# Cell-specific Bioorthogonal Tagging of Glycoproteins

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Alterations in glycoprotein expression and composition are an undisputed corollary of cancer development. Consequently, some of the most faithful tumor biomarkers are heavily glycosylated. Understanding cancer-related changes of the glycoproteome is paramount but hampered by limitations in cellular model systems: Technological advances in mass spectrometry have allowed profiling of glycoproteomes but are often restricted to isolated cells that do not adequately reflect the interaction between tumor and microenvironment. Co-culture systems in vitro or in vivo better reflect the physiological environment but the glycoproteomes of cells from the same host organism cannot be meaningfully discerned to distinguish tumor from associated cells.

Here, we report the development of chemical “Precision Tools” that allow for Bio-Orthogonal Cell-specific Tagging of Glycoproteins (BOCTAG). We equip cells with an artificial metabolic pathway to biosynthesize chemically tagged UDP-GalNAc analogues. Engineered glycosyltransferases accommodate these chemical tags, allowing to selectively study the glycoproteome of transfected cells in the presence of bystander cells. We extensively validate BOCTAG as a strategy for cell-specific imaging in co-culture and to selectively annotate cell-specific glycosylation sites by mass spectrometry. BOCTAG serves to visualize and profile the cancer-specific glycoproteome in co-culture in vivo and in vitro without cell sorting and in secretome, unraveling the importance of glycosylation as a modulator of cellular function.

Recently, we have applied the principles of BOCTAG and other chemical tools to unveil O-GalNAc glycosylation as a modulator of the proteolytic maturation of SARS-CoV-2 spike, majorly influencing the mutational trajectory of variants of concern including Alpha, Delta and Omicron. Our work highlights the outstanding relevance of chemistry for glycobiology.

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