Molecular Visualization with PyMOL: DiSCoBio 2014

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July 8, 2014

1 Introduction

The purpose of this tutorial is the familiarize you with using the PyMOL molecular graphics system. To do so we will be examining three dimensional (3D) structures of MDM2 (mouse double minute 2) in complex with two different molecules: p53 (an anti-tumor molecule), and an inhibitor of the p53-MDM2 complex.

2 Introduction to PyMOL

PyMOL is a program that is used for viewing and manipulating the 3D structures of molecules.

- Launch PyMOL on your computer.

Now, let's load a protein structure to look at. There are many ways that we can load a protein structure in to PyMOL: using the `File > Open` menu, using the `Plugin > PDB Loader Service`, or by using the `fetch` command line tool. We will first need to get a PDB structure file from the PDB website. Open a web browser and go to `http://www.pdb.org/pdb/explore/explore.do?structureId=1YCR`. Click `Download Files` (on the right side of the page, above the picture). Finally click on `PDB File (Text)`. This will download the a file called `1YCR.pdb` in your Downloads folder.

In PyMOL, go to the `File > Open` menu and select `1YCR.pdb`.

- `File > Open...`

To make explaining things easier in this tutorial, we will label certain portions of the user interface as in Figure 1.

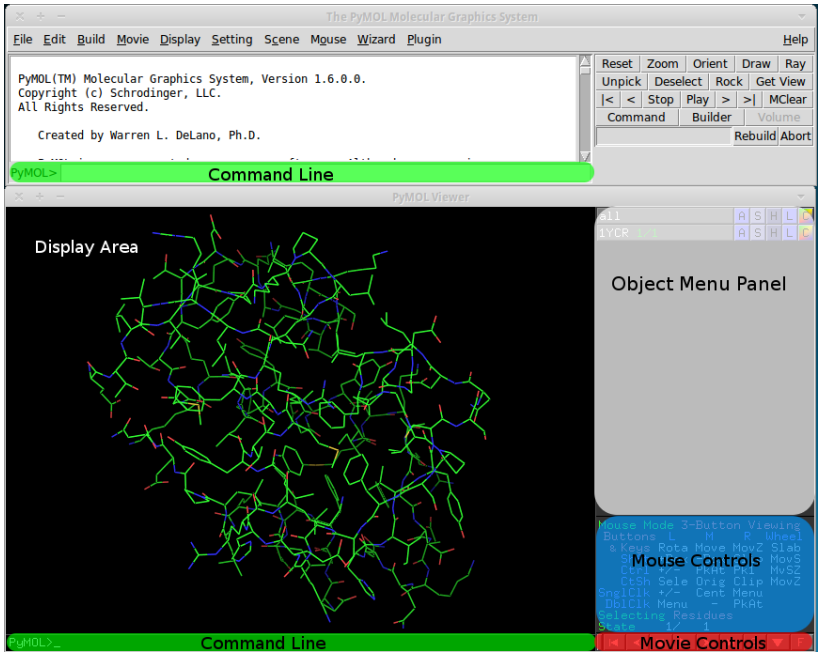


Figure 1: Labeled areas of the PyMOL user interface.

ASHLC In the object menu panel, you may have noticed that next to each object there are 5 letters, **ASHLC**. Clicking each of these letter brings up a menu with commands that can be applied to that object. The five buttons and some of their options are:

A Actions: Renaming or deleting the object, adding or removing hydrogens, or zooming or centering to it.

S Show: Display the molecule in a variety of ways (lines, sticks, cartoon, etc.)

H Hide: Same as the **S** menu, but for hiding representations.

L Label: Label atoms, residues, etc.

C Color: Change the color of atoms.

In this tutorial, commands using these buttons will be given as follows:

- object_of_interest > **A** > Some menu item in the **A**ctions menu

Using the Mouse The primary way that you interact with PyMOL is by using the mouse. While the mouse can be set into a number of different modes, the main button functions are as follows (Figure 2), to rotate the molecule use the left mouse button, to zoom in and out use the left mouse button (click and hold and move the mouse up and down), and to recenter the view, use the center mouse button. There are a wealth of command line tools that can be used in place or as a supplement to the graphical interface, but we will not be discussing them much here. See the PyMOL Wiki for more information (http://www.pymolwiki.org/).

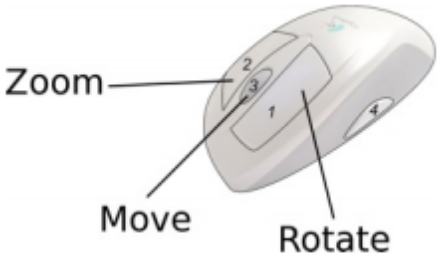


Figure 2: Basic mouse button functions. Adapted from 'Introduction to PyMOL', DeLano Scientific, 2009.

