Introduction to Glycobiology

Date	Subject	Discussion leader
Apr 20	The Glycobiology Landscape: Sugars as Language	Ron Schnaar
Apr 21	Structures & Functions of Glycoproteins	Natasha Zachara
Apr 22	Structures & Functions of Hyaluronan & Proteoglycans	Ron Midura
Apr 23	Structures & Functions of Glycolipids	Ron Schnaar
Apr 24	Protein-Glycan Recognition	Ron Schnaar
Apr 27	Glycan Binding Protein Functions	Natasha Zachara

Faculty

Ron Schnaar, Johns Hopkins <schnaar@jhu.edu>
Natasha Zachara, Johns Hopkins <nzachara@jhmi.edu>
Ron Midura, Cleveland Clinic Foundation <midurar@ccf.org>

JOHNS HOPKINS MEDICINE

Cleveland Clinic Lerner Research Institute

Logistics

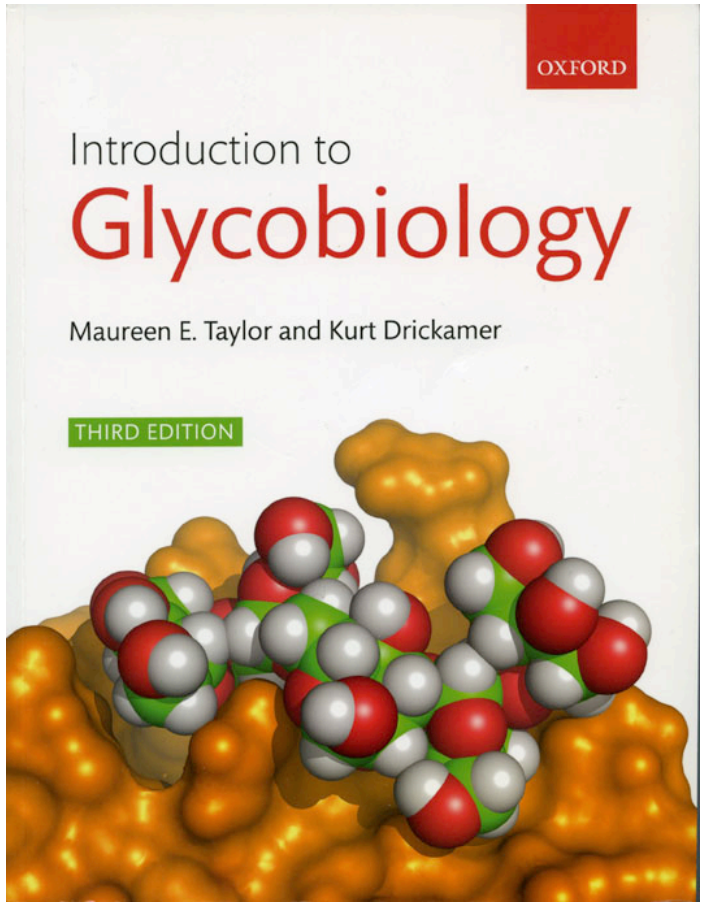
GoToMeeting link (primary): https://www.gotomeet.me/RonaldSchnaar

Zoom *EMERGENCY* link (backup):
https://jhjhm.zoom.us/j/96923102132?pwd=SURoZUpXUHpaOUhFSkhvUzc0NEJhQT09
Password: Neu5Ac

DropBox course materials:
https://www.dropbox.com/sh/c4urzf3m2z0p2vk/AABqEZ1E0D25IbUBX-0yc5LDa?dl=0
Password: Neu5Ac

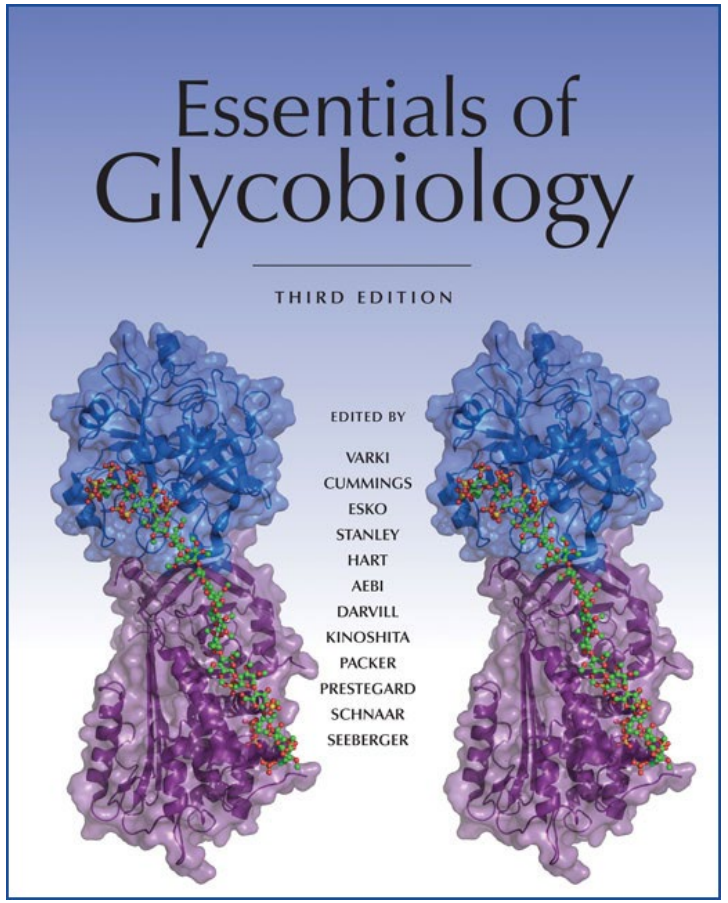
Resources

"Concise, clear introduction"



Oxford University Press, 2011

"Thorough, detailed, comprehensive"



Cold Spring Harbor Laboratory Press, 2017
Content freely available:
http://www.ncbi.nlm.nih.gov/books/NBK310274

*Disclaimer: Dr. Schnaar is a co-editor.
** He put a lot of effort into this and won't make a friggin' cent

NCBI Glycans

https://www.ncbi.nlm.nih.gov/glycans/

Definitions

- Glycobiology – The study of glycan functions related to their structures, recognition, biosynthesis, and molecular context (biological scaffolds, e.g. glycolipids & glycoproteins).
- Glycoscience – Glycobiology & chemistry including chemical synthesis, chemoenzymatic synthesis, metabolic engineering, glycomimetics, 3D structures. Includes glycans in material sciences and plant and bacterial polysaccharides
- Glycomics – Analytical glycoscience & glycan bioinformatics. The "glycome" is the total complement of glycans in a cell or organism

Glycophobia

"The fear of getting involved in understanding any biological processes in which glycans play a major role" (Ajit Varki)

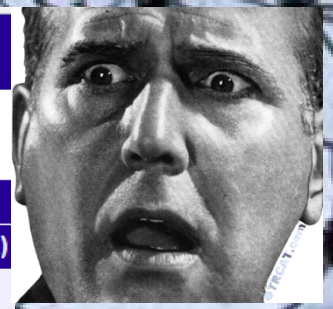
Where does this fear come from?

MonosaccharideDB Home Notation Database

Monosaccharide Query Results

Results 1 - 10 of 776 (Page 1 of 78) • prev • next

Id	Name (MsDB)	Name (CarbBank)
1	b-dglc-HEX-1:5||(2d:1)n-acetyl	b-D-GlcpNAc
2	b-dgal-HEX-1:5	b-D-Galp
3	a-dman-HEX-	
4	b-dglc-HEX-1	
5	b-dman-HEX-	
6	a-dgro-dgal-N	
7	a-lgal-HEX-1:	
8	a-dglc-HEX-1	
9	a-lman-HEX-1	
10	a-dgal-HEX-1	



Invited Commentary

Glycobiology 4:759-767 (1994)

A calculation of all possible oligosaccharide isomers both branched and linear yields 1.05×10^{12} structures for a reducing hexasaccharide: the *Isomer Barrier* to development of single-method saccharide sequencing or synthesis systems

Roger A.Laine

Departments of Biochemis
The Louisiana Agricultura

Special Invited Review *Glycobiology 27*:3-49 (2017)

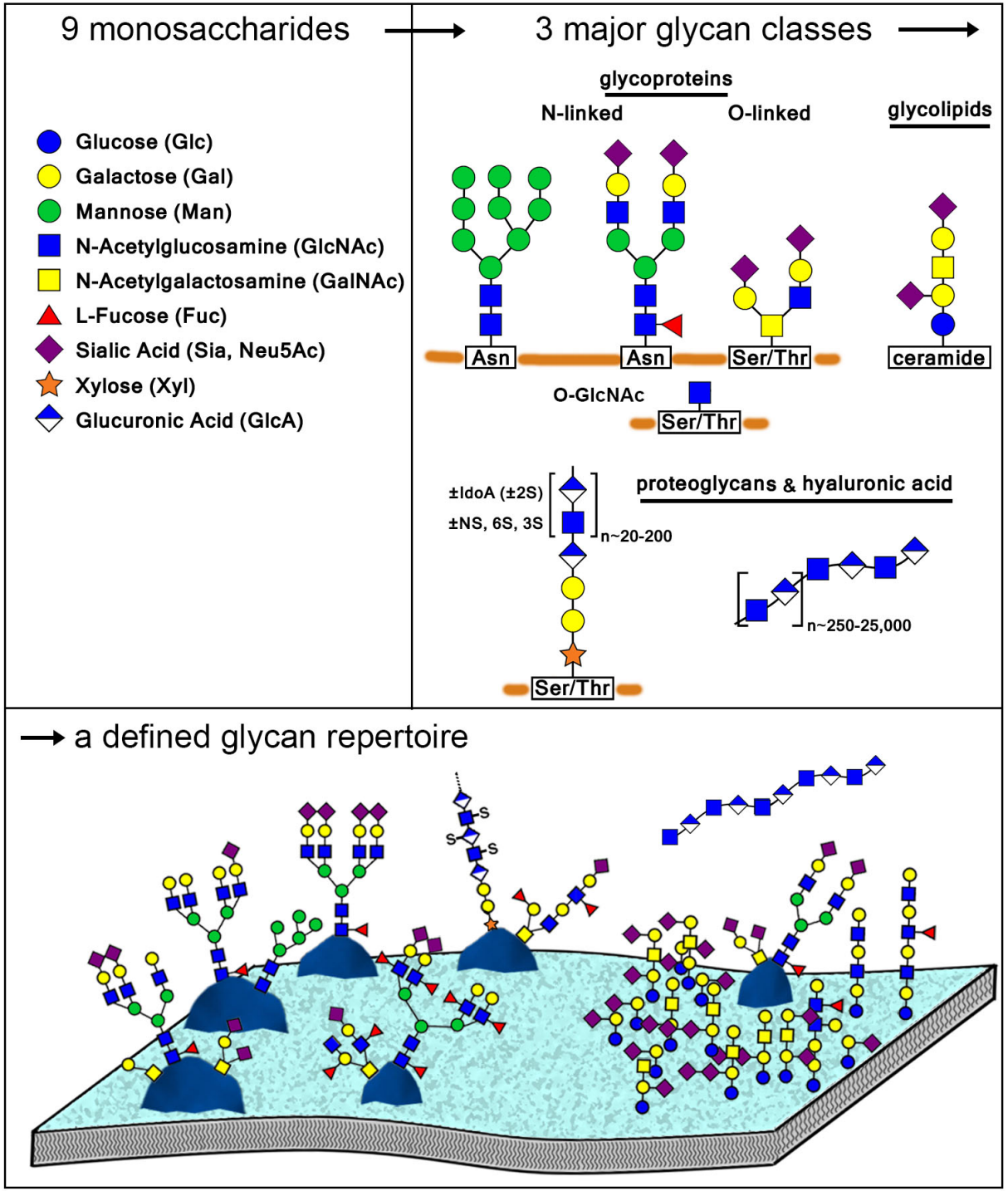
Biological roles of glycans

Ajit Varki

Departments of Medicine and Cellular & Molecular Medicine, Glycobiology Research and Training Center, University of California at San Diego, La Jolla, CA 92093-0687, USA

"....any attempt at being comprehensive is now impractical, and the knowledge base of a single individual cannot do justice to this vast and complex field."

Glycobiology Simplified



Schnaar, J Leuk Biol 99:825, 2016

The Glycobiology Landscape: Sugars as language

- Glycans: the "missing mass" of the biological universe
- Monosaccharides: The alphabet of glycoscience
- The glycosidic bond and glycan nomenclature
- Major carbohydrate classes: sugars, polysaccharides, oligosaccharides and glycans
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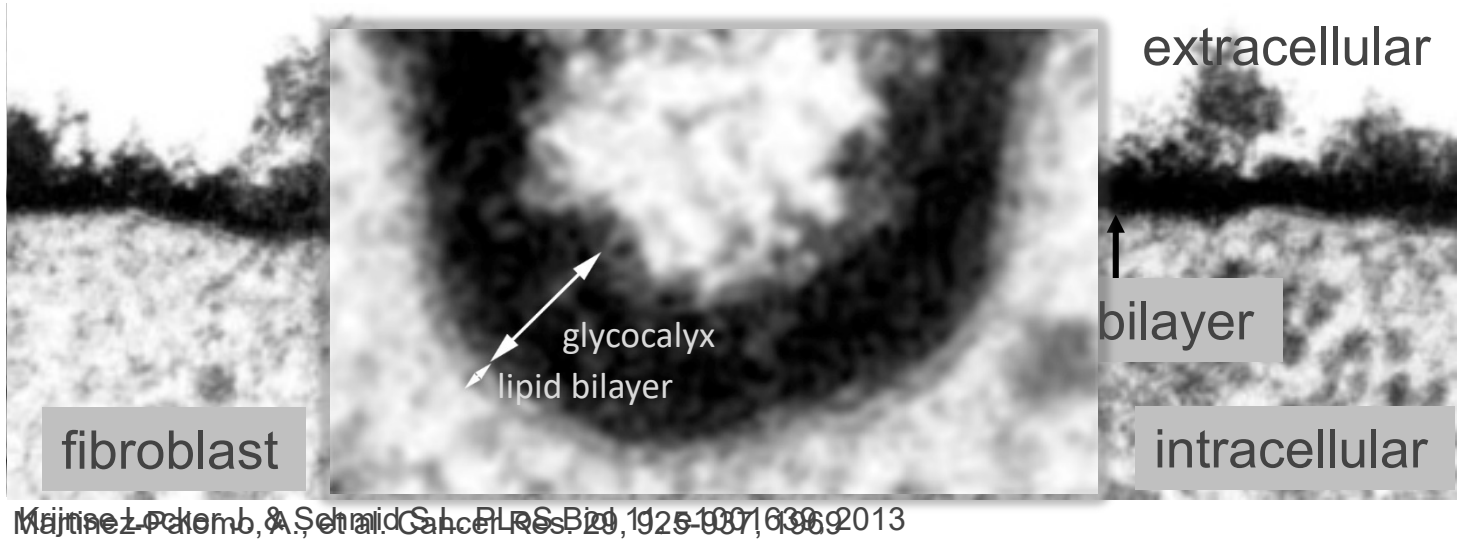
Rosetta Stone, British Museum

The mammalian cell surface

The “glycocalyx” revealed (glycans stained with ruthinium red)

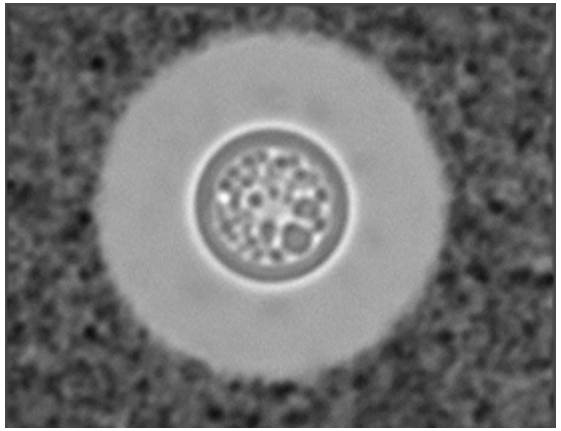


Krijnse Locker J. & Schmid S. L. PLoS Biol. 11: e1001638. 2013
Martinez-Palomo, A., et al. Cancer Res. 29, 1925-1937, 1969

“Every eukaryotic cell is covered with a dense and complex array of glycans.” … in fact…

“Evolution has failed to generate a living cell devoid of surface glycosylation”
- A. Varki

The invisible fungal glycocalyx

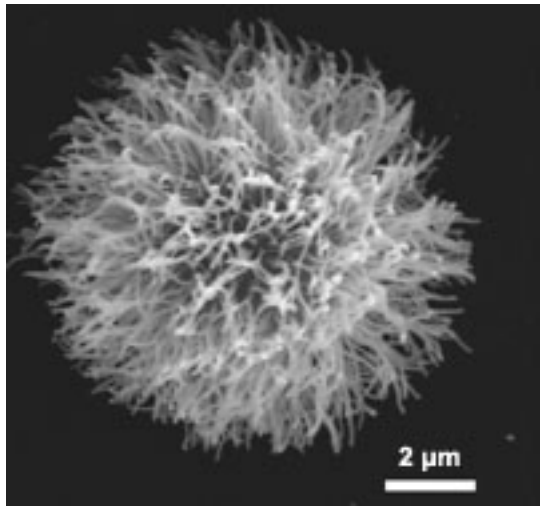


Light (phase) micrograph of *Cryptococcus neoformans* capsule delineated by India ink. The inner circle represents the fungal cell, with the wide outer circle being the capsule.

