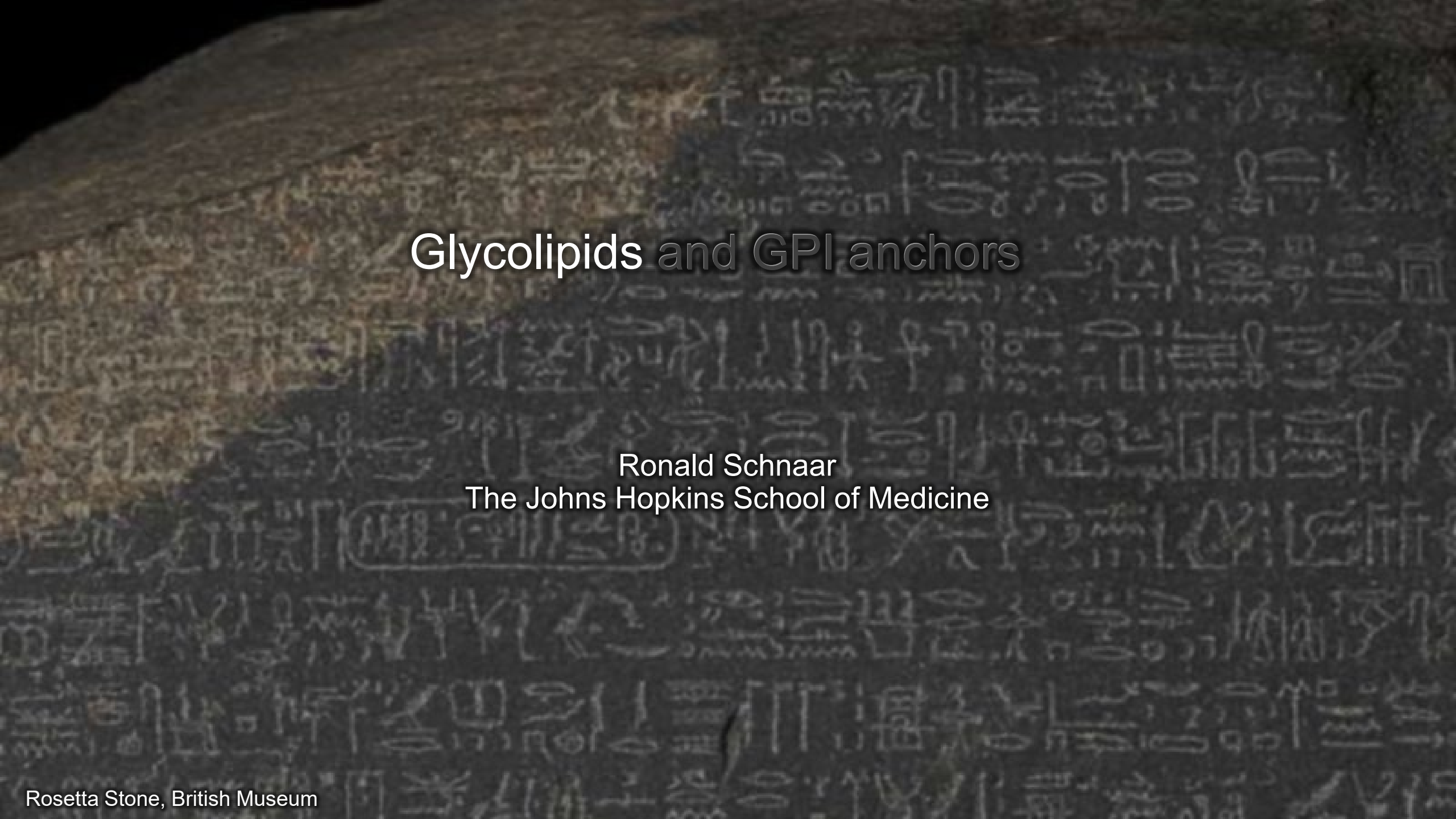
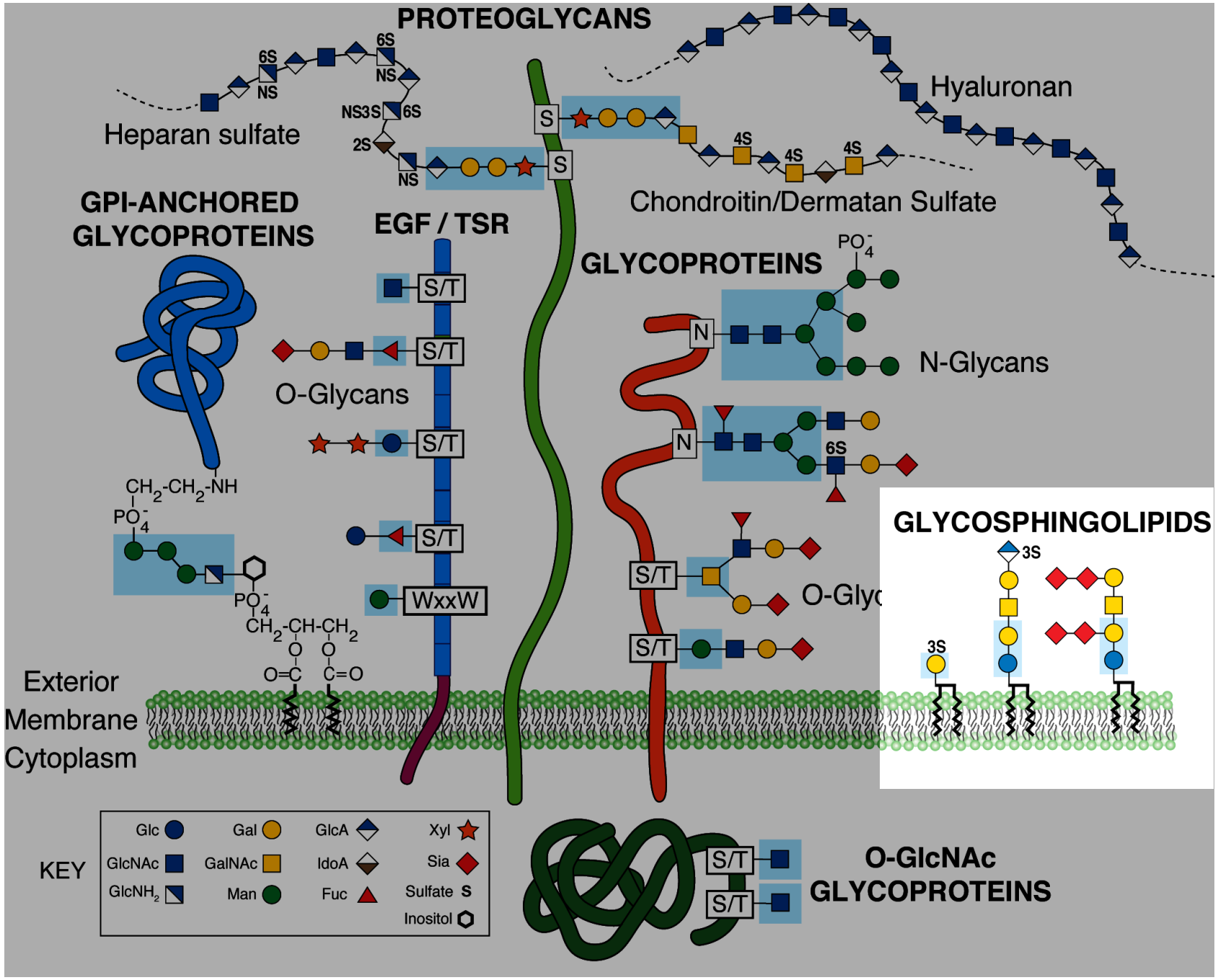
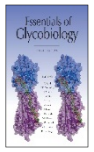


# Glycolipids and GPI anchors

Ronald Schnaar
The Johns Hopkins School of Medicine

Rosetta Stone, British Museum



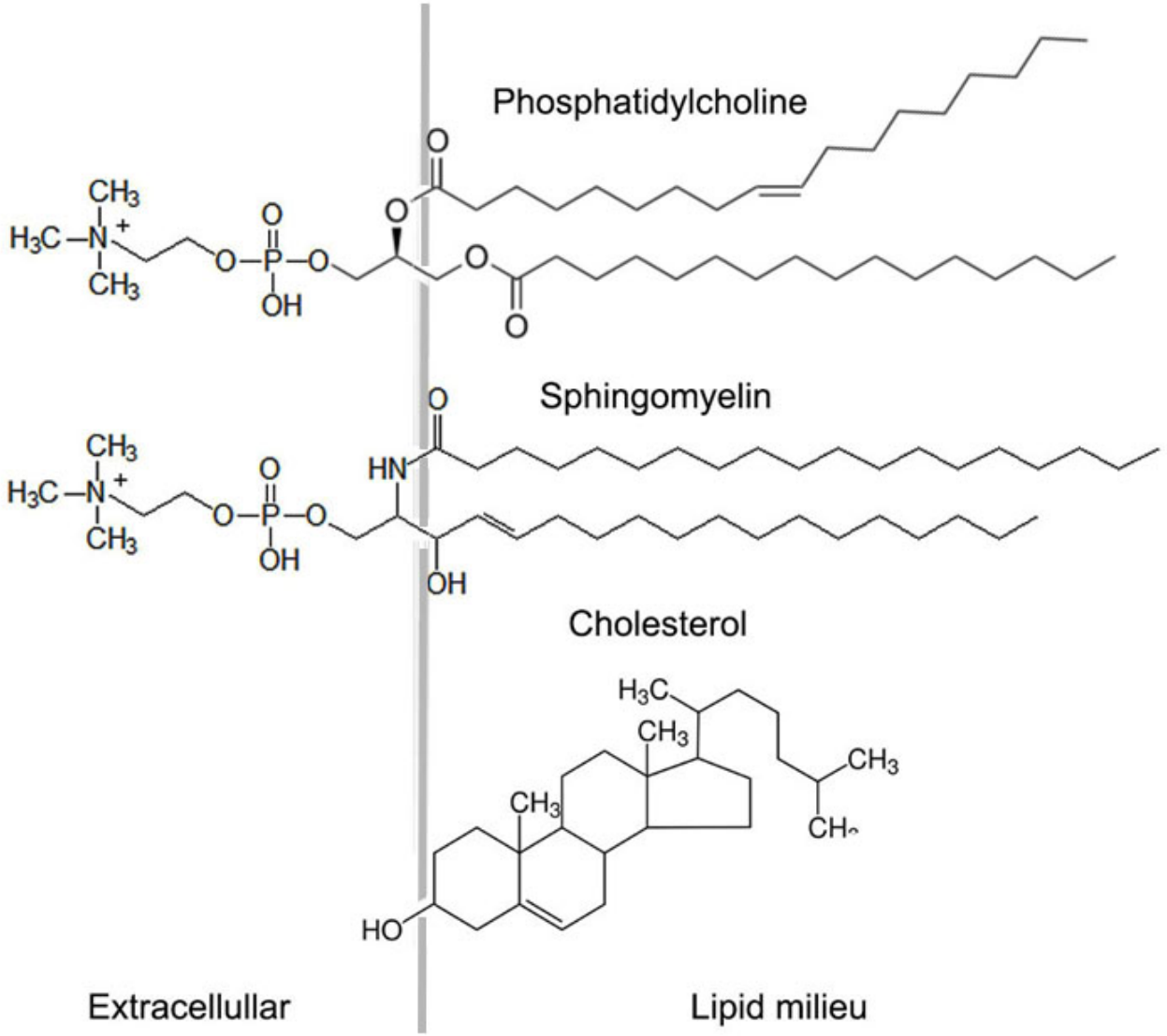



*Essentials of Glycobiology*
Third Edition

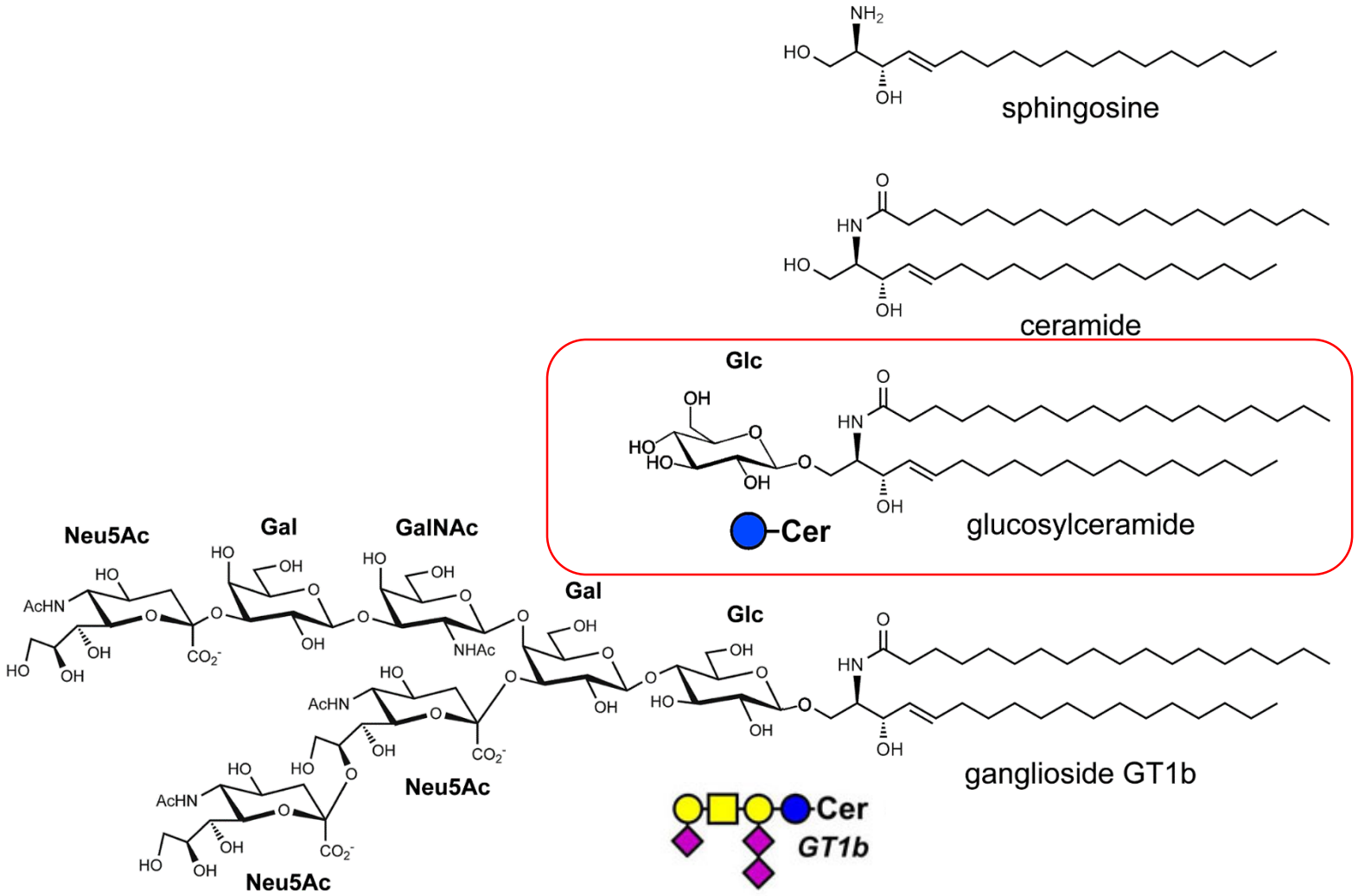
# Major membrane lipid classes

Glycerolipid

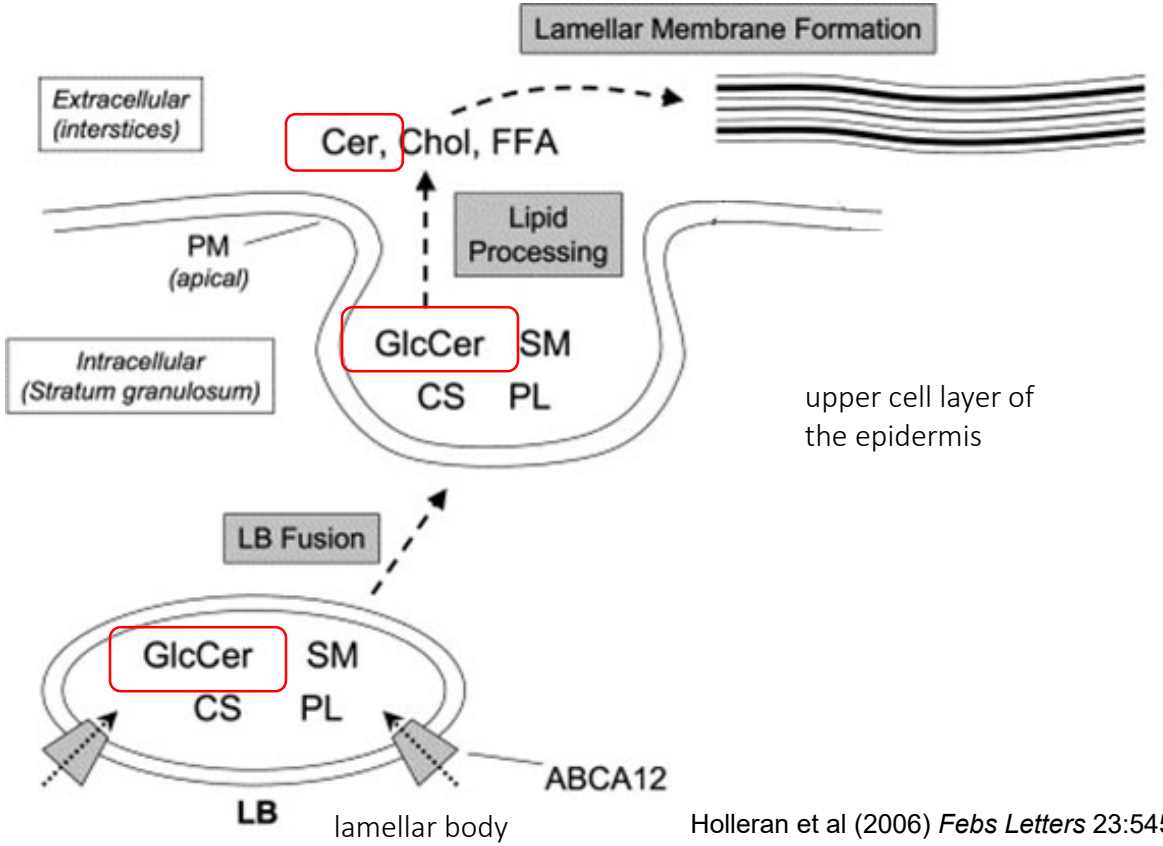
Sphingolipid



# Glycosphingolipids



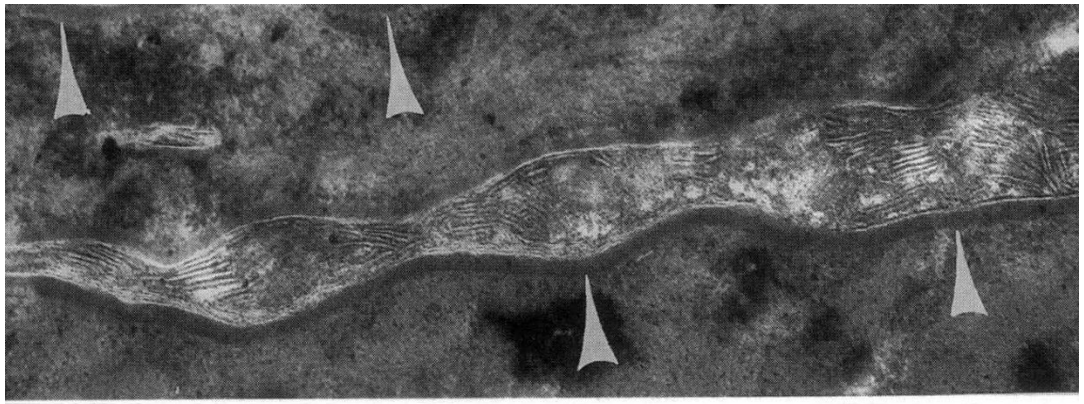
# GlcCer is a key intermediate in skin water barrier formation




Holleran et al (2006) *Febs Letters* 23:5456

Glucosylceramide, sphingomyelin, glycerophospholipids, and cholesterol sulfate, are packaged into lamellar bodies (LB) in the upper epidermis. Fusion with the apical plasma membrane in the uppermost layer of the epidermis (stratum granulosum), releases lipid precursors extracellularly. Enzymatic processing generates the major lipid classes required for epidermal water barrier function.

# GlcCer breakdown by glucocerebrosidase is required to form the skin water barrier

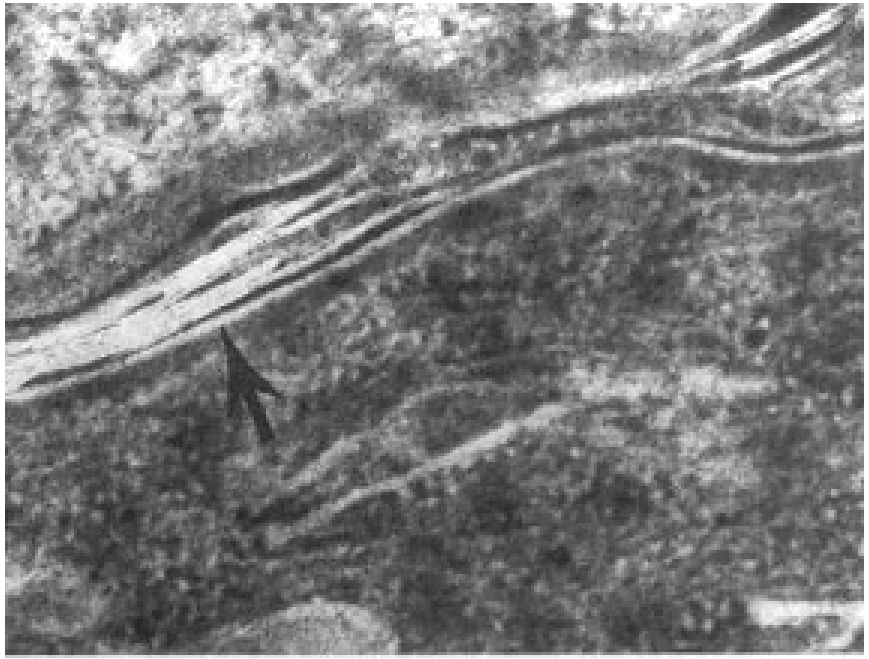


"Gaucher"
mouse skin
(glucocerebrosidase -/-)

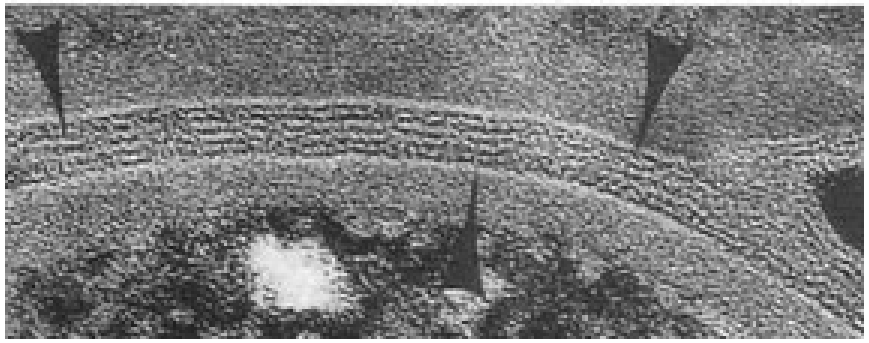
Normal mouse skin

Holleran et al (1994)
*J Clin Invest* 93:1756

# Human skin barrier in severe (type 2) Gaucher disease



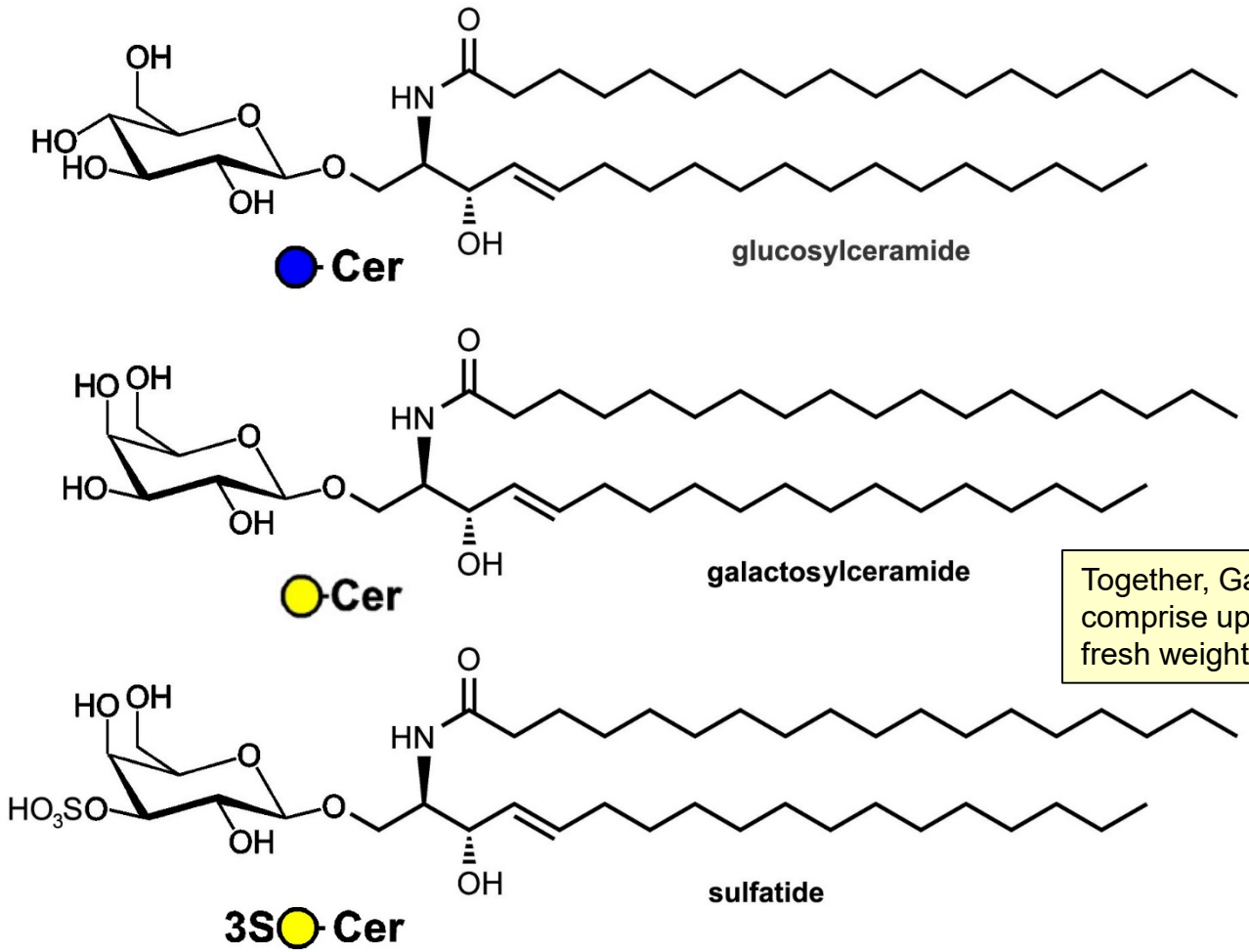
Human type 2 Gaucher disease skin
(glucocerebrosidase-deficient)



