Docking Procedure (Docking of ligands against Main protease of SARS-CoV-2)

Softwares required

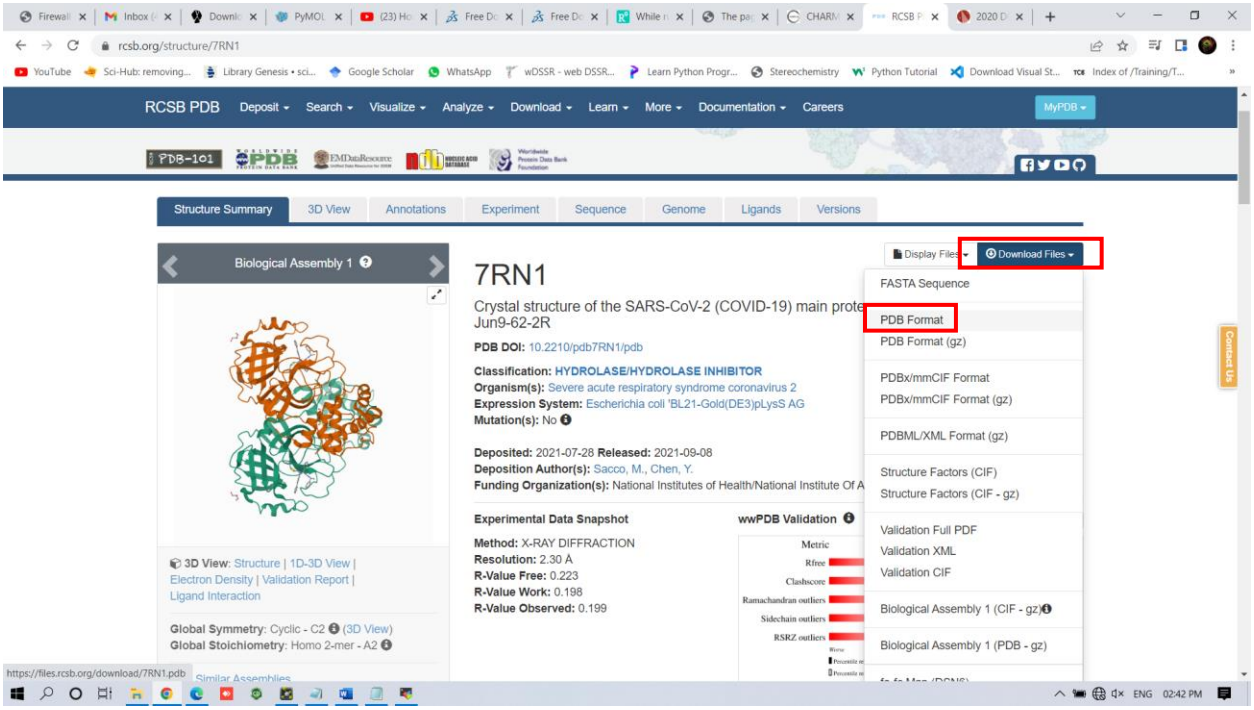
1. AutoDock Tools (https://ccsb.scripps.edu/mgltools/downloads/)
2. Vina (https://vina.scripps.edu/downloads/)
3. PyMOL (https://pymol.org/2/)
4. Discovery Studio (https://discover.3ds.com/discovery-studio-visualizer-download)

I. Introduction

AutoDock Vina is an open-source program for doing molecular docking. Vina uses the PDBQT molecular structure file format. PDBQT files can be generated (interactively or in batch mode) and viewed using MGLTools.

7RN1

Crystal structure of the SARS-CoV-2 (COVID-19) main protease in complex with inhibitor Jun9-62-2R. Download Receptor file from https://www.rcsb.org/structure/7RN1. On the RCSB website enzyme structure is given with 4 letter code. Click on "Downloads Files" and download the "PDB Format" file.



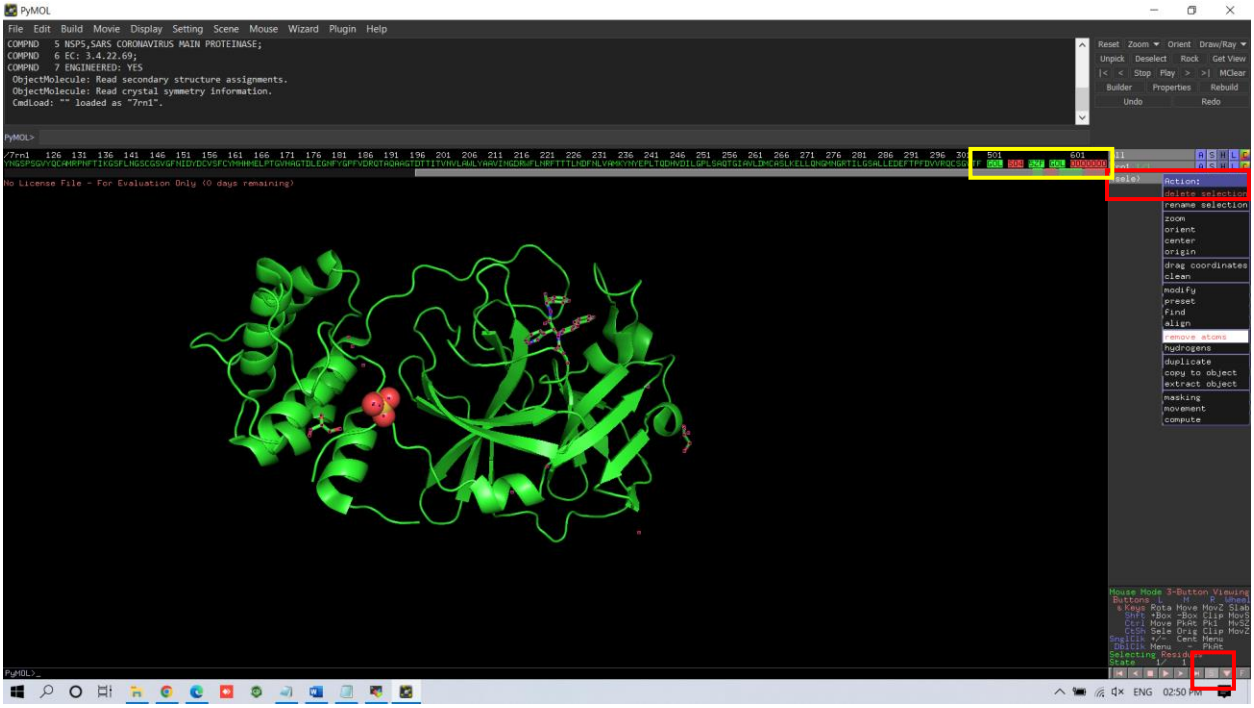
II. Preparation of receptor and ligand file

Read the article related to the PDB file given on the RCSB web to understand protonation states, charges and other important information regarding the receptor and the ligand.
Next, we are going to generate the receptor file and the ligand file.

