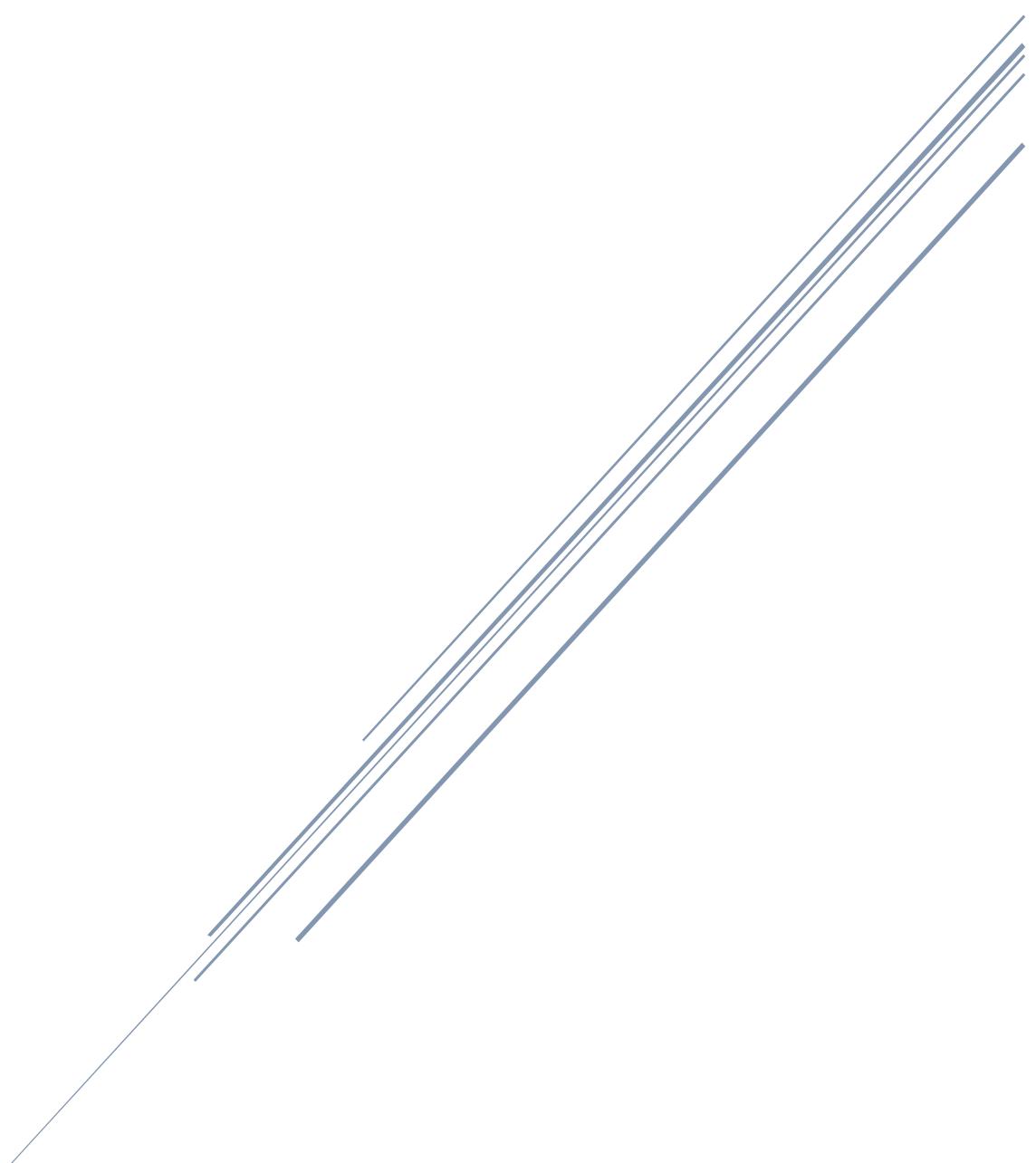


BIOCHEMESTRY 1.1

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La Sapienza
Bioinformatics

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Carbohydrates:

Storage of energy, structural function, nucleic acid components, signal transduction among cells all depend on carbohydrates. Glycoproteins are involved in signal recognition and sorting, in which sugars are covalently linked to a protein. Photosynthesis: chlorophyll and other photosynthetic pigments absorb energy as ATP and NADPH, used to reduce CO₂ and form ATP, starch and sucrose. Glucose is synthesized from CO₂ and H₂O with O₂ being created as a biproduct.

Structure of Monosaccharides:

Carbohydrates as sugars have the form C_nH_{2n}O_n (C_n(H₂O)_n). We also have other monosaccharide categories, polyhydroxy aldehydes have an aldehyde group on their primary carbon, while polyhydroxy ketones have ketone functional group on secondary or tertiary carbon.

Monosaccharides begin in complexity as trioses. This includes glyceraldehyde, and the corresponding ketone, dihydroxyacetone. Sugars all contain many hydroxyl groups. After glyceraldehyde, the hydroxyl group can be added to the right, or the left, and therefore each added carbon of the monosaccharides has two possible sugars.

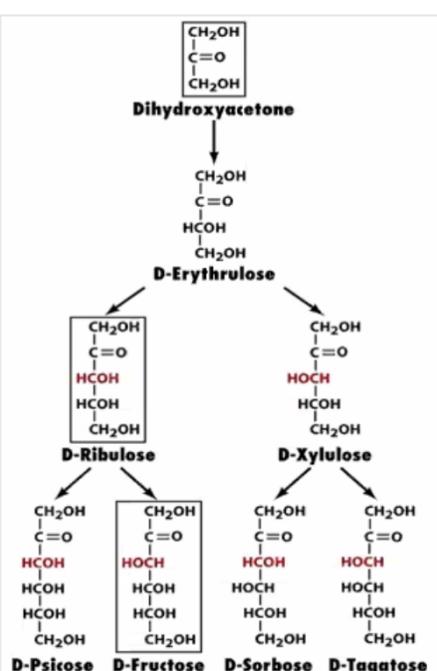
Important: Glyceraldehyde(1) structure. What are the 4 carbon aldoses(2a, 2b), the aldotetroses. D-Ribose (3a), structure, other 3 pentoses not important. Aldohexoses, Glucose(4c), Mannose(4d), Galactose(4g) structure. Mannose is an C2 epimer of glucose, and Galactose is a C4 epimer, so they are easy to remember. Monosaccharides have a range of 3-7 carbon size.

Monosaccharides are chiral molecules, thus the D/L distinction.

Polyhydroxyaldehydes and Polyhydroxyketones

$\text{CH}_2\text{OH} - (\text{CHOH})_n - \text{CHO}$
ALDOSES

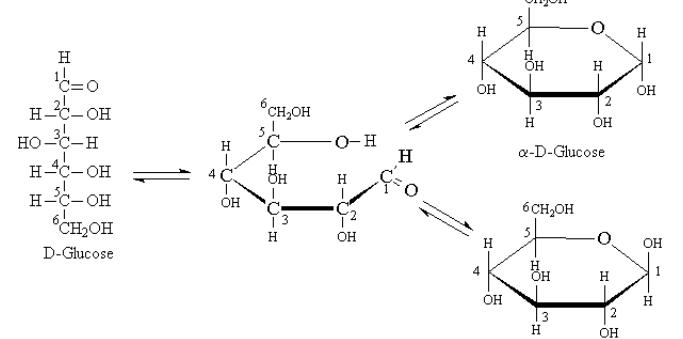
$\text{CH}_2\text{OH} - \text{CO} - (\text{CHOH})_n \text{H}$
KETOSES



Ketoses version of Monosaccharides of importance:

Dihydroxyacetone, D-Erythulose (because it's the only 4 carbon ketose), D-Ribulose, D-Fructose. D-series sugars always have the hydroxyl group on the right for the lowest carbon, (furthest carbon from carbonyl) and we only find D-series in nature.

The number of stereoisomers is 2^n where n is number of asymmetric carbons.



Monosaccharide Derivatives

Hemiacetalic Bonds: Monosaccharides with intramolecular hemiacetalic bonds yield a cyclization of the molecule. This is seen in glucose, which has a disposition, from its hemiacetalic bond, to form a cyclic sugar with a hemiacetal bond linking the alcohol to the aldehyde group. Hemiketal bonds serve a similar purpose, but occur between the linkage of an alcohol group, and a ketone group.

D-Glucose forms two isomers that differ only in the configuration of the hemiacetal or hemiketal carbons. These α and β anomers interconvert between themselves through a process called mutarotation.

Aldohexoses with six member rings are called pyranoses. Aldopentoses and ketoses with five member rings are called furanoses. In the cell, we only find α-D-glucopyranose.

This cyclization is necessary because it makes the glucose less reactive. In the oxidation of glucose, we end with gluconic acid ($\text{CH}_2\text{OH} \rightarrow \text{COOH}$), which can still be cyclized to form delta-gluconolactone.

Oligosaccharides

Important: Structure of lactose, sucrose, maltose, and how to form a glycosidic bond, and how to indicate which hydroxyl group is involved in the glycosidic bond. Multiple monosaccharides are linked through condensation of the oxygen bridge, forming a glycosidic bond. When two glucose molecules undergo dehydration, the disaccharide Maltose is formed.

The most important group of oligosaccharides are disaccharides made up of two residues including Sucrose, lactose, maltose. Distinguishing features of disaccharides are the species involved, location of their oxygen bridge (1→2, 1→4), order of monomers if they are different, and the configuration of the anomeric hydroxyl group of the residues.

The formation of the glycosidic bond comes from the condensation reaction - losing water analogously to the formation of polypeptides, or nucleic acids- of the two species. This is a thermodynamically unfavorable reaction, while the hydrolysis of oligosaccharides is favorable, it is not so favorable that it occurs spontaneously, therefore the species are fairly stable in the body. Therefore, like the phosphodiester bond, the glycosidic bond is meta-stable, controlled by enzyme activity.

The α1 hydroxyl group is oxidized, and the OH- hydroxy group is removed, therefore we have α-D-glucopyranosyl-(1→4)-D-glucopyranose, also known as Maltose. This tells us the bond is between the 1 alpha hydrogen, and the 4 carbon.

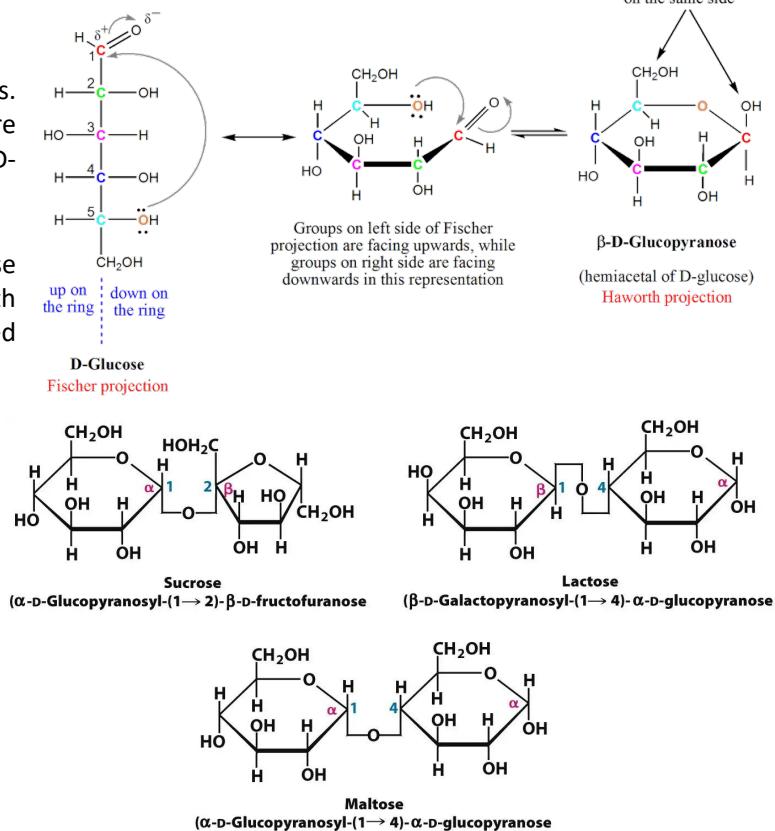
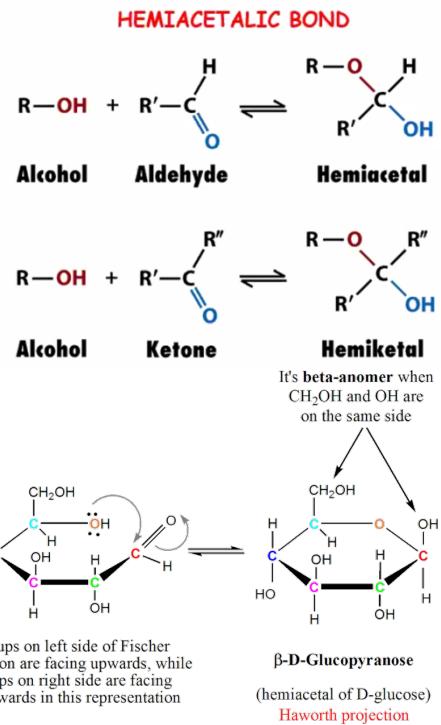
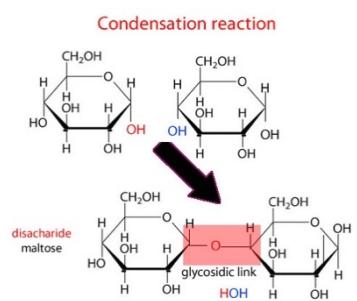


Figure 11.11
Biochemistry, Seventh Edition
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Polysaccharides(Glycans)

We have homopolysaccharides, such as cellulose, glycogen, starch and cellulose, and heteropolysaccharides, made of two or more monomers. Both of these can be linear, and branches structures. Homopolysaccharides play two important roles: energy storage (starch and glycogen) , and structural elements (cellulose and chitin). Starch is formed by amylopectin(branched) , and a-amylase (unbranched). Glycans are complex polysaccharides formed of only glucose subunits, while more specifically glucans are amylose, amylopectin and glycogen because they use α -glucose. Glycogen, like amylopectin is branched, but has much closer branching sites, thus increasing availability of terminal glucose, which can be easily hydrolyzed. Glycogen is highly abundant in muscles and liver cells. Cellulose instead play a structural role and differs from the energy storage homopolysaccharides in that it has a Beta1-4-link between its glucose units. Cellulose having a B1-4 linkage instead of an A1-4 means many animals do not possess the enzymes able to hydrolyze the polysaccharide rendering it indigestible. Chitin is very similar to cellulose and serves a similar structural role in insects, having an acetylated amino group instead of a hydroxyl in carbon-2 of each residue.