Normal human skin

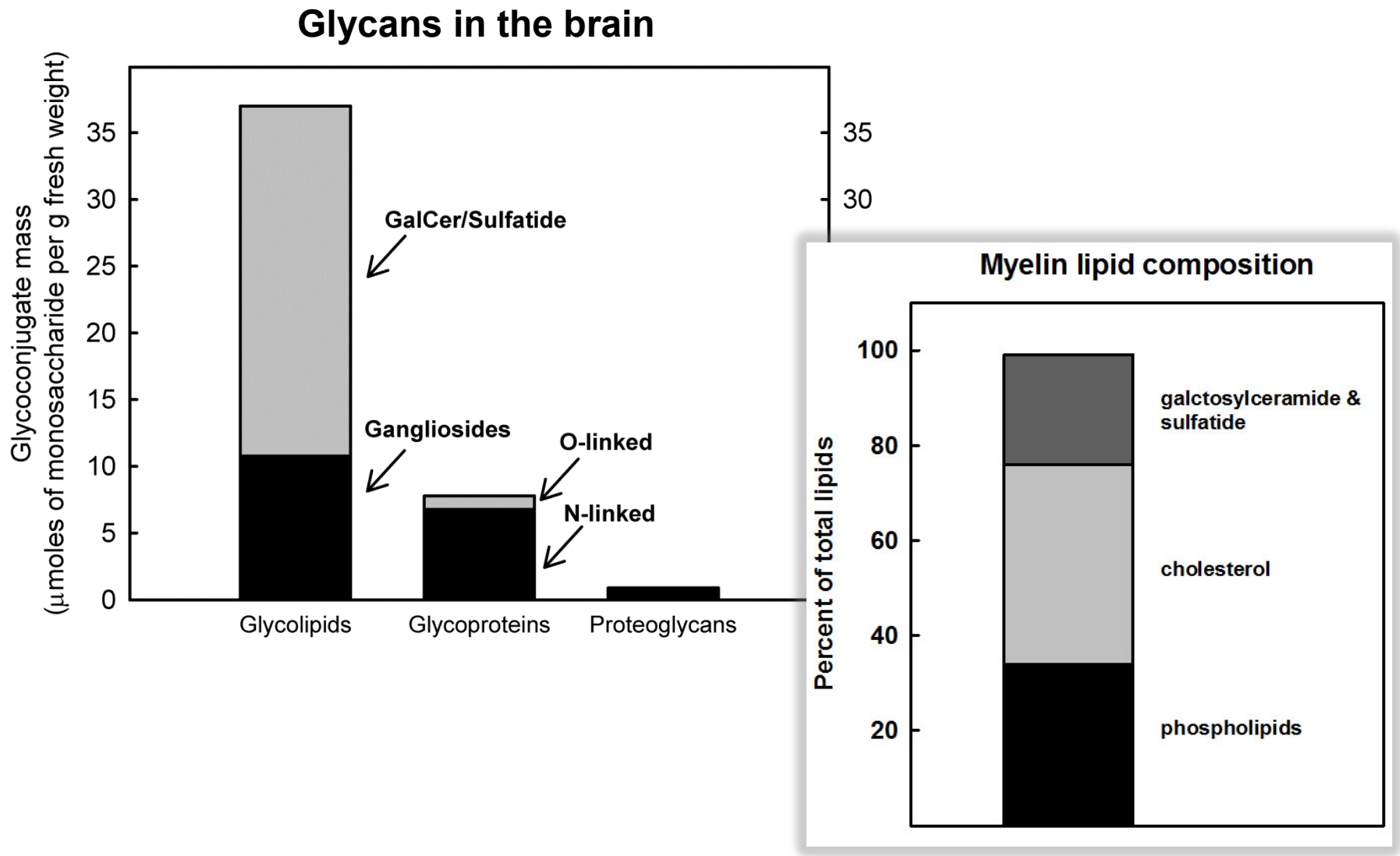
Holleran et al (1994) *J Clin Invest* 93:1756

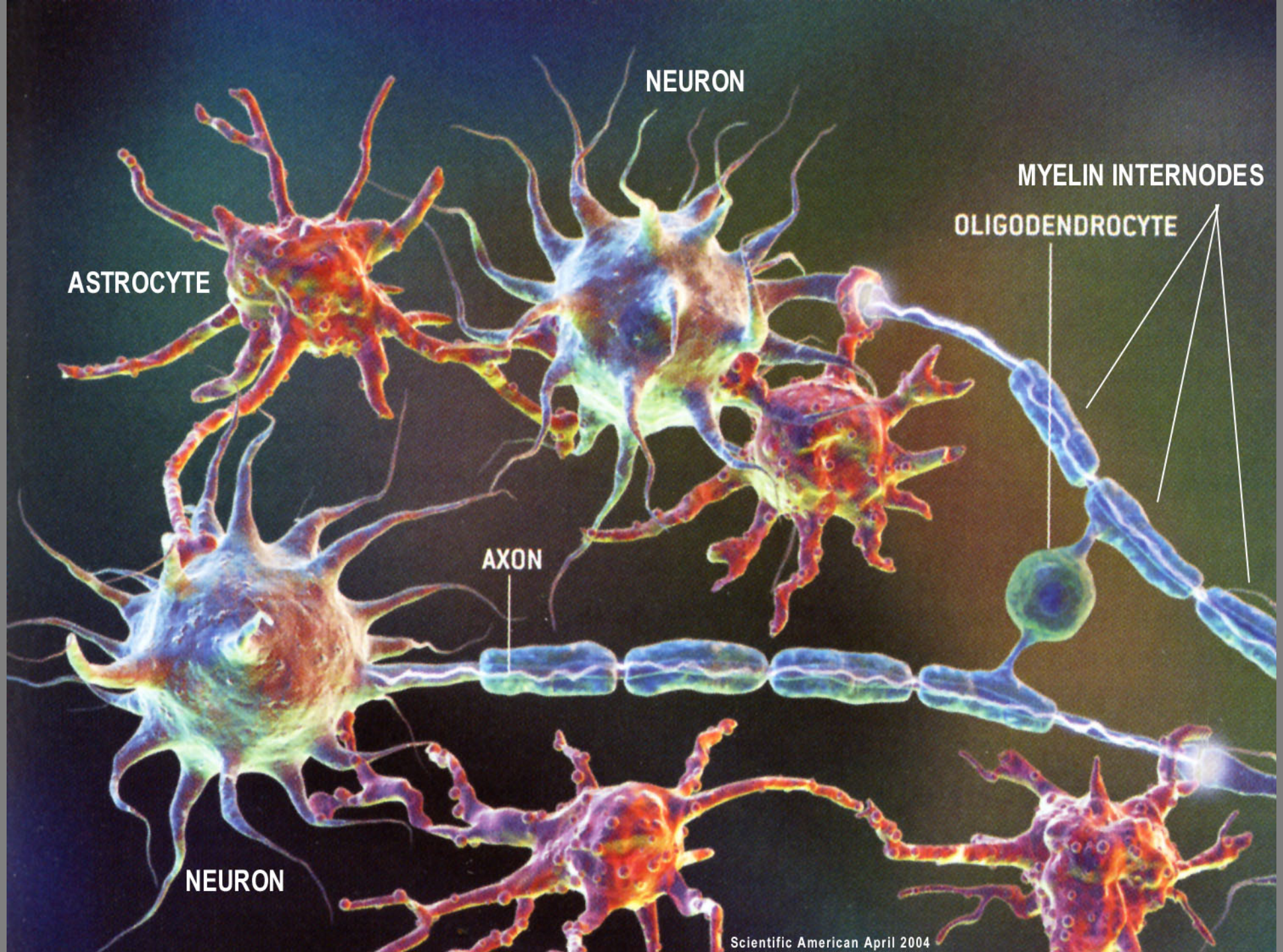
# GalCer and it's 3-sulfated from, sulfatide, are major lipids in the brain



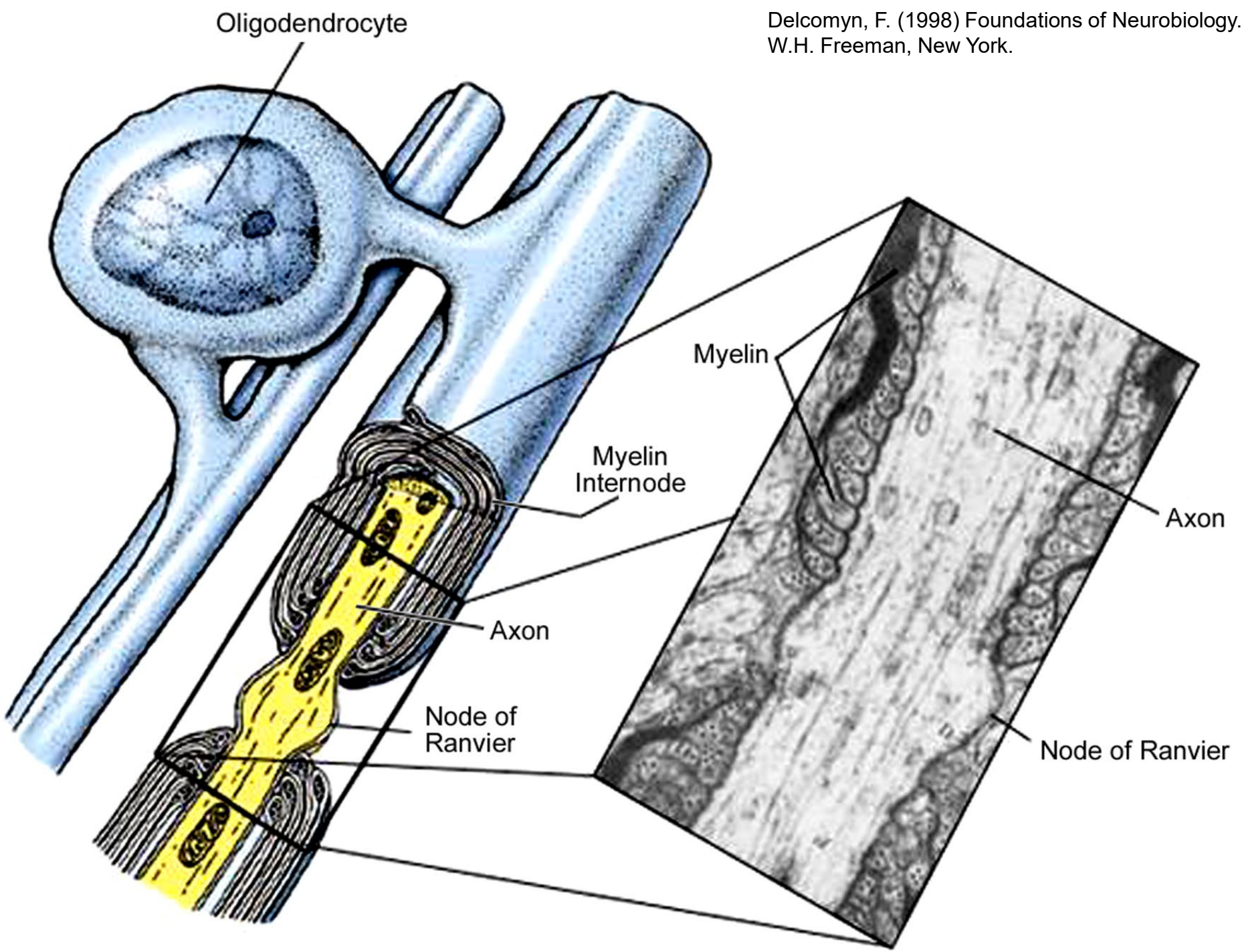

Together, GalCer and sulfatide comprise up to 1.9% of brain fresh weight!

# GalCer & sulfatide are the major glycans in the brain, and major lipids in myelin



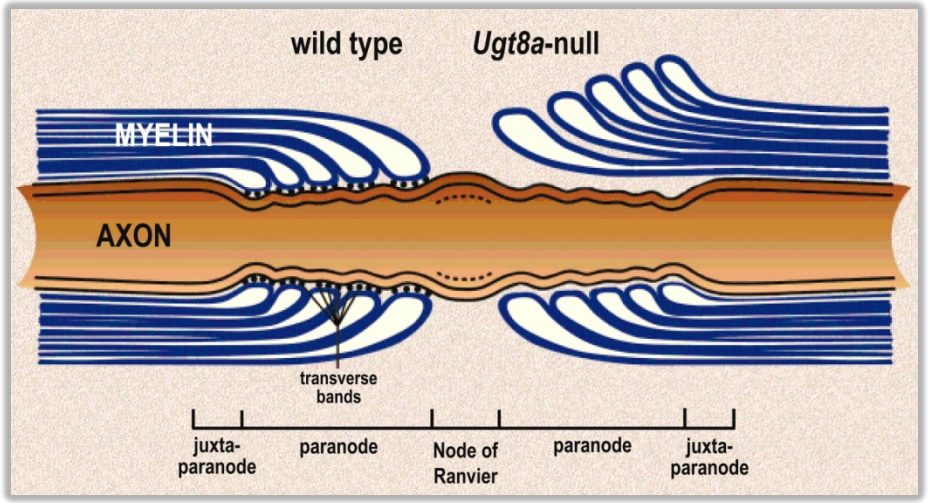

NEURON
MYELIN INTERNODES
OLIGODENDROCYTE
ASTROCYTE
AXON
NEURON
Scientific American April 2004

Delcomyn, F. (1998) Foundations of Neurobiology. W.H. Freeman, New York.

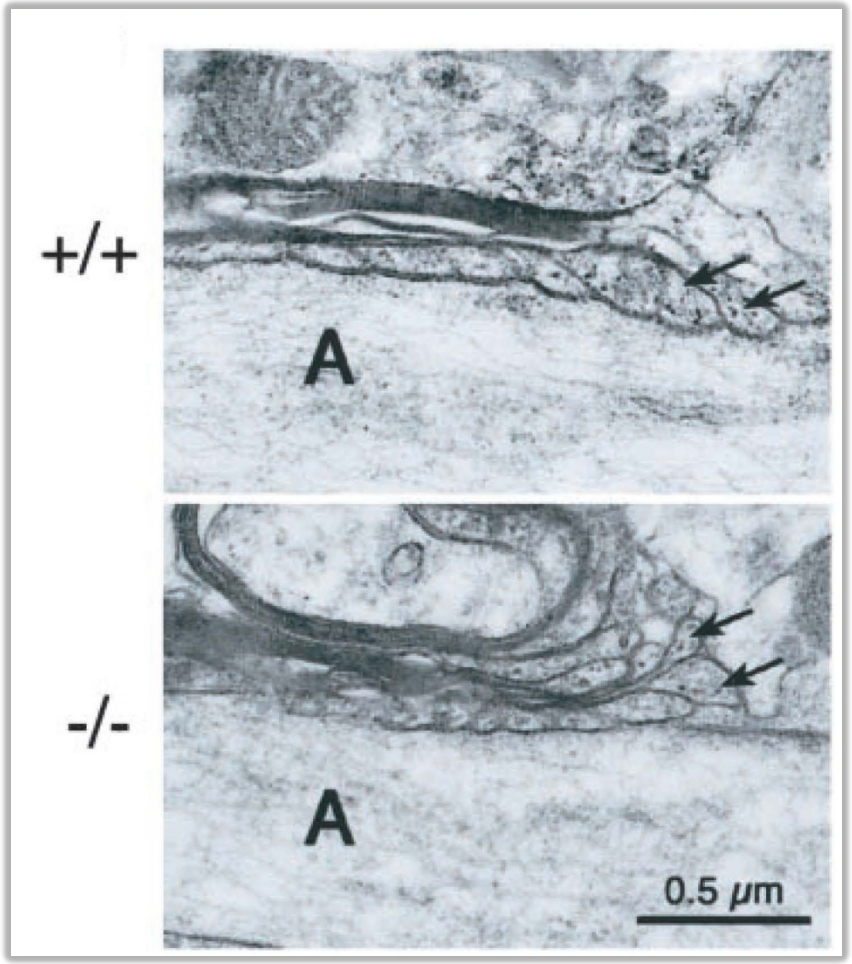


# Mutant mice lacking GalCer synthetase (Ugt8a) or GalCer 3-sulfotransferase (Gal3st1) display myelin defects and associated behavioral deficits

Honke et al (2002) *PNAS* 99:4227




Popko (2000) *Glia* 29:149

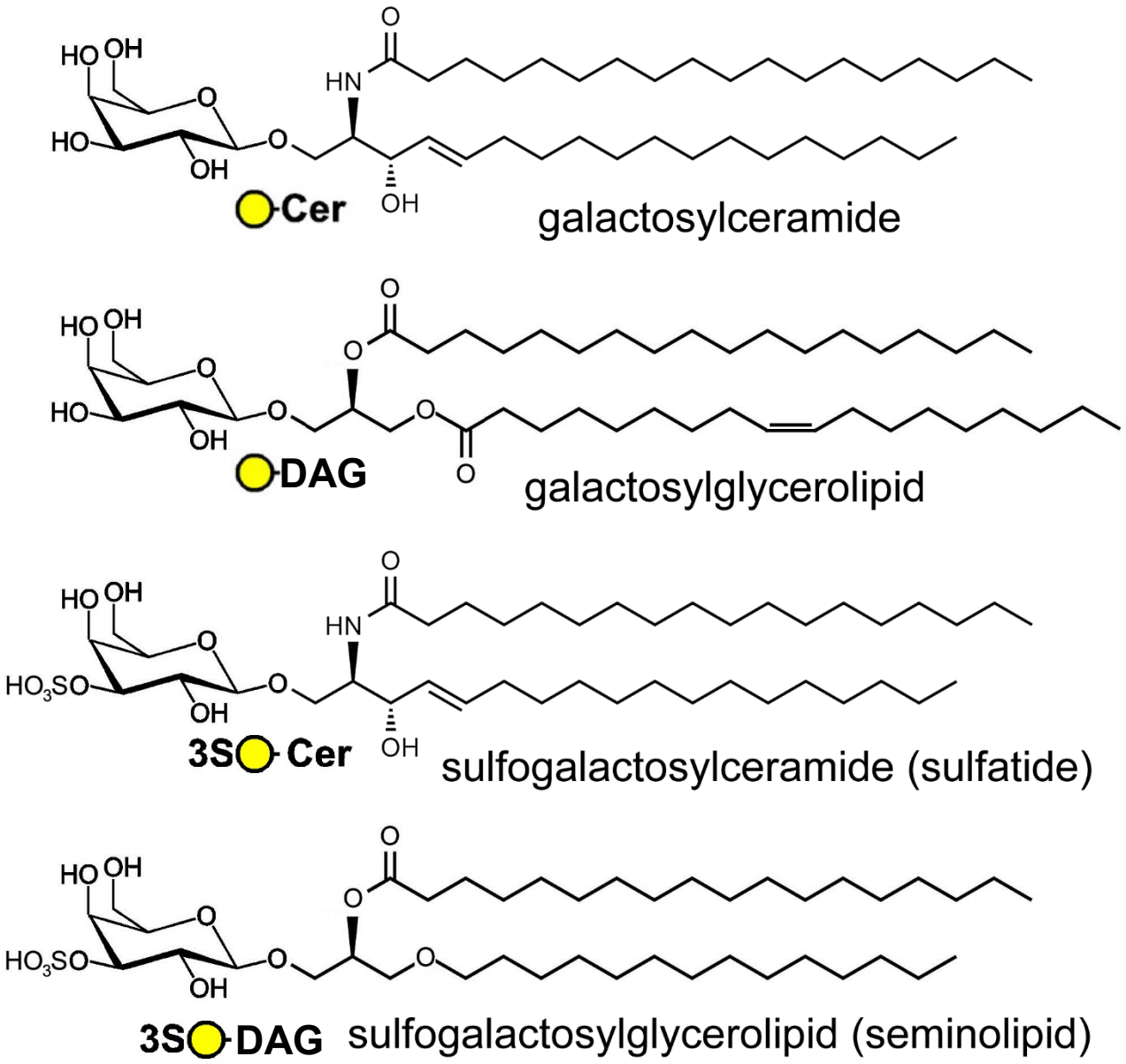



Honke et al (2002) *PNAS* 99:4227

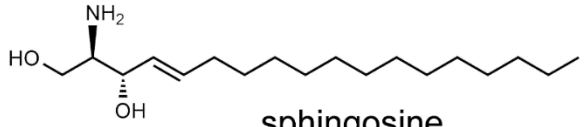
# Rare mammalian glycoglycerolipids

Glycoglycerolipids are not widely distributed in vertebrates, but 3-sulfated galactosyldiacylglycerol (seminolipid) makes up 90% of the glycolipid in mammalian testes.

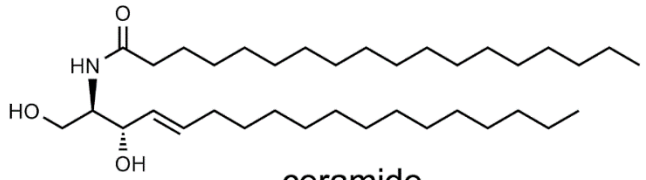
Gal3st1-null males are infertile due to an early block in spermatogenesis.



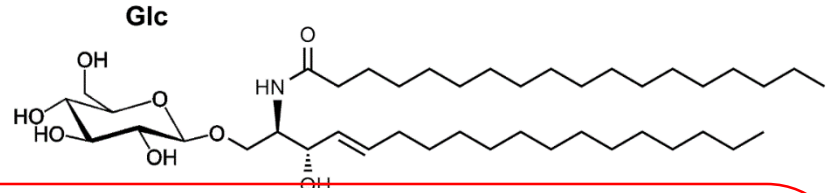
# Glycosphingolipids

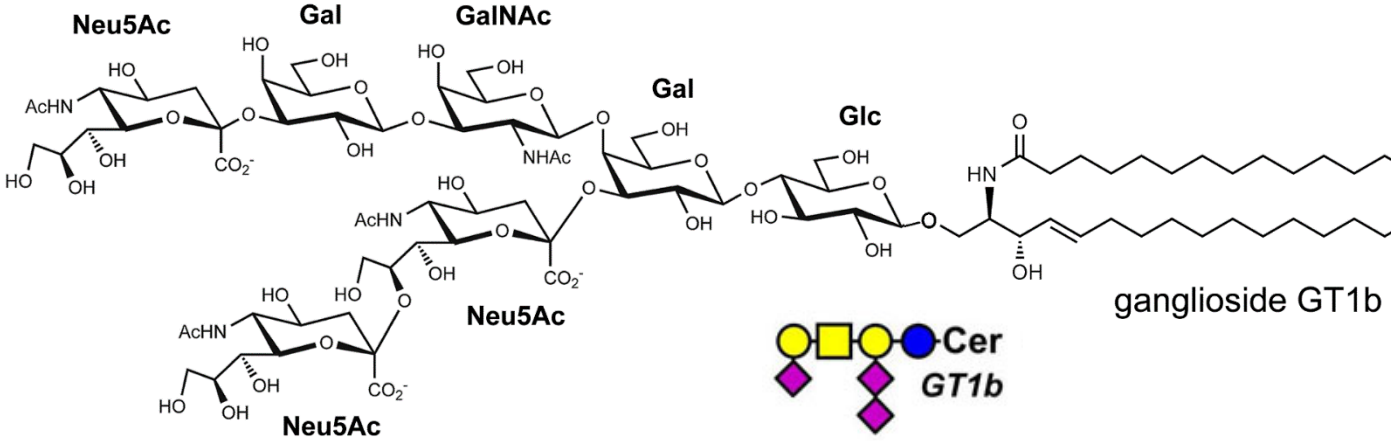



sphingosine



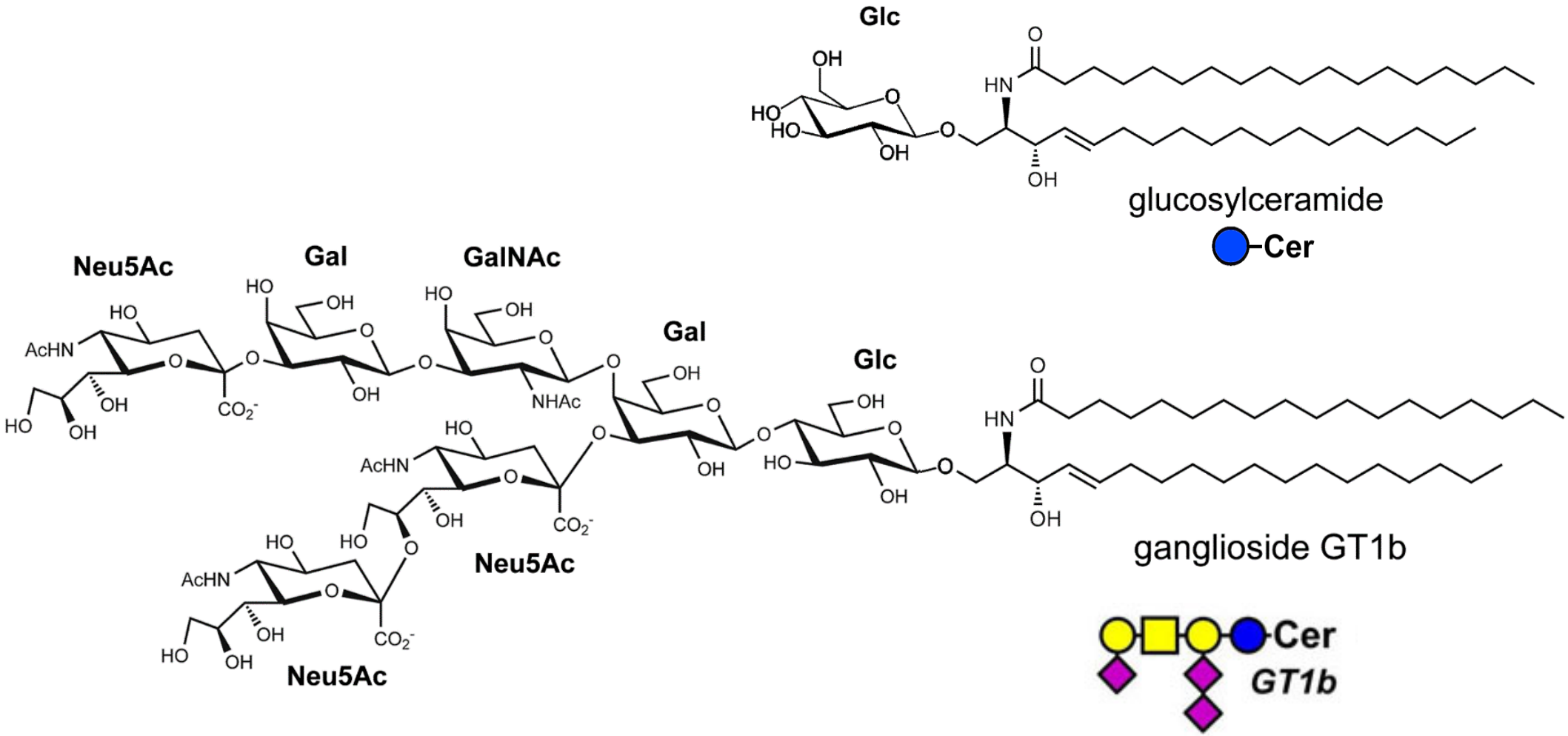

ceramide



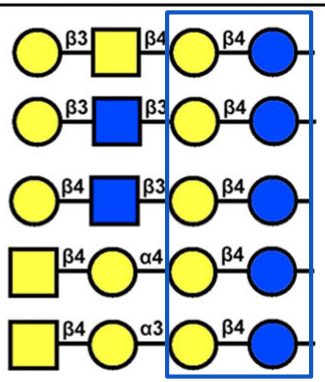

ganglioside GT1b

# Complex mammalian glycosphingolipids are extensions of GlcCer



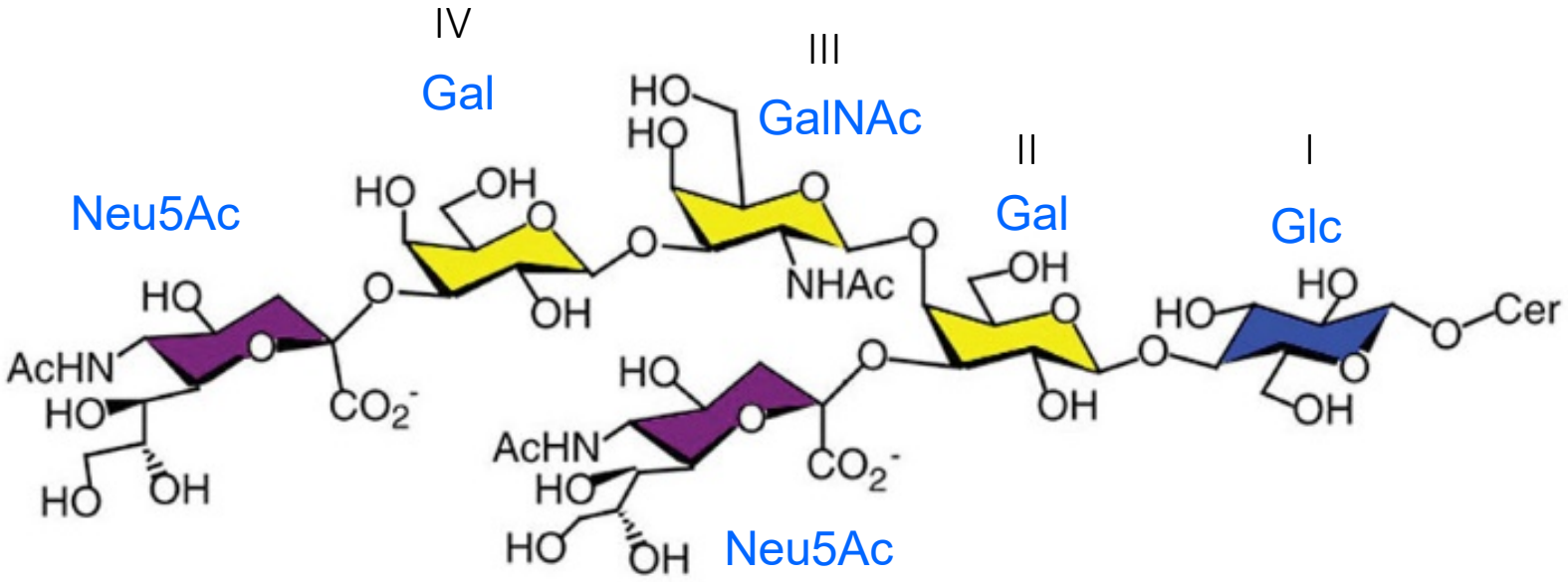
# Families of eukaryotic glycosphingolipids are named for their neutral tetrasaccharide cores

| | | sugars and linkages | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| series | symbol | IV | | III | | II | | I | | symbol nomenclature |
| ganglio | Gg | Gal | β3 | GalNAc | β4 | Gal | β4 | Glc | β1 | $Gg_4Cer$ |
| lacto | Lc | Gal | β3 | GlcNAc | β3 | Gal | β4 | Glc | β1 | $Lc_4Cer$ |
| neolacto | nLc | Gal | β4 | GlcNAc | β3 | Gal | β4 | Glc | β1 | $nLc_4Cer$ |
| globo | Gb | GalNAc | β3 | Gal | α4 | Gal | β4 | Glc | β1 | $Gb_4Cer$ |
| isoglobo | iGb | GalNAc | β3 | Gal | α3 | Gal | β4 | Glc | β1 | $iGb_4Cer$ |



