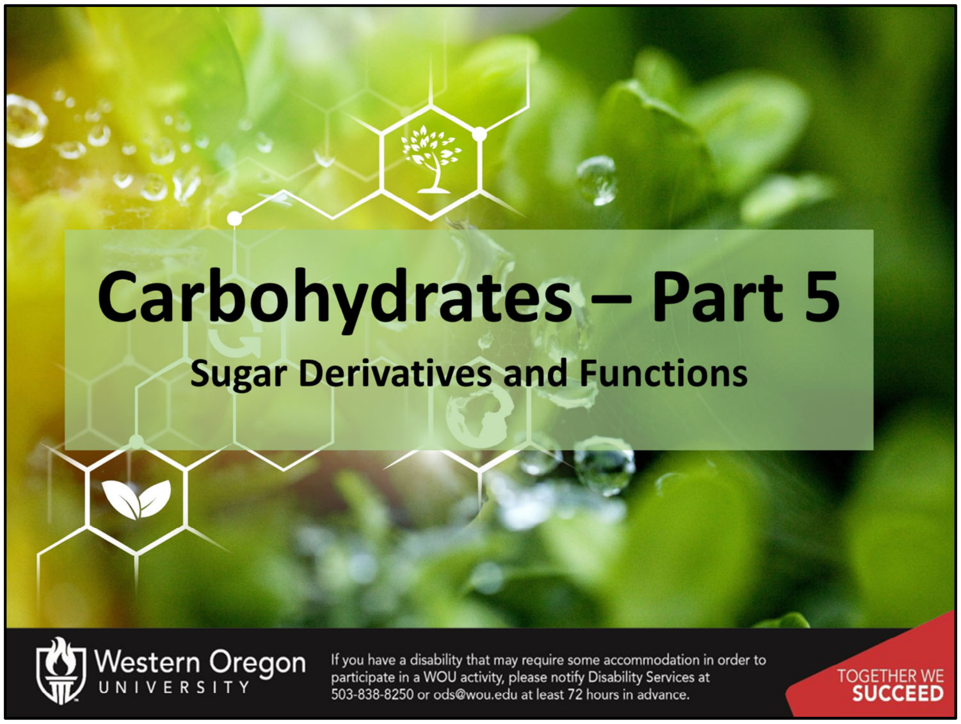
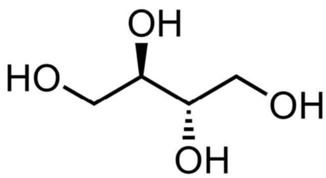


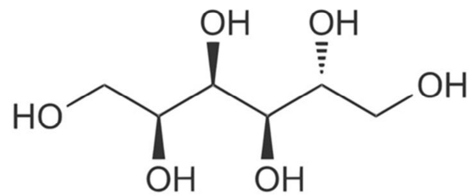
Welcome to part 5 of our carbohydrate series. In this presentation, we will discuss common sugar derivatives and their functions.

Monosaccharide Derivatives

- Monosaccharides can commonly be converted to sugar alcohols and sugar acids

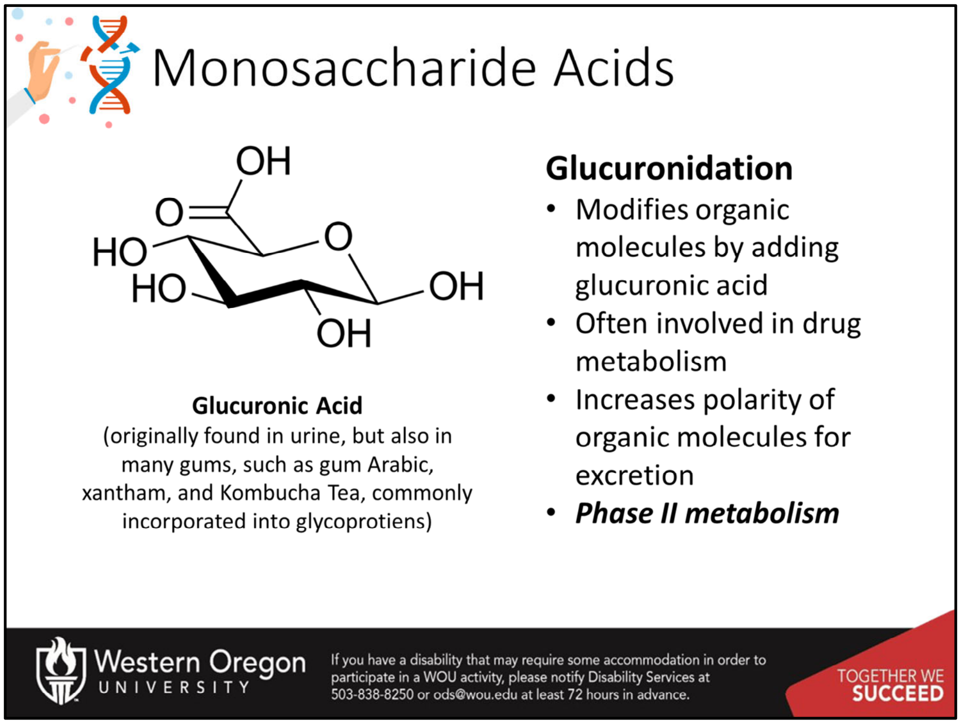


Erythritol – sugar alcohol
(60-70% as sweet as sucrose,
But contributes far fewer
calories when eaten)

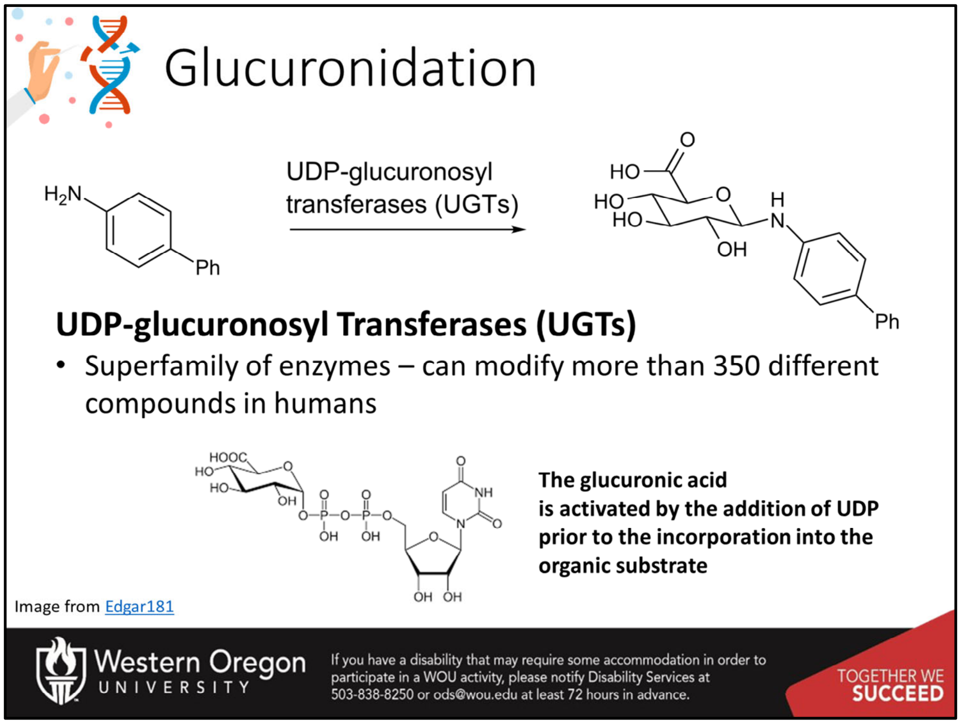


Sorbitol – sugar alcohol
(found naturally in fruits, it is also used as
an artificial sweetener. It is metabolized
more slowly than glucose)

