Chapter 3

Structure & Function

Function flows from structure. In order to understand the function of biomolecules, we must first understand their structures.

Chapter 3

Structure and Function

If we hope to understand function in biological systems, we must first understand structure. At a simple level, we can divide molecules up according to their affinities for water – **hydrophobic** (limited solubility in water), **hydrophilic** (soluble in water) and **amphiphilic** (have characteristics of both hydrophobicity and hydrophilicity). **Hydrophobicity** in biological molecules arises largely because carbon-hydrogen bonds have electrons that are fairly evenly shared (not unlike carbon-carbon bonds). By contrast, the electrons between the oxygen and hydrogen of water are not equally shared. Oxygen has a greater electronegativity, so it holds them closer than hydrogen does. As a consequence, oxygen has what we call a partial negative charge and hydrogen has a partial positive charge.

Virtually all of life on Earth is built upon the biochemistry that arises from the molecular properties described in the preceding paragraph. The biomolecules referred to as lipids are largely water insoluble because they have predominantly carbon-hydrogen bonds with few ionic or hydrogen bond characteristics.

Building Blocks

Biological macromolecules are all polymers of a sort, even fats, in which the fatty acids can be thought of as polymers of carbon. (We will consider fatty compounds - **fats**,

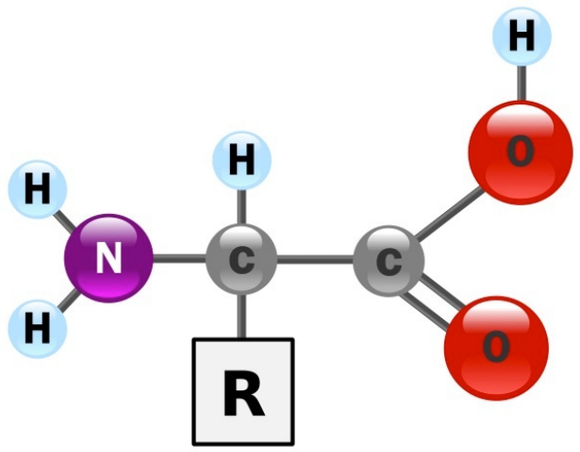
Click HERE for Kevin's YouTube lecture on amino acids

glycerophospholipids, **sphingolipids**, **isoprenoids/ terpenoids** separately).

The remaining categories of biological macromolecules include **proteins**, **nucleic acids**, and polysaccharides. The building blocks of these, respectively, are amino acids, **nucleotides**, and **monosaccharides** (sugars). Of these, the most diverse collection of chemical properties is found among the amino acids.

Proteins

Whereas nucleotides all are water soluble and have the same basic composition (sugar, base, phosphate) and the sugars also are water soluble and mostly contain 5 or 6 carbons (a few exceptions), the amino acids (general structure below) are structurally and chemically diverse. Though all of the amino acids are, in fact, soluble in water, the interactions of their side chains with water differ significantly. This is important, because it is only in the side chains (R-groups) that amino acids differ from each other. Based



Amino Acid Schematic

from Wikipedia

The Amino Alphabet Song

To the tune of "*Twinkle, Twinkle Little Star*"

Lysine, arginine and his
Basic ones you should not miss
Ala, leu, val, ile, and met
Fill the aliphatic set
Proline bends and cys has 's'
Glycine's 'R' is the smallest
Then there's trp and tyr and phe
Structured aromatically

Asp and glu's side chains of R
Say to protons "au revoir"
Glutamine, asparagine
Bear carboxamide amines
Threonine and tiny ser
Have hydroxyl groups to share
These twen-TY amino A's
Can combine a zillion ways

Recorded by David Simmons
Lyrics by Kevin Ahern

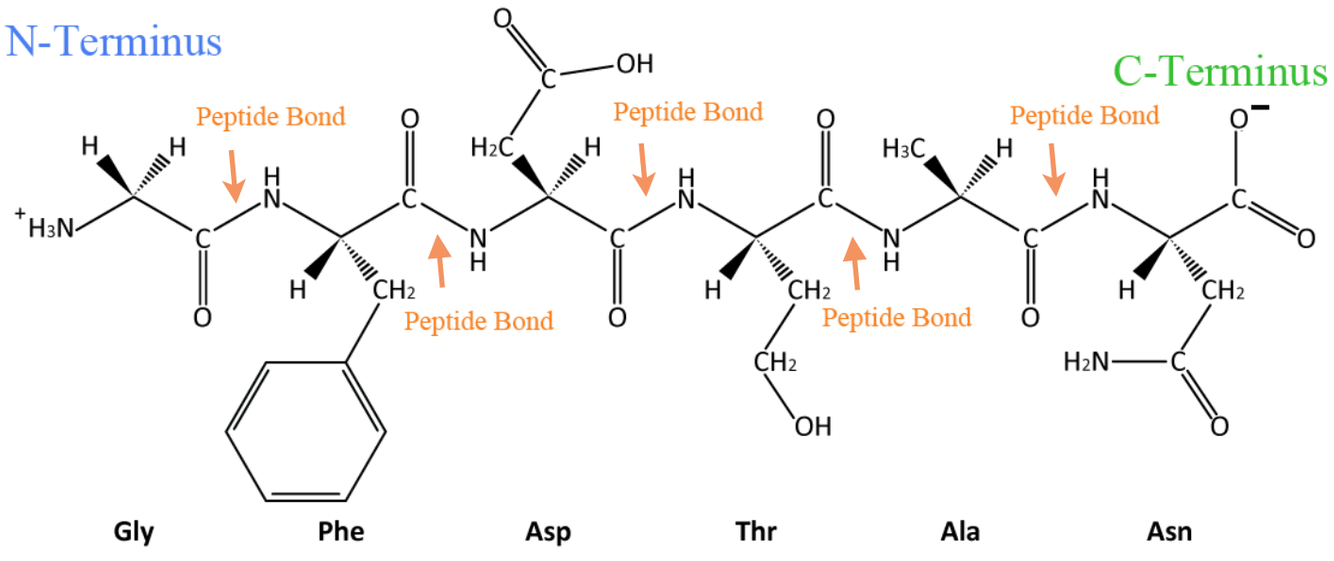
on side chains, we can group the 20 amino acids found in proteins as follows:

- **Aromatic** (**phenylalanine**, **tyrosine**, **tryptophan**)
- **Aliphatic** (**leucine**, **isoleucine**, **alanine**, **methionine**, **valine**)
- **Hydroxyl/Sulfhydryl** (**threonine**, **serine**, tyrosine, **cysteine**)
- **Carboxyamide** (**glutamine**, **asparagine**)
- **R-Acids** (**glutamic acid**, **aspartic acid**)
- **R-Amines** (**lysine**, **histidine**, **arginine**)
- **Odd** (**glycine**, **proline**)

Note that tyrosine has a hydroxyl group and fits into two categories. Note also that biochemistry books vary in how they organize amino acids into categories.

Amino acids are joined to each other by **peptide bonds**. This introduces a slight simplifying aspect to the structure of proteins – one need only consider the positioning of the R-groups around each peptide bond when determining protein structure schematically. Proteins that are in aqueous environments, such as the cytoplasm of the cell, have their amino acids arranged so that those with **hydrophilic side chains** (such as threonine or lysine) predominate on the exterior of the protein so as to interact with water. The **hydrophobic amino acids** in these proteins are found predominantly on the interior. When one examines the structure of proteins in non-aqueous environments, such as the interior of a lipid bilayer, the arrangement is flipped – hydrophobics predominate on the outside where they can interact with the hydrophobic side chains of membrane fatty acids and the hydrophilic amino acids are arranged anyplace where they can contact water. For a protein like **porin**, which provides an interior channel through which water can pass, this is where the hydrophilics are found. For transmembrane proteins, which project through both sides of the membrane, the hydrophilics are found at each point where the polypeptide chain emerges from the membrane.

Click HERE and HERE, for Kevin's Protein Structure lectures on YouTube



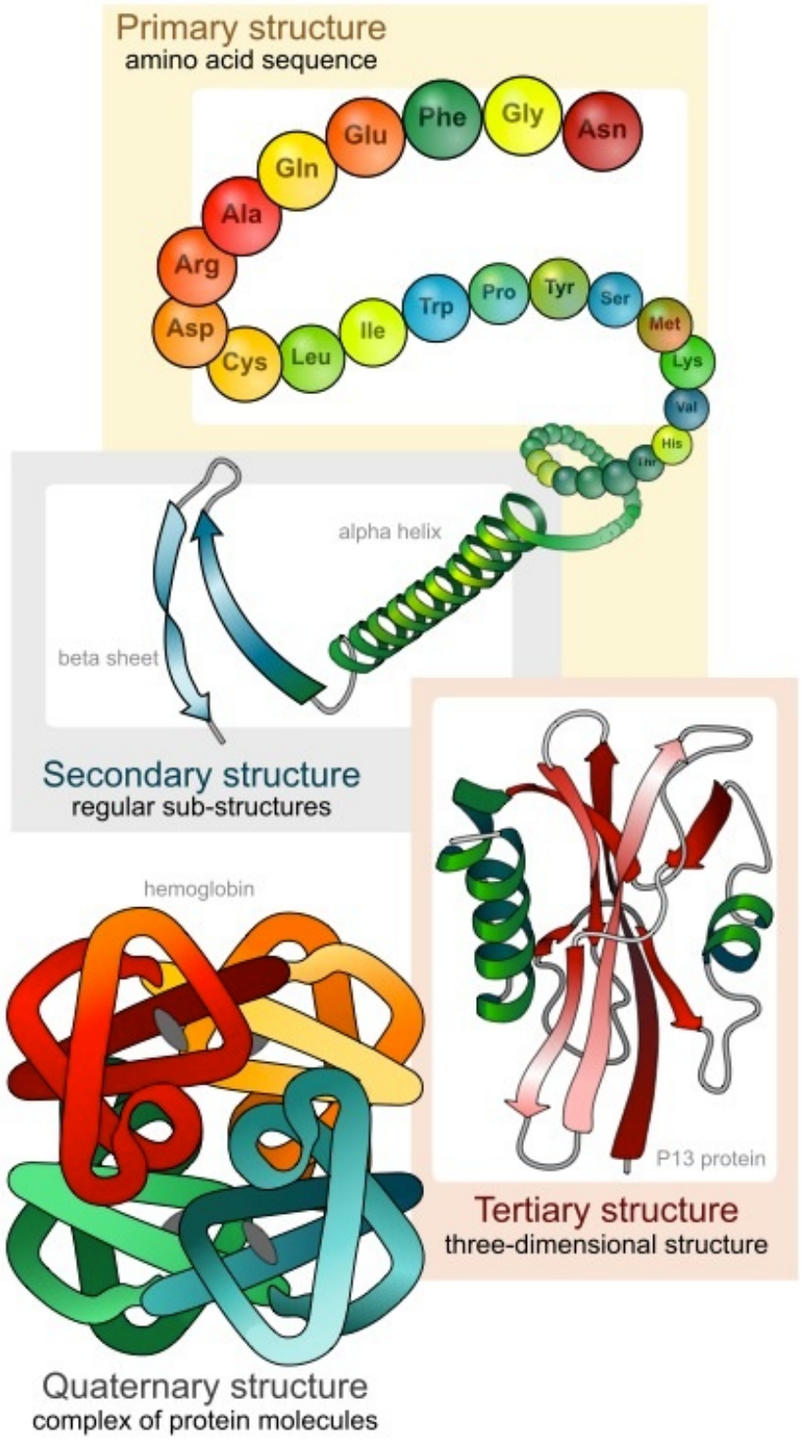
Primary Structure

How do proteins obtain such arrangements of amino acids? As we shall see, the structures of all proteins

ultimately arise from their amino acid sequences. The amino acid sequence is referred to as the **primary structure** and changes in it can affect every other level of structure as well as the properties of a protein. The primary structure of a protein arrived at its current state as a result of mutation and selection over evolutionary time. On a more immediate time scale, 3D protein structure arises as a result of a phenomenon called **folding**. **Protein folding** results from three different structural elements beyond primary structure. They are referred to as **secondary**, **tertiary**, and quaternary structures, each arising from interactions between progressively more distant amino acids in the primary structure.

Secondary Structure

Interactions between amino acids within about ten units of each other give rise to regular repeating



from Wikipedia

structures. These **secondary structures** include the well known **alpha-helix** and **beta strands**. Both were predicted by **Linus Pauling**, **Robert Corey**, and **Herman Branson** in 1951. Each structure has unique features. We use the terms **rise**, **repeat**, and **pitch** to describe the parameters of a helix. The repeat is the number of residues in a helix before it begins to repeat itself. The rise is the distance the helix elevates with addition of each residue. The pitch is the distance between the turns of the helix.

Alpha Helix

The **alpha helix** (left) forms as a result of interactions between amino acids separated by four residues. Interestingly, the side chains of the amino acids in an alpha helix are all pointed outwards from the axis of the helix. Alpha helices have a repeat of 3.6 amino acid residues per turn of the helix, meaning that four turns of the helix have approximately 14 amino acid residues. Hydrogen bonds occur between the C=O of one amino acid and the N-H of

A Beta Sheet

from Wikipedia

another amino acid four residues distant and these help to stabilize the structure (note that the C=O and N-H involved are part of the polypeptide backbone, not the R-groups) Some amino acids have high helix forming tendencies. They include methionine, alanine, leucine, uncharged glutamate, and lysine. Others, such as proline, glycine, and negatively charged aspartate, disfavor its formation.

Beta Strands

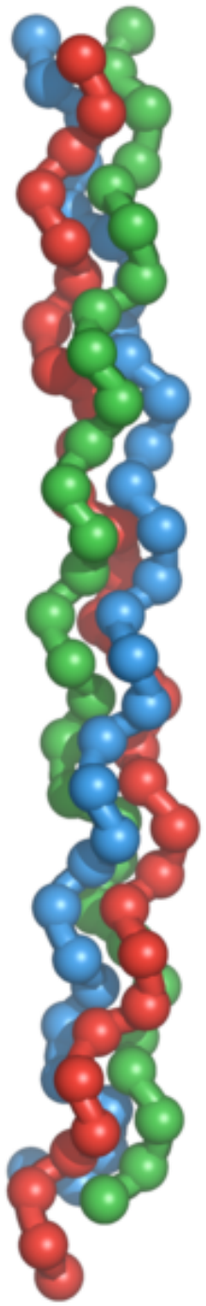
Beta strands are the most fundamental helix, having essentially a 2D backbone of ‘folds’ like those of the pleats of a curtain. Indeed, beta strands can be arranged together to form what are called **beta sheets**. Other regular structures are also known. What determines whether a given stretch of a protein is in a helical or other structure? Here is where the shape and chemistry of the side chains plays a role.

We wouldn’t be too popular
If keratins were globular
Our nails and hair would be as knots
Their structures folded up like clots
No strength they’d have and oh by gosh
They’d rearrange with every wash

Fibrous Proteins

Not all proteins have significant amounts of tertiary or quaternary structure. (As we shall see, these last two levels of structure arise from ‘bends’ in the polypeptide chains and interactions between separate polypeptide chains, respectively.)

Alpha keratin, for example, is what we refer to as a **fibrous protein** (also called **scleroprotein**). Alpha keratin has primary structure and secondary structure, but little tertiary or quaternary structure. Consequently, alpha keratin exists mostly as long fibers, such as are found in hair. **Beta-keratin** is a harder fibrous protein found in nails, scales, and claws. It is made up mostly of



Collagen

from Wikipedia

beta sheets. Proline, which is the least flexible amino acid, due to attachment of the side chain to the alpha-amino group, is less likely to be found in alpha helices, but curiously it is found abundantly in the fibrous protein known as collagen. **Collagen** (previous page) is the most abundant protein in the human body and is the 'glue' that literally sticks us together. How does the inflexibility of proline permit it to be in a helix? The answer is probably the parallel abundance in collagen of glycine, which contains the smallest side group and therefore has the greatest flexibility.

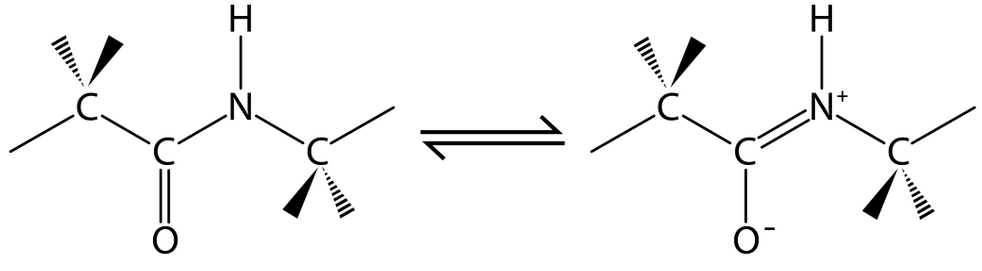
Click HERE and HERE, for Kevin's Protein Structure Lectures on YouTube

An interesting sidelight of the presence of proline in collagen is the chemical modification of prolines, by the addition of hydroxyl groups, after the protein is made. Such '**post-translational modifications**' are not uncommon. Threonine, serine, and tyrosine frequently have their hydroxyl side-chains phosphorylated. Lysines in collagen too are hydroxylated post-translationally. The hydroxylated prolines and lysines play a role in the formation of interchain hydrogen bonds and crosslinking of triple helices during the assembly of collagen fibrils. These bonds provide structural integrity to the collagen. The enzymes that add hydroxyls to proline and lysine require **vitamin C** (**ascorbic acid**) for their activity. Lack of vitamin C leads to the production of weakened collagen fibrils, resulting in a condition called scurvy.

