LECTURE-40

PROTEOMICS: ADVANCES AND CHALLENGES

TRANSCRIPT

In today's lecture we will talk about post-translational modifications, structural proteomics, role of bioinformatics, challenges and future directions of proteomics.

Let's start with PTM. PTMs are vital cellular control mechanism, 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.

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

There are several types of post-translational modifications that can take place at different amino acid residues. The most commonly observed PTMs include phosphorylation, glycosylation, methylation as well as hydroxylation and acylation. Many of these modifications, particularly phosphorylation, serve as regulatory mechanisms for protein action.

PTMs generate tremendous diversity and are extremely important. Many documented effects of PTMs include – change in enzymatic activity, ability to interact with other proteins, sub-cellular localization and targeted degradation

The final structure of functional proteins most often does not correlate directly with the corresponding gene sequence. This is due to the PTMs 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. This further increases the complexity of the proteome as compared to the genome.

Protein phosphorylation acts as a switch to turn on and off protein activity and governs wide range of polypeptides, from transcription factors, enzymes to cell-surface receptors. The reversible phosphorylation of proteins catalyzed by kinases and phosphatases regulates important cellular functions.

Phosphorylation of amino acid residues is carried out by a class of enzymes known as kinases that most commonly modify side chains of amino acids containing a hydroxyl group. Phosphorylation requires the presence of a phosphate donor molecule such as ATP, GTP or other phoshorylated substrates. Serine is the most commonly phosphorylated residue followed by threonine and tyrosine. Removal of phosphate groups is carried out by the phosphatase enzyme and it forms one of the most important mechanisms for regulation of proteins.

Glycosylation involves linking saccharides to proteins in presence of glycosyl transferases enzymes, giving rise to a glycoprotein. Glycosylation play vital role in various biological functions such as antigenicty of immunological molecules, cell division, protein targeting stability and interactions. Aberrant glycosylation forms result into various human congenital disorders. Depending on the linkage between the amino acid and the sugar moiety, there are 4 types of glycosylations; N-linked glycosylation, O-linked glycosylation, C-mannosylation and Glycophosphatidlyinositol 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 aspargine and O-linked glycosylation, which links the sugar moieties to the hydroxyl groups of serine or threonine. Suitable glycosyl transferase enzymes catalyze these reactions. Sugar residues that are attached most

commonly include galactose, mannose, glucose, N-acetylglucosamine, N-acetylgalactosamie as well as fucose.

There is a growing interest in proteomic community to decipher the role of PTMs in various biological contexts. Detection of subtle PTM changes; pose challenge to even advance proteomic techniques. There are many approaches ranging from gel-based techniques, mass spectrometry, microarrays that are currently used to study PTMs. We will discuss some of these techniques one by one.

Protein phosphorylation can be detected using gel-based detection technique. Proteins separated on a 2-DE gel are placed in a fixing solution containing methanol and acetic acid which fixes the protein bands on to the gel and minimizes any diffusion. They are stained using the Pro-Q-diamond staining solution, which selectively stains only phosphoproteins on the gel. The excess stain is washed off with a solution of methanol and acetic acid. The stained gel is scanned at its excitation wavelength using a gel scanner. The gel image obtained shows the protein bands corresponding to only the phosphoproteins present. This image is saved and the gel is removed from the scanner for treatment with the second stain, a procedure known as dual staining. The scanned gel is removed from the scanner and placed in SYPRO-Ruby Red fluorescent dye solution. This dye stains all the protein spots present on the gel thereby providing a total protein image with sensitivity down to nanogram level. Excess dye is then washed off using a solution of methanol and acetic acid. The gel stained with SYPRO-Ruby Red is scanned in the gel scanner at its excitation maxima. The image produced will have more number of spots since all proteins present on the gel are detected. This dual staining procedure provides a useful comparative profile of the phosphoproteins and the total proteins on the gel, which enables detection of the phosphorylated proteins.

Protein phosphorylation is widely detected using immunological or enzymatic techniques. Protein mixture containing phosphorylated as well as other unmodified proteins 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 for further analysis. The separated protein bands are blotted onto a nitrocellulose membrane. These membranes are then probed either by means of specific anti-phospho-amino acid antibodies or more recently, by motif antibodies that specifically bind to proteins having phosphorylation at a particular amino acid residue. This binding interaction can be detected by means of suitably labeled secondary antibodies or by autoradiography using a radioactive probe. Thus, the use of immunoblotting technique has been shown to be extremely effective for detection of PTMs.

