Review Article

Overview of Proteomics

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Abstract

The era of genomics has brought tremendous advancement especially in the fields of medicine and life sciences. Despite the overwhelming growth of information generated from genomics research, a huge gap specifically in relation to genome expression persists, and became increasingly noticeable. This has sparked interest in studies of proteins expression and eventually added limelight to the field of proteomics. Aside from the need to fill the gap, the emergence of proteomics is also deemed to have occurred due to the advancement in capabilities of research techniques, particularly in the separation and identification of proteins. Proteomics has since progressed and is slowly extending into other research as well as applied subspecialties. This brief overview was written to provide a basic and simplified understanding of proteomics in view of its growing interest from newcomers to the area.

Keyword: proteomics

Defining Proteomics

Proteomics is broadly defined as the systematic analysis of the protein expression of a proteome. The term 'proteome' was introduced to describe an entire protein complement expressed by a genome, or by a particular cell or specific tissue type [1,2]. In many cases, the proteome is a virtual reality that arises from accumulation of data obtained from separate analyses of subproteomes, which were done at different times and under different experimental settings and conditions. It is therefore an annotation of all normal/wild type proteins that may be expressed by a genome with complete disregard of the differences of time, conditions or regulatory parameters. Thus, all studies annotating or analyzing the genomic expression in the form of proteins being expressed within a defined proteome or subproteome are termed proteomics.

Why is Proteomics Necessary?

Aside from water, protein is the main constituent of the human body. The body's function is also orchestrated by proteins in the form of its structures, biocatalysts, modulator and transporter molecules, metabolites as well as an energy source at crucial times of requirement. Proteins not only form the human body but operate it. It is also the main site of action of most drugs that are used to manipulate and assert necessary changes to the physiological conditions of the human body as and when required. Perhaps the greatest expectation from proteomics comes from research carried out to identify new protein targets in transformed cell lines and diseased tissues for the actions of specific drugs. Added knowledge on proteins interaction may also drive new initiatives to design better drugs. In order to gain further insight into many diseased conditions, in-depth knowledge and understanding of protein expression and interaction, i.e., the processes that uphold the normal physiological functions of the human body, is increasingly in demand.

Although the synthesis of proteins is directed by the genome, the expression of proteins within a cell cannot be directly predicted from genomic information. It is not always a direct complement of the genome. For example, the proopiomelanocortin gene when expressed in the corticotrophic cells of the anterior pituitary generates adrenocorticotropic hormone and β-lipotropin, but expression of the same gene in the cells of the intermediary pituitary gland produces a different set of hormones comprising α-melanocyte-stimulating hormone, corticotrophin-like intermediary peptide, γ-lipotropin and β-endorphin [3]. This phenomenon which is attributed to differential peptide processing, together with other mechanisms like alternative splicing, mRNA editing, polyadenylation and post-translational modifications, by and large, results in different forms of protein products that all arise from the same genome (Fig. 1). Further additions to this complexity are the facts that different cells or tissues express different genes and many of these genes are not constitutively expressed but rely on the presence of specific modulators. All proteins are also continuously degraded, sorted and may be modified upon interaction with other proteins. Thus, the dynamics of protein expression and interaction within a defined proteome at various stages and conditions, more often than not, could only be unraveled at its level and not possibly deduced from mere genomic information. This has become even clearer when analysis of mRNA

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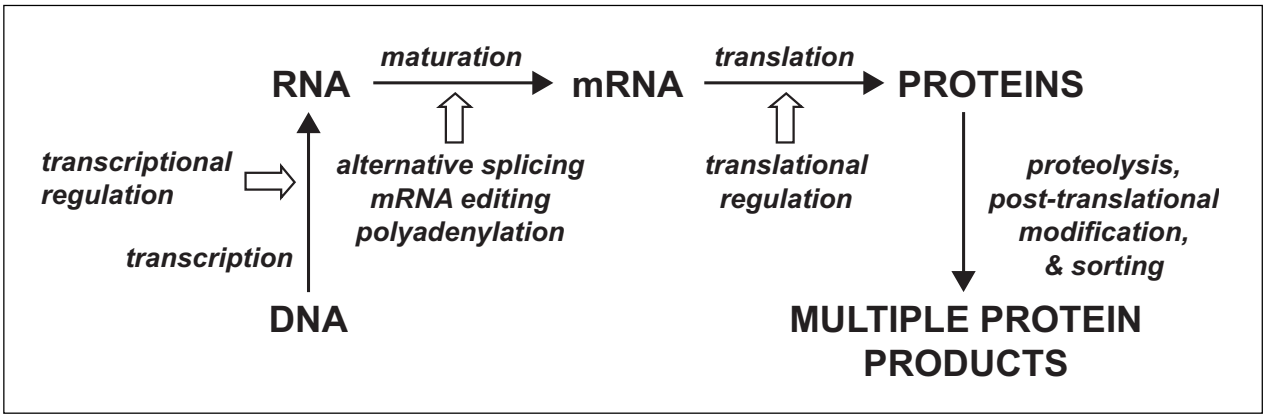

