Creative Proteomics

PROTEOMICS

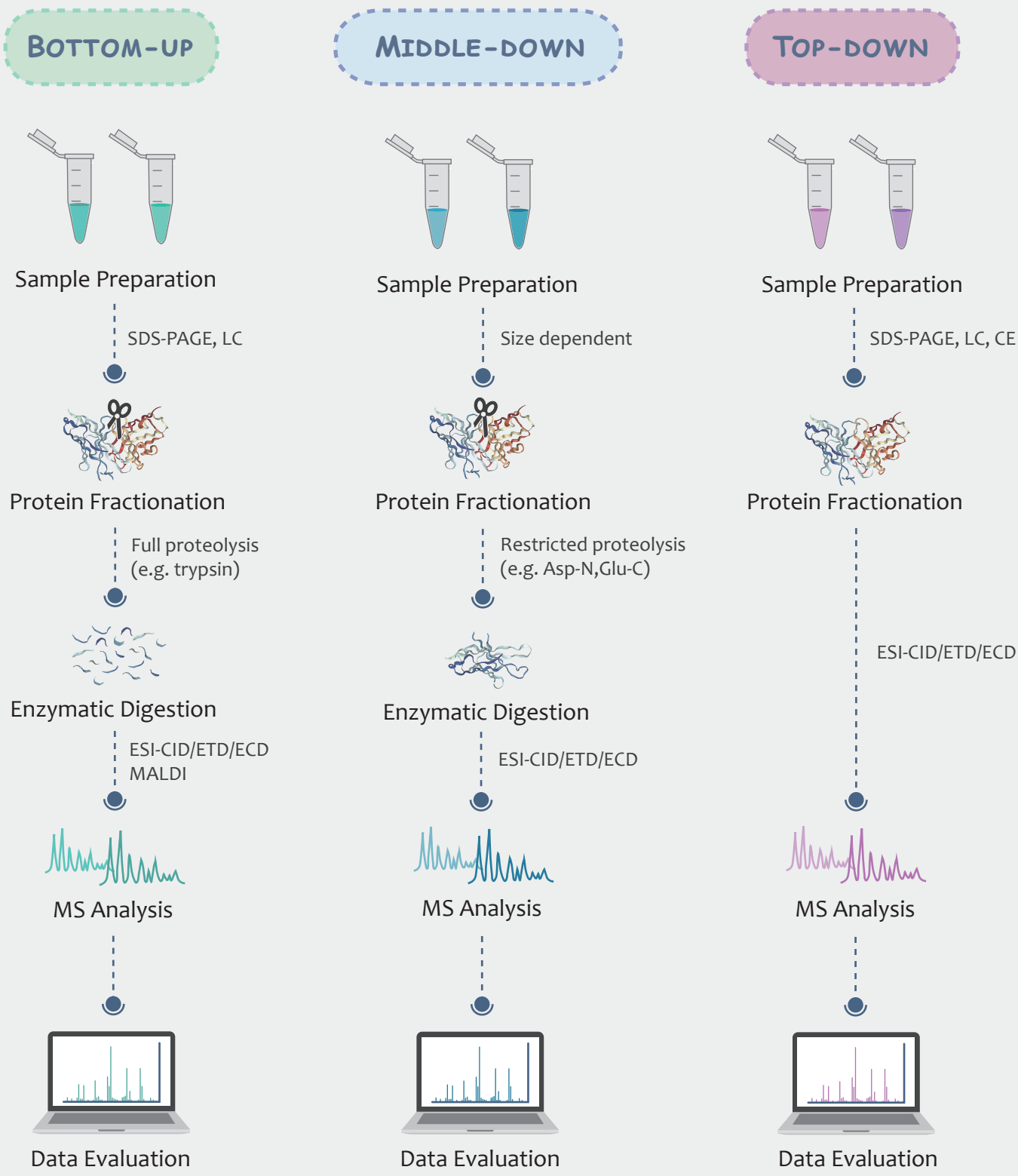
Top-down Middle-down Bottom-up

Bottom-up is a traditional method that digests large fragment mixtures of proteins/enzymes into small fragments of peptides for analysis. It is a mass spectrometry technique widely used in proteomics research.

Middle-down proteomics strategy uses different enzymes to obtain longer peptides. It can analyze and identify several simultaneous posttranslational modifications on longer peptide chains. Compared to bottom-up method, it can analyze a wider range of peptides.

Top-down proteomics strategy does not need the enzyme digestion process, but directly analyzes the complete protein. This technique can provide more accurate and rich biological information of the complete protein.

Workflow



Strategy Comparison

BOTTOM-UP

- ☑ Digest the protein into small peptides
- ☑ Well-developed methods available for protein quantification
- ☑ Higher throughput
- ☒ Loss of natural information leads to the inability to identify protein variants
- ☒ Unable to accurately analyze the association between PTMs

MIDDLE-DOWN

- ☑ This procedure works with 5–20 kDa large polypeptides, produced by limited proteolytic digestion
- ☑ Several simultaneous post-translational modifications on longer peptide chains can be analyzed and identified. Compared with BU method, it can analyze a wider range of peptide segments.
- ☑ Histone post-translational modification identification

TOP-DOWN

- ☑ Does not require the laborious chemical or enzymatic digestion
- ☑ 100% sequence coverage enables full characterization of proteoforms
- ☑ The complete protein was analyzed by mass spectrometry, and the excellent PTM characterization was achieved.
- ☒ Lower throughput

Application

Title:

- Middle-down hybrid chromatography/tandem mass spectrometry workflow for characterization of combinatorial post-translational modifications in histones

Method:

- Combining a RP trap column with subsequent weak cation exchange–hydrophilic interaction LC interfaced directly to high mass accuracy ESI MS/MS using electron transfer dissociation, which enabled automated and efficient separation and sequencing of hypermodified histone N-terminal tails for unambiguous localization of combinatorial PTMs.

Highlight:

- Histone tails from mouse embryonic stem cells were identified in the zeste12 inhibitory gene and 256 combined histone marks in histones H3, H4 and H2A were quantified.
- A total of 713 different combined histone marks were identified in purified histone H3.

https://doi.org/10.1002/pmic.201400084

Title:

- Deep Top-Down Proteomics Using Capillary Zone Electrophoresis-Tandem Mass Spectrometry: Identification of 5700 Proteoforms from the Escherichia coli Proteome

Method:

- Coupling size exclusion chromatography (SEC) and RPLC based protein prefractionation to CZE-MS/MS for deep top-down proteomics of Escherichia coli.

Highlight:

- The platform generated high peak capacity (~4000) for separation of intact proteins, leading to the identification of 5700 proteoforms from the Escherichia coli proteome. The data represents a 10-fold improvement in the number of proteoform identifications compared with previous CZE-MS/MS studies.

https://doi.org/10.1021/acs.analchem.8b00693

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