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The incongruity of validating quantitative proteomics using western blots

Similar to the age-old reviewer request for quantitative PCR validation of RNA-sequencing data, nearly every researcher who uses proteomics technologies has at one time or another been asked by a reviewer to provide 'western blot validation' of their mass-spectrometry-based protein abundance data. We believe that this request demonstrates a lack of awareness amongst the plant biology community about the extraordinary improvements in cost, sensitivity and reliability that the field of mass-spectrometry-based proteomics has made in recent years. Here, as a group of experts in different domains of quantitative plant proteomics, we explain why western blot validations of quantitative proteomics data are both unnecessary and invalid. Furthermore, we invite our colleagues in the plant science community to update their perception of quantitative mass spectrometry as a sensitive and reliable method of protein identification and quantification.

Quantitative proteomics, in its many flavours, offers plant science researchers the ability to quantify thousands of proteins in a single experiment with much greater precision and reliability than western blotting. Both intra- and inter-laboratory studies have established the reproducibility of protein quantification by both targeted[1,2] and untargeted (particularly data-independent acquisition) proteomics[3,4]. By contrast, western blotting has a high rate of failure owing to the inherent stochasticity of in-gel separation, membrane transfer and unspecific antibody binding. Furthermore, these processes – in conjunction with variability in blot development using luminol-based chemiluminescent reagents – render western blot results semiquantitative at best. Mass-spectrometry-based protein quantification is highly reproducible[1–4] and reliable because quantification is typically based on the measurement of multiple spectra and peptides per protein and is accompanied by statistical confidence measures for each peptide and protein.

Another point in favour of quantitative proteomics is the sensitivity of protein detection. For instance, even the very best antibodies cannot detect proteins at below nanogram quantities[5], whereas the latest mass spectrometers can consistently quantify proteins at pico- and femtogram levels using as little as 0.8 ng of input[6]. This is especially important as the plant biology community endeavours to parallel the pursuits of health research in defining and quantifying the cell-type and single-cell proteomes of the plant kingdom[7,8]. Owing to the inherent low protein abundance of single cells, the question of western blot validation becomes a moot point. Finally, sourcing and developing highly specific antibodies is another major challenge in reliably performing western blots. Although monoclonal antibodies are available for a few commonly probed epitopes, researchers frequently use polyclonal antibodies, which have a wide range of specificities – leading to an increased possibility of false-positive results. By contrast, mass spectrometry offers peptide identification with high statistical confidence. In fact, antibody vendors have begun offering mass-spectrometry-validated antibodies, following published guidance from the International Working Group for Antibody Validation[9,10]. So although western blotting offers a visual depiction of a singular protein, its limitations render it an inappropriate validation for quantitative proteomic data.

An obvious advantage offered by quantitative proteomics over western blotting is the greater depth and breadth of understanding made possible in a single experiment. Western blotting permits the detection of a single protein, whereas quantitative proteomics offers information on hundreds (for example, targeted assays) to several thousands of proteins (for example, untargeted analyses), providing a global picture of the cell rather than one limited by the availability of a good antibody. This offers exciting opportunities to benchmark experimentation using previously substantiated data, ultimately leading to more-robust biological insights.

In our considered opinion, unlike other fields, the plant science community has been relatively slow to embrace mass-spectrometry-based proteomic methods, from global proteome profiling to interaction mapping and targeted proteomics. One reason for this is that western blots offer an intuitive and easy means to assay individual proteins without the steep learning curve that is required to successfully carry out a quantitative proteomics experiment. Unfortunately, mass spectrometry equipment and expertise are also not yet as widely available as genomics or microscopy infrastructure (particularly in the Global South), which limits accessibility compared to western blotting. Proteomics technology development has also largely occurred in biomedical fields that have little crossover with plant science, which perhaps explains the relatively slower adoption of the technology by plant biologists.

However, quantitative proteomics is currently seeing a resurgence of interest, with several initiatives (such as the Plant Cell Atlas[7]) enhancing the visibility and accessibility of proteomics to the general plant biologist. Correspondingly, the extraordinary opportunities offered by today's mass spectrometry instrumentation for quantitative proteomics in plant science – from the population to single-cell scales – should not be underestimated. New machine-learning-enabled data analysis software and reagents or consumables are permitting researchers to execute high-throughput workflows faster and at lower cost, and advances in ion mobility separation, tribrid device architectures and ionization lasers (among other hardware improvements) are enabling more-sensitive, precise and reproducible experimentation than ever before. We believe that it is finally time for the plant biology community to retire the western blot as a means to 'validate' quantitative proteomics data and to adopt mass spectrometry as the gold standard for protein abundance measurements.

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Competing interests

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