GIFU UNIVERSITY

iGCORE
Institute for Glyco-core Research
Tokai National Higher Education and Research System

Activity, specificity and disease relevance of N-glycan branching enzymes

Tokai National Higher Education and Research System
Gifu University
Institute for Glyco-core Research（iGCORE)

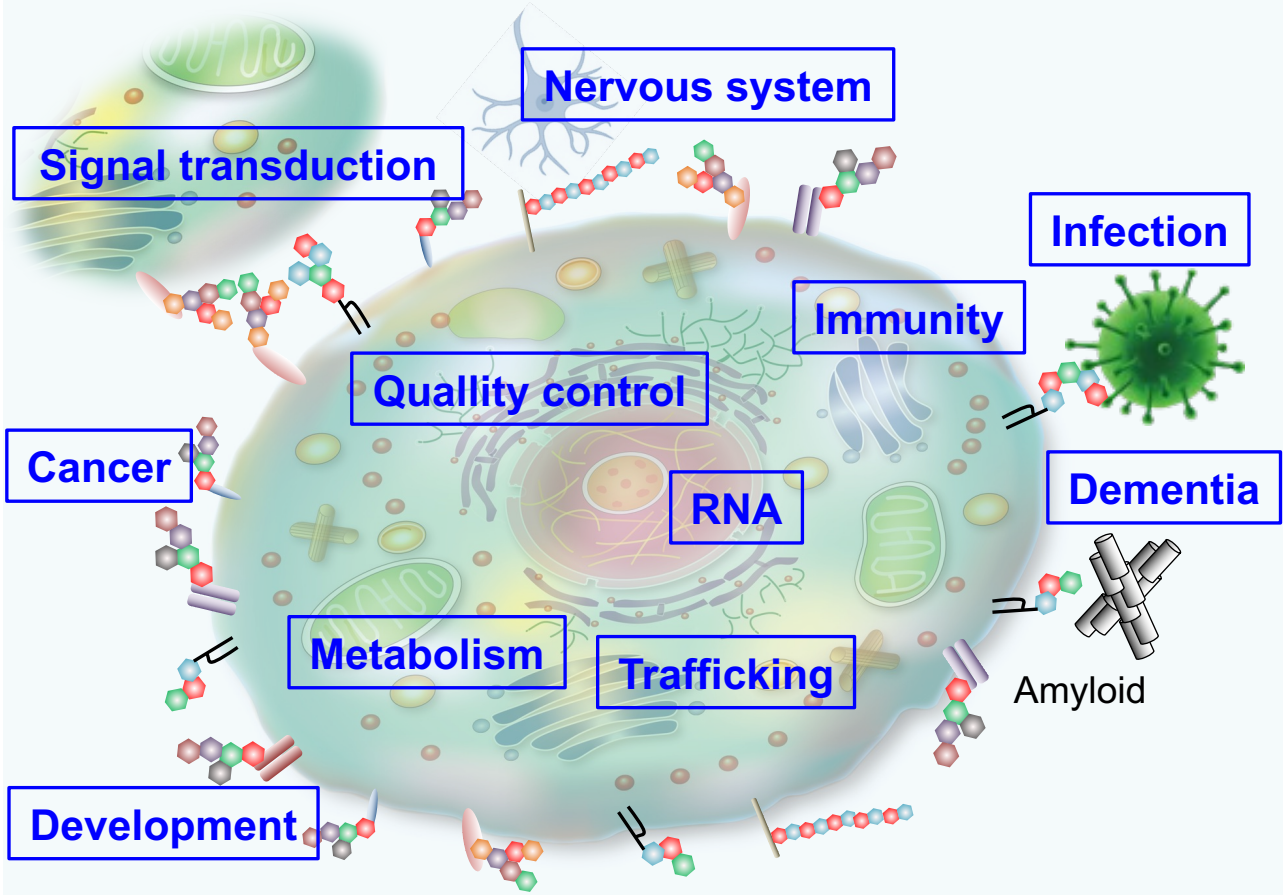
Yasuhiko Kizuka

Background：Protein glycosylation

Glycan and life

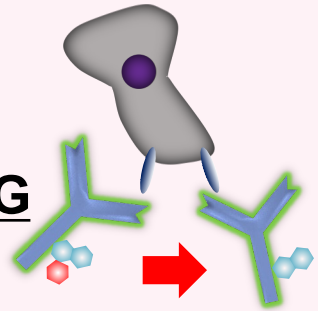
Glycan

- covers all cells
- over half of proteins are glycosylated



Glycans are involved in various phenomena and diseases

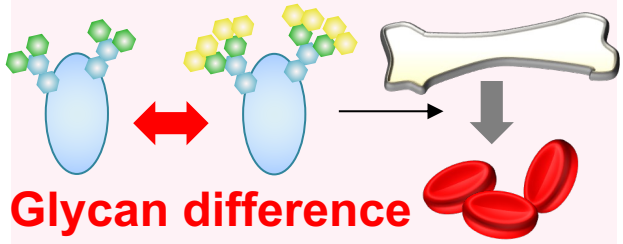
Glycan functions



Glycan modification

ADCC activity
~100 fold (potelligent)

Erythropoietin (EPO)

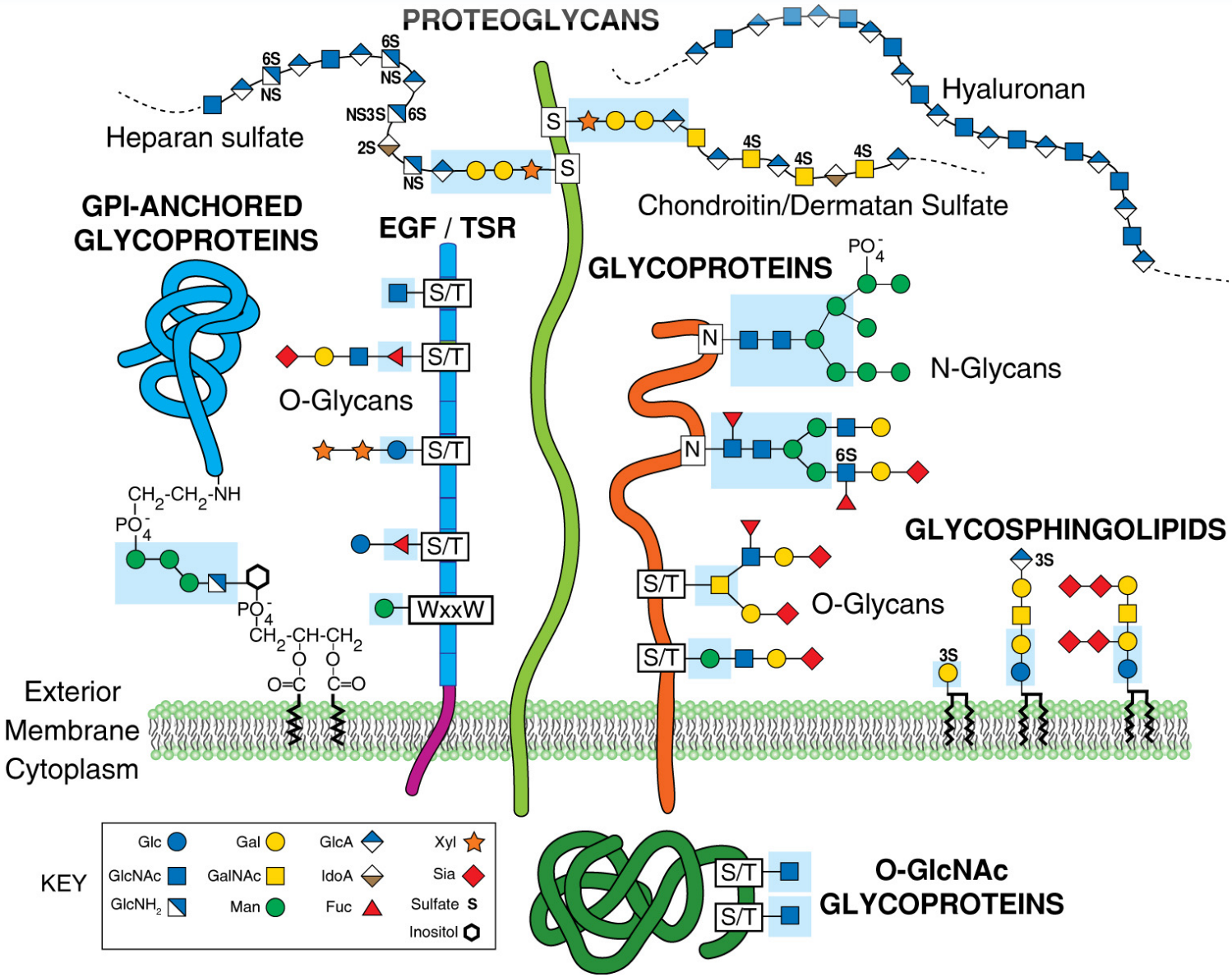


Glycan difference

Difference in stability in blood

Glycan regulates protein functions

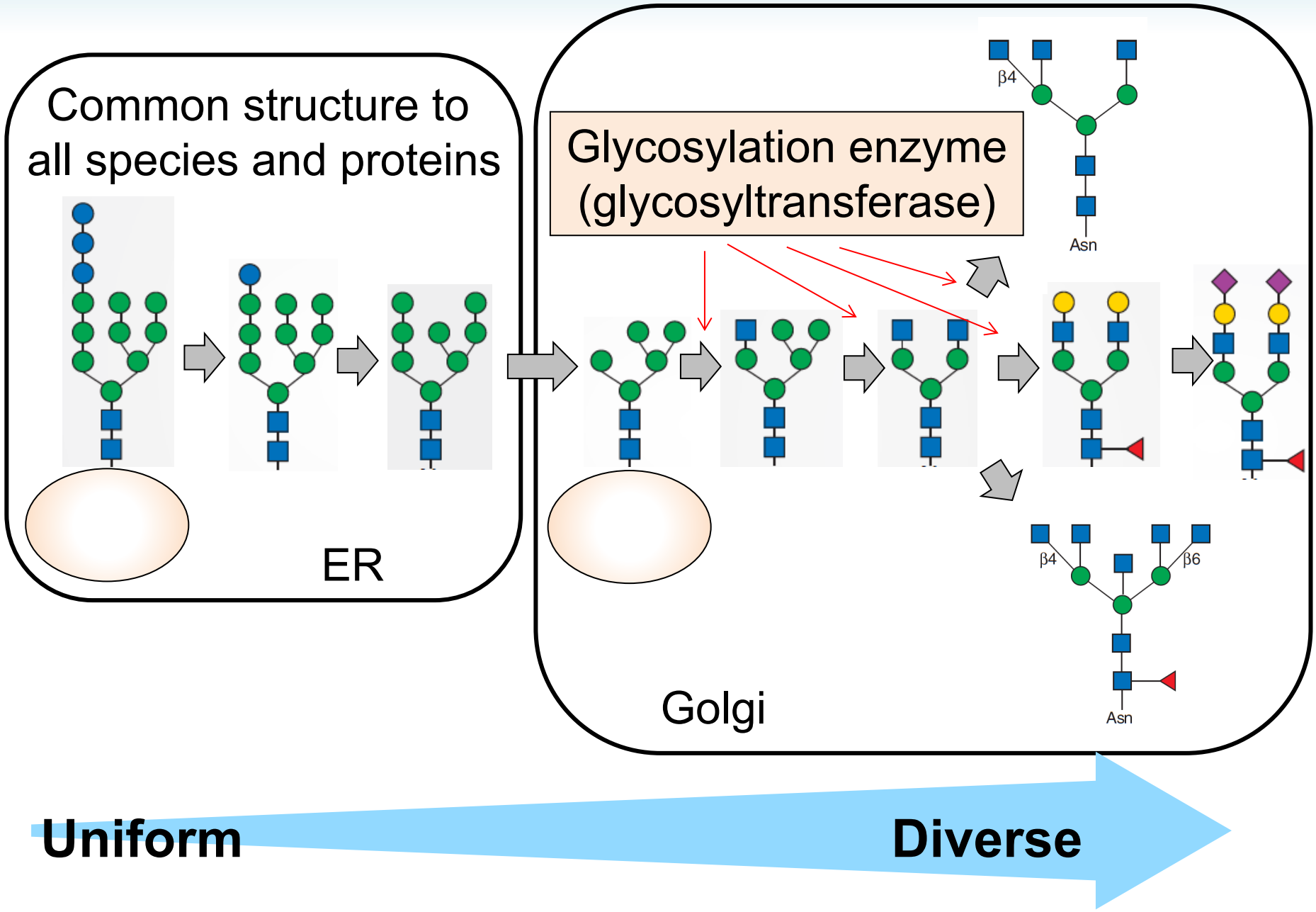
Glycans in mammals



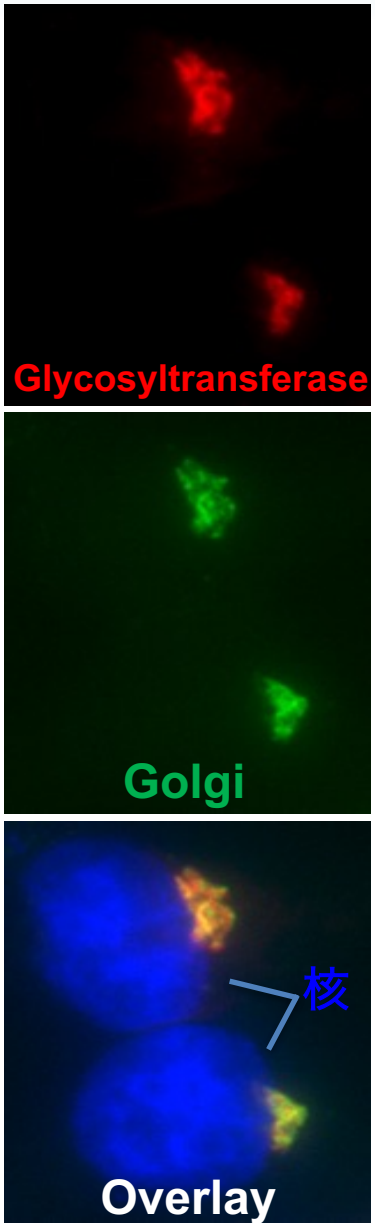
Widely conserved

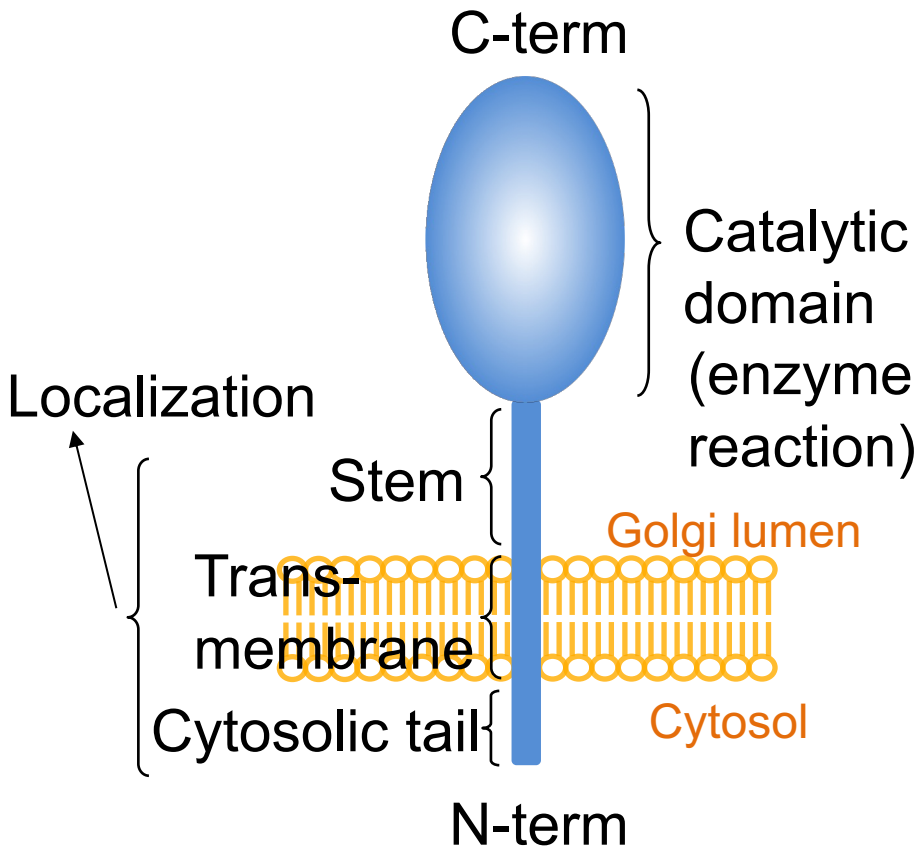
(Essentials of Glycobiology 4th ed.)

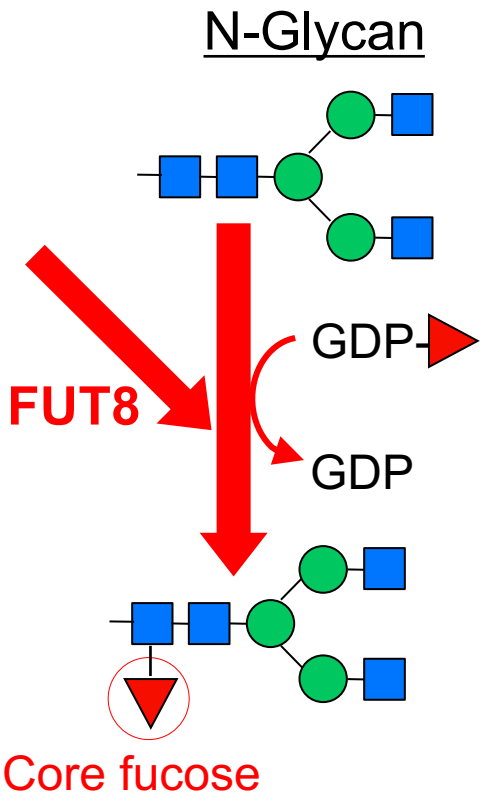
Biosynthetic steps of animal N-glycans



Typical structure of Golgi glycosyltransferase





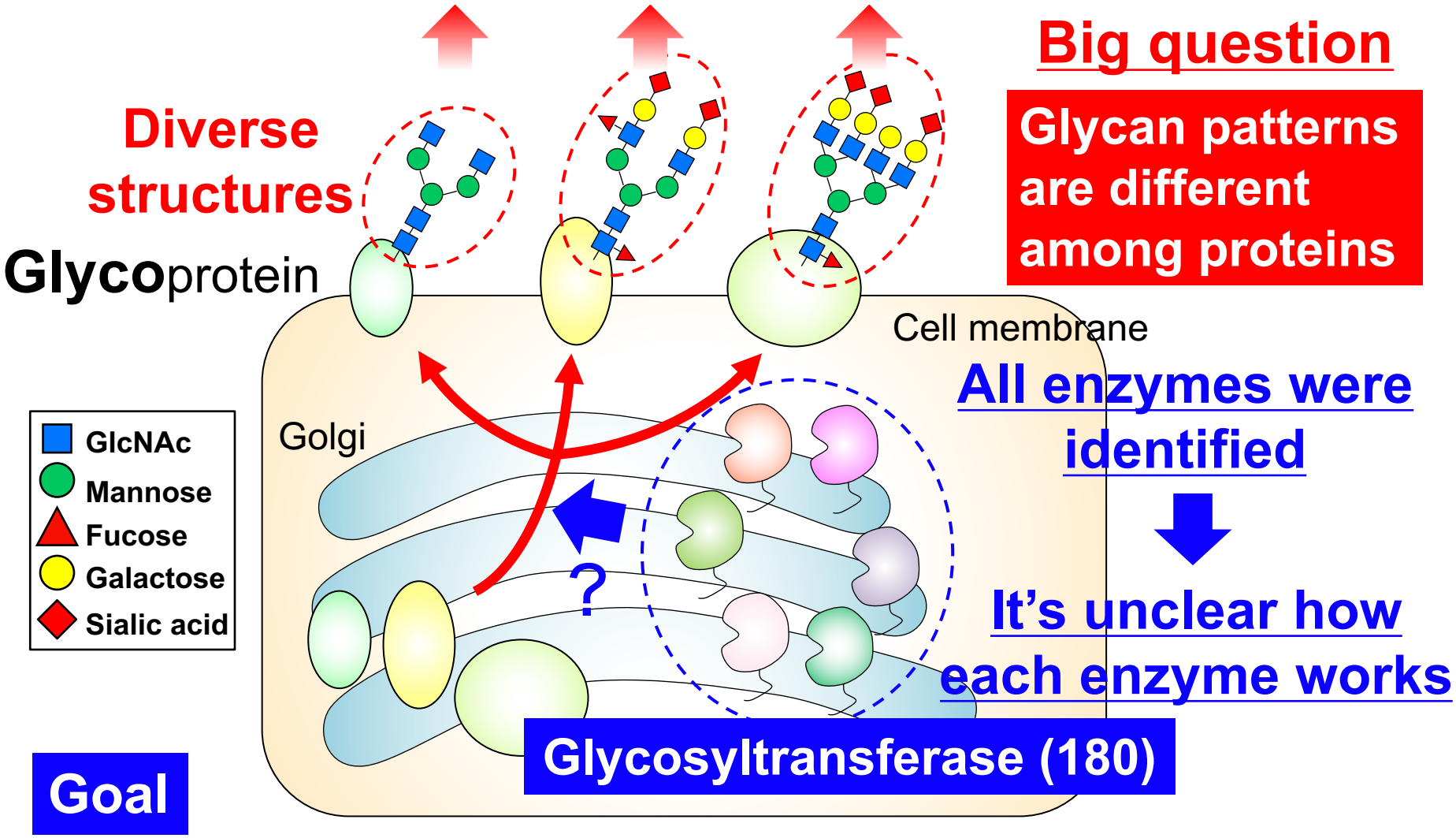


Much is known about sugar transfer reactions, but the localization mechanism is not understood well

