Docking assignment

Name: Pragathi Prasad SRN: PES1UG22BT038

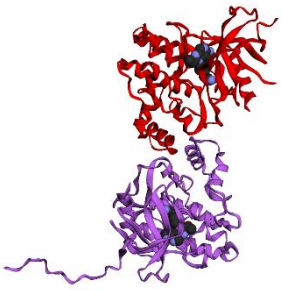
Protein name and PDB ID:

4IVC: JAK1 kinase (JH1 domain) in complex with the inhibitor (TRANS-4-{2-[(1R)-1-HYDROXYETHYL] IMIDAZO[4,5-D] PYRROLO[2,3-B] PYRIDIN-1(6H)-YL} CYCLOHEXYL) ACETONITRILE

About the protein:

Janus kinases (JAKs) are a group of intracellular, non-receptor tyrosine kinases essential in cytokine signalling pathways. This family consists of four kinases: JAK1, JAK2, JAK3, and Tyk2. They are tyrosine kinase receptors linked to a transmembrane domain phosphorylates various STAT (signal transducers and activators of transcription) protein isoforms in response to ligand binding.

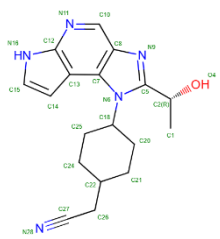
Janus Kinase 1 (JAK1) is a type of protein kinase that plays a key role in mediating the signalling of cytokines and growth factors involved in haematopoiesis and immune function. Dysregulated JAK1 activity has been tied to diseases like leukaemia and lymphoma, given its essential role in regulating cell growth. Overactive JAK1 is similarly linked to autoimmune disorders such as rheumatoid arthritis and inflammatory bowel disease (IBD), where it drives excessive inflammation. Meanwhile, loss-of-function mutations in JAK1 can result in immunodeficiencies. JAK1 inhibitors, such as tofacitinib, are employed to manage conditions marked by overactive JAK1 signalling, providing therapeutic relief for autoimmune and inflammatory disorders.



Source: PDBSum (ebi.ac.uk/thornton-srv/databases/cgi-bin/pdbsum/GetPage.pl)

The JH1 domain of JAK1 is its kinase domain, located at the protein's C-terminal end, and plays a critical role in initiating key cell signalling pathways, particularly in the immune system. This domain phosphorylates tyrosine residues on signalling proteins, activating pathways like JAK-STAT that drive immune responses and inflammation. Due to its link to excessive signalling in autoimmune diseases and cancers, unregulated JH1 activity is a primary target for JAK inhibitors, which help control overactive signalling in these conditions.

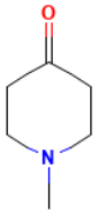
The PDB structure chosen for this docking exercise consists of JAK1 bound to an inhibitor (Trans-4-{2-[(1r)-1-Hydroxyethyl] imidazo[4,5-D] pyrrolo[2,3-B] pyridin-1(6h)-Yl} cyclohexyl) acetonitrile.



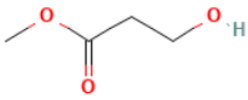
Inhibitor's molecular structure. Source: RCSB PDB (RCSB PDB - 1J6 Ligand) Summary

About the Ligands:

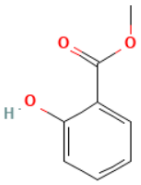
1. 1-Methyl-4-piperidone (PubChem Id: 74049): It a compound consisting of a six-membered piperidine ring with a ketone group positioned at the 4-carbon and a methyl group attached to the nitrogen. It's frequently used as an intermediate in the synthesis of pharmaceuticals, agrochemicals, and other organic compounds, valued for its reactive ketone group, which supports diverse transformations in medicinal chemistry. Its chemical formula is $C_6H_{11}NO$ and molar mass is 113.16 g/mol.



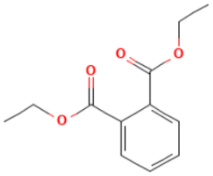
2. Methyl 3-hydroxypropanoate (PubChem Id: 80252): It is an organic ester consisting of a three-carbon chain with a hydroxyl group (-OH) on the second carbon and a methyl ester group (-$COOCH_3$) at the end. It's commonly used as a building block in organic synthesis and in the production of polymers and pharmaceuticals. Its molecular formula is $C_4H_8O_3$ and its molar mass is 104.10 g/mol.



3. Methyl Salicylate (PubChem Id: 4133): It is an organic ester, derived from salicylic acid by replacing its hydroxyl group with a methyl group, is recognized for its distinctive wintergreen aroma. It's widely used in topical analgesics, as well as in flavourings and fragrances. Its molecular formula is $C_8H_8O_3$ and molar mass is 152.15 g/mol



4. Diethyl Phthalate (PubChem Id: 6781): It is a colourless liquid that has a bitter, disagreeable taste. This synthetic substance is commonly used to make plastics more flexible. Products in which it is found include toothbrushes, automobile parts, tools, toys, and food packaging. It can be released fairly easily from these products, as it is not part of the chain of chemicals (polymers) that makes up the plastic. Diethyl phthalate is also used in cosmetics, insecticides, and aspirin. Its molecular formula is $C_{12}H_{14}O_4$ and its molar mass is 222.24 g/mol.



Sources of images:

1-Methyl-4-piperidone | C6H11NO | CID 74049 - PubChem

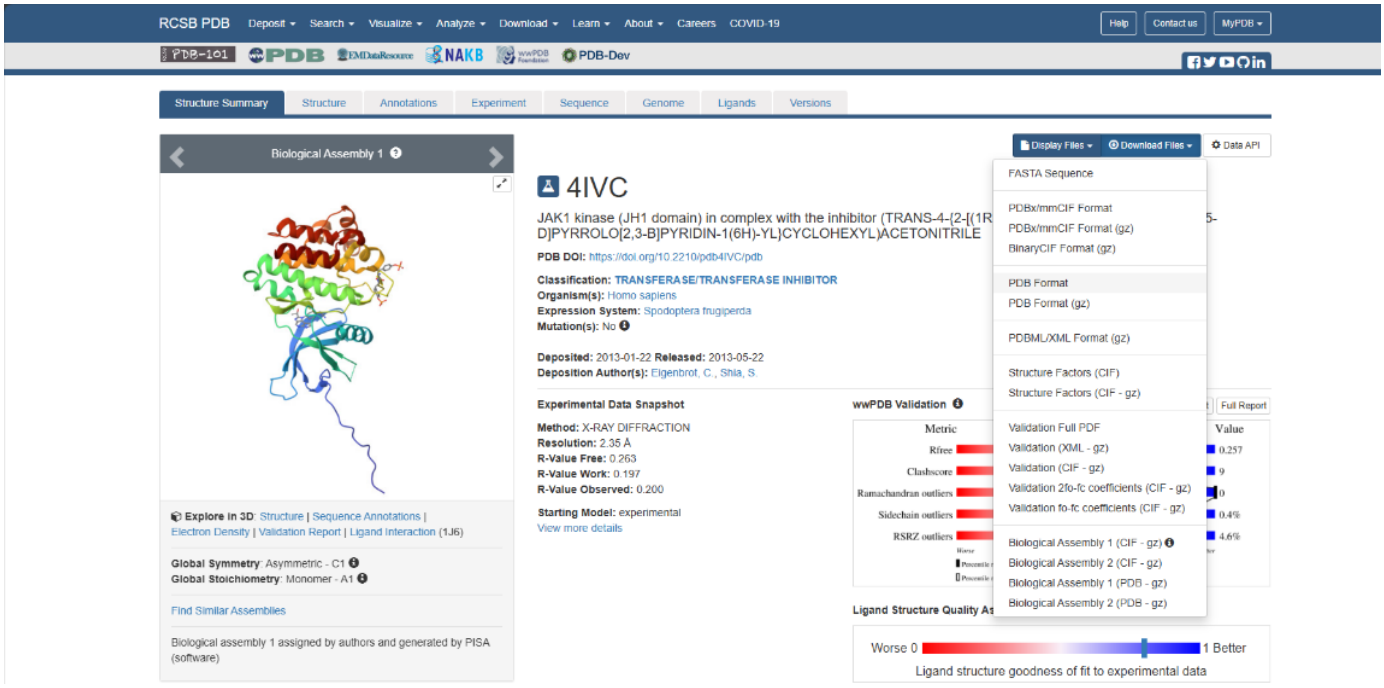
Methyl 3-hydroxypropanoate | C4H8O3 | CID 80252 - PubChem

Methyl Salicylate | C8H8O3 | CID 4133 - PubChem

Diethyl Phthalate | C12H14O4 | CID 6781 - PubChem

The docking procedure:

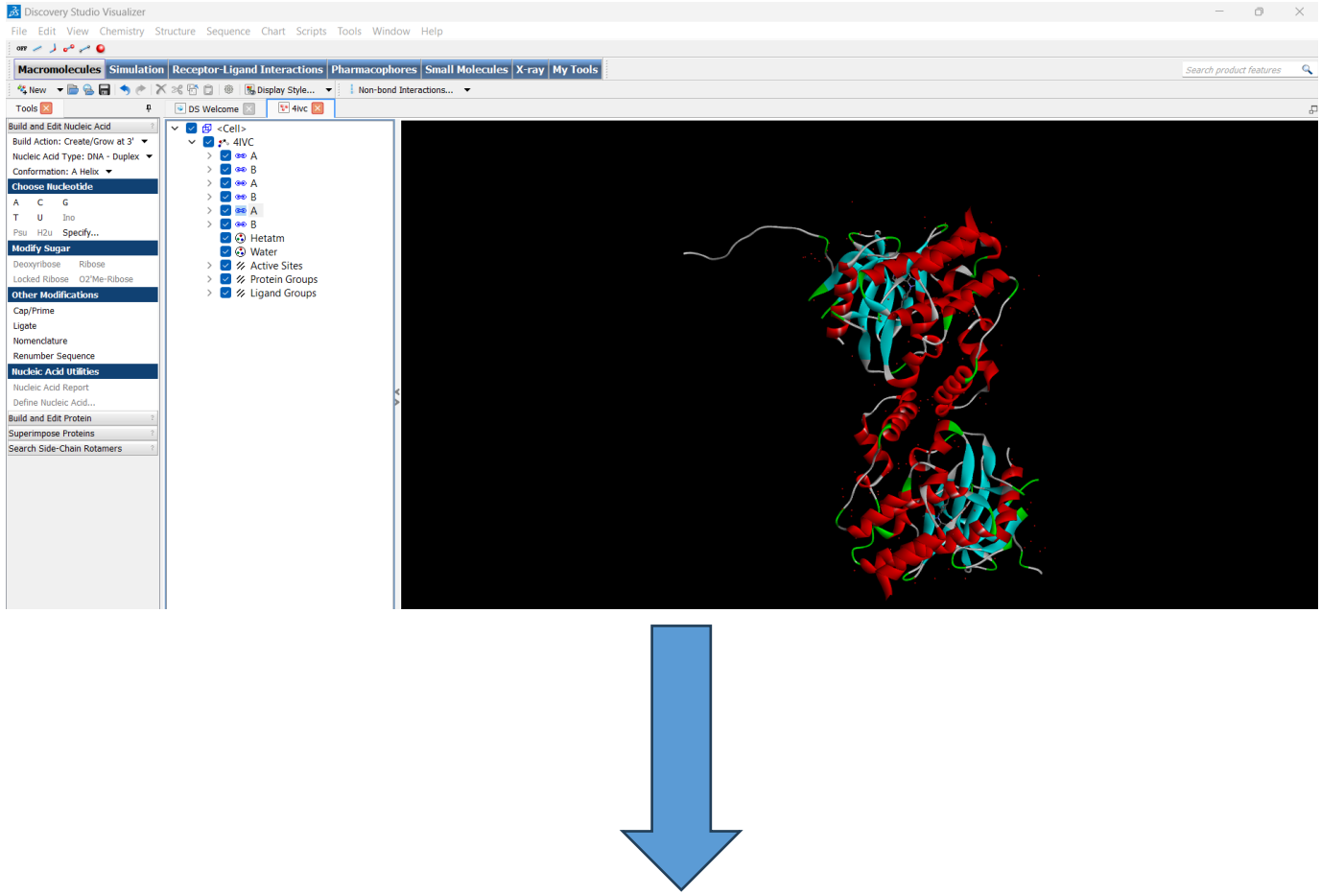
First, the structure of the protein was obtained as a PDB file from the RCSB PDB database. The file was named *4ivc.pdb*

