**Enzyme Science and Technology**
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**Module - IX**
**Enzyme Inhibitor Designing**
**Lecture - 41**
**Inhibitor Designing (Part-III: Computational Approach)**

Hello everyone, this is Dr. Vishal Trivedi from Department of Biosciences and Bioengineering IIT Guwahati. And what we were discussing, we were discussing about the different properties of the enzyme in the course, Enzyme Science and Technology. So, in this context, in this particular module, we are discussing about the different approaches and the way in which you can be able to design the inhibitor for the enzyme.

Now, what we have discussed, we have discussed about the traditional approach where you are actually going to have the no information about the enzyme structure or the inhibitor structure, what you require is you require the enzyme assays and you can be able to screen the different types of inhibitors from the different libraries.

One of the major drawback of the traditional approach is that it will not allow you to improve the inhibitor because you do not know the structure of the inhibitor. So, you do not know which molecule which region of the inhibitor is inhibiting or which region of the inhibitor is important for the interactions. To overcome this particular problem, people have started you know looking at the targeted approach.

So, in the targeted approach, you actually will know the structure of the enzyme, you actually will know the structure of the inhibitors and that is how you can be able to use the different types of computational approaches to you know to see how the inhibitor is binding into the binding pocket and what are the different interactions are involved.

Based on these approaches, you can be able to use either the ligand-based approach where you can actually be know that this is the ligand, what actually fits into the active site. And then you can take that ligand and search the similar ligands into the database and that is how you can be able to identify the some of the potential inhibitors or you can also be able to use the receptor-based approach.

Where you can actually be able to study the receptor binding site and based on the binding site, you can be able to identify the crucial interactions, which is responsible for the receptor to interact with the ligands and considering and keeping those intact interaction intact, you can be able to design the new ligands. Both of these approaches we have discussed in detail in the previous lecture and we have also taken some of the case studies, how you can be able to use the some of these approaches.

Now, in today's lecture, we are going to discuss about the computational approaches, how you can be able to use them for designing the new inhibitors. So, when we talk about the computational approaches, it is well understood that you actually know the structure of the enzyme and you also know the structure of the inhibitors.

If the structure of the enzyme is not known, then you actually have the possibility of designing you know enzyme structure either by the homology modelling or the dynamo modelling. So, prerequisite of the computational based approach is right it should have you should have the enzyme structure as well as the inhibitor structures.

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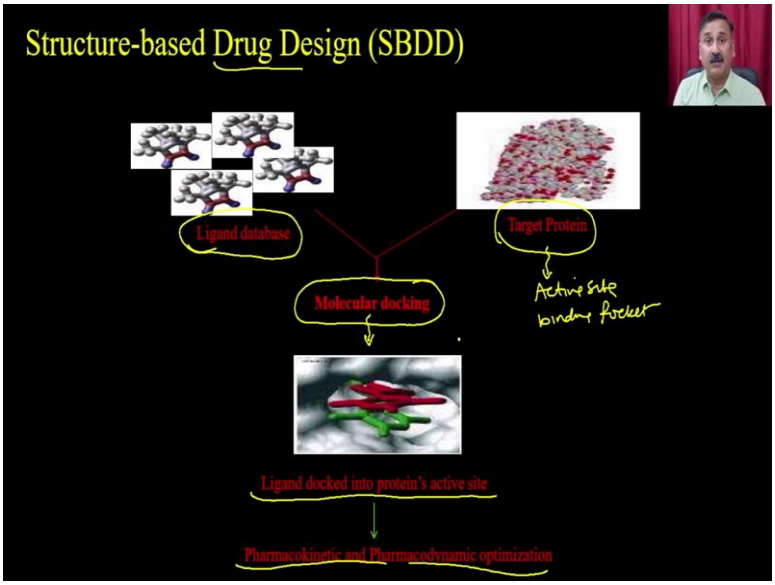


So, in a structure-based drug design or structure-based inhibitor design, what you require is you require a database of the ligands. So, that you can be able to select the different types of ligands from the database and you can test them into the computational approach. You require a target protein and you also should have know the structure of

the target protein. Not only that, you should also know the position of the active site and the binding pockets and all that ok.

And then you can actually be able to put them into a process which is called as the molecular docking. So, within the molecular docking, what it is going to do is it is actually going to see how the ligands are fitting into the active site and the ligands are docked into the protein active site and the pharmacokinetics and the pharmacodynamics optimizations are actually going to be done through that you can be able to know how well these ligands are fitting into the active site. So, the question comes what is docking?

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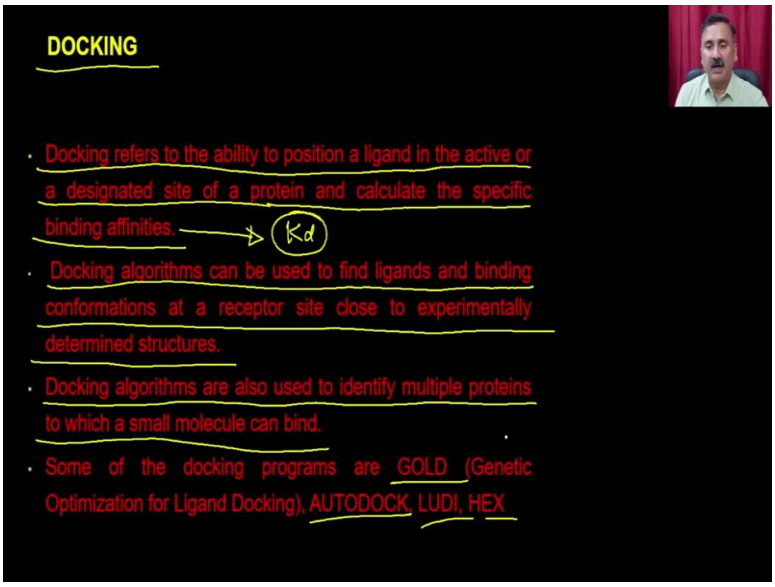


So, what is docking? So, docking refer to the ability of to position a ligand into the active site or a defined designated site of the protein and calculate the specific binding affinities. It means it is actually going to tell you about the affinity constants. Then docking algorithm can be used to find the ligand and the binding conformation at a receptor site close to the experimentally determined structures.

Docking algorithms are also used to identify the multiple proteins to which a small ligand can be used. You have the some of the important docking programs such as Gold or AutoDocks, Ludi and Hex and some of these programs are free and whereas, some of the programs are the licensed programs. So, you can actually be able to use them for performing the docking. And what docking is exactly doing?

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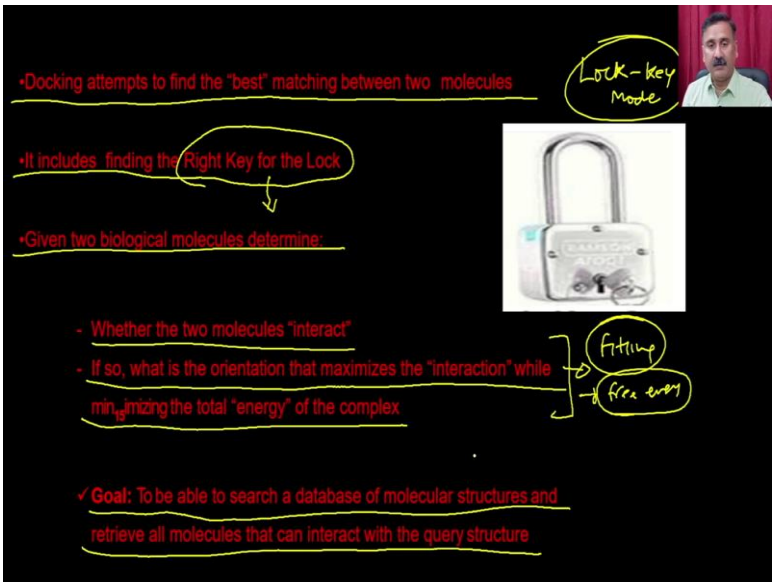


Docking attempts to find the best matching between the two molecule. Remember that the docking is actually works on the lock-and key model. So, remember that lock-and key model, right? So, lock-and key model says that there is a lock and there is a key right? So, if there is a lock there is a always a definite key right?

So, if you can be able to make the match between the two molecules whether it is a lock-and key model or whether the induced fit model so, it includes finding the right key for a lock, right. That is the correct statement ok. Given the two biological molecules you can actually be able to determine whether the two molecules interact with each other.

If so, what is the orientation that maximize the interaction while the minimizing the total energy of the complex because these are the two important parameter. One fitting and the second is the free energy right. So, it should be have the total free energy of the molecule lower and it should have the best fitting. And the goal is to able to search a database of molecular structures and retrieve all structure that can interact with the query structures.

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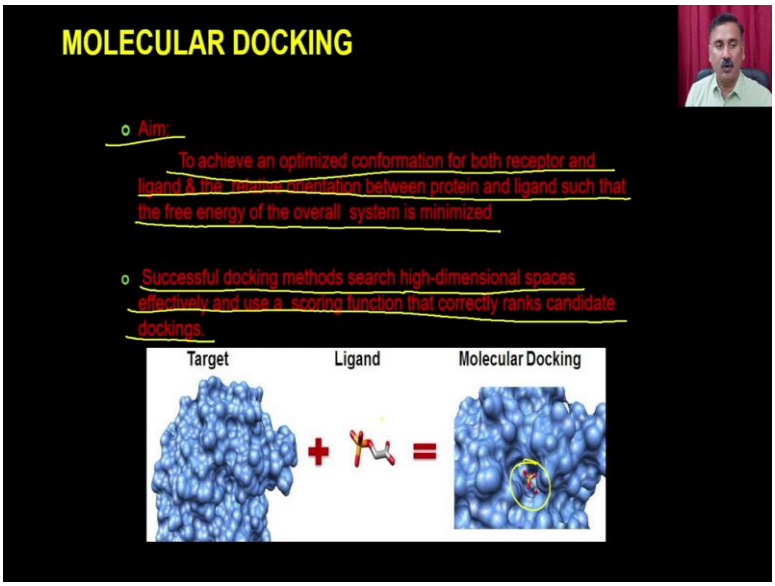


So, the aim of the molecular docking is to achieve an optimized confirmation for both the receptor and the ligand and the relatively orientation between the protein and ligand such as the free energy of the overall system is minimized. Successful docking methods search high dimensional spaces effectively and use a scoring function that correctively rank the candidate docking.

So, you have a target molecules you can have the ligand molecules and with the help of the molecular docking you will able to say whether this particular ligand is fitting into this particular active site or not.

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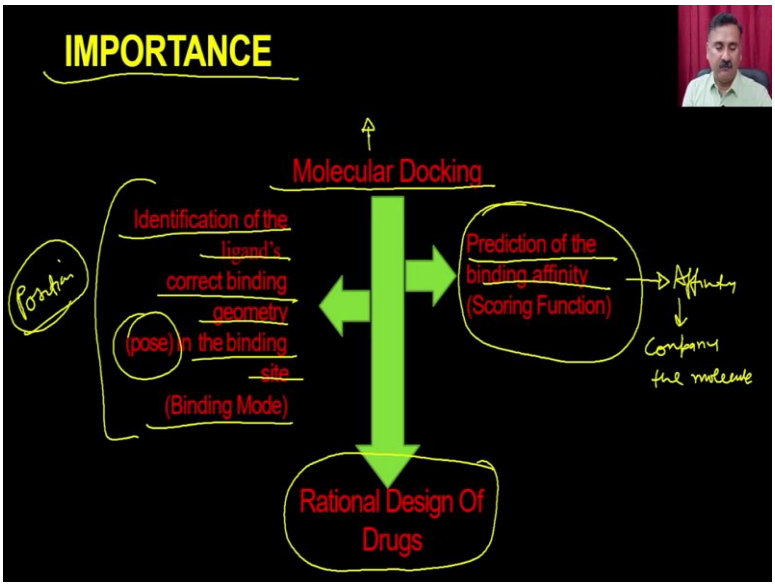


So, what is the importance of the molecular docking? Ok. So, molecular docking is actually going to identify the ligands correct binding geometry or the pose into the binding site or the binding mode; whereas it is actually going to predict the binding affinity or the binding scores right. So, it is first of all it is actually going to say at what position this particular ligand is actually going to go and bind into the target; whereas it also going to tell you the scoring functions.

So, it is actually going to tell you the affinity parameters and that can be used for the comparing the molecules. So, that can be used for comparing the molecules and that is why the molecular docking can be used for rationally designing the target molecules. Now, when you talk about the molecular docking, molecular docking can be performed in two different modes.

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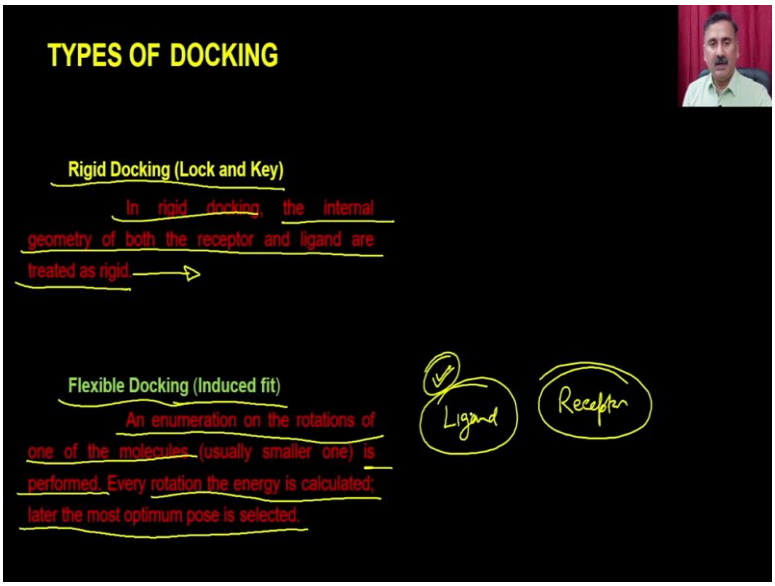


So, you can actually be follow the lock and key model or you can actually be able to follow the induced fit model, which means you can actually be able to do the rigid body docking or you can be able to do the flexible docking. So, in the rigid docking the internal geometry of both the receptor and ligands are treated as a rigid. So, in this case you are actually going to follow philosophy of the lock and key model; whereas in the flexible docking you are actually going to have the induced fit model.

So, an enumeration on the rotation of the one of the molecule is performed. Every rotation the energy is calculated, later the most optimal pose is selected. So, in this case you are actually going to make one of the molecule as the flexible molecule. So, either you can actually be able to use the ligand as a flexible molecule or the receptor as a flexible molecule and that is why you can be able to do the flexible docking. Majority of the other time it is ligand what you are actually going to make this flexible molecule.

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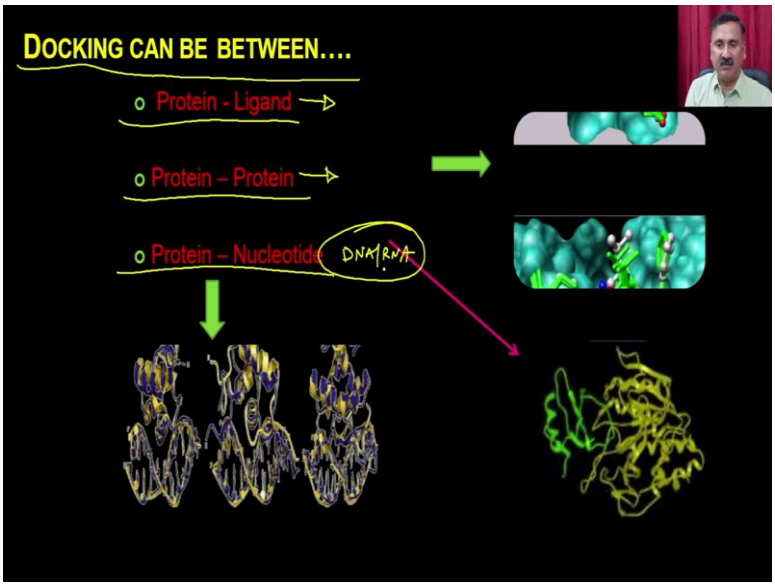


Now, as far as the docking is concerned you can actually be able to do the docking between the different pairs. You can actually do the protein ligand docking, you can actually do the protein-protien docking and you can actually be able to do a protein nucleotide or I will say DNA or RNA docking ok. So, this is the protein DNA RNA docking. So, either of these pair can be used and can be studied in the docking experiments.

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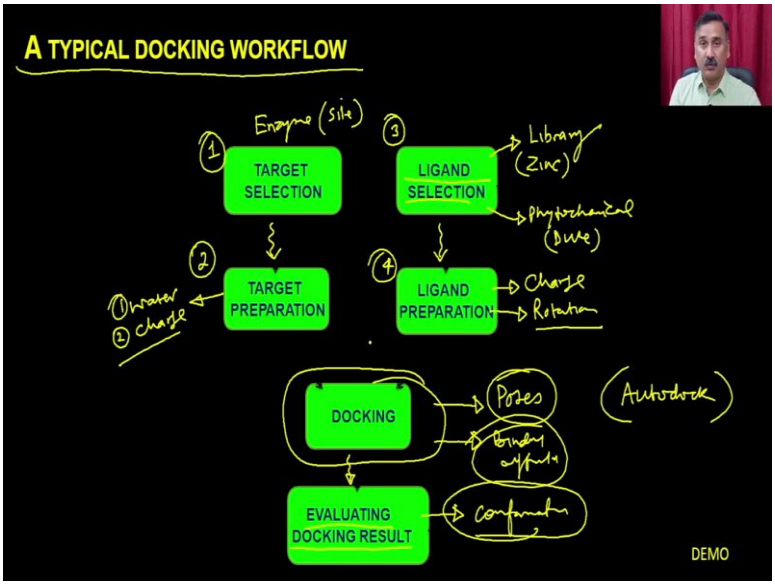

Now, when you want to do a docking experiments you are actually going to follow the different types of work flow. So, first is number 1 you are actually going to do the target selection, which means you are actually going to select the enzyme and not only the enzyme you are actually also going to select the binding site ok. And there are the multiple procedures and multiple steps how you can be able to select the target. Once you have selected the target then you are actually going to prepare the target ok.

This means you are actually going to have the different types of procedures. So, you can actually be able to add the water molecules because remember when we were talking about the protein structure, we said that the protein is always been present in a hydration shell. So, you are actually going to add the water molecule. So, that you are actually going to mimic the real examples. And then you also going to add the charge and other kinds of procedures and that is how your target is actually going to prepared.

On the other hand, you are actually going to prepare the ligand molecules. So, you are going to select the ligands, right. So, you can actually when you want to select the ligands you can actually be able to select the ligands form the different types of libraries. You can actually be able to use from the phytochemical libraries, or you can actually be able to use the chemical libraries for example, you can actually be able to use the molecule from the ZINC data base or the phytochemical data base like that Dukes data base.

Once you have selected the ligands then ligand is also going to prepare. So, exactly just like the target preparations you are also going to prepare the target preparation, ligand preparation. So, in the ligand also you are going to add the charges you are actually going to add or you are also going to define the rotatable bonds and all that and so on because all these are you are going to do the flexible docking.

And then you are actually going to put it into the molecular docking and molecular what the molecular docking is going to do is it is actually going to predict the different poses of the ligand within the target proteins. And that is how it is actually going to give you the information about the binding affinity of each target or each pose, right.

Taking these into account you can be able to do the analysis you can do evaluate the docking results and you are actually going to see the binding confirmations and you are

actually be considering these binding confirmations you can be able to select the right pose and right confirmations.

So, to explain all these procedures we have prepared a small demo where we are actually going to use a software which is called as AutoDock and where the students are explaining how you can be able to do the target selections, how you can be able to prepare the target, how you are going to prepare ligand and all that, how you are going to prepare the grid parameter files, how you are going to prepare the docking parameter files, how you are going to analyze the results and so on.

So, in this demo the students are explaining you different steps of how you can be able to perform the docking with the AutoDock.

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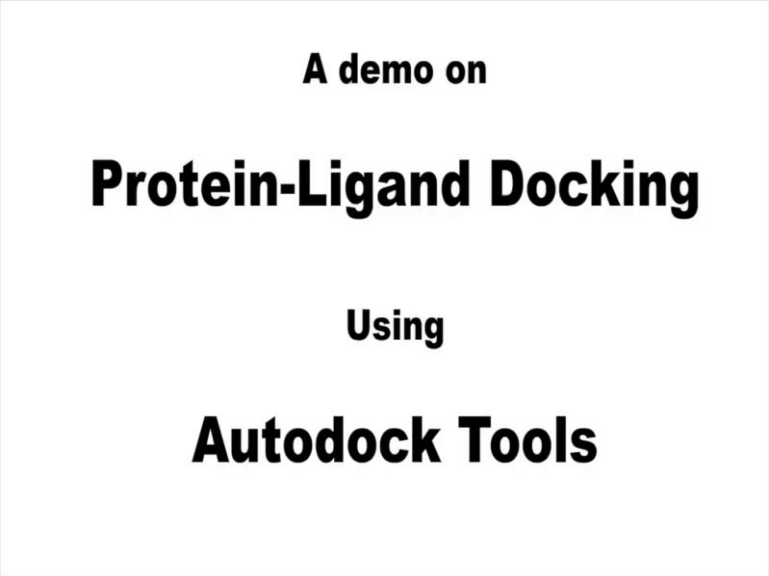


Welcome to the demo on protein ligand docking using AutoDock tools. So, in this demo I will be explaining the steps involved in docking of a ligand to a receptor using AutoDock tools. So, I will start with a flowchart of the steps involved, these are the 10 steps involved in docking.

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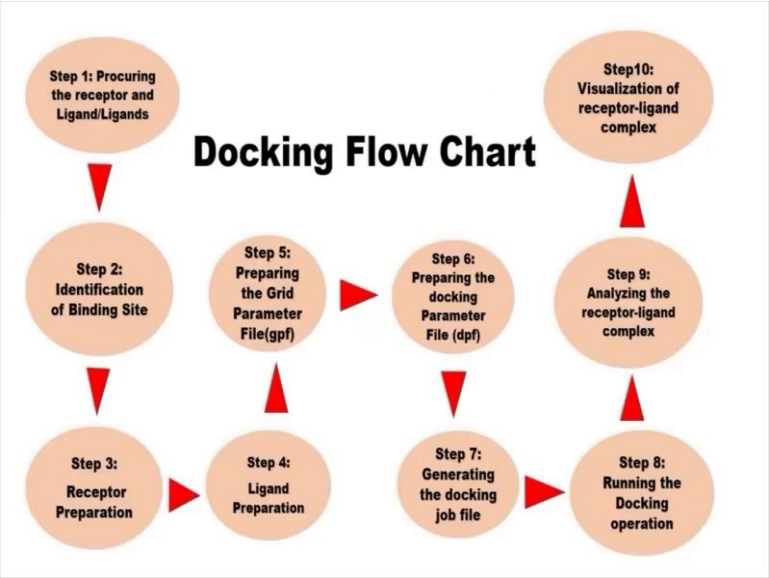


So, will I will be explaining each step in detail and parallely I will be performing each step on screen and explaining them.

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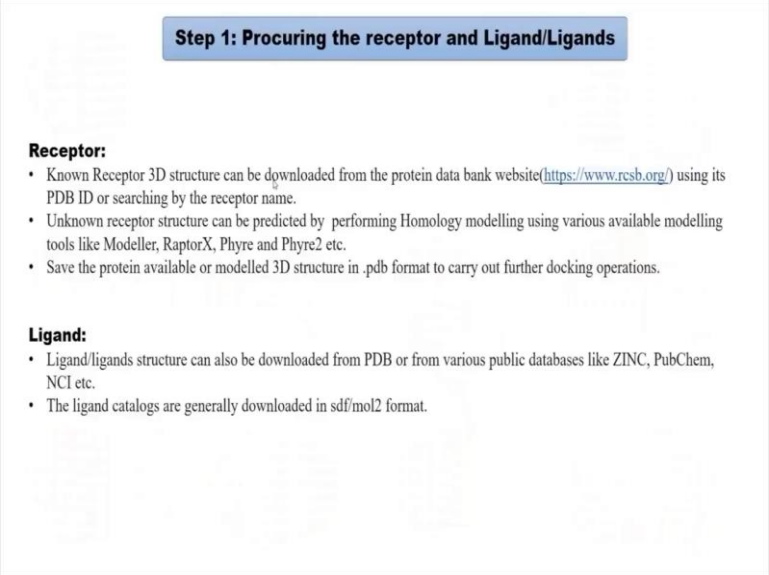


So, will start with the 1st step that is, procuring the receptor and the ligand: So, the receptor can be its PDB structure, 3D structure can be downloaded from the protein data bank site given here and it and the it should be downloaded in PDB format. And the second thing if the structure of the receptor is not known then we can predict it by

modelling the structure using various software's like Modeller, Raptor X or Phyre2 etcetera.

Now, For the ligand we can download the ligand from PDB as well as from various other public databases like ZINC database, SpiderCam, PubChem, NCI and various others are there. And we need to save the ligands in sdf or mol2 format. So, after procuring the receptor and ligand we will move to the next step.

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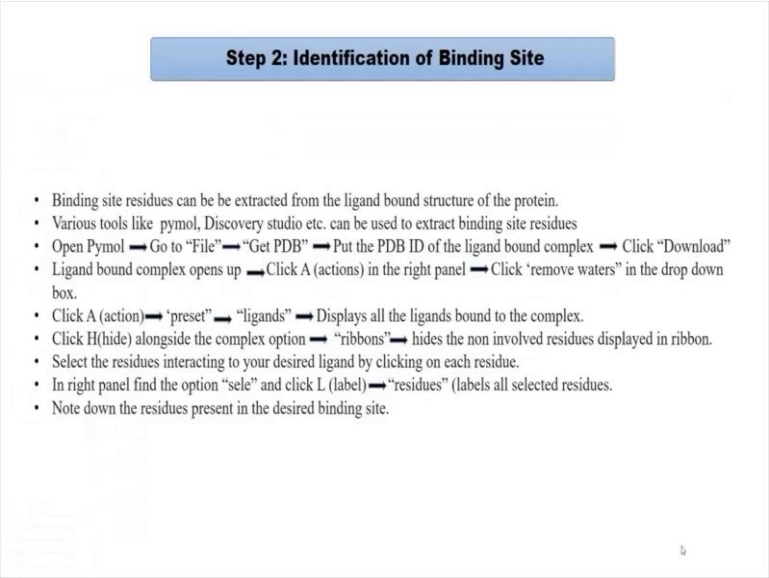


Where, we need to identify the binding site where we need, where we want our ligand to dock into the receptor. So, for that if the binding site is not known then again, we can use various software's like, (Refer Time: 14:52) For identification of the binding site in a protein structure and if the if there is a complex of the ligand with the receptor already available then we can extract the residues which are involved in the interaction. So, I will be showing how to extract those residues using PyMol. So, I will open PyMol here.

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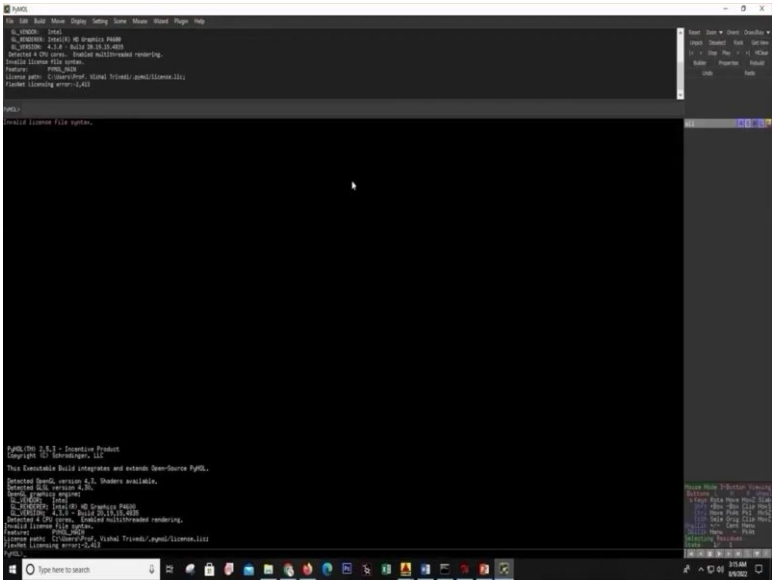

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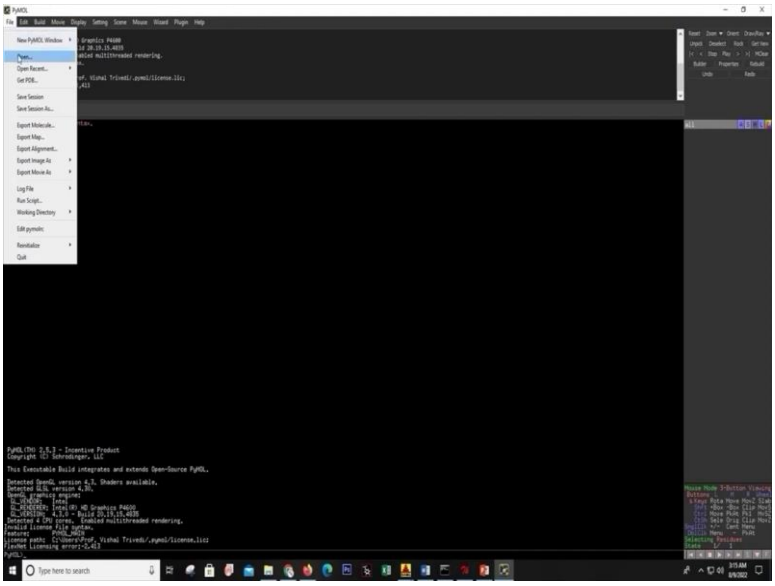

So, in PyMol we will go to file and here we will open the ligand bound structure, which I have already downloaded from PDB.

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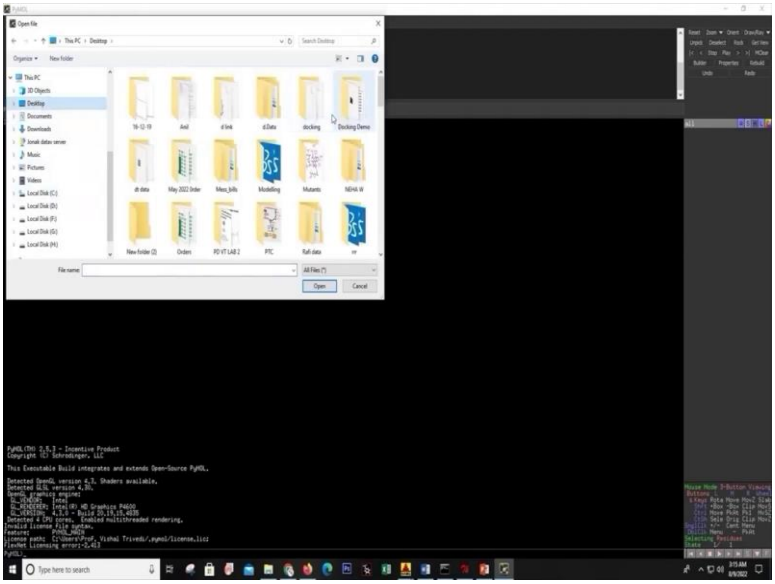

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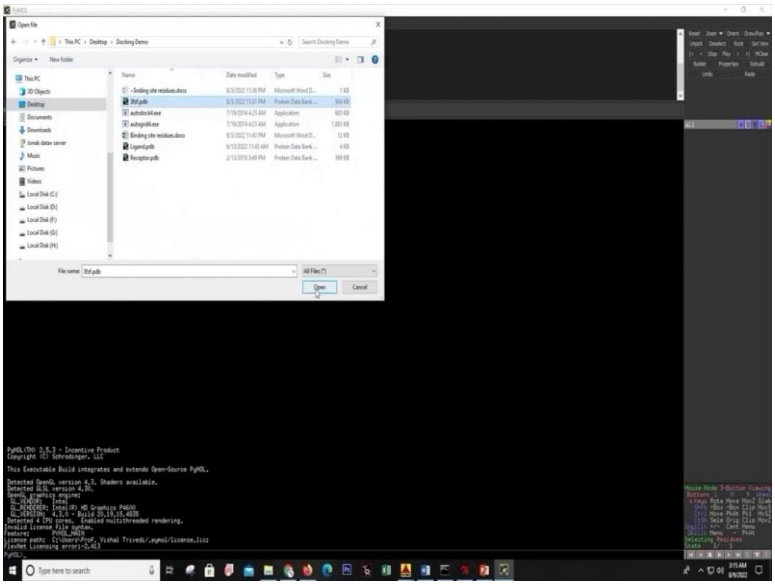

So, here I will be, this is the structure, which I have downloaded.