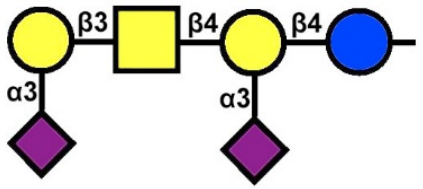

- Lactosylceramide disaccharide core
- Nomenclature based on the next two sugars in the tetrasaccharide core
- Classified as “neutral”, “sulfated” or “gangliosides” (sialylated)
- Nomenclature describes the length of the core and substituents via the sugar number (roman numeral)
- Biosynthesized stepwise by individual glycosphingolipids, some of which are glycolipid-specific, others of which also make glycoprotein glycans

# Nomenclature (example)




Neu5Ac α2-3 Gal β1-3 GalNAc β1-4 (Neu5Ac α2-3) Gal β1-4 Glc β1-1’ Cer

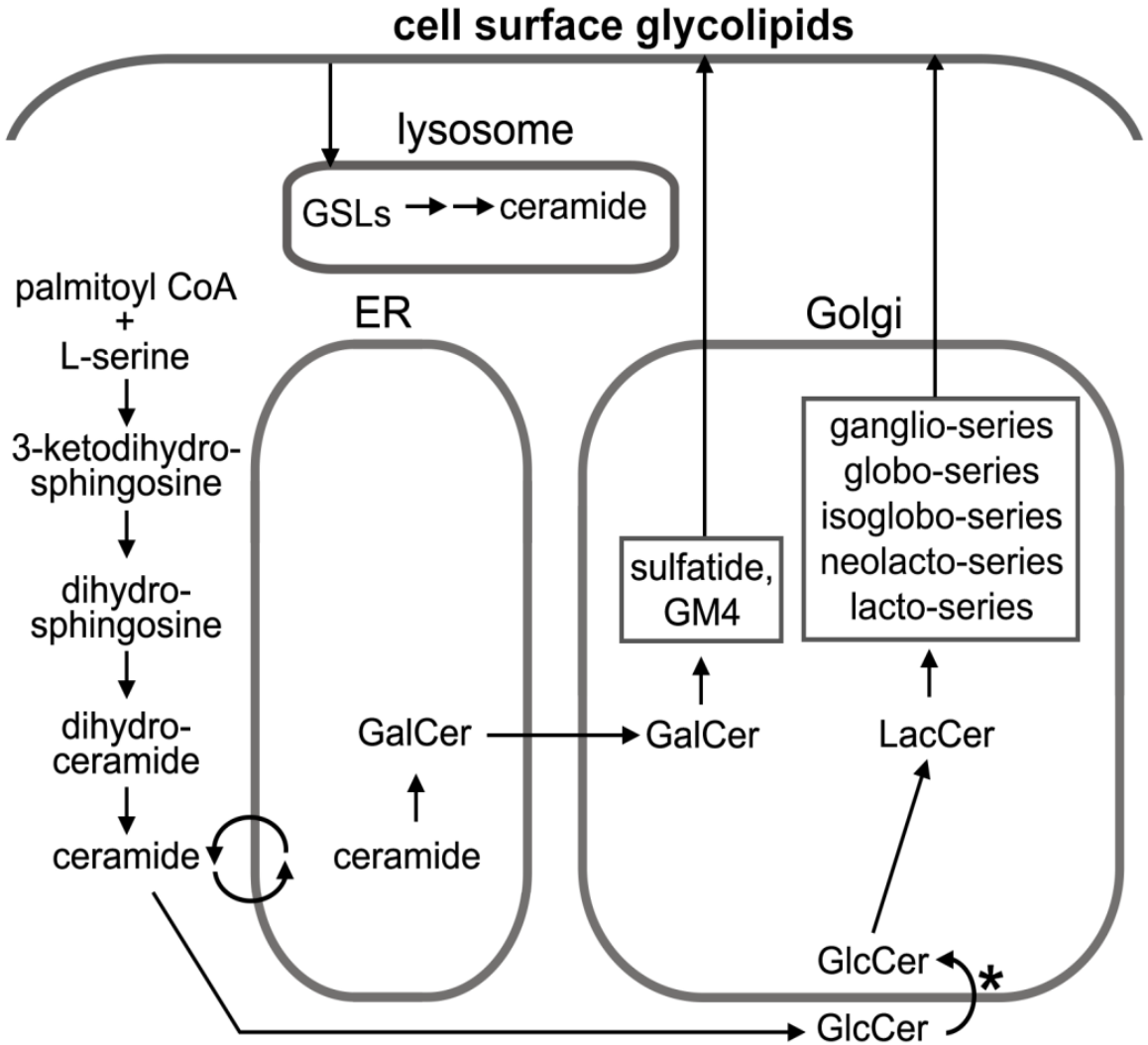
$IV^{3}Neu5Ac,II^{3}Neu5Ac\text{-}Gg_{4}Cer$ Shorthand




Symbol

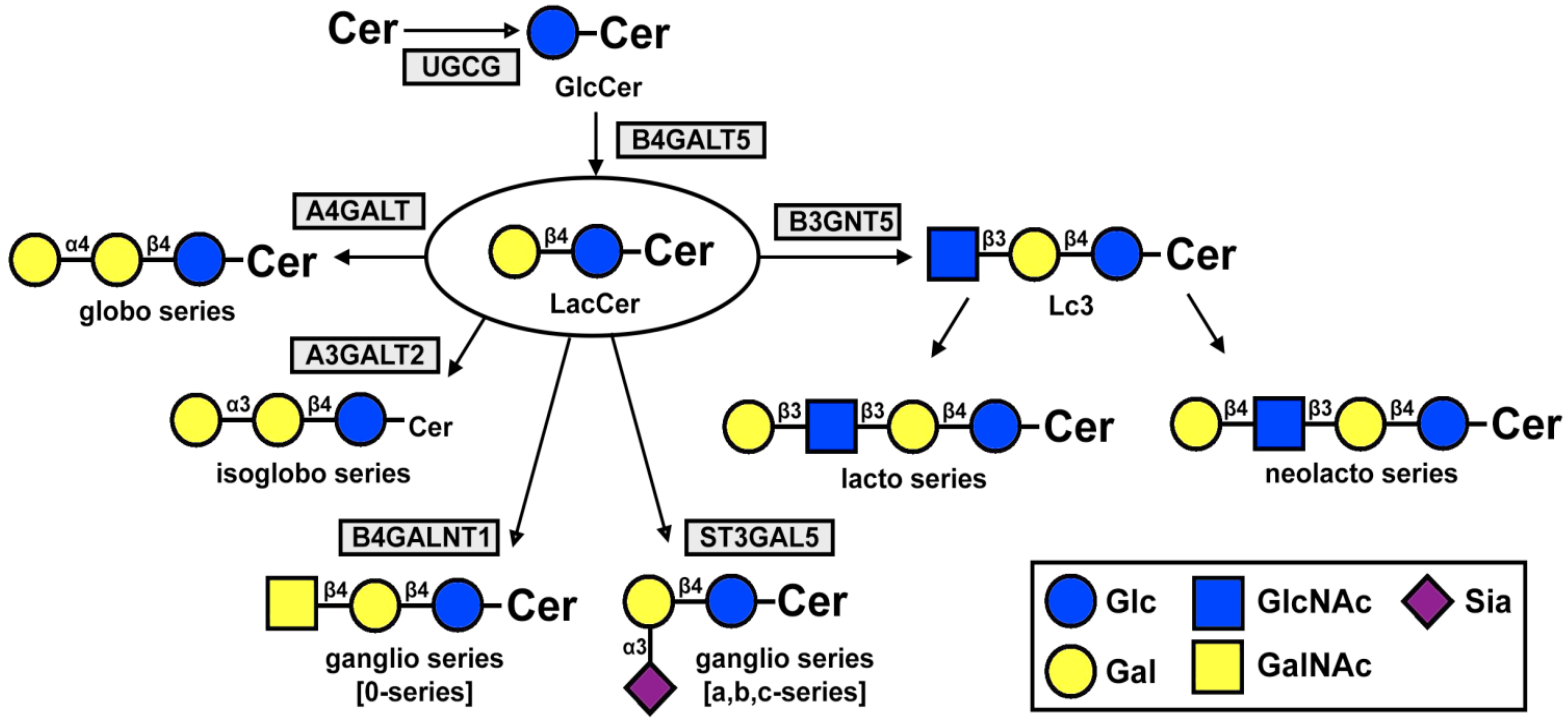
GD1a Common name

# The cellular pathway to glycosphingolipids



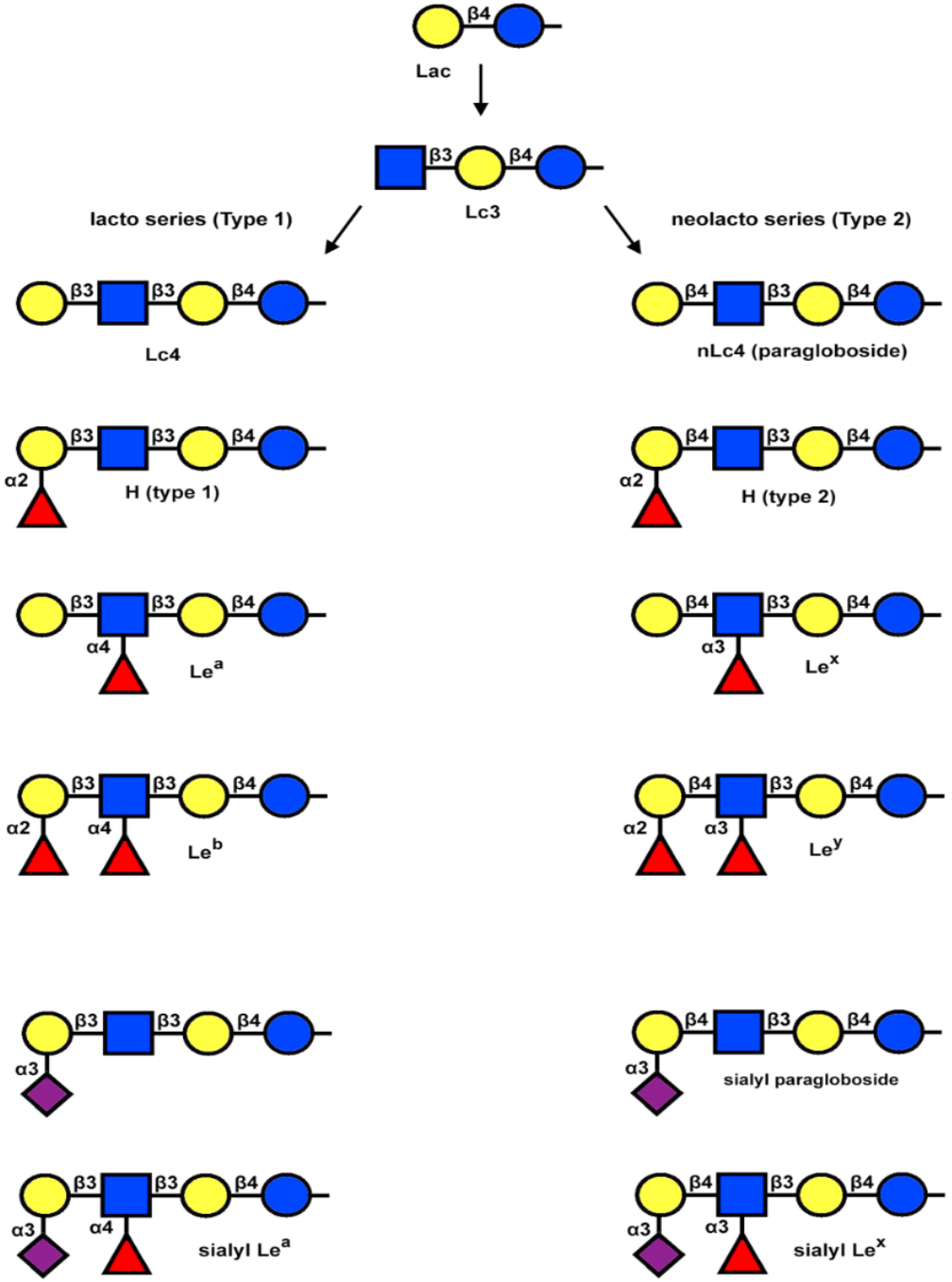

Schnaar (2015) Animal Glycolipids. *eLS.* http://www.els.net

# Vertebrate glycosphingolipids are extensions of LacCer



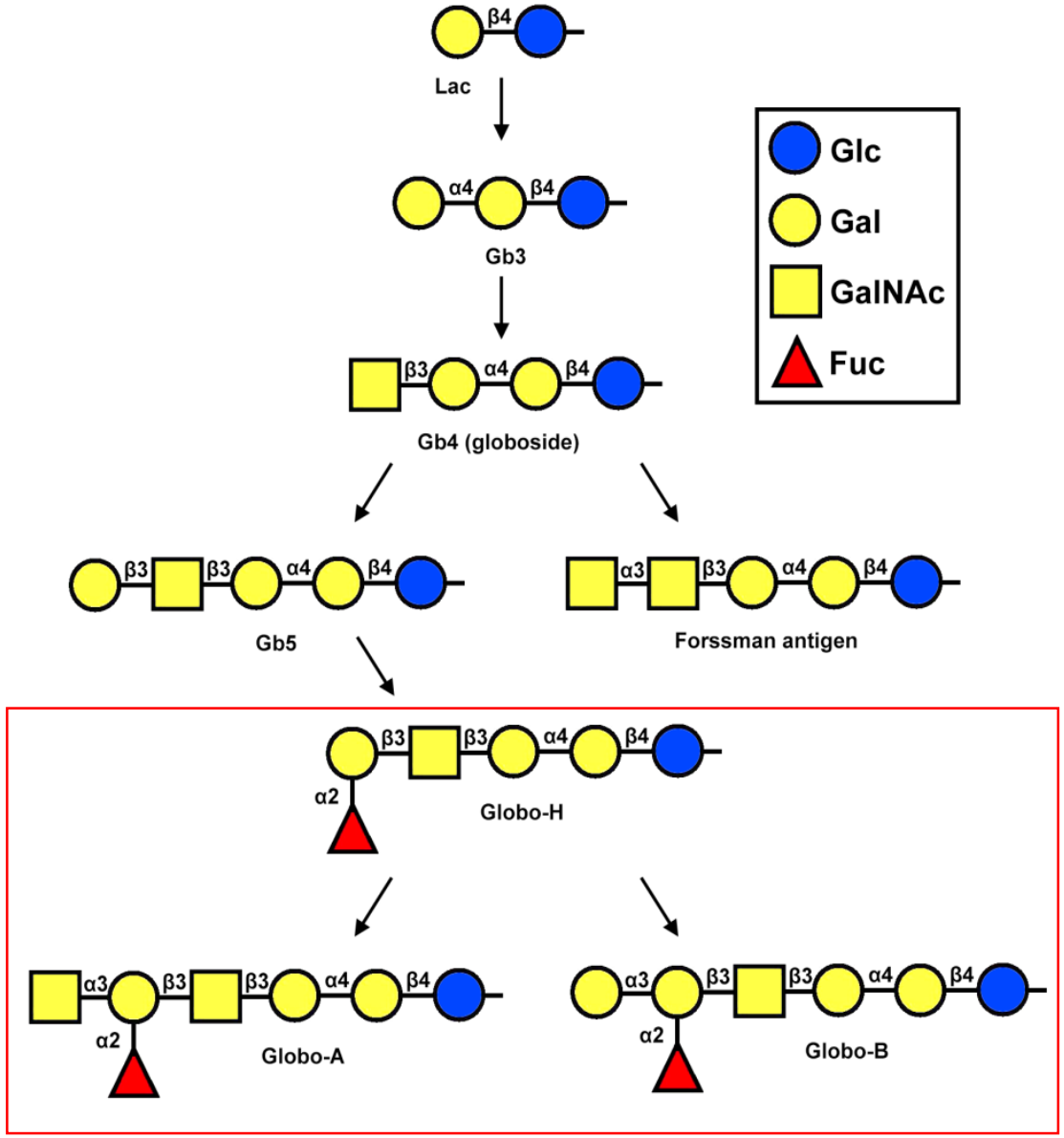

Schnaar (2015) Animal Glycolipids. *eLS.* http://www.els.net

# Lacto and neolacto glycosphingolipids are common on human hemopoietic lineage cells (immune cells)




Schnaar (2015) Animal Glycolipids
*eLS.* http://www.els.net

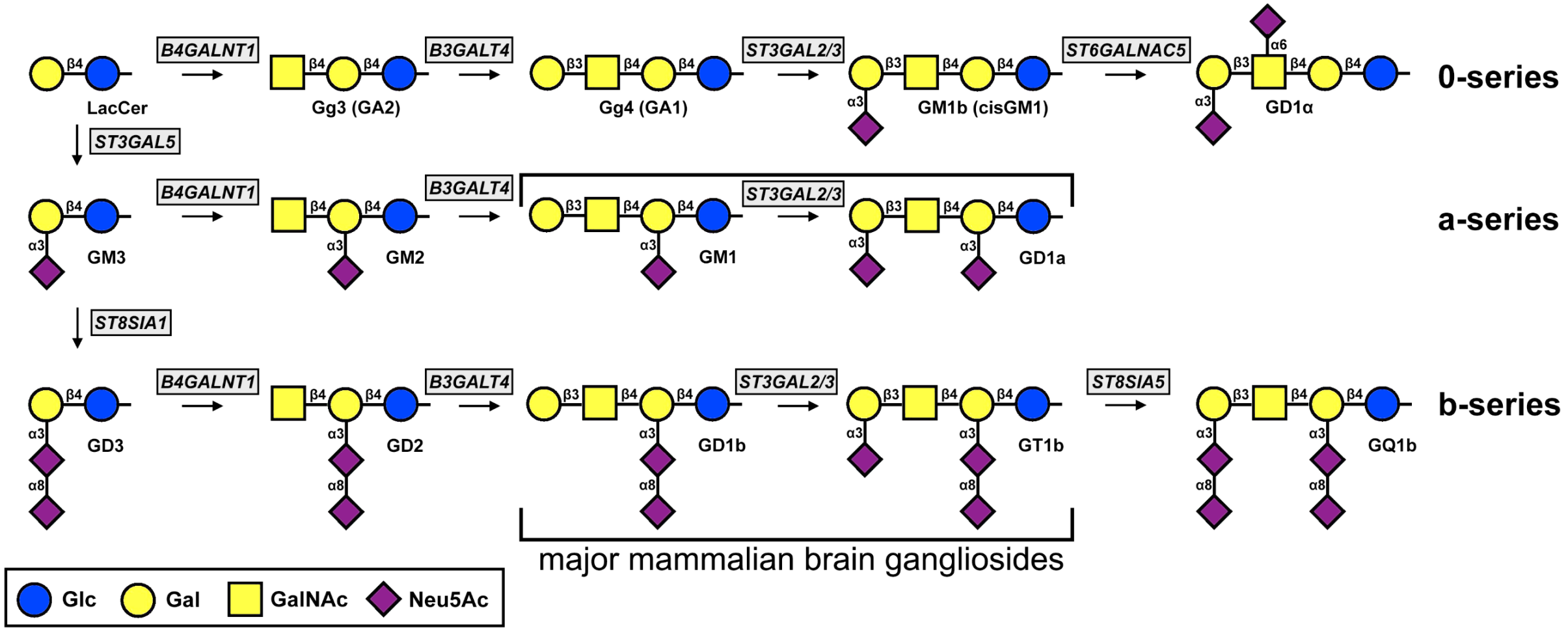
# Globo glycosphingolipids are common on human red blood cells




Blood group glycolipids

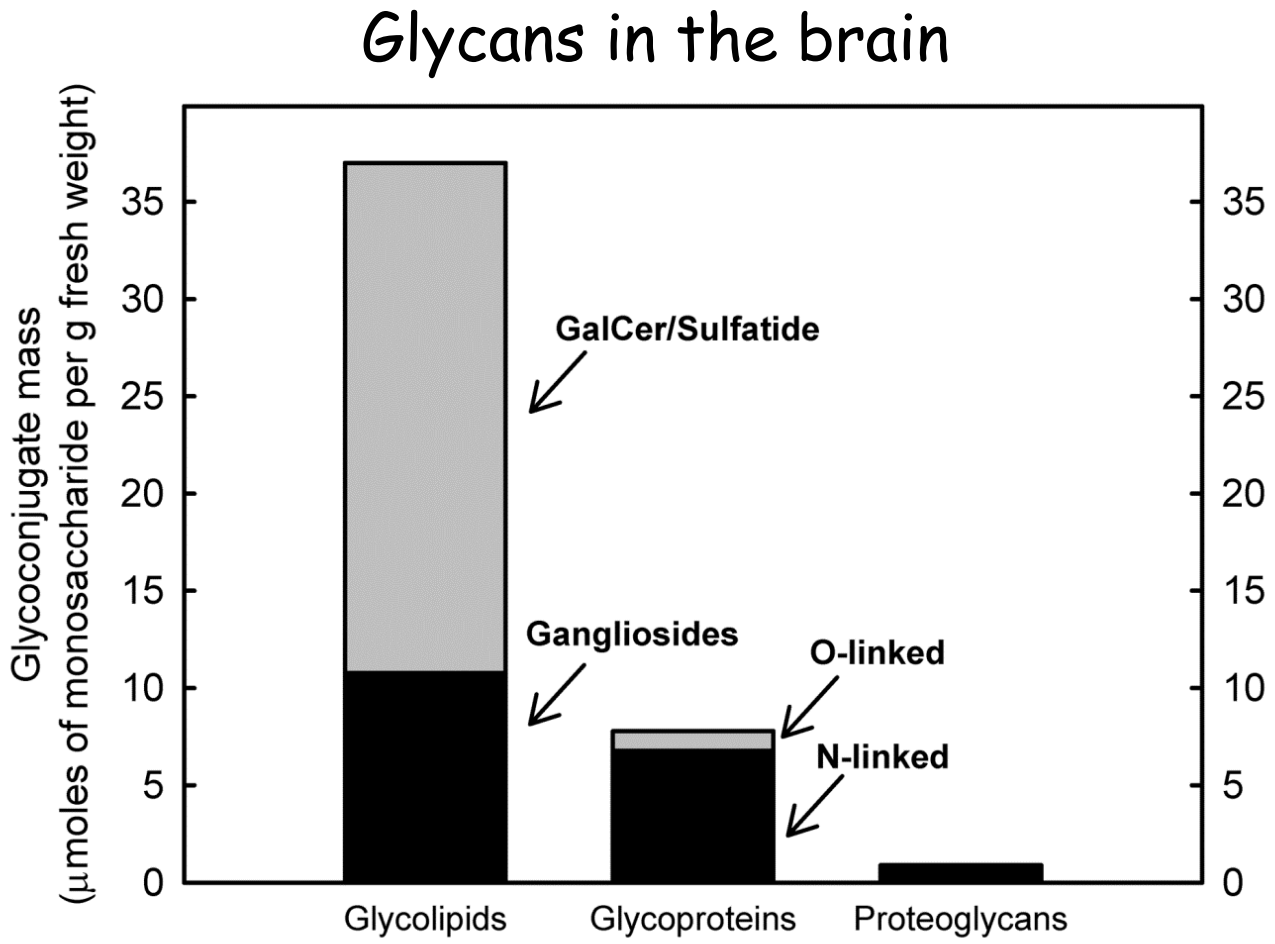
Schnaar (2015) Animal Glycolipids. *eLS.* http://www.els.net

# Gangliosides are ubiquitous in mammals, but are most abundant in the brain



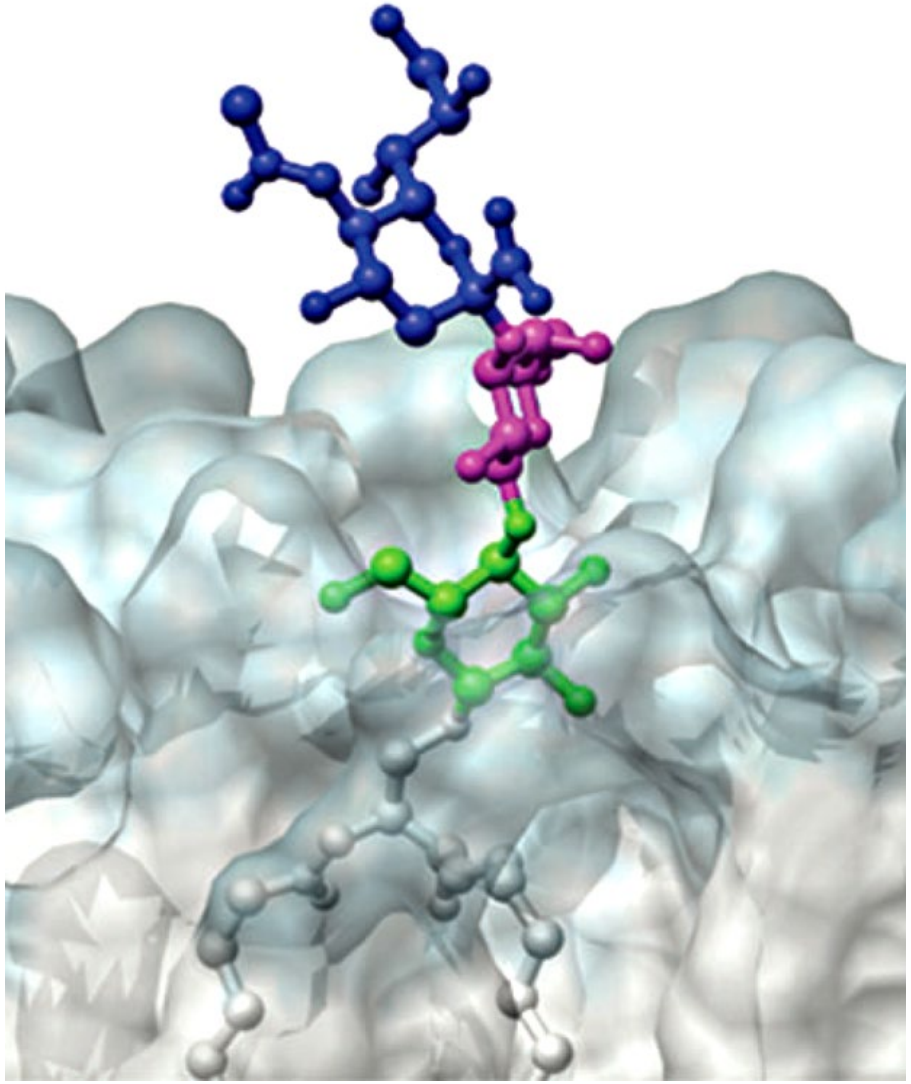

Schnaar (2015) Animal Glycolipids. *eLS.* http://www.els.net

# Gangliosides are the major glycans in nerve cells




Schnaar (2005) Brain glycolipids, in *Neuroglycobiology.* Oxford University Press

