Bioengineering: An Interface with Biology and Medicine
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Lecture – 39
Bioinformatics-II

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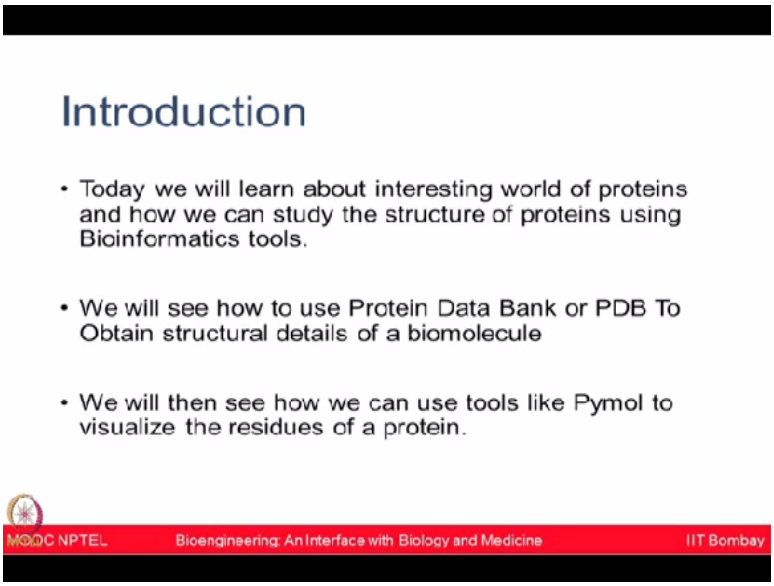

Today, we will learn about the interesting world of proteins and how can we study the structure of proteins using bioinformatics tools. We will see how to use protein data bank or PDB to obtain structural details of a biomolecule. We will then see how can we use tools like PyMOL to visualize the residues of a given protein.

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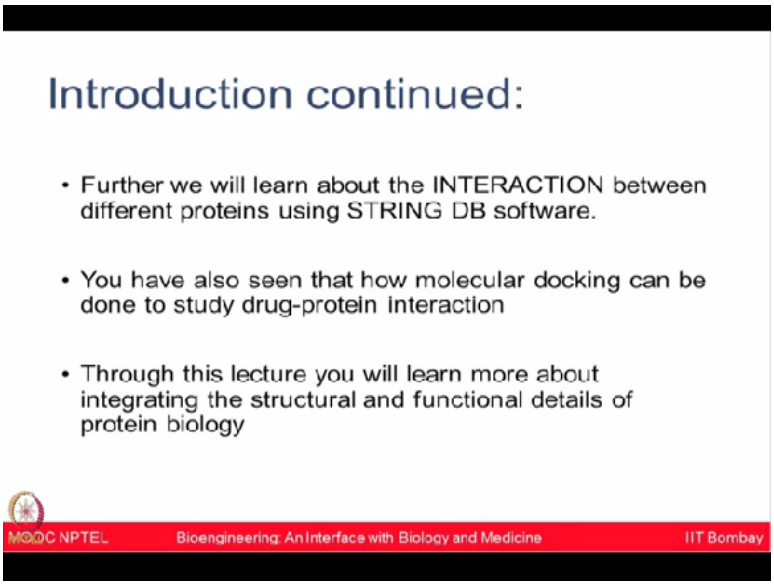

Further we will learn about the interaction between different proteins using STRING DB software. You have also seen that how molecular docking can be performed to study drug-protein interactions. Through this lecture, you will learn more about integrating the structural and functional details of protein biology. (Video Starts 01:05 - Video Ends 21:58)

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Hi. I am Shuvolina Mukherjee. I am a final year PhD student under Professor Sanjeeva Srivastava and I will be the TA for this course.

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Welcome to the MOOK-NPTEL course of Bioengineering, an Interface between Biology and Medicine.

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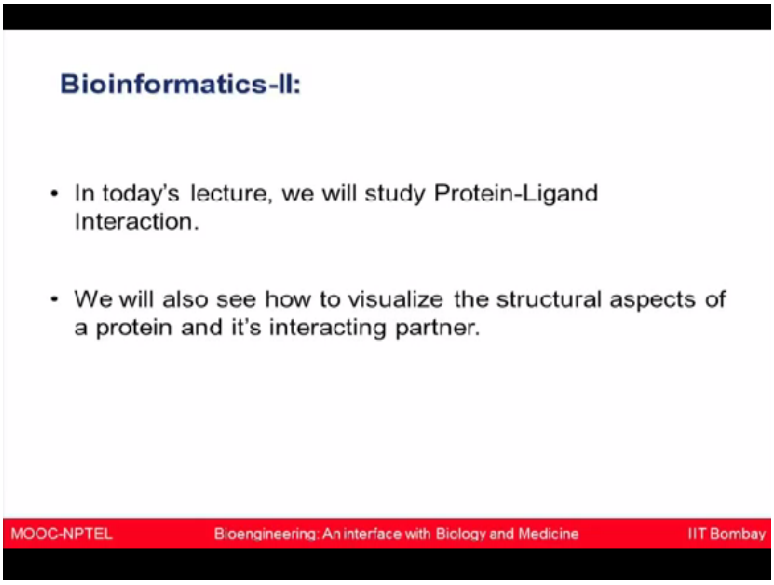

Today in this bioinformatics assignment II, we will study protein and ligand interaction and how to visualize the structural aspects of a protein. Structural bioinformatics helps us to visualize important biological molecules and their residues. We will learn a few tools that can help us in learning about the structural aspects of a biomolecule.

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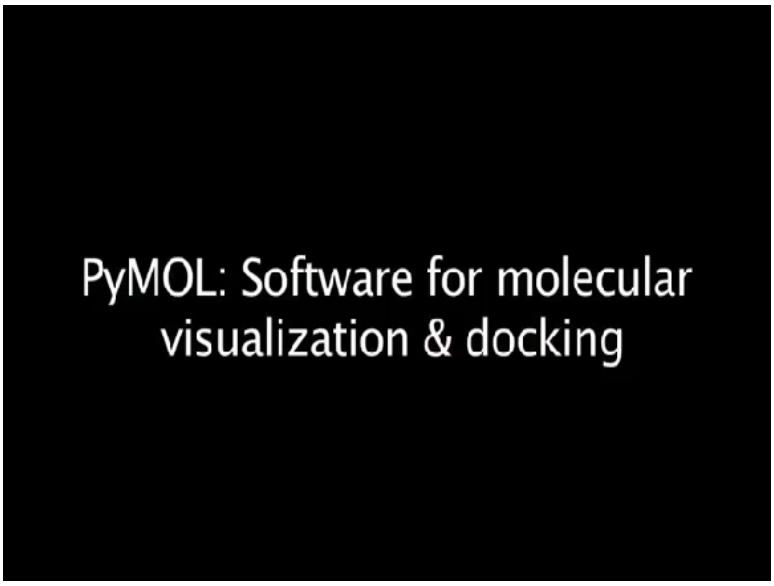

One of them is PyMOL. PyMOL is a molecular visualization system.

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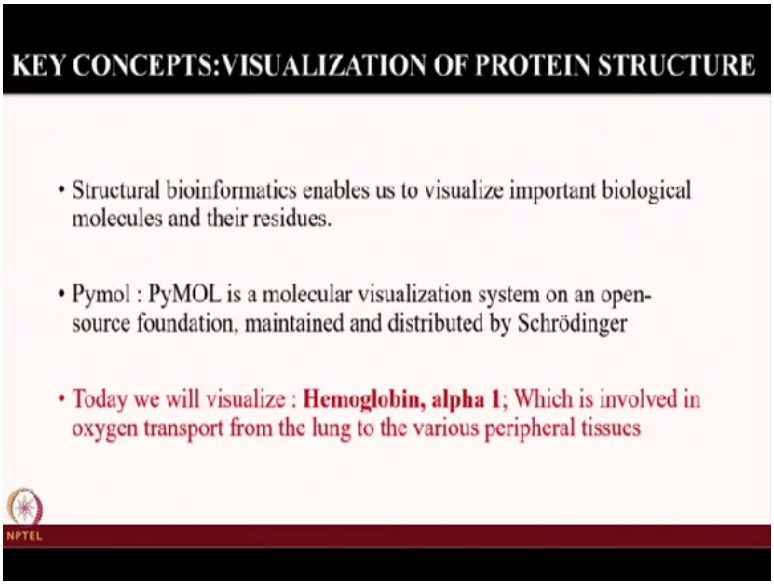

And it is an open source software. We will visualize hemoglobin alpha 1 whose genetic aspects you have already learnt in the previous assignment. As we all know, hemoglobin is an important molecule which is present in the blood and it is an oxygen carrier.

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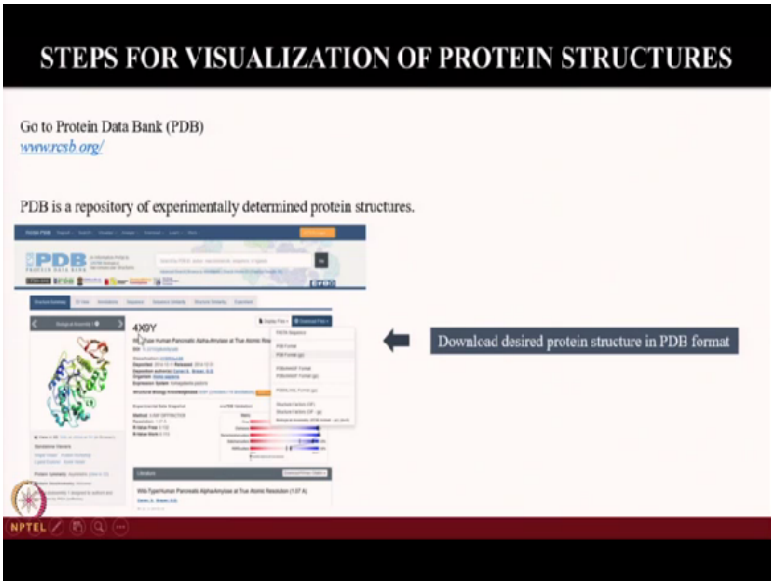

So we will first go the protein data bank. Protein data bank is an open data base for protein structures which are experimentally determined. So we can go and search for any protein in the protein data bank and it will give a unique ID which is also known as the PDB ID of the protein.

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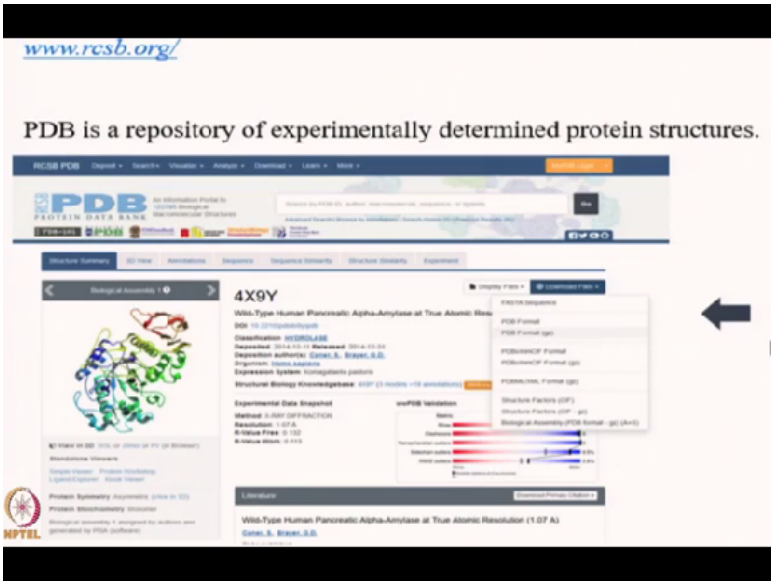

We can go and search for the protein of interest and after that in the download options, you can see various formats of this protein structure. You can download the FASTA sequence, you can also download the PDB format of the protein.

