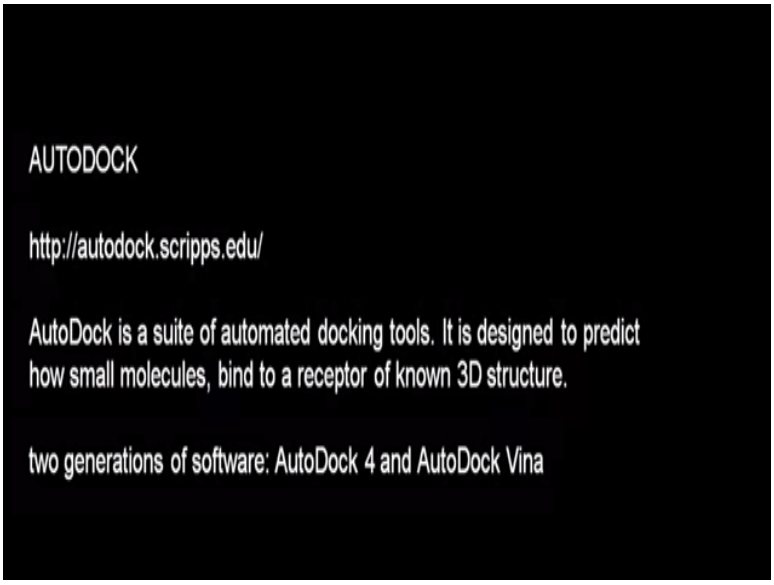
Computer Aided Drug Design
Prof. Mukesh Doble
Department of Biotechnology
Indian Institute of Technology – Madras

Lecture - 37
Docking

Hello everyone. Welcome to the course on Computer Aided Drug Design. Today, we are going to look at docking. We will look at how to use AutoDock.

(Refer Slide Time: 00:24)

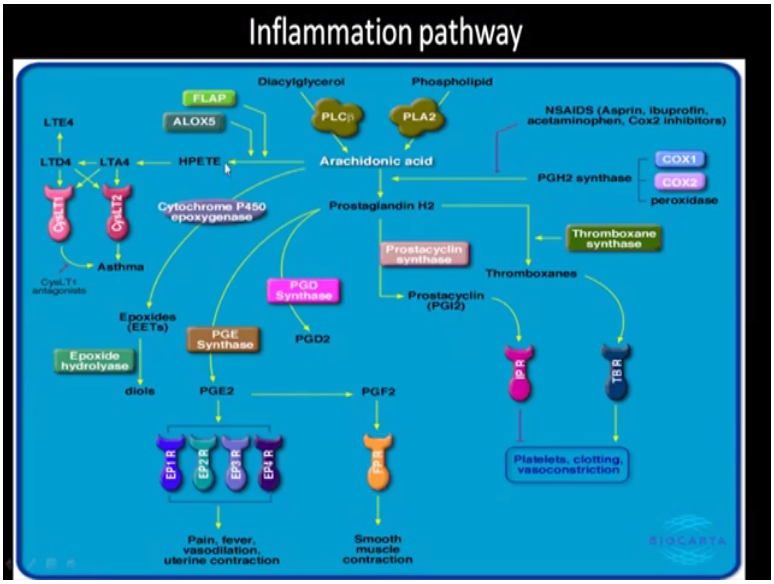


I will show you in an example but you need to later on spend lot of time looking at all the features of the AutoDock software and how to do docking, but I will show you one example. So if you look at AutoDock, this is the website you need to look at which is sort of automated docking tools and it is designed to predict how small molecules, bind to a receptor of known 3D structure. So AutoDock 4 and AutoDock Vina are there.

(Refer Slide Time: 00:53)

And there is another software which is called a Swiss Dock you can use that also, that also does docking and we will look at that as well, okay. So if you want to go to AutoDock, we need to download those files and as you know AutoDock is free software for academic and it got lot of features built into that and, okay.

(Refer Slide Time: 01:23)

