Basics of glycan/lectin drug discovery and new technology for glycan decoding (scGR-seq)

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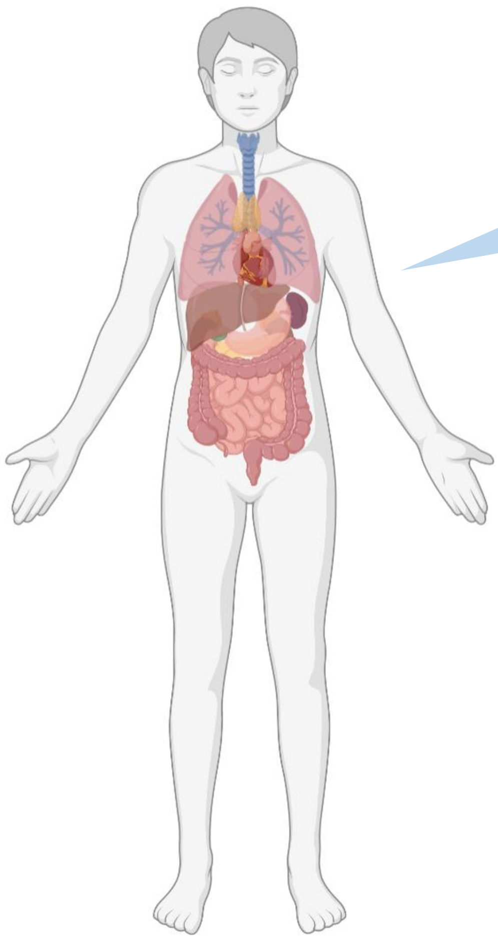
1. Basics of glycan and lectin

2. Trends in glycan and lectin drug discovery

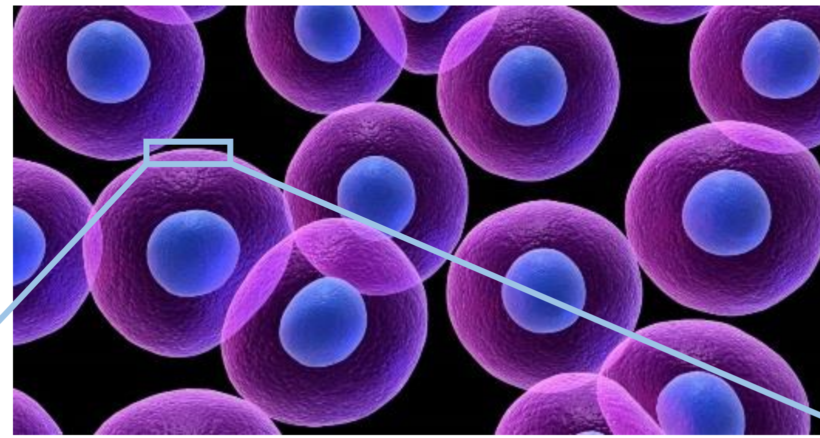
3. Single cell glycan and RNA sequencing (scGR-seq)

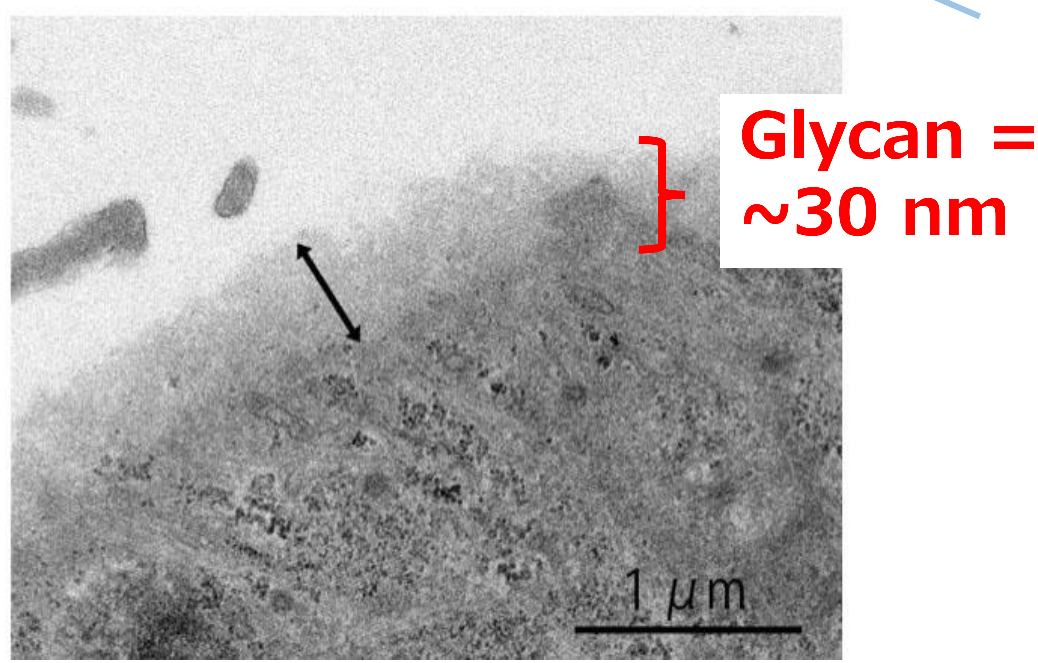
Contents

All cells are coated with glycans



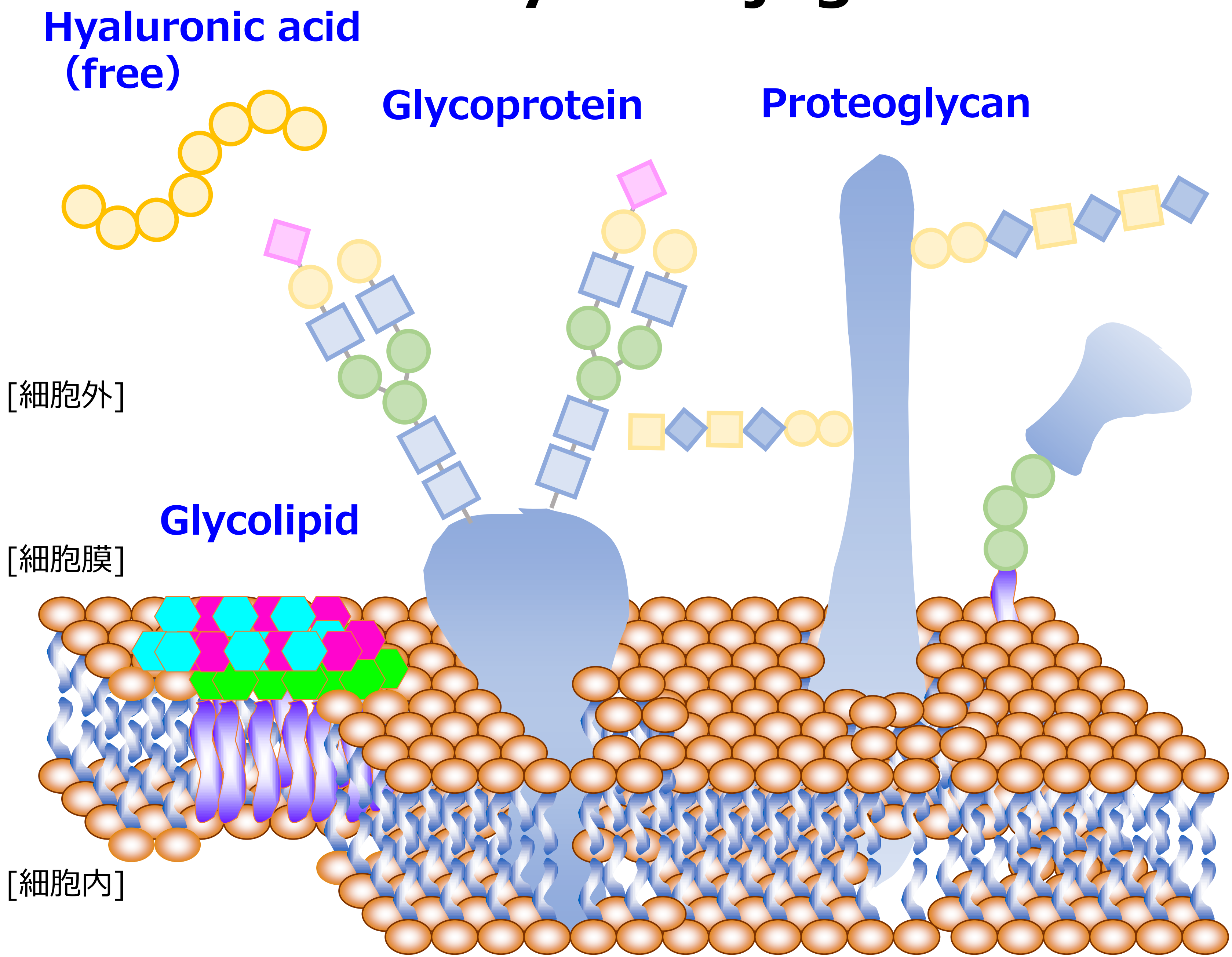
37 trillion cells



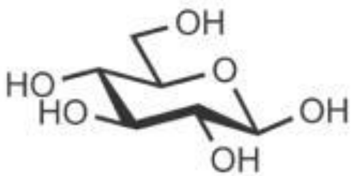


Cancer Res. **29**, 925–937 (1969)

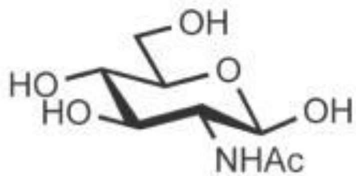
Glycoconjugate



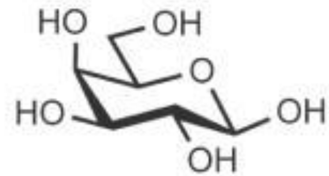
Glycans in human consist mainly of 10 monosaccharides



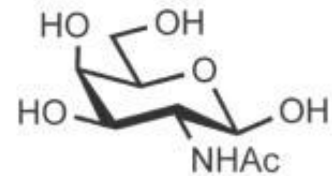
D-Glucose
(Glc)



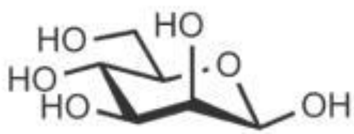
N-Acetyl-D-glucosamine
(GlcNAc)



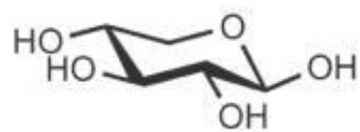
D-Galactose
(Gal)



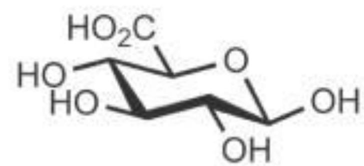
N-Acetyl-D-galactosamine
(GalNAc)



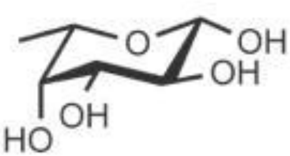
D-Mannose
(Man)



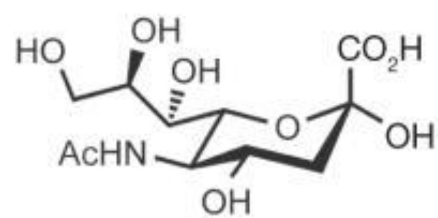
D-Xylose
(Xyl)



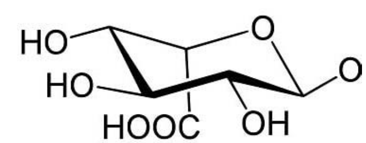
D-Glucuronic acid
(GlcA)



L-Fucose
(Fuc)

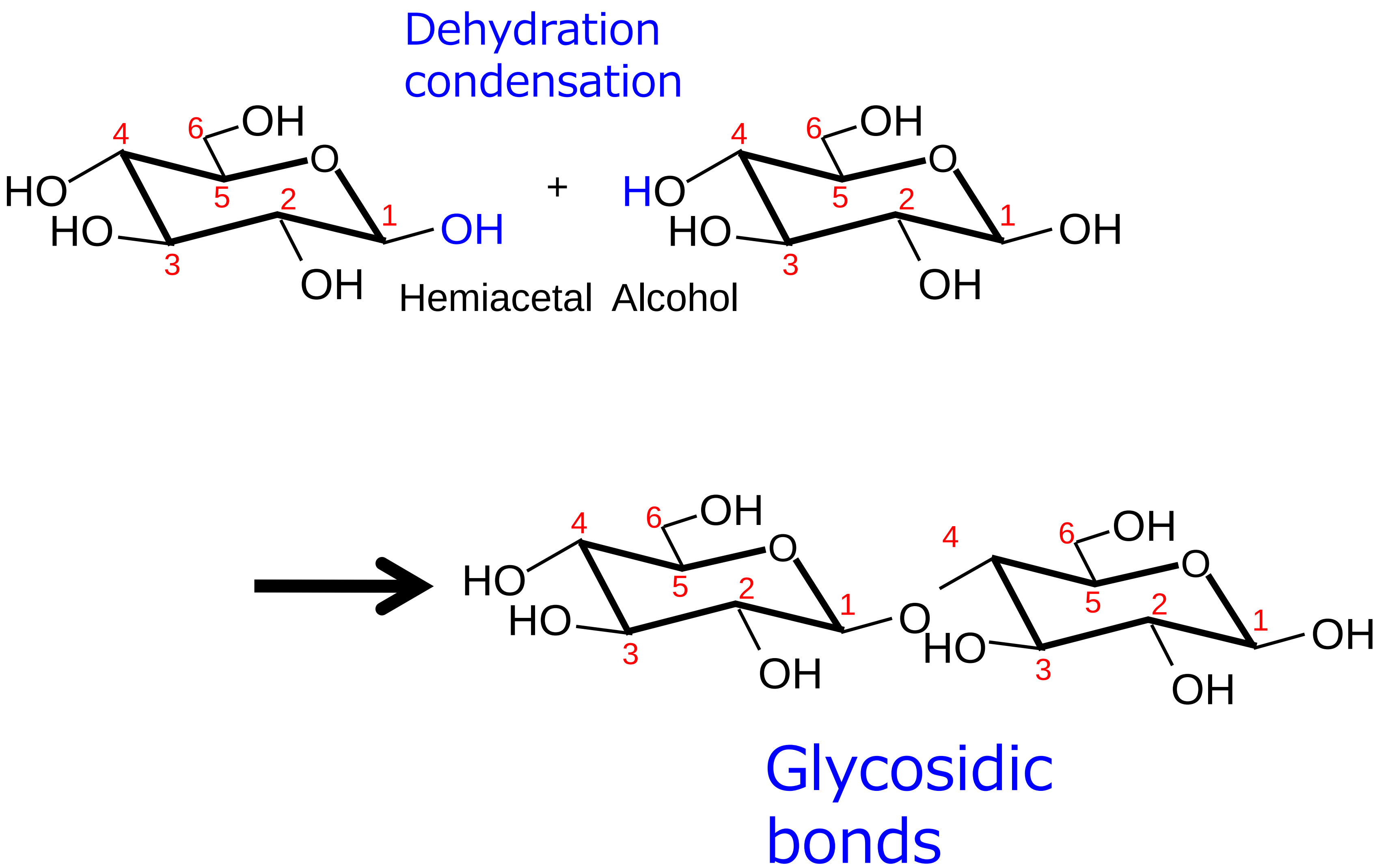


N-Acetylneuraminic acid
(NeuAc)



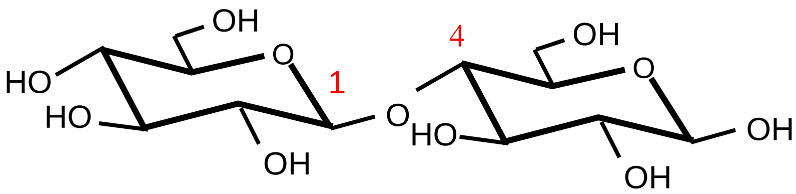
Iduronic acid
(IdoA)

Glycans are composed of monosaccharides linked by glycosidic bonds.

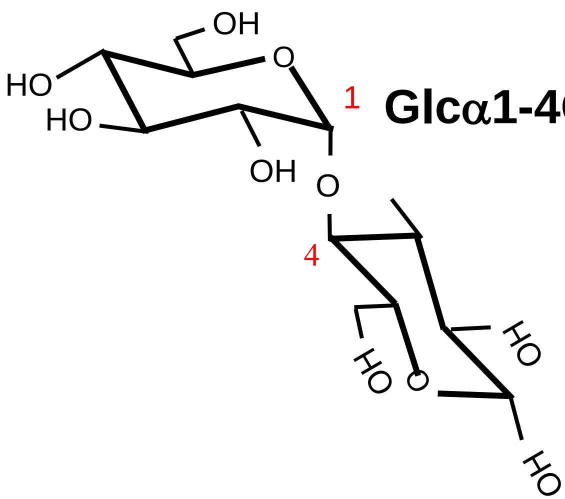


Anomeric and positional isomer (glucose disaccharide)

Glcβ1-4Glc

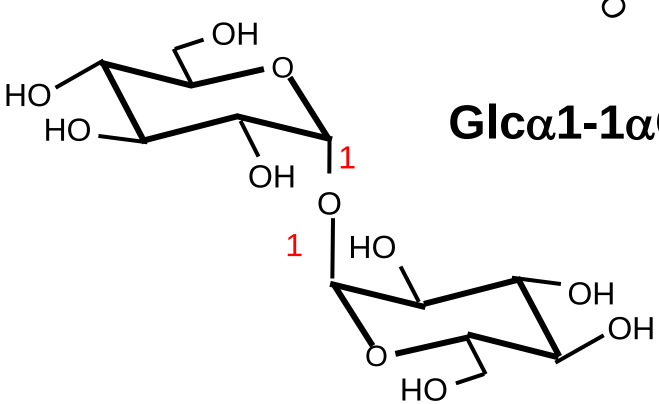


Cellobiose → Cellulose



Glcα1-4Glc

Maltose → Amylose (Starch)



