COMMENTARY

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Chemistry

A chemical ligation approach enables rapid and large-scale glycopeptide enrichment for the comprehensive profiling of protein glycosylation

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Glycosylation is one of the most complex and important post-translational modifications in proteins, playing essential roles in cellular signaling, protein folding, and immune responses [1]. Despite its biological significance, low abundance and high heterogeneity of glycoproteins pose significant analytical challenges. In a recent study published in *National Science Review*, a team of scientists led by Prof. Haojie Lu from Fudan University developed a groundbreaking strategy to address these challenges, offering a robust and scalable method for the comprehensive profiling of protein glycosylation [2].

The novel enrichment strategy, termed HG-TCs, utilizes bioorthogonal ligation with trypsin-cleavable tags to achieve simultaneous identification of multiple glycosylation types, including N-glycosylation, O-GlcNAcylation, O-GalNAcylation, and even the N-glycans (Fig. 1) [2]. This method provides unprecedented efficiency and reproducibility, significantly reducing sample loss and experimental variability. Importantly, the enrichment process is completed in a single tube within 6 hours, offering a streamlined workflow compared to traditional methods.

By applying HG-TCs to HeLa cell samples, the researchers identified over 900 O-GlcNAc sites, 800 N-glycosites, and dozens of O-GalNAc sites with exceptional sensitivity and coverage. Furthermore, the strategy uncovered distinct spatial patterns of glycosylation under oxidative stress, revealing significant differences between nuclear and cytoplasmic compartments. These findings shed light on the dynamic and context-specific roles of glycosylation in stress responses, paving the way for deeper insights into disease mechanisms such as cancer progression and neurodegeneration [1].

This study represents a significant step forward in glycoproteomics research by addressing longstanding technical challenges. The ability to analyze multiple glycosylation types simultaneously within the same sample not only enhances data comparability but also reduces experimental costs and sample requirements. In previous studies, the Click-iG platform also enabled various types of glycosylation analysis at the intact glycopeptide level by integrating biotin-avidin strategies, optimized MS method and a tailored version of pGlyco3 software [3]. Researchers can balance and leverage the strengths of both strategies to achieve

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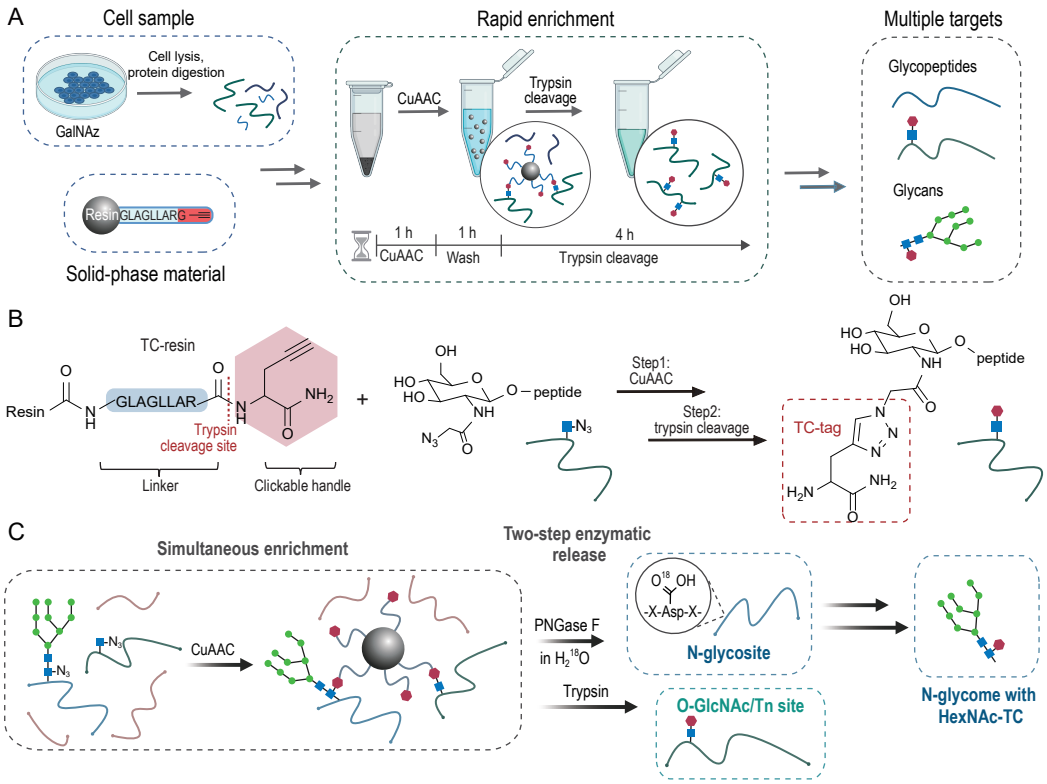


Figure 1 Schematic representation of the HG-TCs workflow. (A) Workflow of the rapid and large-scale enrichment strategy based on TC-resins. (B) Schematic illustration of TC-resins for peptide enrichment. (C) Schematic illustration of simultaneous identification of N-glycosites, O-GlcNAc sites, O-GalNAc sites and N-glycans via a two-step enzymatic release strategy. Reprinted from Ref. [2].

comprehensive and efficient glycopeptide analysis in the future.

The authors highlight the potential of HG-TCs for studying highly dynamic and complex carbohydrate systems such as cancer, allowing not only the mapping of multiple glycosylations but also the simultaneous monitoring of multiple glycosylation alterations. Moreover, the compatibility of HG-TCs with various labeling and analytical platforms makes it a versatile tool for both basic research and clinical applications, such as in combination with enzymatic labeling [4]. The integration of this method with advanced mass spectrometry and quantitative proteomics approaches could enable more comprehensive profiling of glycosylation dynamics.

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