a. Clean the Receptor file

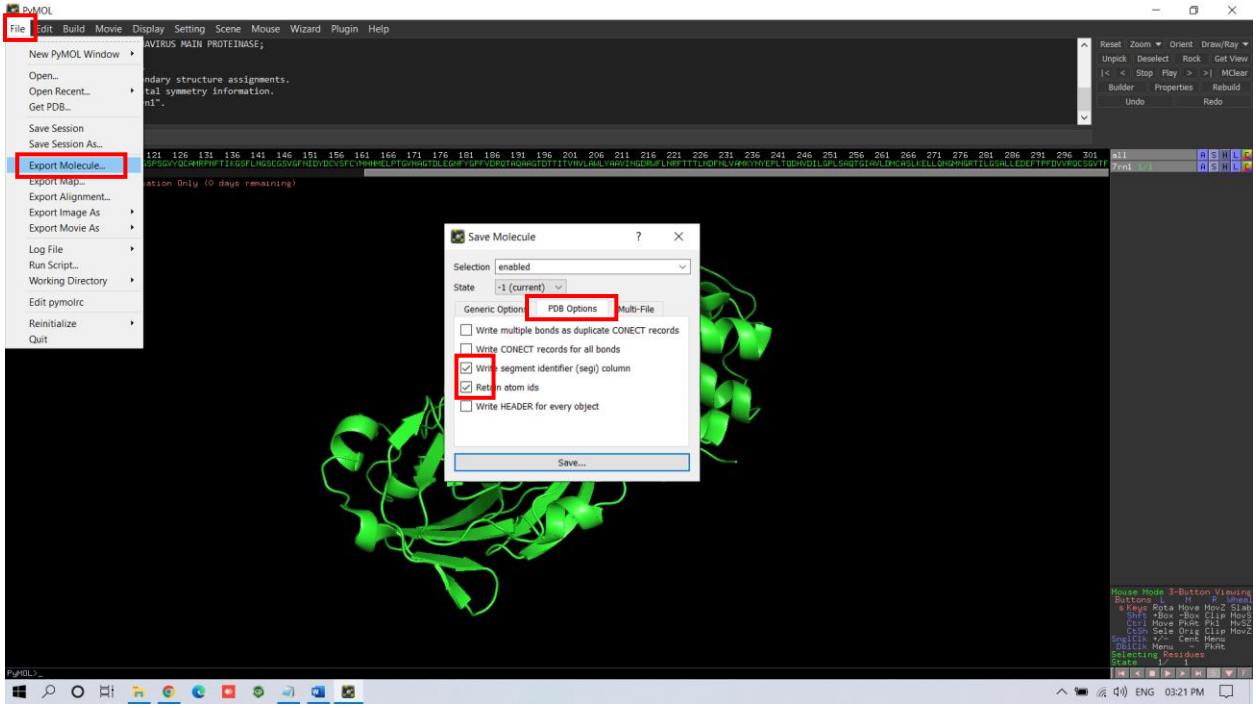
Open the pdb file with PyMOL that has been downloaded from the RCSB. In the right corner of the opened PyMOL window, cilck on "**S**" which will show the sequence of downloaded enzyme along with other heteroatoms present that can be water/ligand/ion. Scroll the bar underneath shown sequence to the end and select all the heteroatoms (other than protein) by clicking on them sequentially like "**GOL,SO4,5ZF,GOL,0000000**". Selected atoms turned to brightened color and

also, they will be selected in structure. Then in the panel present on the right side, beside the "**(sele)**", click on "**A**" which means action, & from the dropdown menu click on "**remove atoms**". Hence all the heteroatoms will be deleted.



Go to File ---> Export molecule.

Tab will appear, click on **pdb options**, check **retain Atom IDs** ---> **save**. select folder in which file should be saved, name it **receptor.pdb**, click **save**.

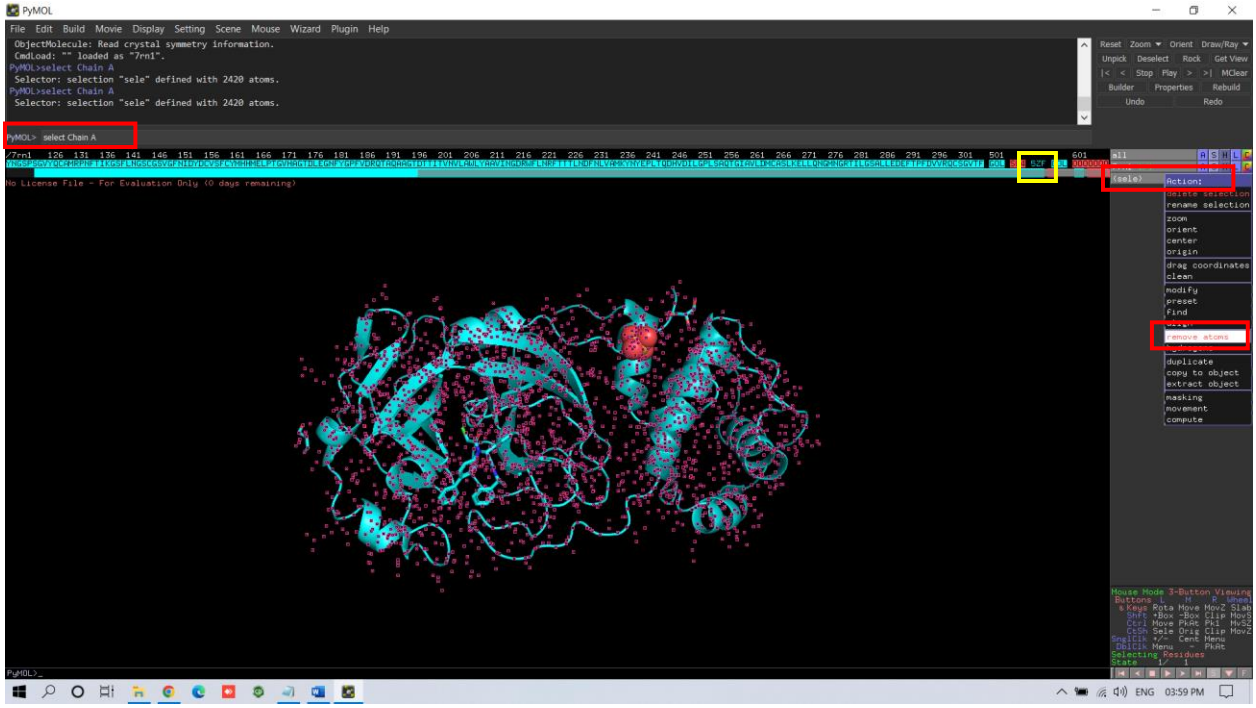


b. Clean the ligand file

Again, Open the pdb file with PyMOL that has been downloaded from the RCSB. Show sequence then type the following command in command line above sequence and press enter

