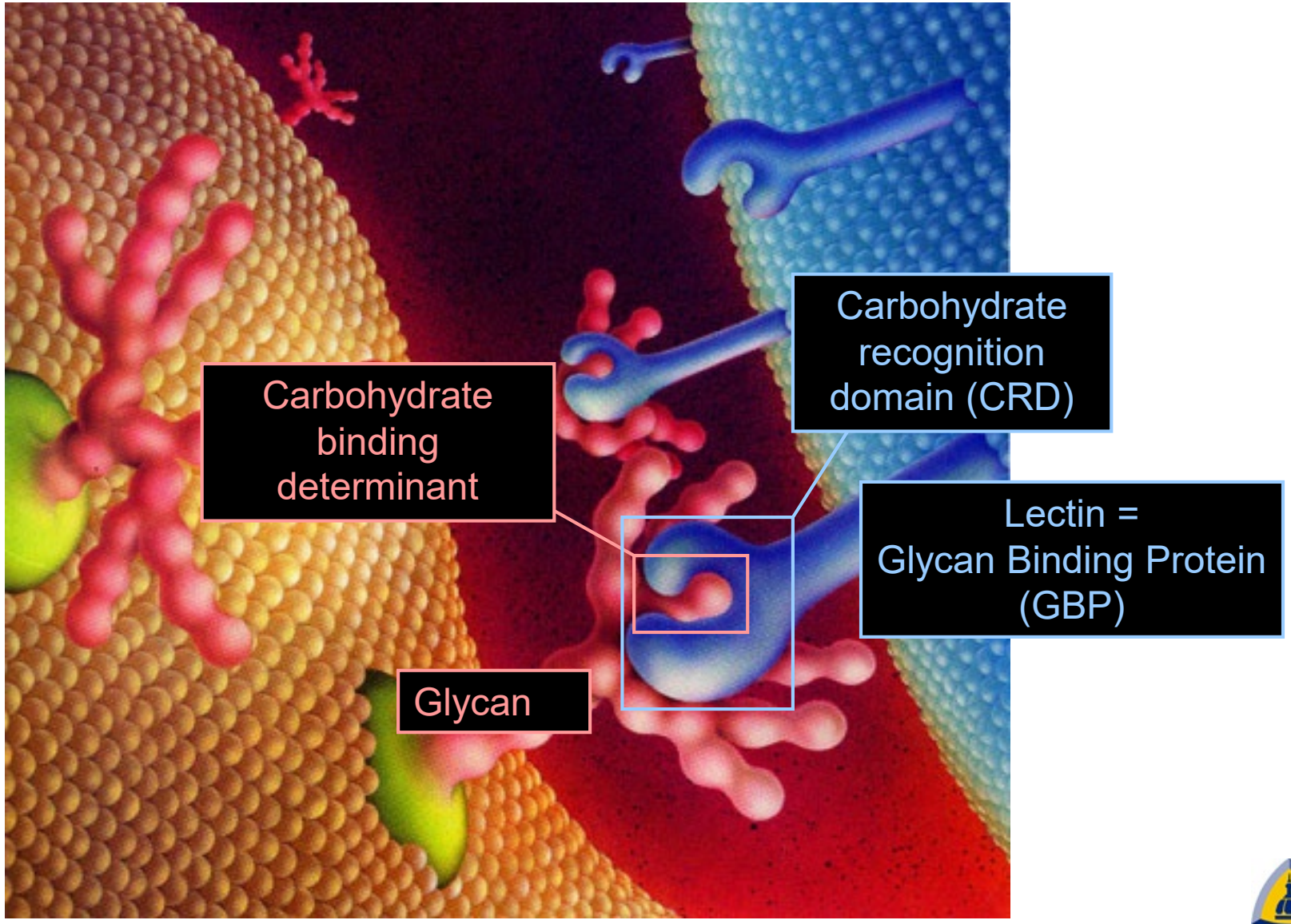
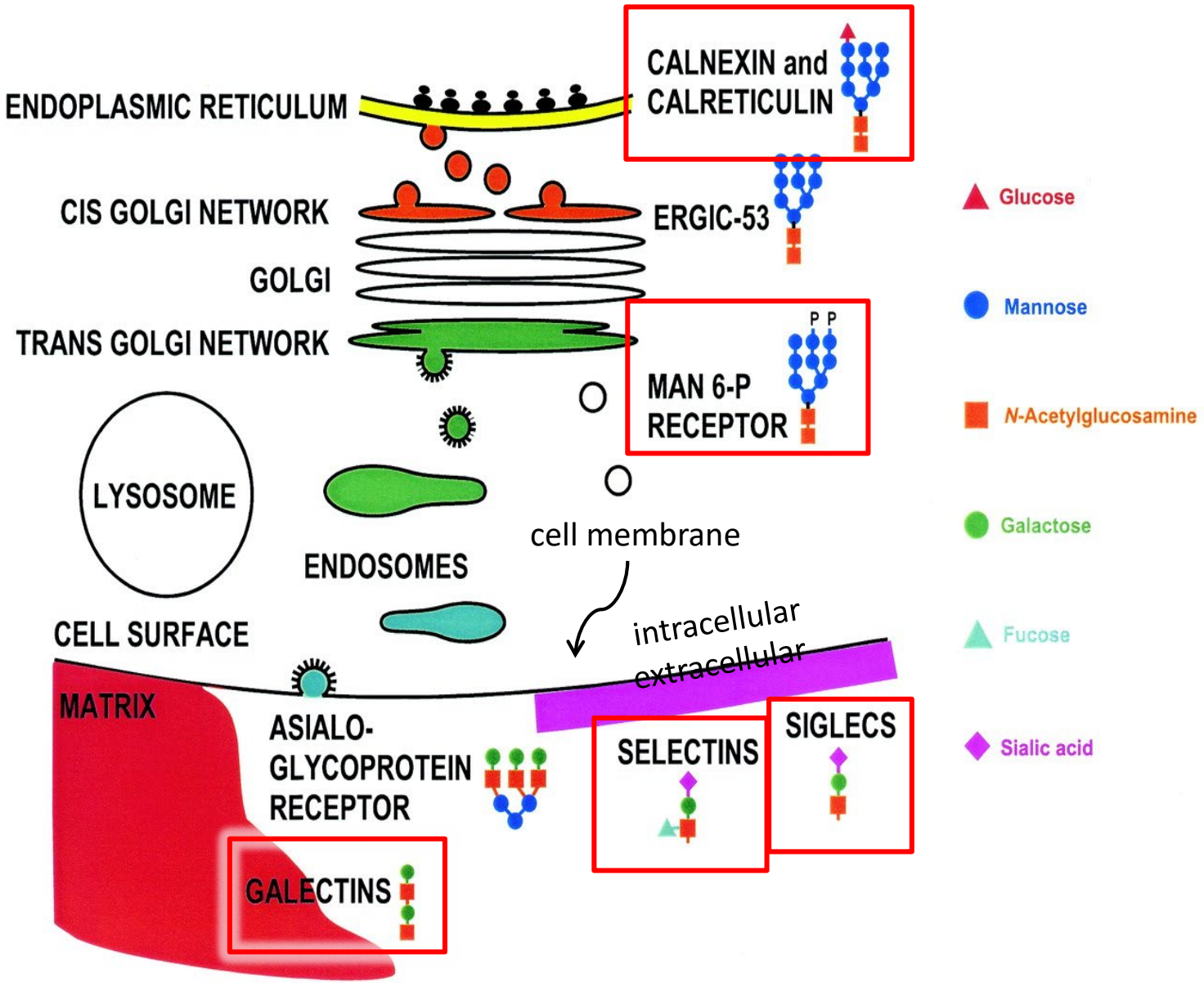
Protein-glycan recognition



Sharon and Lis (1993) Scientific American

JOHNS HOPKINS MEDICINE

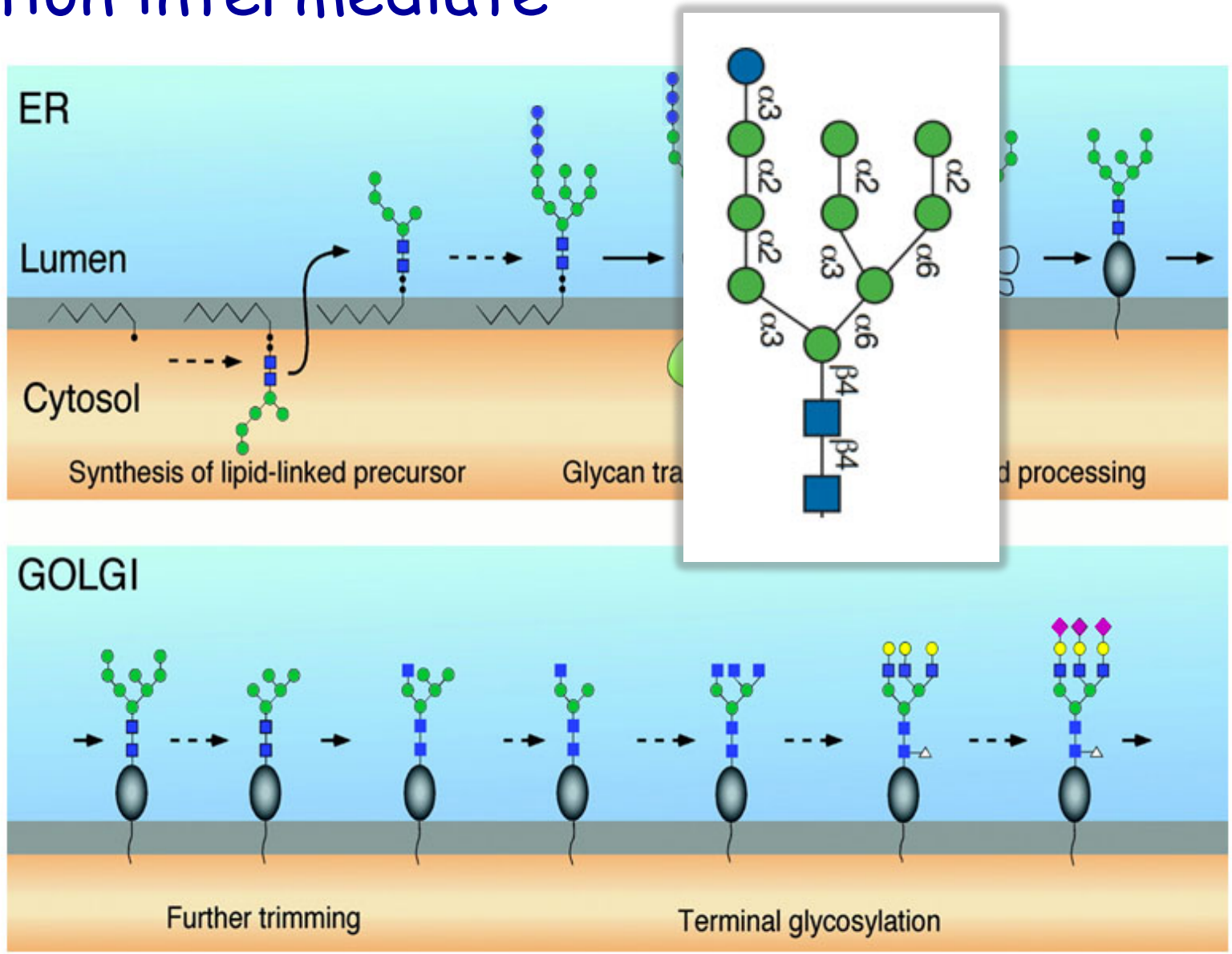
Glycan Binding Protein Functions



Dodd, R.B. and Drickamer, K. (2001) *Glycobiology* **11**, 71R-79R

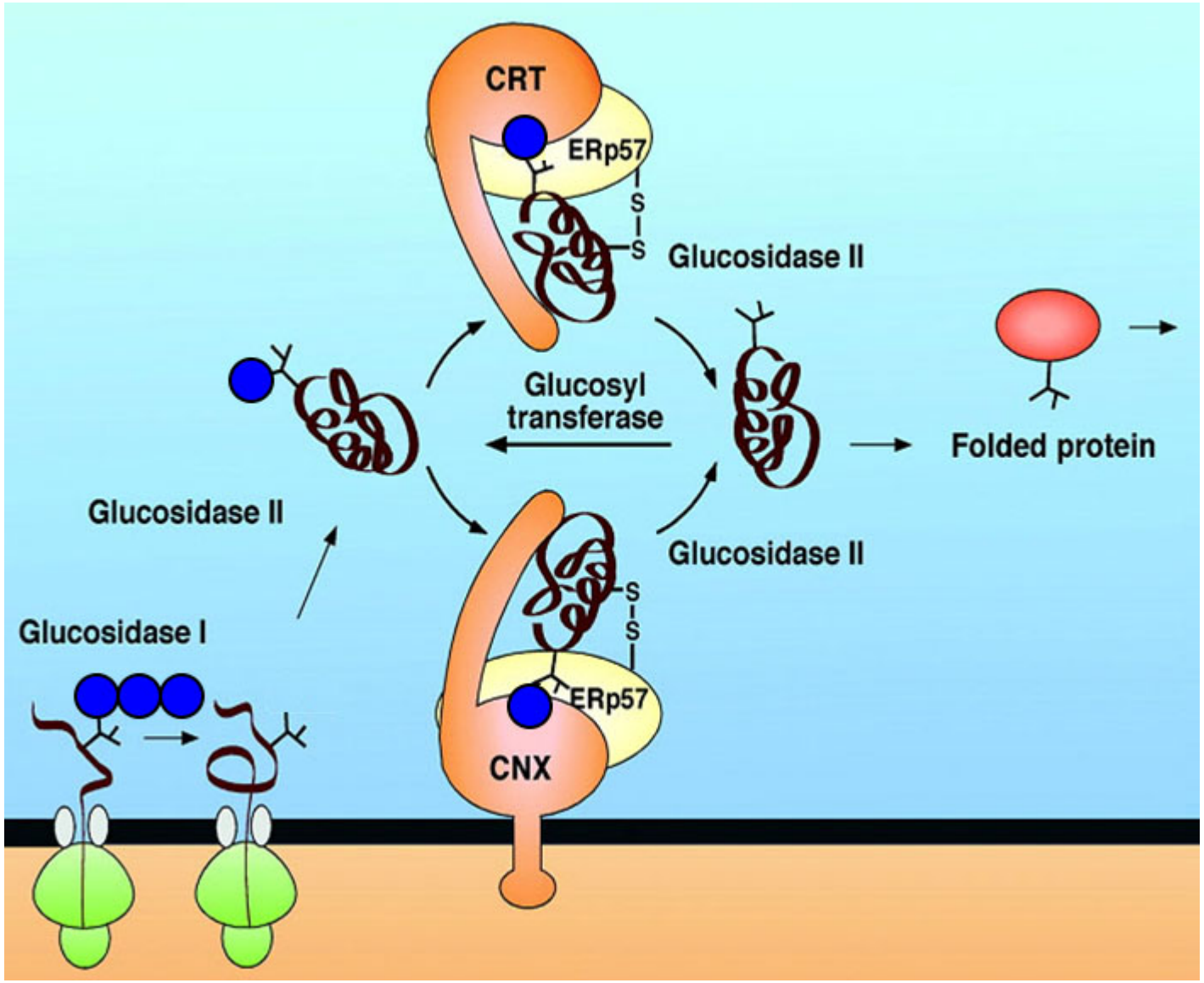
Calnexin (CNX) & Calreticulin (CRT) target an N-glycosylation intermediate

N-linked glycan biosynthesis



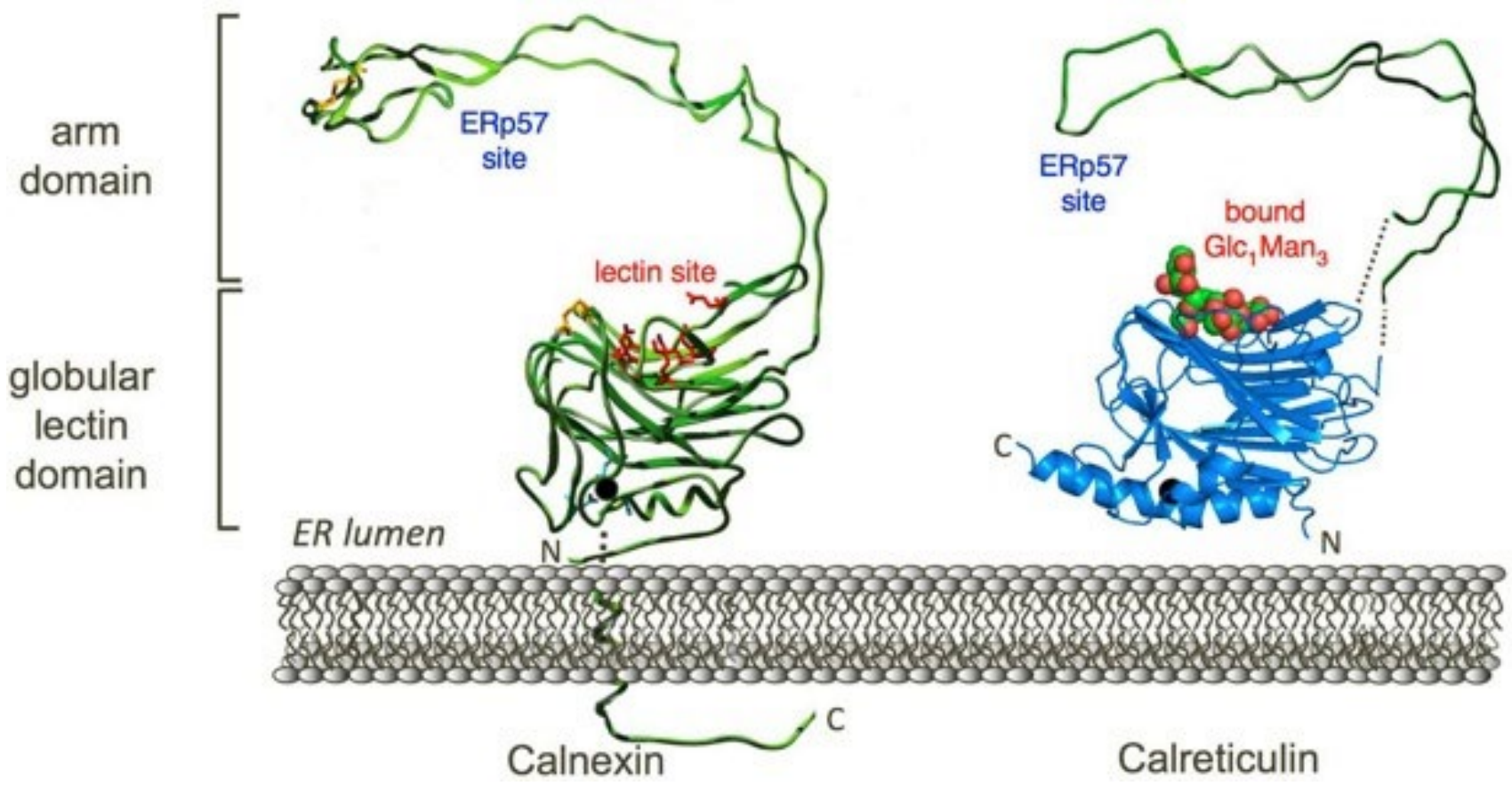
Helenius, A. and Aebi, M. (2001)
Science **291**, 2364-2369

Function of CNX & CRT



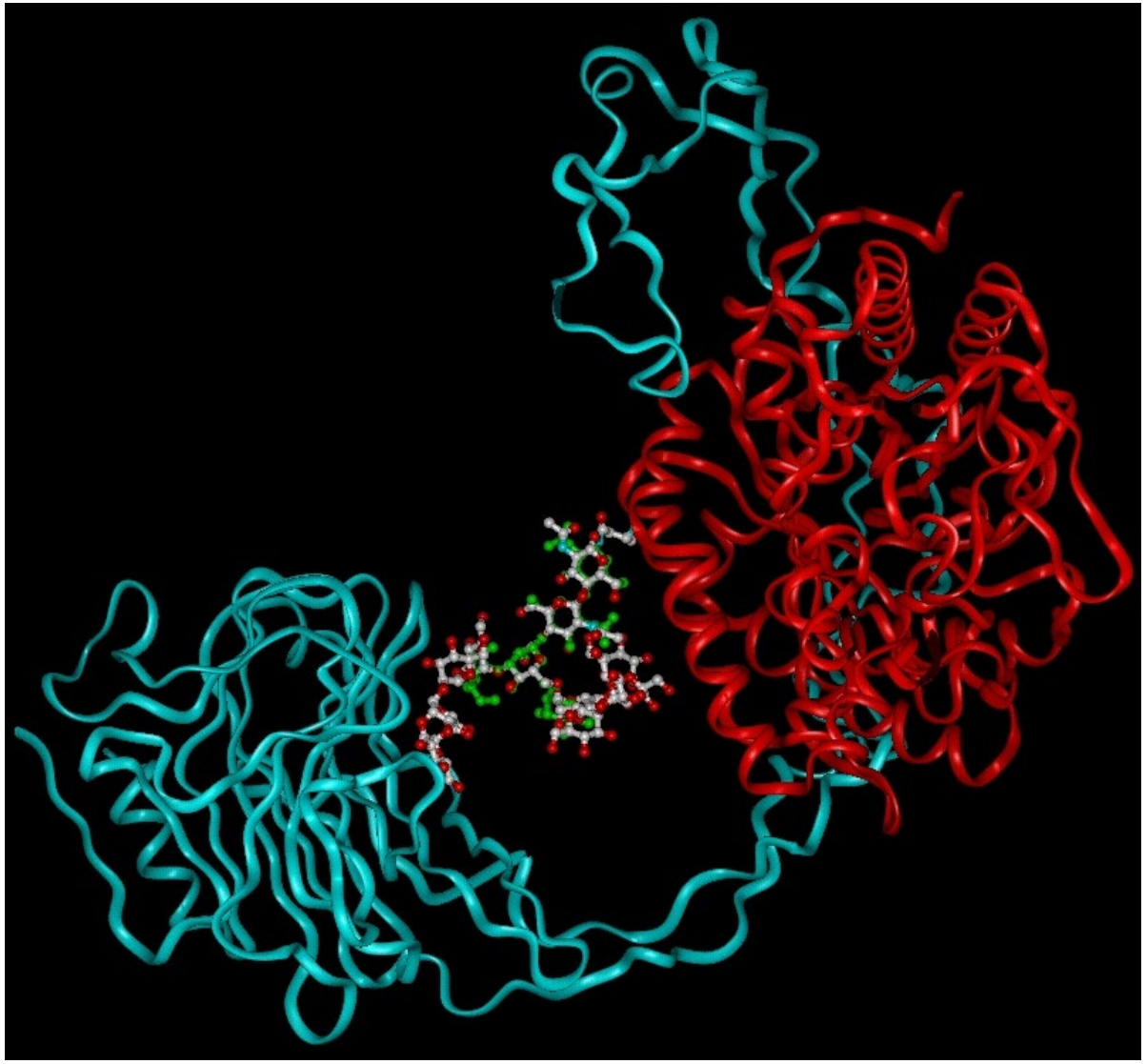
Helenius, A. and Aebi, M.
(2001) *Science* **291**, 2364-2369

Calnexin & Calreticulin structures



Courtesy of Dr. David Williams, University of Toronto

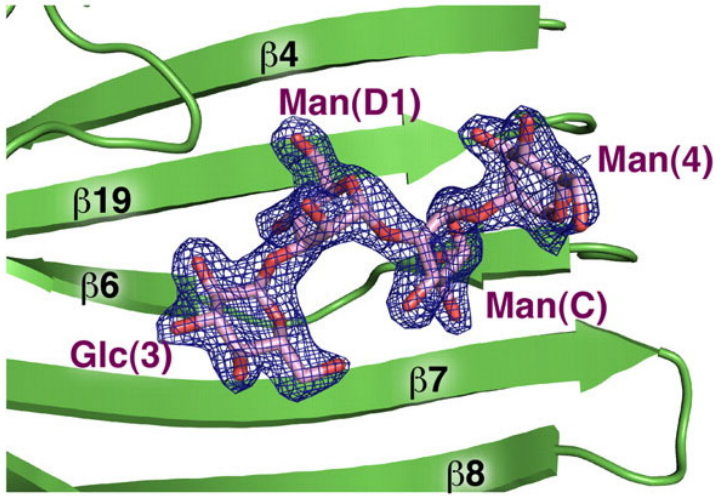
Model of calnexin and a Glc_1Man_9-glycoprotein

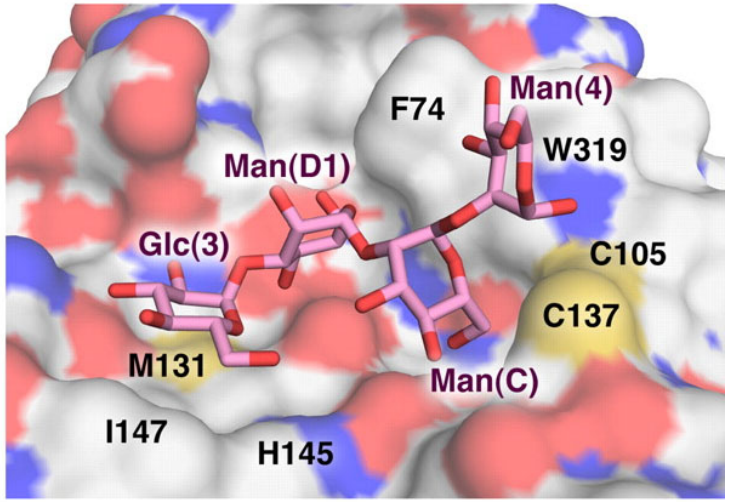


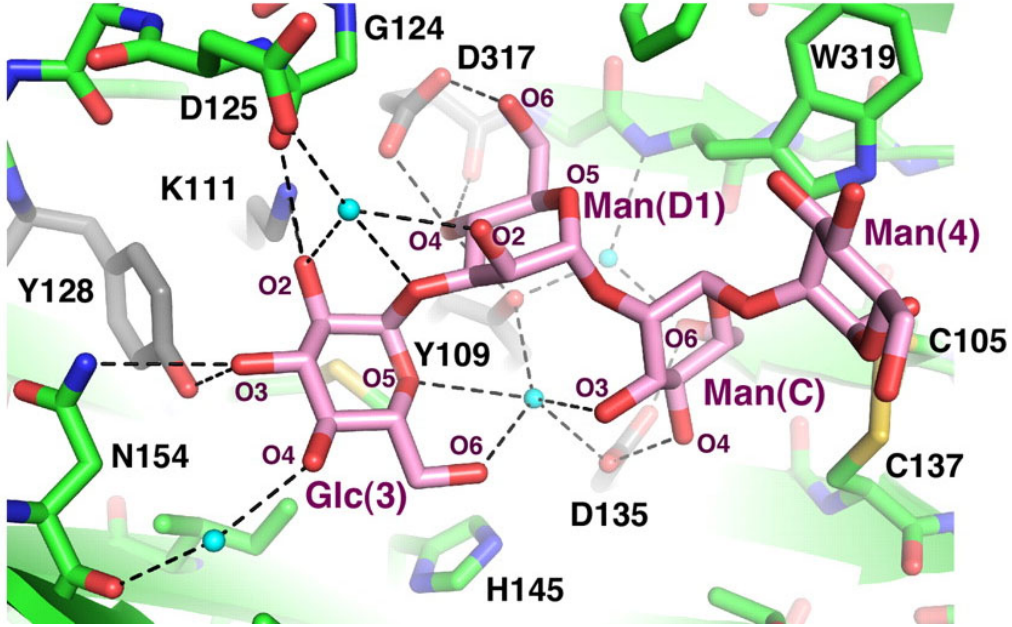
Courtesy of Miroslaw Cygler, McGill University