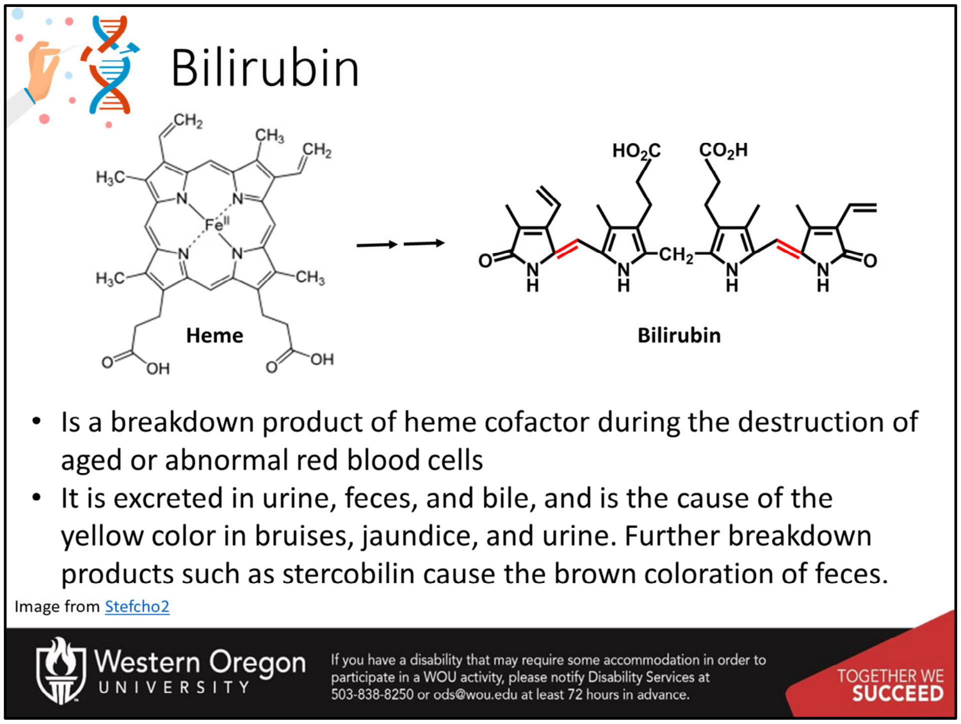
A common alteration that occur with monosaccharides involve the reduction of the aldehyde or ketone functional group into an alcohol, creating a sugar alcohol as shown here with erythritol and sorbitol. Sugar alcohols can be found as natural components of fruits and are typically not absorbed well by the small intestine. They are also metabolized more slowly than glucose, thus, they have been used as artificial sweeteners.



Monosaccharides can also be modified into acids. Glucuronic acid is one of the most important sugar acids in humans. It is commonly involved in the detoxification and removal of foreign substances from the body. In the liver, detoxification reactions add glucuronic acid to drug compounds and other organic substances injested from the diet. This increases their polarity and prepares them for excretion from the body. The process is called glucuronidation and it is an important part of phase II metabolism.



Glucuronidation reactions are mediated by a family of enzymes called UDP-glucuronosyl transferases (UGTs). These constitute a superfamily of enzymes with the ability to modify over 350 different compounds in humans, including drugs like Acetaminophen and Morphine. Note that glucuronic acid must by activated by the addition of UDP prior to being added to the final substrate. We will see this type of activation is required for other sugar metabolic pathways such as glycogen synthesis as well.



The metabolism of bilirubin is another example that utilizes the glucuronidation pathway. Bilirubin is a breakdown product of the heme cofactor of hemoglobin. It accumulates during the breakdown of old or damaged red blood cells.

Glucuronidation of Bilirubin

UDP-glucuronosyl Transferases (UGTs)

- Are utilized during bilirubin metabolism and secretion
- A deficiency in the glucuronosyltransferase for bilirubin can cause disease states with symptoms of **unconjugated hyperbilirubinemia**.

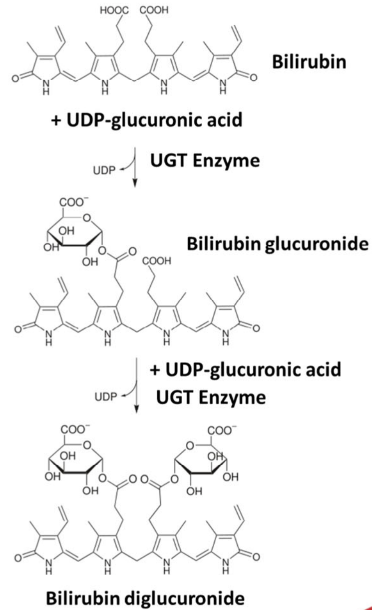


Image modified from Choij

Glucuronidation of bilirubin is required for excretion primarily through the urine and feces. Deficiency in the glucuronidation pathway leads to the accumulation of bilirubin in a condition known as unconjugated hyperbilirubinemia.

Bilirubin Accumulation

Hyperbilirubinemia could be caused by

- increased bilirubin production
- decreased uptake into the liver cells
- impaired conjugation (defect in UGT enzyme)
- interference with the secretion of conjugated bilirubin.

Causes of this accumulation could be from many factors including reduced conjugation with glucuronic acid that impairs excretion.

Crigler Najjar Syndrome

Serious Disease that often causes neonatal death

- Less than 10% activity of bilirubin UGT enzyme
- Unconjugated bilirubin accumumates in blood and brain

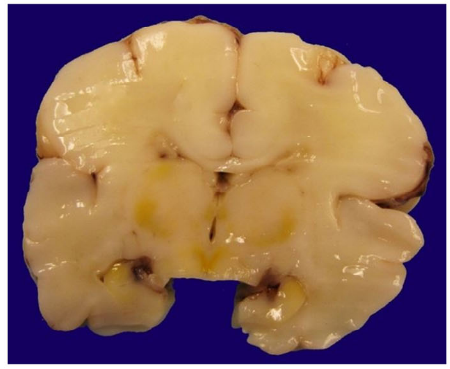
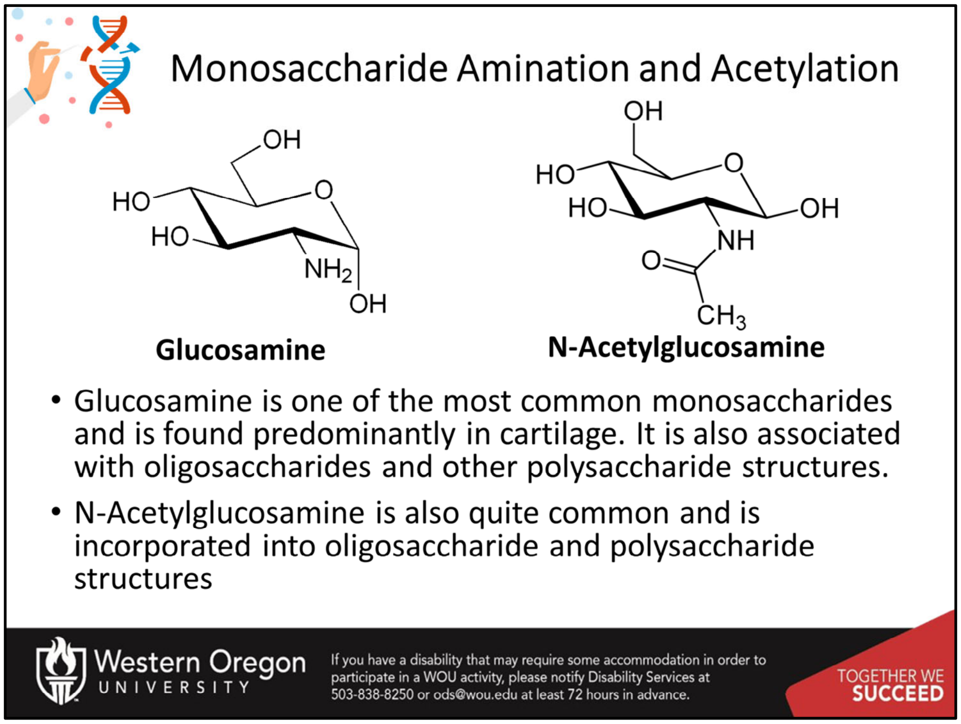
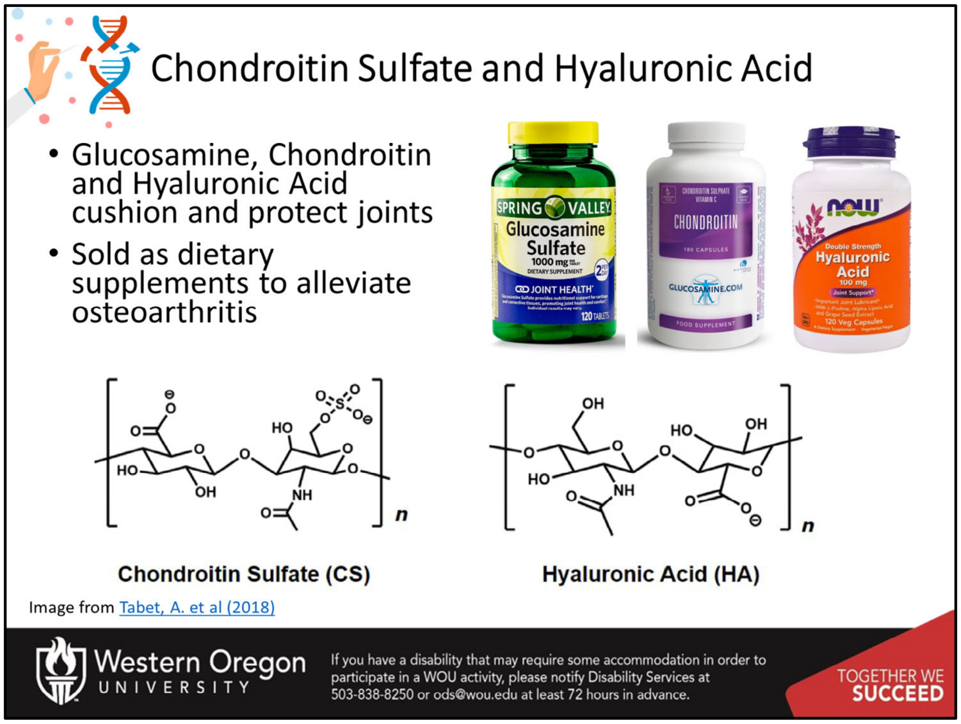