Figure 1: Genomic expression. Due to multiple processing and regulatory mechanisms at different stages of the expression of a gene, multiple protein products may be generated.

expression using methods like serial analysis of gene expression and DNA microarray technology have now shown poor correlation between mRNA and protein expression levels [4-6].

At tandem with the need to perform its studies, the era of proteomics has also evolved from the advancement of techniques used in the identification and separation of proteins. A tremendous leap has generally taken place as proteins can now be identified using mass spectrometry and the separation of proteins by two-dimensional gel electrophoresis (2-DE) is modified and improved. An attribute to the genomic work, compilation of cDNA sequences of proteins are directly translated into putative amino acid sequences. By identifying specific positions of trypsin action, amino acid sequences of polypeptides within the cleavage points of the enzyme are identified. The masses of the putative tryptic peptides of proteins are deciphered from databases, which are then used as references, in the form of peptide mass fingerprints (PMF), for identification of proteins. In the process of identifying a protein, masses of its tryptic peptides, obtained by mass spectrometry analysis, are generally used to search for matching PMFs in the database. Identification is generally achieved when considerable matches are made.

As for the separation of proteins, although the use of 2-DE has been reported as early as 1975 by O'Farrell [7] and Scheele [8], its popularity was only seen approximately twenty years later when the technique was proven to be reproducible and more convenient [9]. This was made possible mainly when the problem of gel gradient drift [10] was remedied with the use of immobilised pH gradient in replacement of carrier ampholytes [11].

Applications of Proteomics

At present, proteomics may be categorized into three different types, i.e., expression proteomics, structural proteomics and functional proteomics, depending on its application. Expression proteomics, which is also called classical proteomics, is the quantitative study of protein expression between samples that differ by some variables using a combination of separation and identification techniques like 2-DE and mass spectrometry. In this case, variables would include comparison between different cells or tissues, stages of a disease, the states of being induced or inhibited, normal or diseased, treated or non-treated and many others. The data obtained from such comparison studies will certainly enhance understanding in the specific areas of medicine or life sciences. Differences in the expression of the proteins that are detected in the analyses of the proteome or subproteome may also form a basis for further investigations to seek improvements in techniques and methodologies that are currently being applied in diagnosis and treatment of patients.

Our studies comparing the serum 2-DE protein profiles of patients with breast and nasopharyngeal cancers with that of matching normal healthy controls are examples of expression proteomics. By comparing the serum protein profiles of normal individuals with that of newly detected and untreated patients with breast cancer, we were able to demonstrate significant differences in the expression of several acute-phase proteins [12]. Identities of the serum proteins were verified by mass spectrometry. The aberrant expression of some of the proteins was validated by ELISA as well as by immunohistochemical staining of the respective lesions of the breast. Unlike that of patients with breast cancer, the sera of patients with nasopharyngeal carcinoma (NPC) demonstrated 2-DE protein profiles similar to that of the controls, with exception of the ceruloplasmin spots, which appeared up-regulated [13]. The enhanced ceruloplasmin expression, which was also validated by ELISA studies, was normalized when the NPC patients were treated. Immunohistochemical analysis of nasopharyngeal lesions of NPC patients demonstrated positive staining for ceruloplasmin only at the malignant areas. These expression proteomics studies may form a basis for further research to investigate whether the differentially expressed proteins may be used as additional biomarkers to aid diagnoses of both the cancers or to monitor the progress of their treatment towards patient's recovery.