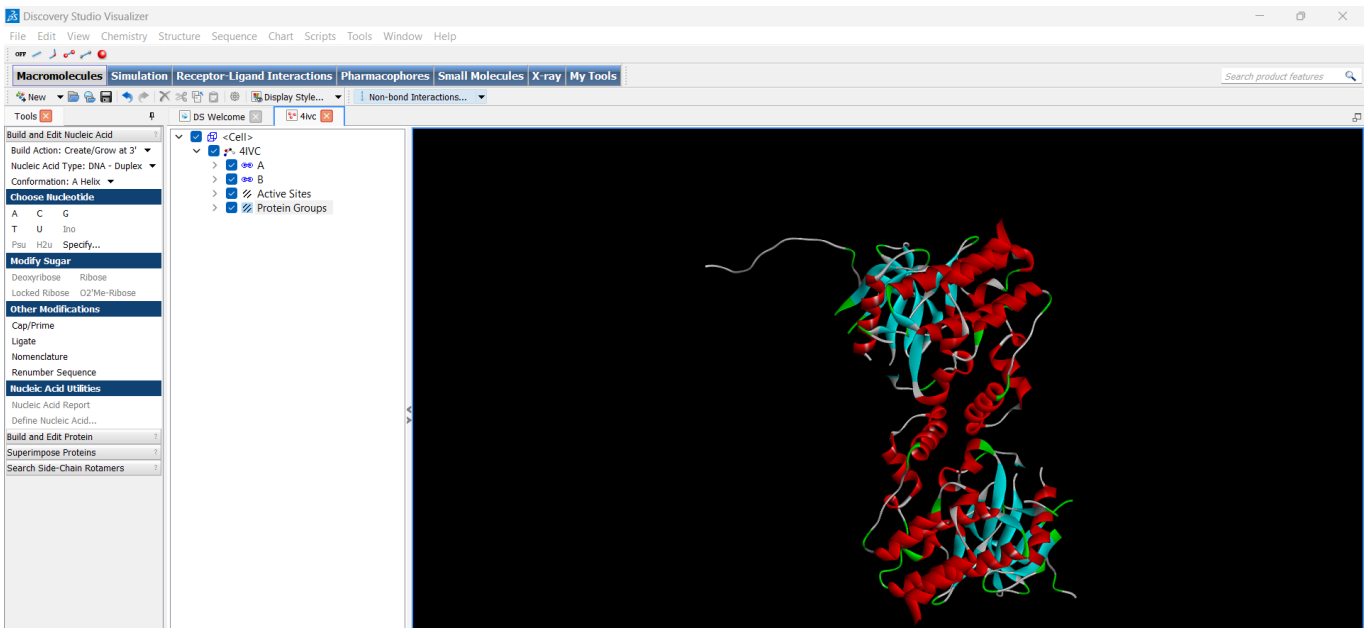


Then, the structure was loaded onto the BIOVIA Discovery Studio visualiser.

The purpose for using Discovery Studio is to obtain the pure protein structure devoid of the ligand, water molecules and any heteroatoms as these can disrupt and interfere with the docking process.

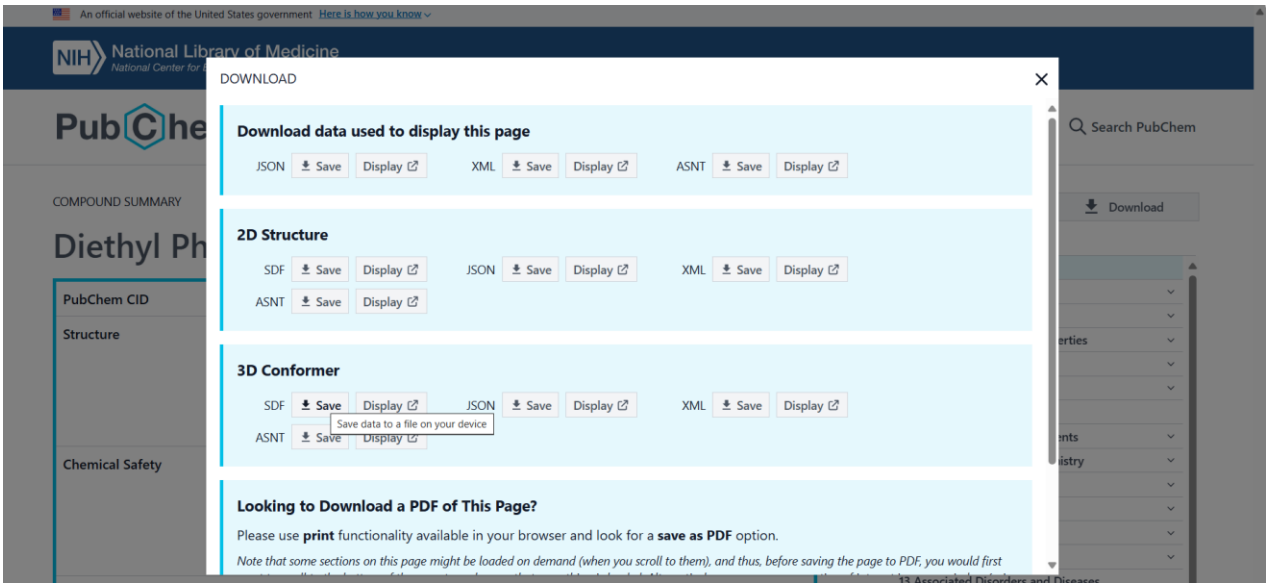
On pressing Ctrl + H, a menu opens up where all these elements can be easily removed. Only the two main chains of the protein, A and B and the active site are retained for the further docking processes.



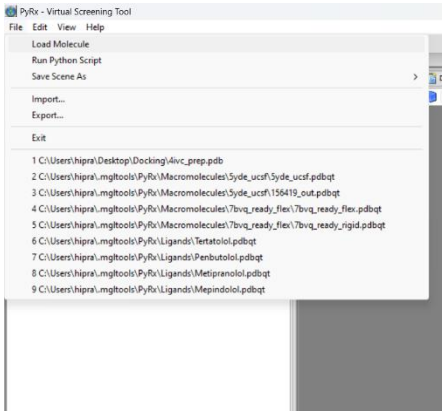


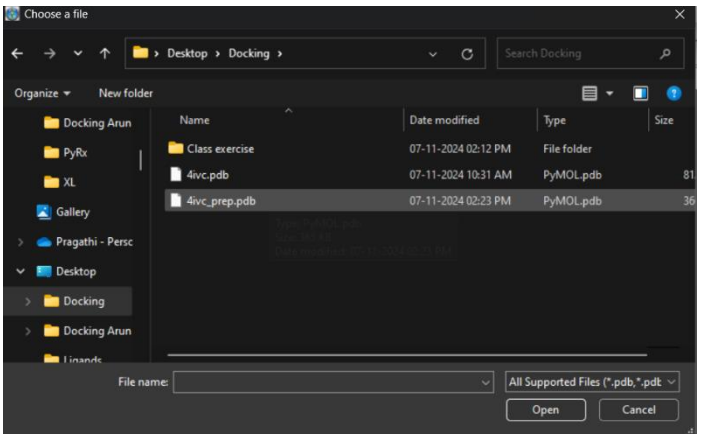
This structure is then saved as *4ivc_prep.pdb*

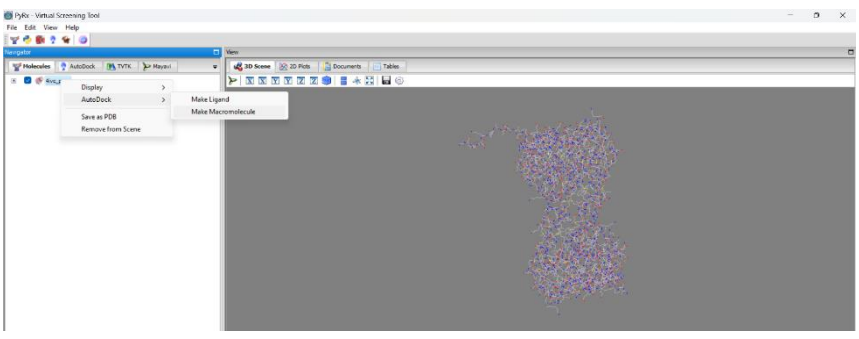
Then the 3D structures of the ligand molecules are downloaded from the PubChem database. They are downloaded as 3D conformers in .sdf file format.



