2019年9月３日
お茶の水女子大　国際交流留学生プラザ多目的ホール

TIAナノバイオサマースクール（糖鎖・レクチン）

糖鎖のシークエンス解析および立体構造解析

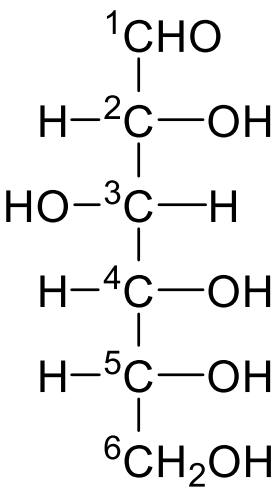
名古屋市立大学大学院薬学研究科
矢木　宏和

Contents

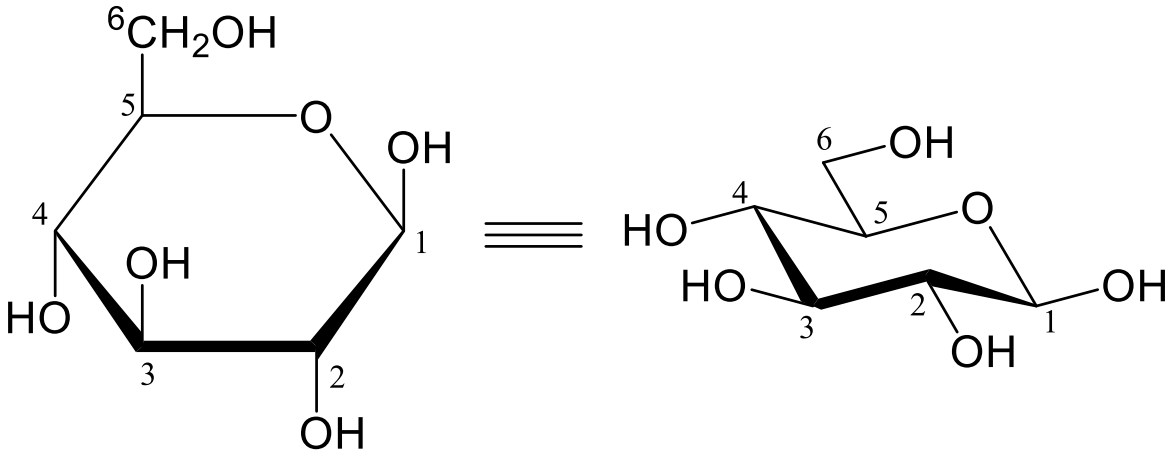
Monosaccharide structure

β-D-Glucose

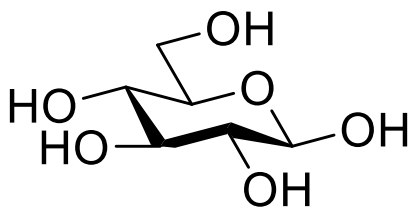
Fischer



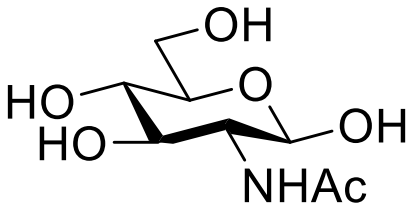
Haworth



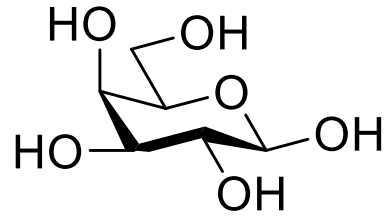
Common monosaccharides found in vertebrates



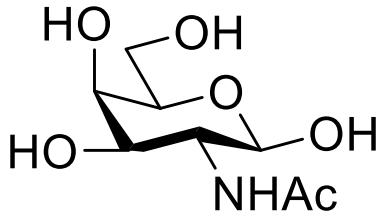
D-Glucose (Glc)



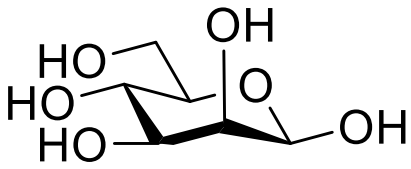
N-acetyl D-Glucosamine (GlcNAc)



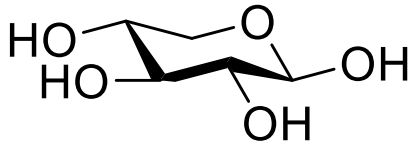
D-Galactose (Gal)



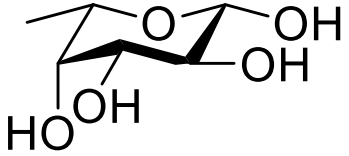
N-acetyl D-Galactosamine (GalNAc)



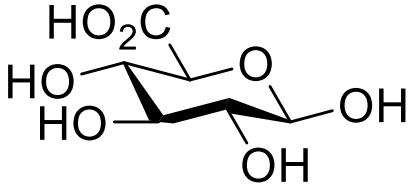
D-Mannonse (Man)



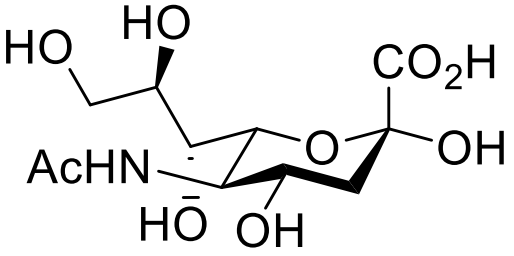
D-Xylose (Xyl)



L-Fucose (Fuc)



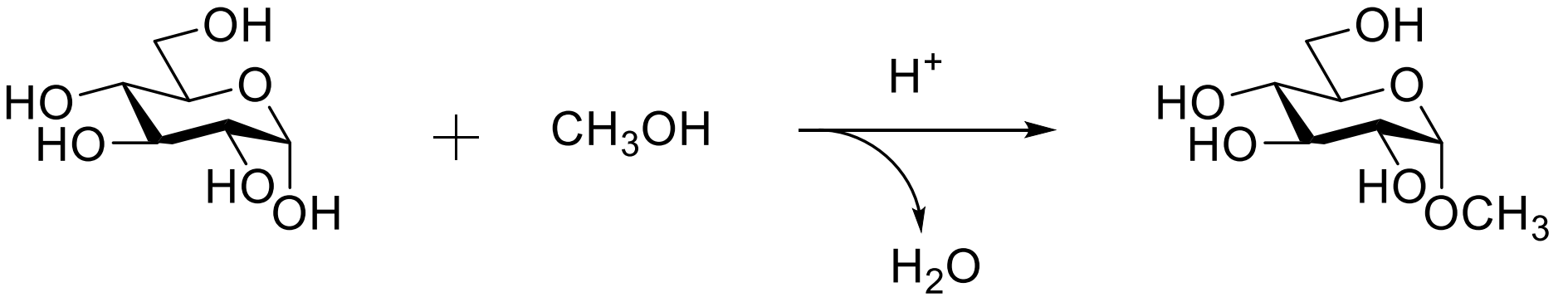
D-Glucronic acid (GlcA)



N-acetylnuraminic acid (NeuAc)

Glycoside formation

グリコシド結合の形成

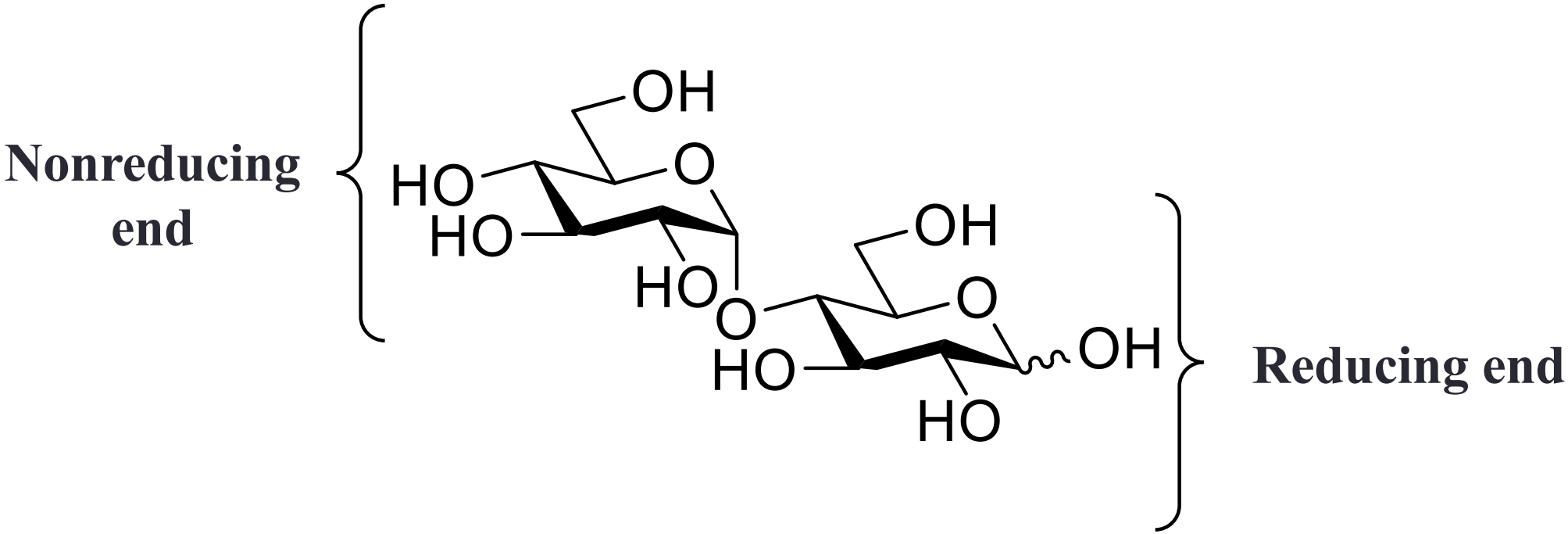


α-D-Glucose

Metyl α-D-Glucose

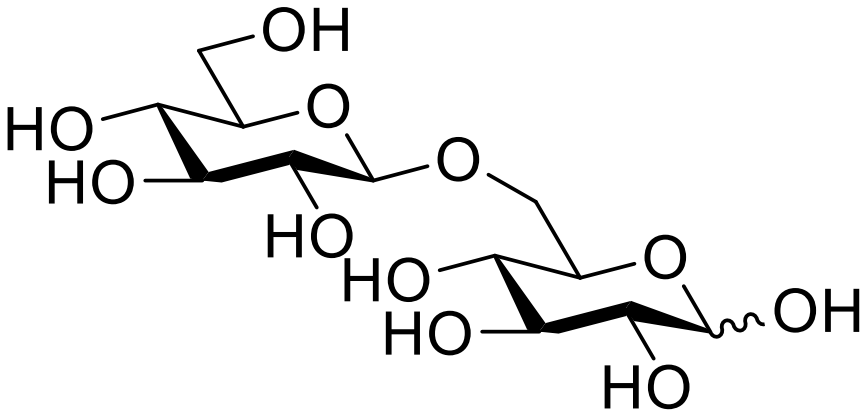
Reducing and nonreducing ends of a disaccharide

糖鎖の末端



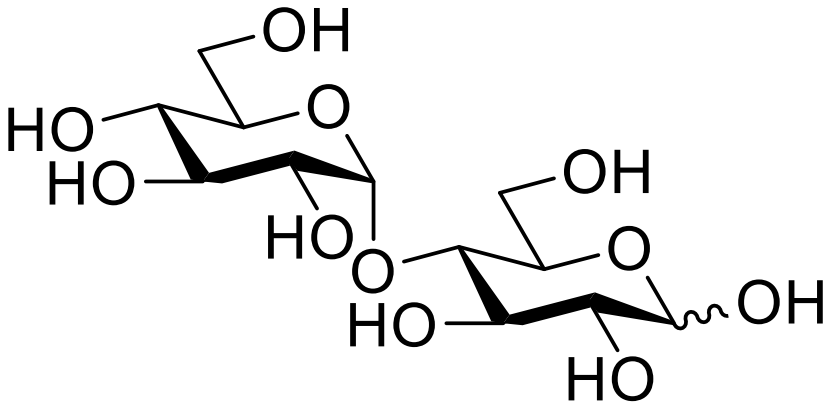
異性体

β1-6 linkage



Gentibiose

α1-4 linkage



Maltose

Isomers

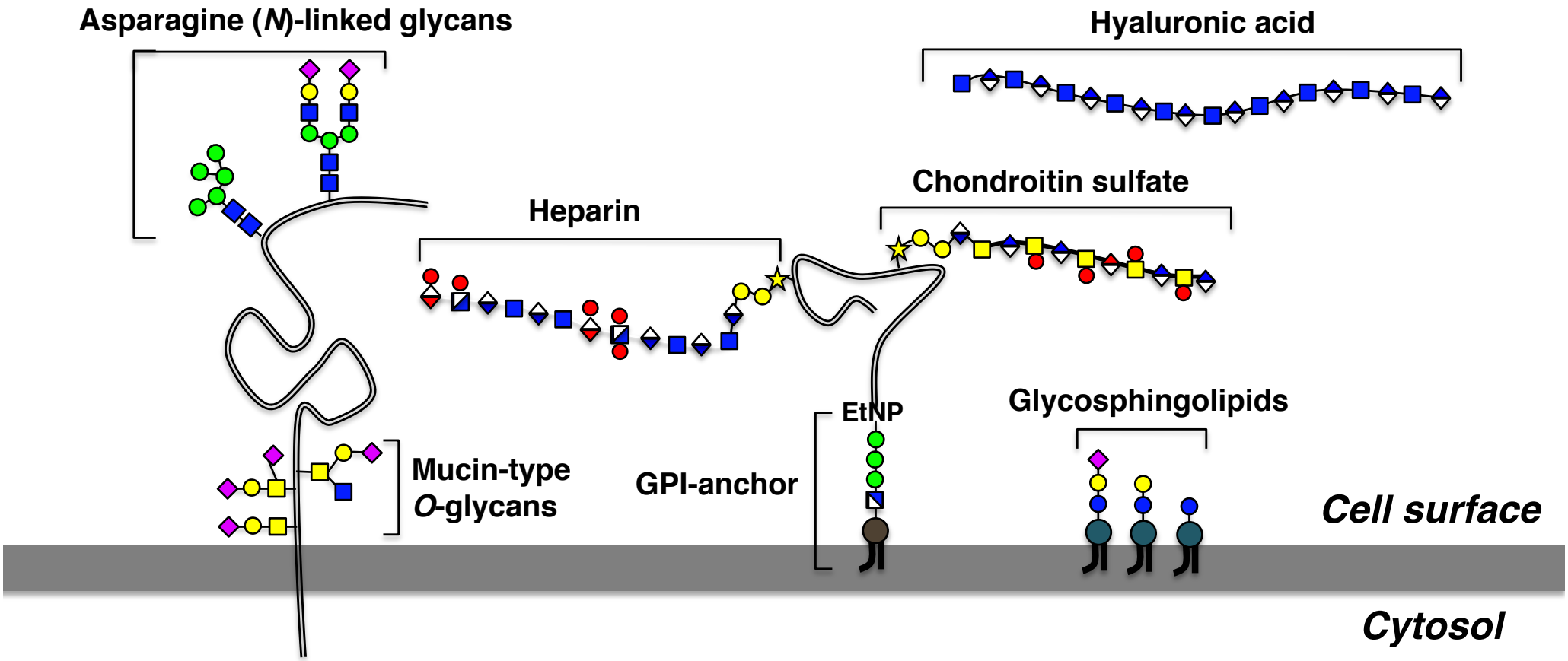
Oligomer	Composition	Possible oligopeptide and oligonucleotide	Possible oligosaccharides
Dimer	AA / AB	1 / 2	11 / 20
Trimer	AAA / ABC	1 / 6	120 / 720
Tetramer	AAAA / ABCD	1 / 24	1424 / 34560
Pentamer	AAAAA / ABCDE	1 / 120	17872 / 2144640

Essentials of Carbohydrate Chemistry and Biochemistry (2003) より引用

Dimers composed of two glucose resides

Glcα1-α1Glc　Glcβ1- β1Glc　Glcα1- β1Glc
Glcα1-2Glc　Glcβ1-2Glc
Glcα1-3Glc　Glcβ1-3Glc
Glcα1-4Glc　Glcβ1-4Glc
Glcα1-6Glc　Glcβ1-6Glc

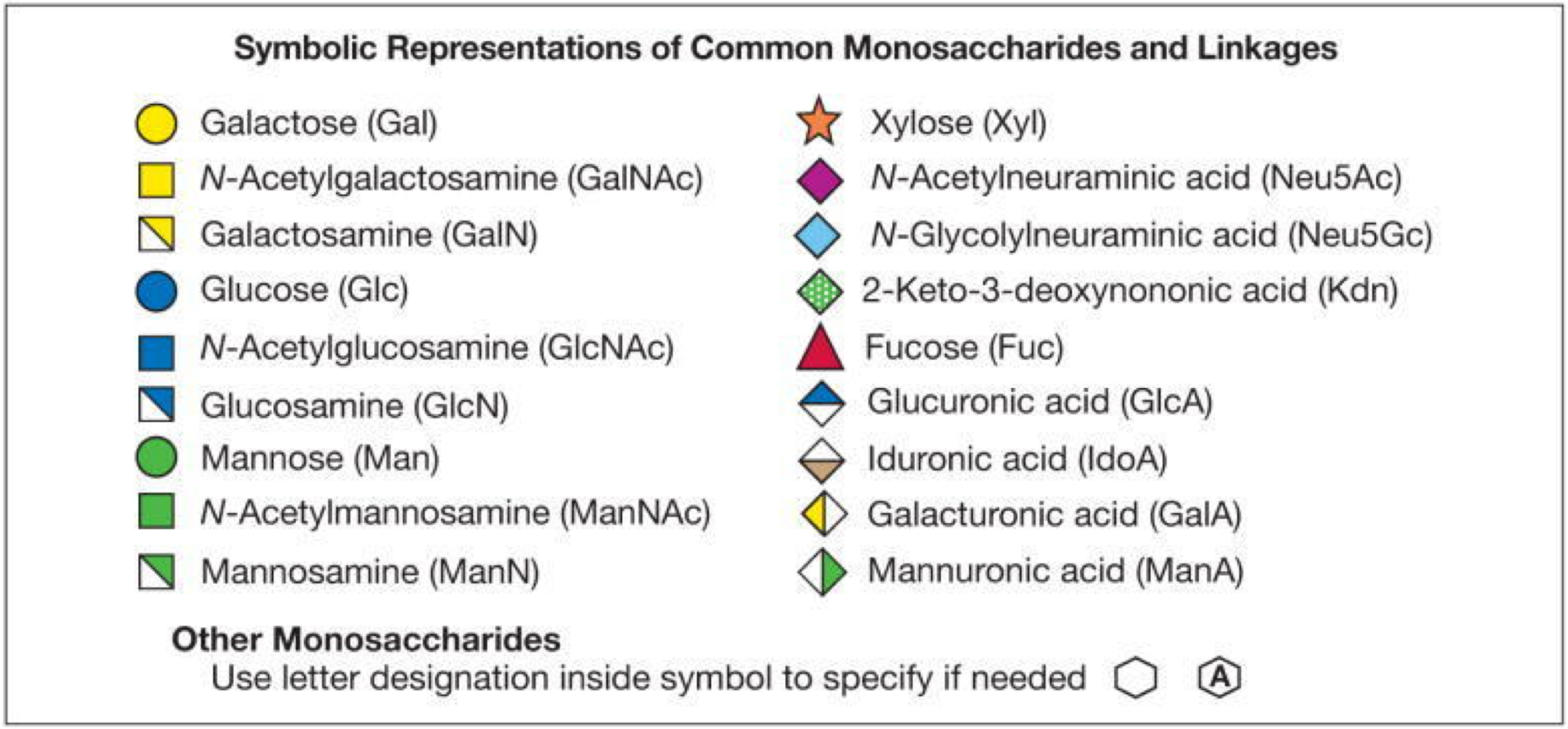
Glycans in Mammals



Basic components of glycans

Glucose (Glc)	*N*-Acetylglucosamine (GlcNAc)	Glucuronic acid (GlcA)
Galactose (Gal)	Glucosamine (GlcN)	Iduronic acid (IdoA)
Mannose (Man)	*N*-Acetylgalactosamine (GalNAc)	Sialic acid (Sia)
Xylose (Xyl)	Sulfate	

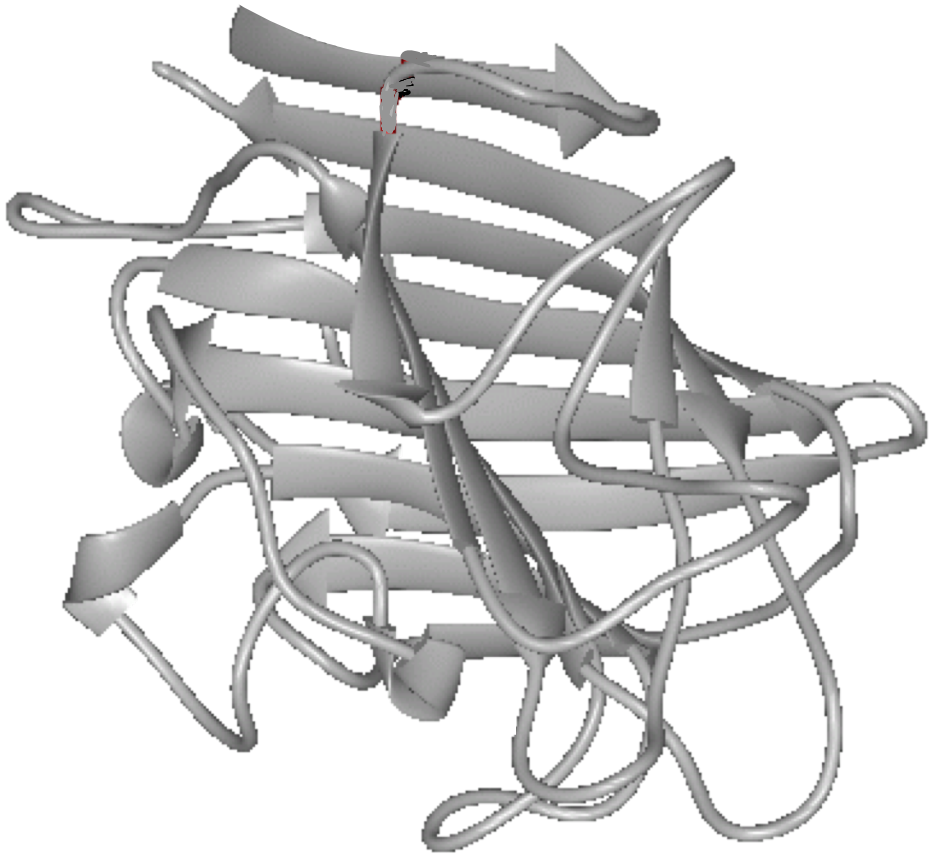
Symbolic representations



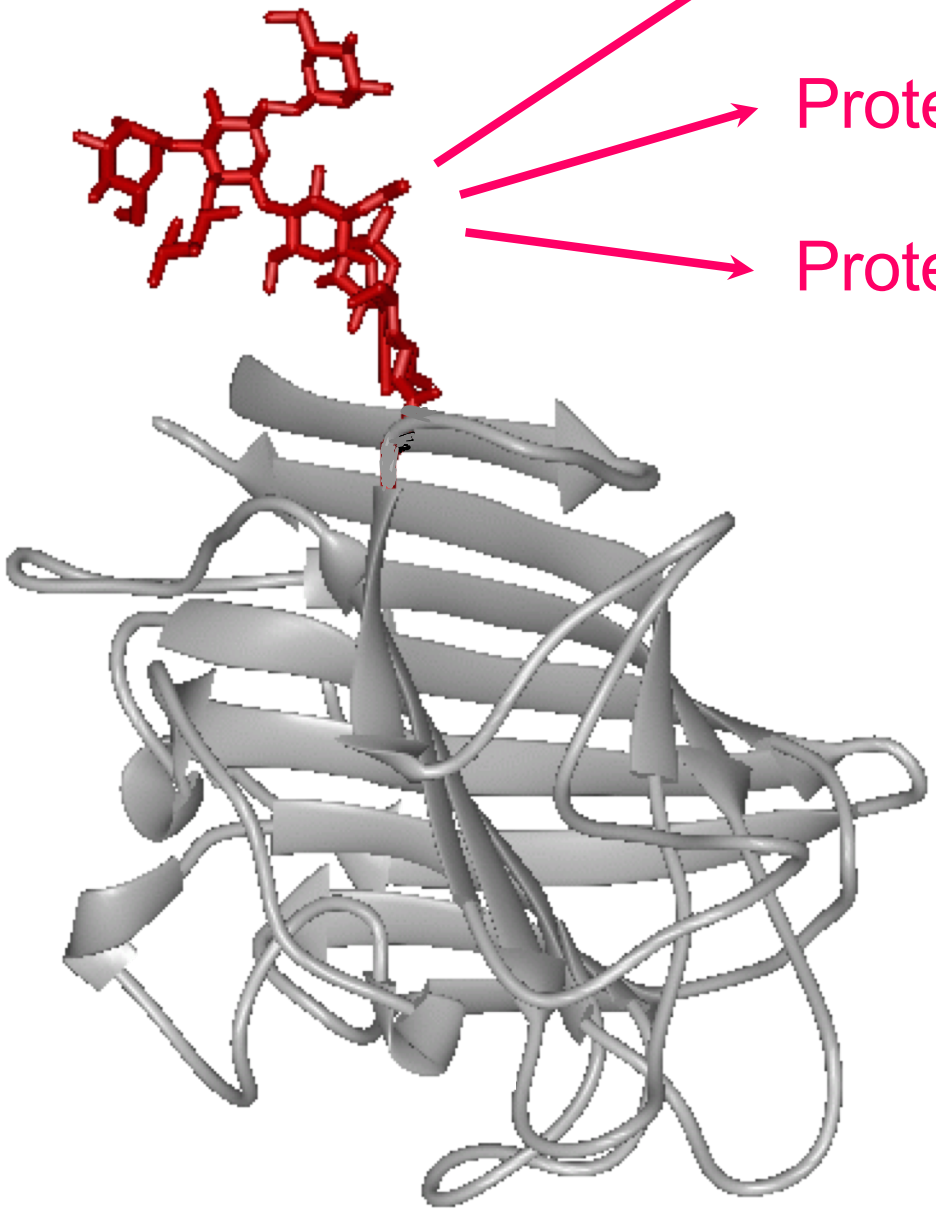
http://www.functionalglycomics.org/static/consortium/CFGnomenclature.pdf

