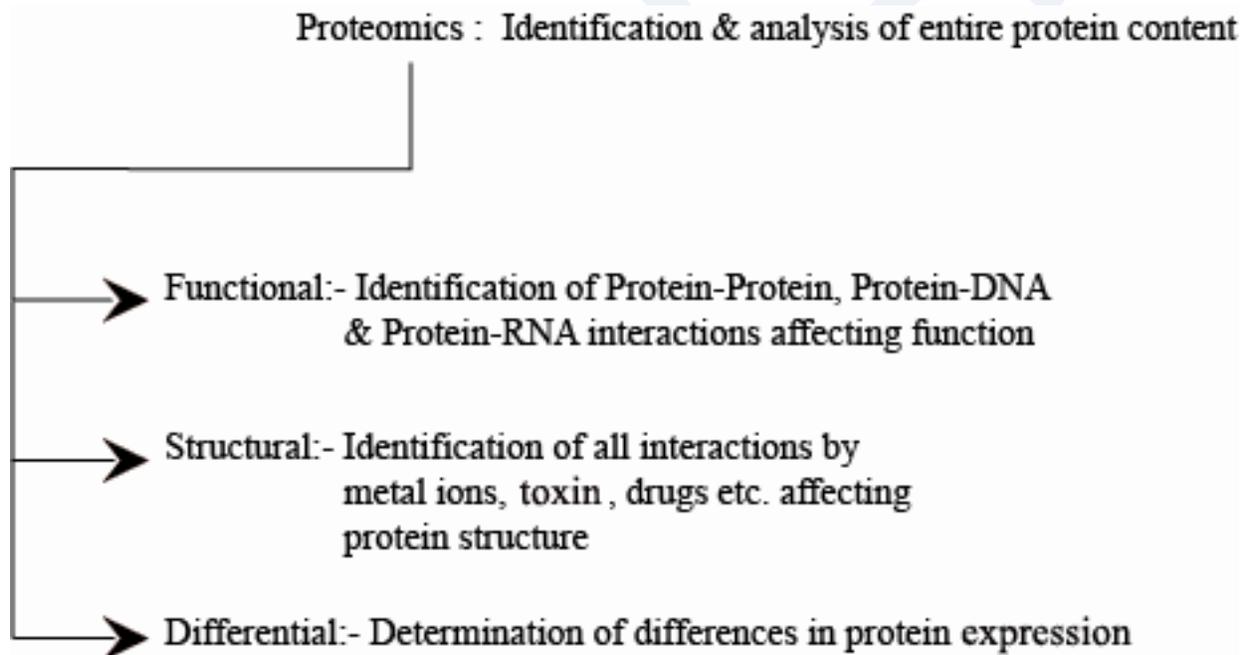## Lecture 1: Introduction and scope of Proteomics

The word "proteome" represents the complete protein pool of an organism encoded by the genome. In broader term, Proteomics, is defined as the total protein content of a cell or that of an organism. Proteomics helps in understanding of alteration in protein expression during different stages of life cycle or under stress condition. Likewise, Proteomics helps in understanding the structure and function of different proteins as well as protein-protein interactions of an organism. A minor defect in either protein structure, its function or alternation in expression pattern can be easily detected using proteomics studies. This is important with regards to drug development and understanding various biological processes, as proteins are the most favorable targets for various drugs. Proteomics on the whole can be divided into three kinds as described below (Fig. 1):
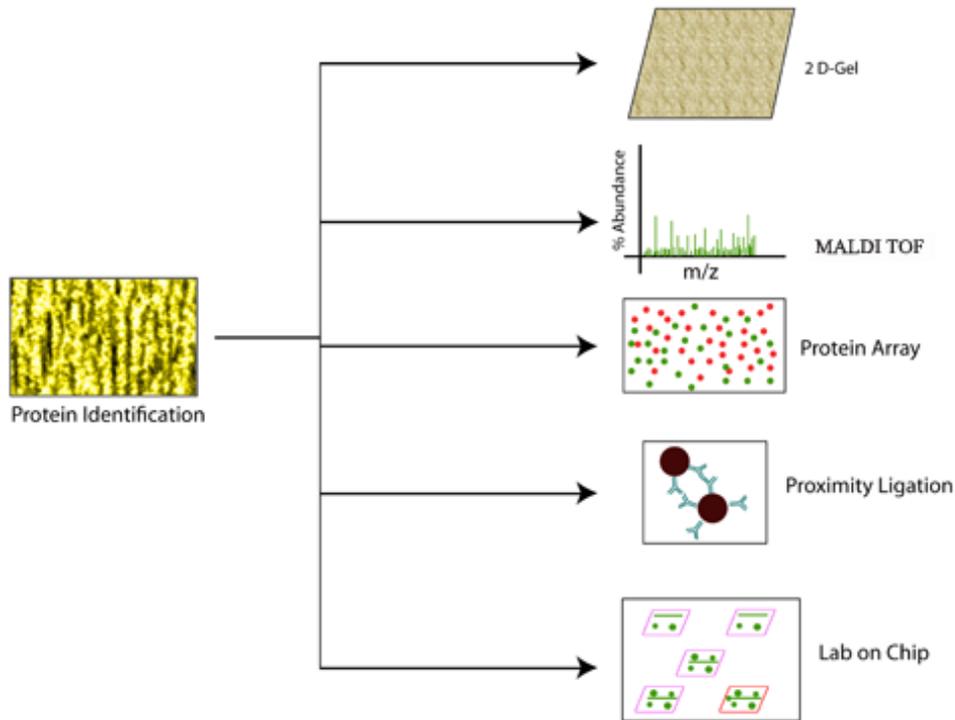
Proteomics : Identification & analysis of entire protein content

