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INSIGHTS INTO PROTEOMICS AND ITS APPLICATIONS

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Abstract:

Proteomics is the large-scale study of proteins, particularly their function and structure on a genome-wide scale. It involves the application of technology for the identification and quantification of overall protein content of a cell, tissue or an organism. Current proteomic tools allow large-scale, high throughput analysis for the detection, identification, and functional investigation of proteome. Advances in protein fractionation and labeling techniques have improved protein identification to include the least abundant proteins. The present review focuses on different types of techniques for the analysis of expressed proteins. The review also discusses applicative perspective of proteomics in the fields of biomedical, agriculture and food.

Keywords: Proteomics, 2-D gel electrophoresis, MALDI-TOF-MS, Isotope-coded affinity tags.

Introduction:

Proteins, which are polymers of amino acids are highly complex structures present in all living organisms. They were first reported in 1902 by Emil Fischer and Franz Hofmeister[1]. Their primary structure is specific amino acids, encoded by the mRNA, which directs the proper folding of the polypeptide chain into the secondary structure. The name Protein is derived from the Greek term, proteios, meaning “the first rank,” and was used for the first time by Berzelius in 1838 to illustrate the importance of these molecules[1].

Proteomics is the large-scale study of proteins particularly their composition, structures, functions, and interactions of the proteins directing the activities of cell[2]. In contrast to genome, proteome is a more accurate indicator of the dynamic state of the cell, tissue, or an organism. Hence proteomics is expected to play a vital role in yielding better

disease markers for early diagnosis and therapy monitoring[4]. Proteomics plays a vital role in many scientific disciplines today, enabling discovery of disease biology and mechanisms, new drug targets and much more. For example, applications for proteomics in the drug discovery process include the identification of biomarkers of human and animal diseases[4]. Proteomics is a challenging field, largely due to the sheer size of the proteome and the volume of data that can be generated by it. Analysis of a full proteome is a very complex process and one of the most effective approaches to this issue is simplification of the starting samples and good experimental design[5].

The Importance of Proteomics[6]:

Proteomics is a relatively recent field; the term was coined in 1994, and the science itself had its origins in electrophoretic separation techniques of the 1970's and 1980's. Studying proteins generates insight on how proteins affect cell processes. Conversely, this study also investigates how proteins themselves are affected by cell processes or the external environment.

Proteins provide intricate control of cellular machinery, and are in many cases components of that same machinery. They serve a variety of functions within the cell, and there are thousands of distinct proteins and peptides in almost every organism. This great variety comes from a phenomenon known as alternative splicing, in which a particular gene in a cell's DNA can create multiple protein types, based on the demands of the cell at a given time.

The goal of proteomics is to analyze the varying proteomes of an organism at different times, in order to highlight differences between them. Thus proteomics analyzes the structure and function of biological systems. For example, the protein content of a cancerous cell is often different from that of a healthy cell. Certain proteins in the cancerous cell may not be present in the healthy cell, making these unique proteins good targets for anti-cancer drugs. The realization of this goal is difficult; both purification and identification of proteins in any organism can be hindered by a multitude of biological and environmental factors:

Proteomics can help to investigate[7]

- when and where proteins are expressed;
- rates of protein production, degradation, and steady-state abundance;
- how proteins are modified (for example, post-translational modifications (PTMs) such as phosphorylation);
- the movement of proteins between subcellular compartments;

- the involvement of proteins in metabolic pathways;
- how proteins interact with one another.

Because Proteomics is growing at a very rapid pace, there is a shift in the field away from a specialized/focused way of conducting studies and towards a more global perspective. A critical aspect to this strategy is planning ahead and in doing so, the most appropriate plans and technologies can be implemented in the most efficient manner. By developing a strategy tailored to understanding a particular proteome, problems and setbacks can be avoided during the study. The first step when utilizing proteomics is to develop a hypothesis specific to the proteome being studied. It is best to choose organisms that already have a great deal of genomic information available, since the genome is always a useful supplement to proteomic information. Once the hypothesis and organism are established, the proper technologies should be chosen; and these technologies should be compatible with whatever biological factors are present (i.e. sample type). Some important and relevant proteomic methods include HPLC, Mass Spectrometry, SDS-PAGE, two-dimensional gel electrophoresis, and in silico protein modeling.