# Gangliosides are embedded in the extracellular leaflet of the plasma membrane with their glycans extending outward



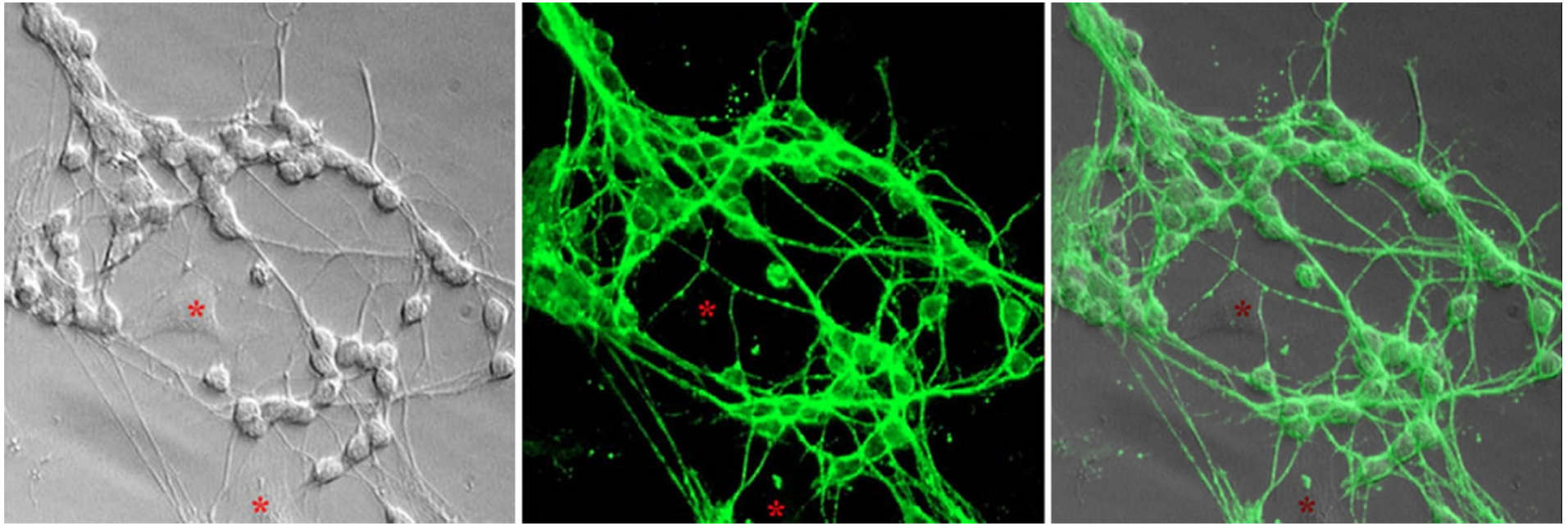
GM3 modeled in the plasma membrane

DeMarco & Woods (2009)
*Glycobiology* 19, 344

# Gangliosides are on all vertebrate cells, but are the dominant glycan structures on nerve cells

Rat cerebellar granule cell axons in culture immunostained for ganglioside GD1a

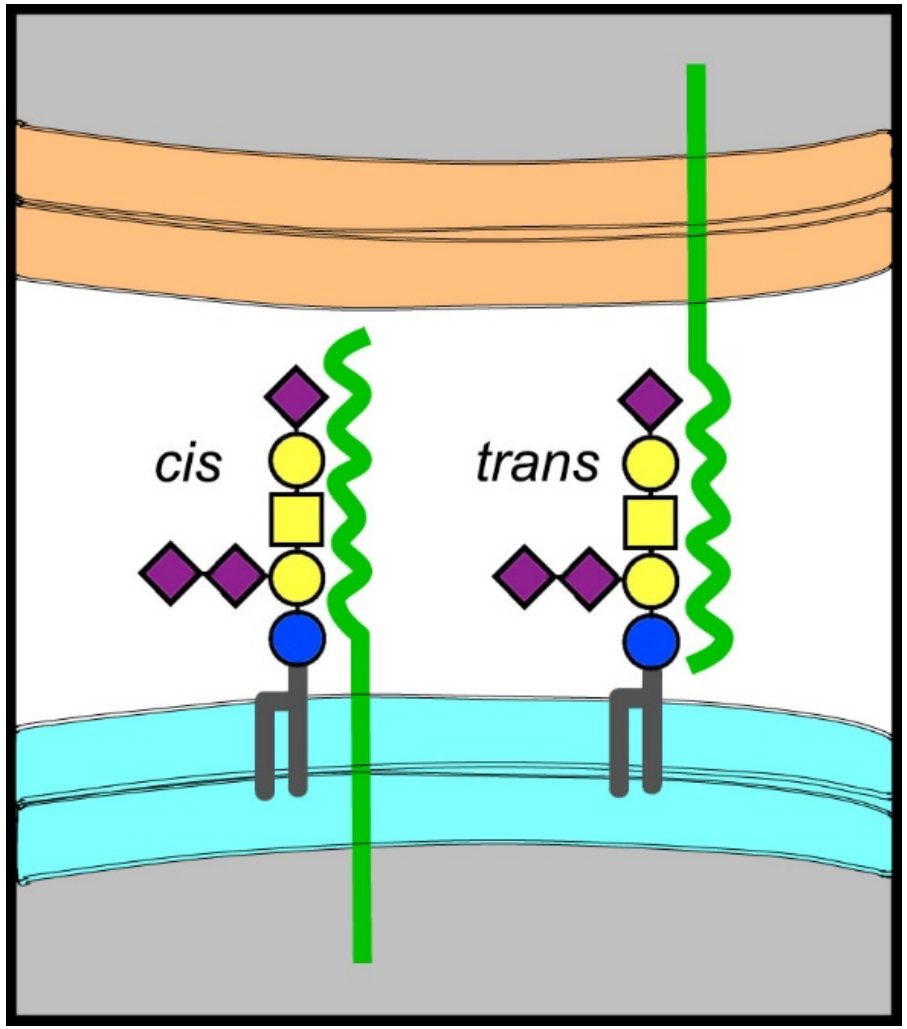
HIC (phase) image | anti-GD1a mAb | overlay image



* non-neuronal cells

# Glycolipid functions: cis and trans

cis regulation via
lateral association

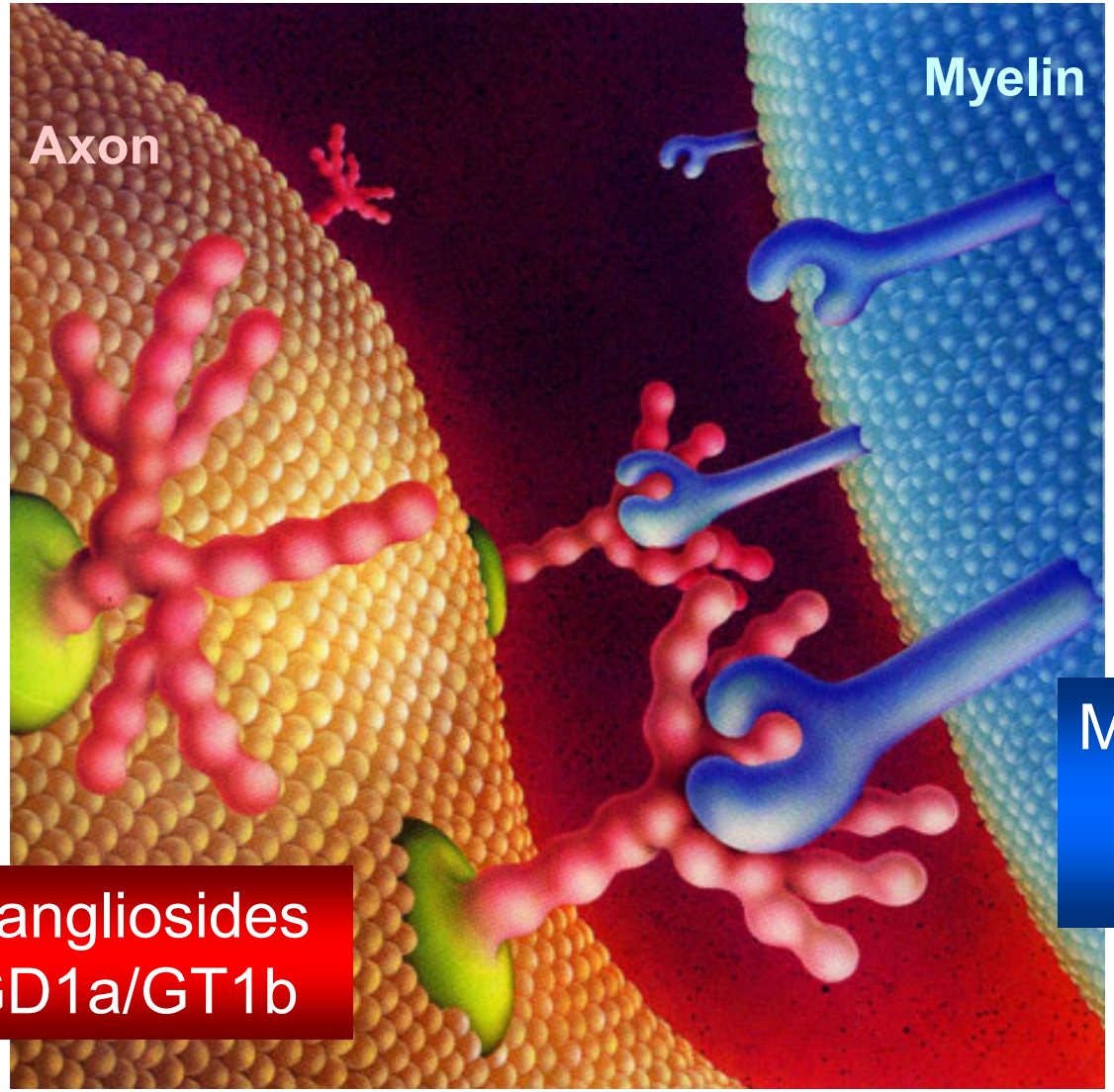



trans regulation
by cell-cell recognition

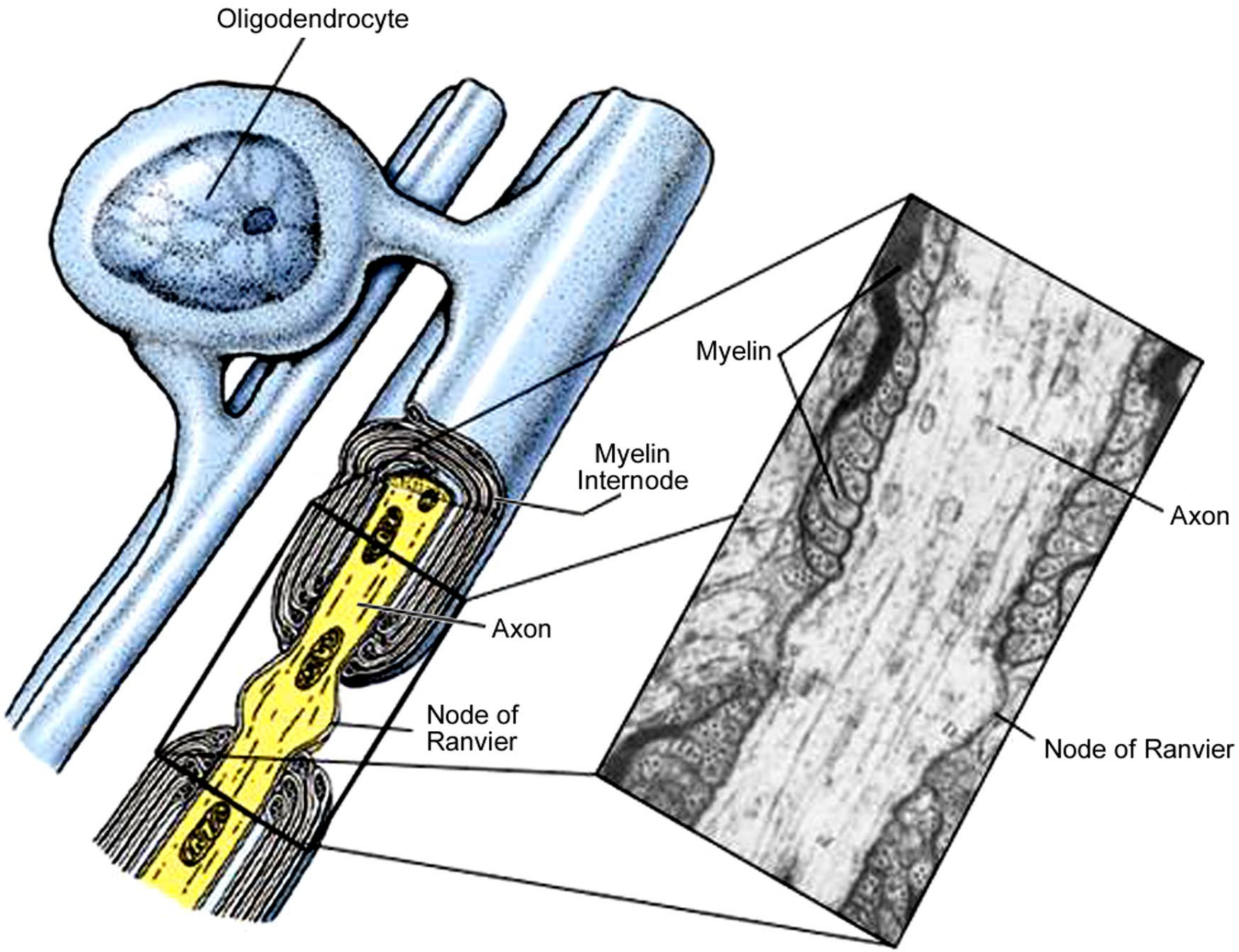
Because of their long unsaturated lipid chains, glycolipids cluster together, along with cholesterol, selected other lipids, and selected proteins, in "lipid rafts"

Schnaar (2016)
*J Mol Biol* 428:4425

# Gangliosides function in axon-myelin interactions

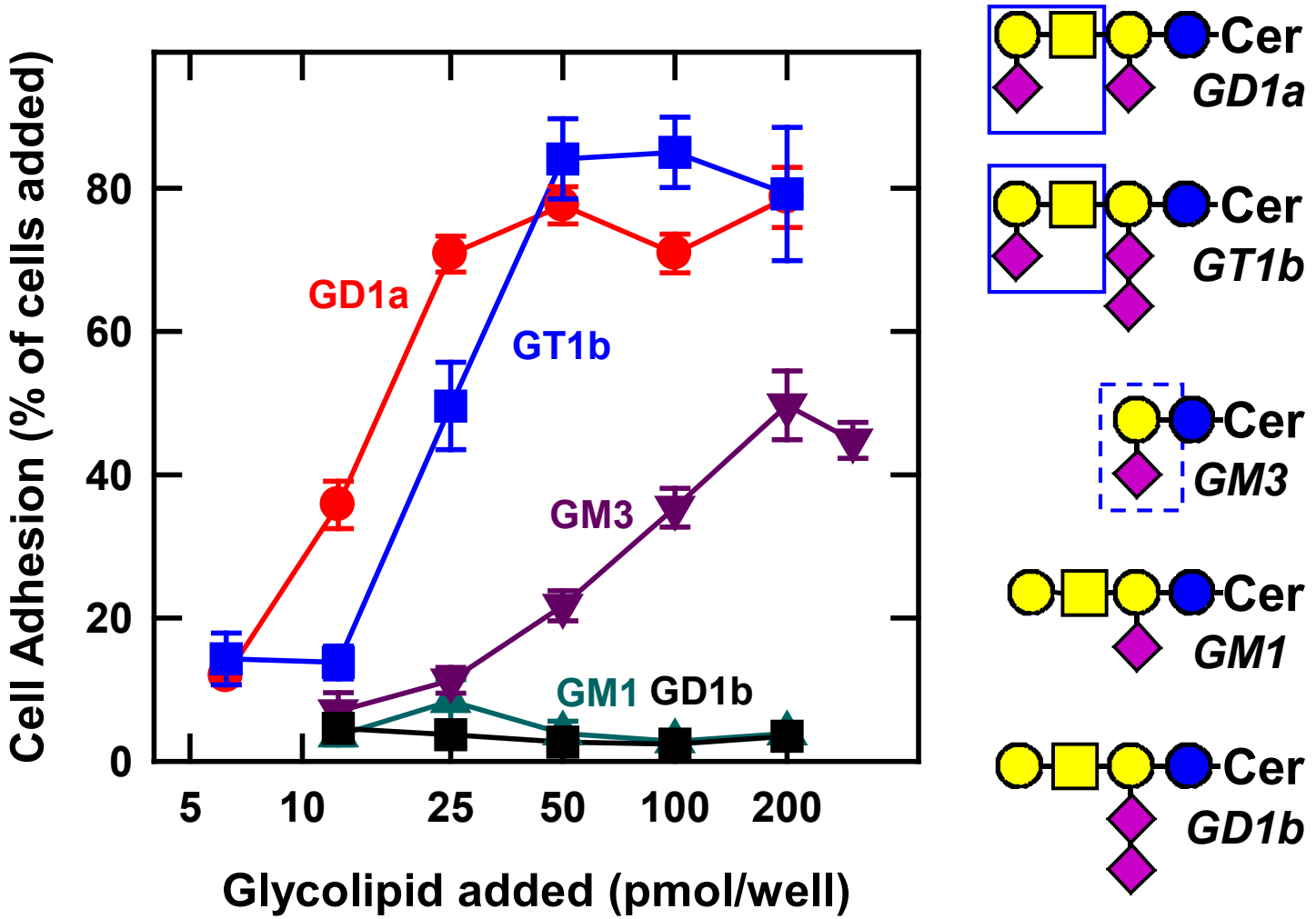



Modified from
Sharon and Lis (1993)
Scientific American



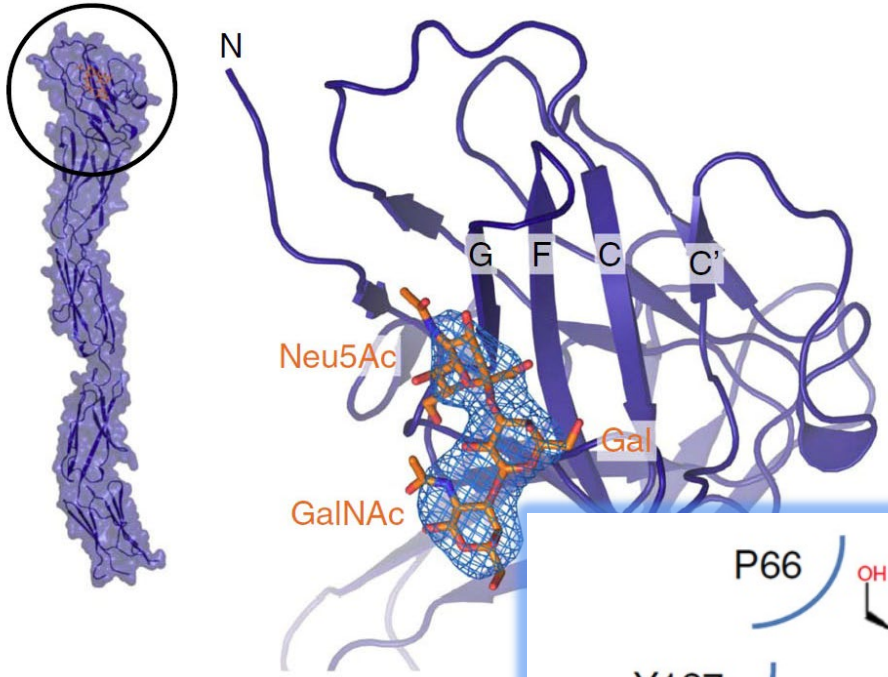

Delcomyn, F. (1998)
Foundations of Neurobiology.
W.H. Freeman, New York.

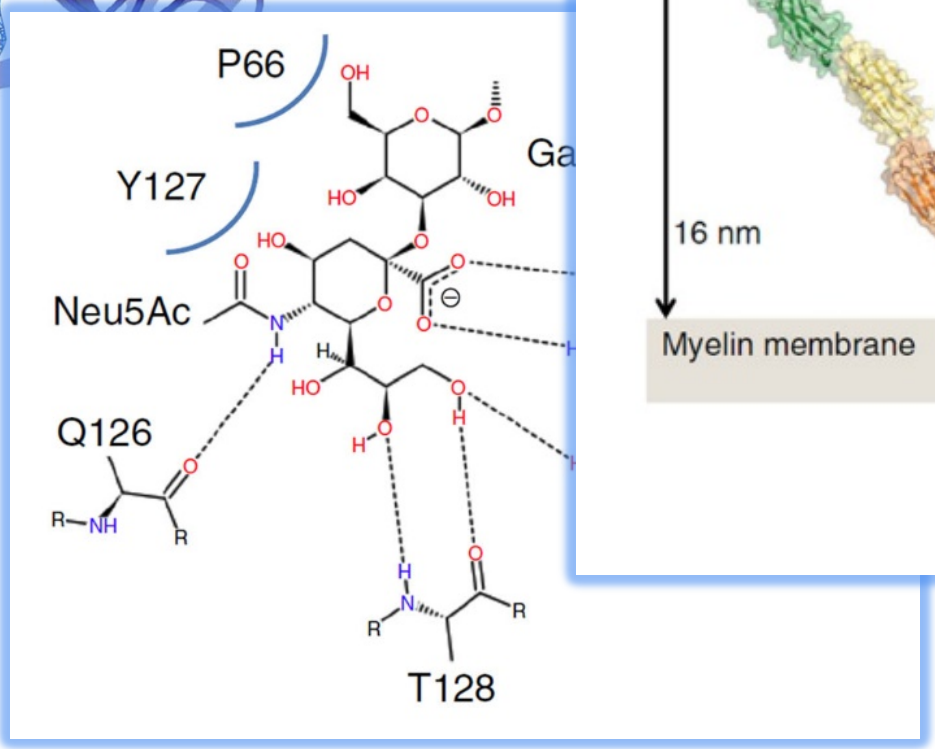
# Myelin-associated glycoprotein binds to GD1a, GT1b

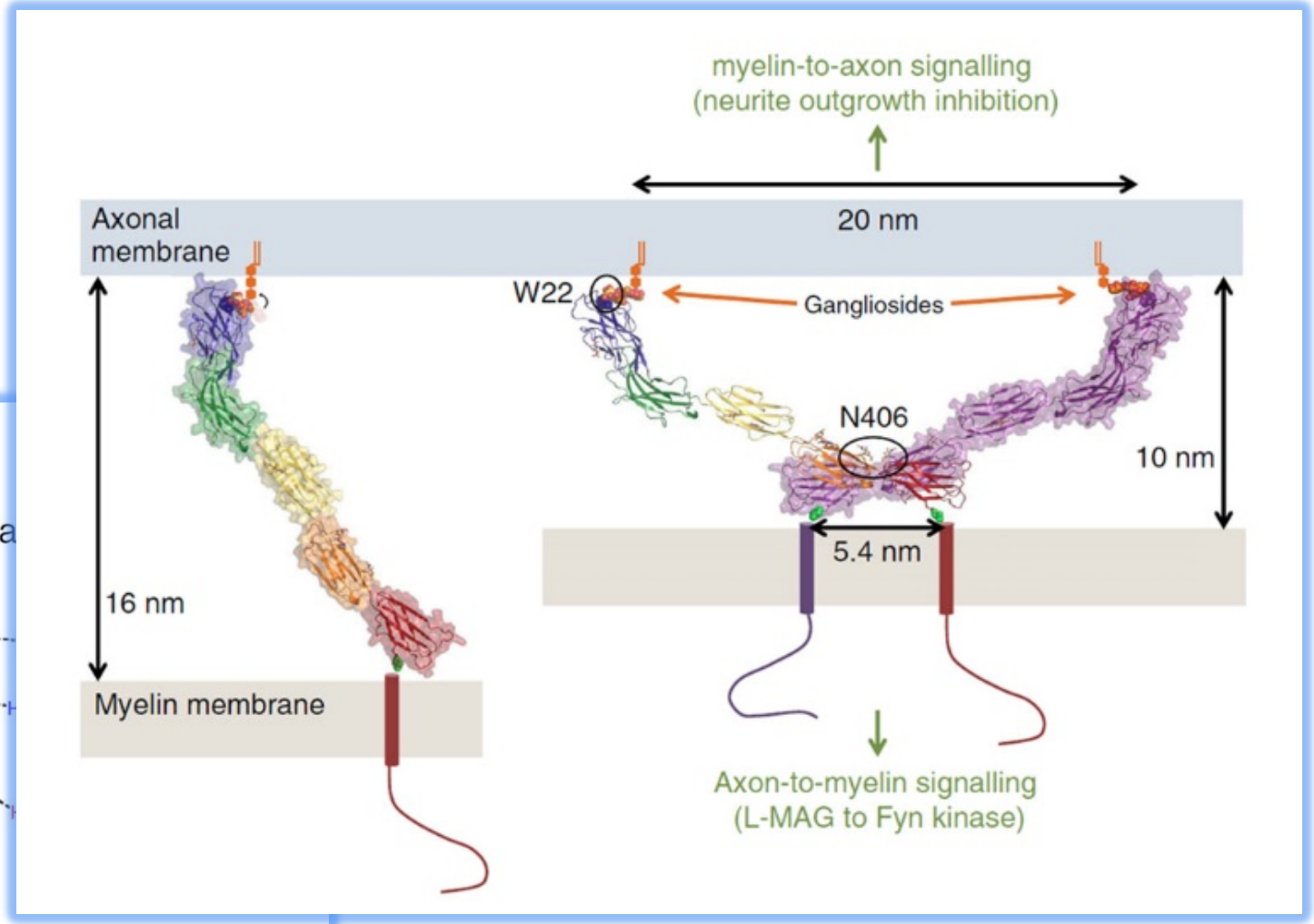



Collins, et al. (1997)
J. Biol. Chem. 272, 16889

# Structural determination of MAG-ganglioside binding

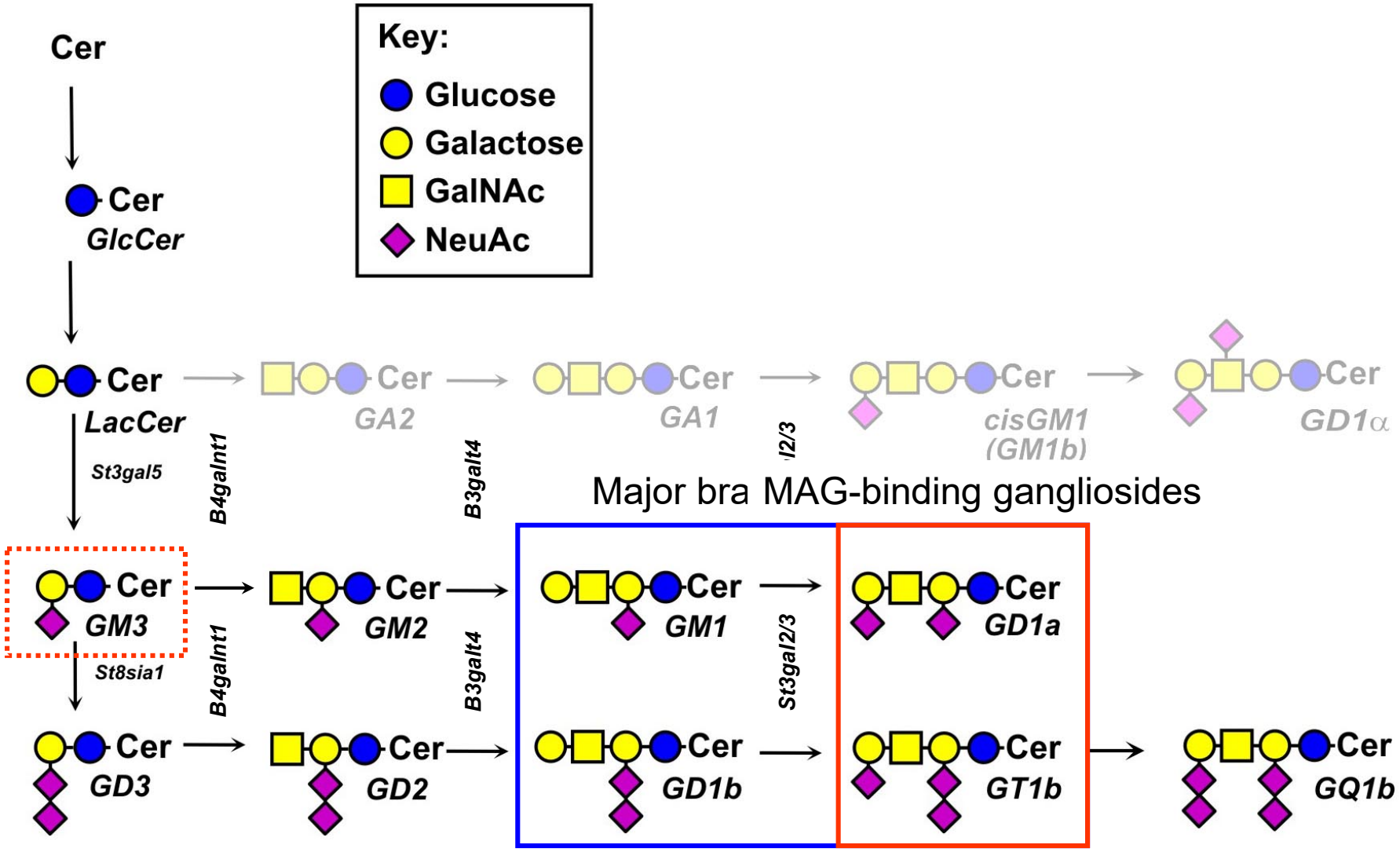


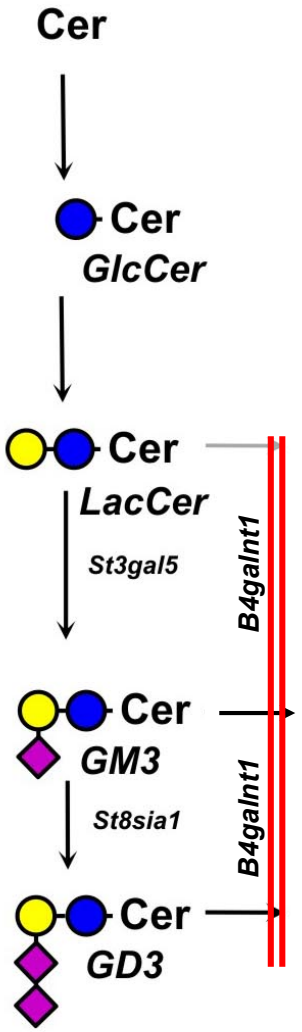

Pronker et al,
*Nature Comm*
7:13584 (2016)