Glycosaminoglycans

Network of proteins and carbohydrates bound together to form glycosaminoglycans, and glycoproteins, as well as fibrous proteins such as collagen(structural), and fibronectin(adhesive property). Glycoaminoproteins (GAG's) are usually linked to proteoglycans, and compose 95% of the extracellular weight, and serve as a lubricant, structure, component in connective tissue, mediator for cell adhesion, and a binding factor for mitogens. GAG's are long disaccharide unit chains and use B1-4 glycosylic bonds between disaccharide units. Because they are negatively charged, they attract cations and water, allowing the matrix to adjust in fluidity, as well as provide mechanical resistance.

Glycoproteins

Glycoproteins are either O-linked: glycans attached to a threonine or serine residue, or N-linked: glycan attached to asparagine amide group. O-linked is used often in eukaryotes as protein localization signals (think Mannose-6-Phosphate for the lysosomal enzymes) while O-linked is often seen in intercellular marking and cell identification(blood groups, cell cortex) Proteoglycans and glycoproteins are both combinations of carbohydrates and proteins. Glycoproteins are conjugated proteins attached to a saccharide lacking a repeating unit, therefore protein \gg carbohydrates. Glycosylated proteins add a recognition signal, which can be readily modified or removed. Binding of saccharides also adds hydrophilicity to the protein and are known as antigens. Antigens allow us to increase complexity of the immune response without synthesizing specific proteins to bind to a specific trigger. Oligosaccharides also contribute to the three-dimensional folding of the protein. Glycogens can be N-Linked or O-linked. Blood group antigens are sugars attached to red blood cells, and determined our ABO blood group.

Lipid and Fats:

Lipids are insoluble in polar solvents, they are hydrophobic, but are soluble in organic solvents. They serve as storage for energy, structural roles in the cell membrane (phospholipids) , and can be biologically active such as hormones (cholesterol, sex hormones) and vitamins (vitamin A, E, K and D). Lipids are the major energy source in animals, and not carbohydrates. Lipids can be classified as Simple or Complex lipids. Complex lipids can be hydrolyzed to fatty acids, and Simple lipids cannot be hydrolyzed. Complex lipids include Triglycerides, Phospholipids (phosphoglycerates, and sphingomyelins), Glycolipids (Gangliosides and cerebrosides) and simple include steroids and terpenes.

Composition of lipids

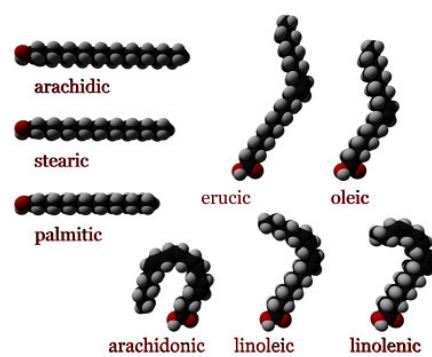
Lipids are mainly composed of fatty acids, which are made of long chain carboxylic acids. These fatty acids have 10-36 carbon atoms in the chain. Longer than this is not called a fatty acid, and only occurs in plants. At the end of the fatty acid is a COH

Source	Cellulose	Starch		Glycogen
		Amylose	Amylopectin	
Subunit	β -glucose	α -glucose	α -glucose	α -glucose
Bonds	1-4	1-4	1-4 and 1-6	1-4 and 1-6
Branches	No	No	Yes (~per 20 subunits)	Yes (~per 10 subunits)
Diagram				
Shape				

group, which is hydrophilic. Saturated fatty acids contain no carbon-carbon double bonds, unsaturated have one or more double bond (mono or poly-saturated). When the double bond appears in the cis conformation creates a kink in the fatty acid, this property contributes to membrane fluidity. More saturated fatty acids create a more rigid membrane.

Saturated vs unsaturated fatty acids

Saturated fatty acids: Palmitic acid (important) and Stearic (Important) have 16 and 18 carbon atoms respectively and can only be added in two carbons. Arachidic has 20. All of these saturated fatty acids are solid at room temperature, such as stearic acid in meat fat.



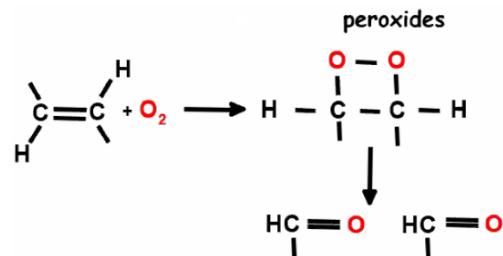
Unsaturated fatty acids: Oleic(18 carbons-c9) (Important) and Linoleic acid (18 carbons-c9c12) and Linolenic (18C-c9c12c) and Arachidonic (20C-c5c8c11c14).

All unsaturated fatty acids of interest have a cis-bond and are therefore liquid at room temperature. Cooking unsaturated acids too high breaks the cis bond and creates the saturated form. The cis bonds appear in unsaturated every three carbons after the 9, so Oleic can be converted to Linoleic by adding a cis bond at c12. Our bodies can only synthesize unsaturated fatty acids with the 9-10 cis bond, therefor we cannot synthesize linoleic, linolenic or arachidonic therefor we have to eat it, and they are there for essential fatty acids. The kink lowers the boiling point of the lipids. Omega designation system tells where the double bond is, omega-3-fatty acid has a double bond at 3.

Arachidonic acid is normally bound in the membrane. But during a stress response, such as a pathogen, arachidonic acid is released by a signal cascade, and can undergo several reaction, including Prostaglandins, thromboxane's, and leukotrienes, which regulate blood clotting, blood pressure. These pathways are the target of NSAID drugs like aspirin, which inhibits Cyclooxygenase temporarily, which blocks the formation of Prostaglandin G2. All NSAID follow this inhibitory pathway of either Prostaglandins or thromboxane's.

Reactivity of fatty acids

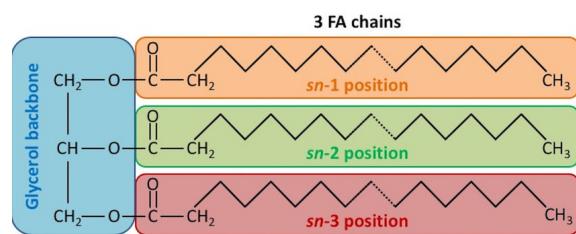
Reduction: saturated fatty acids can be reduced, when cooked, which removes the double bond and turns the unsaturated into saturated.



Oxidation: The oxidation of fatty acids produces peroxides, and the cascade of oxidation is called peroxidation cascade, forming peroxide intermediates which break down the chain, and eventually release active aldehydes which attack cellular components by binding, called free radical or oxidative stress. This peroxidation of the membrane is blocked by vitamin E.

Triacylglycerols

Triacylglycerols are the major source of fats from the diet and are formed by the alcohol binding to the three fatty acids, in the case of triglycerol, a glycerol + three fatty acids yielding a triacylglycerol formed by ester bonds (bond between carboxylic acid and an ester group) as well as 3 molecules of water. This means the oxidation of these reduced carbons on the chain will yield a high amount of energy.



Physical properties: Oils are liquid at room temperature, because they contain mostly unsaturated fatty acids, while fats are usually solid. Both oil and fats are insoluble in water. Tristearin (a fat) contains saturated fatty acids (stearic acid) and is solid at room temp, showing we can have a mixture of saturated and unsaturated, and the state at room temp depends on concentration.

Function of Triacylglycerols: 1) major energy source, due to their ability to be oxidized many times, and are therefore less reduced than carbohydrates and proteins. They can also be stored as Anhydrous, which do not contain a hydroxy group and therefore are much denser energetically -about 6 times more energy per weight than sugars- because sugars are weighted as sugars + water while lipids have no water. 2) Heat production in brown cells by oxidation of triacylglycerol, and 3) protection against temperature change and mechanical stress through adipose tissues.

Complex Lipids

Polar complex lipids are involved in the cell membrane. Phospholipids are the most common membrane lipid, and most are glycerophospholipids. The most common phospholipid is lecithin. We also have sphingolipids related phospholipid molecules.

Phospholipids have a hydrophobic head due to the polarity created by the phosphate group. The fatty acid tails are non-polar and hydrophobic. This difference in polarity by region is key for the assembly of the membrane.

Phospholipids are formed by the attachment of polar heads to the non-polar tails, but the phosphate head can be added to by other amines in specific cases. No matter how the polar head is modified, it maintains its polarity. We don't need to know all the types of polar heads found in glycerophosphates, only that others exist. In the membranes, we never find triglycerides, only phospholipids, due to their polar head and non-polar tails.

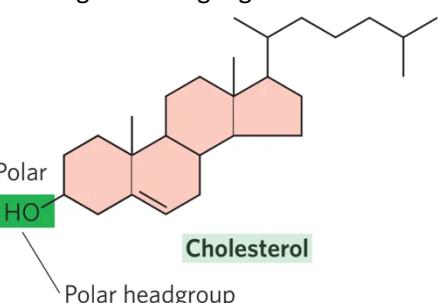
Phospholipases hydrolyze the ester bonds to the glycerol resulting in the release of the free fatty acid into the cell, which happens when we consume phospholipids.

Sphingolipids which differ from Glycerophospholipids, in the alcohol attached, In this case an amino alcohol. This means the second fatty acid is bound to the phosphate head with an amino bond instead of an ester bond. Sphingomyelin is found in the brain very often. We only need to know that we can have both amine bonds, and cerebrosides, which contain sugars attached. Sphingolipids are still amphipathic like phospholipids and play a role in structure of cell membrane.

Liposomes are spontaneously forming vesicles made of self-assembling lipids, such as in plasma membrane. Liposomes are important in drug delivery in the body, increasing bioavailability. Glycolipids are formed by the addition of sugar to the -OH of the lipid head, important in cell marking and messaging. Glycolipids contain one sugar while gangliosides contain Oligosaccharides. That's all we need to know about glycolipids.

Simple Lipids, Steroids

Steroids are simple lipids that contain a fused-ring control system called the steroid nucleus (in pink). Simple lipids cannot be degraded or broken down. Cholesterol is the most abundant steroid, and many other steroids are synthesized from it. Cholesterol is highly hydrophobic, except the one -OH group. When cholesterol is stored, it is converted to the ester form in the blood. Cholesterol functions as a space filler and regulates membrane fluidity, making up 30-40% of the membrane. The steroid nucleus is highly hydrophobic and increases the interaction between neighboring phospholipids, hence more cholesterol increases the rigidity of the membrane. Some cholesterol derivatives include testosterone, cortisol, prednisone which all maintain the steroid nucleus structure, but vary in the hydrophobic -OH to -O and the tail.



Membranes structure:

Membranes are composed of phospholipids, proteins, and cholesterol, as well as carbohydrates in the form of glycolipids and glycoproteins. All cells have a plasma membrane, eukaryotes have many other membranes around membrane bound organelles. Proteins and lipid elements can diffuse freely literally on the membrane, because of the fatty acids, and depends on cholesterol and saturation of fatty acids. On the other hand, we need some rigidity to keep everything in place. This ability to move around while maintaining composure is called the fluid mosaic model.

Membrane lipids

We find membrane lipids, divided into phospholipids: glycerophospholipids, sphingolipids, and glycosphingolipids, as well as simple lipids such as cholesterol. The concentration of these lipids varies greatly depending on the membrane. Plasma membrane has a lot of cholesterol, mitochondria has a lot of phosphatidylcholine. The lipid bilayer is asymmetrical, glycolipids are more likely to be found on the extracellular side, while lipids like phosphatidylinositol and phosphatidylserine are found on the cytosolic side.

Membrane fluidity can be manipulated in response to temperature or other environmental signals, shorter tails are more fluid, and increase in unsaturated fatty acids increase fluidity as well. Membrane lipids can flip-flop alone, but it's extremely slow. Flippases, floppase and scramblase enzymes can instead speed this flip-flop up.

Membrane proteins

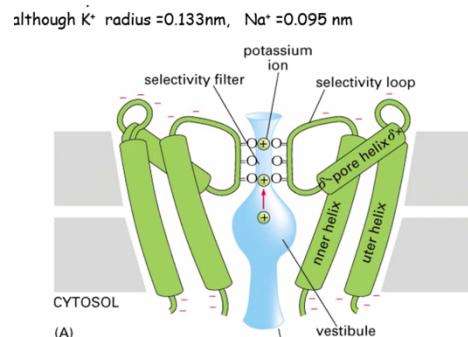
Membrane proteins can be peripheral, or integral. Integral is bound tightly to the interior of the bilayer, while peripheral proteins are lipid, or protein linked. Integral membrane proteins are hard to isolate to study, while peripheral are easier to remove. Membrane proteins serve as transporter, enzymes, receptors, cellular identifiers, cell adhesion, and cytoskeleton connections. Hydrophobic regions cannot cross the membrane and lead to integral membrane proteins.

