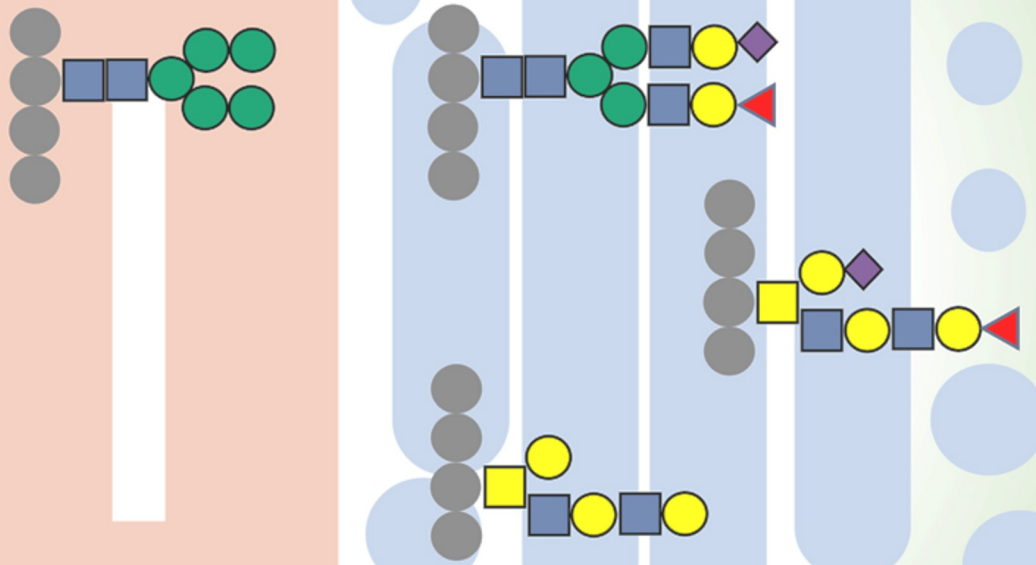
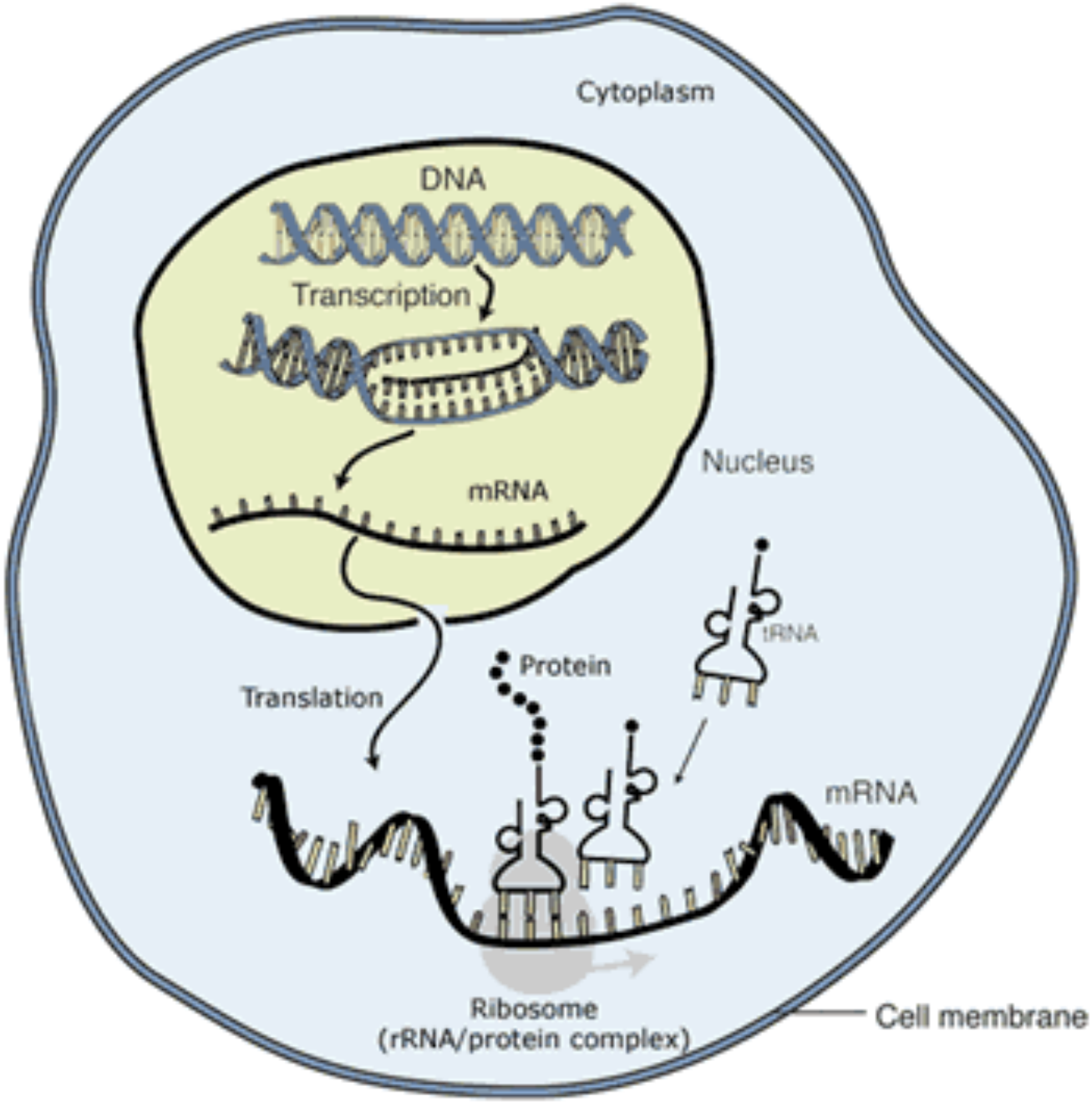
MEDICAL GLYCOBIOLOGY



ATIT SILSIRIVANIT, PH.D.
atitsil@kku.ac.th



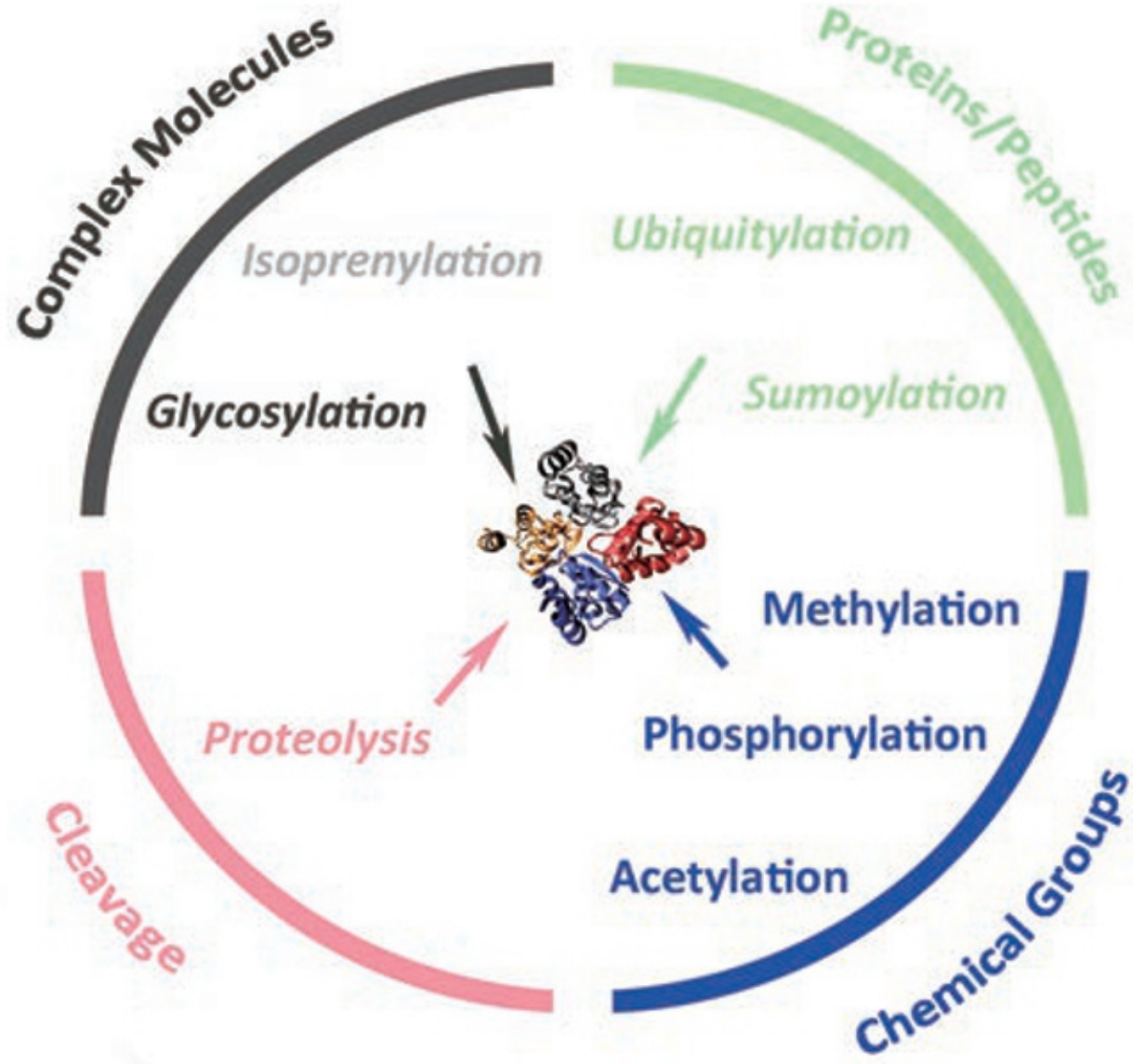
DNA

RNA

Protein

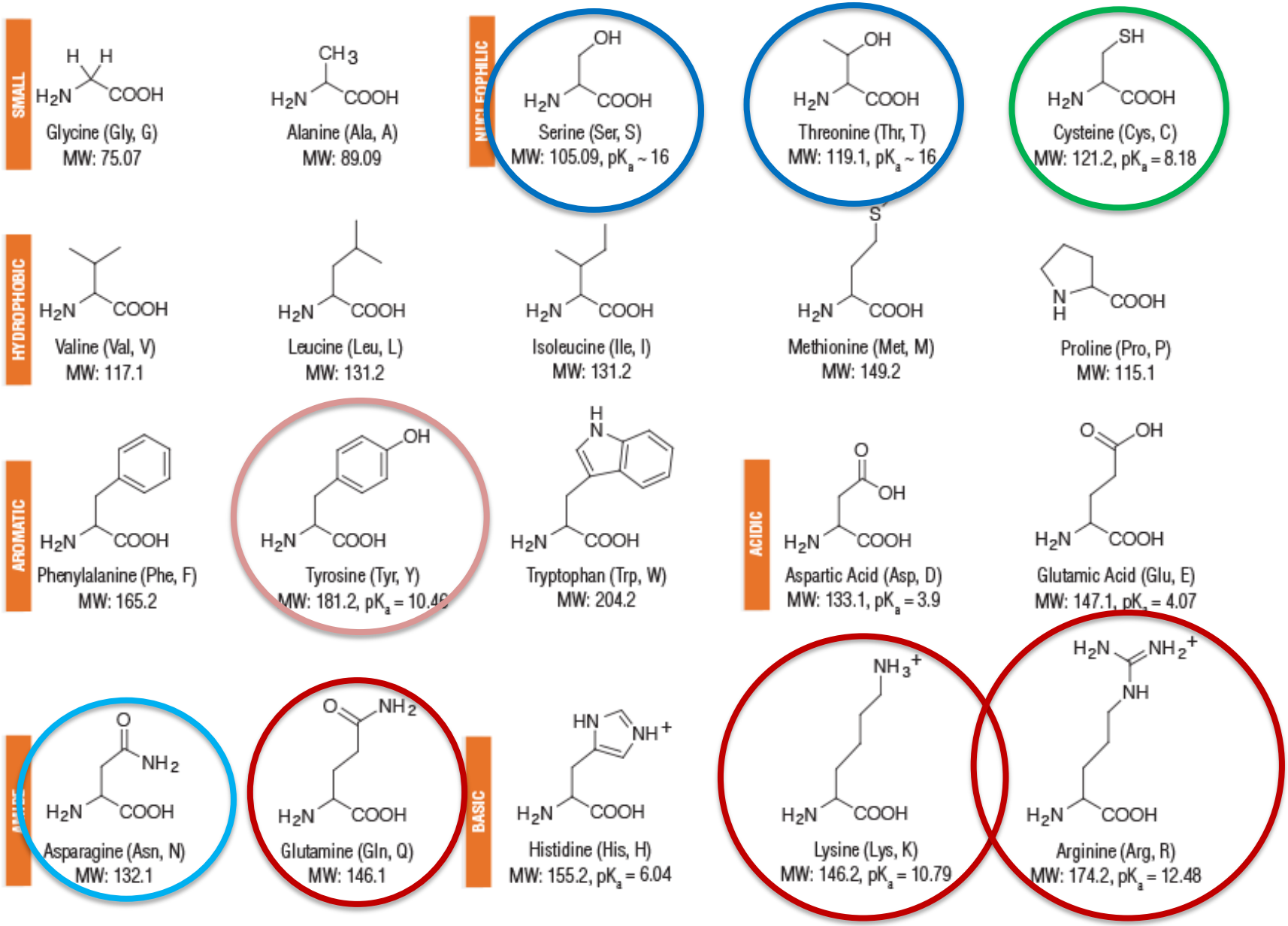
PTMs

Post-translational modifications (PTMs)



