PRINCIPLES OF BIOCHEMISTRY



Jennifer Lanni
Wheaton College, MA

LibreTexts™

Principles of Biochemistry

Principles of Biochemistry

Wheaton College BIO305

This page titled Principles of Biochemistry is shared under a license and was authored, remixed, and/or curated by LibreTexts.

This text is disseminated via the Open Education Resource (OER) LibreTexts Project (https://LibreTexts.org) and like the thousands of other texts available within this powerful platform, it is freely available for reading, printing, and "consuming."

The LibreTexts mission is to bring together students, faculty, and scholars in a collaborative effort to provide an accessible, and comprehensive platform that empowers our community to develop, curate, adapt, and adopt openly licensed resources and technologies; through these efforts we can reduce the financial burden borne from traditional educational resource costs, ensuring education is more accessible for students and communities worldwide.

Most, but not all, pages in the library have licenses that may allow individuals to make changes, save, and print this book. Carefully consult the applicable license(s) before pursuing such effects. Instructors can adopt existing LibreTexts texts or Remix them to quickly build course-specific resources to meet the needs of their students. Unlike traditional textbooks, LibreTexts' web based origins allow powerful integration of advanced features and new technologies to support learning.



LibreTexts is the adaptable, user-friendly non-profit open education resource platform that educators trust for creating, customizing, and sharing accessible, interactive textbooks, adaptive homework, and ancillary materials. We collaborate with individuals and organizations to champion open education initiatives, support institutional publishing programs, drive curriculum development projects, and more.

The LibreTexts libraries are Powered by NICE CXone Expert and were supported by the Department of Education Open Textbook Pilot Project, the California Education Learning Lab, the UC Davis Office of the Provost, the UC Davis Library, the California State University Affordable Learning Solutions Program, and Merlot. This material is based upon work supported by the National Science Foundation under Grant No. 1246120, 1525057, and 1413739.

Any opinions, findings, and conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of the National Science Foundation nor the US Department of Education.

Have questions or comments? For information about adoptions or adaptions contact info@LibreTexts.org or visit our main website at https://LibreTexts.org.

The LibreTexts name, logo, and book covers are trademarked by LibreTexts, Inc. (a 501(c)(3) not-for-profit organization) and protected against unauthorized use.

This text was compiled on 10/08/2026

TABLE OF CONTENTS

Principles of Biochemistry

Licensing

1: Biology and Chemistry Review

1.1: Transcription

1.2: Translation

1.3: Prokaryotic Cells

1.4: Eukaryotic Cells

1.5: Essential elements for life

1.6: The Building Blocks of Molecules

2: Introduction to Biochemistry

2.1: Water

2.2: pH and Buffers

2.3: Functional groups and electronegativity

2.4: Types of Biological Molecules

2.5: Assembly of Biological Macromolecules

3: Amino Acids

3.2: Amino Acid Charges

3.3: Cysteine Chemistry

3.4: Post Translational Modification of Amino Acids

3.1:Structure and Property of the Naturally-Occurring Amino Acids

4: Protein structure and function

4.1: From Amino Acids to Peptides

4.2: Levels of Protein Structure

4.3: Secondary Structure

4.4: Protein folding

4.5: Protein function, domains and cooperativity

4.6: Protein Aggregation

4.7: Prions and Disease

5: Techniques for studying proteins

5.1: Cell Disruption

5.2: Fractionation

5.3: Quantification of Protein Concentration

5.4: Electrophoresis

5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

5.6: Western blot / Immunoblot overview

5.7: Steps in Western blotting

6: Energetics and metabolism

6.1: Endergonic and Exergonic Reactions

6.2: Thermodynamics

6.3: Energy in Metabolism

7: Enzymes, catalysis and kinetics

7.1: Basic Principles of Catalysis

7.2: Derivation of Michaelis-Menten equation

7.3: Mechanisms of Catalysis

7.4: Control of Enzymatic Activity

8: Carbohydrate structure and metabolism

8.1: Carbohydrates overview

8.2: Glycolysis

8.3: Gluconeogenesis

8.4: Fermentation

8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

8.6: Citric Acid Cycle and Related Pathways

8.7: Electron Transport and Oxidative Phosphorylation

8.8: Carbohydrate Storage and Breakdown

9: Lipids and membranes

9.1: Structure and Function - Lipids and Membranes

9.2: Basic Concepts in Membranes

9.3: Transport in Membranes

9.4: Metabolism of Fat

9.5: Fatty Acid Oxidation

9.6: Ketone body production and acidosis

10: Nitrogen metabolism and the urea cycle

10.1: Amino Acid Metabolism

10.2: Amino Acid Catabolism

10.3: Urea Cycle

11: Nucleotide and nucleic acid structure and metabolism

11.1: Structure and Function - Nucleic Acids

11.2: Pyrimidine de novo Biosynthesis

11.3: Deoxyribonucleotide de novo Biosynthesis

11.4: Epigenetic Control- Regulating Access to Genes within the Chromosome

Index

Glossary

Detailed Licensing

This page titled Principles of Biochemistry is shared under a license and was authored, remixed, and/or curated by LibreTexts.

Licensing

A detailed breakdown of this resource's licensing can be found in ***Back Matter/Detailed Licensing***.

This page titled Licensing is shared under a license and was authored, remixed, and/or curated by LibreTexts.

1: BIOLOGY AND CHEMISTRY REVIEW

1.1: Transcription
1.2: Translation
1.3: Prokaryotic Cells
1.4: Eukaryotic Cells
1.5: Essential elements for life
1.6: The Building Blocks of Molecules

This page titled 1: Biology and Chemistry Review is shared under a license and was authored, remixed, and/or curated by LibreTexts.

1.1: Transcription

In both prokaryotes and eukaryotes, the second function of DNA (the first was replication) is to provide the information needed to construct the proteins necessary so that the cell can perform all of its functions. To do this, the DNA is “read” or transcribed into an mRNA molecule. The mRNA then provides the code to form a protein by a process called translation. Through the processes of transcription and translation, a protein is built with a specific sequence of amino acids that was originally encoded in the DNA. This module discusses the details of transcription.

The Central Dogma: DNA Encodes RNA; RNA Encodes Protein

The flow of genetic information in cells from DNA to mRNA to protein is described by the central dogma (Figure 1.1.1), which states that genes specify the sequences of mRNAs, which in turn specify the sequences of proteins.

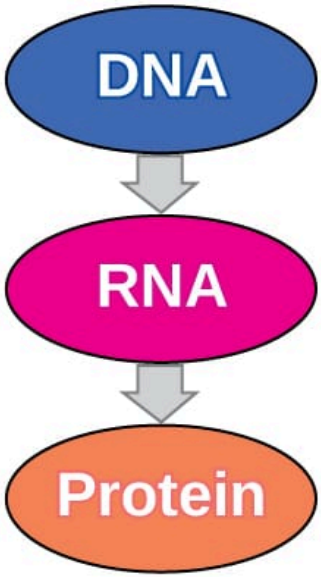


Figure 1.1.1: The central dogma states that DNA encodes RNA, which in turn encodes protein.

The copying of DNA to mRNA is relatively straightforward, with one nucleotide being added to the mRNA strand for every complementary nucleotide read in the DNA strand. The translation to protein is more complex because groups of three mRNA nucleotides correspond to one amino acid of the protein sequence. However, as we shall see in the next module, the translation to protein is still systematic, such that nucleotides 1 to 3 correspond to amino acid 1, nucleotides 4 to 6 correspond to amino acid 2, and so on.

Transcription: from DNA to mRNA

Both prokaryotes and eukaryotes perform fundamentally the same process of transcription, with the important difference of the membrane-bound nucleus in eukaryotes. With the genes bound in the nucleus, transcription occurs in the nucleus of the cell and the mRNA transcript must be transported to the cytoplasm. The prokaryotes, which include bacteria and archaea, lack membrane-bound nuclei and other organelles, and transcription occurs in the cytoplasm of the cell. In both prokaryotes and eukaryotes, transcription occurs in three main stages: initiation, elongation, and termination.

Initiation

Transcription requires the DNA double helix to partially unwind in the region of mRNA synthesis. The region of unwinding is called a transcription bubble. The DNA sequence onto which the proteins and enzymes involved in transcription bind to initiate the process is called a promoter. In most cases, promoters exist upstream of the genes they regulate. The specific sequence of a promoter is very important because it determines whether the corresponding gene is transcribed all of the time, some of the

time, or hardly at all (Figure 1.1.2).

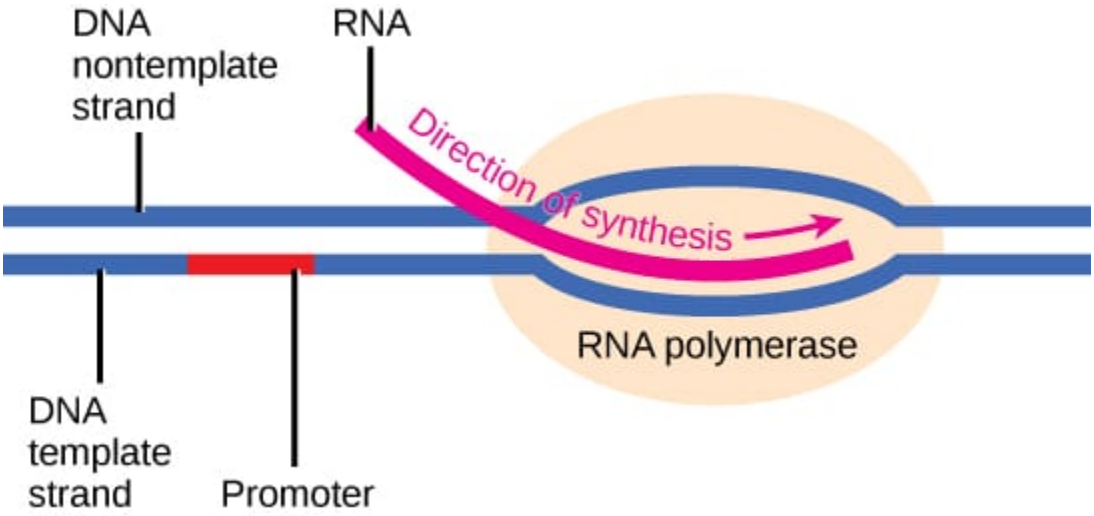


Figure 1.1.2: The initiation of transcription begins when DNA is unwound, forming a transcription bubble. Enzymes and other proteins involved in transcription bind at the promoter.

Elongation

Transcription always proceeds from one of the two DNA strands, which is called the template strand. The mRNA product is complementary to the template strand and is almost identical to the other DNA strand, called the nontemplate strand, with the exception that RNA contains a uracil (U) in place of the thymine (T) found in DNA. During elongation, an enzyme called RNA polymerase proceeds along the DNA template adding nucleotides by base pairing with the DNA template in a manner similar to DNA replication, with the difference that an RNA strand is being synthesized that does not remain bound to the DNA template. As elongation proceeds, the DNA is continuously unwound ahead of the core enzyme and rewound behind it (Figure 1.1.3).

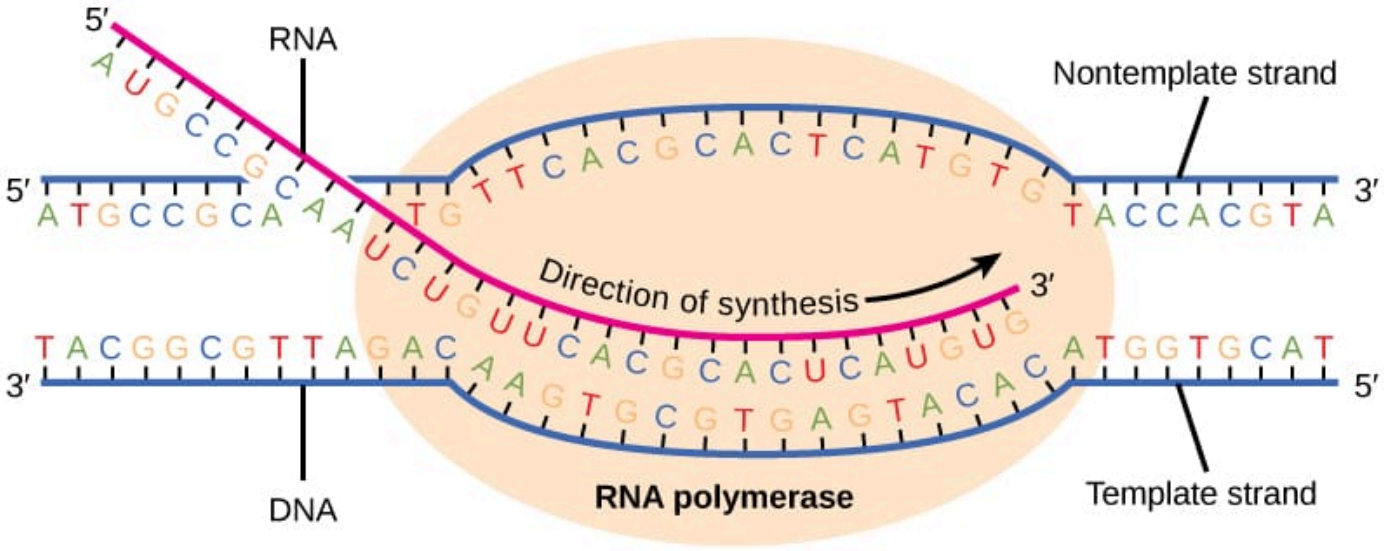


Figure 1.1.3: During elongation, RNA polymerase tracks along the DNA template, synthesizes mRNA in the 5' to 3' direction, and unwinds then rewinds the DNA as it is read.

Termination

Once a gene is transcribed, the prokaryotic polymerase needs to be instructed to dissociate from the DNA template and liberate the newly made mRNA. Depending on the gene being transcribed, there are two kinds of termination signals, but both involve repeated nucleotide sequences in the DNA template that result in RNA polymerase stalling, leaving the DNA template, and freeing

the mRNA transcript.

On termination, the process of transcription is complete. In a prokaryotic cell, by the time termination occurs, the transcript would already have been used to partially synthesize numerous copies of the encoded protein because these processes can occur concurrently using multiple ribosomes (polyribosomes) (Figure 1.1.4). In contrast, the presence of a nucleus in eukaryotic cells precludes simultaneous transcription and translation.

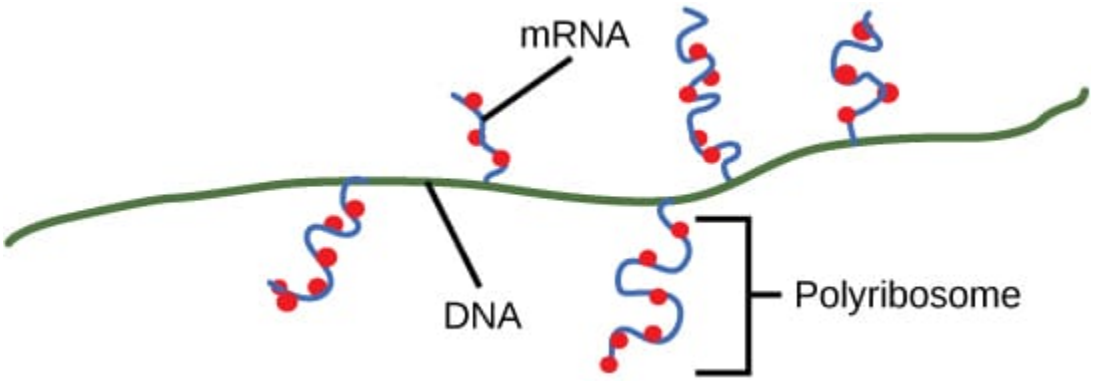


Figure 1.1.4: Multiple polymerases can transcribe a single bacterial gene while numerous ribosomes concurrently translate the mRNA transcripts into polypeptides. In this way, a specific protein can rapidly reach a high concentration in the bacterial cell.

