Macau Talent Program 2018
Introduction to Computational Drug Design
Molecular Visualization Lab

PyMol is a molecular visualization program used to view molecular structures and to make figures. You can download the software for this exercise from our lab copy here:
http://cbbio.cis.umac.mo/shared/csbio2016/
Or if you are using a Linux or MacOSX, you can install the latest version from
https://sourceforge.net/projects/pymol/

1. Getting Started

Open PyMOL by typing `pymol` at the command line (in Linux) or double click the PyMOL icon on your desktop (in Windows), now two windows should open.

The smaller window (The PyMOL Molecular Graphics System) contains the menu bar, some command buttons and a command line. This is called the **Command window.** The larger window is **PyMOL Viewer** where the molecules are displayed.

Figure 1. The PyMOL main display.

To fetch the PDB structure from the PDB database (http://www.rcsb.org/pdb/home/home.do), a convenient command to use the *fetch* command. Type at the Command window "fetch 3NMM".

2. Visualizing Protein Structures

In the Viewer, on the right hand side, you see the object buttons.
The first one contains the object name (**3NMM**) and 5 smaller buttons:
A(actions) **S**(show), **H**(hide), **L**(label) and **C**(colour)
When a molecule is selected in PyMOL, the object button is Grey. Click on 3NMM. What happens? Select the molecule again by clicking the 3NMM button.

Let's get rid of some details:
- Select `A/Hydrogens/remove` will hide the hydrogens which are included in the structure.

- Select `H/nonbonded`. *What happens?*
- Select `H/lines`. *What happens?*

There is no 'undo'!

If you want to see the 'nonbonded' and 'lines' again, you can do find them from the S(show) tab: `S/nonbonded` and `S/lines`.

Hide (H) both (lines and nonbonded) before you continue. Or simply `H/everything`.

Now you try a few different drawing styles or drawing representations:
- To display the protein structure as a Cα trace, select `S/ribbon`.
- To display the protein structure as cartoon, select `S/cartoon`.
- To display the protein structure as spheres, select `S/spheres`.
- To hide the Cα trace, select `H/ribbon`.
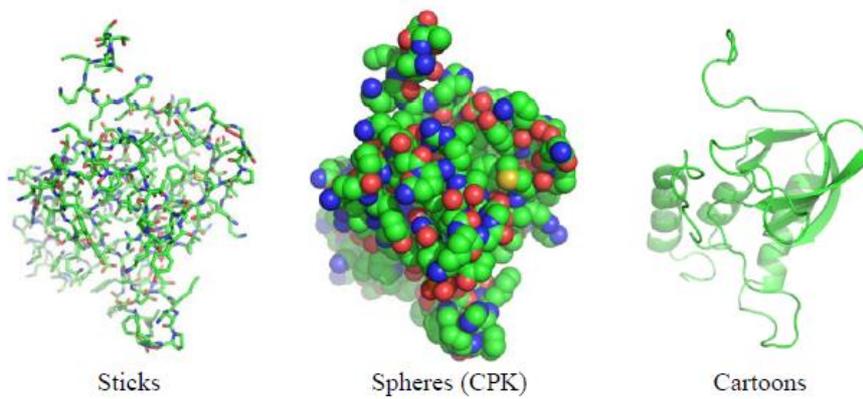
Figure 4. Different drawing styles of protein structure.

3. Adjust the View

You will continue this section with the molecule drawn as cartoon.

Large molecules are difficult to understand as a two-dimensional projection, but it helps if you can move the molecule.

- Hold on the mouse left-button, right-button, and middle-button, try to rotate, move, and zoom the molecule.

- Try to scroll with the mouse wheel button slowly, backward and forward, what happen?
  You can see that back of the molecule dims out. This adjusts the *slab* in the Z-direction. If you scroll too far, the whole molecule is sliced away. This is however a useful way to reduce detail in the figure. Now scroll back so that you can see the full molecule.
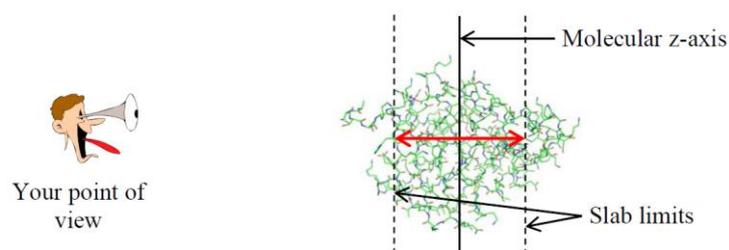
Figure 2. The concept of slab.