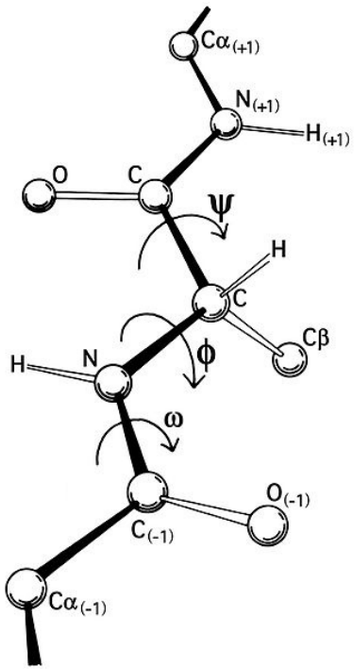
The carbonyl oxygen of the peptide bond can exist in resonance with the C-N bond, giving the peptide bond characteristics of a double bond and imposing limitations for rotation around it. If we treat the peptide bond as a double bond, then the arrangements of adjacent carbon bonds around it can be thought of as being in the *cis* or *trans* configurations. In proteins, not surprisingly, the preferred arrangement of these groups is strongly *trans* (1000/1). Of the 20 amino acids, the one that favors peptide bonds in the *cis* configuration most commonly is proline, but even for proline, the *trans* isomer is strongly preferred.

Ramachandran Plots

Another consequence of considering the peptide bond as a double bond is that it reduces the number of variable rotational angles of the polypeptide backbone. The terms **phi** and **psi** refer to rotational angles about the bonds between the N-alpha carbon



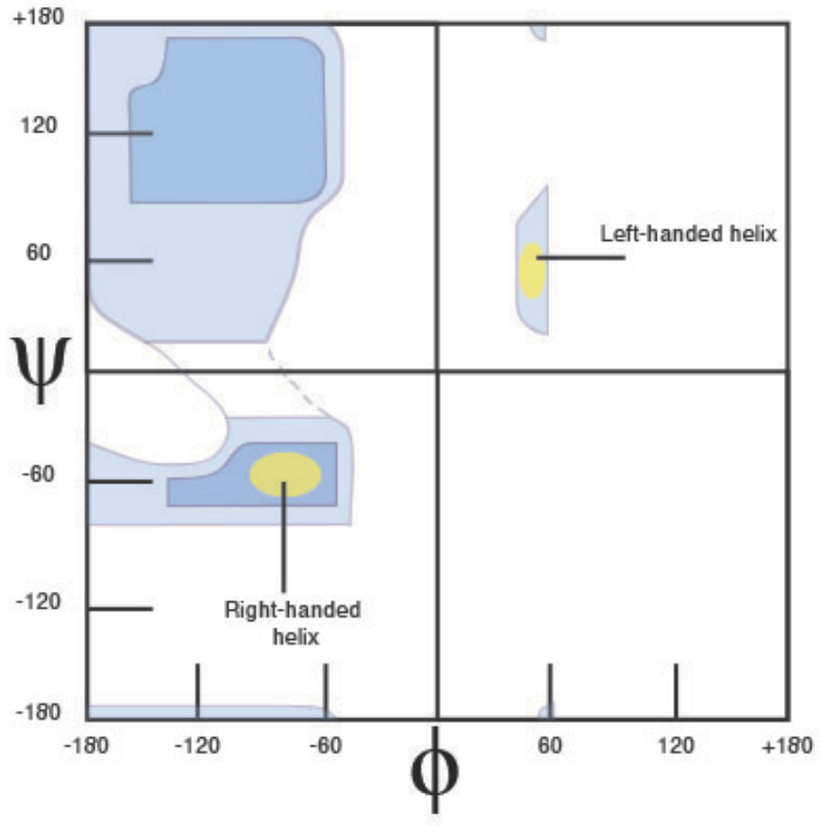
Peptide Bond Resonance



Phi and Psi Angles

from Wikipedia

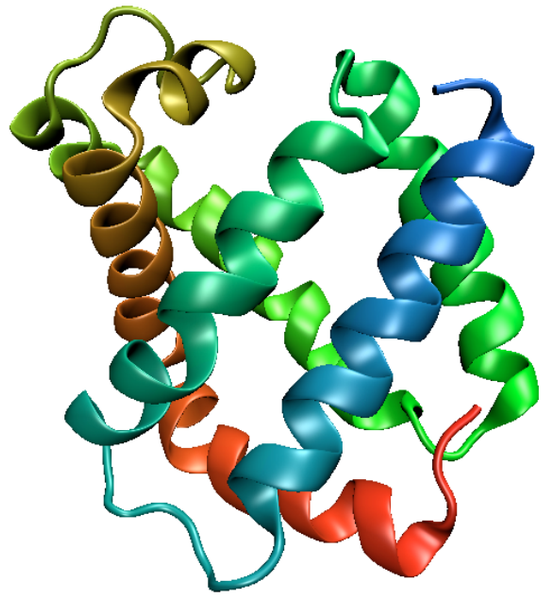
and alpha carbon-carbonyl carbon respectively (previous page). Given the bulkiness of R-groups, the phenomenon of **steric hindrance** and the tendency of close side chains to interact with each other, one might expect to find a bias in the values of phi and psi. Indeed, that is exactly what is observed. Dr. G.N. **Ramachandran** proposed such a result and, in a plot that bears his name, depicted the theoretical likelihood of each angle appearing in a polypeptide. More recent observations of actual phi and psi angles in data from the PDB protein database bear out Dr. Ramachandran's predictions. In the plot above, beta strands fit nicely in the darker blue section at the top and alpha helices fit in the yellow section near the middle.



Ramachandran Plot

Tertiary Structure

In contrast to secondary structures, which arise from interactions between amino acids close in primary structure, **tertiary structure** arises from interactions between amino acids more distant in primary structure. Such interactions are not possible in an endlessly stretching fiber because each amino acid placed between two amino acids causes them to be moved farther away from each other in what is essentially the two dimensions of a secondary structure. For distant amino acids to interact, they must be brought into closer proximity and this



The Structure of Myoglobin

from Wikipedia

O Little Protein Molecule

To the tune of "*O Little Town of Bethlehem*"

Oh little protein molecule
You're lovely and serene
With twenty zwitterions like
Cysteine and alanine

Your secondary structure
Has pitches and repeats
Arranged in alpha helices
And beta pleated sheets

The Ramachandran plots are
Predictions made to try
To tell the structures you can have
For angles phi and psi

And tertiary structure
Gives polypeptides zing
Because of magic that occurs
In protein fol-ding

A folded enzyme's active
And starts to catalyze
When activators bind into
The allosteric sites

Some other mechanisms
Control the enzyme rates
By regulating synthesis
And placement of phosphates

And all the regulation
That's found inside of cells
Reminds the students learning it
Of pathways straight from hell

Recorded by Tim Karplus
Lyrics by Kevin Ahern

requires bending and folding of the polypeptide chain. Proteins with such structures are referred to as 'globular' and they are, by far, the most abundant class of proteins. Indeed, it is in **globular proteins** that we have the most vivid images of the results of folding.

"Folds" in **polypeptides** arise as a result of '**bends**' between regions of secondary structure (such as alpha helix or beta strands). Such structures may be preferred due to incompatibility of a given amino acid side chain for a secondary structure formed by the amino acids preceding it. Bends occur commonly in proteins and proline is often implicated.

Bends do not have the predictable geometry of alpha helices or beta strands and are often referred to as random coils. Thus, even though protein structure can be described easily as regions of secondary structure separated by bends, the variability of bend structures makes prediction of tertiary

structure from amino acid sequence enormously more difficult than identifying/ predicting regions of secondary structure.

Hydrophobic Effect

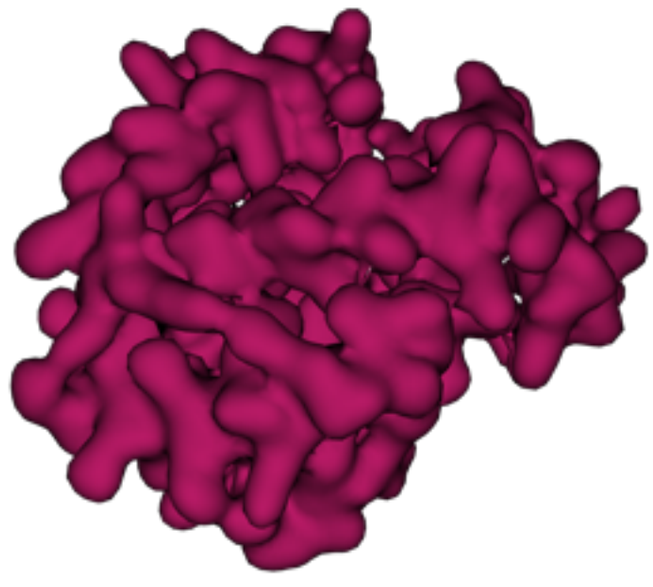
It is at the level of tertiary structure that the characteristic arrangement of hydrophobic and hydrophilic amino acids in a protein occurs. In an aqueous environment, for a protein to remain soluble, it must have favorable interactions with the water around it, hence, the positioning of hydrophilic amino acids externally. Another impetus for the folding phenomenon is a bit harder to understand. It is known as the **hydrophobic effect**. At a chemical level, it makes sense – hydrophobic amino acids will 'prefer' to interact with each other internally and away from water. The driving force for this phenomenon, though, is a bit more conceptually difficult. Consider a bottle containing oil and water. As everyone knows, the two liquids will not mix and instead will form separate layers. A reasonable question might be why they do this instead of one existing as tiny globules inside of the other. The answer to that question, as well as the positioning of hydrophobic amino acids in the interior of water soluble proteins, is the hydrophobic effect.

A hydrophobic effect causes the water on this leaf to assume a spherical shape

from Wikipedia

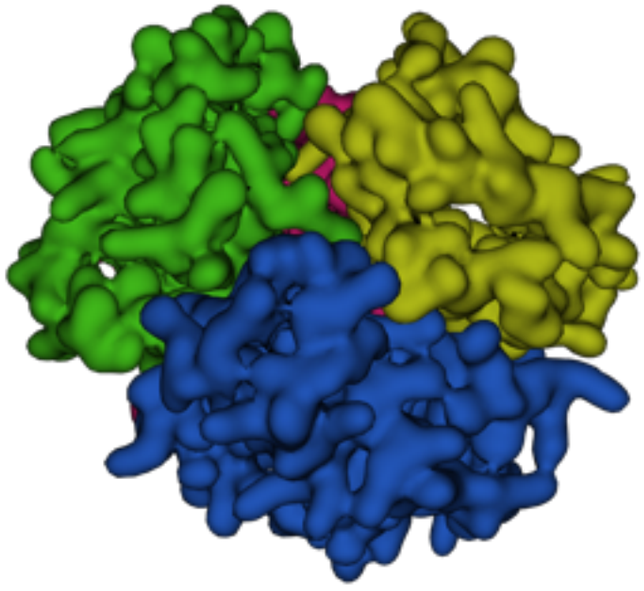
To understand the hydrophobic effect, perform the following experiment – take the water-oil mixture and shake it vigorously. This will force the layers to mix and one will observe that tiny globules of both water and oil can, in fact, be found initially in the layer of each. Over time, though, the tiny globules break up and merge with the appropriate layer. This is due to the phenomenon of entropy and consideration of surface area. First, the sum of the surface area of the embedded tiny globs is far greater than

Interactive 3.1



Oxygenated Myoglobin

Interactive 3.2

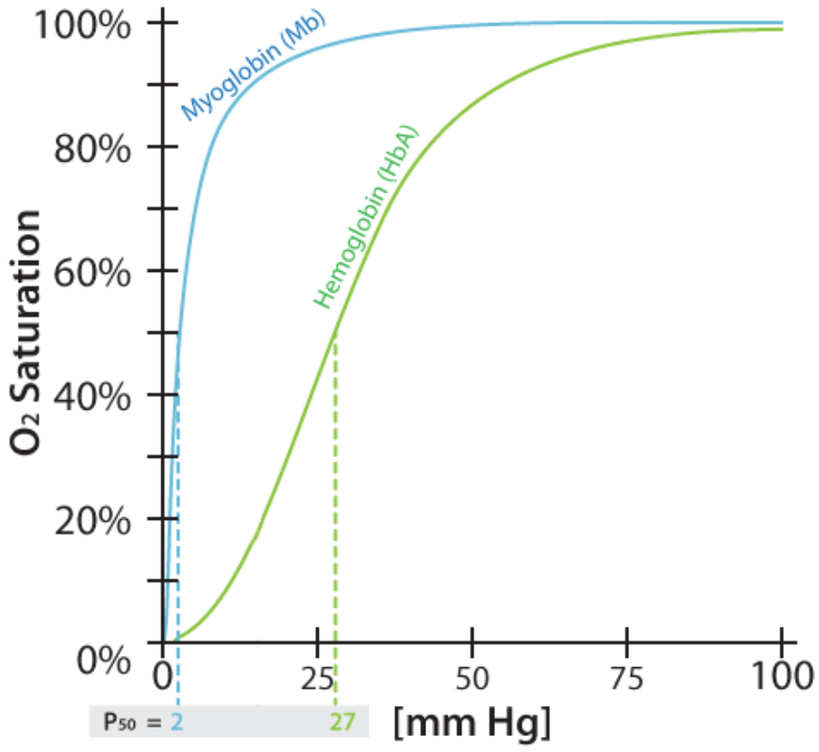


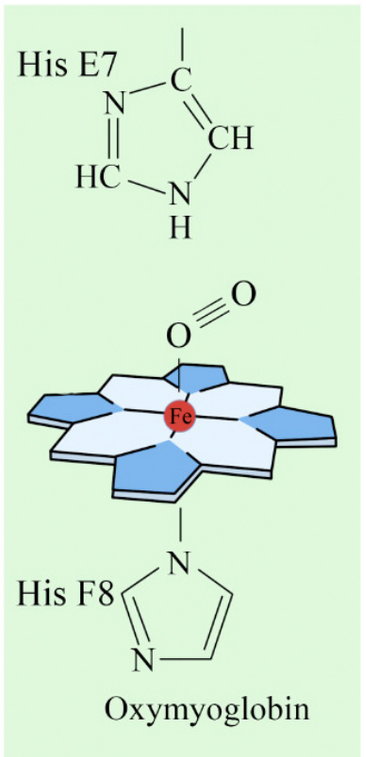
Hemoglobin in the Absence of Oxygen

the area of the region between the two layers after mixing is over. The smaller the globs, the more the surface area of interaction between the oil and the water. The minimum possible surface area of interaction occurs when there are no globs at all – just two layers and nothing else.

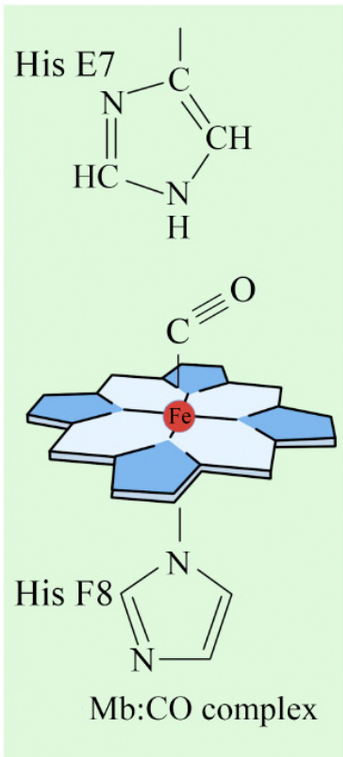
How does this relate to entropy? Interactions between the water-hydrophobic layers causes the molecules at the interface to arrange themselves precisely/regularly so as to minimize their interactions. Ordering thus occurs at the layer interfaces. The maximum amount of ordering occurs when the maximum surface areas of oil and water interact. Small globules give rise to more exposed surface area between the water and hydrophobic layers and, as a consequence, more ordering. Since **entropy** in a closed system tends to increase, it will tend to reduce the amount of ordering, if left alone. Thus, one can increase the ordering on a nanoscopic scale (forming globules) by applying energy in the form of shaking. When left alone, however, the system will increase its disorder by reducing the interactions between hydrophobic groups and hydrophilic ones.

