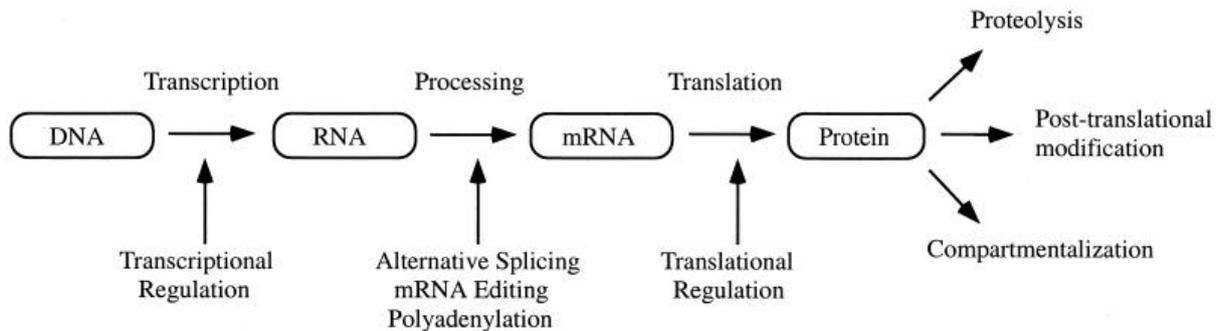


The word “**proteome**” can be defined as the overall protein content or the complete protein pool of an organism encoded by the genome of a cell that is characterized with regard to their localization, interactions, post-translational modifications and turnover, at a particular time. In broader term, Proteomics, is defined as the total protein content of a cell or that of an organism.

The term “**proteomics**” was first used by Marc Wilkins in 1996 to denote the “PROTEin complement of a geNOME”



[A single gene can give rise to multiple gene products. Multiple protein isoforms can be generated by RNA processing when RNA is alternatively spliced or edited to form mature mRNA. mRNA, in turn, can be regulated by stability and efficiency of translation. Proteins can be regulated by additional mechanisms, including posttranslational modification, proteolysis, or compartmentalization]

The proteome in any cell represents a subset of all possible gene products. Not all the genes are expressed in all the cells. It will vary in different cells and tissue types in the same organism and between different growth and developmental stages. The proteome of a given cell or organism is dynamic, which reflect the immediate environment in which it is studied. In response to internal or external cues, proteins can be modified by posttranslational modifications, undergo translocations within the cell, or be synthesized or degraded. Thus, examination of the proteome of a cell is like taking a “snapshot” of the protein environment at any given time. In a word the proteome is dependent on environmental factors, disease, drugs, stress, growth conditions.

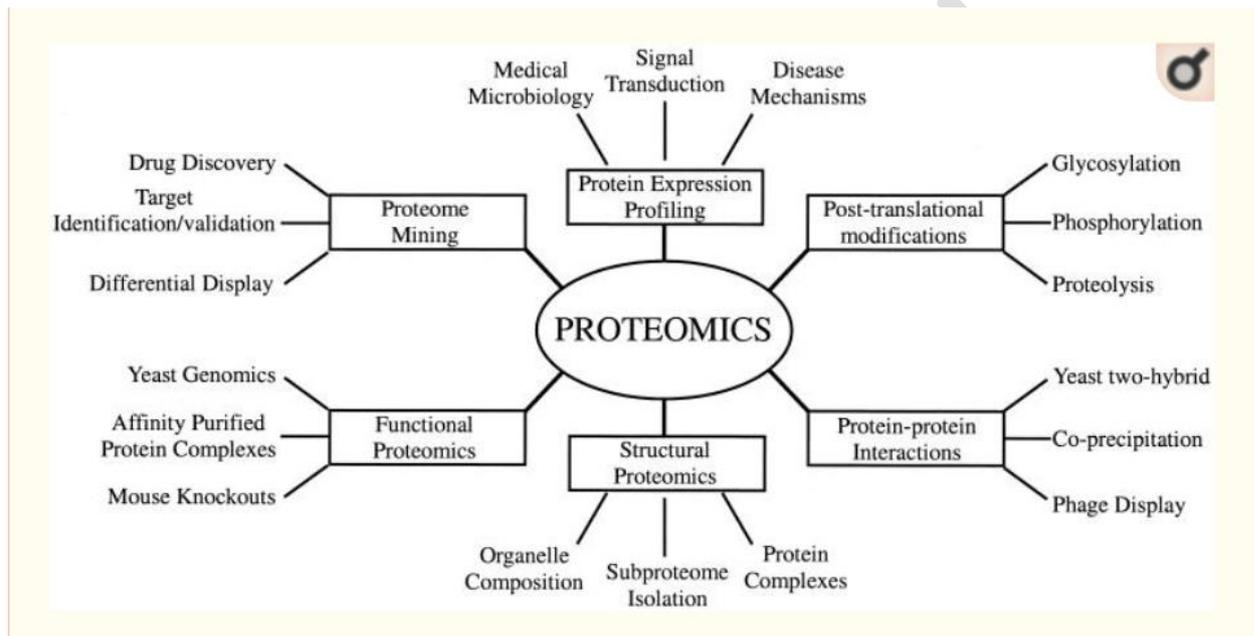
Most of the functional information of genes is characterized by the proteome. The proteome of eukaryotic cells is relatively complex and exhibits extensive dynamic range. Moreover, prokaryotic proteins are responsible for pathogenic mechanisms; however, their analysis is challenging due to huge diversity in properties such as dynamic range in quantity, molecular size, hydrophobicity and hydrophilicity. Proteomics is the characterization of proteome, including expression, structure, functions, interactions and modifications of proteins at any stage.