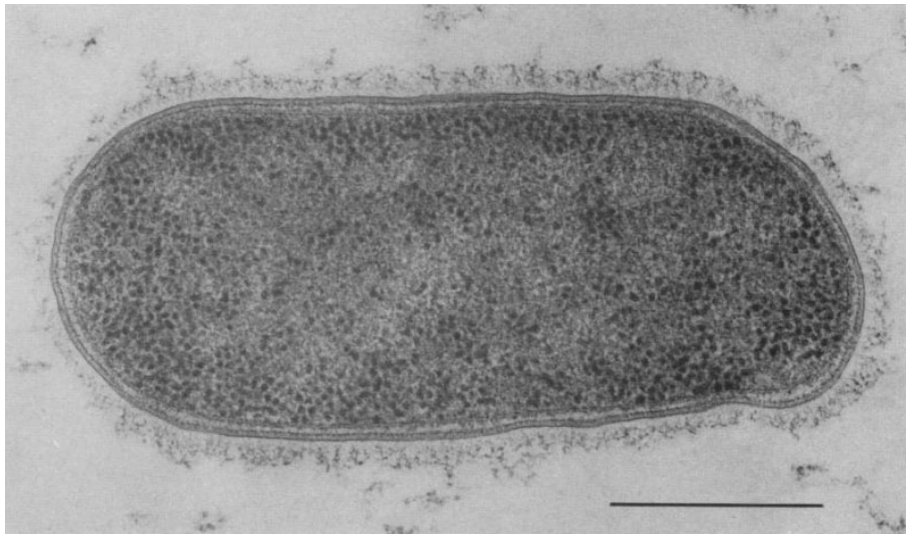
Steenbergen et al. (2003) *Microbes and Infection* 5:667

Scanning electron micrograph of *C. neoformans* yeast cells.

Van Duin et al. (2004) *Antimicrobial Agents and Chemotherapy* 48:2014



The bacterial glycocalyx

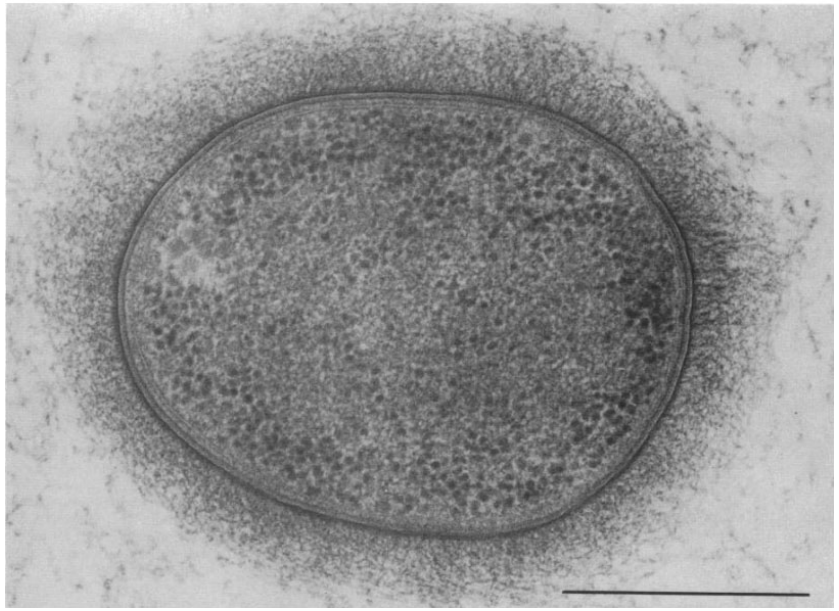


Electron microscopic thin section of *Escherichia coli* K1

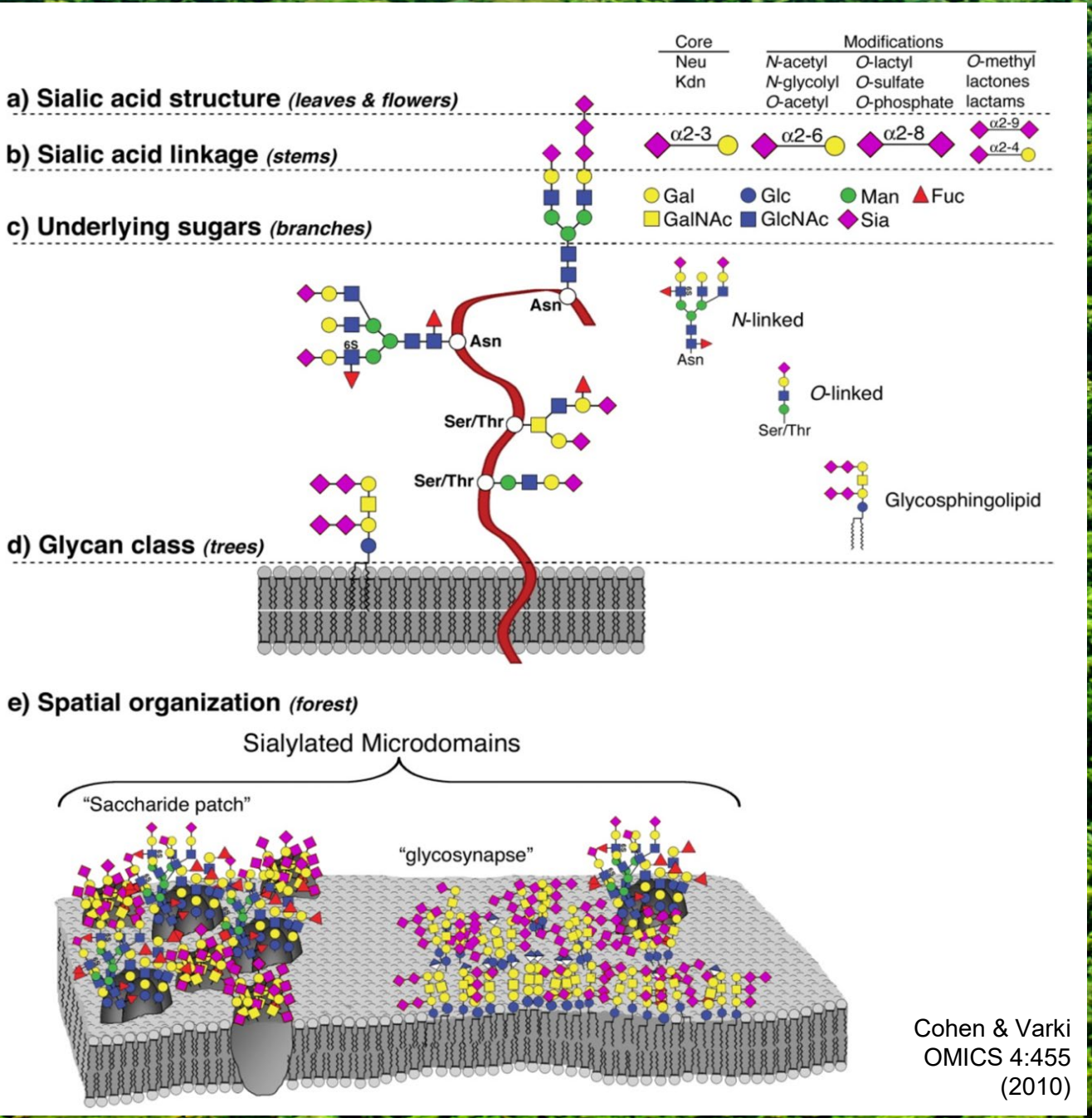
Amako et al. (1988) *J Bacteriol* 170:4960

Electron microscopic thin section of *Klebsiella pneumoniae*

Amako et al. (1988) *J Bacteriol* 170:4960



The cell surface is a forest canopy of glycans



Cohen & Varki
OMICS 4:455
(2010)

based on Ajit Varki, "Perspectives on the Future of Glycobiology," Athens, GA, 14-Mar-2011

The cell surface

Textbook image

Real image

The Glycobiology Landscape: Sugars as language

- Glycans: the "missing mass" of the biological universe
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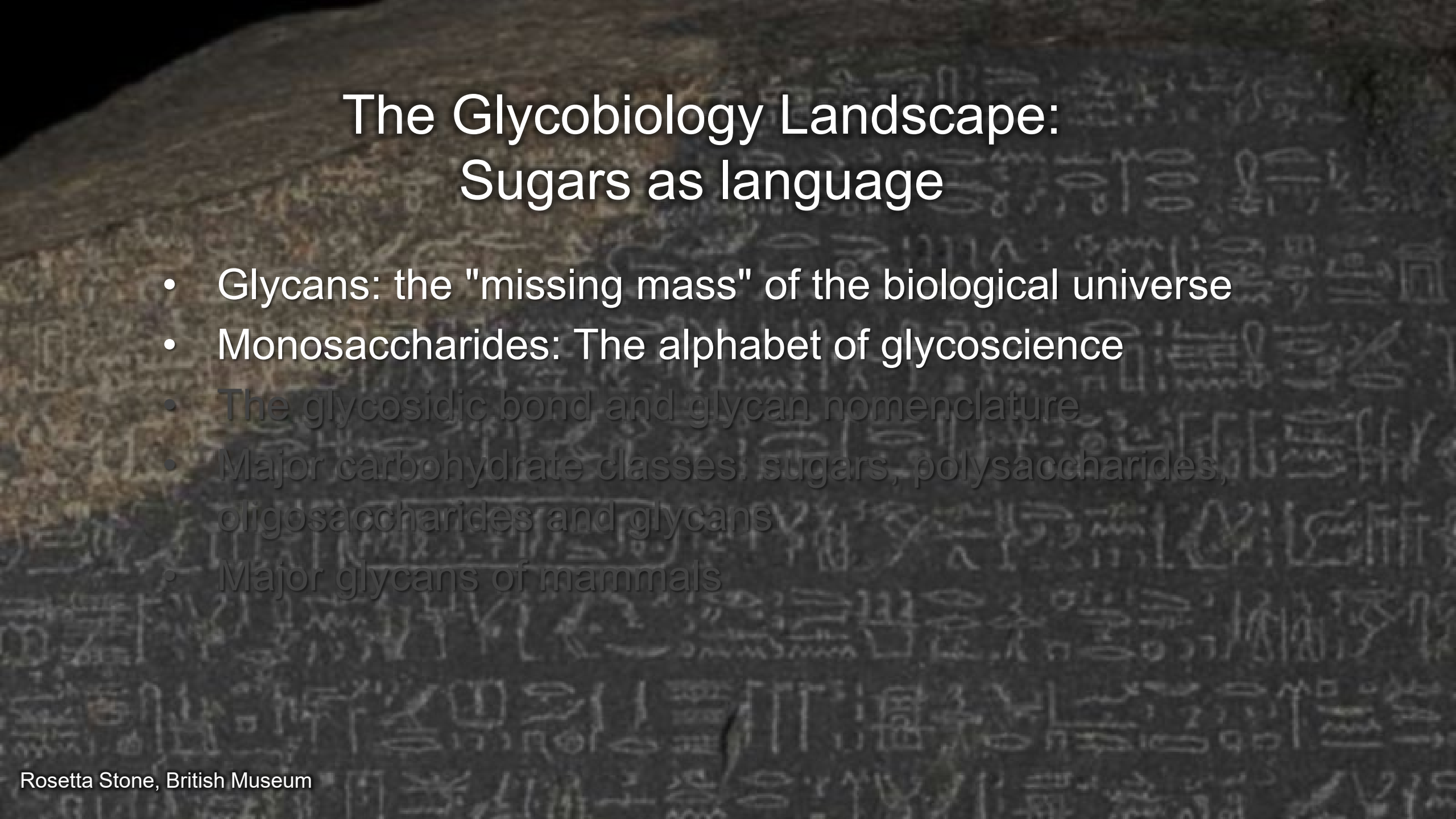
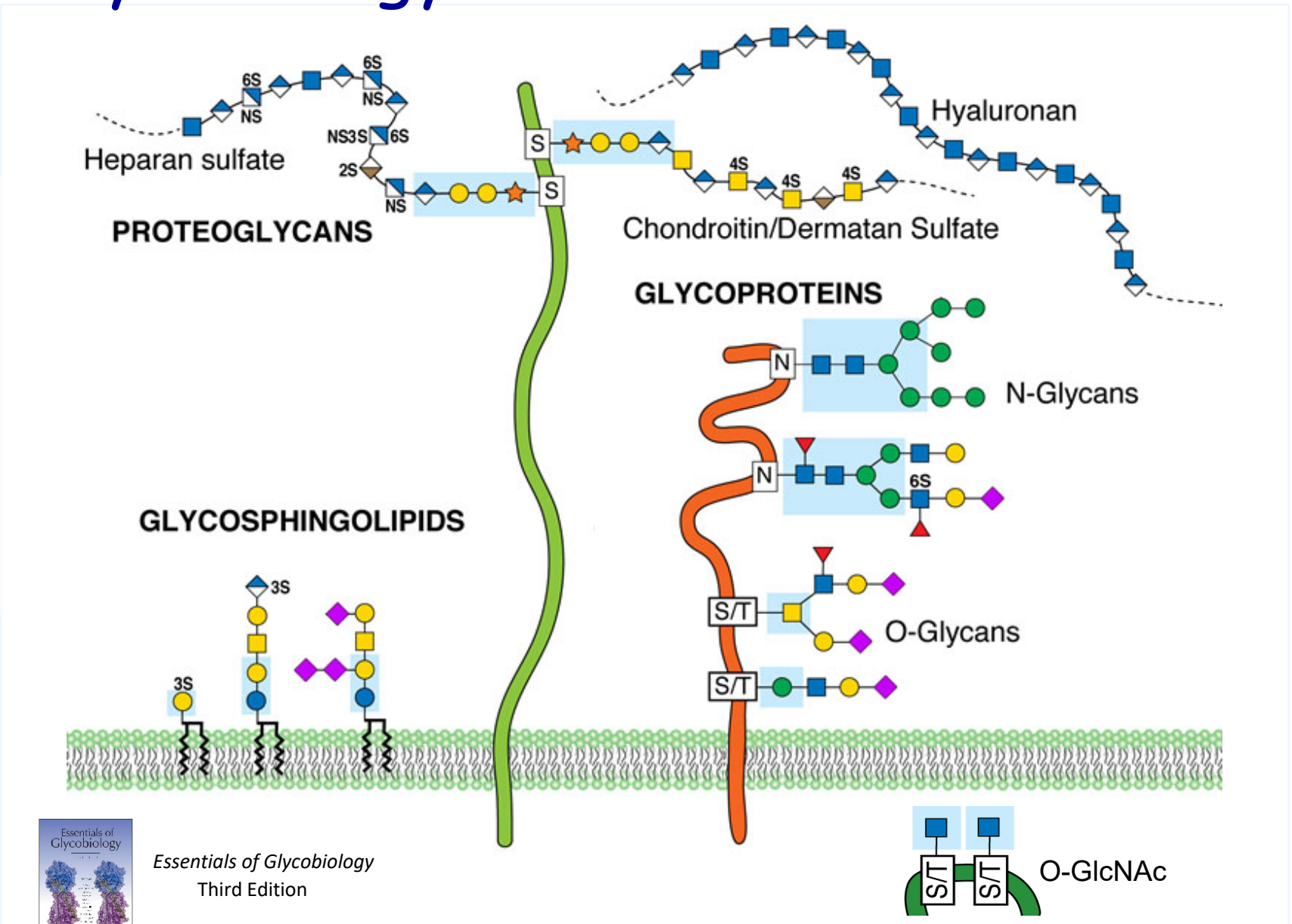


Rosetta Stone, British Museum

Mammalian Glycobiology

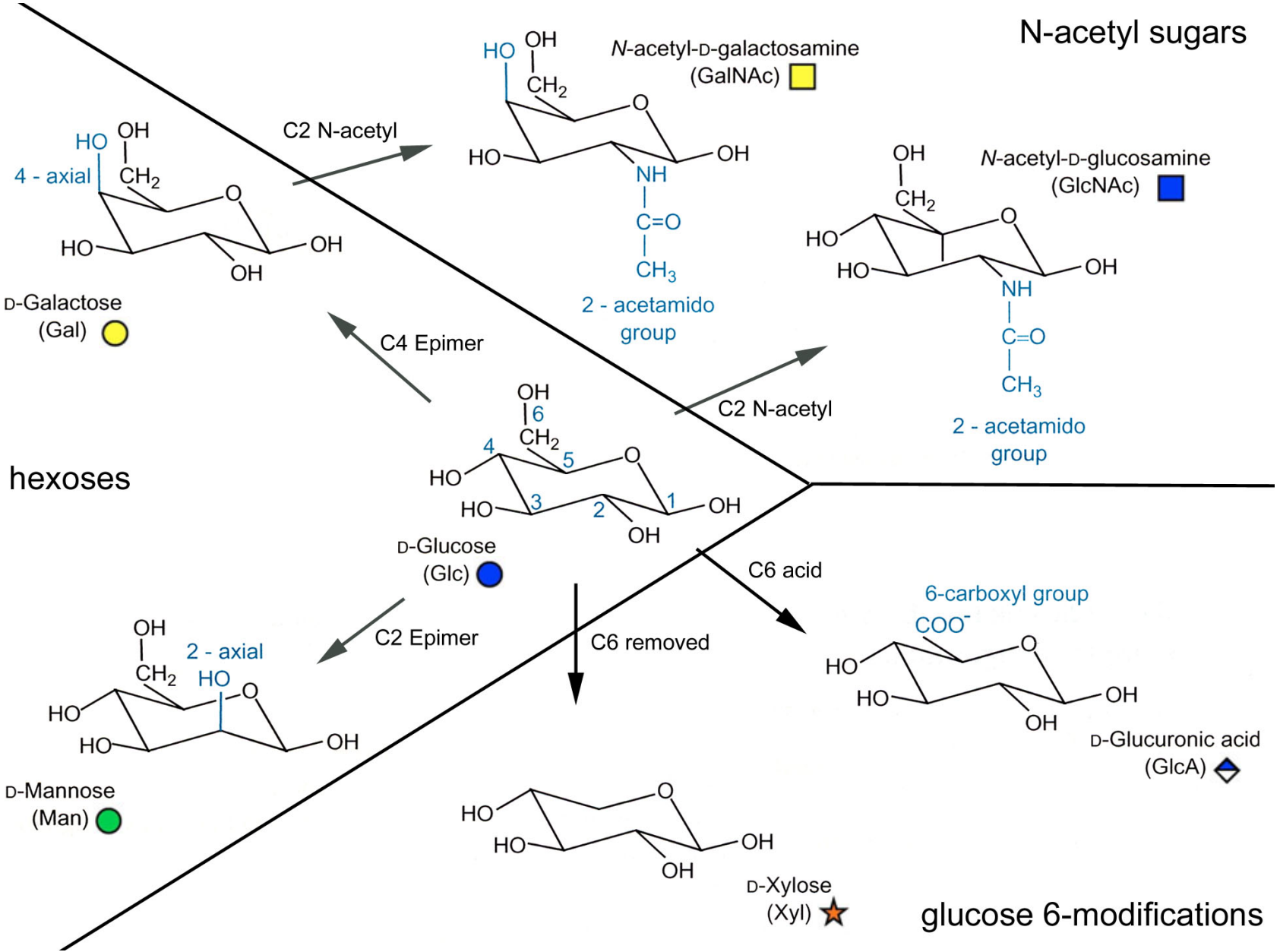
9 monosaccharides

- Glucose (Glc)
- Galactose (Gal)
- Mannose (Man)
- N-Acetylglucosamine (GlcNAc)
- N-Acetylgalactosamine (GalNAc)
- L-Fucose (Fuc)
- Sialic Acid (Sia, Neu5Ac)
- Xylose (Xyl)
- Glucuronic Acid (GlcA)



