional) If you want to show hydrogens on the crystal structure, go to **A**ction for "all" and select "hydrogens" and then select "show". Some examples of hydrogen bonds include:




(https://ww2.chemistry.gatech.edu/~lw26/structure/molecular_interactions/h_bond_functional_groups.jpg)

6. Mutating the residue involved in your chosen interaction.

Go the wizard menu and drop down to "Mutagenesis: Protein" A new set of commands should appear in the menu to the right above those commands for Mouse Mode called "Mutagenesis".

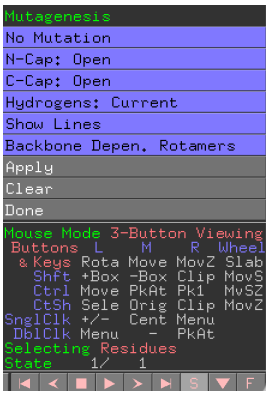



**Figure 12**. Mutagenesis mode.

It will ask you to "Pick a residue . . ." Click on any atom in your chosen residue.

Click on the top line under "Mutagenesis" and slide down the menu that appears to select the change you wish to make in the chosen residue. Careful now because the next click will continue to mutate residues.

Go down the Mutagenesis menu and Click on "Apply." This makes your chosen residue into the one you just chose.

If you are successful, click on "Done" in the Mutagenesis menu.

Here in this example, I will select my residue to be mutated to tyrosine. After configuring the rotamer confirmation using the left/right pink arrows at the lower right of the graphics user interface (Figure 13), click on "Apply" and "Done" to finalize your mutation. Please choose the rotamer that gives the lowest strain. In this case, we selected rotamer 3 (Figure 14). Tabulate the rotomers and their strain values in a table and upload it on Gradescope.