(Tomida et al., *J. Biol. Chem.*, 2020, 298, 102676)

Research question

Biological functions and diseases (cancer, Alzheimer's)



Big question

Glycan patterns are different among proteins

All enzymes were identified

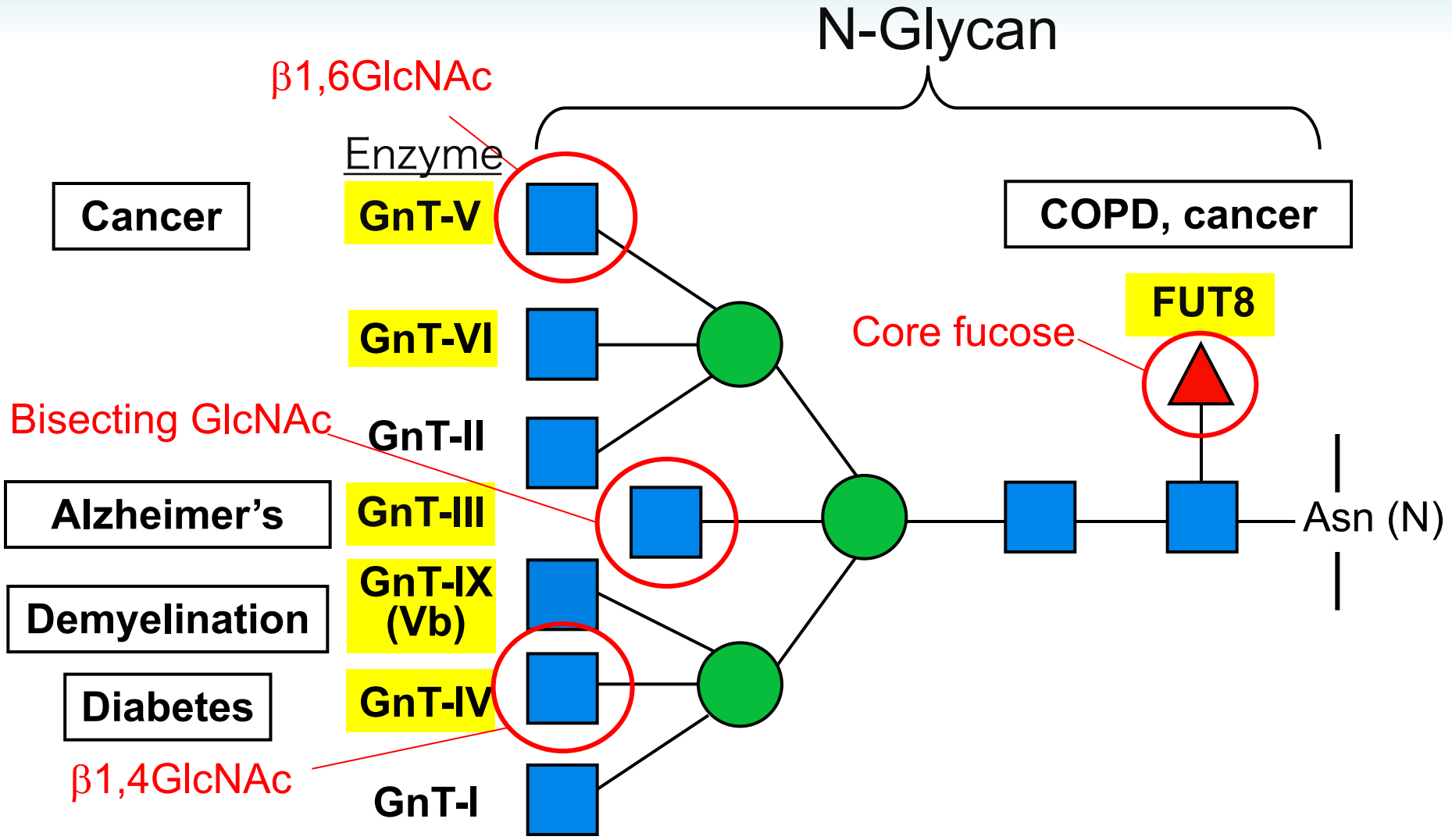
It's unclear how each enzyme works

Glycosyltransferase (180)

Goal

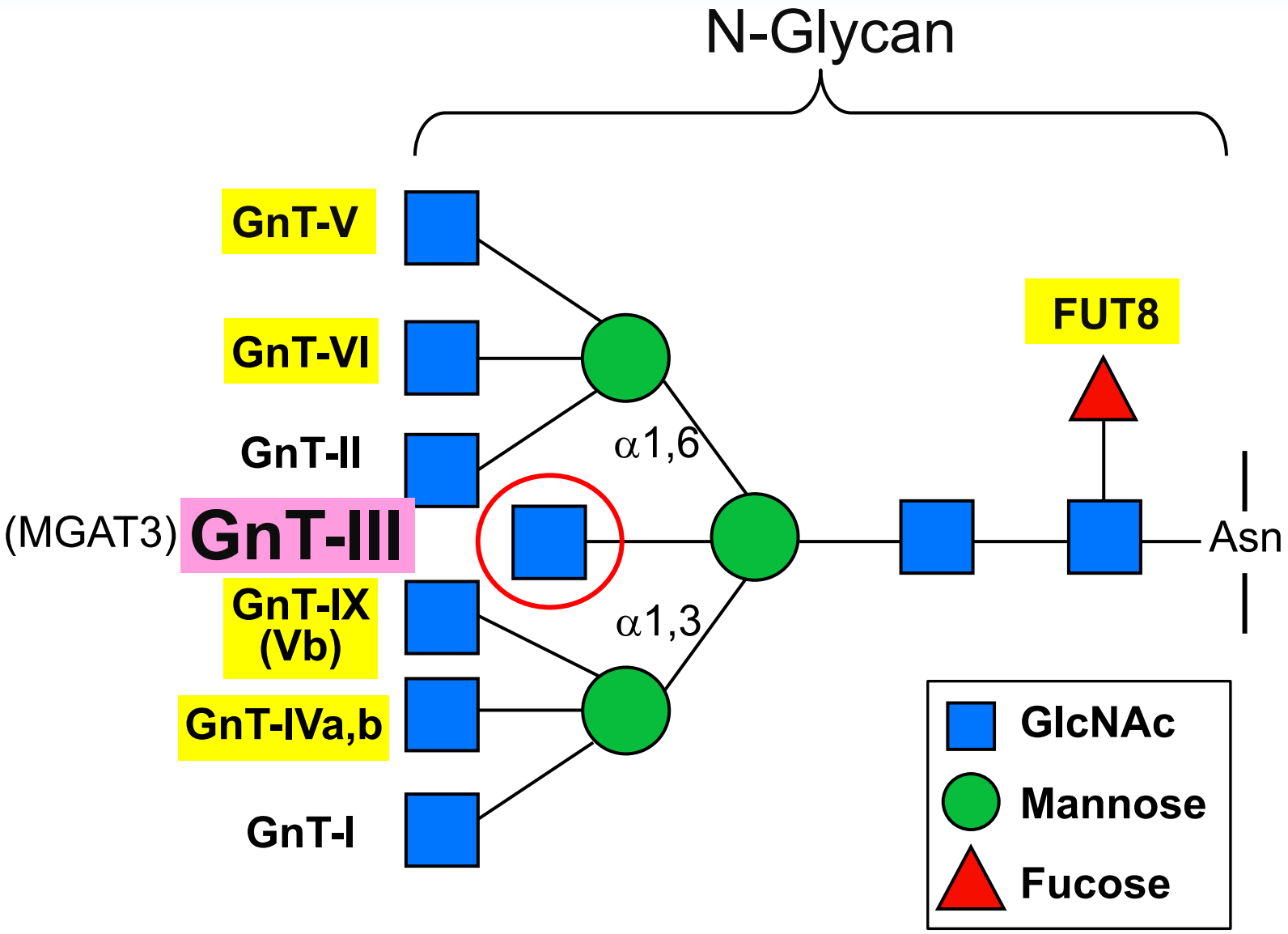
Understanding the mechanisms of protein-dependent glycosylation by glycosyltransferases

Research target: N-glycan branches

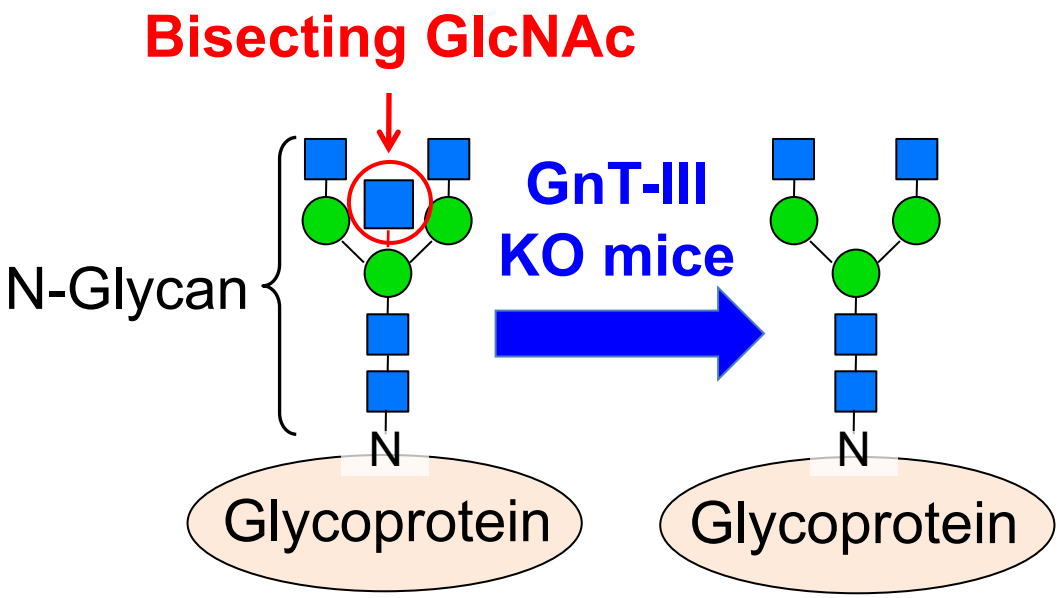


How is each branch synthesized in protein-dependent manners?

Topic 1 : GnT-III



Bisecting GlcNAc and Alzheimer's disease



Alzheimer's Disease (AD) patients

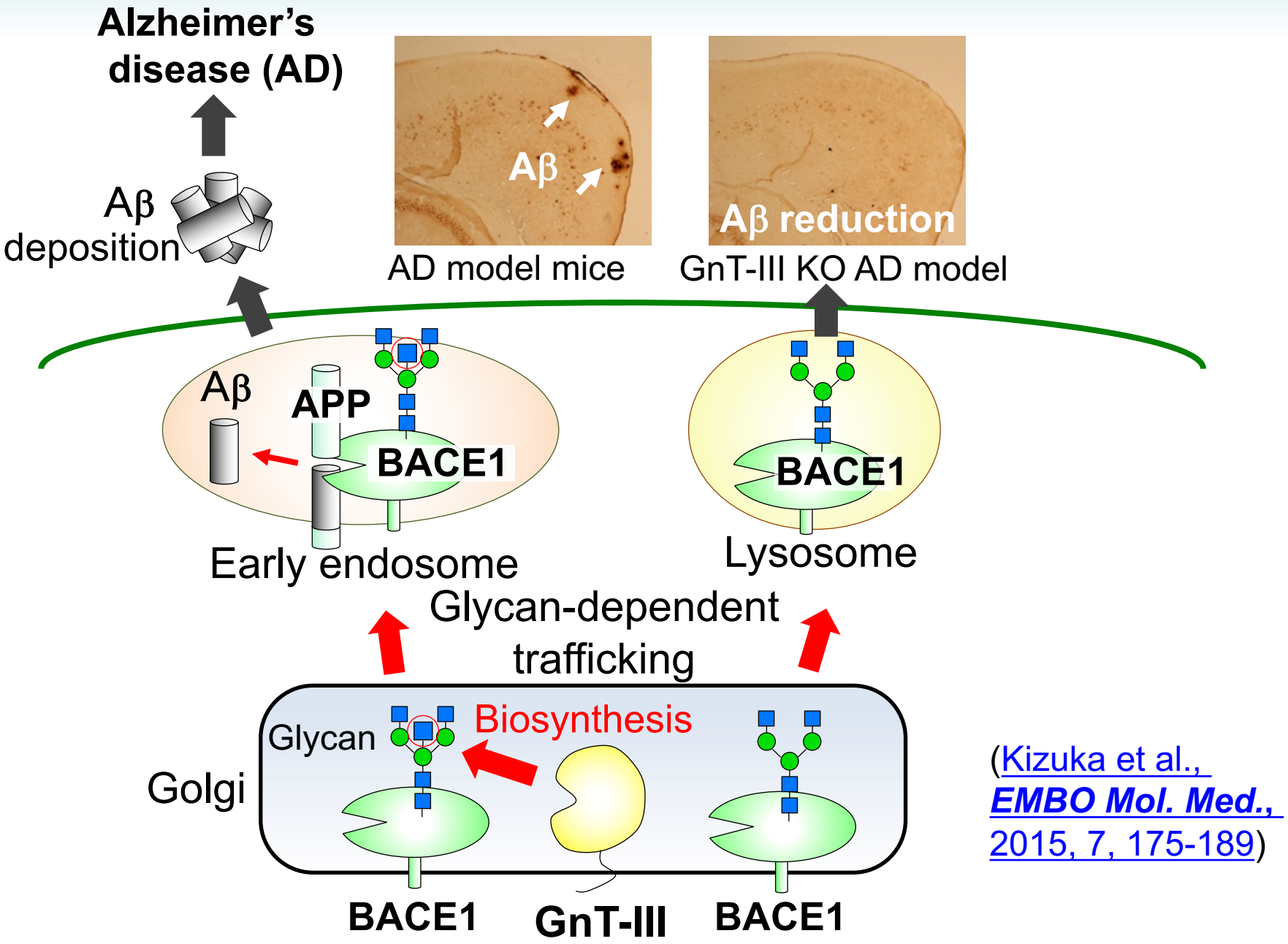
GnT-III mRNA

Control eAD AD

Upregulated

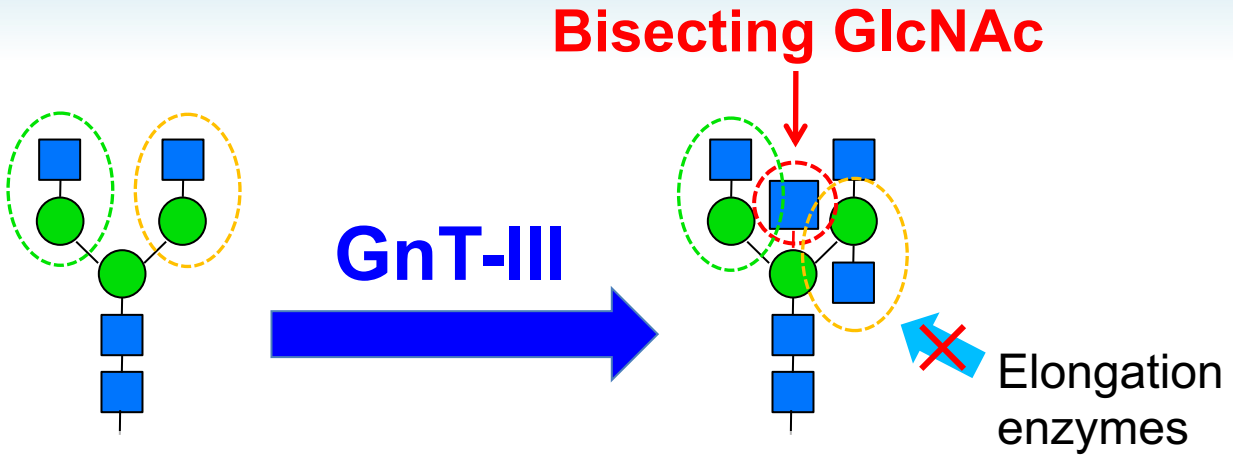
(Akasaka-Manya et al. *Glycobiology* 2010, 20, 99-106)

Bisecting GlcNAc and Alzheimer's disease

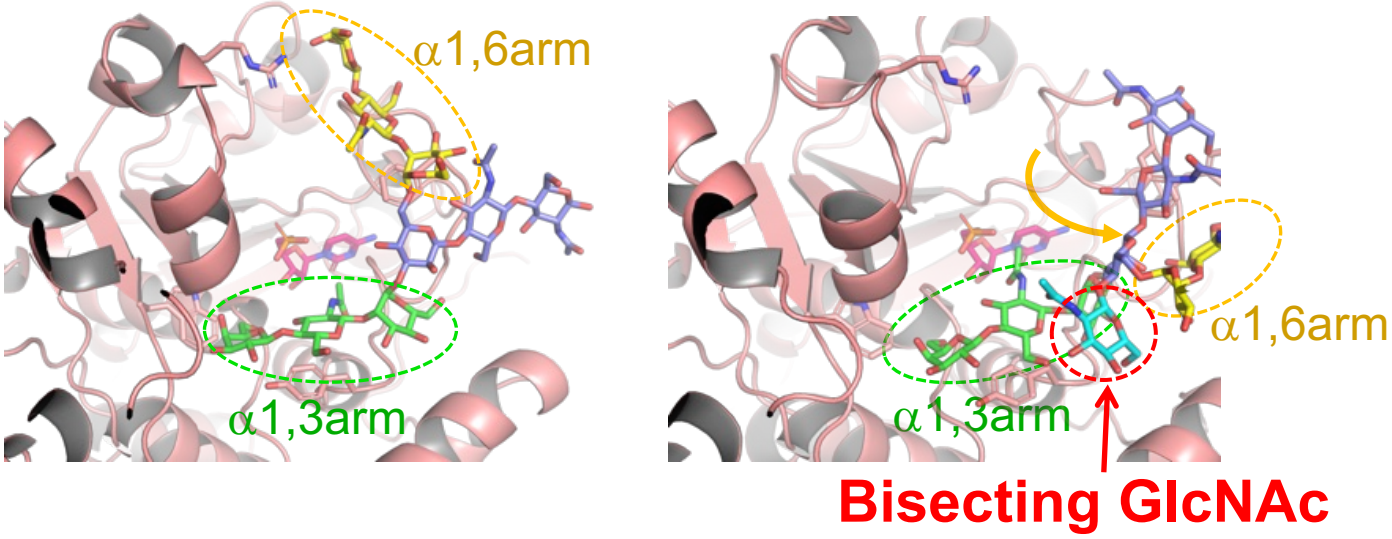


(Kizuka et al., ***EMBO Mol. Med.***, 2015, 7, 175-189)