- It is easier to work if your **center of rotation** is in the area, which you are interested in. `Rotate the molecule`. The center of rotation is now in the center of your structure.

  Click with `the mouse wheel` anywhere in the structure where you would like to put as the center. Now when you rotate, this area is easier to visualize.

- You can set the center of the rotation back to the center of the molecule by selecting from the buttons `(3NMM)→ A(action)/center`.

4. Selecting and Coloring

When you rotate the molecule around you can see that the structure has many loose ends.
These are the N- and C-termini of the protein.
The molecule you are looking at is *Hemoglobin*. It is actually a tetrameric protein; there are four equal monomers. This is difficult to see when everything is in one color.

- `Zoom back` so that you can see the whole molecule.
- Choose `(3NMM)→ C/by ss` to distinguish the secondary structures.
- Try also `(3NMM)→ C/by chain`, which colors the molecule from N- to C- terminus.

Now the backbone is displayed nicely with cartoon drawing. We want to display the last residue of chain A with its sidechain as stick drawing.
- Find out which chain is chain A by clicking on it (note: you have to look at the command window)
- Select the last protein residue in this chain. What is it? (ARG141)
- You get red dots on the molecule, but nothing still happens. You have a new object now: **(sele)**.
- Select on `(sele) → S/lines.` Now you see the selected residue in line representation.

If the backbone of the molecule is shown as a Cα ribbon or cartoon, often you do not want to display the backbone atoms.
- `(sele) → S/Side chain/sticks`
- `(sele) → C/Side chain/by element`
- `(sele) → H(hide)/lines`
Deselect the object by clicking anywhere on the background so that the red dots disappear. Now you see just the side chain sticking from the backbone Cα ribbon.

5. Working with Sequence

If you want to color a particular residue, or for example a whole subunit, finding and selecting on the graphics window is very laborious.

- First remove the previous selection by clicking on **(sele)** `A/delete selection.` This deletes just the viewer object, not the selected residues in the structure!

- On upper Command window select `Display/sequence.`

Now you see all the residues, which are included in the pdb file. You can select residues also from the sequence line. The graphics window shows the selected area on the molecule.

- Center on the object (3NMM)→ A/center.
- `Zoom back` so that you see the whole molecule.
- `Select amino acids in sequence view` by clicking on them one by one. You can also hold down the shift key while clicking and select a wider area at the time.

- `Select the whole first subunit` (until you come to residue HEM and SO4)
- Now colour this subunit which ever colour you like
    (sele) → C/magentas/deeppurple.
- Now save your selection by renaming it
    (sele) → A/rename selection,
    remove 'sele' with backspace and type `subA <Enter>` on the screen window.

There are a lot of **OOOOO** residues in the sequence view. These are the waters associated with the protein. You can ignore them for now. Just scroll over.

Now in the same way, color all the subunits with their own color.

6. Show the Cofactors

When you explain a protein function, displaying the protein peptide is not enough. Some proteins include essential cofactors.

First make sure you do not have an active **(sele)** object. Remove if necessary.
    In the `sequence view`, click on the **HEM** residue.

    Click on (sele) → S/lines.
Now you see the Heme bound into the protein structure.
You can color the Heme in the same way as residues, but you can also keep it the color it was.

7. Scenes

Now you know how to move the molecule, zoom in and out and show different areas with their own colour. The PyMol program does not have an UNDO button, so it is sometimes good to save things before the next step.

Move the molecule so that the four subunits are well visible
- On upper Command window select `Scene/Store F1`
- Next `center on the Heme`, and `turn` the molecule so that the Heme is easy to see.
- `Zoom in`.
- On upper Command window select `Scene/Store F2`
You can now come back to these setting any time by selecting `Scene/recall/F1 or F2`.

These scenes are also saved into the PyMol session when you save it. You can do this from the Command window

```
        File/Save    Session    As...
```
type `Heme_tutorial_yourname`

Now you can come back to this exercise later. Note that PyMol does **not** warn if you quit without saving.

8. Adjust the Selection Types

Whenever you click on the molecule, it selects a residue. You can also ask PyMOL to select a chain for each click. To do this, look at the line "Selecting Residues" at the right bottom of your molecule window, click on it to switch to "Selecting Chains" mode.

Let's remove the other chains from the display and let alone chain A.
- In "Selecting Chains" mode, click on chain B, C, D to put them in (sele). If you select the wrong chain, just click it again to deselect.
- (sele) → H/everything

Now you should see only chain A. Find the first Heme from the sequence of chain A and make an object called Hem1.
- Switch back to "Selecting Residues" mode
- On `sequence view`, select the first `HEM`
- (sele) → A/rename remove 'sele' and type `hem1`
- (hem1) → S/stick
- (hem1) → A/duplicate
- (sel01) → A/rename remove 'sele' and type `nearhem1`
- (nearhem1) → A/modify/around/residues within 4A
- (nearhem1) → S/side chain/lines

Deselect the object by clicking anywhere on the background so that the red dots disappear.

