Proteomics: Principles and Techniques
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Lecture No. # 40
Proteomics: Advances and Challenges

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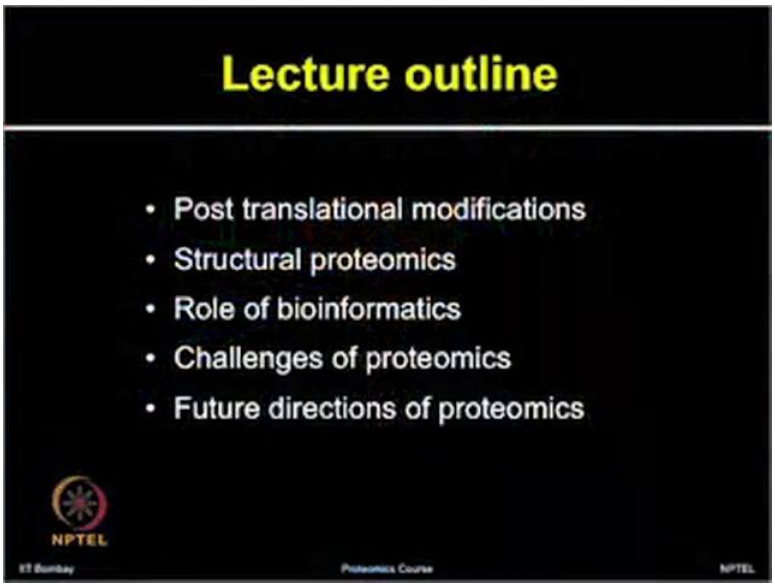

Welcome to the proteomics course; in today's lecture, we will talk about post translational modifications, a structural proteomics, role of bioinformatics, challenges and future direction of proteomics. So, let us start with PTM's post translational modifications are vital cellular control mechanisms known as cellular switches that affect protein properties such as protein folding conformation activity and functions; as a result, they play very important role in various diseases.

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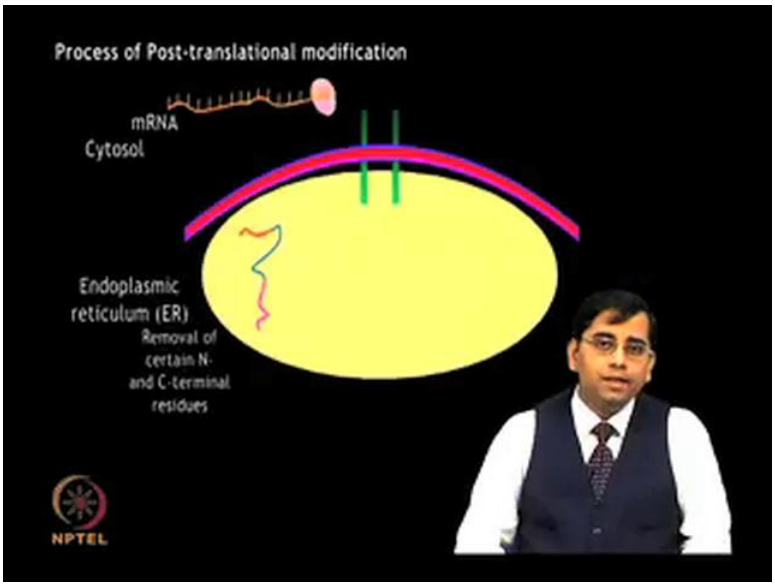

The protein complexity arises due to gene splicing and post translational modification. Once the protein is synthesised by the ribosome from its corresponding MRNA in the cytosol, many proteins get directed towards the endoplasmic reticulum for further modification, certain NLC terminal sequences are often cleaved in the endoplasmic reticulum after which they are modified by various enzymes at specific amino acid residues. These modified proteins then undergo proper folding to give functional proteins due to these modifications the number of proteins are three orders of magnitude higher than the total number of genes encoded in genome.

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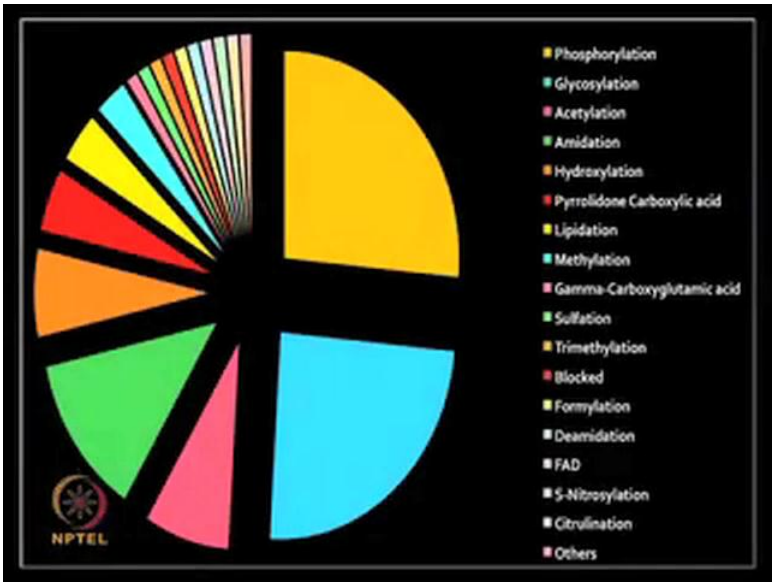

There are several types of post translational modifications that can take place at different amino acid residues. The most commonly observed PTM's include phosphorylation, glycosylation, methylation as well as hydroxylation and acylation. Many of these modifications particularly phosphorylation serves as regular mechanism for the protein action. PTM's generate tremendous diversity and are extremely important.

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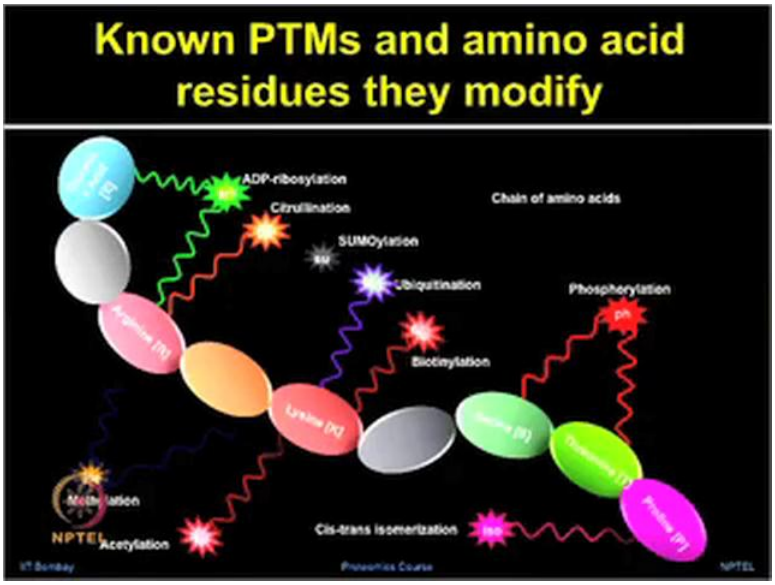

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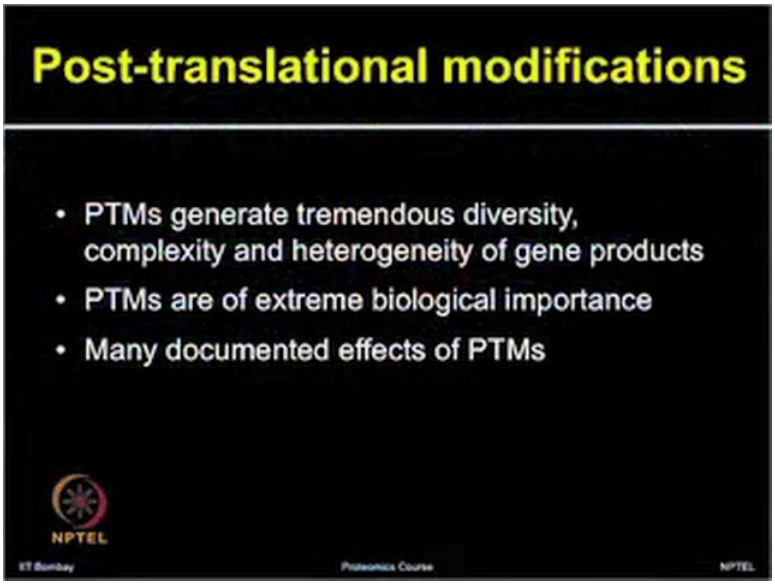

Many documented effects of post translational modification include change in enzymatic activity, ability to interact with other proteins sub-cellular localization and targeted degradation.

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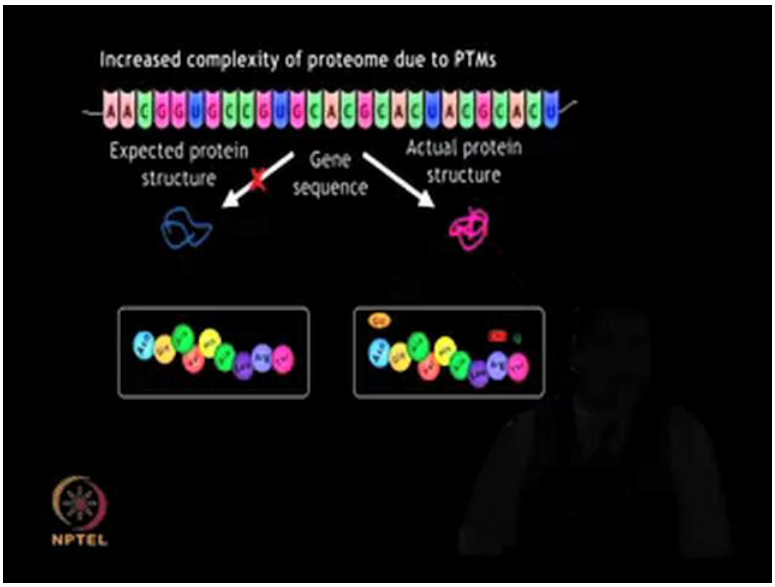

The final structure of functional proteins most often does not correlate directly with the corresponding gene sequence. This is because of PT Ms that occur at various amino acid residues in the protein which cause changes in interactions between the amino acid side chains thereby modifying the protein structure, it further increases the complexity of the proteome as compared to the genome. The protein phosphorylation acts as a switch to turn on or turn off the protein activity and governs wide range of polypeptides from transcription factors enzymes to cell surface receptors.

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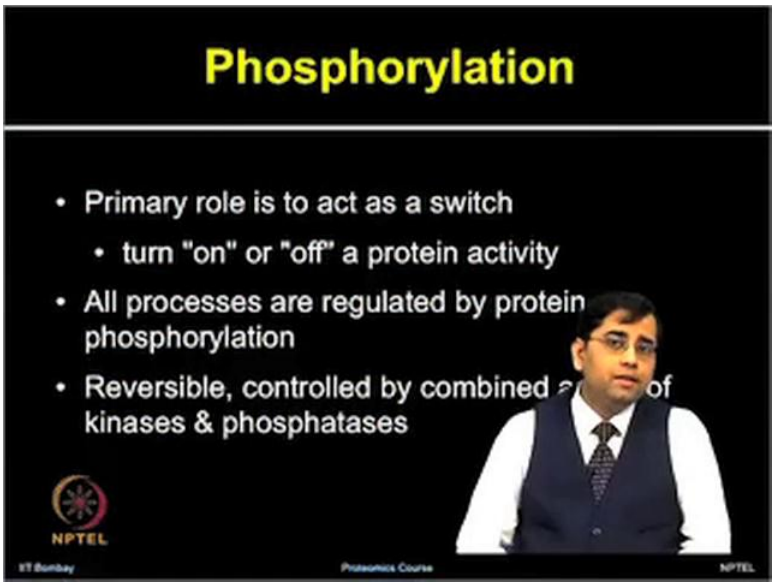

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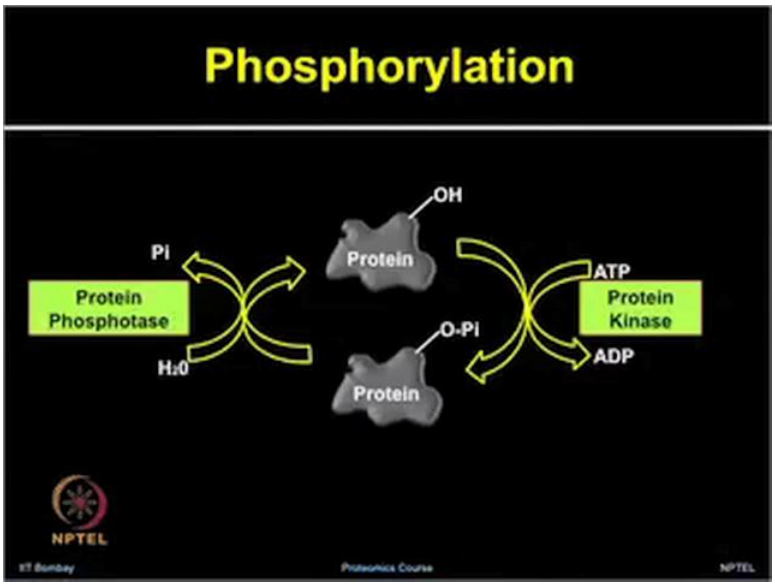

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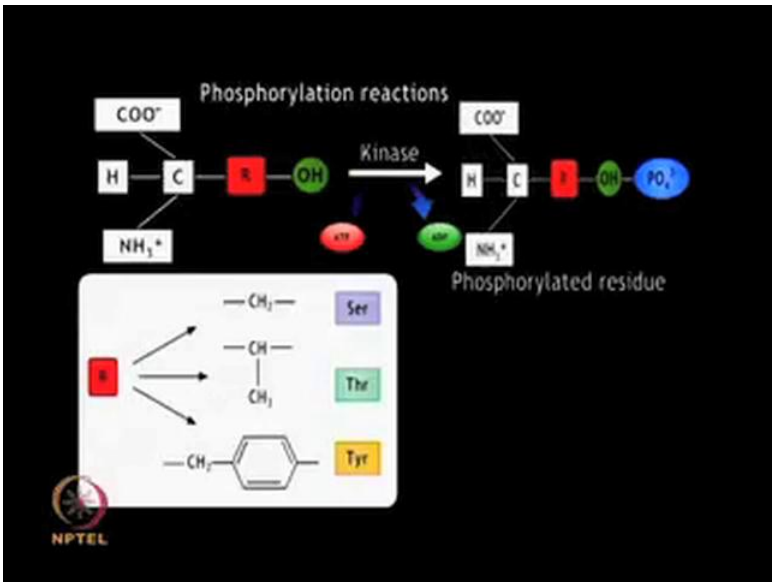

The reversible phosphorylation of proteins catalyzed by kinases and phosphatages regulates important cellular function. Phosphorylation of amino acid residues is carried out by a class of enzymes known as kinases that most commonly modified side chains of amino acids containing a hydroxyl group. Phosphorylation requires the presence of a phosphate donor molecule such as ATP , GTP or other phosphorylated substrates. Serine is the most commonly phosphorylated residue followed by threonine and tyrosine. The removal of phosphate groups is carried out by phosphatase enzyme and it forms one of the most important mechanisms for protein regulation. Glycosylation involves leaking saccharides to proteins in presence of glycosyl transferases enzymes giving rise to a glycoprotein.

