1. Concept of Glycobiology

Learning object

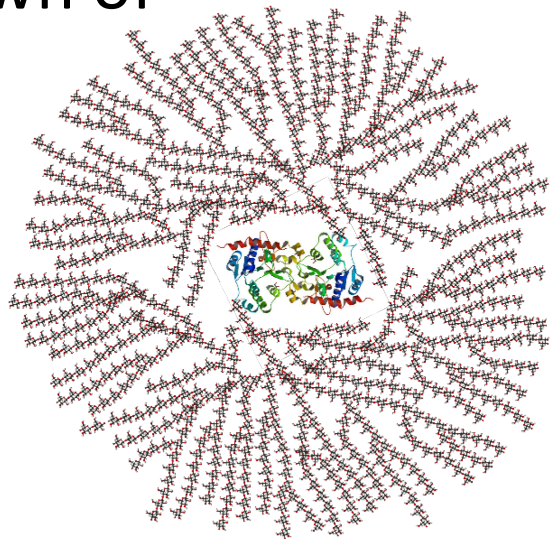
- The classes of glycoconjugates and their locations in eukaryotic cells
- The type of function served by glycosylation

- The challenge of glycobiology is to define the biological functions of sugars attached to proteins and membranes and to determine how these functions are carried out.
- Although this challenge is ongoing, the principles that describe how protein- and lipid- linked sugars mediate biological processes are becoming clear.
- The goal of this lecture is to illustrate the themes that underlie the functions of glycoproteins and glycolipids by highlighting well-understood examples of how the carbohydrate portions of these molecules work.

1.1 The field of glycobiology encompasses the multiple functions of sugars attached to proteins and lipids

Energy metabolism in cells

Pathway to abstract energy from breakdown of glucose and glycogen



Glycogen is even linked to protein core

The function of sugar molecules as forms of energy for storage and transport is generally considered to fall outside the field of glycobiology.

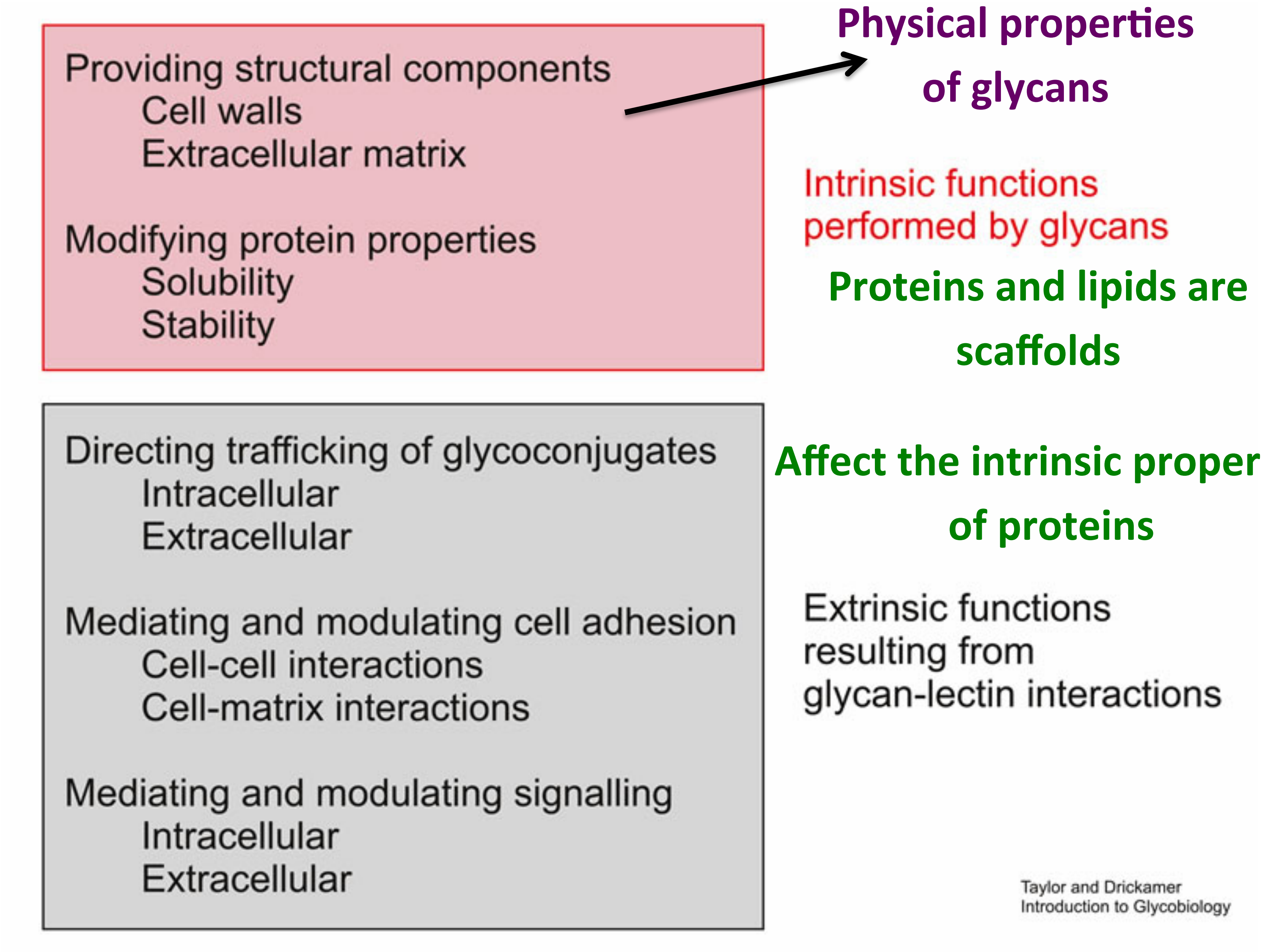
Glycoconjugates: mono-, oligo-, or polysaccharides are attached to proteins or lipids

Generally complex heteropolymers, rather than repeating homopolymers, such as glycogen and amylose

The sugars of glycoproteins and glycolipids are referred as Glycans

No single predominant function of protein- and lipid-linked glycans.

Similar to proteins, which serve diverse functions as enzymes, hormones, transporters and structural elements.



Providing structural components
- Cell walls
- Extracellular matrix

Modifying protein properties
- Solubility
- Stability

Physical properties of glycans

Intrinsic functions performed by glycans

Proteins and lipids are scaffolds

Affect the intrinsic properties of proteins

Directing trafficking of glycoconjugates
- Intracellular
- Extracellular

Mediating and modulating cell adhesion
- Cell-cell interactions
- Cell-matrix interactions

Mediating and modulating signalling
- Intracellular
- Extracellular

Extrinsic functions resulting from glycan-lectin interactions

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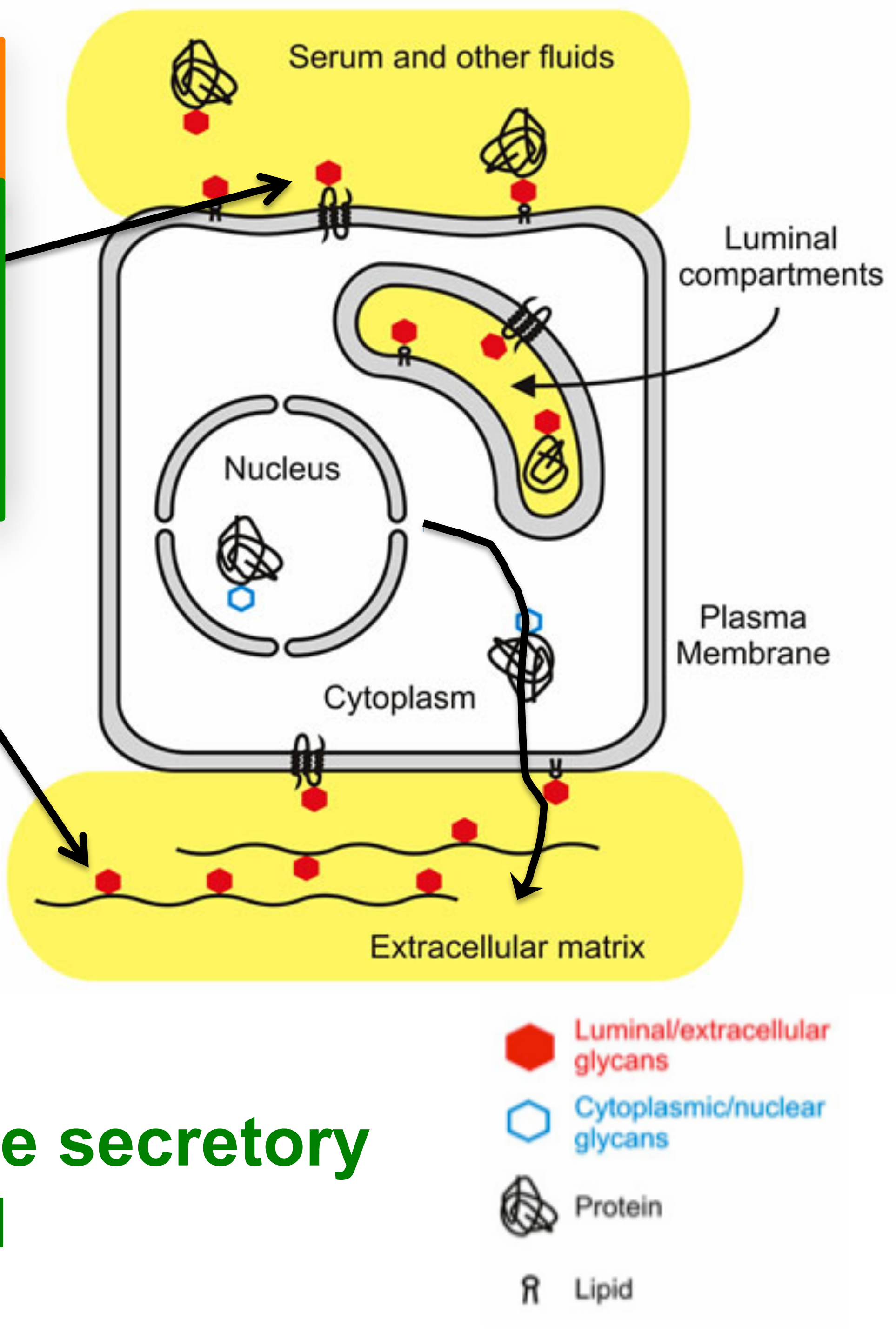
1.2 There are three major classes of glycoconjugates

Those attached to lipids

Those attached to proteins through N (N-linked)

O-linked glycan

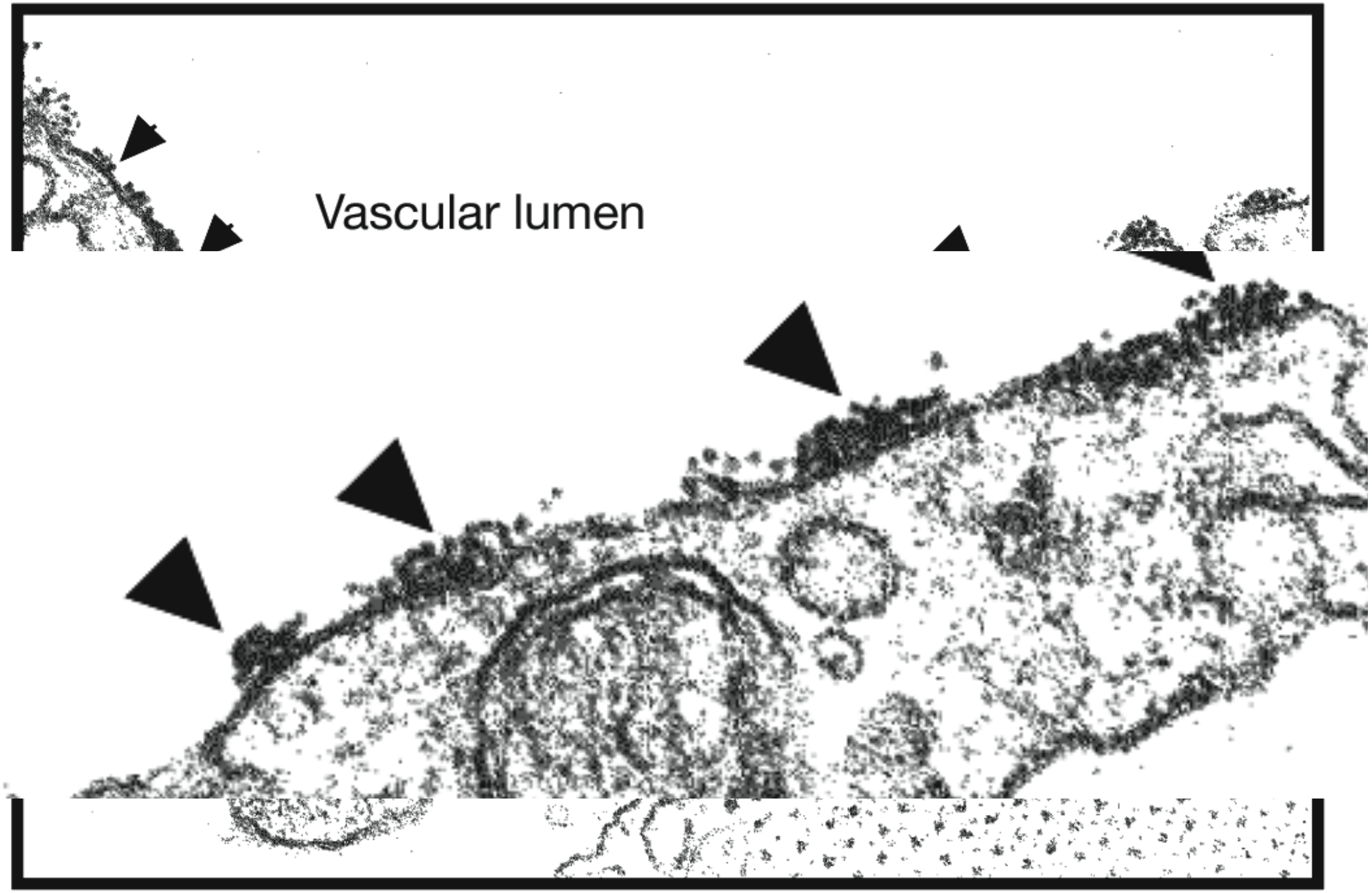
Reflects the historical availability of serum glycoproteins



An integral part of the secretory machinery of the cell

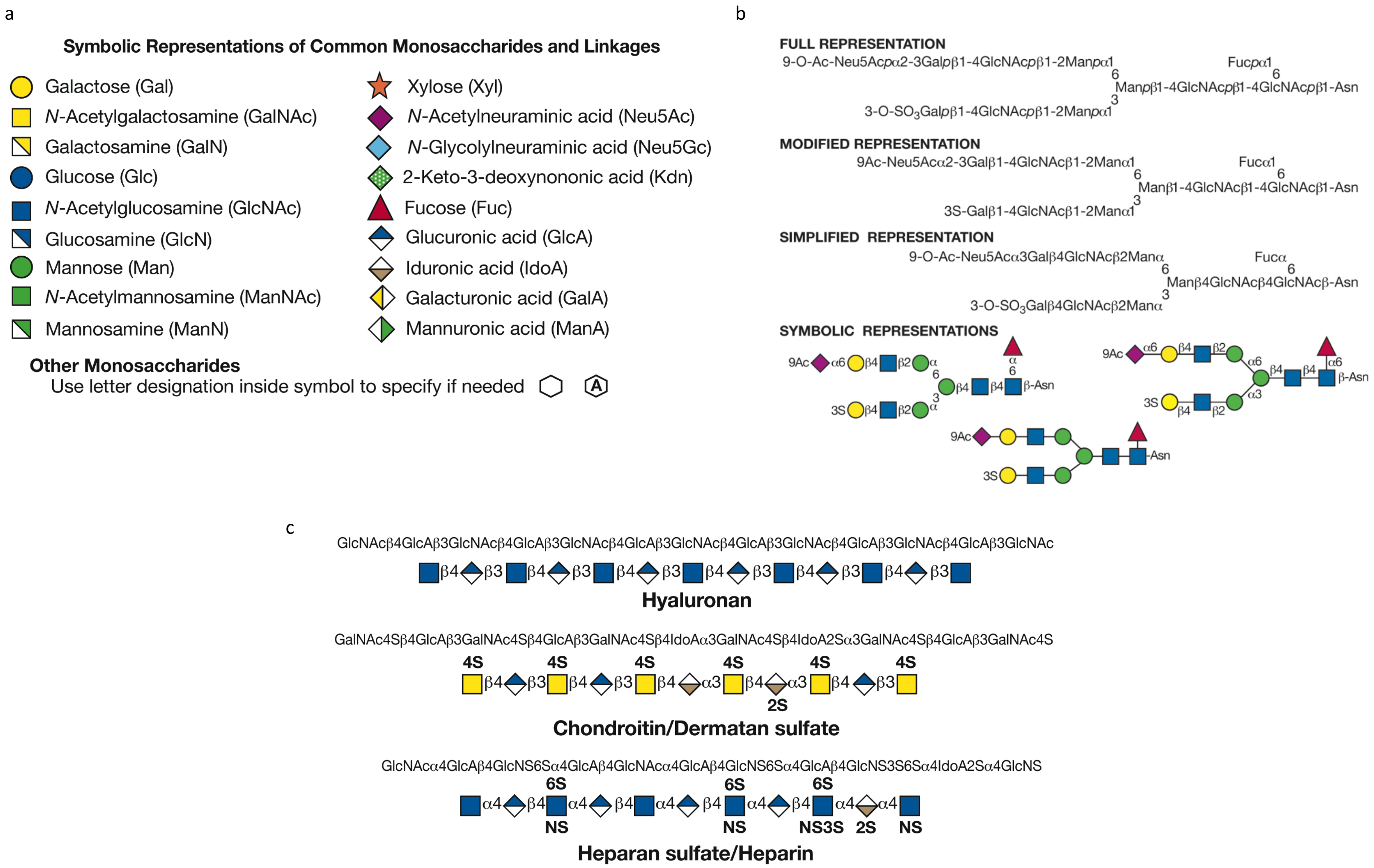
Electron micrograph of endothelial cells from a blood capillary

