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Quantification and structural characterization of glycans and glycopeptides by TQMS: the energy-resolved oxonium ion monitoring (Erexim) platform

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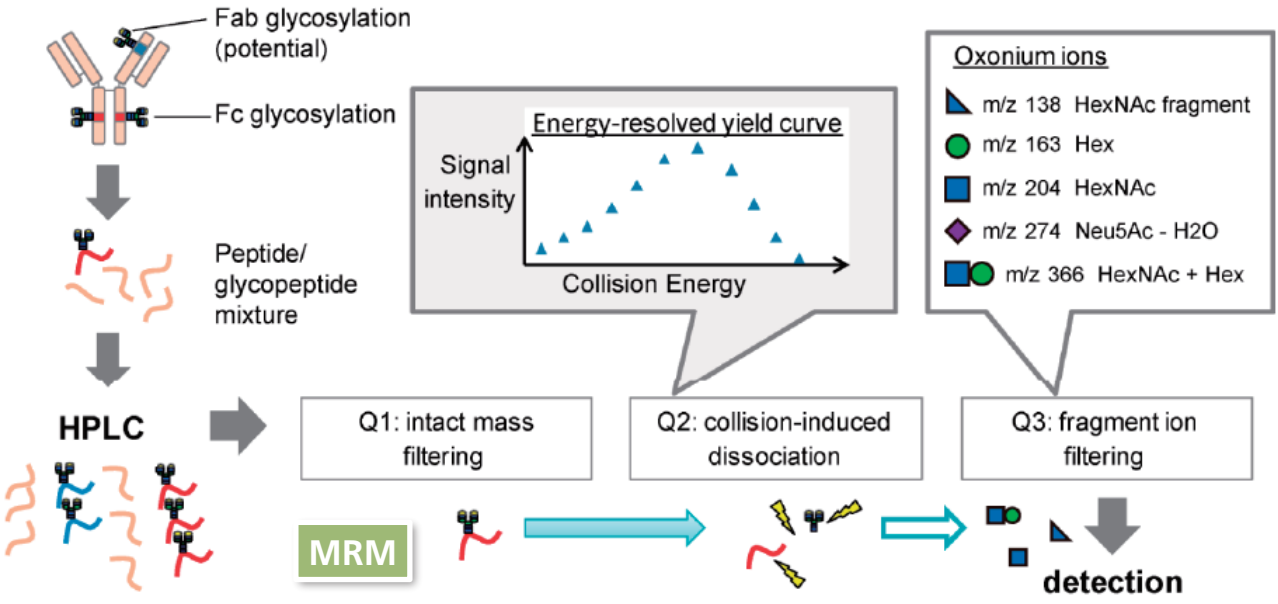
Introduction

We have previously reported the method of using a triple quadrupole mass spectrometer to reproducibly monitor glycan-derived low molecular weight ions (oxonium ions) to acquire, within milliseconds, a profile of fragmentation patterns at a range of CID collision energies. This, which we termed the energy-resolved oxonium ion monitoring (Erexim) profile, reflects the glycan structure in such a way that isobaric structures can be clearly distinguished and assigned. Here we have developed new software to fully support Erexim data acquisition and analysis. Moreover, we acquired the Erexim profile database using purified glycan standards to enable glycan structure identification by pattern matching. The Erexim platform thus gives solution to both structural characterization and quantitative analysis of glycan and glycopeptides.

Energy-Resolved Oxonium Ion Monitoring (Erexim)



- *m/z* 138 is the universal product ion for quantitative profiling of glycopeptides and labelled glycan by MRM
- Energy-resolved MRM enables differentiation of isobaric structures

Figure 1 Overview of Erexim platform

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Materials and Methods

2-aminopyridine (PA) -labeled glycan standards were purchased for 45 different N-glycan structures. These include structural isomers such as linkage difference of sialic acids. For glycopeptide analysis, commercially available purified human IgG was digested with trypsin and glycopeptide fraction derived from the Fc region was collected by negative selection using Supel-Select HLB SPE cartridge (Sigma Aldrich).
Both PA-labeled glycans and glycopeptides were separated on reverse-phase HPLC and measured by Shimadzu LCMS-8050 instrument under conditions summarized in Table 1. Quantitative profiling of glycan heterogeneity was performed by acquiring MRM for monitoring *m/z* 138 as the common product ion that occur from all glycans. The Erexim profiles were acquired by series of transitions with the same combination of Q1 and Q3 mass filters at different collision energies.