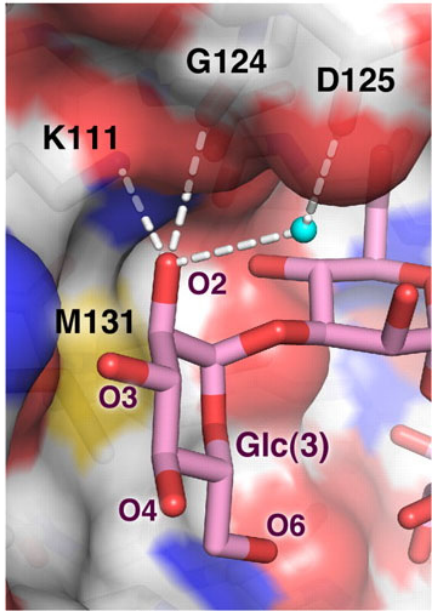
Molecular basis of recognition

precisely oriented hydrogen bonds – direct and through bound water



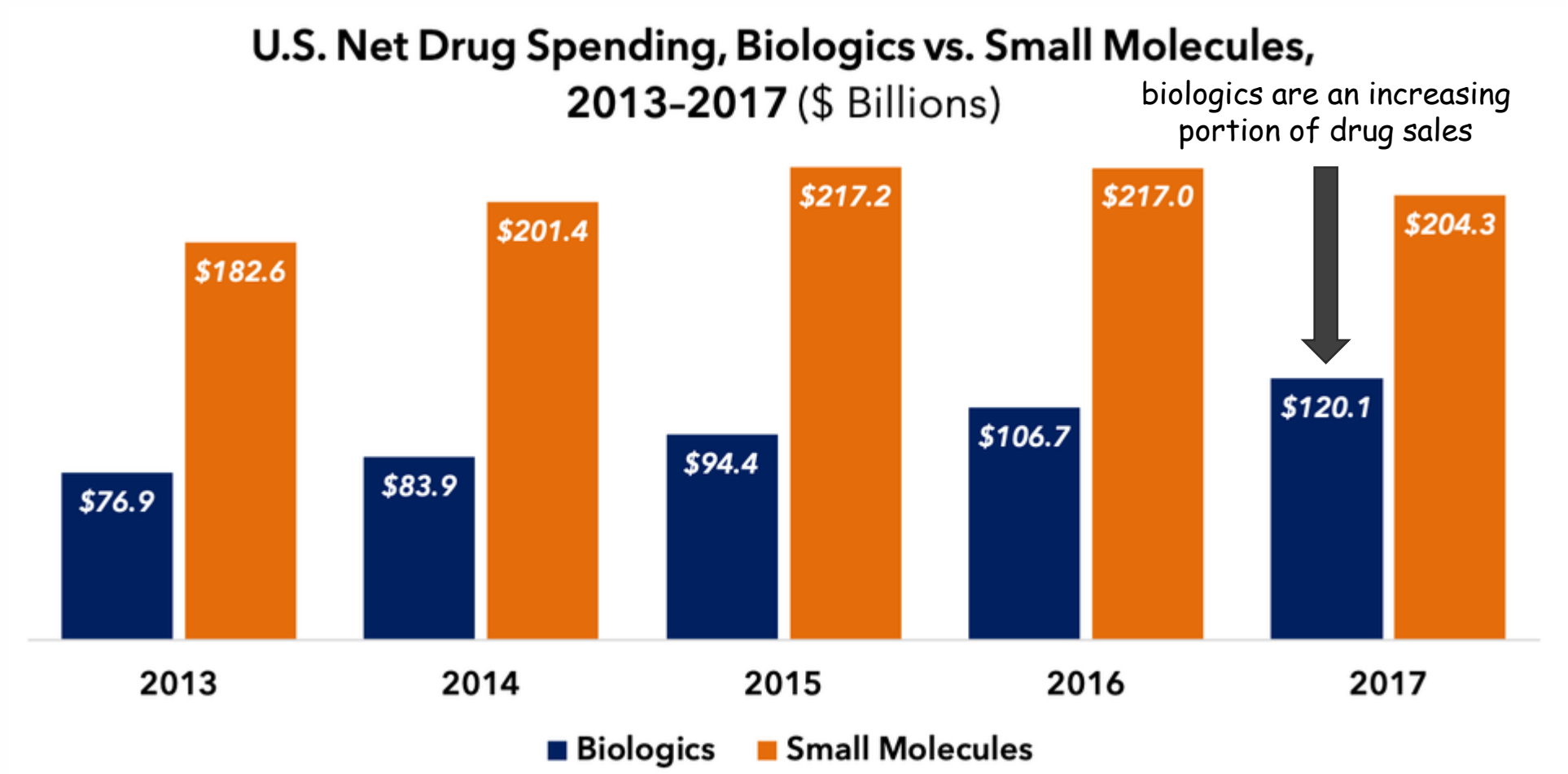






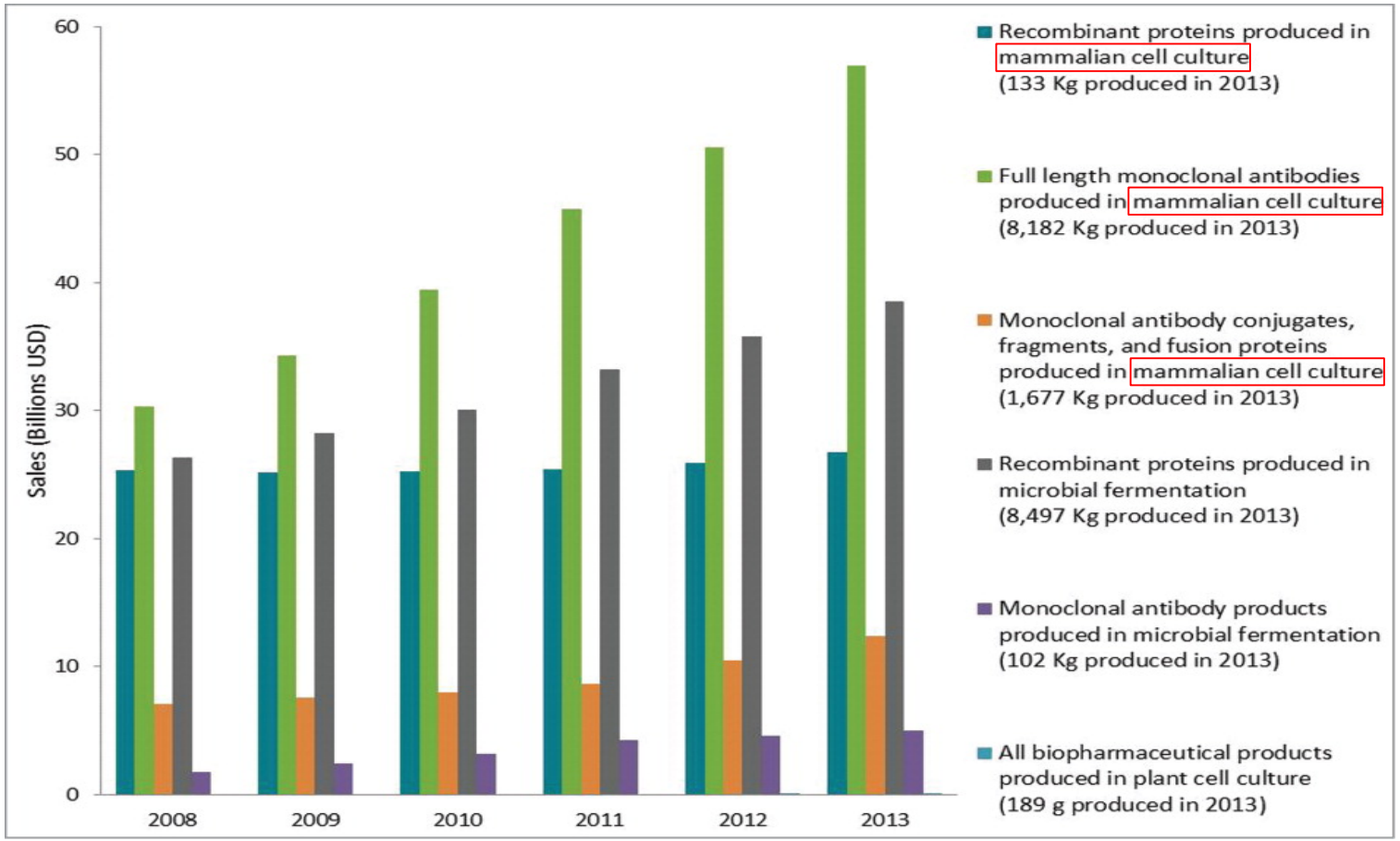
Kozlov et al. (2010)
J. Biol. Chem. 285:38612

N-linked glycoprotein folding is big business



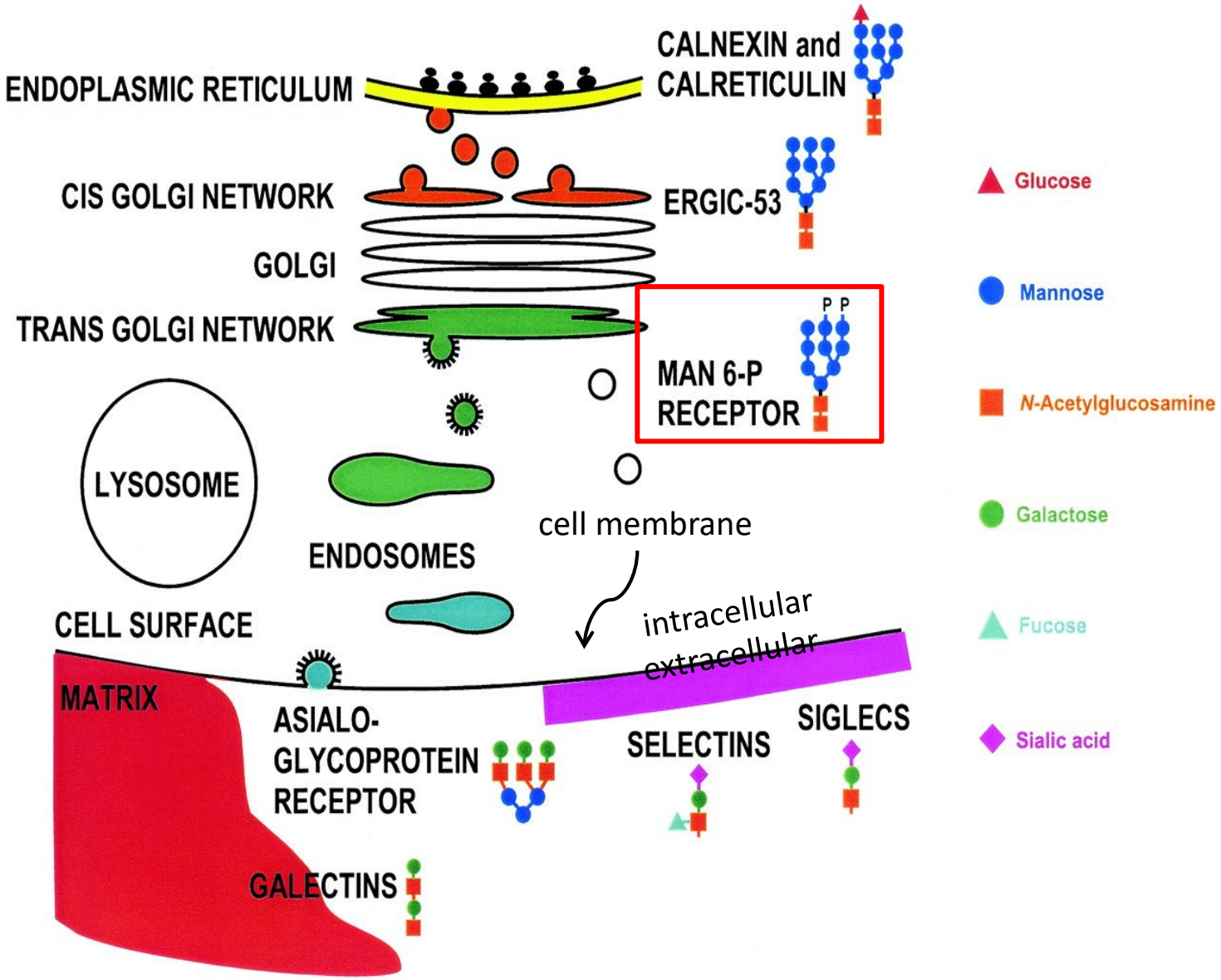
https://www.forbes.com/

Most biologics are N-linked glycoproteins



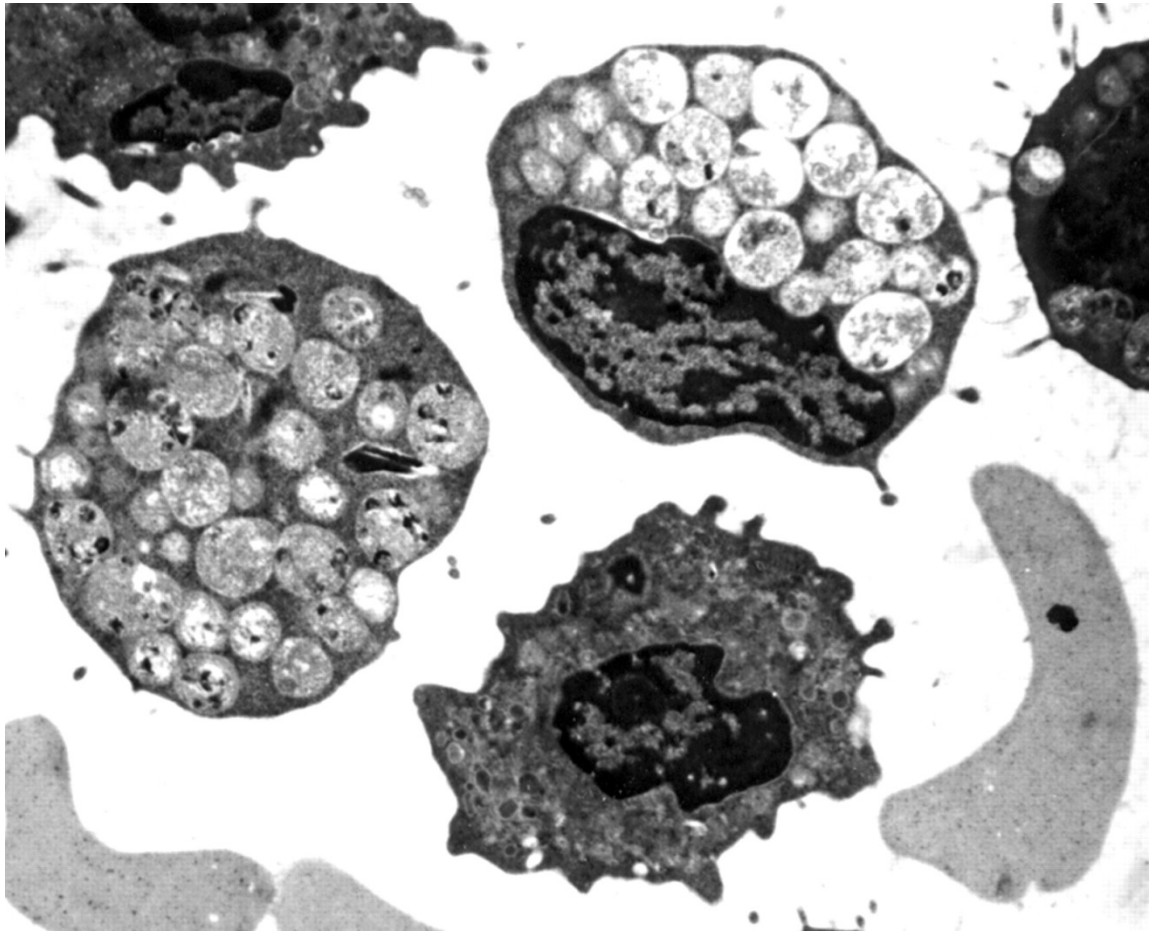
Ecker, et al (2015) *MAbs* 7,9

Glycan Binding Protein Functions



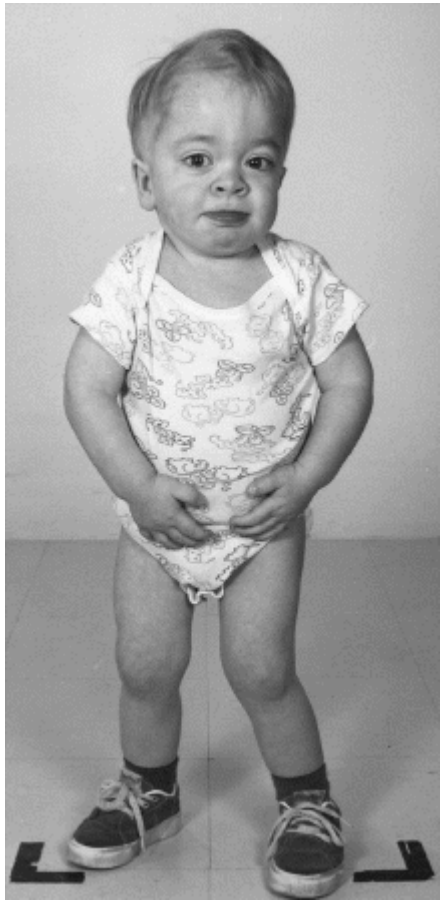
Dodd, R.B. and Drickamer, K. (2001) *Glycobiology* **11**, 71R-79R

I-cell disease: a multi-component severe lysosomal storage disease

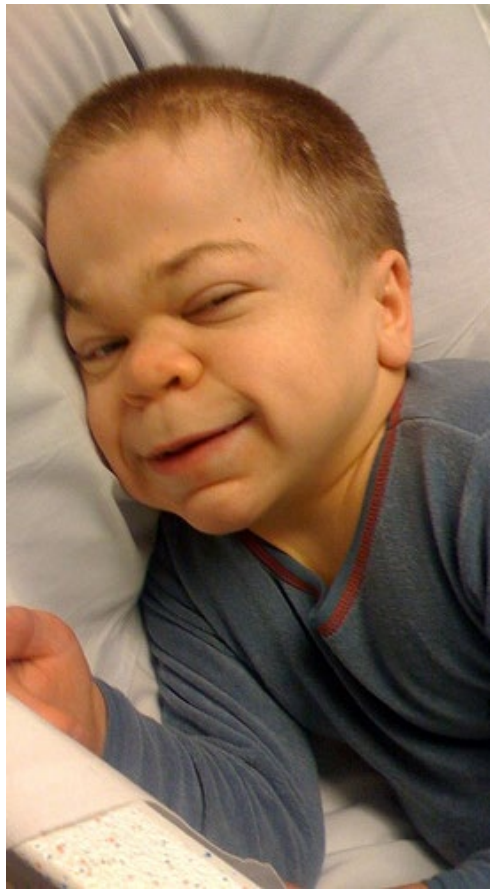


I-cell lyphocytes, van der Meer et al (2001) *J Clin Pathol* 54:724

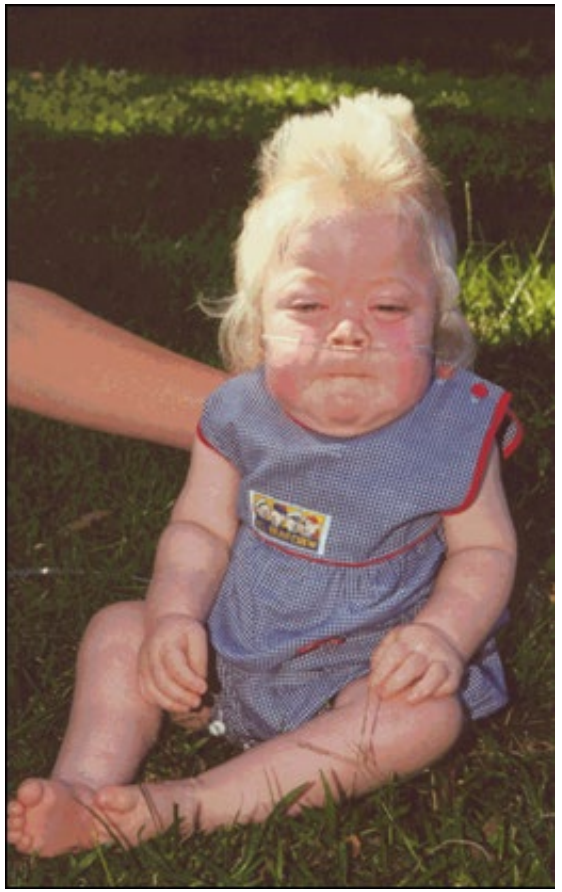
Lysosomal storage diseases are often due to faulty glycan catabolism



Hurler
(α-L iduronidase)



Hunter
(iduronate-2-sulfatase)

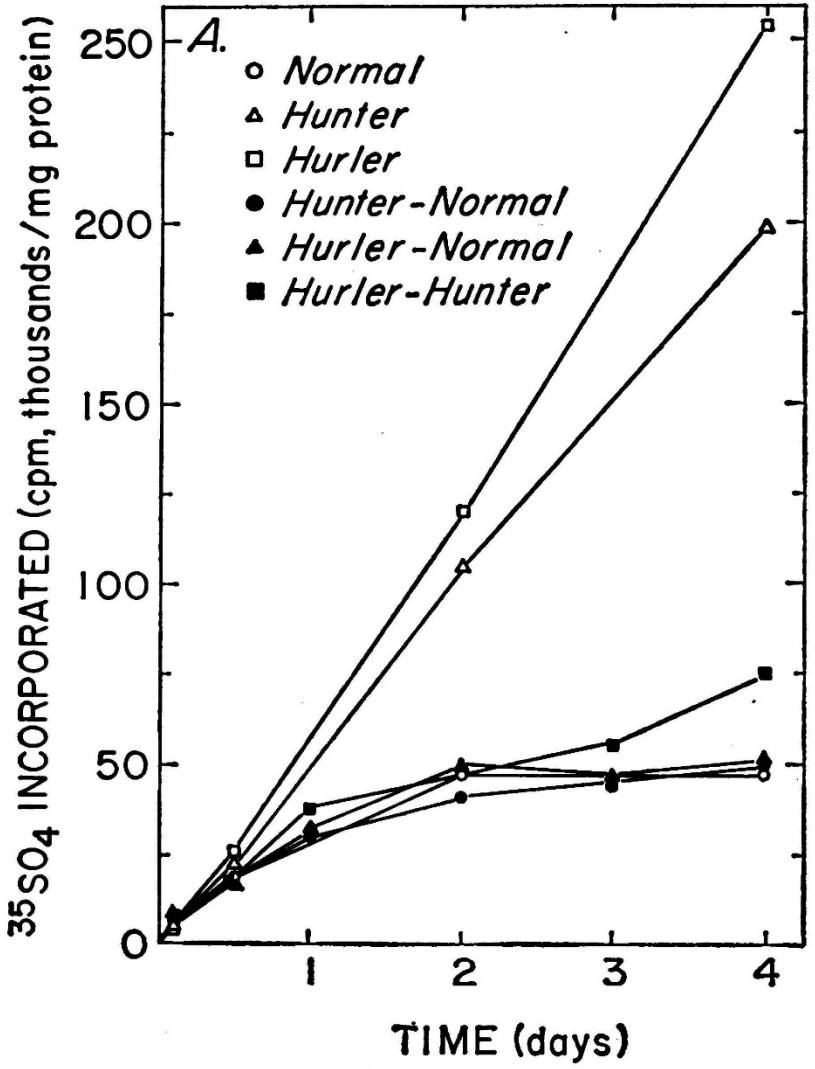


I-cell
(GlcNAc phosphotransferase)

Fibroblasts from Hunter & Hurler patients build up glycosaminoglycans

Co-incubation with normal fibroblasts, or with each other, corrects the buildup…

Correction is via a soluble factor (direct contact not required)



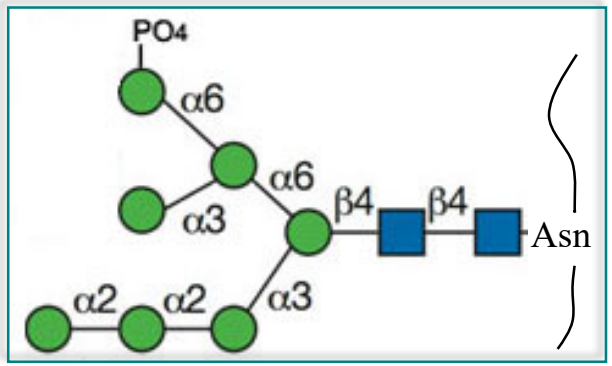
Fratantoni et al (1968) *Science* 162:170

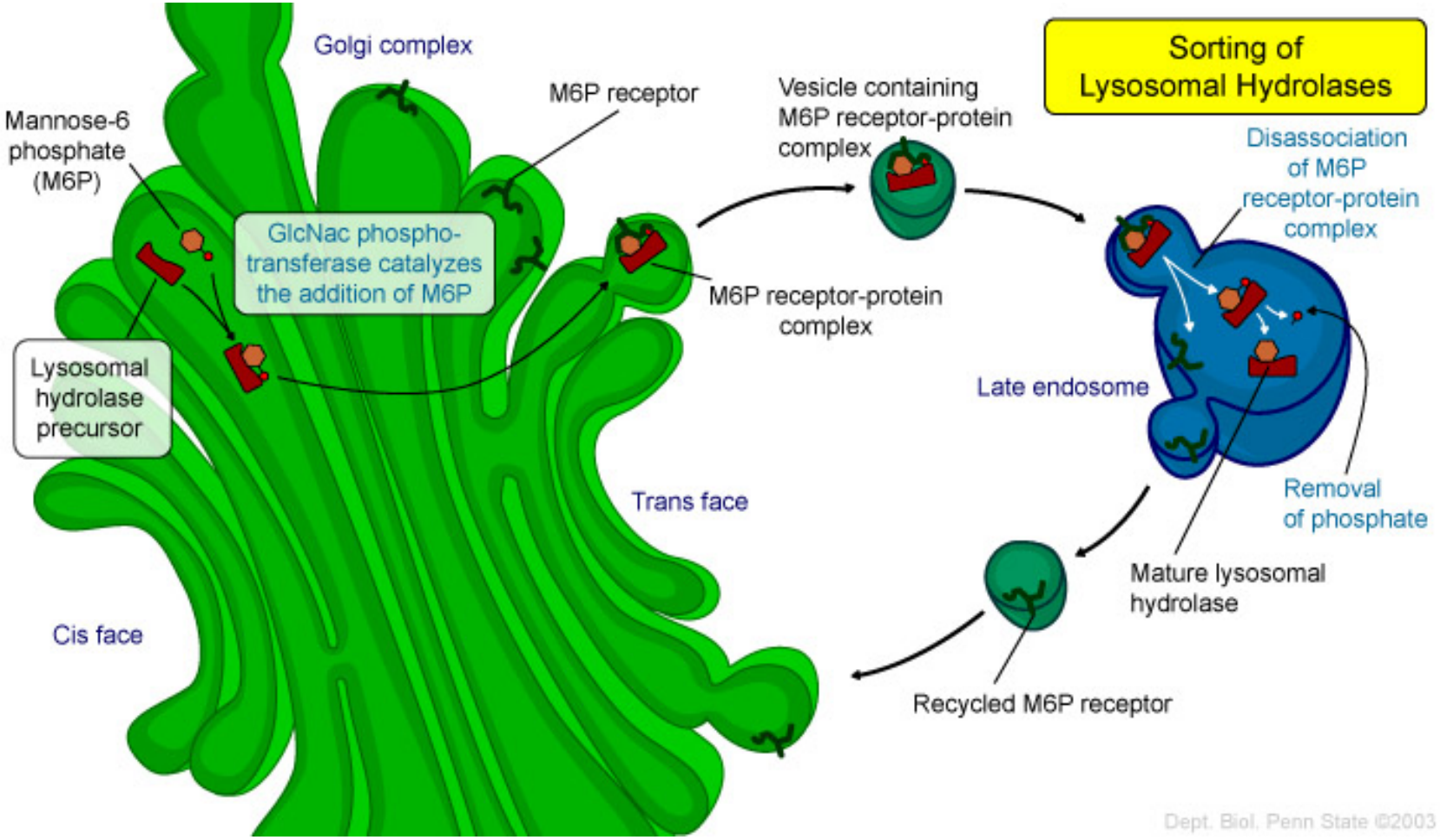
Corrective factor properties

- Lysosomal hydrolases in the medium are taken up by cells
- Periodate treatment of the hydrolases inhibits uptake
- Uptake is blocked by mannose
- Uptake is blocked more efficiently mannose-6-phosphate
- Phosphatase pre-treatment of hydrolases blocks uptake
- Normal lysosomal hydrolases have mannose-6-phosphate residues on their N-linked glycans
- Fibroblasts do not take up I-cell lysosomal hydrolases
- I-cell patients are deficient in addition of 6-linked phosphate to mannose on N-linked glycans

