Inorganic Chemistry of Life Principles & Properties
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Lecture - 11
Techniques used inorganic chemistry life

Welcome you back to the next lecture on the Inorganic Chemistry of Life. We have been going through the some background studies one of the background study was the biological molecules, biological processes. The other background study that I have explained using coordination chemistry aspects, the third component that I need to complete before I go into any enzyme or is the what kind of techniques are being used in gazing the biological inorganic chemistry.

So, the earlier 2 components you need to use my slides along with some information from the regular text book; similarly true with this one too, but this is going to be mostly on the techniques. So, I will not be explain the physical chemistry behind that or physics behind that, but I will explain mainly what kind of an application is used in is relevant in the context of the metalloproteins and metalloenzymes.

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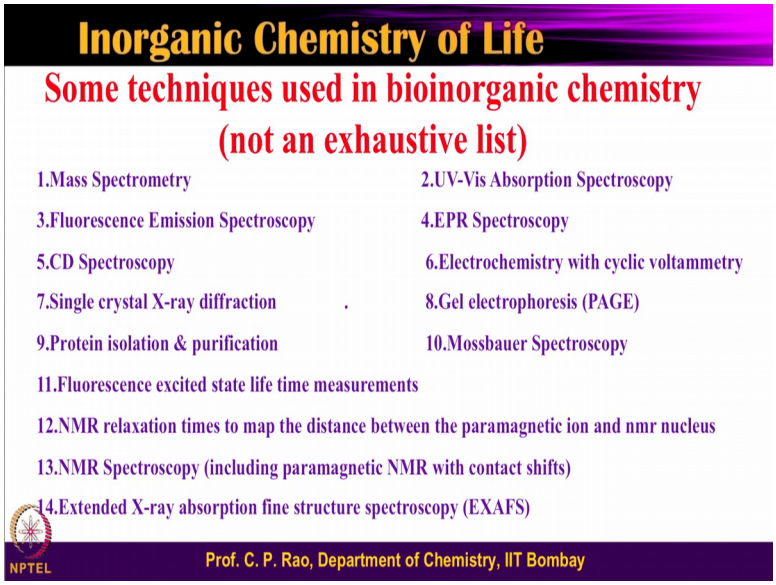

Let us look at ones this particular slide here and if you look at this particular slide; a huge list of items there is a list of item more than 1 dozen or and I say that it is not an

exhaustive list. So; that means, that many different kinds or techniques are being used in gazing the metal ion its complexation in biological inorganic chemistry.

For example, mass spectrometry some of these you may have heard, some of these you may not have heard also I will not give much in the principles, but I will give how the utility of these with some example or something mass spectrometry. Obviously, what does it do? It gives you the mass are the of the protein or metalloprotein, when you isolate protein the protein there could be a protein impurities, therefore impurity can also be found from the mass spectrometry then we will say the impurity is present.

The fluorescence emission spectroscopy what will it do? You have an electronic exaltation, but you will not look the exaltation part, you look at the emission part. So, when it returns back to the ground state it emits certain light in the form of radiation and that is what you study which is referred as the fluorescence emission spectroscopy. In the CD spectroscopy and absorption spectroscopy are very similar and you know in this UV-Vis absorption spectroscopy you are looking at the electronic transitions going from the ground electronic level to the excided electronic levels.

And that is what is the one which gives absorption data and now the same kind of absorption in presence of a dissymmetry or asymmetry kind of a situation and in such a situation you get; the light being absorbed is not uniform, is absorbed one some components are absorbed more than the other.

So, generally the plane polarize light can be can be talked in terms the 2 circularly polarized lights which are referred as a right circularly polarized light and left circularly polarized light of which one of the component could be absorbed more than the other; you will get the due to the dissymmetry and that will give the CD spectroscopy.

Therefore, the CD spectroscopy is an extension of the absorption spectroscopy, the feature that you do not see here, you will see in the CD spectroscopy. That will give you the dissymmetry parameters; you can have a negative and positive and you do not have anything called negative and positive in UV visible absorption spectroscopy. And then when you have a electrons in d shell some of the electrons may be unpaired in such a kind of situation, you will have the electron paramagnetic resonance too.

In the organic case, most of the molecules the electrons are paired therefore, they are diamagnetic unless you have a radical you will not have any pr; but in organic complexes there are so many metal centers under certain conditions are the other; the d electron are not paired unpaired d electrons can lead to the paramagnetism.

And you can also try to get the single crystal of these enzymes; in our case is this there the metal enzymes. And look at their atomic positions using then single crystal X-ray diffraction, I will tell you little bit more details bit later and there are many other techniques like gel electrophoresis, protein isolation purification and for ion case we can use something called Mossbauer spectroscopy which is a nuclear based spectroscopy it is gamma ray radiation spectroscopy.

The transitions or in the nuclear transitions not in the electronic transitions and the fluorescence excited state life time; how long an electron sits in the excited state these kind of characteristics will explain you the what the surroundings are, how a protein when you say if it is a particular group which is giving the fluorescence and there is some surrounding the rest of the protein; how the rest of the protein is influencing that particular species; all these can be studied.

You can also study a NMR spectroscopy; NMR spectroscopy what we study in general is called the diamagnetic NMR, but here we can see is paramagnetic NMR too.

Because iron 2 plus iron 3 plus then cobalt 2 plus all are these cobalt 3 plus they have a paramagnetism, the paramagnetism will shift and giving the contact shifts this. NMR relaxation times can also be used to find the distance between the centre and the species which is interacting with it. You can have something called extended X-ray absorption fine structure spectroscopy which is referred as E X A F S; EXAFS; this will be giving us the information from with respect to the metal center the primary coordination sphere, but not so, much of the other information; not like.