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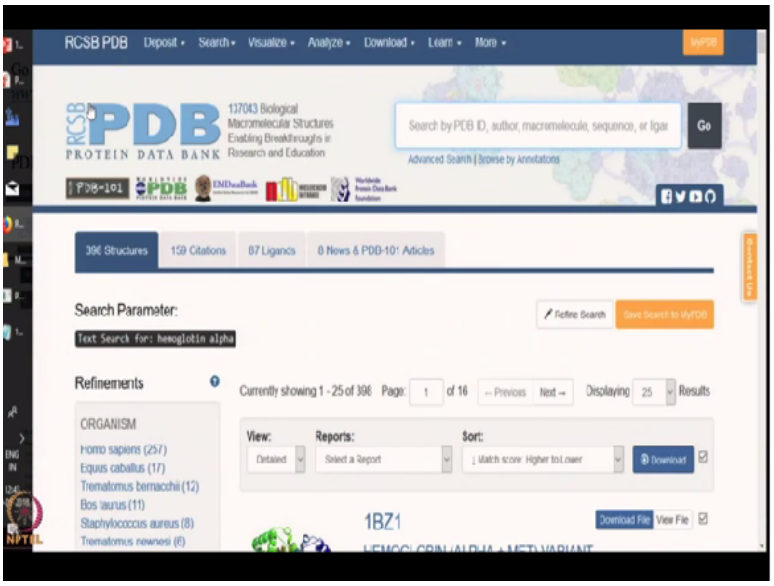

Let us just now directly go to the PDB. Now if you go to the protein data bank, you can see there are many tabs. So here you will search for hemoglobin alpha.

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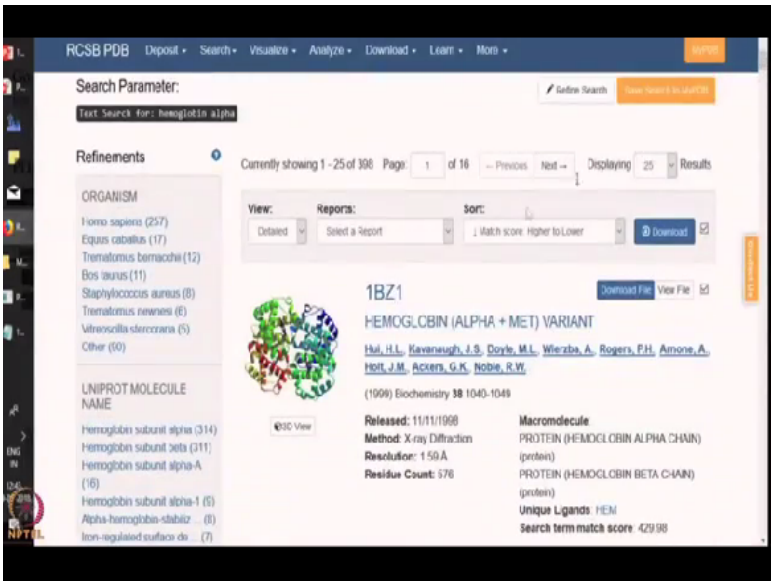

Immediately after the search you can see there will be various structures of hemoglobin available.

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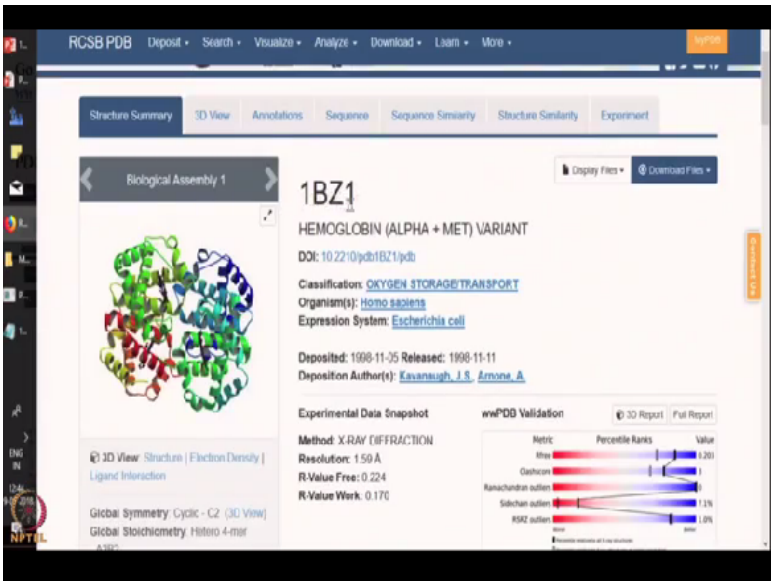

Let us click on the first one, 1BZ1. Now if we click on 1BZ1, we will get all the information available on this molecule. So you can see various information. We can see the 3D structure as well. So let us just go and see how the molecule looks like.

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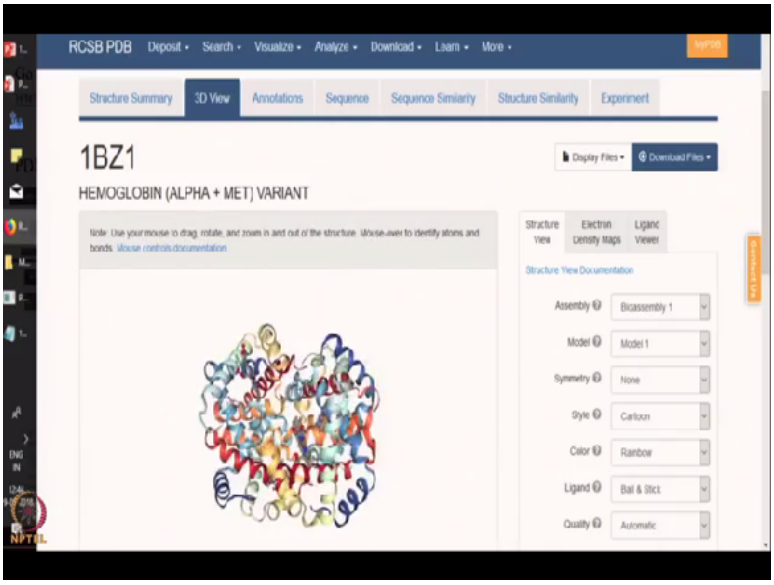

So you can see PDB has an inbuilt interface which will help you in visualizing the structure of the molecule. You can also see there are 3 tabs, structural view, electron density map and ligand view.

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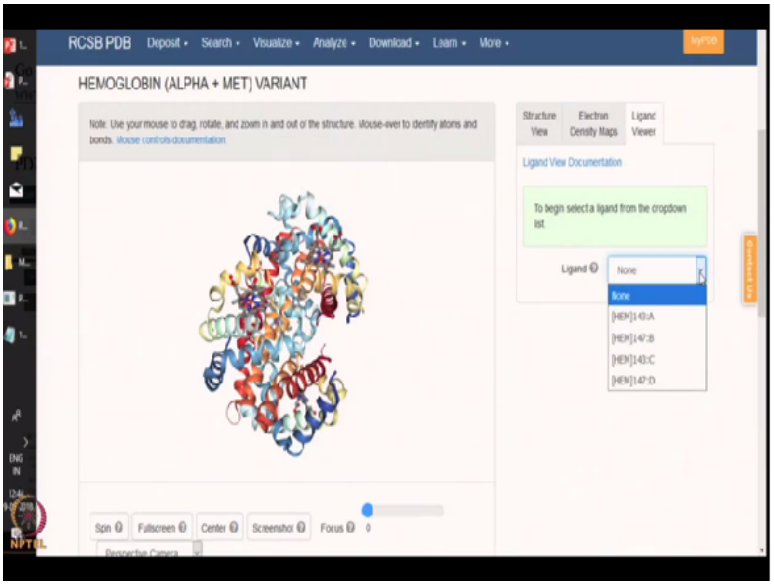

So you can see there are lot of ligands bound to the hemoglobin molecule.

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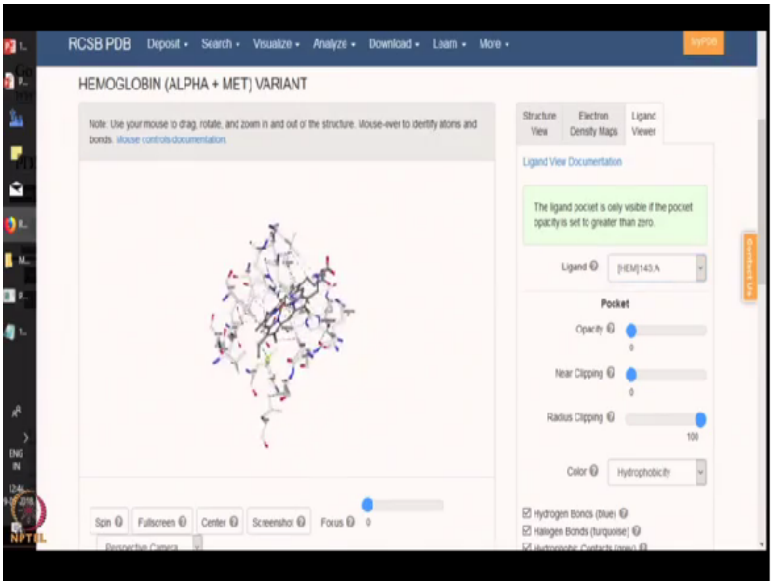

So you can click on it and it will give you a ligands view of the molecule.

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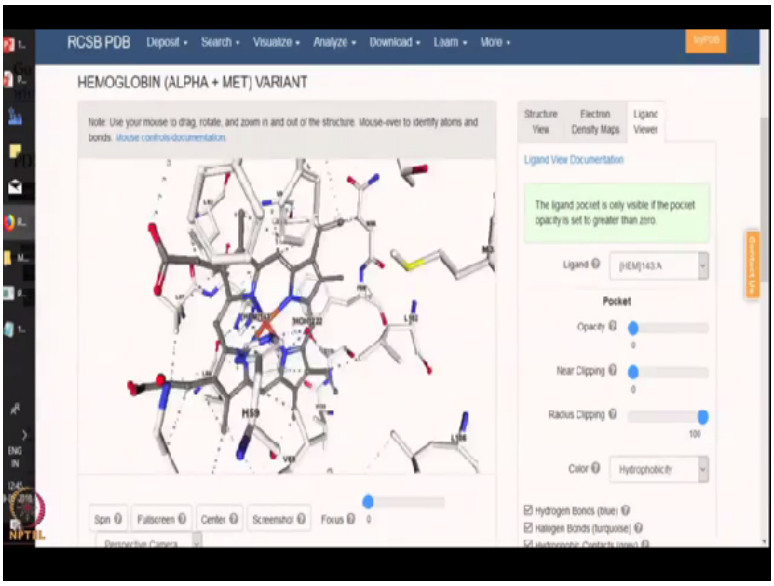

Here you can zoom in and see the residues. You can see the heme residues bound to the hemoglobin alpha subunit.

