Proteomics: Principles and Techniques
Prof. Sanjeeva Srivastava
Department of Biosciences and Bioengineering
Indian Institute of Technology, Bombay

Module No# 09
Lecture No # 09
Sample Preparation for Proteomics Application

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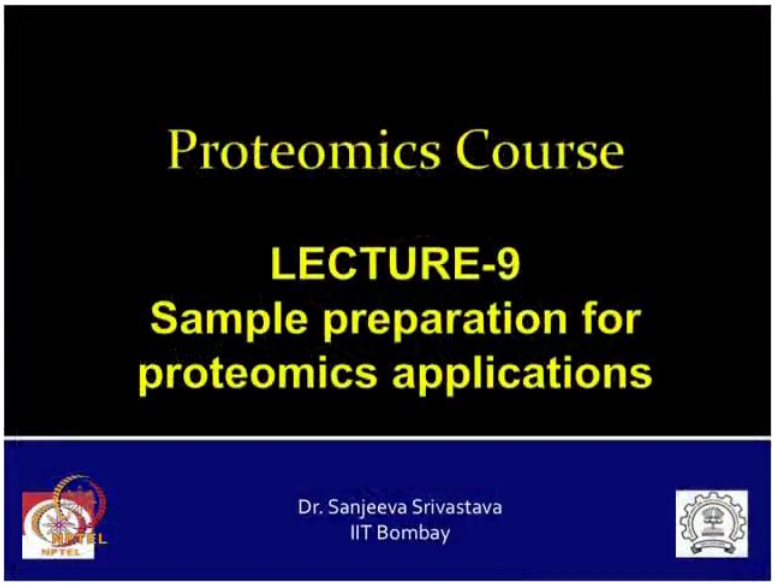

Welcome to the proteomics course, in today's lecture we will talk about sample preparation for proteomics applications. As you know a very good sample is essential to perform a very good experiment and specially when you want to characterize thousands of protein in a given experiment. It is very essential that you start with a very good sample. An ideal sample preparation step ensures that you have no contamination you havevery good protein yield and no interfering substances are present in your extract. Often each type of biological sample, pose its own challenges.

For example, if you are working on bacterial cultures, working on plants, working on human samples, different body fluids such as serum, urine, saliva or several spinal fluids, each sample type bring its own unique composition and its own challenges. Now, when you are performing a sample preparation you have to be very cautious that what sample you are processing, you cannot follow a generic protocol you have to optimize the

conditions depending upon your sample. An ideal sample preparation will ensure that you have all the protein present in your sample without different type of contaminants such as nucleic acids salts and other interfering components.

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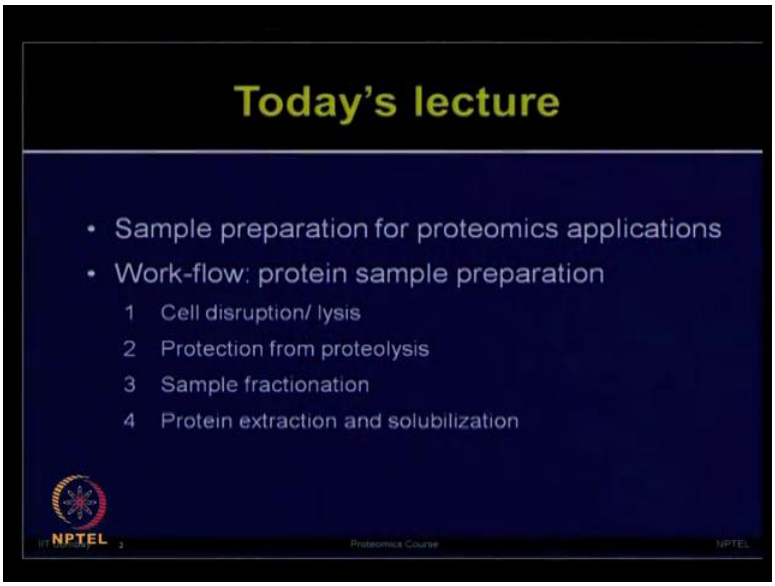

In today's lecture we will talk about the sample preparation for proteomics application, I will give you a work flow for protein sample preparation. Such as the first step, how to disrupt the cell how to lysed them, how to protect the cells during the lysis step. So that, there is no proteolysis occurring how to fractionate the samples. Often you need to simplify the proteome you need to ensure that, the sample is simple enough to show the good protein throughout the proteome coverage. So, to obtain a comprehensive proteome coverage, often it is important that you should pre-fractionate your samples, protein extraction and solubilization these are another very essential component.

Now when we are talking about the sample preparation, this is a quite generic for different type of proteomic application whether one want to use for gel based approaches or gel free approaches. So, whether you want to use two dimensional electrophoresis dige or different type of gel based application or you want to perform mass spectrometry and different type of label free techniques. You have to ensure that you are starting with a very good protein extract, but sample solubilization and some other components are

added more when you are performing the gel based proteomics.

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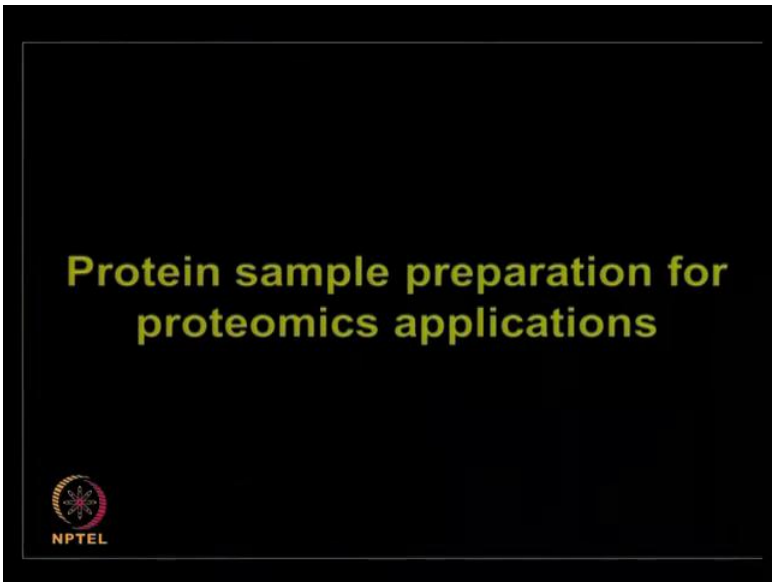

So, during this lecture I will talk to you about different type of components which are essential to make a good sample preparation for proteomic applications, I will give you stepwise workflow. So, let us start workflow for proteomic sample preparation so, the proteome is very complex whether you want to perform your proteomic analysis for the whole organism. It means you want to know all the proteins present in a given organism or in a tissue or in body fluids or in different type of cells proteomics can be global or it can be very targeted or expression based.

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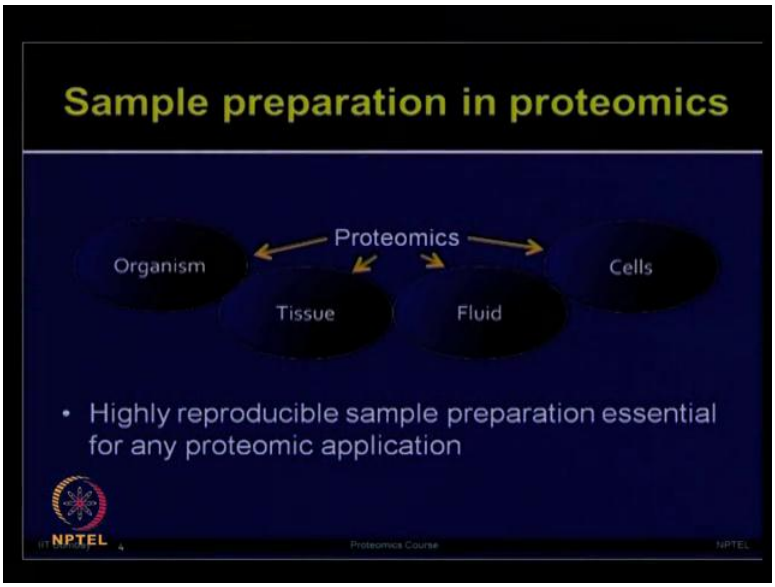

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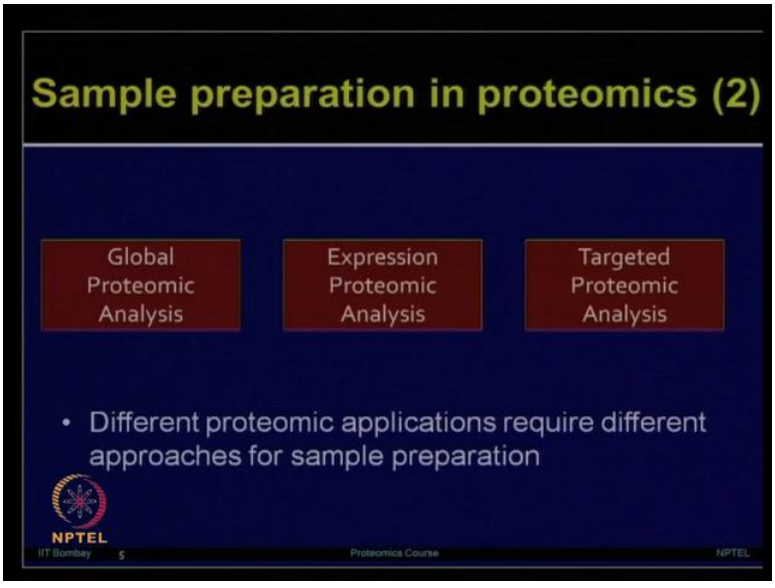

So, a very highly reproducible, samples are very important for performing comparative proteomic analysis. If you want to know the difference in your sample as compared to the controls, you need to ensure that your sample preparation is very reproducible. If you introduce the multi facts to begin with then obviously, you are not going to identify the reproducible biological changes. So, let me give you three different terminology here for

proteomic analysis, one is global proteome analysis, expression proteome analysis and targeted proteome analysis.

When I am talking about global proteome analysis, it means your aim is to characterize all the proteins present in the given sample. Expression proteome analysis, it means you are mainly interested to look for those changes which are bit due to any chemical or your treatment those are induced either going up or down the protein amount is changing. So, the protein expression analysis, that is most commonly used for various type of clinical and different studies. Targeted proteomic analysis, if you are very focused for a given organelle or a given sample type often you had like to know what is happening in that particular proteome for example, mitochondria proteome.

So, one need to try different type of strategies when thinking about performing a sample preparation, what is your objective? Whether you want to global profiling or you want to do expression profiling. In either case you need to track all the proteins present in that particular target sample.