Crystallography will give the entire thing; so these are some of the thing. We can also look at the redox properties of these; so the electrochemistry with cyclic voltammetry. So, these are some techniques which I would say are absolutely important in this context. Let me see some of them I can explain you with some detail in terms of their application if not the basic principles of this. Let us look at the first example is the Circular Dichroism.

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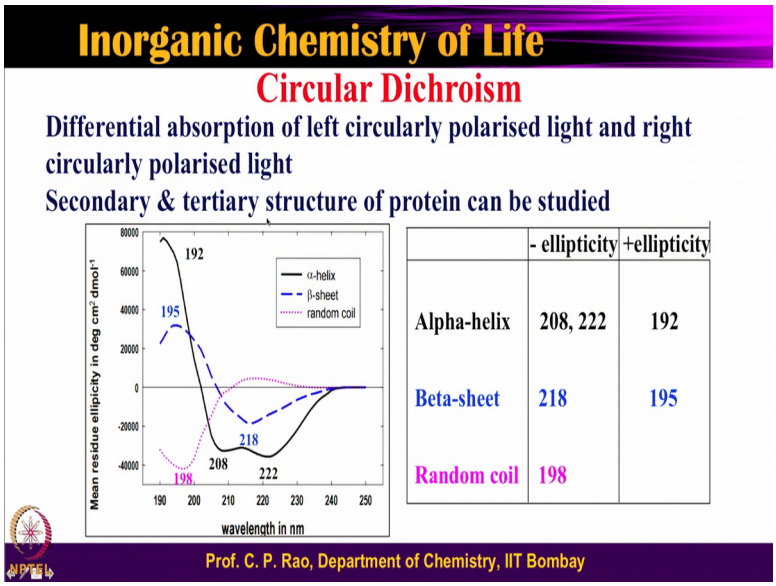

So, in case of the proteins what are we interested in? We are interested in secondary structures, tertiary structures etcetera. What are the secondary and tertiary structures? We already studied in the earlier classes that there is alpha-helix, beta-sheet and random coil.

And you can see that the alpha helix has got this kind of a the CD signature and if you look at that beta-sheet; this is a different kind of CD signature and random coil it has a different kind of thing. So; that means, now if you take a protein or metalloprotein and measure its CD spectrum, you can try to identify the alpha helical components, beta sheet components, random coil components.

Their ways and means by which you can deconvolute and get each those things too ok example is shown over there these alpha helix has got, these are the kinds of the bands at which ellipticity city is found minus ellipticities found plus; the beta sheet and random coil too.

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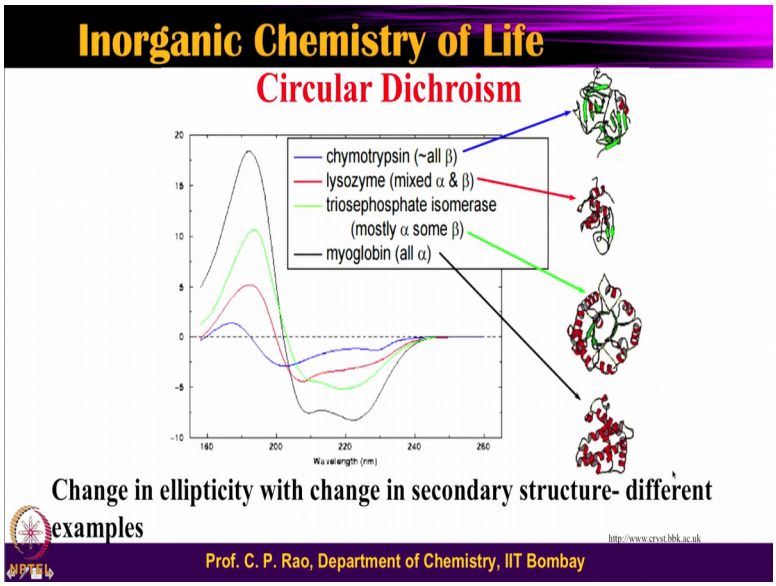

So, if you apply these to a protein system as you can see the different kinds of protein shown over here chymotrypsin has a beta sheet which is having this blue kind of thing, you can see this blue curve; lysozyme which is a red which got both alpha and beta you see that that one and these 2 peaks. So, therefore, giving both the alpha and beta kind of thing triosephosphate isomeras is; mostly alpha and beta.

And this you can see again very similar to this up there and then you see myoglobin mostly is alpha. So, the myoglobin is same from here as you can see. So, you have different kinds of proteins having greater beta, greater alpha, beta and alpha combined; alpha more, beta less beta more or alpha less all these kinds of things can be characterized by the CD spectroscopy.

So, CD spectroscopy is very strong method which will help us to explain the protein secondary structures. Now when you add some ligand to this particular protein and what happens? So, what kind of things happen in beta sheets, alpha structures; all these can be identified by this circular dichroism; how will we do that? You take your enzyme protein then you add some small molecule or a metal complex or something and then see that what kind of a changes are coming in alpha and beta and random coil.

So, you can take as an example take an apoprotein how do we get apoprotein? A metallo protein minus metal ion what is minus metal ion? You use some chelator that chelator can take away the metal ion then you have a apoprotein. And this apoprotein you take

and start adding the metal ion or metal salt or a metal complex and that will go in seat in that metal position and that will change certain secondary structures you can monitory too.

