

# A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MS system

**IMSC 2012** PTu-041

Shuuichi Nakaya, Yuzo Yamazaki  
Global Applications Development Center, Shimadzu  
Corporation.

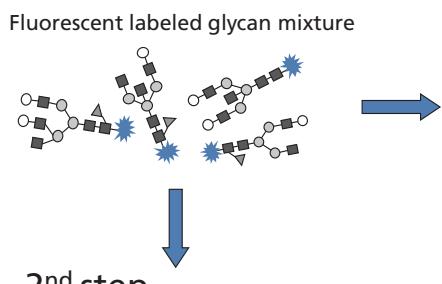
<sup>1</sup>Nishinokyo Kuwabara-cho, Nakagyo-ku, Kyoto  
604-8511, Japan

## A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MS<sup>n</sup> system

# Introduction

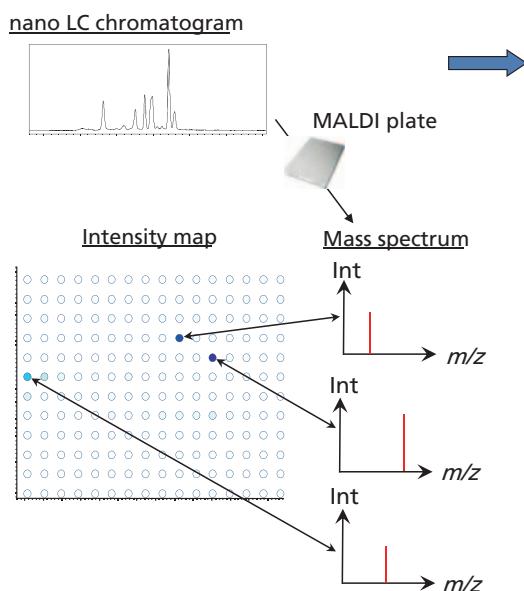
Analysis of glycosylation is one of the indispensable approaches for a development of antibody-drug and biomarker discovery, because a large number of proteins in eukaryotes are glycosylated and they play various roles in physiological function like a molecular recognizing. Therefore, a well-established workflow for characterization of glycosylation has been a one of the growing demands.

The author and co-workers has reported a system for identification of glycan structures using an observational MS<sup>n</sup> spectral library obtained by MALDI-QIT-TOF MS<sup>1), 2)</sup>. In this study, we will demonstrate a practical workflow combined with the spectral library and off-line separation system for the glycan structure characterization.



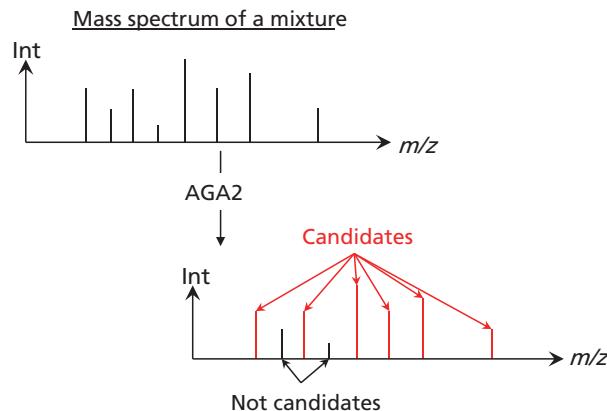
### 2<sup>nd</sup> step

An aliquot of the remaining sample is provided to a nano HPLC and eluted glycans are automatically fractionated onto a MALDI plate. A MS spectrum is generated for each fraction, and the intensity maps of the mass values which were listed in the 1st step are created.



### 1<sup>st</sup> step

Small aliquot of a glycan sample is subjected to MALDI MS analysis, a list of candidate precursor ions is generated by the "Accurate Glycan Analyzer 2 (AGA2)".



### 3<sup>rd</sup> step

MS<sup>n</sup> analysis is performed semiautomatically on all the found precursors in each well and isomer structures are identified by AGA2.