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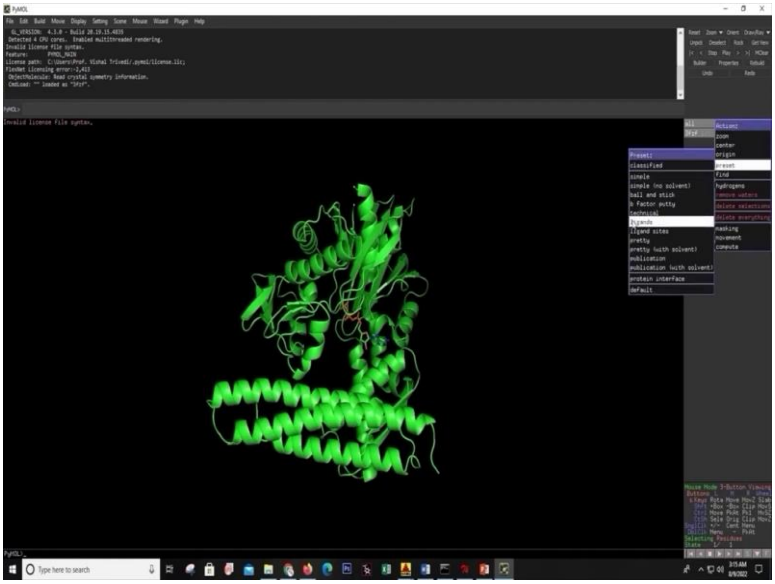

So, here we can see that the ligand is bound to this receptor here and now we have to, we need to know the residues, which are involved in the interaction. So, here in the right panel we will go on in front of all it is written as A where we will click A means action and here, we will go to Preset and then click on Ligands.

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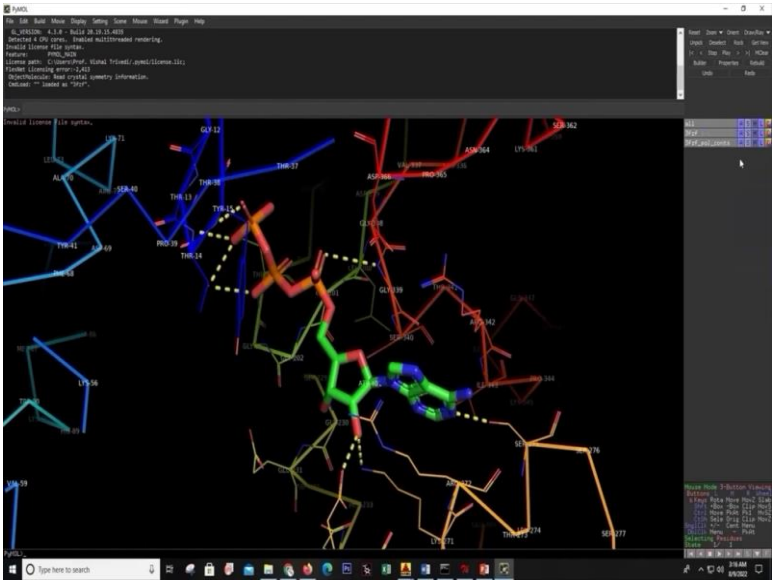

So, it will zoom up the ligand on the screen along with the interact along with the bonds and interactions. So, now we need to label the residues. So, that we can know what are the residues involved. So, here in front of this receptor name we will click on L; that

means, label and we will click on residues. So, it will label all the residues. Now, again we will go to All and here we will click on H; that means, hide and then click on ribbon.

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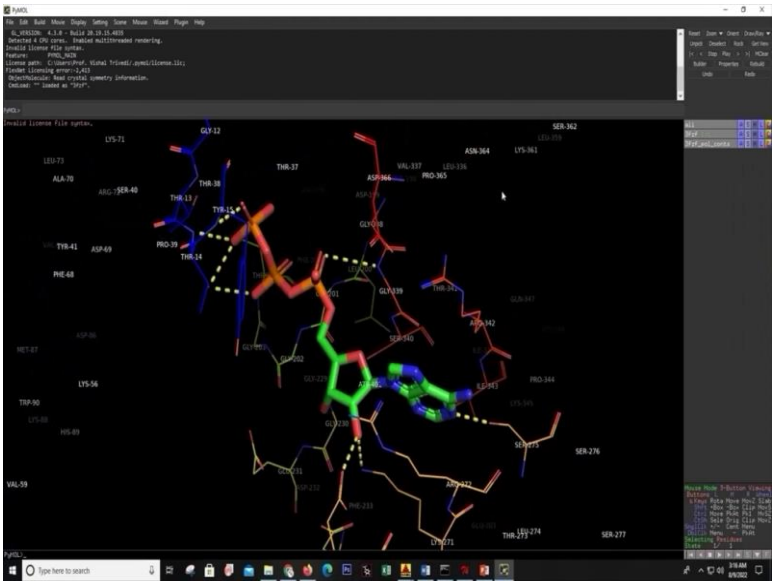

So, it will hide the residues which are not involved in the interaction. Now, we can just rotate this structure and note down all the residues, which are showing interactions. So, I have already noted down the residues, which are showing interaction.

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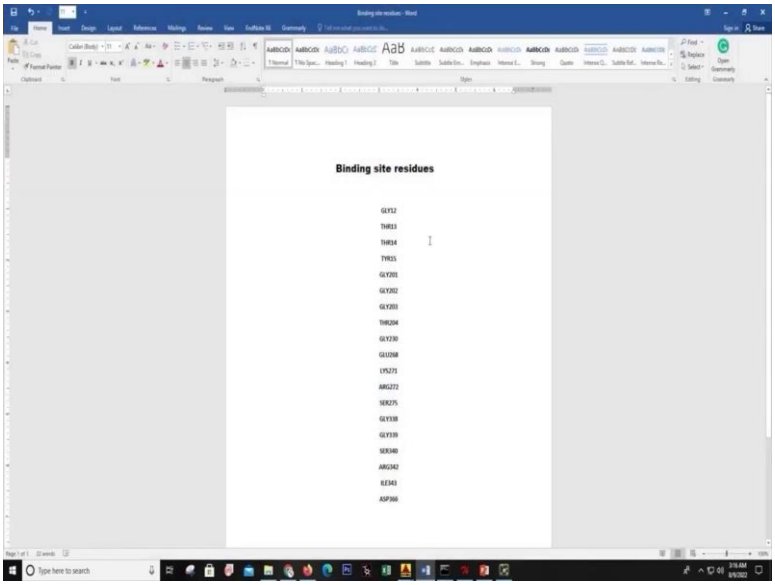


So, these are the residues involved in the interaction of this ligand to this receptor. So, now we will move to the next step, which is the preparation of the receptor for dock.

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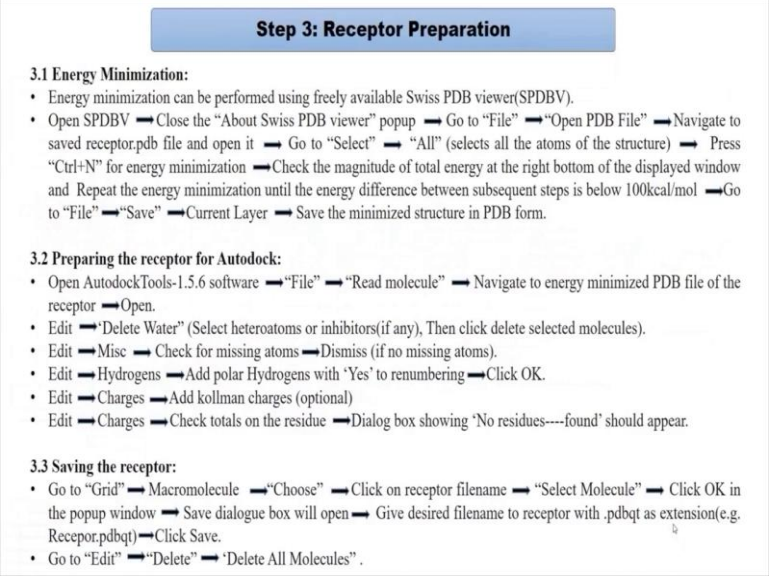


So, before going into AutoDock we need to minimize the energy of the receptor. So, that it shows better binding. It is in it is most stable state. So, now we can do the energy minimization of the receptor using Swiss Pdb-Viewer. So, I will open Swiss Pdb-Viewer here.

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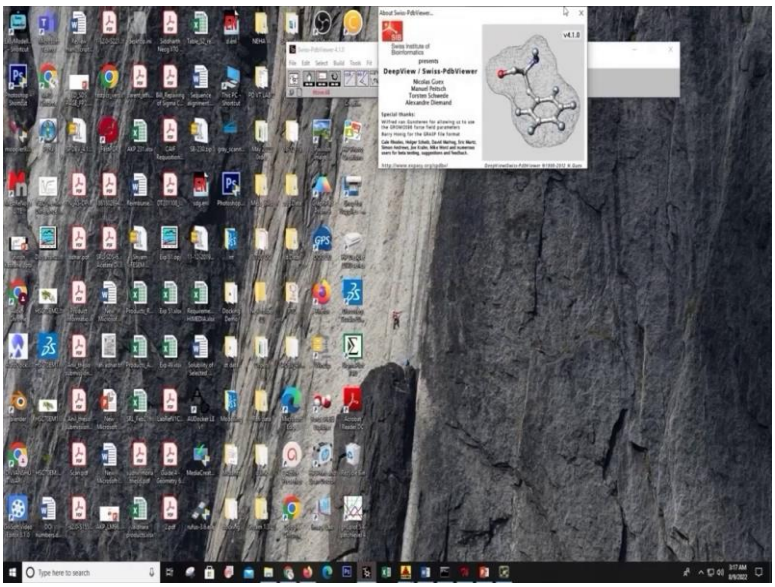

So, this is the Swiss Pdb-Viewer window.

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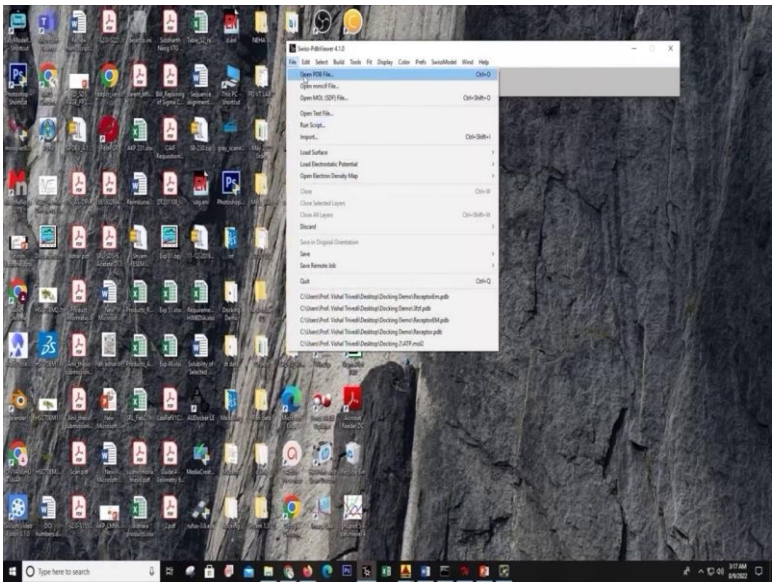

Here we will click on File and then Open PDB File.

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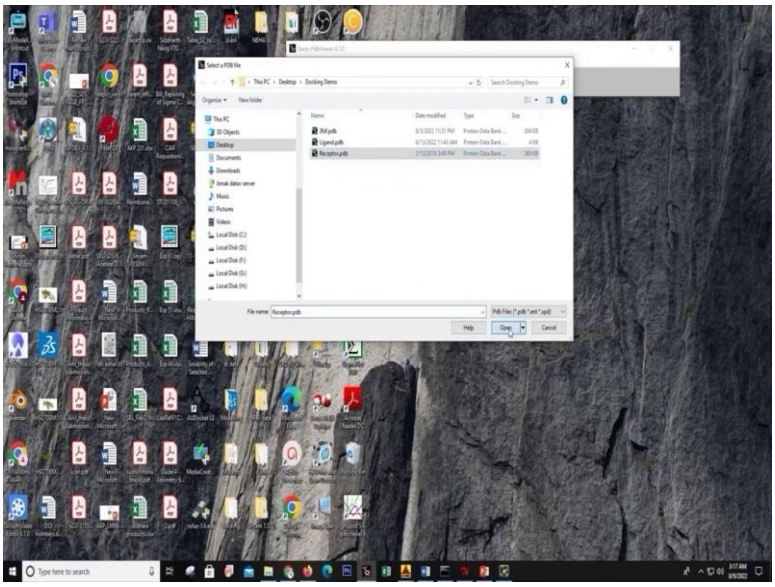

So, here this is our receptor. I will open this structure here in PDB well this.

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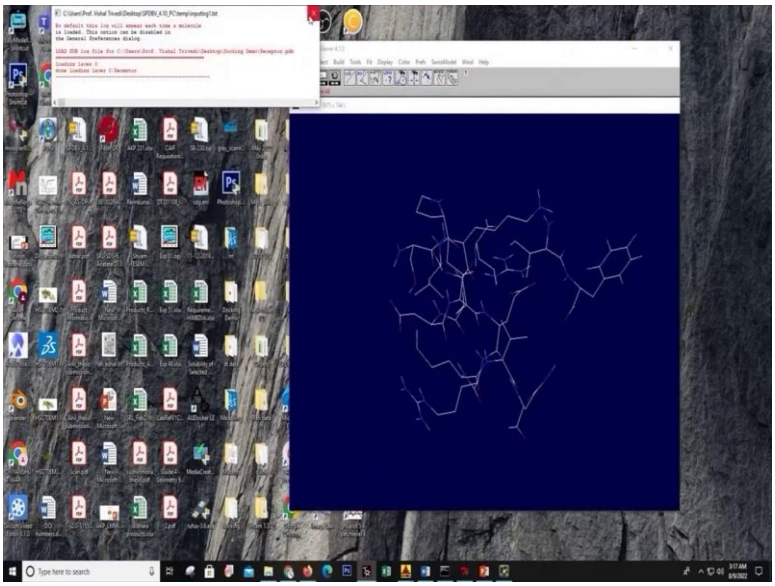

It will show a pop up. We can close this and then we can go on Select and then click on All.

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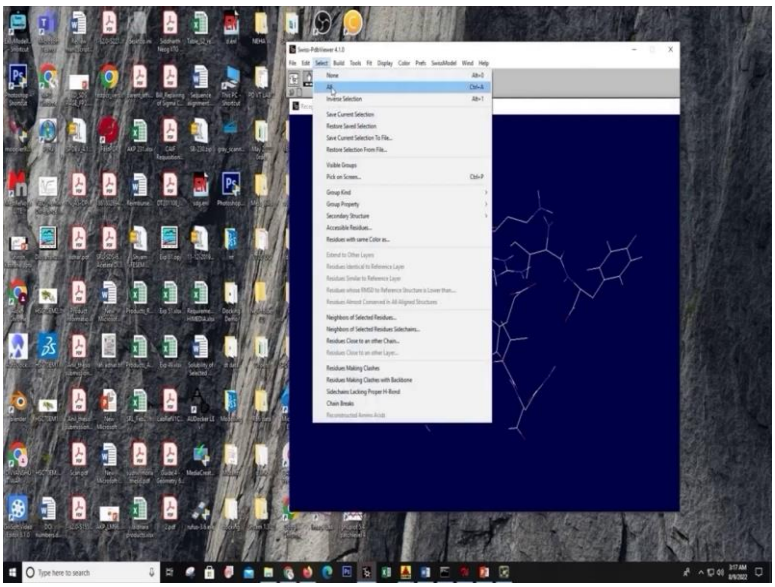

So, it will select the complete molecule and then we can just press Control N which will perform the energy minimization of this structure. So, after perform pressing Control N, here it will show the progress of the energy minimization process and then after the process is complete a pop up will come up showing the energy of this complete the global energy of this receptor molecule.

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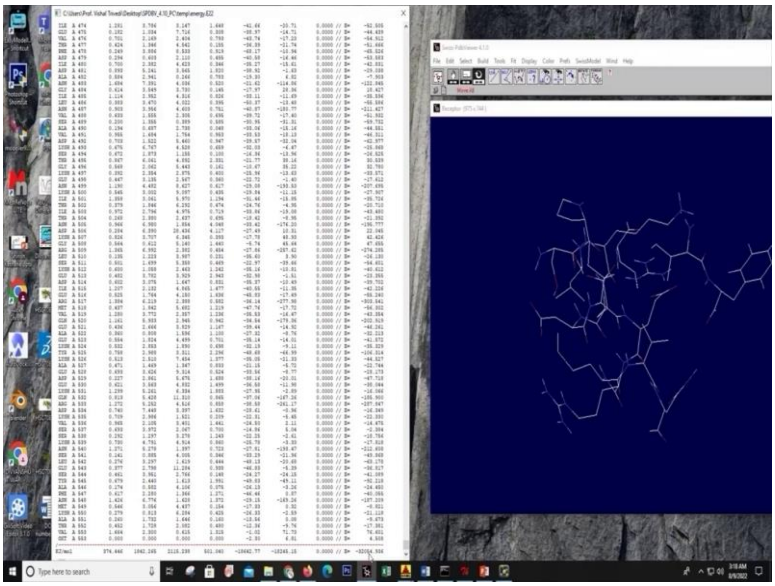

So, we will see the global energy of the receptor molecules here. So, here in this new window, which opened up at the right bottom it will show the energy of this receptor which is minus 32054. Now, we have to repeat this step. We have to press Control N again. So, that it is further minimizes structure.

So, I press Control N and again it is showing the progress and then we have to compare the energy of this new layer with the previous layer. And we have to keep doing this control repeatedly repetitively. We need to press Control N until the energy difference between the two consequent layers is less than 100.

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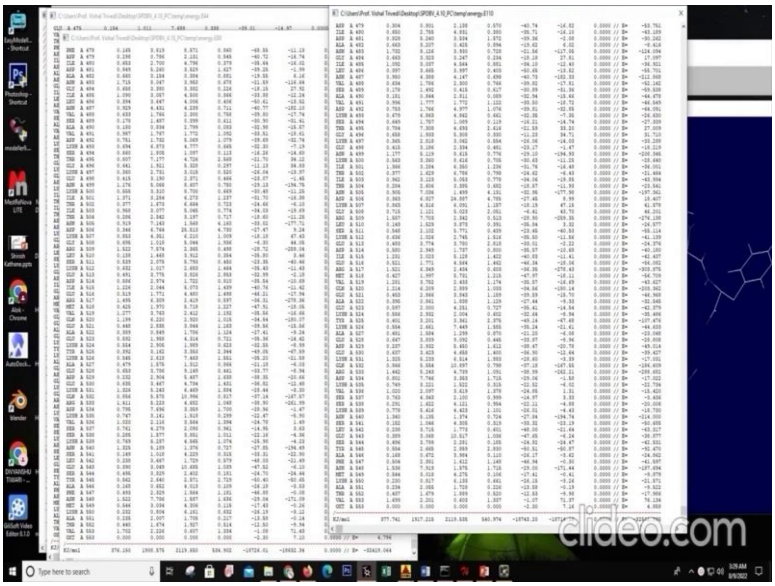

So, here the next; so, after performing several energy minimization steps now we can see the energy difference between this layer and the previous layer is less than 100. So, now we can just click on this Layer. And now we will click on File.

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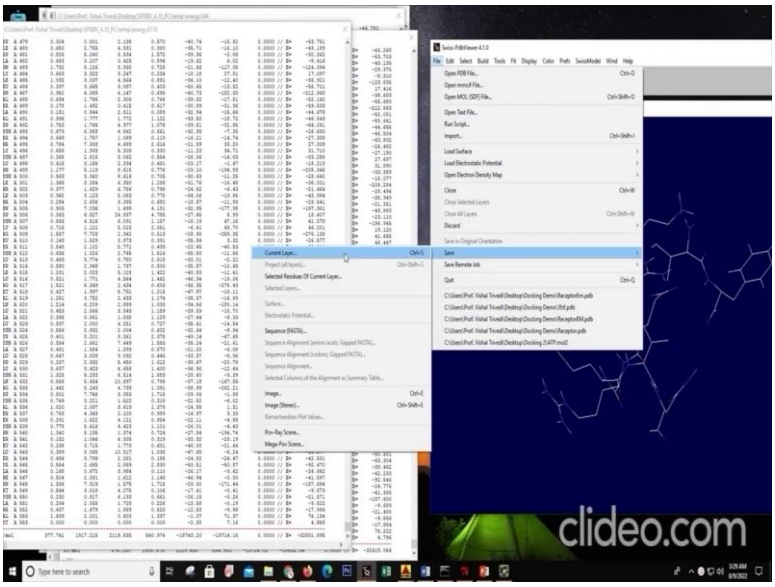

And then Save and then Current Layer.

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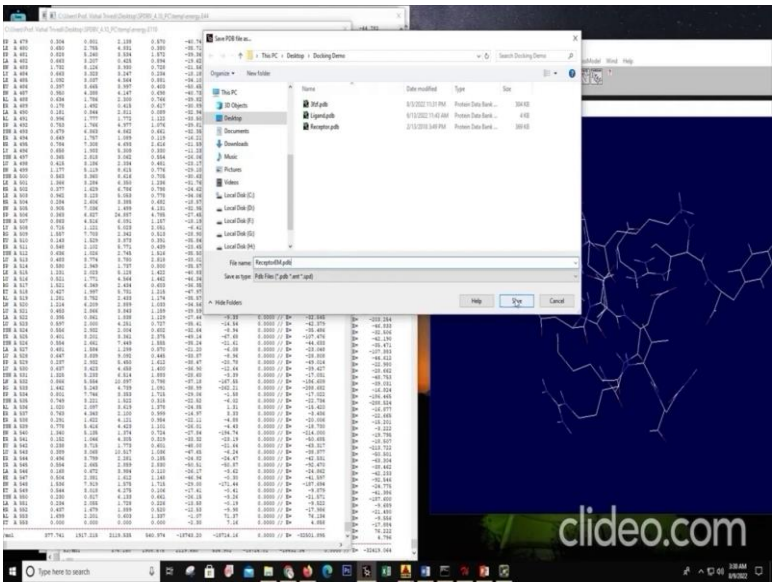


So, that it will save the most minimum energy layer of this receptor. Now, we can just give its name ReceptorEM; that means, energy minimized dot pdb. And I will Save this. Now, we can close this. Swiss Pdb-Viewer and after the energy minimization of the receptor we need to prepare this in AutoDock. So, we will open AutoDock now.

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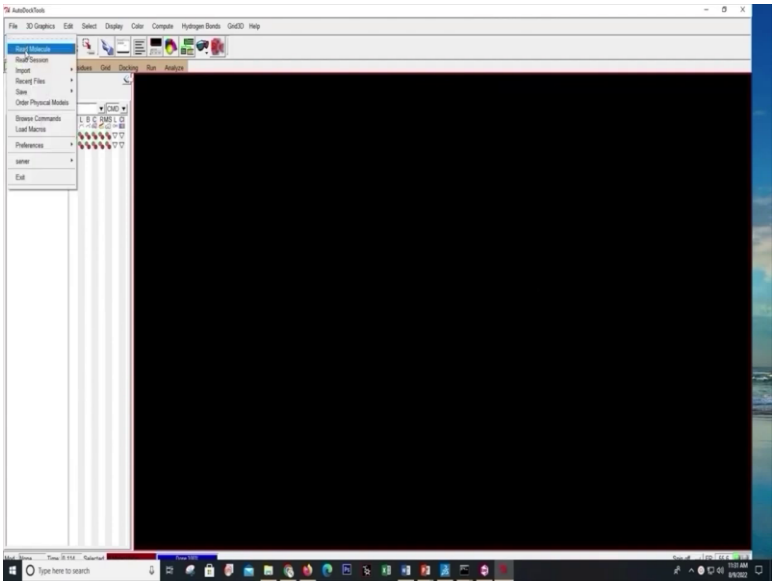
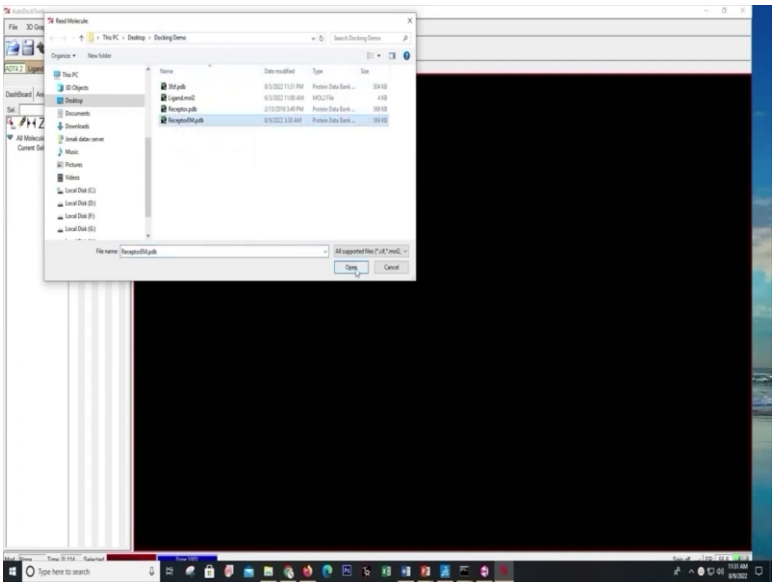


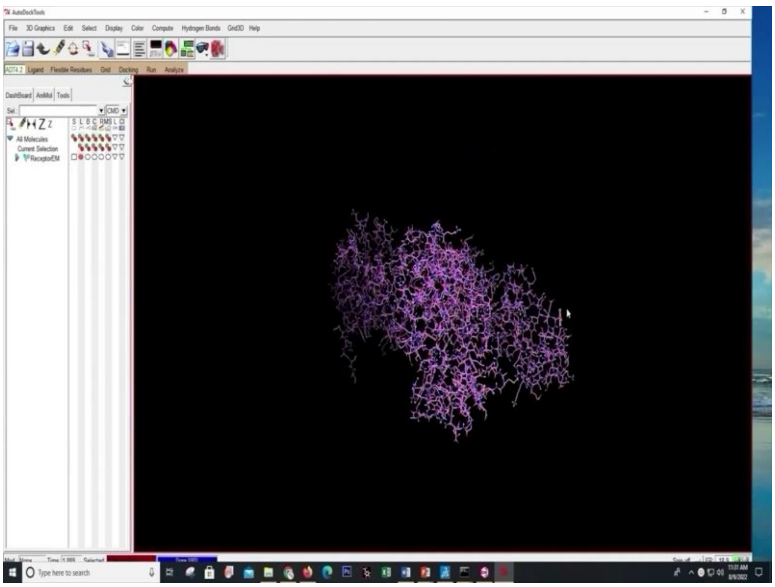
Now, we will open our receptor energy minimization receptor in AutoDock. We will go to File then Read Molecule.

(Refer Slide Time: 20:07)



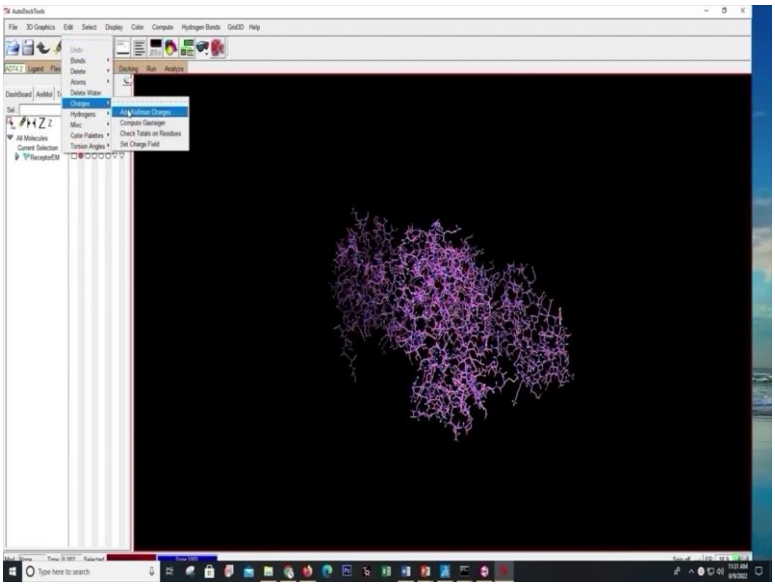
And then we will select the receptor, energy minimization receptor from the same folder. So, I will select this ReceptorEM dot pdb and open it.

(Refer Slide Time: 20:18)



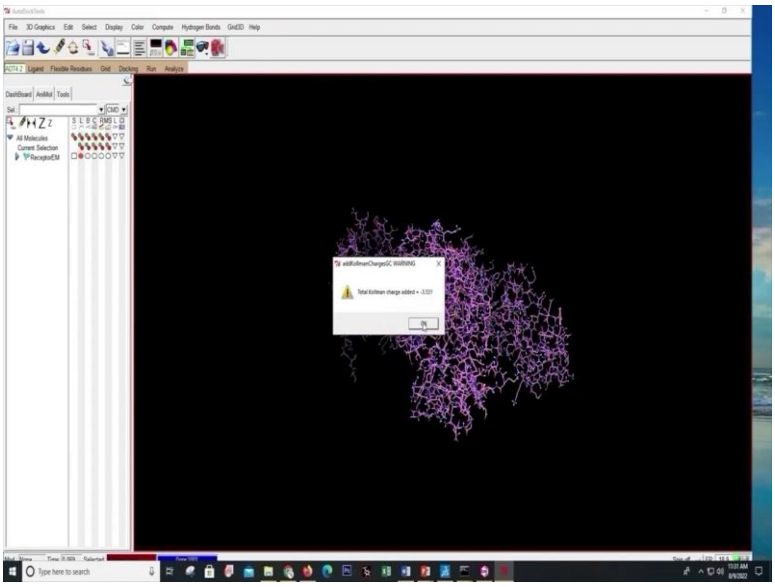
So, this will the receptor will be displayed on the AutoDock window and then after that we will go to Edit and then we will Delete Water. So, that all the water molecules are deleted and the it will not interfere in the interactions and then again we will go to Edit.

(Refer Slide Time: 20:36)



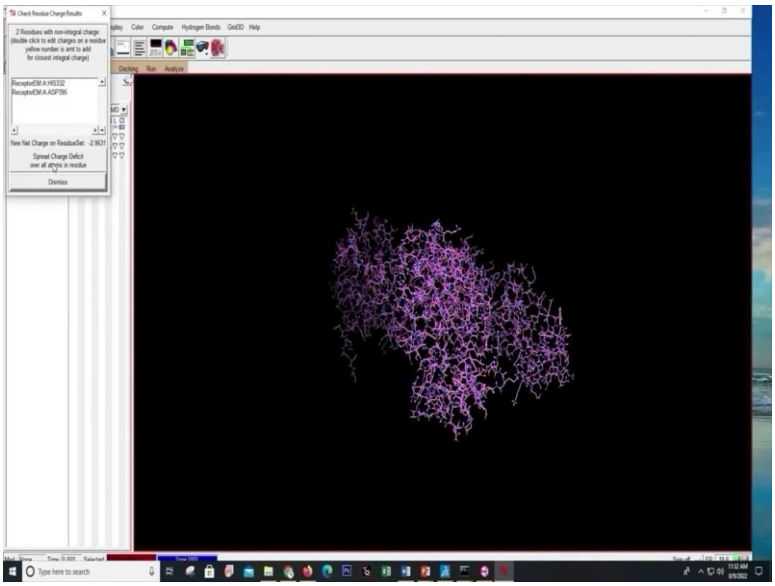
And then go to Charges and we will click on Add Kollman Charges.