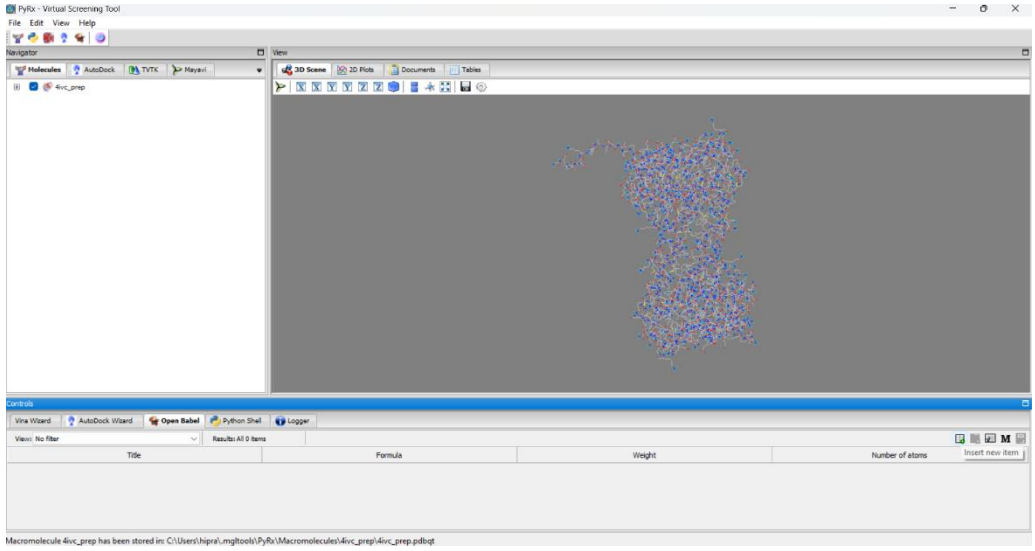
Then , the prepared molecule is loaded onto PyRx. The molecule is assigned as a macromolecule upon right clicking on option in the left screen pane and selecting the option (“Make macromolecule”) from the AutoDock options’ side menu.

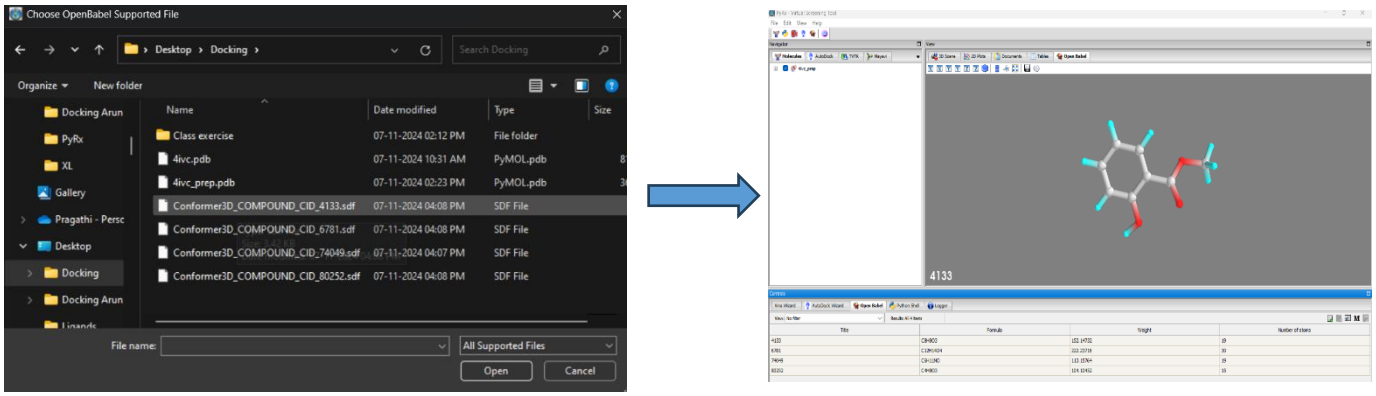




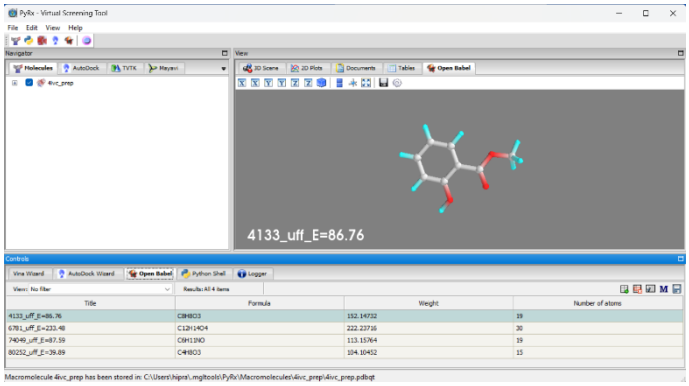


Then, the OpenBabel option is selected in the tab below and the ligands are loaded on clicking the green plus sign.

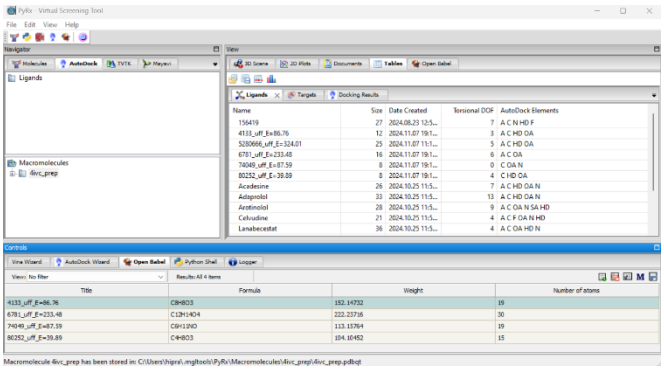




The ligand structures were all minimised and converted to AutoDock ligand format (pdbqt)

