Experimental Biochemistry
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Lecture – 51
Analysis of the Structure of Protein Ligand Complex

In the experimental class, we have seen how we can actually determine and quantify the protein ligand interaction. So, the protein we have taken there was HSA and 2 ligands we have taken one is curcumin and the other is Gallic acid. So now, we will study the actual structural background or rather how the docking or how the interaction takes place. Now talking about docking I will not go into detail. So, rather I will touch the peripheral part and we will show you how we can actually see or how we can actually structurally analyze the protein ligand interaction.

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Now, I will show you just the structure of the ligands, the structure of the protein and protein ligand complex.

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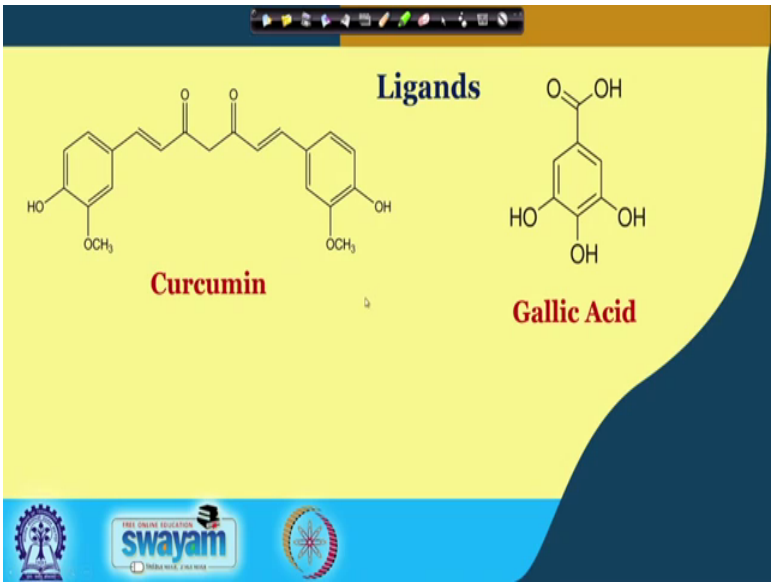

So now we have taken 2 ligands. First ligand is curcumin and the second ligand is Gallic acid. So, the structure of this two compounds more of most of you might have been familiar with. Now this curcumin have this 2 aromatic rings out here this one is number 1 and this 1 is number 2 ok. And this Gallic acid has only one aromatic ring ok. And now, we will see how the structure looks when you take it on a computational level ok.

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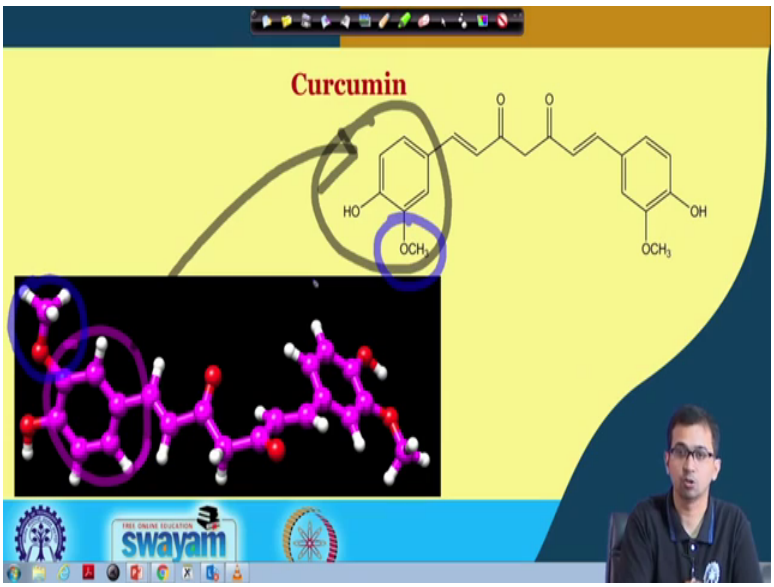

So, talking about this one the first thing we can actually see is about curcumin. So, this is the structure of curcumin. And when we take curcumin when we analyze this in any

software in a computer using any sort of software ok, let it be Gaussian or any sort of software. This is the ball and stick model of curcumin ok. So, here we can see. So, this one is actually one of the rings of curcumin. This has been marked in purple ok. As you can see now this ring actually corresponds to this structure ok. So, this structure we can actually correlate with this one. Why? Because if we take into account of this moiety this moiety and this moiety this is the O CH 3 part and this one is also the O CH 3 part ok.