The gel-based approaches are convenient to use, it has a few drawbacks with regards to limit of detection, membrane proteins, localization of modification sites, robustness, sensitivity and gel-to-gel reproducibility. Characterization of these resolved proteins subsequently requires other technique such as mass spectrometry to identify the proteins.

The shotgun based MS methods have accelerated identification of proteins and PTMs from complex mixtures. Protein mixture is digested with proteolytic enzyme such as trypsin, and resultant peptides are analyzed by MALDI-TOF or LC-MS/MS. Top down mass spectrometry involves analysis of intact proteins using high-resolution MS techniques. High-resolution MS platforms such as FTICR-MS, Orbitrap-MS with PTM friendly dissociation techniques such as Electron capture dissociation (ECD) 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 seine and threonine residues. The modified protein of interest is digested into smaller peptide fragments using a suitable enzyme like trypsin. This digest is then mixed with a suitable organic matrix such as a-cyano-4-hydroxycinnamic acid, sinapinic acid etc. and then spotted on to a MALDI plate.

The target plate containing the 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 flight tube at different rates. The lighter ions move rapidly and reach the detector first while the heavier ions migrate slowly. The ions are resolved and detected on the basis of their m/z ratios and a mass spectrum is generated.

Identification of PTMs by MS largely lies in the interpretation of results. Comparison of the list of observed peptide masses from the spectrum generated with the expected peptide masses enables identification of those peptide fragments that contain any PTM due to the added mass of a modifying group. In this hypothetical example, two peptide fragments are found to have different m/z values, differing by 80 daltons and 160 daltons. It is known that the added mass of a phosphate group causes an increase in m/z of 80 daltons. Therefore, this principle of mass difference enables detection of modified fragments.

Affinity based enrichments, immunopurification and metal affinity chromatography are commonly employed for the purification of proteins containing specific PTM. Immobilized metal affinity chromatography (IMAC) and metal oxide affinity resins such as TiO_2, Fe_3O_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 will interact specifically with proteins having the PTM of interest. Immobilized metal affinity chromatography columns containing ions such as Ga^{3+}, Zn^{2+}, Fe^{3+} or TiO_2 specifically chelate the phosphorylated proteins. Unwanted proteins are removed by washing the column with a suitable buffer solution after which the phosphorylated protein of interest is eluted out by modifying the buffer solution.

The protein purified by liquid chromatography is then subjected to tryptic digestion followed by analysis using tandem mass spectrometry. Here we demonstrate the use of MALDI-TOF-TOF-MS for resolution of the generated ion fragments. Separation is based on the flight time of the ions and greater resolution 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/z values.

Metabolic labeling methods such as SILAC is used for label-based quantification of PTMs. However, this strategy can only be used for the living cells. Other chemical labeling techniques such as iTRAQ is also used for PTM quantification. Another technique, protein microarray, is one of the versatile platforms for HT screening of PTMs. Kinases have been used in a 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 suitably coated array surface. To this, kinase enzyme and gamma P-32 labeled ATP are then added and the array is incubated at 30oC. 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 wherein a photographic film is placed in contact with the array surface. The radioactive emissions from the phosphate label present at the phosphorylated protein sites strike the film. Upon development, the positions at which phosphorylation has occurred can be clearly determined. Thus proteome chip technology offers a useful platform for detection of phosphporylated proteins.

Antibodies specific to phosphorylated serine, threonine or tyrosine residues as well as motif antibodies can be immobilized on to a suitably coated microarray surface and used for detection of PTM. The complex protein mixture containing modified and unmodified proteins is labeled with a suitable fluorescent tag molecule and added to the array surface. Specific binding interactions occur between the phosphorylated proteins and their corresponding antibodies.

The array is then 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 to detect the fluorescent tag of the bound proteins. This method offers sensitive and simultaneous detection of large number of post translationally modified proteins.

For glycosylation studies different types of microarray platforms such as carbohydrate array, lectin arrays, glycoprotein arrays and other array formats have been used. Studying PTMs still remains challenging. Mny advanced 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.