Eukaryotic RNA Processing

The newly transcribed eukaryotic mRNAs must undergo several processing steps before they can be transferred from the nucleus to the cytoplasm and translated into a protein. The additional steps involved in eukaryotic mRNA maturation create a molecule that is much more stable than a prokaryotic mRNA. For example, eukaryotic mRNAs last for several hours, whereas the typical prokaryotic mRNA lasts no more than five seconds.

The mRNA transcript is first coated in RNA-stabilizing proteins to prevent it from degrading while it is processed and exported out of the nucleus. This occurs while the pre-mRNA still is being synthesized by adding a special nucleotide “cap” to the 5' end of the growing transcript. In addition to preventing degradation, factors involved in protein synthesis recognize the cap to help initiate translation by ribosomes.

Once elongation is complete, an enzyme then adds a string of approximately 200 adenine residues to the 3' end, called the poly-A tail. This modification further protects the pre-mRNA from degradation and signals to cellular factors that the transcript needs to be exported to the cytoplasm.

Eukaryotic genes are composed of protein-coding sequences called exons (*ex*-on signifies that they are *ex*pressed) and *int*ervening sequences called introns (*int*-ron denotes their *int*ervening role). Introns are removed from the pre-mRNA during processing. Intron sequences in mRNA do not encode functional proteins. It is essential that all of a pre-mRNA’s introns be completely and precisely removed before protein synthesis so that the exons join together to code for the correct amino acids. If the process errs by even a single nucleotide, the sequence of the rejoined exons would be shifted, and the resulting protein would be nonfunctional. The process of removing introns and reconnecting exons is called splicing (Figure 1.1.5). Introns are removed and degraded while the pre-mRNA is still in the nucleus.

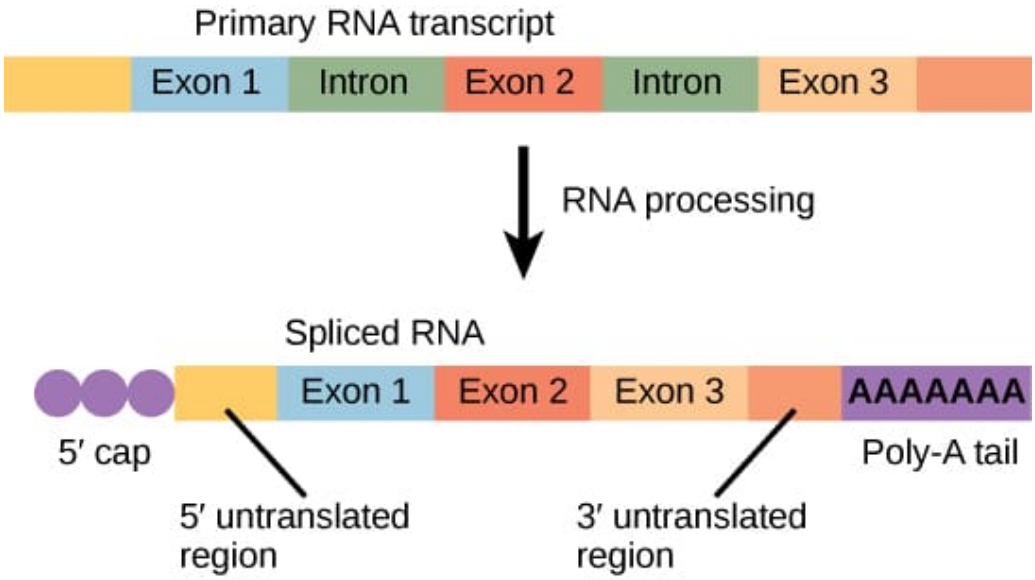


Figure 1.1.5: Eukaryotic mRNA contains introns that must be spliced out. A 5' cap and 3' tail are also added.

Summary

In prokaryotes, mRNA synthesis is initiated at a promoter sequence on the DNA template. Elongation synthesizes new mRNA. Termination liberates the mRNA and occurs by mechanisms that stall the RNA polymerase and cause it to fall off the DNA template. Newly transcribed eukaryotic mRNAs are modified with a cap and a poly-A tail. These structures protect the mature mRNA from degradation and help export it from the nucleus. Eukaryotic mRNAs also undergo splicing, in which introns are removed and exons are reconnected with single-nucleotide accuracy. Only finished mRNAs are exported from the nucleus to the cytoplasm.

Glossary

exon
: a sequence present in protein-coding mRNA after completion of pre-mRNA splicing

intron
: non–protein-coding intervening sequences that are spliced from mRNA during processing

mRNA
: messenger RNA; a form of RNA that carries the nucleotide sequence code for a protein sequence that is translated into a polypeptide sequence

nontemplate strand
: the strand of DNA that is not used to transcribe mRNA; this strand is identical to the mRNA except that T nucleotides in the DNA are replaced by U nucleotides in the mRNA

promoter
: a sequence on DNA to which RNA polymerase and associated factors bind and initiate transcription

RNA polymerase
: an enzyme that synthesizes an RNA strand from a DNA template strand

splicing
: the process of removing introns and reconnecting exons in a pre-mRNA

template strand
: the strand of DNA that specifies the complementary mRNA molecule

transcription bubble
the region of locally unwound DNA that allows for transcription of mRNA

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 1.1: Transcription is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 9.3: Transcription by OpenStax is licensed CC BY 4.0.

1.2: Translation

The synthesis of proteins is one of a cell's most energy-consuming metabolic processes. In turn, proteins account for more mass than any other component of living organisms (with the exception of water), and proteins perform a wide variety of the functions of a cell. The process of translation, or protein synthesis, involves decoding an mRNA message into a polypeptide product. Amino acids are covalently strung together in lengths ranging from approximately 50 amino acids to more than 1,000.

The Protein Synthesis Machinery

In addition to the mRNA template, many other molecules contribute to the process of translation. The composition of each component may vary across species; for instance, ribosomes may consist of different numbers of ribosomal RNAs (rRNA) and polypeptides depending on the organism. However, the general structures and functions of the protein synthesis machinery are comparable from bacteria to human cells. Translation requires the input of an mRNA template, ribosomes, tRNAs, and various enzymatic factors (Figure 1.2.1).

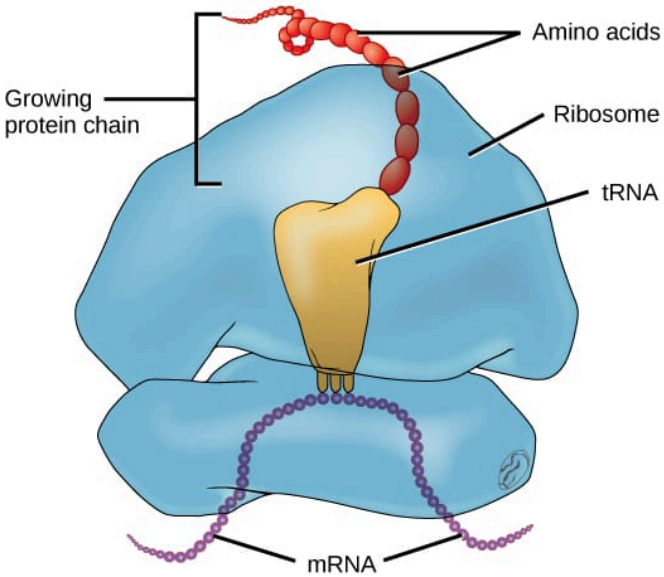


Figure 1.2.1: The protein synthesis machinery includes the large and small subunits of the ribosome, mRNA, and tRNA. (credit: modification of work by NIGMS, NIH)

In *E. coli*, there are 200,000 ribosomes present in every cell at any given time. A ribosome is a complex macromolecule composed of structural and catalytic rRNAs, and many distinct polypeptides. In eukaryotes, the nucleolus is completely specialized for the synthesis and assembly of rRNAs.

Ribosomes are located in the cytoplasm in prokaryotes and in the cytoplasm and endoplasmic reticulum of eukaryotes. Ribosomes are made up of a large and a small subunit that come together for translation. The small subunit is responsible for binding the mRNA template, whereas the large subunit sequentially binds tRNAs, a type of RNA molecule that brings amino acids to the growing chain of the polypeptide. Each mRNA molecule is simultaneously translated by many ribosomes, all synthesizing protein in the same direction.

Depending on the species, 40 to 60 types of tRNA exist in the cytoplasm. Serving as adaptors, specific tRNAs bind to sequences on the mRNA template and add the corresponding amino acid to the polypeptide chain. Therefore, tRNAs are the molecules that actually "translate" the language of RNA into the language of proteins. For each tRNA to function, it must have its specific amino acid bonded to it. In the process of tRNA "charging," each tRNA molecule is bonded to its correct amino acid.

The Genetic Code

To summarize what we know to this point, the cellular process of transcription generates messenger RNA (mRNA), a mobile molecular copy of one or more genes with an alphabet of A, C, G, and uracil (U). Translation of the mRNA template converts nucleotide-based genetic information into a protein product. Protein sequences consist of 20 commonly occurring amino acids; therefore, it can be said that the protein alphabet consists of 20 letters. Each amino acid is defined by a three-nucleotide sequence called the triplet codon. The relationship between a nucleotide codon and its corresponding amino acid is called the genetic code.

Given the different numbers of "letters" in the mRNA and protein "alphabets," combinations of nucleotides corresponded to single amino acids. Using a three-nucleotide code means that there are a total of 64 (4 × 4 × 4) possible combinations; therefore, a given amino acid is encoded by more than one nucleotide triplet (Figure 1.2.2).

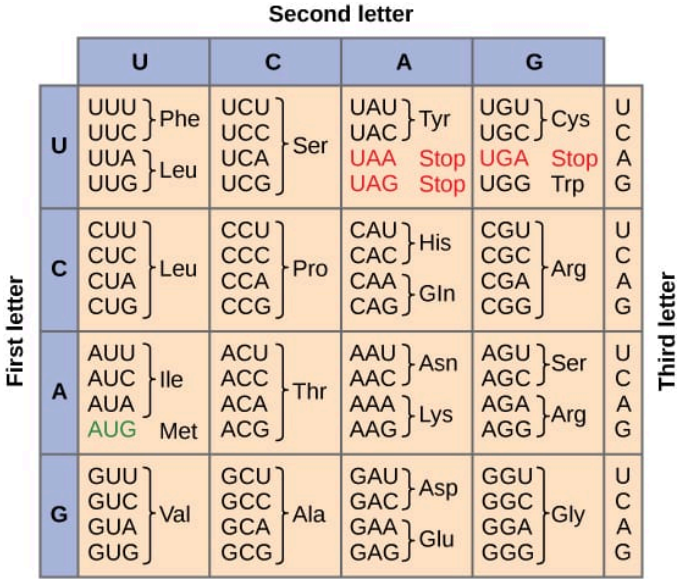


Figure 1.2.2: This figure shows the genetic code for translating each nucleotide triplet, or codon, in mRNA into an amino acid or a termination signal in a nascent protein. (credit: modification of work by NIH)

Three of the 64 codons terminate protein synthesis and release the polypeptide from the translation machinery. These triplets are called stop codons. Another codon, AUG, also has a special function. In addition to specifying the amino acid methionine, it also serves as the start codon to initiate translation. The reading frame for translation is set by the AUG start codon near the 5' end of the mRNA. The genetic code is universal. With a few exceptions, virtually all species use the same genetic code for protein synthesis, which is powerful evidence that all life on Earth shares a common origin.

The Mechanism of Protein Synthesis

Just as with mRNA synthesis, protein synthesis can be divided into three phases: initiation, elongation, and termination. The process of translation is similar in prokaryotes and eukaryotes. Here we will explore how translation occurs in *E. coli*, a representative prokaryote, and specify any differences between prokaryotic and eukaryotic translation.

Protein synthesis begins with the formation of an initiation complex. In *E. coli*, this complex involves the small ribosome subunit, the mRNA template, three initiation factors, and a special initiator tRNA. The initiator tRNA interacts with the AUG start codon, and links to a special form of the amino acid methionine that is typically removed from the polypeptide after translation is complete.

In prokaryotes and eukaryotes, the basics of polypeptide elongation are the same, so we will review elongation from the perspective of *E. coli*. The large ribosomal subunit of *E. coli* consists of three compartments: the A site binds incoming charged tRNAs (tRNAs with their attached specific amino acids). The P site binds charged tRNAs carrying amino acids that have formed bonds with the growing polypeptide chain but have not yet dissociated from their corresponding tRNA. The E site releases dissociated tRNAs so they can be recharged with free amino acids. The ribosome shifts one codon at a time, catalyzing each

process that occurs in the three sites. With each step, a charged tRNA enters the complex, the polypeptide becomes one amino acid longer, and an uncharged tRNA departs. The energy for each bond between amino acids is derived from GTP, a molecule similar to ATP (Figure 1.2.3). Amazingly, the *E. coli* translation apparatus takes only 0.05 seconds to add each amino acid, meaning that a 200-amino acid polypeptide could be translated in just 10 seconds.

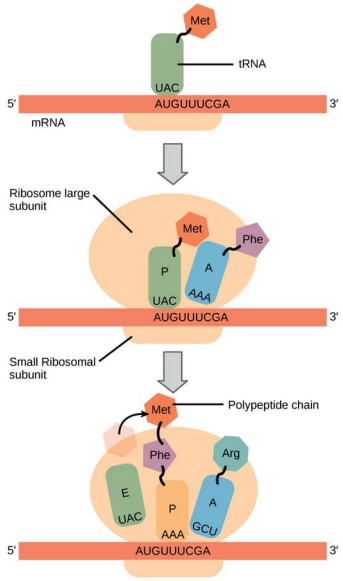


Figure 1.2.3: Translation begins when a tRNA anticodon recognizes a codon on the mRNA. The large ribosomal subunit joins the small subunit, and a second tRNA is recruited. As the mRNA moves relative to the ribosome, the polypeptide chain is formed. Entry of a release factor into the A site terminates translation and the components dissociate.

Termination of translation occurs when a stop codon (UAA, UAG, or UGA) is encountered. When the ribosome encounters the stop codon, the growing polypeptide is released and the ribosome subunits dissociate and leave the mRNA. After many ribosomes have completed translation, the mRNA is degraded so the nucleotides can be reused in another transcription reaction.

CONCEPT IN ACTION

Transcribe a gene and translate it to protein using complementary pairing and the genetic code at this site.

Summary

The central dogma describes the flow of genetic information in the cell from genes to mRNA to proteins. Genes are used to make mRNA by the process of transcription; mRNA is used to synthesize proteins by the process of translation. The genetic code is the correspondence between the three-nucleotide mRNA codon and an amino acid. The genetic code is "translated" by the tRNA molecules, which associate a specific codon with a specific amino acid. The genetic code is degenerate because 64 triplet codons in mRNA specify only 20 amino acids and three stop codons. This means that more than one codon corresponds to an amino acid. Almost every species on the planet uses the same genetic code.

The players in translation include the mRNA template, ribosomes, tRNAs, and various enzymatic factors. The small ribosomal subunit binds to the mRNA template. Translation begins at the initiating AUG on the mRNA. The formation of bonds occurs between sequential amino acids specified by the mRNA template according to the genetic code. The ribosome accepts charged tRNAs, and as it steps along the mRNA, it catalyzes bonding between the new amino acid and the end of the growing polypeptide. The entire mRNA is translated in three-nucleotide "steps" of the ribosome. When a stop codon is encountered, a release factor binds and dissociates the components and frees the new protein.