Glcα1-1αGlc

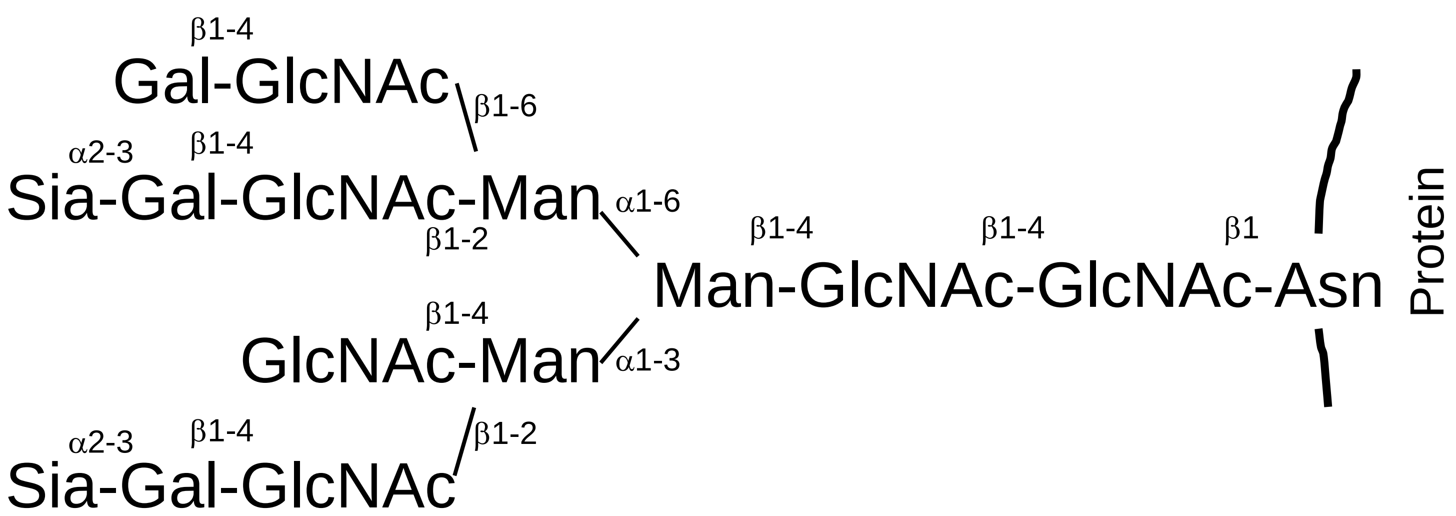
Trehalose

Glycans are composed of branch structures

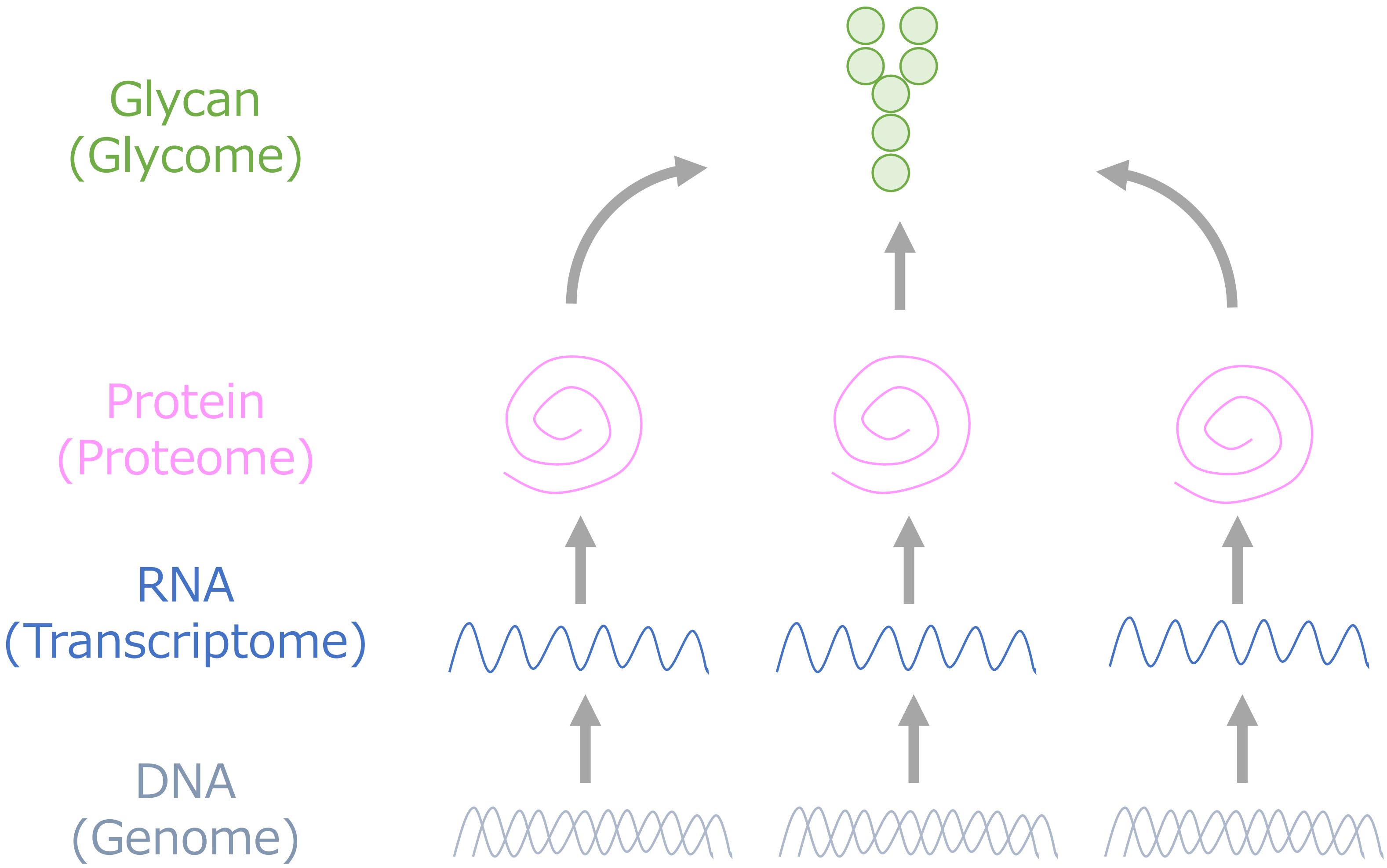
Nucleic acid A-T-G-C-A-T-G-C-A-T-G-C-A-T

Protein Met-Ala-Arg-Gly-Thr-Ser-Glu-Asp

Glycan



Glycans are secondary products of genes and are synthesized by various proteins

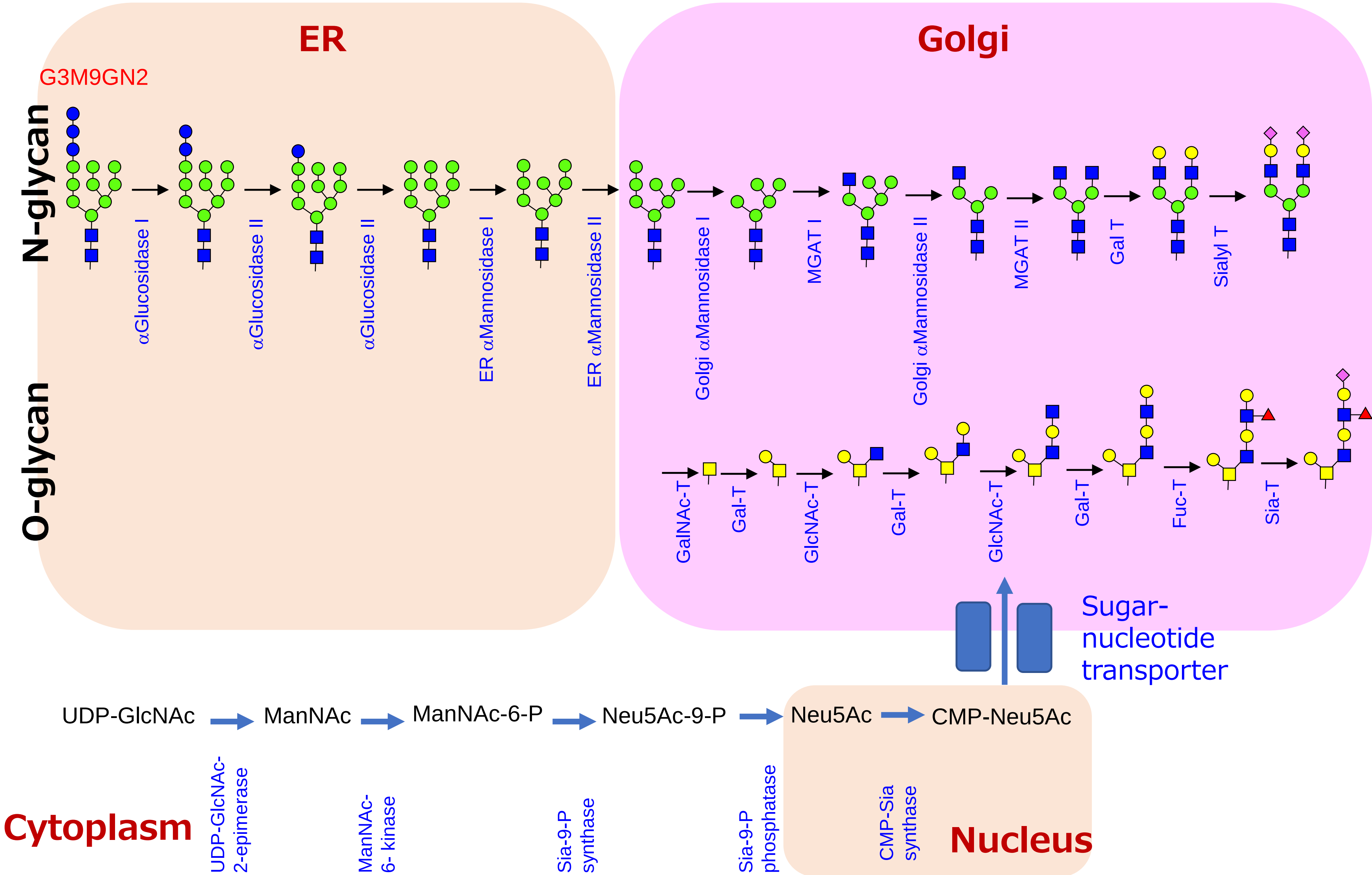


Glycan structure is difficult to determine from gene expression alone

Glycogenes involved in glycan synthesis

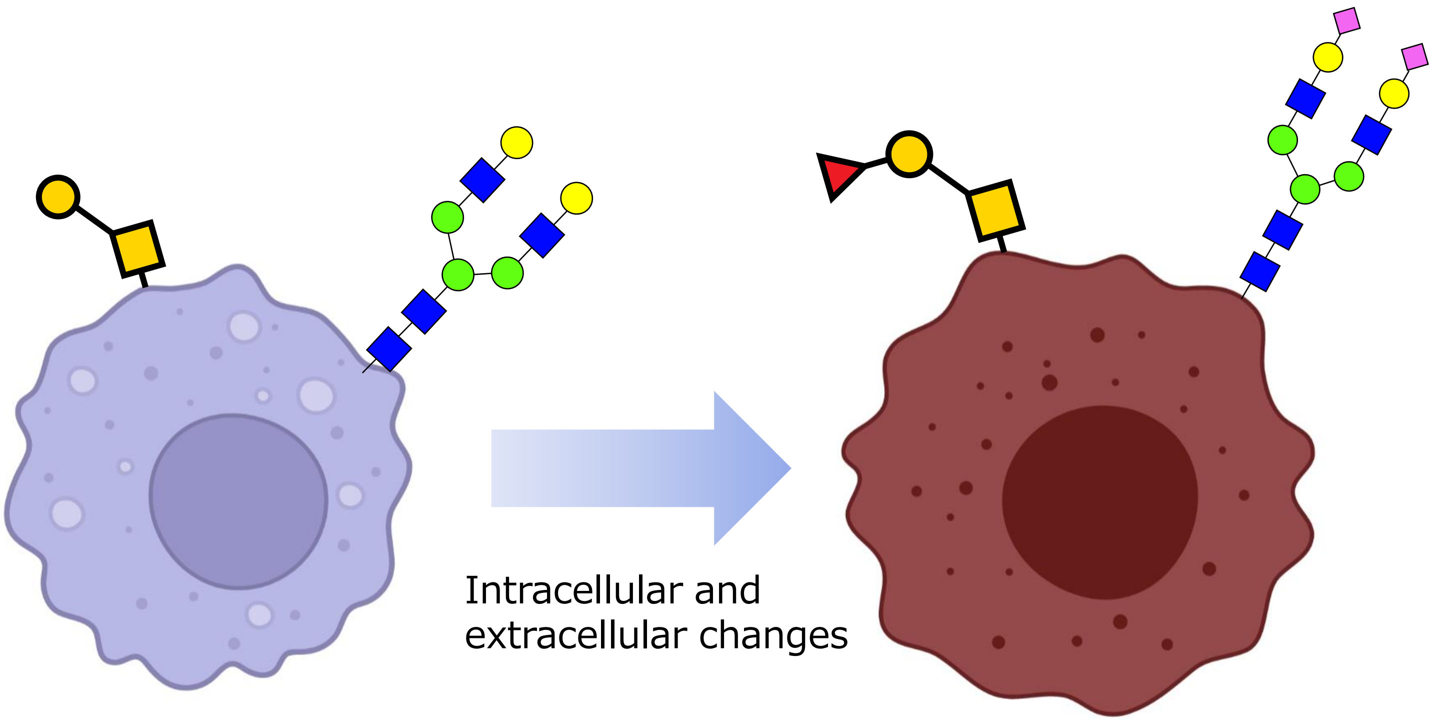
Category	Property
Glycosyltransferase	Enzymes that transfer a monosaccharide
Sulfotransferase	Enzymes that transfer sulfate
Epimerase	Enzyme that epimerizes a monosaccharide
Glycosidase	Enzymes that hydrolyze glycans
Sugar-nucleotide synthase	Enzymes that synthesize sugar nucleotides, which are the materials for glycans
Sugar-nucleotide transporter	Molecules that transport sugar nucleotides, the materials for glycans, into the ER and Golgi lumen

Overview of glycoprotein glycan biosynthesis process

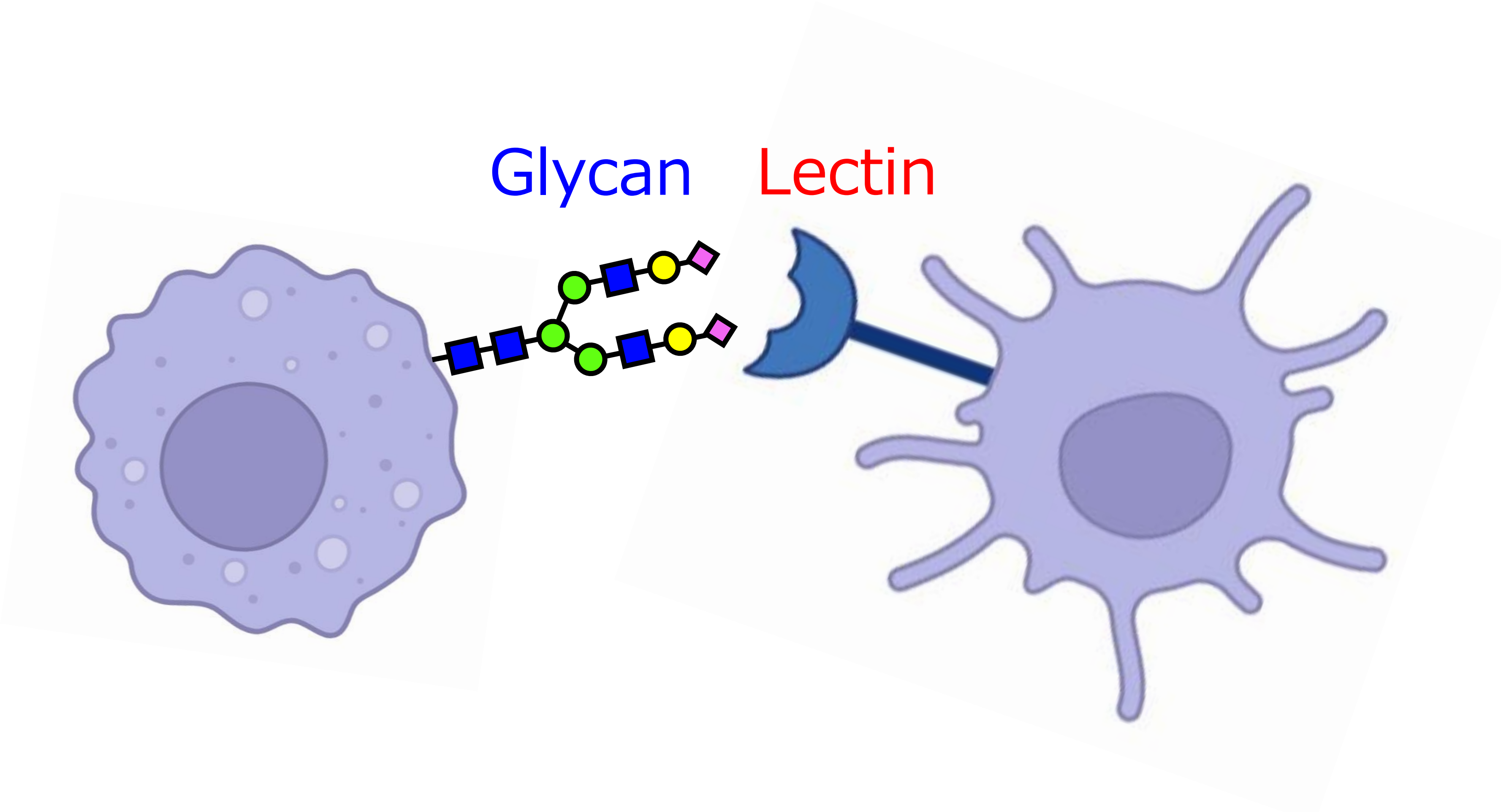


Glycans are cell signature

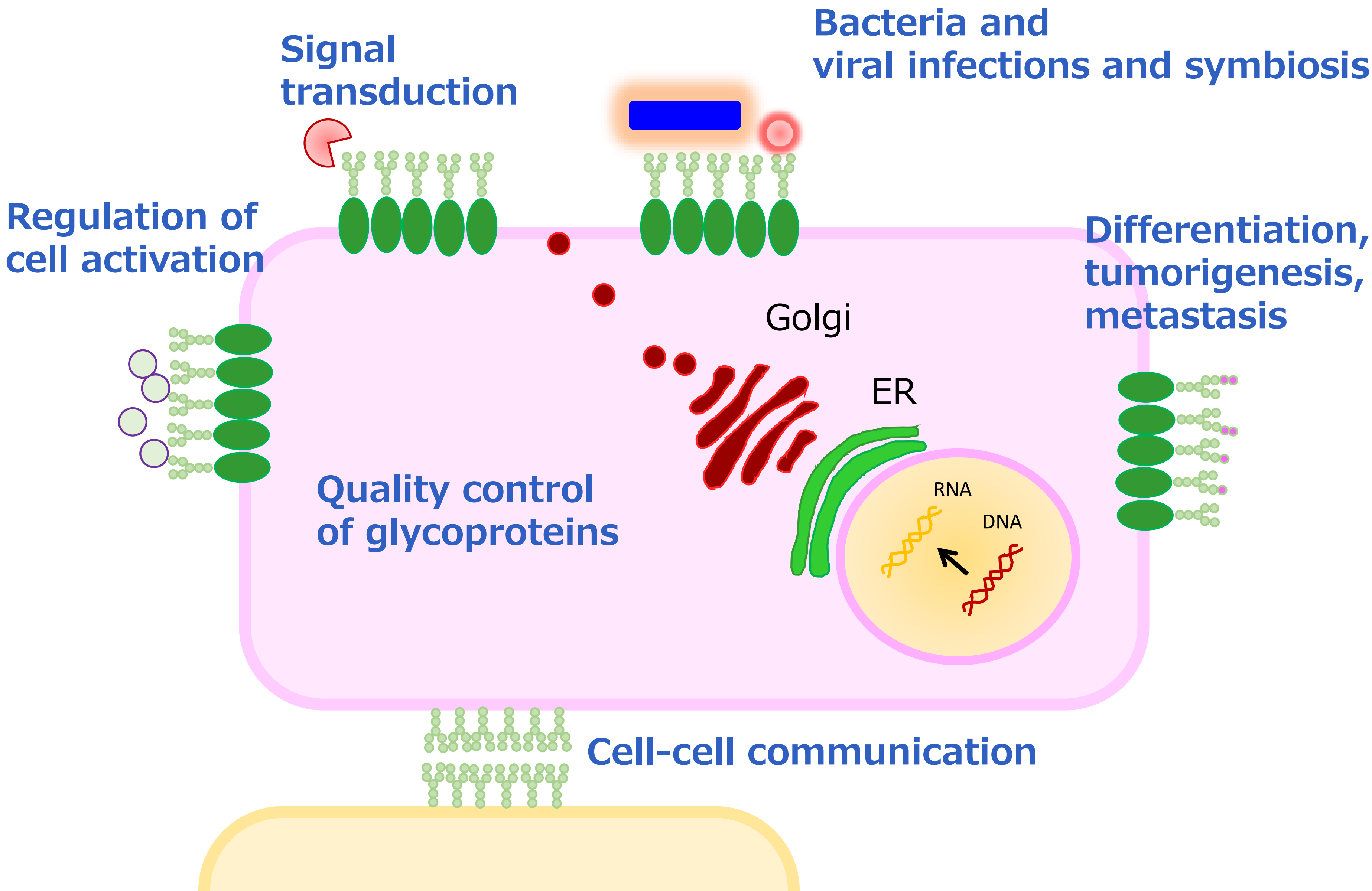
Change with cell type and cell state (differentiation/de-differentiation, tumorigenesis, inflammation, aging)



Glyco-codes are recognized by lectins and mediate multicellular communication



Glycan-lectin interactions mediate various life phenomena



What is lectin?