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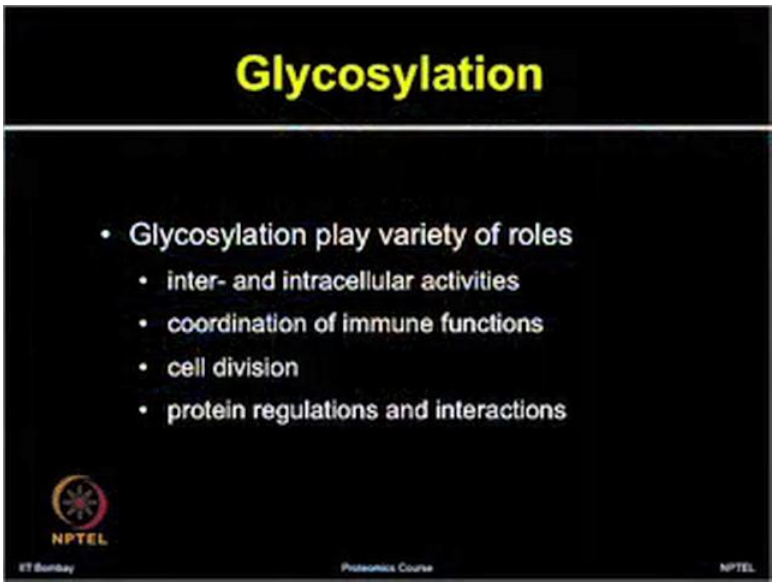

Glycosylation play vital role in various biological functions, such as antigenicity of immunological molecules cell division protein targeting stability and interactions. The aberrant glycosylation forms resulting to various human congenital disorders.

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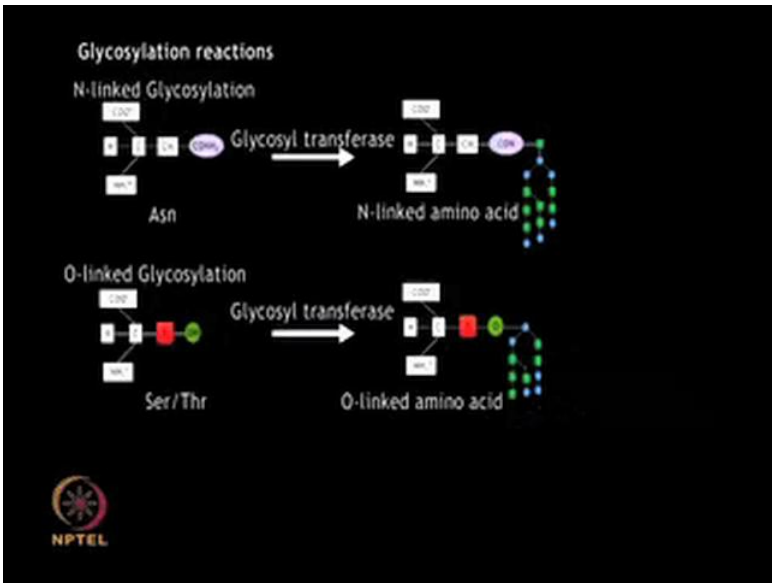

Depending on the linkage between the amino acid and the sugar moiety. There are four types of glycosylation and N linked glycosylation, O linked glycosylation, C mannosylation and glycophosphatidylinositol anchored GPI attachments. Glycosylation involves the enzymatic addition of saccharide molecules to amino acid side chains.

This can be of two types N linked glycosylation, which links sugar residues to the amide group of asparagine and O linked glycosylation which links the sugar moieties to the hydroxyl group of serine or threonine. The glycosyl transferase enzymes catalyze these reactions sugar residues that are attached most commonly include galactose, mannose, glucose and acetyl glucosamine etcetera. There is a growing interest in proteomics community to decipher the role of PTMs in various biological contexts.

Detection of subtle PTM changes post challenge to even advanced proteomic techniques there are many approaches ranging from gel based techniques mass spectrometry, microarrays that are currently used to a steady post translational modification. We will discuss some of these techniques one by one.

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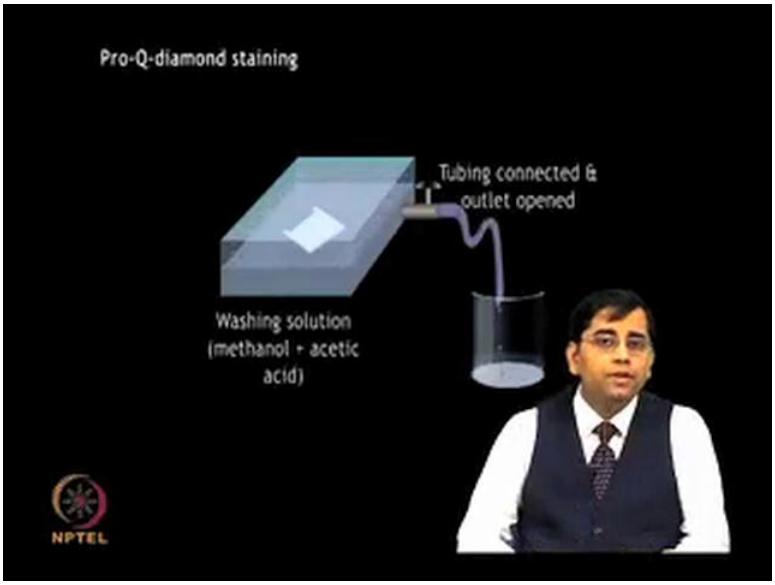

The protein phosphorylation can be detected using gel based detection technique, protein separated on a two d gel are placed in effective solution containing methanol and acetic acid which fixes the protein bands on to the gel and minimizes any diffusion.

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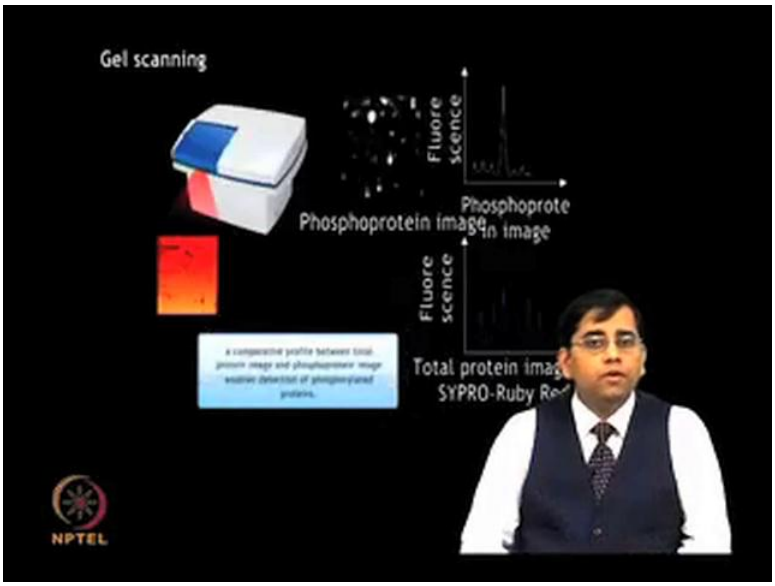

They are stained using pro q diamond staining solution which selectively stains only phosphoproteins on the gel, excess stain is washed off with solution of methanol and acetic acid. The stain gel is a scanned at it excitation and emission wavelength using gel scanner. The gel image obtained shows the protein bands corresponding to only the phosphoproteins which are present image can be saved and gel is removed from the scanner for treatment with the second stain.

A procedure known as dual staining this gel is placed in sypro ruby a red fluorescent dye solution, then dye stains all the protein spots present on the gel thereby providing a total protein image where sensitivity down to nanogram level. Excess dye can be washed off using methanol and acetic acid the gel is stained with sypro ruby red it is scanned in gel scanner at it is excitation maxima. Image produced will have more number of spots since all the proteins present on the gels can be detected, this dual staining procedure provides a useful comparative profile of the phosphoproteins and total proteins on the gel and enables detection of phosphorylated proteins.

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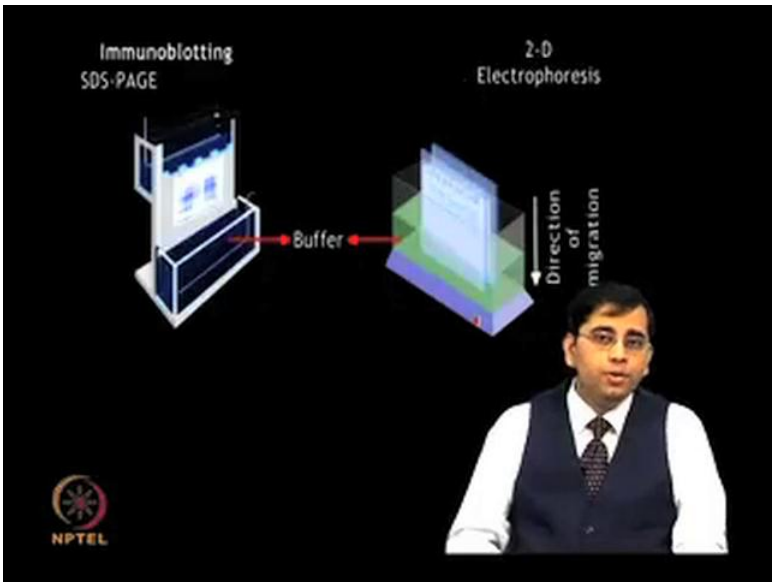

Proteins phosphorylation is widely detected using enological or enzymatic techniques protein mixture containing phosphorylated as well as other modified forms can be separated by electrophoresis. SDS page and two-dimensional gel electrophoresis are most commonly used for protein separation, these separated proteins on the gel are used further analysis.

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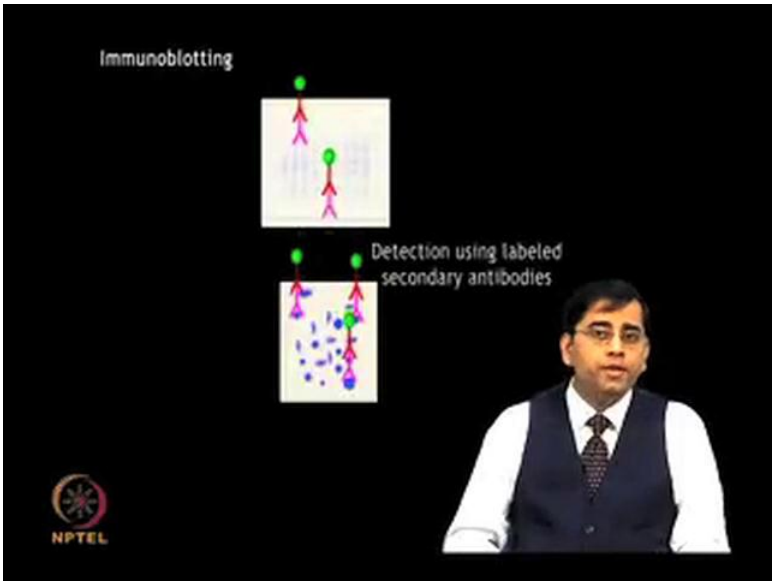

The separated protein bands are blotted onto a nitrocellulose membrane those membranes are then probed either by means of a specific entire phosphoamino acid

antibodies or more recently by using motif antibodies that specifically bind to proteins having phosphorylation at a particular amino acid residue.

This binding interaction can be detected by using suitably labeled secondary antibodies or by autography using radioactive probes. Therefore, use of immune blotting techniques has been shown to be extremely effective for detection of post translational modifications. The gel based approaches are convenient to use however, it has few drawbacks with regards it is limit of detection, membrane proteins localization of modification sites robustness, sensitivity and gel to gel reproducibility.

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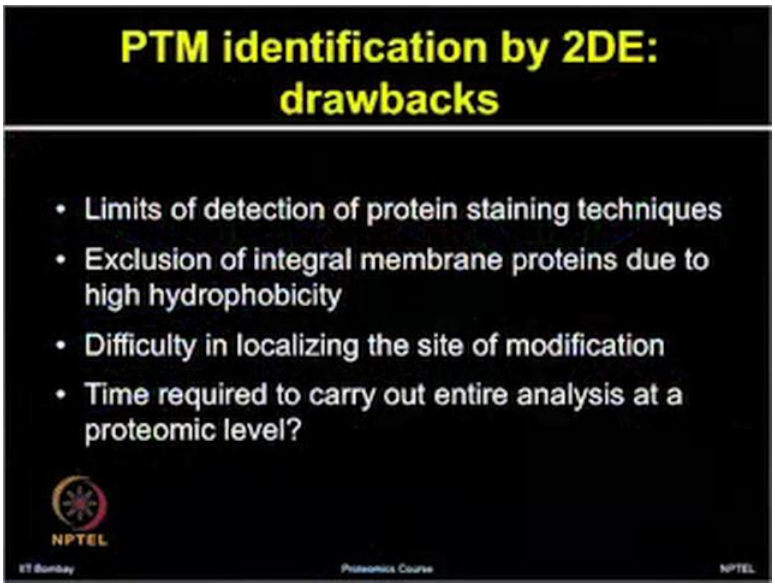

Characterization of these resolved proteins subsequently requires other techniques, such as mass spectrometry to identify the proteins. The shotgun based mass spectrometry methods have accelerated identification of proteins and post translational modifications from complex mixtures, protein mixture is digested with proteolytic enzyme such as trypsin and result in peptides can be analyzed by MALDI TOF or LC MS MS the top down mass spectrometry involves analysis of intake protein using high resolution mass spectrometry techniques.