Lysosomal hydrolase trafficking

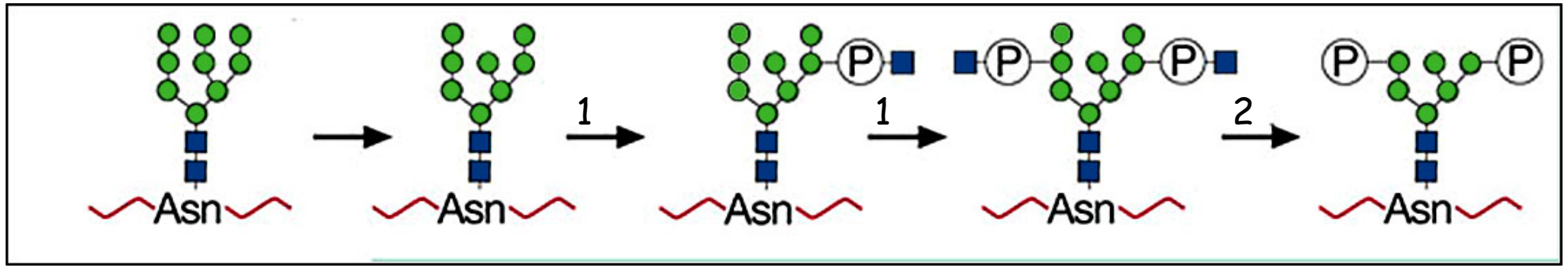
Unique glycosylation of lysosomal enzymes "tags" them for trafficking to the lysosome via the Man-P receptor(s)





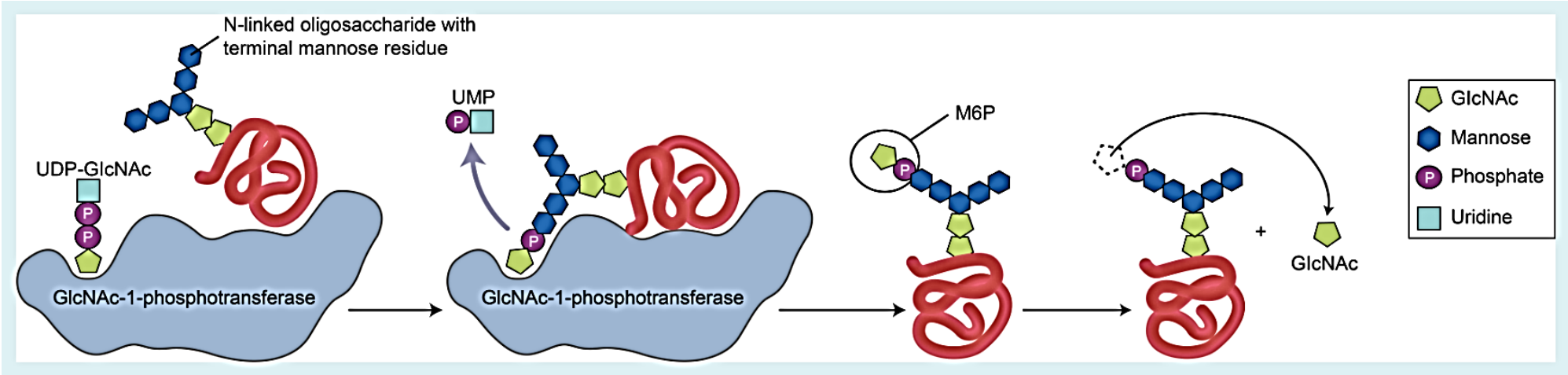
https://wikispaces.psu.edu/display/230/Intracellular+Compartments-+ER+and+Golgi+Complex

Targeting proteins to the lysosome via mannose-6-phosphate



1. UDP-*N*-acetylglucosamine:lysosomal-enzyme *N*-acetylglucosamine-1-phosphotransferase

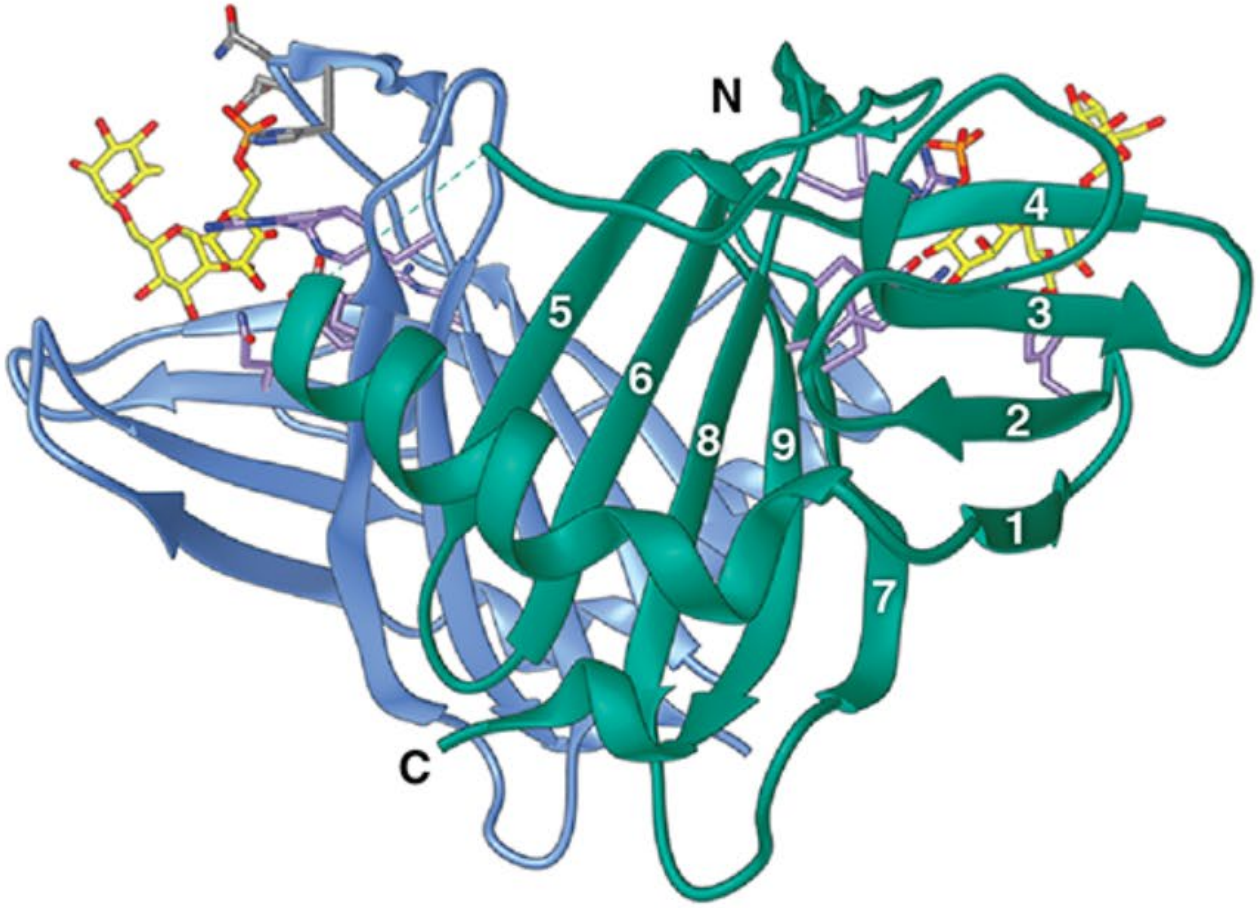
2. α-N-acetylglucosaminyl-1-phosphodiester glycosidase

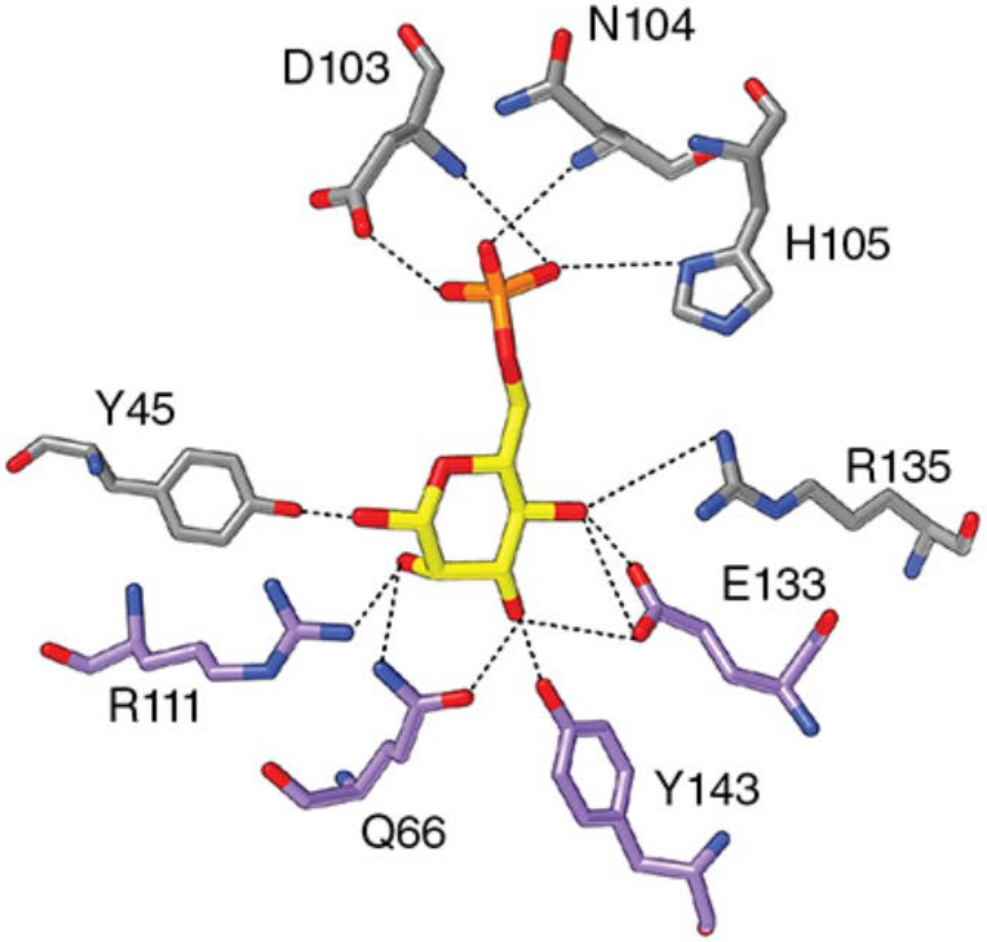


mutated in I-cell disease

Kang et al. *NEJM* 362:677 (2010)

P-type lectin structure





Therapeutic opportunities!

structure: Dahms et al (1989) *J Biol Chem* 264:12115

Source: D. Valle, S. Antonarakis, A. Ballabio, A. Beaudet, G.A. Mitchell. The Online Metabolic and Molecular Bases of Inherited Disease.

Glycobiology goes Hollywood

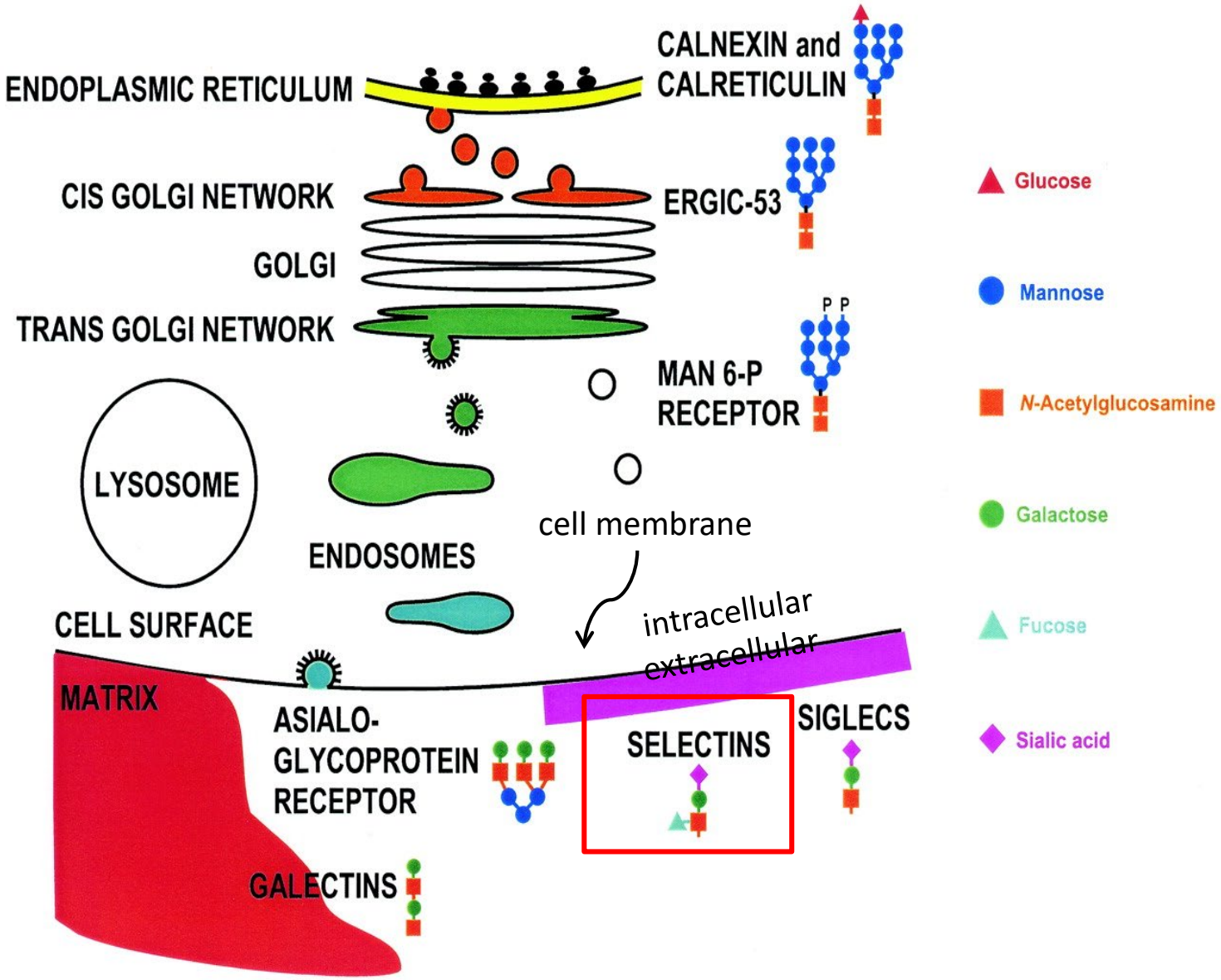
William Canfield and John Crowley, Novazyme (http://blog.newok.com)

H Ford and B Fraser (2010) *Extraordinary Measures*, CBS Films, Los Angeles, CA, USA

Harrison Ford, Glycobiologist

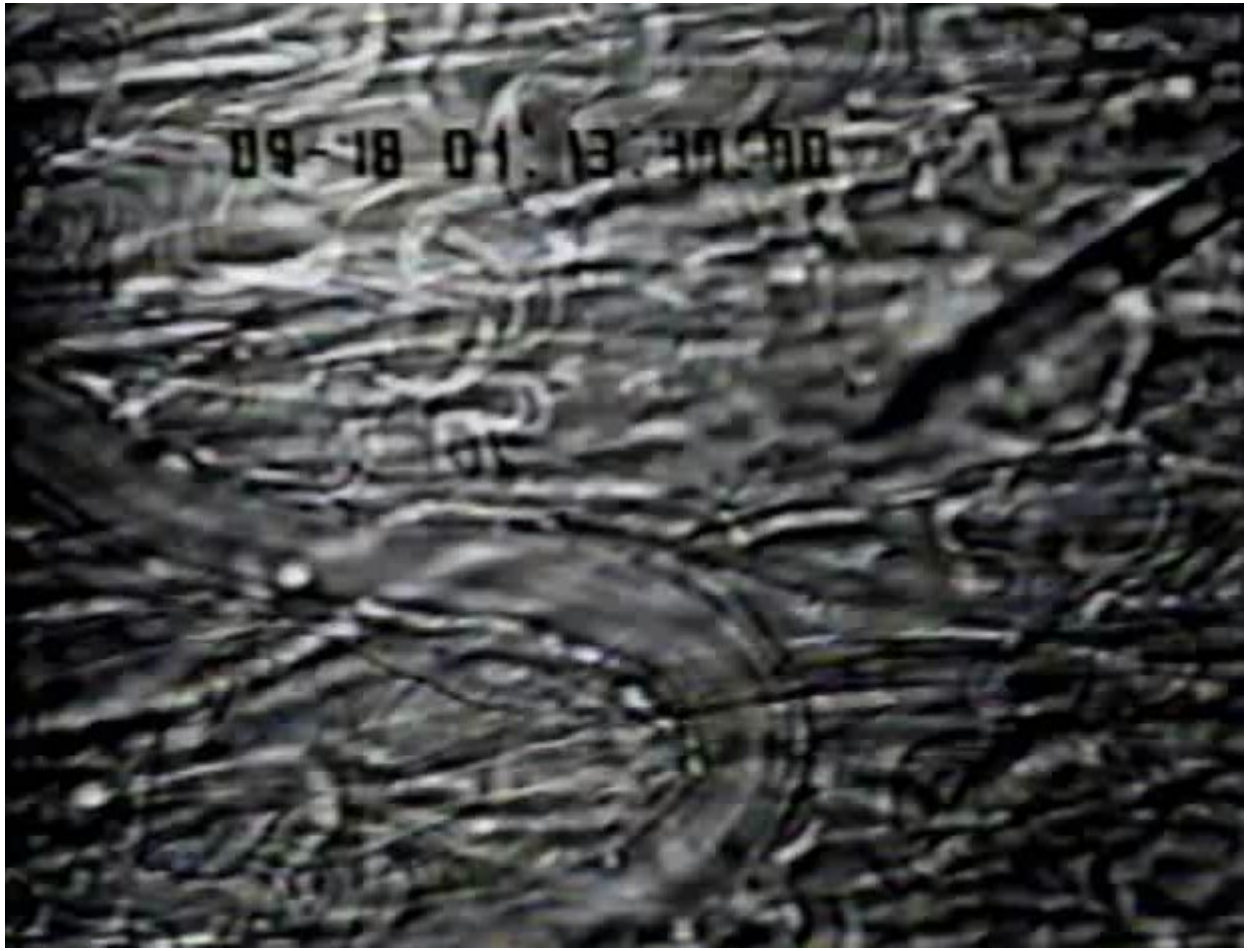
“What the matter, Sal, not up on your glycobiology?

Glycan Binding Protein Functions



Dodd, R.B. and Drickamer, K. (2001) *Glycobiology* **11**, 71R-79R

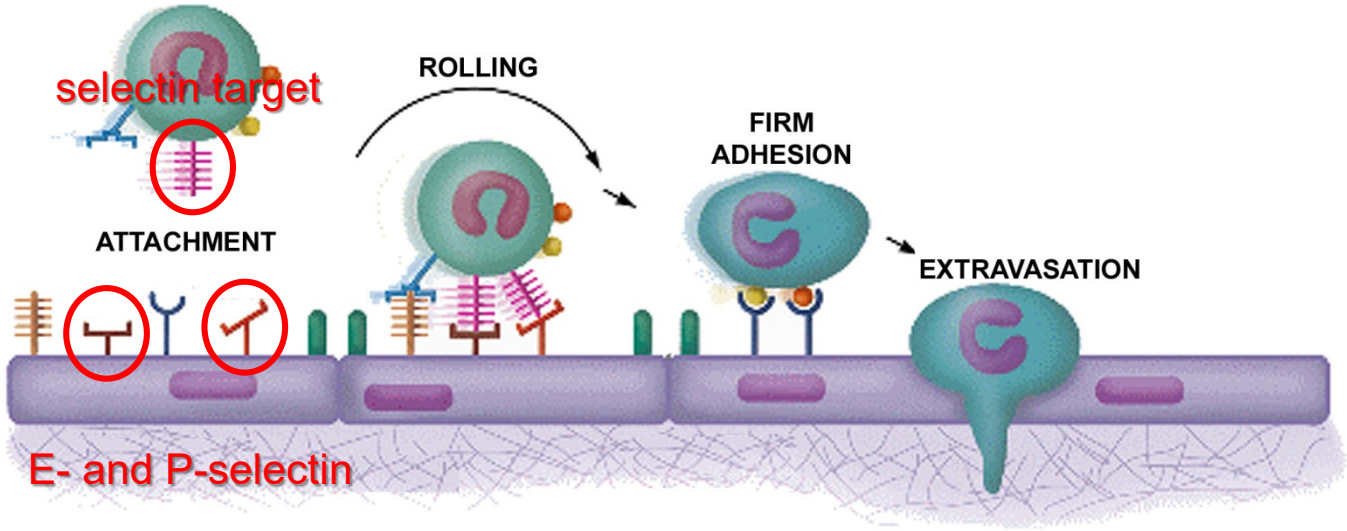
Leukocyte trafficking

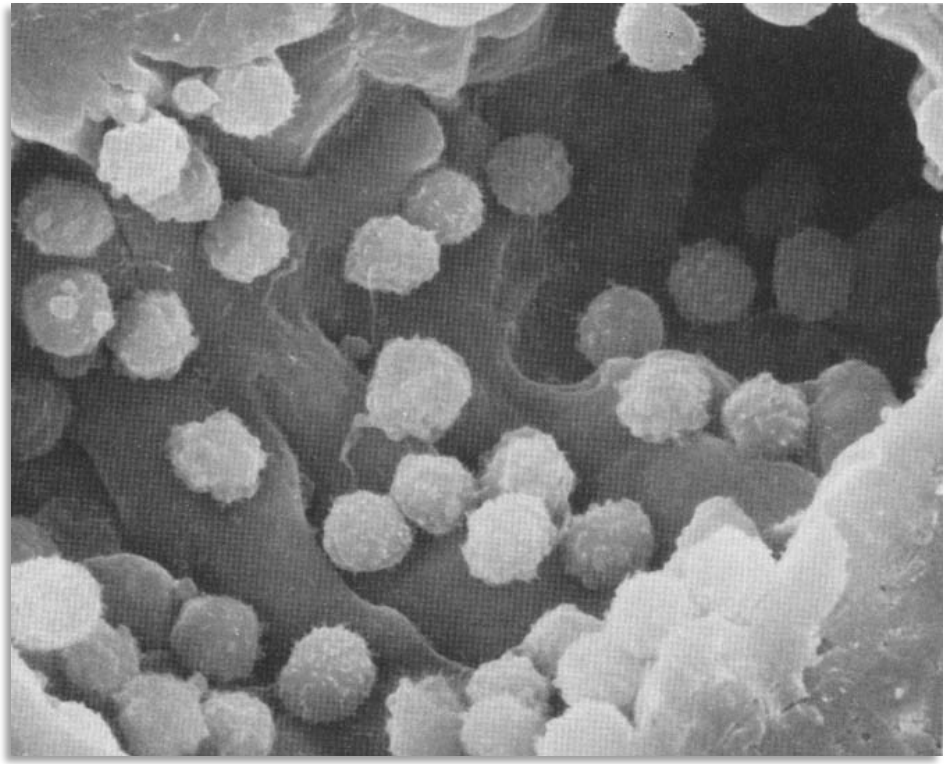


thanks to: Michael Lawrence, University of Virginia

Leukocyte trafficking

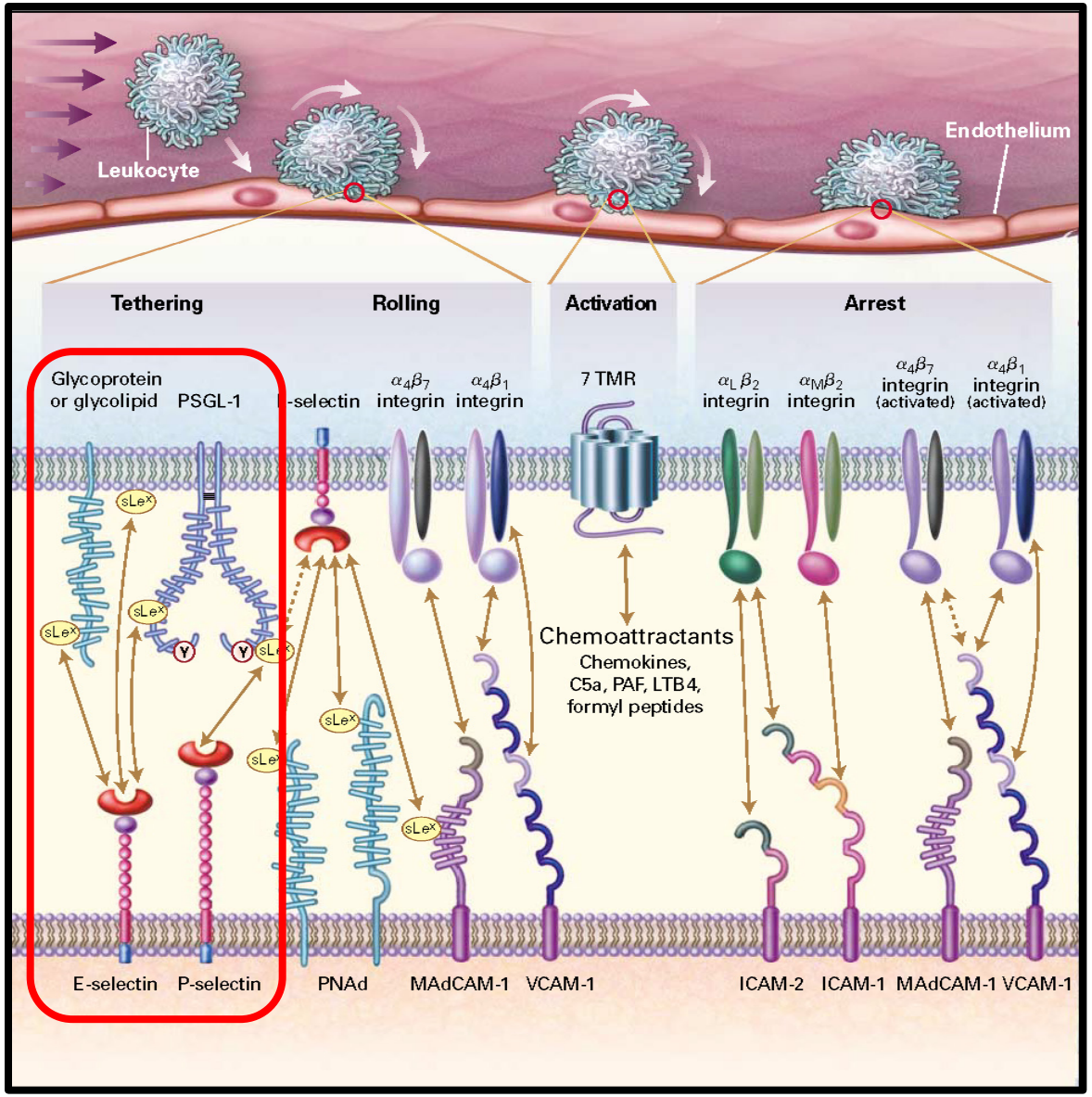
The first step in leukocyte extravasation: E-Selectin and P-Selectin on the endothelium bind to pre-existing target glycans on neutrophils