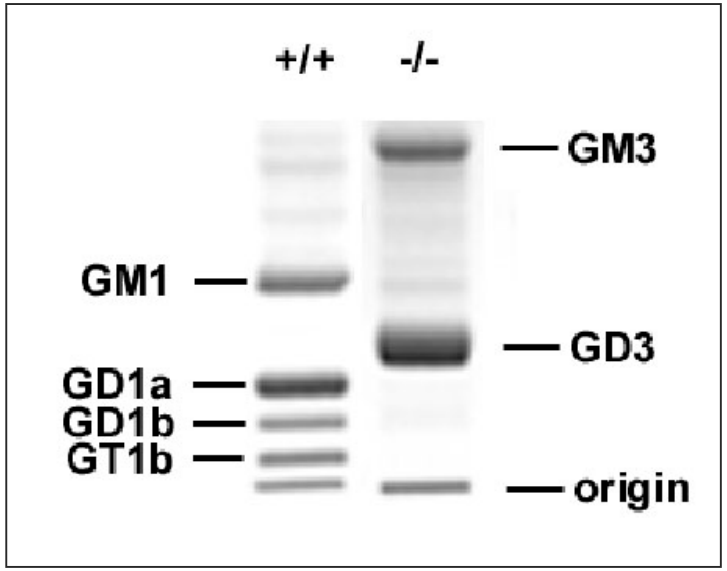
# Brain ganglioside biosynthesis




Major bra MAG-binding gangliosides

# Mouse genetic model of complex ganglioside depletion (*B4galnt1*-null)



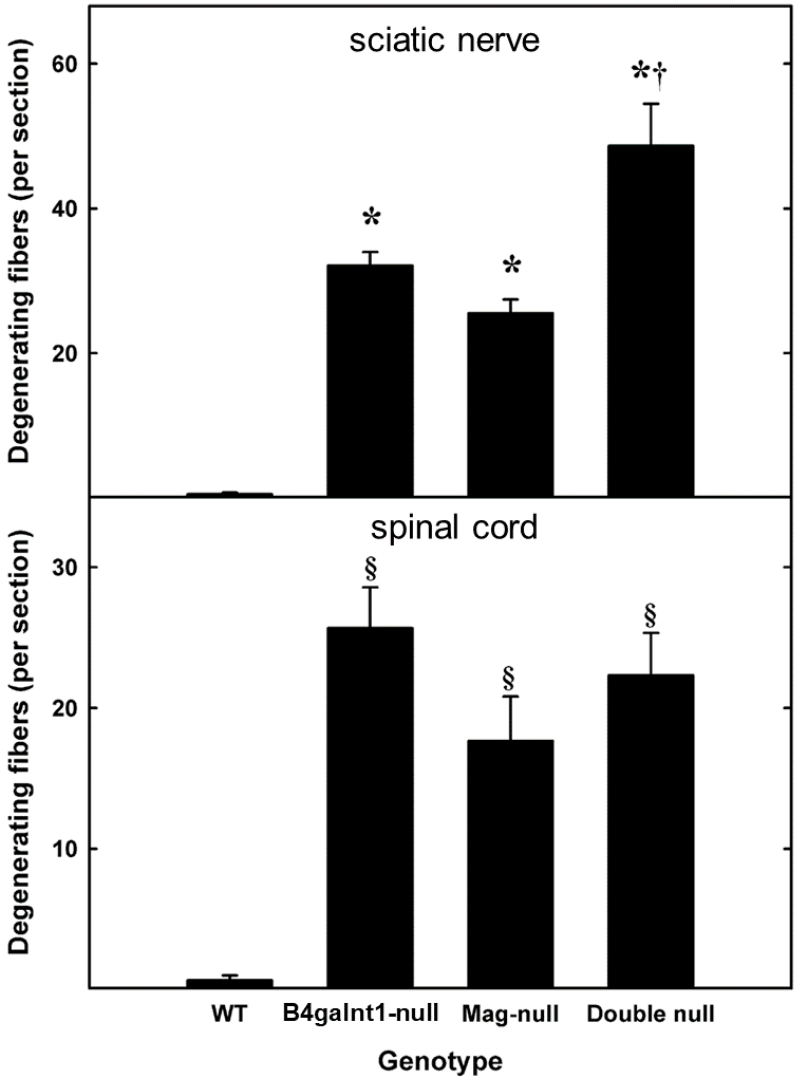

Bierfreund & Sandhoff

- altered nerve conduction velocity
- progressive peripheral neuropathy of the axonal type
- enhanced susceptibility to toxic axonopathy
- molecular disruption at Nodes of Ranvier
- cognitive deficits
- reduced hippocampal plasticity
- seizure susceptibility

Reviewed in Schnaar, *Adv Carb Chem Biochem* 76:113 (2019)

Liu et al, *J Clin Invest* 103:497 (1999)

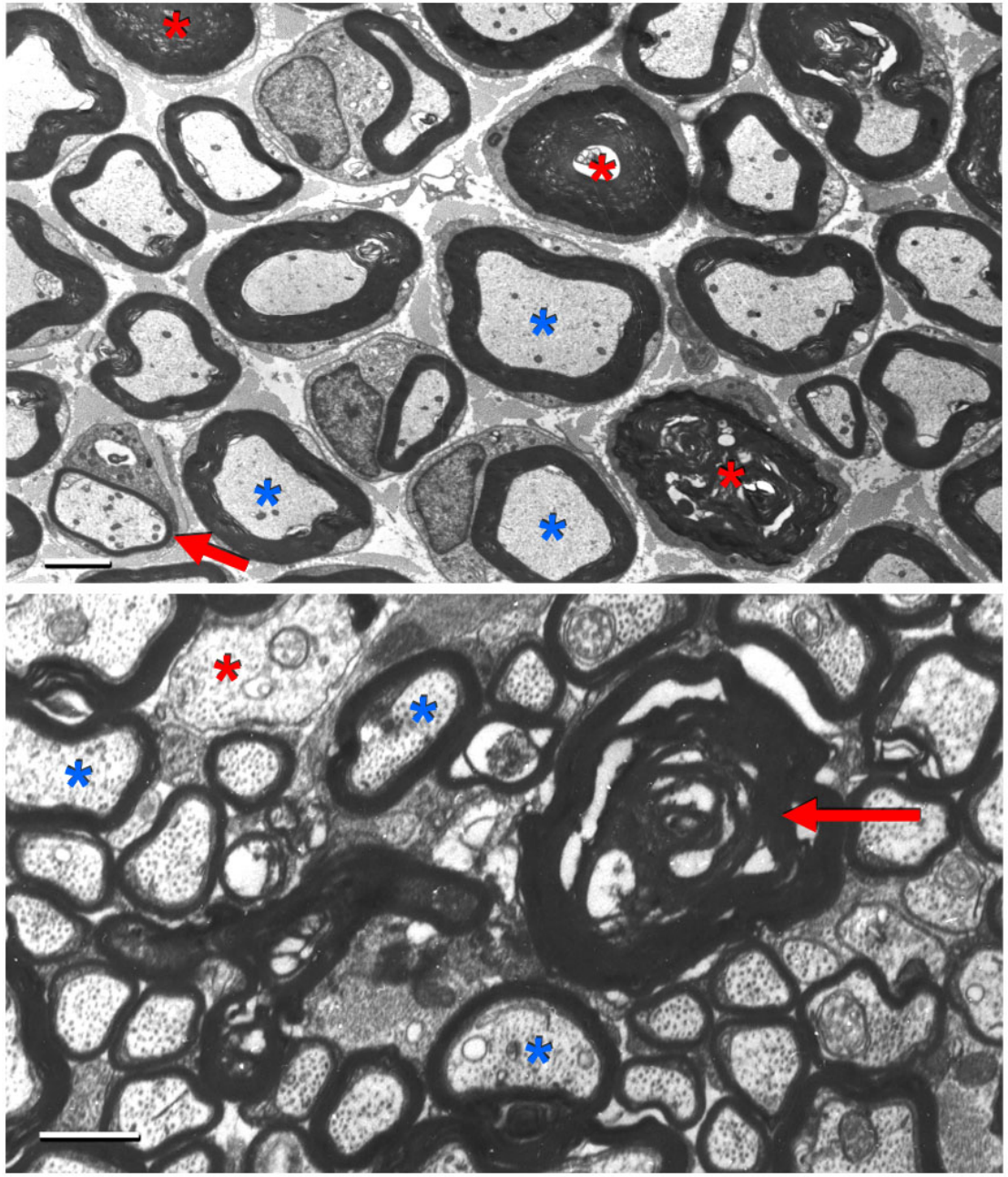
# Axon degeneration in *B4galnt1*-null mice




Pan et al. *Exp Neurol* 195, 208 (2005)

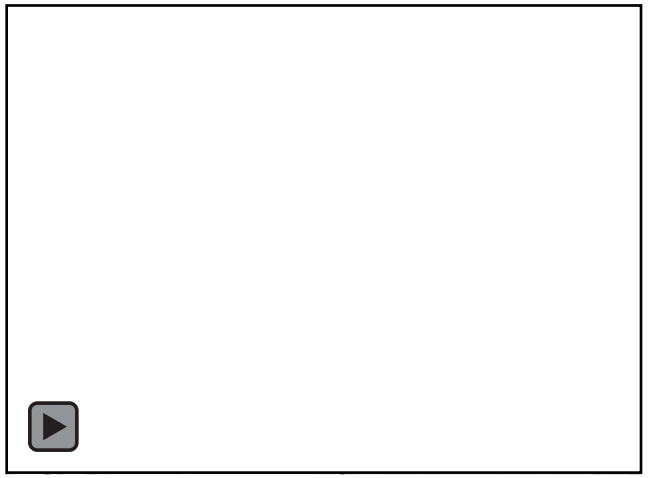
Peripheral Axons

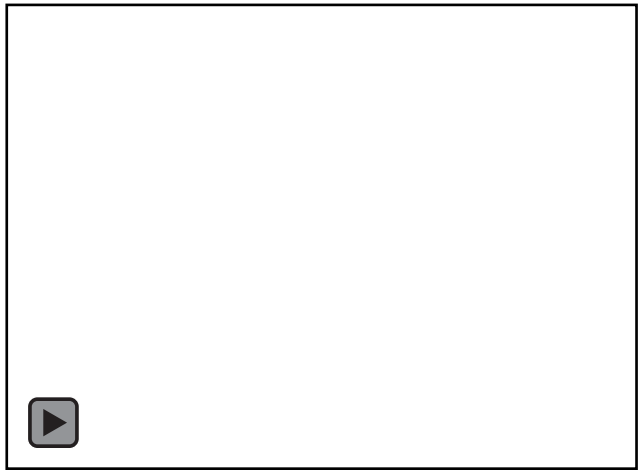
Central Axons

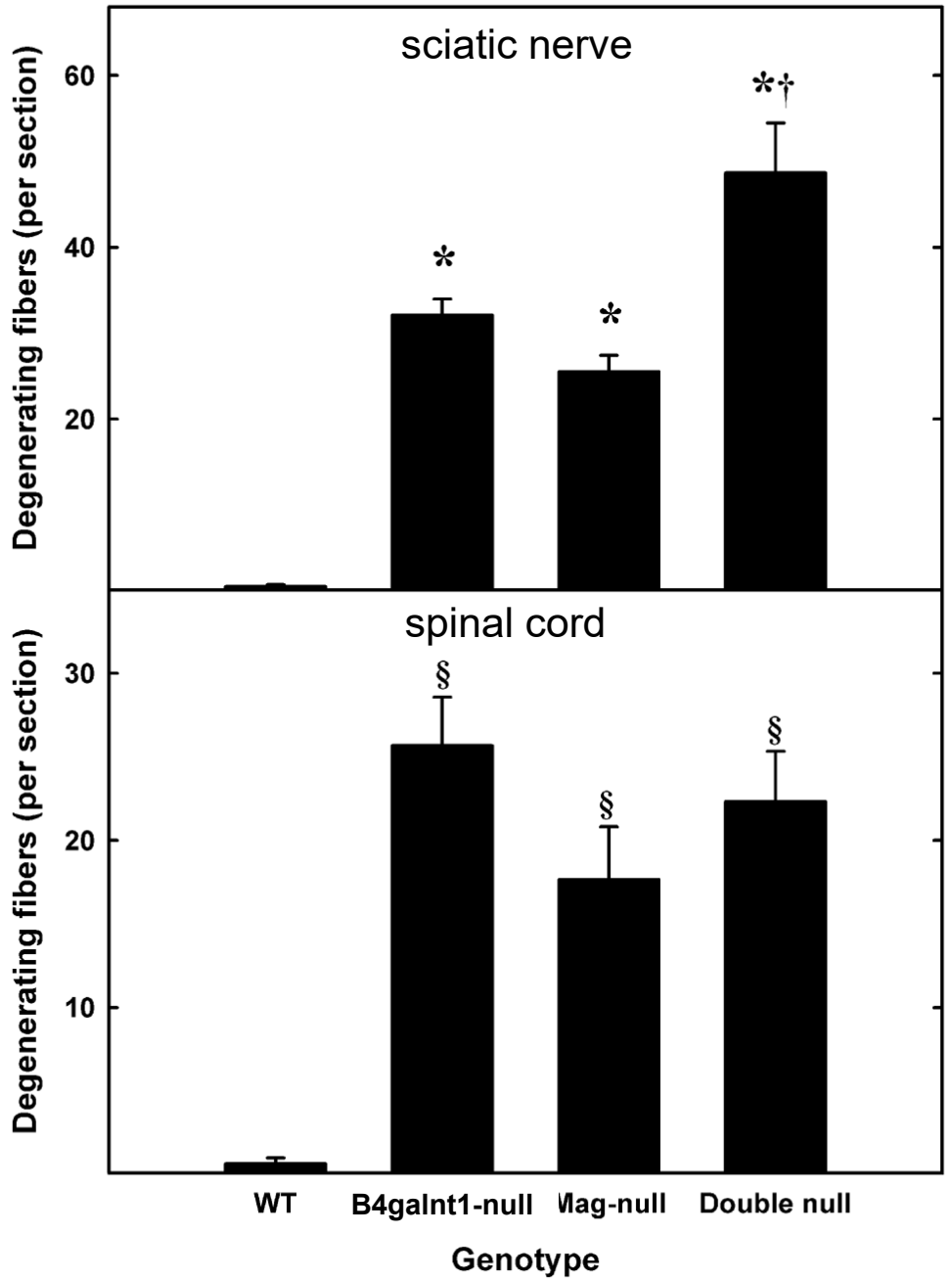


Sheikh et al. (1999) PNAS 96, 7532

# Axon degeneration in *B4galnt1*-null mice

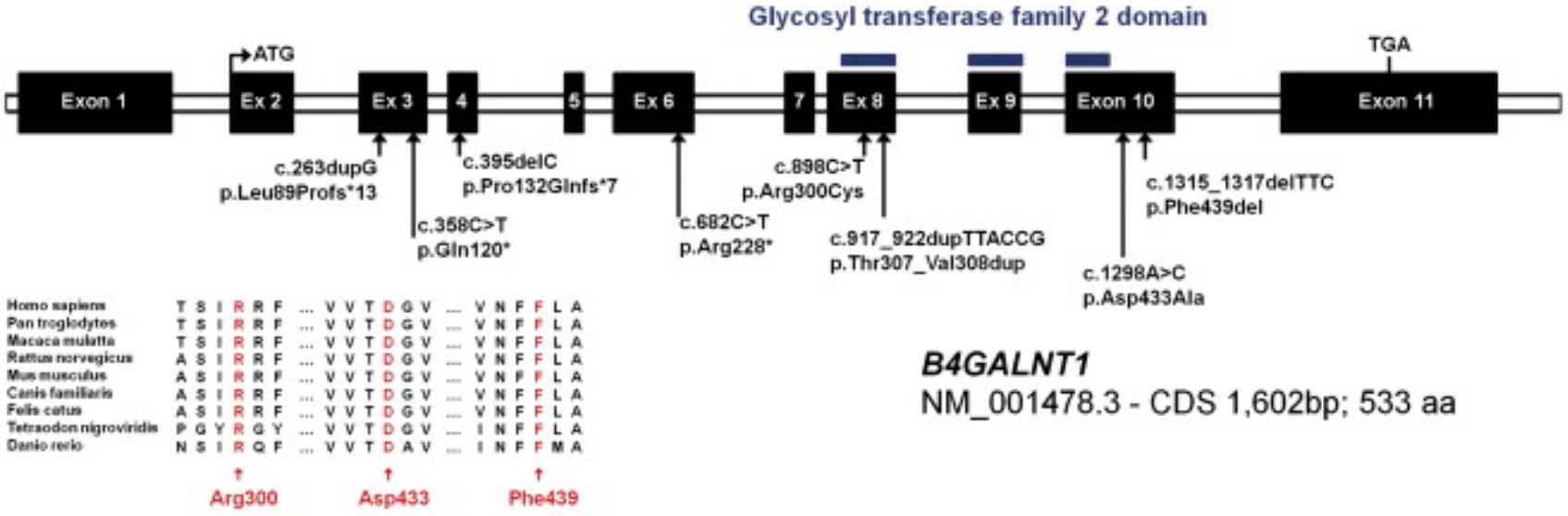







Pan, et al. (2005)
Exp. Neurol. 195, 208

# Congenital B4GALNT1 mutations result in complex hereditary spastic paraplegia.




"All subjects had predominant spasticity of the lower limbs..."

"After a mean disease duration of 32 years, four subjects were confined to a wheelchair or bedridden"

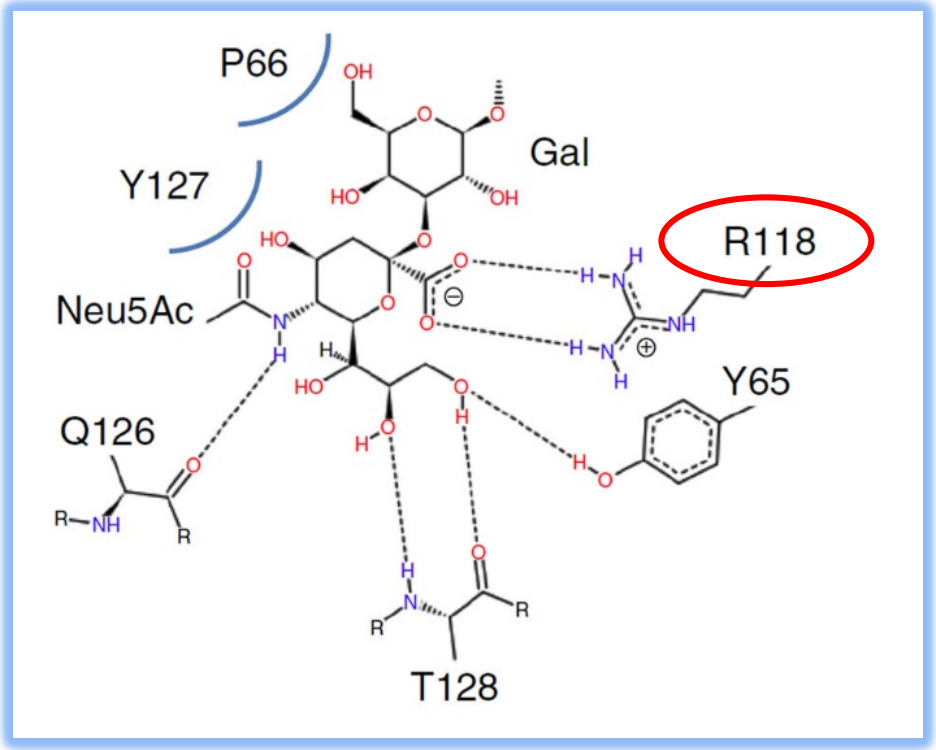
"…peripheral neuropathy predominantly of the axonal type in four families"

"Mild to moderate cognitive impairment and developmental delay was noted in all individuals, often preceding motor deficits. Cognitive dysfunction was noted to worsen with time in some subjects."

Boukhris et al. (2013)
Am J Hum Genet.
93:118-23

# A human mutation of the *MAG* gene at R118 recapitulates some of the deficits of *B4GALNT1* mutations

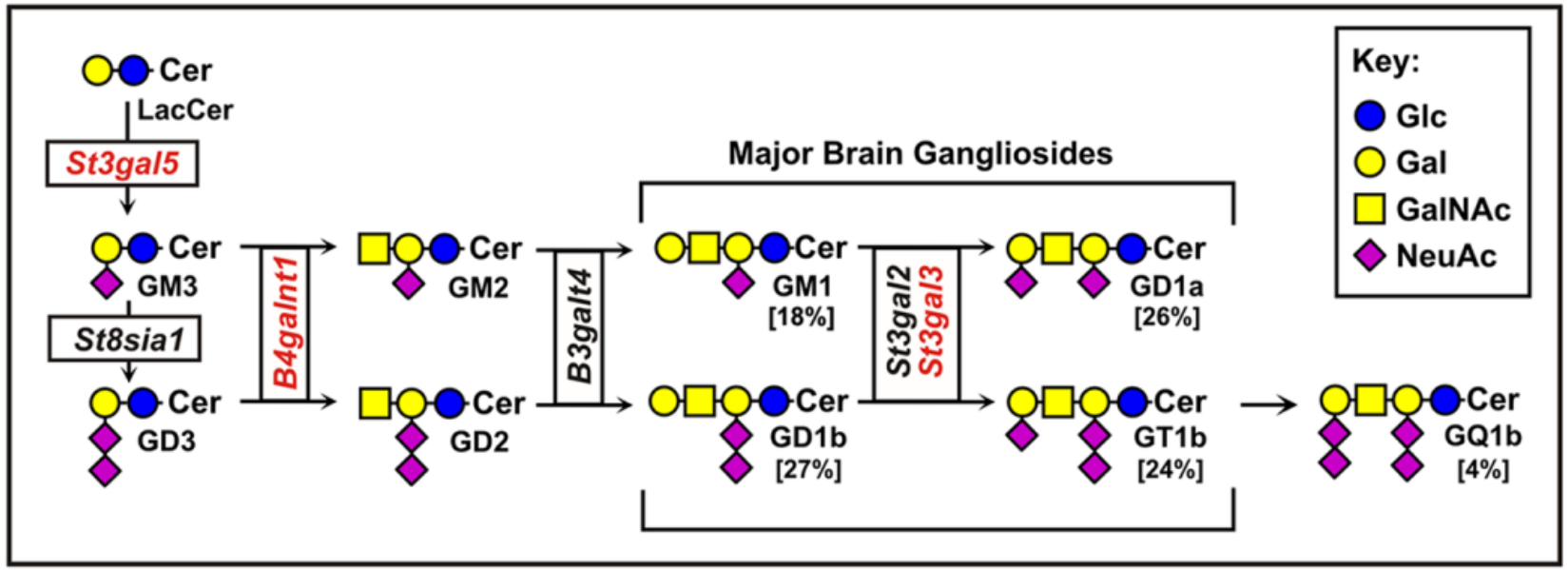
p.Arg118His




- Two siblings
  - Patient 1: Hyporeflexive, mild ataxia, waddling gait, axonal sensorimotor polyneuropathy, demyelinating features
  - Patient 2: Mild learning disabilities, hyporeflexive, wheelchair-bound, axonal sensorimotor polyneuropathy, demyelinating features

Roda et al, *Ann Clin Transl Neurol* 3:650 (2016)

# Human mutations responsible for congenital disorders (red)




ST3GAL5: Seizures with severe motor and intellectual disability.

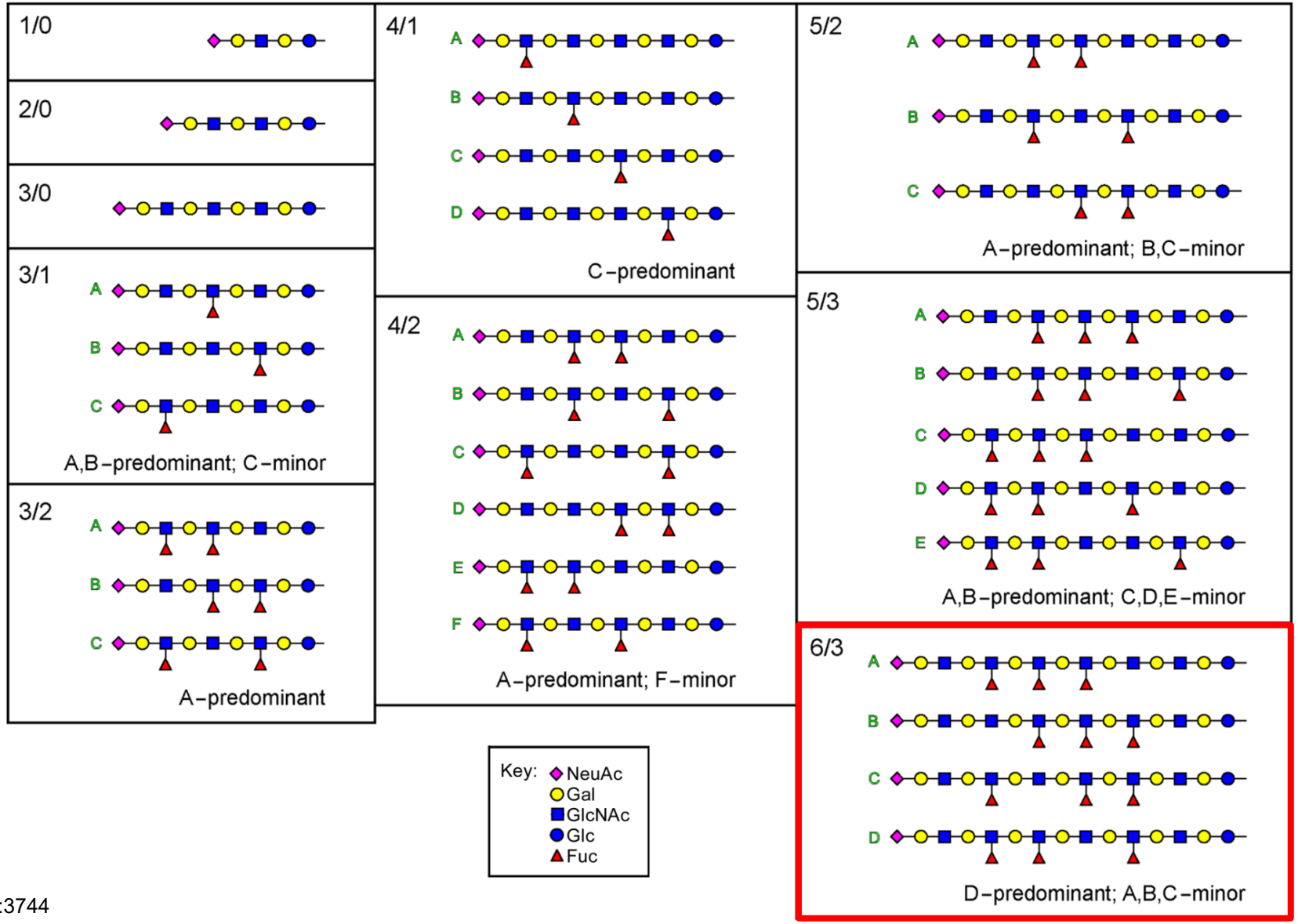
B4GALNT1: Hereditary spastic paraplegia with intellectual disability.

ST3GAL3: Cognitive disability, infantile seizures, developmental regression.

Li & Schnaar
*Prog Mol Biol Transl Sci.*
2018;156:63-82.