Glycan function of therapeutic antibody and biologics

"Naked" protein



Glycan function of therapeutic antibody and biologics

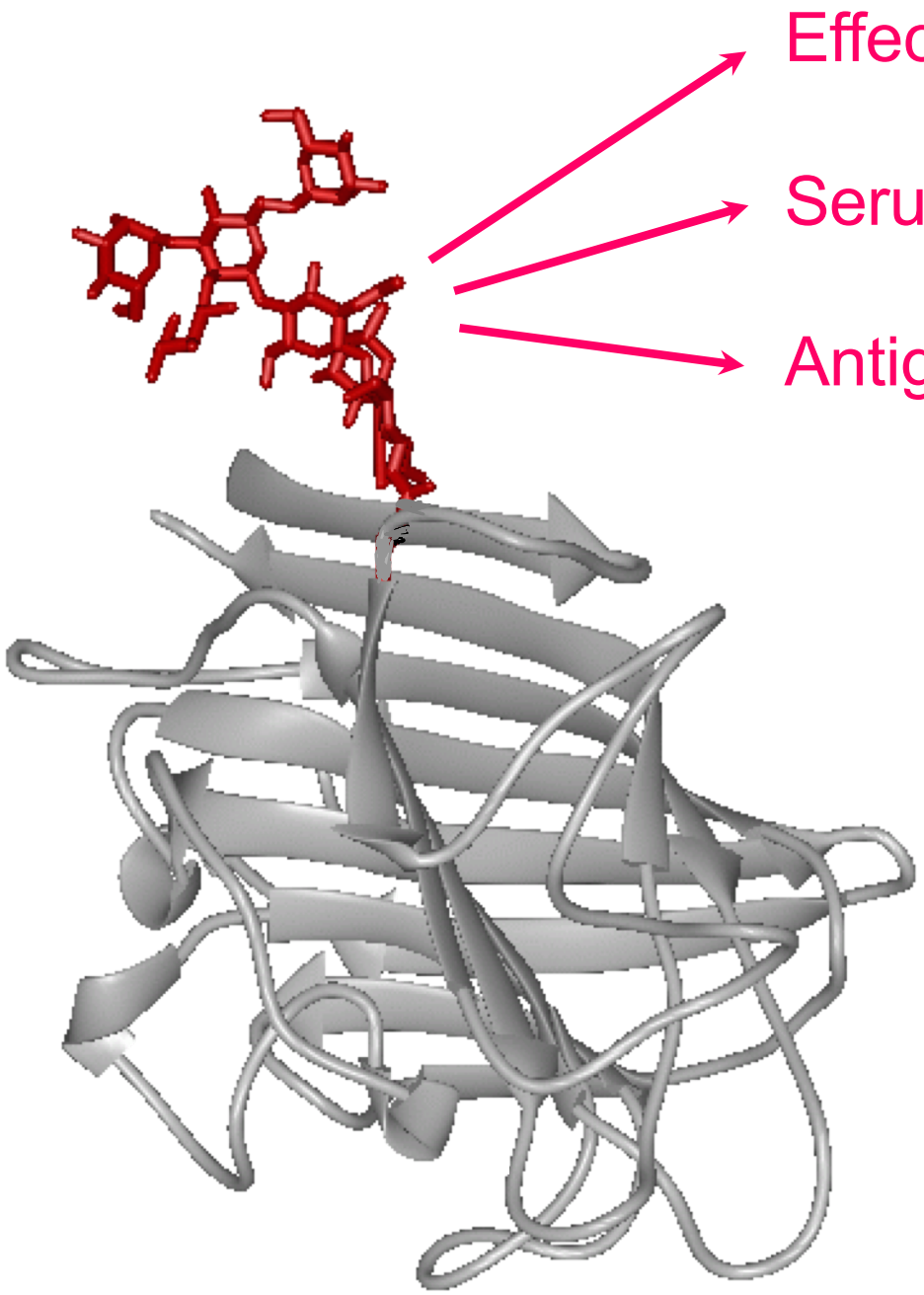


Cell-Cell Communication

Protein Quality Control

Protein Solubility & Stability

Glycan function of therapeutic antibody and biologics

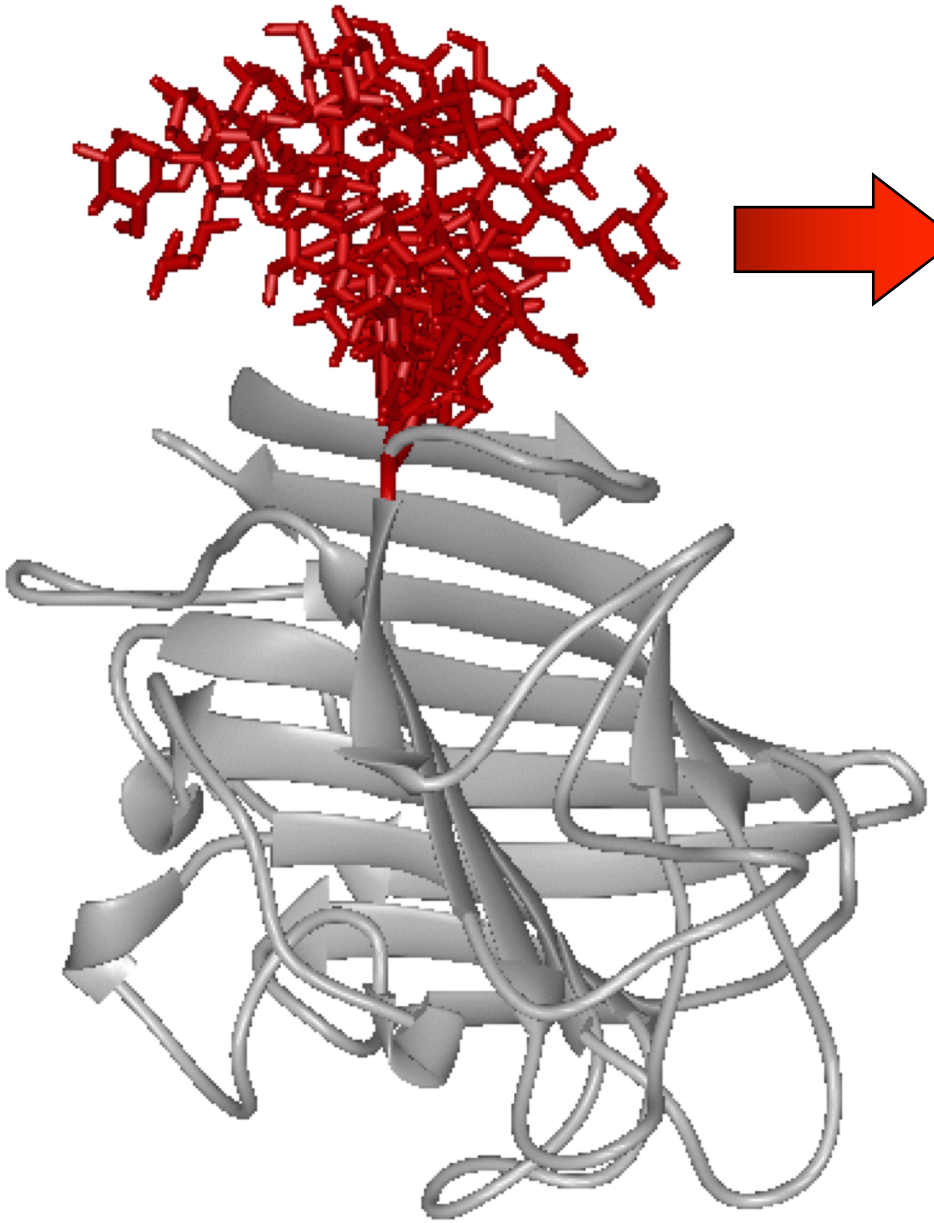


Effecter functions

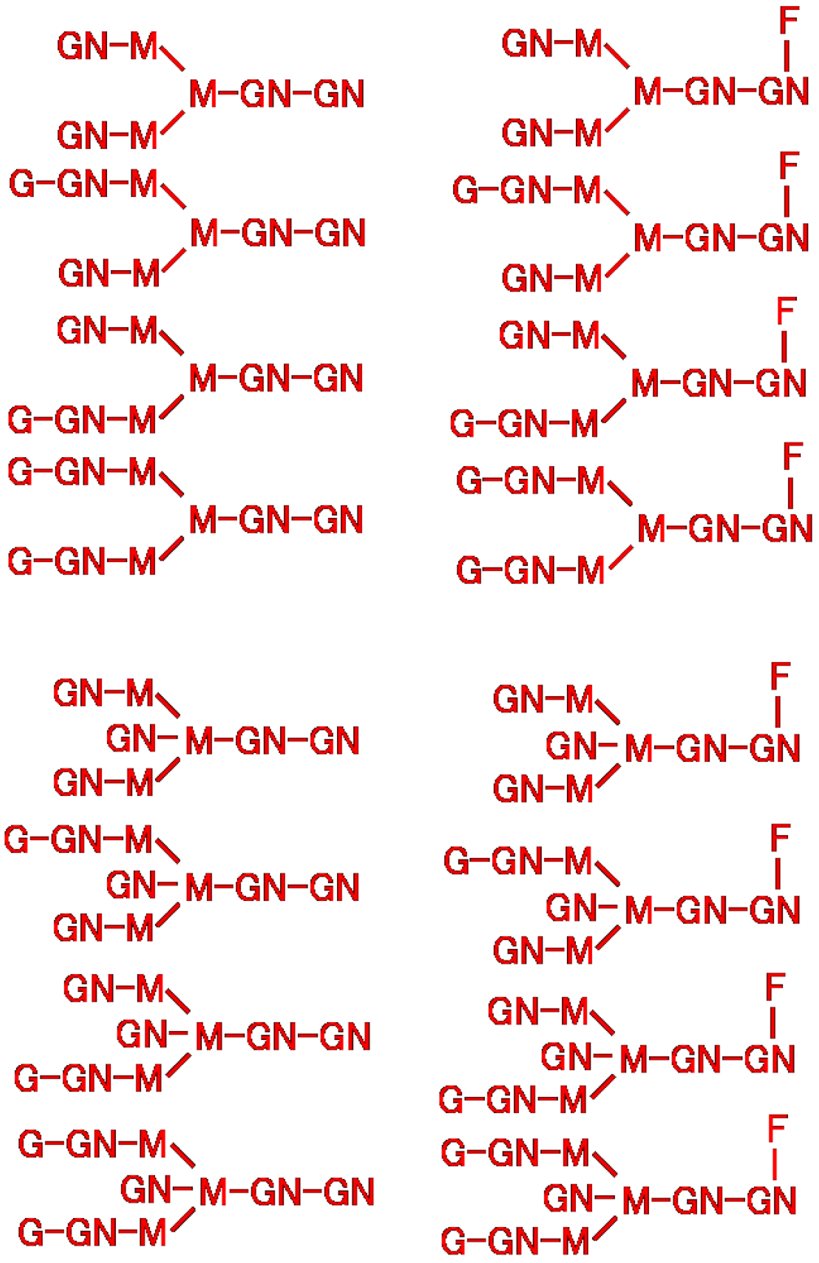
Serum half-life

Antigenicity

Mobility

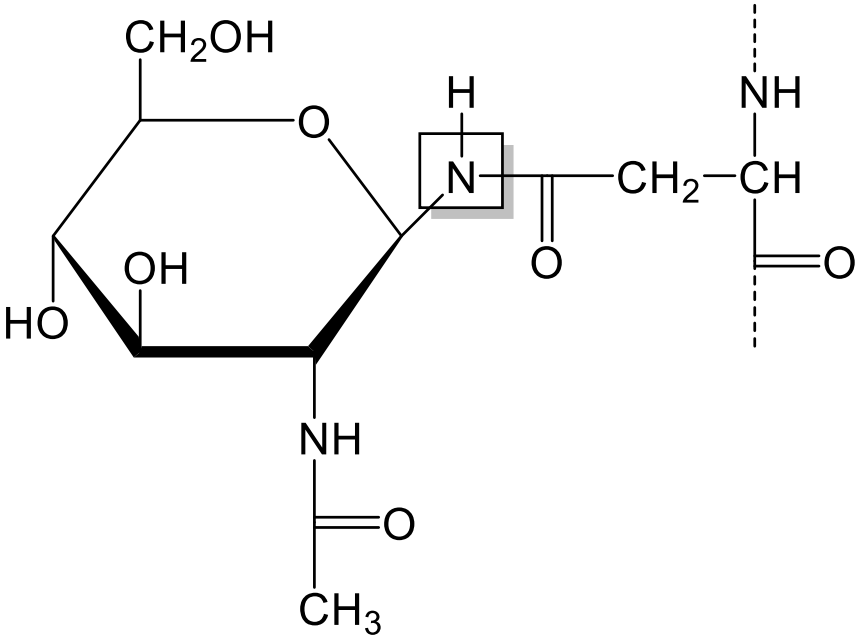


Heterogeneity

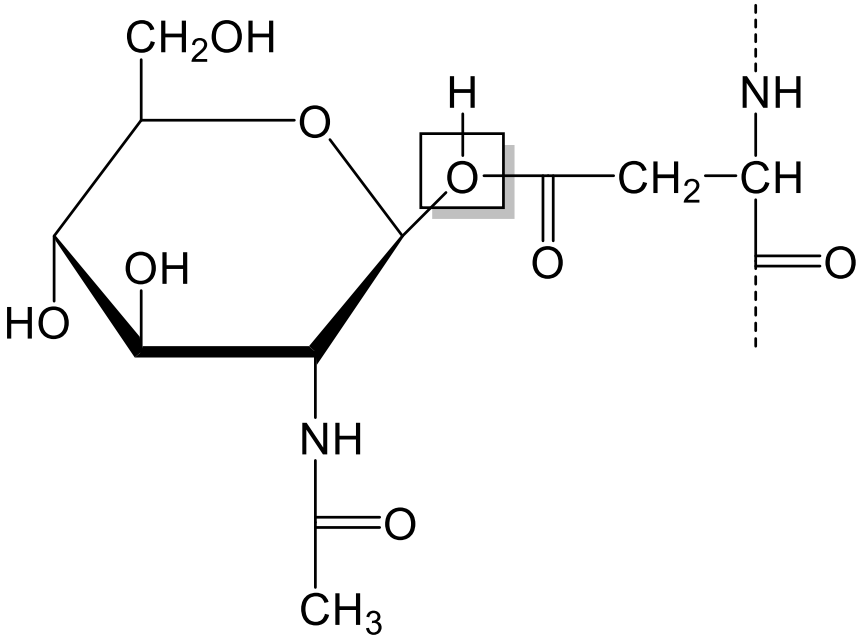


Glycoprotein glycans

▪ ***N*-linked glycans**
（Asn）

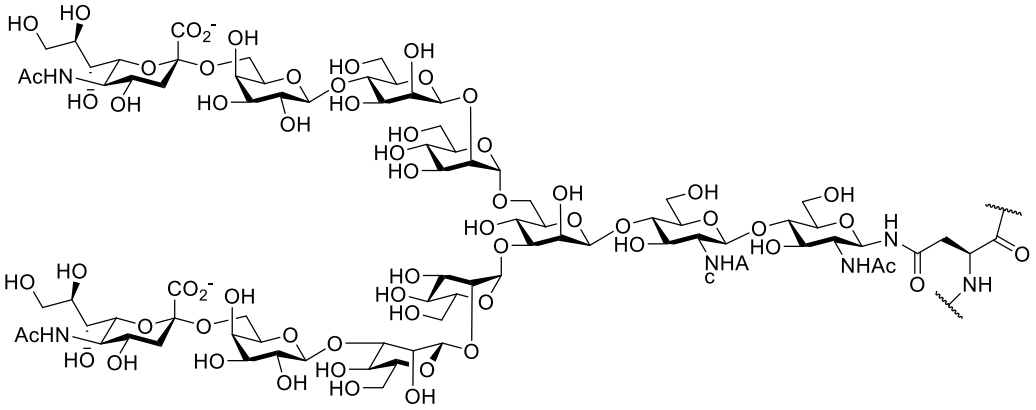


▪ ***O*-linked glycans**
（Ser/Thr）

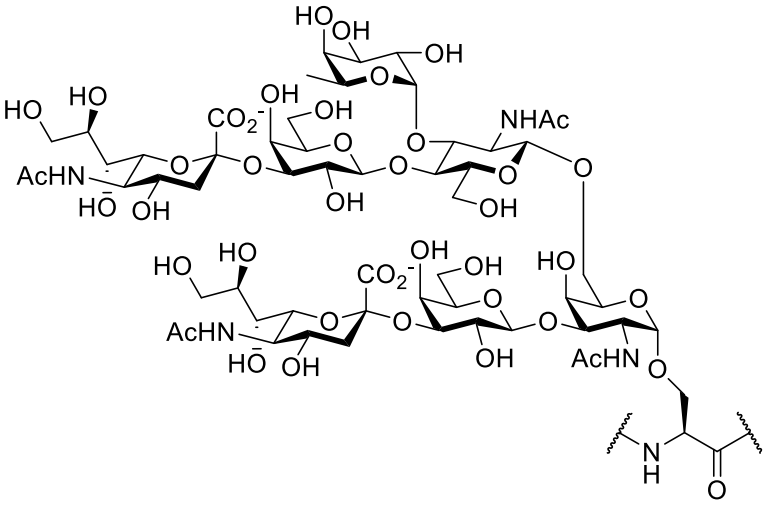


Examples of typical N- and O-linked glycans

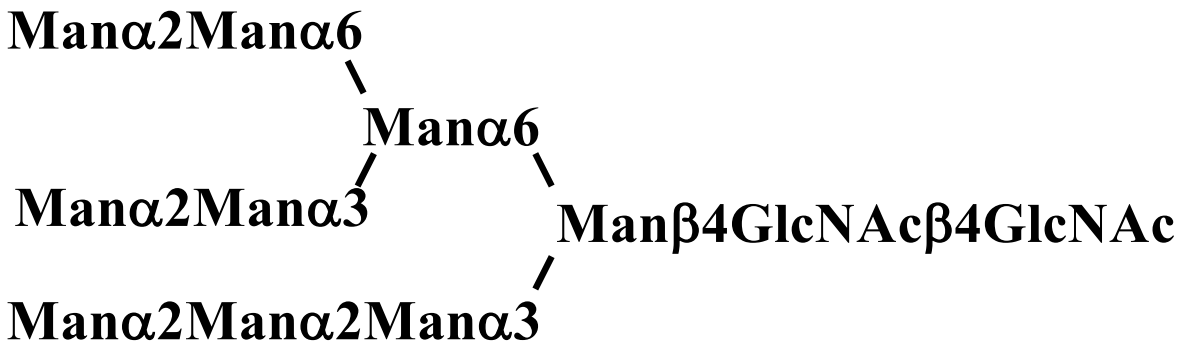
N-linked glycan



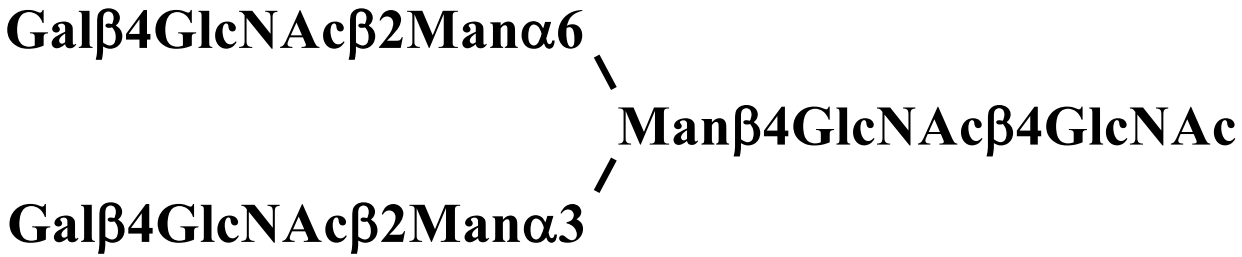
O-linked glycan



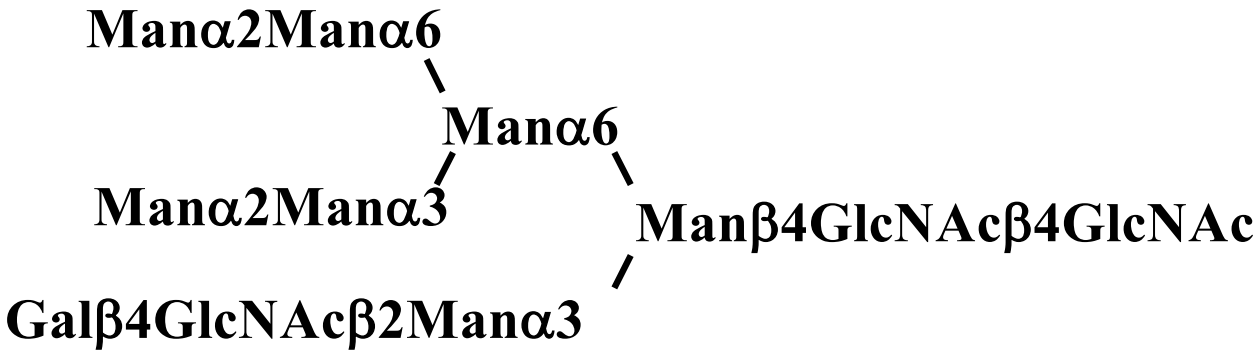
Classification of *N*-linked glycans



High-mannose型



Complex型



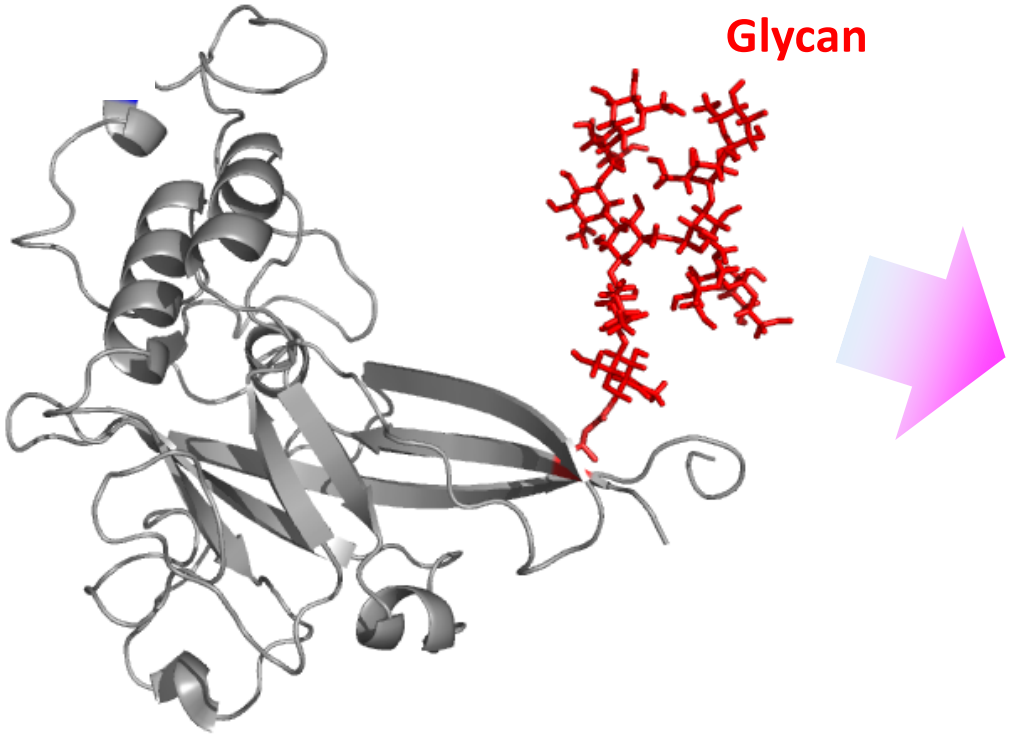
Hybrid型

Classification of *O*-linked glycans

Type	Structure	Type	Structure
Core 1	Galβ1-3GAlNac	Core 4	GalNAcβ1 ↖ 6 GalNAcβ1-3GalNAc
Core 2	GalNAcβ1 ↖ 6 Galβ1-3GalNAc	Core 5	GalNAcα1-3GalNAc
Core 3	GalNAcβ1-3GalNAc	Core 6	GlcNAcβ1 ↖ 6 GalNAc

Sugar chains

- Protein solubility and stability
- Structural integrity of protein functional sites
- Cell-cell communication

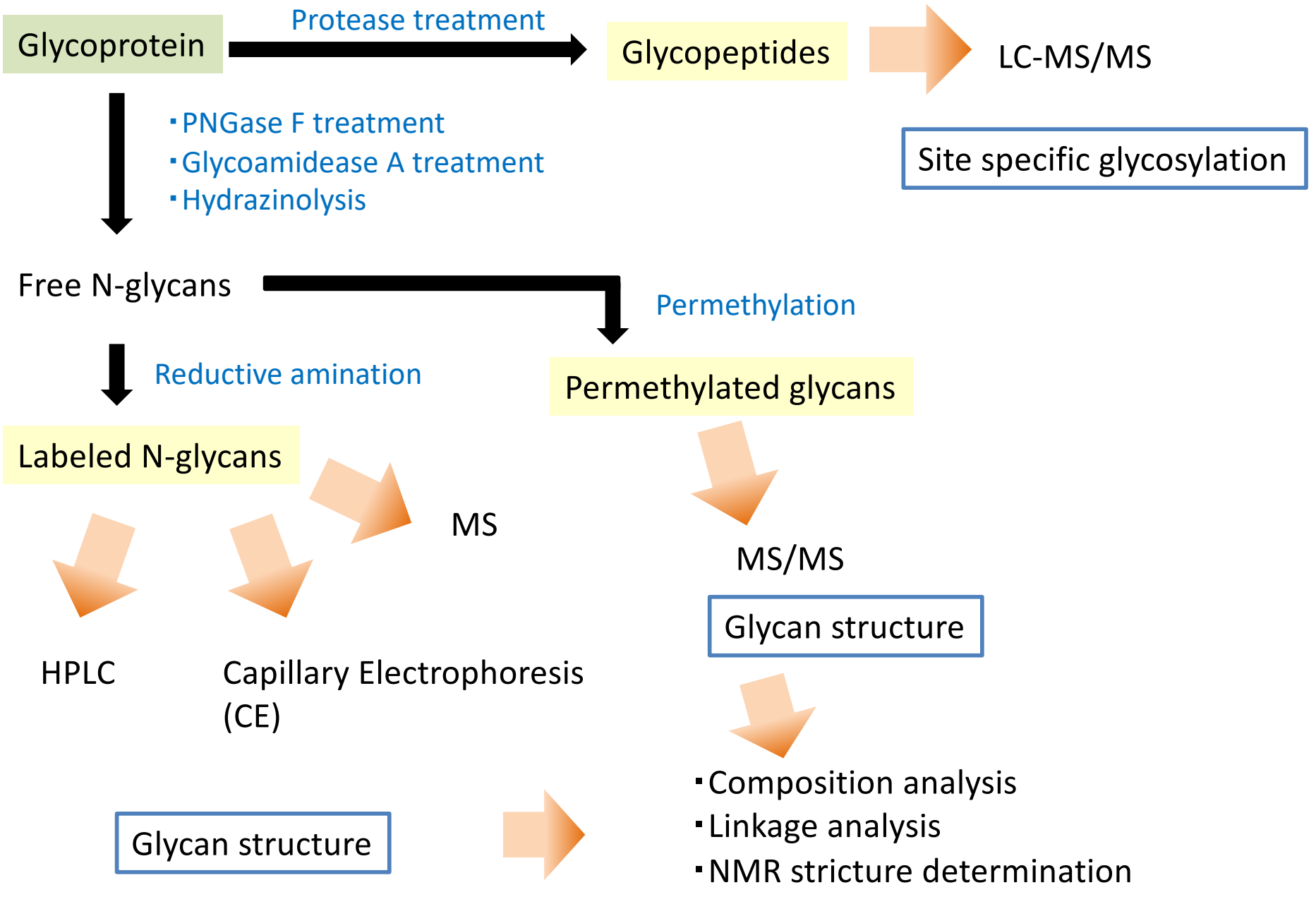


- Highly branched structures
- Microheterogeneity
- Conformational fluctuations

Such structural complexity, diversity, and fluctuation hamper the structural biology-based approaches for understanding the function of glycoprotein as well as oligosaccharides.

Contents

Scheme of N-glycan structural analyses



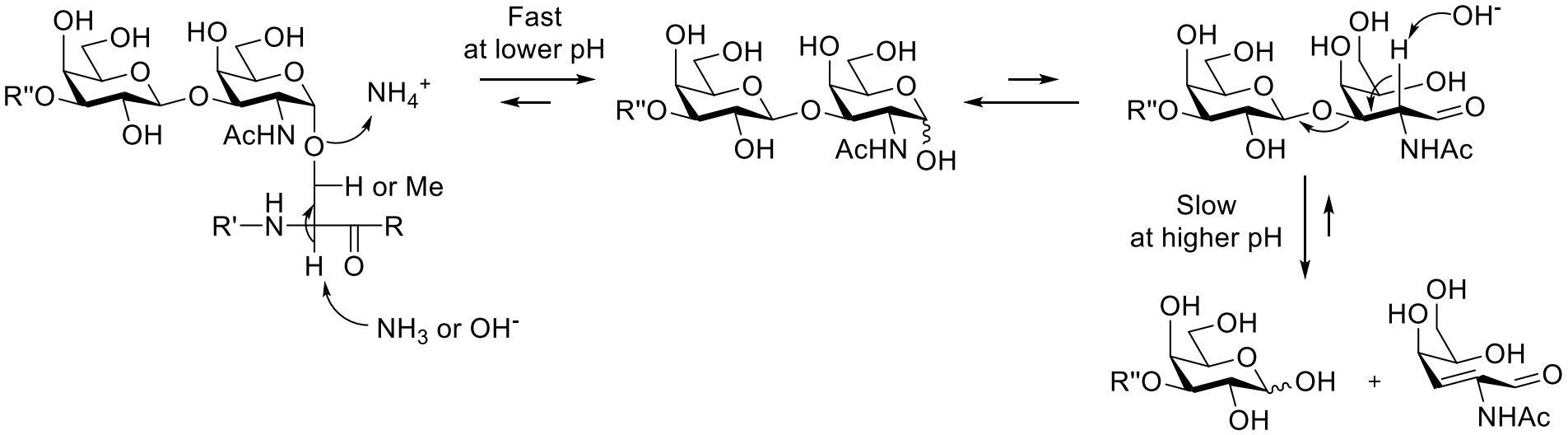
Comparison of analytical methods for N-glycans

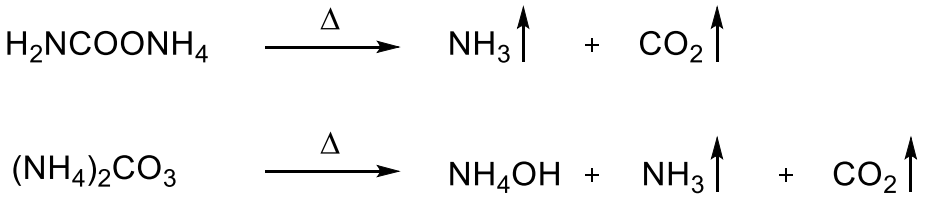
	HPLC		CE		MS	
Detection	Fluorescence	MS	Fluorescence	MS	MS	MS^n
Analysis time	long		rapid		rapid	middle
Sensitivity	◎	○	◎	○	○	△
Discrimination of isomeric product	◎	◎	○	○	×	△
Identification of isomeric product	◎	△	△	△	×	○
Index of determination of glycan structures	Elution position	Molecular mass	Elution position	Molecular mass	Molecular mass	Fragmentation
Database or analytical web application	・GALAXY ・Glycobase		Glycostore		・GlycoMod ・jCGGDB	・Glycan Mass Spectral DataBase

N-glycan-releasing methods

	Hydrozynolysis	peptide:N-glycanase F (PNGase F)	glycoamidase A
	Chemical reaction（hydrazine）	Enzyme reaction（recombinant protein） optimal pH 7-8	Enzyme reaction（Extract of alamond seeds） optimal pH 4
Merit	・Application for crude sample (Cells and tissues)	・Direct glycan-releasing from glycoproteins	・Possible for releasing to core α1,3 fucosylation
Demerit	・Since N-acetyl and N-glycoryl gropus are removed by hydrazinolysis, reacetylation is nessesary for sialylated glycans (Undistinguishable for molecular species of sialic acid) ・Production of Byproducts	・Uncleavable to core α1,3 fucosylated oligosacchairdes	・Uncleavable to whole glycoproteins (cleavable to glycopepetides)

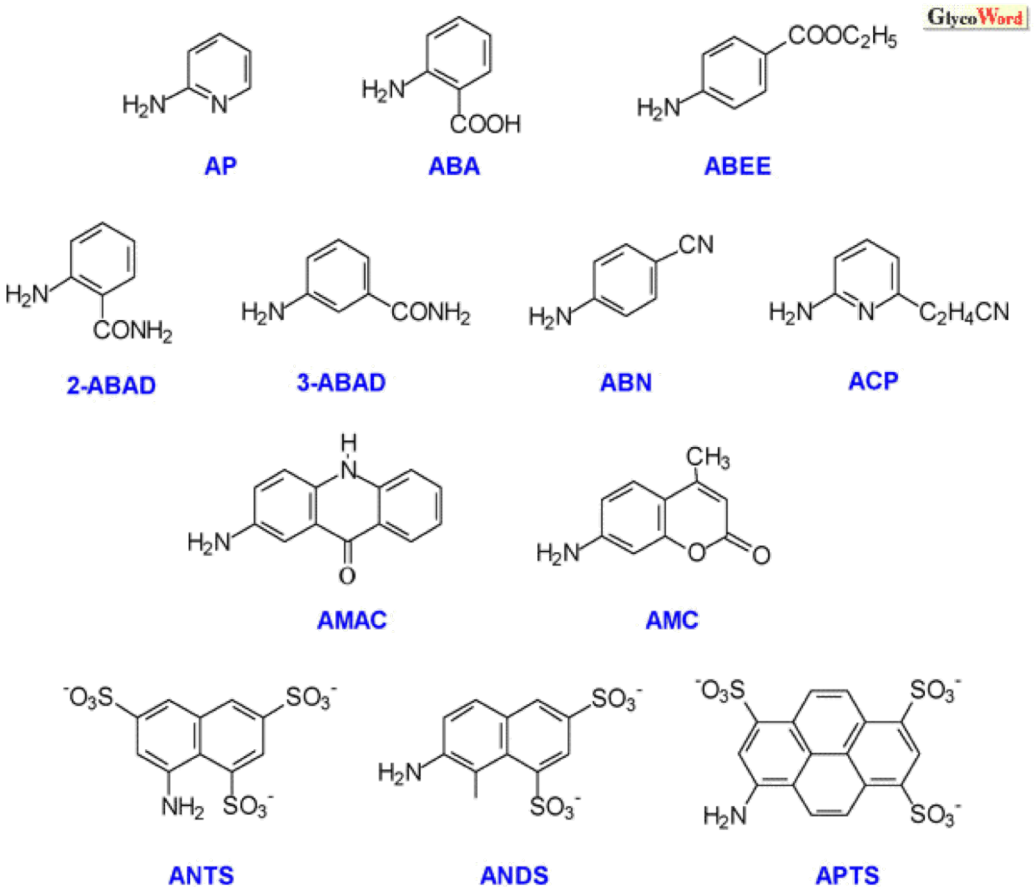
O-glycan-releasing method





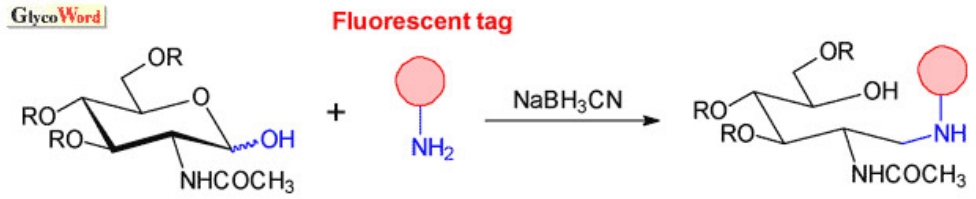
β-Elimination in common O-glycoside linkages with Ser or Thr residues in alkaline conditions and a plausible mechanism of subsequent peeling reaction.