(Refer Slide Time: 20:39)



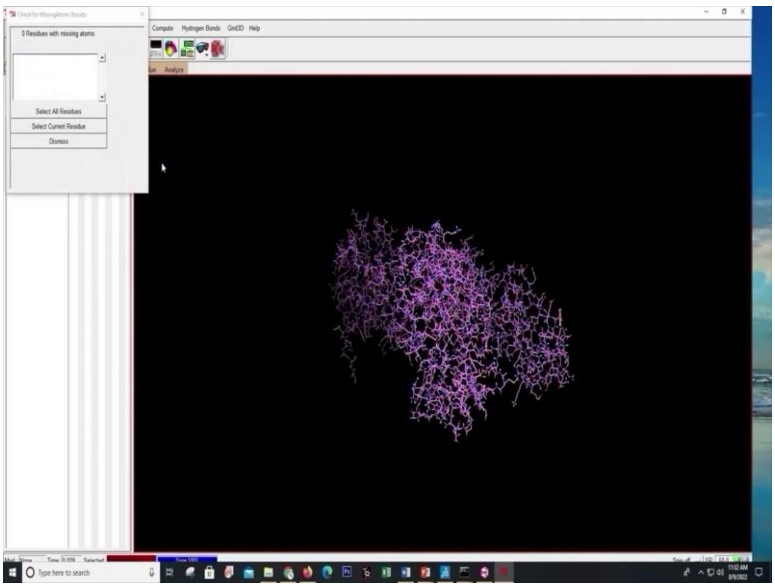
So, it will show how many Kollman charges have been added just we will click OK and after that we have to go on Edit and again, we have to go on Charges and then Check Totals on Residues.

(Refer Slide Time: 20:53)



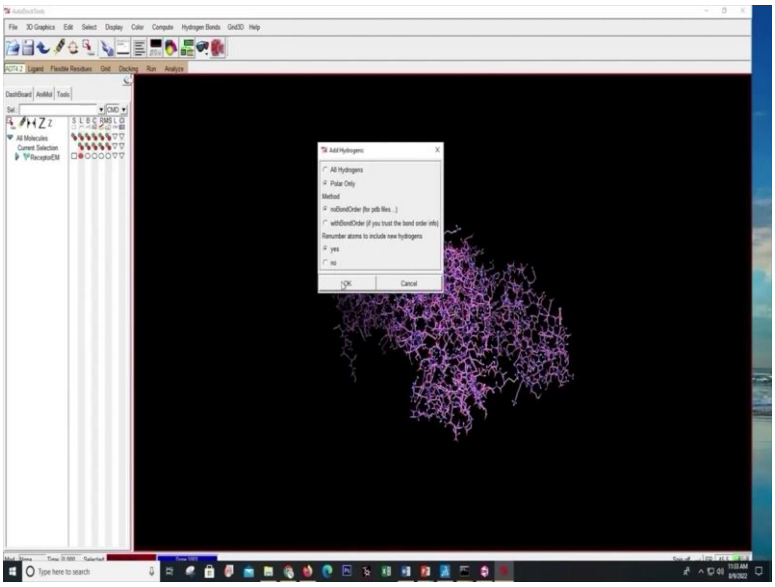
So, it is showing there is 2 Residues with non-integral charges. We just have to click on the Spread Charge Deficit over all atoms in residue. So, it will spread all the charges and neutralize it and then we will Dismiss it and we will again check the charge total charges on the residue Check Totals on Residue. So, it will it has is to it should show no residues with non-integral charges found. So, we will click OK and after that we will go again go to Edit and then MISC miscellaneous and there here we will click on Check for Missing Atoms.

(Refer Slide Time: 21:30)



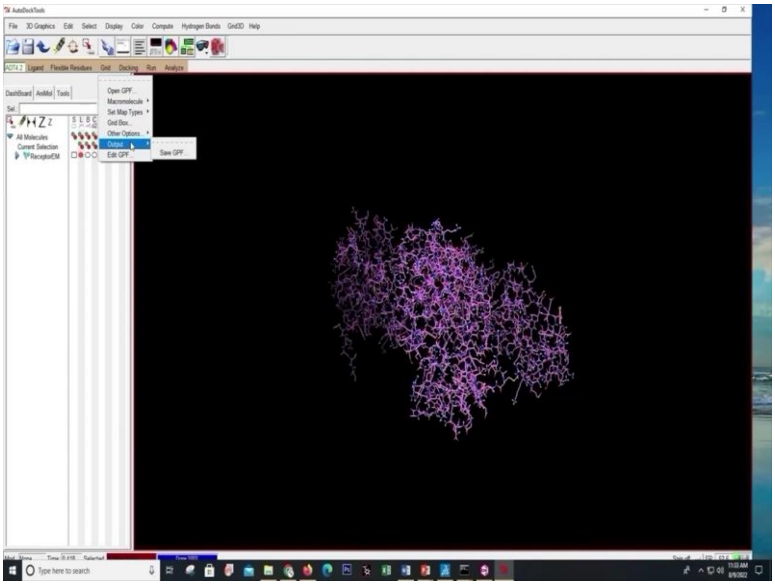
So, it should show 0 Residues with missing atoms. So, now we will click on Dismiss.

(Refer Slide Time: 21:36)



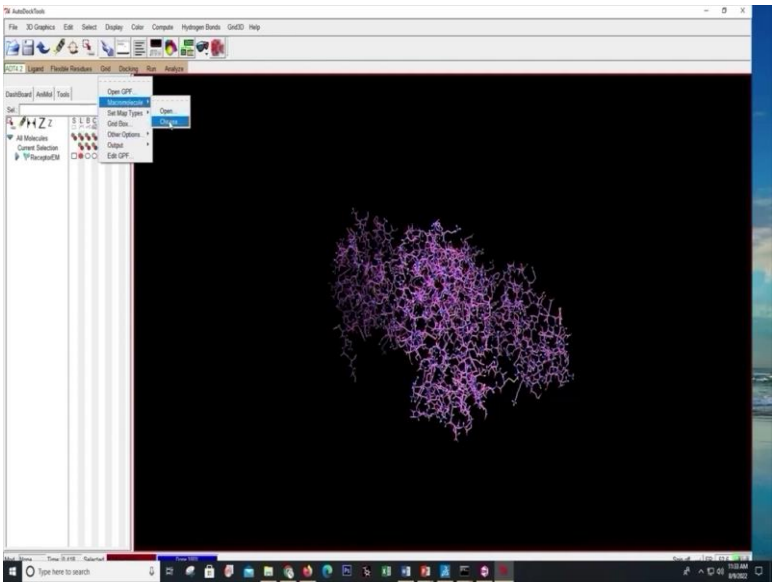
And after that we will again go to Hydrogens and then we will click on Add and here we have to select Polar Only and in the method, it will be no bond order and in the Renumber atoms to include new hydrogens we should we have to check Yes and then we will click Ok.

(Refer Slide Time: 22:04)

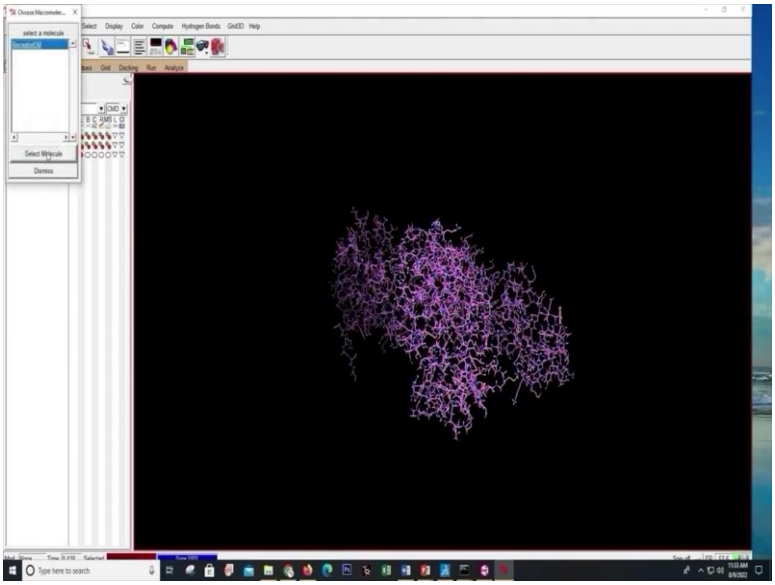


So, after that we will save our receptor in PDBQT format for that we need to go on Grid and then Macromolecules.

(Refer Slide Time: 22:15)

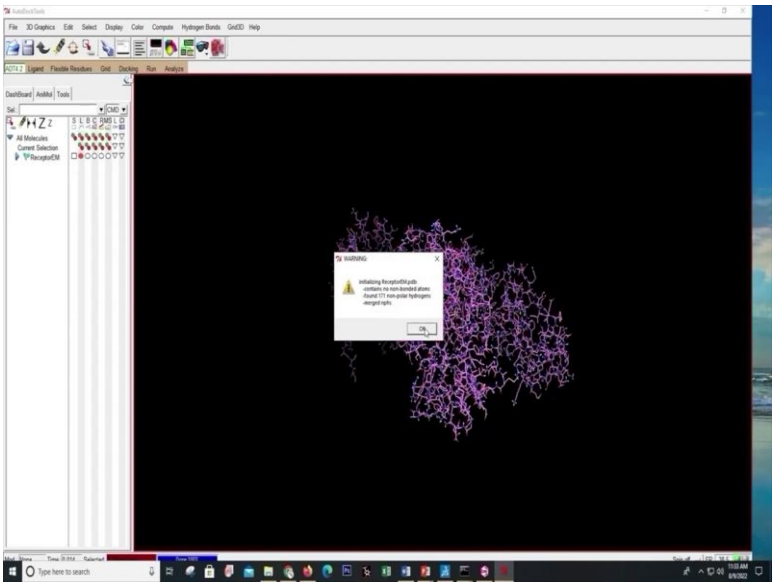


(Refer Slide Time: 22:18)



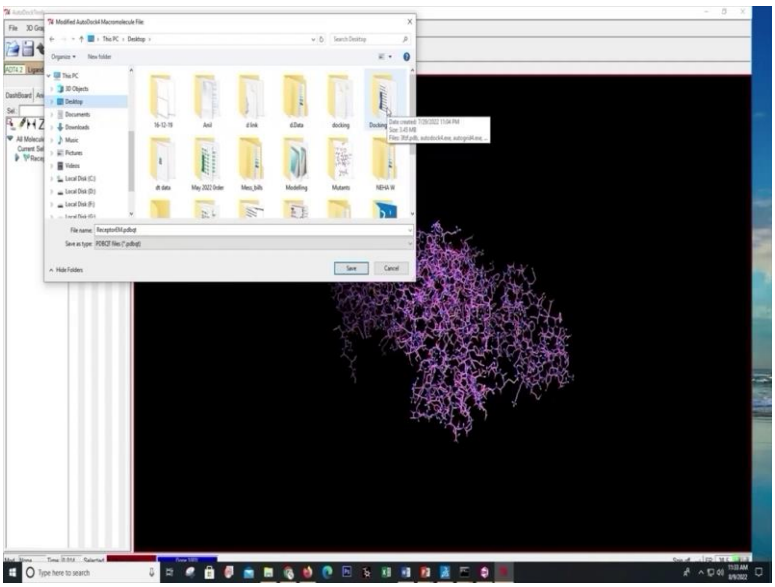
And then Choose and then we will choose this receptor.

(Refer Slide Time: 22:23)



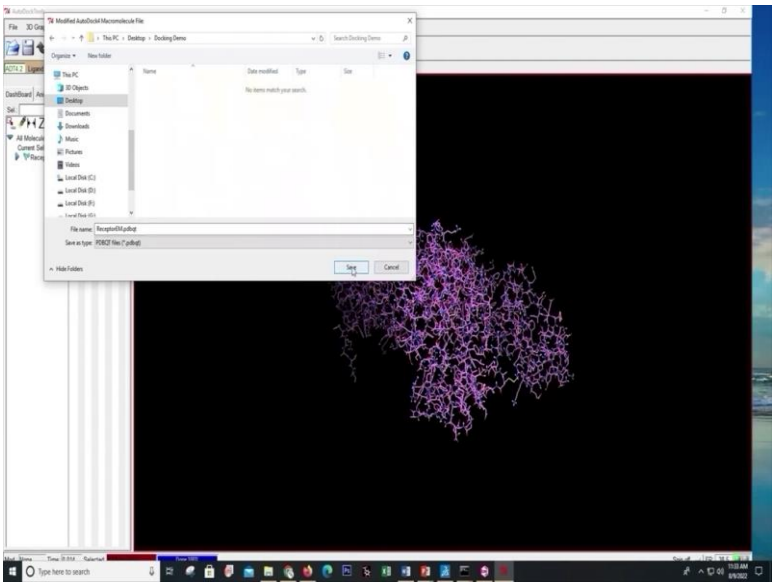
So, it will show the some pop up we have to just click Ok and then a save dialog box will open.

(Refer Slide Time: 22:29)



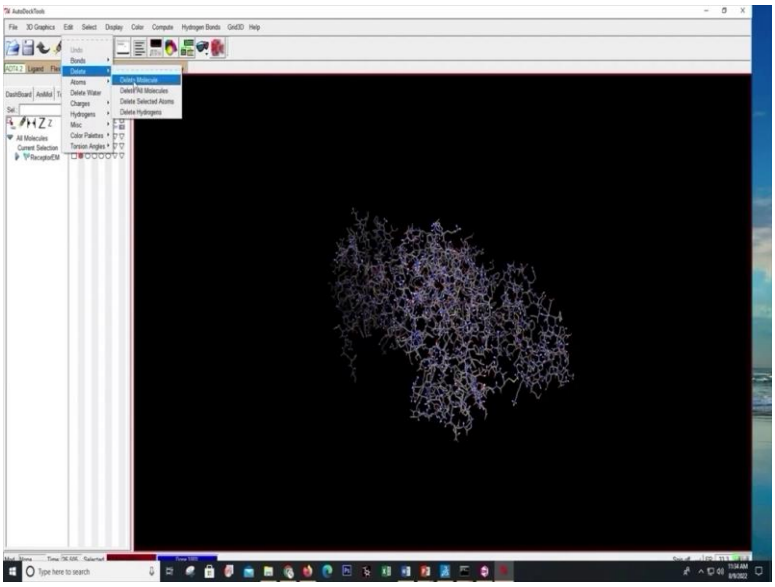
Then we have to go to the same folder and save this in save the receptor repaired receptor in pdbqt format.

(Refer Slide Time: 22:40)



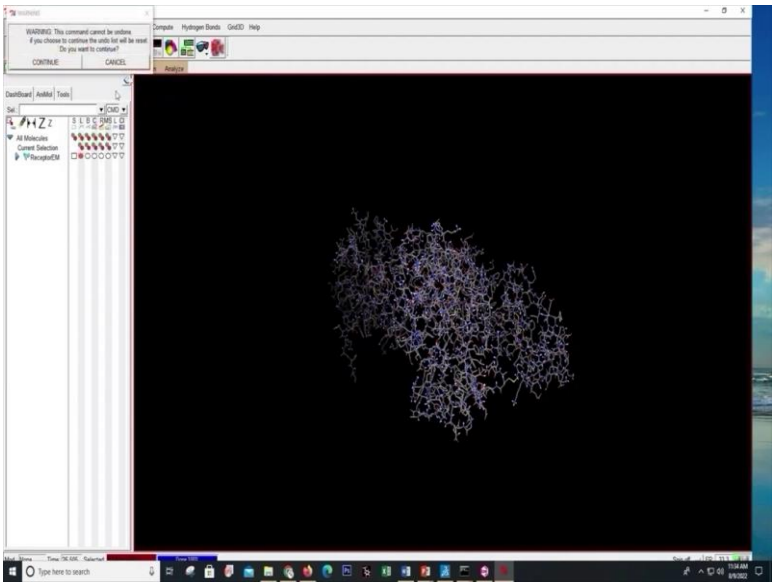
So, it is already written here ReceptorEM dot pdbqt we just have to click Save.

(Refer Slide Time: 22:50)



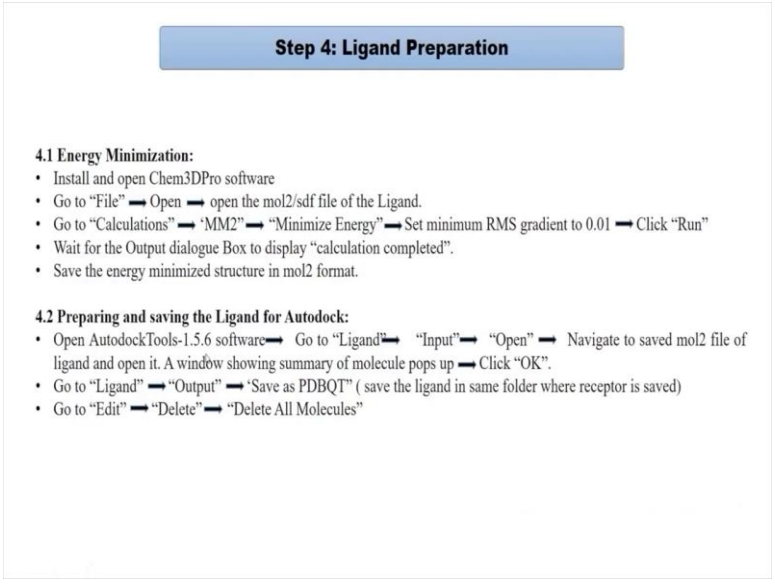
So, now our receptor is prepared we will go to Edit and then we will go to Delete and Delete All Molecules.

(Refer Slide Time: 22:54)



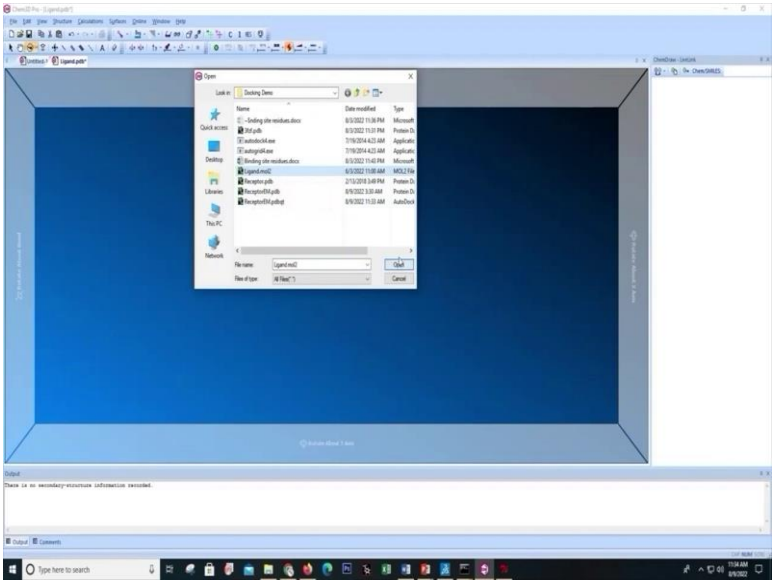
Now, we can minimize the AutoDock window.

(Refer Slide Time: 23:07)



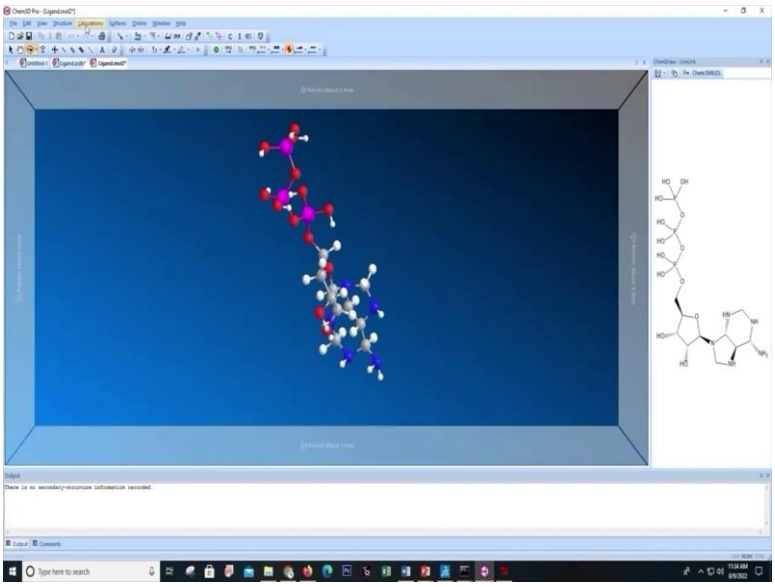

Now, the next step is preparation of the ligand. So, this is the next step again here we have to first minimize the energy of the ligand and then prepare it in AutoDock and convert it in PDBQT format. So, for energy minimization of the ligand we will be using Chem3D Pro.

(Refer Slide Time: 23:25)



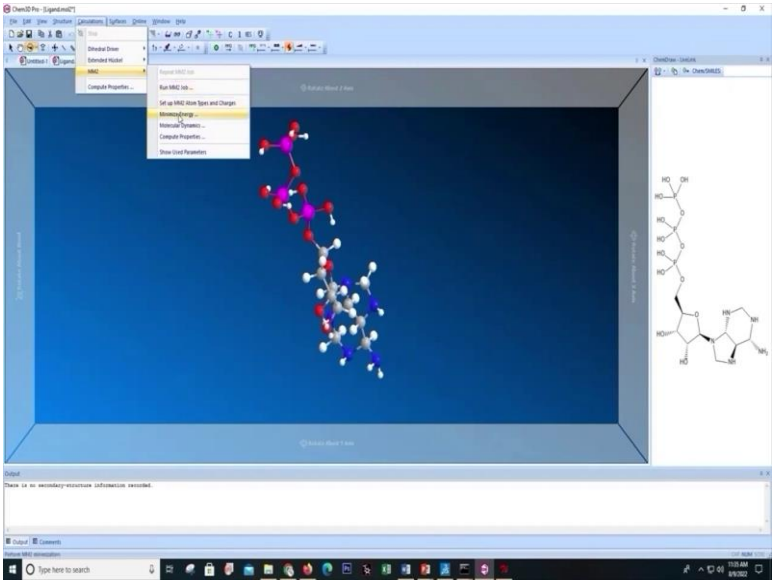
So, here I have opened Chem3D Pro this is Chem3D Pro window here we will go to File and then Open in the same folder the same folder is open here we will select the Ligand dot mol2 file here.

(Refer Slide Time: 23:41)



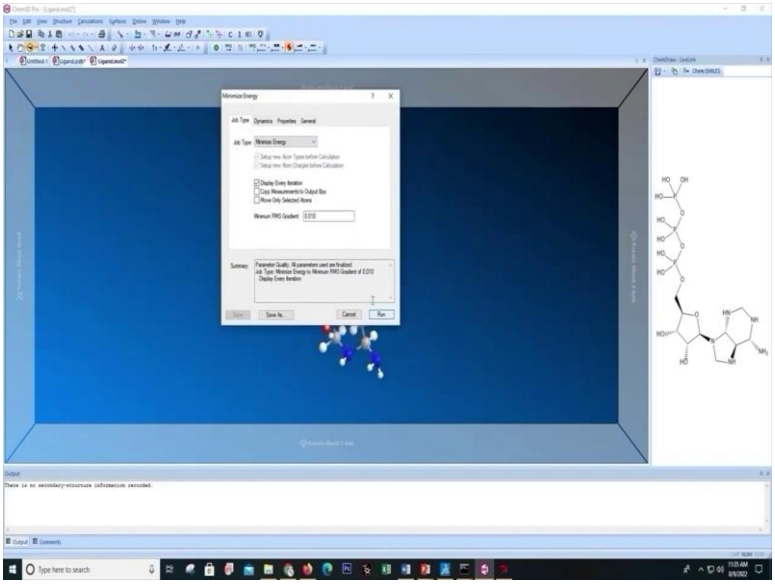
So, this will open up the ligand here you can see the ligand it displays on the screen after that you have to go to Calculations.

(Refer Slide Time: 23:46)



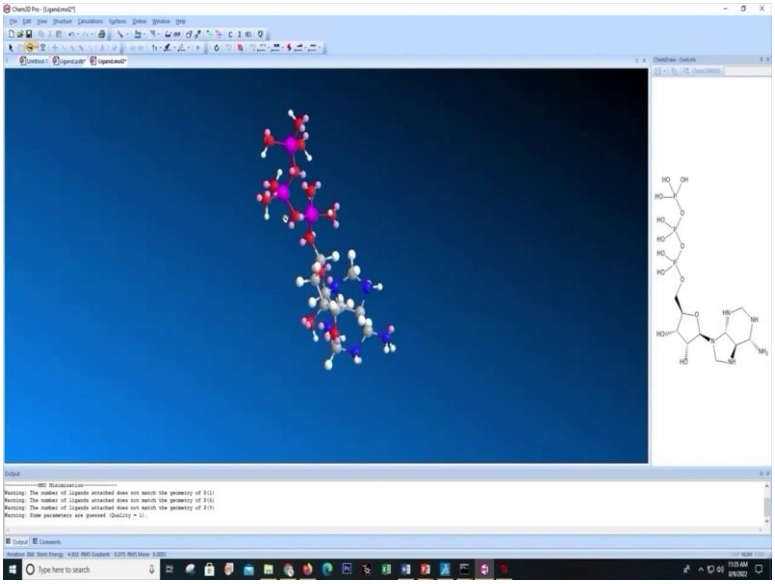
Then MM2 and then we have to click MinimizeEnergy.

(Refer Slide Time: 23:53)



So, pop up will open up we have to just click run with these default parameters. So, the energy minimization process will start.

(Refer Slide Time: 23:59)

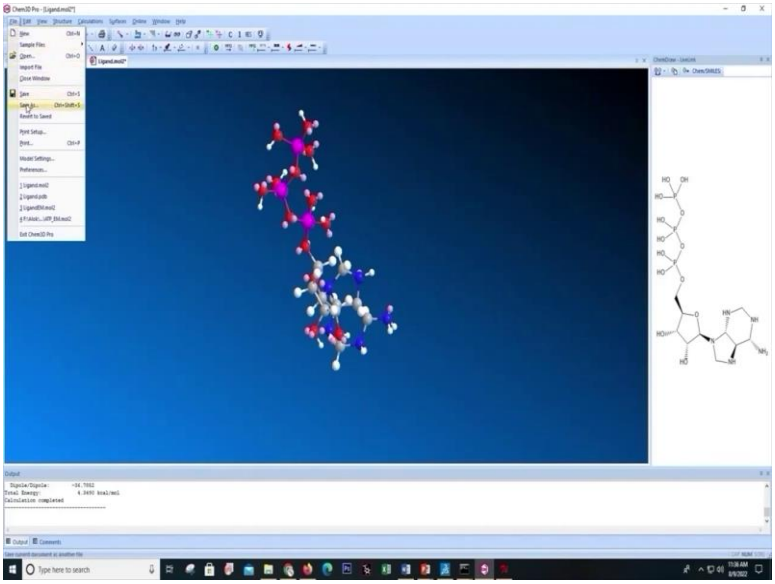


We can see the atoms and bonds moving in space which shows that the energy minimization step is in progress. So, after the energy minimization is done here in the lower panel it will show calculation completed alongside it will show the energy of the ligand the minimized energy of the ligand.

So, it will take few seconds 10 to 12 seconds to energy minimize that is depends on the size of the molecule as well. So, we have to wait for few seconds and then we can save the energy minimized structure of the ligand as well. So, still the energy minimization step is running.

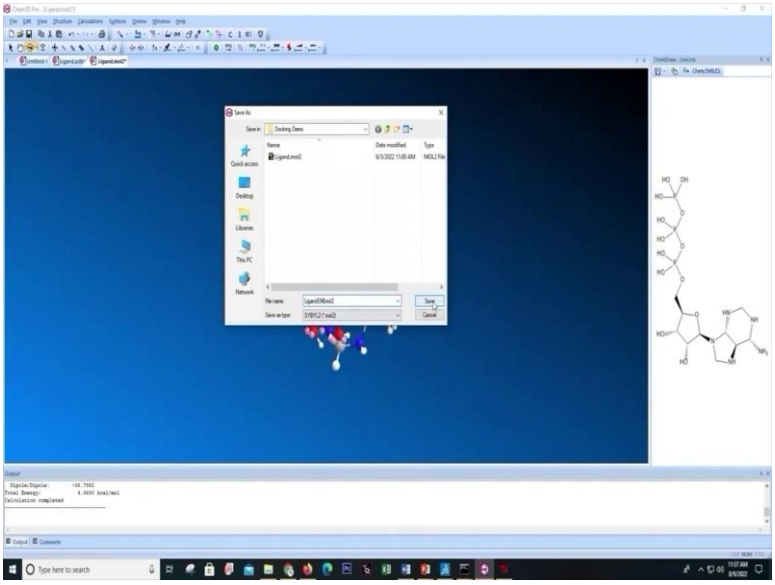
So, for energy minimization of small molecules Chem Pro Chem3D Pro is better and for bigger molecules like a protein molecule that is Swiss Pdb-Viewer which I showed earlier is considered better. Now, you can see that still the energy minimization is running and the atoms and bonds are getting arranged in their minimum energy conformation. Ok, now we can see here in the lower panel it is showing calculation completed along with the energy of the ligand.

(Refer Slide Time: 25:35)



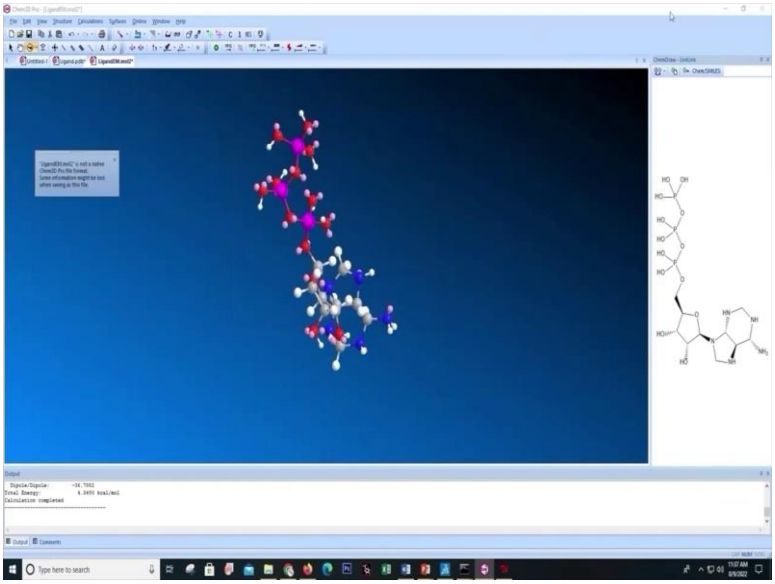
So, now we will save it will go to File will go to Save as.

(Refer Slide Time: 25:39)



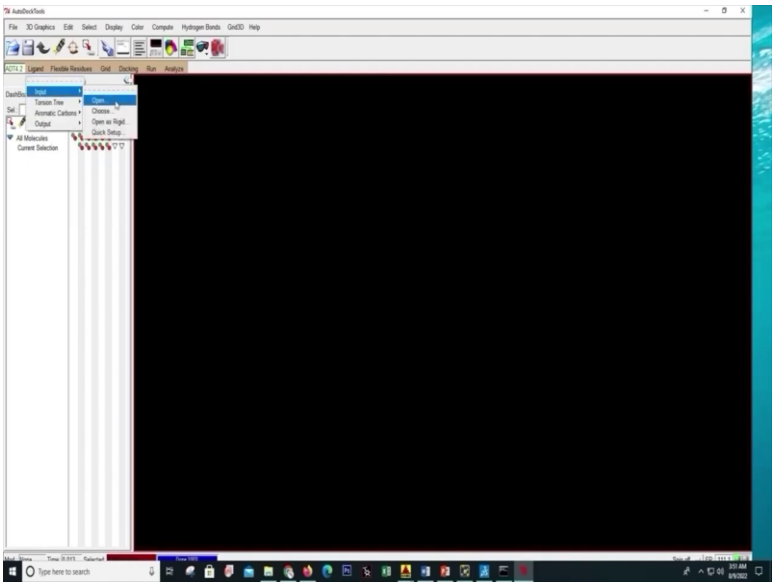
And here we will just change the name of the ligand. So, that you can have a different file I will write LigandEM dot mol2 and then Save it.