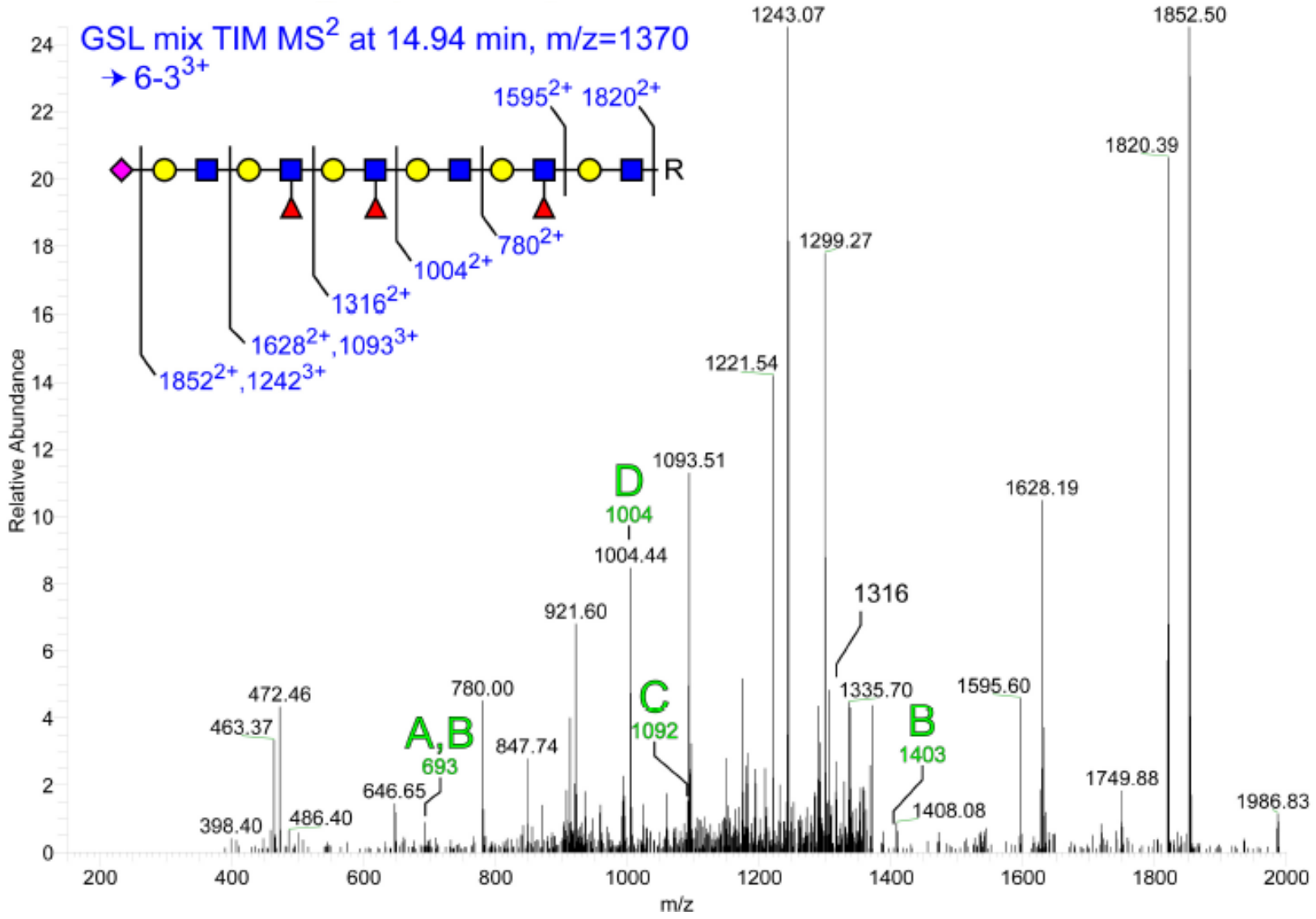
# Functional glycosphingolipids in human neutrophils

monosialylated glycosphingolipids isolated from ~$10^{10}$ human neutrophils



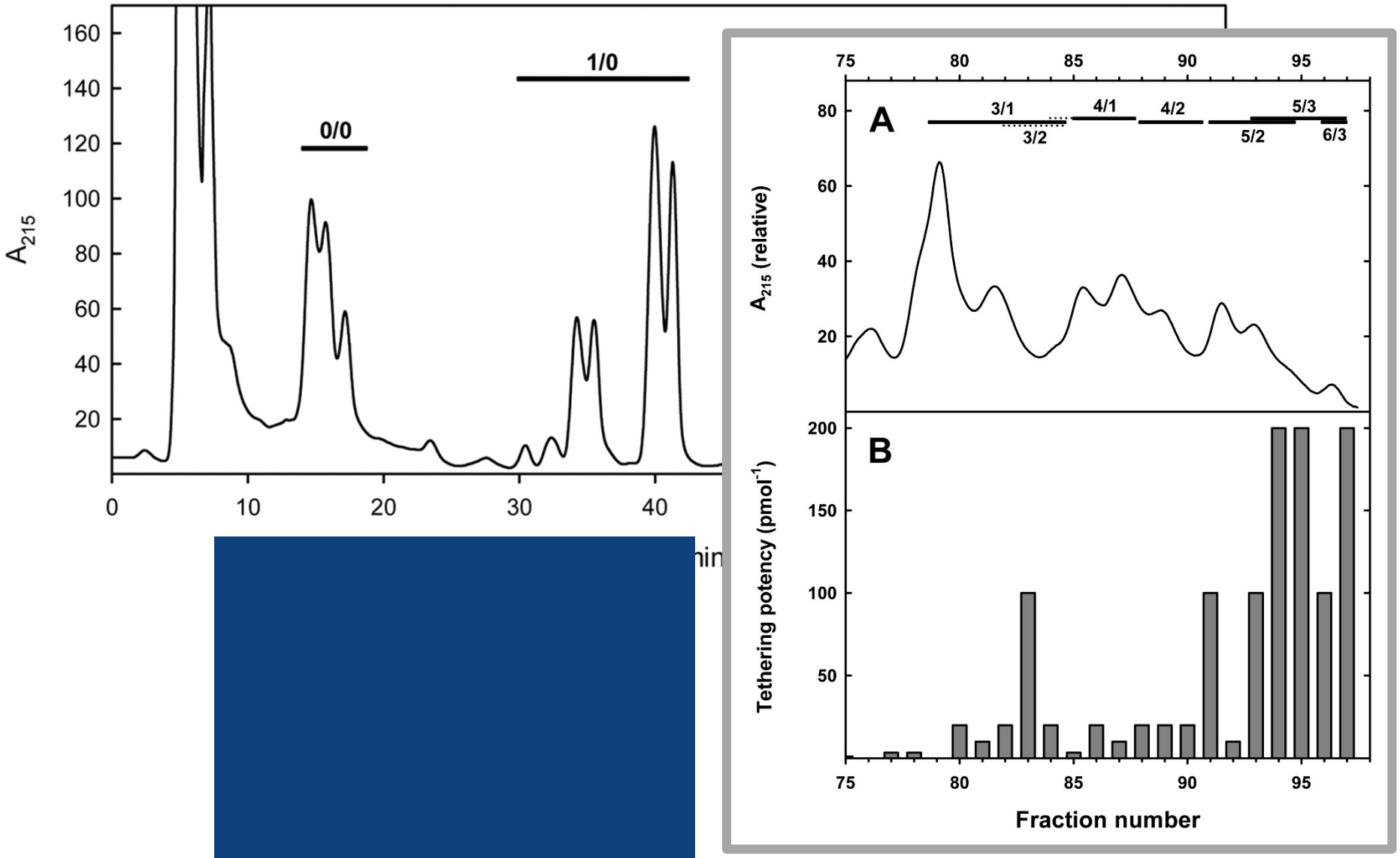

Nimrichter et al (2008) *Blood,* 112:3744

# Neolactoseries monsialylated glycolipids on human neutrophils




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

# Resolution & testing of human neutrophil gangliosides

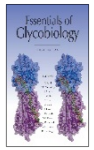



Nimrichter et al (2008) *Blood,* 112:3744

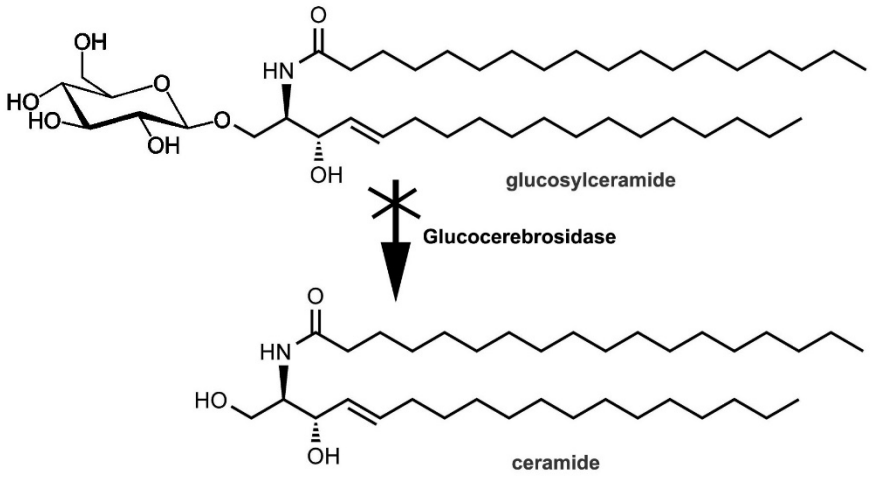
# Defects in glycolipid degradation

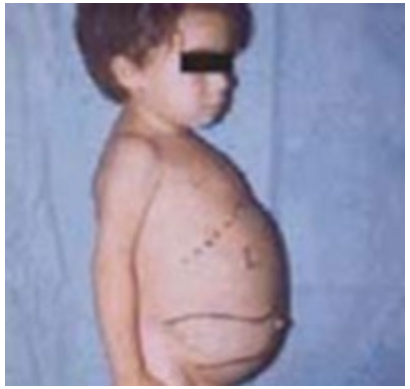
| Disease (glycolipid) | Enzyme or protein deficiency | Clinical symptoms |
|---|---|---|
| Tay–Sachs (GM2) | β-hexosaminidase A | neurodegeneration, death by 4 years |
| Sandhoff (GM2) | β-hexosaminidase A and B | neurodegeneration, death by 4 years |
| GM1 gangliosidosis (GM1) | β-galactosidase | progressive neurological disease and skeletal dysplasia in severe infantile form |
| Fabry* (Gb3) | α-galactosidase | severe pain, angiokeratoma, corneal opacities, death from renal or cerebrovascular disease |
| Gaucher's* (GlcCer) | β-glucoceramidase | hepatosplenomegaly<br>neurodegeneration (severe form only) |
| Krabbe (GalCer) | β-galactoceramidase | early onset with progression to severe mental and motor deterioration |
| Metachromatic leukodystrophy (sulfatide) | arylsulfatase A (cerebroside sulfatase) | mental regression, peripheral neuropathy, seizures, dementia |
| Saposin deficiency (many) | saposin precursor | neurodegeneration, death by 4 years |

*enzyme replacement available



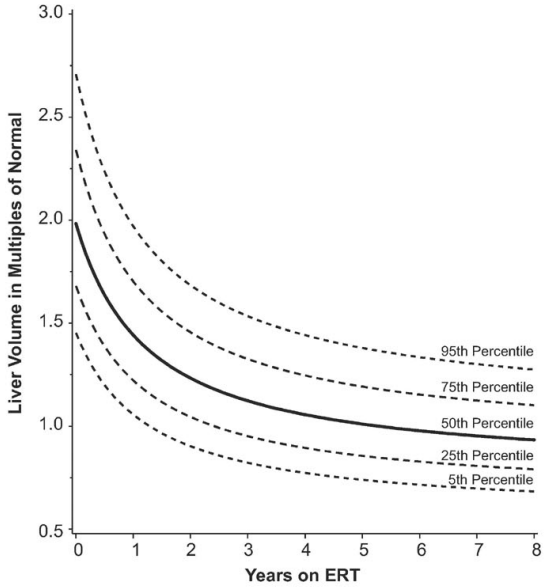
# Gaucher disease – cause, symptoms and treatment

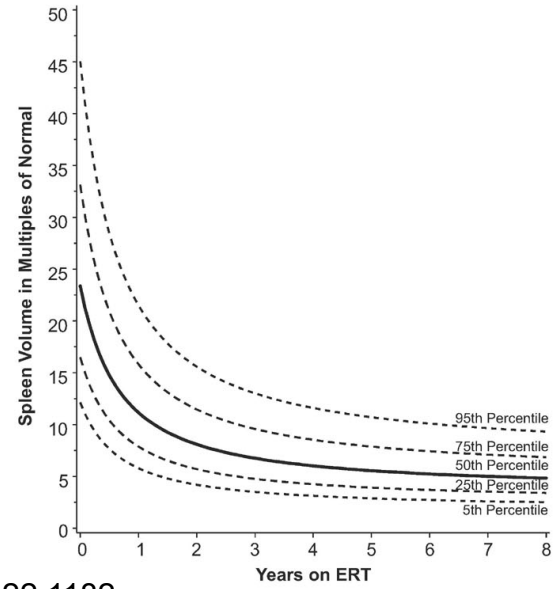


Hepatosplenomegaly: Grossly enlarged liver and spleen
http://www.gauchercare.com/

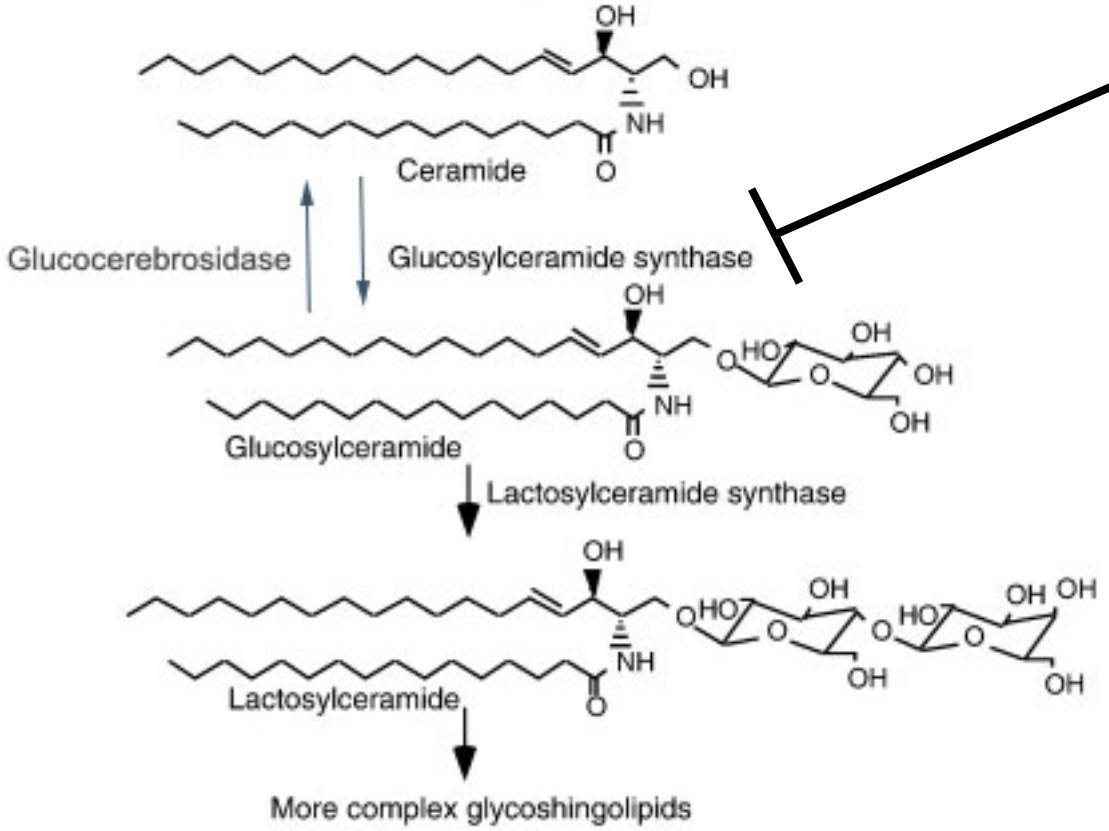
The cure: enzyme replacement therapy

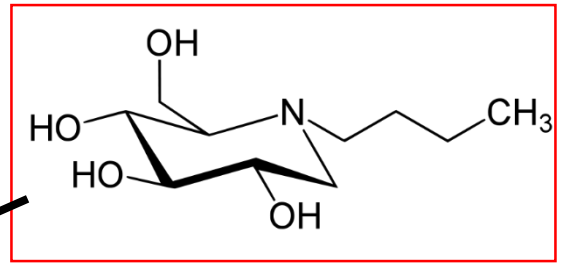


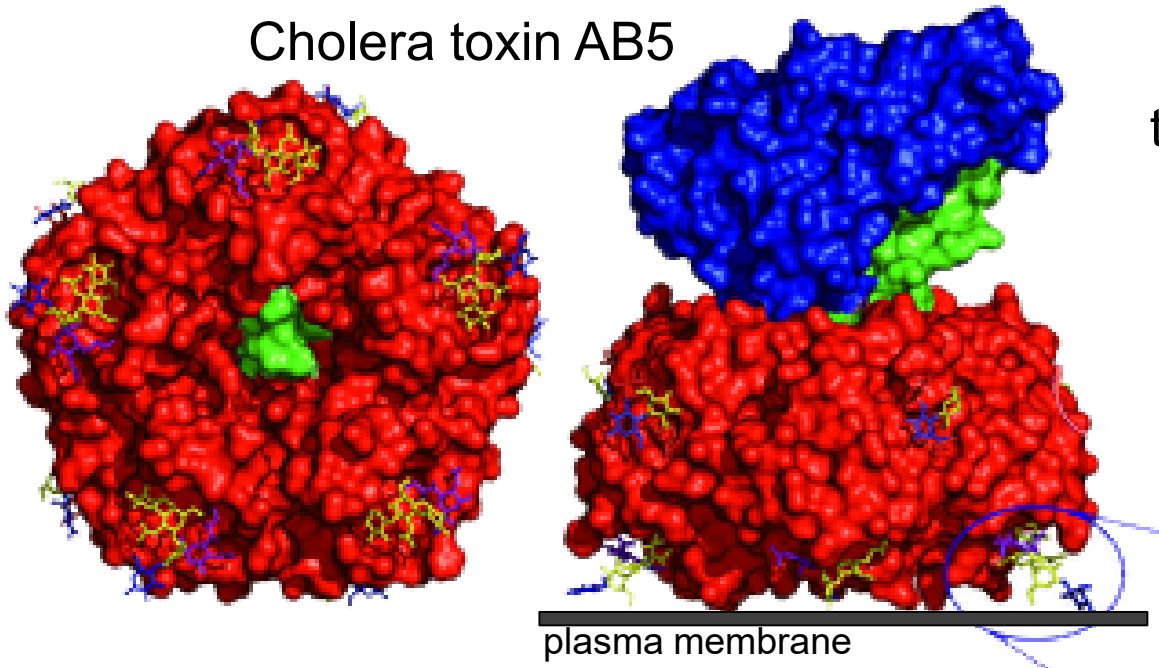

Andersson et al.. (2008) *Pediatrics* 122:1182

# A new direction – substrate reduction therapy



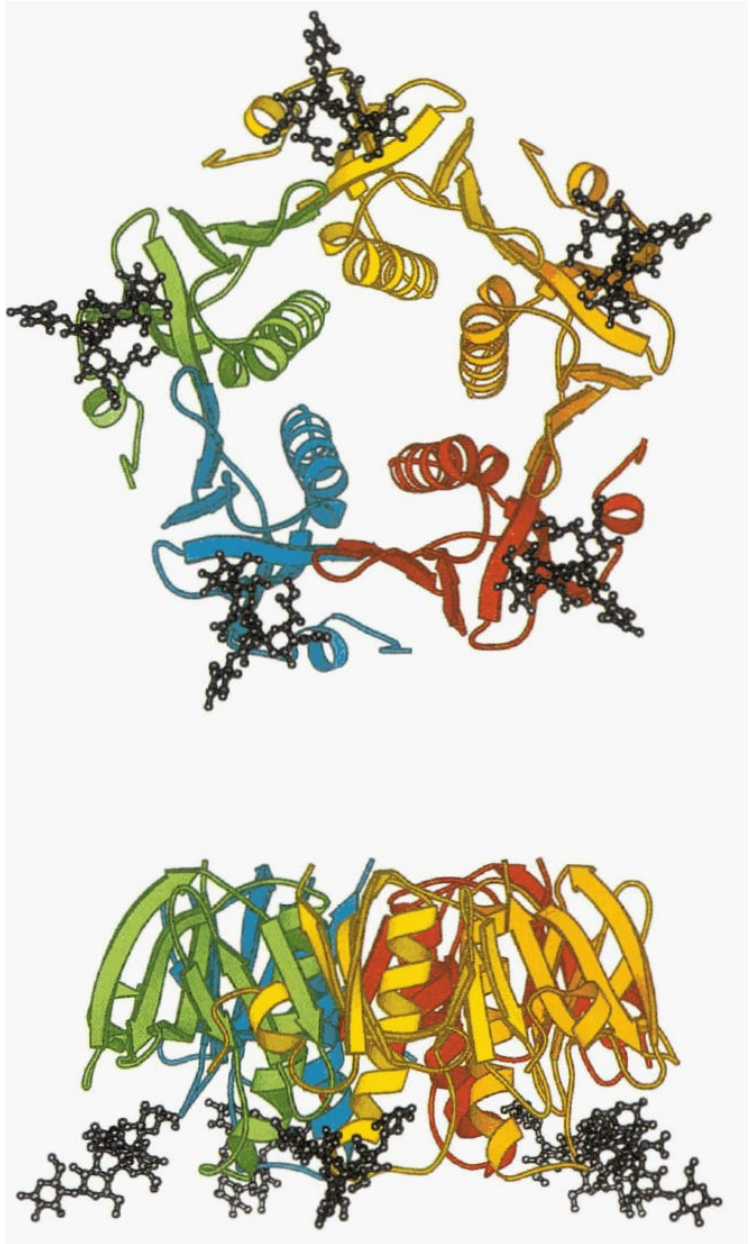




n-butyl-deoxynojirimycin =
Miglustat = Zavesca®



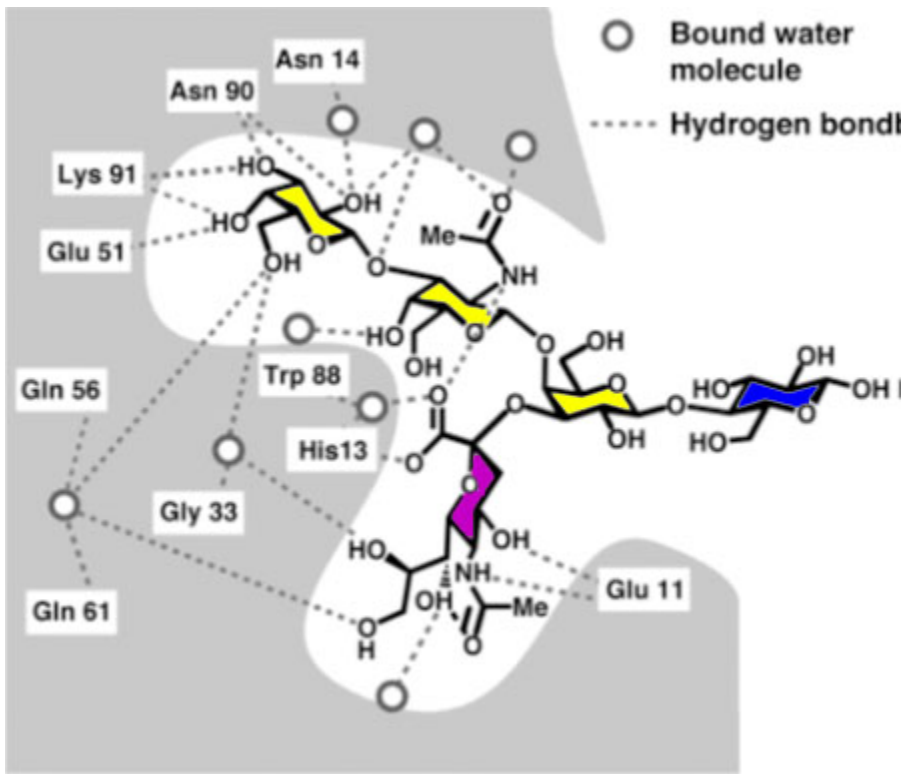

## Bacterial toxins that bind to glycolipids

- Shigatoxin (verotoxin) binds to the neutral glycosphingolipid $Gb_3$ (Galα1-4Galβ1-4Glcβ1-Cer)
- Cholera and E. Coli enterotoxins bind to the ganglioside GM1 (Galβ1-3GalNAcβ1-4(NeuAcα2-3)Galβ1-4Glcβ1-Cer)
- Tetanus and botulinum neurotoxins toxins bind to nervous system gangliosides with different specificities

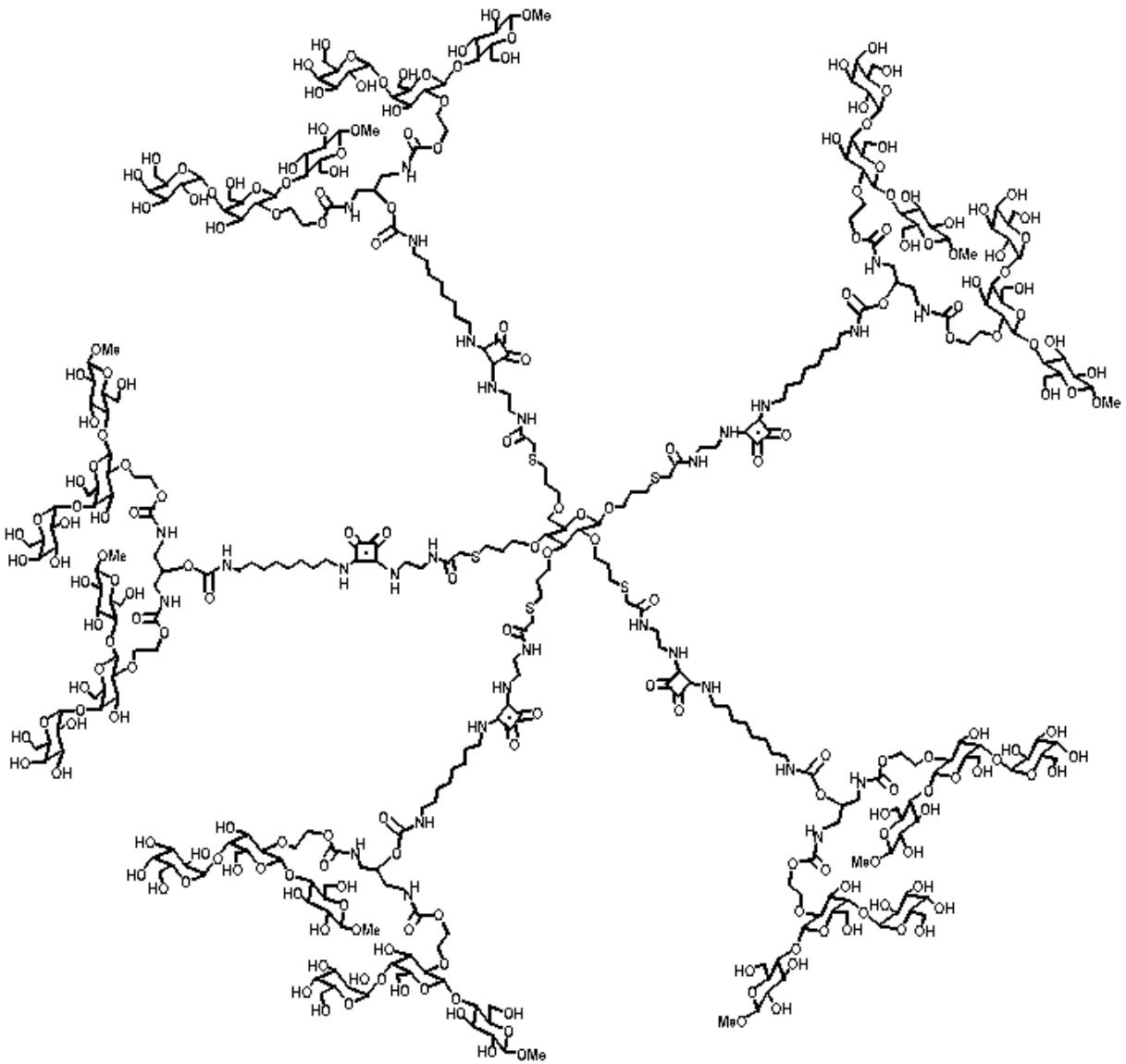


Merritt et al (1994) *Protein Sci* 3:166

Cholera toxin B-subunit evolved to engage both "arms" of GM1 (Gal β1-3 GalNAc and Neu5Ac)

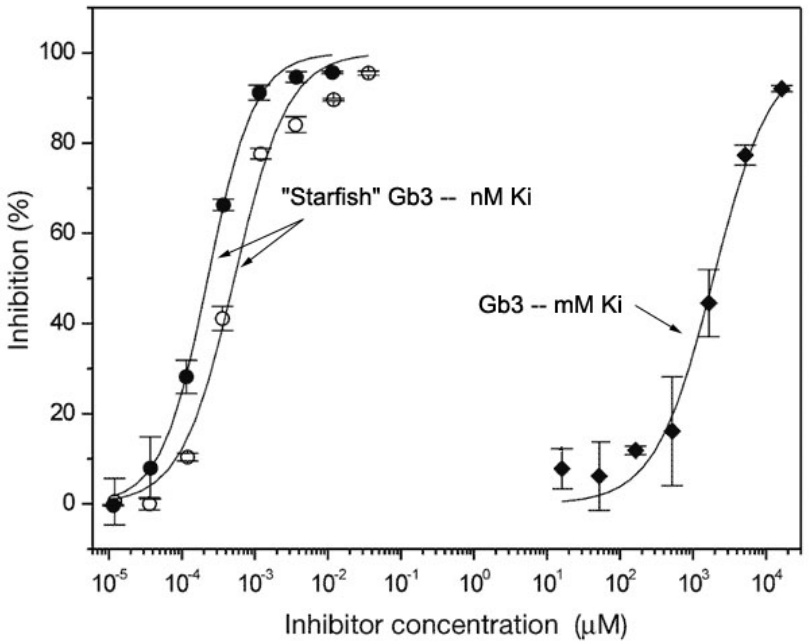
# Multivalent glycan toxin inhibitor



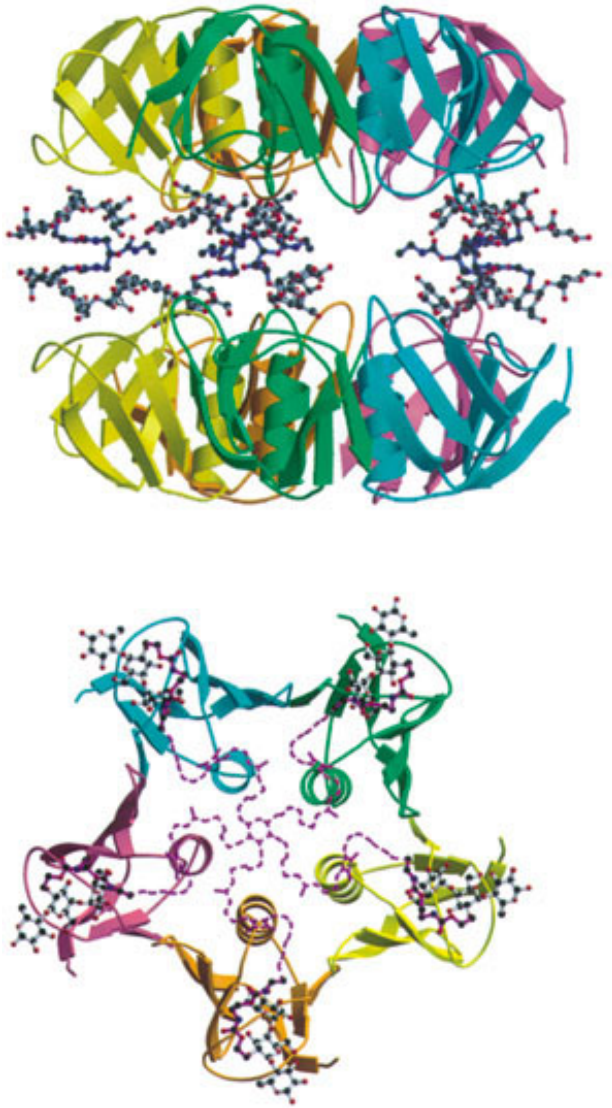
Taking advantage of the AB5 structure of vertotoxin, and its Gb3 specific binding, Kitov et al synthesized a "starfish" shaped inhibitor with five arms, each carrying two copies of Gb3.

