

## N-Glycan analysis on monoclonal antibodies using MALDI-Q-IT-TOF

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### Abstract

Monoclonal antibodies (MAbs) are increasingly being used for the treatment of cancer or other autoimmune diseases. Correct glycan structure of MAbs is essential for functional and biological activity of the related Mab such as identification of antigens triggered by immune system cells, regulation of the signaling activities and physicochemical properties of produced therapeutics etc. Therefore, rapid monitoring of glycan structure is very critical for therapeutic drug production, biosimilar drug production and their quality control processes.

The principal of this study is the characterization of glycan structure of MAbs. In our study, glycan structure of trastuzumab, used as a drug for the treatment of breast cancer, was characterized. N-glycans were enzymatically released from Trastuzumab protein structure by trypsin digestion and PNGase F (Peptide:N-Glycosidase F) cleavage. Moreover, special buffer exchange and Solid Phase extraction procedures were used for the clean-up purposes prior to MALDI-TOF-MS (Matrix Assisted Laser Desorption/Ionization-Time of Flight-Mass Spectrometry) detection.

After designing appropriate sample treatment and optimization, GO, GOF, G1, G1F, GOF-GN glycans were clearly detected by MALDI-Q-IT-TOF MS without any labelling or using any additional chromatographic separation methods.

The current talk deals with the theoretical and experimental aspects of MALDI-Q-IT-TOF MS for the study of glycans on monoclonal antibodies. Sample preparation procedures and tips for MALDI-TOF-MS measurements will be addressed and the original data obtained from MALDI-Q-IT-TOF MS will be presented.

Glycosylation is a critical attribute for development and manufacturing of therapeutic monoclonal antibodies (mAbs) in the pharmaceutical industry. Conventional antibody glycan analysis is usually achieved by the 2-aminobenzamide (2-AB) hydrophilic interaction liquid chromatography (HILIC) method following the release of glycans. Although this method produces satisfactory results, it has limited use for screening a large number of samples because it requires expensive reagents and takes several hours or even days for the sample preparation. A simple and rapid glycan analysis method was not available. To overcome these constraints, we

developed and compared 2 ultrafast methods for antibody glycan analysis (UMAG) that involve the rapid generation and purification of glycopeptides in either organic solvent or aqueous buffer followed by label-free quantification using matrix-assisted laser desorption/ionization-time of flight mass spectrometry. Both methods quickly yield N-glycan profiles of test antibodies similar to those obtained by the 2-AB HILIC-HPLC method. In addition, the UMAG method performed in aqueous buffer has a shorter assay time of less than 15 min, and enables high throughput analysis in 96-well PCR plates with minimal sample handling. This method, the fastest, and simplest as reported thus far, has been evaluated for glycoprofiling of mAbs expressed under various cell culture conditions, as well as for the evaluation of antibody culture clones and various production batches. Importantly the method sensitively captured changes in glycoprofiles detected by traditional 2-AB HILIC-HPLC or HILIC-UPLC. The simplicity, high speed, and low cost of this method may facilitate basic research and process development for novel mAbs and biosimilar products.

(MALDI) source, a Quadrupole Ion Trap (QIT) and a Reflectron Time of Flight (reToF) Mass Spectrometer (MS) has been developed. Computer simulations have been carried out to model different methods for efficiently introducing MALDI ions into the QIT and for subsequently extracting ions from the QIT to a dual-stage gridless reToF. The parameters for the system were optimized to get near 100% trapping efficiency and the highest mass resolution. A prototype instrument was built to realize the performance predicted from the computer simulations. Methods for the near orthogonal laser irradiation of a sample, fast switching of large amplitude Radio Frequency (RF) voltages and a gridless dual-stage reflectron were developed. These new developments were combined with a number of other already known techniques to provide the facility to perform high efficiency ion trapping and MS/MS and higher (MS<sup>n</sup>) analysis. High mass spectra for m/z up to 16,000 have been obtained, and MS<sup>3</sup> experiments have been successfully carried out. A sensitivity of 0.1 fmol has been achieved at a mass resolution exceeding 3,000.

Therapeutic monoclonal antibodies (mAbs) are glycoproteins produced by living cell systems. The glycan moieties attached to the proteins can directly affect protein stability, bioactivity, and immunogenicity. Therefore, glycan variants of a glycoprotein product must be adequately analyzed and

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controlled to ensure product quality. However, the inherent complexity of protein glycosylation poses a daunting analytical challenge. This review provides an update of recent advances in glycan analysis, including the potential utility of lectin-based microarray for high throughput glycan profiling. Emphasis is placed on comparison of the major types of analytics for use in determining unique glycan features such as glycosylation site, glycan structure, and content.

## **Biography**

She received her BSC degree in Biochemistry, Faculty of Science from Ege University in 2006. She is currently doing her master thesis about "Glycan Analysis of Monoclonal Antibodies" and she will be graduated at 2017 summer. At the same time she is working as a scientific researcher at the Center for Drug Research & Development and Pharmacokinetic Applications (ARGEFAR) since 2008. Her professional experience includes usage of HPLC, LC MS, MALDI TOF MS techniques. Her research interest covers physicochemical characterization of biosimilar drugs and biopharmaceutics.