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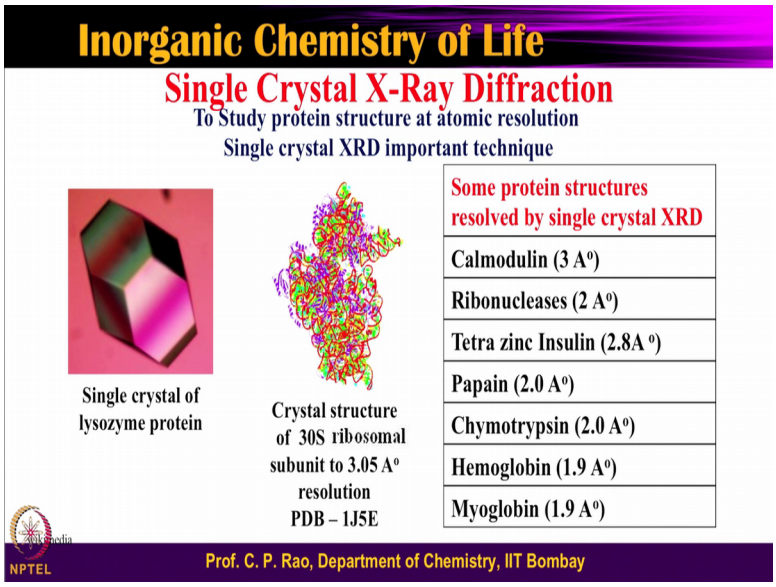

Let us go to the another technique, so another technique that I am trying to give you here is that single crystal X-ray diffraction. So, for this the criteria is the enzymes should give a good diffractable single crystal ok. Single crystal means the whole thing is one single piece and developed along its axis of x, y and z

So, integrated that it give builts if this; so one of the example is shown here is the lysozyme protein when you crystallize. A very nice phases you can see; so nice single crystal and there is a structure shown here which is a crystal structure the 30S ribosomal unit and this is to 3.05 Angstrom resolution; I will come to the resolution part in a while. I told in one of the earlier lectures; the protein data bank will give you the crystal structure or crystal structure coordinates and then using their coordinates, you can build the crystal structure.

As you can see here the 1 J 5 E is a code; this code if you go into PDB site and give this code, then you will get the structure. Just like you give your the train booking PNR and find the status of your PNR status when you go into the railway website you get that.

And similar if you go to the PDB website and give this number, a unique number you will get the structure. There are large number of crystal structures unknown several; 100s of crystal structures of metalloenzyme crystal structures are known maybe to 1000s also. Some examples given calmodulin, the bracket value is the resolution is the 3 angstrom resolution, ribonuclease 2 angstrom resolution, tetra zinc insulin 2.8 Angstrom, papain 2 Angstrom, chymotrypsin 2, hemoglobin 1.9, myoglobin 1.9.

Do you know whether this number should be greater to have a greater resolution? These number should be smaller to have a greater resolution. When I ask this particular question there in the regular class I get an answer for many people the right answer the right answer is the smaller the value; the greater the resolution is.

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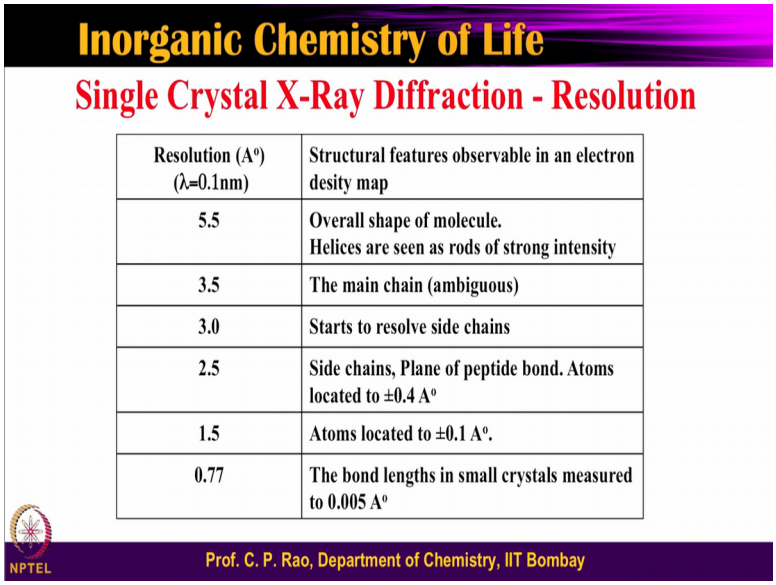

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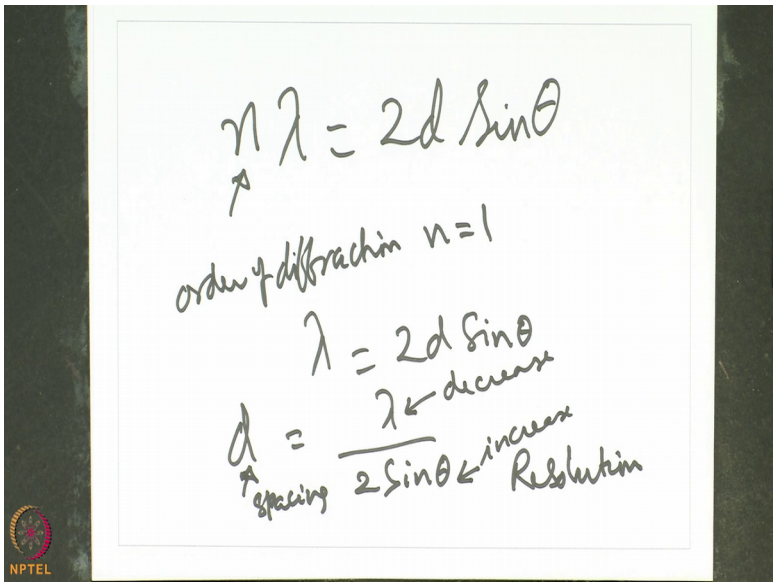