Image from Hamza, A. (2019)

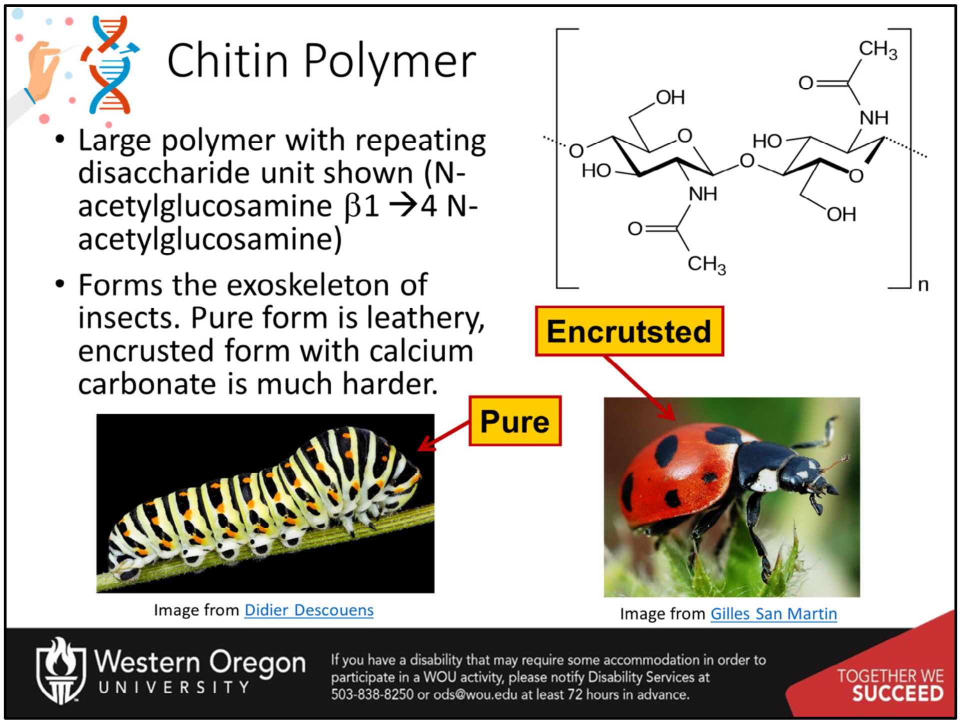
Babies born with Crigler Najjar Syndrome have less than 10% activity of the UGT enzyme required for bilirubin metabolism. This results in the accumulation of bilirubin in brain tissue, as the nonpolar compound can cross the blood-brain barrier. Death usually occurs in infancy.



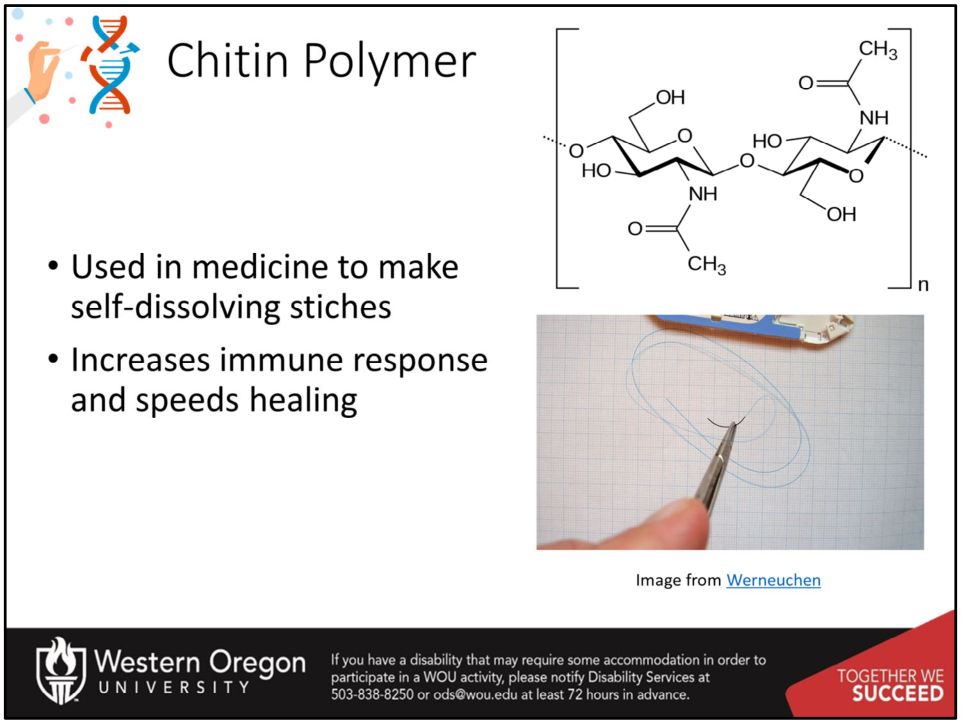
Monosaccharides may also be altered via amination and acetylation. Common metabolites include glucosamine, with amination occurring at position 2 of D-glucose. Acetylation of the amine position produces N-Acetylglucosamine. These are common metabolites within the body and are found predominantly in joints, where they provide cushioning and support. Glucosamine and other polysaccharides built from N-acetylglucosamine are commonly taken as dietary supplements for osteoarthritis and joint pain. Yearly sales of these supplements routinely generate millions of dollars with the anticipation that sales could reach $1.5 billion by 2026.



These supplements include the polymers, Chondroitin Sulfate which has a repeating disaccharide core unit made up of beta-D-glucuronic acid and N-acetylgalactosamine sulfate, and Hyaluronic Acid composed of beta-D-glucuronic acid and N-acetylglucosamine. Note that the core structural linkages are in the beta conformation, like cellulose. Fibrous, structural support carbohydrates usually have beta linkages, while energy storage/food molecules have alpha-linkages. Note that chondroitin and hyaluronic acid have many positions for hydrogen bonding and tend to swell and hold water. This water retention is thought to contribute significantly to their joint cushioning abilities. Hyaluronic Acid is also used in many skin care products, as it has the ability to hold 1,000 times its weight in water. Thus it serves as a great moisturizer.