Florescence labeling of oligosaccharides



ABA:2-Aminobenzoic acid
2-ABAD:2-Aminobenzamide
3-ABAD:3-Aminobenzamide
ABEE:Ethyl *p*-aminobenzoate
ABN:*p*-Aminobenzonitrile
ACP:2-Amino-6-cyanoethylpyridine
AMAC:2-Aminoacridone
AMC:7-Amino-4-methylcoumarin
ANTS:8-Aminonaphthalene-1,3,6-trisulfonic acid
ANDS:7-Aminonaphthalene-1,3-disulfonic acid
AP:2-Aminopyridine
APTS:8-Aminopyrene-1,3,6-trisulfonic acid

Reductive amination



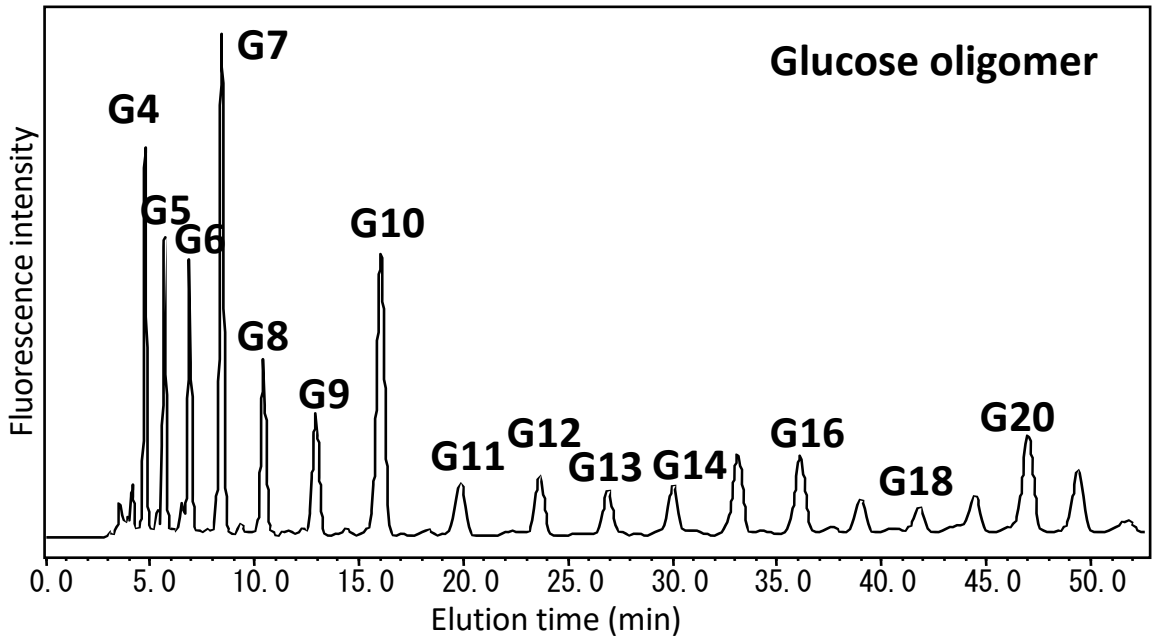
吉年正宏, 鈴木茂生: 糖の高感度分析法 -Update-. Glycoword. GT-C03.
https://www.glycoforum.gr.jp/glycoword/glycotechnology/GT-C03upJ.html

Separation of oligosaccharides by HPLC

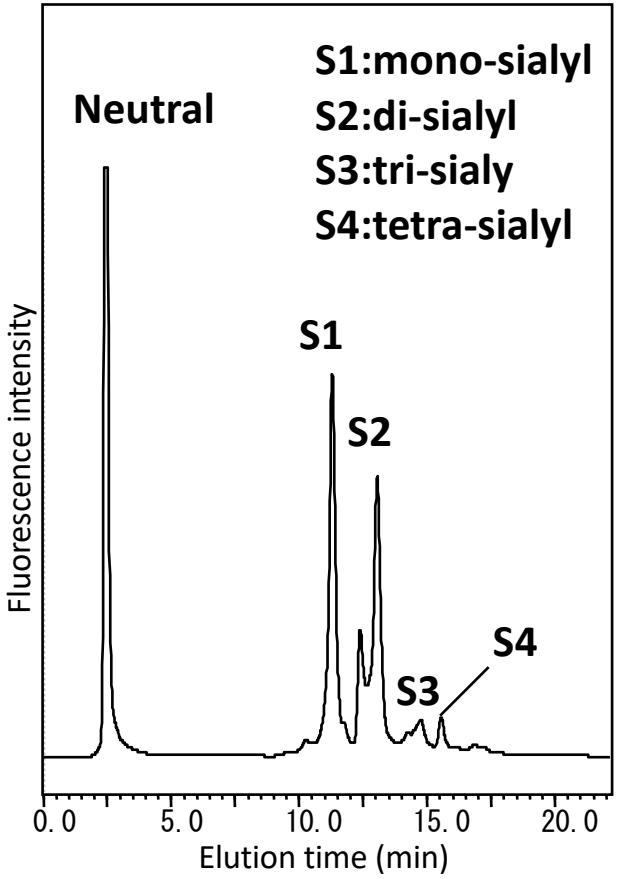
Separation modes	Anion exchange column	Normal phase column	Reverse phase column
Species	・DEAE ・mono Q	・amide ・amino ・cellurose	・ODS ・C30
Principal	According to negative charge degree such as number of sialic acid residues and sulfate groups	Separation is carried out using hydrogen bonds between the resin and sugar chains.	Separation is carried out using hydrophobic interaction between the resin and sugar chains.

Examination of glycosylation profiles

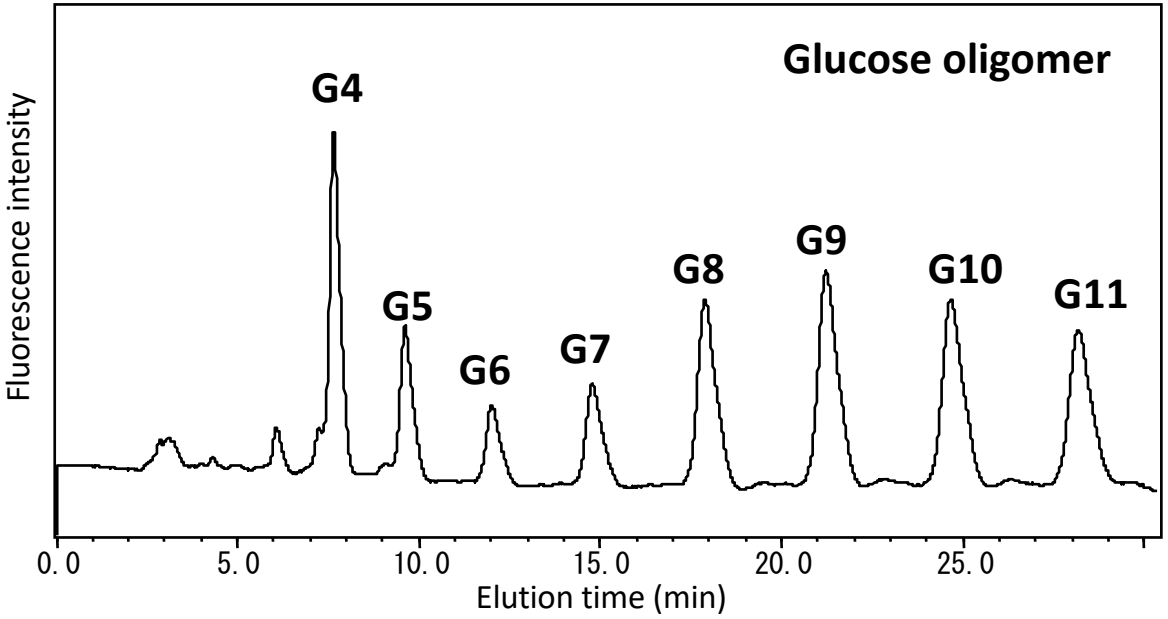
ODS column



DEAE column



Amide column



Identification of glycan structures by HPLC

- Coinjection with standard glycans
- Evaluation by mass spectrometric data

Comparison with elution accumulated data

GALAXY(http://www.glycoanalysis.info/)

Over 500 data of PA-N-oligosaccharides

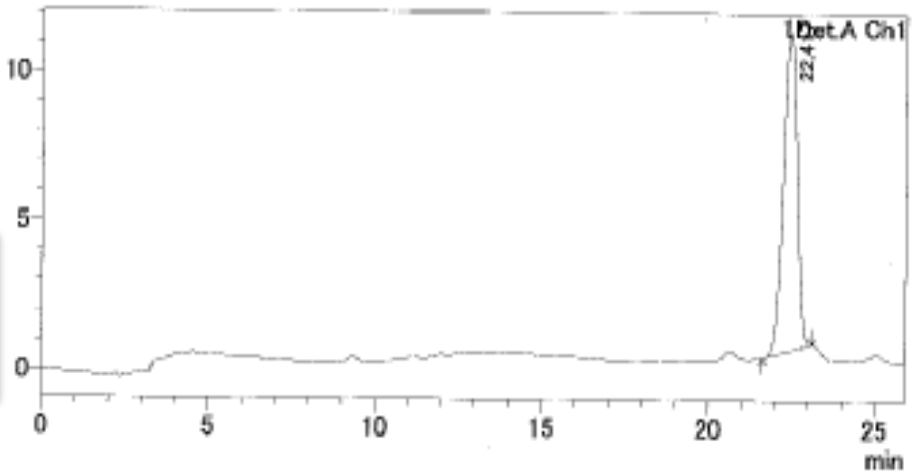
GALAXY
Glycoanalysis by the three axes of MS and chromatography.

Glycobase(http://glycobase.nibrt.ie/glycobase/show_nibrt.action)

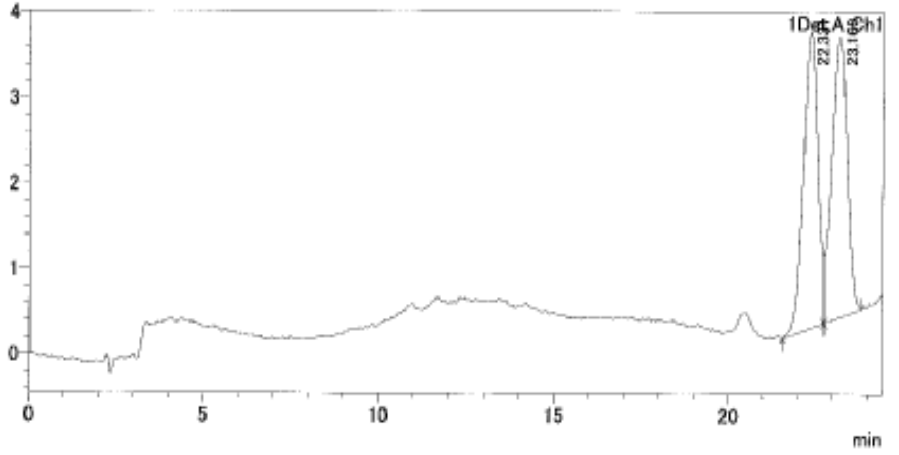
Over 675 data of AB-oligosaccharides (containing O-glycans)

GLYCOBASE 3.1
NATIONAL INSTITUTE FOR BIOPROCESSING RESEARCH AND TRAINING

Consistence between standard and sample



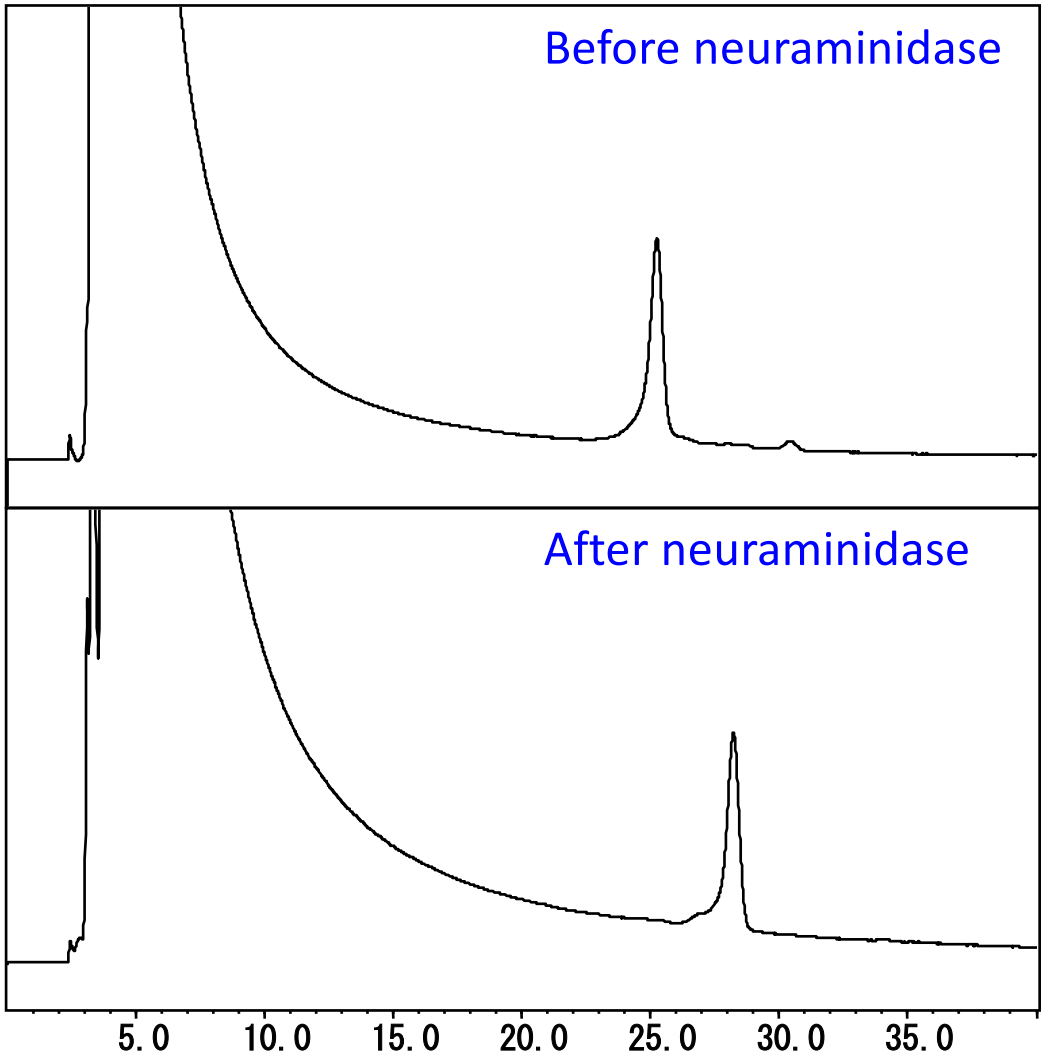
Inconsistence between standard and sample

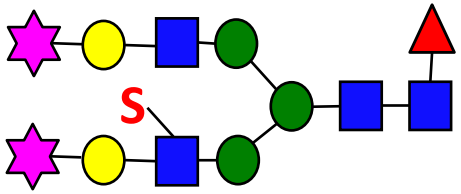


Incase of unknow oligosaccharide which is not registered in database

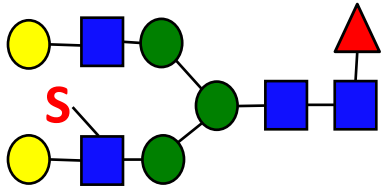
↓

Estimation/identification by the enzyme treatment





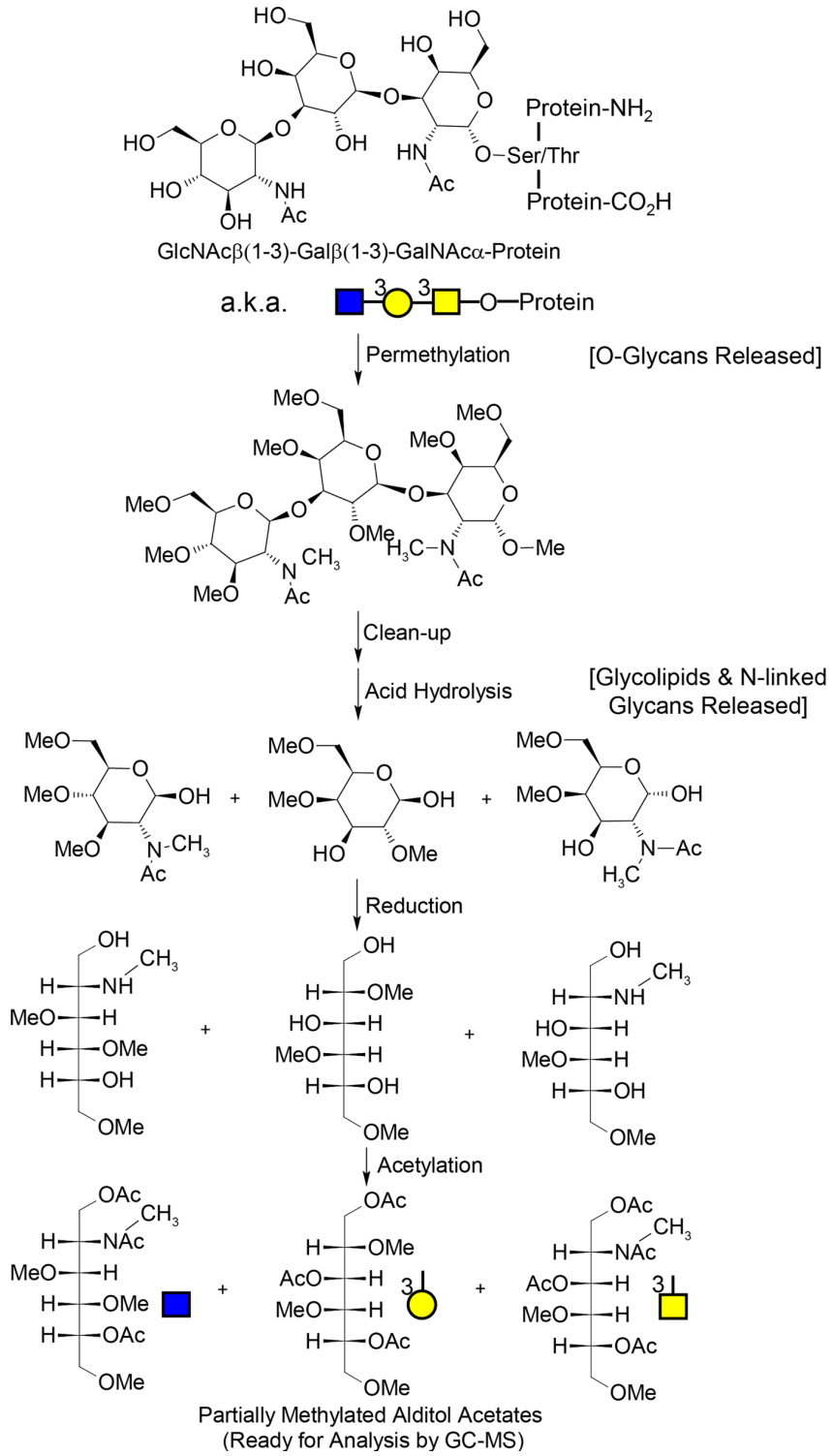
Two Neu5Ac residues were released by neuraminidase treatment



Composition and linkage analyses

The CCRC Spectral Database for
Partially Methylated Alditol Acetate

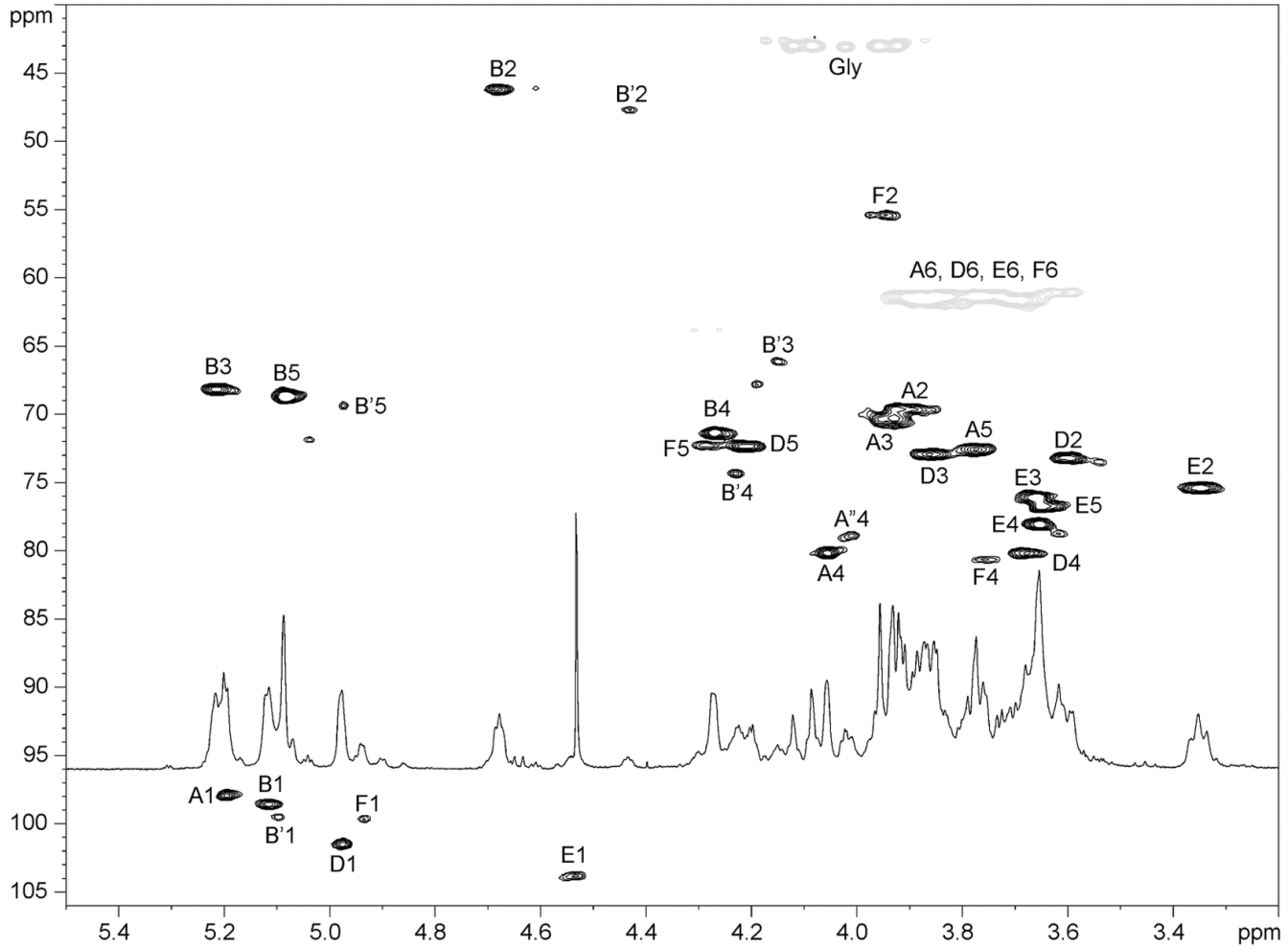
https://www.ccrc.uga.edu/specdb/ms/pmaa/pframe.html



Ferdosi S, Ho TH, Castle EP, Stanton ML, Borges CR (2018) Behavior of blood plasma glycan features in bladder cancer. PLoS ONE 13(7): e0201208. https://doi.org/10.1371/journal.pone.0201208

Structural identification by NMR

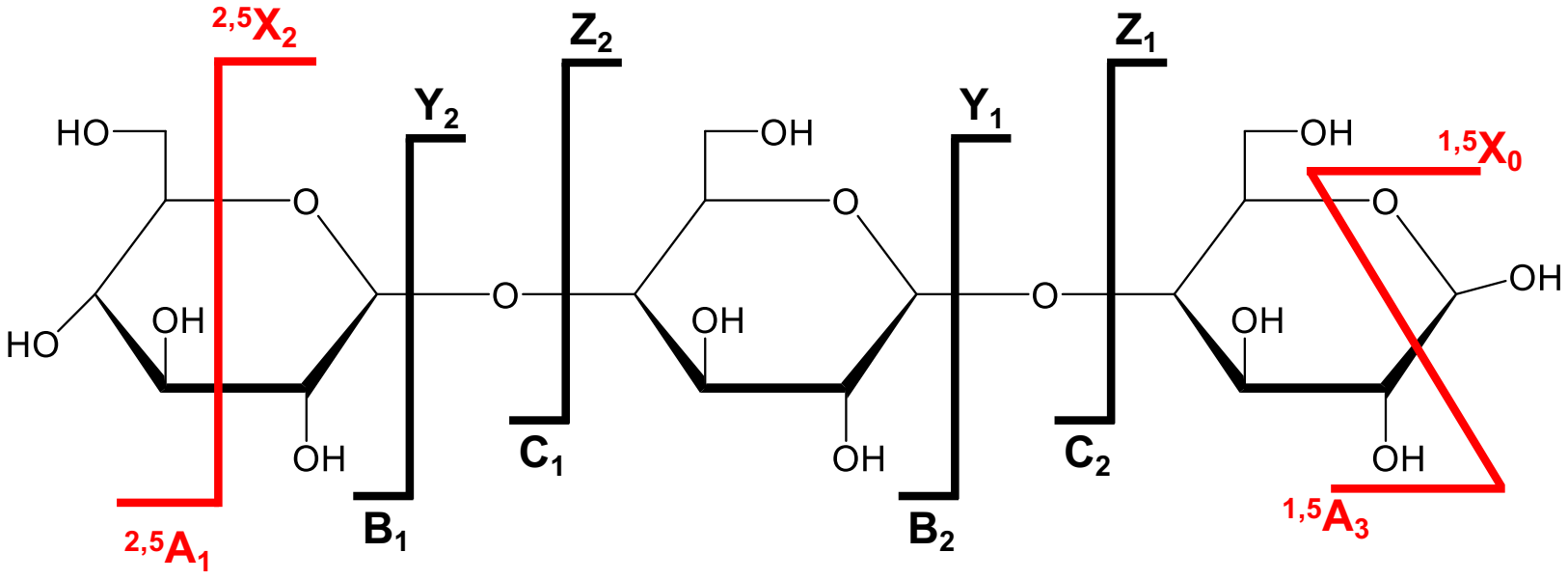
H-^{13}C HSQC spectrum of the VPS-PS with ^{1}H NMR trace.



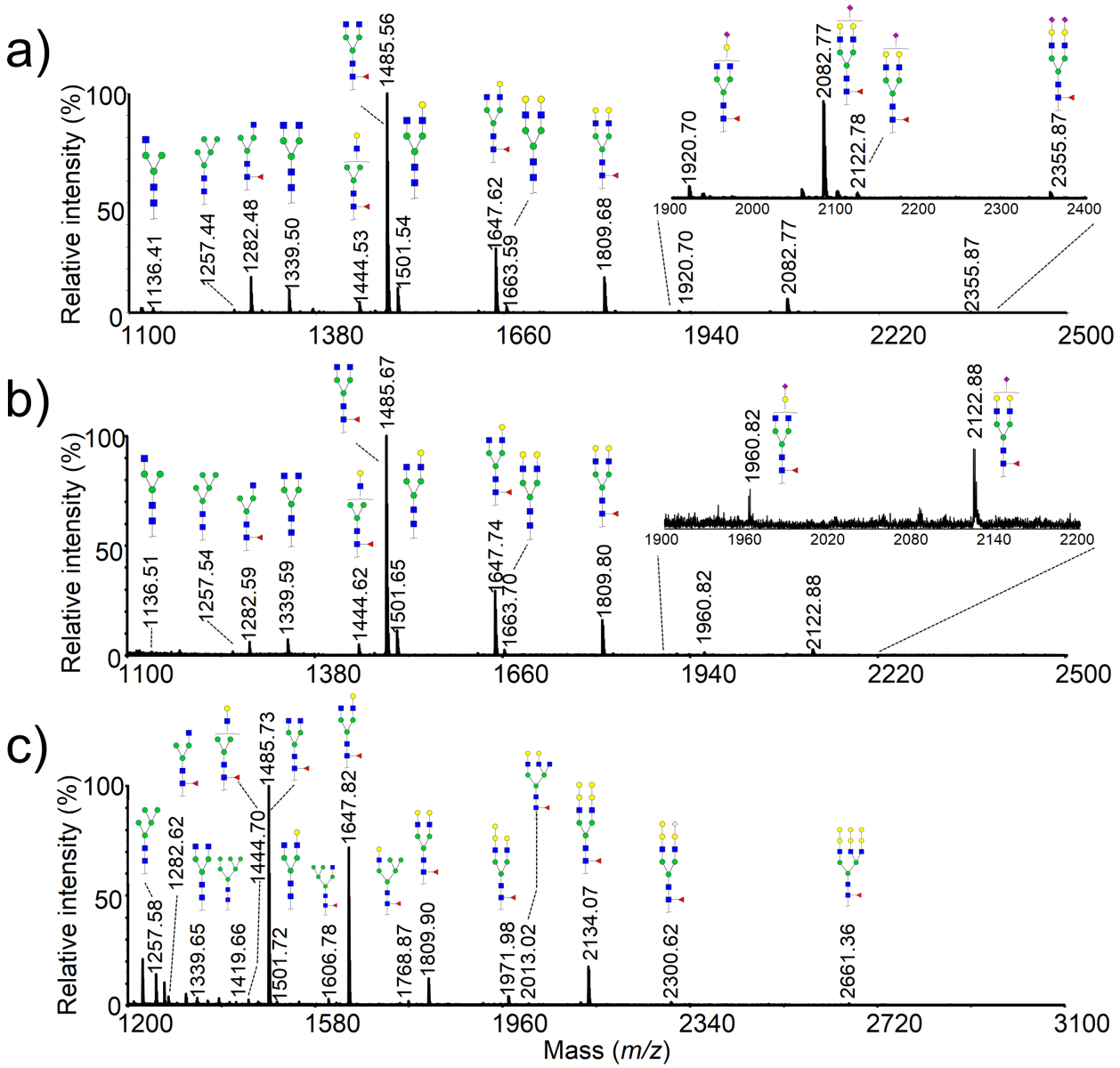
Yildiz F, Fong J, Sadovskaya I, Grard T, Vinogradov E (2014) Structural Characterization of the Extracellular Polysaccharide from *Vibrio cholerae* O1 El-Tor. PLoS ONE 9(1): e86751. https://doi.org/10.1371/journal.pone.0086751

Mass spectrometric analysis

The following figure illustrates the general nomenclature scheme for glycan fragments.