Courtesy of George E. Palade (NP 1974)

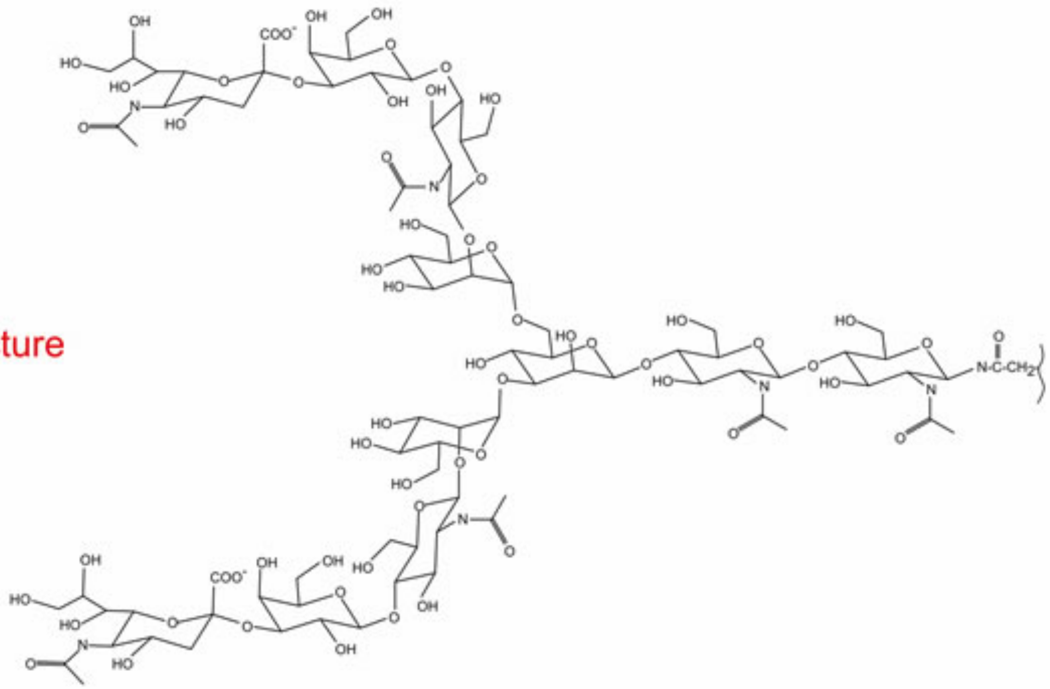


decorated with particles of cationized ferritin

Monosaccharide symbol set



Complete chemical structure

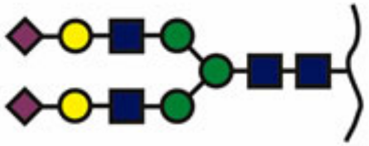


Word structure

NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1
$^{6}_{3}$Manβ1-4GlcNAcβ1-4GlcNAcβ1-Asn
NeuAcα2-3Galβ1-4GlcNAcβ1-2Manα1

Symbol representation

◆ NeuAc
■ GlcNAc
○ Gal
● Man



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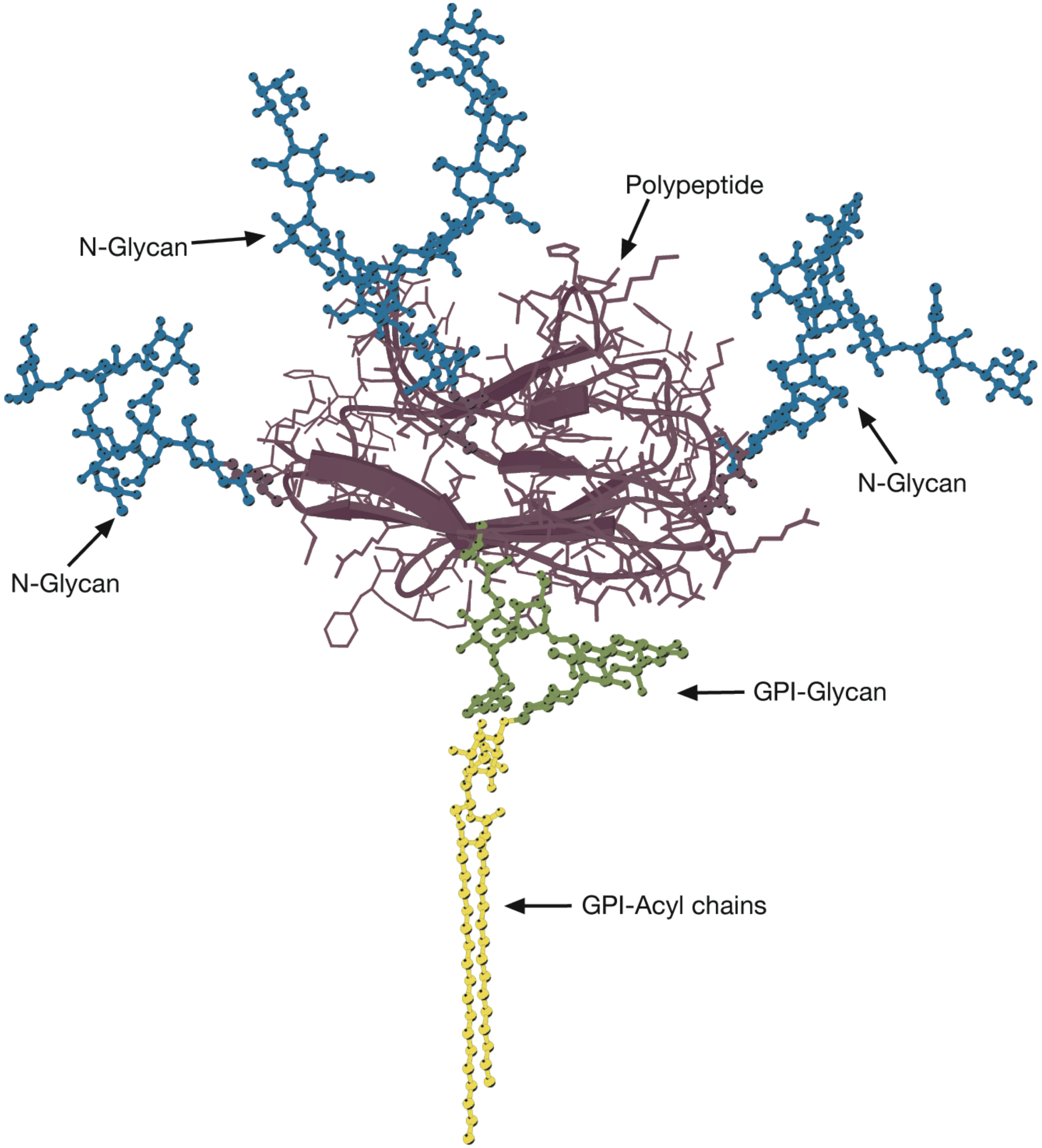
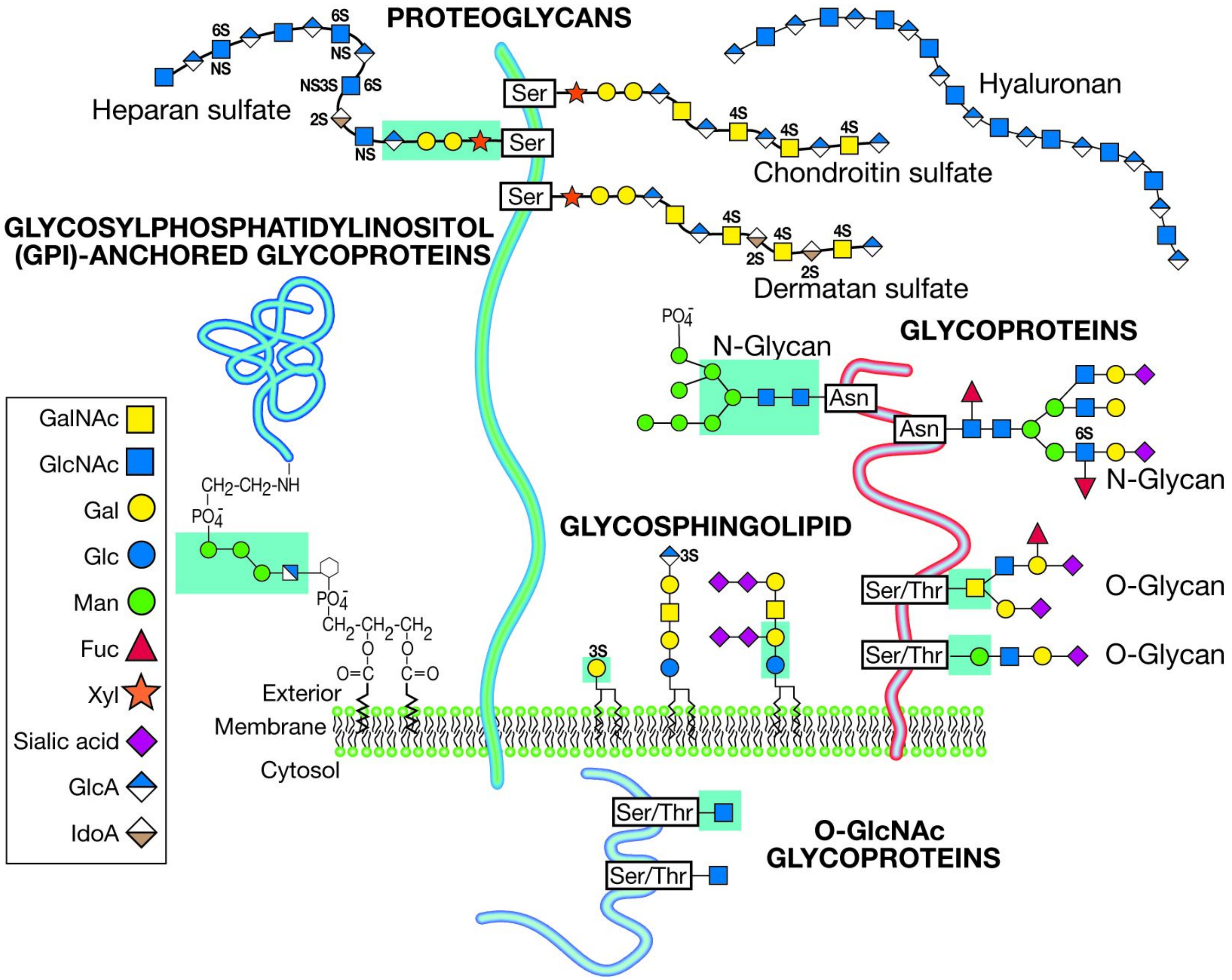
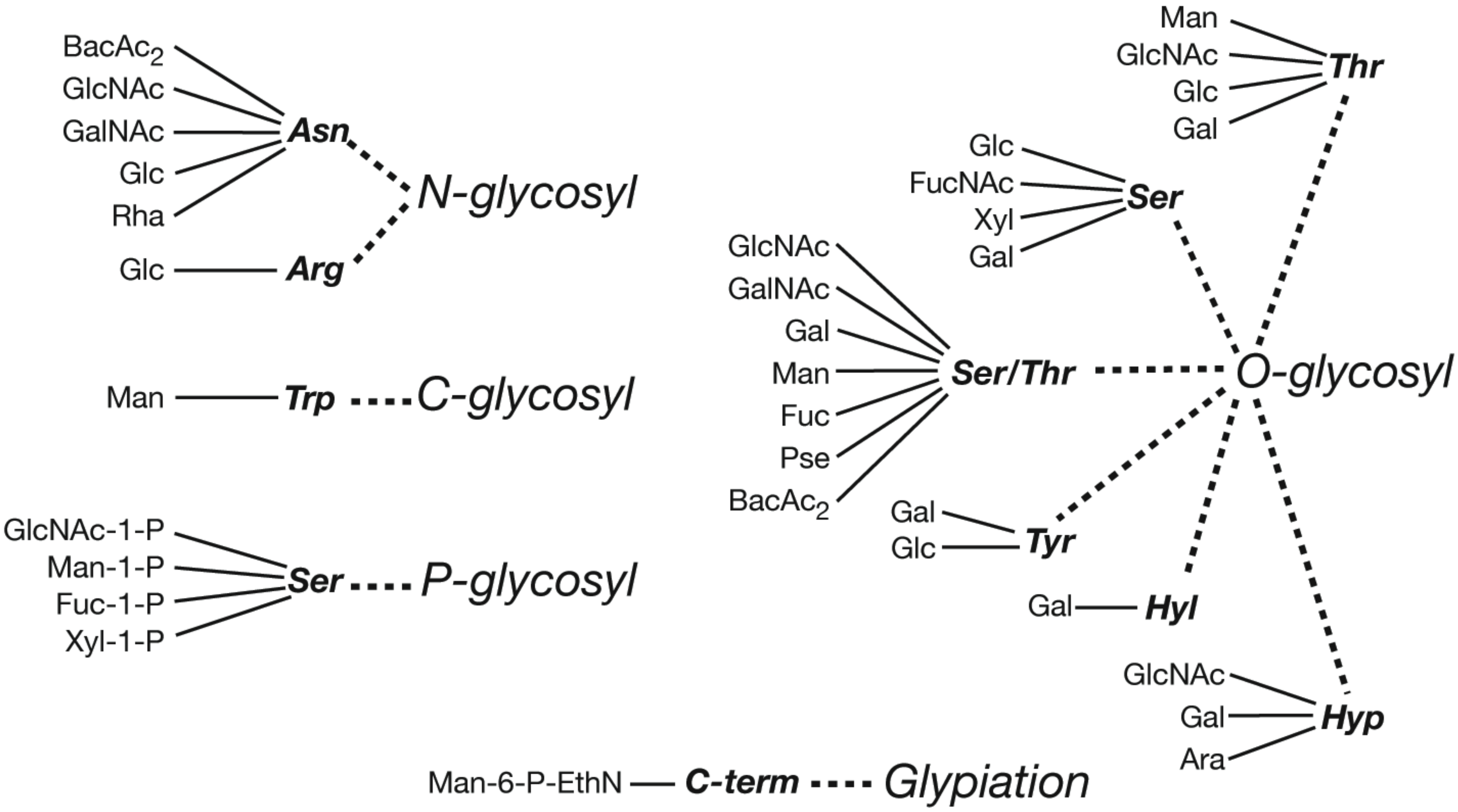
Schematic representation of the Thy-1 glycoprotein



