Structural Biology
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Lecture – 43
Demonstration of COOT

Hi everyone, welcome again to the course of structural biology. For a long time, in around 30 classes, we have discussed structural biology techniques. Now we are working through visualization. In the beginning, I talked about how visualization is very important. And in this module, we are continuing with how visualized and developed to the current stage, and we have also talked about the pdb file, which we discussed in the last class of the cryo-electron microscopy module how that pdb and mtz files are being used.

Today we are going to introduce a visualization software which is visualization software. It is much more than that. So, we will discuss visualizing the platform, the other parts it offers us, and how this can be used. So, welcome to the 3D visualization using the software called Coot.

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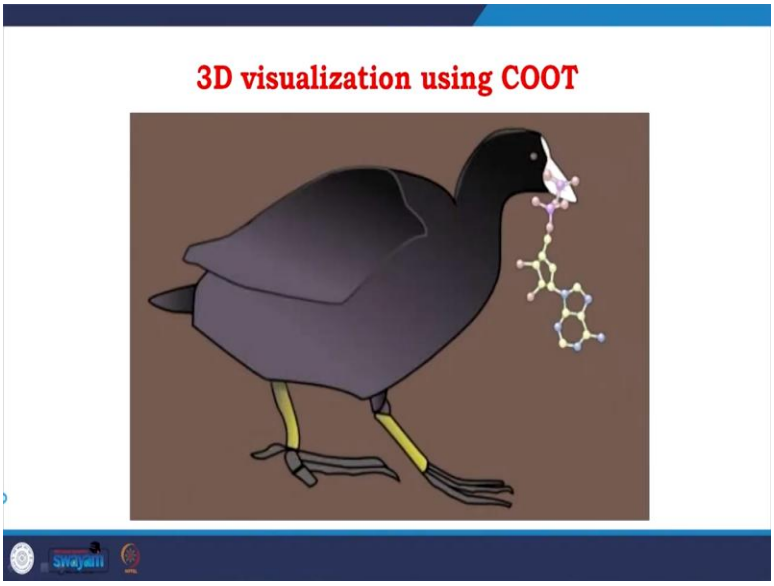

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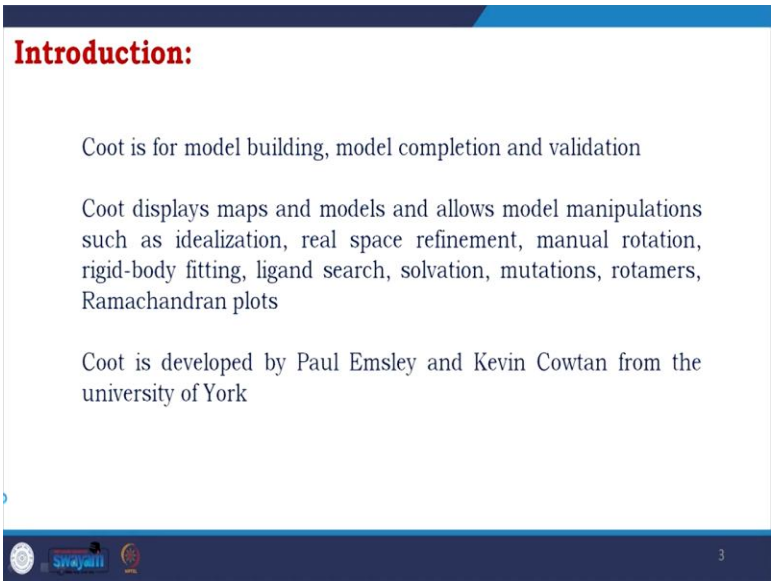

Coot is for model building, model completion, and validation. So, it is not only to visualize but to build the model to look at the level of the process, the journey to complete it, and, more importantly, to validate. Coot displays maps and models and allows model manipulations such as idealization, real space refinement, manual rotation, rigid body fitting, ligand searching, solvation, mutations, rotamers, Ramachandran plots, and what not? Paul Emsely and Kevin Cowtan develop Coot at the University of York.

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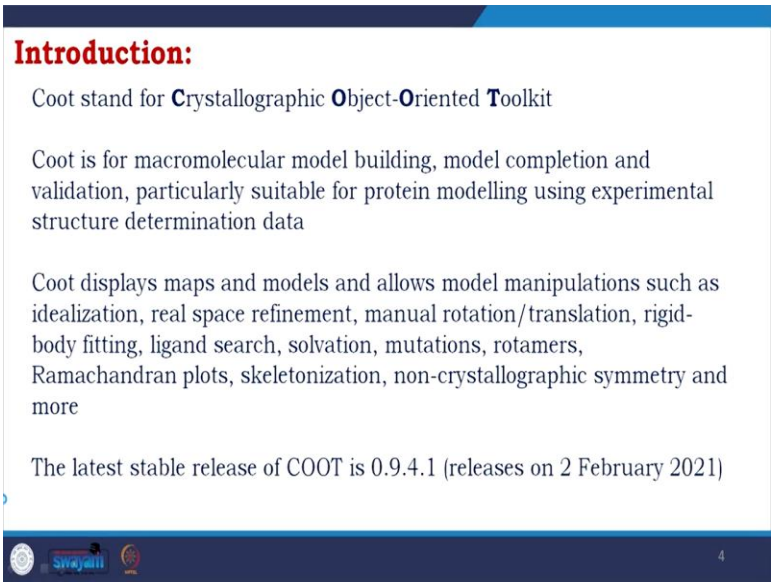

Coot, Coot is the name of a small bird that I have sown in the introductory slide. But why the name came to Coot because Coot stands for Crystallographic Object-Oriented Toolkit. If you remember, the history of determination of the 3-dimensional structure of macromolecules is

initiated and proceeded with crystallographic structures. Coot started with the visualization modeling of the experimental data and the electron density data we got from the experiment of X-ray crystallography. Then we do the model building, and Coot plays a very important role.

So, as I told Coot is for macromolecular model building, model completion, and validation, particularly suitable for protein modeling using experimental structure determination data. Coot displays maps, which is one of the very prominent nature of Coot that it helps you see the electron density map. So, Coot displays maps and models and allows model manipulations such as idealization, real space refinement, manual rotation, translation, rigid body fitting, ligand search, salvation, mutation, rotamers, Ramachandran plots, skeletonization, non-crystallographic symmetry and much more. The latest stable release of COOT is 0.9.4.1, released on the second of February 2021.

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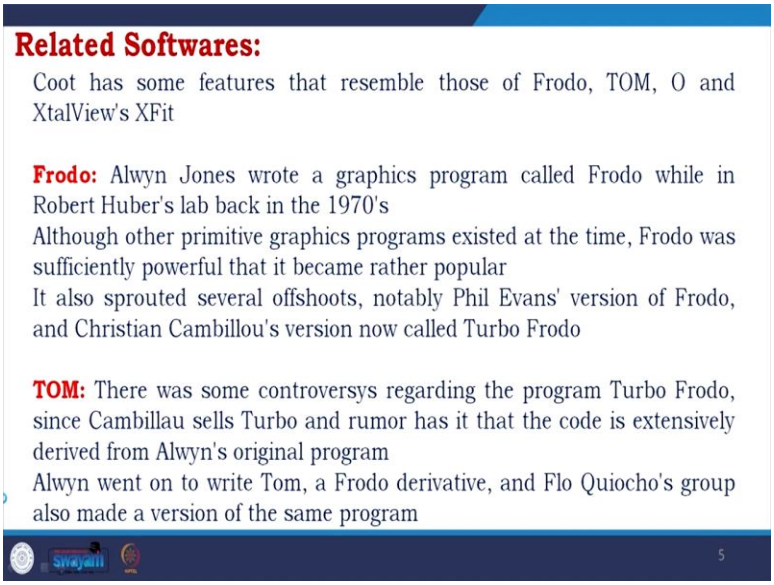

Coot has features resembling those of the softwares like Frodo, Tom, and O Crystalviews module called crystal feet. When we talk about them, it talks about where we are with the Coot. Frodo is written by Alwyn Jones, who wrote the first kind of graphics program while in Robert Huber's lab back in the 1970's one of the initial visualization programs.

Although other primitive graphic programs existed at that time, Frodo was sufficiently powerful and became very popular. It also sprouted several offshoots, notably Phil Evans' version of Frodo

and Christian Cambillou's version, now called Turbo Frodo. Then comes Tom; there was some controversy regarding the program of Turbo Frodo because Cambillau sells Turbo, and rumor has that the code he claimed about the ownership of himself is derived from Alwyn's original Frodo program. Alwyn went on to write Tom, a Frodo derivative, and Flo Quiocho's group also made a version of the same program.