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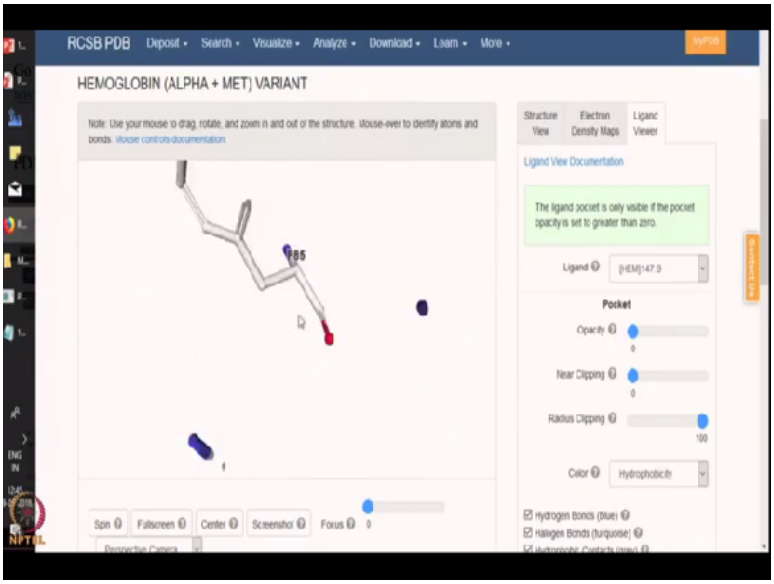

You can click on the next one and again PDB will give you the exact location of the molecule. Thus protein data bank is an excellent repository wherein you can find out the structural details of a protein.

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We can further zoom in and find out a lot of other details like we can find out the hydrophobic residues, the bridged hydrogen bond, the metal interactions and so on.

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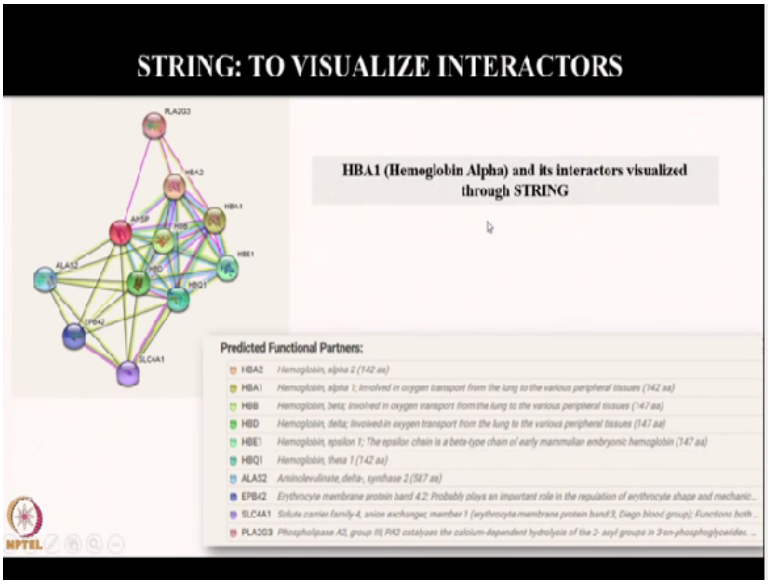

Now moving on, we will explore another tool called the STRING. So the string is like a network. So string means meshwork and in this, the string means a visualization of all the interacting partners of a particular molecule. In this case, it is hemoglobin alpha. So let us now go and see how STRING can help us in identifying interacting partners of hemoglobin alpha.

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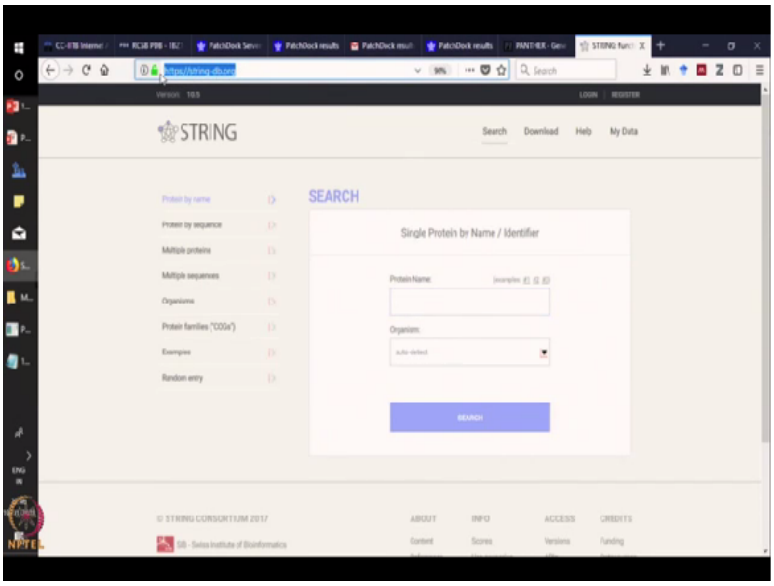

We will type for STRING DB and we will get an interface like this. Here we can directly type hemoglobin alpha.

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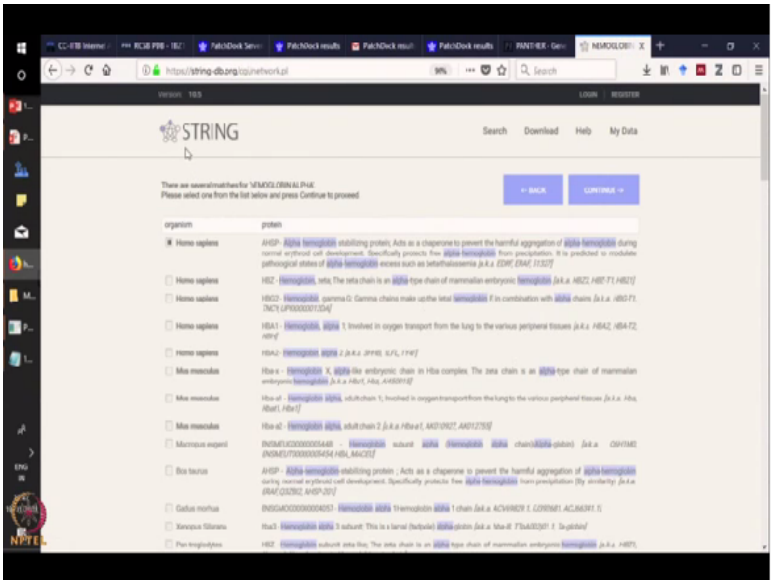

Now we can see that STRING has found out several matches for hemoglobin alpha. So we have hemoglobin alpha, we have zeta, gamma, but this is the most specific search that comes on the top and the species of our interest is of course human, Homo sapiens. So we will click on it.

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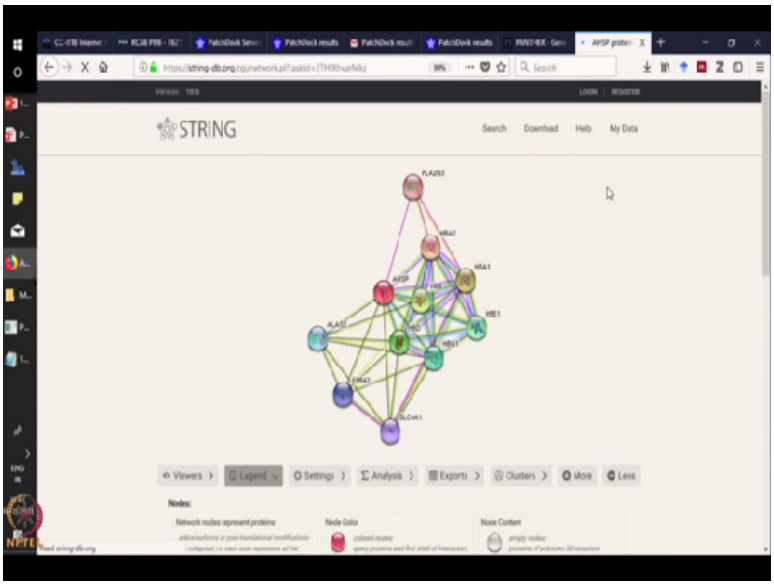

As you can see, this is what STRING has generated. So this is our molecule of interest. There is hemoglobin alpha.

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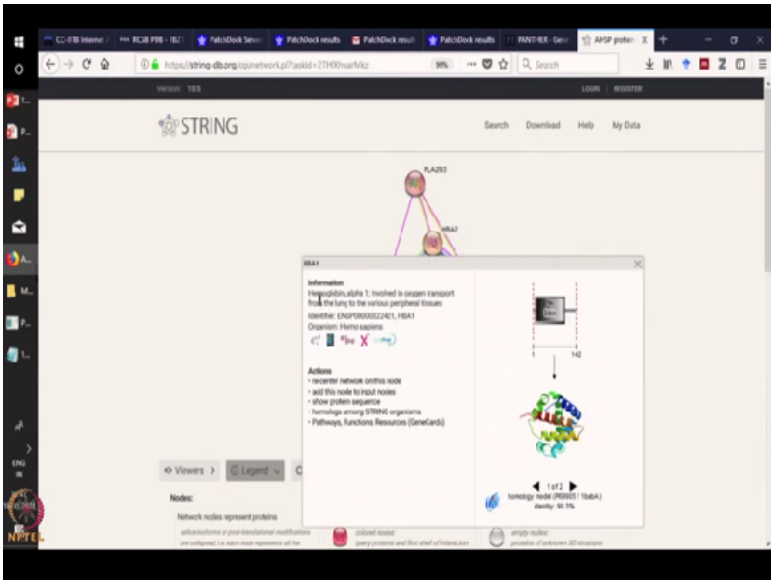

So if you click on it, STRING will give you all the information related to this protein. As you can see, it is determining the structural and functional aspects simultaneously. It is involving oxygen transport from lung to the various peripheral tissue, just the way we learnt. Furthermore, if you want to know what are the proteins that are closely interacting, you can click on the near molecules.

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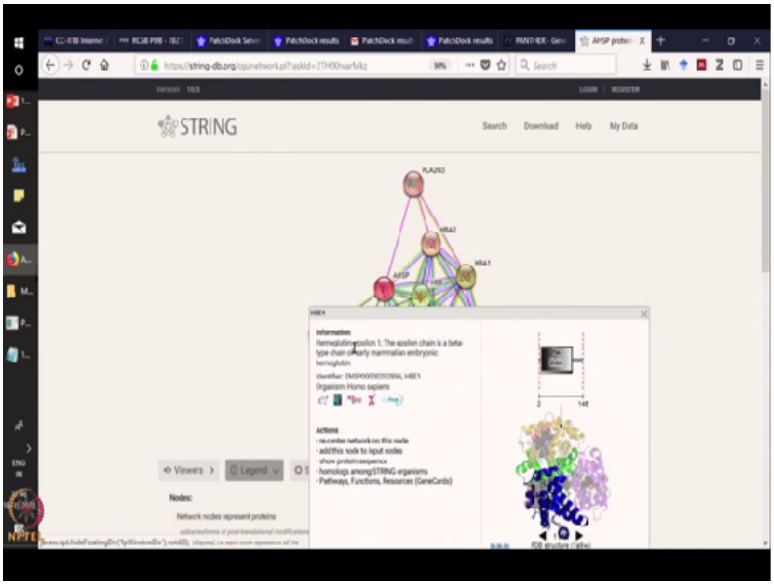

This is again another molecule of hemoglobin only, epsilon chain. If you want to know some interactors that are interacting but in a distant way that means it is interacting through other partners. Then you can click on these molecules. Let us see what it does.