Results

Software overview

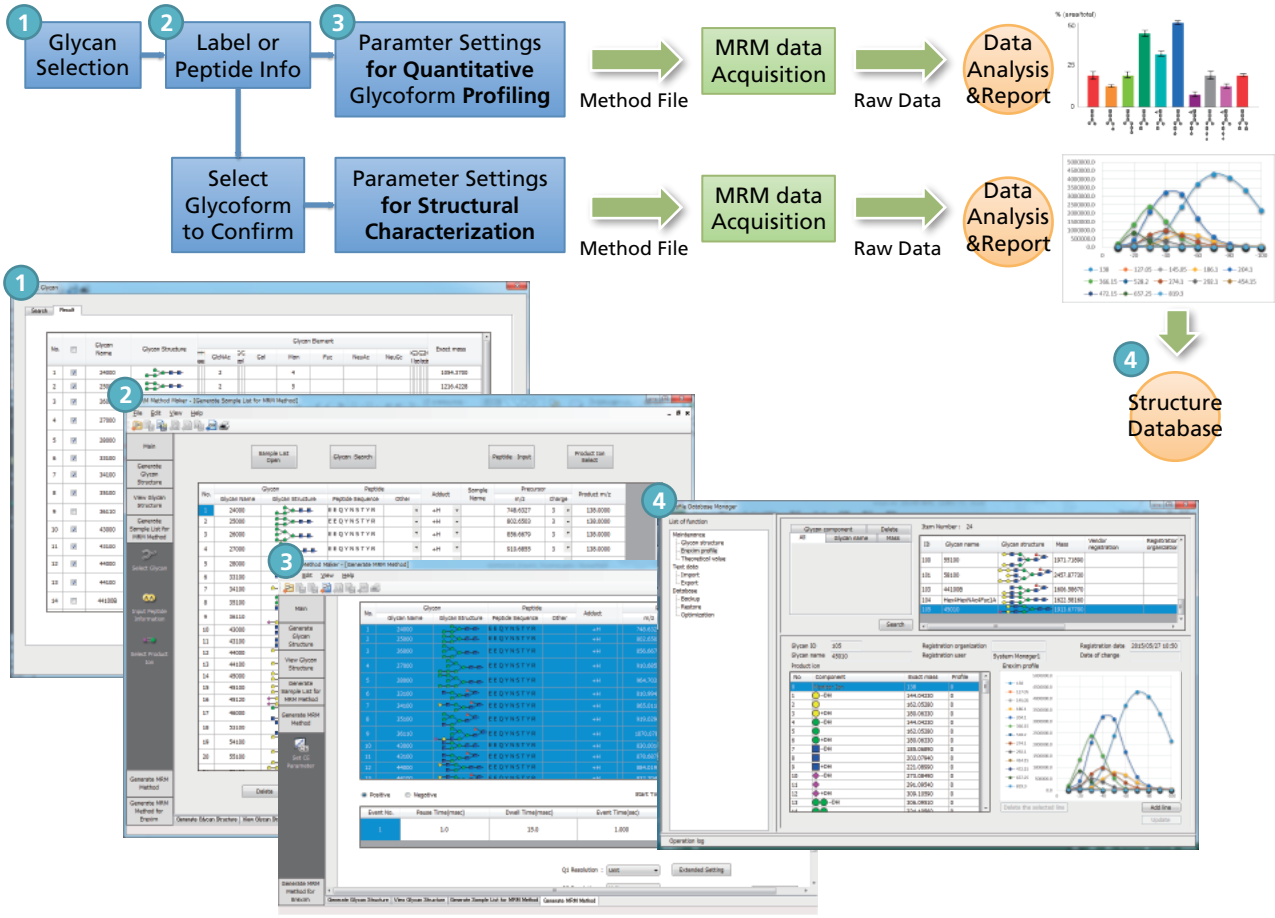

Figure 2 Overview of the Erexim platform with screenshots of analysis software. First, glycan structures of interest are selected from the structure database to produce a list of glycan structures. Since this platform is compatible with both labelled glycan and glycopeptides, modifications on glycan are specified either as amino acid sequence or as any given derivative so that the software calculates the mass shift automatically to produce a list of m/z combinations for MRM. After entering further conditions for data acquisition, such as dwell time, CE, etc., method file will be produced that can be used directly for analysis.

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Table 1 HPLC conditions for separation of glycopeptides and PA-labelled N-glycan

Column	: Aeris Peptide 1.7µ 150mm×2.1mm (Phenomenex)
Mobile phase A	: 0.1% Formic Acid in Water
Mobile phase B	: 0.1% Formic Acid in Acetonitrile
Flow rate	: 0.3 mL/min
Time program	: B conc.2%(0-1 min) -30%(7 min) - 98%(7.1-9 min) - 2%(9.1-12 min)
Injection vol.	: 20 µL
Column temperature	: 40 °C

High Speed Mass Spectrometer
Ultra Fast Scanning
- 30,000 u / sec.
Ultra Fast Polarity Switching
- 5 msec.
Ultra Fast MRM
- Max. 555 transitions /sec