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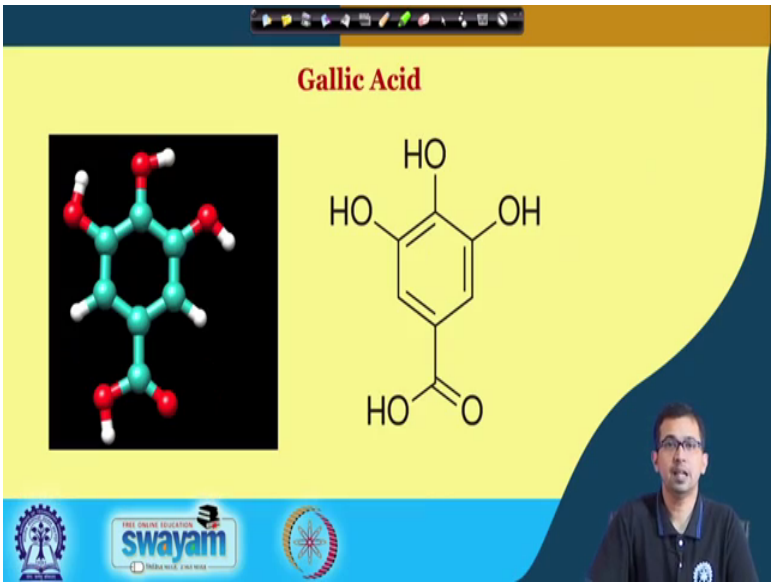

Now, let us move to the next molecule. The next molecule is actually Gallic acid out here we can see that this Gallic acid has 3 OH groups 3 OH groups and one cooh group. Generally, this type of molecules are known as your polyphenolic compounds ok. So, first we have seen the structure of curcumin and next we have seen the structure of Gallic acid.

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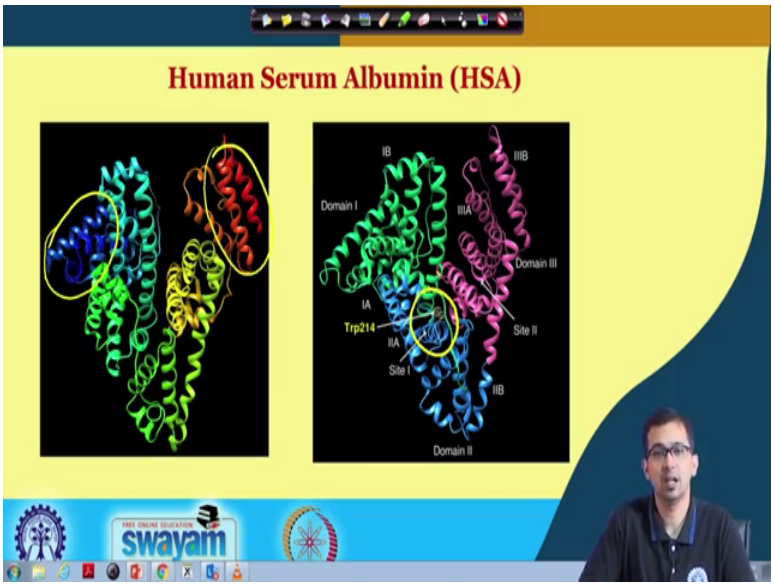

Now, this is the human serum albumin. Well this looks pretty good in terms of color combination, but; however, the structure is a bit complex here we can see several helices are there ok. This one is one of the alpha helix this one is one of the alpha helix. So, this HSA compound have this different types of alpha helices as we can see.

Now generally these human serum album protein have this heart shape structure ok. And this structure has several domains and out here there is one tryptophan residue. So, important thing about this protein is that it has only one tryptophan residue.