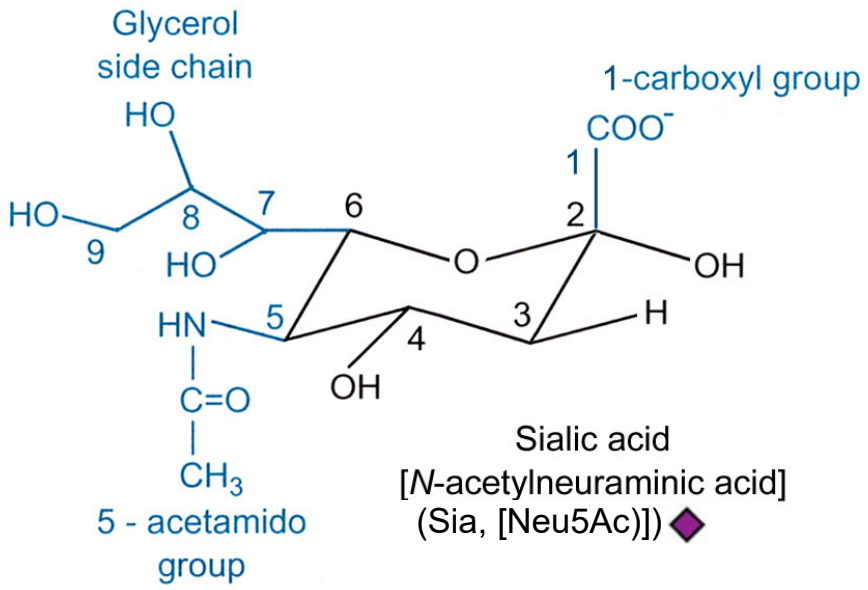
Essentials of Glycobiology
Third Edition

Seven sugars closely related to glucose

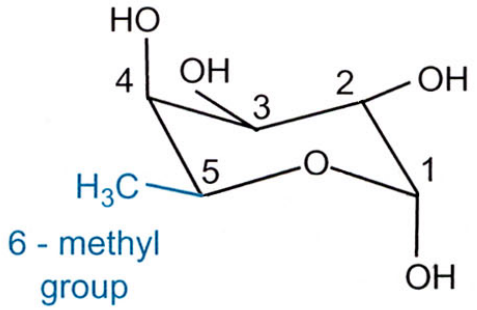


Taylor & Drickamer (2011)
Introduction to Glycobiology, 3rd edition

Two important terminal sugars



Sialic acid
[*N*-acetylneuraminic acid]
(Sia, [Neu5Ac]) ◆



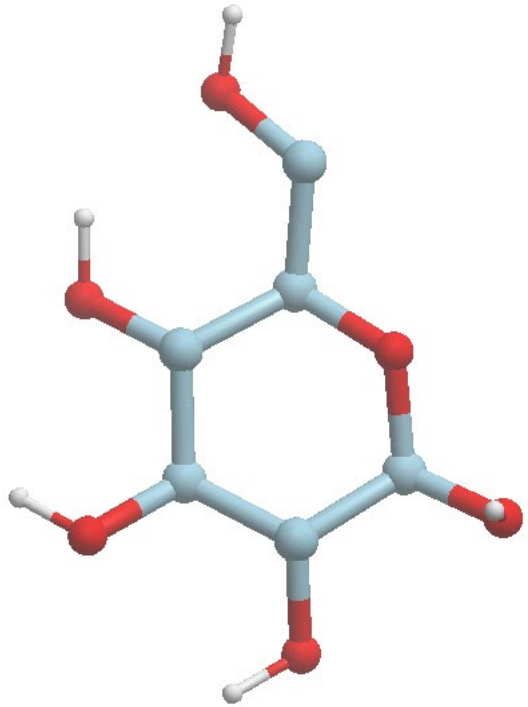
L-Fucose
(Fuc) ▲

(6-deoxy-L-galactose)

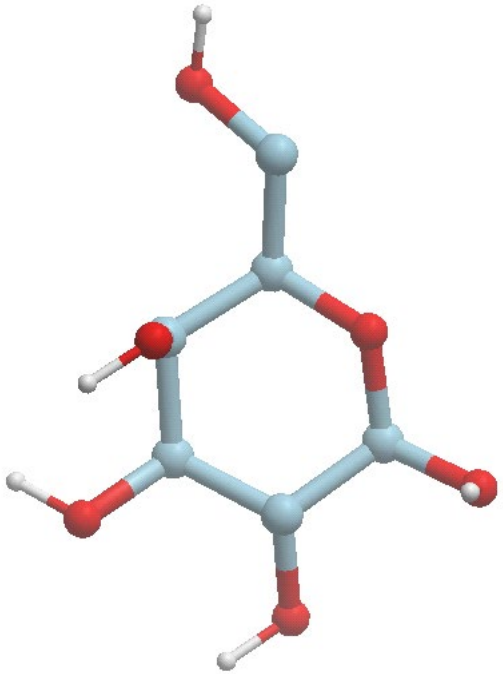
Taylor & Drickamer (2011)
Introduction to Glycobiology, 3rd edition

Sugars as language: Strategic placement of molecular recognition determinants

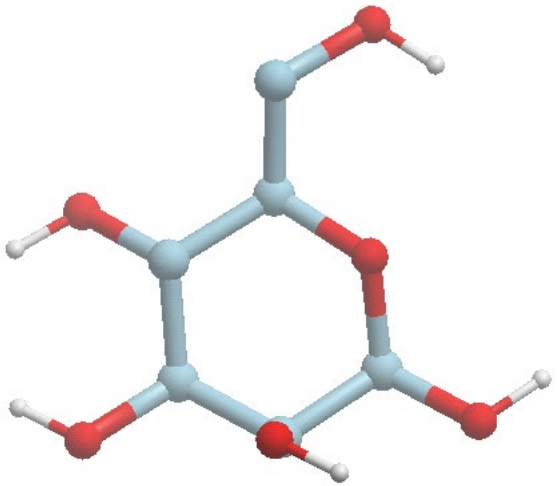
Glucose



Galactose

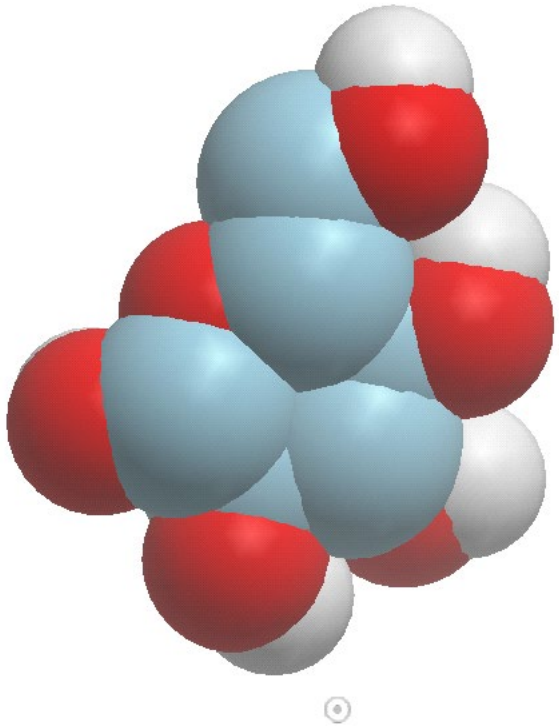


Mannose

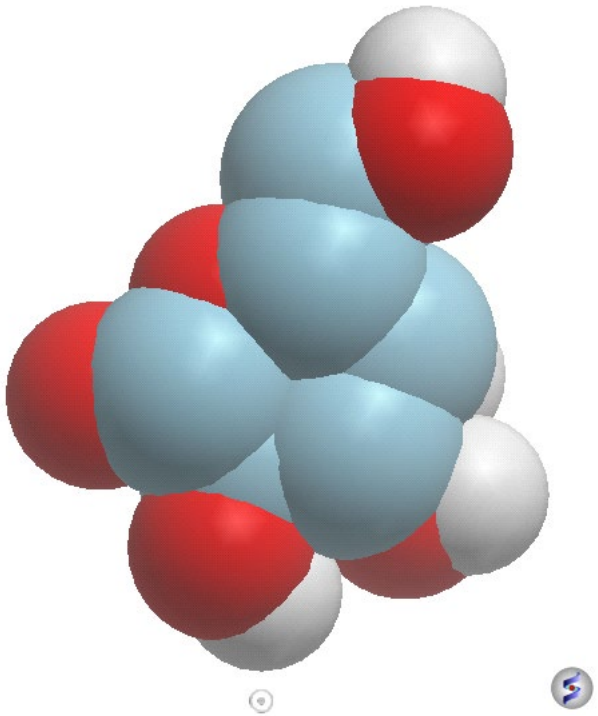


Sugars as language: Strategic placement of molecular recognition determinants

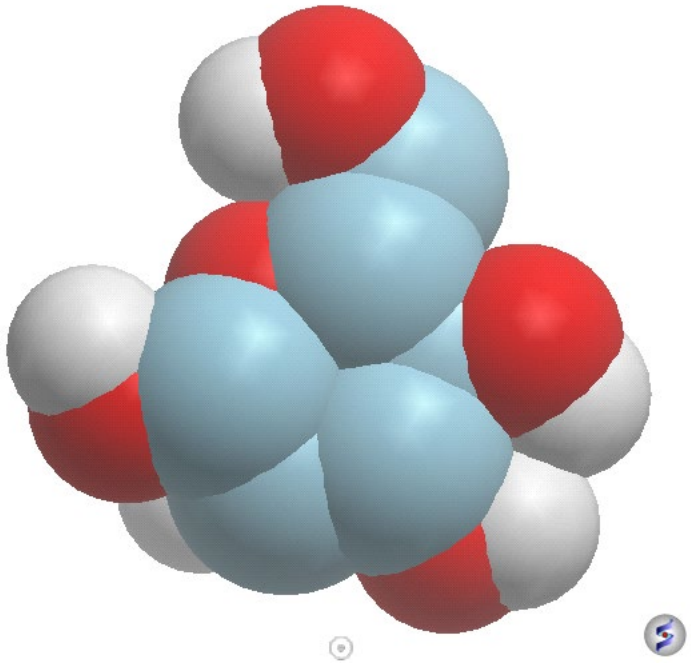
Glucose



Galactose

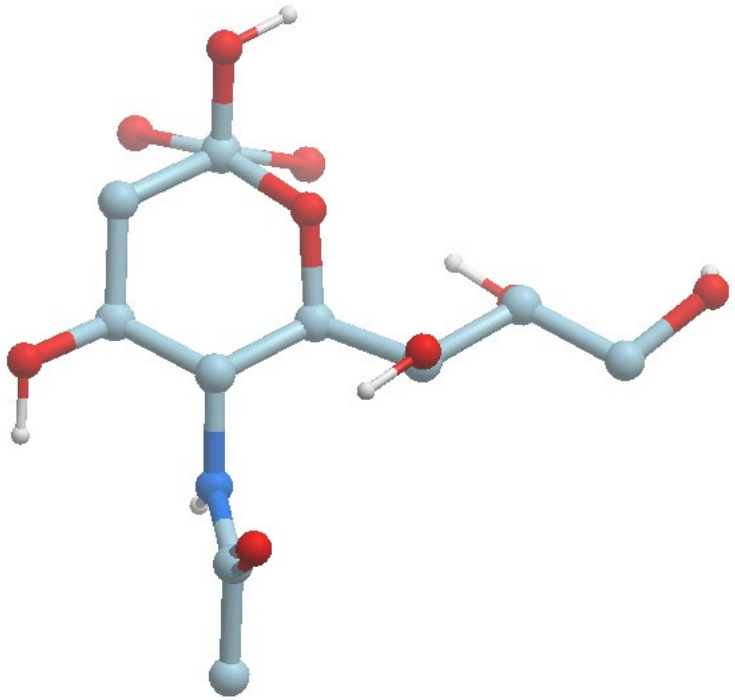


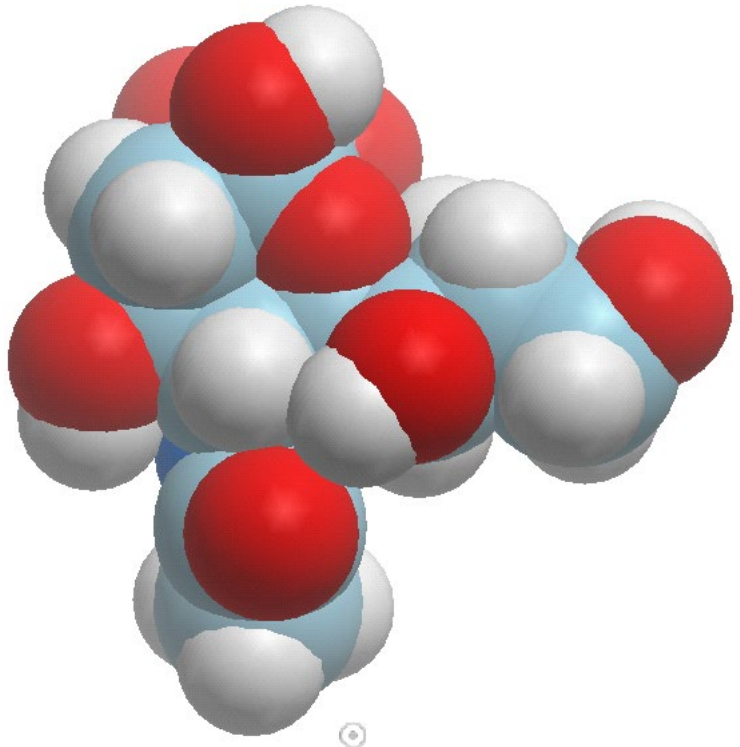
Mannose



Sugars as language: Strategic placement of molecular recognition determinants

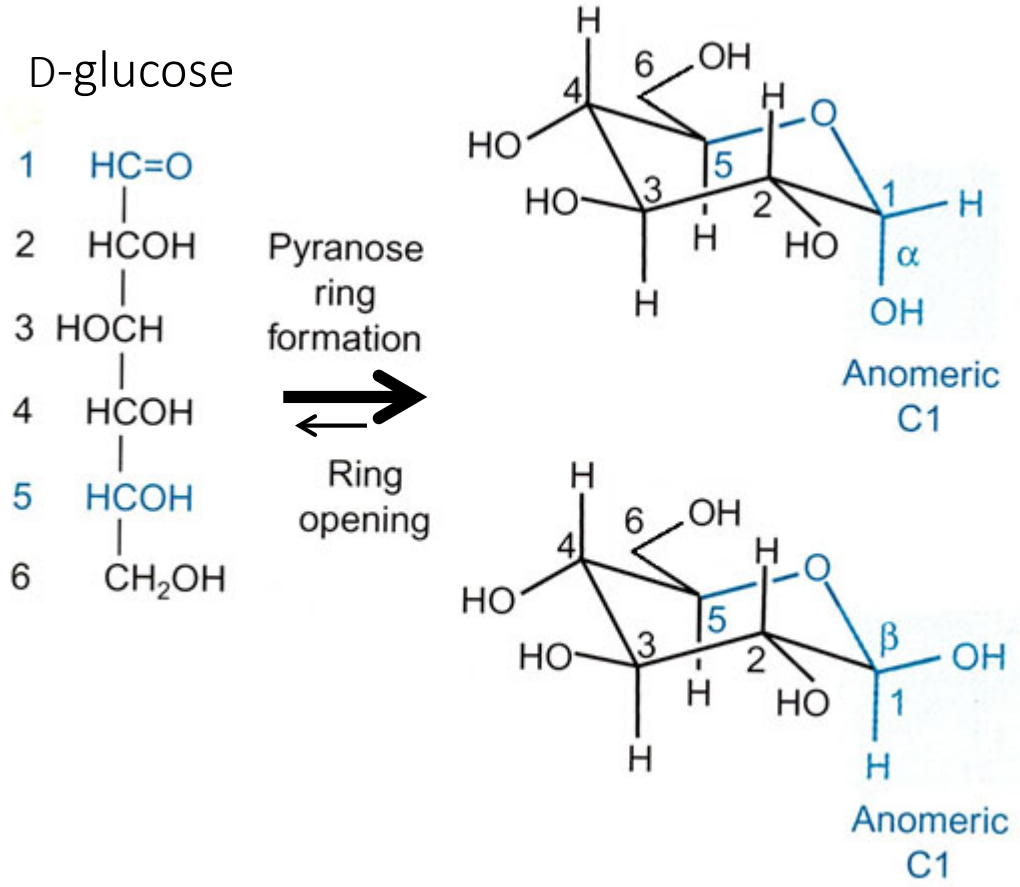
Sialic Acid (Neu5Ac)





The anomeric carbon – α or β

- Glycobiologists seldom think of sugars as linear molecules
- Pentoses and hexoses are predominantly in their ring forms at equilibrium
- Only free sugars equilibrate between open chain and ring forms; when sugars are linked to one another (or an aglycone) their ring form and anomeric configuration are fixed
- There are two possible configurations at the anomeric carbon, α and β