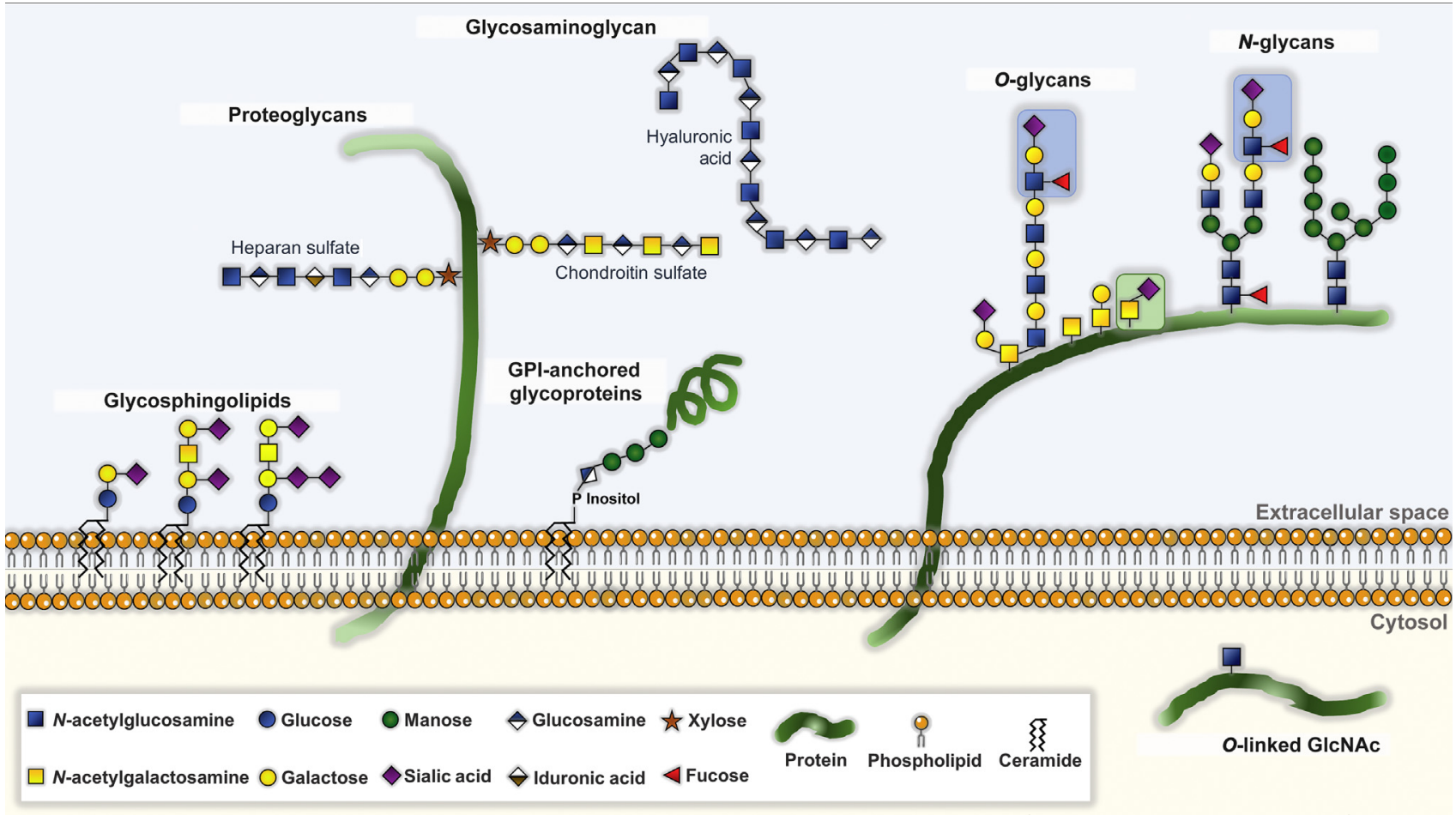
Wang YC, Cell Resaerch 2014

PTM is a modification on a particular amino acid.



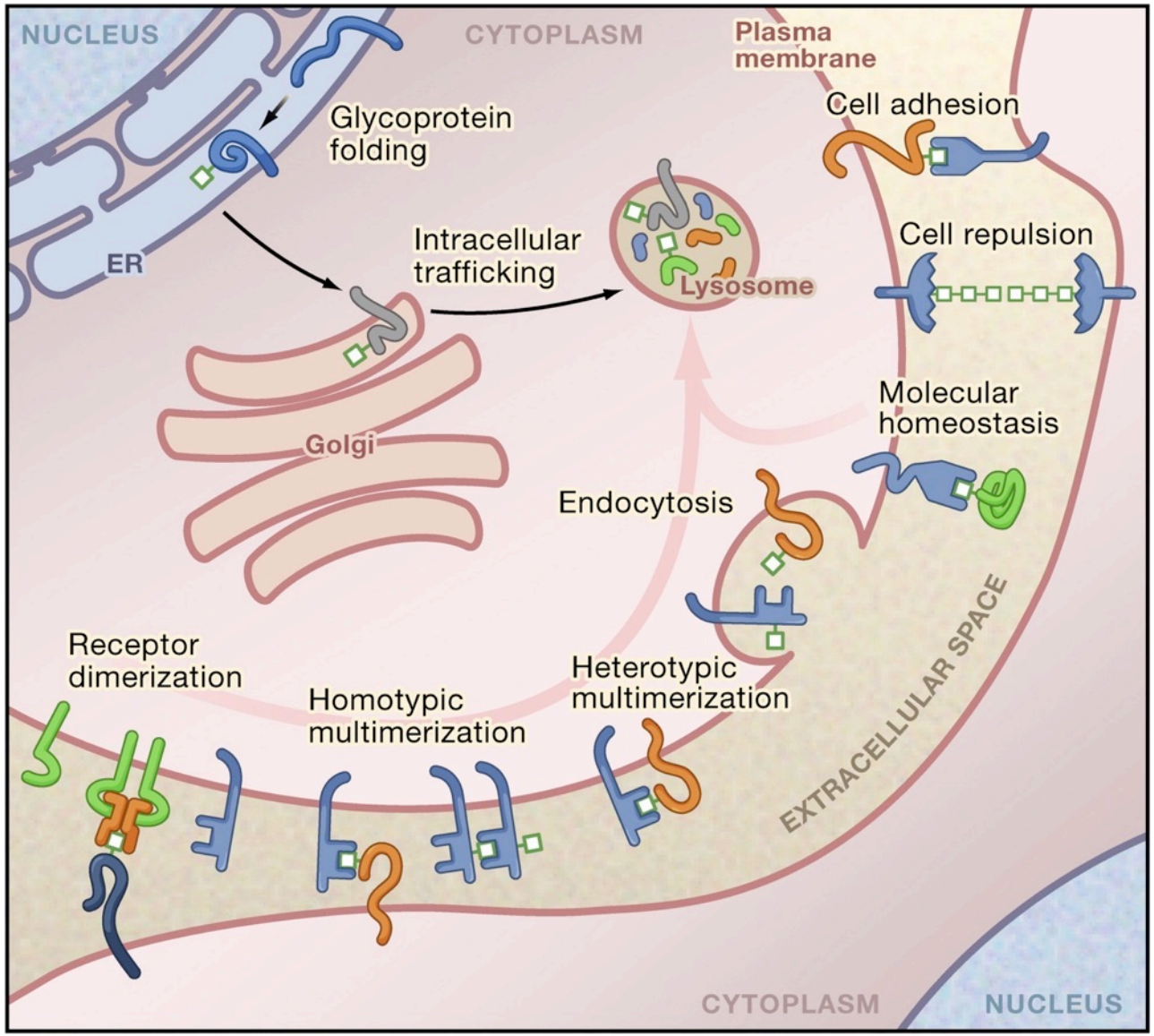
Glycosylation

Glycosidic-linkage between **sugar** and **proteins/lipids** catalyzed by enzymatic reactions. Glycosylation produces different types of glycans that are typically attached to cellular proteins and lipids.



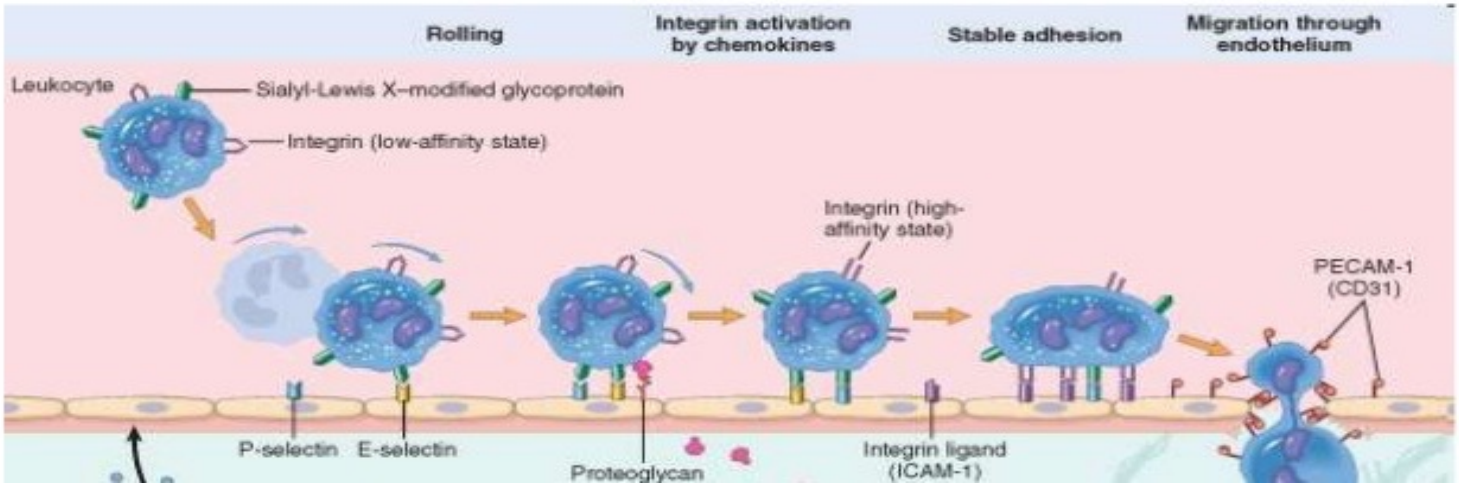
Costa AF, Trand in Cancer, 2020

Regulation of cellular mechanisms by glycans

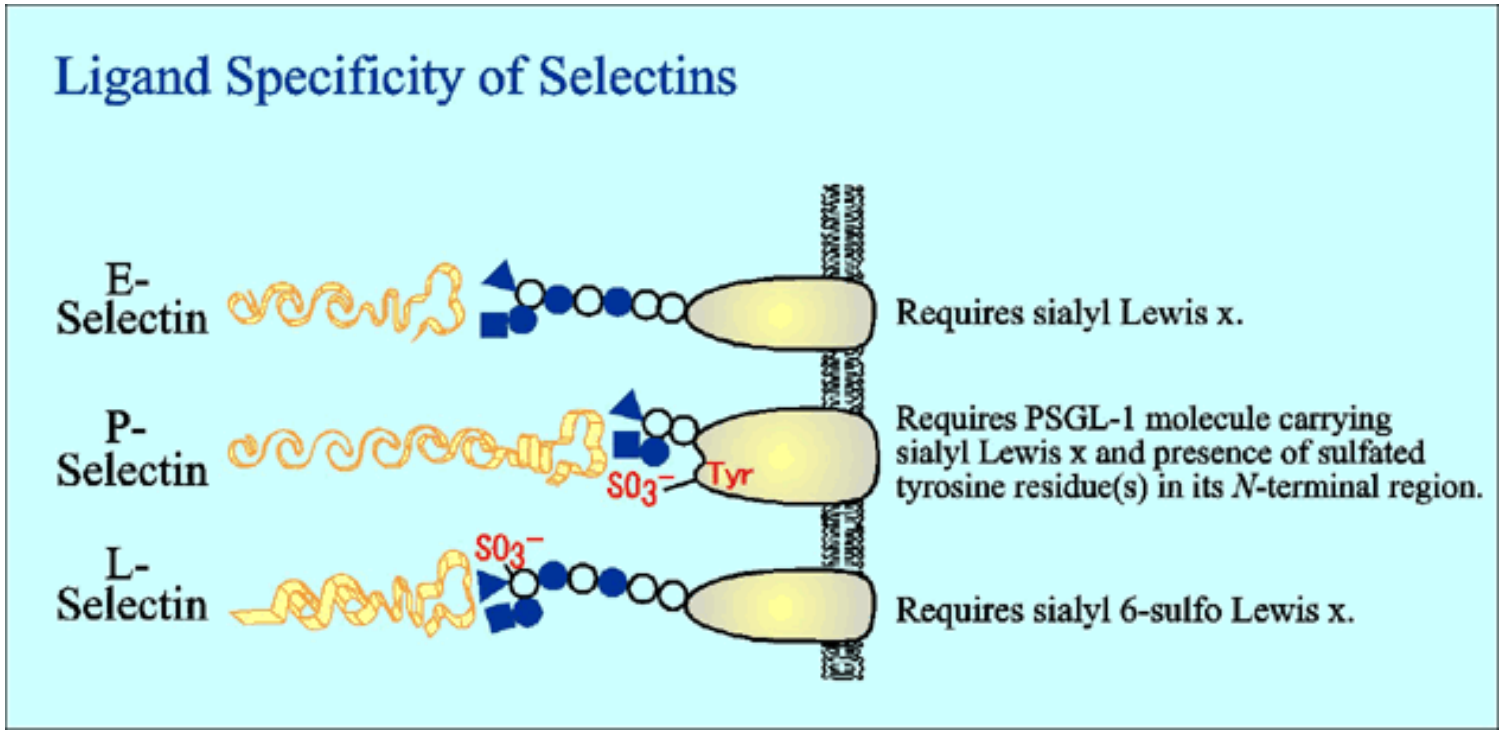


- Cell adhesion
  - Lewis antigen
  - WBC: endothelium
- Self/Non-self recognition
  - Blood groups
  - Toll-like receptor
- Molecular trafficking and clearance
  - Asialoglycoprotein receptors in hepatocytes, Kupffer cells and macrophages
  - RBC aging
- Receptor activation
  - FGF, Wnt, TGF-beta, EGF
- Endocytosis
  - Glut2, EGRR, TGFR endocytosis

