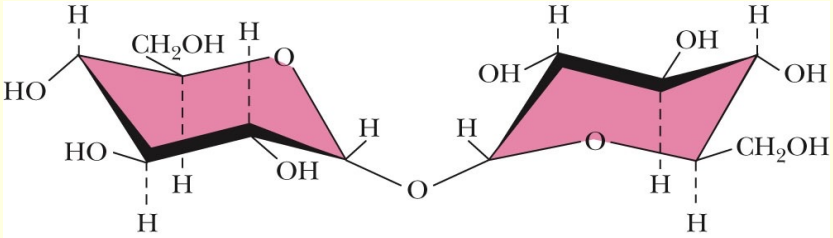
Trehalose – A Natural Protectant for Bugs

- Insects use an open circulatory system to circulate "hemolymph" (insect blood). The "blood sugar" is **trehalose**, an unusual, nonreducing disaccharide.
- Trehalose may act as a natural cryoprotectant, protecting the insect from damage due to freezing temperatures.

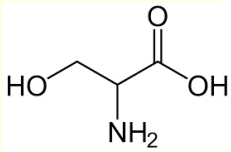


1

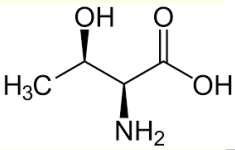
1

Glycoproteins

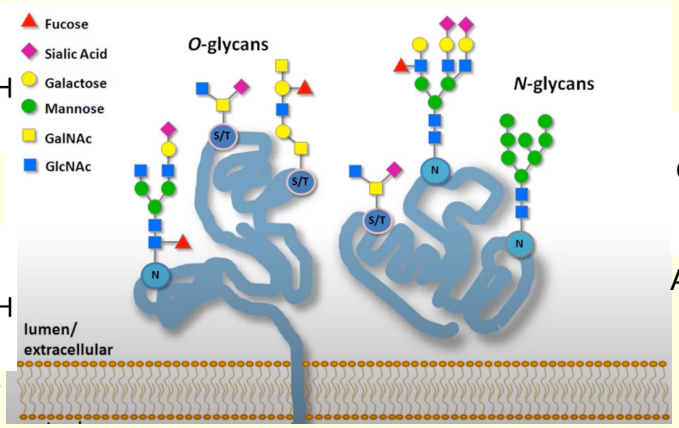
How do saccharides link to the protein?

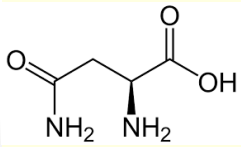


Serine/Ser/S



Threonine/Thr/T



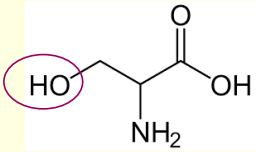


Asparagine/Asn/N

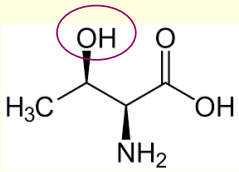
- **O**-Linked glycosylation
- **N**-Linked glycosylation

2

O-Linked glycosylation

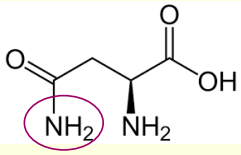


Serine/Ser/S



Threonine/Thr/T

N-Linked glycosylation



Asparagine/Asn/N

生物化學一 Chapter 7-9

Sugar, lipid, and membrane protein

鄭惠春 (生物資訊與結構生物所)

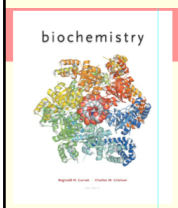
TA: 黃豊元

請假：請與助教聯絡（lydianng27@gmail.com）

成績：33%（5% 上課點名，28% 期中考）

上課時間異動

Wk(date)	Chapter/Topic	
07 (10/15)	7/Carbohydrates and the Glycoconjugates of Cell Surfaces	8:30-9:50
(10/18)	7/Carbohydrates and the Glycoconjugates of Cell Surfaces	8:30-9:50
08(10/22)	7/Carbohydrates and the Glycoconjugates of Cell Surfaces	8:00-9:50
(10/25)	8/Lipids	8:00-9:50
09 (10/29)	8/Lipids	8:30-9:50
(11/01)	8/Lipids	8:30-9:50
10 (11/05)	No Class	
(11/08)	9/Membranes and Membrane Transport	8:30-9:50
11 (11/12)	9/Membranes and Membrane Transport	8:30-9:50
(11/15)	2nd Exam Ch. 7, 8, 9 (p.203-323)	8:00-9:50



CENGAGE Learning

Reginald H. Garrett
Charles M. Grisham

www.cengage.com/chemistry/garrett

Chapter 7
Carbohydrates and the
Glycoconjugates of Cell Surfaces

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Molecular Biochemistry I

Carbohydrates

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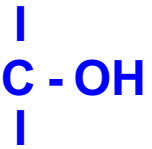
Rensselaer

Essential Question

- What are the structure, chemistry, and biological functions of carbohydrates?

Carbohydrates (glycans) have the following basic composition:

$(CH_2O)_n$ or



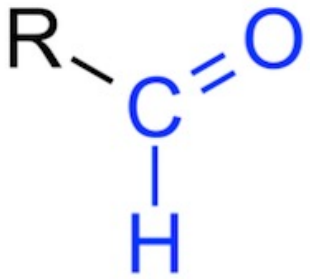
Only true for monosaccharides

- Monosaccharides - simple sugars with multiple OH groups. Based on number of carbons (3, 4, 5, 6), a monosaccharide is a triose, tetrose, pentose or hexose.
- Disaccharides - 2 monosaccharides covalently linked.
- Oligosaccharides - a few monosaccharides covalently linked (usually 2-10).
- Polysaccharides – polymers of many monosaccharides.

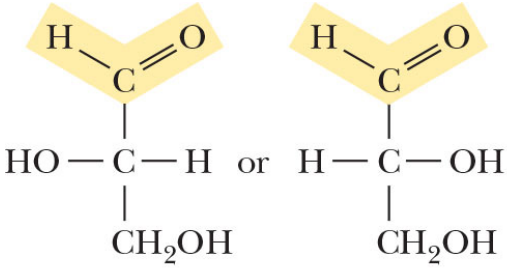
Monosaccharides

Monosaccharides Are Aldehydes or Ketones with Multiple Hydroxyl Groups

Aldehyde Functional Group



R is a hydrocarbon group that is attached to C=O



L-Isomer D-Isomer

Glyceraldehyde

(Aldose)

12

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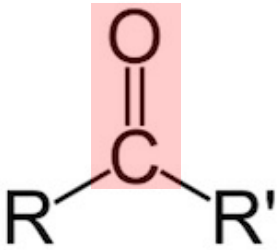
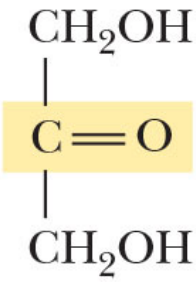
Monosaccharides Are Aldehydes or Ketones with Multiple Hydroxyl Groups

Ketone Functional Group



R and R' are hydrocarbon groups that are attached to C=O



Dihydroxy-acetone

(ketose)

13

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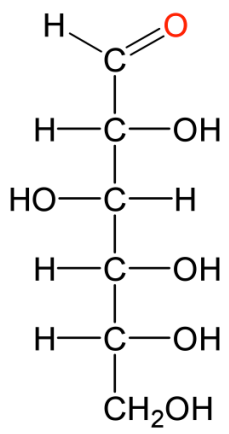
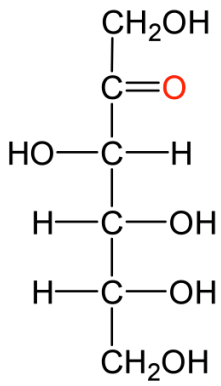
Monosaccharides

Aldoses (e.g., glucose) have an aldehyde group at **C1**.



D-glucose

Ketoses (e.g., fructose) have a keto group, usually at **C2**.



D-fructose

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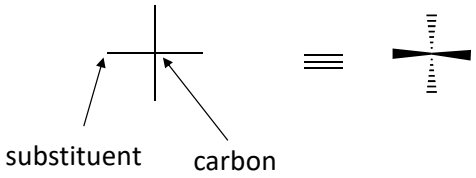
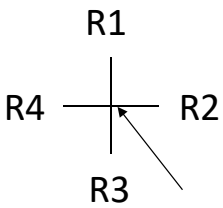
Fischer Projections

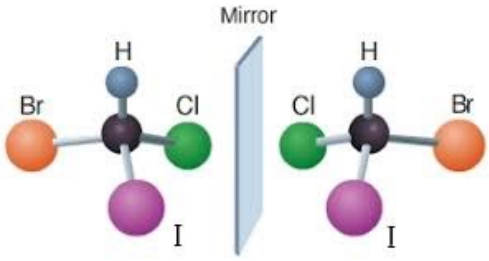
Representation of a three-dimensional molecule as a flat structure. Tetrahedral carbon represented by two crossed lines:

1. **horizontal** line is coming out of the plane of the page (toward you)
2. **vertical** line is going back behind the plane of the paper (away from you)



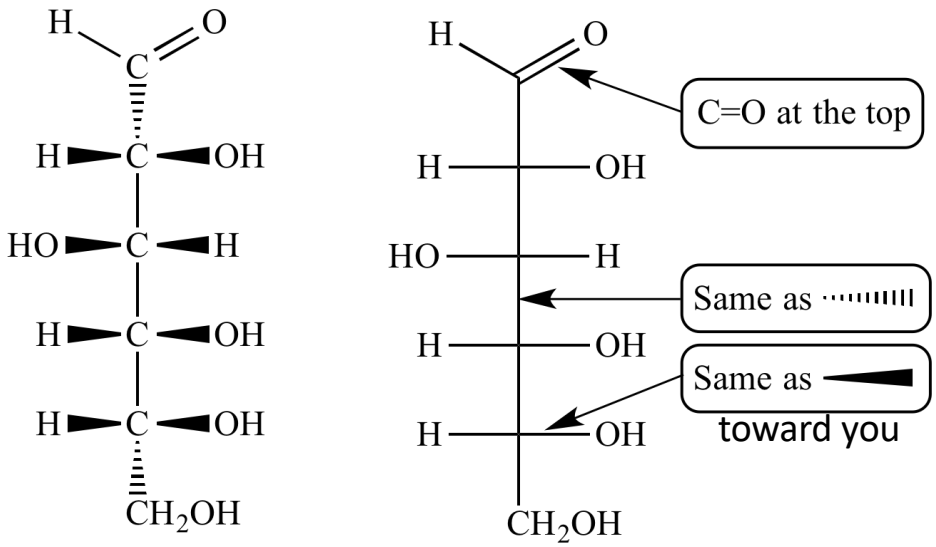


If R1≠R2≠R3≠R4, the center carbon is chiral.



15

Fisher projection of D-glucose



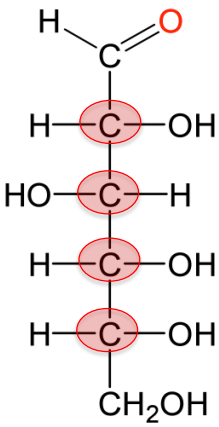
Fisher projection

Image source: http://web.chem.ucla.edu/~harding/IGOC/F/fischer_projection.html

16

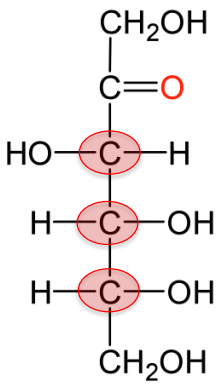
How many chiral carbons?

Aldoses



D-glucose

Ketoses



D-fructose

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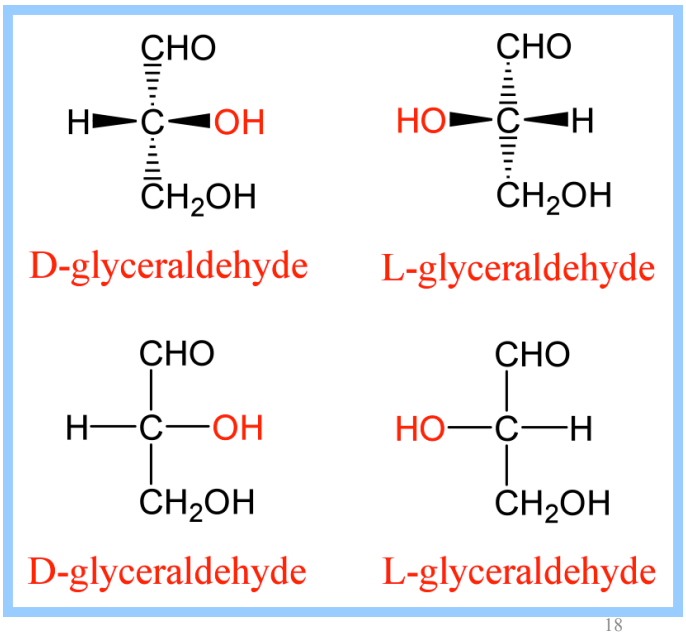
D vs L Designation

D (dextro-) & L (levo-) designations are based on the configuration about the single asymmetric C in glyceraldehyde.

The lower representations are Fischer Projections.



18

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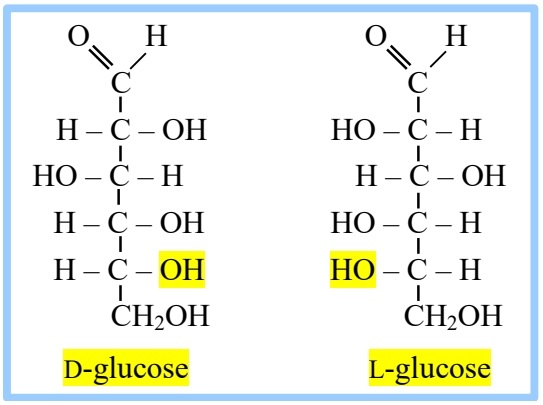
Sugar Nomenclature



For sugars with more than one chiral center, **D** or **L** refers to the asymmetric **C** farthest from the aldehyde or keto group.

Most naturally occurring sugars are D isomers.

19

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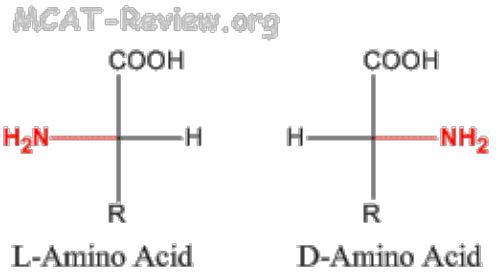
L-amino acids are the more common in nature, and are the type found in proteins.

D-amino acids are less common in nature, and are never found in proteins.

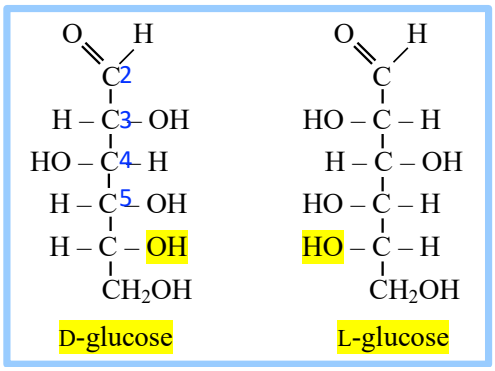


Sugar Nomenclature

D & L sugars are **mirror images** of one another.

They have the same name, e.g., D-glucose & L-glucose.

Other stereoisomers have unique names, e.g., glucose, mannose, galactose, etc.

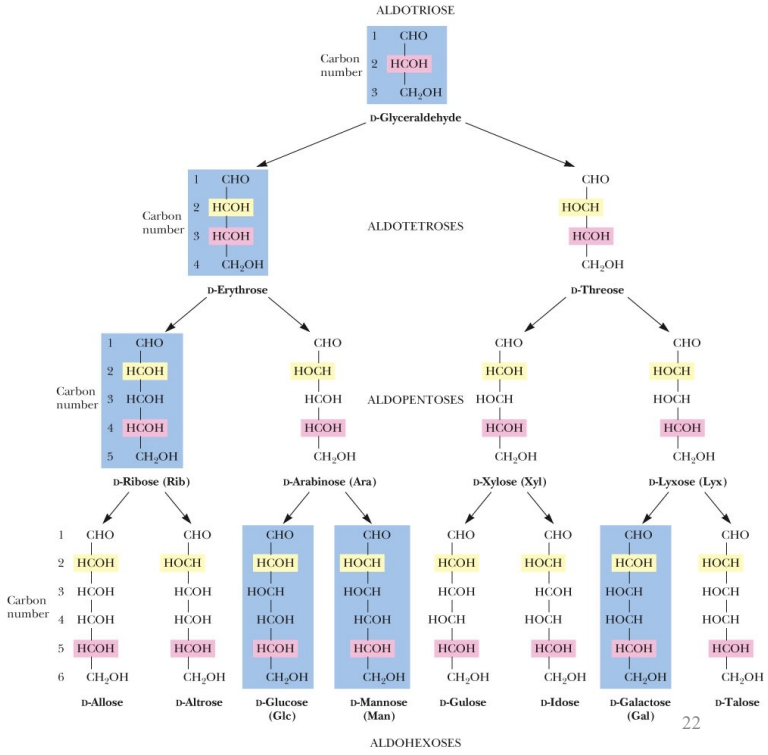


For aldose, the number of stereoisomers is 2^{n-2}, where (n-2) is the number of chiral centers. (n:C的總數)

The 6-C aldoses (**aldohexose**) have 4 chiral centers. Thus there are 16 stereoisomers (8 D-sugars and 8 L-sugars).

D-Aldoses

The structures and stereochemical relationships of D-aldoses with three to six carbons.



22

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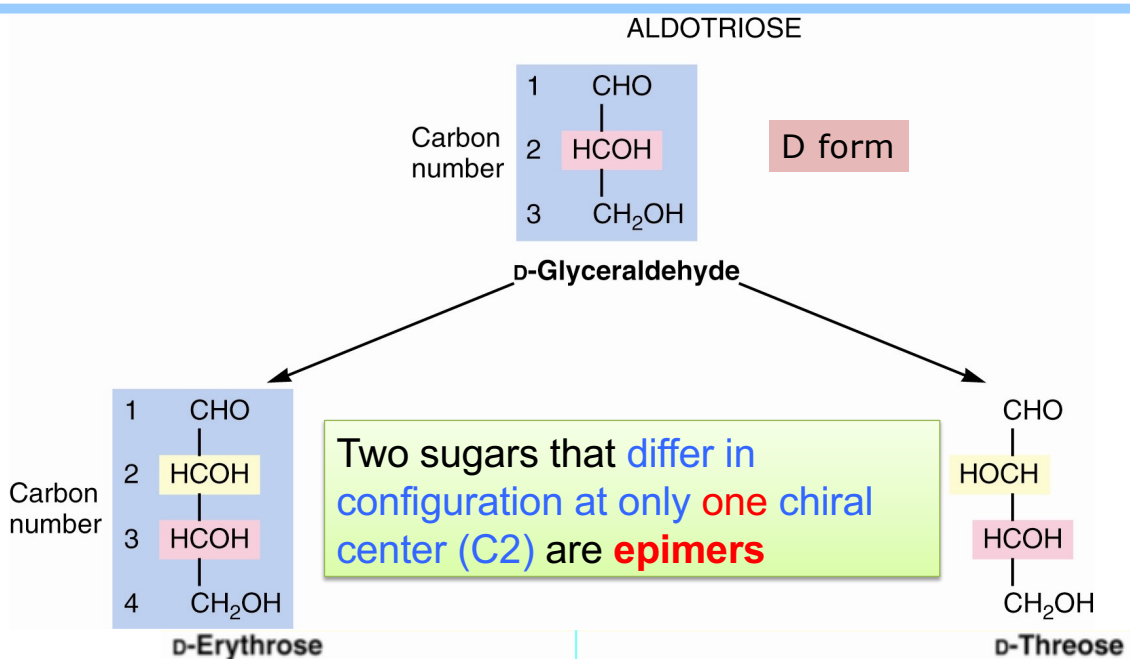
Structure and Chemistry of Monosaccharides



Two sugars that differ in configuration at only one chiral center (C2) are **epimers**

23

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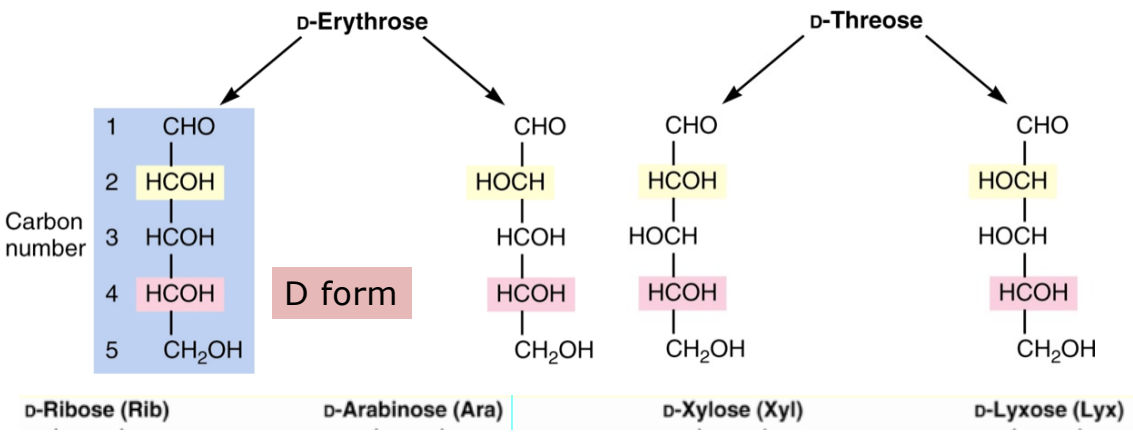
Structure and Chemistry of Aldopentoses

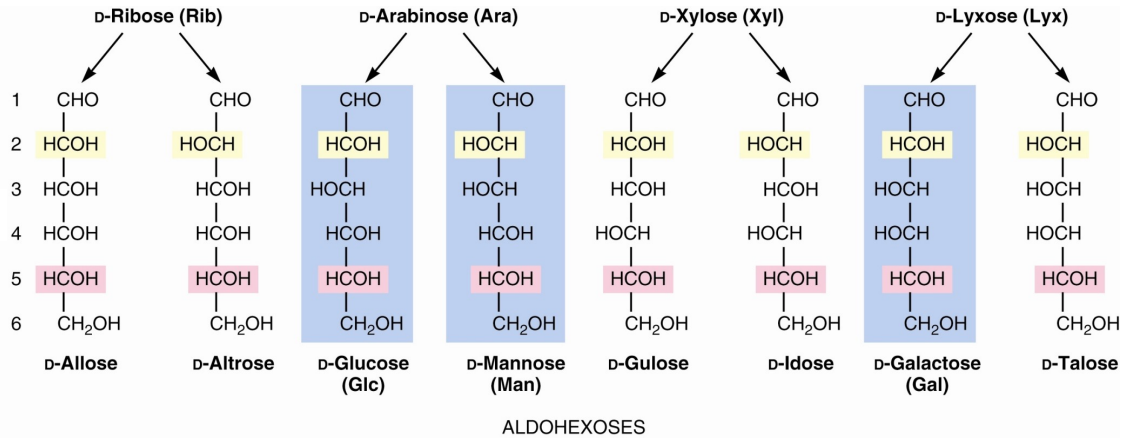


核糖　　阿拉伯糖　　木糖

24

24

Structure and Chemistry of Aldohexoses



葡萄糖　　半乳糖

26

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Stereochemistry Review I

- D,L designation refers to the configuration of the highest-numbered asymmetric center
- D,L optical activity only refers the stereocenter of interest back to D- and L-glyceraldehyde
- All structures in Figures 7.2 and 7.3 are D-sugars
- **D**-sugars predominate in nature

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Stereochemistry Review II

- Stereoisomers that are nonsuperimposable mirror images of each other are **enantiomers** (鏡像異構物) 全部chiral centers不同
- Pairs of isomers that have opposite configurations at one or more chiral centers but are NOT mirror images are **diastereomers** (非鏡像異構物)部分chiral centers不同
- Any 2 sugars in a row in Figure 7.2 and 7.3 are diastereomers
- Two sugars that differ in configuration at only one chiral center are **epimers** 1個chiral center不同