1. General term for proteins that bind to glycans

2. Named lectin after the Latin word *legere*, "to select".

3. First discovered in 1888 by Peter Hermann Stillmark in Russia from castor bean seed extract (ricin)

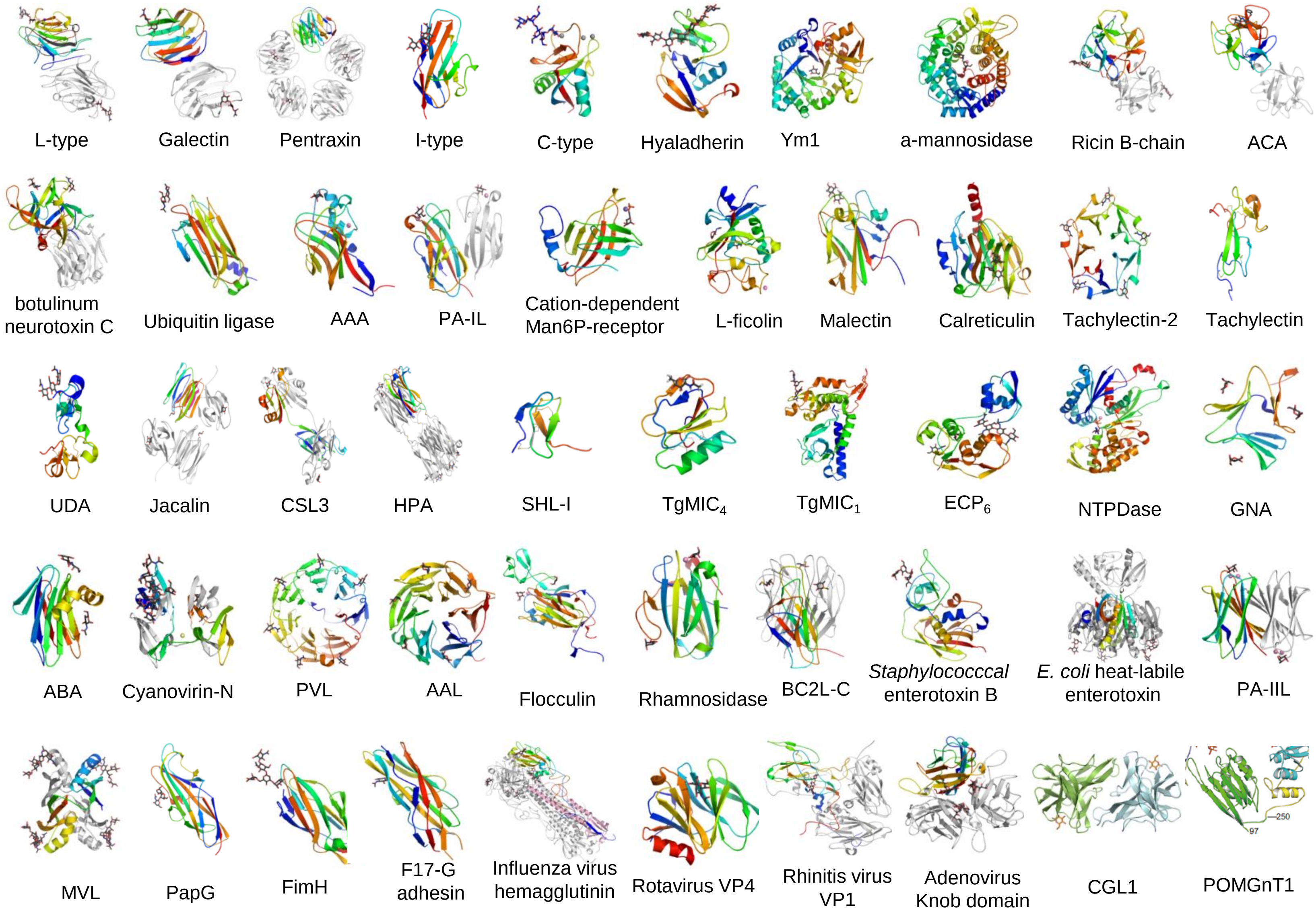
4. Present in all organisms from viruses to mammals

5. More than 150 lectins have been known in humans, but in fact many proteins such as cytokines also have binding activity to glycans

Various protein structures have sugar-binding activity

Generally contain β-sheet

Fujimoto et al. Methods Mol Biol 2014



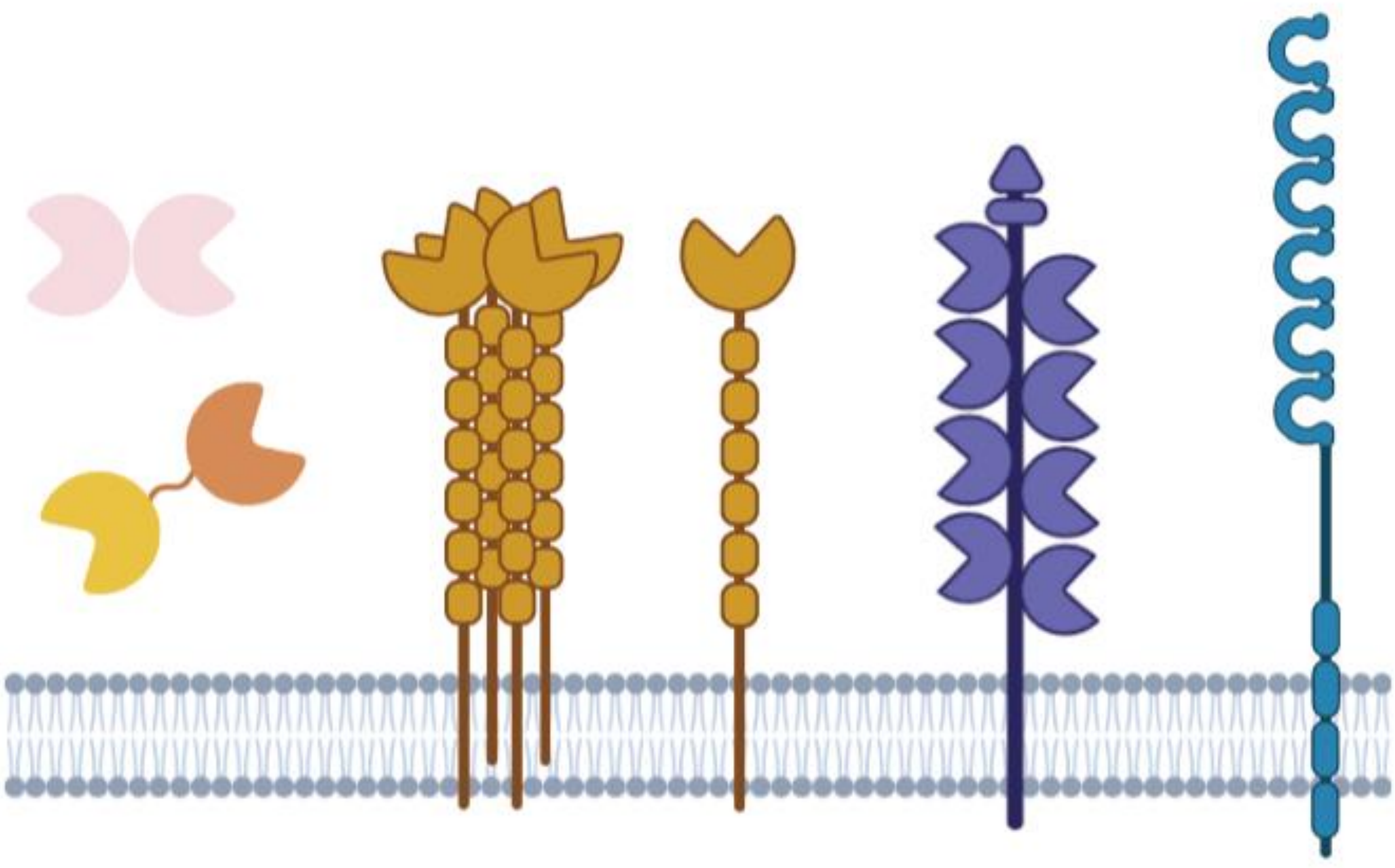
Major lectins present in humans

Galectin
14 member

C-type lectin
～100 member

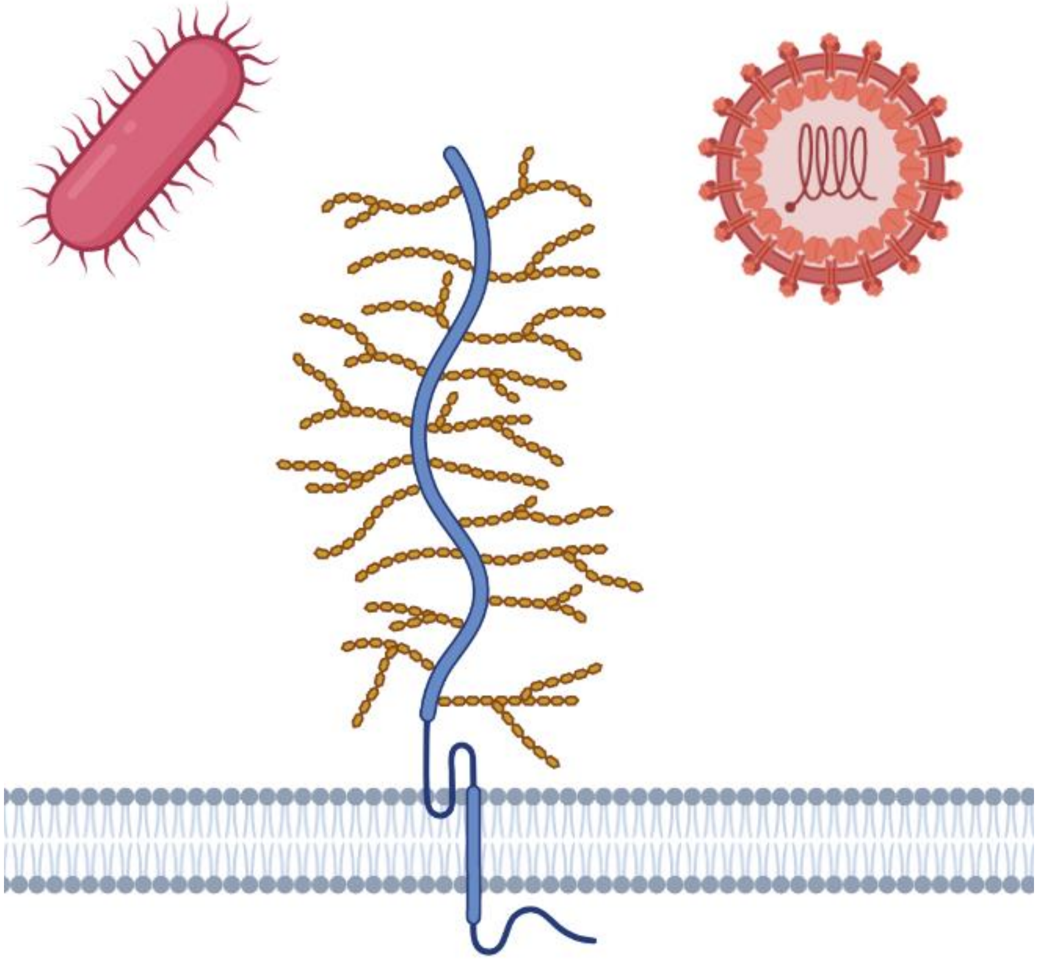
P-type lectin
1 memer

SIGLEC
14 memer



Many pathogens infect through binding to host glycans

Virus
Bacteria
Funji



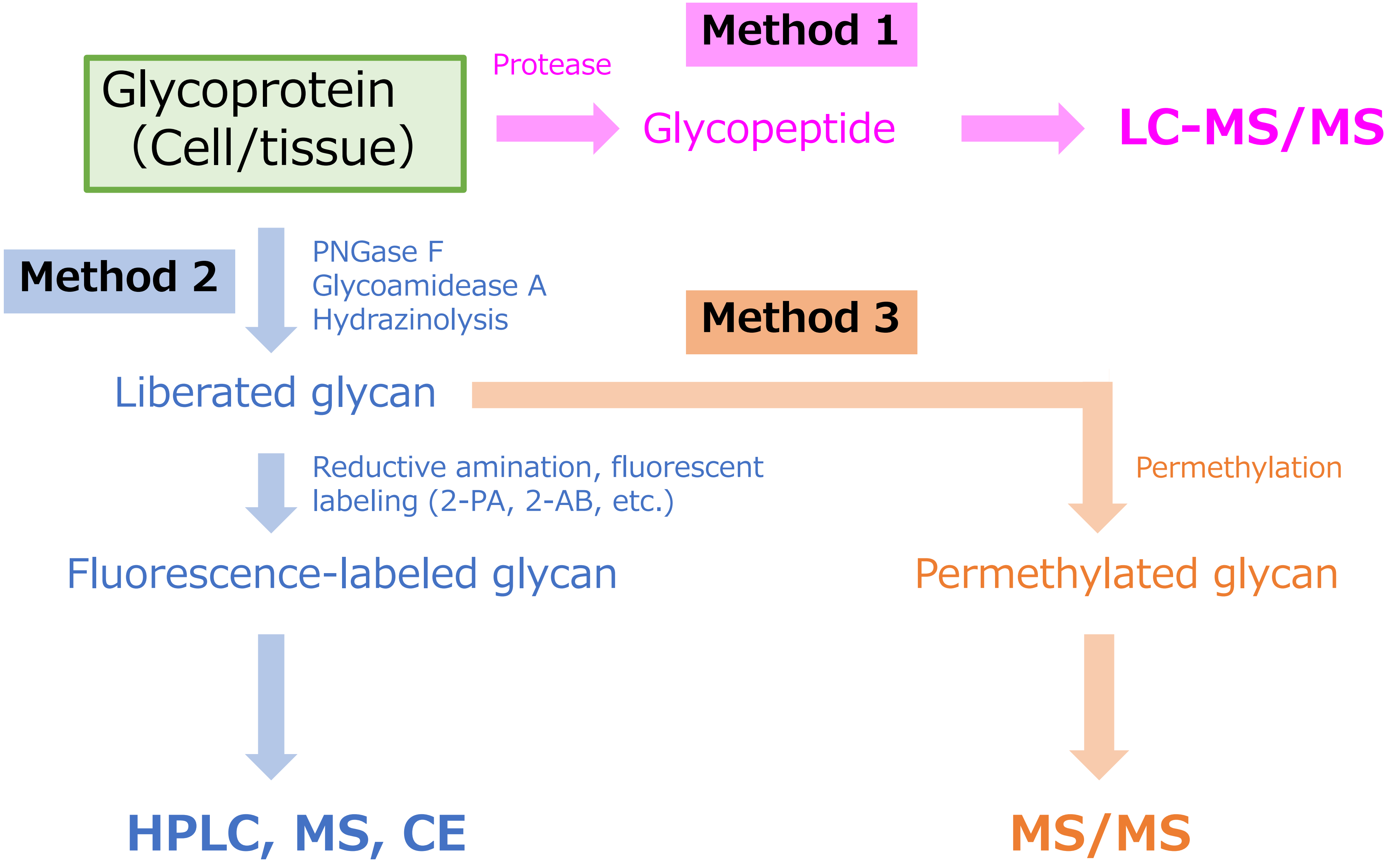
Representative examples

Class	Name of pathogen	Glycan ligands
Virus	SARS-CoV-2	GAG、Glycolipid
	Influenza virus A/B	Sialic acid
	Norovirus	Blood group
	Coronavirus OC43/HKU1	O-acetyl Sia
Bacteria	*Yersinia enterocolitica* SubB	Sia
	Escherichia Coli F17a-G	GlcNAc
	Clostridium botulinum HA	Gal/GalNAc
	Salmonella typhi toxin B5	Sia
	Vibrio cholerae AB toxin	GM1

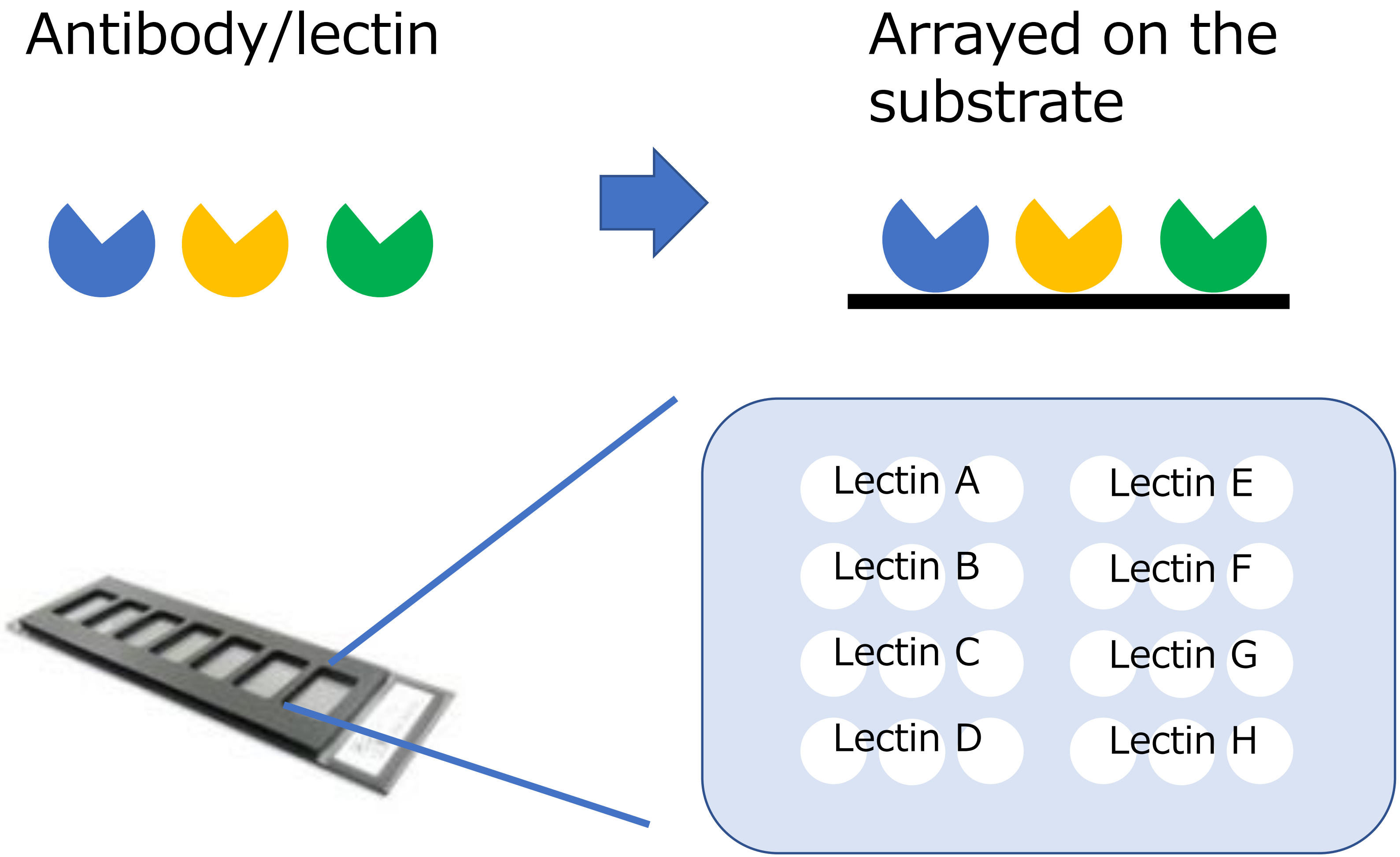
General methods of glycan analysis

1. Mass spectrometry
2. HPLC
3. Capillary electrophoresis
4. NMR
5. Antibody/lectin staining
6. Antibody/lectin microarray

