Mass Spectrometry and Proteomics: Principle, Workflow, Challenges and Perspectives

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Principle

Mass Spectrometry (MS) is a method that can analyze a variety of chemical and biological compounds, from products of chemical synthesis or degradation to biological building blocks and their products, including proteins, nucleic acids, lipids, and glycans. Within the MS field, different specialized fields have emerged. They have been based mainly on the chemical or biological entities that are analyzed, including: proteomics, lipidomics, glycomics and others. Proteomics analyzes proteins, peptides, aglycans, protein interactions, or post-translational modifications (PTMs).

Proteomics is the study of the proteome which is a whole protein complement in a cell or organism at any given time. The proteome of an organism or even a single type of cells is much more complex than its corresponding genome. This is mostly due to alternate splicing and PTMs which affect virtually all proteins. The proteome differs from cell-to-cell and from time-to-time with composition depending on the physiological or pathological state of cells or organisms. Understanding that proteins are the actual effectors of biological functions has been essential part of biochemistry for over a hundred years [1]. Due to proteomes complexity, their analysis is extremely challenging. Therefore modern biochemical technologies with improved separation and identification methods have been introduced. MS became the core for advanced methods in proteomic experiments. Integration of MS with a variety of other analytical methods made it possible to examine virtually all types of samples derived from tissues, organs and organisms.

Workflow

The workflow in a proteomics experiment involves sample fractionation by 1, 2, n biochemical approaches, followed by enzymatic digestion (usually trypsin), peptide extraction, and MS analysis [2-6]. When the peptide mixture is analyzed by MALDI-MS, the proteins of interest are identified using a procedure named peptide mass fingerprinting. Alternatively, the peptide mixture is further fractionated by HPLC on different columns (usually reverse phase HPLC or LC), followed by ESI-MS analysis. The combination of LC and ESI-MS is usually named LC-MS/MS; and analysis of a protein using this approach provides not only the protein identity, but also sequence information for that particular protein [5,7-9]. In addition to qualitative information provided by MALDI-MS or LC-MS/MS analysis, MS may also provide quantitative information about a particular protein [4,10-12]. Another dimension to identification and characterization of proteins is added due to the intensive PTMs of proteins. It is a great challenge to fully identify the PTM pattern at any given time in cells, tissues and organisms and MS-based proteomics became the method of choice for their detection and characterization. PTMs are important to virtually all biological processes. For example, glycosylation enhances many biological processes such as cell-cell recognition and influences proteins biological activity, while phosphorylation is a reversible and common PTM that plays a role in controlling and modifying the majority of cellular processes [13]. Another important modification of proteins is the formation of disulfide bridges, which play significant role in maintaining the correct function of proteins [14,15]. Additional stable or transient modifications in proteins which include, acetylation, ubiquitination and methylation, which when combined with stable or transient protein-protein interactions (PPIs), add an additional level of complexity to proteomics approaches [13,16,17].

Challenges

Key parameters of MS-based proteomics experiments include sensitivity, resolution, dynamic range and mass accuracy. Therefore, various elements need to be taken into consideration during a typical MS-based proteomics experiment. As such, the challenges in proteomics should always be kept in mind when designing a proteomics experiment. The first challenge in these experiments is always sample fractionation which, except for bodily fluidic samples, is generally performed by some kind of electrophoresis. Electrophoresis is a time-consuming procedure and a major source of errors. The second challenge is MS-based sample analysis. Due to the instrument capabilities and specifications, different MS instruments will provide different types of information, as well as different numbers of identified proteins per experiment. However, sample fractionation and MS analysis can be easily adjusted. A one-time analysis of a very complex proteome investigated on a high-resolution (Orbitrap) or high-definition ion mobility (SYNAPT) instrument can usually be compensated for by multiple fractionation of the proteome, followed by multiple analysis of those samples by an older-generation MS instrument. However, none of these combinations or adjustments can be successful unless we address the third and the most important challenge in proteomics: bioinformatics. MS analysis can produce a great deal of data, but only a small percentage contains useful and/or meaningful information. Most of the data is, however, of lower quality and not useful. Therefore, development of specialized software that allows for the identification and characterization of proteins from unusable, lower quality data will greatly enhance the outcome of a proteomics experiment.

Perspectives

Many proteomes are already characterized. However, proteomes are not static and a deeper look at proteomics must be taken, from a dynamic perspective. For example, during the development (or even the cell cycle), different genes are constantly turned on or off and various proteins are produced and destroyed. As such, 1) various proteomes exist at a particular time-point within a cell, tissue, organ or organism, 2) various PTMs exist at the same proteins, depending on the developmental stage of the cell, tissue, organ or organism, 3) various stable and transient PPIs are constantly formed and destroyed.

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Therefore, even if we think that we know the proteomes for that cell, tissue, organ or organism, we know just a little portion about that proteome: the static proteome and the perspective leans towards investigation of the dynamic proteomes.

References