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Journal of Chromatography & Separation Techniques

Mini Review

Cutting-edge Proteomics: Techniques and their Applications

Sravani Neelapu*

Department of Analytics, Indian Institute of Science, Bengaluru, Karnataka, India

ABSTRACT

Proteomics involves the application of technology to determine the content and size of the total protein content of a cell, tissue, or organism. It replaces other "omics" techniques such as genomics and transcriptomics to explain the identity of an organism's proteins and to identify the structure and functions of a particular protein. Proteomics is practically complex because it involves the analysis and classification of the total protein signatures of a gene. Mass spectrometry devices with LC-MS-MS and MALDI-TOF/TOF are widely used in current proteomics. However, the use of Proteomics facilities, including software for equipment, databases, and the need for skilled personnel, significantly increases costs, thus limiting their widespread use, especially in developing countries. Furthermore, the protein is highly dynamic due to the complex control systems that control the expression levels of the proteins. This review attempt to explain the various proteomics approaches, recent developments and their application in research and analysis

Keywords: Proteomics; Genomics; Crystallization; Mass spectrometry

INTRODUCTION

Proteomics are crucial for early diagnosis, diagnosis and monitoring of disease progression. Furthermore, it also plays a key role in drug development as target molecules. Proteomics is a feature of a protein that includes expression, structure, functions, interactions, and modification of proteins at any stage [1]. The protein undergoes fluctuations over time, in response to cell and cellular and external stimuli. Proteomics in eukaryotic cells is complicated by post-translational modifications, which arise in different ways at different sites [2].

Proteomics is the most important method for understanding genetic function, although it is more complex compared to genetic. Fluctuations in the level of genetic expression are determined by the analysis of a transcriptome or protein to differentiate between two biological states of the cell. Microwave chips were developed for large-scale analysis of the entire transcript. However, growth synthesis of mRNA cannot be measured directly by microarray. Proteins affect biological function and their levels depend not only on the corresponding mRNA levels but also on host translation control and regulation. Therefore, proteomics is considered to be the most relevant data set to classify the biological system.

ADVANCED TECHNIQUES OF PROTEOMICS

Protein microarray: Protein microarrays, also known as protein chips, are a growing class of proteomics techniques capable of detecting high-output from a sample in small amounts. Protein microarrays can be classified into three categories; Analytical protein microarray, functional protein microarray and reverse-phase protein microarray.

Analytical protein microarray: The most common type of analytical protein microarray is antibody microarray. Direct protein labelling is used to detect proteins after antibody capture. These are often employed to determine the degree of protein expression and their binding associations. Antibody microarray for high-throughput protein screening of cancer cells for differential protein expression in tissues generated from oral squamous cell carcinoma. Protein profiling of bladder cancer has also been done using an antibody array [3]. The staphylococcal enterotoxin, cholera toxin, Bacillus globigi, and Bacillus resin were all identified using a microelectric immunoassay. To discover cellular signalling pathways and characterise plant kinases using protein microarrays, analytical and experimental

Correspondence to: Sravani Neelapu, Department of Analytics, Indian Institute of Science, Bengaluru, Karnataka, India, E-mail: sravanineelapu@gmail.com

Received: 28-Feb-2022, Manuscript No. JCGST-22-17149; **Editor assigned:** 02-Mar-2022, PreQC No. JCGST-22-17149 (PQ); **Reviewed**: 22-Mar-2022, QC No. JCGST-22-17149; **Revised:** 31-Mar-2022, Manuscript No. JCGST-22-17149 (R); Published: 11-Apr-2022, DOI: 10.35248/2385-5495.22.13.476.

Citation: Neelapu S (2022) Cutting-edge Proteomics: Techniques and their Applications. J Chromatogr Sep Tech. 13:476.

methodologies have been established. Arabidopsis has a class of Mitogen-Activated Protein Kinases (MAPKs). MAPKs are highly preserved single-transducers and universal molecules in plants that respond to a wide range of extracellular stimuli.

Functional protein microarray: Purified protein is used to produce functional protein microarrays, which allow researchers to investigate a variety of interactions such as protein–DNA, protein–RNA, protein–protein, protein–drug, protein–lipid, and enzyme–substrate interactions. Functional protein microarray was originally used to investigate the substrate selectivity of protein kinases in yeast. Thousands of proteins' functions were studied using a functional protein microarray. Calmodulin-like proteins and Calmodulin substrates were discovered while studying the protein-protein interaction in A. thaliana.

Reverse-phase protein microarray: Cell lysates obtained from different cell states are mounted on a nitrocellulose slide, which is then tested with antibodies against the target proteins. Subsequently, antibodies are detected with fluorescent, chemiluminescent, and colorimetric tests. For protein sizing, reference peptides are printed on slides. These microarrays are used to identify a mutated or dysfunctional protein that indicates a specific disease. Analysis of hematopoietic stem cell and primary leukemia specimens by reverse-phase protein microarray has been found to be highly reproducible and reliable for large-scale analysis of phosphorylation status and acute myelogenous leukemia cells and human cells expressed proteins. By monitoring apoptosis, DNA damage, cell-cycle management, and signalling pathways, the reverse-phase protein microarray technique has been tested for quantitative analysis of phosphoproteins and other cancer-related proteins in Non-Small Cell Lung Cancer (NSCLC) cell lines.