Common classes of animal glycans



Glycan-protein linkages reported in nature



BacAc$_2$
GlcNAc
GalNAc
Glc
Rha
Asn

Glc
Arg

N-glycosyl

Man
Trp
C-glycosyl

GlcNAc-1-P
Man-1-P
Fuc-1-P
Xyl-1-P
Ser
P-glycosyl

Man
GlcNAc
Glc
Gal
Thr

Glc
FucNAc
Xyl
Gal
Ser

GlcNAc
GalNAc
Gal
Man
Fuc
Pse
BacAc$_2$
Ser/Thr

O-glycosyl

Gal
Glc
Tyr

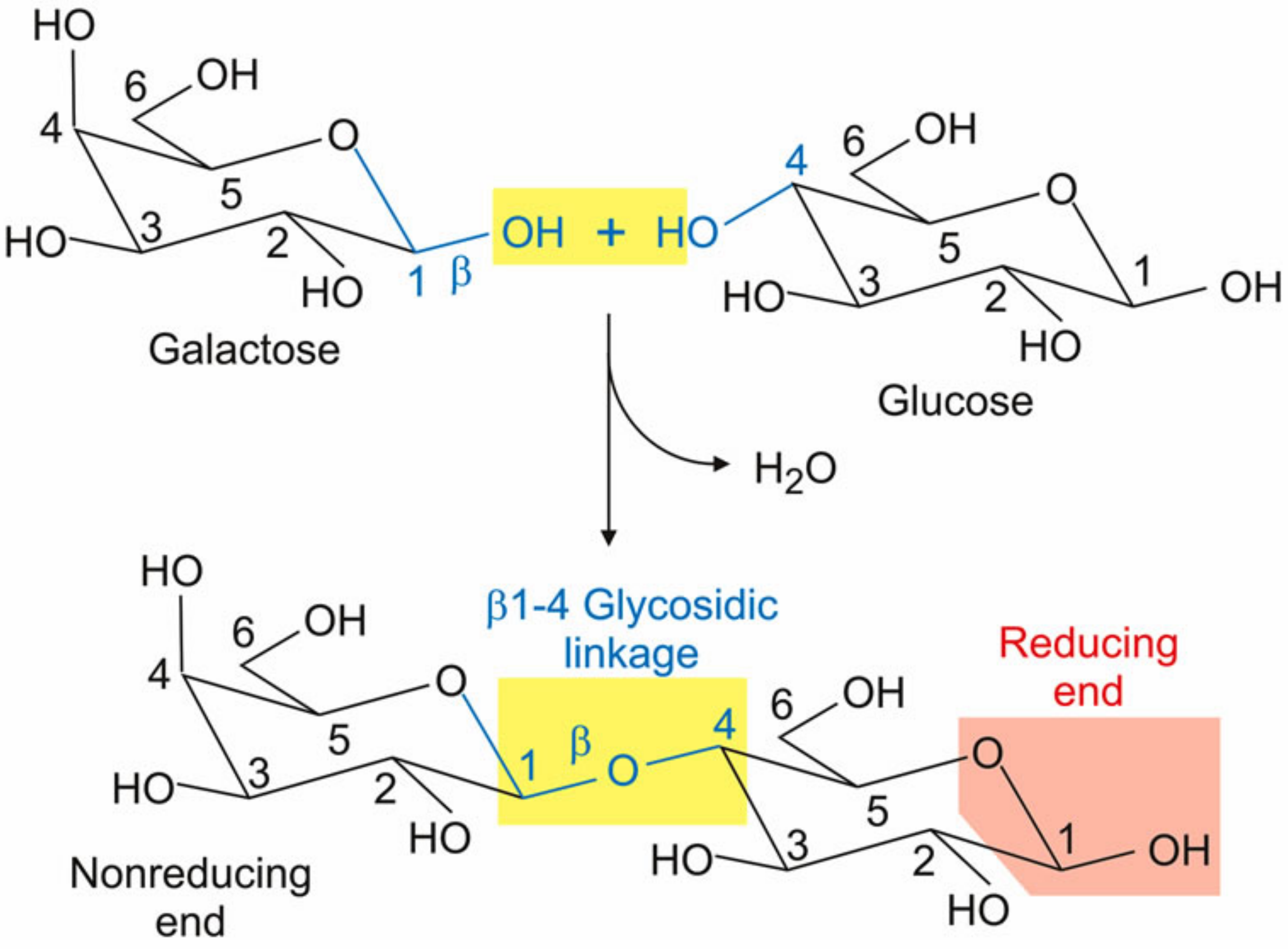
Gal
Hyl

GlcNAc
Gal
Ara
Hyp

Man-6-P-EthN
C-term
Glypiation

1.3 Formation of glycosidic linkages requires energy and is catalysed by specific enzymes

Figure 1.8 Formation of a glycosidic linkage



Galβ1-4Glc = Lactose

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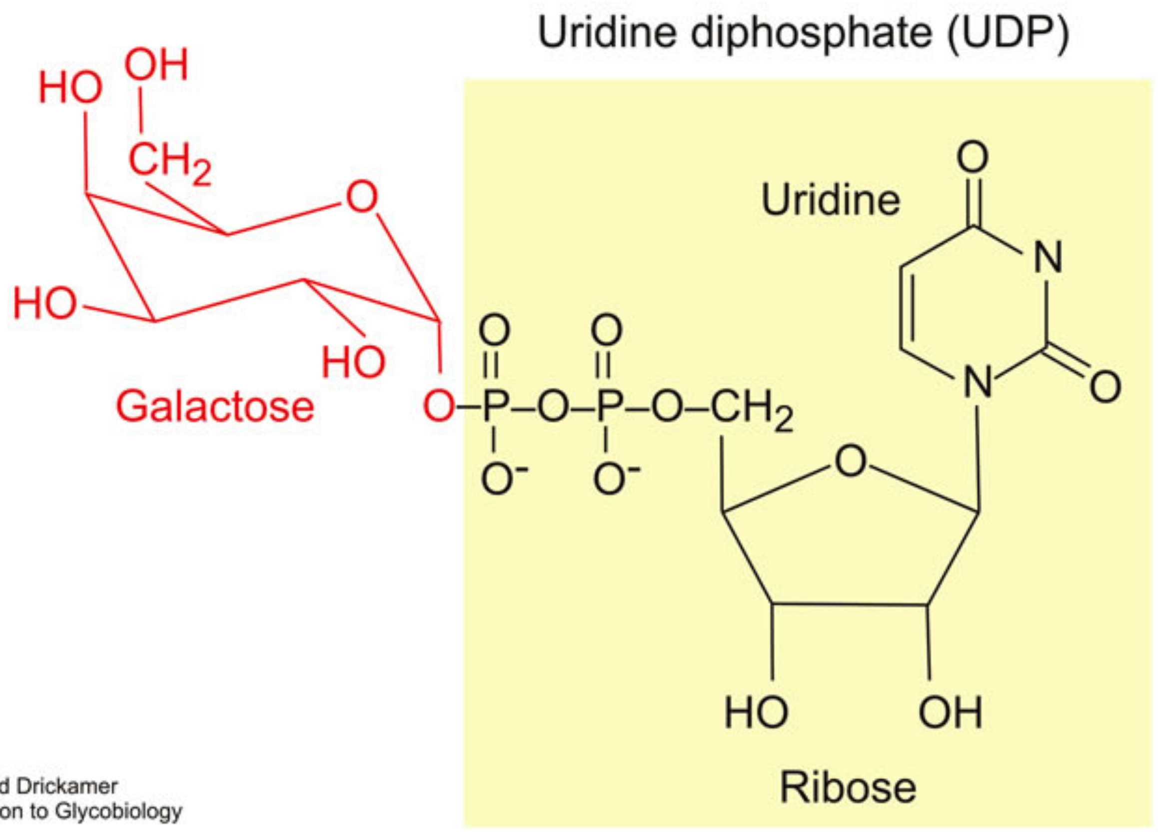

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Gal + UDP + 2ATP → UDP-Gal + 2ADP + $2P_i$

Creation of glycosidic bond

UDP-Gal + Glc → Galβ1-4Glc + UDP

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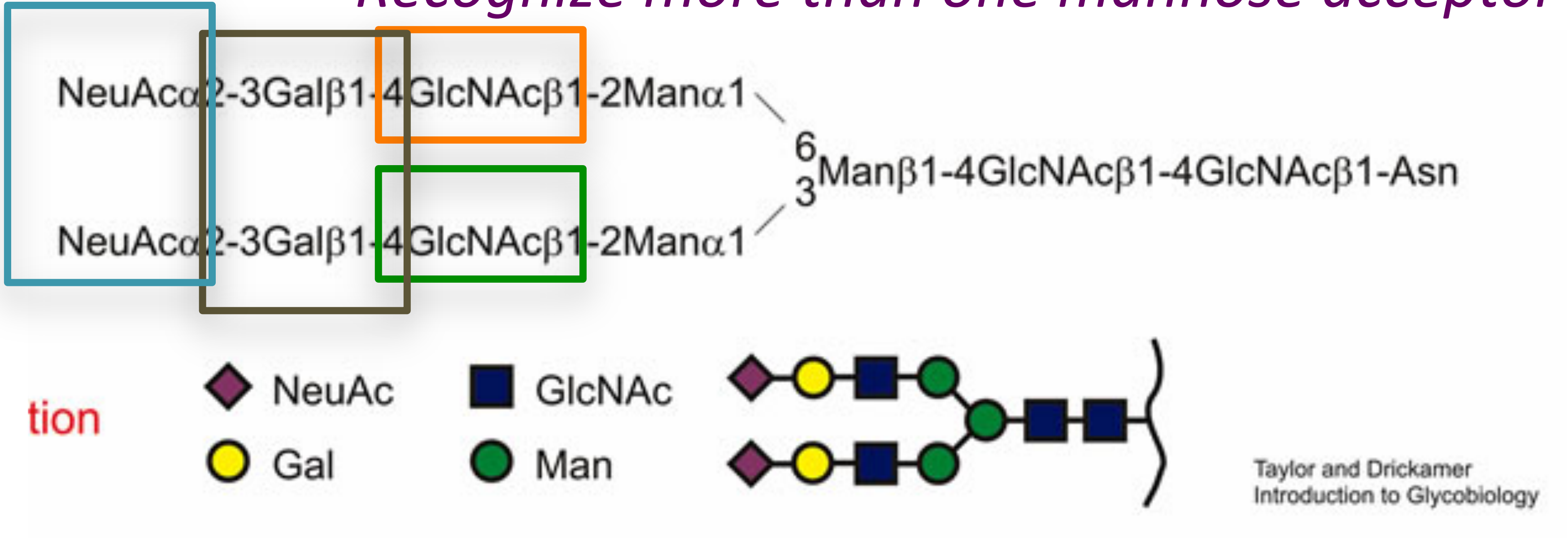
UDP-galactose: glucose β(1,4)-galactosyltransferase

Different transferases for synthesis of lactose and N-acetyl-lactosamine (Galβ1-4GlcNAc)

One enzyme-one linkage rule

Recognize more than one mannose acceptor



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But,

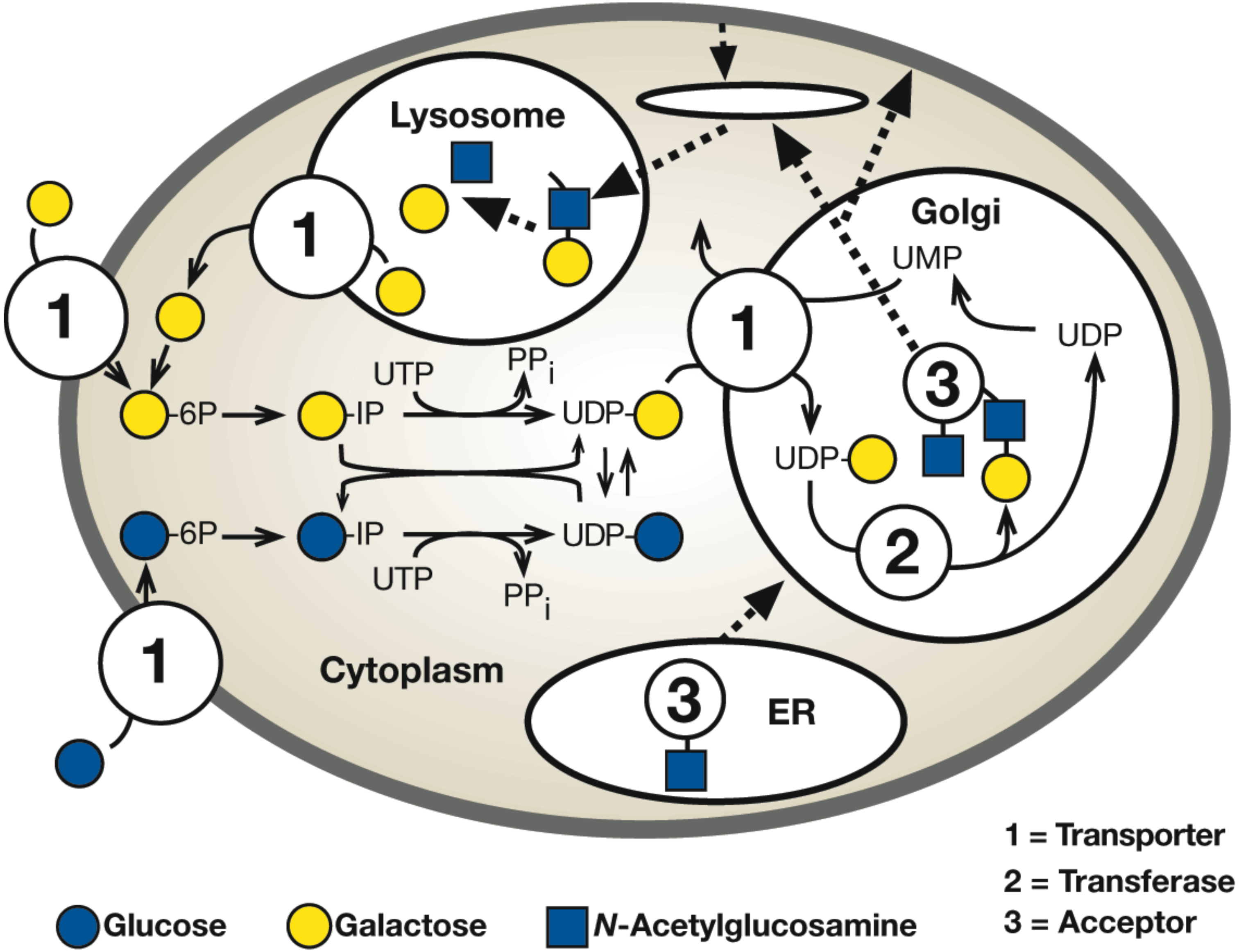
There are often several different glycosyltransferases with similar or overlapping specificities.

Six sialyltransferases can each add sialic acid in 2-3linkage to galactose

Breaking of glycosidic linkages requires enzymes, but does not require the input of energy.

Glycosidases catalyse the energetically favourable hydrolysis of glycosidic linkages
Specific, but Sialidase does not discriminate between α*2-3 and* α*2-6 linked NeuAc*

Biosynthesis, utilization, and turnover of a common monosaccharide



Essentials of Glycobiology
Second Edition

Chapter 1, Figure 8

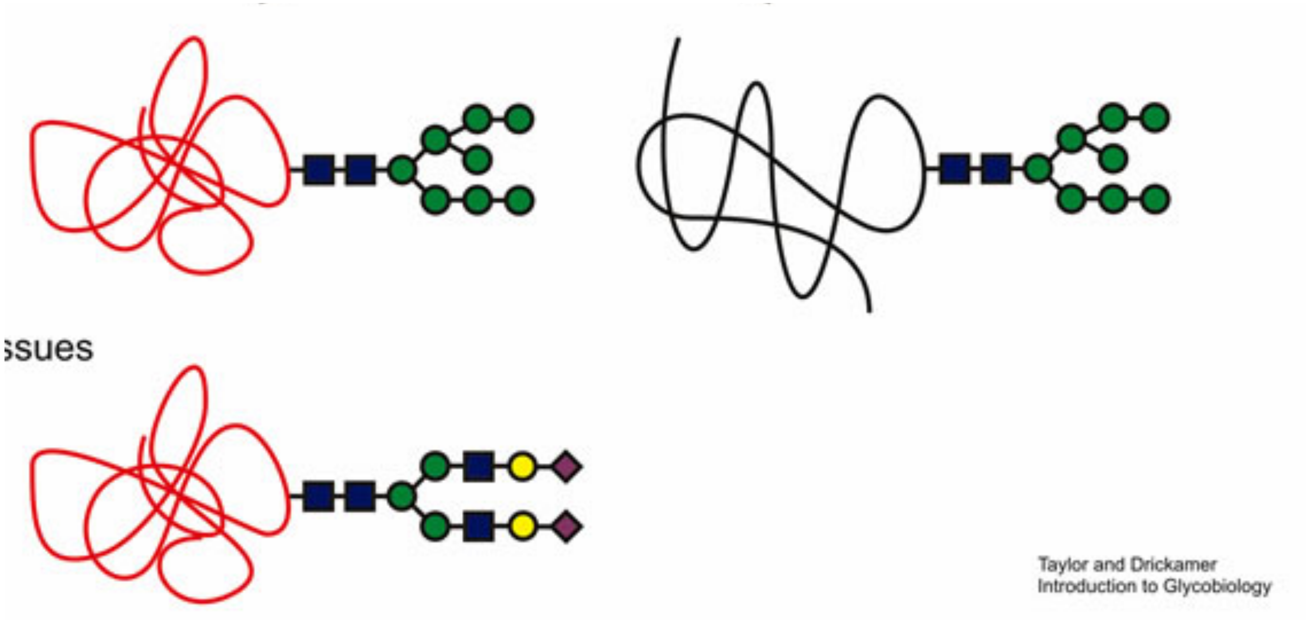
1.4 Understanding structure-function relationships for glycans can be more difficult than for other classes of biopolymer

Proteins share two common features that unify the study of their properties, in spite of their diverse biological roles

Each protein is synthesized as an identical copy by translation of an mRNA template, encoded in the genome

The activity of a protein results from formation of a precisely folded three-dimentional structure

Glycans are assembled without a template through a series of individually catalysed rxns, resulting not unique structures



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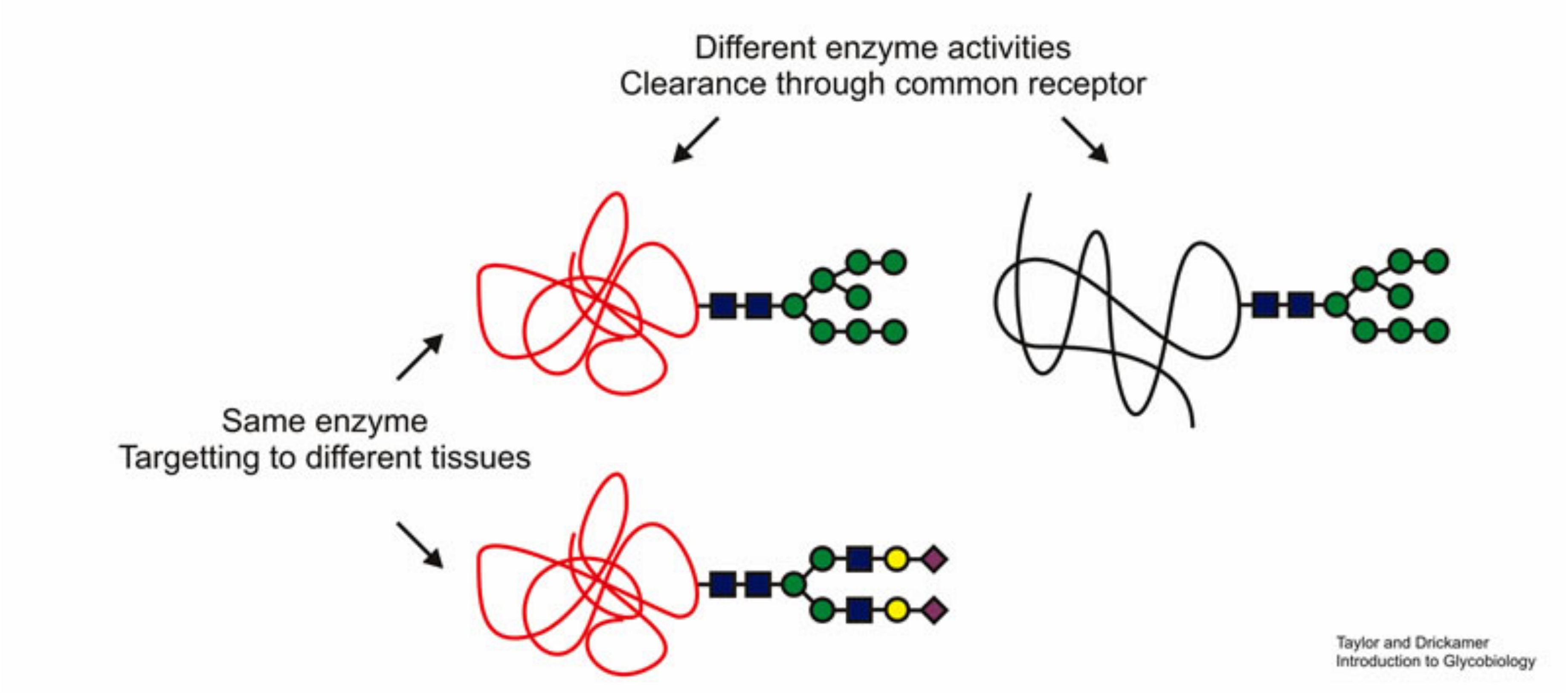
Glycans often appear to lack a discrete, folded structure.