Structural proteomics are studies whose goal is to map out all free proteins or protein complexes that are present in a specific cellular organelle. This involves attempts to identify all proteins within an organelle or a multiprotein complex, determine where they are located, and characterize all protein-protein interactions. Establishment of subproteomes by isolating specific subcellular organelles or protein complexes by purification can greatly simplify analysis of structural proteomics [14]. Identifying the proteins and activities localized to specific cellular compartments provides insight on the structural organization of the cell. Analysis of the nuclear pore complex of yeast is a good example of this subtype of proteomics [15]. The data obtained has helped piece together the overall architecture of yeast cells and explain how expression of certain proteins contributes to the unique characteristics of a cell.

Functional proteomics is a broad term generally used to define many specific, directed proteomic approaches, usually under non-denaturing conditions and smaller subset of proteins. In some studies, specific subproteomes are isolated by affinity chromatography and analyzed for their protein expression. This could include the isolation of protein complexes or the use of protein ligands to isolate specific types of proteins or glycoproteins. This approach, which seems to have attracted more interest in recent years, allows a selected group of proteins to be analyzed and characterized in their native forms, and can provide important information about disease mechanisms, protein signaling or protein-drug interactions.

More recently, the use of protein microarrays as an approach in functional proteomics has been explored [16]. Microarrays, generated by spotting biomolecules on a solid surface at high spatial density, offer miniaturized, robust platforms for high-throughput study of proteins. Two different forms of protein microarrays have been described. The abundance-based microarrays measures the abundance of specific biomolecules using analyte-specific reagents such as antibodies, while the function-based microarrays examines protein functions by printing a collection of target proteins on the array surface and assessing their interactions and biochemical activities.

A good example of functional proteomics is the use of protein microarrays in the form of chip-based antibodies to screen labeled sera from patients with prostate cancer for potential biomarkers [17]. A capture microarray containing 184 antibodies targeting serum proteins, proteins previously detected in sera of cancer patients and intracellular proteins were used. From this, five proteins were demonstrated to be able to discriminate prostate cancer serum from control. Four of these serum proteins were those that had been previously associated with prostate cancer.

Proteomics Technology

The technology of proteomics is generally divisible into three different stages involving separation, identification and quantification of proteins.

Separation of Proteins

Proteomics analysis by mass spectrometry may be carried out on proteins that are separated by one-dimensional gel electrophoresis (1-DE). The technique is simple to perform, reproducible and can generally separate proteins that differ by a few kDa. Nevertheless, the resolving power of 1-DE is rather limited and thus, the technique is usually carried out on protein mixtures after some stages of purification. Separation of proteins by 1-DE occurs on the basis of differences of molecular mass.

For more complex mixtures of proteins such as a crude cell lysate, high-resolution 2-DE is a better choice. 2-DE separates proteins on the basis of their net charge in the first dimension and molecular mass in the second dimension. Despite being labor-intensive and time consuming, the technique has become a popular choice among researchers mainly due to its high resolving power and reproducibility. The technique has the ability to resolve proteins that have undergone some form of post-translational modification like phosphorylation [18] or glycosylation [19]. The primary application of 2-DE is in protein expression profiling. The appearance and disappearance of spots, usually detected by subjecting the gels to silver staining, provide information on differential protein expression while the intensity of the spots provides quantitative information on protein expression levels.

Although 2-DE is generally performed using a standard protocol, modifications may be required depending on samples to be analyzed. Analysis by 2-DE generally requires samples that are denatured, free of contaminants (mainly salt) and at optimal loading concentration. Denaturation of proteins is important so as to prevent oxidation, aggregation and protein-protein interaction. Under native conditions, proteins exists in different conformations, may aggregate and interact with other proteins and therefore cannot be analyzed by 2-DE. Too much salt in the sample disturbs isoelectric focusing and leads to streaky patterns. For comparative purposes, all samples are to be kept and treated similarly as proteins are continuously degraded and the rate of degradation of protein varies at different conditions.