Since there are multitudes of sample type, sample preparation, and analytical technology combinations possible, it is obvious why careful planning from a broad-based proteomic perspective is critical. By planning upfront, an efficient proteomic study can be conducted and when the efforts of many broad-based proteomic studies are taken together, understanding the proteome in its entirety becomes a realistic possibility.

Classification of Proteomics: Based on the protein response proteomics are classified into different groups.

1. Expression proteomics:

Expression proteomics is used to study the qualitative and quantitative expression of total proteins under two different conditions. The normal cell and treated or diseased cell can be compared to understand the protein that is responsible for the stress or diseased state or the protein that is expressed due to disease[8]. 2-D gel electrophoresis and mass spectrometry technique can be used to observed the protein expressional changes, in tumour tissue, as compared with normal tissue[9,10].

2. Structural proteomics:

Structural proteomics helps to understand three dimensional shape and structural complexities of functional proteins Structural proteomics can give detailed information about the structure and function of protein complexes present in

a specific cellular organelle. It is possible to identify all the proteins present in a complex system such as membranes, ribosomes, and cell organelles and to characterise all the protein interactions that can be possible between these proteins and protein complexes[11].

3. Functional proteomics:

Functional proteomics deals with understanding the protein functions as well as unveiling molecular mechanisms within the cell then depend on the identification of the interacting protein partners[12,13].

Steps in isolation of proteins for Proteomic Analysis. The following steps are involved in analysis of proteome of an organism[14]:

1. **Purification of proteins:** This step involves extraction of protein samples from whole cell, tissue or sub cellular organelles followed by purification using density gradient centrifugation, chromatographic techniques (exclusion, affinity etc.)

2. **Separation of proteins:** 2D gel electrophoresis is applied for separation of proteins on the basis of their isoelectric points in one dimension and molecular weight on the other. Spots are detected using fluorescent dyes or radioactive probes.

3. **Identification of proteins[15]:**

Protein identification using mass-spectrometry involves using one of two established methodologies such as peptide mass mapping (or fingerprinting) and peptide sequencing. Both techniques use a proteolytic enzyme to specifically cleave the proteins into peptide fragments of a suitable length (mass) for mass spectrometric analysis. Peptide mass mapping involves an initial separation of the proteins by gel electrophoresis (one or two-dimensional) followed by an in situ protein digestion. Protein spots on the gel are visualized and quantitated by staining and then excised for subsequent digestion followed by mass spectral analysis by MALDI-TOF. Observed accurate mass values acquired by the TOF analyzer provide a "fingerprint". This 18 peptide fingerprint can be compared to a predicted list of peptides derived from the choice of protease used and their corresponding masses for all proteins in a database. The classic example of this would be the cleavage of Cytochrome C with trypsin, an enzyme that cleaves the peptide bond on the carboxy terminal side of arginine and lysine (except when adjacent to proline). With a sufficient number of peptide masses a match is usually found. Generally, five or more accurate masses are required to identify

a protein with enough confidence and three or four for a more ambiguous match. Two-dimensional electrophoresis (2DE) is hampered by some limitations, these are mainly attributed to its limited dynamic range. Low abundant proteins, low and high mass proteins, membrane proteins and proteins with extreme isoelectric points all present a challenge for 2DE. For MALDI-MS analysis, typically the individual protein spots from the gel are treated with a protease and each subsequent digest is spotted individually onto the MALDI plate. In this fashion the method can be considered as being performed off-line. The operator can visualize the MALDI plate and sample/matrix crystals can be picked out and targeted by the laser. A number of crystals from one protein digest can be hit maximizing the number of ions obtained from the sample spot. This has the effect of increasing the probability of detecting some peptide ions that may have their signal suppressed by the presence of more readily ionizable peptides in the sample crystal. This process can then be repeated for each individual protein digest spotted onto the MALDI plate. In ESI, as the technique inherently involves a continuous stream of liquid then sample introduction to the mass spectrometer is performed on-line. Each individual digest from the protein spot excised from the gel (possibly containing many peptides from the original protein) could be infused as a liquid sample stream directly into the instrument, however in practice this is rarely done. Separation of individual peptides from the digest can be achieved by using liquid chromatography thus limiting the effects of readily ionizable peptides suppressing the signal from peptides that are not so readily ionizable ('ion suppression'). It could be argued that chromatographic separation prior to sample deposition on MALDI plates would also be beneficial and indeed this process is being introduced into MS proteomics[18]. However, it involves the use of careful automation for the collection of the chromatographic eluent together with a subsequent increase in the number of sample spots required for analysis and hence increasing the workload.