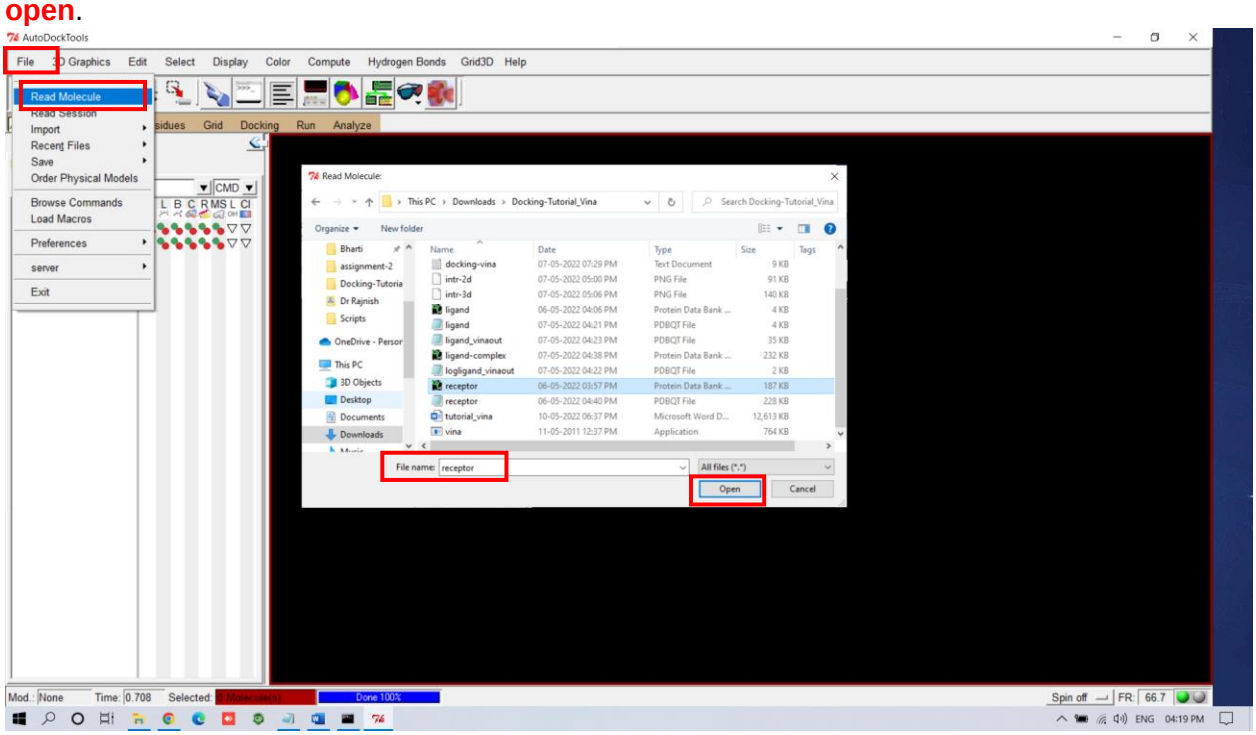
⇨ **select Chain A**

All the structures will be selected, unselect the ligand by clicking on its name "**5ZF**". Delete the selected atoms as done previously. Now you should be left with ligand structure only & save the structure as "**ligand.pdb**" in the same manner as you saved receptor file.

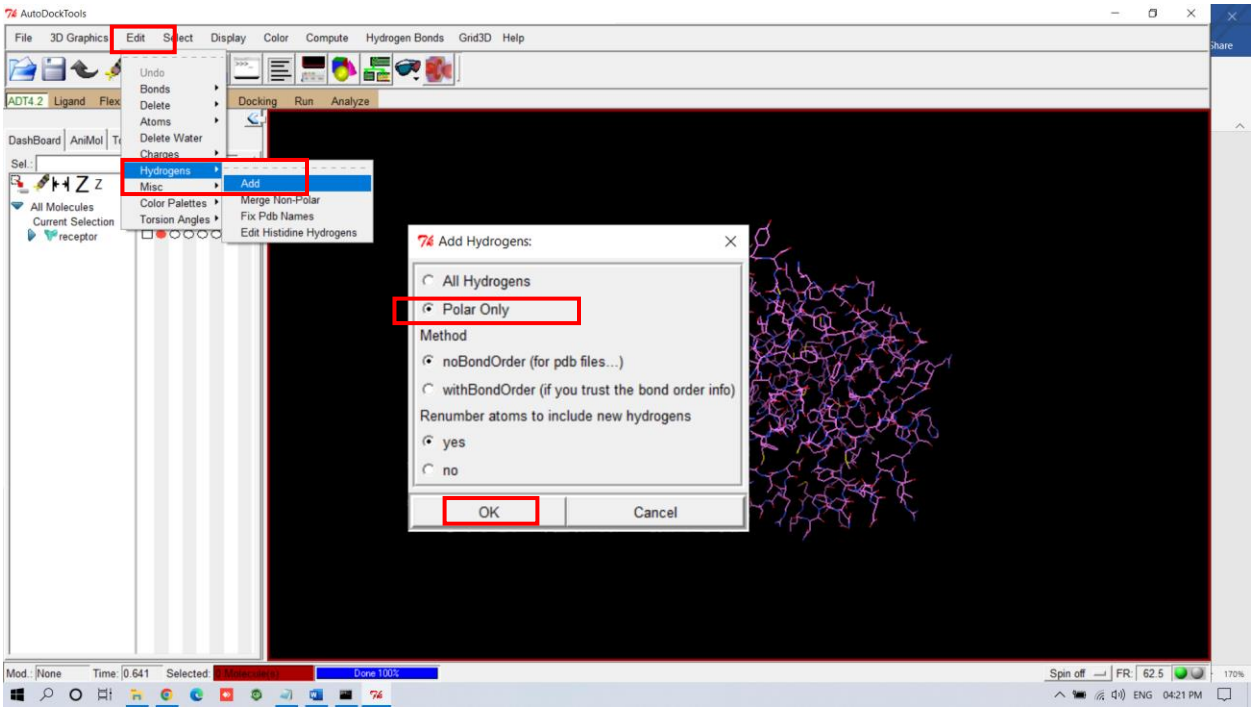


c. Preparing the receptor file in ADTools

Go to **file** ---> **Read molecule**. Choose the file "**receptor.pdb**" i.e. saved from PyMOL and click **open**.



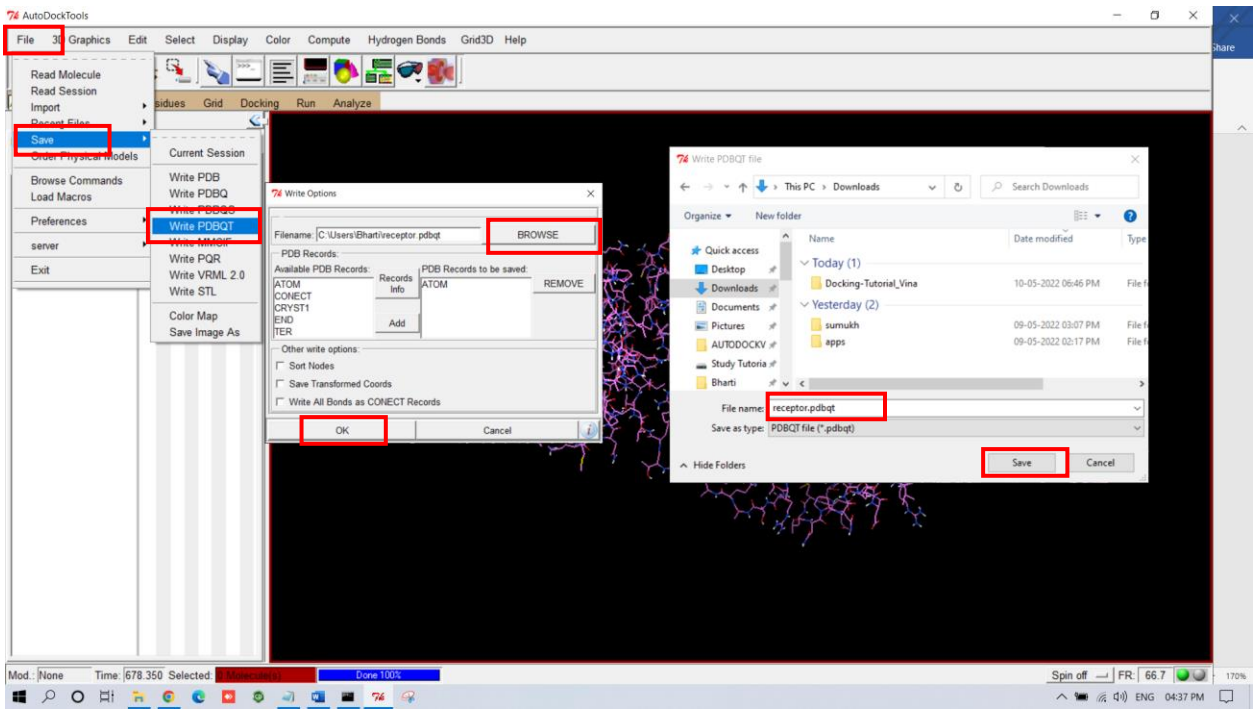
Go to **Edit** ---> **Hydrogens** ---> **Add**. then check "**Polar Only**" and click **OK**.