(Refer Slide Time: 25:51)



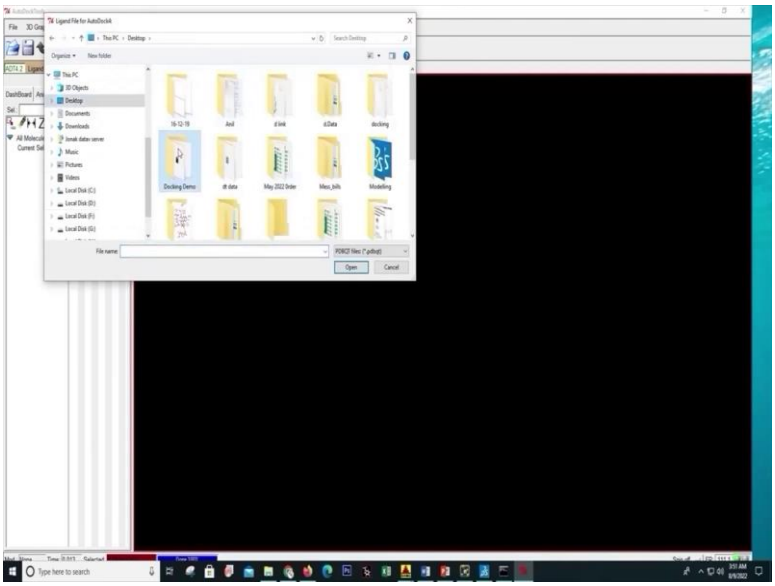
So, the energy minimization of the ligand is done after that we have to prepare the ligand in the we have to prepare ligand in AutoDock tools. So, for this again we will open AutoDock.

(Refer Slide Time: 26:04)



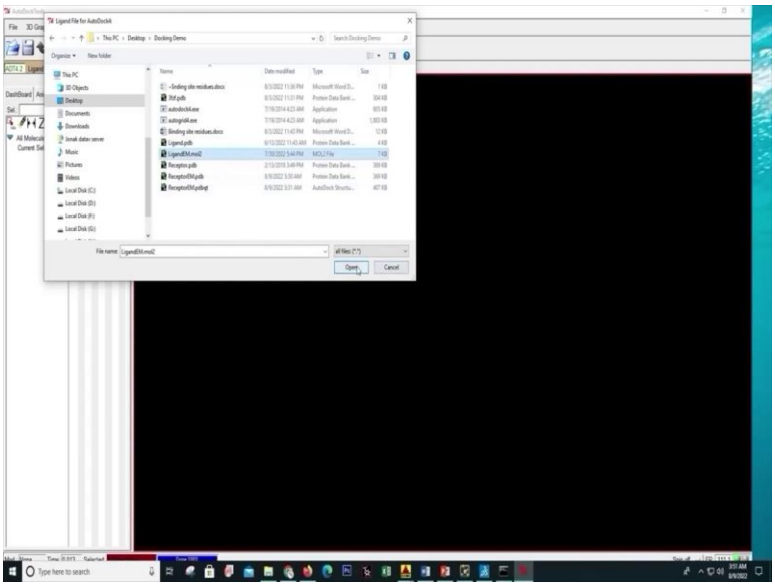
And here any AutoDock will go on Ligand and then Input and then click on Open.

(Refer Slide Time: 26:11)



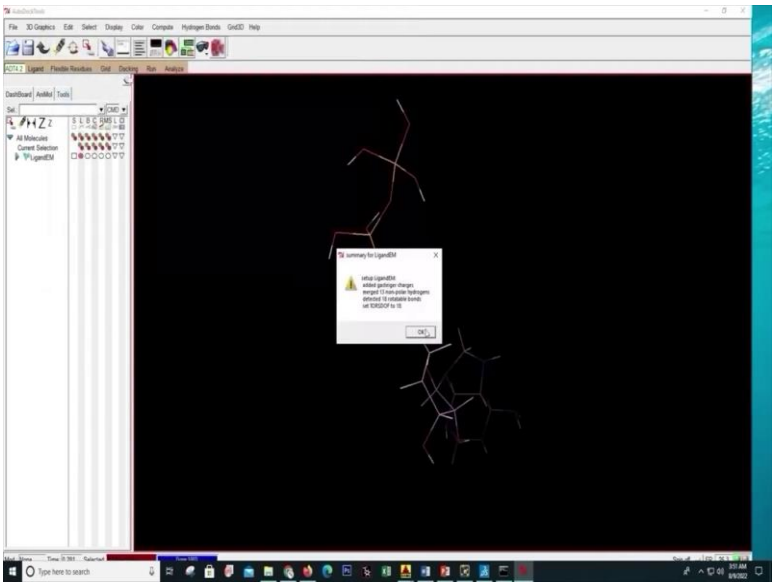
Then we will navigate to the folder where we saved our energy minimized ligand.

(Refer Slide Time: 26:16)



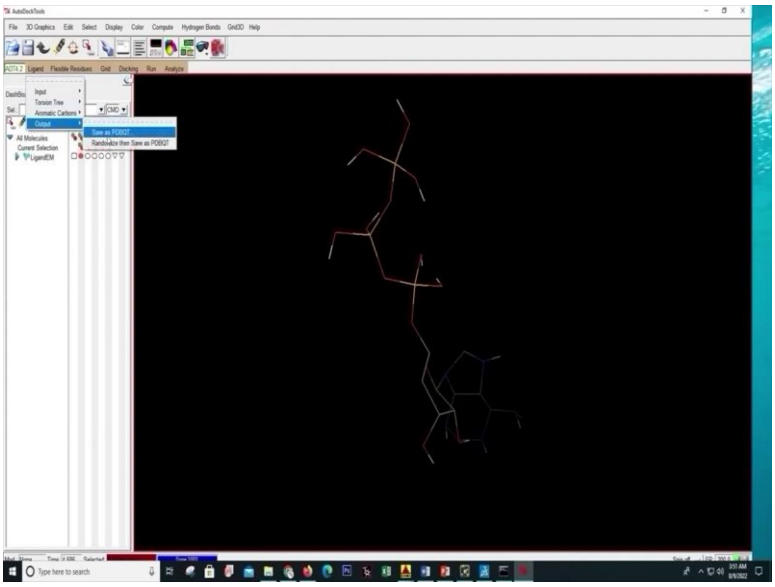
And open it in AutoDock. So, this is our energy minimized ligand in mol2 format.

(Refer Slide Time: 26:23)



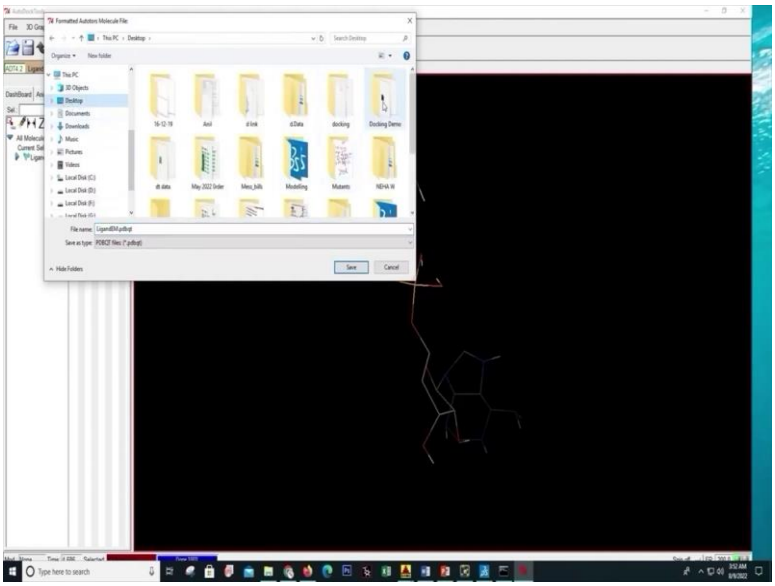
If I will open it in AutoDock it will show the ligand summary will click Ok.

(Refer Slide Time: 26:29)



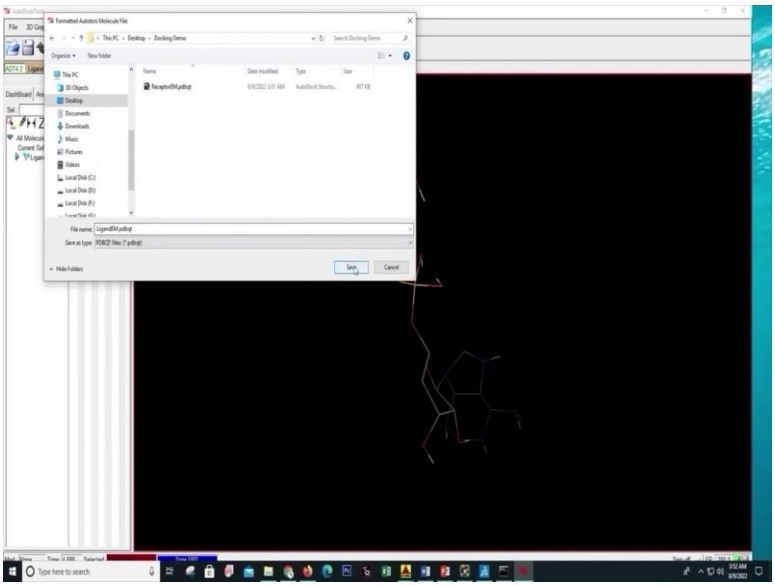
And after that we will go to Ligand again and then go to Output in Output will click on Save as PDBQT.

(Refer Slide Time: 26:36)



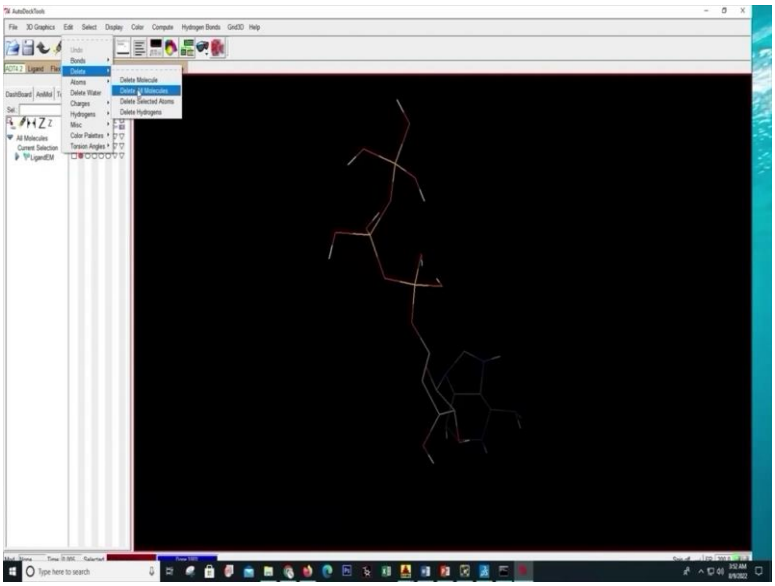
And now we will save this ligand in the same folder in PDBQT format.

(Refer Slide Time: 26:41)



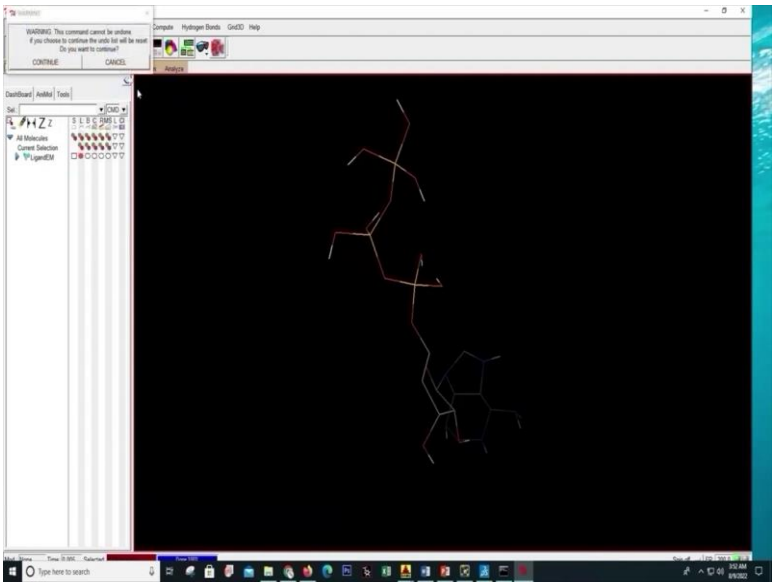
So, it is already written as LigandEM dot pdbqt will just Save it.

(Refer Slide Time: 27:49)



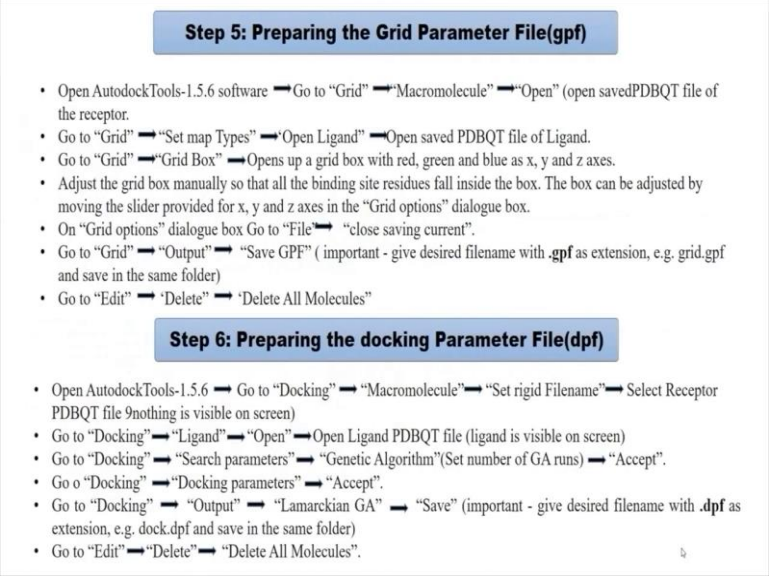
Now, again we will go to Edit Delete and then Delete or All Molecules.

(Refer Slide Time: 26:53)



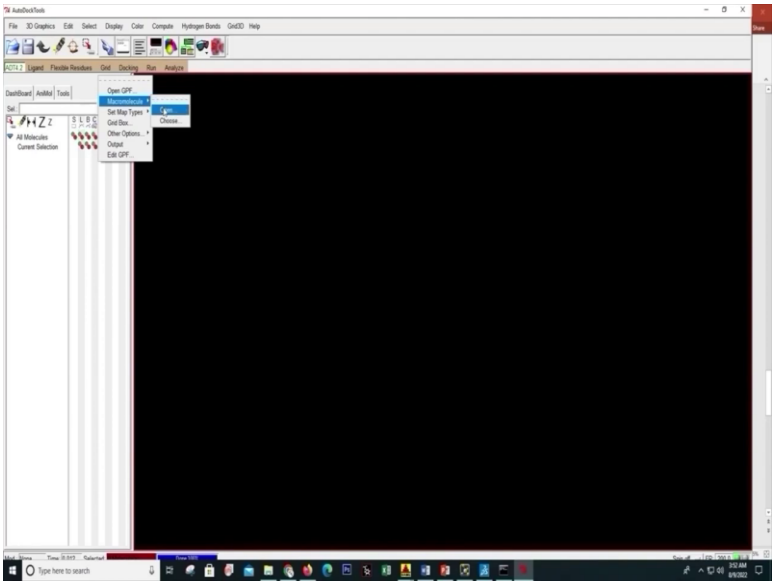
Now, the ligand preparation is also done.

(Refer Slide Time: 27:04)



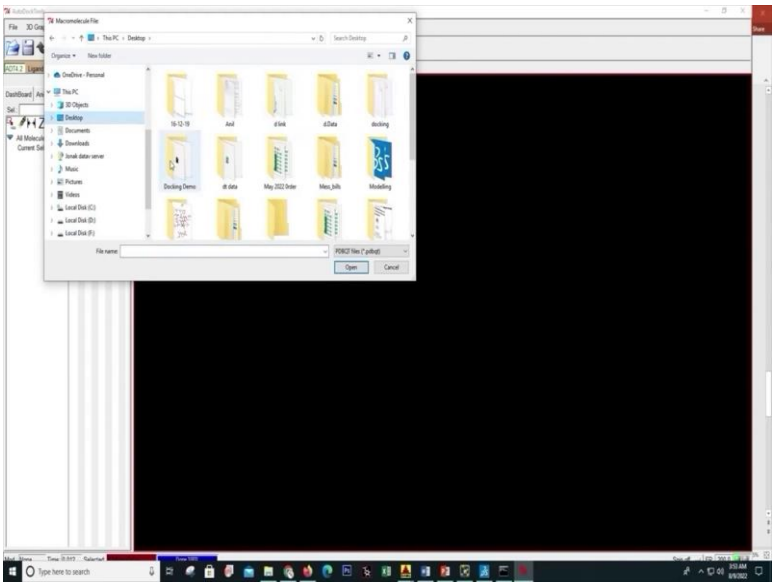

Now, we will move to our next step that is the preparing the grid parameter files. It contains the information about the coordinates of the grid and that grid will define where we need to dock or ligand. So, we will put our grid at the site which we have extracted from the PyMol. So, again for this we will go to in the AutoDock, we will open AutoDock here.

(Refer Slide Time: 27:27)



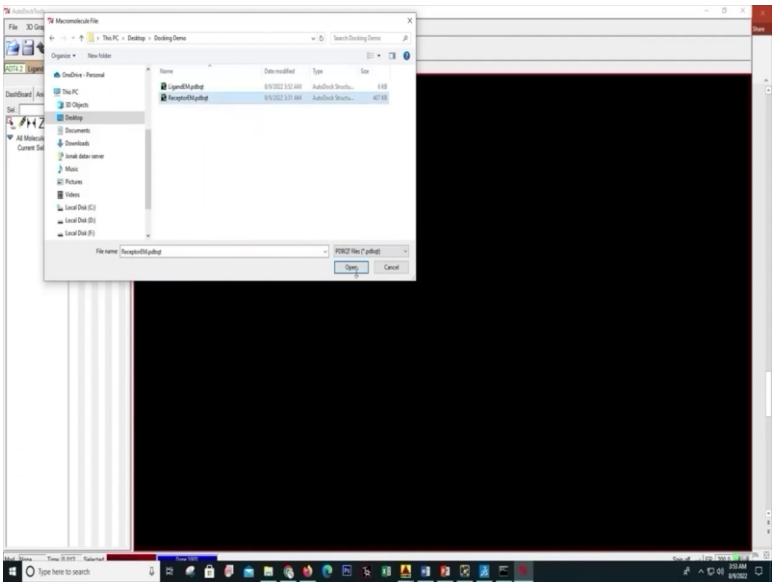
And now we will go to Grid and in grid we will go to Macromolecule and then Open.

(Refer Slide Time: 27:35)



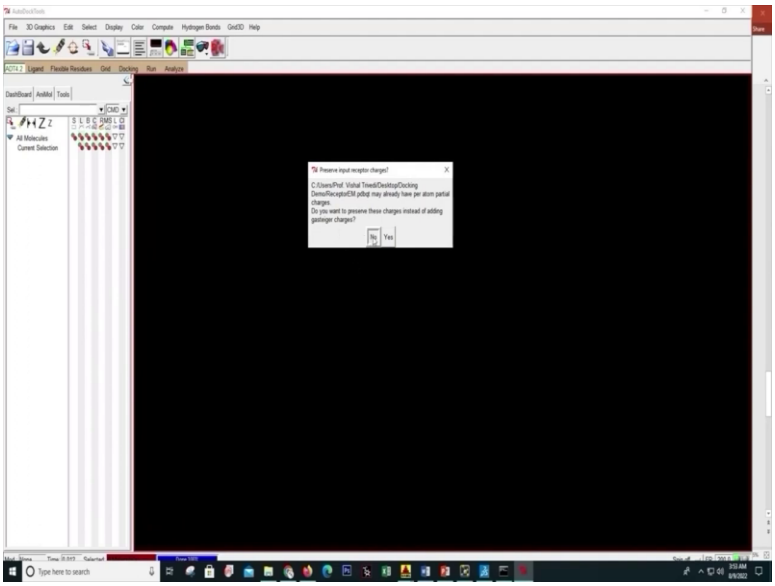
So, we will open our macromolecule saved in PDBQT format.

(Refer Slide Time: 27:40)



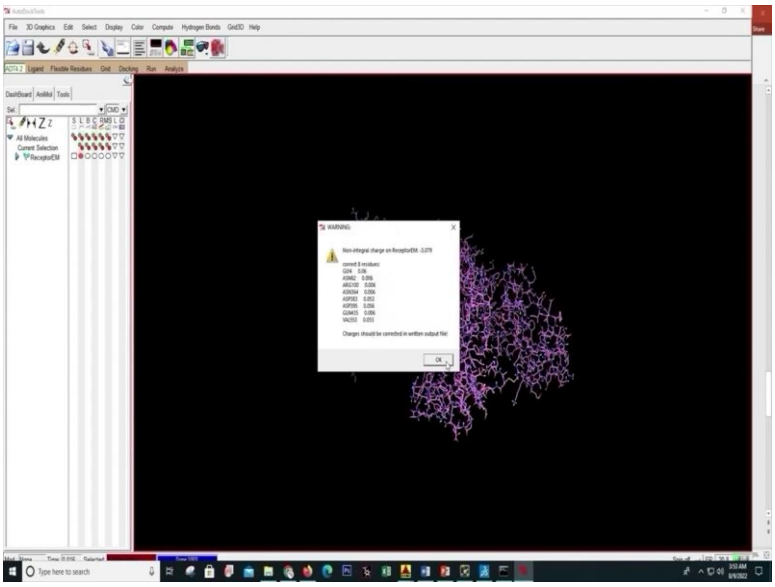
That is ReceptorEM dot pdbqt will select this and Open it here.

(Refer Slide Time: 27:46)



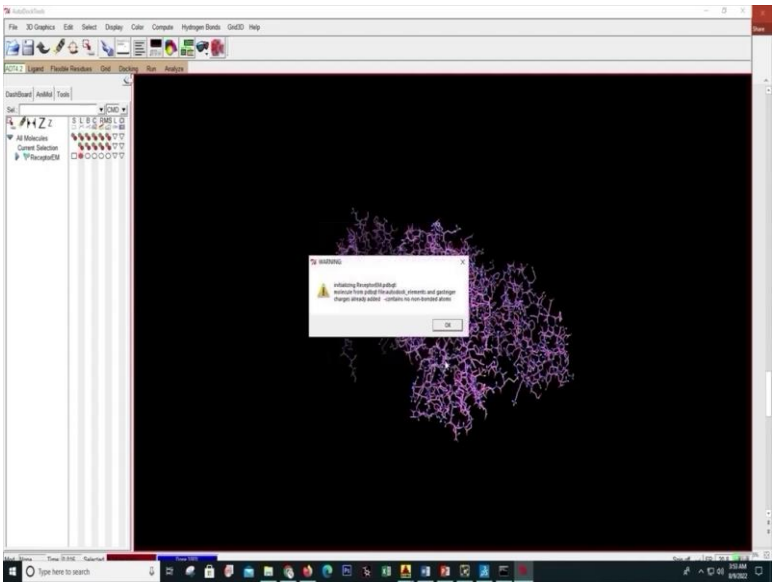
So, we will click No on the pop up.

(Refer Slide Time: 27:57)

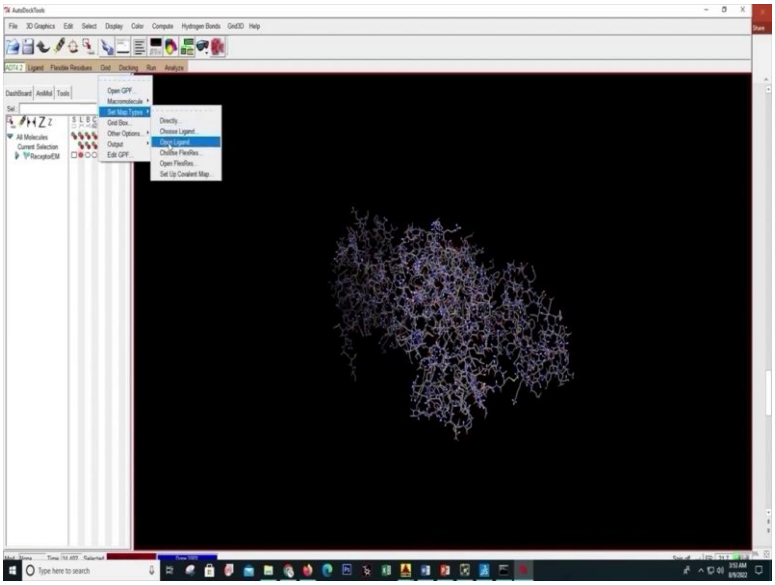


And then it will show it is showing like we need to correct some charges on the residues that will be done later will be just press Ok.

(Refer Slide Time: 28:04)

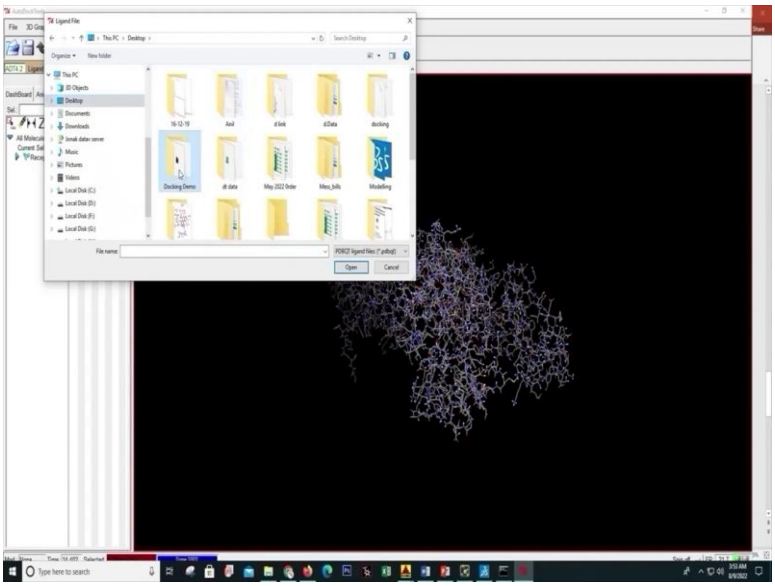


(Refer Slide Time: 28:07)



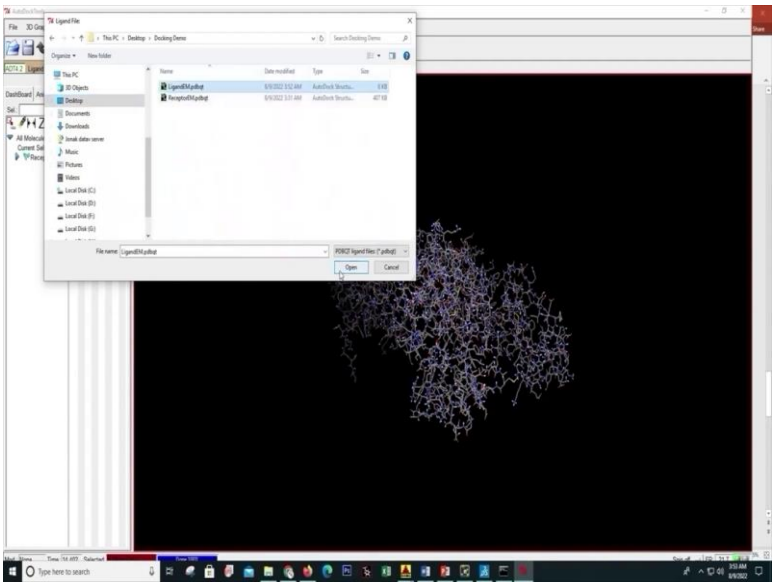
And after that we will again go to Grid and then we will go to Set Map Types and then we will click on Open Ligand.

(Refer Slide Time: 28:15)



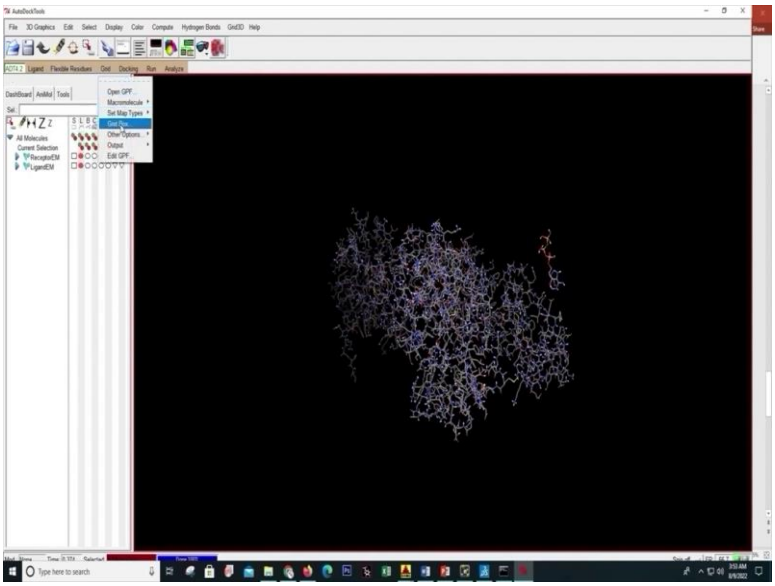
And here we will open our ligand in dot pdbqt format from the same folder.

(Refer Slide Time: 28:20)



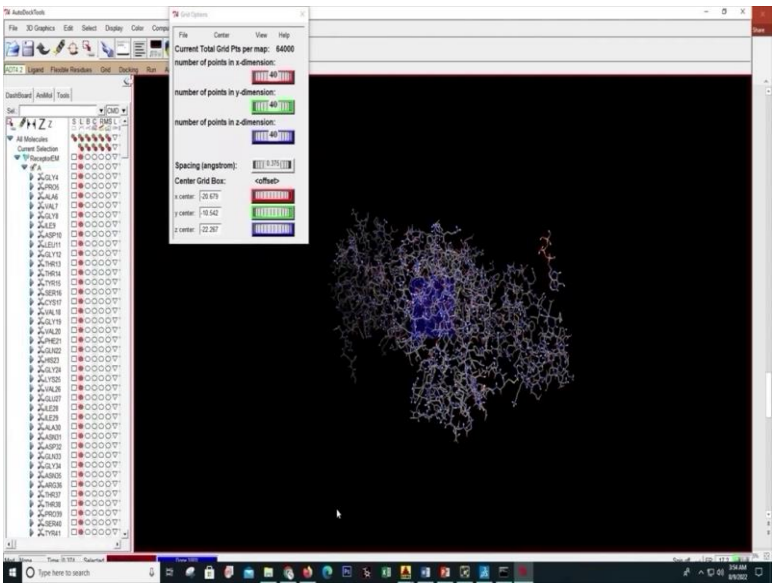
So, this is LigandEM dot pdbqt format we will Open it.