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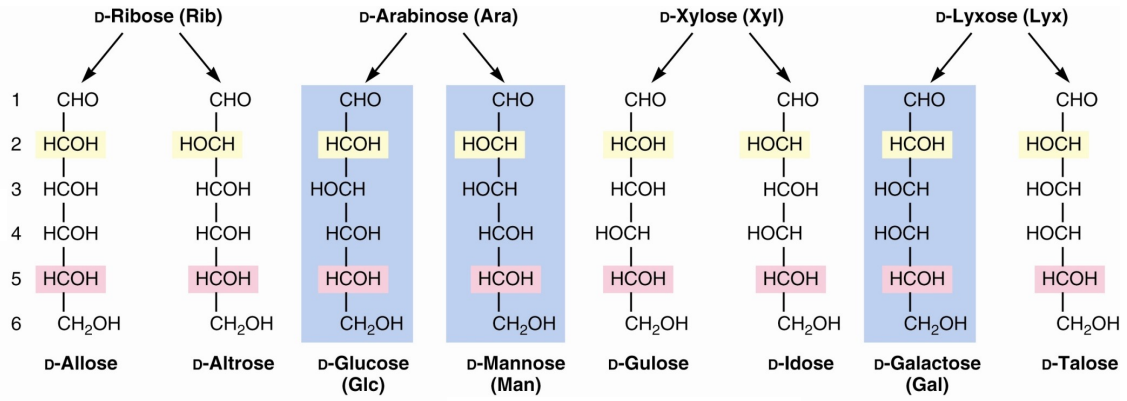
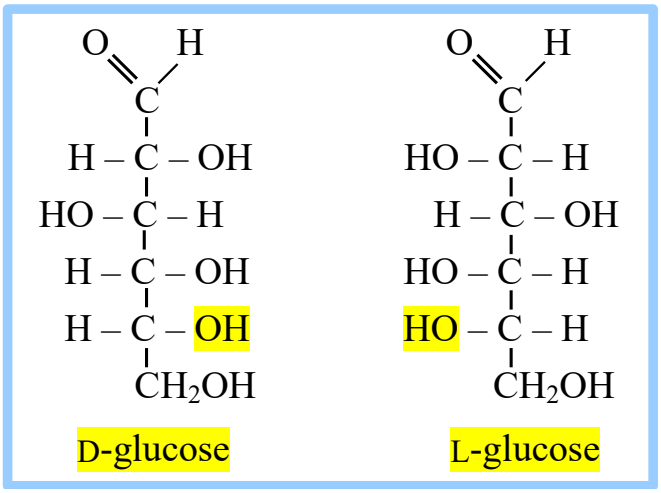
Structure and Chemistry of Aldohexoses



- diastereomers (非鏡像異構物) 部分chiral centers不同
  Any 2 sugars in a row are diastereomers
- epimers 1個chiral center不同
  D-glucose and D-mannose

30

30

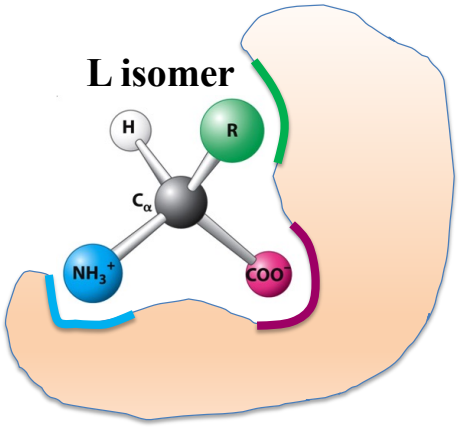


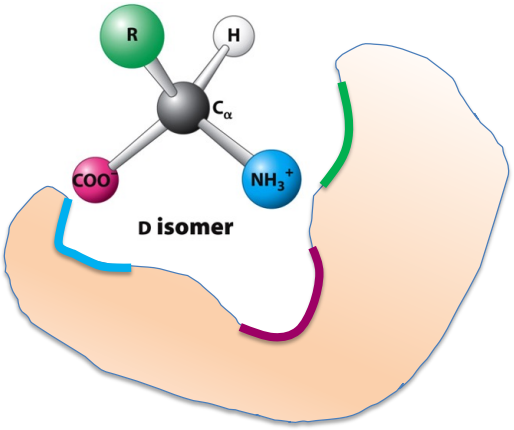
- enantiomers (鏡像異構物) 全部chiral centers不同
  D-glucose and L-glucose

32

32

Stereochemistry is important!





✓Shape
✓ Local chemical environment
→**Strong** interaction

Weak interaction

33

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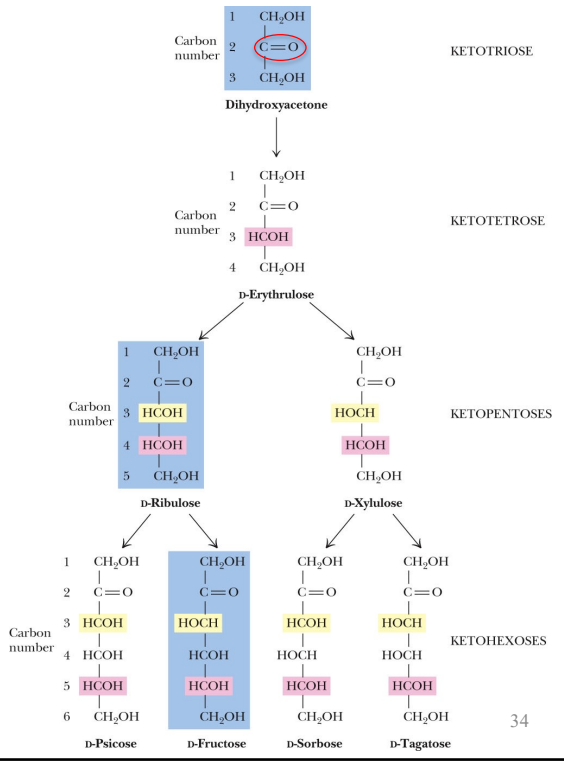
The Ketoses



The structures and stereochemical relationships of D-ketoses with three to six carbons.

34

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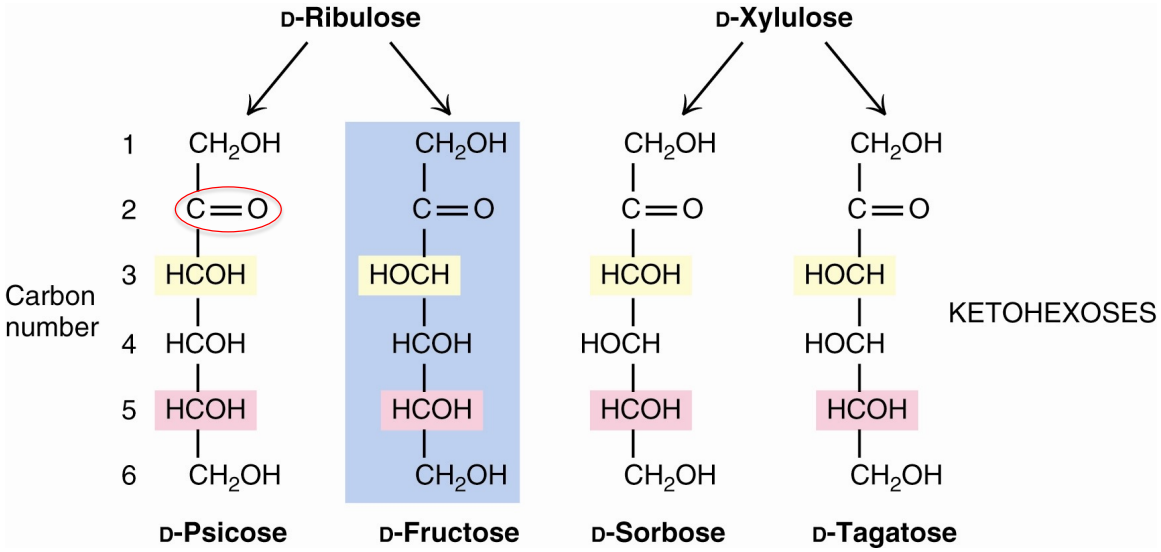
Structure and Chemistry of ketohexoses



Ketoses have **fewer** chiral centers and **fewer** stereoisoformers. ($2^{(n-3)}$, n is the # of carbons)

35

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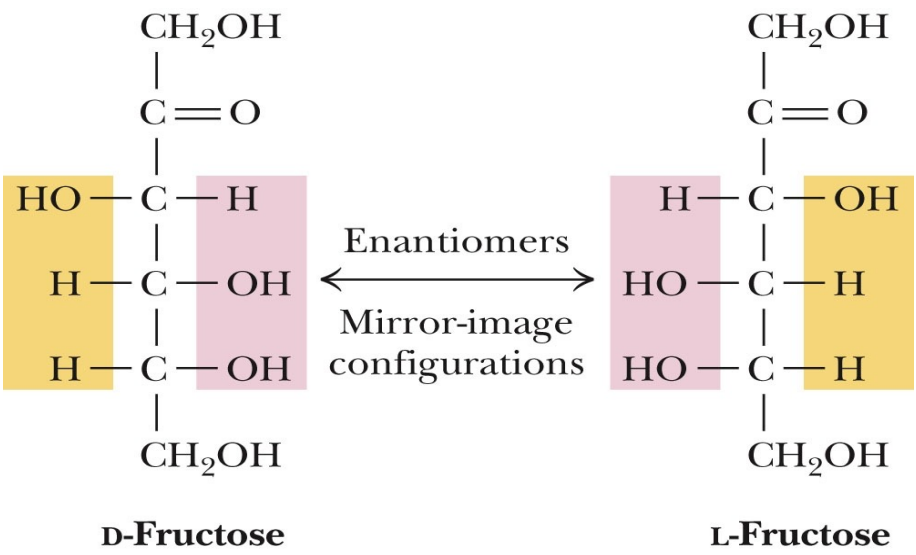
D- and L-Forms of a Monosaccharide are ***Mirror Images*** of Each Other



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Cyclization of monosaccharides

37

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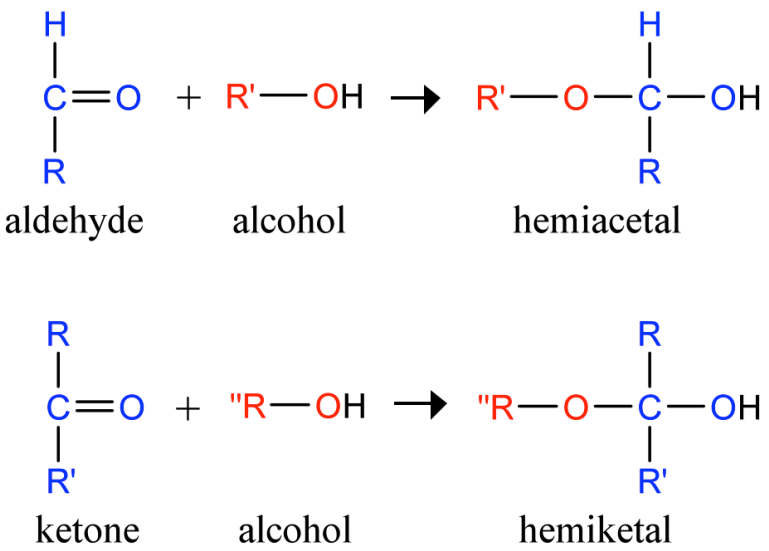
Hemiacetal & hemiketal formation

An aldehyde can react with an alcohol to form a hemiacetal.

A ketone can react with an alcohol to form a hemiketal.



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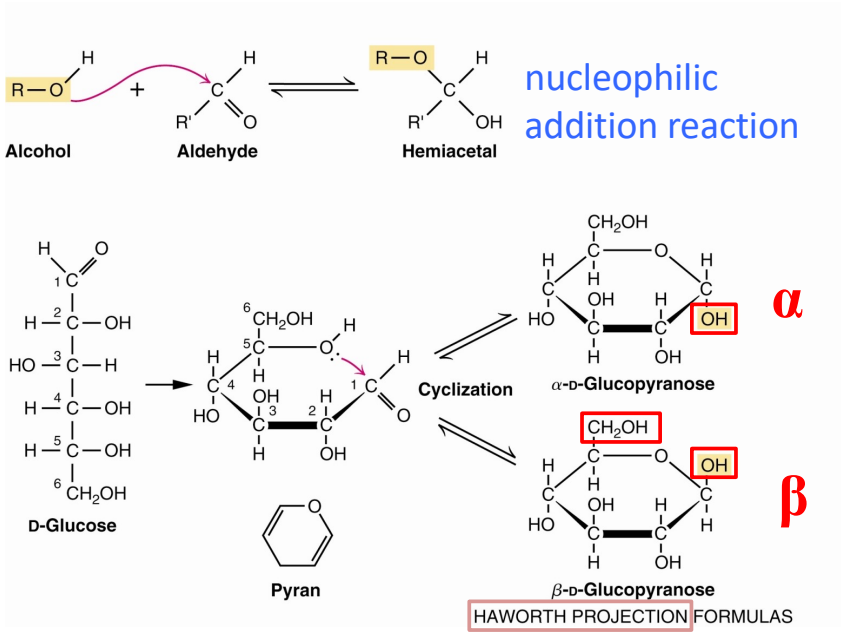
Cyclic monosaccharide structures and anomeric forms

- Glucose (an aldose) can cyclize to form a cyclic **hemiacetal**
- Fructose (a ketose) can cyclize to form a cyclic **hemiketal**
- When hemiacetals and hemiketals are formed, the carbonyl carbon atom becomes an asymmetric center
- Isomers of monosaccharides that differ only in their configuration about that asymmetric carbon are called **anomers**

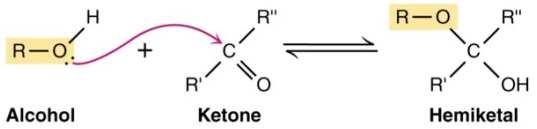
39

The linear form of **D-glucose** undergoes an intramolecular reaction to form a cyclic **hemiacetal** – with an **anomeric** carbon.

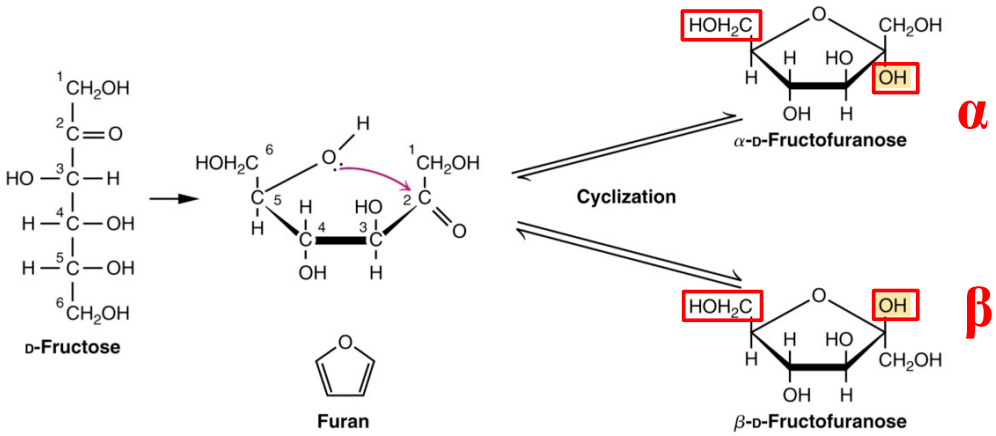


40

The linear form of **D-fructose** undergoes an intramolecular reaction to form a cyclic **hemiketal** – with an **anomeric** carbon.



nucleophilic addition reaction



HAWORTH PROJECTION FORMULAS

41

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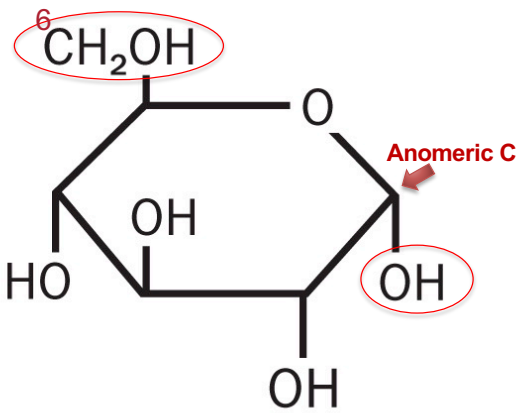
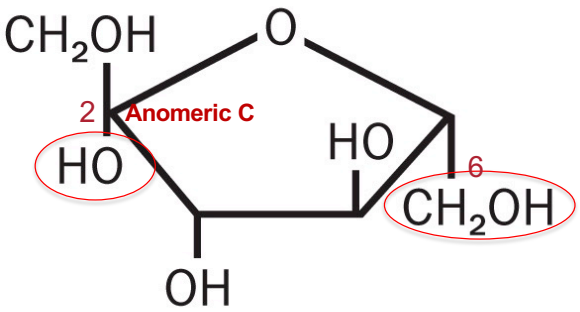
Glucose



Alpha-glucose

Fructose

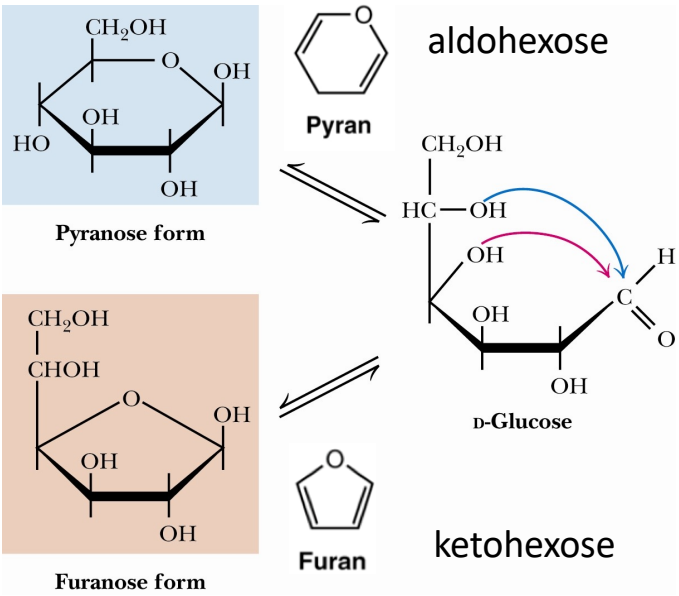


beta-fructose

42

42

Simple sugars can cyclize in two ways, forming either furanose or pyranose structures.



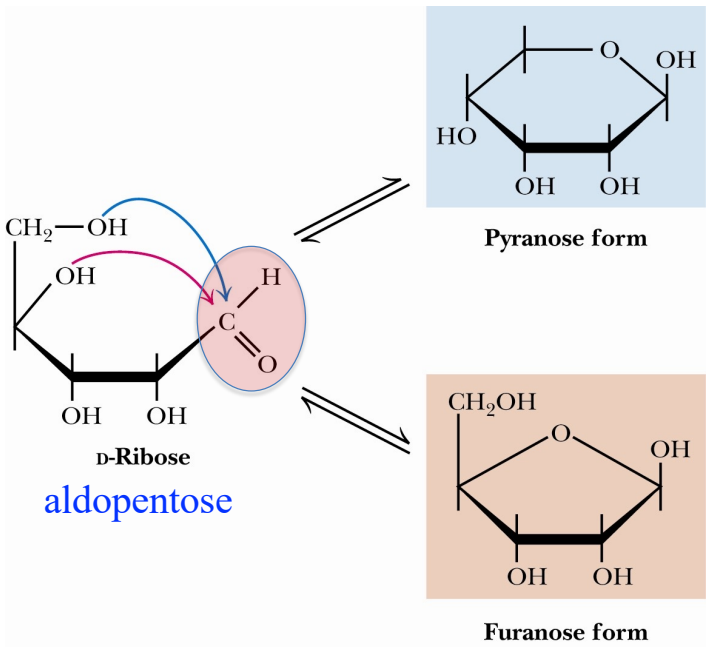
In general, **aldohexoses** prefer the **pyranose** form, while ketohexoses prefer the furanose form.

5- and 6-membered rings are stable. 3, 4, 7-membered rings are not due to a high ring strain.

43

43

Simple sugars can cyclize in two ways, forming either furanose or pyranose structures.



For ribose, furanose is the major form in nature

44

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Cyclic monosaccharide structures and anomeric forms

- Cyclic monosaccharides that differ only in the carbonyl C are called **anomers**

A simpler rule:

In the Haworth drawing

- **α**: C6 CH2OH and OH at the anomeric carbon are on the opposite side of the ring
- **β**:C6 CH2OH and OH at the anomeric carbon are on the same side of the ring

45

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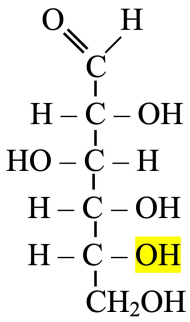
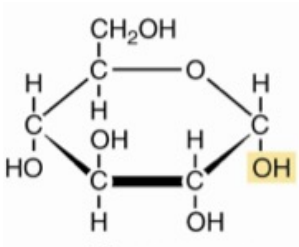
β–D-Glucopyranose is the dominant form of glucose

<<1%



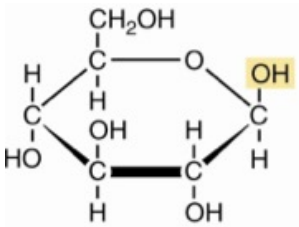
D-glucose

~36%



α–D-Glucopyranose

~64%

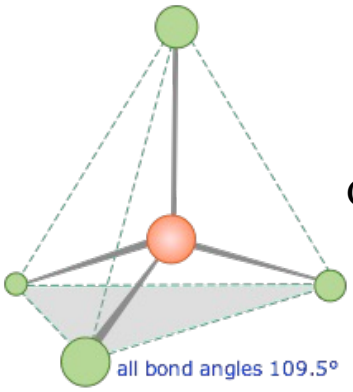


β–D-Glucopyranose

Why??

46

46



C-C-C dihedral angle=109.5°

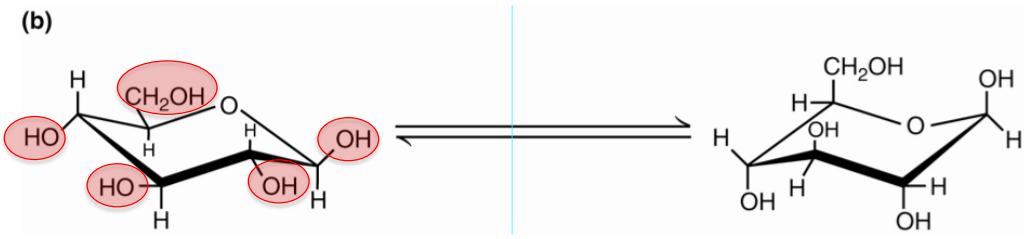
Because of the tetrahedral nature of carbon bonds, pyranose sugars actually assume a "chair" or "boat" configuration.

http://s409yan.blogspot.tw/2009_05_01_archive.html

Chair and boat conformations

- **Bulky** groups are more stable at the **equatorial** position than axial position.
- Chair conformations are more stable than boat conformations.

Two possible chair conformations of β-D-glucose



Note the axial and equatorial substituents.

Of all the D-aldohexoses, β-D-glucose is the only one that can have **all its bulky groups in equatorial positions.**

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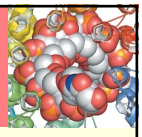
Monosaccharide Derivatives



- A variety of chemical and enzymatic reactions produce derivatives of the simple sugars
- Some of the most common are:
  - Sugar acids
  - Sugar alcohols
  - Deoxy sugars
  - Sugar esters
  - Amino sugars
  - Acetals, ketals, and glycosides

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Monosaccharide Derivatives

Reducing sugars are sugars with free anomeric carbons - they will reduce oxidizing agents, such as peroxide, ferricyanide $[Fe(CN)_6]^{3-}$ and some metals (Cu^{2+} and Ag^{+}).