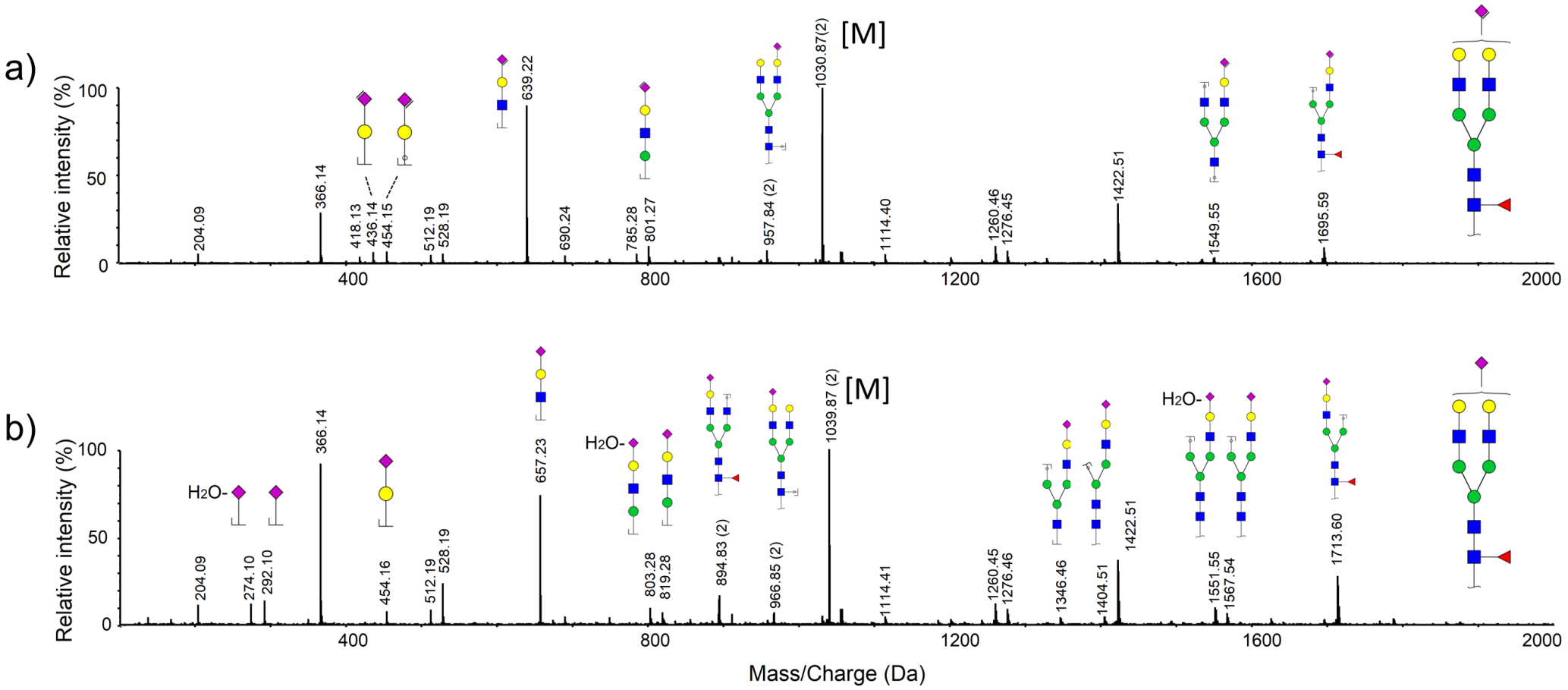
MALDI-TOF MS spectrum of N-glycans enzymatically released from the biosimilar of cetuximab and cetuximab



a) native N-glycans before mild alkali treatment (pH 10 ammonium hydroxide); b) native N-glycans of the biosimilar after mild alkali treatment; c) native N-glycans from the cetuximab. The cartoons of possible structures of glycans were adapted from Glycoworkbench and structure is depicted following the CFG notation.

Liu S, Gao W, Wang Y, He Z, Feng X, Liu B-F, et al. (2017) Comprehensive N-Glycan Profiling of Cetuximab Biosimilar Candidate by NP-HPLC and MALDI-MS. PLoS ONE 12(1): e0170013. https://doi.org/10.1371/journal.pone.0170013

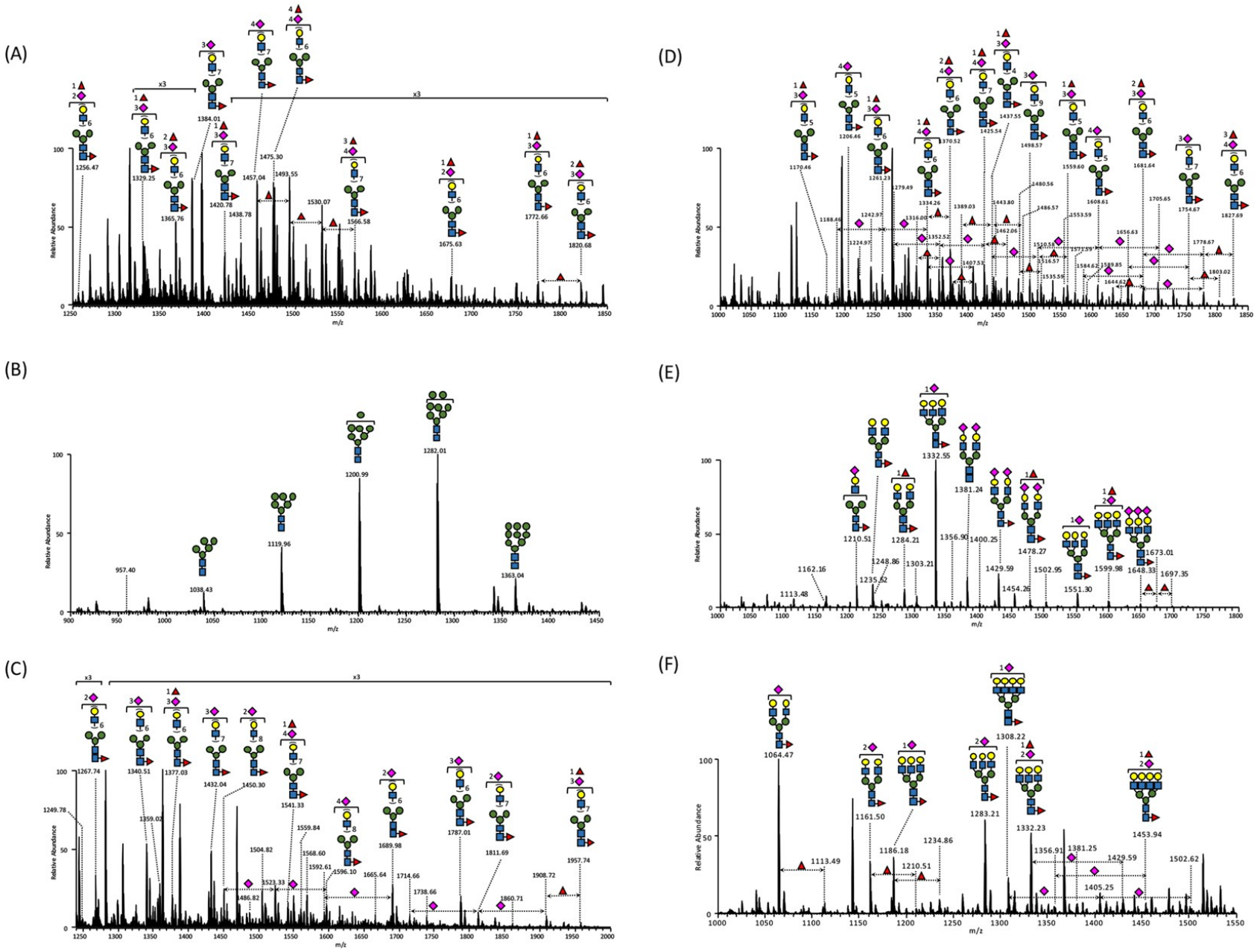
NanoLC-ESI-MS/MS spectrum of native glycans



MS/MS spectra of *m/z* 2060 with chemical composition of $GlcNAc_4Man_3Gal_2NeuAcLac_1$; b) MS/MS spectra of *m/z* 2078 with chemical composition of $GlcNAc_4Man_3Gal_2NeuAc_1$.

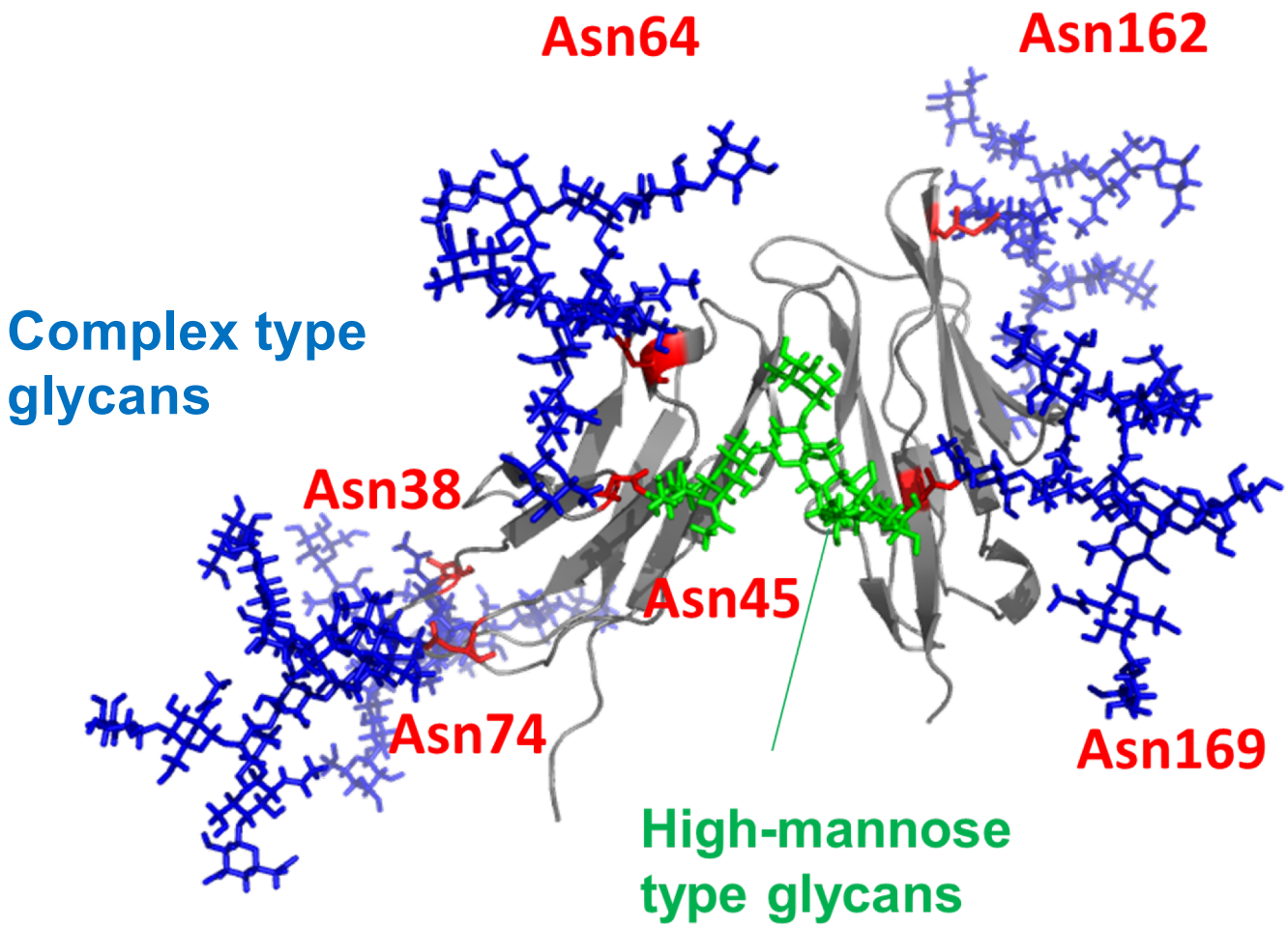
Liu S, Gao W, Wang Y, He Z, Feng X, Liu B-F, et al. (2017) Comprehensive N-Glycan Profiling of Cetuximab Biosimilar Candidate by NP-HPLC and MALDI-MS. PLoS ONE 12(1): e0170013. https://doi.org/10.1371/journal.pone.0170013

MS profiling of site-specific glycoforms of the serum sFcγRIIIb,



H. Yagi et al. *Sci. rep.* ,9: 2719, 2018

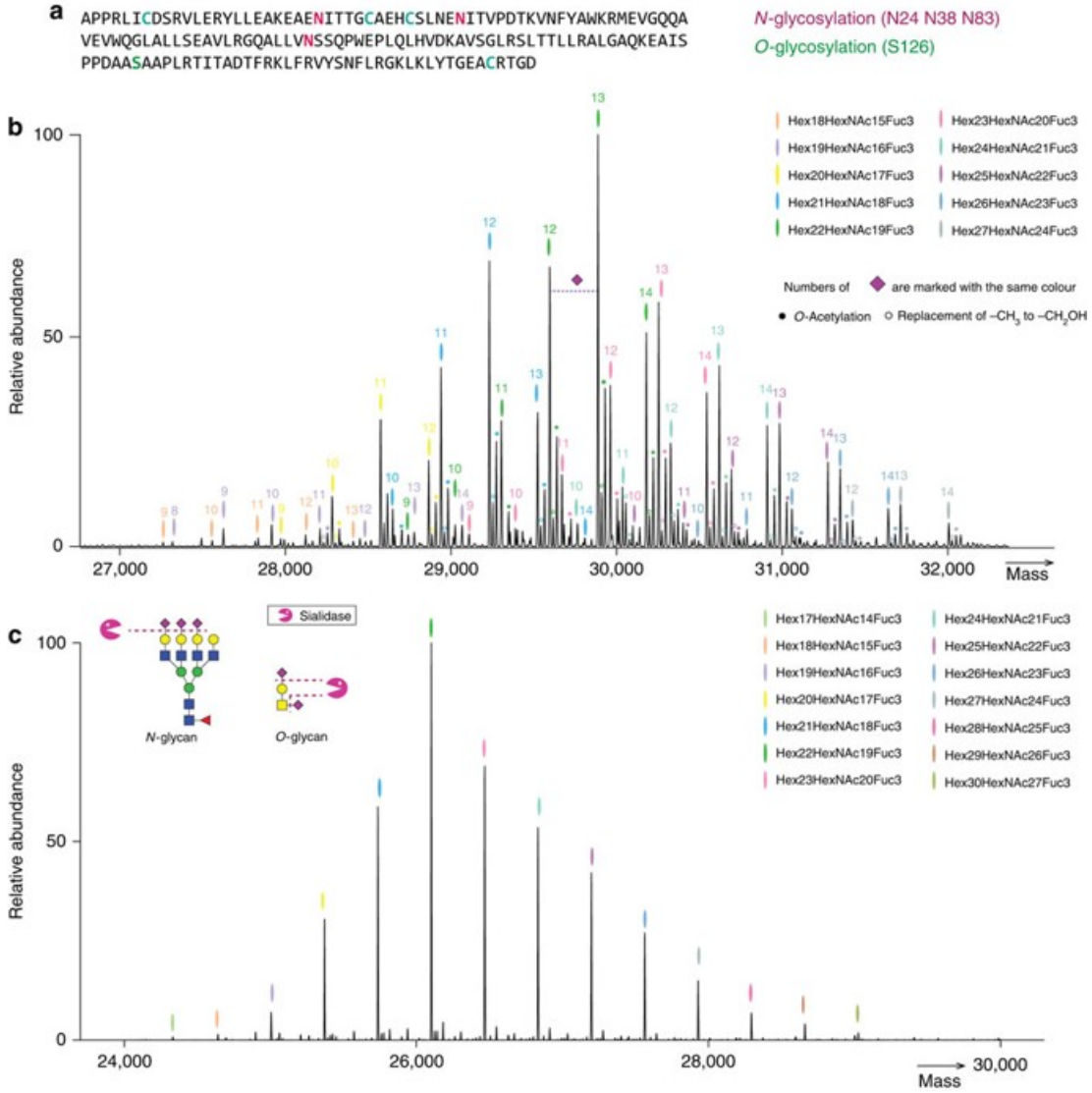
Molecular model of sFcγRIIIb with *N*-glycans on the basis of our LC-MS/MS data.



H. Yagi et al. *Sci. rep.* ,9: 2719, 2018

Native mass analysis

MS can be used to measure the stoichiometry and composition of protein complexes, the presence of small molecules

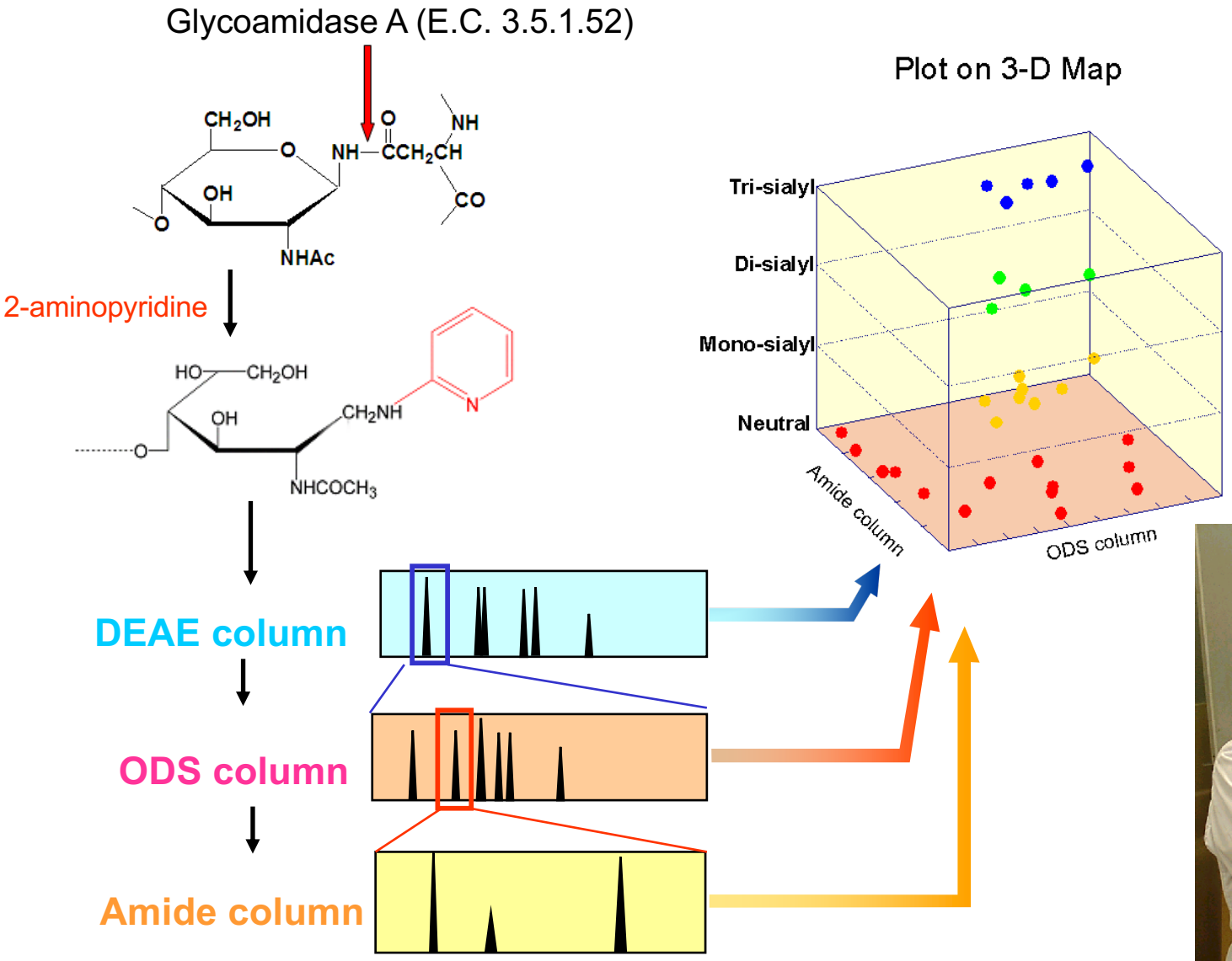


(**a**) Schematic of the rhEPO backbone sequence and its reported PTM sites. (**b**) The zero-charge deconvoluted native MS spectrum of rhEPO.

Yang, Y., Liu, F., Franc, V. *et al.* Hybrid mass spectrometry approaches in glycoprotein analysis and their usage in scoring biosimilarity. *Nat Commun* **7,** 13397 (2016). https://doi.org/10.1038/ncomms13397

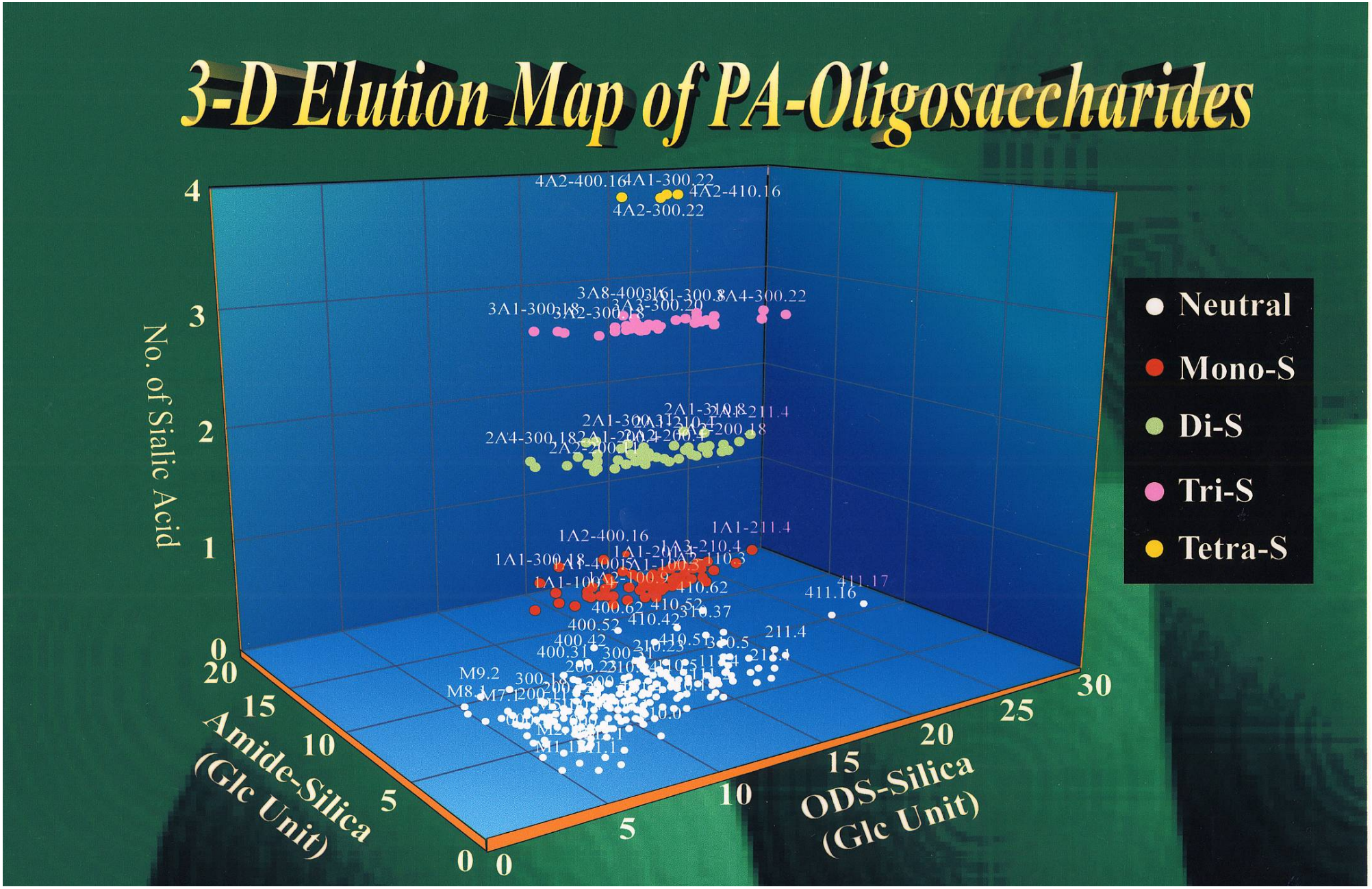
Detail information of N-glycans structural analysis by using HPLC mapping method

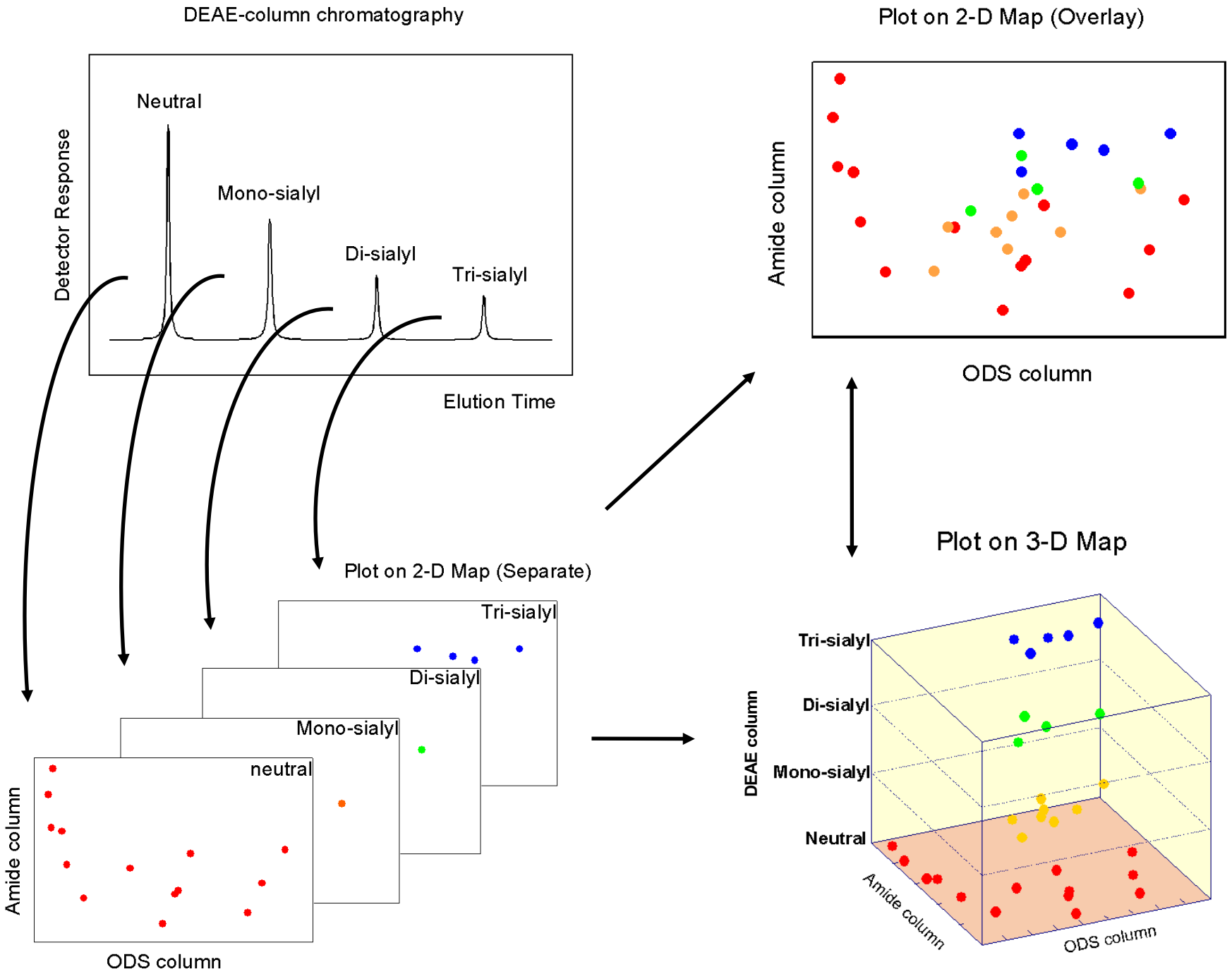
The multi-dimensional HPLC mapping technique