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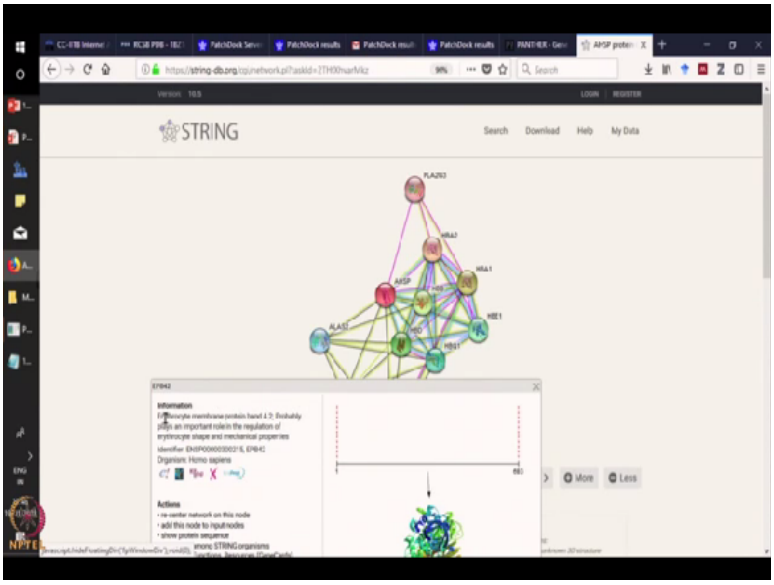

As you can see, this is also a protein that is related to the blood only, erythrocyte membrane protein band. So in this way, you can query for 1 protein and get to know a lot of other interacting partners of this protein through STRING.

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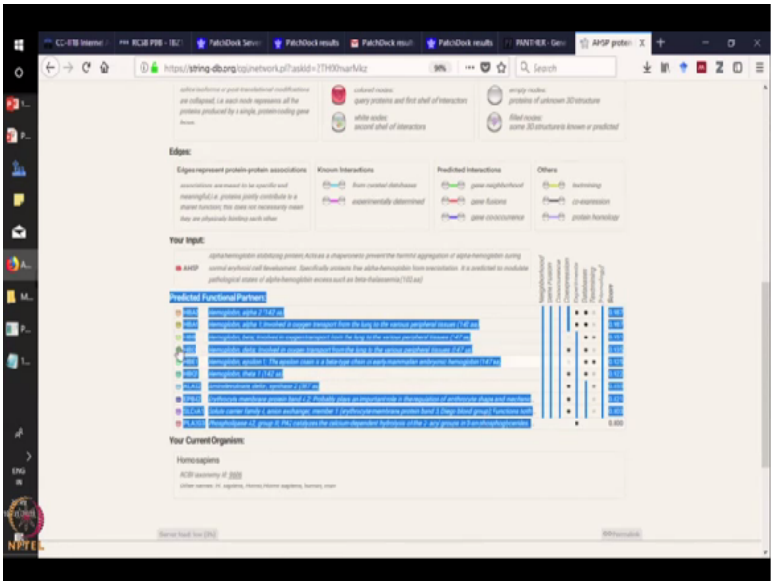

Furthermore, we can get a list of all the interacting partners of this protein. So you can click on it and save this interacting partners. This information we will further use for probing the ontology or doing some molecular based classification in the next part of the assignment. So we just saw how to use STRING. Now what we will do is, we will try to find out more on these molecules that we just found out our interacting with hemoglobin alpha 1. Using another simple online tool called the PANTHER.

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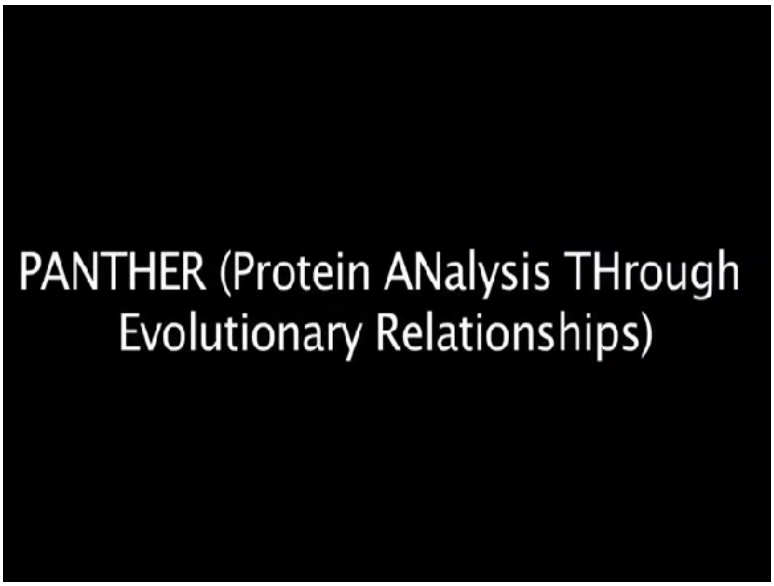

So the PANTHER is again another gene ontology software which will use several statistical parameters and help you to know more about the molecule. Let us now go to PANTHER.

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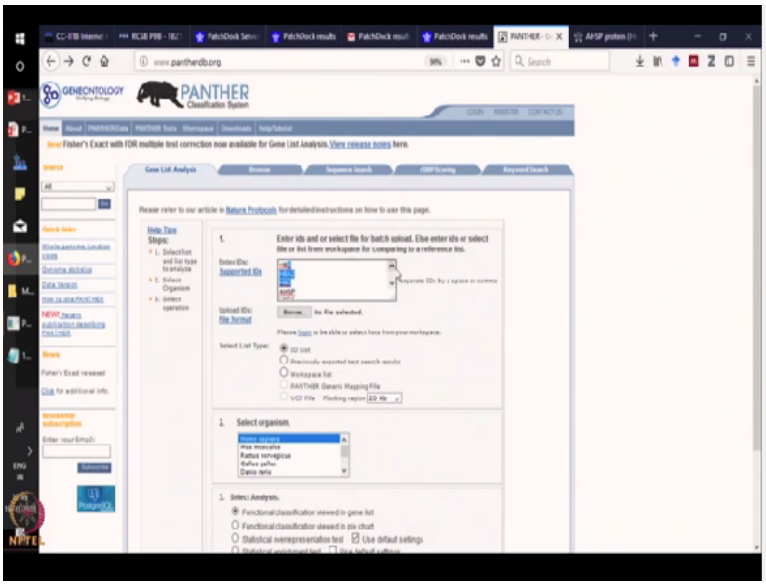

So I have now inserted all the list of the interacting partners of hemoglobin alpha which we just found out from STRING. Again the species that we will select is Homo sapiens. If you want, you can select something else if you have some other protein of interest from other organisms. So I have imported the list from STRING. This is the list of interactors that we just found out from STRING and we have to select the species of interest which is Homo sapiens in our case. We can select the list that we just found out from STRING and we can query it in PANTHER. We will select our species which is Homo sapiens and we will submit it.

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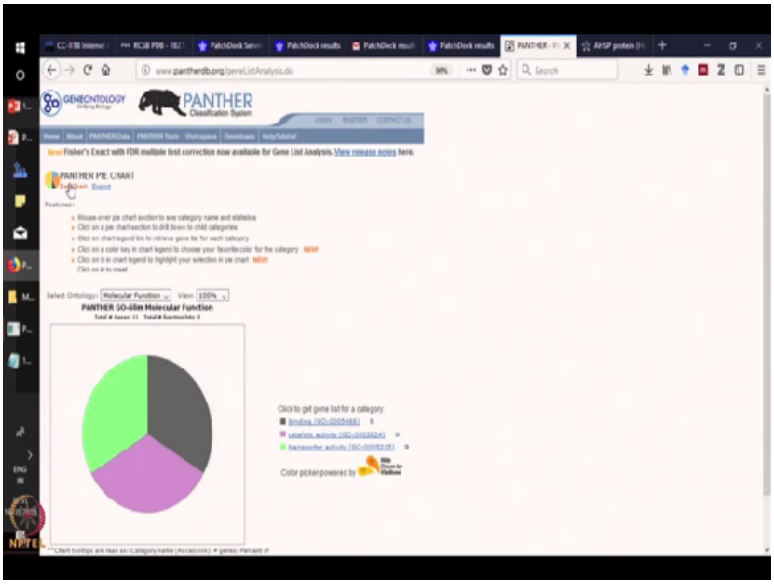

Here as you know, PANTHER just gave you the molecular function of all these molecules and most of the molecules that were interacting with hemoglobin alpha chain are involved in binding,

catalytic activity and transporter activity. If you want to know some other details, you can also click on the other tabs that are available in PANTHER.

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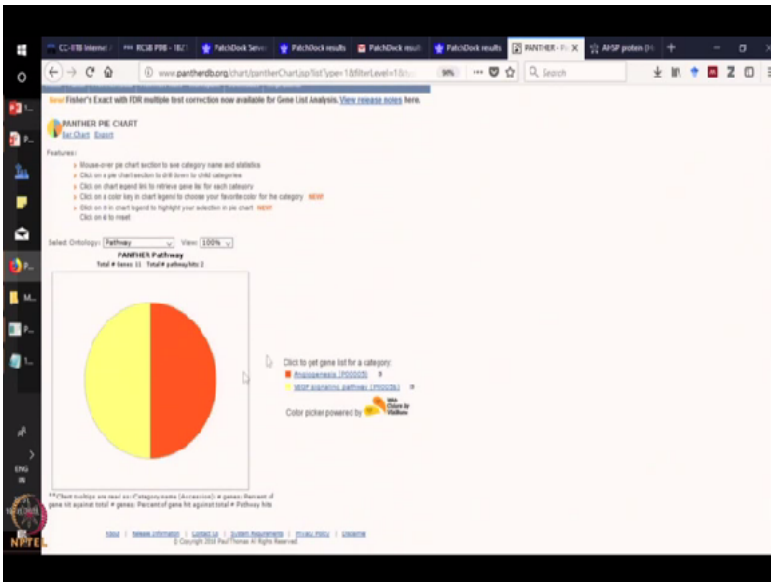

For example, let us see what happens when we click on the pathways. Now you can see majority of these molecules are involved in angiogenesis which is development of new blood vessels and VEGF signalling pathway.

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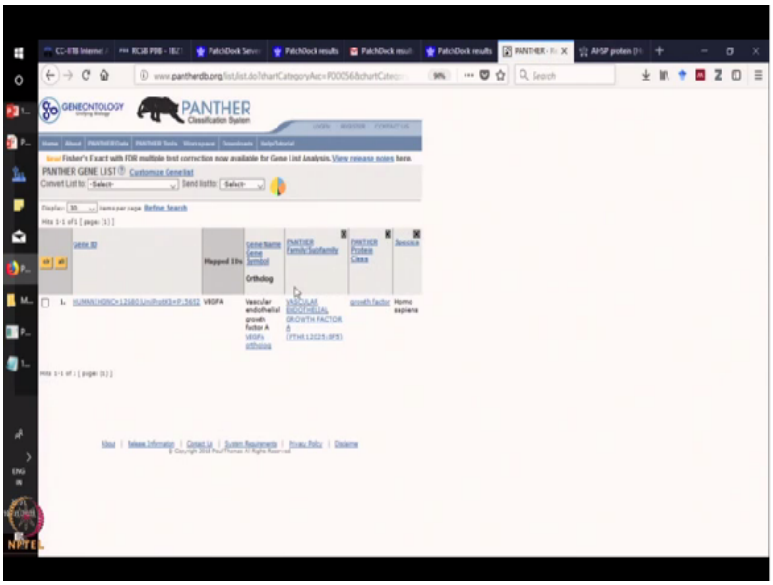

Now if you click on the VEGF signalling pathway, it will also give you details of the vascular endothelial growth factor pathway. Thus using a very simple set of tools, we can now know so much more about the protein that we are interested to know about.

