**PROTEOMICS**

**TOP-DOWN** | **MIDDLE-DOWN** | **BOTTOM-UP**

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**Sample Preparation**

**Enzymatic Digestion**

**Protein Fractionation**

**MS Analysis**

**Data Evaluation**

**TOP-DOWN**

Digest the protein into small peptides
Well-developed methods available for protein quantification
Higher throughput

**MIDDLE-DOWN**

This procedure works with large peptides, produced by limited proteolytic digestion.
Several simultaneous post-translational modifications on longer peptide
Beeper can be analyzed and identified. Compared with MS method, it can analyze a wider range of peptide segments.
Matrix-assisted post-translational modification identification.

**BOTTOM-UP**

Digest the protein into small peptides
Well-developed methods available for protein quantification
Higher throughput

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**Strategy Comparison**

**TOP-DOWN**

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

**MIDDLE-DOWN**

Restricted proteolysis
This procedure works with large peptides, produced by limited proteolytic digestion.
Several simultaneous post-translational modifications on longer peptide
Beeper can be analyzed and identified. Compared with MS method, it can analyze a wider range of peptide segments.
Matrix-assisted post-translational modification identification.

**BOTTOM-UP**

Enzymatic digestion (e.g. trypsin)
Well-developed methods available for protein quantification
Higher throughput

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**Application**

**Title**: Top-Down Proteomics Using Acrylate Zone Electrophoresis Tandem Mass Spectrometry: Identification of Tumor Proteins from the SKBR-3 Breast Carcinoma Line

**Method**: Combining a HP trap column with subsequent weak cation-exchanger hydrophilic interaction columns interfaced directly to high mass accuracy Q-Exactive XACT, the proteome was successfully deconvoluted, which enabled the identification of proteins from the skin and breast-related tumor. A total of 107 different combined fraction sets were identified in parallel in the same time.

**Highlight**: The platform generated high peak capacity (~9000) for separation of intact proteins, leading to the identification of 86 proteoforms from the SKBR-3 breast carcinoma. The data representation is 10-fold improvement in the numbers of protein identifications compared with previous LC-MS/MS studies.

**Title**: Middle-down hybrid chromatography/tandem mass spectrometry followed by separation of monofunctional post-translational modifications in keratins

**Method**: Combining a 1.5% organic/water gradient with a 13% organic/water gradient, the proteins were separated into different fractions based on the type and degree of post-translational modifications.

**Highlight**: The method allows for the separation of proteins with different post-translational modifications, enabling the identification of specific modifications in keratin proteins.

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**Workflow**

**Sample Preparation**

**Protein Fractionation**

**Enzymatic Digestion**

**MS Analysis**

**Data Evaluation**

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**BOTTOM-UP**

**MIDDLE-DOWN**

**TOP-DOWN**

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