

Proteomics

[Science](#), [Biology](#)



Tracing the footprints of Proteomics – To compare and study the techniques used in proteomics since the last decade. Abstract: Proteomics is a study of the proteome of an organism. The last few decades have seen a rapid progress in the development of this field. This paper attempts to compare and contrast the way in which proteomics studies are performed today as opposed to those performed ten years ago and analyse its future implications. The thrust of research while studying biology at a molecular level initially was focused specifically on the genomes of various organisms. As scientists discovered the intricacies of genes and their functionalities, the attention was soon drawn towards the end result of the central dogma of molecular biology, namely, the proteins, produced through translation of RNAs. Therefore, to study the proteins produced in an organism, referred to as the proteome, not just as products of a genome, but more importantly how they interact and bring about changes at the macro level, the field of proteomics has emerged. (1)

Proteins play a pivotal role in carrying out various functions in a body at the structural and dynamic levels. Proteins as enzymes and hormones regulate the vital metabolic processes and as structural components provide stability to the cellular components. The knowledge obtained through the study of these systems gives an insight into the overall functioning of the living organisms. In spite of having similar genetic blue prints, the protein expression in various organisms are regulated differently through diverse networks of protein-protein interactions.

Hence, proteomics provides an understanding about these regulatory processes and establishes the differences and similarities between the evolutionary pathways of the organisms by grouping them under phylogenetic trees. Further, drugs can be developed for specific diseases by designing structural analogues of proteins responsible for diseased conditions after elucidating their structures, which can then up or down regulate metabolic processes.

Thus, the study of proteins plays an essential part of researches carried out in other related fields of study such as developmental and evolutionary biology and drug designing. (1)(2) Since the invention of the 2-Dimensional Gel Electrophoresis in the 1970s, which is considered to be the stepping stone of modern day protein studies, scientists have been constantly striving to develop new and potent methods to study proteomics.

Thus, this paper is an attempt to identify and compare these techniques which have been used and improved over the last decade. The popular and preferred procedure to study the proteome of an organism comprises of three major steps, isolation, separation on 2-D gel and analysis through a mass spectrometer. Most of the improvements revolve around this basic protocol. 2-D gel electrophoresis was one of the first methods which were used to analyse the proteome of an organism. In this technique, the protein is separated on the basis of its charge and size.

The proteins are first separated on the basis of their different charges in the 1st dimension, following which they are separated on the 2nd dimension on the basis of their molecular weight. The gel or map provides a graphical

representation of each protein after separation and hence they can be distinguished individually. However, the reproducibility of the results obtained through such an analysis has not been satisfactory. Till date there are constant efforts being made to improve the efficacy of this technique, such that a large number of proteins could be separated at the same time.

The first 2-D separation which was carried out by using the electrophoresis buffer and starch gel, the improvements which followed gave rise to the foundation of modern day 2-D separation, which was combining two 1-d techniques involving separation on the basis of pH using isoelectric focusing (IEF) and using SDS-Page for separation on the basis of molecular weight after the samples have been prepared specifically using various reagents such as Urea (as a chaotrope to solubilise) and DTT (to break di-sulphide linkages without fragmentation into peptides), in a suitable buffer (3).

Further, for certain segments of proteins which were hydrophobic in nature, like those found in the cell membrane, it was discovered that special reagents such as thiourea, sulfobetaine and tributyl phosphine which are classified as chaotropes, surfactants and reducing agents respectively, assisted their solubility during sample preparation before running them on the gel. Another notable extension of 2-D separation was the use of IPG strips, which had different pH gradients. These strips were made available commercially and drastically contributed to the convenience of the technique.

Also, experiments were carried out using a number of such strips to increase the range of pH, hence successfully accommodating a large number of

proteins(4). Nevertheless, such a method, although successful, was human-error prone and hence the results on the varied from each other in majority of cases. To overcome this, a number of replicates of the gel had to be prepared and therefore demanded a lot of labour. To overcome this barrier, the differential gel electrophoresis technique DIGE was developed. In this method, the proteins are labelled with fluorescent dyes prior to electrophoresis.

The fluorophores are joined via an amide linkage to the amino acid lysine and therefore the proteins can be resolved together on the same gel through distinguished patterns of fluorescent emissions (5). Further advancement of the standard 2-D gel analysis was to incorporate automation to the technology, however the room for automation to analyse the results was limited due to the inability of a computer to distinguish between the different patterns. Differentiating a spot of protein on a gel, its intensity and to separate it from a background still remains an overwhelming task for the computer.

The next step in proteome analysis is protein identification using mass spectrometry (MS). One of the most compelling problems of using MS to study biomolecules such as proteins was the inability to obtain ions of sufficiently large size which would effectively lead to their identification. Since the development of Electron Spray ionization and MALDI (Matrix assisted Laser Desorption Ionization) this drawback of MS was overcome and today the combination of these ion sources with different mass analysers e.g.

MALDI-TOF/TOF, ESI Q-TOF and ESI triple quadrupoles are used widely in proteomics. Identification of a protein is carried out through a process referred to as peptide mass fingerprinting (PMF). In this technique, proteins that have been separated on a 2-D gel are excised and digested into peptides using proteases such as trypsin. The digested peptides, when subjected to study in a MS, give a characteristic m/z spectrum. The protein can be identified when this data correlates to the data in protein databases; compared using softwares based specific algorithms.