Despite many improvements, 2-DE is still limited in its ability to resolve proteins that are large, hydrophobic or those of extreme acidity or basicity [20]. A eukaryotic cell lysate protein mixture may be too complexed to be

completely resolved in a single 2-D gel [21]. Similarly, it is also impossible to resolve all proteins of samples with broad dynamic range, like plasma [22], in a single 2-DE gel. Due to these limitations of 2-DE, several alternative approaches have been used. One approach is to subject the entire protein sample mixture to trypsin digestion and then purify the peptides before subjecting them to analysis by mass spectrometry. Several methods have been used to purify the tryptic peptides including liquid chromatography [23], capillary electrophoresis [24] and a combination of ion-exchange chromatography and reverse-phase chromatography [25]. However, these methods require considerable effort, time and computing power for data analysis.

Identification of Proteins

Progress in peptide analysis using mass spectrometry has advanced very rapidly in recent years. It is currently the state of the art technique used in the identification of proteins in proteomics analysis. Prior to the use of mass spectrometry, proteins were identified by Western blotting and binding recognition using specific antibodies or on the basis of their N-terminal sequences or Edman sequencing. While the earlier technique is dependent on availability of antibodies, the latter technique is restrictive and cannot be applied on proteins that are N-terminally blocked. Nevertheless, N-terminal sequencing is still a viable alternative to mass spectrometry despite its waning application. In addition, an alternative protocol for the sequencing of proteins, in the form of mixed peptide sequencing, has been developed for protein identification purposes [26].

Before analysis using a mass spectrometer, proteins are initially subjected to peptide cleavage, usually with trypsin (Fig. 2). For proteins that are separated using gel electrophoresis, trypsin digestion is usually performed "in-gel" [27] as it is more efficient for sample recovery than other methods like electroblotting or electroelution [28]. Before being analyzed by mass spectrometry, the peptide fragments recovered following in-gel digestion need to be concentrated and purified to remove contaminants such as salts, buffers and detergents, which interfere with mass spectrometric analysis [29]. This is usually performed using miniaturized chromatography columns in the form of ZipTips, which purify proteins on the basis of reverse-phase, ion exchange or affinity chromatography principles. Alternatively the samples can also be subjected to high performance liquid chromatography.