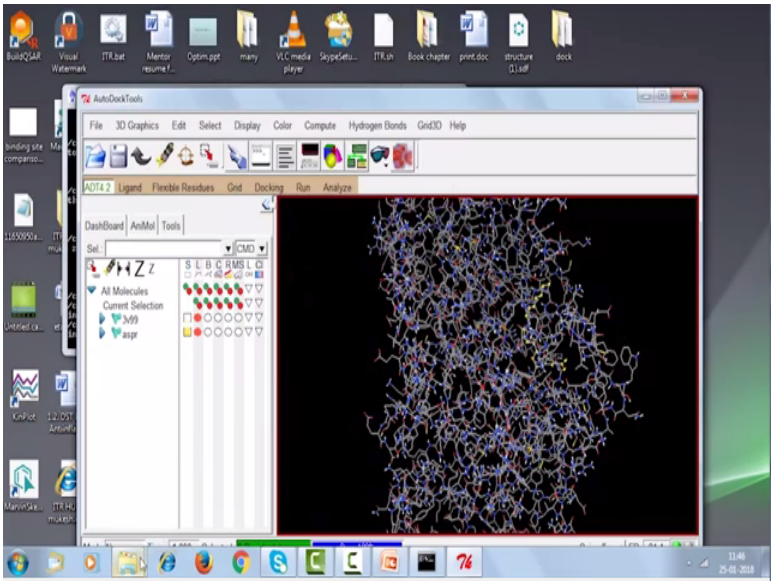


So before it gets loaded, let me tell you something about the inflammation pathway, the Arachidonic acid pathway, I have been talking about that for a long time and okay. So the Arachidonic acid pathway we have the Arachidonic acid which is produced by Phosphor and with these enzymes and from phospholipids.

Now the Arachidonic acid goes down into the Prostaglandin pathway or the leukotriene pathway. So the Prostaglandin pathway we have enzymes like Cyclooxygenase 2 which converts Arachidonic acid into PGH2 and the PGH2 is converted by another enzyme called PGE Synthase which leads to pain, fever, vaso, dilation and so on. Plus, simultaneously Arachidonic acid goes into the leukotriene pathway where the enzyme called the lipoxygenase 5 converts these Arachidonic acid into 5 HETE and so on.

So generally the focus of the drug discovery has been targeting this COX2 enzyme or targeting this PGE Synthase enzyme or targeting the lipoxygenase enzyme because lipoxygenase is also involved in Asthma and so on or simultaneously targeting. So we will look at this particular target and we will look at docking of molecules as you know molecules like Aspirin which is called NSAIDS where or Ibuprofen or there are selective Cyclooxygenase drugs are there in the market.

(Refer Slide Time: 03:08)



Okay. Let us go back to our AutoDock. So we do that. So this is the screen, so either we can download the that means that called Fetch from where, so when you say Fetch from where, we give the PDB that is Protein Data Bank ID then you get the Protein or if you have the protein already then we can if it is already downloaded we can just read the molecules so we can say read the molecules, so I have it and submit and the 5- lipoxygenase is okay-- is called a 3v99 okay, it is a 3v99 file.

So we can download that if you want or we can download from the net also. So this particular, yeah this particular protein, so we can use the mouse button to move over both sides this way and this way, rotate it or then we can enlarge it smaller using that gap other button, okay. So it is got two okay portions. It is got A as well as the B so we can delete one of them, the B especially. Before doing that we need to prepare this protein, we cannot just start docking.

So first we say Edit and then we say Delete and then sorry delete water okay so all the water that is present because when the protein is crystallized there are lot of water molecules that maybe inside, after all protein is enzyme, so all the water molecules also be shown which may get deleted. And after that I will now try to delete and as I said earlier we have A and B residue so we will try to delete the B. So what do we do is we say Select and then a Select from String okay select from string okay.

Then we go to change as you can see A and B. So we will select B okay then we say Add and then I will say Edit then we say Delete and then delete the selected atoms, okay. So as you can see in this picture, okay delete selected atoms, continue. Okay, so these now deleted, only A is there and this is what we are going to use one as a target in our study. Now we need to add hydrogen because as you know when protein is crystallized hydrogens are not visible in the X-ray, so we need to add. So the software will add hydrogen based on the valence and so on.

So say Edit and then you say Hydrogen okay then we say Add, okay then we say S, it will add hydrogen okay. Now we need to add charges to these various elements okay. So we say Edit and then we say charges you can see the charges then we say Kollman Add Kollman Charges, protein Kollman charges is so much for the entire protein then again we say and Edit charges, we say Edit charges then will say calculate a Gasteiger charges, computer Gasteiger charges.

Okay, I talked about Gasteiger charges long back in the course okay so this is the protein Gasteiger charges. Okay, then I will say Edit Atoms; assigning a 84 type okay this is just assigning okay. Now we need to save this file because lot of things have been done, this is the correct protein, so when you download a protein from the PDB it is not the correct protein for

docking, we need to delete water, we need to add hydrogen, we need to remove residue which are of no use and then we need to leave a software calculate charges and so on.

Okay now we need to save this file because this is the correct file okay, Save and then say the format in AutoDock is PBDQT file so we save in the PBDQT form okay then we say it is not known then we say okay, so the file is saved and so I have created default as you can see here 3v99, I have created a default folder and that is called a doc so we can preference, in Preferences we can say set as you can see everything startup directories everything is there.

So all the results all the reading automatically go there, okay, so the preference is considered to that, you understand. Now we say Select, Select from String, Residue Sets, okay Residue Sets, ligands. There are some ligands from which the protein has been crystallized okay so set ligand okay, wait a minute-- okay, Residue Sets select and from string Residue Sets then so the Ligand okay then it is done okay. Then this list then ligand, ligand input open.

