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Cutting-Edge Technologies Driving Quantitative Mass Spectrometry

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Editorial

Proteomics approaches can include detection and quantification of the peptides, proteins, and specific post-translational modifications (PTMs) present at a certain cellular state, as well as the proteins coming together in specific organelles or macromolecular protein complexes. Proteomics is a diverse field that can be partitioned into general and quantitative workflows. Quantitative proteomics is focused on determining the amount of protein or protein PTM in a sample. The biological question should be considered prior to performing any proteomics experiment in order to determine the most appropriate sample preparation, sequencing, and data analysis method necessary to answer the question. Proteins are highly dynamic and mass spectrometers are sensitive to sequencing higher abundant proteins over lower abundant PTMs; therefore, the choice of sample preparation and sequencing workflows are critical. This methods collection describes approaches to quantify certain types of proteins using cutting-edge MS methods and provides a data analysis workflow for protein networks.

Peptidomics is the study of peptides naturally generated during normal cellular processes as well as under disease conditions, and this approach was historically limited to antibody-based quantification until liquid chromatography coupled mass spectrometry and peptide extraction processes advanced to their current state[1]. These advancements, along with the application of heat inactivation of endogenous proteases, now enable the identification and quantification of hundreds of intracellular peptides. In Correa et al.[2], the authors describe a sample preparation workflow, which includes heat inactivation, peptide extraction under mild conditions to preserve peptide bonds, and relative quantification through stable isotope labeling of amines. The authors have developed an inexpensive approach that could enable the identification and quantification of native and biologically active peptides from cell culture and tissue specimens. This approach could be used to discover disease-specific peptide biomarkers, antimicrobial peptides, or targets for future therapeutic development.

Advances in mass spectrometric and bioinformatics approaches now enable the detailed characterization of proteomes from diverse sources of biological material[3]. Deciphering the biological significance from the proteomic expression patterns in these complex samples remains

a significant challenge in the field. To address this challenge, Vanderwall et al. introduce JUMPn, a systems biology program and its associated workflow that organizes proteomic data into modules (i.e., protein complexes) that connect protein co-expression clusters with related protein-protein interaction (PPI) network nodes[4]. The authors evaluate the effectiveness of this tool using existing proteomic datasets as well as simulated data and show its utility for facilitating biological interpretation of complex proteome datasets. Their results show that this approach can be used to measure the transcription factor and kinase cascade activation, demonstrating its utility for identifying drivers of human disease, and for other studies attempting to determine biological mechanisms from deep proteome data.

Inositol pyrophosphates (PP-InsPs) are found in all eukaryotic cells where they function as metabolic signaling molecules and regulate diverse processes[5]. Historically, due to the physical properties and low abundance of PP-InsPs, analysis has been limited to the use of radioactive tracers, which are expensive and require special care and storage. As an alternative, Qiu et al. have developed a protocol allowing high-throughput, absolute quantitation of inositol pyrophosphates from cell culture or tissue source material by capillary electrophoresis coupled electrospray ionization mass spectrometry (CE-ESI-MS)[6]. In their report, they show how this approach can be used to profile all PP-InsPs present in a specimen, resolving species and identifying regioisomers in order to identify spatial and temporal changes under diverse conditions and disease states.

A large proportion of cellular processes are controlled through protein phosphorylation and errors in these signaling pathways are associated with many different disease states[7]. However, there are still significant gaps in understanding the complete mechanisms that regulate protein phosphatases and kinases in different cell types and under various conditions. To address these gaps in understanding, Smolen et al. introduce a novel workflow utilizing phosphatase inhibitor beads coupled with mass spectrometry (PIB-MS)[8]. Using a non-selective phosphoprotein phosphatase inhibitor, microcystin-LR, coupled tosepharose beads, the authors are able to enrich these target proteins. Additionally, by using mild wash conditions to remove non-specific containments, they were also able to capture protein interactors. They show how this approach can be used to identify and quantify phosphoprotein phosphatases, their phosphorylation status, and potential protein-protein interactions in both cell culture and tissue.

The advancements in sample preparation methods, such as strategies for quantifying endogenous peptides, enrichment of phosphatases and kinases, protein interactions, and inositol pyrophosphates, is expanding the knowledge base for protein dynamics in biological processes. The advancement of mass spectrometers such as data independent acquisition in contrast to data dependent acquisition allows for greater depth of sequencing capacity to identify peptides in such a way that the higher abundant peptides do not mask lower abundant peptides during data collection, which allows other classes of proteins to be identified. The timsTOF mass spectrometers will help drive the future forward by incorporating the ion mobility in addition to liquid chromatography as an added separation of peptides to allow even deeper sequencing and greater chance of detecting peptides from the sample preparation strategies described in this collection. The field of proteomics will continue to move forward based on new sample preparation methodologies,

advancement in mass spectrometers for data acquisition, and bioinformatics analysis tools, such as JUMPn, to understand the biological complexities and function.

Disclosures

The authors have nothing to disclose.

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