Dr. Noriko Takahashi

Prog. Nucl. Magn. Reson. Spectrosc. **56, 346-359 (2010)**

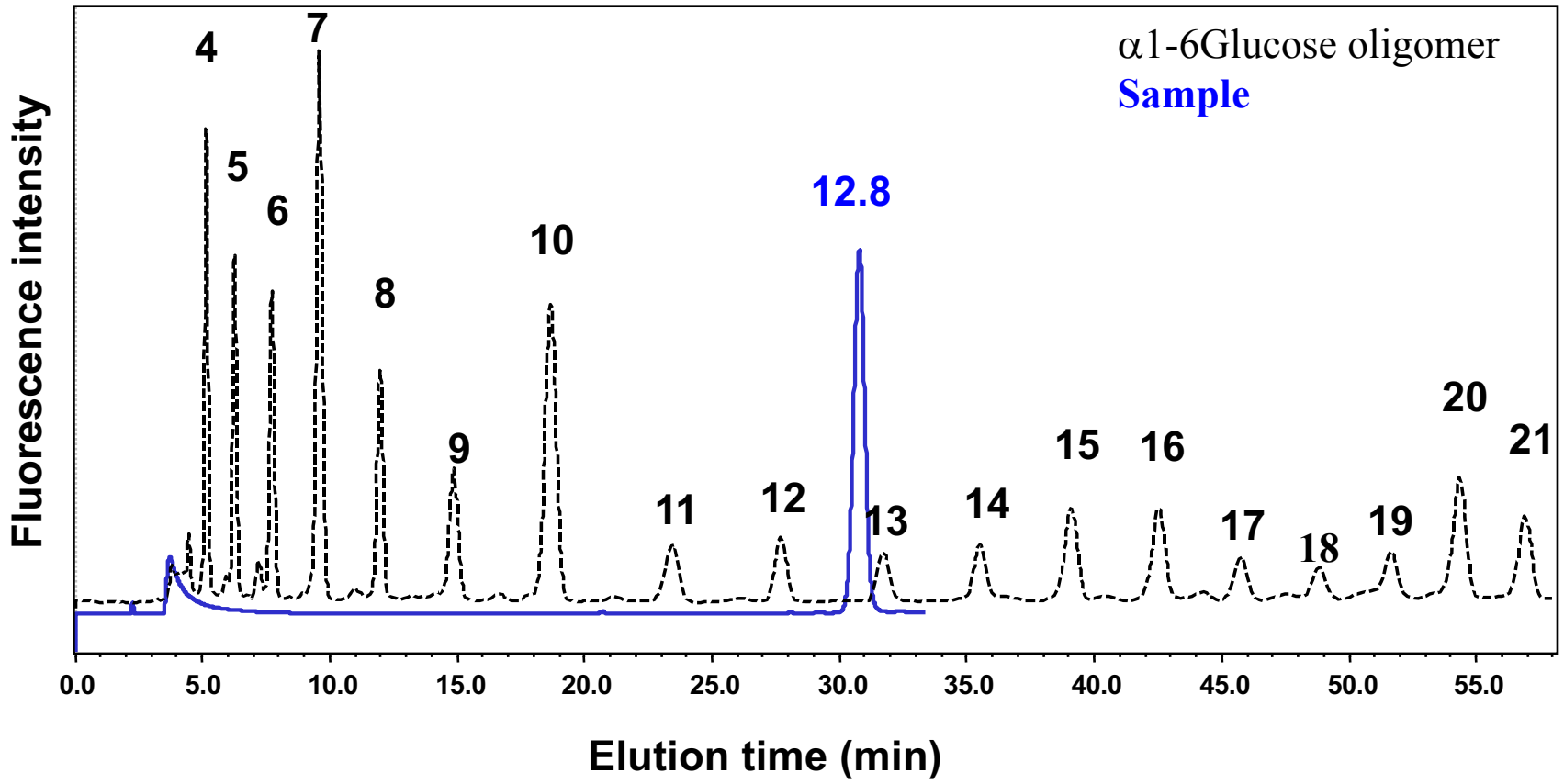

3-D Elution Map of PA-Oligosaccharides
No. of Sialic Acid
Amide-Silica (Glc Unit)
ODS-Silica (Glc Unit)
Neutral
Mono-S
Di-S
Tri-S
Tetra-S
4A2-400.16
4A1-300.22
4A2-410.16
4A2-300.22
3A1-300.18
3A2-300.18
3A3-300.20
3A4-300.22
2A4-300.18
1A2-400.16
1A1-211.4
1A1-300.18
1A3-210.4
410.62
411.17
411.16
400.62
410.52
410.37
400.52
410.42
400.42
211.4
400.31
M9.2
M8.1
M7.1


DEAE-column chromatography
Neutral
Mono-sialyl
Di-sialyl
Tri-sialyl
Detector Response
Elution Time
Plot on 2-D Map (Overlay)
Amide column
ODS column
Plot on 2-D Map (Separate)
Tri-sialyl
Di-sialyl
Mono-sialyl
neutral
Amide column
ODS column
Plot on 3-D Map
Tri-sialyl
Di-sialyl
Mono-sialyl
Neutral
DEAE column
Amide column
ODS column

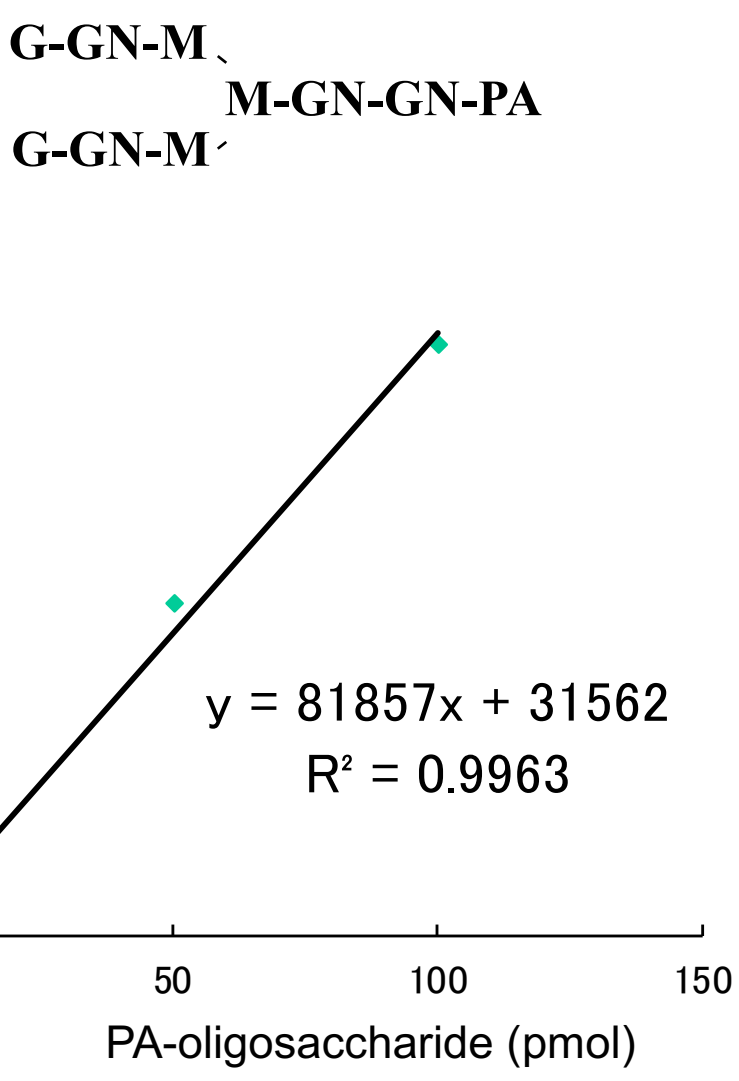
The elution position of each peak is expressed in glucose units (gu).

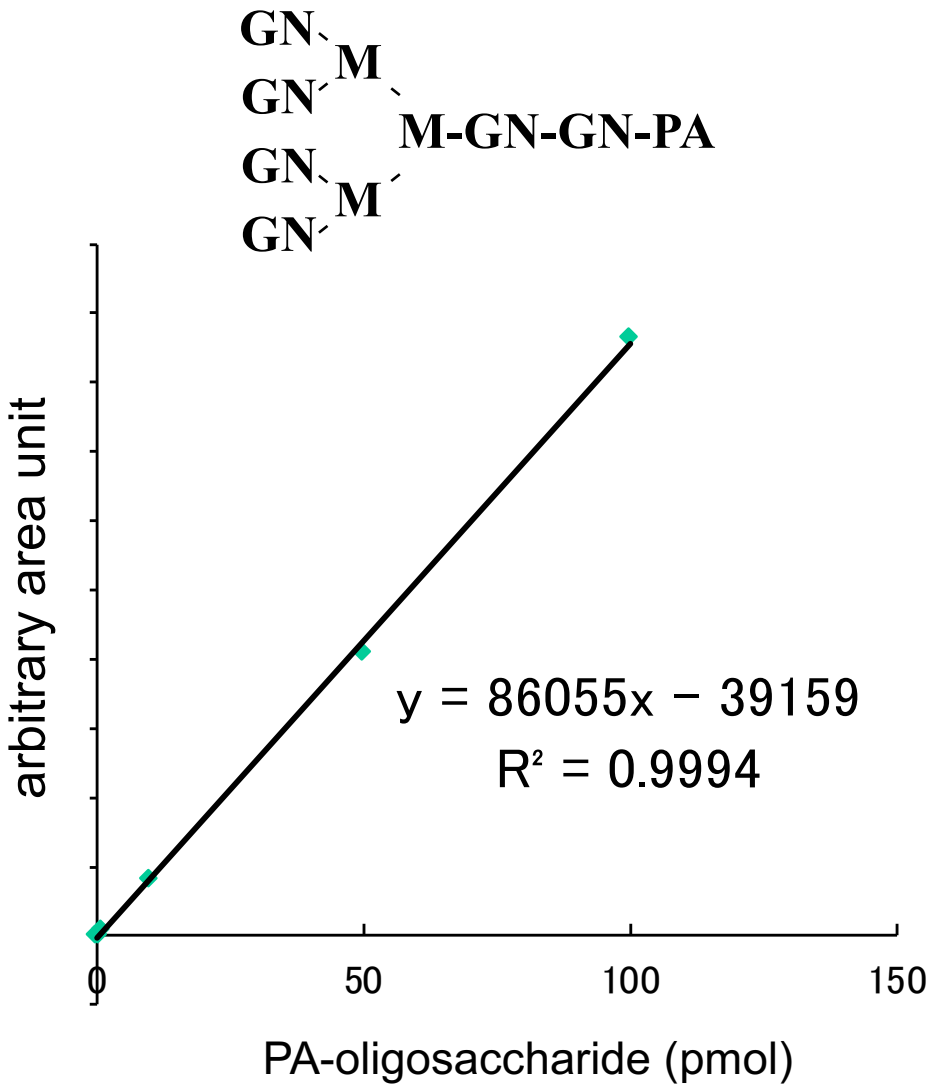
The elution positions of peaks in an unknown glycan pool are assigned an overall gu value by comparison with the standard α1-6glucose oligomers.

N-glycosylation profiles on ODS cplumn

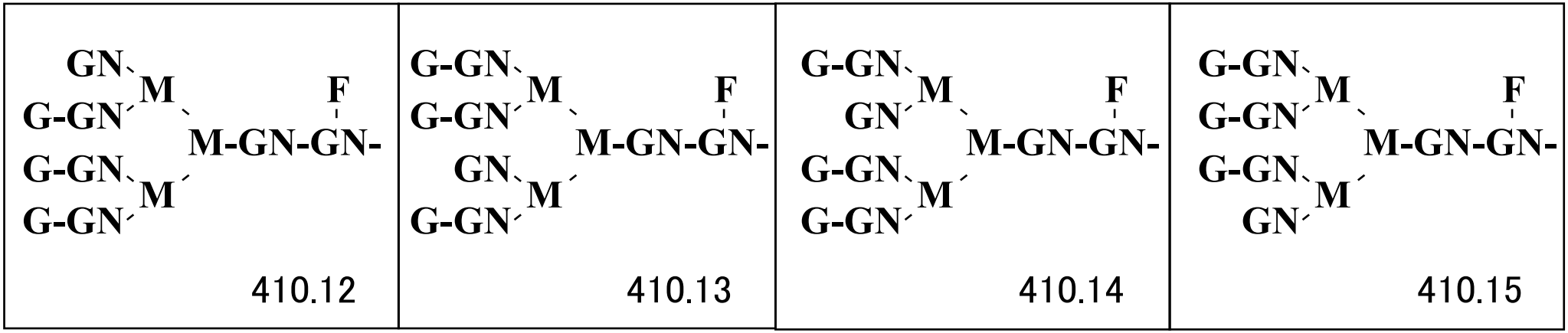


HPLC peak areas of PA-glycans can show a linearity plot from 0.1 to 100 pmol (in a quantitative manner)



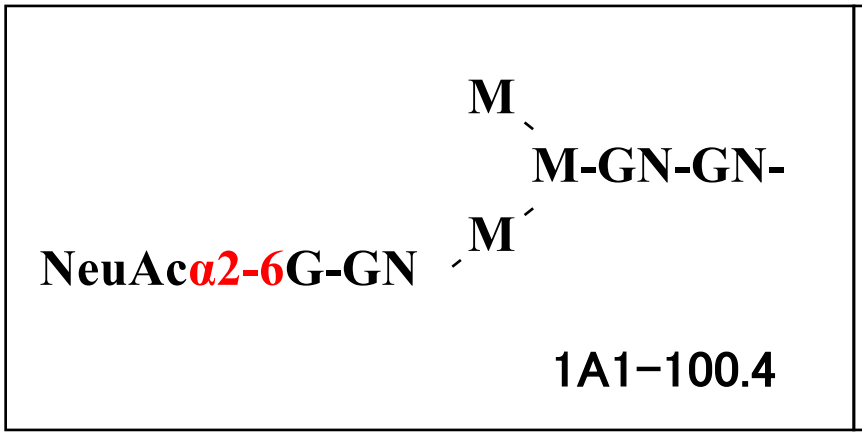


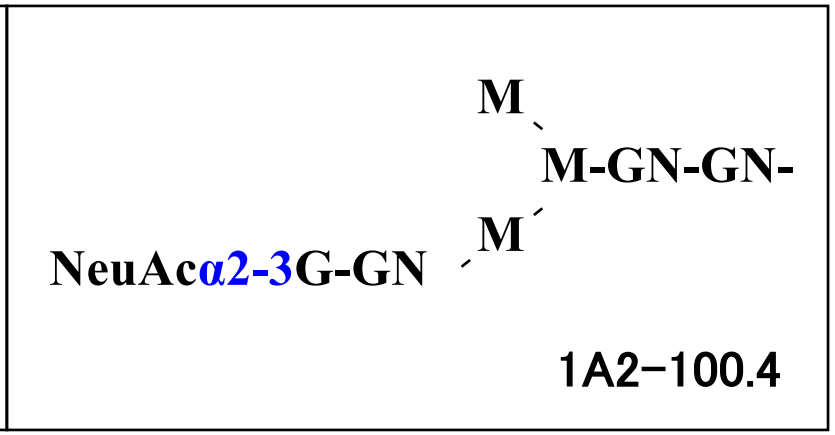
HPLC-based discrimination of glycol-isomers



ODS : 14.1	ODS : 13.8	ODS : 13.7	ODS : 12.5
Amide : 9.5	Amide : 9.3	Amide : 9.2	Amide : 8.9

Distinguish α2-6 from α2-3!





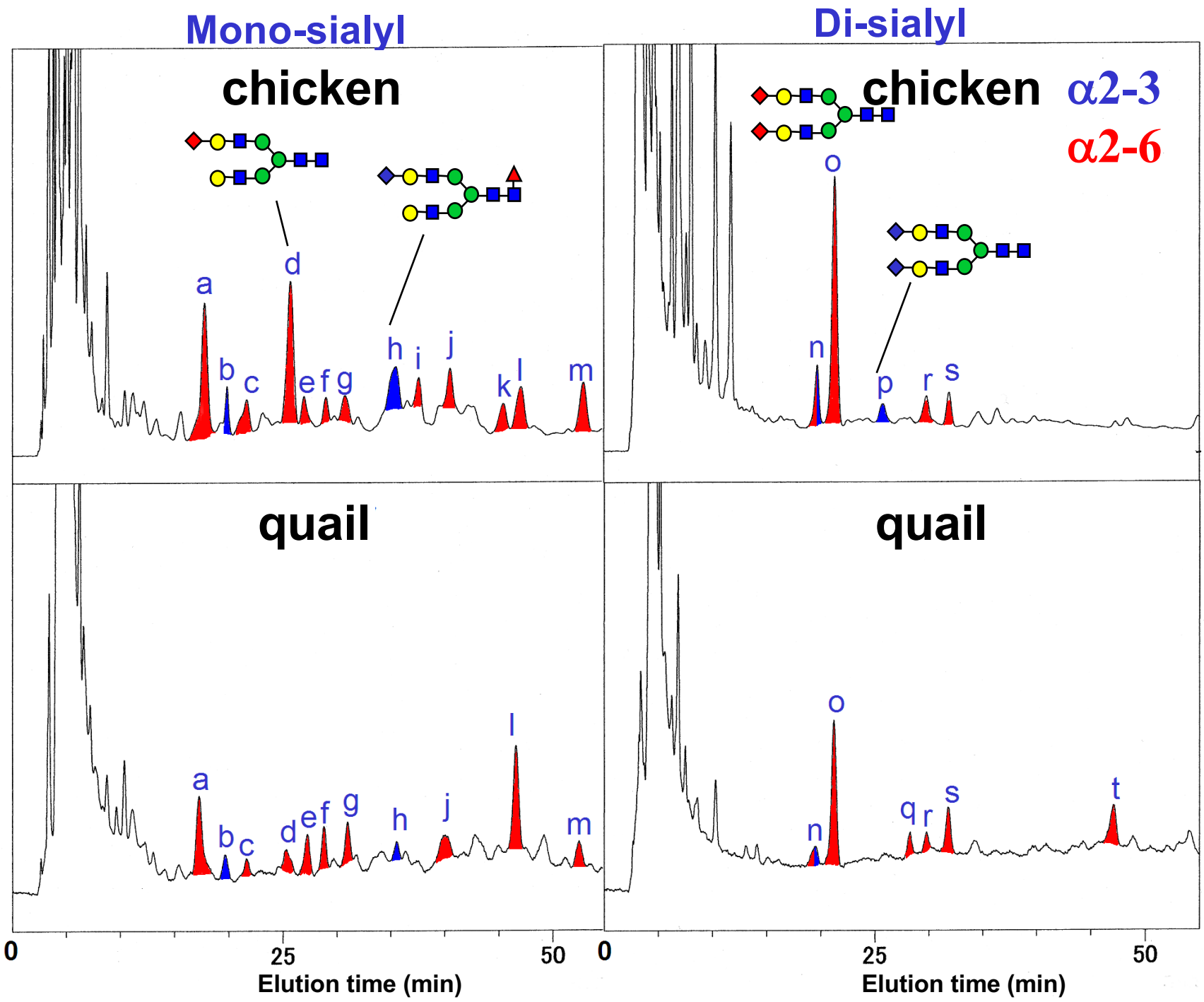
ODS :7.8

Amide : 6.0

ODS : 9.1

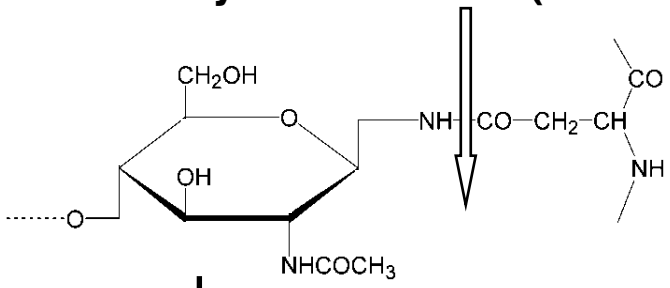
Amide : 5.4

Expression of α2-6 sialylated *N*-glycans in avian intestines

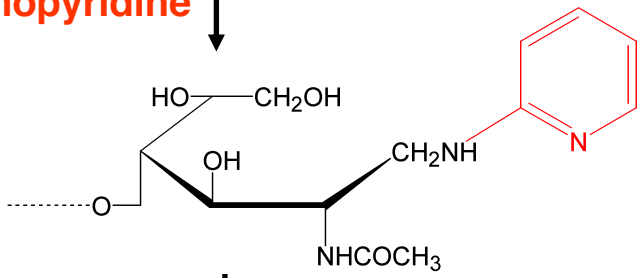


A principal of HPLC mapping method

Glycoamidase A (E.C. 3.5.1.52)



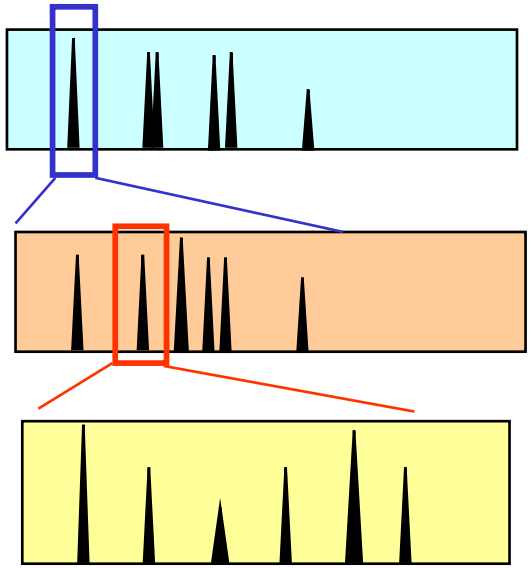
2-aminopyridine



Anion-exchange column

ODS column

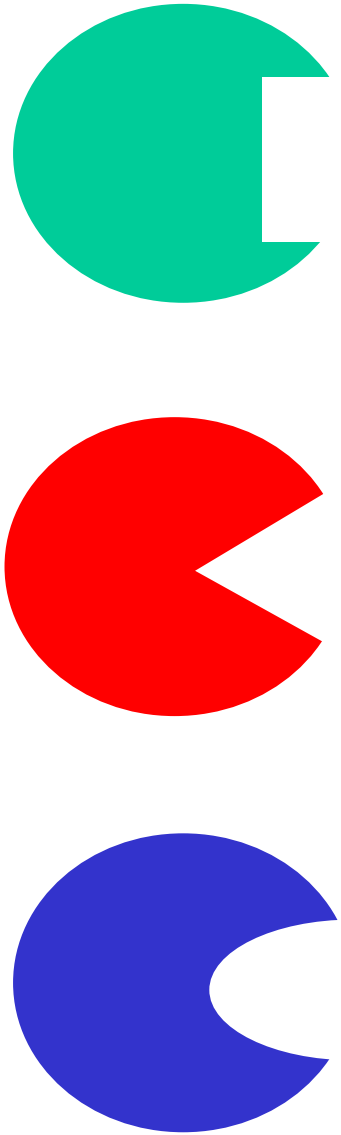
Amide column



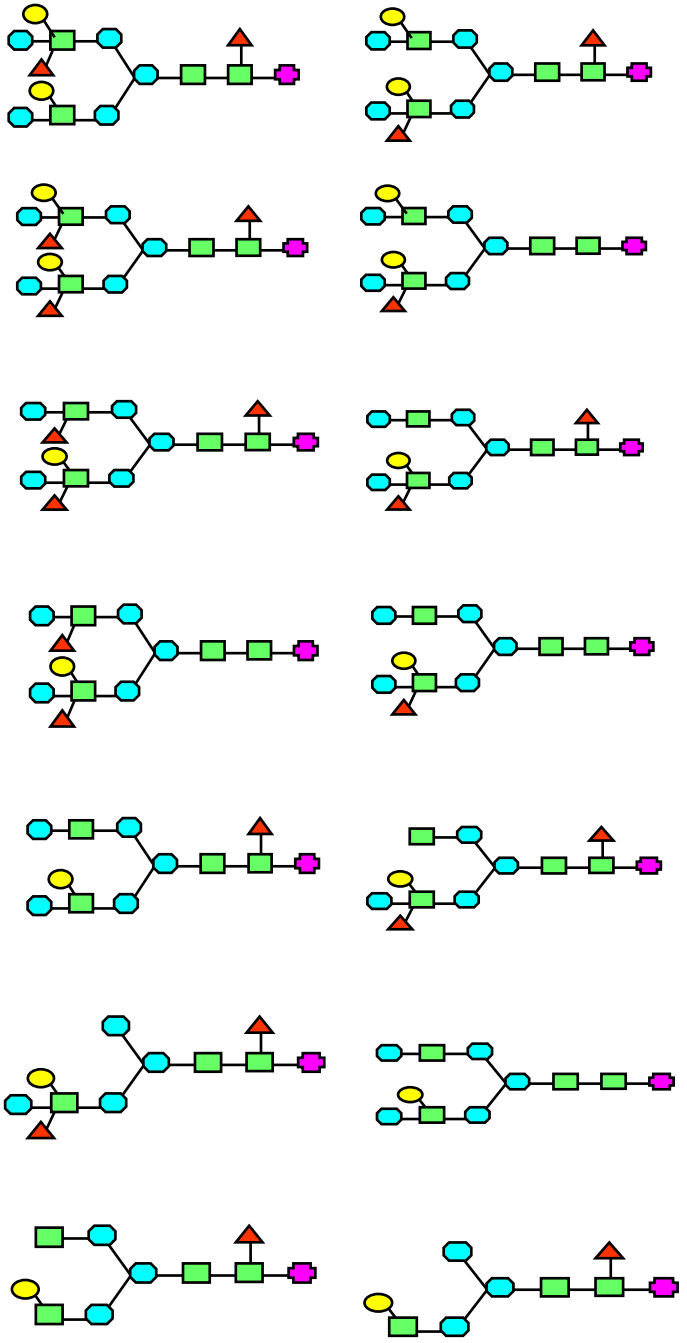
Sugar Library

The HPLC mapping method enable us to collect the standard oligosaccharides according to HPLC separation.

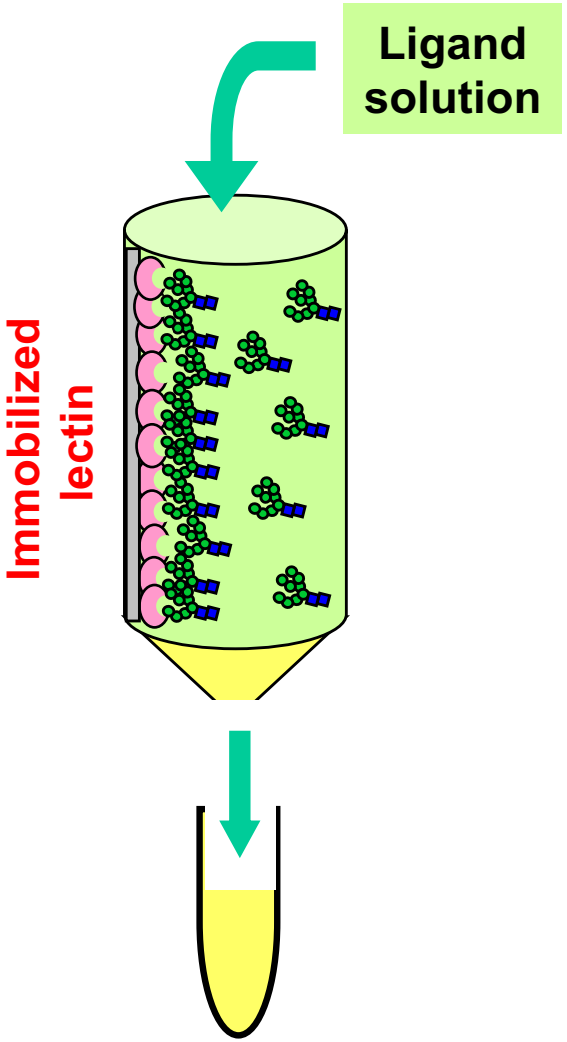
Lectin＝Glycan binding protein

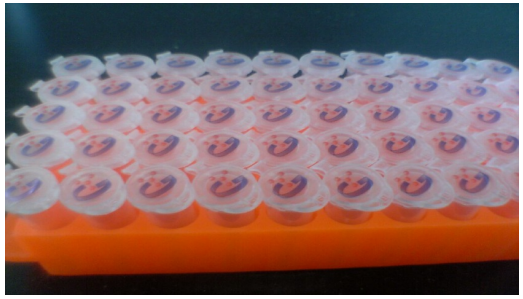


Multiple structures

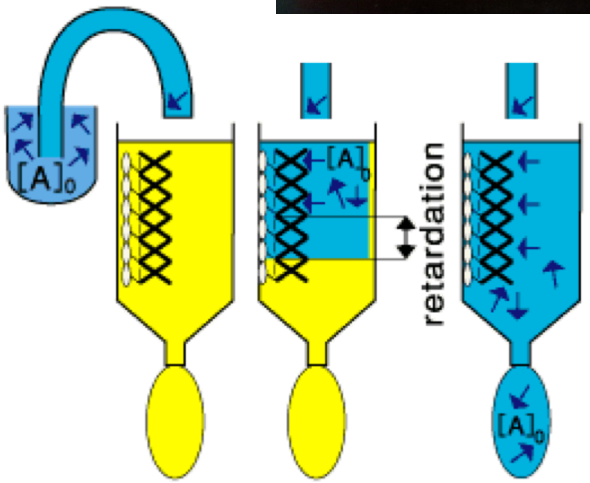


Systematic analysis of sugar chain-protein interactions by frontal affinity chromatography (FAC) method

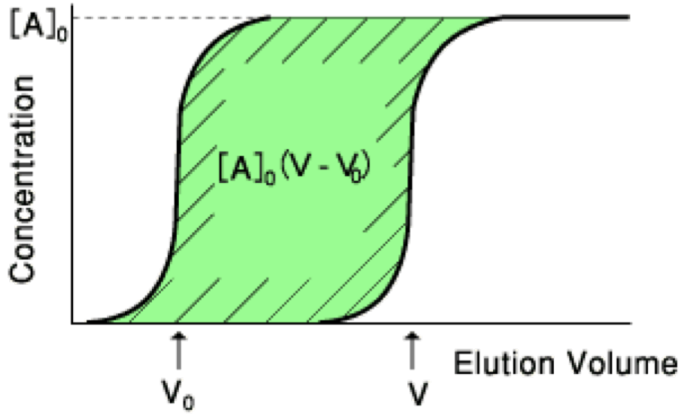




Library of PA-glycans



GlycoWord



$$K_d = \frac{[A]\,[B]}{[AB]} = \frac{B_t}{V-V_0} - [A]_0 \qquad (1)$$

$$\frac{1}{[A]_0\,(V-V_0)} = \frac{K_d}{B_t} \cdot \frac{1}{[A]_0} + \frac{1}{B_t} \qquad (2)$$

$$K_d = \frac{[A]\,[B]}{[AB]} = \frac{B_t}{V-V_0} \qquad (3)$$

平林淳: フロンタル分析を利用する糖-タンパク質相互作用解析. Glycoword. GT-C07. https://www.glycoforum.gr.jp/glycoword/glycotechnology/GT-C07J.html

レクチンアフィニティカラムからのPA化糖鎖の溶出プロファイル

		$V-V_0$	K_d
LNFP-I	Galβ1-3GlcNAcβ1-3Galβ1-4Glc－PA Fucα1-2	0.18ml	0.17mM
LNT	Galβ1-3GlcNAcβ1-3Galβ1-4Glc－PA	0.16	0.19
LNnT	Galβ1-4GlcNAcβ1-3Galβ1-4Glc－PA	0.096	0.32
GM1	Galβ1-3GalNAcβ1-4Galβ1-4Glc－PA NeuAcα2-3	0.048	0.63
GA1	Galβ1-3GalNAcβ1-4Galβ1-4Glc－PA	0.052	0.58
Gb4	GalNAcβ1-3Galα1-4Galβ1-4Glc－PA	0.024	1.3