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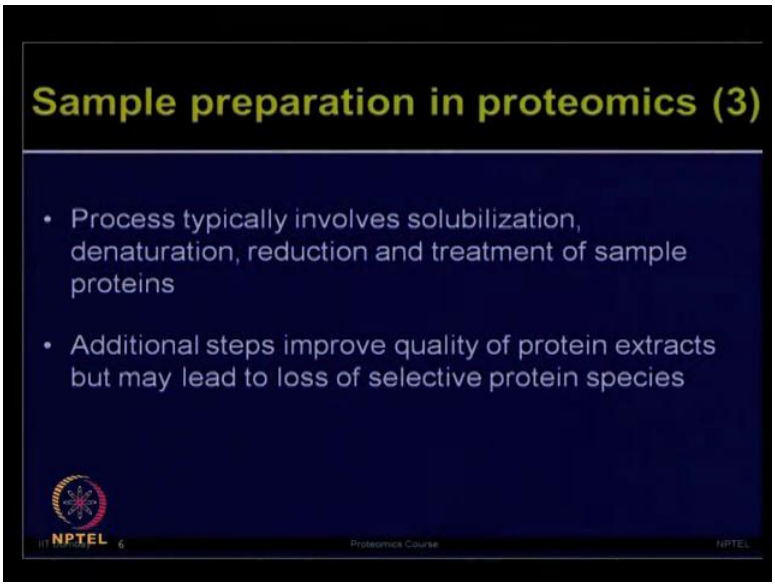

Now, when you are looking at targeted proteome analysis, you just want to pre-fractionate your sample in such a way that only that particular component is isolated and

then all the proteins from that organelle or cell is being extracted. So, different type of strategies need to be used to perform these types of proteomic analysis. Now all of the sample processing involve solubilization, denaturation, reduction and treatment of sample proteins. But you need to involve additional steps depending upon the type of samples and your type of objective. So that, the protein quality the protein extract can be improved.

And while you are doing this you have to be very cautious that when you are performing various steps and sequential type of extraction you may also lose a small fraction of the proteins.

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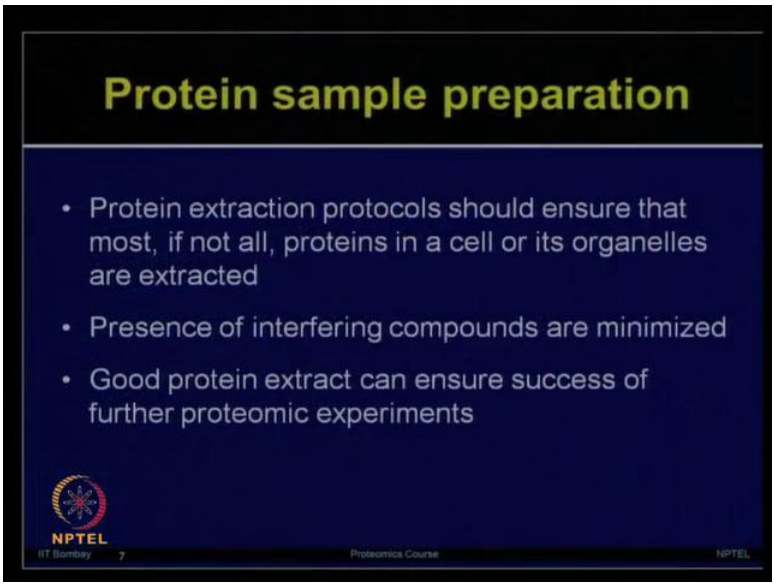

So, one has to be careful when adding various additional steps, during the sample preparation. Now protein extraction protocols they need to ensure that most, if not all the proteins in a cell or its organelle are extracted the presence of interfering compounds should be minimized. So, if you have optimized a very good protein extraction procedure, that should ensure that you have a very wide proteome coverage and that is ultimately going to determine the success of you proteomic experiment.

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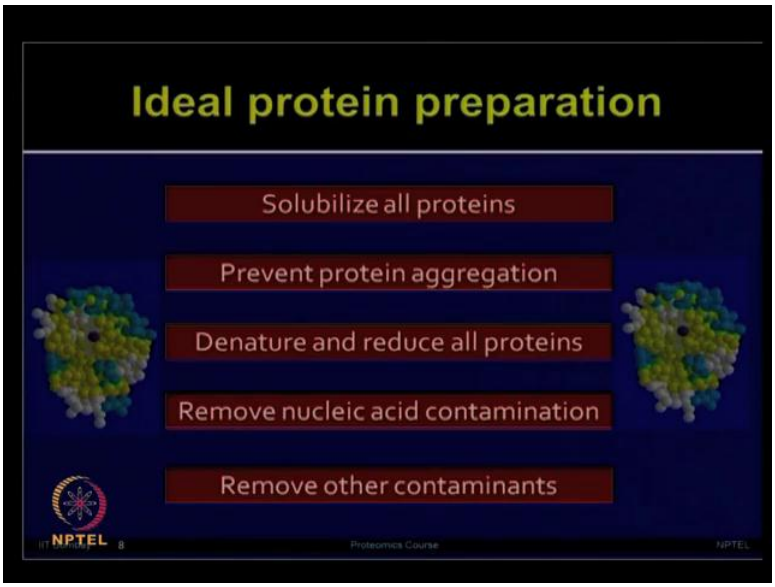

So, ideal protein preparation steps involve solubilizing all the proteins present in a given mixture, preventing protein aggregation, denaturing and reducing all the proteins which are present in that mixture. Removal of nucleic acid and other contaminants as well as removing salt and some other small interfering components. Again depending upon your sample type you may have to think what are different type of interfering components could be present in that sample type. If you are talking about plant roots you may have to get rid of phenolic components.

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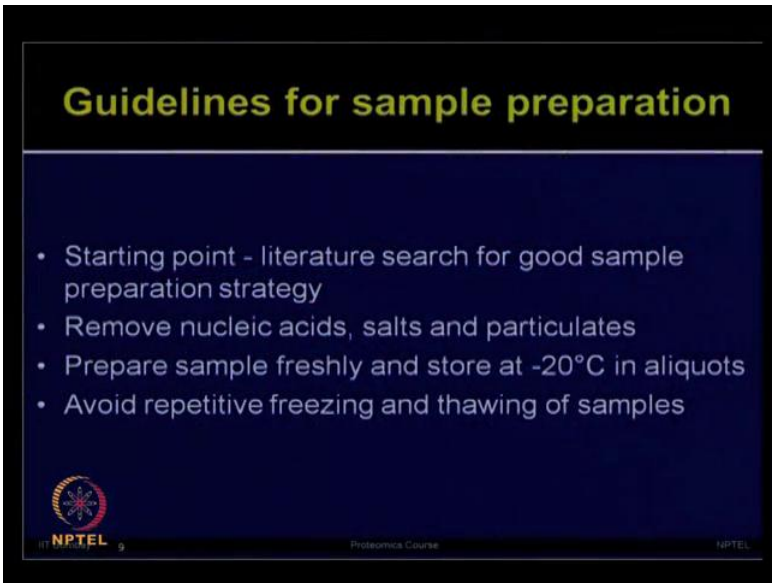

Similarly, you have to think a specific sample types and what could be the major contaminants present in that sample. For example, serum that is rich in lot of salt components you need to get rid of those. So, an ideal protein preparation should involve all other steps as I mentioned previously. Some giving you the guidelines for sample preparation, starting point one should start with finding a good reference from the literature as a starting point and then try to modify the protocol depending upon the objective of that experiment. You need to ensure that, you remove nucleic acids salts and different particulates prepare the samples as freshly as possible and store it in minus 20 degree in a small aliquots. You should avoid repetitive freezing and thawing steps of sample.

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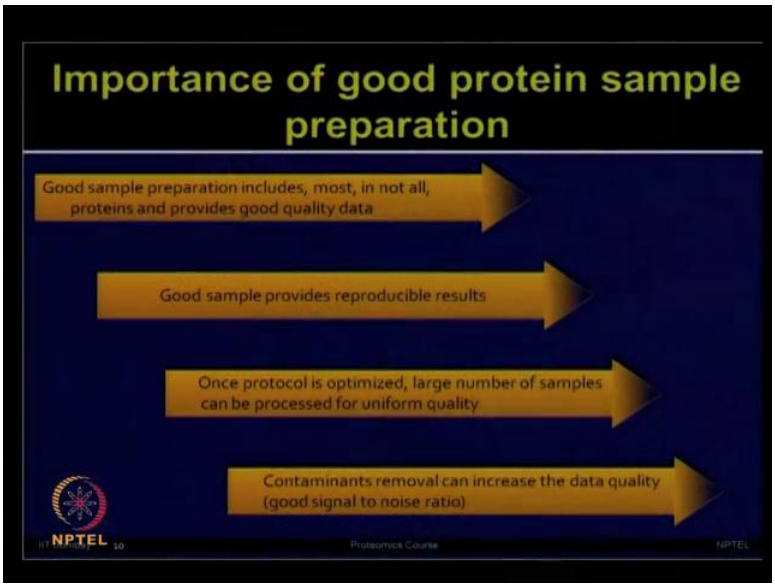

So, why a good protein sample preparation is important? A good protein sample preparation includes all the proteins present in that mixture. And it is going to provide you high quality data, because there will be less interference from the artifacts. A good sample will provide the reproducible results, you have to perform biological replicates and technical replicatesof a given experiment. So, a good sample will provide very reproducible result once you have optimized a protein extraction protocol then you can apply the same protocol for the large studies. For example, if you are performing a clinical trial study on 200 patients.

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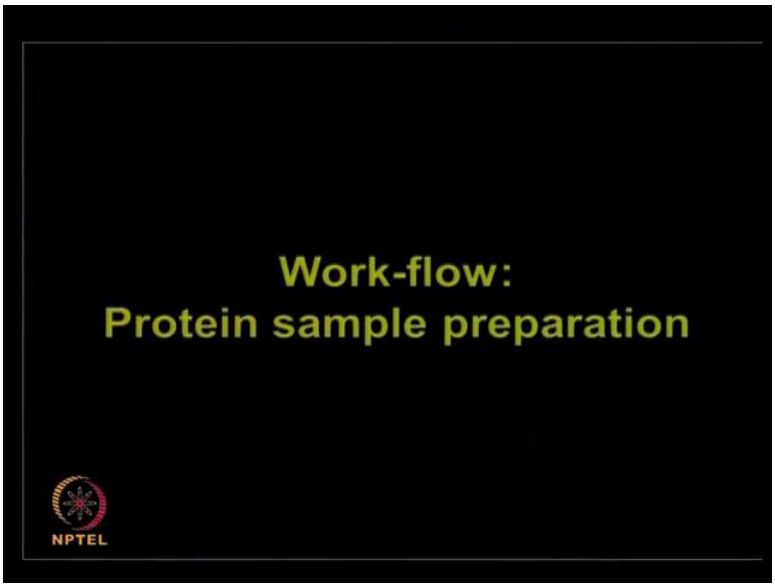

So, once you have optimized the protocol with a small population, then the same protocol can be applied for large number of sample so, that is going to make sure the success of the clinical studies. Now, if you are able to remove lot of contaminants or artifacts present in your sample, then your signal to noise ratio will improve. You have to literally see your good signal and do not have to worry too much how to remove the background and the noise. Some giving you the workflow of protein sample preparation.