Taylor & Drickamer (2011) Introduction to Glycobiology, 3rd edition

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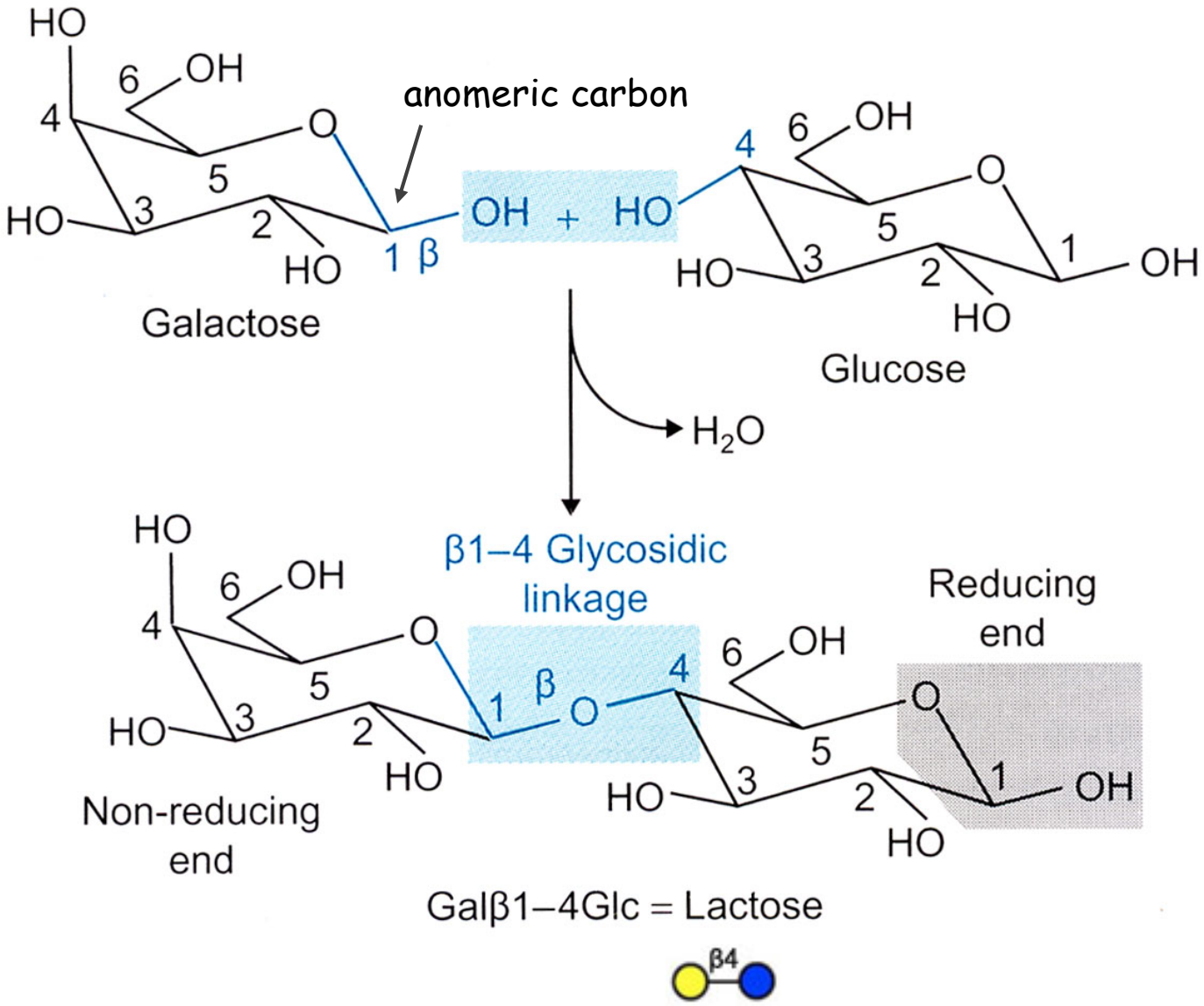
Rosetta Stone, British Museum

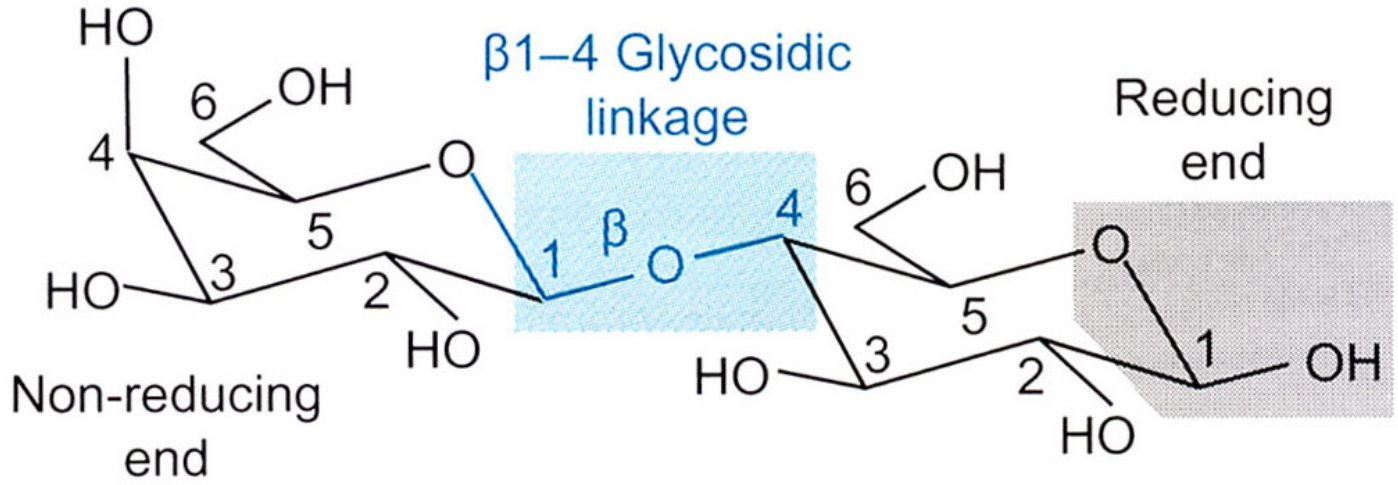
Sugars and linked via glycosidic bonds



When a glycosidic bond is formed the anomeric configuration is “locked”

Taylor & Drickamer (2011) Introduction to Glycobiology, 3rd edition

Glycan nomenclature

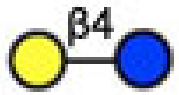


Taylor & Drickamer (2011) Introduction to Glycobiology, 3rd edition

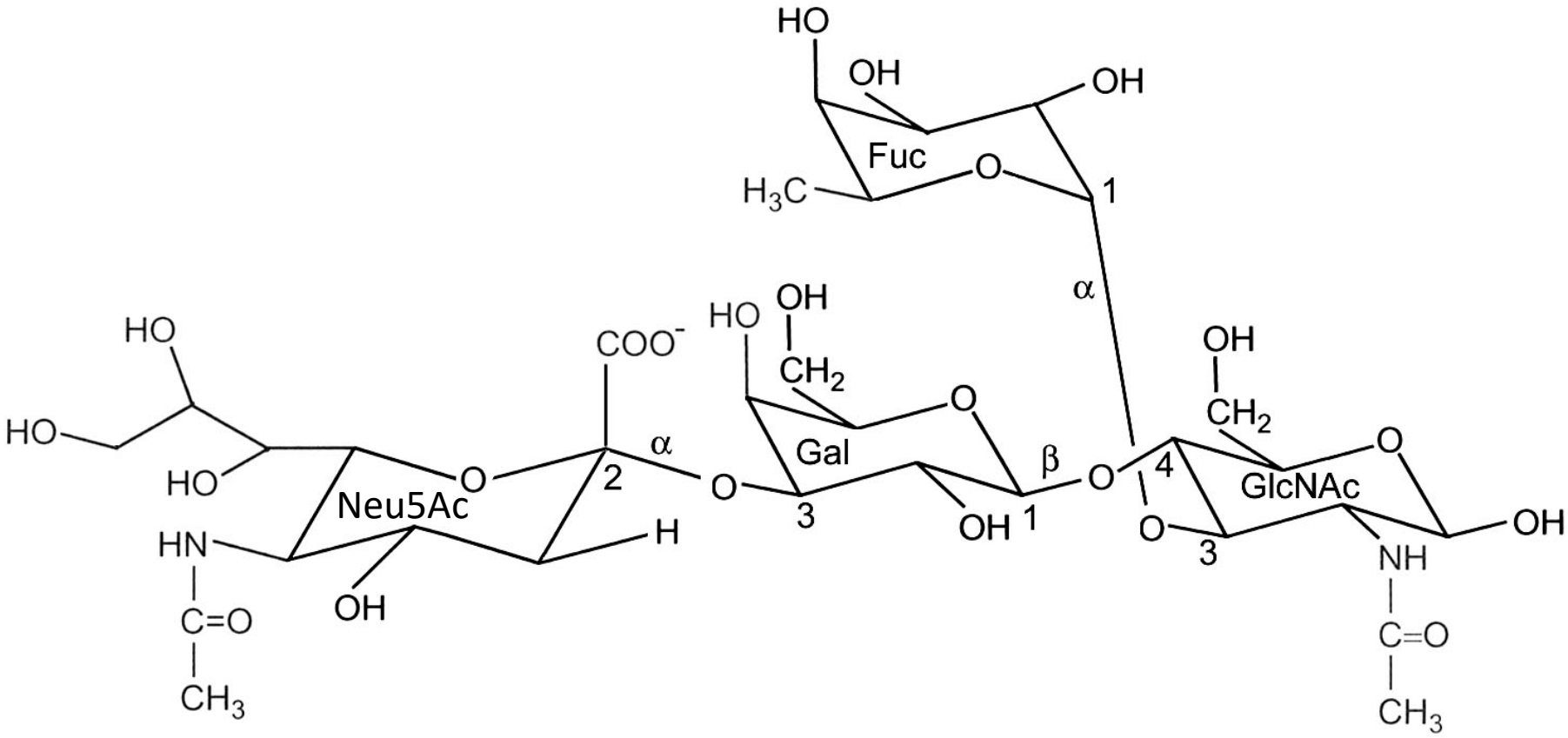
Nomenclature:

-Name the non-reducing (left-most) sugar (Gal)
-Name the anomeric configuration (β)
-Name the anomeric carbon number (1)
-Name the substituted carbon number (4)
-Name the substituted sugar (Glc)

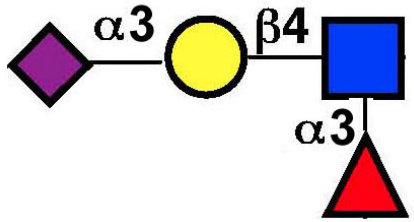
RESULT: Gal β1-4 Glc



Branched glycan nomenclature



Neu5Ac α2-3 Gal β1-4 (Fuc α1-3) GlcNAc



Symbol nomenclature for glycans

Glycan Symbol Nomenclature

Neutral Hexoses - Circles; N-Acetylhexosamines - Squares

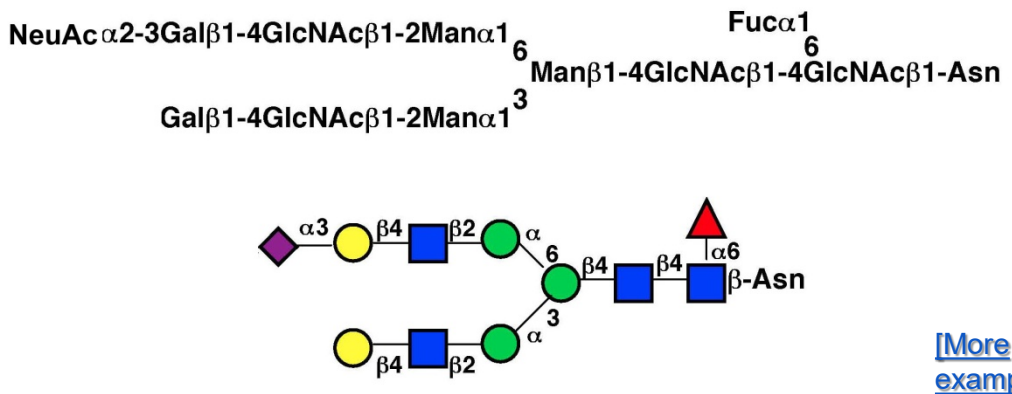
- Galactose stereochemistry: Yellow (255,255,0)
- Glucose stereochemistry: BLUE (0,0,250)
- Mannose stereochemistry: GREEN (0,200,50)

Deoxysugar - Triangle; Penose - Star

- Fucose: RED (250,0,0)
- Xylose: (5-pointed star) ORANGE (250,100,0)

Acidic Sugars (Diamonds)

- Neu5Ac: PURPLE (125,0,125)
- GlcA: BLUE (0,0,250)/Upper segment



[More examples]

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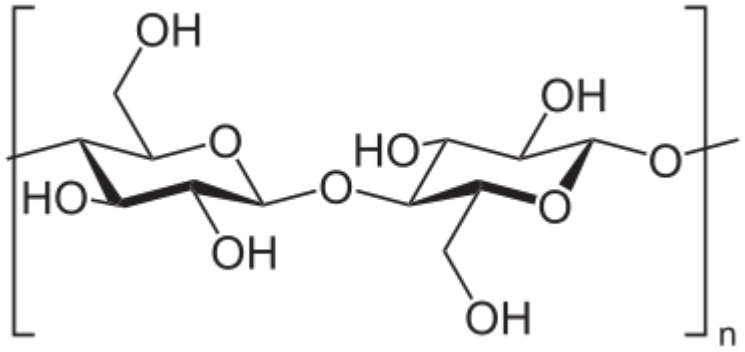
Rosetta Stone, British Museum

Sugars, polysaccharides, oligosaccharides and glycans

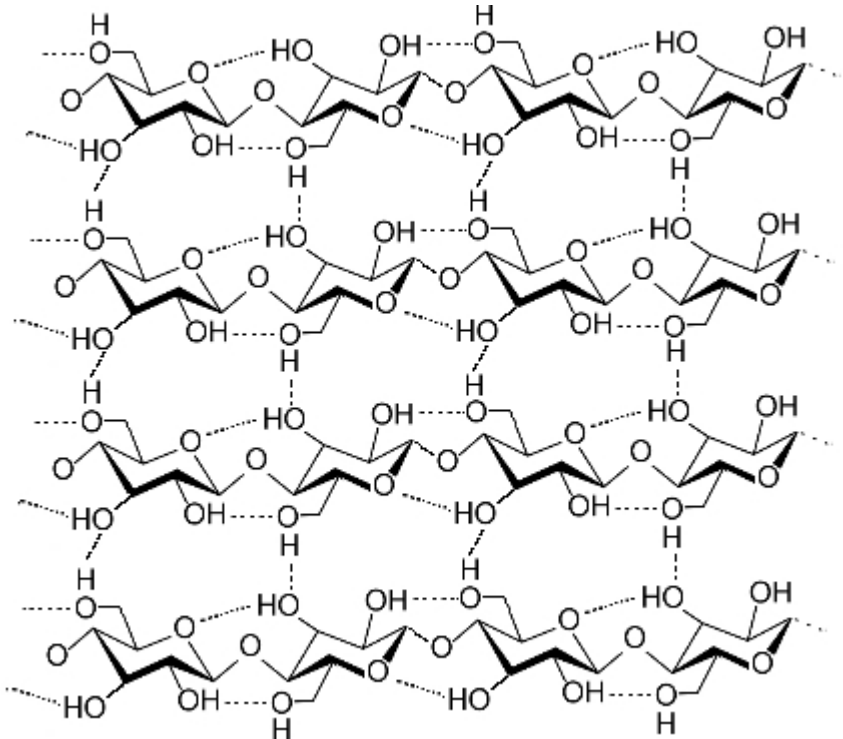
- Sugars: often from plants or plant polysaccharides
  [glucose, sucrose (glucose α1-2 fructose), maltose (Glc α1-4 Glc), lactose (Gal β1-4 Glc)]
- Polysaccharides: linear or branched polymers of a single sugar
  [cellulose, amylose (starch), glycogen, chitin]
- Oligosaccharides: groups of monosaccharides (typically 3 or more) in linear or branched glycosidic linkage
  [Seldom found as free structures – most often components of glycans]
- Glycans: Oligosaccharides on proteins or lipids
  [glycoproteins, glycolipids, proteoglycans]

Polysaccharide: cellulose



$(\beta 1\text{-}4\ Glc\ \beta 1\text{-}4\ Glc)_n$

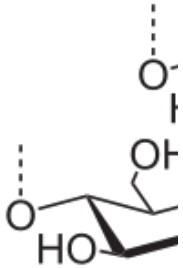
Most abundant organic compound on Earth. ~90% of cotton fiber, 50% of wood, 33% of all plant matter.