Glossary

codon
: three consecutive nucleotides in mRNA that specify the addition of a specific amino acid or the release of a polypeptide chain during translation

genetic code
: the amino acids that correspond to three-nucleotide codons of mRNA

rRNA
: ribosomal RNA; molecules of RNA that combine to form part of the ribosome

stop codon
: one of the three mRNA codons that specifies termination of translation

start codon
: the AUG (or, rarely GUG) on an mRNA from which translation begins; always specifies methionine

tRNA
: transfer RNA; an RNA molecule that contains a specific three-nucleotide anticodon sequence to pair with the mRNA codon and also binds to a specific amino acid

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 1.2: Translation is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 9.4: Translation by OpenStax is licensed CC BY 4.0.

1.3: Prokaryotic Cells

Learning Objectives

- Name examples of prokaryotic and eukaryotic organisms
- Compare and contrast prokaryotic cells and eukaryotic cells
- Describe the relative sizes of different kinds of cells
- Explain why cells must be small

Cells fall into one of two broad categories: prokaryotic and eukaryotic. Only the predominantly single-celled organisms of the domains Bacteria and Archaea are classified as prokaryotes (pro- = “before”; -kary- = “nucleus”). Cells of animals, plants, fungi, and protists are all eukaryotes (ceu- = “true”) and are made up of eukaryotic cells.

Components of Prokaryotic Cells

All cells share four common components: 1) a plasma membrane, an outer covering that separates the cell’s interior from its surrounding environment; 2) cytoplasm, consisting of a jelly-like cytosol within the cell in which other cellular components are found; 3) DNA, the genetic material of the cell; and 4) ribosomes, which synthesize proteins. However, prokaryotes differ from eukaryotic cells in several ways.

A prokaryote is a simple, mostly single-celled (unicellular) organism that lacks a nucleus, or any other membrane-bound organelle. We will shortly come to see that this is significantly different in eukaryotes. Prokaryotic DNA is found in a central part of the cell: the nucleoid (Figure 1.3.1).

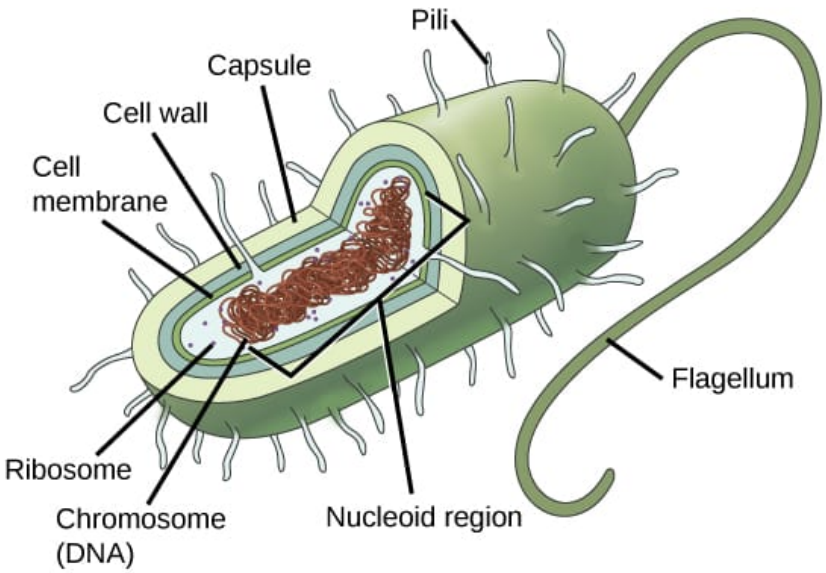


Figure 1.3.1: This figure shows the generalized structure of a prokaryotic cell. All prokaryotes have chromosomal DNA localized in a nucleoid, ribosomes, a cell membrane, and a cell wall. The other structures shown are present in some, but not all, bacteria.

Most prokaryotes have a peptidoglycan cell wall and many have a polysaccharide capsule (Figure 1.3.1). The cell wall acts as an extra layer of protection, helps the cell maintain its shape, and prevents dehydration. The capsule enables the cell to attach to surfaces in its environment. Some prokaryotes have flagella, pili, or fimbriae. Flagella are used for locomotion. Pili are used to exchange genetic material during a type of reproduction called conjugation. Fimbriae are used by bacteria to attach to a host cell.

Career Connection

Microbiologist: The most effective action anyone can take to prevent the spread of contagious illnesses is to wash his or her hands. Why? Because microbes (organisms so tiny that they can only be seen with microscopes) are ubiquitous. They live on doorknobs, money, your hands, and many other surfaces. If someone sneezes into his hand and touches a doorknob, and afterwards you touch that same doorknob, the microbes from the sneezer's mucus are now on your hands. If you touch your hands to your mouth, nose, or eyes, those microbes can enter your body and could make you sick.

However, not all microbes (also called microorganisms) cause disease; most are actually beneficial. You have microbes in your gut that make vitamin K. Other microorganisms are used to ferment beer and wine.

Microbiologists are scientists who study microbes. Microbiologists can pursue a number of careers. Not only do they work in the food industry, they are also employed in the veterinary and medical fields. They can work in the pharmaceutical sector, serving key roles in research and development by identifying new sources of antibiotics that could be used to treat bacterial infections.

Environmental microbiologists may look for new ways to use specially selected or genetically engineered microbes for the removal of pollutants from soil or groundwater, as well as hazardous elements from contaminated sites. These uses of microbes are called bioremediation technologies. Microbiologists can also work in the field of bioinformatics, providing specialized knowledge and insight for the design, development, and specificity of computer models of, for example, bacterial epidemics.

Cell Size

At 0.1 to 5.0 μm in diameter, prokaryotic cells are significantly smaller than eukaryotic cells, which have diameters ranging from 10 to 100 μm (Figure 1.3.2). The small size of prokaryotes allows ions and organic molecules that enter them to quickly diffuse to other parts of the cell. Similarly, any wastes produced within a prokaryotic cell can quickly diffuse out. This is not the case in eukaryotic cells, which have developed different structural adaptations to enhance intracellular transport.

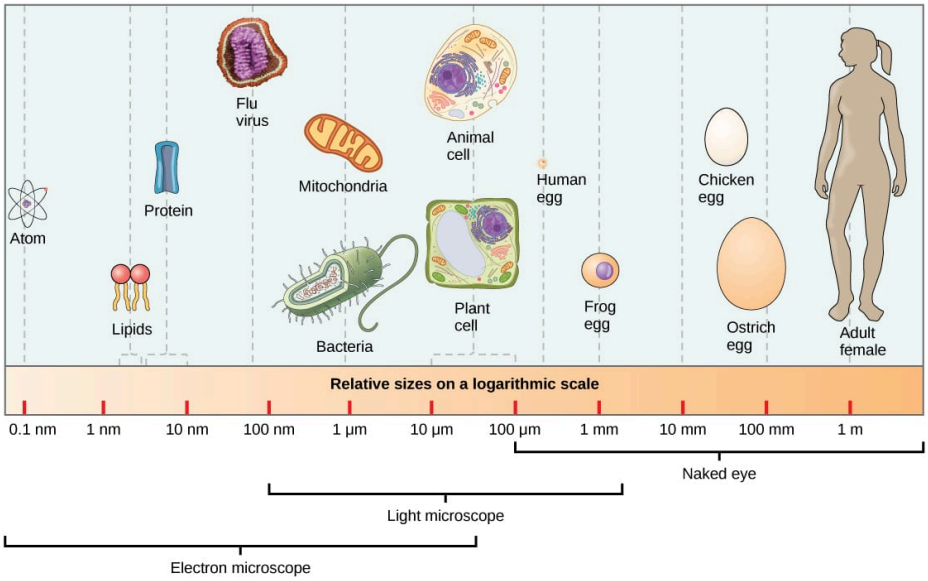


Figure 1.3.2: This figure shows relative sizes of microbes on a logarithmic scale (recall that each unit of increase in a logarithmic scale represents a 10-fold increase in the quantity being measured).

Small size, in general, is necessary for all cells, whether prokaryotic or eukaryotic. Let's examine why that is so. First, we'll consider the area and volume of a typical cell. Not all cells are spherical in shape, but most tend to approximate a sphere. You may remember from your high school geometry course that the formula for the surface area of a sphere is $4\pi r^2$, while the formula for its volume is $4\pi r^2/3$. Thus, as the radius of a cell increases, its surface area increases as the square of its radius, but its volume increases as the cube of its radius (much more rapidly). Therefore, as a cell increases in size, its surface area-to-volume ratio decreases. This same principle would apply if the cell had the shape of a cube (Figure 1.3.3). If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion required for the increased volume. In other words, as a cell grows, it becomes less efficient. One way to become more efficient is to divide; another way is to develop organelles that perform specific tasks. These adaptations lead to the development of more sophisticated cells called eukaryotic cells.

Art Connection

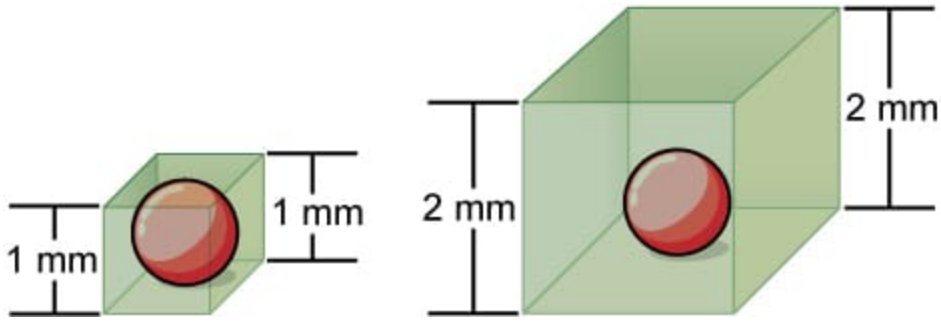


Figure 1.3.3: Notice that as a cell increases in size, its surface area-to-volume ratio decreases. When there is insufficient surface area to support a cell's increasing volume, a cell will either divide or die. The cell on the left has a volume of $1mm^3$ and a surface area of $6mm^2$, with a surface area-to-volume ratio of 6 to 1, whereas the cell on the right has a volume of $8mm^3$ and a surface area of $24mm^2$, with a surface area-to-volume ratio of 3 to 1.

Prokaryotic cells are much smaller than eukaryotic cells. What advantages might small cell size confer on a cell? What advantages might large cell size have?

Summary

Prokaryotes are predominantly single-celled organisms of the domains Bacteria and Archaea. All prokaryotes have plasma membranes, cytoplasm, ribosomes, and DNA that is not membrane-bound. Most have peptidoglycan cell walls and many have polysaccharide capsules. Prokaryotic cells range in diameter from 0.1 to 5.0 µm.

As a cell increases in size, its surface area-to-volume ratio decreases. If the cell grows too large, the plasma membrane will not have sufficient surface area to support the rate of diffusion required for the increased volume.

Art Connections

[link] Prokaryotic cells are much smaller than eukaryotic cells. What advantages might small cell size confer on a cell? What advantages might large cell size have?

[link] Substances can diffuse more quickly through small cells. Small cells have no need for organelles and therefore do not need to expend energy getting substances across organelle membranes. Large cells have organelles that can separate cellular processes, enabling them to build molecules that are more complex.

Glossary

nucleoid
: central part of a prokaryotic cell in which the chromosome is found

prokaryote
: unicellular organism that lacks a nucleus or any other membrane-bound organelle

Contributors and Attributions

- Connie Rye (East Mississippi Community College), Robert Wise (University of Wisconsin, Oshkosh), Vladimir Jurukovski (Suffolk County Community College), Jean DeSaix (University of North Carolina at Chapel Hill), Jung Choi (Georgia Institute of Technology), Yael Avissar (Rhode Island College) among other contributing authors. Original content by OpenStax (CC BY 4.0; Download for free at http://cnx.org/contents/185cbf87-c72...f21b5eabd@9.87).

This page titled 1.3: Prokaryotic Cells is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 4.2: Prokaryotic Cells by OpenStax is licensed CC BY 4.0.

1.4: Eukaryotic Cells

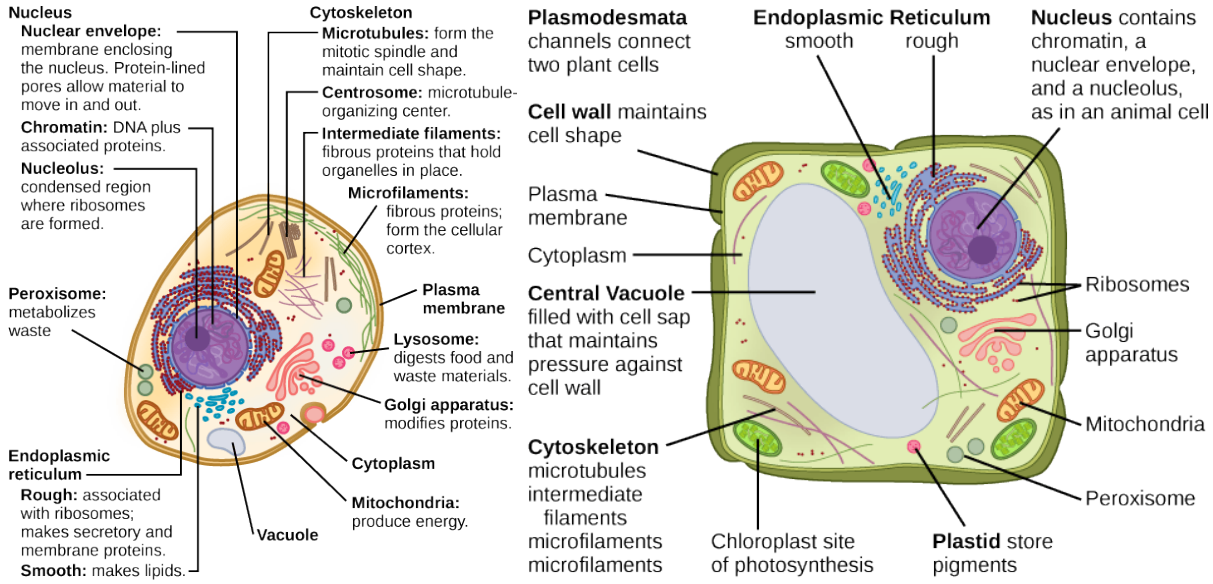
Learning Objectives

- Describe the structure of eukaryotic cells
- Compare animal cells with plant cells
- State the role of the plasma membrane
- Summarize the functions of the major cell organelles

Have you ever heard the phrase “form follows function?” It’s a philosophy practiced in many industries. In architecture, this means that buildings should be constructed to support the activities that will be carried out inside them. For example, a skyscraper should be built with several elevator banks; a hospital should be built so that its emergency room is easily accessible.

Our natural world also utilizes the principle of form following function, especially in cell biology, and this will become clear as we explore eukaryotic cells (Figure 1.4.1). Unlike prokaryotic cells, eukaryotic cells have: 1) a membrane-bound nucleus; 2) numerous membrane-bound organelles such as the endoplasmic reticulum, Golgi apparatus, chloroplasts, mitochondria, and others; and 3) several, rod-shaped chromosomes. Because a eukaryotic cell’s nucleus is surrounded by a membrane, it is often said to have a “true nucleus.” The word “organelle” means “little organ,” and, as already mentioned, organelles have specialized cellular functions, just as the organs of your body have specialized functions.

At this point, it should be clear to you that eukaryotic cells have a more complex structure than prokaryotic cells. Organelles allow different functions to be compartmentalized in different areas of the cell. Before turning to organelles, let’s first examine two important components of the cell: the plasma membrane and the cytoplasm.



(a) (b)

Figure 1.4.1***:*** *These figures show the major organelles and other cell components of (a) a typical animal cell and (b) a typical eukaryotic plant cell. The plant cell has a cell wall, chloroplasts, plastids, and a central vacuole—structures not found in animal cells. Plant cells do not have lysosomes or centrosomes.*

Exercise 1.4.1

If the nucleolus were not able to carry out its function, what other cellular organelles would be affected?

