**Principal Investigator: Junfeng Ma**
**Grant Title: Characterization of tyrosine O-GlcNAcylation**

Abstract
*Objectives*: O-linked β-N-acetylglucosamine modification (O-GlcNAcylation) modifies many Ser/Thr sites on thousands of proteins in dozens of species. Despite the functional importance, deep and accurate O-GlcNAc analysis at the proteome scale has been challenging for many years. Very recently, we developed a highly integrated strategy for site-specific O-GlcNAc proteomics analysis. By benchmarking that approach on cancer cells, we found that in addition to confirming known sites and discovering many novel sites of Ser/Thr modification, O-GlcNAc modification was even present on over a hundred of tyrosine residues on proteins. As a newly revealed glycosylation form, a lot of unknowns remain. As one endeavor, we strive to further refine glycoproteomics apporaches for precision analysis of O-GlcNAcylated proteins, including tyrosine O-GlcNAcylated proteins.
*Methods*: In brief, we developed chemoenzymatic labeling/click chemistry-based analytical workflows for glycoproteomics by utilizing four cleavable bioorthogonal probes, including photocleavabe-biotin-alkyne (PC-biotin-alkyne), dialkoxydiphenylsilane-biotin-alkyne (DADPS-biotin-alkyne); 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl-biotin-alkyne (Dde-biotin-alkyne), and diazobenzene-biotin-alkyne (Diazo-biotin-alkyne). The analytical performance of these probes was rigorously evaluated with synthetic O-GlcNAc peptides and then benchmarked by using mouse brain lysates for O-GlcNAc proteomics.
*Results*: The workflows yielded an unprecedented O-GlcNAc proteome depth in the mouse brain (1). In total, 2906 O-GlcNAc sites were unambiguously assigned on 878 proteins. Among them, 1611 sites were newly identified, including 138 O-GlcNAcylated tyrosine residues. Besides offering valuable technical insights and helping guide the selection/development of O-GlcNAc proteomics methods for future studies, our work provides an invaluable resource for functional elucidation of protein O-GlcNAcylation in brain biology and offers critical insights into tyrosine O-GlcNAcylation. Furthermore, we explored (tyrosine) O-GlcNAcylation in other model systems, which further support our notion that tyrosine O-GlcNAcylation exists in multiple types of samples. Last but not least, we found that tyrosine O-GlcNAcylation plays a regulatory role. Related manuscripts are under preparation. Again we appreciate the kind support from the Mizutani Foundation that helped with development of new tools and applications of them to explore (tyrosine) O-GlcNAcylation.

(1) Chunyan Hou, Hemeng Zhang, Jingtao Deng, Xiaoxin Wang, Stephen Byers, Moshe Levi, Daniel T.S. Pak, Kelley W. Moremen, Huadong Pei, Gerald W. Hart, Junfeng Ma. Mol Cell Proteomics. 2025 Oct;24(10):101064. doi: 10.1016/j.mcpro.2025.101064.