QUANTITATIVE ANALYSIS OF SITE-SPECIFIC N-GLYCAN STRUCTURES IN HUMAN IMMUNOGLOBULINS

Weiwei Wang[a,b,c], Karli R Reiding[a,b], Albert J.R. Heck[a,b]

[a] Biomolecular Mass Spectrometry and Proteomics, Bijvoet Center for Biomolecular Research and Utrecht Institute for Pharmaceutical Sciences,
Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands
[b] Netherlands Proteomics Center, Utrecht, The Netherlands
[c] School of Pharmaceutical Science, Shanghai Jiao Tong University, Shanghai, China
w.wang1@uu.nl

Immunoglobulins (Igs), are crucial to the immune system and are classified into five classes: IgG, IgM, IgA, IgE, and IgD, each with distinct functions and locations in the body. All Igs share a characteristic Y-shaped structure, with Fc domains containing one or more N-glycosylation sites. These sites, harboring diverse glycan structures, play key roles in Igs' functionalities, such as interacting with Fc receptors or maintaining stability. The functionality of glycans is determined by their precise structures, including branches and linkages. Although site-specific glycan compositions have been studied by mass spectrometry, the structural details remain largely unexplored. Our work offers a comprehensive analysis of glycan structures at specific sites across Igs, providing new insights into their role in immune modulation.
Briefly, We developed an advanced HILIC-LC-MS/MS method [1] that enables site-specific quantitative structural analysis of glycans on immunoglobulin-specific glycopeptides, allowing for comprehensive analysis across all immunoglobulins.
Key findings from our study include:
1. Recombinant Igs display a wider variety of glycan structures compared to those from human biofluids, with distinct quantitative glycan signatures characteristic of particular Ig classes and subclasses, irrespective of source.
2. Igs from the same donor show unique glycan structural signatures differing across various biofluids.
3. Within a single serum sample, different molecular forms of Igs present distinctive glycan structural signatures.
4. The glycan structures of Igs exhibit several conserved features: most remain stable over extended periods, far exceeding the half-lives of the Igs themselves. Unlike other glycoproteins in human serum, which are predominantly produced by liver cells and show monosialylated glycans on both 6-branch and 3-branch structures, Igs consistently display a primarily 3-branch monosialylated glycan structure.
Our research offers pioneering insights into the quantitative glycan structural patterns at specific sites of endogenous immunoglobulins, enhancing our understanding of their function in the human body and offering a glycosylation blueprint for the production of therapeutic antibodies.

References:
1. Wang W, Maliepaard JCL, Damelang T, Vidarsson G, Heck AJR, Reiding KR. *ACS Cent Sci.* **2024**, *10(11),* 2048-2058.