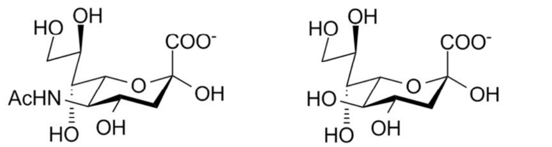


Chitin is another structural support molecule that is common to the insect world. It is a sugar polymer composed of repeating beta-N-acetylglucosamine (1→4) N-acetylglucosamine disaccharide units. Chitin forms the exoskeleton of insect. In its pure form it forms a leathery texture like that found in the skin of a caterpillar. Combined with calcium carbonate, it forms and encrusted chitin that is very hard, and is used in the shells of many beetles and other insects.



Within the medical field, we use the chitin polymer (or a synthetically modified version of it) to make self-dissolving thread used for stitches. In addition to dissolving over time, this material attracts the immune response of the host and helps to speed the healing of wounds.

Monosaccharide Acids



N-Acetylneuraminic acid
Neu5Ac

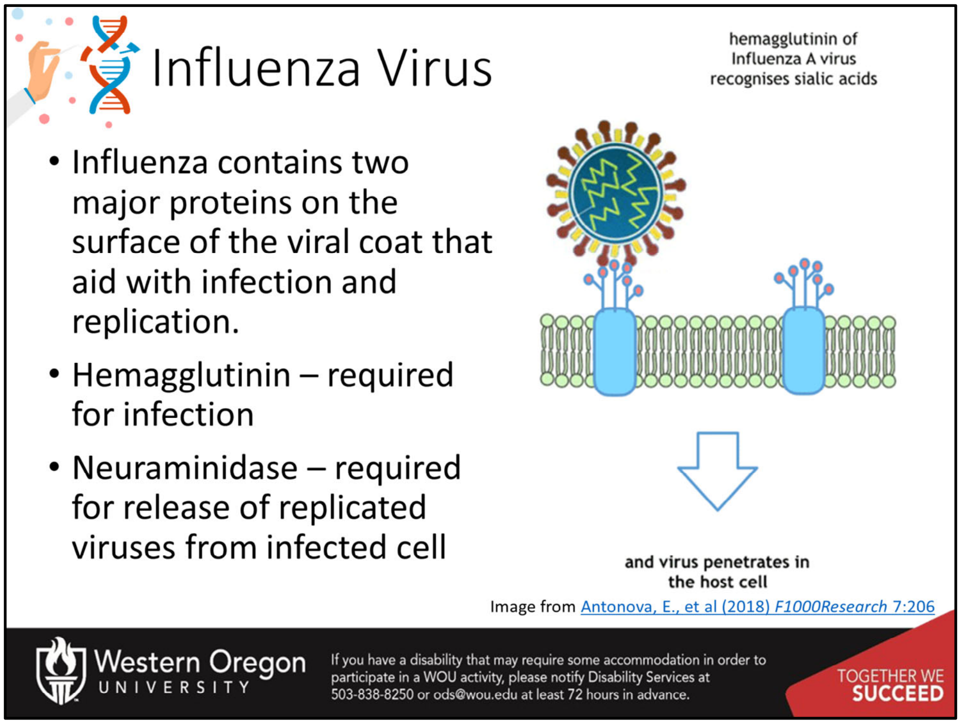
2-Keto-3-deoxynonic acid
Kdn

Sialic Acids

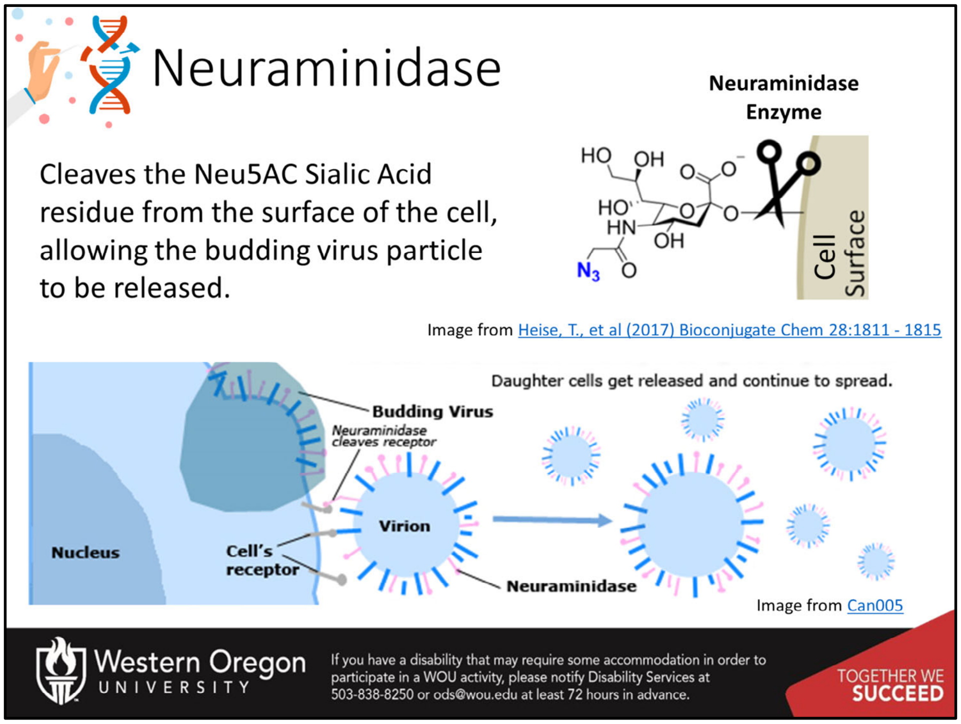
- Sialic Acids include 43 sugar derivatives, 2 common derivatives are shown above
- Commonly found as components of oligosaccharide chains in glycolipids and glycoproteins
- These residues impart a negative charge to glycoproteins

Image from Glycoform

Sialic acid derivatives of monosaccharides are also common, with 43 different derivatives. Two of the most common ones, N-acetylneuraminic acid and 2-keto-3-deoxynonic acid are shown. These types of residues are commonly found in glycolipids and glycoproteins where they impart a negative charge to those molecules.



Sialic acid recognition sequences can also be exploited by infectious agents such as the influenza virus to gain entry into their host targets. Two proteins on the surface of the influenza virus interact with sialic acid residues on the host cell to help the virus infect the cell, and then once propagated, help the virus release itself from the infected cell to continue the infectious cycle. These are the hemagglutinin and neuraminidase proteins. The hemagglutinin protein recognized sialic acid residues of glycoproteins in the upper respiratory tract to mediate cellular infection.