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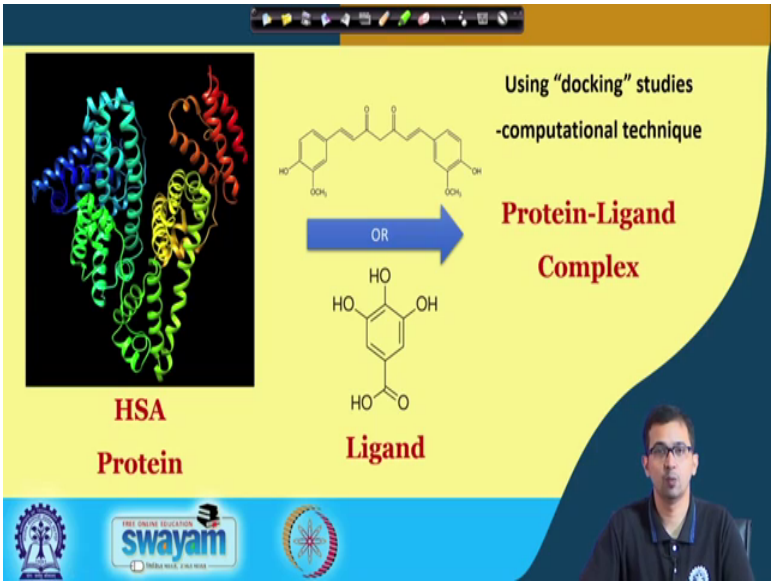

Now, what we have done we have taken this HSA protein and we have used 2 different ligands. So, this part what we have done we have done this actually using experimental techniques; however, on the contrary on this stage what we will do, we will take some computational tools generally known as docking studies. So, docking studies many of you might have heard or many of you might not be familiar with ok.

So, docking studies basically takes into account of this protein structure. And it basically places either one of the ligands out here that is either curcumin or Gallic acid it places here and it gives the structure of the protein ligand complex. Now we will see how this protein ligand complex looks like.

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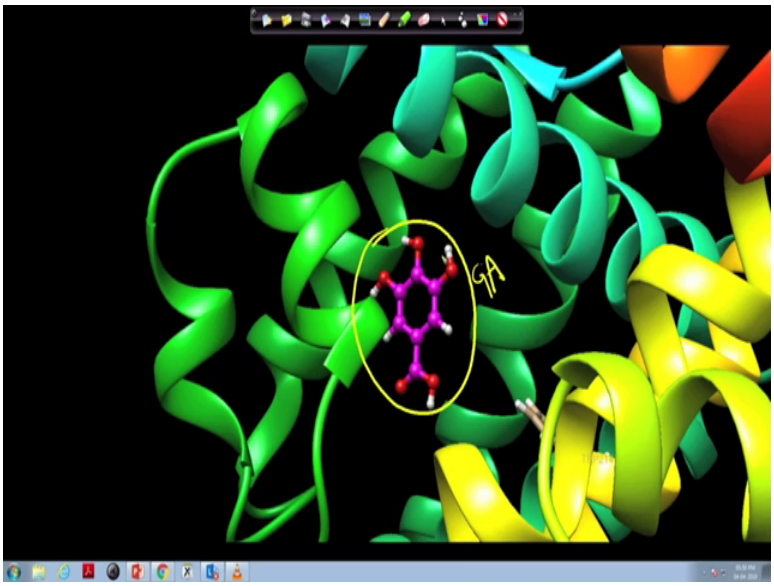

So, this is a structure of human serum albumin protein ok. So now, what we can do? We can actually play this small movie here in which we can actually see the structure of this protein. But there are two different types of molecule out here. And one of this molecule as we can see is a very familiar molecule which is our Gallic acid. So, this is the Gallic acid molecule ok. So, Gallic acid basically attaches to this region fine. Now if you play the structure and here we can see that Gallic acid attaches to the protein around these helices ok.

So, if we just pause this one for a while, and if we see this we can see that this is a protein and there are several alpha helices as we have discussed previously. And this one is a tryptophan residue out here the tryptophan residue is something out here ok. And this

one is the Gallic acid moiety. Now Gallic acid attaches to this part of the protein ok. There are several parts, but Gallic acid specifically attaches to this part of the protein. Now let us play this structure once again. So, if we are interested in knowing the distance between the tryptophan residue and the Gallic acid.