Peptide sequencing involves the production of fragment ion spectra by tandem MS (or by sequential MS experiments using an ion trap). Peptides are collided with gas molecules inside the mass spectrometer causing fragmentation along the polypeptide backbone. This usually results in the formation of two fragments, one containing the N-terminus and the other the C-terminus. N-terminal fragments are known as b-ions and are numbered starting from the n-terminus. C-terminal fragments are known as y-ions and are numbered starting from the C-terminus.

Techniques Involved In Characterization of Proteins[16]:

In proteomic analysis both analytical and bio-informatics tools were used to characterize protein structure and functions. Analytical techniques such as 2-D gel electrophoresis, MALDI-TOF-MS were used. In case of bio-informatics numbers of software tools were used.

1) 2-D gel electrophoresis[17,18]:

In 2-D gel electrophoresis, protein samples are first resolved based on charge, in a step called isoelectric focusing, and then based on molecular weight. The result is an image in thousands of small spots, each representing a protein. A good 2-D gel can resolve one thousand to two thousand protein spots, which appear after staining, as dots in the gel. 2-D gel electrophoresis technique is mainly used to compare two similar samples to find specific protein differences.

The development of 2-DE is commonly associated with the birth of proteomics – it is at the heart of proteomic research. 2-DE separates proteins in two steps, according to their two independent properties: the first dimension is isoelectric focusing (IEF), in which proteins are separated according to their isoelectric points (pI), that is until they reach a stationary position where their net charge is zero; the second dimension is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), which separates proteins according to their molecular mass. The high-resolution of 2-DE results from the fact that first and second separations are based on two independent protein parameters. The combination of these two orthogonal separation techniques in 2-DE resolves proteins into spots (each spot being a protein isoform with specific pI and MW as its coordinates), and this map of protein spots can be considered as the "protein fingerprint" of that sample two such fingerprints from different cellular states can be compared to each other in order to identify proteins of relevance to that particular state or phenotype.

This technique has several limitations including limited reproducibility, and a smaller dynamic range as well as being not suitable for high throughput analysis. Also, certain proteins including those that are in low abundance, acidic, basic, hydrophobic, very large, or very small are difficult to separate using this technique.

3) MS analysis:

Mass spectrometry is an analytical technique that produces spectra of the masses of the atoms or molecules comprising a sample of material. The spectra are used to determine the elemental or isotopic signature of a sample,

the masses of particles and of molecules, and to elucidate the chemical structures of molecules, such as peptides and other chemical compounds. Mass spectrometry works by ionizing chemical compounds to generate charged molecules or molecule fragments and measuring their mass to charge ratios. This technique has shown tremendous application in study of protein of diseased cells. Bio molecules and synthetic polymers have low volatility and are thermally unstable, which has limited the use of MS as a means of characterization[19].

4) Maldi-TOF-MS:

Matrix Assisted Laser Desorption/Ionisation is a soft ionization technique used in spectrometry, for analysis of bio molecules like DNA, protein, peptides. This technique can be used for the rapid and sensitive analysis of biomolecules. One of the main applications of MALDI-TOF-MS is in the identification of proteins, by peptide mass fingerprinting (PMF)[20]

Principle[21]:-

The sample for MALDI is uniformly mixed in a large quantity of matrix. The matrix absorbs the ultraviolet light (nitrogen laser light, wavelength 337 nm) and converts it to heat energy. A small part of the matrix (down to 100 nm from the top outer surface of the Analyte in the diagram) heats rapidly (in several nano seconds) and is vaporized, together with the sample.