General methods for structural analysis of glycoprotein glycans



Antibody/lectin microarray

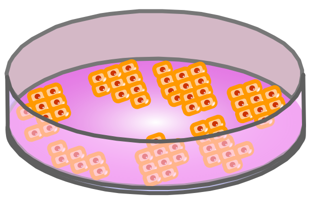


Highly sensitive acquisition of glycan profiles from reaction patterns with lectins and antibodies

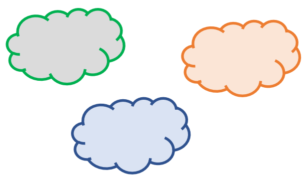
Analytical flow of microarray analysis

Cell

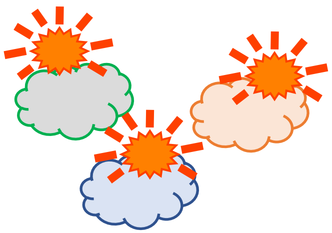
~10 million cells



Protein extraction



Fluorescence labeling

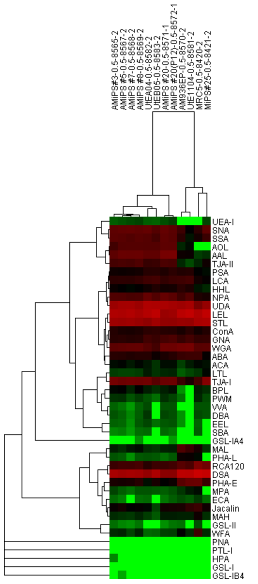


50 ng = ~10^3 cells

React with lectin microarray

Scan

Statistical analysis



Contents

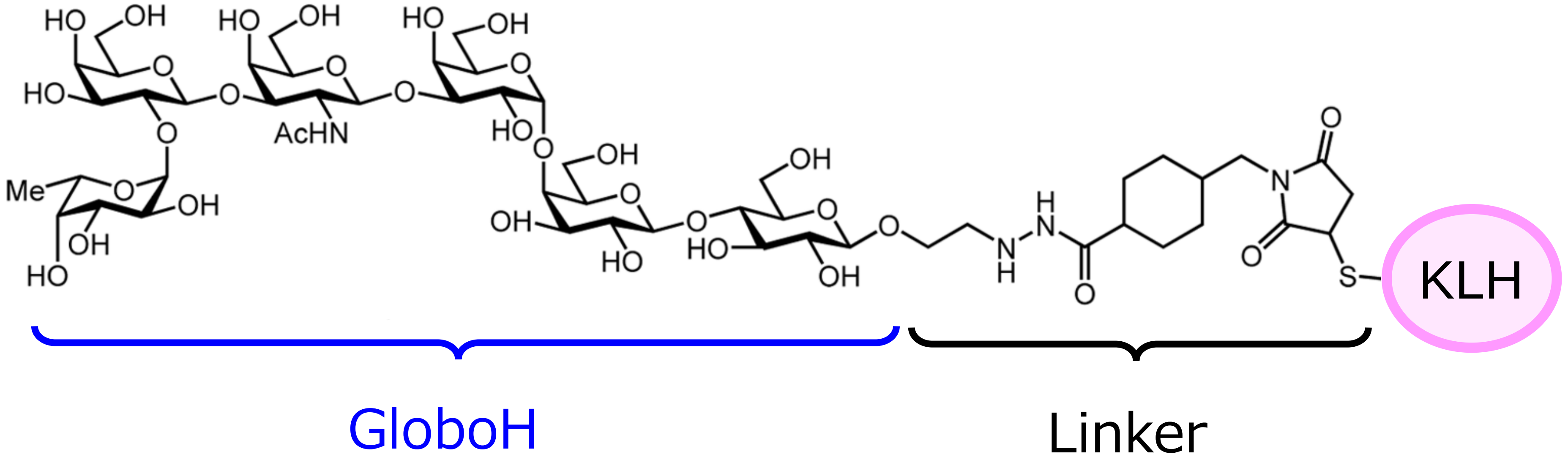
Glycan/lectin-related drugs

Classification	Example
Glycans	Hyaluronic acid（Joint function improver)、low molecular weight heparin fragment（Fondaparinux, FDA approval in 2001, anti-coagulant)
Glycopeptide antibiotics	Vancomycin（FDA approved, antibiotic）、Teicoplanin（FDA approved, antibiotic)
Glycan vaccine	Globo H–KLH/QS-21 vaccine（Adagloxad simolenin, Phase III ongoing, triple-negative breast cancer)
Glycosidase inhibitor	Sialidase inhibitor（oseltamivir/zanamivir, influenza, FDA approval in 1999)
Lectin inhibitor	Pan-selectin inhibitor（Rivipansel, FDA approval in 2020, sickle cell disease）、galectin inhibitor（TD139, idiopathic pulmonary fibrosis)
Anti-lectin antibody	CD33 antibody（Gemtuzumab ozogamicin, FDA approval in 2017、lymphoma)
Anti-glycan ligand antibody	CD24Fc（Phase III ongoing, viral pneumonia)
Anti-glycan antibody	Dinutuximab (FDA approval in 2015, high-risk neuroblastoma)
Anti-glycoprotein antibody	Sotrovimab（FDA approval in 2021、COVID19）、anti-MUC16/CA125（Oregovomab, Phase III ongoing, ovarian cancer)
Glycan-targeted CAR-T	Anti-GD2 CAR NKT（Phase I ongoing, neuroblastoma)
Antibody-enzyme (sialidase) conjugates	Anti-HER2-sialidase conjugates（breast cancer)
Glycosyltransferase inhibitor	GlcCer synthetic enzyme inhibitor (Genz-112638, Diabetes treatment drugs)
Adjuvant	LPS (Cancer)

Globo H–KLH/QS-21 vaccine

1. GloboH is highly expressed in breast cancer (stem) cells and is involved in the progression of cancer.
2. Drug for treatment of metastatic breast cancer with 5-year survival rate of 27% or less.
3. Under development by OBI Pharma (a Taiwanese company) and currently in clinical studies (Phase 3)

OBI-822 (OBI pharma)

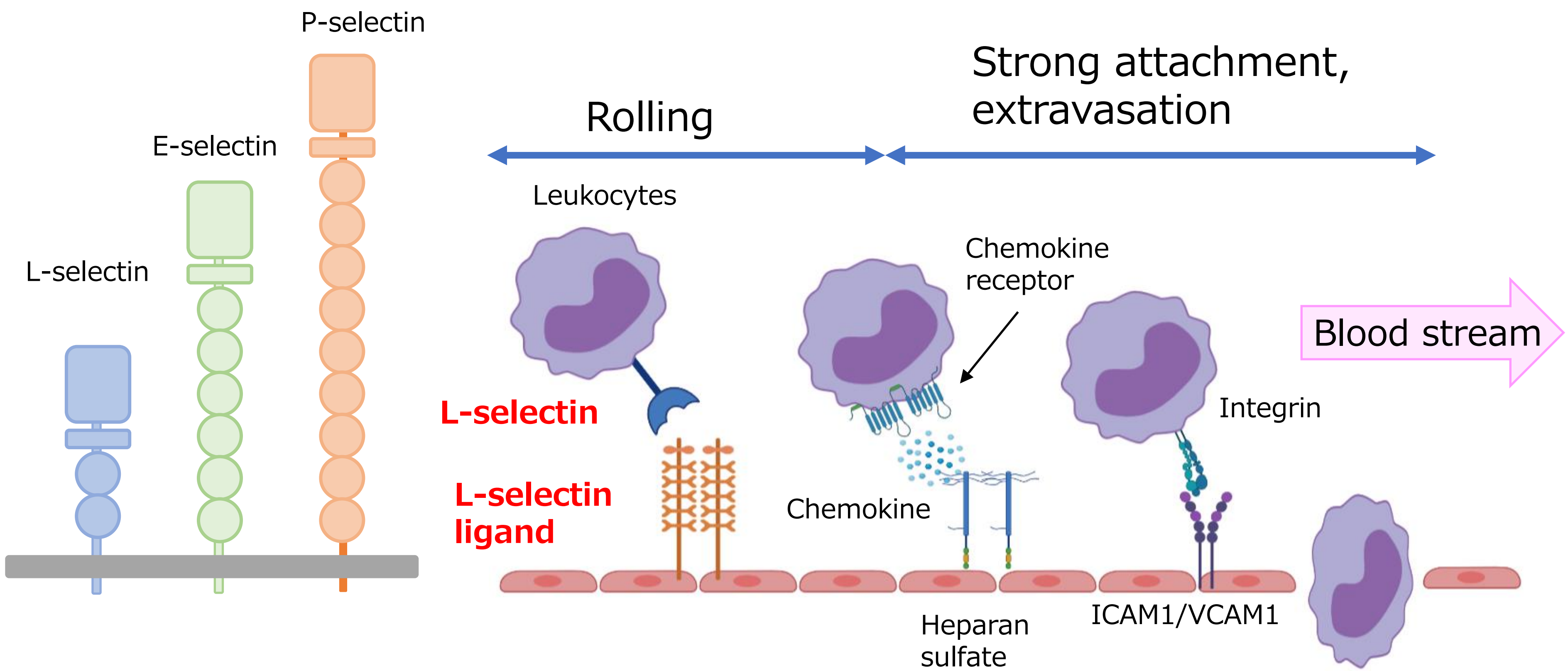


Danishefsky et al. Acc. Chem. Res. 2015, 48, 3, 643–652

Selectin

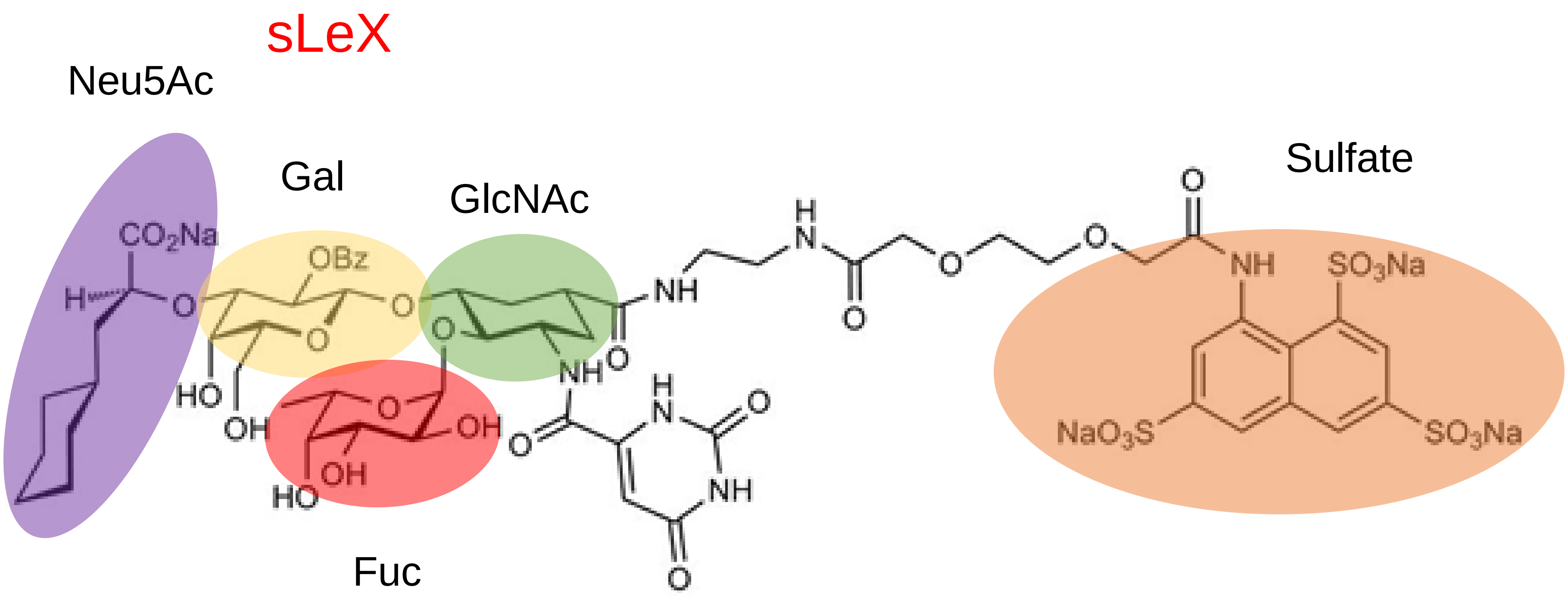
- A member of C-type lectins involved in the adhesion of leukocytes to endothelial cells.
- Involved in leukocyte migration to inflammatory sites and high endothelial venules

  L-selectin : expressed on leukocytes
  E-selectin : expressed on endothelial cells
  P-selectin : expressed on platelets



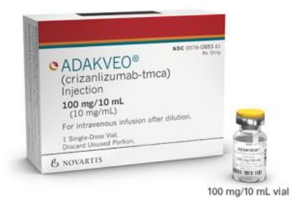
Selectin inhibitors have been expected to be anti-inflammatory agents

Rivipansel : pan-selectin inhibitors

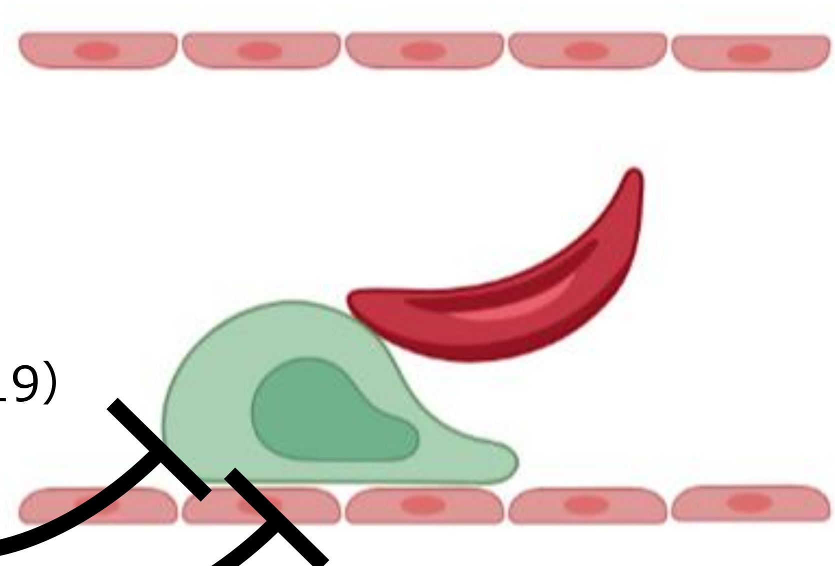


Pan-selectin inhibitor : Rivipansel Glycomimetics

FDA approval in 2020 for effective treatment of vascular occlusion caused by sickle cell disease.



Crizanlizumab
(P-selectin inhibitor,
FDA approval in 2019)



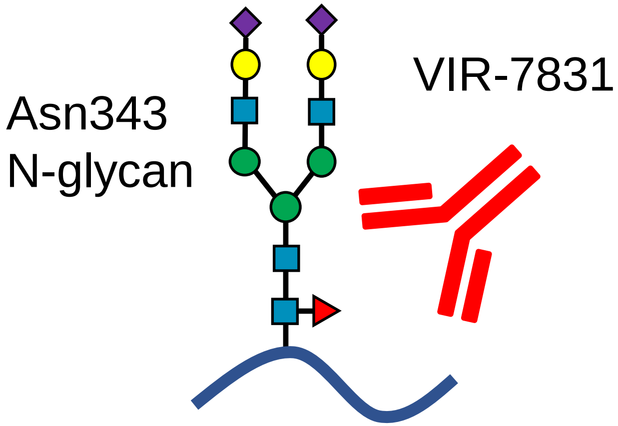
Rivipansel

Inhibits adhesion of leukocytes to vascular endothelial cells

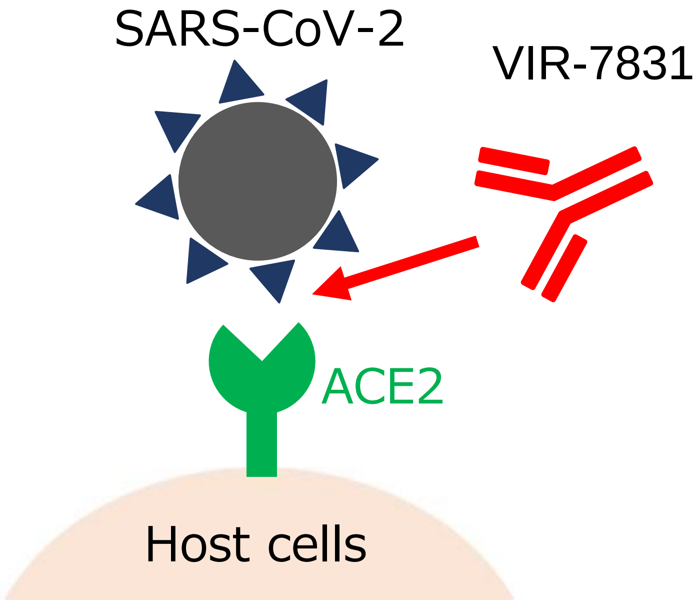
Sotrovimab VIR-7831

- Recognizes core fucose and neighboring peptides of N-glycans modified with Asn343, which is highly conserved in the receptor binding domain (RBD) of SARS-CoV-2 S protein
- Shows neutralizing activity, ADCC activity, and ADCP activity
- Developed by Vir Biotechnology, Inc. and GSK (approved by FDA and EUA in 2021)
- Effective against omicron strains

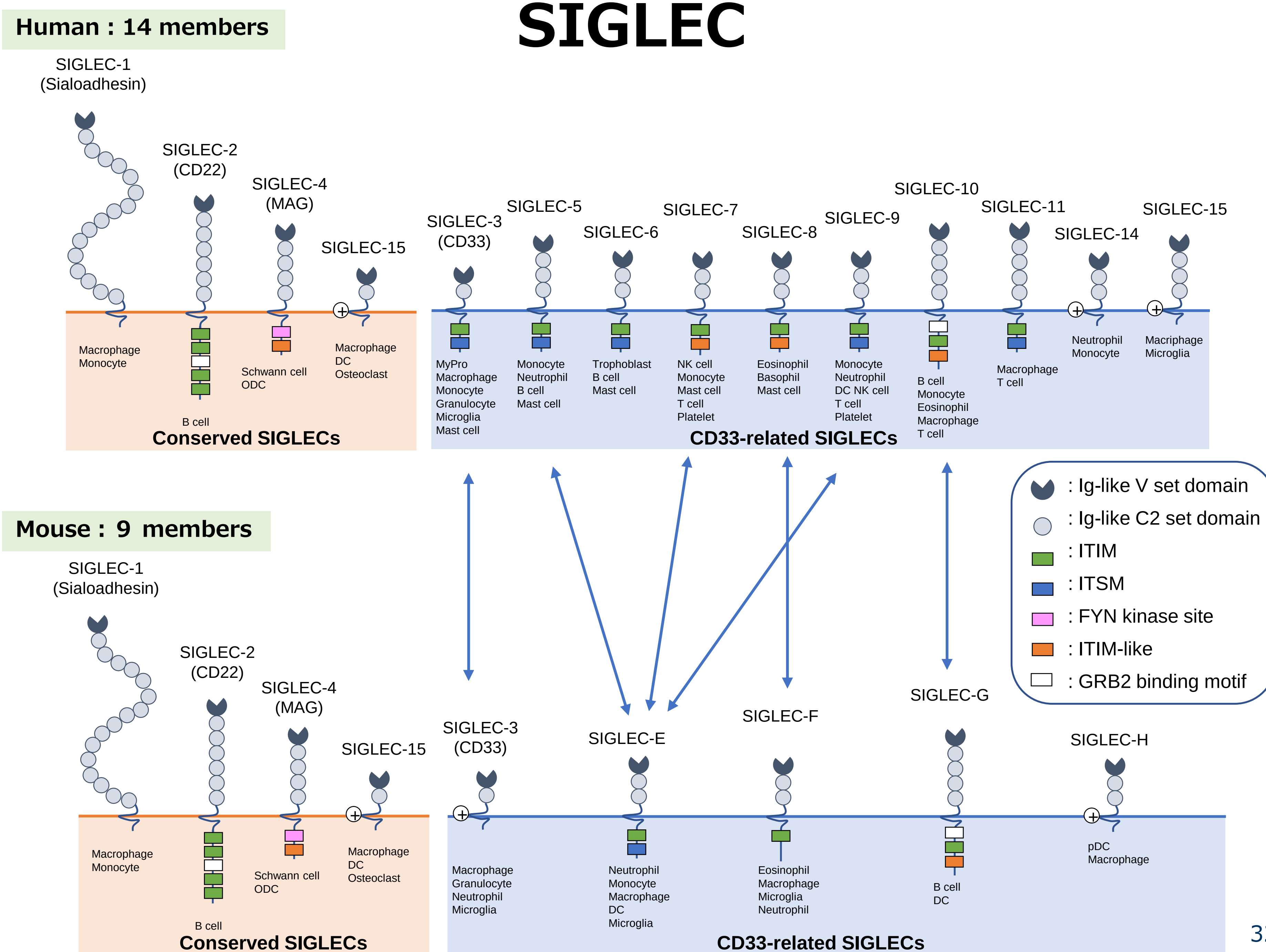
Recognizes N-glycans and neighboring peptides of Asn343, which are highly conserved in S proteins