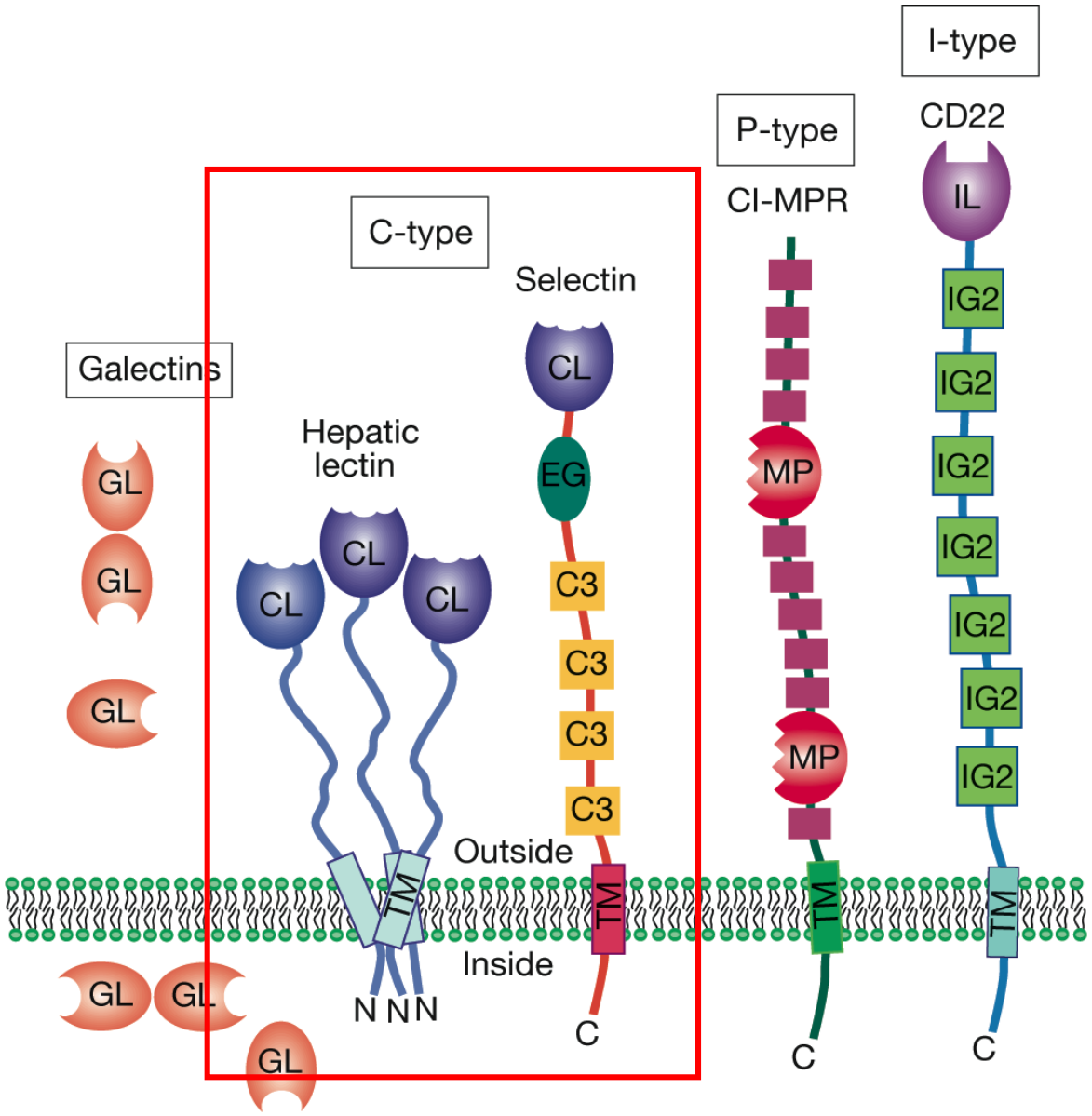
R&D Systems (1996)

Three-step paradigm of leukocyte extravasation

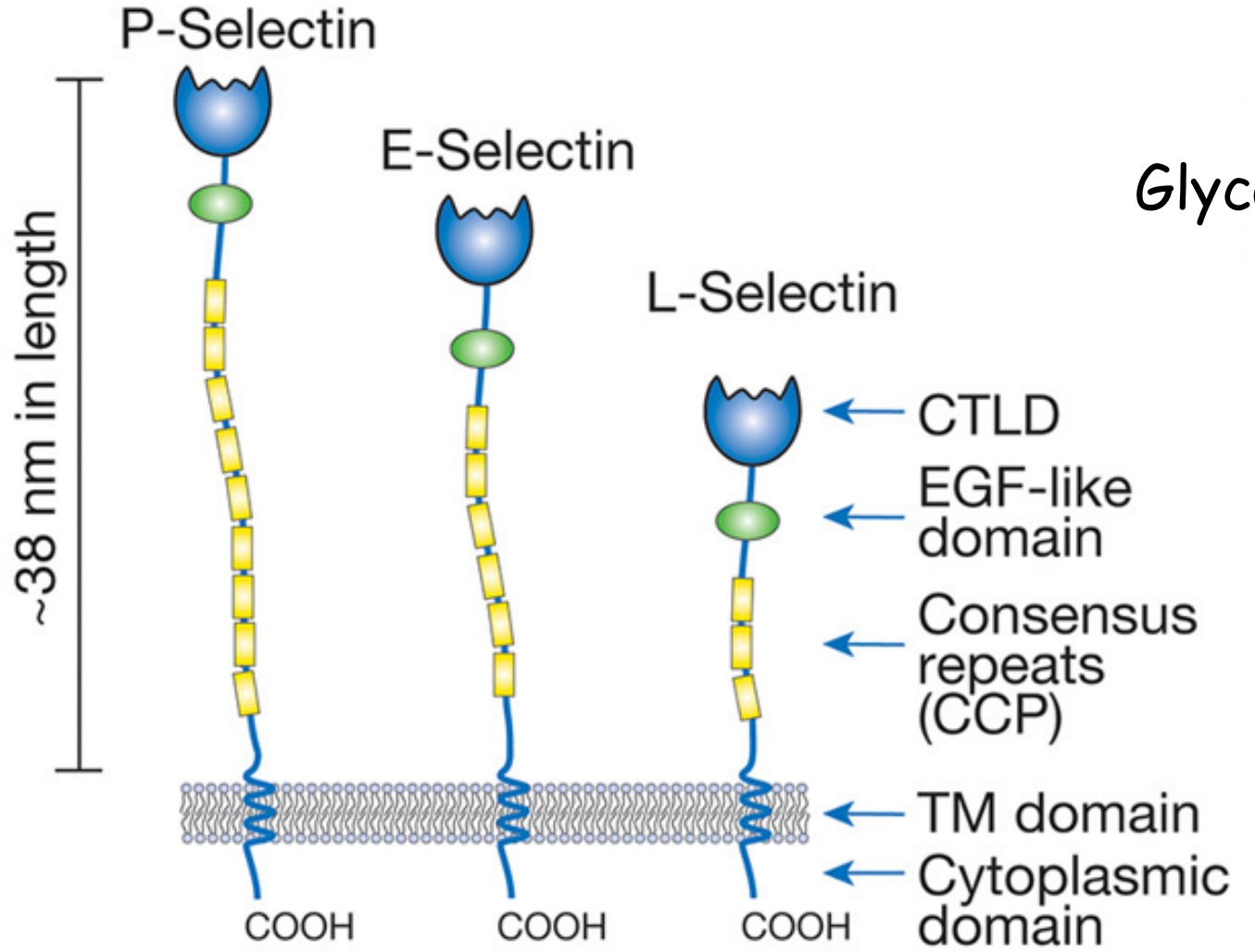


von Andrian & Mackay CR, *NEJM* 343:1020 (2000)

Mammalian lectin families



Selectin C-type lectin subfamily

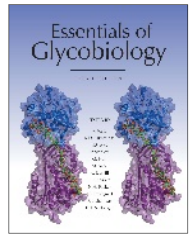


– endothelial cells –

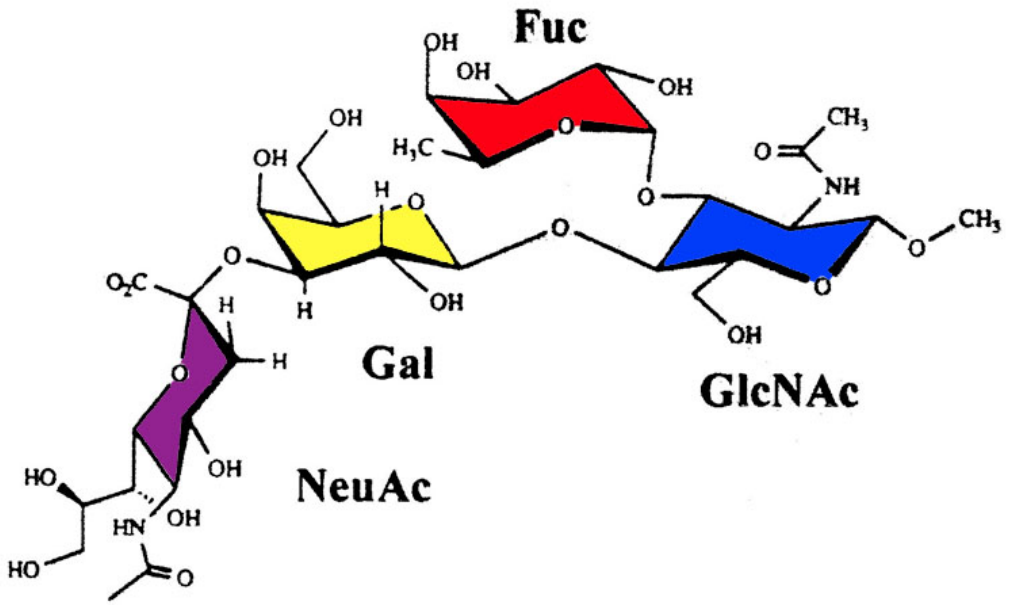
Glycan Array Specificity

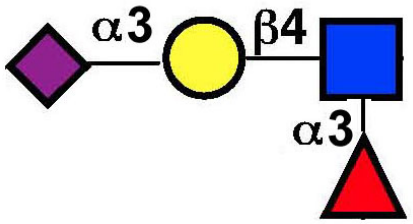
E-Selectin

P-selectin



Sialyl Lewis X – Selectin minimal binding determinant

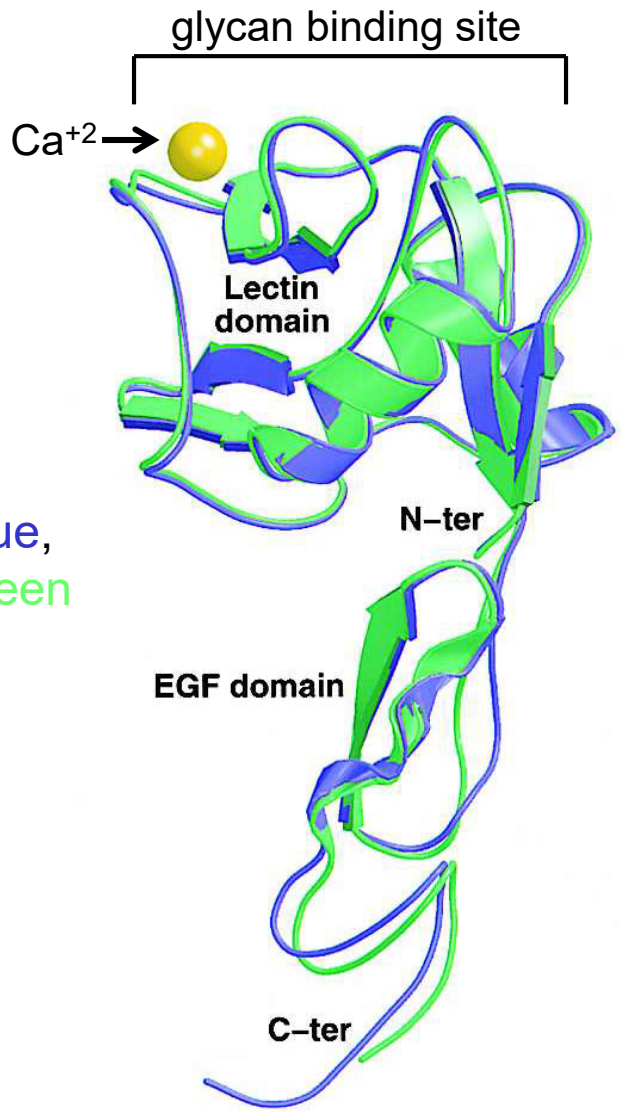


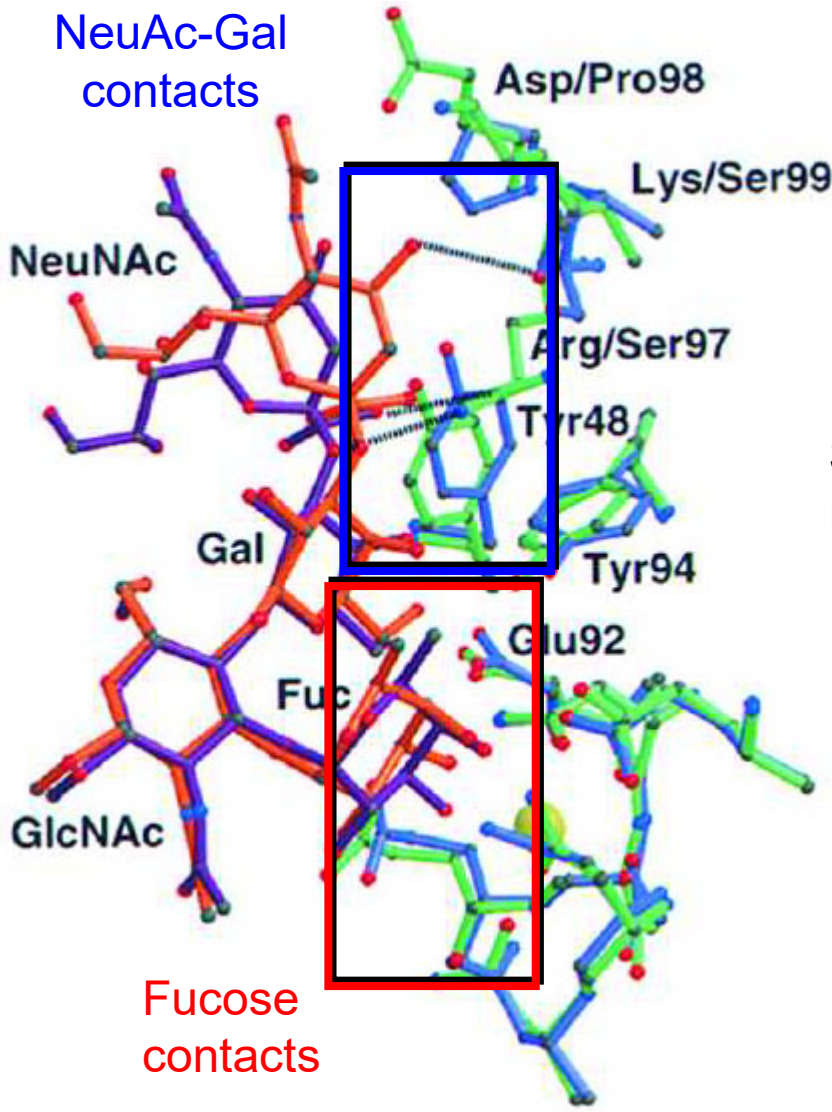


Sialyl Lewis x (SLe^x) – NeuAc α2-3 Gal β1-4 (Fuc α1-3) GlcNAc

E- and P-selectin binding sites

P-selectin blue,
E-selectin green

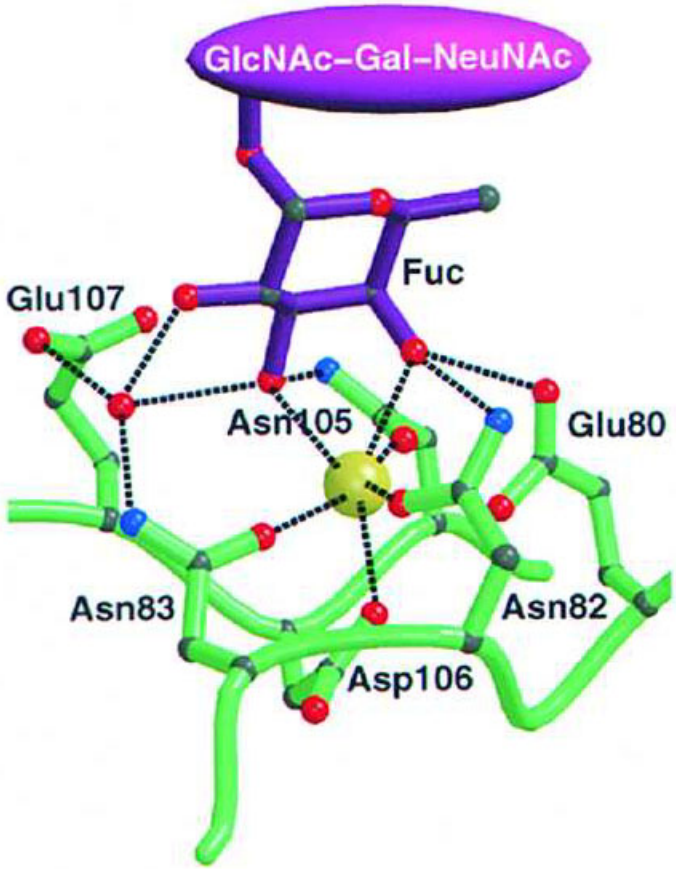




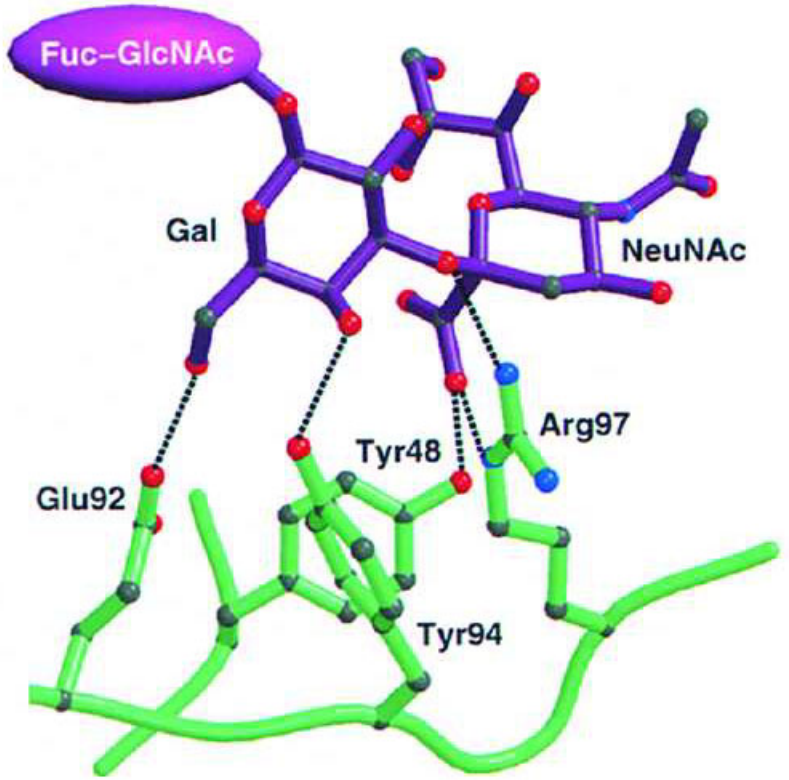
SLe^x binding to:
P-selectin (orange)
E-selectin in purple

Somers W.S., et al. (2000) *Cell* **103**, 467

E-selectin binding site



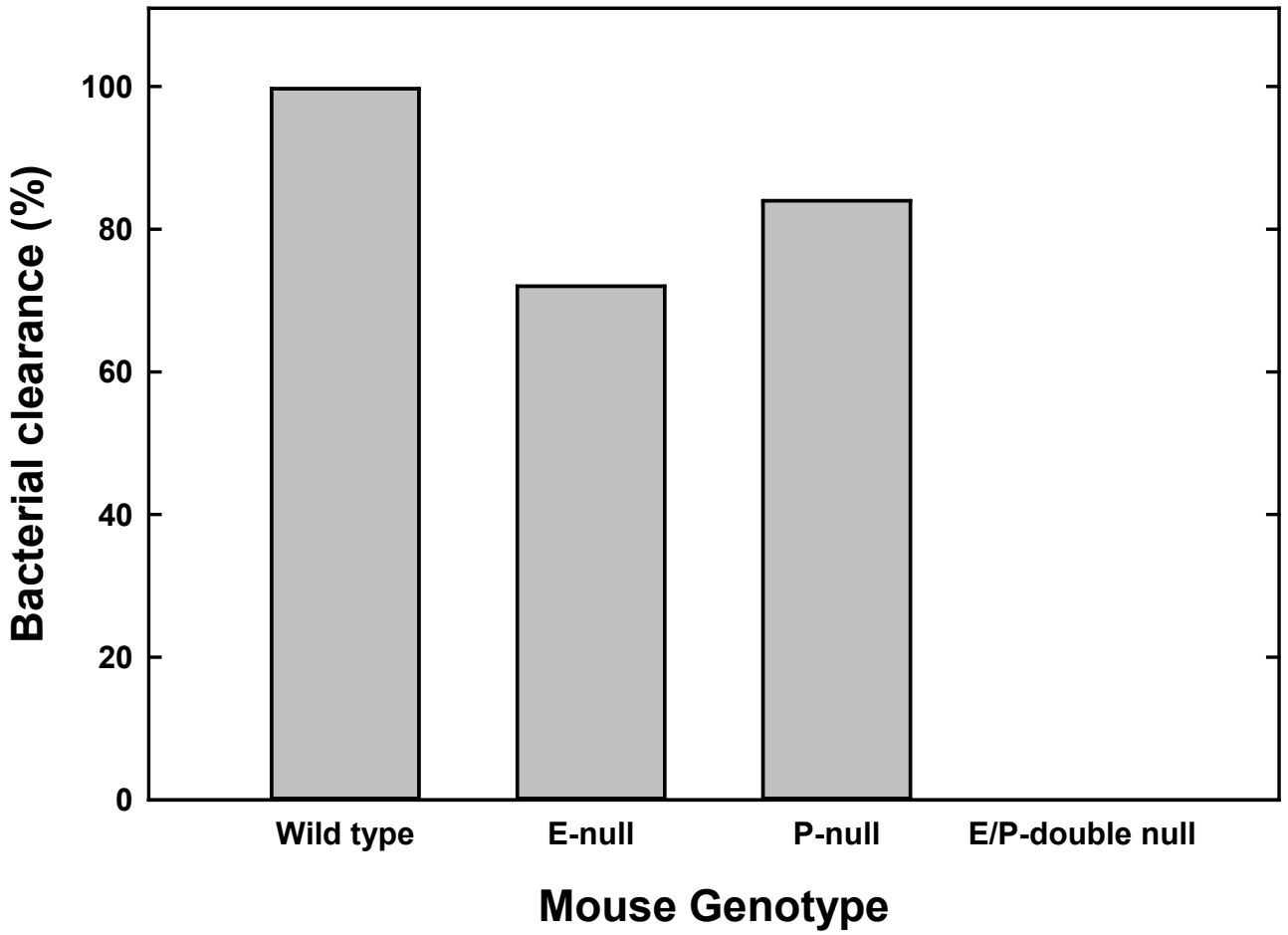
Fucose binding detail



NeuAc-Gal binding detail

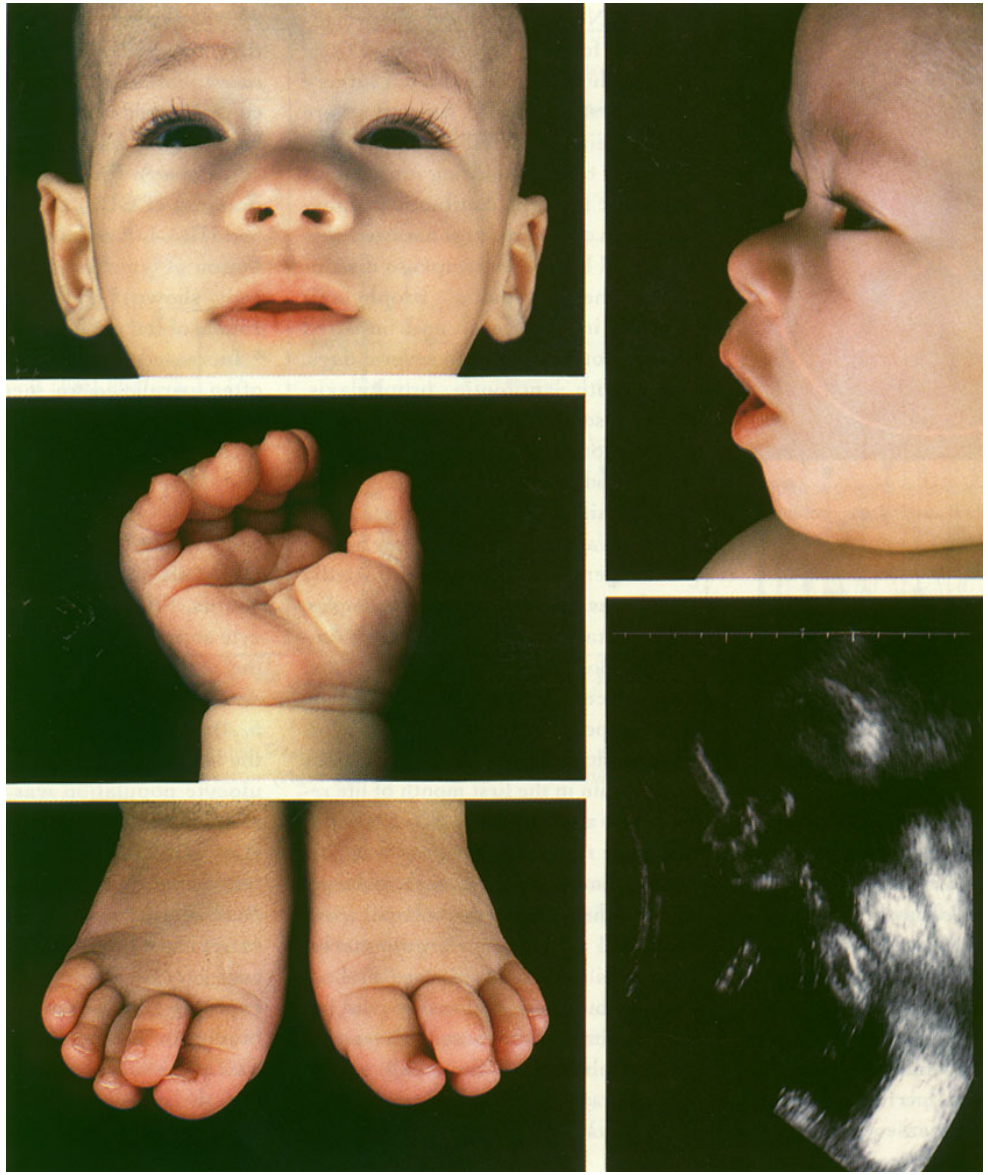
Somers W.S., et al. (2000) *Cell* **103**, 467-479