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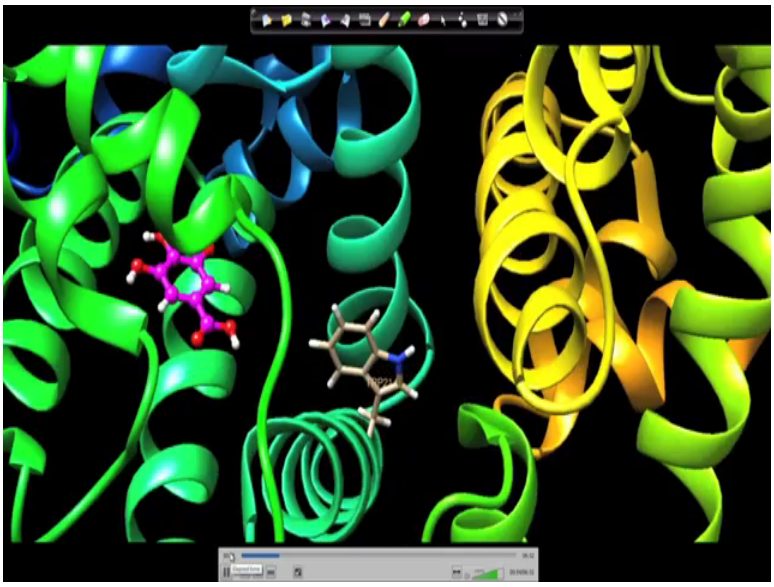

We can actually easily do it by measuring the bond length or basically this is not the actual bond length. Since there are no such bonds, but this is the distance between the 2 atoms ok. So, here we have selected one of the atoms out here, that is one of the atoms we have selected is our nitrogen atom of tryptophan. And the second atom we have selected is the oxygen on the OH group oxygen of Gallic acid and we are measuring the distance.

So, let us see the distance the distance is around 10.4 Armstrong. So, disturbs the average distance ok. So, this is the average distance of the Gallic acid from our tryptophan residue of HSA. Now an interesting thing is that Gallic acid attaches to this part of the protein ok; however, there might be some sort of interaction. So, there are might be some sort of attractive interaction with stabilizes this Gallic acid to this region of the protein. Since, we cannot see any sort of covalent bondage. So, there some be might be some sort of non covalent bondages which actually stabilizes this moiety in this part of the protein.

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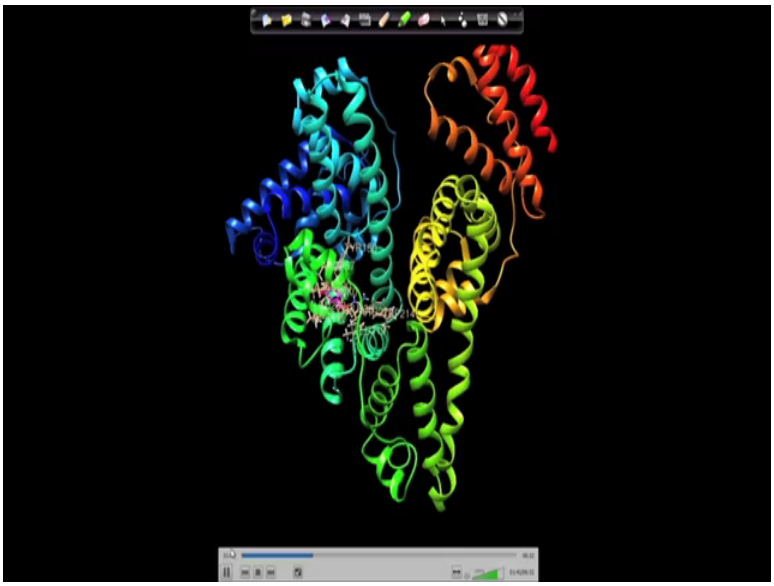

So, for that we actually have actually selected a few local regions around this Gallic acid moiety. And these are the protein residues. We have labeled it and in order to visualize it properly. We will have to first omit basically neglect the ribbon structure. In order to see the residues amino acid residues we have to remove the ribbon structure we have removed it and now we can see the nearby residues around this Gallic acid.

