CHEM 537
Carbohydrate Biochemistry and Glycobiology
Part II: Oligosaccharides & Polysaccharides

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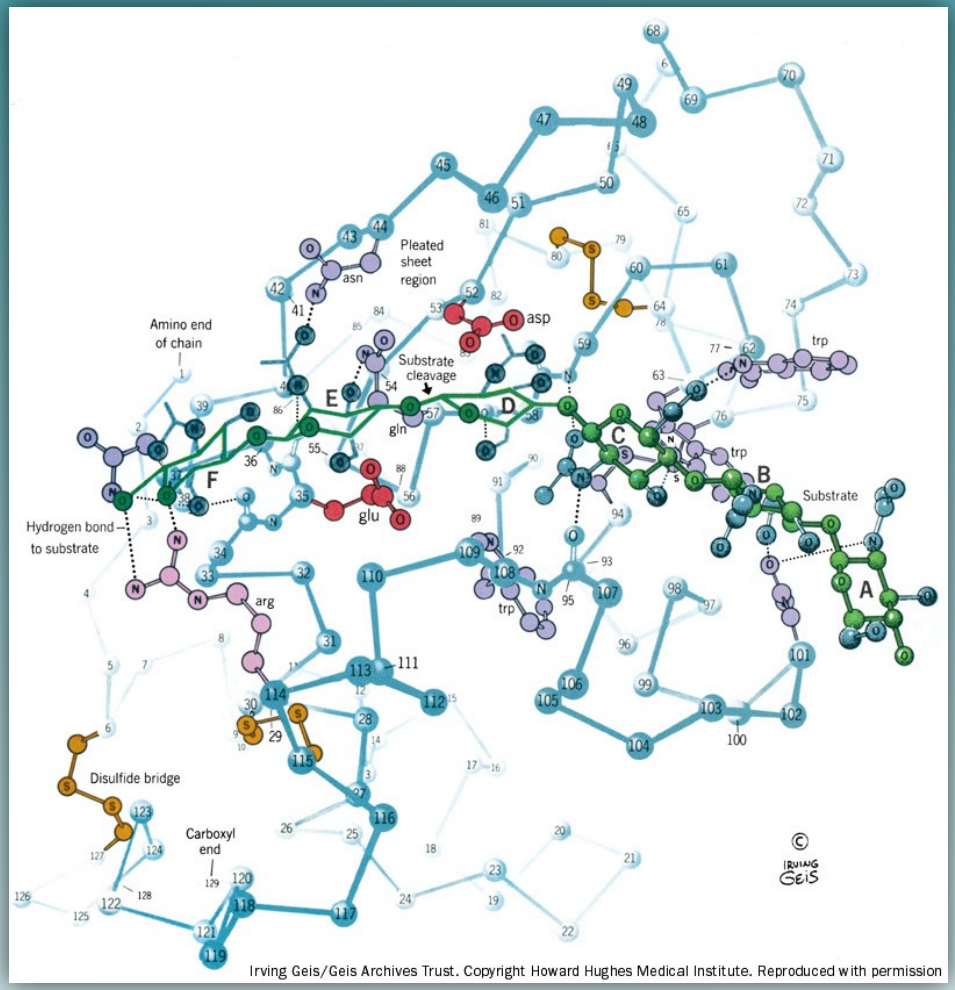
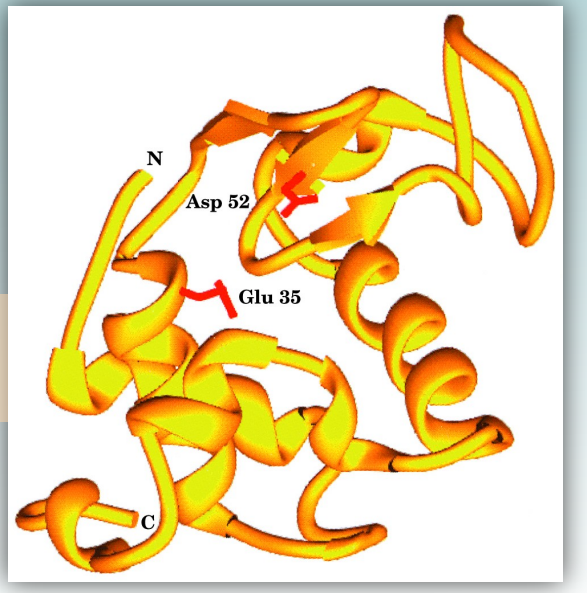
Slide Set 2c