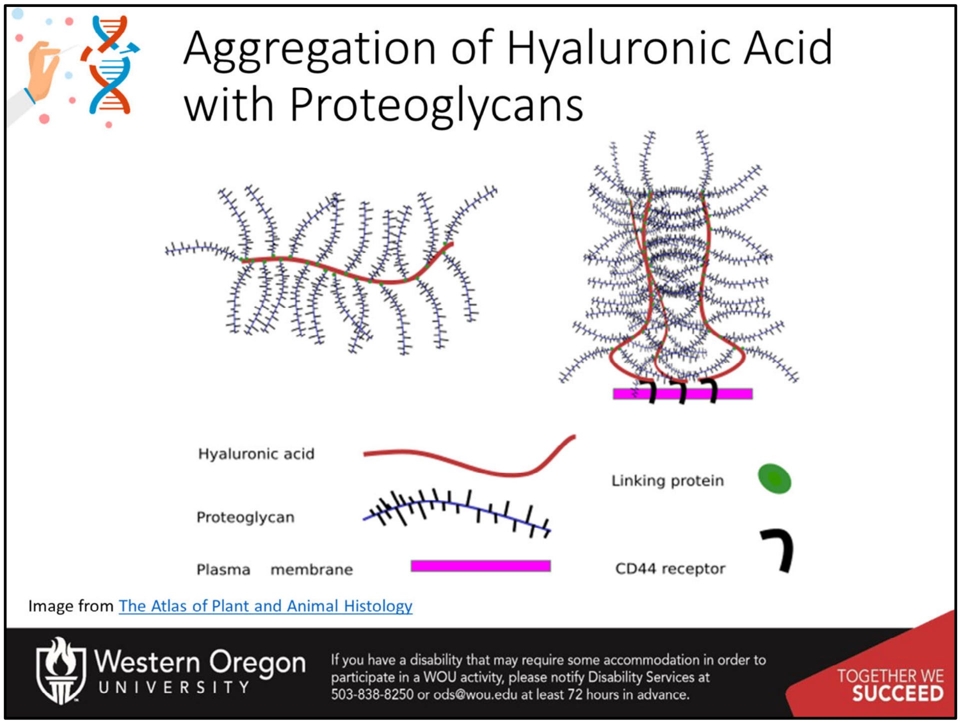


Once the virus has infected the cell, it replicates and prepares new viruses to continue the infectious cycle. The virus directs the host cell to express the viral coat proteins in the plasma membrane of the host cell. The viral genome is then replicated, packaged, and associates with the regions of the plasma membrane containing the viral proteins. New virus then bud off from the host cell. For these new buds to be released as new viral particles, the action of the influenza neuraminidase enzyme is required. The neuraminidase cleaves a sialic acid residue from the cell surface, freeing the newly budded virus from the cell. Inhibition of this enzyme blocks the creation of new infectious particles and is a drug target for influenza treatments, such as oseltamivir.

Proteoglycans vs Glycoproteins

	Proteoglycans	Glycoproteins
Structure	Protein covalently attached to one or more glycosaminoglycan chain	Oligosaccharide chains covalently attached to proteins
Location	Connective Tissue	Cell Surface
Function	Combine with collagen to form cartilage, modulation of cellular development	Cell-to-cell recognition, signaling, communication
Carbohydrate Content	50-60%	10-15%
Charge	Negatively Charged	Charge varies
Significance	Water associated with proteoglycans helps provide the cushion function of cartilage.	Carbohydrate modifications are essential for proper protein function. Changes in carbohydrate patterns are common in cancer.

Protein-sugar complexes can be divided into two major classes: the proteoglycans and the glycoproteins. Thus far, we have mainly discussed glycoproteins and their role in cell-cell communication and identification. Glycoproteins are 85-90% protein and only 10 – 15% carbohydrate whereas, proteoglycans have much larger sugar component (roughly 50 – 60% of the polymer is carbohydrate). Proteoglycans tend to be negatively charged due to the incorporation of sugar acids, whereas glycoproteins have more charge influence from the protein core structure and can vary in charge potential. Proteoglycans are found predominantly within connective tissue where they combine with collagen to form cartilage. The ability of proteoglycans to become extensively hydrated aid in their cushioning function within joints. Glycoproteins, on the other hand, are typically embedded in the plasma membrane where they serve as receptors, signaling molecules and channels to aid cell-cell communication, recognition and signaling.



Hyaluronic acid commonly forms complex aggregates with proteoglycans in cartilage. Typically, these complexes are held together with intramolecular forces such as hydrogen bonding and dipole-dipole interactions, rather than covalently being attached to the protein components.

Glycoproteins

- Oligosaccharides are attached to proteins through specific amino acid linkages:
- N-linked attachment
- O-linked attachment

Glycoproteins, on the other hand, are proteins that are covalently linked with their oligosaccharide components. The linkages occur via nitrogen or oxygen mediated bond formation.

O-linked Glycosylation

- Predominantly at Ser and Thr alcohol groups.
- Process occurs mainly in the Golgi
- Has many functions:
  - trafficking of cells in the immune system,
  - recognition of foreign material,
  - controlling cell metabolism,
  - changing protein stability, and
  - regulating protein activity.

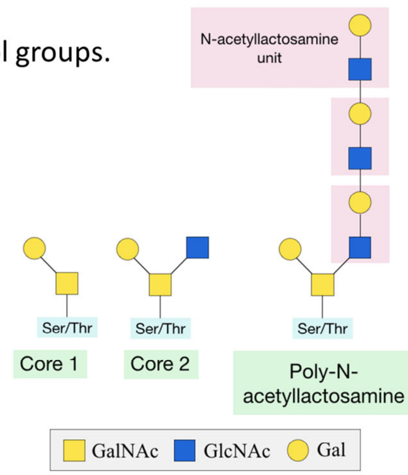
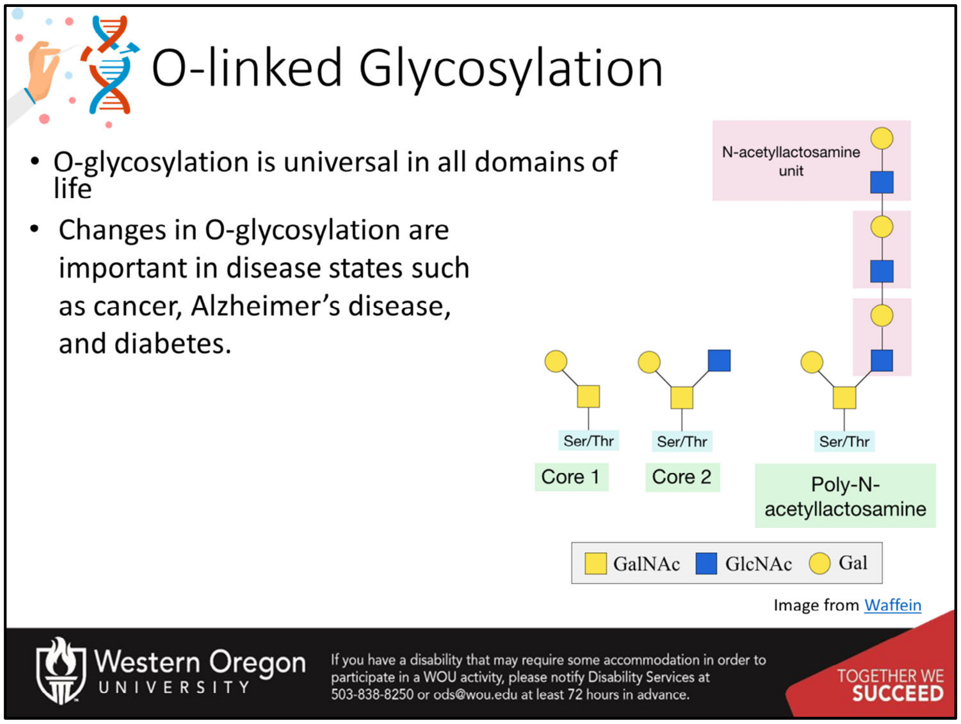


Image from Waffein

O-linked glycosylation occurs mainly at Ser and Thr alcohol functional groups. Glycoproteins are typically translated into the rough endoplasmic reticulum where they can be processed and packaged for transport to the plasma membrane. Enroute to the plasma membrane, the oligosaccharide component of the glycoprotein is post-translationally linked to the protein core structure within the golgi apparatus. The oligosaccharide component of the protein can have many different functions. It can traffic cells to their final destination, be a required component of cell-cell recognition, help control cellular metabolism, or change physical attributes of the protein, such as stability or reactivity.



O-linked glycosylation occurs widely throughout the major domains of life, from bacteria to higher order plants and animals. Changes or alterations in glycosylation patterns often result in disease states such as cancer, diabetes and Alzheimer's disease. This underscores the importance of glycosylation in protein function and activity.

Mucins

- are a group of heavily *O*-glycosylated proteins that line the gastrointestinal and respiratory tracts to protect these regions from infection
- Changes in mucins are important in numerous diseases, including cancer and inflammatory bowel disease.