Lipid linked proteins can bind through hydrophobic interactions, or covalent bonds -involving Cystine. Peripheral proteins interact directly with one of the phospholipids in the membrane, through a covalent bond. An example of peripheral proteins is cytochrome-c. Glycoproteins serve as cellular markers in the cells glycocalyx. The oligosaccharides of glycoproteins can be recognized and exploited by receptors on other cell surfaces or viruses.

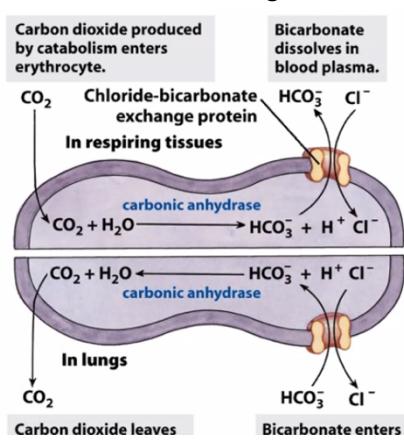
Glycocalyx is the fuzzy coat around animal cells, consisting of carbohydrates in the form of glycolipids and glycoproteins. Protein scaffolds bind to the actin filaments inside the cell, providing correct placement as well as mechanical support.

Transport across membranes

Hydrophobic small molecules, as well as uncharged polar molecules can freely cross the membrane, while large polar molecules, or charged ions need to be transported by specific proteins. Components can enter the cell through passive transport or active transport, that is with or against the concentration gradient. Passive transport can be in simple diffusion(O_2 , CO_2 , H_2O) of nonpolar hydrophobic molecules, or facilitated diffusion, in which we need a transporter, but no energy is required. Active transport requires energy, because it's against the gradient such as the case of the sodium potassium pump.



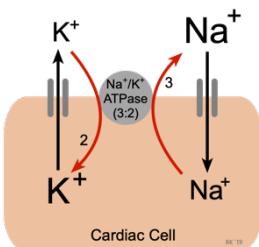
Passive transporters can be divided into carriers, or channels, differing mostly in the rate of diffusion, where channels are much faster and less specific, while carriers are specific for a certain molecule or related family. Channels refer mainly to the transport of ions and give the membrane its semipermeability. Channels are allosteric, with a conformation change induced by voltage, ligand, or mechanical activation. Voltage gated channels are specific and selective for the size of the ion, too big cannot enter, and too small cannot connect the specific amino acids inside, which induce the confirmational change called the specificity of the pore. Ligand-gated channels open in response to the binding of the specific ligand and are nonspecific when open. Acetylcholine stimulates pentamers and allows the entrance of Na^+ into the cell to activate the action potential in neurons. Even in the case of molecules that can simply diffuse, we can actively transport them in cases such as H_2O to increase the turnover rate, seen in aquaporins, water selective (H_3O^+ not allowed) pores made of alpha helices at a higher rate.



Carriers instead are stereospecific, and saturable, meaning they have a maximum rate of import, which channels do not have. Carriers also undergo allosteric rearrangement, but there is never an open channel all the way through the membrane, meaning one side opens and the other closes. GLUT1 is a 12-alpha helix integral membrane protein that imports glucose into the cell, and forms glucose-6-phosphate, maintaining the higher concentration outside the cell. Carbon dioxide is also transported out of the cell

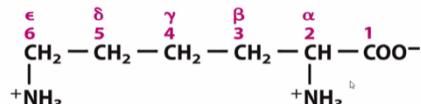
through facilitated diffusion, by converting it into bicarbonate, which can be solvated into the blood. From the blood, it is transported to the lung, where the reaction is reversed, creating again carbonic acid, where it is freely released out of the lung.

Active transport moves molecules against concentration gradient. This is achieved by maintaining membrane potential, through ion pumps. In the cytosol, ATPases keep the concentration of potassium high, and sodium low, the opposite of the extracellular concentration. The Na-K pump is the most important in biology, which costs one ATP and pumps out 3Na⁺, while pumping in 2K⁺ per cycle. Large molecules enter through endocytosis, in vesical bundles, broken into phagocytosis(large) and pinocytosis(small).



Proteins:

Amino Acids

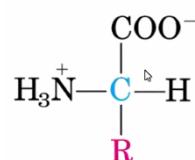


Lysine

Proteins are macromolecules formed of H, C, O and N, with different combinations called amino acids. Amino acids follow the same structure, with an R group being variable in different amino acids. N composes 16% of the dry weight in the organism, and therefore excess amino acids are eliminated, through urine. In addition, sulfur, and phosphate atoms in variable amounts. Proteins can be classified as enzymes, transporters, storage, defense, structural, contractor, signal transducer, and gene regulator. These different functions depend on the sequence of different amino acids, all only differing by their R constituents. Amino acids also appear in L and D confirmations due to the chirality of their central carbon. In humans, there are only L amino acids, and in bacteria, only D. Carbon-1 is the carbon in the carboxyl group, COO⁻, so when numbering we start there, and continue down the R chain.

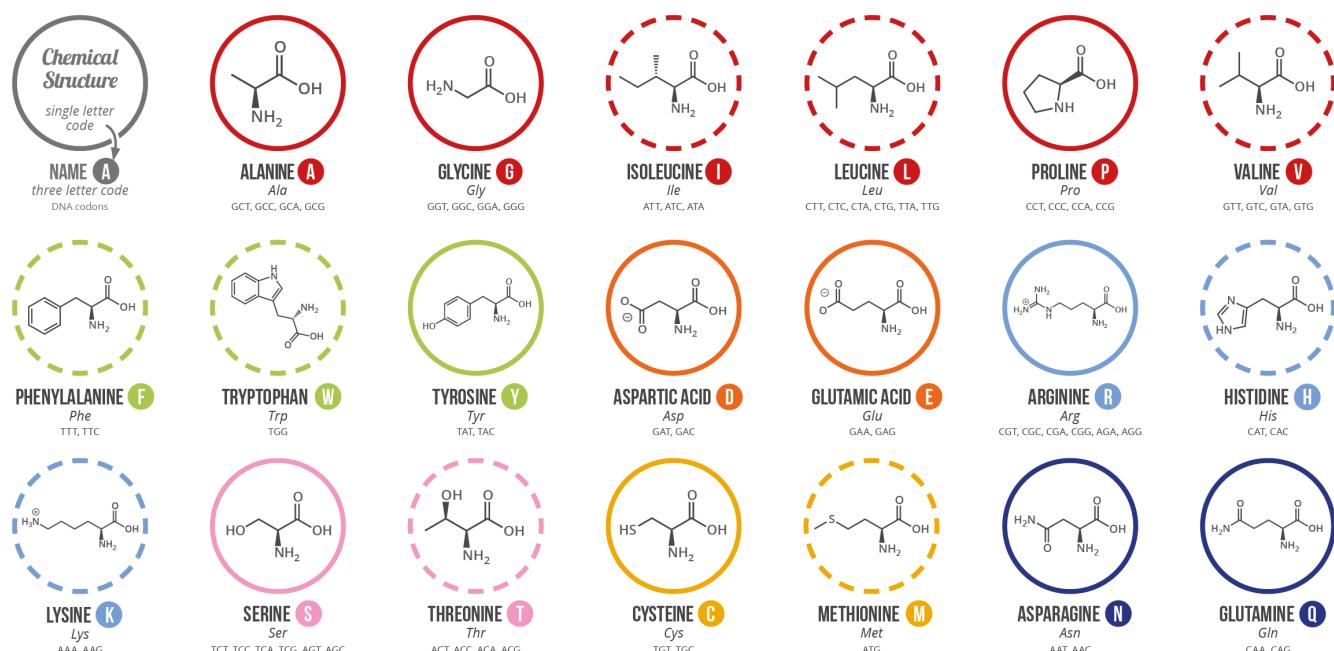
Amino acids can be grouped into 5 broad categories:

- Nonpolar, aliphatic (Alanine, Glycine, Proline, Valine, Leucine, Isoleucine, Methionine)
- Aromatic (Phenylalanine, Tryptophan, Tyrosine)
- Polar, uncharged (Serine, Threonine, Cysteine, Asparagine, Glutamine)
- Positively charged (Lysine, Arginine, Histidine)
- Negatively charged: (Aspartate, Glutamate)



AMINO ACIDS ARE THE BUILDING BLOCKS OF PROTEINS IN LIVING ORGANISMS. THERE ARE OVER 500 AMINO ACIDS FOUND IN NATURE - HOWEVER, THE HUMAN GENETIC CODE ONLY DIRECTLY ENCODES 20. 'ESSENTIAL' AMINO ACIDS MUST BE OBTAINED FROM THE DIET, WHILST NON-ESSENTIAL AMINO ACIDS CAN BE SYNTHESISED IN THE BODY.

Chart Key: ● ALIPHATIC ● AROMATIC ● ACIDIC ● BASIC ● HYDROXYLIC ● SULFUR-CONTAINING ● AMIDIC ○ NON-ESSENTIAL ○ ESSENTIAL



Note: This chart only shows those amino acids for which the human genetic code directly codes for. Selenocysteine is often referred to as the 21st amino acid, but is encoded in a special manner. In some cases, distinguishing between asparagine/aspartic acid and glutamine/glutamic acid is difficult. In these cases, the codes asx (B) and glx (Z) are respectively used.

Nonpolar and aliphatic side chains are generally found on the inside of a folded protein, where they are shielded from the water due to them being hydrophobic. Nonpolar aromatic side chains are even more hydrophobic and can ionize at high pH. Aromatic amino acids are also the most highly conjugated compounds, absorbing light at high wavelengths therefore they are easy to detect. Polar side chains can form multiple H bonds with water and are therefore often found on the surface of the folded protein. Positively charged basic side chains are often involved in proton transfer enzymatic catalysis and are also found on the outside of the protein where they can be hydrated. Finally, negatively charged sidechains are similarly positioned on the outside, but are found mainly in their negatively charged form because their titration curve is so low.

We can measure the absorption of UV in a lab to determine the amino acid composition of a protein, because different amino acids absorb at different wavelengths. Cysteines are the only amino acid that can form a disulfide bridge, a disulfide bond, also the only example of a covalent bond forming in the amino acids. Reducing agents remove the disulfide bridge in the laboratory, and give the tertiary, and 4th structure stability.

Amino acid protonation

Other uncommon amino acids exist, and usually occur from the modification of specific amino acids, such as the addition of a -OH group (hydroxyproline) or addition of a carboxyl(carboxyglutamate) or even the addition of a whole group (selenocysteine). Amino acids can also be phosphorylated, binding to the hydroxyl group to form an ester bond. This means it can only occur in serine, threonine, and tyrosine. We can also add methyl, acetyl, or adenylyl groups.

Amino acids can exist in three forms, the cationic, anionic, and Zwitterionic form. These conformations allow for different bonds, and therefore the addition (protonation, left) or removal (deprotonation, right) of protons in the environment can influence the structure of the amino acid. The protonation reaction is used in the lab and also in the body, to release oxygen in the blood. The nonionic form(first and last) has no charged groups, while the zwitterionic form(middle) has protonated amino group, and a negatively charged carboxyl group. Each amino acid has a buffering power (pI), and we can use this to test the composition of proteins.

Amino acids are stronger acids than simple carboxylic acids and have a lower pKa. This is because the affinity to release the protons on the -COOH group is decreased by the electron withdrawing property of the amino group. We also have to consider the protonated vs deprotonated forms of the amino acid, in their protonated form, they have a low pKa and therefore are stronger acids than the deprotonated form. Glutamate for example has three possible H⁺ to release, and so it has 4 forms, following the trend that the further from the amino group going first.

Conjugated Proteins

Peptides derive unique properties based on their sequence such as:

- serve as hormones, and pheromones
- Neuropeptides
- Antibiotics
- Protection, such as against toxins

We can also see different modifications affecting the structure and function of proteins, such as the addition of sugar, lipid attachments, phosphorylation, generally called conjugation of the proteins.

Proteins are polypeptides, that is covalently linked amino acids through a phosphodiester bond and form cofactors, coenzymes, prosthetic group (covalent attachments). Protein size varies hugely based on length of chain, as well as number of chains.