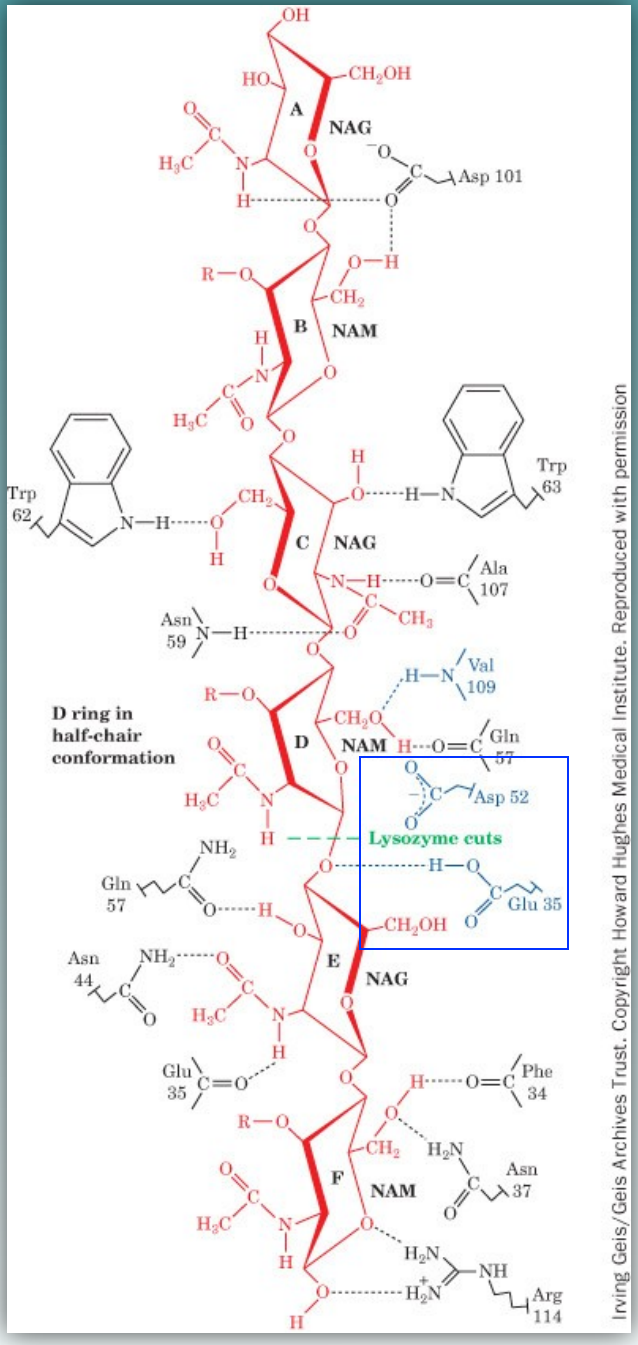
Chapters 11 & 23: *Biochemistry*, Voet/Voet, 4rd edition, 2011
Introduction to Glycobiology, Taylor/Drickhamer, 3rd edition, 2011



$(NAG)_6$ binding by HEW lysozyme. The enzyme contains a prominent cleft that accommodates binding of the hexasaccharide. Hexasaccharide residues are denoted A-F, with cleavage occurring between residues D and E.

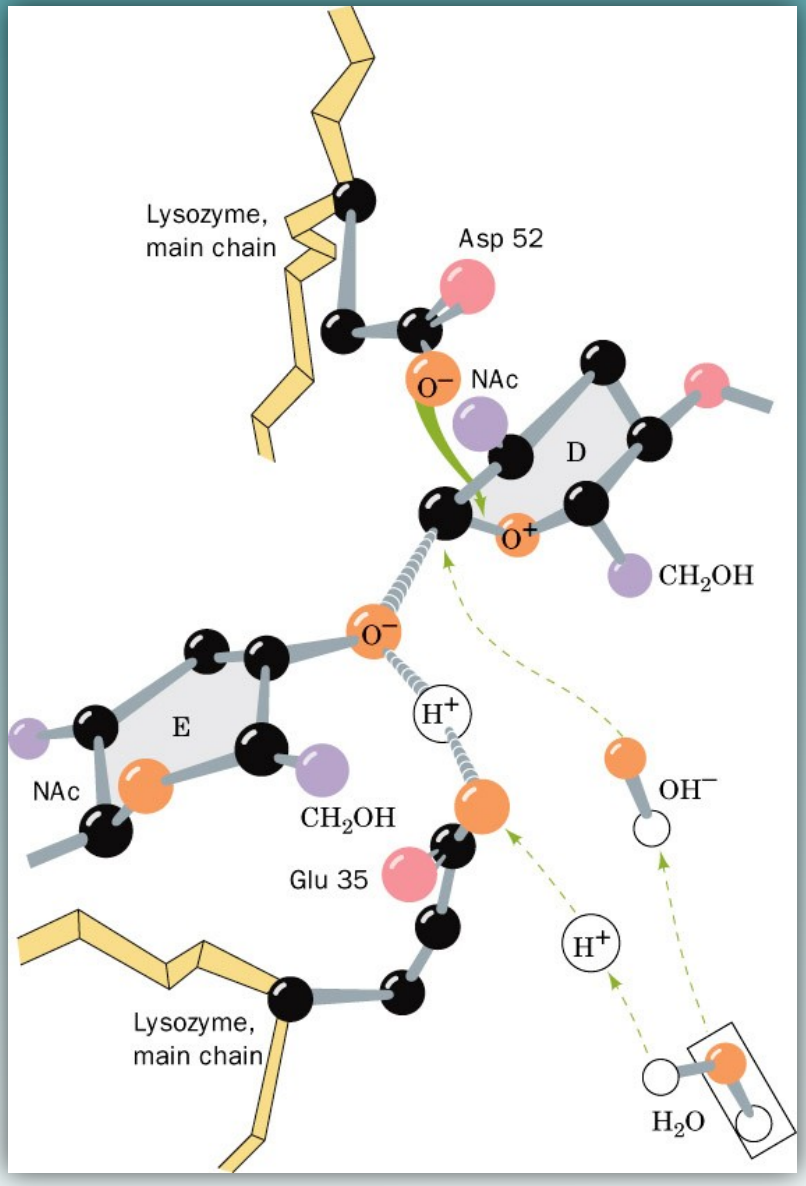


Ribbon diagram of HEW lysozyme



Lysozyme interactions with substrate.

Multiple H-bonds secure the (NAG-NAM)$_3$ hexasaccharide to the catalytic site, positioning the NAM-NAG glycosidic linkage joining residues D and E in proximity to the acidic side-chains of Glu 35 and Asp 52. The enzyme uses these acidic functionalities to hydrolyze the intrinsically acid-labile acetal.



The Phillips mechanism of glycoside hydrolysis by lysozyme. Glu 35 protonates the linkage oxygen. The resulting D-ring oxonium cation is stabilized by proximity to the negatively-charged Glu 52 side-chain and by enzyme-induced distortion of the D-ring. Upon release of the E-ring, solvent H_2O provides OH^- and H^+ that react with the oxonium ion and re-protonate Glu 35, respectively. Note that in this mechanism, the Glu 35 side-chain is protonated, and the Glu 52 side-chain is deprotonated in the active form of the enzyme.

