



Functional glycomics: unveiling the role of protein glycosylation

Wei Huang (黄蔚)

*Shanghai Institute of Materia Medica
Chinese Academy of Sciences*

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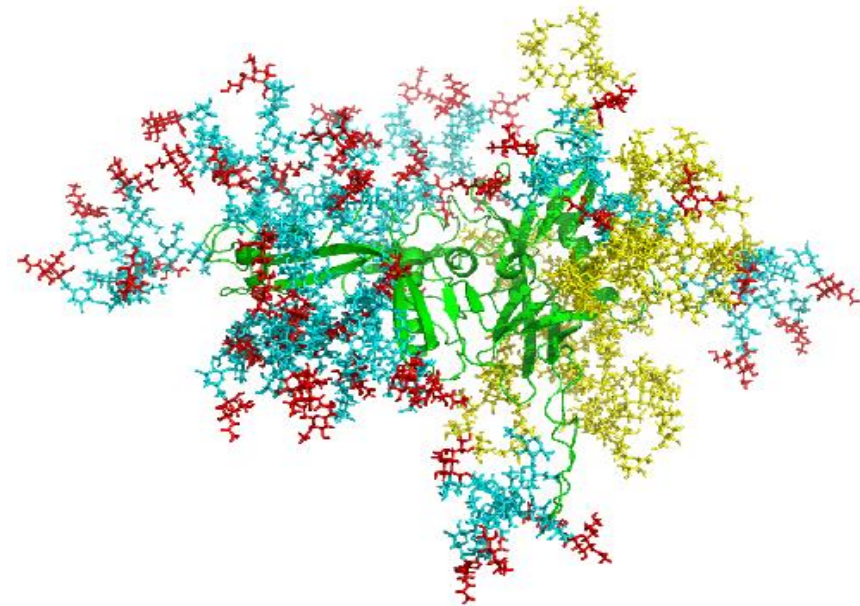
Background

Protein glycosylation

is a most common post-translational modification of protein.

More than 50% mammalian proteins are glycoproteins.

More than 70% clinical therapeutic protein drugs are glycoproteins.



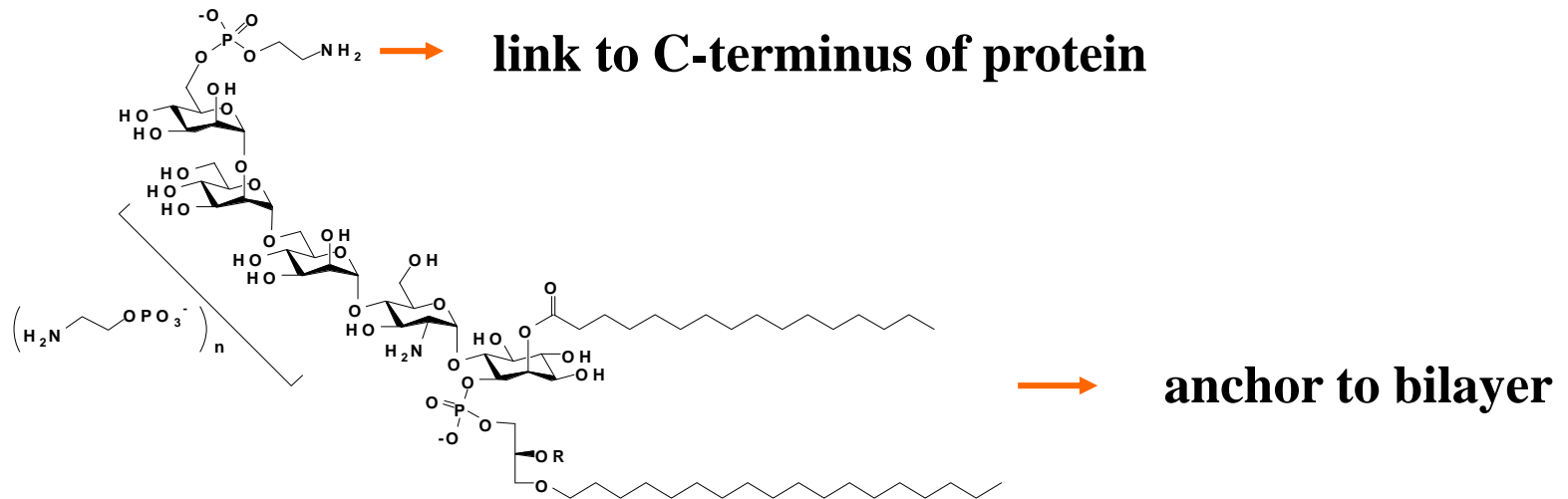
HIV-1 gp120

Protein glycosylation forms

N-glycan: Asn-linked oligosaccharide

O-glycan: Thr/Ser-linked oligosaccharide

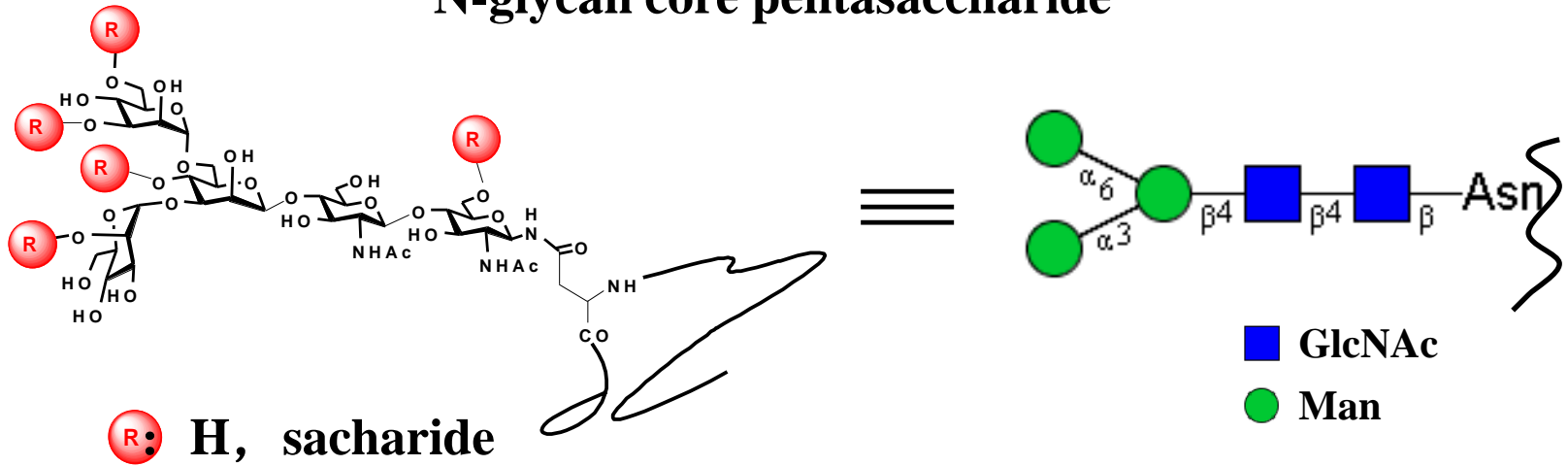
GPI anchor:



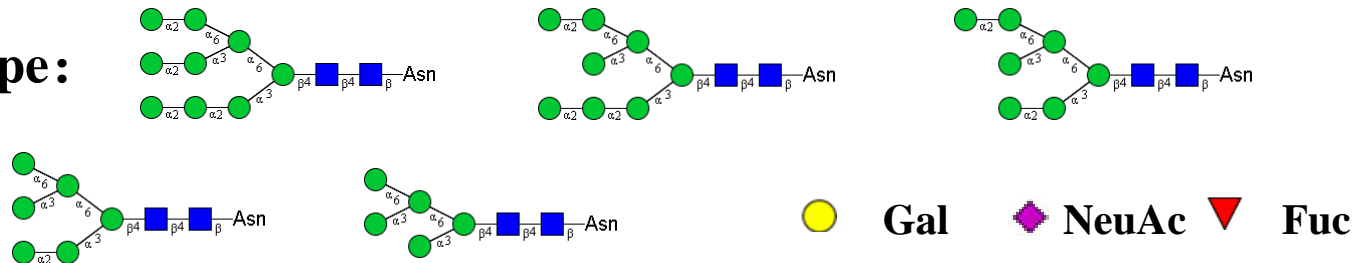
Proteoglycan: protein-linked polysaccharide

N-glycan structures

N-glycan core pentasaccharide

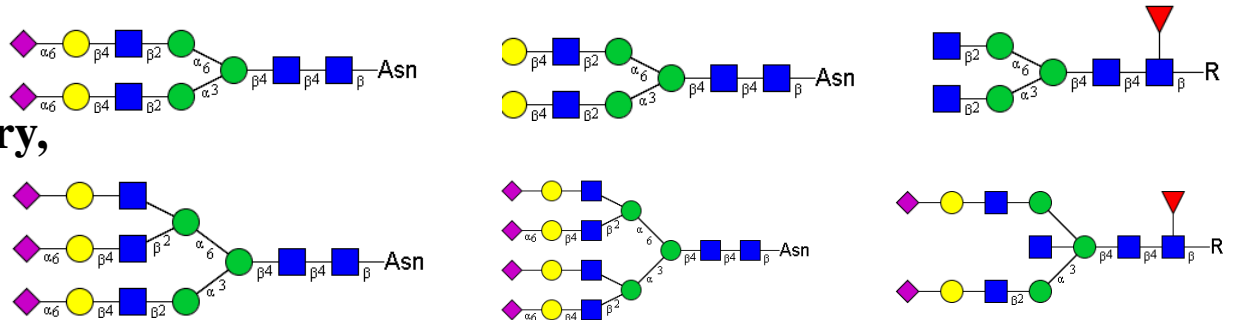


High-mannose type:

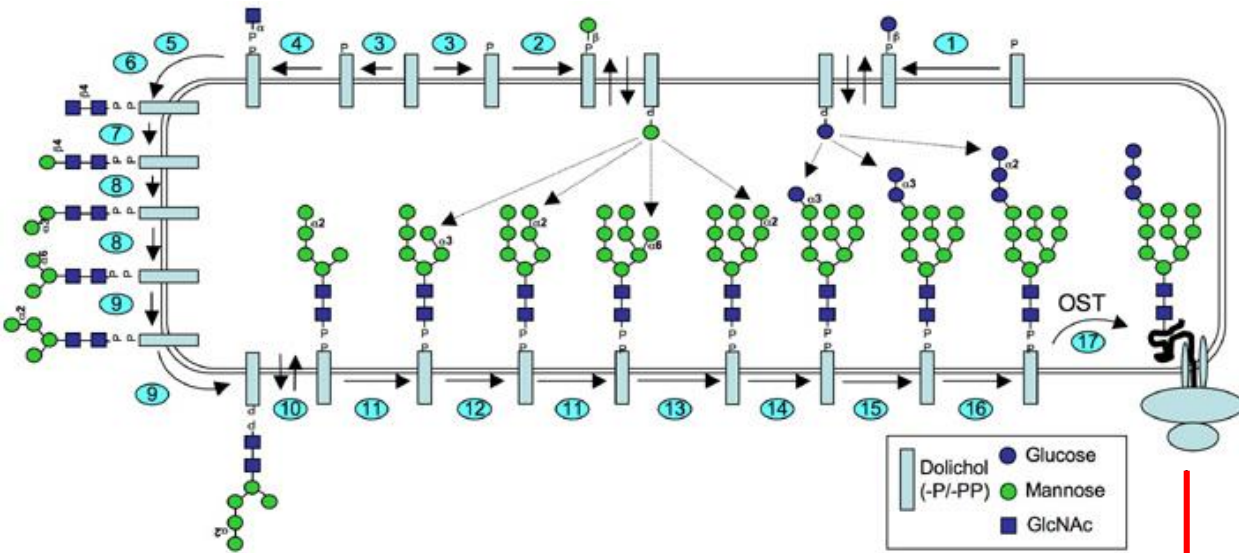


Complex type:

(biantennary, triantennary, tetraantennary, bisecting, fucosylated)

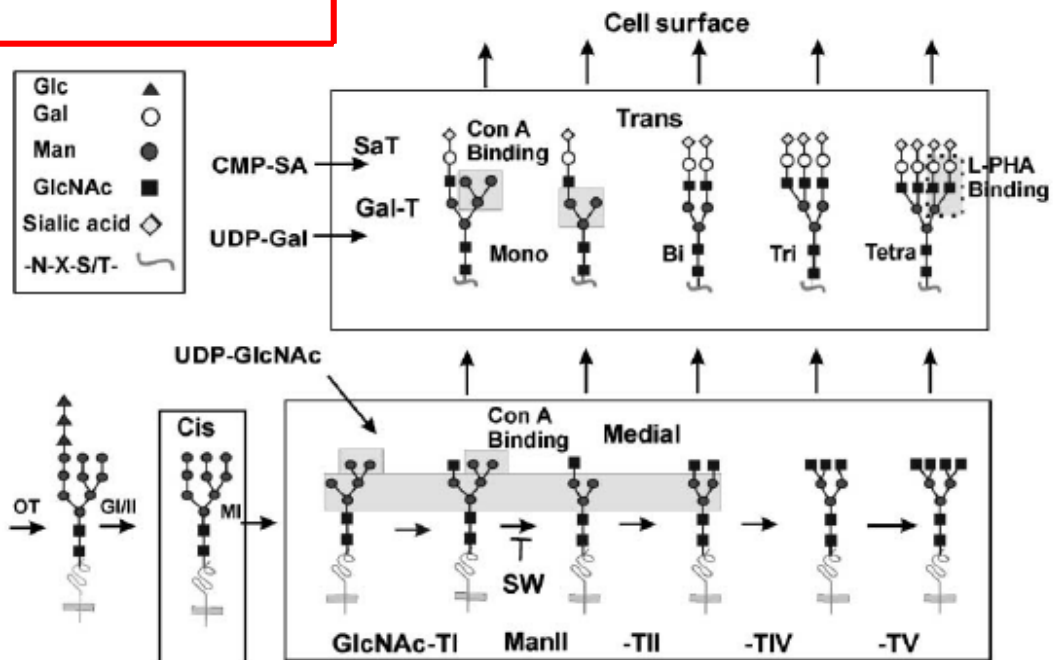


Biosynthesis of N-glycan



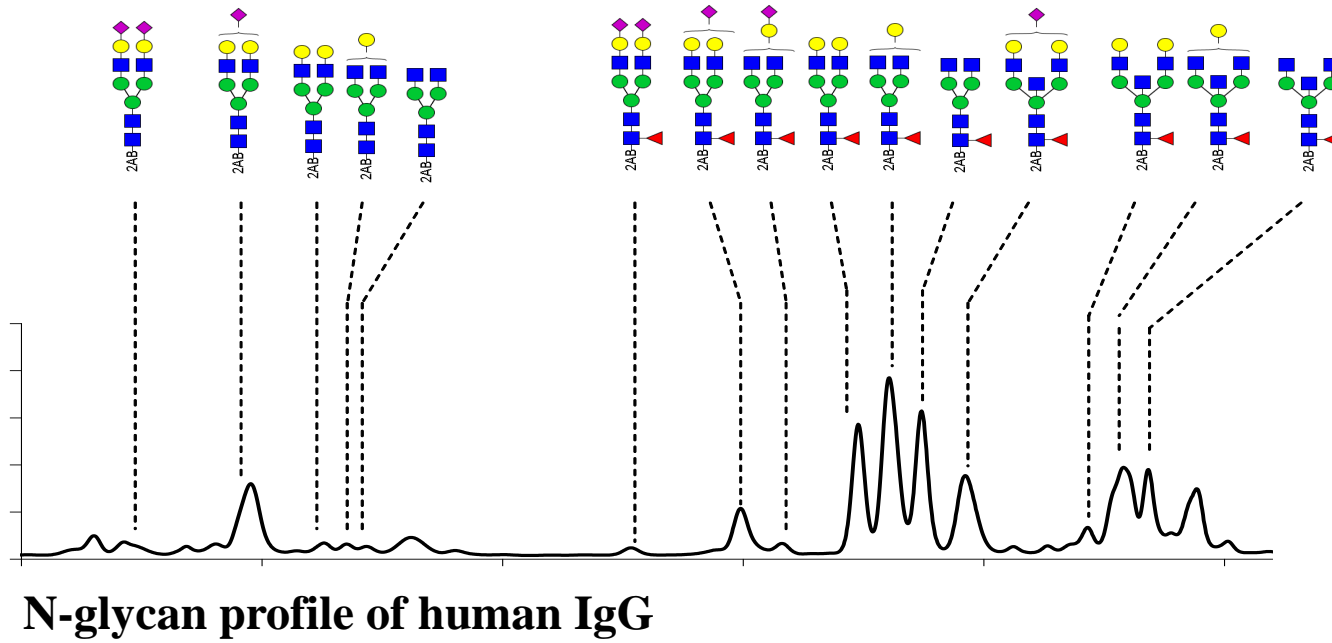
Endoplasmic Reticulum

Golgi



Glycosylation control?

Native glycoprotein: highly heterogeneous



N-glycan profile of human IgG



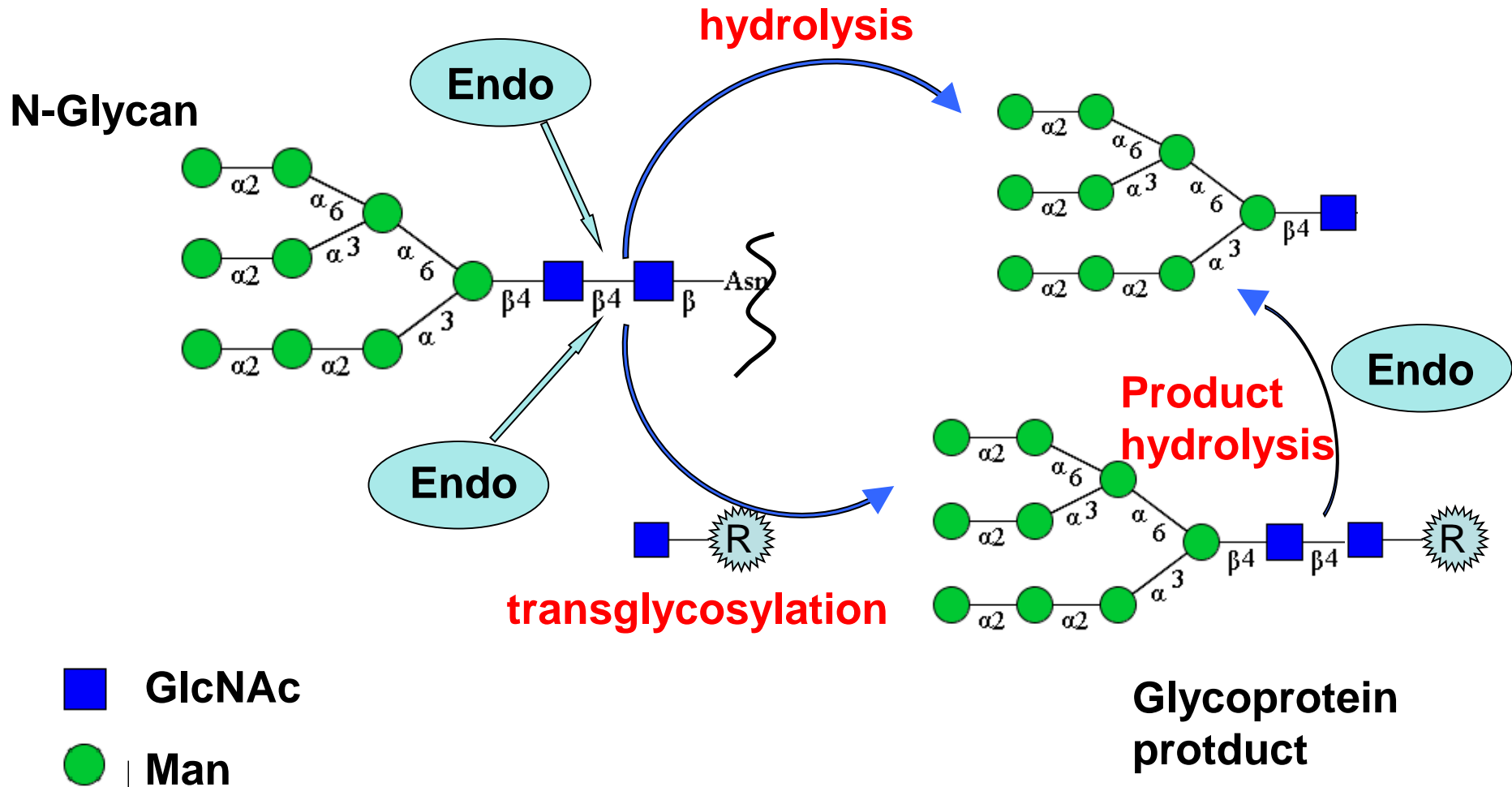
Function

Glycomics



Method

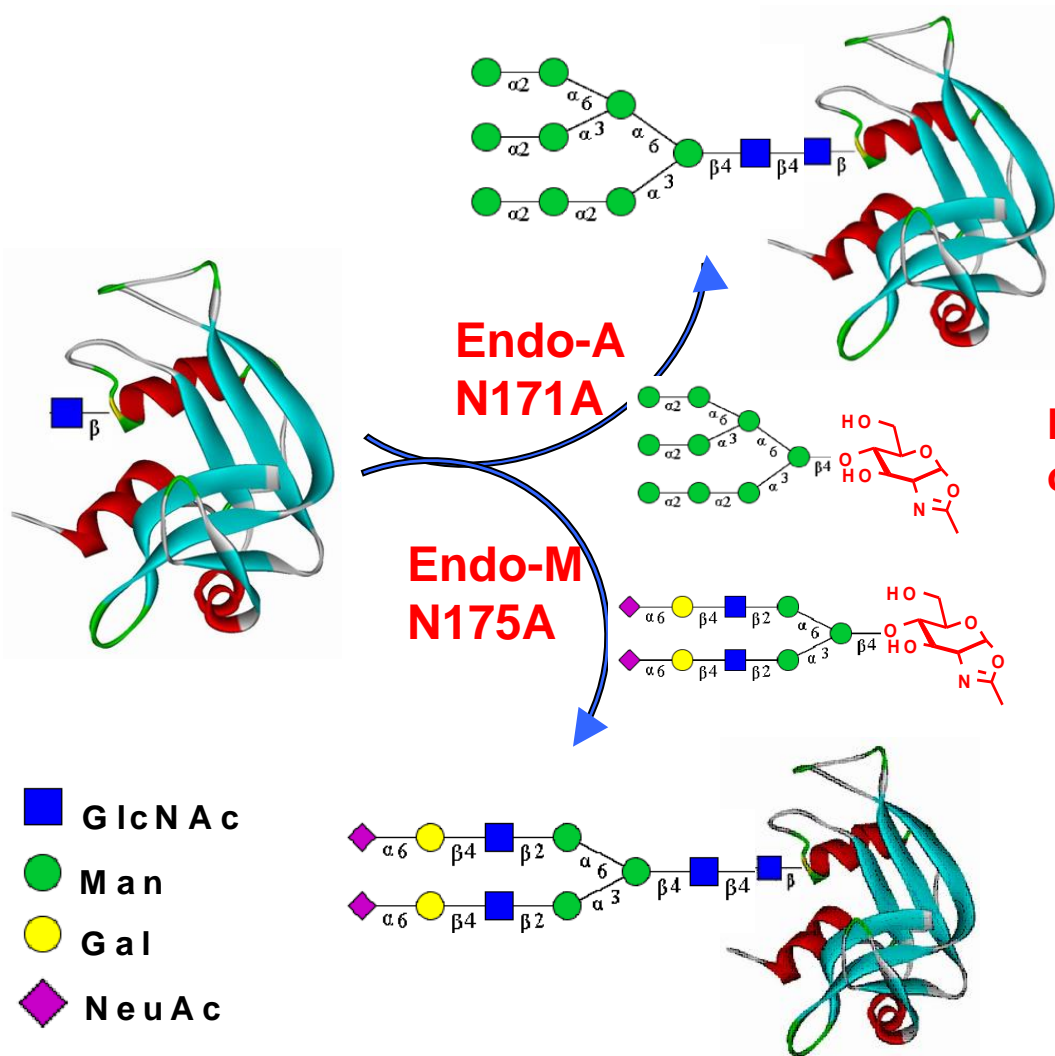
Transglycosylation activity of Endo-glycosidases