In the oil water mixture, this causes the tiny globs to break up and produce the two layers we are familiar with because this is the minimum surface area that can be made between the two layers and thus the least ordering. In proteins, hydrophobic amino acid side chains are 'shielded' from water by placement internal to the protein, thus also reducing interfaces between hydrophobic residues and water. In both cases, entropy





Oxymyoglobin



Mb:CO complex

O_2 and CO Binding to Heme

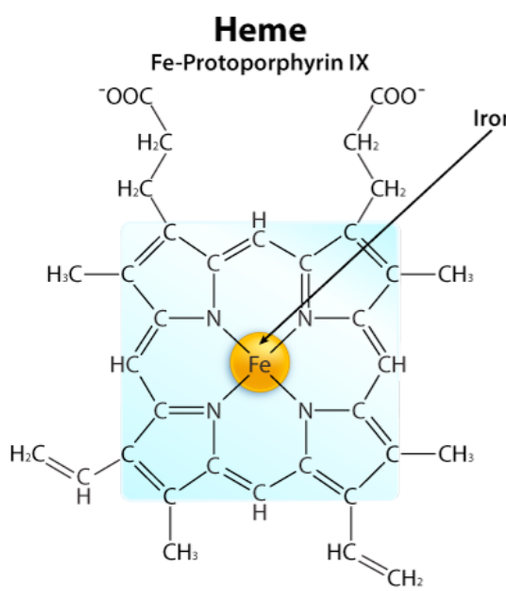
is increased, due to the reduced organization of the layers. Once formed, the interactions between the hydrophobic amino acid side chains helps to stabilize the overall protein structure.

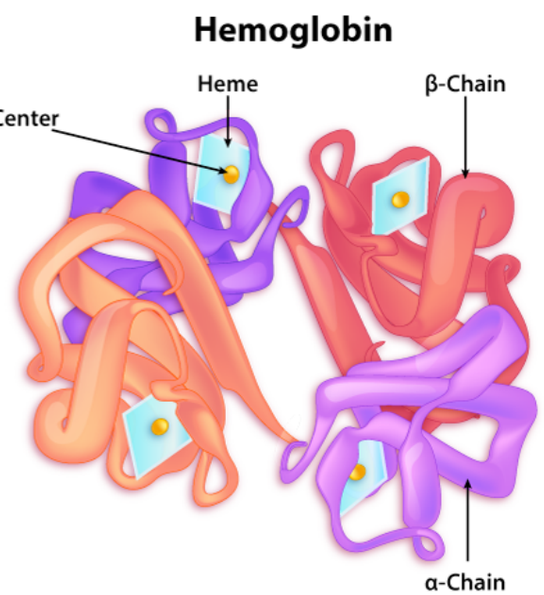
Quaternary Structure

The last level of protein structure we will consider is that of **quaternary structure**. In order to have quaternary structure, a protein must have multiple polypeptide subunits because the structure involves the arrangement of those subunits with respect to each other. Consider **hemoglobin**, the oxygen-carrying protein of our blood. It contains two identical subunits known as alpha and two other identical ones known as beta. These are arranged together in a fashion as shown on the previous page. By contrast, the related oxygen storage protein known as myoglobin only contains a single subunit. Hemoglobin has quaternary structure, but myoglobin does not. Multiple subunit proteins are common in cells and they give rise to very useful properties not found in single subunit proteins. In the case of hemoglobin, the multiple subunits confer

Click HERE and HERE for Kevin's Hemoglobin lectures on YouTube

My blood has a proclivity
For co-op-ER-a-TIV-it-y
It's 'cause when in the lung environs
Ox-y-GEN binds to the irons
And changes hemoglobin's fate
Out of a T to the R-State





the property of cooperativity – variable affinity for oxygen depending on the latter's concentration. In the case of enzymes, it can impart **allosterism** – the ability to have the activity of the enzyme altered by interaction with an effector molecule. We will discuss allosterism in detail in the next chapter.

Other Protein Structural Features

Not everything found in a protein is an amino acid. Proteins frequently have other chemical groups, known as prosthetic groups, bound to them, that are necessary for the function of a protein. Examples include the **porphyrin** ring of heme in myoglobin and hemoglobin that carries an iron so that oxygen can be bound. Metals are frequently employed by enzymes in their catalysis. Several **vitamins** (referred to as **coenzymes**), such as **thiamine** (B_1) and **riboflavin** (B_2) are modified and chemically bound to enzymes to help them perform specific catalytic functions.

Cooperativity

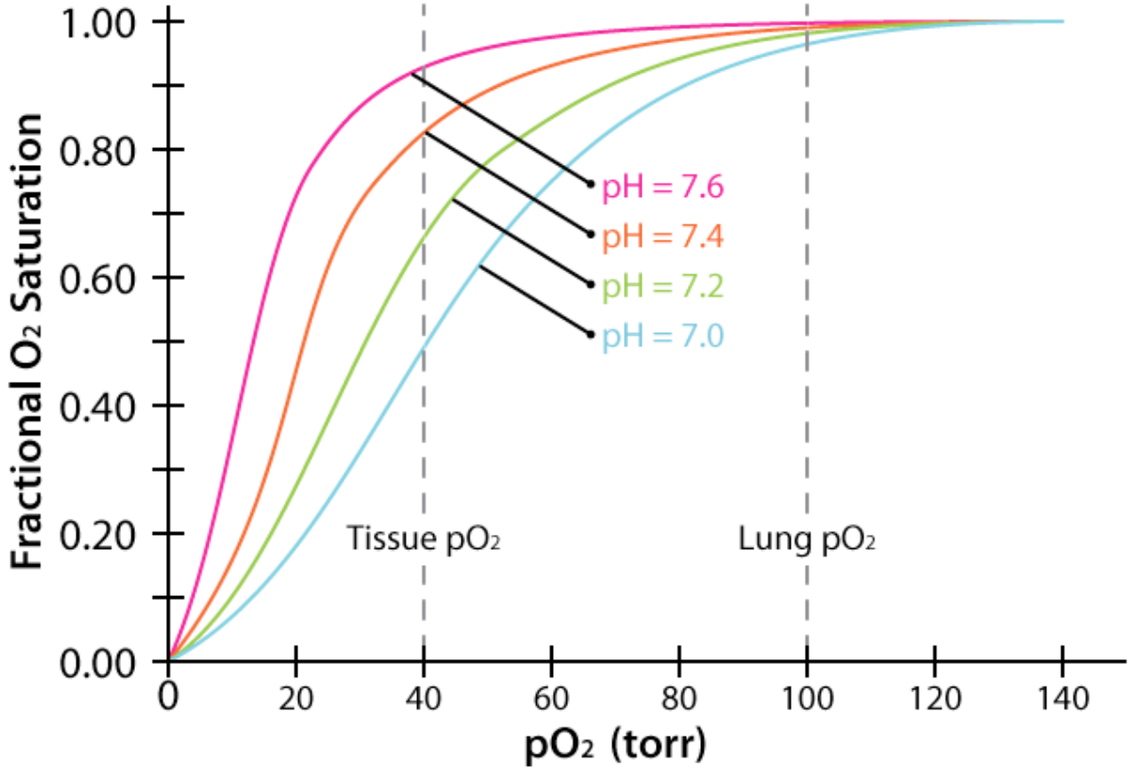
An interesting and important aspect of some proteins is the phenomenon of **cooperativity**. Cooperativity refers to the fact that binding of one ligand molecule by a protein favors the binding of additional molecules of the same type. Hemoglobin, for example, exhibits cooperativity when the binding of an oxygen molecule by the iron of the heme group in one of the four subunits causes a slight conformation change in the subunit. This happens because the heme iron is attached to a histidine side chain and binding of oxygen 'lifts' the iron along with the histidine ring (also known as the imidazole ring).

Since each hemoglobin subunit interacts with and influences the other subunits, they too are induced to change shape slightly when the first subunit binds to oxygen (a transition described as going from the T-state to the R-state). These shape changes favor each of the remaining subunits binding oxygen, as well. This is very important in the lungs where oxygen is picked up by hemoglobin, because the binding of the first oxygen molecule facilitates the rapid uptake of more oxygen molecules. In the tissues, where the oxygen concentration is lower, the oxygen leaves hemoglobin and the proteins flips from the R-state back to the T-state.

Cooperativity is only one of many fascinating structural aspects of hemoglobin that help the body to receive oxygen where it is needed and pick it up where it is abundant. Hemoglobin also assists in the transport of the product of cellular respiration (carbon dioxide) from the tissues producing it to the lungs where it is exhaled. Let us consider these individually.

Bohr Effect

The Bohr Effect was first described over 100 years ago by Christian Bohr. Shown graphically (above left), the observed effect is that hemoglobin's affinity for oxygen decreases as the pH

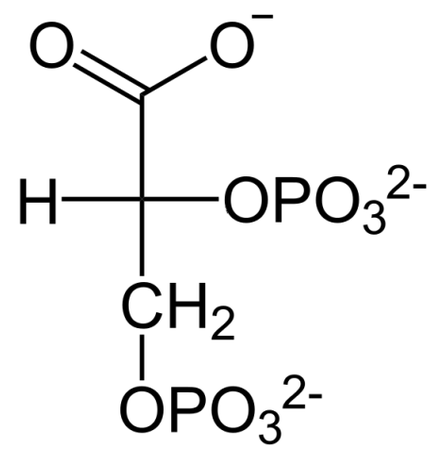


The Bohr Effect

decreases and/or as the concentration of carbon dioxide increases. Binding of the protons by histidine helps to facilitate structural changes in the protein and also with the uptake of carbon dioxide. Physiologically, this has great significance because actively respiring tissues (such as contracting muscles) require oxygen and release protons and carbon dioxide. The higher the concentration of protons and carbon dioxide, the more oxygen is released to feed the tissues that need it most.

2,3 BPG

Another molecule affecting the release of oxygen by hemoglobin is 2,3 bisphosphoglycerate (also called 2,3 BPG or just BPG). Like protons and carbon dioxide, 2,3 BPG is produced by actively respiring tissues, as a byproduct of glucose metabolism. The 2,3 BPG molecule fits into the 'hole of the donut' of adult hemoglobin. Such binding of 2,3 BPG favors the T (tight) state of hemoglobin, which has a reduced affinity for oxygen. In the absence of 2,3 BPG, hemoglobin can exist in the R (relaxed) state, which has a high affinity for oxygen.



2,3 BPG

Fetal Hemoglobin

Adult hemoglobin releases oxygen when it binds 2,3 BPG. This is in contrast to fetal hemoglobin, which has a slightly different configuration ($\alpha_2\gamma_2$) than adult hemoglobin ($\alpha_2\beta_2$). Fetal hemoglobin has a greater affinity for oxygen than maternal hemoglobin, allowing the fetus to obtain oxygen effectively from the mother's blood. Part of the reason for fetal hemoglobin's greater affinity for oxygen is that it doesn't bind 2,3 BPG.

Another significant fact about 2,3 BPG is that its concentration is higher in the blood of smokers than it is of non-smokers.

Hemoglobin's Moving Around

To the tune of "*Santa Claus is Coming to Town*"

Oh isn't it great
What proteins can do
Especially ones that bind to O2
Hemoglobin's moving around

Inside of the lungs
It picks up the bait
And changes itself from T to R state
Hemoglobin's moving around

The proto-porphyrin system
Its iron makes such a scene
Arising when an O2 binds
Pulling up on histidine

The binding occurs
Cooperatively
Thanks to changes qua-ter-nar-y
Hemoglobin's moving around

It exits the lungs
Engorged with O2
In search of a working body tissue
Hemoglobin's moving around

The proton concentration
Is high and has a role
Between the alpha betas
It finds imidazole

To empty their loads
The globins decree
"We need to bind 2,3BPG"
Hemoglobin's moving around

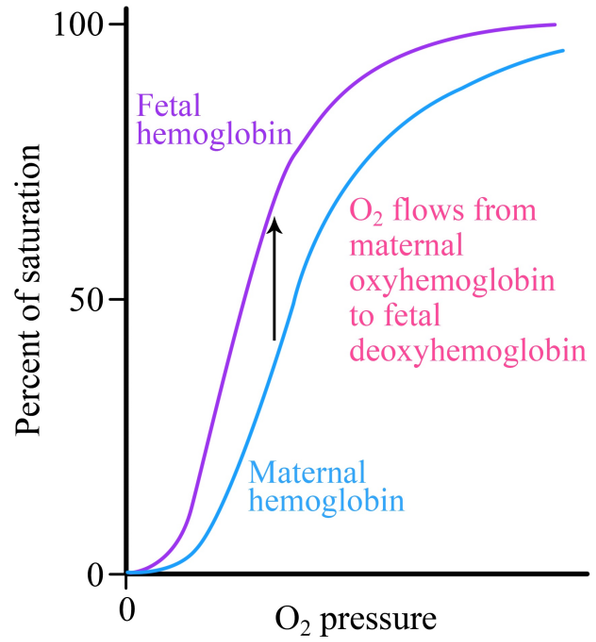
The stage is thus set
For grabbing a few
Cellular dumps of CO2
Hemoglobin's moving around

And then inside the lungs it
Discovers ox-y-gen
And dumps the CO2 off
To start all o'er agin

So see how this works
You better expect
To have to describe the Bohr effect
Hemoglobin's moving around

Recorded by Tim Karplus
Lyrics by Kevin Ahern

Consequently, hemoglobin in a smoker's blood spends more time in the T state than the R state. That is a problem when it is in the lungs, where being in the R state is necessary to maximally load the hemoglobin with oxygen. A high blood level of 2,3 BPG is one of the reasons smokers have trouble breathing when they exercise – they have reduced oxygen carrying capacity.



Fetal Hemoglobin Binding of O_2

Last, though it is not related directly to 2,3 BPG, smokers have another reason why their oxygen carrying capacity is lower than that of non-smokers. Cigarette smoke contains carbon monoxide and this molecule, which has almost identical dimensions to molecular oxygen, competes effectively with oxygen for binding to the iron

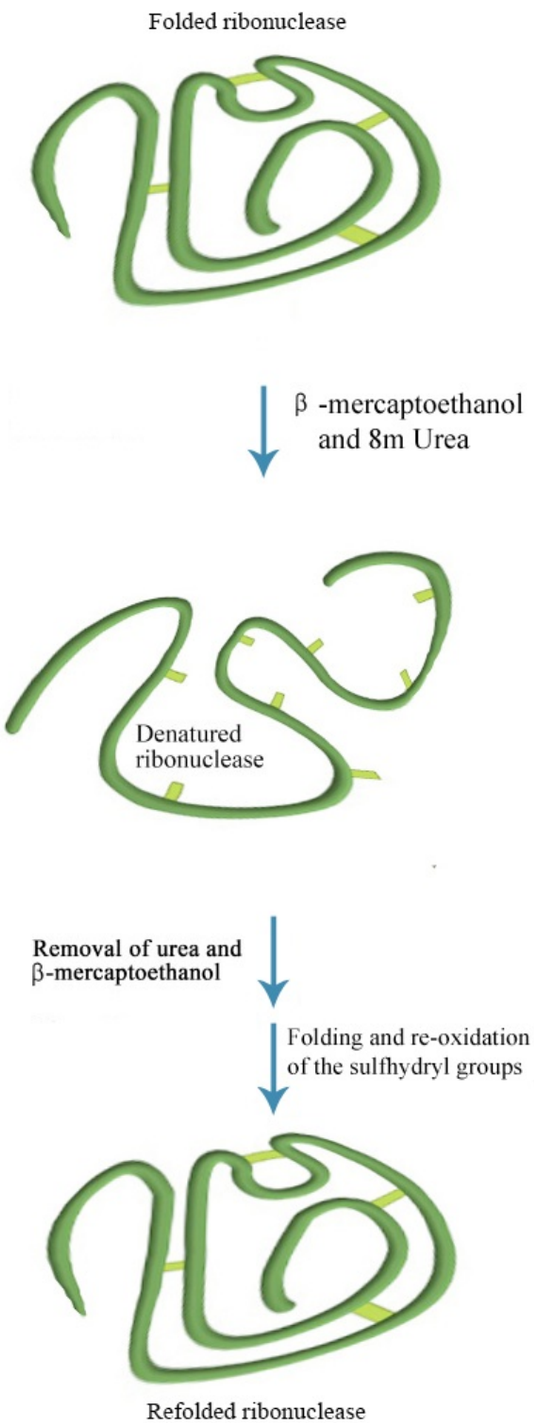
atom of heme. Part of carbon monoxide's toxicity is due to its ability to bind hemoglobin and prevent oxygen from binding.