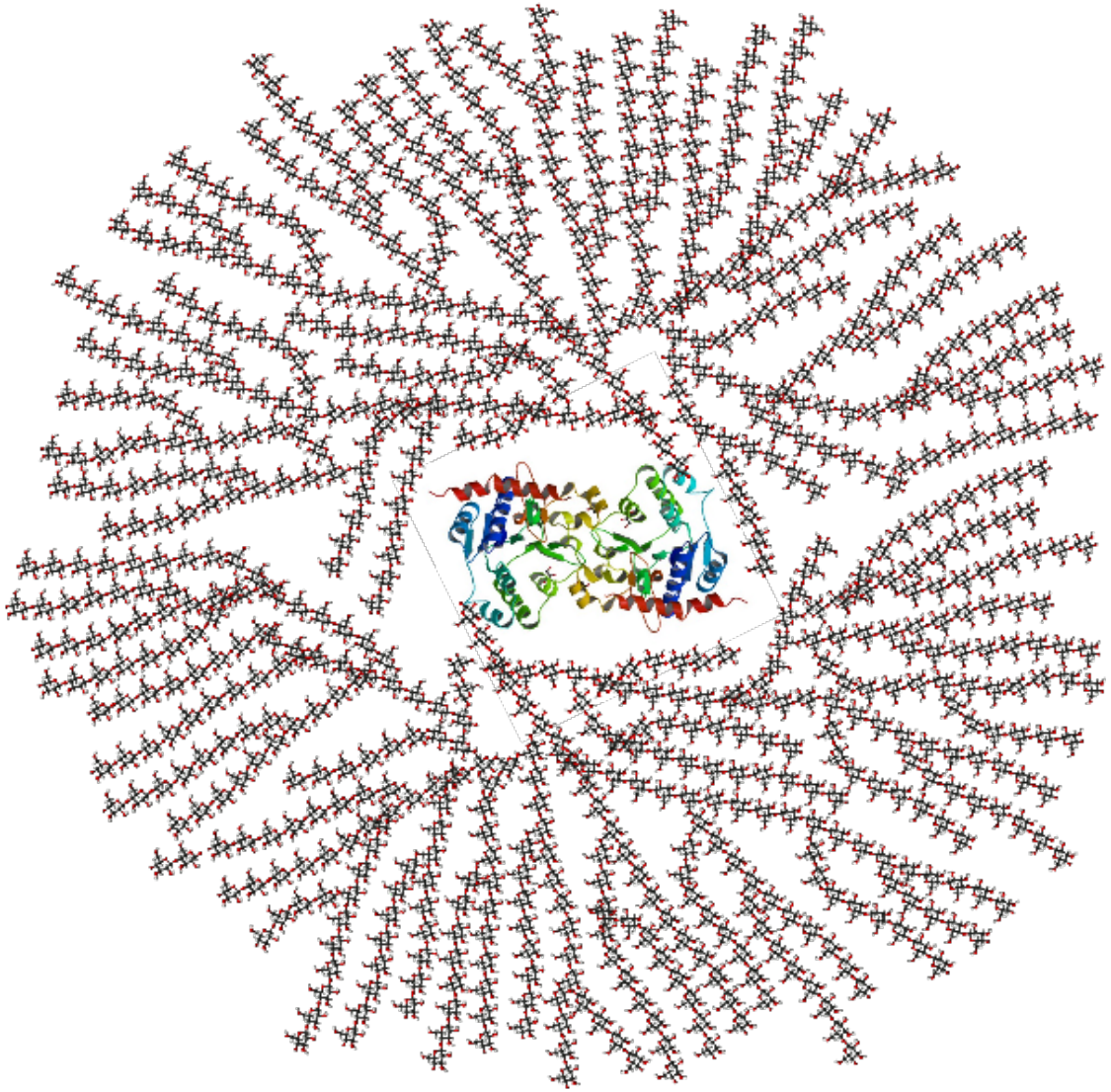


Intra- and inter-strand hydrogen bonding provides strength

http://en.wikipedia.org/wiki/Cellulose

Polysaccharide: glycogen





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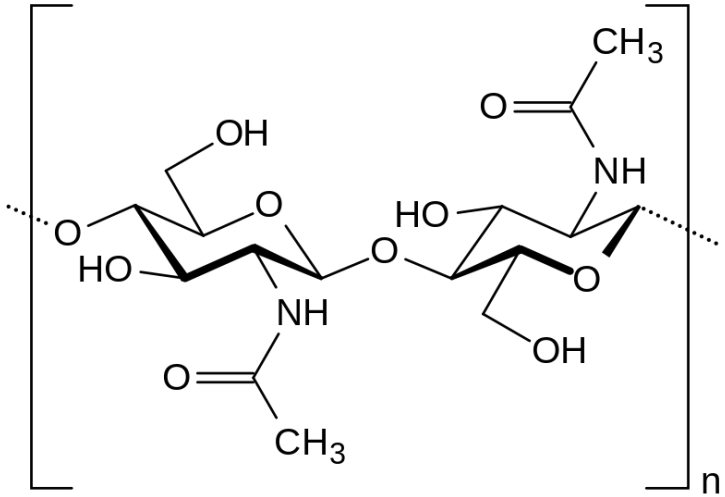
http://en.wikipedia.org/wiki/Glycogen

Polysaccharide: chitin



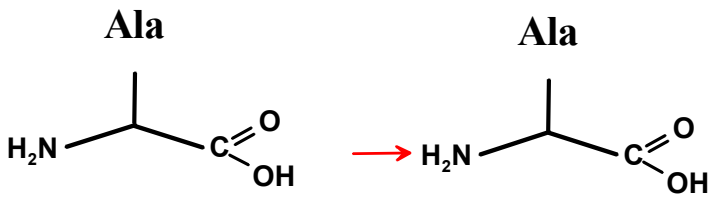
$(\beta 1\text{-}4\ GlcNAc\ \beta 1\text{-}4\ GlcNAc)_n$

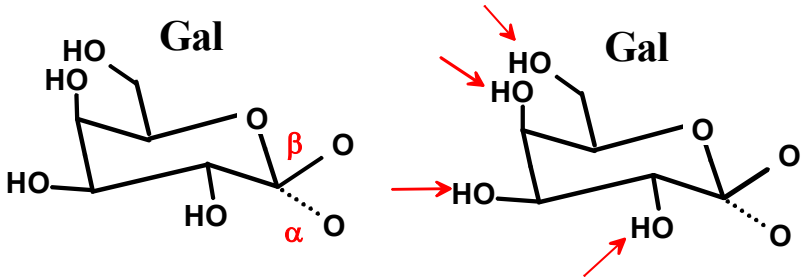
Among the most abundant of organic molecules on Earth. Main component of the cell walls of fungi and exoskeletons of arthropods such as insects and crustaceans

http://en.wikipedia.org/wiki/Chitin

Oligosaccharide comparative molecular diversity

	Polypeptides	Glycans
Building blocks	amino acids	monosaccharides
Number of different monomers	20 common	9 common
Linkage sites per monomer	1	3-4
Possible linkage configurations	1	2
Possible homodimer structures	1	6-8
Linkage modes	linear	linear or branched





Oligosaccharide comparative molecular diversity

Three different amino acids (Ala, Ser, Tyr) – 6 structures

Ala-Ser-Tyr
Ala-Tyr-Ser
Ser-Ala-Tyr
Ser-Tyr-Ala
Tyr-Ala-Ser
Tyr-Ser-Ala

Three different sugars (Glc, Gal, Man) – 1,056 structures …

Oligosaccharide comparative molecular diversity

Three different sugars (Glc, Gal, Man) – 1,056 structures

Man a1-4 Gal a1-3 Glc b1
Man a1-6 Gal a1-3 Glc b1
Man b1-2 Gal a1-3 Glc b1
Man b1-3 Gal a1-3 Glc b1
Man b1-4 Gal a1-3 Glc b1
Man b1-6 Gal a1-3 Glc b1
Man a1-2 Gal a1-4 Glc b1
Man a1-3 Gal a1-4 Glc b1
Man a1-4 Gal a1-4 Glc b1
Man a1-6 Gal a1-4 Glc b1
Man b1-2 Gal a1-4 Glc b1
Man b1-3 Gal a1-4 Glc b1
Man b1-4 Gal a1-4 Glc b1
Man b1-6 Gal a1-4 Glc b1
Man a1-2 Gal a1-6 Glc b1
Man a1-3 Gal a1-6 Glc b1

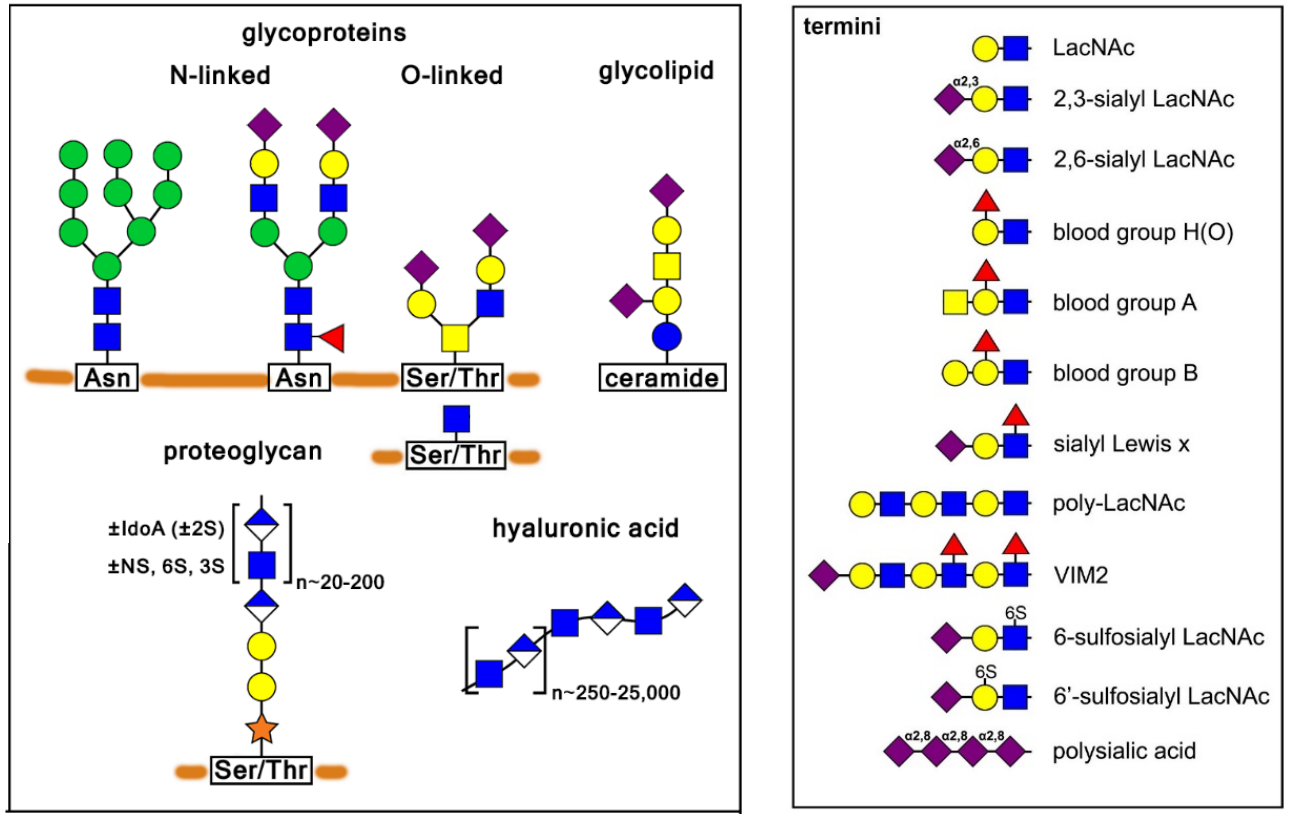
Using the 9 monosaccharides of human glycans, ~10^{12} (one trillion)* hexasaccharides can be drawn. Why don't we quit now and work on something else?

*Laine Glycobiology 4:759 (1994)

Glycan diversity is biosynthetically constrained and analytically tractable

- 209 human glycosyltransferases
- ~3,000 glycoprotein and glycolipid "glycan determinants"
- ~4,000 proteoglycan pentasaccharide determinants

theme & variation



Schnaar, "Glycobiology Simplified", J Leuk Biol 99:825, 2016

Cummings, "The repertoire of glycan determinants in the human glycome" Molecular Biosystems 5:1087, 2009

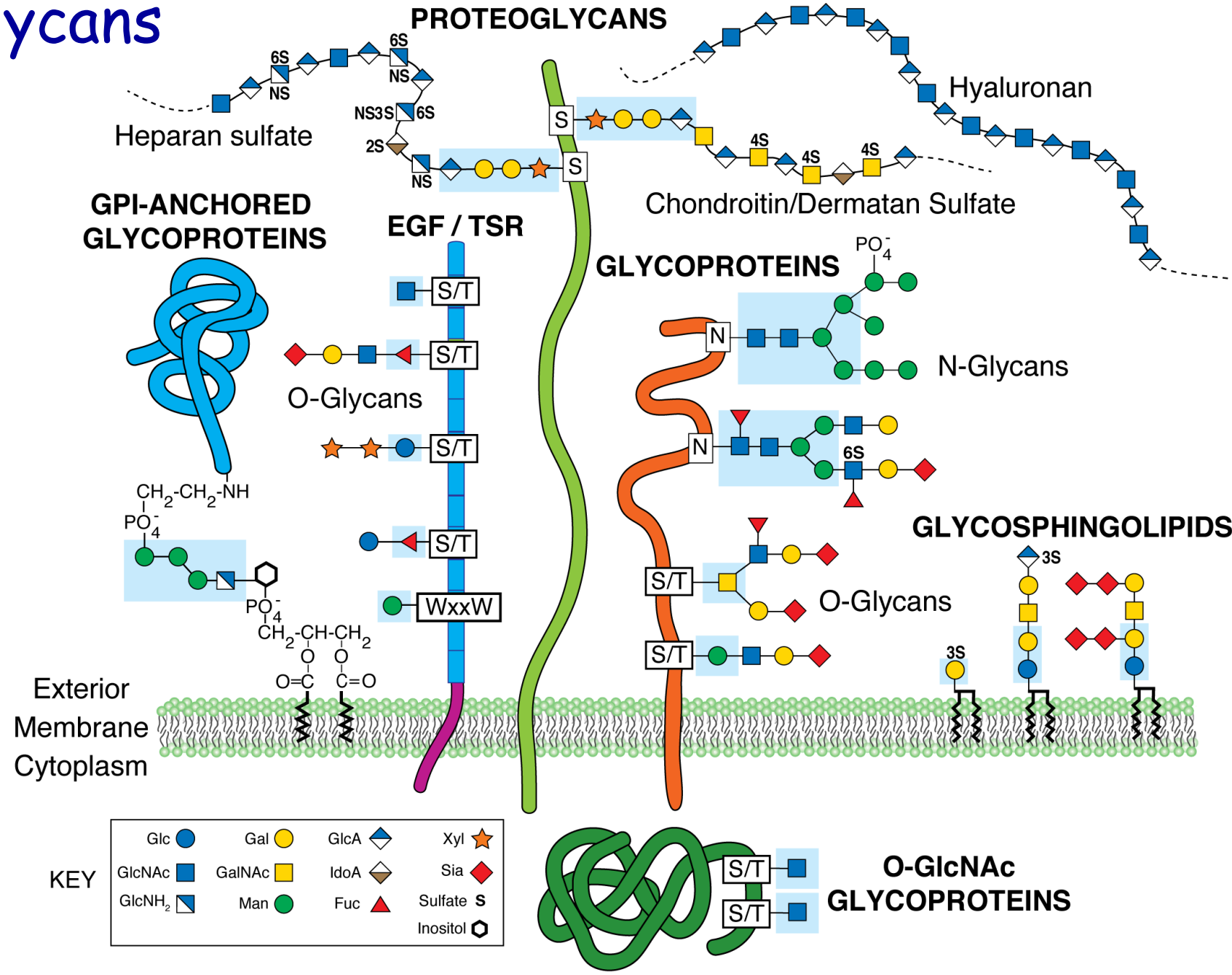
The Glycobiology Landscape: Sugars as language

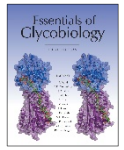
- Glycans: the "missing mass" of the biological universe
- Monosaccharides: The alphabet of glycoscience
- The glycosidic bond and glycan nomenclature
- Major carbohydrate classes: sugars, polysaccharides, oligosaccharides and glycans
- Major glycans of mammals

Rosetta Stone, British Museum

Mammalian glycans

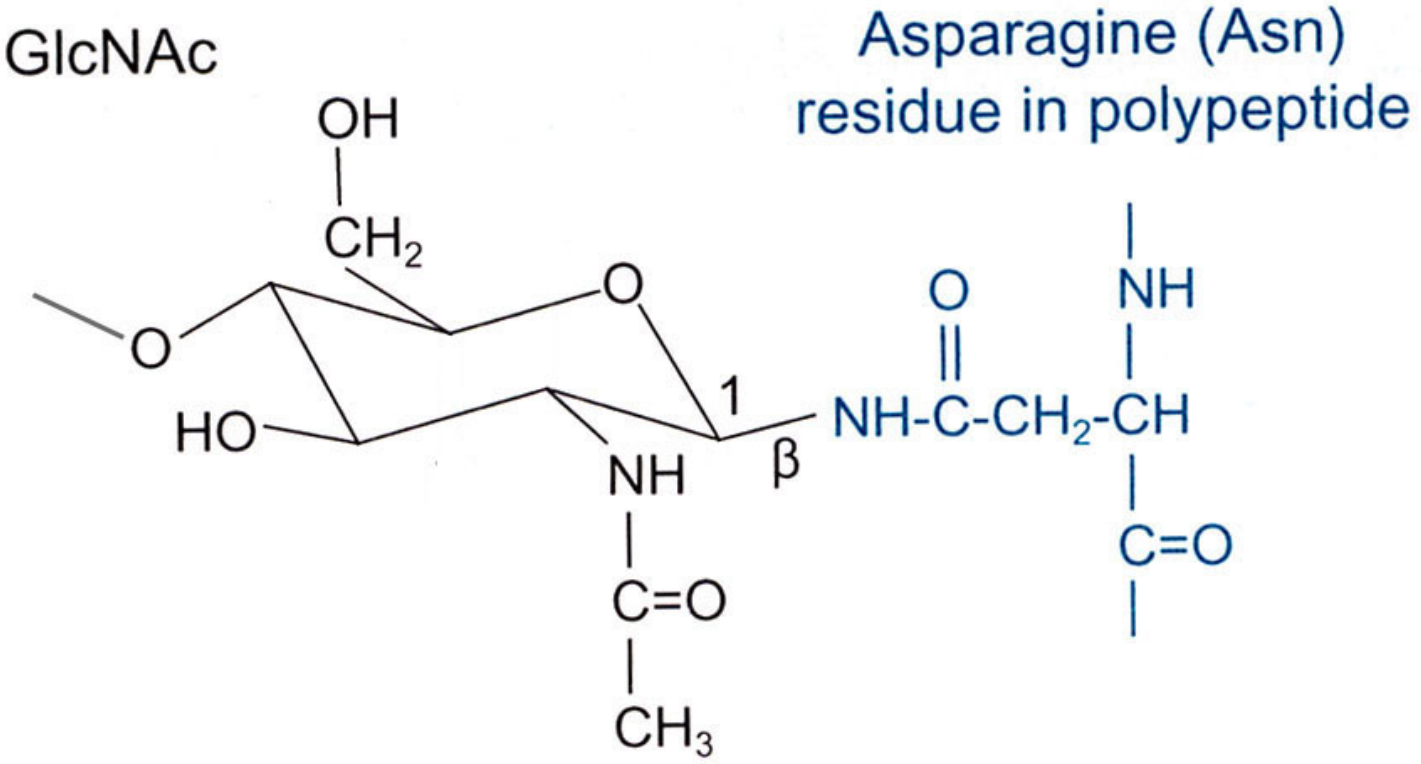
- Glycoproteins
  - N-linked
  - O-linked
  - GPI-anchored
  - O-GlcNAc





