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Perspective

Quantitative Proteomics: A Technique for Determining the Amount of Proteins

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DESCRIPTION

Quantitative proteomics is a method of measuring the quantity of proteins in a material using analytical chemistry. The procedures for identifying proteins are the same as in conventional proteomics, with the addition of quantification as a new dimension. Quantitative proteomics, rather than merely providing lists of proteins found in a given sample, provides information about the physiological differences between two biological samples. This method may be used to compare samples from healthy and sick individuals, for example. Two-dimensional gel electrophoresis or mass spectrometry is the most common method for quantitative proteomics. However, a recently developed quantitative dot blot analysis method can quantify both the absolute and relative quantities of individual proteins in a sample in a high throughput manner, opening up a new avenue for proteome study. MS technique can identify and quantify alterations, unlike 2-DE, which requires MS for downstream protein identification.

Spectrophotometry-based quantification: Spectrophotometric techniques can be used to quantify the concentration of a specific protein in a sample. A spectrophotometer may be used to measure the OD at 280 nm of a protein, which can then be used in conjunction with a standard curve test to quantify the content of Tryptophan, Tyrosine, and Phenylalanine. This approach, however, is not the most accurate since protein composition varies significantly, and this method would be unable to measure proteins that do not include the aforementioned amino acids. Due to the likelihood of nucleic acid contamination, this technique is also incorrect. The Biuret, Lowry, BCA, Bradford, and Full spectrum UV/VIS techniques are among the more accurate spectrophotometric approaches for protein measurement.

2D electrophoresis for quantification: Two-dimensional gel electrophoresis is one of the most used quantitative proteomics methods, with benefits and drawbacks. 2-DE gives information on the intact protein's amount, charge, and mass. It has limits when it comes to analysing proteins bigger than 150 kDa or smaller than 5 kDa, as well as proteins with limited solubility. The sensitivity of quantitative MS is higher; however it does not offer information about the complete protein. Traditional 2-DE based on post-electrophoretic dye staining has limitations in that technique requires at least three technical replicates to ensure consistency. The use of fluorescence-based protein labelling prior to separation in difference gel electrophoresis has enhanced the precision of quantification as well as the sensitivity of protein detection. As a result, DIGE is the most widely used method for studying proteomes using 2-DE.

Mass spectrometry-based quantification: One of the most used methods for quantitative proteomics is mass spectrometry (MS), which has both advantages and downsides. Although quantitative MS has a better sensitivity, it can only offer limited information on intact proteins. To understand global proteome dynamics in populations of cells or individual cells, quantitative MS has been utilised for both discovery and targeted proteomic research. Isotope-coded affinity tags, which employ two reagents with heavy and light isotopes, respectively, plus a biotin affinity tag to alter cysteine-containing peptides, were first created in the 1990s. This technique was used to label entire Saccharomyces cerevisiae cells, and it helped build the groundwork for quantitative proteomics by combining it with mass spectrometry. Isobaric mass tags, which are also employed for single-cell protein analysis, have supplanted this method.

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