Research Tools

Parallel Measurements by Fluorescence and Mass Spectrometry for Absolute Quantification of Proteins

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The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing a method for improved quantification of chromatographically separated materials such as proteins.

Overview

Mass spectrometry (MS) has become the principal tool for routine identification of proteins and their post-translational modifications (PTMs). Quantification of the amounts of proteins and PTMs is required to further understand the dynamics and subtleties of biological systems. MS-based techniques for quantifying amounts of materials may employ gas-phase measurements of the proteins and PTMs. However, highly variable peptide and protein ionization efficiencies often complicate quantification using gas-phase measurements. The effects of other components in the solution or supporting matrix surrounding the proteins or PTMs and instrument platform-specific mass spectrometer biases may be introduced, further complicating cross-platform quantification comparisons.

There are two strategies for high-throughput quantitative MS-based proteomics: label and label-free analysis. Each strategy is divided into various techniques that provide relative and/or absolute quantification of peptide and protein abundances. Techniques that utilize chemical labels or synthesized standards require the use of expensive isotopic reagents. Chemical labeling may introduce bias due to labeling chemistry, labeling efficiency and sample recovery, and provides only relative quantification. Isotope dilution techniques can provide both relative and absolute quantification, but require extensive method development and optimization. Label-free quantification techniques suffer from limited dynamic ranges and usually are used for relative quantification only. An improved method for quantification of chromatographically separated materials to overcome limitations of existing methods is needed.

The Invention

UW–Madison researchers have developed an improved method for MS-based quantification using an integrated electrospray emitter and fluorescence detector. Solution-phase measurements are employed to overcome the limitations in standard quantification techniques using measured intrinsic fluorescence to quantify selected amino acids. The inventors have developed a label-free technique that exploits the property of intrinsic fluorescence exhibited by tryptophan-containing peptides and proteins as a means to provide both relative and absolute quantification of proteins and peptides in complex mixtures identified through tandem mass spectrometry.

Integration of the emitter and detector permits quantification on a single stream of analyte while placement of the fluorescence detector immediately before the electrospray emitter provides improved correlation between the measurements and reduction of chromatographic dead volume. A continuous capillary may be used to provide not only the electrospray emitter, but also the liquid chromatography column. This combination allows effective analysis of extremely small amounts of material at nanoliter flow rates and a significant reduction in dead volume, which may cause loss of chromatographic resolution and sensitivity.

Applications

- Electrospray ionization mass spectrometry for quantification of proteins

Key Benefits

- Expands peptide and protein quantification dynamic range by one to two orders of magnitude
- Provides both relative and absolute quantification
- Improves correlation between the readings of mass spectrometry instruments
- Reduces chromatographic dead volume
- Requires little modification to existing standard nanoflow liquid chromatography electrospray ionization mass spectrometry platforms
- Provides a small device comprised of low-cost components

Additional Information

For More Information About the Inventors

- Joshua Coon
- Lloyd Smith

Tech Fields

- Analytical Instrumentation, Methods & Materials : Mass spectrometry
- Research Tools : Detection

For current licensing status, please contact Jennifer Gottwald at jennifer@warf.org or 608-960-9854