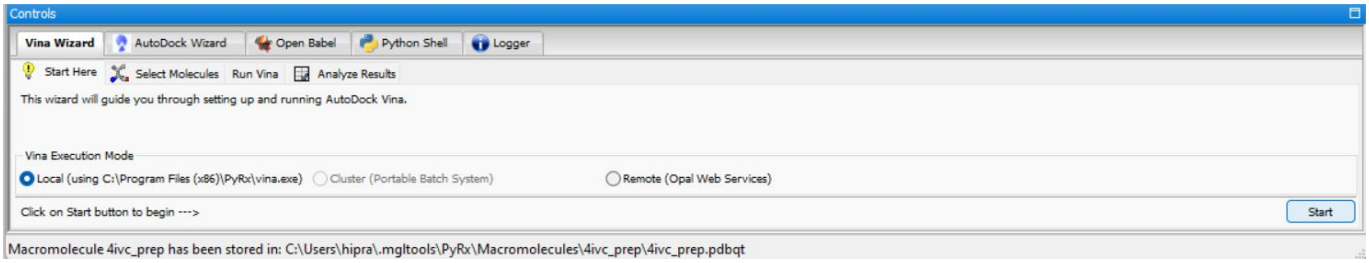


Minimisation

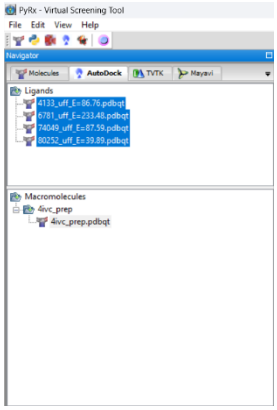


Converted to pdbqt

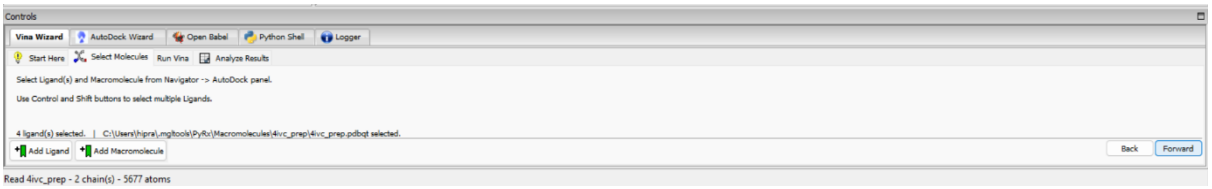
Click on start in the Vina wizard menu.

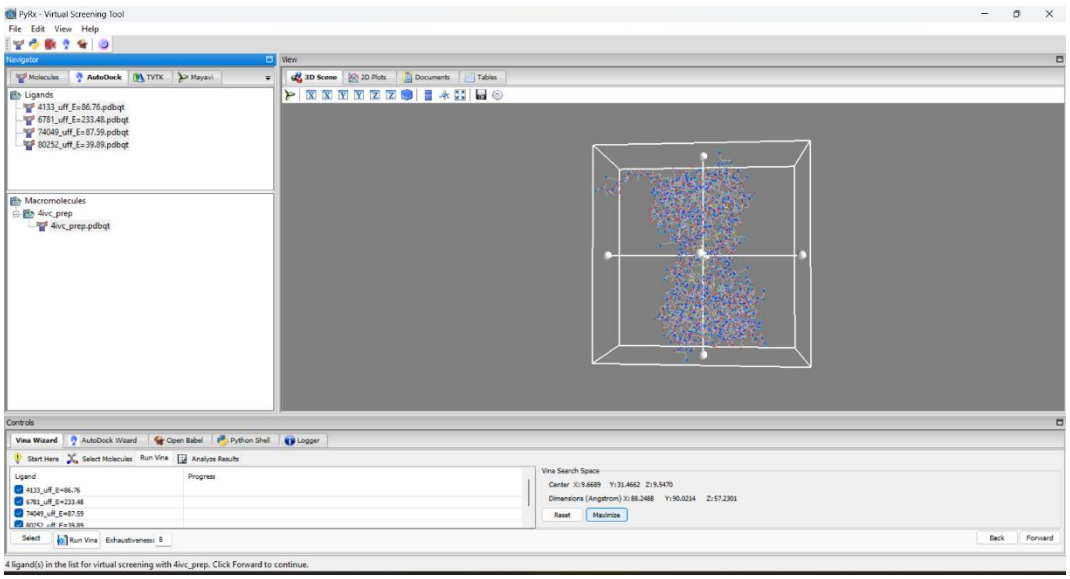


Then, go to the AutoDock menu where the ligand molecules are available. Select all ligands by clicking on the first, pressing shift and clicking on the last ligands.

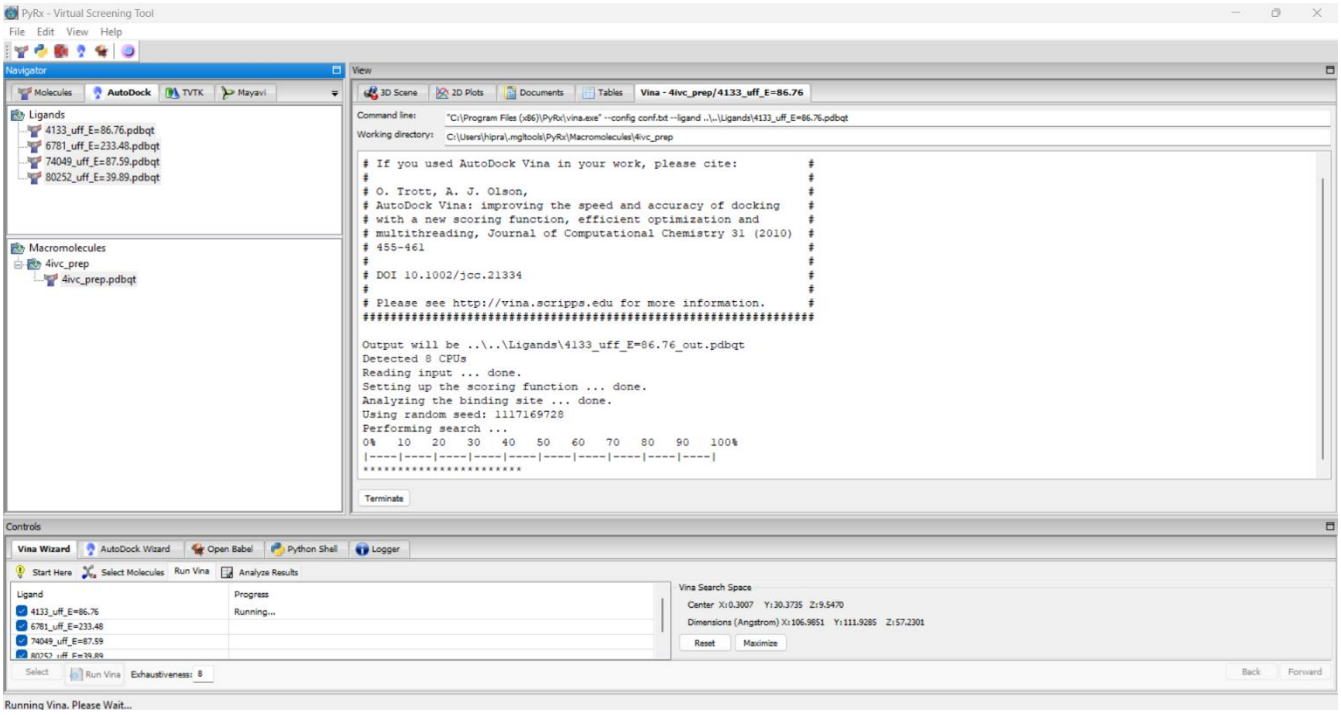


Then, click on the Forward option in the Vina wizard. It will fit a grid box around the protein molecule which is a pre-requisite to begin the docking process. Click on maximise to the molecule, although, manual adjustments are recommended for better results.