Denaturation

For proteins, function is dependent on precise structure. Loss of the precise, folded structure of a protein is known as denaturation and is usually accompanied by loss of function. Anyone who has ever worked to purify an enzyme knows how easy it is for one to lose its activity. A few enzymes, such as ribonuclease, are remarkably stable under even very harsh conditions. For most others, a small temperature or pH change can drastically affect activity. The reasons for these differences vary, but relate to 1) the strength of the forces holding the structure together and 2) the ability of a protein to refold itself after being denatured. Let us consider these separately below.

Forces Stabilizing Structure

Amino acids are linked one to the other by peptide bonds. These covalent bonds are extraordinarily stable at neutral pHs, but can be broken by hydrolysis with heat under acidic conditions. Peptide bonds, however, only stabilize primary structure and, in fact, are the only relevant force responsible for it. Secondary structure, on the other hand, is generally stabilized by weaker forces, including hydrogen bonds. Hydrogen bonds are readily disrupted by heat, urea, or guanidinium chloride.



Denaturation and Renaturation of Ribonuclease

Forces stabilizing tertiary structure include ionic interactions, disulfide bonds, hydrophobic interactions, metallic bonds, and hydrogen bonds. Of these, the ionic interactions are most sensitive to pH changes. Hydrophobic bonds are most sensitive to detergents. Thus, washing one's hands helps to kill bacteria by denaturing critical proteins they need to survive. Metallic bonds are sensitive to oxidation/reduction. Breaking disulfide bonds requires either a strong oxidizing agent, such as performic acid or a strong reducing agent on another disulfide, such as mercaptoethanol or dithiothreitol.

Quaternary structures are stabilized by the same forces as tertiary structure and have the same sensitivities.

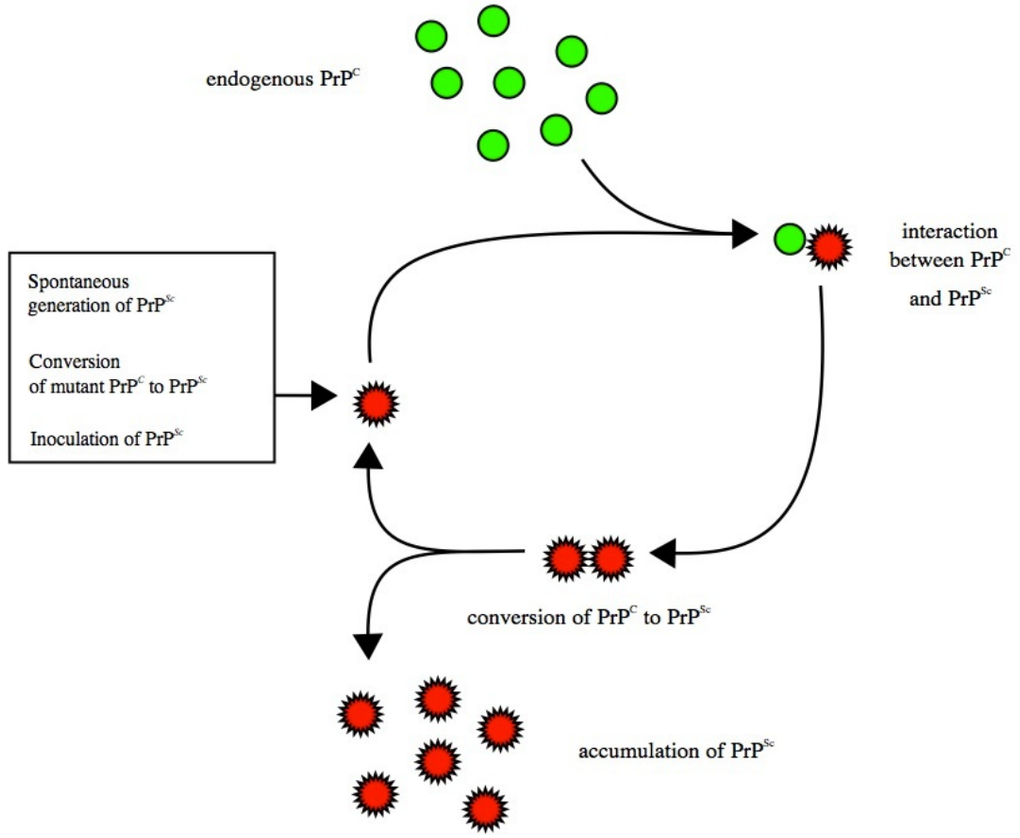
Refolding Denatured Proteins

All of the information for protein folding is contained in the primary structure of the protein. It may seem curious then that most proteins do not refold into their proper, fully active form after they have been denatured and the denaturant is removed. A few do, in fact, refold correctly under these circumstances. A good example is bovine ribonuclease (also called RNase). Its catalytic activity is very resistant to heat and urea. However, if one treats the enzyme with mercaptoethanol (which breaks disulfide bonds) prior to urea treatment and heating, activity is lost, indicating that the covalent disulfide bonds help stabilize the overall enzyme structure. If one allows the enzyme mixture to cool back down to room temperature, over time some enzyme activity reappears, indicating that ribonuclease can re-fold under the proper conditions.

There are not very many ways
Inactivating RNase
It's stable when it's hot or cold
Because disulfides tightly hold
If you desire to make it stall
Use hot mercaptoethanol

Irreversible Denaturation

Most enzymes, however, do not behave like ribonuclease. Once denatured, their activity cannot be recovered to any significant extent. This may seem to contradict the idea that folding information is inherent to the sequence of amino acids in the protein. It does not. The reason most enzymes can't refold



from Wikipedia

Prion Protein Misfolding

properly is due to two phenomena. First, normal folding may occur as proteins are being made. Interactions among amino acids early in the synthesis are not "confused" by interactions with amino acids later in the synthesis because those amino acids aren't present as protein synthesis starts. In many cases, the proper folding of newly made polypeptides is also assisted by special proteins called chaperones. Chaperones bind to newly made proteins, preventing interactions that might result in misfolding. Thus, early folding and the assistance of chaperones

eliminate some potential “wrong-folding” interactions that can occur if the entire sequence was present when folding started.

Denatured full-length polypeptides have many more potential wrong folds that can occur. A second reason most proteins don’t refold properly after denaturation is probably that folding, like any other natural phenomenon, is driven by energy minimization. Though the folded structure may have a low energy, the path leading to it may not be all downhill. Like a chemical reaction that has energies of activation that must be overcome for the reaction to occur, folding likely has peaks and valleys of energy that do not automatically lead directly to the proper fold. Again, folding during synthesis leads the protein along a better-defined path through the energy maze of folding that denatured full-length proteins can’t navigate.

I think that if I chanced to be on
The protein making up a prion
I’d twist it and for goodness sakes
Stop it from making fold mistakes

Prions and Misfolding

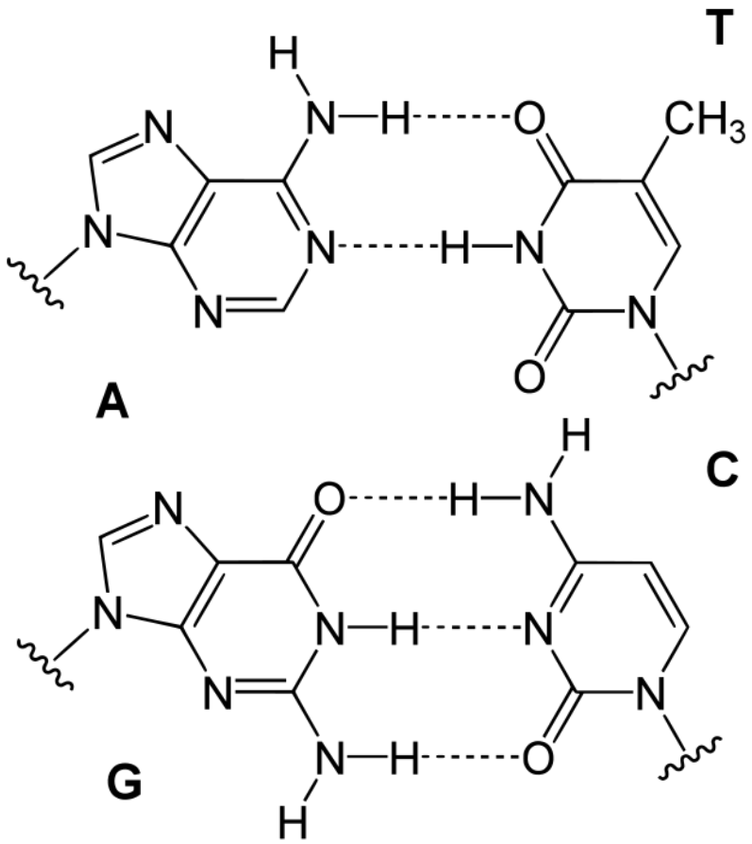
Folding and the stability of folded proteins is an important consideration for so-called “infectious” proteins known as prions. These mysterious proteins, which are implicated in diseases, such as mad cow disease and the related human condition known as Creutzfeldt-Jakob disease, result from the improper folding of a brain protein known as PrP. The misfolded protein has two important properties that lead to the disease. First, it tends to aggregrate into large complexes called amyloid plaques that damage/destroy nerve cells in the brain, leading ultimately to dementia and loss of brain function.

Second, and probably worse, the misfolded protein “induces” other copies of the same protein to misfold as well. Thus, a misfolded protein acts something like a catalytic center and the disease progresses rapidly. The question arises as to how the PrP protein misfolds to begin with, but the answer to this is not clear. There are suggestions that exposure in the diet to misfolded proteins may be a factor, but this is disputed. An outbreak of mad cow disease in Britain in the 1980s was followed by a rise in the incidence of a rare form of human Creutzfeld-Jakob disease called variant CJD (v-CJD), lending some credence to the hypothesis. It is possible that misfolding of many proteins occurs sporadically without consequence or observation, but if PrP misfolds, the results are readily apparent. Thus, Creutzfeld-Jakob disease may ultimately give insights into the folding process itself.

Nucleic Acids

Determination of the structure of the most common form of DNA, known as the B form, was one of the most important scientific advances of the 20th century. Using data from **Rosalind Franklin**, **James Watson** and

Click HERE for Kevin’s YouTube lecture on Nucleic Acid Structure

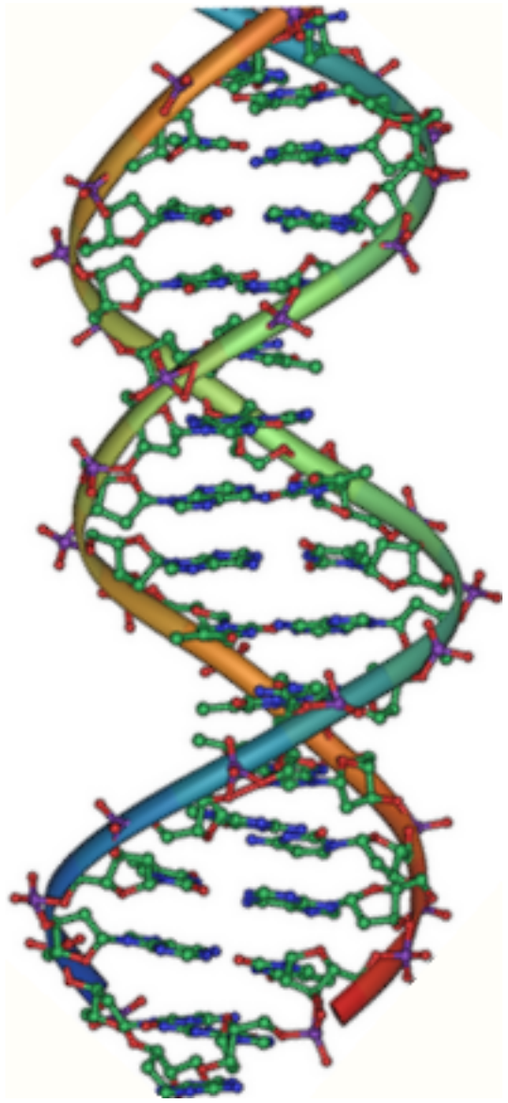


A-T Base Pairs (Top) and G-C Base Pairs (Bottom)

Francis Crick initiated the modern era of molecular biology with their paper in the April 25, 1953 issue of Nature. Arguably, that single page paper has had more scientific impact per word than any other research article ever published. Today, every high school biology student knows the double helical structure in which G pairs with C and A pairs with T. The DNA molecule is a polymer of nucleoside monophosphates with **phosphodiester bonds** between the phosphate and the 5' end of one deoxyribose and the 3' end of the next one. In the B form the DNA helix has a repeat of 10.5 base pairs per turn, with sugars and phosphate forming the covalent "backbone" of the molecule and the adenine, guanine, cytosine, and thymine bases oriented in the middle where they form the now familiar base-pairs that look like the rungs of a ladder.

Hydrogen bonds help to hold the base pairs together, with two hydrogen bonds per A-T pair and three hydrogen bonds per G-C pair. The two strands of a DNA duplex run in opposite directions. The 5' end of one strand is paired with the 3' end of the other strand and vice-versa at the other end of the duplex. The B form of DNA has a prominent major groove and a minor groove tracing the path of the helix (shown at left). Proteins, such as transcription factors bind in these grooves and access the hydrogen bonds of the base pairs to "read" the sequence therein.

Other forms of DNA besides the B form are known. One of these, the 'A' form, was identified by Rosalind



DNA Double Helix

Major Groovy

To the tune of "*Feelin' Groovy*"

The DNA forms
A and B
Have bases
Complementary
Despite the similarities
They differ in their
Major groovies
Nanananananana major groovies

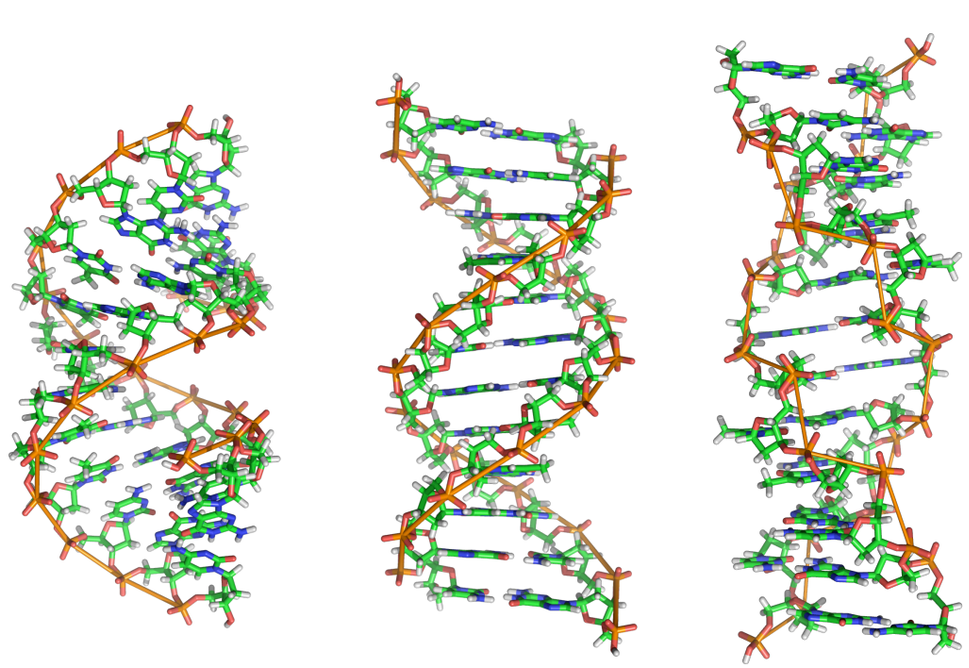
Transcription factors
With their bindin'
Cause DNA to
Start unwindin'
Holding it
Aggressively
By forming bonds
In major groovies
Nanananananana major groovies

For proteins, the key
To sequence I-D
Is hydrogen bonding, each base pair unique
Purine, pyrimidine patterns discrete
In DNA's most
Major groovy
Nanananananana major groovy

Recorded by David Simmons
Lyrics by Kevin Ahern

Franklin in the same issue of Nature as Watson and Crick's paper. Though the A structure is a relatively minor form of DNA and resembles the B form, it turns out to be important in the duplex form of RNA and in RNA-DNA hybrids. Both the A form and the B form of DNA have the helix oriented in what is termed the right-handed form.