Inhibits SARS-CoV-2 binding to ACE2 receptors

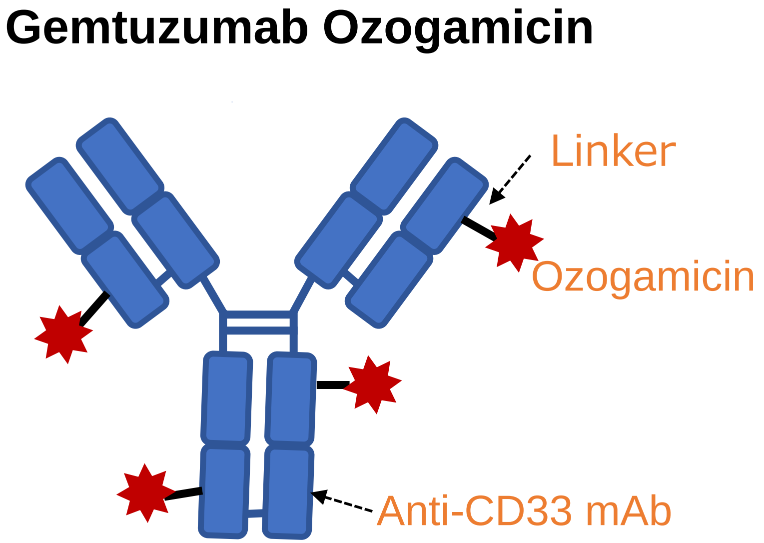


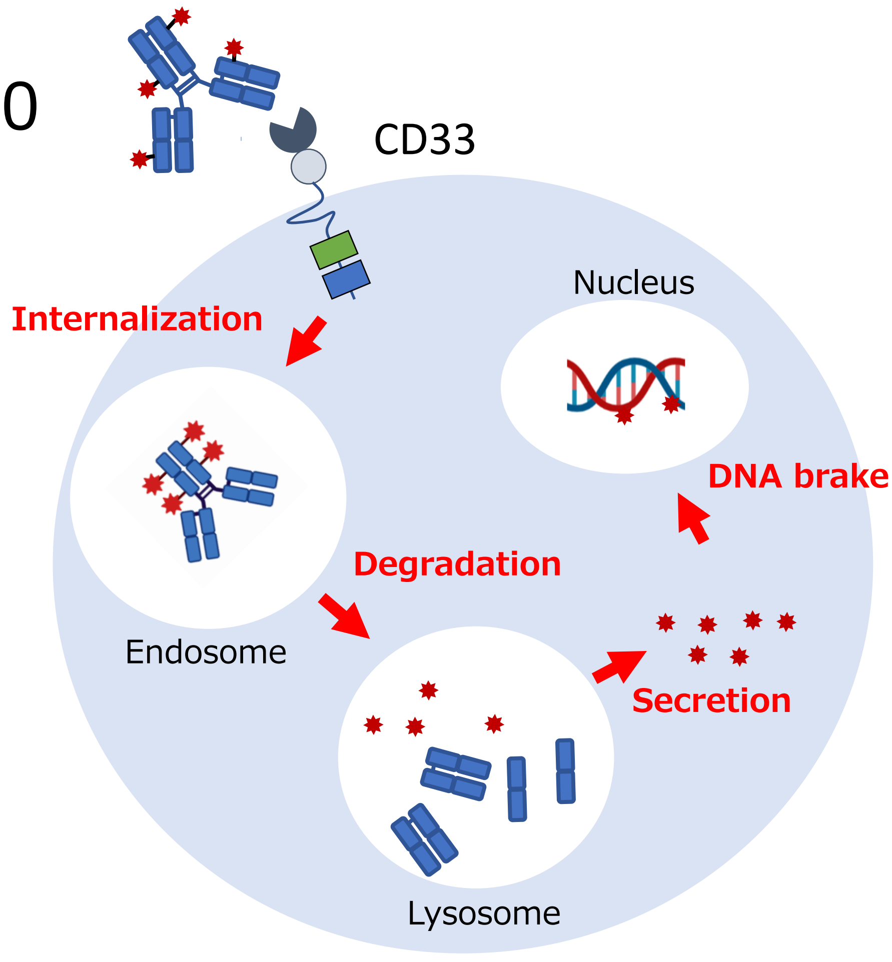
SIGLEC


Human : 14 members
SIGLEC-1
(Sialoadhesin)
SIGLEC-2
(CD22)
SIGLEC-4
(MAG)
SIGLEC-15
Macrophage
Monocyte
B cell
Schwann cell
ODC
Macrophage
DC
Osteoclast
Conserved SIGLECs
SIGLEC-3
(CD33)
SIGLEC-5
SIGLEC-6
SIGLEC-7
SIGLEC-8
SIGLEC-9
SIGLEC-10
SIGLEC-11
SIGLEC-14
SIGLEC-15
MyPro
Macrophage
Monocyte
Granulocyte
Microglia
Mast cell
Monocyte
Neutrophil
B cell
Mast cell
Trophoblast
B cell
Mast cell
NK cell
Monocyte
Mast cell
T cell
Platelet
Eosinophil
Basophil
Mast cell
Monocyte
Neutrophil
DC NK cell
T cell
Platelet
B cell
Monocyte
Eosinophil
Macrophage
T cell
Macrophage
T cell
Neutrophil
Monocyte
Macriphage
Microglia
CD33-related SIGLECs
: Ig-like V set domain
: Ig-like C2 set domain
: ITIM
: ITSM
: FYN kinase site
: ITIM-like
: GRB2 binding motif
Mouse : 9 members
SIGLEC-1
(Sialoadhesin)
SIGLEC-2
(CD22)
SIGLEC-4
(MAG)
SIGLEC-15
Macrophage
Monocyte
B cell
Schwann cell
ODC
Macrophage
DC
Osteoclast
Conserved SIGLECs
SIGLEC-3
(CD33)
SIGLEC-E
SIGLEC-F
SIGLEC-G
SIGLEC-H
Macrophage
Granulocyte
Neutrophil
Microglia
Neutrophil
Monocyte
Macrophage
DC
Microglia
Eosinophil
Macrophage
Microglia
Neutrophil
B cell
DC
pDC
Macrophage
CD33-related SIGLECs

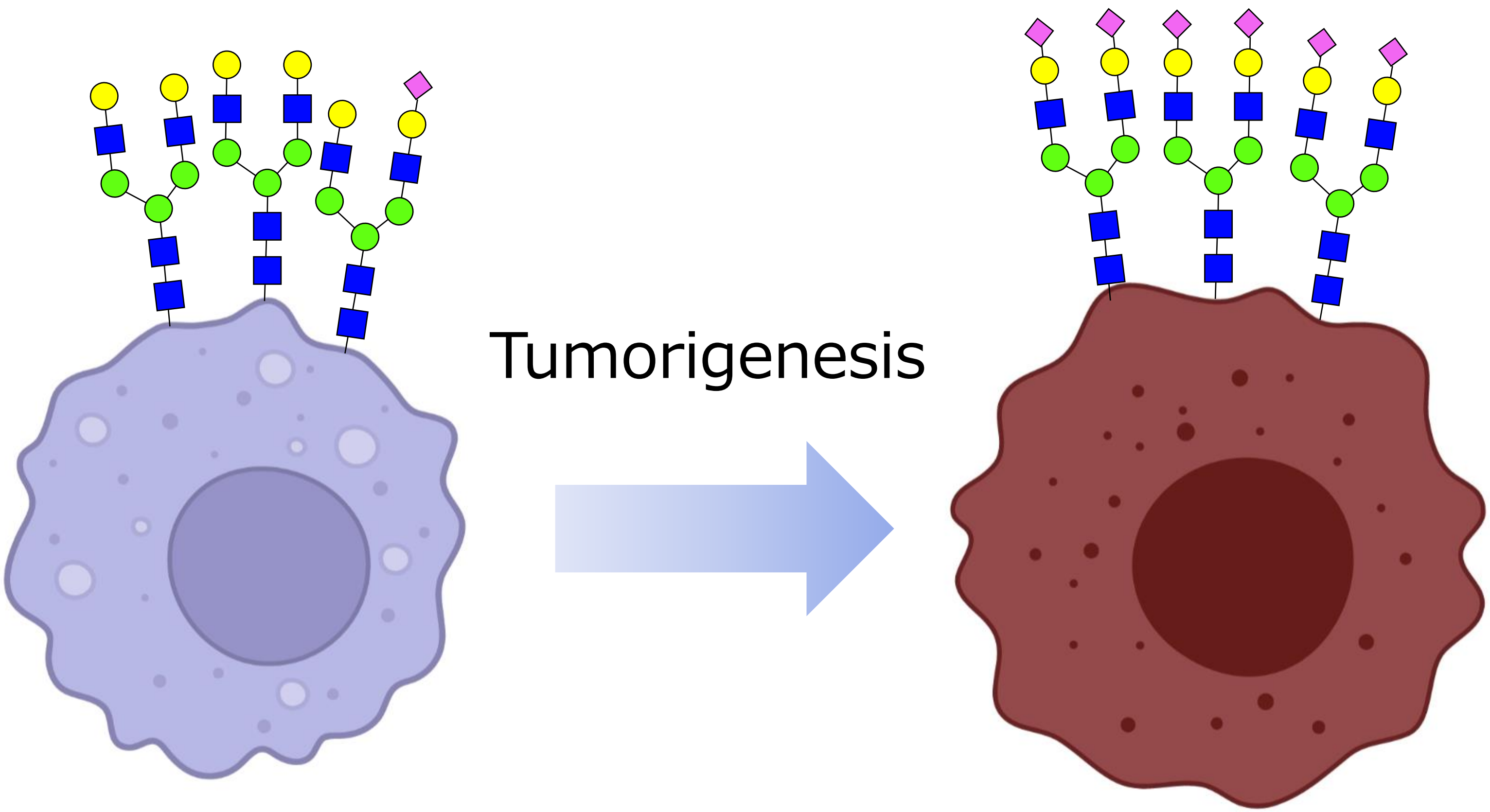
Anti-CD33 antibody-drug conjugates（ADC）：Gemtuzumab Ozogamicin

- SIGLEC-3（CD33）is expressed on myeloid cells
- Therapeutic drugs of acute myeloid leukemia (AML)
- Approved fron FDA in 2000

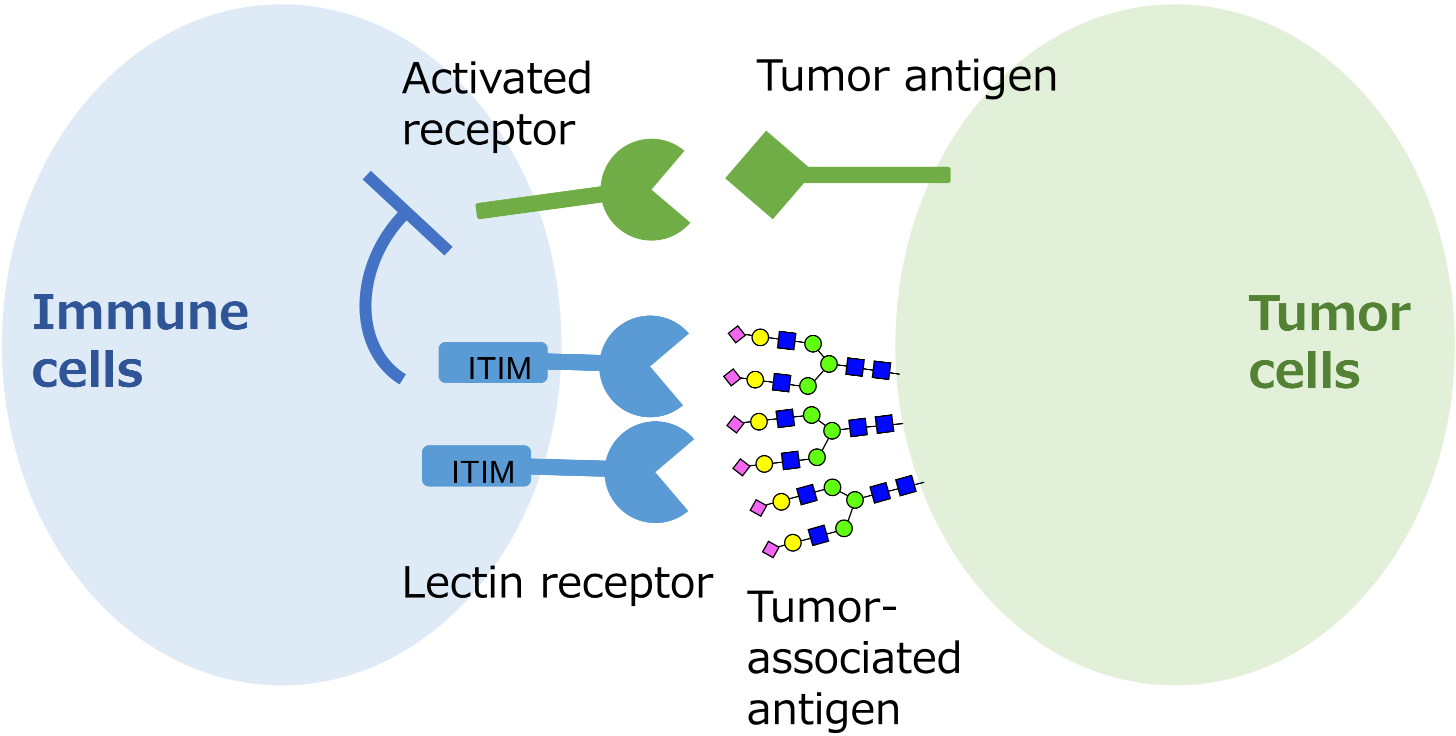




Sialylation is enhanced by tumorigenesis



Glyco-immune checkpoints

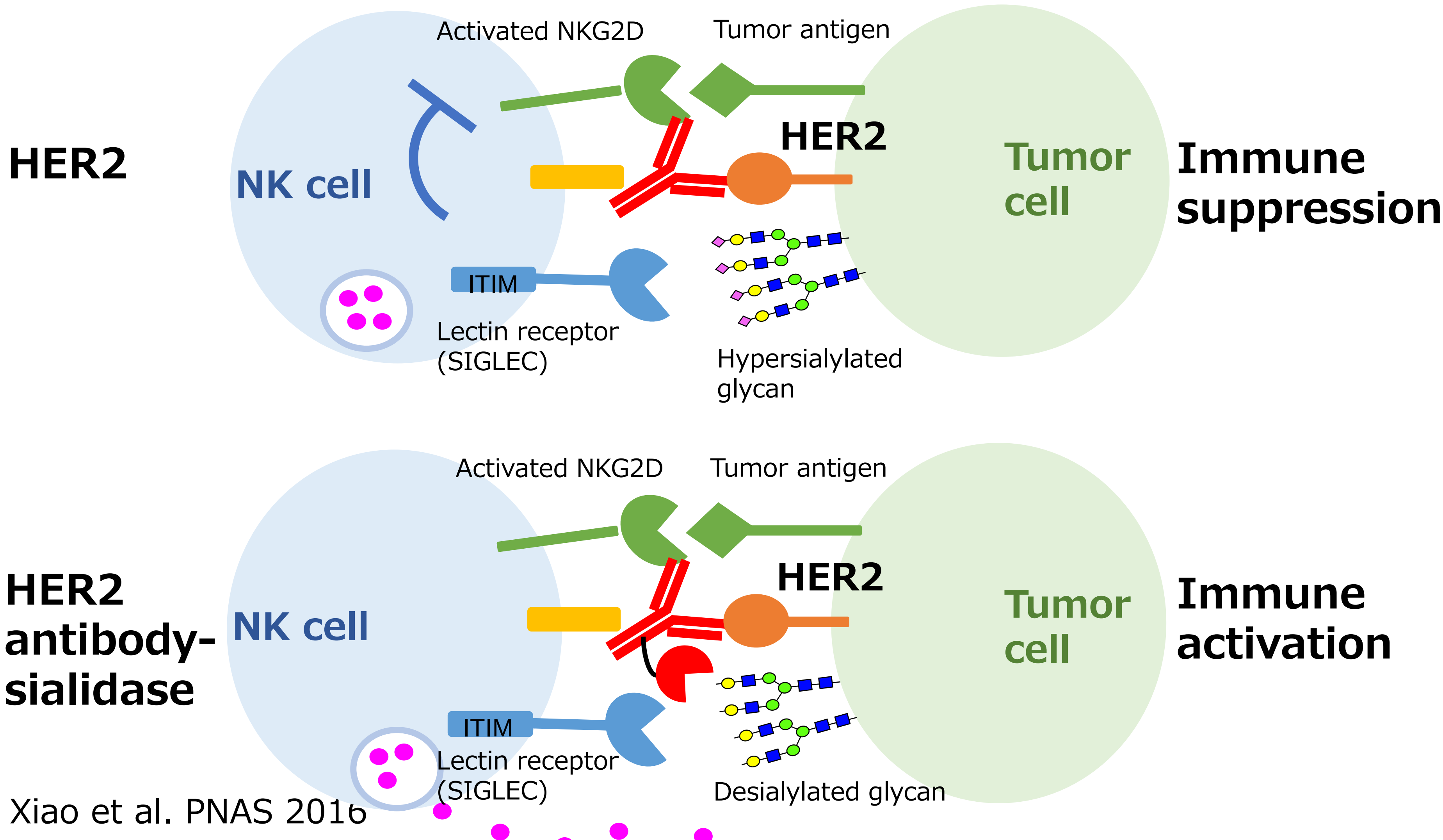


・**Lectin receptors (e.g., SIGLEC) interact with tumor-associated glycans and induce immunosuppression**

・**Tumor cells express glycans that differ from those of normal cells, and escape from immune reaction**

Desialylation of tumor cells by antibody-sialidase complex → enhance NK cell-dependent ADCC

Research is ongoing at Palleon Pharmaceuticals for commercialization.