Endo-glycosidase for glycan remodeling

Glycosynthases:

Mutants of EndoA/M, lack hydrolytic activity but possess transglycosylation activity using oxazolines

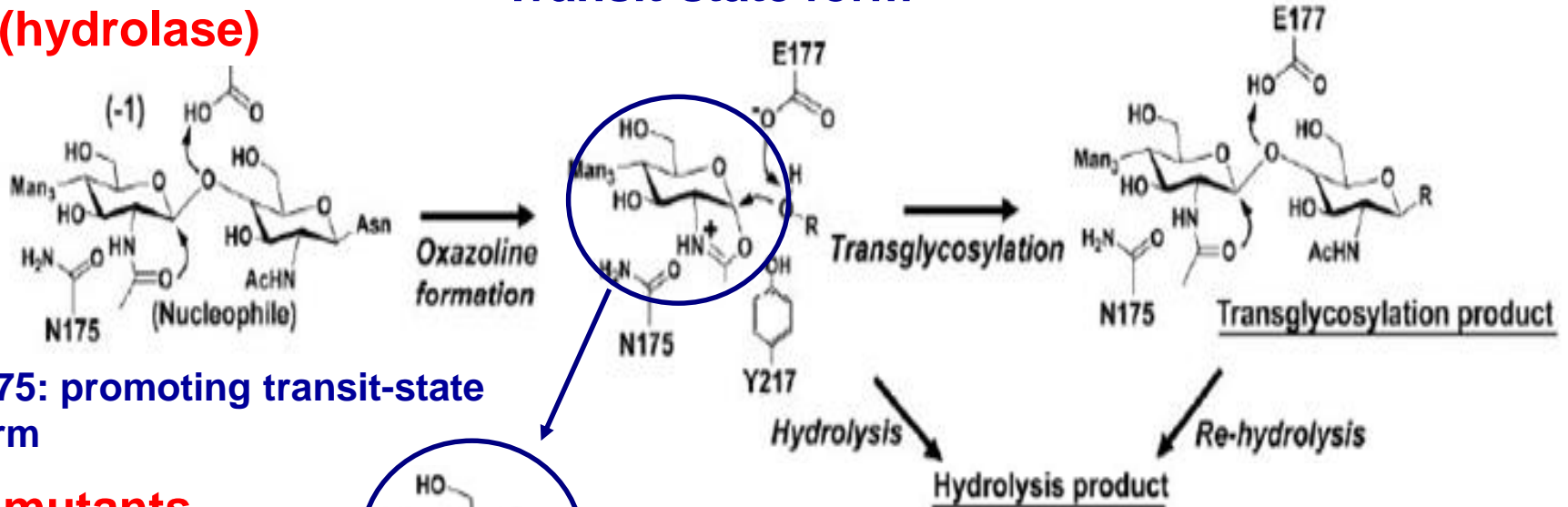


J. Biol. Chem. **2008**, 283, 4469
J. Am. Chem. Soc. **2009**, 131, 2214
J. Biol. Chem. **2010**, 285, 511
ChemBioChem **2010**, 12, 932

Mechanism of glycosynthase-glycan oxazoline system

**A: wide-type
(hydrolase)**

Transit-state form



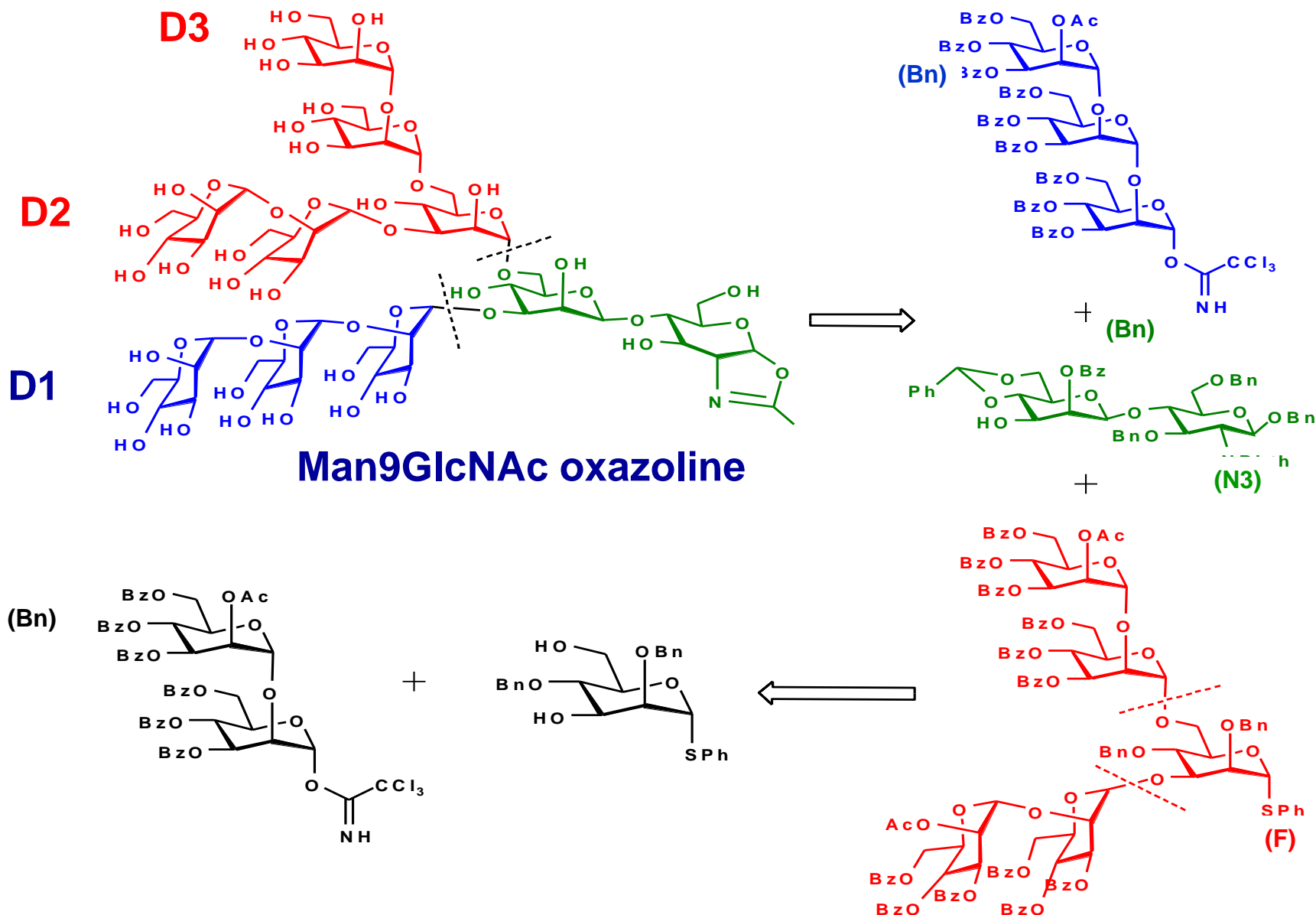
N175: promoting transit-state form

**B: mutants
(glycosynthase)**



N175A: lost hydrolytic activity

Total synthesis of Man9GlcNAc oxazoline (~70 steps)



Nature resource N-glycan



300 egg yolks (3.3 L)

- i) added 1.5 L water, stirred at rt for 1h
- ii) lyophilization

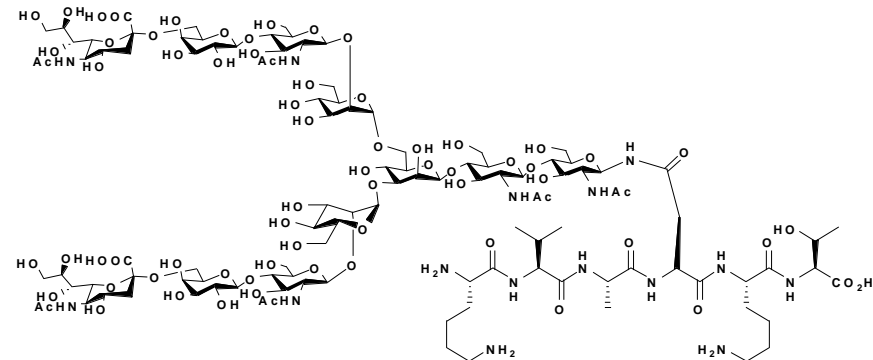
Yolk powder (2.1 Kg)

- i) Et₂O wash (6 L x 2), 70% acetone wash (6 L)
- ii) 40% acetone extraction (3 L x 2)
- iii) drying of extracted solution

Yolk extract powder (36.7 g)

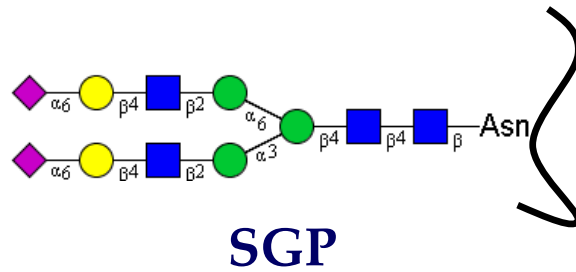
active carbon/celite (2:1) column,
eluted by 25% MeCN

SGP (1.9 g)

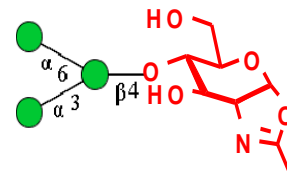
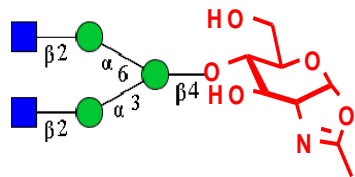
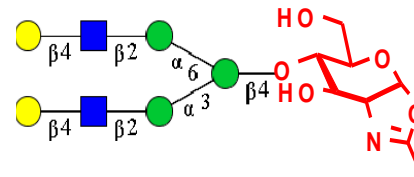
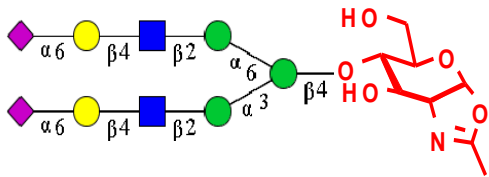


Sialyglycopeptide (SGP)

One-pot synthesis of N-glycan oxazolines from SGP



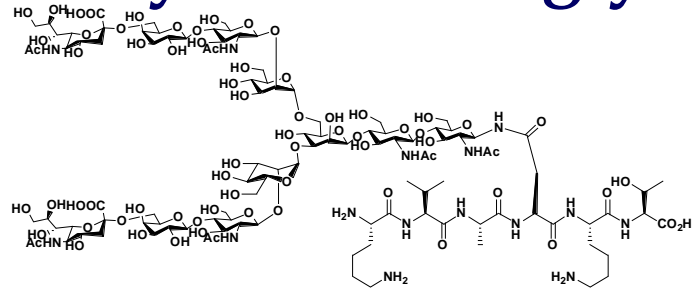
- 1) NeuAcase (Galactosidase, GlcNAcase)
- 2) Endo-M
- 3) DMC/Et3N