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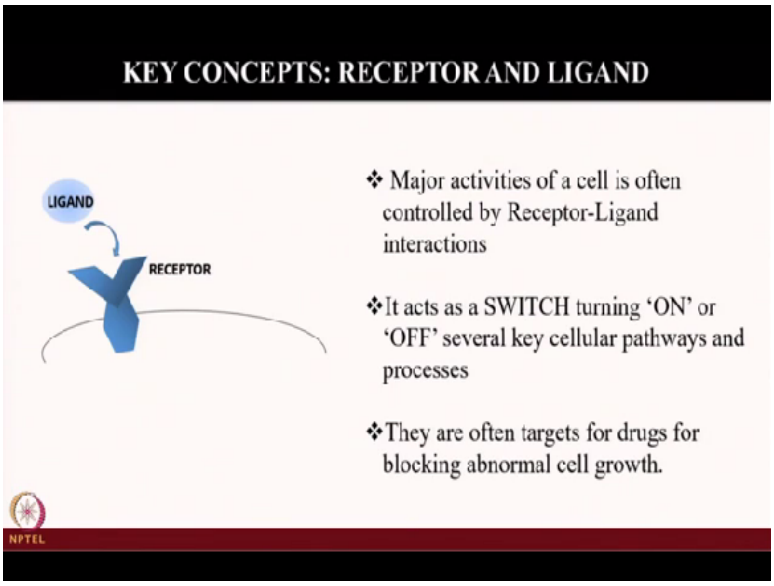

Furthermore, let us learn about and ligand. Now major activities of a cell are often controlled by Receptor-Ligand interactions. Moving on, let us learn about receptor and ligand. Major activities of a cell are often controlled by Receptor-Ligand interactions. It acts as a switch turning on or off several key cellular pathways and processes. As you can see in this small cartoon, this is a ligand and when it binds to a receptor, many many cellular processes gets started.

Sometimes it is switched on, sometimes it is switched off. Why are we suppose to know about this? Because these are often targets for drugs and abnormal cell growth. Ligand receptor interaction are very attractive targets for drugs for sometimes blocking abnormal cell growth, cell proliferation, cancer, etc.

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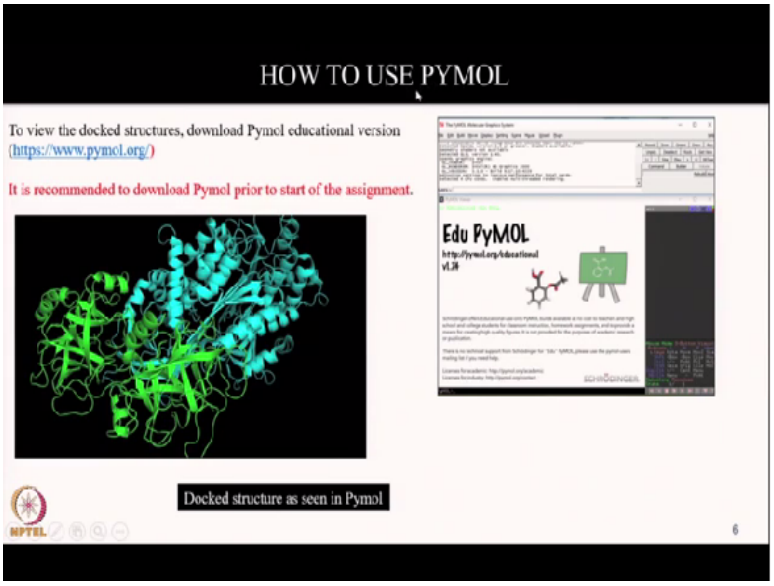

We will now perform a simple bioinformatic exercise called the molecular docking. PyMOL is a software which can help us not only visualize the structure of a molecule but it can also help us in performing molecular docking. So if we take 2 molecules, we can see the structure. Also we can use PyMOL to see the molecular docking. First let us see how we can use PyMOL for finding out the structure of a particular protein.

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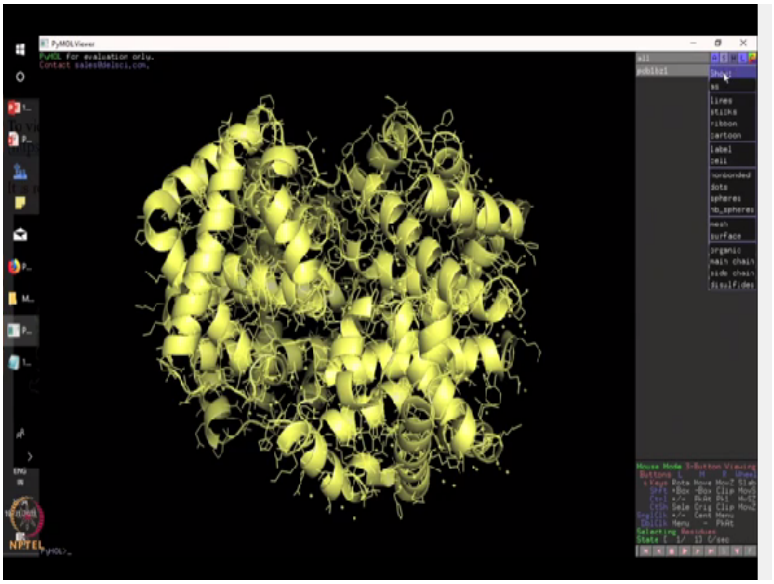

For that let us see, if you query in PDB, we will get the PDB file of hemoglobin alpha. As you can see, this is the hemoglobin alpha molecule. PDB 1BZ1. So this is the structure of the hemoglobin molecule. There are a lot of options in PyMOL by which we can personalize the structure. You can visualize whatever you want to. If you click here, you can see there are lot of

options for different visualization.

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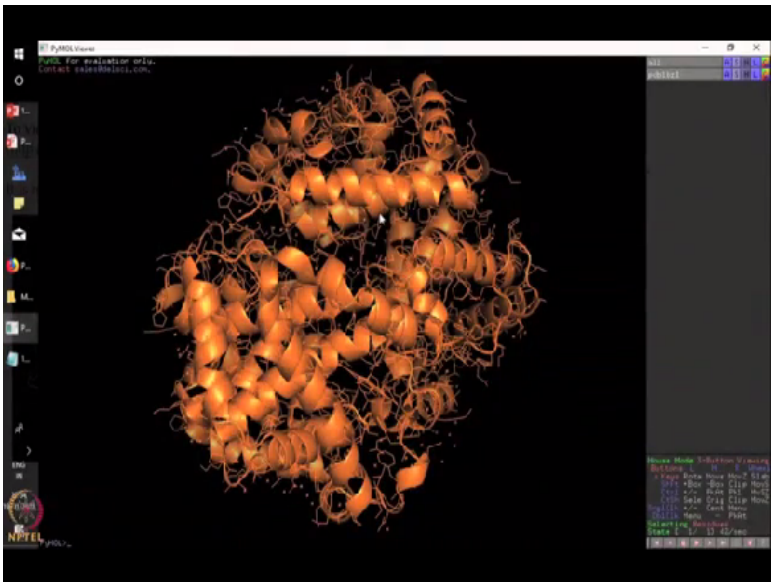

So if you want to see lines, if you want to see ribbon, you can also colour. So now you can see, this is how it looks.

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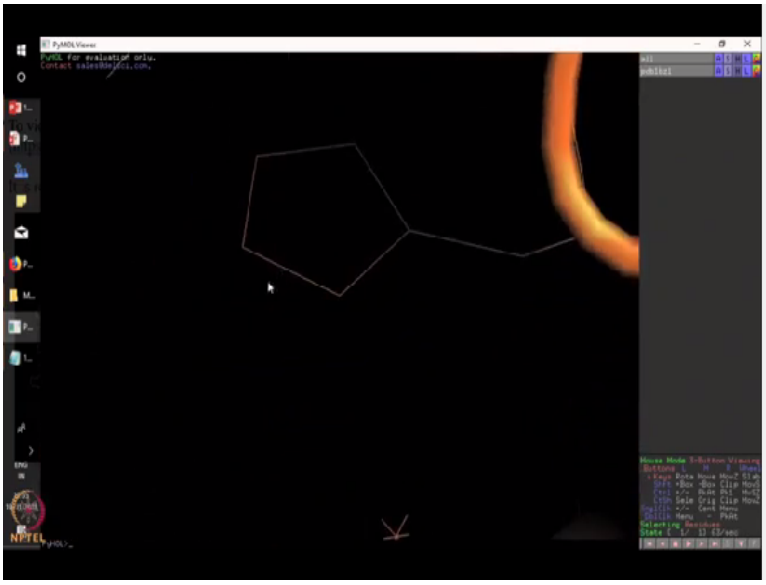

If you click on a particular aspect, it will tell you about the residues which also you can personalize like you can colour, you can show, you can see the different atoms. It can zoom the particular atom that you want to. So this kind of personalization feature makes PyMOL a very attractive software to learn for several bioinformatic and structural analysis.

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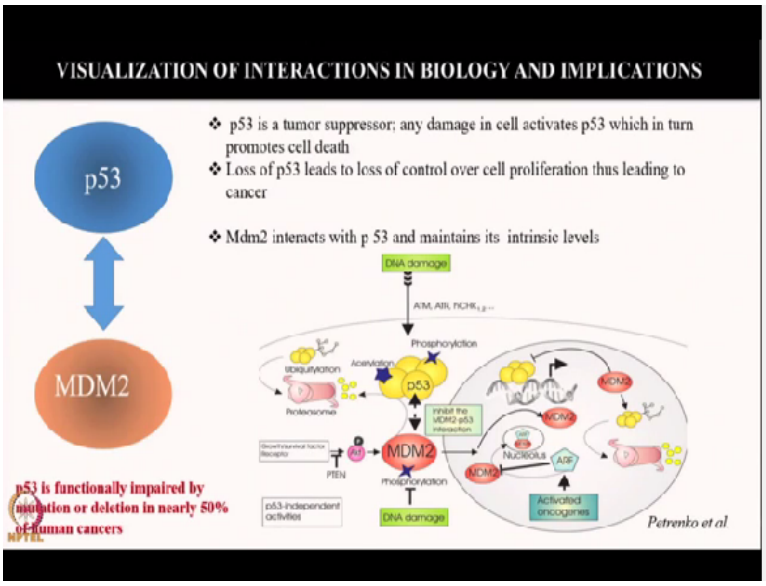

We will now do a small exercise by which we will learn about 2 very important molecules using molecular docking. And we will see how important sometimes this visualization is for understanding several biological processes. We will learn about p53 and MDM2 interaction using molecular docking. So p53 is a tumor suppressor and any damage in the cell activates p53 which in turn promotes the cell to die.

Sometimes p53 can lead a lot of complications. P53 if not present, can lead to cell proliferation and cancer. In the cell, there is another interacting partner of p53 called the MDM2. So MDM2 normally regulates the level of p53 in the cell, thereby keeping a tab on the cells' functioning. In abnormal cases, MDM2 and p53 control gets disrupted and the cells often become cancerous. P53 is also functionally impaired in nearly 50% of human cancer.