The Phillips (strain) mechanism has been recently challenged by a covalent mechanism through the use of fluorinated substrates (Stephen Withers; see Voet/Voet, 4th edition, 2011, pages 523-525).

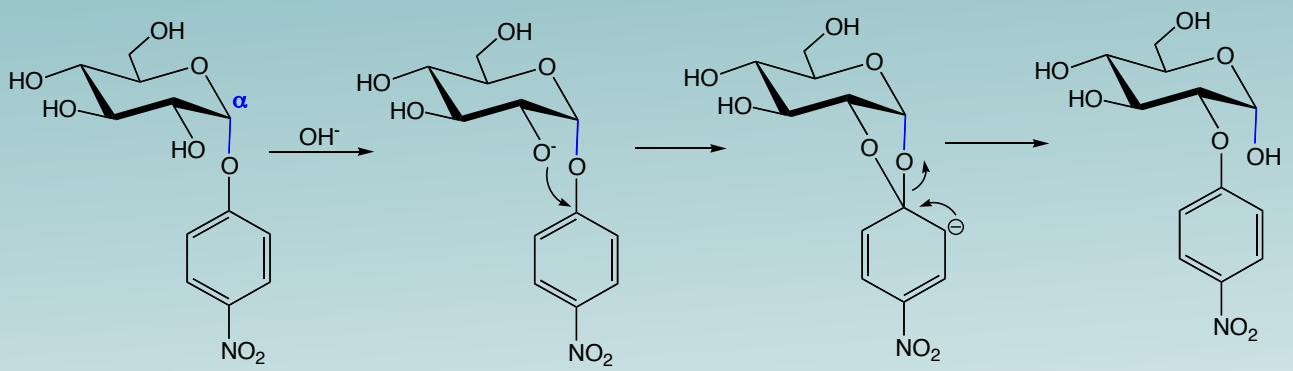
Comparison of protease and glycosidase mechanisms

Chemical hydrolysis of an amide (peptide) bond can be acid- or base-catalyzed. Proteases utilize the same chemistry, albeit at enhanced rates: serine proteases exploit the base mechanism (catalytic triad), whereas aspartic proteases exploit the acid mechanism.

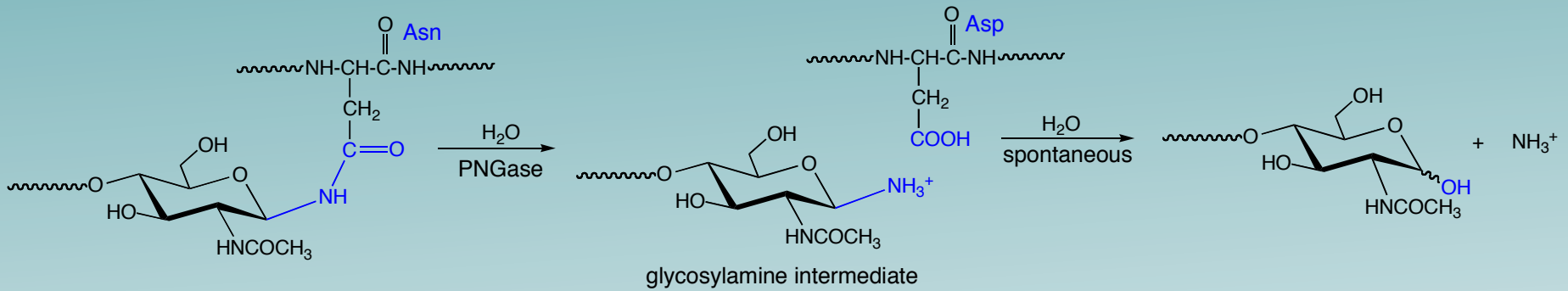
Chemical hydrolysis of a glycosidic linkage (acetal/ketal) involving an alkyl aglycone is only acid-catalyzed. All known glycosidases that act on these types of substrates utilize acid catalysis. It is unlikely that a glycosidase will ever be discovered that utilizes a base mechanism for the hydrolysis of oligo- or polysaccharides (enzymes do not disobey the rules of solution chemistry).

Are there cases where glycosidic linkages can be hydrolyzed under basic conditions?