(Refer Slide Time: 28:26)



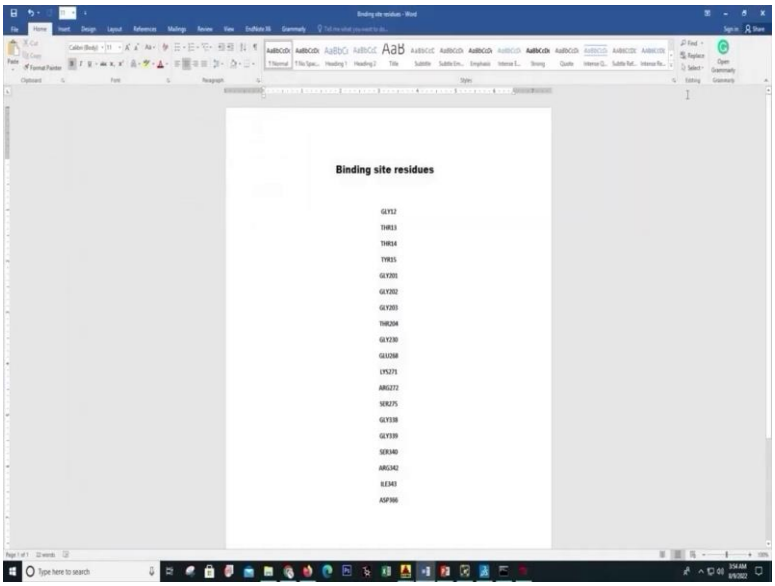
So, the ligand has opened up here and now we will move to the again go to the grid and click on Grid Box.

(Refer Slide Time: 28:34)




So, here this window this window here we can define the coordinates of our grid box. So, for this first what we will do we will select the residues which are which were involved in the interaction.

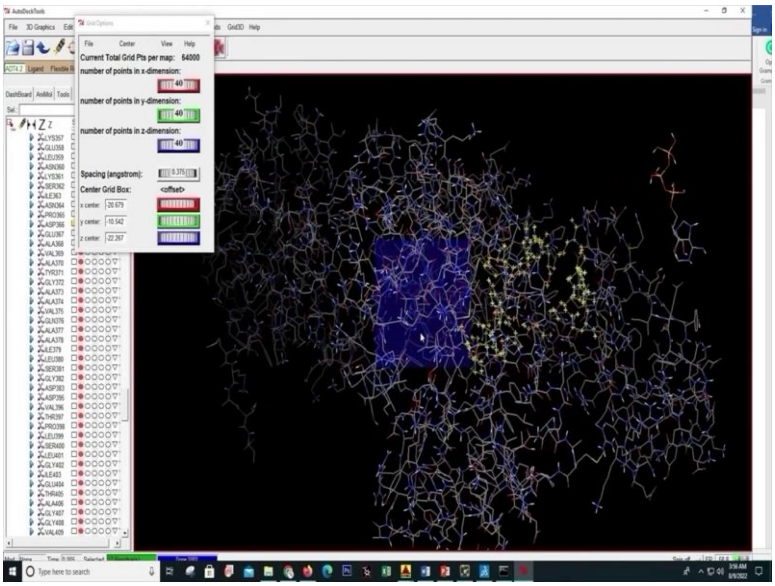
(Refer Slide Time: 28:53)




So, we will just open this window and then we will select each residues one by one one by one. So, here you can see we will start with glycine 12 and then threonine13, threonine14, tyrosine 15 then it is glycine 201. So, This is glycine 201 glycine 202 and similarly we will select all the residues here.

So, when we select these residues, these residues will be selected will be displayed in yellow so, that we can easily adjust our grid box to contain all the residues in the grid box. So, now glycine glutamine 268, glycine 271, arginine 272, serine 275 then we need to go to 338 now. So, this is glycine 338, glycine 339, arginine 342 and then isolation 343 and the last residue is ASP366. So, this is 366 now we will maximize this.

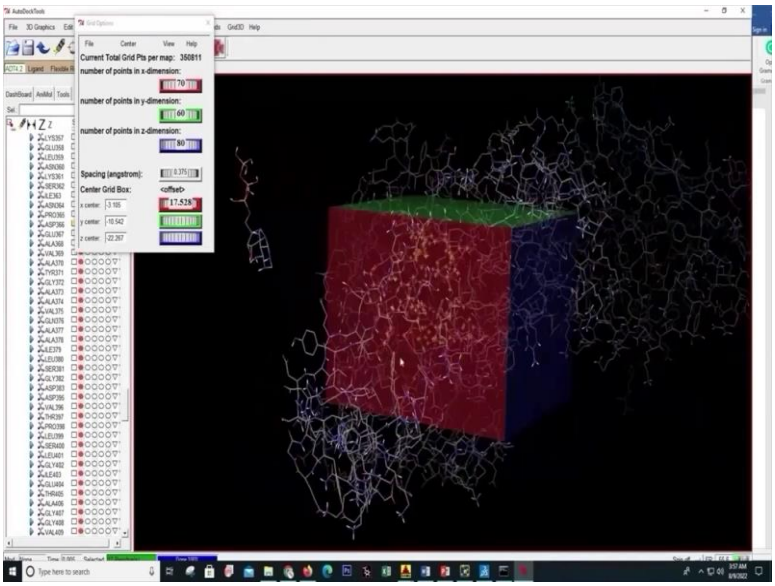
(Refer Slide Time: 30:53)




Now you can see the residues, which we have selected is displayed in yellow. Now we can just with the upper sliders we can move the size we can adjust the size of the grid box and with the lower sliders we can just move the grid box you can change its coordinate. So, first we will move the grid box towards the (Refer Time: 31:19) binding site.

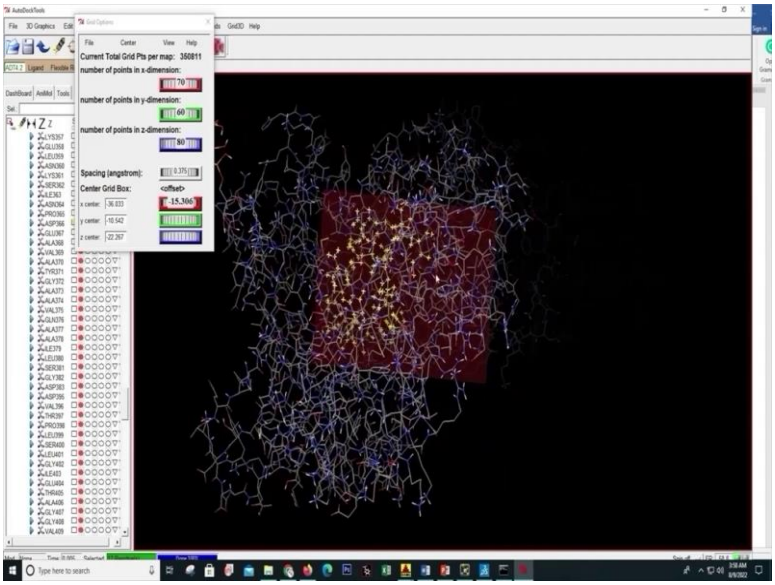
So, that all the residues are contained in this. Now, we will just increase the size all three dimensions. So, this is now I will use the green slider. So, this will increase it vertically and then we can just rotate this molecules this molecule like this and we can increase this size.

(Refer Slide Time: 31:50)



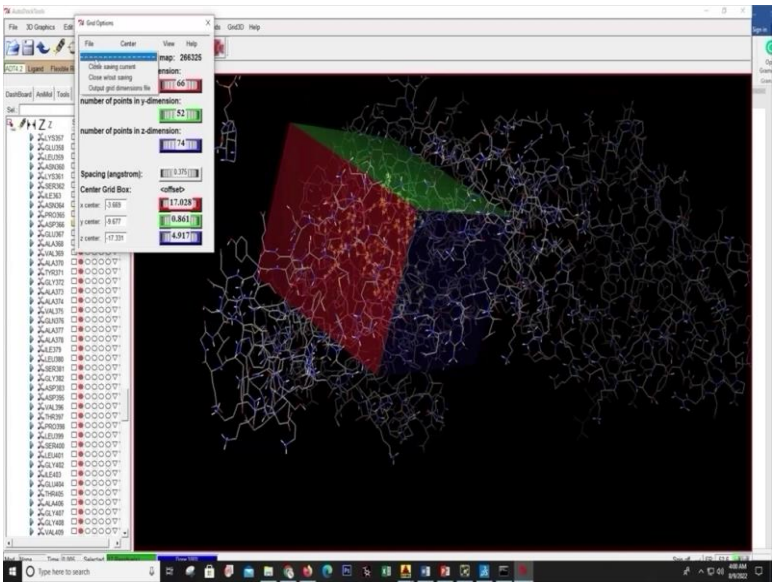

So, we can see the we can rotate this in all direction and just see all the residues are contained in the grid box or not. So, here we see it is there.

(Refer Slide Time: 32:20)




Now, we can just move our grid box little this side because it is containing some unwanted residues as well.

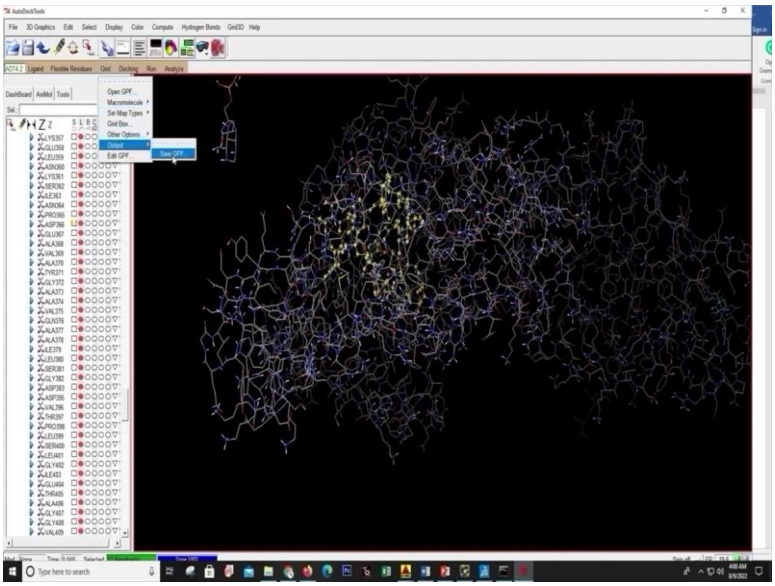
(Refer Slide Time: 32:47)




So, we move to more than to this side. Now, we will again rotate the molecule and see the grid box has moved too far. So, we will just bring it back using the red slider. We will try to keep the residues in the middle so, that you can adjust it properly. So, now, we just reduce the size of the grid box. So, that it does not contain the unwanted residues and again rotate. You can see this side some residues are move out.

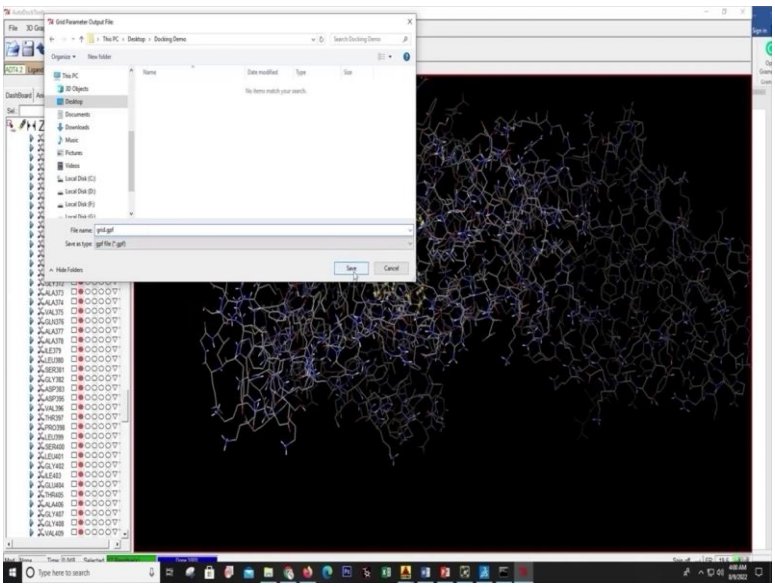
So, again we will increase the size. And we can move it little this side. So, now we can see the residues are almost inside you can increase this little bit more and then we can just rotate it in all directions. You can see all the residues are inside only. You can reduce the size this way little bit and then move it. Now, we can see all the residues are inside the grid box. So, we will just go to File and Close saving current.

(Refer Slide Time: 35:03)



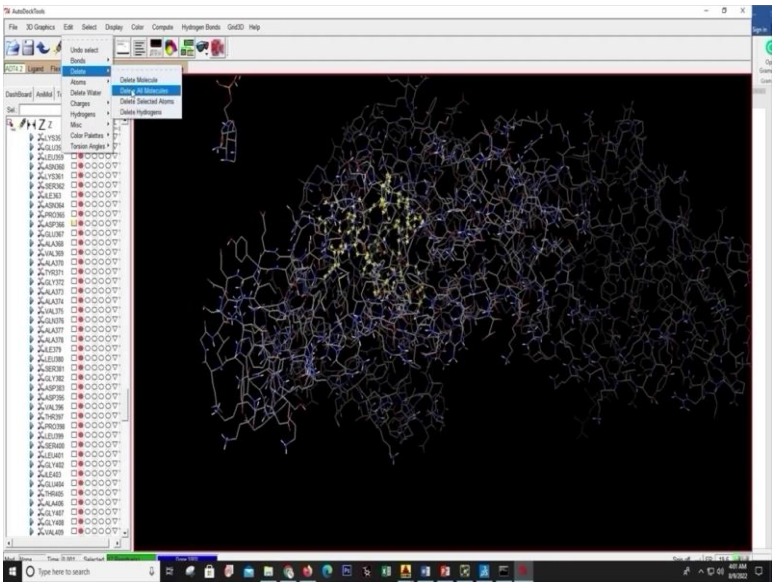
So, the grid, grid parameters we have set and now we need to save this grid parameter. So, we will go to Grid and then we will go to Output and then Save GPF.

(Refer Slide Time: 35:20)

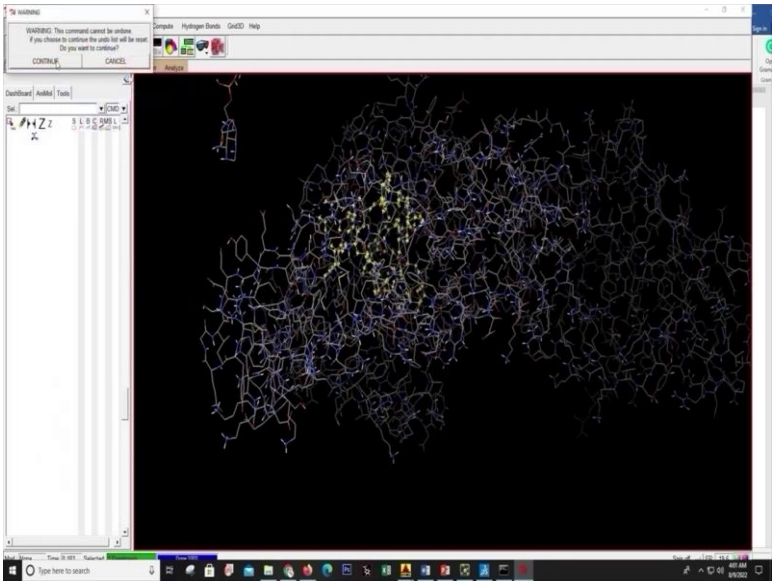


GPF means grid parameter file. We need to save this in the same folder we have saved all other files. You can write it like grid dot gpf it is important to write dot gpf in this file. Now, I will save this.

(Refer Slide Time: 35:43)

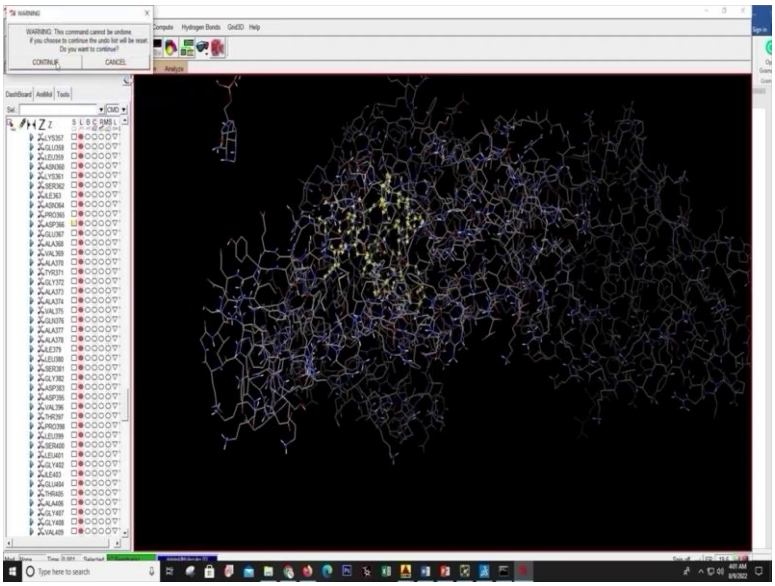


(Refer Slide Time: 35:48)



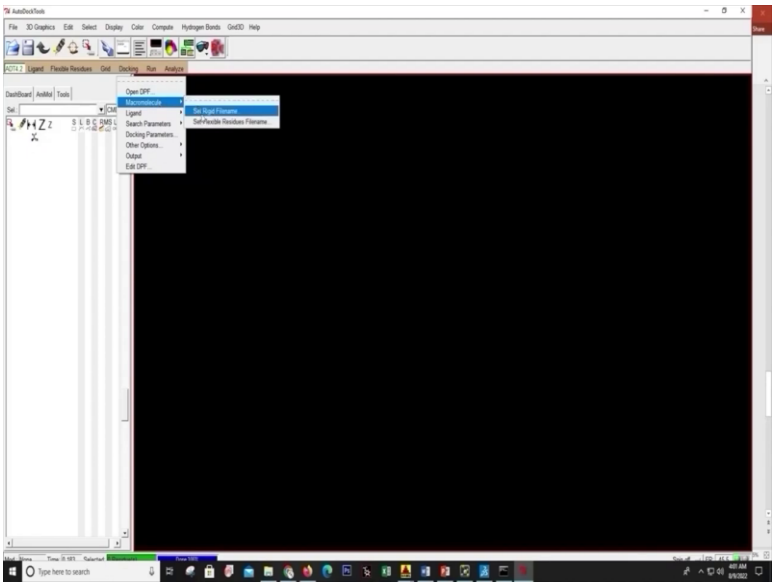
And after that again we will go to Edit then Delete.

(Refer Slide Time: 35:48)



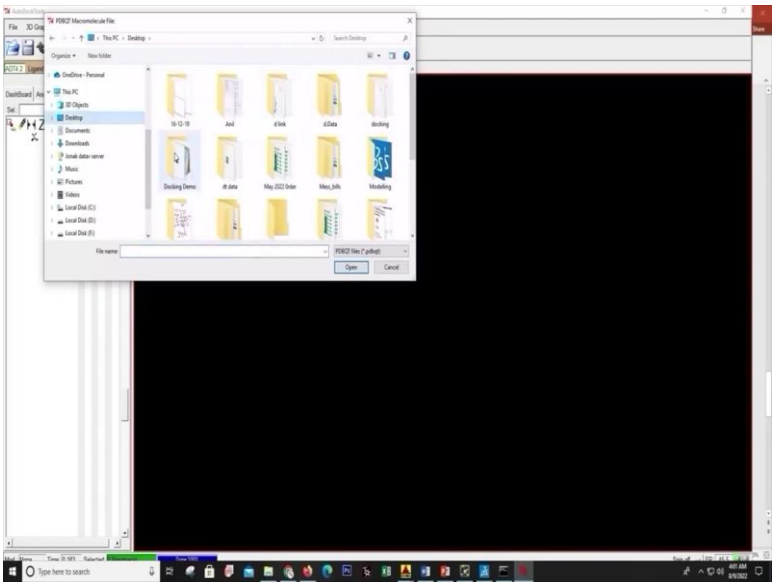
And then Delete all molecules. So, it will delete everything. Now, we will go to the next step, which is the preparing the docking parameter file. So, now we will prepare the docking parameter file that is dot gpf file. So, for that again we will go to AutoDock.

(Refer Slide Time: 36:05)



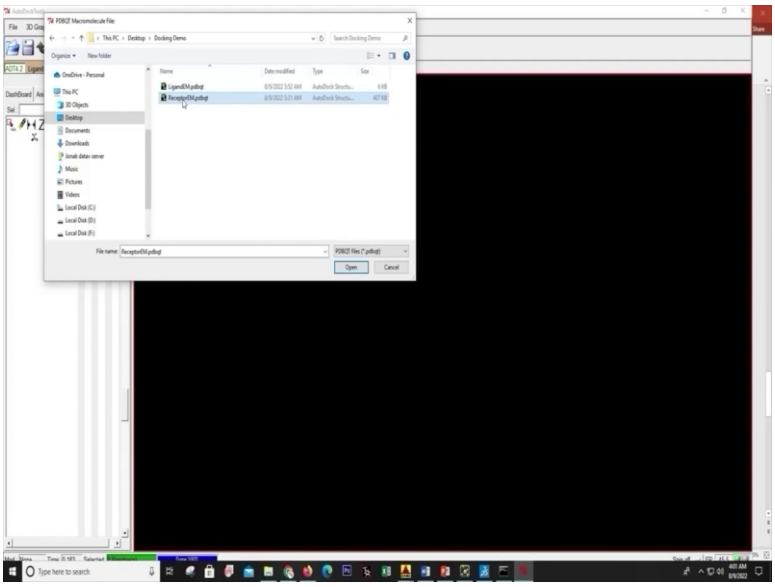
And then we will go to Docking here and then docking will go to Macromolecule and that Set Rigid Filename.

(Refer Slide Time: 36:15)



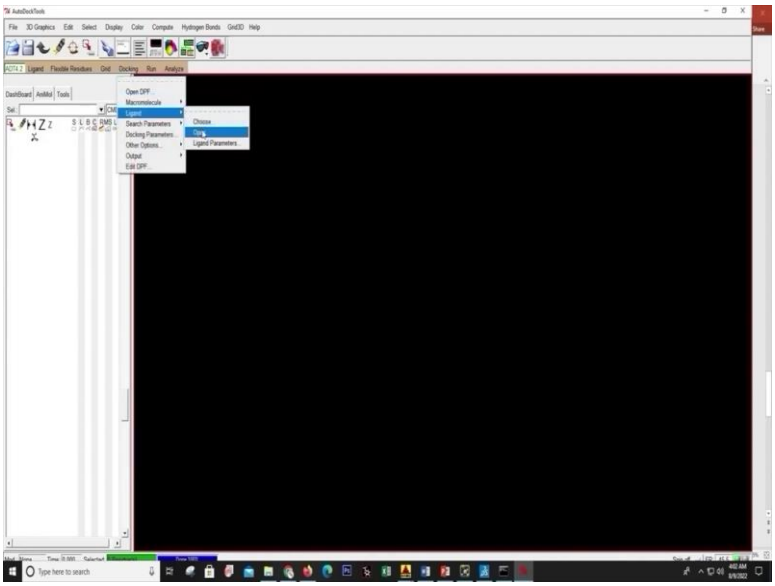
So, again we will go to the same folder.

(Refer Slide Time: 36:19)



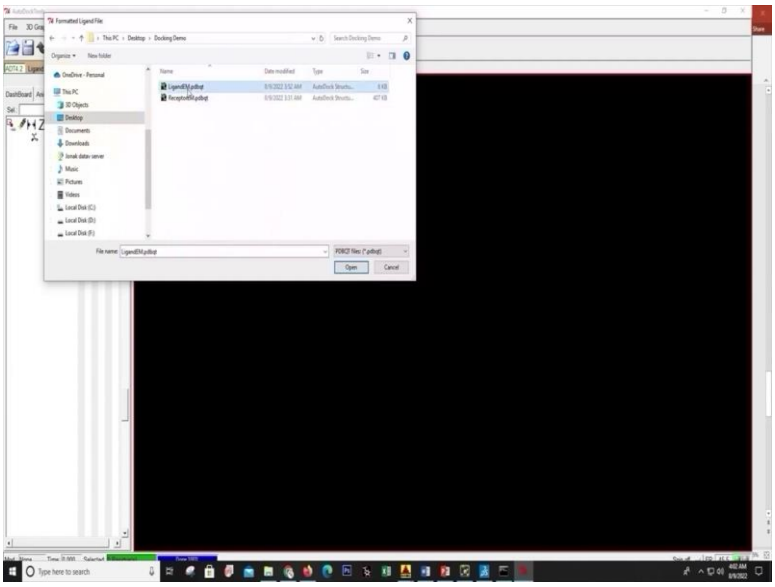
And here we will save our in for rigid file name the receptor in this docking is rigid; that means, it will not change its conformations conformation. So, we will select the receptor energy minimize receptor and then Open it. And nothing will be there on the screen, but its fine.

(Refer Slide Time: 36:43)



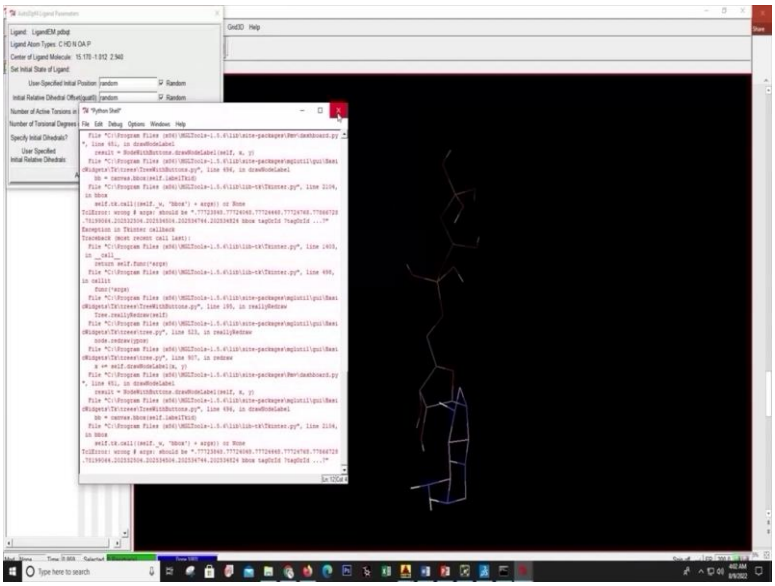
Then we will go to docking and then Ligand and Open.

(Refer Slide Time: 36:47)



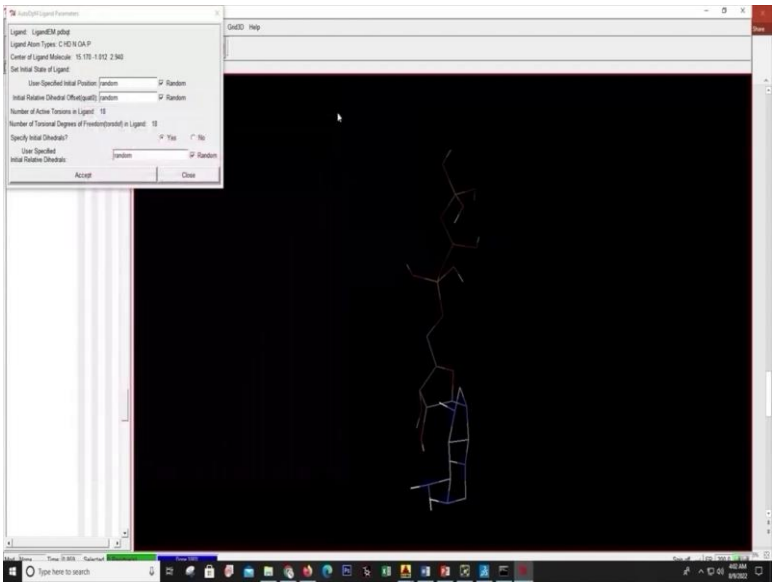
Then we will open the ligand from the same folder em dot pdbqt format.

(Refer Slide Time: 36:55)



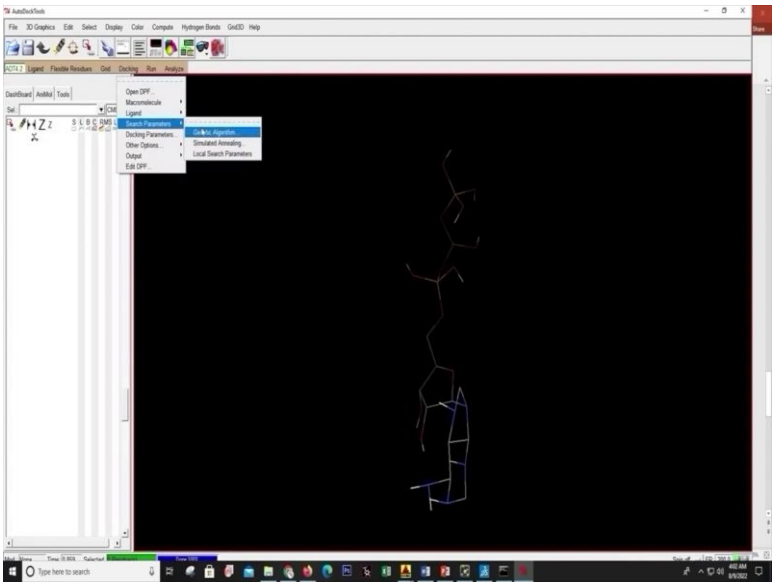
So, it will open up a pop up.

(Refer Slide Time: 36:59)



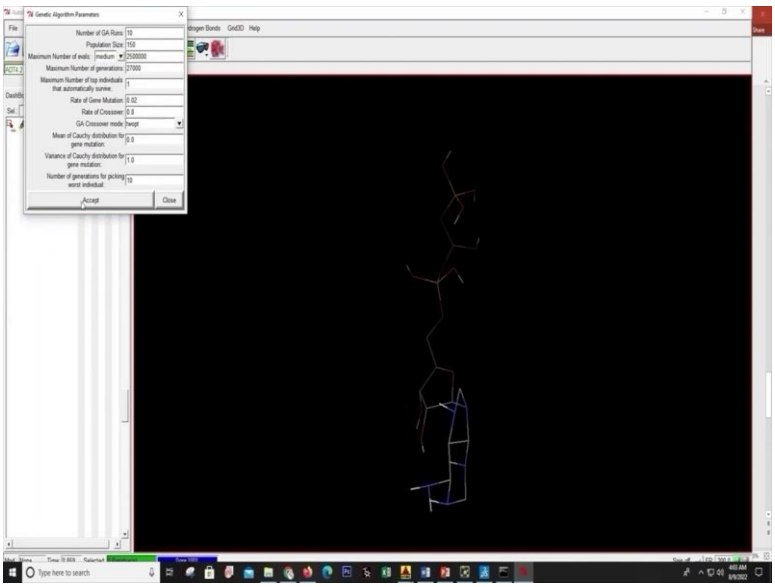
Here we need to and it will also show the ligand on screen. So, here we will press on Accept.

(Refer Slide Time: 37:12)



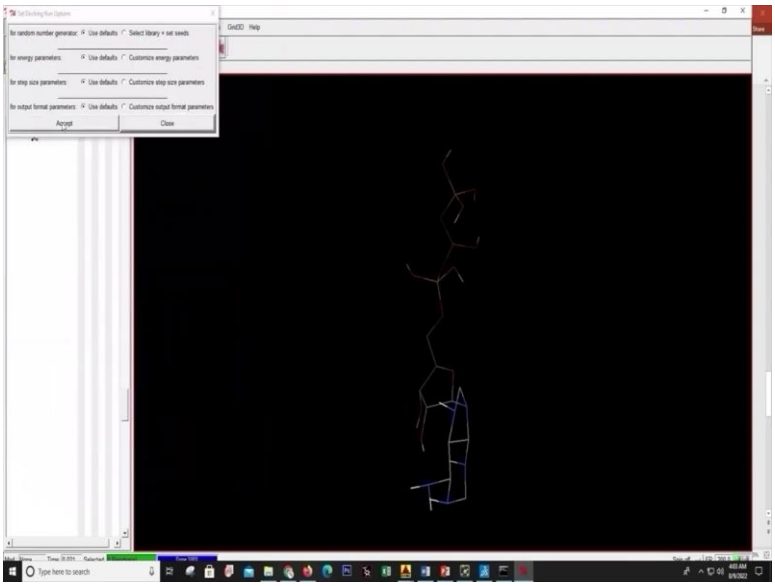
After that we will again go to Docking and in docking we will click on Search Parameters.