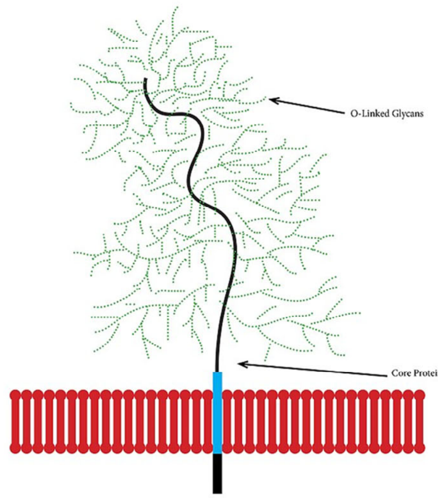
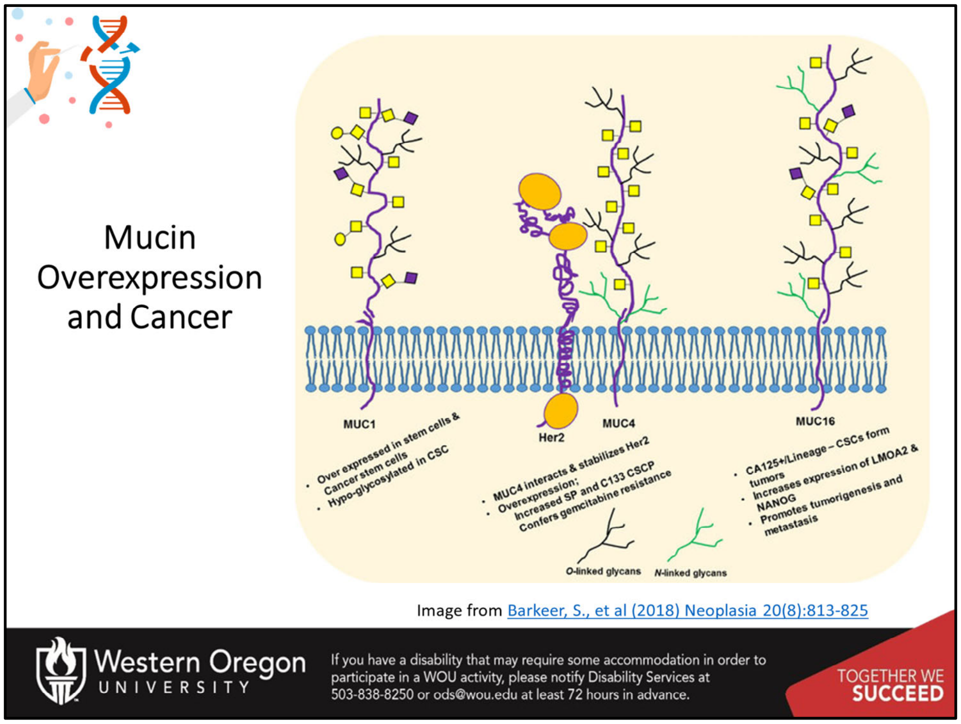
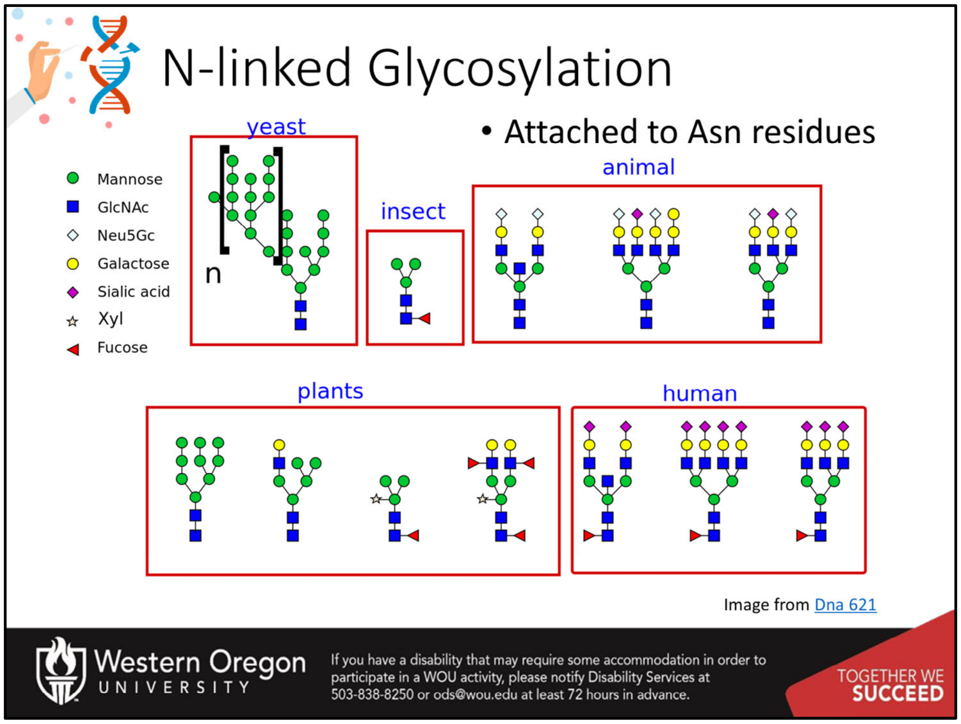


Image from [Jcastr07]

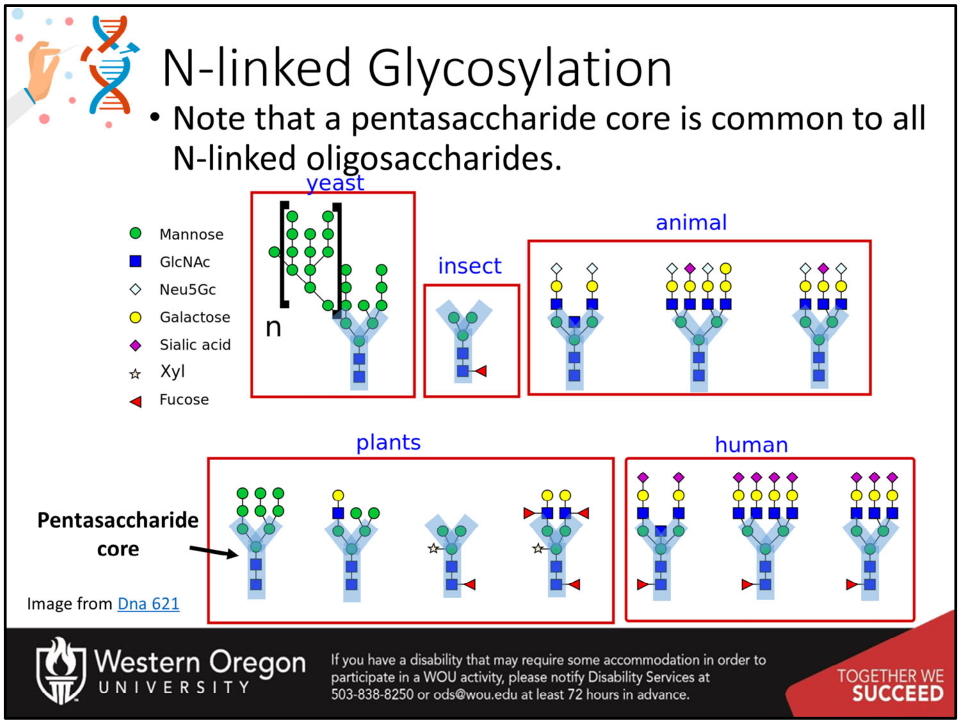
An example of a protein class that is heavily modified by O-linked glycosylation are the mucins. The mucins are a primary component of mucus and may be embedded in the plasma membrane of epithelial cells that line the gastrointestinal and respiratory tract, as well as other ducts, glands and locations that secrete mucus. The mucins can also be secreted as free floating proteins into the extracellular matrix within these locations. Due to their carbohydrate component, they can be heavily hydrated creating a gel-like surface that protects and lubricates epithelial cell surfaces that are exposed to the external environment. They also aid in the protection of these regions from infection by trapping bacteria, viruses and other microbes within the viscous mucus layer. Alterations in mucin structure have been linked to disease states such as inflammatory bowel disease and cancer.