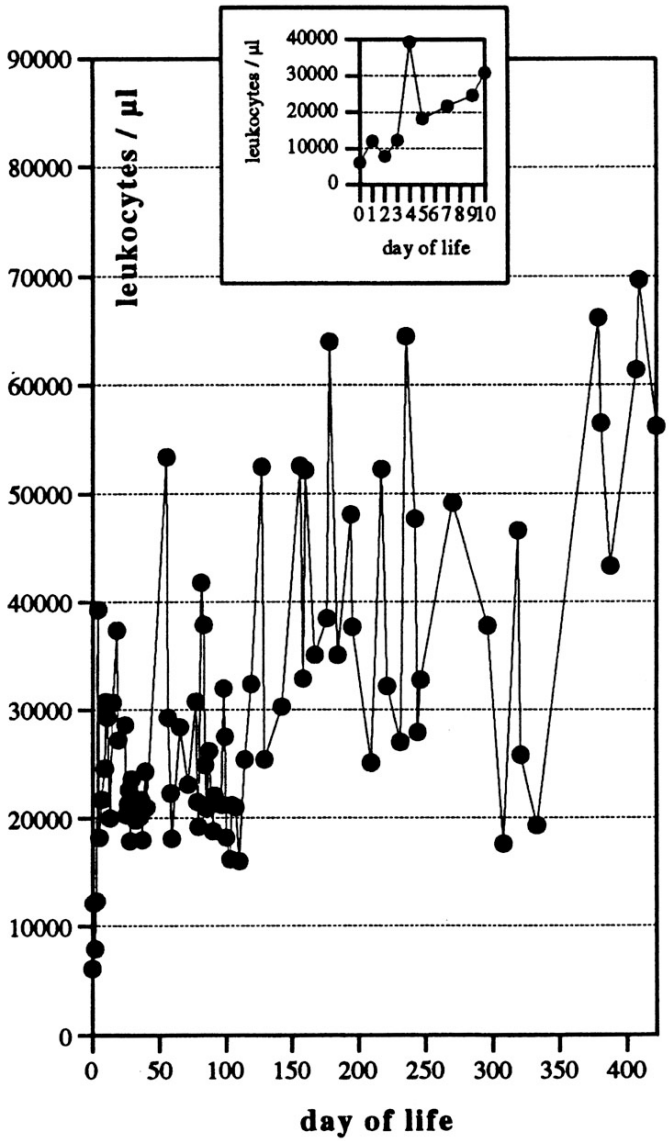
E- and P-selectin cooperate in inflammation



Bullard, et al. (1996) *J. Exp. Med.* **183**, 2329-2336

Leukocyte adhesion deficiency (LAD) Type II





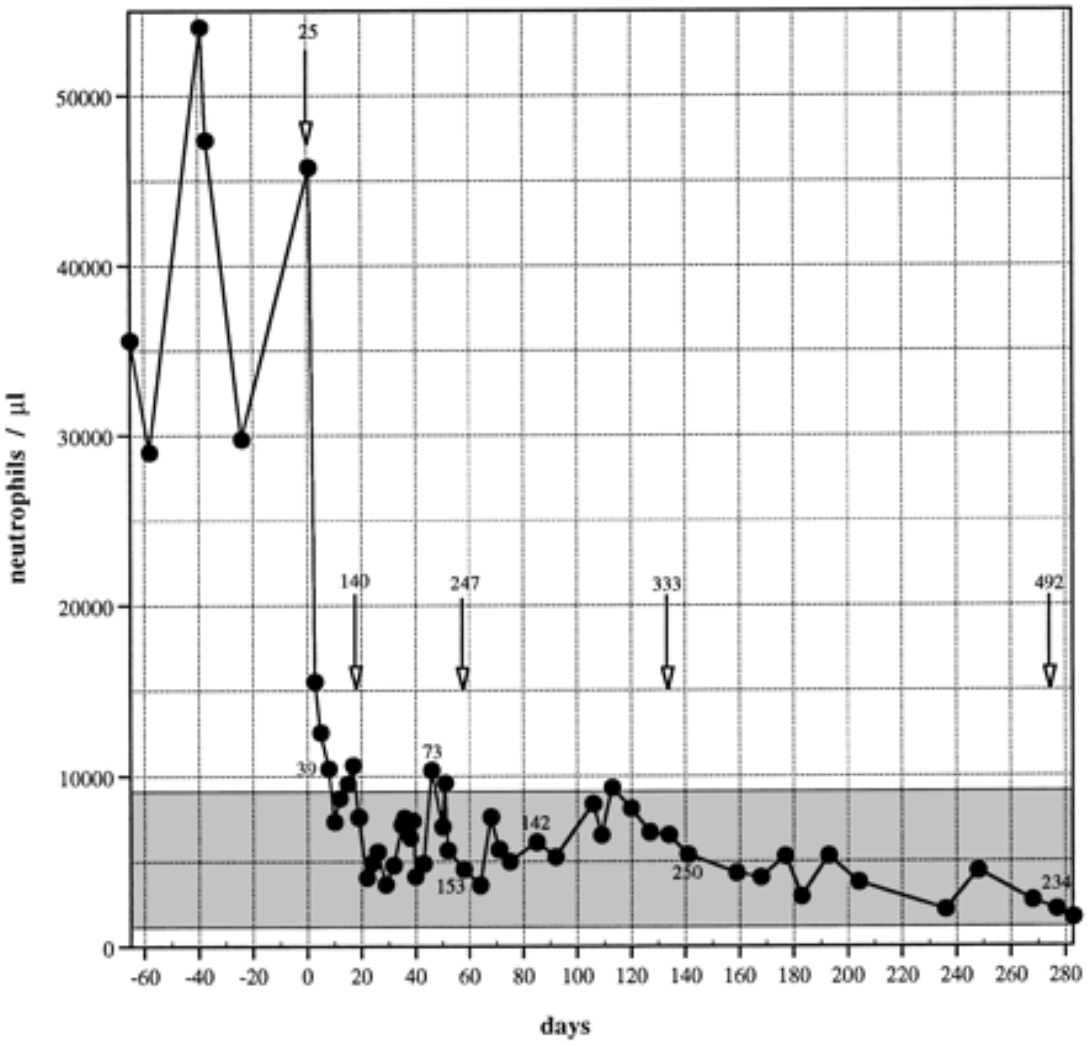
Marquardt, et al. (1999)
J. Pediatr. 134, 681

Human blood group antigens and Selectin ligands

Fuc α1-2 Gal β1-	O (H) Blood Type
Fuc α1-2 Gal β1- | GalNAc α1-3	A Blood Type
Fuc α1-2 Gal β1- | Gal α1-3	B Blood Type
Gal β1-	Bombay Phenotype
NeuAc α2-3 Gal β1-4 GlcNAc- | Fuc α1-3	Selectin ligand
NeuAc α2-3 Gal β1-4 GlcNAc-	LAD Type II

Oral fucose therapy for LADII

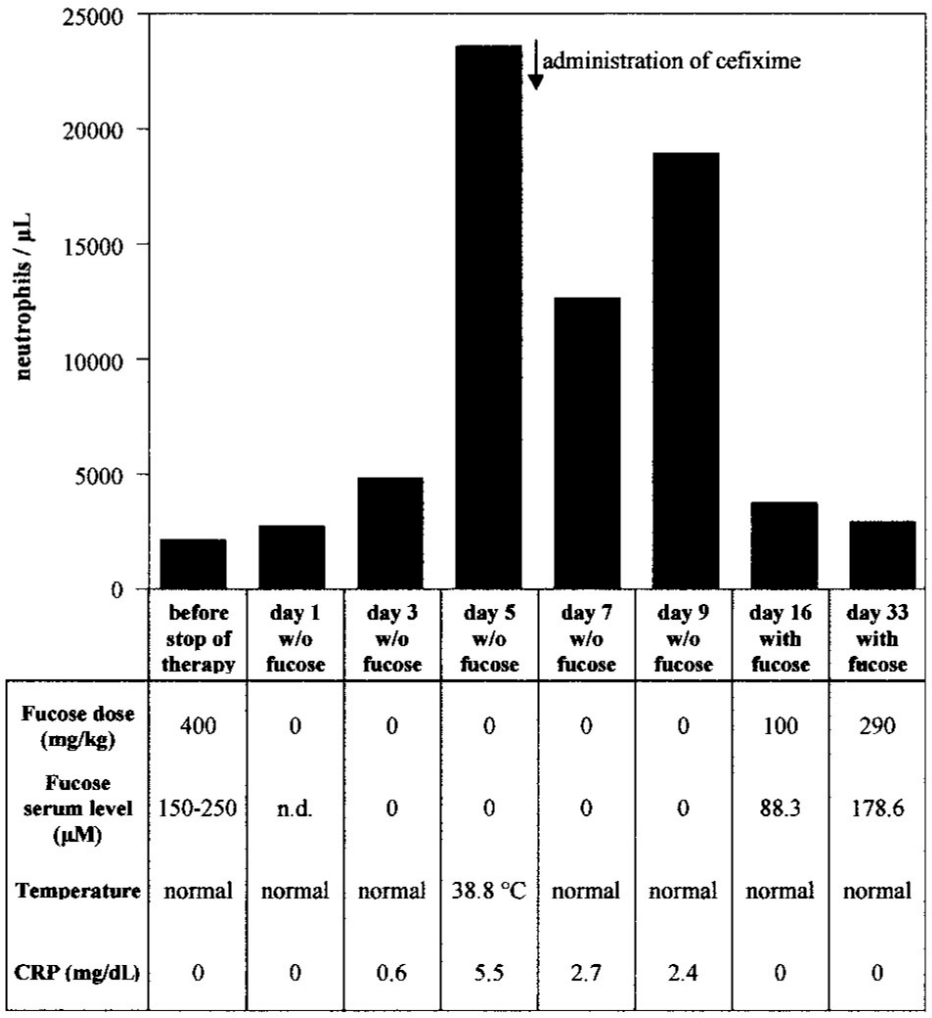
"Within days of starting fucose therapy, neutrophil levels returned to the normal range .... there were no further infections and antibiotic prophylaxis was discontinued."



Marquardt, et al. (1999) Blood 94, 3976

Oral fucose therapy for LADII

Reversible correction of leukocyte adhesion deficiency in an LADII patient taking oral fucose



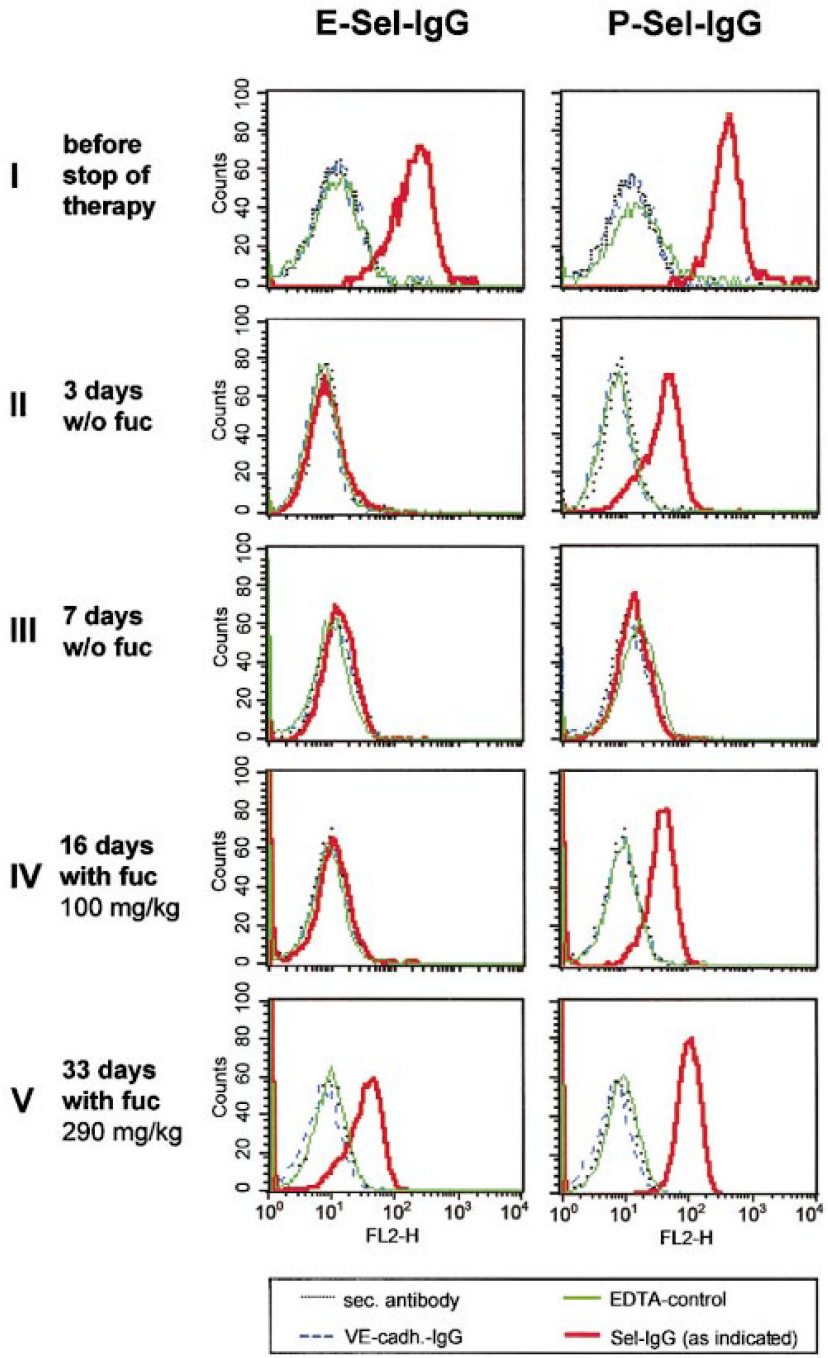
	before stop of therapy	day 1 w/o fucose	day 3 w/o fucose	day 5 w/o fucose	day 7 w/o fucose	day 9 w/o fucose	day 16 with fucose	day 33 with fucose
Fucose dose (mg/kg)	400	0	0	0	0	0	100	290
Fucose serum level (µM)	150-250	n.d.	0	0	0	0	88.3	178.6
Temperature	normal	normal	normal	38.8 °C	normal	normal	normal	normal
CRP (mg/dL)	0	0	0.6	5.5	2.7	2.4	0	0

Figure 1. Peripheral neutrophil counts and other therapy parameters during discontinuation and resumption of fucose therapy. Peripheral neutrophil counts, fucose doses, serum fucose levels, body temperature, and C reactive protein (CRP) were recorded for each time point as indicated.

Luhn, et al. (2001)
Blood 97, 330-332

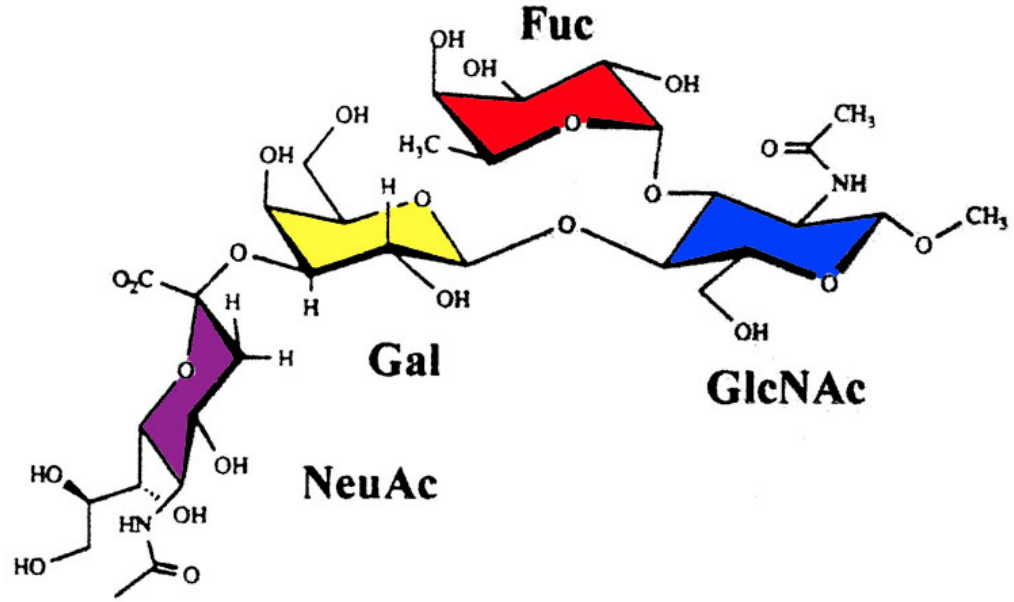
Oral fucose therapy for LADII

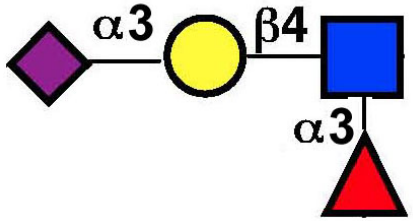
Reversible correction of selectin adhesion of neutrophils from a LADII patient taking oral fucose



Endogenous selectin ligands

Although SLex is a <u>minimal</u> binding determinant, it is not, by itself, the endogenous ligand

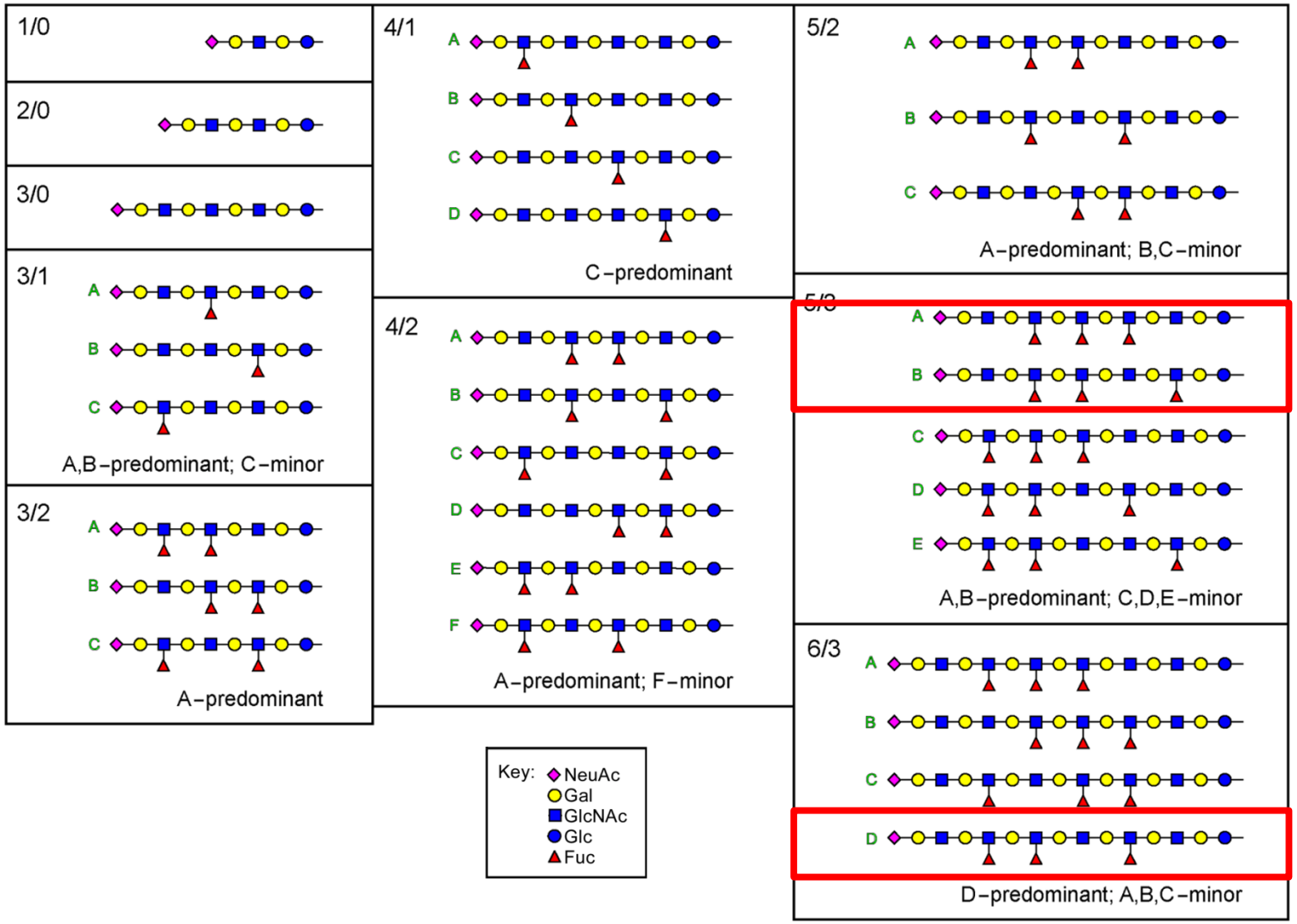




Sialyl Lewis x (SLe^x): NeuAc α2-3 Gal β1-4 (Fuc α1-3) GlcNAc

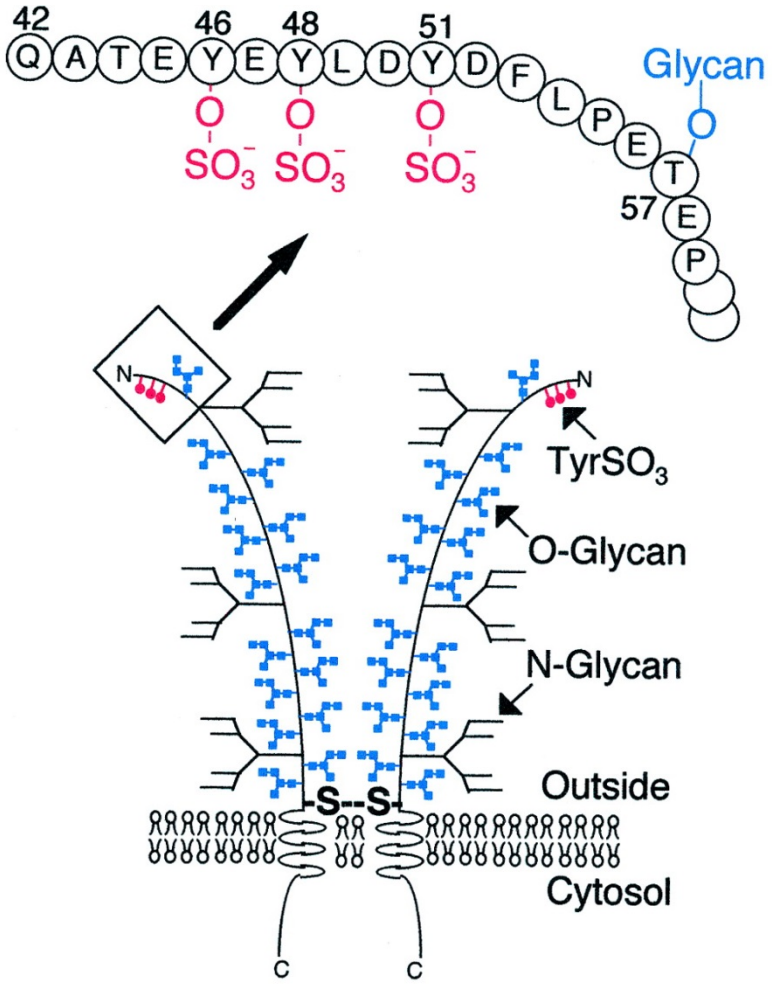
Endogenous human E-selectin ligands

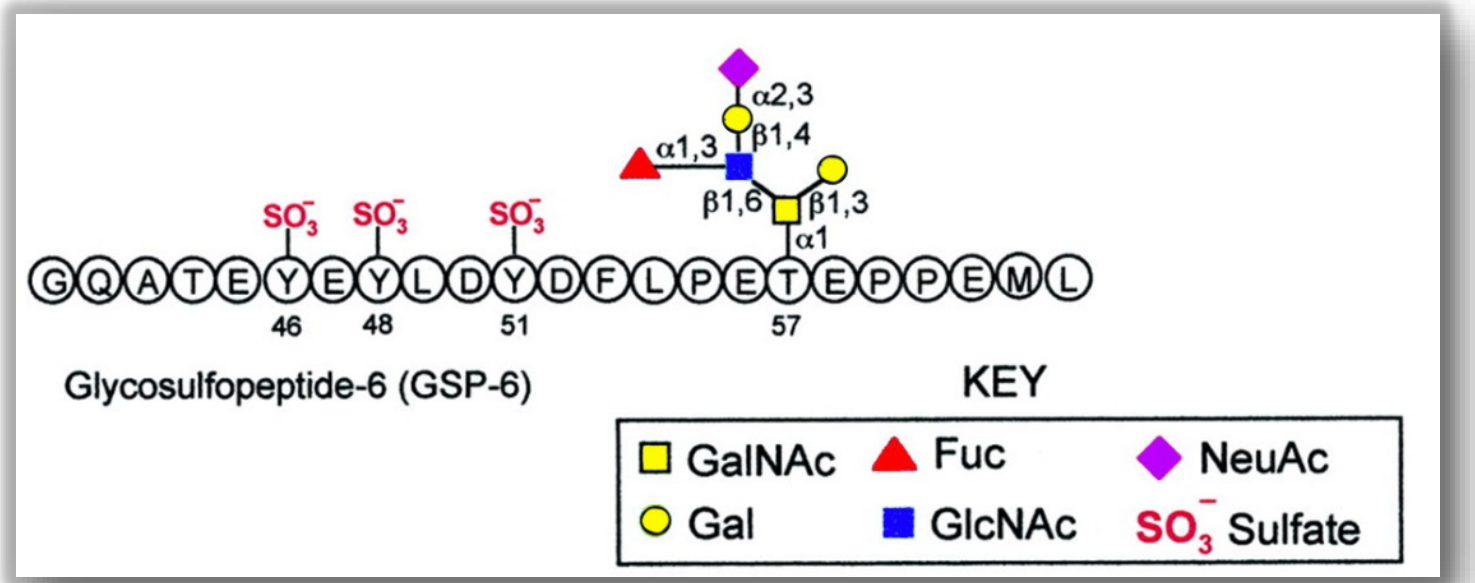
Long VIM2-epitope poly-LacNAc gangliosides