However, to extrapolate a proteins role in metabolism, it is also necessary to identify how the protein is modified after translation. Post translation modification plays an important role in acting like a regulating switch; modifications such as phosphorylation play an important in processes such as cell signalling. The main drawback while analysing a phosphorylated protein through MS was its signal suppression. To rectify this issue, high performance separation techniques such as HPLC were conjugated with the MS; LC-MALDI-MS is an example of such a combination (6).

Further extension of the protein mass fingerprinting was the development of shotgun proteomics, to specifically do away with the disadvantages of a standard 2-D gel analysis. This technique is based on separation of peptides obtained after protease digestion, using multidimensional chromatography. It is necessary that the two dimension of this multidimensional separation done using chromatography are orthogonal in nature, i. e. using two different properties of a protein similar to a 2-D gel separation which uses pI and mass.

Separating proteins using reversed phase, based on hydrophobicity, and Strong cation exchange, using the charged state of the peptides is an example of separation in two dimensions. Although the PMF approach provided a successful identification process to recognize the proteins present in a proteome, it was also necessary to study the changes in protein expression in response to a stimulus. To achieve this, the technique called ICAT was developed which protein mixtures from after isolation were modified such that they can be differentiated on the basis of mass from one cellular location to another.

In ICAT, this modification is done using a cysteine with an isotope labelled biotin tag. Today, the efforts to develop new technologies are directed towards automation in sample preparation and effective interfacing with other techniques. Interfacing has been achieved more successfully with ESI than MALDI owing to its ability of operating with a continuous flow of liquid (7). Sample from organisms contain thousands of proteins, to effectively separate certain important proteins such as disease biomarkers from this mixture, is a highly demanding task.

Further, effective proteolytic digestion can be challenging when the proteins of interest are present in low quantities. Therefore, before a sample of protein can be effectively analysed there are a number of steps to be performed which are prone to human error and are laborious. The development of Micro-fluidic system as an interface with the mass spectrometer such as ESI provides the option of automating this process and hence making proteome analysis more effective less time-consuming.

Therefore, such a chip based technology has a clear advantage over the traditionally used methods due its improved probability of obtaining the protein of interest, reduced consumption of reagents and accelerated reaction time. The micro fluidic chips can be directly coupled to an ESI- MS using a pressure driven or electro-osmotic flow. Thus, such a system where there is a direct interface is called an on-line setup. On the other hand, such a setup cannot be achieved in MALDI where a mechanical bridge is created between the micro-fluidic chip and the Mass spectrometer.

The first step of a proteome analysis, i. e. sample purification is carried out using a hydrophobic membrane integrated into an inlet channel of a polyimide chip. Separation of proteins from the sample can be achieved either using a capillary electrophoresis (CE) or a liquid chromatographic (LC) method. CE is usually preferred over LC due as it provides a faster separation and can be coupled to an electric pump. Proteolytic digestion is carried out on the solid surface of the chips, where the enzymes are immobilized.

Thus, such a chip provides a platform for the automation of the initial steps of a proteomic study, and more studies are still being performed to increase the efficacy of this approach (8). To conclude, over the last decade, there has been a rapid progress in the techniques used to study proteomics. The direction of progress has also shed a light on the importance of proteomics and the implications if would have in the coming years. Studies on evolution have benefitted a great deal with the development of techniques like ICAT

which enhances quantitative and comparative studies of the different proteomes.

In the field of medicine and drug discovery, the application of these techniques, paves the road for discovery of novel biomarkers for specific diseases in a quicker and less complicated manner. Further, it would also assist vaccine development by identifying specific antigens for a disease. The developments of micro-fluidic chips have opened the door for new diagnostics techniques by characterizing effectively the protein responsible for a diseased state. Such an approach has already been employed to study the proteins produced in the body in a cancerous state.

Therefore, as more researchers and academics adapt these with these applications, many more improvements would soon evolve. References: 1. Anderson, L. , Matheson, A. and Steiner, S. (2000). “ Proteomics: applications in basic and applied biology. ” Current Opinion in Biotechnology Vol: 11: pp. 408–412. 2. Pazos, F. and Valencia, A. (2001). “ Similarity of phylogenetic trees as indicator of protein protein interaction. ” Protein Engineering Vol: 14: no 9: pp. 609-614. 3. Klose, J. (2009). From 2-D electrophoresis to proteomics. ” Electrophoresis Vol: 30: pp. 142–149. 4. Herbert, B. (1999). “ Advances in protein solubilisation for two-dimensional electrophoresis. ” Electrophoresis Vol: 20: pp. 660- 663. 5. Alban, A. , David, S. , Bjorkesten, L. , Andersson, C. , Sloge, E. , Lewis, S. and Currie, I. (2003). “ A novel experimental design for comparative two-dimensional gel analysis: Two-dimensional difference gel electrophoresis incorporating a pooled internal standard. Proteomics Vol: 3: pp. 36–44. 6. Reinders, J. , Lewandrowski, U. ,

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