The MALDI TOF process is a two-phase procedure-

1. Ionization Phase
2. Time of Flight Phase

Ionization Phase:

Initially, the samples are fixed in a crystalline matrix in a target plate and are bombarded by a laser. The sample molecules vaporize into the vacuum while being ionized at the same time. High voltage is then applied to accelerate the charged particles.

Time of Flight Phase:

1. **In the linear mode,** particles will impinge upon the linear detector within a few nanoseconds after ionization. Higher mass molecules will arrive later than lighter ones. Flight time measurement make it possible to

determine molecule masses directly. Each peak in the spectrum corresponds to the specific mass of the particle along the time axis, starting with the ionization moment.

2. **In the reflector mode**, the particles are diverted so that they fly towards a second detector. In addition to extending the flight distance, the reflector also focuses the masses.

   The combination of these two effects makes for higher resolution than in the linear mode. The net result is a generation of a mass spectrum which is compared with those of well-characterized moieties available in the reference library database to identify the isolate.

Advantages[22]

- MALDI-TOF MS is a promising analytical method with high sensitivity and accuracy, which determine biomolecules including proteins, glycolipids, carbohydrates, and oligonucleotides.
- MALDI-TOF MS can detect the biological activity of enzymes responsible for the modification of antibiotic molecules, which is not possible with genetic techniques.
- MALDI-TOF MS is a relevant tool for the detection of antibiotic resistance and opens new avenues for both clinical and experimental microbiology.
- MALDI-TOF MS is capable of detecting hundreds of proteins in an individual sample with high accuracy, and hence, is an indispensable technique for protein-based biomarker discovery.

Components of MALDI-TOF (Figure 1)

1. Ion source:-A major component of MS instrumentation which fragments the sample into an ionic form for further detection.

2. Matrix Assisted Laser Desorption ionization (MALDI):-MALDI is an efficient ionization source for generating gas-phase ions of peptides & proteins for mass spectrometric detection.

3. Mass analyser:-The mass analyser resolves the ions produced by the ionization source on the basis of their mass to charge ratios. Various characteristics such as resolving power, accuracy, mass range & speed determine the efficiency of these analysers.

4. Time of flight (TOF):-This is a mass analyser in which the flight time of the ion from the source to the detector is correlated to the m/z of the ion.

5. Flight tube:-Connecting tube between the ion source & detector within which the ions of different size & charge migrate to reach the detector .

6. Reflectron:-The reflectron acts as an ion mirror & extends the flight length without increasing the instrument size. The reflection compensates for the initial energy spread of ions having the same mass.

7. Reflectron detector:-Detects the ions reflected by ion mirror. This over all setup improves the resolution.

8. Detector:-The ion detector determines the mass of ions that are resolved by the mass analyzer and generates data which is then analysed. The election multiplier is the most commonly used detection technique.

Advances in Proteomics:

1. Isotope-coded affinity tags (ICAT)

2. Isobaric Tags for Relative and Absolute Quantification (iTRAQ)

3. Absolute Quantification (AQUA)

4. ESI-Q-IT-MS

5. SELDI-TOF-MS

1. Isotope-coded affinity tags (ICAT): It is a gel- free method for quantitative proteomics that relies on chemical labelling reagents. These chemical probes consist of three elements i.e. defined amino acid side chain, an isotopically coded linker, and a tag for the affinity isolation of labelled proteins/peptides[17]. The isotope coded linker can be heavy (deuterium labeled) or light (hydrogen labeled). For ICAT quantification, proteins are collected from the two samples to be compared and one is labeled with the light tag and the other with the heavy tag. The samples are then mixed, digested with a protease, and purified via affinity chromatography. The purified peptides are analyzed by mass spectrometry and the light- to heavy-ratios for each peptide indicate the relative amount of protein in each sample.

The next step is automated correlation with protein sequence data banks using algorithms and permutations, to identify the protein from which the sequenced peptide originated and thus identify the protein. A combination of all results generated on the chromatogram by the mass spectrometer; and analysis of the ICAT reagent-labelled peptides determines the relative quantities as well as the sequence identities of the components of protein mixtures in a single automated operation.