High resolution, MS platforms such as fticr MS orbitrap MS with PTM friendly dissociation techniques, such as electron capture dissociation and electron transfer dissociation etd are commonly used. PTMs can be detected by means of mass spectrometry due to the unique fragmentation patterns of phosphorylated serine and

threonine residues. The modified protein of interest is digested into small peptide fragments using trypsin, this digest is then mixed with a suitable organic matrix such as alpha cyano four hydroxyl cinnamic acid sinapinic acid etcetera.

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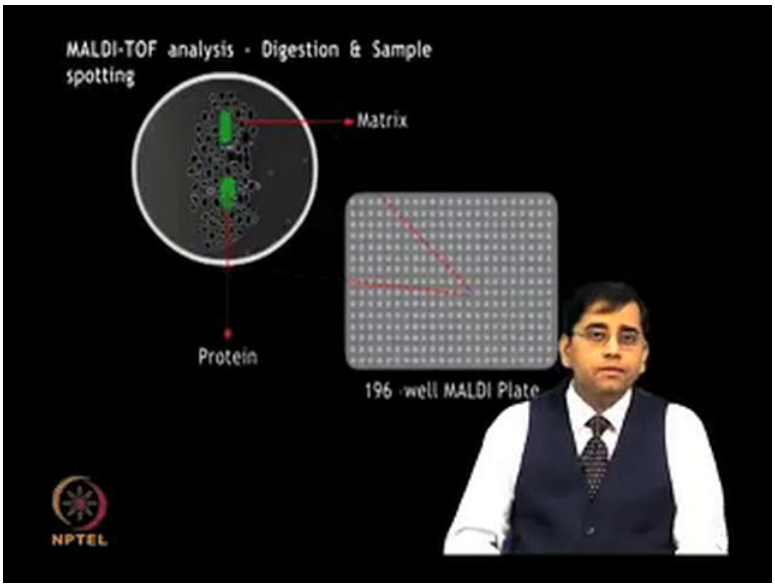

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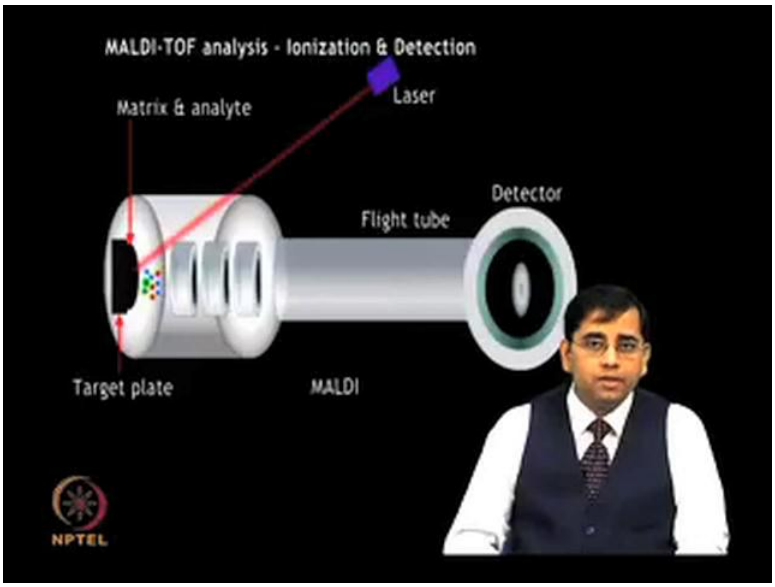

And then it is spotted onto MALDI plate the target plate containing spotted matrix and analyte is placed in a vacuum chamber with high voltage and short laser pulses are applied. The laser energy gets absorbed by the matrix and is transferred to the analyte molecules which undergo rapid sublimation resulting in gas phase ions, these ions are

accelerated and travel through the fly tube at different fields the lighter ions move rapidly and reach the detector first while the heavier ions migrate slowly.

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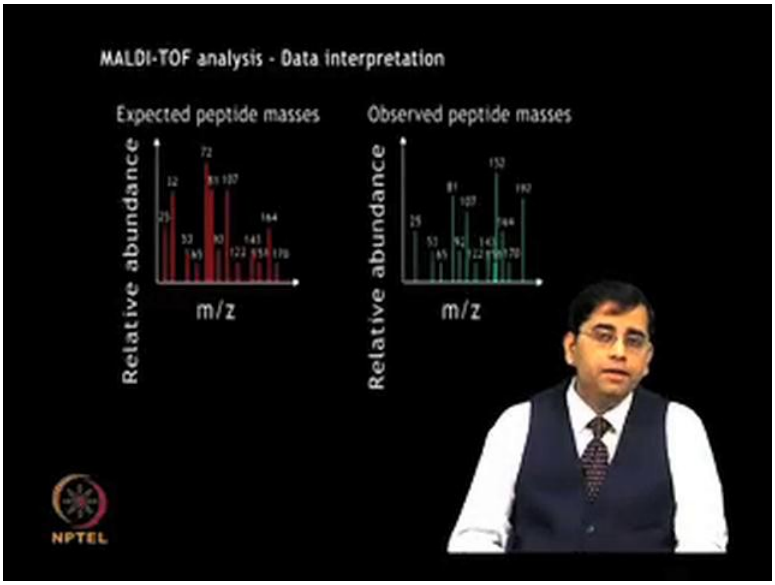

The ions are resolved and detected on the basis of their m by z ratio and a mass spectrum is generated. Identification of post translational modification by MS largely lies in interpretation of results.

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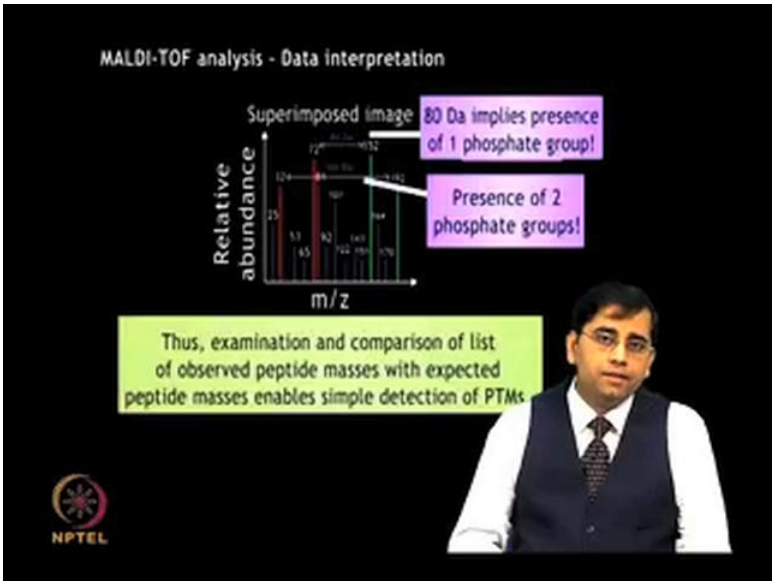

Comparison of list of the observed peptide masses from the spectrum generated with expected peptide masses enables the identification of those peptide fragments that contain any post translational modification due to added mass of a modifying group.

In this hypothetical example two peptide fragments are found to have different m by z values differing, by 80 daltons and one 60 daltons. It is known that the added mass of a phosphate group causes an increase in m by z of 80 daltons therefore, this principle of mass difference enables the detection of modified fragments. Affinity based enrichment immune purification and metal affinity chromatography are commonly employed for purification of proteins containing specific PTMs.

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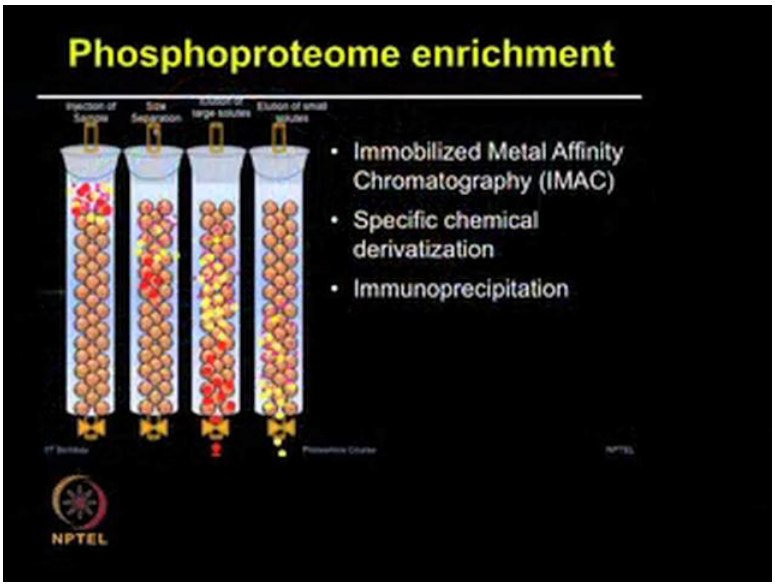

Immobilized metal affinity chromatography IMAC elemental oxide affinity resin such as titanium dioxide fe 3 or 4 are also commonly used for the enrichment of phosphoproteins. A protein phosphorylation experiment is shown here, the complex protein sample is loaded onto a miniaturized affinity column which interacts a specifically with proteins having the post translational modification of interest. The IMAC chromatography columns containing ions such as zinc iron titanium dioxide specifically kylate the phosphorylated proteins.

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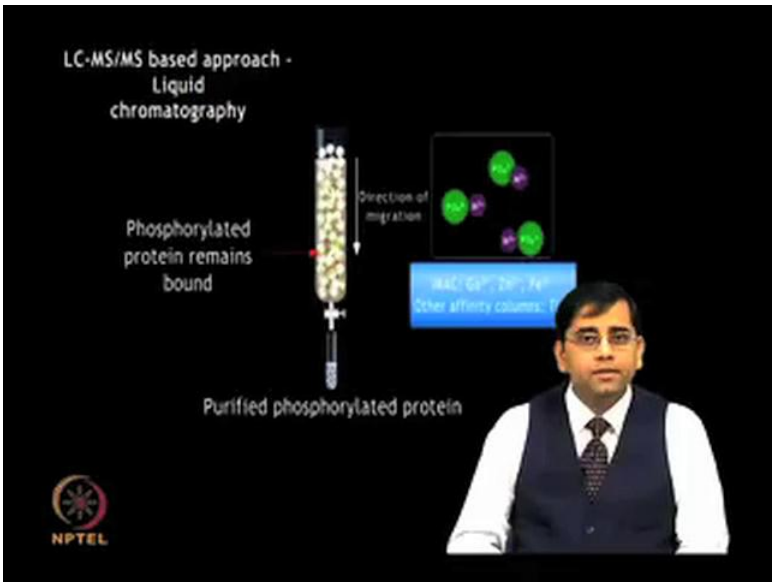

The unwanted proteins are removed by washing the column with a suitable buffer solution, after which the phosphorylated protein of interest can be eluted out by modifying the buffer solution.

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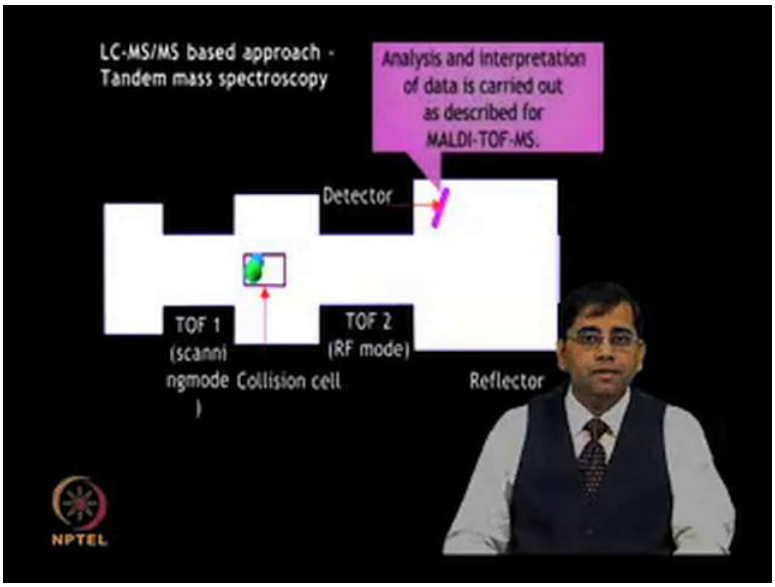

The protein purified by liquid chromatography is then subjected to tryptic digestion followed by analysis using tandem mass spectrometry. Here, I have demonstrated the use of MALDI TOF TOF MS for resolution of the generated ion fragments, separation is based on the fly time of the ions and greater is achieved due to the presence of two mass

analyzers. The peptide ion spectrum generated is analyzed by comparing it with the expected spectrum thereby, allowing determination of modified peptides having different m by z values. The metabolic labeling methods such as silac is used for label based quantitation of PTMs. However, this strategy can only be used for the living cells other chemical labeling methods such as itraq is also used for PTM analysis.