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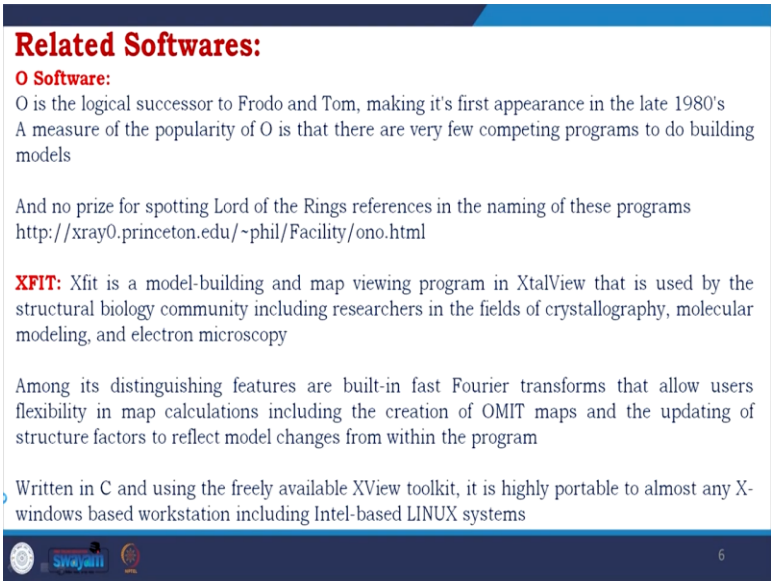

Coming to O, O is the logical successor of Frodo and Tom, making the first appearance in the late 1980's a measure of popularity of O is that there were very few competing programs to do building models at that time. And it is not surprising that Alwyn named it O for his influence of many rings to refer to it to acknowledge it. And you could get O if it is still valid and call in this link.

XFIT is a model-building and map-viewing program in crystal view. As I mentioned, it is a crystal view model used by structural biologic community including researchers in crystallography, molecular modeling, and electron microscopy. Among its distinguishing features is built-in first Fourier transforms that allow user flexibility in map calculation, including the creation of OMIT maps.

And updating structure factors to reflect model changes from within the program. So. you could do the automatic refinement and all written in C using the freely available XViwe toolkit.It is

highly portable to almost any X-windows based workstation, including Intel-based LINUX system.

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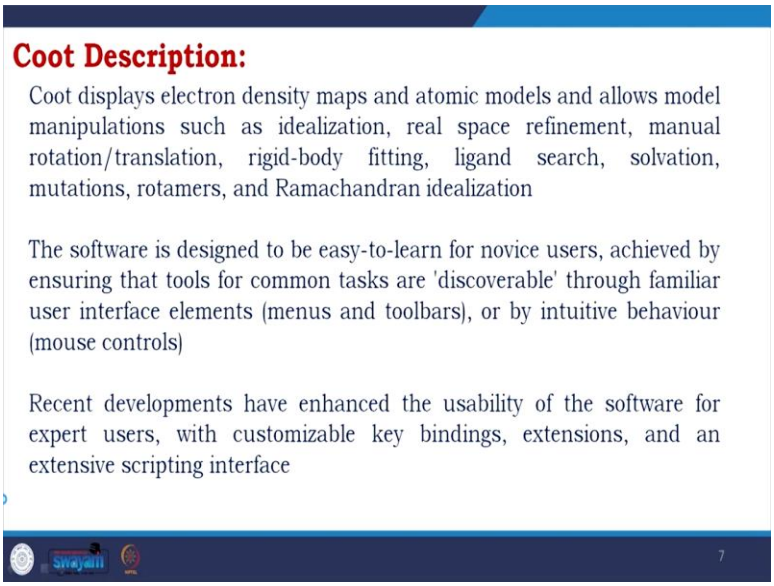

So these are the ones behind, so we have coot displays, electron density maps, and atomic models that allow model manipulation. We discussed idealization, real space refinement, manual rotation translation, rigid body fitting, ligand search, salvation, mutation, rotamers, and Ramachandran idealization. The software is designed to be easy to learn for novice users actually by ensuring that tools for common tasks are discoverable through familiar user interface elements, menus, and toolbars or by intuitive behavior like using the mouse.

So, this is very important and applied to any of the visualizations software, especially to Coot and all which help you to do further manipulation more you play more you learn. So, they are giving you the initial comments. I will describe how to do that and install it, but you who Coot have learned it even better than me as my students who are regular user coot you know to do with more efficiency than me.

So, it is more you have to play on that whatever I am talking about. Recent developments enhance the software's usability for expert users with customizable key bindings extensions and an extensive scripting interface.

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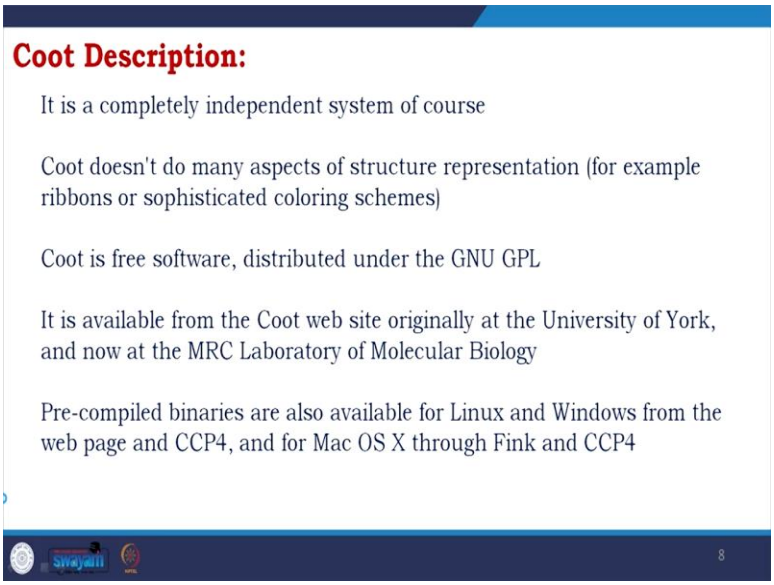

It is a completely independent system coot does not do many aspects of structuring representation. I am trying to say that we discussed different visualization software in the next class. We will talk about PyMOL to make many publication-quality figures that are Chi Mira VMD. They produce beautiful figures. Coot is not making beautiful figures.

But it would help you develop the concept and the idea so that you could make a good figure by designing it from Coot going to that other software. Coot is free software distributed under the GNU GPL. It is available from the Coot website originally at the University of York, where the inventors were, and now at the MRC Laboratory of Molecular Biology. Pre-compiled binaries are also available for Linux and Windows from the web page and CCP4 and for Mac users through Fink and CCP4.

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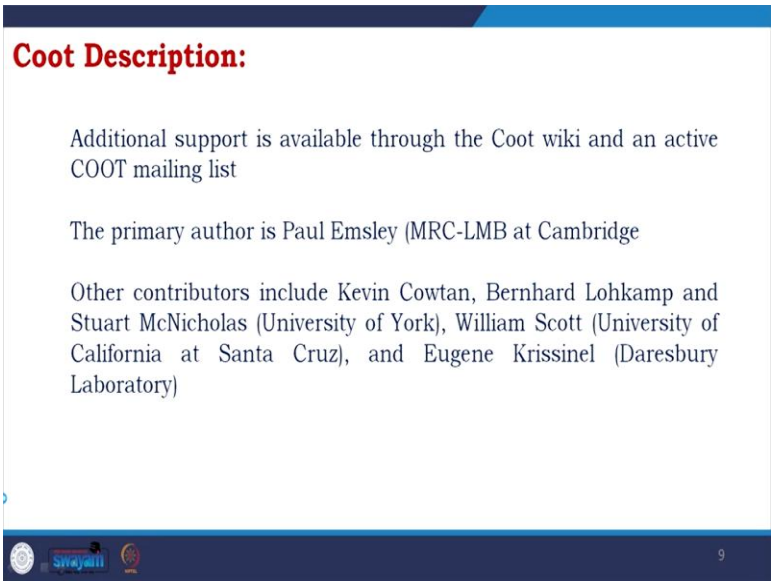

Additional support is available through coot wiki, an active Coot mailing list. They are very helpful; you mail them any problem and get answers. Also, if you are part of the mailing list, you will see other users and new users like you are facing a problem which will be good for you to learn things that you do not think or you do not face the problem, but others are facing. So, it is good to be part of the Coot mailing list.

As I told the primary author is Paul Emsley. Other contributors include Kevin Cowtan, Bernhard Lohkamp, and Stuart McNicholas, University of York, Williams Scott University of California at Santa Cruz, and Eugene Krissinel Daresbury laboratory.

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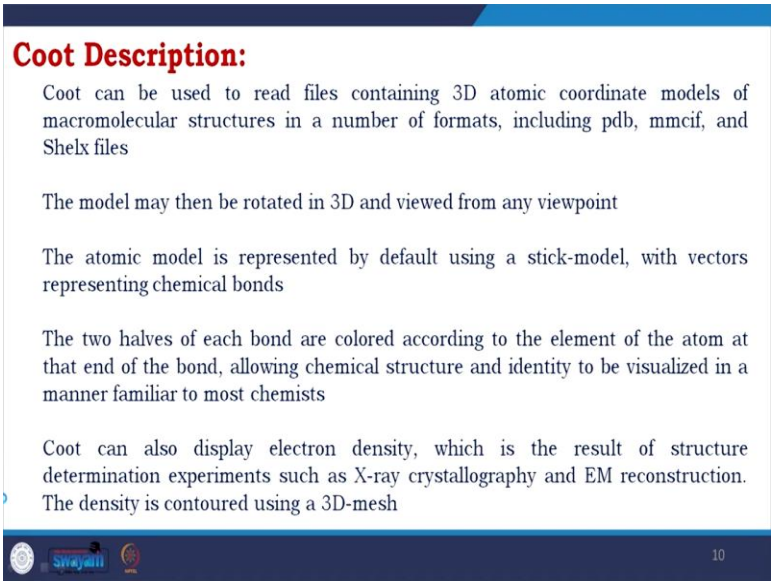

Coot can be used to read files containing 3D atomic coordinate models of macromolecular structure in several formats, including pdb, which we have discussed in detail, which go in the format, go in the coordinates, and understand how to use mmcif. Again we talked about mmcif and selects readable files in Coot. The model may then be rotated in 3D and viewed from any viewpoint. By default, the atomic model uses a stick model with vectors representing chemical bonds. I will show you them at the time of the demonstration.