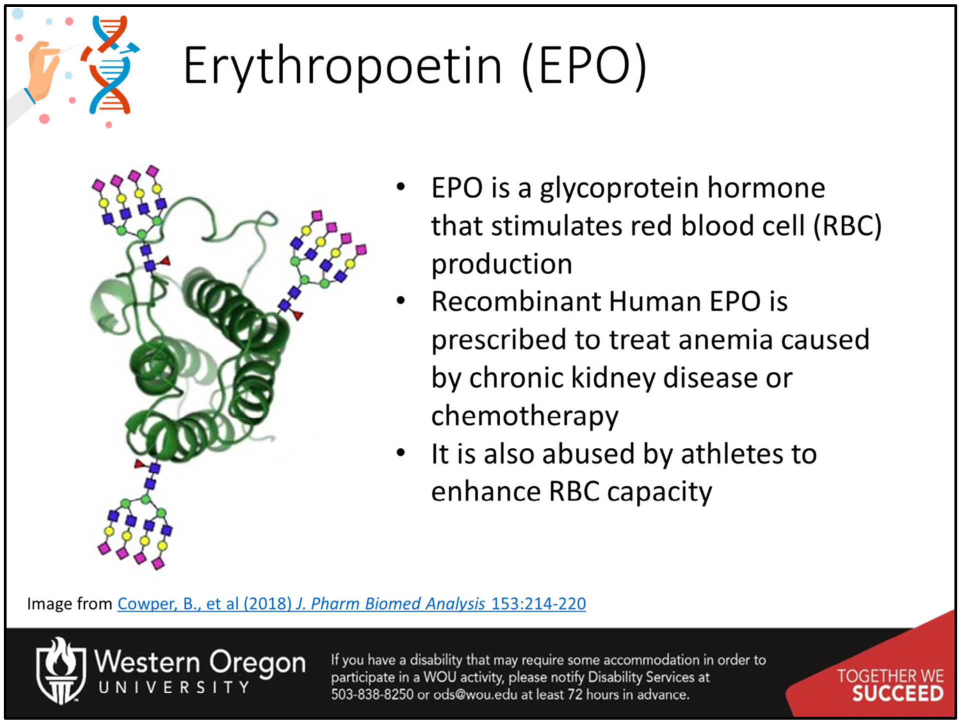
For example, mucins have been found to be overexpressed in many tumor types. In these situations, overexpressed proteins are often under glycosylated. Overexpression can also lead to the stabilization/activation of other signaling pathways such as the Her2-mediated growth stimulatory pathway. Overexpression of other mucins may play a role metastasis or the spread of tumor cells from one location to another.



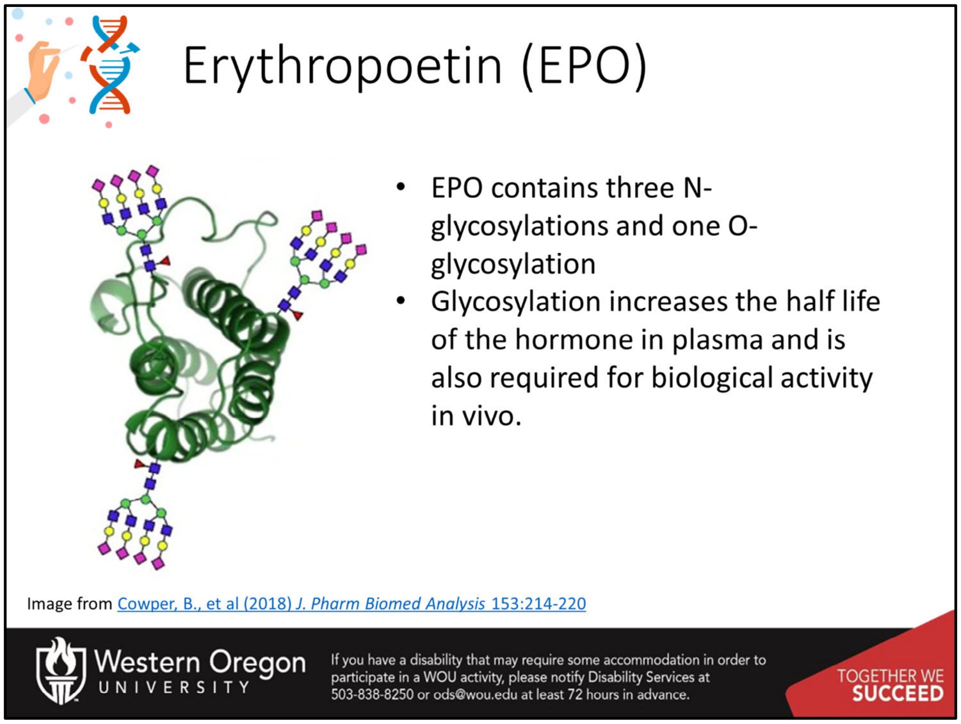
In addition to O-linked oligosaccharides, sugars can be ligated to proteins via amine functional groups as well. This commonly occurs on asparagine residues within the protein core.



The pentasacharide core, containing two N-acetylglucosamine and 3 mannose residues shown in this diagram, is required for N-linked glycosylation to occur. In contrast to O-linked glycosylation, N-linked glycosylation occurs in eukaryotes as well as the archaea domain, but very rarely in eubacteria.



An example of a protein that has N-linked glycosylation is the Erythropoetin protein (EPO). This glycoprotein serves as a hormone that stimulates red blood cell production. It is commonly prescribed to treat anemia caused by kidney disease or cancer chemotherapy. It is also abused by athletes requiring endurance training.



EPO has three N-linked glycosylations and one O-linked glycosylation that contribute to its function. Glycosylation increased the half-life of the protein in plasma and is also required for the biological activity of the hormone.

Immune System Function

- *N*-linked glycans on an immune cell's surface will help dictate that migration pattern of the cell,
- e.g. immune cells that migrate to the gut lumen have specific glycosylations that favor homing to that site

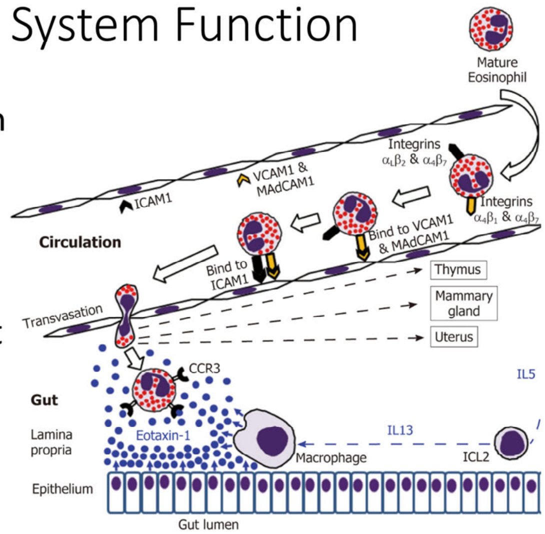
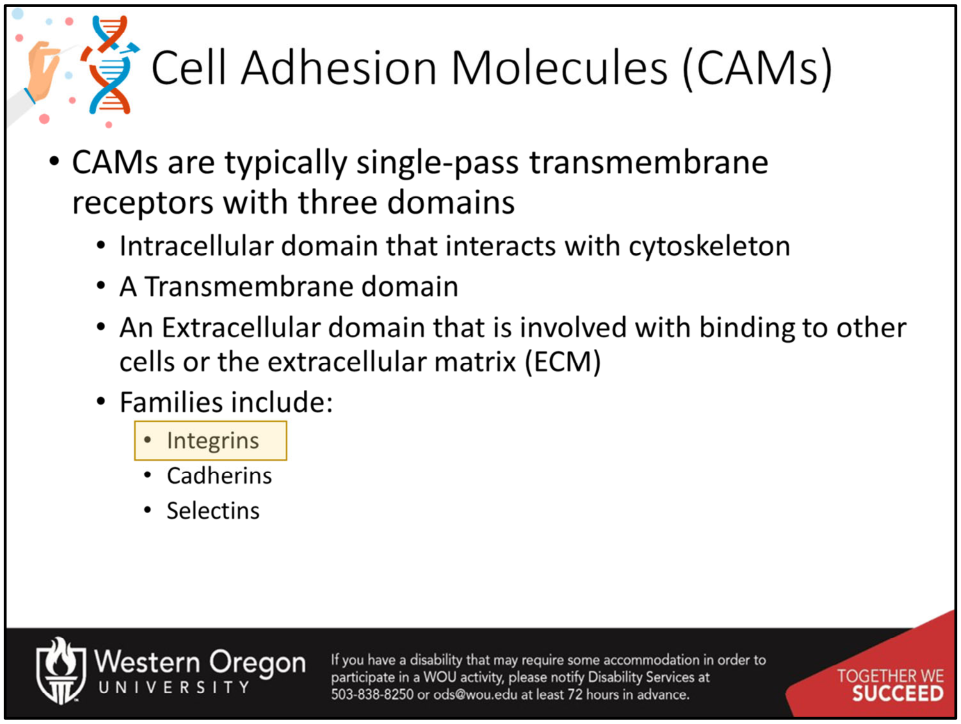
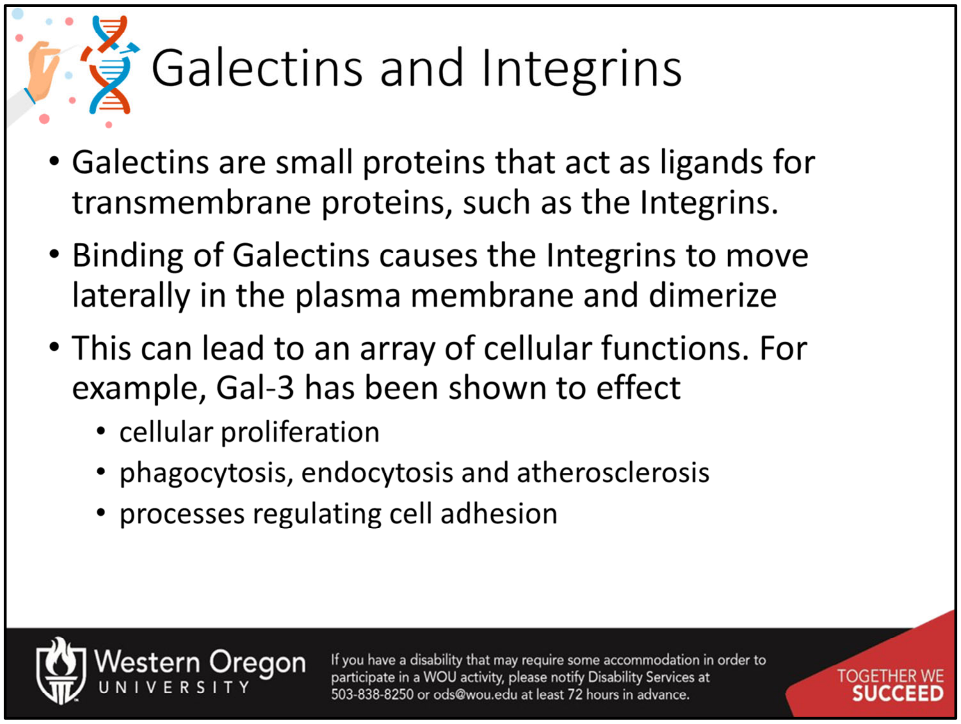


