CHEM 537
Carbohydrate Biochemistry and Glycobiology
Part III: Glycobiology, Glycoproteins & Glycoconjugates

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Slide Set 3a

Chapters 11 & 23: *Biochemistry*, Voet/Voet, 4th edition, 2011
Introduction to Glycobiology, Taylor/Drickhamer, 3rd edition, 2011

Glycobiology: Definitions and terminology

Glycobiology: studies of the structures and functions of sugars attached to proteins and lipids.

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

Glycoproteins and glycolipids: proteins and lipids to which carbohydrate is covalently attached; the mechanism of attachment is enzyme-catalyzed *in vivo*.

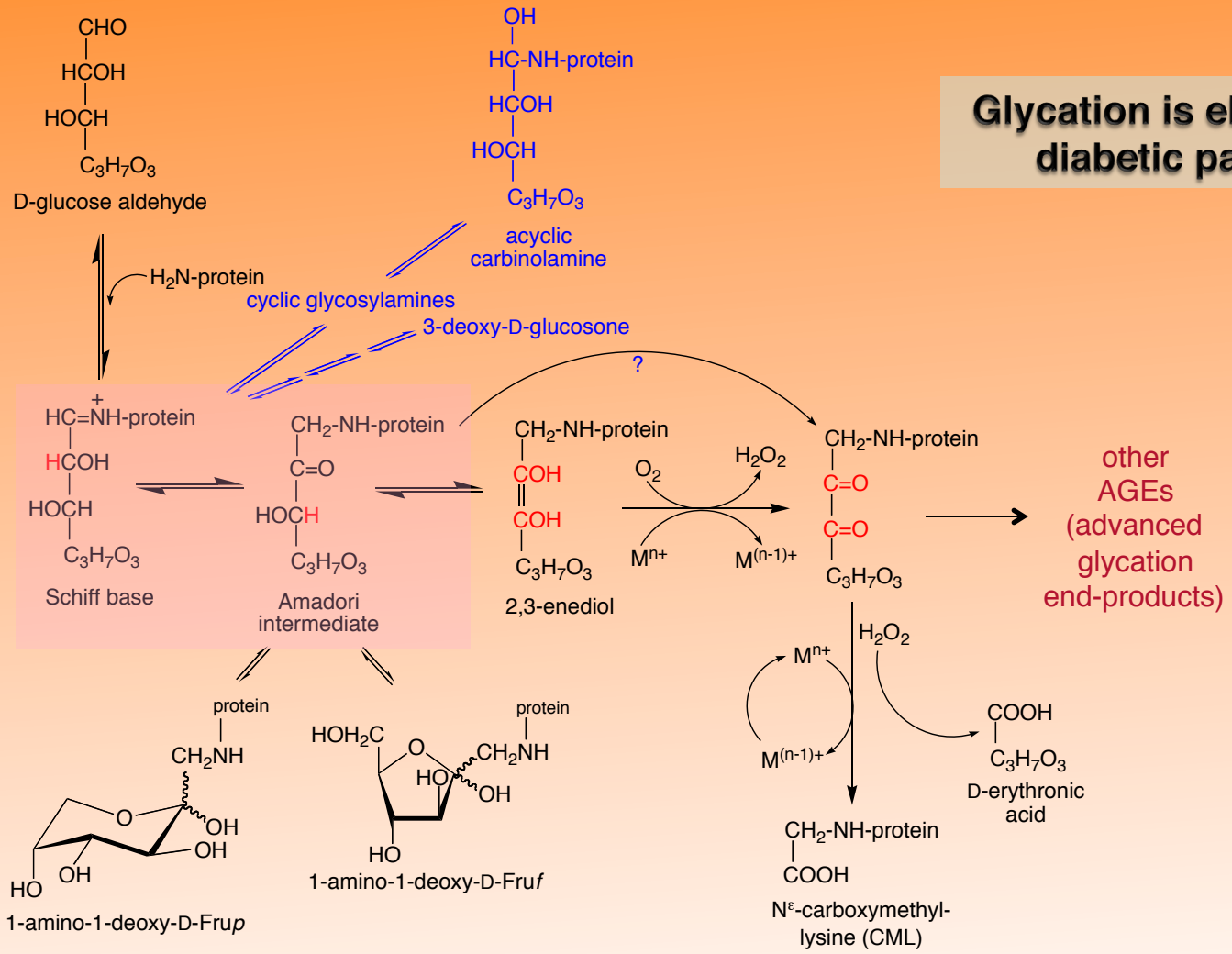
Glycan: the carbohydrate component of glycoproteins and glycolipids.

Glycosylation and glycation

Glycosylation: enzyme-catalyzed covalent modification of proteins and lipids; involves specific sugar donors such as nucleotide and dolichol sugars, and glycosyltransferases; glycosylated products have specific structures and biological functions.