3 Exploring the MDM2-p53 Complex

Let's look at the secondary structure of the complex that we loaded by showing the complex as cartoons.

- 1YCR > **S** > cartoon

Now we can see that there are several α-helices in the structure. It may not be immediately obvious, but there are actually two different proteins in this structure (MDM2 and p53). Color each chain a different color to see them.

- 1YCR > **C** > by chain > by chain(e. c)

Now MDM2 is shown in green and p53 is shown in blue as shown in Figure 3.

Now we will use the **Sequence Viewer** to select the residues belonging to p53. Open the sequence viewer by through the `Display` Menu.

- `Display` > Sequence

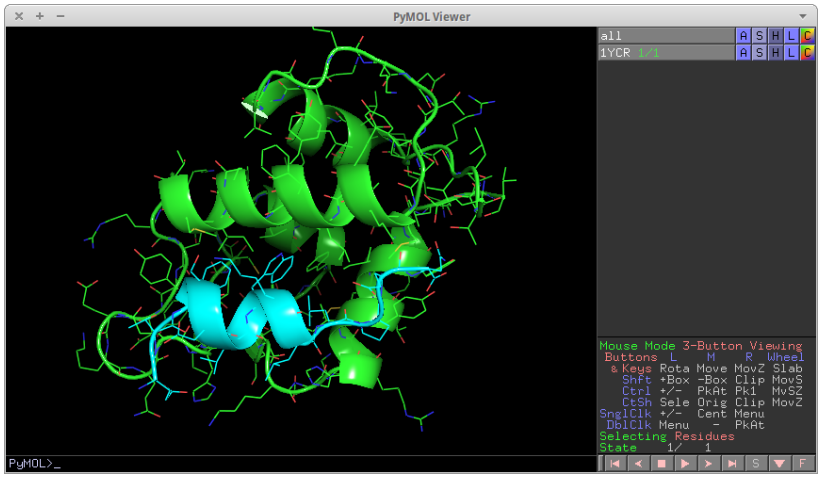


Figure 3: 1YCR colored by chain. MDM2 is shown in green and p53 is shown in blue.

Figure 4: A portion of the sequence viewer with the p53 residues selected.

You can also open the Sequence Viewer by clicking the `S` in the Movie Controls in the bottom right of the window. Use the grey slider bar and scroll all the way to the right until you see the blue residues.

- Click and drag over the (blue) residues in chain B to select them.

It should look like Figure 4. You should notice that the atoms belonging to p53 are highlighted in pink squares in the display area.

You may also have noticed that a new object has appeared in the object pane called `(sele)`. This is a selection object that contains the selected atoms (the p53 residues in this case). Now let's separate the two chains.

- (sele) > **A** > extract to object

This removes the selected residues from the original object (1YCR) and creates a new object called `obj01`. Rename it something more useful.

Figure 5: The selected interface residues of p53.

- obj01 > **A** > rename object
- This will bring a display where you can delete the current name and rename it to **p53**.

By clicking on objects in the object pane, you can hide and show them in the interface.

3.1 The MDM2-p53 interface

Now look at the residues in the binding interface. In the sequence view, click on residues 19, 23, and 26 from the p53 object (Figure 5).

Show the interface residues as sticks and hide the rest of p53.

- (sele) > **S** > sticks
- p53 > **H** > lines
- p53 > **H** > cartoon

We can also show the surface of MDM2 to get a better feeling for the shape of the binding site (Figure 6).

- 1YCR > **A** > surface

Now we can see that MDM2 has a deep binding groove that the three p53 residues nestle into. Next we will look at how inhibitors of this complex bind to MDM2.

Save your session PyMOL gives you the ability to save your work and later pick up exactly where you were. To do this, we will save our session to a file.

- File > Save Session As...
- Save the session to 1YCR_session_1.pse

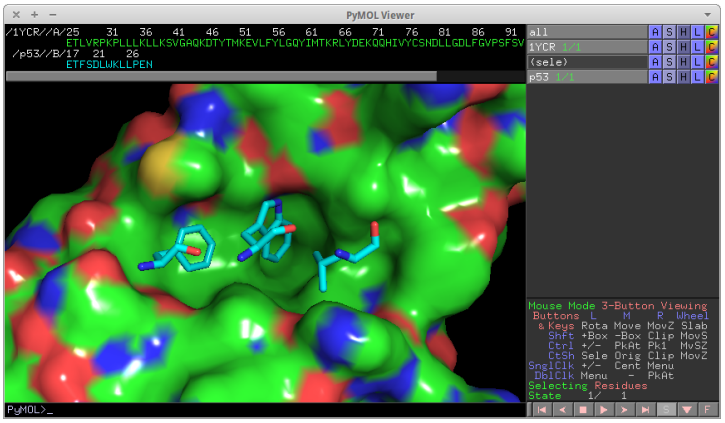


Figure 6: The critical interactions of MDM2 (surface) and p53 (sticks).