Image from [Loktionov, A. (2019) World J Gastroenterol. 25(27):3503-3526]

N-linked glycosylation also plays a role in the trafficking of immune cells . Immune cells floating through the blood stream adhere to signal proteins on the endothelial lining. For example, immune cells that migrate to the gut lumen have specific glycosylation patterns that favor homing to that location.



Cellular adhesion is also facilitated by protein glycosylation. One class of glycoproteins involved in this process are the Cell Adhesion Molecules (CAMs). These are typically single-pass transmembrane proteins that contain an intracellular domain that interacts with the cytoskeleton of the cell, and an extracellular domain involved with binding to the extracellular matrix components or neighboring cells. The major different families of this protein class are the integrins, the cadherins, and the selectins.



We will focus on a subset of integrins that bind to a small family of ligands known as the galectins. The binding of galectins with their associated integrins cause the integrins to move laterally within the plasma membrane until they dimerize with another galectin-bound integrin. This can then activate an array of cellular responses, including cellular proliferation, phagocytosis, endocytosis, and cellular adhesion. These can influence disease states, such as coronary artery disease, by increasing atherosclerotic lesions.

Gal-3 and β1-Integrin Activation

- Gal-3 cannot bind to β1-Integrin unless the neuramidase enzyme (NEU) cleaves a sialic acid residue from the Integrin glycosyl-group
- Gal-3 binds as a homodimer to β1-Integrin causing lateral movement of integrin within the membrane until it can dimerize with another β1-Integrin receptor

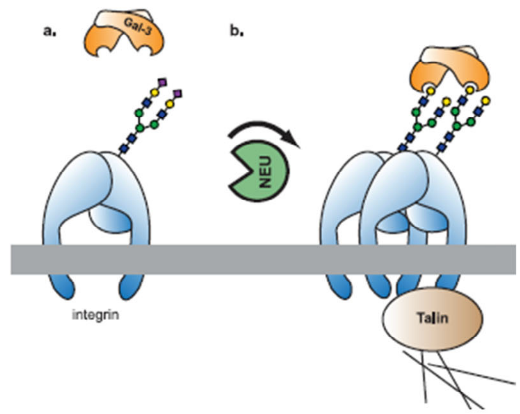
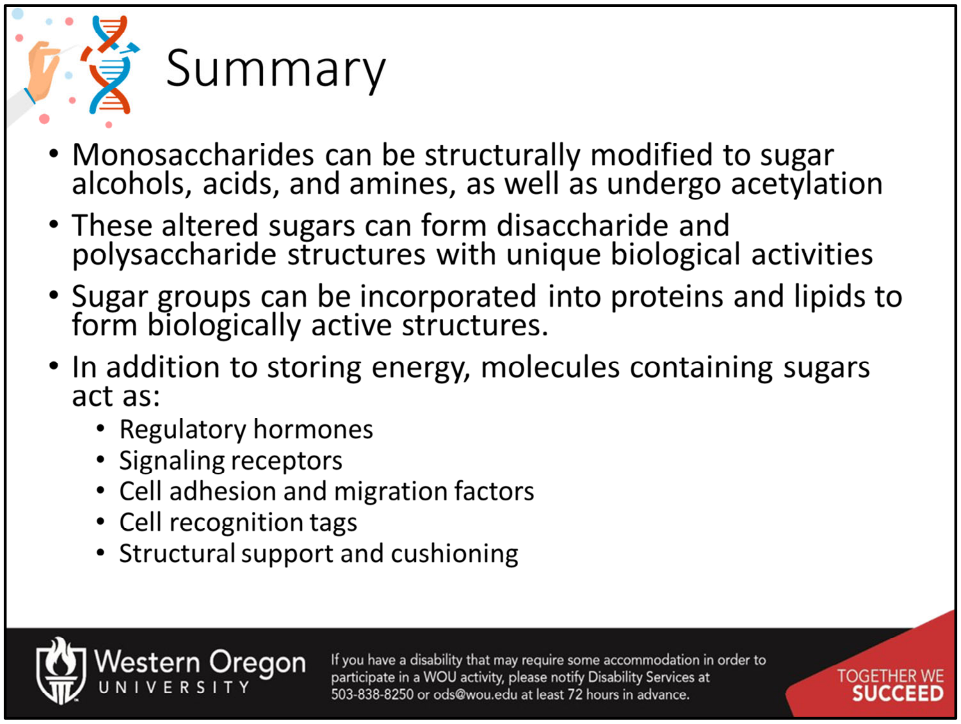


Image from [Yang, E.H., et al (2017) *PLOS ONE* DOI.org/10.1371](DOI.org/10.1371)

This slide shows the activation of beta-1-integrin by the gal-3 ligand. Note that the gal-3 ligand cannot bind with the integrin unless a neuraminidase enzyme cleaves the sialic acid residue from the integrin glycosyl functional group. Gal-3 binding can then lead to the dimerization of the integrin receptor and further activation of downstream pathways.



Overall, in this section, you have been exposed to some of the unique chemical modifications that sugars can undergo in vivo and how these altered sugars can be combined to form oligosaccharide and polysaccharide structures with diverse functions. You have also learned about the integration of carbohydrate structures into proteins and lipids, with a focus on glycoproteins, and proteoglycans. We will come back and talk further about glycolipids when we introduce lipid structures more fully in a later chapter. It is clear that carbohydrates play a diverse and important role in cellular function, supplying energy resources, as well as contributing to cell-cell communication, signaling, identification and trafficking, and structural support and cushioning.