Functional:- Identification of Protein-Protein, Protein-DNA & Protein-RNA interactions affecting function

Structural:- Identification of all interactions by metal ions, toxin , drugs etc. affecting protein structure

Differential:- Determination of differences in protein expression

**Figure 1:** Types of Proteomics: Functional, structural and differential proteomics.

# Techniques Involved in Proteomics Study

Some of the very basic analytical techniques are used as major proteomic tools for studying the proteome of an organism. We shall study most of these techniques as we progress in the course. The initial step in all proteomic studies is the separation of a mixture of proteins. This can be carried out using Two Dimensional Gel Electrophoresis technique in which proteins are first of all separated based on their individual charges in 1D. The gel is then turned 90 degrees from its initial position to separate proteins based on the difference in their size. This separation occurs in $2^{nd}$ dimension hence the name 2D. The spots obtained in 2D electrophoresis are excised and further subjected to mass spectrometric analysis of each protein present in the mixture.

Apart from charge and size of proteins there are number of other intrinsic properties of proteins that can be employed for their separation and detection. One of these techniques is Field Flow Fractionation (FFF) which separates proteins based on their mobility in presence of applied field. The difference in mobility may be attributed to different size and mass of proteins. The applied field can be of many types such as electrical, gravitational, centrifugal etc. This technique helps in determining different components in a protein mixture, different conformations of protein, their interaction with other proteins as well as some organic molecules such as drugs (Fig. 2).

**Figure 2.** Techniques involved in protein identification during proteomic analysis.

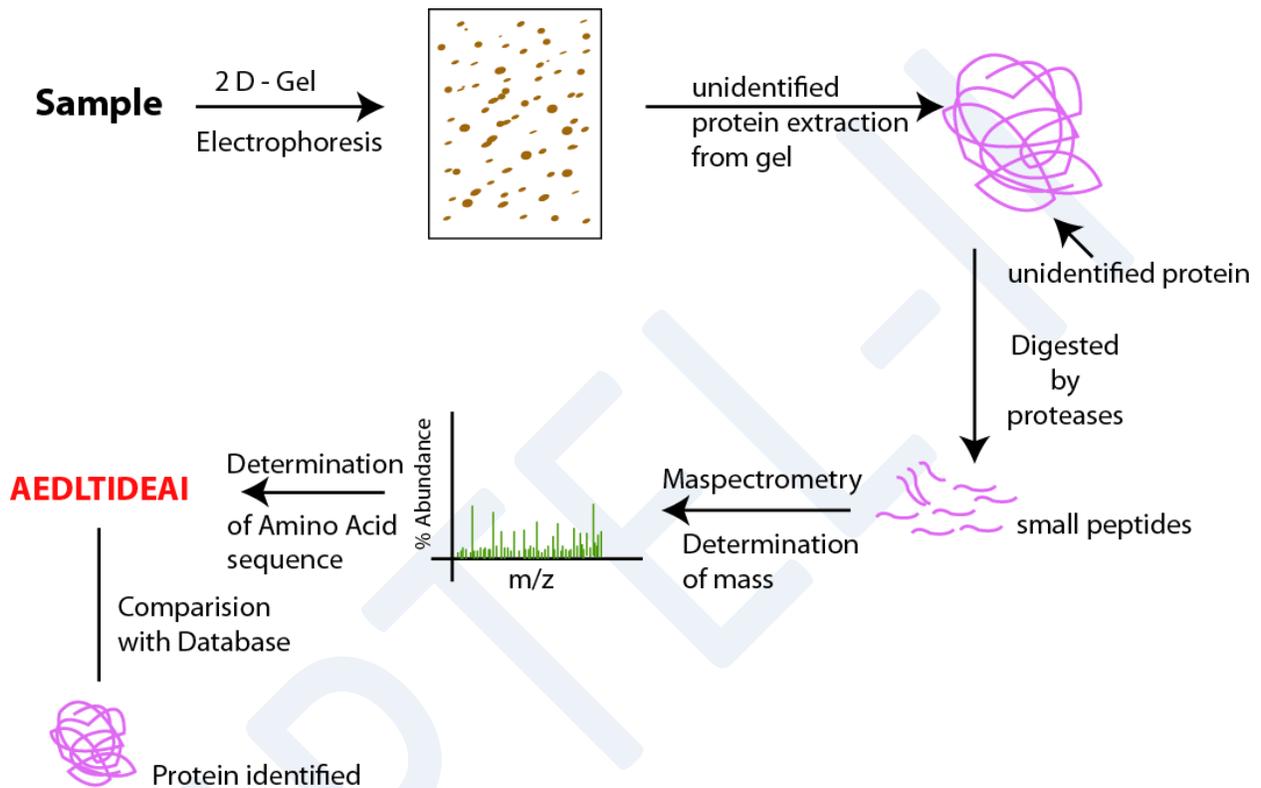## Steps in Proteomic Analysis

The following steps are involved in analysis of proteome of an organism as shown in Fig. 3:

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:* The separated protein spots on gel are excised and digested in gel by a protease (e.g. trypsin). The eluted peptides are identified using mass spectrometry.