Novel principles must be defined to describe the ways that glycans function

The functions of the protein and glycan portions of many glycoproteins can be independent of each other.



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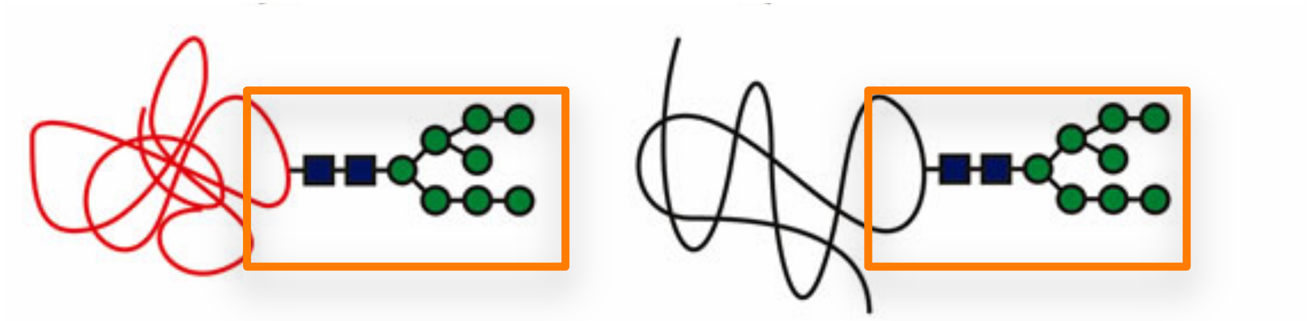
Glycans serve as tags that can be recognized and used to direct glycoprotein trafficking.

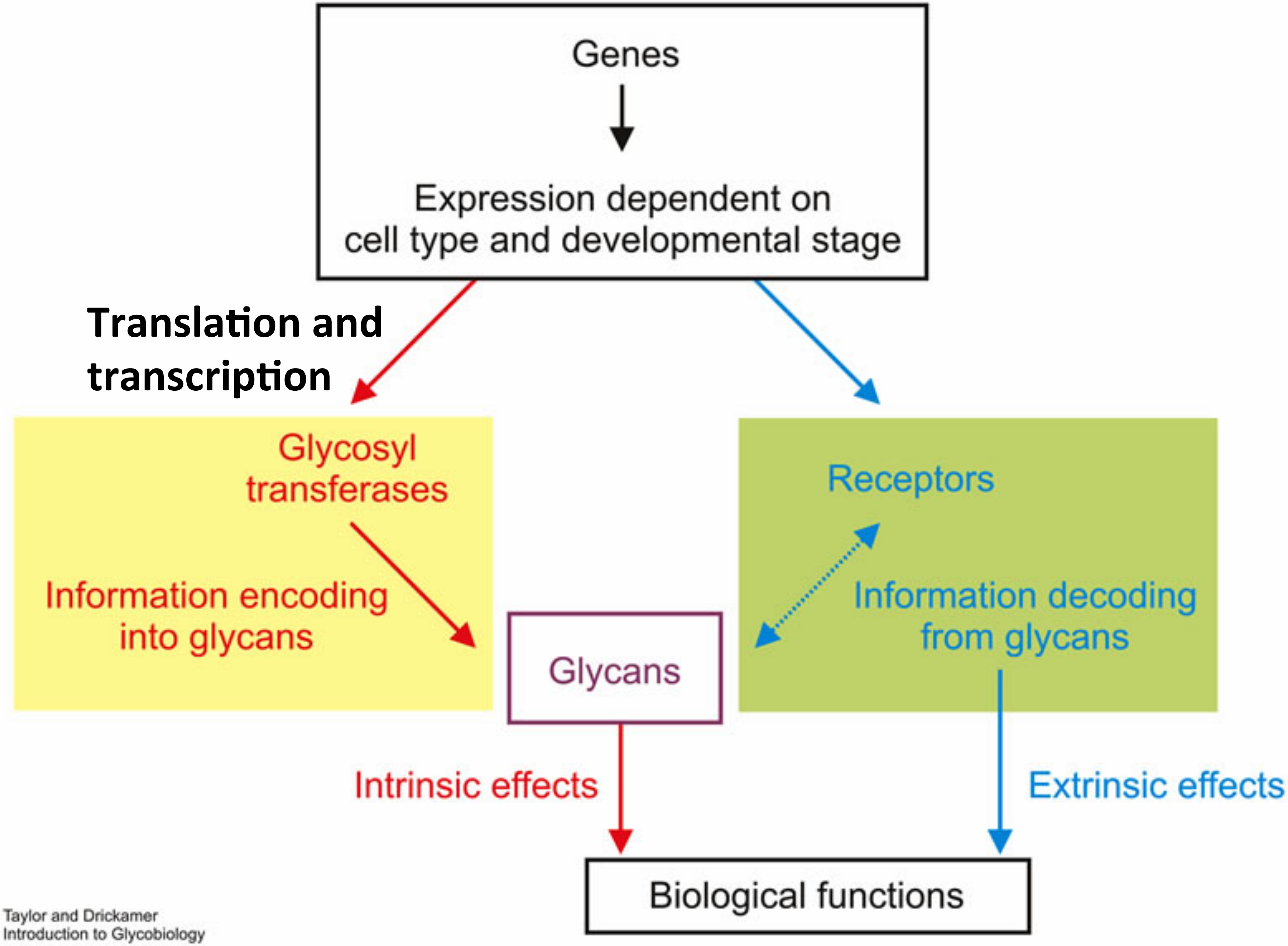
Glycoproteins in the secretory pathway of eukaryotic cells are subjected to a series of quality-control checks.



Hold the proteins in the appropriate luminal compartments during this process.

May have essentially no role once the protein has reached the cell surface

1.5 Glycan structures are encoded indirectly in the genome



Genes

Expression dependent on
cell type and developmental stage

Translation and transcription

Glycosyl transferases

Information encoding into glycans

Glycans

Receptors

Information decoding from glycans

Intrinsic effects

Extrinsic effects

Biological functions

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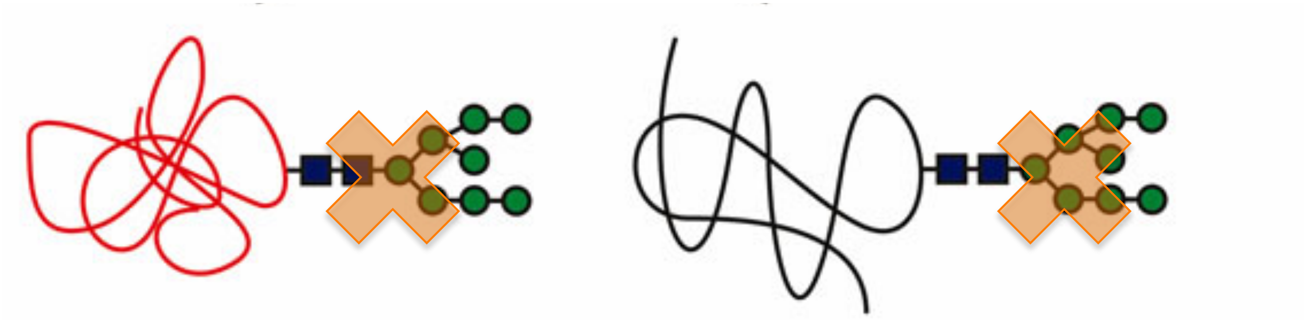
Knockout mice: glycosyltransferases have been eliminated



Complete elimination of any of the classes of glycoconjugates is fatal to the organism at an early stage in development--------critical biological functions for the organism

Cells lacking any of these classes of glycoconjugates are viable

2. Conformation of oligosaccharide

Learning object

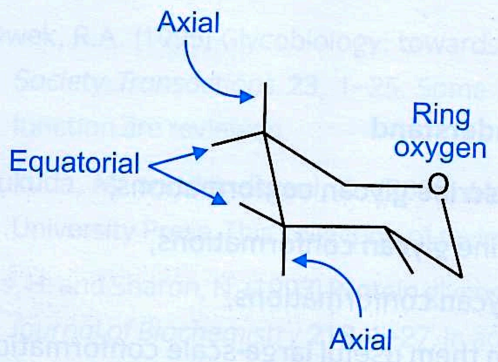
- Definition of torsion angles used to describe glycan conformations
- Nature of the interactions that determine glycan conformations
- Experimental methods used to analyse glycan conformations
- How linkages in polysaccharides confer on their large-scale conformations

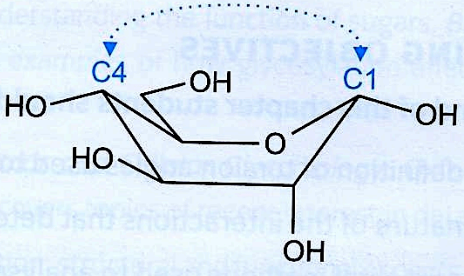
- Relate the structures to functions
- Proteins with different sequences have distinct tertiary structures or folds
- Sequences of proteins dictate their 3D structures and these structures determine their functions
- Limited knowledge of the conformations that glycans assume
- Similar principles relating structure to function have not been easy to establish

sequence

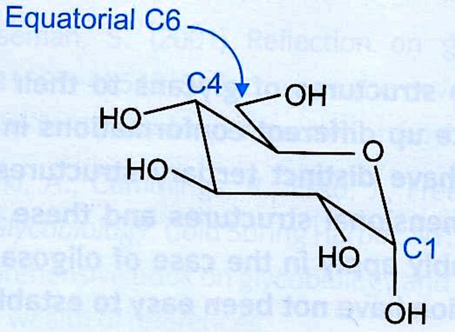
Structure (as branched)

Conformation
Arrangement of oligosaccharide in 3D space

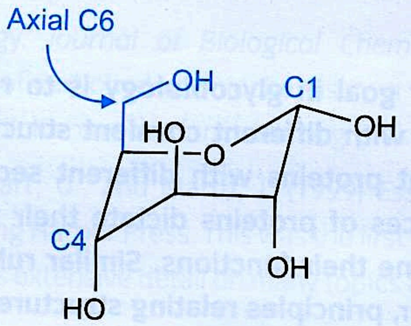




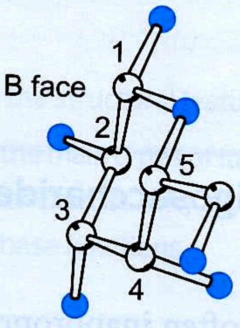
Boat conformation



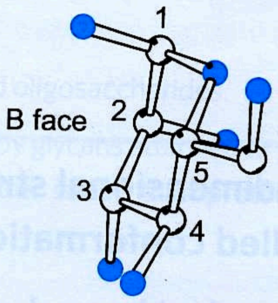
$^4C_1$ Chair conformation



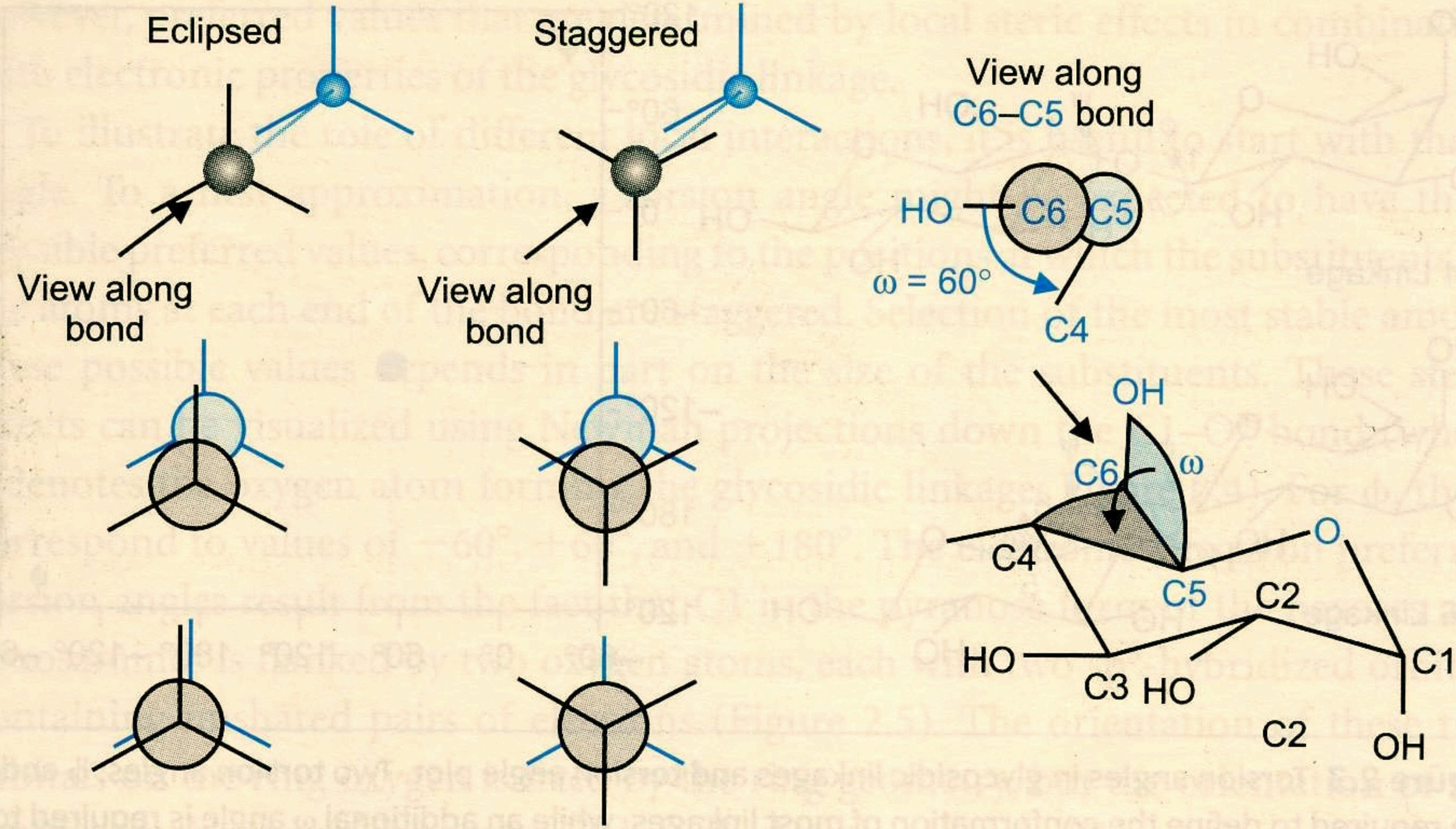
$^1C_4$ Chair conformation



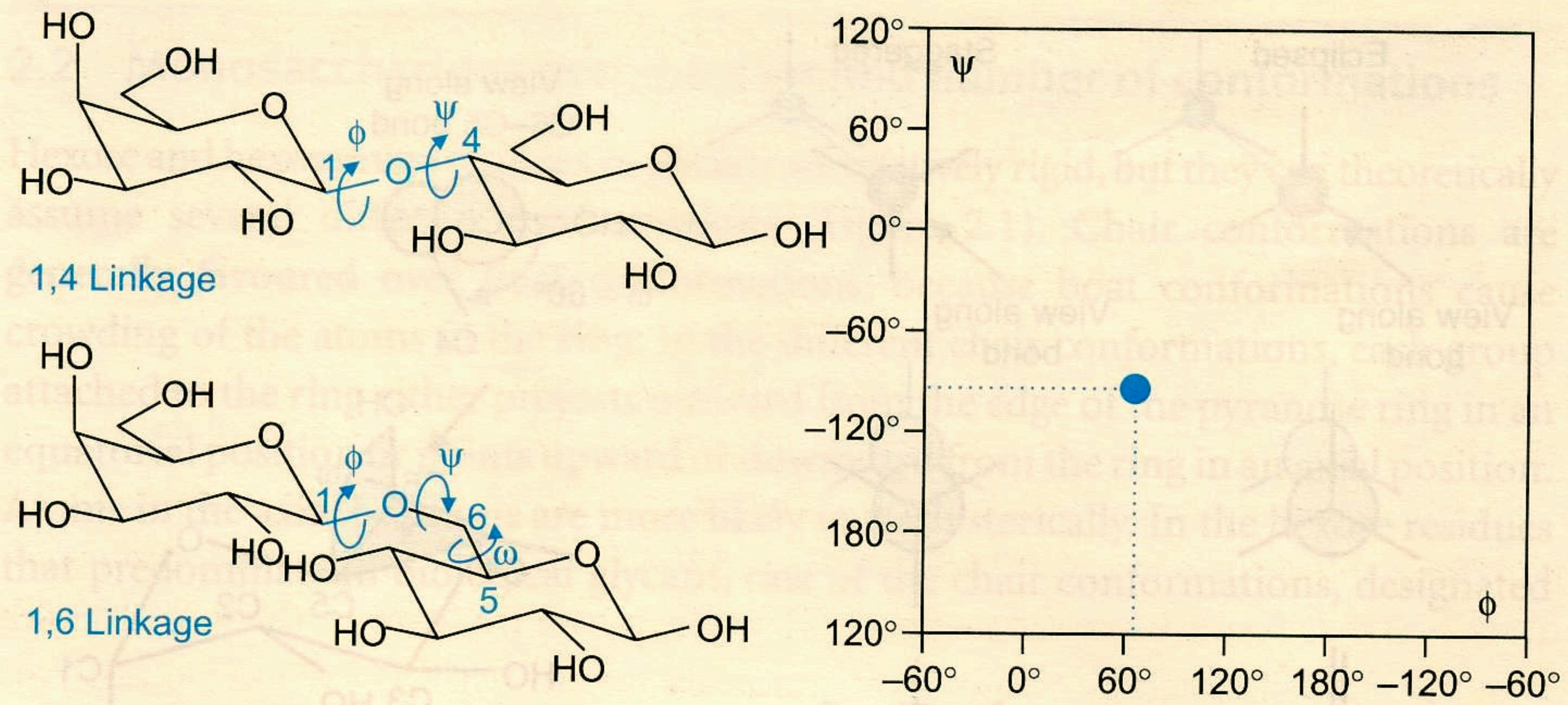
β-Gal



α-Man


Eclipsed
Staggered
View along bond
View along bond
View along C6–C5 bond
HO
C6
C5
ω = 60°
C4
OH
C6
ω
C4
C5
O
C2
HO
C1
C3
HO
C2
OH