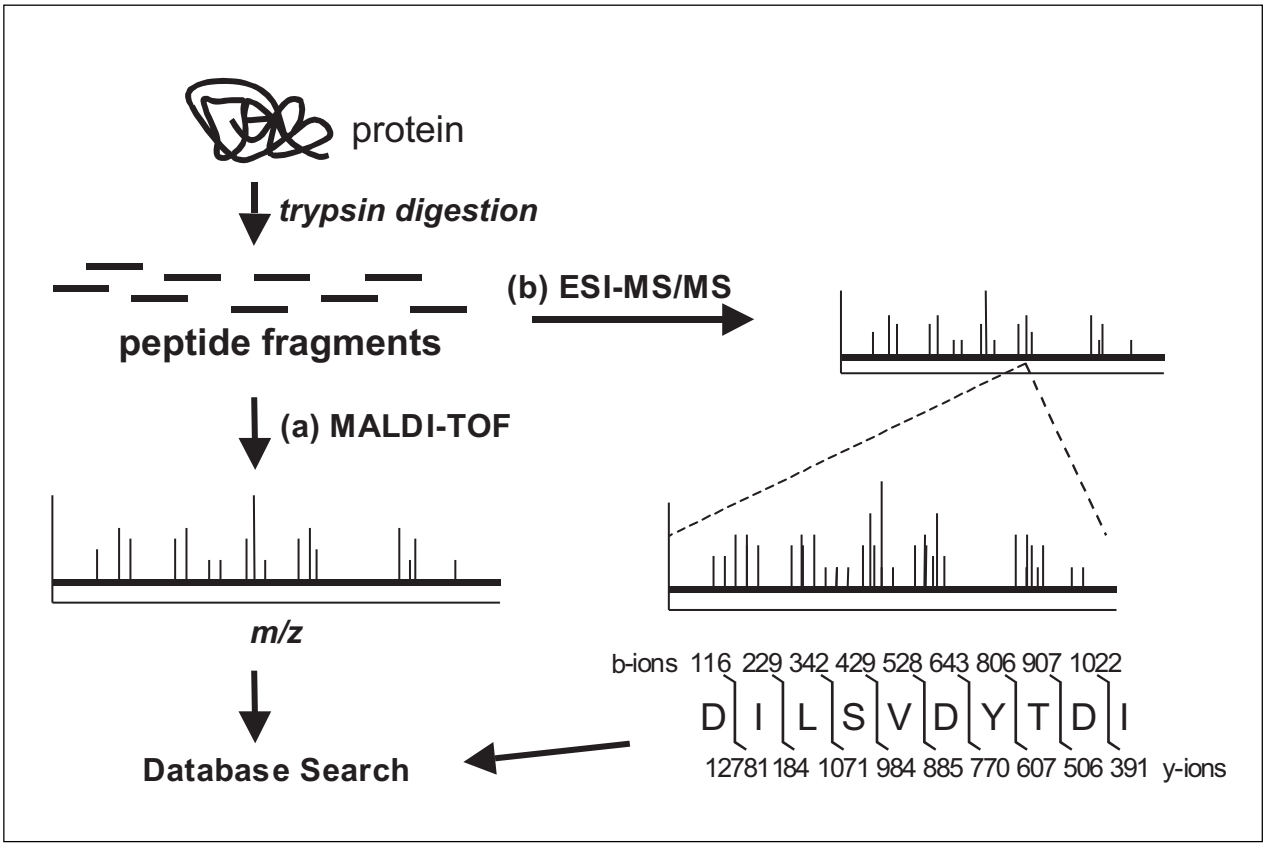

Figure 2: Mass spectrometric identification of proteins. Analyses of tryptic peptide fragments by (a) MALDI-TOF and (b) ESI-MS/MS generate data in the form of peptide mass fingerprints and sequence of amino acids, respectively. Proteins may be identified by search of the peptide mass fingerprints or amino acid sequence in protein databases (Table 1).

Analysis of the concentrated peptide fragments by mass spectrometry generates data in the forms of peptide mass fingerprints or amino acid sequences. In peptide mass fingerprinting, a mass fingerprint or map of peptides is derived from mass spectrometric analysis, e.g., by using MALDI-TOF, of the trypsin-digested peptides (Fig. 2a). By searching protein databases, the molecular masses of this set of peptides are then measured and compared against the theoretical molecular masses of known proteins cleaved with the same protease in the database. Identification is achieved when many of the molecular masses match. It is often not to find matches for all the peptides as some may have been covalently modified (e.g., glycopeptide) or resisted cleavage.

In the second approach, a tandem mass spectrometer (MS/MS), which combines two mass analyzers or two mass analysis steps, is indirectly used to determine the amino acid sequence of a peptide. In this approach, e.g., by using ESI-MS/MS, a peptide ion is further activated using a gas-phase collision to break peptide bonds creating a ladder of fragment ions (Fig. 2b). Peptide fragmentation which maintains the charge on the N-terminus is designated b-ion, whereas fragmentation which maintains the charge on the C-terminus is designated a y-ion. The fragment ions produced in the dissociation of peptides reflect the sequence of amino acids and thus can be used to search theoretical sequence databases. Table 1 demonstrates some of the common databases available in the internet that can be searched for peptide mass fingerprints or sequencing.

The two main stages involved in mass spectrometry are sample ionization and mass analysis. Mass spectrometry requires protein samples to be charged and in dry form. For this purpose, the highly concentrated, purified and digested protein samples are initially converted to desolvated ions either by matrix-assisted laser desorbtion/ionization (MALDI) or electrospray ionization (ESI). In MALDI, the sample is incorporated into matrix molecules and then subjected to irradiation by a laser [30], while in ESI, the formation of desolvated ions occur when a liquid sample evaporates as it flows through a microcapillary tube that is induced with a potential difference [31]. The biggest advantage of MALDI is that sample application can be performed using a robot so that the entire process including data collection and analysis can be automated. As such, MALDI is more suitable for ionization of peptide samples in high throughput studies. Lately, the use of surface-enhanced laser desorbtion/ionization (SELDI) in proteomics analysis is also becoming popular [32]. Unlike MALDI or ESI, SELDI is usually used to analyze complex protein mixtures directly without the need of sample preparation and purification steps.