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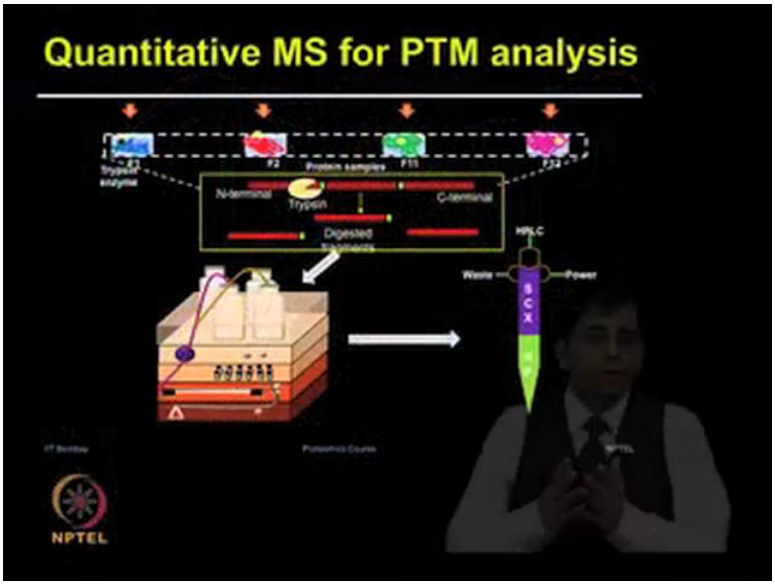

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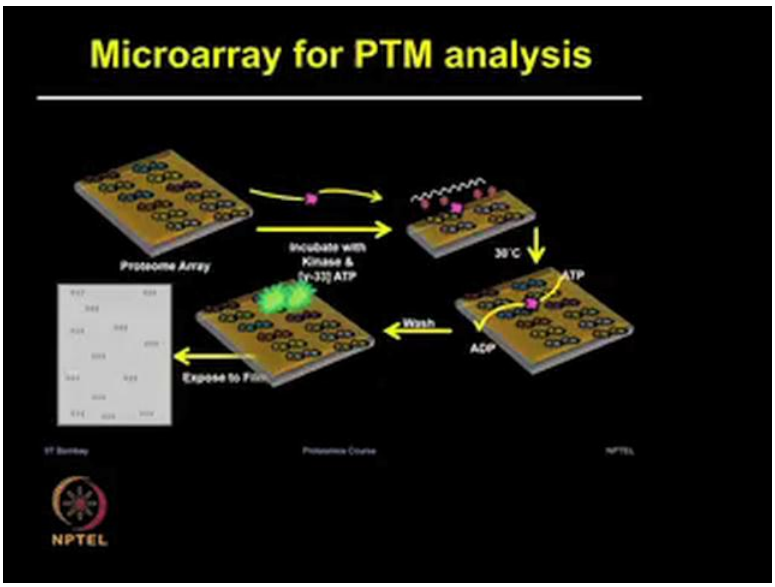

Another technique protein microarrays is one of the versatile platforms for high throughput screening of post translational modifications. Kinases have been used in

number of ways to analyze protein phosphorylation, PTMs can be detected on protein arrays by using kinase assay. Potential substrates for protein phosphorylation are immobilized on a suitable coated array surface kinase enzyme and gamma P32 labeled ATP are then added and array is incubated at 30 degree centigrade.

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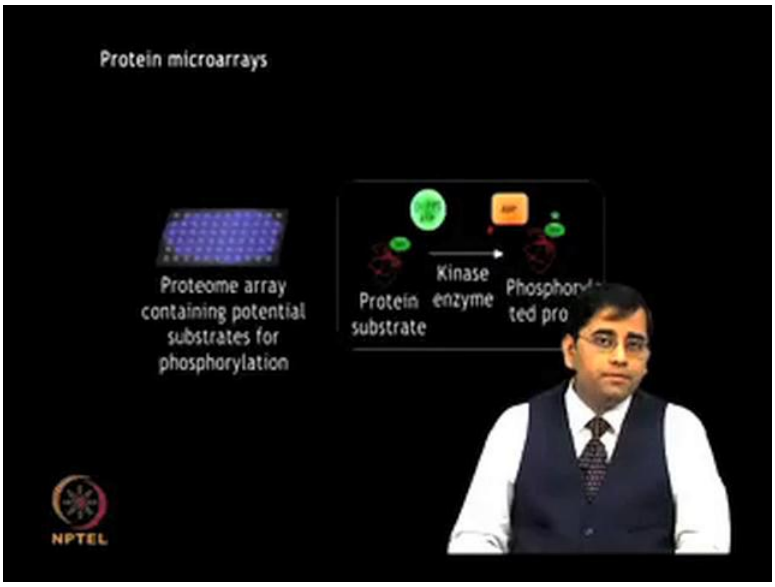

The phosphorylation reaction occurs at those sites containing proteins that can be modified. After sufficient incubation excess unbound ATP and enzyme are washed off the array surface detection is carried out by means of autoradiography, where in a photographic film is placed in contact with the array surface. The radioactive emissions from the phosphate label present at the phosphorylated protein sides strike the film upon development the positions at which phosphorylation has occurred can be clearly determined.

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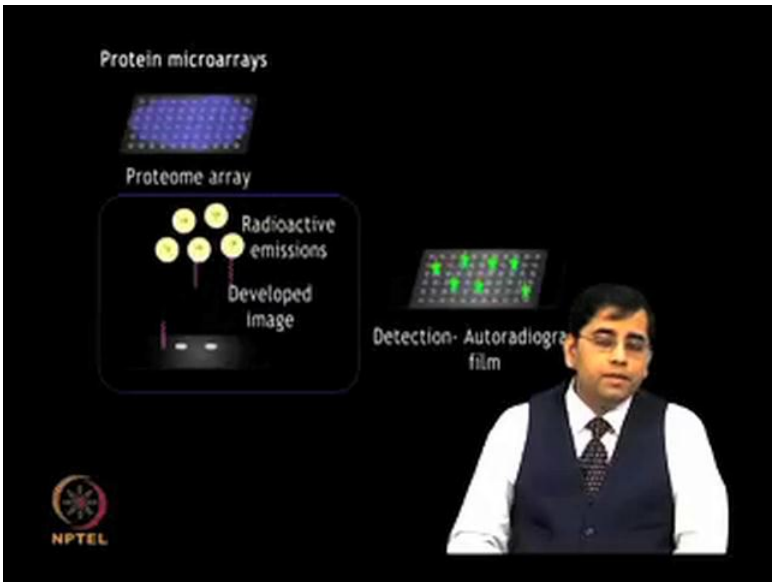

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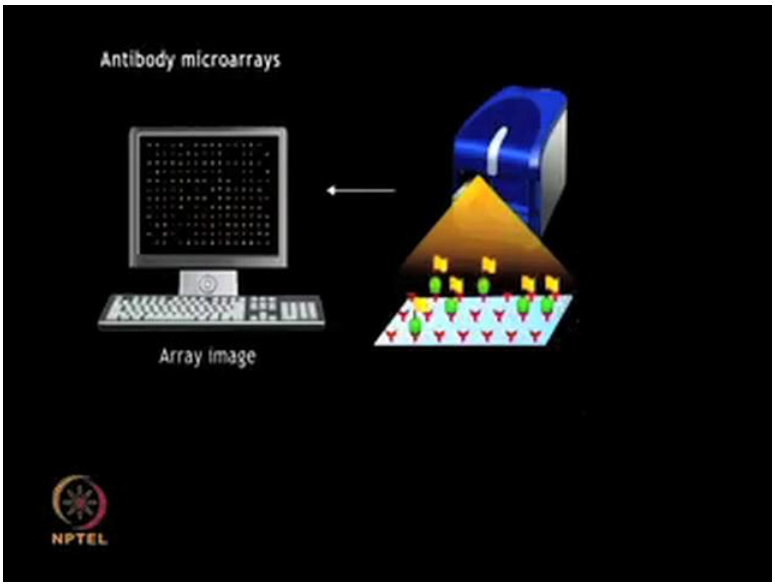

Thus, proteome chip technology offers a useful platform for detection of phosphorylated proteins, antibodies specific to phosphorylated serine, threonine or tyrosine residues as well as motif antibodies can be immobilized onto a suitably coated micro array surface and used for detection of post translational modification. The complex protein mixture containing modified and unmodified proteins is labeled with a suitable fluorescent tag molecule and added to the array surface.

A Specific binding interactions occur between the phosphorylated proteins and their corresponding antibodies, arrays washed to remove any excess unbound proteins from the surface. This is followed by scanning of the array using a microarray scanner at a suitable wavelength do detect the fluorescent tag of bound proteins. This method offers sensitive and simultaneous detection of large number of post translationally modified proteins.

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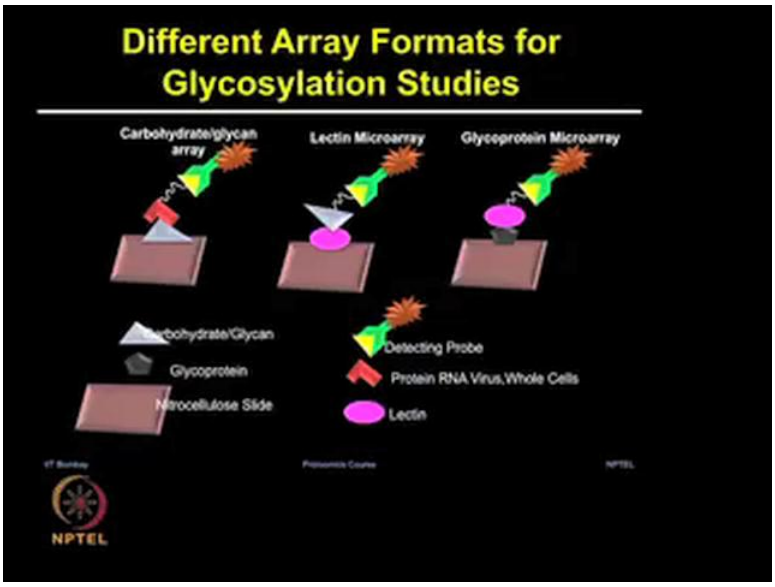

For glycosylation studies different types of micro array platforms such as carbohydrate array, lectin arrays, glycoprotein arrays and other array formats have been used.

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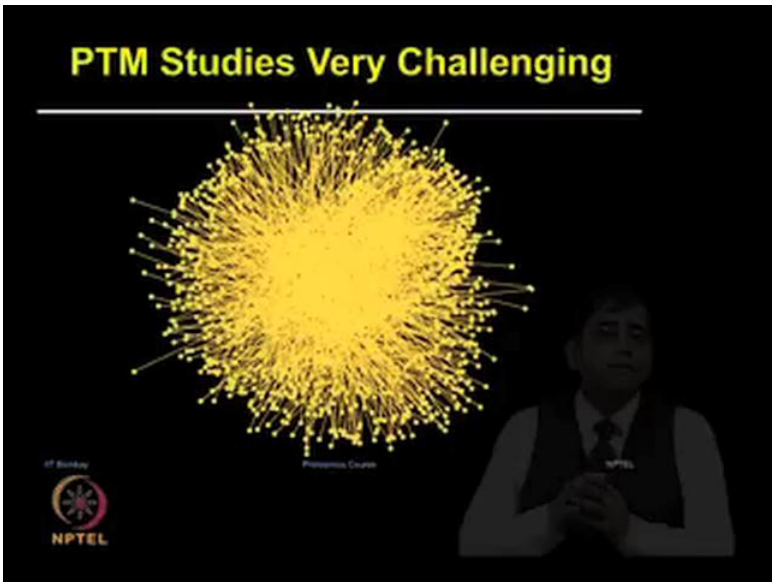

Studying PTM's is still remains challenging many advance proteomic technologies have attempted to bridge this gap. However, no single technique can be solely relied for screening all the PTMs in a given biological question. The genome wide approach to determine and predict the atomic resolution three dimensional structures of protein which is known as structure proteomics aims to provide better understanding of protein structure function relationship, as well as new rational for structural biology. The technical advances in protein structure determination by X-ray crystallography nuclear magnetic resonance NMR spectroscopy imaging technologies and computational methods are very helpful to annotate the structure and biochemical function of protein on a genome-wide scale.

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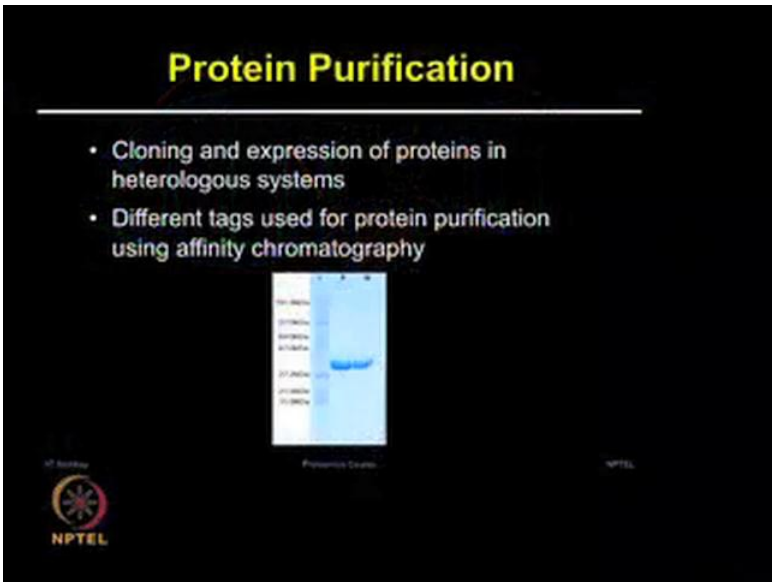

Protein purification is required for the structural analysis, the first step in determining a protein structure using X-ray crystallography involves protein purification. Advances in molecular biology have allowed cloning and expression of proteins in heterologous system for example, E Coli S cerevisiae and various tag such as histidine six tag, glutathione S transferase , GST and maltose binding protein MBP. These tags have been used for the affinity chromatography based protein purification the large scale protein production involve TDS cloning expression and purification steps.

Over expression in host cell encounters several drawbacks such as incorrect folding inappropriate post translational modifications and formation of insoluble aggregates and inclusion bodies. A large number of approaches such as refolding chromatography which chaperones and cell free expression system for protein production have been used to overcome these problems. The X-ray crystallography method provides information on 3D structure of well diffracting protein crystals in a very short time.