Bisecting GlcNAc is a key for glycan elongation

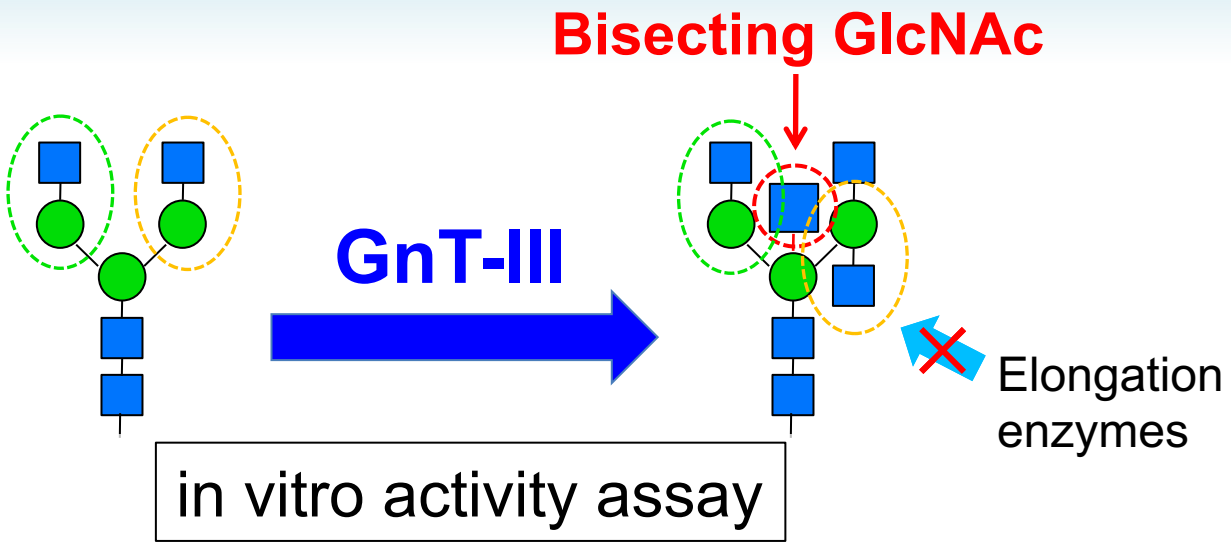


MD (Molecular dynamics) simulation

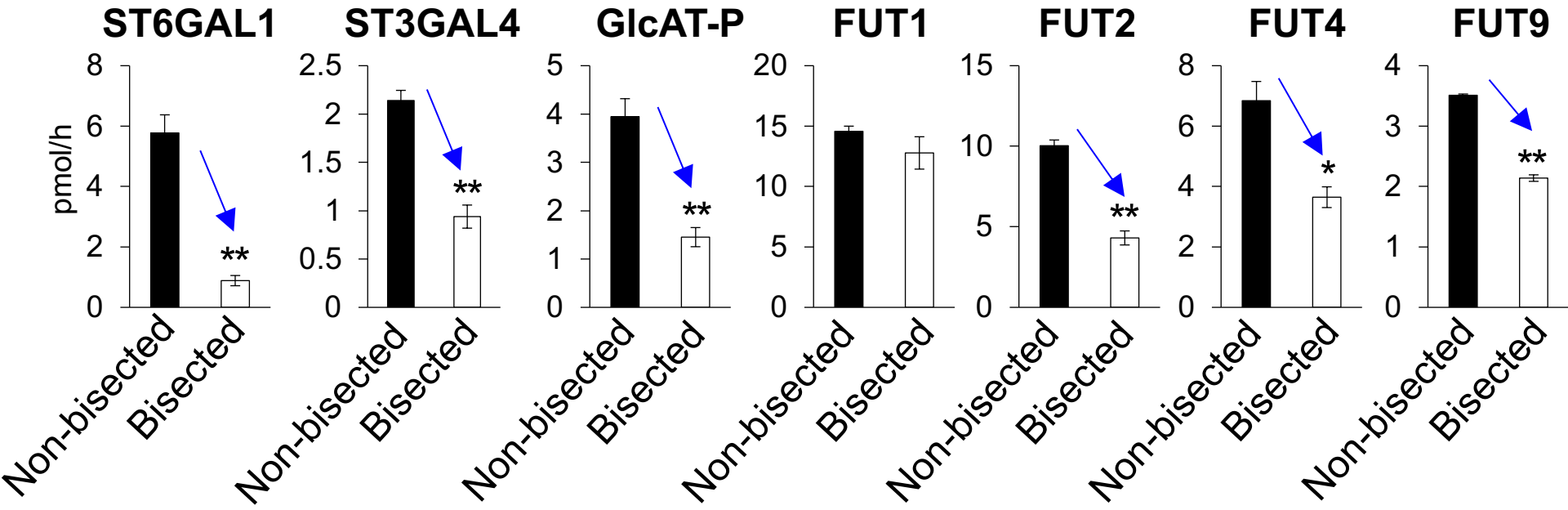


(Nakano et al., ***Mol. Cell. Proteomics***, 2019, 18, 2044-2057)

Bisecting GlcNAc is a key for glycan elongation

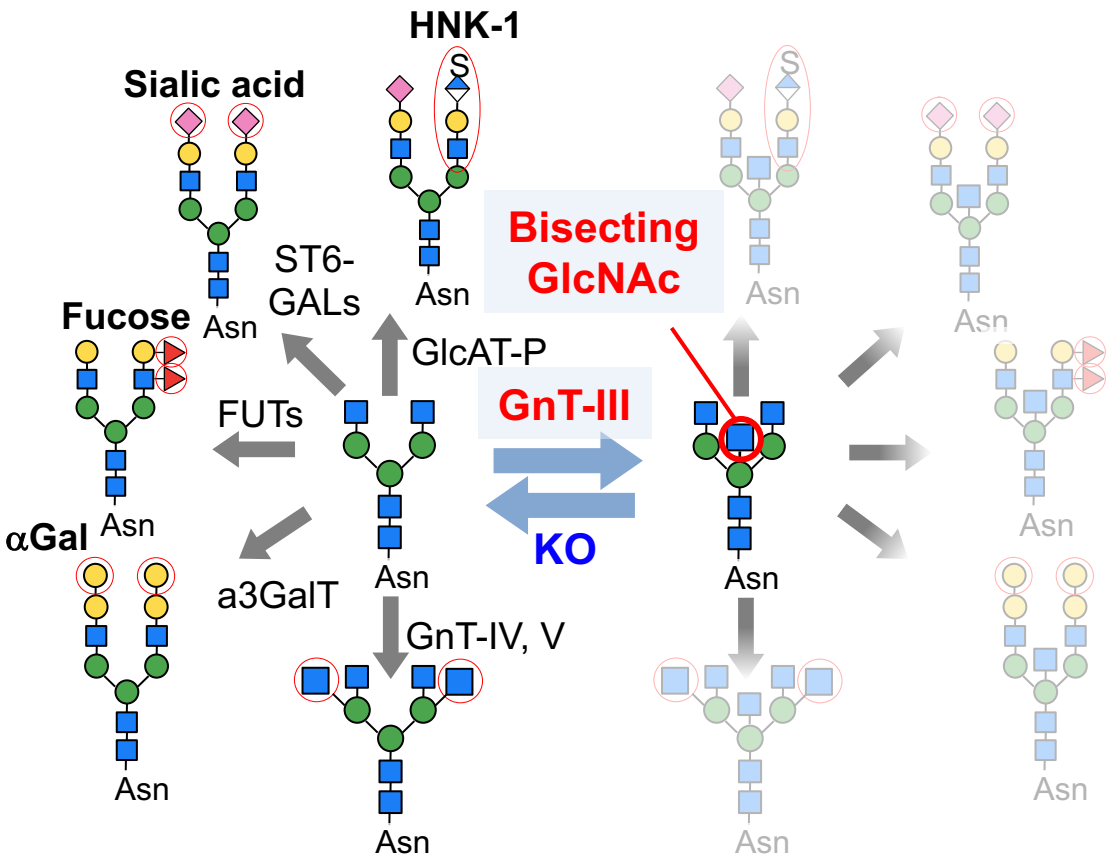


Sialyltransferase Glucuronyltransferase Fucosyltransferase

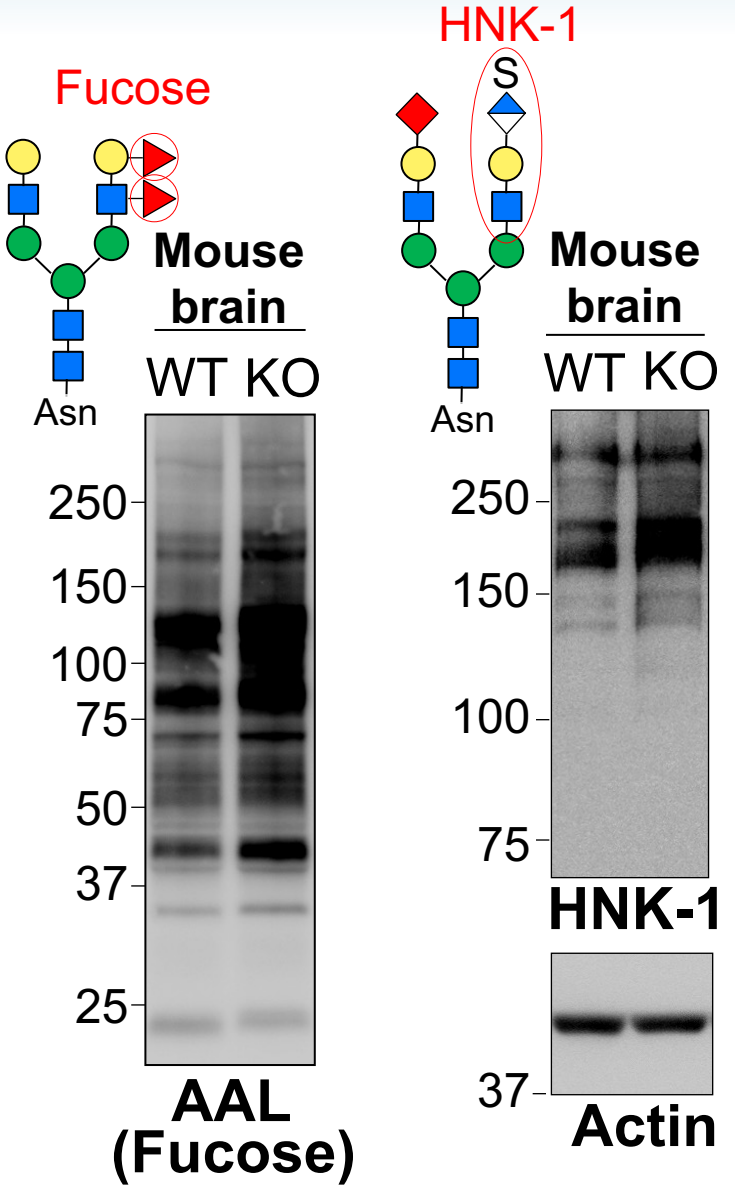


(Nakano et al., ***Mol. Cell. Proteomics***, 2019, 18, 2044-2057)

Bisecting GlcNAc suppresses terminal modifications



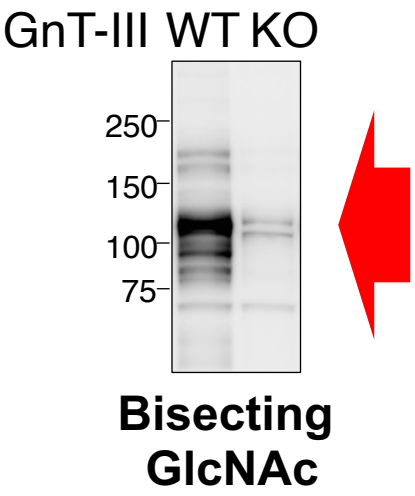
Terminal structures are increased in GnT-III KO

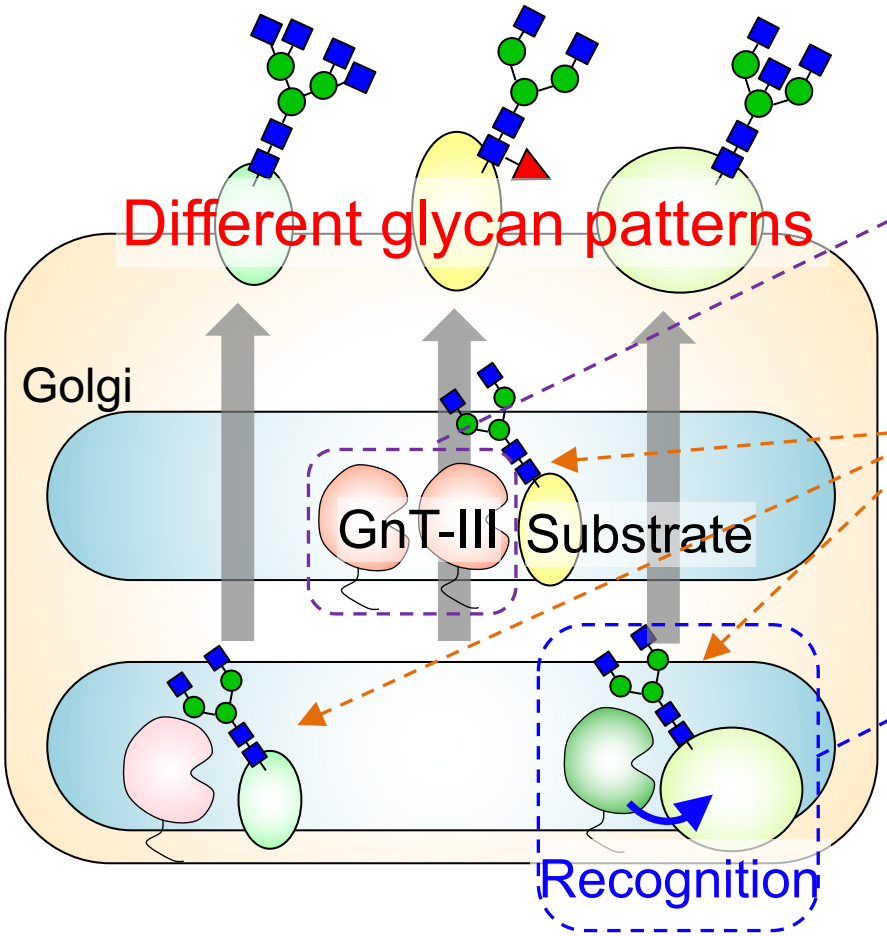


(Nakano et al., ***Mol. Cell. Proteomics***, 2019, 18, 2044-2057)

Question : protein selectivity of GnT-III action

Protein-selective glycosylation





GnT-III's

1. Amount

➡ mRNA, protein (secretion, degradation)

2. Location

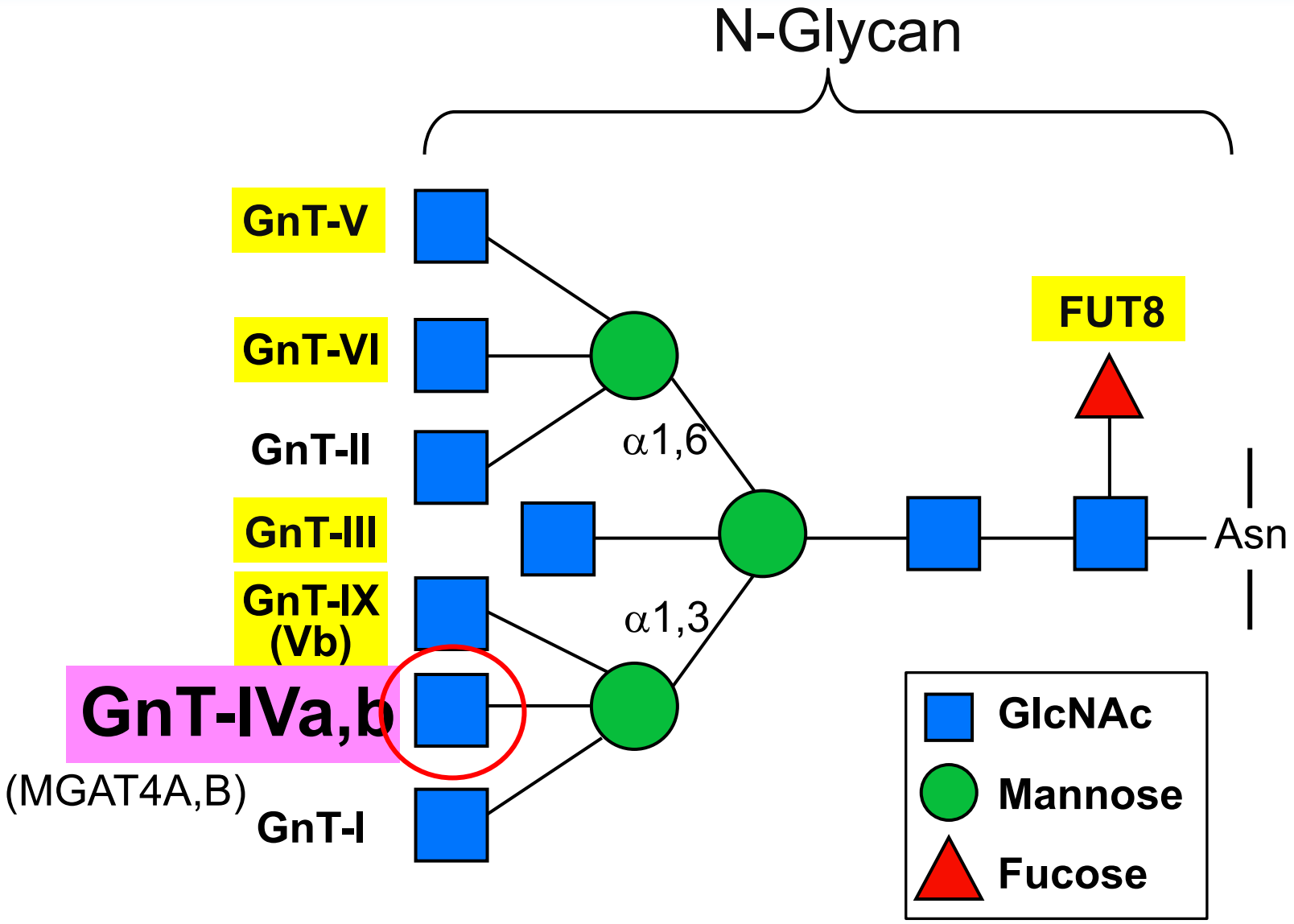
➡ Fine localization in Golgi

3. Reaction

➡ Recognition of polypeptide

Understanding biological functions and diseases

Topic 2 : GnT-IV



GnT-IVa (*MGAT4A*) and type2 diabetes

High expression of
GnT-IVa in pancreas

⇩

Insulin secretion
Control of blood glucose

Prof. Ohtsubo
(Kumamoto)

(Ohtsubo et al., *Cell*, 2005, 123, 1307-1321)
(Ohtsubo et al., *Nat. Med.*, 2011, 17, 1067-1075)

Diabetic phenotype of
GnT-IVa KO mice

Glucose transporter-2 in pancreas
→ enhanced endocytosis

↓

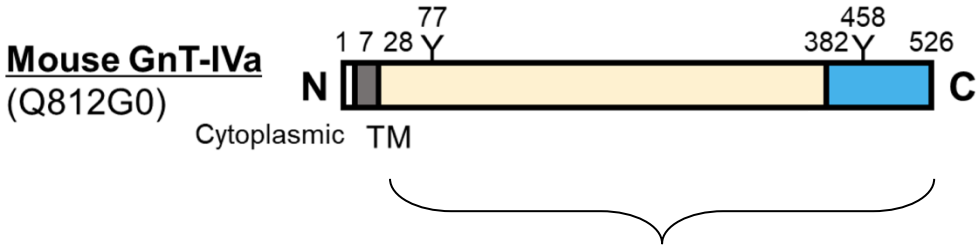
Type2 diabetes

Unclear points

Structure and specificity
of GnT-IVa

→Structural analysis

in collaboration with Dr. Nagae
(Osaka Univ.)