These redox reactions oxidize the sugar to a **sugar acid**.

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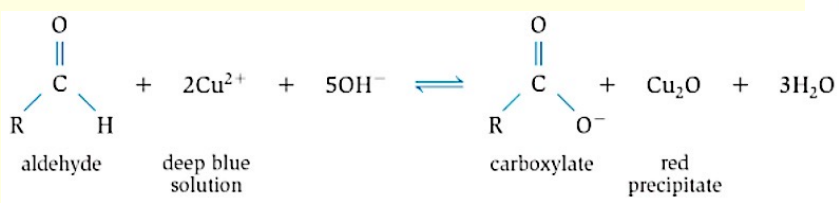
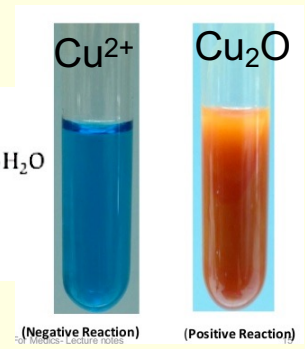
Oxidation of D-**glucose** to **sugar acids**

C1 of aldoses can be selectively oxidized to the carboxylic acid (*aldonic acids*) with Br_2 , Ag(I) (Tollen's test 銀鏡反應), or Cu(II) (Benedict's reagent).

Benedict's Test (本氏液)

Reducing sugars are oxidized by the copper ion in solution to form a carboxylic acid and a reddish precipitate of copper (I) oxide.



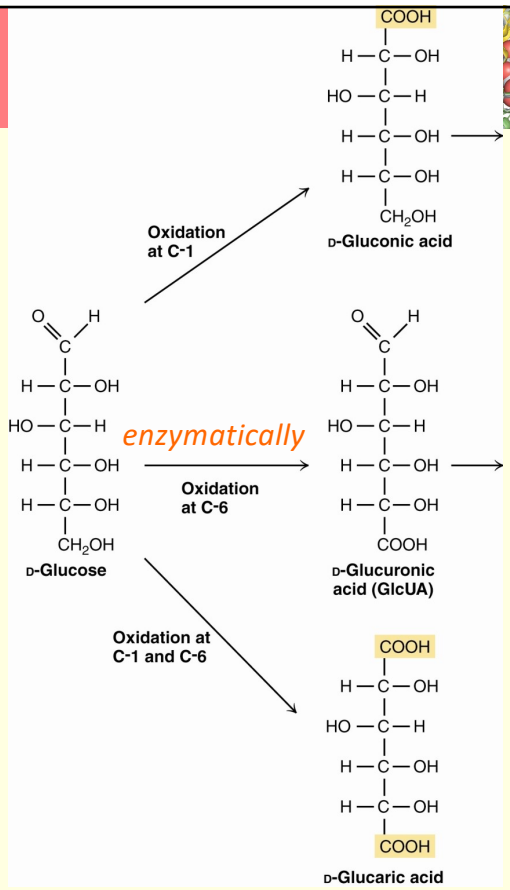


53

53

Reducing sugars:
carbohydrates that can be oxidized to aldonic acids.

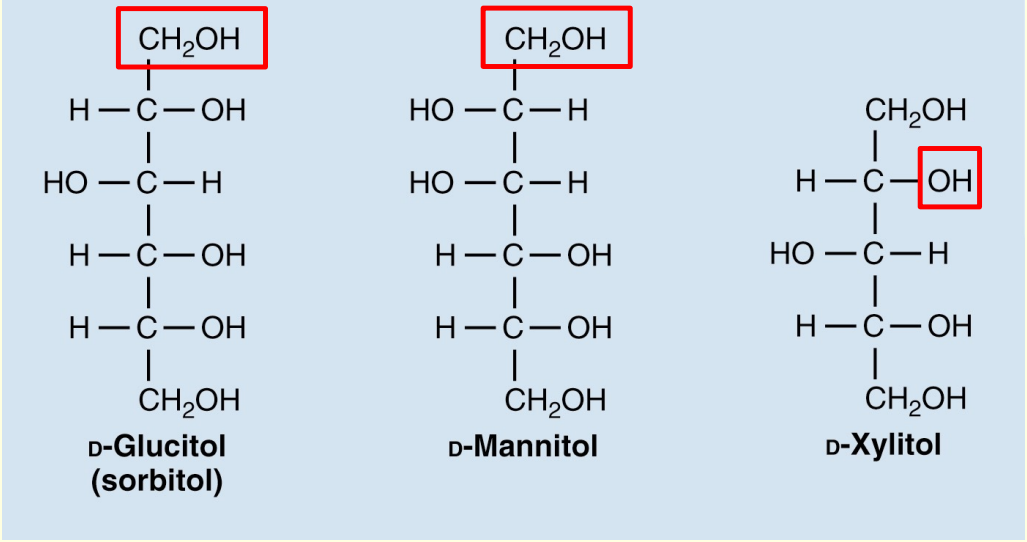
sugar acid - the aldehyde at C1, or OH at C6 is oxidized to a carboxylic acid; e.g., gluconic acid (C1), glucuronic acid (C6), glucaric acid (C1+C6)



Glucose is a reducing sugar - so these redox reactions were the basis for diagnostic tests for blood sugar in the old days.

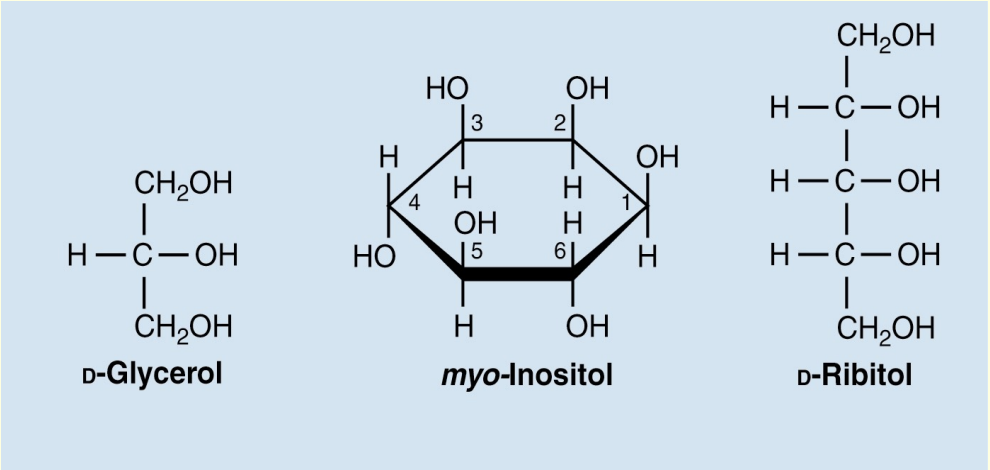
L-Xylulose accumulation in the urine in patients with pentosuria （戊醣尿症）, due to a deficiency in L-xylulose reductase. Since L-xylulose is a reducing sugar like D-glucose, pentosuria patients had been wrongly diagnosed in the past to be diabetic.

Sugar alcohols are formed by mild reduction of sugars



(from glucose) (from mannose) (from xylose)

Sugar Alcohols



Structures of some sugar alcohols.
Glycerol and myo-inositol are components of lipids.

Sugar Alcohols are Sweeteners

Sugar alcohols such as sorbitol, mannitol, and xylitol sweeten many "sugarless" gums and candies.

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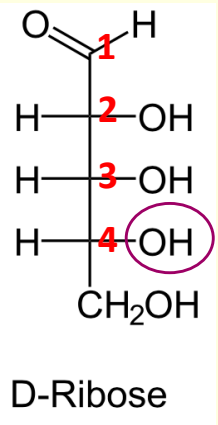
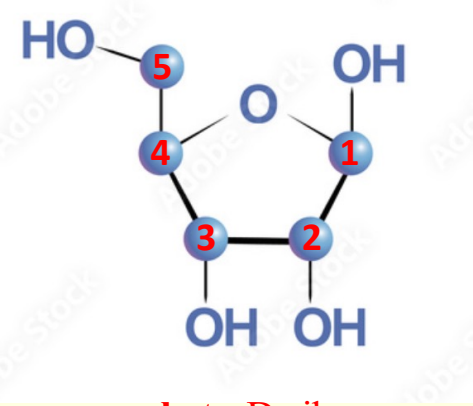
D-ribose

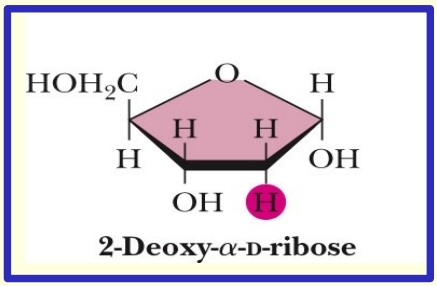




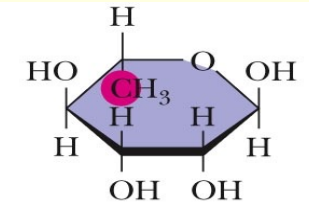
beta-D-ribose

Deoxy Sugars

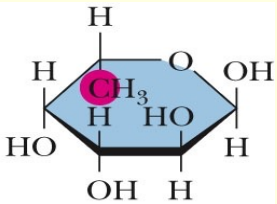
- Deoxy sugars are monosaccharides with one or more **hydroxyl groups** replaced by hydrogens
- **2-Deoxy-D-ribose** is a constituent of **DNA**
- Rhamnose is a component of ouabain, a toxic "cardiac glycoside"
- Fucose is a component of some cell walls



2-Deoxy-α-D-ribose



α-L-Rhamnose (Rha)



α-L-Fucose (Fuc)

Hydrogen and carbon atoms in red are "deoxy" positions.

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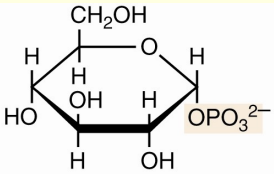
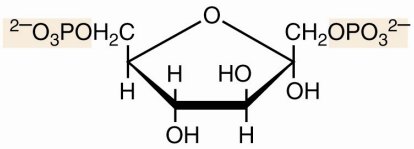
Sugar Esters

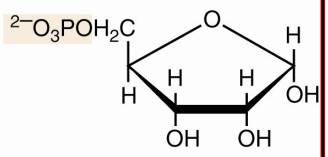
- **Phosphate esters** of glucose, fructose, and other monosaccharides are important metabolic intermediates
- The ribose moiety of nucleotides such as ATP and GTP is phosphorylated at the **5' -position.**



α-D-Glucose-1-phosphate



α-D-Fructose-1,6-bisphosphate



α-D-Ribose-5-phosphate

Several sugar esters important in **metabolism**.

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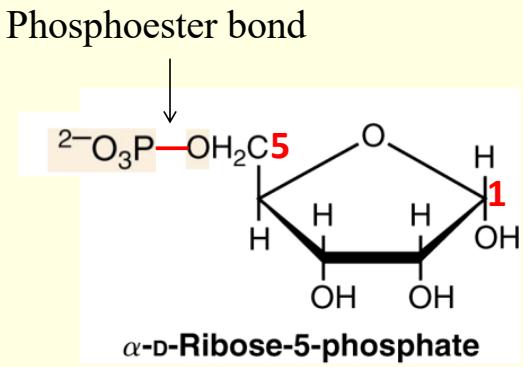
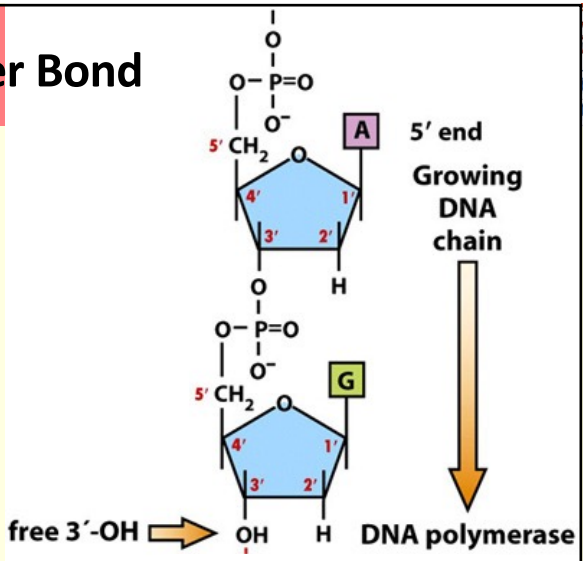
Phosphate esters and nucleotides

D-Ribose-5-phosphate is the precursor for the synthesis of nucleotides.



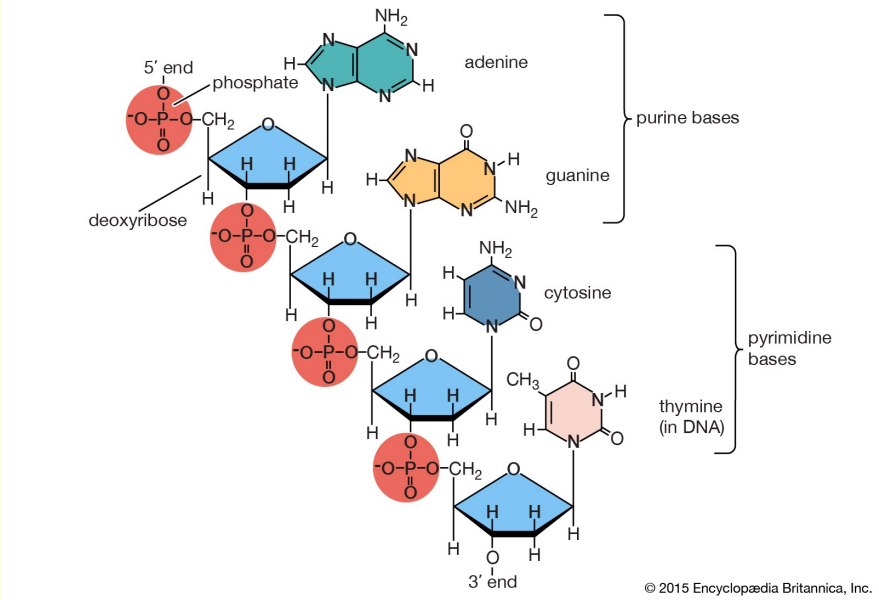
Formation of Phosphodiester Bond

DNA polymerase catalyzes formation of **phosphodiester bond** between the free 3'-OH group of new chain & the alpha phosphate of the dNTP (in this case dTTP)



http://rowdysites.msudenver.edu/~churchcy/BIO3600/bio3600_images/phosphodiester_bonds.htm

Structure of a single-stranded DNA



Phosphodiester bonds link dNTPs into a single-stranded DNA.

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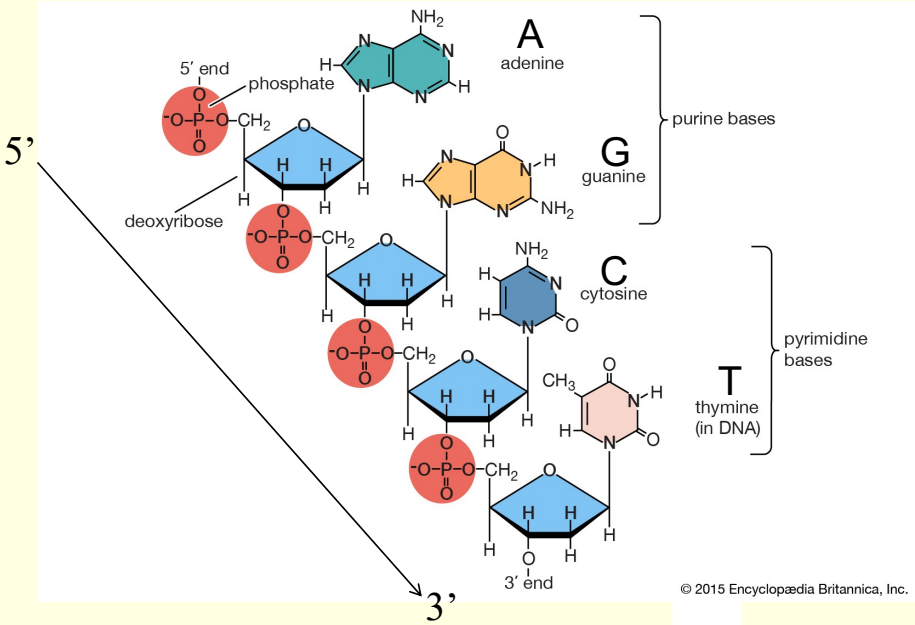
Structure of a single-stranded DNA



DNA sequence reads from the 5’ to 3’ end. $^{5'}$AGCT $^{3'}$

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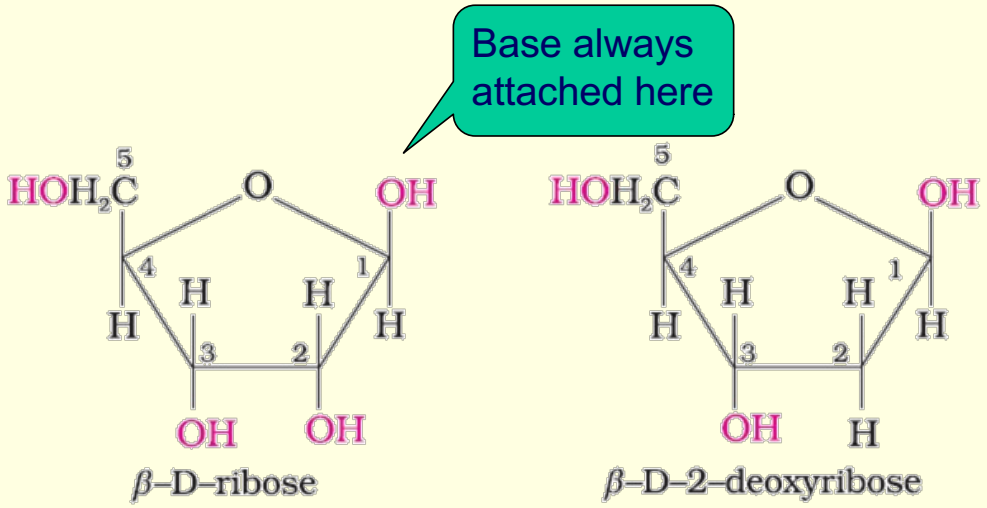
β-configuration of sugars in RNA & DNA



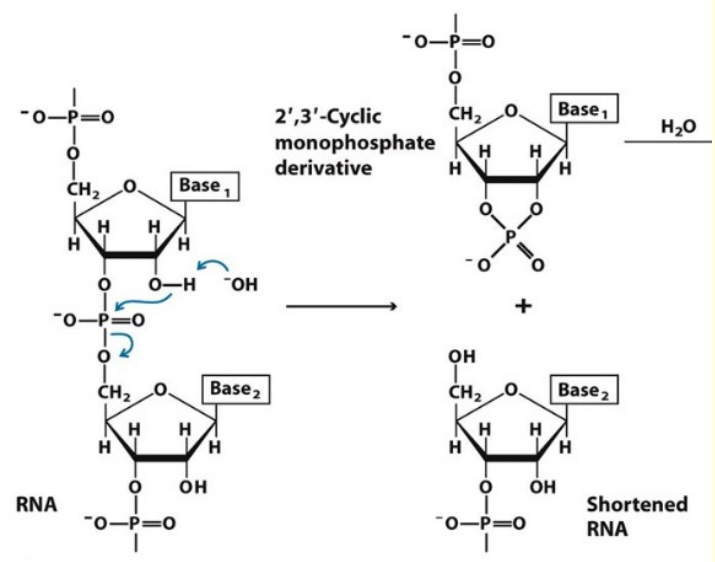
RNA疫苗為什麼需要冷鏈？

廠牌	國家	保存溫度
Pfizer BioNTech (BNT輝瑞疫苗)	美國 / 德國	-80~-60°C
Moderna (莫德納疫苗)	美國	-25~-15°C
AstraZeneca (AZ疫苗)	英國	2~8°C
Johnson & Johnson (嬌生疫苗)	美國	2~8°C
高端疫苗	台灣	2~8°C
聯亞疫苗	台灣	2~8°C

參考資料：

衛生福利部疾病管制署-疫苗簡介

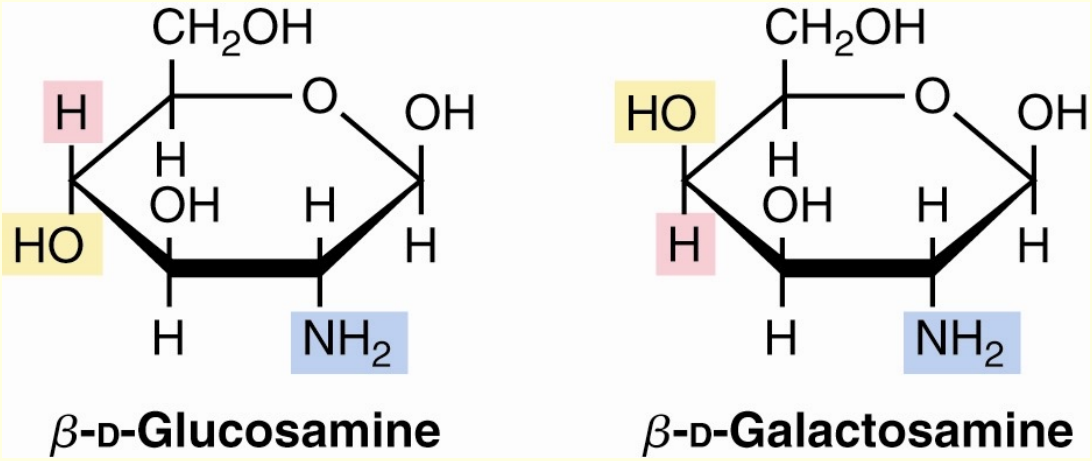
RNA is hydrolyzed under **basic** conditions



http://biology.stackexchange.com/questions/24786/why-deoxyribose-for-dna-and-ribose-for-rna

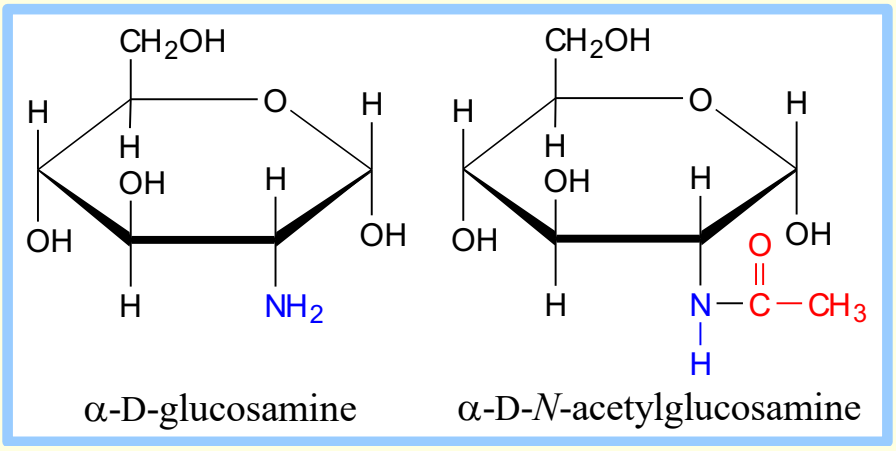
Amino Sugars

- Sugars with an **amino** group at **C-2** are **amino sugars**.
- They are found in many oligosaccharides and polysaccharides.



葡萄糖胺

Sugar derivatives- amino sugar

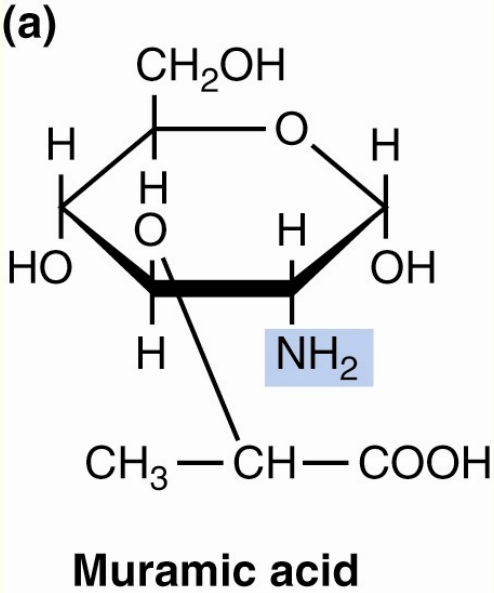


The amino group may be acetylated, as in *N*-acetylglucosamine.