Structural Proteomics

The genome-wide approach to determine and predict the atomic resolution 3D structures of protein, which is known as "structural proteomics", aims to provide better understanding of protein structure-function relationship and new rationale for structural biology. 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 proteins on a genome-wide scale. Protein purification for structural analysis. The first step in determining a protein structure using x-ray crystallography involves protein purification. Advancement in molecular biology have allowed cloning and expression of proteins in heterologous systems (e.g. *E. coli*, *S. cerevisiae*) and various tags [His6-tag, glutathione-S-transferase (GST) and maltose binding protein (MBP)] have been used for the affinity chromatography based protein purification. The large-scale protein production involves tedious cloning, expression and purification steps. Over-expression in host cells encounters several drawbacks such as incorrect folding, inappropriate PTMs and formation of insoluble aggregates and inclusion bodies. A number of approaches such as Refolding chromatography with chaperones and cell-free expression system for protein production have been used to overcome these problems.

X-ray crystallography based on traditional diffractometer or synchrotron radiation presents one of the most powerful technique for protein structure determination at atomic resolution. X-ray crystallography method provides information on 3D structure of well diffracting protein crystals in a very short time. The basic crystallography set-up used in X-ray diffraction 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 the crystallization buffer at constant temperature. This process takes few days to several weeks. The protein crystals are irradiated with x-rays producing diffraction patterns that ultimately provides amplitudes and positions of scattered waves.

Multiple Isomorphous Replacement (MIR) and Multi-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 HT, this view is changing and evolution of X-ray crystallographic screening for drug discovery is moving rapidly. Various proteases have been examined structurally. The aspartic proteases or acid proteases primarily contain β-sheets and utilize two Asp residues to catalyze proteolysis at low pH. 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.

Nuclear Magnetic Resonance (NMR) spectroscopy is one of the techniques of choice for protein structure determination. This is a useful technique to measure proteins in their native state, characterize protein–protein and protein-DNA interactions, as well as determination of protein dynamics. NMR has been employed for the proteomics and initial target selection. Screenings by NMR identify lead compounds, which are capable to inhibit protein–protein interactions, a very challenging task in drug discovery. It is able to determine 3D structures, and employed in upstream processes of the drug discovery pipelines.

As the name suggests, the elements of Nuclear Magnetic Resonance are: nuclear (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 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.

Advancement in NMR techniques such as Two-dimensional (2D) NMR techniques, Transverse Relaxation Optimization Spectroscopy (TROSY) for applications with biological macromolecules, provides much better sensitivity, line width, and enables resolving resonance overlap for larger proteins, complexes, and membrane proteins. NMR field is still evolving. Rapid & promising developments at various fronts are likely to improve the speed and quality of data and structure determination. Use of both X ray crystallography and NMR method allows to obtain structural information from a wider range of proteins than either method alone. NMR is rapid, non-destructive, uses small amount of sample and does not requires tedious sample preparation such as making crystals and large amounts of purified protein samples. On other hand, X-ray crystallography has the advantage of defining ligand-binding sites with more certainty.

Imaging technologies such as electron microscopy (EM), and electron tomography (ET) are also used to obtain crucial information regarding protein subunit shape, contacts and proximity. When the size of protein crystal is insufficient for the X-ray diffraction analysis but they are visible in light microscope at several hundredfold magnification, EM represents a good alternative to obtain low-resolution images. Electron crystallography determines the structure of macromolecular complexes or membrane proteins, which are difficult to crystallize, in the 2D crystal state by cryo-transmission EM imaging. Although the resolution obtained from electron crystallography is often lower than X-ray crystallography, it is useful for the analyses of protein structure embedded in a native membrane environment.

Electron tomography technique is capable to provide 3D images at molecular resolution with best possible preservation of the specimen. Application of ET to obtain 3D view of the proteome of single, unstained cells in frozen state has been demonstrated. Another means of imaging biological samples with molecular resolution is by atomic force microscopy (AFM). The imaging techniques have demonstrated their application in structural proteomics and utility as potential alternative of X-ray crystallography but these techniques are time consuming, laborious and needs to be automated for HT use. Development of hybrid approaches for Electron tomography and maximum resolution will advance the structural proteomics research.

Bioinformatics and Proteomics

Complementary approach to structural proteomics is computational method simulations. To predict the structure and biological function of an uncharacterized protein, computational methods 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 structural homology to another protein with a known function

Recent advancement in proteomic and other omics technologies allow large-scale analysis of biological samples, and generate an unprecedented amount of digital data.