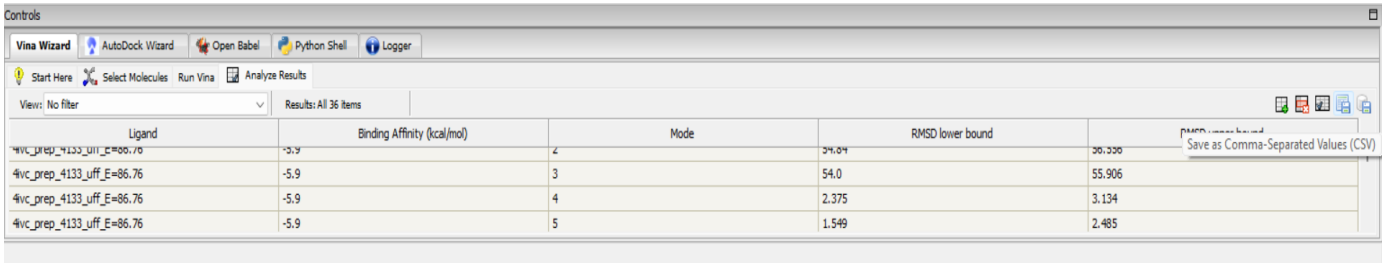


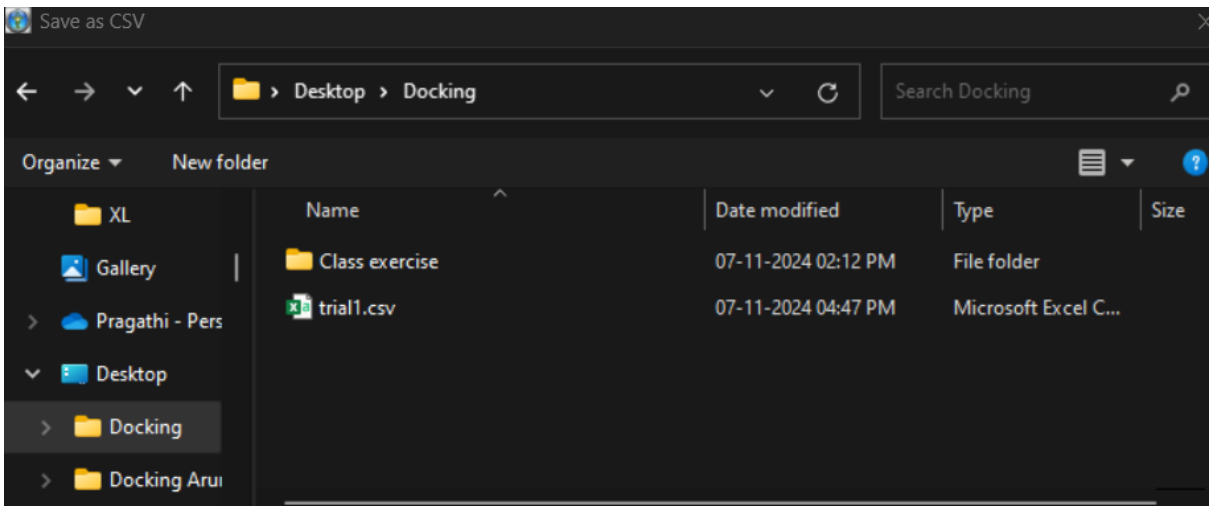


After adjusting, click the Forward option again to initiate the docking process.

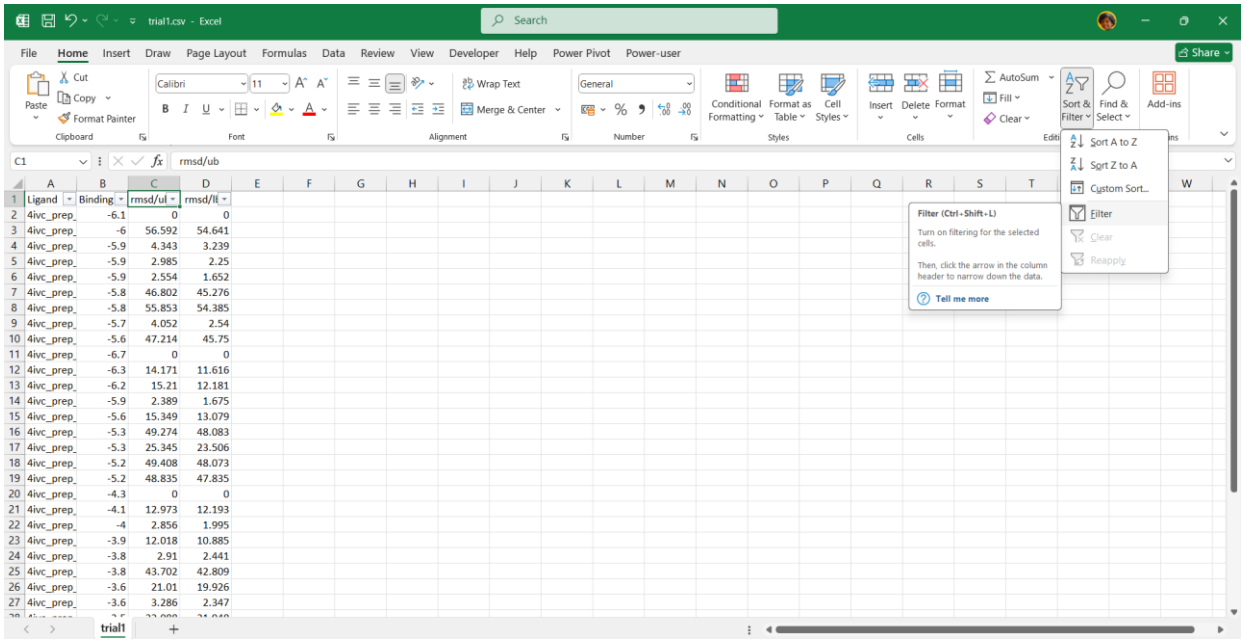


After the docking process is finished, the Vina wizard will show a table consisting of the following columns: Ligand, Binding affinity (kcal/mol), Mode, RMSD lower and upper bound. Click on the blue logo in the right side and save the table a s .csv file. Since this is the first trial, save as *trial1.csv*





The .csv file is then opened in Microsoft Excel or any spreadsheet software. Click on the first cell of the column and select the Filter option from the Sort and Filter icon in the Home tab.