2.1 Torsion angles are used to describe conformations of glycans

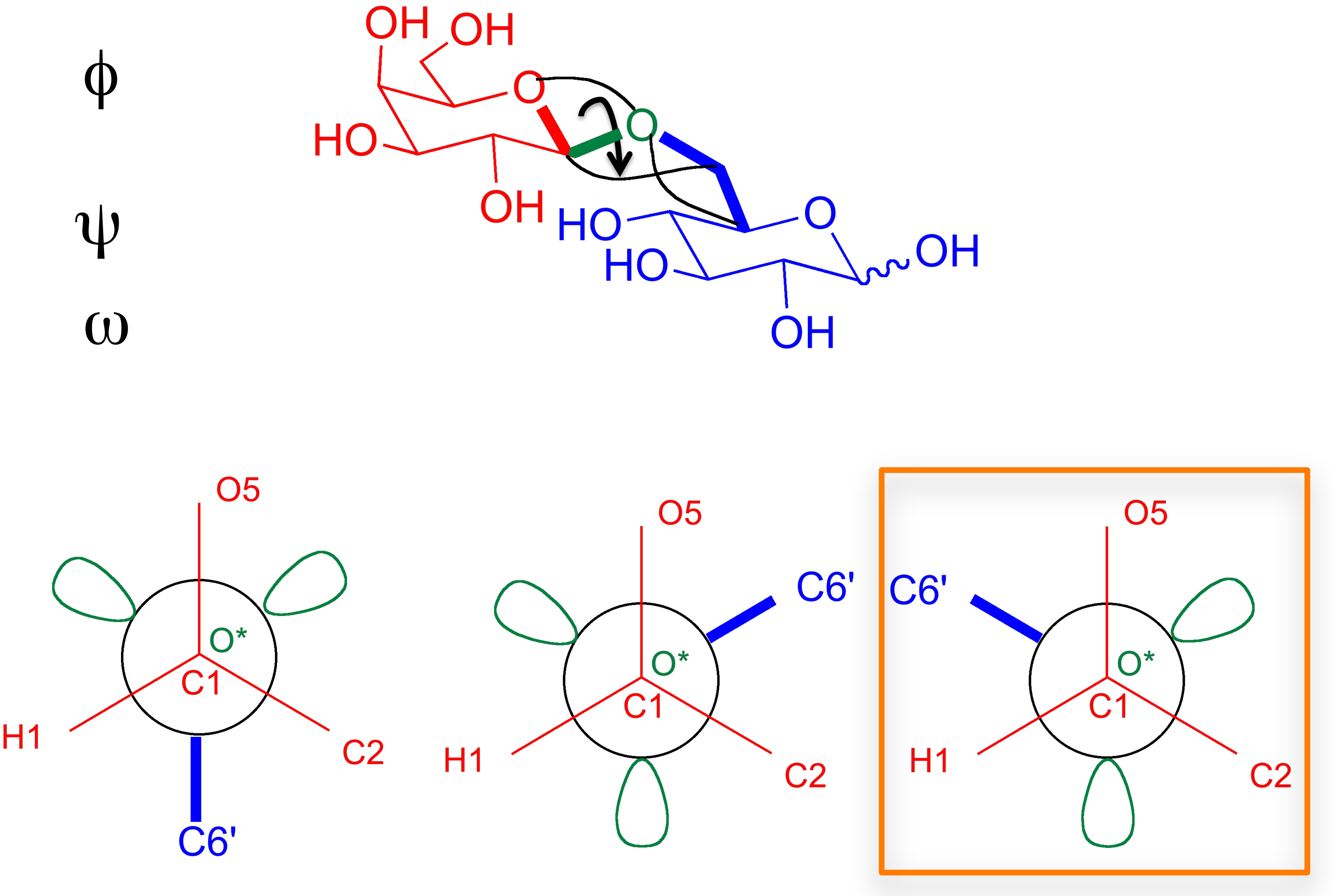

HO
OH
O
φ
ψ
OH
1
O
4
O
HO
HO
HO
OH
HO
1,4 Linkage
HO
OH
O
φ
ψ
HO
1
O
6
ω
HO
HO
O
5
1,6 Linkage
HO
OH
HO
120°
60°
0°
−60°
−120°
180°
120°
ψ
φ
−60°
0°
60°
120°
180°
−120°
−60°

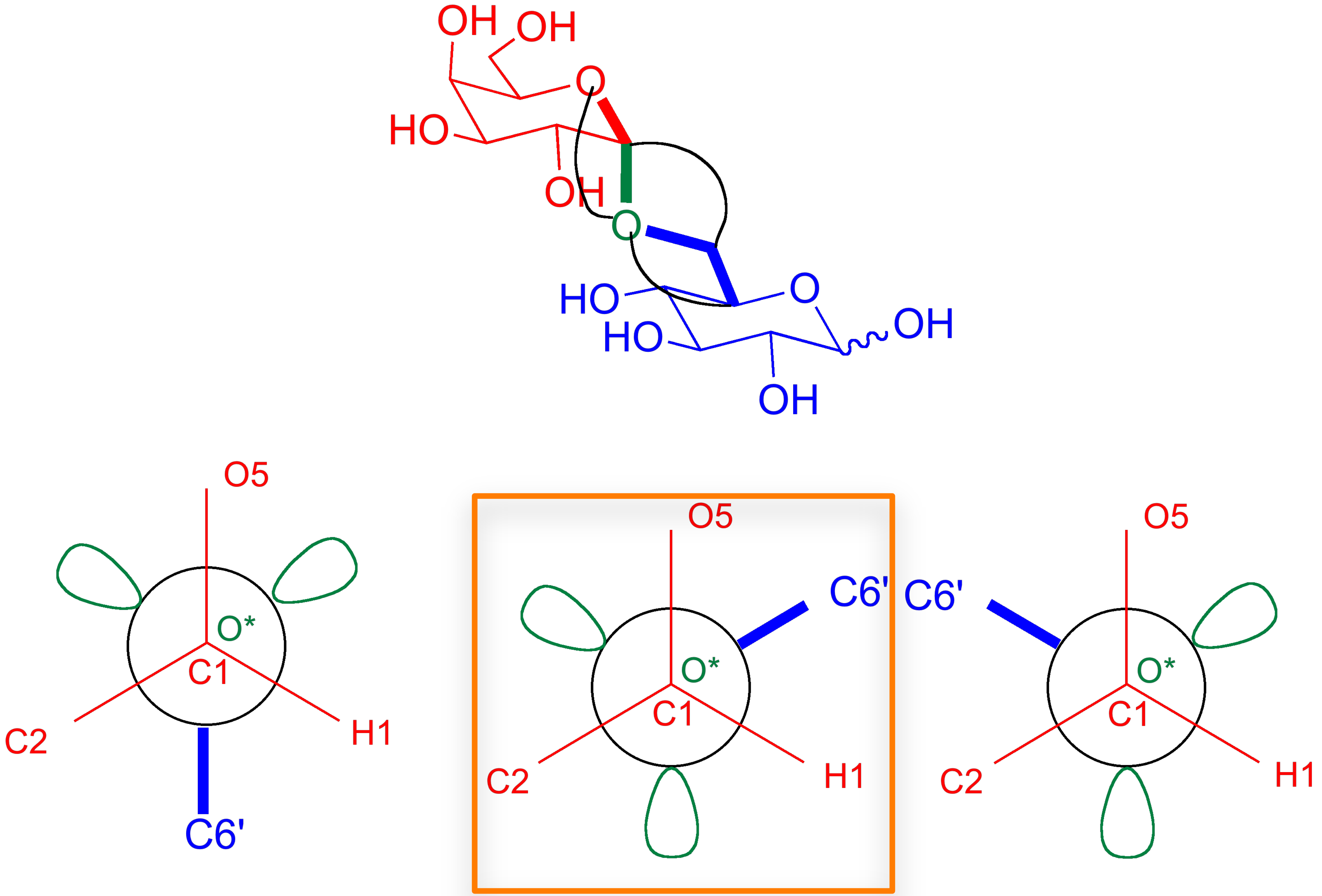
2.2 Local steric and electronic interactions limit the possible conformations of glycosidic linkages