These stand in contrast to another form of DNA, known as the Z form. Z-DNA, as it is known, has the same base-pairing rules as the B and A forms, but instead has the helices twisted in the opposite direction, making a left-handed helix (see figure on previous page). The Z form has a sort of zig-zag shape, giving



A, B, and Z Forms of DNA

from Wikipedia

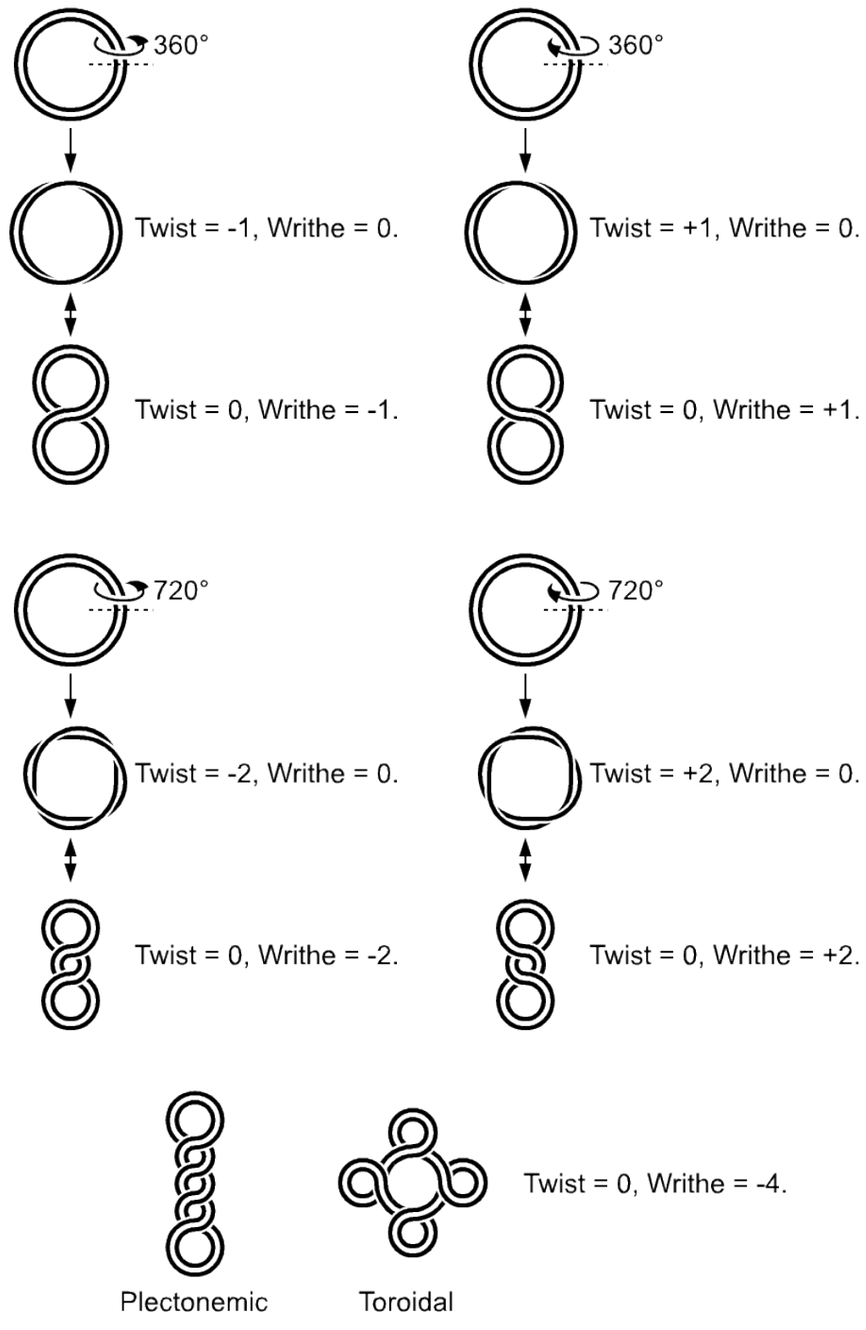
It's taught in school of DNA
The race for structure underway
Gave rise to competition huge
Along with data subterfuge
In view of this we should extend
New authorship to make amends
A fairer order could be picked
Franklin, Wilkins, Watson, Crick

rise to the name Z DNA. In addition, the helix is rather stretched out compared to the A and B forms. Why are there different forms of DNA? The answer relates to both superhelical tension and sequence bias. Sequence bias means that certain sequences tend to favor the "flipping" of B form DNA into other forms. Z DNA forms are favored by long stretches of alternating Gs and Cs. Superhelical tension will be discussed below.

Superhelicity

Short stretches of linear DNA duplexes exist in the B form and have 10.5 base pairs per turn. Double helices of DNA in the cell can vary in the number of base pairs per turn they contain. There are several reasons for this. For example, during DNA replication, strands of DNA at the site of replication get unwound at the rate of 6000 rpm by an enzyme called helicase. The effect of such local unwinding at one place in a DNA has the effect increasing the winding ahead of it. Unrelieved, such 'tension' in a DNA duplex can result in structural obstacles to replication.

Such adjustments can



Topoisomers of DNA

from Wikipedia

B-DNA

To the tune of "*Y-M-C-A*"

Phosphates
Are in nucleotides
I say phosphates
Cover bases inside
I say phosphates
Span the 5 and 3 primes
There's no need - to - be - real - mixed - up

Bases
Carry info you see
I say bases
Are all complement'ry
I say bases
Like A,T,G and C
They have got - to - be – all - paired - up

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
It's got everything
A polymerase needs
When you melt all the A's and T's

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
You can make RNAs
With a po-ly-mer-ase
Just by pairing up U's with A's

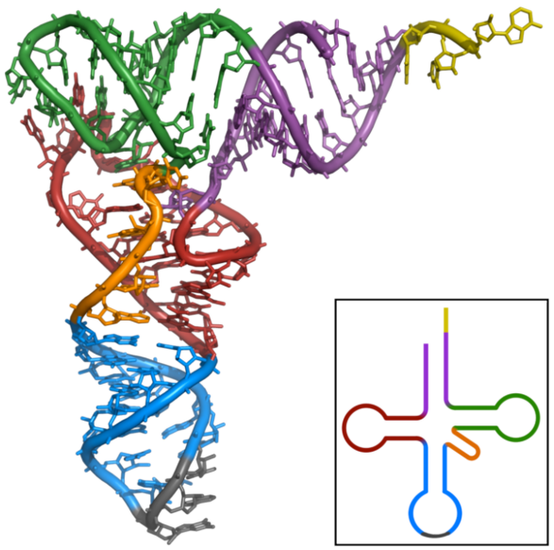
Proteins
Full of amino A's
I say proteins
Come from mRNAs
I say proteins
Require tRNAs
There is more – you - need - to – trans-late

Codons
Like our friend U-A-C
I say codons
Come in clusters of three
I say codons
Have one base wobble –ee
Now you can - go - forth - and - tran-slate

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
With those hydrogen Bs
And right-hand he-li-ces
Anti-par-a-llel fives and threes

It's fun to play with some B-DNA
It's got a boatload of G-C-T-A
With those hydrogen Bs
And right-hand he-li-ces
Anti-par-a-llel fives and threes

Recorded by Tim Karplus
Lyrics by Kevin Ahern



3D tRNA Structure (left) and
2D projection (right)

from Wikipedia

occur in three ways. First, tension can provide the energy for 'flipping' DNA structure. Z-DNA can arise as a means of relieving the tension. Second, DNA can 'supercoil' to relieve the tension. In this method, the strands of the duplex can cross each other repeatedly, much like a rubber band will coil up if one holds one section in place and twists another part of it. Third, enzymes called **topoisomerases** can act to relieve or, in some cases, increase the tension by adding or removing twists in the DNA.

RNA Structures

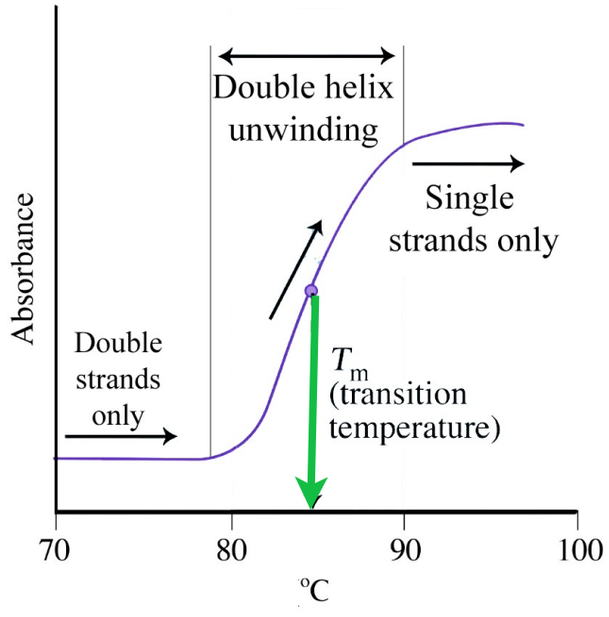
With respect to structure, RNAs are more varied than their DNA cousins. Created by copying regions of DNA, cellular RNAs are synthesized as single strands, but they often have self-complementary regions leading to "fold-backs" containing duplex regions. The structure of **tRNAs** and **rRNAs** are excellent examples. The base-pairing rules of DNA are the same in RNA (with U in RNA replacing the T from DNA), but in addition, base pairing between G and U can also occur in RNA. This latter fact leads to many more possible duplex regions in RNA that can exist compared to single strands of DNA.

RNA structure, like protein structure, has importance, in some cases, for catalytic function. Like random coils in proteins that give rise to tertiary structure, single-stranded regions of RNA that link duplex regions give these molecules a tertiary structure, as well. Catalytic RNAs, called ribozymes, catalyze important cellular reactions, including the formation of peptide bonds. DNA, which is usually present in cells in strictly duplex forms (no tertiary structure, per se), is not known to be involved in catalysis.

RNA structures are important for reasons other than catalysis. The 3D arrangement of tRNAs is important for enzymes that attach amino acids to them to do so properly. Small RNAs called siRNAs found in the nucleus of cells appear to play roles in both gene regulation and in cellular defenses against viruses. The key to the mechanisms of these actions is the formation of short fold-back RNA structures that are recognized by cellular proteins and then chopped into smaller units. One strand is copied and used to base pair with specific mRNAs to prevent the synthesis of proteins from them.

Denaturing Nucleic Acids

Like proteins, nucleic acids can be denatured. Forces holding duplexes together include hydrogen bonds between the bases of each strand that, like the hydrogen bonds in proteins, can be broken with heat or urea. (Another important stabilizing force for DNA arises from the stacking interactions between the bases in a strand.) Single strands absorb light at 260 nm more strongly than double strands (hyperchromic effect), allowing one to easily follow denaturation. For DNA, strand separation and strand hybridization are important aspects of the technique known as the polymerase chain reaction (PCR). Strand separation of DNA duplexes is accomplished in the method by heating them to boiling. Hybridization is an important aspect of the method that requires complementary single strands to "find" each other and form a duplex. Thus, DNAs (and RNAs too) can renature readily, unlike most proteins. Considerations for efficient hybridization (also called annealing) include temperature, salt concentration, strand concentration, and magnesium ion levels.



Hyperchromic Effect

Carbohydrates

The last class of macromolecules we will consider structurally here is the carbohydrates. Built of sugars or modified sugars, carbohydrates have several important functions, including structural integrity, cellular identification, and energy storage.

Monosaccharides

Simple sugars, also known as monosaccharides, can generally be written in the form $C_x(H_2O)_x$. It is for this reason they are referred to as carbo-hydrates. By convention, the letters 'ose' at the end of a biochemical name flags a molecule as a sugar. Thus, there are glucose, galactose, sucrose, and many other '-oses'. Other descriptive nomenclature involves use of a prefix that tells how many carbons the sugar contains. For example, glucose, which contains six carbons, is described as a hexose. The following list shows the prefixes for numbers of carbons in a sugar:

- Tri- = 3
- Tetr- = 4
- Pent- = 5
- Hex- =6
- Hept- = 7
- Oct- = 8

Other prefixes identify whether the sugar contains an aldehyde group (aldo-) or a ketone (keto) group. Prefixes may be combined. Glucose, which contains an aldehyde group, can be described as an aldo-hexose. The list that follows gives some common sugars and some

descriptors.

- Ribose = aldo-pentose
- Glucose = aldo-hexose
- Galactose = aldo-hexose
- Mannose = aldo-hexose
- Glyceraldehyde = aldo-triose
- Erythrose – aldo-tetrose
- Fructose = keto-hexose
- Ribulose = keto-pentose
- Sedoheptulose = keto-heptose
- Dihydroxyacetone = keto-triose

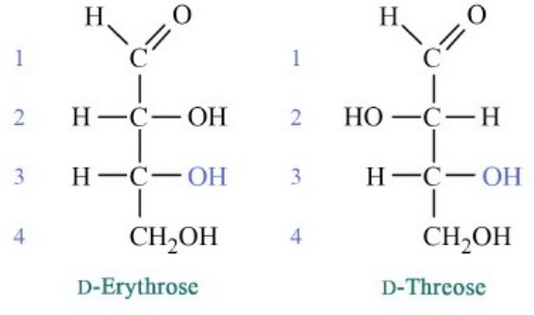
Stereoisomer Nomenclature

Sugars of a given category (hexoses, for example) differ from each other in the stereoisomeric configuration of their carbons. Two sugars having the same number of carbons (hexoses, for example) and the same chemical form (aldoses, for example), but differing in the stereoisomeric configuration of their carbons are called diastereomers. Biochemists use D and L nomenclature to describe sugars, as explained below.

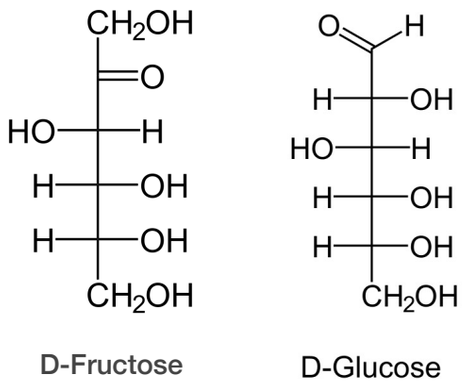
D-sugars predominate in nature, though L-forms of some sugars, such as fucose, do exist. The D and L designation is a bit more complicated than it would appear on the surface. To determine if a sugar is a D-sugar or an L-sugar, one simply examines the configuration of the highest numbered asymmetric carbon. If the hydroxyl is written to the right, it is a D-sugar. If the hydroxyl is on the left, it is an L-sugar. That part is simple. The confusion about D and L arises because L sugars of a given name (glucose, for example) are mirror images of D sugars of the same name. The figure on the previous page shows the structure of D- and L-glucose. Notice that D-glucose is not converted into L-glucose simply by flipping the configuration of the fifth carbon in the molecule. There is another name for sugars that are mirror images of each other. They are called **enantiomers**. Thus, L-glucose and D-glucose are enantiomers, but **D-Erythrose** and **D-Threose** are diastereomers.

Click HERE and HERE for Kevin's YouTube lectures on Carbohydrate Structure

Sugars of 5-7 carbons can fairly easily form ring structures (called **Haworth structures**). For aldoses like glucose, this involves formation of a hemi-acetal. For ketoses like fructose, it involves formation of a hemi-ketal. The bottom line for both is that the oxygen that was part of the aldehyde or the ketone group is the one that becomes a part of the ring. More important than the oxygen, though, is the fact that the carbon attached to it (carbon #1 in aldoses or #2 in

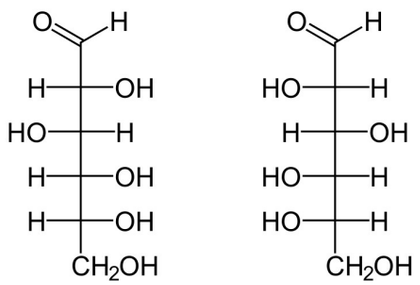


Diastereomers



D-Fructose D-Glucose

Ketose and Aldose

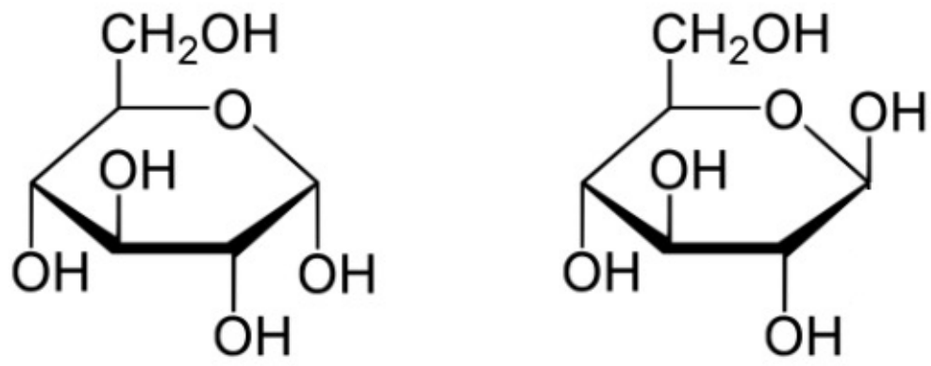


D-Glucose L-Glucose

Enantiomers

ketoses) becomes asymmetric as a byproduct of the cyclization. This new asymmetric carbon is called the **anomeric carbon** and it has two possible configurations, called alpha and beta.