How do we understand? I am sure you know that n lambda is to 2 d sin theta; n is order of diffraction, then you take this as 1. So, so n is equal to 1; then this will becomes lambda is 2 d sine theta and d is this spacing; so, lambda by 2 sin theta.

So, this will tell you about the spacing also is referred as the resolution; so smaller these value is the better the crystal structure resolution is. So, let us look at this table for couple of minutes and on the left side column, you have the resolution in Angstrom; 5.5 Angstrom, 3.5 Angstrom, 3 Angstrom, 2.5, 1.5, 0.77 Angstrom ok.

So, look at this; this is the kind of information that you can resolve at 5.5 angstrom; you can see overall shape of the molecule in this case the protein and you can see some helices are there, some rods etcetera, but not very clarity. Then you go to the 3.5; reduce the value of the this one d value; 3.5 means increase the resolution; increasing the resolution is by decreasing the d value.

So, how do you decrease the d value here; is the d value is can be decreased by decreasing this one or increasing this one. So, you can increase or decrease; that means, lambda radiation that you have used. So, you can increase decrease the radiation that is use molybdenum has got the lowest value of the lambda and increase the denominator. So, the denominator is these 2 sin theta so; that means, the reflections that you collect should be increased ok. So, if you increase that one then you totally d value will decrease; the d value decrease in means the resolution is this.

So, when you come to the 3.5; you will start seeing the main chain, but not very great clarity then you go to the 3 Angstroms, you will start seeing the each of the amino acid residue. You have seen in the previous class the protein is combined from the amino acids condensation, through the peptide bonds; one after there you can see c alpha, c alpha, c alpha kind of things residues and that is what it means by.

So, if go to 3 or below then you will start seeing the resolving the side chains and the peptide bonds etcetera. Then you go to the 1.5; you can start finding the each of the atom you go of course, the 0.77; you get very highly resolved. So, far there are no structures with 0.77 protein structures, the small molecule structures there are millions are there 0.77, but for proteins you have the lowest probably is 1 point; 11.2 in the recent days, but not lower than that. So; that means, you can get the to some extent; the each atoms very well resolved, but not to the plus 0.1 kind of a thing.

So, what is how do we understand this in a different in a in a way. So, it is like let us say we have a huge class room; class room having let us say 20 rows and the teacher is sitting at the center in the at the near the black board and so, you know the first row; the front row will be very close by the last row is very far and obviously, the way that the teacher can see the last row student is not so, clear; with the one below they can show little better, one front towards him better and those rows which are very close to the teacher; teacher can monitor, teacher can see observe what are all the students are doing with more clarity.

So, exactly the same; so, you as you decrease the d value the resolution increases the greater the resolution is the better the structure is and that kind of thing one can that. So, to do all these; the proteins have to be either isolated or proteins have be synthesized.

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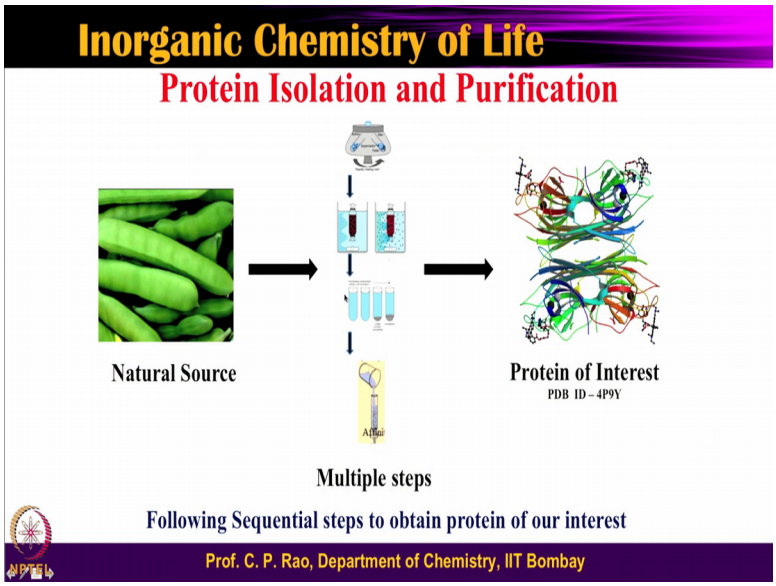

You can isolate in fact, lot of natural resources you can take and then do some steps of the biochemical processes, at the end you can get the protein; several steps or there and the protein of interest you can get the structure to if crystallize as a told in the earlier.