Chemoenzymatic synthesis of glycoconjugates

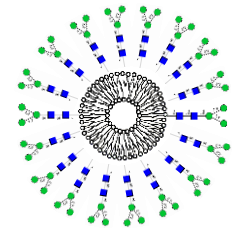
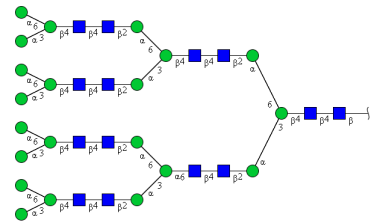
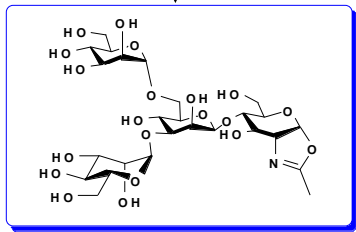


extraction



Chemical total synthesis

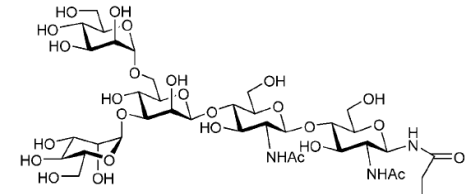
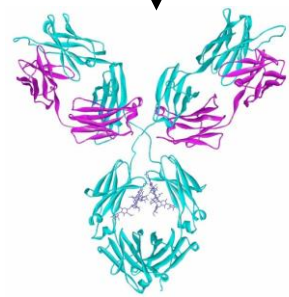
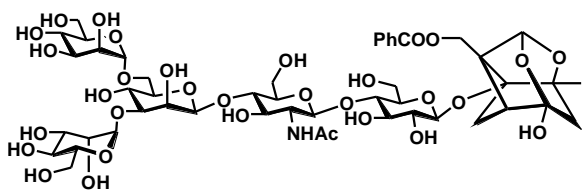
One-pot synthesis



Glyco-clusters
Glyco-dendrimers

N-glycan oxazoline

Glyco-liposome



Ac-W-M-E-W-D-R-E-I-N-N⁺-Y-T-S-L-I-H-S-L-I-E-E-S-Q-N-Q-Q-E-K-N-E-Q-E-L-L-NH₂

Glyco-natural products

Glycoproteins

Glycopeptides

Results

Application-1

Glycan remodeling of antibody drugs



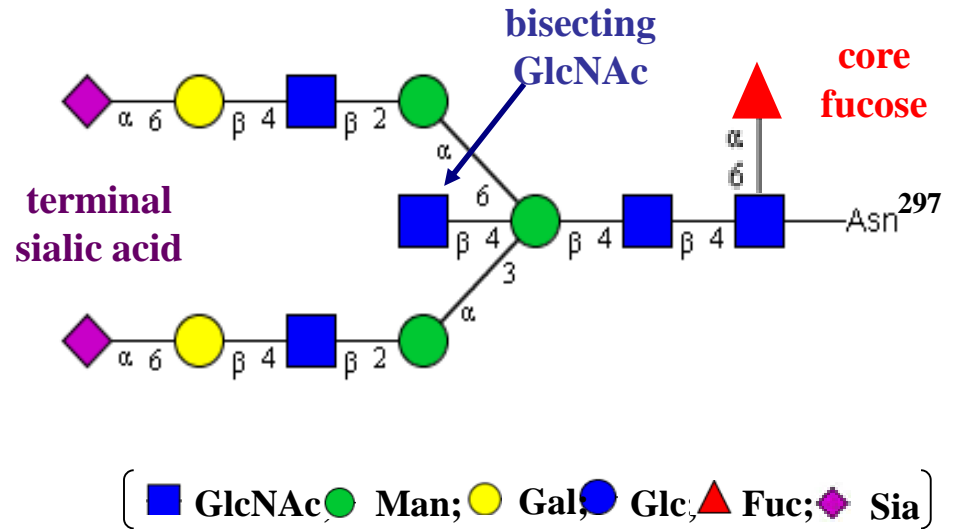
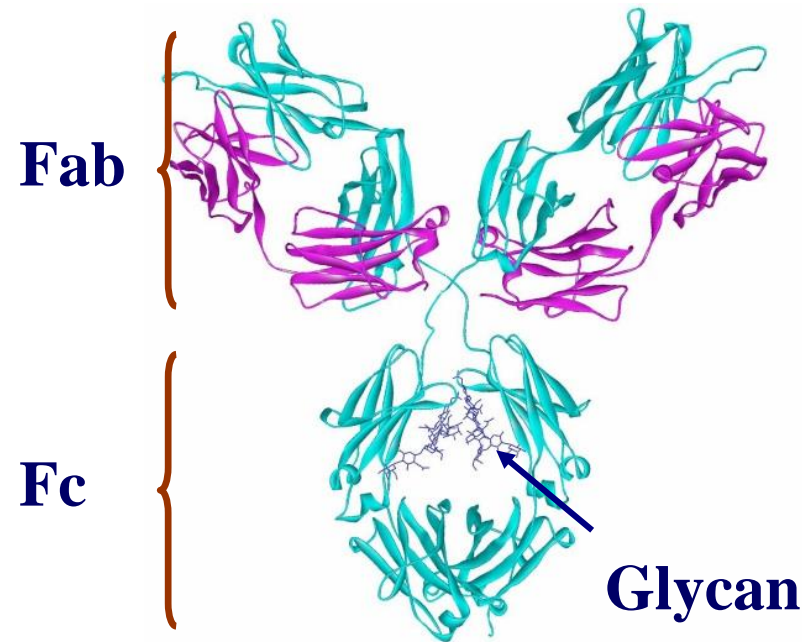
Antibody glycosylation

IgG Antibody

Required for ADCC activity

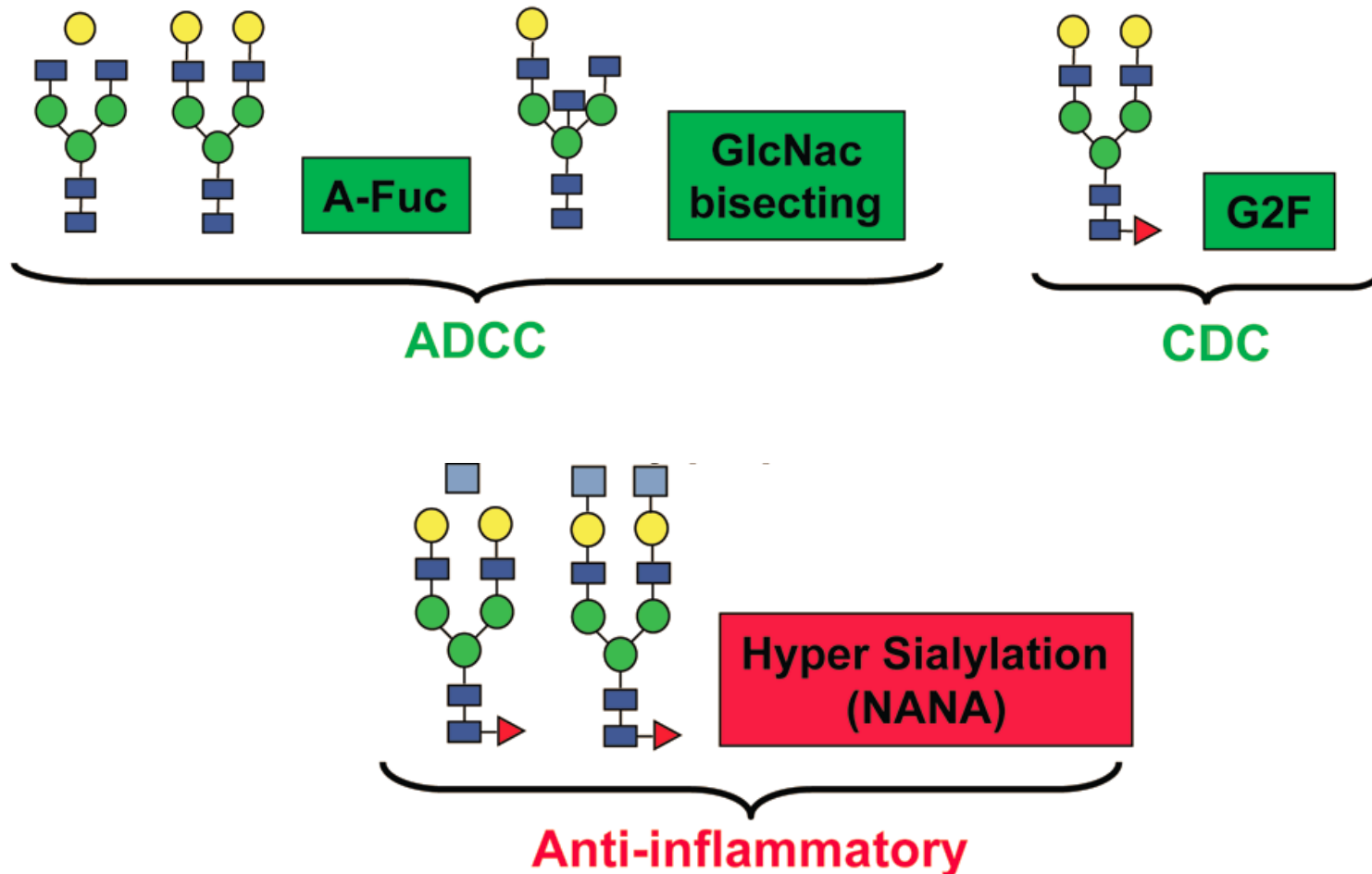


Fc glycan structure



New-generation glycoengineered antibody drugs

Engineered glycoforms of Abs for enhanced functions:



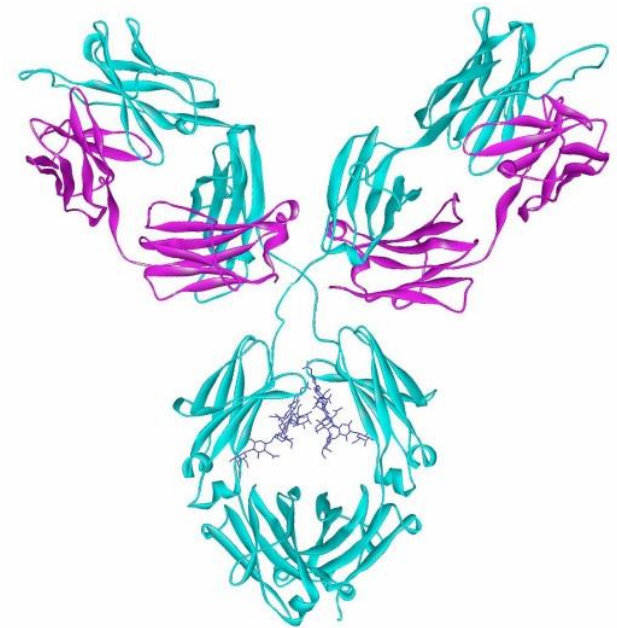
Glycosynthases for IgG glycosylation remodeling

Requirements:

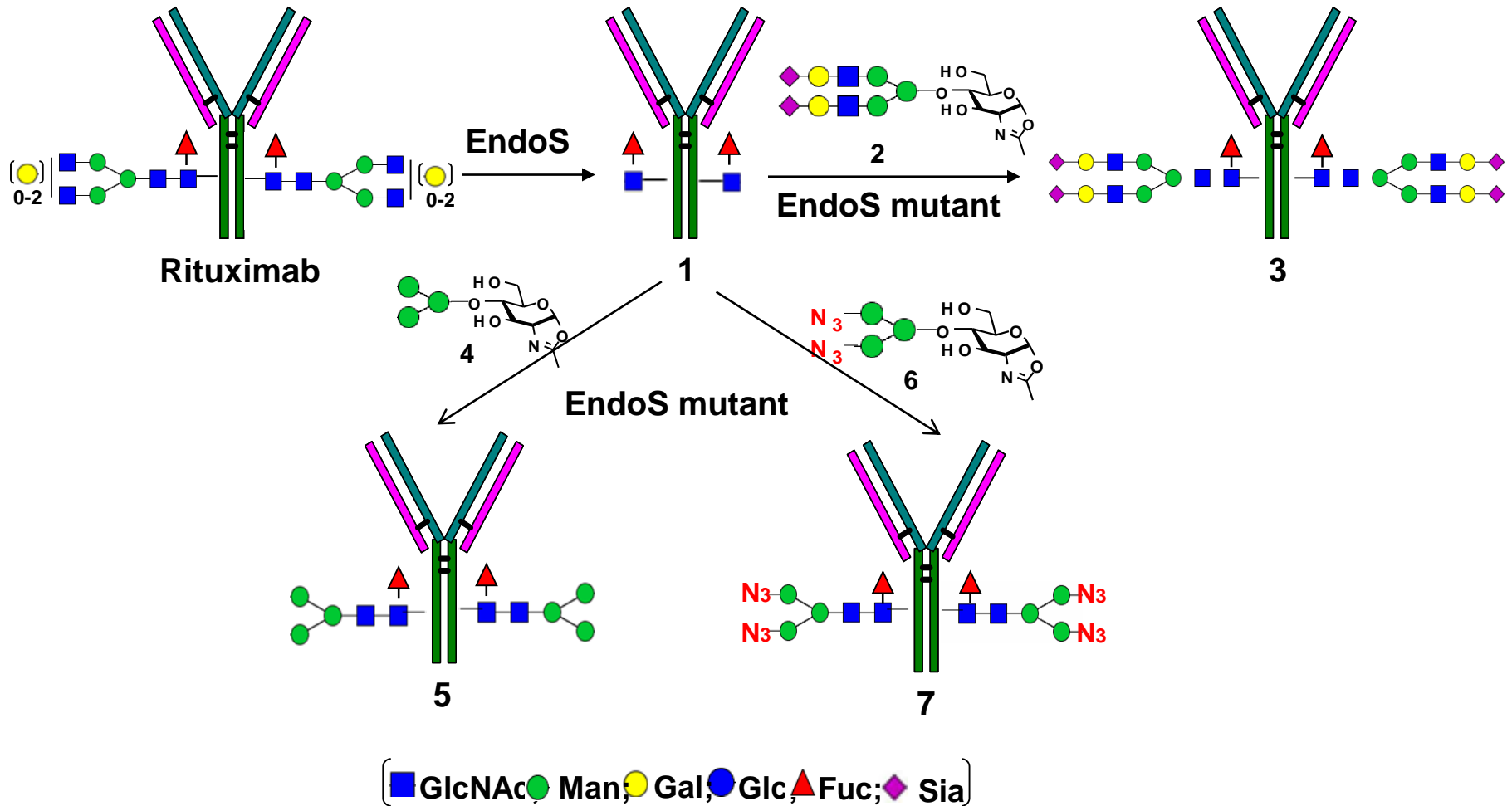
- 1) core-fucosylated or non-fucosylated GlcNAc-IgG as acceptors
- 2) wide substrate specificity in glycan
- 3) good k_{cat}/k_m for both donor and acceptor substrates

Endo S from *Streptococcus pyogenes*

Glycosynthases: **EndoS mutants**

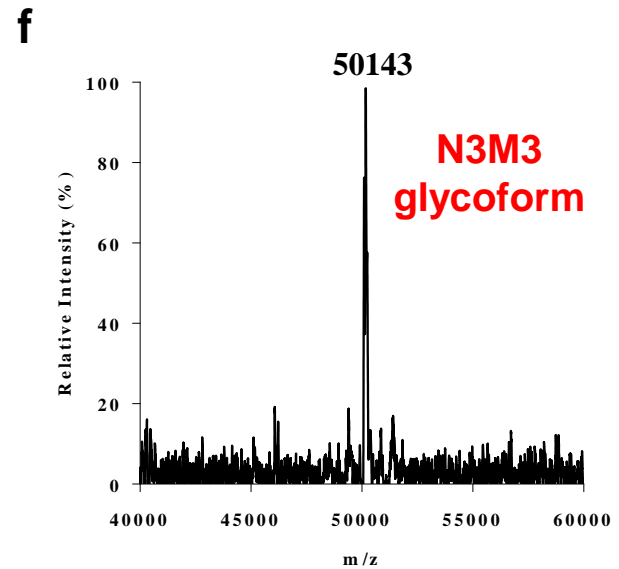
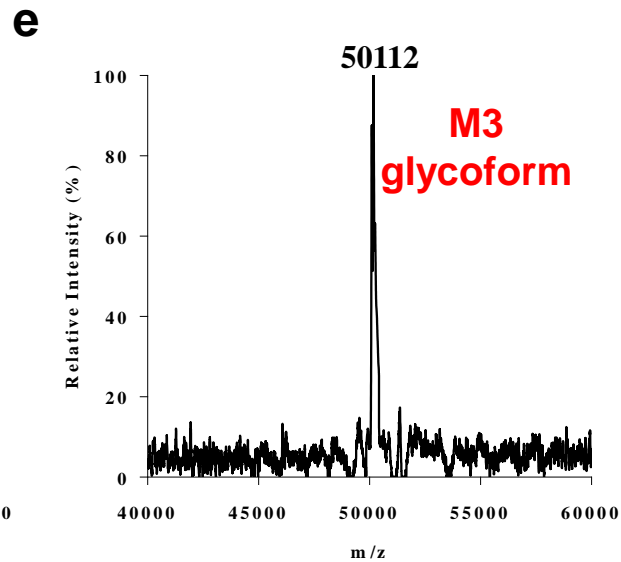
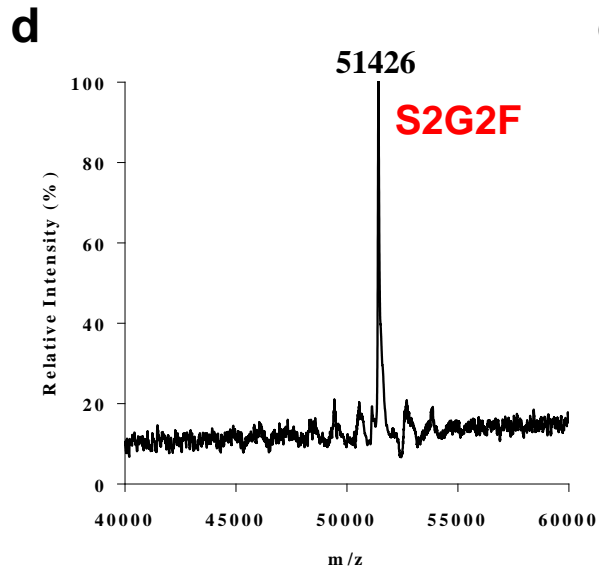
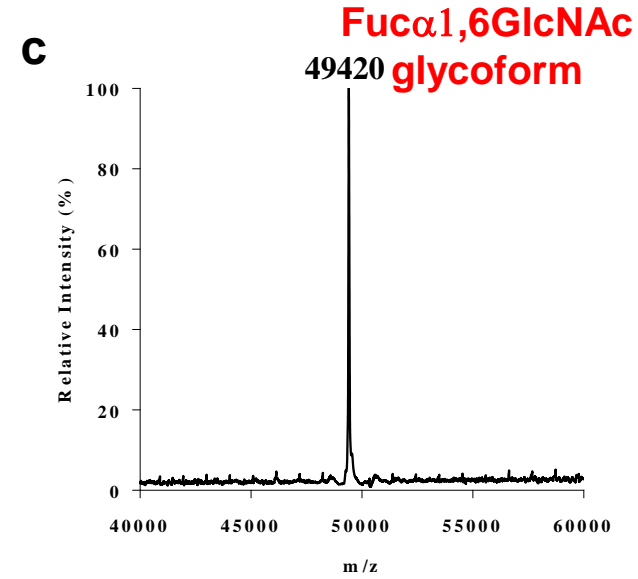
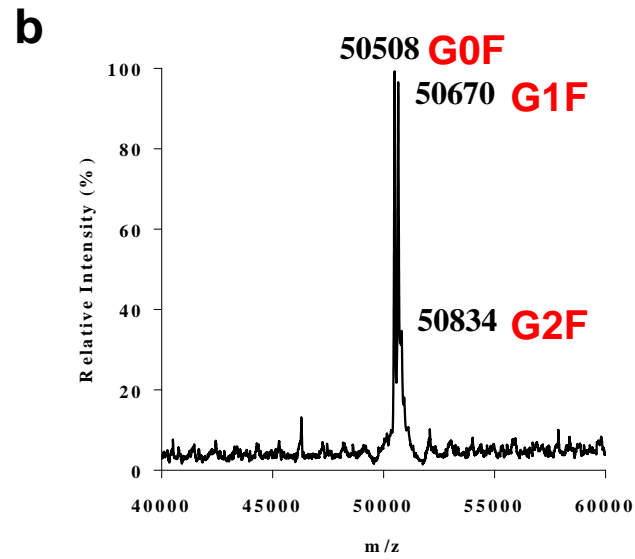
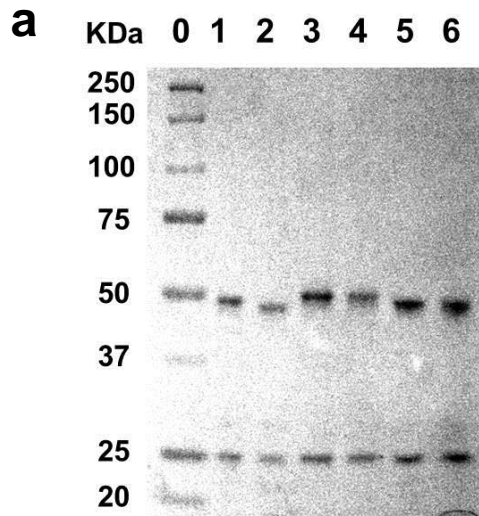