You now have an object, which contains the Heme and all residues, which are within 4 Å from it.
This most likely contains all residues involved in Heme coordination. You can now center on the **(hem1)** –object and zoom in. Rotate the molecule to see better. Remember to center on the object if rotation is difficult.
Can you find out which residue interacts with the center of the heme? (HIS87)
Display this residue with sticks to see better.
The heme FE atom and the HIS-NE2 atom is so close that PyMOL mistakenly displayed a bond between them.

9. Measure Distances

Sometimes it is informative to show coordination or hydrogen bonds in a picture. This is best done in PyMol by measuring distances.

Delete any (sele) objects you might have. Display the protein in cartoon, display the heme as sticks.
`Select Lysine residue number 61`. This is easiest to do from the sequence view.
Rename this object to K61, so that it does not get lost with further selections.
- (sele) → A/rename remove 'sele' and type `K61`
- (K61) → A/center and zoom in.

You can see that this residue is close to a heme carboxyl (i.e.. COO group).

In the Command window `Wizard/Measurement`

Click on the `lysine residue end` and the closest `oxygen` on the heme.

The distance should now be displayed.

Click `Done` on the lower right when you do not want to measure more distances.

You now have a new object called **measure01**

How close was the K61 residue from the heme?

Often the numbers are not necessary in a picture.

- `(measure01)` → `H/labels` to hide the numbers.

You can also change the colour of the line from

- `(measure01)` → `C...`

Center on the (hem1) object and select a position, which shows the interaction well. You can also change the slab if you want to but be careful not to clip away too much! Zoom in so that you fill the display window. Save this view to a new Scene from the Command window:

`Scene/Store/F3`

10. Save the View and Generate Images

Although black background works well on a computer screen, in printed pictures lighter background is better.

From the Command window choose

`Display/background/white.`

Do you need to change the color of the measurement, which you just made? Are other colors visible?

If you change these, you need to save again:

`Scene/Store/F3`

On the Command window, you can now go through all the nice views you saved:

`Scene/Recall/F1`
`Scene/Recall/F2`
`Scene/Recall/F3`

Choose one of these views for a figure.

Now we will render the figure surfaces to look nicer. This takes more computer power on the screen so it is practical to do only as a final step.

If your PyMOL is a Windows version, please jump directly to the box below.

On the Command window prompt, write `ray <Enter>`.

Wait for the computer to do the calculation; do not move the molecule!
This will render your figure with the screen resolution. If you would want higher resolution, you can try for example:

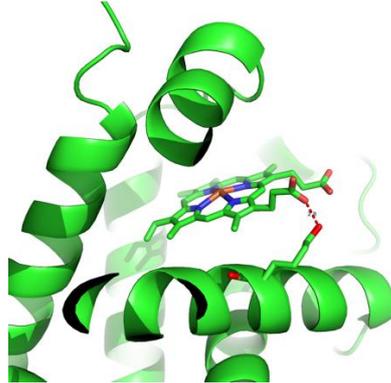On the Command window prompt, write `ray 1000 <Enter>`.

What is your image size now?

As soon as you click on the Viewer window, you image quality is set back to the working mode.

Save the rasterised image in the Command window (without touching the graphics window):
- `File/Save image as/png`
- Give a file name, `Heme.png`

Open your image file, does it look ok?

Figure 3. A sample image rendered in PyMOL.

Note for Windows User:

If you get an error "Ray-Error: ray tracing is not supported in this PyMOL build", you can use shader rendered image instead, the quality of which is close to ray trace quality. On the Command window prompt, type:
- set use_shaders
- png Heme.png, 4000, 3000

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11. Now Your Turn to Practice

Please perform the following **for chain A** using PyMOL, and answer the questions:
1. How many atoms are there in one Heme group? (Hint: After you select one Heme group, do A/compute/atom count)

2. What is the closest atom of the protein to the Heme in chain A? Measure the distance between the two closest atoms from the protein and the Heme.

3. The place where Heme binds is a protein pocket. Visualize the protein as *Surface* and Heme as both *spheres* and *sticks*, adjust the sphere_scale to 0.25 by using the command:
   set sphere_scale, 0.25
   Sample of the image is shown below. Generate your version of the image!