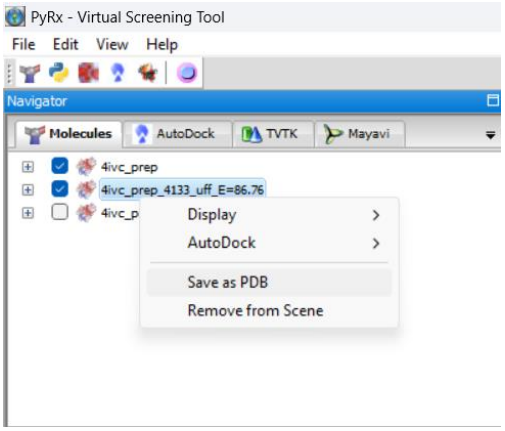
Ligand	Binding	rmsd/ul	rmsd/ll
4ivc_prep_	-6.1	0	0
4ivc_prep_	-6	56.592	54.641
4ivc_prep_	-5.9	4.343	3.239
4ivc_prep_	-5.9	2.985	2.25
4ivc_prep_	-5.9	2.554	1.652
4ivc_prep_	-5.8	46.802	45.276
4ivc_prep_	-5.8	55.853	54.385
4ivc_prep_	-5.7	4.052	2.54
4ivc_prep_	-5.6	47.214	45.75
4ivc_prep_	-6.7	0	0
4ivc_prep_	-6.3	14.171	11.616
4ivc_prep_	-6.2	15.21	12.181
4ivc_prep_	-5.9	2.389	1.675
4ivc_prep_	-5.6	15.349	13.079
4ivc_prep_	-5.3	49.274	48.083
4ivc_prep_	-5.3	25.345	23.506
4ivc_prep_	-5.2	49.408	48.073
4ivc_prep_	-5.2	48.835	47.835
4ivc_prep_	-4.3	0	0
4ivc_prep_	-4.1	12.973	12.193
4ivc_prep_	-4	2.856	1.995
4ivc_prep_	-3.9	12.018	10.885
4ivc_prep_	-3.8	2.91	2.441
4ivc_prep_	-3.8	43.702	42.809
4ivc_prep_	-3.6	21.01	19.926
4ivc_prep_	-3.6	3.286	2.347

Then, click on the drop down in the rmsd/ub or rmsd/lb (Root mean square upper/ lower bound) cell and deselect all options. Then select only 0. 0 implies that the docking pose aligns exactly with the reference structure, the average distance between atoms of the predicted binding pose and a reference structure is 0.

	A	B	C	D	E
1	Ligand	Binding Affini	rmsd/ub	rmsd/lb	
2	4ivc_prep_4133_uff_E=86.76	-6.1	0	0	
11	4ivc_prep_6781_uff_E=233.48	-5.2	0	0	
20	4ivc_prep_74049_uff_E=87.59	-4.3	0	0	
29	4ivc_prep_80252_uff_E=39.89	-3.7	0	0	

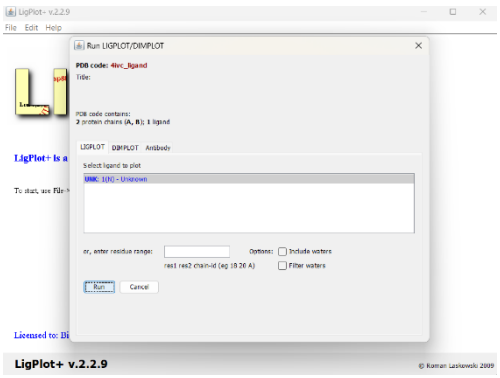
The Ligand option having the lowest binding energy is the most suitable ligand for binding with the protein as the bond between them will very stable. Lower the binding energy, more stable is the structure.

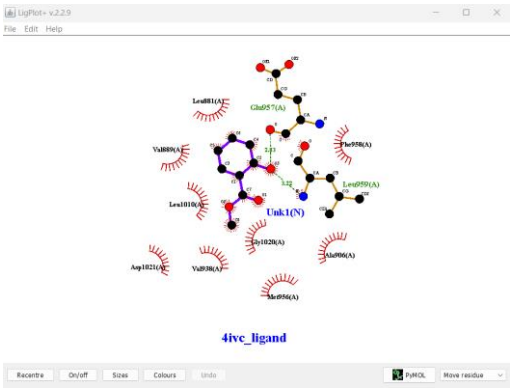
On PyRx, right click on the ligand with the lowest binding energy and save as *4ivc_docked1.pdb*



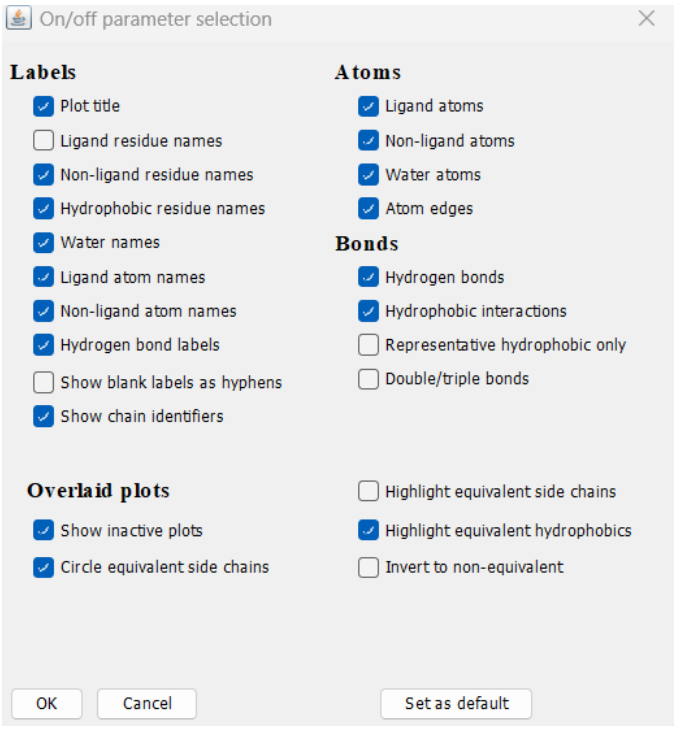
Then, open PyMol and load both and ligand and prepared pdb molecule structures (*4ivc_ligand1.pdb* and *4ivc_prep.pdb)*. This gives the final result of the docking as we can observe the region where the ligand has been docked. N the right side of the screen, click on Zoom → Zoom all to get the overall proper view. Click on File → Export molecule. Save as *4ivc_ligand.pdb.*