α-Aryl glucopyranosides: base-labile

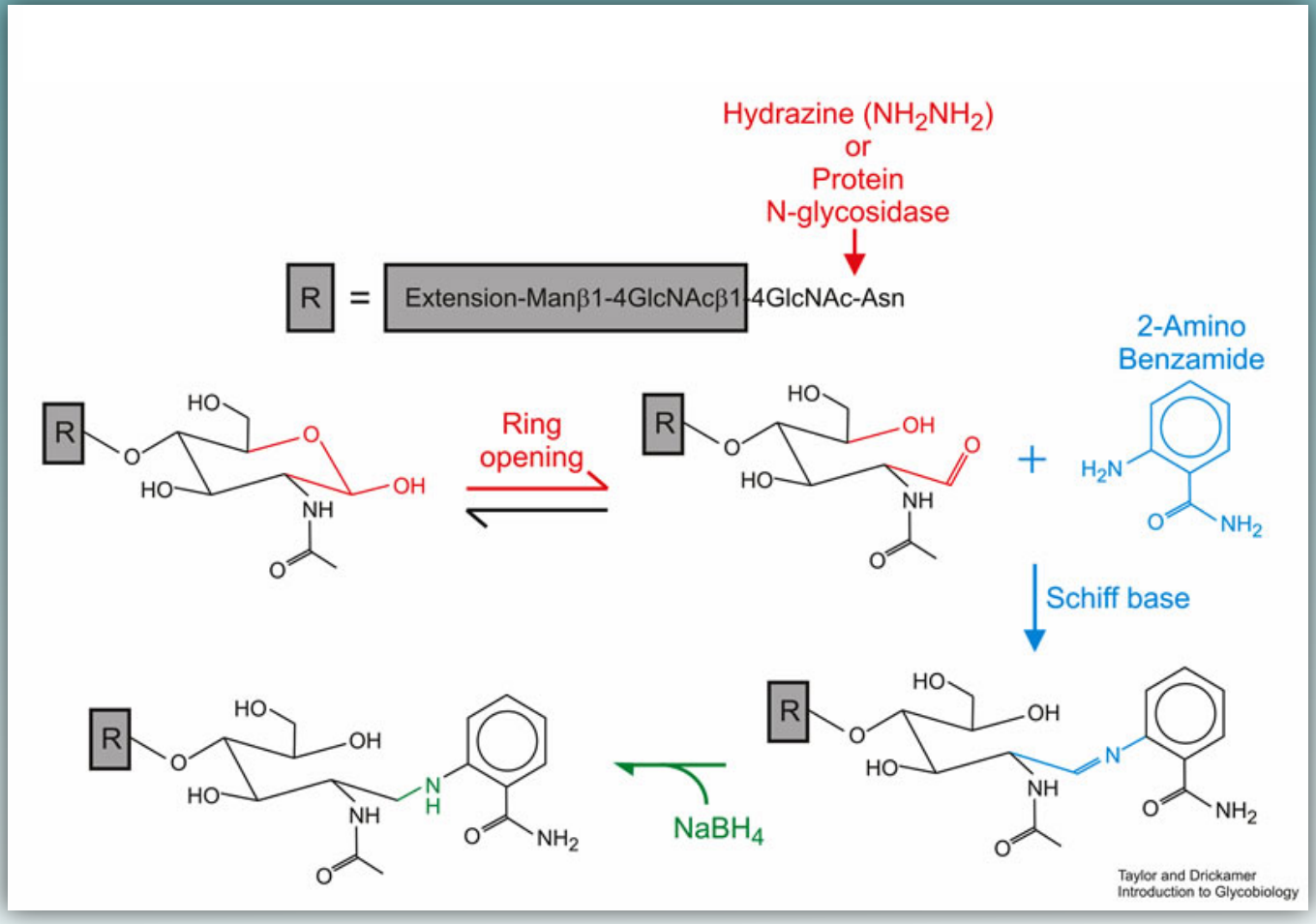


Hydrolysis of the *N*-glycoside bond of *N*-glycans by peptide *N*-glycanase (PNGase)

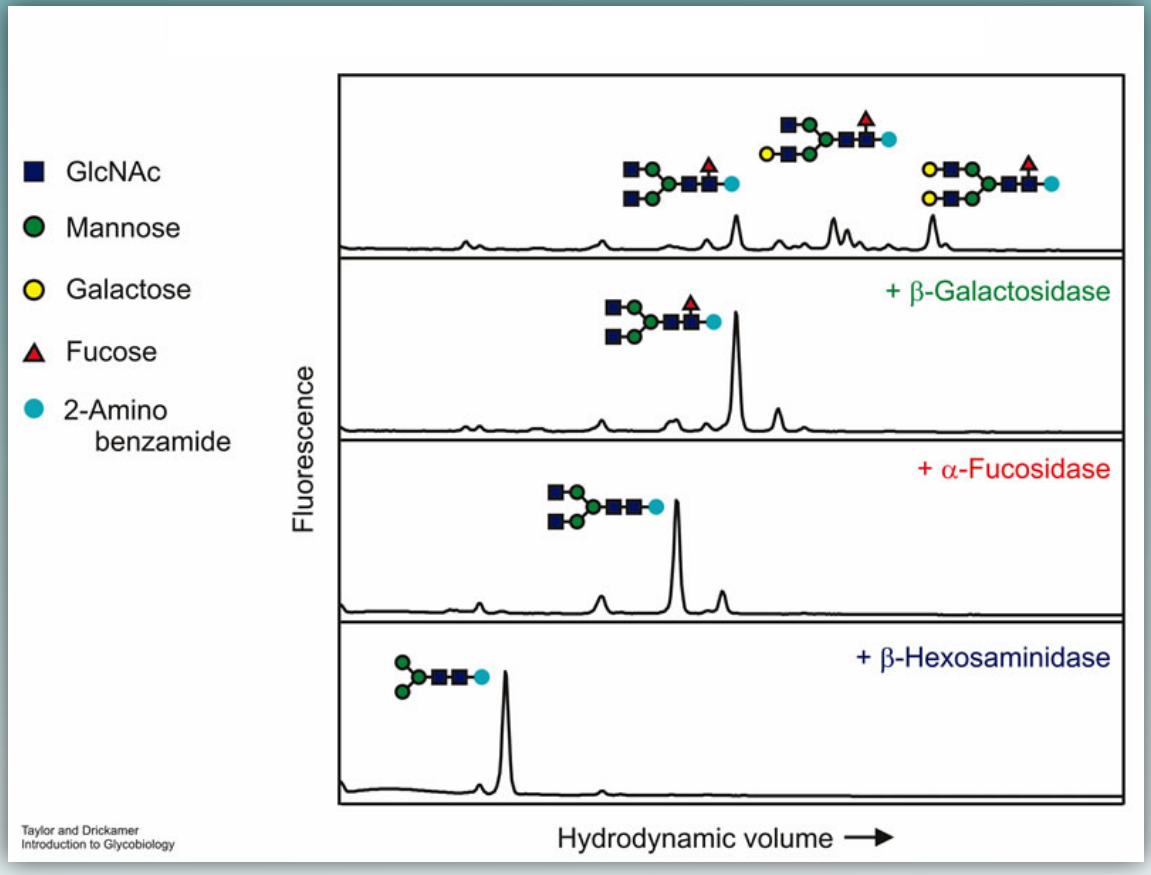


Results in the release of the intact *N*-glycan from the protein. **The released *N*-glycan has a free reducing end available for derivatization.**