Nimrichter et al (2008) *Blood,* 112:3744

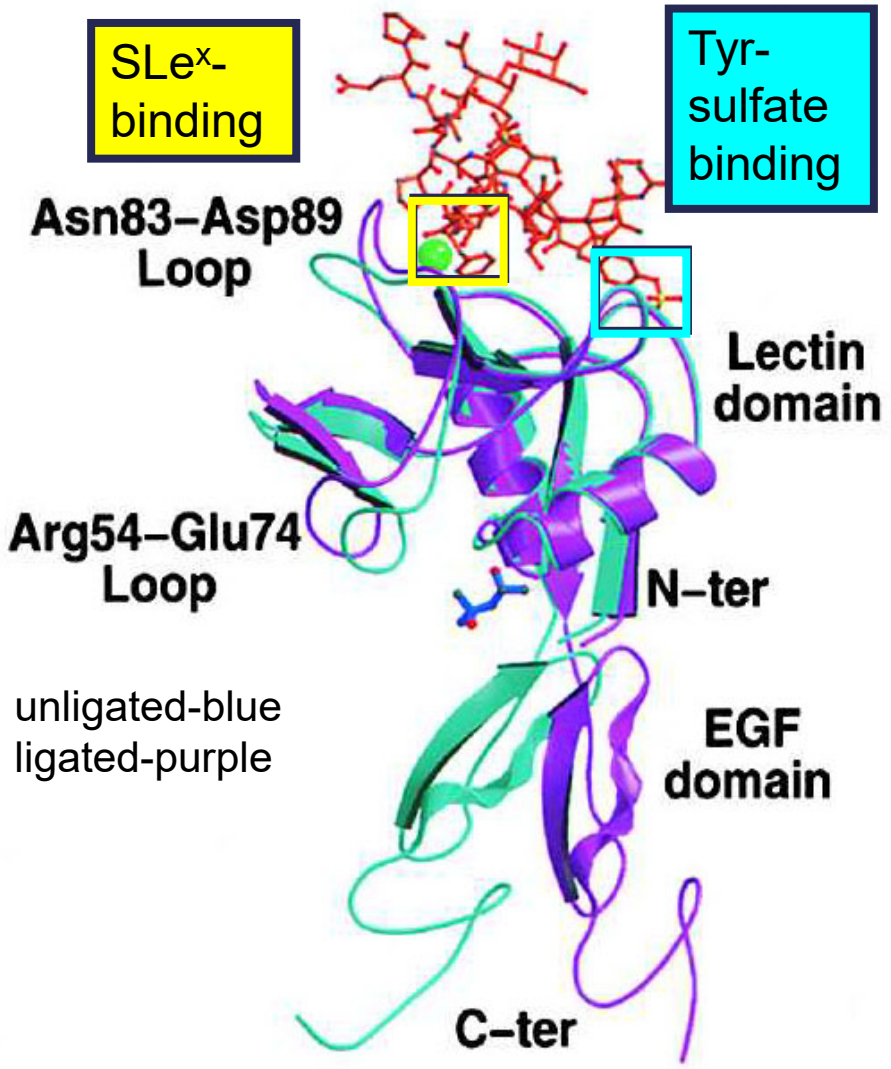
Endogenous P-selectin ligand: PSGL-1

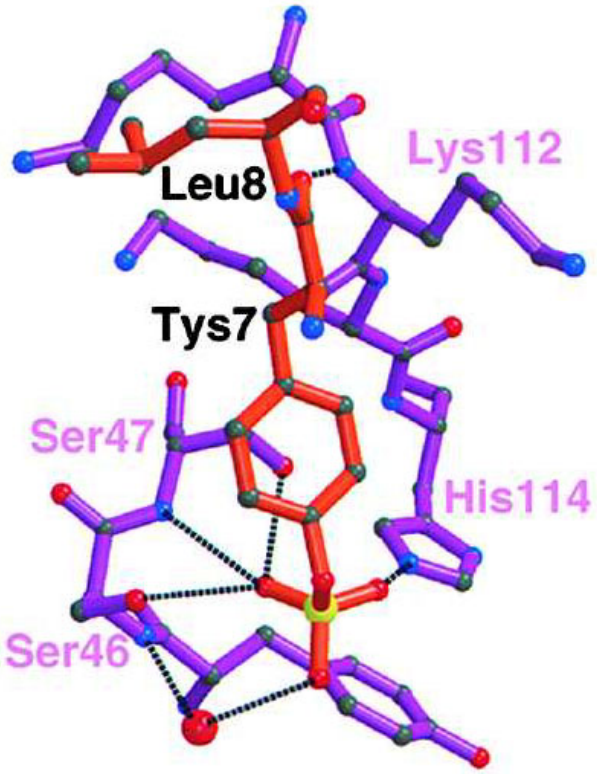




Leppanen, A., et al. (1999) *J. Biol. Chem.* **274**, 24838-24848

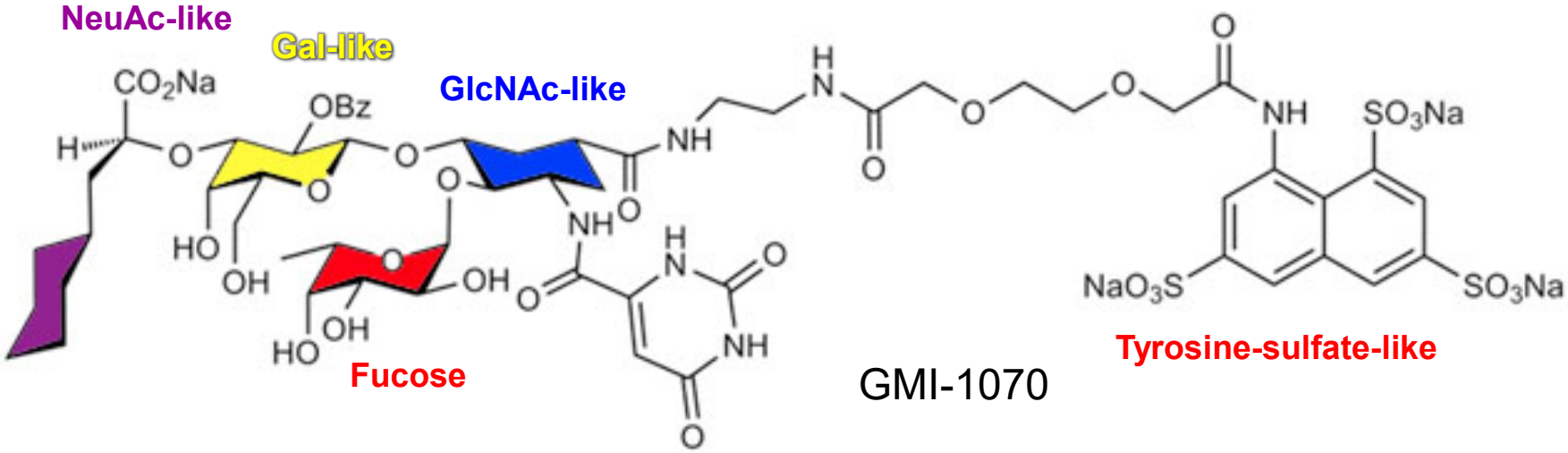
PSGL-1 binding to P-selectin

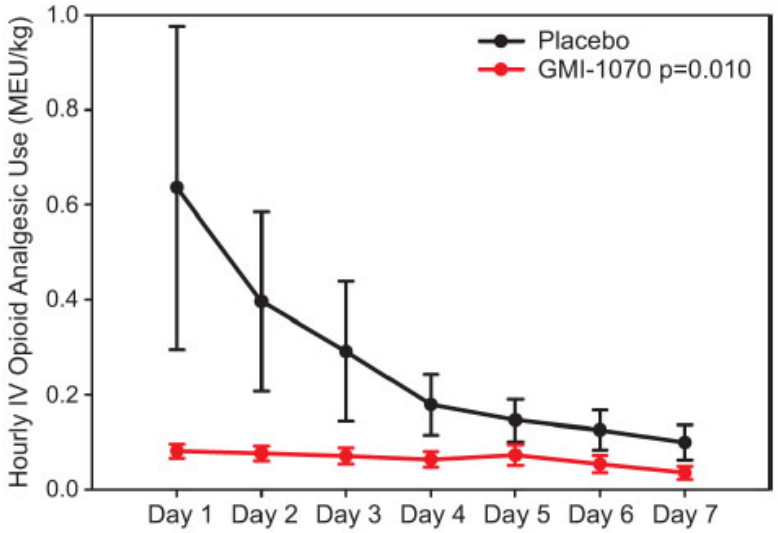




Somers W.S., et al. (2000) *Cell* **103**, 467-479

Anti-inflammatory Selectin mimetic

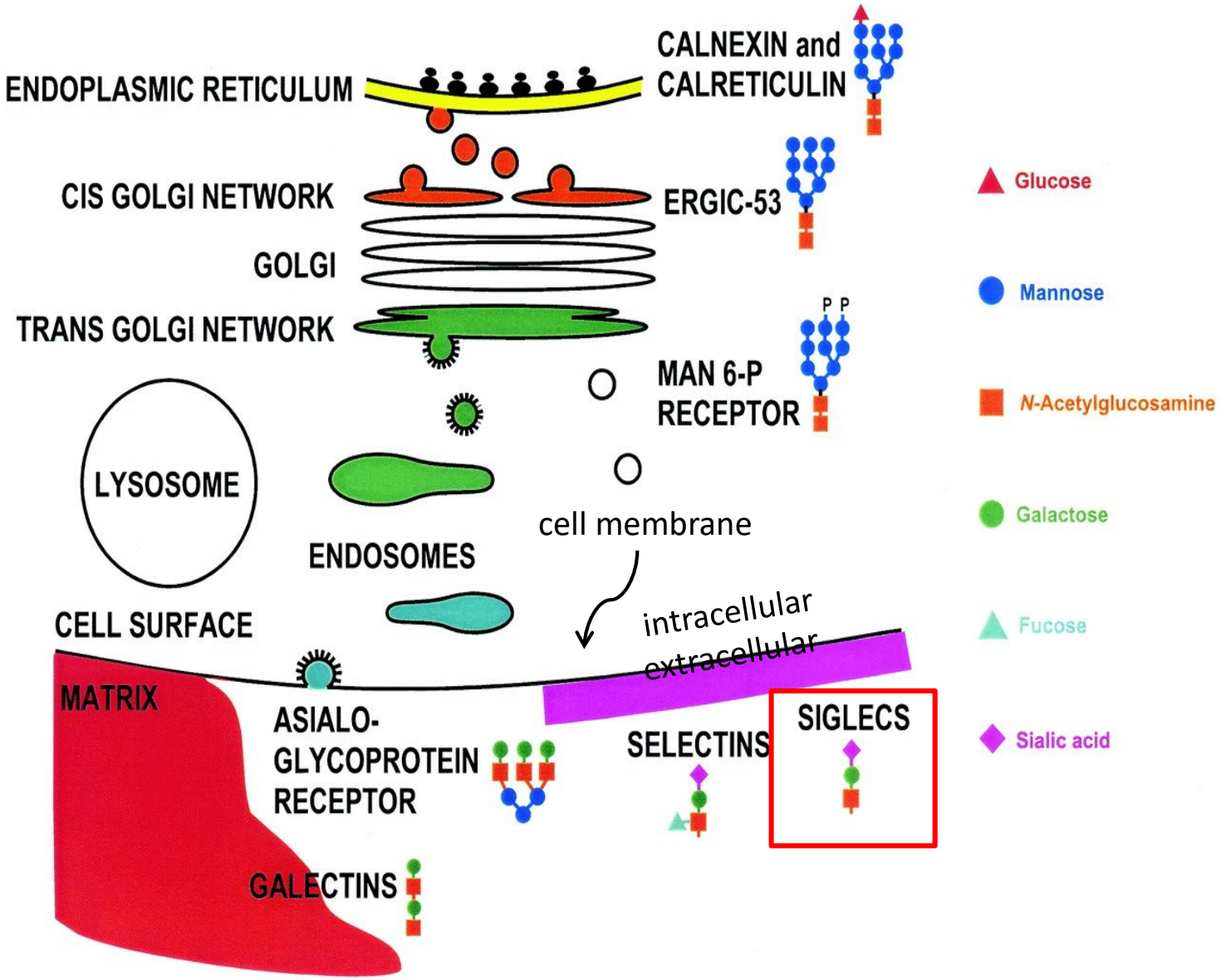




Phase 2 human trial effect in vaso-occlusive crisis

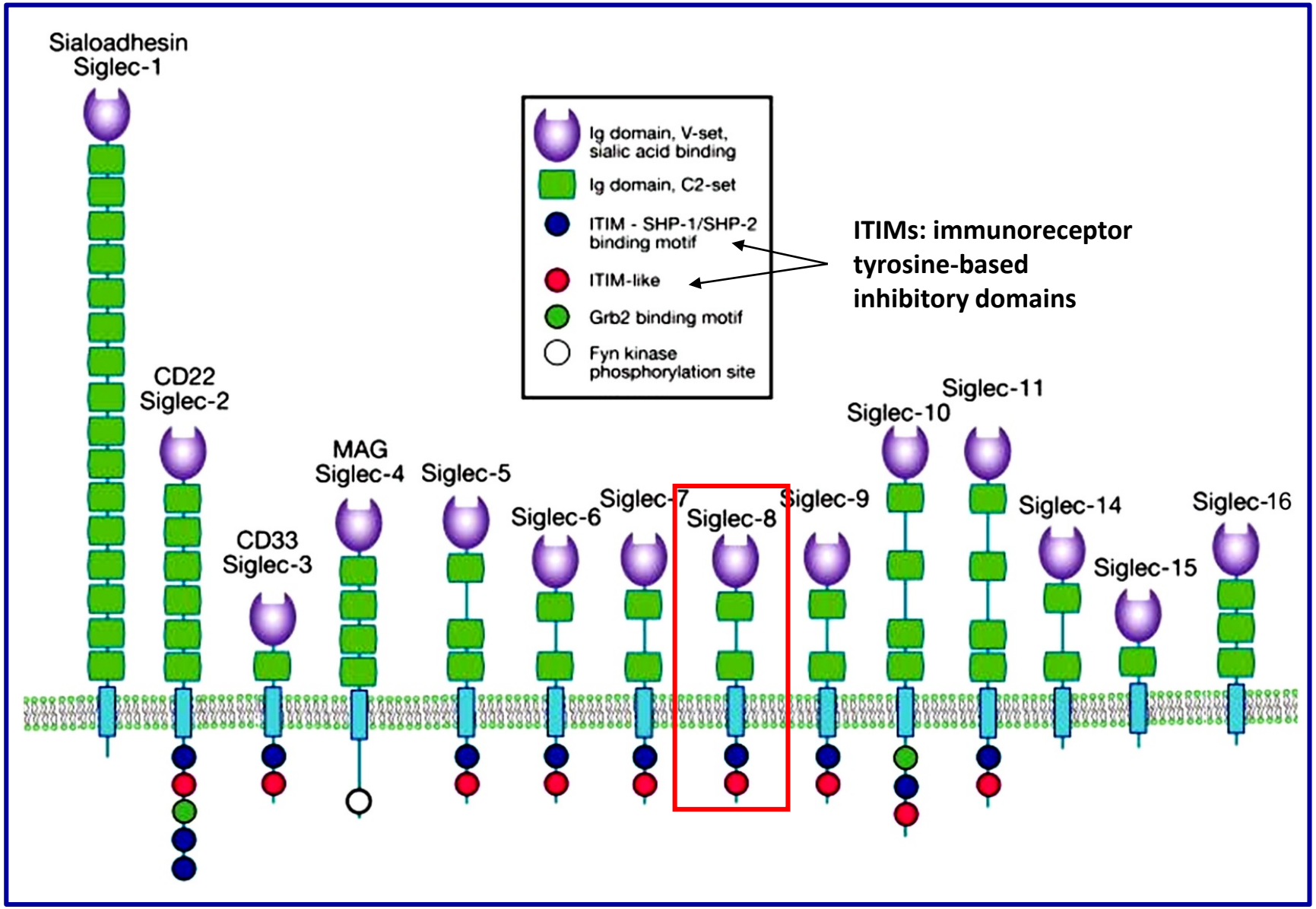
Telen et al (2015) *Blood* 125:2656

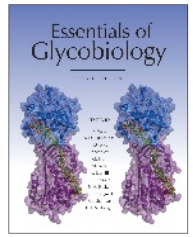
Glycan Binding Protein Functions



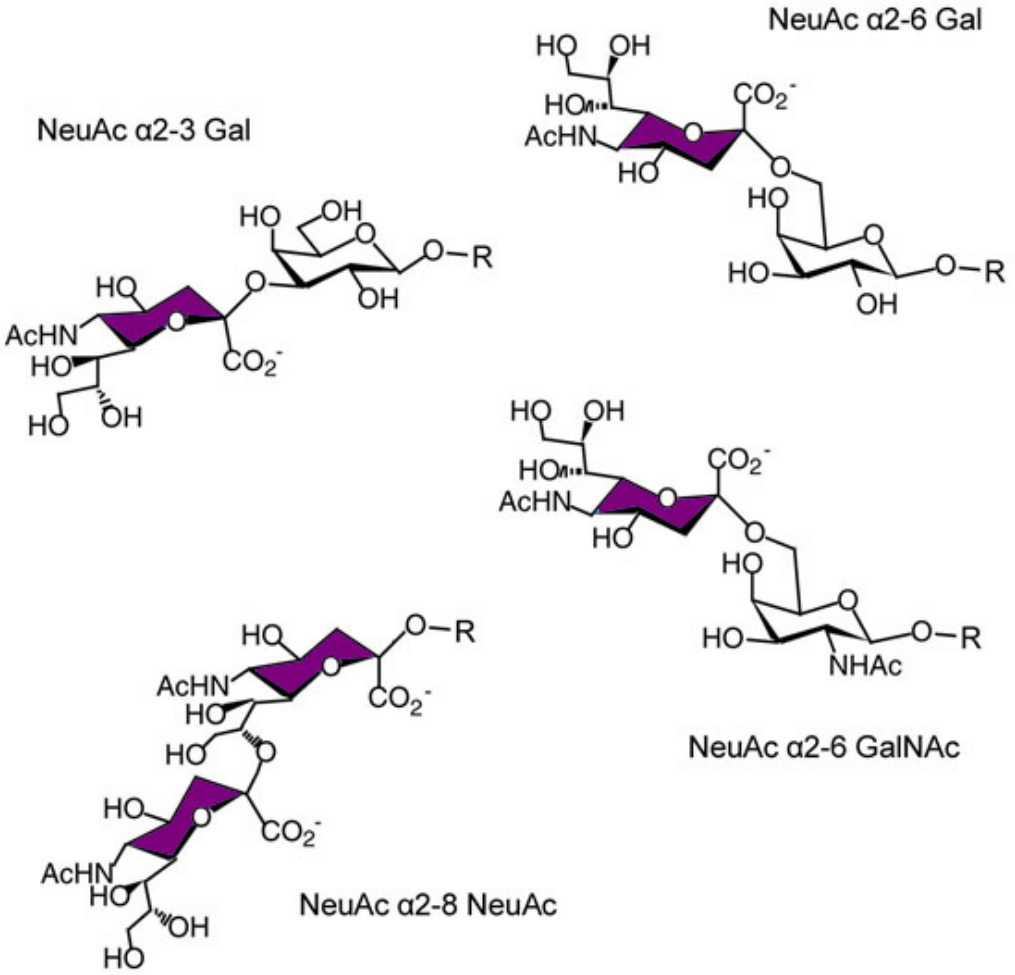
Dodd, R.B. and Drickamer, K. (2001) *Glycobiology* **11**, 71R-79R

Human sialic acid-binding immunoglobulin-like lectins





Siglecs display diverse sialic acid specificities



Human Siglec	Sialoside preference
Siglec-1 (Sialoadhesin)	NeuAcaα2-3Galβ1-4GlcNAc
Siglec-2 (CD22)	NeuAcα2-6Galβ1-4GlcNAc
Siglec-4 (MAG)	NeuAcα2-3Galβ1-3GalNAc
Siglec-15	NeuAcα2-6GalNAc
Siglec-3 (CD33)	NeuAcα2-3Galβ1-4GlcNAc
Siglec-5	NeuAcα2-8NeuAc / NeuAcα2-6Gal(Nac) / NeuAcα2-3Gal
Siglec-6	NeuAcα2-6GalNAc
Siglec-7	NeuAcα2-8NeuAcα2-3Galβ1-4GlcNAc
Siglec-8	NeuAcα2-3(6-SO3-)Galβ1-4GlcNAc
Siglec-9	NeuAcα2-3Galβ1-4(Fucα1-3, 6-SO3-)GlcNAc
Siglec-10	NeuAcα2-6Galβ1-4GlcNAc
Siglec-11	NeuAcα2-8NeuAc
Siglec-14	NeuAcα2-6GalNAc NeuAcα2-8NeuAc
Siglec-16	NeuAca2-8NeuAc

http://functionalglycomics.org

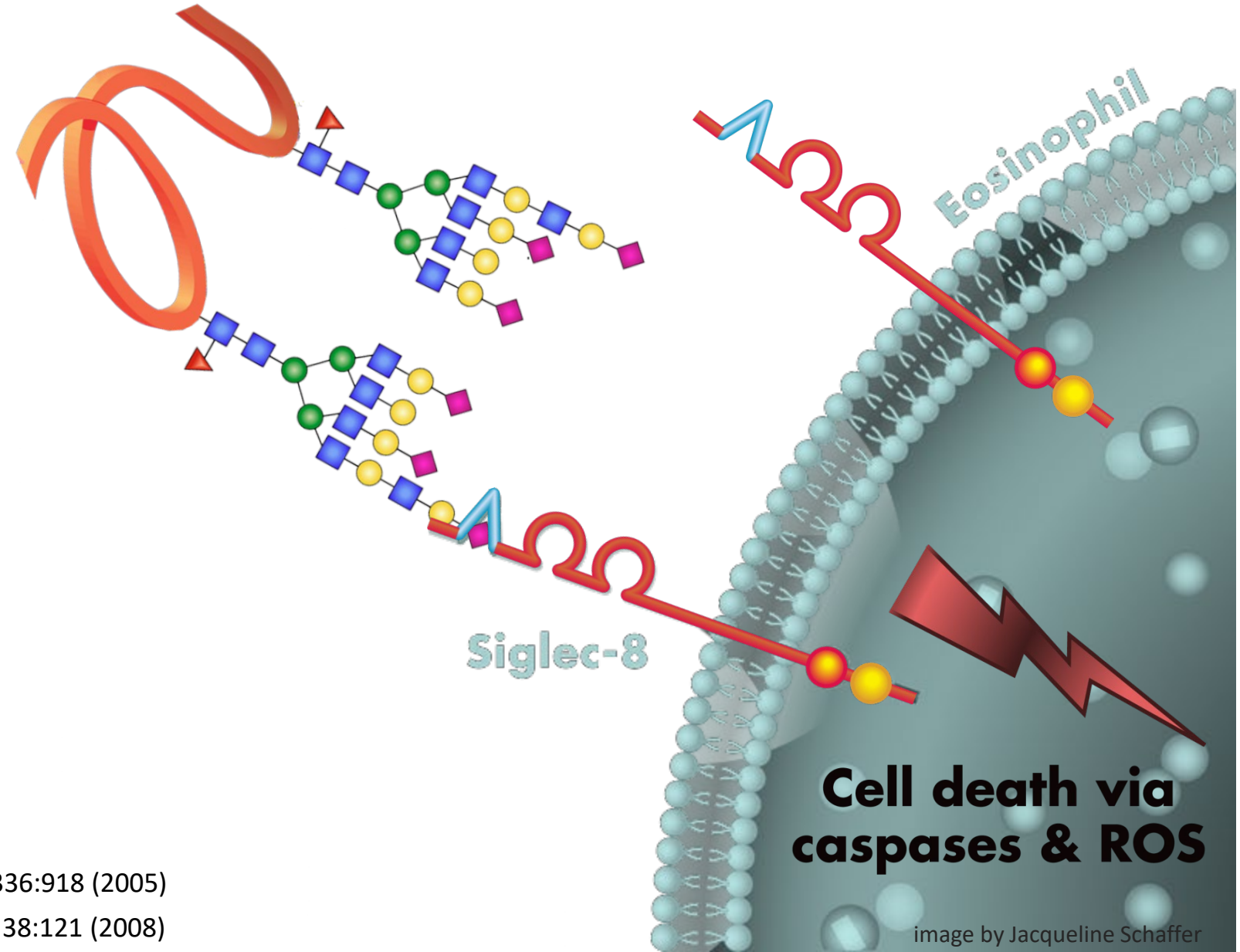
Magesh et al *Curr Med Chem* 18,3537 (2011)
Crocker et al *Nature Rev Immunol* 7,255 (2007)

Human Siglecs are mostly expressed in overlapping subsets of immune cells

Human Siglecs	Primary distribution
• Sialoadhesin (Siglec-1)	macrophages
• CD22 (Siglec-2)	B-lymphocytes
• CD33 (Siglec-3)	myeloid progenitors / monocytes / macrophages / DC
• MAG (Siglec-4)	oligodendrocytes / Schwann cells
• Siglec-5	[neutrophils / monocytes / B cells / macrophages]
• Siglec-6	trophoblasts / B cells
• Siglec-7	monocytes / NK cells / T cells / DC
• Siglec-8	eosinophils / basophils / mast cells
• Siglec-9	neutrophils / monocytes [NK cell / B cell / T cell]
• Siglec-10	B-cells / NK cells / monocytes / eosinophils
• Siglec-11	macrophages
• Siglec-14	[neutrophils / monocytes / B cells / macrophages]
• Siglec-15	macrophages/dendritic cells
• Siglec-16	macrophages

Varki and Angata *Glycobiology* 16, 1R (2006)
Angata, et al. *FASEB J.* 20, 1964 (2006)
Angata et al. *Glycobiology* 17, 838 (2007)
Cao et al. *Eur J. Immunol* 38, 2303 (2008).