Now we can read ligand okay, whatever ligand you want to read, if you want to dock for example, I am thinking about the Aspirin so we can read it as a molecule file, Aspirin in the form of molecule file, okay click this molecule file open. Okay as you can see here Aspirin is there, do you understand? So Aspirin is there and its data is molecule file. Now ligand has to be saved as PBD QT of course and because AutoDock regenerating in that format only it will be QT, so ligand output then save as PDB QT so to be saved in the default folder Save.

Okay now we need to now create the location where we need to do the docking. And now this particular PBD particular protein has Arachidonic acid bound to and that is how it is been crystallized—we can look at where the Arachidonic acid is there so we can say Select, you can say Select From String then we can say then we say Residue Set, ligand then we say, so Arachidonic acid will be there as you can see here so we can read that also, we can say display residues, display residues, yeah you can see that. As you can see events are there okay, Arachidonic acid is here okay that is there, okay.

Now we need to create the grid that is the location where we want to do the docking. Okay, so the command here is grid macro molecule choose, then we say 3V99, select molecule, so you can save it as PBDQT again, okay so again it is saved same place okay we want to replace it, Yes, we say. Now, okay no problem. Now we say grid—Grid box. Okay. So as you can see here it is created a grid box but I am I want to make the grid box in this region okay so I can then move it here.

So I can spacing I can move it here, I see on that active site and I want my Aspirin to do bind to that active site. So as you can see I am moving the grid box here right, okay. And I can also look here it and spacing-- it comes down here, so I want to push it up, I want to move it up here, yeah I want to move it up here okay. We want to still move it this side right? Yeah. So now if you look, now we can move this bigger also if you want and so we can make this bigger or we can make this smaller and okay so depending upon how much region you want to cover in your, okay.

So we feel that this would the entire region where we want our Aspirin to dock and then looking that the different energy levels, okay then we say File and choose, okay it is created that gird okay, so docking can happen there. Now we say Grid output, so we want to output file to be saved say gpf okay filename we could give same 3v99 and gpf that is Grid parameter file that is called grid parameter file, okay. Now the output from the grid will come in GLG file, Grid Log File, okay then we say Run and AutoGrid.

So, okay we say Browse then you will have the AutoGrid, exe file should come there okay then we will see Grid Parameter File will come here everything and then say it is automatically makes a Grid Log File where result would be grids will come. Okay then you say Launch, okay then it will make the Grid Box this will take little bit fine. Once the Grid has been created this box will close and then after that we have to run AutoDock okay.

And so there will be a GLG file which will have the results of the GLG file which will have the results of your gridding, you can have a look at that file as well after that we will run the AutoDock, okay. So again when you will run the AutoDock we can give confirmation and for the

ligand to take into the active site, okay. And generally we have to do 100 confirmation at least but for our convenience we will just run 10 confirmations okay.

And then see how it looks like. So in 10 confirmation means the Aspirin will be placed at 10 different locations inside at the grid box, it is in active site the Grid box is created in active site and then it calculates the non-bonded interactions, okay things like Van der Waal nitrogen, electrostatic and then it gives you the binding energy. So from those binding energy, you can select whatever you want to.

So assume that, it will take little bit more time so, that is the problem, depending upon how big is your grid box and how big is your ligand and how big is your protein, it can take lot of time and how fine is your mesh, it can take lot of time in the calculations, so we need to wait, that is why we have computer which maybe faster running and to do this type of job, okay. Okay once we have done that, what we do is—okay once we have done that say, docking.

So docking macro molecule, residue file, set residue filename, so we select the protein, add which is your protein then we open and then we say docking, select ligand and then we say choose and then this is aspirin, select ligand, okay then accept and then will say docking then will say output okay. This is the (()) (20:39) algorithm method, let us not go into that okay. Okay then here we give the dpf that is Docking Parameter File using 3V99 and dpf, okay, save. Okay.

After that we say run, AutoDock, okay so here again we need to give AutoDock exe file as you can see here we have the AutoDock.exe just like we had AutoGrid we have AutoDockc4 exe file, we give that and then we say browse then this is the docking parameter file, this will open so automatically DLG file is there then we just click on the Launch. And once the docking is completed it will stop and then we can look at the DLG file that is Docking Log File that gives you the answer, okay.

So that is launched okay and then it does the job. Okay so if you look at the results of the docking log file like I said DLG file docking log file you look at the results, okay here it is. So

this is how output comes in, this is automatic docking flexible ligand, the ligand is flexible and your macro molecule is also flexible which is 4- in your AutoDock 4.

