Goals of the course:
- understand the biochemistry of sugars and oligosaccharides
- learn the approaches used to analyze carbohydrate content and structure
- appreciate the structural diversity of oligosaccharides in different biological systems
- understand the function of oligosaccharides in different organisms
- appreciate the current research frontiers in areas of glycobiology and the biomedical relevance of oligosaccharides

General lecture format:
glycan type/biological system:
1) structure
2) biosynthesis
3) function
4) research frontiers

Required and recommended reading material
Voet and Voet (3rd edition) - Chapter 8
“Essentials of Glycobiology” (2nd Edition) - Chapters 2, 8, 9, 15, 16, 20, 21, 39, 47 (available online at PubMed under “Books”)

Review and journal articles

Today’s lecture...
- goals of the course
- review of the syllabus and general lecture format
- recommended and required reading material
- chemical background of sugars and polysaccharides
- oligosaccharide diversity
- key concepts in oligosaccharide biosynthesis
Carbohydrate = saccharide = “sugar”

- most abundant biological molecule
- Chemical simple: C, H, O - \((CH_2O)_n\)
- Basic unit: monosaccharide
- Monosaccharide covalently link together to form polysaccharide
- Structurally more heterogeneous than protein and nucleic acids: size & composition
- Biological functions: Multiple - energy source, structural materials, mediate many key recognition events between proteins and cells.

Monosaccharides are aldoses or ketoses

D-Glucose
D-Ribulose

\((CH_2O)_n\)

Monosaccharides are aldoses or ketoses

aldoses
ketoses

Fischer projections
According to the Fischer convention, D-sugars are the mirror images of L-sugars.

D-sugar vs. L-sugar

All D-sugars have the same absolute configuration at the asymmetric center farthest removed from their carbonyl group.

Epimers

The sugars that differ only by the configuration around one C-atom.

D-glucose and D-mannose are epimers.
Sugars with a 6- and 5-membered ring are known as pyranose and furanose, respectively

- internal strain of 3 and 4-membered rings makes them less stable than the linear strains

Cyclic sugars

Hydroxyl groups and either the aldehyde or ketone groups of monosaccharides can react intramolecularly to form cyclic sugars

\[ R \text{--OH} + R' \text{--C\text{-O}} \rightleftharpoons R \text{--O--C\text{-H}} \]

Alcohol        Aldehyde       Hemiacetal

\[ R \text{--OH} + R' \text{--C\text{-O}} \rightleftharpoons R \text{--O--C\text{-R'}} \]

Alcohol        Ketone        Hemiketal

Haworth projections are convenient formulas to represent the configuration of sugar substituents

D-Glucose (linear form) \quad \alpha-D-Glucopyranose (Haworth projection)

D-Fructose (linear form) \quad \alpha-D-Fructofuranose (Haworth projection)

Sugars with a 6- and 5-membered ring are known as pyranose and furanose, respectively

- the cyclization of a monosaccharide renders the carbonyl carbon asymmetric

Cyclic sugars have two anomeric forms (α and β)

- in solution, D-glucose is a mixture of the β anomer (63.6%) and the α anomer (36.4%)
  (linear form is present in very small amounts)
- the pure anomers have unique chemical properties (i.e. optical rotation)
Sugars are often modified

- Oxidation: 1). aldehyde group → carboxylic acid group = aldonic acid (e.g. gluconic acid)  
  2). primary alcohol group → carboxylic acid group = uronic acid (e.g. glucuronic acid)

- Reduction: carbonyl group → polyhydroxy alcohols = alditol (e.g. glycerol)

- Deoxygenation of hydroxyl group: Replace hydroxyl group by H. (e.g. β-D-2-deoxyribose (DNA))

- Amino sugars: Replace hydroxyl group by amino group. (e.g. D-glucosamine, D-galactosamine)

- Methylation of hydroxyl group: occurs in nature, and also an approach for structural analysis

- Esterification of hydroxyl group: phosphate-, sulfate-

Sugars can adopt different conformations

- overall conformation of sugars is not planar: carbon centers have an sp3 hybridization

- substituents can be either axial or equatorial; β-D-glucose is the only sugar that can have all 5 of its non-H substituents in the equatorial position

- the equatorial position of OH or CH₂OH substituents is more sterically favored

Conformation: (e.g. chair or boat) shifts readily since no bonds are broken

Configuration: (e.g. α and β anomers) shift between anomers is slow in solution since bonds are broken and re-formed

Epimerization: (e.g. C2 epimers, glucose vs. mannose) does not occur without appropriate enzyme

Glycosidic bonds

- the bond that links two monosaccharides (technically, it is the bond connecting the anomic carbon to the other molecules)

C₅₋₆ – OR’ = glycosidic bond
There are basically 2 things we need to keep track of:
1) the **anomeric configuration** of the “glycosidic linkage”
2) the **identity of the carbon** on the next sugar that shares the bridging oxygen

- naming begins with the sugar farthest from the “reducing terminus” of the oligosaccharide
- the “reducing sugar” is the sugar that still contains a free anomeric carbon

![Galactose and Glucose](image)

**Lactose**

O-β-D-galactopyranosyl-(1→4)-D-Glucopyranose

**Examples of glycosidic bonds**

N-glycosidic bonds link the anomeric carbon and an amine, occurring the link between the sugars and bases of DNA and RNA
How are glycosidic linkages and oligosaccharides represented?