Glycan as adhesion molecules



WBC
& endothelial
adhesion



Glycan as blood group antigens

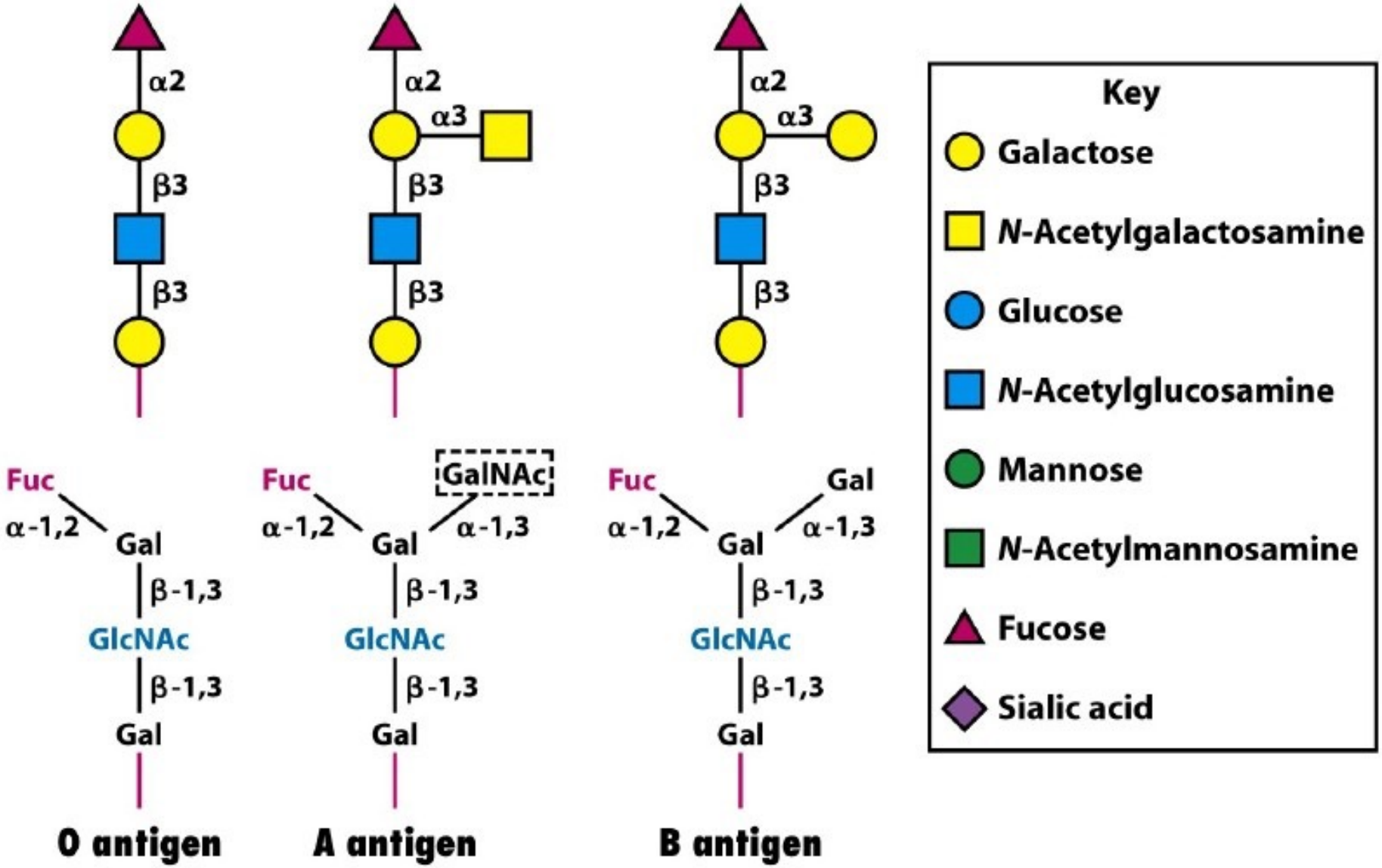
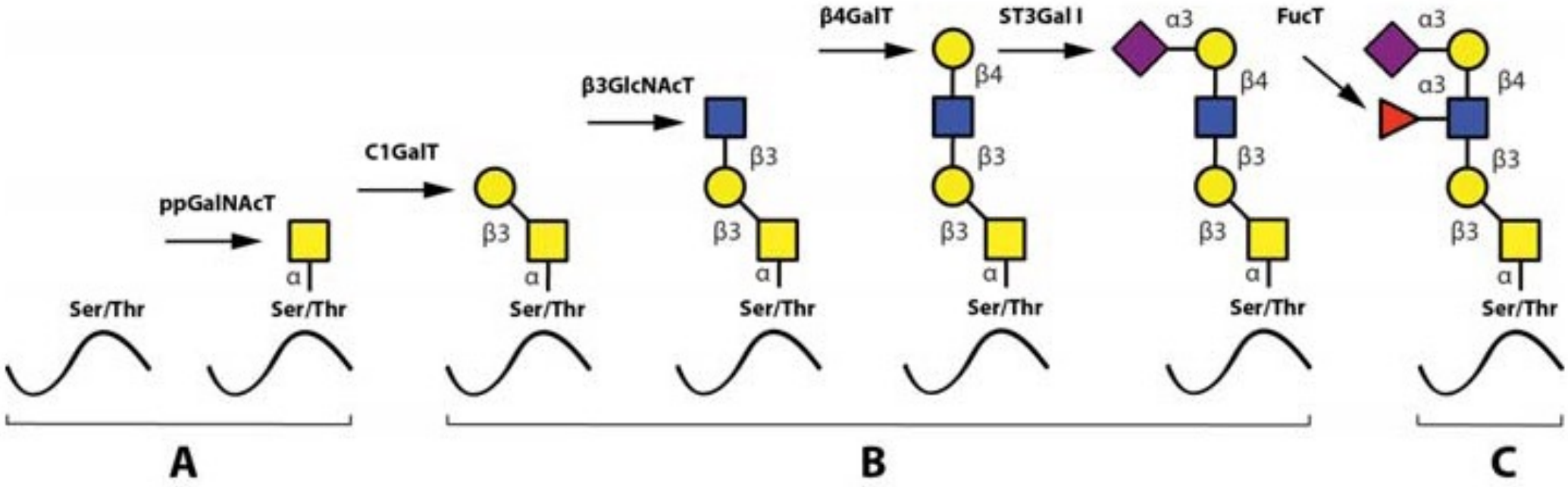


Figure 11-18
Biochemistry, Sixth Edition

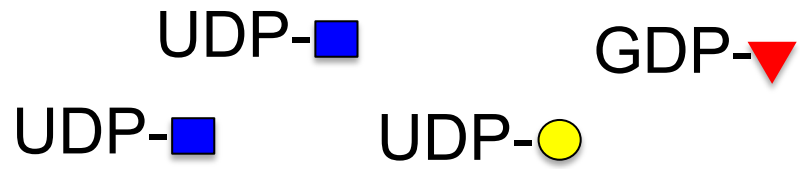
Sequentially adding & removal of monosaccharide to form oligosaccharide in ER & golgi (major)



https://www.glycopedia.eu

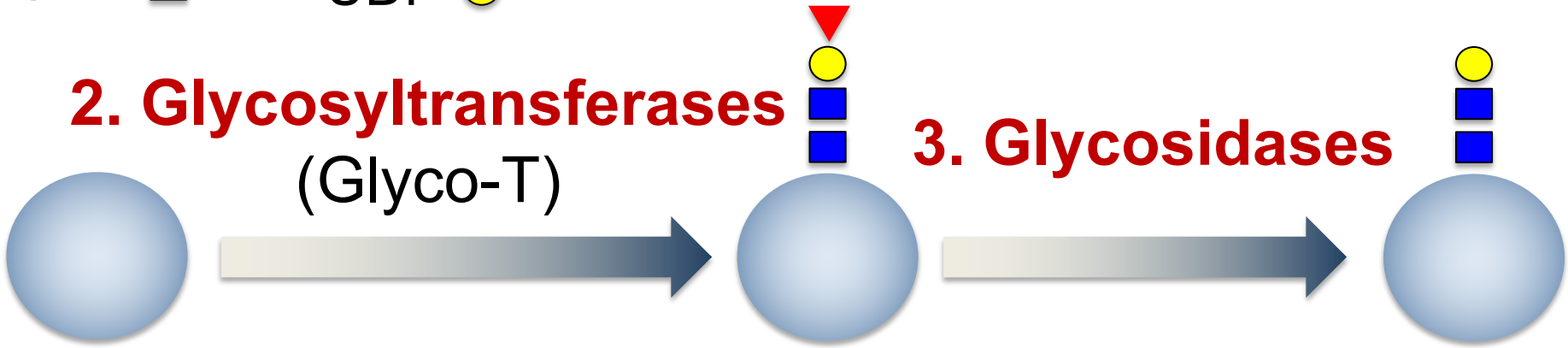
Glycosylation

4. Sugar-donors



2. Glycosyltransferases

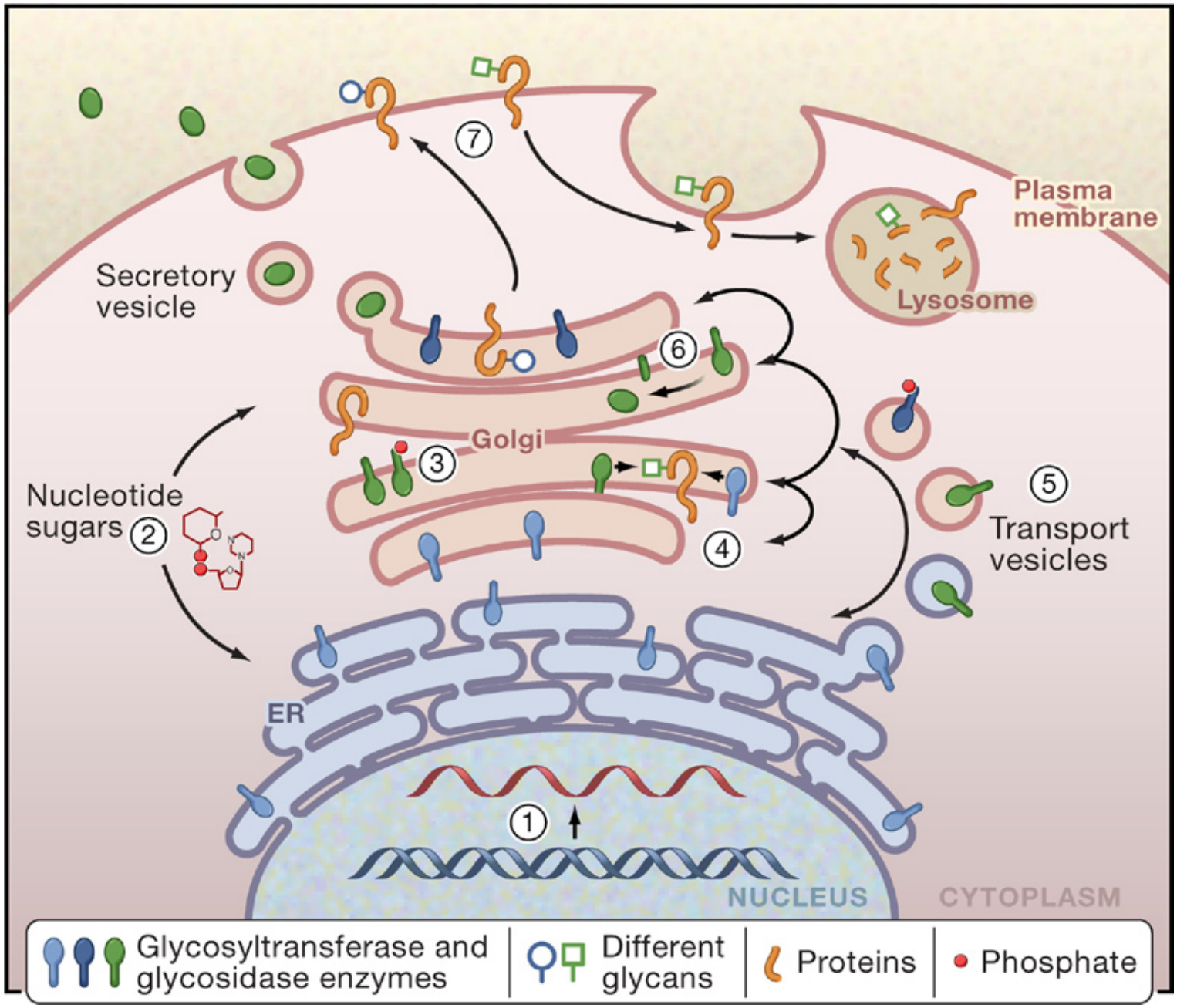
(Glyco-T)



3. Glycosidases

1. Acceptors

(Proteins & Lipids)



Human:

~ 200 Glyco-Ts (add)

ER, Golgi, Cytoplasm (OGT)

~ 80 glycosidases (remove)

ER, Golgi, membrane, lysosome, etc.

Table 2 Glycosyltransferase and glycosidase enzymes.

Enzymes
Glycosyltransferase families
1. Alpha-1,2-glucosyltransferases (ALG10)
2. Alpha-1,3-glucosyltransferases
3. Alpha 1,4-glycosyltransferases
4. Beta-1,3-glucuronyltransferases (B3GAT)
5. Beta 3-glycosyltransferases
6. Beta 4-glycosyltransferases
7. Collagen beta(1-O)galactosyltransferases (COLGALT)
8. Dolichyl D-mannosyl phosphate dependent mannosyltransferases
9. Exostosin glycosyltransferase family (EXT)
10. Fucosyltransferases (FUT)
11. Glucosaminyl (N-acetyl) transferases/xylosyltransferases (GCNT, XYLT)
12. Glycogen phosphorylases (PYG)
13. Glycosyltransferase family 2
14. Glycosyltransferase family 6
15. Glycosyltransferase family 8
16. Glycosyltransferase family 90
17. Glycosyl transferases group 1 domain containing
18. Mannosyl-glycoprotein N-acetylglucosaminyltransferases (MGAT)
19. O-linked N-acetylglucosaminyltransferases
20. Polypeptide N-acetylgalactosaminyltransferases (GALNT)
21. Sialyltransferases
22. STT3 oligosaccharyltransferase catalytic subunits (STT3)
23. UDP-glucose ceramide glucosyltransferases
24. UDP-glucose glycoprotein glucosyltransferases (UGGT)
25. UDP glucuronosyltransferases (UGT)
26. UDP-N-acetylglucosaminyltransferase subunits