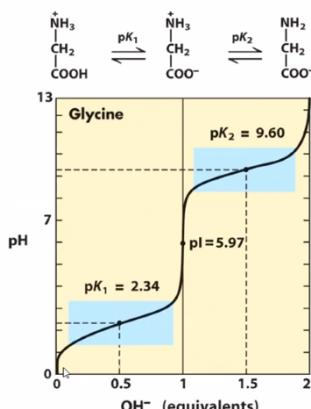
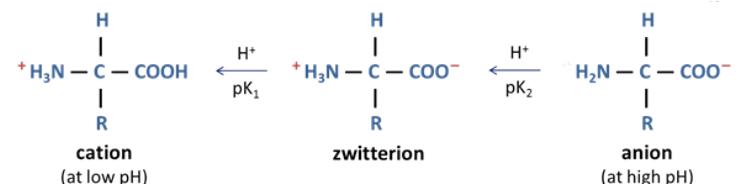
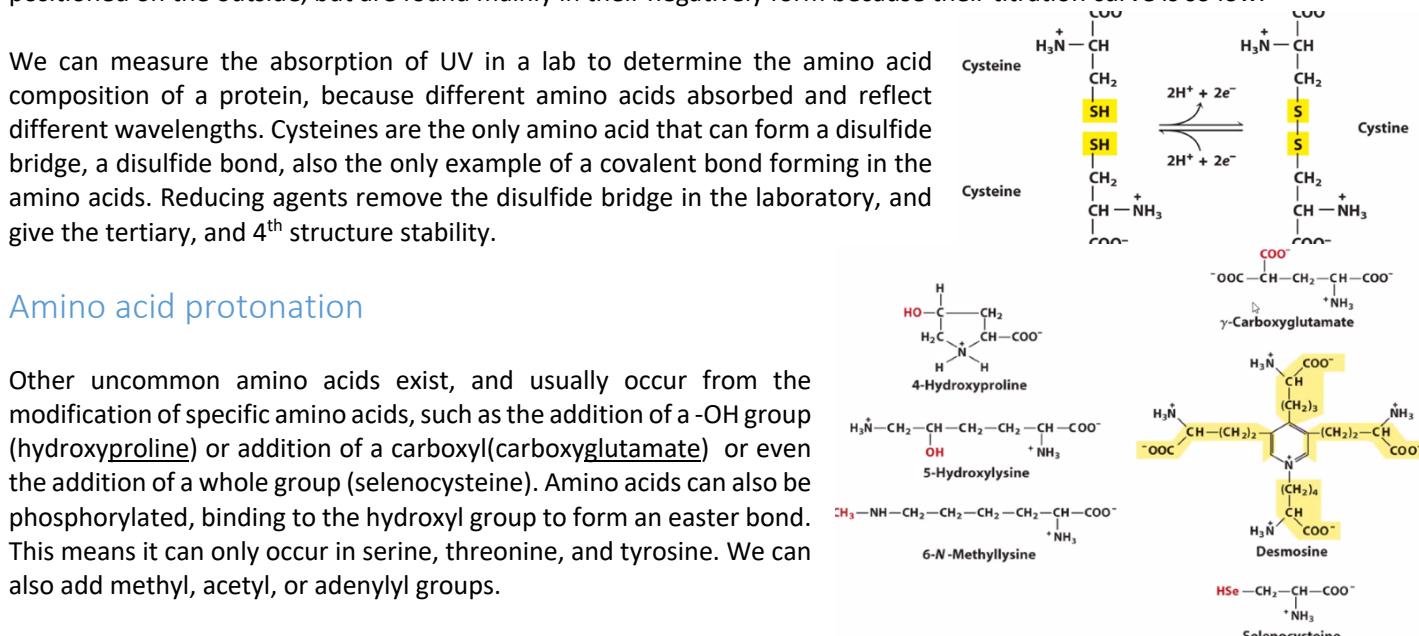
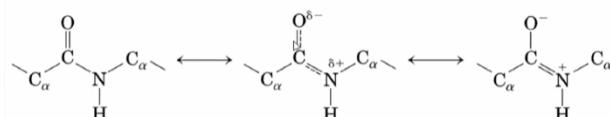


table 5-4

Conjugated Proteins		
Class	Prosthetic group(s)	Example
Lipoproteins	Lipids	β_1 -Lipoprotein of blood
Glycoproteins	Carbohydrates	Immunoglobulin G
Phosphoproteins	Phosphate groups	Casein of milk
Hemoproteins	Heme (iron porphyrin)	Hemoglobin
Flavoproteins	Flavin nucleotides	Succinate dehydrogenase
Metalloproteins	Iron Zinc Calcium Molybdenum Copper	Ferritin Alcohol dehydrogenase Calmodulin Dinitrogenase Plastocyanin

Protein structures:



Peptide Bonds: Primary structure

Protein synthesis takes place in the ribosomes, where RNA is used as the template, - something that doesn't exist in polysaccharide synthesis. The aminoacyl-tRNA begins the correct anticodon to the RNA that binds with the RNA, and also contains the next polypeptide required for the chain. This chain is elongated through the formation of a peptide bond, through the elimination of water. The amino terminal(NH₂) is written on the left, and the carboxyl(COOH) on the right, meaning the polypeptide has a directionality.

The peptide bond has a bond length between a single and a double bond, giving it a partial double bond property, and therefore can be written as a resonance hybrid bond as a 1.5, one solid, one dashed line. Therefore, this bond cannot rotate, which is the key take away. The formation of the peptide bond varies based on the pH of the medium, because of the protonated and deprotonated forms still exist, but we have to count the charge of every peptide to determine if the protein is charged or not, based only on the R group.

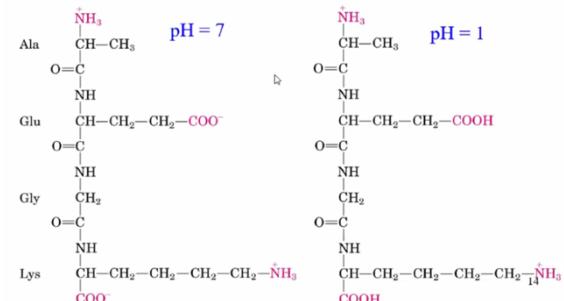
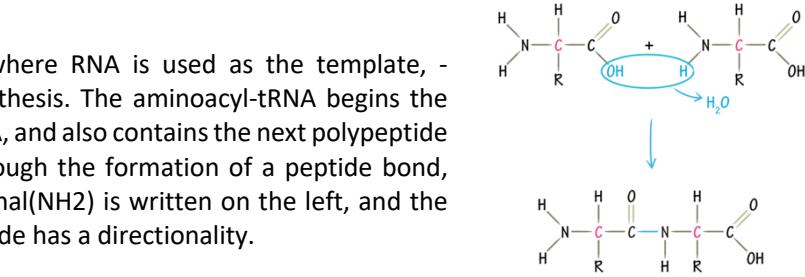
Studying proteins

Biological peptides vary greatly in size, so it's hard to generalize anything about them. They can exist as huge chains or as small as two. Dipeptides are linkages between only two peptides, but they can still be relevant in biology such as aspartame, a common sugar substitute. Another example of a small peptide is Glutathione (GSH), a tripeptide, which fights against radical stress, antioxidant properties. GSH oxidizes free reactive oxygen species by forming disulfide bonds such as free radicals and peroxides. We can also see the formation of disulfide bonds between two amino acid chains such as in insulin. Hemoglobin is an example of a large polypeptide.

Chromatography allows us to measure the concentration of proteins in a solution. Size chromatography uses a porous gel material, in which different size proteins reach different levels of penetration through the median. Ion-exchange chromatography allows the differentiation based on charge, that is positively charged polypeptides cannot penetrate the charged gel, while negatively charged can pass through the negatively charged median. We can also artificially attach charged groups to proteins in order to study them. Lastly, we have affinity chromatography, which is based on antibody antigen interactions, in which the ligand that can bind to a target protein is added to the solution, and then the mixture can be analyzed based on bonding ability to the ligand. These methods can be stacked to work more efficiently, but we must know the basic properties of the protein in order to use them. Gel electrophoresis is the most common way of separating proteins based on molecular weight, which depends on the protein's molecular weight, where the smaller proteins flow faster to the bottom, and the larger ones remain at the top. We can also use isoelectric focusing, in which proteins are added to a tube with a gradient of pH (created by ampholytes), in which the proteins move towards the region corresponding to their isoelectric point, according to their ionization (cation, zwitterionic, anionic etc.). The combination of isoelectric point, and molecular weight electrophoresis yields the segregation of proteins without mistake.

Secondary Structure

As we have seen, the primary structure of a protein is the amino acid sequence of polypeptides, linked by peptide bonds. The secondary structure instead refers to the regular and recurring special organization of the polypeptide chain, or the local polypeptide confirmation. The secondary, tertiary, and even quaternary structure are determined by the specific amino acids in the polypeptide chain. We must recall the formation of the partial double bond between the C-N in every amino acid, which absolutely does not allow free rotation. Therefore, the variation in bond angles between the C-N(omega) and C-C(Psi) -that is the peptide torsion



Structure	ϕ	ψ
right-handed α helix	-57°	-47°
left-handed α helix	+57°	+47°
parallel β sheet	-119°	+113°
anti-parallel β sheet	-139°	+135°

angles of each amino acid- determine its secondary structure, which are either classified as alpha-helix or beta-sheets. Torsional angles of proteins backbones, we see specific Psi and Omega angles in specific secondary structures.

A-Helix are stabilized only by hydrogen bonds, in which every peptide-N donates 1 hydrogen bond. This happens because every peptide bond generates an electric dipole, origination from the difference in polarity between N-H and C-O groups. Overall, the a-helix gains a net dipole moment with the partial+ on the N-terminus, and parietal- on the C-terminus. Amphipathic a-helices expose one side of to the solvent and another to the protein core, therefore exposing the hydrophilic group and hiding the hydrophobic groups. Alpha helices can be left-handed, or right-handed, just like DNA.

B-sheets can be parallel, or antiparallel and are stabilized again by hydrogen bonds. While a-helices form a compact structure, b-sheets increase the space taken. Antiparallel is usually favored because it decreases steric hinderance between the sheets. The hydrogen bonds are formed again between the carbonyl group, and the N-H. B-sheets in conglomerates can form b-barrels, important in transmembrane proteins, especially, allowing large channels to be formed.

B-turns connect 2 antiparallel b-sheets, are located on the protein surface. The beta turns connect two beta helices, often in a b-barrel. The propensity to form a-helices, b-sheets, and b-turns is highly variant by amino acid, and we can use the data to predict the secondary structures.

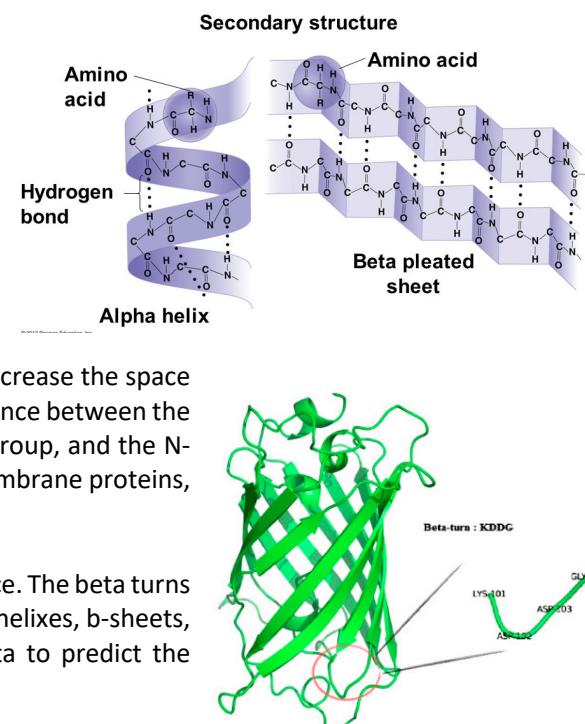
Proteins are the most versatile macromolecules in the cell, and their function is completely determined by its structure. The functional diversity results from the chemical variability of r-chains, the flexibility of the polypeptide chain, and the massive diversity of folding patters a polypeptide can create.

One of the main functions of proteins is binding. Binding is the process of molecular recognition, in which the ligand is bound by the protein. Binding is essential in signal transduction, transcriptional control, and inhibitors. Catalysis is another important function, in which a specific class of proteins called enzymes decreases the activation energy of a reaction in order to speed up the rate of reaction. Switch proteins are proteins who undergo a confirmational change depending on ligand binding, or environmental conditions. Switch proteins are important in signal transduction, and gene regulation. Structural proteins represent the major structural elements in living systems. They can associate with themselves, or other cellular elements, and form the mechanical resisting structures such as bones and connective tissues.

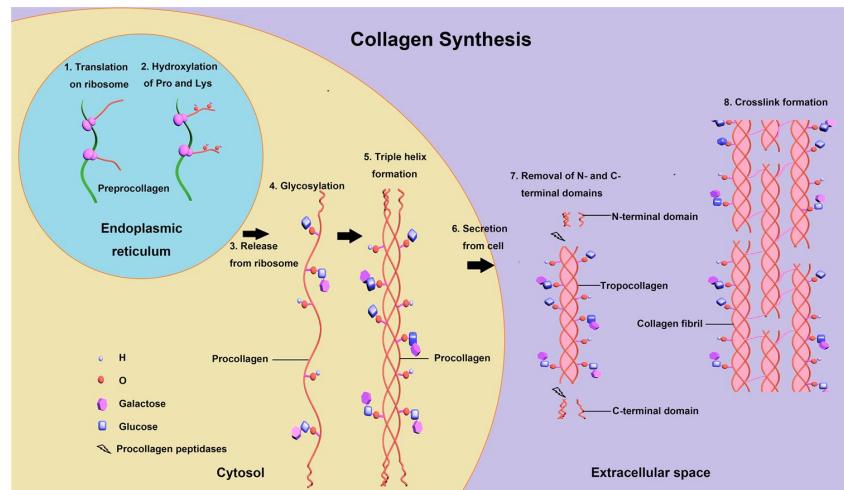
Fibrous Proteins

Proteins can be classified as globular or fibrous, that is circular or elongated. Fibrous proteins are insoluble and strong, and structural proteins are generally fibrous. Collagen is one of the most abundant fibrous proteins in structure protein classes. Collagen is broken into four types:

- Type I: 90% of the total collagen, found in connective tissue such as skin, tendons, bones
- Type II: Found in hyaline cartilage, and invertebrate disks
- Type III: Found in healing wounds, skin, intestine
- Type IV: Found in basal lamina and eye lens



Tropocollagen is the structural unit of collagen, which forms into 3 polypeptide chains, wrapped around each other in a left-handed confirmation. The three chains have a characteristic 3 amino acid pseudo-repetitive sequence of Gly-X-Y where x and y are often Hyp. The triple helix of collagen is post translationally modified. The 3 chains in the supercoiling pf the triple helix, are stabilized by H-bonds and lysine/hydroxylysine crosslinks. For this reason, tropocollagen, made of the 3 polypeptide chains, is formed into pre-pro-collagen in the cell, ready to be exported when a signal is received. Every collagen helix is left-handed, but its supercoiling is right-handed. In the cytosol, the pre-pro-polypeptide undergoes post-translational processing where three major modifications are made to the pre-pro-polypeptide for it to become pro-collagen.