Glycosylation remodeling of Rituximab

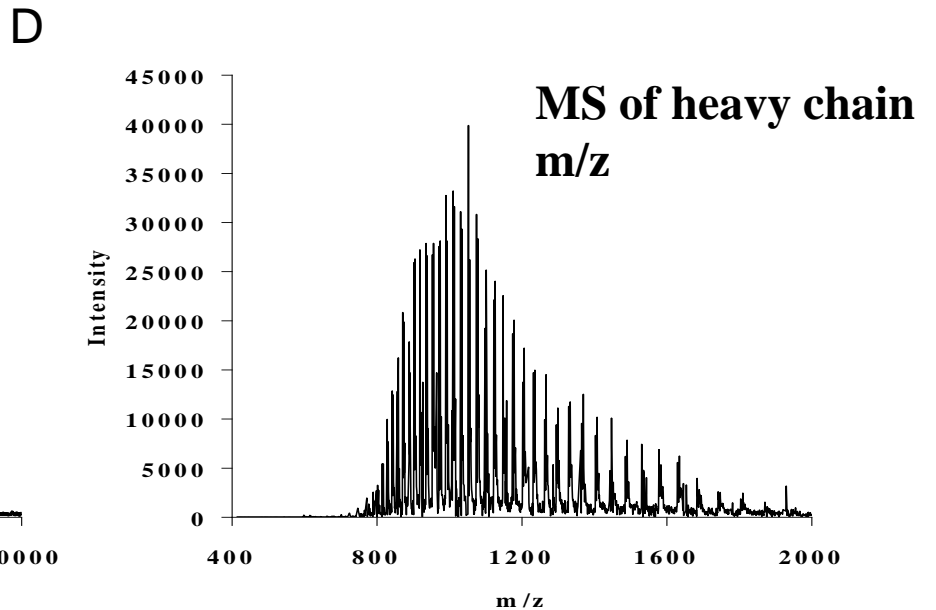
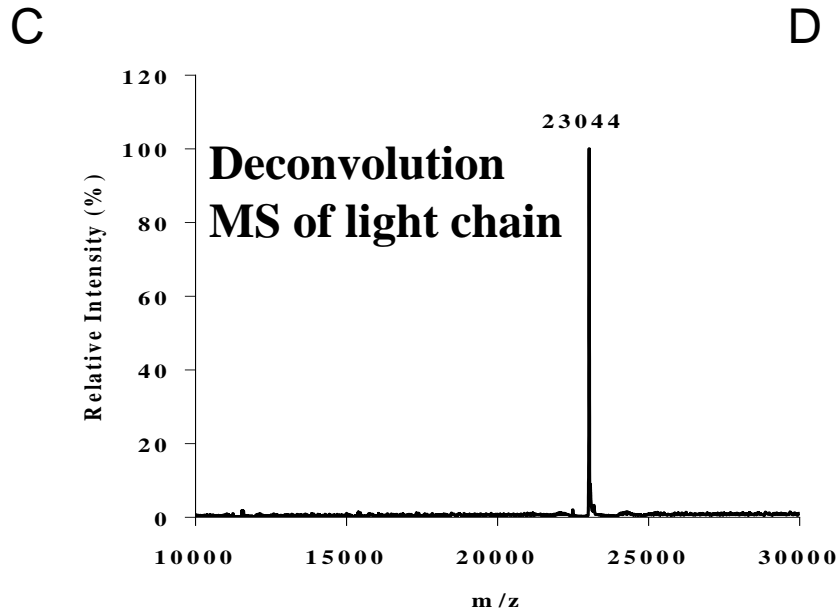
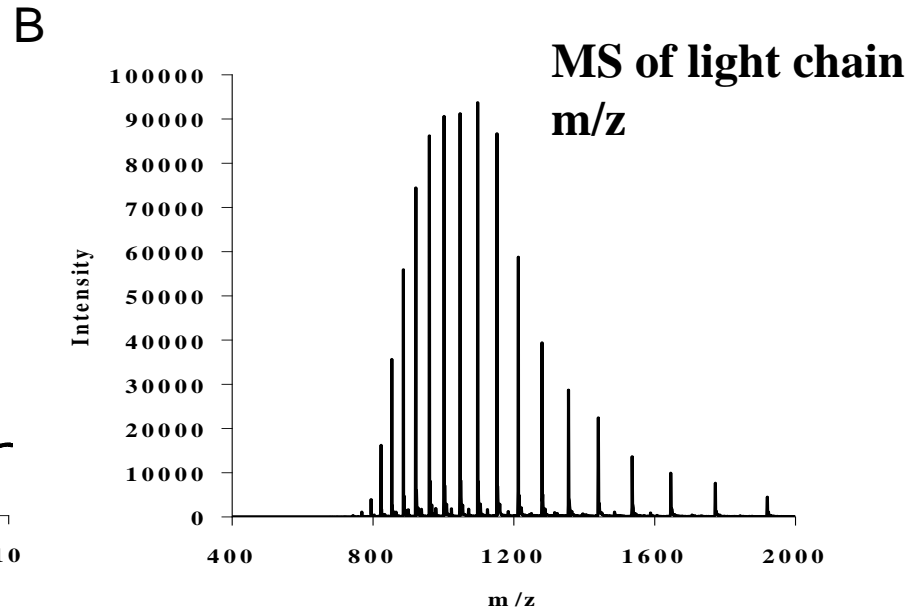
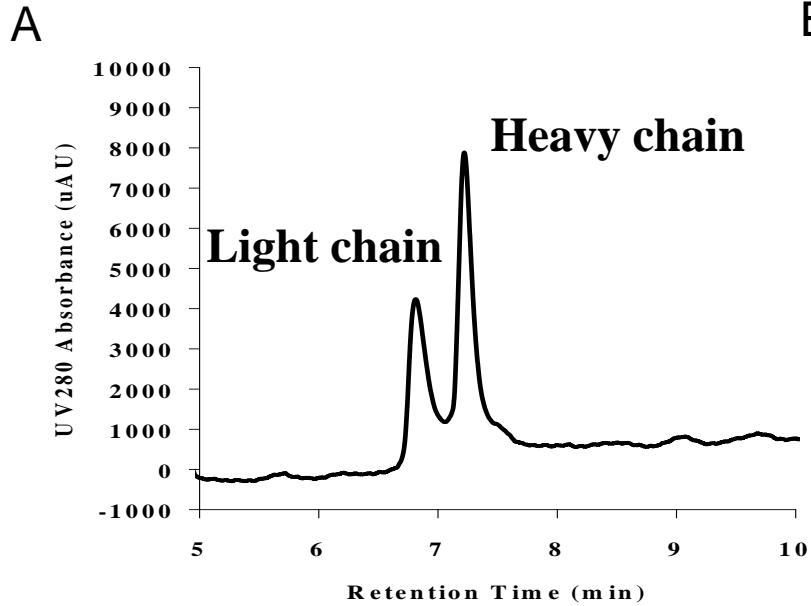


