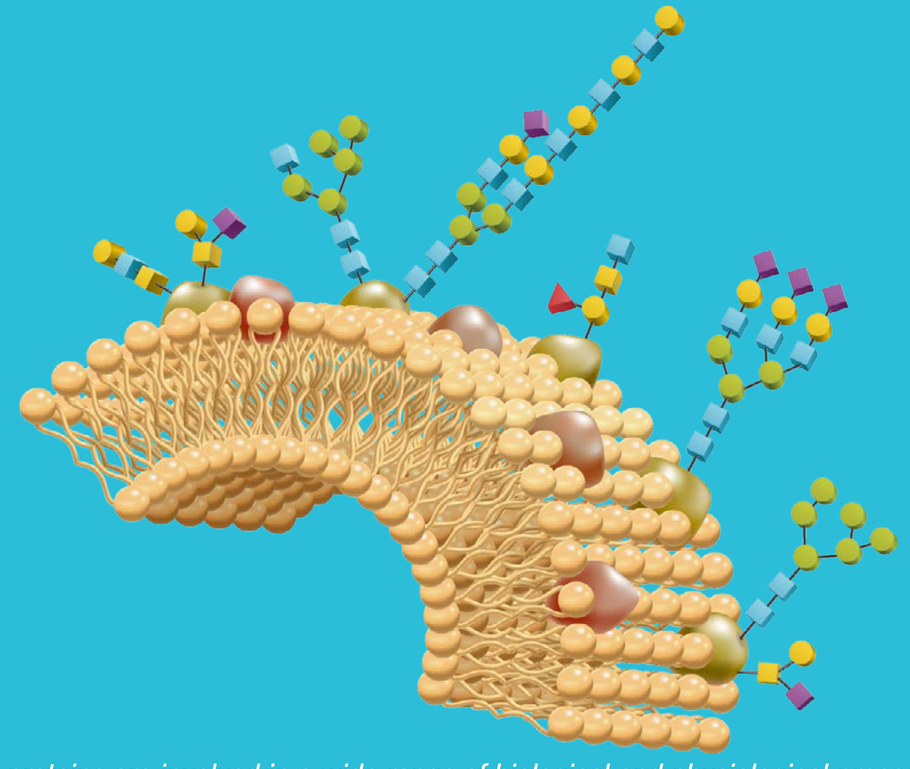
Top Four Things to Know about Glycan Analysis by Mass Spectrometry

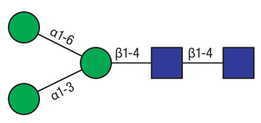


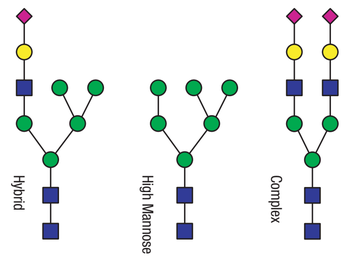
Glycan-modified proteins are involved in a wide range of biological and physiological processes including recognition and regulatory functions, cellular communication, gene expression, cellular immunity, growth and development. Glycans are investigated as biomarkers, in order to understand changes related to complex organelle development, and as part of therapeutic protein drug development.

1 The two most common types of glycans are *N*-linked and *O*-linked.

Core Structure for N-linked Glycans

N-linked glycosylation occurs via the amide group of asparagine in the tripeptide sequence Asn-X-Ser/Thr, or much less frequently Asn-X-Cys, where X can be any amino acid except proline. All *N*-linked glycans have the same core structure, 3 mannose and 2 *N*-acetylglucosamine residues, referred to as the trimannosyl core. It should be noted that the core structure can be fucosylated in some cases for vertebrates by an α1-6 linkage to the reducing terminal GlcNAc. In plants and many invertebrates this can also occur via an α1-3 linkage.