The Plasma Membrane

Like prokaryotes, eukaryotic cells have a plasma membrane (Figure 1.4.2), a phospholipid bilayer with embedded proteins that separates the internal contents of the cell from its surrounding environment. A phospholipid is a lipid molecule with two fatty acid chains and a phosphate-containing group. The plasma membrane controls the passage of organic molecules, ions, water, and oxygen into and out of the cell. Wastes (such as carbon dioxide and ammonia) also leave the cell by passing through the plasma membrane.

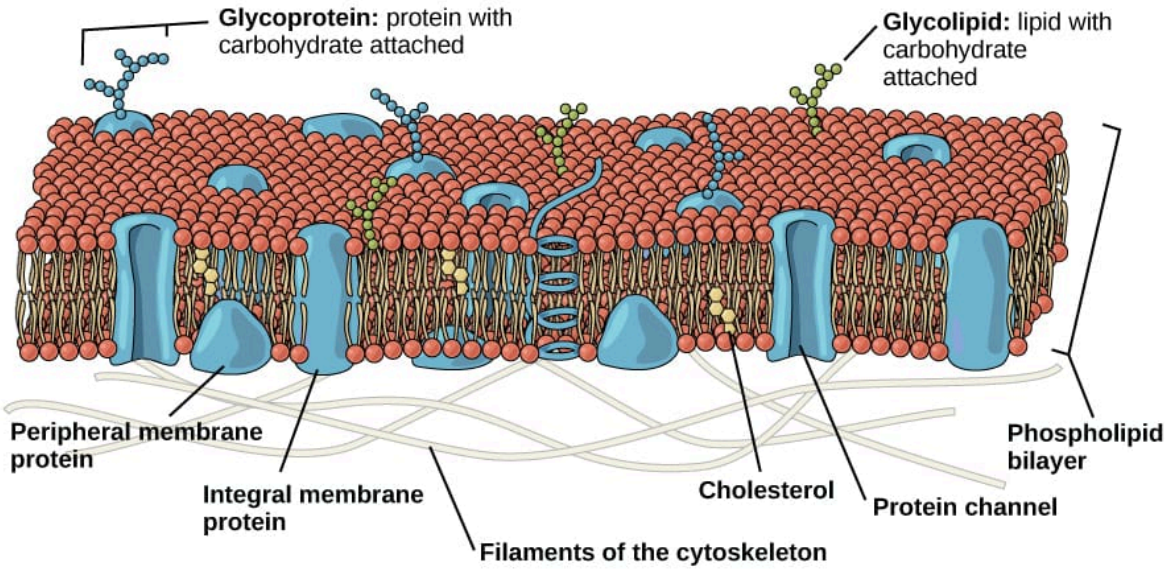


Figure 1.4.2: The eukaryotic plasma membrane is a phospholipid bilayer with proteins and cholesterol embedded in it.

The plasma membranes of cells that specialize in absorption are folded into fingerlike projections called microvilli (singular = microvillus); (Figure 1.4.3). Such cells are typically found lining the small intestine, the organ that absorbs nutrients from digested food. This is an excellent example of form following function. People with celiac disease have an immune response to gluten, which is a protein found in wheat, barley, and rye. The immune response damages microvilli, and thus, afflicted individuals cannot absorb nutrients. This leads to malnutrition, cramping, and diarrhea. Patients suffering from celiac disease must follow a gluten-free diet.

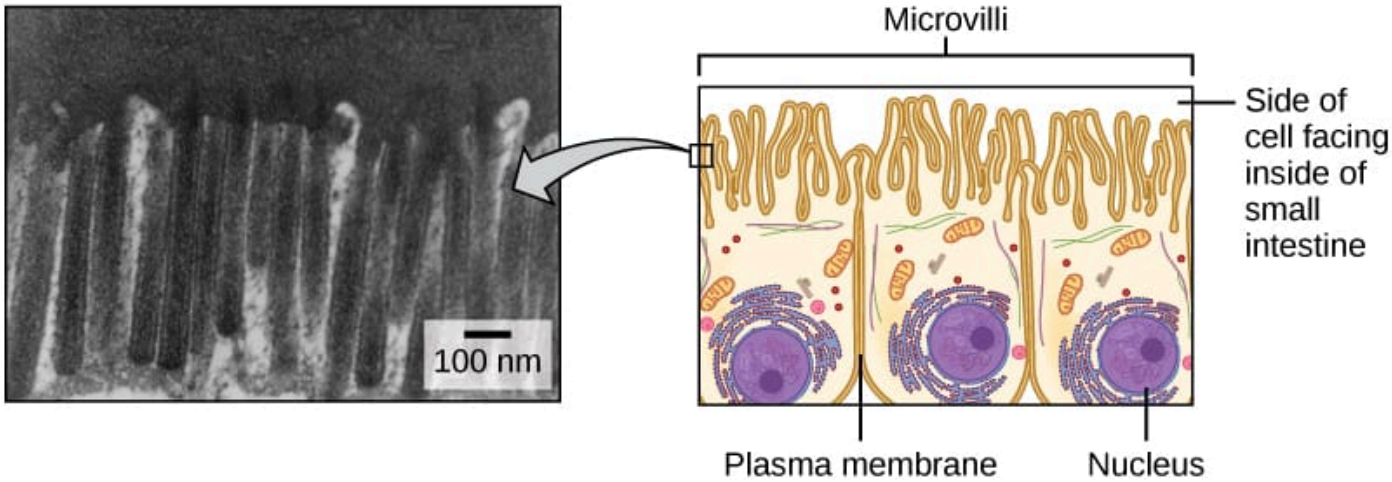


Figure 1.4.3: Microvilli, shown here as they appear on cells lining the small intestine, increase the surface area available for absorption. These microvilli are only found on the area of the plasma membrane that faces the cavity from which substances will be absorbed. (credit "micrograph": modification of work by Louisa Howard)

The Cytoplasm

The cytoplasm is the entire region of a cell between the plasma membrane and the nuclear envelope (a structure to be discussed shortly). It is made up of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals (Figure 1.4.1). Even though the cytoplasm consists of 70 to 80 percent water, it has a semi-solid consistency, which comes from the proteins within it. However, proteins are not the only organic molecules found in the cytoplasm. Glucose and other simple sugars, polysaccharides, amino acids, nucleic acids, fatty acids, and derivatives of glycerol are found there, too. Ions of sodium, potassium, calcium, and many other elements are also dissolved in the cytoplasm. Many metabolic reactions, including protein synthesis, take place in the cytoplasm.

The Nucleus

Typically, the nucleus is the most prominent organelle in a cell (Figure 1.4.1). The nucleus (plural = nuclei) houses the cell's DNA and directs the synthesis of ribosomes and proteins. Let's look at it in more detail (Figure 1.4.4).

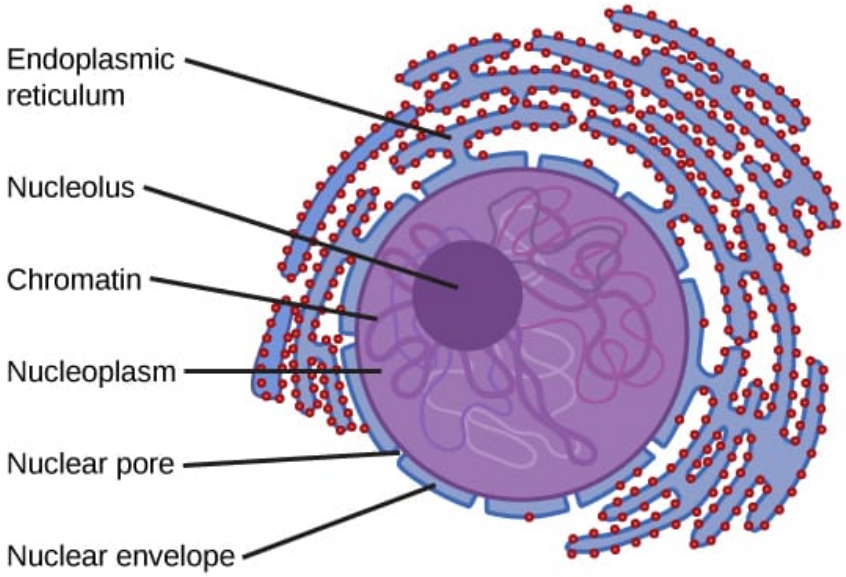


Figure 1.4.4: The nucleus stores chromatin (DNA plus proteins) in a gel-like substance called the nucleoplasm. The nucleolus is a condensed region of chromatin where ribosome synthesis occurs. The boundary of the nucleus is called the nuclear envelope. It consists of two phospholipid bilayers: an outer membrane and an inner membrane. The nuclear membrane is continuous with the endoplasmic reticulum. Nuclear pores allow substances to enter and exit the nucleus.

The Nuclear Envelope

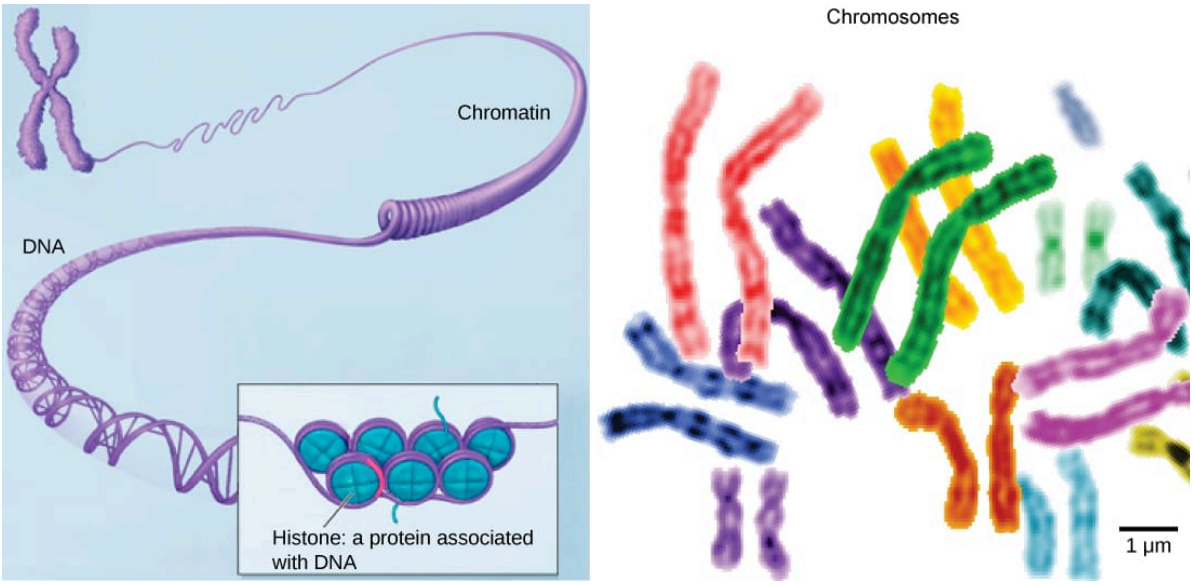
The nuclear envelope is a double-membrane structure that constitutes the outermost portion of the nucleus (Figure 1.4.4). Both the inner and outer membranes of the nuclear envelope are phospholipid bilayers.

The nuclear envelope is punctuated with pores that control the passage of ions, molecules, and RNA between the nucleoplasm and cytoplasm. The nucleoplasm is the semi-solid fluid inside the nucleus, where we find the chromatin and the nucleolus.

Chromatin and Chromosomes

To understand chromatin, it is helpful to first consider chromosomes. Chromosomes are structures within the nucleus that are made up of DNA, the hereditary material. You may remember that in prokaryotes, DNA is organized into a single circular chromosome. In eukaryotes, chromosomes are linear structures. Every eukaryotic species has a specific number of chromosomes in the nuclei of its body's cells. For example, in humans, the chromosome number is 46, while in fruit flies, it is eight. Chromosomes are only visible and distinguishable from one another when the cell is getting ready to divide. When the cell is in the growth and maintenance phases of its life cycle, proteins are attached to chromosomes, and they resemble an unwound, jumbled bunch of threads. These unwound protein-chromosome complexes are called chromatin (Figure 1.4.5); chromatin describes the

material that makes up the chromosomes both when condensed and decondensed.



(a) (b)

Figure 1.4.5***:*** *(a) This image shows various levels of the organization of chromatin (DNA and protein). (b) This image shows paired chromosomes. (credit b: modification of work by NIH; scale-bar data from Matt Russell)*

The Nucleolus

We already know that the nucleus directs the synthesis of ribosomes, but how does it do this? Some chromosomes have sections of DNA that encode ribosomal RNA. A darkly staining area within the nucleus called the nucleolus (plural = nucleoli) aggregates the ribosomal RNA with associated proteins to assemble the ribosomal subunits that are then transported out through the pores in the nuclear envelope to the cytoplasm.

Ribosomes

Ribosomes are the cellular structures responsible for protein synthesis. When viewed through an electron microscope, ribosomes appear either as clusters (polyribosomes) or single, tiny dots that float freely in the cytoplasm. They may be attached to the cytoplasmic side of the plasma membrane or the cytoplasmic side of the endoplasmic reticulum and the outer membrane of the nuclear envelope (Figure 1.4.1). Electron microscopy has shown us that ribosomes, which are large complexes of protein and RNA, consist of two subunits, aptly called large and small (Figure 1.4.6). Ribosomes receive their “orders” for protein synthesis from the nucleus where the DNA is transcribed into messenger RNA (mRNA). The mRNA travels to the ribosomes, which translate the code provided by the sequence of the nitrogenous bases in the mRNA into a specific order of amino acids in a protein. Amino acids are the building blocks of proteins.

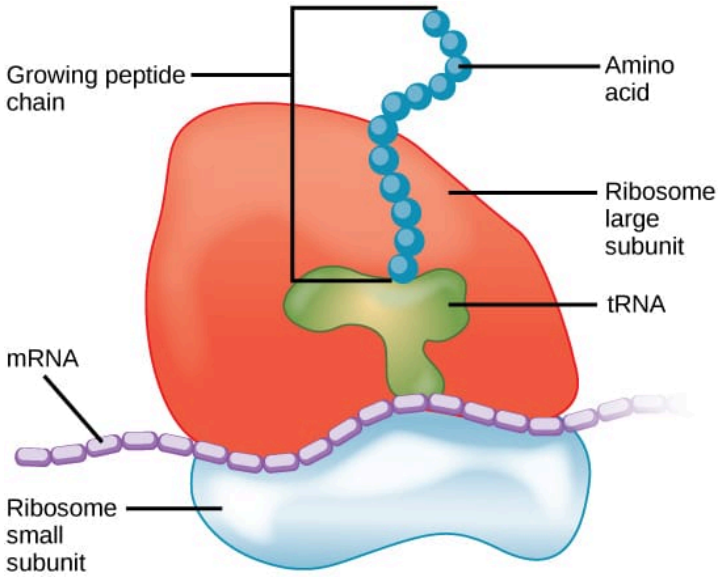


Figure 1.4.6: Ribosomes are made up of a large subunit (top) and a small subunit (bottom). During protein synthesis, ribosomes assemble amino acids into proteins.

Because proteins synthesis is an essential function of all cells (including enzymes, hormones, antibodies, pigments, structural components, and surface receptors), ribosomes are found in practically every cell. Ribosomes are particularly abundant in cells that synthesize large amounts of protein. For example, the pancreas is responsible for creating several digestive enzymes and the cells that produce these enzymes contain many ribosomes. Thus, we see another example of form following function.