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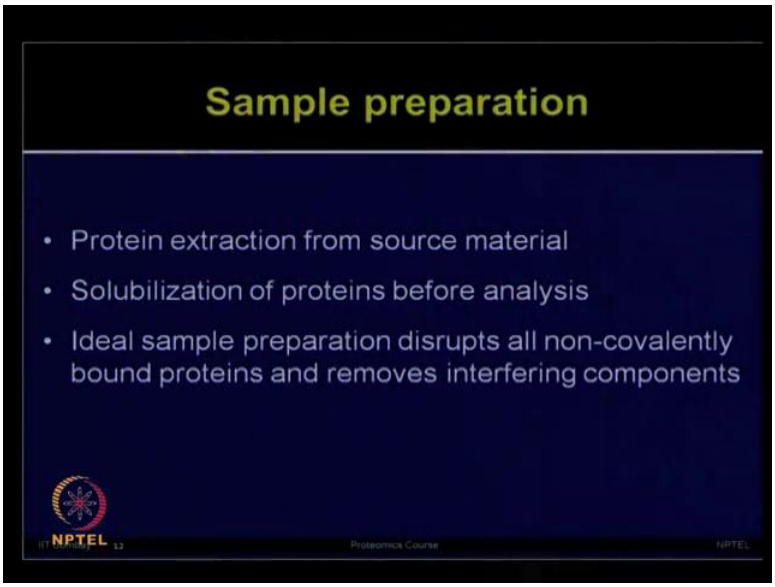

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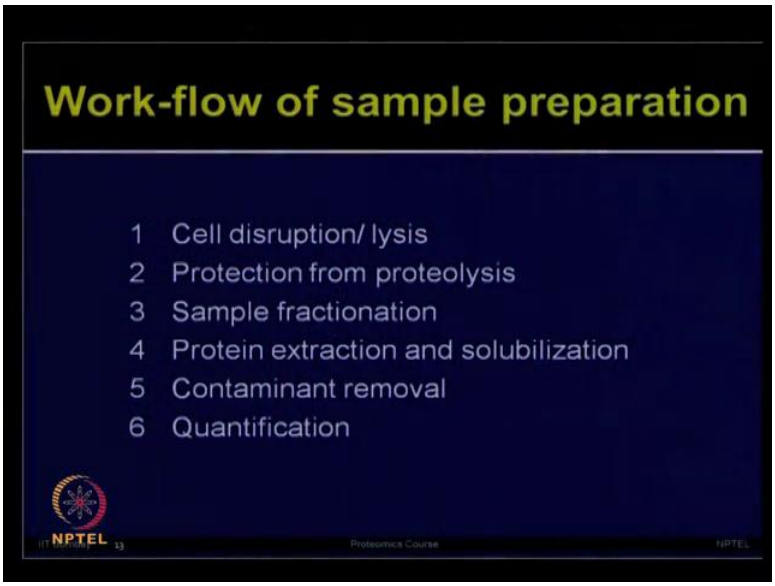

Let us go step by step sample preparation the protein extraction should be performed from source material and then you need to solubilize the proteins before starting analysis. The ideal sample will disrupt all the non covalent bonds present with the proteins and it will remove the other interfering compound. So, the work flow of sample preparation would involve first cell disruption or lysis, second protection from the proteolysis. Third

sample fractionation, fourth protein extraction and solubilization, fifth removal of contaminants and sixth quantification. Obviously, this work flow can be modified depending upon your sample type and few steps can be moved in that sequence.

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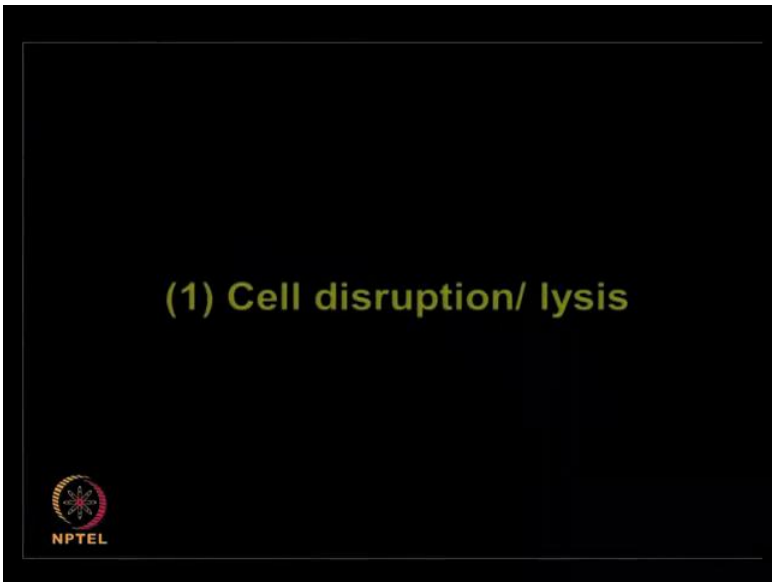

So, let us follow this workflow and during this workflow, I will give you some examples of different type of samples, how to extract protein from those and what type of challenges these samples are going to impose. So, cell disruption or lysis, the lysis is very important step because first of all you had like to break open the cells and remove all the cell components outside, so that you can get good protein yield. So, cell disruption or lysis is very important and it is often challenging, because all the sample types you cannot lysed with the same type of method.

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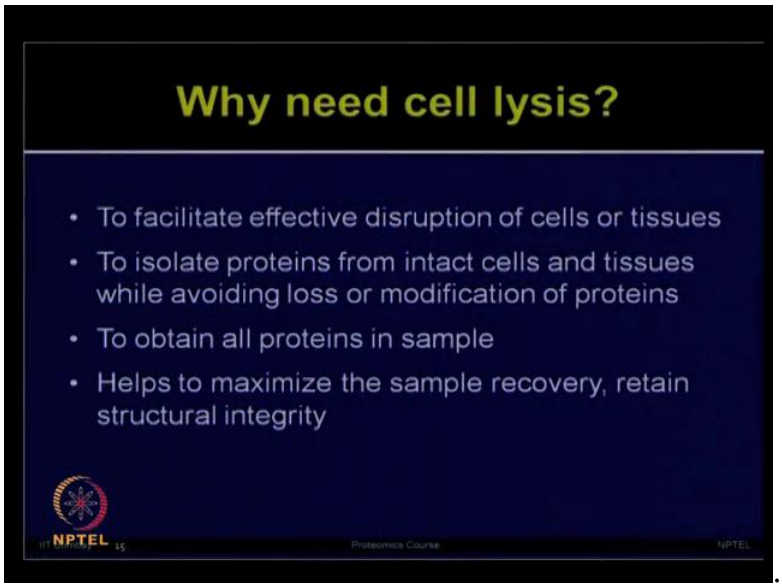

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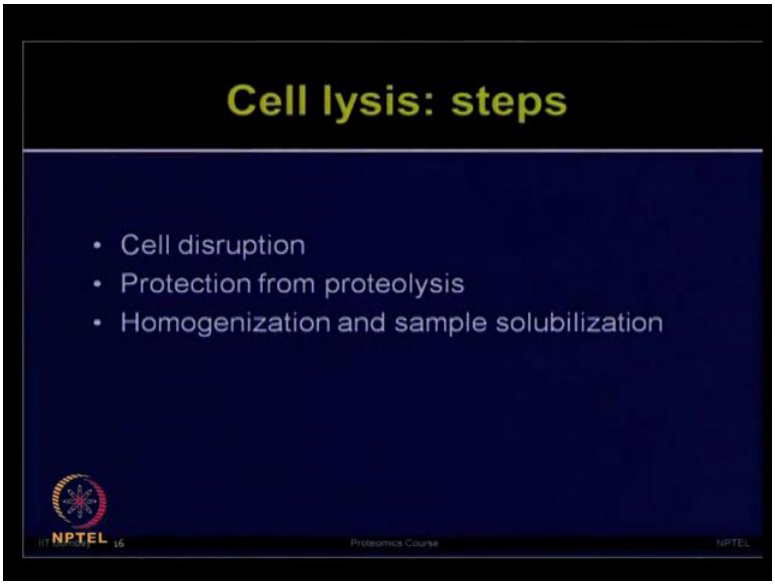

So, the lysis strategy has to be modified depending upon the sample type. So, why need cell lysis? To facilitate the effective disruption of cell or tissue to isolate the proteins from intact cells and tissue. While avoiding a loss or modification of proteins to obtain all the proteins which are present in a given sample and to help to maximize the sample recovery and retain the structural integrity. So, cell lysis is very important due to all of

these factors, the different steps are involved in cell lysis. You need to disrupt the cell protect from the proteolysis during the lysis step, homogenize and solubilize your sample. I will describe all of this in more detail.

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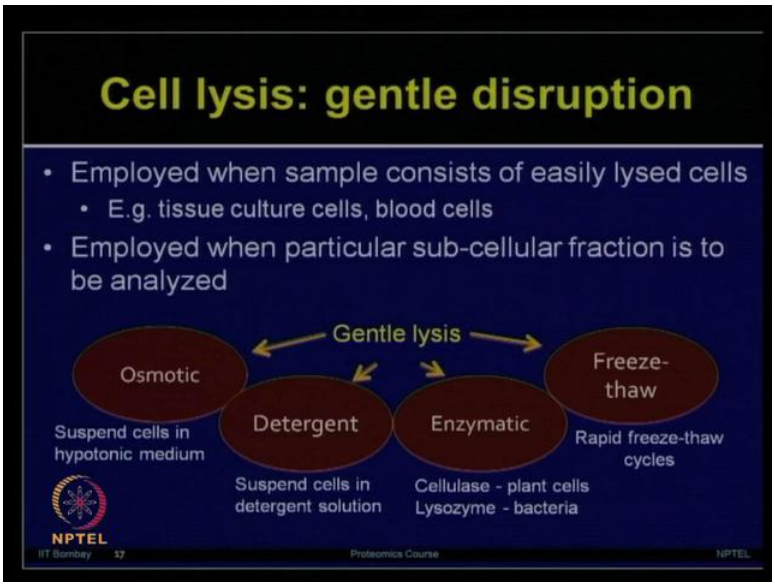

So, cell lysis can be performed with the gentle way or in harsh condition depending upon the type of cells you want to disrupt. When you are employing gentle disruption method, you need to think that you have to break open all the cellular components. So, your gentle disruption should be efficient enough to disrupt the cell. So, if you are looking at those cell type which can be easily lysed such as blood cells or cultured cell then you can involve the gentle disruption methods. The different type of lysis methods available such as osmotic lysis, in which one can suspend the cell in hypotonic medium, detergent lysis you need to suspend the cells in detergent solution.

Enzymatic lysis, if you are using plant cell for example, one can use cellulase enzyme if you are using bacterial sample, one can use lysozyme. There are different type of enzymes present which are used for enzymatic lysis. Freeze thaw that is one of the very commonly used method for gentle disruption which involves rapid freezing and thawing cycle.You need to cool your sample in a very very cold condition for example, liquid nitrogen and then immediately putting the boiling water by while doing this rapid

freezing thawing the cells will break open.

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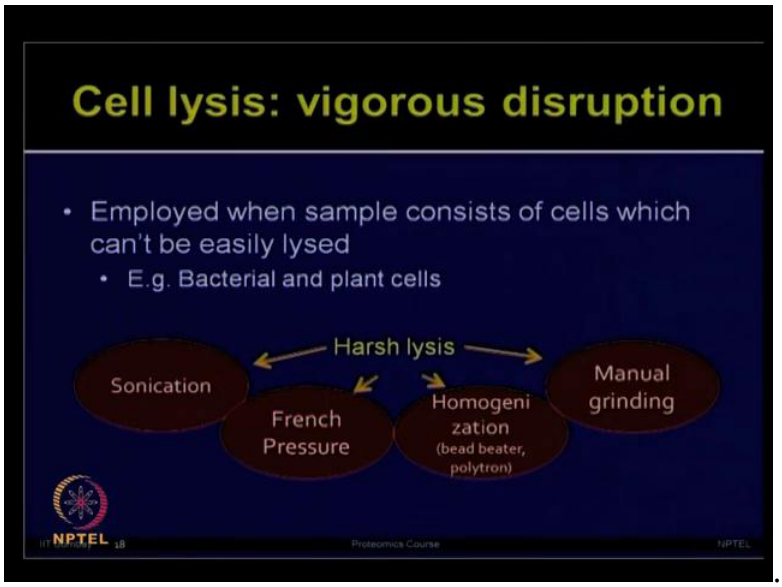

Now, there are different type of challenges being imposed by different type of cells. If the cells are very difficult to lysed, very difficult to break open, then you need to involve vigorous disruption methods such as sonication, French pressure, homogenization or manual grinding. We will talk about different type of cell lysis methods and some of its principle involved as we go along with more specific type of samples. But in all the samples preparation mostly people use manual grinding or homogenization by using bead beater or polytron homogenizers. So, these are very commonly used homogenization methods.