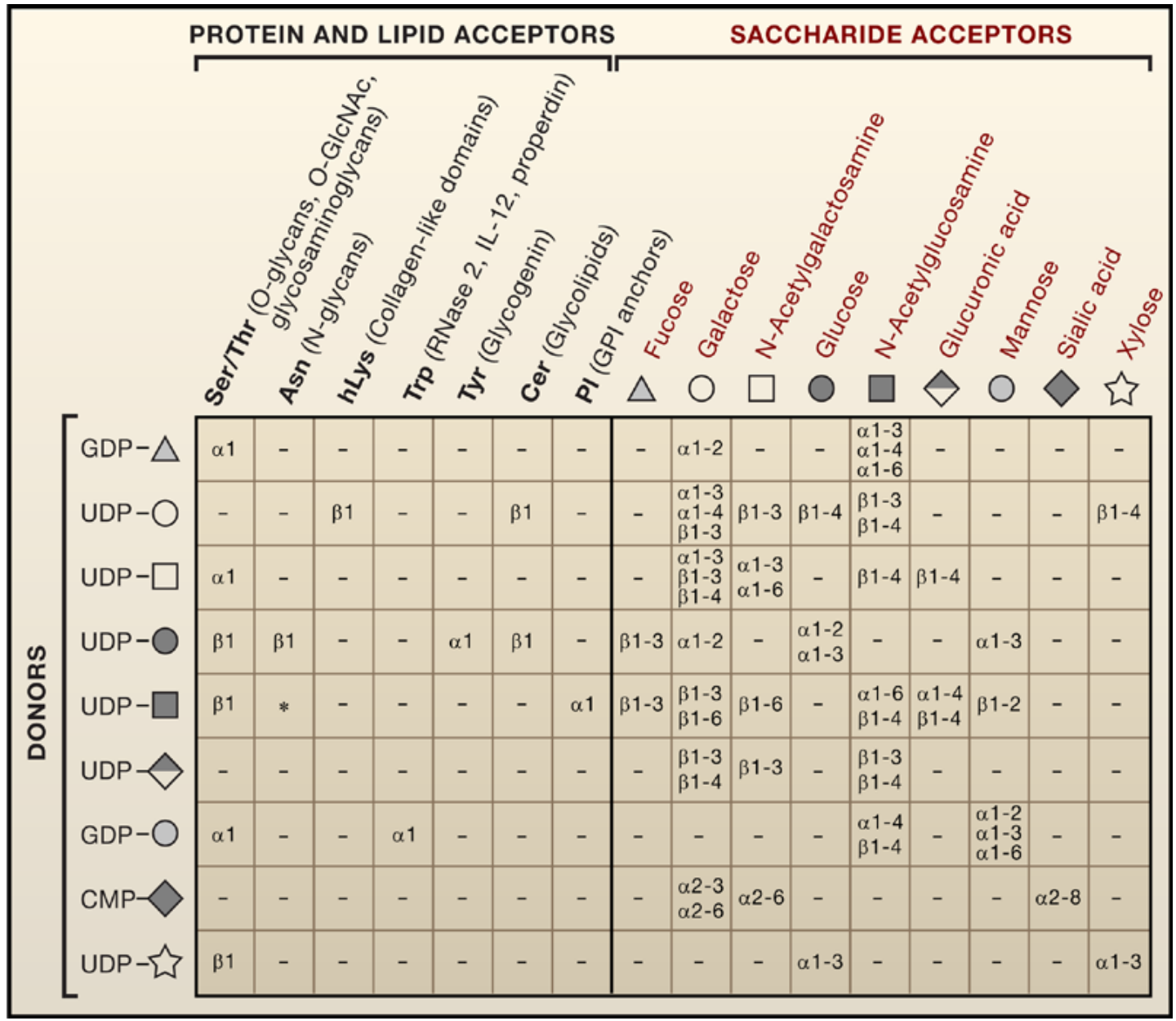
Human Glyco-Ts and glycosidases

Glycosidase (glycoside hydrolase) families

1. Alpha-L-fucosidases (FUCA)
2. Amylases alpha (AMY)
3. Chitinases (CHI)
4. Galactosidases alpha (GLA)
5. Galactosidases beta (GLB)
6. Glycoside hydrolase family 1
7. Glycoside hydrolase family 31
8. Heparanases (HPSE)
9. Hexosaminidases (HEX)
10. Hyaluronidases (HYAL)
11. Lysozymes (LY)
12. Mannosidases
13. Neuraminidases (NEU)

Silsirivanit A. Advance in Clinical Chemistry, 2019

Nucleotide sugar-donors and specific acceptors

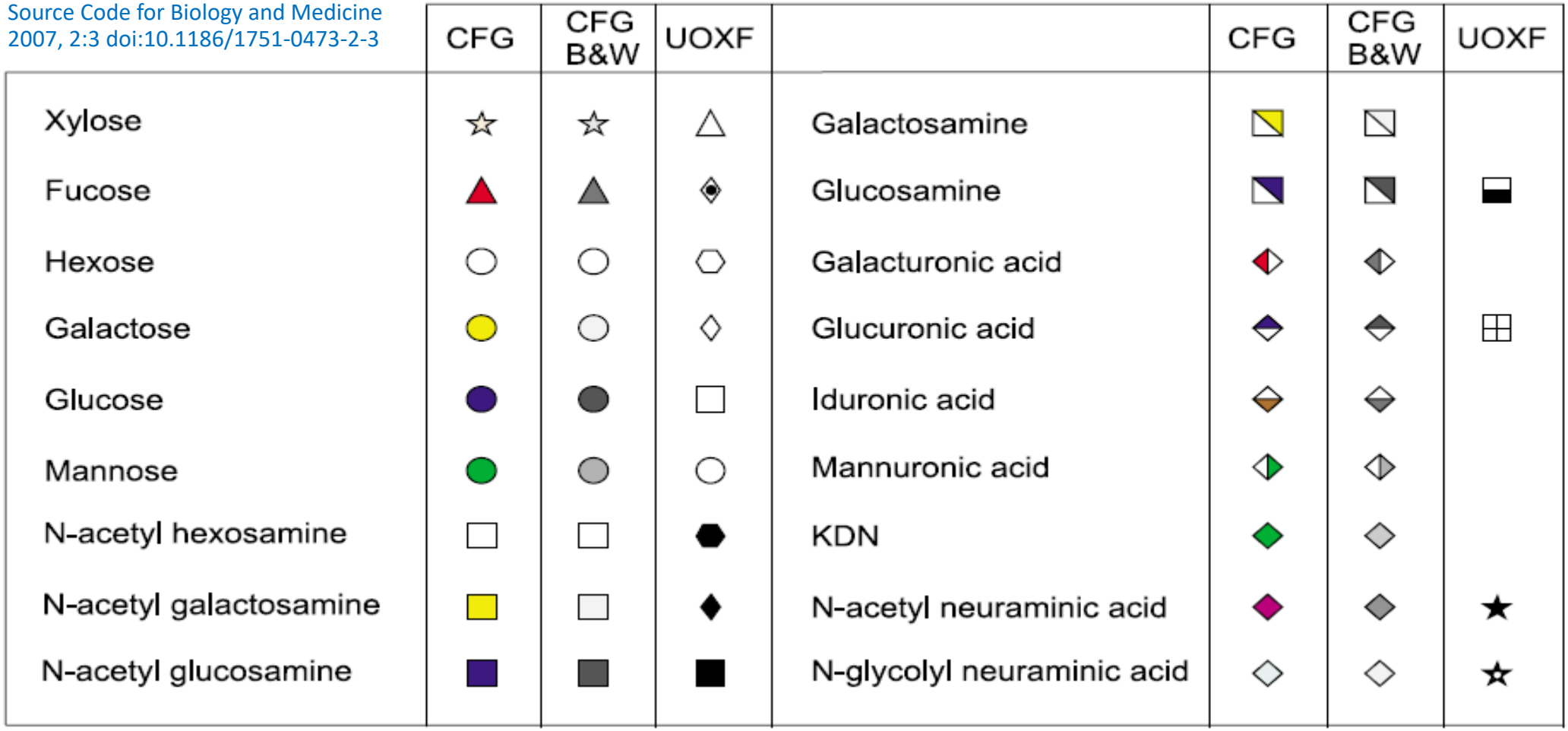


DONORS	PROTEIN AND LIPID ACCEPTORS							SACCHARIDE ACCEPTORS								
	Ser/Thr (O-glycans, O-GlcNAc, glycosaminoglycans)	**Asn** (N-glycans)	**hLys** (Collagen-like domains)	**Trp** (RNase 2, IL-12, properdin)	**Tyr** (Glycogenin)	**Cer** (Glycolipids)	**PI** (GPI anchors)	Fucose	Galactose	N-Acetylgalactosamine	Glucose	N-Acetylglucosamine	Glucuronic acid	Mannose	Sialic acid	Xylose
GDP–△	α1	–	–	–	–	–	–	–	α1-2	–	–	α1-3 α1-4 α1-6	–	–	–	–
UDP–○	–	–	β1	–	–	β1	–	–	α1-3 α1-4 β1-3	β1-3	β1-4	β1-3 β1-4	–	–	–	β1-4
UDP–□	α1	–	–	–	–	–	–	–	α1-3 β1-3 β1-4	α1-3 α1-6	–	β1-4	β1-4	–	–	–
UDP–●	β1	β1	–	–	α1	β1	–	β1-3	α1-2	–	α1-2 α1-3	–	–	α1-3	–	–
UDP–■	β1	*	–	–	–	–	α1	β1-3	β1-3 β1-6	β1-6	–	α1-6 β1-4	α1-4 β1-4	β1-2	–	–
UDP–◆	–	–	–	–	–	–	–	–	β1-3 β1-4	β1-3	–	β1-3 β1-4	–	–	–	–
GDP–◐	α1	–	–	α1	–	–	–	–	–	–	–	α1-4 β1-4	–	α1-2 α1-3 α1-6	–	–
CMP–◆	–	–	–	–	–	–	–	–	α2-3 α2-6	α2-6	–	–	–	–	α2-8	–
UDP–☆	β1	–	–	–	–	–	–	–	–	–	α1-3	–	–	–	–	α1-3

Ohtsubo, Cell, 2006

Symbolic representation of monosaccharides

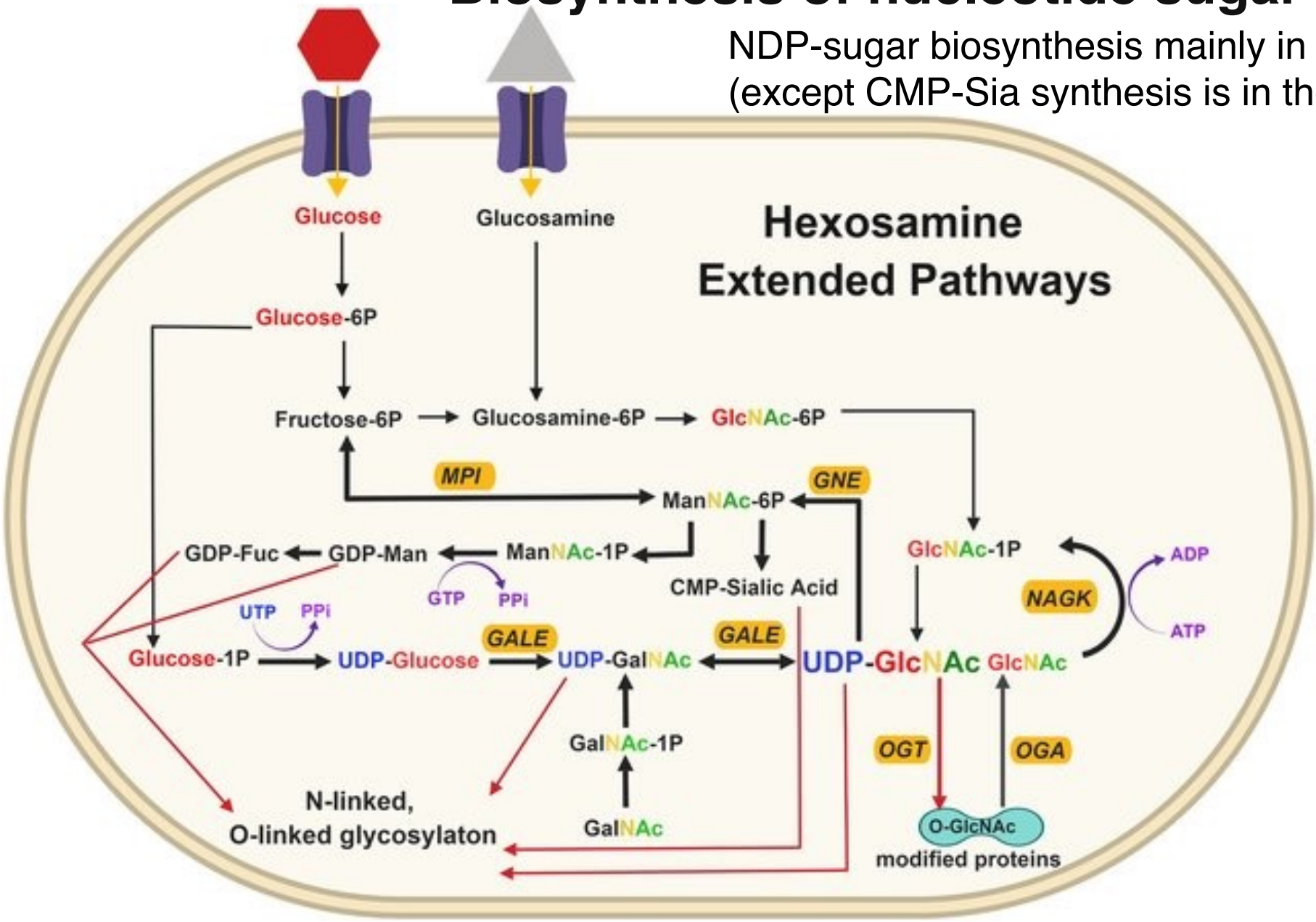
Source Code for Biology and Medicine
2007, 2:3 doi:10.1186/1751-0473-2-3



Symbolic representation of monosaccharides with geometric shapes as described in the notations used by the Consortium for Functional Glycomics (CFG) and the Oxford Glycobiology Institute (UOXF).

Biosynthesis of nucleotide sugar donors

NDP-sugar biosynthesis mainly in cytoplasm
(except CMP-Sia synthesis is in the nucleus).