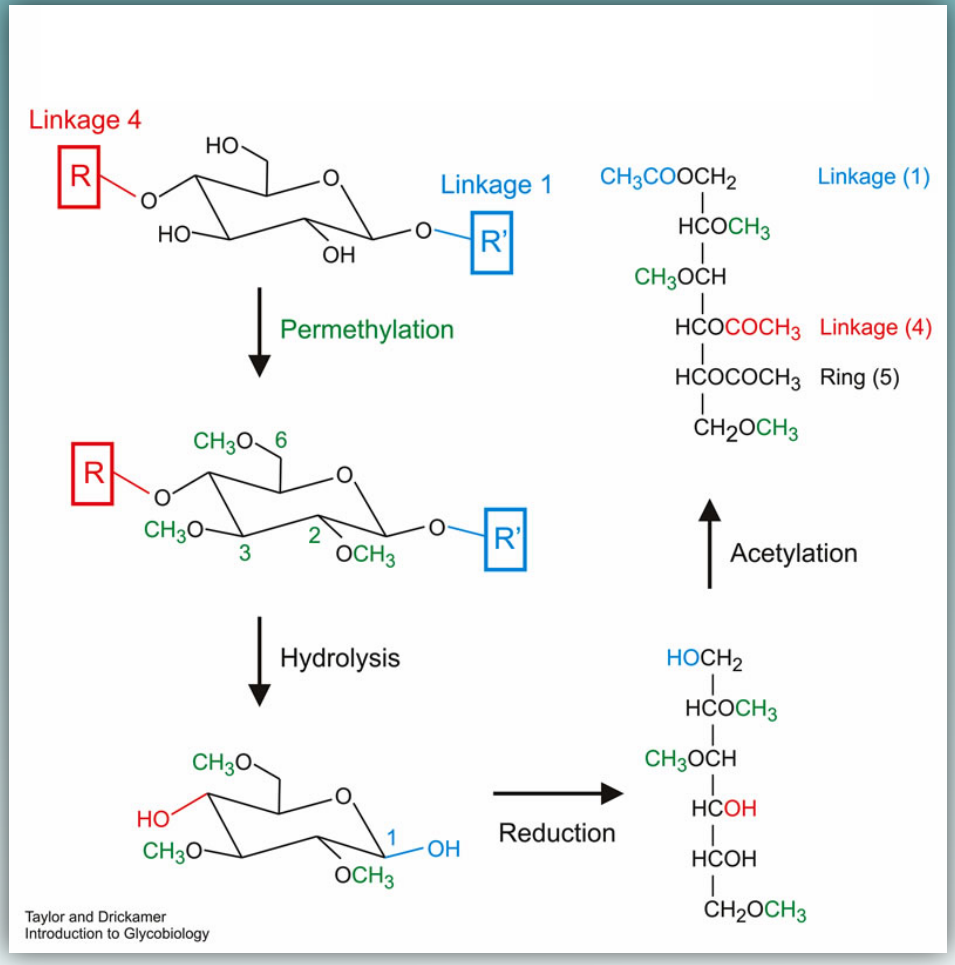
Tagging the reducing ends of released *N*-glycans to facilitate spectrophotometric detection



Sequential glycosidase treatment of an end-labeled glycan: HPLC analysis of cleavage products



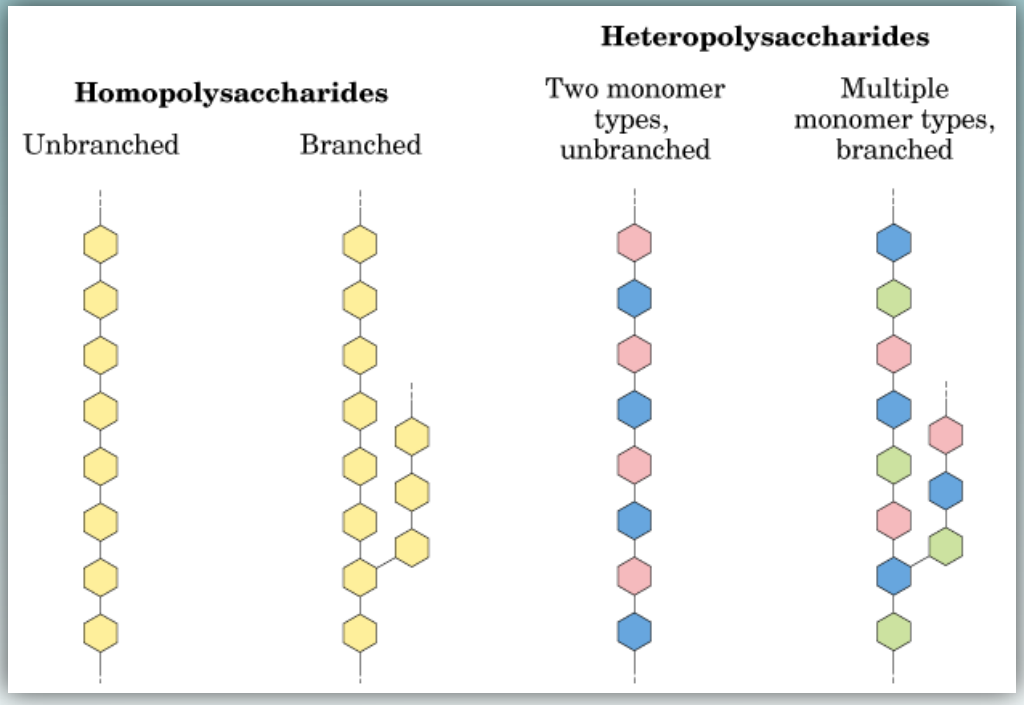
Linkage analysis using permethylation: Determining how the consituent monosaccharides of an oligosaccharide are linked together (linkage regiochemistry)



Polysaccharides

Polysaccharides are formed by linking multiple monosaccharides together via *O*-glycosidic linkages. They can have molecular weights > 1 x 10^6 Da. There are two basic structural types:

- **Homopolysaccharides**: comprised of only one type of monosaccharide; linkages may not be homogeneous (examples: cellulose, starch, glycogen)
- **Heteropolysaccharides**: comprised of more than one type of monosaccharide (examples: hyaluronic acid, glycosaminoglycans)



Functions: energy storage; structural support; protective; cell identification.