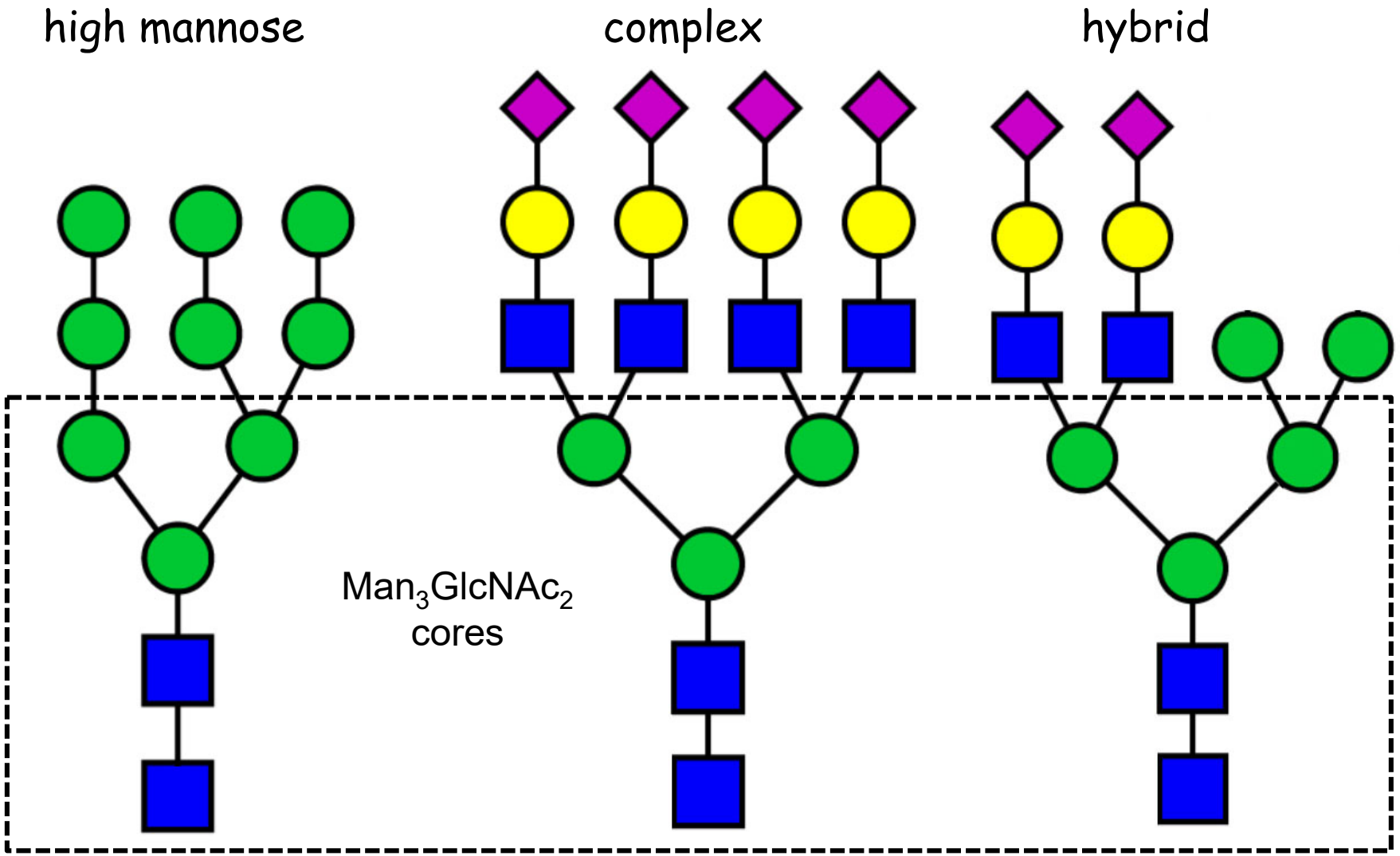
Essentials of Glycobiology
Third Edition

N-linked glycoprotein glycans



Taylor & Drickamer (2011) Introduction to Glycobiology, 3rd edition

Families of N-linked glycan structures



With terminal saccharide diversity

Neu5Ac α2-6 Gal β1-4 GlcNAc---

Neu5Ac α2-3 Gal β1-4 GlcNAc---

Gal α1-3 Gal β1-4 GlcNAc---

4-SO_3-GalNAc β1-4 GlcNAc---

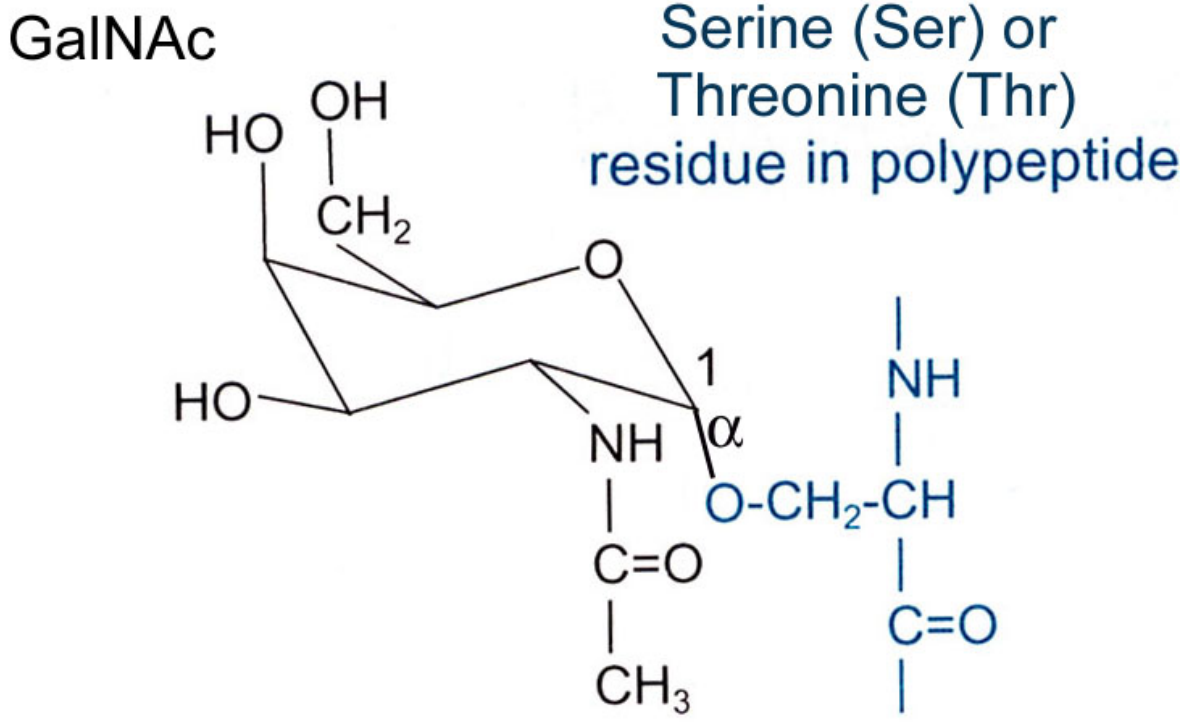
(-Gal β1-4 GlcNAc β1-3-)$_n$ = polylactosamine repeats (n may be >9)

(-Neu5Ac α2-8-)$_n$ = polysialic acid (n may be >50)

Fuc residues (e.g. Fuc α1-2 Gal; Fuc α1-3 GlcNAc; Fuc α1-4 GlcNAc)

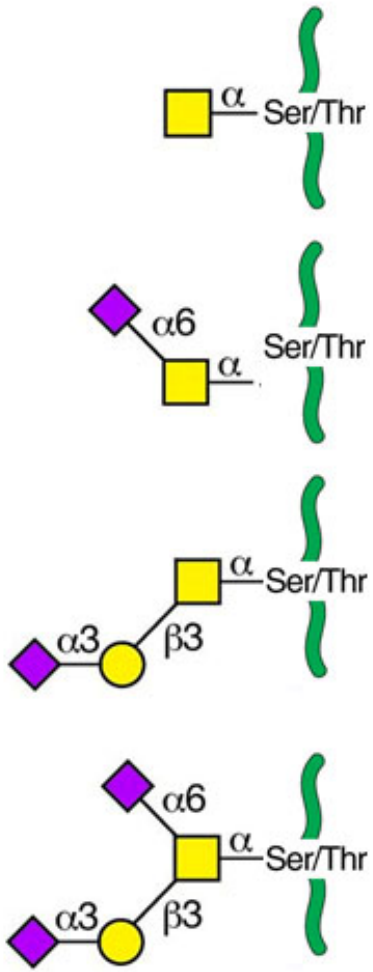
Terminal Saccharide Diversity is key to molecular recognition

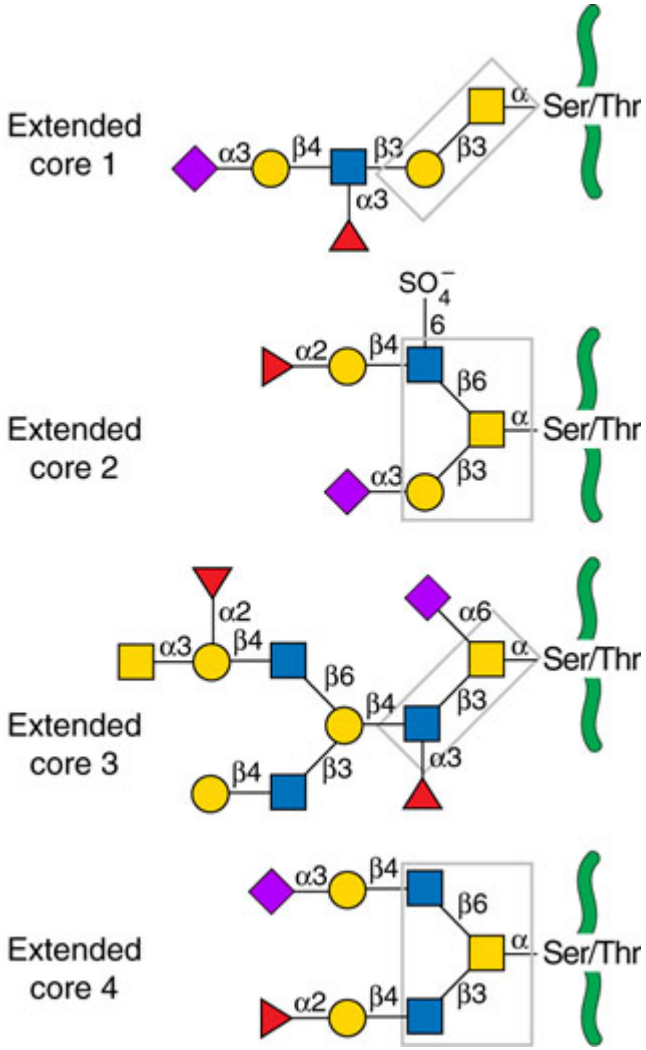
O-linked glycoprotein glycans

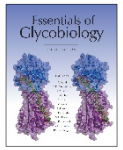


Taylor & Drickamer (2011) Introduction to Glycobiology, 3rd edition

O-linked glycan structures



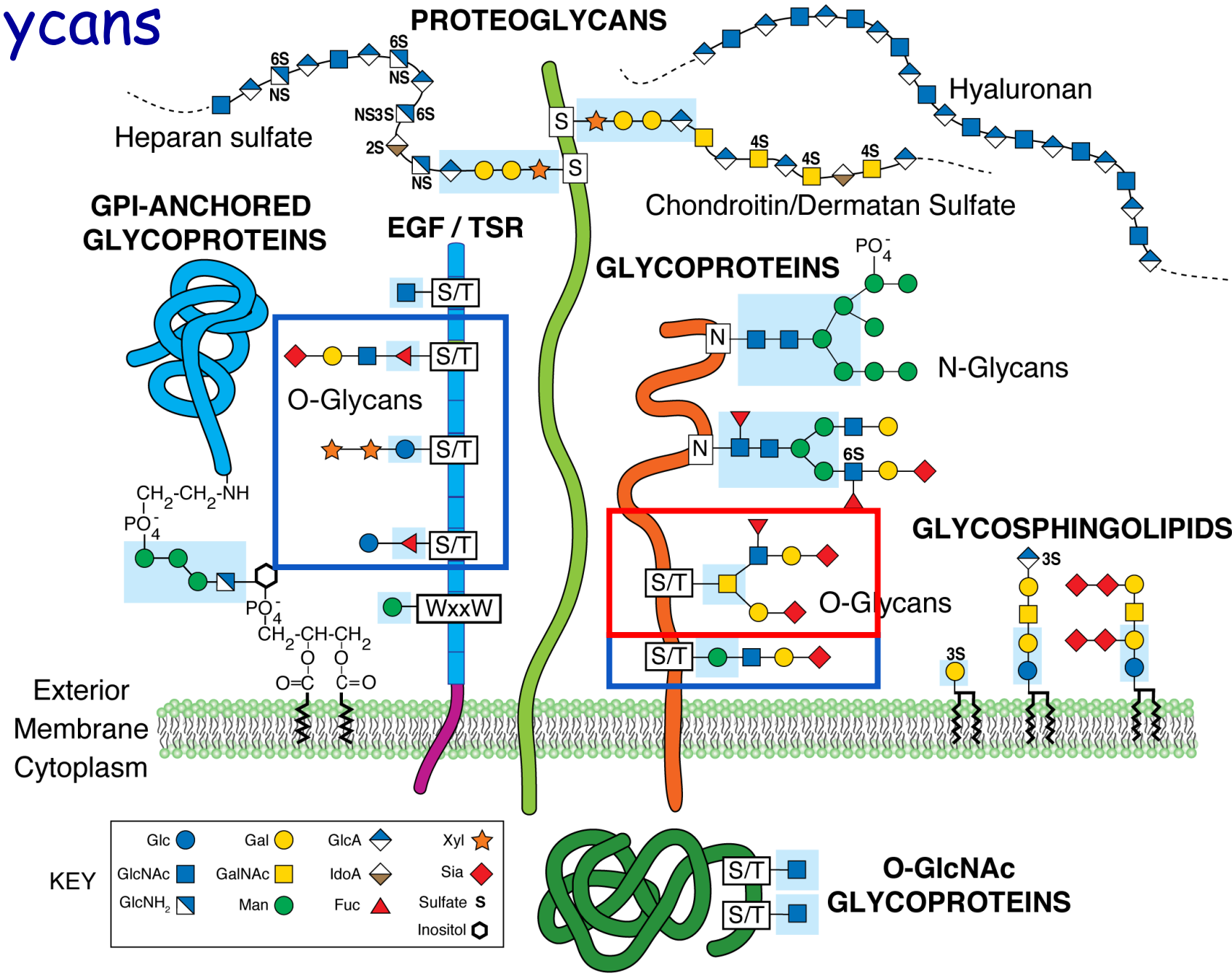




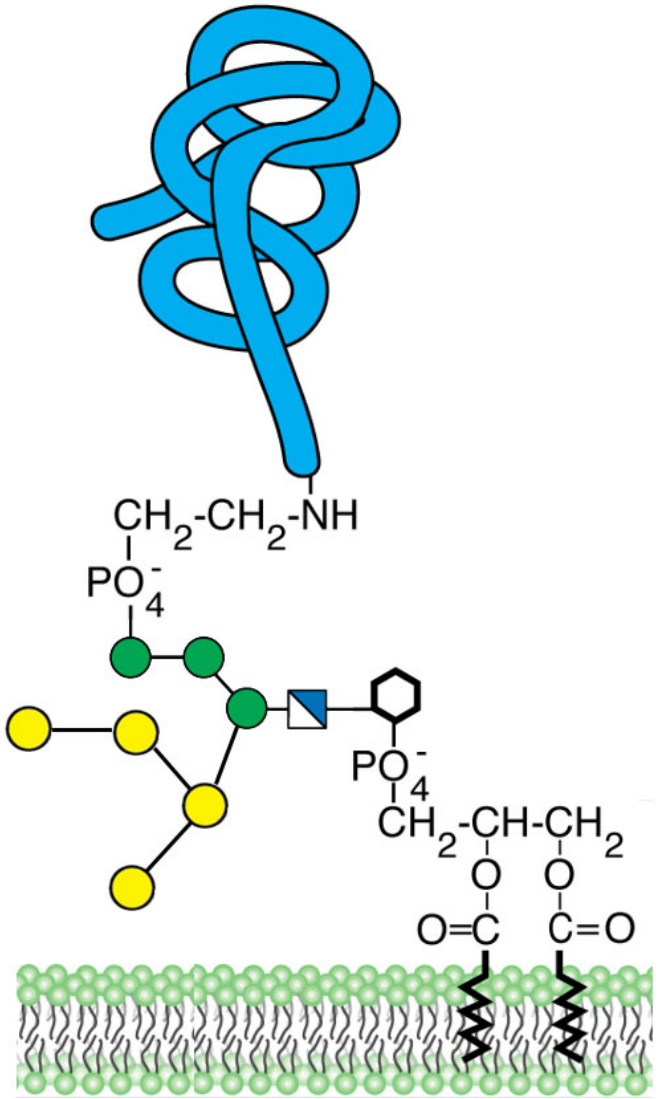
Essentials of Glycobiology
Third Edition

