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Metal Labeling of Biopolymers for High Precision Quantification
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Summary

The introduction of mass spectrometry (MS) into life science and biotechnology provided a powerful tool for the identification of proteins. However, qualitative analysis is not the only part of the proteomics analysis, and the demand for quantitative information is becoming more and more frequent for applications concerning the status of biological systems. Different MS-based techniques have been developed for quantification of peptides and proteins using stable isotope labeling.

Some examples of these techniques are isotope-coded affinity tags (ICAT), isobaric tags for relative and absolute quantitation (iTRAQ) and Tandem Mass Tags (TMT). In addition to these post-harvest labeling techniques, metabolic labeling strategy such as stable isotope labeling by amino acids in cell culture (SILAC) was also developed. All these quantification strategies show their power when the labeled samples are analyzed by MS coupled with liquid chromatogram (LC) techniques.

Since all these labeling strategies are designed for molecular MS analysis, they suffer several drawbacks related to these analytical techniques. The absolute quantification requires more elaborated strategies where usually standards of the target species must be employed for calibration due to the structure-dependent response of molecular MS. And also very low abundant proteins in the complex samples call for very high sensitivity and wide dynamic range measurements.

To deal with these challenges, Metal-coded affinity tagging (MeCAT) was developed and patented in Prof. Dr. Linscheid's group as a new labeling reagent for absolute and sensitive quantification of proteins and peptides. Instead of labeling biomolecules with isotopes, MeCAT labels were devised to label peptides and proteins with lanthanide ions that are loaded into 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) complexes. These reagents allow tracking labeled species by ICP-MS taking advantage of its high sensitivity, multiplexing capability and structure independent response for quantification purposes. In addition, the structures of the labeled biomolecules can be clarified by molecular MS. However, due to the size of the DOTA complexes and spatial hindrance in peptides and proteins, some of the active sites are inaccessible, which limits its wide application.

In this work, a new MeCAT label is presented for improving the labeling efficiency and reducing the steric hindrance, which shows the capability of quantifying the proteins in real samples.

The proposed approach labels thiol residues in peptides and proteins following a two-step labelling based strategy including *in situ* click chemistry reaction for quantification. Advantages in terms of labeling efficiency have been demonstrated in comparison with other DOTA-based labels, such as MeCAT-Mal and MeCAT-IA. The improvement of the labeling efficiency, even at the protein level, is an important characteristic for application to complex samples. The absence of washing steps or clean-up during the labeling process avoids sample losses or contaminations and brings robustness to the approach.

The employed label showed compatibility with ESI-MS techniques allowing clear identification of the labeled peptides and proteins. Moreover, different approaches for the analysis of the labeled samples have been applied such as, liquid chromatography separations or gel electrophoresis followed by mineralization and direct infusion ICP-MS, both with intact and digested proteins.

In addition to the application to standard peptide and protein, Ln-MeCAT-Click approach was further utilized to quantify the cysteine-containing proteins during the heat shock in *Escherichia coli*. During the application, two parallel experimental approaches, where both elemental and molecular MS techniques were involved, were utilized in a complementary way.

ESI-MS and ESI-MS/MS analyses allowed the identification and relative quantification of cysteine-containing proteins labeled with Ln-MeCAT-Click using Proteome Discoverer. The use of LA-ICP-MS to specifically detect the Ln-MeCAT-Click labeled proteins with high sensitivity is of great interest for complex real samples, where the target species can be sentitively followed by different experimental protocols. This excellent response allows the determination of low abundant proteins that were not detectable by ESI-MS.

Finally, not only identification but also relative quantification of peptides labeled with Ln-MeCAT-Click was proved using specific characteristic fragments. In general, the quantification results obtained by MS/MS fragmentation (CID, IRMPD, HCD) agreed with those obtained by ICP-MS and from the full scan spectra of the parent ions by ESI-MS. In comparison with Ln-MeCAT-Mal and Ln-MeCAT-IA, peptides labeled with Ln-MeCAT-

Click can also produce the reporter ions with enough intensity for quantification by ESI-MS/MS (CID) for the first time.

The concept of peptide quantification using the elemental reporter ions produced by high energy HCD fragmentation was also proved. In these preliminary experiments, the results agree with those from ICP-MS and precursor ion from ESI-MS. It can be regarded as complementary to established methods for protein and peptide quantification.

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