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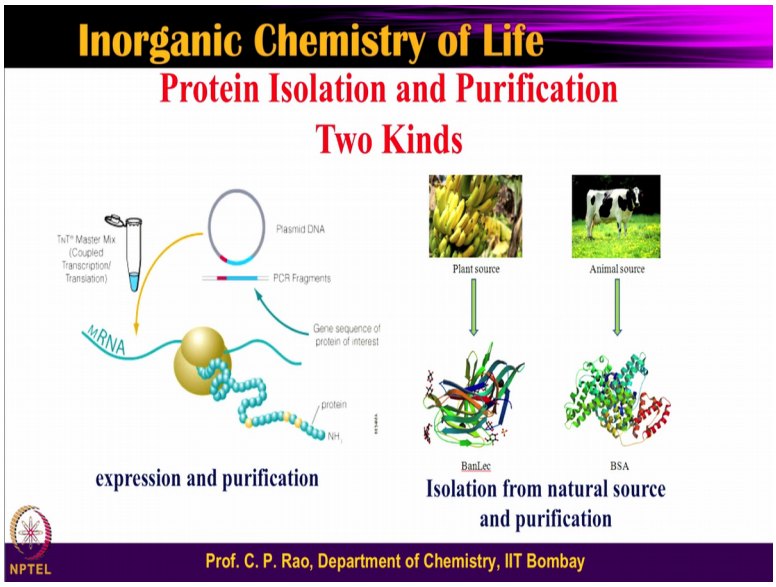

So, there are several steps involved in that; it is not only that you can take another example 2 and you can use the plant source or the animal source you can get this corresponding protein out and in some cases you can also make by using the plasmid. So, if you know the protein what you want; you can generate the corresponding plasmid or

the DNA sequence and that DNA sequence is made into the plasmid and this plasmid is integrated into the bugs and then you can you can generate that particular protein which is called the expression.

So, you can express and get the protein or you can extract and get the protein. So, the proteins can be obtained either by expression or by extraction in this.

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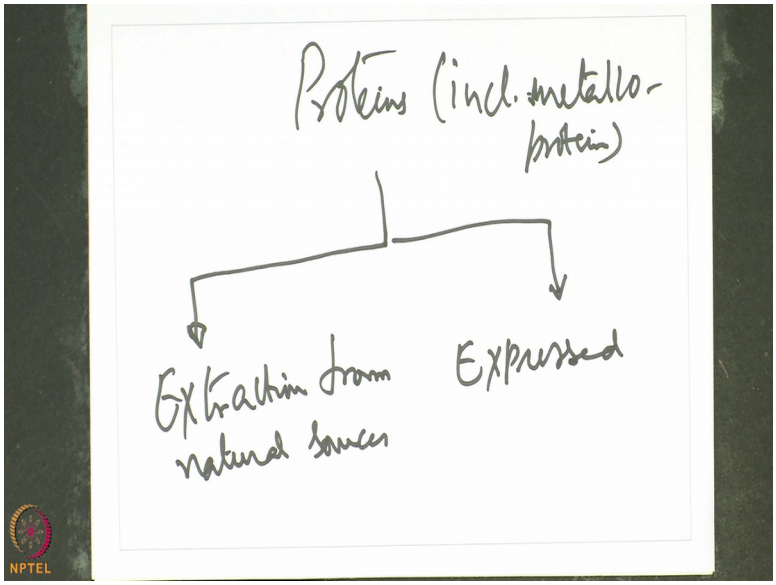

So, the proteins it can be metalloprotein does not matter protein; so including metalloprotein. So, these can be obtained either by extraction from natural sources and it can also be expressed.

So, for express for expression you need to get the what the what is the protein sequence that you want and that protein sequence to be translated into DNA sequence and then make a plasmid and put into a live bug and express and then of course, you have to purify. So, purification methods I will explain in a while ok.

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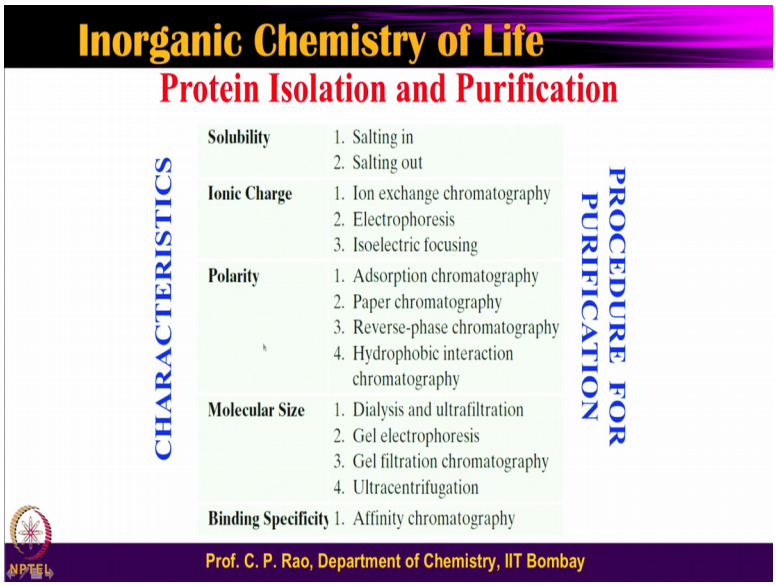

So, put in isolation and purification as I said that the proteins can be obtained either from extraction, from the natural source or by expression on these ones. The different characteristics that solubility characteristics, ionic charges, protein will have all of these properties.

And these properties will determine what method you want to used for either for isolation of a purification ok; their polarity, their molecular size, the binding specificity etcetera. Solubility; you can make it soluble, you can make it insoluble by using the salts in and out kind of things, ionic charge; ion exchange chromatography, electrophoresis, isoelectric focusing all of these are the different techniques when you have ironic charges present on the; on these proteins.