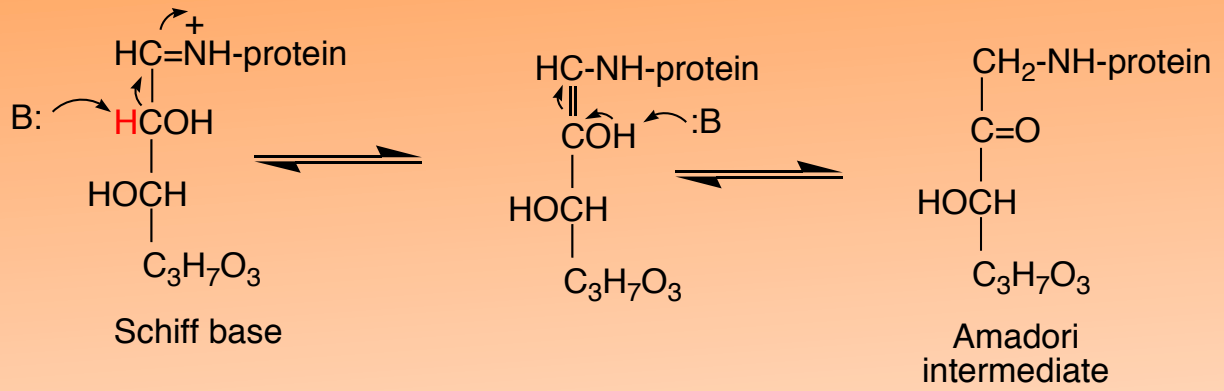
Glycation: chemical modification of proteins that occurs *in vivo*; spontaneous, non-enzyme-catalyzed; products are heterogeneous in structure and often deleterious to the organism.

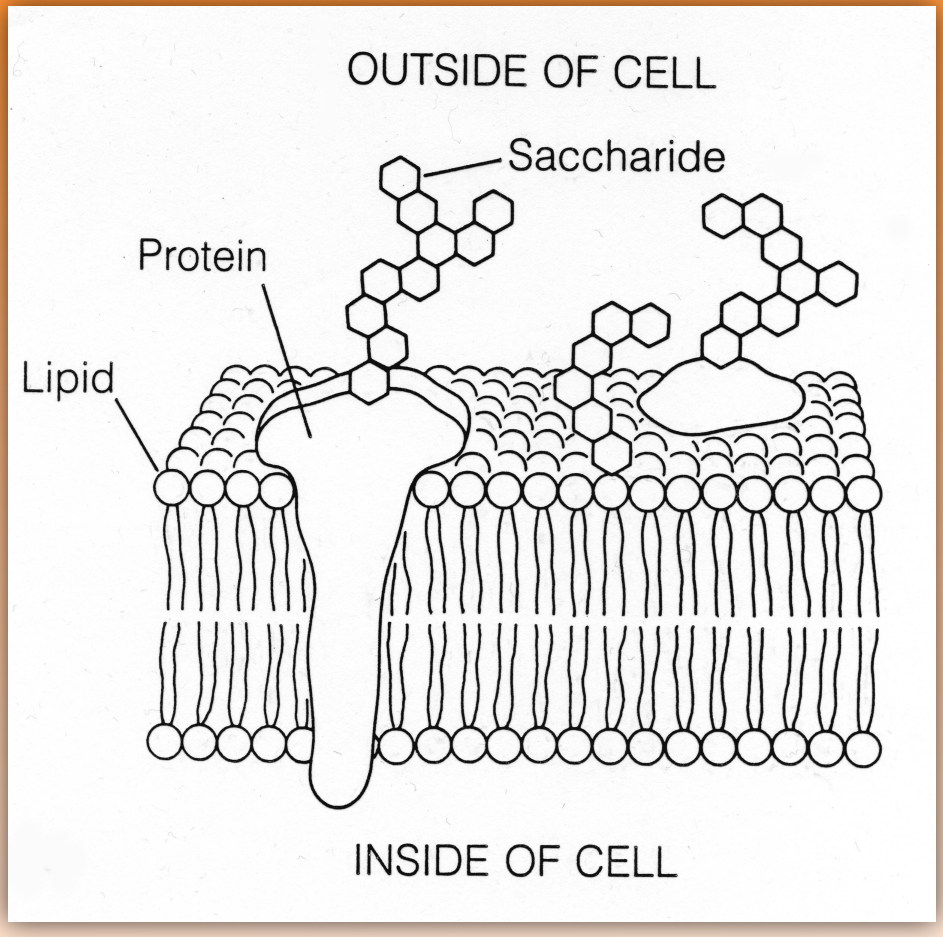
Protein glycation is not enzyme-catalyzed.



Glycation is elevated in diabetic patients.