(Refer Slide Time: 37:19)



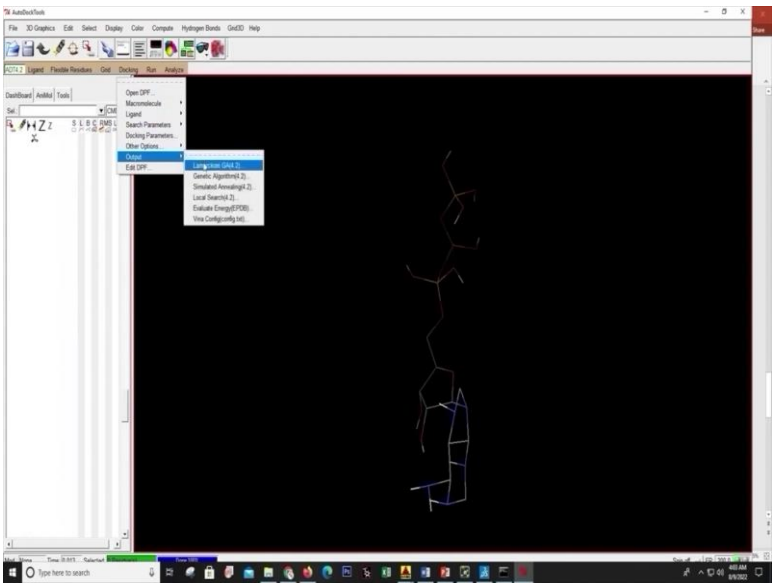
In search parameters we will click on Genetic Algorithm. Here it is here we can set the GA runs like how many conformations of the ligand we need to we need the auto dock to create and then dock. So, it is set to 10 it can be set to 50 100 depending upon the requirement. Then we will press Accept and then after that we will again click on Docking.

(Refer Slide Time: 37:54)



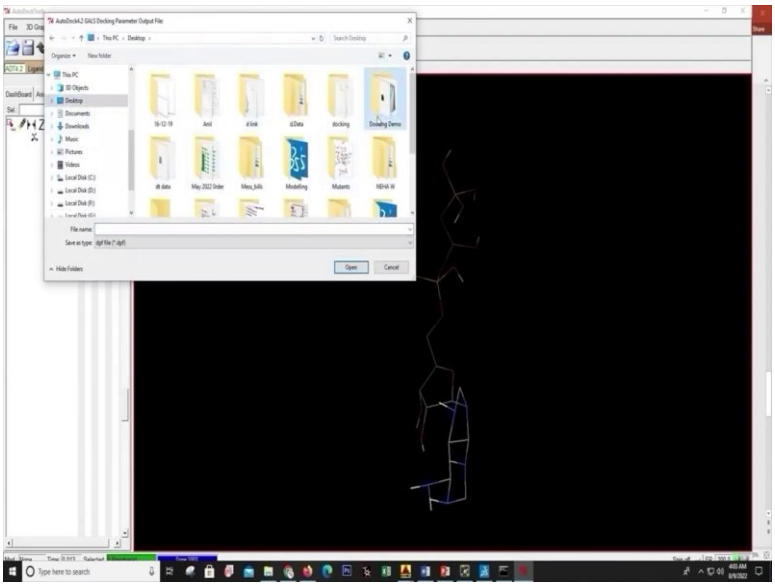
And then we will click on Docking Parameters. It will open up a pop up we have to just click on Accept and after that.

(Refer Slide Time: 38:02)



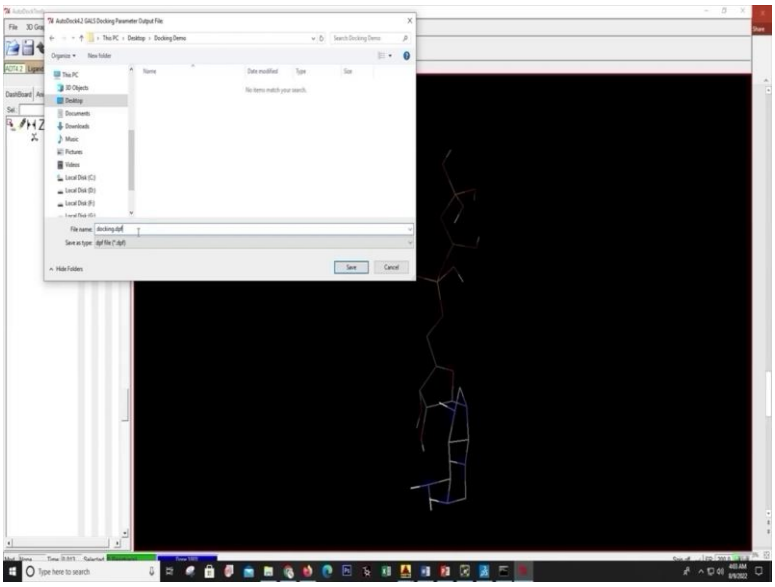
Again, we will go to Docking and then we will Save the files we will click on Output and then click on Lamarckian GA.

(Refer Slide Time: 38:08)



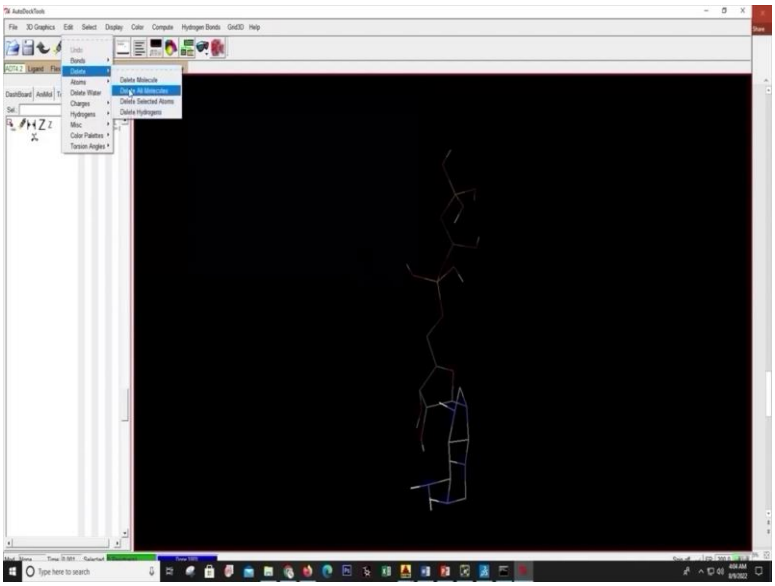
And we will navigate to the same folder.

(Refer Slide Time: 38:12)



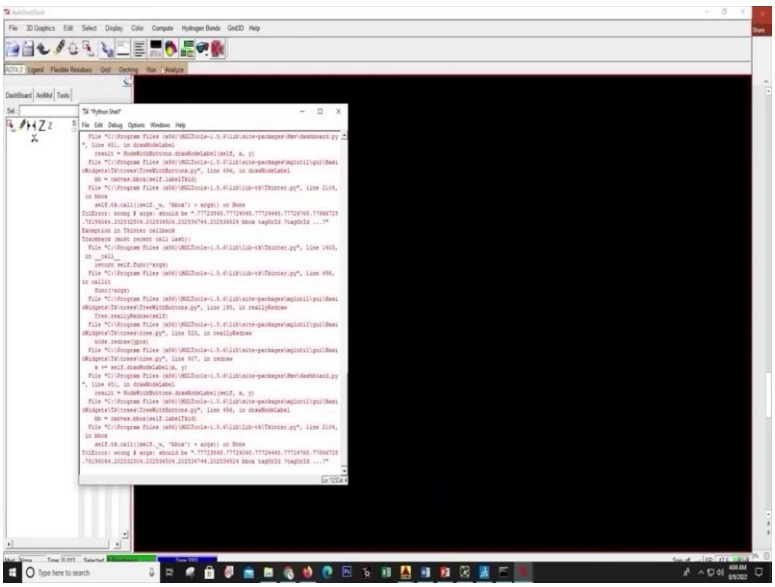
And here we will save the file in dock dot dpf format. So, I will write dock docking dot dpf, which is what again important to write the dot dpf. So, that it will be easily read by auto docking.

(Refer Slide Time: 38:39)



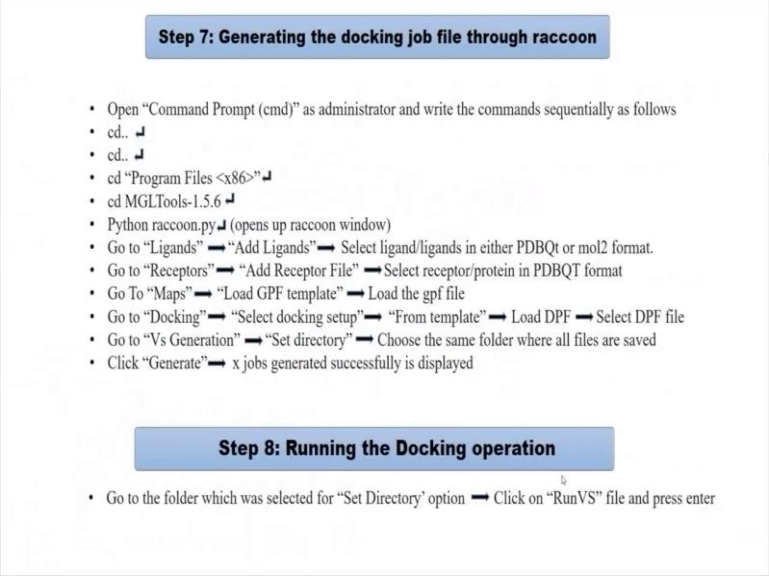
I will save it and after that again we will go to Edit and then Delete and Delete All Molecules.

(Refer Slide Time: 38:46)



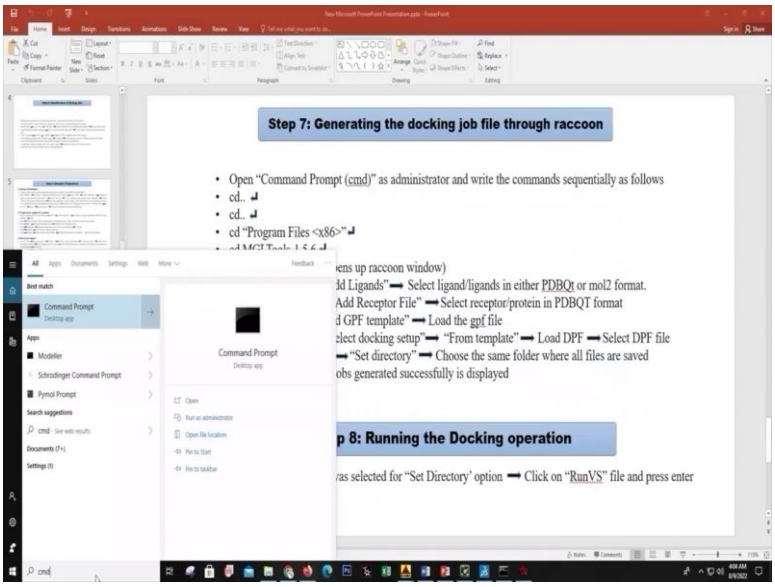
And then now our preparation part is done.

(Refer Slide Time: 38:53)



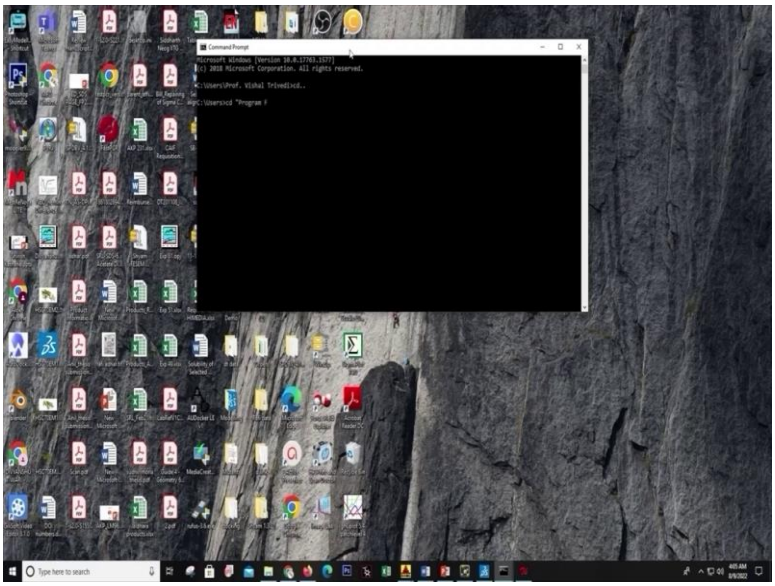

So, we will go to the next step that is generating the docking job file through raccoon. So, we need to run raccoon here raccoon is another software which comes embedded with auto dock. So, to open this raccoon we need to go to command prompt.

(Refer Slide Time: 39:13)



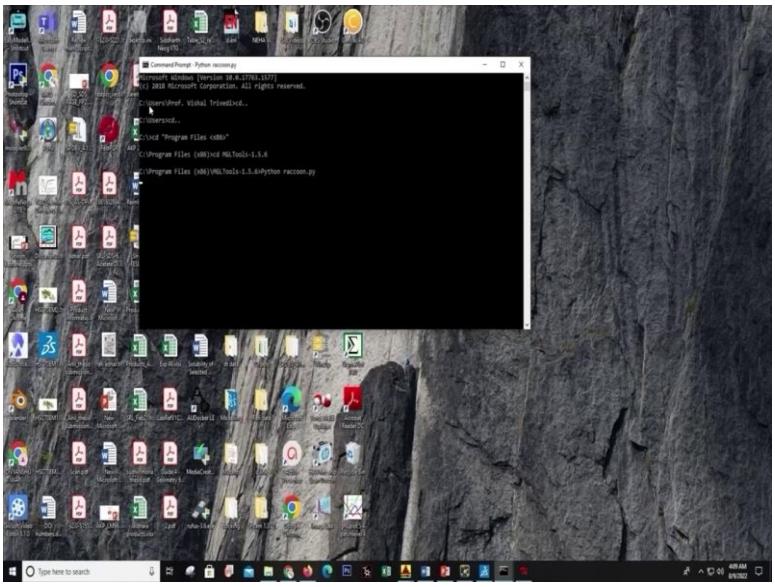

I will open command prompt here. I will type cmd.

(Refer Slide Time: 39:19)



And so, this is the Command Prompt window. So, in command prompt we have to go to the folder where there we have saved the our where the AutoDock is installed. So, here I will go to that folder I will press cd dot dot and then I will write cd space in brackets.

(Refer Slide Time: 39:49)

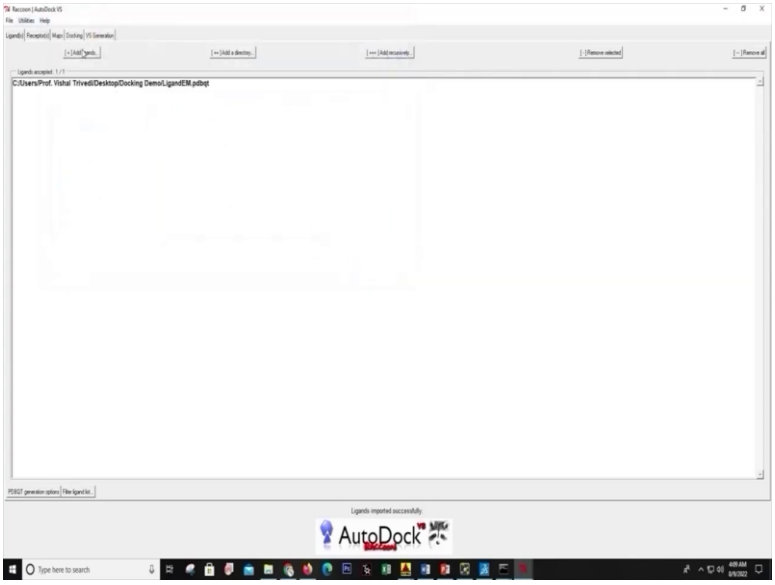


I will have to write the path of this is the Command Prompt window. Now, first we need to the active folder is different. So, first we need to go to the c drive and then open the folder where our AutoDock is installed. So, I will type cd dot dot and again cd dot dot now c drive is active. Now, I will go to the that folder. So, we have to type the exact

pathway here cd space braces on Program Files x86. Now I will close the braces now press enter.

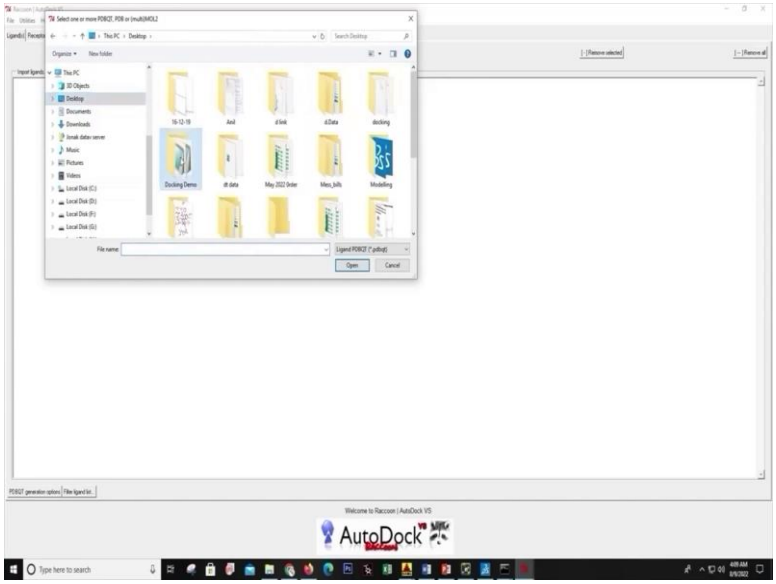
So, now the program files folder is active we will move to the MGL Tools. We need to type the correct spelling and correct pathway otherwise it will show some error. Click Enter and then after this we will the raccoon is embedded in this folder only. So, just we will type Python space raccoon we need to type the correct spelling of raccoon dot py.

(Refer Slide Time: 41:11)



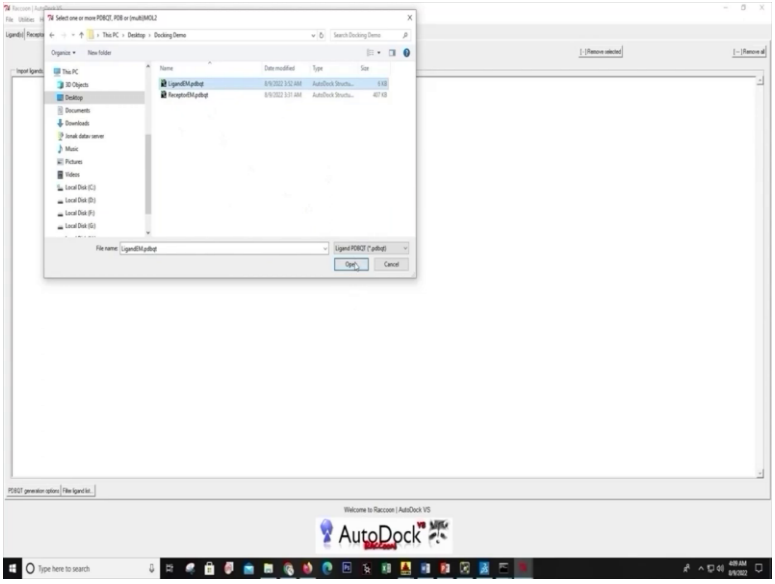

Then and then pressing enter after pressing enter our raccoon window will open up this is the raccoon window. Now, here we will perform our docking. So, first we will select the ligand we will go to add ligand.

(Refer Slide Time: 41:21)



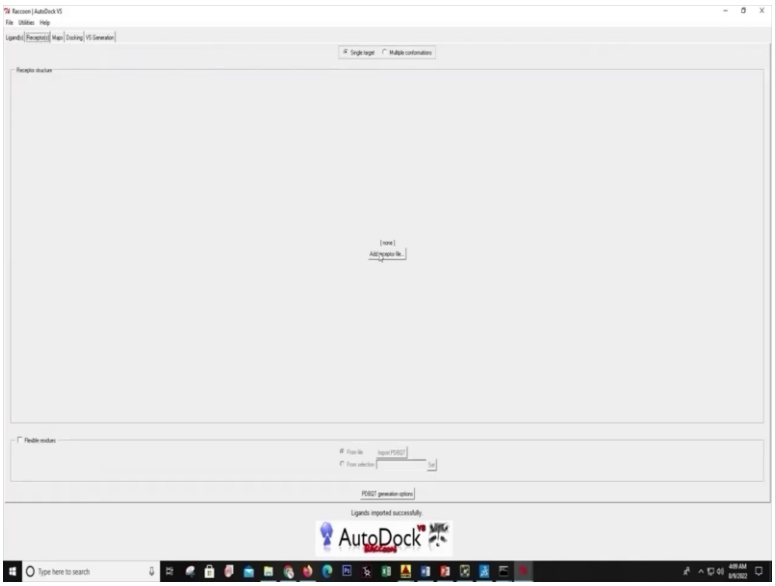
And we will select the ligand here from the same folder.

(Refer Slide Time: 41:24)



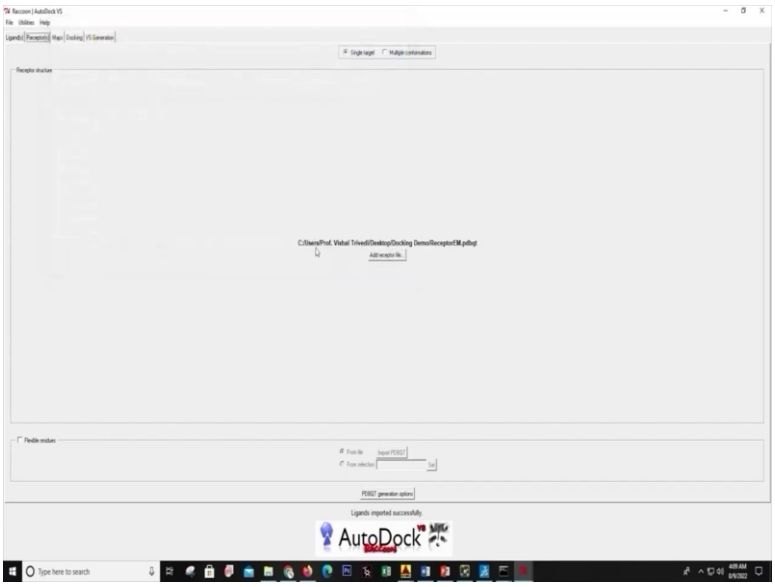
So, this is our energy minimized Ligand in pdbqt format. So, I will I have added that and then we will click on Receptors.

(Refer Slide Time: 41:33)



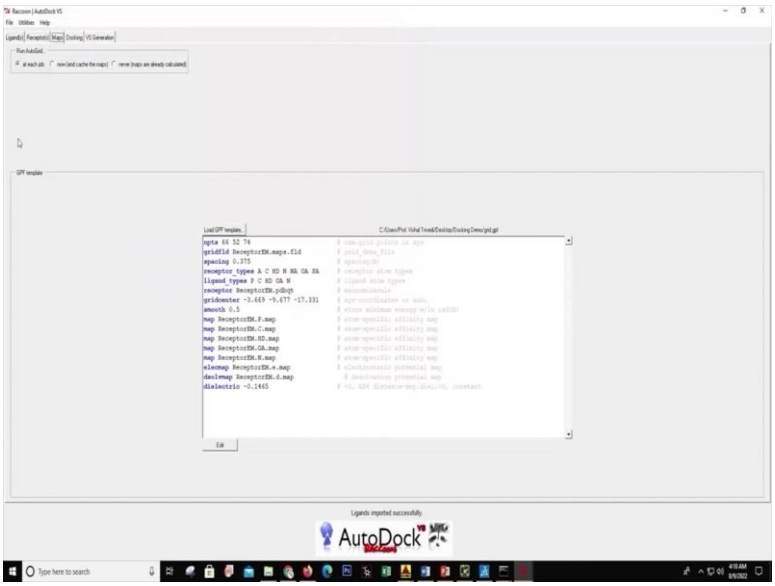
And in receptor we will Add receptor file.

(Refer Slide Time: 41:44)



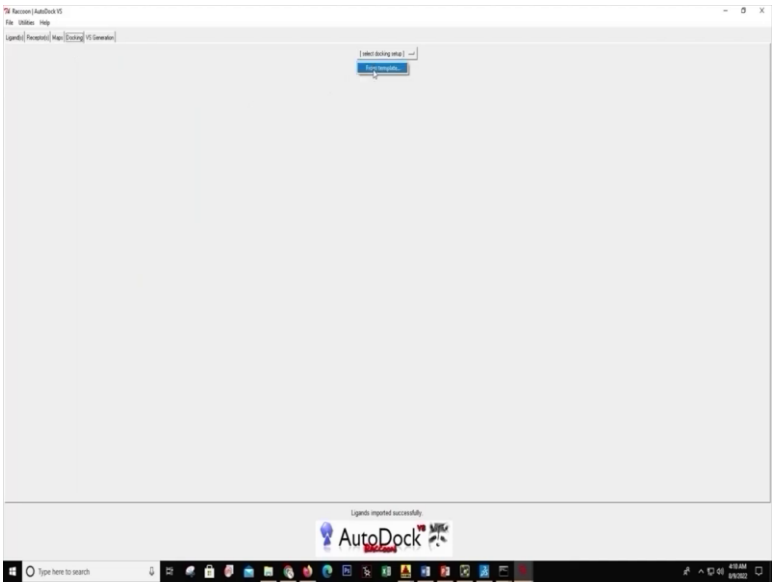
And then we will go to the same folder and then click on our receptor file and open it in here. So, it will add the receptor file in raccoon.

(Refer Slide Time: 41:49)



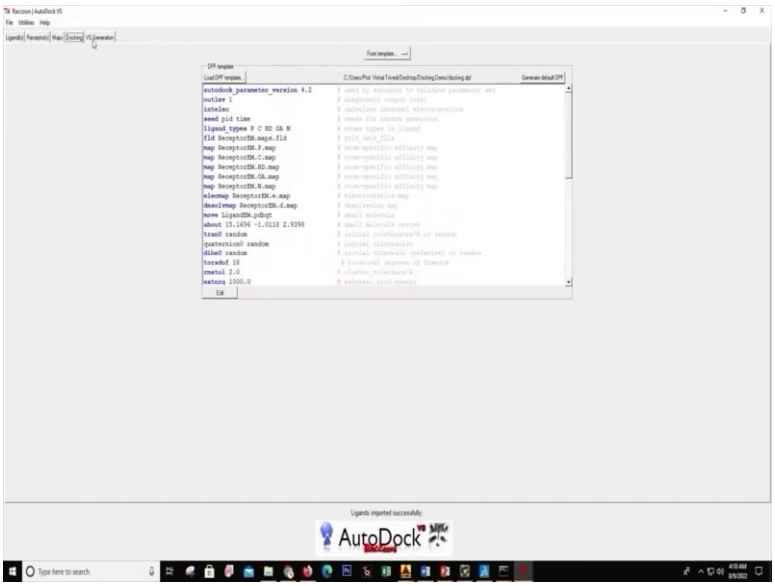
Then we will go to Maps in maps we need to load the our gpf grid parameter file. So, I will click on Load GPF template and again in the same folder here we have saved here it is grid dot gpf is there we will just add it here. Now, after that we will go to Docking.

(Refer Slide Time: 42:07)



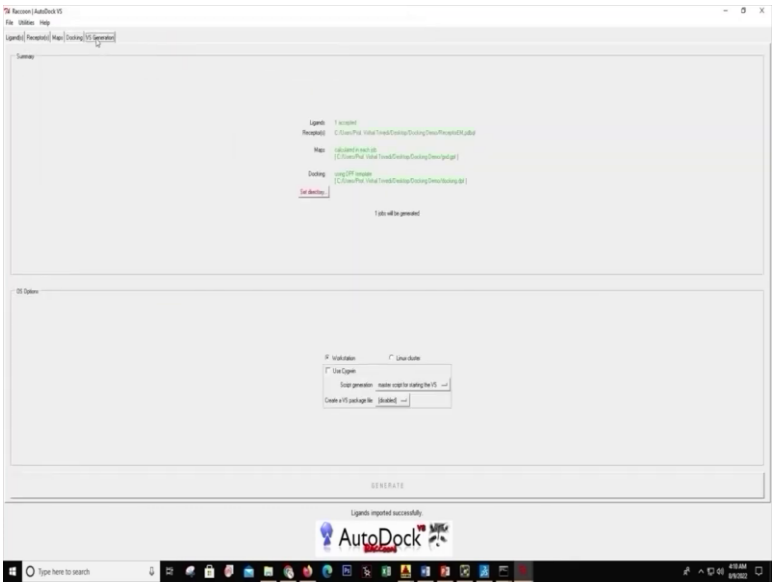
And docking will click on select docking set setup from template.

(Refer Slide Time: 42:12)



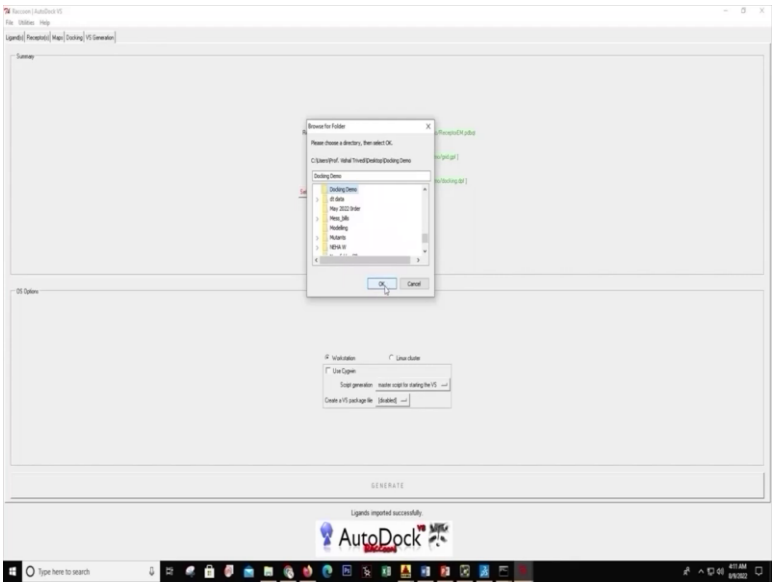
And here Load DPF template. Now, here we will select the dpf file the docking parameter file, which we created which is in the same folder. So, now the dpf file is also open. Now, we will click on VS generation.

(Refer Slide Time: 42:29)



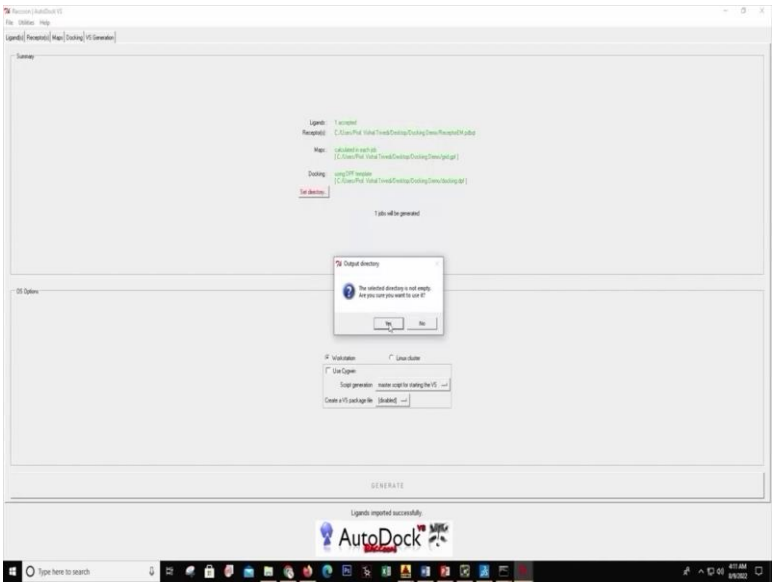
So, it will open up another window. Now, here we need to set the directory where the output will be saved.