- symbols are used to represent sugars instead of Haworth projections or chair diagrams

**Symbolic Representation**

Glycosidic bonds are shortened to convey only the position of the anomic carbon and the carbon of the next sugar in the structure

Glucose\(\alpha_1,4\) Galactose = Glc\(\beta_4\)Gal

The diversity of oligosaccharides is enormous and gives these structures the capacity to mediate many important biological functions

- the diversity of oligosaccharide structures stems from properties inherent to sugars themselves:
  * multiple monosaccharides
  * several configurations
  * many derivatives
  * multiple possible linkages
  * branching
The Complexity of Carbohydrates

\[ C_6(H_2O)_6 \]

\[ C_6H_{12}O_6 \]

\[ \beta-D-Glucose (Glc) \]

The Complexity of a Dipeptide

Ala → Gly
Ala → Gly

\[ \begin{align*}
\text{Gal-1} & \rightarrow \text{Glc-1} = \text{Glc-1} \rightarrow \text{Gal} \\
\text{Gal-1} & \rightarrow \text{Glc-2} = \text{Glc-1} \rightarrow \text{Gal} \\
\text{Gal-1} & \rightarrow \text{Glc-3} = \text{Glc-1} \rightarrow \text{Gal} \\
\text{Gal-1} & \rightarrow \text{Glc-4} = \text{Glc-1} \rightarrow \text{Gal} \\
\text{Gal-1} & \rightarrow \text{Glc-6} = \text{Glc-1} \rightarrow \text{Gal}
\end{align*} \]
How is the diversity of oligosaccharides generated?

Key concepts in the biosynthesis of oligosaccharides:

1) Glycosylation is a non-template derived phenomenon
2) Nucleotide-linked monosaccharides or lipid-linked sugars are the high energy donors for most glycosylation reactions
3) Oligosaccharide biosynthesis is ultimately tied to pathways of sugar metabolism
4) Oligosaccharide biosynthesis requires the concerted action of multiple enzymes and proteins
5) Oligosaccharide biosynthesis is compartmentalized within the cell

Glycosylation is a non-template derived phenomenon
- the presence of certain sugars within a given oligosaccharide chain is not determined by a pre-defined plan (like transcription and translation) but depends on multiple factors:
  - expression level of glycosylation enzymes
  - availability of substrates
  - localization of glycosylation enzymes

Nucleotide-linked monosaccharides or lipid-linked sugars are the high energy donors for most glycosylation reactions
Different nucleotides are utilized for the biosynthesis of nucleotide-sugars

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Activated form</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose</td>
<td>UDP-sugar</td>
</tr>
<tr>
<td>Galactose</td>
<td></td>
</tr>
<tr>
<td>N-Acetylglucosamine</td>
<td></td>
</tr>
<tr>
<td>N-Acetylglactosamine</td>
<td></td>
</tr>
<tr>
<td>Glucuronic acid</td>
<td></td>
</tr>
<tr>
<td>Idoxylic acid</td>
<td></td>
</tr>
<tr>
<td>Mannose</td>
<td>G6P-sugar</td>
</tr>
<tr>
<td>Fucose</td>
<td></td>
</tr>
<tr>
<td>Sialic acid</td>
<td>CMP-Sia</td>
</tr>
</tbody>
</table>

- the biosynthesis of nucleotide-sugars takes place in the cytosol with the exception of CMP-sialic acid (nucleus)

Reactions:
1. \[ \text{Sugar} + \text{NTP} \rightarrow \text{Sugar-P} \rightarrow \text{Sugar-NDP} \]
2. \[ \text{Sugar(A)-NDP} \rightarrow \text{Sugar(B)-NDP} \]
3. \[ \text{Sugar(A)-NDP} + \text{Sugar(B)-1-P} \rightarrow \text{Sugar(B)-NDP} + \text{Sugar(A)-1-P} \]

Nucleotide-linked monosaccharides or lipid-linked sugars are the high energy donors for most glycosylation reactions

- glycosidic bonds require 16 kJ/mol of free energy input under physiological conditions; this free energy is acquired through the conversion of monosaccharides to nucleotide-sugars
- NDP is a good leaving group, this facilitates the reaction with the acceptor sugar alcohol of a second sugar (in cells, this reaction is catalyzed by glycosyltransferases)

Lactose synthase: a unique glycosyltransferase

- specificity of enzyme changes from N-acetylglucosamine to glucose in the presence of α-lactalbumin (mammary glands)

Examples of dolichol-linked sugars include dol-PP-glucose and dol-PP-mannose

- the biosynthesis of dolichol-linked sugars takes place within the membrane of the endoplasmic reticulum
Oligosaccharide biosynthesis is ultimately tied to cytosolic pathways of sugar metabolism

Oligosaccharide biosynthesis is compartmentalized within the cell

Nucleotide-sugar transporters utilize an antiport mechanism

- nucleotide diphosphates are converted to nucleotide monophosphates by specific enzymes within the Golgi lumen

Biosynthesis of oligosaccharides within the endoplasmic reticulum and Golgi requires the concerted action of glycosidases, glycosyltransferases and nucleotide-sugar transporters

Oligosaccharide biosynthesis is compartmentalized within the cell

- formation of oligosaccharides can occur within the endoplasmic reticulum (both sides on the membrane) the Golgi apparatus, the plasma membrane and the cytosol
The Golgi apparatus is the main site of glycosylation in eukaryotic cells.