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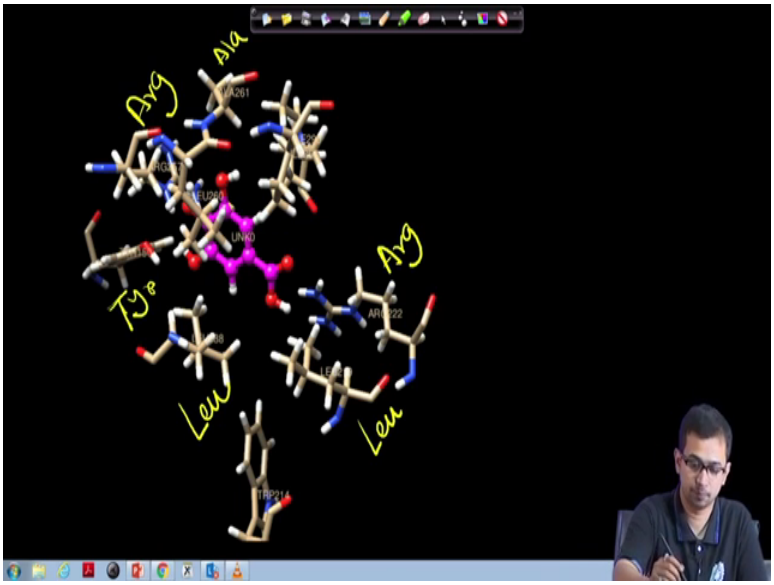

So, if we pause this for a while and if we analyze this what we can see is actually this type of structure there are tyrosine residues ok. Now, there are alanine residues there is

arginine residues and this unk part is basically our Gallic acid moiety ok. And here is also arginine residue fine and here is leucine residues and here is also leucine ok. So, one important thing we can see out here that, there are polar residues and one aromatic residues this one is your tyrosine and the number if we tend the residue number of recent written just next to it.

So, out here we can see there a polar as well as non-polar and aromatic residues around this Gallic acid moiety. Now, if we visualize this structure from different angles we can actually see that these residues actually have shielded this Gallic acid moiety, which was basically signifies that there are some sort of week interactions might be prevailing out here. So, what are the interactions might be prevalent out there.

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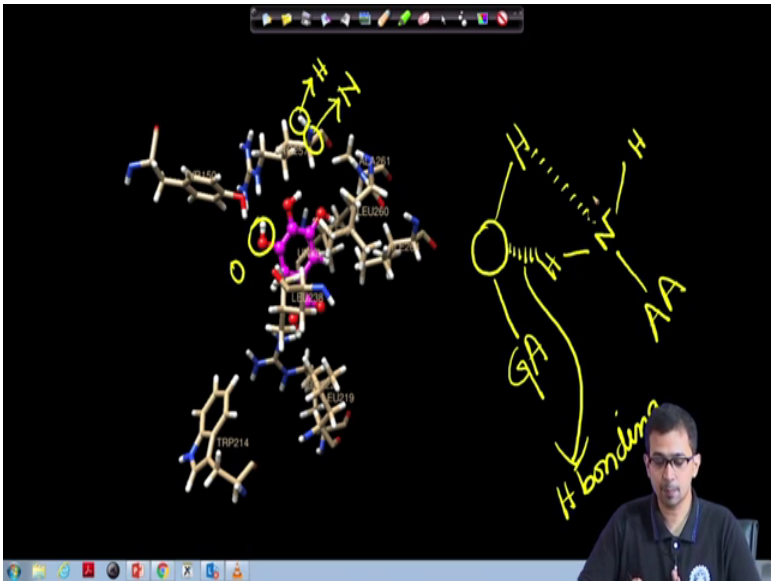

So, since this Gallic acid has this rate part the great part is actually the oxygen this one is the oxygen and these green parts are actually nitrogen ok. So, and this is the white part is actually hydrogen. So, what happens in this case if here is oxygen atom is attached here, and this is OH this is for Gallic acid ok. And out here there is any amino acid residues whose n h 2 is the out here nh or any sort of residue.

So, there might be some sort of. So, hydrogen bonding interaction might take place out here ok. Again this might interact with nitrogen also for hydrogen bonding. So, what I am trying to portray out here. So, the interaction which actually take place during protein ligand interaction might be hydrogen bonding.