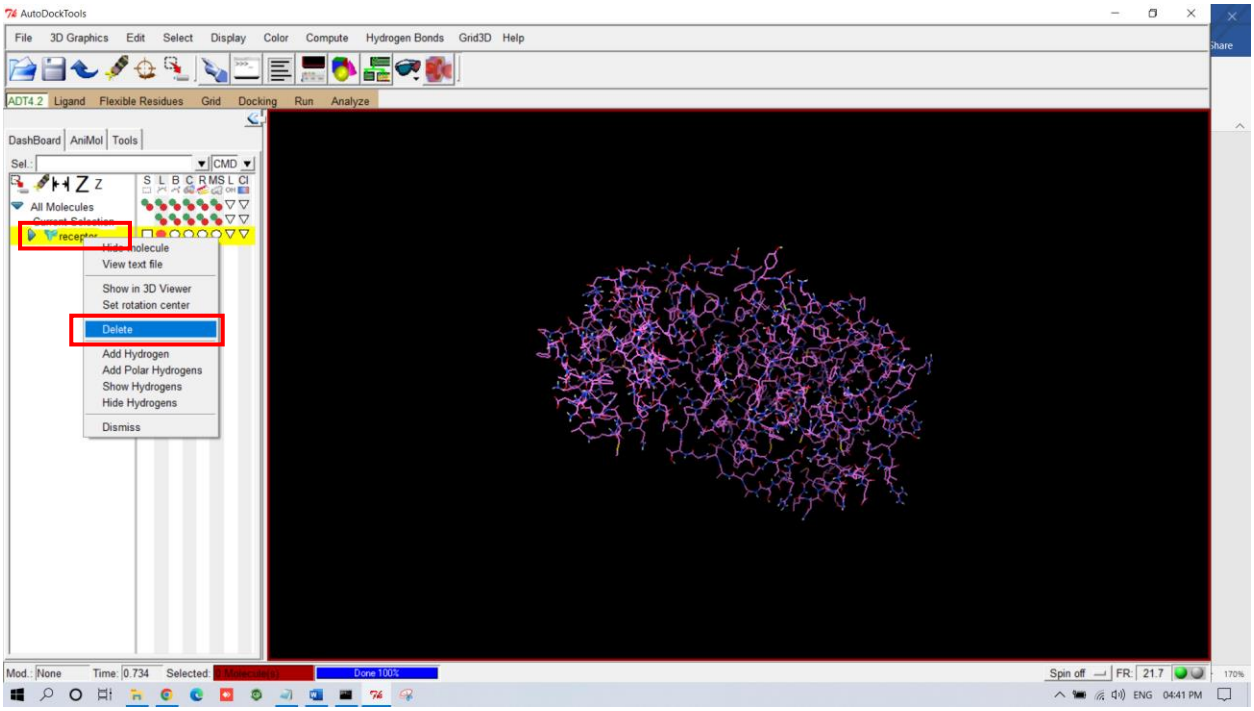
Similarly, Go to **Edit** ---> **Atoms** ---> **Assign AD4 type**

Go to **Edit** ---> **Charges** ---> **Add Kollman Charges**. Total charge added will appear on the tab ---> click **OK**

Go to **file** ---> **Save** ---> **Write PDBQT**. Choose the directory where the file has to be save and name it "**receptor.pdbqt**" and **save**.

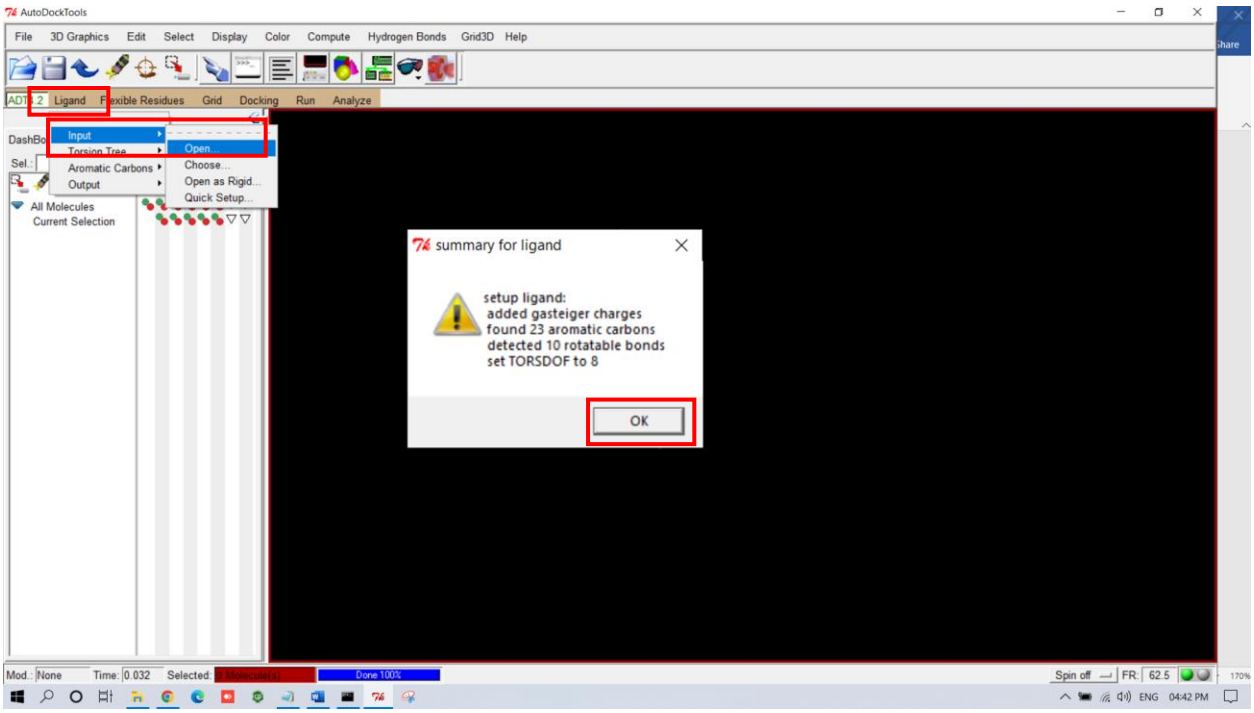


Then **Delete** the receptor from left side panel by right click.



