

Proteome and Proteomics: from Single Protein to Whole Body



Author : Dr. Leonid Tarassishin (Editor - In - Chief of JPGR)

Affiliation: Albert Einstein College of Medicine, Bronx, NY

Received : Nov 19, 2013; **Accepted :** Dec 15, 2013; **Published :** Jan 30, 2014;

Proteome is the entire set of proteins produced by a cellular system or an organism. **Proteomics** is the large-scale study of a proteome, which practically means an analysis of many proteins in parallel. Recently, it was proposed to separate Proteomics into **Expression** and **Functional** or Interaction (Cell Map) **Proteomics** which is the global analysis of protein expression and study of protein complexes and signaling pathways (virtually, protein interaction) accordingly [1].

Protein analysis started after Laemmli created the technique for protein separation by electrophoresis in SDS-polyacrylamide gel [2]. The next step, which allows the identification of the proteins, became Towbin's Western blotting which is when proteins are transferred to the nitrocellulose (or PVDF) membrane following detection with a specific antibody [3].

Another important method (Functional Proteomics) became the protein microarray for the identification of many proteins using the microchip, a solid support (glass slide, nitrocellulose membrane, beads, microtitre plate) to which an array of capture proteins is bound. The detection is usually performed with a specific probe which is labeled with a fluorescent dye following registration by a laser scanner [4].

Expression Proteomics is based on 2-dimensional (2-D) gel electrophoresis and mass spectrometry. 2-D gel electrophoresis allows the separation of thousands of proteins by isoelectrofocusing followed by electrophoresis in gradient polyacrylamide gel [5]. Another useful technique is Differential In-Gel Electrophoresis (DIGE) which is when proteins labeled with up to three different fluorescent dyes, run on the same 2-D gel and are individually scanned at appropriate fluorescent wavelengths [6].

Mass spectrometry (MS) appeared with the great possibility of identifying and quantifying proteins in complex mixtures using a minimal amount of biological specimens [7]. In fact, MS has a long history which started more than a century ago with the experiments of J.J.Thomson on ray particles. But the first analysis of amino acids and peptides using MS was performed by C.O. Anderson in 1958. The modern technique of MS is conditioned by the findings of A.J. Dempster, F.W.Aston, H.Dehmelt, W.Paul, J.B.Fenn, M.Karas, F. Hillenkamp, and K.Tanaka who developed the ion trap method, electrospray ionization, matrix-assisted laser desorption ionization and soft laser desorption.

Mass spectrometry is a powerful and sensitive technique which is used to detect, identify and

quantitate molecules by measuring the mass-to-charge ratio (m/z). This process consists of the conversion of molecules into gaseous ions, which then separate according to m/z values in magnetic or electric fields. This is then followed by their detection and analysis by appropriate software.

In Proteomics, the two most common approaches used are: peptide mass fingerprinting and tandem mass MS sequencing. Additionally, liquid chromatography helps to separate the proteins before MS. This technique can be included into so called gel-free methods which also involve a combination of affinity capture and isotope labeling (Isotope-Coded Affinity Tag /ICAT).

At present, MS is widely used in oncology, neurology, endocrinology, immunology, infection diseases and other areas of biology and medicine. In Proteomics, it is used to identify a protein from the mass of its peptide fragments, to determine the protein structure, functions and interactions, to detect post-translational modifications, quantitate the proteins, check the enzymatic reactions, et cetera.

The latest achievement in MS is Mass Spectrometry Imaging (MSI) [8]. This very powerful method allows the examination of bimolecular patterns in cells, tissues and even in the whole body. Practically, this is direct imaging of tissue (cells etc.) based on the ion signals produced by rastering a laser across the sample and recording the produced mass spectra. Three major desorption-ionization techniques used in MSI are: Matrix-Assisted Laser Desorption Ionization (MALDI), Secondary Ion Mass Spectrometry (SIMS), and Desorption ElectroSpray Ionization (DESI). The spatial information can be obtained in two ways - microprobe or scanning probe and microscope. In microprobe mode, mass spectrum from small localized areas collects until the whole sample is analyzed and then the total image is reconstructed. This is a relatively easy method which is compatible with all six mass spectrometers, but the resolution is limited by the profile of the ionization beam. In microscope mode, the special detector records time-of-flight (m/z) as well as surface position of the ion in x and y coordinates. The microscope mode is compatible only with a TOF analyzer or dispersing magnetic sector analyzer, but it gives a very high spatial resolution.

The newest step in MSI is creating 3D imaging which is able to give a full picture of the proteins as well as their interactions in biological specimens [9].

Thus, it has been a long road for what began as the study of a single polypeptide and has culminated as the study of the whole body. Is it the end? I hope not, as I believe we will definitely have new discoveries in the field of Proteomics.

A future project could be to create the **Proteome Catalog** and **Atlas** which would characterize all proteins in the human body in norm and pathology. It would be great to quickly check the protein spectrum, find the changes in some of them, and make a fast diagnosis based on this result!

Despite the huge financial pressures today, I still hope that science (particularly, biomedical science) will live in the near future. I remember performing my first Western blotting in a simple home-made apparatus just shortly after the publication of Towbin's article. And I believe that young investigators will be just as excited as I was when I saw my first protein gel. In fact, I am sure that the new generation of scientists will develop new methods and ideas, so that Proteomics will have an even brighter future.

7. Yates J.R.III. (2011) A century of mass spectrometry: from atoms to proteomes. *Nature Methods*, 8, 633-637.

8. Caprioli R.M., Farmer T.B., Gile J. (1997) Molecular imaging of biological samples: localization of peptides and proteins using MALDI-TOF MS. *Anal. Chem.*, 69, 4751-4760.

9. Seeley E.H. and Caprioli R.M. (2012) 3D imaging by mass spectrometry: a new frontier. *Anal. Chem.*, 84, 2105-2110.

References

1. Grant S.G.N., Blackstock W.P. (2001) Proteomics in neuroscience: from protein to network. *J. Neurosci.*, 21, 8315-8318.

2. Laemmli U.K. (1970) Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature*, 227, 680-685.

3. Towbin H., Staehelin T., Gordon J. (1979) Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA*, 76, 4350-4354.

4. Stoevesandt O., Taussig M.J., He M. (2009) Protein microarrays: high-throughput tools for proteomics. *Expert Rev. Proteomics*, 6, 145-157.

5. O'Farrell P.H. (1975) High resolution two-dimensional electrophoresis of proteins. *J. Biol. Chem.*, 250, 4007-4021.

6. Unlu M, Morgan ME, Minden JS. (1997) Difference gel electrophoresis: a single gel method for detecting changes in protein extracts. *Electrophoresis*, 18, 2071-2077.