The two halves of each bond a colored according to the element of the atomic at that end of the bond allowing chemical structure and identity to be visualized in a manner familiar to most chemists. Coot can also display electron density resulting from structure determination experiments such as X-ray crystallography and EM reconstruction. The thickness is contoured using a 3D mesh.

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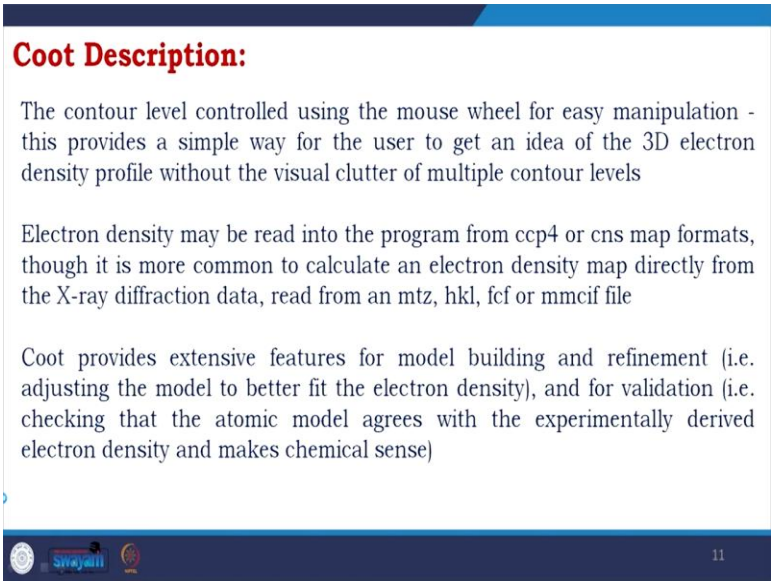

The contour level is controlled using the mouse will. I will show you again that for easy manipulation, this provides a simple way for the user to get an idea of the 3D electron density profile without the visible clutter of multiple contour levels. So, you rotate the mouse, and we will see where the electron density is high and where it is low, and all these ideas could be developed.

Electron density may be read into the program from ccp4 or cns map formats, though it is more common to calculate an electron density map directly from an X-ray diffraction data read from an mtz, hkl, fcf, or mmcif file. Coot provides extensive features for model building and refinement, adjusting the model to fit the electron density better like you have the electron density you are developing the model manually.

Now when you work manually, you make mistakes that could be corrected because Coot has the library. So, when you do the refinement, I have talked about these. This is energy minimization. So, it will push the whole structure towards lower energy based on the bond, angle, and dihedral data, which are already there in the library. The library comes from ccp4. So, Coot provides extensive features for model building and refinement. And for validation, check whether the atomic model agrees with the experimentally derived electron density and makes chemical sense.

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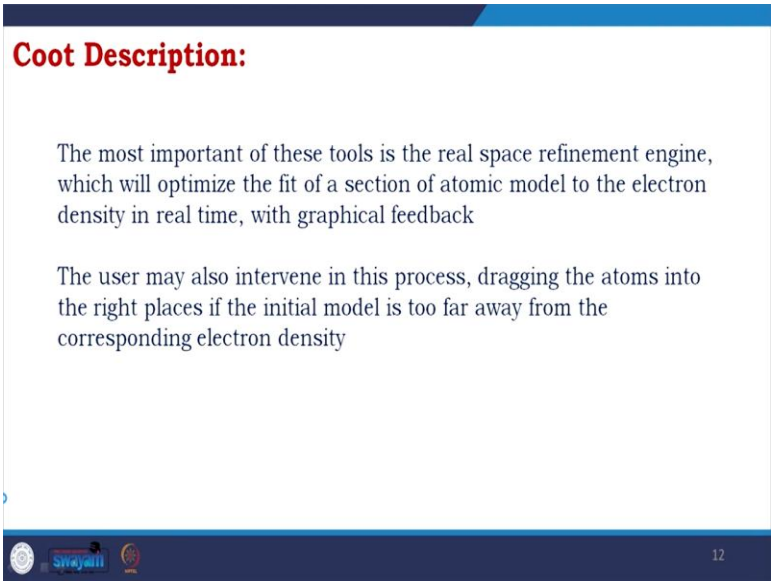

The most important of these tools is the real space refinement engine which will optimize the fate of a section of an atomic model to the electron density in real-time with graphical feedback. That is what I am talking about. You have the electron density, build a model, and then allow the refinement. It will automatically fix your errors based on the values in the library.

The user may also intervene in dragging the atoms into the right places if the initial model is too far away from the corresponding electron density. So, one that is automated based on the library, but then as a user, you have sense and could always utilize it.

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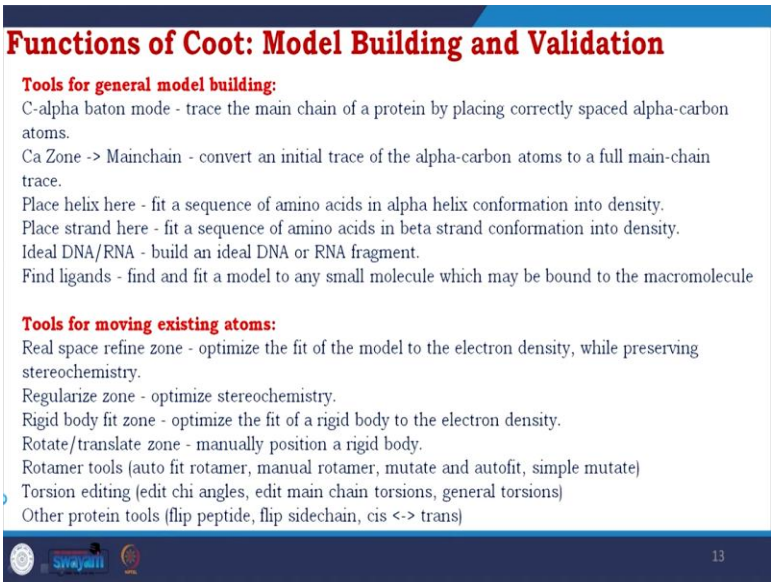

The functions of Coot are majorly distributed into model building and model validation. So, this is also what you could understand: the visualization software visualization interface is there, but visualizing is the least important thing. The part of the model building has majorly again divided into three parts tools for the general model building where C alpha baton mode, C alpha zone, and all these how the helix are their fit.

How the strand or their ideal DNA RNA finding ligands all those generalized programs are there. The tools for moving existing atoms and how we could do that. So, you have real space refine zone, regularized zone, rigid body fit zone, rotate translate zone, rotamer tools, Tersion editing tools like chi and other you know torsional angles and other protein tools like flip peptide, flip sidechain, cis to trans transformation and all these things.

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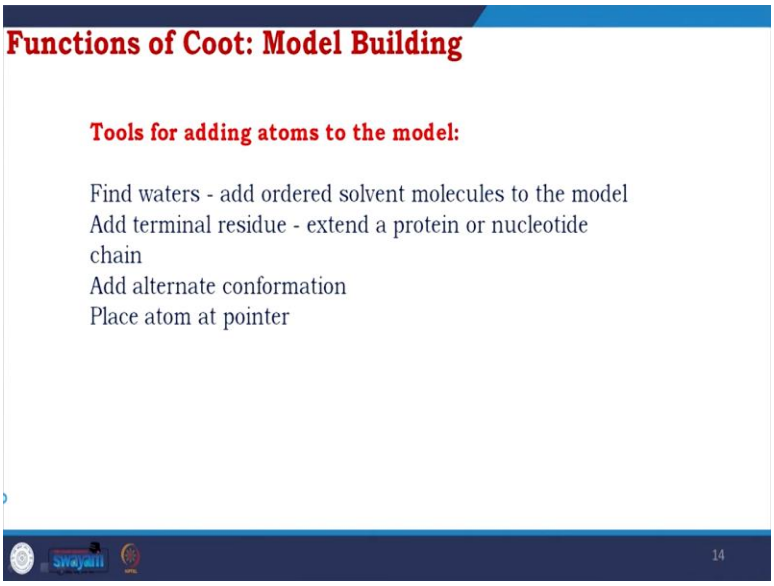

Then there are tools for adding atoms to the model; find waters because you have the electron density map, and the waters are represented like spherical blobs. So, you get it; you put water there; when you want to extend the chain, you use add terminal residue and alternate confirmation when you see that there is the existence of 2 or more confirmations and place the atom at pointer I will show you all of them when I will go to the demonstration part.