The proteome also fluctuates from time to time, cell to cell and in response to external stimuli. Proteomics in eukaryotic cells is complex due to post-translational modifications, which arise at different sites by numerous ways.

Proteomics is crucial for early disease diagnosis, prognosis and to monitor the disease development. Furthermore, it also has a vital role in drug development as target molecules.

Proteomics is one of the most significant methodology to comprehend the gene function although, it is much more complex compared with genomic.

Fluctuations in gene expression level can be determined by analysis of transcriptome or proteome to discriminate between two biological states of the cell. Proteins are effectors of biological function and their levels are not only dependent on corresponding mRNA levels but also on host translational control and regulation. Thus, the proteomics would be considered as the most relevant data set to characterize a biological system.



Types of Proteomics and their application to Biology

The complete identification of all proteins in a genome will aid the field of structural genomics in which the ultimate goal is to obtain 3-D structures for all proteins in a proteome. This is necessary because the functions of many proteins can only be inferred by examination of their 3-D structure.

Protein modifications is one of the most important applications of proteomics will be the characterize by posttranslational modifications as Proteins can be modified post translationally in response to a variety of intracellular and extracellular signals. For example, protein phosphorylation is an important signalling mechanism and dis regulation of protein kinases or phosphatases can result in oncogenesis. By using a proteomics approach, changes in the modifications of many proteins expressed by a cell can be analysed simultaneously.

Protein localization and compartmentalization is one of the most important regulatory mechanisms as the mis-localization of proteins is known to have profound effects on cellular function (e.g., cystic fibrosis). Proteomics aims to

identify the subcellular location of each protein. This information can be used to create a 3-D protein map of the cell, providing novel information about protein regulation.

The fundamental importance in biology is the understanding of protein-protein interactions. The process of cell growth, programmed cell death, and the decision to proceed through the cell cycle are all regulated by signal transduction through protein complexes. Proteomics aims to develop a complete 3-D map of all protein interactions in the cell. One step toward this goal was recently completed for the microorganism *Helicobacter pylori*. Using the yeast two-hybrid method to detect protein interactions, 1,200 connections were identified between *H. pylori* proteins covering 46.6% of the genome. A comprehensive two-hybrid analysis has also been performed on all the proteins from the yeast *S. cerevisiae*.