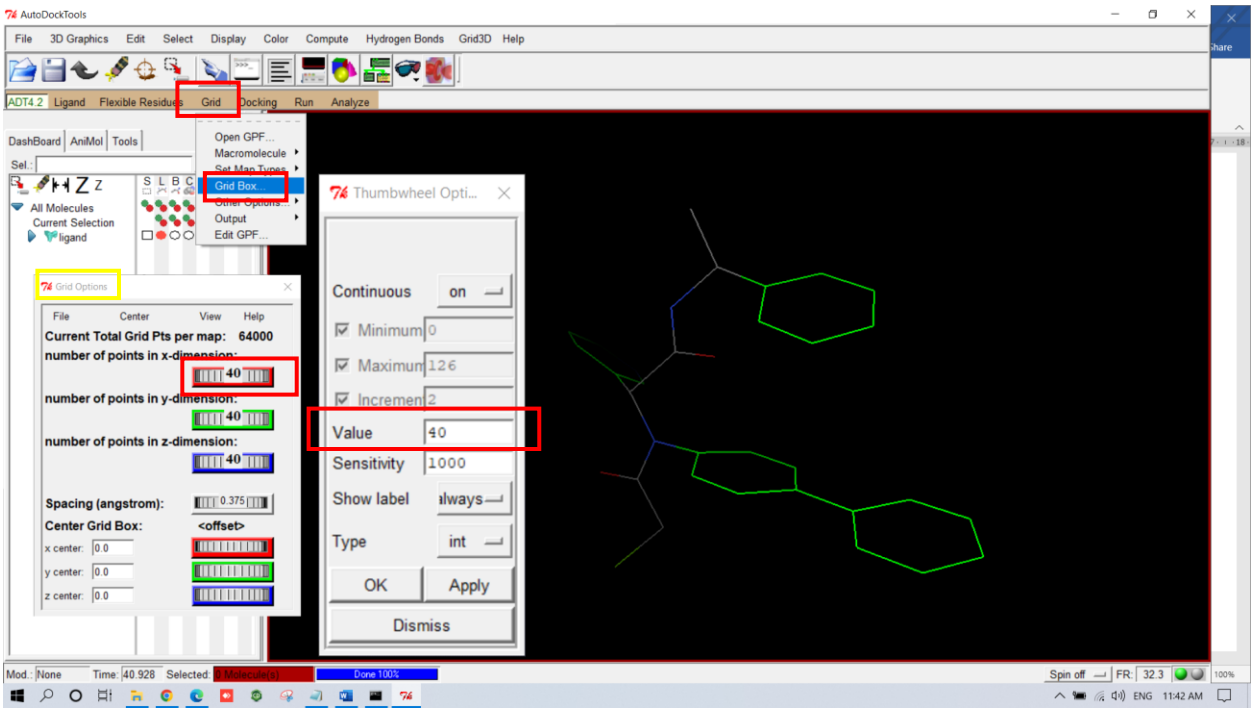
d. Preparing the ligand file

Go to **Ligand** ---> **Input** ---> **Open**. Choose the file "**ligand.pdb**" that was saved from PyMOL and click open. Press **Ok** for the popup of ligand summary. then agian go to **ligand** ---> **Output**---> **Save as PDBQT**. name it as "**ligand.pdbt**" and save in the same folder as of receptor file.