Subsequent to their conversion to molecular ions, peptides are then subjected to mass analysis using the mass analyzers in mass spectrometers. Analysis is usually accomplished on the basis of the mass-to-charge (m/z) ratio of a peptide ion in vacuum. There are currently three main types of mass analyzers. The time-of-flight (TOF) instrument is among the simplest mass analyzers, which measures the m/z ratio of an ion by measuring the time required for it to traverse the length of a flight tube [29]. The quadrupole analyzer consists of four parallel metal rods through which the gas phase ions have to achieve a stable trajectory. The analyzer is operated by the application of a voltage to create an electric field that acts to transmit all ions or as mass filter to allow the transmission of a certain m/z ratio [33]. If multiple quadrupoles are combined, they can be used as a tandem mass spectrometer to obtain information on the amino acid sequence of a peptide. Quadrupoles can also be combined with the TOF mass analyzers to generate the hybrid type quadrupole-TOF tandem mass spectrometer [34]. However, even more sensitive and accurate are the ion trap mass analyzers (e.g., Fourier transform ion cyclotron resonance mass spectrometer), which have the ability to store ions and then selectively eject them from the ion trap unlike the quadrupole mass analyzer in which ions are discarded before analysis begins [35,36]. These mass analyzers also have the unique ability to perform multiple stages of mass spectrometry.

Quantification of Proteins

In 2-DE, in order to compare the expression of proteins between different samples, gels are often scanned and subjected to image analysis. The evaluation and comparison of the complex 2-DE profiles with the eye is impossible. For a proper evaluation, it is important to acquire the image as a grey-scale TIFF file with adequate resolution. Gels with visible spots have to be scanned in

Table 1: Common databases for peptide search in the internet.

Site name	URL	Information
MASCOT	www.matrixscience.com	Peptide mass fingerprinting & Sequencing
PeptIdent	www.expasy.ch/tools/peptident	
BLAST	www.ncbi.nlm.nih-gov/BLAST	
PepFrag	www.proteometrics.com	
FindMod	www.expasy.ch/tools/findmod	Post-translational modification

transmission mode while blot membranes are scanned in reflectance mode. Dedicated scanners are required to scan proteins that are labeled with fluorescent dyes or radioisotopes.

Quantification of individual protein spots can be performed by using computerized image analysis. Many image analysis softwares are currently available. PDQuest, ImageMaster, MELANIE and Progenesis are among the common softwares widely used by researchers. All of these softwares have the capability to quantify the expression of peptide spots in the form of volume (i.e., optical density x area in square mm of the region) subsequent to a background intensity correction. Some are sophisticated enough to match gels, correct gel distortion and perform automated quantification of annotated proteins. Also currently available is software solely meant for the quantitative image analysis of 2-DE gels that simultaneously separate two distinct protein samples that are labeled with different spectrally resolvable fluorescent dyes [37]. Because samples are distinctively labeled, differential expression of proteins can be detected directly using this method. Besides computerized image analysis, quantification can also be performed using mass spectrometers [38]. However, this requires the creation of internal standards, which must be chemically similar to the molecule that is to be measured by mass spectrometry, for each molecule to be measured.

Challenges for Proteomics

The last decade has seen an explosion of data derived from proteomics research as well as tremendous improvements in the technology of proteomics. Despite these advancements, proteomics technology is still restrictive in several aspects. Unlike genomics, proteomics lacks a "PCR-like" technique, which could be useful for studies on proteins of low abundance. Many important proteins that may be targets for drugs such as transcription factors, regulatory proteins and protein kinases are low-copy proteins. Proteomics analysis is generally dependent on theoretical databases that are derived mainly from genomics studies and these are not always available for certain organisms and plants. The existing reference proteome maps are also not complete. For example, quite a number of proteins that are detected in the human plasma, which are provided with primary accession numbers, are yet to be identified and validated. Proteomics is also faced with problems like post-translational modifications of proteins and the analysis of proteomes with a broad dynamic range of proteins (e.g. plasma proteins has an estimated concentration range of ten orders of magnitude [22]) although substantial progress has been made to tackle the problems. Despite these shortcomings and within the boundaries of its limitations, proteomics remains a major impact to scientists at the turn of this century mainly because of its role in enlightening the basis of life sciences in terms of proteins expression and interaction, and this cannot be obtained from the study of individual or groups of proteins.

