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Review

A review of the role of chemical modification methods in contemporary mass spectrometry-based proteomics research

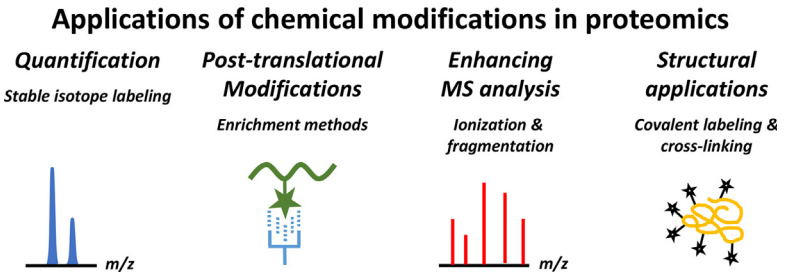
Alexander Leitner

Department of Biology, Institute of Molecular Systems Biology, ETH Zurich, Auguste-Piccard-Hof 1, 8093 Zurich, Switzerland

HIGHLIGHTS

- Chemical modification reactions serve multiple purposes in proteomics research.
- Quantification using isotopic and isobaric tags is one of the main applications.
- Attachment of affinity tags is mainly used to study posttranslational modifications.
- Chemical modifications can enhance peptide ionization and fragmentation.
- Structural proteomics applications include covalent labeling and cross-linking.

GRAPHICAL ABSTRACT



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ABSTRACT

For more than two decades, mass spectrometry has been a central technology for proteomics research. During this time, the use of chemical reactions to introduce tags for quantification, affinity enrichment and other uses has facilitated the comprehensive profiling of the proteome by mass spectrometry in many ways. In the last decade, the introduction of more powerful instruments and more sophisticated data acquisition and data analysis routines has made some strategies obsolete, while making other approaches practically feasible only now. Here, the current status of chemical tagging strategies in proteomics is reviewed, with a particular emphasis on proteome quantification workflows, strategies for the enrichment of (post-translationally) modified proteins and peptides, and methods for structural proteomics.

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E-mail address: leitner@imsb.biol.ethz.ch.

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Non-standard abbreviations

AMP	adenosine monophosphate
BE	β-elimination
CID	collision-induced dissociation
COFRADIC	combined fractional diagonal chromatography
CuAAC	copper-catalyzed azide-alkyne cycloaddition
DiART	deuterium-labeled isobaric amine reactive tag
DTT	dithiothreitol
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ESI	electrospray ionization
HNA	4-hydroxynonenal
ICAT	isotope-coded affinity tag
IPTL	isobaric peptide termini labeling
iTRAQ	isobaric tag for relative and absolute quantitation
LFQ	label-free quantification
MA	Michael addition
mTRAQ	amine-modifying tag for relative and absolute quantitation
NHS	N-hydroxysuccinimide
PTM	post-translational modification
SIL	stable isotope labeling
TAILS	terminal amine isotopic labeling of substrates
TCEP	tris(2-carboxyethyl)phosphine
TMPP	tris(2,4,6-trimethoxyphenyl)phosphonium
TMT	tandem mass tag

1. Introduction: why chemical modifications?

Proteomics aims to comprehensively characterize the protein complement of the genome in biological systems of various complexity, for example cells or tissues. Compared to the static nature of the genome, the proteome is highly dynamic and regulated by changes in protein expression and degradation, post-translational modifications (PTMs), subcellular localization and interactions of proteins with other proteins and other biomolecules. These and other features make the profiling of the proteome much more challenging (and also lower throughput) than genome and transcriptome sequencing. Nevertheless, researchers in the proteomics field have also witnessed extraordinary improvements in analytical methodology. In particular, this applies to mass spectrometry, the de *facto* standard instrumental technique in modern proteomics research. Compared to instrumentation dating back even ten years ago, state-of-the-art mass spectrometers offer at least an order of magnitude higher sensitivity and scan speeds. These advances have made it possible to identify a large fraction of the proteins present in lower organisms such as yeast in just an hour of analysis time [1], and enabled the experimental verification of about three quarters of the predicted human proteome [2].

Proteomics was - and to some extent still is - connected to basic protein chemistry and thus to the (bio)chemical manipulation of proteins, only at a much larger scale. A typical proteomic workflow typically contains several chemical or biochemical transformation steps, for example the cleavage of disulfide bonds by a reducing agent, the capping of free thiol groups by an alkylating reagent, or the enzymatic cleavage of peptide bonds to generate peptides that are more accessible to high throughput chromatographic separation and MS analysis than intact proteins. In addition to these "generic" sample handling steps, chemical modification reactions have been used in protein chemistry and proteomics research to facilitate the analytical characterization of proteins. The concept of the isotope coded affinity tag (ICAT) [3] introduced at the end of the 1990s by Aebersold and co-workers is a prototypical example (Fig. 1): The derivatization of a functional group in a protein (in this case, the free thiol group in cysteines) was used to attach an affinity tag that enabled the enrichment of Cys-containing peptides from the total peptide pool. This helped to reduce the sample complexity and therefore enabled more proteins to be characterized (although at lower sequence coverage), because upon digestion, most proteins yield at least one Cys-containing peptide suitable for identification by mass spectrometry. At the same time, the linker region in the ICAT reagent was used for differential stable isotope labeling; the reagent was prepared in two forms, one with a natural isotope composition, and one for which all hydrogen atoms in the spacer