Types of Proteomics

Protein expression proteomics

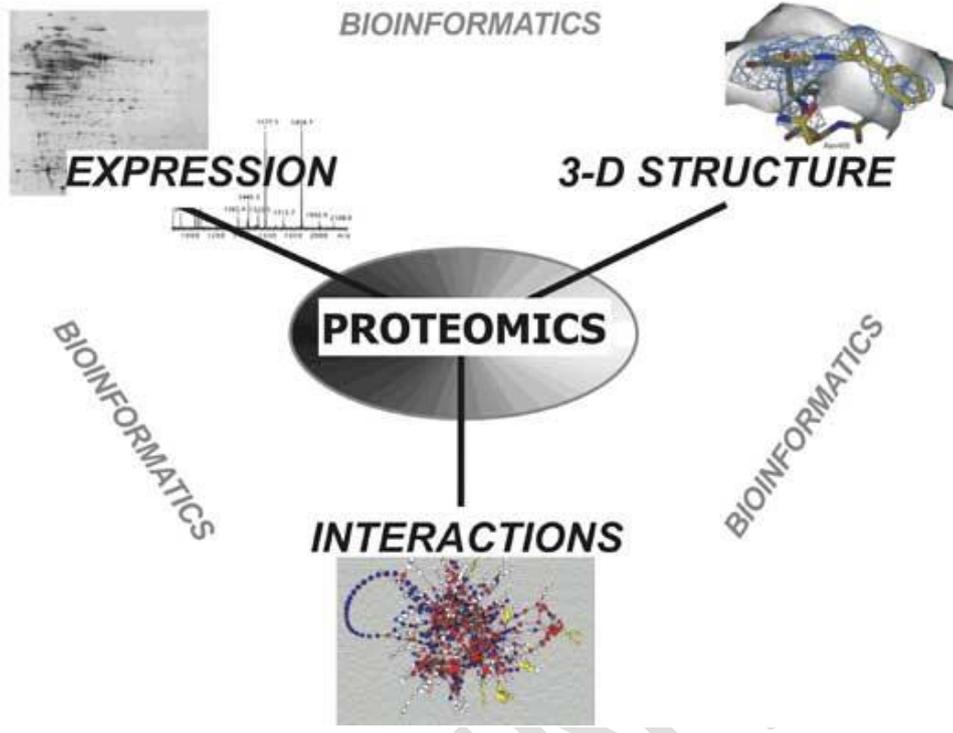
The quantitative study of protein expression between samples that differ by some variable is known as expression proteomics. In this approach, protein expression of the entire proteome or of sub proteomes between samples can be compared. Information from this approach can identify novel proteins in signal transduction or identify disease-specific proteins.

Structural proteomics

The structure of protein complexes or the proteins present in a specific cellular organelle are known as "cell map" or structural proteomics. Structural proteomics attempts to identify all the proteins within a protein complex or organelle, determine where they are located, and characterize all protein-protein interactions. An example of structural proteomics was the recent analysis of the nuclear pore complex. Isolation of specific subcellular organelles or protein complexes by purification can greatly simplify the proteomic analysis. This information will help to understand the overall architecture of cells and explain how expression of certain proteins gives a cell its unique characteristics.

Functional / Interactions proteomics

"Functional proteomics" is a broad term for many specific, directed proteomics approaches. In some cases, specific sub-proteomes are isolated by affinity chromatography for further analysis. This could include the isolation of protein complexes or the use of protein ligands to isolate specific types of proteins. This approach allows a selected group of proteins to be studied and characterized and can provide important information about protein signalling, disease mechanisms or protein-drug interactions.



CONFIDENTIAL

The conventional **techniques for purification of proteins** are chromatography based such as

1. ion exchange chromatography (IEC),
2. size exclusion chromatography (SEC) and
3. affinity chromatography

For analysis of selective proteins,

1. enzyme-linked immunosorbent assay (ELISA) and
2. western blotting can be used.

These techniques may be restricted to analysis of few individual proteins but also incapable to define protein expression level.

Sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE), and **two-dimensional gel electrophoresis (2-DE)** and **two-dimensional differential gel electrophoresis (2D-DIGE)** techniques are used for separation of complex protein samples.

Protein microarrays or chips have been established for high throughput and rapid expression analysis; however, progress of a protein microarray enough to explore the function of a complete genome is challenging.

The diverse proteomics approaches such as **mass spectrometry (MS)** have **developed to analyze the complex protein mixtures with higher sensitivity**.

Additionally, **Edman degradation** has been developed to **determine the amino-acid sequence of a particular protein**.