Akella *et al* BMC Biology, 2019

Glycosidic linkages in oliogo/polysaccharides

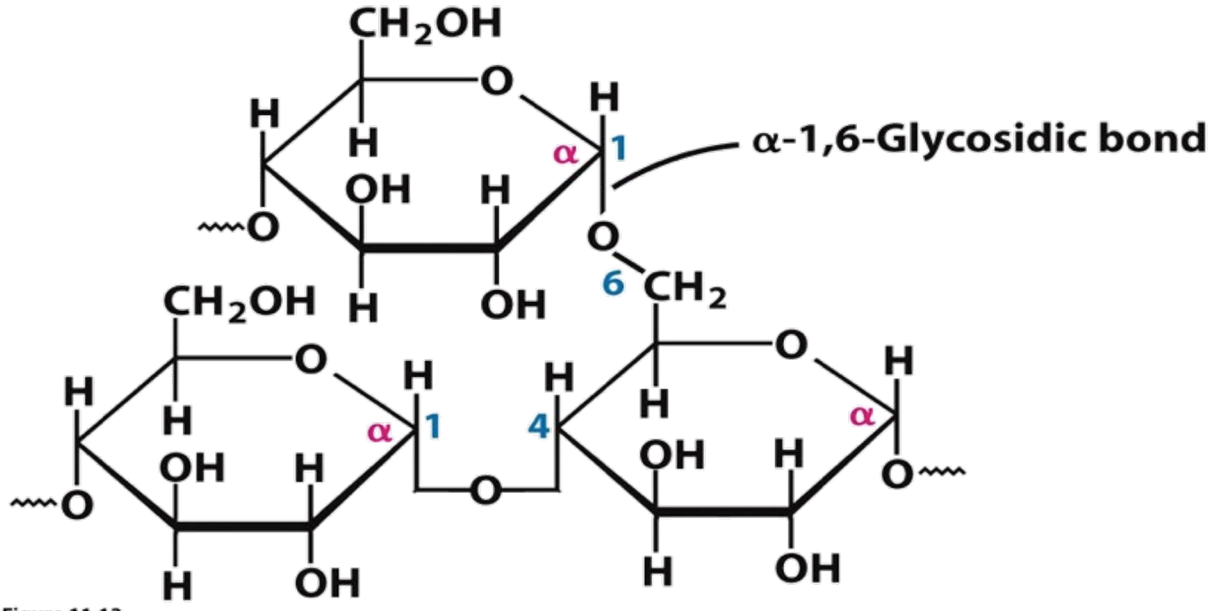
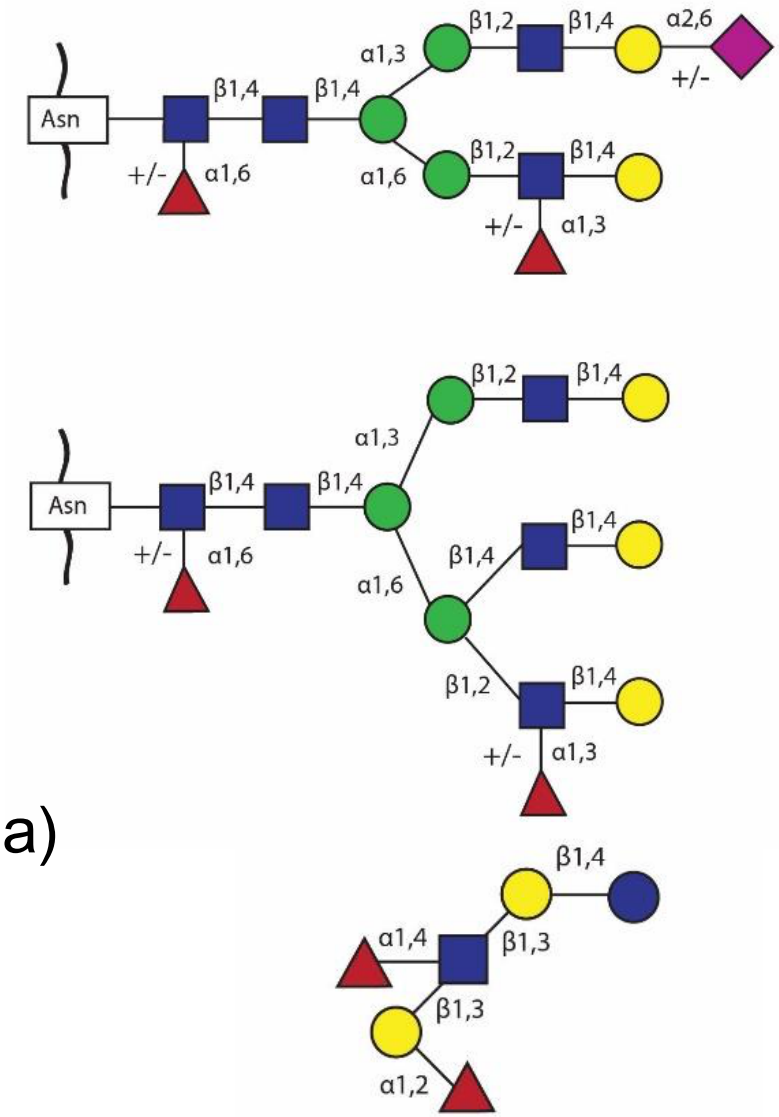


Figure 11.13
Biochemistry, Seventh Edition



Lacto-N-difucosylhexaose

Monosaccharide
Structure of monosaccharides (beta, alpha)
Position of linkage (C-2, -3, C-4, C-6)

↓

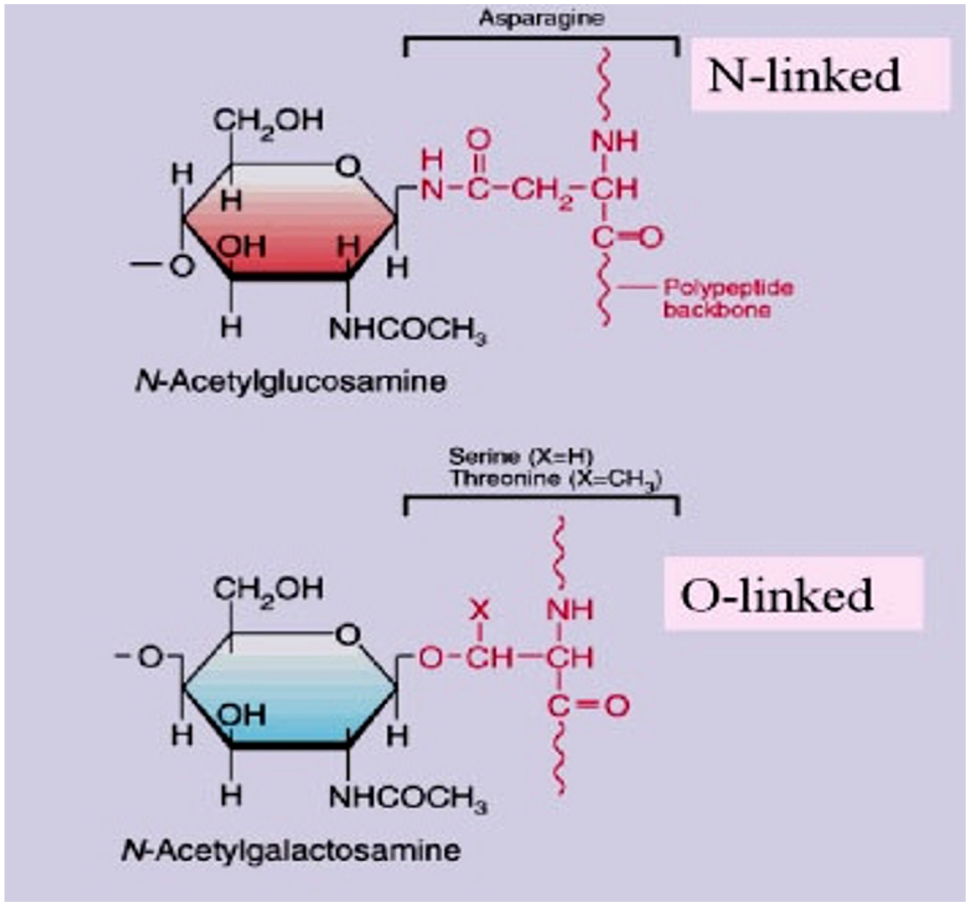
High Complexity
No template

Orczyk-Pawiłowicz M, Nutrients 2020

Protein Glycosylation

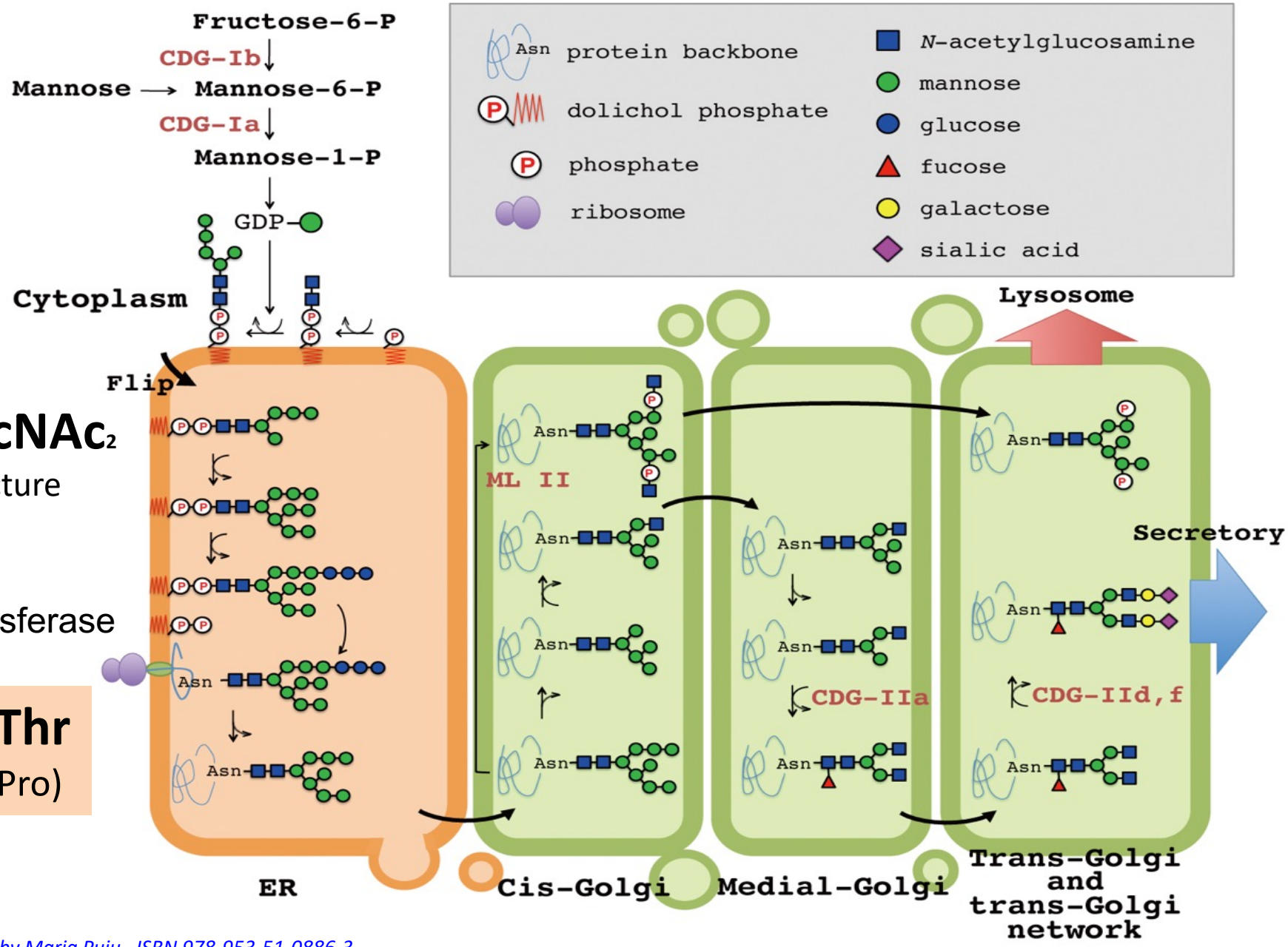
Protein glycosylation encompasses:

1) N-glycans (N-linked glycosylation): Asn, Gln (rare)
2) O-glycans (O-linked glycosylation): Ser,The, Pro-OH+Tyr (rare)



Asn : N-glycosidic bond
1st sugar: GlcNAc
Ser/The : O-glycosidic bond
1st sugar: Any
Tryp (rare) : C-liked mannosylation

N-linked glycosylation: from ER through Golgi



$Glc_3Man_9GlcNAc_2$

14-sugar structure

OST

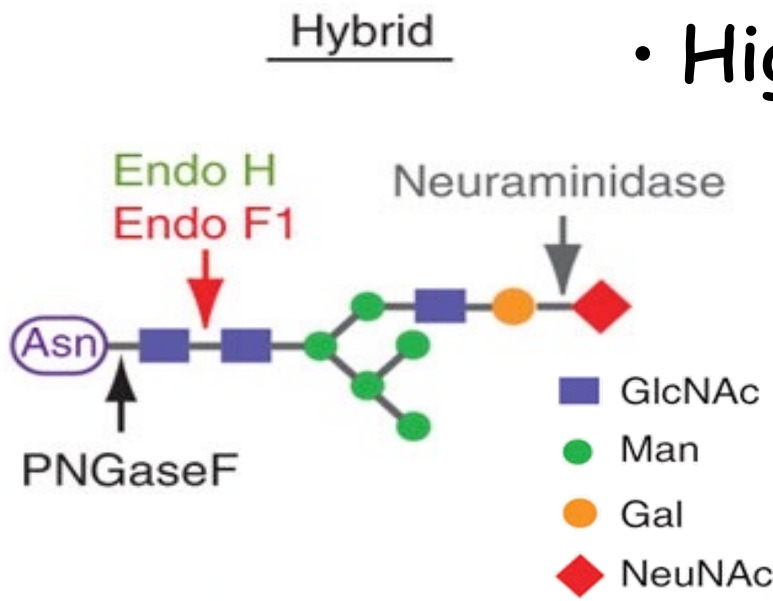
oligosaccharyltransferase

Asn-X-Ser/Thr

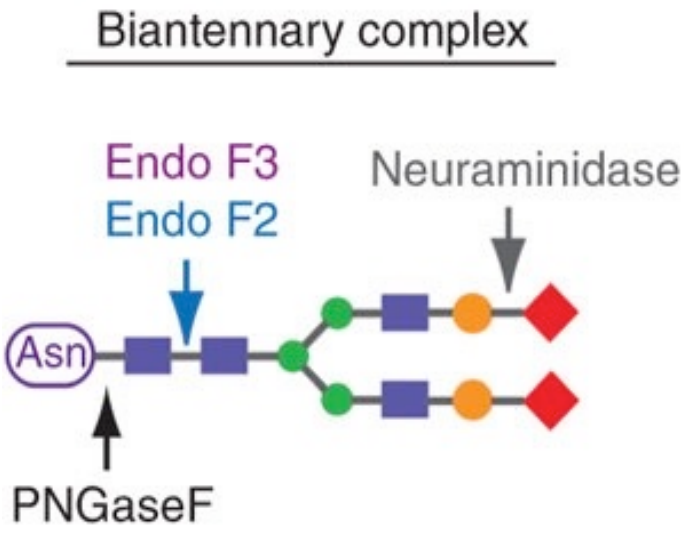
(X = AA except Pro)