So we see lot of setup default parameters chosen that is mostly in the top okay then lot of parameters for the atom—for each type of atom what that parameter that it has chosen and then as go down, down, down, and then what type of equation we selected for some of the non-bonded interactions, you can see here, okay r raised to the power 12 (()) (23:23) and 6 and selected okay or some of the non-bonded interactions, what type of equations has taken as you can see here, okay.

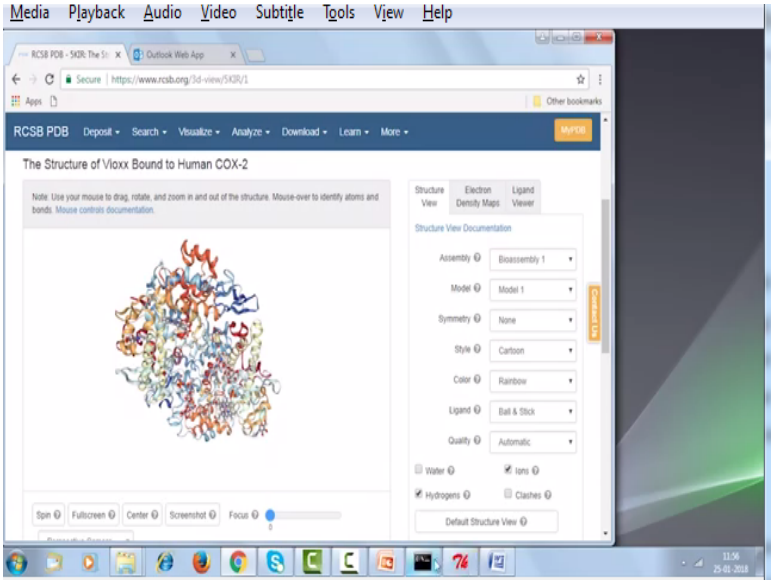
And then finally it will give for, as you can see how many grid points (()) (23:45) dimension and just go down, down, down. So we can do a detailed understanding of what is happening and then you start doing run, Run =1 that is here I have given 10, normally it is good to do 100, here I have given 10 for fast as you can see Run = 2 it gives you estimated free energy of binding, Run = 3 and like that 10 different confirmations location inside the active site and it calculates the non-bonded interactions, okay.

And finally, it will give all the 10 results and then it will place them with the lowest binding energy to the highest binding energy, okay yeah. As you can see here, yeah so as you can see here -5.56 is the binding energy that is the best we got in this one. You can run 4-grid run, you can run 5-grid run and so on, so this is the best for location confirmation to ligand into that. Now when results are fluctuated and as you can see – out of that 10 runs 6 runs and the ligand took confirmation to match this particular binding energy, okay.

And 3, 3 of those confirmation came up with this, only one came up with this so-- and most favorable confirmation and most favorable binding into the active site this is -5.42. So we looked at them Aspirin binding to lipoxygenase enzyme, lipoxygenase is in the leukotriene pathway, Arachidonic acid inflammatory and which is leading to leukotrienes which has got a relation to Asthma bronchitis okay.

So and using AutoDock you are able to look at how Aspirin binds to this particular enzyme called 3V99 which is obtained from the Protein Data Bank and this particular enzyme also has Arachidonic acid inside when it was crystallized, okay. We can select different lipoxygenase also if you look go to PDB database you will see that it may have many, many blocks available in that.

(Refer Slide Time: 27:09)



Depending upon your requirement what you want to do, so for example just like 3V99 which has got Arachidonic acid that is a 3V98, okay so you can look at different enzymes Aspirin binding to that, this is like I showed how to do that, you can also do that okay. So we can take download this enzyme then need to look at the water, adding hydrogen, adding charges all those things we need to do before that is called the preparation, enzyme preparation.

Once you have done the enzyme preparation you get the ligand okay. And then this is the COX-2, VIoxx Bound Human COX-2, okay. And so once you have prepared the protein you save it as PDBQT then you take your ligand like Aspirin or whatever it is and then you, okay then you again you can get it as a molecule file then you save it as PBDQT and then you prepare your grid box around the active site.

Like I showed you and big rectangular grid box and once you know the active site you can prepare it where the docking is going to take place, once that is prepared the Grid Parameter File-

GLG and is given and the results will be in GLG Grid Log File. After that you handle AutoDock, AutoDock.exe then again here you give the docking parameter file and the result will come in docking log file, DLG.

So as we showed you, we can see the results and then I gave in this example only 10 okay so it will show 10 result of the docking okay and you can see the binding result here okay the best one is given here -5.56 and your run code is second best and third best in all that. You know that and then you can also cluster and clustering -5.42 most of the-- hence give this particular binding energy 6 of them out of 10 okay so the chances are this is the most preferred confirmation into the active site, okay.

And Swiss Dock, okay. So this is how AutoDock is run, okay. Thank you very much for your time.