Relatively large as a
glycosyltransferase catalytic domain

⇩

multiple domains??

(Nagae et al., ***Commun. Biol.***, 2022, 5, 695)

GnT-IVa has a lectin domain

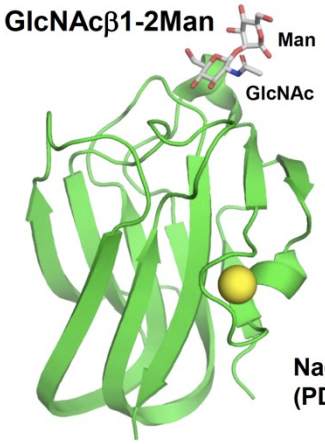
Phyre2 server (prediction of protein fold)

(Kelley et al., *Nat. Protocols*, 2015, 10, 845-858)

C-term region is similar to a bacterial lectin NagH

#	Template	Alignment Coverage	3D Model	Confidence	% i.d.	Template Information
1	c2ls6A_	Alignment		99.0	14	**PDB header:**hydrolase **Chain:** A: **PDB Molecule:**hyaluronoglucosaminidase; **PDBTitle:** solution nmr structure of a non-canonical galactose-binding cbm32 from2 clostridium perfringens
2	c5vcmA_	Alignment		97.7	15	**PDB header:**transferase **Chain:** A: **PDB Molecule:**alpha-1,6-mannosyl-glycoprotein 2-beta-n- **PDBTitle:** alpha-1,6-mannosyl-glycoprotein 2-beta-n-acetylglucosaminyltransferase2 with bound udp and manganese

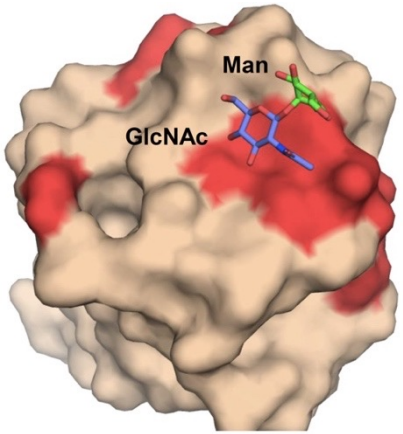
N-term region is similar to GnT-II



NagH CBM32
(PDB code: 2WDB)

Binds to GlcNAc

Sugar-binding domain of clostridium glycosidase

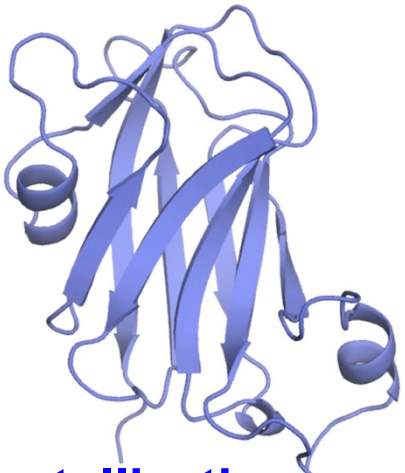


Residues conserved between NagH and GnT-IV (red)

GnT-IV C-term is likely a GlcNAc-binding lectin

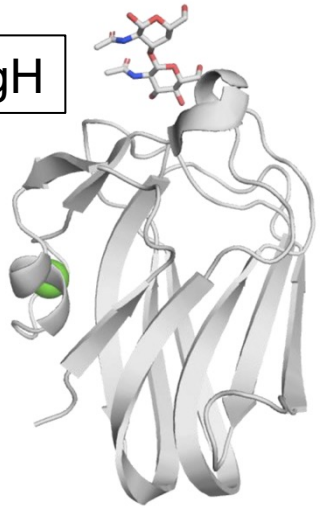
Crystal structure of the lectin domain

GnT-IVa lectin domain



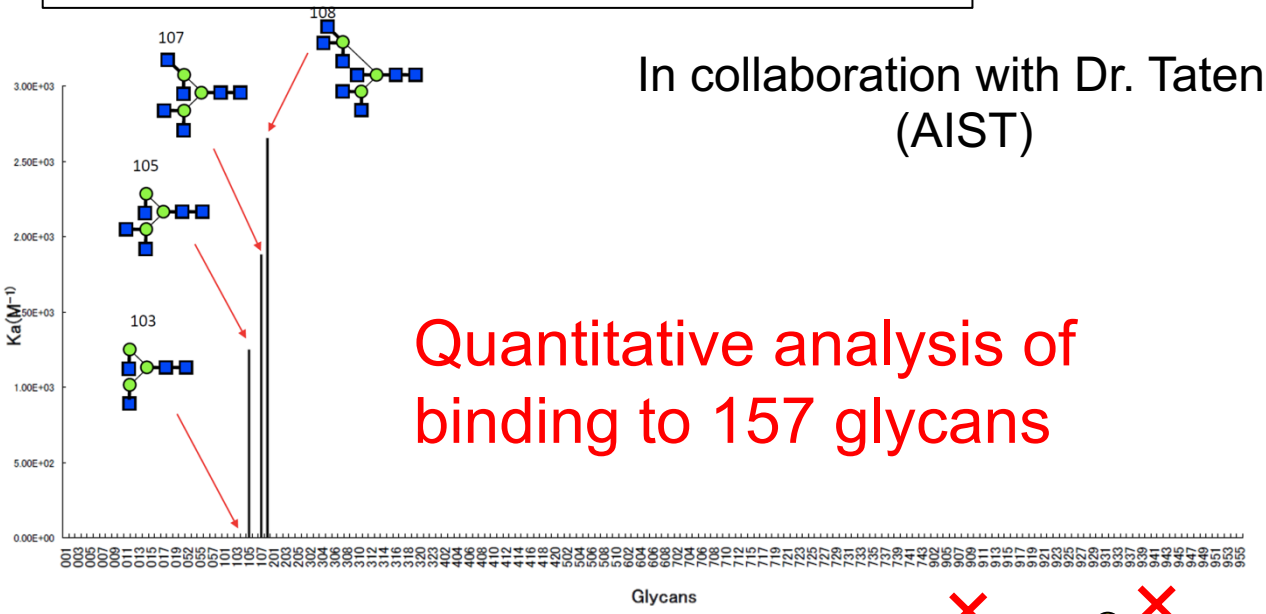
Crystallization was succeeded

NagH



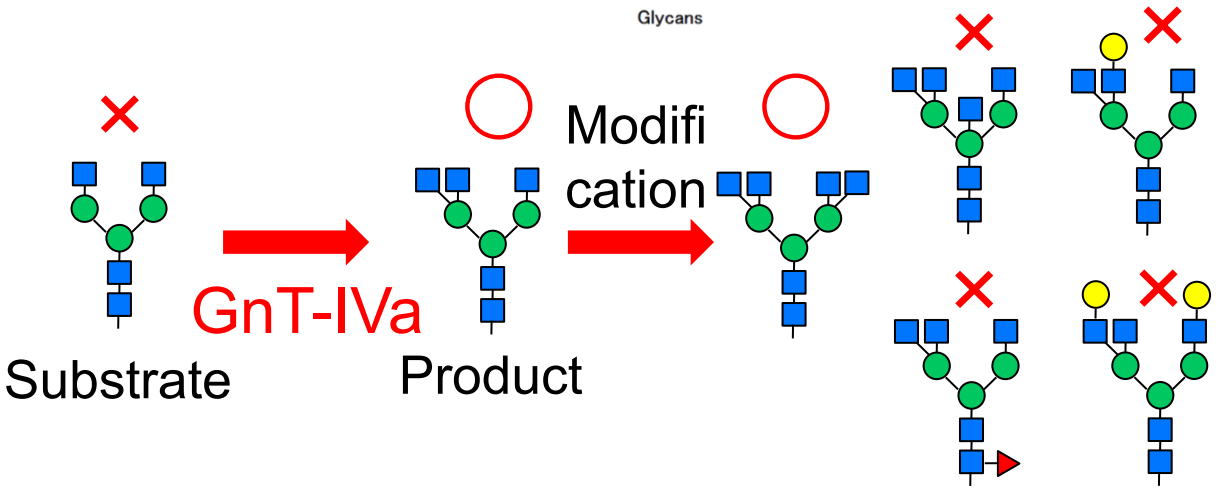
→ Similar to NagH

Frontal affinity chromatography



In collaboration with Dr. Tateno (AIST)

Quantitative analysis of binding to 157 glycans

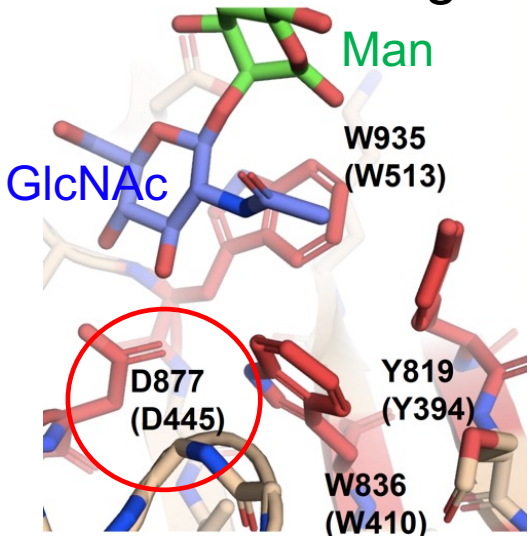


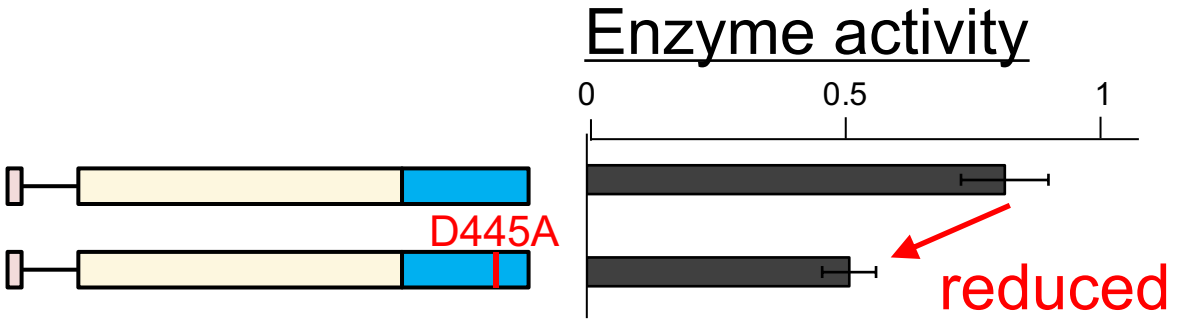
Lectin domain recognizes the product glycan

(Nagae et al., ***Commun. Biol.***, 2022, 5, 695)

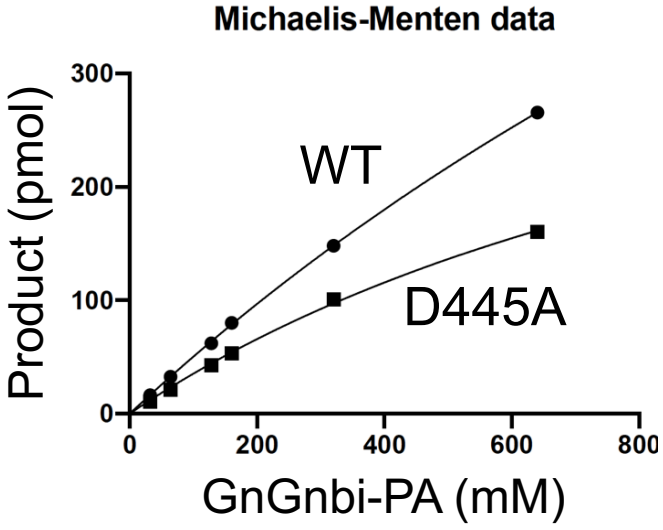
Lectin domain is required for enzyme activity

Glycan binding domain of NagH





Lectin domain is required for full activity

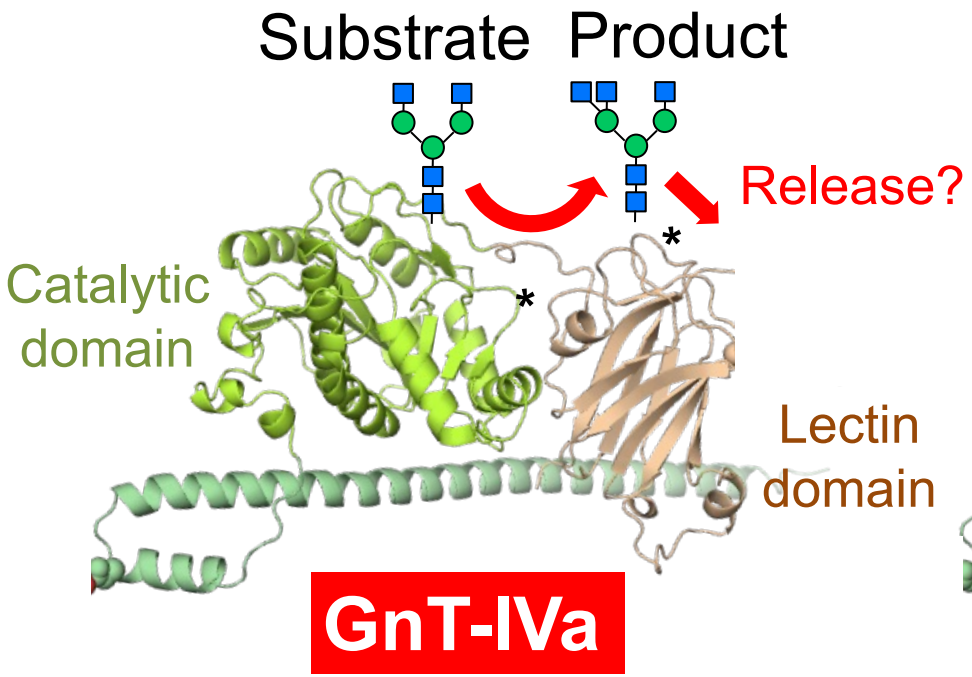


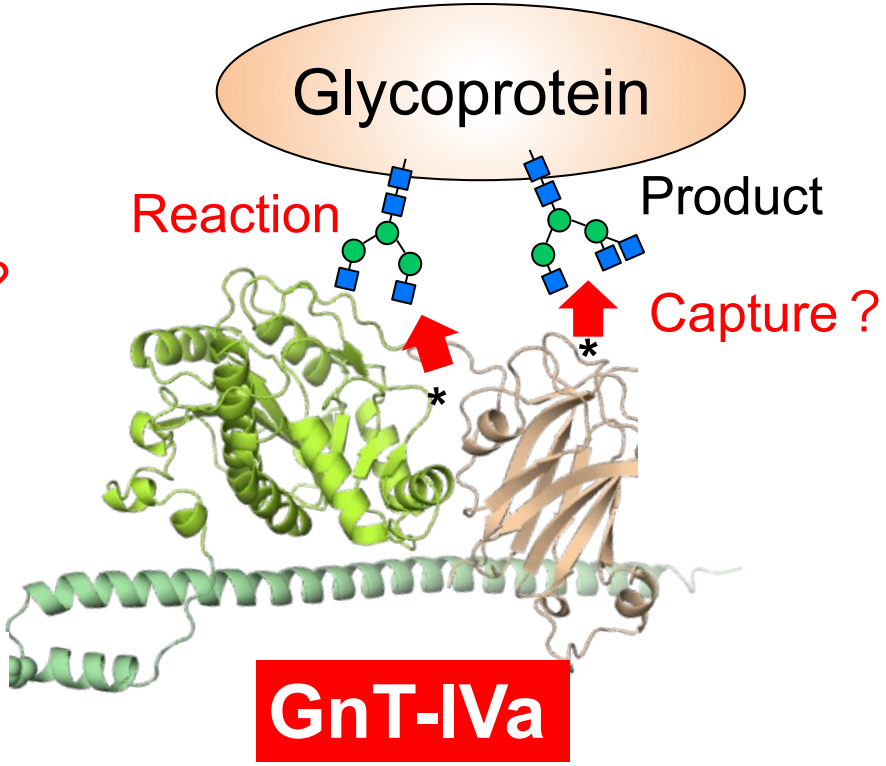
	Km for donor (mM)	Km for acceptor (μM)
WT	3.24	2444
D445A	3.95	1251

similar **Higher affinity of the mutant (roughly half Vmax)**

(Nagae et al., ***Commun. Biol.***, 2022, 5, 695)

Two models

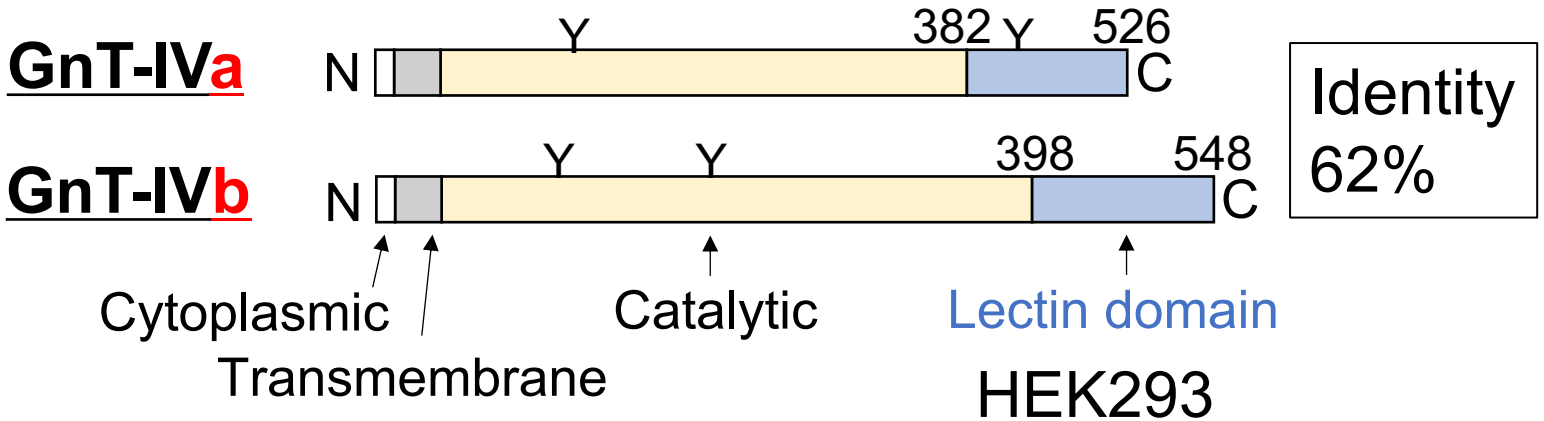




Acceleration of sugar transfer reaction itself

Easy to modify Multiple glycosylation sites in one protein

GnT-IVa, -IVb : Why two isozymes?



Identity 62%

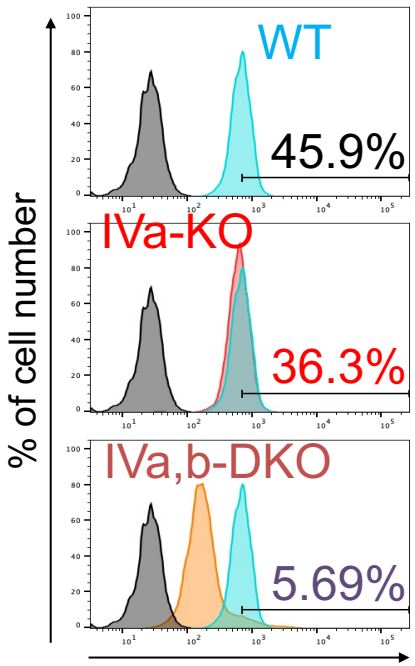
Tissue distribution

GnT-IVa (*MGAT4A*) — Limited tissue (pancreas, etc)

GnT-IVb (*MGAT4B*) — Ubiquitous

(Yoshida et al., *Glycoconj. J.*, 1998, 15, 1115-1123)

HEK293



Both are functional within the same cell

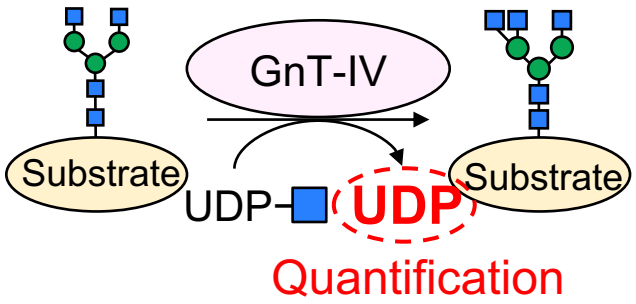
Different roles?