You can use polarity based one to adsorption chromatography, paper chromatography, reverse phase, hydrophobic interaction chromatography etcetera. So, there are variety of these kind of a techniques there again can be used for separating or purifying both ways molecular size. So, there are proteins when you isolate; there are other protein also come along with that, so you need to filter them off, remove them off, take them out. So, this can be done by dialysis and ultrafiltration; then gel electrophoresis, gel filtration chromatography; ultracentrifugation.

So, there are various kinds of things are there and you can also use affinity chromatography; where your protein will be bind bound to the column very effectively

with some affinity; all other proteins will come out; so, you can do; so all these procedures for purification. So, you can use these characteristics of proteins both for isolation purpose as well as for the purification purpose too; one of the example is shown here is Size Exclusion Chromatography.

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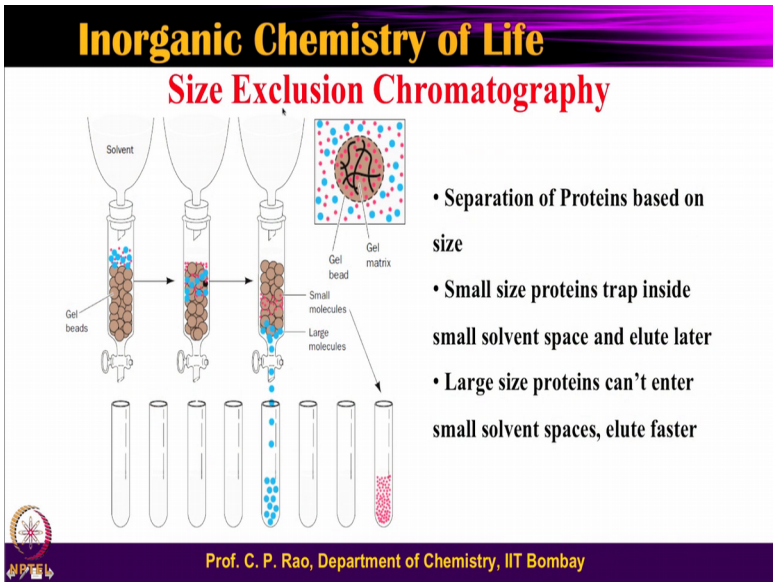

That means there is some sizes are held, some sizes are left out. So, that is what is referred as a size exclusion chromatography; you have your the protein which was already isolated by extraction let us say or by some other method and then that particular you know mixture is passed through some kind of a beads; some kind of a beads.

And these beads will have some specific size or the pore ok. So, separation of the proteins based on the size is called size exclusion chromatography. So, it is based on the size and you exclude certain sizes and the certain sizes proteins are captured. So, small size protein it trapped proteins trap inside and small solvent space and elute later; later on. And the large size proteins, they will not enter into the ports; if the ports are small then therefore, you can get these things out. So, the largest ones will come out and the small.

So, therefore, there is a hierarchy; so you can separate different sizes of the proteins which are probably impurities in your protein and they can be separated out and you are interest in a particular protein. So, therefore, this is dependent on the size principle and the beads are dependent on the on the size of the pore principal. So, size of the protein

size of the pore; so if the when there is size of the protein and the size of the pore are comparable and they are trapped a smaller their trapped. And they when the size of the protein is bigger than that, they cannot be trapped; they will go out you can see the blue color here and the protein and that is going out; that means, that is bigger than the bigger than this particular the bead size.

So, therefore, that will go out. So, that will basically go out you see that; so, so, therefore, based on the size of the protein you can separate that is called size exclusion chromatography So, the size of the protein versus the size of the beads that you have the pore size; the post size of the beads the beads having a pore size.

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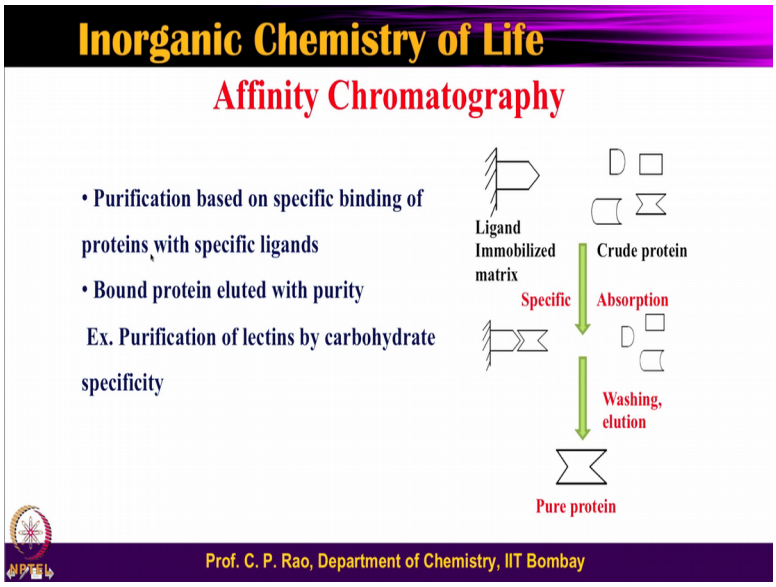

Now, you can also purify this proteins by using affinity kind of a chromatography. So, affinity; so what is affinity? Means it should have the liking affinities liking; so the more and the more liking. So, liking with what? The protein is liking with the column material; with the column material has a liking towards the protein, the protein and the column material will form a complex and you will get retained then the remaining will go out ok.

