# Rapid and quantitative glyco-analysis by MALDI using isotopically labelled glycans

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Numerous diseases are known to involve changes in glycosylation. Altered glycosylation is a universal feature of cancer cells and some glycan structures are well-known markers for tumours and tumour progression.[1] Also, the biopharmaceutical industry is particularly interested in the glycosylation of monoclonal antibodies and other therapeutical proteins as glycans can be important determinants of their biological activity and therapeutic efficacy as well as in the immunogenicity of the protein. As a result, methods for the comprehensive analysis of protein glycosylation and glycan composition are of interest to the scientific community.

Owing to its high sensitivity at low concentrations, mass spectrometry is often used in the analysis of the resulting complex mixtures.[2] However, the signal intensity of particular analytes is dependent, amongst many other factors, on the physical properties of the analyte, making any relative quantification very difficult. Identification of glycans common to two samples and their relative quantification may be facilitated by use of derivatisation of the glycan mixtures to incorporate isotopic tags.[3] However, the introduction of additional steps (labeling and washing procedures) make these methods time-consuming and less accurate, providing only semi-quantitative results and difficulting the high-throughput. There exists an unmet need for improved methods for rapidly and easily analysing the content of released glycan mixtures.

We present here the use of stable isotopologues of individual N-glycans as standards in matrix assisted mass spectrometry for the quantitative analysis of protein glycosylation. Following a chemo-enzymatic approach, a library of $^{13}$C-labeled N-glycans has been prepared. The glycoprotein of interest can be spiked with a mixture of these standards and directly analysed by MALDI, after the corresponding glycan release, in a quantitative way using the isotope dilution analysis principle. This method of analysis may have utility both in the identification of glycan markers associated with particular disorders and diseases and for the monitoring and control of glycosylation in biopharmaceutical manufacturing, as it provides a rapid and easily automated method for the determination of the absolute content of glycans in a sample.

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[2] Morelle, W.; Michalski, J. C. *Nat. Protoc.* **2007**, *2*, 1585.
[3] Ruhaak, L. R.; Zauner, G.; Huhn, C.; Bruggink, Am. M.; Deelder, A. M.; Wuhrer, M. *Anal. Bioanal. Chem.* **2010**, *397*, 3457.