4 Inhibitor of MDM2-p53

Now we will examine the structure of MDM2 bound to an inhibitor. In this section, we will go a little faster because you already know many of the commands.

- Download 3LBK from the PDB website
- Load it into PyMOL

In this structure, there a number of water molecules (red crosses). We will not be using them, so let's remove them.

- 3LBK > **A** > remove waters

You may have noticed that the two proteins are not next to each other.

- all > **A** > zoom

Before we can really compare these two structures, we must align them. PyMOL makes aligning two structures quite easy.

- 3LBK > **A** > align > to molecule > 1YCR

Note: When performing alignments, PyMOL creates an 'alignment object' called something like 'aln_3LBK_to_1YCR'. This object displays lines between atoms that were aligned in the two structures. We will not be using it so you can safely delete this object.

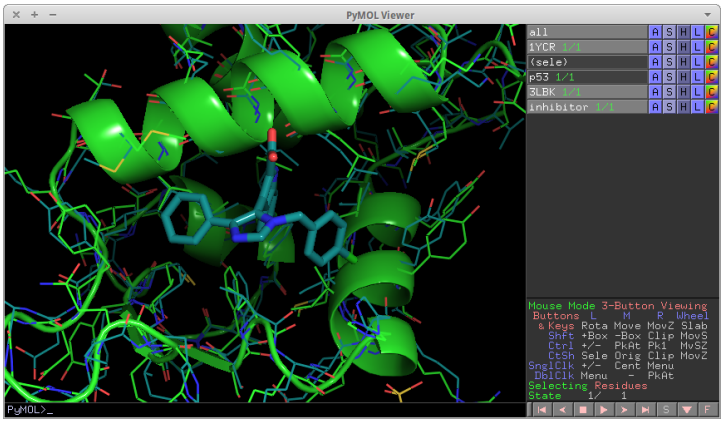


Figure 7: The inhibitor bound structure of MDM2 (3LBK) aligned to 1YCR.

- aln_3LBK_to_1YCR > **A** > delete

Hide the surface of 1YCR and you can see that the protein structures are very similar. As before we will now extract the inhibitor from the 3LBK structure (Figure 7).

- Extract the inhibitor (named 'K23' in the sequence viewer)
- Show it as sticks
- Rename it to **inhibitor**.

If we examine the inhibitor and the p53 residues, we can see that they overlap very closely on the tryptophan and the two hydrophobic phenyl rings overlap the leucine and phenyl amino acids nicely.

- Save your session again to 1YCR_session_2.pse

5 Multi-State Objects

So far we have only dealt with single state molecules. PyMOL can also handle multi-state objects like sets of compounds, MD frames, NMR structures.

- Load in an NMR solution structure of unbound MDM2 (PDB: 1Z1M).
- Align it to 1YCR

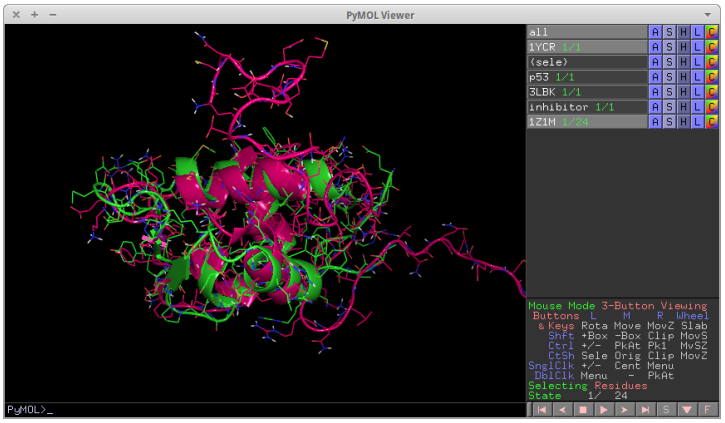


Figure 8: NMR structure of unbound MDM2 (1Z1M) aligned to 1YCR.