Mechanism of formation of the Amadori intermediate during protein glycation

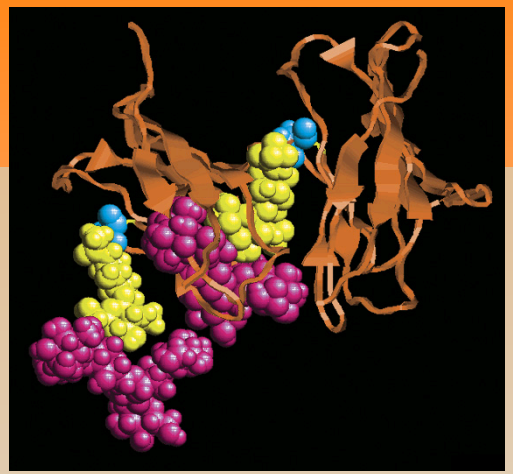




Glycoconjugates associated with plasma membranes (glycoproteins and glycolipids): asymmetric distribution of glycan chains on the extracellular side of the membrane.

The extracellular location allows specific glycan interactions with biomolecules, cells, viruses.

Glycoproteins



Protein glycosylation affects:

- thermodynamic stability
- biological half-life
- cellular localization
- biological activity

Protein glycosylation is under enzymatic control:

- glycosylation of a particular protein can differ by cell type, growth stage, metabolic activity, and substrate availability, resulting in different isoforms that differ by glycosylation only.
- glycosylation differences produce **glycoforms** characterized by their **microheterogeneity** (a conserved protein component but different glycan components)

Nearly all eukaryotic secreted and membrane-associated proteins are heavily glycosylated; glycosylation is the most common post-translational modification of proteins; ~50% of proteins in the human body are glycosylated.

Two major forms of protein glycosylation: ***N*-linked** glycans and ***O*-linked** glycans

As a general rule, prokaryotes do not glycosylate proteins.

Some functions of protein glycosylation

Structural: *O*-glycosylation of mucins results in an open, extended structure.

Recognition: *N*- and *O*-glycosylation of membrane proteins promote cell identity and adhesion (leukocyte rolling, immune system recognition).

Protein degradation: Slow cleavage of *N*-linked glycans can serve as a timing device for initiating protein proteolysis.

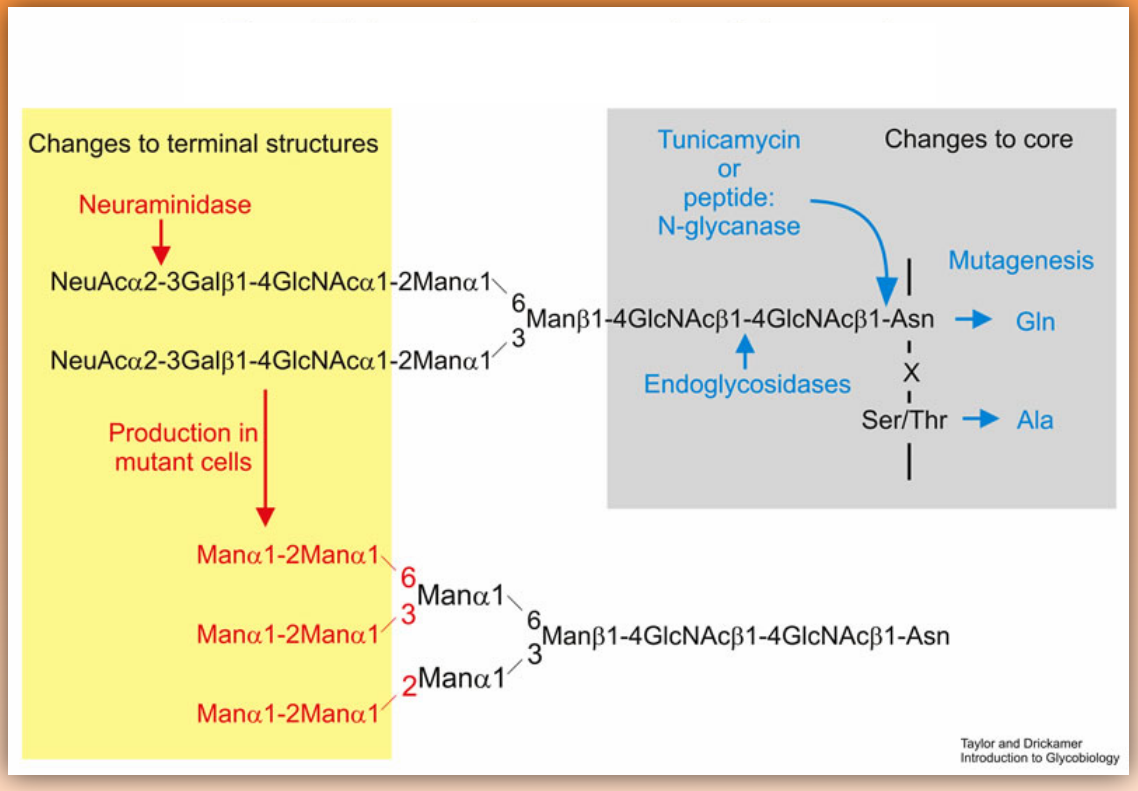
Protein stability: *N*-linked glycans can increase protein stability by enhancing water activity around the protein, "magnifying" the influence of the hydrophobic effect.