1. The signal peptide on the N-terminal is removed
2. The lysine and proline residues get additional hydroxyl groups added to them via hydroxylase enzymes which require vitamin C as a cofactor
3. Glycosylation of the selected hydroxyl groups on lysine with galactose and glucose b

Three of the hydroxylated and glycosylated pro-a-chains assemble by twisting into a triple helix by zipper-like folding. The triple helix configuration is 3 left-handed helices twisted into a right-handed coil. Once outside the cell, the registration peptides are cleaved and tropocollagen is formed by procollagen peptidase. These tropocollagen molecules gather to form collagen fibrils, via covalent cross-linking by lysyl-oxidase which links hydroxylysine and lysine residues. Multiple collagen fibrils form into collagen fibers. Collagen may be attached to cell membranes via several types of protein, including fibronectin and integrin. A-keratin forms helices, forming structural proteins. Keratin is essential in the formation of the cytoskeleton, specifically in intermediate filaments. Keratin is responsible for forming structures like hair.

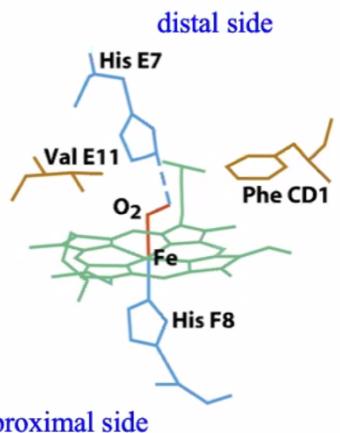
Hemoglobin:

Hemoproteins are proteins formed of specific globular proteins, containing a globin, and a prosthetic group, with the specific conjugation heme group, which can bind oxygen. Hemoglobin is found in the red blood cells, transports O₂, while myoglobin also binds oxygen, but is present mainly in the muscles, for the purpose of depositing and reserving O₂ when oxygen levels drop. Neuroglobins and cytoglobin are less common, neuroglobin is an oxygen storage in the brain, while cytoglobin is found in all cells but use is not clear. Cytochromes are located in mitochondria and are used in the electron transport chain.

Myoglobin is a single amino chain, and can work only as a O₂ storage, cannot transport O₂. Hemoglobin instead is a tetramer, made of two types of chains, a and b. Hemoglobin can also change its affinity for oxygen, allowing it to be a transporter as well as a storage. Globin's likely all came from the same ancestral globin chain. Fluids in the body are made of about 45% red blood cells, and 55% plasma. Hemoglobin, the protein that transports O₂ and CO₂, is found in red blood cells, and transports O₂ to the tissues, and bring CO₂ back to the lungs for excretion. Oxygen is essential for the electron transport chain, as it is the final acceptor for electrons. Without O₂, the electron transport chain doesn't work.

Heme Structure

Myoglobin and hemoglobin share the same prosthetic group, called heme. Heme is formed of 4 protodopor IX and becomes heme with the bonding of the Fe. The Fe forms 6 bonds, 4 with nitrogen, one with the oxygen, and one with the hydrogen. The Iron is in a deeply hydrophobic pocket of the heme, called the heme pocket. These pockets protect the iron, which is important because the Fe, must be oxidized to ferric iron, forming methemoglobin when oxygen is bound. Antioxidants are used to stop oxidation stress and keeping the Fe safe in the heme pocket insures this. The iron can bind the oxygen directly, as well as the His F8 on the globular region. The attached oxygen can also bind to another

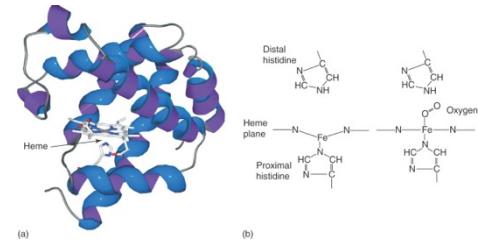


globular domain, but this time attached to His-E7. The connection with His-E7 increases the affinity to the oxygen, allowing more oxygen to bind, and conjugatively decreasing the affinity of CO₂ due to steric hinderance. This is a selective advantage, when His-E7 is bound, oxygen is more likely to be bound, and CO₂ is less likely to interfere.

Hemoglobin changes its color on the basis if it is bound to oxygen or not. This is due to the oxidation state of the Fe, where the bright red color appears when the hemoglobin is full of oxygen, fading to a brown when the oxidation state of the iron has reached 3+. Pulse oximeter measures the absorption spectrum of hemoglobin in inferred, and therefore the oxygen saturation of the blood.

Myoglobin

$$Y_{O_2} = \frac{[O_2]}{K_D + [O_2]} = \frac{pO_2}{p_{50} + pO_2}$$

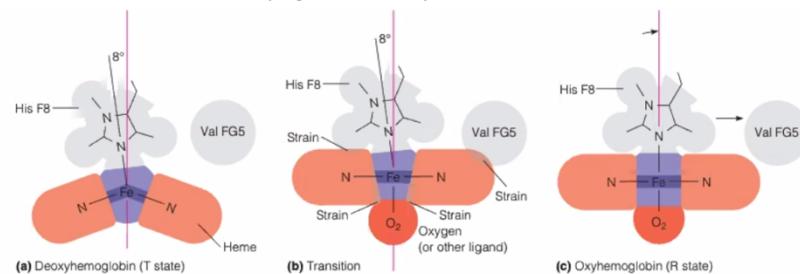


Myoglobin has an ellipsoidal shape, with 8 alpha helices, indexed from A-H, containing the loops labeling the helices it's connected to. These alpha helices composed 80% of the amino acids in the protein, while the other 20% are connecting the loops.

Helix E and F form the hydrophobic pocket, with help from B, C, G, H, and CD and FG loops. The saturation function of hemoglobin is defined by a sharp increase and a gradual plateau, where the concentration of O₂ increases with saturation of O₂(duh). In the equation, the saturation can be calculated from p₅₀, a measurement of the affinity of Mb to O₂, and pO₂ is the atmospheric saturation. Inside tissues, the pressure is too low to consistently hold oxygen, but in the capillary's and lungs the Partial pressure allows for full saturation. In this way, myoglobin only acts as a storage for oxygen when the oxygen levels drop, for example in a working muscle, so that when the oxygen level drops, the partial pressure drops, and myoglobin disassociates from its bound oxygen to use in the muscle. For this reason, myoglobin is important, but not vital for life.

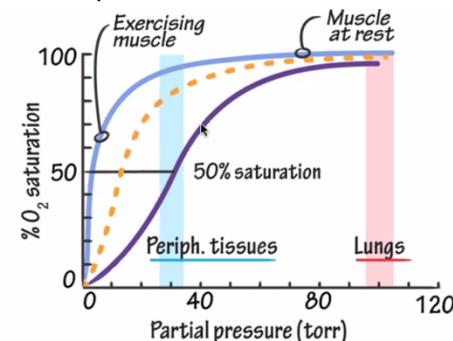
Hemoglobin

Hemoglobin, because it contains 4 subunits, can change its affinity for oxygen, and can therefore - unlike myoglobin- transport oxygen. Hemoglobin is made of two α-chains, and two β-chains, each chain being very structurally similar to myoglobin, but are slightly shorter than Mb chain, as well as containing 7-helixes in the β chain. Another important fact is that hemoglobin essentially forms two identical dimers, each made of a β and an α chain, which are formed by 35 rigidly bound residues, while the bonds between the two dimers is only 19 and contributes to its dynamics.



This means the hemoglobin can switch between two conformations, deoxyHb (T-tense-state) and oxyHb (R-relax-state), a change modulated by the binding of oxygen, and can be covalently modified to rotate the two dimers, releasing or binding to the oxygen. T-state favors the release of the oxygen, and R-state favors the binding of the oxygen. The changing between states opens up the donut hole inside the Hb, exposing bonding sites for the oxygen. In the T-state, the iron is inside the globulin molecule, but slightly off the proximal plane, and in the R-state the iron is displaced, dragging the His-F8 with it, displacing α1β2 and α2β1 interface, breaking ionic bonds and rotating the molecule 15 degrees. This means the pressure of oxygen controls the state of the hemoglobin, not that the hemoglobin regulates the oxygen pressure.

Compared to the Mb, Hb exhibits much more control over its binding of oxygen for the complex interactions between the domains. Therefore we have to consider the cooperation coefficient when calculating the saturation. In the case, a positive n relates positive cooperativity, such as in the case of hemoglobin. In the transition state, the cooperation coefficient is at its highest for hemoglobin and n=3, but usually in high affinity or low affinity state n=1. Therefore, the binding of oxygen, up to 4 oxygens favors the conversion to the R-state, and the release of oxygens, down to zero favors the return to the T-state.



Myoglobin

- Higher O₂ affinity
- Responds to muscle's O₂ needs

Hemoglobin

- Cooperative binding = sigmoidal curve
- Responds to O₂ availability

$$Y_{O_2} = \frac{[O_2]^n}{K_D + [O_2]^n} = \frac{pO_2^n}{p_{50}^n + pO_2^n}$$

n = 1: the system is non cooperative and ligand binding is simple (hyperbolic)

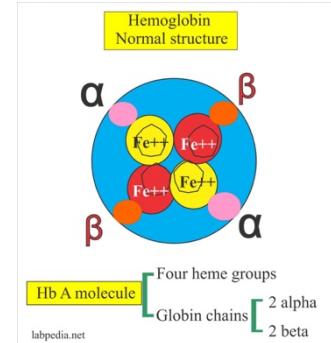
n > 1: displays positive cooperativity (sigmoid)

n < 1: displays negative cooperativity (biphasic)

O₂-binding kinetics summary:

- Four subunits, so four O₂-binding sites
- O₂ binding is cooperative meaning that each subsequent O₂ binds with a higher affinity than the previous one

- Similarly, when one O₂ is dissociated, the other three will dissociate at a sequentially faster rate.
- Due to positive cooperativity, a single molecule is very rarely partially oxygenated.
- There is always a combination of oxygenated and deoxygenated hemoglobin molecules. The percentage of hemoglobin molecules that remain oxygenated is represented by its oxygen saturation.
- O₂-binding curves show hemoglobin saturation as a function of the partial pressure for O₂.



Hb Effectors

Two models exist for the method which state shifts occur. Sequential says it happens one at a time, each Hb chair binds an oxygen, while the symmetrical/concerted model says in zero oxygen we have all T-state, and when oxygen is added, they all become R-state at the same time. Hemoglobin are homotropic regulators, meaning the molecule that induces an allosteric change is the same target molecule it is targeted to bind, while heterotopic change conformation by the binding of another molecule(L2) that is not the L1(target).

Hb plays another important role, removing CO₂ back to the lungs after respiration in the tissues. The path of CO₂ can take two path, either it can directly bind Hb (20-30%) after O₂ is released, or it can be transported as bicarbonate ion (70-80%), a change catalyzed by carbonic anhydrase, where it can enter the blood and then is transformed back to CO₂ after entering the lungs. This process releases a proton when bicarbonate is formed increasing the pH of the blood. A small amount can be solubilized directly in the blood, but more than 7% becomes toxic. This system is necessary to expunge CO₂ to block buildup from the massive amounts of CO₂ produced in glycolysis/Krebs.

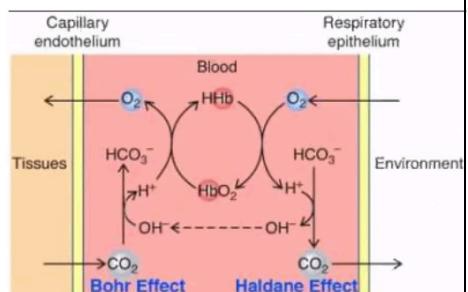
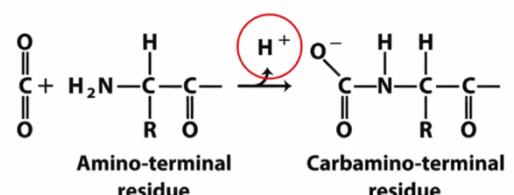
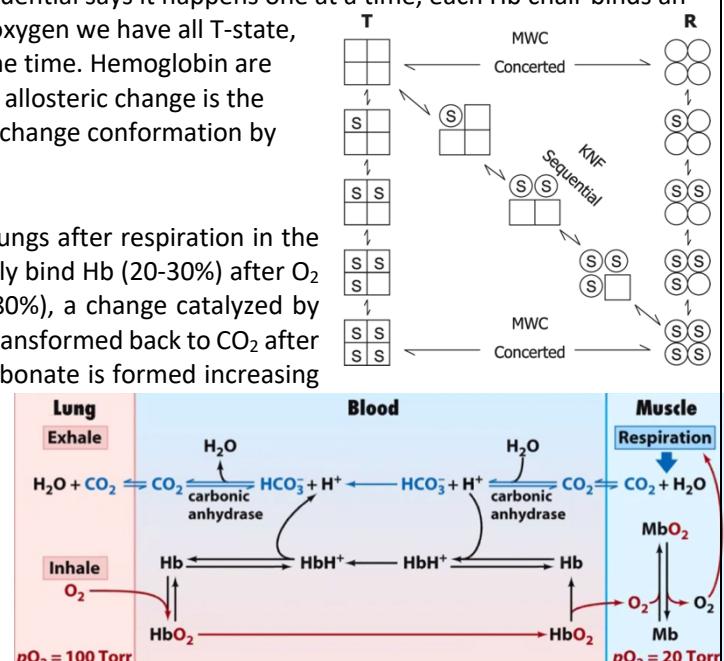
The effect that CO₂ and pH have on oxygenation of Hb in the lungs is called the Bohr effect, and induces deoxygenation in tissues. Protons can directly bind hemoglobin as a negative, heterotopic effector -binding at several different amino acid residues. Proton release, as well as the binding of O₂ influences the quaternary structure of Hb, favoring the transition from T-state to R-state. This means in an acidic pH, where protons bind, Asp-94 will form hydrogen bonds, causing a favoring of the T state over R.