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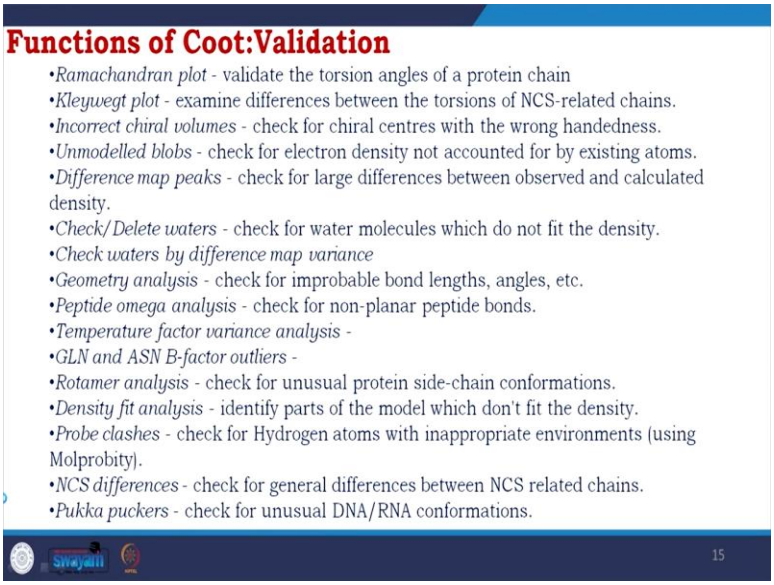

In the validation part, there are lots of programs. Ramachandran is there, Kleywegt plot is there incorrect chiral volumes, unmodelled blobs, difference map peaks, check to delete waters, check waters by difference map variance, geometric analysis, peptide omega analyses, GLN and ASN in the glutamine and asparagines, B factored out layers, rotamer analysis, density fit analysis,

probe clashes, NCS differences, Pukka puckers all type of analysis would be available in this coot software.

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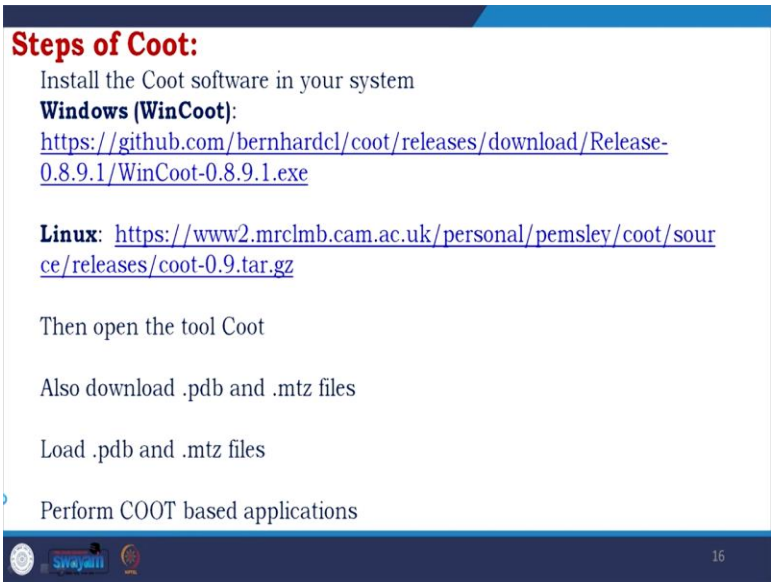

How we will use Coot, first, you have to install the Coot software in your system. If it is Windows, this is the link I gave the exe file. If it is Linux, then the file would be available here. Once you have installed a coot software, you have to open the tool coot. In addition, you have to download as I told pdb. Pdb represents the coordinate, and mtz represents the map because we will have the electron density map if you remember. The X-ray exit crystallographer got the electron density map and then developed the model.

So, the model would be represented in the pdb format, whereas the mtz file is represented in the electron density map. You have to load those .pdb and .mtz files. I will show you again that, and then you have to perform all the Coot-based applications.

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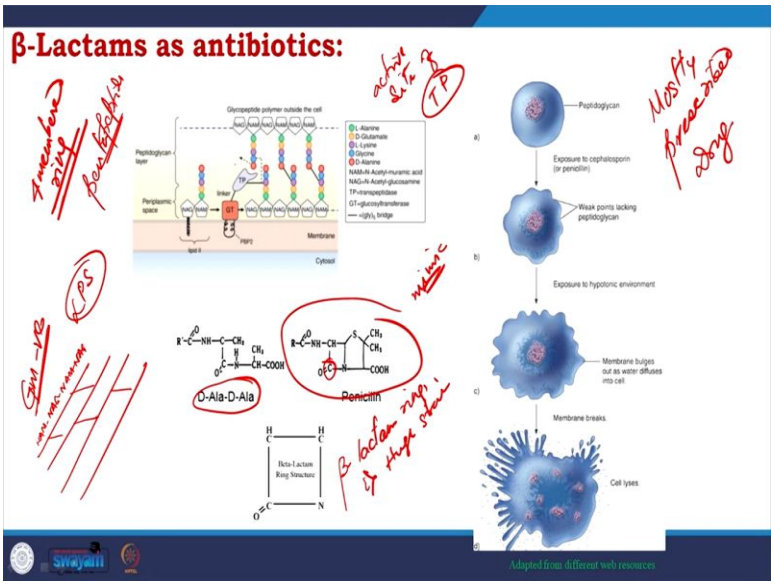

So, in today's demonstration, I will talk about protein-ligand interaction. But I tried to make it a story so that in addition to looking at how I am using Coot, you could also understand how science could be done. So, I picked up a few structures of an enzyme called beta-lactams from Mycobacterium tuberculosis. So, first, I will talk about why I have chosen them and what my goal is. What do I want to show you?

And then, in the next part, as I told you, I will demonstrate how I would analyze those structures using that Coot software. So, as I said beta-lactams the story starts with a drug called beta-lactams. Beta lactams are drugs that are, and I would say, mainly prescribed drugs by medical doctors. It is given or prescribed around 75 to 80% of all the antibiotics we prescribe.

So, if you go to a doctor, you say you have a headache and all other types of apparent problems. If a doctor will give you 3 medicines, 2 of them have to be beta-lactam; why? Because beta-lactam had a structure like that, penicillin is the first representative of the beta-lactam that Alexander Fleming invented; you probably all know about the story, but I would talk about the chemistry if you see there is a 4 membered ring; this is called typical beta-lactam ring.

There are two things; one, if you look at the ring, is the 4-membered ring. If you put your imagination into biology, I have discussed biological macromolecules, proteins, RNA, DNA, carbohydrates, and lipids. You will never say you will ever get any 4-membered ring containing

compound; why? Because there is a lot of strain. So, these have been a huge strain, so that is something followed by if you look at a D-Ala D-Ala.

What is a D-Ala D-Ala if you remember, gram-negative bacteria have layers of lippo polysaccharides, which are independent. If they are independent, they will not work together and are not strong. So, they want to work together and to do that, and they do so. This is the layer of NAM NAG. What are NAM and NAG? N-acetylmuramic acid, N-acetylglucosamine. So, they want to cross-link, you know they want to cross-link.

And its pentapeptide does the cross-linking; where it cross-links, there is D-Ala D-Ala. Now so, the enzyme which takes part in the cross-linking is called trans peptide because the cross-linking involves this D-Ala, D-Ala in the active site of the enzyme transpeptidase. They have to bind D-Ala D-Ala. And if you look at D-Ala, D-Ala, and the structure of penicillin chemically, these 2 structures are very similar.

So, penicillin mimics this, and this binds to the active site of transpeptidase. The enzyme transpeptidase is here; what is the result? So, the first thing is because of the chemical similarity between D-Ala, D-Ala, and penicillin or beta-lactams. They successfully bind to the active side of the enzyme transpeptidase. I remember discussing the strain because of the 90-degree angle in a 4-membered ring.

When penicillin or other beta-lactams bind successfully on that active site of the enzyme transpeptidase because of the angle strain, the ring wants to open, and the opening is enzymatically happened by a serine residue week attacks the carbon of the beta-lactam ring there occurs a very, very rare phenomenon what are the rear phenomena? The rare phenomenon is forming a covalent bond between an enzyme and the substrate.

I am sure you guys memorize the definition of a catalyst. And if you remember that definition, you will understand what I am talking about? I am talking about how an enzyme starts a reaction and makes the reaction rapid but enzyme never takes part in the response directly. So, now you

see an exception here where the enzyme takes part directly in the response. And because of that, because of the formation of the covalent bond, it becomes an E reversible kind of innovation.

So, now transpeptidase is arrested, and cross-linking is prohibited. It is not the date of the bacteria, but the bacterial cell layers are not cross-linked. So, they are weak now when exposed to the hypertonic environment because the last layers are not assertive. The membered bulges out as water diffuses in the cell, the cell lies, and then the bacteria dies. So, this is a phenomenal mechanism. And that is why as I told beta-lactams are mainly prescribed drugs. So, now you think about the relation of these beta-lactams with Coot? I am coming.

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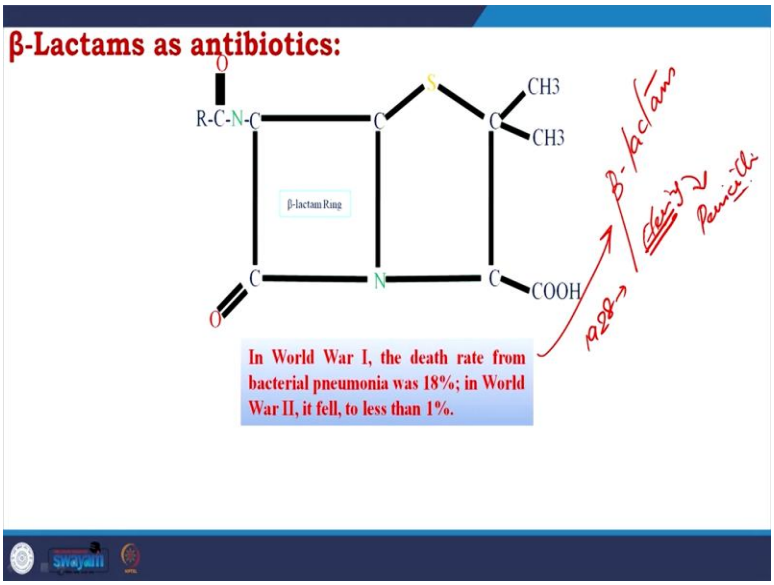

So, as I said, beta-lactams as an antibiotic were wonderful. In World War I, the death rate from bacterial pneumonia was around 18%. When we did not have beta-lactams, then in 1928, Fleming invented penicillin. It was not even a drug; he invented penicillium; it was given like the fungus is grown, and then squeeze the juice came and it was given to the wound. And you know how dramatic the effect is, as you can easily understand.