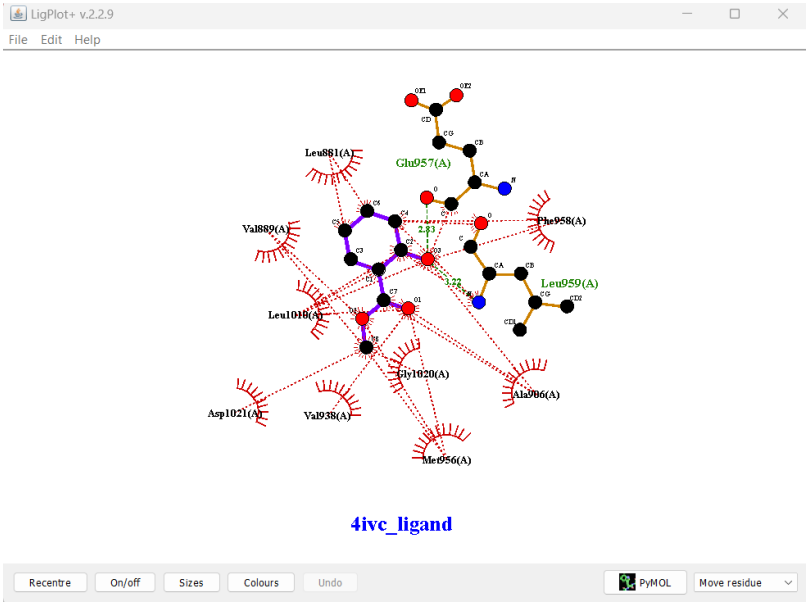
To visualise the 2D interaction of the ligand in ligplot+, run the *LigPlus.jar* file. Click on open → PDB file → Browse → *4ivc_ligand.pdb.* Click on the run option.





Click on the On/Off option on the bottom left side and disable Ligand residue names and enable Hydrophobic interactions.





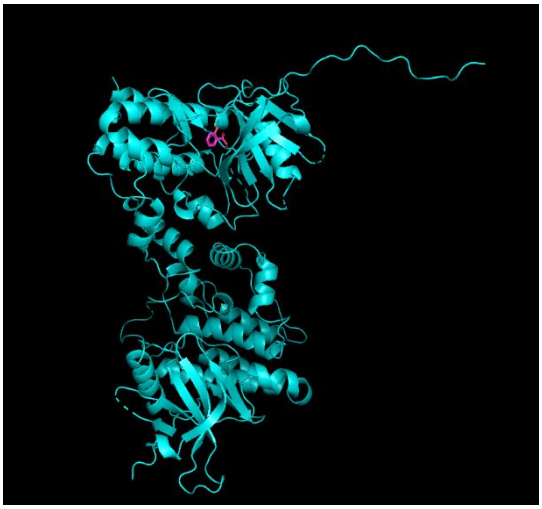
The green coloured amino acids indicate that they are directly bound to the ligand whereas the ones with the pointed red structures and red lines indicate they are bound to the ligand by hydrogen bonds.

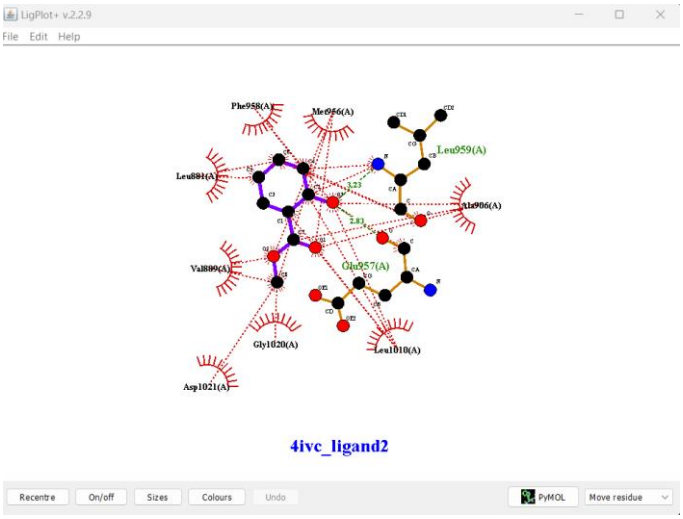
In this trial, the ligand Methyl Salicylate has shown to have the lowest binding affinity , therefore the most stable bond is between this and the protein.

Similarly, conduct 2 more trials using PyRx to see if there are any changes in trends in the binding energy of the ligands to the protein and if the most stable ligand changes. In order to prevent interference, delete the macromolecule and start from the beginning.

Trial 2 results:

Ligand	Binding	rmsd/ul	rmsd/lb
4ivc_prep_4133_uff_E=86.76	-6.1	0	0
4ivc_prep_6781_uff_E=233.48	-5.6	0	0
4ivc_prep_74049_uff_E=87.59	-4.6	0	0
4ivc_prep_80252_uff_E=39.89	-3.9	0	0

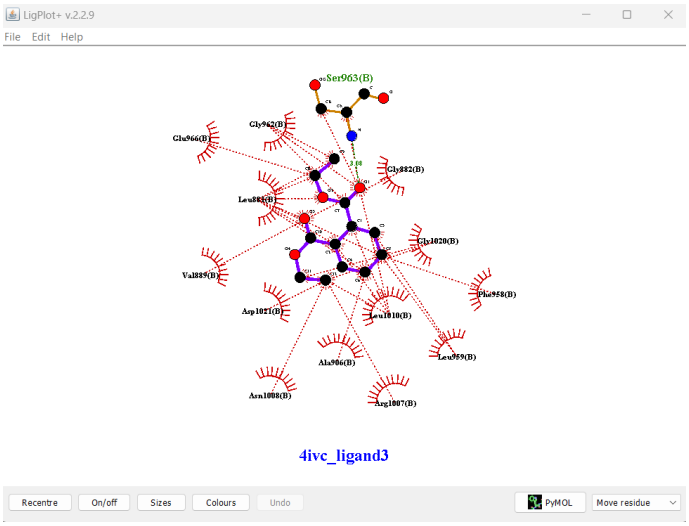




In this trial too, the ligand Methyl Salicylate has shown to have the lowest binding affinity, therefore the most stable bond is between this and the protein.

Trial 3 results

1	Ligand	Binding	rmsd/ul	rmsd/lb
2	4ivc_prep_4133_uff_E=86.76	-6.1	0	0
11	4ivc_prep_6781_uff_E=233.48	-6.8	0	0
20	4ivc_prep_74049_uff_E=87.59	-4.2	0	0
29	4ivc_prep_80252_uff_E=39.89	-3.6	0	0



Unlike the previous trials, in this trial, the ligand Diethyl Phthalate has shown to have the lowest binding affinity, a deviation from the previous results.

Summary:

Trial No.	1	2	3
Binding affinity	-6.1	-6.1	-6.8
Ligand name	Methyl Salicylate	Methyl Salicylate	Diethyl Phthalate

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