Orientation in assemblies: protein glycosylation can affect their interactions to form larger assemblies (*e.g.,* membrane signaling complexes)

Glycoproteins and glycolipids on plasma membranes mediate cell identity, communication, adhesion and/or growth.

Most oligosaccharides attached to proteins extend away from the protein's surface and probably do not affect protein structure significantly (we think).

Experimental methods to modify protein glycosylation patterns

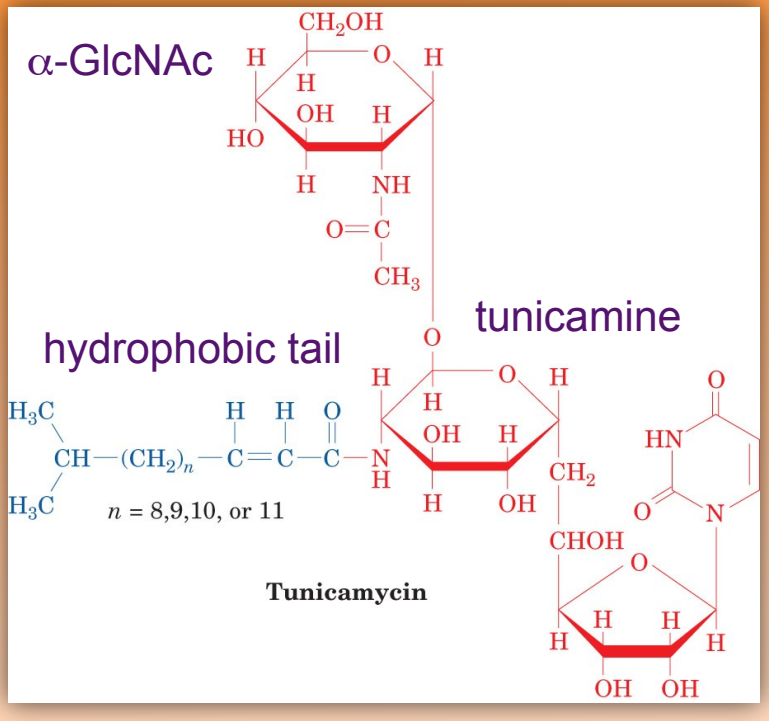


Peptide *N*-glycanase (PNGase): Cleaves at the GlcNAc-Asn attachment point, liberating the full *N*-glycan *in vitro*.

Tunicamycin: a small molecule inhibitor of the initial step of protein *N*-glycosylation (dolichol-P stage); prevents *N*-glycosylation *in vivo*. **Mutagenesis** can achieve the same effect, although the protein is modified.

Endo- and exo-glycosidases trim existing oligosaccharides *in vitro*.

Protein expression in different organisms/cells can modify glycosylation patterns *in vivo*.



In eukaryotes, tunicamycin inhibits the GPT translocase involved in the biosynthesis of GlcNAc-linked dolichol pyrophosphate (an early event in protein *N*-glycosylation). Tunicamycin is widely used to inhibit glycoprotein translocation and processing.

Tunicamycins are natural products isolated from *Streptomyces*. They vary in the structure of the fatty acid hydrophobic tail.

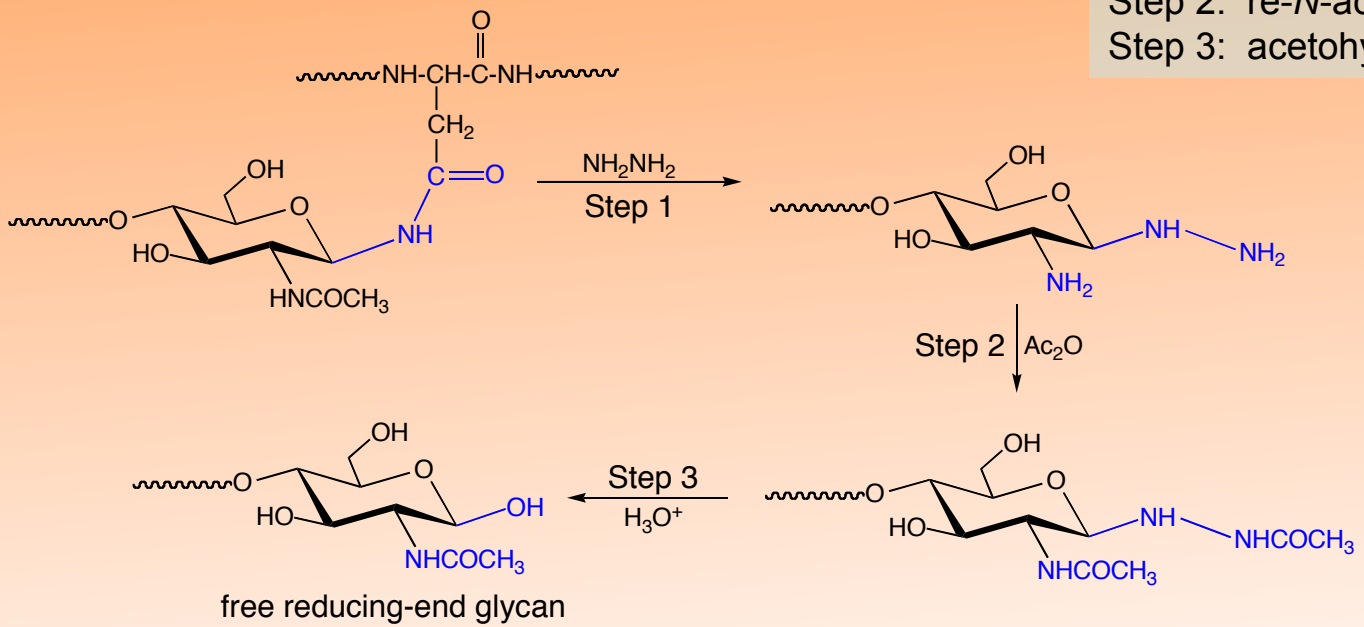
Xu *et al. Biochemistry* **2004**, *43*, 13248-13255