From the First World War to the Second World War, the ferocity of the war increased, and more dangerous weapons were used people had more serious wounds. Still, the death from the wound, which was 18%, was reduced to less than 1%, and that was the launch of the drug beta-lactams, which then in there got popular and the first drug that got universal use before that and still now

there are drugs or alternative medicines around the world Chinese have their own herbal medicine India have their ayurveda there are other countries they have their local practices.

But this is the first drug, beta-lactams, which got universal popularity. What is the result of this popularity? I talked about the difference between the death rate of World War I and II, but second, it created the pharma industry concept. Today, when you are standing in the COVID era, you see the fight between big pharma industries making vaccines for COVID and all these things. All those pharma industries were coming up with one or the other derivative of a beta-lactams.

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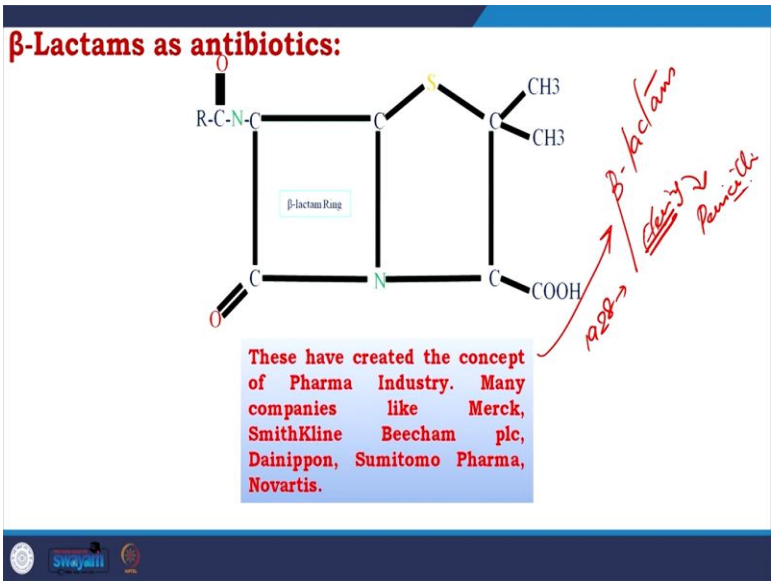

So, companies like Mreck, SmithKline, Beecham, Dainippon, Sumitomo Pharma, and Novartis, which are big giants now, started with beta-lactams in 1928. Fleming invented penicillin.

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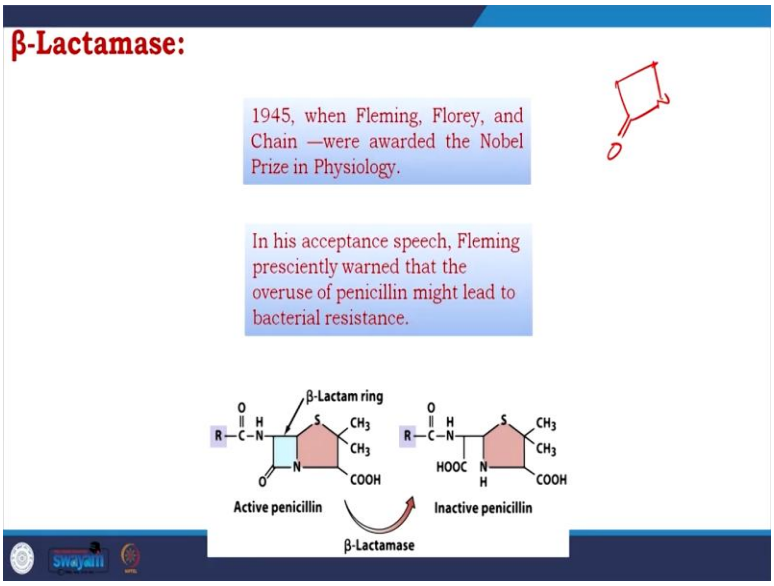

In 1945 Fleming, Florey, and Chain were awarded the Nobel Prize in Physiology for innovating the first universal antibiotic and getting their mechanism. So, Fleming, as you all know, inventory but Florey and chain was from Oxford University. They developed a group to perform the molecular characterization, and that group successfully came up with the molecular characterize the duration of the beta-lactams.

So, from 1928 to 1945, 18 years, you know, imagine you are the one who invented the world's first antibiotic. In these 18 years, you have the right to any funding because pharma industries are interested, and big funding agencies are interested. You should do a lot of work. Fleming did not do significant work in between, so he did not perform. He talked about that in his Nobel Prize acceptance speech.

In his acceptance speech, Fleming very clearly warned that the overuse of penicillin might lead to bacterial resistance the first time the world heard about the thing bacterial resistance. Still, you know in between as I told already people are get the magic bullet. A lot of scientists are trying to find a magic bullet against bacteria. They consider beta-lactamase is the magic bullet. So, the solution was there.

There was the usability proof I talked about in World War I and II. The difference then money was there because as it became a universal drug, the demand was increasing worldwide all those

companies; it was said that if someone had invested 1 dollar at the time in the beta lactamase-related industry, the minimum profit was 1000 dollars. So, money was there, and automatically power was there.

So, when activity, power, and money come together, a poor scientist cannot do anything. But it was proven that what Alexander Fleming thought in 1945 was very true. And currently, the whole world is at stake of existence because of anti-bacterial resistance.

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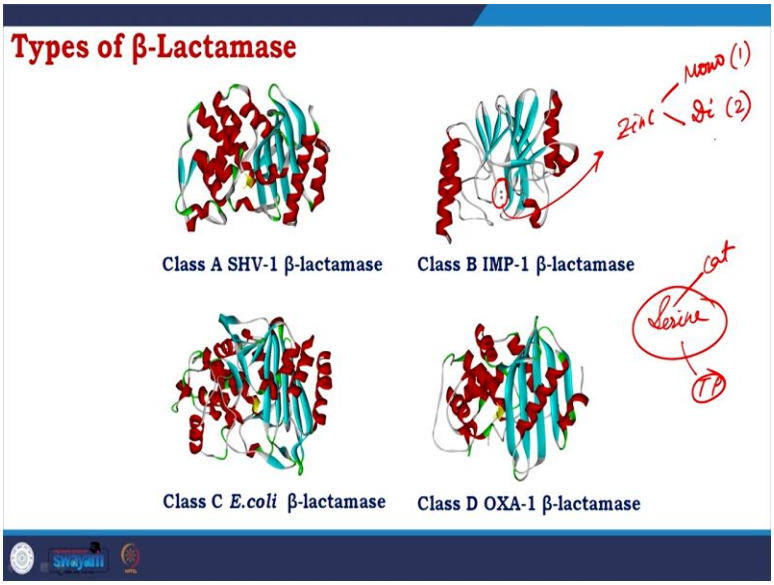

So there are different types of beta-lactamase, so they are included in classes A, B, C, and D, and I have given representative that in class A I have given SHV - 1 beta-lactamase is in class B IMP – 1 beta-lactam in class C the E. coli beta-lactamase and Class D the OXA – 1 beta-lactamase. Class A, C, and D walk through the serine residue as the catalytic residue just I have talked about in transpeptidase, whereas in class B they have these two dots are zinc. Should they have Mono zinc, Di zinc, and one or two zinc?

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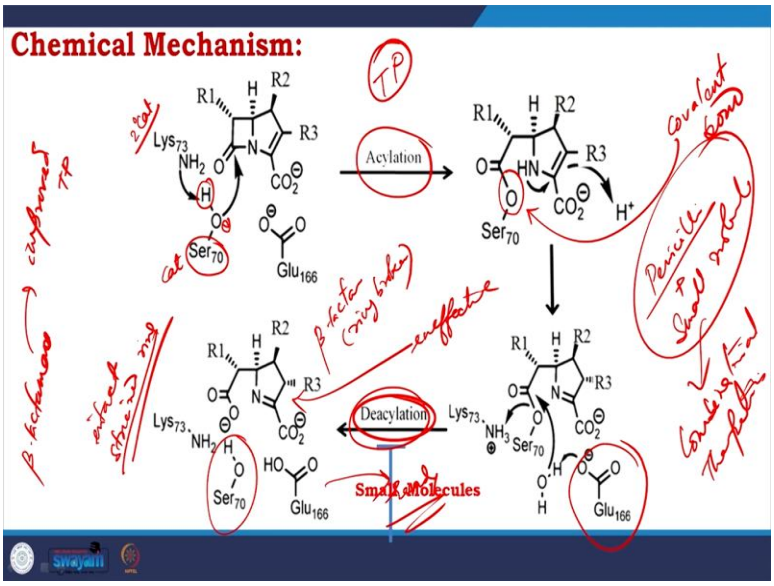

In this demonstration, I discuss tuberculosis beta-lactamase, a Class A. So, I am showing the chemical mechanism of how class A works as I told the walk through the catalytic residue serine. This attack occurs when the other residue, the lysine, is called a secondary catalytic residue. It takes the proton, making it negative so it attacks. This makes the formation of a covalent bond.