The manual grinding is performed by using Mortar Pestle often it is very efficient, but if you have very less sample and you want to avoid any contamination. Or you have some samples which are going to pose challenges with Mortar Pestle, then you need to use the electronic homogenizers. Then sonication and French pressures are more used when you are applying bacterial and yeast or different type of those cells which are difficult to open. We will talk about these in more detail in the more specific example when we talk about how to extract the proteins from bacterial sample.

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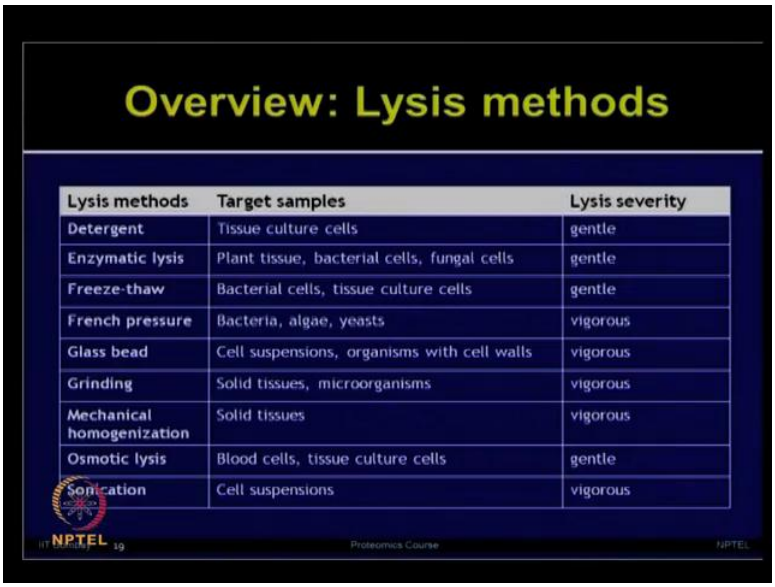

So, now I will give you the overview of lysis methods, we have talked about all of these methods briefly I am giving you an overview now. One can use detergents if your target example is tissue culture cells, this is going to provide gentle lysis method. Enzymatic lysiscan be used for plant tissues bacterial cells, fungal cells etcetera, this is again a gentle lysis method. Freeze thawing can be used for bacterial cells or cultured cells and this is again a gentle lysis method. French pressure is often applied on bacteria, algae, yeast which is a vigorous lysis method. Glass beads are used with cell suspensions or organism with cell wall, this is again a vigorous lysis method.

Grinding of solid tissues and microorganism is another vigorous lysis method. The mechanical homogenization of solid tissues is another vigorous lysis method, osmotic lysiscan be used for blood samples and tissue culture cell which is a gentle lysis method. Sonication can be used for cell suspensions and other bacterial samples which is another vigorous lysis method. I hope these methods give you some options to lysed your cells effectively.

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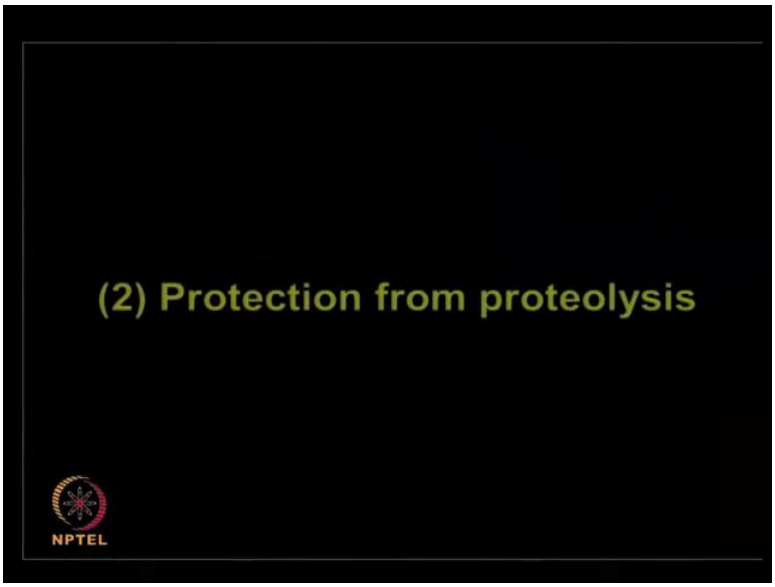

Now, you have tried to break open your cells and release all the protein components and other cellular components present inside the cell. But often during this process of grinding a lysis you made degrade some of the protein and you need to ensure that you have to protect your protein from the proteolytic activities of various enzyme.

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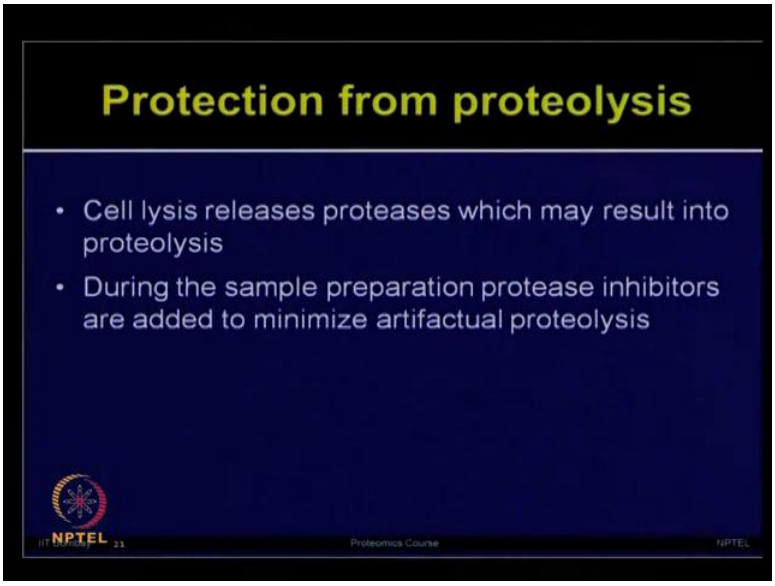

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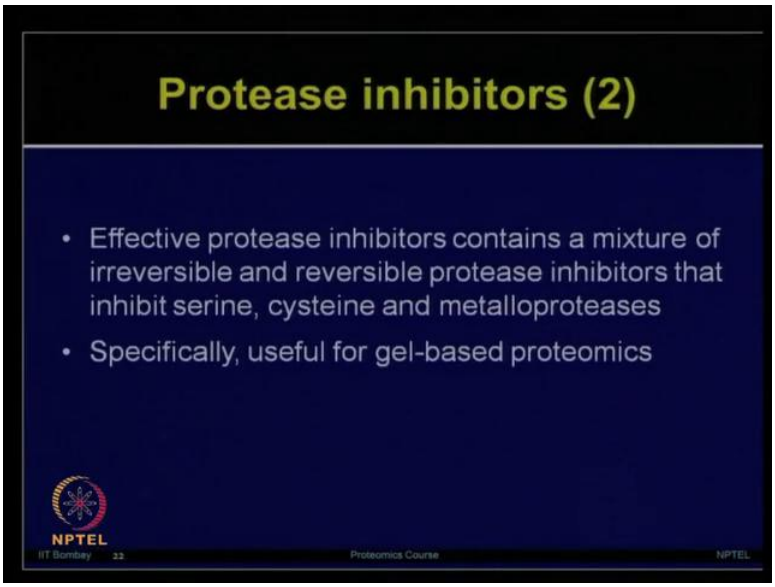

So, protection of your protein components from the proteolytic step is very important during the protein sample preparation. So, cell lysis will release various proteases which may result into proteolysis. During the sample preparation one can use different type of protease inhibitors which can minimize the artifactual proteolysis. The effective protease inhibitors contain a mixture of different type of protease inhibitors, irreversible and reversible which inhibits serine cysteine and different metalloproteases. This step is more important when you are preparing your samples for gel based proteomic applications.

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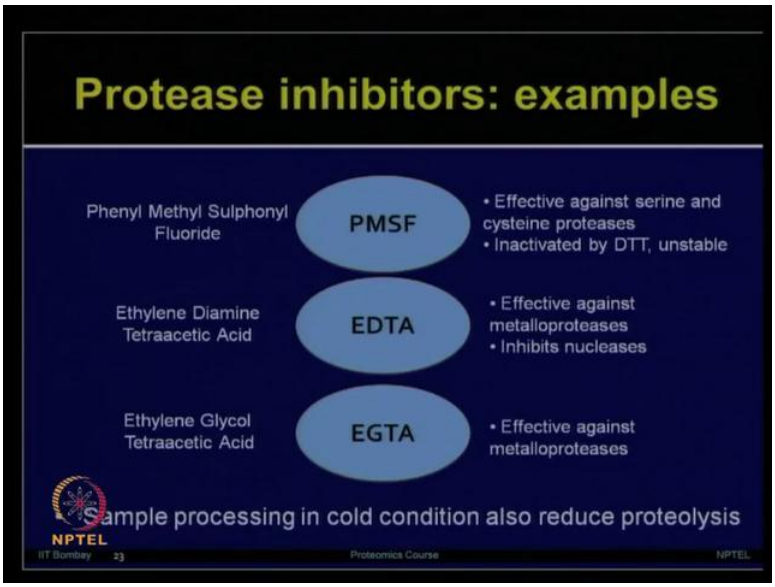

Now, I will give you few specific examples of protease inhibitors such as phenyl methyl sulfonyl fluoride P M S F which is a very effective against serine and cysteine protease. It can be inactivated by D T T and it is unstable when you are preparing the samples for gel based applications. E D T Aethylene diaminetetraacetic acid, it is effective against the specifically metalloproteases, it inhibits nucleases as well. So, E D T A can serve both purpose of protecting from proteolysis and also partial inhibition of nuclear activity. E G T A or ethylene glycol tetraacetic acid, it is also effective against metalloproteases.

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Now, when you are making your sample preparation for proteomic applications it is very important that you process the samples in the cold conditions to reduce any proteolysis step. Now, this proteolysis artifacts can be minimized by using cold conditions during the grinding as well as during the centrifugation esters. Now, let us talk about sample fractionation, as I mentioned in the beginning depending upon your applications, you need to decide a strategy effective way of obtaining all the proteins. So that you can study your biological problem in an effective way.

For example, if you are looking at a particular organelle a proteome analysis for a given particular organelle then you need to fractionate your sample or if you are looking for a white proteome coverage. But the proteome is posing lot of challenge to provide all the proteins mixture separation simultaneously then we need to do the fractionation to reduce the complexity of sample.