Mechanism of hydrazine-mediated cleavage of an *N*-glycan from a glycoprotein

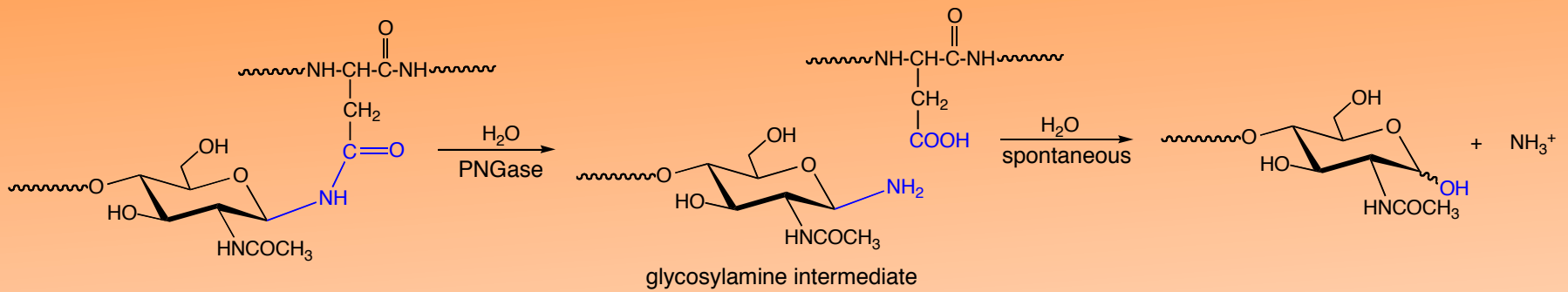
The chemical mechanism by which hydrazine cleaves the *N*-glycoside linkage of *N*-glycans is not completely understood.

A proposed reaction scheme:

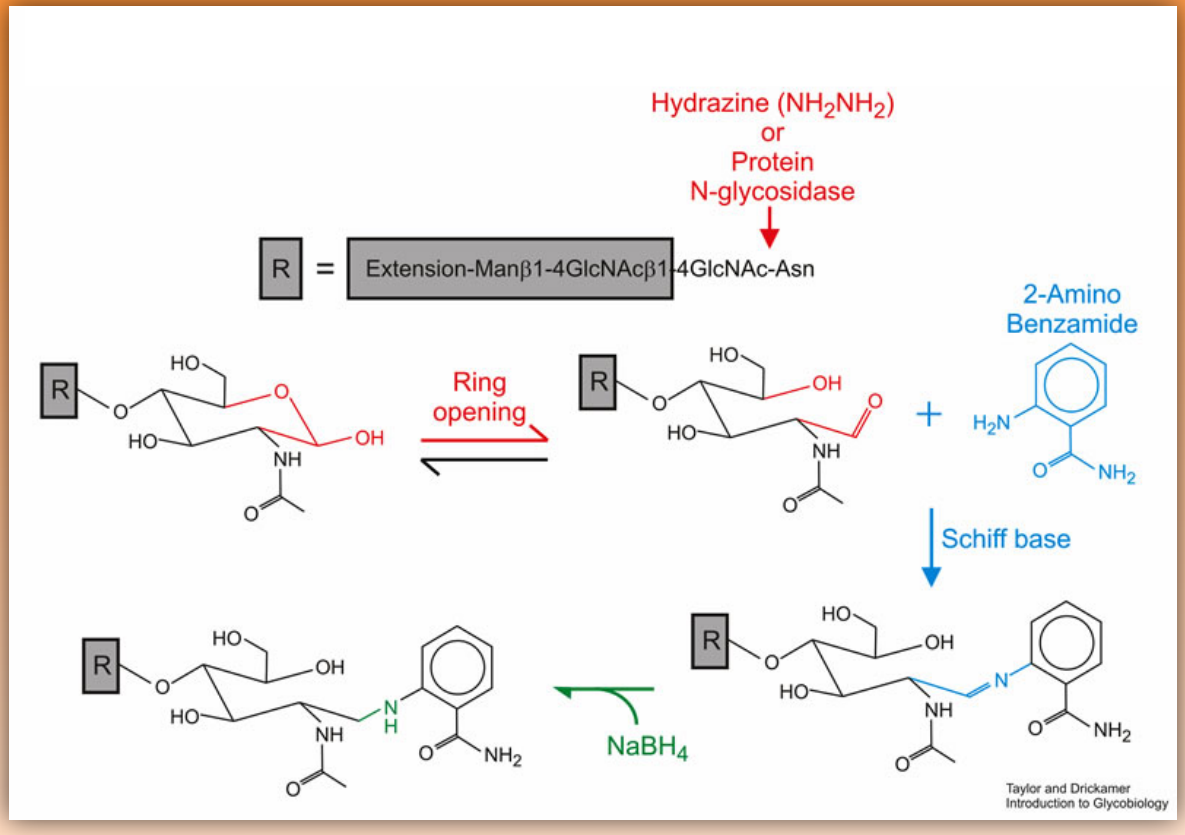
Step 1: hydrazinolysis
Step 2: re-*N*-acetylation
Step 3: acetohydrazone cleavage



Hydrolysis of the *N*-glycoside bond of *N*-glycans by peptide *N*-glycanase



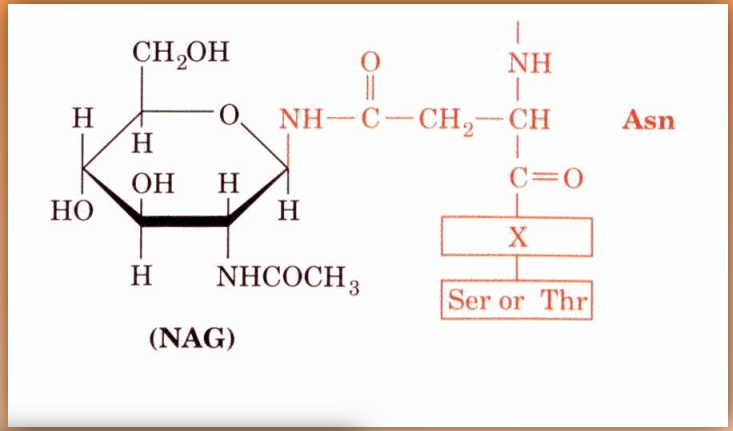
Results in release of the intact *N*-glycan from the protein; the *N*-glycan has a free reducing end available for subsequent derivatization.

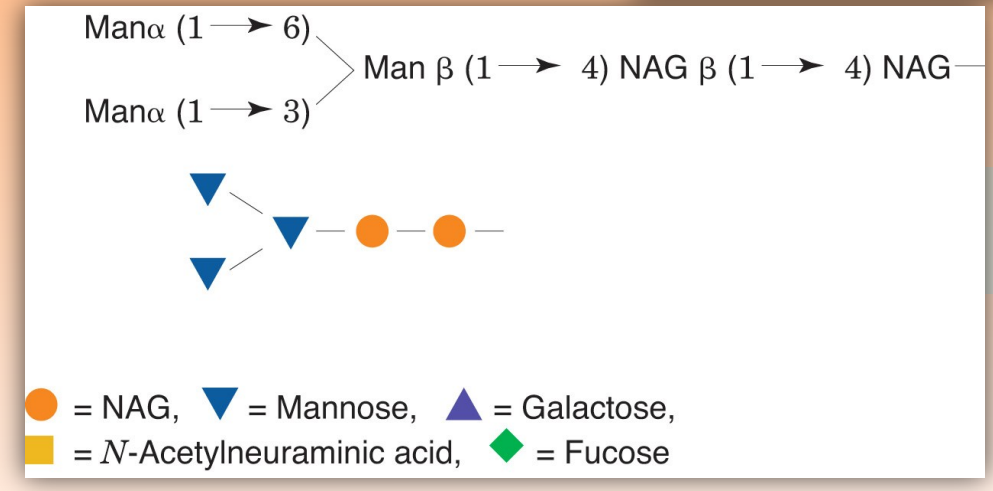


Use of hydrazine or PNGase to release an intact *N*-glycan from a glycoprotein. Subsequent chemical tagging of the released oligosaccharide at the reducing end with a fluorescent probe facilitates analysis by HPLC.