Ligation of Siglec-8 induces eosinophil apoptosis



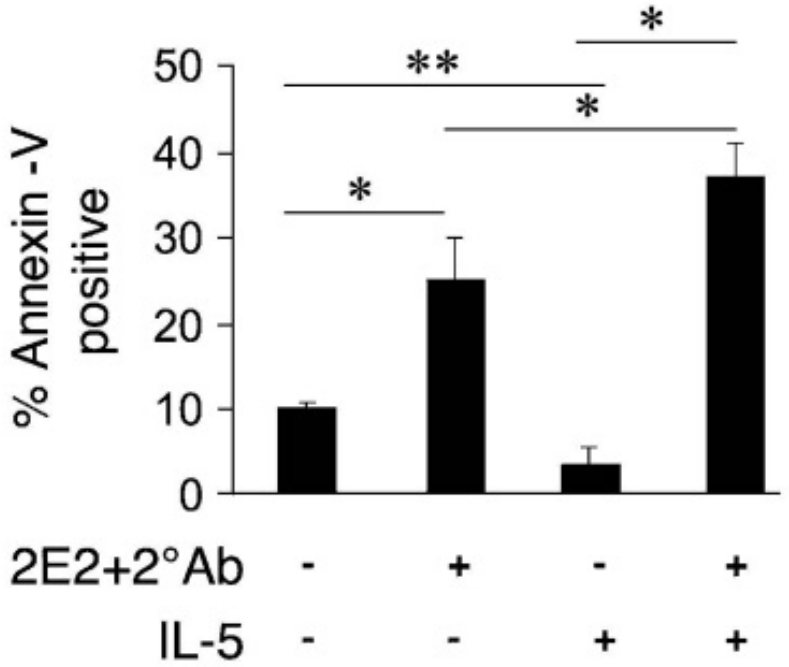
Nutku et al *Blood* 101:5014 (2003)

Nutku et al *Biochem Biophys Res Comm* 336:918 (2005)

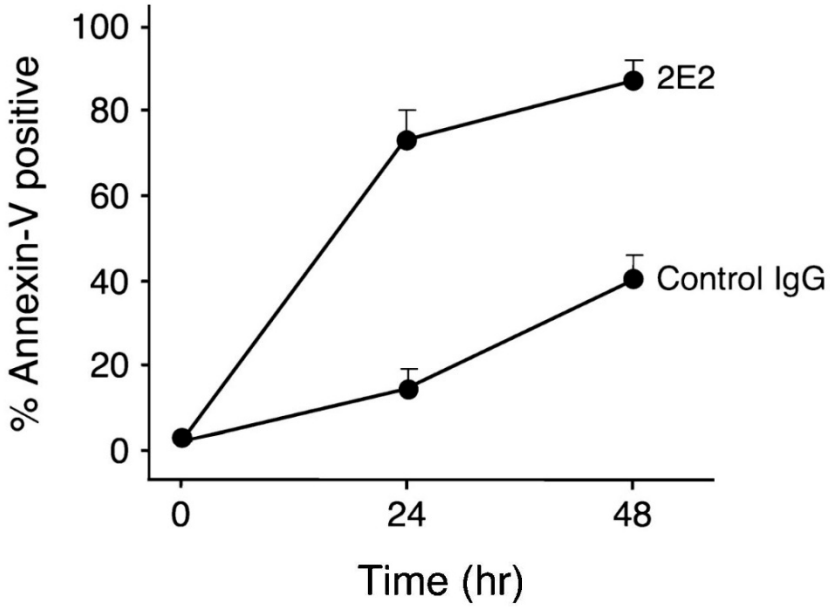
Nutku-Bilir et al *Am J Respir Cell Mol Biol* 38:121 (2008)

Ligation of Siglec-8 induces eosinophil apoptosis

Crosslinking Siglec-8 on primary human eosinophils induces apoptosis. IL-5 activation enhances death

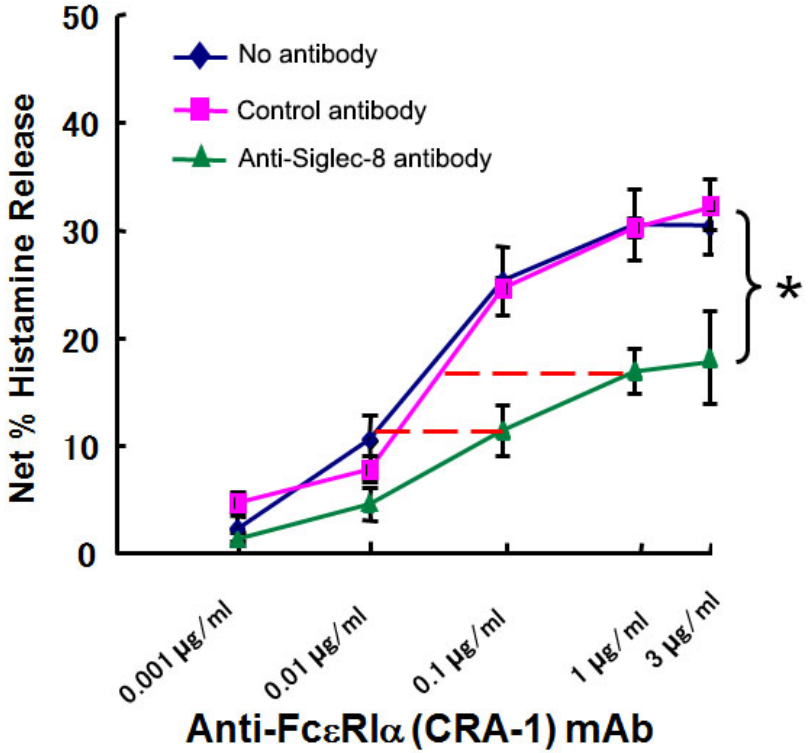


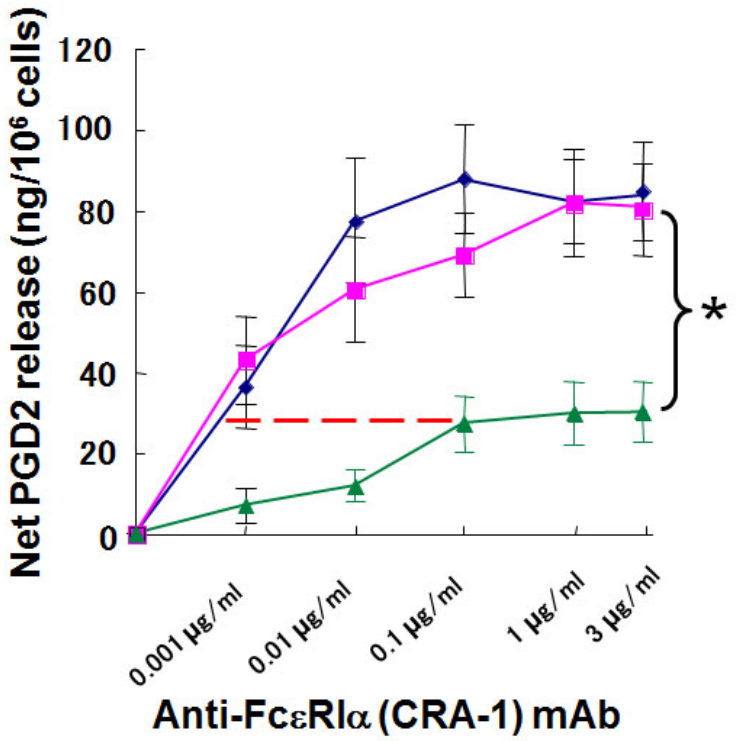
Late-phase (endogenously activated) human eosinophils are even more sensitive to Siglec-8 crosslinking



Nutku-Bilir et al (2008) *Am J Respr Cell Mol Biol* 38:121

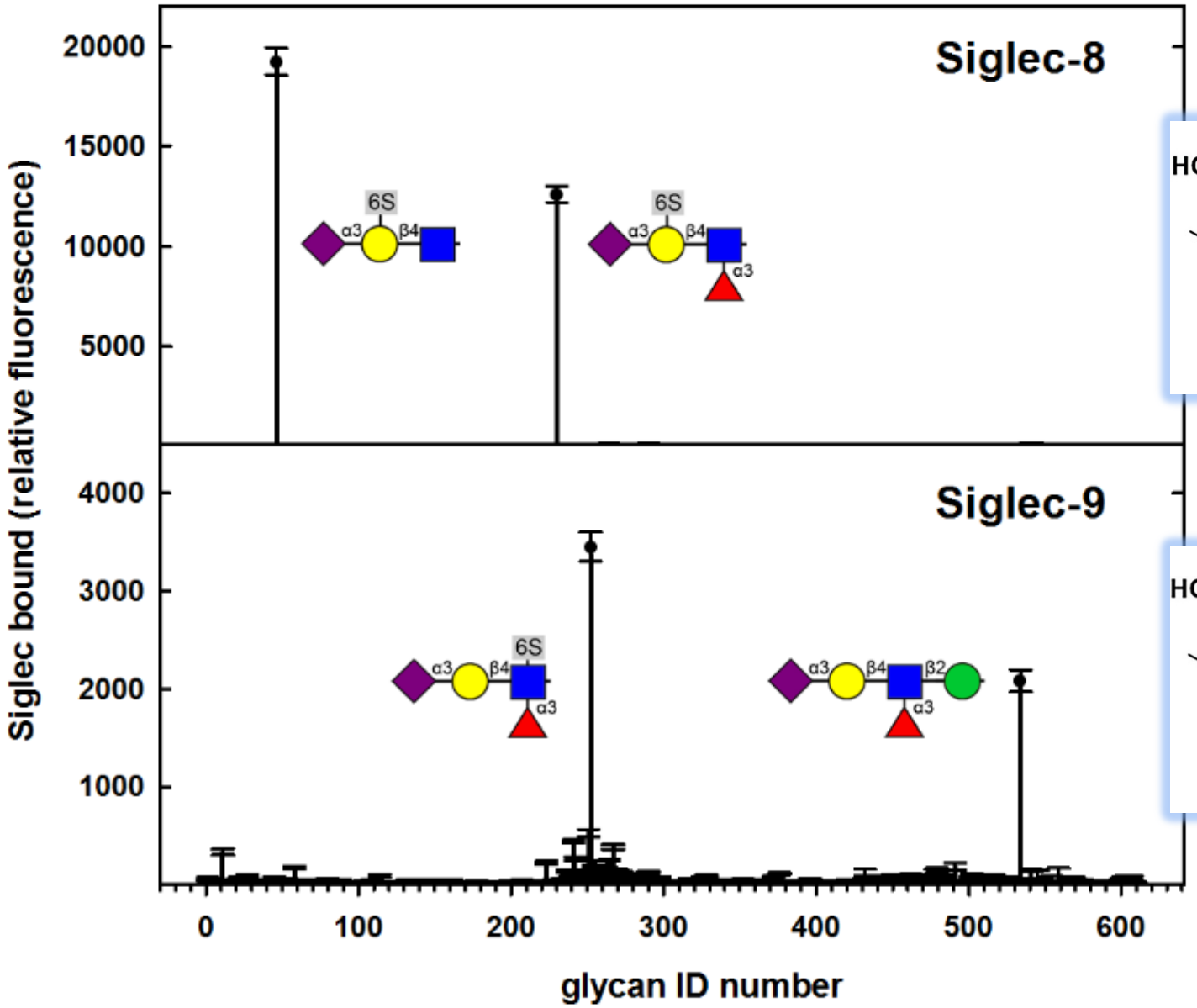
Ligation of Siglec-8 blocks mediator release from primary human *mast cells*

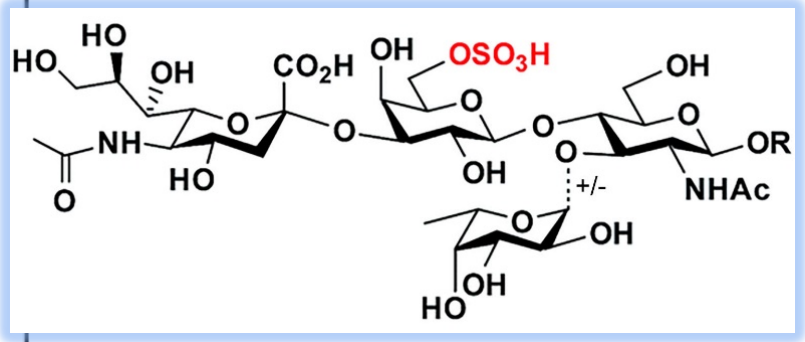




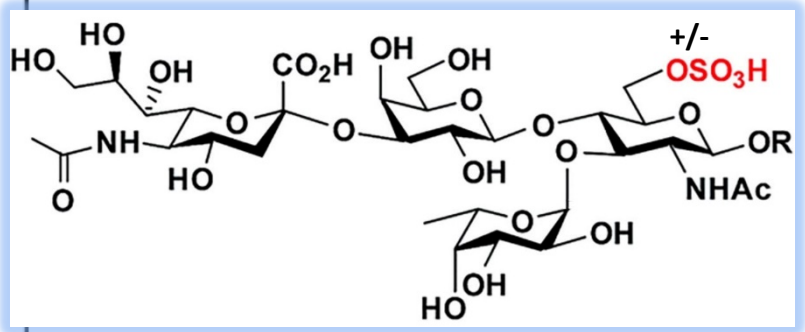
Yokoi et al *J. Allergy Clin. Immunol.* 121:499 (2008)

Glycan array binding of Siglec-8 (and Siglec-9)





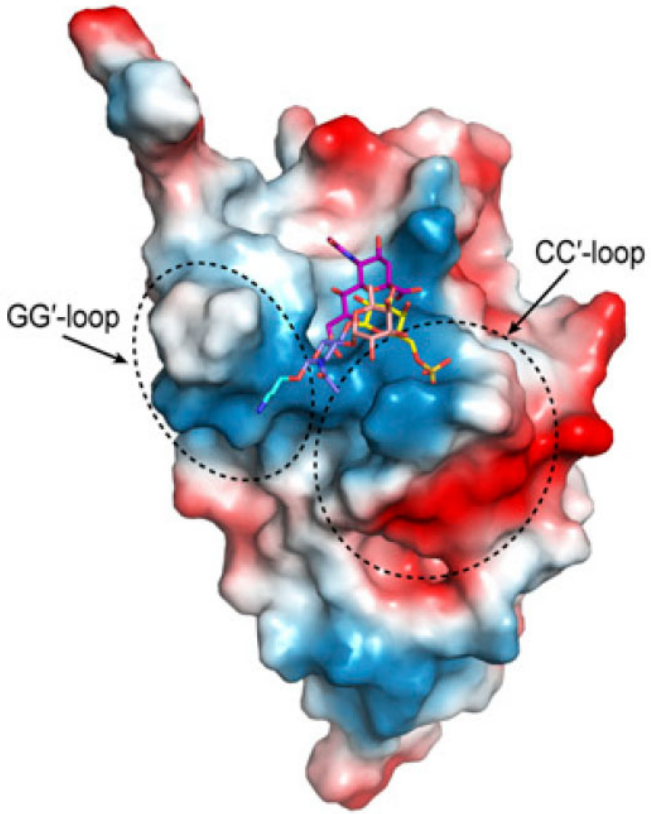
6'S-Sia-LacNAc (or Lex)

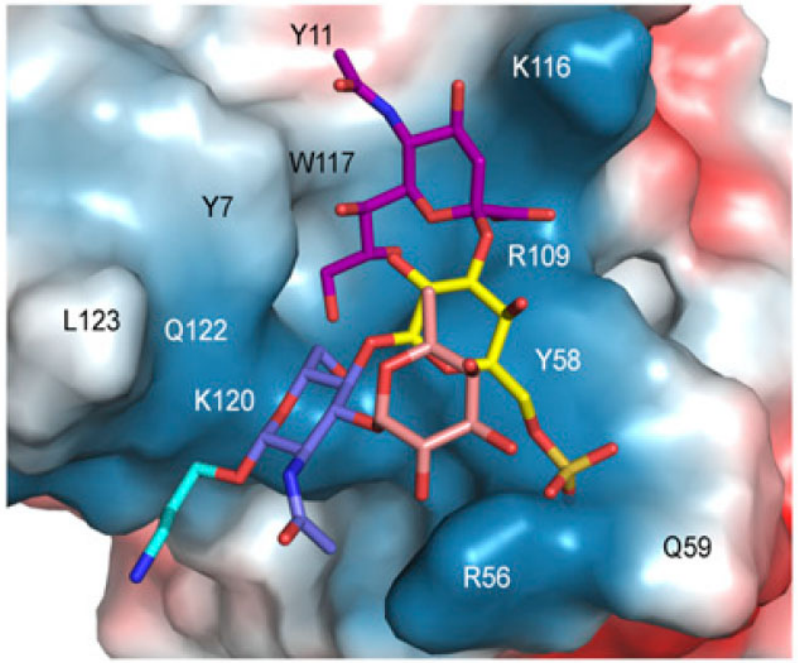


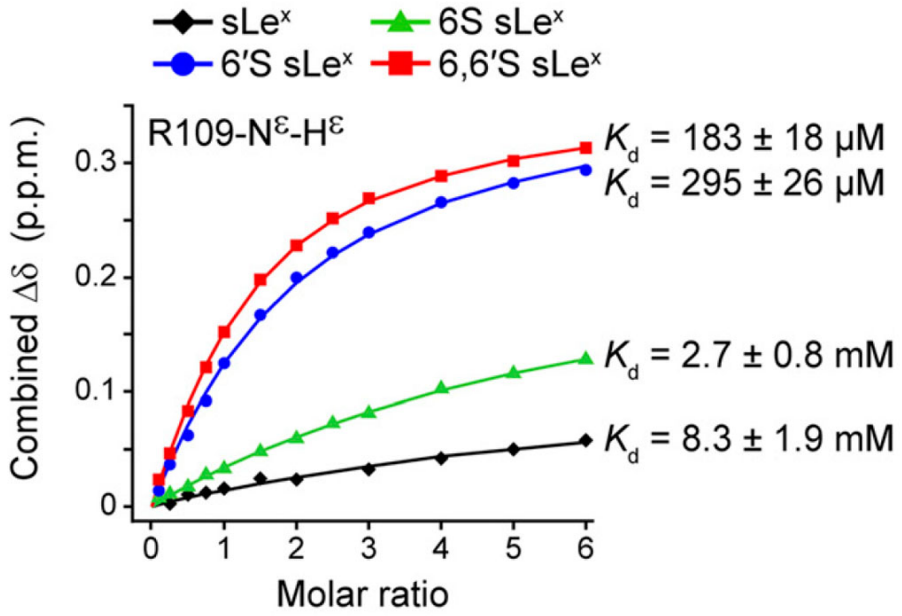
6S-Sia-LacNAc (or Lex)

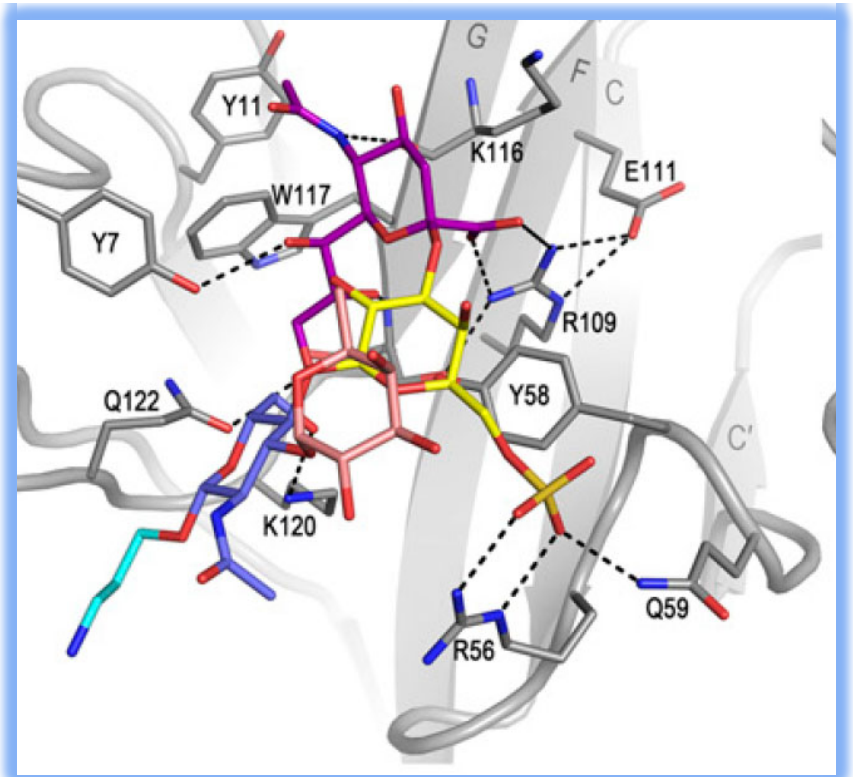
Yu H et al *Glycobiology* 27:657 (2017)

NMR spectroscopy reveals the basis for Siglec-8 glycan binding



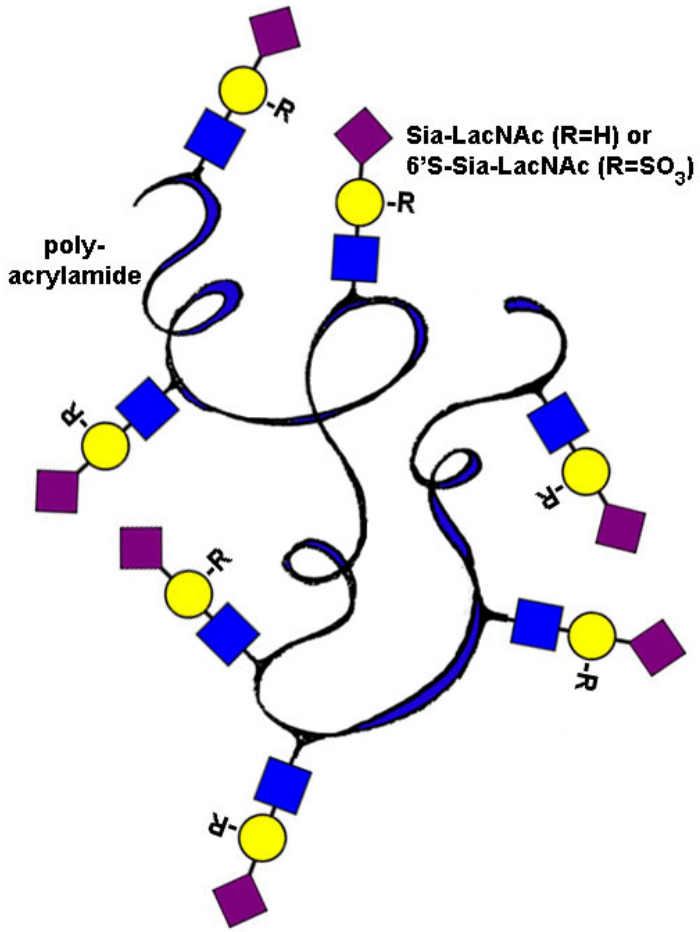


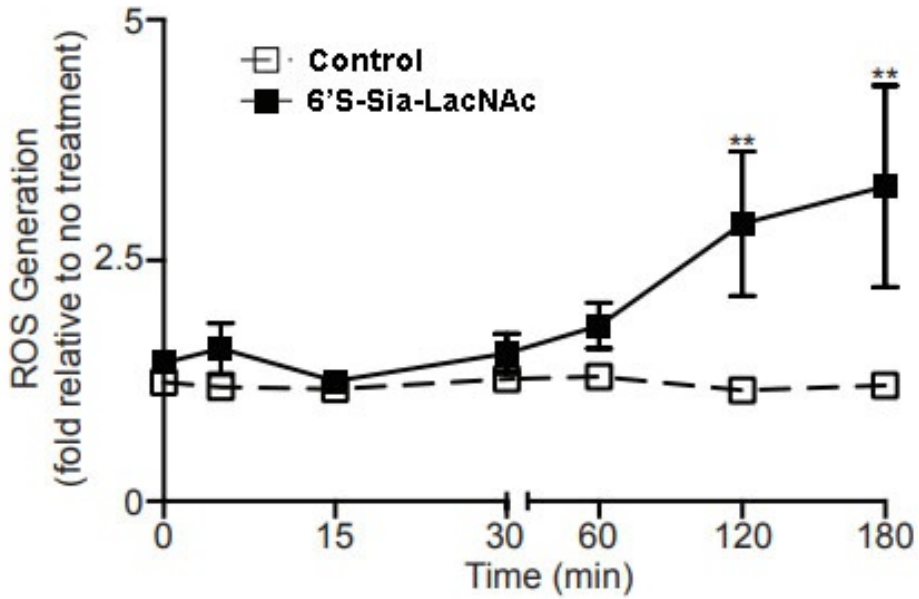


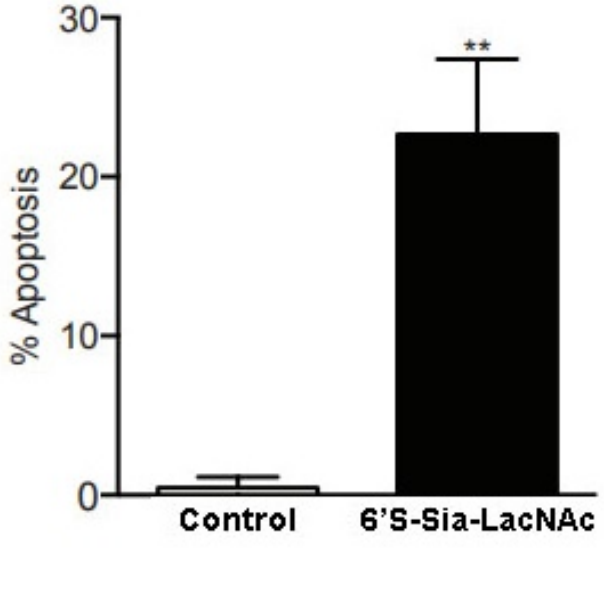


Pröpster et al *Proc Natl Acad Sci USA* 113,E4170 (2016)

Polyvalent 6'S-Sia-LacNAc induces apoptosis in activated human eosinophils

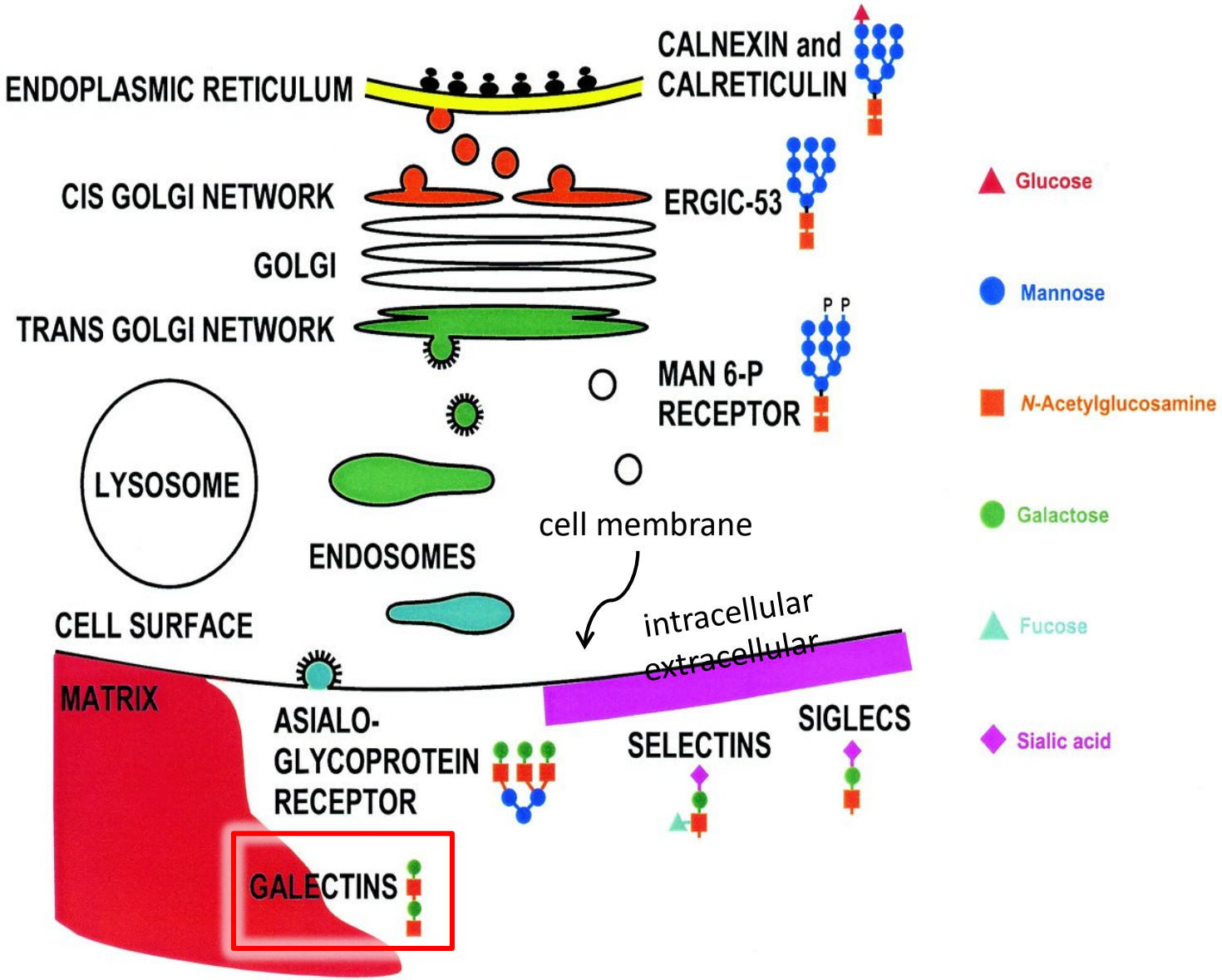






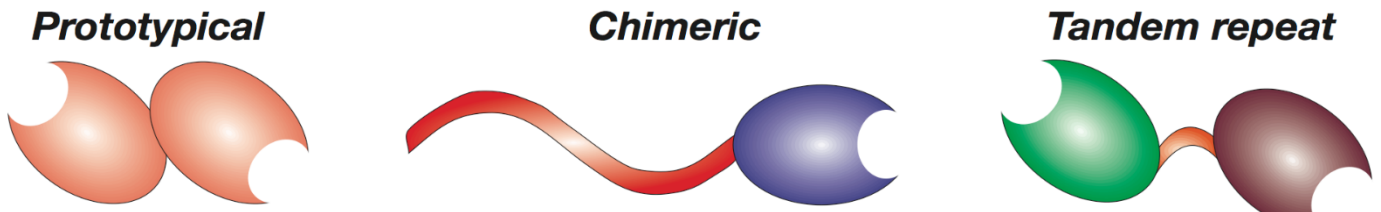
Carroll et al *J Allergy Clin Immunol 141,2196* (2018)

Glycan Binding Protein Functions



Dodd, R.B. and Drickamer, K. (2001) *Glycobiology* **11**, 71R-79R

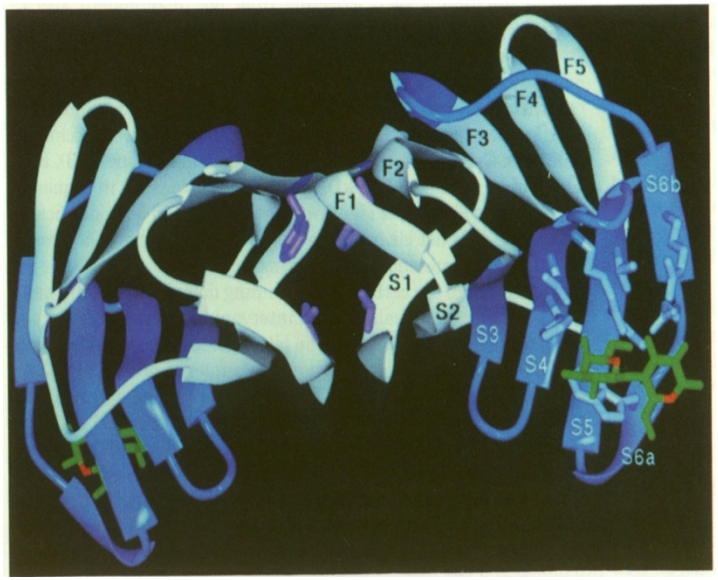
Galectin family of soluble glycan binding proteins