2. Isobaric Tags for Relative and Absolute Quantification (iTRAQ):

This is a non- gel- based technique used to quantify proteins[16]. Here primary amines of peptides and proteins are labelled by isobaric reagents and the quantitative changes are determined by tandem mass spectrometry. The isobaric tagging reagents consist of a unique charged reporter group, a peptide reactive group, and a neutral balance group.

iTRAQ helps in studying the progress of metabolism of a particular drug or the progressive concentration of certain biomarkers at different stages of a particular disease. Also, multiplexing ability is another advantage. On the other hand, iTRAQ reagents are extremely costly and also extremely sensitive to contamination. Moreover, sophisticated software is required for analyzing iTRAQ data. Another disadvantage is the variability arising due to the inefficient enzymatic digestion[23].

3. Absolute Quantification (AQUA):

AQUA, studies the absolute quantification of proteins and their modification states. Covalent modifications can be used to prepare synthetic proteins. These modifications are chemically identical to naturally occurring posttranslational modifications. These types of peptides used to quantify the posttranslational modified proteins after proteolysis with the help of tandem mass spectrometer.

4. ESI-Q-IT-MS[24]:

Micro electrospray ionization (ESI)-Quadrupole ion trap (QIT) Time of flight (TOF) mass spectrometer (MS) has a very good resolution. In ESI ionization proteins are ionized in solution and carry multiple charge state. The advantage of using ESI-QTOF analysis for protein mass determination is that due to the high charge state of proteins their m/z measurements is typically less than 2000 and the TOF detector has a very good mass accuracy in this scan range. This results in more accurate mass measurements for proteins in ESI-QTOF.

5. SELDI-TOF-MS:

The technique Surface-enhanced laser desorption/ionization (SELDI) is used for the analysis of protein mixtures, it is an ionization method combined with mass spectrometry. SELDI is typically used with time-of-flight mass spectrometers and is used to detect proteins in clinical samples; to compare protein levels with and without a disease can be used for biomarker discovery[25].

Proteomics and biomarker discovery[26]:

With the recent emergency of novel technologies such as genomics- and proteomics-based approaches, the field of biomarker discovery, development and application has been the subject of intense interest and activity.

The term "biomarker" is used in many scientific fields, and hence can be defined in many different ways. In general, a biomarker is a measurable indicator that correlates to specific biological or disease state. Clinical biomarker can be defined as a characteristic that is objectively measured and evaluated as an indicator of normal biological process, pathogenic process, or pharmacologic responses to a therapeutic intervention. Besides screening for the early diagnosis, biomarkers can be used for the classification and staging of diseases in order to design targeted treatments, monitoring of a treatment response and disease reoccurrence detection. Generally, ideal biomarkers have to be reliable, highly specific and sensitive, easily measurable (i.e. minimally invasive), as well as low-cost. Biomarkers may be genes, proteins, small molecules or metabolites. A wide variety of methods have been applied to find new biomarkers. With the completion of genome sequencing projects, the was a hope to uncover common disease-associated gene variants by means of large-scale genome-wide association studies. However, while SNPs indicate the potential for disease susceptibility, it is the activity of the resultant protein that actually measures it. In other words, using proteins as biomarkers has an advantage over employing DNA and mRNA for that purpose, as proteins are responsible for the biological complexity of the corresponding physiological phenotypes. Traditionally, biomarker discovery was focused on detection and quantitative measurement of single proteins. However, soon it became clear that the predictive utility of a single protein biomarker might be limited. Alternatively, a panel of proteins may be utilized to evaluate the level of perturbation of a biological system. With the rapid development of proteomic techniques it became possible to search for protein biomarkers in complex biological samples in a high-throughput manner.

The road to biomarker discovery has two major components:

1) Differential proteome profiling for potential biomarkers;

2) Validation of these candidate biomarkers over a large set of samples, assisted by extensive statistical analysis.

Quantitative proteomic approaches that are used for biomarker discovery can be divided into three major categories: (A)protein profiling; (B) 2-DE combined with MS analysis, (C) shotgun MS-based quantitative proteomics

Identification of proteomic patterns, or protein profiling strategy has generated great enthusiasm in the field of biomarker discovery.

