# INTACT GLYCOPEPTIDE ANALYSIS OF RECOMBINANT PROTEIN FROM CHO CELLS

Qiong Wang, Johns Hopkins University
qwang54@jhu.edu
Sandra Chough, Johns Hopkins University
Michael J. Betenbaugh, Johns Hopkins University
Hui Zhang, Johns Hopkins University

Key Words: intact glycopeptide analysis, CHO, recombinant proteins, mass spectrometry, glycoproteomics

The quality of recombinant glycoproteins including antibodies and other biologics is dictated by their glycan profiles. What is missing is how to analyze these glycans rapidly for process improvement and control applications. Conventional glycan analysis involves the release of glycans, which rarely captures the glycan site-specific information. Intact glycopeptide analysis in which glycans are retained on the peptide provides insights into the glycan structure and the glycosylation site information simultaneously. This information can reveal additional details about site occupancy and cellular glycosylation of proteins. Avoiding glycan release and some modifications and labeling steps in our intact glycopeptide analysis can result in a shorter sample preparation time than conventional glycan analysis methods. Compared to peptide mapping using LC-MS to decipher protein amino acid sequence in proteomics, this analysis focuses on glycopeptide profiling following protease-digestion. With the aid of LC-MS/MS, we are able to obtain targeted glycoprotein sequence information, glycan profiles and glycan distribution at specific sites. Here we present the application of glycopeptide analysis for model AMBIC and other proteins from CHO-GS and CHO-K1 cells. The site-specific glycosylation patterns of our model proteins EPO-Fc and EPO are characterized. Further, we examine the impact of media formulation and additives on the glycan profiles for these proteins.

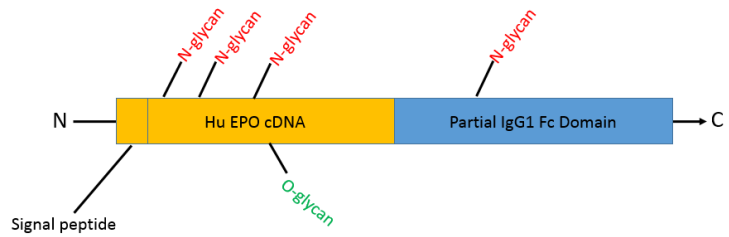


*Figure 1. Schematic representation of N- and O-glycosylation sites in EPO-Fc fusion protein.*