Protein selectivity? (almost the same specificity toward glycans)

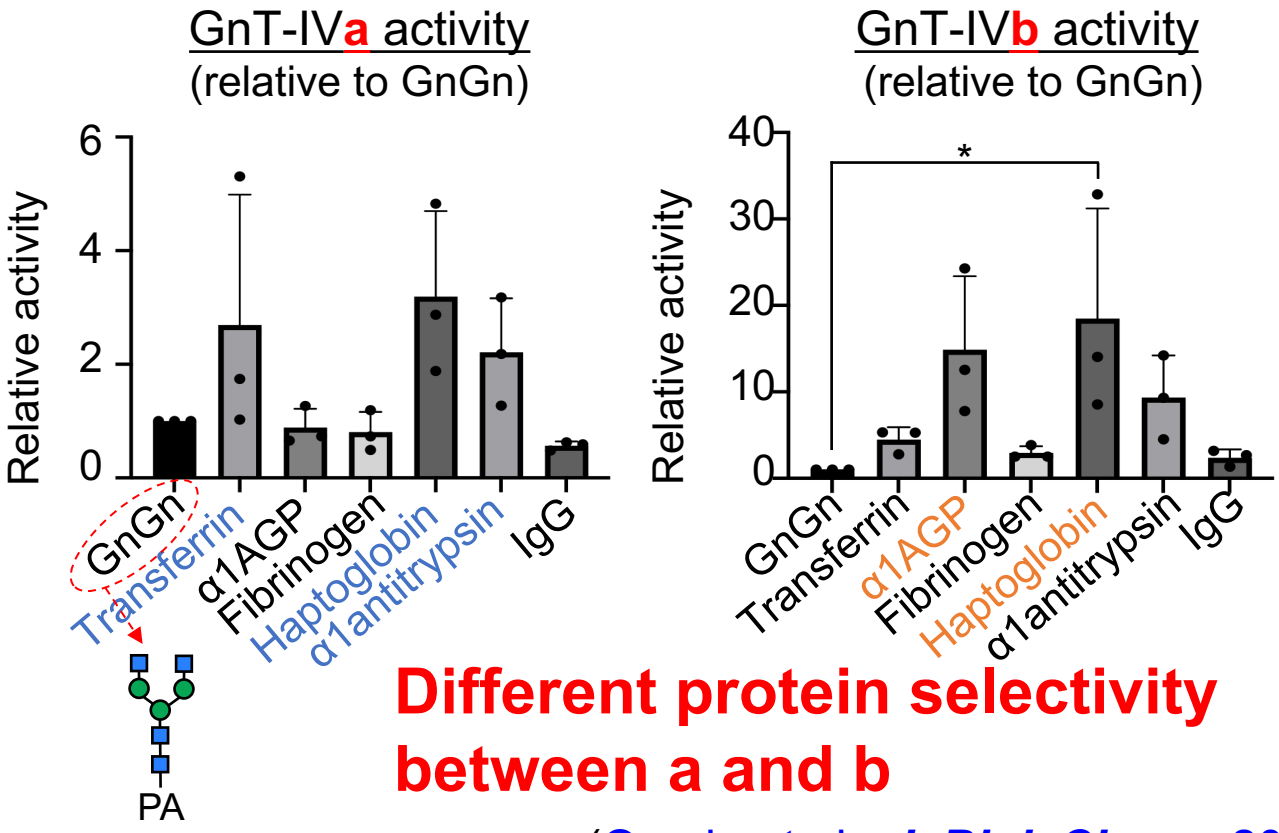
(Osada et al., ***J. Biol. Chem.***, 2022, 298, 102400)

Enzyme activity toward glycoproteins

UDP-Glo assay



Activity toward
6 glycoproteins



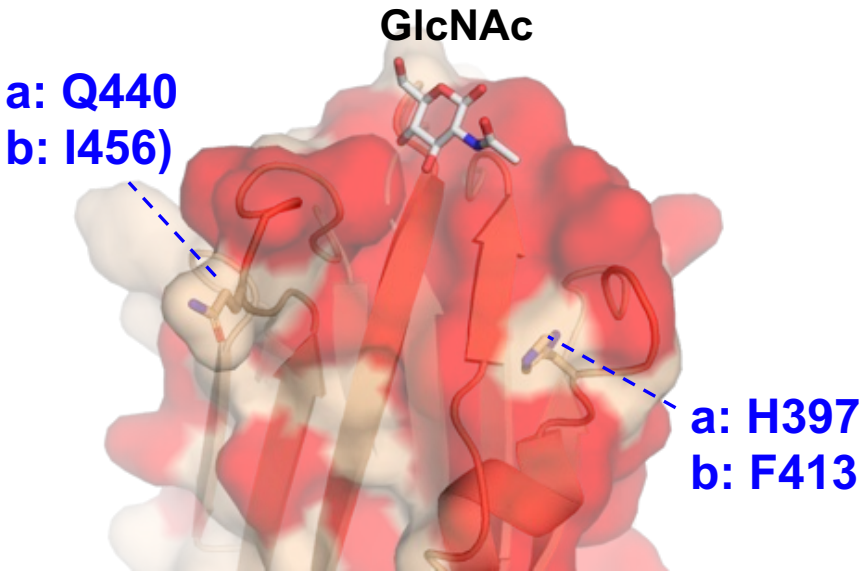
Different protein selectivity between a and b

(Osada et al., ***J. Biol. Chem.***, 2022, 298, 102400)

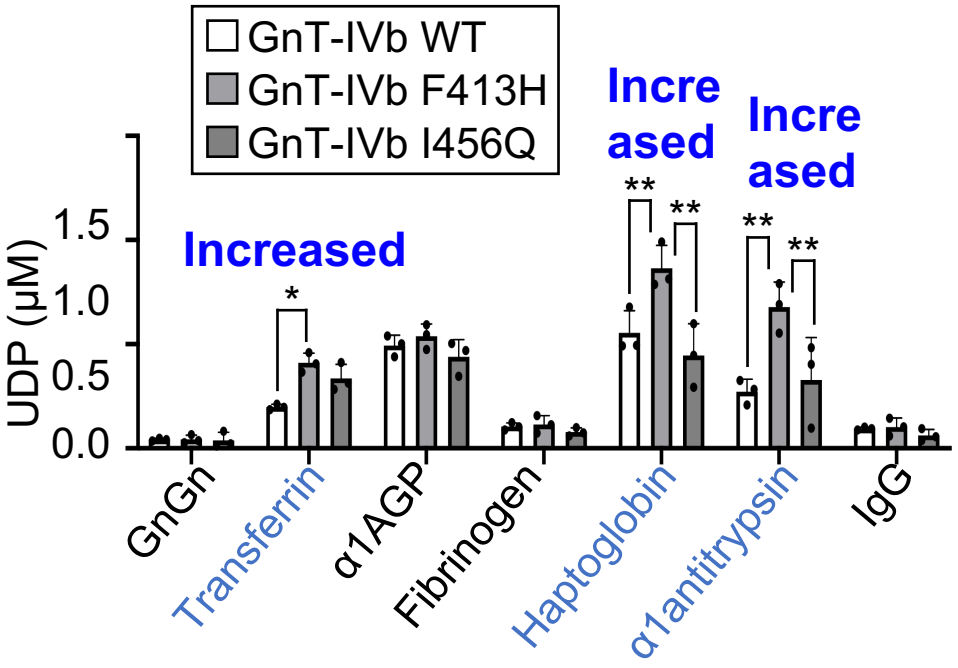
Lectin domain regulates protein selectivity

Structure of lectin domain

Red : conserved between a and b



UDP-Glo assay

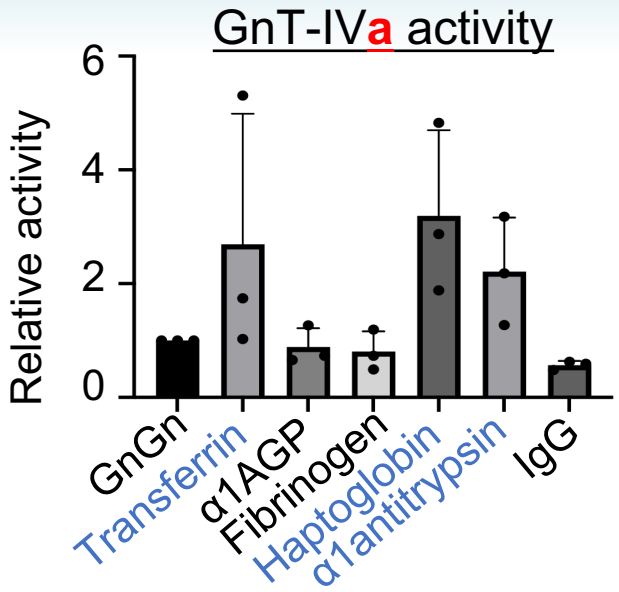


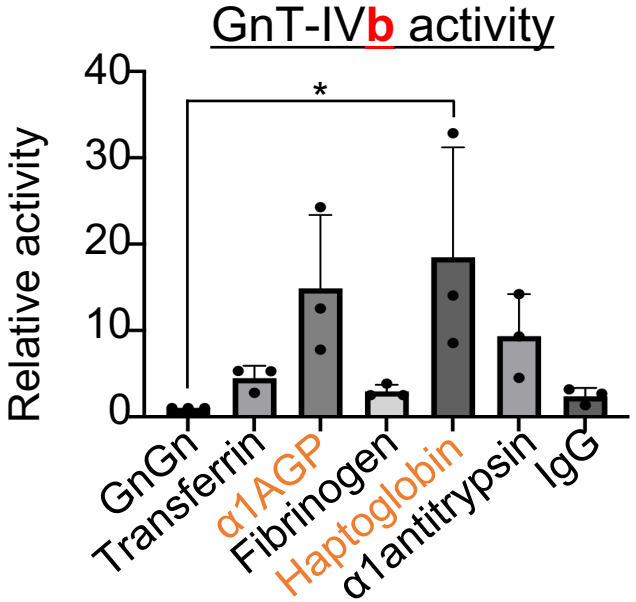
2 unconserved amino acids

➡ **Mutant IVb was purified whose 2 amino acids were replaced by IVa type residues.**

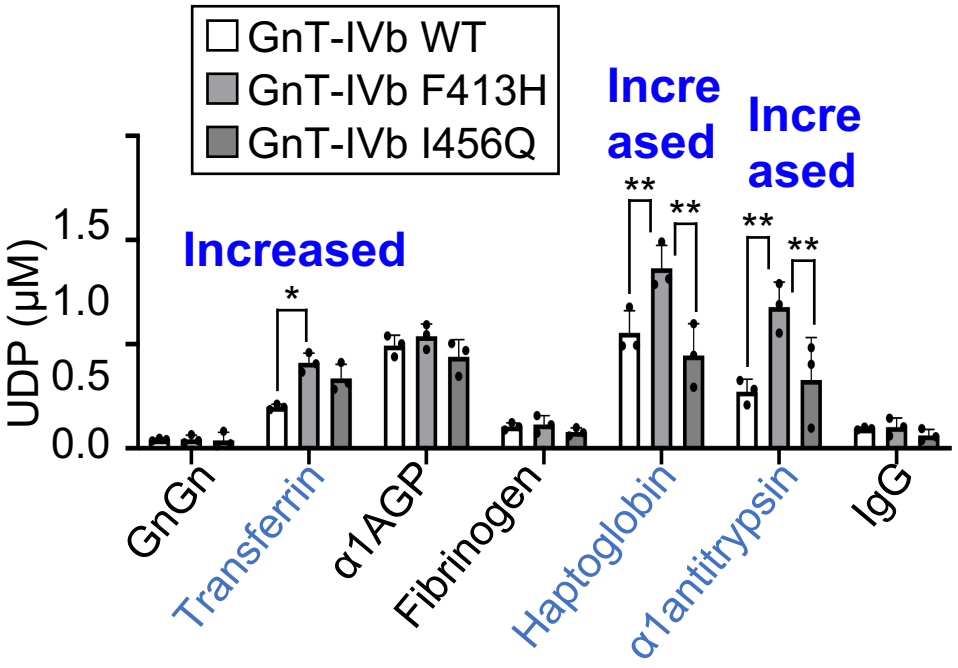
(Osada et al., ***J. Biol. Chem.***, 2022, 298, 102400)

Lectin domain regulates protein selectivity





UDP-Glo assay

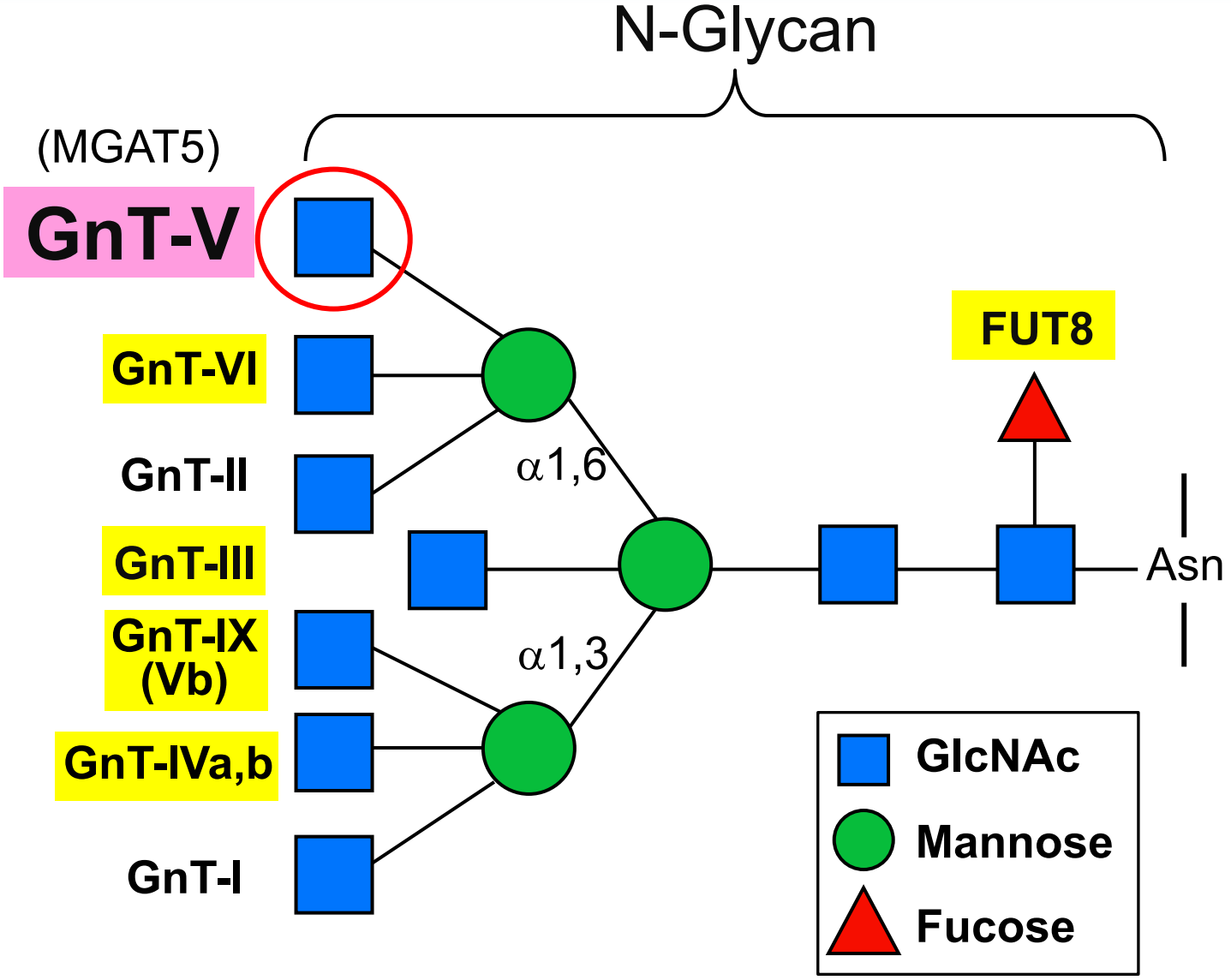


Lectin domain mutant of GnT-IVb F413H shows IVa-like selectivity

GnT-IVa,b has different protein selectivity regulated by lectin domain

(Osada et al., ***J. Biol. Chem.***, 2022, 298, 102400)

Topic 3 : GnT-V

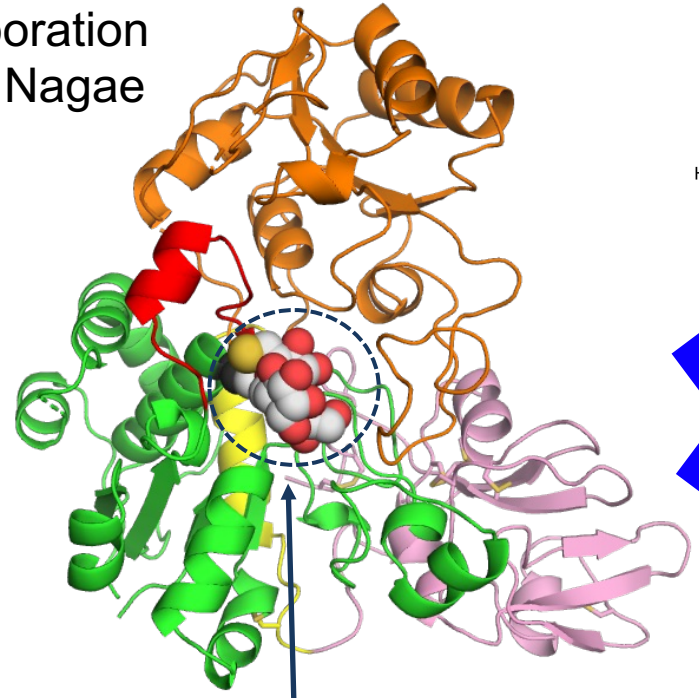


GnT-V inhibitor is a promising drug candidate against cancer

3D structure of GnT-V (MGAT5)

GnT-V structure

in collaboration with Dr. Nagae

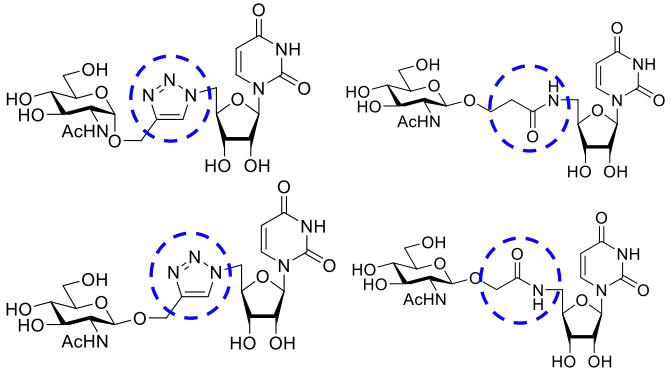


Substrate analog

Mechanism of glycan recognition

(Nagae# and Kizuka#, ***Nat. Commun.***, 2018, 9, 3380)

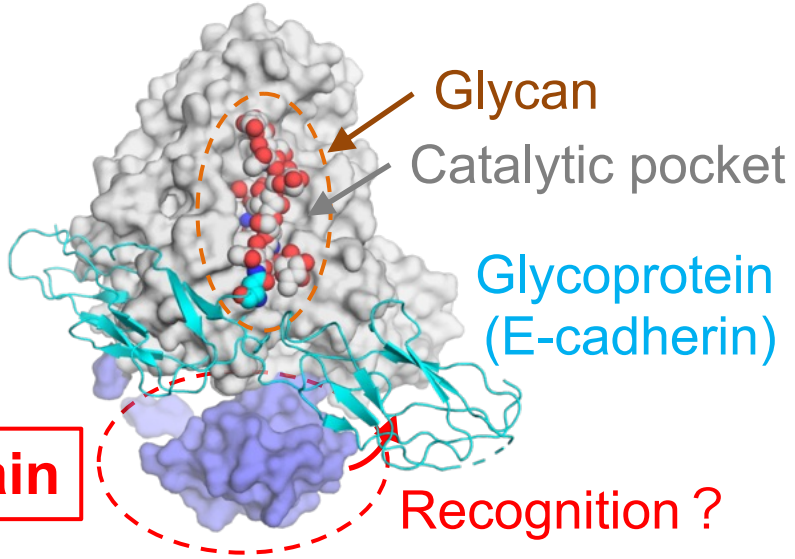
Structure-based inhibitor design



Chemistry
Prof. Ando
Dr. Tanaka
(Gifu Univ.)