φ

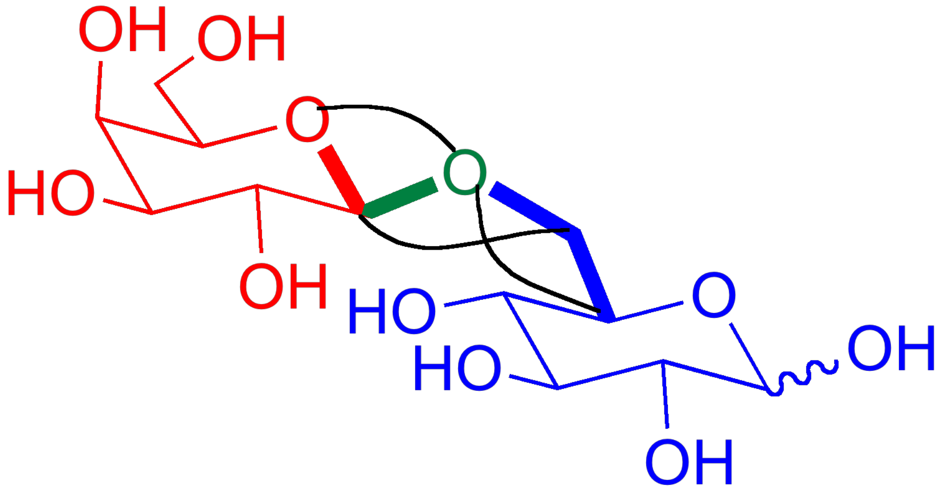
ψ

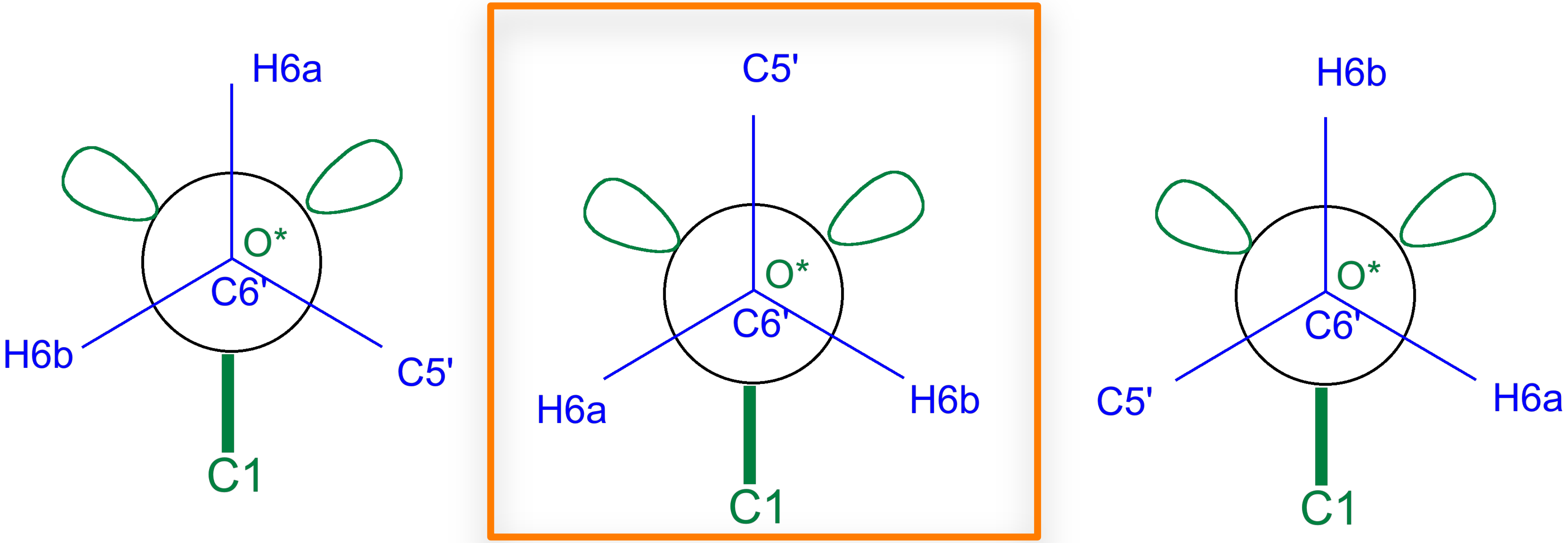
ω

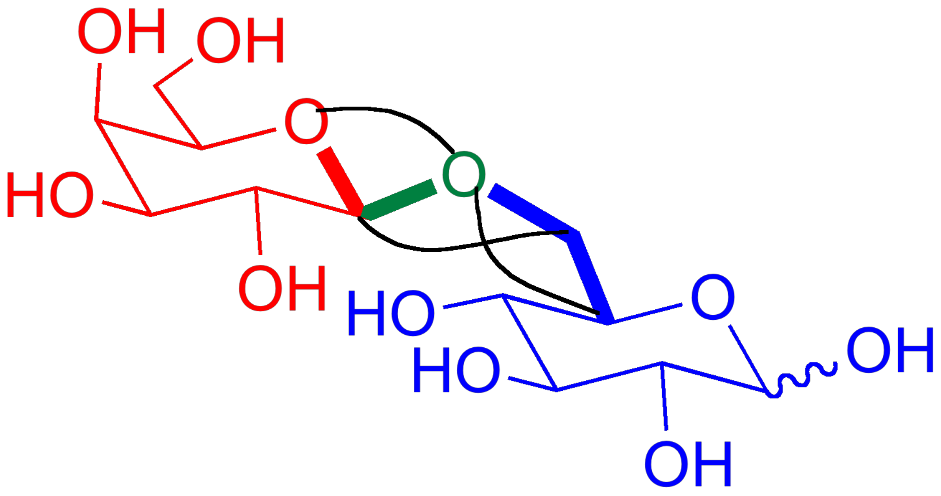

OH
OH
O
HO
O
OH
HO
O
HO
OH
OH
O5
O*
C1
H1
C2
C6'
O5
C6'
O*
C1
H1
C2
C6'
O5
O*
C1
H1
C2


OH
OH
O
HO
OH
O
HO
HO
O
OH
OH
O5
O*
C1
C2
H1
C6'
O5
C6'
O*
C1
C2
H1
C6'
O5
O*
C1
C2
H1

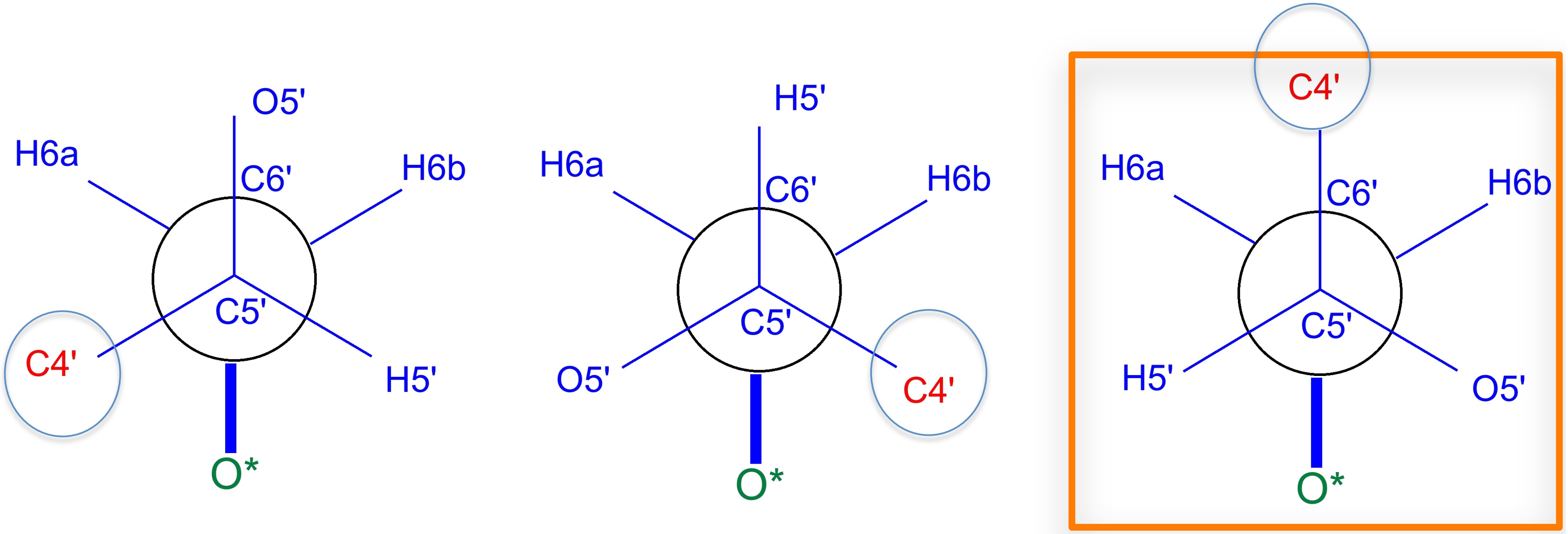
ψ


OH
OH
O
HO
OH
O
HO
O
HO
OH
OH

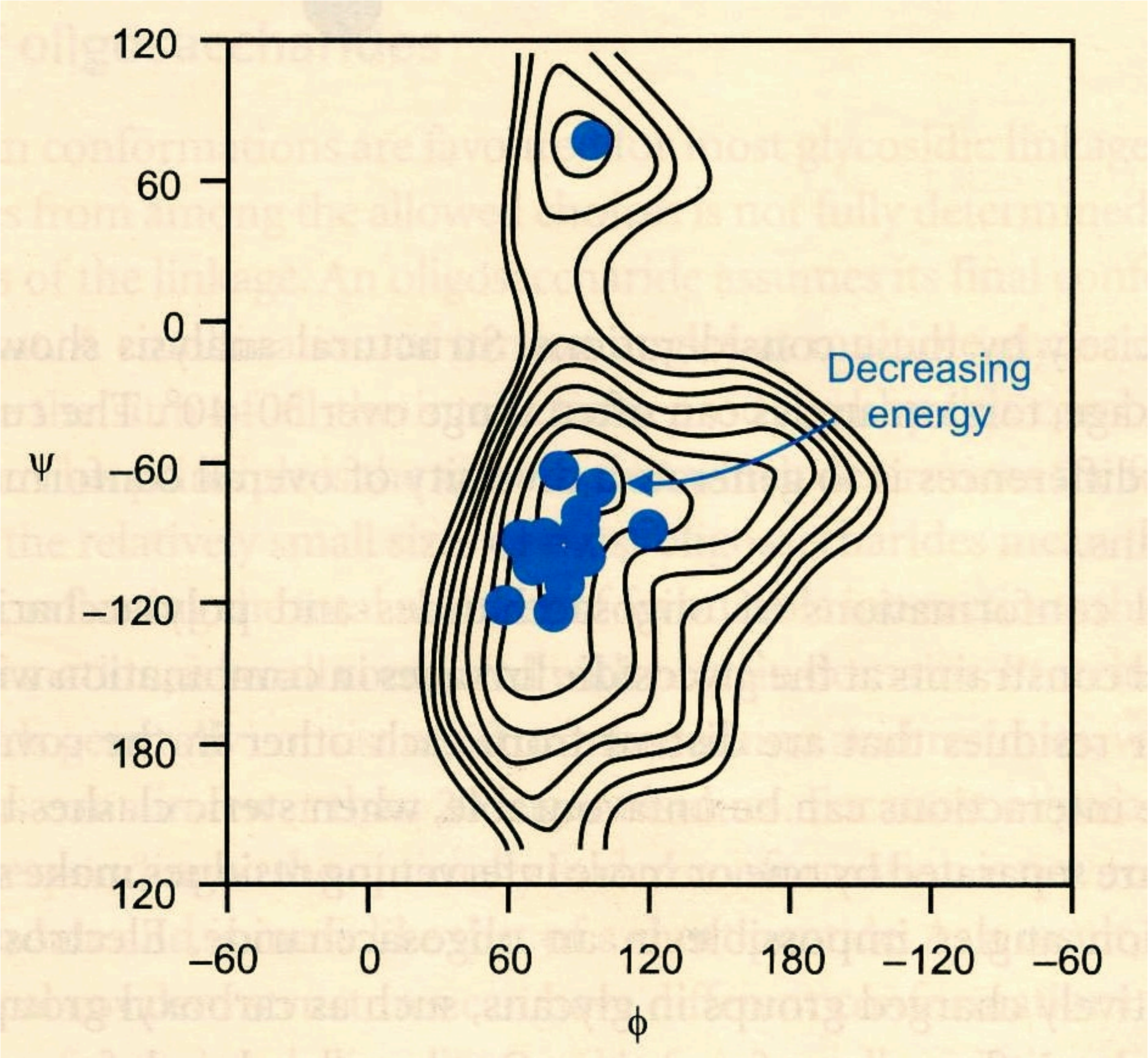

H6a
O*
C6'
H6b
C5'
C1
C5'
O*
C6'
H6a
H6b
C1
H6b
O*
C6'
C5'
H6a
C1


OH
OH
O
HO
O
OH
HO
O
HO
OH
OH

ω


O5'
H6a
C6'
H6b
C4'
C5'
H5'
O*
H5'
H6a
C6'
H6b
O5'
C5'
C4'
O*
C4'
H6a
C6'
H6b
H5'
C5'
O5'
O*

Energy contours for various Manα1-3Man disaccharides. Both primary and secondary energy minima are present

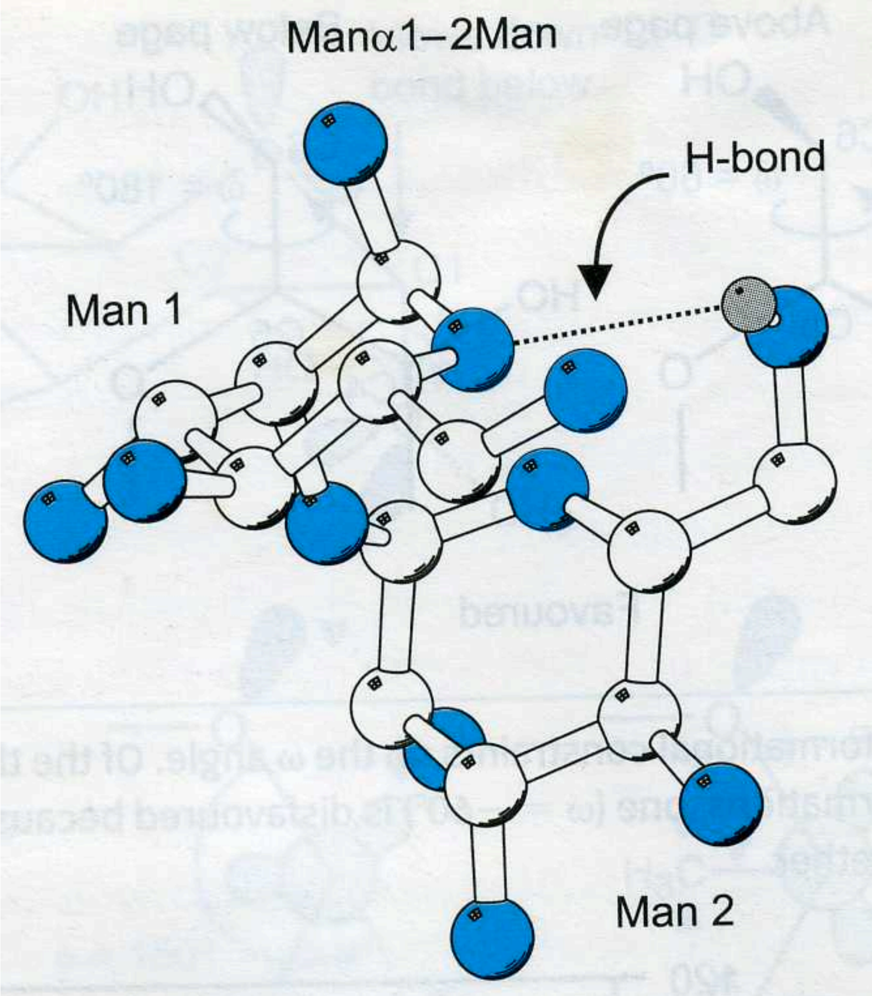


2.3 The conformation of an oligosaccharide is influenced by interactions between hexoses that are distant from each other in the covalent structure

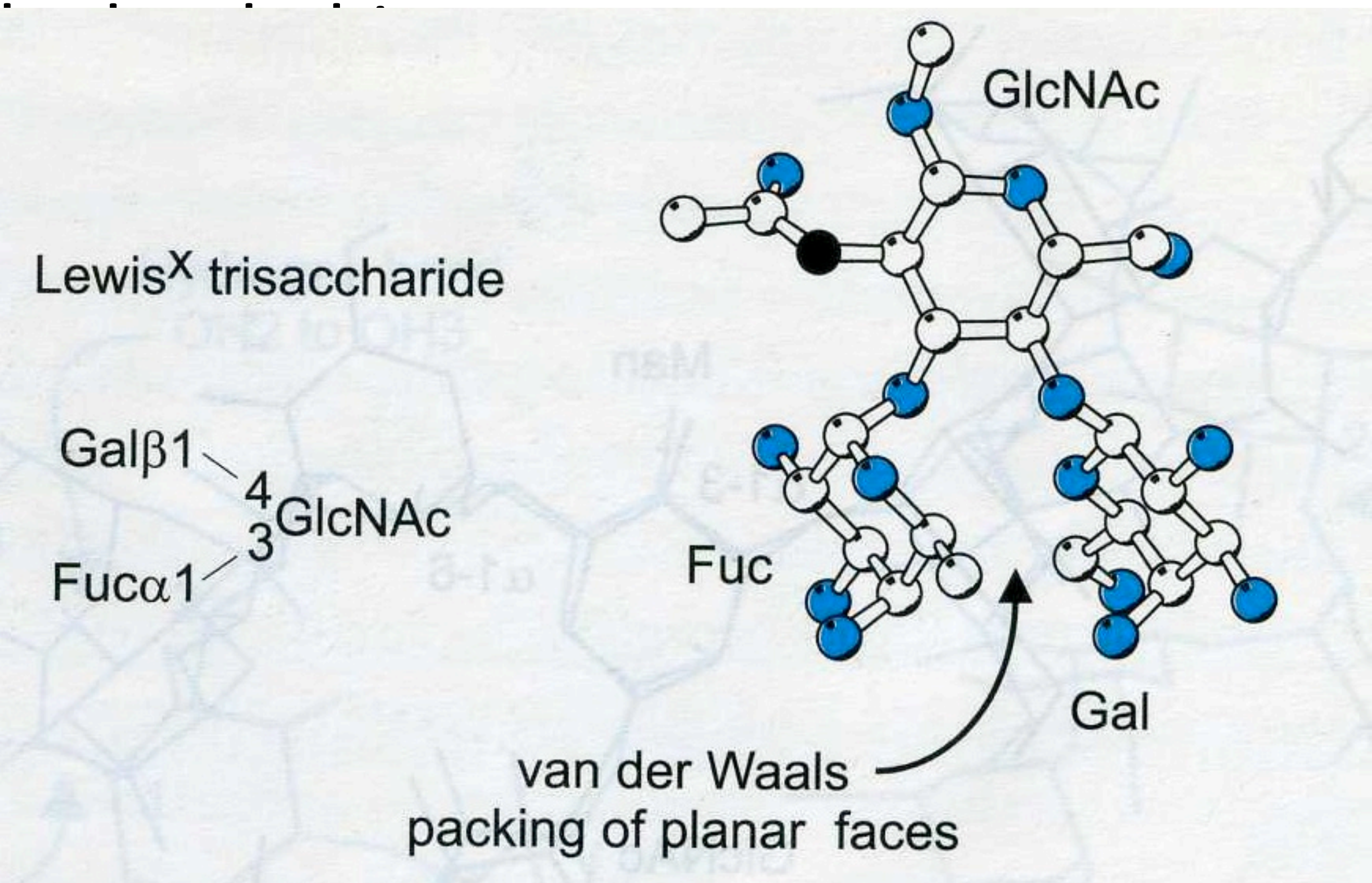
- Rigid pyranose ring, exo-anomeric effect, and steric factors
- only a few conformations will be favoured
- But the torsion angles are not fixed precisely
- For any particular linkage, torsion angles can often range over $30\text{-}40^{o}$
- The cumulative effect of such small differences is to generate a diversity of overall conformations even for identical glycans

- Overall conformations of sugars are determined by local constraints at the glycosidic linkages
- in combination with interactions between sugar residues that are distant from each other in the covalent structure
- Some are unfavourable: when steric clashes between hexose residues that are separated by one or more intervening residues make some combinations of torsion angles impossible
- Electrostatic repulsion between negatively charged groups

- hydrogen bonds stabilize selected conformations of individual glycosidic linkages
- Potential hydrogen bond donor/acceptors



- van der Waals interactions, between adjacent residues or those that are further apart
- Amphiphilic character of hexoses, B face
- obic

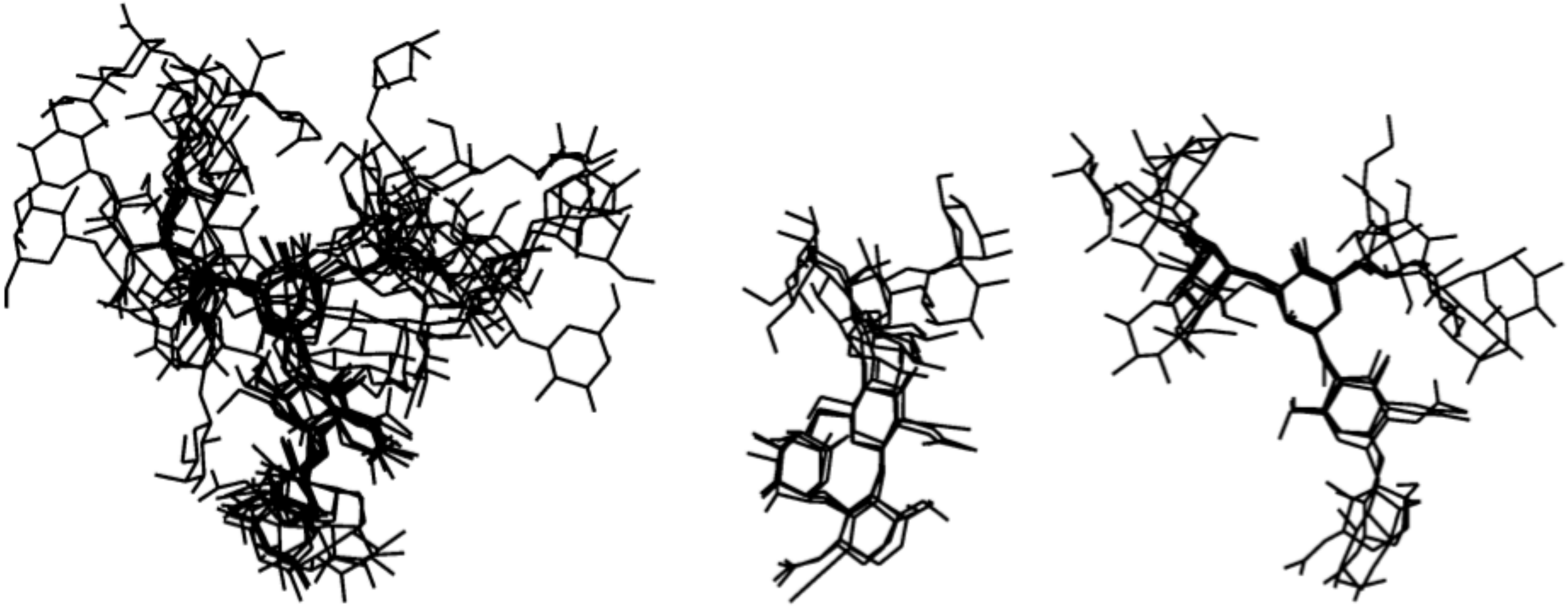


2.4 Cooperative interactions determine the overall folds of oligosaccharides

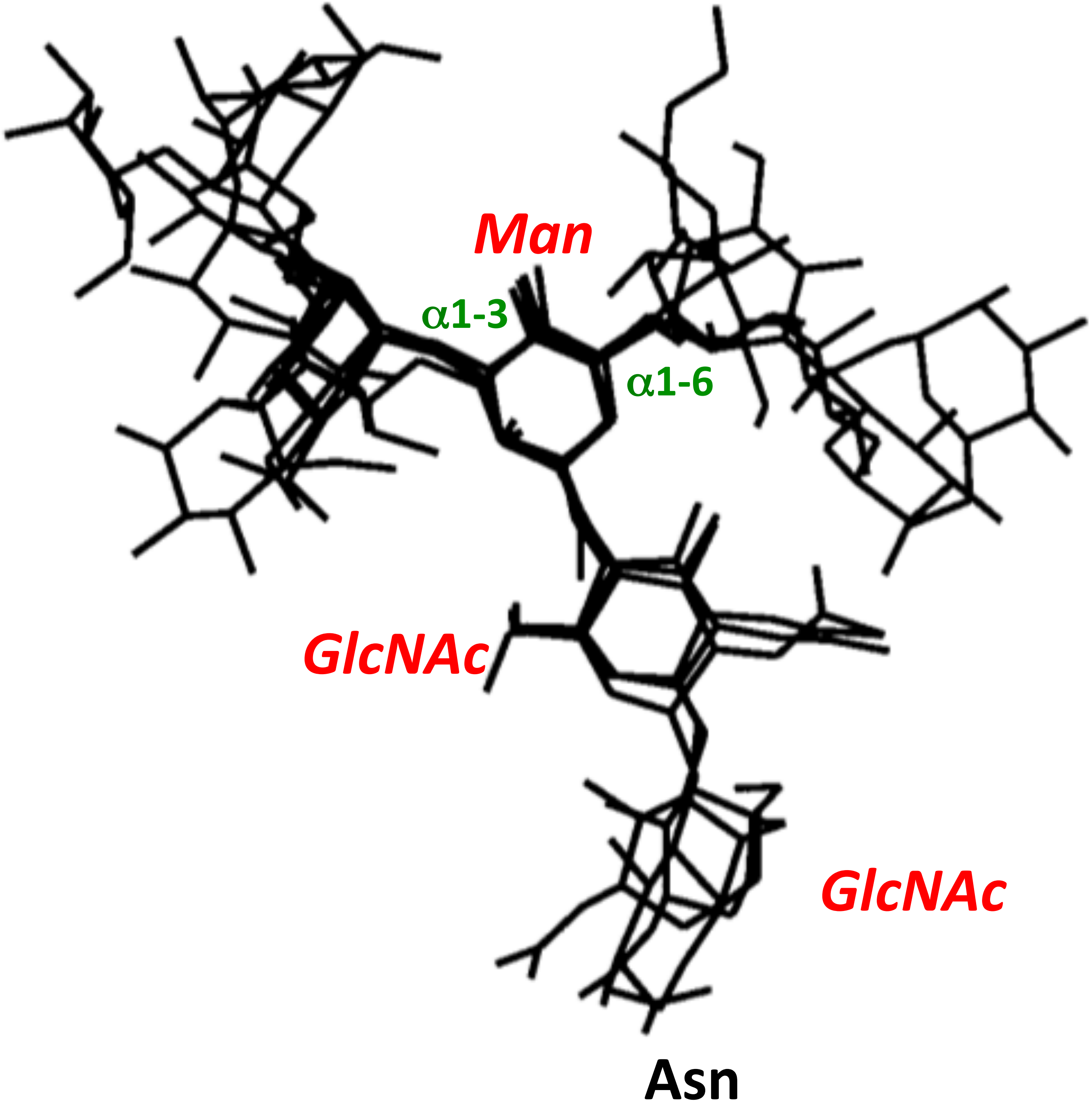
- Combination of various interactions
- Same as protein folding
- Relatively small size of oligosaccharide
- Protein >50 AA; sugar <20
- Energy well corresponding to the optimally folded conformation is not nearly as deep as for a protein
- More like a short peptide