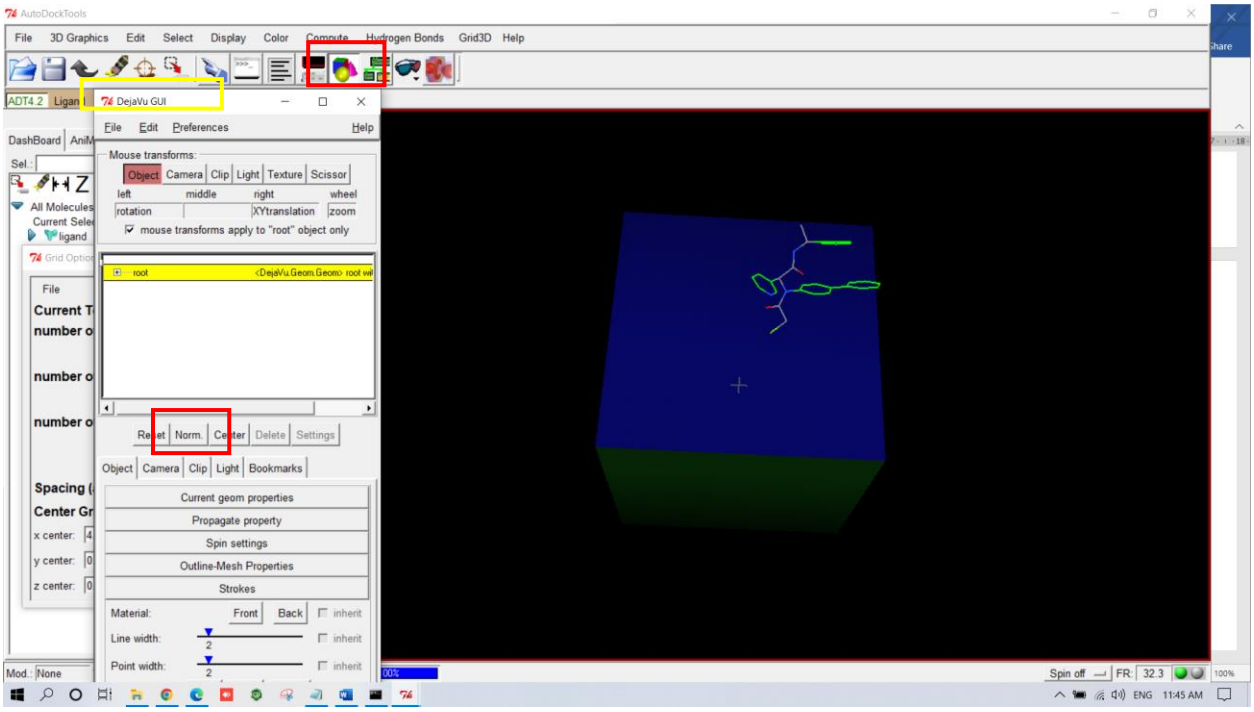


III. Generation of Grid box

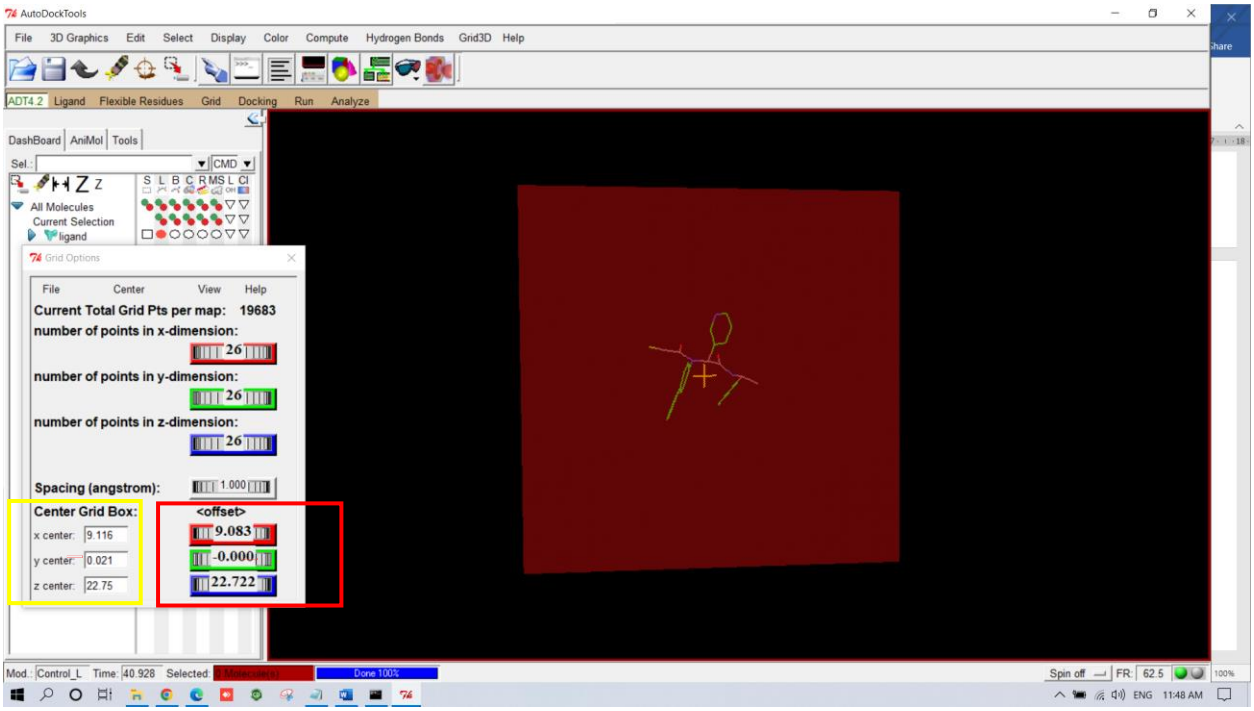
Go to **Grid** ---> **Grid Box**. Tab will be open with **Grid Options**. Adjust the x,y and z dimension upto 26, by clicking on the number change the "**Value**" to 26 in the tab opened for all the three dimensions. keep the "**Spacing**" value 1.



Now look for box, if not visible, decrease the size of ligand structure by dragging with 2 fingers. and try to place ligand on screen such that box appears. To change the box visual, in the menu bar, click "**DejaVu GUI**" symbol (containing cube,sphere and cone), a tab will open, click "**Norm**" present in the near middle of the tab and close it.



Then try to center the grid box around the ligand, for that drag the red button in the line of x center either left or right. likely do the center the ligand in y and z direction also. no atom of ligand should lie out of box in any direction. note down the dimension of grid and center in all direction. Close the ADTools.



Write a configuration text file named "**config.txt**" using notepad which contain receptor, ligand and output filenames with dimensions as follow:

receptor = rec.pdbqt
ligand = ligand.pdbqt
center_x = 9.083
center_y = -0.000
center_z = 22.722
size_x = 26
size_y = 26
size_z = 26
exhaustiveness = 8
out = ligand_vinaout.pdbqt

IV. Docking

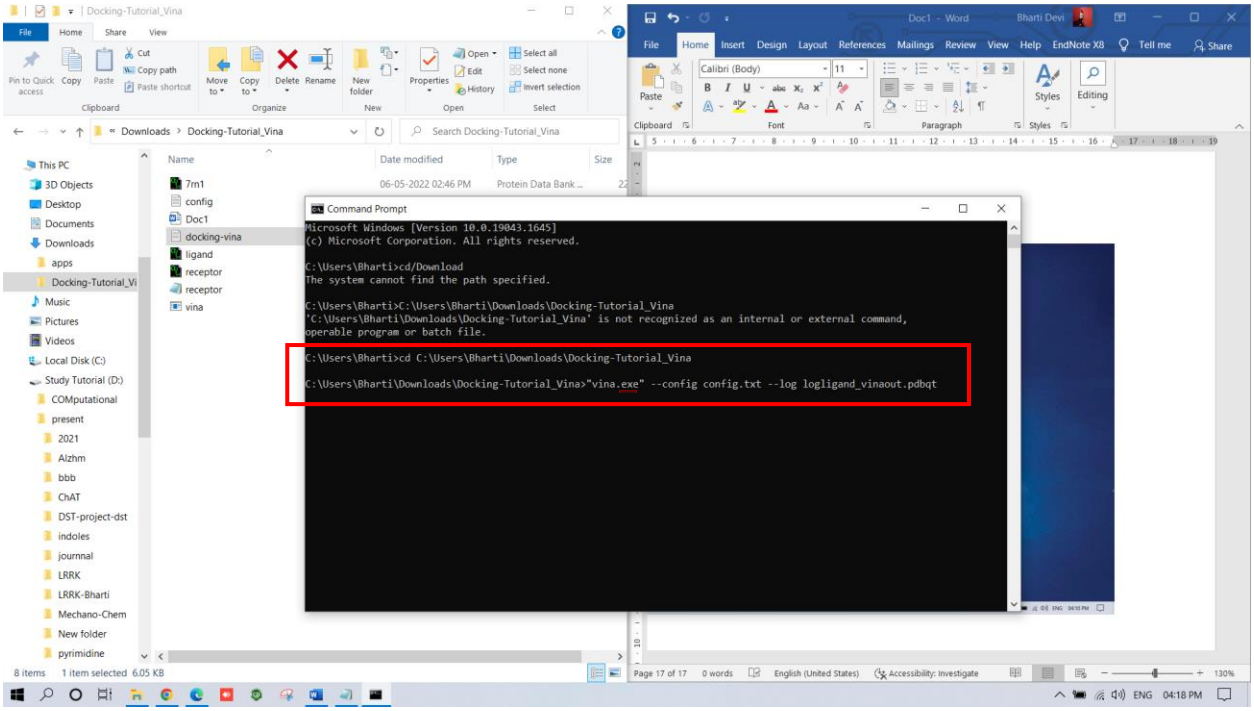
Go the folder where vina is installed for example if the folder is:- C:\Program Files (x86)\The Scripps Research Institute\Vina. Copy the "vina" application file into your working folder where all the files are saved by you. Before running the dock program check you have following files required:

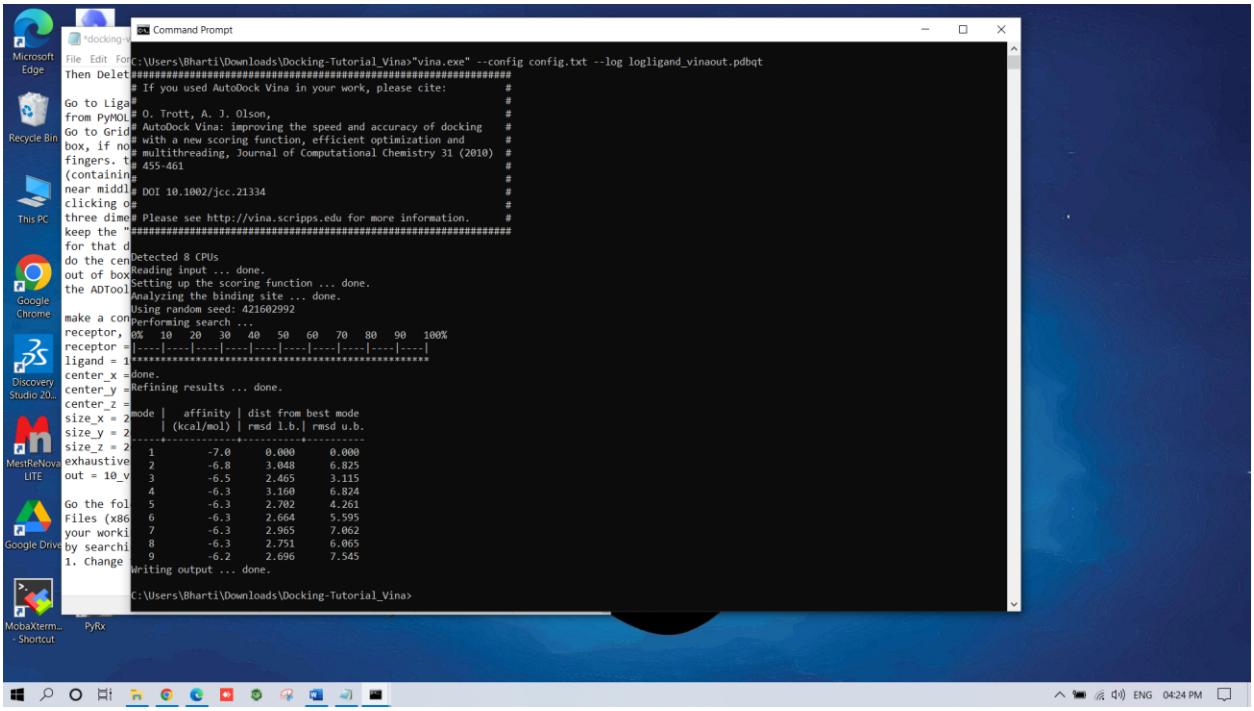
1. receptor.pdbqt
2. ligand.pdbqt
3. config.txt
4. vina