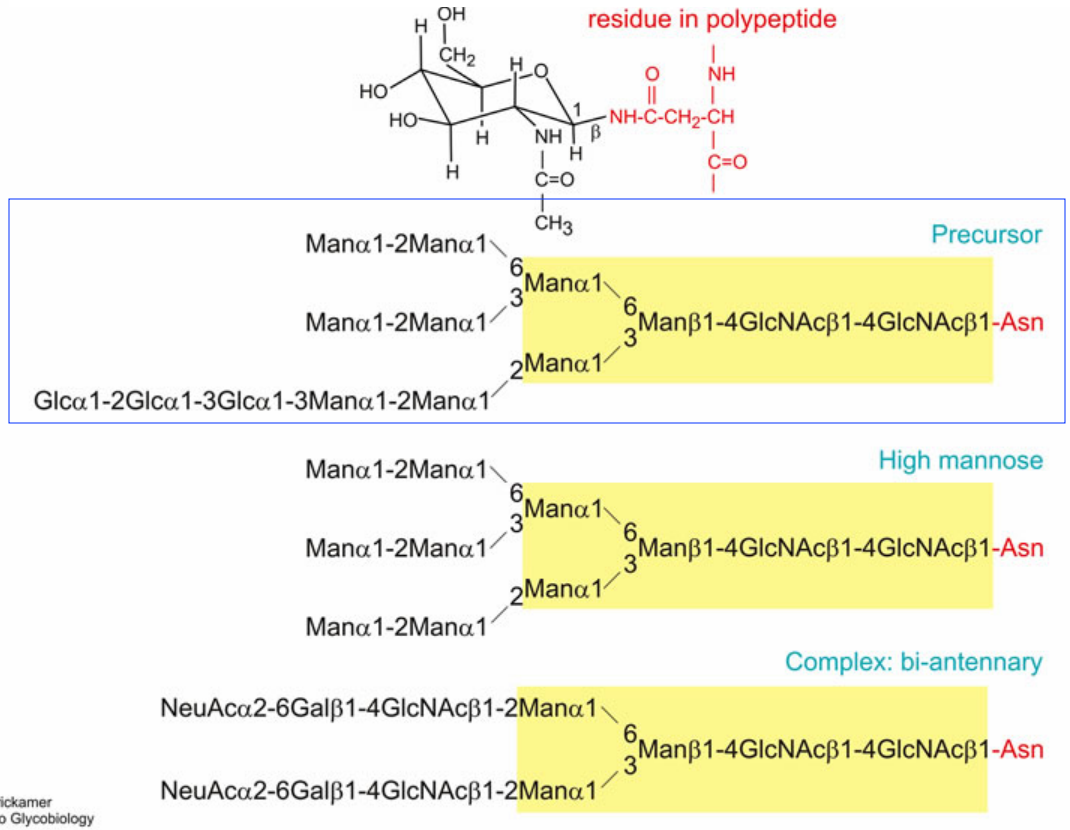
N-Linked Glycoproteins and *N*-Glycans

N-Glycosylation involves a **consensus sequence**:
GlcNAc is β-linked to the amide nitrogen of an Asn sidechain
Consensus tripeptide sequence = Asn-X-Ser or Thr (X ≠ Pro / Asp)