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References

1. Wilkins MR, Sanchez JC, Cooley AA, Appel RD, Humphery-Smith I, Hochstrasser DF, Williams KL. Progress with proteome projects: why all proteins expressed by a genome should be identified and how to do it. Biotechnol Genet Eng Rev 1995; 13: 19-50.
2. Wasinger VC, Cordwell SJ, Cerpa-Poljak A, Yan JX, Cooley AA, Wilkins MR, Duncan MW, Harris R, Williams KL, Humphery-Smith I. Progress with gene product mapping of the Mollicutes: Mycoplasma genitalium. Electrophoresis 1995; 16: 1090-1094.
3. Raffin-Sanson ML, de Keyzer Y, Bertagna X. Proopiomelanocortin, a polypeptide precursor with multiple functions: from physiology to pathological conditions. Eur J Endocrinol 2003; 149: 79-90.
4. Anderson L, Seilhamer J. A comparison of selected mRNA and protein abundances in human liver. Electrophoresis 1997; 18: 533-537.
5. Gygi SP, Rochon Y, Franza BR, Aebersold R. Correlation between protein and mRNA abundance in yeast. Mol Cell Biol 1999; 19: 1720-1730.
6. Ideker T, Thorsson V, Ranish JA, Christmas R, Buhler J, Eng JK, Bumgarner R, Goodlett DR, Aebersold R, Hood L. Integrated genomic and proteomic analyses of systematically perturbed metabolic network. Science 2001; 292: 929-934.
7. O'Farell PH. High resolution two-dimensional electrophoresis of proteins. J Biol Chem 1975; 250: 4007-4021.
8. Scheele GA. Two-dimensional gel analysis of soluble proteins. Characterization of guinea pig exocrine pancreatic proteins. J Biol Chem 1975; 250: 5375-5385.
9. Anderson NG, Anderson NL. Twenty years of two-dimensional electrophoresis: past, present and future. Electrophoresis 1996; 17: 443-453.
10. Westermeier R, Naven T. Expression Proteomics In: Proteomics in practice, a laboratory manual of proteome analysis. New York: Wiley 2002; 11-57.
11. Bjellqvist B, Pasquali C, Ravier F, Sanchez JC, Hochstrasser D. A non linear wide-range immobilized pH gradient for two-dimensional electrophoresis and its definition in a relevant pH scale. Electrophoresis 1993; 14: 1357-1365.