This approach attempts to identify molecular signatures, or unique features within mass spectral profiles, that would characterize biological samples. Mass spectra of complex mixture is generated and protein profiles (peak intensities) are compared.

Applications of Proteomics[27,28]:

Proteomics is currently used in various biological fields, such as Oncology (Tumour biology), Bio-medicine, Agriculture and Food Microbiology.

1. Disease biomarkers:

One of the most common applications of proteomics is the search to identify novel biomarkers of disease, particularly cancer. Early detection of disease is essential in determining the best course of treatment options and possible outcomes. A challenge for successful biomarker identification is obtaining the appropriate samples for analysis. Zangar's group has applied a proteomics-based approach to both the identification and analysis of biomarkers for breast cancer using LC-MS/MS and ELISA microarrays[28.]

Understanding the proteome, the structure and function of each protein and the complexities of protein-protein interactions will be critical for developing the most effective diagnostic techniques and disease treatments in the future. An interesting use of proteomics is using specific protein biomarkers to diagnose disease. A number of techniques allow to test for proteins produced during a particular disease, which helps to diagnose the disease quickly. Techniques include western blot, immunohistochemical staining, enzyme linked immunosorbent assay (ELISA) or mass spectrometry. The following are some of the diseases that have characteristic biomarkers that physicians can use for diagnosis-:

Alzheimer's disease

In Alzheimer's disease, elevations in beta secretase create amyloid/beta-protein, which causes plaque to build up in the patient's brain, which is thought to play a role in dementia. Targeting this enzyme decreases the amyloid/beta-protein and slows the progression of the disease. A procedure to test for the increase in amyloid/beta-protein is

immunehistochemical staining, in which antibodies bind to specific antigens or biological tissue of amyloid/beta-protein.

Heart disease

Heart disease is commonly gauged using several key protein based biomarkers. Standard protein biomarkers for cardiovascular diseases include interleukin-6, interleukin- 8, serum amyloid A protein, fibrinogen, and troponins. Cardiac troponin I (cTnI) increases in concentration within 3 to 12 hours of initial cardiac injury and remains elevated days after an acute myocardial infarction. A number of commercial antibody based assays as well as other methods are used in hospitals as primary tests for acute heart infarction.

Toxicoproteomics:

The pharmaceutical industry is focused on developing drugs with superior safety profiles. While animal safety studies clearly make up the bulk of preclinical product testing, there is a substantial need to develop more predictive tools to aid in the early assessment of product liabilities. One such tool that has been of great interest is toxicoproteomics, utilizing global protein expression techniques to identify key proteins and pathways in biological systems that change in response to novel chemical entities.

Oncology:

Oncology deals with study of tumor cell and tumor metastasis. Analysis of protein expressions correlated to the metastatic process helps to understand the mechanism of metastasis and thus facilitate the development of strategies for the therapeutic interventions and clinical management of cancer.

Agricultural applications:

The applications of proteomics in plant based research is still in budding stage. Proteomics aids in study of plant-insect interactions that help identify candidate genes involved in the defensive response of plants to herbivore.

Food Microbiology:

Proteomics plays an important role in food technology for characterisation and standardisation of raw materials, process development, and detection of batch-to batch variations as well as quality control of the final product. It also aids in determining level of food safety, especially regarding biological and microbial safety and the use of genetically modified foods.