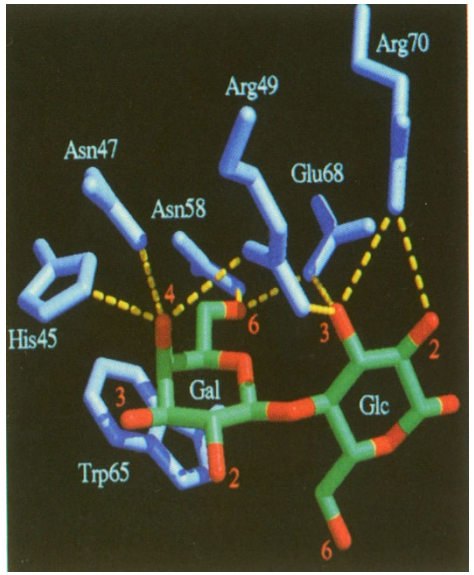


Galectin-1
Galectin-2
Galectin-7
Galectin-10
Galectin-13
Galectin-14

Galectin-3

Galectin-4
Galectin-8
Galectin-9
Galectin-12



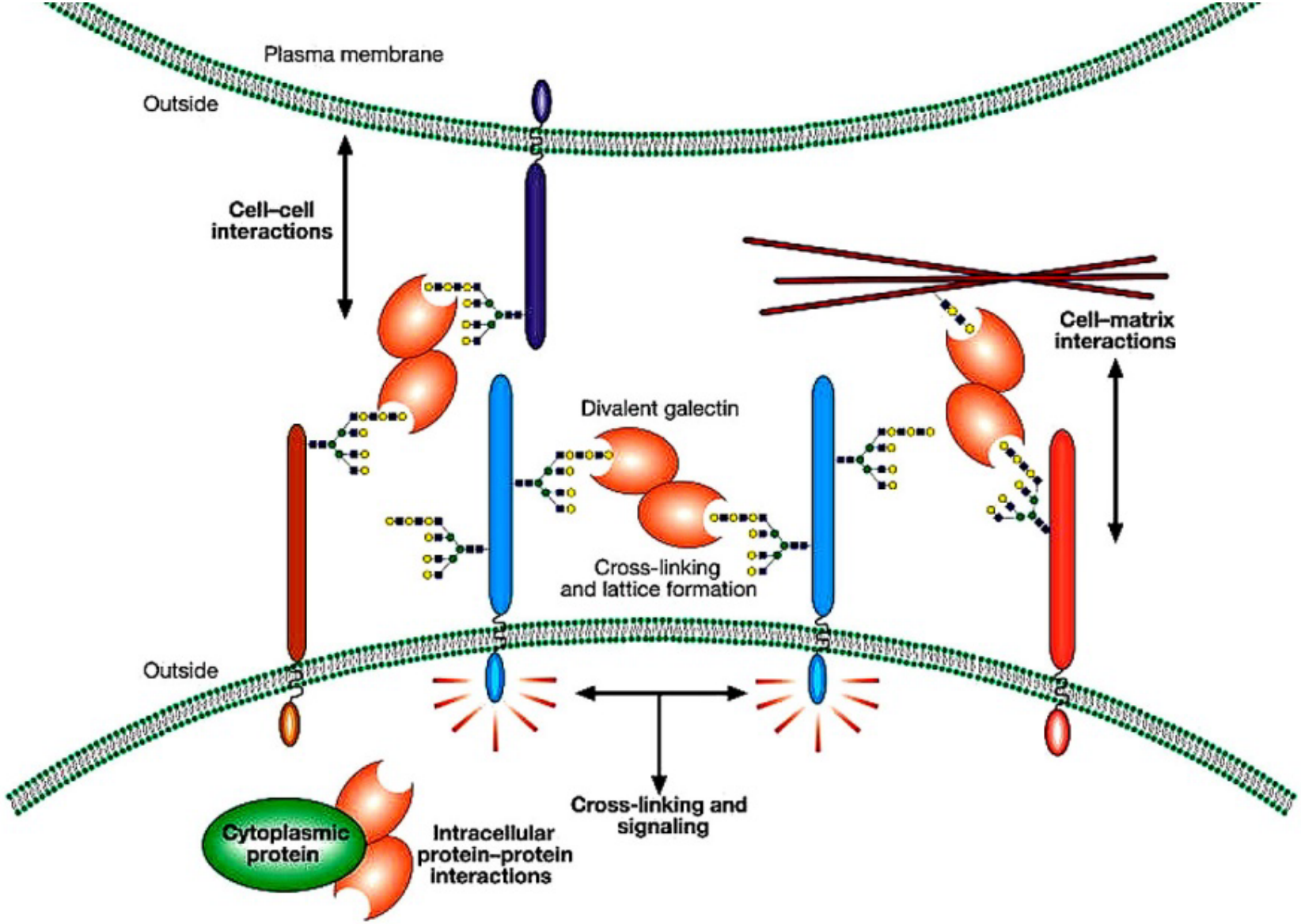


Galectin Specificity

Galectin-2, Barondes, et al. (1994) J. Biol. Chem. 269:20807

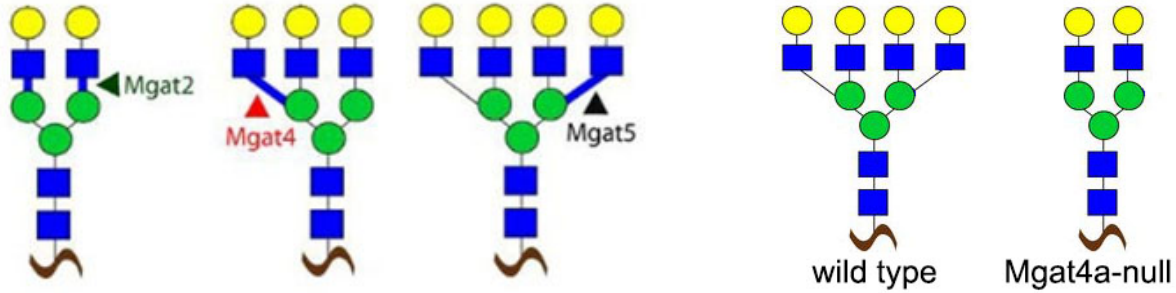
Essentials of Glycobiology
Second Edition

Galectin-mediated cell surface lattice formation



Galectin function revealed in glycosyltransferase (*Mgat4a*) null mice

N-glycan branching

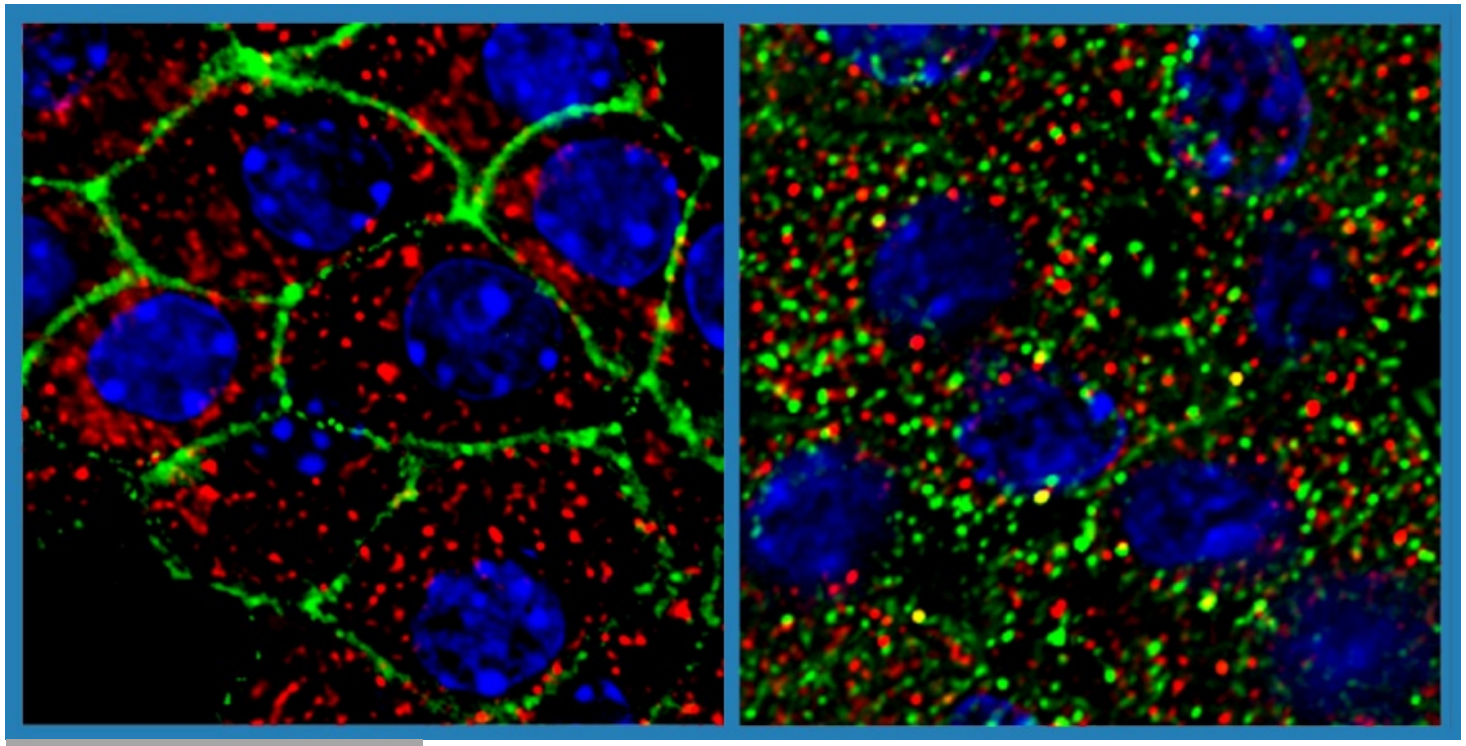


12 Months of Age, Standard Chow	Wild-Type (n = 15)	*Mgat4a* Null (n = 15)
Glucose (fasting) (mg/dl)	108.9 ± 7.72	168.6 ± 10.14 (p = 0.0001)
Glucose (fed) (mg/dl)	193.2 ± 8.12	249.4 ± 15.41 (p = 0.0029)
Insulin (fasting) (ng/ml)	1.023 ± 0.127	0.651 ± 0.087 (p = 0.0239)
Insulin (fed) (ng/ml)	4.389 ± 0.361	1.673 ± 0.121 (p = 0.0001)
Free fatty acid (fasting)	1.10 ± 0.03	1.660 ± 0.050 (p = 0.0001)
Free fatty acid (fed)	0.62 ± 0.06	0.680 ± 0.040
Triglyceride (mg/dl)	102.6 ± 8.93	158.5 ± 18.75 (p = 0.0174)
AST (IU/l)	96.52 ± 6.58	170.8 ± 14.21 (p = 0.0003)
ALT (IU/l)	87.39 ± 6.60	173.2 ± 10.57 (p = 0.0001)
Lipase (U/l)	60.63 ± 4.81	52.82 ± 3.80
Total cholesterol (mg/dl)	190.9 ± 15.00	176.6 ± 14.32
HDL cholesterol (mg/dl)	168.0 ± 7.84	152.0 ± 12.57
Body weight (g)	47.98 ± 1.92	57.98 ± 2.27 (p = 0.0031)

Ohtsubo et al (2005)
Cell 123, 1307

The galectin lattice keeps the pancreatic β cells glucose transporter (Glut-2) on the cell surface

Reduced N-glycan branching results in loss of cell surface Glut-2 in pancreatic β cells, and diminished glucose-mediated insulin release

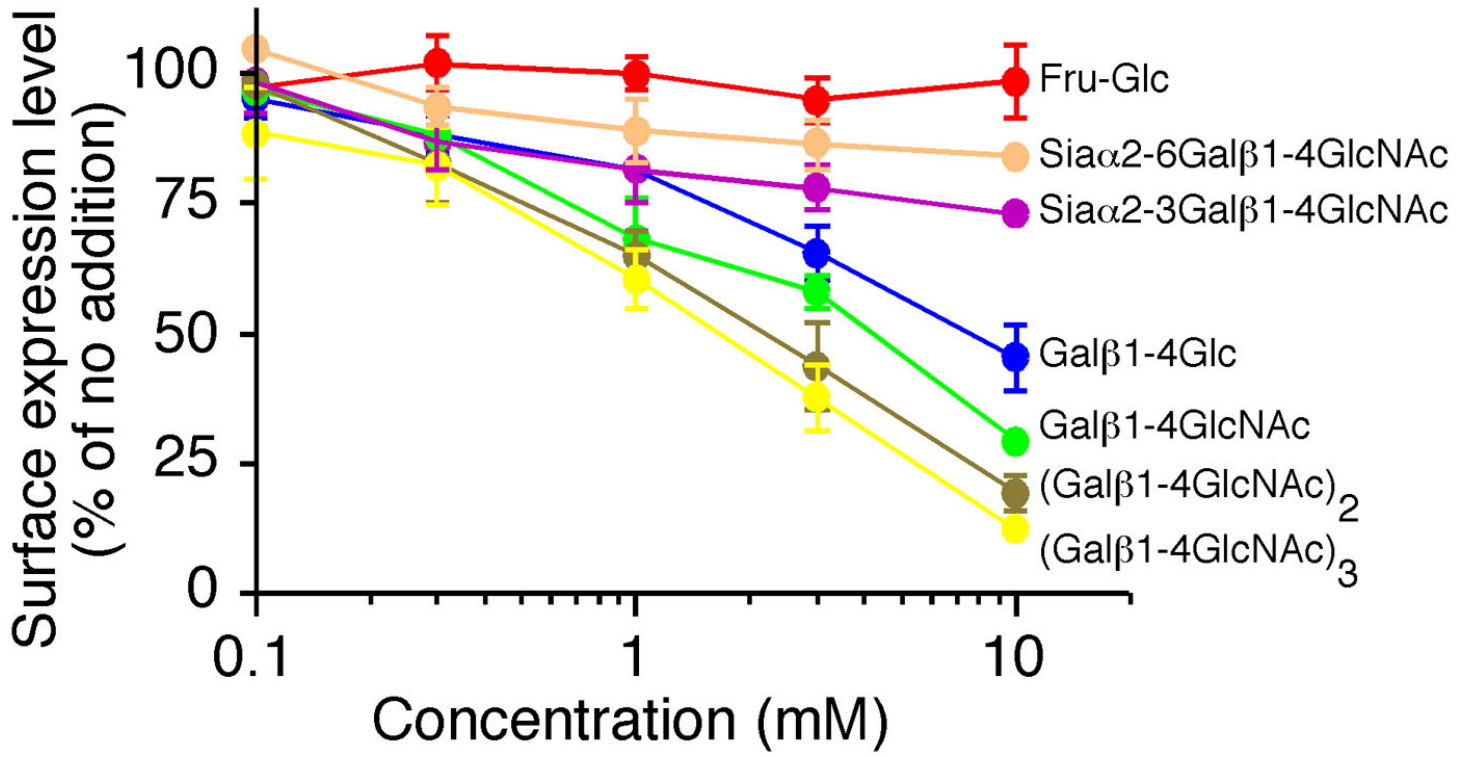


Glut-2; insulin; DNA

Ohtsubo et al (2005) *Cell* 123, 1307

The galectin lattice keeps the pancreatic β cells glucose transporter (Glut-2) on the cell surface

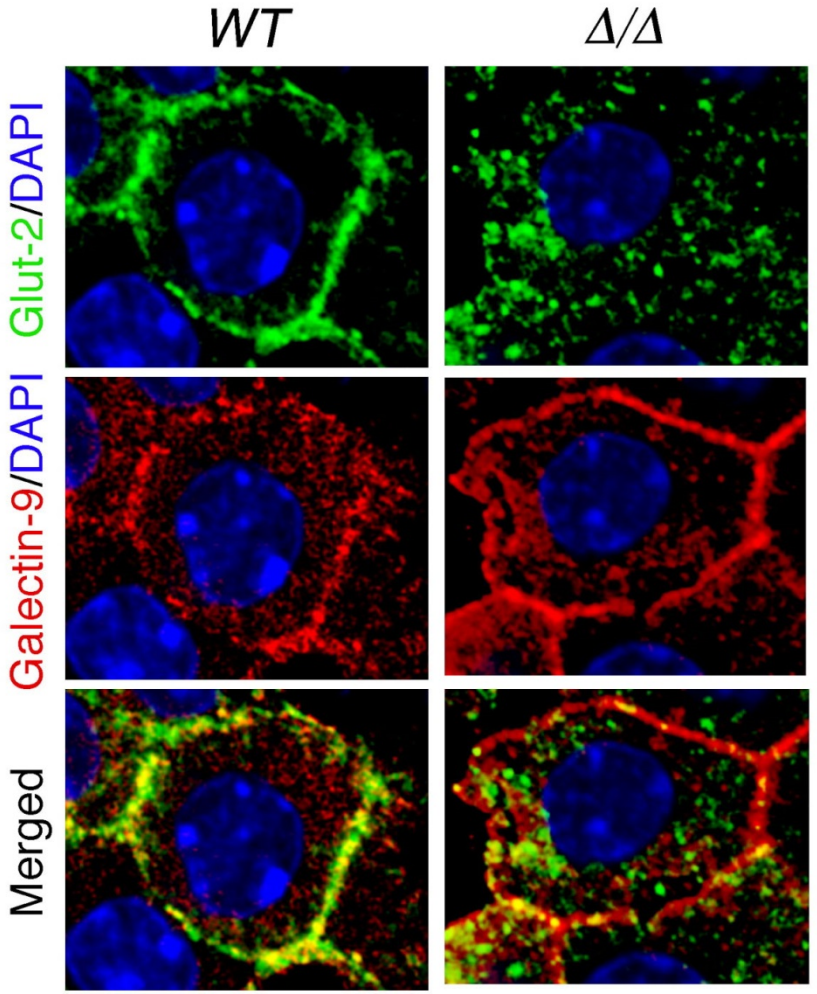
Galectin-inhibiting sugars diminish cell surface expression of Glut-2 in isolated pancreatic β cells

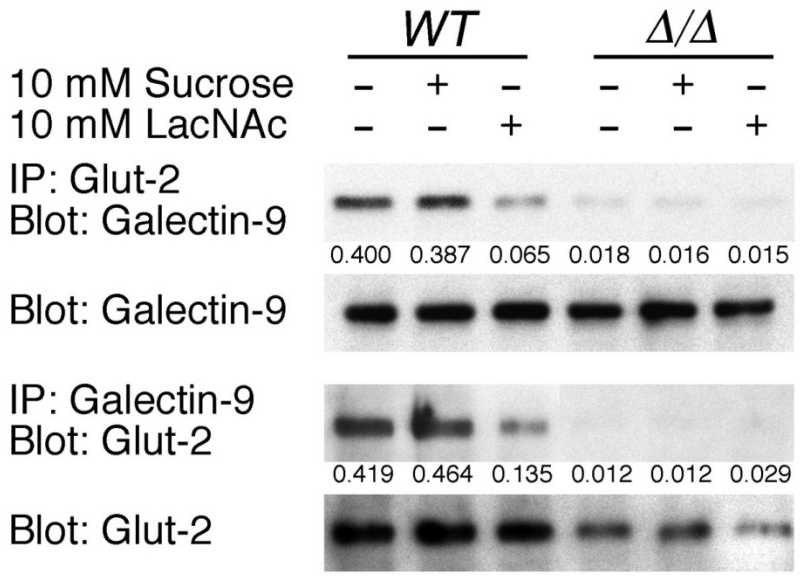


Ohtsubo et al (2005) *Cell* 123, 1307

The galectin lattice keeps the pancreatic β cells glucose transporter (Glut-2) on the cell surface

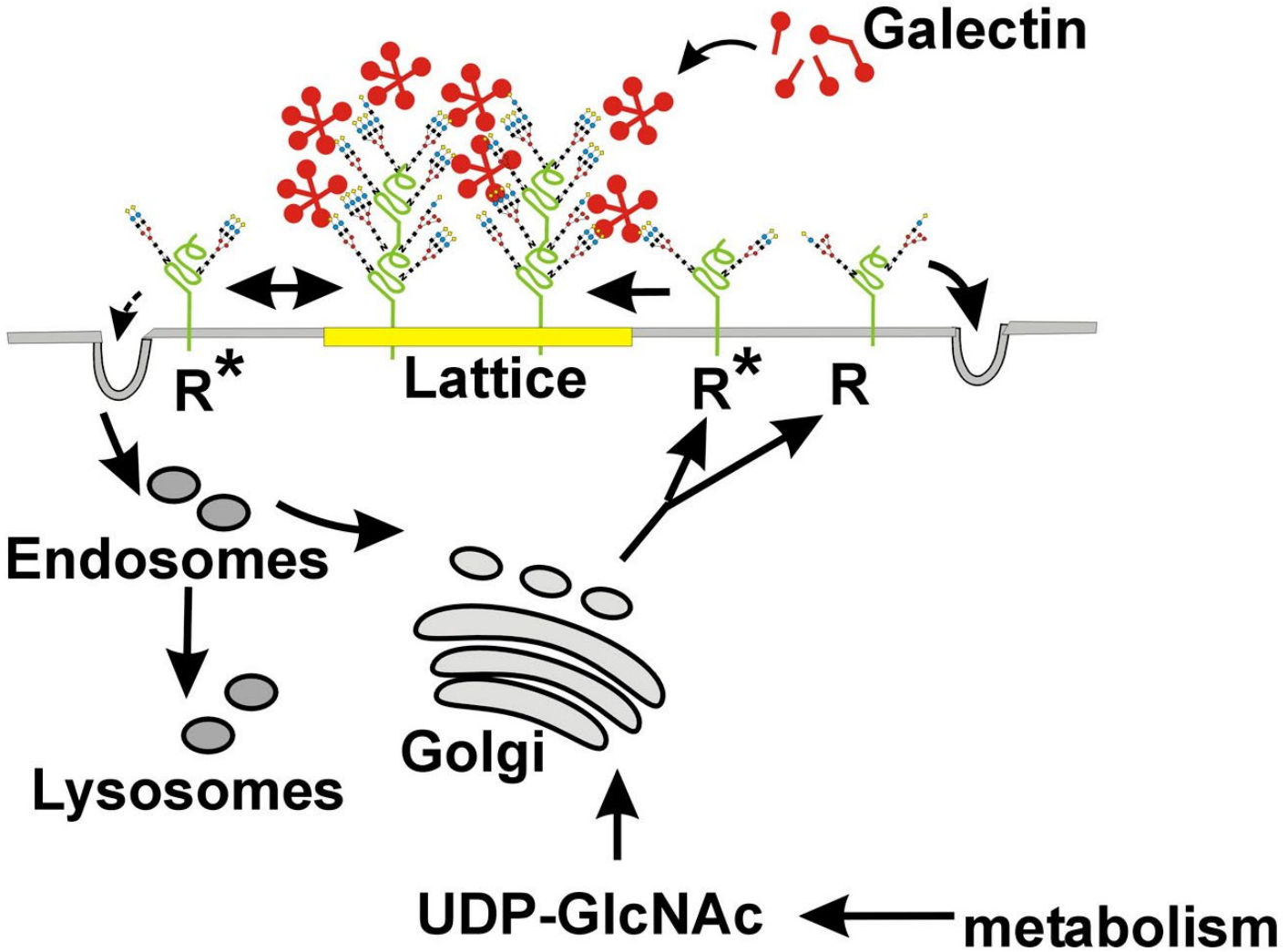
Galectin-9 co-localizes with and co-immunoprecipitates with Glut-2





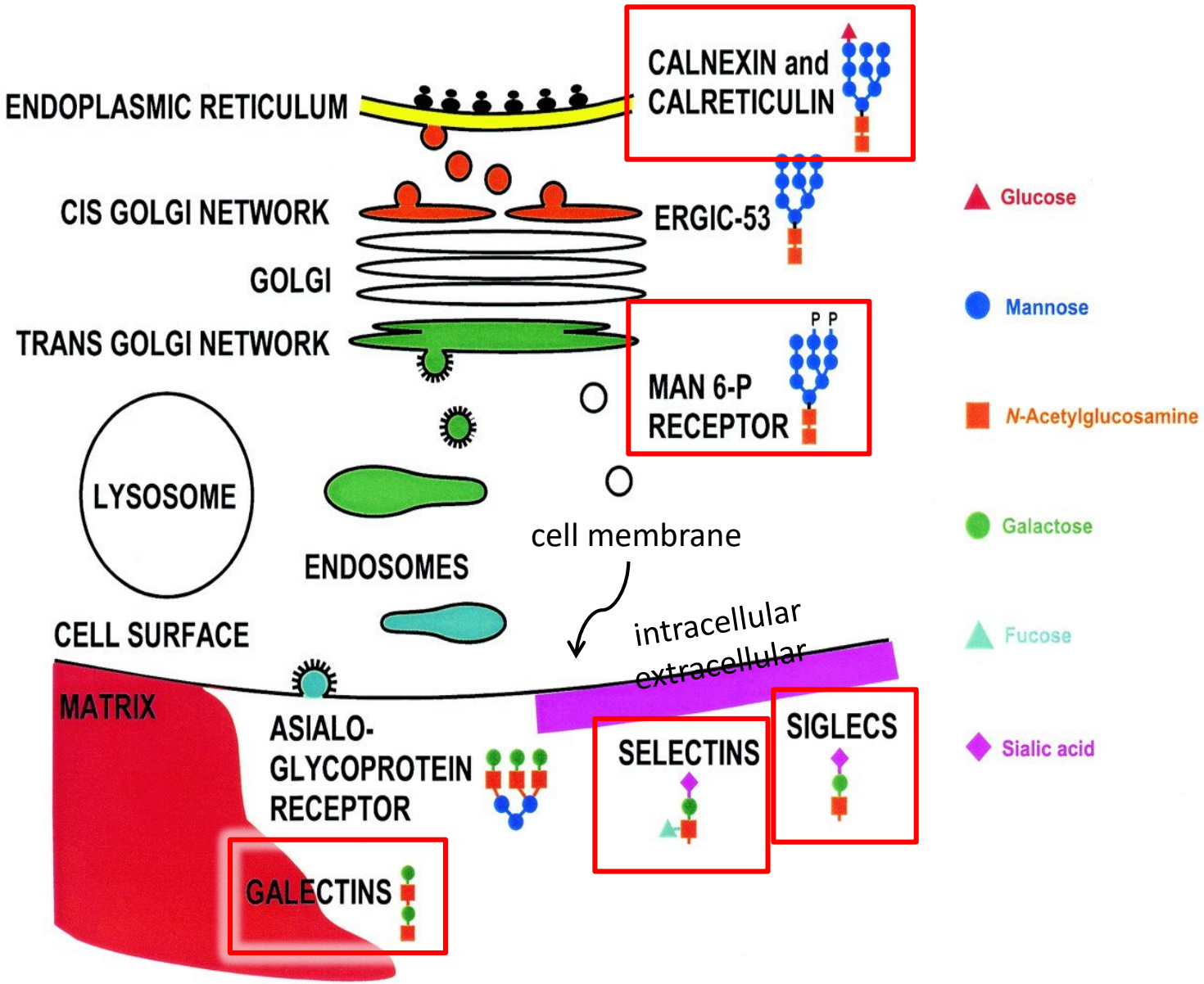
Ohtsubo et al (2005) *Cell* 123, 1307

Metabolic control of cell surface protein expression via galectin to N-glycan engagement



Lau et al (2007) *Cell* 129, 123

Glycan Binding Protein Functions



Dodd, R.B. and Drickamer, K. (2001) *Glycobiology* **11**, 71R-79R