GlycoWord

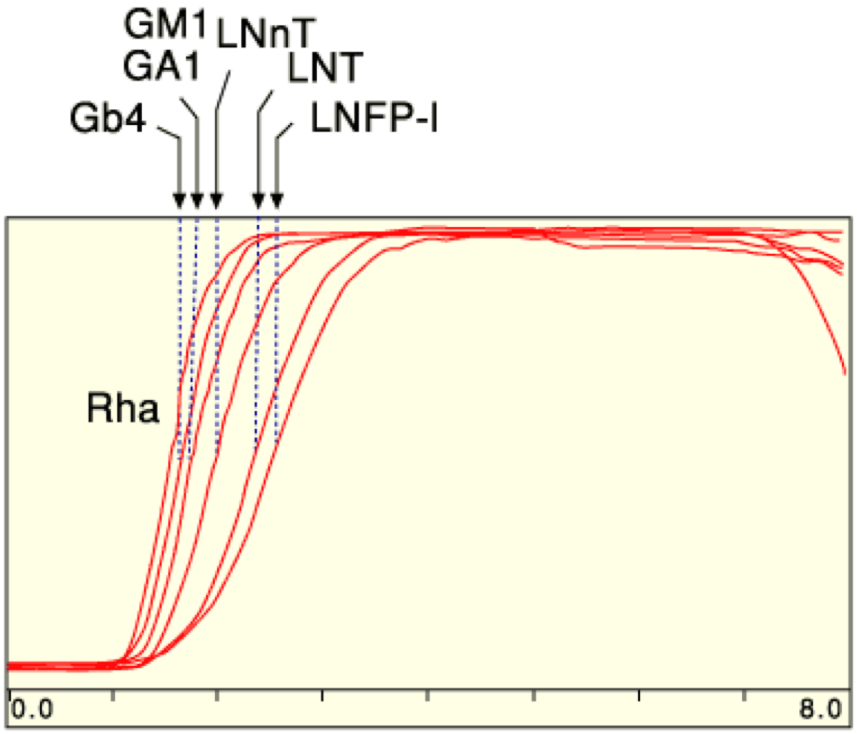
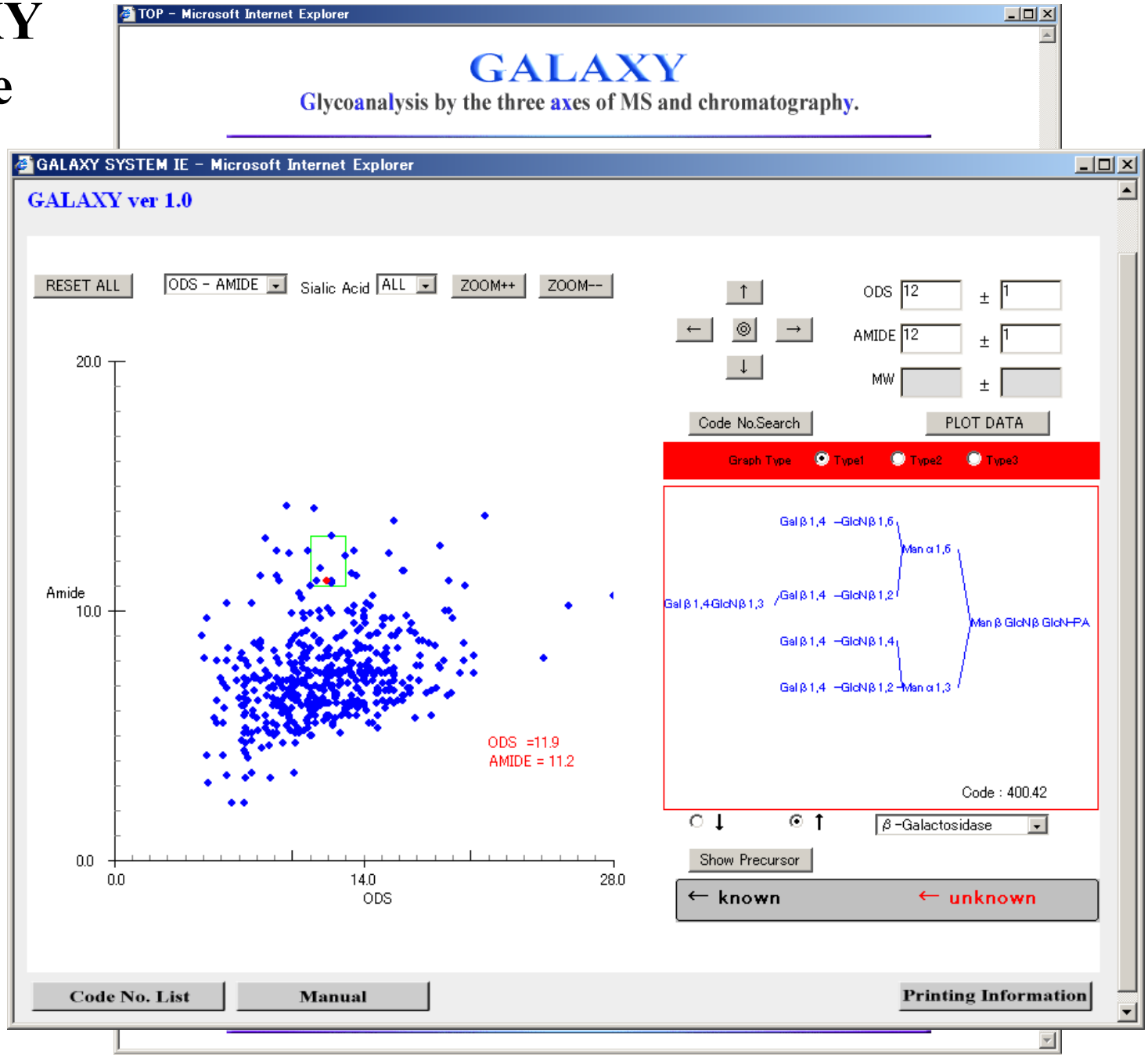


図.3
FACの解析例：C. elegansガレクチンLEC-6を固定化したカラム（7.44 mg/mlゲル）に糖脂質由来のオリゴ糖溶液（ピリジルアミノ化体、10 nM）6種を2 mlのサンプルループを介して0.25 ml/minの流速で注入する。V0はラムノースの溶出位置として求めている。各オリゴ糖に対するKd値はp-アミノフェニルラクトシドの濃度変化解析からBtを求め、FACの基本式（1）から算出している。

平林淳: フロンタル分析を利用する糖-タンパク質相互作用解析. Glycoword. GT-C07. https://www.glycoforum.gr.jp/glycoword/glycotechnology/GT-C07J.html

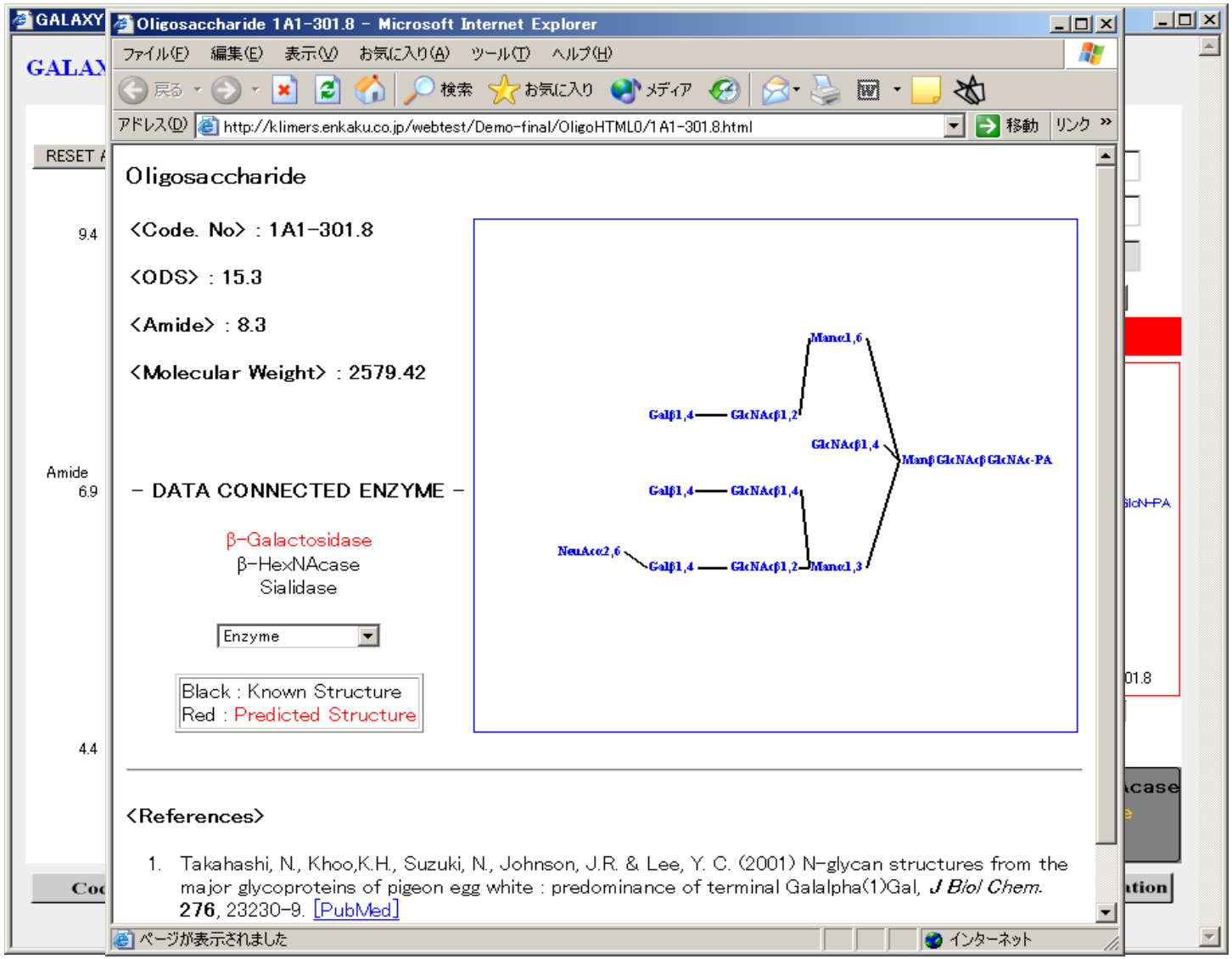
GALAXY database

http://www.glycoanalysis.info/

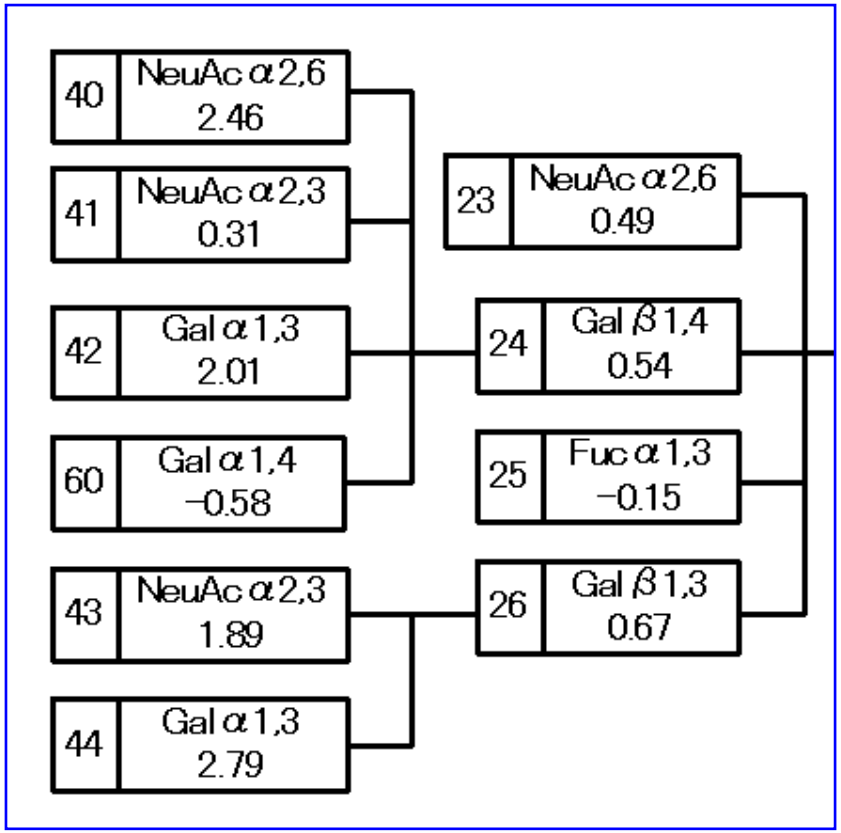


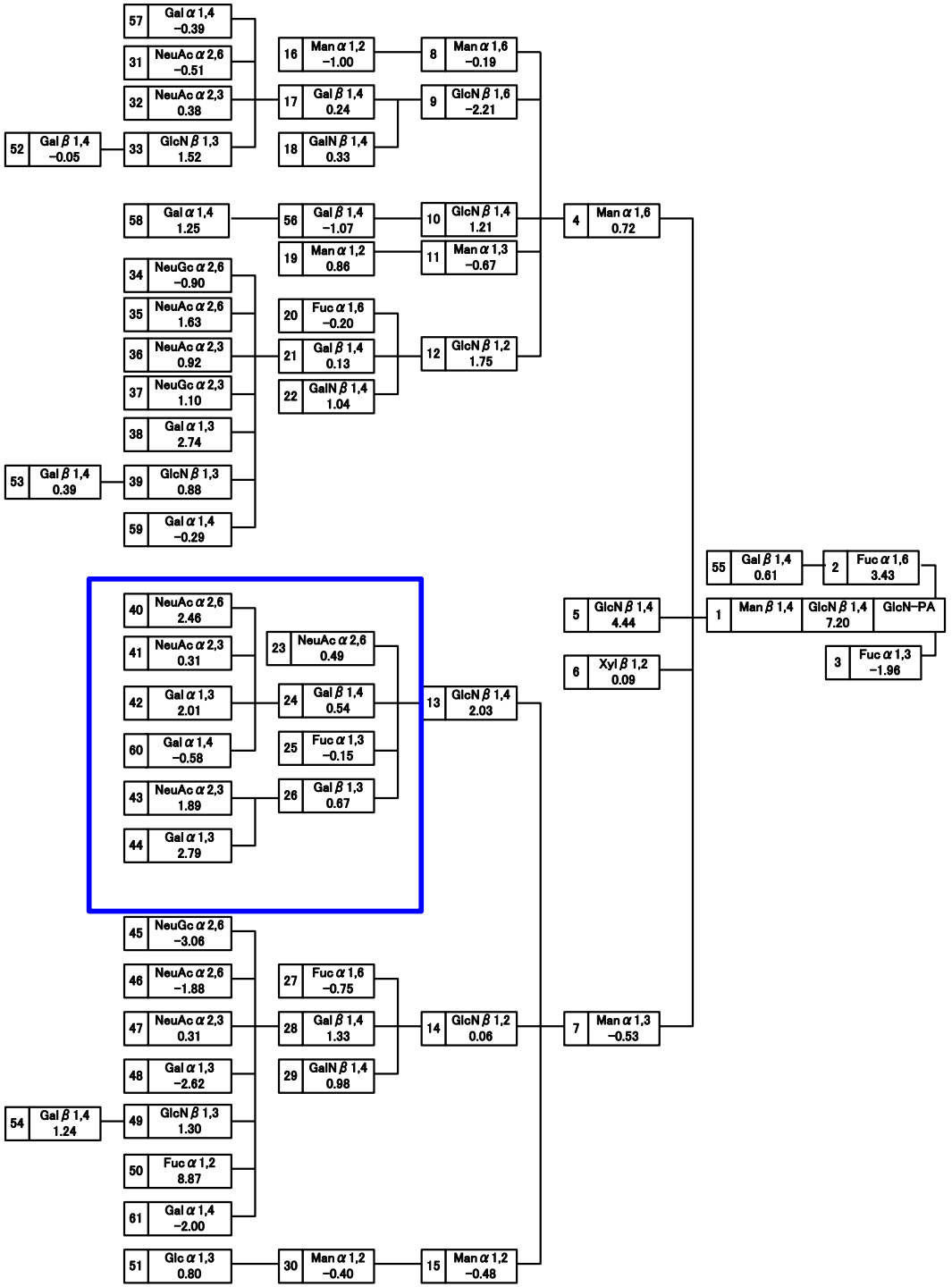
Trends Glycosci. Glycotech. 15, 235-251 (2003)

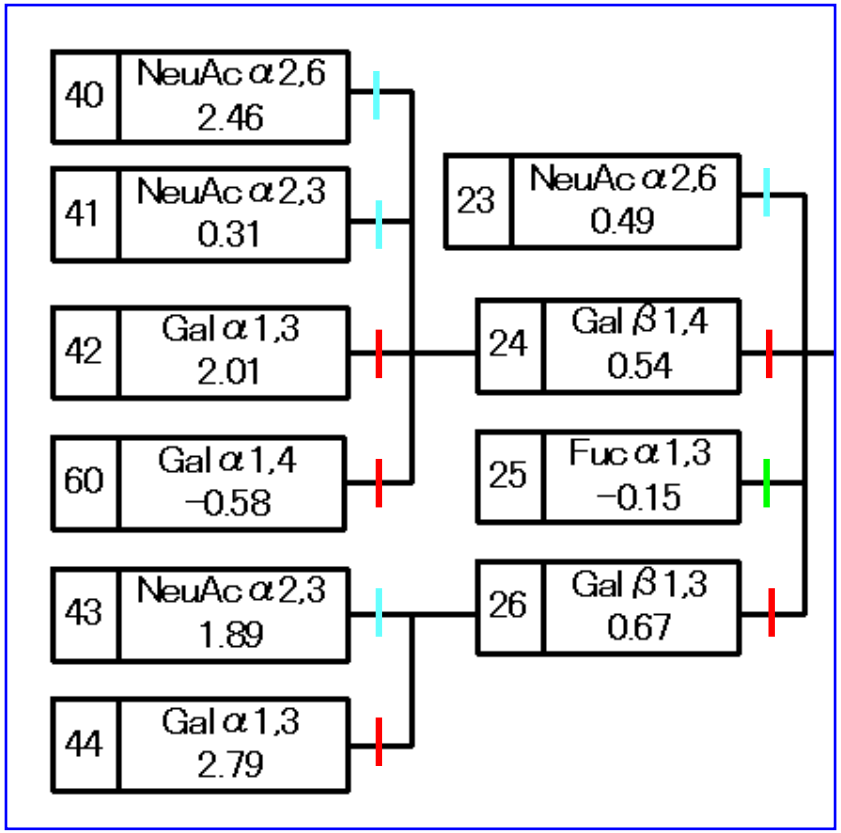
Information page for the individual N-glycans

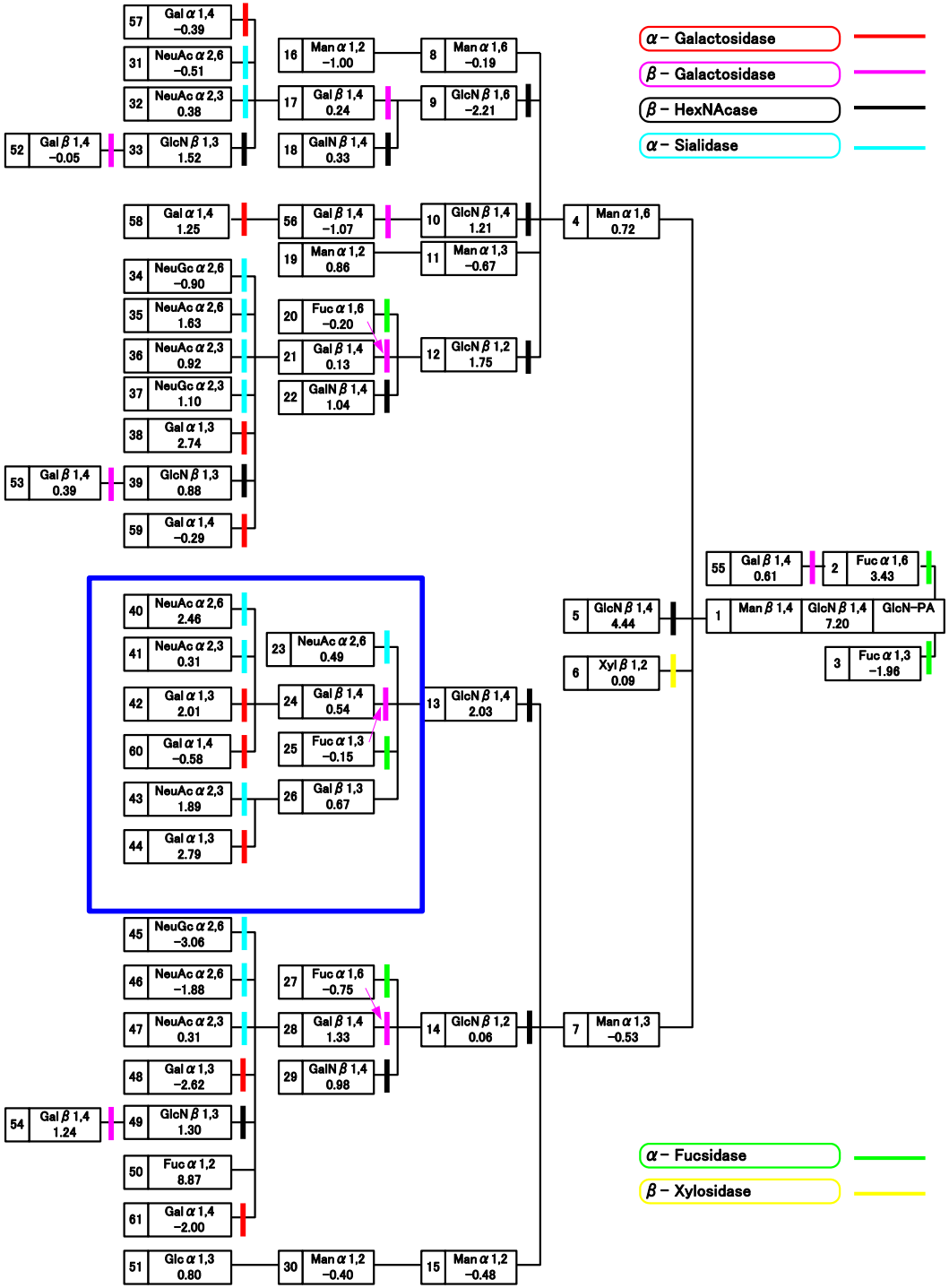


The GlycoTree diagram

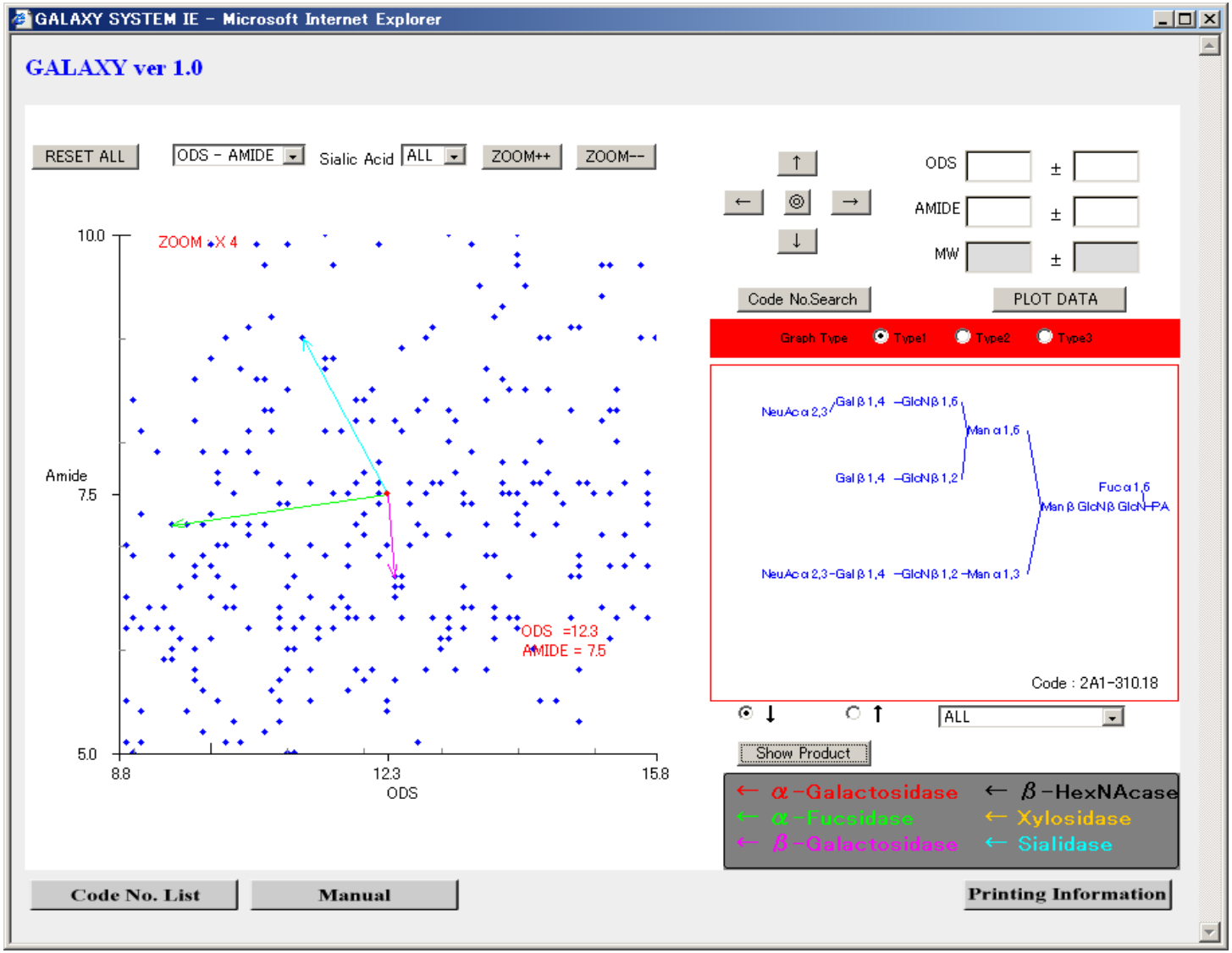




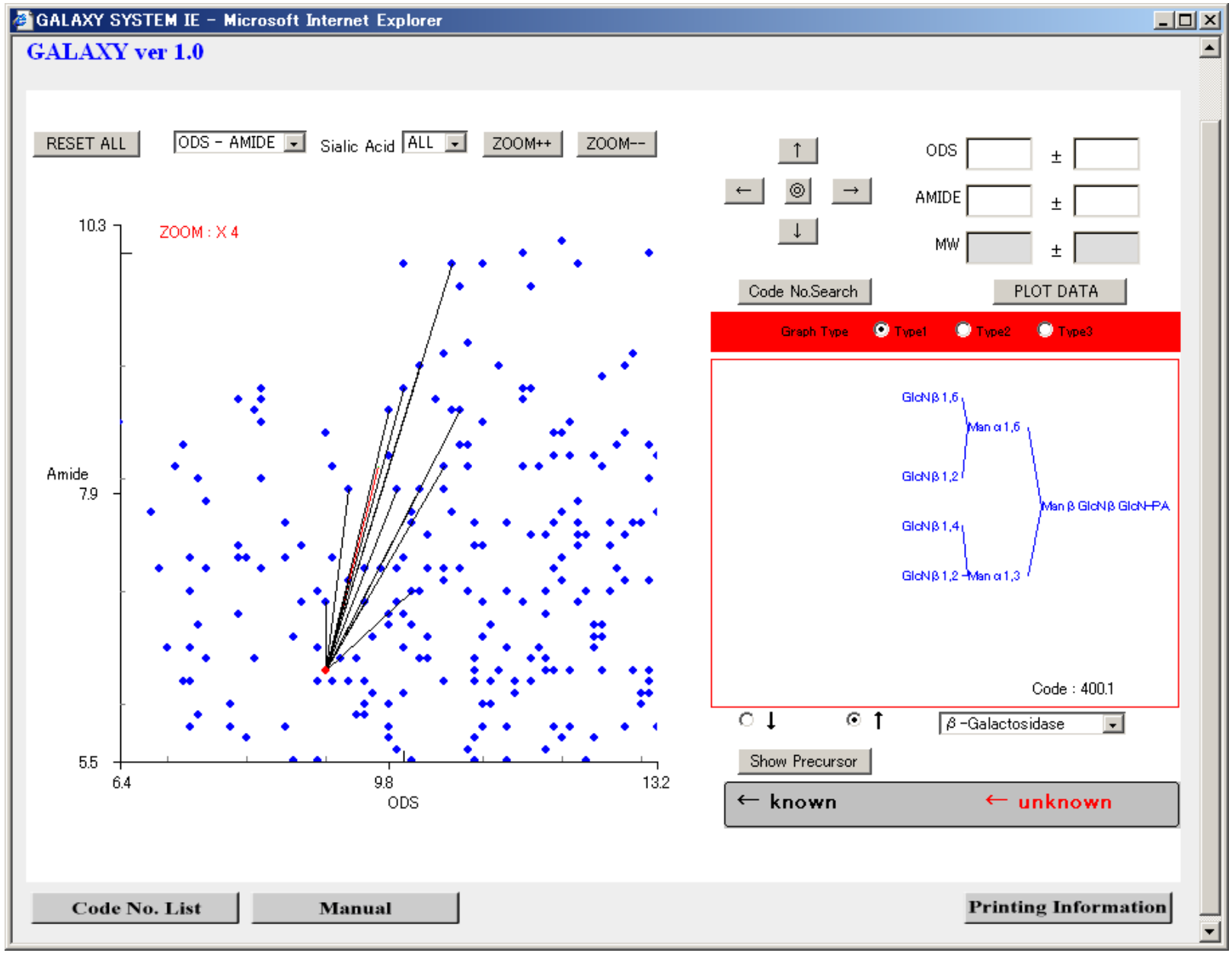

40 NeuAc α2,6 2.46
41 NeuAc α2,3 0.31
42 Gal α1,3 2.01
60 Gal α1,4 −0.58
43 NeuAc α2,3 1.89
44 Gal α1,3 2.79
23 NeuAc α2,6 0.49
24 Gal β1,4 0.54
25 Fuc α1,3 −0.15
26 Gal β1,3 0.67