Muramic acid

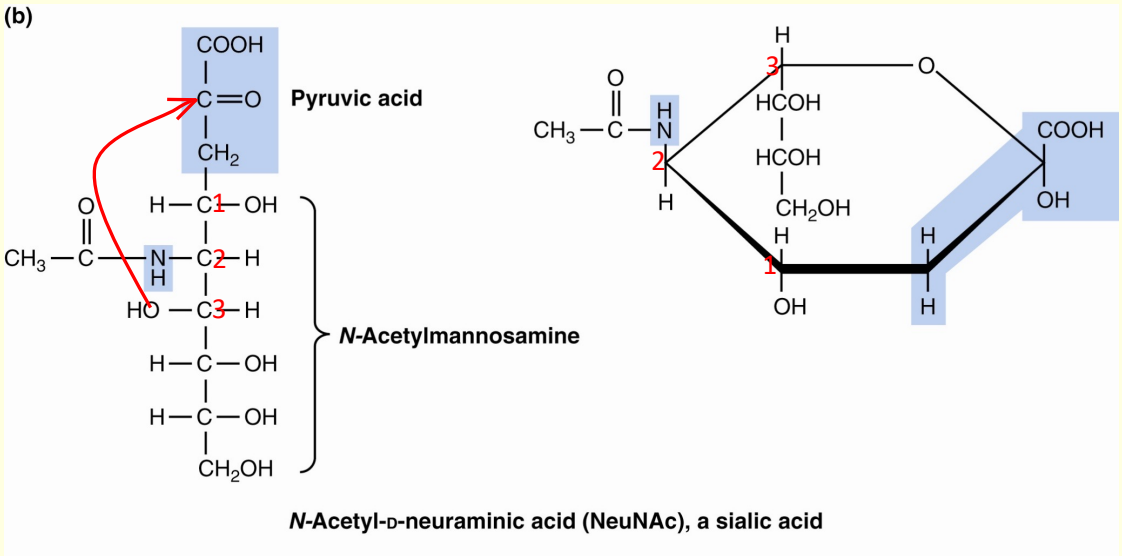
Muramic acid is a component of the polysaccharides of cell membranes of higher organisms and also bacterial cell walls.

Muramic acid is a glucosamine linked to a 3-carbon acid at C-3. (*Murus* is Latin for "wall".)

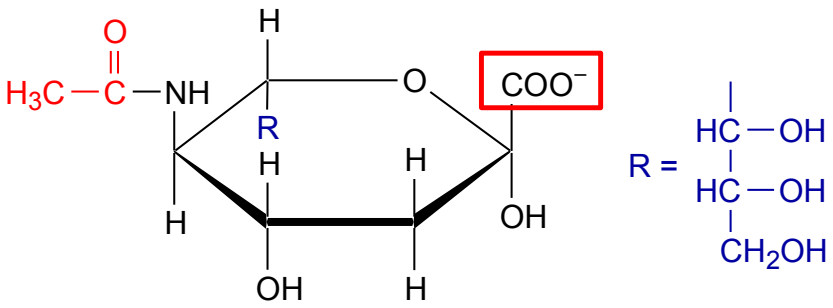


Sialic acids

The N-acetyl and N-glycolyl derivatives of neuraminic acid (first isolated from neural tissues) are known as **sialic acids**.



- **Sialic acid** is a generic term for a family of derivatives of neuraminic acid with a nine-carbon backbone.
- It is also the name for the most common member of this group, *N*-acetylneuraminic acid (Neu5Ac, pKa 2.6).



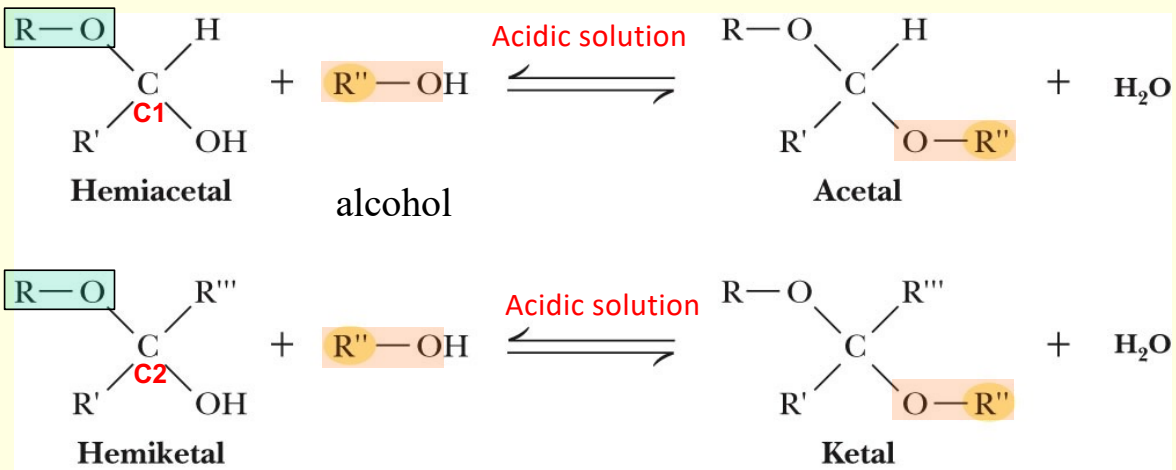
N-acetylneuraminate (sialic acid)

N-acetylneuraminic acid, also called **sialic acid**, is often found as a **terminal residue** of oligosaccharide chains of glycoproteins.

Sialic acid imparts **negative charge** to glycoproteins, because its carboxyl group tends to dissociate a proton at physiological pH, as shown here.

Reaction for polysaccharide formation

Acetals and Ketals



Acetals and ketals can be formed from hemiacetals and hemiketals, respectively. These reactions are examples of *dehydration syntheses*. In this sense, they are similar to the reactions of amino acids to form peptides.

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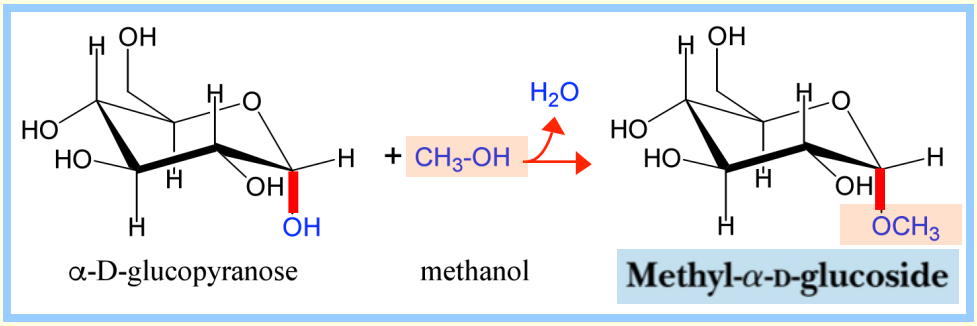
Glycosidic Bonds

E.g., methanol reacts with the **anomeric OH** on glucose to form methyl glucoside (methyl-glucopyranose).



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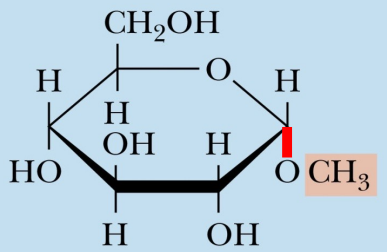
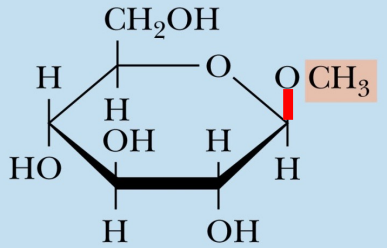
Glycosides

The pyranose and furanose forms of monosaccharides react with alcohols in dehydration synthesis reactions to form **glycosides**, **with retention of the α- or β-configuration** at the anomeric carbon.

The new bond formed between the anomeric carbon and the oxygen of the alcohol is called a **glycosidic bond**.



Methyl-α-D-glucoside



Methyl-β-D-glucoside

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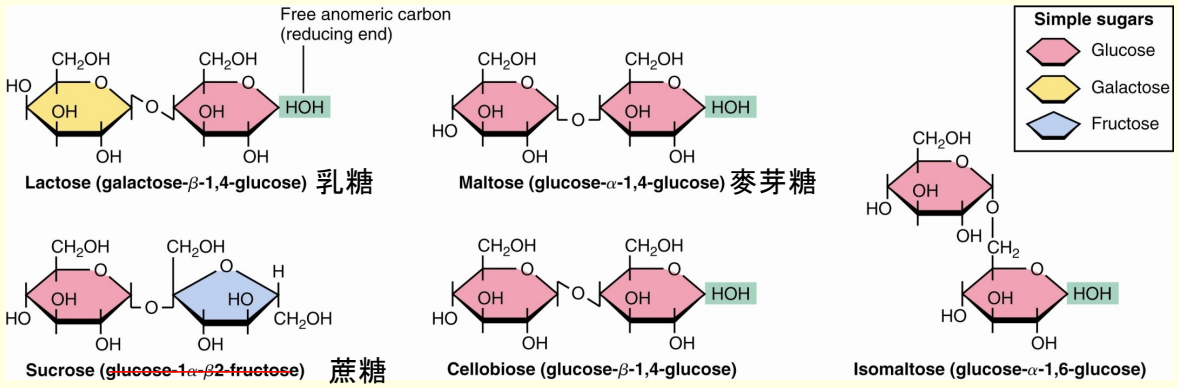
Disaccharides

What are the Structure and Chemistry of Oligosaccharides?

- Two monosaccharides linked by a **glycosidic bond**
- Each unit in an oligosaccharide is termed a residue
- Each of these (except for sucrose) possesses **one free unsubstituted anomeric carbon**, and is thus a **reducing sugar**
- Sucrose is not a reducing sugar – it does not have a free anomeric carbon

Structure and Chemistry of **Disaccharides**



Glucose-α-1,β-2-fructose

not a reducing sugar

Note the configurations at the anomeric carbons.
How are the residues linked?
Glucose residues are abundant in oligosaccharides.

7.3 What Are the Structure and Chemistry of Oligosaccharides?

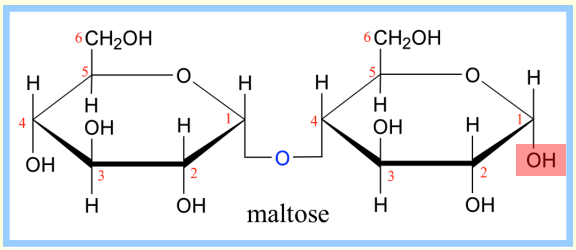
Know the important features

- Be able to identify anomeric carbons and reducing and nonreducing ends
- Note carefully the nomenclature of links. Be able to recognize alpha(1,4), beta(1,4) linkages, etc., in structures.

Maltose

- A cleavage product of starch, is a disaccharide with an α(1→ 4) glycosidic link between C1-C4 OH of 2 glucoses.
- It is the α anomer.
- Hydrolyzed by **maltase**.

Non-reducing end

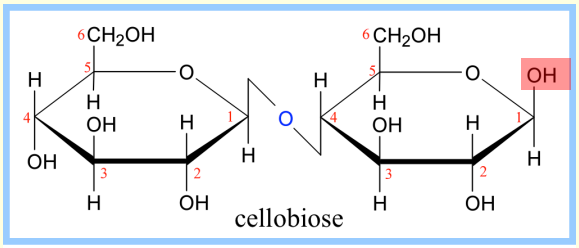


reducing end

Free anomeric carbon

Cellobiose

- a product of cellulose breakdown, is the otherwise equivalent β anomer (O on C1 points up).
- The **β(1→ 4)** glycosidic linkage is represented as a zig-zag.
- Hydrolyzed by **cellobiase**.



Free anomeric carbon
Reducing end

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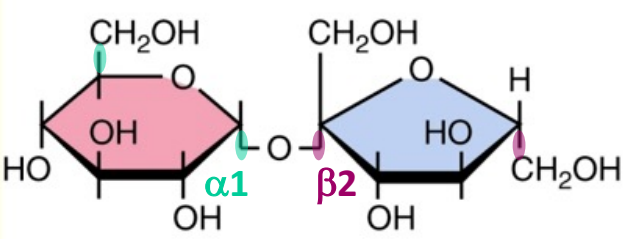
Other **disaccharides** include:

- **Sucrose**, common table sugar, has a glycosidic bond linking the anomeric hydroxyls of glucose & fructose.

Because the configuration at the anomeric C of glucose is α (O points down from ring), the linkage is **α1→β2**.

Hydrolyzed by **invertase**, also called **sucrase**.



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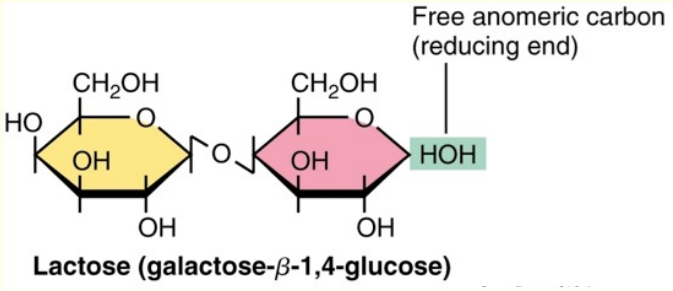
Other **disaccharides** include:

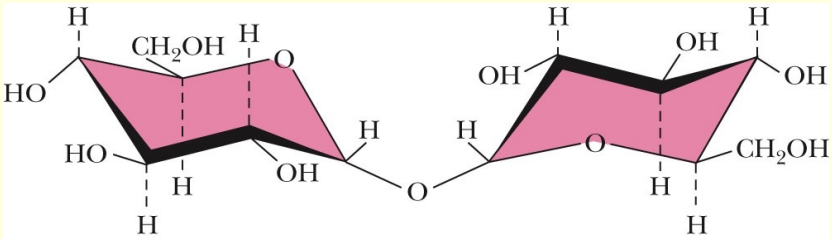
- **Lactose**, milk sugar, is composed of galactose & glucose, with β(1→4) linkage from the anomeric OH of galactose.
- Lactose is hydrolyzed by **lactase**.

Lactose intolerance (乳糖不耐症,小腸中缺乏lactase)



Trehalose – A Natural Protectant for Bugs

- Insects use an open circulatory system to circulate "hemolymph" (insect blood). The "blood sugar" is **trehalose**, an unusual, nonreducing disaccharide.
- Trehalose may act as a natural cryoprotectant, protecting the insect from damage due to freezing temperatures.

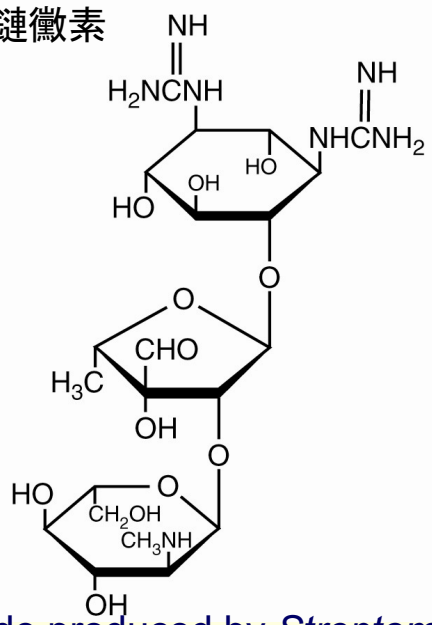


A Variety of Higher Oligosaccharides Occur in Nature

- First antibiotic against tuberculosis (肺結核)
- Inhibit protein synthesis
- Aminoglucoside

Streptomycin (a broad-spectrum antibiotic)

鏈黴素



Streptomycin is an oligosaccharide produced by *Streptomyces griseus.*

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Polysaccharides
(also called glycans)

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What Are the Structure and Chemistry of **Polysaccharides**?

Functions: storage, structure, recognition

- **Homopolysaccharide** – a polysaccharide that contains only one kind of monosaccharide
- **Heteropolysaccharide** – a polysaccharide made of several monosaccharides
- Starch and glycogen are storage molecules
- Chitin and cellulose are structural molecules
- Cell surface polysaccharides are recognition molecules

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Starch

A plant storage polysaccharide

One large polymer has a much lower osmotic pressure than a bunch of monomeric molecules.

- A polymer of glucose
- Two forms: amylose and amylopectin
- Most starch is 10-30% amylose and 70-90% amylopectin
- Branches in amylopectin every 12-30 residues
- Amylose has alpha(1→4) links, one reducing end
- The branches in amylopectin are α(1→6).

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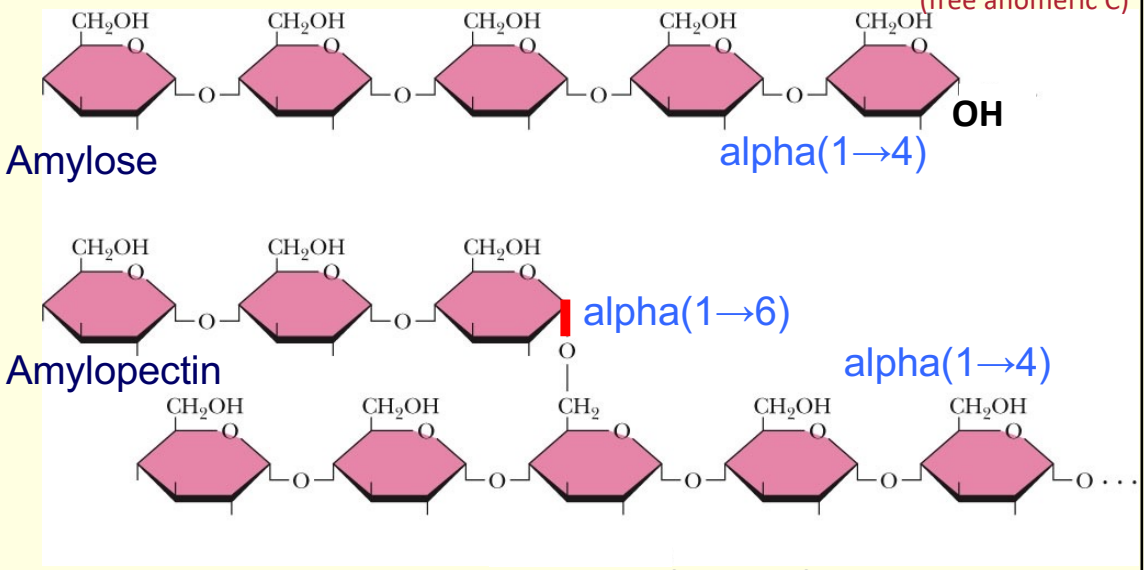
Amylose and Amylopectin are energy storage molecules in plants



- Amylose and amylopectin are two forms of starch.
- Amylopectin is highly branched, with branches occurring every 12 to 30 residues.

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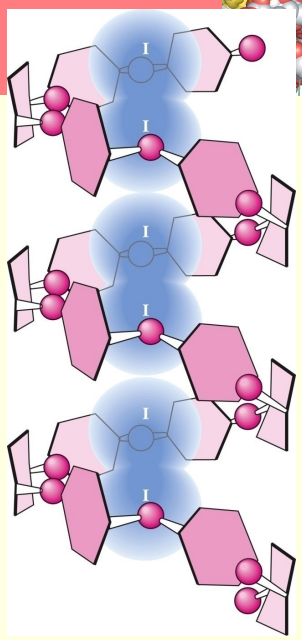
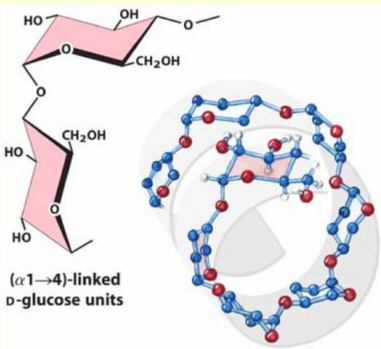
The Structure of Amylose

- Amylose is poorly soluble in water, but forms micellar suspensions
- In these suspensions, amylose is helical
- Iodine (I_2) fits into the helices to produce a blue color





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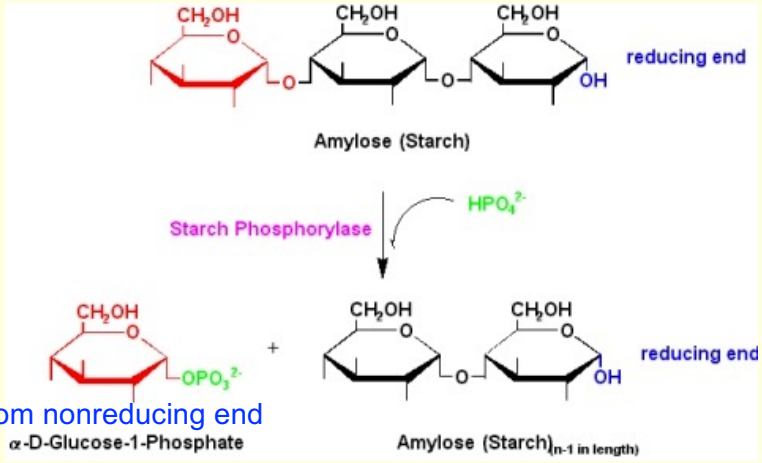
The Phosphorylase Reaction Releases Glucose Units for Metabolic Energy



- The **starch phosphorylase** reaction cleaves glucose residues from amylose, producing **α-D-glucose-1-phosphate**, an energy source for the organism.
- Phosphorylases are enzymes that catalyze the addition of a phosphate group from an inorganic phosphate to an acceptor.

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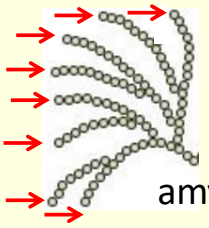
Why Branching in Starch?

Consider the phosphorylase reaction

- Phosphorylase releases **glucose-1-P** products from the amylose or amylopectin chains
- The more branches, the more sites for phosphorylase attack
- Branches in amylopectin provide a mechanism for quickly releasing glucose units for energy metabolism

amylose



amylopectin

100

100

米食、升糖指數(GI)

林俐岑營養師的小天地

名稱	直鏈澱粉(%)	製作成品	升糖指數
在來米(秈米)	20-30%	碗粿、蘿蔔糕	稍低
蓬萊米(粳米)	15-20%	一般米飯	中
糯米	6%以下	湯圓、麻糬、油飯、粽子、傳統飯糰等	高

支鏈多，分解快，GI值較高

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Glycogen

The glucose storage device in animals

- Glycogen constitutes up to 10% of liver mass and 1-2% of muscle mass
- Glycogen is stored energy for the organism
- Only difference from amylopectin: even more highly branched
- Alpha(1,6) branches every 8-12 residues
- Like amylopectin, glycogen gives a red-violet color with iodine

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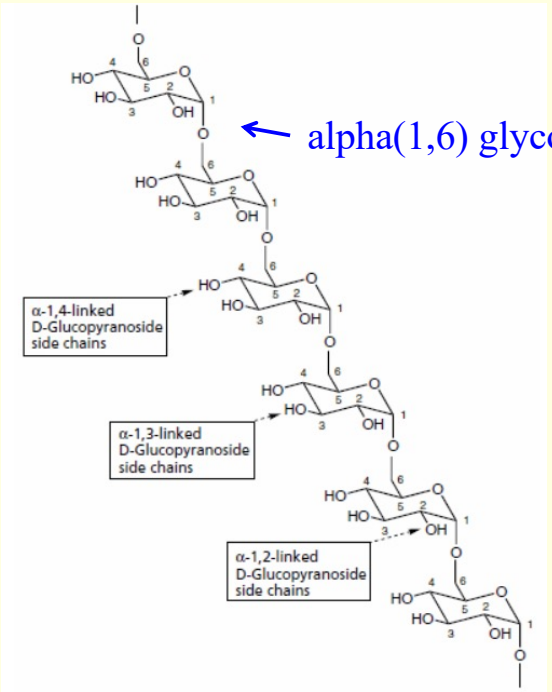
Dextrans

Yeast and bacteria make dextran as a storage polysaccharide.