(Refer Slide Time: 42:37)



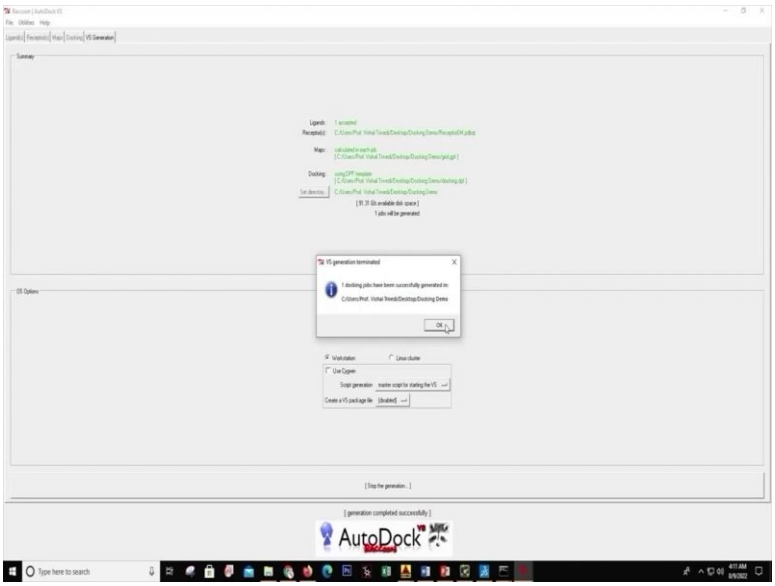
So, we will set directory we will choose the same folder here also. So, we will go on Desktop and desktop we will go to the folder which we created. So, this is the Docking Demo folder I will select that folder.

(Refer Slide Time: 43:08)



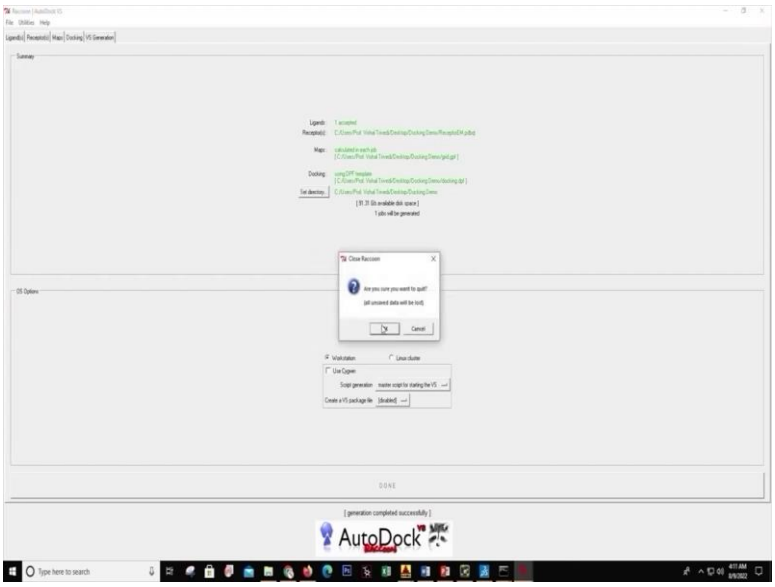
It will say that folder is not empty that is not too worry we will just click on Yes.

(Refer Slide Time: 43:14)



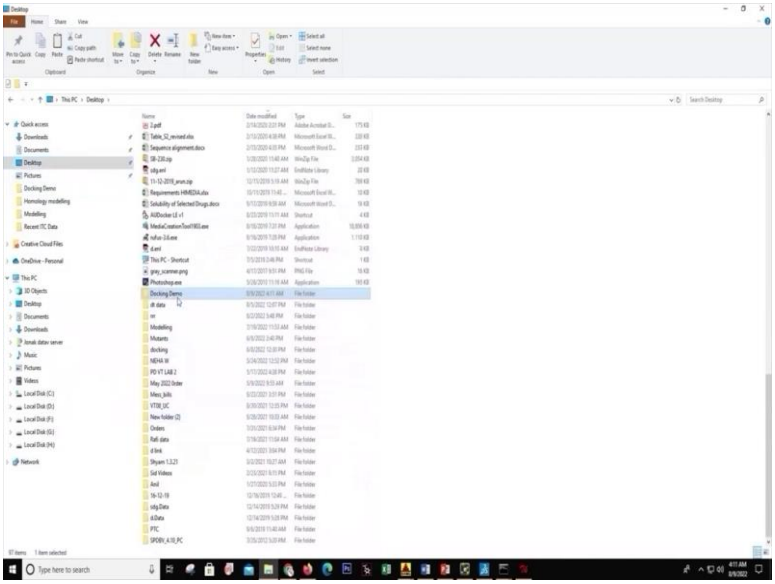
Now, we will click here on generate. So, it will generate a job for docking. It will show up pop up one docking job has been successfully generated in this folder you click on Ok.

(Refer Slide Time: 43:29)

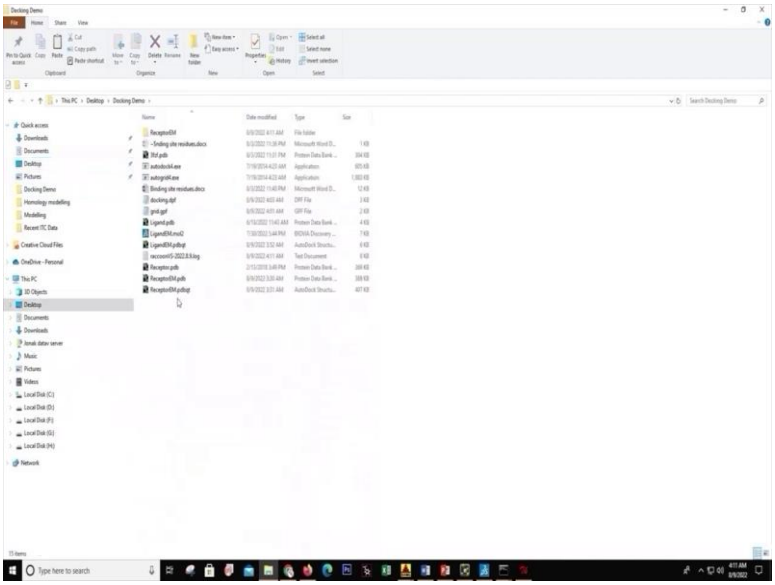


And then now we can close the raccoon here.

(Refer Slide Time: 43:35)

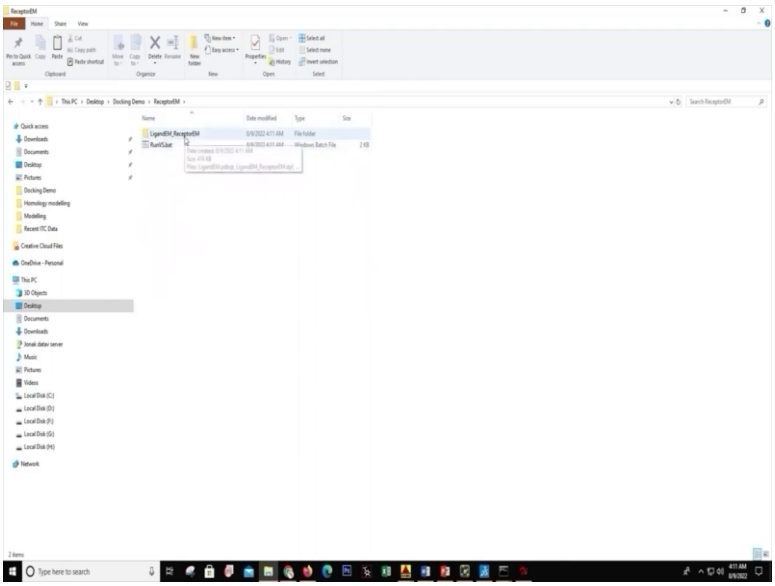


(Refer Slide Time: 43:40)



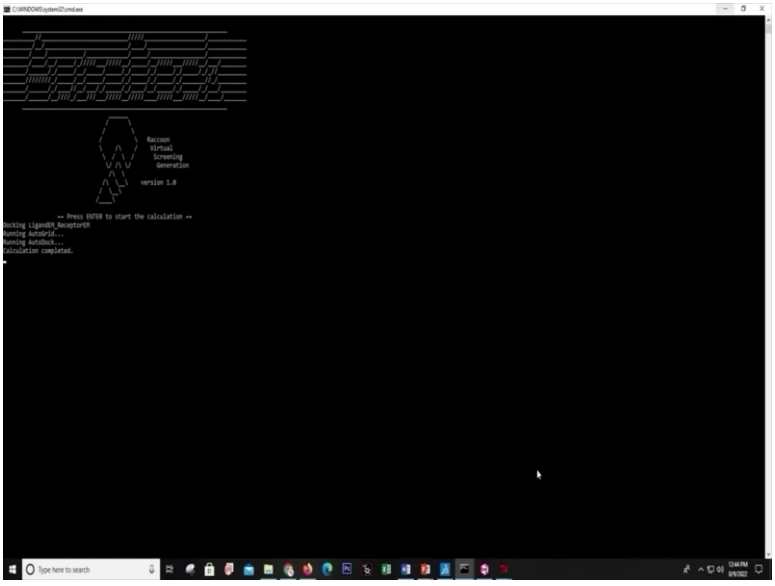
And after that we will go to the that folder and then here we can see a new folder has been created as ReceptorEM.

(Refer Slide Time: 43:45)



And then here we can see one folder ReceptorEM underscore ReceptorEM; that means, LigandEM underscore ReceptorEM; that means, this ligand is being docked to this and the output will be saved in this folder. And one more file is there run VS dot bat we have to click on this file and then a Command Prompt will open up.

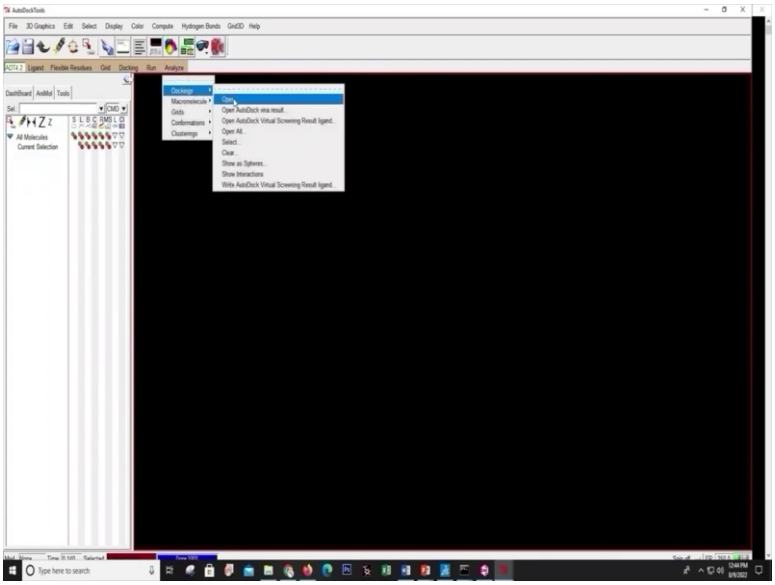
(Refer Slide Time: 44:07)




And then it is saying press enter to start just we need to press Enter and the docking job will start here and now we have to wait for some time. So, that the docking is completed.

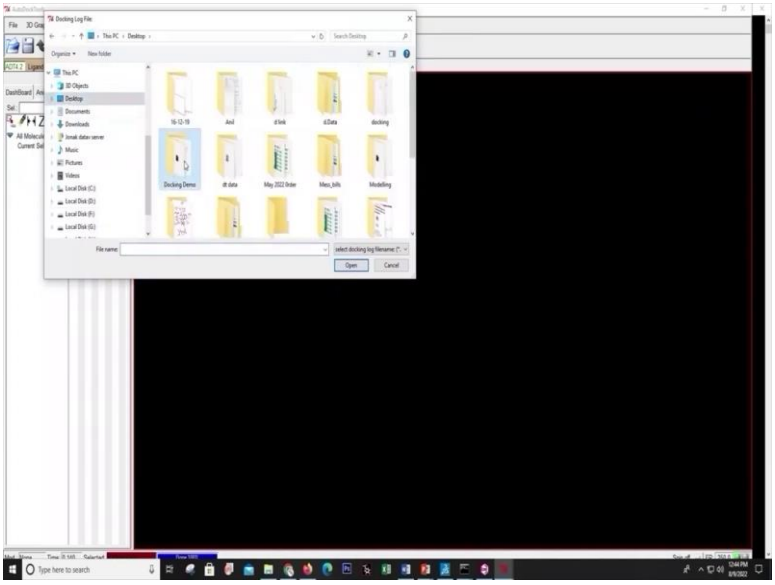
See here our docking is completed. So, now, we will go to the analysis of the dock complexes for that we will open AutoDock.

(Refer Slide Time: 44:32)



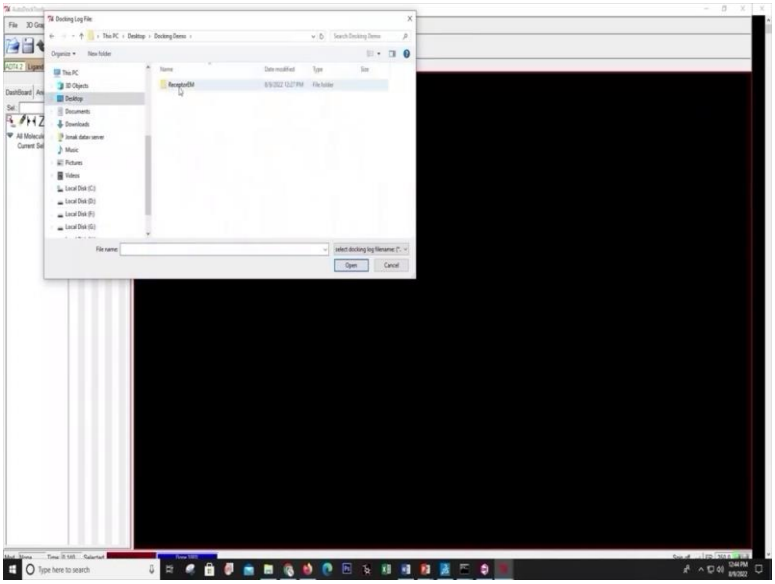
And here we will go to analyze and then click on docking and open.

(Refer Slide Time: 44:36)

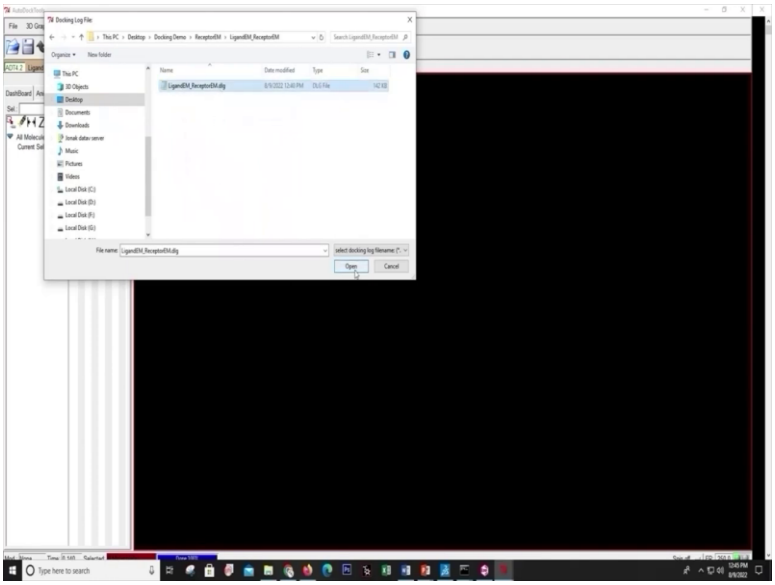


And then we will move to the same folder here.

(Refer Slide Time: 44:40)

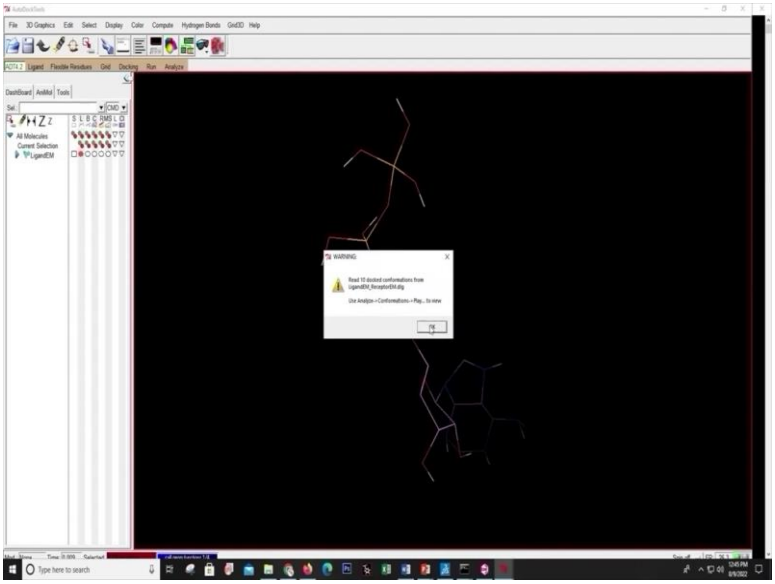


(Refer Slide Time: 44:42)



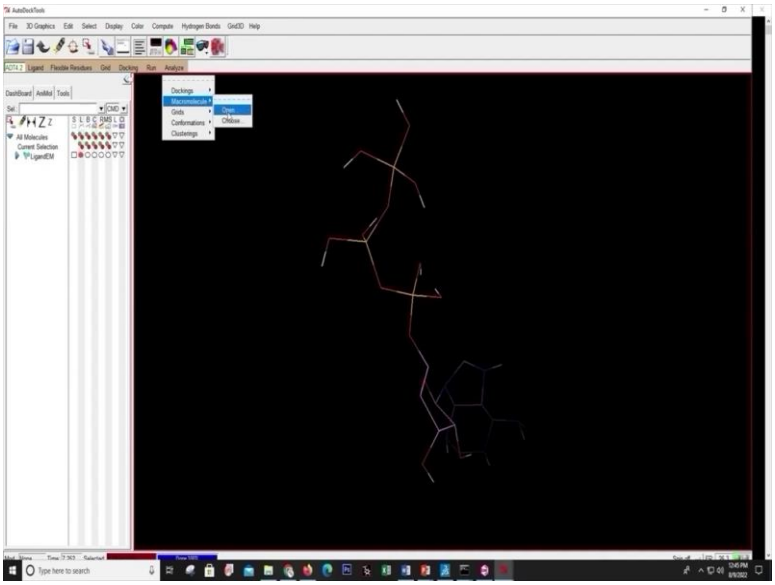
We can see dot dlg file has been created open this file in AutoDock.

(Refer Slide Time: 44:48)



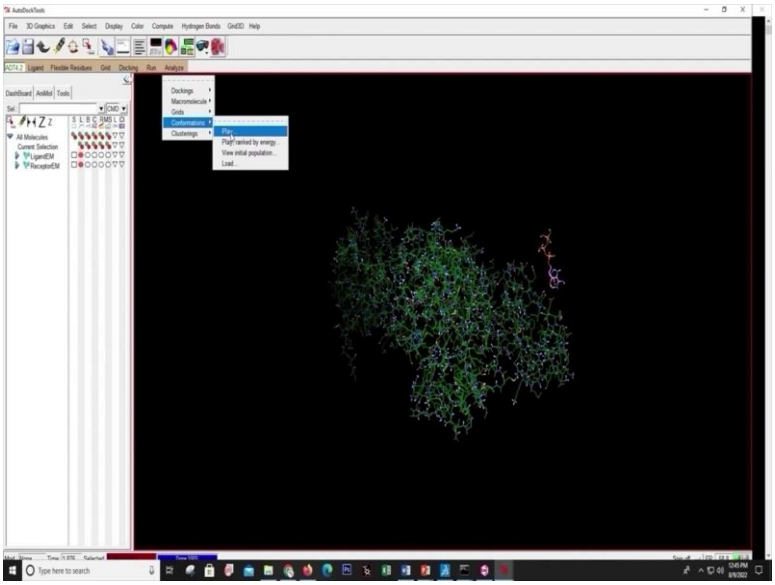
So, it is showing Read 10 dock confirmations from the ligand receptor dot dlg we will click Ok.

(Refer Slide Time: 44:56)



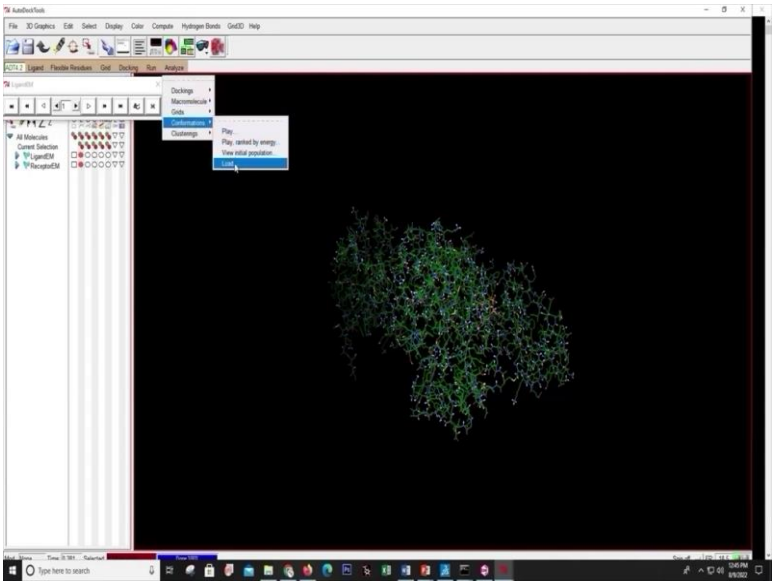
And after that again we will go to Analyze. And on Macromolecule we will click on Open. So, it will automatically open.

(Refer Slide Time: 45:02)



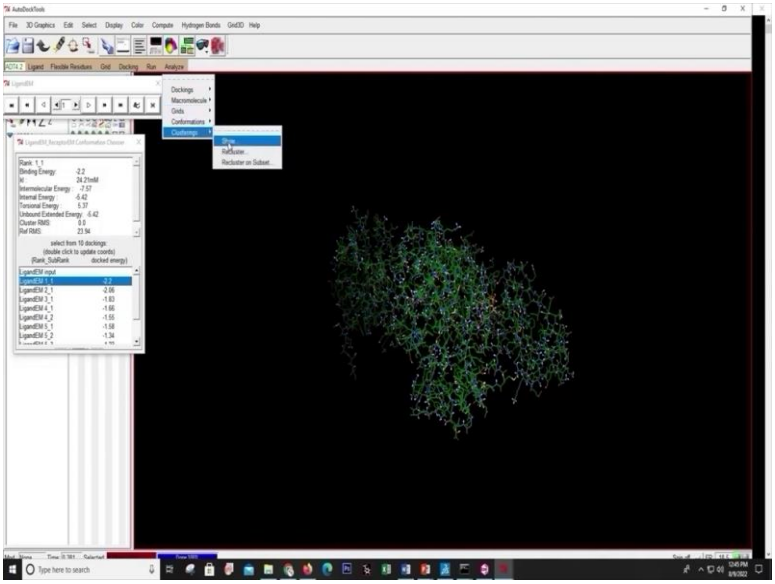
The macromolecule we have selected and after that again we will go to Analyze the Confirmations we will click on Play.

(Refer Slide Time: 45:10)



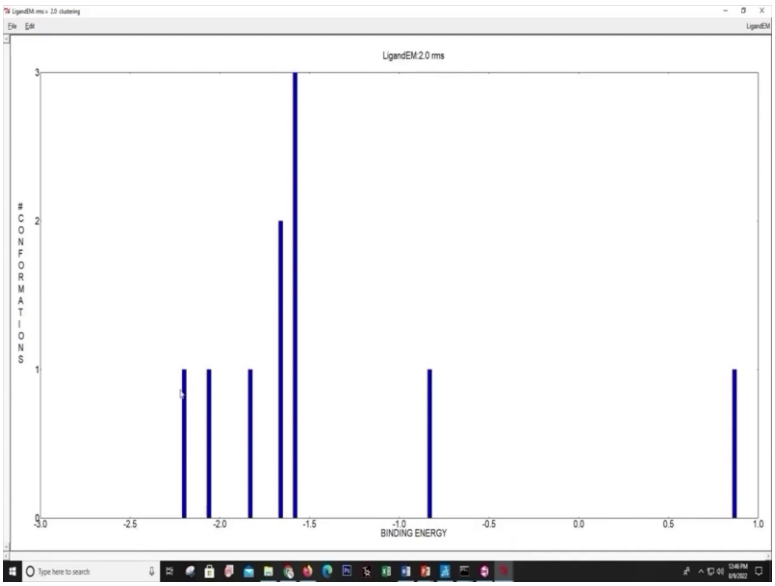
So, it will open another pop-up which will be used later for saving the complex after that again we will go to Confirmations and click on Load.

(Refer Slide Time: 45:17)



So, here it will show the binding energy of the confirmations. So, this is the confirmation with the lowest binding energy that is minus 2.2. So, now, we will again go to analyze and click on Clustering and click on Show.

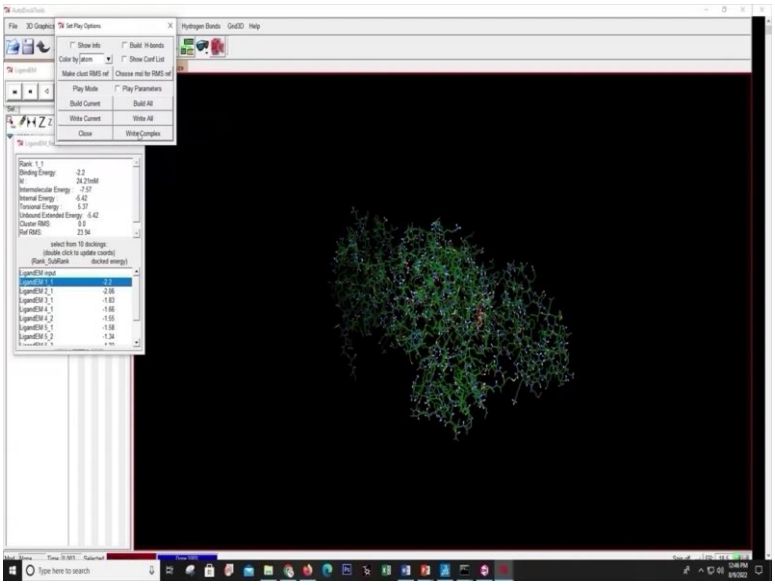
(Refer Slide Time: 45:36)




So, it will open up a graph that will show the graph between number of confirmations and the binding energy. So, number of confirmation in each clusters and binding energy. So, here we can see the cluster with the lowest binding energy is having only one confirmations whereas, this cluster this is having three confirmations. So, now, we will

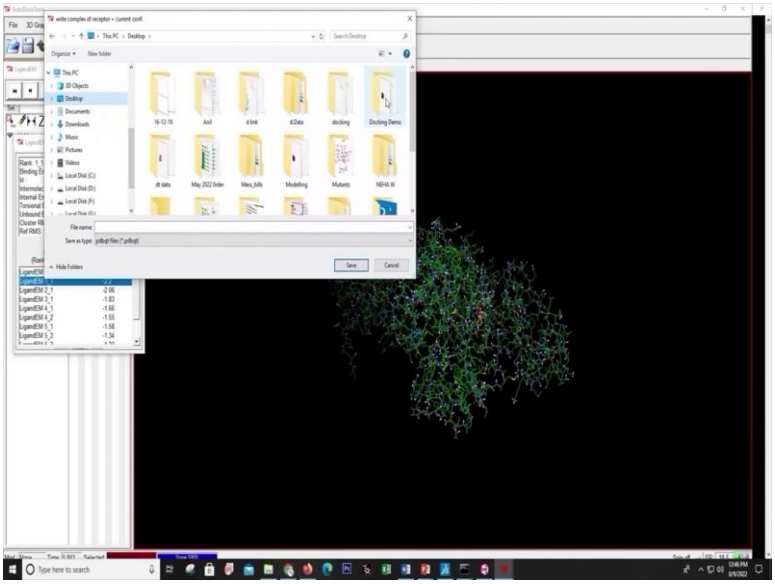
save the confirmation with the lowest binding energy. So, for that we need to select this confirmation with less binding energy first and then click on this icon.

(Refer Slide Time: 46:11)



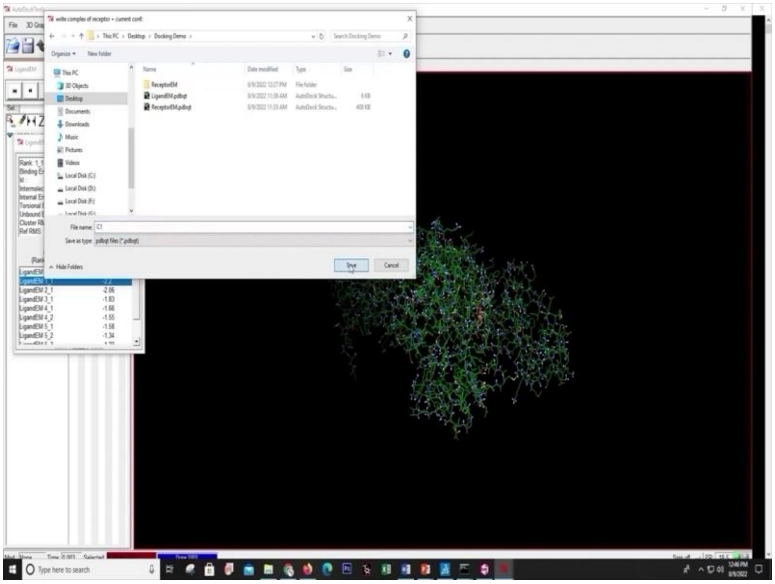
So, it will open up another window here we will click on Write Complex.

(Refer Slide Time: 46:16)



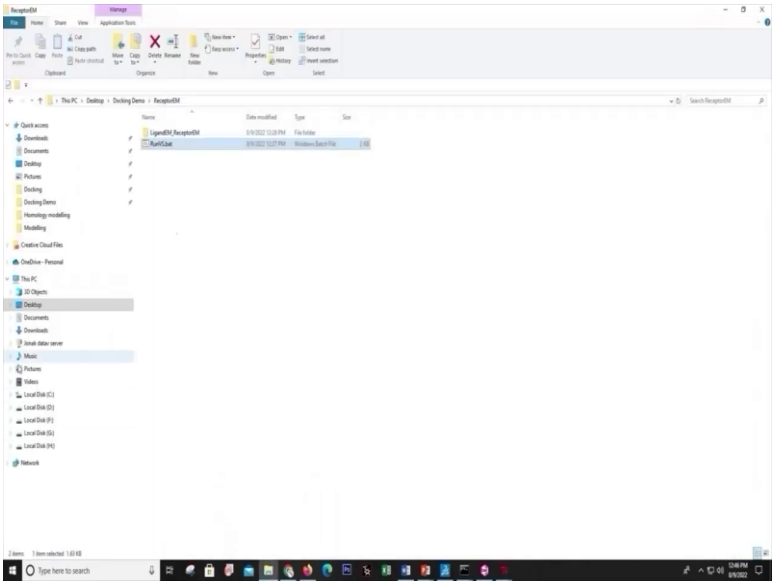
And then we will save this in the same folder.

(Refer Slide Time: 46:21)

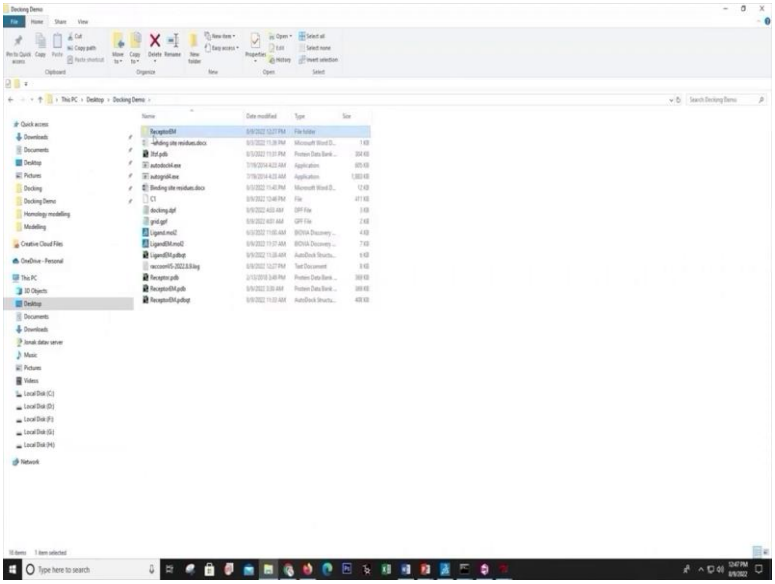


So, here I will write C1 it is complex 1 and then click on Save it will be saved in dot pdbqt format.