Mitochondria

Mitochondria (singular = mitochondrion) are often called the “powerhouses” or “energy factories” of a cell because they are responsible for making adenosine triphosphate (ATP), the cell’s main energy-carrying molecule. ATP represents the short-term stored energy of the cell. Cellular respiration is the process of making ATP using the chemical energy found in glucose and other nutrients. In mitochondria, this process uses oxygen and produces carbon dioxide as a waste product. In fact, the carbon dioxide that you exhale with every breath comes from the cellular reactions that produce carbon dioxide as a byproduct.

In keeping with our theme of form following function, it is important to point out that muscle cells have a very high concentration of mitochondria that produce ATP. Your muscle cells need a lot of energy to keep your body moving. When your cells don’t get enough oxygen, they do not make a lot of ATP. Instead, the small amount of ATP they make in the absence of oxygen is accompanied by the production of lactic acid.

Mitochondria are oval-shaped, double membrane organelles (Figure 1.4.7) that have their own ribosomes and DNA. Each membrane is a phospholipid bilayer embedded with proteins. The inner layer has folds called cristae. The area surrounded by the folds is called the mitochondrial matrix. The cristae and the matrix have different roles in cellular respiration.

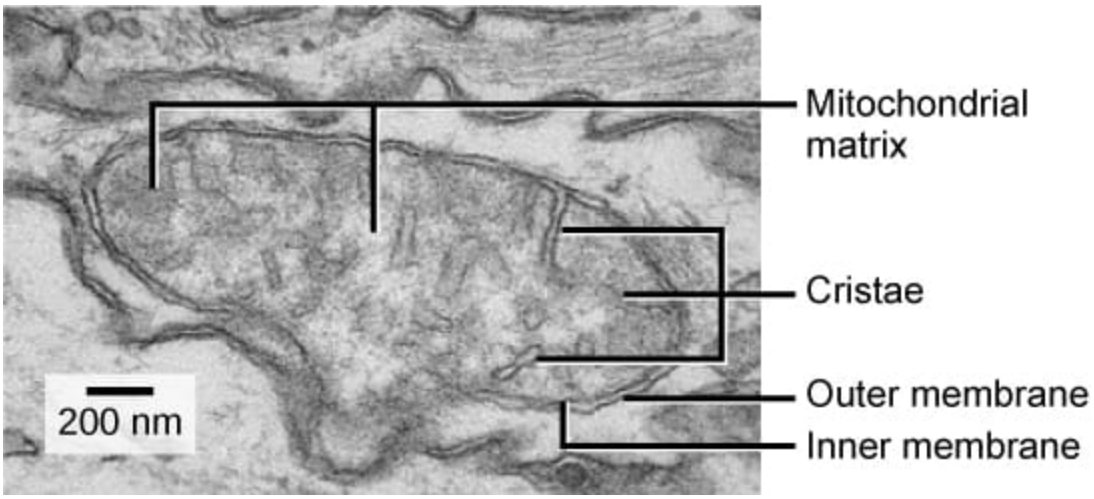


Figure 1.4.7: This electron micrograph shows a mitochondrion as viewed with a transmission electron microscope. This organelle has an outer membrane and an inner membrane. The inner membrane contains folds, called cristae, which increase its surface area. The space between the two membranes is called the intermembrane space, and the space inside the inner membrane is called the mitochondrial matrix. ATP synthesis takes place on the inner membrane. (credit: modification of work by Matthew Britton; scale-bar data from Matt Russell)

Peroxisomes

Peroxisomes are small, round organelles enclosed by single membranes. They carry out oxidation reactions that break down fatty acids and amino acids. They also detoxify many poisons that may enter the body. (Many of these oxidation reactions release hydrogen peroxide, H_2O_2, which would be damaging to cells; however, when these reactions are confined to peroxisomes, enzymes safely break down the H_2O_2 into oxygen and water.) For example, alcohol is detoxified by peroxisomes in liver cells. Glyoxysomes, which are specialized peroxisomes in plants, are responsible for converting stored fats into sugars.

Vesicles and Vacuoles

Vesicles and vacuoles are membrane-bound sacs that function in storage and transport. Other than the fact that vacuoles are somewhat larger than vesicles, there is a very subtle distinction between them: The membranes of vesicles can fuse with either the plasma membrane or other membrane systems within the cell. Additionally, some agents such as enzymes within plant vacuoles break down macromolecules. The membrane of a vacuole does not fuse with the membranes of other cellular components.

Animal Cells versus Plant Cells

At this point, you know that each eukaryotic cell has a plasma membrane, cytoplasm, a nucleus, ribosomes, mitochondria, peroxisomes, and in some, vacuoles, but there are some striking differences between animal and plant cells. While both animal and plant cells have microtubule organizing centers (MTOCs), animal cells also have centrioles associated with the MTOC: a complex called the centrosome. Animal cells each have a centrosome and lysosomes, whereas plant cells do not. Plant cells have a cell wall, chloroplasts and other specialized plastids, and a large central vacuole, whereas animal cells do not.

The Centrosome

The centrosome is a microtubule-organizing center found near the nuclei of animal cells. It contains a pair of centrioles, two structures that lie perpendicular to each other (Figure 1.4.8). Each centriole is a cylinder of nine triplets of microtubules.

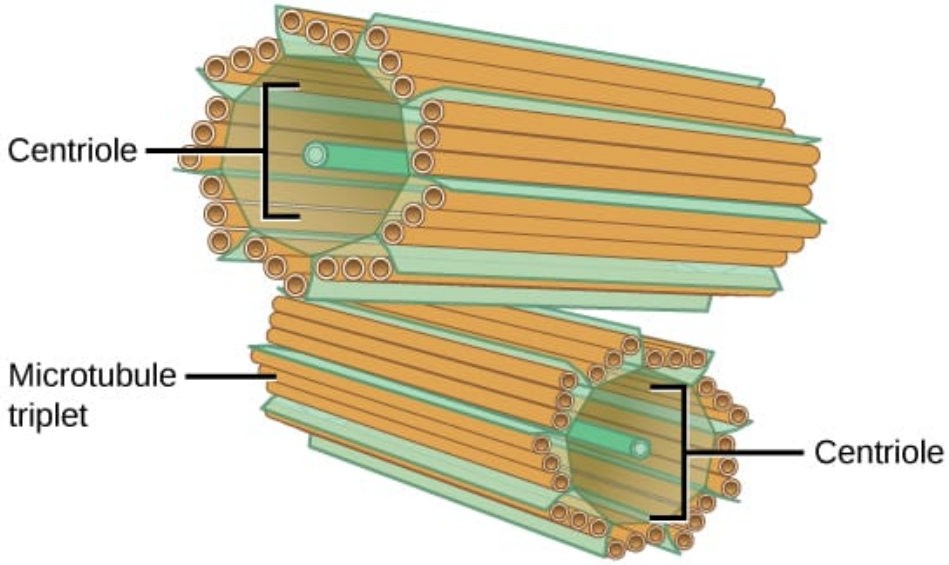


Figure 1.4.8: The centrosome consists of two centrioles that lie at right angles to each other. Each centriole is a cylinder made up of nine triplets of microtubules. Nontubulin proteins (indicated by the green lines) hold the microtubule triplets together.

The centrosome (the organelle where all microtubules originate) replicates itself before a cell divides, and the centrioles appear to have some role in pulling the duplicated chromosomes to opposite ends of the dividing cell. However, the exact function of the centrioles in cell division isn't clear, because cells that have had the centrosome removed can still divide, and plant cells, which lack centrosomes, are capable of cell division.

Lysosomes

Animal cells have another set of organelles not found in plant cells: lysosomes. The lysosomes are the cell's "garbage disposal." In plant cells, the digestive processes take place in vacuoles. Enzymes within the lysosomes aid the breakdown of proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles. These enzymes are active at a much lower pH than that of the cytoplasm. Therefore, the pH within lysosomes is more acidic than the pH of the cytoplasm. Many reactions that take place in the cytoplasm could not occur at a low pH, so again, the advantage of compartmentalizing the eukaryotic cell into organelles is apparent.

The Cell Wall

If you examine Figure 1.4.1b, the diagram of a plant cell, you will see a structure external to the plasma membrane called the cell wall. The cell wall is a rigid covering that protects the cell, provides structural support, and gives shape to the cell. Fungal and protistan cells also have cell walls. While the chief component of prokaryotic cell walls is peptidoglycan, the major organic molecule in the plant cell wall is cellulose (Figure 1.4.9), a polysaccharide made up of glucose units. Have you ever noticed that when you bite into a raw vegetable, like celery, it crunches? That's because you are tearing the rigid cell walls of the celery cells with your teeth.

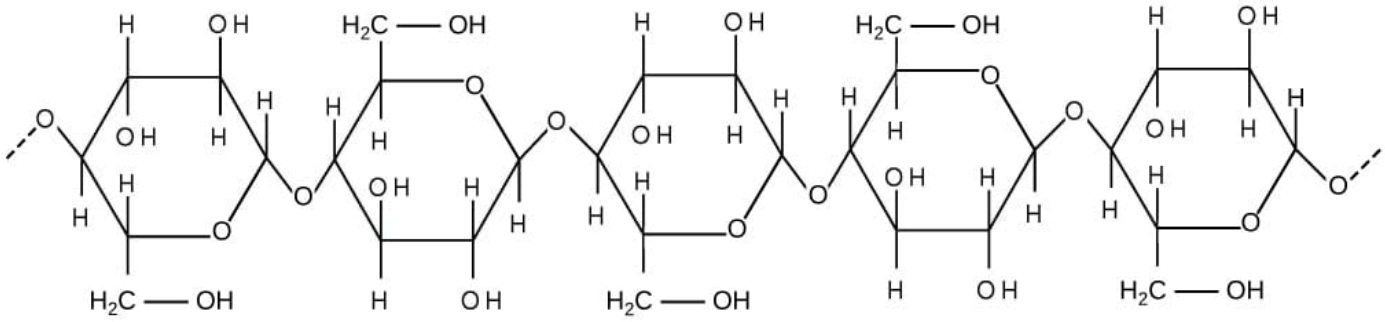


Figure 1.4.9: Cellulose is a long chain of β-glucose molecules connected by a 1-4 linkage. The dashed lines at each end of the figure indicate a series of many more glucose units. The size of the page makes it impossible to portray an entire cellulose molecule.

Chloroplasts

Like the mitochondria, chloroplasts have their own DNA and ribosomes, but chloroplasts have an entirely different function. Chloroplasts are plant cell organelles that carry out photosynthesis. Photosynthesis is the series of reactions that use carbon dioxide, water, and light energy to make glucose and oxygen. This is a major difference between plants and animals; plants (autotrophs) are able to make their own food, like sugars, while animals (heterotrophs) must ingest their food.

Like mitochondria, chloroplasts have outer and inner membranes, but within the space enclosed by a chloroplast's inner membrane is a set of interconnected and stacked fluid-filled membrane sacs called thylakoids (Figure 1.4.10). Each stack of thylakoids is called a granum (plural = grana). The fluid enclosed by the inner membrane that surrounds the grana is called the stroma.

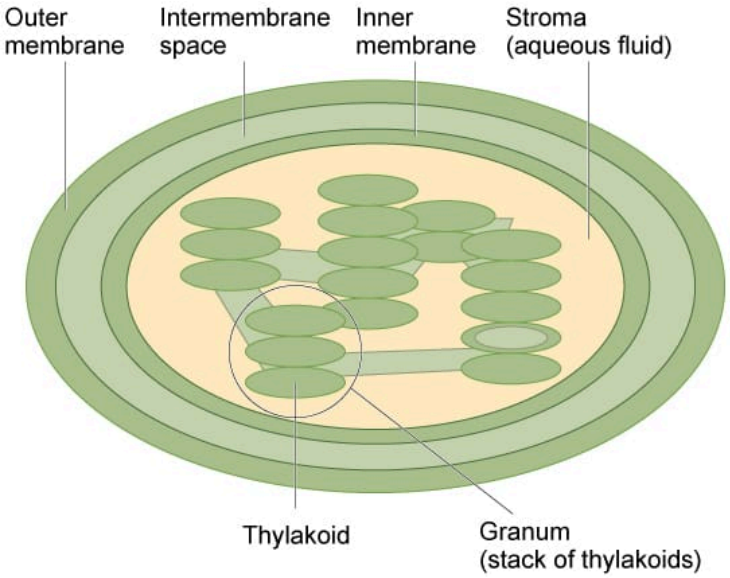


Figure 1.4.10: The chloroplast has an outer membrane, an inner membrane, and membrane structures called thylakoids that are stacked into grana. The space inside the thylakoid membranes is called the thylakoid space. The light harvesting reactions take place in the thylakoid membranes, and the synthesis of sugar takes place in the fluid inside the inner membrane, which is called the stroma. Chloroplasts also have their own genome, which is contained on a single circular chromosome.

The chloroplasts contain a green pigment called chlorophyll, which captures the light energy that drives the reactions of photosynthesis. Like plant cells, photosynthetic protists also have chloroplasts. Some bacteria perform photosynthesis, but their chlorophyll is not relegated to an organelle.

Evolution Connection

EndosymbiosisWe have mentioned that both mitochondria and chloroplasts contain DNA and ribosomes. Have you wondered why? Strong evidence points to endosymbiosis as the explanation.

Symbiosis is a relationship in which organisms from two separate species depend on each other for their survival. Endosymbiosis (endo- = "within") is a mutually beneficial relationship in which one organism lives inside the other. Endosymbiotic relationships abound in nature. We have already mentioned that microbes that produce vitamin K live inside the human gut. This relationship is beneficial for us because we are unable to synthesize vitamin K. It is also beneficial for the microbes because they are protected from other organisms and from drying out, and they receive abundant food from the environment of the large intestine.

Scientists have long noticed that bacteria, mitochondria, and chloroplasts are similar in size. We also know that bacteria have DNA and ribosomes, just as mitochondria and chloroplasts do. Scientists believe that host cells and bacteria formed an endosymbiotic relationship when the host cells ingested both aerobic and autotrophic bacteria (cyanobacteria) but did not destroy them. Through many millions of years of evolution, these ingested bacteria became more specialized in their functions, with the aerobic bacteria becoming mitochondria and the autotrophic bacteria becoming chloroplasts.

The Central Vacuole

Previously, we mentioned vacuoles as essential components of plant cells. If you look at Figure 1.4.1b, you will see that plant cells each have a large central vacuole that occupies most of the area of the cell. The central vacuole plays a key role in regulating the cell's concentration of water in changing environmental conditions. Have you ever noticed that if you forget to water a plant for a few days, it wilts? That's because as the water concentration in the soil becomes lower than the water concentration in the plant, water moves out of the central vacuoles and cytoplasm. As the central vacuole shrinks, it leaves the cell wall unsupported. This loss of support to the cell walls of plant cells results in the wilted appearance of the plant.

The central vacuole also supports the expansion of the cell. When the central vacuole holds more water, the cell gets larger without having to invest a lot of energy in synthesizing new cytoplasm.

Summary

Like a prokaryotic cell, a eukaryotic cell has a plasma membrane, cytoplasm, and ribosomes, but a eukaryotic cell is typically larger than a prokaryotic cell, has a true nucleus (meaning its DNA is surrounded by a membrane), and has other membrane-bound organelles that allow for compartmentalization of functions. The plasma membrane is a phospholipid bilayer embedded with proteins. The nucleus's nucleolus is the site of ribosome assembly. Ribosomes are either found in the cytoplasm or attached to the cytoplasmic side of the plasma membrane or endoplasmic reticulum. They perform protein synthesis. Mitochondria participate in cellular respiration; they are responsible for the majority of ATP produced in the cell. Peroxisomes hydrolyze fatty acids, amino acids, and some toxins. Vesicles and vacuoles are storage and transport compartments. In plant cells, vacuoles also help break down macromolecules.

Animal cells also have a centrosome and lysosomes. The centrosome has two bodies perpendicular to each other, the centrioles, and has an unknown purpose in cell division. Lysosomes are the digestive organelles of animal cells.