SDS-PAGE and LC-MS monitoring of remodeled Rituximab

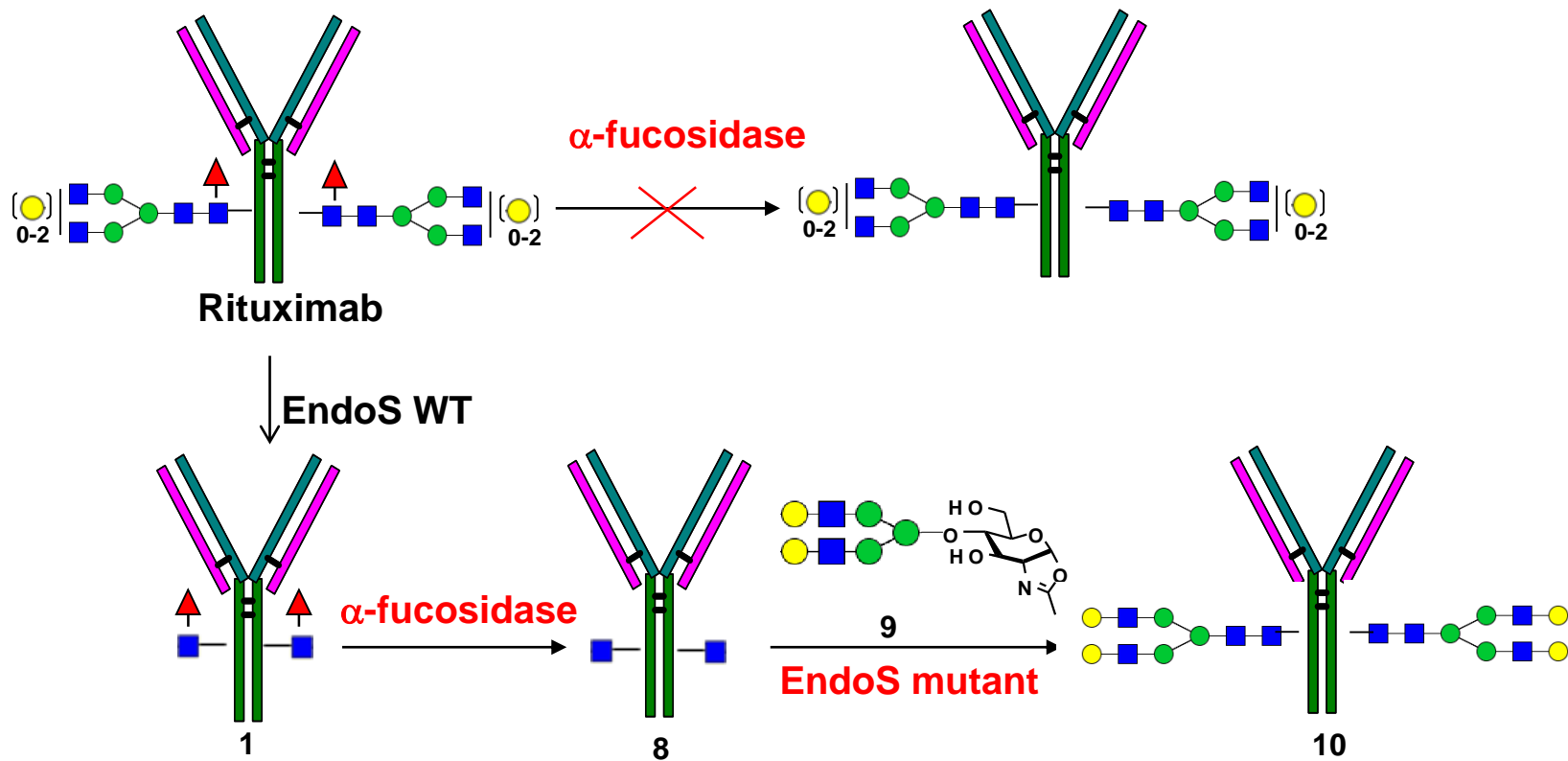
Results

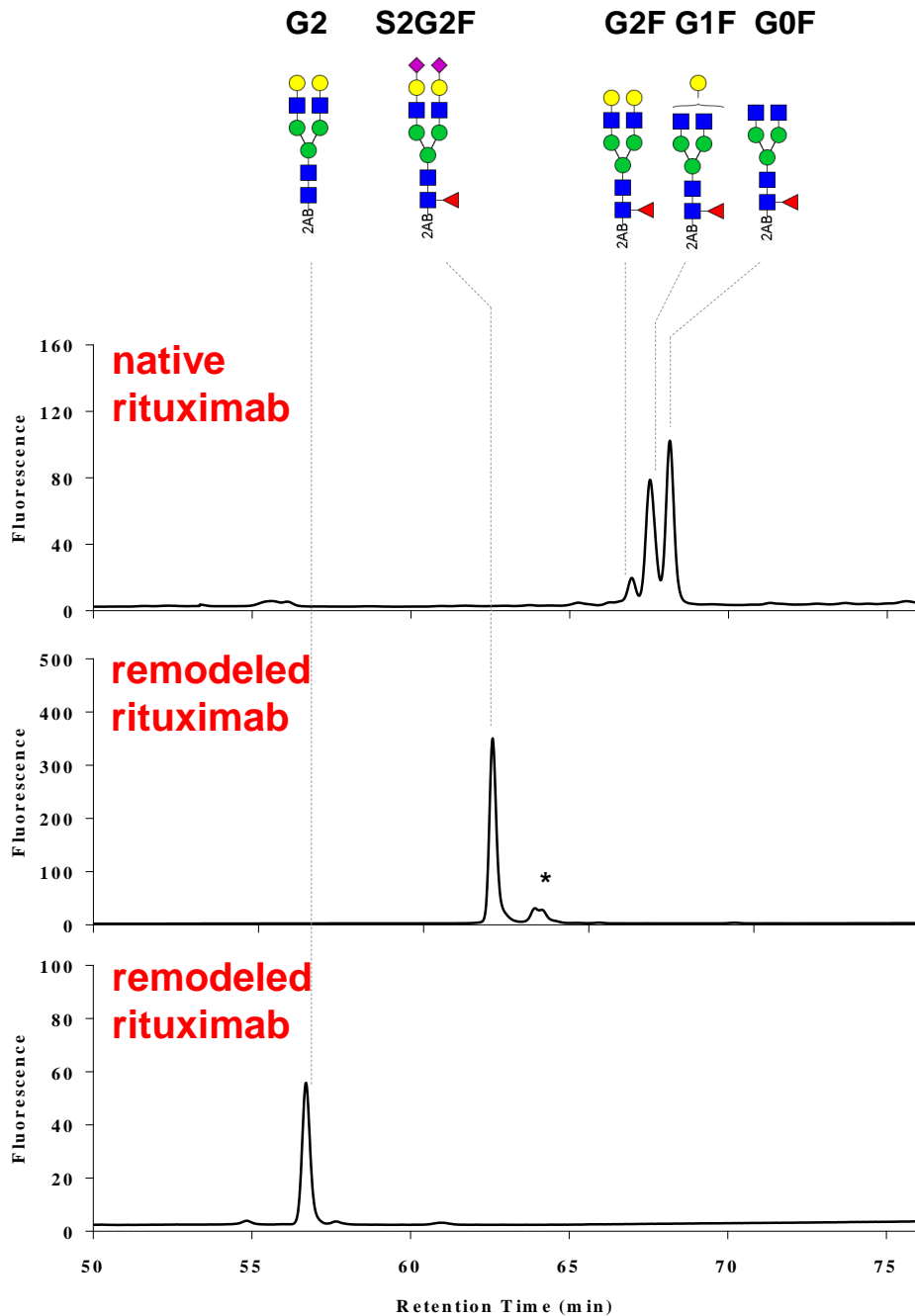


LC-MS of reduced IgG



Glycosylation remodeling of non-fucosylated Rituximab

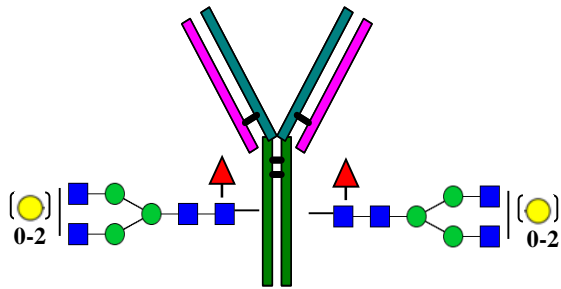




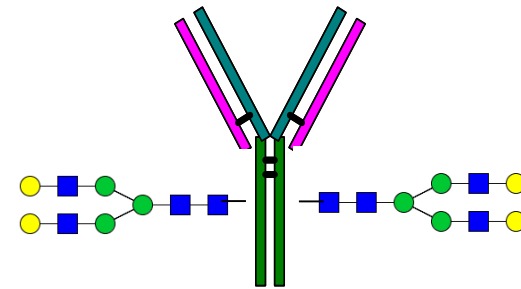
Glycan analysis of remodeled rituximab glycoforms:

from heterogeneous to homogeneous

FcgIIIa receptor binding assay



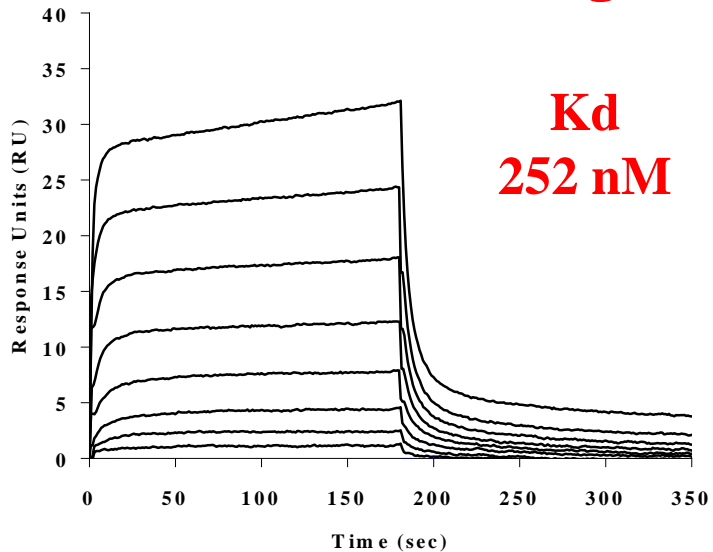
Rituximab



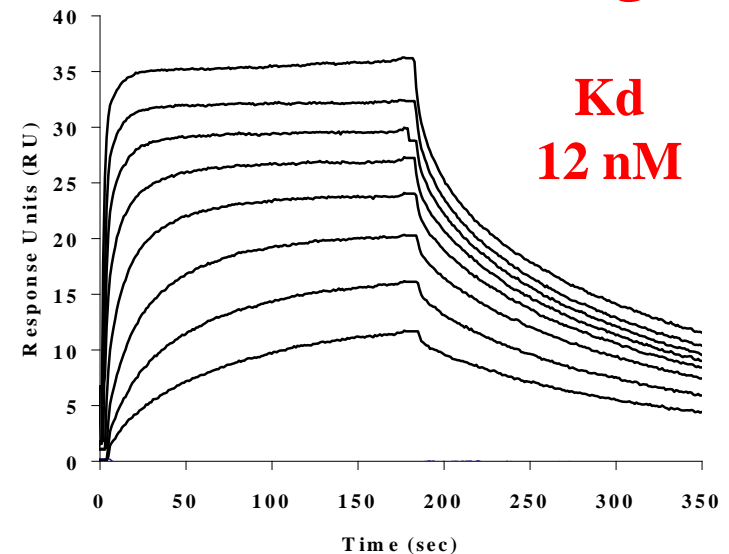
Defucosylated Rituximab

FcgIIIa receptor binding by SPR

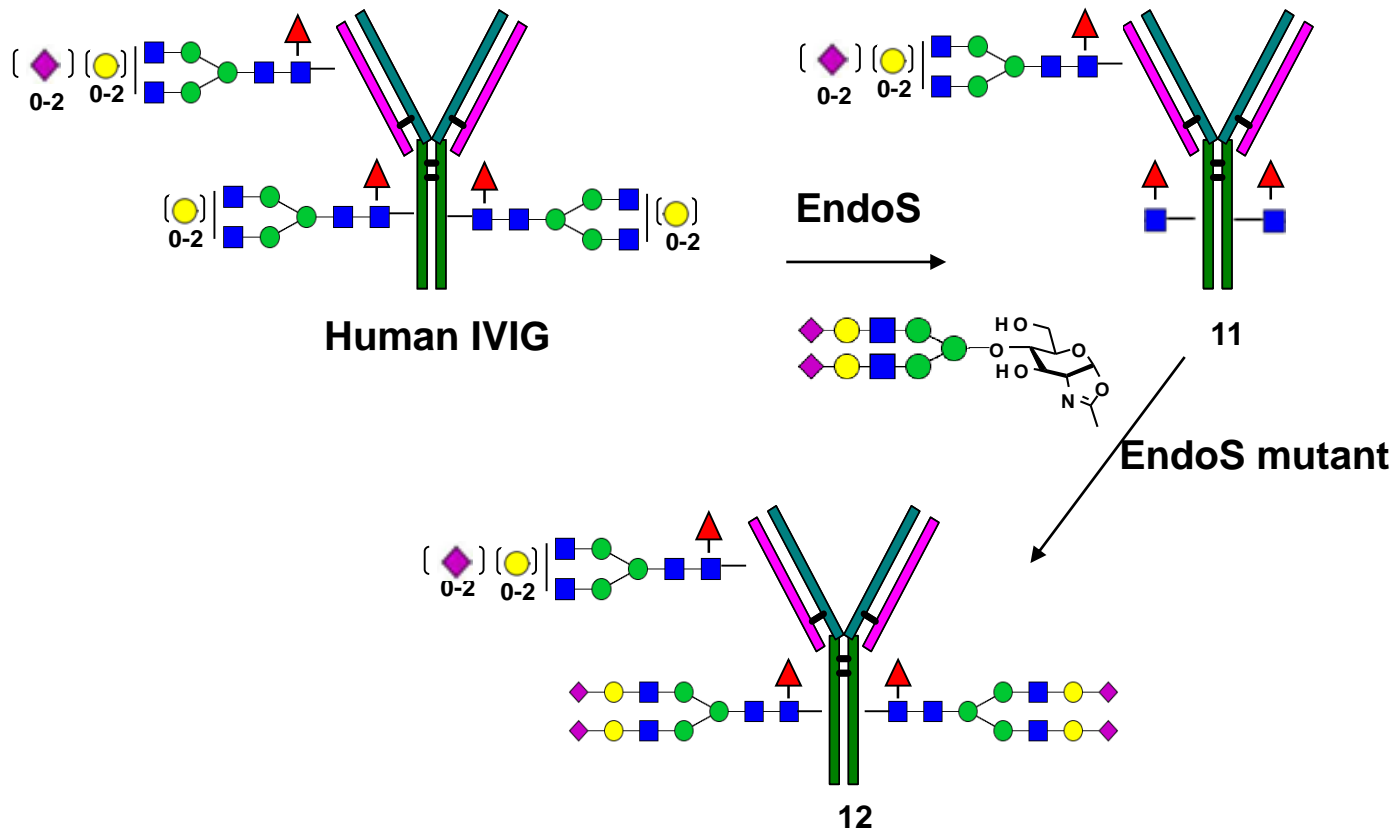
before remodeling



after remodeling



Glycosylation remodeling of human Intravenous Immunoglobulin (IVIg)