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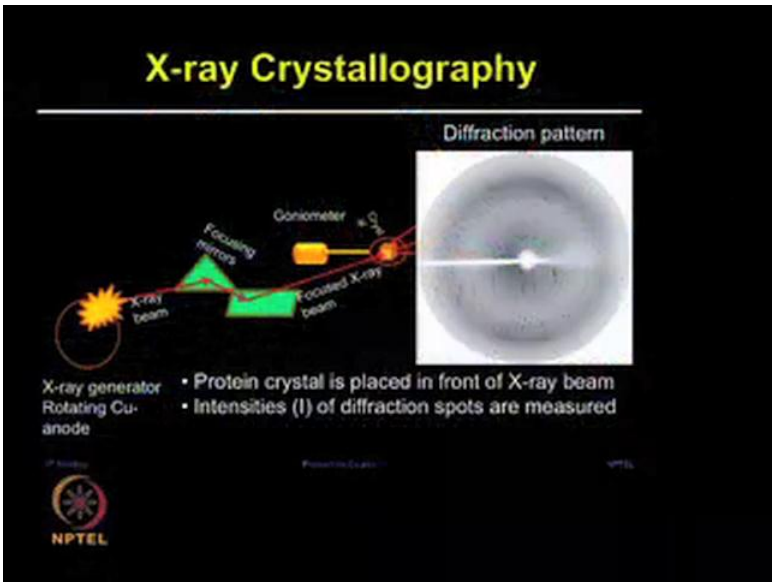

The basic crystallography set up used in XRD is shown here, in this technique the purified protein sample is first crystallized, for crystallization protein solution is mixed with crystallization buffer and crystallization drop is equilibrated with equilibration buffer at constant temperature.

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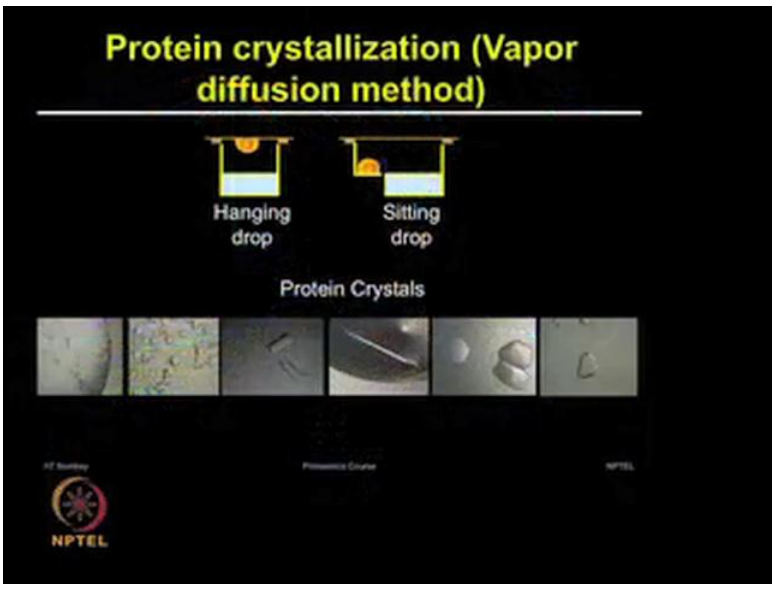

This process takes few days to several weeks the protein crystals are irradiated with x-rays producing diffraction patterns that ultimately, provides amplitudes and position of scattered waves, multiple isomorphous replacement MIR and multiple wavelength

anomalous dispersion MAD methods are primarily used to resolve the structure in form of an electron density map. Although to date the process of protein crystallization has not been amenable to high throughput.

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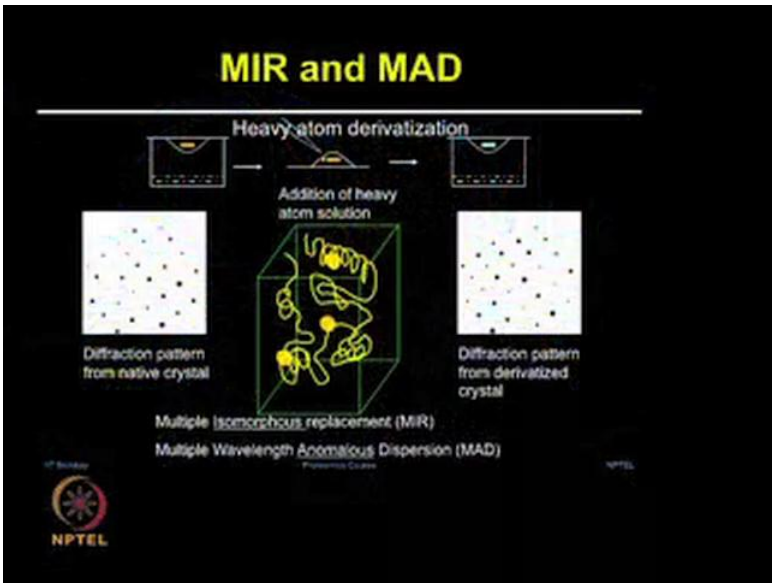

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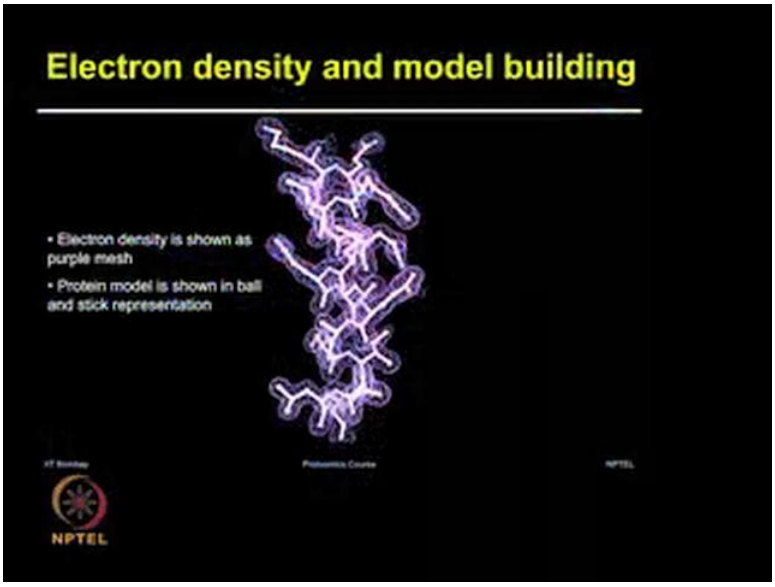

However, this view is changing and evolution of XRD screening for drug discovery is rapidly moving, various proteases have been examined structurally by using XRD the

aspartic proteases or acid proteases primarily, contained beta sheets and utilized two aspartic residues to catalyze proteolysis at low ph.

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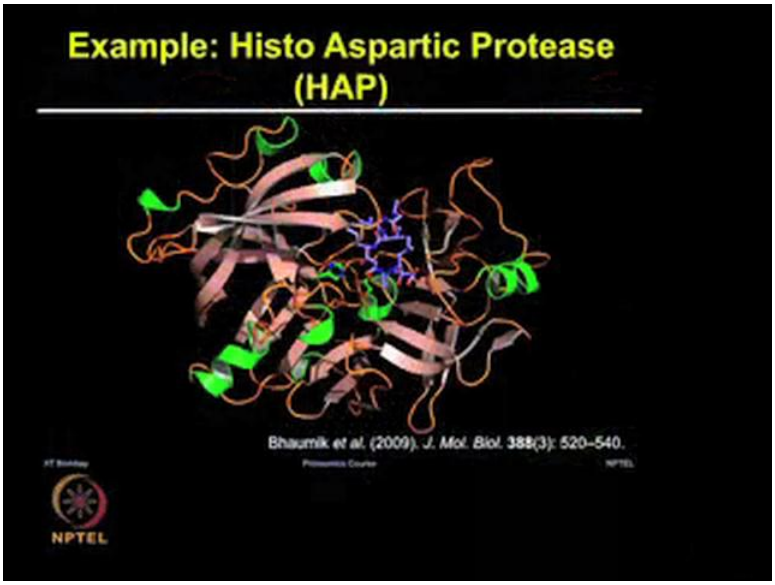

Broadly they are divided into two groups two domain pepsin like proteases and dimeric retroviral proteases, one of the solved structure of histo aspartic protease HAP and HAP pepstatin a complex is shown here. In a structure biology nuclear magnetic resonance NMR spectroscopy is one of the techniques of choice for protein structural determination. This is a useful technique to measure proteins in their native state, characterized protein protein and protein DNA interactions as well as determination of protein dynamics.

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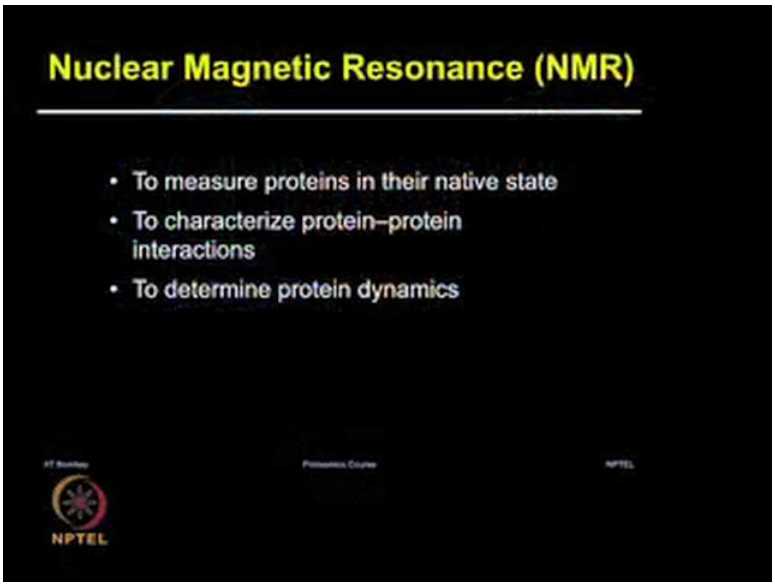

NMR has been employed for the proteomics and initial target selection. Screening by NMR identify lead compounds which are capable to inhibit protein protein interactions, which is a very challenging task in drug discovery. It is able to determine three dimensional structures and employed in enough stream processes of the drug discovery pipelines.

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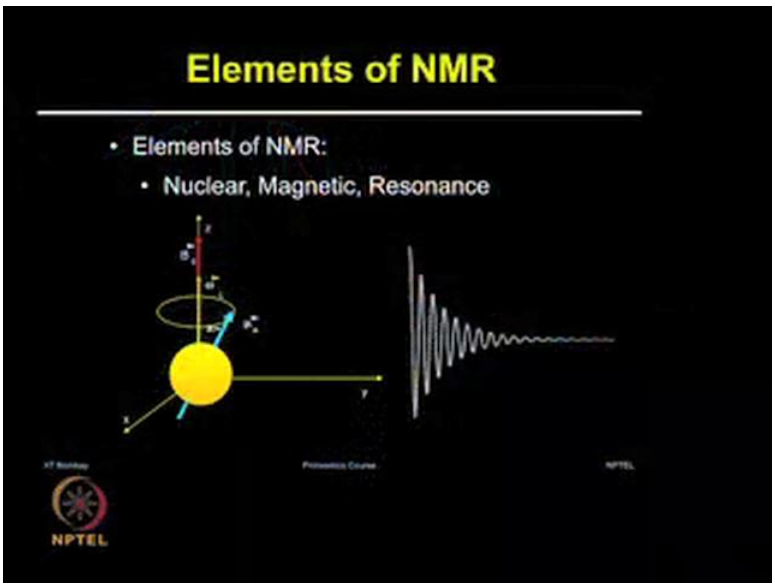

As the name suggests the elements of nuclear magnetic resonance are nuclear the physical phenomenon which involves the nuclei of atoms, magnetic ,effect in a magnetic

field and resonance which is absorption of energy at a defined frequency. The simplest NMR spectrometer can provide a spectra, which is suitable to determine the presence or absence of some functional groups through chemical shift data. It can also provide evidence through coupling constant data and conformational relationships.

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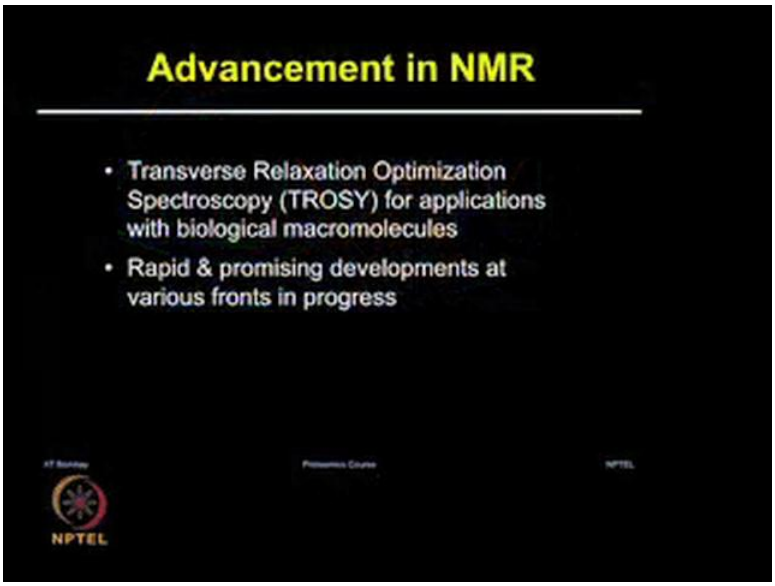

Advancement in NMR techniques such as two dimensional NMR techniques transverse relaxation optimization spectroscopy TROSY for applications with biological macromolecules provides much better sensitivity line width and enables resolving resonance overlaps for larger proteins complexes as well as membrane proteins. NMR field is still evolving rapid and promising developments at various fronts are likely to improve the speed and quality of data and structure determination.