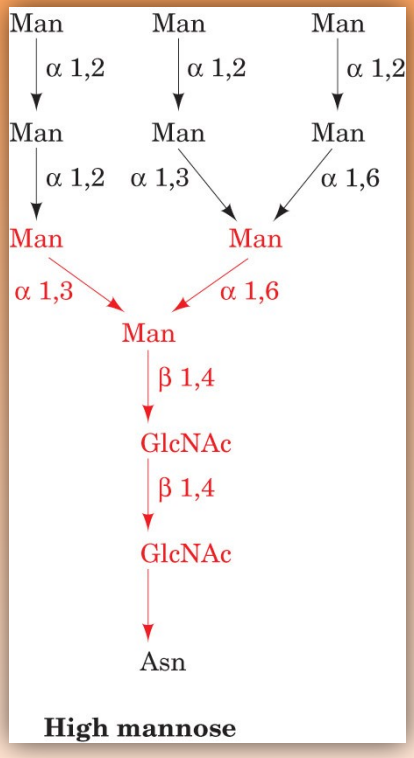


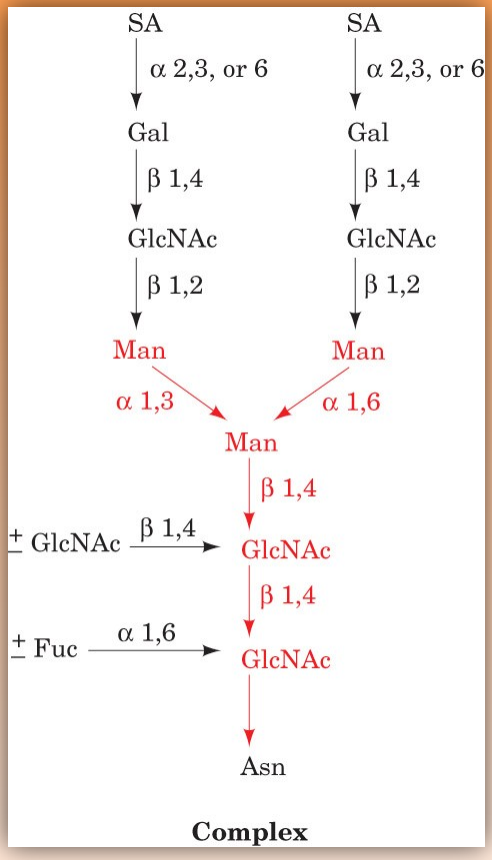
***N*-linked glycans contain a common pentasaccharide core: $(Man)_3(GlcNAc)_2$**

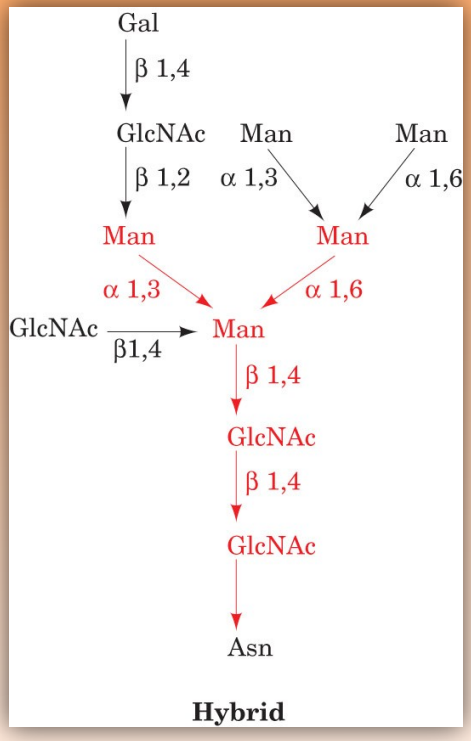


The $GlcNAc_2Man_3$ "core" pentasaccharide is common to all *N*-linked glycans. The two Man branch points in this core pentasaccharide give rise to the 1,3 and 1,6 arms of the *N*-glycan. The $GlcNAc_2Man_9Glc_3$ oligosaccharide is the **biological precursor** in the construction of all *N*-glycans *in vivo*.

Three main classes of N-glycans



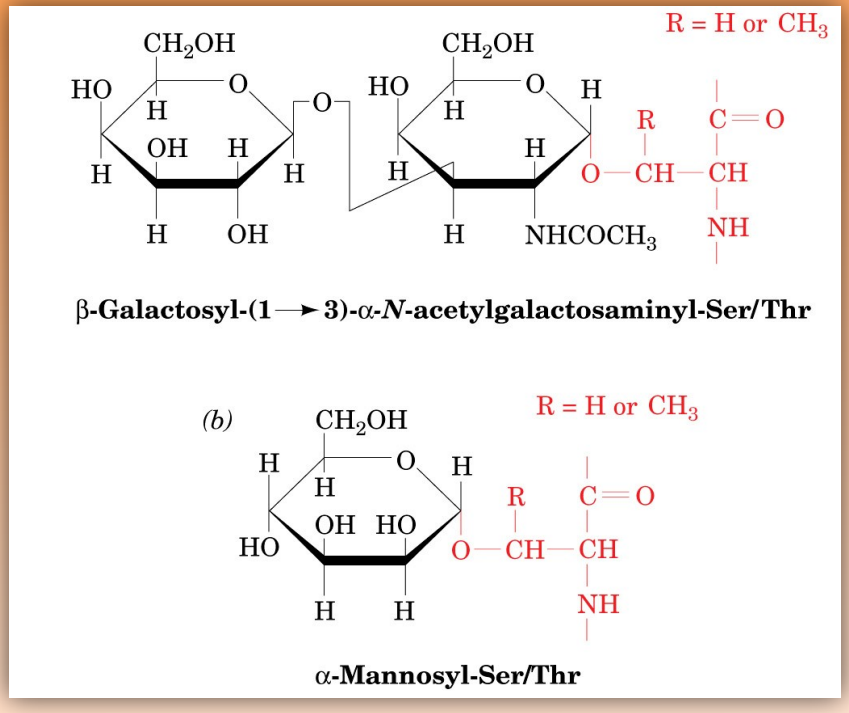




O-Linked Glycoproteins and *O*-Glycans

O-Glycosylation

β-D-Galactopyranosyl-(1,3)-*N*-acetyl-D-galactosamine α-linked to the side-chain OH group of either Ser or Thr.



***O*-Glycosylation is often structural (*e.g.*, in proteoglycans and mucins). Heavy *O*-glycosylation forces the protein to adopt an extended conformation.**