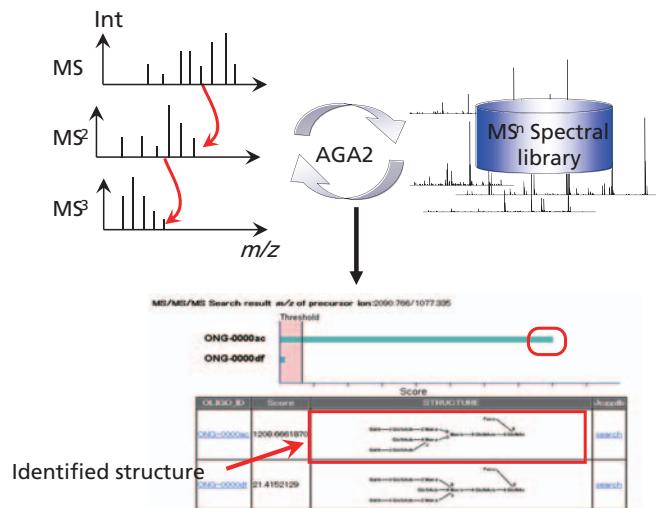


Fig. 1 NBS Biomarker Discovery System

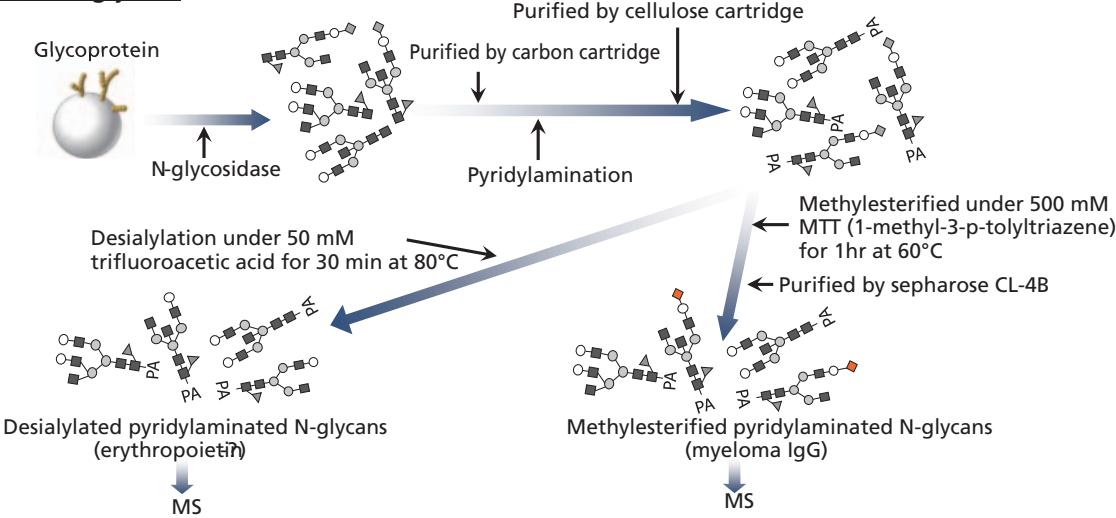
## A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MS<sup>n</sup> system

# Analysis of N-glycans released from human myeloma IgG and human erythropoietin- $\alpha$

To identify proteins associated with insulin resistance in adipocytes *in vitro*, differential proteome analysis using the NBS method was performed in 3T3-L1 adipocytes in which insulin resistance was induced by TNF-alpha or

dexamethasone (Fig. 2a). The relative quantification and the identification of differentially expressed proteins were performed using LC-MALDI-TOF MS (Fig. 1b and 2a).