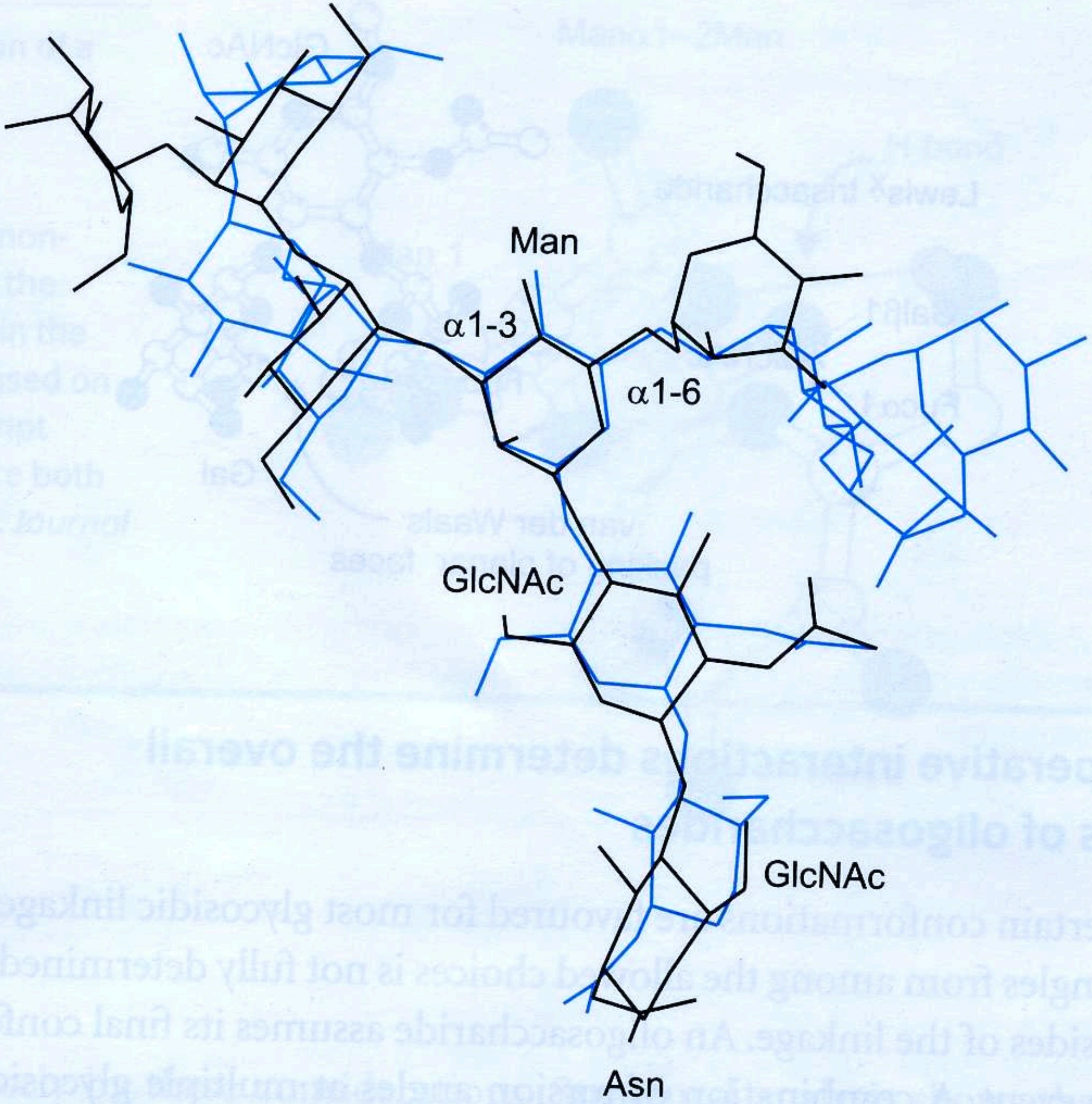
Oligosaccharides with identical covalent structures can have different conformations

A statistical analysis of N- and O-glycan linkage conformations from crystallographic data



R. A. Dwek, et al. Glycobiology 1999, 9, 343–352.

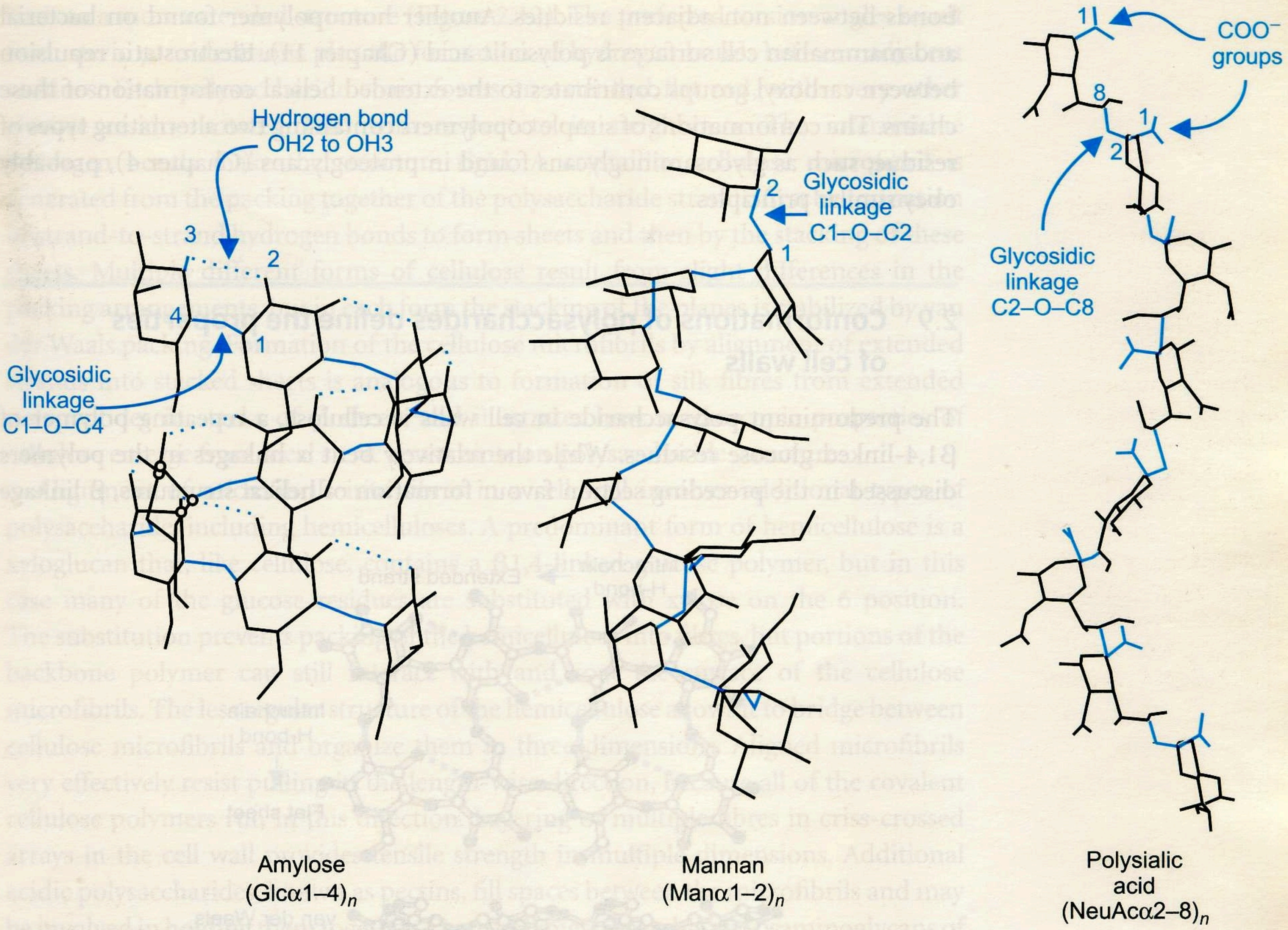

Man
α1-3
α1-6
GlcNAc
GlcNAc
Asn


Man
α1-3
α1-6
GlcNAc
GlcNAc
Asn

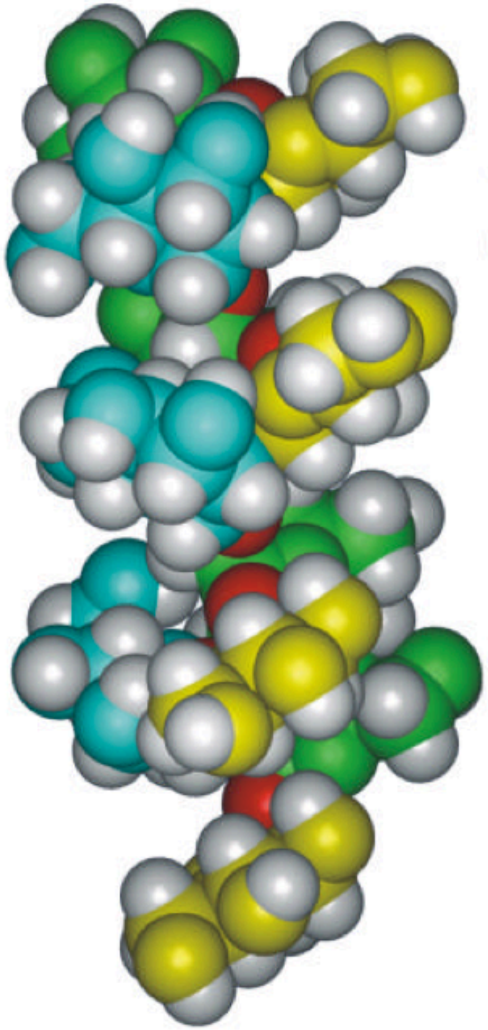
2.5 Oligosaccharide conformations are dynamic

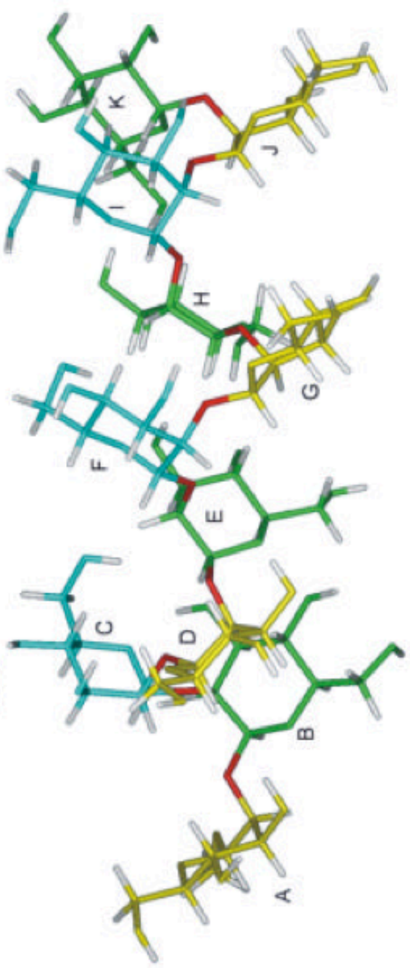
2.6 Short- and long-range interactions also determine the conformations of polysaccharides

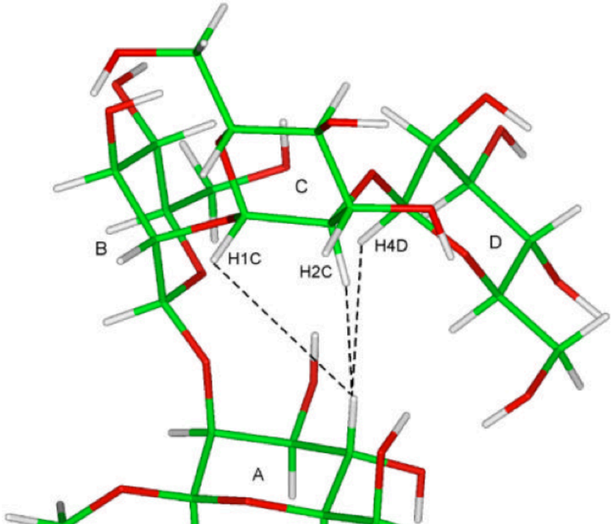
- Longer chains consisting of repeating sugar residues in identical linkage
- Specific combination of torsion angles are repeated over and over again
- Cumulative effect

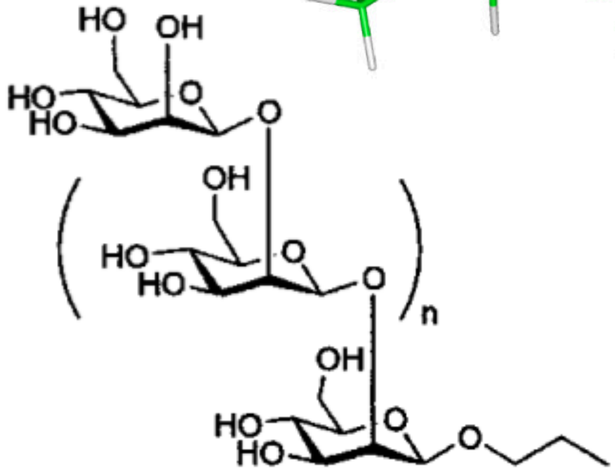

Hydrogen bond
OH2 to OH3
3
2
4
1
Glycosidic
linkage
C1–O–C4
2
Glycosidic
linkage
C1–O–C2
1
1
COO⁻
groups
8
1
2
Glycosidic
linkage
C2–O–C8
Amylose
(Glcα1–4)$_n$
Mannan
(Manα1–2)$_n$
Polysialic
acid
(NeuAcα2–8)$_n$