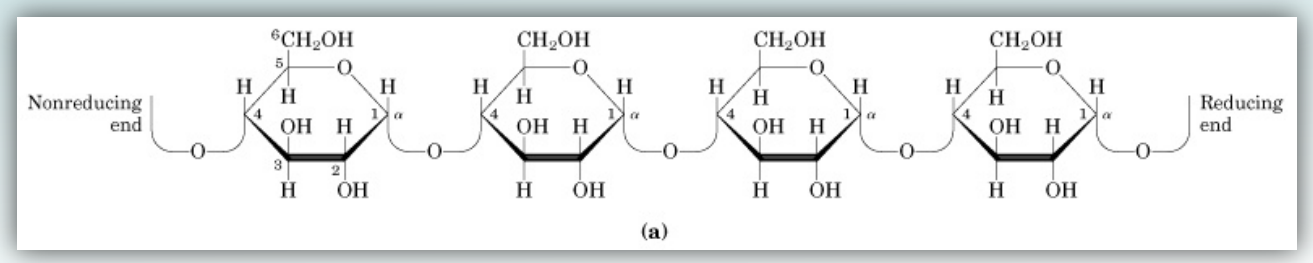
Polysaccharide purification and analysis

1. **Purification by affinity chromatography**: carbohydrate binding proteins = **lectins**
   - ♦ concanavalin A: specifically binds α-D-glucopyranosyl and α-D-mannopyranosyl residues
   - ♦ agglutinin: specifically binds sialic acid residues
   - ♦ MBP (mannose binding protein): specifically binds terminal α-D-mannopyranosyl residues

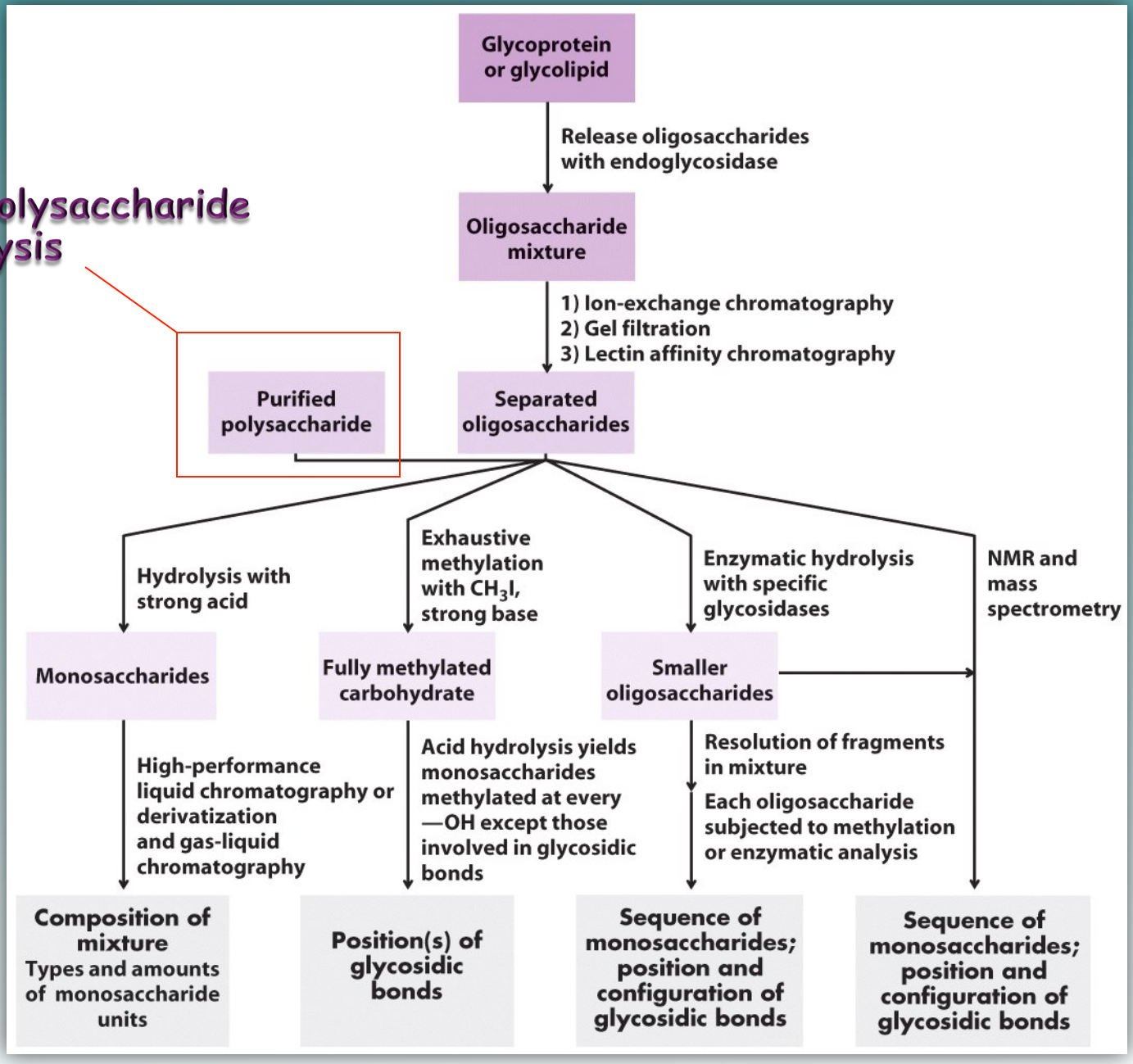
2. **Methylation analysis** (composition and linkage positions)
   Methyl ethers are resistant to acid hydrolysis.
   Procedure: Exhaustively methylate the polysaccharide; hydrolyze at low pH - the free OH groups in the released monosaccharides mark the positions of glycosidic bonds in the intact polysaccharide; reduce and acetylate; separate the resulting alditol derivatives chromatographically; identify the structures of the alditol derivatives using appropriate alditol standards.