- Show as cartoon

You may notice that this structure differs from the 1YCR structure of MDM2 is several ways. One is that it is longer (contains more amino acids) and there there are hydrogens. Hide the non-polar hydrogens.

- 1Z1M > **H** > hydrogens > nonpolar

To flip through the different structures either use the left/right arrow keys, or click the forward/back buttons in the Movie Controls. From this we can see that there is a fair amount of variability in the unbound structure of MDM2 (Figure 8).

- Save your session as 1YCR_session_3.pse

6 Making Publication Quality Images

Now that we have our structures set up, let's make some pretty pictures of them.

- Disable all except 1YCR, p53, and inhibitor
- Show the surface for 1YCR

The process of making nice figures is called **ray tracing**. In PyMOL, there is a button in the upper right called `Ray` to do this. (You can also accomplish the same thing by entering the `ray` command into the command line.) Rotate the view so you can see how the inhibitor overlays the p53 residues. Now make a ray traced image with the default settings.

- Hit the `Ray` button
- Save the image: `File` > Save Image As > PNG...
- Save it to ray_traced_default.png

An example is shown in Figure 9(a).

Now we will change a number of settings that will improve the quality of the image.

- `Display` > Background > White
- `Display` > Color Space > CMYK (for publications)
- `Display` > Quality > Maximum Quality

To get a better idea about the chemistry of the inhibitor, turn on the valences setting so we can see where there are double bonds.

- `Display` > Show Valences

We are also going to shut off the shadows that the ray tracing produces.

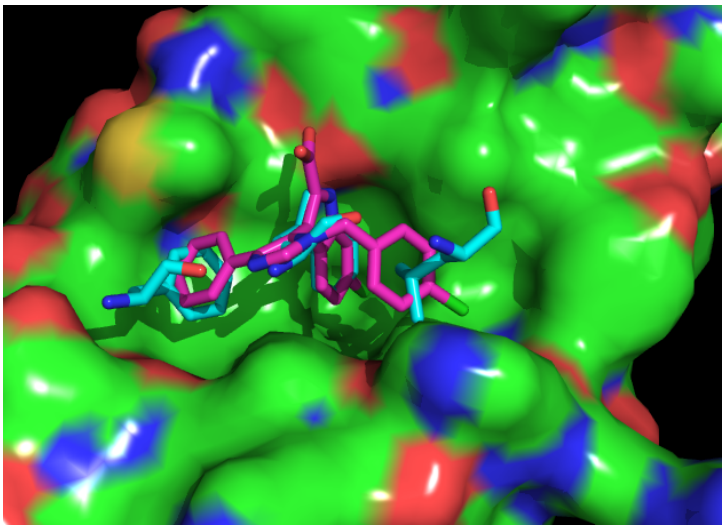
- `Setting` > Rendering > Shadows > None

Finally, ray trace and save the new image produced with the improved settings. My image is shown in Figure 9(b).

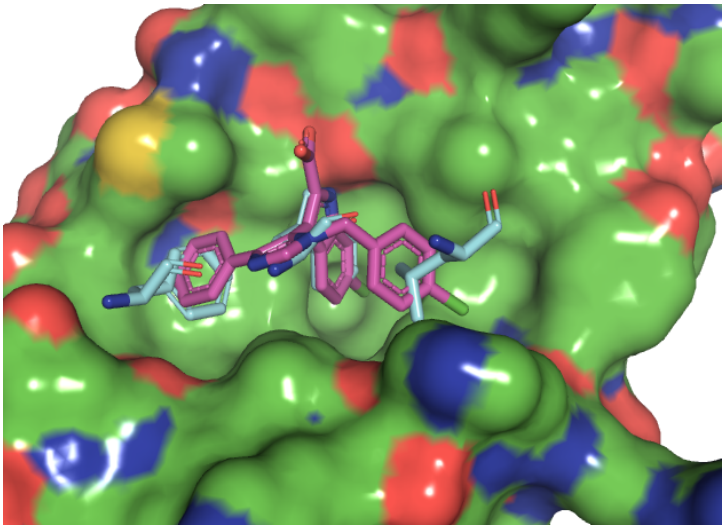
- Hit the `Ray` button
- Save it to ray_traced_improved.png

7 Conclusion

This tutorial meant to show you some of the main features of the PyMOL molecular graphics system. This is by no means an exhaustive tutorial but it should serve to get you started. One of the most powerful things about PyMOL is the command line. Every single command that you used through a menu can also be done through the command line (as well as many more). This is very useful for doing things like automatically processing PDB files with PyMOL.



(a)



(b)

Figure 9: Ray traced image with (a) default and (b) improved settings.