Figure 3 LCMS-8050 triple quadrupole mass spectrometer

Sensitivity and Repeatability of Quantification

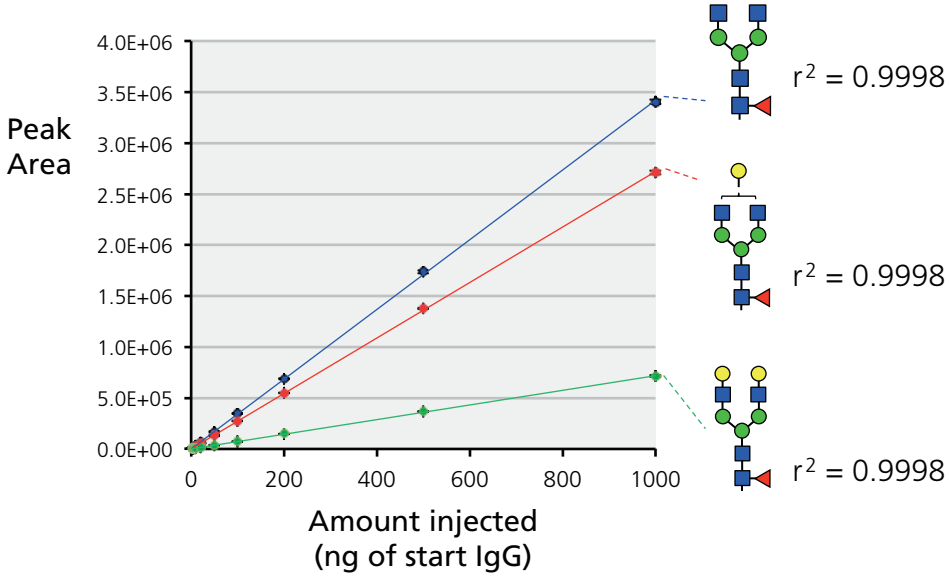

Figure 4 Commercially available monoclonal IgG sample was digested with trypsin and glycopeptides were subjected to quantitation by MRM of product ion *m/z* 138. High linearity was observed for 2 – 1000 ng of start IgG amount.

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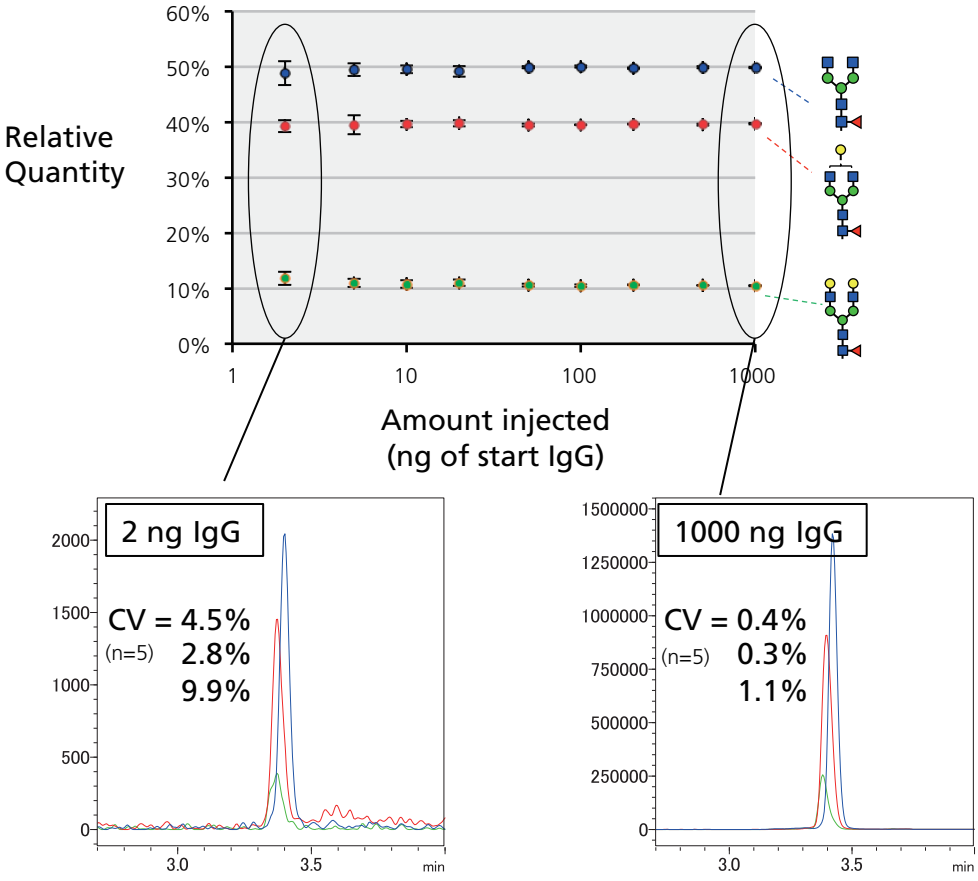

Figure 5 For each measurement, quantitites of glycopeptides were expressed as percentages relative to the sum of all glycopeptides combined. Even at injection amount as small as 2 ng IgG, relative quantities were reproducible with good CV.