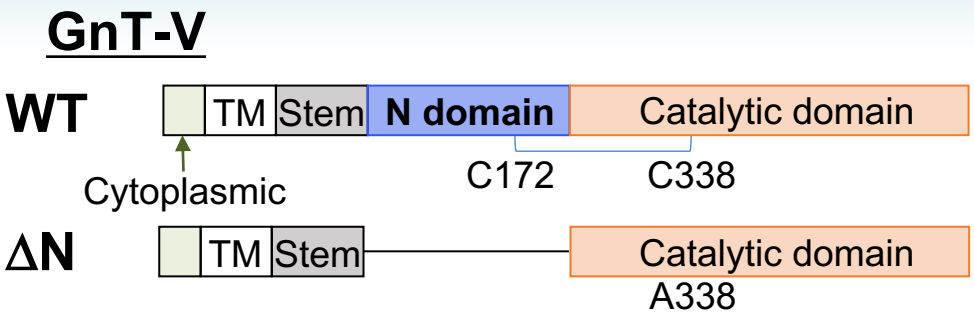
(Vibhute, et al., ***BBA Gen. Subj.***, 2021, 1866, 130118)

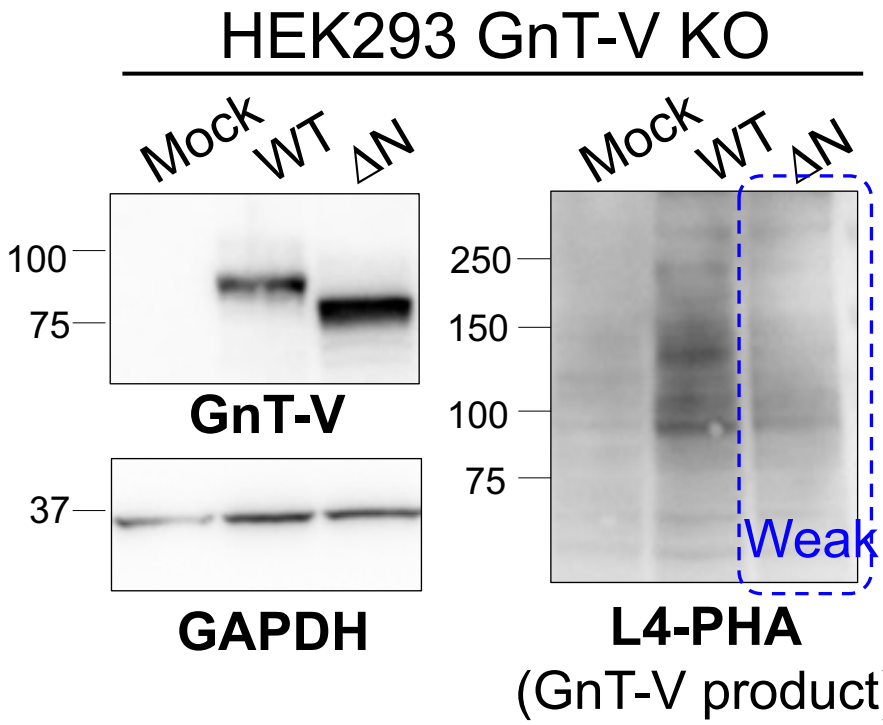
Recognition of substrate protein

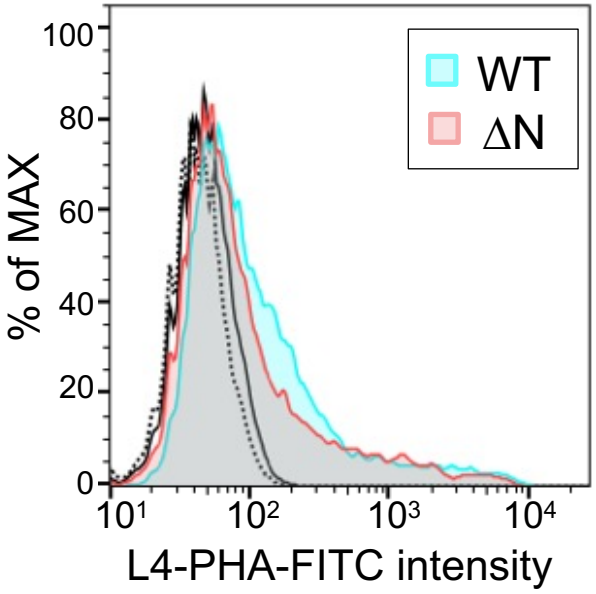


(Osuka et al., ***J. Biol. Chem.***., 2022, 298, 101666)

ΔN shows reduced activity in cells



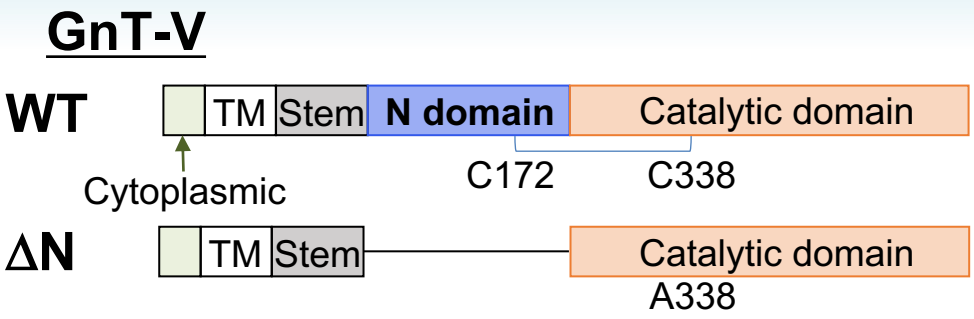


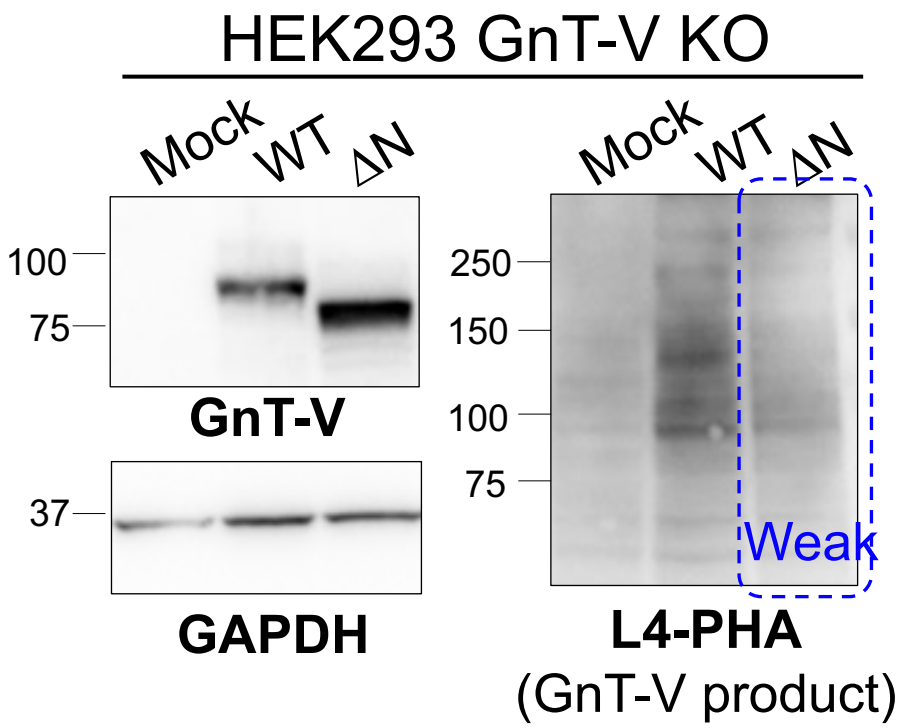


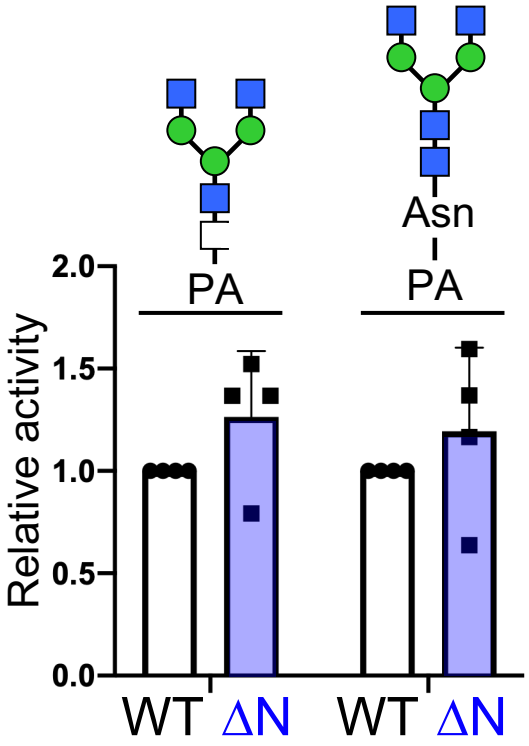
N domain is required for efficient glycosylation in cells

(Osuka et al., ***J. Biol. Chem.***., 2022, 298, 101666)

ΔN shows reduced activity in cells





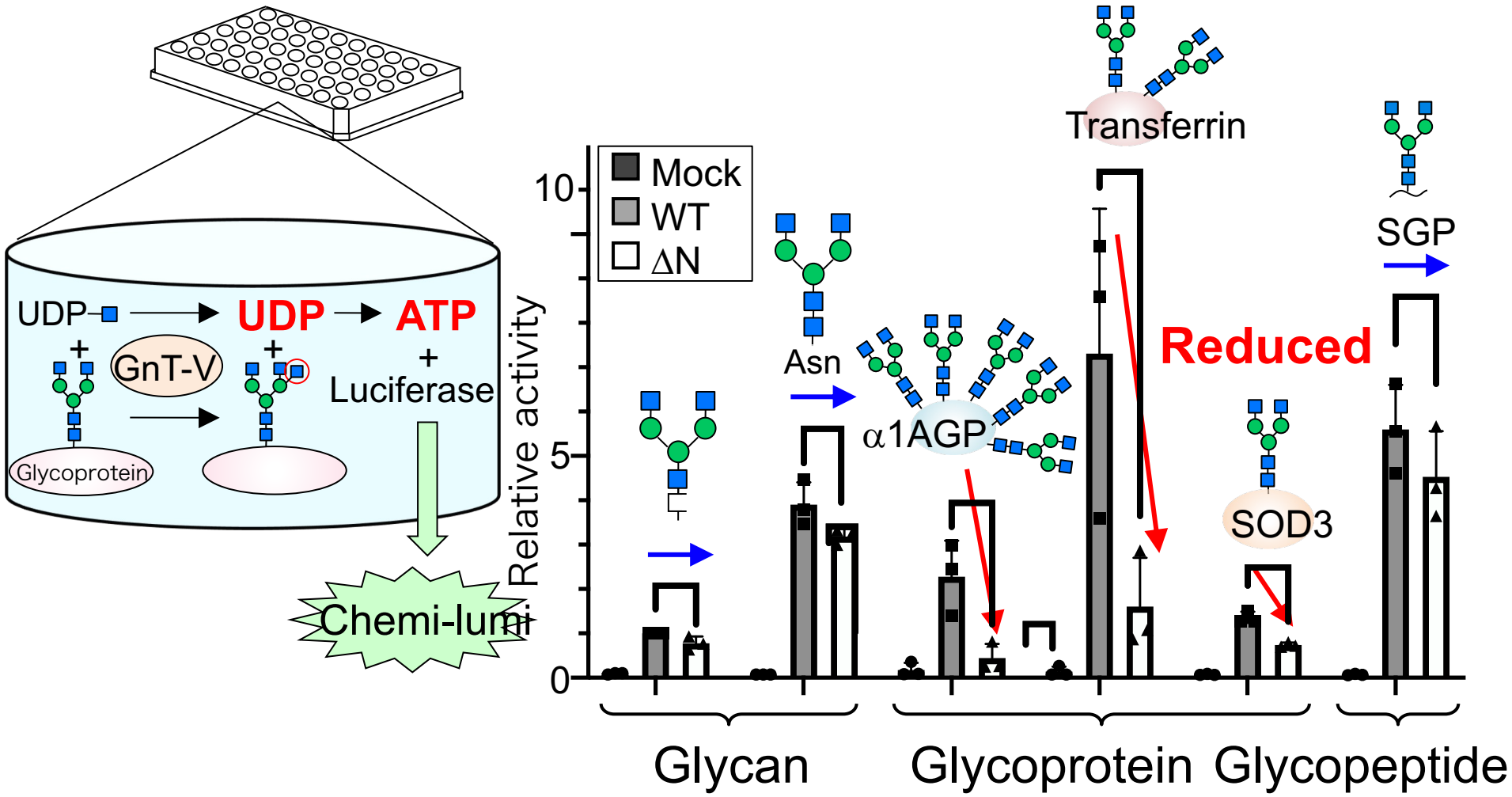


GnT-VΔN fully retains activity toward glycans

N domain is required for efficient glycosylation in cells

(Osuka et al., ***J. Biol. Chem.***., 2022, 298, 101666)

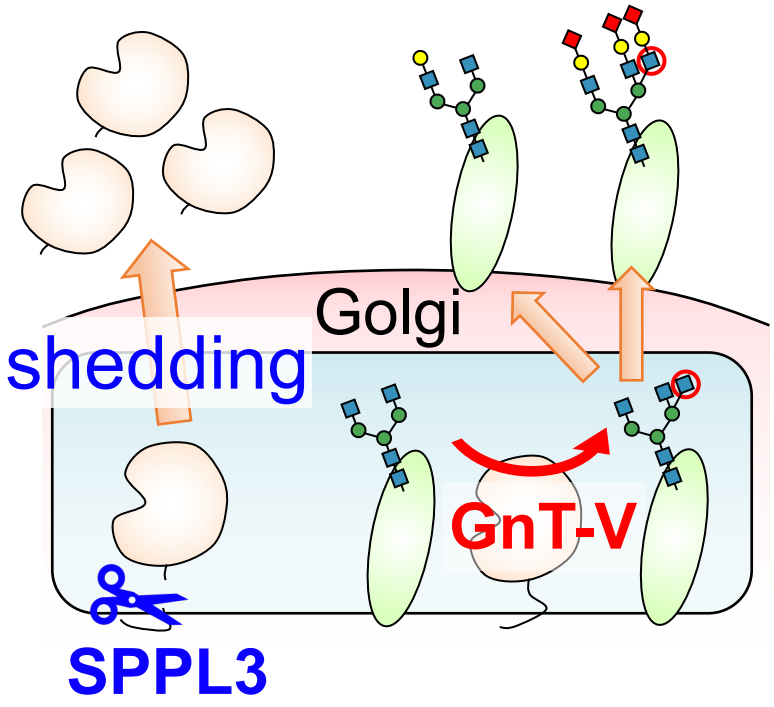
Activity assay toward glycoproteins (UDP-Glo)



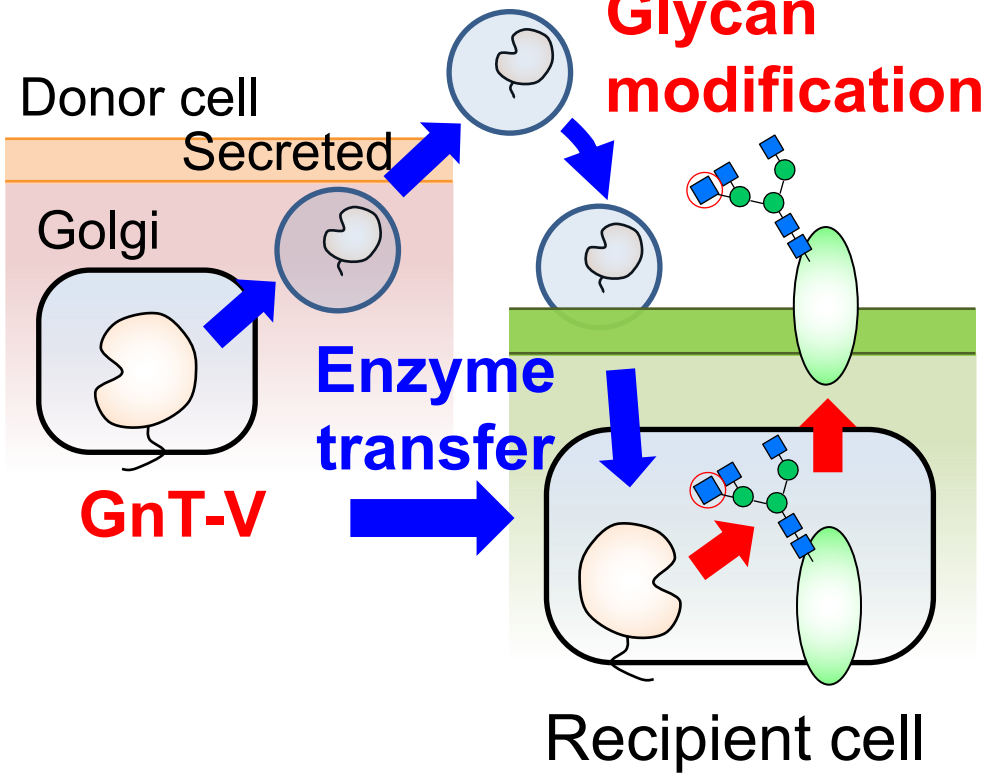
N domain is required for activity toward glycoproteins

(Osuka et al., ***J. Biol. Chem.***., 2022, 298, 101666)

Regulation of the levels of GnT-V protein



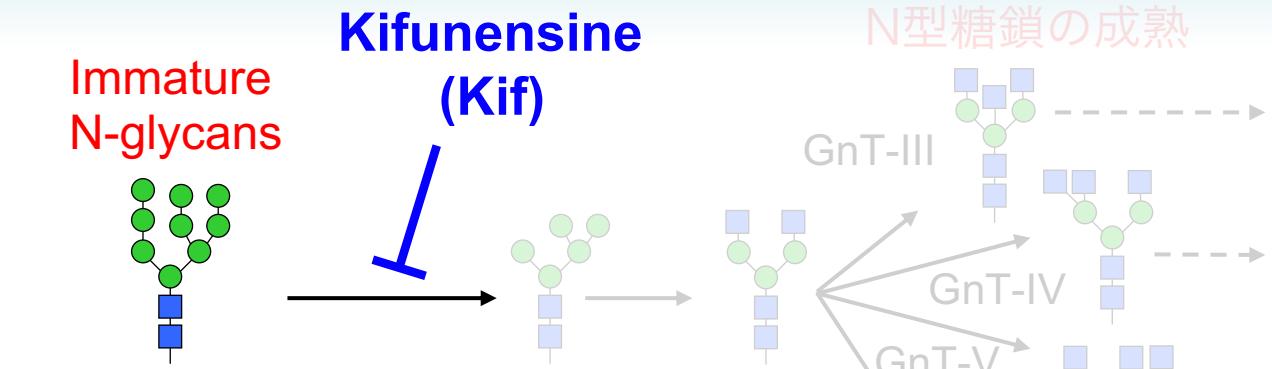
Small extracellular vesicle (sEV) (exosome etc.)