Plant cells and plant-like cells each have a cell wall, chloroplasts, and a central vacuole. The plant cell wall, whose primary component is cellulose, protects the cell, provides structural support, and gives shape to the cell. Photosynthesis takes place in chloroplasts. The central vacuole can expand without having to produce more cytoplasm.

Art Connections

[link] If the nucleolus were not able to carry out its function, what other cellular organelles would be affected?

[link] Free ribosomes and rough endoplasmic reticulum (which contains ribosomes) would not be able to form.

Glossary

cell wall
rigid cell covering made of cellulose that protects the cell, provides structural support, and gives shape to the cell

central vacuole
large plant cell organelle that regulates the cell's storage compartment, holds water, and plays a significant role in cell growth as the site of macromolecule degradation

centrosome
region in animal cells made of two centrioles

chlorophyll
green pigment that captures the light energy that drives the light reactions of photosynthesis

chloroplast
plant cell organelle that carries out photosynthesis

chromatin
protein-DNA complex that serves as the building material of chromosomes

chromosome
structure within the nucleus that is made up of chromatin that contains DNA, the hereditary material

cytoplasm
entire region between the plasma membrane and the nuclear envelope, consisting of organelles suspended in the gel-like cytosol, the cytoskeleton, and various chemicals

cytosol
gel-like material of the cytoplasm in which cell structures are suspended

eukaryotic cell
cell that has a membrane-bound nucleus and several other membrane-bound compartments or sacs

lysosome
organelle in an animal cell that functions as the cell's digestive component; it breaks down proteins, polysaccharides, lipids, nucleic acids, and even worn-out organelles

mitochondria
(singular = mitochondrion) cellular organelles responsible for carrying out cellular respiration, resulting in the production of ATP, the cell's main energy-carrying molecule

nuclear envelope
double-membrane structure that constitutes the outermost portion of the nucleus

nucleolus
darkly staining body within the nucleus that is responsible for assembling the subunits of the ribosomes

nucleoplasm
semi-solid fluid inside the nucleus that contains the chromatin and nucleolus

nucleus
cell organelle that houses the cell's DNA and directs the synthesis of ribosomes and proteins

organelle
compartment or sac within a cell

peroxisome
small, round organelle that contains hydrogen peroxide, oxidizes fatty acids and amino acids, and detoxifies many poisons

plasma membrane
: phospholipid bilayer with embedded (integral) or attached (peripheral) proteins, and separates the internal content of the cell from its surrounding environment

ribosome
: cellular structure that carries out protein synthesis

vacuole
: membrane-bound sac, somewhat larger than a vesicle, which functions in cellular storage and transport

vesicle
: small, membrane-bound sac that functions in cellular storage and transport; its membrane is capable of fusing with the plasma membrane and the membranes of the endoplasmic reticulum and Golgi apparatus

Contributors and Attributions

- Connie Rye (East Mississippi Community College), Robert Wise (University of Wisconsin, Oshkosh), Vladimir Jurukovski (Suffolk County Community College), Jean DeSaix (University of North Carolina at Chapel Hill), Jung Choi (Georgia Institute of Technology), Yael Avissar (Rhode Island College) among other contributing authors. Original content by OpenStax (CC BY 4.0; Download for free at http://cnx.org/contents/185cbf87-c72...f21b5eabd@9.87).

This page titled 1.4: Eukaryotic Cells is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 4.3: Eukaryotic Cells by OpenStax is licensed CC BY 4.0.

1.5: Essential elements for life

The diverse environments and ecosystems on Earth have a wide range of conditions in terms of temperature, available nutrients, acidity, salinity, and energy sources. Prokaryotes are very well equipped to make their living out of a vast array of nutrients and conditions. To live, prokaryotes need a source of energy, a source of carbon, and some additional nutrients.

Macronutrients

Cells are essentially a well-organized assemblage of macromolecules and water. Recall that macromolecules are produced by the polymerization of smaller units called monomers. For cells to build all of the molecules required to sustain life, they need certain substances, collectively called **nutrients**. When prokaryotes grow in nature, they obtain their nutrients from the environment. Nutrients that are required in large amounts are called macronutrients, whereas those required in smaller or trace amounts are called micronutrients. Just a handful of elements are considered macronutrients—carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur. (A mnemonic for remembering these elements is the acronym *CHONPS*.)

Why are these macronutrients needed in large amounts? They are the components of organic compounds in cells, including water. Carbon is the major element in all macromolecules: carbohydrates, proteins, nucleic acids, lipids, and many other compounds. Carbon accounts for about 50 percent of the composition of the cell. Nitrogen represents 12 percent of the total dry weight of a typical cell and is a component of proteins, nucleic acids, and other cell constituents. Most of the nitrogen available in nature is either atmospheric nitrogen (N_2) or another inorganic form. Diatomic (N_2) nitrogen, however, can be converted into an organic form only by certain organisms, called nitrogen-fixing organisms. Both hydrogen and oxygen are part of many organic compounds and of water. Phosphorus is required by all organisms for the synthesis of nucleotides and phospholipids. Sulfur is part of the structure of some amino acids such as cysteine and methionine, and is also present in several vitamins and coenzymes. Other important macronutrients are potassium (K), magnesium (Mg), calcium (Ca), and sodium (Na). Although these elements are required in smaller amounts, they are very important for the structure and function of the prokaryotic cell.

Micronutrients

In addition to these macronutrients, prokaryotes require various metallic elements in small amounts. These are referred to as micronutrients or trace elements. For example, iron is necessary for the function of the cytochromes involved in electron-transport reactions. Some prokaryotes require other elements—such as boron (B), chromium (Cr), and manganese (Mn)—primarily as enzyme cofactors.

Contributors and Attributions

CC licensed content, Shared previously

- Biology. **Provided by**: OpenStax CNX. **Located at**: http://cnx.org/contents/185cbf87-c72e-48f5-b51e-f14f21b5eabd@10.8. **License**: *CC BY: Attribution*. **License Terms**: Download for free at http://cnx.org/contents/185cbf87-c72...f21b5eabd@10.8

This page titled 1.5: Essential elements for life is shared under a license and was authored, remixed, and/or curated by LibreTexts.

- 6.10: Needs of Prokaryotes has no license indicated.

1.6: The Building Blocks of Molecules

At its most fundamental level, life is made up of matter. Matter occupies space and has mass. All matter is composed of elements, substances that cannot be broken down or transformed chemically into other substances. Each element is made of atoms, each with a constant number of protons and unique properties. A total of 118 elements have been defined; however, only 92 occur naturally, and fewer than 30 are found in living cells. The remaining 26 elements are unstable and, therefore, do not exist for very long or are theoretical and have yet to be detected.

Each element is designated by its chemical symbol (such as H, N, O, C, and Na), and possesses unique properties. These unique properties allow elements to combine and to bond with each other in specific ways.

Atoms

An atom is the smallest component of an element that retains all of the chemical properties of that element. For example, one hydrogen atom has all of the properties of the element hydrogen, such as it exists as a gas at room temperature, and it bonds with oxygen to create a water molecule. Hydrogen atoms cannot be broken down into anything smaller while still retaining the properties of hydrogen. If a hydrogen atom were broken down into subatomic particles, it would no longer have the properties of hydrogen.

At the most basic level, all organisms are made of a combination of elements. They contain atoms that combine together to form molecules. In multicellular organisms, such as animals, molecules can interact to form cells that combine to form tissues, which make up organs. These combinations continue until entire multicellular organisms are formed.

All atoms contain protons, electrons, and neutrons (Figure 1.6.1). The only exception is hydrogen (H), which is made of one proton and one electron. A proton is a positively charged particle that resides in the nucleus (the core of the atom) of an atom and has a mass of 1 and a charge of +1. An electron is a negatively charged particle that travels in the space around the nucleus. In other words, it resides outside of the nucleus. It has a negligible mass and has a charge of –1.

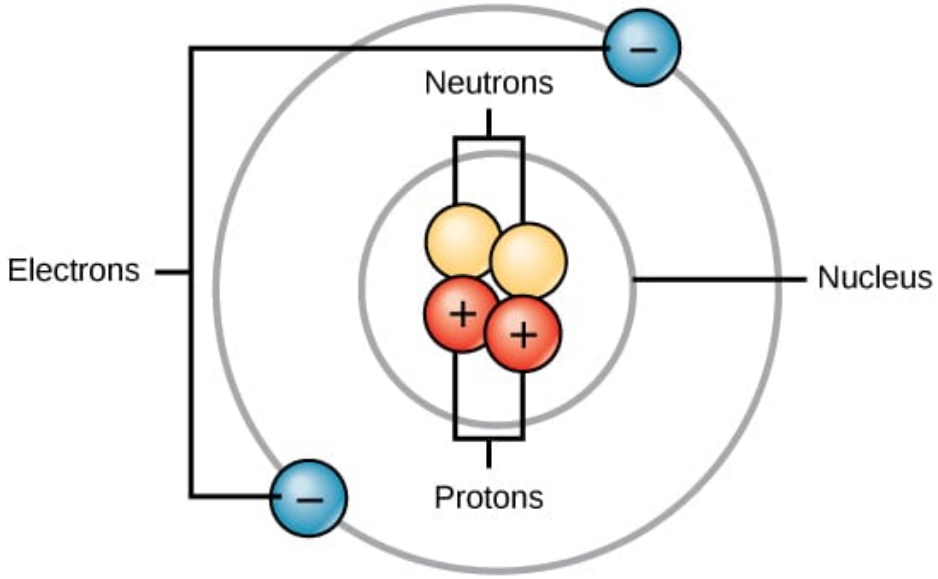


Figure 1.6.1***:*** *Atoms are made up of protons and neutrons located within the nucleus, and electrons surrounding the nucleus.*

Neutrons, like protons, reside in the nucleus of an atom. They have a mass of 1 and no charge. The positive (protons) and negative (electrons) charges balance each other in a neutral atom, which has a net zero charge.

Because protons and neutrons each have a mass of 1, the mass of an atom is equal to the number of protons and neutrons of

that atom. The number of electrons does not factor into the overall mass, because their mass is so small.

As stated earlier, each element has its own unique properties. Each contains a different number of protons and neutrons, giving it its own atomic number and mass number. The atomic number of an element is equal to the number of protons that element contains. The mass number, or atomic mass, is the number of protons plus the number of neutrons of that element. Therefore, it is possible to determine the number of neutrons by subtracting the atomic number from the mass number.

These numbers provide information about the elements and how they will react when combined. Different elements have different melting and boiling points, and are in different states (liquid, solid, or gas) at room temperature. They also combine in different ways. Some form specific types of bonds, whereas others do not. How they combine is based on the number of electrons present. Because of these characteristics, the elements are arranged into the periodic table of elements, a chart of the elements that includes the atomic number and relative atomic mass of each element. The periodic table also provides key information about the properties of elements (*Figure* 1.6.2)—often indicated by color-coding. The arrangement of the table also shows how the electrons in each element are organized and provides important details about how atoms will react with each other to form molecules.

Isotopes are different forms of the same element that have the same number of protons, but a different number of neutrons. Some elements, such as carbon, potassium, and uranium, have naturally occurring isotopes. Carbon-12, the most common isotope of carbon, contains six protons and six neutrons. Therefore, it has a mass number of 12 (six protons and six neutrons) and an atomic number of 6 (which makes it carbon). Carbon-14 contains six protons and eight neutrons. Therefore, it has a mass number of 14 (six protons and eight neutrons) and an atomic number of 6, meaning it is still the element carbon. These two alternate forms of carbon are isotopes. Some isotopes are unstable and will lose protons, other subatomic particles, or energy to form more stable elements. These are called radioactive isotopes or radioisotopes.

ART CONNECTION

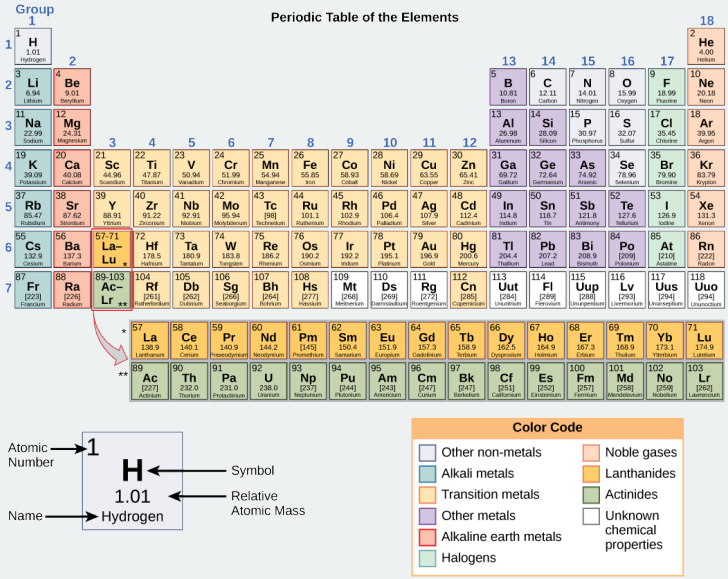


Figure 1.6.2: Arranged in columns and rows based on the characteristics of the elements, the periodic table provides key information about the elements and how they might interact with each other to form molecules. Most periodic tables provide a key or legend to the information they contain.

Chemical Bonds

How elements interact with one another depends on how their electrons are arranged and how many openings for electrons exist at the outermost region where electrons are present in an atom. Electrons exist at energy levels that form shells around the nucleus. The closest shell can hold up to two electrons. The closest shell to the nucleus is always filled first, before any other shell can be filled. Hydrogen has one electron; therefore, it has only one spot occupied within the lowest shell. Helium has two electrons; therefore, it can completely fill the lowest shell with its two electrons. If you look at the periodic table, you will see that hydrogen and helium are the only two elements in the first row. This is because they only have electrons in their first shell. Hydrogen and helium are the only two elements that have the lowest shell and no other shells.

The second and third energy levels can hold up to eight electrons. The eight electrons are arranged in four pairs and one position in each pair is filled with an electron before any pairs are completed.

Looking at the periodic table again (Figure 1.6.2), you will notice that there are seven rows. These rows correspond to the number of shells that the elements within that row have. The elements within a particular row have increasing numbers of electrons as the columns proceed from left to right. Although each element has the same number of shells, not all of the shells are completely filled with electrons. If you look at the second row of the periodic table, you will find lithium (Li), beryllium (Be), boron (B), carbon (C), nitrogen (N), oxygen (O), fluorine (F), and neon (Ne). These all have electrons that occupy only the first and second shells. Lithium has only one electron in its outermost shell, beryllium has two electrons, boron has three, and so on, until the entire shell is filled with eight electrons, as is the case with neon.

Not all elements have enough electrons to fill their outermost shells, but an atom is at its most stable when all of the electron positions in the outermost shell are filled. Because of these vacancies in the outermost shells, we see the formation of chemical bonds, or interactions between two or more of the same or different elements that result in the formation of molecules. To achieve greater stability, atoms will tend to completely fill their outer shells and will bond with other elements to accomplish this goal by sharing electrons, accepting electrons from another atom, or donating electrons to another atom. Because the outermost shells of the elements with low atomic numbers (up to calcium, with atomic number 20) can hold eight electrons, this is referred to as the octet rule. An element can donate, accept, or share electrons with other elements to fill its outer shell and satisfy the octet rule.

When an atom does not contain equal numbers of protons and electrons, it is called an ion. Because the number of electrons does not equal the number of protons, each ion has a net charge. Positive ions are formed by losing electrons and are called cations. Negative ions are formed by gaining electrons and are called anions.

For example, sodium only has one electron in its outermost shell. It takes less energy for sodium to donate that one electron than it does to accept seven more electrons to fill the outer shell. If sodium loses an electron, it now has 11 protons and only 10 electrons, leaving it with an overall charge of +1. It is now called a sodium ion.