In different modules we have discussed different bioinformatics tools and software for analyzing proteome and systems level investigations using 2DE, MS, Microarrays and SPR.

Computational challenges associated with proteomic studies have recently emerged as some of the most critical and limiting factors in this rapidly evolving discipline.

Bioinformatics tools have been widely used for protein sequence analysis. It is also used for protein motif detection and Epitope Prediction, Active site determination, Determination of trans-membrane domains and Identification of DNA binding residues.

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 prospected usage. At the logical level, the tables, attributes of the tables and data-types are defined. At the View level the views and appearance of the database are defined. A typical biological database can be characterized by its "Type" and its "Tools". The "Type" defines the category of data that it includes, such as sequence, domains or structure. This implies that the particular database's most prominent feature includes either sequences, domains or structure and it is primarily used for their analysis. The analysis tools defines the platforms that the site will provide for gaining an insight into the protein data. For extracting the protein information from a database, users can give a variety of input terms. These can be: Unique ID, Molecular Name, Amino-acid sequence, Keyword, Literature, Gene, Taxonomy. 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, annotations of the protein, name and description of the gene that transcribes them, ID of the same protein in other relevant databases, Details of the experiment conducted for characterizing proteins, Details of the Protein's secondary structure, Details of the organism which was used as a source for obtaining the protein and Citations of research conducted to obtain this protein

Database analysis tools - different kinds of analysis can be conducted on a given protein sequence. The query can be the protein name, sequence or any other identifier of the protein. Various kinds of results output can be obtained. Identify protein from sequence, Identify physico-chemical properties, molecular weight, iso-electric point, sequence tag information; Similarity search algorithms such as versions of BLAST, FASTA and Multiple Sequence Alignment; Finding conserved and variable domains in the protein to study its evolutionary relationships with other; Molecular modeling and visualization tool, Secondary and tertiary structure prediction and structure analysis, Biological text analysis such as bio-medical acronyms, gene-protein synonyms

Database Mining in Proteomics and Visualization Tools - Collective improvement in any research fields can be accelerated by sharing scientific data among different research groups across the world, seeing as it allows other researchers to access, validate and reanalyze one's findings and correlate the results with their own observations. Data management is critical when using high-throughput proteomic techniques. Several Internet databases have been established to collect the proteomic datasets.

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 the various research groups worldwide, on the other hand we can also deposit our datasets, which may be useful for a broad range of researchers working in similar fields. Presently, the broad field of DELSA encompasses biological sciences, ecology, environmental sciences, evolution, genomics, computer sciences, cyber-infrastructure, management, health sciences, and policies for global distribution.

Challenges of Proteomics

Let's discuss some of the challenges of proteomic technologies and current status of this field.

Gel-based proteomics: Gel-based technological approaches are routinely used in proteomics research used for protein separation, characterization as well as quantification. Major challenges associated with gel-based proteomics includes; poor reproducibility, limited sensitivity and dynamic range (10^3-10^4), less coverage of complex proteome, low-throughput, biasness in analysis process, time consuming and highly dependent on performer's technical skill.

The poor reproducibility of classical 2DE owing to the extensive gel-to-gel variations has been partially resolved by the introduction of DIGE. In recent years, the detection approaches for gel-based proteomic techniques have also improved tremendously to capture low-abundance protein biomarkers in different biological fluids. Apart from the traditional CBB or silver staining, more sensitive and superior staining agents reagents

(post-electrophoresis Epicoccone fluorescent dyes like Lightning Fast and Deep purple as well as pre-electrophoresis fluorescent dyes such as CyDyes have been introduced to increase the dynamic range and coverage in gel-based proteomics.

MS-based proteomics: MS-based proteomics encounters the following biological problems while analysis of huge nos. of proteins; Fragile nature of proteins; substantial loses occur during sample collection and processing steps, presences of multiple isoforms of single protein, wide dynamic range of protein concentrations in biological fluids; presence of high-abundance proteins masking low-abundance marker proteins.

Additionally, technological limitations associated with most of the commonly used MS-based approaches include: typical dynamic ranges of only 10^2–10^4, inadequate coverage of whole proteome unless sample is fractionated extensively, low-throughput and issues of robustness and cost, over fitting the data, machine fluctuation, instrument noise and contaminants in spectrum and lack of standard procedure for analysis and interpretation of MS and MS/MS spectrum.