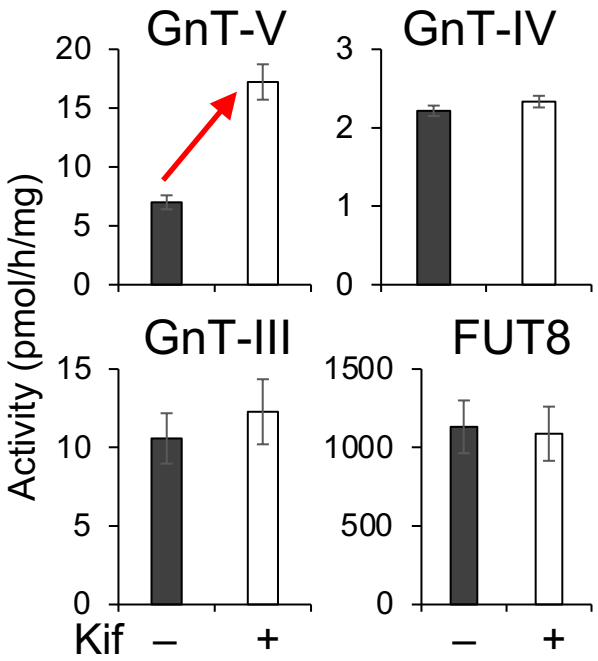
GnT-V is cleaved and secreted (Hirata, et al., ***Commun. Biol.***, 2022, 5, 743)

GnT-V is present in sEV (Hirata, et al., ***iScience***, 2022, 26, 105747)

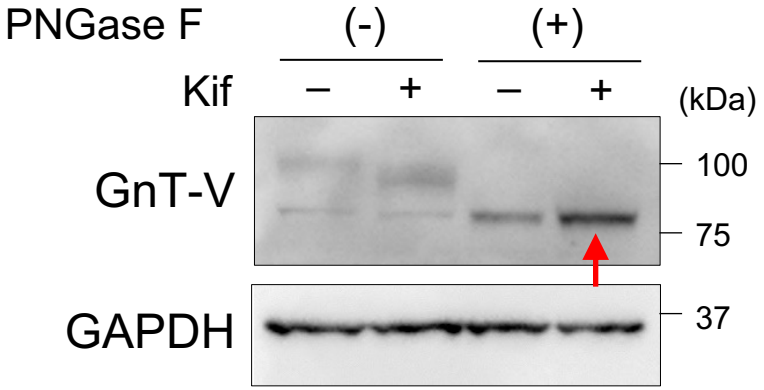
GnT-V protein is increased by blocking N-glycan maturation



Activity of cell lysate



Western blot of cell lysate

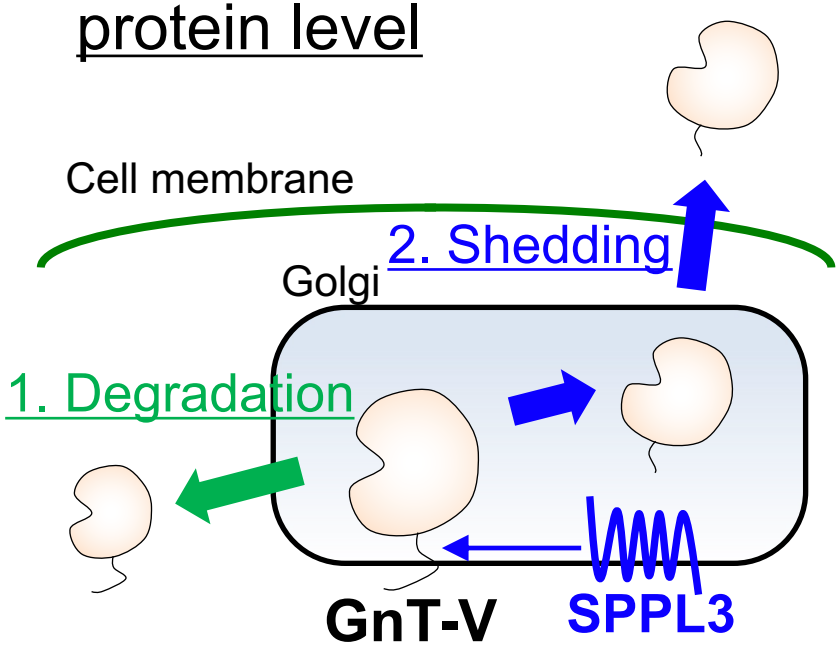


GnT-V is increased by blocking N-glycan maturation

(Hirata, et al., ***Commun. Biol.***, 2022, 5, 743)

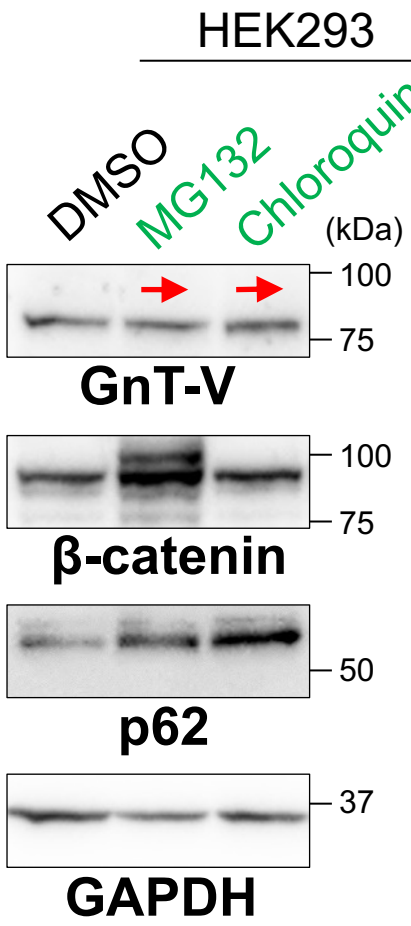
SPPL3-mediated cleavage of GnT-V

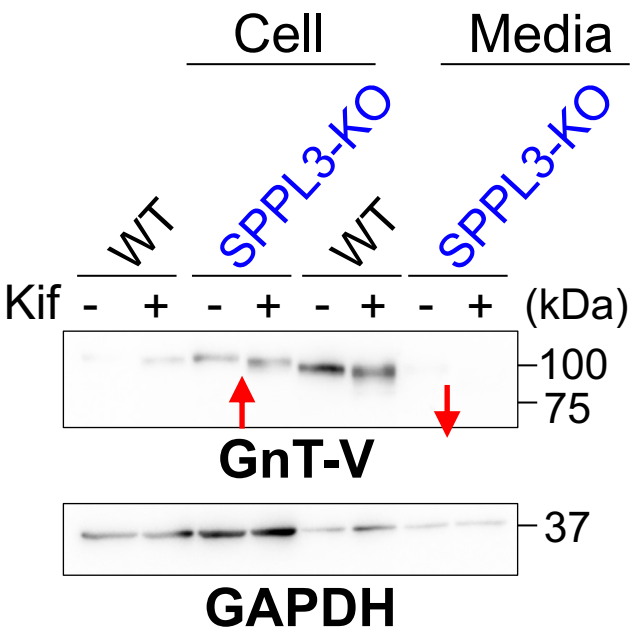
Regulation of the GnT-V protein level



Which regulates the level of GnT-V protein dominantly?

MG132: proteasome inhibitor
Chloroquine: lysosome inhibitor

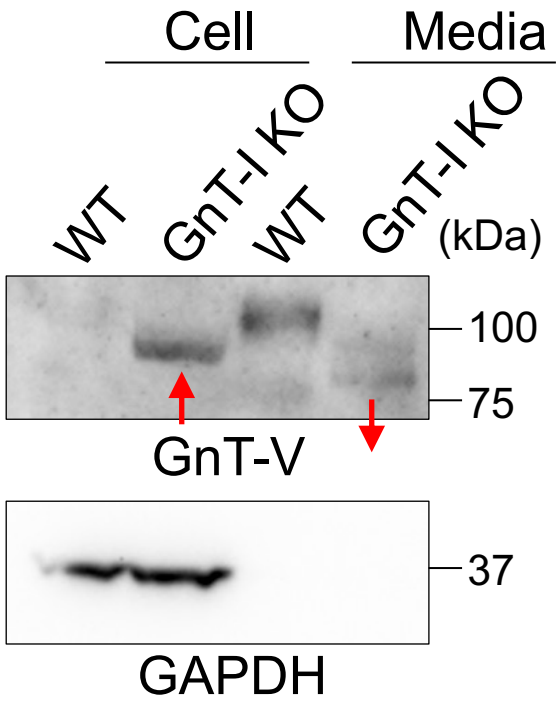




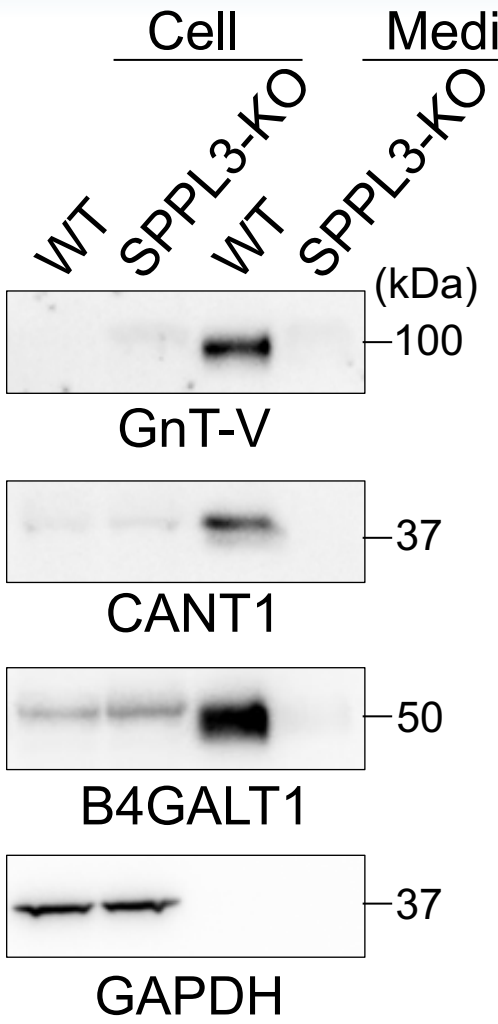
SPPL3-mediated shedding dominantly regulates the GnT-V protein level

(Hirata, et al., ***Commun. Biol.***, 2022, 5, 743)

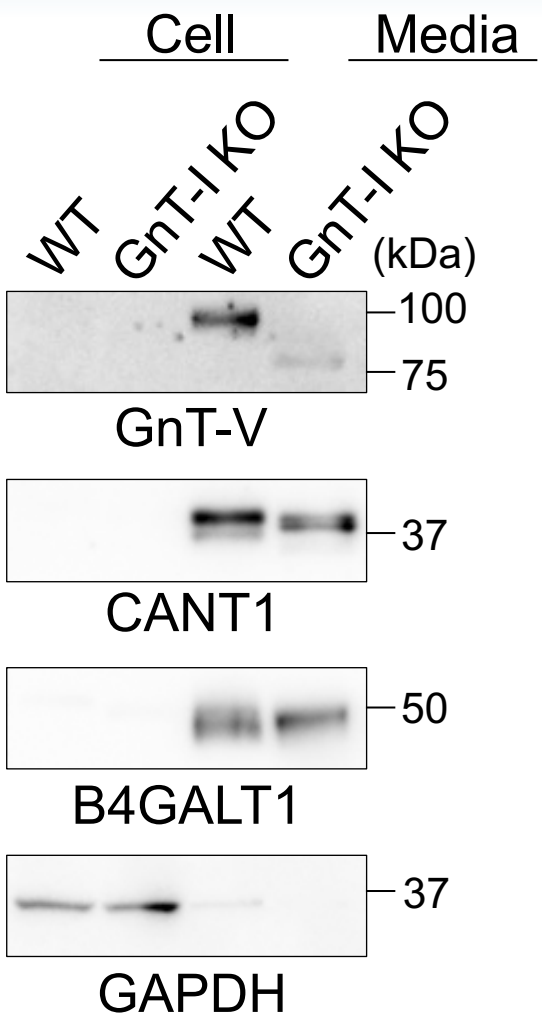
Reduced GnT-V shedding by blocking N-glycan maturation



Reduced SPPL3-dependent secretion



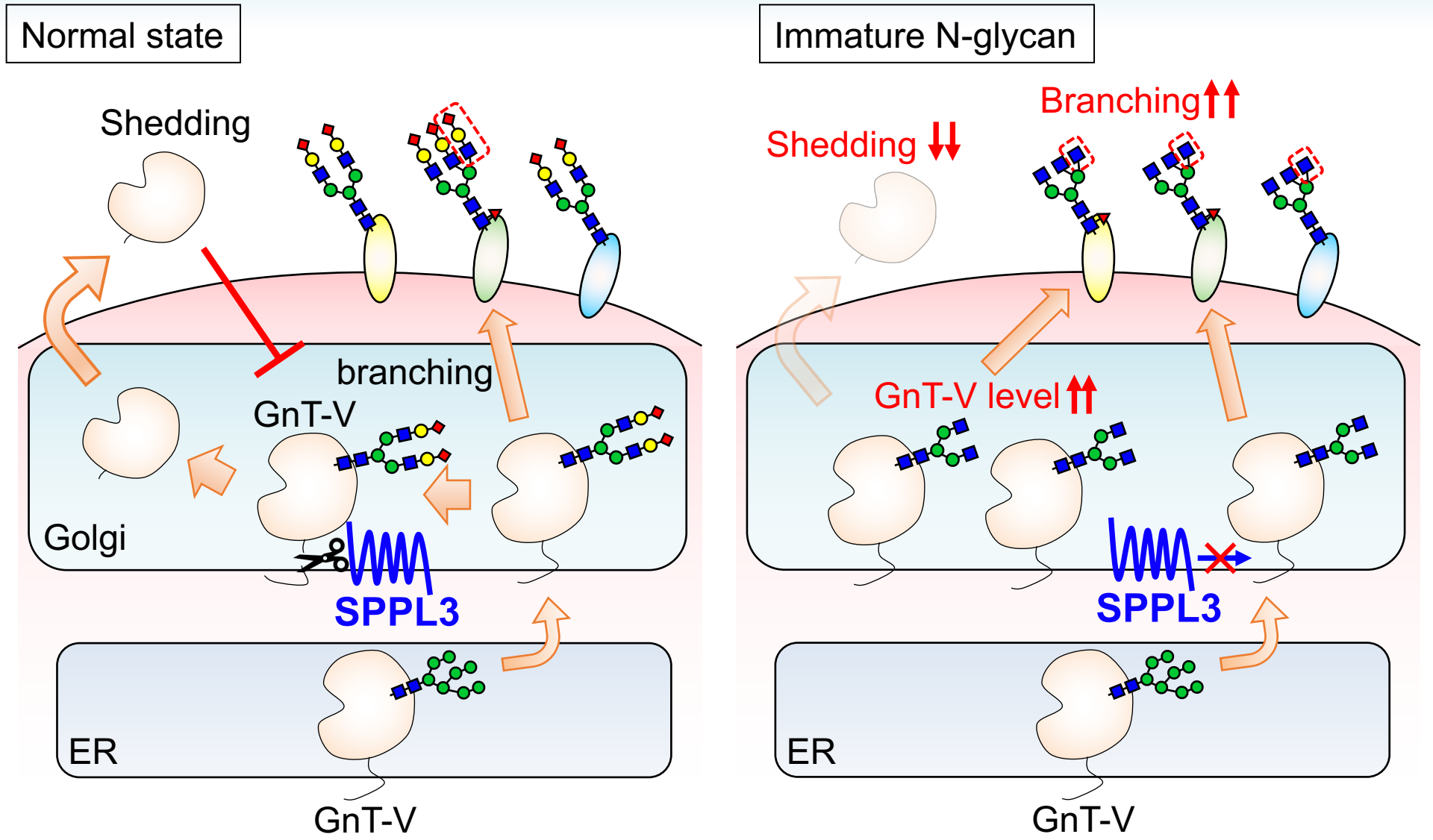
All are SPPL3 substrates



The largest reduction in GnT-V

(Hirata, et al., ***Commun. Biol.***, 2022, 5, 743)

Mechanism of control of GnT-V secretion

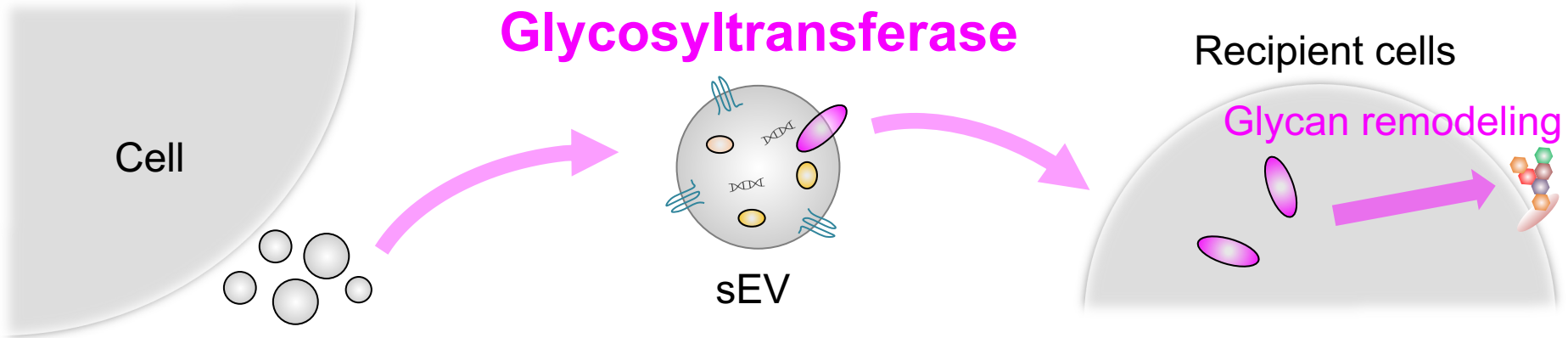


Amount of GnT-V protein depends on cellular N-glycan maturity

(Hirata, et al., ***Commun. Biol.***, 2022, 5, 743)

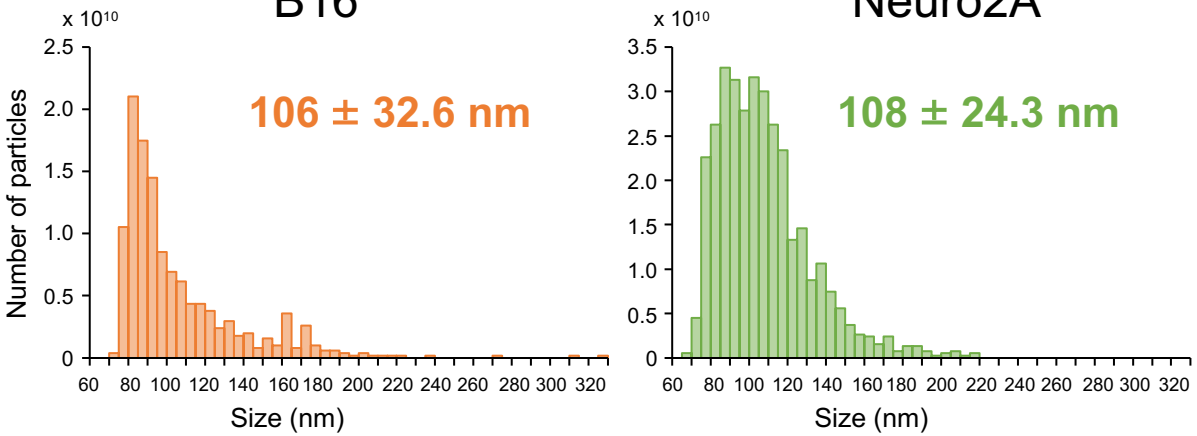
Glycosyltransferase activity in sEV

Hypothesis

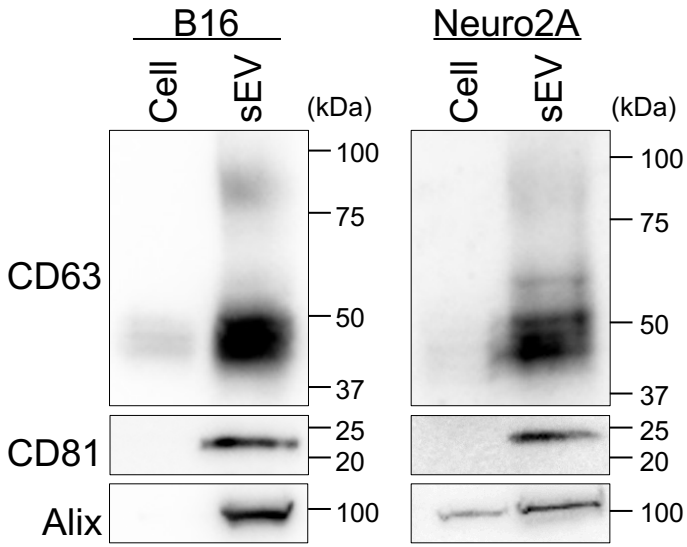


Preparation of sEVs

Vesicle size (qNano)