3. **Enzymatic degradation** (glycosidases) (composition, linkage configuration, sequence)
   - ♦ exoglycosidases: hydrolyze terminal (non-reducing end) residues of a polysaccharide
   - ♦ endoglycosidases: hydrolyze internal residues of the polysaccharide

4. **NMR spectroscopy**: linkage configuration; sequence; conformational properties



Summary of polysaccharide analysis



Extracellular polysaccharides

Connective tissue (cartilage, skin, tendons, blood vessel walls) consist of collagen and elastin (protein-based) fibers embedded in a viscous, gel-like matrix called ground substance.

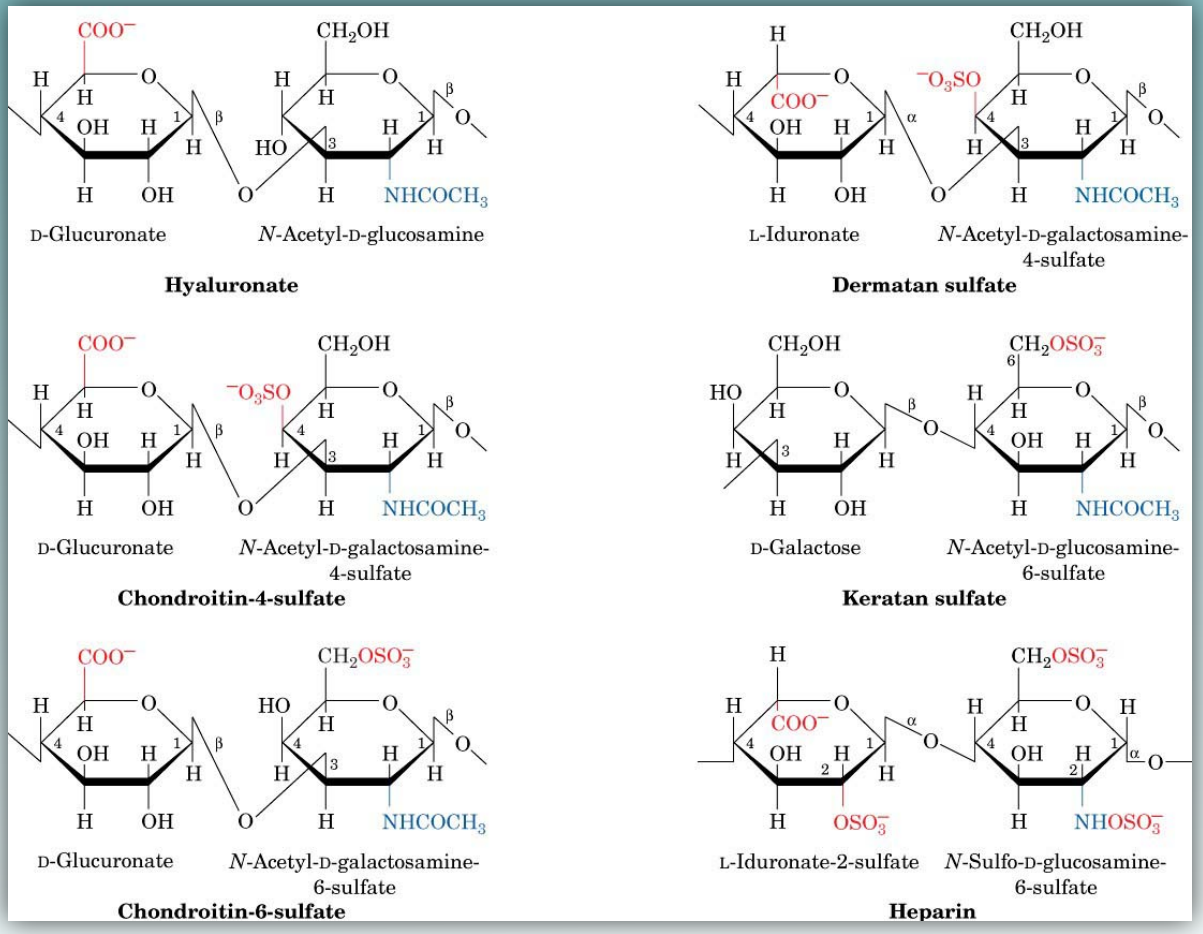
Composed largely of glycosaminoglycans (GAGs) – the most abundant heteropolysaccharides. Unbranched structures contain derivatives of GlcNAc, GalNAc, and uronic acids (*e.g.*, D-glucuronic and L-iduronic acids); backbones consist of repeating disaccharide units.