Kitov et al. (2000) *Nature* 403:669

# Multivalent glycan toxin inhibitor




"Starfish"Gb3 blocks verotoxin binding at sub-nM concentrations – a million times more potent than Gb3



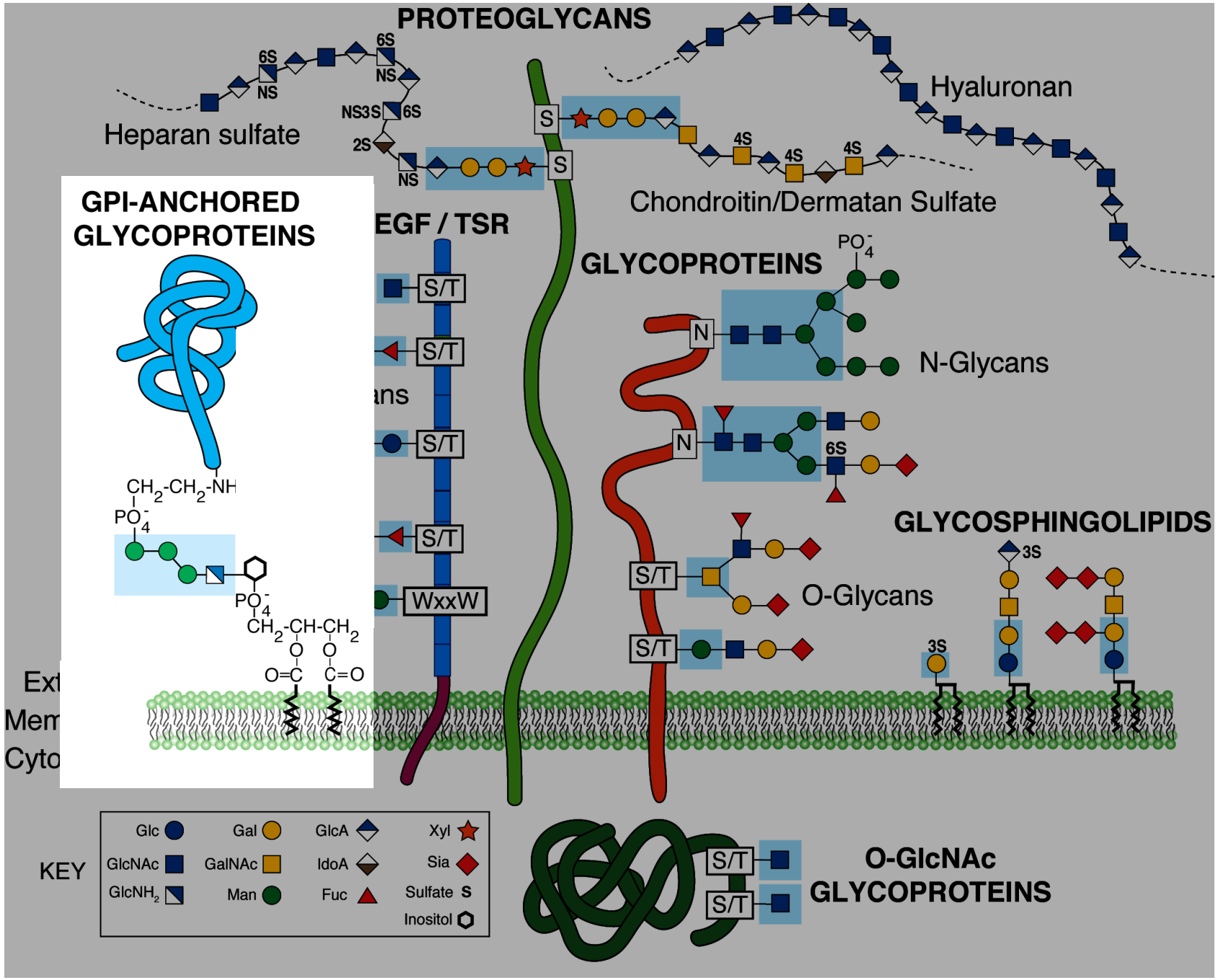
"Starfish"Gb3 crystallized with verotoxin – a pentomeric sandwich

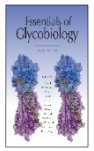
Kitov et al. (2000) *Nature* 403:669

# Glycolipids and GPI anchors

Ronald Schnaar
The Johns Hopkins School of Medicine

Rosetta Stone, British Museum



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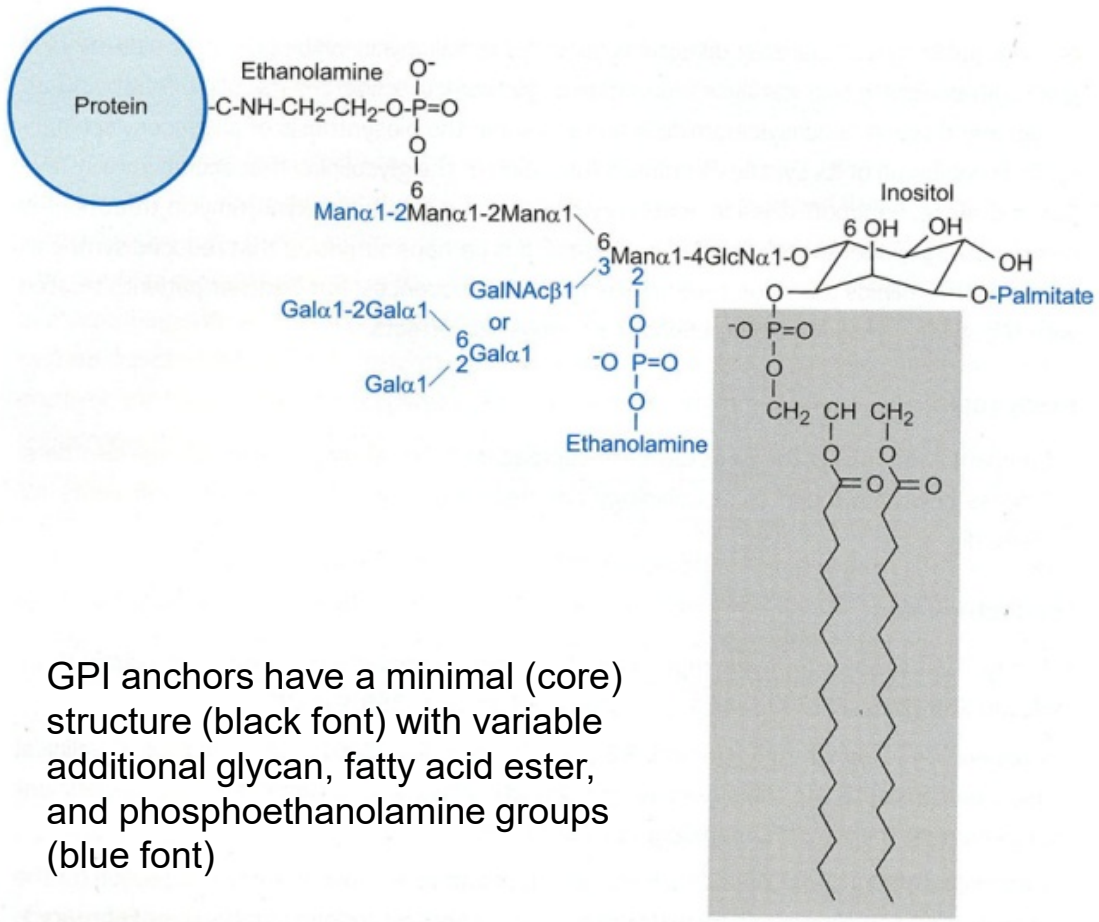
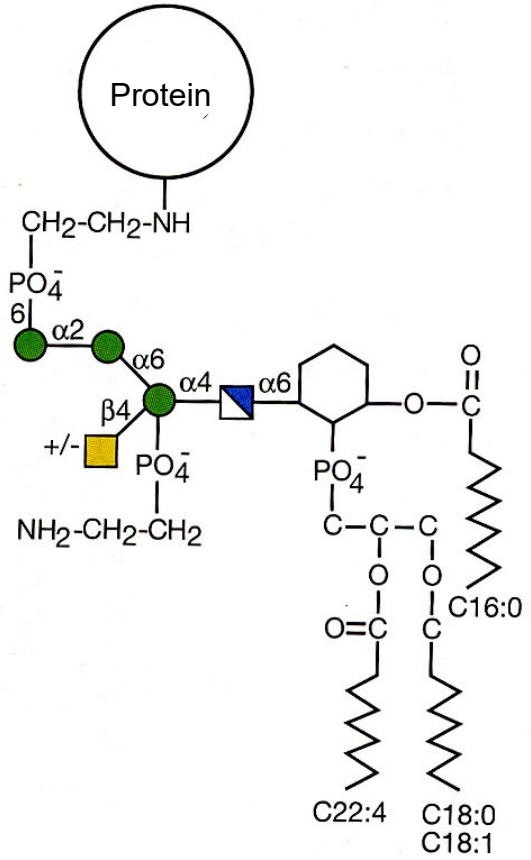
# GPI anchored proteins

A small functionally diverse group of proteins are attached to the cell surface by a glycosylphosphatidylinositol (GPI) anchor



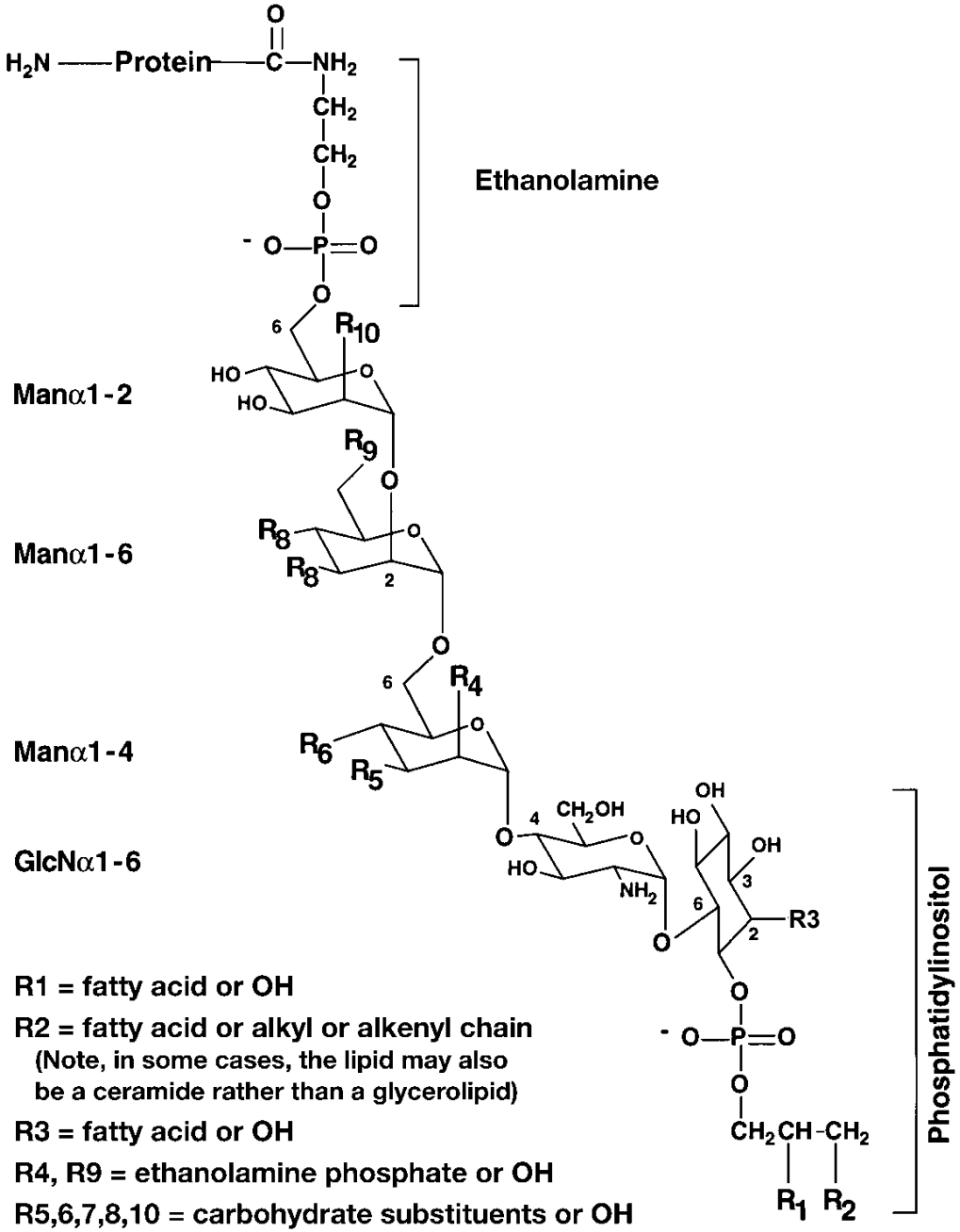




GPI anchors have a minimal (core) structure (black font) with variable additional glycan, fatty acid ester, and phosphoethanolamine groups (blue font)

Taylor and Drikamer (2011) *Introduction to Glycobiology*

# GPI anchor characteristics

- Rare glucosamine (free amine)
- Phosphodiesters at each end
- Diverse core modifications, especially in parasites



# A select group of proteins are GPI anchored*

## Mammals

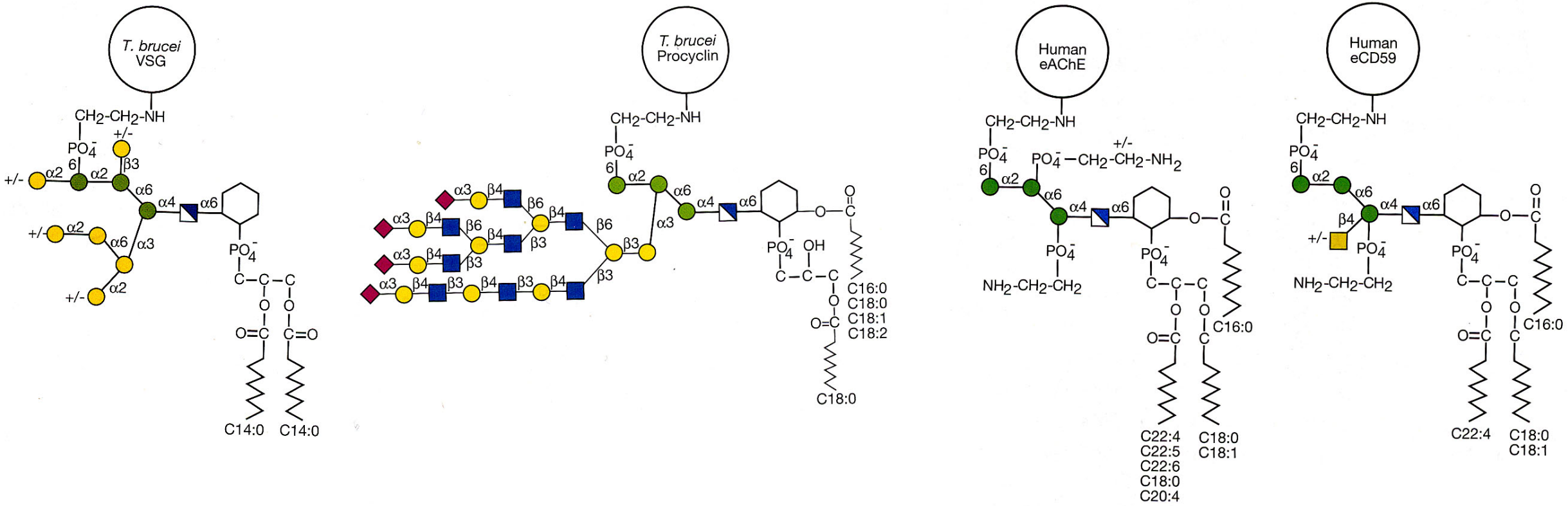
| | |
|---|---|
| Erythrocyte CD59 and decay acceleration factor (DAF) | complement regulation |
| Alkaline phosphatase | cell-surface hydrolase |
| 5′-Nucleotidase | cell-surface hydrolase |
| Renal dipeptidase | cell-surface hydrolase |
| Trehalase | cell-surface hydrolase |
| Neural cell adhesion molecule 120 (NCAM-120) | adhesion molecule |
| Neural cell adhesion molecule TAG-1 | adhesion molecule |
| CD58 | adhesion molecule |
| FcγIII receptor | Fc receptor |
| Ciliary neurotrophic factor receptor (CNTFR) α subunit | neural receptor |
| Glial-cell-derived neurotrophic factor receptor (GDNFR) α subunit | neural receptor |
| CD14 | LPS receptor |
| Prion protein (PrP) | unknown |
| Glypican family of GPI-anchored proteoglycans | extracellular matrix component |

## Parasites

| | |
|---|---|
| *Trypanosoma brucei* variant surface glycoprotein (VSG) | protective coat |
| *Leishmania major* promastigote surface protease (PSP) | bound complement degradation |
| *Trypanosoma cruzi* GPI-anchored mucins | host cell invasion |
| *Plasmodium falciparum* merozoite surface protein 1 (MSP-1) | erythrocyte invasion |
| *Toxoplasma gondii* surface antigen 1 (SAG-1) | host cell invasion |
| *Entamoeba histolytica* GPI proteophosphoglycans | virulence factor |

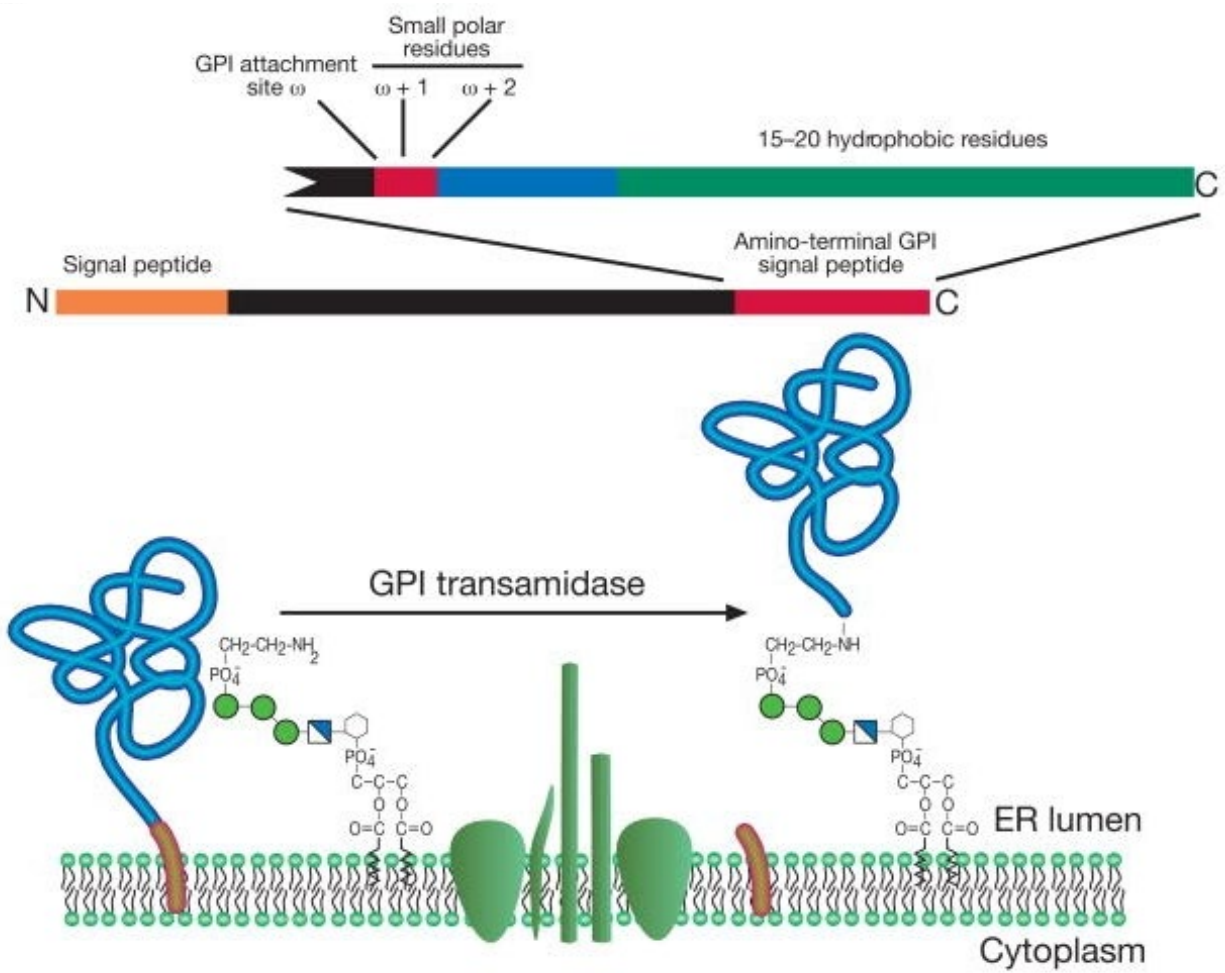
*plus some in yeast, plants, some other species

# Variety is the spice of… GPI anchors



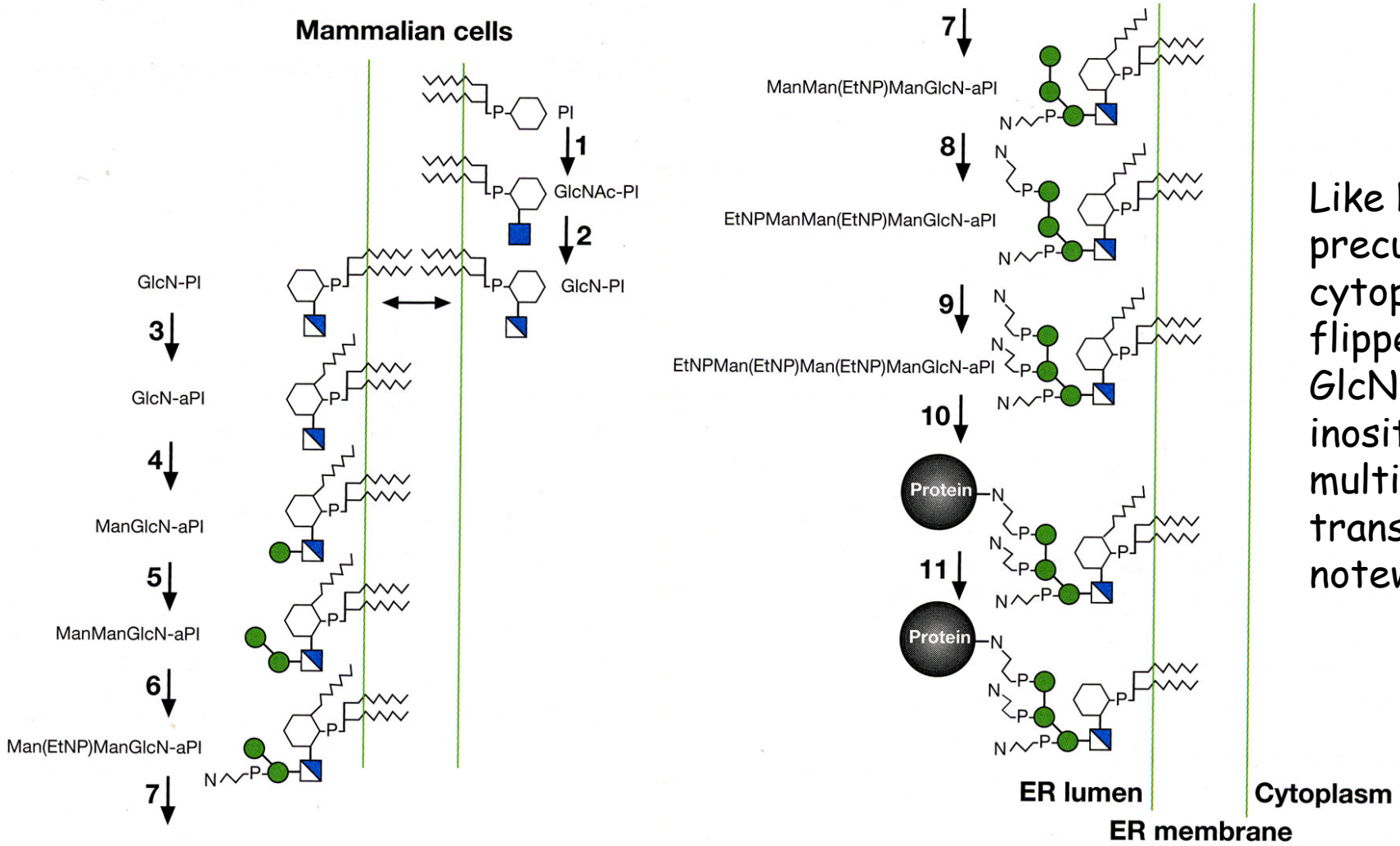
# GPI anchor installation

In the ER, preassembled GPI anchors are transferred en bloc via amide linkage to an amino acid near the carboxy terminus of a nacient protein, releasing a C-terminal fragment.




Although there is no “consensus sequence” for GPI transfer, likely sites are identified by their structural features.