α − Galactosidase
β − Galactosidase
β − HexNAcase
α − Sialidase
α − Fucsidase
β − Xylosidase
57 Gal α1,4 −0.39
31 NeuAc α2,6 −0.51
32 NeuAc α2,3 0.38
52 Gal β1,4 −0.05
33 GlcN β1,3 1.52
16 Man α1,2 −1.00
17 Gal β1,4 0.24
18 GalN β1,4 0.33
8 Man α1,6 −0.19
9 GlcN β1,6 −2.21
58 Gal α1,4 1.25
56 Gal β1,4 −1.07
19 Man α1,2 0.86
10 GlcN β1,4 1.21
11 Man α1,3 −0.67
4 Man α1,6 0.72
34 NeuGc α2,6 −0.90
35 NeuAc α2,6 1.63
36 NeuAc α2,3 0.92
37 NeuGc α2,3 1.10
38 Gal α1,3 2.74
53 Gal β1,4 0.39
39 GlcN β1,3 0.88
59 Gal α1,4 −0.29
20 Fuc α1,6 −0.20
21 Gal β1,4 0.13
22 GalN β1,4 1.04
12 GlcN β1,2 1.75
40 NeuAc α2,6 2.46
41 NeuAc α2,3 0.31
42 Gal α1,3 2.01
60 Gal α1,4 −0.58
43 NeuAc α2,3 1.89
44 Gal α1,3 2.79
23 NeuAc α2,6 0.49
24 Gal β1,4 0.54
25 Fuc α1,3 −0.15
26 Gal β1,3 0.67
13 GlcN β1,4 2.03
55 Gal β1,4 0.61
2 Fuc α1,6 3.43
5 GlcN β1,4 4.44
1 Man β1,4 GlcN β1,4 GlcN−PA 7.20
3 Fuc α1,3 −1.96
6 Xyl β1,2 0.09
45 NeuGc α2,6 −3.06
46 NeuAc α2,6 −1.88
47 NeuAc α2,3 0.31
48 Gal α1,3 −2.62
54 Gal β1,4 1.24
49 GlcN β1,3 1.30
50 Fuc α1,2 8.87
61 Gal α1,4 −2.00
27 Fuc α1,6 −0.75
28 Gal β1,4 1.33
29 GalN β1,4 0.98
14 GlcN β1,2 0.06
7 Man α1,3 −0.53
51 Glc α1,3 0.80
30 Man α1,2 −0.40
15 Man α1,2 −0.48

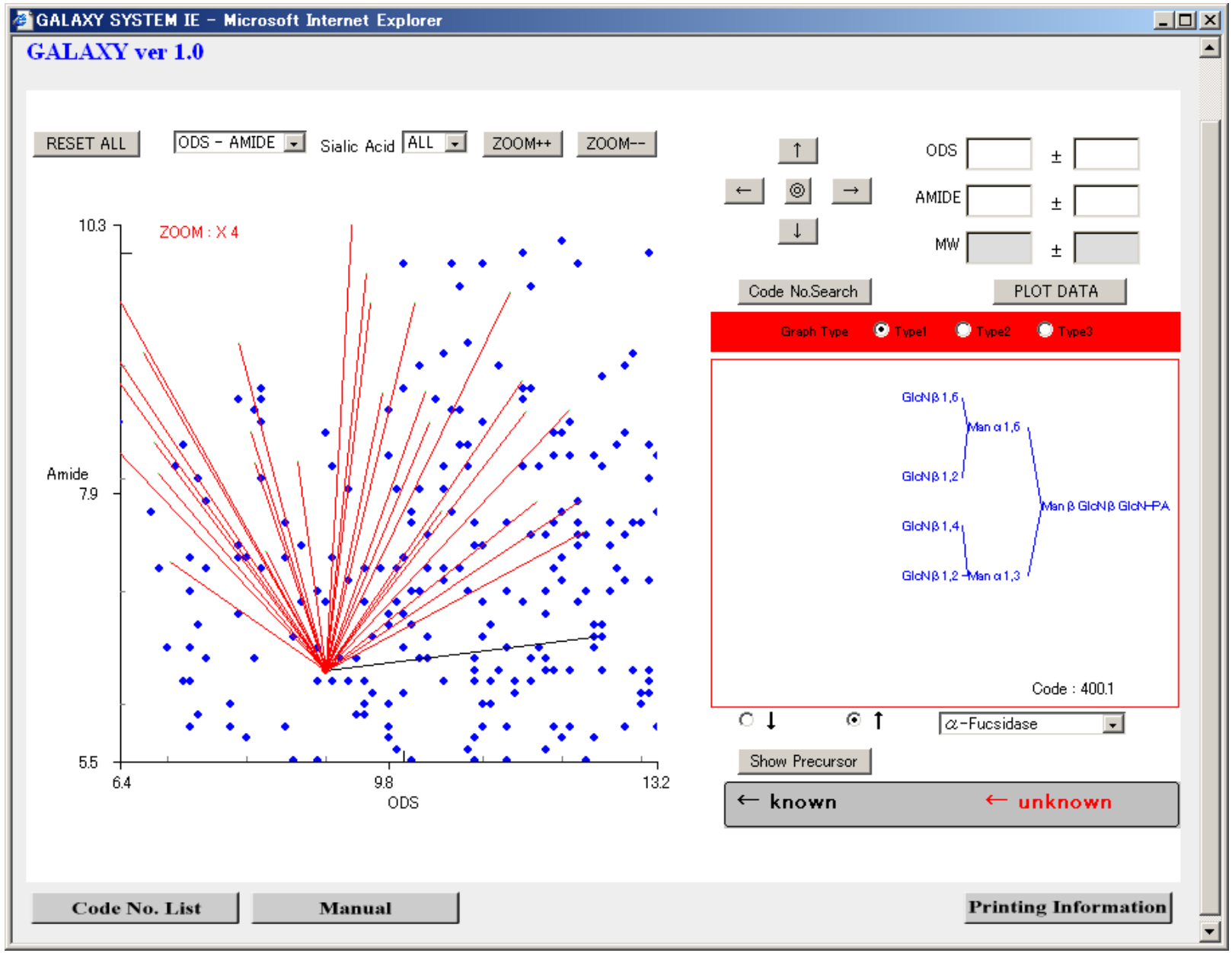
Display of products resulting from glycosidase treatments



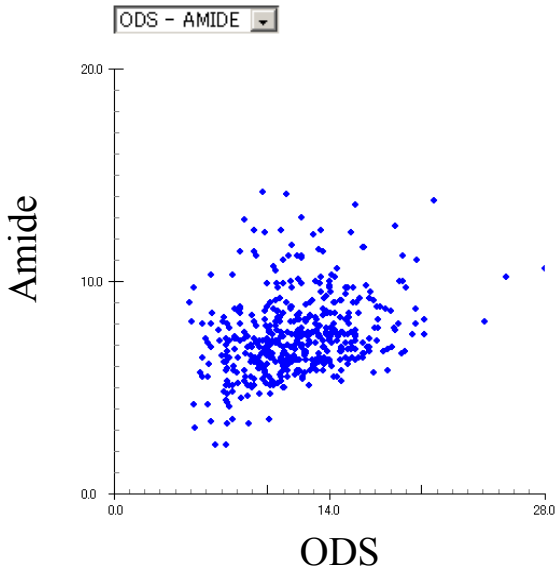
Prediction of digestion precursors of a selected N-glycan



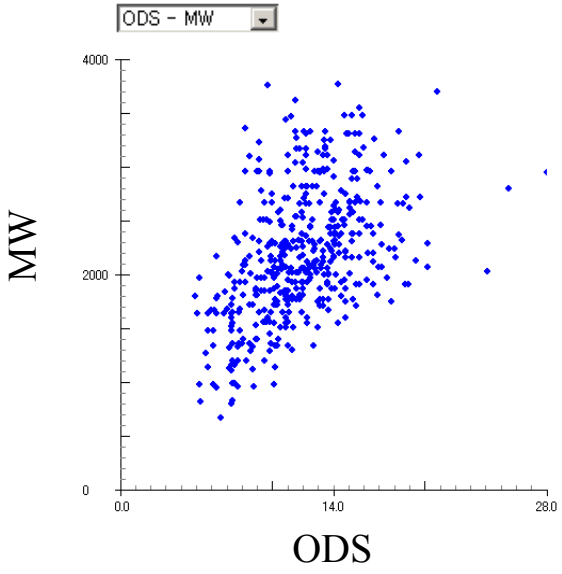
Prediction of digestion precursors of a selected N-glycan



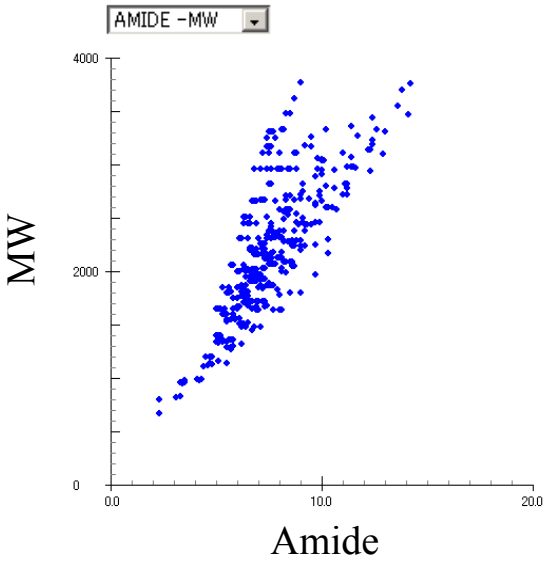
Graph selection from the three types of combination of the axes



ODS-Amide



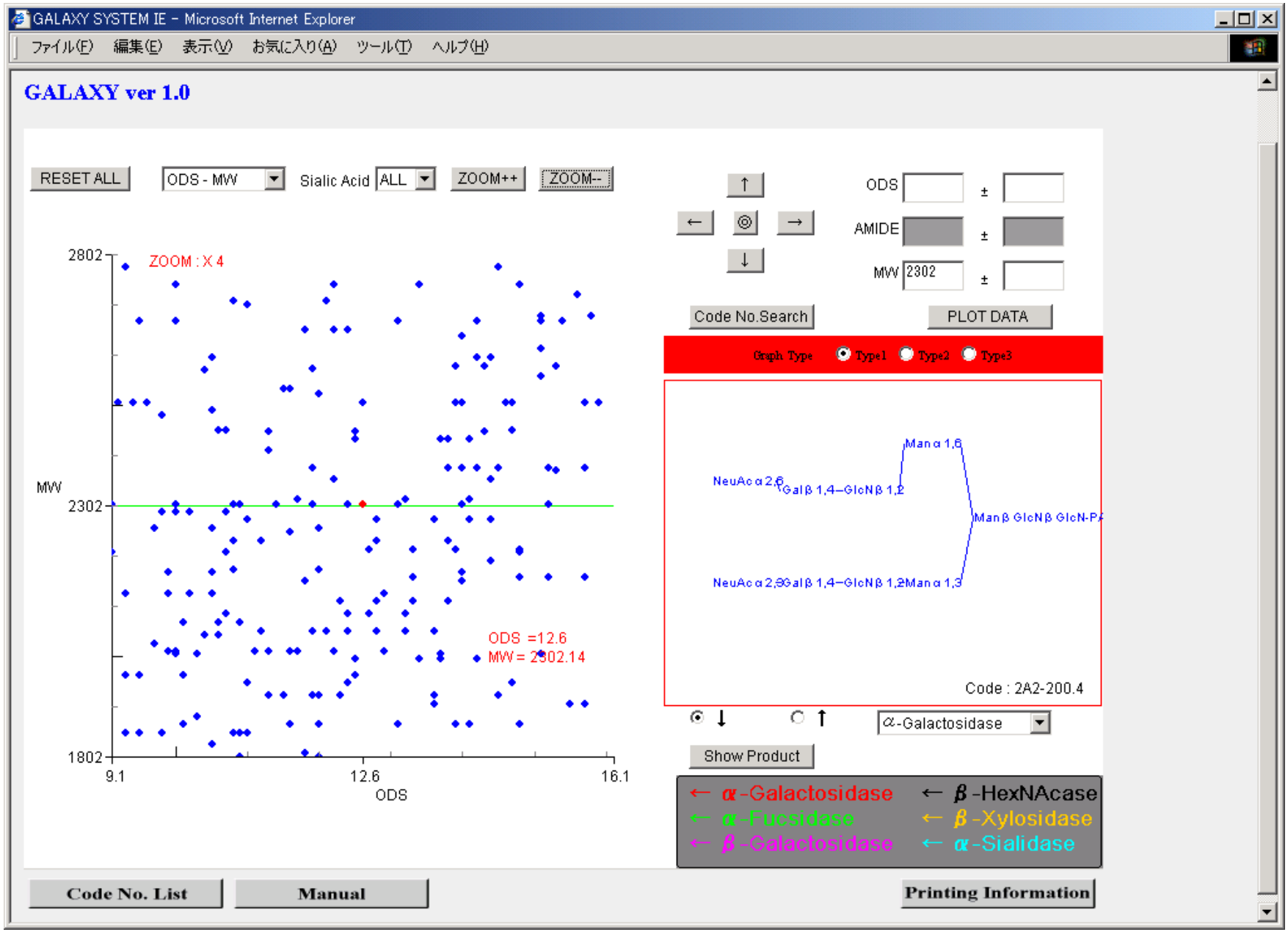
ODS-MW



Amide-MW

Trends Glycosci. Glycotech. **15, 235-251 (2003)**

MW 2302 ?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NeuAcα2,6Galβ1,4-GlcNβ1,2
Manβ GlcNβ GlcN-PA
NeuAcα2,3Galβ1,4-GlcNβ1,2Manα1,3
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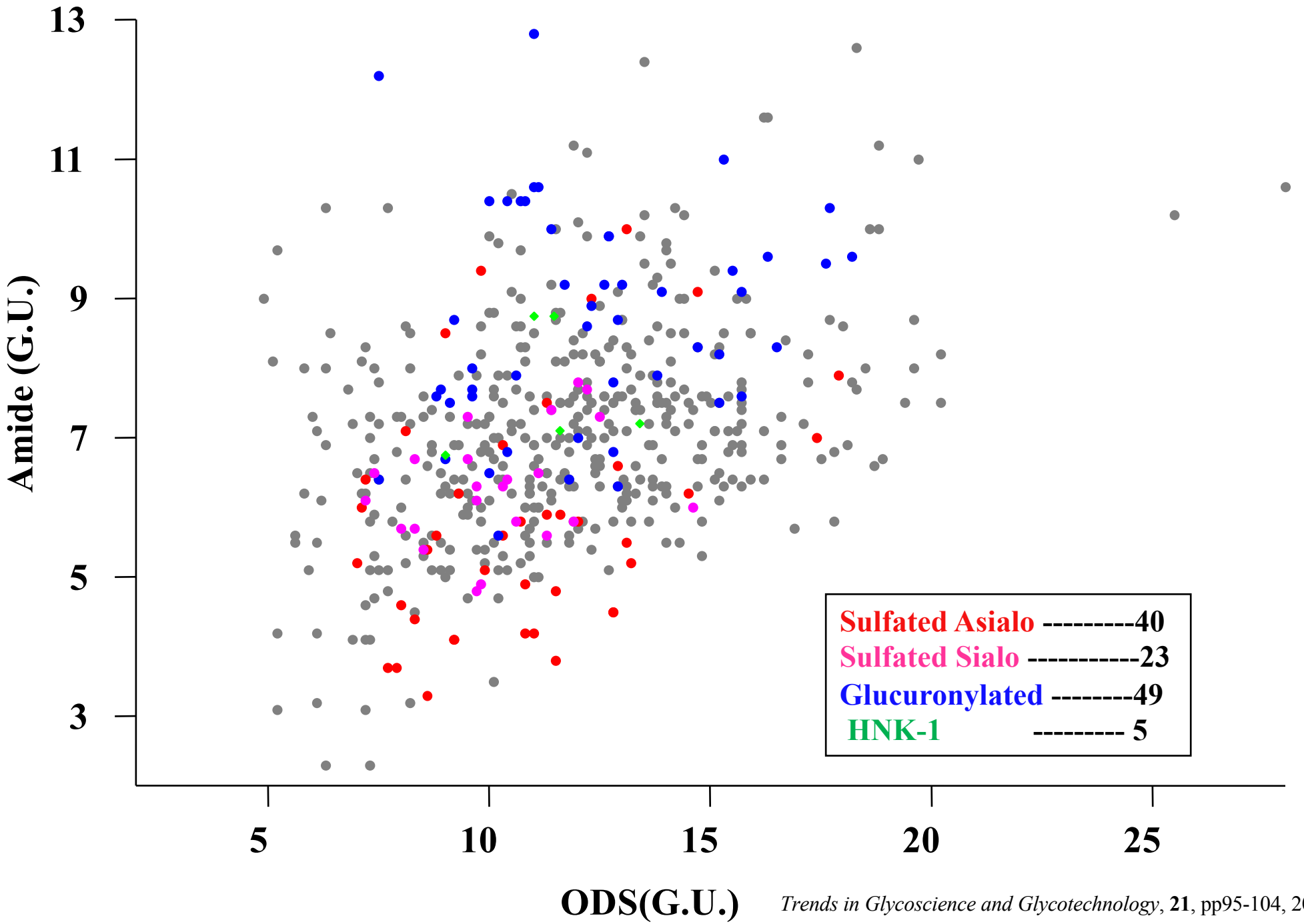
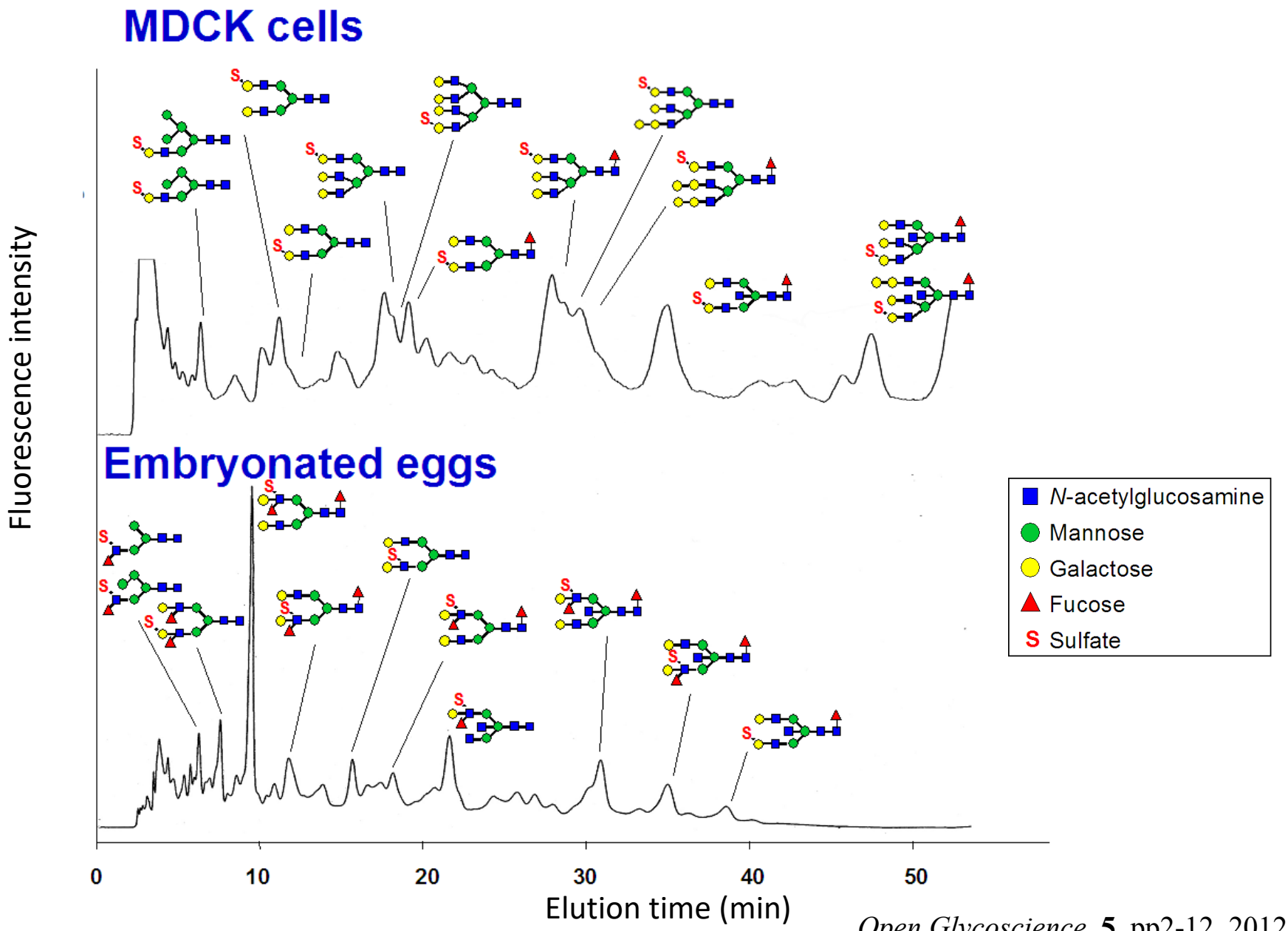
← α-Fucsidase
← β-Xylosidase
← β-Galactosidase
← α-Sialidase
Code No. List
Manual
Printing Information

Expanded HPLC map including sulfated oligosaccharides



Trends in Glycoscience and Glycotechnology, **21**, pp95-104, 2009

N-glycosylation profiles derived from two different influenza A viruses grown in MDCK cells and embryonated eggs



Open Glycoscience, **5**, pp2-12, 2012

Contents

I. Introduction

- Chemical character

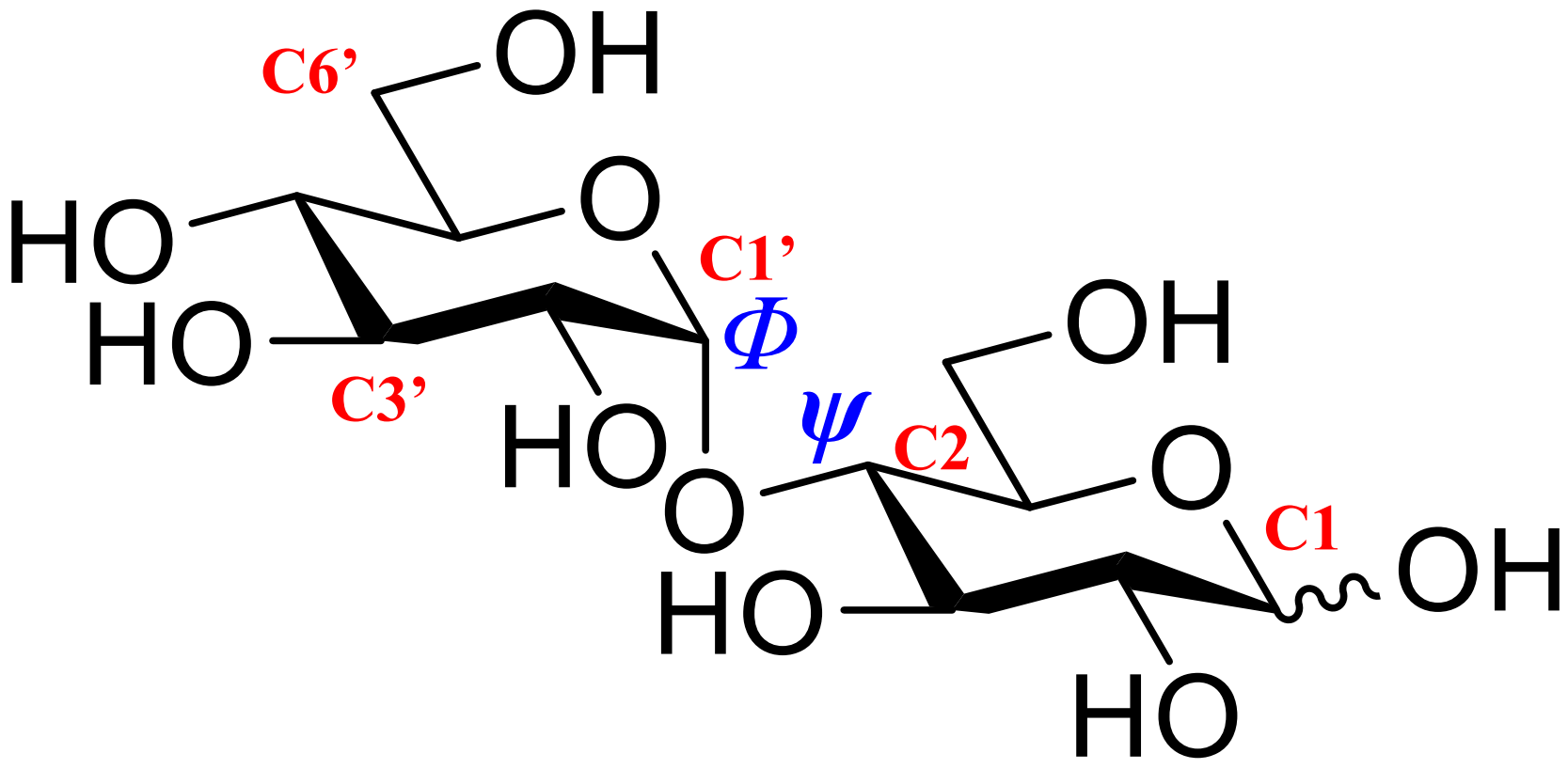
II. Sequence analysis

- Released glycan analysis
- Mass spectrometric analysis
- HPLC mapping method

III. Conformational analysis

- **Digest for conformational analysis**
- **Our recent topics**

Conformation analysis



Conformations of saccharide linkages- information available

X-ray crystallography –

Most oligosaccharides and glycoproteins either do not crystallize or give no resolvable electron density for the glycan. Glycans that can be seen are incomplete.

→ average properties of linkages

Nuclear Magnetic Resonance Spectroscopy –

Experimental structural parameters (inter-nuclear distances and torsion angles) averaged on a msec timescale.

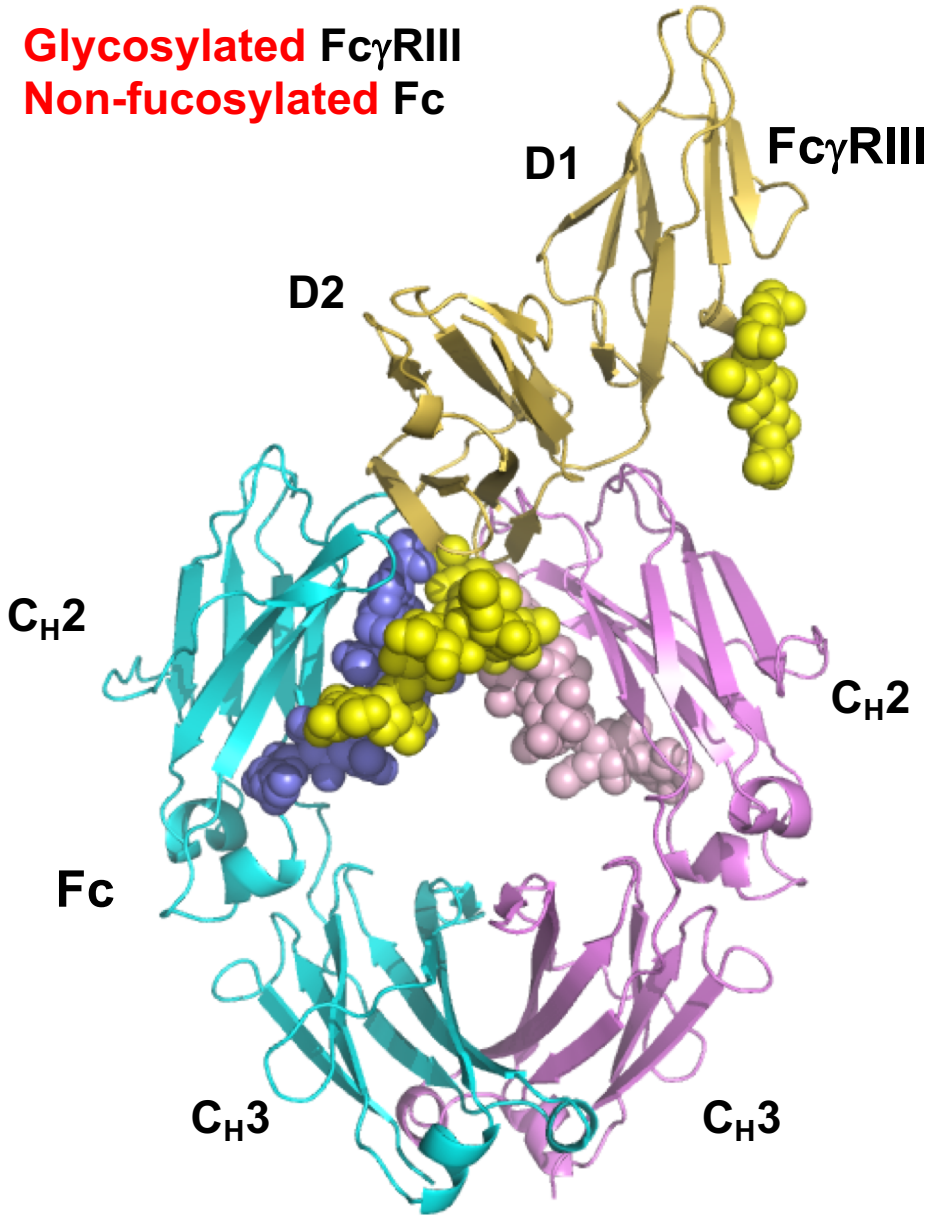
→ a single well-defined conformation as an average structure.

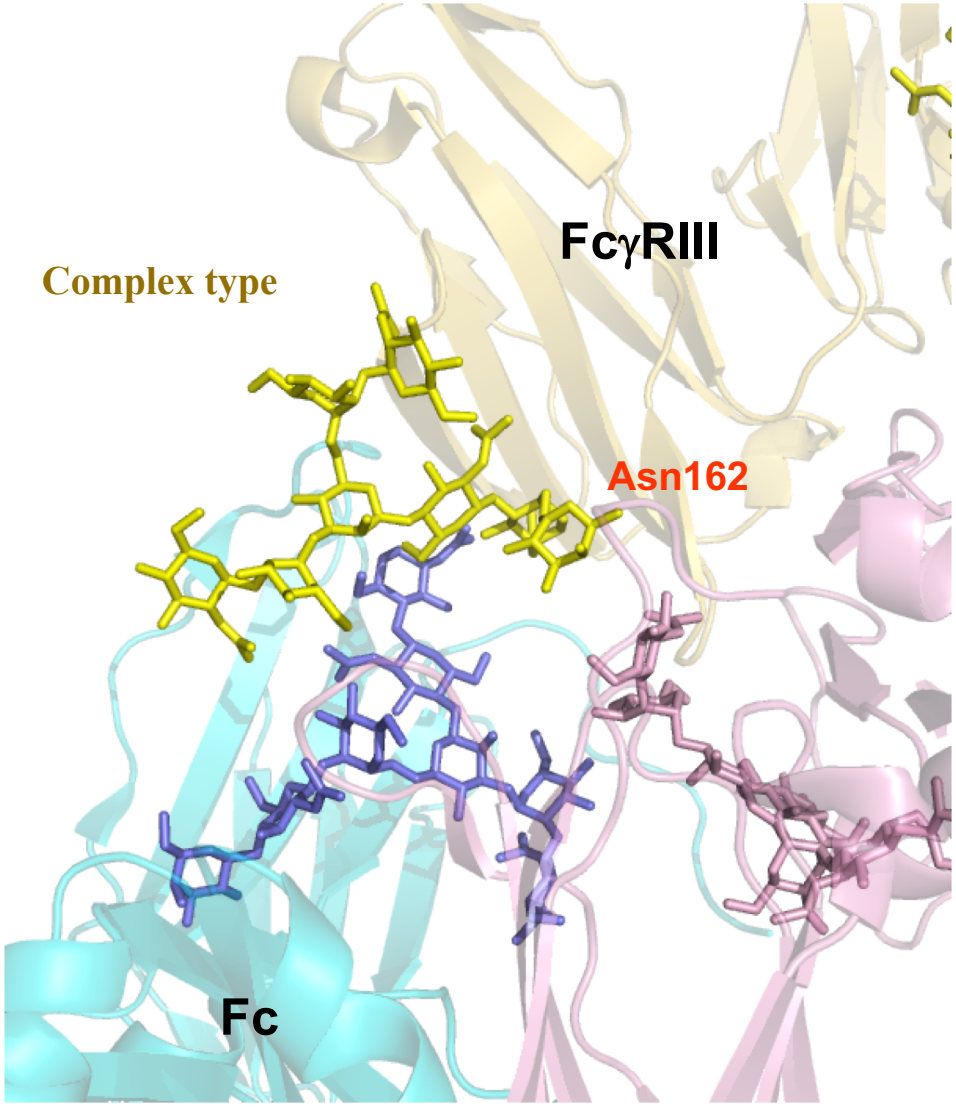
Molecular Dynamics Simulations –

Theoretical dynamic structures on a nsec timescale.