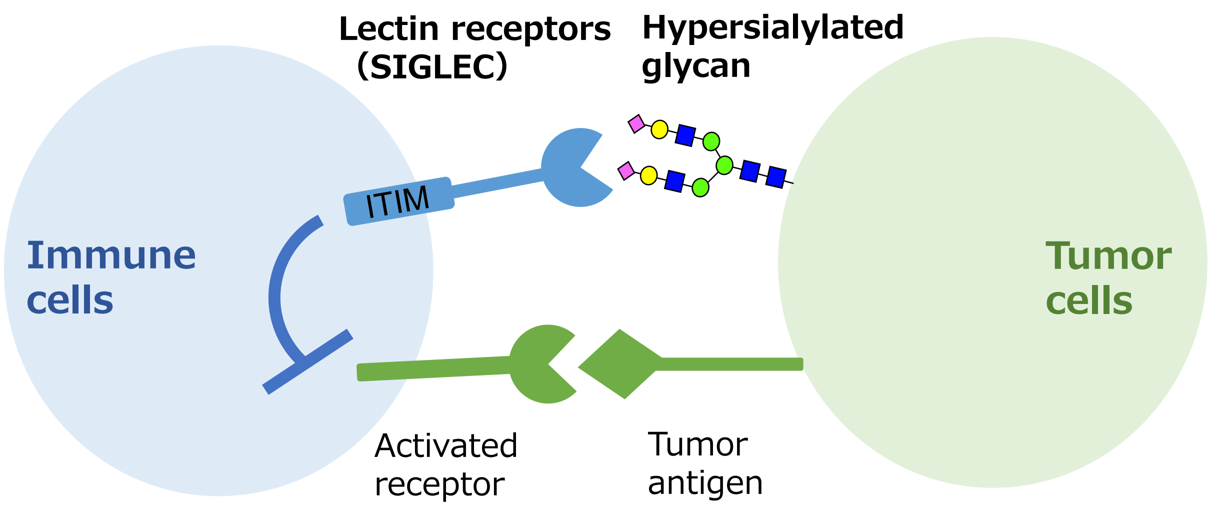


Xiao et al. PNAS 2016

SIGLEC-targeted therapy

1. SIGLEC antibody
2. Recombinant SIGLEC ligand
3. Glycan-coated nanoparticles
4. Anti-SIGLEC ligand antibody
5. SIGLEC decoy
6. Antibody-sialidase complex

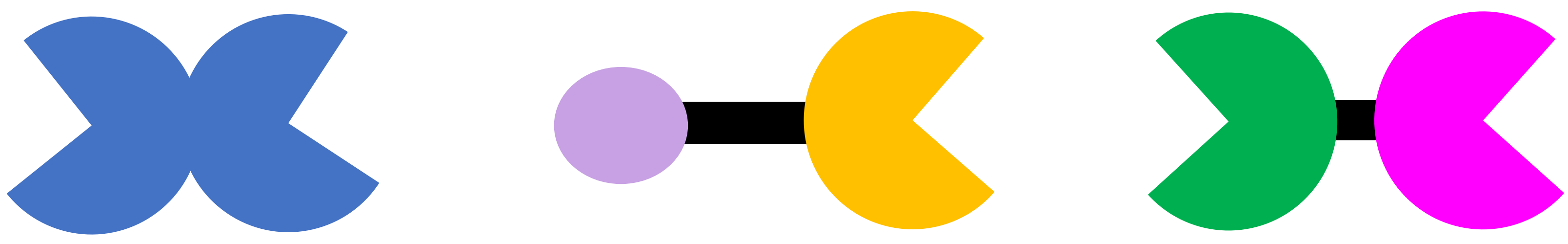
Inhibits SIGLEC-glycan ligand interactions and removes glycan immune checkpoints



Galectin

・Secreted lectins, of which 14 members exist in human
・Classified into three major groups according to domain structure: proto, chimera, and tandem repeat.
・Bind to β-galactoside

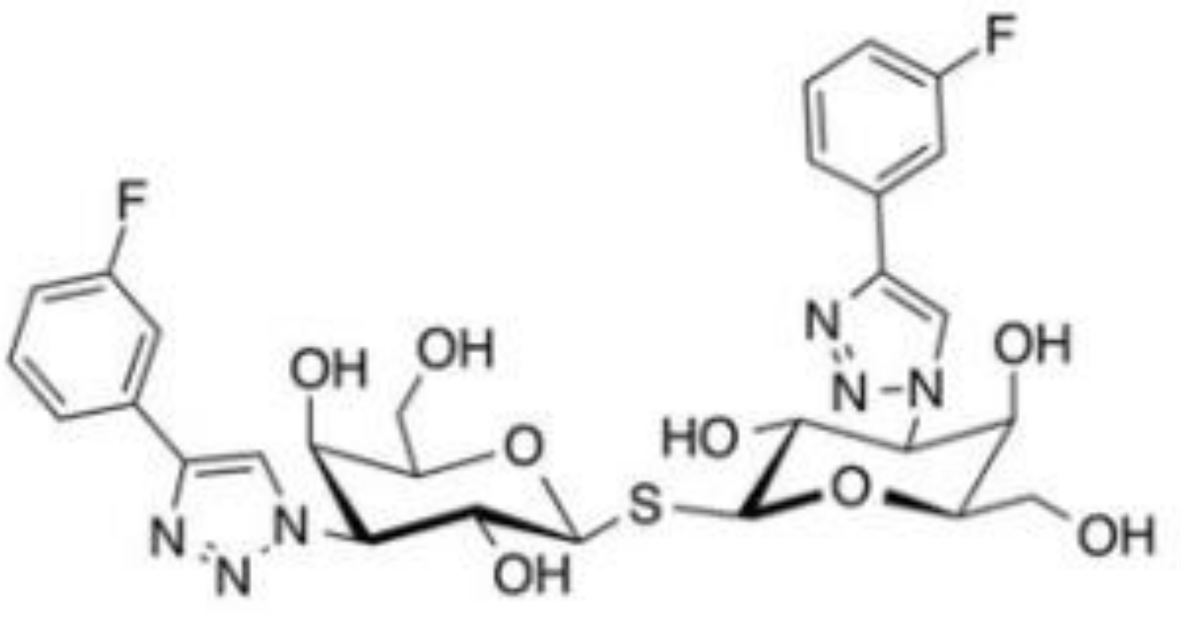
Proto | Chimera | Tandem-repeat



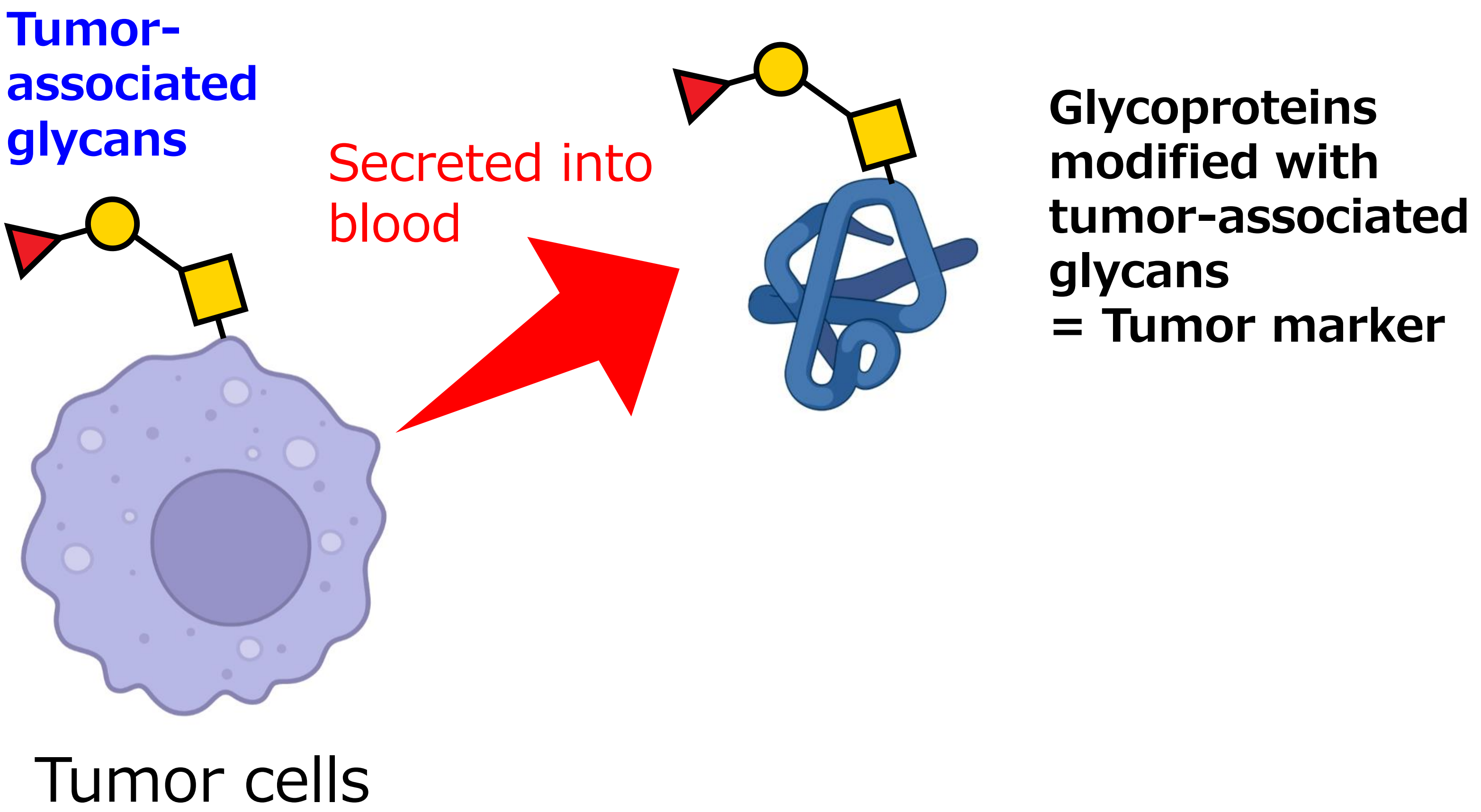
Galectin-1,2,5,7, 10,11,13,14 | Galectin-3 | Galectin-4,6,8,9,12

Olitigaltin TD139

- Galectin 3 ligand (β-thiodigalactoside)
- It specifically inhibits galectin 3, which is highly expressed in the lungs and is associated with inflammation, and is effective in treating idiopathic pulmonary fibrosis.
- Currently under development by Galecto, Inc. for the treatment of COVID19 (phase2/3)



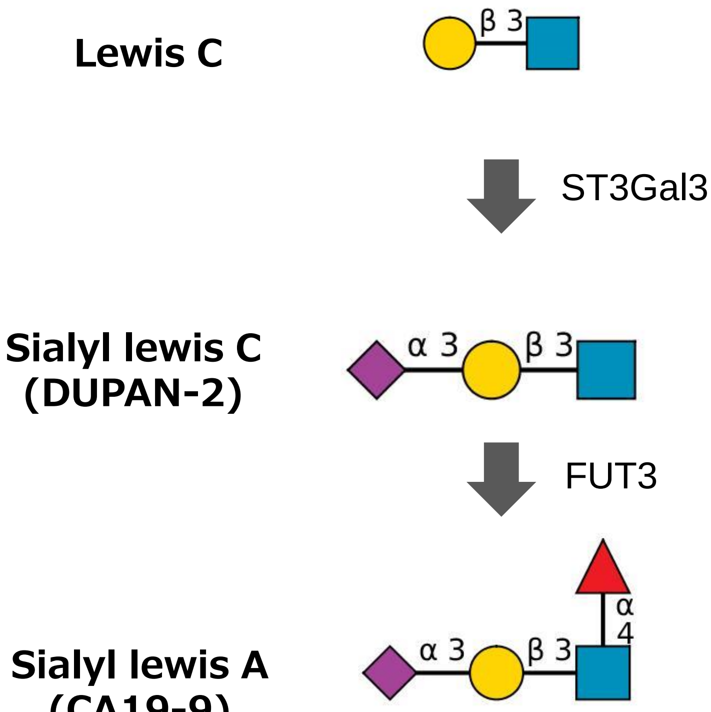
Glycan-related diagnostics



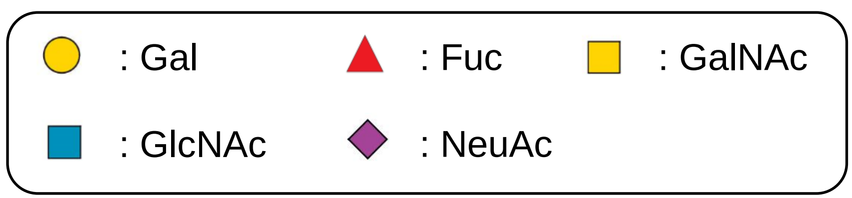
Glycan-related diagnostics

Classification	Example
Anti-glycan antibody	**CA19-9（sLeA, pancreatic cancer)** DUPAN-2（sLeC, pancreatic cancer) STN（sTn, gastric cancer) SLX（sLeX、ovarian cancer)
Anti-glycoprotein antibody	CEA（digestive cancer) CA125（ovarian cancer) CA72-5（ovarian cancer, breast cancer) PSA（prostate cancer)
Lectin-anti-glycoprotein antibody	**AFP-L3（hepatocellular carcinoma ）** M2BPGi（liver fibrosis)

Synthetic pathway of pancreatic cancer marker (DUPAN-2, CA19-9)



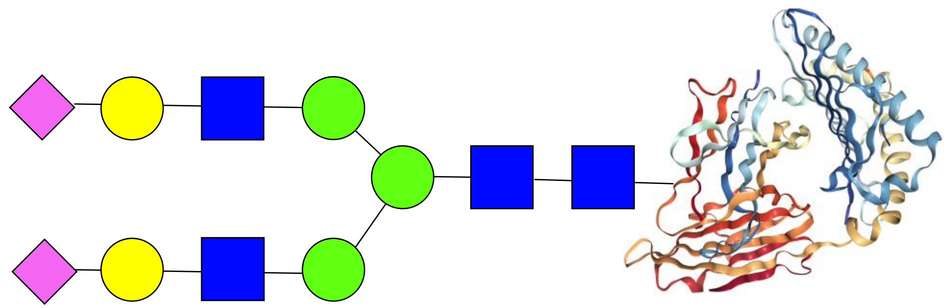
10% of Japanese are Lewis negative



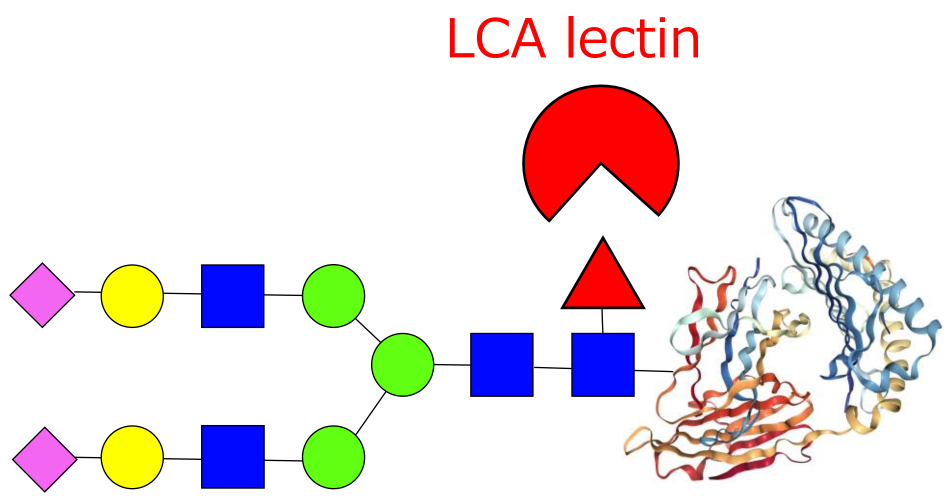
Hepatocellular carcinoma marker（AFP-L3）

An N-glycan modified on alpha-Fetoprotein (AFP) is core-fucosylated in hepatocarcinoma

AFP
Elevated by liver inflammation



AFP-L3
Elevated specifically in hepatocellular carcinoma



Automated diagnostic system, μTASWako, has been commercialized from Fujifilm Wako

Subjects and solutions for glycan/lectin drug discovery

(Subject)

1. No progress has been made in the search for new glycan targets
2. The overall picture of the glycan-receptor interaction network is not yet clear, and regulators cannot be strategically developed.
3. No progress has been made in the application of early diagnostic agents

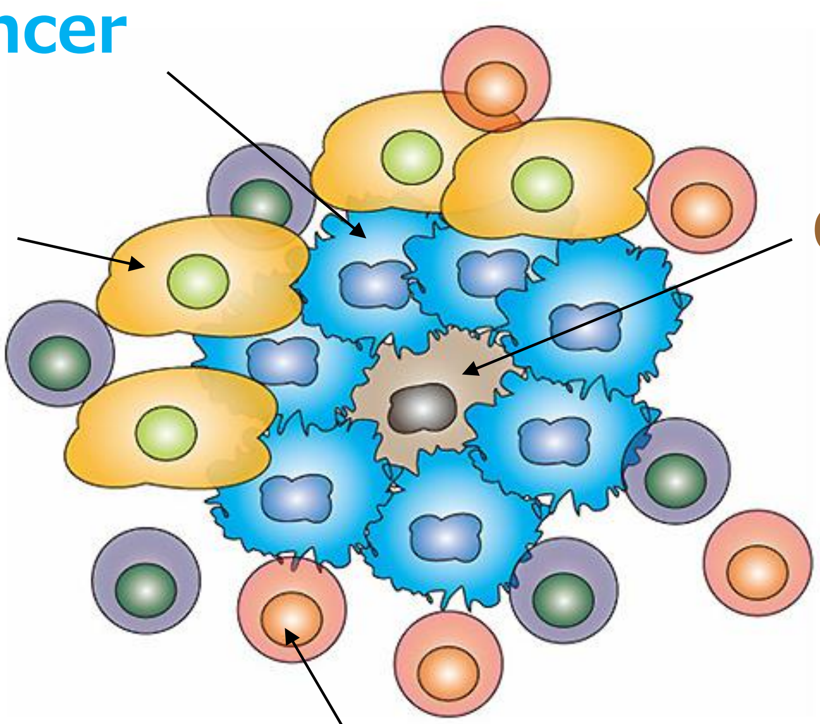
(Solutions)

1. Search for glycan targets using new glycan analysis technology
2. Development of regulatory drugs by elucidating the glycan-lectin interaction network
3. Search for glycan markers expressed in disease at early stage

Contents

1. Basics of glycan and lectin
2. Trends in glycan and lectin drug discovery
3. Single cell glycan and RNA sequencing (scGR-seq)

Tumor microenvironment



Consist of various cell types

Conventional glycan analytical methods

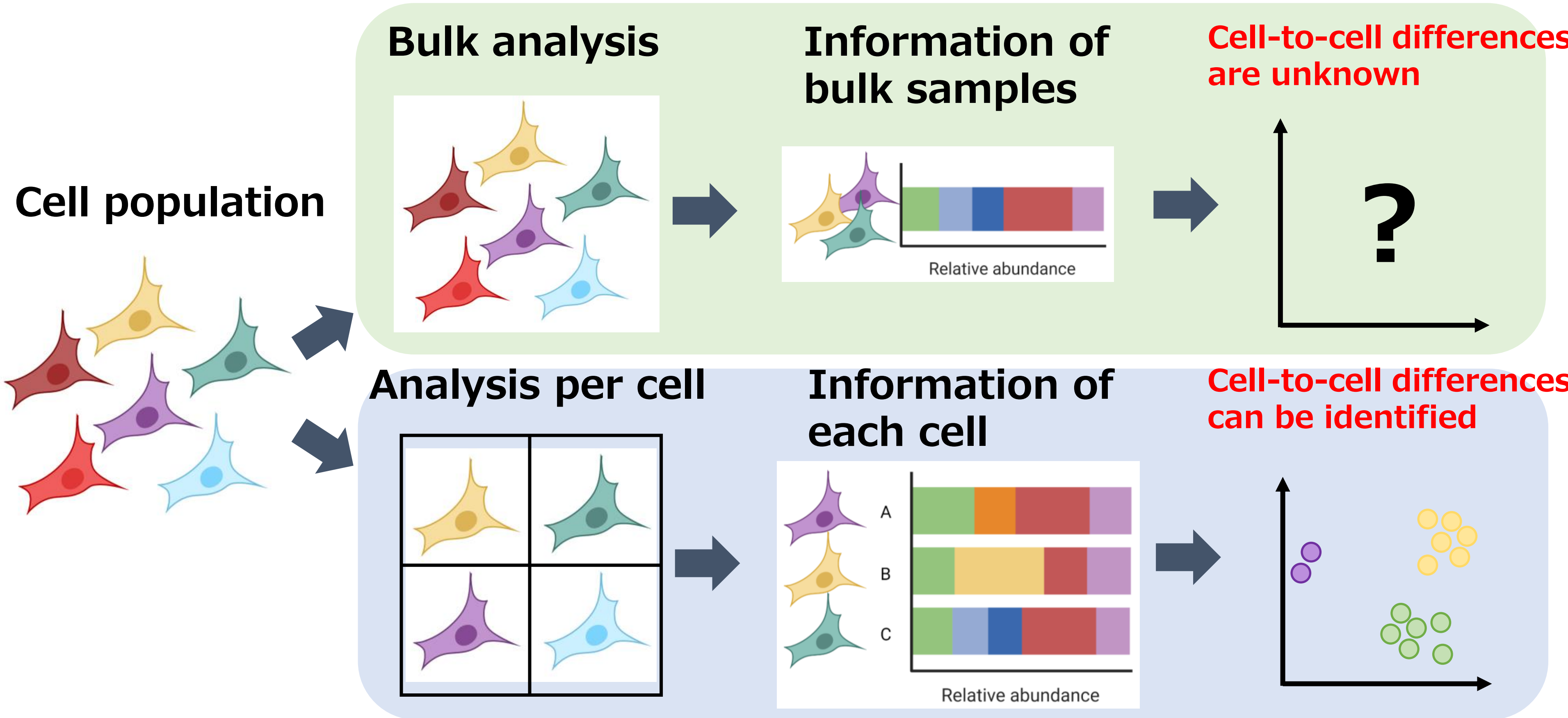
(Subject)

1. Conventional glycan analytical technology cannot obtain glycan information of individual cells constituting tumor tissues.
2. Because cells are destroyed for analysis, other omics information cannot be obtained at the same time.