Open the command prompt by searching from task bar and open it.

1. Change the folder to working folder
cd C:\Users\Bharti\Downloads\Docking-Tutorial_Vina

2. Run the docking program
"vina.exe" --config config.txt --log logligand_vinaout.pdbqt

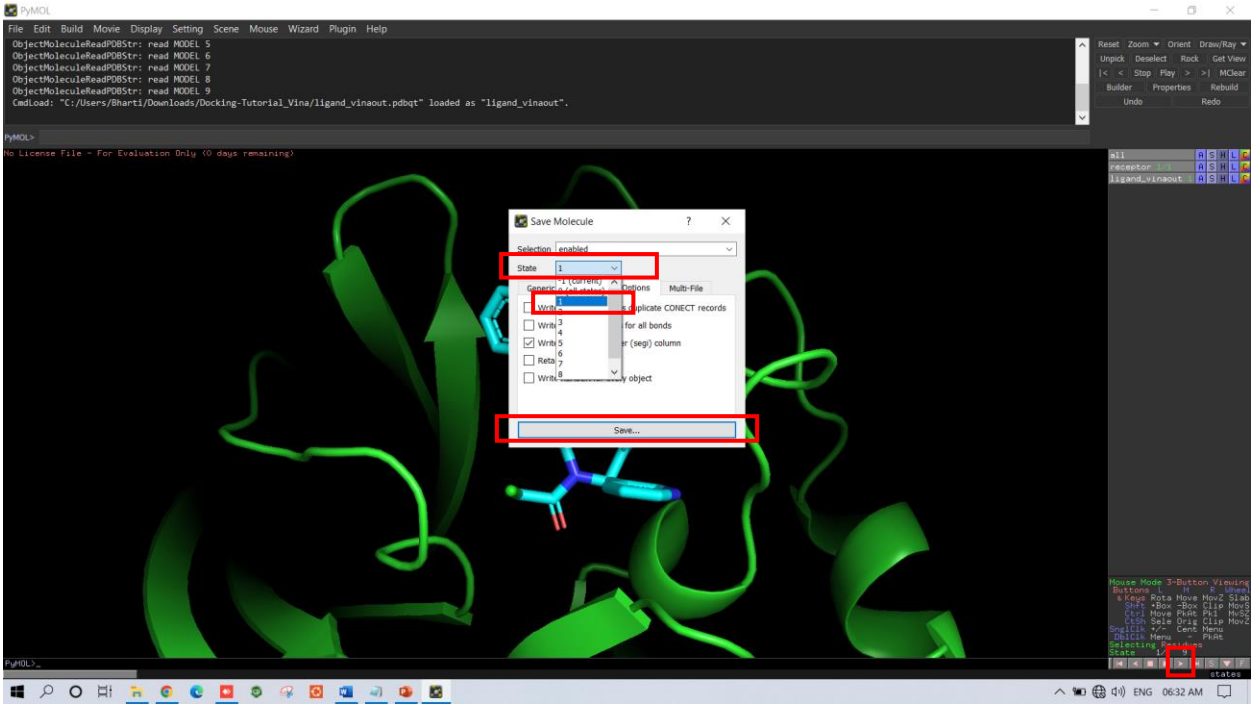




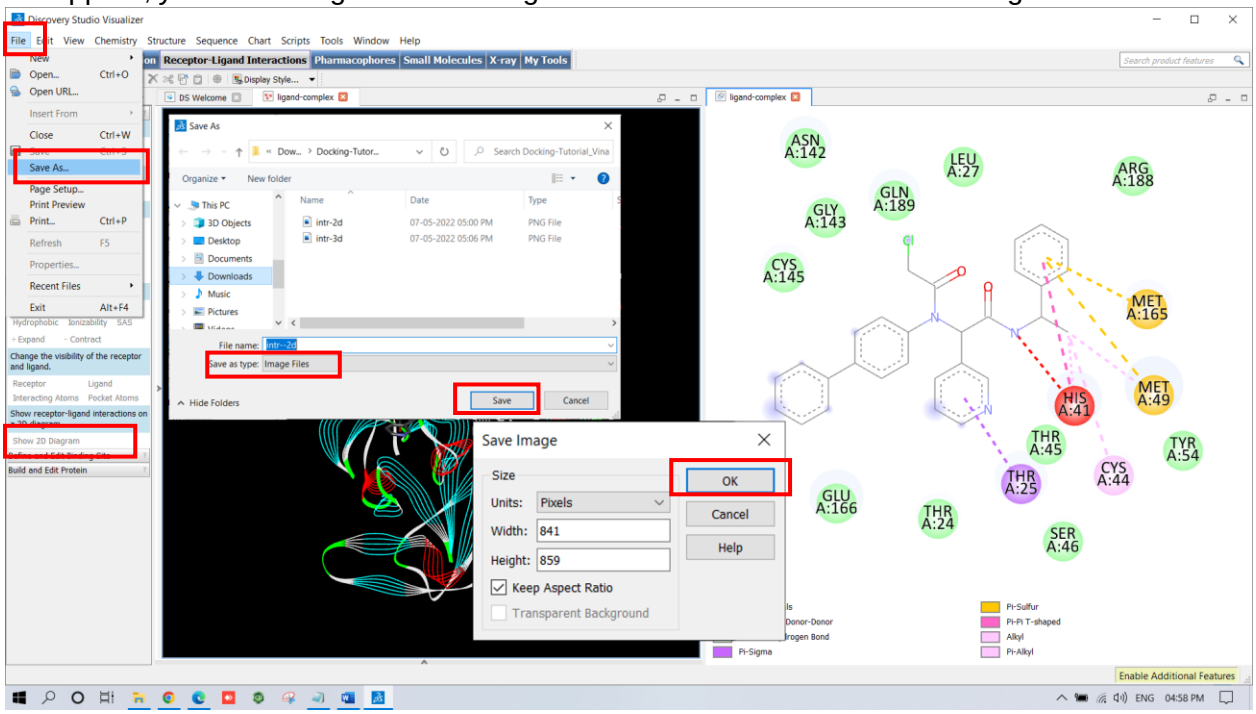
After the completion of docking two files will be generated
1. **ligand_vinaout.pdbqt** (contain ligand poses)
2. **logligand_vinout.txt** (contain dock score of ligand poses)