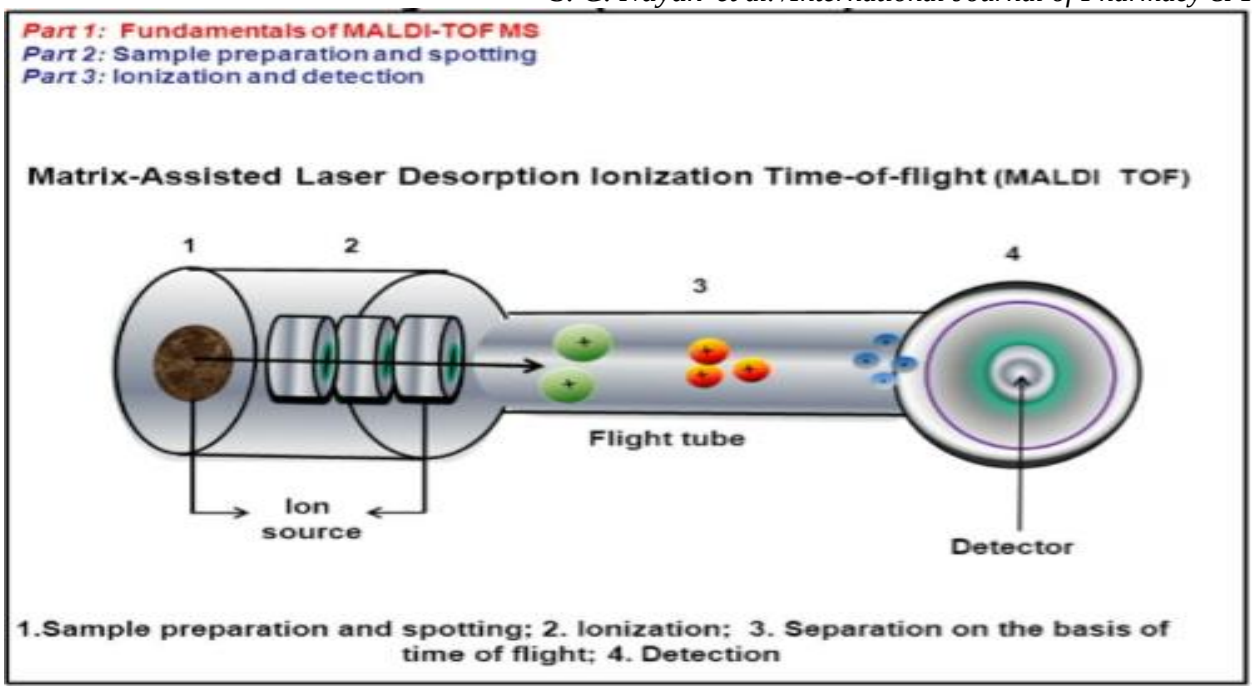

Figure1: Diagrammatic representation of MALDI-TOF (https://www.google.com).

Environmental research:

With the development of the global industry and growth of human population, thousands of man-made chemicals are annually released to the environment by transport, industry, agriculture and other human activities. Traditionally, the pollution status of aquatic or terrestrial ecosystem has been assessed by the chemical analysis of environmental samples (e.g. water, soil). However, given the large number, complexity and in some cases low toxicity thresholds of the chemicals present, chemical analysis alone is not able provide a satisfying assessment of the environmental quality of an ecosystem.

The application of proteomic approaches in environmental research can be divided into two groups: 1) study of proteomic response to stress, which provides information on the mechanism of the response and helps to understand why some organisms could resist extreme environmental stress 2) screening environmental samples by a proteomic method to monitor exposure to certain pollutants.

References:

1. Anderson N.G., Anderson N.L., Electrophoresis 17 (1996) 443-453.
2. Anderson N.L., Anderson N.G., Electrophoresis 19(1998) 1853-1861.
3. Blackstock W.P., Weir M.P., Treands in Biotechnology 17(1999) 121-127.