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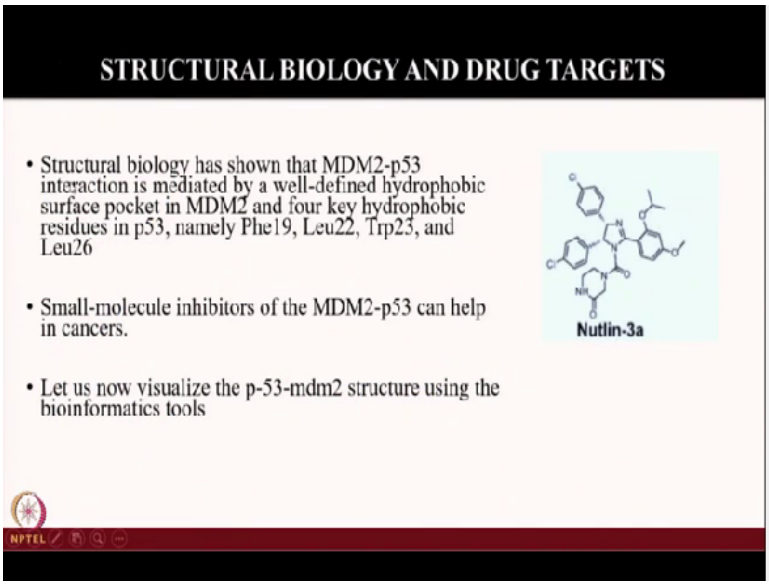

Structural biology has shown that MDM2-p53 is interacting via a hydrophobic surface pocket using these residues, amino acid residues. Also structural elucidation of this interaction led to the discovery of small molecules like Nutlin-3a which can help in cancer. So now let us see this interaction using the bioinformatics tools. Now let us visualize p53 and MDM2 structure using the bioinformatics tools.

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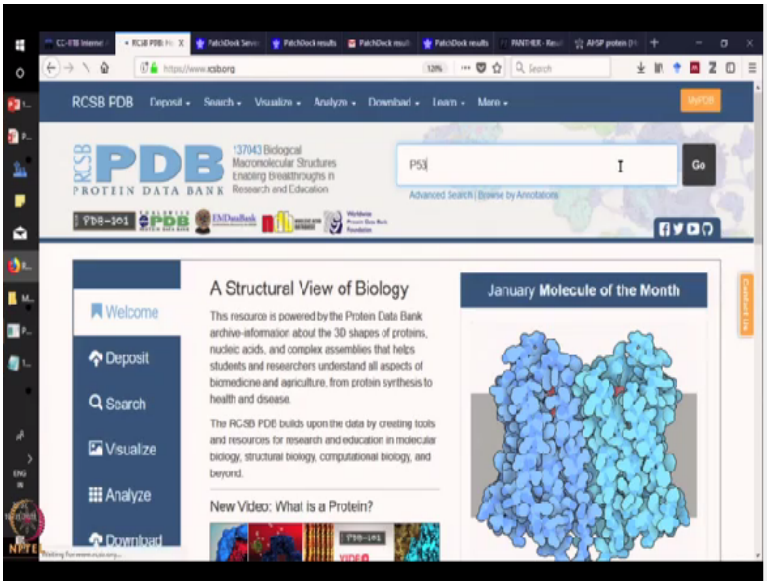

Here going to the home page of PDB, if you type p53, go to PDB and type p53.

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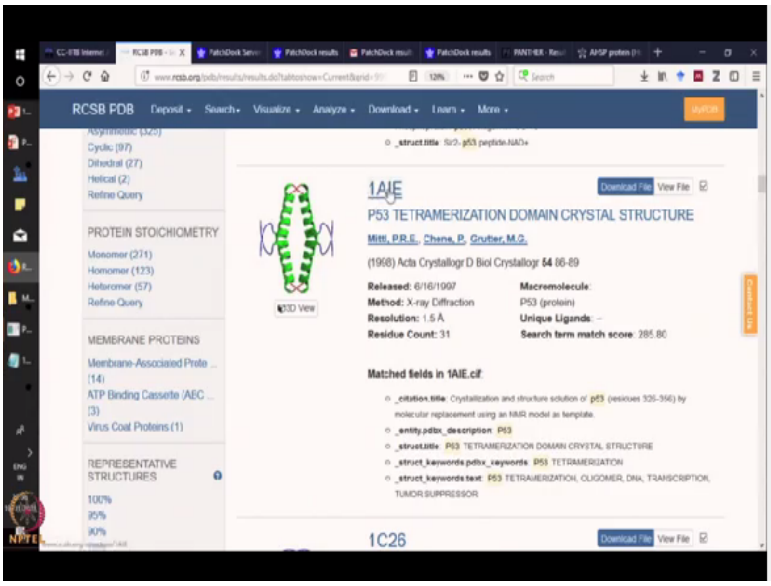

You can find various structures of p53.

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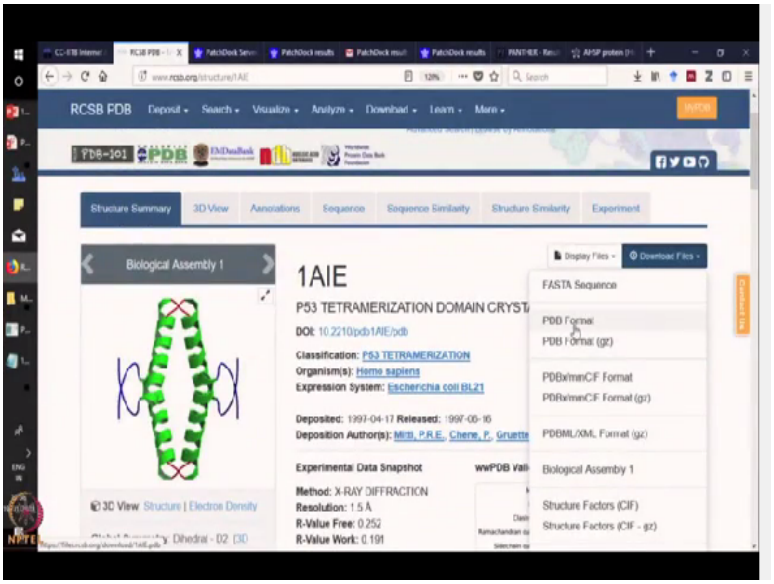

If you click on p53 structure, then you will get the option to download these molecules in the PDB format and as mentioned earlier in the lecture, PDB format can be used in PyMOL and we can do the molecular docking.

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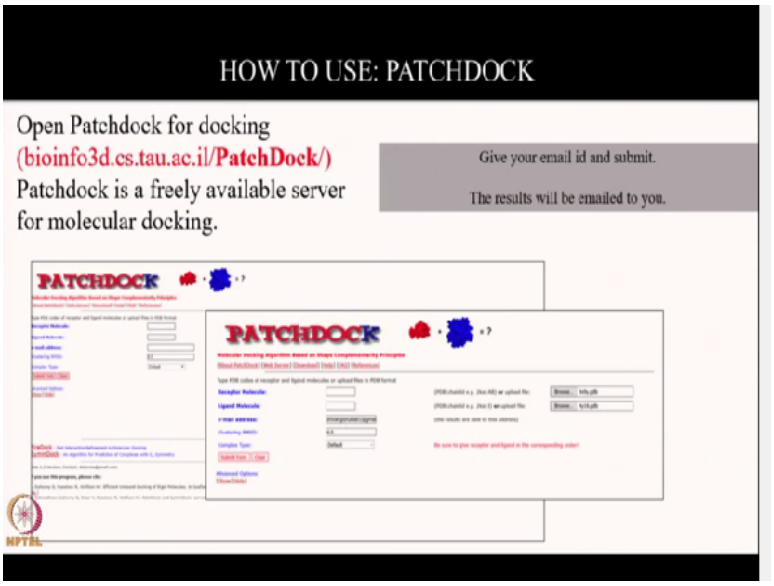

Patchdock is an open source software freely available which can help you in performing molecular docking. So in Patchdock, we have different tabs. We have to fill up a receptor molecule and we have to fill up the details of the ligand molecule. We have to give the email id and the results will be directly mailed to you through Patchdock service system.

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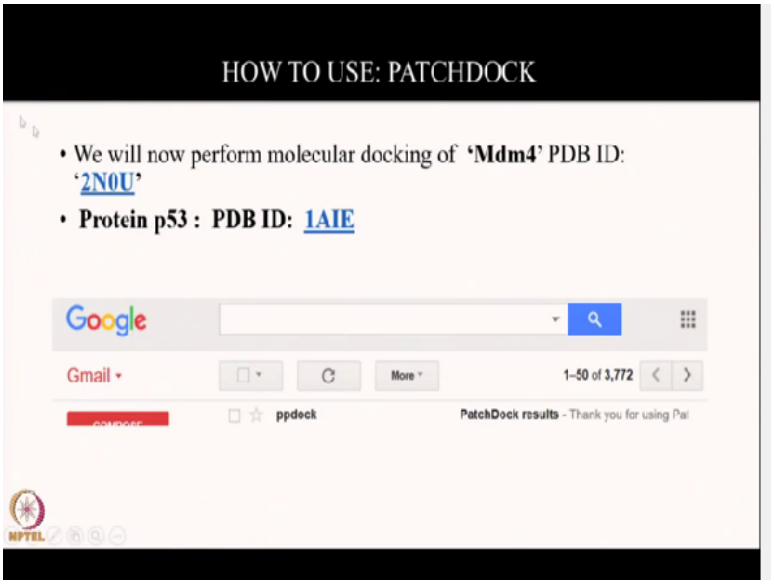

We have found out the 2 interacting partners that is MDM and P53 and we have also now searched for the IDs of these 2 interacting partners. So now what we will do is, we will use these 2 IDs and we will find out how the molecular interaction is taking place through Patchdock.

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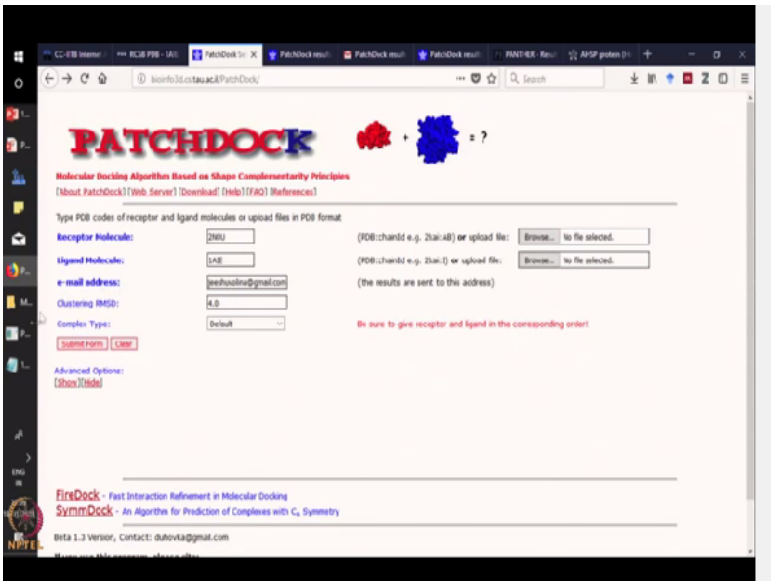

As you can see now we have come to the interface of Patchdock. Here we have inserted the IDs for MDM and p53 and we have given the email ID.

