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Chemical methods for the proteome-wide identification of posttranslationally modified proteins

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Thousands of proteins are subjected to posttranslational modifications that can have dramatic effects on their functions. Traditional biological methods have struggled to address some of the challenges inherit in the unbiased identification of certain posttranslational modifications. As with many areas of biological discovery, the development of chemoselective and bioorthogonal reactions and chemical probes has transformed our ability to selectively label and enrich a wide variety of posttranslational modifications. Collectively, these efforts are making significant contributions to the goal of mapping the protein modification landscape.

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Introduction

Posttranslational modifications (PTMs) augment the chemical diversity of proteins, which is otherwise typically limited by the naturally occurring amino acids and the underlying genetic code. A wide array of enzymatic and chemical modifications, ranging from simple side-chain oxidations to the addition of large polysaccharides, has been shown to dramatically alter the biochemical and biophysical properties of proteins. Despite the documented importance of individual PTMs in cellular biology, the proteome-wide identification of specific PTM substrates remains challenging. PTMs can be substoichiometric, dynamically regulated and heterogeneous in their structure, making their analysis and identification particularly difficult. During the past two decades, chemical methods have significantly contributed to the identification and analysis of posttranslationally modified proteins. In general, these techniques can be broadly classified into two categories, which both enable the installation of visualization or affinity tags:

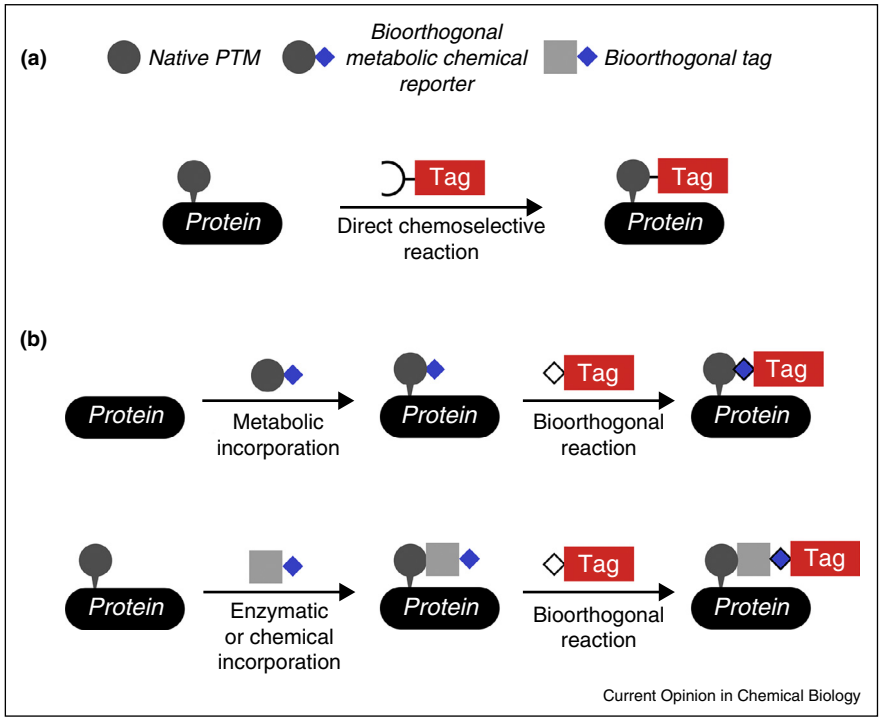
1) Methods that use chemoselective reactions to exploit the unique chemical-reactivity of the posttranslational modifications themselves (Figure 1a).
2) Strategies to install abiotic probes (chemical reporters, Figure 1b) that can be selectively subjected to a range of bioorthogonal reactions (Figure 2).

In parallel, the development of more sensitive mass spectrometers and increasingly sophisticated computer-algorithms for peptide identification has enabled the routine proteomic identification of thousands of proteins from individual complex-samples. Coupled with complementary ionization chemistries and quantitative methods, mass spectrometry can offer high sensitivity, modification-site identification and the ability to quantify changes in PTM occupancy. Here, we review the currently available chemical-strategies to enrich and identify posttranslationally modified proteins in mammalian cells and highlight their recent applications. For a more comprehensive review of the particular advantages of chemical reporters or the scope of bioorthogonal chemistries, we direct readers to excellent summaries elsewhere [1,2]. We end with a summary of future challenges and opportunities that when met will expand the utility of chemical approaches for the study of specific PTMs.