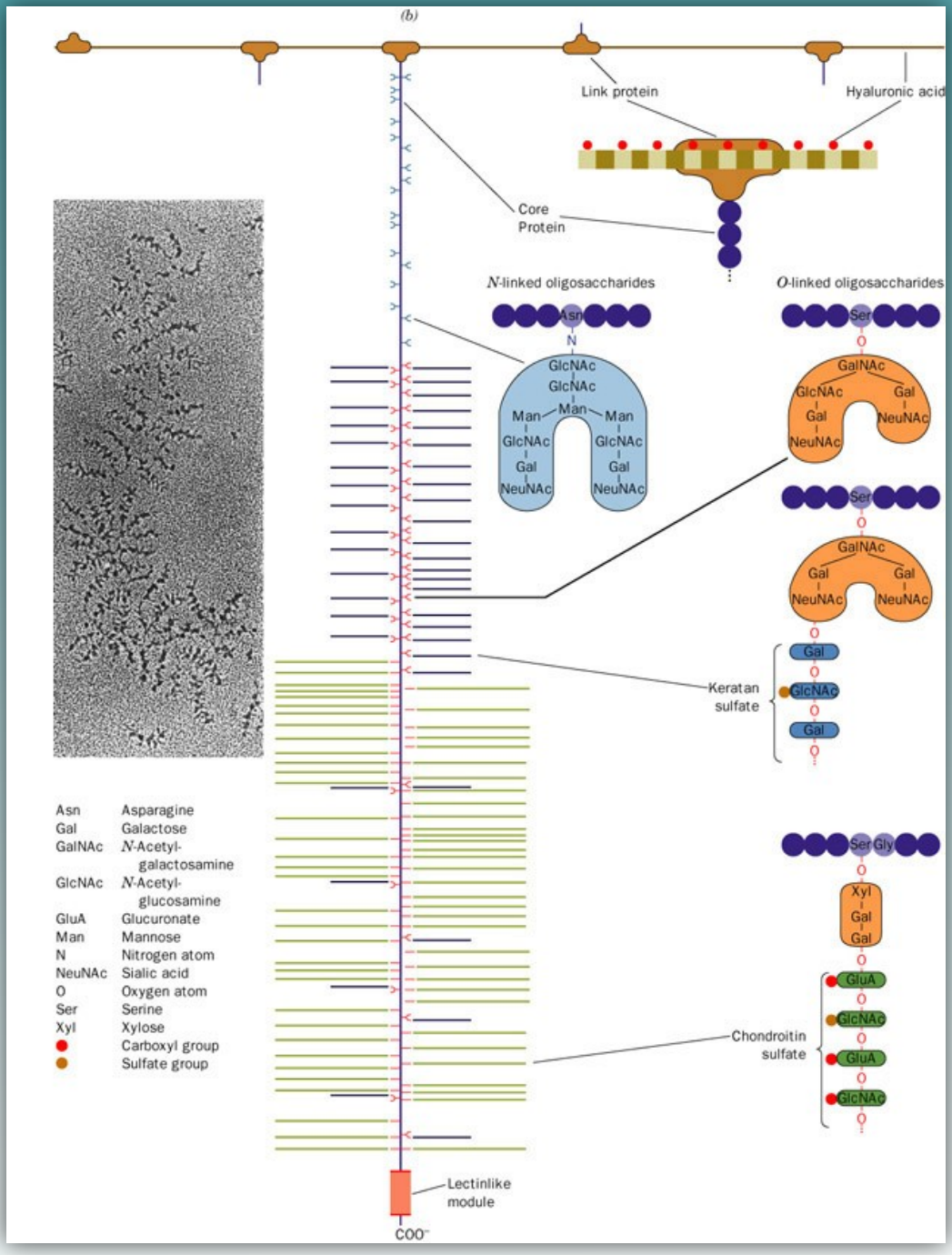
- Highly negatively charged, due primarily to the presence of sulfate esters
- Located primarily on the surface of cells or in the extracellular space
- Extended conformation imparts high viscosity to extracellular solutions
- Low compressibility – ideal for lubricating joints
- Highly viscous and elastic
- Rigidity provides structural integrity to cells and provides passageways between cells, allowing for cell migration.

Some glycosaminoglycans are linked to core proteins in the extracellular matrix, producing proteoglycans. Proteoglycans are heterogeneous protein/polysaccharide complexes with molecular weights $>10^7$ Da.

Extracellular polysaccharides

GAGs are comprised of repeating disaccharide subunits.





Proteoglycan structure.

An electron micrograph showing a central strand of hyaluronic acid (left), and the bottlebrush model of the proteoglycan aggrecan (right).

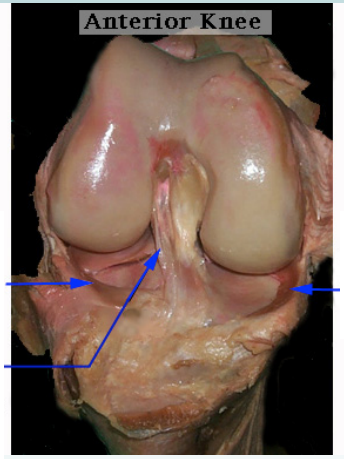
Core protein is *O*- and *N*-glycosylated. The glycans are decorated with keratan and chondroitin sulfates.

Extracellular polysaccharides

	GAG	Localization	Comments
A	hyaluronate	synovial fluid (lubricates joints); vitreous humor (eye fluid); extracellular matrix of loose connective tissue	large polymers; shock absorbing; totally non-sulfated
B	chondroitin sulfate	tendons, cartilage, bone, heart valves	most abundant GAG; structural support for extracellular matrix
	heparin sulfate	basement membranes; components of cell surfaces	contains higher acetylated glucosamine than heparin
	heparin	component of intracellular granules of mast cells lining the arteries of the lungs, liver and skin	more sulfated than heparin sulfates; prevents blood clotting; most negatively-charged polyelectrolyte in tissues
	dermatan sulfate	skin, blood vessels, heart valves	extracellular matrix of skin
C	keratan sulfate	cornea, bone, hair cartilage aggregated with chondroitin sulfates	may be fucosylated

A

B



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