So, so example is the purification of lectins; a lectins have got certain affinity towards a particular specific carbohydrate and you make a column which is having that kind of a carbohydrate immobilized on that; attached to that. So, such a column you use and pass the solution; then all the other protein will go out and only that particular protein will get

trapped in that, only that particular protein will get trapped in that; so therefore, that is what is call the affinity kind of thing.

So, and then use another medium to detach this protein to come out of this. So, you see that let us say this is your column; which has some molecule or we can call it is a ligand and this particular ligand, will have a specific interaction; a specific affinity towards another protein. This is not just a size characteristic, it is a chemical characteristics.

It is basically a chemical kind of affinity not just simple size; do not take it as size, the previous one as based on size. The pore of the beads size not here and this please do not take this as a size characteristic; though it will show somewhat similar to that. So, purification based on the specific binding of the proteins with specific ligands or specific molecules which are attached to the to the stationary phase ok; so, bound proteins eluted with this etcetera.

So, this crude protein has got this kind of a protein, this kind of a protein, this kind of protein, this kind of protein. For example, now you pass through this only one this kind of protein will be specifically absorbed and all other things have come out. So, the this can be taken out and this is still retained on the column and then use by elution and then get back get back this one. So, that is what is referred as the so you can also have a electrophoresis.

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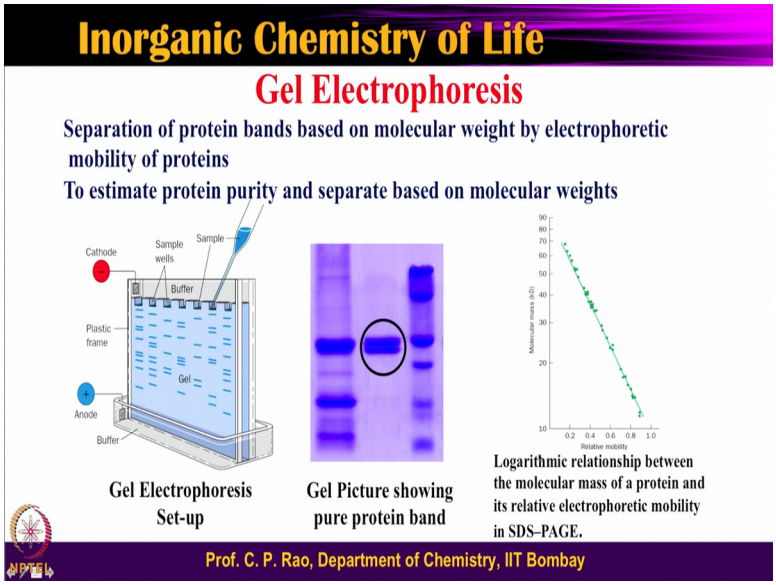

So, so one is by pore size of the bead; another is by the affinity this one is by the electrophoresis. What is electrophoresis? Obviously, you are applying a voltage and you have anodic, cathodic kind of thing. So, so based on the voltage the charge on that, the opposite charge will move towards that and proteins have in certain size, their molecular weight will vary. So, therefore, some of them will move fast, some of them will move slow.

So, therefore, moving fast, moving slow under the electric field is called the electrophoresis. So, therefore you have proteins different proteins have got different molecular weights and they will make; as you can see they will come like a bands. So, gel picture showing the pure protein band and that is the one actual have and these are some other cases markers.

So, you can identify the molecular weight too. So, you can in fact, get the logarithmic relationship between the molecular weight of the protein and the electrophoresis mobility. So, this mobility and the molecular weight standard is known from this; you can find out the molecular weight of your protein too ok.

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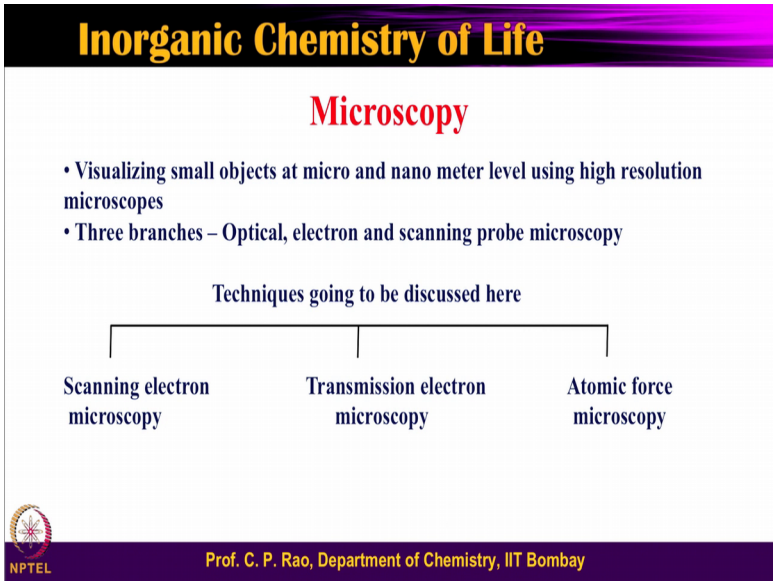

So, haven got the protein you can also look at the protein microscopic feature; features. So, you can visualize these small objects at micro and nano meter level; using a higher resolution microscopes and the kind of techniques uses scanning electronic microscopy, transmission electron microscopy, atomic force microscopy; as this scope of this course

does not permit; I am not going into the details in this but I will show you example and try to impress you on how well it can be used.