   Analysis of protein molecules is usually carried out by MALDI-TOF (Matrix Assisted Laser Desorption Ionization-Time of Flight) based peptide mass fingerprinting.

Determined amino acid sequence is finally compared with available database to validate the proteins.

Several online tools are available for proteomic analysis such as Mascot, Aldente, Popitam, Quickmod, Peptide cutter etc.



**Figure 3.**Overview of steps involved in proteomic analysis

**Applications of Proteomics:**

Proteomics has broad applications in all the aspects of life sciences including several practical applications as drug development against several diseases.  Difference in expression protein expression profile of normal and diseased person may be analyzed for target protein.  Protein to gene may be predicted (Fig.4). Once protein/gene is identified, function may be predicted. This can help in disease management/drug development.

# From Protein To Gene
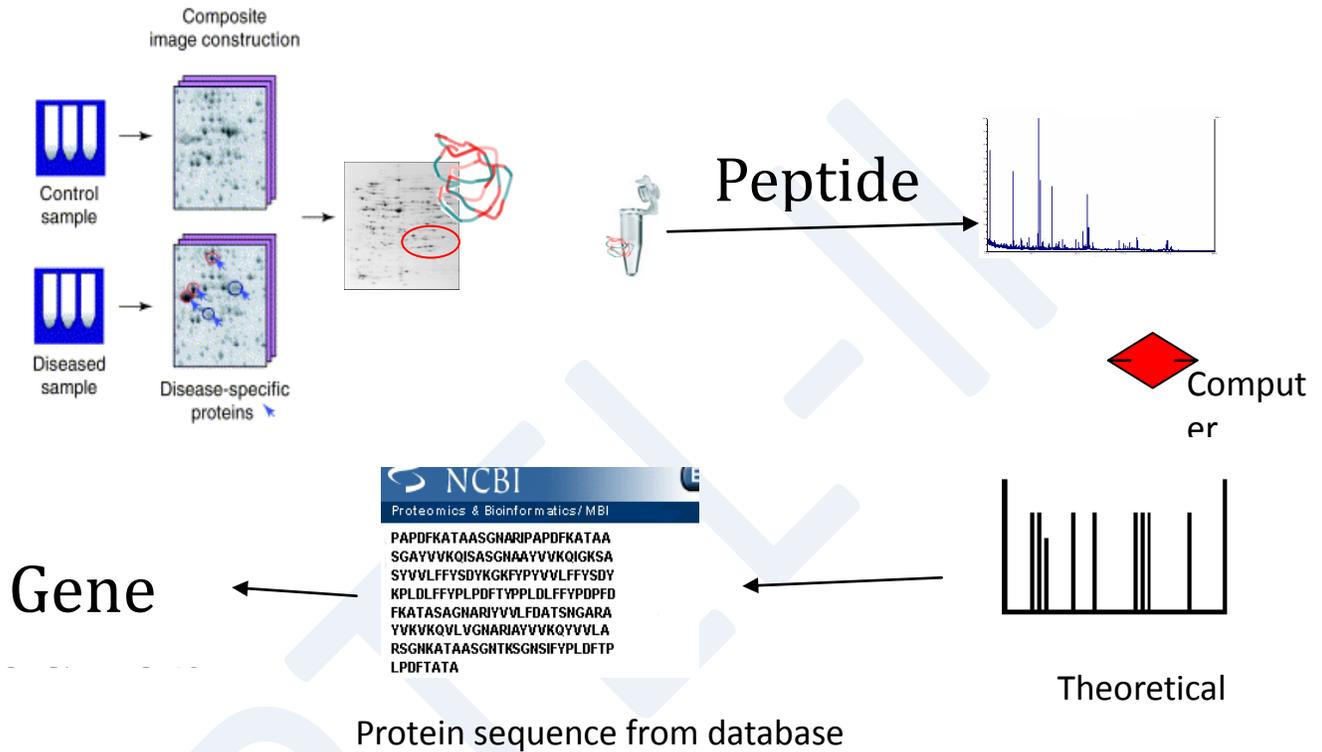


**Figure 4** : Proteomic analysis-protein to gene sequence

Whole Genome Sequences of several organisms have been completed but genomic data does not show how proteins function or how these proteins are involved in biological processes. Gene codes for a protein by at several occasion proteins are modified after synthesis (several types of post-translational modifications) for functional diversification. In the current course, we shall study several applications of proteomics in coming lectures.