V. Analysing the results

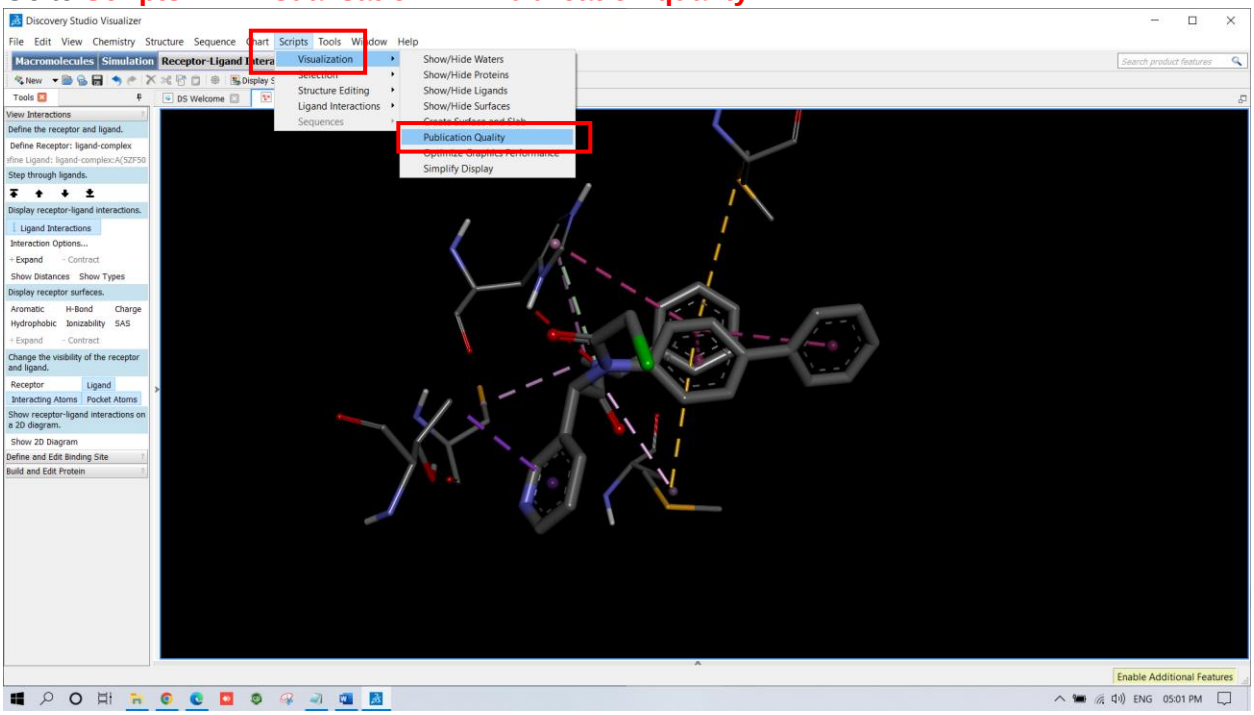
Open the **receptor.pdbqt** in PyMOL and then **ligand_vinaout.pdbqt**. See all the poses by clicking the next button in right corner of PyMOL window. We will save the first pose of ligand with receptor in pdb format to visualise the interactions. Go to **file**---> **Export molecule**. a tab will open, Change the "**State**" to **1**, and check the "**retain atom IDs**" and save the file as **ligand-complex.pdb**.



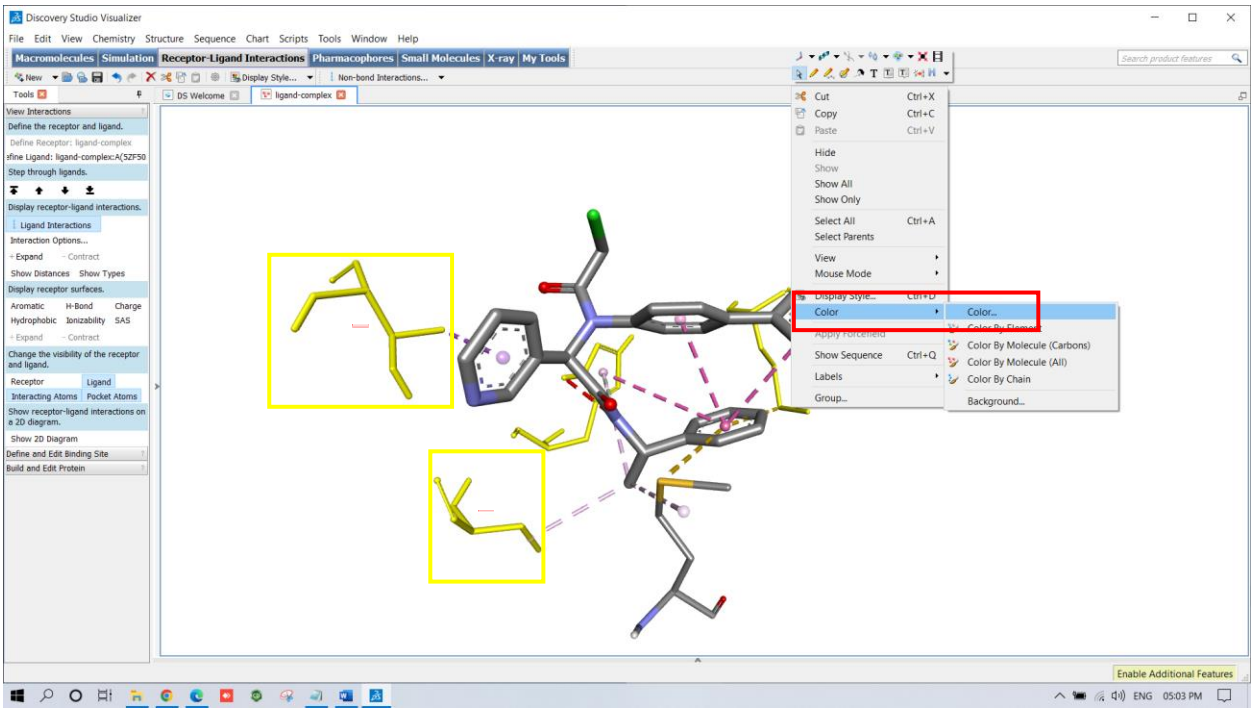
Open the complex file with discovery studio. In the left panel, click "**Show 2D diagram**". Go to **File** ---> **Save As**. Name the file and change the **file type** to **image files** and **save**. then, a tab will appear, you can change the size image and click **OK**. the 2d interaction diagram will be saved.



Go to **Scripts** ---> **Visualisation** ---> **Publication quality**.



Next, in the left panel, click "**ligand Interactions**" which will come up with a 3D interaction digram of ligand with amino acids of receptor. then by pressing **ctrl** and **shift** together **double click** on all the amino acids to select them. Right click on the structure, drop down menu will be open. there, click **color**---> **color**, choose any color you wish to give to amino acids and press **ok**.



Further for naming the amino acids, select them and right click, now select labels from the drop down menu ---> **add**. A tab will appear, select **amino acid** for "**object**", **3-letter & ID#** for Attribute, select any specific size, font, color for label and click **OK**. then for saving this 3d interaction

diagram Go to menu present on the window click save icon, give the file name and change the file type to image files and save.