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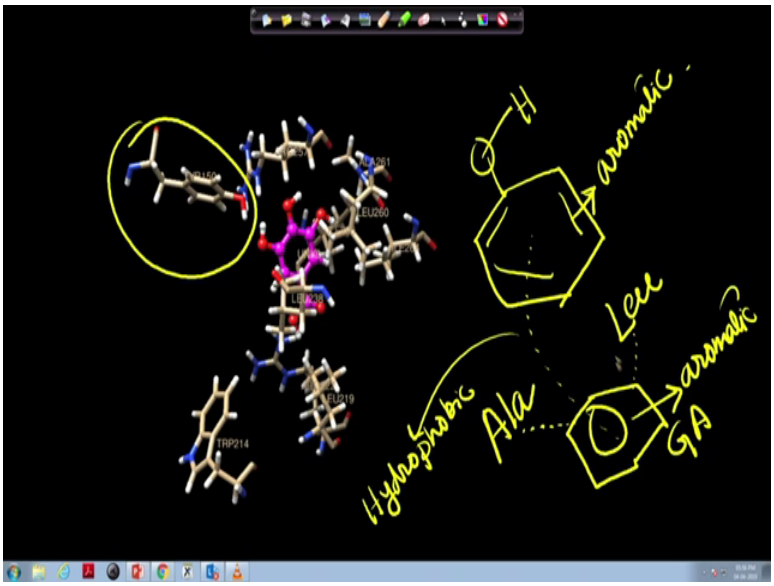

But again apart from hydrogen bonding there is this tyrosine residue ok. So, tyrosine residue has an aromatic part. So, tyrosine residue is basically like this. And, this and this automatic part and apart from this there is alanine residue alanine and also leucine. So, this all are aromatic. So, what happens actually in case of Gallic acid, in Gallic acid we can see there is one aromatic moiety aromatic part in Gallic acid.

So, there might be some sort of what we can say hydrophobic or aromatic interactions out there. And also hydrophobic interaction might exist between alanine and also leucine. So, out there what we can actually suggest that apart from actually you can see the structure apart from hydrogen bonding there might be some sort of hydrophobic interaction which basically stabilizes this Gallic acid structure within those residues ok. So, we can also see the tryptophan residues moving around here. So, these are all the 3 dimensional structures there are various structures.

So, this part we can see that several residues are surrounding this Gallic acid and this residues are kept at a distance of around 3 Youngstorm from Gallic acid. So, you can actually measure we can actually estimate the number of residues around it ok. So, these are the images or these are the video taken from various angles in the softer ok. So now, what we can do we can actually see the structure of Gallic acid within the protein moiety again we are back with the primary structure.

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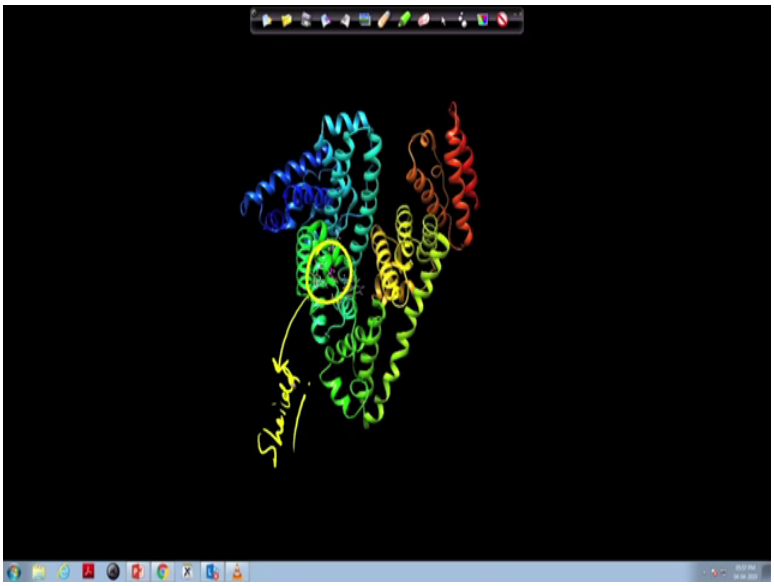

And one important thing out here if we can see that the Gallic acid present here if shielded ok. So, the Gallic acid is shielded out here. So, we cannot actually see the Gallic acid. So, as we have discussed in the experimental part that, during the uv absorbance since our molecule that is the our point of more interest the ligand is actually getting shielded. So, it is absorbance might decrease. So, the absorbance on Gallic acid decreases as we have already seen. Now this is another molecule and as we can we have seen previously this molecule is actually curcumin ok; however, the point of attachment of curcumin is somewhat different than that of Gallic acid ok.

So, curcumin attaches in this cliff ok. In this cliff the curcumin attaches out here curcumin attaches in the cliff again nearby the alpha helices. So, different ligands have different regions of attachment in the protein ok. And we have selected the tryptophan residue ok. This tryptophan of HSA is actually tryptophan 2 1 4 fine. So, this tryptophan residue you know measuring the distance between those curcumin and typtophan for that we first select a residue or particular atom of curcumin. And the tryptophan residue and now we are interested in making a distance a distance.