(Refer Slide Time: 46:39)

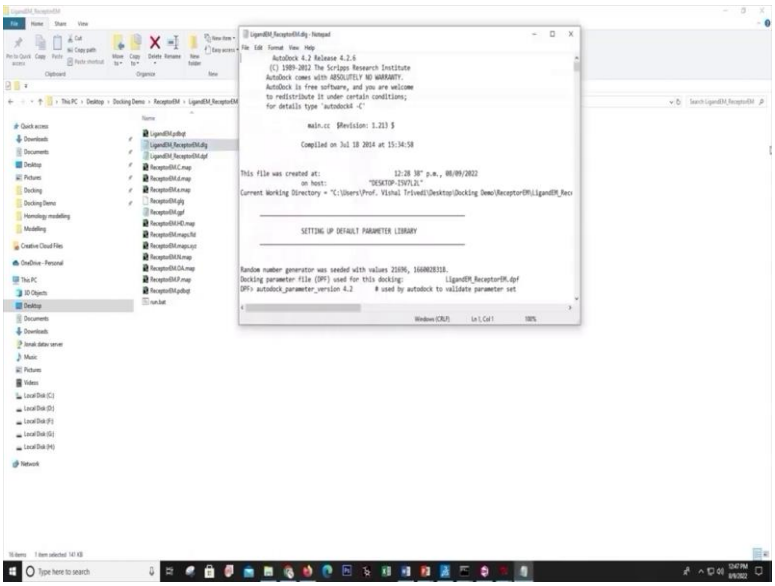


(Refer Slide Time: 46:43)

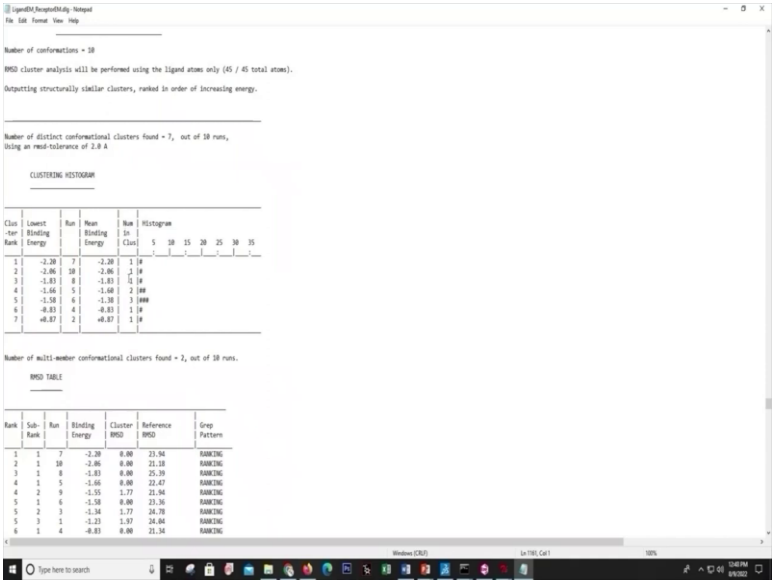


So, now what we can do we can open the folder which we created and then here we can open the new folder created by AutoDock and here we can see the dot dlg file we can open it in notepad.

(Refer Slide Time: 46:51)

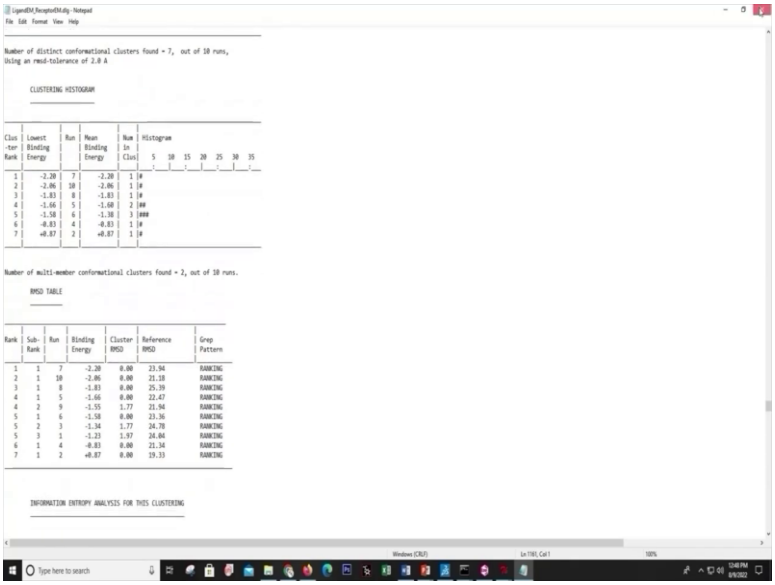


(Refer Slide Time: 46:55)



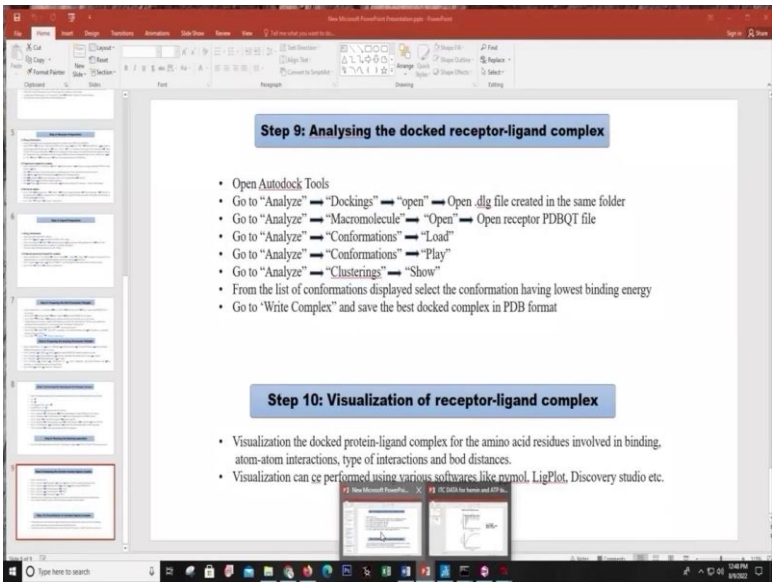

And see the binding energy of in the list of binding energy of all the confirmations it will be at the last. So, this is so, this is run 5 then run 6 after run 10 it will show the table of binding energies. So, here it is this is the table showing the binding energy the run number the mean binding energy of the cluster and the cluster, how many confirmations are there in each cluster.

(Refer Slide Time: 47:44)



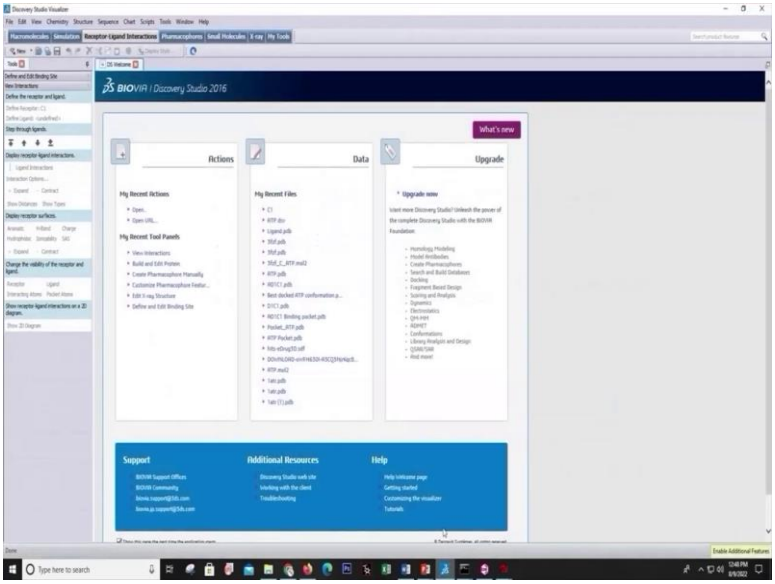

So, this we can just copy and paste in excel or in notepad for our reference.

(Refer Slide Time: 47:59)

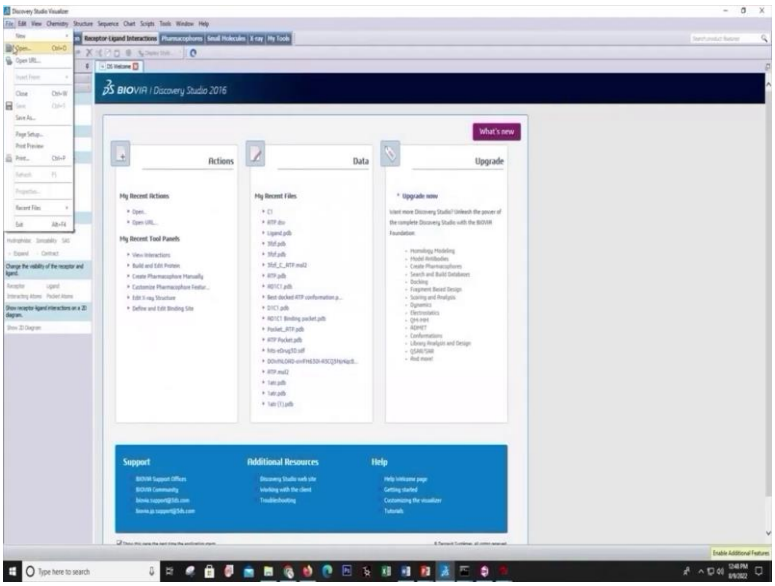



Now, will close this dot dlg file now we have analyzed the binding energy of the receptor of the complex then the next step is the visualization of the receptor ligand complex. So, this can be done by various softwares like PyMol LIGPLOT or Discovery Studio. So, here I will be showing it in Discovery Studio. So, I will open Discovery Studio here.

(Refer Slide Time: 48:19)

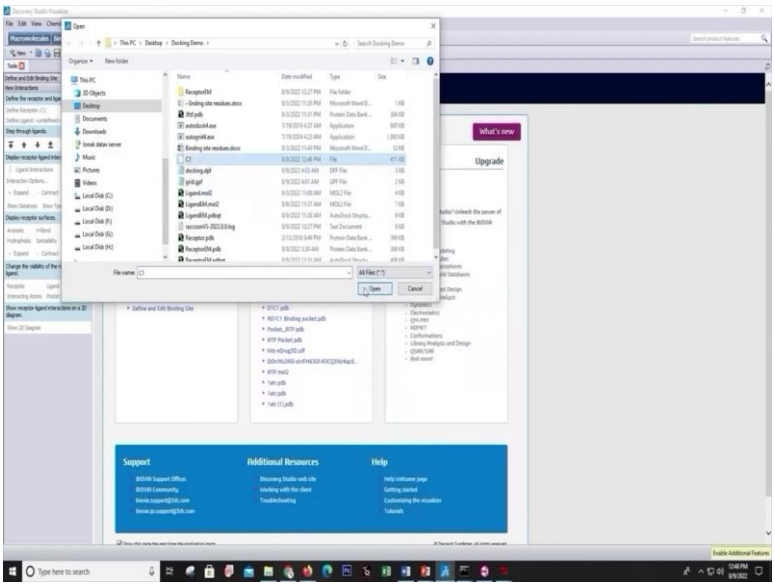


(Refer Slide Time: 48:21)



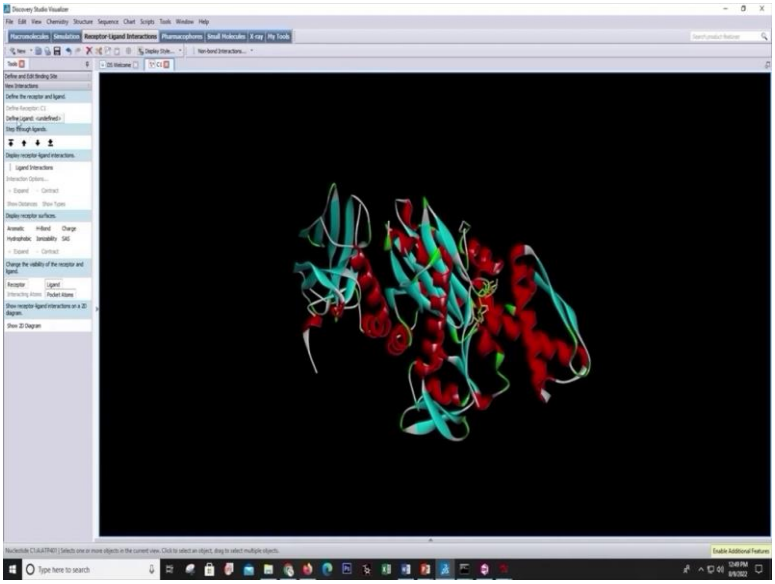

And then I will go to File then Open.

(Refer Slide Time: 48:23)



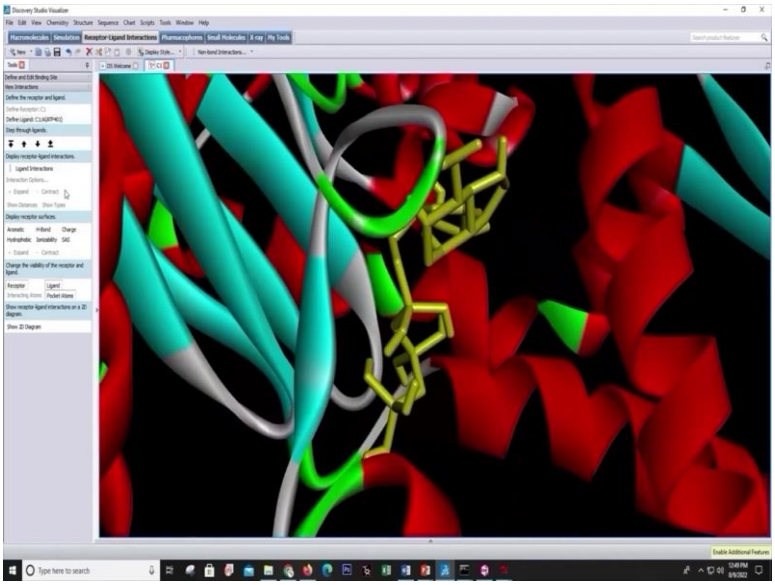

Then I will open and thus C1 which we saved. So, it will open in Discovery Studio.

(Refer Slide Time: 48:40)



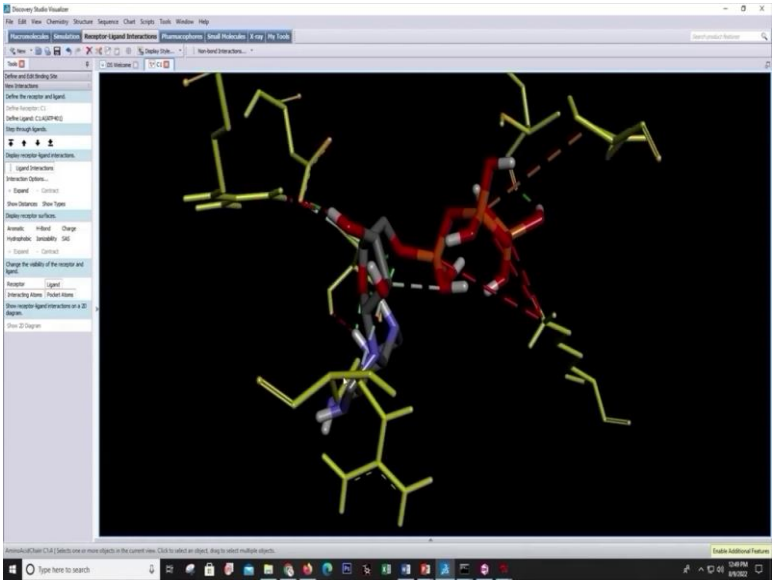
So, here we can see that the ligand is bound here this is the ligand. So, we can click on this ligand to select it will be displayed in yellow and then we will click on define ligand.

(Refer Slide Time: 49:02)



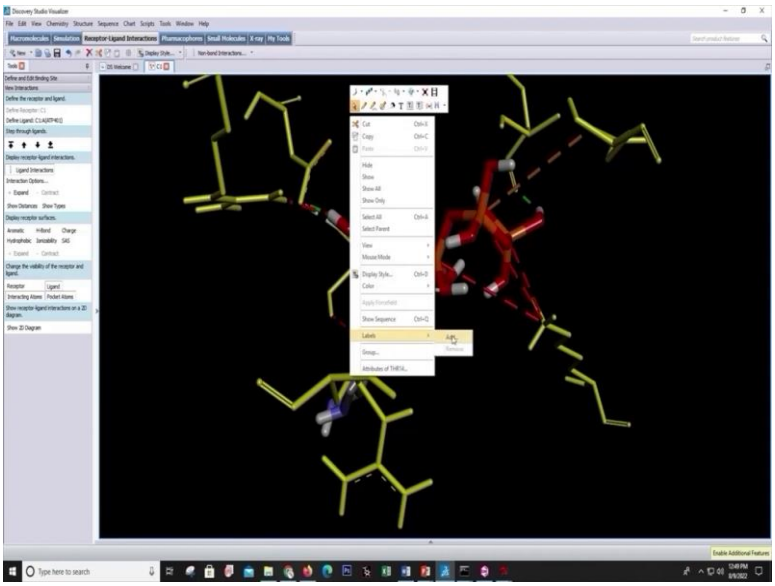
So, it will zoom the ligand here and then we can click on Ligand Interactions.

(Refer Slide Time: 49:08)



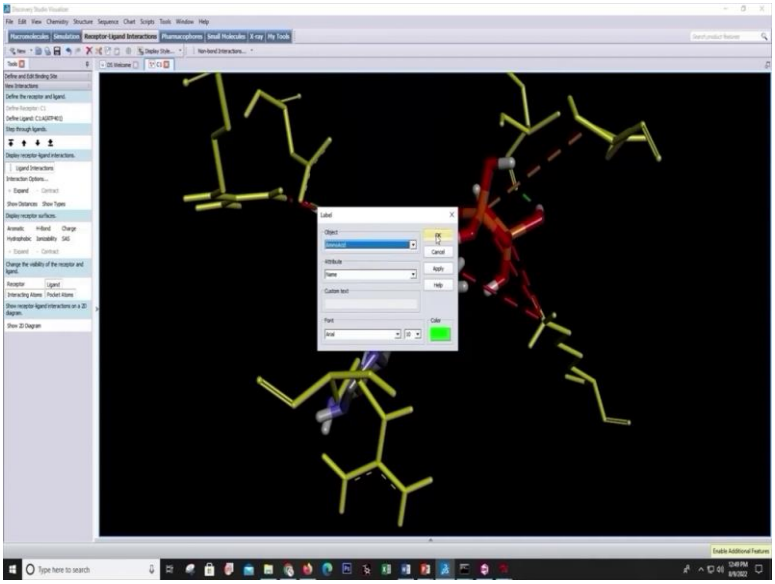
So, it will show the ligand amino acids which are interacting with the ligand then you can just double click on residue and again double click. So, that all the residues will be selected.

(Refer Slide Time: 49:23)



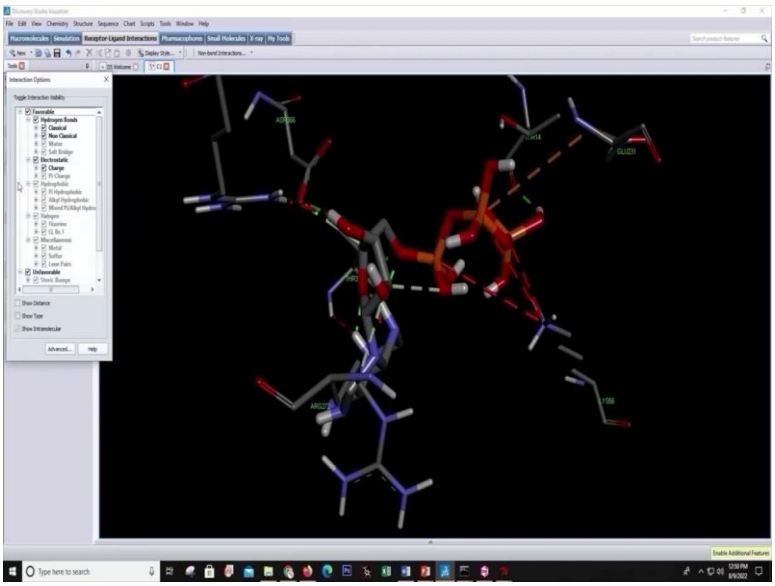
Then we will right click go to Labels and then click Add.

(Refer Slide Time: 49:27)



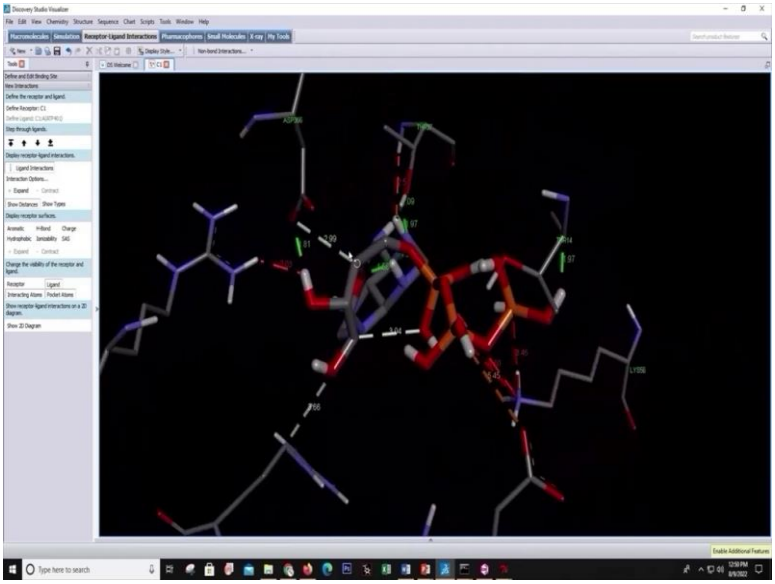
And here in place of Atom we can select AminoAcid we will click Ok. So, it will the it will label the amino acids which are involved in the interactions we can go to Interaction Options here.

(Refer Slide Time: 49:45)



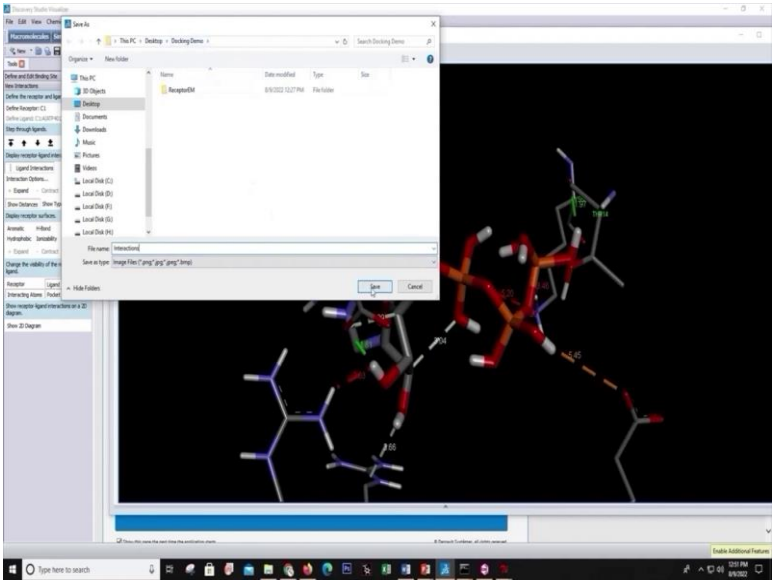
And then it is showing what are the interaction present like Hydrogen Bonds Electrostatic and Unfavorable bonds here we will click on Show Distance. So, it will show the distances of the bonds.

(Refer Slide Time: 50:00)



Now we can analyze this type which type of the green bonds are hydrogen bonds and the orange ones are electrostatic bonds and the red ones are few unfavorable bonds also. Because of these unfavorable bonds we find the banding energy is higher that is only minus 2 point something. So, now, we can save this is structure as well we can take the image from here and then we will go to File Save as we will select here as image file.

(Refer Slide Time: 50:39)



And we can thus give a file name to it and then Save it in the same folder.

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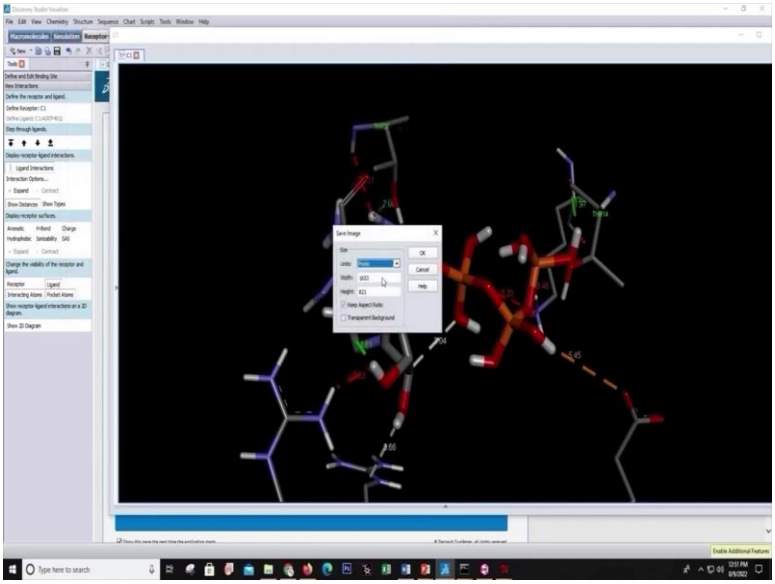
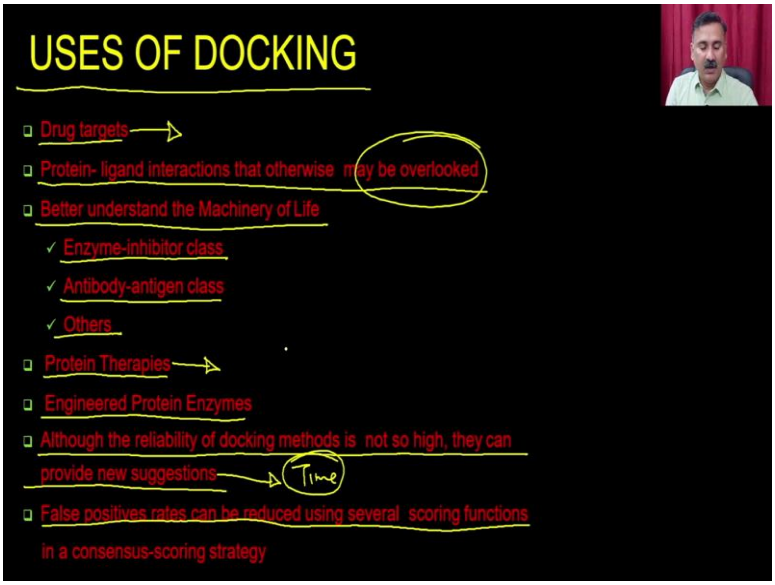


So, this is all with docking of a ligand with receptor.

So, thank you.

So, I hope that you have understood the different steps and the students have very in a detail they have explained how you can be able to perform the different task to perform docking.

(Refer Slide Time: 51:23)

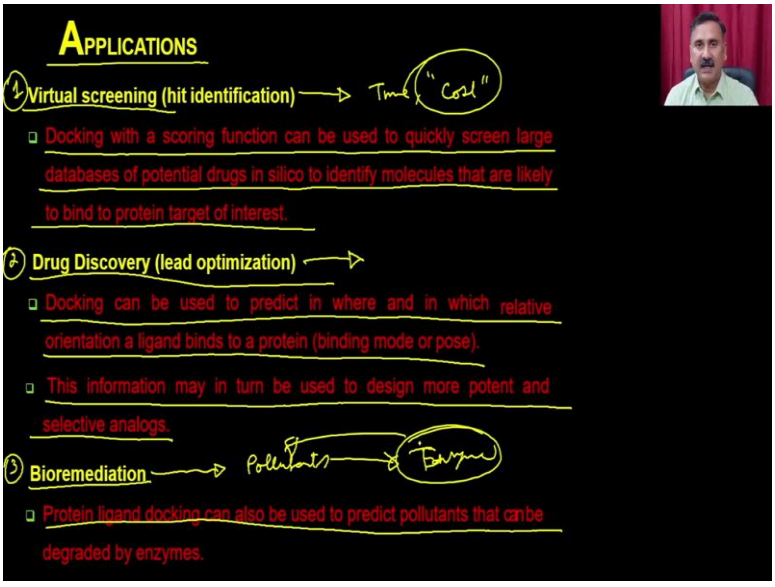


Now what are the usage of the docking right you can actually be able to use to develop the target right. So, you can be able to define the drug targets you can be able to study the protein ligand interaction, which is very difficult to perform under the non silico conditions.

You can actually better understand the machinery of the life you can actually be able to understand the enzyme inhibitor class you can actually be able to identify the (Refer Time: 51:47) antigen complex and other type of complexes you can actually be able to use the protein therapies. So, you can actually be able to define the different types of targets.

You can actually be able to use the molecular docking to even you know engineered the proteins and that is how you can be able to use them for more efficient working although the reliability of the docking method is not so, high they can be provide new suggestions. So, they can actually one of the major idea is that actually going to reduce the time and the false positive rate can be reduced using the several scoring functions in a consensus scoring strategy.

(Refer Slide Time: 52:25)




So, what are the applications of the docking? So, applications are first application is that they are going to be used in the virtual screening. So, docking with a scoring function can be used to quickly screen the large database of protein drugs in silico to identify the molecule that are likely to bind the protein target of the interest.

And this is actually going to reduce the time it is actually going to reduce the it is actually going to make the cost effective and it is going to answer a lot of questions. Because in a single day you can be able to screen 10 different types of targets you can actually be able to define screen the thousands of molecules to know whether these molecules are what is the probability of these molecules and that you can do in a virtual screening. Number 2 you can actually be able to use the drug discovery.

So, you can if you suppose you have a lead then you can actually be able to do the lead optimizations. So, docking can be used to predict in where and which the relative orientation of a ligand binding into the proteins. This information may be in turn be used to design the more potent and the selective analogs. So, drug discovery is also a very very important aspect of the docking.

And then the 3rd is bioremediations. So, protein ligand docking can also be used to predict the pollutant that can be degraded by the enzyme. So, by the bioremediations you can actually be able to select the pollutants which are actually going to be targeted by the enzyme and that is why you can be able to use the specific enzyme to reduce the level of that particular pollutant and this is very very relevant when we talk about the applications of the enzyme.

So, when we talk about the application of enzyme you will understand that there are so many enzymes which are available to you know reduce the level of pollutants. So, what we have discussed in this particular module we have discussed about how you can be able to design the inhibitors.

What we have discussed we have discussed that enzyme has definite areas to bind the substrate or the inhibitor and that area is called as the active site in some cases you can also have the allosteric site where the enzyme is also going to interact and inhibit the enzyme.

And as far as the inhibitor designing is concerned, we can have the multiple approaches you can have the traditional approach where you are actually going to use the enzyme assay as a screening criteria and you can be able to test the different types of inhibitors.

And that is how you can be able to select the inhibitors, which is inhibiting the enzyme apart from that you can also have the targeted approach where you can actually have the

ligand based approach receptor based approach or the computational approaches to design the different types of inhibitors. So, with this I would like to conclude my lecture in our subsequent lecture we are going to discuss some more aspects of the enzymes.

Thank you.