12. Doustjalali SR, Yusof R, Yip CH, Looi LM, Pillay B, Hashim OH. Aberrant expression of acute-phase reactant proteins in sera and breast lesions from patients with malignant and benign breast tumors. Electrophoresis 2004; 25: 2392-2401.
13. Doustjalali SR, Yusof R, Govindasamy GK, Bustam AZ, Pillay B, Hashim OH. Patients with nasopharyngeal carcinoma demonstrate enhanced serum and tissue ceruloplasmin expression. J Med Invest 2006; 53: 20-28.
14. Blackstock WP, Weir MP. Proteomics: quantitative and physical mapping of cellular proteins. Trends Biotechnol 1999; 17: 121-127.
15. Rout MP, Aitchison JD, Suprapto A, Hjertaas K, Zhao Y, Chait BT. The yeast nuclear pore complex: composition, architecture, and transport mechanism. J Cell Biol 2000; 148: 635-651.
16. LaBaer J, Ramachandran N. Protein microarrays as tools for functional proteomics. Curr Opin Chem Biol 2005; 9: 14-19.
17. Miller JC, Zhou H, Kwekel J, Cavallo R, Burke J, Butler EB, Teh BS, Haab BB. Antibody microarray profiling of human prostate cancer sera: antibody screening and identification of potential biomarkers. Proteomics 2003; 3: 56-63.
18. Lewis TS, Hunt JB, Aveline ID, Jonscher KR, Louie DF, Yeh JM, Nahreini TS, Resing KA, Ahn NG. Identification of novel map kinase pathway signaling targets by functional proteomics and mass spectrometry. Mol Cell 2000; 6: 1343-1354.
19. Wu J, Lenchik NJ, Pabst MJ, Solomon SS, Shull J, Gerling IC. Functional characterization of two-dimensional gel-separated proteins using sequential staining. Electrophoresis 2005; 26, 225-237.
20. Gorg A, Obermaier C, Boguth G, Harder A, Scheibe B, Wildgruber R, Weiss W. The current state of two-dimensional electrophoresis with immobilized pH gradients. Electrophoresis 2000; 21: 1037-1053.
21. Celis JE, Gromov P. 2D protein electrophoresis: can it be perfected? Curr Opin Biotechnol 1999; 10: 16-21.
22. Anderson NL, Anderson NG. The human plasma proteome: history, character and diagnostic prospects. Mol Cell Proteomics 2002; 1: 845-867.
23. Link AJ, Eng J, Schieltz DM, Carmack EB, Mize GJ, Morris DR, Garvik BM, Yates JR. Direct analysis of protein complexes using mass spectrometry. Nat Biotecnol 1999; 17: 676-682.
24. Figeys D, Corthals GL, Gallis B, Goodlett DR, Ducret A, Corson MA, Aebersold R. Data-dependent modulation of solid-phase extraction capillary electrophoresis for the analysis of complex peptide and phosphopeptide mixtures by tandem mass spectrometry: application to endothelial nitric oxide synthase. Anal Chem 1999; 71: 2279-2287.
25. Opitech GJ, Lewis KC, Jorgenson JW, Anderregg RJ. Comprehensive on-line LC/LC/MS of proteins. Anal Chem 1997; 69: 1518-1524.
26. Rosenfeld J, Capdevielle J, Guillemot JC, Ferrara P. In-gel digestion for proteins for internal sequence analysis after one- or two-dimensional electrophoresis. Anal Biochem 1992; 203: 173-179.
27. Courchesne PL, Luethy R, Patterson SD. Comparison of in-gel and on-membrane digestion methods at low to sub-pmol level for subsequent peptide and fragment-ion mass analysis using matrix-assisted laser-desorbtion/ionization mass spectrometry. Electrophoresis 1997; 18: 369-381.
28. MacDonald JA, Borman MA, Muranyi A, Somlyo AV, Hartshorne D, Haystead TA. Identification of the endogenous smooth muscle myosin phosphatase-associated kinase. Proc Natl Acad Sci USA 2001; 98: 2419-2424.
29. Yates JR. Mass spectrometry and the age of the proteome. J Mass Spectrom 1998; 33: 1-19.
30. Karas M, Hillenkamp F. Laser desorbtion ionization of proteins with molecular masses exceeding 10,000 daltons. Anal Chem 1988; 60: 2299-2301.
31. Fenn JB, Mann M, Meng CK, Wong SF, Whitehouse CM. Electrospray ionization for mass spectrometry of large biomolecules. Science 1989; 246: 64-71.
32. Merchant M, Weinberger SR. Recent advancements in surface-enhanced laser desorbtion/ionization-time of flight-mass spectrometry. Electrophoresis 2000; 21: 1164-1167.
33. Yates JR. Protein structure analysis by mass spectrometry. Methods Enzymol 1996; 271: 351-377.
34. Shevchenko A, Chernushevich I, Ens W, Standing KG, Thomson B, Wilm M, Mann M. Rapid de novo peptide sequencing by a combination of nanoelectrospray, isotopic labeling and a quadrupole/time-of-flight mass spectrometer. Rapid Commun Mass Spectrom 1997; 11: 1015-1024.
35. Jonscher KR, Yates JR. The quadrupole ion-trap mass spectrometer - a small solution to a big challenge. Anal Biochem 1997; 72: 4266-4274.
36. Martin SE, Shabanowitz J, Hunt DF, Marto JA. Subfemtomole MS and MS/MS peptide sequence analysis using nano-HPLC-ESI Fourier-transform ion cyclotron resonance mass spectrometry. Anal Chem 2000; 72: 4266-4274.
37. Swatton JE, Prabakaran S, Karp NA, Lilley KS, Bahn S. Protein profiling of human postmortem brain using 2-dimensional fluorescence difference gel electrophoresis (2-D DIGE). Mol Psychiatry 2004; 9, 128-143.
38. Jiang H, English, AM. Quantitative analysis of the yeast proteome by incorporation of isotopically labeled Leucine. J Proteome Res 2002; 1: 345-350.