Now, is actually 12.63 youngstrom as we can see. Now this distance is quiet the higher than that of the Gallic acid, which shows that Gallic acid binds to a region near the tryptophan residue. This information might be important if we measure the fluorescence emission spectra ok; however, here also we have seen that curcumin is shielded. Now we

are interested in finding the stabilizing force of curcumin within this cliff of the protein ok.

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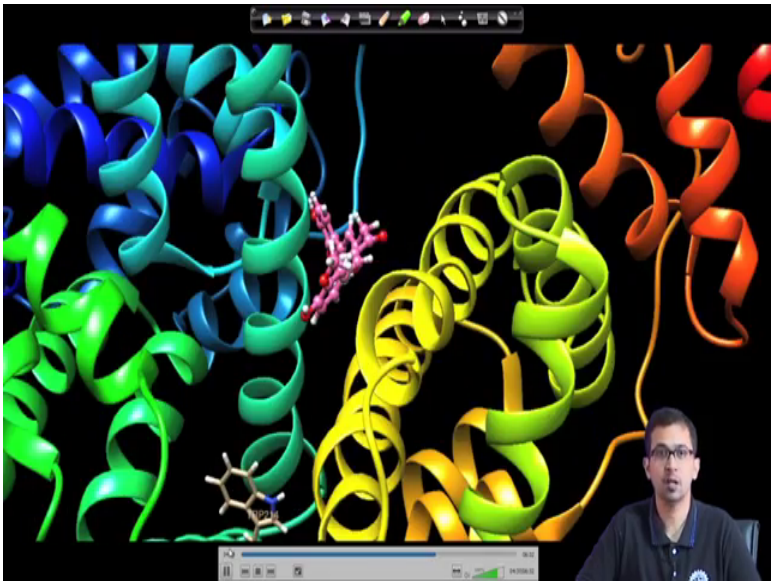

For that what we need to do we need to select this curcumin for selection we need to first allow curcumin to bind to the region of this protein. And select the nearby region that is the region around 3 Armstrong from this curcumin. And these are the residues and we can label this one and it seems a bit cumbersome and for that we actually neglect and we actually hide the ribbon structure of this protein.

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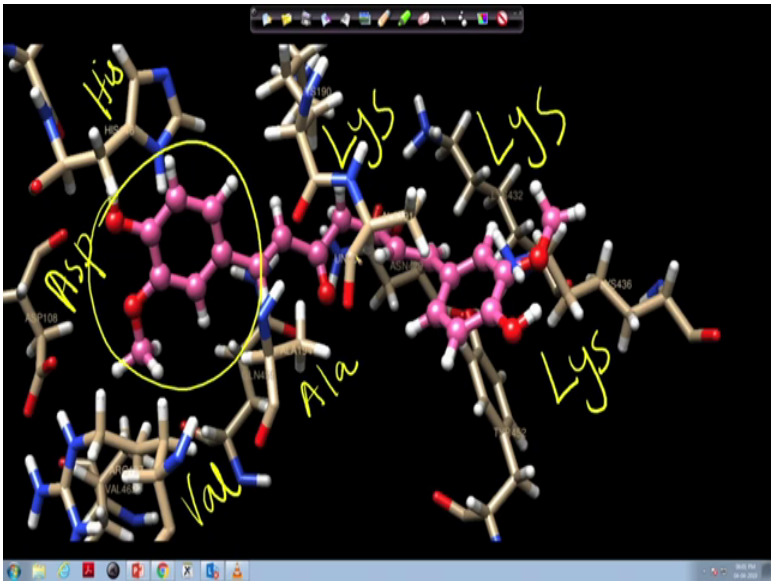

And then if we see this one we can basically see that in similarly here also here also this one is actually curcumin. So, this one is actually our curcumin ring ok. And nearby we can see alanine and here is valin and here is aspartate, here is histidine ok. And there is also another ring where we can see lysine here is another lysine and here is another lysine.

So, what we can say from here, that there are polar residues as well as nonpolar residues surrounding this curcumin. And now let us just go through this structure and if we see the residues around it we can actually say that there are.