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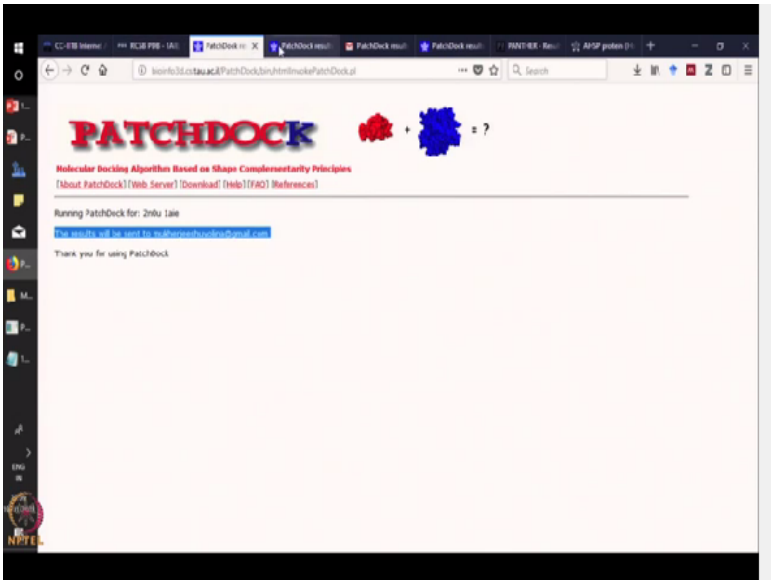

If we submit the form, then Patchdock will give you this response that this result will be sent to you via email.

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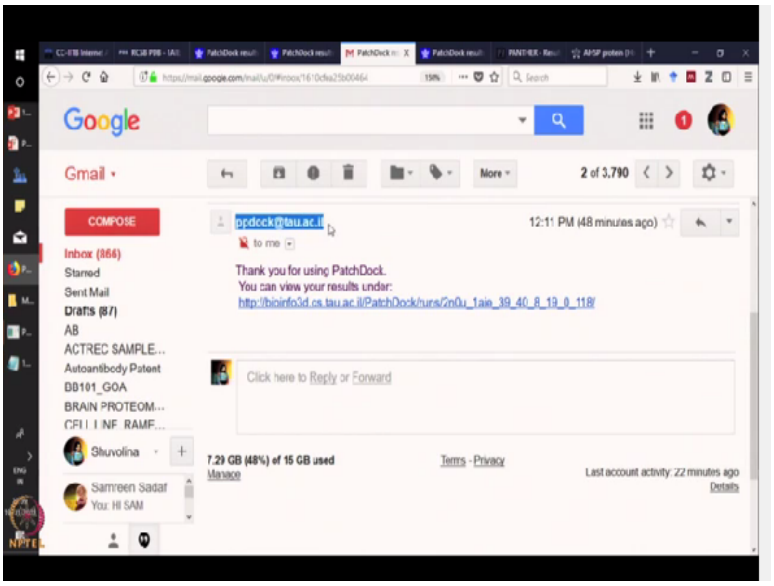

If you open your email, you will get the result from Patchdock.

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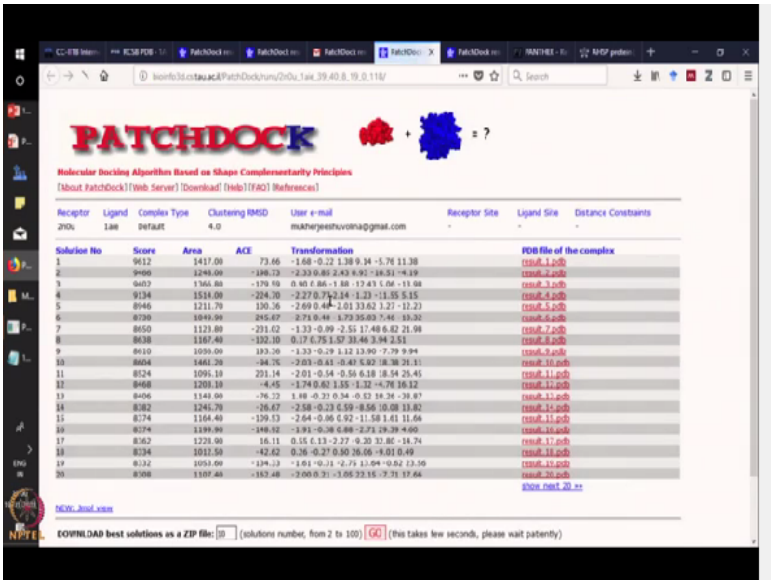

Let us open and see how the result looks like. Now as you can see, this is your receptor molecule and this is your ligand molecule. Also it will tell you how the score of the interaction is. This will tell you that this is the maximum score for this kind of interaction and according to the score, they will give you different results. So the score indicates the fit of the ligand and receptor.

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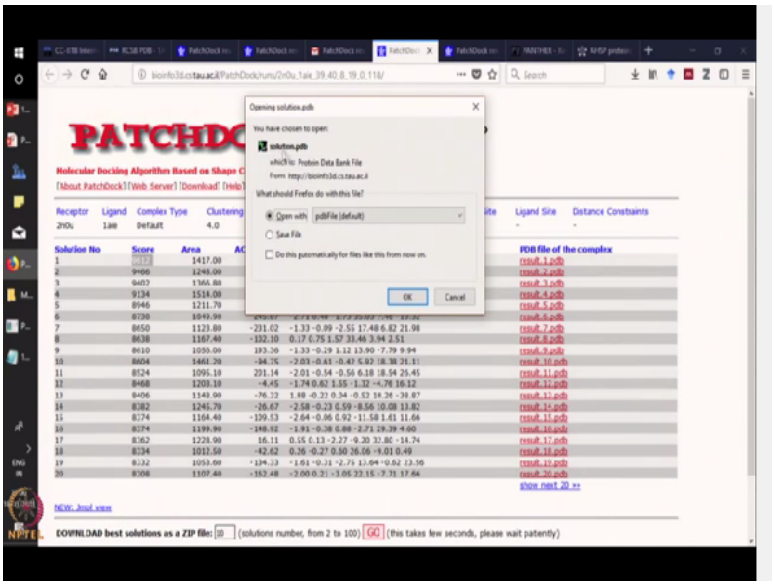

So if we click here, we will again find the interaction file.

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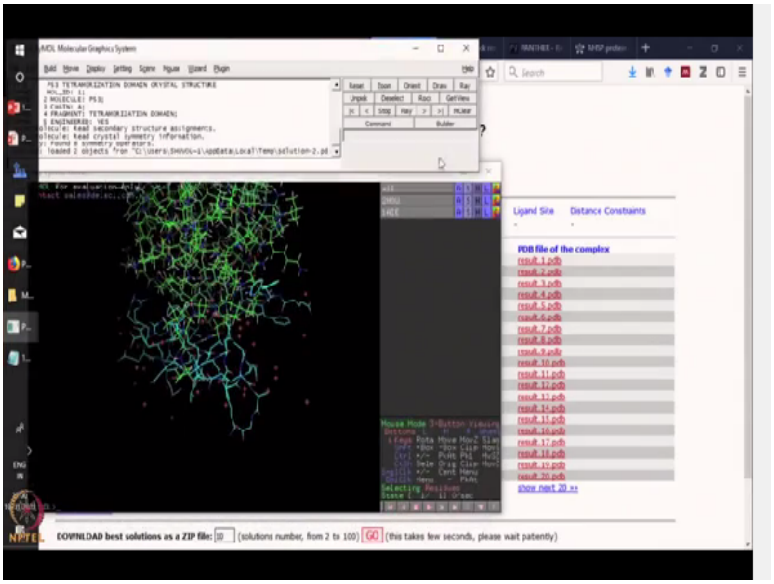

So if you open it, as you can see, the visualization will happen through PyMOL. So 2 files will open. The PyMOL molecular graphic system wherein you will see there will be various details of the structure mentioned.

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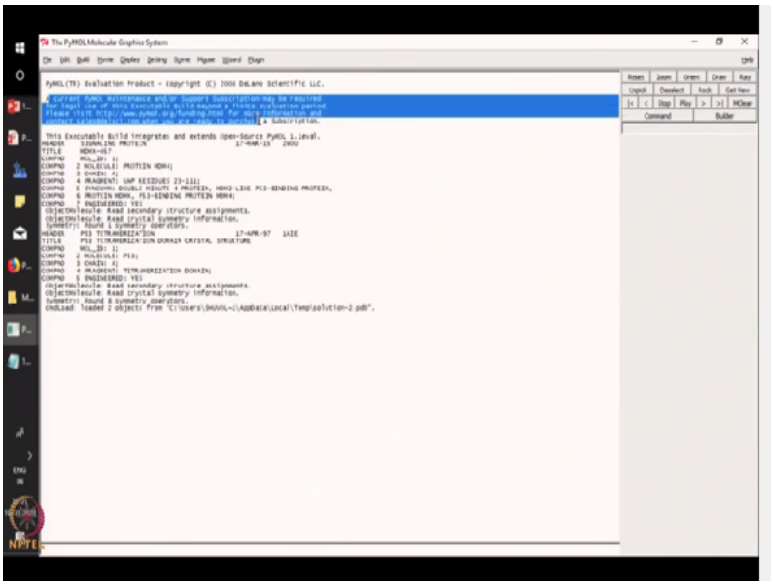

So you can see we are talking about these 2 molecules that is MDM family protein and p53. And they will also tell you that how, which structure it is reading. Now for visualization, of course, we will see how these molecules look like.

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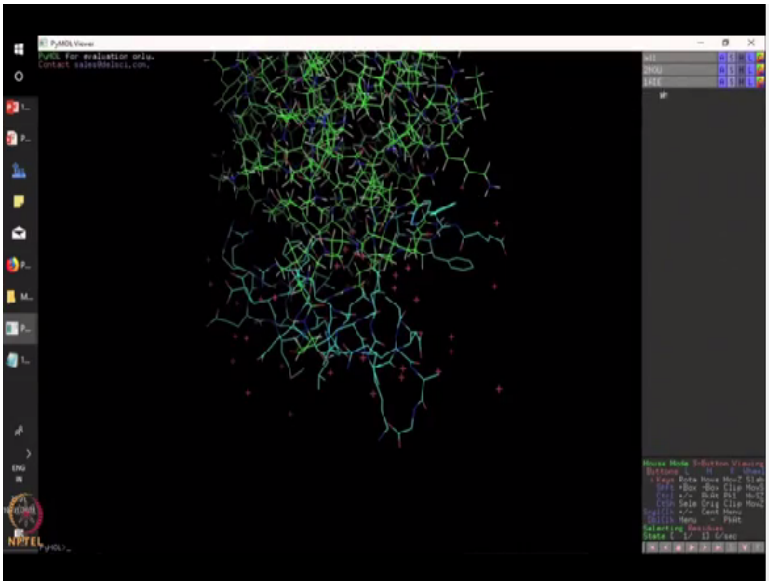

As you can see, there are 2 molecules, one is MDM and one is p53. And it is a 3D structure that you can see. And you can find out which residues it is interacting. Now you can personalize your interacting molecules. You can colour it using your choice of colour.

