Automated High-Throughput Permethylation for Glycosylation Analysis Using MALDI-TOF-MS

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Abstract
For most therapeutic glycoproteins, the glycosylation patterns correlate strongly with the clinical safety and efficacy profiles. In biological tissues these patterns can also correlate with the state of health or disease of the individual. Given this, there is an increasing interest in accurately characterising changes in glycosylation — for example in Quality by Design (QbD) studies throughout biopharmaceutical development.

To perform these studies, reliable systems for high-throughput (HT) glycomics are needed. Despite many advances in glycosylation analysis there are still problems with current technologies, including high cost per sample, low sample throughput and high labour intensity.

This poster presents “LongFlow” — a system developed at Ludger for reliable HT glycomics. The “LongFlow” system is made up of flexible, modular technologies for semi-automated processing of glycans from a variety of clinical and bio-therapeutic samples as shown in Scheme 1. The emphasis of this poster will be on Automated and HT Permethylation of N- and O-glycans analysed by Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF-MS).

Introduction
Permethylation is the most popular derivatization of carbohydrates for MS detection, as it enhances ionization efficiency and stabilizes the ionic species. This modification is the addition of methyl substituent to all of the hydroxyl and N-acetyl groups. Permethylation also methyl esterifies the carboxy function on the sialic acid.

The conventional in-solution technique is labour intensive with long turn around times, which prompted us to develop a microplate based permethylation kit which delivers a cost effective, automated and HT method for QbD and biomarker studies. (See Scheme 2)

However, the reliability of glycans quantitation using this method is challenging and to improve the reliability we have:

a) automated sample preparation and clean-up,
b) automated HT permethylation for glycan identification,
c) automated data acquisition on MALDI-TOF-MS and semi-automated data analysis

d) use a heavy isotope 13C permethylated IgG standard for calibration and relative quantitation of IgG samples (Fig-1).

Methods
To demonstrate our automated and HT technology, we successfully applied it to various sample subsets and the methods for N- and O-glycan release and derivation are outlined as follows:

Glycan release: N-glycans from IgG1 monoclonal Antibody (mAb) standard and recombinant human erythropoietin (rHepO) were released using Peptide N Glycosidase F enzyme and cleaned up using a LudgerCleanTM Pre-Permethylation Clean-up Plate (LC-PERMT96). A manual chemical release method (Hydrazinolysis) was used for release of O-glycans from rHepO.

Glycan derivation: The released and purified N-glycans from IgG1 mAb standard (Fig-2) and rHepO (Fig-3) were permethylated using LudgerTag™ permethylation microplate kit (LT-PERMT-96). Both the permethylation and post-derivation sample clean up steps were performed on the a liquid handling robot and then analysed by flexAnalysis software from Bruker.

The O-glycans released from rHepO were split into two aliquots to show comparability and orthogonality between two techniques:
a) Ultrahigh Performance Liquid Chromatography (UHPLC) and b) MALDI-TOF-MS.

Sample processing and preparation for both methods was performed using the liquid handling robot. Pool 1 samples were reductively aminated with LudgerTag 2-AB labelling kit (LT-KAB-VP24), cleaned up using LudgerClean T1 cartridge (LC-T1-46) and analysed by UHPLC (Fig-4a). Pool 2 samples were permethylated using the LT-PERMT-96 kit and analysed by MALDI-TOF-MS (Fig-4b).

Results
Fig-1: MALDI-TOF-MS spectrum of the 12C and 13C permethylated human-IgG N-glycan standards from Ludger (Ct13Cp13C-IgG-02 and Cmp13Cp13C-IgG-02). 12C was spiked with 13C on the same sample to show the comparison of relative quantities of the major IgG N-glycans. The mass values shown in the spectra are [M+Na]+ of permethylated glycans, with 13C permethylated masses in parentheses.

Fig-2: MALDI-TOF-MS spectrum of the permethylated N-glycans from rHepO.

Fig-3: MALDI-TOF-MS spectrum of the permethylated N-glycans from rHepO.

Fig-4a: Typical UHPLC profile of 2-AB labelled biosimilar rHepO depicting the major O-glycans. Note: Processing 96 samples on UHPLC takes a minimum of 48 hours.

Fig-4b: MALDI-TOF-MS spectrum of the permethylated rHepO, depicting the O-glycans. Note: Automated data acquisition for 96 samples on MALDI-TOF-MS takes under 1 hour.

Conclusions
• We have developed and optimized a new permethylation technique which exploits the use of a liquid handling robot to enable higher throughput and automated glycosylation analysis of N- and O-glycans analysed by using MALDI-TOF-MS.

• This HT permethylation technique is compatible to UHPLC results (see Fig 4a and 4b) and it gives a reliable overview of the glycosylation profile in a short timeframe.

• The advantages of permethylated glycans analysis using the MALDI-TOF-MS are: enhanced signal due to increased ionization efficiency, good sensitivity, and rapid analysis. You can process hundreds of samples per day i.e., on average one sample takes less than a minute to process, so 96 samples can be processed in under 1 hour on MALDI-TOF-MS compared to UHPLC which takes more than 48 hours to run 96 samples.

• Therefore we conclude that our microplate based, automated and HT permethylation technique delivers the most cost effective, fast and reliable method for QbD and biomarker studies.

References

• Wuhrer, M, Glycoconjugate journal, 2013, DOI: 10.1007/s10719-012-9376-3


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Thank you for viewing my poster. If you’d like a copy or want to know more about our glycomics workflows, then please email: archana.shubhakar@ludger.com. Thanks, Archana.