The chlorine atom has seven electrons in its outer shell. Again, it is more energy-efficient for chlorine to gain one electron than to lose seven. Therefore, it tends to gain an electron to create an ion with 17 protons and 18 electrons, giving it a net negative (–1) charge. It is now called a chloride ion. This movement of electrons from one element to another is referred to as electron transfer. As Figure 1.6.4 illustrates, a sodium atom (Na) only has one electron in its outermost shell, whereas a chlorine atom (Cl) has seven electrons in its outermost shell. A sodium atom will donate its one electron to empty its shell, and a chlorine atom will accept that electron to fill its shell, becoming chloride. Both ions now satisfy the octet rule and have complete outermost shells. Because the number of electrons is no longer equal to the number of protons, each is now an ion and has a +1 (sodium) or –1 (chloride) charge.

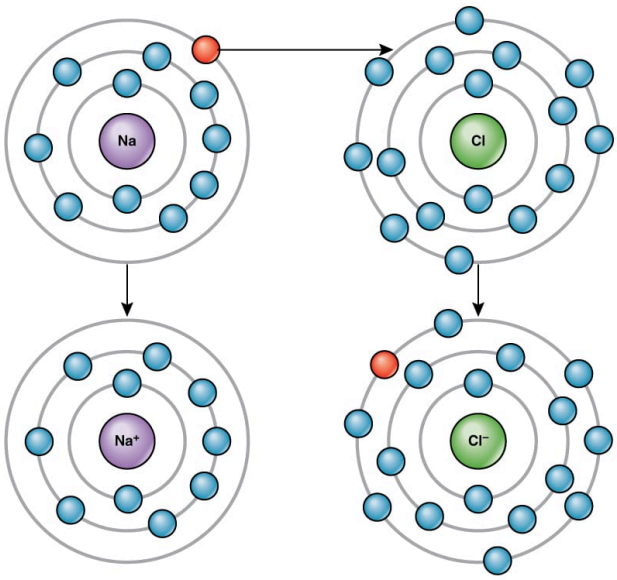


Figure 1.6.4: Elements tend to fill their outermost shells with electrons. To do this, they can either donate or accept electrons from other elements.

Ionic Bonds

There are four types of bonds or interactions: ionic, covalent, hydrogen bonds, and van der Waals interactions. Ionic and covalent bonds are strong interactions that require a larger energy input to break apart. When an element donates an electron from its outer shell, as in the sodium atom example above, a positive ion is formed. The element accepting the electron is now negatively charged. Because positive and negative charges attract, these ions stay together and form an ionic bond, or a bond between ions. The elements bond together with the electron from one element staying predominantly with the other element. When Na^+ and Cl^- ions combine to produce NaCl, an electron from a sodium atom stays with the other seven from the chlorine atom, and the sodium and chloride ions attract each other in a lattice of ions with a net zero charge.

Covalent Bonds

Another type of strong chemical bond between two or more atoms is a covalent bond. These bonds form when an electron is shared between two elements and are the strongest and most common form of chemical bond in living organisms. Covalent bonds form between the elements that make up the biological molecules in our cells. Unlike ionic bonds, covalent bonds do not dissociate in water.

The hydrogen and oxygen atoms that combine to form water molecules are bound together by covalent bonds. The electron from the hydrogen atom divides its time between the outer shell of the hydrogen atom and the incomplete outer shell of the oxygen atom. To completely fill the outer shell of an oxygen atom, two electrons from two hydrogen atoms are needed, hence the subscript "2" in H_2O. The electrons are shared between the atoms, dividing their time between them to "fill" the outer shell of each. This sharing is a lower energy state for all of the atoms involved than if they existed without their outer shells filled.

There are two types of covalent bonds: polar and nonpolar. Nonpolar covalent bonds form between two atoms of the same element or between different elements that share the electrons equally. For example, an oxygen atom can bond with another oxygen atom to fill their outer shells. This association is nonpolar because the electrons will be equally distributed between each oxygen atom. Two covalent bonds form between the two oxygen atoms because oxygen requires two shared electrons to fill its outermost shell. Nitrogen atoms will form three covalent bonds (also called triple covalent) between two atoms of nitrogen because each nitrogen atom needs three electrons to fill its outermost shell. Another example of a nonpolar covalent bond is found in the methane (CH_4) molecule. The carbon atom has four electrons in its outermost shell and needs four more to fill it. It gets these four from four hydrogen atoms, each atom providing one. These elements all share the electrons equally, creating four nonpolar covalent bonds (Figure 1.6.5).

In a polar covalent bond, the electrons shared by the atoms spend more time closer to one nucleus than to the other nucleus. Because of the unequal distribution of electrons between the different nuclei, a slightly positive ($\delta+$) or slightly negative ($\delta-$) charge develops. The covalent bonds between hydrogen and oxygen atoms in water are polar covalent bonds. The shared electrons spend more time near the oxygen nucleus, giving it a small negative charge, than they spend near the hydrogen nuclei, giving these molecules a small positive charge.

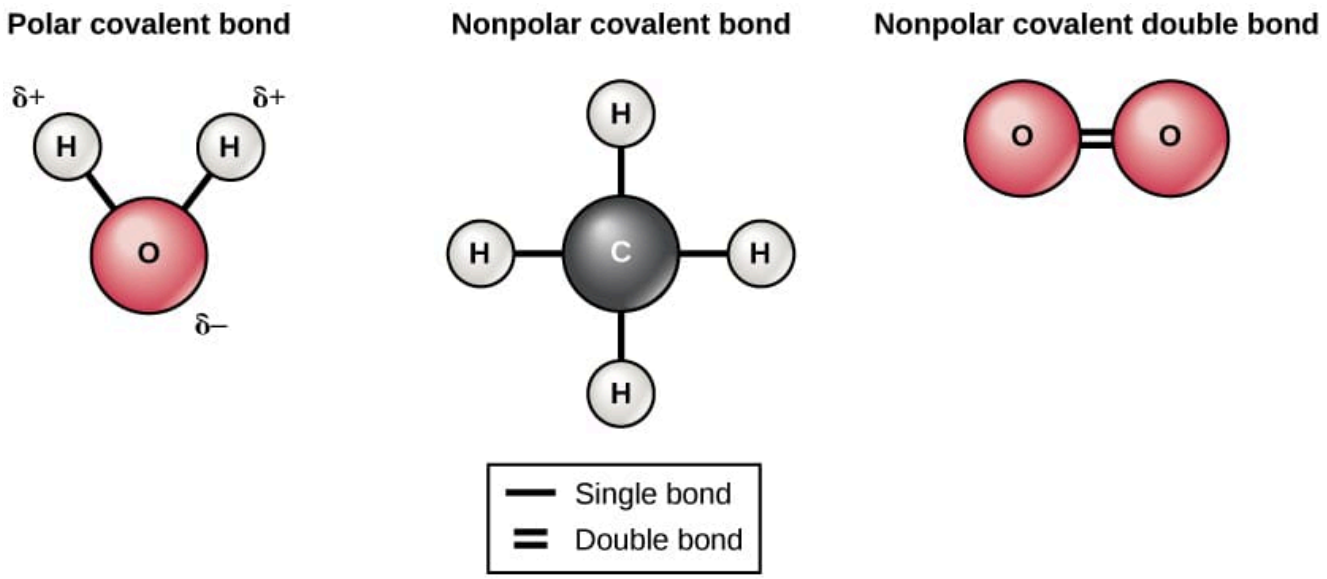


Figure 1.6.5: *The water molecule (left) depicts a polar bond with a slightly positive charge on the hydrogen atoms and a slightly negative charge on the oxygen. Examples of nonpolar bonds include methane (middle) and oxygen (right).*

Hydrogen Bonds

Ionic and covalent bonds are strong bonds that require considerable energy to break. However, not all bonds between elements are ionic or covalent bonds. Weaker bonds can also form. These are attractions that occur between positive and negative charges that do not require much energy to break. Two weak bonds that occur frequently are hydrogen bonds and van der Waals interactions. These bonds give rise to the unique properties of water and the unique structures of DNA and proteins.

When polar covalent bonds containing a hydrogen atom form, the hydrogen atom in that bond has a slightly positive charge. This is because the shared electron is pulled more strongly toward the other element and away from the hydrogen nucleus. Because the hydrogen atom is slightly positive (δ+), it will be attracted to neighboring negative partial charges (δ–). When this happens, a weak interaction occurs between the δ+ charge of the hydrogen atom of one molecule and the δ– charge of the other molecule. This interaction is called a hydrogen bond. This type of bond is common; for example, the liquid nature of water is caused by the hydrogen bonds between water molecules (Figure 1.6.6). Hydrogen bonds give water the unique properties that sustain life. If it were not for hydrogen bonding, water would be a gas rather than a liquid at room temperature.

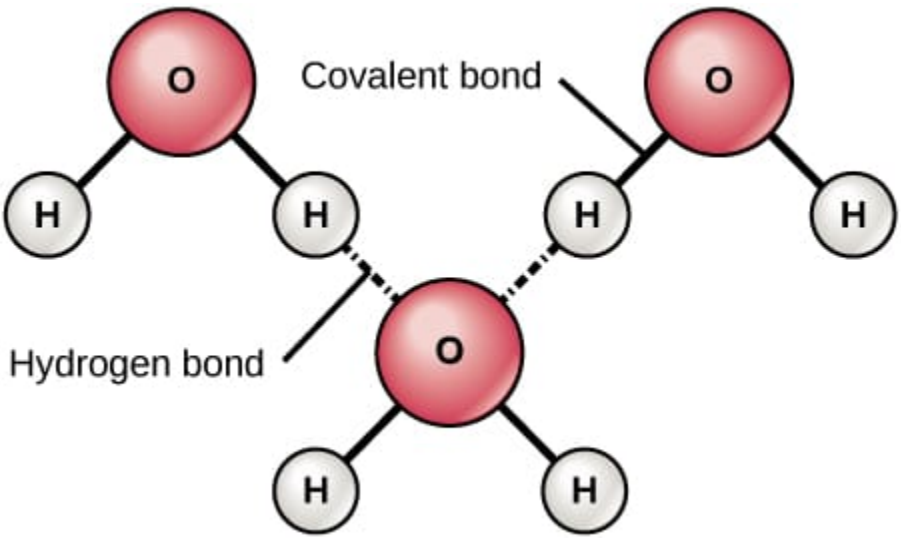


Figure 1.6.6: Hydrogen bonds form between slightly positive (δ+) and slightly negative (δ–) charges of polar covalent molecules, such as water.

Hydrogen bonds can form between different molecules and they do not always have to include a water molecule. Hydrogen atoms in polar bonds within any molecule can form bonds with other adjacent molecules. For example, hydrogen bonds hold together two

long strands of DNA to give the DNA molecule its characteristic double-stranded structure. Hydrogen bonds are also responsible for some of the three-dimensional structure of proteins.

van der Waals Interactions

Like hydrogen bonds, van der Waals interactions are weak attractions or interactions between molecules. They occur between polar, covalently bound, atoms in different molecules. Some of these weak attractions are caused by temporary partial charges formed when electrons move around a nucleus. These weak interactions between molecules are important in biological systems.

Matter is anything that occupies space and has mass. It is made up of atoms of different elements. All of the 92 elements that occur naturally have unique qualities that allow them to combine in various ways to create compounds or molecules. Atoms, which consist of protons, neutrons, and electrons, are the smallest units of an element that retain all of the properties of that element. Electrons can be donated or shared between atoms to create bonds, including ionic, covalent, and hydrogen bonds, as well as van der Waals interactions.

Glossary

anion
: a negative ion formed by gaining electrons

atomic number
: the number of protons in an atom

cation
: a positive ion formed by losing electrons

chemical bond
: an interaction between two or more of the same or different elements that results in the formation of molecules

covalent bond
: a type of strong bond between two or more of the same or different elements; forms when electrons are shared between elements

electron
: a negatively charged particle that resides outside of the nucleus in the electron orbital; lacks functional mass and has a charge of –1

electron transfer
: the movement of electrons from one element to another

element
: one of 118 unique substances that cannot be broken down into smaller substances and retain the characteristic of that substance; each element has a specified number of protons and unique properties

hydrogen bond
: a weak bond between partially positively charged hydrogen atoms and partially negatively charged elements or molecules

ion
: an atom or compound that does not contain equal numbers of protons and electrons, and therefore has a net charge

ionic bond
: a chemical bond that forms between ions of opposite charges

isotope
: one or more forms of an element that have different numbers of neutrons

mass number
: the number of protons plus neutrons in an atom

matter
: anything that has mass and occupies space

neutron
: a particle with no charge that resides in the nucleus of an atom; has a mass of 1

nonpolar covalent bond
: a type of covalent bond that forms between atoms when electrons are shared equally between atoms, resulting in no regions with partial charges as in polar covalent bonds

nucleus
: (chemistry) the dense center of an atom made up of protons and (except in the case of a hydrogen atom) neutrons

octet rule
: states that the outermost shell of an element with a low atomic number can hold eight electrons

periodic table of elements
: an organizational chart of elements, indicating the atomic number and mass number of each element; also provides key information about the properties of elements

polar covalent bond
: a type of covalent bond in which electrons are pulled toward one atom and away from another, resulting in slightly positive and slightly negative charged regions of the molecule

proton
: a positively charged particle that resides in the nucleus of an atom; has a mass of 1 and a charge of +1

radioactive isotope
: an isotope that spontaneously emits particles or energy to form a more stable element

van der Waals interaction
: a weak attraction or interaction between molecules caused by slightly positively charged or slightly negatively charged atoms

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 1.6: The Building Blocks of Molecules is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 2.1: The Building Blocks of Molecules by OpenStax is licensed CC BY 4.0.

2: INTRODUCTION TO BIOCHEMISTRY

2.1: Water
2.2: pH and Buffers
2.3: Functional groups and electronegativity
2.4: Types of Biological Molecules
2.5: Assembly of Biological Macromolecules

This page titled 2: Introduction to Biochemistry is shared under a license and was authored, remixed, and/or curated by LibreTexts.

2.1: Water

Do you ever wonder why scientists spend time looking for water on other planets? It is because water is essential to life; even minute traces of it on another planet can indicate that life could or did exist on that planet. Water is one of the more abundant molecules in living cells and the one most critical to life as we know it. Approximately 60–70 percent of your body is made up of water. Without it, life simply would not exist.

Water Is Polar

The hydrogen and oxygen atoms within water molecules form polar covalent bonds. The shared electrons spend more time associated with the oxygen atom than they do with hydrogen atoms. There is no overall charge to a water molecule, but there is a slight positive charge on each hydrogen atom and a slight negative charge on the oxygen atom. Because of these charges, the slightly positive hydrogen atoms repel each other and form the unique shape seen in Figure 2.1.6. Each water molecule attracts other water molecules because of the positive and negative charges in the different parts of the molecule. Water also attracts other polar molecules (such as sugars), forming hydrogen bonds. When a substance readily forms hydrogen bonds with water, it can dissolve in water and is referred to as hydrophilic ("water-loving"). Hydrogen bonds are not readily formed with nonpolar substances like oils and fats (*Figure* 2.1.1). These nonpolar compounds are hydrophobic ("water-fearing") and will not dissolve in water.



Figure 2.1.1: As this macroscopic image of oil and water show, oil is a nonpolar compound and, hence, will not dissolve in water. Oil and water do not mix. (credit: Gautam Dogra)

Water Stabilizes Temperature

The hydrogen bonds in water allow it to absorb and release heat energy more slowly than many other substances. Temperature is a measure of the motion (kinetic energy) of molecules. As the motion increases, energy is higher and thus temperature is higher. Water absorbs a great deal of energy before its temperature rises. Increased energy disrupts the hydrogen bonds between water molecules. Because these bonds can be created and disrupted rapidly, water absorbs an increase in energy and temperature changes only minimally. This means that water moderates temperature changes within organisms and in their environments. As energy input continues, the balance between hydrogen-bond formation and destruction swings toward the destruction side. More bonds are broken than are formed. This process results in the release of individual water molecules at the surface of the liquid (such as a body of water, the leaves of a plant, or the skin of an organism) in a process called evaporation. Evaporation of sweat, which is 90 percent water, allows for cooling of an organism, because breaking hydrogen bonds requires an input of energy and takes heat away from the body.