And the type of reaction is called Acylation. If you remember, I talked about transpeptides, transpeptides are used to go through this tape, and because of these covalent bonds, they are arrested. Now beta-lactamase is an improved transpeptidase. So, it has a second step; it contains a residue called glutamate where the O minus attacks and takes the proton of a catalytic water molecule which attacks and breaks the bond.

So, the beta-lactam ring is broken now, and we know that beta-lactamase activities because of that intact strain ring. So, in the absence of that ring now, it is ineffective, but the enzyme is free if you look at the serine. So, the enzyme is ready to go for another attack. And that is why the registration happened the resistance, and the second reaction step is called de Acylation. So, as drug designers, we always try to target this de Acylation step.

We try to get a small molecule that would prevent the de Acylation step or at least slow it down. So, it would be slowed down, and then you have the penicillin or other beta-lactamase. So, penicillin plus this small molecule is called combinatorial therapeutics. So, as I told you in the

next phase of my demonstration, you will learn how to use Coot, hear the story of drug designing, and learn how we help up high-resolution structured data to go for drug designing.

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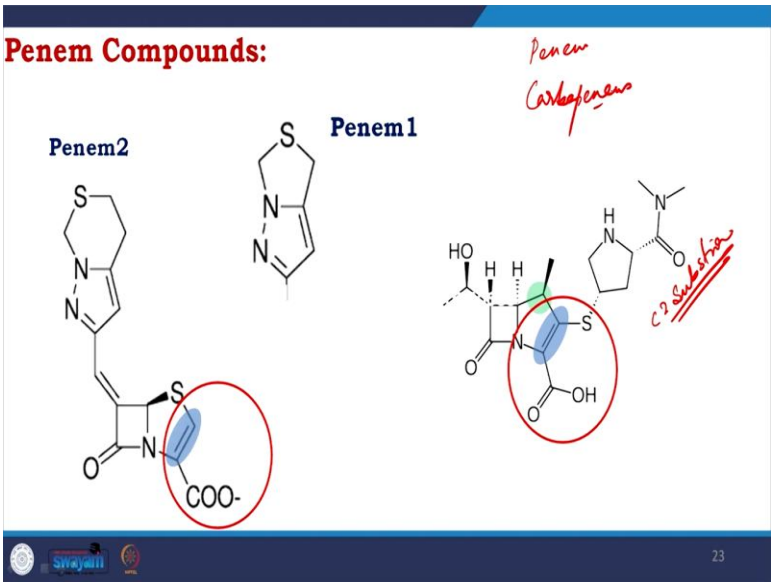

So, I am talking about some compounds; these are carbapenems, and these are penems. The penems are the drugs we designed carbapenems are already established, but many things are still to be understood. And the understanding will lead you to further innovation of more potent, novel new, generation therapeutics. So, if you look at these portions as identical, they have what we call C 2 substitution in Carbapenem here; the substitution is not there. Here the substitution is in a different place, so we are showing you how the penem and carbapenems are further investigated.

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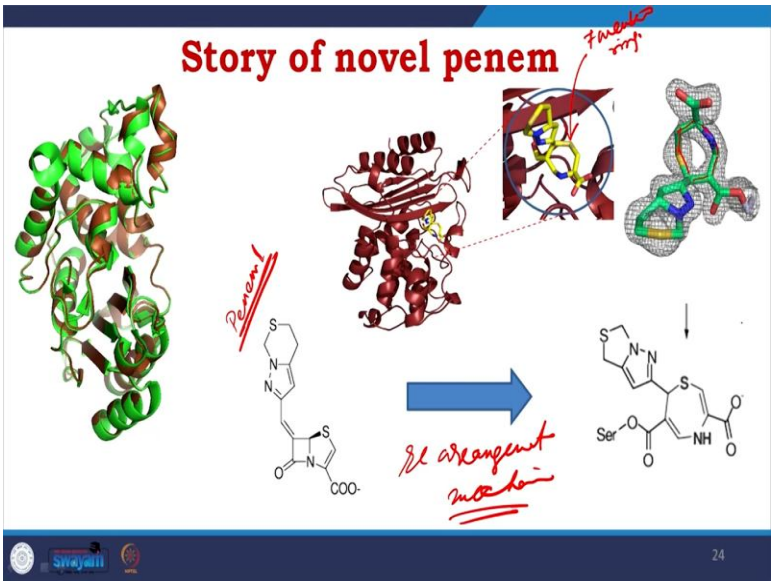

So, as I told the story of the novel penem, the structure was solved. And when the active site was doomed, to everyone's surprise, there was a 7-membered ring. Again, if you return to their biological macromolecules, you do not get any 7-membered rings. So, the development of a 7-membered ring is surprising. And if you look at the high-resolution structure, you will see that in 1, 2, 3, 4, 5, 6, and 7, the 7-membered ring is present and intact.

So, how this 7-membered ring happened? Now if you come to the original, the penem 1, the conversion from this to this is a re-arrangement mechanism. If you look from the point of view of bacteria, they were exposed to this drug, but in the presence of the enzyme, this becomes a modified drug. So, it is difficult for them to develop resistance and this drug, whatever they are used. We have no incidence of resistance reported yet. So, this is one story I want to explain. Another story is about Carbapenem, a novel carbapenem new carbapenem which is called tebiepenem.

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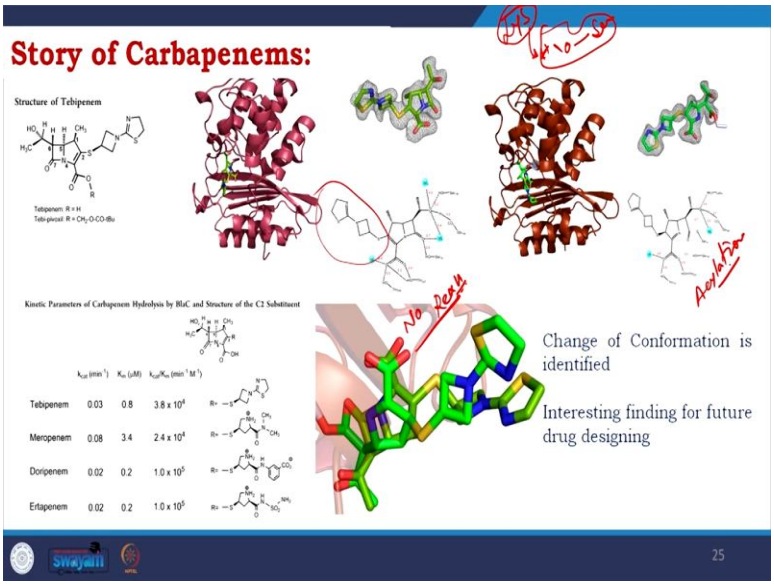

Carbapenem has a very interesting story. If you look at them, they have changed in structure. But if you so, there are Tebiepenem, Meropenem, Doripanem, Ertapenem, and others like Bayapanem, Imipenem, and Panypenam, Bridgeupenem. But when you look at the enzyme, you will see that this part is mostly out of the system. And you did not get a lot of interaction with the protein, and you understand what I mean.

So, what I am trying to say is that the C 2 substituted portion is different from all the drugs. The rest of the part is the same. And if C 2 substituted part of no interacts and then all those compounds, all those drugs should behave equally or similarly. But there is a significant difference; I am showing this difference in beta-lactams from tuberculosis. However, the trend is similar in other beta-lactamases 2; why has this difference developed?

To understand that what I did this is my work, so what I did I tried to make trapping of the compound before the reaction and after the response. You will say we understand after the reaction and how before the reaction what we. If you remember, a lysine takes the proton from the serine. We muted that lysine so the serine proton was intact, and the reaction did not happen.

So, we get two states: where the reaction does not happen and where Acylation happened. So, there are very similar candidates to compare, and very compare them, we see the change in conformation. So that tells us we have identified the change of conformation. So, this is an

exciting finding for future drug design because people have long wondered why those drugs behave differently with the C 2 substitution when the C 2 substitutes have nothing to interact with the protein. Now we see that there is a significant change of conformation upon the reaction, giving a platform for further investigation.