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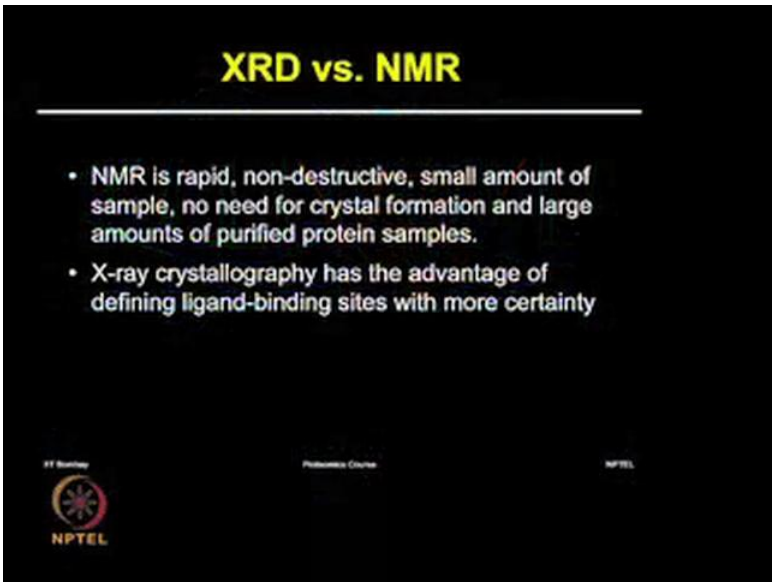

Use of both XRD and NMR method allows to obtain the structural information from a wider range of proteins then either methods alone NMR is rapid non-destructive uses small of amount of sample and does not require TDS sample preparation, such as making crystals and large amounts of purified protein samples. On other hand XRD it has the advantage of defining the ligand binding sites with more certainty, imaging techniques such as electron microscope and electron tomography are also used to obtain crucial information regarding protein subunit shape contacts and proximity.

When the size of protein crystal is insufficient for X-ray diffraction analysis but, they are visible in light microscope at several hundred fold magnification, electron microscopy represents a good alternative to obtain low resolution images.

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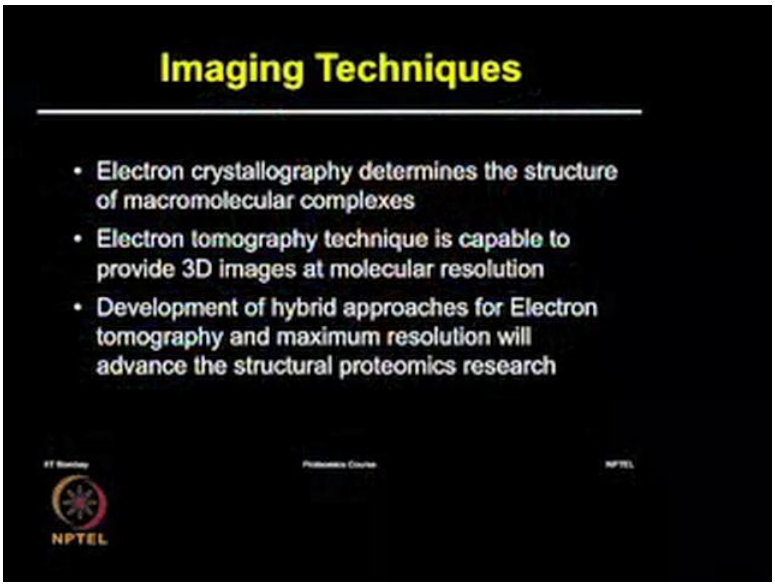

Electron crystallography determines the structure of macromolecular complexes or membrane proteins which are difficult to crystallize in the 2D crystal state by cryo transmission electron microscopy imaging. Although, the resolution obtained from electron crystallography is often lower than X-ray crystallography.

However, it is useful for the analysis of protein structure embedded in a native membrane environment. Electron tomography technique is capable to provide three dimensional images at molecular resolution with best possible preservation of the specimen. Application of electron tomography to obtain three dimensional view of the proteome of single unstained cell in frozen state has been demonstrated. Another means of imaging biological samples with molecular resolution is by using atomic force microscopy or AFM.

The imaging techniques have demonstrated their applications in a structure proteomics and utility as potential alternative of X-ray crystallography but these techniques are time consuming laborious and needs to be automated for high throughput use. Development of hybrid approaches for electron tomography and maximum resolution will advance the structure proteomics research. The complimentary approach to structure proteomics is computational method simulations to predict the structure and biological function of an uncharacterized protein computational method rely on structural homology of unknown protein from proteins with known structure and biological function.

By relying on such methods for structure function correlations it is possible to predict biochemical function of uncharacterized proteins based on structure homology to another protein with a known function. Recent advancement in proteomics and other omic technologies allow large scale analysis of biological samples and generate an unprecedented amount of digital data. In different modules we have discussed different bioinformatic tools and software for analyzing proteome and system level investigation using two dimensional electrophoresis mass spectrometry, microarray and surface plasmon resonance.

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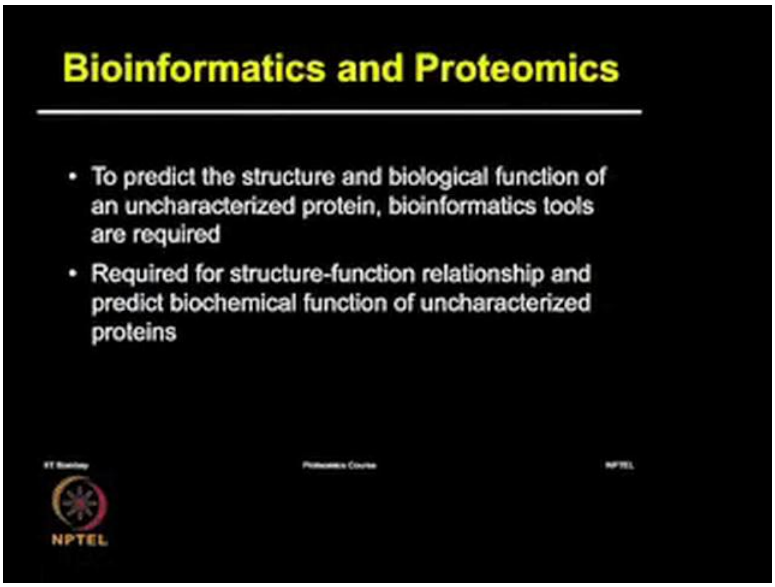

Computational challenges associated with proteomic studies have recently emerged as some of the most critical and limiting factors in this rapidly evolving discipline. Bio informatic tools have been widely used for protein sequence analysis, it is also used for protein motif detection and epitope prediction; active site determination determining transmembrane domains as well as identification of DNA binding residues.

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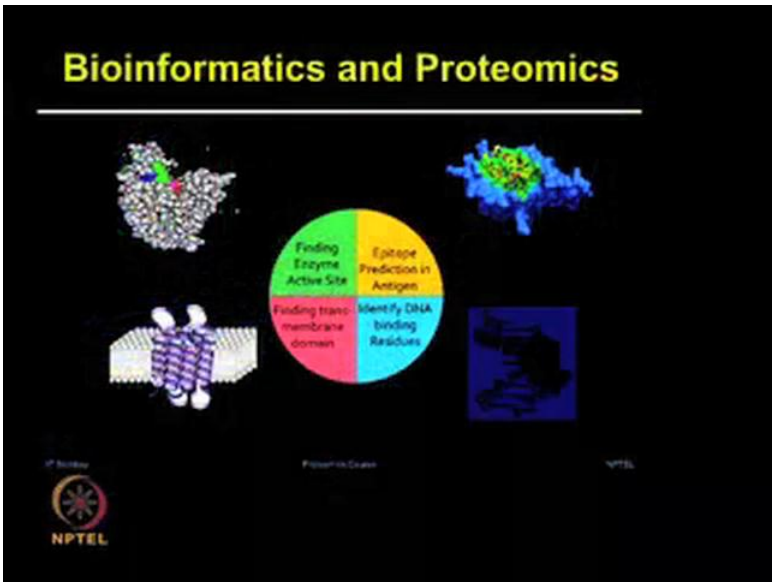

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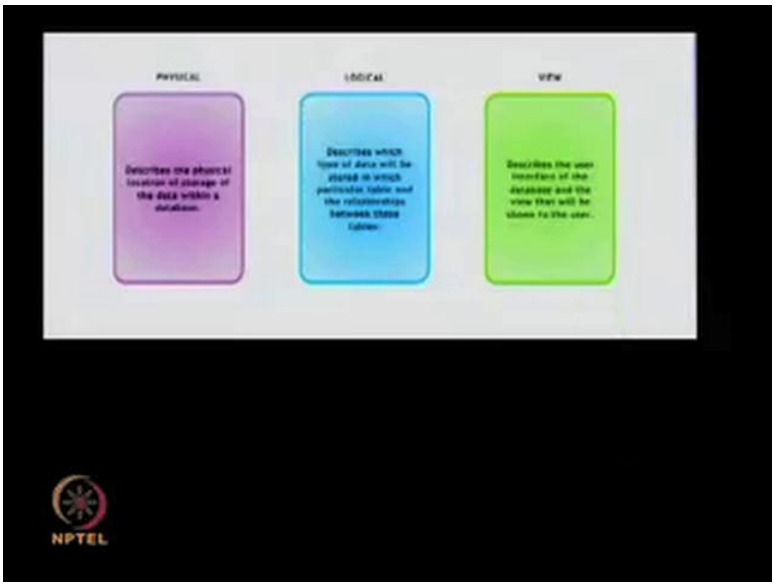

Database designing is done at various levels such as physical logical and conceptual at the physical level the purpose of the database is defined which is in accordance with the proposed usage at the logical level, the tables attribute of the tables and data types are defined at the view level the views and appearances of the databases are defined.

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A typical biological database can be characterized by it is type and it is tool the type defines the category of data that it includes such as sequence domains or structure. This implies that the particular database is most prominent feature includes either sequences, domains or structure and it is particular used for their analysis. The analysis tool defines the platforms that the site will provide for gaining an insight into the protein data.

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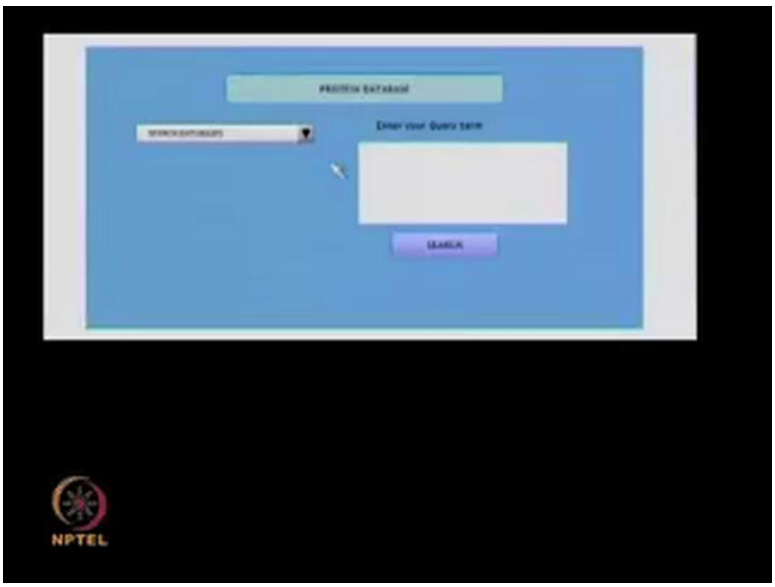

For extracting the protein information from database users can give a variety of input terms, these can be unique ID molecular name amino acid, sequence, keyword, literature gene, taxonomy etcetera.

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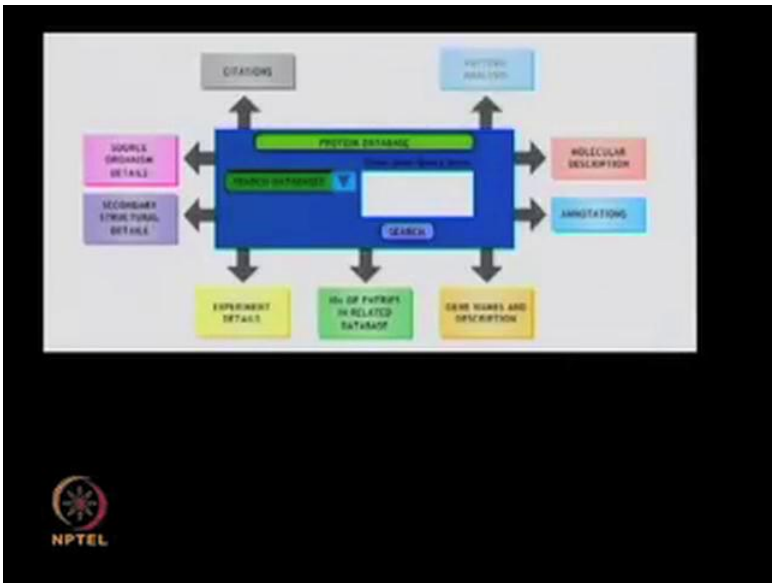

Once the user submits the query the output can be of multiple formats the generalized information that users can obtain from protein databases is general description of the protein molecule. The generalized information that users can obtain from protein databases is general description of the protein molecule annotation of the protein name and description of the gene that transcribe them. ID of the same protein in other relevant databases details of the experiment conducted for characterizing proteins, details of proteins secondary structures, details of the organism which was used as a source for obtaining the protein and citations of research conducted.

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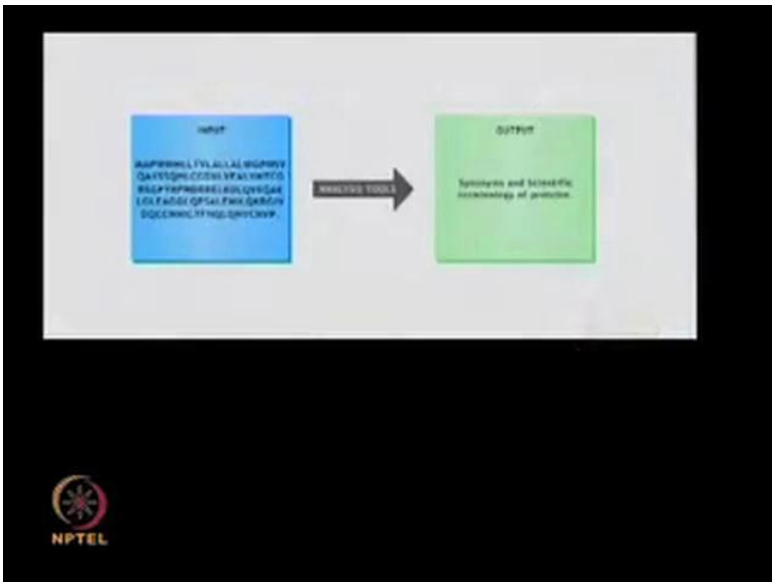

Database analysis tools there are different kinds of analysis can be conducted on a given protein sequence the query can be protein name, sequence or any other identifier of the protein. Various kinds of results output can be obtained identity of protein from sequence, identify physicochemical properties, molecular weight, isoelectric point, sequence tag information. Similarly, search algorithms such as versions of blast fasta and multiple sequence alignment. Finding conserved and variable domains in the protein to study its evolutionary relationship with other proteins molecular modeling and visualization tool; secondary and tertiary structure prediction and structural analysis, biological text analysis such as biomedical acronyms, gene, protein synonyms etcetera.