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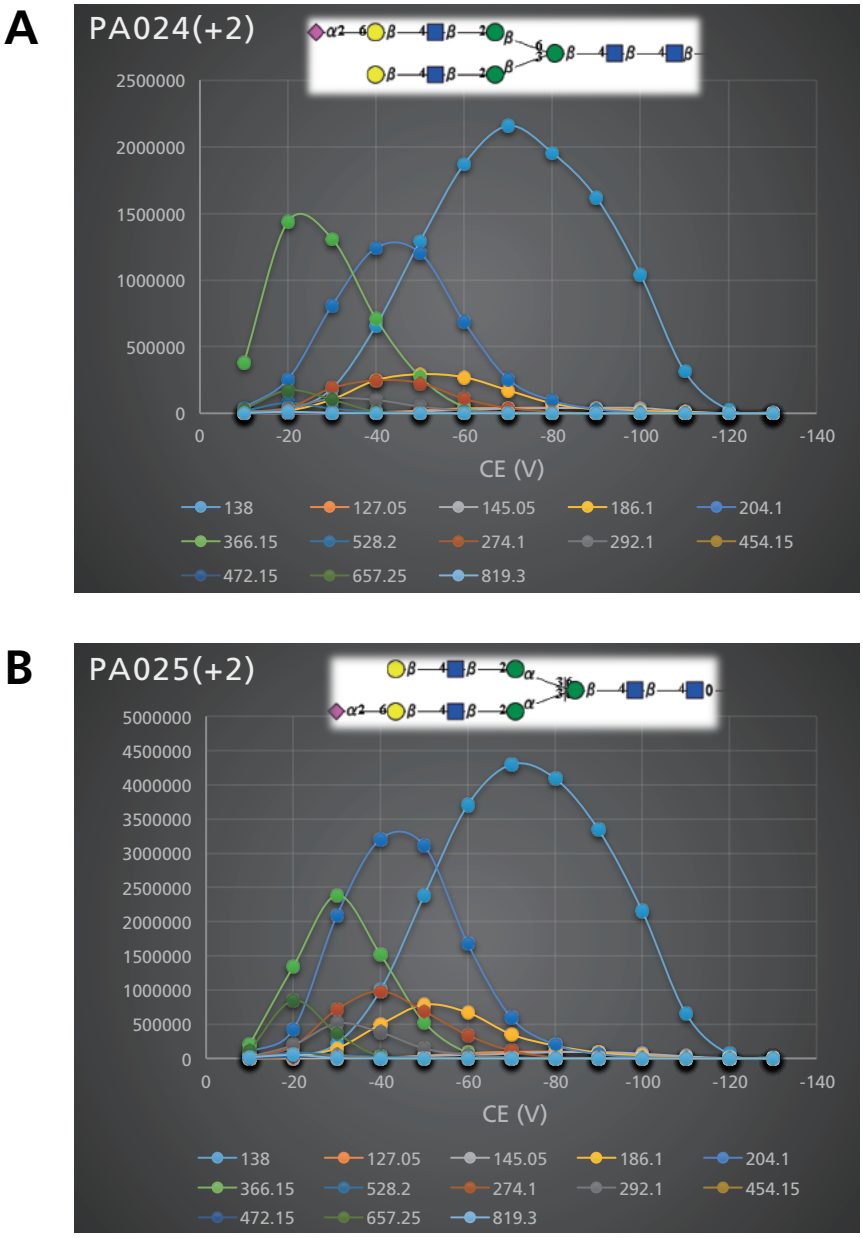

Figure 6 Two purified, isobaric PA-labelled glycans were analyzed by Erexim for structural characterization. The charts above show the peak area of each product ion at steps of CID collision energies, where **A** and **B** represent the glycan with terminal sialic acid occurring on α1-6 and α1-3 branch, respectively.
By examining the differences in these "Erexim profiles", it is possible to differentiate and identify the two very similar structures. Specifically, we observed that product ion *m/z* 366 (Gal-GlcNAc) reaches maximum at higher CE in profile B and that intensity of *m/z* 657 (NeuAc-Gal-GlcNAc) was higher in profile **B**. This observation is in agreement with theoretical prediction that bonds in α1-3 branch are slightly more labile than corresponding bonds in α1-6 branch.

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Conclusions

- Erexim platform is a versatile platform for glycoform profiling, which offers both accurate quantification and in-depth structural characterization using triple quadrupole mass spectrometer.
- Sample can be glycopeptides or (labelled) glycan.
- We are currently strengthening our database to ultimately enable identification of glycans detected from unknown samples.

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