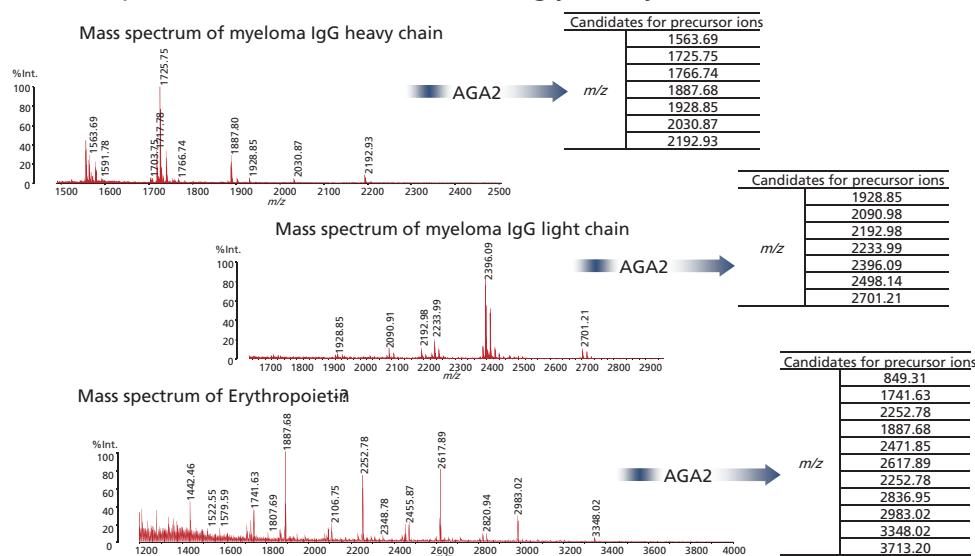
### I) Preparation of N-glycans



Human myeloma IgG (Carbiochem) was separated by 1D SDS-PAGE and treated with N-glycosidase (Takara Bio Inc.) in gel. On the other hand, Human erythropoietin- $\alpha$  (Carbiochem) was treated with N-glycosidase in solution.

Released N-glycans were pyridylminated. Additionally, sialic acids of N-glycans from IgG were methylesterified, and N-glycans from erythropoietin were desialylated.

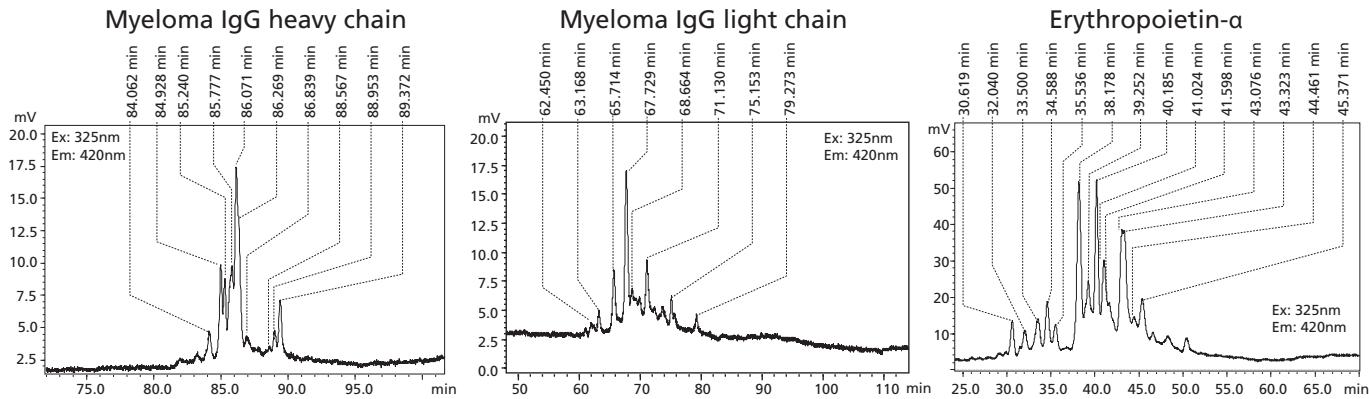
### II) MS analysis of each sample and detection of candidate N-glycans by AGA2



7, 7, and 11 signals were generated from MS analysis of the methylesterified N-glycans of IgG heavy chain, light chain, and desialylated N-glycans of erythropoietin by AGA2, respectively.

# A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MS<sup>n</sup> system

### III) Separation of isomers using nano HPLC



N-glycans were separated by the carbon column (Hypercarb KAPPA Capillary column, Length: 100 mm, ID 180um; Thermo scientific) under the linear gradient of 90% CH3CN solution containing 0.1% formic acid. The total flow rate of the nano HPLC was set at 1 uL/min. Each

fraction was spotted onto a MALDI plate with the MALDI matrix solution (2.5 mg/mL DHBA in 80% EtOH, containing 5 mM NaCl). Separated N-glycans were detected by the Laser induced fluorescent detector (ZETALIF 2000 He/Cd Laser; Picometrics).