(Solution)

By developing a technology for simultaneous analysis of glycan and RNA in each cell, we can elucidate the glycans of individual cells that constitute the tumor microenvironment and the overall picture of the glycan-lectin interaction network.

Advantages of single cell analysis



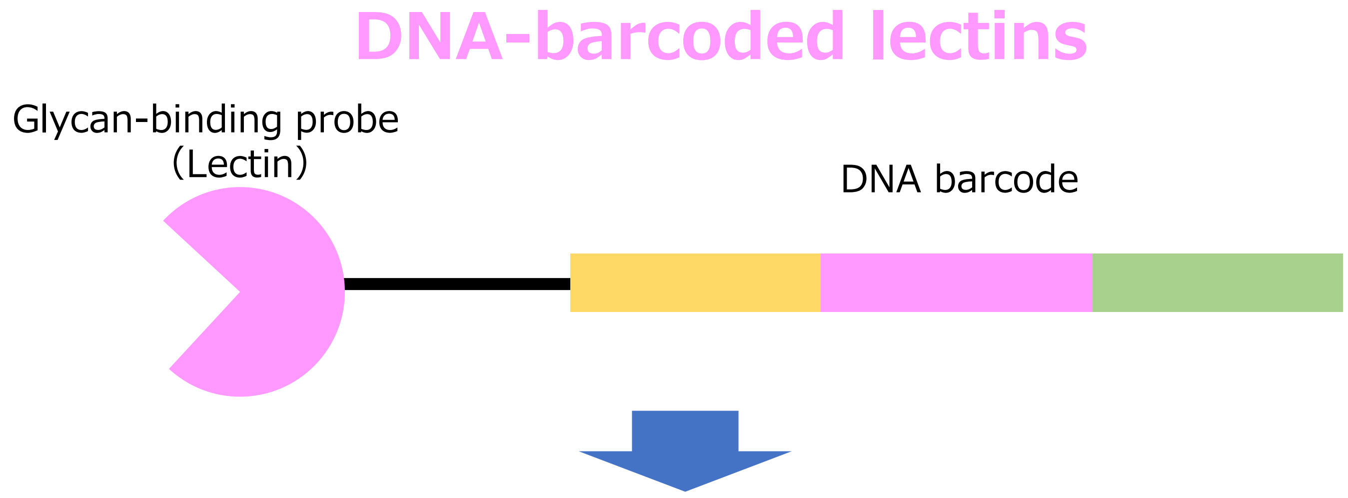
1. Analysis of heterogeneity within a cell population (identification of cell subpopulations)
2. Analysis of cellular response of each cell type
3. Identification of surface markers of rare cells

Why is it so difficult to obtain glycan information for each cell?

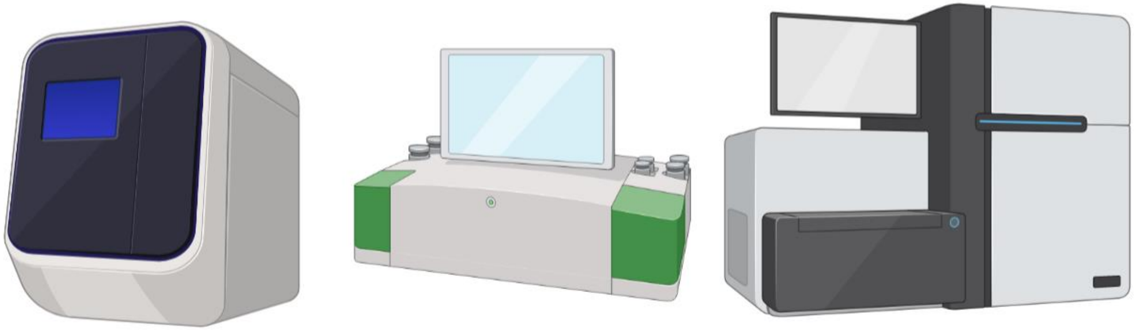
↓

Because glycan information cannot be amplified

Conversion of glyco-code to genetic-code

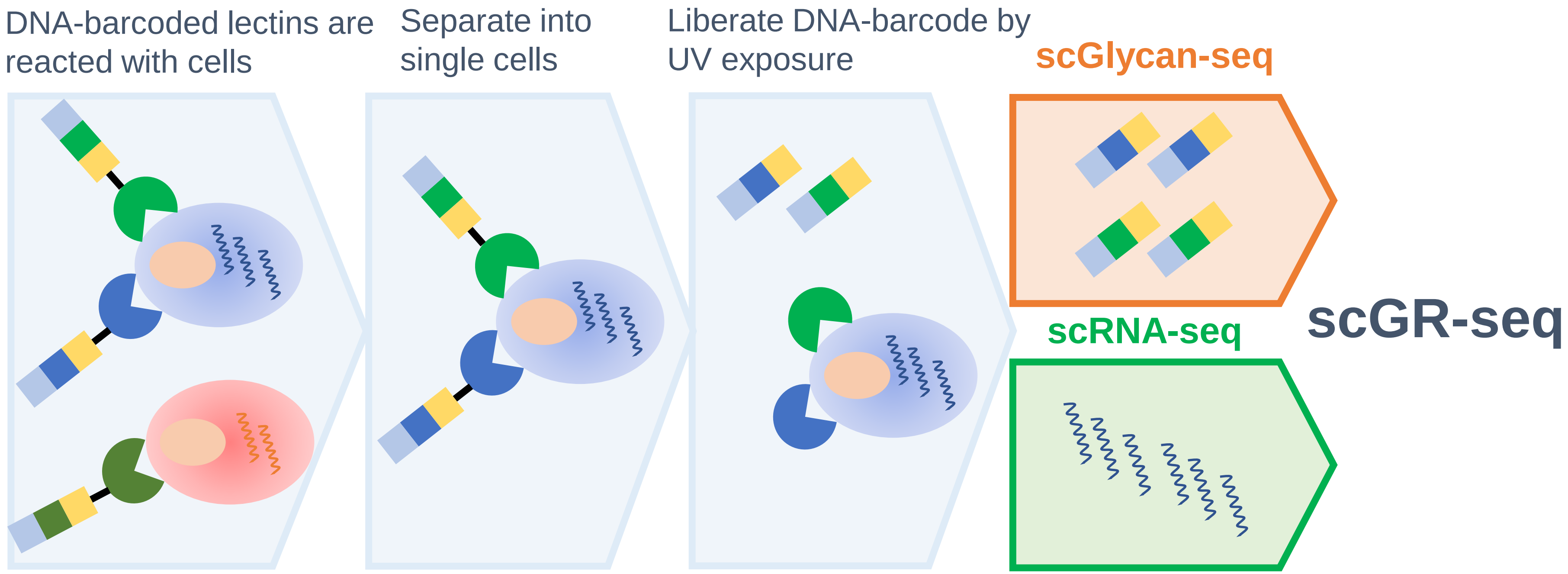


Glyco-code can be converted to genetic-code, amplified by PCR, and analyzed by highly-sensitive gene analytical systems such as NGS



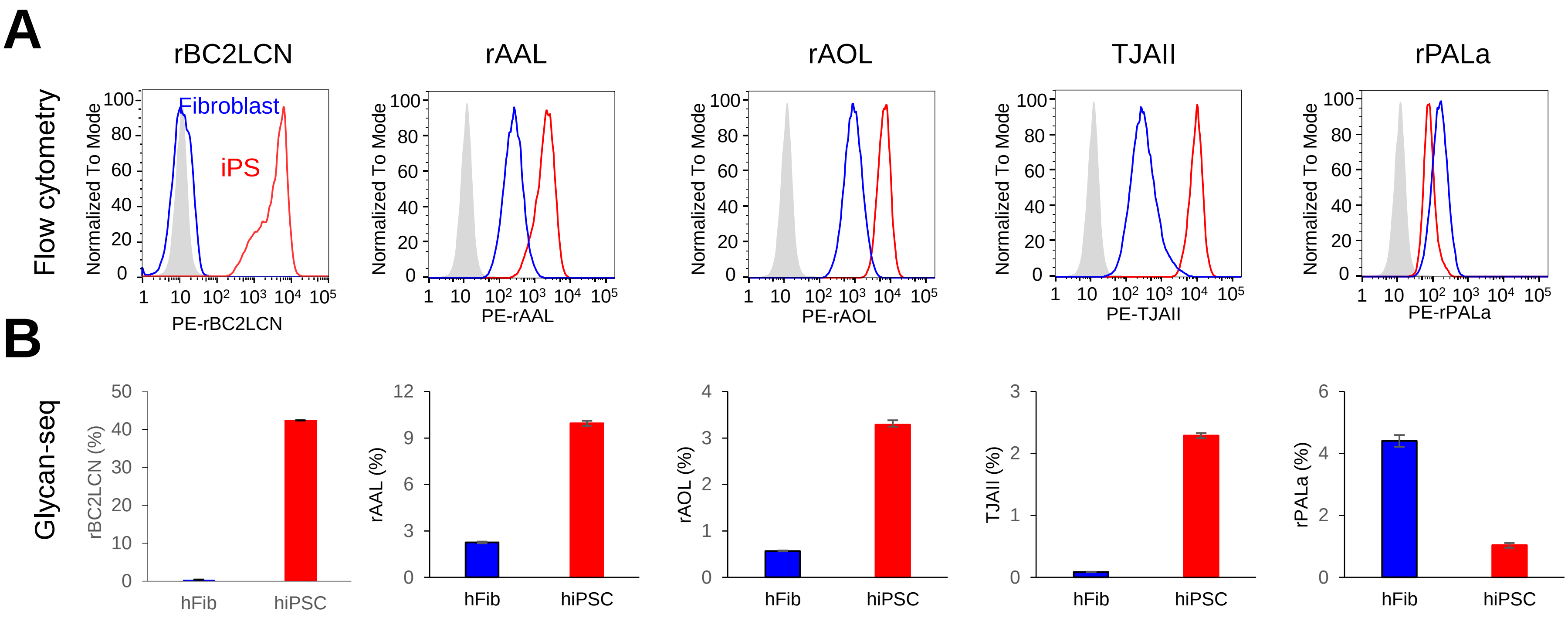
Single cell glycan and RNA profiling technology（scGR-seq）

Protocol summary



Minoshima et al. iScience 2021
Odaka et al. STAR Protocols 2022

Glycan-seq analysis of iPS and fibroblast

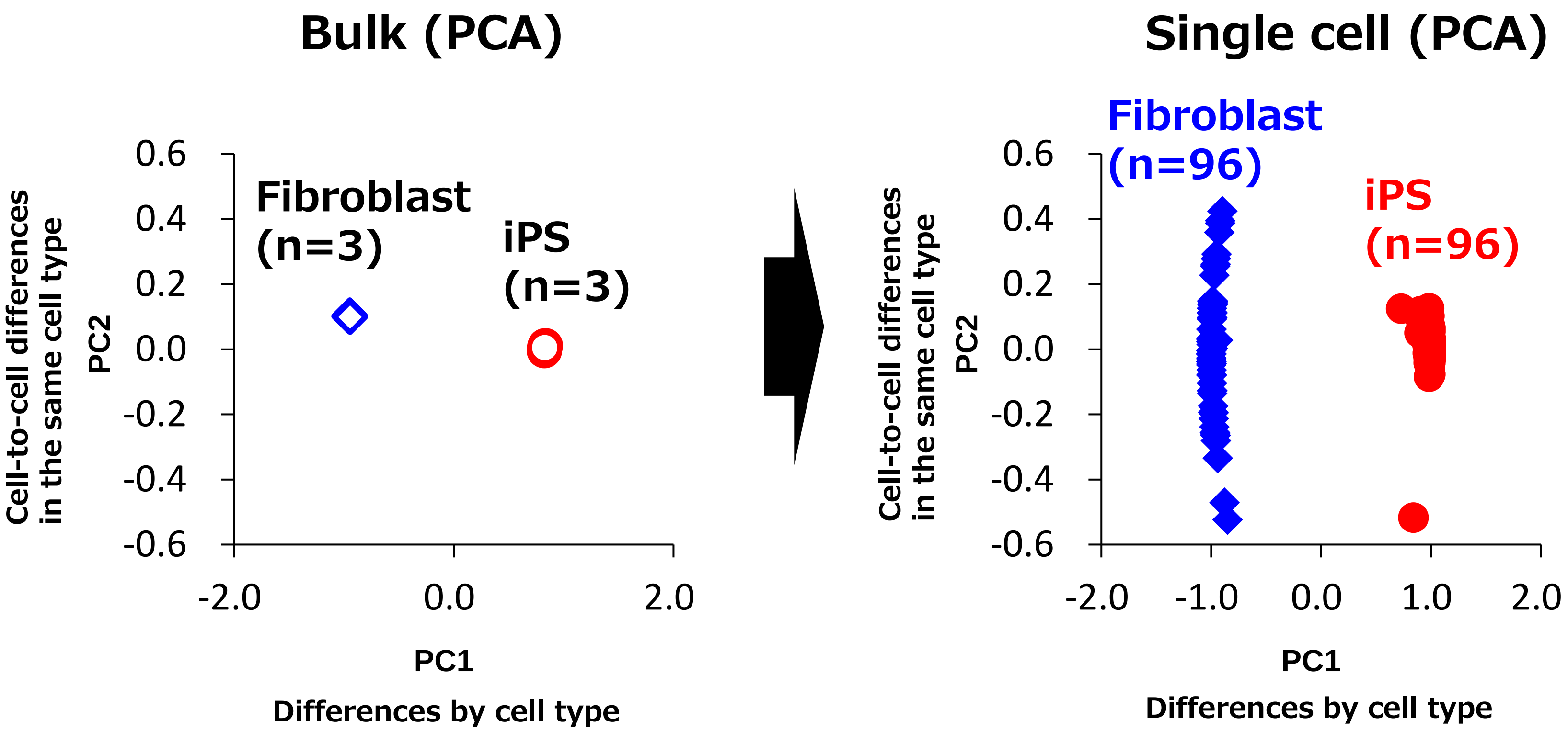


Glycan-seq data agree well with flow cytometry data

Minoshima et al. iScience 2021

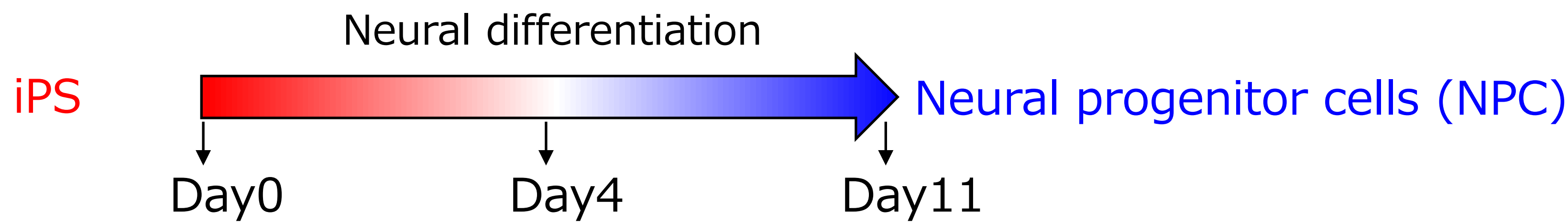
Bulk and single cell Glycan-seq of iPS and fibroblast

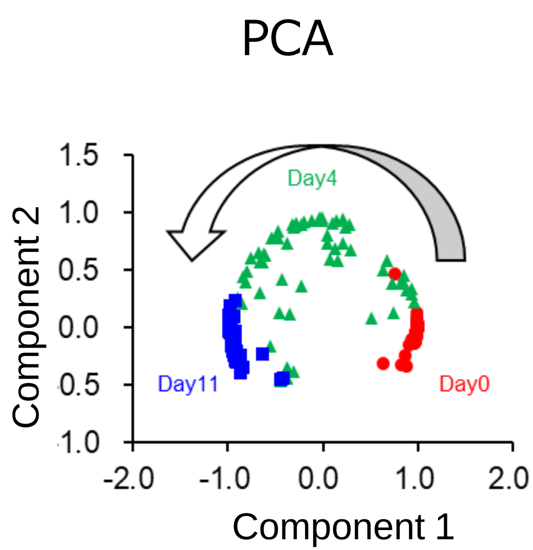
Minoshima et al. iScience 2021

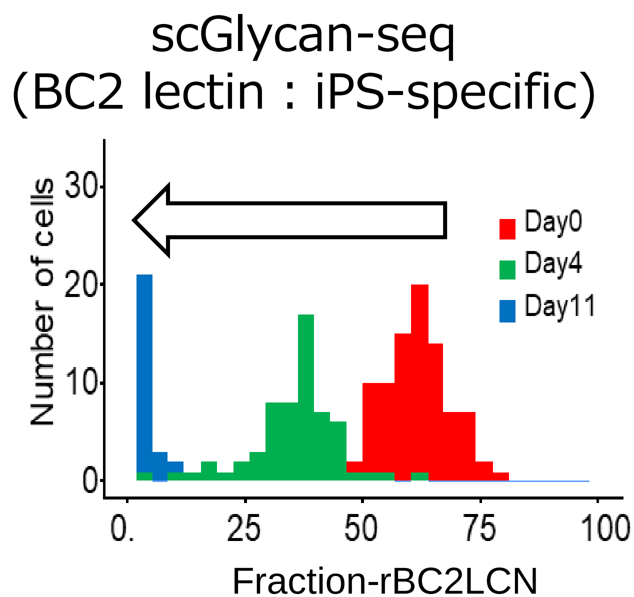


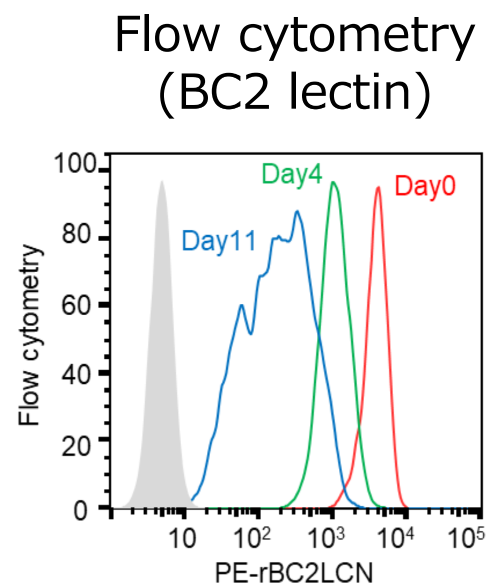
Differences in glycan profiles of each cell can be determined by scGlycan-seq.