Solution structures of β-1,2-Mannopyranan Cell Wall Antigens

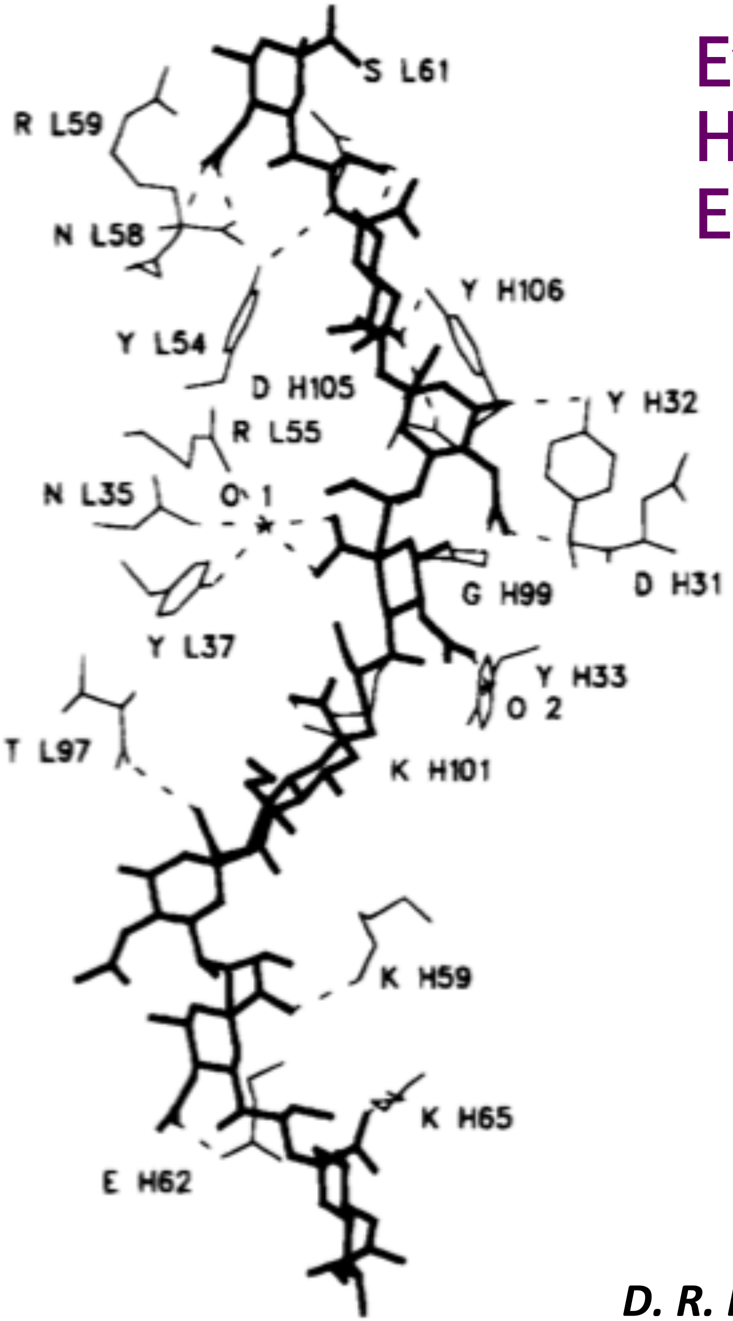








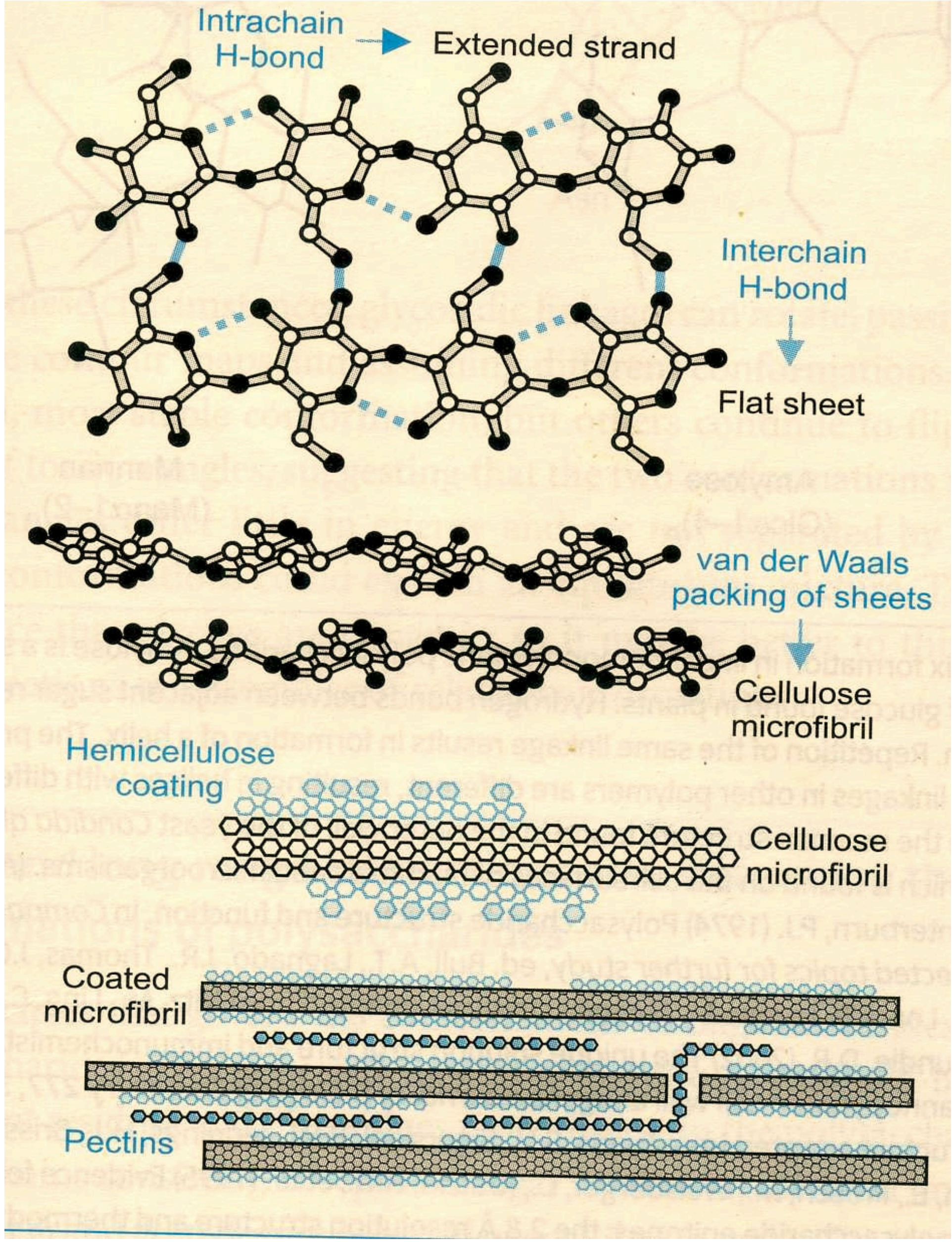
D. R. Bundle, et al. J. Bio. Chem. 2002, 277, 3440–3446

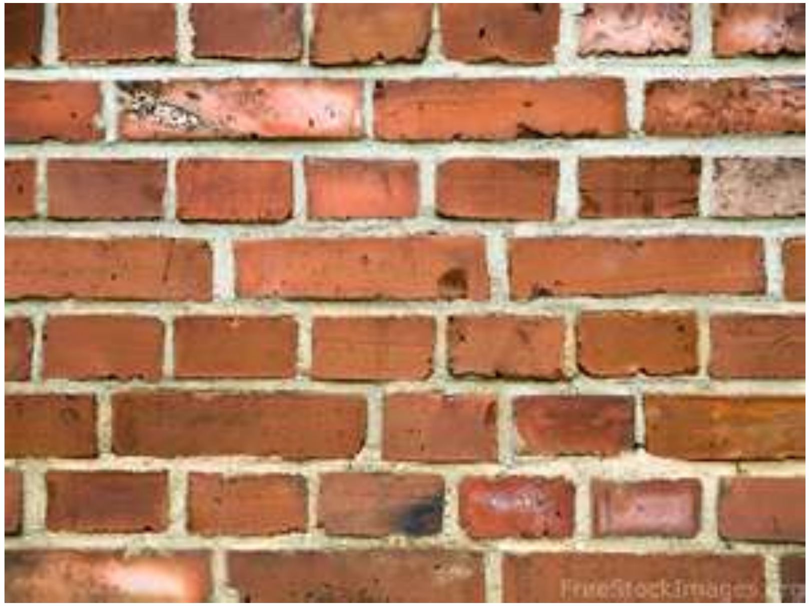


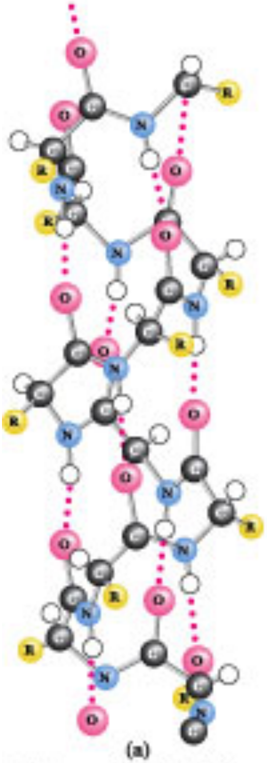
Evidence for the Extended Helical Nature of Polysaccharide Epitopes.

D. R. Bundle, Biochemistry 1995,34, 6737-6744

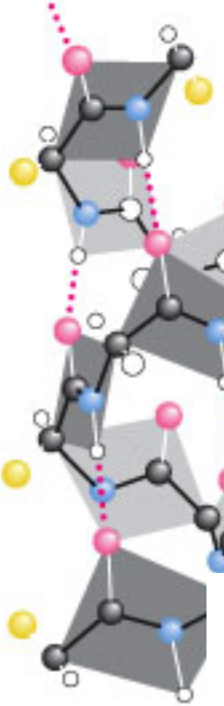
2.7 Conformations of polysaccharides define the properties of cell walls


Intrachain H-bond
Extended strand
Interchain H-bond
Flat sheet
van der Waals packing of sheets
Cellulose microfibril
Hemicellulose coating
Cellulose microfibril
Coated microfibril
Pectins

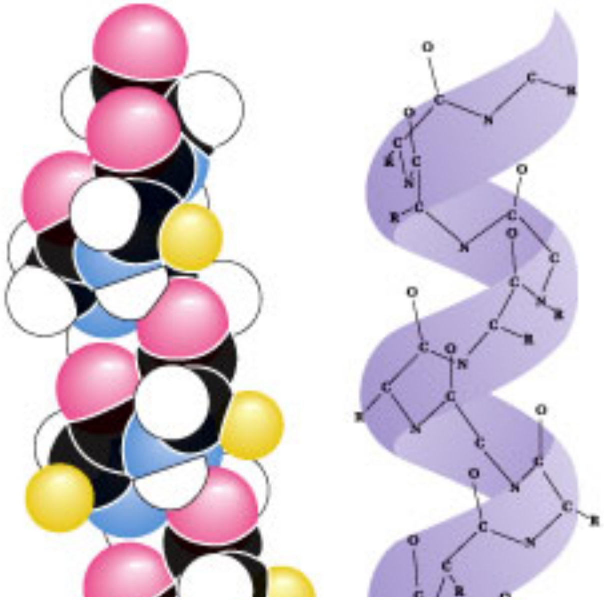


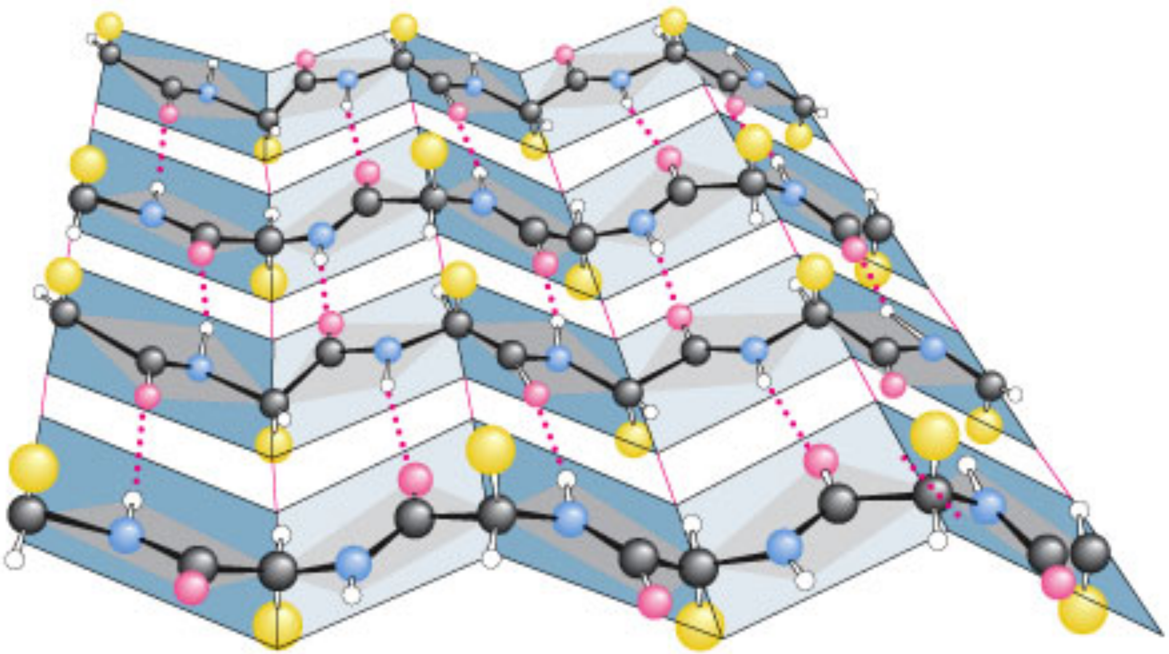


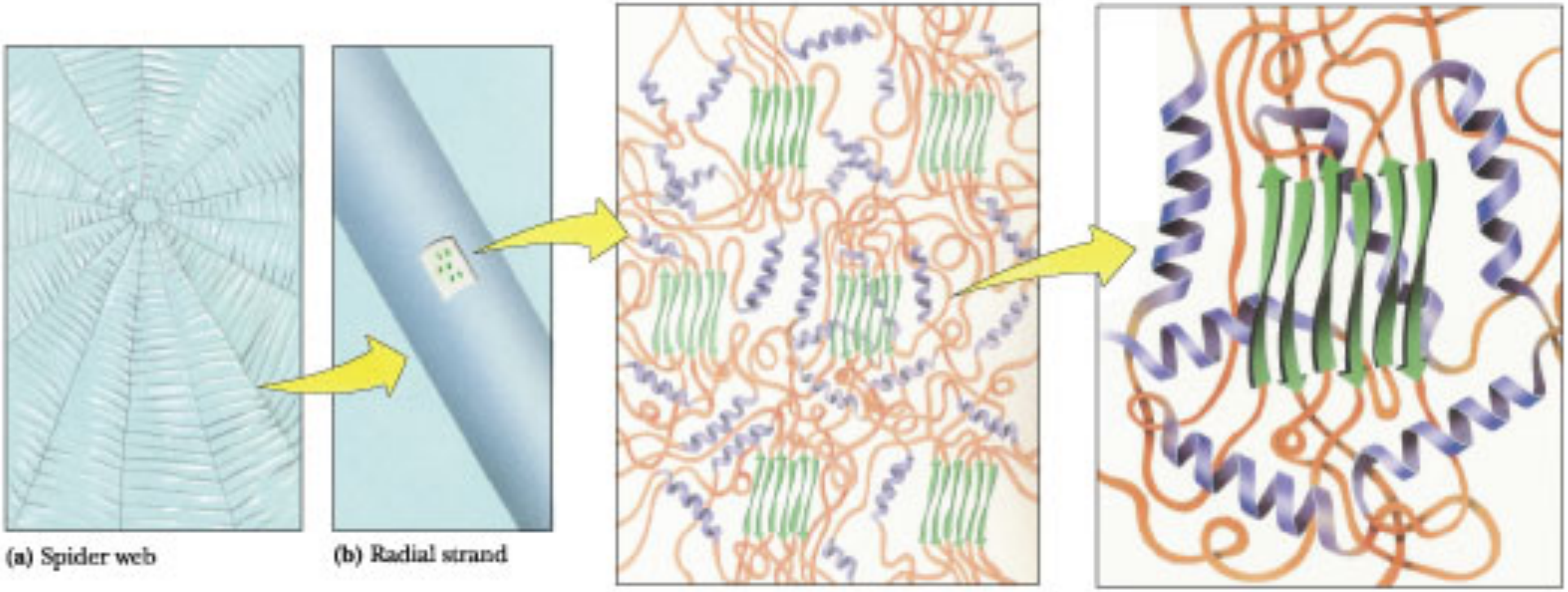
(a)
Hydrogen bonds stabilize
the helix structure.



(b)
The helix can be v
as a stacked array
peptide planes hi
at the α-carbons a
approximately par
to the helix.







(a) Spider web

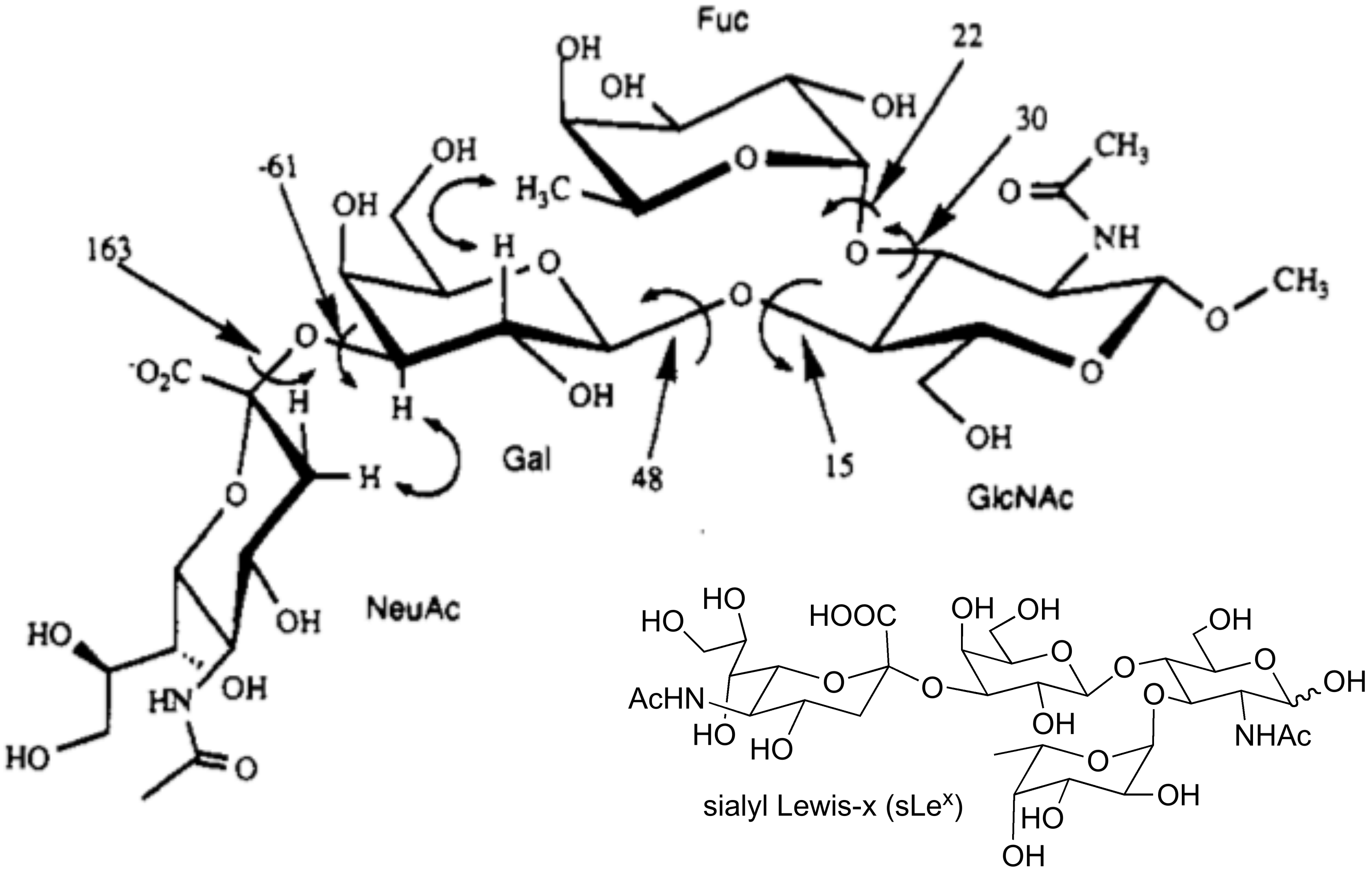
(b) Radial strand

(c) Ordered β-sheets surrounded by disordered α-helices and β-bends.

(d) β-sheets impart strength and α-helices impart flexibility to the strand.

2.8 The conformations of oligosaccharides can be analysed by X-ray crystallography and nuclear magnetic resonance

Conformation of Sialyl Lewis X in aqueous solution



sialyl Lewis-x (sLe^x)

C.-H. Wong, *et al.* *J. Am. Chem. Soc.* **1992,** 114, 5452-5454