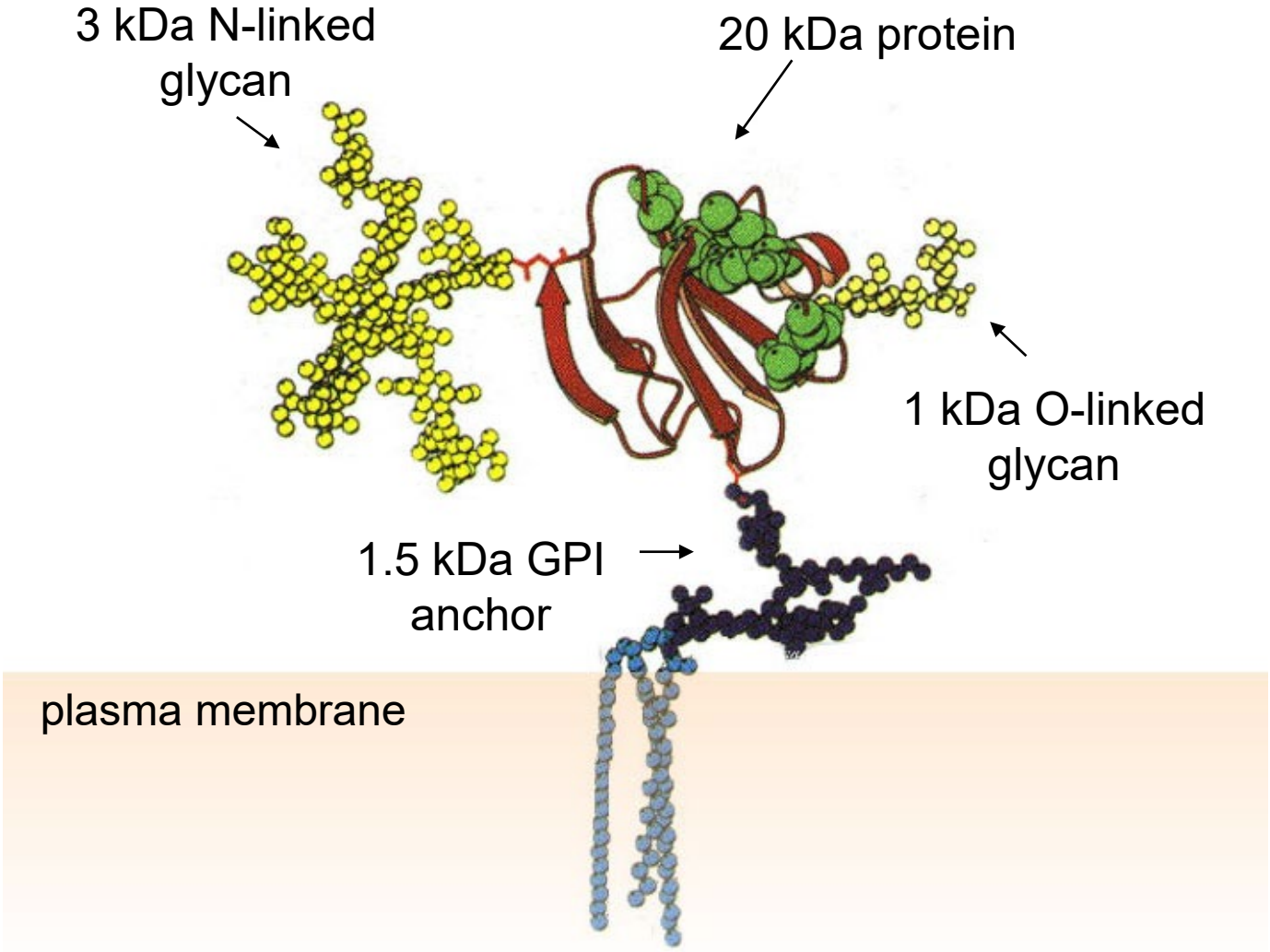
Mammalian glycans

- Glycoproteins
  - N-linked
  - O-linked
  - GPI-anchored
  - O-GlcNAc

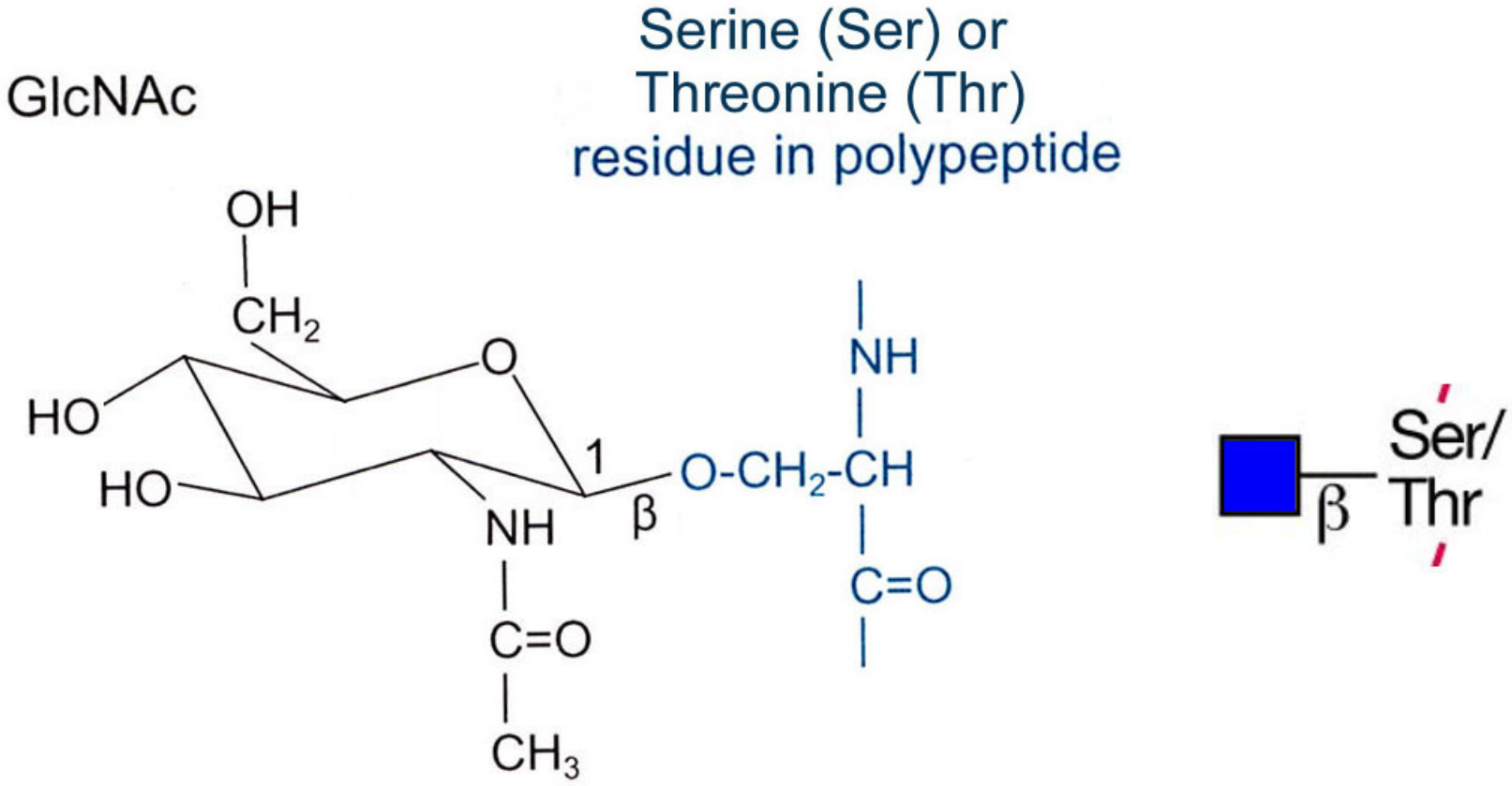


Glycophosphatidylinositol (GPI) anchor





O-GlcNAc

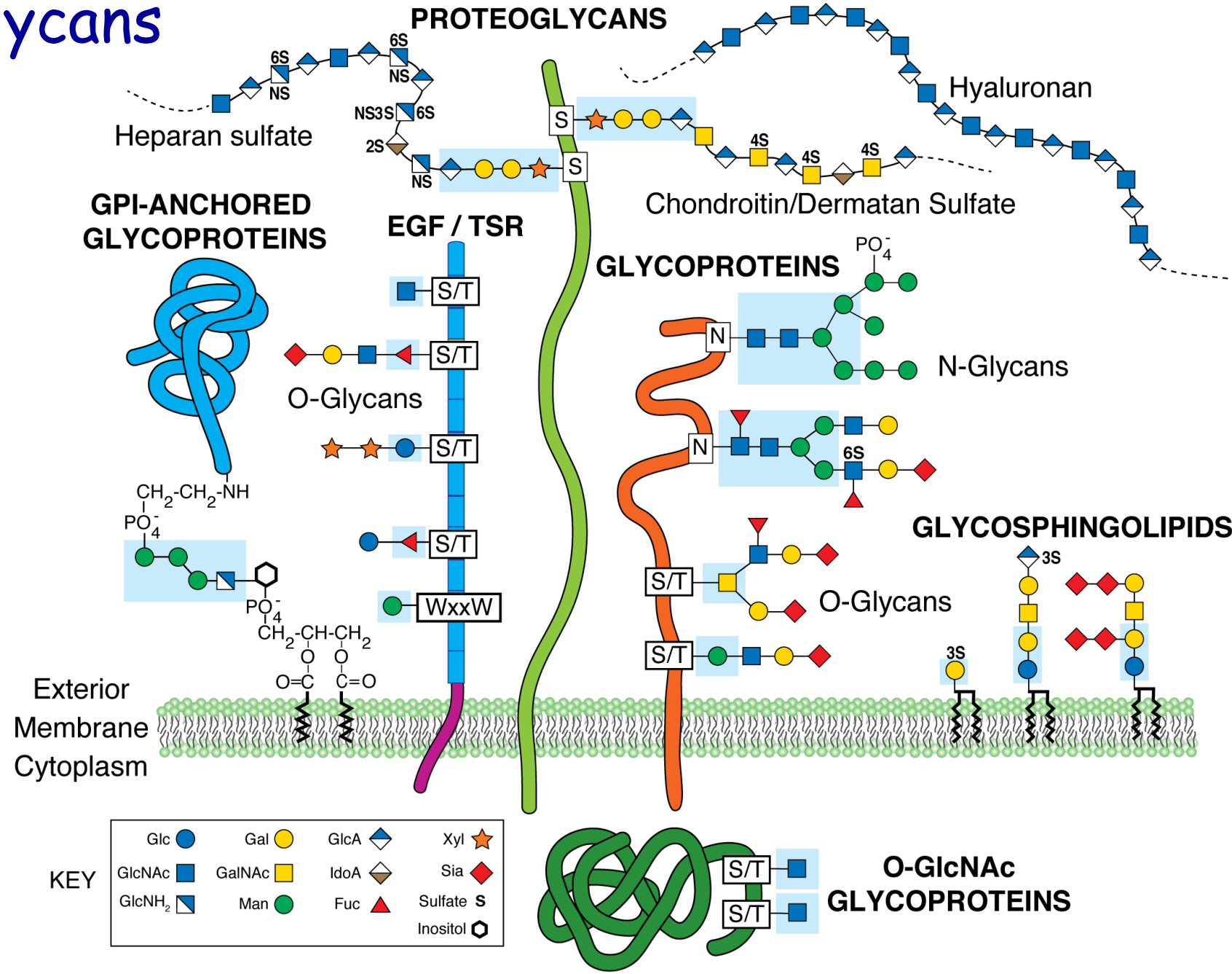


Note: No further sugar substitutions have been confirmed
Highly dynamic post-translational modification!

modified from Taylor & Drickamer (2011) Introduction to Glycobiology, 3rd edition

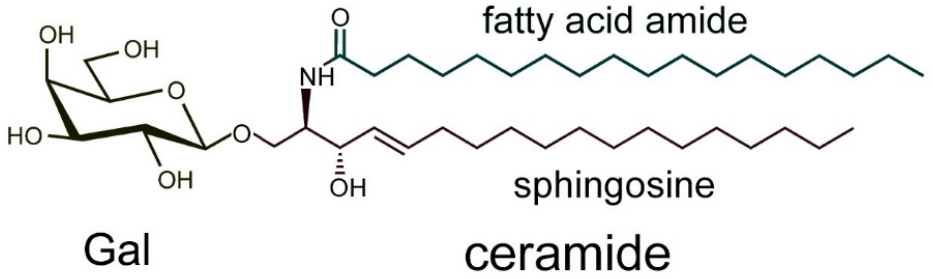
Mammalian glycans

- Glycoproteins
- Glycolipids
- Proteoglycans

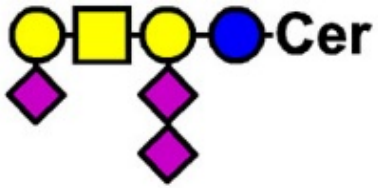


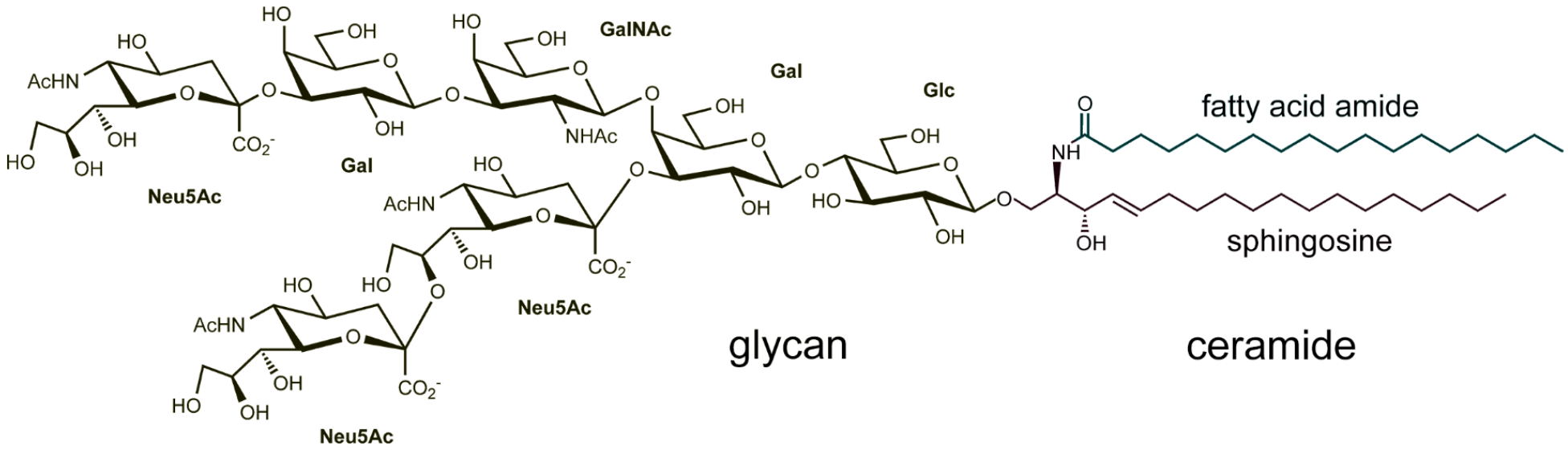
Glycosphingolipids

GalCer



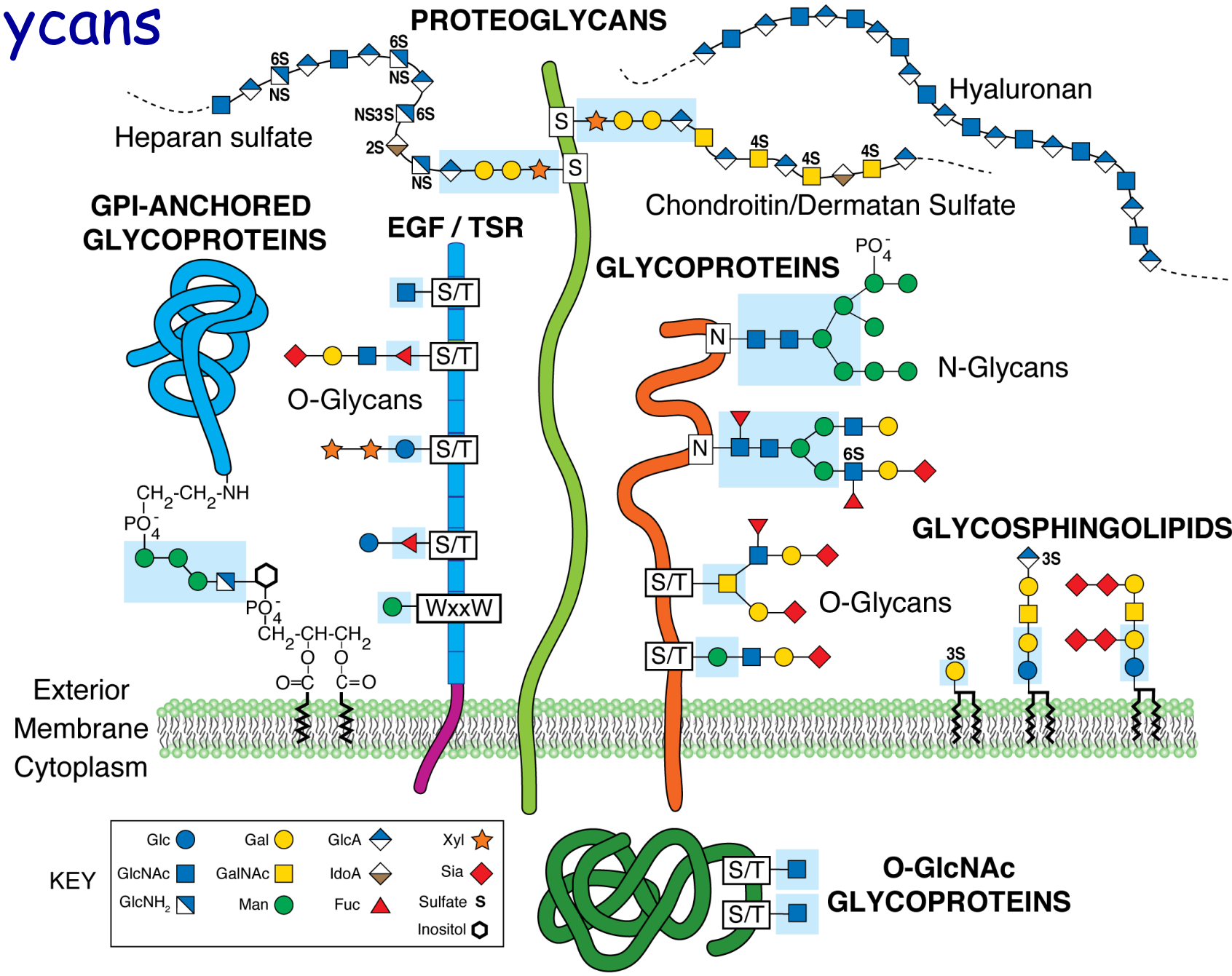
Ganglioside GT1b





Mammalian glycans

- Glycoproteins
- Glycolipids
- Proteoglycans

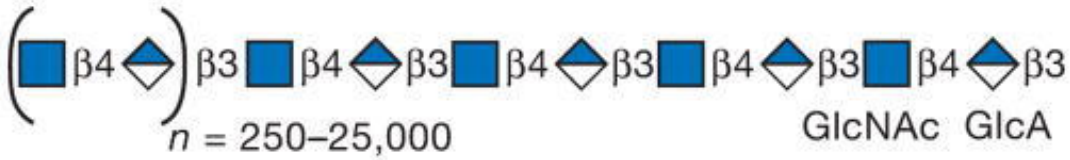


Glycosaminoglycans & Proteoglycans

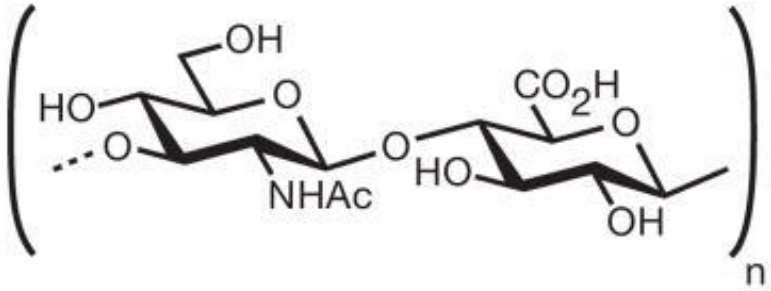
- GLYCOSAMINOGLYCANS (GAG's)
  - long linear glycans made of characteristic repeating disaccharides
  - hyaluronic acid is the only "stand-alone" GAG, other GAG's are constituents of …
- PROTEOGLYCANS
  - GAG's on proteins
  - Defined by their repeating disaccharide units
  - GAG's on proteoglycans are sulfated