scGlycan-seq
(iPS vs iPS-derived neural progenitor cells)



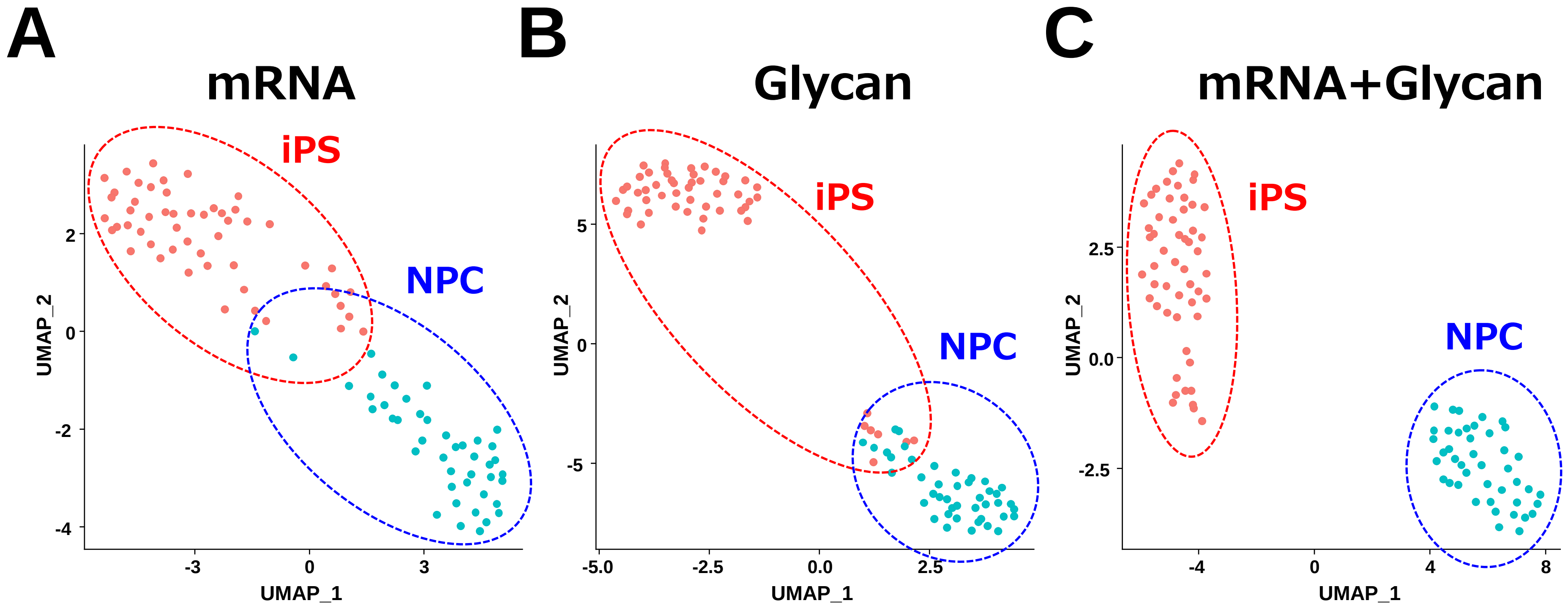






Alteration of glycan profiles of each cell can be quantitatively analyzed

Cell classification of iPS and NPCs by UMAP



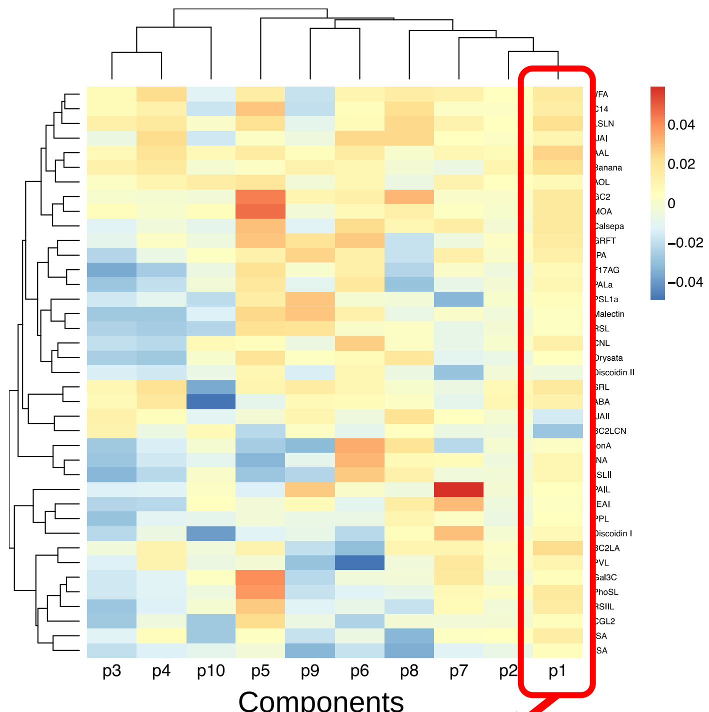
Minoshima et al.
iScience 2021

The combination of the two omics information more accurately discriminated the two types of cells

Correlation analysis of gene and lectin groups by PLS

Correlated genes and lectins are grouped

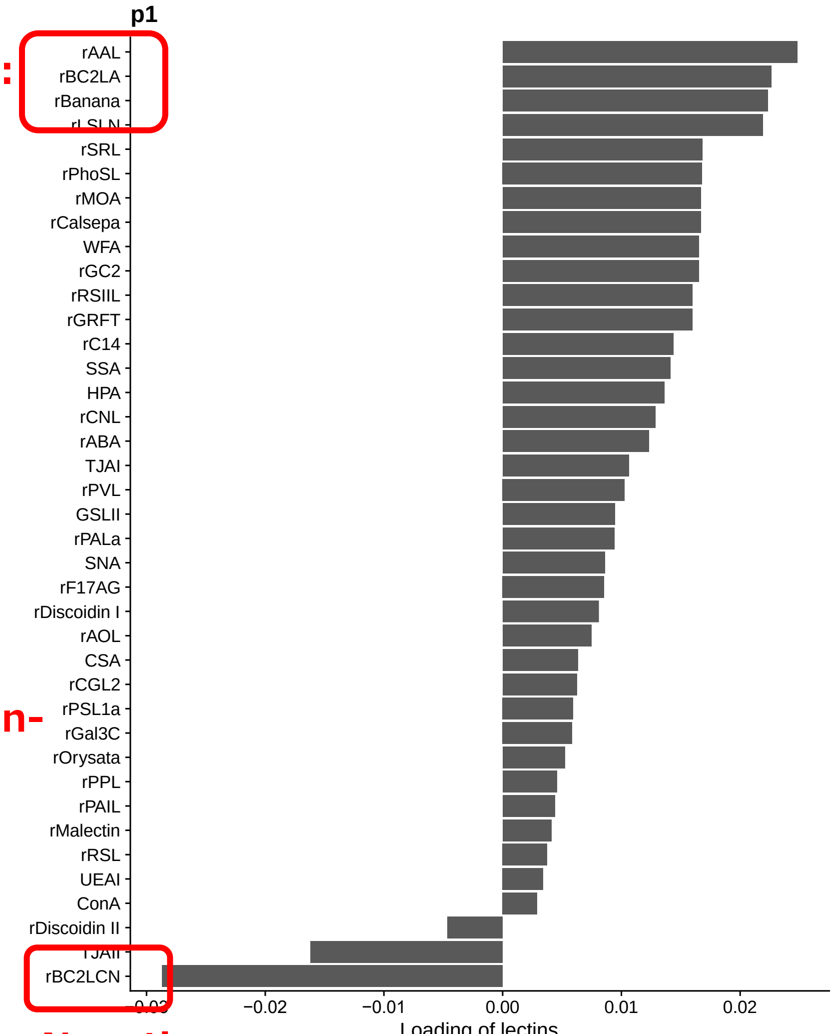
A



Lectin Correlation

B

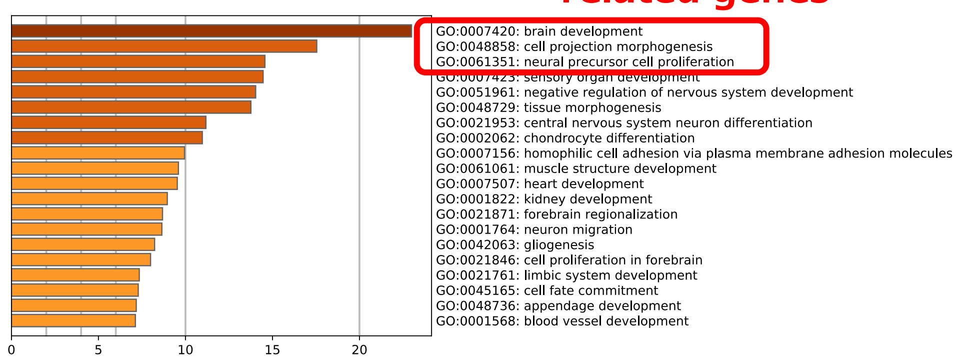
Positive : rAAL、rBanana



Negative : rBC2LCN

C **GO enrichment analysis**

Neural differentiation-related genes



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Lectin-binding profile and glycogene expression of each cell can be acquired

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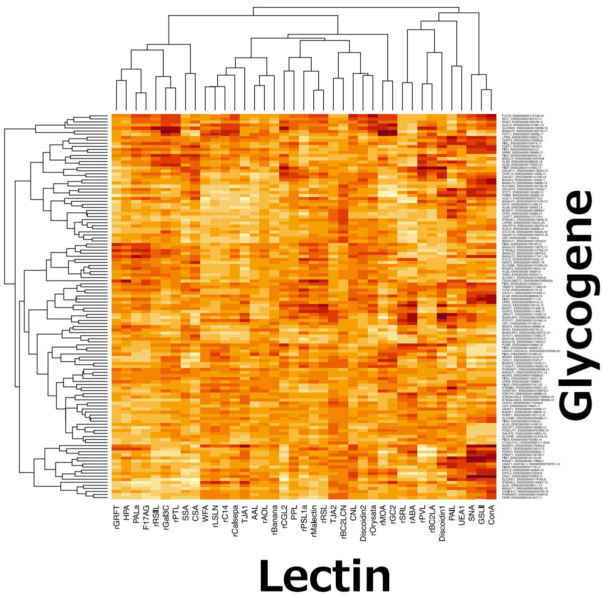
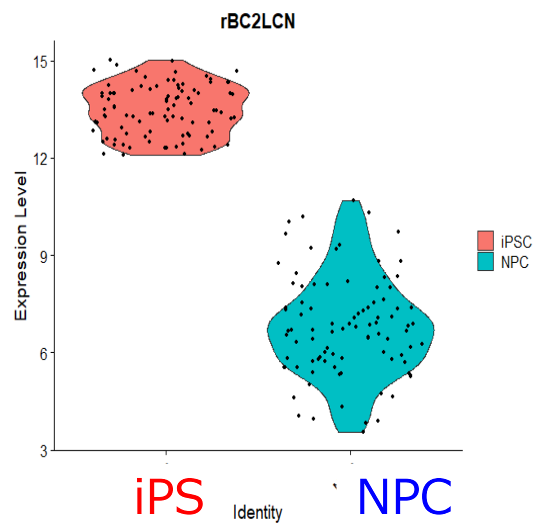
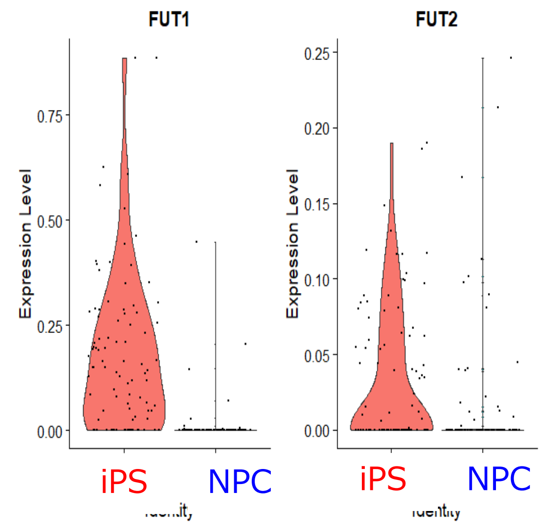


α1,2fucose-specific lectin（rBC2LCN）



α1,2fucosyltransferase



Combined data of glycogene expression and lectin reactivity enable more accurate analysis of glycans in individual cells

Pseudotime analysis using the data of scGR-seq

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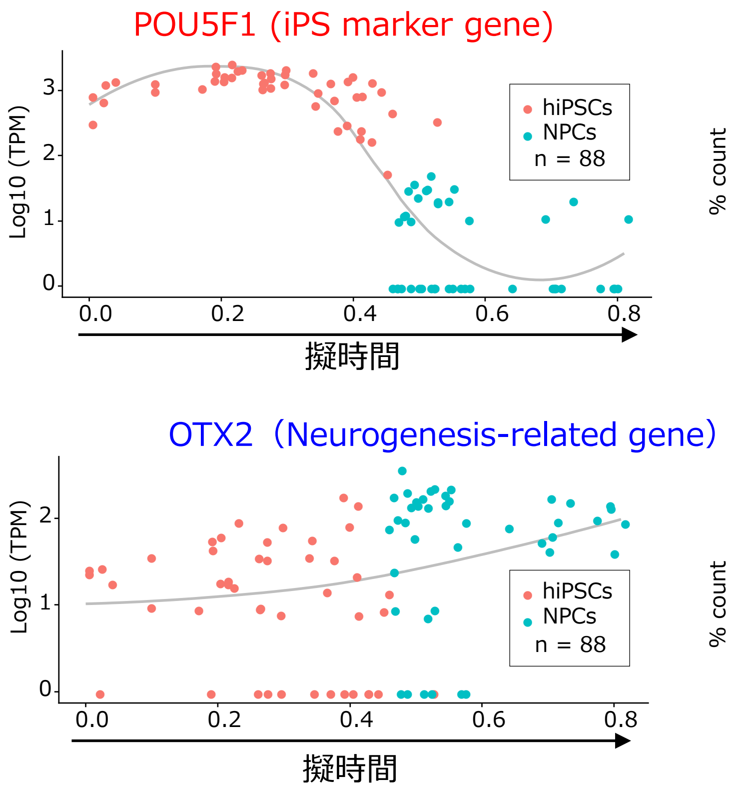
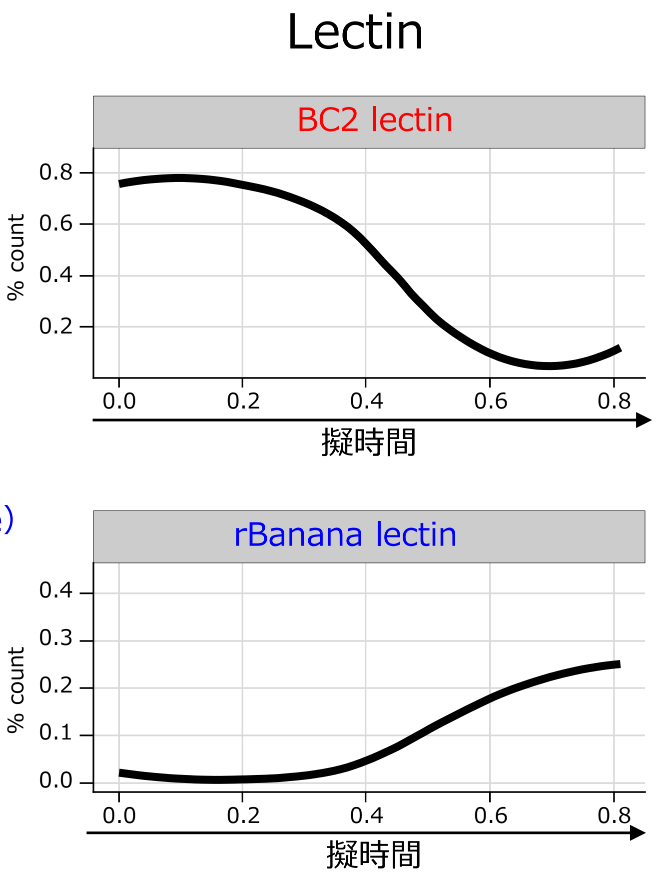




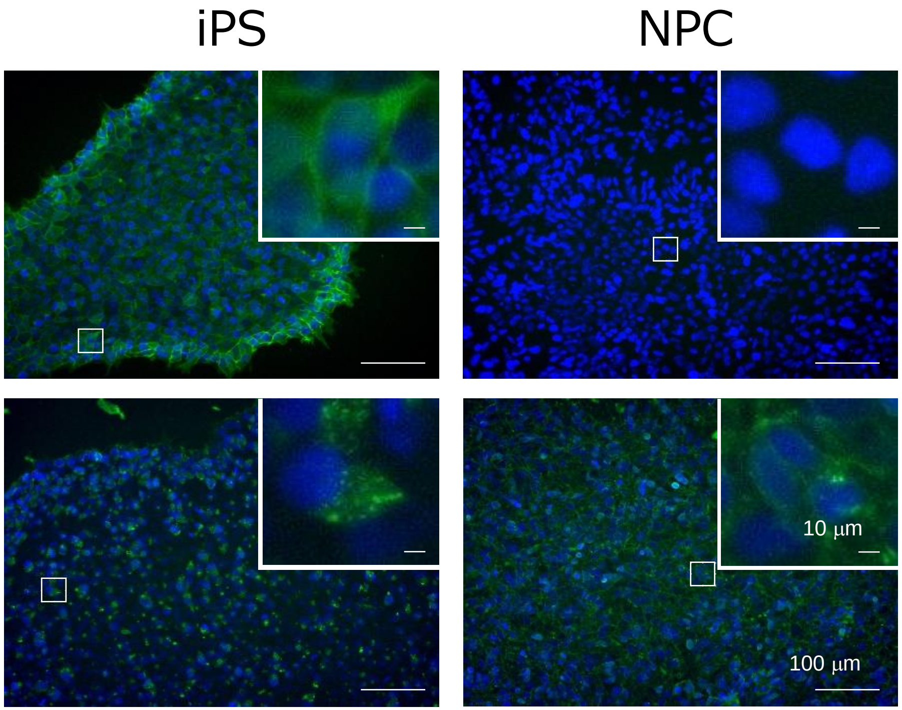
Lectins that correlate with changes in gene expression can be searched

Identification of glycan marker probes for undifferentiated and differentiated cells

Lectin staining

BC2 lectin
(known iPS marker probe)

rBanana
(new neural progenitor cell marker probe)



Green: lectin staining, *blue*: nuclear staining.

Cell surface glycan marker probes can be identified

Conclusion

We developed bulk and single cell glycan profiling technologies by sequencing using DNA-barcoded lectins (scGlycan-seq).

We also developed simultaneous profiling method of glycan and RNA in single cells (scGR-seq)

Advantage of scGR-seq

1. Glycans can be analyzed by NGS, the same instrument used for gene expression analysis.

2. We can obtain glycan profile of each cell in tissues containing various cell types at once.

3. We can analyze the relationship between the glycome and the transcriptome in each cell.

References

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2. Glycan Profiling by Sequencing to Uncover Multicellular Communication: Launching Glycobiology in Single Cells and Microbiomes.
Oinam L. and Tateno H.
Front. Cell Dev. Biol. 2022 in press. doi: 10.3389/fcell.2022.919168

3. Glycan profiling of the gut microbiota by Glycan-seq.
Oinam L, Minoshima F, Tateno H.
Isme Commun. 2022; 2(1). doi: org/10.1038/s43705-021-00084-2

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