4. M. M. Elrick, J. I. Walgren, M. D. Mitchell, D.C. Thompson, Proteomics: Recent Applications and New Technologies Basic & Clinical Pharmacology & Toxicology2006,98, 432–441.
5. J. B. Klein, V. Thongboonkerd, Overview of Proteomics Thongboonkerd V, Klein JB (eds): Proteomics in Nephrology. Contrib Nephrol. Basel, Karger, 2004, vol 141, 1–10.
6. Proteomics – advances, applications and the challenges that remain R. Aebersold and B. F. Cravatt Trends in Biotechnology Vol. 20 No. 12 (Suppl.), 2002 1-2.
7. Bilal A., Madiha B., Muhammad A., Mohsin K., Muhammad H., Proteomics: Technologies and Their Applications Journal of Chromatographic Science, Volume 55, Issue 2, 1 February 2017, Pages 182–196.
8. Pandey A., Fernandez M.M., Stehen H., Blagoev B., Nielsen M.M., Roche S., Mann M. Lodish H.F., Journal of Biological Chemistry 275(2000) 38633-38639.
9. Hinsby A.M., Olsen J.V., Bennett K.L., Mann M., Molecular and Cell Proteomics 2 (2003) 29-36.
10. Banks R.E., Dunn M.J., Hochstrasser D.F., Sanchez J.C., Blackstock W., Pappin D.J., Selby P.J., Lancet 356 (2000) 1749-1756.
11. Yokoyama S., Hirota H., Kigawa T., Yabuki T., Shirouzu M., Terada T., Ito Y., Massuo Y., Kuroda Y., Nishimura Y., Kyogoku Y., Miki K., Masui R., Kuramitsu S., Nature Structural and Molecular Biology 7 (2000) 943-945.
12. Gavin A.C., Bosche M., Krause R., Grandi P., Marzioch M., Bauer A., Nature 415 (2002) 141-147.
13. Ho Y., Gruhler A., Heilbut A., Bader G.D., Moore L., Adams S.L., Millar A., Taylor P.,Bennett K., Boutilier K., Yang L., Wolting C., Donaldson I., Schadoeff S., Nature 415 (2002) 180-183.
14. C. H. Lee, A Simple Outline of Methods for Protein Isolation and Purification, Endocrinol Metab 2017;32:18-22.
15. Kalpana Joshi, Dada Patil, Innovative Approaches in Drug Discovery, Scientific Word Journal Jan2017 pg.273-294.
16. Zieske L.R., Journal of Experimental Botany 57(2006) 1501-1508.
17. Mikkelsen Susan, Cortón Eduardo, Bioanalytical Chemistry. John Wiley & Sons, Inc. p. 224 (2004).
18. Sparkman David O., Mass spectrometry desk reference. Pittsburgh: Global View Pub. 2000.

19. Wu K.J., Shaler T.A., Becker C.H., Analytical Chemistry 66 (1994) 1637-1645.
20. MALDI-TOF mass spectrometry proteomic based identification of clinical bacterial isolates A. Panda, S. Kurapati, J. Samantaray, A. Srinivasan, and S. Khalil Indian J Med Res. 2014 Dec; 140(6): 770–777.
21. An introduction to mass spectrometry based proteomics—Detection and characterization of gonadotropins and related molecules A. T. Kicman, M. C. Parkin, R. K. Iles, Molecular & Cellular Endocrinology, 260-262,(2) Jan 2017, 212-227.
22. M. W. Duncan, D. Nedelkov, R. Walsh, S.J. Hattan, Application Of MALDI Mass Spectrometry in Clinical Chemistry, Clinical Chemistry 62:1 2016 134-143.
23. Muth T., keller D., Puetz S.M., Maetens L., Sickmann A., Boehm A.M., Proteomics 10 (2010) 1223-1225.
24. Chapter 5 - Small RNA proteome as disease biomarker: An incognito treasure of clinical utility Author links open overlay panel Jyoti Roy Neha Jain Garima Singh Basudeb Das Bibekanand Mallick AGO-Driven Non-Coding RNAs Codes to Decode the Therapeutics of Diseases 2019, Pages 101-136.
25. Electrospray Ionization Mass Spectrometry: A Technique to Access the Information beyond the Molecular Weight of the Analyte s. Banerjee & S. Mazumdar, International Journal of Analytical Chemistry 2012, 9Aug, 1-30.
26. Li J., Zhang Z., Rosenzweig J., Wang Y.Y., Chan D.W., Clinical Chemistry 48 (2002) 1296-1304.
27. A. N. Bhatt, R. Mathur, A. Farooque, A. Verma, and B. S. Dwarakanath, "Cancer biomarkers—current perspectives," The Indian Journal of Medical Research, 2010 vol. 132, no. 8,129–149.
28. Proteomic Approaches for the Discovery of Biofluid Biomarkers of Neurodegenerative Dementias B. C. Carlyle, B. A. Trombetta and S. E. Arnold MDPI 32 2018 (6), 1-12.

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