A small but significant difference from starch and glycogen

- If you change the main linkages between glucose from alpha(1,4) to alpha(1,6), you get a new family of polysaccharides - dextrans
- Branches can be alpha-(1,2), (1,3), or (1,4)

Structure of Dextran



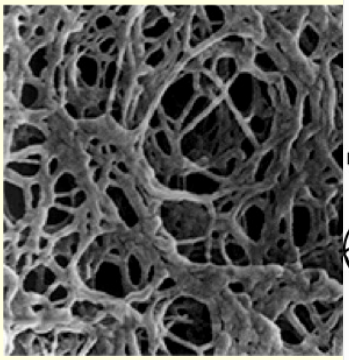
Roles for Dextrans

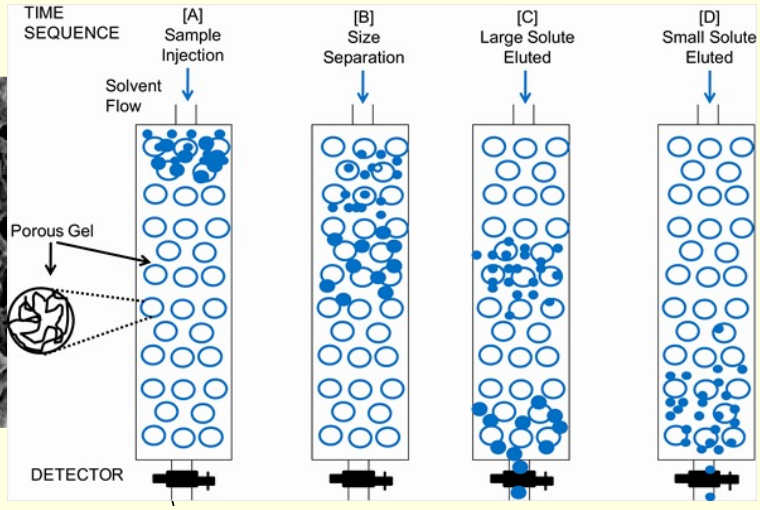
- Dextrans formed by bacteria are components of dental plaque
- Dextrans in plaque presumably provide protection for oral bacteria

- Cross-linked dextrans are used as "Sephadex" gels in column chromatography
- These gels, used to separate biomolecules on the basis of size, are up to 98% water!

Gel filtration (size exclusion) chromatography

- 一種將溶液中蛋白質分子按其大小(在某些情況下為分子量)分開的實驗技術
- 可以用於純化蛋白質





Structural Polysaccharides: cellulose

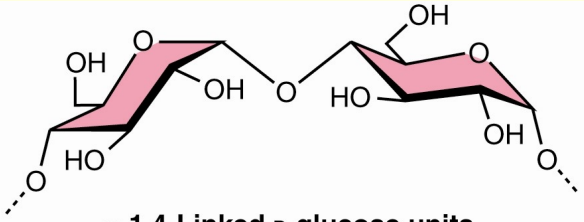
- The composition of structural polysaccharides is similar to storage polysaccharides
- But small structural differences greatly influence properties
- Starch and glycogen linkages consist primarily of α(1→4) linkages.
- Cellulose consists of **β(1→4)** linkages

Cellulose Provides Physical Structure and Strength to Plants

- Cellulose is a structural polysaccharide
- It is the most abundant natural polymer in the world
- It is found in the cell walls of nearly all plants
- The wood and bark of trees are insoluble, highly organized structures formed from cellulose and lignin
- Cotton is almost pure cellulose
- Cotton acetates, made from the action of acetic anhydride on cellulose, are used in dresses, lingerie, and other clothing

The Structural Difference Between Amylose and Cellulose

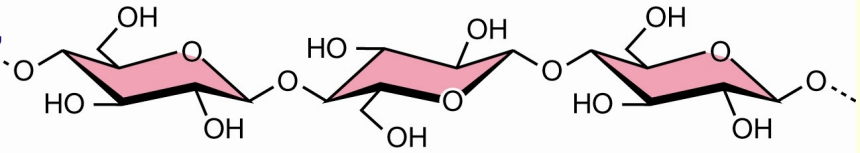
Amylose prefers a helical conformation (due to its bent α(1→4) linkages.



α-1,4-Linked D-glucose units

(a)

Cellulose, with β(1→4) linkages, can adopt a fully extended conformation.

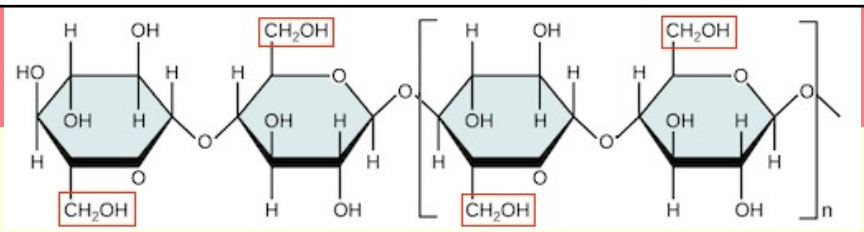


β-1,4-Linked D-glucose units

(b)

110

110



Cellulose, a major constituent of plant cell walls, consists of long linear chains of glucose with β(1→4) linkages.

Every other glucose is flipped over, due to β linkages.

This promotes intra-chain and inter-chain H-bonds and van der Waals interactions, that cause cellulose chains to be straight & rigid, and pack with a crystalline arrangement in thick bundles - microfibrils.

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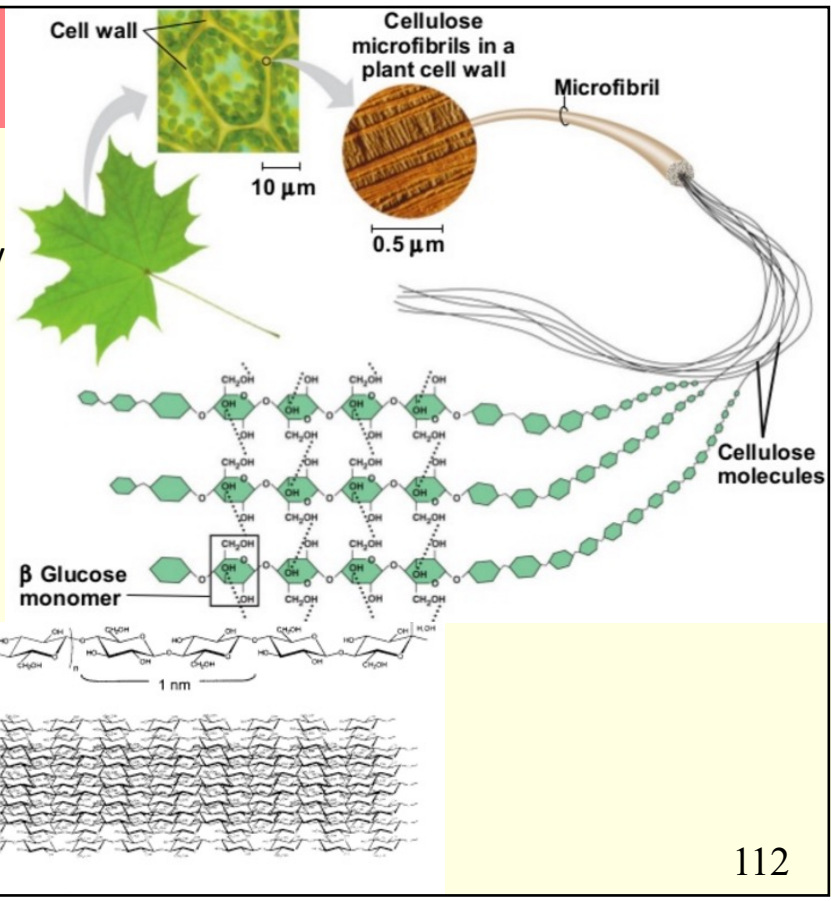
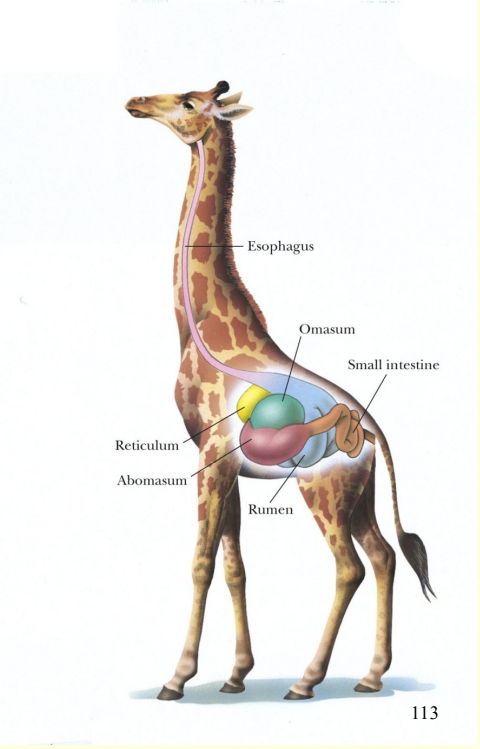
Celluloses impart strength and rigidity to plant cell walls. Osmotic swelling is prevented.



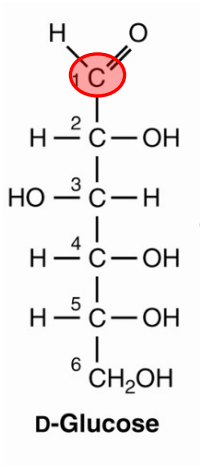
How Do Ruminant Animals Digest Cellulose?

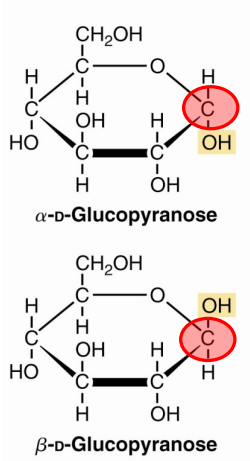
Giraffes, cattle, deer, and camels are ruminant animals that are able to metabolize cellulose, thanks to bacterial cellulase in the rumen, a large first compartment in the stomach of a ruminant.

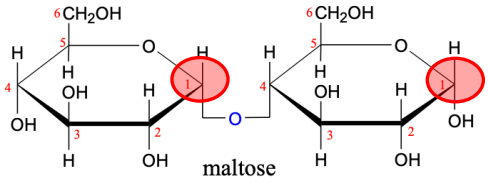
瘤胃(反芻動物的第一胃)



Anomeric carbons and glycosidic bonds







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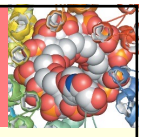
Other Structural Polysaccharides



- **Chitin** – found in the exoskeletons of crustaceans, insects and spiders, and cell walls of fungi
- It is similar to cellulose, but C-2s are N-acetyl
- Cellulose strands are parallel; chitins can be parallel or antiparallel

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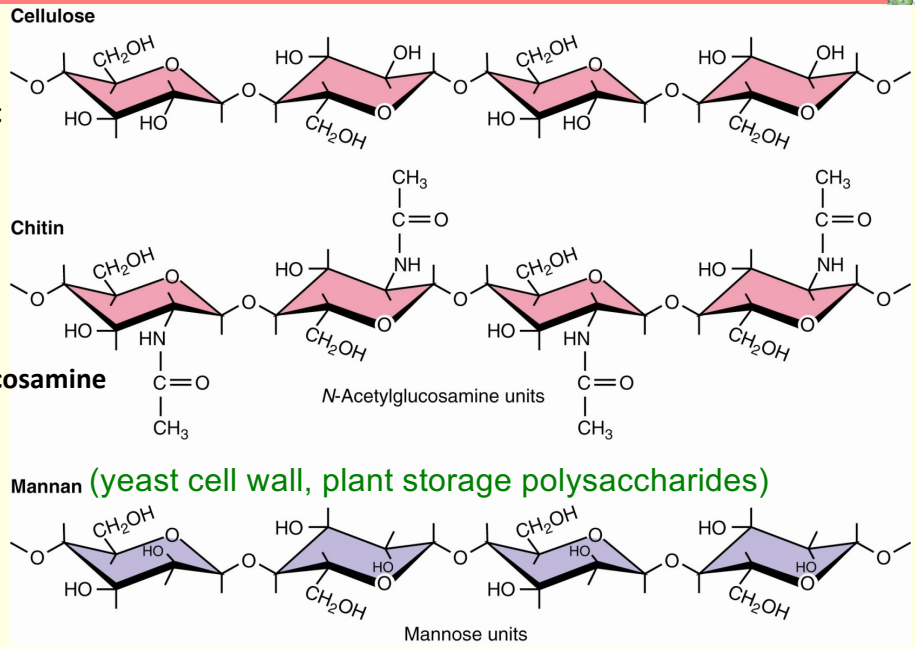
Structures of cellulose, chitin and mannan

β1-4

glucose unit



β1-4

N-acetylglucosamine

Mannan (yeast cell wall, plant storage polysaccharides)

β1-4

mannose

Like cellulose, chitin and mannan form and pack together efficiently, taking advantage of multiple hydrogen bonds.

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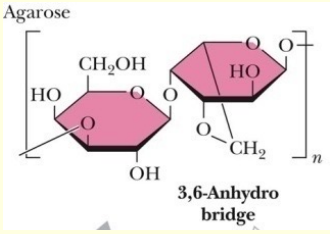
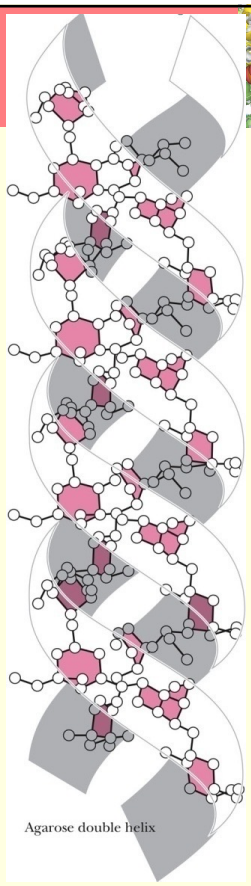
The Structure of Agarose in Solution

- Agarose, a component of agar obtained from marine red algae, is a chain of alternating D-galactose and 3,6-anhydro-L-galactose.



- Agarose gels are used in laboratories to separate biomolecules on the basis of size



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Glycosaminoglycans

Glycosaminoglycans are linear polymers of **repeating disaccharides**.

The constituent monosaccharides tend to be **modified**, with carboxylate groups ($-COO^-$), amino groups ($-NH_2$), and sulfate groups ($-SO_3^-$), etc.

One unit is an amino sugar and at least one unit contains acidic group(s).

Glycosaminoglycans tend to be **negatively charged**, because of the prevalence of acidic groups.

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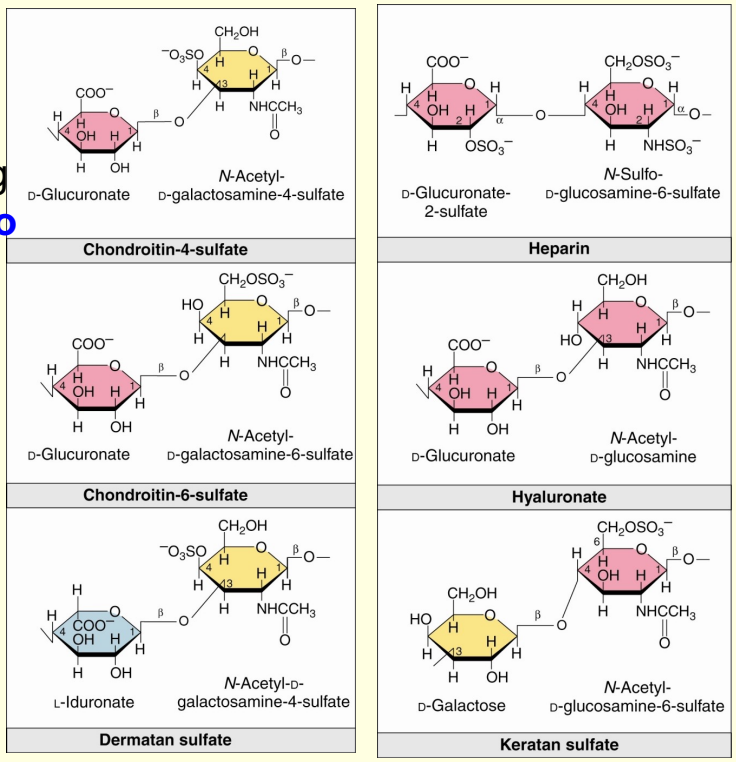
Glycosaminoglycans – Linear Chains of Repeating Disaccharides (heteropolysaccharides)

Glycosaminoglycans are linear chains of repeating disaccharides with **amino sugars and negative charges.**

Glycosaminoglycans are components of **proteoglycans**.



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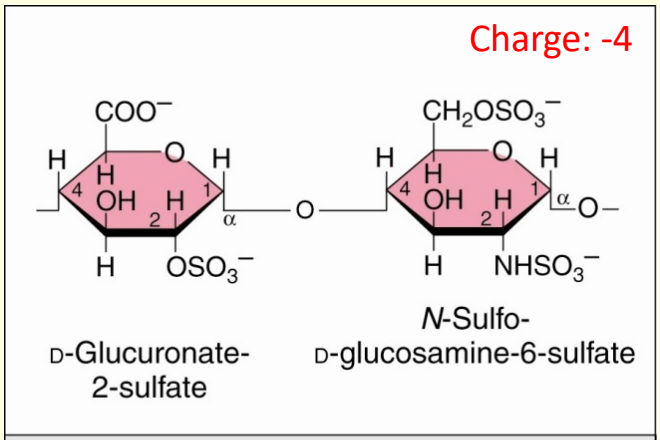
Heparin

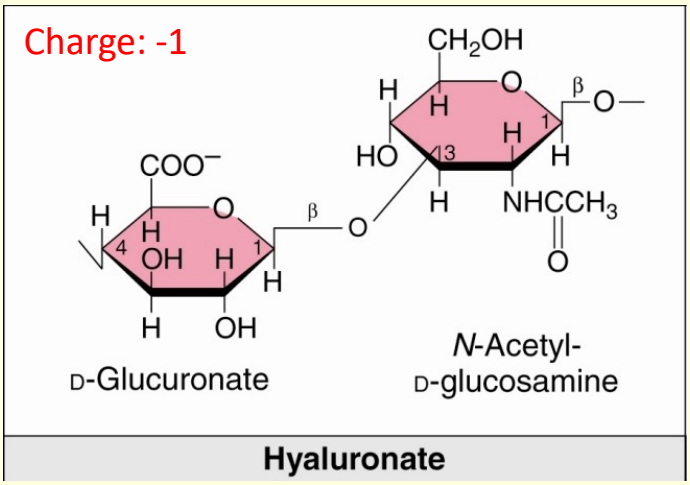
Heparin, with a very high negative charge, is a natural anticoagulant.



Heparin

Hyaluronate (玻尿酸)

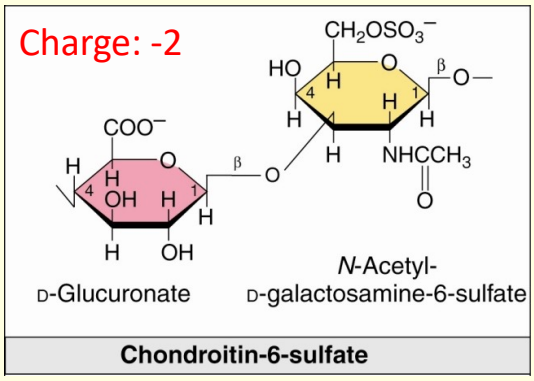
Hyaluronates (consisting of up to 25,000 disaccharide units) are components of the vitreous humor (玻璃體) of the eye and of synovial fluid（關節液）, the lubricant fluid of the body’s joints



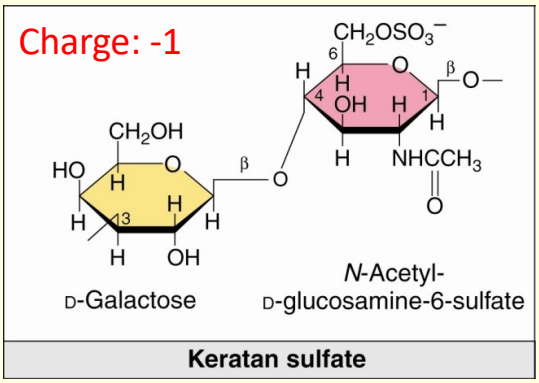
Hyaluronate

Chondroitins and keratan sulfate

Chondroitins and keratan sulfate are found in tendons, cartilage, and other connective tissue

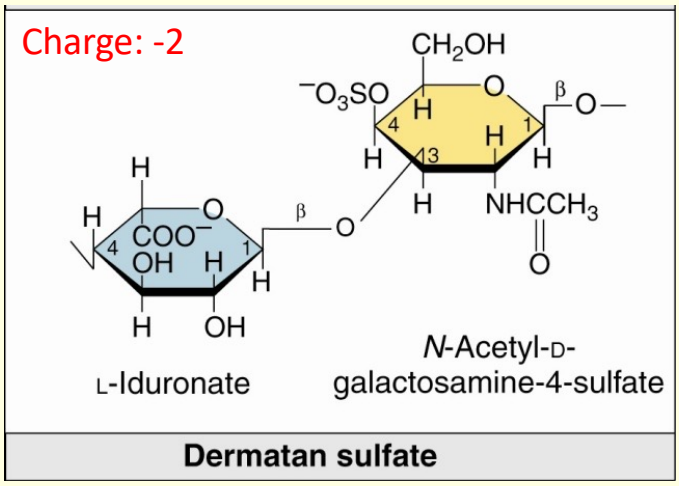


(軟骨素)



Dermatan sulfate

- Dermatan sulfate is a component of the extracellular matrix of skin
- Glycosaminoglycans are constituents of proteoglycans (discussed later in this chapter)



Peptidoglycan:
polysaccharide of bacterial cell walls

胜肽聚醣

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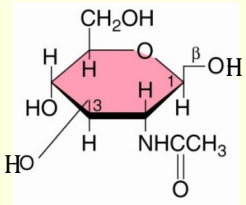
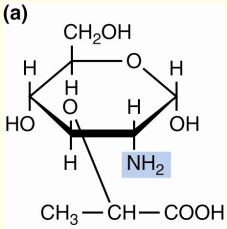
- Peptidoglycan is the polysaccharide of bacterial cell wall.
- Gram stain identifies peptidoglycan structures.
- Bacteria are classified as Gram-positive or Gram-negative.
- Joined to a tetrapeptide, the backbone of peptidoglycan is a β1-4 linked polymer of N-acetylglucosamine (NAG) and N-acetylmuramic acid (NAM) units.