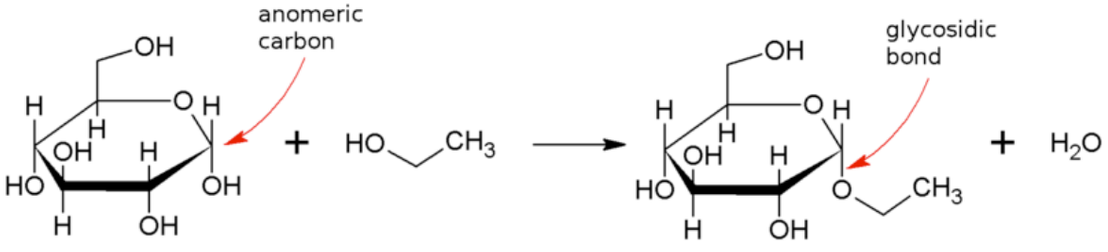
A solution of glucose will contain a mixture of alpha and beta forms. Whether the alpha or the beta arises upon cyclization is partly determined by geometry and partly random. Thus, one can find a bias for one form, but usually not that form exclusively. A given molecule of sugar will flip between alpha and beta over time. A requirement for this is that the hydroxyl on the anomeric carbon is unaltered, thus facilitating flipping back to the straight chain form followed by recyclization. If the hydroxyl becomes chemically altered in any way (for example, replacement of its hydrogen by a methyl group), a **glycoside** is formed. Glycosides are locked in the same alpha or beta configuration they were in when the modification was made. Glycosides are commonly found in nature. **Sucrose**, for example , is a di-glycoside – both the



α-D Glucose (left) and β-D Glucose (right)

glucose and the fructose have had their anomeric hydroxyls altered by being joined together.

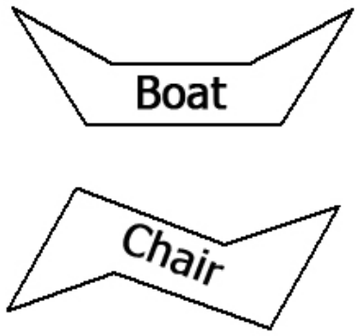
The last considerations for sugars relative to their structure are their chemical reactivity and modification. The aldehyde group of aldoses is susceptible to oxidation, whereas ketoses are less so. Sugars that are readily oxidized are called '**reducing sugars**' because their oxidation causes other reacting molecules to be reduced. Reducing sugars can easily be identified in a chemical test. Chemical modification of sugars occurs readily in cells. As we will see, phosphorylation of sugars occurs routinely during metabolism. Oxidation of sugars to create carboxyl groups also can occur. Reduction of aldehyde/ketone groups of sugars creates what are called sugar alcohols, and other modifications, such as addition of sulfates and amines also readily occur.



Glycoside Formation

Boat/Chair Conformations

Independent of stereoisomerization, sugars in ring form of a given type (such as glucose) can "twist" themselves into alternative conformations called **boat** and **chair**. Note that this rearrangement does not change the relative positions of hydroxyl groups. All that has changed is the shape of the molecule. As shown for glucose, one can see that the beta-hydroxyl of glucose is closer to the CH_2OH (carbon #6) in the boat form than it is in the chair form. Steric hindrance can be a factor in favoring one configuration over another.

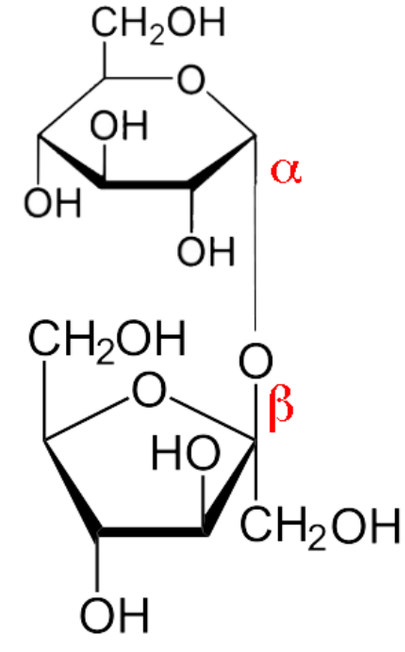


Disaccharides

Sugars are readily joined together (and broken apart) in cells. Sucrose (right), which is common table sugar, is made by joining the anomeric hydroxyl of alpha-D-glucose to the anomeric hydroxyl of beta-D-fructose. Not all **disaccharides** join the anomeric hydroxyls of both sugars. For example, **lactose** (milk sugar) is made by linking the anomeric hydroxyl of **galactose** in the beta configuration to the hydroxyl of carbon #4 of glucose.

Oligosaccharides

The term '**oligosaccharide**' is used to describe polymers of sugars of 5-15 units, typically. Oligosaccharides are not commonly found free in cells, but instead are found covalently attached to proteins, which are then said to be glycosylated. Oligosaccharides attached to proteins may be N-linked (through asparagine) or O-linked (though serine or threonine). **O-linked sugars** are added only in the Golgi apparatus while **N-linked sugars** are attached starting in the endoplasmic reticulum and then completed in the Golgi.



Sucrose

Oligosaccharides often function as identity markers, both of cells and proteins. On the cell surface, glycoproteins with distinctive oligosaccharides attached establish the identity of each cell. The types of oligosaccharides found on the surface of blood cells is a determinant of blood type.

Hark the Sucrose

To the tune of "*Hark the Herald*"

Carbohydrates all should sing
Glory to the Haworth ring

Anomeric carbons hide
When they're in a glycoside

Glucopyranose is there
In the boat or in the chair

Alpha, beta, D and L
Di-astere-omer hell

Alpha, beta, D and L
Di-astere-omer hell

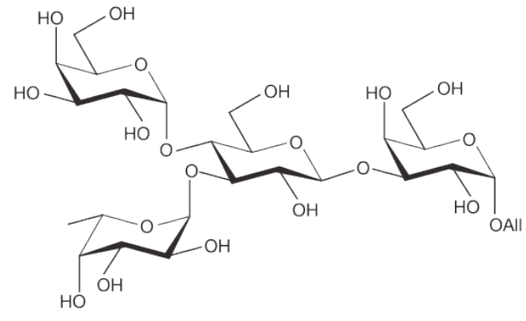
Recorded by Tim Karplus
Lyrics by Kevin Ahern

The oligosaccharides that are attached to proteins may also determine their cellular destinations. Improper glycosylation or sugar modification patterns can result in the failure of proteins to reach the correct cellular compartment. For example, inclusion cell (I-cell) disease arises from a defective phosphotransferase in the Golgi. This enzyme normally catalyzes the addition of a phosphate to a mannose sugar attached to a protein destined for the lysosome. In the absence of a functioning enzyme, the unphosphorylated glycoprotein never makes it to the lysosome and is instead exported out of the cell where it accumulates in the blood and is excreted in the urine. Individuals with I-cell disease suffer developmental delays, abnormal skeletal development, and restricted joint movement.

Click HERE and HERE for Kevin's YouTube lectures on Carbohydrate Structure

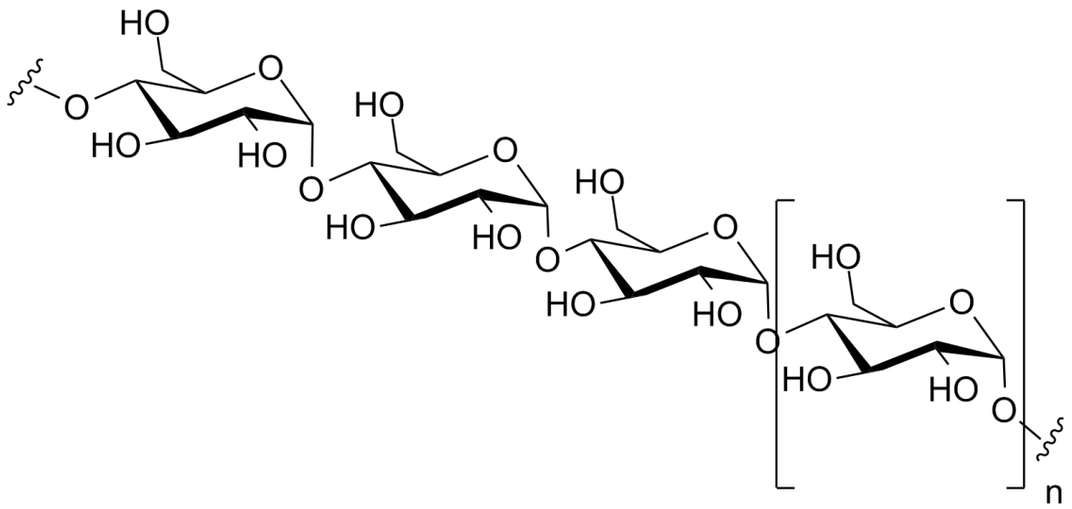
Polysaccharides

Polysaccharides, as their name implies, are made by joining together many sugars. The functions for polysaccharides are varied. They include energy storage, structural strength, and lubrication. Polysaccharides involved in energy storage include the plant polysaccharides, amylose and amylopectin. The polysaccharide involved in energy storage in animals is called glycogen and it is mostly found in the muscles and liver.



An oligosaccharide

from Wikipedia



Amylose

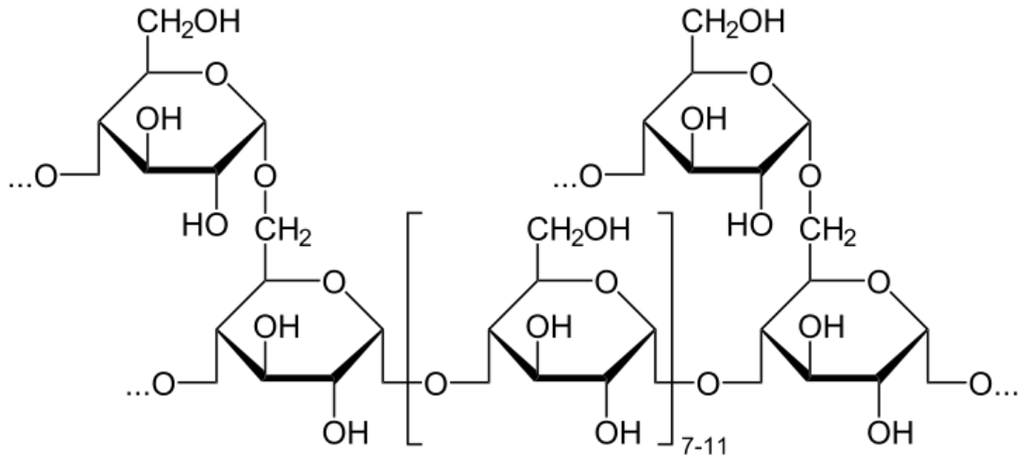
Amylose/Amylopectin

Amylose is the simplest of the polysaccharides, being comprised solely of glucose units joined in an alpha 1-4 linkage. Amylose is broken down by the enzyme alpha-amylase, found in saliva.

Amylopectin is related to amylose in being composed only of glucose, but it differs in how the glucose units are joined together. Alpha 1-4 linkages predominate, but every 30-50 residues, a 'branch' arises from an alpha 1-6 linkage. Branches make the structure of amylopectin more complex than that of amylose.

Glycogen

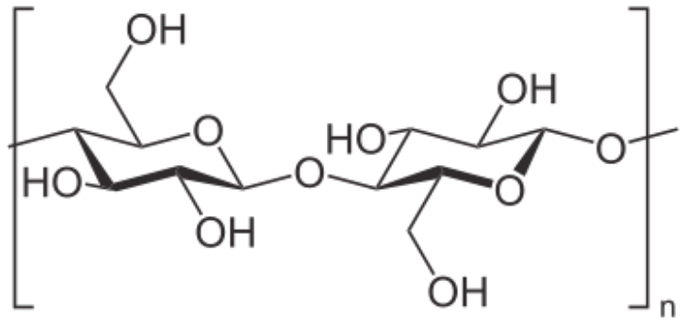
Glycogen is a polysaccharide that is physically related to amylopectin in being built only of glucose and in having a mix of alpha 1-4 and alpha 1-6 bonds. Glycogen, however, has many more alpha 1-6 branches than amylopectin, with such bonds occurring about every 10 residues. One might wonder why such branching occurs more abundantly in animals than in plants. A plausible explanation is based on the method by which these molecules are broken down. The breakdown of these polysaccharides is catalyzed by enzymes, known as **phosphorylases**, that clip glucose residues from the ends of glycogen chains and attach a phosphate to them in the process, producing **glucose-1-phosphate**. More highly branched polysaccharides have more ends to clip, and this translates to more glucose-1-phosphates that can be removed simultaneously by numerous phosphorylases. Since glucose is used for energy by muscles, glucose concentrations can be increased faster the more branched the glycogen is. Plants, which are immobile do



Glycogen Structure

from Wikipedia

not have needs for such immediate release of glucose and thus have less need for highly branched polysaccharides.



Repeating structure of cellulose

from Wikipedia

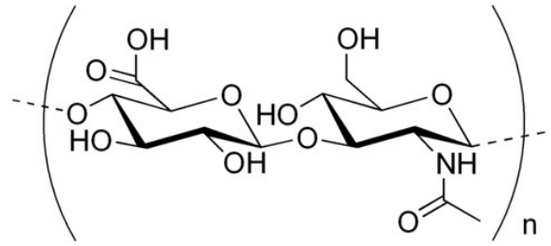
Cellulose

Another important polysaccharide containing only glucose is **cellulose**. It is a polymer of glucose used to give plant cell walls structural integrity and has the individual units joined solely in a beta 1-4 configuration. That simple structural change makes a radical difference in its digestibility. Humans are unable to break down cellulose and it passes through the digestive system as roughage. Ruminant animals, such as cattle, however have bacteria in their rumens that contain the enzyme **cellulase**. It breaks the beta 1-4 links of the glucoses in cellulose to release the sugars for energy.

Another polysaccharide used for structural integrity is known as chitin. **Chitin** makes up the exoskeleton of insects and is a polymer of a modified form of glucose known as N-acetyl-glucosamine.

Glycosaminoglycans

Yet another category of polysaccharides are the **glycosaminoglycans** (also called **mucopolysaccharides**), some examples of which include **keratan sulfate**, **heparin**, **hyaluronic acid** (right), and **chondroitin sulfate**. The polysaccharide compounds are linked to proteins, but differ from glycoproteins in having a much larger contingent of sugar residues and, further, the sugars are considerably more chemically modified. Each of them contains a repeating unit of a disaccharide that contains at least one negatively charged residue. The result is a polyanionic substance that, in its interactions with water, makes for a “slimy” feel. Glycosaminoglycans are found in snot, and in synovial fluid, which lubricates joints. Heparin is a glycosaminoglycan that helps to prevent blood from clotting.



The repeating unit in a glycosaminoglycan

from Wikipedia

Lipids and Membranes

Lipids are a broad class of molecules that all share the characteristic that they have at least a portion of them that is hydrophobic. The class of molecules includes fats, oils (and their

Hyaluronic Acid

To the tune of "*Rudolph the Red-Nosed Reindeer*"

Hyaluronic Acid
Acting almost magically
Placed just beneath the kneecap
Lubricating the debris

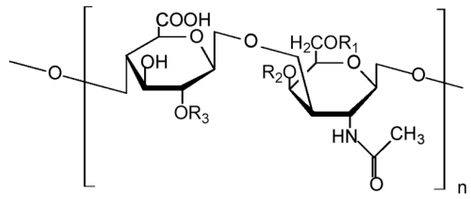
Better than joint replacement
Simple as 1-2-3
If it can stop the aching
You will get to keep your knee

When the pain is getting bad
Try not to be sad
Just go out and have a talk
With your orthopedic doc

Beg him to use the needle
To not do so would be a crime
Hyaluronic acid
Workin' where the sun don't shine

Recorded by David Simmons
Lyrics by Kevin Ahern

substituent fatty acids), steroids, fat-soluble vitamins, prostaglandins, glycerophospholipids, and sphingolipids. Interestingly, each of these can be derived from acetyl-CoA.



Monomeric unit of chondroitin sulfate. Chemical structure of one unit in a chondroitin sulfate chain.