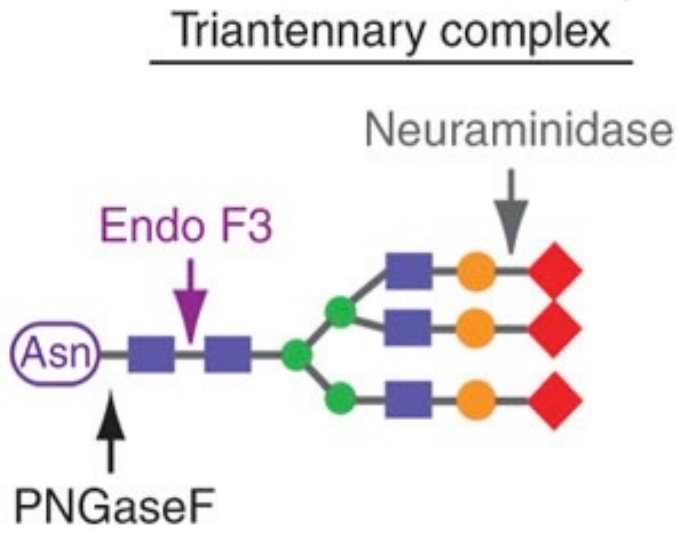
"Genetic Disorders", *book edited by Maria Puiu , ISBN 978-953-51-0886-3*

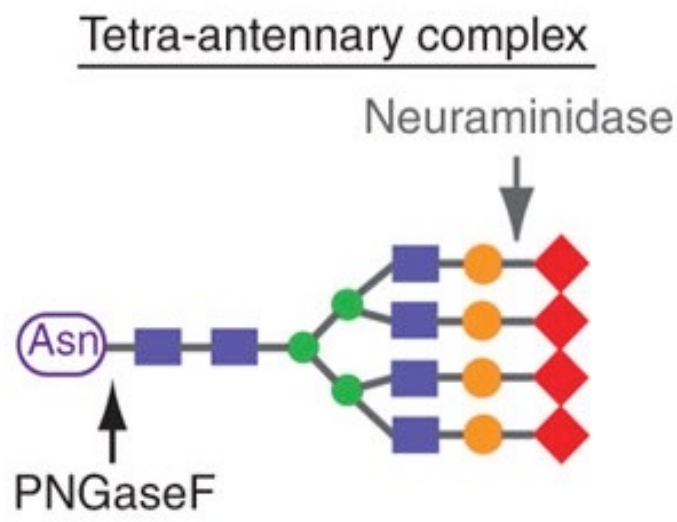
N-linked glycans



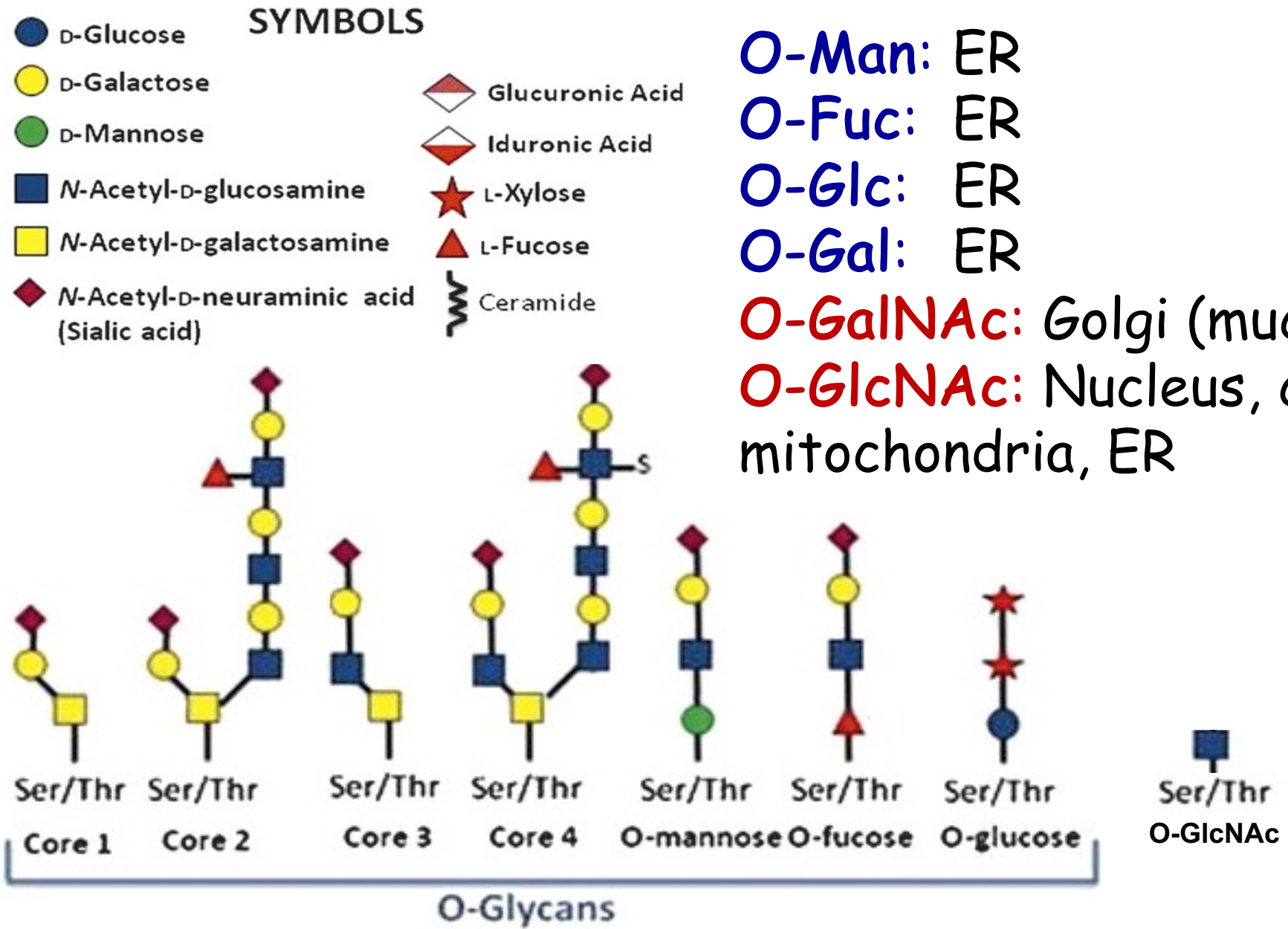
- High mannose type
- Hybrid type
- Complex type







O-linked glycan



O-Man: ER

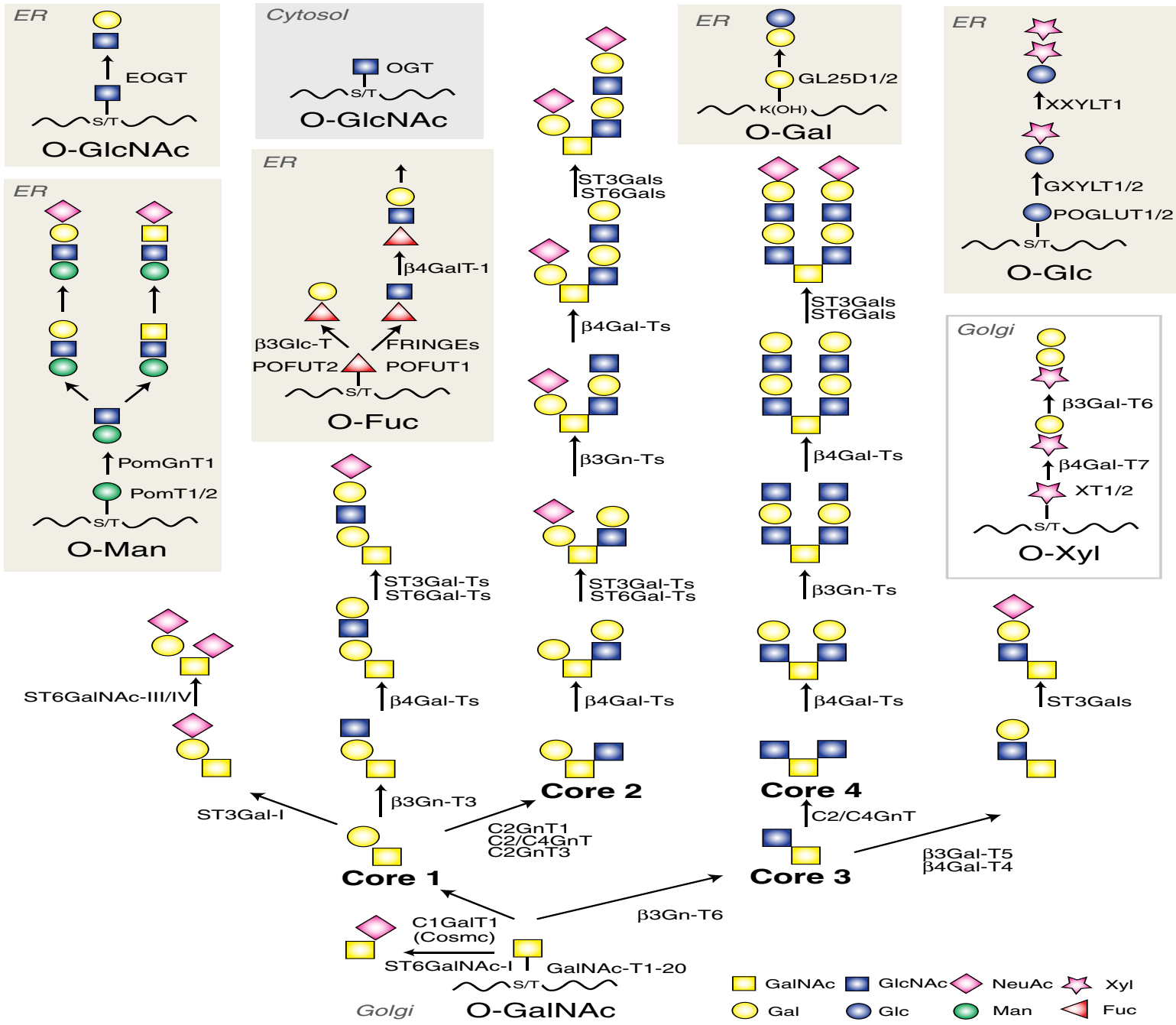
O-Fuc: ER

O-Glc: ER

O-Gal: ER

O-GalNAc: Golgi (mucin type)

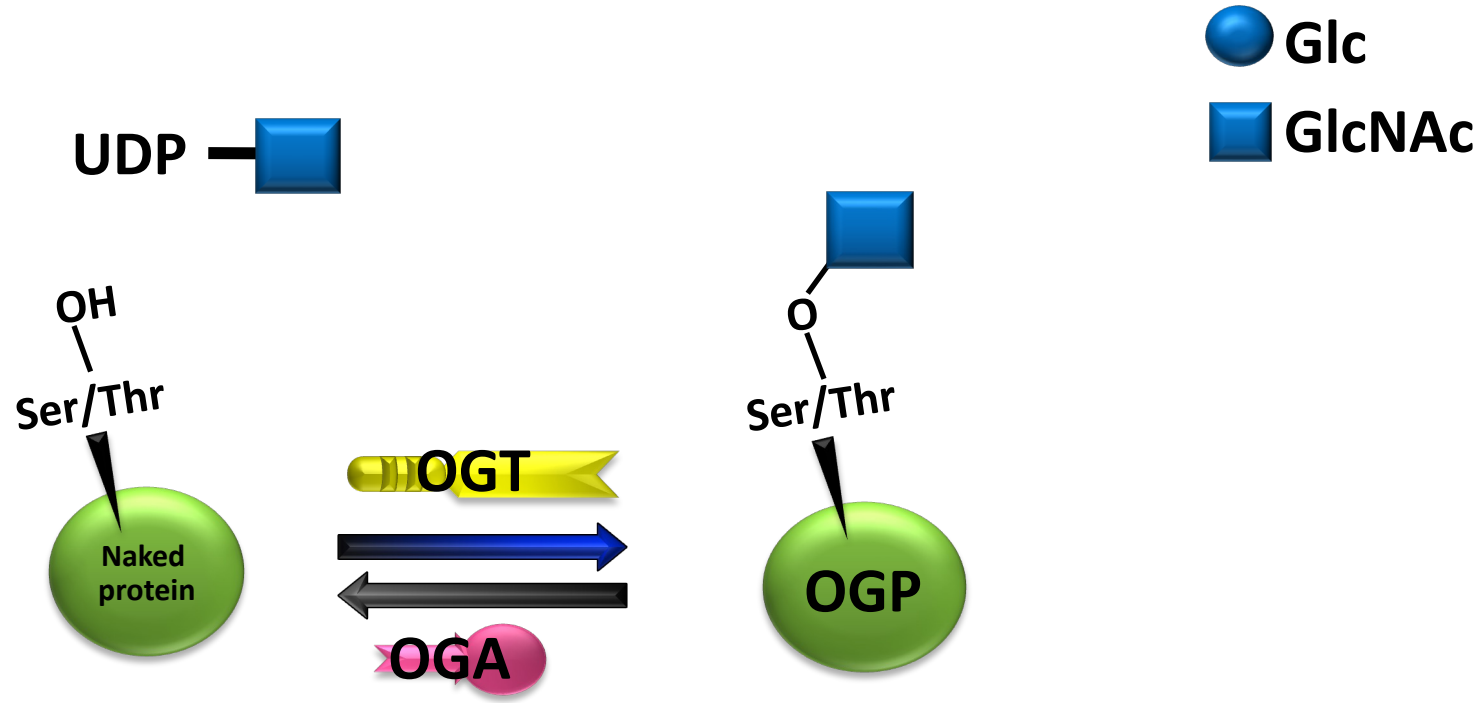
O-GlcNAc: Nucleus, cytoplasm, mitochondria, ER