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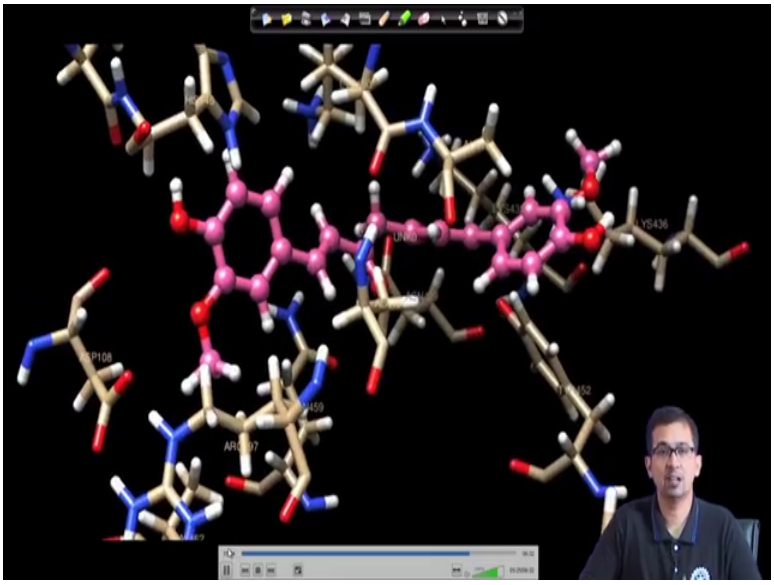

In a similar way hydrophilic as well as hydrophobic interaction which basically stabilizes this curcumin at this point ok. Similarly, what we can see there is another tyrosine residue out here. So, this tyrosine residue actually suggests this tyrosine residue actually suggest that aromatic part of curcumin, that curcumin has also an aromatic ring 2 aromatic rings basically. There might be some sort of hydrophobic interaction between this aromatic ring and this tyrosine residue. Since this tyrosine residue is located quite close to one of the aromatic rings of curcumin. So, as we have seen these were the stabilizing interactions will basically stabilizers this circumin in HSA.

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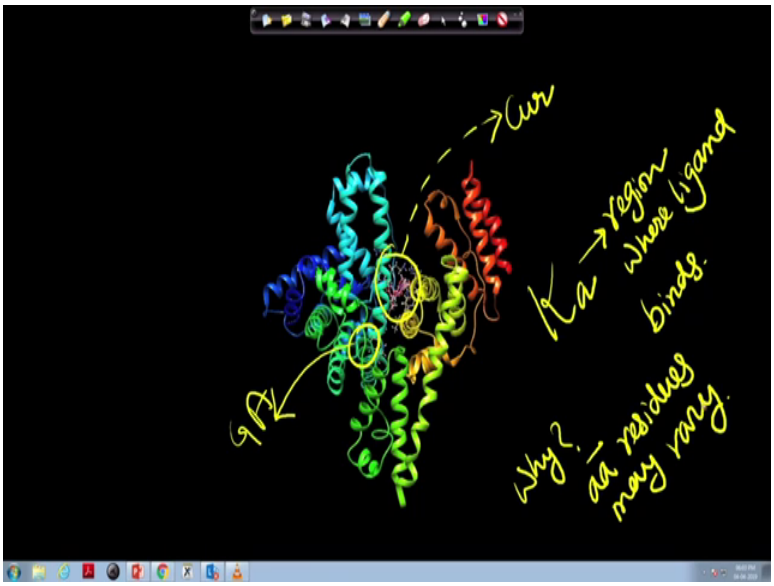

However, one important thing out here is that for both HSA and Gallic acid what we can see is that for Gallic acid HSA it attaches to a region around this tryptophan. So, it attaches out here. This is a region where Gallic acid attaches; however, this is the region where curcumin attaches. So, the region of attachment in a protein varies for different ligands. From this way what we can actually estimate the extent of interaction actually varies. So, what we can say the extent of interaction given by ka or (Refer Time: 17:41) constant depend on the region where ligand binds. Why? Because, as we know depending upon the various regions amino acid residues may vary.

So, in different region there might be different sorts of amino acid residues. And depending upon the different types of amino acid residues this Ka value varies. So, in this study what we have seen we have actually seen how the interaction between Gallic acid and curcumin can be visualized using computational tools. These are quite at a advanced level and these are not actually in your syllabus which was studying out here; however, for a better for a advanced study I am showing you this one and this might be helpful in analyzing the experimental techniques.