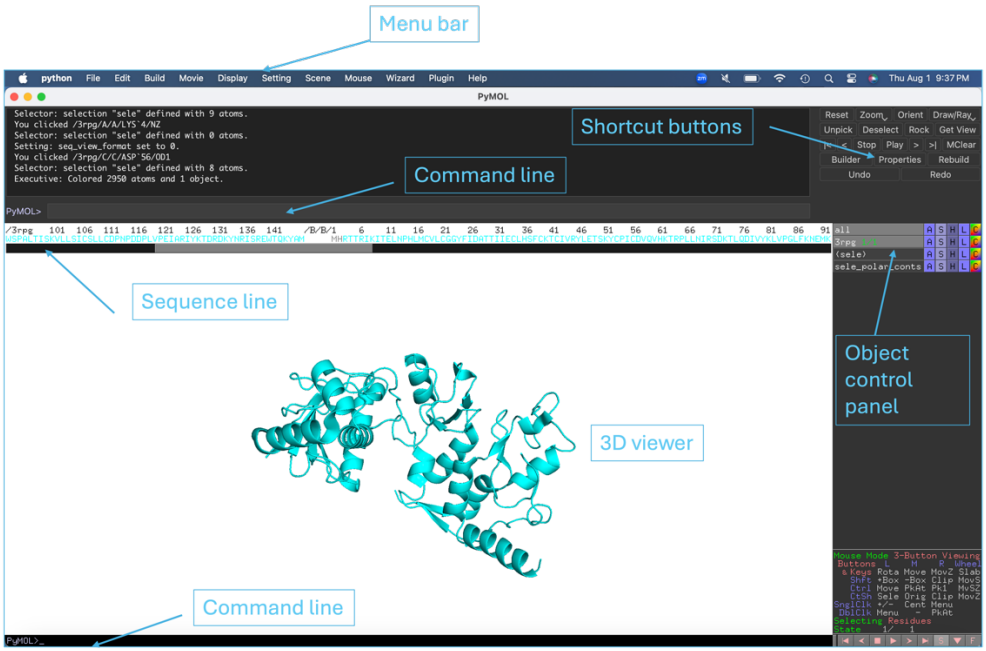
PyMOL exercise

You will look at the 3D structures of three interacting proteins including RING2 (chain C) and BMI1 (chain B), both being part of human polycomb repressive complex 1-like, which plays a role in histone and gene regulation as describe by Wang et al. (2004) in “Role of histone H2A ubiquitination in polycomb silencing”. These two proteins interact as a heterodimer with the E2 enzyme UbcH5c (chain A) and the complex structure has been crystallized by Bentley et al. (2011) “Recognition of UbcH5c and the nucleosome by the BMI1/RING1b ubiquitin ligase complex” (PDB ID 3RPG). Now you will analyze how these proteins interact with each other.

The PyMOL Window has the following parts:



- Menu bar – Pull down menus
- Command line – written commands and scripting for experienced users
- Sequence line – if a sequence is displayed as Residue codes, it shows 1-letter code
- Viewer - molecular graphic window, where the molecule is visualized
- Shortcut buttons – common commands (e.g., Draw/Ray)
- Object Control Panel – Object name and 5 Object buttons
- Each object can be controlled using the **A**(action), **S**(show), **H**(hide), **L**(label) and **C**(color).

Open the Protein Data Bank (PDB) https://www.rcsb.org/ webpage.

Write **3RPG** in the search field and perform the search to find the structure with PDB ID 3RPG. Click on the **3RPG** -link and select **Download Files – PDB Format** to save the structure on your computer in an appropriate folder.

Open PyMOL

Open the structure in PyMOL: In the Menu bar, select **File/Open/**3RPG.pdb from the directory where your save the file.

Zoom and center, and tips:

At all times, to unselect everything, click on the background of the 3D viewer.

In PyMOL you can move around to visualize the protein. In these instructions we use the following abbreviations:

- Left Mouse Button = LMB
- Middle Mouse Button (mouse wheel) = MMB
- Right Mouse Button = RMB

You can move the molecule

Hold on the LMB and drag the cursor in the window

Now you can zoom in

Hold on the RMB and drag the cursor in the window

You can adjust z-direction

Scroll (Careful, not too fast!) with the mouse wheel

You can center your molecule, center rotation on molecule center, automatically zoom

3RPG → select A/center

3RPG → select A/zoom

Change background color

Menu bar **Display/Background/White**

Tips

To color, be careful to use contrast colors for easier visualization, and do not use blue (nitrogen color) or red (oxygen color).

When creating a picture, less is better, keep visible only what is necessary for your picture. E.g., most of the time, hydrogens are hidden if they are included in the structure. Always use white background.

The PyMOL Viewer automatically shows the secondary structure of protein as one-color cartoon and all the water as red crosses, which is not very informative in a large molecule.

Change the protein view:

Change representation:

3RPG → select H/everything

3RPG → select S/ribbon

Under **S(**Show), you can find ribbon and cartoon styles for secondary structure, and several atom styles e.g., lines and sticks.

Color by chain:

3RPG → select C/by chain/by chain (elem C)

You can also color the whole protein complex in one color or only select some residues to color those in another color.

Find interactions between chain:

Display sequence

On Menu bar, Display/Sequence

On Menu bar, Display/Sequence Mode/Chain Identifiers

What happens?

Find interaction

Select chain C

New object **(sele)** is created

What happens?

(sele) → A/find/polar contacts/to other atoms in object

New object created sele_polar_conts

sele_polar_conts → A/rename

The prompt appears on the upper left in your viewer window. Remove "**sele_polar_conts"**with backspace, write Contacts-ChainCToOther and **<Enter>**

What happens?

You can search protein-protein interactions, DNA-protein interactions, protein-ligand interactions in a similar way. You can find polar interactions within chain, with ligands, water, etc.

To visualize interactions

3RPG → S/lines

Label important residues

Unselect everything by clicking on the background of the 3D viewer

On Menu bar Display/Sequence Mode/Residue code

Select Glu33 in Chain B and Lys65 in Chain C by scrolling the Sequence line

New object **(sele)** is created

(sele) → L/Label/residues

(sele) → S/sticks

(sele) → A/zoom

What happens?

Unselect everything, click on the background of the 3D viewer

You can select residues by clicking them in the 3D viewer or in the Sequence line.

Save:

Save a session:

It is recommended to save session regularly, since there is no undo button, and PyMOL can crash.

File/Save Session As …

Select the right folder and give a name e.g., pymol_exercise

Save a scene:

Move around to create a picture of labelled interaction.

On Menu bar, select **Scene/Store/F1**

You can **Recall** the scene later by clicking **F1 on the keyboard** or **the 3D viewer**

Create high quality picture:

Choose the **Draw/Ray button** on Shortcuts button panel.

Click **Reset** to set size of picture to your Pymol window size

Change 150 DPI to **300 DPI**

Click on **Ray (slow)**

Save image to a file: give a name and save in an appropriate folder.

You have learned the basics of PyMol now and it is time to have a Group meeting and start working on the project work!