To overcome the technological challenges different novel and amalgamated approaches have emerged in last few years; the most promising advancements includes large scale quantitative proteomics: culture-derived isotope tags (CDIT) and Super-SILAC based technologies; Multiplexing: Tandem Mass Tag (TMT) and iTRAQ (8 plex), Quantitative accuracy: label-free LC-MS/MS, Low sample consumption and large-scale analysis: Chip-based and Nano-LC-MS. Sensitive quantification of proteins within complex mixtures-biomarker discovery: multiple reaction monitoring (MRM) MS and MS/MS, Large-scale biomarker discovery: Label-free LC-MS/MS quantitative proteomics

Array-based proteomics: Array-based proteomics (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 its HT capabilities, array-based proteomics attracted tremendous attention in clinical research, it has quite a few technological challenges including; Protein array designing difficulties (acquisition,

arraying, and stable attachment of proteins to array surfaces and detection of interacting proteins), miniaturization of assays and protein dehydration, non-specific 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 (SPR, SPRi, ellipsometry-based and Interference-based techniques, and microcantilevers) which depend on the measurement 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 in protein/antibody microarrays. 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 detections techniques are very promising and potential candidates for real-time measurements of low-abundance analytes and protein-protein interactions, issues regarding sensitivity and specificity remain to be addressed further. Additionally, costly fabrication techniques, morphological anomalies of sample spots and insufficient knowledge regarding the exact working principles of the label-free biosensors often restrict their 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 (Carbon nanotubes and nanowires, silicon nanowire field effect transistor, quantum dots and gold nanoparticles and nanocages), which has led to the establishment of a novel analytical platform known as 'nanoproteomics'.

Presently, nanoproteomics is at a proof-of-principle concept level and have the following limitations; Toxicity, biosafety and biocompatibility issues associated with the use of nanostructured materials, inadequate knowledge on the precise mechanism of action for the regularly used nanomaterials, insolubility in biologically compatible buffers and

conditions, presence of metallic impurities and lack of standard protocol for determining degree of purity of synthesized nanotubes and nanowires.

Biomarker discovery; Detection of low-abundance proteins: Biomarkers are biomolecules that can be used for early disease detection, discrimination between the different diseases or different stages/severity of same disease, as well as aid in monitoring disease progression. Despite various advancements, still there are multiple biological and technological challenges for the existing proteomics technologies commonly used in disease biomarker discovery. The formidable limitations include pre-analytical variation at sample collection, handling and storage process, complexity of biological sample due to the dynamic range of protein concentration, presence of high-abundance proteins masking low-abundance marker proteins, presence of high levels of salts and other interfering compounds in most of the biological specimens, insufficient sensitivity of the detecting 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 SCX (separation), ICAT, iTRAQ, TMT (labeling), nanoparticles like nanowires and nanotubes and quantum dots (signal amplification and enrichment of low-abundance proteins) have evolved 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 is discussed with a colleague Cynthia Goh and shown in video clip.

Future of Proteomics

During the last decade this emerging filed 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 a global level and establishment of effective data repositories accessible to the proteomic researchers across the world.

In 2010 Human Proteome Organization (HUPO) has launched a global Human Proteome Project (HPP), which is designed to map the entire human protein encoded by the genome.

Let's now discuss some of the targeted initiatives. Human Liver Proteome Project is the first initiative of the human proteome project for human organs/tissues with an intention of generation of comprehensive protein atlas of the liver and an international liver tissue network, collection and distribution of normal liver samples and Validation of new discoveries

HUPO Brain Proteome Project (BPP) focuses on the revelation of the brain-related proteome focusing on: Understand neurodegenerative diseases and aging and identification of prognostic and diagnostic biomarkers.

Human Kidney and Urine Proteome Project (HKUPP) aims to understand kidney functions, mechanisms of Chronic Kidney Diseases (CKDs) at a protein level and discover biomarkers and target molecules for new therapeutics of kidney diseases.

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 evident from recent 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 the space of emerging proteome level research, it can be anticipated that in coming future some amicable solutions for the existing limitations associated with the burgeoning 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 proteomics and enthused you for research in proteomics area.

Thank you!