→ a conformational amassable of the structure if it is assumed that the theory is correct.

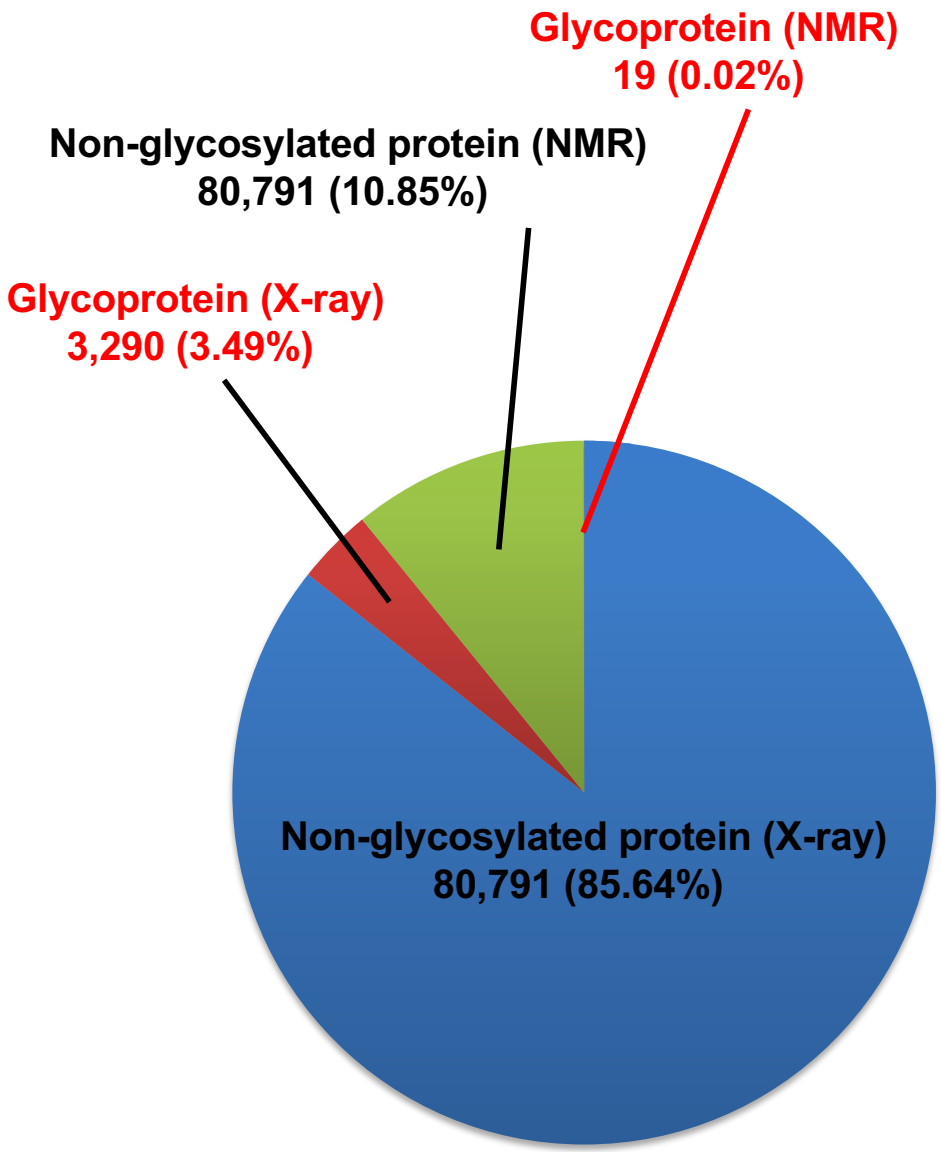
Crystal structures of IgG1-Fc/FcγRIII complex

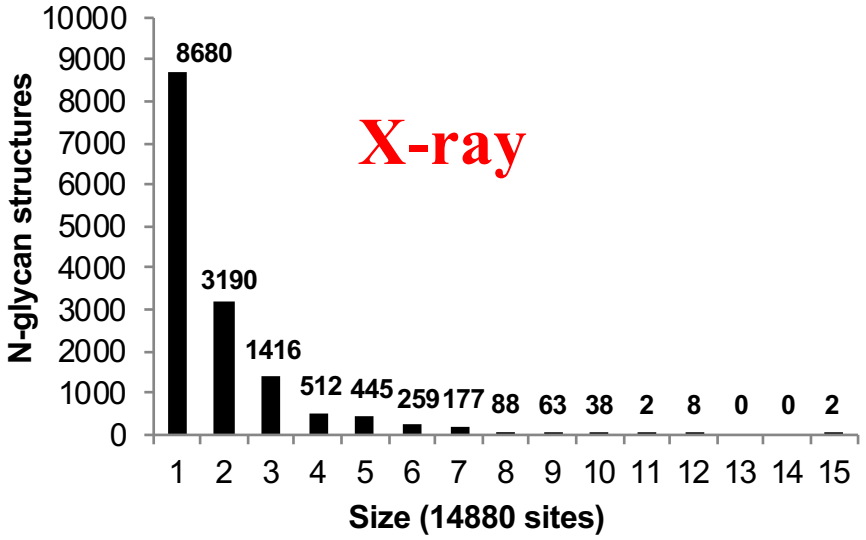


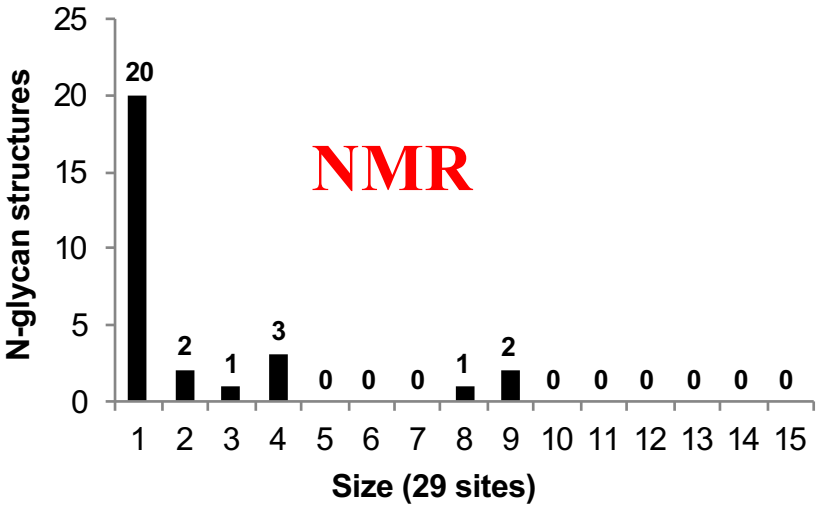


Mizushima *et al.* Genes Cells. 2011 Nov;16(11):1071-80. doi: 10.1111/j.1365-2443.2011.01552.x.

Statistics of N-linked glycoproteins from PDB (94,336 structures, 2013.10.02)

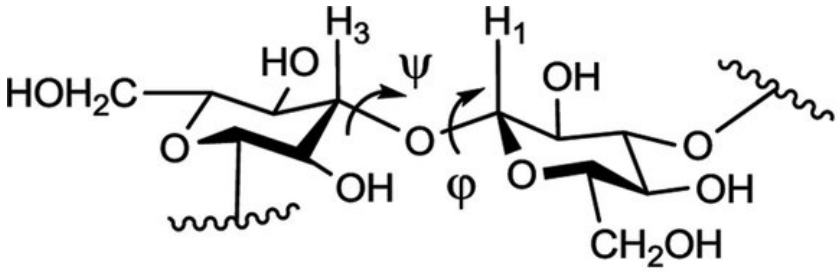






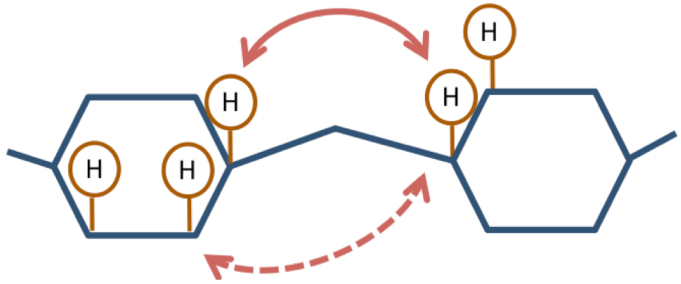
Nuclear Magnetic Resonance Spectroscopy

J coupling :Dihedral angles

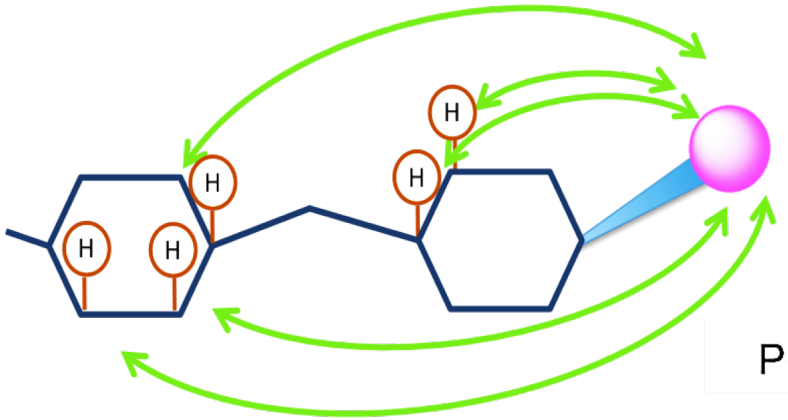


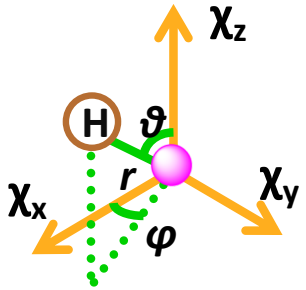
$$\left.\begin{array}{l} J\varphi = {}^3J(C_3\text{-}H_1) \\ J\psi = {}^3J(C_1\text{-}H_3) \end{array}\right\} \quad {}^3J(\text{C-H}) = 5.5\cos^2\theta - 0.7\cos\theta + 0.6$$

Nuclear Overhauser effect (NOE) < 5 Å



Pseudocontact Shift (PCS) < 40 Å





$$\text{PCS} = \frac{1}{12\pi \cdot r^3}\left[\Delta\chi_{ax}(3\cos^2\theta - 1) + \frac{3}{2}\Delta\chi_{rh}\sin^2\theta \cdot \cos 2\phi\right]$$

MD simulation

Multiscale modeling of glycosaminoglycans from disaccharide to polysaccharide is necessitated by their size and heterogeneity

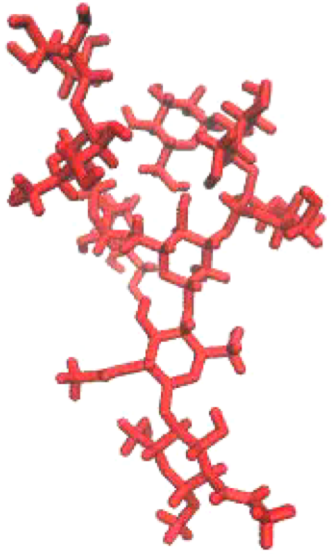
$$E = \sum_{bonds} k_b(l - l_0)^2 + \sum_{angles} k_a(\theta - \theta_0)^2 + \sum_{torsions} \frac{V_n}{2}[1 + cos(n\emptyset - \emptyset_0)]$$

Harmonic oscillator-like bonding, angular, torsional terms

$$+ \sum_{j=1}^{N-1} \sum_{i=j+1}^{N} \varepsilon_{i,j} \left[\left(\frac{\gamma_{0ij}}{\gamma_{ij}} \right)^{12} - 2 \left(\frac{\gamma_{0ij}}{\gamma_{ij}} \right)^{6} \right]$$ van der Waals

$$+ \sum_{j=1}^{N-1} \sum_{i=j+1}^{N} \frac{q_i q_j}{4\pi\varepsilon_o \gamma_{ij}}$$ electrostatic

$$+ \sum_{j=1}^{N-1} \sum_{i=j+1}^{N} \left[\frac{C_{ij}}{\gamma_{ij}^{12}} - \frac{D_{ij}}{\gamma_{ij}^{10}} \right]$$ hydrogen bonding



Paramagnetic NMR-Validated Molecular Dynamics Simulation

Experiment

Nuclear Magnetic Resonance (NMR)

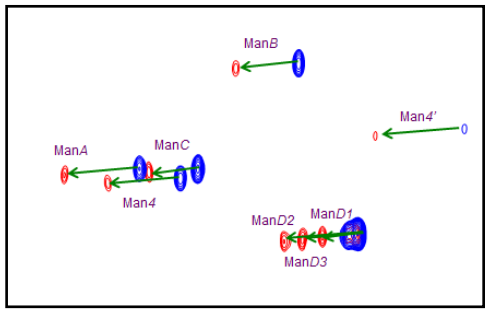
Information averaged over dynamic conformational ensemble

Computational Calculation

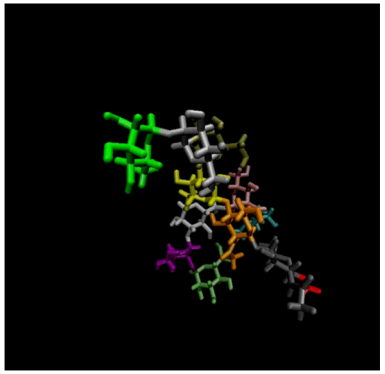
Molecular Dynamics Simulation (MD)

Results depending on force field, initial state and simulation time

3D Structural Information



3D Structural Model



Compare

Disagree

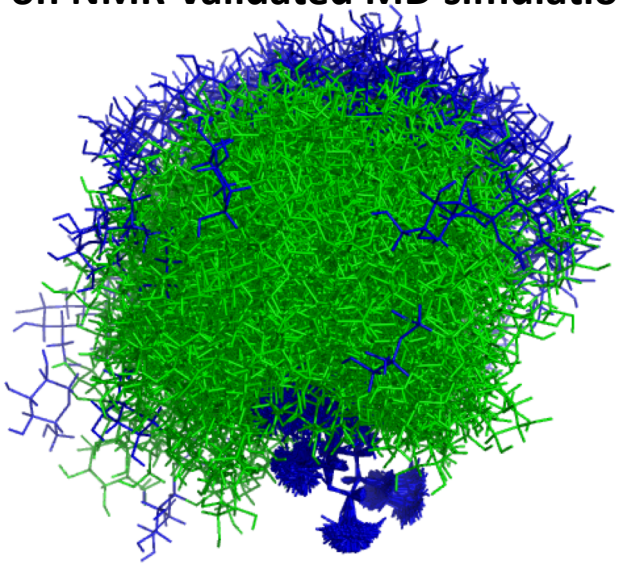
Agree

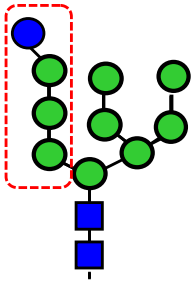
Validated conformational ensemble

The combination between NMR and MD data enable us to obtain validated conformational ensemble.

Conformational dynamics of GM9 dodecamer

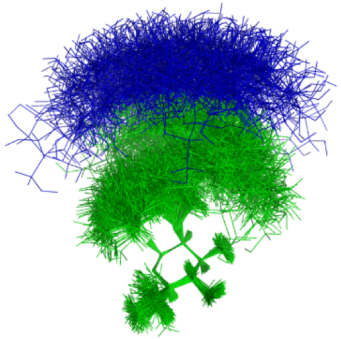
GM9 conformational ensemble based on NMR-validated MD simulation

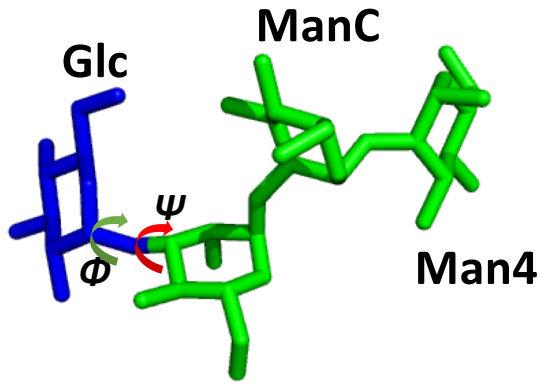




Glc-ManD1-ManC-Man4

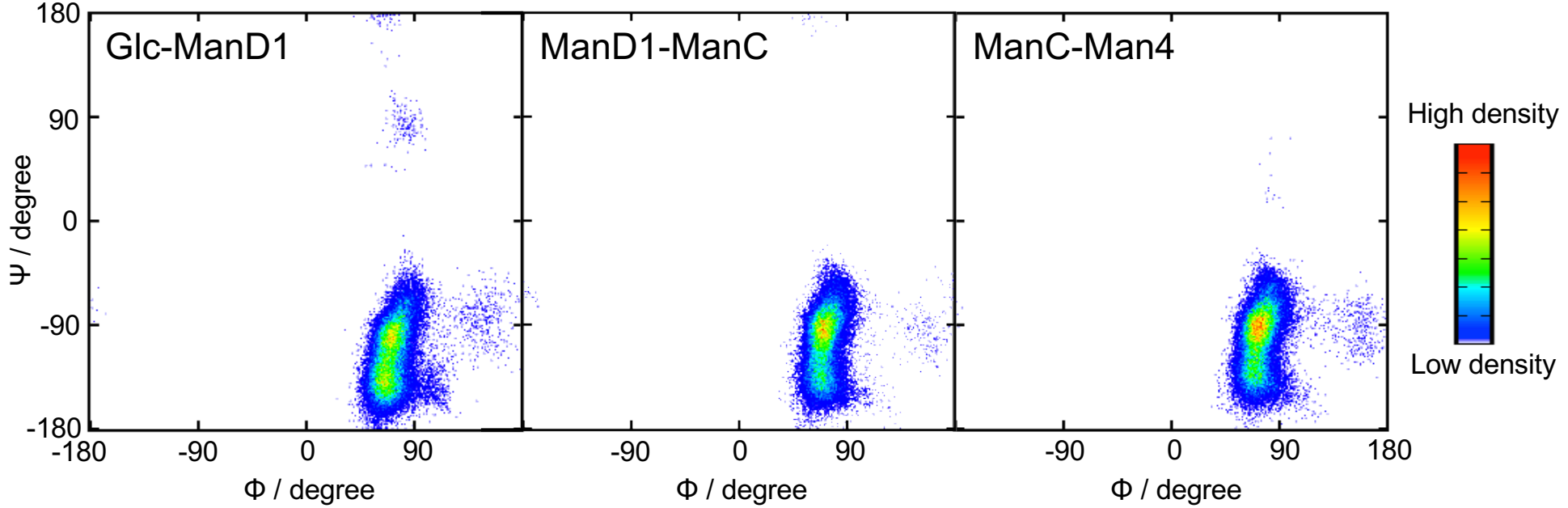
Conformational dynamics of trisaccharide on GM9





Φ : O_5-C_1-O_1-C'_X
Ψ : C_1-O_1-C'_X-C'_{X-1}

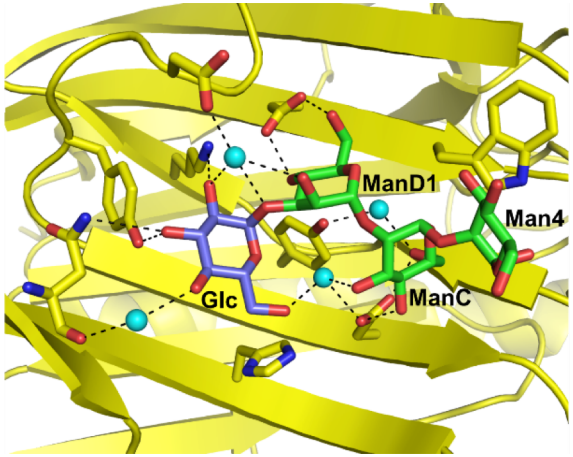
Density maps of glycosidic linkage torsion angles



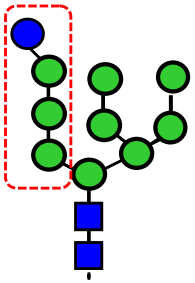
Suzuki et. al. Chembiochem . 2017 Feb 16;18(4):396-401. doi: 10.1002/cbic.201600595

The carbohydrate recognition by the ER chaperone calreticulin involves an induced-fit mechanism

3D-structural models of the sugar-binding mode of calreticulin

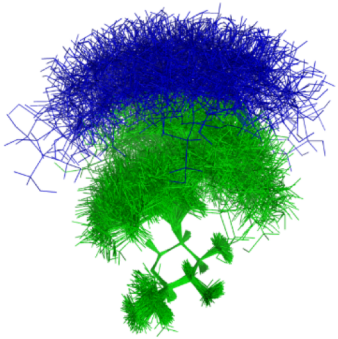


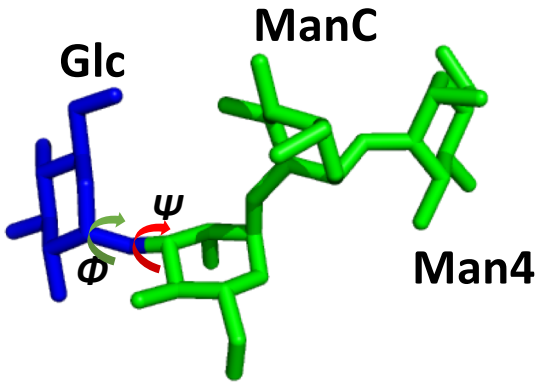
Kozlov, G.; et al, J. Biol. Chem. 2010, 285, 38612-38620



Glc-ManD1-ManC-Man4

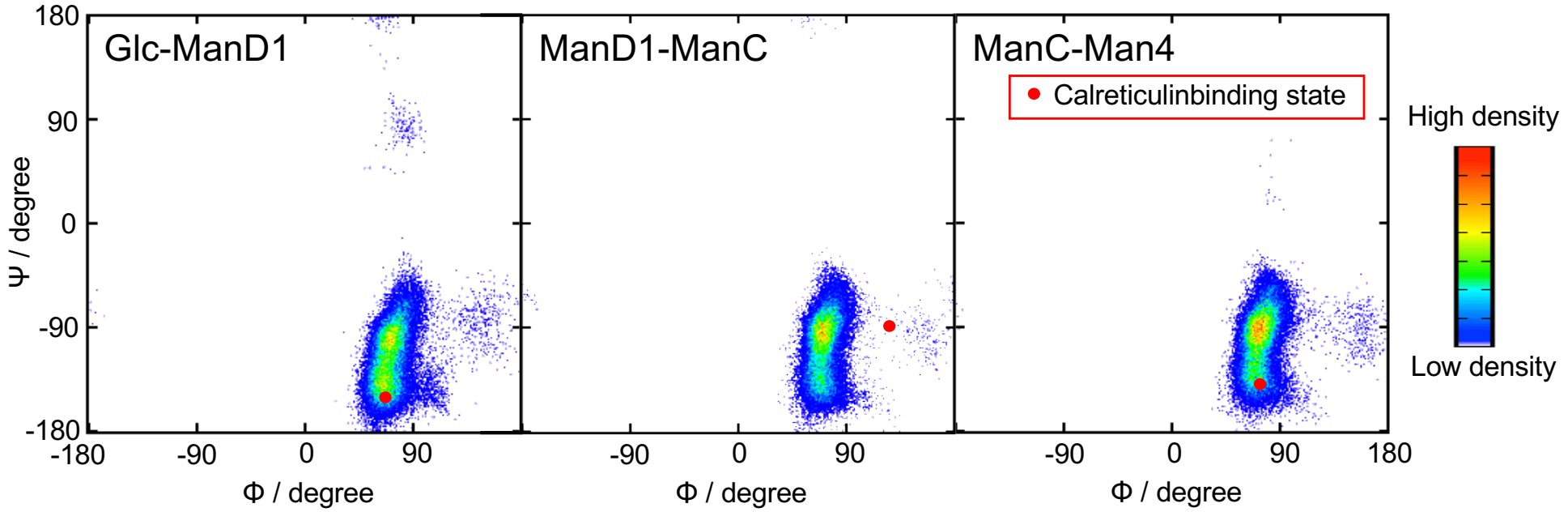
Conformational dynamics of trisaccharide on GM9





Φ : O_5-C_1-O_1-C'_X
ψ : C_1-O_1-C'_X-C'_{X-1}

Density maps of glycosidic linkage torsion angles



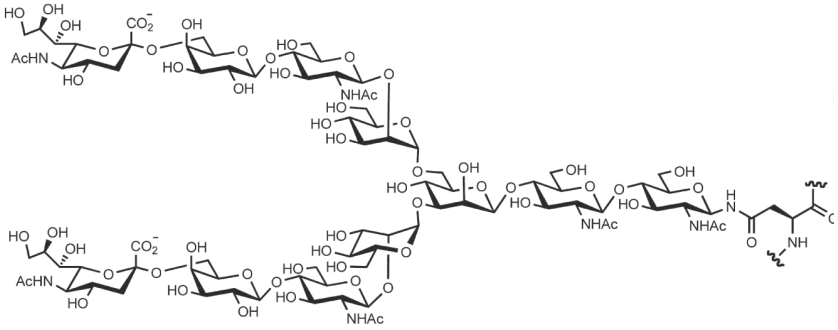
Suzuki et. al. Chembiochem . 2017 Feb 16;18(4):396-401. doi: 10.1002/cbic.201600595

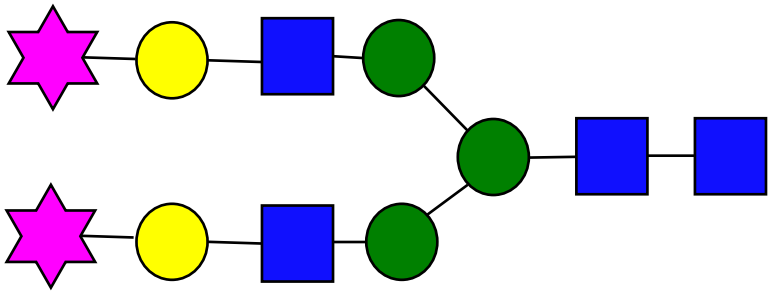
Take home message!

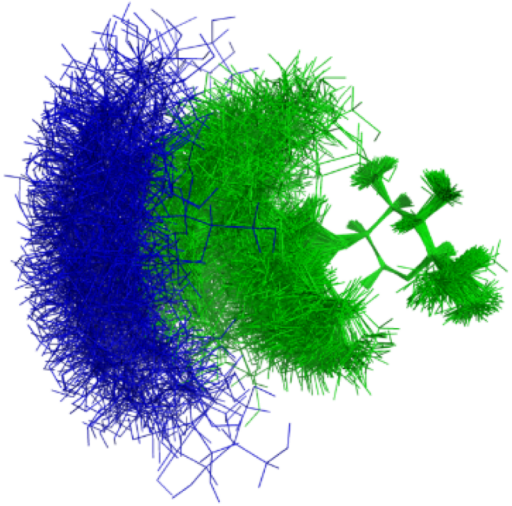
糖鎖の構造解析をした際に、どこまでの詳細構造
をキャラクタライズしているかのかを理解しておくことが重要！

$Hex_5NexNAc_4Sia_2$

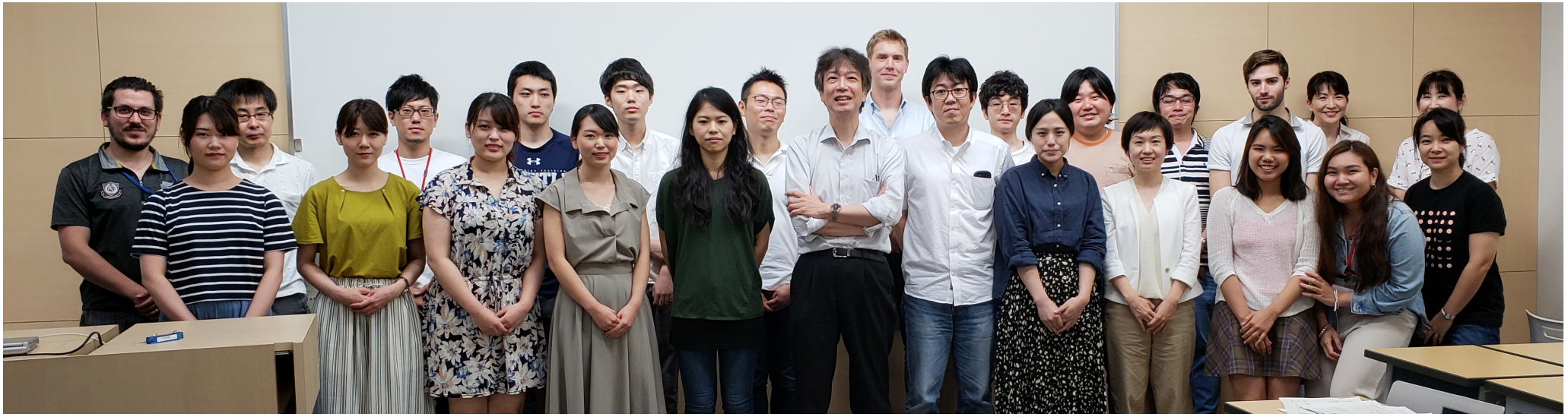
$Gal_2Man_3GlcNAc_4Neu5Ac_2$







Acknowledgement



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