Application-2

Glycan and protein folding

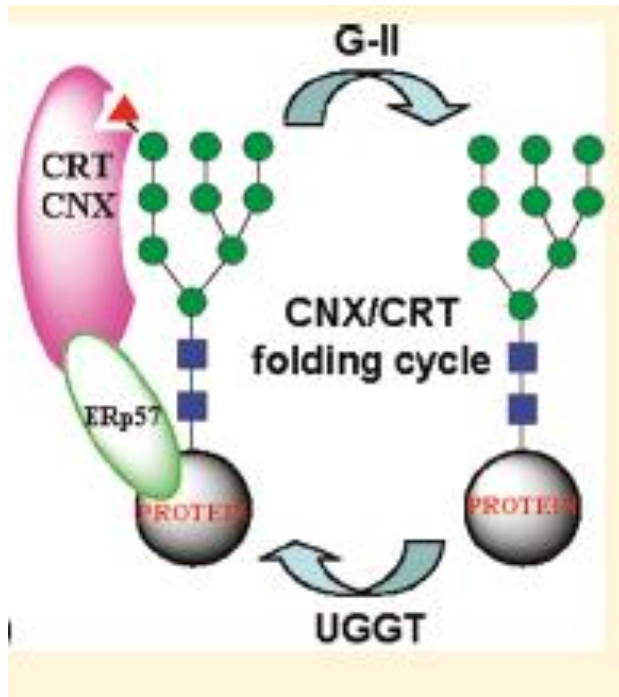


GlcMan9GlcNAc2 glycan and protein folding

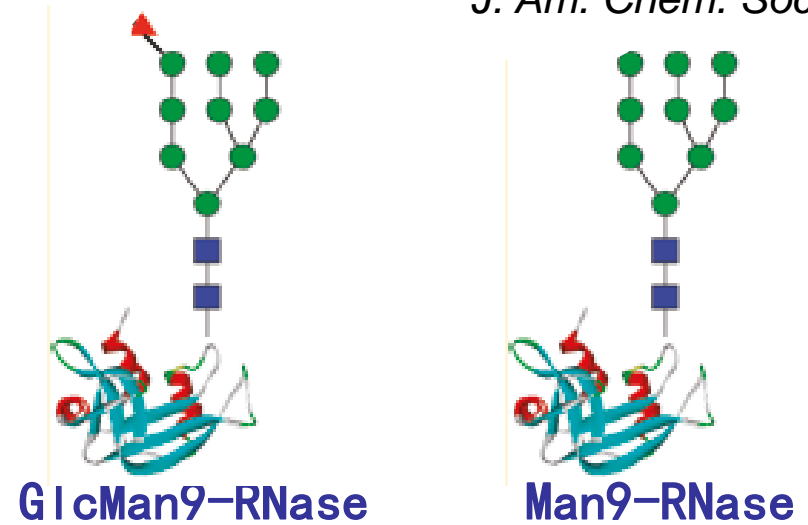
J. Am. Chem. Soc. 2009

J. Am. Chem. Soc. 2011

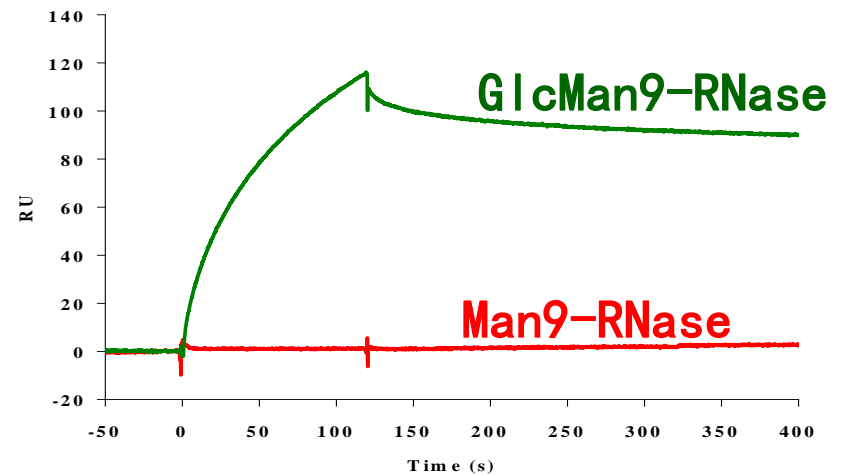
Chaperone CRT/CNX and protein folding



- GlcNAc
- Man
- ▲ Glc



CRT binding

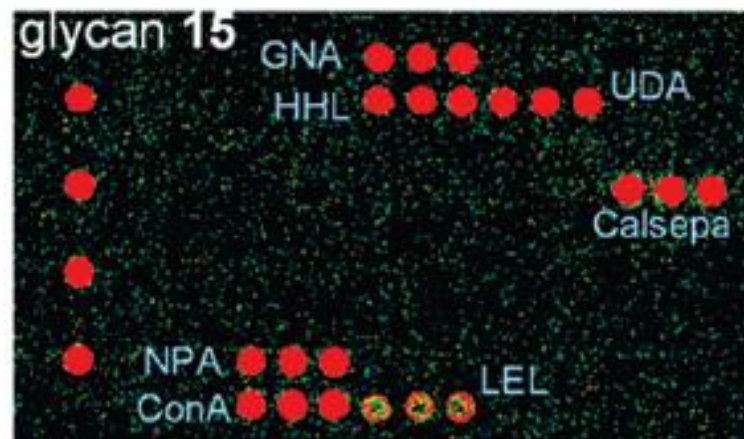
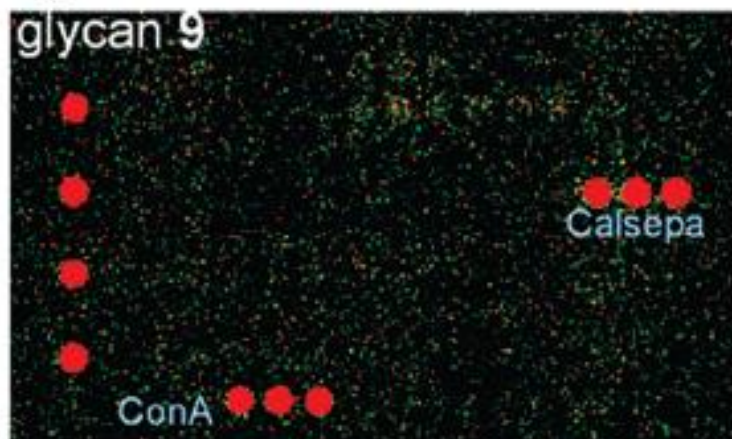
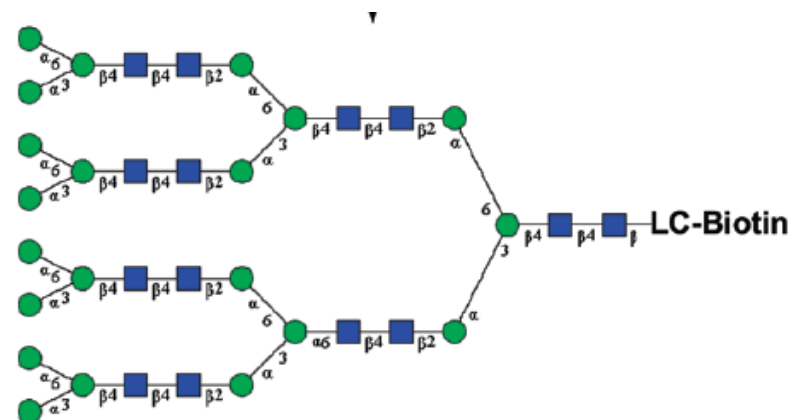
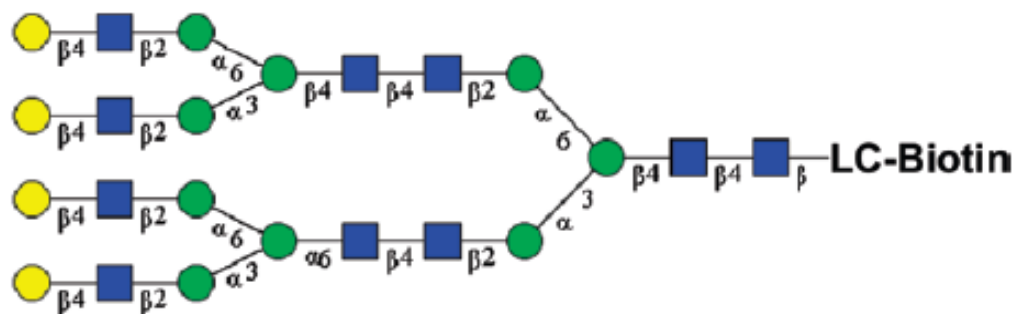


Application-3

Glyco-cluster and lectin microarray



Glyco-cluster as a probe for molecular recognition



Probing lectin binding property: Lectin Microarray

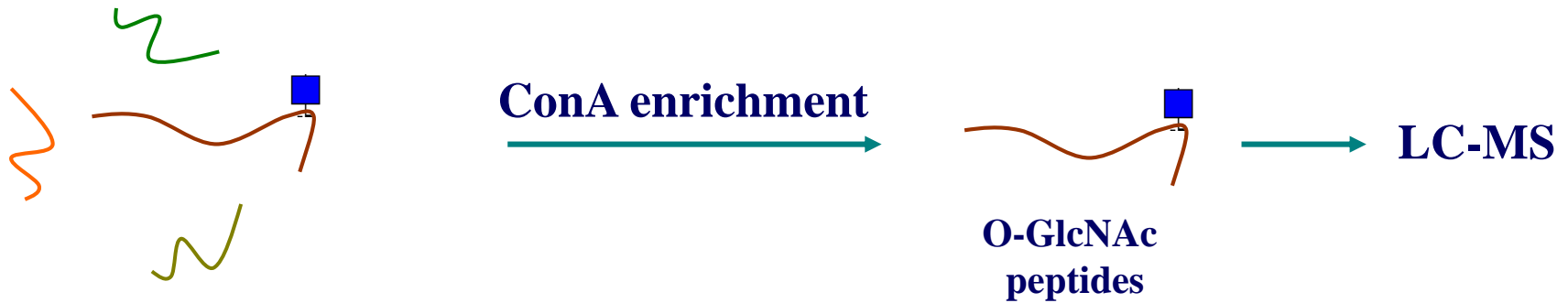
Application-4

O-GlcNAcylation identification

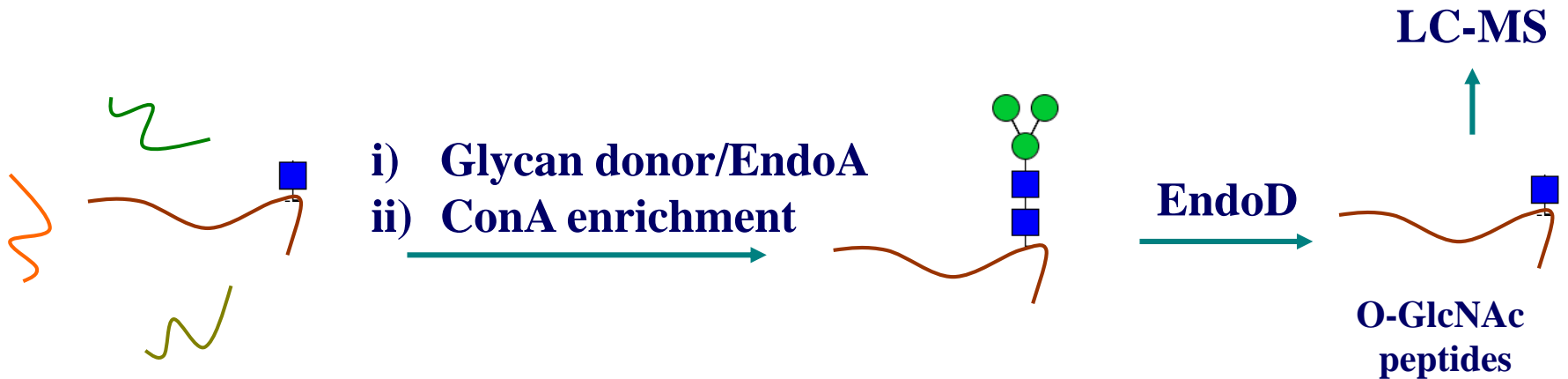


O-GlcNAcylation identification

Traditional method:



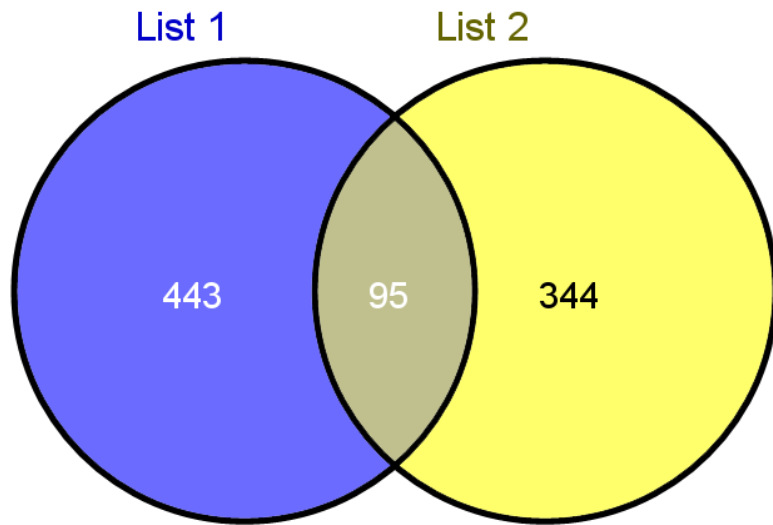
Chemoenzymatic label method:



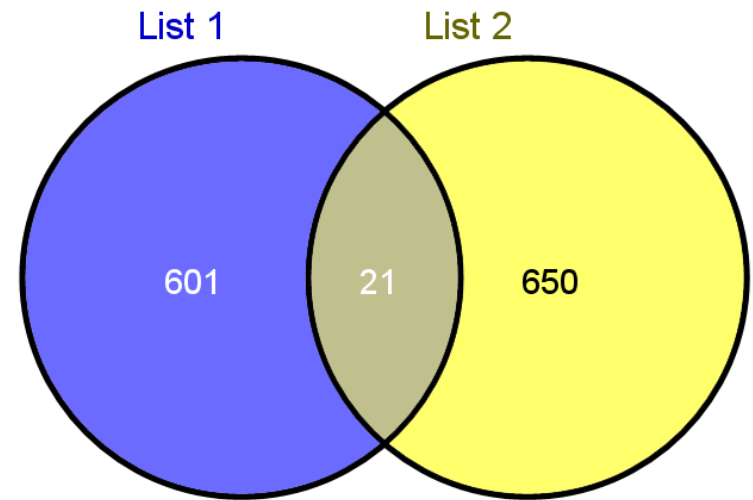
O-GlcNAcylation identification

List1: traditional method

List2: chemoenzymatic label method



identified O-GlcNAc glycoproteins



identified O-GlcNAc glycopeptides

Acknowledgement

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Prof. Zhi-jie Liu
Prof. Fei Xu

University of Maryland

Prof. Lai-Xi Wang
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Shu-Quan Fan

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