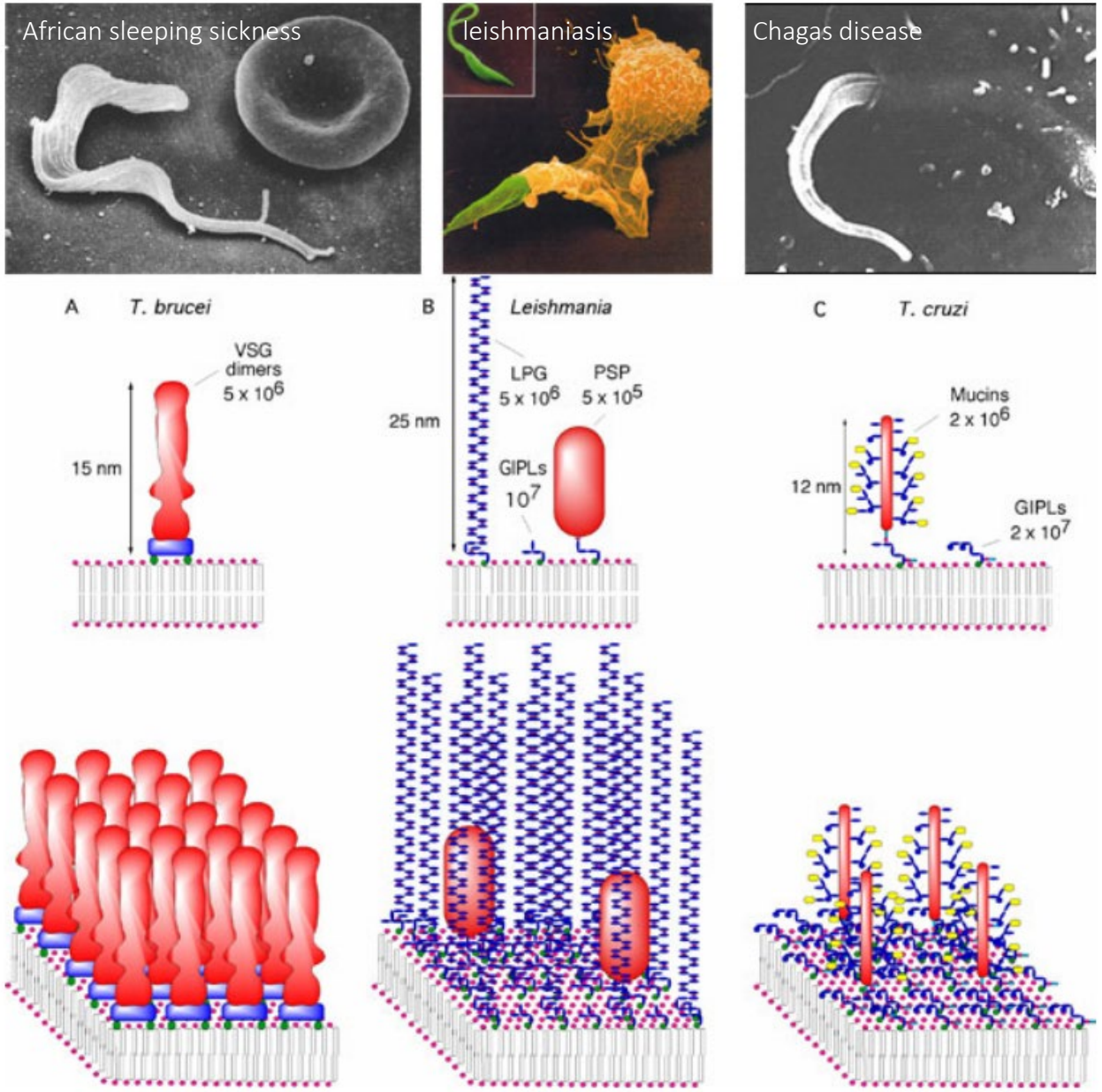
# GPI anchor biosynthesis




Like N-linked glycan biosynthesis, the precursor starts assembly on the cytoplasmic face of the ER, then is flipped inside for further processing. GlcNAc transfer and deacetylation, inositol fatty acid esterification, and multiple ethanolamine phosphate group transfers by separate enzymes are noteworthy.

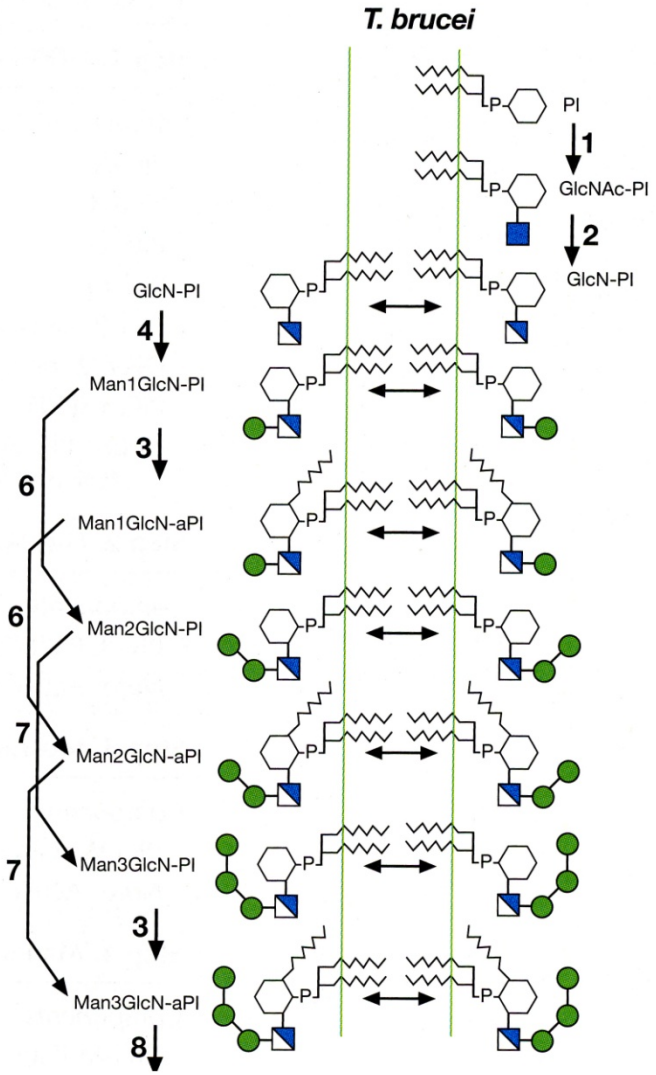
# Parasite GPI anchors

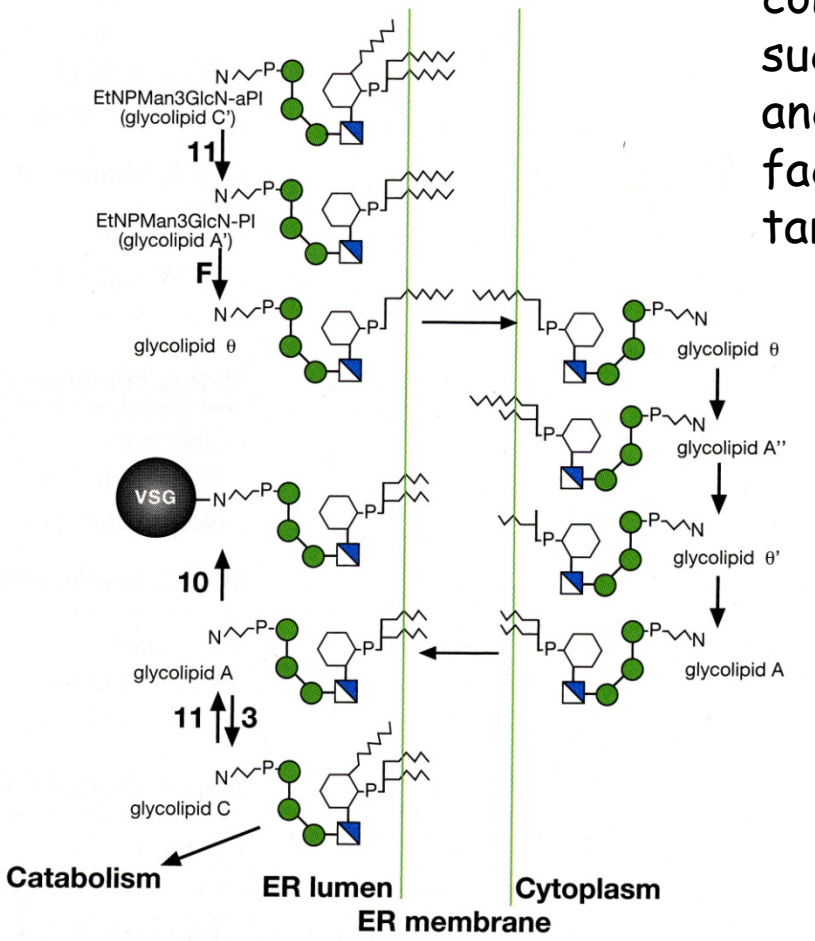
GPI anchor synthesis has been studied most extensively in parasites, which express millions of GPI-anchored proteins and glycans on the surface of each cell.




http://www.lifesci.dundee.ac.uk/people/mike-ferguson

# Parasite GPI anchor biosynthesis




GPI anchor synthesis in parasites is complex with some interesting twists, such as fatty acid remodeling. Since GPI anchored coat proteins are virulence factors, they are being exploited as targets for therapy.

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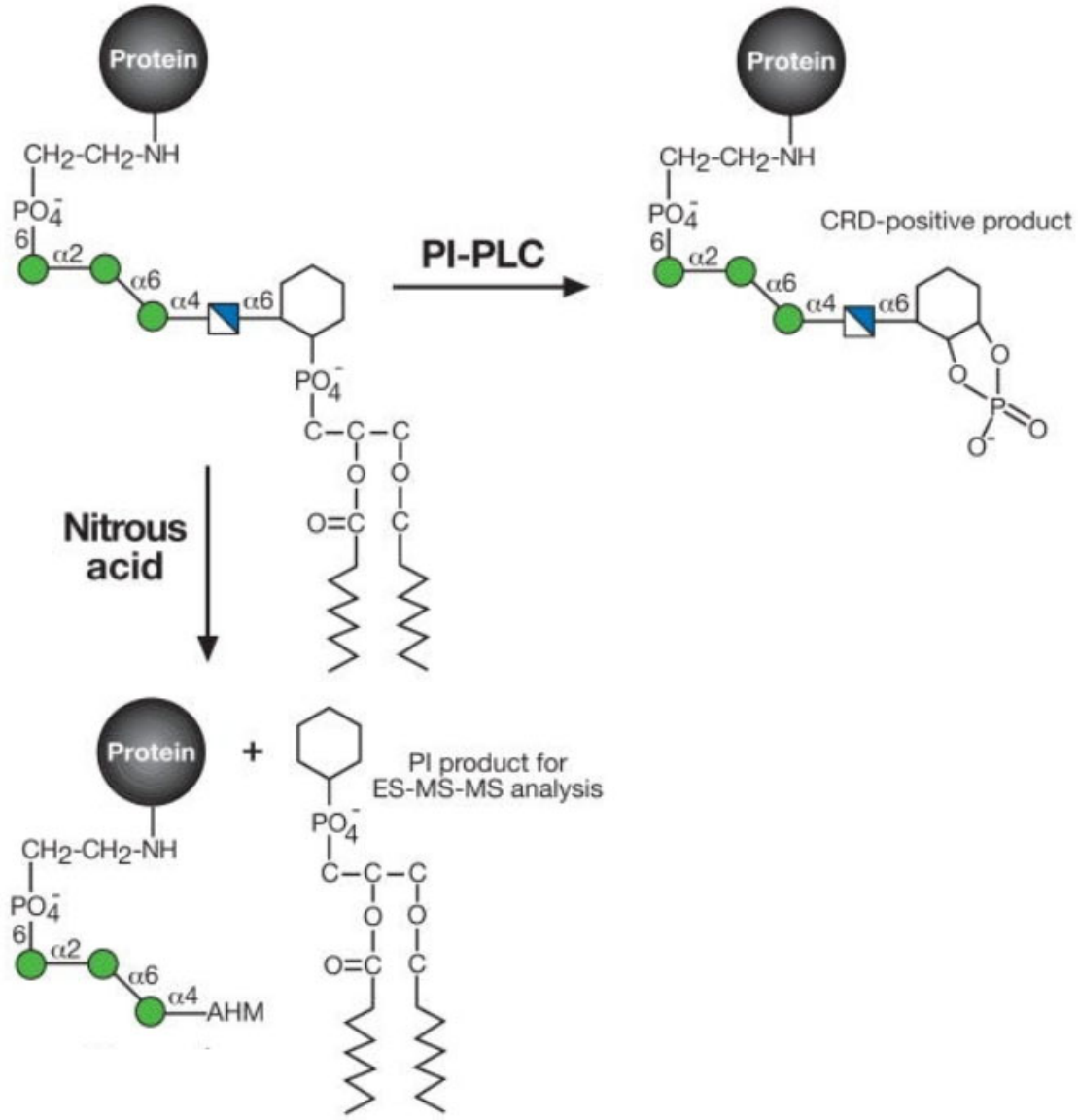
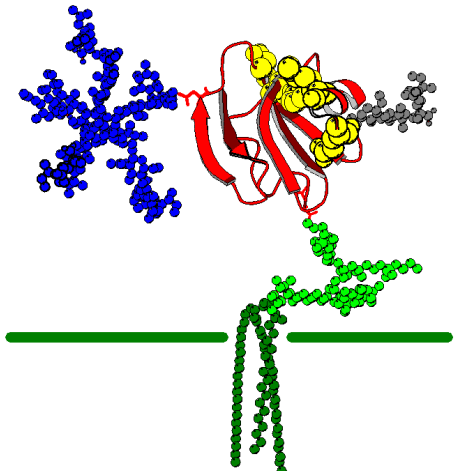
# GPI anchored protein release

GPI anchors are released under biologically compatible conditions using bacterial phosphatidylinositol-specific phospholipase C (PIPLC), or decomposed by nitrous acid cleavage at the glucosamine



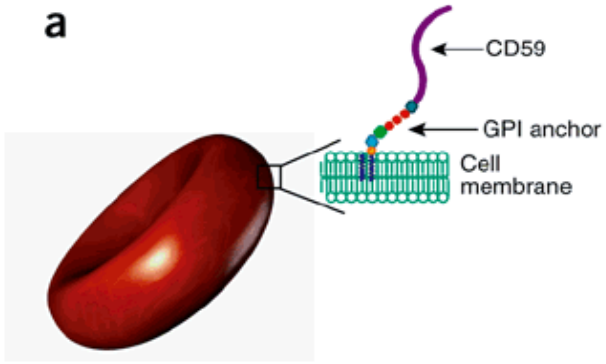
# Clinical correlation: Paroxysmal nocturnal hemoglobinuria

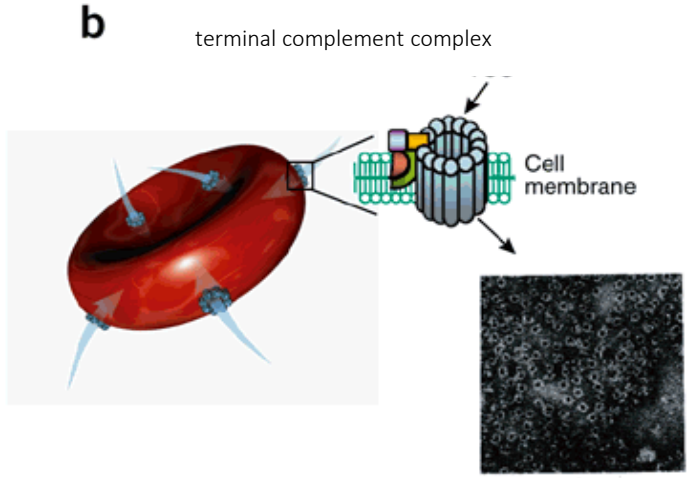


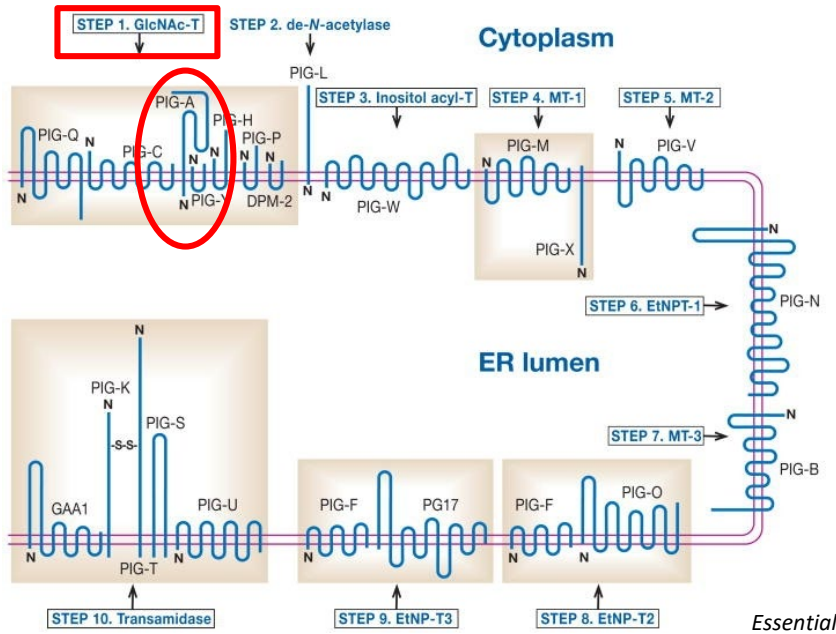
CD59, a GPI-anchored "complement defense protein"

http://www.bioch.ox.ac.uk/glycob/archive/

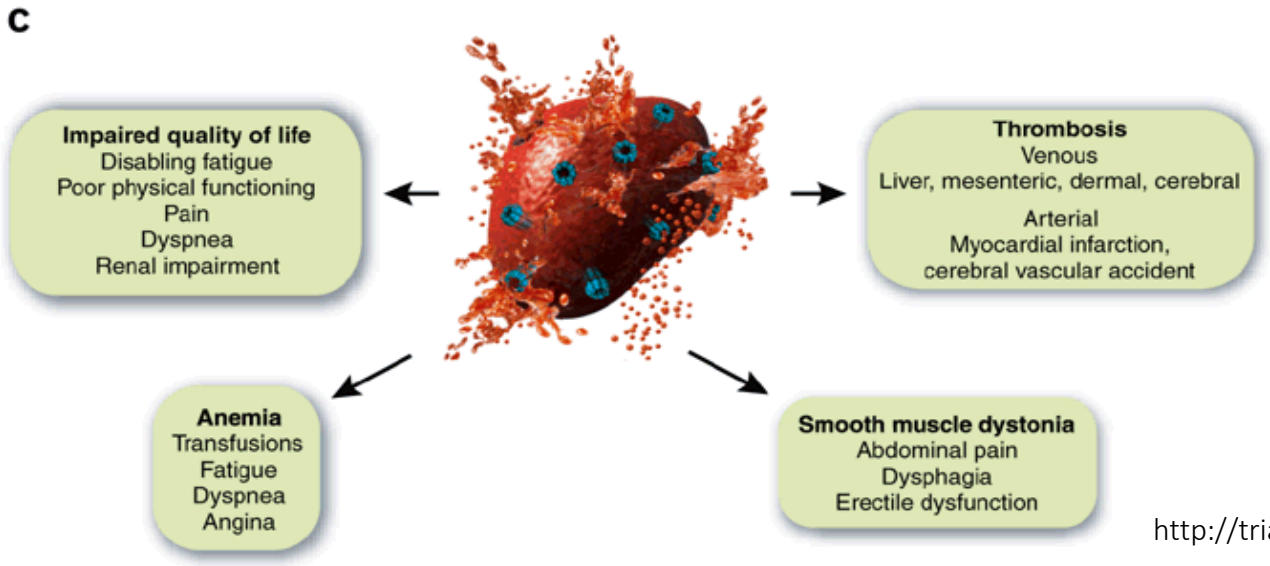
Somatic PIG-A mutation results in failure to transfer GlcNAc to phosphatidylinositol & loss of GPI-anchored complement control proteins



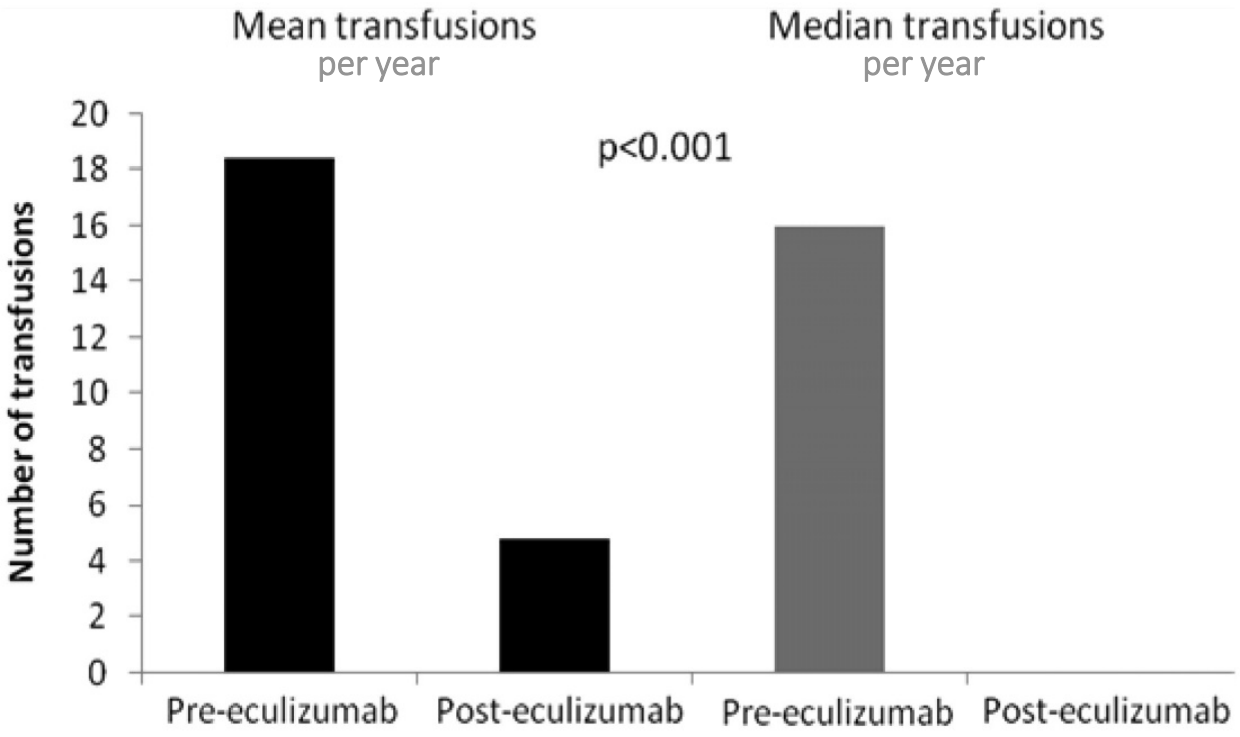



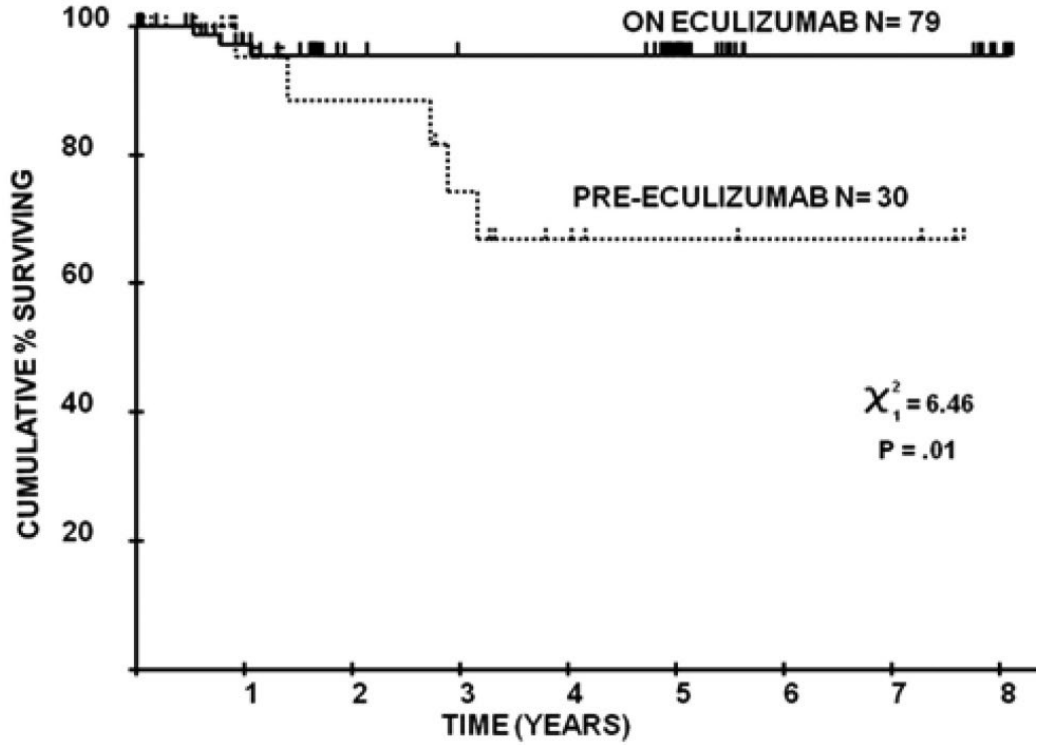

*Essentials of Glycobiology*



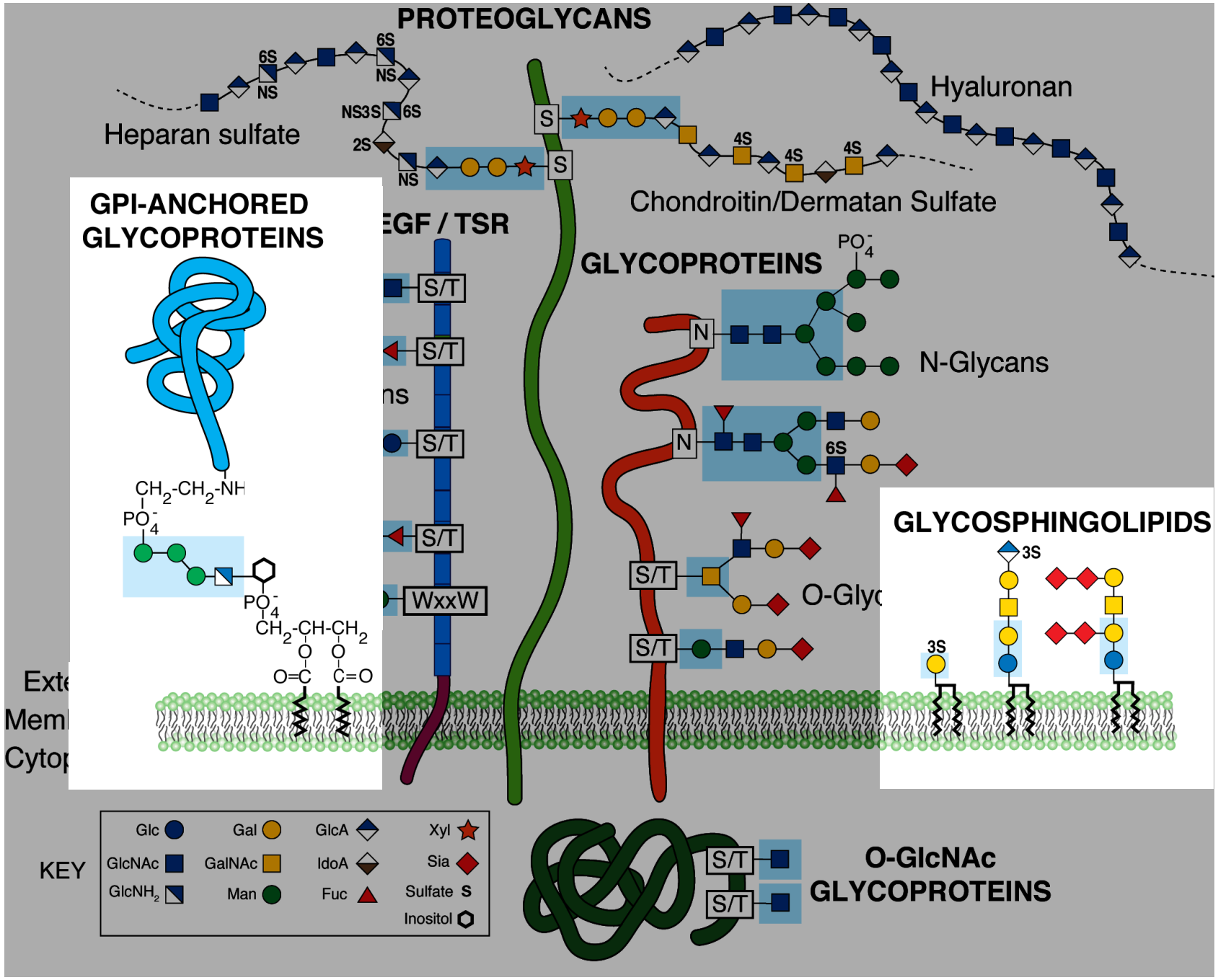

http://trialx.com/

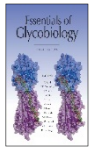
# Anti-complement protein 5 treatment improves outcomes




Kelly R J et al. *Blood* (2011)117:6786



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