CO₂ can also bind Hb, in a manner inversely related to the binding of O₂, binding to the carbamate group on the globulin chain, forming carbaminohemoglobin. This reaction also increases H⁺, contributing to the Bohr effect, as well as forming an additional salt bridge that further stabilizes the T-state, and promoting the release of O₂. The Bohr effect ensures that tissue is sufficiently supplied with O₂ when CO₂ and pH increase.

Another important effector is lactic acid, when oxygen is not present, pyruvate will -instead of becoming acetyl-CoA- becomes lactic acid. Lactic acid decreasing the pH, further increasing the signal process of the Bohr effect, therefor lactate is a negative heterotrophic effector for Mb.

The Haldane effect is the opposite of Bohr effect, that is it stabilizes the R-State, increasing the amount of bound O₂. These two opposite processes are controlled by the binding of oxygen. The Haldane effect also reduces the CO₂ content, and increasing the O₂ decreases the formation of carbinamine compounds, as well as the release of H⁺ ions from the hemoglobin.

Although CO₂ and H⁺ are the main effectors, other effectors such as 2,3-bis-phosphoglycerate, a modified form of 1,3-BPG an intermediate in glycolysis. This transformation occurs can happen in any cell but mainly occurs in red blood cells. 2,3-BPG binds 1:1 with hemoglobin, only when Hb is in the T-form, and stabilizes it through cross links between the beta chains, and is therefore a negative heterotopic effect, increasing the release of oxygen. 23-BPG is mainly released when the environmental oxygen partial



pressure decreases, such as happens in an increased altitude. This may seem counterintuitively at first, but the decreased affinity towards Hb in fact increases the amount of oxygen in the tissues, BECAUSE in normal conditions, all of the oxygen isn't released, so an increase in release rate means more reaches the tissues. In these ways, the body can control Hb activity and therefore oxygen release in the tissues both instantly through the Bohr effect, and slowly through increase in conversion of 13-BPG to 23-BPG, as well as the increase in transcription.

Effectors of O₂ binding review:

- Small molecules that influence the O₂-binding capacity of hemoglobin are called effectors (allosteric regulation)
- Effectors may be positive or negative; homotropic or heterotropic effectors
- Oxygen is a homotropic positive effector
- Positive effectors shift the O₂-binding curve to the left; negative effectors shift the curve to the right.
- From a physiological view, negative effectors are beneficial since they increase the supply of oxygen to the tissues.

Temperature also influences the affinity of hemoglobin for oxygen, by altering the structure of globin. As metabolism increases, temperature increases and Hb loses affinity for oxygen, causing more oxygen to be released, while the decreases in temperature in the blood promotes the binding of oxygen by Hb. In summary, hemoglobin affinity for oxygen is decreased by lowering pH, increasing temperature, increasing CO₂ concentration. CO is produced by incomplete combustion of carbon, is colorless and odorless and has 250x higher affinity for Hb than oxygen, making it extremely dangerous for humans, lethal at 70% carbon monoxide Hb association in blood.

Physiological hemoglobin

Adult hemoglobin has 2 a and 2 b subunits, and called HbA, while we also produce HbA₂ and is the minor form of hemoglobin in adults, only 2-3%. There also exist HbF and HbE(embryotic). The differences are in the b-chains, while the a-chains are always present. In HbF(fetal) there are 2 gamma(y) chain instead the B chains and is able to bind with more affinity to oxygen than the HbA, allowing the fetus to absorb maternal oxygen from Hb CO, BPG, Cl- A. HbF achieves this because the gamma chain cannot be protonated like the HbA can, therefore 23-BPG cannot bind.

Pathological hemoglobin's are defined as anomaly's in the primary structure due to a mutation in the genes encoding for the, resulting in a mutated protein prion. This mutation only affects individuals who have a mutation on both the alleles for a specific chain(a or b), since the subunits are assembled after translation. The most famous and well-studied is sickle cell anemia, in which a single nucleotide mutation causes Val to be translated instead of glutamine, in the beta chain. This change, between a hydrophobic for a hydrophilic amino acid creates a misshaped HbA molecule, called HbS. HbS is much easier to aggregate inside cells compared to the globular HbA, causing the red blood cell to form into a sickle shape instead of a disc. This causes rapid hemolysis of sickle cells, needing to be replaced every 10-20 days compared to 4 months, leading to severe anemia, being exhausted much easier. Sickled cells also create clots much more easily than disc cells in the capillaries, and increases blood viscosity. Sickle cell disease induces a resistance to malaria, even in heterozygotes who do not experience the negative effects of sickle cell, the infection rate is decreased by 10. Thalassemia refers to the lack of production of the alpha or beta chain and must affect more than one of the four alleles coding for a specific chain, therefore thalassemia can be classified as minor (1/4 mutant), intermediate (2/4) and major (3-4/4).

Hemoglobin can also be glycosylated, through a non-enzymatic reaction between glucose and the amino terminal group of the valine on the b-chain. Glycated hemoglobin reflects the blood glucose concentration in the individual, allowing researchers to determine if the patient has had a hyperglycemic episode in the past, as glucose binds for a long time.

Enzymes:

Enzymes do not fundamentally change the energy levels of the reactants and products, they only decrease the activation energy needed to begin the spontaneous reaction, thus increasing the rate of the reaction. The enzymes do not modify the equilibrium constant K, only accelerate it, and are unchanged after the reaction finishes. Enzymes are also effective at minimal concentrations compared to substrate concentration.

Classes of enzymes

Enzymes usually end in -ase, where the stem gives us some indication of the enzymes use. Some enzymes were named by their broad function before the specific function was found, such as pepsin, involved in digestion, or lysozyme for its ability

to lyse bacteria cell walls. For this confusion, IUBMB has adopted a system of naming and classifying enzymes based on the type of reaction they can work in. The first four digits classify the classification, but also has a systematic name. Hexokinase for example called E.C. 2.7.1.1. or ATP:glucose phosphotransferase, where 2 indicates the class(transferease):

- Oxidoreductases: Oxidation-Reduction reactions
- Transferases: Transfers a functional group
- Hydrolases: Hydrolytic Cleavage
- Lyases: adds or removes a group, or bond through electron rearrangement
- Isomerases: Intermolecular rearrangement
- Ligases: join two molecules

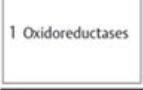
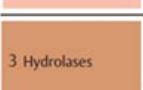
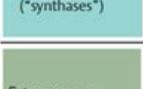
Properties of enzymes

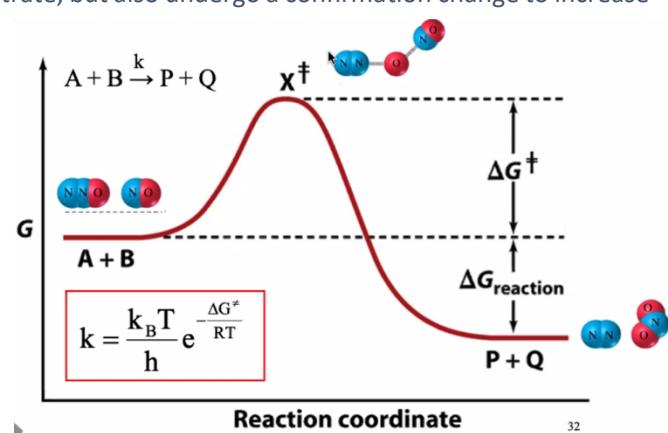
Enzymes bind to a specific substrate at the active site, and the compounds formed are called the products. Therefore, we can have the substrate binding step, then the catalytic step. Generally, biological catalysts increase the rate of the reaction by a factor of 10^6 to 10^{14} , work in milder reaction condition compared to chemical catalysts, meaning normal temp and pressure or close to it, while chemical catalysts often require high temps and extreme pH. Biological enzymes also have greater reaction specificity compared to chemical enzymes, due to their geometric complementation, plus their electronic complementation in the form of charges and bonds. The major bonds driving enzyme substrate binding are weak, non-covalent bonds such as hydrogen, hydrophobic, electrostatic and van der Waals. The enzyme also likely exhibits induced fit pairing, that is when the substrate begins to bind, the enzyme will change confirmations in order to bind more tightly around the substrate molecule.

Enzymes can be simple proteins or conjugated proteins, which contain cofactors or coenzymes. Cofactors are usually metal ions which help in the active site or substrate recognition, while coenzymes are co-substrates or prosthetic groups. Coenzymes are water soluble vitamins, which must be taken from the diet as they cannot be synthesized. Coenzymes are responsible for the specificity of the reaction, while apoenzymes (inactive enzymes) are responsible for the specificity of the substrate. The active site contains six important properties:

- It binds to the substrate: The active site is a cavity/cleft/surface on the enzyme containing a catalytic group.
- It stabilizes the substrate, by stabilizing the transition state (lowering free energy) the transition energy is reduced
- It establishes a new microenvironment; active sites usually create non-polar environments where bonds can be formed and broken more easily, unless water is directly involved in the reaction, it is actively excluded
- It makes up a small portion of the enzyme
- It binds the substrate reversibly, through non-covalent forces, hydrogen, Van Der Walls and not covalent usually
- It has structural complementary to its corresponding substrate, but also undergo a confirmation change to increase the fit (induced fit)

Enzymes catalyze thermodynamically favorable ($\Delta G < 0$) reactions, in which the final product has less free energy than the reactants. DG does not provide information about the rate of the reaction, only the free energy change. In a chemical reaction, bonds are broken and formed through favorable collisions. In unfavorable collisions, the wrong portions of the molecules interact, and therefore the bonds cannot be broken and formed even if the free energy would have been reduced. In favorable collision, the molecules are provided with sufficient energy to overcome their free activation energy. The transition state is the condition in which the old bonds are still formed, and the new bonds are also forming, requiring energy entropy is increased. After the transition state is reached, the reaction proceeds rapidly. The speed of the reaction depends on the number of molecules that have the sufficient energy content for their

Class	Reaction type	Important subclasses
1 Oxidoreductases		Dehydrogenases, Oxidases, peroxidases, Reductases, Monoxygenases, Dioxygenases
2 Transferases		C1-Transferases, Glycosyltransferases, Aminotransferases, Phosphotransferases
3 Hydrolases		Esterases, Glycosidases, Peptidases, Amidases
4 Lyases ("synthases")		C-C-Lyases, C-O-Lyases, C-N-Lyases, C-S-Lyases
5 Isomerases		Epimerases, cis/trans isomerases, Intramolecular transfersases
6 Ligases ("synthetases")		C-C-Ligases, C-O-Ligases, C-N-Ligases, C-S-Ligases



collisions to give rise to the reaction. Depending on the free activation energy, and the collision rate, a small number of molecules will be able to reach the transition state at any given time (slow reaction).

In this way we can increase the number of molecules who achieve this favorable collision by 1) increasing temperature, which increases the collision rate due to increased movement rate, or by 2) using a catalyst, which changes the orientation of the collisions in order to create more favorable collisions, and fewer unfavorable because the free activation energy in the active site is lower. More accurately, enzymes allow the transition energy to lower by generating a new pathway to the bond formation. The activation energy can be composed by many small steps up and down, and their sum energy required is the free activation energy. Some of these steps however may be much more difficult to achieve in terms of free energy content and are then called rate limiting steps.

First order reactions involve the change of substance A → B, such as a half-life problem. First order biological reaction are complicated by the fact that they are usually reversible, so we need to know the concentration of A, B as well as the equilibrium constant K. Second order reactions instead involve more than two molecules, often, two molecules must come together to form a product A+B → C. This is illustrated by Hb binding to O₂, we need to know the concentration of Mb, O₂ as well as rate constant K. Binding of a substrate to an enzyme to form an enzyme-substrate complex is also a second order process.

The rate enhancement of an enzyme is the numerical increase in efficiency in the rate of reaction due to the enzyme and can be measured by the time to $K_{\text{catalyzed}}/K_{\text{uncatalysed}}$. This means that the rate of forwards and backwards reactions will be increased, so while the speed of reaching the equilibrium concentration is increased, the actual concentration at equilibrium will remain unchanged.