Sialyl-Ts
Fuc-Ts
Xyl-Ts

Gal-Ts
GlcNAc-Ts
Man-Ts
GalNAc-Ts

O-GlcNAc modification



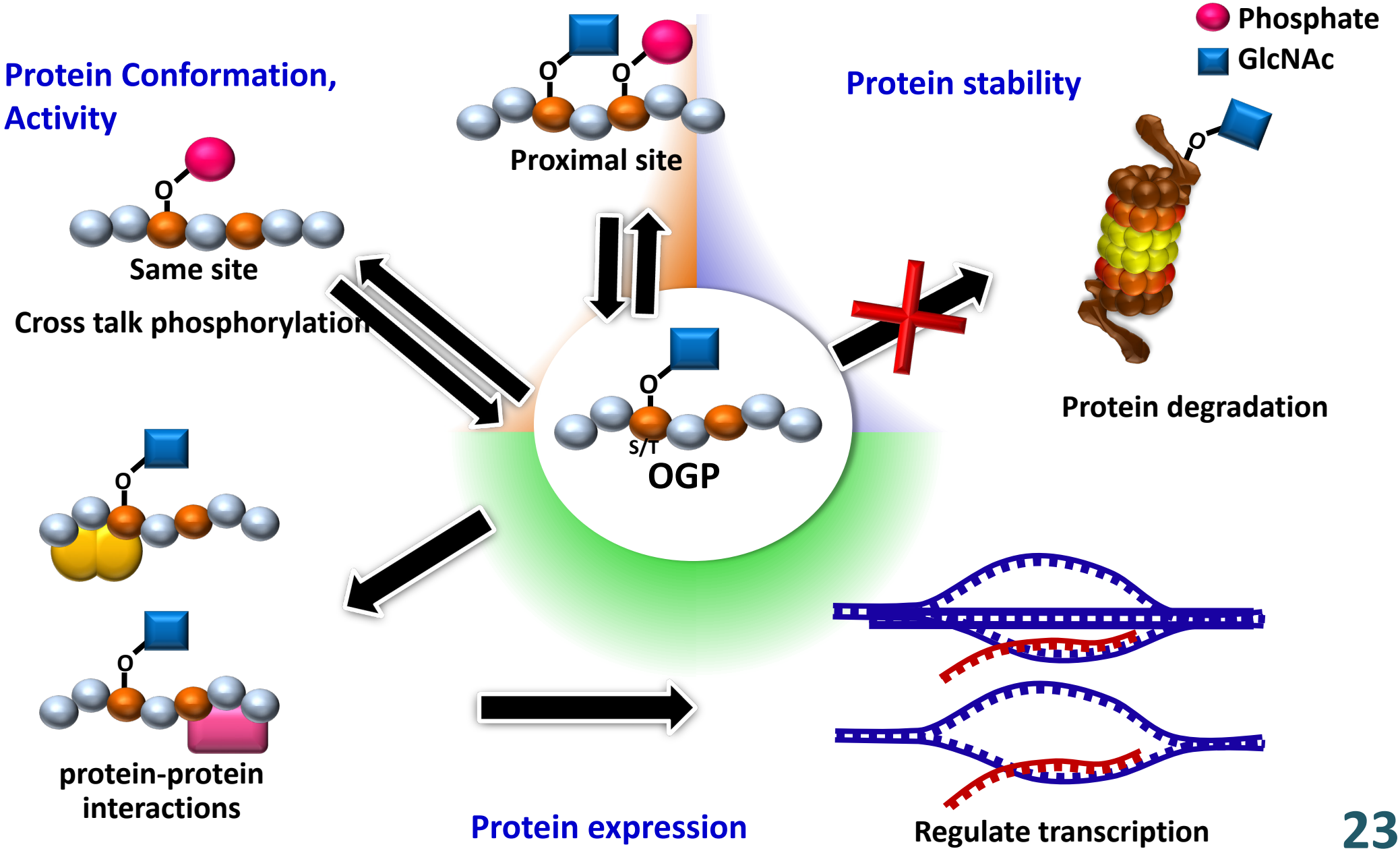
O-GlcNAc cycling

OGT: O-linked β-N-acetylglucosaminyl transferase

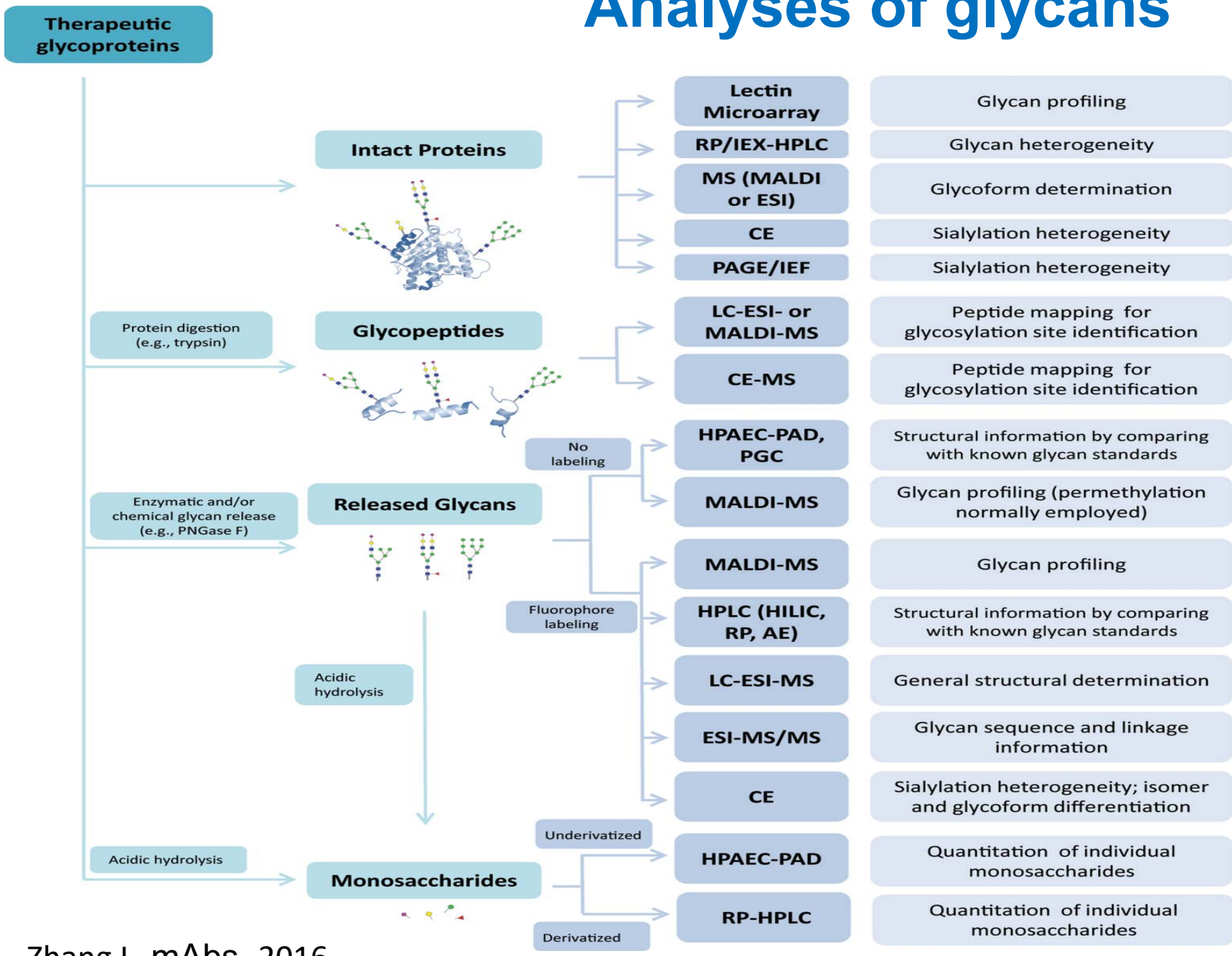
OGA: β-N-acetylglucosaminidase(O-GlcNAcase)

OGP: O-GlcNAcylated protein

Biological roles of O-GlcNAcylation



Analyses of glycans

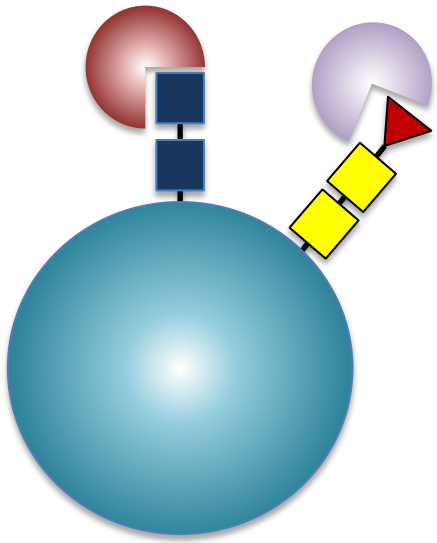


Lectin-based

MS/MS

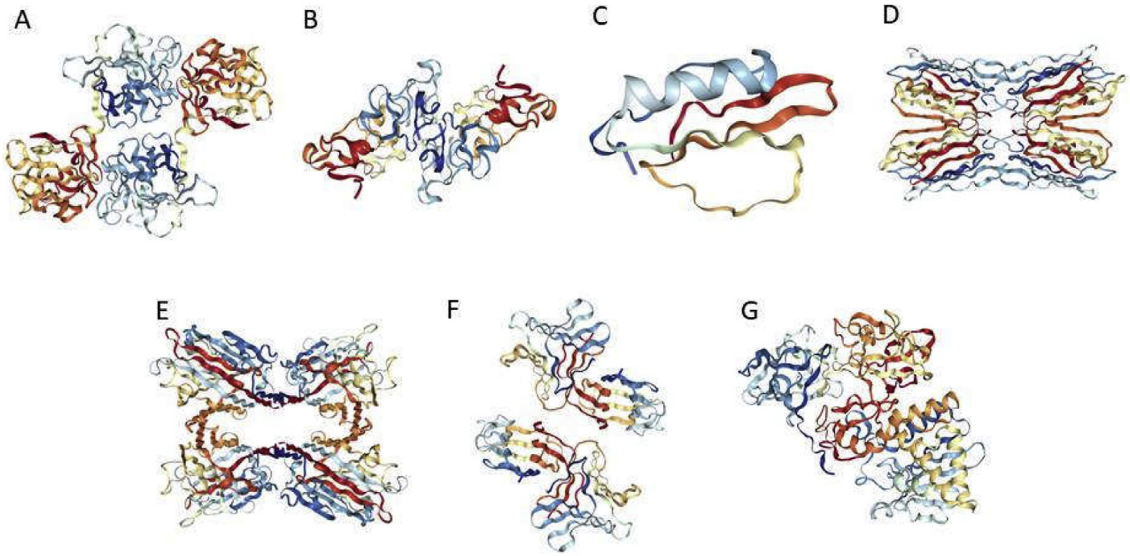
Zhang L. mAbs, 2016

Analysis of glycans and glycoproteins



Lectins

- Sugar-binding proteins
- Binds specific sugar structure
- (like antibody)
- Found in animals & plants
- Applications in science, medicine and technology



Crystal structures of
(A)amaranthin lectin
(B)WGA
(C)pumpkin lectin
(D)jacalin lectin
(E)SBL
(F)Galanthus nivalis agglutinin (GNA)
(G) ricin

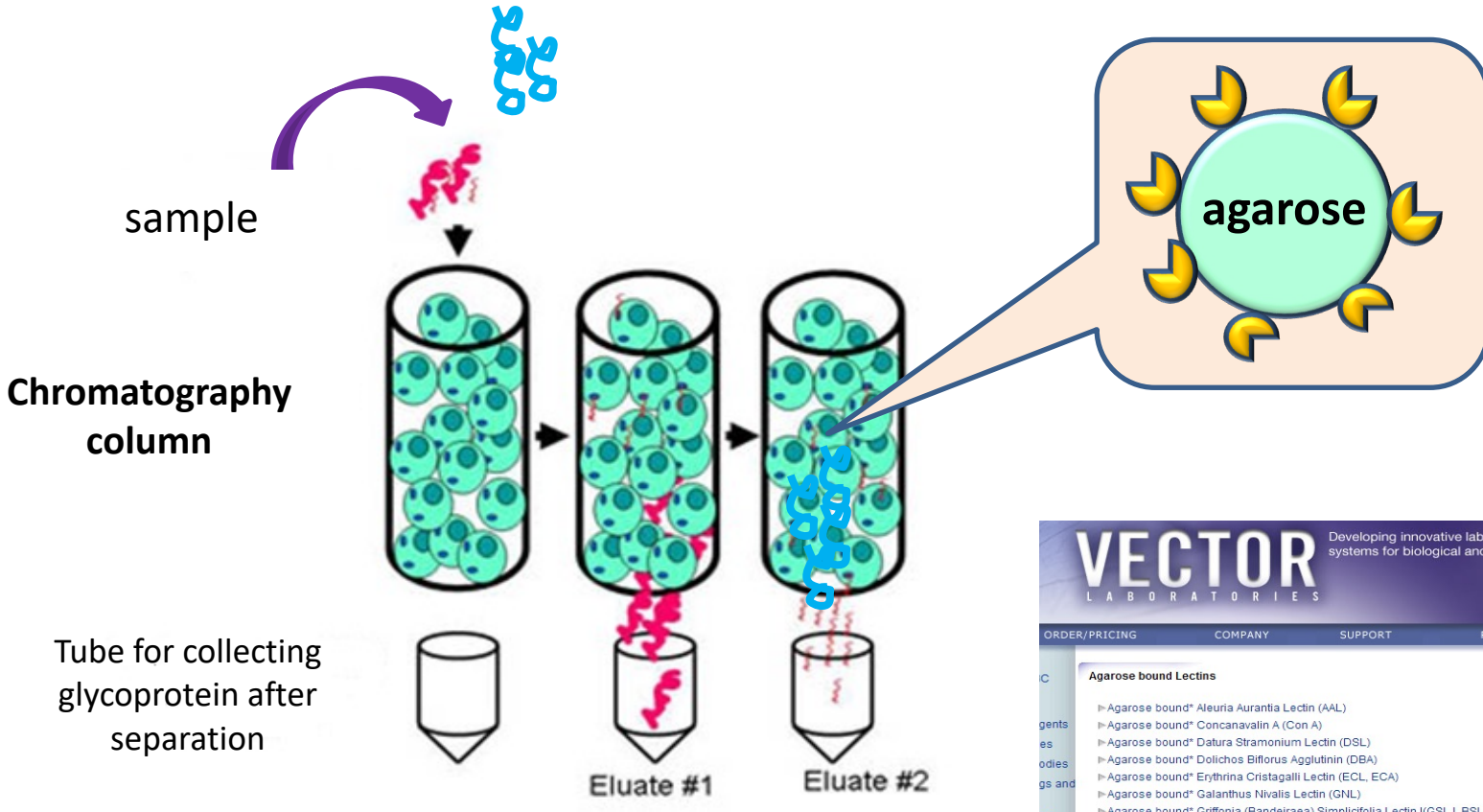
Plant lectin as a tool for analysis of glycans