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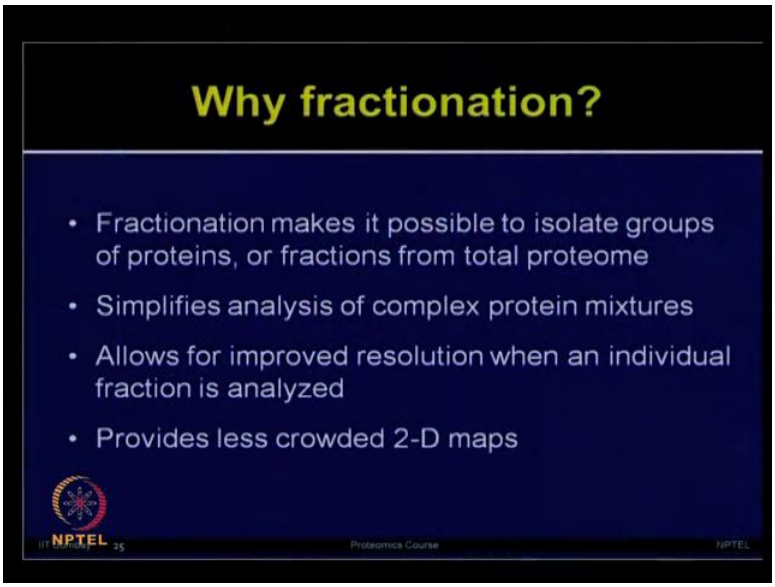

So, why to perform fractionation? The sample fractionation makes it possible to isolate the group of proteins or fractions from a given total proteome. By doing fractionation you can simplify the analysis of complex proteome mixture. This method can further allow the improved resolution of proteomic data and then when you are obtaining the data from individual fractions and putting it together, it is going to be more informative. When you are applying your protein samples on 2-D gel or other type of gel based proteomic technique, this will provide you less crowded protein map. So, again fractionation can serve a good need in both gel based and gel free proteomic applications.

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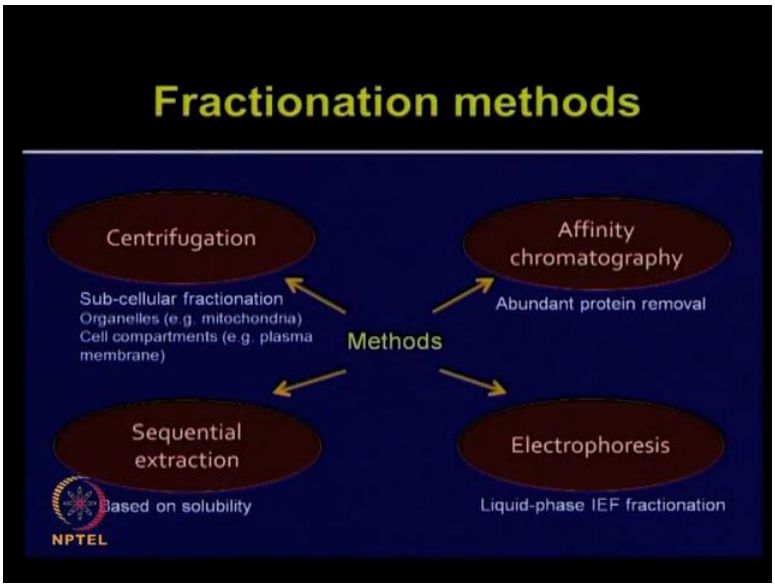

So, let us talk about different type of fractionation methods available. Simplest for looking for the organelle or a specific type of proteome, people use different ways of centrifugation, ultra centrifugation. When you are looking for subcellular fractionation of organelle such mitochondria or chloroplast or cell compartments such as plasma membrane. Different type of chromatography methods can be used for fractionation . If you are interested in looking at all the serum proteins, but there are certain protein which are highly abundant in the serum such as serum albumin protein. So, by using affinity chromatography methods, one can remove those highly abundant proteins so that all the proteins in that given mixture can be well resolved.

Now, affinity chromatography methods can be used for fractionation in different context, sequential extraction which is again going to simplify your proteome. It is based on solubility and different type of chemicals are used so that, in a sequential way one can extract the proteins. Few proteins may be more soluble in one particular composition of reagents and other proteins are more soluble in different solubalization buffer. So, by apply different type of recipe of chemicals one obtain the bigger coverage of whole proteome in doing the sequential extraction. Electrophoresis can be used for fractionation for example,if you are using gel free method directly you want to extract the protein and want to analyze that using mass spectrometry.

So, rather than applying the whole sample directly with the liquid chromatography, one can first simply the proteome by using isoelectric focusing and do the I E F process liquid phase itself. So, liquid I E F fractionation can simplify the proteome based on the isoelectric point. In the next lectures when I will talk about specific type of gel based and free techniques, I will describe you about a new method being used is off gel fractionation which is simplifying the step of isolating proteins. Separating proteins based on their isoelectric point in liquid phase. So, one can collect the fractions of different isoelectric point p I range and then further perform the proteomic analysis.

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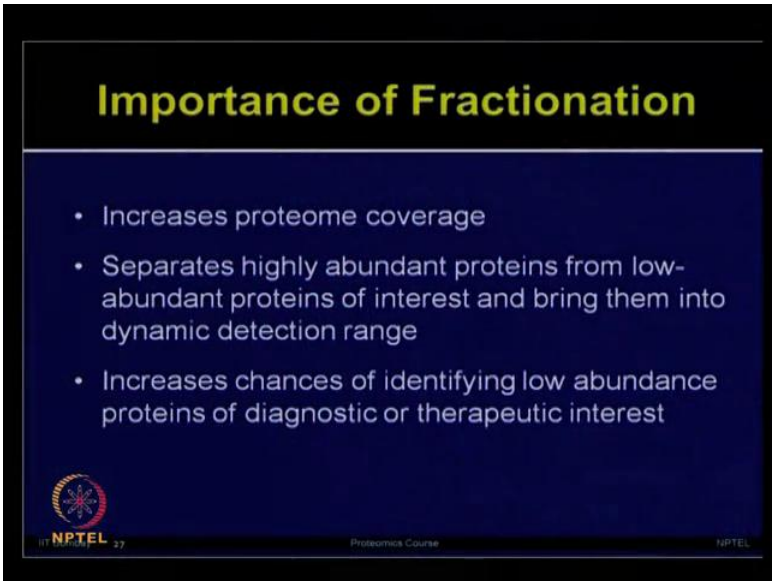

So, we have talked about different type of fractionation strategy. Now, what is the significance term fractionation methods? The first most important thing to like to increase the proteome coverage, you would like to analyze as many proteins as possible from that given sample. And effective fractionation method separates highly abundant proteins from the low abundant proteins of interest and bring them into the dynamic detection range. Often the low abundant proteins could be the proteins of interest which are being masked by the highly abundant proteins. If you are able to separate the high abundant proteins then probably you are able to increase the dynamic detection range and bring everything in the same range. Fractionation can also increase the chances of identifying low abundance proteins of diagnostic or therapeutic interest.

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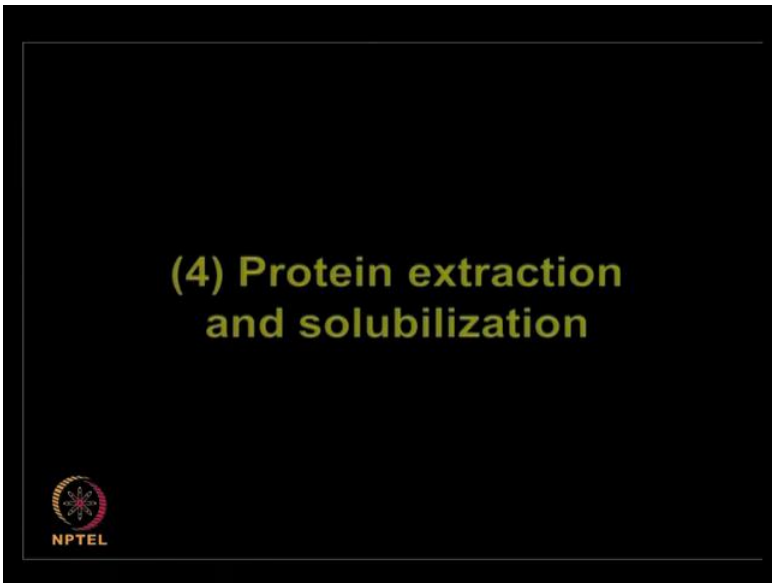

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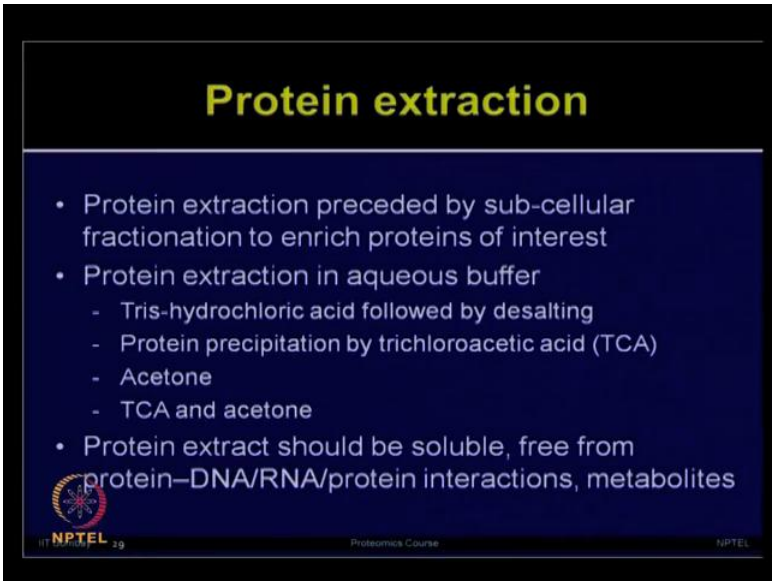

So far, we have talked about how to perform the cell lysis then how to protect proteins. For the proteolysis we have then looked at different type of strategies people involved to fractionate the proteome. And now let us talk about protein extraction and solubalization now, this step will be more towards talking about gel based proteomics where solubalization will be more important. So, protein extraction after performing the sub-

cellular fractionation so that the proteins can be enriched which is you are going to be analyzing in your experiment.

So, protein extraction in the aqueous buffer, one can follow different type procedure either you would tress hydrochloric acid followed by the desalting method. Protein precipitation by trichloroacetic acid, or T C A or acetone alone or trichloroacetic acid and acetone. I will give you more specific composition and recipe when I will talk to you about specific type of examples, how to perform protein extraction for serum bacteria and plants.

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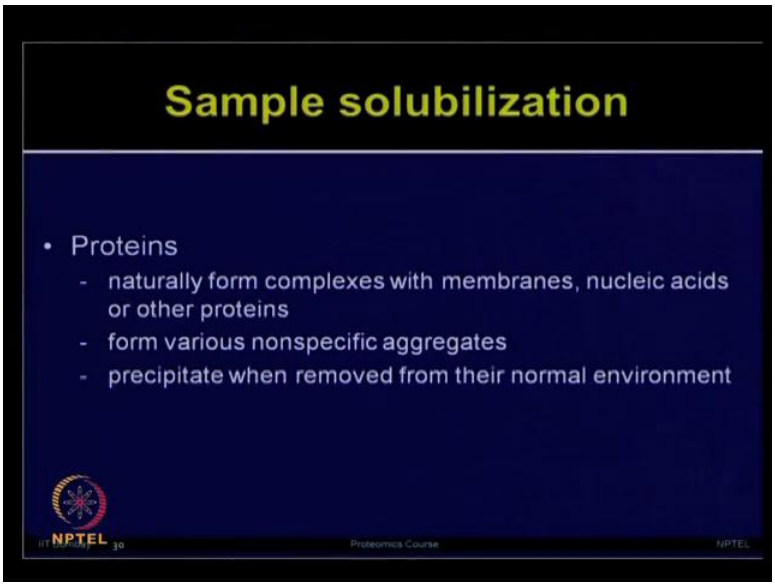

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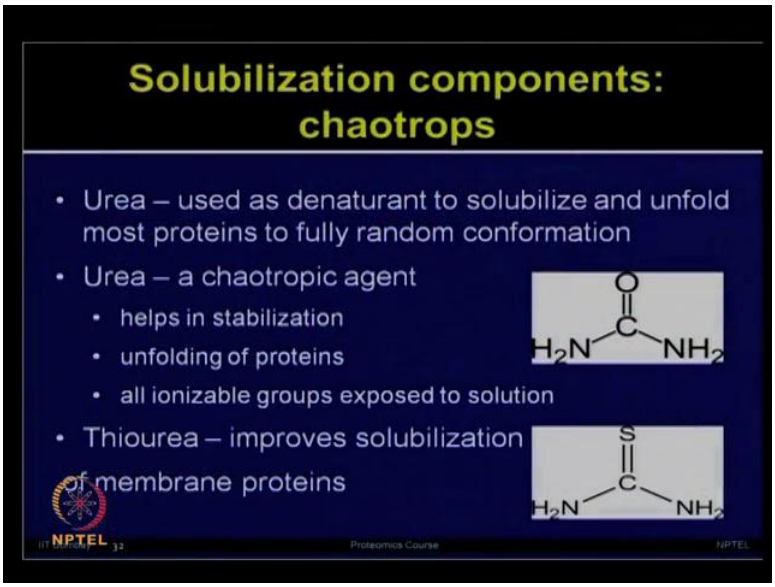

So, protein extracts should be soluble, it should be free from protein to protein interactions, protein to D N Aor protein RNA interaction. Similarly, there are different type of other cellular components present and those should be effectively removed. No metabolites should be interfering in your protein extract. Sample solubalization is important because proteins naturally form complexes with membranes nucleic acids as well as other proteins. So, to avoid all of these issues sample solubalization is very important that the components being used in solubalization.