### IV) Characterization of N-glycans by MS<sup>n</sup> analysis combined with AGA2

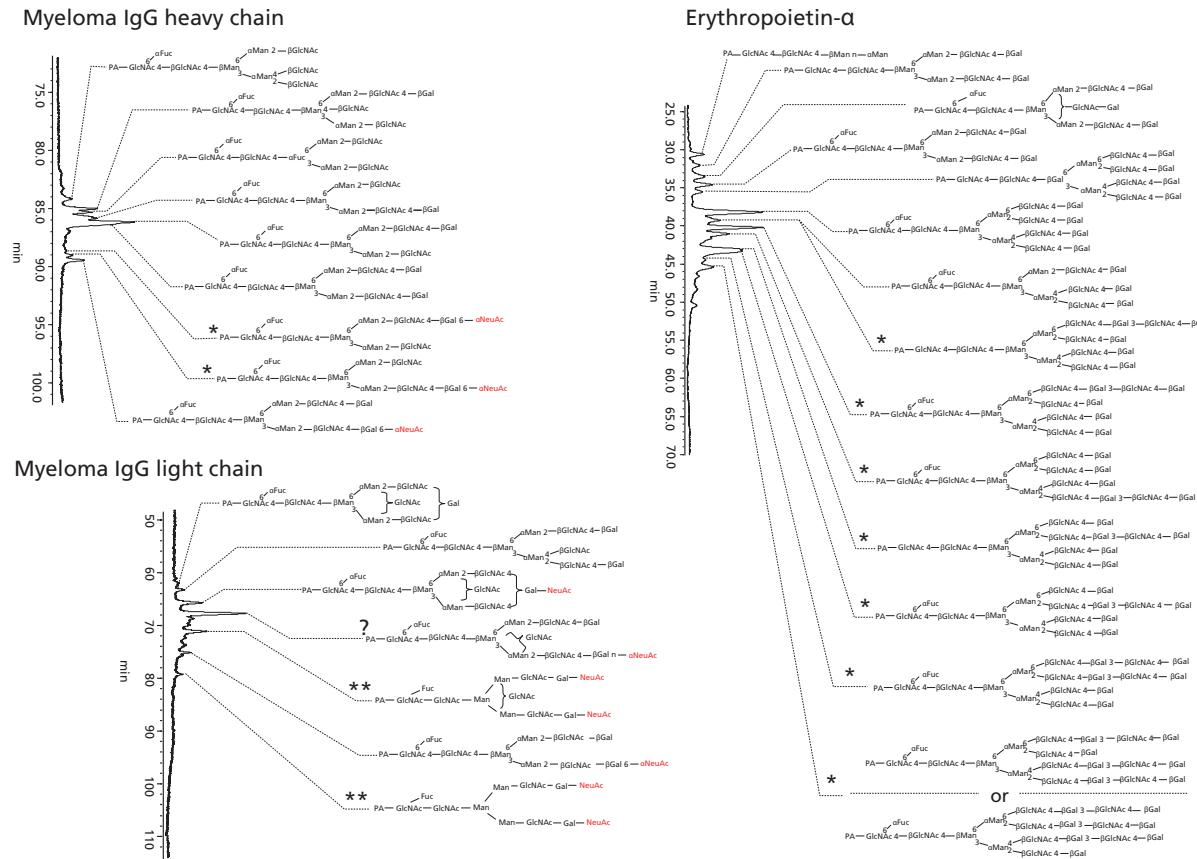


Fig. 3 Identification and functional analysis of novel adipokine

## A workflow for identification of isobaric isoforms of glycans using off-line MALDI-MS<sup>n</sup> system

## Conclusions

- We identified various glycan isomers using our workflow.
- Our workflow could be applicable to various areas of research where screening of glycans is required.

## References

- 1) A. Kameyama et.al. Anal. Chem., 77, 4719-4725 (2005)
- 2) A. Kameyama et.al. J. Proteome. Res., 5, 808-814 (2006)

---

First Edition: September, 2012



Shimadzu Corporation

[www.shimadzu.com/an/](http://www.shimadzu.com/an/)

For Research Use Only. Not for use in diagnostic procedures.

The content of this publication shall not be reproduced, altered or sold for any commercial purpose without the written approval of Shimadzu. The information contained herein is provided to you "as is" without warranty of any kind including without limitation warranties as to its accuracy or completeness. Shimadzu does not assume any responsibility or liability for any damage, whether direct or indirect, relating to the use of this publication. This publication is based upon the information available to Shimadzu on or before the date of publication, and subject to change without notice.

© Shimadzu Corporation, 2012