FROM THE DIRECTOR’S DESK

Posttranslational modifications (PTMs) contribute to proteome diversity, altering the function, abundance and/or subcellular localization of proteins. Mass spectrometry allows us to identify different PTMs based on the mass change of specific amino acids, using top-down\(^1\) or bottom-up\(^2\) approaches. This is a powerful discovery approach that contributes to our understanding of molecular changes induced during a specific biological process, in response to pathological changes or drugs. However, there are three aspects that need to be taken in consideration. First, enrichment of the target prote in will increase the chances of identifying the putative PTM. Mass spectrometry-based proteomics is used for the identification and quantification of thousands of proteins from tissue, cell culture or biofluid sample. Two or more peptides is enough for identification and quantification purposes, but it is not enough for PTM identification. To identify PTMs requires maximizing the sequence coverage of the targeted protein. Immunoprecipitation, subcellular fractionation and/or chromatography can be used to enrich/purify the protein of interest from tissue, cell culture or biofluid. The purified protein is then subjected to tandem mass spectrometry. Second, PTMs play an important role under specific biological conditions or due to environmental changes. Therefore, the target protein needs to be purified under those specific biological conditions or environmental changes in order to capture the modified protein molecules and reduce the dilution effect that unmodified molecules may exert. Lastly, it is crucial to take in consideration specific proteases based on the sequence of the target protein and the lability of the PTM during sample preparation. Rest assured that, at IMSU, we will work with you the details of the experimental design. We have the tools and expertise to help you reach your research goals.

TECHNICAL DISCUSSION – PTMS

Posttranslational modifications (PTMs) are often less abundant on peptides compared to their unmodified counterparts. In order to overcome this, PTM peptides must be enriched prior to analysis. Enrichment of phosphorylated peptides commonly takes place using TiO$_2$ affinity chromatography or immobilized metal ion affinity chromatography (IMAC). Both techniques operate on a similar principle to cation exchange chromatography, but the metal-based resins offer a more selective interaction with phosphate groups. The two techniques have been demonstrated to be complementary and can, therefore, be combined to maximize phosphoprotein coverage.

Some PTMs, like glycosylation, are enriched prior to protein digestion. Lectin affinity columns take advantage of the naturally specific interaction between the lectin Concanavalin A and abundant glycan monosaccharides in order to generate a highly enriched glycoprotein sample. Other lectins can be used to enrich for specific glycans, such as O-linked. Enrichment strategies also exist for a wide variety of other PTMs such as methylation, acetylation, ubiquitination, etc.

In standard proteomic data analysis, each peptide corresponds to a single mass. When investigating PTMs, modifications may or may not be present on an amino acid, adding to the pool of potential masses. Additionally, multiple PTMs can exist in the same peptide. It is important to use high resolution-accurate mass instruments with long gradient separations to confidently pinpoint the exact mass of every measured peptide and ensure that all of the mass variations are accounted for. PTMs like glycosylation add an additional component to the analysis. These modifications exist in multiple different forms, with multiple molecular weights. Modifying these PTMs with a tag of consistent weight, employing specialized fragmentation methods, and utilizing software dedicated to a specific PTM are all common when tackling variable mass PTMs.

IMSU SUGGESTED ARTICLES

Some recent reviews of glycosylation analysis and the application of PTM studies to Alzheimer’s research.