Database mining in proteomics and visualization tools collective improvement in any research field can be accelerated by sharing scientific data among different research probes across the world. Seeing as it allows other researches to access validate and reanalyze once finding and correlate the results with their own observations.

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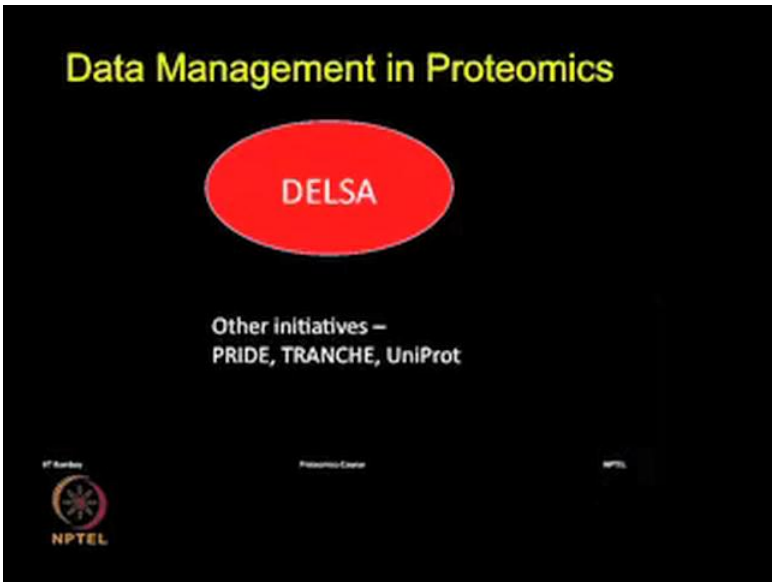

Data management is critical when you using high throughput proteomic techniques several internet databases have been established to collect the proteomic data sets. Data enabled life sciences alliance DELSA is a timely and important initiative to create a common data bank where on one hand we can access the huge data set generated by various research groups, on other hand we can also deposit our data sets which may be useful for a wide range of researches working in similar fields. At present the brought field of DELSA in compasses biological sciences, ecology, environmental sciences, evolution, genomic and proteomics, computer sciences, cyber infrastructure, management , health sciences and policies for global distribution. Let us discuss some of the challenges encountered by various proteomic technologies.

Let us start with gel based proteomics, the gel based technological approaches are routinely used in proteomics research primarily, used for protein separation characterization as well as quantitation. Major challenges associated with gel based proteomics includes poor reproducibility, limited sensitivity and dynamic range of 10 to the power 3 to ten to the power 4 and less coverage of complex proteome, low throughput, biasness in, analysis process, time consuming and highly dependent on performers technical skill.

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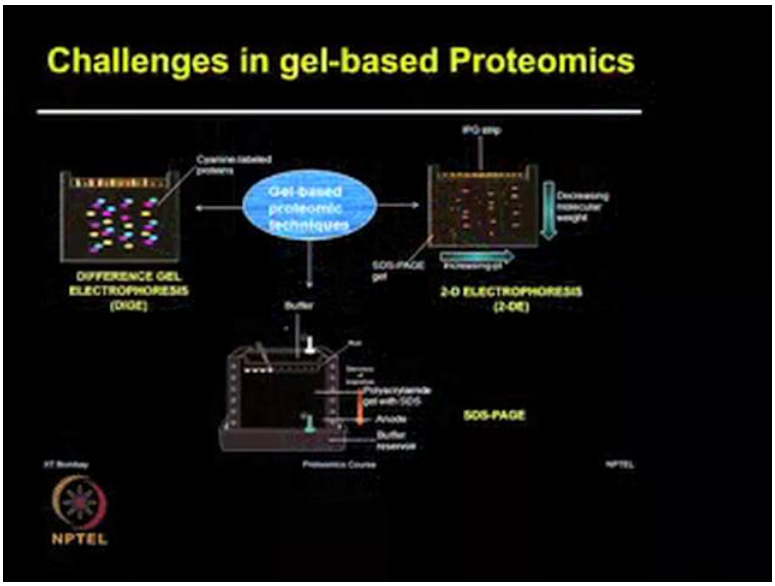

The poor reproducibility of classical 2DE owing to the extensive gel to gel variations has been partially, resolved by the introduction of advanced two dimensional difference in gel electrophoresis. In recent years the detection approach is for gel based proteomic techniques have also improved tremendously to capture the low abundance protein biomarkers in different biological fluids. Apart from the traditional coomassie brilliant blue or silver staining more sensitive and superior staining reagents post electrophoresis, a pico cone fluorescent dyes like lighting fast and deep purple as well as pre electrophoretic fluorescent dyes, such as cyanine dyes have been introduced to increase the dynamic range and coverage in gel-based proteomics.

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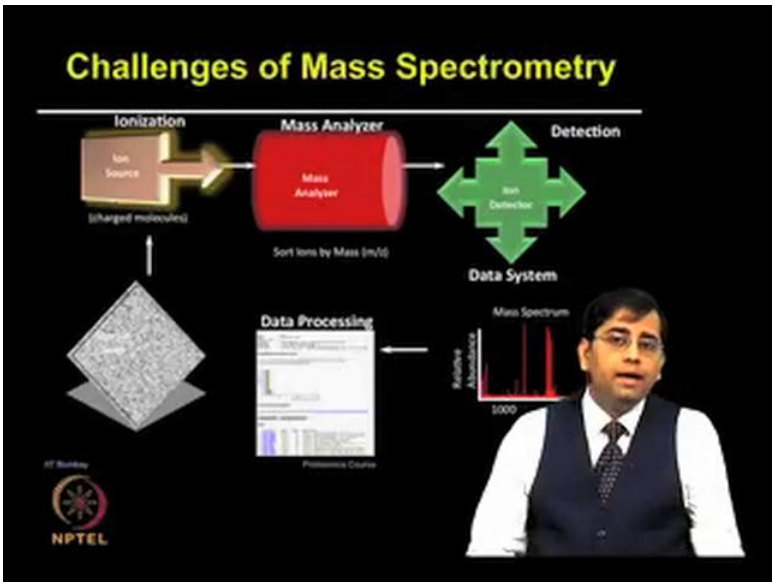

Mass spec based proteomics the MS based proteomics encounters the following biological problems while analysis of huge number of proteins. Fragile nature of proteins substantial losses occurring during the sample collection and processing steps presence of multiple iso forms of single protein the wide dynamic range of protein concentration in biological fluids, presence of high-abundance proteins masking low abundant marker proteins additionally the technological limitations associated with most of the commonly used MS based approaches.

Include typical dynamic range of only 10 to the power 2 to then to the power 4 inadequate coverage of whole proteome unless sample is fractionated intensively, low throughput and issues of robustness and cost or fitting the data machine fluctuation instrument noise and contaminants in a spectrum and lack of standard procedure for analysis and interpretation of MS and MS MS spectrum, to overcome these technological challenges different novel and amalgamated approaches have emerged in last few years.

The most promising advancements include large scale quantitative proteomics culture derived isotope tags and super-silac based technology. Multiplexing tandem mass tags TMTs and itraq 8 plexing quantitative accuracy label-free LC MS MS. Low sample consumption and large scale analysis chip based and nano LC MS sensitive quantitation of proteins within complex pictures biomarker discovery multiple reaction monitoring MR MS large scale biomarker discovery etcetera.

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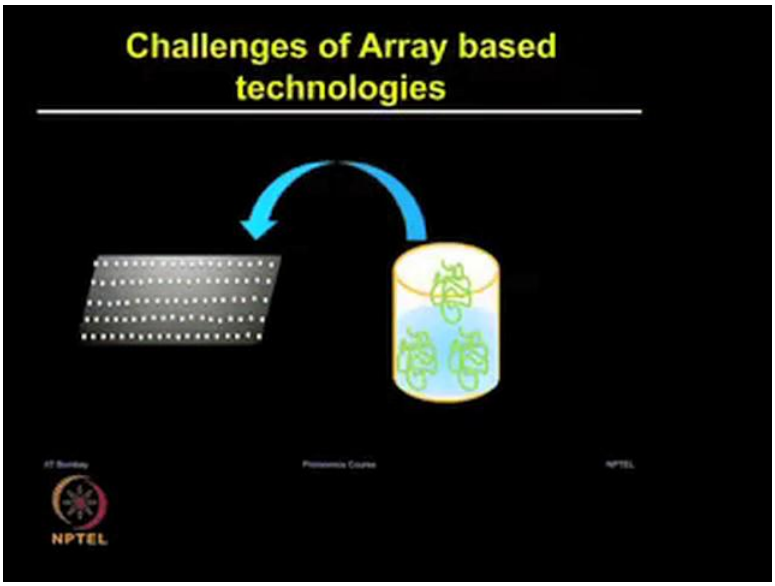

Array-based proteomics, array-based proteomics such as protein and antibody microarrays on which thousands of discrete proteins are printed provide an important platform for large scale functional analysis of the proteome. Although, due to it is high throughput capabilities array-based proteomics have attracted tremendous attention in clinical research. However, it has quite a few technological challenges, the challenges include protein printing acquisition array and a stable attachment of proteins to array surfaces and detection of interacting proteins for biomarkers miniaturization of assays, and protein dehydration, nonspecific binding, unavailability of highly specific antibodies against all the proteins that comprise the complex proteome and lack of direct correlation between protein abundance and activity.

Label-free detection techniques label-free detection approaches surface plasmon resonance, SPRI ellipsometry based and interference based techniques and microcantilevers, which dependent on measurements of an inherent property of the query itself, such as mass and dielectric property are capable of multiplexed detection, which is the central requirement for high throughput proteomics applications particularly, it is relevant for protein antibody microarrays.

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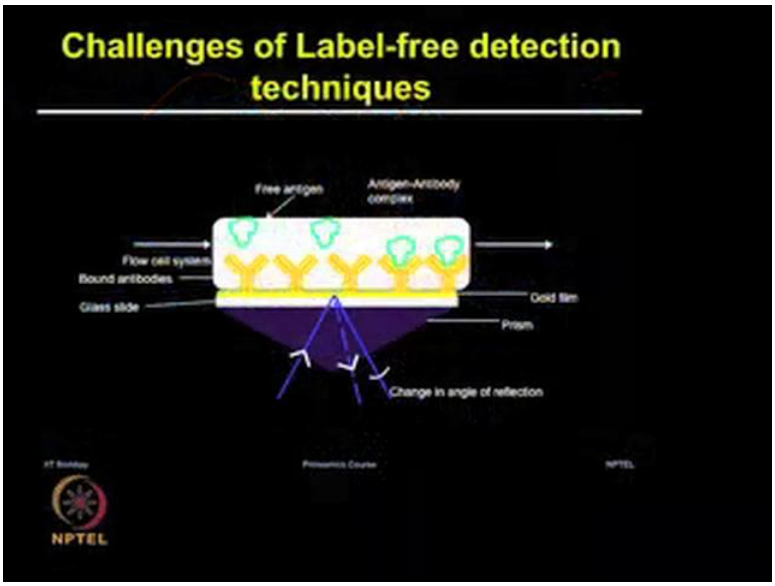

Now-a-days label-free detection techniques are gaining popularity due to their simplicity, real time detection, elimination of the necessity of secondary reactants and lengthy labeling process. Although, label-free detection techniques are very promising and potential candidates for real time measurements of low-abundance analytes and protein-protein interactions issues regarding sensitivity and specificity remains to be addressed in future.

Additionally costly fabrication techniques, morphological anomalies of sample spots and insufficient knowledge regarding the exact working principle of label-free biosensor often restrict, they are use in practical clinical applications. Nanoproteomics in order to circumvent multiple technical limitations associated with sensitivity, dynamic range, detection time and multiplexing. Proteomics has integrated nanotechnological approaches such as, carbon nanotubes and nanowires, silicon nanowire field, effect transistor, quantum dots, gold nanoparticles, nanocages etcetera which has lead to the establishment of a novel analytical platform known as nanoproteomics.

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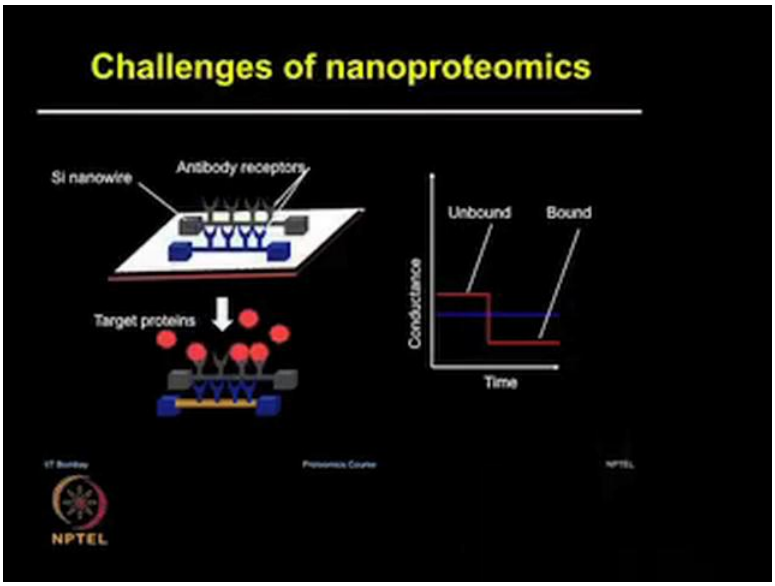

Presently, nano proteomics is at a proof of principle concept level and having following limitations toxicity, biosafety and biocompatibility issues associated with the use of nano structured materials. Inadequate knowledge on precise mechanisms of action for the regularly used nano materials, in solubility in biologically compatible buffers and condition presence of metallic impurities and lack of standard protocols for determining degree of purity of synthesized nano tubes and nano wires. Biomarker discovery detection of low-abundance proteins; biomarkers biomolecules that can be used for early disease detection discrimination between different diseases or different stages or severity of same disease as well as aits in monitoring disease progression.