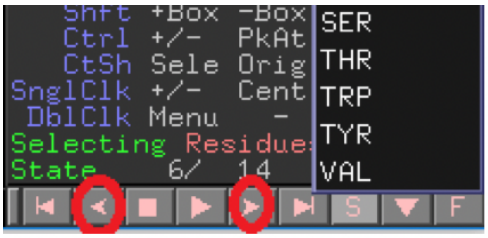



**Figure 13**. Left/right pink arrows for adjusting rotamers.

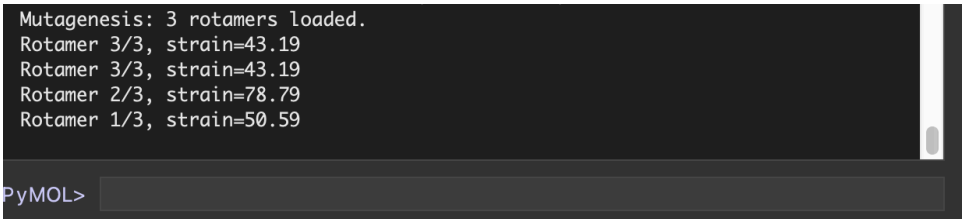



**Figure 14.** On the top of your interface, the different rotamers and its associated strain values will be shown.

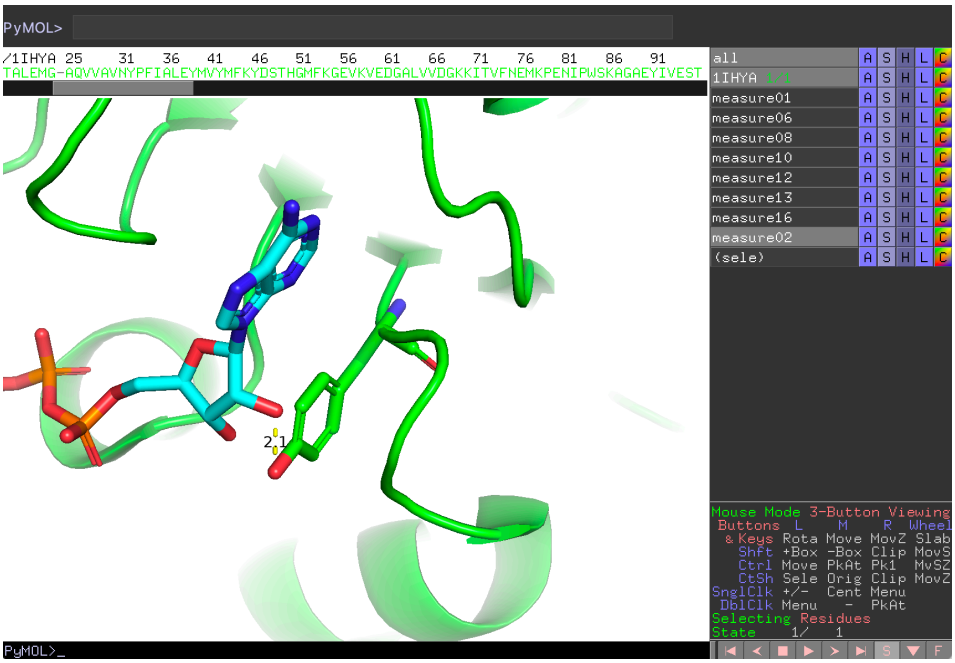



**Figure 15**. Bond distance between the same atom of the ligand and a newly "mutated" residue on the protein.

Go through the procedure in Step #6 above to use the Measurement menu and measure the distance from the same atom of the ligand and an atom of your newly "mutated" residue. Be sure to select "Distances" instead of polar neighbors. Click on first atom them the second to get the distance between the two.

Take a screen shot of the new measurement (Figure 15). Upload it on Gradescope.

7. Alignment of two 3D structures

In this example, I will align GAPDH from a different species, spinach (PDB code **2PKR**) with GAPDH from spiny lobster (PDB code **1IHY**).

If you are starting from a new PyMOL session, type in the command line:

```
fetch 2PKRA
fetch 1IHYA
```

If you are continuing in the same session, you only need to fetch **`2PKRA`**.

Remove water molecules by clicking on A > remove waters, if desired.

Re-color the two molecules in different colors.

To align the two molecules together, click on A of one of the molecule (e.g. 1IHYA) and select align > to molecule > name of other molecule (in this case it is 2PKRA).

The two proteins should now be aligned (Figure 16). Take a screen shot and upload on Gradescope.

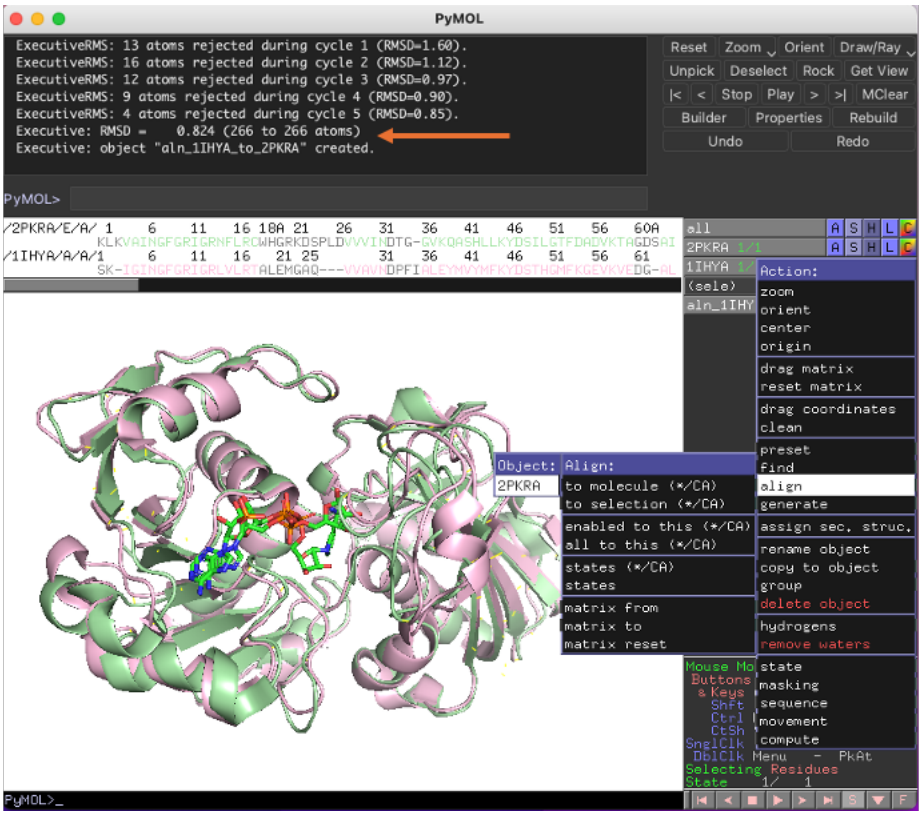



**Figure 16**. Aligned proteins after entering the alignment command. After entering the alignment command, 2PKRA and 1IHYA will now be aligned. The RMSD score indicated by the orange arrow indicates how close a fit the two structures are.

Check the RMSD score generated by the alignment.
The RMSD (root mean square deviation) is a measure of the degree of structural overlap between two protein structures, and is an average measure of how far apart an α-Carbon (Cα) atom is in one structure is from the Cα atom from the other structure after they are aligned. Alignments with RMSD < 2.5 - 3.0 Å are reasonable alignments.

In our case, the RMSD is 0.824, so it is < 1 Å (see the orange arrow in Figure 16).