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Commentary

A Brief Note on Bottom-Up Proteomics

Francesco Andelli*

Department of Genomics, University Hospital of Modena and Reggio Emilia, Via del Pozzo 71, 41124 Modena, Italy

DESCRIPTION

Proteolytic digestion of proteins before mass spectrometry analysis is a typical approach for identifying proteins and characterising their amino acid sequences and post-translational changes. Top-down proteomics is a popular alternative workflow in proteomics, in which intact proteins are purified before being digested and/or fragmented in a mass spectrometer or by 2D electrophoresis. Bottom-up proteomics is a reasonably easy and reliable method of estimating the protein composition of a given sample of cells, tissues, or other biological materials. Proteases are used in most proteomic investigations to breakdown proteins into peptides with a known terminal. The mass to charge ratio and anticipated sequence of the peptides are then utilised to infer information about the proteins in the sample using the MS/MS equipment. The term "bottom-up" proteomics refers to any experimental setups that begin with the analysis of peptides from whole protein digests and use a protein database to characterise the open-reading frame from which the peptide originated.

Bottom-up proteomics involves enzymatic digestion of the crude protein extract, followed by one or more dimensions of peptide separation using liquid chromatography coupled to mass spectrometry, a process known as shotgun proteomics. Peptides can be identified and multiple peptide identifications integrated into protein identification by comparing the masses of proteolytic peptides or their tandem mass spectra with those predicted from a sequence database or annotated peptide spectral in a peptide spectral library. Bottom-up proteomics takes advantage of peptides benefits over proteins: they're easier to separate by reversed-phase liquid chromatography (RPLC), ionise well, and fragment more predictably. This corresponds to a robust technology that allows for high-throughput analysis, allowing thousands of proteins to be identified and quantified from complex lysates. Proteomics fundamental technologies today are bottom-up techniques with data-dependent acquisition workflows. Selected reaction monitoring (SRM), for example, is a new data collecting strategy that improves the quantification accuracy and repeatability of bottom-up proteomics research.

The broad use of protease digestions is a characteristic of bottom-up proteomics, although it has downsides. When it comes to shotgun techniques, trypsin is the gold standard, accounting for over 96% of the data sets submitted in the Global Proteome Machine Database. Furthermore, only a small percentage of these peptides produce meaningful fragmentation ladders. As a result, current bottom-up proteomics provides a skewed and limited view of the whole proteome in a particular sample, akin to "tunnel vision" of the proteome.

Small peptide sequence information is often enough to assign proteins to clusters, but not necessarily enough to identify proteoforms. Protein isoform and PTM identification without prior knowledge is relatively limited in bottom-up proteomics, despite being possible through mass shift measurements. There is greater front-end separation of peptides relative to proteins and higher sensitivity for high throughput bottom-up methods than for (non-gel) top-down approaches. Protein sequence coverage by recognised peptides is restricted, labile PTMs are lost, and the origin of duplicated peptide sequences is ambiguous. Recently, middle-down proteomics, which combines bottom-up and top-down proteomics, has gotten a lot of attention since it can be used to analyse huge protein fragments while also avoiding redundant peptide sequences.

The extraction of proteins from the biological matrix, removal of non-protein contaminants such as DNA, sugars, and lipids, removal of residual salts that may form adducts during ionisation, and protein fractionation to reduce sample complexity are common methodologies for bottom-up proteomic sample preparation. Bottom-up proteomics makes use of unfolded proteins, which make it easier for proteases to access amino acids, resulting in a higher number of peptides for MS analysis. Because of its high peak capacity, repeatability, and resilience, RP-HPLC is employed in most bottom-up proteomic investigations for distinguishing proteolytically produced peptides. To provide large-scale proteome coverage in a given time, achieve higher analytical throughput, and cover a broad dynamic protein concentration range, including trace amounts of distinct proteins, both bottom-up and top-down proteomics approaches are completely reliant on employed separation technologies.

Correspondence to: Francesco Andelli, Department of Genomics, University Hospital of Modena and Reggio Emilia, Via del 41124 Modena, Italy, E-mail: Francesco@12mreu.it Pozzo 71,

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