

A Winning Combination

Integrated strategy allows for better identification, characterization of proteins

Researchers at Pacific Northwest National Laboratory used resources at the Department of Energy's EMSL to develop an innovative approach that integrates two fundamental proteomic strategies for protein identification and characterization by mass spectrometry. The approach overcomes the limitations of the traditional top-down and bottom-up strategies, allowing for high-throughput analysis of protein isoforms and genetic variance through amino acid modifications, such as acetylation and phosphorylation.

Bottom-up sequencing is the analysis of trypsin-digested proteins and is the basis for contemporary proteomics. However, researchers are limited by the bottom-up approach because complete sequence coverage of proteins is rarely achieved.

Top-down analysis involves direct analysis of intact proteins without digestion. But it suffers from limited throughput and has a

low success rate when used with online liquid chromatography-tandem mass spectrometry and on-line fraction collection. By combining top-down and bottom-up approaches, scientists can achieve more precise identification and characterization of protein isoforms and combinatorial post-translational modifications. The researchers demonstrated their integrated strategy by coupling reversed-phase liquid chromatography with a 12-Tesla Fourier transform ion cyclotron resonance mass spectrometer and on-line fraction collection. They identified modified proteins by applying intact protein masses with "bare" proteins from the bottom-up analysis of the collected fractions. Integrated proteomics opens up new areas in the profiling of potential biomarkers that may be used to diagnose disease.

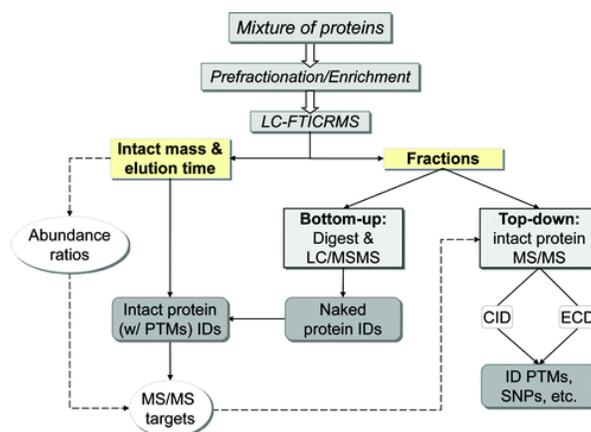
Scientific Impact: The integrated strategy is a more comprehensive approach to protein identification and profiling, and it allows for high-throughput characterization of intact proteins and their modifications. This work also supports EMSL's goal to predict biological functions from molecular and chemical data.

Societal Impact: An integrated top-down bottom-up strategy could play a critical role in identifying and characterizing biomarkers of cancer, cardiovascular disease, neurological disease, diabetes, and autism.

For more information, contact EMSL Communications Manager Mary Ann Showalter (509-371-6017).

Reference: Wu Si, M Lourette, N Tolic, R Zhao, E Robinson, AV Tolmachev, RD Smith, and L Pasa-Tolic. 2009. "An Integrated Top-Down and Bottom-Up Strategy for Broadly Characterizing Protein Isoforms and Modifications." *Journal of Proteome Research* 8(3):1347-1357. DOI: 10.1021/pr800720d

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Integrated top-down and bottom-up strategy for characterizing protein isoforms and modifications