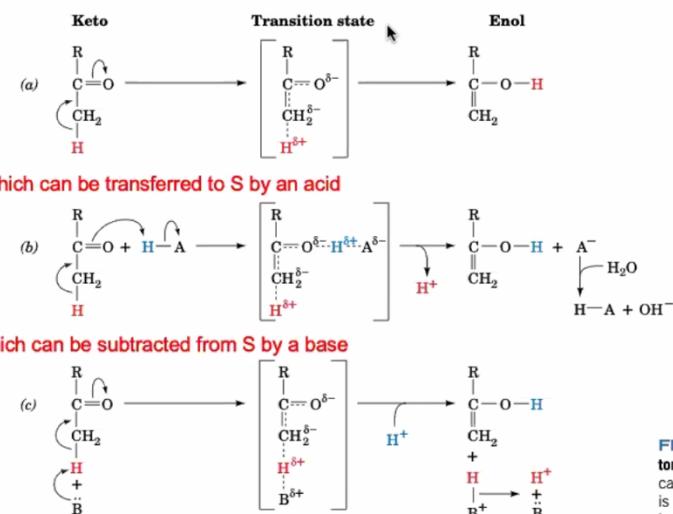
Mechanisms of enzymes

Enzymes rely on six main strategies to significantly increase rate enhancement:

- 1) Electrostatic catalysts: Increase the molecules preference to the transition state through complementary non-covalent bonds (H-bonds, charge-charge interactions)
- 2) Induced Fit: distortion of the substrate and/or the active site in a way that reduces the activation energy
- 3) Catalysis by approximation/DeltaS^{**}: Referring to the specific orientation and proximity the favorable collision must make, this strategy binds 1+ substrates in a way that maximizes its S^{**} in order to make favorable collision more likely for the multi-substrate reaction
- 4) Covalent Catalyst: Altering the reaction pathway to create intermediate states
- 5) General Acid/Base catalysis: Proton transfer from molecule (nucleophilic or electrophilic attack)
- 6) Metal Ion Catalysis: Involvement of metal ion in catalysis. A metal ion is an electrophile and 1) may stabilize a negative charge on an intermediate, shielding charges; 2) by attracting electrons from water, renders water more acidic (prone to lose a proton) in a redox reaction; 3) may bind to substrate and reduce activation energy

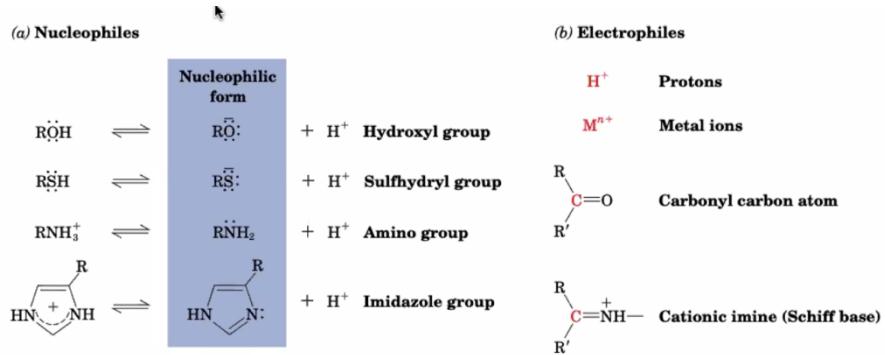
Acid-base Catalysis GABC:

In acid catalysis transfer the H⁺ ion from the catalyst (acid) to the substrate, while in base catalysis the H⁺ ion is transferred from the substrate to the catalyst (base). We see this in Keto-Enol tautomerization, in which an acid can form the enol form from the keto form by transferring a proton to the enol, and a base can subtract a proton to form an enol. Different amino acid side chains can have variable pKa depending on their environment. We can see this example in digestion, where pepsin works at low pH, and trypsin works at high pH, both serving the same function of breaking bonds.



Covalent Catalysis:

Accelerates the reaction by a transient formation of a catalyst-substrate covalent bond. The bond is formed by a nucleophilic group on the catalyst with the electrophilic group on the substrate, therefore it can also be called nucleophilic catalysis. Most of the enzymes using this catalysis technique are called serine proteases, because serine is the amino acid involved in the formation of the peptide bond. In this way the serine residue participates in the catalytic mechanism of covalent catalysis. Most enzymes using this technique are found in digestion, but it can be found in any enzyme which relates to the breaking of the peptide bond.



The size of the cleft must be larger for aromatic substrates such as pepsin, as well as the charge of the active site must be complementary to the substrate, trypsin's have negatively charged active sites because they receive arginine and lysine.

Enzyme Kinetics:

Factors influencing enzyme activity are pH, temperature, concentration of enzyme[E], concentration in substrate[S], presence of inhibitors or activators.

pH and Temperature

Each enzyme has an optimal pH that it functions at, usually a very narrow range. If we differ from this range, efficiency decreases, and can even change the enzymes structure, reversibly or irreversibly. The variation of pH can cause changes in the active site or change the structure of the substrate due to changes in protonation. If the change in pH is subtle, the changes will likely be reversible, but if the changes are large, the structure of the enzyme can denature resulting in an irreversible change. Enzymes optimum pH value likely corresponds to the environment in which they are found, lysosomes enzymes work optimally at low, acidic pH.

Temperature can affect different enzymes in different ways, either directly influencing the reaction speed due to more energy in the system, or it can result in denaturing of the enzyme. As temperature increases, enzyme activity reaches a point called the optimum temperature, after which it denatures. Most enzymes have an optimum around 30-35C, after which they begin to denature. After 70C, we see complete denaturation, except in some cold-blooded fish (30C) or thermophilic bacteria which still work optimally at 100C.

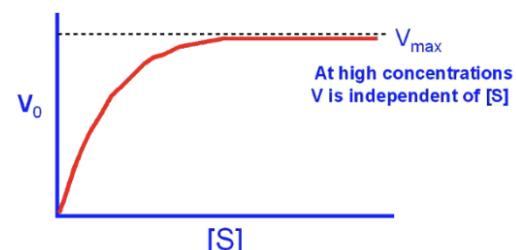
Michaelis-Menten Equation

Enzyme concentration[E] is proportionally related to velocity of the reaction[V], when [S] increase, V increase. Therefore, it is possible to calculate the concentration of an enzyme in a sample by tracking the V of the reaction and comparing it to the known reaction rate at a set concentration. In this case, the saturation of substrate[S] must be greater than [E].

At low [S], V is proportional to S in a first order reaction. At high S however, V becomes independent of S as it approaches saturation limit, indicated by V_{Max} , the maximum velocity the reaction can get to. In this case, the reaction now follows 0 order reaction kinetics, dependent only on concentration of the enzyme [E], that is, when V stops increasing, the enzyme is fully saturated with the substrate and cannot increase its speed anymore.

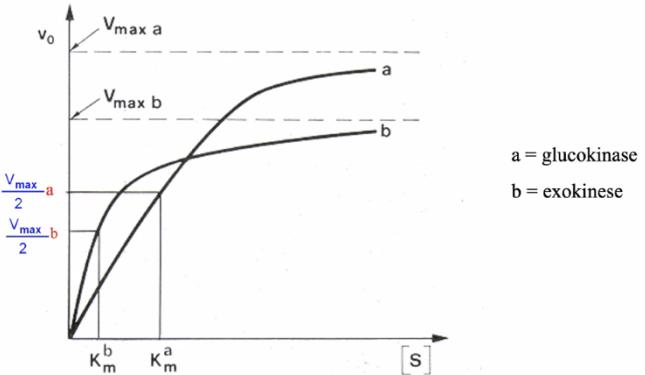
The kinetic equation of an enzyme can be calculated using the Michaelis-Menten equation, telling us the rate of enzyme-complex formation(k1) and the enzyme product disassociation(k2):

$$\begin{array}{c}
 \text{E} + \text{S} \xrightleftharpoons[k_{-1}]{k_1} \text{ES} \xrightarrow{k_2} \text{E} + \text{P} \\
 \boxed{v = \frac{V_{\text{MAX}} [\text{S}]}{K_M + [\text{S}]}} \quad \boxed{V_{\text{MAX}} = k_2 \cdot E_T \quad \text{maximal velocity (dimensions: M·s⁻¹)}} \\
 \boxed{K_M = \frac{k_{-1} + k_2}{k_1} \quad \text{Michaelis constant (dimensions: M)}}
 \end{array}$$



- When $[S] \ll K_m$, the velocity of the reaction depends on K_m and V_{max}
- When $[S] \gg K_m$, K_m becomes less important and the velocity only depends on K_{max}
- When $V = V_{max}/2$, $[S] = K_m$

K_m (called the Michaelis constant) corresponds to the substrate concentration at which the reaction V is equal to half of the V_{max} . K_m is the equilibrium constant of the disassociation reaction of the ES complex, indicating the affinity of the enzyme for the substrate, low values indicate high affinity for the substrate, vice versa. K_m is also the substrate concentration at which V is equal to $\frac{1}{2}(V_{max})$.

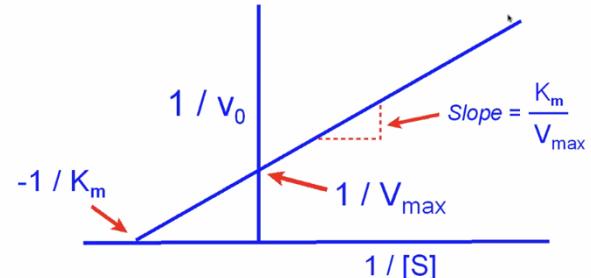


In practice, K_m is a constant specific for each enzyme that gives us an idea of the relative efficiency with which different substrates are transformed (specificity), as well as an idea about the catalytic efficient of the enzyme. Additionally, it estimates the intercellular concentration of the substrate, because it is calculated through the V_{max} . It is an important measure of the effects of inhibitors and activators. We can look at the relationship between different enzymes in a biological pathway above, a and b give the same product, while a is present in all cells, and b is present only in the liver, indicating these enzymes play different roles in the body. b has a lower K_m , and thus a higher affinity for glucose, meaning in low concentration of glucose, the first enzyme to be activated will be exokinase, however if we continue to accumulate glucose $[S]$ in the blood, glucokinase will become activated, still forming glucose-6-phosphate, but this time for the formation of glycogen instead of pyruvate. This check system reduces glucose buildup, each enzyme has a different affinity, and will work at different substrate concentrations.

Lineweaver-Burk Plot

$$\frac{1}{V_0} = \frac{K_m}{V_{max}} \times \frac{1}{[S]} + \frac{1}{V_{max}}$$

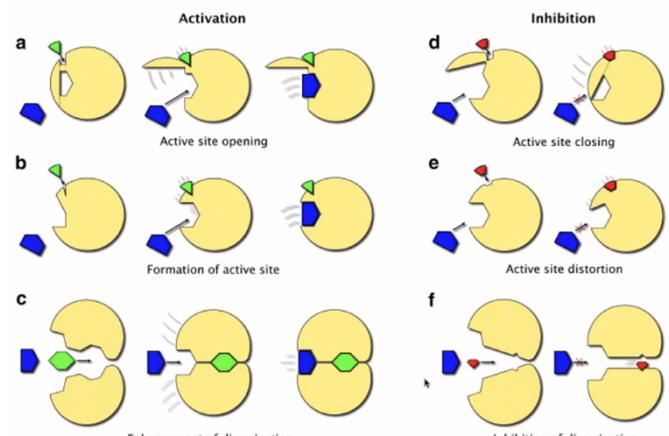
Another plot to study enzyme kinetics is the Lineweaver-Burk plot, in which the inverse of the reaction velocity is used instead of V such that resulting in a straight line, where the y intercept is $1/V_{max}$, the slope is K_m/V_{max} , and the x intercept is $-1/K_m$. This method can be used to record the time of a reaction in a faster way, plotting the results linearly. It also allows us to quickly find the V_{max} and the K_m , making it easier to work with visually. The Burk plot measures the rate of the reaction at different concentrations of the substrate, with few measurements.



Effector Molecules:

Enzyme activity can be regulated in two main ways:

- 1) Regulate availability of the enzyme: Induction/repression of synthesis, or rate of degradation (Fast)
- 2) Regulate Activity: Allosteric regulation, Association-disassociation with regulatory proteins, Reversible modifications(phosphorylation), Irreversible activation by proteolysis (Slow)



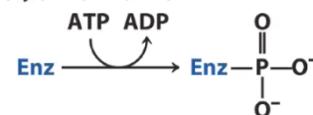
Allosteric effectors change the confirmation of the enzyme, homotropic allosteric effectors are the same molecule as the substrate, while heterotropic effectors are different than the substrate. In Hb, oxygen is a homotropic effector that stabilizes the R, while CO2 is a heterotropic effector stabilizing the other T. Heterotropic allosteric modulators are regulatory molecules that is not the enzymes substrate, which can be an activator or inhibitor.

Cooperative effect increases the affinity of the enzyme for the substrate following the binding of the substrate itself, such as hemoglobin (autocatalytic effectors). Another important regulatory mechanism is the feedback inhibition loop, in which the first enzyme in a pathway is a regulator for the entire pathway to avoid unneeded accumulation. In this way, an enzyme early in the pathway is inhibited by the end product, switching the pathway on or off without the need for other molecules to communicate.

Reversible covalent modifications can also regulate enzyme activity, an addition or removal of a phosphate group is a widely used the body. Phosphate added to Tyr is usually an activator, for Ser, Thr, and His it can activate or inhibit. Kinases add the phosphate group, and phosphates remove it. Covalent modifications also include acetylation, glycosylation, or ubiquitination.

Covalent modification (target residues)

Phosphorylation (Tyr, Ser, Thr, His)



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Inhibitors

Inhibition of an enzyme means the inhibitor will be bound instead of the substrate, or in addition to the substrate, decreasing the turnover number of the enzyme. The majority are drugs as well as toxins are enzyme inhibitors, by studying the kinetics we can determine the effectiveness of the drugs in vitro. Inhibitors can be subdivided into two classes:

- Reversible: Reduce activity by bonding reversible competitive inhibitors (bind active site), non-competitive inhibitors (bind somewhere other than active), or mixed-type inhibitors (can bond both)
- Irreversible or Inactivators: Bind so tightly the enzymes activity is permanently blocked, the only solution is to destroy the enzyme and make a new one

Reversible inhibition does not form covalent bonds between enzyme and inhibitor, meaning when the inhibitor is removed, the enzyme goes back to full activity. These inhibitors can bind free enzymes, or the enzyme-substrate complex, or even both. Competitive inhibition involves direct competition between the substrate and the inhibitor for the active site. Competitive inhibitors decrease the rate of the reaction by decreasing the fraction of enzymes available for binding to the substrate. The inhibition depends on the concentration of [S] and of inhibitor [I] and can be reversed simply by increasing substrate concentration therefore with competitive inhibition, V_{max} is unchanged, but more [S] is required. Competitive inhibitors have a structure similar to the substrates structure, with important chemical differences that stop the normal reaction from occurring. An example is when methanol enters the body, producing the toxic product formaldehyde. This can be treated by increasing the concentration of ethanol, which has a similar structure and instead forms acetaldehyde from the same alcohol dehydrogenase enzyme.

