ENHANCING GLYCOPROTEOME PROFILING: EVALUATING AND COMBINING ENRICHMENT TECHNIQUES FOR COMPLEX BIOLOGICAL SAMPLES

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Glycosylation is a fundamental and widespread post-translational modification where sugars are covalently attached to proteins, typically through asparagine (N-glycan) or serine/threonine (O-glycan) side chains. Unlike proteins, glycans are not directly encoded by the genome but are dynamically regulated by glycosyltransferases and glycosidases. This complexity makes glycosylation analysis particularly challenging using mass spectrometry, as glycosylated peptides exhibit structural and occupancy heterogeneity and often suffer from reduced ionization efficiency. Consequently, glycopeptide enrichment has become an indispensable strategy to improve the depth and accuracy of glycosylation characterization in biological research.

This study systematically compares glycopeptide enrichment techniques for complex biological samples, including cells and biofluids, addressing practical considerations like sample volume and time. We evaluate iSPE®-HILIC, a widely used ZIC-HILIC resin, and a phosphorylcholine enrichment (PCE) strategy, which utilizes a related resin. While iSPE®-HILIC has shown promise in serum glycoproteomics, its application to cell samples requires further investigation. Similarly, the glycoproteomic potential of PCE, primarily used for CRP enrichment, remains unexplored. By combining iSPE®-HILIC and PCE in a sequential enrichment workflow, we demonstrate a significant improvement in glycoproteome coverage in both HEK293 cells and plasma, using high-performance Orbitrap Eclipse mass spectrometer. Our results show that the iSPE-first followed by PCE strategy increased the detection of unique N-glycopeptides by more than fourfold compared to unenriched samples, even in unfractionated digests. This study underscores the importance of optimizing glycopeptide enrichment strategies to achieve comprehensive, unbiased profiling of the glycoproteome, thereby enabling more precise and detailed insights into the roles of glycosylation in biological systems. Furthermore, we explore a novel HILIC-based material, BioSPE™ GlycaClean, showing a ~5-fold increase in glycopeptide identification in single analyses, highlighting its potential for targeted glycan enrichment.

This work not only expands the toolkit for glycoproteomic analysis but also provides substantial improvements in the sensitivity, breadth, and accuracy of glycopeptide identification. It sets the stage for more robust and expansive glycoproteomic studies, with potential applications in biomarker discovery, disease mechanism exploration, and therapeutic development.

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