Exosome markers

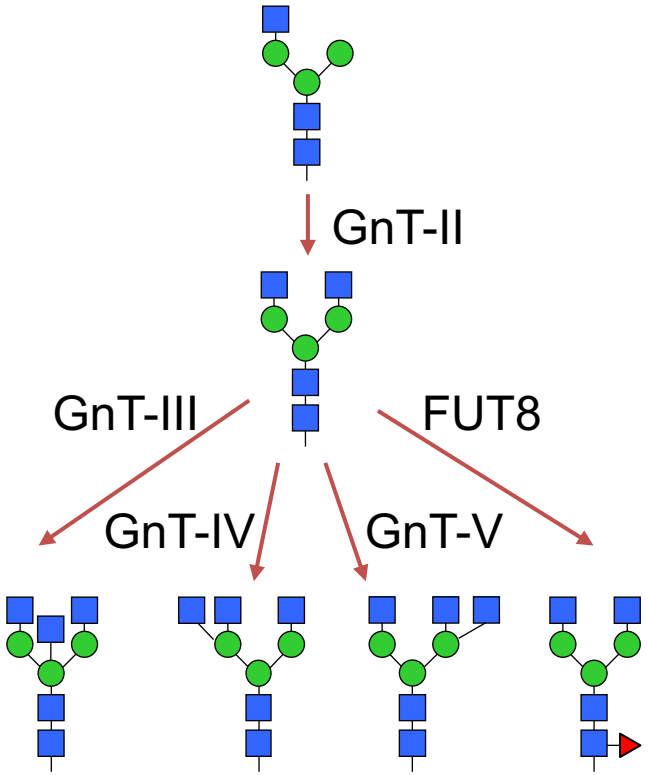


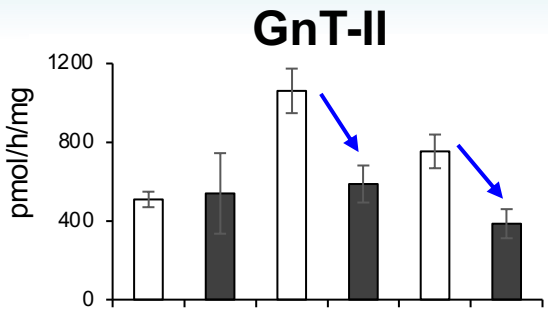
Exosome-rich vesicles were prepared

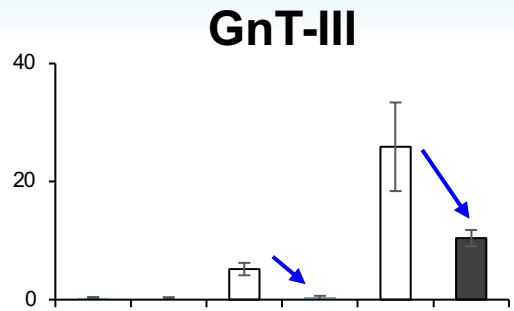
(Hirata, et al., ***iScience***, 2022, 26, 105747)

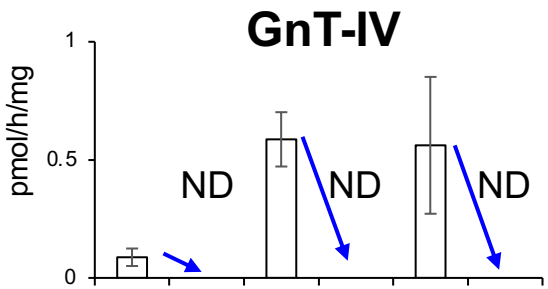
Activity of glycosyltransferase in sEVs

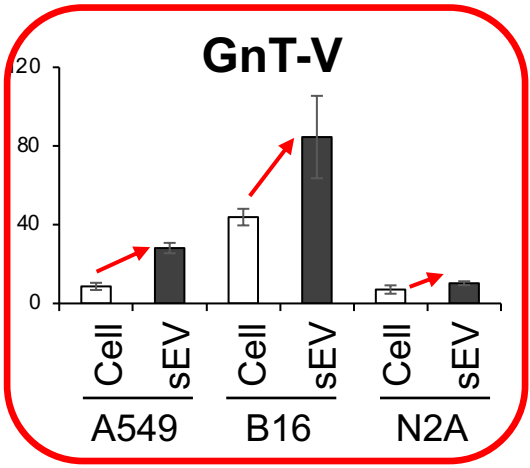
N-glycan branching enzymes

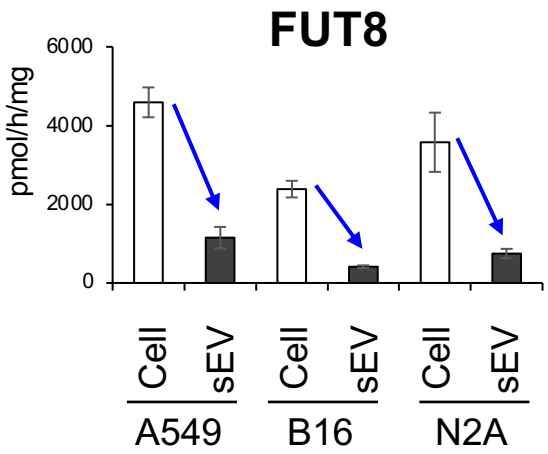








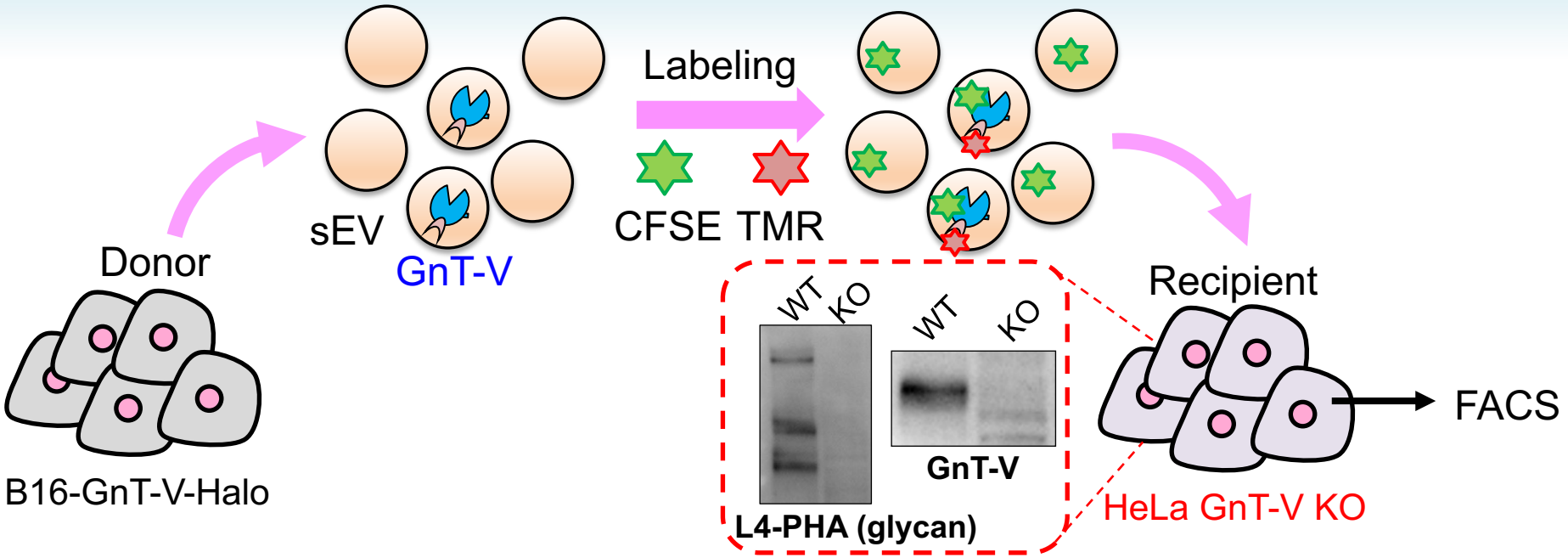




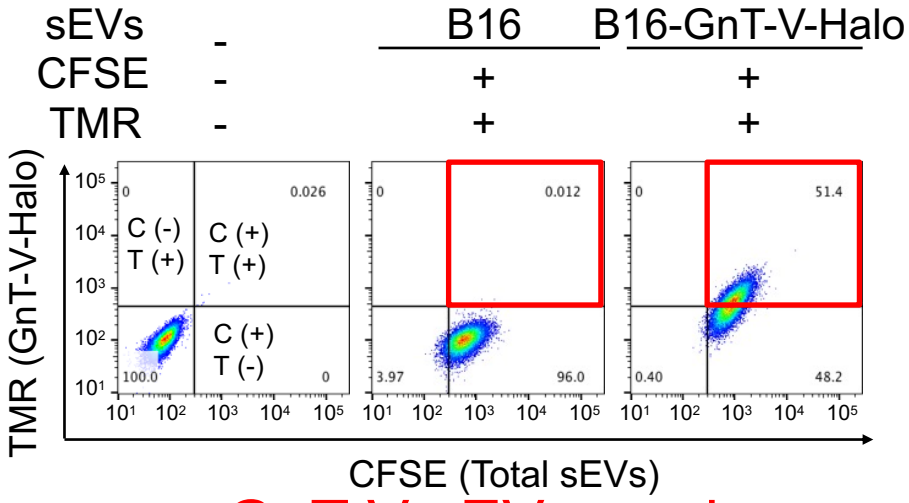
GnT-V activity is selectively enriched in sEVs

(Hirata, et al., ***iScience***, 2022, 26, 105747)

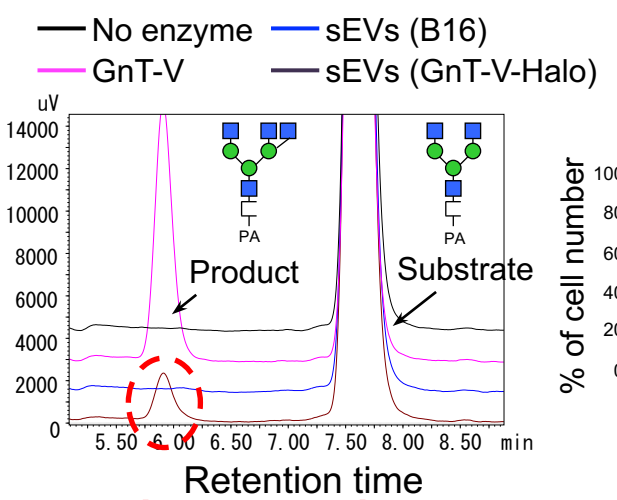
GnT-V-rich sEVs are incorporated to remodel glycans



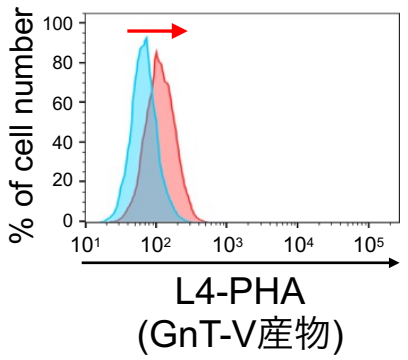
GnT-V incorporation



GnT-V activity (recipient)



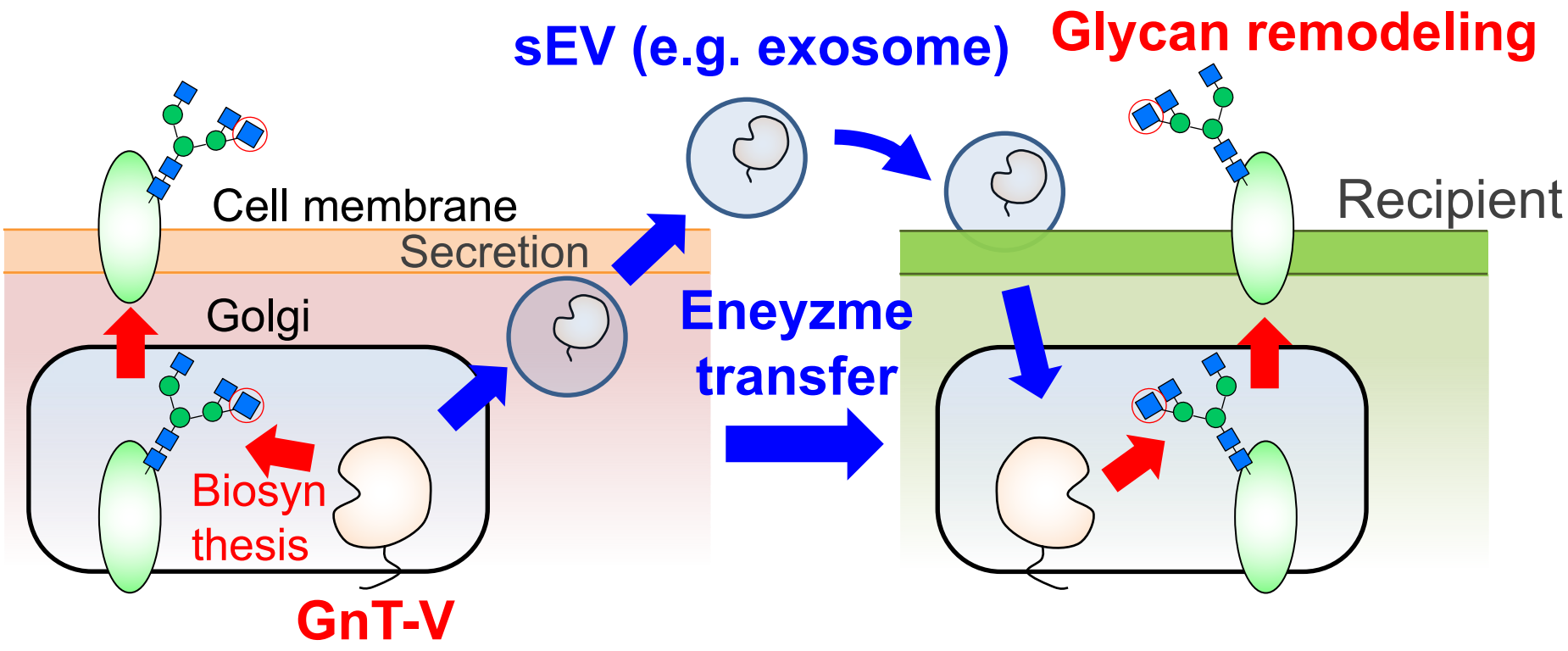
Glycan change (recipient)



GnT-V-sEVs are incorporated to produce glycans

(Hirata, et al., ***iScience***, 2022, 26, 105747)

Cell-to-cell transfer of GnT-V mediated by sEV



A possible regulation mechanism of glycan expression without gene expression

(Hirata, et al., ***iScience***, 2022, 26, 105747)

Summary

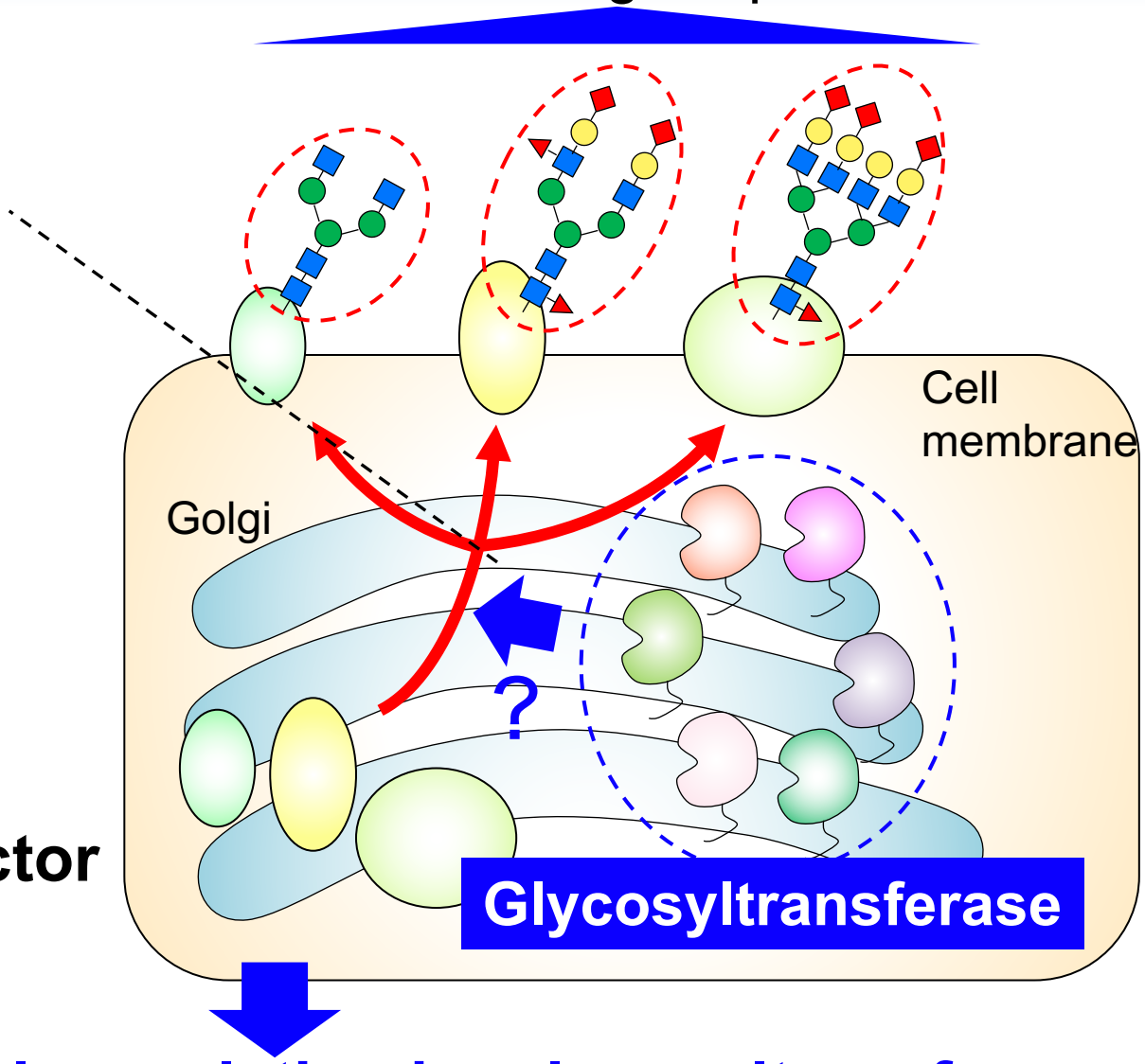
Disease and biological phenomenon

Biological event

- 3D structure
- Specificity

Regulation mechanism

- Localization
- Secretion
- Regulatory factor



Perspective

Protein-selective glycosylation by glycosyltransferase and its medical application

Acknowledgment

Lab member

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AIST

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