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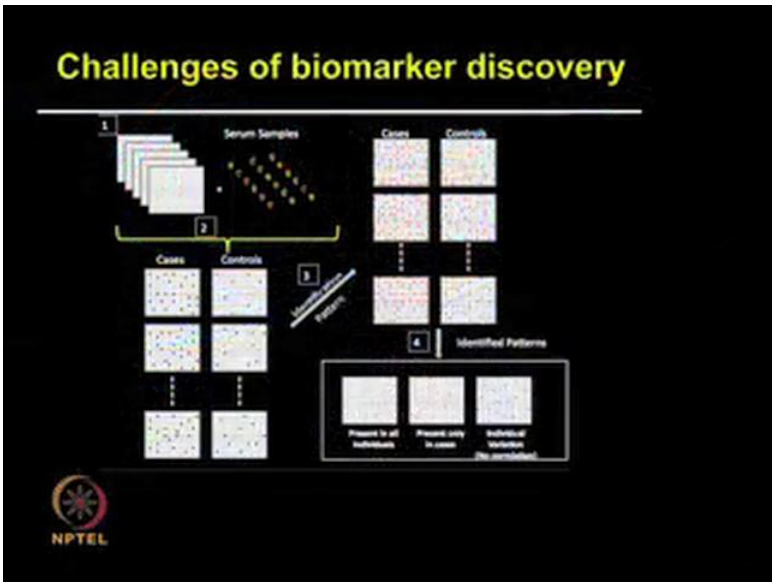

Despite various advancements they still there are multiple biological and technological challenges for the existing proteomic technologies commonly, used in disease biomarker discovery. The formidable limitation include pre-analytical variation at sample collection handling and storage process, complexity of biological samples due to the dynamic range of protein concentration, presence of high-abundance proteins, masking low-abundance marker proteins, presence of high salt level and other interfering components in most of the biological samples insufficient sensitivity of the detection technology and lack of throughput and multiplexed detection ability, to overcome the basic technological limitations a combination of separation detection and labeling strategies such as a strong caution ion exchange for separation icat itraq TMT etcetera.

For labeling, nanoparticles like nano wires, nano tubes and quantum dots, single amplificat ion and enrichment of low-abundance proteins have evolve to effectively enrich this discipline and provides an attractive opportunity for sensitive multiplexed detection of low-abundance disease specific protein biomarkers.

How to convert your proteomic discovery into products services and business ventures the practical insights and experiences from practicing science entrepreneurs let discuss with a colleague and shown in following video clip. So, you have an invention in sometimes, we scientist think that you know that is the most of important part that actually let me, just talk either ideas have diamond. I show already know there is many

ways of measuring bio molecular interaction. So, in the an it is all about execution can you actually get it into something that will become a product that we will sell because, So, let us talk a little bit about how science becomes a product basically, we said that science is not the famous technology and it is not the famous as a product and we talked about what are the differences, and the typical pitfall the we scientist have is to do what is called technology push. I invented this device the world is dying to have this device or sometime they say if you build it they will come actually, that is not really true right, because people do not really know what is the best thing out there.

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Until already available how they are going to know like what you have in your mind and what your about. But even if you already have the device it is really trying to understand that is world really feel like they need it and would like to they needed enough to put money to buy it and that is called the market pull. What does the market really need? That is right. Sure. Yes

So, in my diffraction sensor example, we have that in my lab and I said I am, I mean instrument developer. We build instruments and I have never done anything commercial before, because I feel like well I do not know they does does everybody just make one of this it was not until a colleague challenge me and say like look if you have that I will use it that I really realize oh there is a need for it.

But, now science does not become a product fast in this commonly or just get yourself an exercise when you have a new science development how long is it to take to get it to the market and you are may be do it the other way around look at anything around you and try to see when was the revolutionary science developed and what you find is probably, we will talking fifty years and that is so long time.

So, why is it long time well, it is because there was probably the many issues but, part of it is the there is nobody pushing to create the product. Yes fundamental science was published but, based on it there is need when people actually realized and then push it to developing product it took a long time right, so many time.

Well in but, we do not speak the same language the person making products and person writing the papers do not even see eye to eye and in fact, for many for the first part of my career I was a basic scientist, but I wanted to help company so, I would go to companies and say yes well, I am a surface comes I know how to solve the that is a simple problem. It is just in my paper but, nobody reads my papers. So, it is like that you the scientist who was interested in creating a company is the best conjugate to think about your science, look at the market and then see how you create that product. So, that is really is I mean understanding if you understand that knowledge benefit society only if you have a tangible object or process or something.

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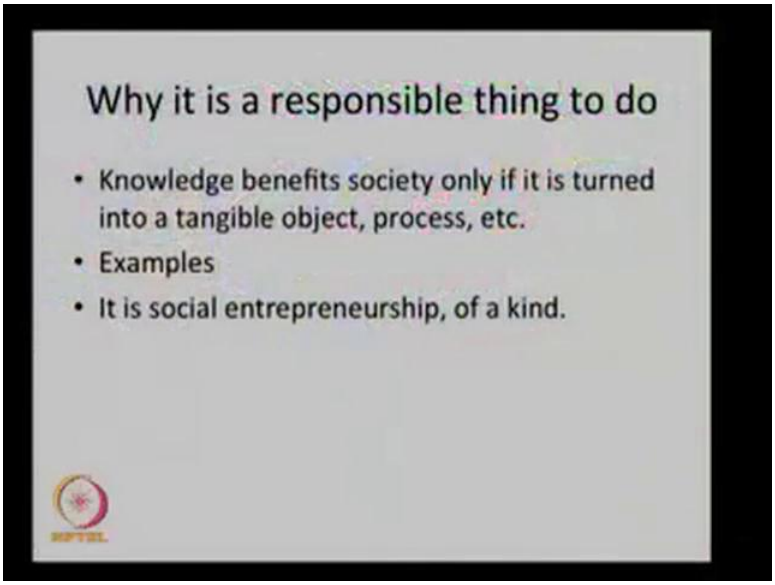

So, what will be your message to the students who are just beginner in the innovative in the lab.

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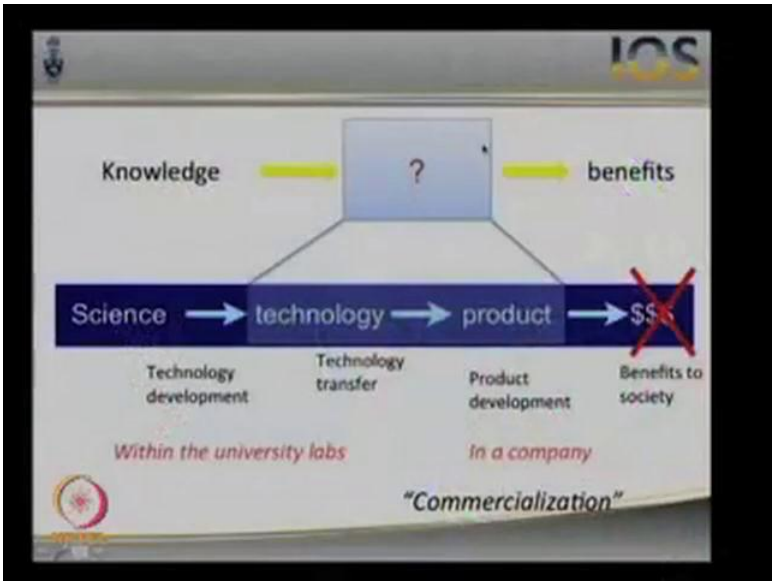

Well So, there is a lot of talk about invention likely, invention is about knowledge becoming benefits to society, for us scientist this path is I think clear because nobody talks about the path, but in my mind the path is clear we are all in the left hand here as engaging a knowledge generating this basic science, benefits to society is on the right hand side. So, the question is how do you get from science to benefits to society and may as I said you turn it first to technology you turn it then into product that benefits to society to turn science to technology is really gap in there.

And I think that is best done within universities within the basic researchers because we are the once who know best what the science is. So, hopefully in even if you are wanting to become a professor instead of founding a company being aware of the fact that there is this translation process and being aware of the needs of the market well actually, move this thing closer because we would like to make sure that instruments get there faster. So, if you just think for example, that MRI which is a very useful instruments magnetic resonance. The concept has been around since, 1930 the first NMR was in 1930 the MRI went to the market in 1986 that is you know 50 years.

If you can reduce that by 5 years that 5 years less of suffering is actually, be a good thing. So, we should all do this translation part. So, I hope with the lady as probably now,

we are in the year of omic research where lot of things are happening in high throughput level and transforming some of these technologies sooner in the market can actually revolutionize lot of fundamental issue. Yes that is right. Well, and it is you are facilitating researcher or you are going directly to the public

Question really is to keep your eyes open to say what is needed by somebody else and then you can ask the question now, how can I get what I know to what is needed? Sure. So, thank you very much Cynthia for very stimulating discussion and we got perspective of a scientist and entrepreneur, thank you very much.

During the last decade this emerging field has propelled its growth eventually, in every aspect of modern biological research. Impending future of this promising research area will highly depend on the collaborative initiatives at global level and establishment of effective data repositories accessible to the proteomics researches across the world.

In 2010 human proteome organization hupo has launched a global human proteome project HPP this project is design to map the entire human proteins encoded by the genome. Let us now, discuss some of the targeted focused initiatives the human liver proteome project this is the first initiative of human proteome project for human organ, tissues with an intention of generation of comprehensive protein atlas of the liver and international liver tissue network. Collection and distribution of normal liver sample and validation of new discoveries, human plasma proteome project analysis of the protein constituents of human plasma and serum; human brain proteome project BPP focuses on the revolution of the brain related proteomic alteration focusing on understanding neurodegenerative disease aging and identification of prognostic and diagnostic biomarkers.

Human kidney and urine proteome project aims to understand kidney function mechanism of chronic kidney disease at a protein level and discover biomarkers and target molecules for new therapeutics of kidney disease.

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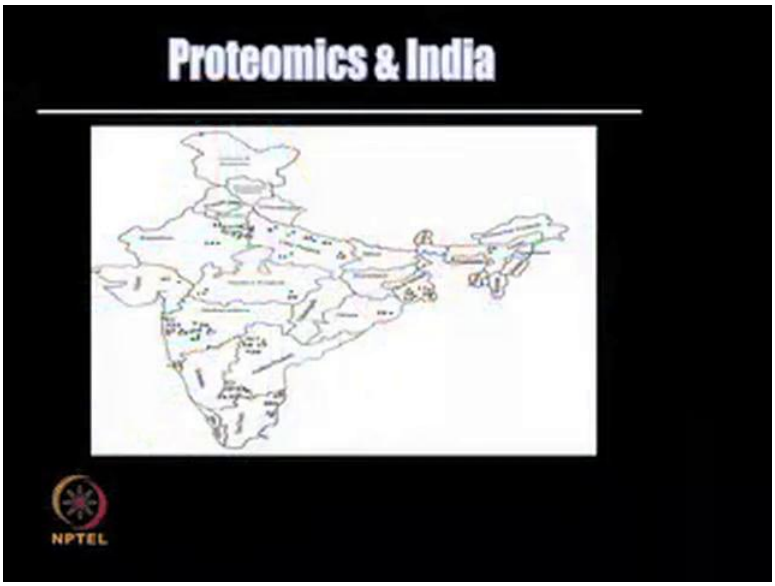

Over the last decade proteomics research is progressing in different regions of India with a considerable interest. India is playing an increasingly significant role in global genomics and proteomics research and development. As it is evident from research publications and patents, Indian government is also supporting the basic and applied proteomics as well as other omics based research and multiple national and international funding agencies are providing investments on existing and new research projects considering this space of emerging proteome level research.

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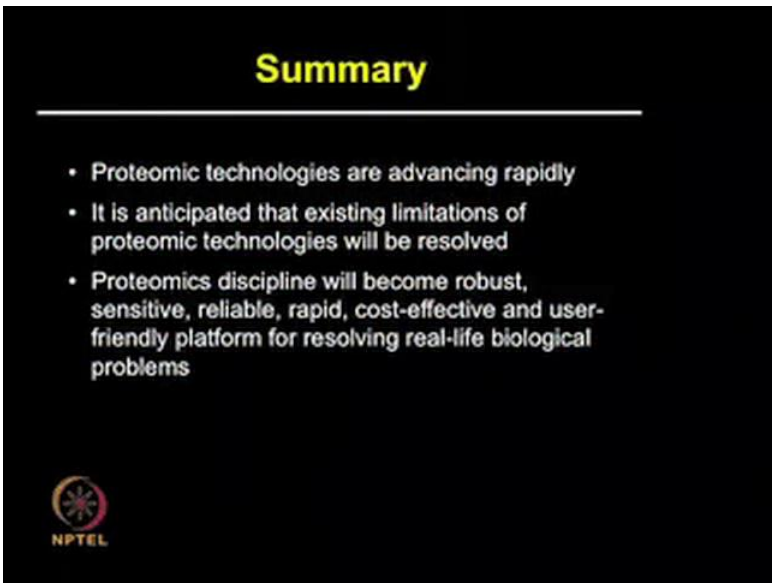

It can be anticipated that in coming future some amicable solution for the existing limitation associated the bergionic field of proteomics will come forward through worldwide research initiatives and this discipline will become more robust, sensitive, reliable, rapid, cost-effective and user friendly for resolving real-life biological problems. Hope this course has given you foundation for proteomic concepts and enthused you for research in proteomics area thank you.