N-Acetyl-D-glucosamine



Muramic acid

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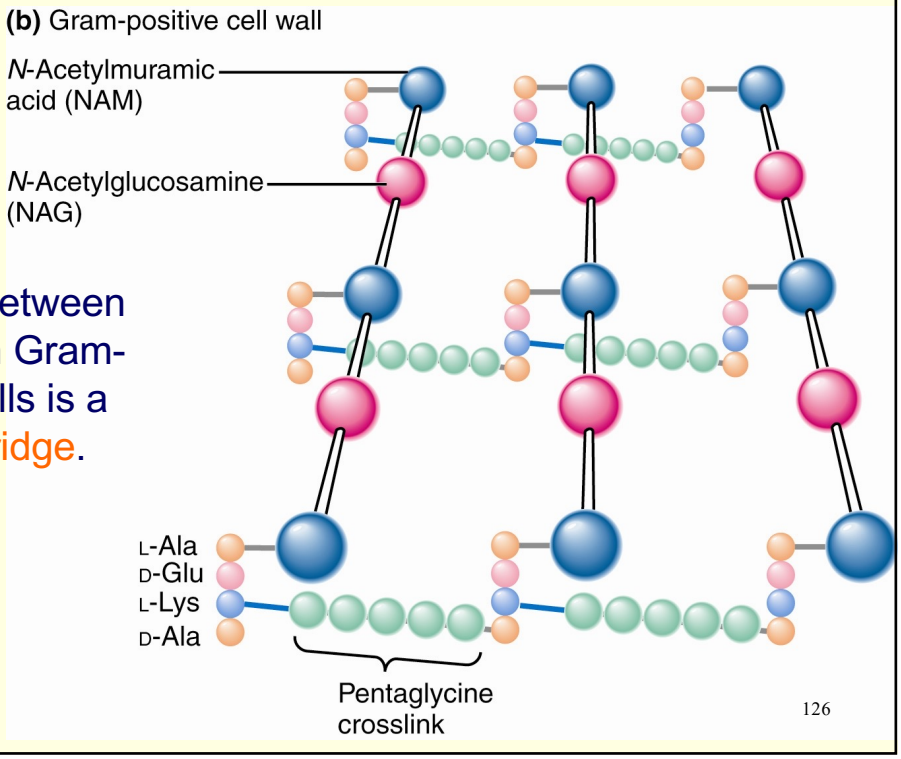
The Structure of Gram-positive Peptidoglycan



The crosslink between tetrapeptides in Gram-positive cell walls is a pentaglycine bridge.

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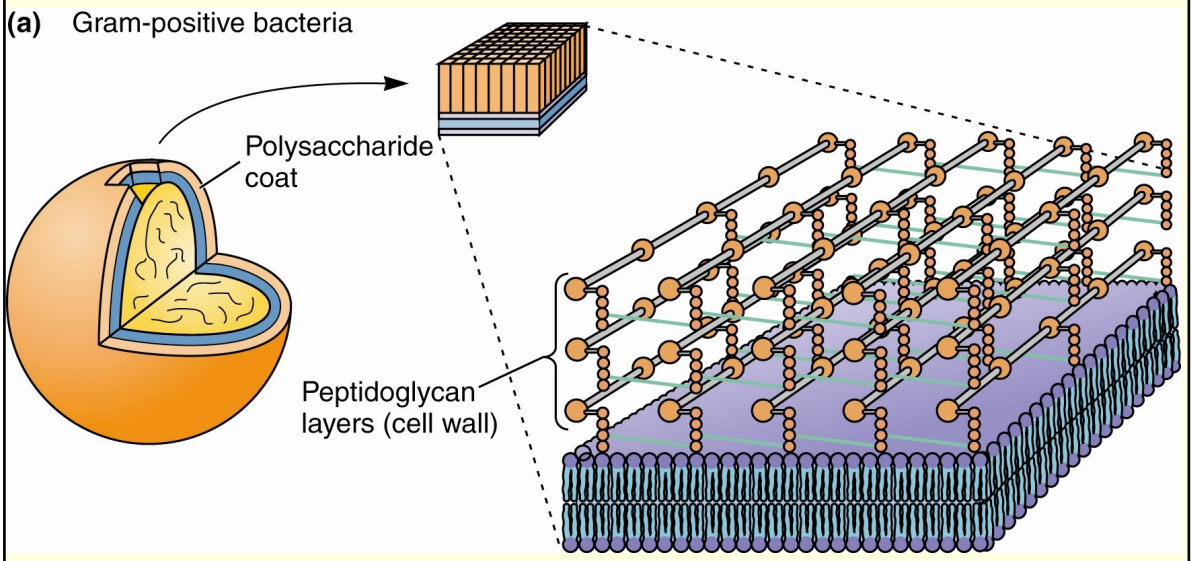
The Cell Wall of Gram-positive Bacteria



The Gram-positive cell wall consists of a single bilayer membrane with a peptidoglycan coat.
The peptidoglycan layer (25 nm) reacts with Gram stain.

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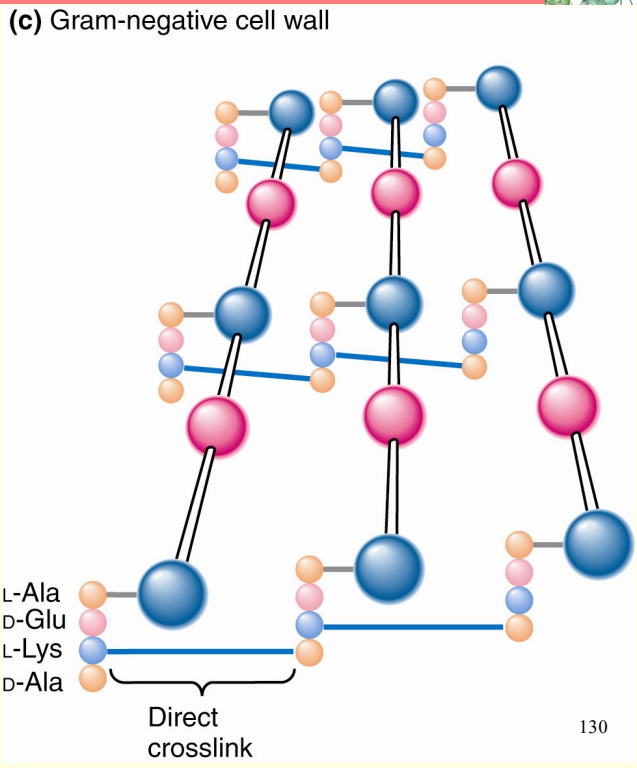
The Structure of Gram-negative Peptidoglycan

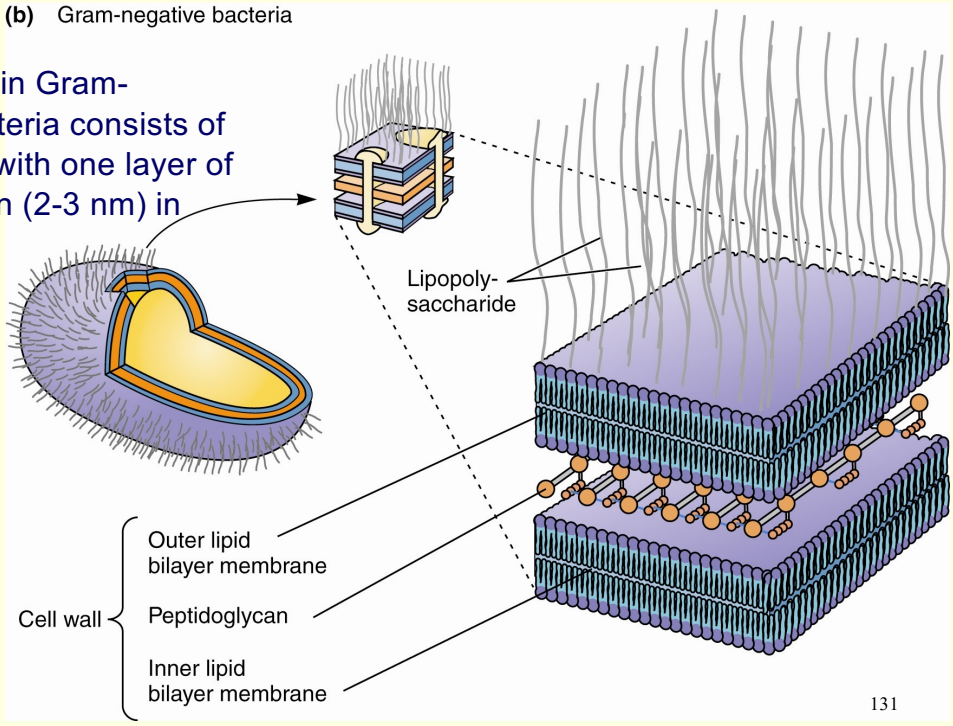
Note the direct crosslinks between tetrapeptide units

In Gram-negative cell walls, the linkage between tetrapeptide segments involves a direct amide bond.



The Cell Wall of Gram-negative Bacteria

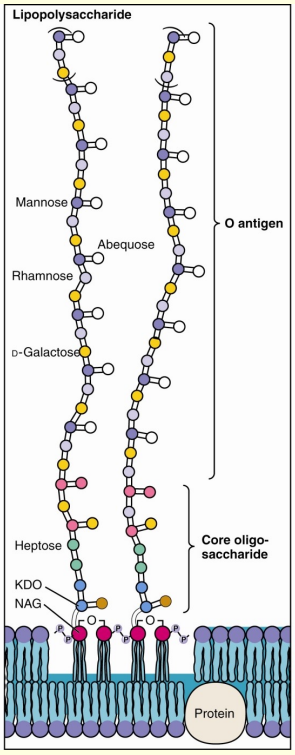
The cell wall in Gram-negative bacteria consists of two bilayers with one layer of peptidoglycan (2-3 nm) in between.



The Structure of Lipopolysaccharide

Lipopolysaccharide consists of a lipid group (anchored in the outer membrane) joined to a polysaccharide made up of long chains with various repeating structures.

- O-antigens are very diverse and recognize host cells.
- Known as endotoxins
- Induce strong immune responses



What Are **Glycoproteins**, and How Do They Function in Cells?

Animals Display a Variety of Cell Surface Polysaccharides

- Animal cell surfaces contain an incredible diversity of glycoproteins and proteoglycans
- These polysaccharide structures regulate cell-cell recognition and interaction
- The uniqueness of the "information" in these structures is determined by the enzymes that synthesize these polysaccharides

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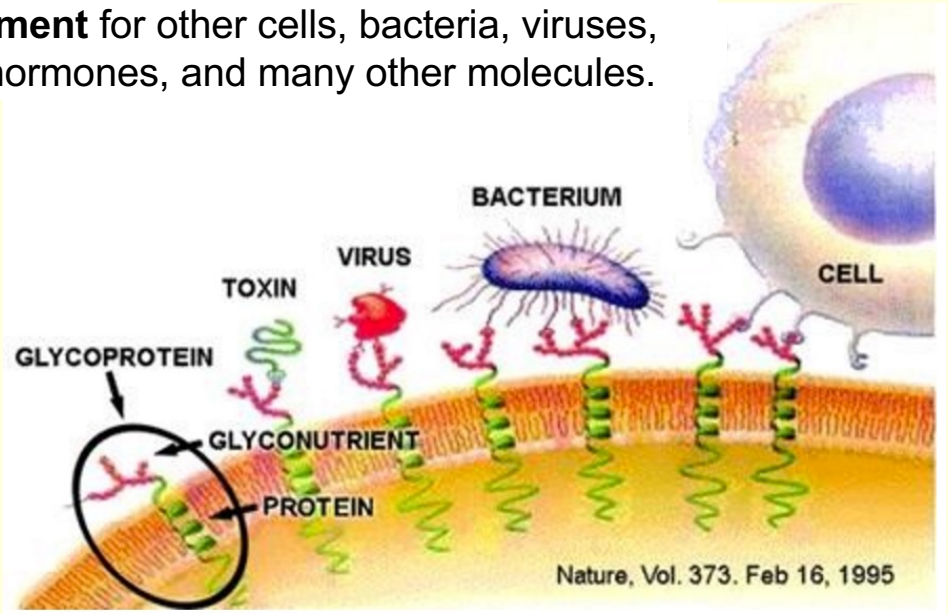
Glycoproteins are mostly transmembrane or extracellular

Cell surface carbohydrates serve as points of **attachment** for other cells, bacteria, viruses, toxin, hormones, and many other molecules.



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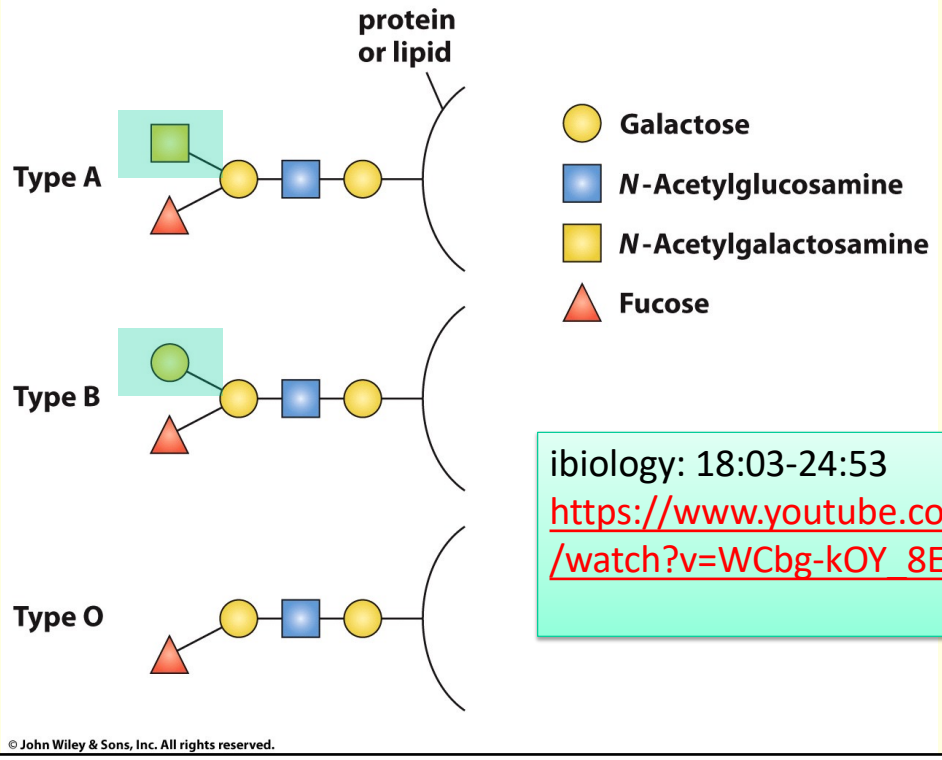
ABO blood group system



ibiology: 18:03-24:53
https://www.youtube.com/watch?v=WCbg-kOY_8E

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Drug Research Finds a Sweet Spot

Drug	Description	Manufacturer
Cerezyme (imiglucerase)	This enzyme degrades glycolipids, compensating for an enzyme deficiency that causes Gaucher's disease.	Genzyme Cambridge, MA
Vancocin (vancomycin)	A very potent glycopeptide antibiotic that is typically used against antibiotic-resistant infections. It inhibits synthesis of peptidoglycan in the bacterial cell wall.	Eli Lilly Indianapolis, IN
Vevesca (OGT 918)	A sugar analog that inhibits synthesis of the glycolipid that accumulates in Gaucher's disease.	Oxford GlycoSciences Abingdon, UK
GMK	A vaccine containing ganglioside GM2; it triggers an immune response against cancer cells carrying GM2.	Progenics Pharmaceuticals Tarrytown, NY
Staphvax	A vaccine that is a protein with a linked bacterial sugar; it is intended to treat *Staphylococcus* infection.	NABI Pharmaceuticals Boca Raton, FL
Bimosiamose (TBC1269)	A sugar analog that inhibits selectin-based inflammation in blood vessels.	Texas Biotechnology Houston, TX
GCS-100	A sugar that blocks action of a sugar-binding protein on tumors.	GlycoGenesys Boston
GD0039 (swainsonine)	A sugar analog that inhibits synthesis of carbohydrates essential to tumor metastasis.	GlycoDesign Toronto, Canada
PI-88	A sugar that inhibits growth factor–dependent angiogenesis and enzymes that promote metastasis.	Progen Darra, Australia

A variety of diseases are being successfully treated with sugar-based therapies. Several carbohydrate-based drugs are either on the market or in various stages of clinical trials. Some are enzymes, whereas others are glycoconjugates.

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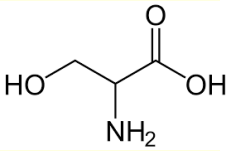
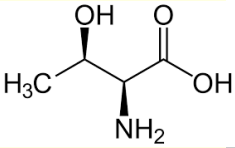
Glycoproteins

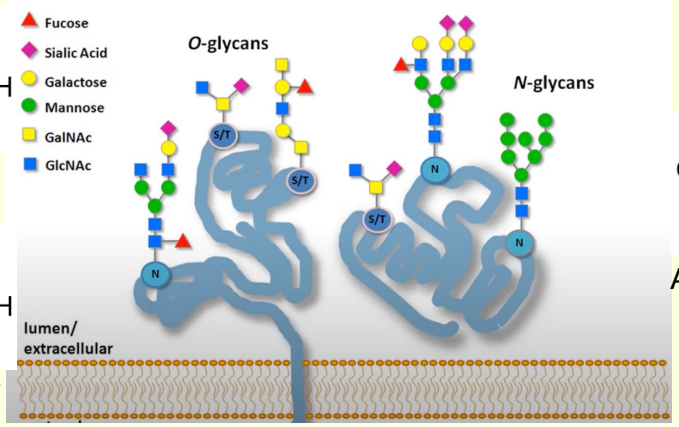
How do saccharides link to the protein?

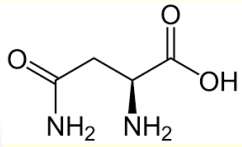


Serine/Ser/S



Threonine/Thr/T

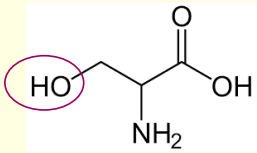




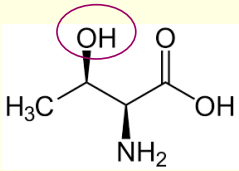
Asparagine/Asn/N

- **O**-Linked glycosylation
- **N**-Linked glycosylation

O-Linked glycosylation

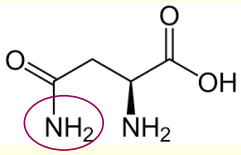


Serine/Ser/S



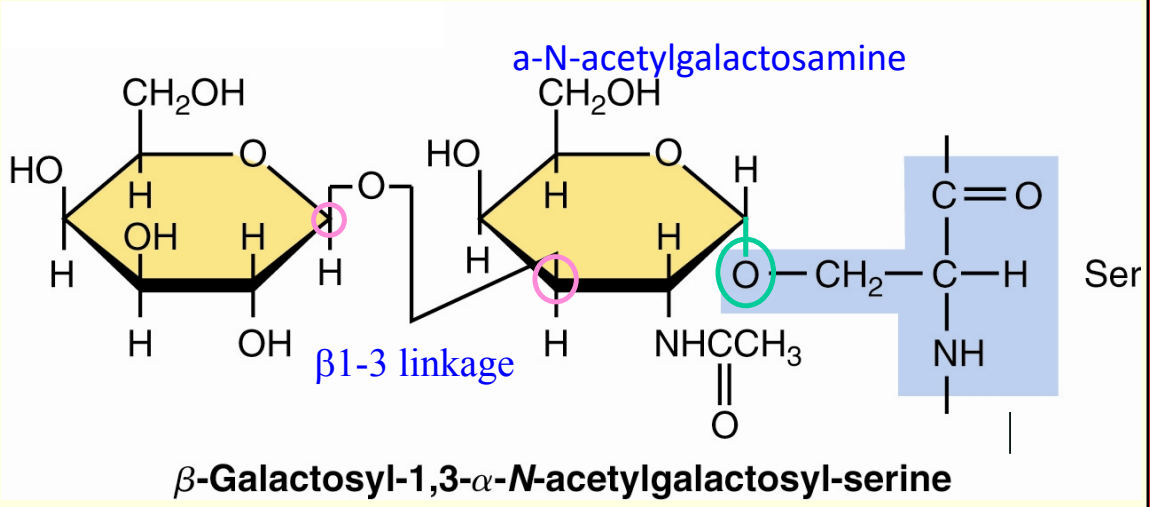
Threonine/Thr/T

N-Linked glycosylation



Asparagine/Asn/N

The Structure of O-Linked Saccharides



The carbohydrate moieties of glycoproteins link to a protein via a **glycosidic bond** between the **anomeric carbon** of a sugar residue and a **serine or threonine hydroxyl group**.

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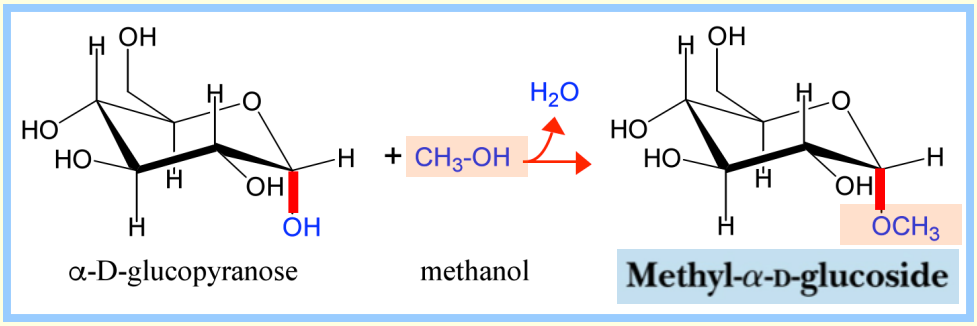
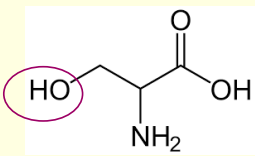
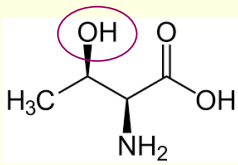
Glycosidic Bonds

E.g., methanol reacts with the anomeric C on glucose to form methyl glucoside (methyl-glucopyranose).





Serine/Ser/S



Threonine/Thr/T

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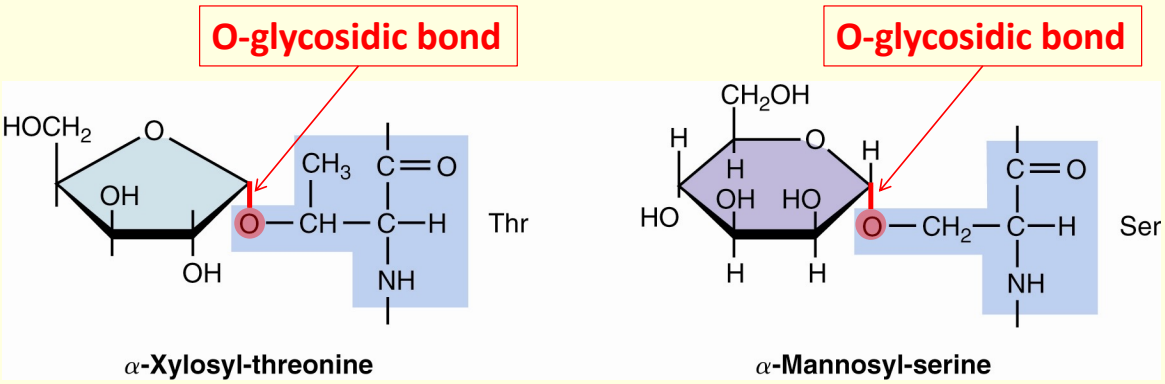
The Structure of O-Linked Saccharides



The carbohydrate moieties of glycoproteins may be linked to the protein via **serine** or **threonine** residues.

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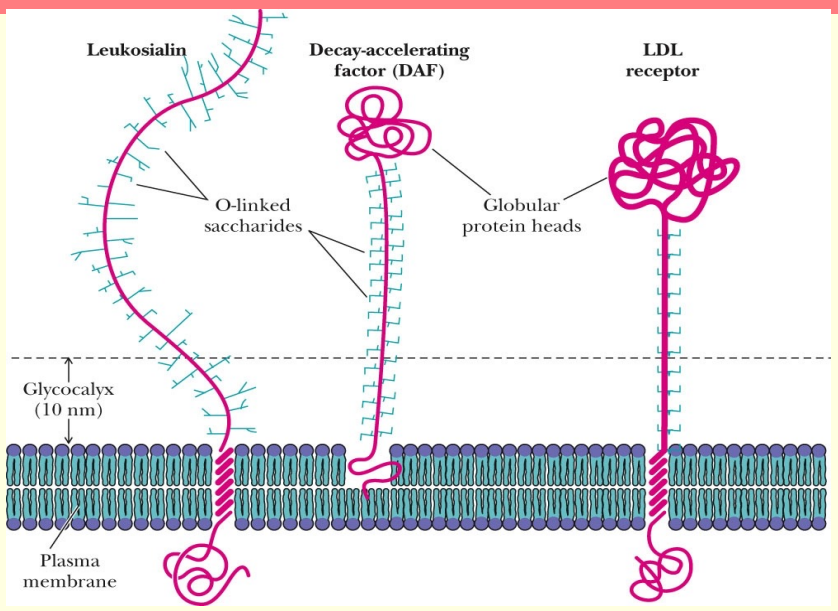
O-linked Glycoproteins



The O-linked saccharides often adopt **extended conformations** to lift the functional domains of these proteins above the membrane surface.