Fatty Acids

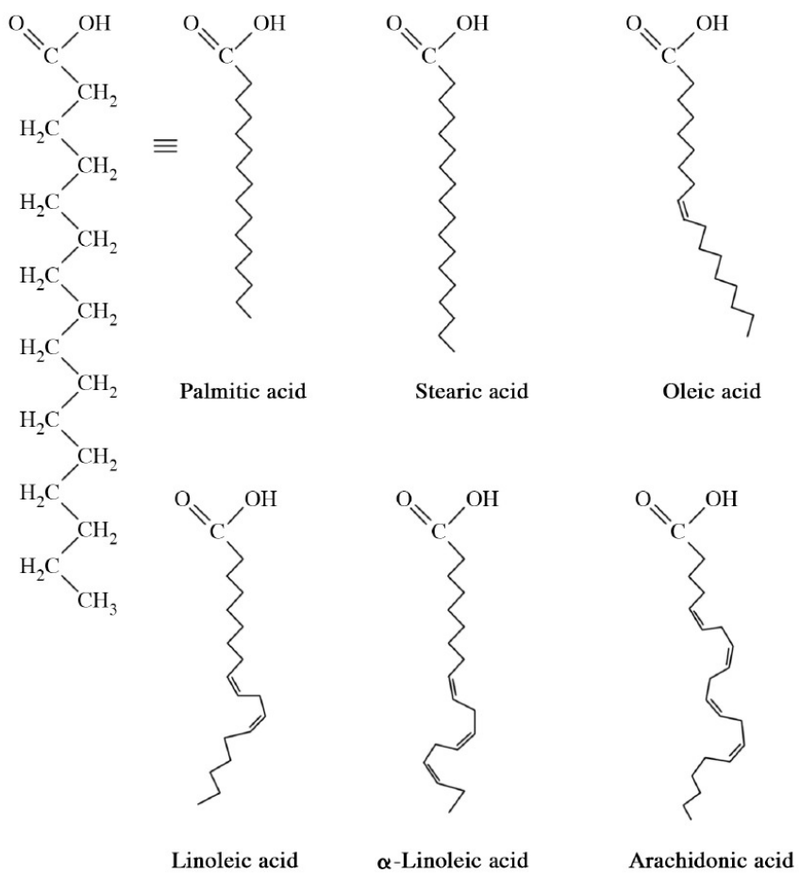
Arguably, the most important lipids in our cells are the fatty acids, because they are components of all of the other lipids, except some of the steroids and fat-soluble vitamins. Consisting of a carboxyl group linked to a long aliphatic tail, fatty acids are described as either saturated (no double bonds) or unsaturated (one or more double bonds). Fatty acids with more than one double bond are described as polyunsaturated. Increasing the amount of unsaturated fatty acids (and the amount of unsaturation in a given fatty acid) in a fat decreases its melting temperature. This is also a factor in membrane fluidity. If the melting temperature of a fat is decreased sufficiently so that it is a liquid at room temperature, it is referred to as an oil. It is worth noting that organisms like fish, which live in cool environments, have fats with more unsaturation. This is why fish oil is a rich source of polyunsaturated fatty acids.

Click HERE and HERE for Kevin's YouTube lectures on Lipids and Membranes.

	Number of Carbon Atoms	Unsaturation	Formula	Melting Point (°C)
Palmitoleic	16	$16:1-\Delta^{9}$	$CH_3(CH_2)_5CH=CH(CH_2)_7CO_2H$	-0.5
Oleic	18	$18:1-\Delta^{9}$	$CH_3(CH_2)_7CH=CH(CH_2)_7CO_2H$	16
Linoleic	18	$18:2-\Delta^{9,12}$	$CH_3(CH_2)_4CH=CH(CH_2)CH=CH(CH_2)_7CO_2H$	-5
Linolenic	18	$18:3-\Delta^{9,12,15}$	$CH_3(CH_2CH=CH)_3(CH_2)_7CO_2H$	-11
Arachidonic	20	$20:4-\Delta^{5,8,11,14}$	$CH_3(CH_2)_4CH=CH(CH_2)_4(CH_2)_2CO_2H$	-50

Important Unsaturated Fatty Acids

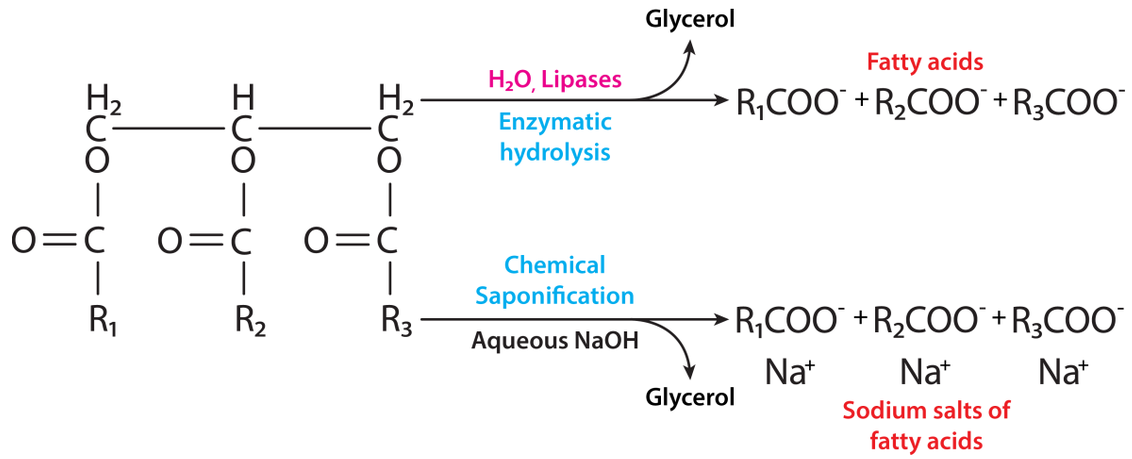
Biochemically, the double bonds found in fatty acids are predominantly in the *cis* configuration. So-called *trans* fats arise as a chemical by-product of partial hydrogenation of vegetable oil (small amounts of *trans* fats also occur naturally). In humans, consumption of trans fats raises low density lipoprotein (LDL) levels and lowers high density lipoprotein (HDL) levels. Each is thought to contribute to the risk of developing coronary artery disease. The most common fatty acids in our body include palmitate, stearate, oleate, linolenate, linoleate, and arachidonate. Fatty acids are numbered by two completely different schemes. The delta numbering scheme has the carboxyl group as #1, whereas the omega number scheme starts at the other end of the fatty acid with the methyl group as #1. Fatty acids are described as essential if they must be in the diet (can't be synthesized by the organism). Animals, including humans, cannot synthesize fatty acids with double bonds beyond position delta 9, so linoleic and linolenic acids are considered essential in these organisms.



Fatty Acids

In animal cells, fats are the primary energy storage forms. They are also known as triacylglycerols, since they consist of a glycerol molecule esterified to three fatty acids. Fats are synthesized by replacing the phosphate on phosphatidic acid with a fatty acid. Fats are stored in the body in specialized cells known as adipocytes. Enzymes known

as lipases release fatty acids from fats by hydrolysis reactions. Of the various lipases acting on fat, the one that acts first, triacylglycerol lipase, is regulated hormonally.



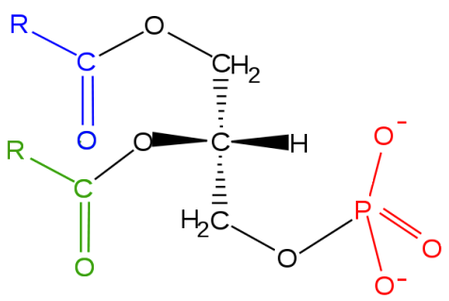
Hydrolysis of Fat

Membrane Lipids

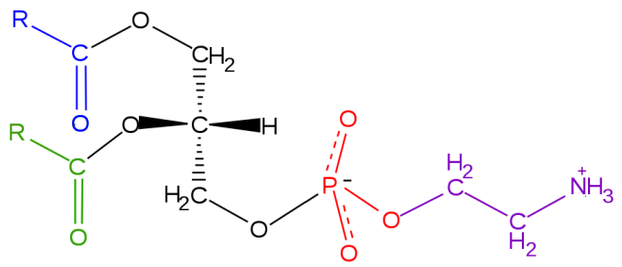
The predominant lipids found in membranes are glycerophospholipids (phosphoglycerides) and sphingolipids. The former are related to fats structurally as both are derived from phosphatidic acid. Phosphatidic acid is a simple glycerophospholipid that is usually converted into phosphatidyl compounds. These are made by esterifying various groups, such as ethanolamine, serine, choline, inositol, and others to the phosphate. All of these compounds form lipid bilayers in aqueous solution, due to the amphiphilic nature of their structure.

Though structurally similar to glycerophospholipids, sphingolipids are synthesized completely independently of them, starting with palmitic acid and the amino acid serine. The figure on the right shows the structure of several sphingolipids. LIke the glycerophospholipids, sphingolipids are amphiphilic, but unlike them, they may have simple (in cerebrosides) or complex (in gangliosides) carbohydrates attached at one end. Most sphingolipids, except sphingomyelin, do not contain phosphate.

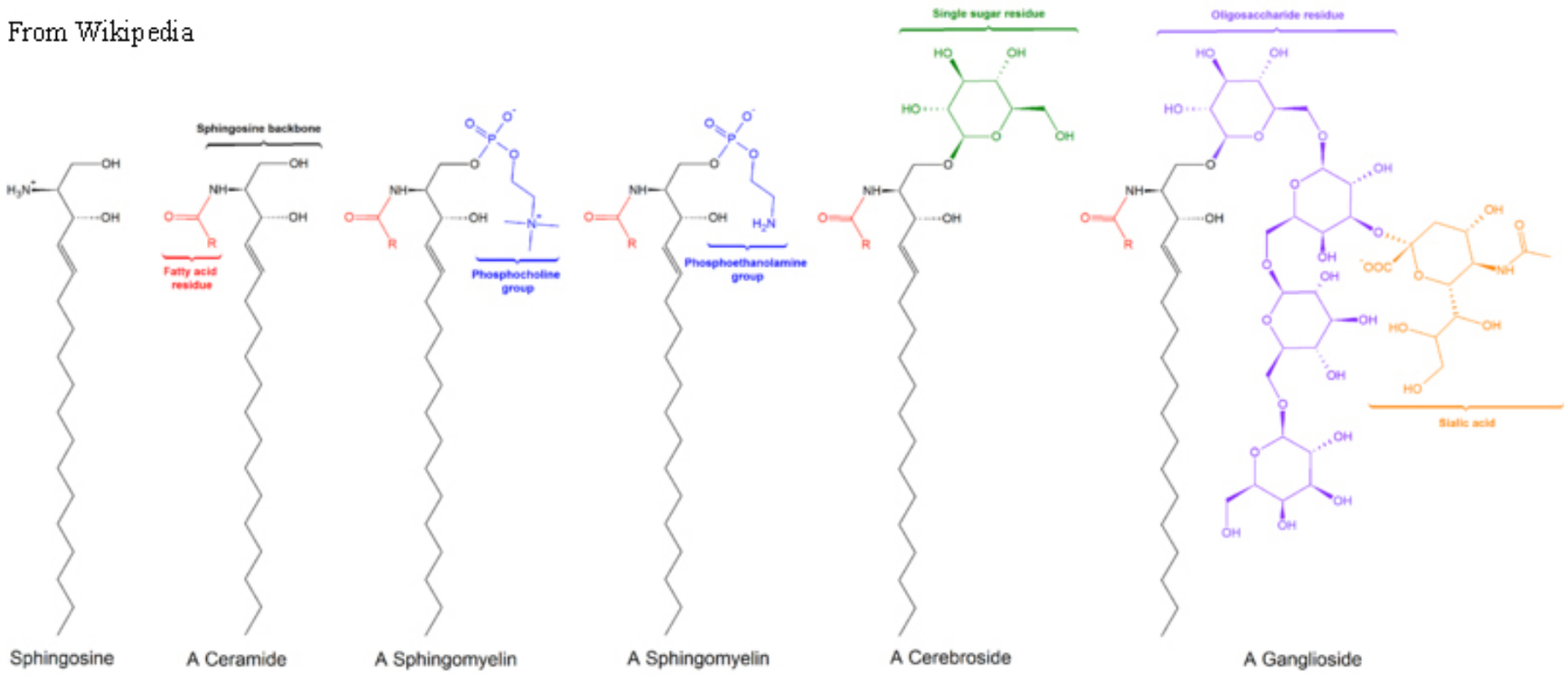
Steroids, such as cholesterol are also found in membranes. Cholesterol, in particular, may play an important role in membrane fluidity. Membranes can be thought of a being more “frozen” or more “fluid.” Fluidity is important for cellular membranes. When heated, membranes move from a more “frozen” character to that of a more “fluid” one as the temperature rises. The mid-point of this transition, referred to as the Tm, is influenced by the fatty acid composition of the lipid bilayer compounds. Longer and more saturated fatty acids will favor higher Tm values, whereas unsaturation and short fatty acids will favor lower Tm values. Interestingly, cholesterol does not change the Tm value, but



Phosphatidic Acid



Phosphatidyl Ethanolamine

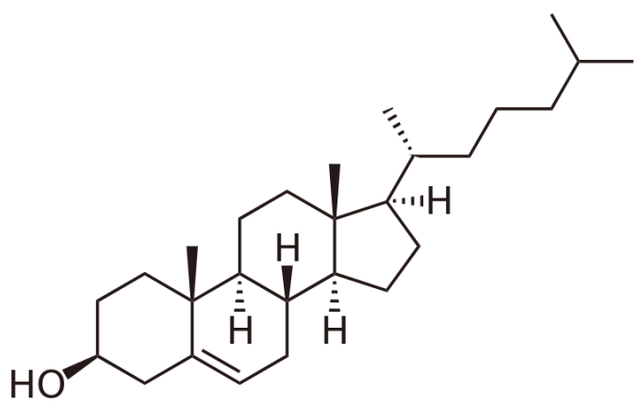


The Structures of Several Sphingolipids

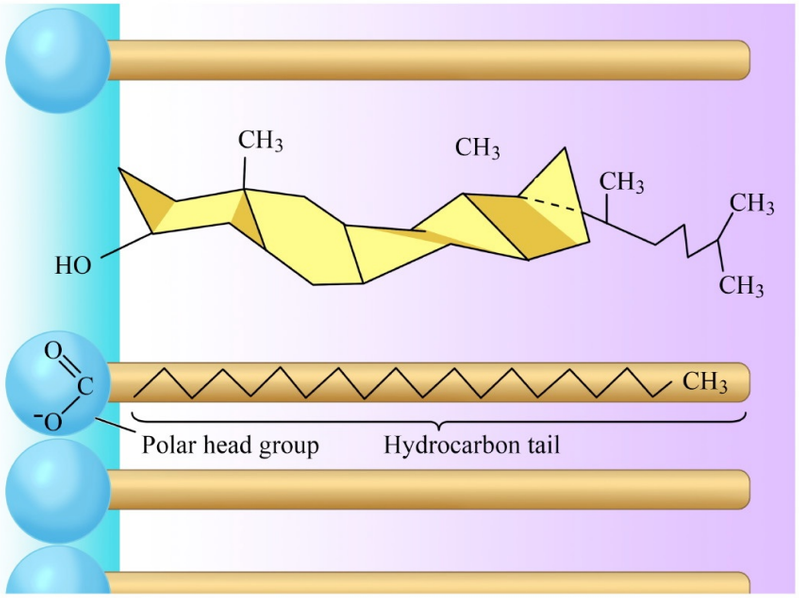
instead widens the transition range between frozen and fluid forms of the membrane.

Lipid Bilayers

The membrane around cells contains many components, including cholesterol, proteins, glycolipids, glycerophospholipids and sphingolipids. The last two of these will, in water, form what is called a lipid bilayer, which serves as a boundary for the cell that is largely impermeable to the movement of most materials across it. With the notable exceptions of water, carbon dioxide, carbon monoxide, and oxygen, most polar/ionic compounds require transport proteins to help them to efficiently navigate across the bilayer. The orderly movement of these compounds is critical for the cell to be able to 1) get food for energy; 2) export materials; 3) maintain osmotic balance; 4) create gradients for secondary transport; 5) provide electromotive force for nerve signaling; and 6) store energy in electrochemical gradients for ATP production (oxidative phosphorylation or photosynthesis). In some cases, energy is required to move the substances (active transport). In other cases, no external energy is required and they move by diffusion through specific cellular channels.