Isotope-coded affinity tag (**ICAT**) labeling,

stable isotope labeling with amino acids in cell culture (**SILAC**) and

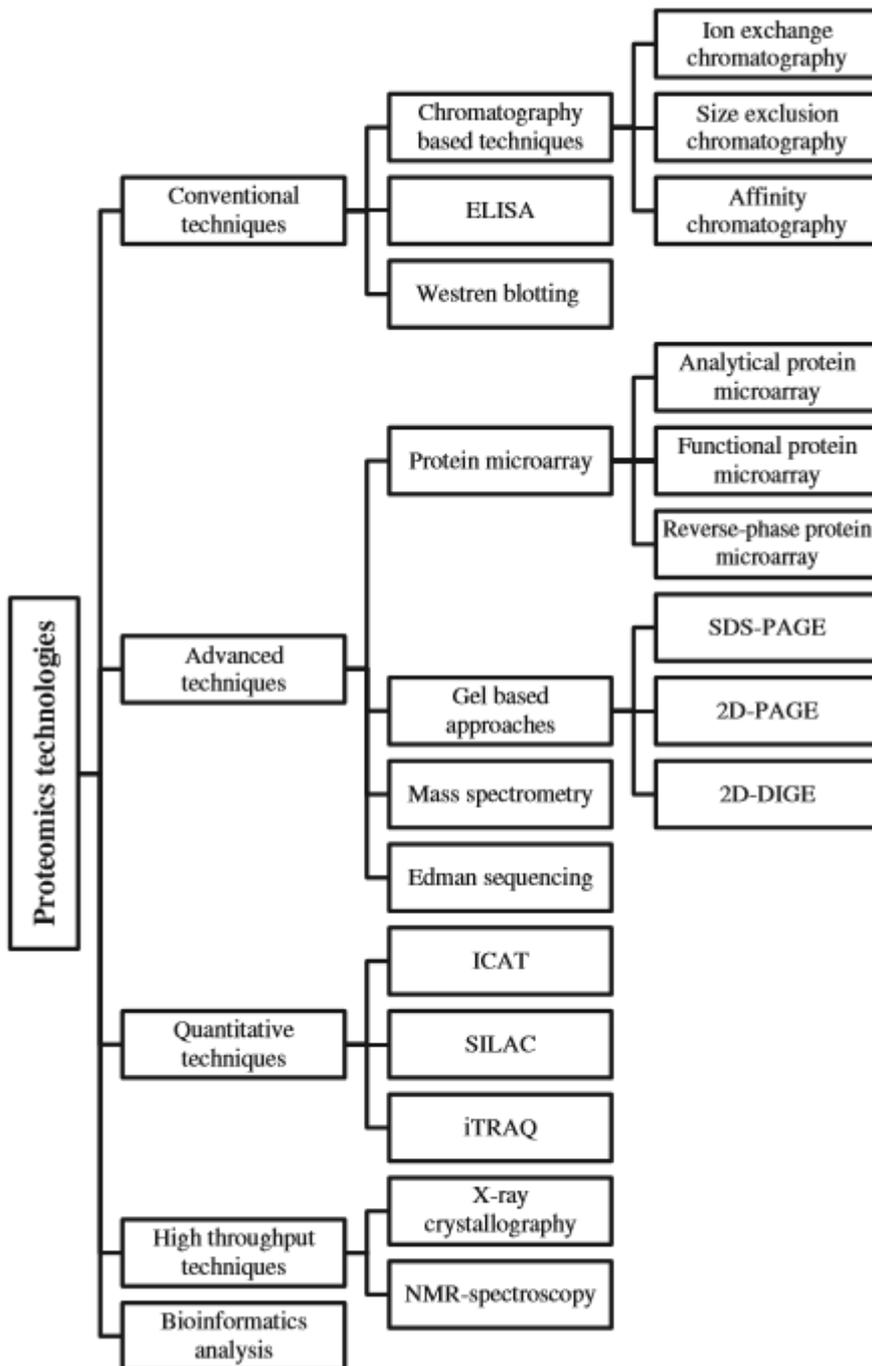
isobaric tag for relative and absolute quantitation (**iTRAQ**) techniques have recently developed for quantitative proteomic.

X-ray crystallography and nuclear magnetic resonance (NMR) spectroscopy are two major high-throughput techniques that provide three dimensional (3D) structure of protein that might be helpful to understand its biological function.

With the support of high-throughput technologies, a huge volume of proteomics data is collected.

Bioinformatics databases are established to handle enormous quantity of data and its storage. Various bioinformatics tools are developed for 3D structure prediction, protein domain and motif analysis, rapid analysis of protein-protein interaction and data analysis of MS. The alignment tools are helpful for sequence and structure alignment to discover the evolutionary relationship.

Proteome analysis provides the complete depiction of structural and functional information of cell as well as the response mechanism of cell against various types of stress and drugs using single or multiple proteomics techniques.



An overview of Proteomic Technique