A recent review by Vidova and Spacil provides an excellent primer for targeted mass spectrometry-based proteomics. In targeted mass spectrometry, the mass spectrometer is tuned to track specific peptide(s) derived from a protein of interest. The peptide(s) serve as surrogates for protein identification and quantitation. This is in contrast to acquiring information on all peptides as would be done in global proteomics. Targeted mass spectrometry generates highly sensitive, quantitative information and can serve as an excellent validation test, one which is highly complementary to western blot analysis.

TARGETED MASS SPECTROMETRY

Targeted mass spectrometry for proteomics is most commonly performed through one of two, similar techniques; Single Reaction Monitoring (SRM) (MRM if multiplexed) or Product Ion Scan (PRM if multiplexed). The publication’s Figure 1, shown to the right, presents a graphical depiction. Both techniques isolate the molecular weight corresponding to the target peptide, break the peptide into fragment ions, and monitor the fragment ion intensities over time. It is in the latter step that the methods differ. SRM monitors only a single fragment ion, while Product Ion Scan measures all fragments ions. SRM is more sensitive, but Product Ion Scan provides a higher degree of identification confidence and multiple channels for quantitative measurement.

PEPTIDE SELECTION

Section 3 from the publication details the method development for designing and validating a targeted MS method. To begin, a peptide or handful of peptides generated by the protein of interest are validated for their specificity to the target protein and their ability to be detected. A recombinant protein standard is the best sample to use for initial target peptide curation, as it is free of interfering ions. The recombinant standard is run via global proteome analysis, which has the benefit of identifying peptides that are most highly expressed. These peptides are then checked for specificity against the corresponding protein database in order to ensure that they are only generated by the target protein. Once a handful of potential peptides are selected, they are evaluated through Product Ion Scan. Peptides which produce low intensity, irreproducible, or convoluted signals are rejected.
The target protein is then spiked into the biological background derived from real samples in order to evaluate the matrix effects on potential peptides and to catalog any interfering peptides. Interfering peptides may show some common fragments with the target peptide, but it is near impossible to observe multiple, coeluting fragment ions with the same retention time as the recombinant standard which do not belong to that standard. Peptides which are obfuscated by the sample matrix are rejected.

ADDITIONAL CONSIDERATIONS

It is important to note that post-translational modifications change the mass of the target peptide and can lead to erroneous quantification. Modified peptides can be tracked alongside their unmodified counterparts in order to correct for this, but the modifications must be identified prior to beginning the evaluation. Some peptides may require additional processing steps such a deglycosylation.

If only general, relative quantitation is desired (comparing the same peptide between two samples) then real samples can now be measured and peak areas between the samples can be compared to observed protein fold change. However, for more accurate quantitation (relative or absolute) a quantitative assay needs to be developed.

RECENT APPLICATIONS

The publication’s Table 2 demonstrates many recent applications utilizing targeted mass spectrometry. These include connecting protein abundance to genetic variation, monitoring protein interaction signaling pathways, and disease biomarker discovery/verification.

PROJECT SUPPORT

The IMSU strives to offer competitive pricing. For collaborative projects, we offer a 30%-50% discount depending on the agreement and the extent of collaboration. Collaborative details can be discussed during the initial consultation. Support is also available for high impact projects with the potential for external funding.

This newsletter is based on the article by Vidova and Spacil

Vidova V, Spacil Z. “A review on mass spectrometry-based quantitative proteomics: Targeted and data independent acquisition”. – Analytica Chimica Acta. 2017. 964:7-23