Cholesterol

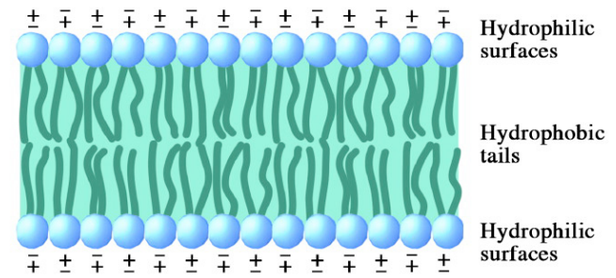


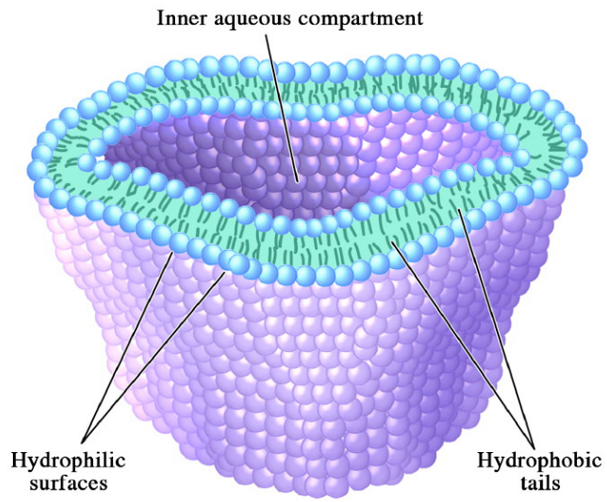
Cholesterol in a Lipid Bilayer

The spontaneous ability of these compounds to form lipid bilayers is exploited in the formation of artificial membranous structures called liposomes. Liposomes have some uses in delivering their contents into cells *via* membrane fusion.

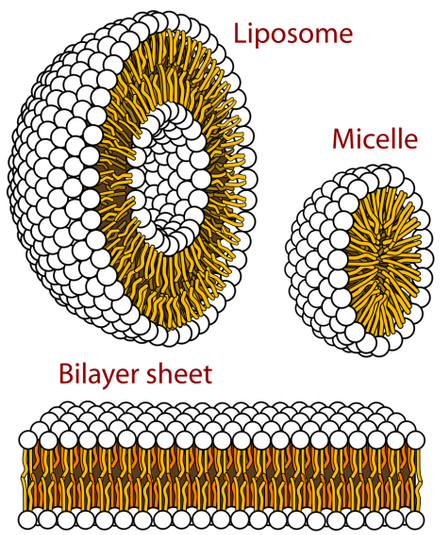
Membrane Proteins

Other significant components of cellular membranes include proteins. We can put them into several categories. Integral membrane proteins are embedded in the membrane and project through both sides of the lipid bilayer. Peripheral membrane proteins are embedded in or tightly associated with part of the bilayer, but do not project completely through both sides. Associated membrane proteins are found near membranes, but may not be embedded in them. Their association may arise as a result of interaction



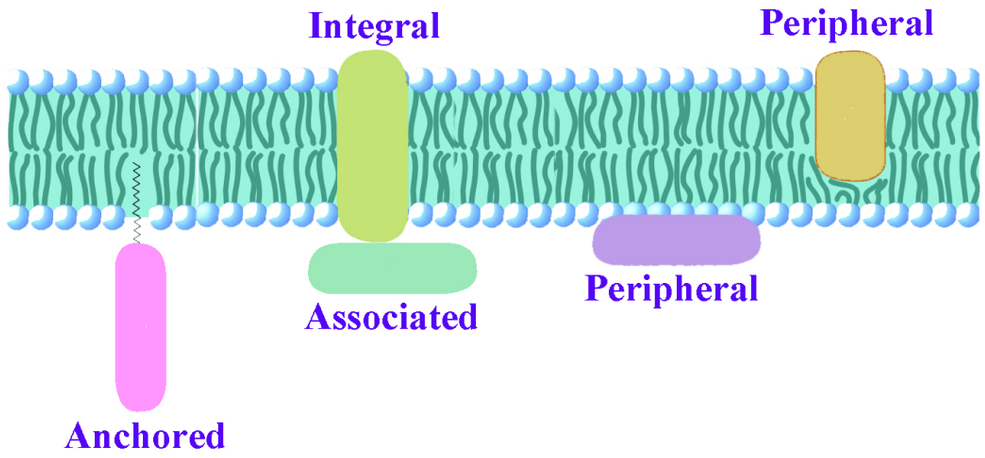


Lipid bilayer structures



with other proteins or molecules in the lipid bilayer. Anchored membrane proteins are not themselves embedded in the lipid bilayer, but instead are attached to a molecule (typically a fatty acid) that is embedded in the membrane.

The geometry of the lipid bilayer is such that is hydrophobic on its interior and hydrophilic on the exterior. Such properties also dictate the amino acid side chains of proteins that interact with the



Types of Membrane Proteins

bilayer. For most membrane proteins, the polar amino acids are found where the protein projects through the bilayer (interacting with aqueous/polar substances) and the non-polar amino acids are embedded within the non-polar portion of the bilayer containing the fatty acid tails.

Glycolipids and glycoproteins play important roles in cellular identification. Blood types, for example, differ from each other in the structure of the carbohydrate chains projecting out from the surface of the glycoprotein in their membranes.

Cells have hundreds of membrane proteins and the protein composition of a membrane varies with its function and location. Mitochondrial membranes are among the most densely packed with proteins. The plasma membrane has a large number of integral proteins involved in communicating information across the membrane (signaling) or in transporting materials into the cell.

Click HERE and HERE for Kevin's YouTube lectures on Membrane Transport.

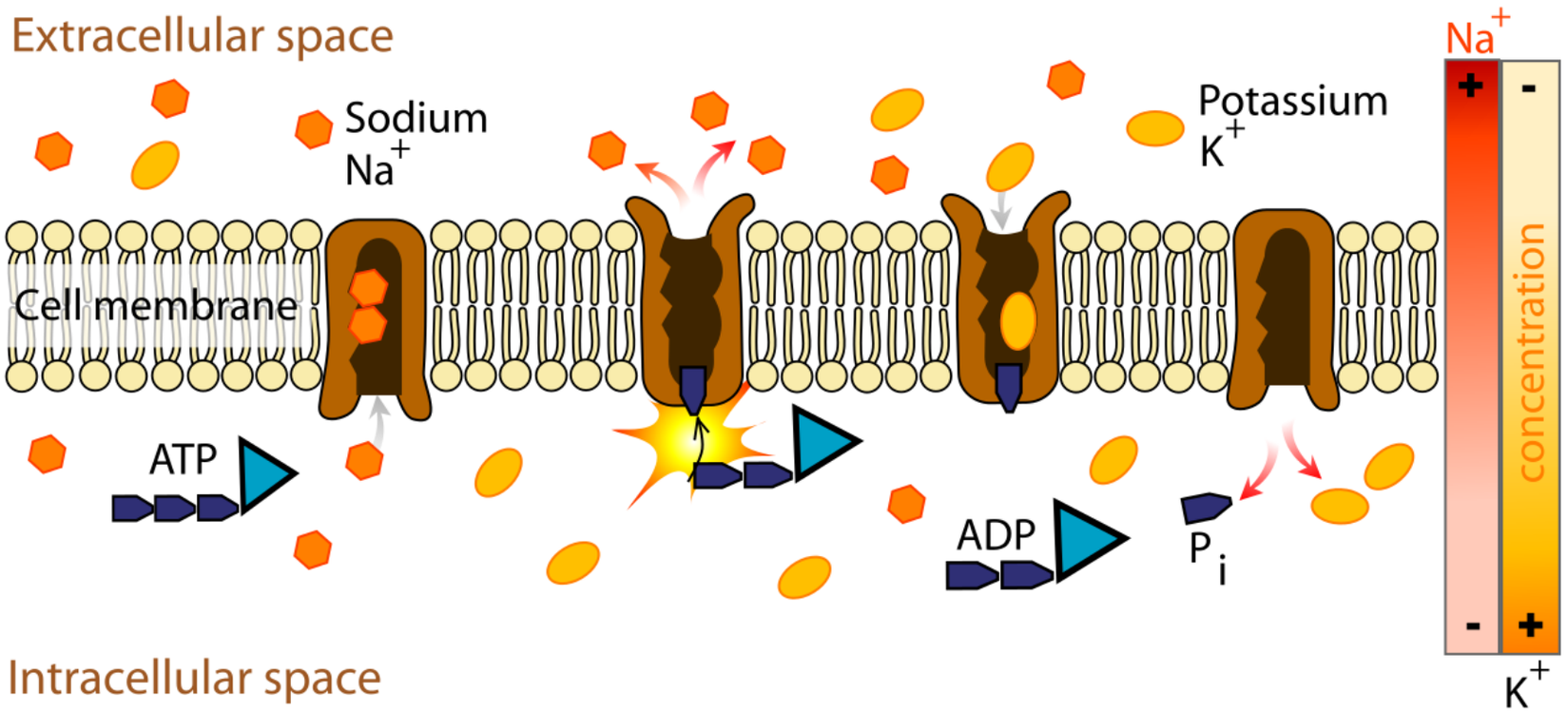
Membrane Transport

Materials, such as food and waste must be moved across a cell's lipid bilayer. There are two means of accomplishing this - passive processes and active processes. Passive processes have as their sole driving force the process of diffusion. In these systems, molecules always move from a higher concentration to a lower concentration. These can occur directly across a membrane (water, oxygen, carbon dioxide, and carbon monoxide) or through special transport proteins (glucose transport proteins of red blood cells, for example). In each case, no cellular energy is expended in the movement of the molecules. On the other hand, active processes require energy to accomplish such transport. A common energy source is ATP (see Na^+/K^+ ATPase), but many other energy sources are employed. For example, the sodium-glucose transporter uses a sodium gradient as a force for actively transporting glucose into a cell. Thus, it is important to know that not all active transport uses ATP energy. Proteins, such as the sodium-glucose transporter that move two molecules in the same direction across the membrane are called symporters (also called synporters). If the action of a protein in moving ions across a membrane results in a change in charge, the protein is described as

electrogenic and if there is no change in charge the protein is described as electro-neutral.

Na^+/K^+ ATPase

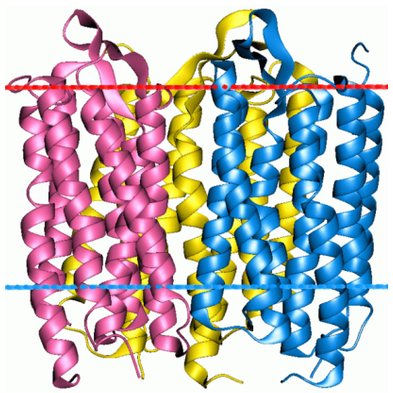
Another important integral membrane protein is the Na^+/K^+ ATPase (previous page), which transports sodium ions out of the cell and potassium ions into the cell. The protein, which is described as an anti-port (molecules moved in opposite directions across the membrane) uses the energy of ATP to create ion gradients that are important both in maintaining cellular osmotic pressure and (in nerve cells) for creating the ion gradients necessary for signal transmission. The transport system moves three atoms of sodium out of the cell and two atoms of potassium into the cell for each ATP hydrolyzed.

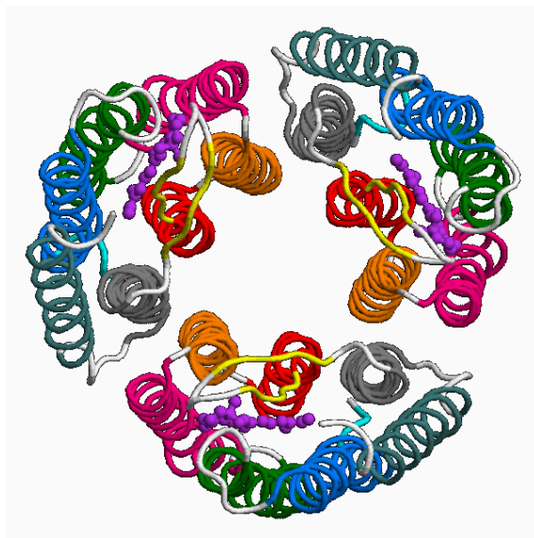


Sodium-Potassium ATPase

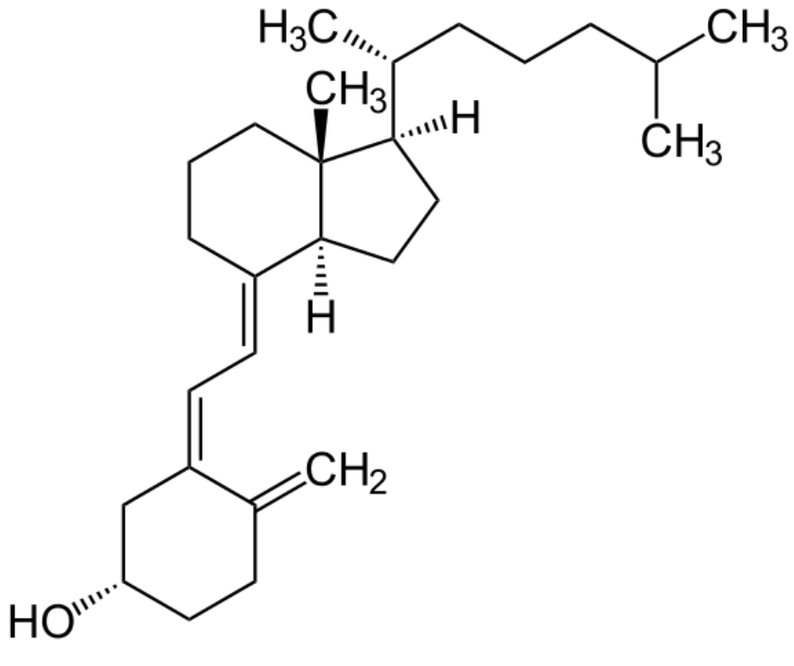
Bacteriorhodopsin

An interesting integral membrane protein is bacteriorhodopsin. The protein has three identical polypeptide chains, each rotated by 120 degrees relative to the others. Each chain has seven transmembrane alpha helices and contains one molecule of retinal (Vitamin A) buried deep within each cavity (shown in purple in lower figure at left). Vitamin A is light sensitive and isomerizes rapidly between a *cis* and a *trans* form in the presence of light. The changing conformation of the





Bacteriorhodopsin

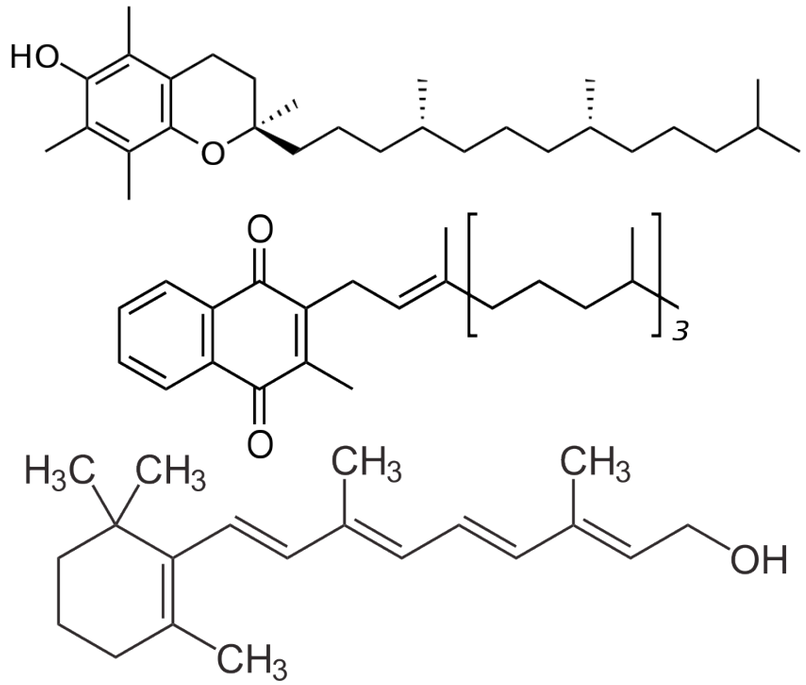


Vitamin D

vitamin A is used to transport protons through the protein and out of the bacterium, creating a proton gradient across the cell membrane, which is used ultimately to make ATP. It is not too difficult to imagine engineering an organism (say a transparent fish) to contain bacteriorhodopsin in its mitochondrial inner membrane. When light is shone upon it, the bacteriorhodopsin could be used to generate a proton gradient (much like electron transport does) and power oxidative phosphorylation. Such a fish would be partly photosynthetic in that it would be deriving energy from light, but would differ from plants in being unable to assimilate carbon dioxide in a series of "dark reactions."

Fat-Soluble Vitamins

Other lipids of note include the fat-soluble vitamins - A, D, E, and K. Vitamin A comes in three primary chemical forms, retinol (storage in liver), retinal (role in vision), and retinoic acid (roles in growth and development). Vitamin D (cholecalciferol) plays important roles in the intestinal absorption of calcium and phosphate and thus in healthy bones. Derived from ultimately from cholesterol, the compound can be synthesized in a reaction catalyzed by ultraviolet light. Vitamin E (tocopherol) is the vitamin about which the least is known. It consists of a group of eight fat-soluble compounds of which the alpha-isomer has the most biological activity. Vitamin K (the name comes from the German for coagulation vitamin) is essential for blood clotting. It is used as a co-factor for the enzyme that modifies prothrombin to increase its affinity for calcium, allowing it to be positioned closer to the site of a wound.



Top to Bottom - Vitamins E, K, and A