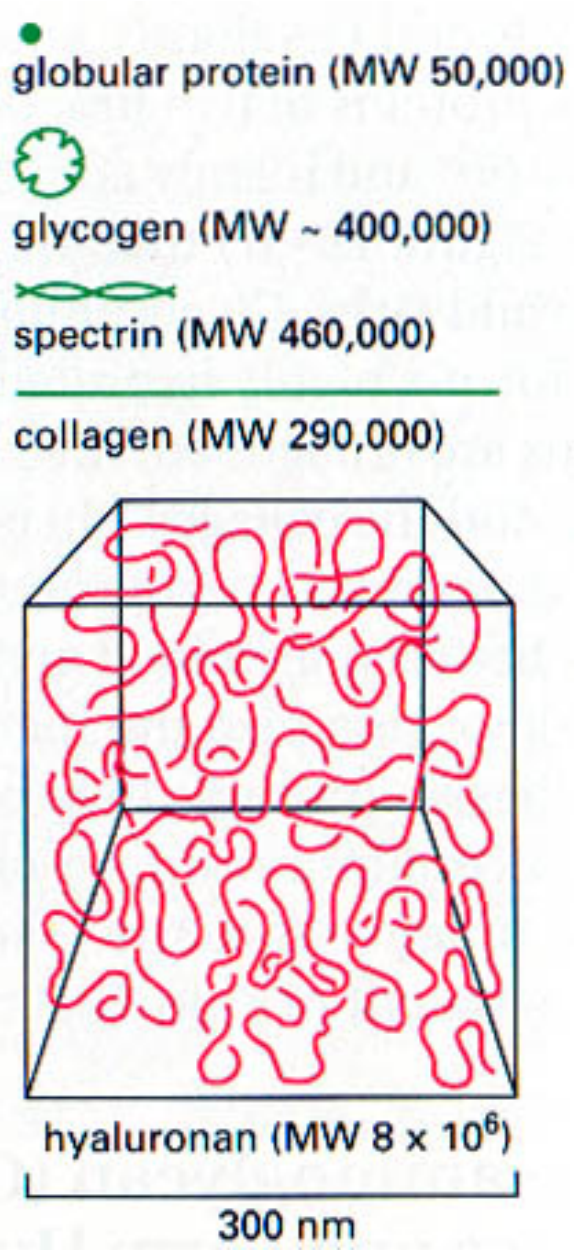
Hyaluronic acid

a space filling & signaling molecule

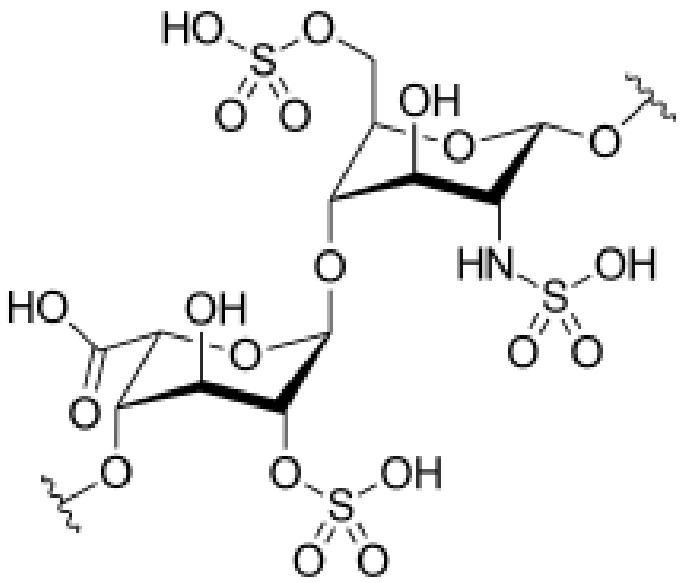


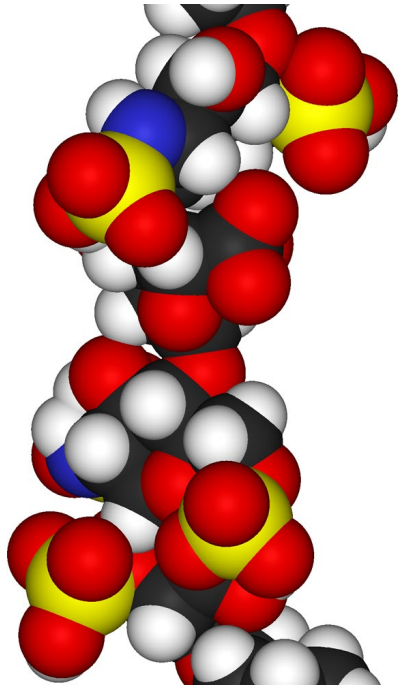
$(GlcU\ \beta4\ GlcNAc)_n$

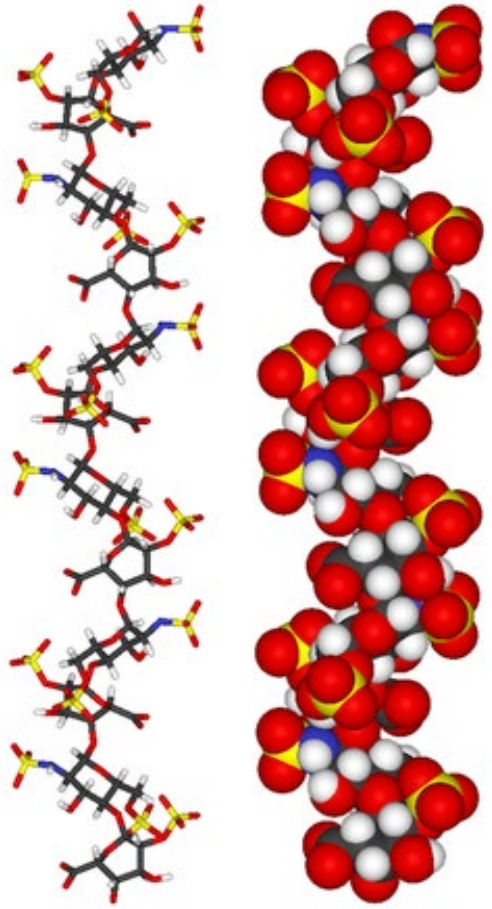


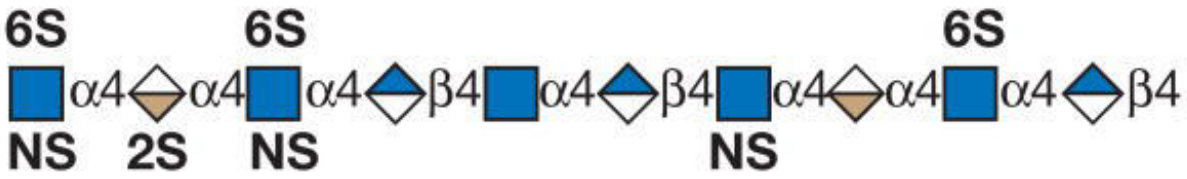


Proteoglcans –masters of post-polymerization modifications



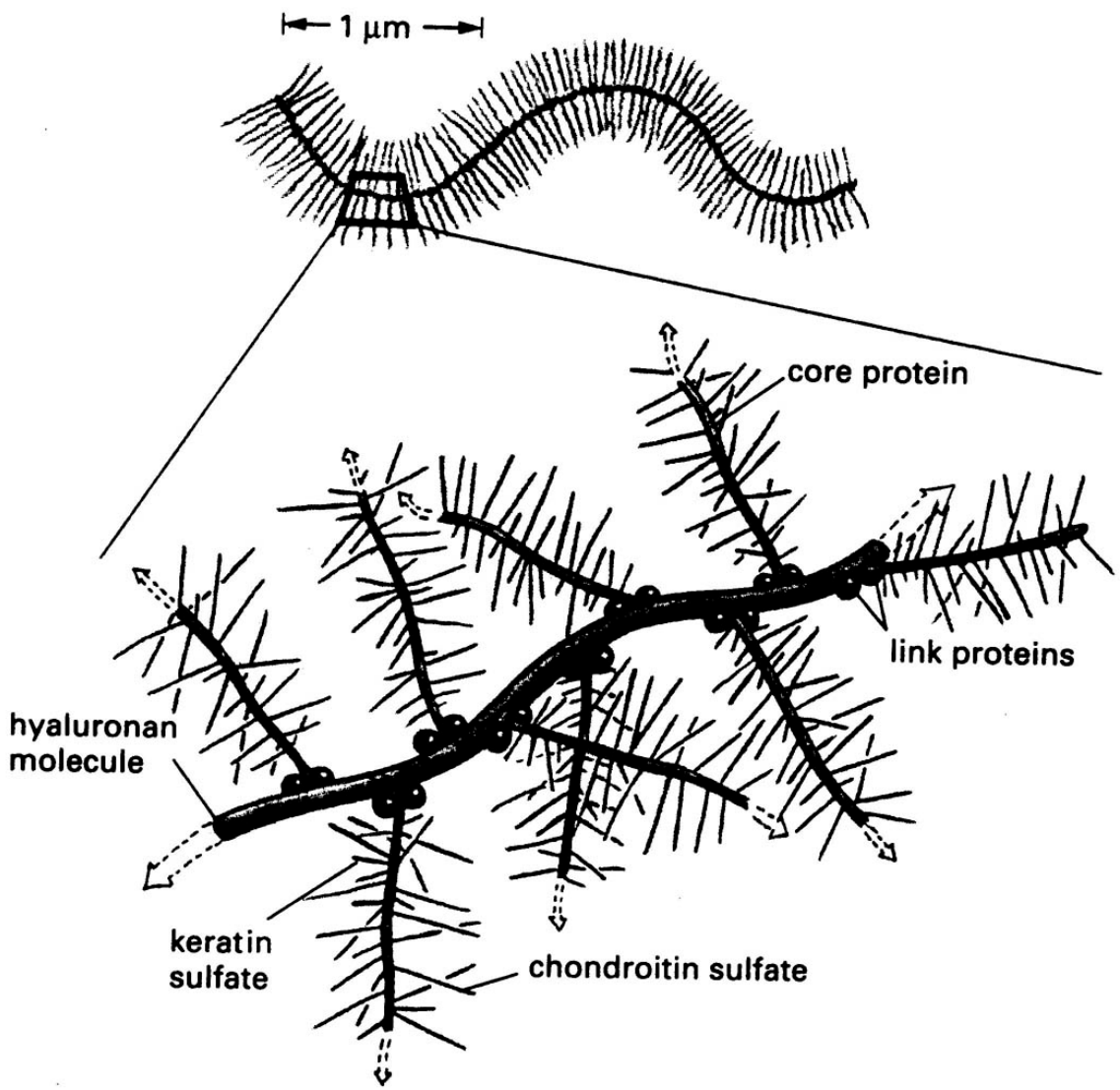


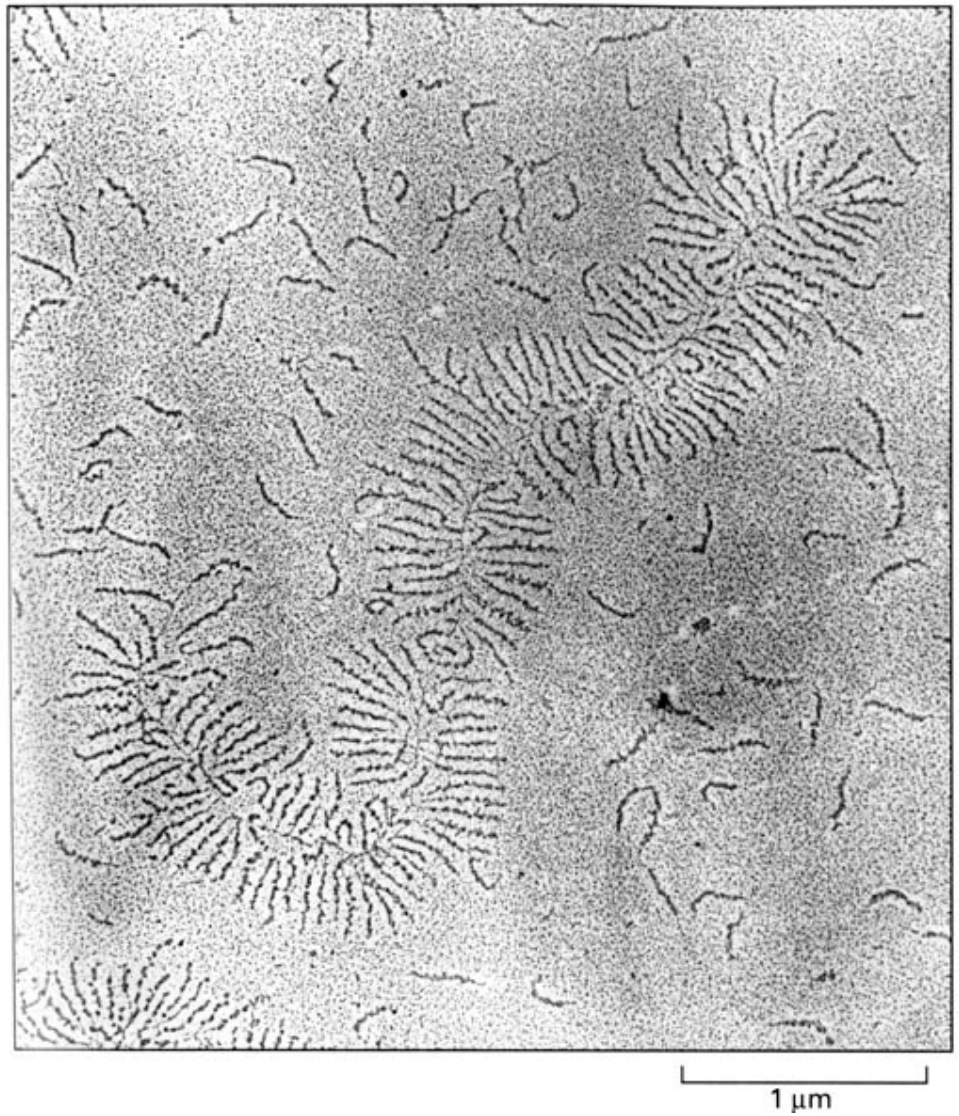




Heparan sulfate/heparin (HS)

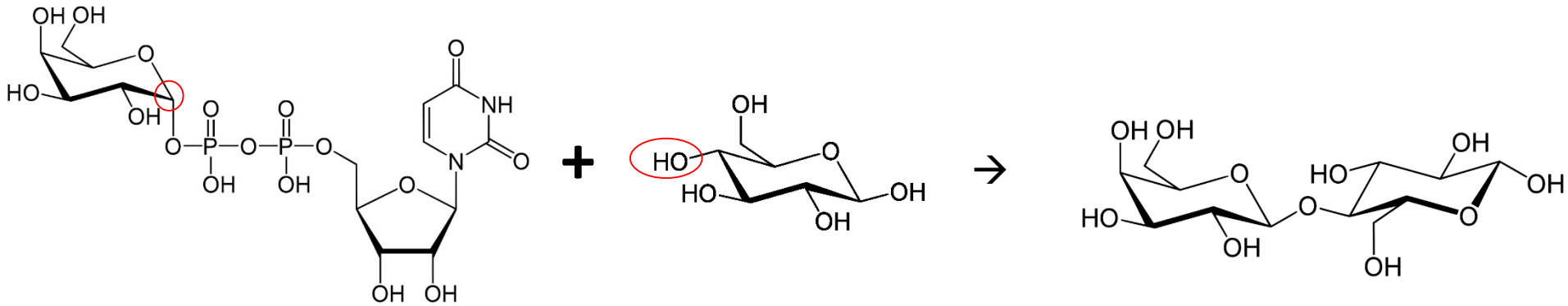
Proteoglycans – masters of molecular complexing





Principles of glycan biosynthesis

- Activated nucleotide sugar donors
- >200 glycosyltransferases (human genome)



UDP-Gal:glucose β4 galactosyltransferase

Activated Sugars:	UDP-Glc	UDP-GlcA
	UDP-Gal	UDP-Xyl
	GDP-Man	GDP-Fuc
	UDP-GlcNAc	CMP-NeuAc
	UDP-GalNAc	

Modes of glycan biosynthesis

- Glycoproteins
  - N-Linked – en-bloc preconstructed core, trimming, terminal elaboration
  - O-Linked – stepwise sugar by sugar addition
  - O-GlcNAc - dynamic transferase/glycosidase
  - GPI Anchor – en-bloc preconstructed core, elaboration
- Glycolipids
  - Stepwise sugar-by-sugar addition
- Proteoglycans
  - Stepwise sugar-by-sugar addition (core and repeating dissaccharide)
  - Post-polymerization modifications

Mammalian glycans

- Glycoproteins
- Glycolipids
- Proteoglycans