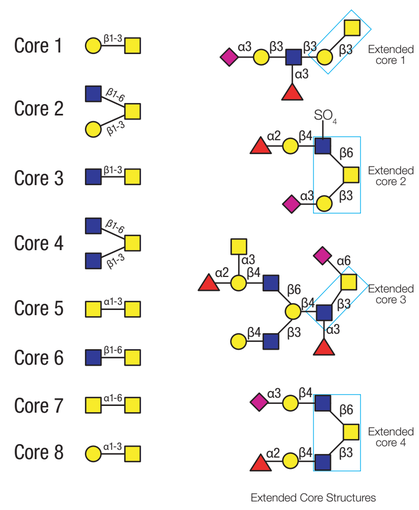




Types of *N*-linked Glycans

Core Structures for O-linked Glycans

O-linked glycosylation occurs through the hydroxyl group on either the serine or threonine. No consensus sequence is required for glycosylation, although amino acid preferences have been described. There are 8 different core structures for *O*-linked glycans. Cores 1-4 occur much more commonly while cores 5-8 have very restricted expression. Additionally each core structure can be elongated with additional Gal, GalNAc, or GlcNAc residues or capped with sialic acid (NeuAc/NeuGc), Fuc or sulfate.



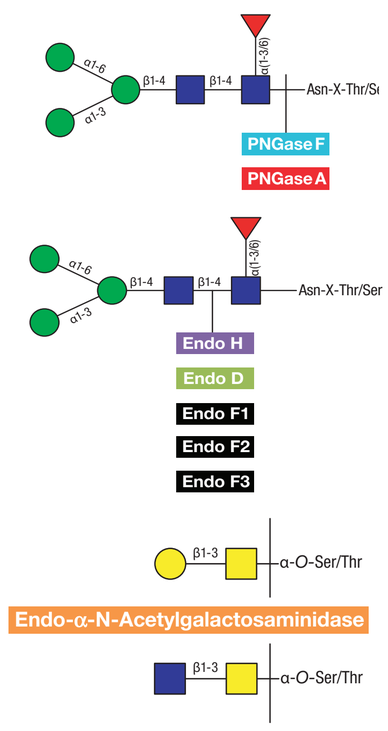
2 There are two methods to remove the glycan from the protein: enzymatically and chemically.

Enzymes Used for Deglycosylation

PNGase F releases all *N*-linked glycans (high mannose, hybrid and complex) except those that contain an α1-3 linked fucose at the reducing terminal GlcNAc. In this case, PNGase A is used. PNGase F and PNGase A cleave the glycan between the innermost GlcNAc and the asparagine, leaving aspartic acid in place of the asparagine. It should be noted that PNGase A may work better on glycopeptides than on intact glycoproteins, although each glycoprotein may be different.

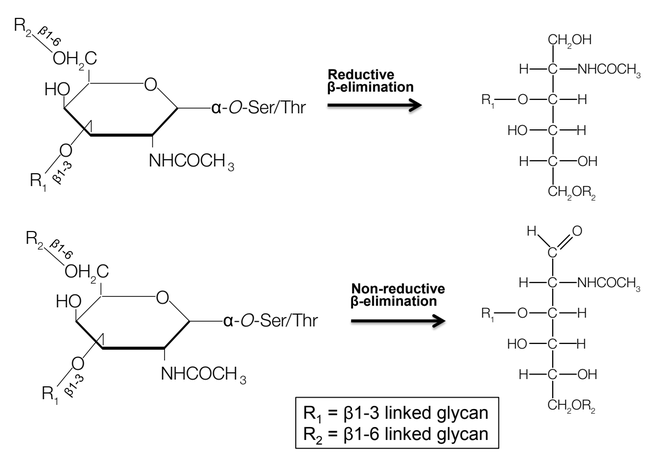
Endo H, D, F1, F2 and F3 cleave *N*-linked glycans between the two GlcNAc residues within the trimannosyl core such that one GlcNAc remains attached to the asparagine residue. Endo H specificity is toward high-mannose and some hybrid glycans, but does not cleave complex glycans. Endo D cleaves only complex glycans but must have the 2 position of the α1-3 linked core mannose unsubstituted. Endo F1 cleaves only high-mannose and hybrid glycans. Endo F2 cleaves only biantennary complex glycans. Endo F3 cleaves only triantennary complex glycans or core-fucosylated biantennary complex chains.