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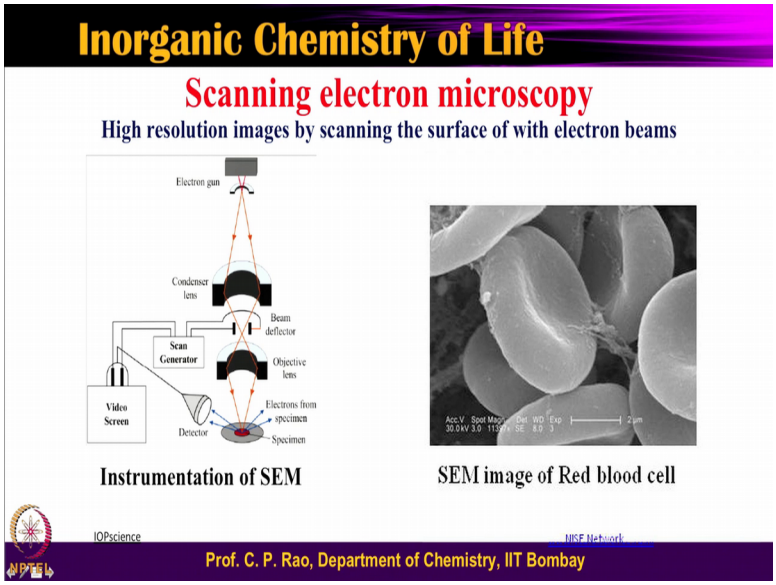

For example, scanning electron microscope this is how the basic configuration they scanning electron microscope looks like and we are not going to the details and you can even micrograph this cell and that is has today as come from microscopy which was not there the 30, 40 years ago.

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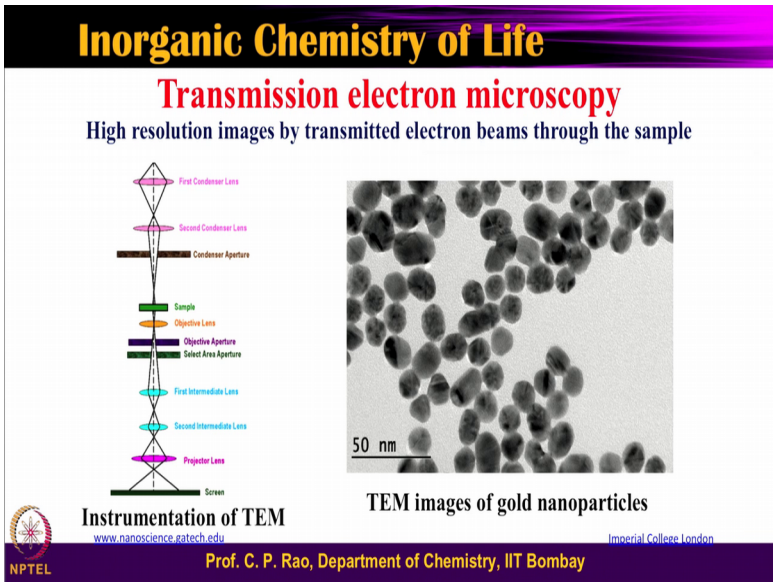

You can also use transmission electron microscopy and again this is a configuration of the microscope; do not worry about this and the here this is TEM image of gold nanoparticles and the same nanoparticles can be coated with protein. Therefore, when it is coated with the protein, when it is not coated with the protein there their characteristics will differ; the transmission electron microscopy which is in short form called as tem characteristics will differ; therefore, we can say that the protein is coated or not coated on this term.

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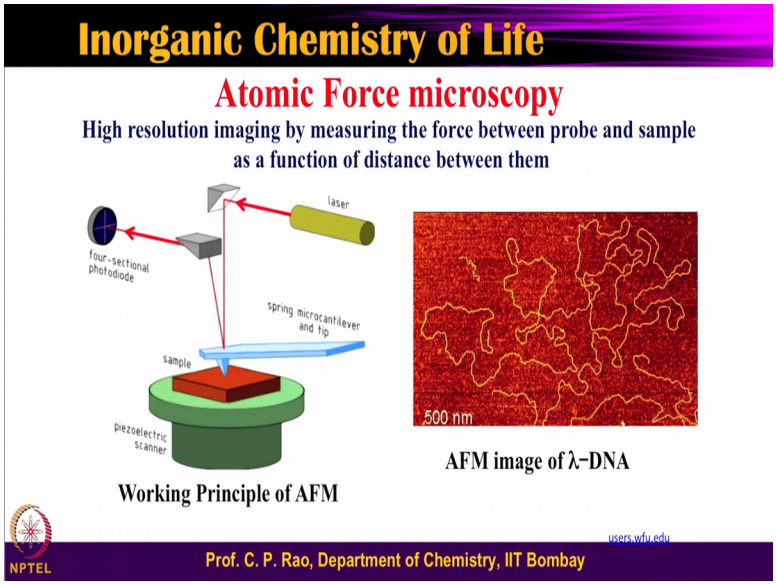

So, we can look at another microscopy which is very commonly used; atomic force microscope in this, in fact you can get the DNA; see the DNA structure very nicely you can see the DNA structure. So, uncertain surface you have up to about 500 nanometres kind of a scale; so very nice.

So, using Scanning Electron Microscopy transmission called SEM; Transmission Electron Microscopy called TEM Atomic Force Microscopy called AFM; we can study the microscopic structures or the protein. Protein plus metal ion, protein plus small complex; metalloenzyme plus something else or protein plus another protein complex; all of these can be studied at the microscopic level and some spectroscopy methods I will explain in the next class so.

Thank you very much.