Gel-based approaches

Sodium dodecyl sulfate-polyacrylamide gel electrophoresis: SDS-PAGE is a high-resolution technology for separating proteins according to their size, thereby facilitating the approximation of molecular weight. Proteins can move with an electric field in a medium having an unequal pH from their isoelectric point. Different proteins in the mixture migrate at different speeds according to the ratio between its charge and mass. However, the addition of sodium dodecyl sulfate reduces the proteins, so that they are completely separated by molecular weight.

Two-dimensional gel electrophoresis: Two-dimensional polyacrylamide gel electrophoresis (2D-PAGE) is an efficient and reliable method for separating proteins based on their mass and charge. 2D-PAGE can fix ~5,000 different proteins in a row depending on the gel size. Proteins are separated by charge in the first dimension and by mass in the second dimension. 2-DE has been successfully applied for the evaluation of post-translational modifications, mutation proteins and metabolic pathways.

Quantitative techniques

ICAT labeling: ICAT is an isotopic labeling method in which chemical labeling factors are used to quantify proteins. ICAT also expands the range of analyzable proteins and allows precise quantification and systematic identification of proteins from complex compounds. ICAT reagents are labeled peptides, isotopically coded linker and have an associative tag for isolation of reactive groups.

Stable Isotopic Labeling with Amino Acids in Cell Culture (SILAC): SILAC is a MS based quantitative proteomics technique based on metabolic labelling of the entire cellular proteome. The proteomes of various cells cultivated in cell culture are labelled with "light" or "heavy" forms of amino acids and differentiated using mass spectrometry [4]. The SILAC was created as a quick way to analyse gene expression regulation, cell signalling, and post-translational changes. In cell culture, SILAC is also an important approach for secretory pathways and secreted proteins.

Isobaric tag for relative and absolute quantitation: ITRAQ is a multiplex protein labeling technique for protein quantification based on tandem mass spectrometry. This technique is based on labeling a protein with isobaric tags (8-plex and 4-plex) for relative and absolute size. This technique involves the labeling of N-terminus and side chain amine proteins, which are cleaved by liquid chromatography and finally analyzed by MS. Finding genetic control is essential to understanding the disease mechanism, so using ITRAQ is the right method to help simultaneously determine and calculate protein size.

X-ray crystallography: X-ray crystallography is the most preferred technique for the three-dimensional structure determination of proteins. The most refined crystallization samples are exposed to X-rays and subsequent scattering patterns are processed to produce information about the recurring unit size that forms the crystal and crystal packing symmetry. X-ray crystallography has a wide range of applications to study the virus system, protein-nucleic acid complexes, and immune complexes. Furthermore, the three-dimensional protein structure provides detailed information on the enzyme mechanism, drug designing, site-direct mutagenesis, and elaboration of the protein-ligand interaction.

High-throughput techniques

Mass spectrometry: MS is used to measure mass ratio (m/z), thus helping to determine the molecular weight of proteins. The whole process consists of three steps. The molecules must be converted to gas-phase ions in the first stage, which is a challenge for the living molecules in the liquid or solid phase. The second step is the separation of ions based on m/z values in the presence of electric or magnetic fields in a compartment called a mass analyzer. Finally, the sum of the separated ions and each species with a specific m/z value is measured. Commonly used ionization methods include Matrix-Assisted Laser Dehydration Ionization (MALDI), Surface Enhanced Laser Dehydration/Ionization (SELDI) and Electro Spray Ionization (ESI).

NMR spectroscopy: NMR is a leading tool for research on the molecular structure, folding and behavior of proteins. Structural diagnosis by NMR spectroscopy usually involves different steps, each using very specific techniques [5]. Samples are made and measurements are made following detailed procedures to confirm the structure. Protein formation is fundamental in many areas of research, such as structure-based drug design, homology modeling, and functional genomics.

Bioinformatics analysis

Bioinformatics is an important component of proteomics; therefore, its implications are gradually increasing with the advent of high-output methods based on powerful data analysis. This new and emerging field is showcasing novel algorithms to handle huge and varied proteomics data and to move forward towards the innovation process.

CONCLUSION

Proteomics-based technologies are used in a variety of research settings, including the detection of various diagnostic markers, vaccine candidates, pathogenicity mechanisms, changes in expression patterns for the interpretation of functional protein pathways in various disorders, as well as the response to diverse signals. Over the past few years, there have been tremendously useful advances in the field of proteomics. Techniques are faster, smoother and provide greater protein coverage. Furthermore, the combination of these technologies has led to success in the purification, analysis, characterization, quantification, sequencing and structural analysis and bioinformatics analysis of large numbers of proteins in all types of eukaryotic and prokaryotic organisms. All fields of biology have benefited from the increasing use of proteomics techniques.

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