Endo-α-N-Acetylgalactosaminidase cleaves Core 1 and Core 3 *O*-linked disaccharides attached to serine or threonine residues. However, the core structures must be unmodified for the enzyme to be effective. Various exoglycosidases can be used to remove attached monosaccharide residues in order to generate a core structure that can be removed by Endo-α-N-Acetylgalactosaminidase.



Chemical Deglycosylation Methods

There are no universal enzymes to remove all *O*-linked glycans and thus, chemical methods are most frequently used for this class of glycans. The two most popular approaches are reductive and non-reductive β-elimination. In reductive β-elimination, glycans are released in the presence of a strong reducing agent which minimizes the peeling of the released glycans by converting the monosaccharide at the reducing terminal of the glycan to its alditol, usually GalNAc-ol. The non-reductive β-elimination procedure keeps the reducing end intact, providing an aldehyde suitable for various labeling strategies. The difference between the reduced and non-reduced forms is two mass units. Unbiased chemical release of *O*-linked glycans can also be achieved by treatment with hydrazine under mild conditions. More vigorous hydrazinolysis will release *N*-linked glycans. Hydrazinolysis requires careful attention to the quality of the reagents and the optimization of conditions in order to minimize side reactions.



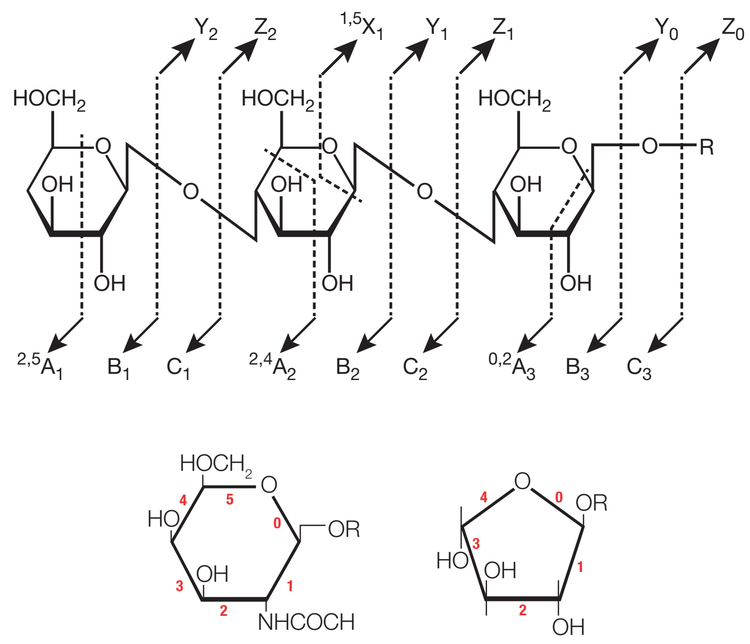
3 Mass values of common glycan residues.

Common Monosaccharides				
Symbols	Monosaccharide	Abbreviation	Residue Mass	Permethylated Residue Mass
□	*N*-Acetylgalactosamine	GalNAc	203.0794	245.1263
■	*N*-Acetylglucosamine	GlcNAc	203.0794	245.1263
●	Mannose	Man	162.0528	204.0998
●	Glucose	Glc	162.0528	204.0998
○	Galactose	Gal	162.0528	204.0998
▲	Fucose	Fuc	146.0579	174.0892
★	Xylose	Xyl	132.0423	160.0736
◆	*N*-Acetylneuraminic Acid	NeuAc	291.0954	361.1737
◇	*N*-Glycolylneuraminic Acid	NeuGc	307.0903	391.1842
◆	Glucuronic acid	GlcA	176.0321	218.0790
◆	Iduronic acid	IdoA	176.0321	218.0790

http://www.functionalglycomics.org/static/consortium/Nomenclature.shtml

Common Substituents		
Substituent	Abbreviation	Mass
Phosphate	P	79.9663
Sulfate	S	79.9568
Acetate	Ac	42.0106

4 Nomenclature shows how glycans fragment in a mass spectrometer.



Domon and Costello Nomenclature for Describing CAD MS/MS Fragmentation of Glycans

Domon, B.; Costello, C.E. A systematic nomenclature for carbohydrate fragmentations in FAB-MS/MS spectra of glycoconjugates. *Glycoconj J* 1988, 5, 397-409.

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