Let us discuss one by one first of all let us talk about chaotrops, urea and thiourea. Urea is used asdenaturant which can solubilize and unfold, most of the proteins to fully random conformations. Urea is chaotropic agent which helps in a stabilization of the proteins and unfolding proteins specific that all the ionizable groups are exposed to the solution. Thiourea improves solubalization of membrane proteins more specifically, mostly both urea and thiourea are both met together during the solubalization step.

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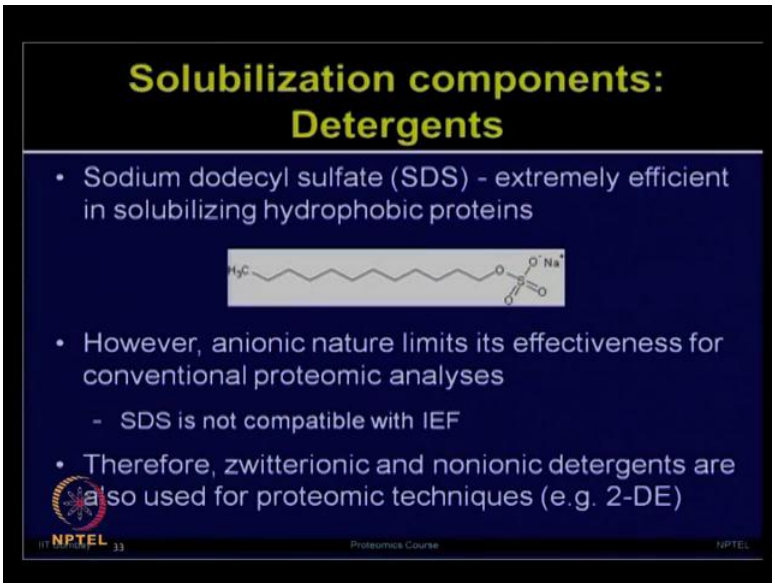

The different type of detergents which are also used in solubalization such as S D S or sodium dodecyl sulphate which is a very efficient solubilizing hydrophobic proteins. If you want to solubilize hydrophobicprotein S D S can be very effectively used. But due to its anionic nature it limits its effectiveness for the conventional proteomic analysis, the S D Sthe anionic detergent is not compatible for isoelectric focusing. So, if you are preparing your protein preparation to perform two dimension electrophoresis,S D S should be avoid from the sample solubalization.

If your objective is to extract the protein and separate that on S D S page then S D S is very useful. So, when if you want to do the 2-DE or dige or different type of other advanced gel based proteomic application where you cannot use S D S. So, zetaionics and non ionic detergents are used for such application.

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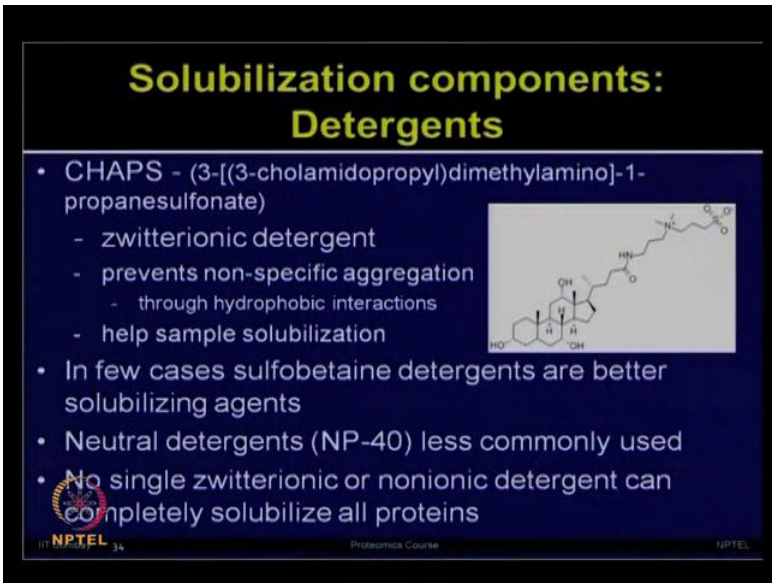

CHAPS, one of the zetaionics detergent is most commonly used detergent used in protein solubalization when your objective is to perform two dimension electrophoresis experiments. It prevents nonspecific aggregations through the hydrophobic interactions and it helps in sample solubalization, depending upon your sample type different type of detergents could be useful. In few cases A S B-14 or sulfobetaine detergents they are better solubilizing agents, you also have options of using neutral detergents such as N P-40, although they are less commonly used.

So, one cannot provide you a list most effective solubalization agents, no single tutorionicor non ionic agent can completely solubilize all the proteins. So, depending upon your sample type and if you know your sample is enrich specific type of proteins, you need to try different type of detergents.

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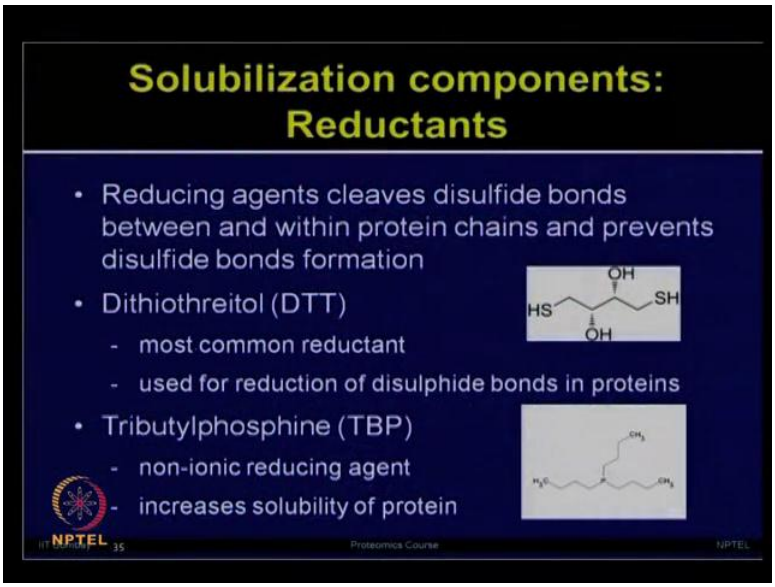

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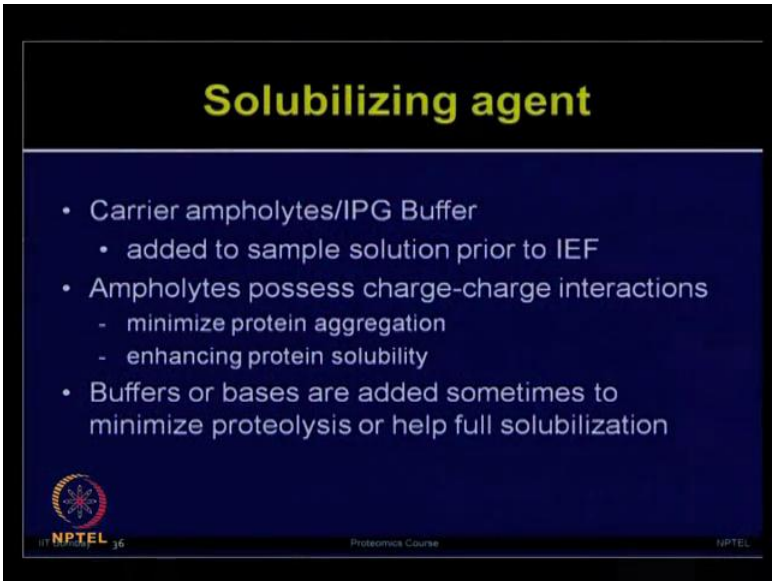

Now, let us talk about reductants. In the solubalization reducing agents cleave the disulphide bonds which are present between and within the protein chains and it prevents the disulphide bond formation. Most commonly used reductants are dithiothreitolD T Tor beta mercaptoethanol, these are used for reduction of disulphide bonds which are present in the proteins. Tributylphosphine or T B P, it is one of the non ionic reducing agent.

Another very commonly used reducing agent when aim is to increase solubility of the proteins often it I used in the 2-DE based, chill based proteomic application.

If your aim is to perform isoelectric focusing from your samples, the solubilizing agent include the carrier ampholytes or immobilized p h gradient buffers. These are added in the sample solutions prior to the isoelectric focusing ester. Which we will talk in the next lectures, when we talk about different steps involved in performing a gel based proteome experiment.

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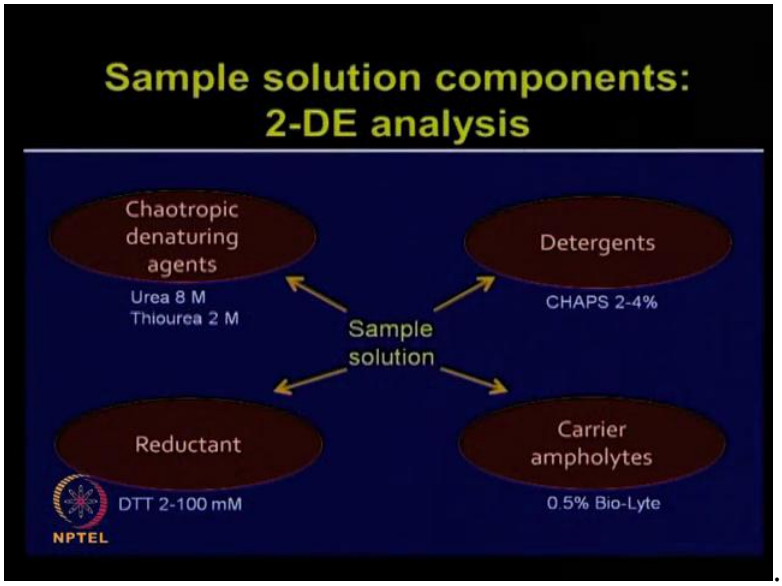

The ampholytes poses charge to charge interactions they minimize protein aggregation and enhance the protein solubility. Different buffers and bases are added which sometime minimize proteolysis and also help in the complete solubalization of proteins. So, if your aim is to perform a two dimensional electro forces experiment, the sample solution involves chaotropic denaturing agents such as urea 8 molar thiourea2 molar. Detergents such as chaps are most commonly used, it could be between 2 to 4 percent, commonly used reductant include D T Tor beta mercaptoethanol2 to 100milllimolar and carrier ampholytes in the concentration of 0.5 percent of biolyte.