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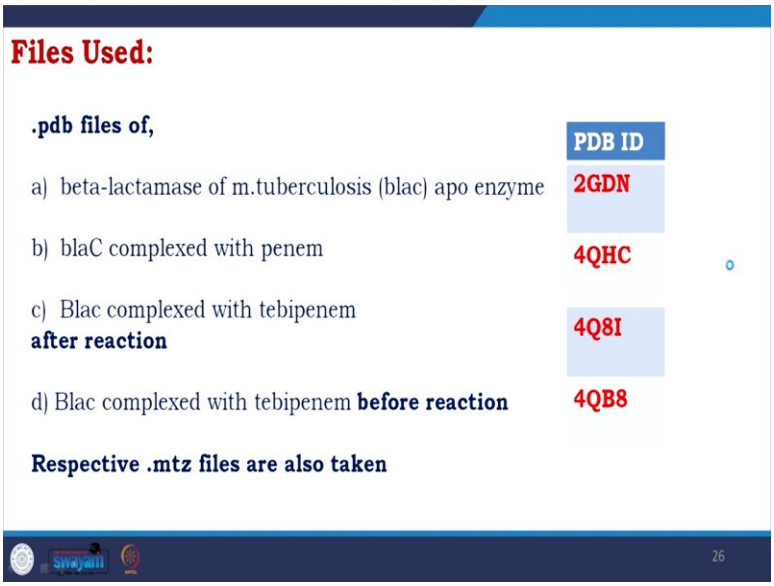

So, to do that, I take the pdb files up beta-lactamase from tuberculosis apo enzyme like the enzyme where no substrate is there. Then blaC the enzyme complex with the penem I talked about the compound with rearranging and mixing is 7 membered rings. So, we will see that then blaC complex with tebipenem but after reaction and blaC complex with tebipenem before the reaction, and we also take respective .mtz files.

So, for the PDB, we have taken 2GDN, 4QHC, 4Q8I, and 4QB8; you could download them from rcsb, as I have shown before, and you could easily follow what I am doing. So, then I will take you to the demonstration part.

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So, this is the interface where you will see the use of Coot; there is another interface where all the scripts are also coming. So, there are two interfaces in this interface we will see all the changes we are making. So, if you have a file, go to open coordinates because we will open the coordinate files. So, we will go to the file and get the pdb. So, this representation coot is a very descriptive interface with lots of in a library and all.

So, whatever you know format there, they always get some errors. So, we agree to correct them, and this is the representation you will see that, as I told earlier in PyMOL, Chi Mira, and VMD, you will see much better representations. But Coot is what it would help us to function. So, here you get open coordinate auto open MTZ open MTZ, mmcif, fcf, phs is open Map import CIF dictionary which is very good.

If you remember, in the previous class, when we discussed the pdb file, I showed you the difference between atoms and hetero atoms. The atoms comprise all those standard amino acids, nucleic acids, lipids, carbohydrates, and all water, and all hetero atoms are what you are developing a new drug or something. So, the library information is absent whenever a new drug is coming.

Whereas for a standard amino acid for a nucleic acid and all the libraries present, you have to provide the software the library or the dictionary of the new compound. That would be done by using the import CIF dictionary. So, there are other ones also; you could directly fetch the pdb from the internet, the electron density map, and all from the internet and all these things.

Also, you have many edit ones; you could copy molecules, you could copy fragments, replace residue, replace fragments, renumber residue, change chain IDs, merge molecules, bond colors, restraints, map color, map parameter, bond parameters, skeleton parameter, residue info, background color some of them have even multiple options and all. Then you could calculate many things you could model feed refine, which is working with the model. This is one of our good handy tools.

But then there are other modeling tools you have SSM, LSQ their alignment once we will use them to mutate residue range, align and mutate, and move molecule here, fit loop, fit loop by doing Ramachandran help, fit loop by database search, maps sharpening, blurring, map skeleton, NCS is non-crystallographic symmetry base maps, frames, scripting you could have introduced script here you could run a script, you could do ligand bind, you could do rcrane launch a lot of options. So, you have this molecule.

Now you see that, and it would be better if we introduce the corresponding map. So, we introduced the map. And now you see these were the original resources on which the coordinate and pdb files were built. Now you go to and click anywhere you could have seen them; as I told when you start moving your mouse, you will see that the label is getting higher, which you would go and see when you are doing that you know when you are doing it is the level is increasing decreasing map level. See, it is enhancing the number.

So, you could do them. Also, you could find mistakes made by a crystallographer by looking at the map. Before that, you must know about the map; the map is colored so that the electron density is there when this is normal, and the atomic model correctly builds up the color blue. There is electron density when it is built somewhere, but if you do not model it accurately, you will get green.

When somewhere you should not model, but you modeled, there is a chance of getting red density. So, we call it traffic; when you should go, you get the green light right. Here also when you should put some model, you get green color on the map when you should not like here, you should not map it here, but you mapped it here, you get red like in the traffic light you should not go, but you break the rule now you obtain fined.

So, here also, the find means you have the wrong structure. So, what is the possibility if you look here? That is the beauty of Coot software while looking at the electron density map. Now you Coot imagine yourself not only as a crystallographer but you should also work as a crystallographer. So, see here the option is rotamers; if you click the rotamer, you get a lot of options of rotamers; see, the rotamers are created in different places.

And among them, something is coming here. And this is one of the most possible and correct confirmations. So, the crystallographer made a mistake. Now using Coot software, you can identify the mistake; that is the beauty of Coot. It is not only a visual interface, but it helps you work like a real-time crystallographer. So, I could make this point here now. I will show you some cool things.

One is interaction, so let us go to the interaction between the amino acids. How to measure go to environmental distance so the residue environment does not take bumps to make this distance around 3.8 or 4 and level the atom. Now if you go somewhere, you see those interactions, but the better thing is you can continue it by clicking the set bar now; you could click this shift and see all the interactions.

By doing that, you could make it a tour of the whole structure without doing much, you know, work that is also a beautiful property of Coot. Here you get all those numbers; you will see the numbers and the atoms like OG1181 threonine. So, the oxygen of the threonine is 181. If you know, you could have read that it had 3 angstrom interaction with the water molecule and a 3.4-angstrom interaction with the threonine next.

And with that nitrogen backbone of the threonine. So, all of these you could have understood in that way. So, what next? Let us go to that one other structure. So, load coordinate of the penem structure. Penem structure is there; now, if you remember, all four structures come from the same protein. So, they are the same amino acids. So, you could have compared what to do to compare them.

Go to calculate, go to SSM superpose and then keep the first one, the 2gdn .pdb and compare it like align the other one, the other one will move that gdn and will be intact and apply and now you get that perfect alignment; if you see in some places, you will get beautiful alignments. Now when you see the alignment, the difference in some areas is different. The differences are because of the small movement of the amino acid site chains because of the complex formation.

So, let us look at how the protein interacts with the substrate. This is the substrate, and if you remember, I told you, see this is the 7-membered ring; this is the penem here you see a seven-membered ring. Now how it interacts with the protein, you see that, so now you see that seven-membered ring 1, 2, 3, 4, 5, 6, 7, you know the protein have a lot of interactions. So, because of this interaction, you see changes in the position of the amino acids.

So that is what you could find how they are different, how they are interacting, all these things you could get from here. Another one remembers I talked about that if you see a green density, you could expect something that was not modeled in the so this is display manager in the display manager, you will see all the structure. So, if I close that structure I modeled, you see that there is nothing here, but if I again put that, you see there is a good phosphate.

So, the phosphate was not correctly modeled in the first structure the EPA structure, which is why they get a big green density. You could also see that the phosphate has moved the movement because this phosphate is very close to the substrate. So, when the substrate comes, it pushes the phosphate, which has a nearby conformation like the same position but a little bit moved.

So, all these things, another thing is if you have, you see time, you do not get a map for the other structure because the map belongs to the GDN. So, you could move a coordinate, but you cannot move the electron density. What more could we do? We could do many things; as I said, I could only talk about a few. So, I could show you one of them that we could do that maybe mutation.

So, what have we learned now? We have learned how to load the PDB file we have learned how to load the electron density map. We have seen a difference in the map; when it is normal, it shows blue color; when it is wrong, it is either red color or green color; when something has to be put somewhere, but people have not modeled it, it will get green when they model it, but it should not be model there they would get red color.

Now we will see that mutation; you all know who are working or following this course, that mutation has a considerable role. What is mutation? The mutation is the change of the amino acids. So, when doing structure understanding, mutation plays a considerable role. And here, you could do mutations very easily. There are two options mutate and autofit are simple mutate. Simple mutate you will do when you do not have an electron density map.

When you have an electron density map, you will mutate and autofit. You will see that all the options are given when you click here. Suppose you want to do alanine. So, you see that that

alanine mutation happened. If I change the mutation again, going back to thyroxine, we have to get the protein molecule you want to mutate. So, mutate autofit. If you're going to put thyroxine, you will see that thyroxine appears.

But if you remember, the thyroxine was a little different in a position; now, it automatically adopts where the map is. What else could we do? We could show how you could use rotamers; as this is where you want to get the other rotamers, you will get 1, 2, 3, 4. 4 different rotamer conformations; we do not include them it. We could also do regularized zone. Regularize zone you click into one atom.

And then you choose a shown we are connected, and you click on the other amino acid; if I go for one structure, it would be easier. So, you see that they are all covalently linked. So, to do regularize zone, you click here, and then you click here, you know the alteration the structure itself tries to regularize, and to do regularize, it makes small changes. So, you will see the changes which are observed here.

So, if you do regularized zone, you get them; you see regularization in the bonds, regularization in the angles, regularization in the planes, regularization in the chiral, and non-bonded interaction; all types of regularization happened. You could accept it; you could reject it. Also, if you want to delete this, you could come and do the delete option then you will get a lot of options.

You want to delete any residue or monomer; you want to delete water, you want to delete atom, you want to delete side chain, the zone you want to delete side chain, zone chain, and hydrogens in residue delete zone; suppose you put water, and then you click it will be deleted but this optional go. But if you keep deleting active, you delete this and see this is deleted. Now you could delete that, and you could continue deleting.