Non-competitive inhibitors instead bind a specific site other than the active site, still allowing the substrate to bind but also inducing a confirmational or chemoelectrical change which blocks the enzymes function in some way. In the case of non-competitive inhibition, the inhibitor binds directly to the Michaelis complex. It is therefore not important the concentration of the substrate, the inhibitor will always bind the complex. In non-competitive inhibition, K_m is unchanged but V_{max} decreases.

Mixed inhibition can both bind to the active site, or to another site which stops the reaction. In mixed inhibition, the enzyme can bind all forms of the enzyme, changing K_m and V_{max}, because it inhibits activity based on concentration of substrate and without concern for concentration.

Irreversible inhibitors bind to a function group with a strong, covalent bond, and thus cannot be removed without denaturing the enzyme. An example of an irreversible inhibitor is aspirin, which binds to COX-1 or COX-2 permanently deactivating the cyclooxygenase, blocking the pro-inflammatory response. Penicillin inhibits the formation of crosslinks in the peptidoglycan wall of bacteria by permanently deactivating the enzyme.

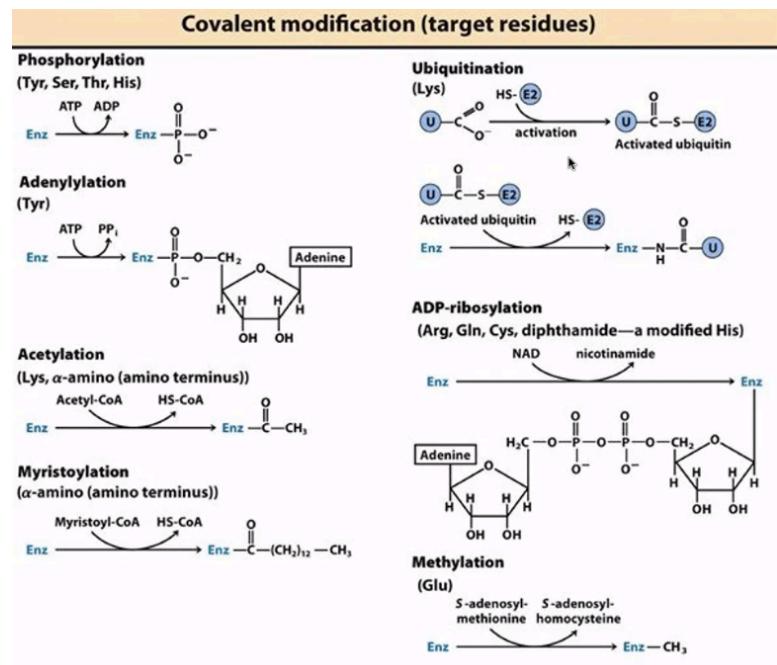
Suicide inhibitors are recognized at the active site, and can start the reaction, but will not be able to form the products, instead transforming into an irreversible inhibitor that binds to the enzyme catalyzing this transformation. These are also called mechanism base inactivators, as they are usually human made and extremely targeted.

Activators

Allosteric activators also change the active sites confirmation, but this time increasing the sites affinity for the substrate. Heterotrophic allosteric activation can also expose the active site or increase dimerization.

Phosphorylation is also commonly found especially when attached to Tyrosine. Other covalent modifications that control the activity of the enzyme are summarized on the right.

Another important activation mechanism is the proteolytic activators, who exist in their inactive form and require hydrolytic cleavage of one or more peptide bonds, converting the enzyme to its active form. The inactive form is referred to as the pro form, procaspase. These mechanisms are largely found in digestive pathways, because these digestive enzymes capable of cleaving the peptide bond are dangerous to the cell, and thus must be formed in their inactive form, and activated only when it has reached its correct location. Trypsin are usually the signal molecule to start this proteolysis cascade.



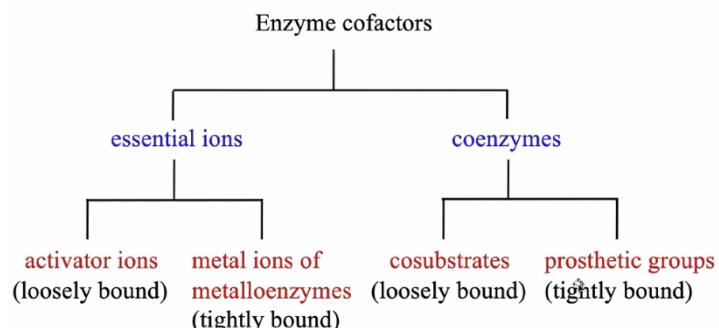
Insulin is also produced in the form preproinsulin, made of signal sequence, b-chain, c-chain and a-chain. First the signal sequence is cleaved to form proinsulin, and after the formation of the disulfide bridge between b and a, the c-chain is later cleaved off to find the final form of insulin.

Enzyme Cofactors/Vitamins

Most enzymes, 2/3 are conjugated, and require other cofactors such as essential ions, or coenzymes. Coenzymes include water soluble vitamins, compromising most coenzymes. Water soluble vitamins are found in foods, as vitamins or precursors (provitamins). Vitamins are essential for the body, required in very small amounts. Vitamins also regulate the metabolism by acting as coenzymes, but do not provide energy. Some vitamins are synthesized in the body, mainly thanks to the intestinal flora. Vitamins were named as vital + amines, K. Funk proposed some compounds must be in our diet, that we cannot synthesize in the correct amount, and therefore many diseases such as rickets, and scurvy were caused by the deficiency of these essential cofactors used by enzymes.

Vitamins are classified as:

- Water soluble: polar vitamins, including Vitamin group B, and vitamin C important coenzymes. B compromised a large family of coenzymes involved in metabolism, while C is involved in hydroxylation. Most of these water-soluble enzymes need to be post translationally modified in order to become active coenzymes.
- Liposoluble: Nonpolar vitamins, including Vitamin A, D, K, E each involved in a specific function in the body



Symbol	Name	Role
B ₁	thiamine	carbohydrate metabolism
B ₂	riboflavin	redox metabolism
B ₃	niacin	redox metabolism
B ₅	panthotenic acid	Krebs cycle, fatty acid & cholesterol metabolism
B ₆	pyridoxine pyridoxamine pyridoxal	amino acid metabolism
B ₇	biotin	gluconeogenesis, Krebs cycle, fatty acid & amino acid metabolism
B ₉	folic acid	C metabolism
B ₁₂	cobalamin	C & H metabolism
C	ascorbic acid	hydroxylation

Vitamin deficiencies generally come from not consuming enough in the diet, but can also be caused by insufficient intestinal absorption, increased demand (such as in pregnancy), and prolonged drug therapy.

Vitamin A (retinol) is essential for eyesight; its active form (retinal) is part of rhodopsin—the protein with which our eyes convert light to neural impulses that are ultimately translated to images in the brain. Deficiency in vitamin A leads to impaired vision, especially night blindness.

Vitamin B₁ (Thiamine) is activated by forming thiamine pyrophosphate through the thiamine-ATP transferase, forming the functional coenzyme. Thiamine pyrophosphate plays an important role in cleavage of bonds adjacent to the carbonyl group. Two of these important reactions are decarboxylation of α -keto acids, and in chemical rearrangement, when one aldehyde is moved from one carbon to another. The transfer (carboxylation) carboxyl group is seen in pyruvate dehydrogenase activated transformation from pyruvate to acetyl-CoA, using TPP as a coenzyme. Deficiency in B1 causes Beri-Beri.

Vitamin	Name	Active Form (co-factor)	Biochemical Function	Physiological/cellular Role
A	β -carotene	Retinal	Conversion of light to neural signals	Sight
		Retinoic Acid	Growth Factor	Tissue growth
B₁	Thiamine	Thiamine diphosphate (TPP, TDP)	• Decarboxylation • Decarbonylation	• Energy production from carbohydrates • Nucleotide synthesis
B₂	Riboflavin	FADH ₂	Oxidation-Reduction	• Energy production from foodstuff • Lipid breakdown & synthesis
B₃	Niacin	NADH	Oxidation-Reduction	• Energy production from foodstuff • Lactic fermentation • Lipid synthesis
		NADPH	Oxidation-Reduction	• Synthesis of lipids, nucleotides & neurotransmitters • Antioxidation • Detoxification of drugs & toxins • Anti-pathogen action

Vitamin B₂ (Riboflavin) is in the FMN form in the vitamin form and becomes FAD in its active form. Riboflavin becomes phosphorylated by riboflavin kinase, forming FMN with the addition of a phosphate group, with an adenine phosphate base, added by FAD diphosphatase, forming FAD. FAD is generally in their oxidized form, but can be reduced to FADH₂, an important electron carrier, the transfer of which can be semiquinone or intermediate, that is together or one at a time. FAD and FMN are coenzymes of flavin dehydrogenases. FAD is also involved in the formation of reduced glutathione, crucial in maintaining Hb and the iron ion in the reduced form, by protecting it when hydrogen peroxide concentrations increase in the blood.

Vitamin B₃ (Niacin or Nicotinic Acid) is a family of acids containing NAD and NADP, produced from nicotinic acid precursor. In some species tryptophan is degraded into nicotinic acid. The coenzyme forms are NAD⁺ and NADP⁺, formed by the addition of a phosphate group to the 2-carbon ADD MORE. NAD⁺ and NADP⁺ are acceptors of two electrons and one proton in dehydrogenation reactions. A large variety of dehydrogenases use these coenzymes in metabolic pathways. The oxidized (coenzyme) form of the vitamins has a different light absorption wavelength; therefore, we can understand the course of the reaction by looking at the percent of wavelengths being absorbed. NADP⁺ is involved mainly in the biosynthesis of fatty acids, while NAD⁺ is involved in oxidation especially in the mitochondria. NADPH is also a powerful antioxidant, especially in reducing glutathione, involved in the detoxification of peroxides. B3 can be eaten in meats, beans and a deficiency of it causes Pellagra, dry weak skin.

Vitamin B₅ (Pantothenic Acid) is converted into coenzyme A, an acyl carrier involved in many metabolic mechanisms in the body. Pantothenic acid binds an acetate group, as well as a phosphate group to form coenzyme A. CoA is involved in most reaction in B-oxidation in the mitochondria, but B5 can also form into ACP, involved in most of the lipid biosynthesis pathways.

Vitamin B₆ (Pyridoxine) is composed of a family of related pyridoxine vitamins which all form into PLP. PLP is important in the removal of the α -amino group in aminotransferases, and transaminase. Mechanism of transamination follows the ping-pong kinetic mechanisms, in which the amino group of the amino acid is transferred to the pyridoxal phosphate, releasing ketoacid. In the second step, α -ketoglutarate reacts with pyridoxamine phosphate, forming glutamate. This mechanism is only allowed by PLP, derived from B6. PLP also is important in synthesis of GABA.

Vitamin	Name	Active Form (co-factor)	Biochemical Function	Physiological/cellular Role
B₅	Pantothenic Acid	Coenzyme A	Acyl Transfer	• Energy production from foodstuff • Fatty acid synthesis
B₆	Pyridoxine	Pyridoxal Phosphate (PLP)	• Transamination • Racemization • Decarboxylation • β / γ -Elimination	• Amino acid breakdown • Glycogen breakdown
B₇	Biotin	Biotin	Carboxylation	• Glucose & fatty acid synthesis • Leucine synthesis
B₉	Folic Acid	Tetrahydrofolate (THF)	One-Carbon Group Transfer	Amino Acid & nucleotide synthesis
B₁₂	Cobalamin	Coenzyme B ₁₂	• Intramolecular Rearrangements • Methyl transfer	• Nucleotide synthesis • Amino acid metabolism • Fatty acids breakdown • Folic acid regeneration
C	Ascorbic Acid	Ascorbic Acid	Proline Hydroxylation Reduction	Collagen synthesis Antioxidation
D	Calciferol	Calcitriol	Gene expression	Bone growth

Vitamin B₇ (Biotin), forming Biocytin when binding with the amino group of lysine. Biocytin is a prosthetic group for enzymes that catalyzes the carboxyl group transfer reaction and ATP-dependent carboxylation reactions. Biotin also plays a role in activating carboxylases which require biocytin.

Vitamin B₉ (Folic acid) forms into tetrahydrofolate, with folate as an intermediate. Folate is involved in synthesis, repair and methylation of DNA. After reduction of folate to form tetrahydrofolate (THF) serves as a coenzyme in the C1 metabolisms. Reduction of Folic acid leads to a deficiency in DNA synthesis. Specifically, THF is a donor of one carbon units in a variety of metabolic reactions in which carbon is consumed.

Vitamin B₁₂ (Cobalamin) is formed of a corrin ring system similar to the prosthetic group of Hb. The active form of the vitamin is 5'-Deoxyadenosyl-cobalamin when bound to a cobalt ion as well as a benzimidazole ribonucleotide, or a Methyl-cobalamin. The former is involved in the synthesis of DNA, RNA, and methionine, while methyl cobalamin is involved in fatty acid metabolism.

Vitamin C (Ascorbic acid) acts in the strengthening of collagen structure, the principal protein of connective tissues (skin, cartilage, bone, etc.). Vitamin C deficiency leads to unstable connective tissues, which results in **scurvy**. Vitamin C is synthesized in many animals, but not in humans.