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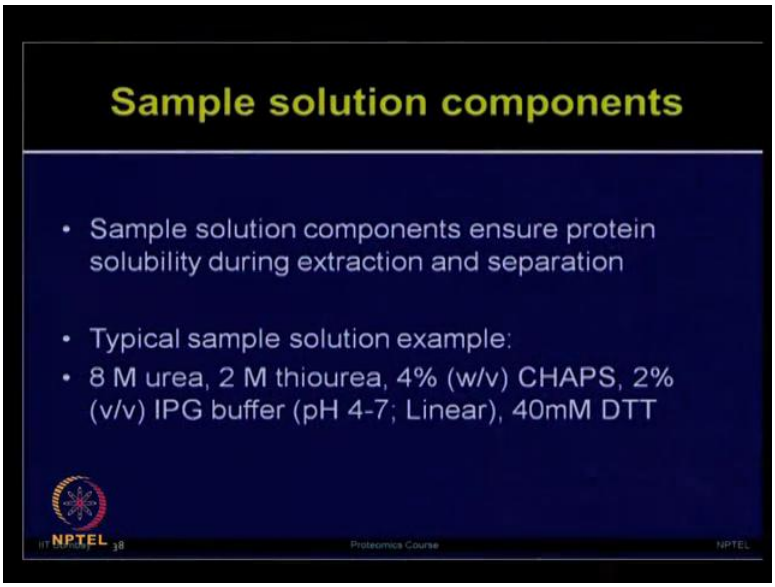

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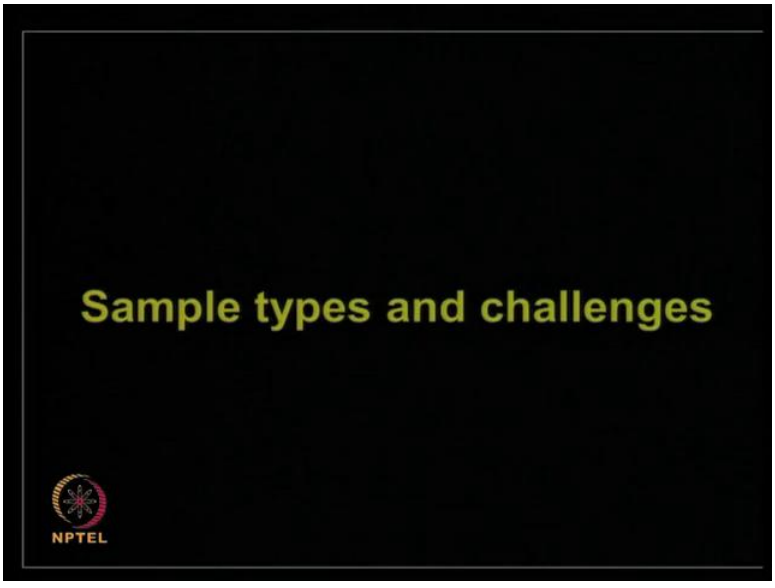

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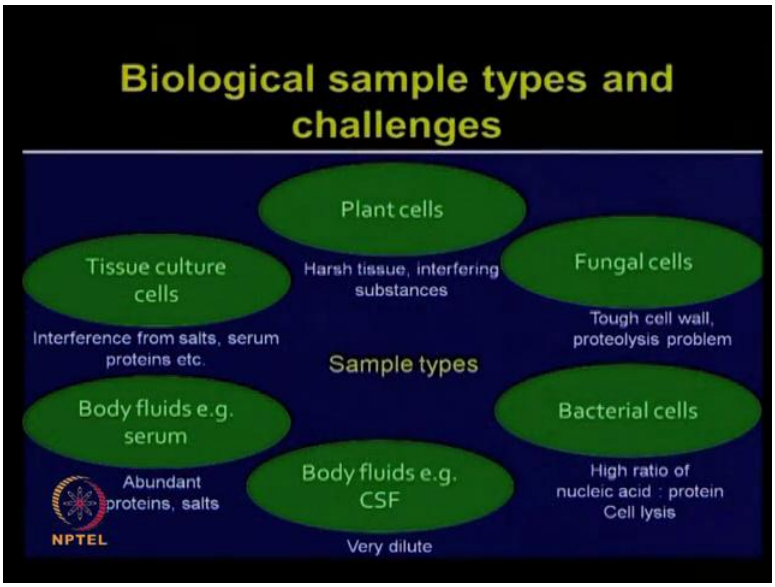

So, the sample solution components insure that the protein solubility is good during the extraction and protein separation. A typical sample solution for the gel based 2-d E application includes 8 molar of urea,2 molar of thiourea, 4 percent CHAPS,2 percent I P Gbuffer,40millimolar of D T T.As well as few are they are small components depending upon your sample type so, as I am giving you an overview of how to prepare a very good sample. Let me also introduce you to different type of challenges being imposed by different sample types. So, if you are using the tissue culture grown cells, you have to grow in a medium which will be rich in different components including salt and serum proteins.

So, when need to get rid of those component, if you want to perform a good sample preparation from the tissue culture cells. If you are interested in plant cells to extract the protein those are very hard tissue and there are various interfering substances present there. So now, let us another salts, now you need to get rid of those interfering substances fungal cell such a least or other type of fungus if you are interested in performing proteomic applications on these samples, you need to back open the very tough cells. So, the proteolysis problem also occurs in these samples. The bacterial cells they have high ratio of nucleic acid to protein and cell lysis also very tedious. Body fluids such as cerebrospinal fluid they are very dilute. So, if you want to perform proteomic

experiments on C S F, you need to concentrate your samples. Body fluid such as serum those are very rich in abundant proteins as well as salt. So, you need to get rid of those abundant protein such as serum albumin protein and remove the interfering salts.

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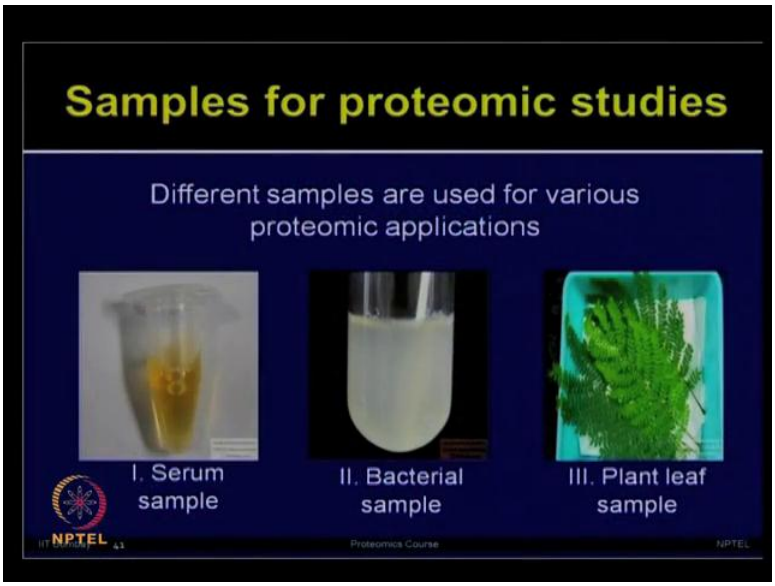

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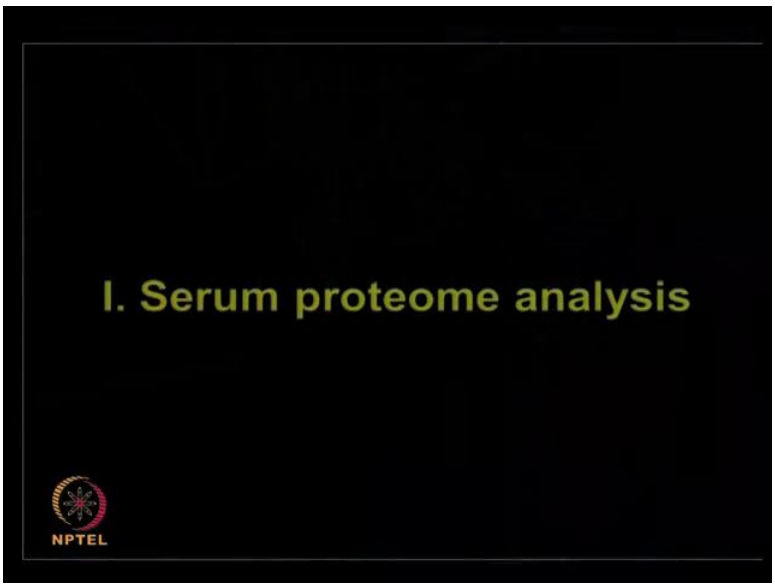

Solution although the sample diversity is very much samples are very complex I will still

try to take three representative example. One the serum sample obtained from human, second the bacterial sample which will be taken from bacillus species and third the plant leaf sample by showing you the protein extraction and solubalization methods. For these three different type of samples, I will try to give you the diversity and different type of methods being used to perform various type of proteomic applications. Surfaces with the serum proteome analysis, but before we talk about how to perform the serum proteome analysis it means all the proteins which are present in the human serum.

First of all how to obtain the samples? How to store these samples? How to minimize various type of preanalytical variations? That is one of the very important consideration.

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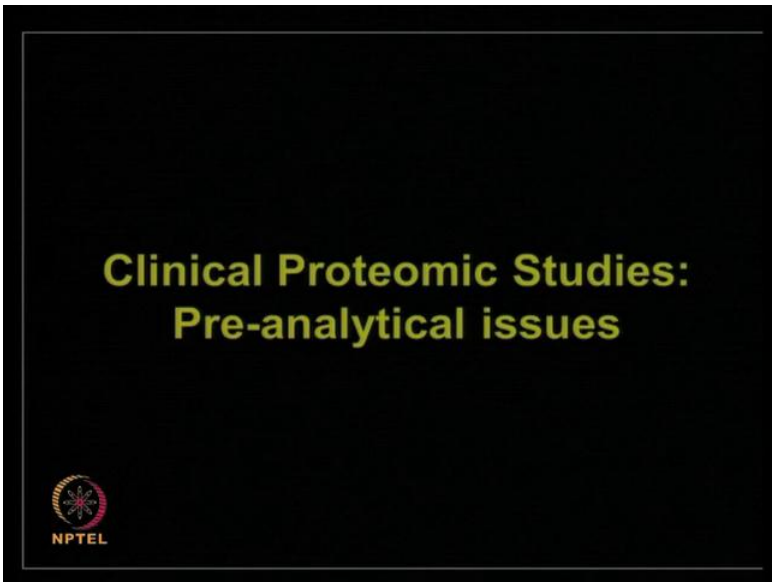