So, you could add alternative confirmation. So, for any amino acid side chains, if you fill that, it has to conformation you take had alternative needed confirmation split all of a single residue or split a single residue at C alpha; you could take the choice, and you see you get two

confirmations. Now you could settle up in this confirmation or that confirmation or that confirmation in any way, or you could also do it manually.

Now I will bring the third molecule; you remember this one is the penem. Let us also take a look; red density here means it would not be there; it should be seen; it should be here. So, you want to delete it; you want to delete water, you delete it, then you go to rotamer and search for rotamer; you do not get it but get the closer one. So, you accept it, and then you could have a rotated translation you could do by rotating on the X.

So, X translation, Y translation, Z translation, X rotation, Y rotation, Z rotation, or you could have another method when becoming an expert. You could push control; you could have directly pushed that residue, but when doing that, you would be confident you would be a good user. Otherwise, if you are not a good user, you should not do it; you should take the help of the software; like see, even if I was doing it for a long time, you still make a mistake and do not accept it.

So, in this way, you have to keep working on and finding the better ones, but when you are working in a difficult zone of the protein, what I mean by the difficult zone of the protein what I mean is when the electron density is not good, no program is helping you to do that you are doing the thing with the help of your experience then what it is advisable is in after minor changes you should go for a cycle of refinements more you perform refinement better electron density you start to get.

So, we have shown some of the changes in some of the factors like mutation, like deletion of water molecule alternate conformation rotamer, and all these things we could also do other related things like edit backbone torsions, the torsions angles; you could edit the chi angles the side chains and all this you could also perform as I talked about there are validation softwares. So, you could go valid Ramachandran plot of that 2 GDN.

So, when you do that, you get the preferred region 96.96% allowed region 1.9 portions of five amino acids in the output layer 3. When you do that for another penem, you get 243 on the

preferred region, 7 in the allowed, and 2 in the out layers. Now let us introduce the other ones, so this is the pdb of Michael is menten complex of the blaC tabipenem. Again this comes in lighter blue, and you want to calculate SSM superpose.

You keep 2gdn unmoved, and you want to move the mm. So, now three structures are together, and you can see more exciting differences here. So, how to go to where the ligand is so to go to the ligand, you go to the draw, go to atom, and then you choose that perfect pdb like tab underscore mm this pdb, and then you get the chain to see here the residue starts with 41, do you know why it starts with 41.

If you remember, I always talked about the thin that the initial residues of the protein are generally not integrated part of the structure. So that is why they are loose and do not provide good diffraction. So, it is difficult to solve them, or sometimes we cut out that portion like here. So that we make our overall protein more rigid; more rigid protein means more stable protein and more protein production.

This is also one type of protein engineering that we are going to discuss in our last module here, we are talking about that one is structured based drug designing; this is one of the perfect examples of structure-based drug designing, and along with that, we will also talk about protein engineering. So, if you go there in the pdb file, you see waters are their amino acids are there, and in between, you will see that there are phosphates, and something which is not common in most cases the not common thing is the drug ligand.

See here, and this is Michaelis Menten complex because the 4-membered ring is perfectly intact. Now if I again introduce to another one that teb C and I do the SSM Superpose. So, everyone is now in that similar position, and we go again to the ligand. Now you will understand what I talked about. If we go to the display manager and put them out of the system, we only have the two tebiepenem.

You could clearly see that, and we could also bring the mtz file of any of that autopenem mtz, and so we get the mtz file, we close the mtz file you see as I was talking about. Now you cannot

align them because the pdb file moved. So, you could only see them with respect to the initial map because they have aligned based on that structure. So you can move the pdb file. You cannot move the mtz file.

So, again, if you come back to the ligand as we were discussing, you will see a significant difference in the movement of the ligand when it was unreacted and when it was reacted. So, as I was talking, if you remember what we have seen and what I talked about, you will also understand that this part is not interacting with a protein, because if you measure the environmental distance you already have set up and everything is there.

Now you come here and clearly see that there are very few interactions after the C 2. There are few interactions with the water molecules. Still, they have no interaction, so when they have no interaction, that proves the discussion happened in the literature that if the C 2 substituted portion does not interact with the protein, how do they make the difference how different C 2 interaction in Carbapenem, in Tebiepenem, Dorbipenem, Bayapenem, Meropenem, Ertapenem how they are coming up with different KKDM and KM values.

Many people have asked those questions, but there is no evidence of an answer. But now, if you look at these where you see that for the one structure, the 4-membered ring is intact, whereas in the other structure, the four-membered if for some moment to make it more; clear if I take out this you will see this now here you see that this is intact whereas this is broken and when the ring is broken you know this carbon is here right.

Now this is very close to the serine. So, very interestingly, if you note here there are three serine, you see one serine belongs to the covalent bond, so this is near to the carbon; the distance is 1.37 angstrom which clearly says that these have to be a covalent bond. But the other two serine are for every additional two belonging to the complex, which does not react with the tebipenem. Now understand what I am saying when the s is interacting and forming a covalent bond

It is in one conformation, whereas when it is not forming, it is in 2 conformations; why the answer you will get if you look at here this serine interaction you will see here there is a lysine.

The lysine is holding it, whereas in the other case, it is an alanine. So, because the side chine is lost now, it cannot hold the serine, and then the serine is not flexible. So, this gives us the in-depth mechanism of how the ligand binding change the conformation of the lysine go and hold the serine to a particular position.

And now the serine not only loses the proton but becomes ready to do the reaction. So, this is not only showing us but also giving us the mechanism; as I told the distance; if you compare the third ligand to the penem one, you will see that the penem again goes in different directions. So, those movements are critical changes that were not apparently observed in normal biochemical analysis.

But when you compare all those structures, you get the movements of those small molecules and will find that movement in the amino acids. All those are complex; when you get 2gdn, you will see that the complex one is in 1 place and the EPO enzyme amino acid is in other places. So, these give you introspective to in-depth knowledge about the chemistry behind the enzymatic mechanism.

So, in short, we have seen many options; as I told you have to play more to know more, but through this file you could have used the pdb file for the coordinate mtz file for the electron density map. You could also use fcf or phs formats. You could also import a CIF dictionary for the small molecules; you could perform a lot of operations through edit through calculating in the draw, you could have drawn a lot of views in the measure, you could have put distance, angel, you could have level them, you could have put the pointer to make measure the distance and all these things.

And invalidate, we have a lot of options to validate different things we have talked about; you could have used the scroll wheel also for an alternative view, or you could have used the read the header remarks if you remember I talked about that in last class in the pdb option when you go to the pdb there are a lot of remarks header and all you could have to get them here, you could work with ligand, you could isolated mol probity dots for the ligand, isolated coot ligand dots, isolated Coot all-atom contact dots it is find a new ligand jiggle fit ligand.

And all those you-know formats and all you could do. Also, you could get a lot of extensions which help you get more options. So, that is one word the preliminary use of Coot; as I told this is not enough. But this source gives the power; you could have compared each water molecule, you could have compared the distance, the moments, the electron density map, the density is correct or not.

You could become a real crystallographer real structural biologist working on electron density data, but even if you are not having your data could download the mtz file; you could download the electron density map from crystallography and the electron density map electron microscopic and practice this part. The computational part is an integrated part of structure development. And only software platforms like Coot could give you, as I told you, there are other ones, but at this moment, it is Coot that is used by the entire combinative.

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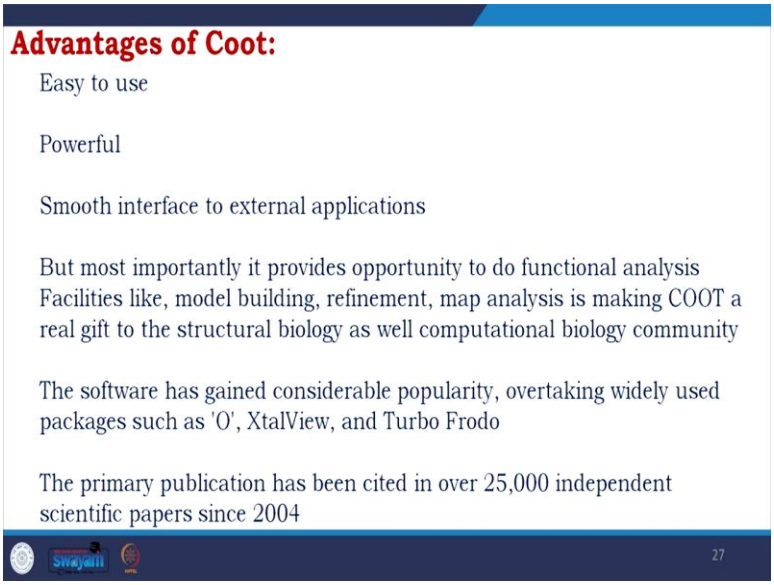

So, I would conclude this session by saying that one of these is Coot easy to use, is powerful, and has a smooth interface to external applications. Still, most importantly, it provides an opportunity to do functional analysis facilities like model building refinement and map analysis, making Coot a real gift to the structural biologist and computational biology community. The software has

gained considerable popularity, already overtaking widely used packages such as O crystal view and Turbo Frodo.

The primary publication has been cited in over 25,000 independent scientific papers since 2004, proofing the metal. Because I have designed this course for newcomers, the young dreamers, I request you to download this software and use it more and more; it is impossible to understand and learn any software without practice in hand. So, I have given you some of the essential clues, but you could do your best by doing more and more practice. But if we that any questions, please feel free to contact us. Thank you very much.