Analytical Instrumentation, Methods & Materials


# Labeling Peptides with Basic Functional Groups to Improve Mass Spectrometric Analysis

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Inventors: Joshua Coon, Brian Frey, April Jue, Casey Krusemark, Lloyd Smith

**The Wisconsin Alumni Research Foundation (WARF) is seeking commercial partners interested in developing methods of labeling proteins and peptides to improve sequence identification during mass spectrometry.**

## Overview

Protein characterization has become an integral part of modern biology, even inspiring a new discipline known as proteomics. Mass spectrometry (MS) is the most widely used technique to characterize proteins. To utilize mass spectrometry for protein sequence identification, the proteins or peptides undergo a process known as ion fragmentation. Tandem MS is the breaking apart of the proteins by ion fragmentation followed by the measuring of the mass-to-charge ratio (*m/z*) via MS.

Since its development in 2004, an ion fragmentation technique known as electron transfer dissociation (ETD) has become one of the preferred methods for peptide fragmentation. ETD uses electron transfer from radical anions to peptide cations to create c- and z-type fragments. The resultant set of fragments, or product ions, can be analyzed using MS to determine the complete amino acid sequence of the peptide.

The charge density of the precursor cation may be the most critical parameter for successful protein or peptide sequencing. Fragmentation by ETD decreases as *m/z* increases and precursor peptide cations with high *m/z* values and low charge density are unlikely to generate sufficient fragmentation to enable sequence assignment. Incorporating additional charge sites on peptides to increase their charge density could improve fragmentation and enhance sequencing.

## The Invention

UW–Madison researchers have developed methods of labeling proteins and peptides with tertiary amines or other functional groups to enhance ion fragmentation and improve identification of target molecules. Labeling the proteins or peptides allows them to acquire more charges during electrospray ionization mass spectrometry. The more highly charged ions fragment more extensively during ETD. This leads to more sequence information and thus improved protein identification. It also helps to locate the sites of post-translational modifications (PTMs).

The methods utilize a chemical labeling strategy that reacts a tagging reagent with one or more carboxylic acid groups of the target molecule. The tagging reagent comprises a binding group that can react with the target peptide and a functional group that can improve the fragmenting characteristics of the target. The functional group may be a tertiary amine or other functional group having high gas-phase basicity. Alternatively, the functional group may comprise a protected amine, phosphonium group or sulfonium group. The tagging reagent also may contain one or more stable isotopes, allowing a single mass spectrometric analysis of labeled peptides or proteins to provide precise relative quantification as well as improved protein identification.



## Applications

- Mass spectrometric identification of proteins and peptides

- Relative quantification of proteins and peptides

## Key Benefits

- Labeling allows twice as many peptides to be identified from a complex proteomics sample.
- Labeling reactions have been optimized to yield a nearly pure product after a simple three-step methodology.
- Carboxylic acid groups are blocked by the amidation reaction, making this method particularly useful for analyzing proteins with phosphate groups as post-translational modifications.
- Use of isotopic variants enables precise relative quantification of labeled proteins or peptides.
- Methods are particularly applicable to non-ergodic fragmentation techniques such as electron capture dissociation (ECD) and ETD.
- Increased fragmentation helps to locate the sites of post-translational modifications (PTMs).

## Additional Information

**For More Information About the Inventors**

- Lloyd Smith
- Joshua Coon

**Related Technologies**

- WARF reference number P06069US describes isotopic tags for relative quantification of peptides and proteins.

**Tech Fields**

- Analytical Instrumentation, Methods & Materials : Mass spectrometry

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