Glycosylation

There are two major types of protein glycosylation that modify large numbers of protein substrates in metazoan and plant cells [3]. Proteins localized to the secretory pathway and the cell surface, or secreted into the extracellular space, can be modified by oligosaccharide structures (e.g., N-linked and mucin O-linked glycosylation). Additionally, intracellular proteins can be substrates for the addition of the single monosaccharide N-acetyl-glucosamine, termed O-GlcNAc modification. Bioinformatic studies suggest that more than 50% of mammalian proteins are glycosylated, and these glycans play diverse cellular roles. Extracellular glycans can alter the half-life of circulating proteins and mediate cellular contacts with proteins, neighboring cells and pathogens, while intracellular glycosylation can affect protein localization and signal transduction. The inherent limitations in characterizing these diverse modifications, prompted the development of the Staudinger ligation and the first azide-bearing metabolic chemical reporter, N-azdioacetyl-mannosamine (Figure 3a), for the detection of sialic acids

Figure 1



Chemical methods to tag and enrich posttranslationally modified proteins. **(a)** Some posttranslational modifications (PTMs) can be specifically reacted with enrichment tags using chemoselective reactions. **(b)** Bioorthogonal reactions in combination with chemical reporters enable the installation of affinity tags. Chemical reporters can either be incorporated into PTMs using cellular metabolism or appended to existing PTMs using enzymes or selective chemical reactions.

[4••]. Together with the creation of other bioorthogonal reactions, this simple idea was extended to other azide-analogs and aklyne-analogs of monosaccharides for the detection of mucin O-linked glycoproteins (Figure 3b), fucose-modified glycans (Figure 3c) and O-GlcNAcylated proteins (Figure 3d) [5].

Many types of cancer display altered levels of both sialic acid and mucin O-linked glycans. Treatment of highly metastatic prostate cancer with Ac_4ManNAz (Figure 3a) allowed for the identification of cell-surface proteins, the majority of which were implicated in cell motility, migration and invasion, supporting a potential role for sialic acid in metastasis [6]. In a separate study, a prostate cancer cell-line was treated with Ac_4GalNAz to enable the enrichment and identification of 29 cell-surface glycoproteins, including many proteins involved in cell adhesion [7]. More recently, Ac_4GalNAz (Figure 3b) was used in combination with iTRAQ (isobaric tag for relative and absolute quantitation) to enrich and compare the secreted mucin glycoproteins from two different CHO cell-lines [8], demonstrating that these chemical reporters could be used in the future to compare cellular glycoproteins from two states (e.g., cancer vs. normal tissue).

O-GlcNAc modification of serine and threonine residues is an abundant modification of proteins in the cytosol, nucleus and mitochondria. Unlike cell-surface glycosylation, O-GlcNAcylation is a dynamic modification that plays critical roles in cellular responses to changes in metabolism and stress, particularly in diseases such as cancer, diabetes and neurodegeneration [9]. The first metabolic chemical reporter of O-GlcNAcylation was peracetylated *N*-azidoacetyl-glucosamine (Ac_4GlcNAz, Figure 3d) [10], which was recently used to identify over one-thousand potentially O-GlcNAcylated proteins from a single cell-line and confirm the modification sites on 80 of these substrates [11]. To provide improved signal-to-noise in the CuAAC bioorthogonal reaction, the alkyne-analog *N*-pentynyl-glucosamine (GlcNAlk, Figure 3d) has also been developed and revealed the O-GlcNAc modification of the ubiquitin ligase NEDD4 [12•]. This chemical reporter was also used to identify O-GlcNAcylated proteins associated with diabetic retinopathy [13]. As an alternative to metabolic methods, a chemoenzymatic method has been developed (Figure 3e). Specifically, incubation of cell-lysates with an engineered β-1,4-galactosyltransferase and azide-containing or ketone-containing UDP-donor sugar results in modification of O-GlcNAc residues [14••]. The resulting