

A Brief Note on Quantitative Proteomics

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EDITORIAL NOTE

Quantitative proteomics is a method of quantifying the number of proteins in a material using analytical chemistry. Protein identification methods are identical to those used in general proteomics, with the addition of quantification as a new dimension. Quantitative proteomics, rather than only 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 ill people, for example. Two-dimensional gel electrophoresis (2-DE) or mass spectrometry is the most used methods for quantitative proteomics. However, a newly discovered approach of quantitative dot blot (QDB) analysis can assess both the absolute and relative quantities of specific proteins in a sample in a high-throughput manner, opening up a new avenue for proteomic study. MS technique can identify and quantify alterations, unlike 2-DE, which requires MS for downstream protein identification.

In the medical world, quantitative proteomics has a variety of uses especially in medication and biomarker development. Due to the time-consuming nature of labelling and separating proteins using these methods, as well as the more global analysis of protein quantification, LC-MS/MS techniques have begun to supplant more traditional approaches such as western blot and ELISA. Mass spectrometry techniques are more sensitive to differences in protein structure, such as post-translational modification, and can thus measure various protein changes. Quantitative proteomics, on the other hand, can get around these problems by merely requiring sequencing data.

Quantification using spectrophotometry: Spectrophotometric methods 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 widely, 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 approach is also imprecise. The Biuret, Lowry, BCA, and Bradford techniques are among the most accurate spectrophotometric methodologies for protein measurement.

Quantification using two dimensional electrophoresis: Two-dimensional gel electrophoresis (2-DE) is one of the most used quantitative proteomics methodologies, with benefits and drawbacks. 2-DE offers information regarding 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 entire protein. Traditional 2-DE based on post-electrophoretic dye staining has limitations in that technique requires at least three technical replicates to ensure repeatability. The use of fluorescence-based protein labelling prior to separation in difference gel electrophoresis has boosted 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.

Quantification using mass spectrometry: One of the most used tools for quantitative proteomics, mass spectrometry, has both advantages and downsides. Although quantitative MS has a better sensitivity, it can only offer limited information on intact proteins.

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