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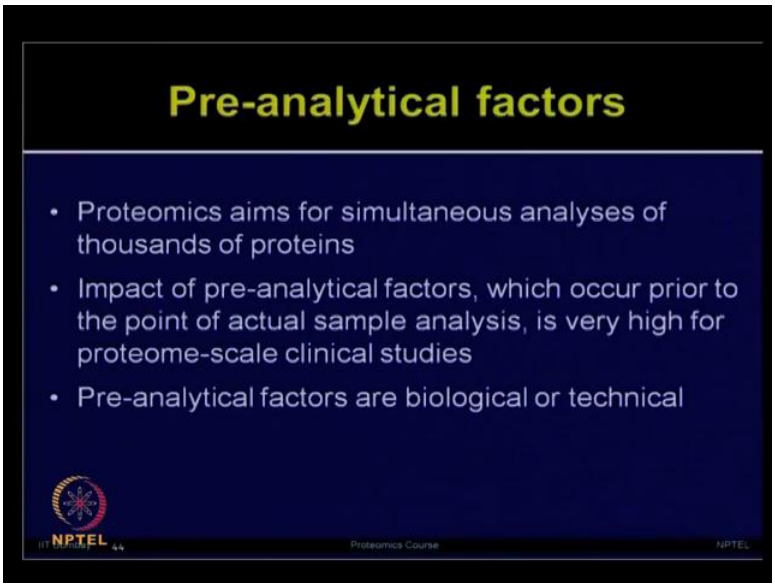

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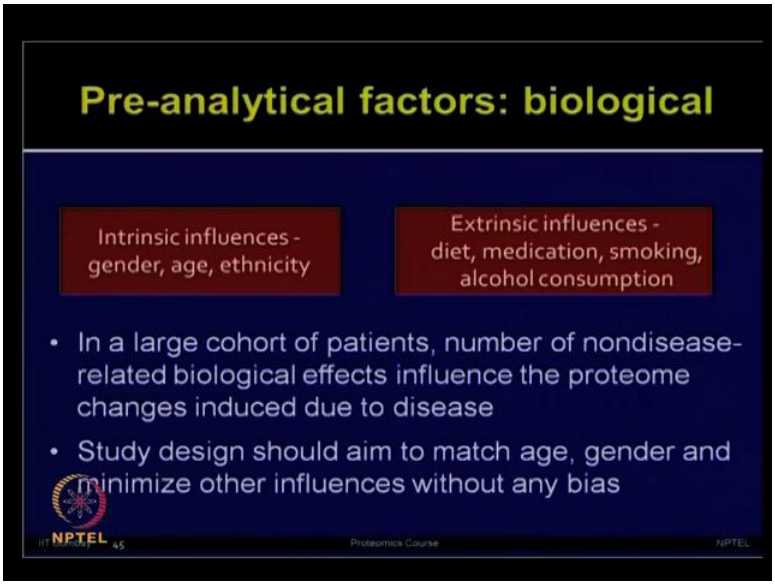

So, before we talk about how to really process the sample let us talk about different type of clinical issues involved in these type of samples for the preanalytical factors. So, proteomic, most of the applications are going to aim for simultaneous analysis of thousands of proteins of given clinical sample whether it is serum saliva urine C S F or tissue. The impact of preanalytical factors which occur prior to the point of actual sample

analysis is very high for the proteome scale clinical studies. The preanalytical factors could be due to biological variations or it could be due to the technical artifacts.

So, your studies could be influenced due to intrinsic factors or due to the extrinsic factors. The intrinsic influences include gender, age, ethnicity, the extrinsic influences include diet, medication, smoking, alcohol consumption etcetera. So, when you are designing clinical study, you need to ensure that you have buyers with the intrinsic factors. You should try to segregate you population with the different type of age, gender, try to in the discovery phase, try to minimize these type of variations. And try to perform your analysis with a narrow range of age group and different type of gender groups in the same ethnicity. But when you want to validate your samples then you need to extend your analysis to the different age type ethnicity and gender.

Try to avoid the extensive influences such as diet, smoking alcohol different type of drug medication, these are going to alter the proteome and your discovery process will be influenced by these factors. So, on the large cohort of patients, the number of non disease related biological effects will influence the proteome changes induced due to the disease. So, the study design should aim to match the age, gender and minimize the other influences without any bias. Often it is very useful to involve statistician before you are designing these type of experiments and thinking about different preanalytical factors before you actually perform your experiment is often going to determine, how successful your analysis going to be.

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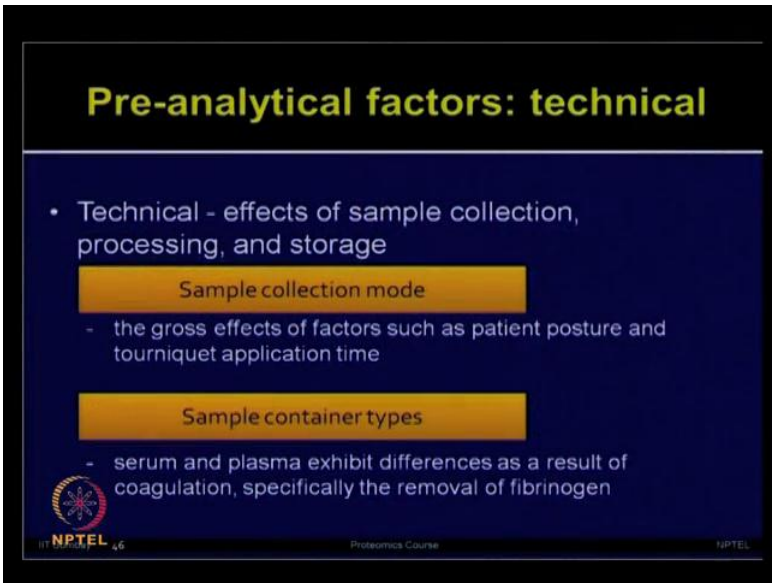

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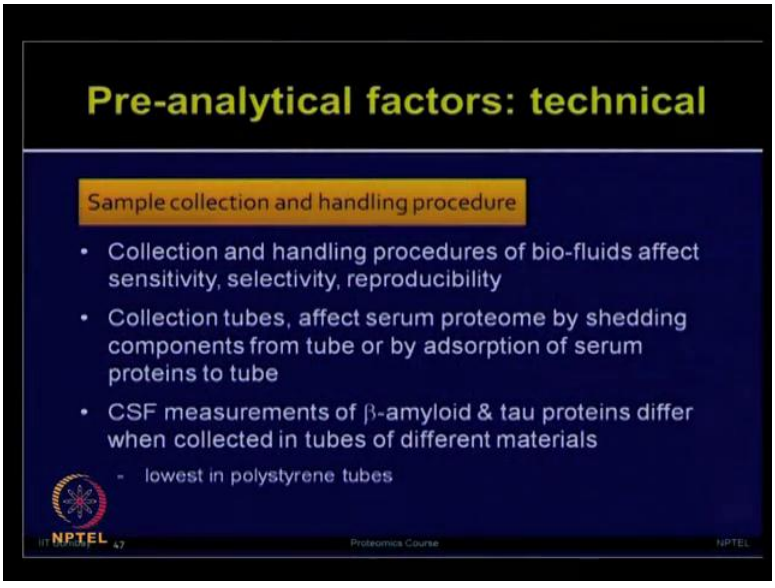

Now, we have looked at different type of biological preanalytical factors. then there are different type of technical artifacts, how to collect the samples, how to process the sample and how to store those the sample collection mode. The gross effect of factors such as the patient posture and the tourniquet application times, these are very important. Sample container types when you were collecting the samples such as serum and plasma,

they exhibit differences as the result of coagulation specifically the removal of the fibrinogen. So, sample collection and handling procedure one has to pay attention. The collection and handling procedure of bio-fluids will affect the sensitivity, selectivity and the reproducibility of the experiment.

Collection tubes in which you are collecting your serum sample often is going to influence the analysis. If you were using different type of tubes material, the shedding components from the tube or adsorption of the serum proteins to the tubes will in some way influence the proteome analysis.

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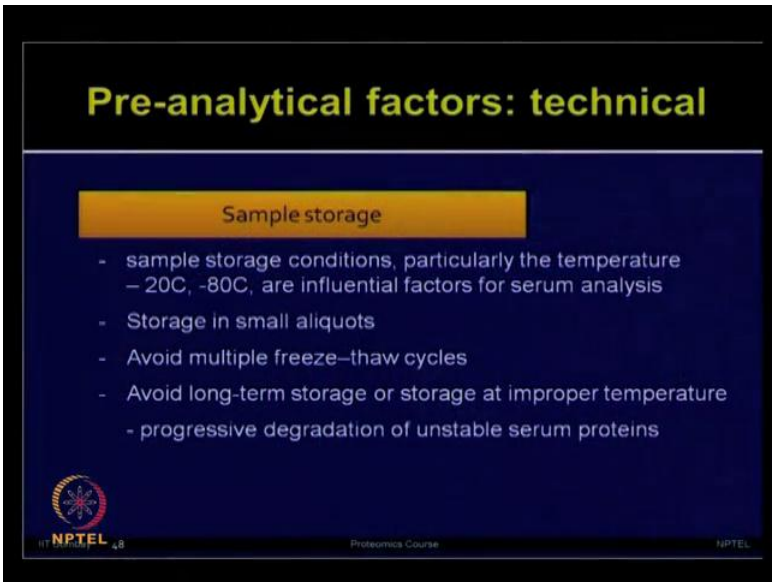

The cerebral spinal fluid measurement of different proteins such as beta amyloid and tau proteins, when people analyze this in different tubes of different materials they found that they have different type of effects and the effects were lowest in the polystyrene tubes. So, with this discussion it is very important to understand that one need to avoid different type of sample tubes being used for collecting your baliscus samples. Sample storageis another very crucial factor whether you are storing your chemical samples in minus 20 degree centigrade or minus 80 degrees.

How quickly have you saved all the clinical samples or how much delay was there before

the sample was collected and stored. All of these are small variations actually influence the sample analysis, the proteome analysis later on. So, avoid multiple phase thawing of your samples, store the samples in a small aliquots so that, you do not have to freeze thaw the whole samples together. Avoid very long term storage or storage at the improper temperature, try to use as freshly store sample as possible because if the samples are used. And stored for the longtime the progressive degradation of unstable serum proteins may occur.

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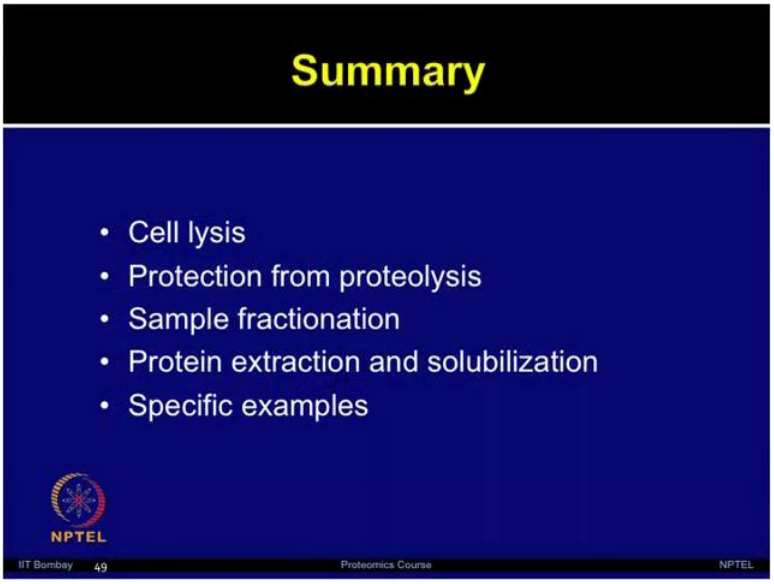

So, we will continue our discussion on how to perform the protein extraction and proteome analysis of serum and different type of baliscus samples in the next lecture. But in summary today you have learnt about how to perform the cell lysis? How to protect the cell from the proteins, from the proteolysis? By adding various type of protease inhibitors, sample fractionation methods. How to use protein extraction and solubalization for effective protein solubalization? And now we are talking about specific examples, we will continue our lecture in the next class on serum proteome analysis followed by bacterial and plant, protein, and proteome analysis. Thank you.