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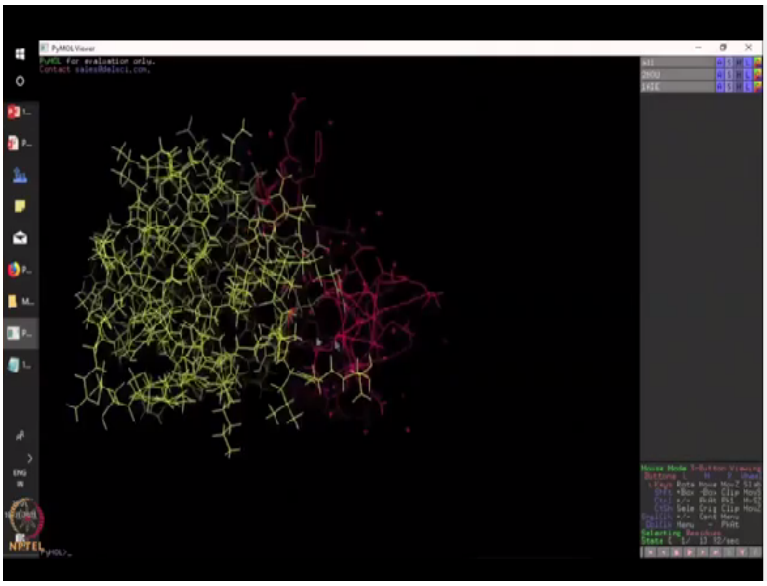

As you can see now, I have coloured one of the molecule in yellow so that this, it is visualized in a better way. Furthermore, I will colour the next molecule using another colour. So now this whole complex is very clearly visualized and you can zoom in and you can see which other residues that are closely interacting.

So if you click on the individual residues, you can also click on the individual residues and you can colour them as well like by the element or by the molecules. So if you want, you can visualize these as well. Thus Patchdock offers a simple docking system wherein you can see very important ligand receptor binding.

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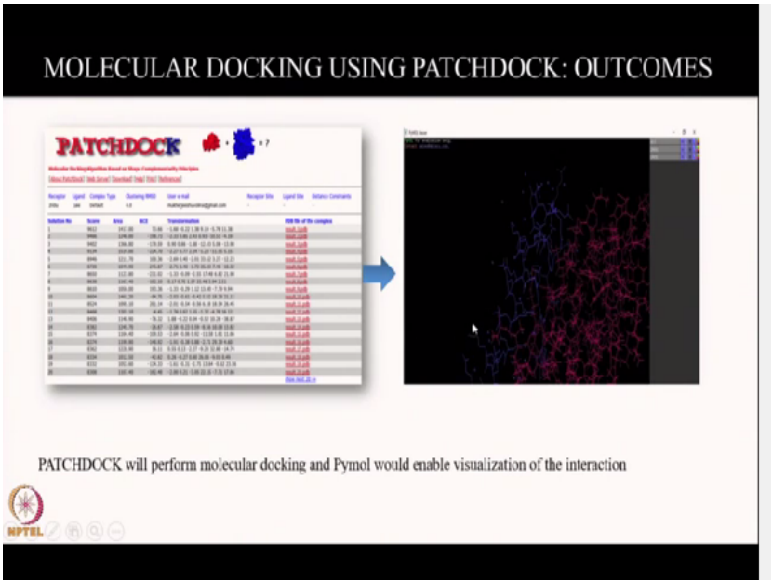

Molecular docking, we have just finished using Patchdock and we could see how the p53 and MDM interacts.

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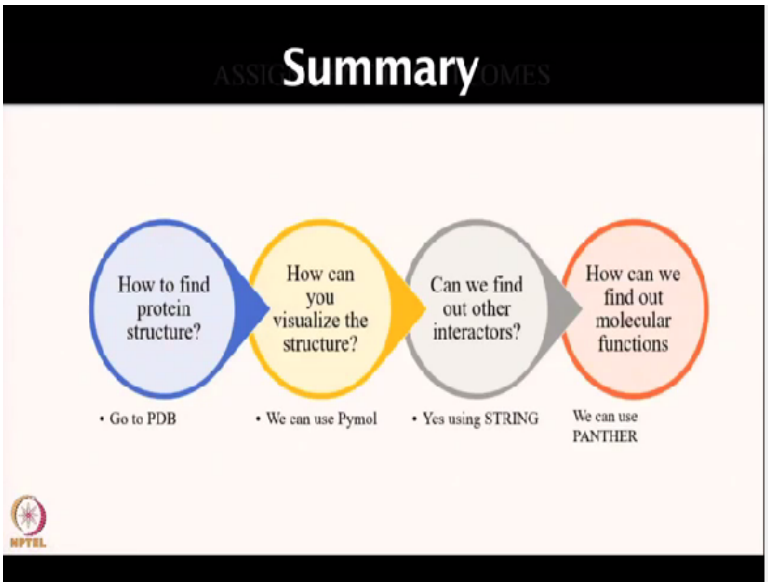

Thus in this assignment, we could perform how to identify the structure of a protein. Now we can query for any protein and go to the protein data bank and find out more about the structure. We could also visualize a particular protein like we visualized hemoglobin alpha 1 and we also visualized p53 and MDM using PyMOL. We also found out other interactors of a particular protein using the software STRING.

So STRING give us a list of proteins that also interact with the query protein. Using the list of interacting proteins, we could also find out molecular functions and pathway details using a simple tool called the PANTHER. Thus we could learn a lot of important features of a particular protein using simple bioinformatics tools like PyMOL, STRING, PDB and PANTHER. Thank you.

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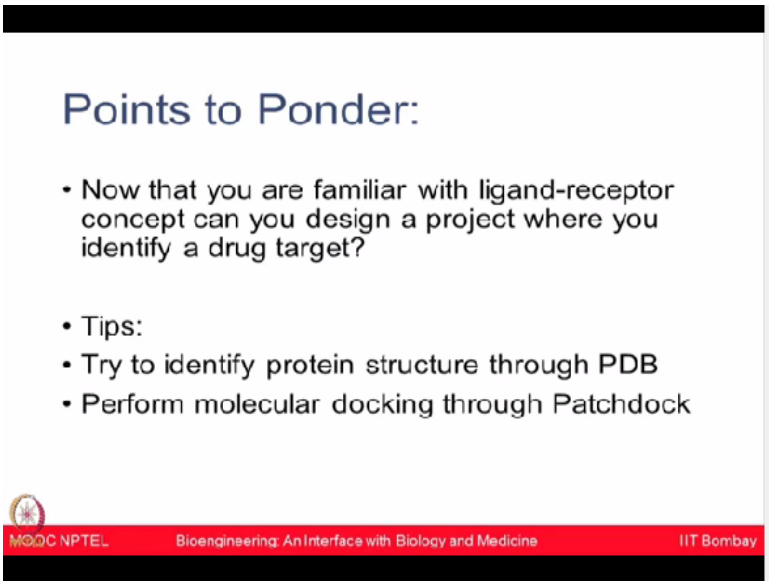

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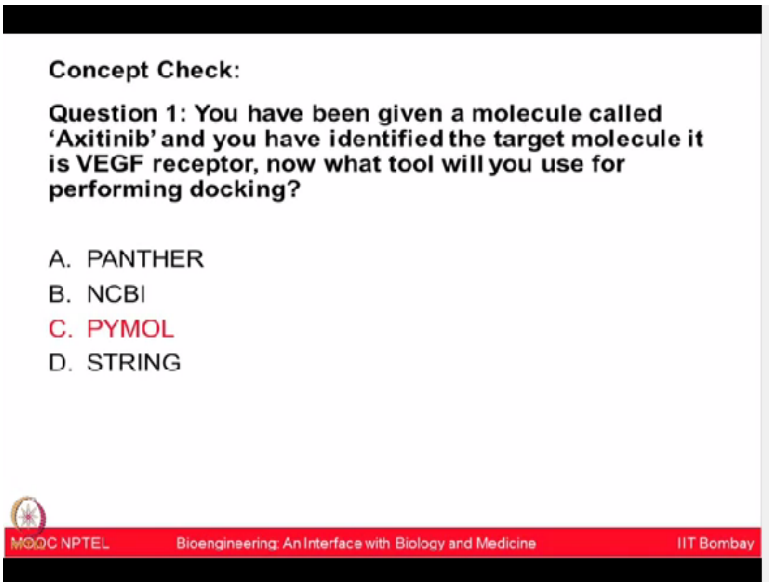

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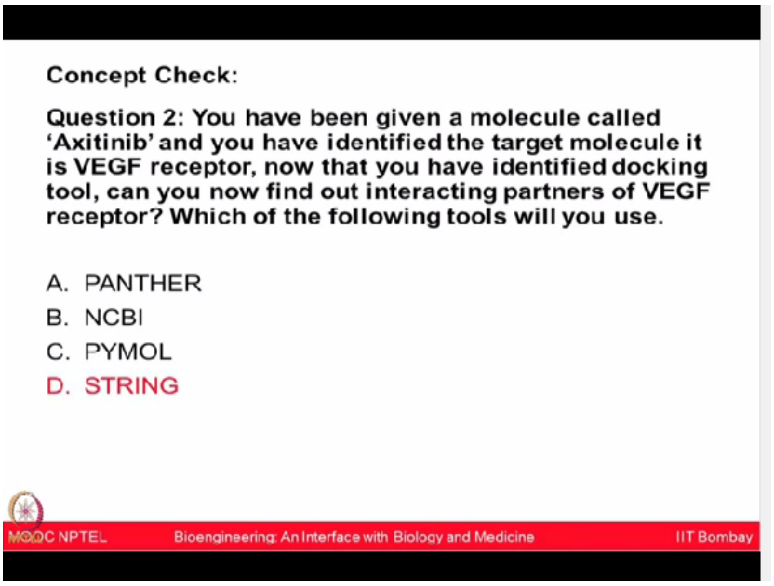

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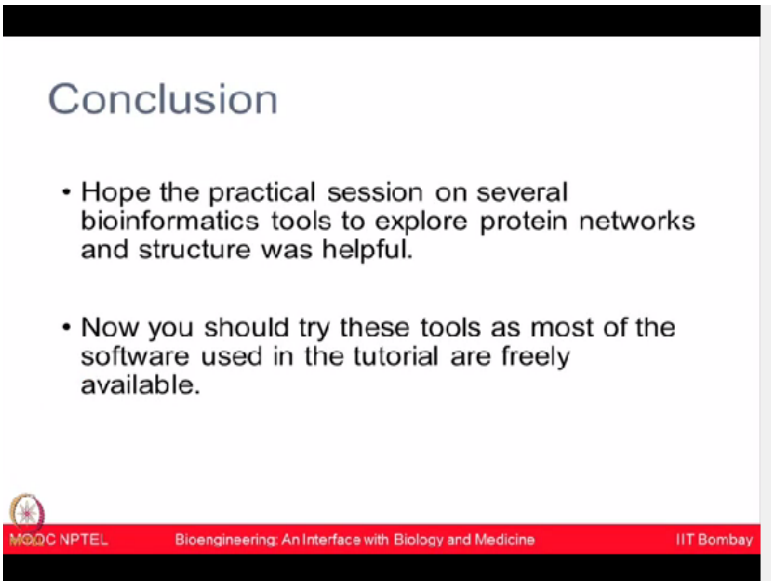

In conclusions, hope today's practical session on several bioinformatics tools to explore protein networks and structure was helpful. Now you should try some of these tools yourself. Most of them are freely open access available and then you will get to learn many interesting biological questions which can be studied and addressed even at your home, even at your own terminal by using these softwares. Thank you very much.

(Refer Slide Time: 23:21)

References

Open Access Softwares as mentioned below:

STRING DB: https://string-db.org/

PANTHER: http://pantherdb.org/

PyMol: https://pymol.org/2/

PatchDock: http://bioinfo3d.cs.tau.ac.il/PatchDock/