Mass spectrometry–based targeted proteomics

A brief overview of mass spectrometry technology for targeted proteomics applications is presented.

A number of technologies can be used to study proteomes, but arguably none is more powerful than mass spectrometry. There are two fundamentally different mass spectrometry–based strategies for analyzing proteomes: discovery-based identification and targeted quantification.

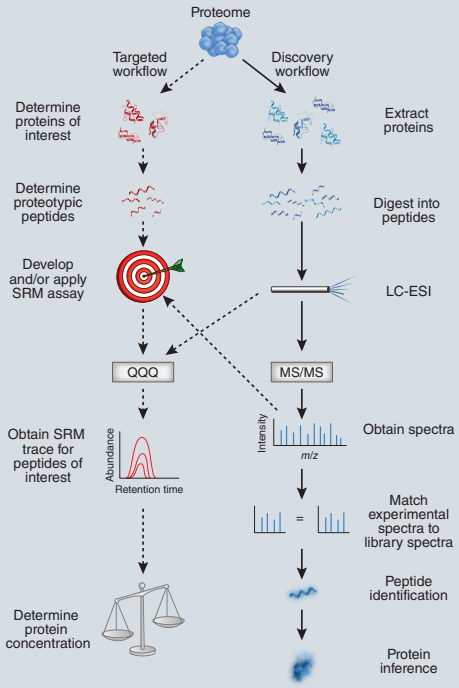
To discover or to target

With a discovery-based strategy, the goal is usually to identify as many proteins as possible. The goal of a targeted proteomics experiment is to monitor a select few proteins of interest with high sensitivity, reproducibility and quantitative accuracy.

In a standard workflow for either type of experiment, proteins are extracted from a sample using biochemical methods, and a protease is used to snip the protein into peptides at defined amino acid residues. The peptides are separated by liquid chromatography, and the fractions are analyzed by electrospray ionization coupled to mass spectrometry. The mass spectrometer measures and reports the mass-to-charge (m/z) ratio of the peptide ions.

In a typical discovery-based experiment, peptide ions are automatically selected in the mass spectrometer for fragmentation on the basis of their signal intensities. Fragmentation generates rich but complex tandem mass spectra for each peptide sequence. The experimental spectra are matched to library spectra to infer the peptide sequences and, by extension, the proteins—a process involving sophisticated bioinformatics tools and careful scrutiny of the results.

In a targeted workflow, the mass spectrometer is programmed to detect specific peptide ions derived from proteins of interest. This requires more up-front investment than a discovery-based experiment, but once a reliable assay is generated for a specific protein, analysis of the mass spectrometry data is relatively straightforward.



Discovery-based versus targeted proteomics workflows using mass spectrometry. LC-ESI, liquid chromatography–electrospray ionization; MS/MS, tandem mass spectrometry.

An old technology made new

The triple quadrupole mass spectrometer (QQQ) was developed more than 30 years ago for small-molecule analysis. It operates as a dual mass filter that allows molecular ions of predetermined masses to be selected for fragmentation in the instrument. In recent years the use of the QQQ for targeted proteomics applications has escalated as methodological advances have made the technology more widespread.

In a targeted proteome analysis, peptide ions travel into the first QQQ mass filter, which can be programmed to select specific 'precursor' ions (on the basis of their m/z ratio) for fragmentation. In the second mass filter, target 'product' ions are selected and then guided to the detector for quantification, resulting in a trace of signal intensity versus retention time for each precursor ion–product ion pair. This process is called selected reaction monitoring (SRM) or multiple reaction monitoring (MRM).

SRM 'assays' are generated by defining a signature set of peptide fragment coordinates. A detectable precursor ion–product ion pair is referred to as a 'transition', and several suitable transitions constitute an SRM assay for detection and quantification of a target peptide and, by extension, the target protein. By spiking the sample with heavy isotope–labeled reference peptides, it is possible to achieve absolute quantification of the targeted peptides. The SRM technique is best suited for analyzing about 50–100 proteins concurrently.

A major bottleneck has been the development of robust SRM assays for reliable protein detection and quantification. Not all peptides are equally analyzed by mass spectrometry: some are better separated, ionized and detected than others owing to their physicochemical properties. Peptide sequences must also be carefully chosen to ensure that they uniquely represent one of the targeted proteins. Several prediction tools and methods have been recently developed to identify these 'proteotypic' peptides, often using information from discovery-based experiments. Once proteotypic peptides have been chosen, the optimal SRM transitions must be determined and rigorously validated. However, once generated, SRM assays can be reproducibly deployed across samples and across laboratories.

Next-generation targeted proteomics

SRM is the most mature mass spectrometry–based technology for targeted proteome analysis, but new methodologies that obviate the need for laborious SRM assay optimization are on the horizon. With an approach called SWATH, complex mass spectra generated by data-independent acquisition (in which peptides are selected for fragmentation without regard to signal intensity) are queried for the presence of specific peptides using libraries of qualified peptide fragment spectra. With another new approach called parallel reaction monitoring, all transitions are monitored in parallel in a single analysis. Continuing methods and software development will be key for bringing targeted proteomics technology to biologists.

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