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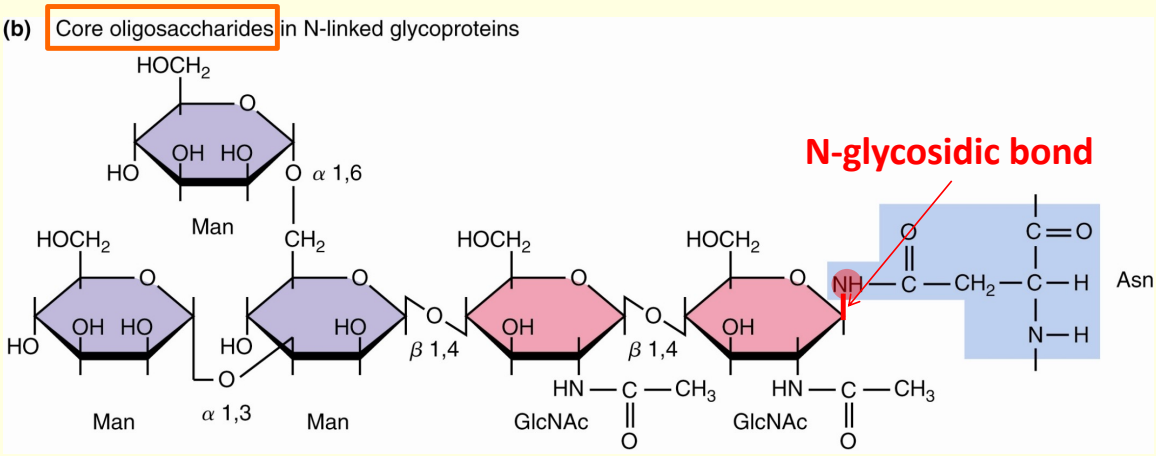
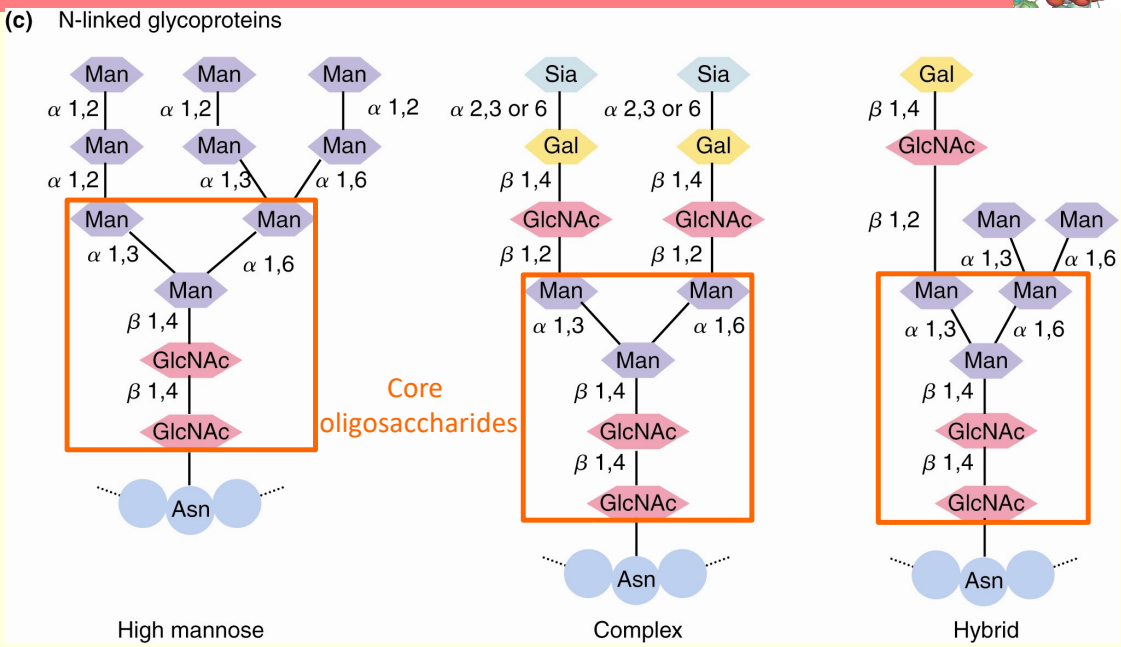
The Structure of N-Linked Saccharides



The carbohydrate moieties of glycoproteins may be linked to the side-chain amide of **asparagine.**

3 types of N-linked glycoproteins



Hybrid: combining structures found in the high mannose and complex saccharides.

Functions of N-linked Oligosaccharides

Many functions known or suspected

- Oligosaccharides can alter the chemical and physical properties of proteins
- Oligosaccharides can stabilize protein conformations and/or protect against proteolysis
- Cleavage of monosaccharide units from N-linked glycoproteins in blood targets them for degradation in the liver

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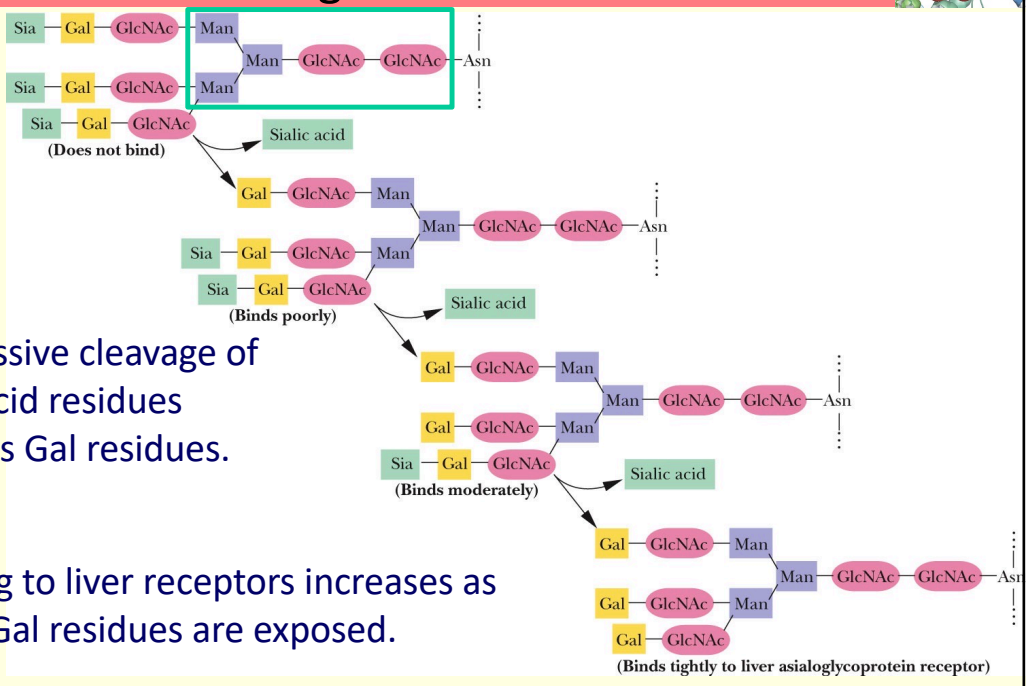
Oligosaccharide Cleavage Can Serve as a Timing Device for Protein Degradation



Progressive cleavage of sialic acid residues exposes Gal residues.

Binding to liver receptors increases as more Gal residues are exposed.

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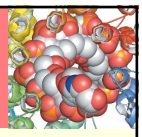
N-Linked Oligosaccharides Help Proteins to Fold by Both Intrinsic and Extrinsic Effects



- One-third of proteins encoded by the human genome are made at and then exported from the ER
- Nearly all are glycosylated at Asn residues
- The N-linked oligosaccharides on these proteins are bulky, amphipathic modifications that *intrinsically* enhance solubility and alter folding energetics via carbohydrate-protein interactions, affecting conformational preferences near the glycosylation sites

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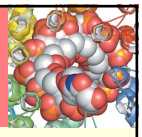
N-Linked Oligosaccharides Help Proteins to Fold by Extrinsic Effects



- In this network, proteins with native conformation selectively transit the ER to the Golgi, whereas misfolded proteins are retained in the ER and/or degraded.
- This quality control pathway begins with addition of a 14-residue oligosaccharide to Asn sites (next figure).
- Stepwise cleavage of Glucose residues from these glycans directs the protein to a series of glycan-binding proteins that promote and assess folding, exporting folded proteins and degrading misfolded proteins

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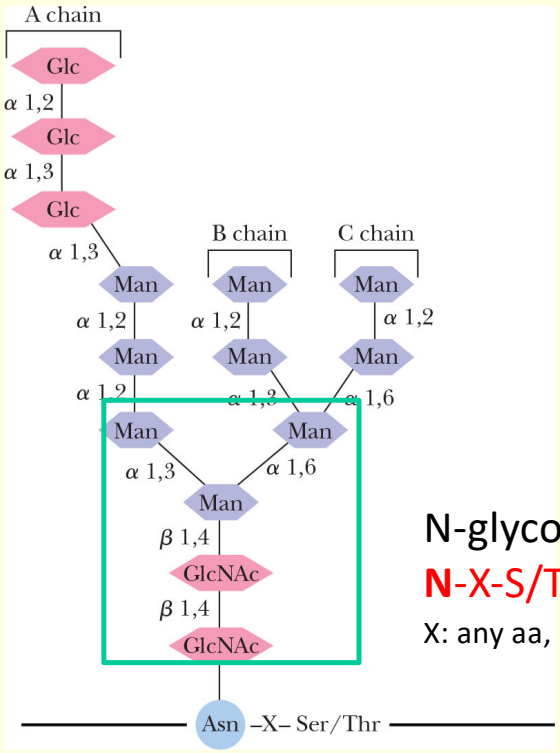
N-Linked Oligosaccharides Help Proteins to Fold by Both Intrinsic and Extrinsic Effects

Glycosidase (glucosidase) hydrolyzes these **glucose** residue



N-glycosylation motif:

N-X-S/T

X: any aa, except for P

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N-Linked Oligosaccharides Help Proteins to Fold by Both Intrinsic and Extrinsic Effects

- Several human diseases have been directly linked to dysfunction of glycoproteostasis (醣蛋白生成分解平衡態) and consequent retention of aggregated and misfolded proteins in the ER
  - Cystic fibrosis 囊狀纖維化
  - Congenital hypothyroidism 先天性甲狀腺功能低下症
  - LDL receptor defects and hypercholesterolemia 高膽固醇血症

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Proteoglycans

- Proteoglycans are proteins that are heavily glycosylated.
- The basic proteoglycan unit consists of a "core protein" with one or more covalently attached **glycosaminoglycan** chains
- They are components of the cell membrane and the **glycocalyx** (a "sugar shell" around the cell)
- They typically consist of proteins with one or two types of glycosaminoglycan

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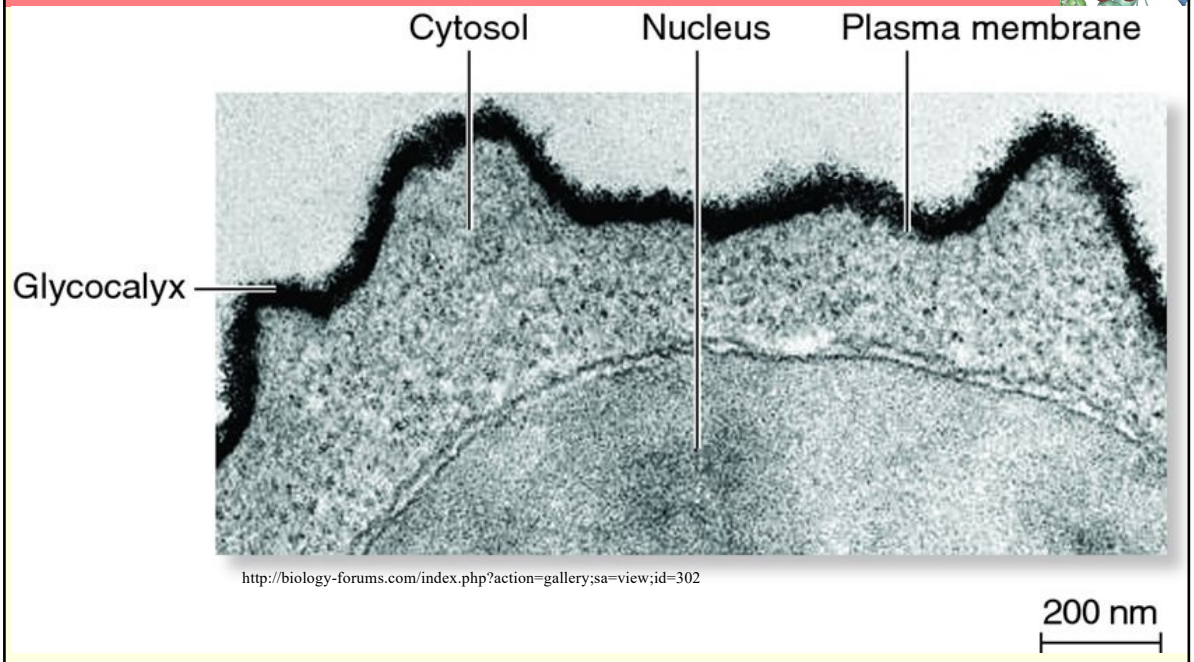
Glycocalyx coats an animal cell.



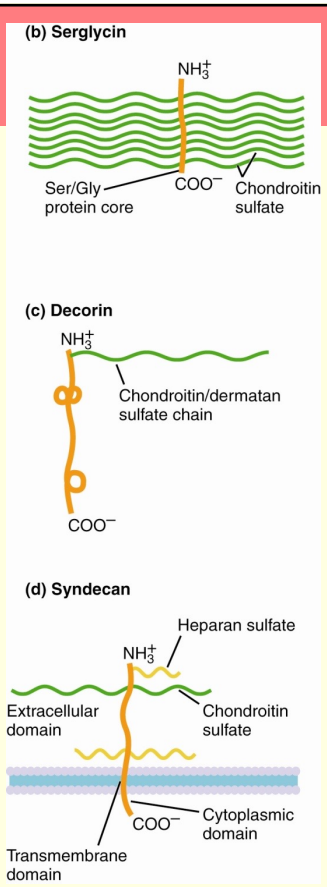
http://biology-forums.com/index.php?action=gallery;sa=view;id=302

a lymphocyte, a type of white blood cell, stained with ethidium red, which emphasizes the thick carbohydrate layer that surrounds the cell.

Structures of Proteoglycans

The carbohydrate groups of proteoglycans are predominantly **glycosaminoglycans O-linked to serine** residues.

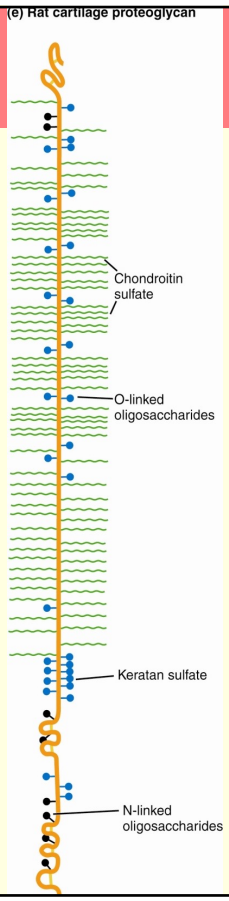
Proteoglycans include both soluble proteins and integral membrane proteins.



rat cartilage proteoglycan

This molecule is highly hydrated.

Compression of cartilage (as in walking) squeezes water out of the cartilage tissue to cushion the joint. Water is reabsorbed when the stress due to compression diminishes.



Functions of Proteoglycans

- **Modulation of cell growth processes**

  Binding of growth factor proteins by proteoglycans in the glycocalyx provides a reservoir of growth factors at the cell surface

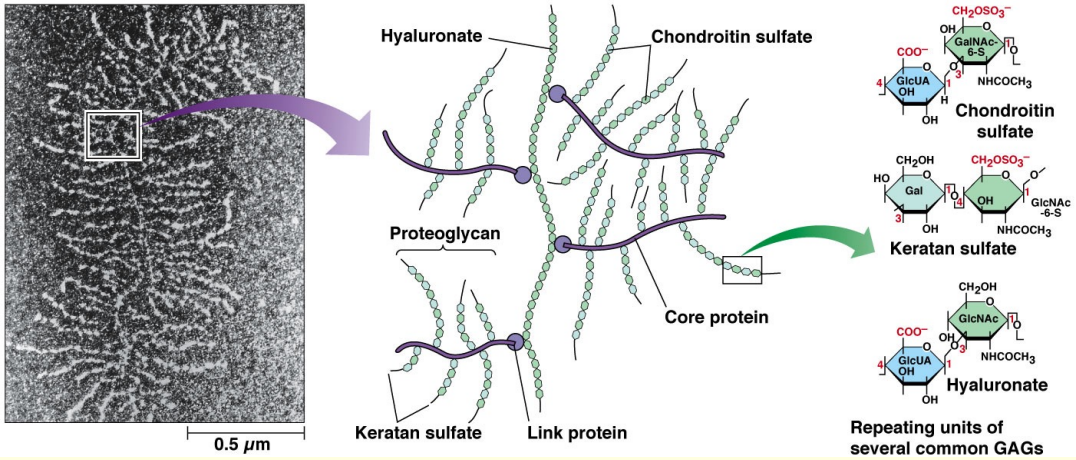
- **Cushioning in joints**

  Cartilage matrix proteoglycans absorb large amounts of water. During joint movement, cartilage is compressed, expelling water.

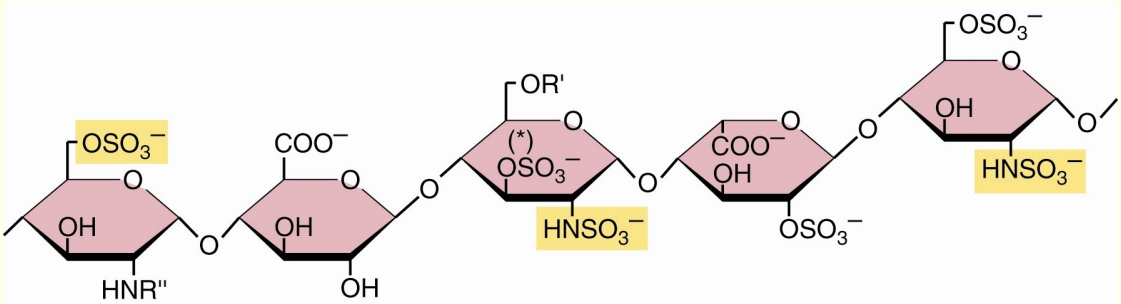
Proteoglycans are composed of a core protein to which glycosaminoglycans (GAGs) are attached.

GAGs consist of repeating disaccharide subunits: one is often an amino sugar (N-acetylglucosamine or N-acetylgalactosamine; usually with an attached **sulfate** group) and the other is a sugar or sugar acid.

Chondroitin sulfate, keratan sulfate, heparan sulfate and hyaluronate are the most common GAGs.



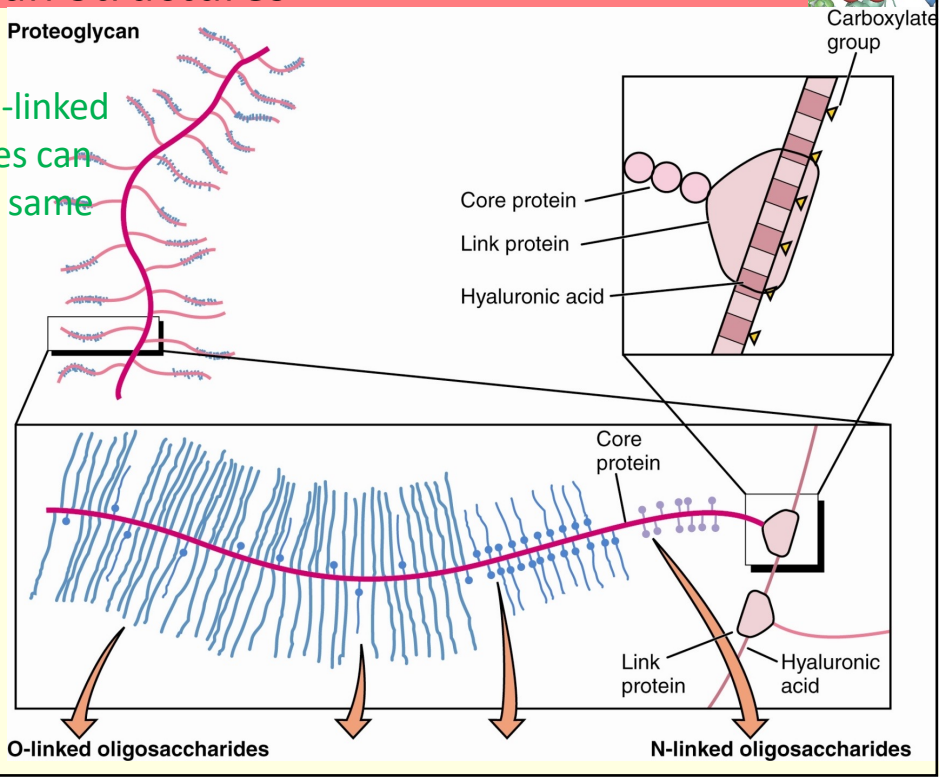
Heparin Inhibits Cell Proliferation



- has anticoagulant properties.
- inhibits cell proliferation in a process involving internalization of the glycosaminoglycan moiety and its migration to the cell nucleus.

Hyaluronate Forms the Backbone of Proteoglycan Structures

O-linked and N-linked oligosaccharides can co-exist on the same proteoglycan.



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Carbohydrates Provide a Structural Code

- Individual sugar units are the "letters" of the **sugar code**, and the "words" and "sentences" of this code are synthesized by **glycosyltransferases** (catalyze the formation of glycosidic bonds), **glycosidases** (catalyze the hydrolysis of glycosidic bonds), and other enzymes

- The total number of permutations for a six-unit polymer formed from an alphabet of 20 hexose monosaccharides is 1.44×10^{15}!

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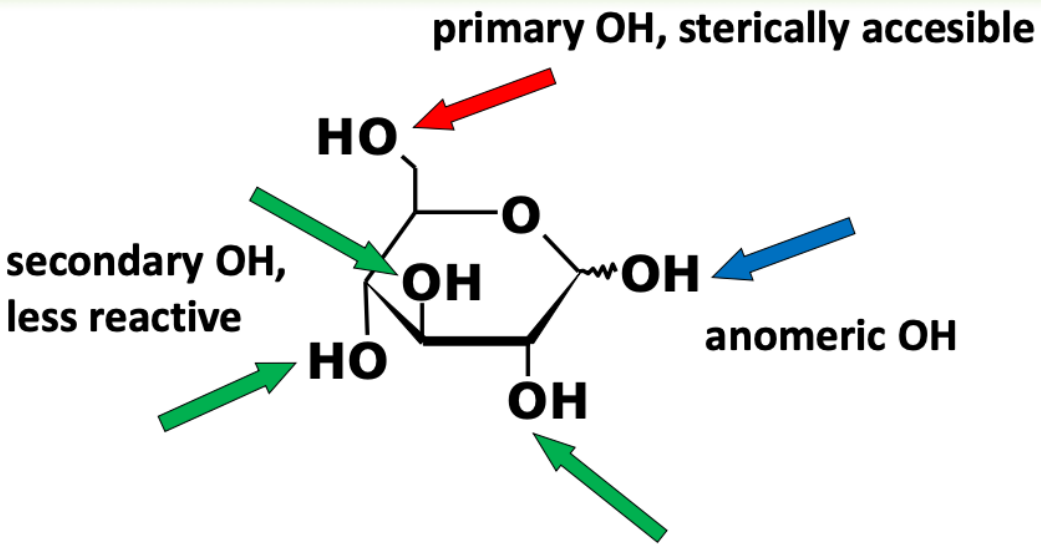
Structural Diversity

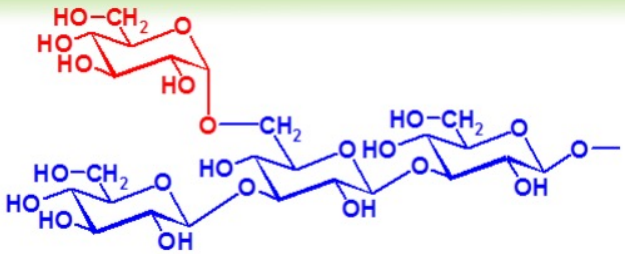
ICT PRAGUE



- bonding at different linkage positions
- site-specific substitution
- branching

Branching

ICT PRAGUE



- a unique feature among all biopolymers
- a pronounced effect on affinity
- an increase of selectivity
- glycoside cluster effect

Winterburn PJ, Phelps CF (*Nature* 236, 147 (1972)): „carbohydrates are ideal for generating compact units with explicit informational properties, since the permutation on linkages are larger than can be achieved by amino acids, and, uniquely in biological polymers, branching is possible“.