Groups	Lectins	Abbreviations	Preferred Sugar Specificity
Fucose	*Ulex europaeus I*	UEA I	αFuc
Mannose/Glucose	Concanavalin A	Con A	αMan, αGlc
	Lens culinaris	LCA, LcH	αMan, αGlc
	Pisum sativum	PSA	αMan, αGlc
GlcNAc	*Griffonia (Bandeiraea) simplicifolia II*	GSL II	α or βGlcNAc
	Wheat Germ	WGA	GlcNAc
	Succinylated Wheat Germ	sWGA	GlcNAc
	Datura stramonium	DSL	(GlcNAc)2-4
	Lycopersicon esculentum	LEL, TL	(GlcNAc)2-4
	Solanum tuberosum	STL, PL	(GlcNAc)2-4
Gal/GalNAc	*Ricinus communis I*	RCA I	Gal
	Griffonia (Bandeiraea) simplicifolia I	GSL I	αGal, αGalNAc
	Dolichos biflorus	DBA	αGalNAc
	Sophora japonica	SJA	βGalNAc
	Soybean	SBA	α>βGalNAc
	Vicia villosa	VVL, VVA	GalNAc
	Wistera floribunda	WFA, WFL	GalNAc
	Jacalin	Jacalin	Galβ3GalNAc
	Peanut	PNA	Galβ3GalNAc
	Erythrina cristagalli	ECL, ECA	Galβ4GlcNAc
Sialic acids	*Maackia amurensis*	MAL II	A2,3Sia
	Sambacus nigra	SNA	α2,6Sia
Complex glycan	*Phaseolus vulgaris*	PHA-E	Galβ4GlcNAcβ2Manα6 (GlcNAcβ4) (GlcNAcβ4Manα3) Manβ4
	Phaseolus vulgaris	PHA-L	Galβ4GlcNAcβ6(GlcNAc β2Manα3)Manα3

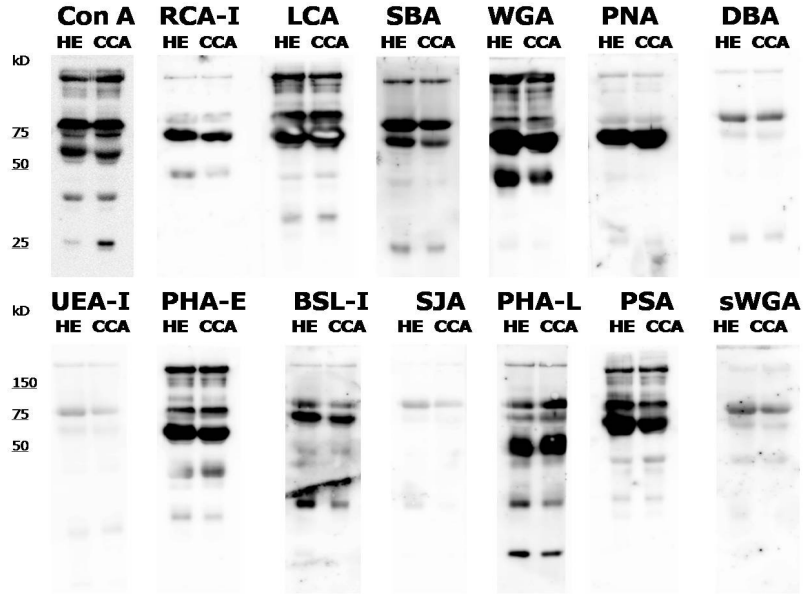
Lectin-based techniques

1. Lectin affinity chromatography
2. Lectin blotting
3. Lectin microarray
4. Ab-Lectin micro array
5. Lectin-captured ELISA
6. Lectin histo/cytochemistry staining
7. Etc.

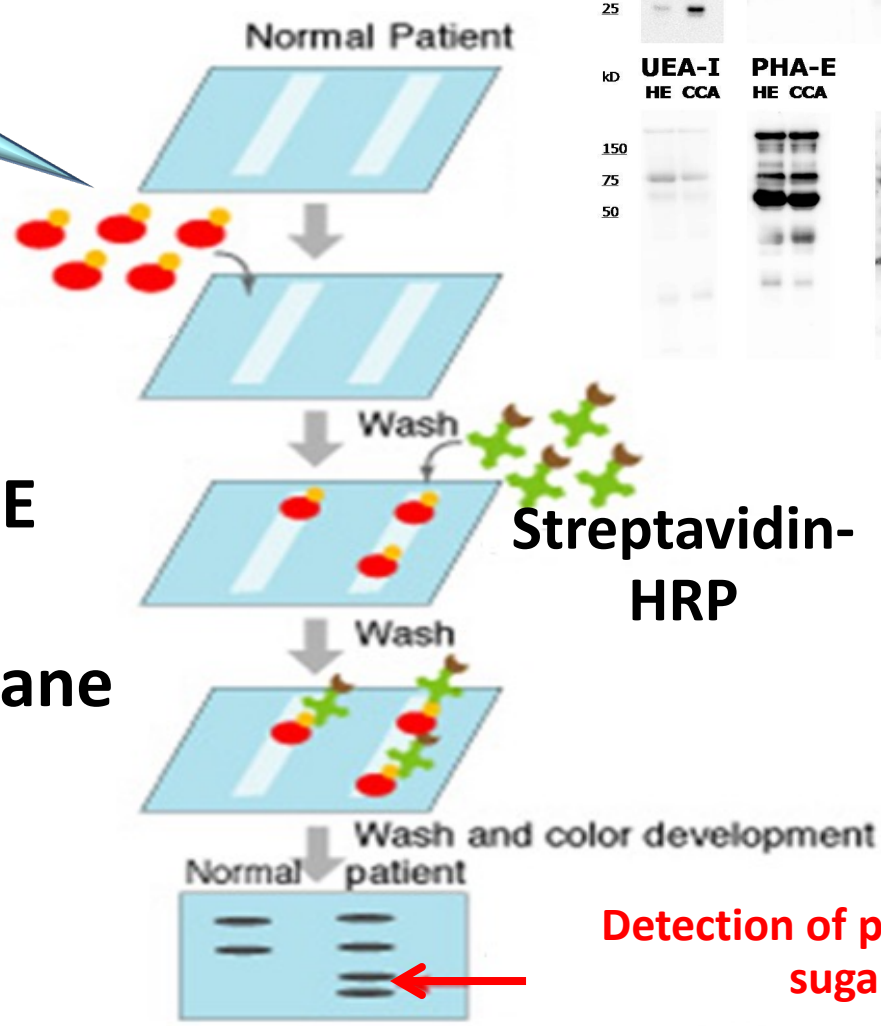
Lectin affinity chromatography



Lectin blotting



Biotin-conjugated lectin



Streptavidin-HRP

SDS-PAGE/2D-PAGE

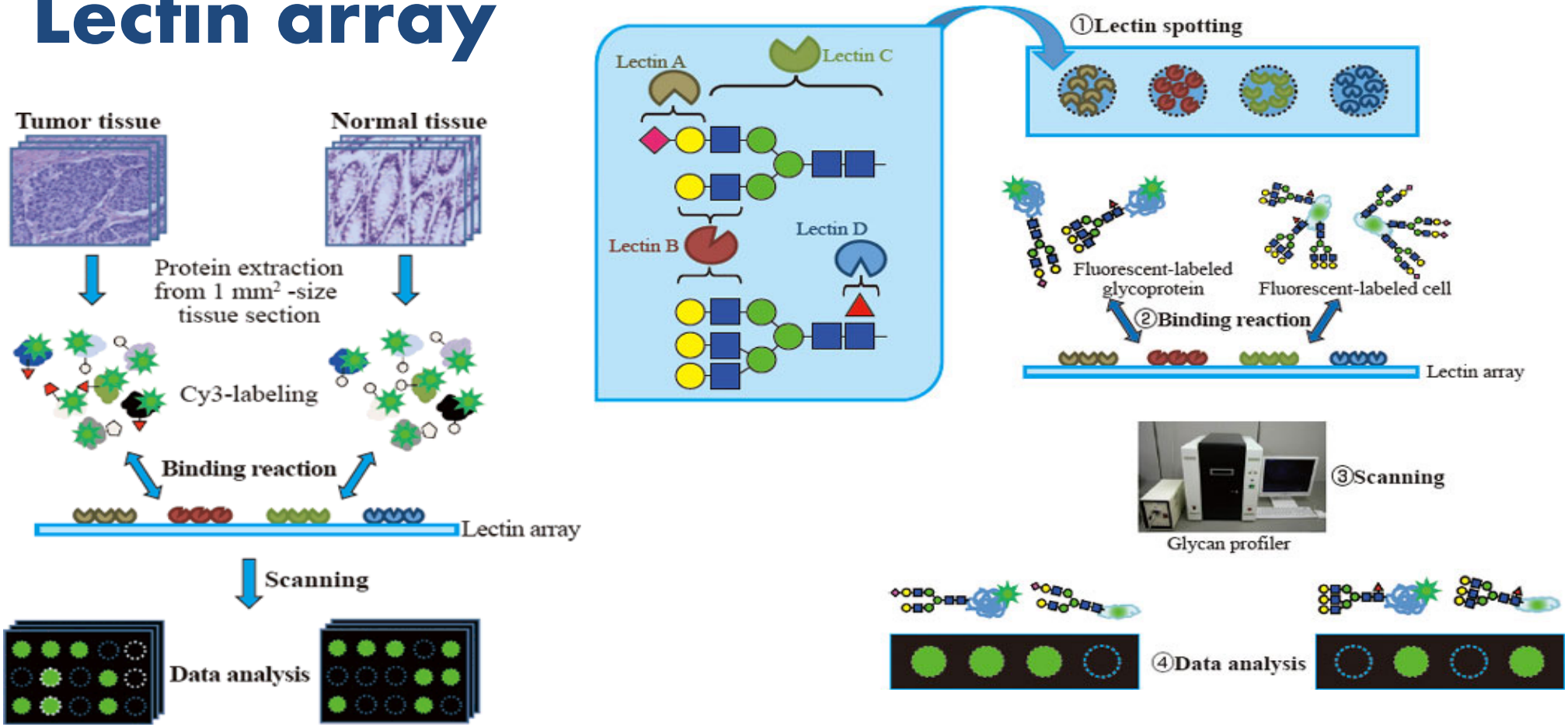
Blotting to membrane

Probe with Lectin

Detection of patient specific chain sugar molecule

https://ruo.mbl.co.jp/jutaku/english/sugar_chain_marker.html

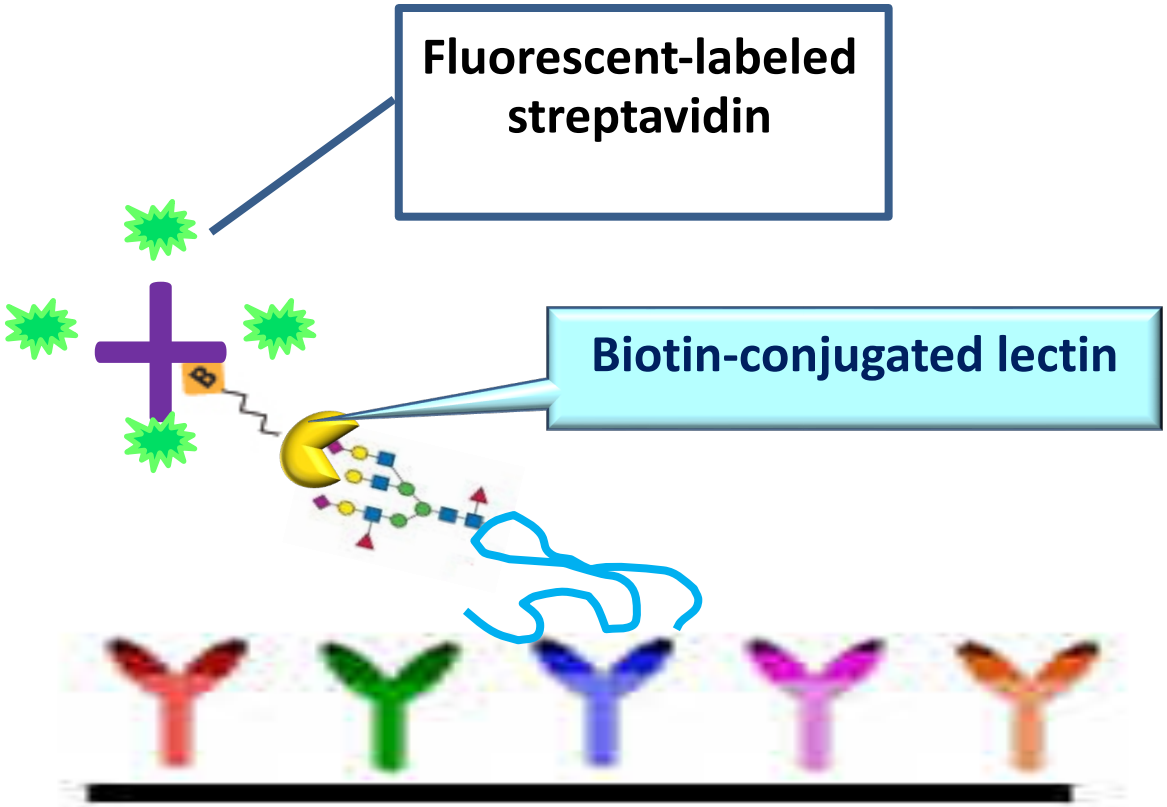
Lectin array

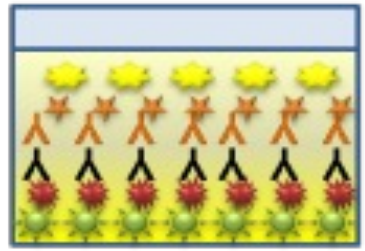


Lectin array is a practical approach to profile glycans expressed on proteins and cells by means of lectins, as decoder molecules, each which shows different sugar-binding specificity. Thus, distinct sugar-binding patterns will be obtained for different cells and glycoproteins.

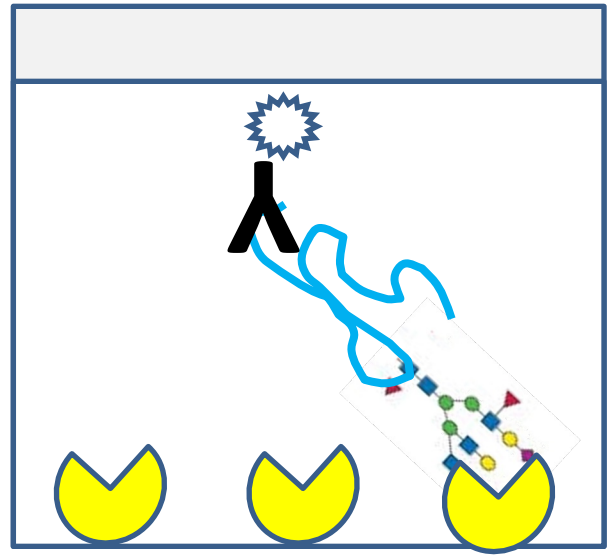
http://www.aist.go.jp/aist_e/aist_today/2009_31/feature/feature_03.html

Lectin-antibody array





Lectin -captured ELISA



Lectin-histo/cytochemistry staining

HRP-streptavidin