Lectins are proteins that bind carbohydrates with high specificity and high affinity

TABLE 7.1 Specificities and Functions of Some Animal Lectins

Lectin Family	Carbohydrate Specificity	Function
Calnexins	Glucose	Ligand-selective molecular chaperones in ER
C-type lectins	Variable	Cell-type specific endocytosis and other functions
ERGIC-53	Mannose	Intracellular routing of glycoproteins and vesicles
Galectins	Galactose/lactose	Cellular growth regulation and cell–matrix interactions
Pentraxins	Variable	Anti-inflammatory action
Selectins	Variable	Cell migration and routing

Sugars are the "letters" of the **sugar code**. Lectins are the translators of the sugar code. Many processes, such as cell migration, cell-cell interactions, immune responses, and blood clotting, depend on information transfer using this code.

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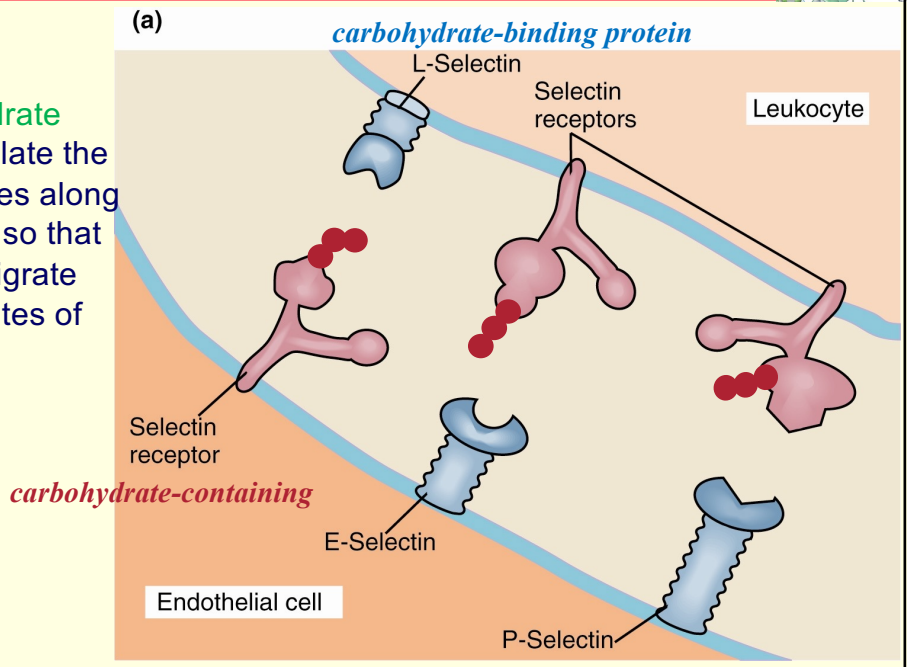
Selectin proteins modulate the inflammatory response

Selectin-carbohydrate interactions modulate the rolling of leukocytes along the vascular wall, so that leukocytes can migrate efficiently to the sites of inflammation.



The interactions of selectins with their receptors.

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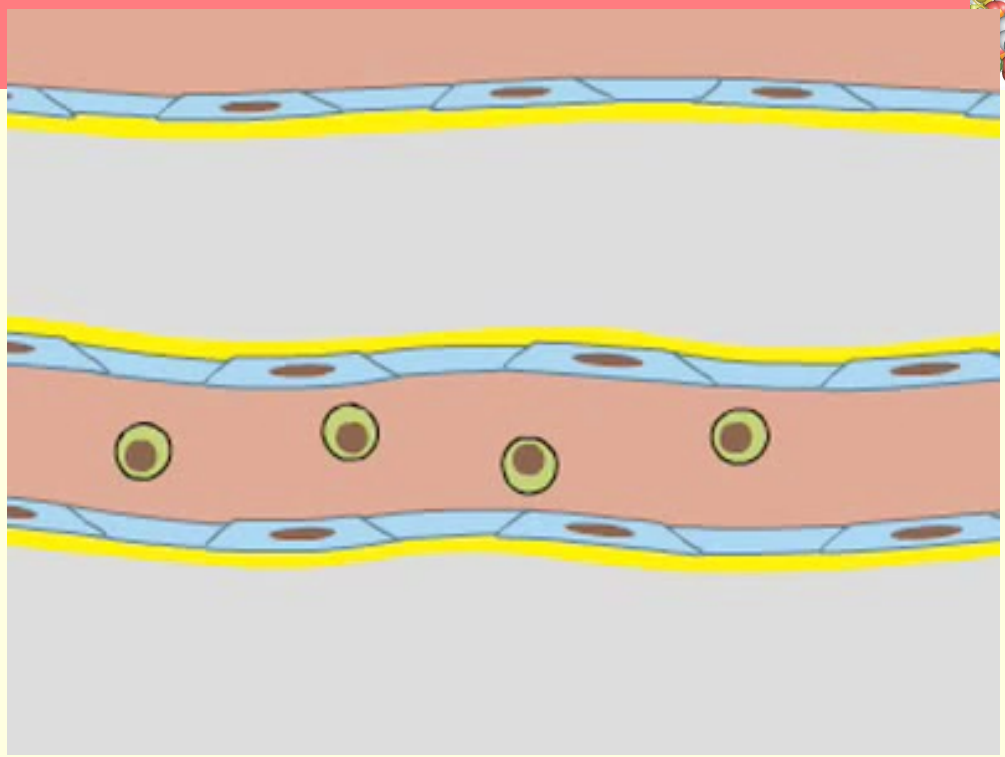
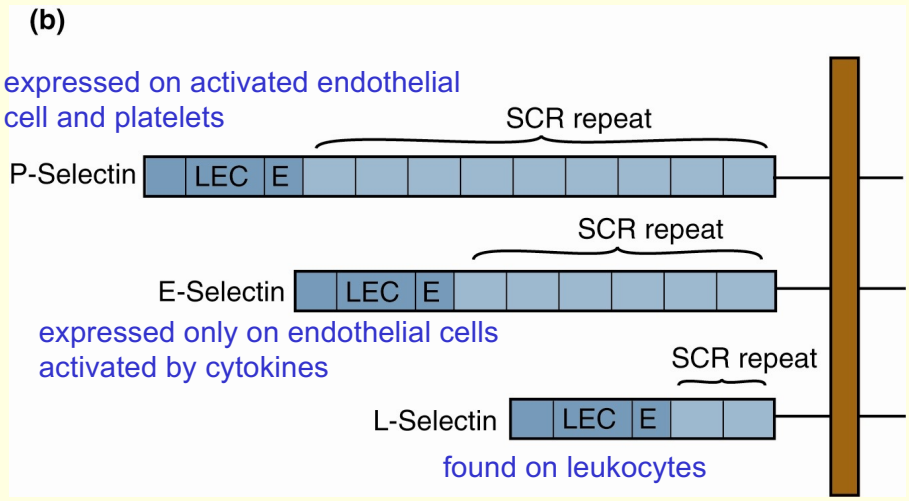


Source: https://www.youtube.com/watch?v=-_wddQWG32A

Selectin proteins modulate the inflammatory response



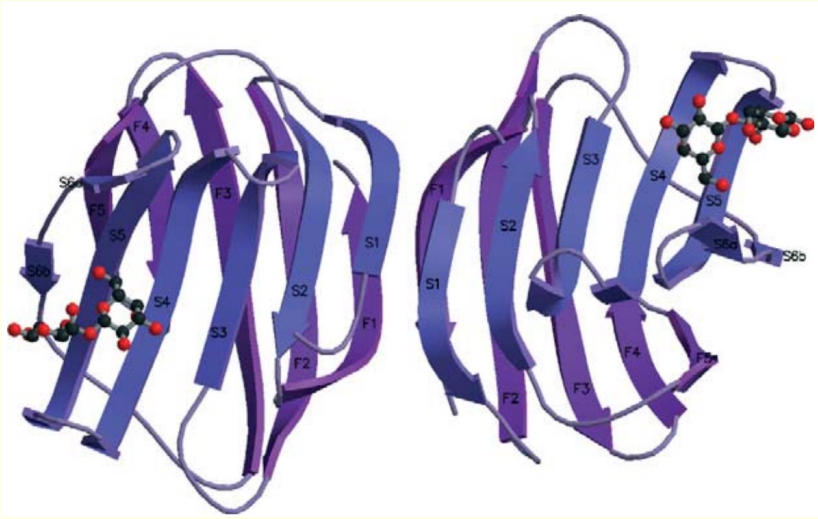
The primary structure of the selectin family of adhesion proteins. **LEC is the lectin domain**.

E: EGF domain
SCR: short consensus repeat

Galectins are Mediators of Inflammation, Immunity, and Cancer

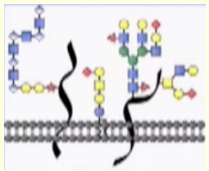
- Galectins are a family of proteins with carbohydrate recognition domains (CRDs) of about 135 residues
- Galectins bind β-galactosides specifically
- Human galectin-1 is a dimer of antiparallel β-sandwich subunits (Figure 7.41a)
- Lactose binds at opposite ends of the dimer
- H-bond networks maintain the structure of the galactose-binding sites.

Galectins are Mediators of Inflammation, Immunity, and Cancer



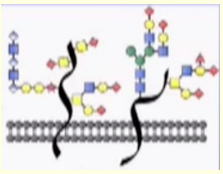
Structure of the galectin-1 dimer. The lactose-binding sites are at opposite ends of the dimer.

Cell surface glycans are varied as physiological conditions change



Physiological change

Sugar composition & structure



State 1

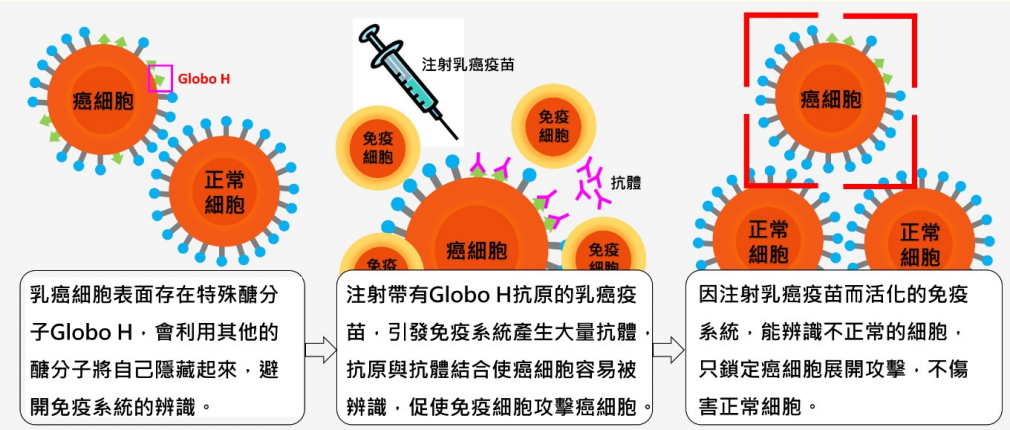
Embryonic stem cell

Healthy cell

State 2

Differentiated cell

Cancer cell



癌症疫苗作用機制，以乳癌疫苗為例 。（圖 / 陳聖昌提供）

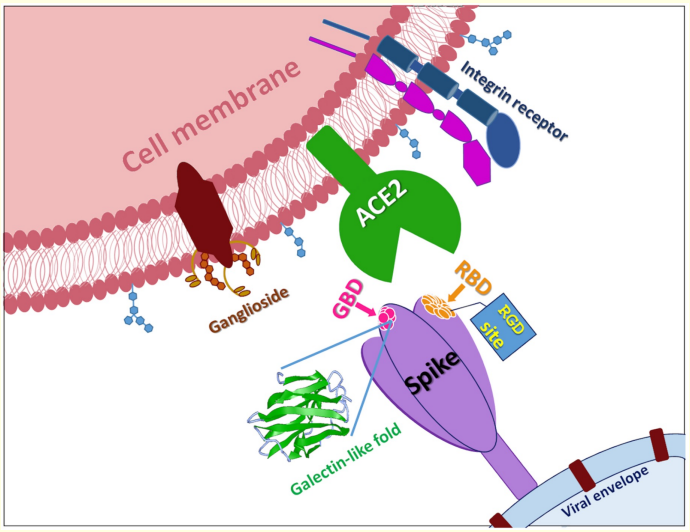
https://scitechvista.nat.gov.tw/Article/C000003/detail?ID=b0e07007-6677-445b-a7eb-8700643d4a99

- 1983年日本箱守仙一郎（Hakomori Sen-itiroh）教授在人體乳癌細胞表面上發現寡醣分子Globo H 有大量表現，之後其他科學家在卵巢癌、攝護腺癌、肺癌等癌細胞表面上，也發現類似情形。

- 因此翁啟惠團隊認為Globo H具有足夠的專一性，將Globo H 製成疫苗的抗原，當癌症病患注射此種疫苗後，體內的免疫系統會開始活化，並產生會和癌細胞的Globo H結合的抗體，使得癌細胞容易被辨識，如此一來，人體本身的免疫機制就會鎖定這些癌細胞展開攻擊，消滅癌細胞及防止癌症復發。

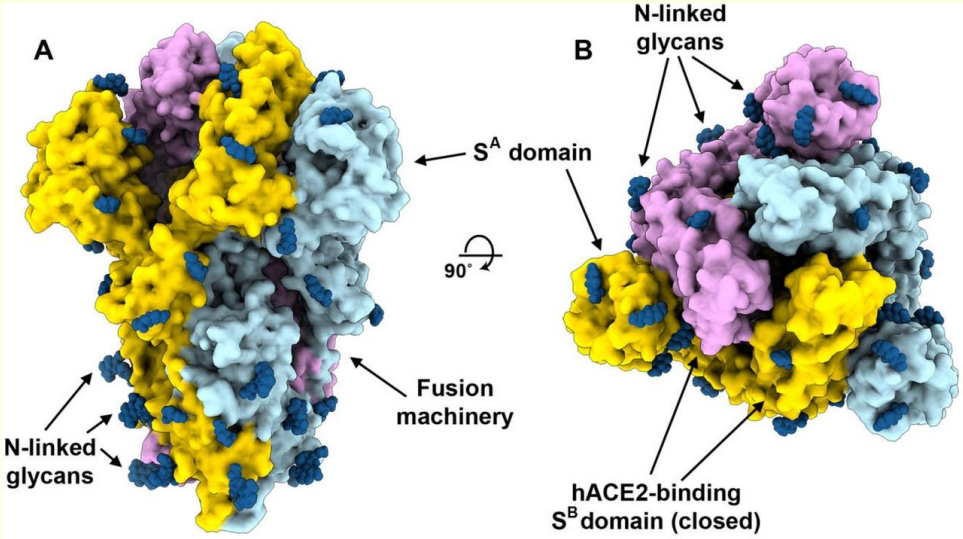
https://scitechvista.nat.gov.tw/Article/C000003/detail?ID=b0e07007-6677-445b-a7eb-8700643d4a99

Spike protein is important for Sars CoV-2 infection



S protein at the surface of the virus particle recognizes ACE2 receptor on host cells.

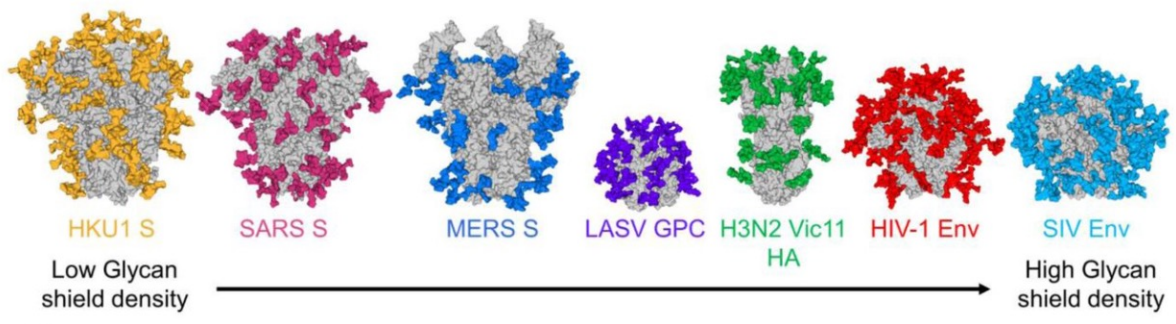
S protein is shield with N-linked glycans



Glycosylation sites, sugar composition & structure are determined by cryo EM and mass spectrometry.

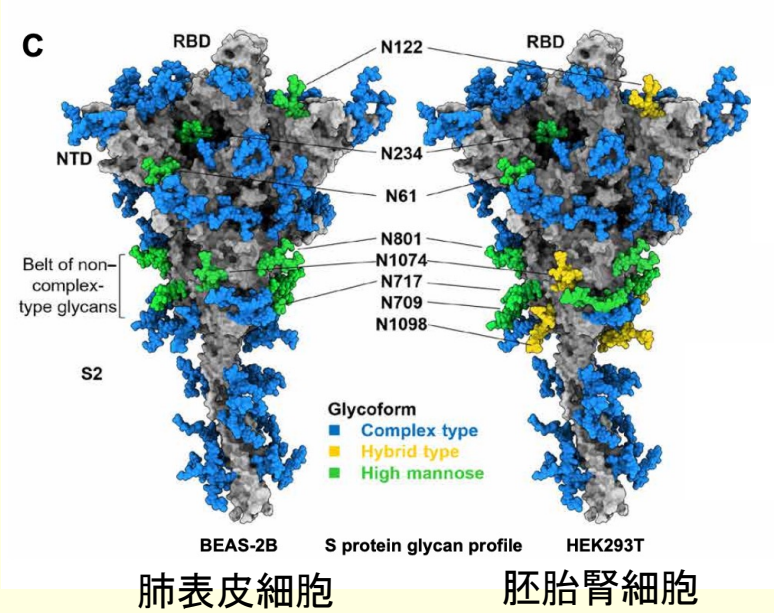
Cell (2020) 181:281

Virus evades the immune system by glycosylation.



Spike proteins are highly glycosylated.

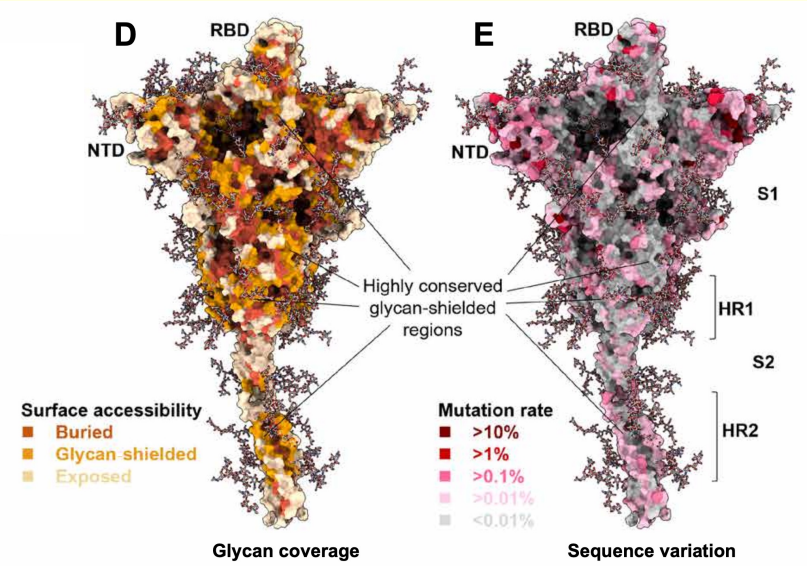
S protein from lung epithelial cells contains more sialylated complex-type glycans



肺表皮細胞　　胚胎腎細胞

Sci. Transl. Med. **14**, eabm0899 (2022)

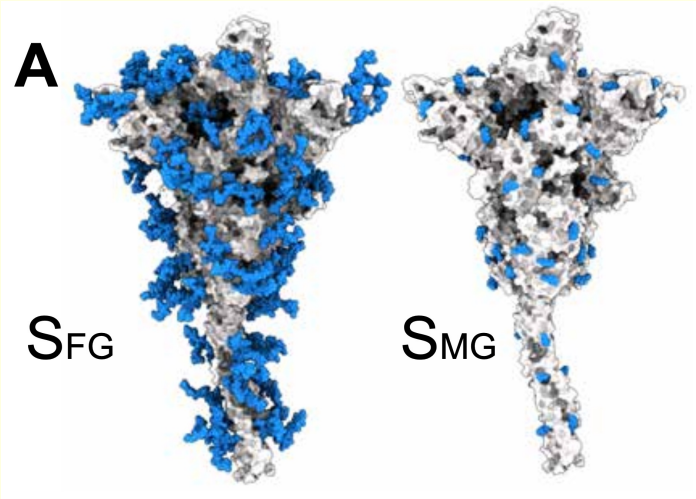
Highly conserved epitopes in S protein are largely shielded by glycans



Sci. Transl. Med. **14**, eabm0899 (2022)

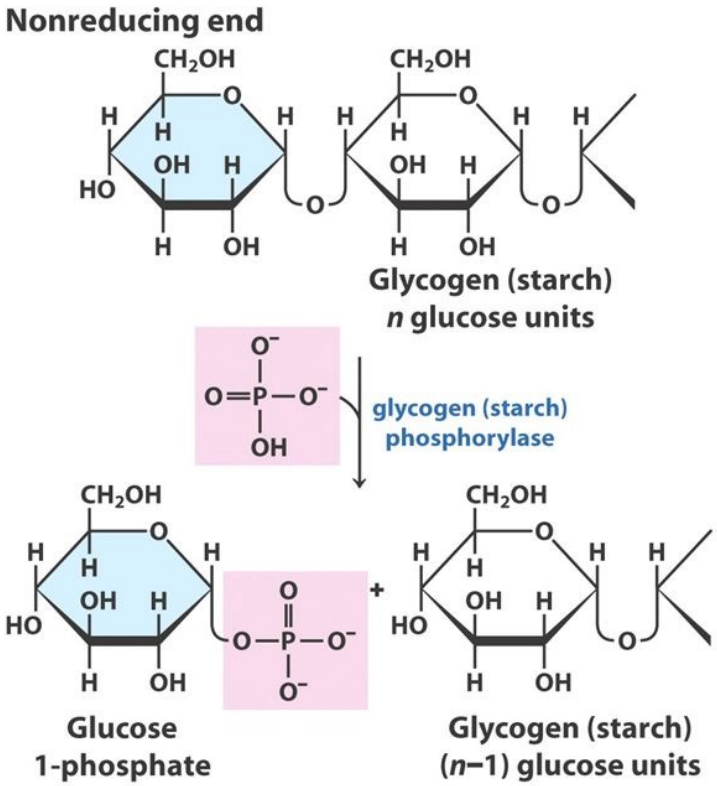
S_{MG} vaccination elicits stronger immune responses than SFG in mice.

SMG was developed as a vaccine.



Sci. Transl. Med. **14**, eabm0899 (2022)

Glycogen Phosphorylase



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1st Quiz

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