Conversely, as molecular motion decreases and temperatures drop, less energy is present to break the hydrogen bonds between water molecules. These bonds remain intact and begin to form a rigid, lattice-like structure (e.g., ice) (Figure 2.1.2**a**). When frozen, ice is less dense than liquid water (the molecules are farther apart). This means that ice floats on the surface of a body of water (Figure 2.1.2**b**). In lakes, ponds, and oceans, ice will form on the surface of the water, creating an insulating barrier to protect the animal and plant life beneath from freezing in the water. If this did not happen, plants and animals living in water would freeze in a block of ice and could not move freely, making life in cold temperatures difficult or impossible.

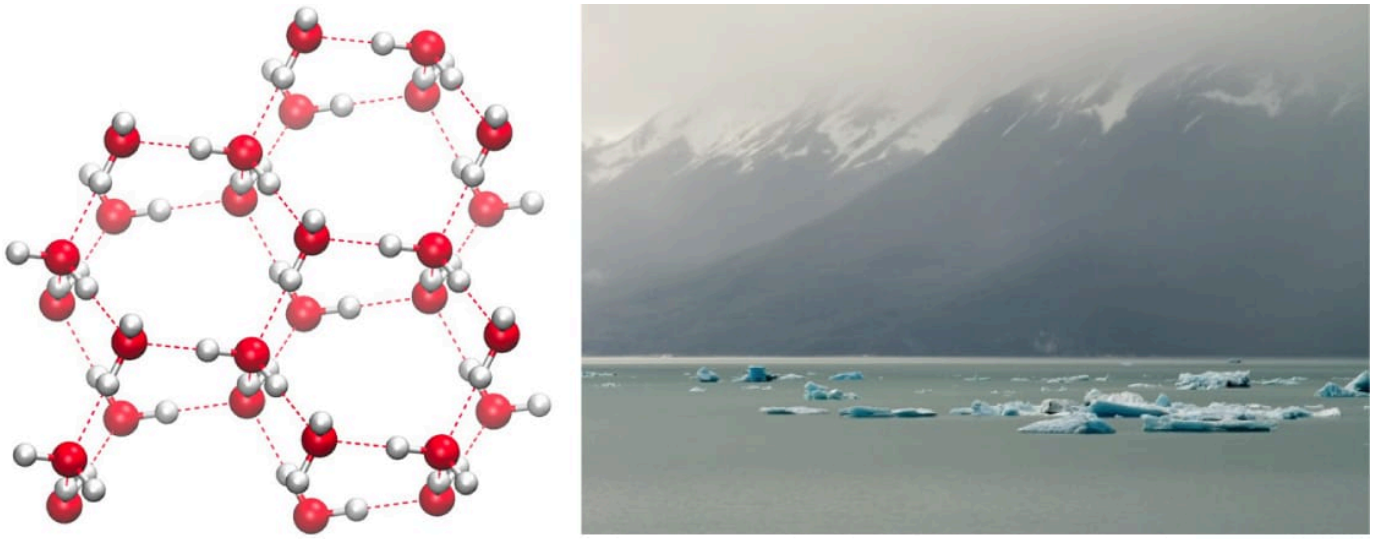


Figure 2.1.2: (a) The lattice structure of ice makes it less dense than the freely flowing molecules of liquid water. Ice's lower density enables it to (b) float on water. (credit a: modification of work by Jane Whitney; credit b: modification of work by Carlos Ponte)

CONCEPTS IN ACTION

Click here to see a 3-D animation of the structure of an ice lattice. (credit: image created by Jane Whitney using Visual Molecular Dynamics (VMD) software [1])

Water Is an Excellent Solvent

Because water is polar, with slight positive and negative charges, ionic compounds and polar molecules can readily dissolve in it. Water is, therefore, what is referred to as a solvent—a substance capable of dissolving another substance. The charged particles will form hydrogen bonds with a surrounding layer of water molecules. This is referred to as a sphere of hydration and serves to keep the particles separated or dispersed in the water. In the case of table salt (NaCl) mixed in water (Figure 2.1.3), the sodium and chloride ions separate, or dissociate, in the water, and spheres of hydration are formed around the ions. A positively charged sodium ion is surrounded by the partially negative charges of oxygen atoms in water molecules. A negatively charged chloride ion is surrounded by the partially positive charges of hydrogen atoms in water molecules. These spheres of hydration are also referred to as hydration shells. The polarity of the water molecule makes it an effective solvent and is important in its many roles in living systems.

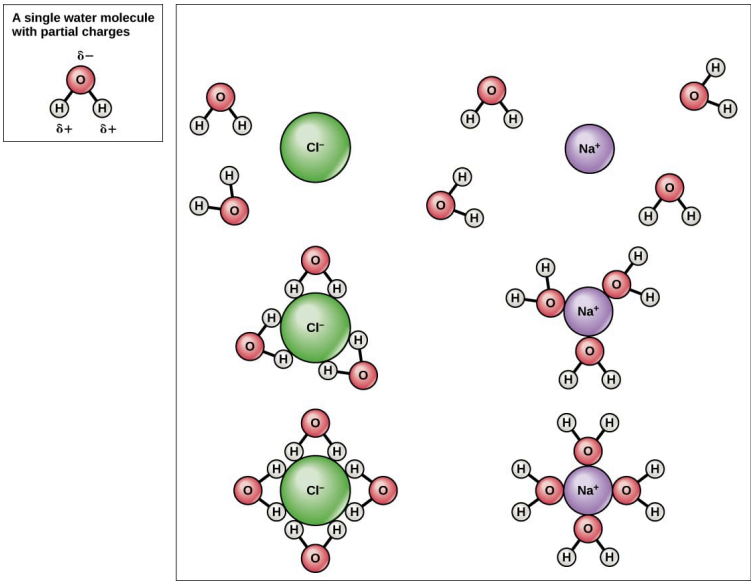


Figure 2.1.3: When table salt (NaCl) is mixed in water, spheres of hydration form around the ions.

Water Is Cohesive

Have you ever filled up a glass of water to the very top and then slowly added a few more drops? Before it overflows, the water actually forms a dome-like shape above the rim of the glass. This water can stay above the glass because of the property of cohesion. In cohesion, water molecules are attracted to each other (because of hydrogen bonding), keeping the molecules together at the liquid-air (gas) interface, although there is no more room in the glass. Cohesion gives rise to surface tension, the capacity of a substance to withstand rupture when placed under tension or stress. When you drop a small scrap of paper onto a droplet of water, the paper floats on top of the water droplet, although the object is denser (heavier) than the water. This occurs because of the surface tension that is created by the water molecules. Cohesion and surface tension keep the water molecules intact and the item floating on the top. It is even possible to “float” a steel needle on top of a glass of water if you place it gently, without breaking the surface tension (Figure 2.1.4).

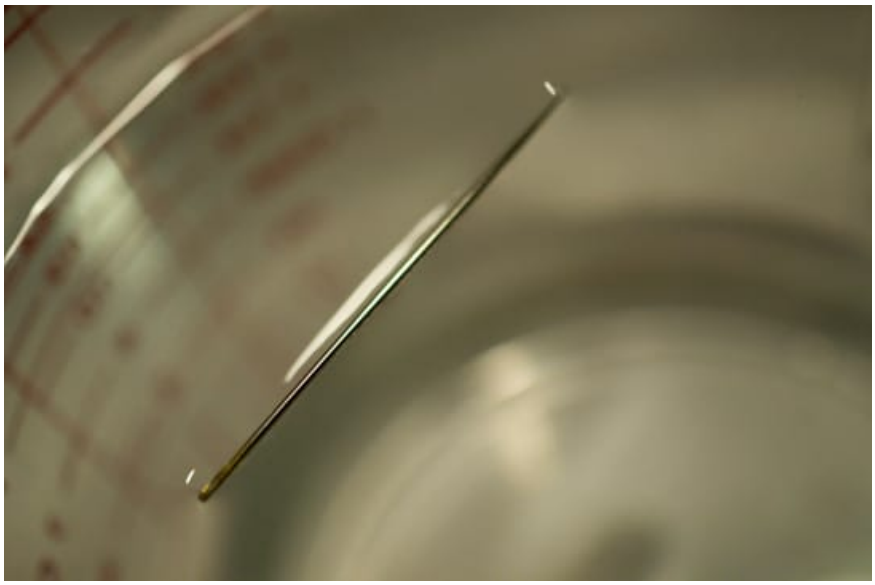


Figure 2.1.4: The weight of a needle on top of water pulls the surface tension downward; at the same time, the surface tension of the water is pulling it up, suspending the needle on the surface of the water and keeping it from sinking. Notice the indentation in the water around the needle. (credit: Cory Zanker)

These cohesive forces are also related to the water's property of adhesion, or the attraction between water molecules and other molecules. This is observed when water "climbs" up a straw placed in a glass of water. You will notice that the water appears to be higher on the sides of the straw than in the middle. This is because the water molecules are attracted to the straw and therefore adhere to it.

Cohesive and adhesive forces are important for sustaining life. For example, because of these forces, water can flow up from the roots to the tops of plants to feed the plant.

CONCEPT IN ACTION

To learn more about water, visit the U.S. Geological Survey Water Science for Schools: All About Water! website.

Buffers, pH, Acids, and Bases

The pH of a solution is a measure of its acidity or alkalinity. You have probably used litmus paper, paper that has been treated with a natural water-soluble dye so it can be used as a pH indicator, to test how much acid or base (alkalinity) exists in a solution. You might have even used some to make sure the water in an outdoor swimming pool is properly treated. In both cases, this pH test measures the amount of hydrogen ions that exists in a given solution. High concentrations of hydrogen ions yield a low pH, whereas low levels of hydrogen ions result in a high pH. The overall concentration of hydrogen ions is inversely related to its pH and can be measured on the pH scale (Figure 2.1.5). Therefore, the more hydrogen ions present, the lower the pH; conversely, the fewer hydrogen ions, the higher the pH.

The pH scale ranges from 0 to 14. A change of one unit on the pH scale represents a change in the concentration of hydrogen ions by a factor of 10, a change in two units represents a change in the concentration of hydrogen ions by a factor of 100. Thus, small changes in pH represent large changes in the concentrations of hydrogen ions. Pure water is neutral. It is neither acidic nor basic, and has a pH of 7.0. Anything below 7.0 (ranging from 0.0 to 6.9) is acidic, and anything above 7.0 (from 7.1 to 14.0) is alkaline. The blood in your veins is slightly alkaline (pH = 7.4). The environment in your stomach is highly acidic (pH = 1 to 2). Orange juice is mildly acidic (pH = approximately 3.5), whereas baking soda is basic (pH = 9.0).

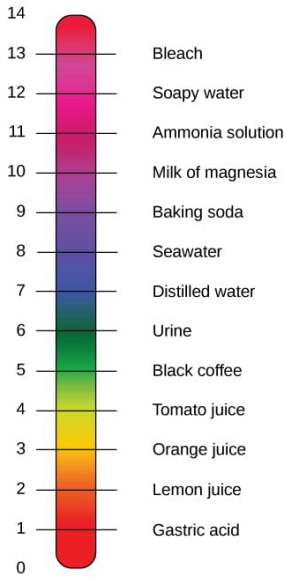


Figure 2.1.5: The pH scale measures the amount of hydrogen ions (H^+) in a substance. (credit: modification of work by Edward Stevens)

Acids are substances that provide hydrogen ions (H^+) and lower pH, whereas bases provide hydroxide ions (OH^-) and raise pH. The stronger the acid, the more readily it donates H^+. For example, hydrochloric acid and lemon juice are very acidic and readily give up H^+ when added to water. Conversely, bases are those substances that readily donate OH^-. The OH^- ions combine with H^+ to produce water, which raises a substance's pH. Sodium hydroxide and many household cleaners are very alkaline and give up OH^- rapidly when placed in water, thereby raising the pH.

Most cells in our bodies operate within a very narrow window of the pH scale, typically ranging only from 7.2 to 7.6. If the pH of the body is outside of this range, the respiratory system malfunctions, as do other organs in the body. Cells no longer function properly, and proteins will break down. Deviation outside of the pH range can induce coma or even cause death.

So how is it that we can ingest or inhale acidic or basic substances and not die? Buffers are the key. Buffersreadily absorb excess H^+ or OH^-, keeping the pH of the body carefully maintained in the aforementioned narrow range. Carbon dioxide is part of a prominent buffer system in the human body; it keeps the pH within the proper range. This buffer system involves carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) anion. If too much H^+ enters the body, bicarbonate will combine with the H^+ to create carbonic acid and limit the decrease in pH. Likewise, if too much OH^- is introduced into the system, carbonic acid will rapidly dissociate into bicarbonate and H^+ ions. The H^+ ions can combine with the OH^- ions, limiting the increase in pH. While carbonic acid is an important product in this reaction, its presence is fleeting because the carbonic acid is released from the body as carbon dioxide gas each time we breathe. Without this buffer system, the pH in our bodies would fluctuate too much and we would fail to survive.

Summary

Water has many properties that are critical to maintaining life. It is polar, allowing for the formation of hydrogen bonds, which allow ions and other polar molecules to dissolve in water. Therefore, water is an excellent solvent. The hydrogen bonds between water molecules give water the ability to hold heat better than many other substances. As the temperature rises, the hydrogen bonds between water continually break and reform, allowing for the overall temperature to remain stable, although increased energy is added to the system. Water's cohesive forces allow for the property of surface tension. All of these unique properties of water are important in the chemistry of living organisms.

The pH of a solution is a measure of the concentration of hydrogen ions in the solution. A solution with a high number of hydrogen ions is acidic and has a low pH value. A solution with a high number of hydroxide ions is basic and has a high pH value. The pH scale ranges from 0 to 14, with a pH of 7 being neutral. Buffers are solutions that moderate pH changes when an acid or base is added to the buffer system. Buffers are important in biological systems because of their ability to maintain constant pH conditions.

Footnotes

1. 1 Humphrey, W., Dalke, A. and Schulten, K., "VMD—Visual Molecular Dynamics", *J. Molec. Graphics*, 1996, vol. 14, pp. 33-38. http://www.ks.uiuc.edu/Research/vmd/

Glossary

acid
: a substance that donates hydrogen ions and therefore lowers pH

adhesion
: the attraction between water molecules and molecules of a different substance

base
: a substance that absorbs hydrogen ions and therefore raises pH

buffer
: a solution that resists a change in pH by absorbing or releasing hydrogen or hydroxide ions

cohesion
: the intermolecular forces between water molecules caused by the polar nature of water; creates surface tension

evaporation
: the release of water molecules from liquid water to form water vapor

hydrophilic
: describes a substance that dissolves in water; water-loving

hydrophobic
: describes a substance that does not dissolve in water; water-fearing

litmus paper
: filter paper that has been treated with a natural water-soluble dye so it can be used as a pH indicator

pH scale
: a scale ranging from 0 to 14 that measures the approximate concentration of hydrogen ions of a substance

solvent
: a substance capable of dissolving another substance

surface tension
: the cohesive force at the surface of a body of liquid that prevents the molecules from separating

temperature
: a measure of molecular motion

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 2.1: Water is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 2.2: Water by OpenStax is licensed CC BY 4.0.

2.2: pH and Buffers

Source: BiochemFFA_1_3.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Water organization

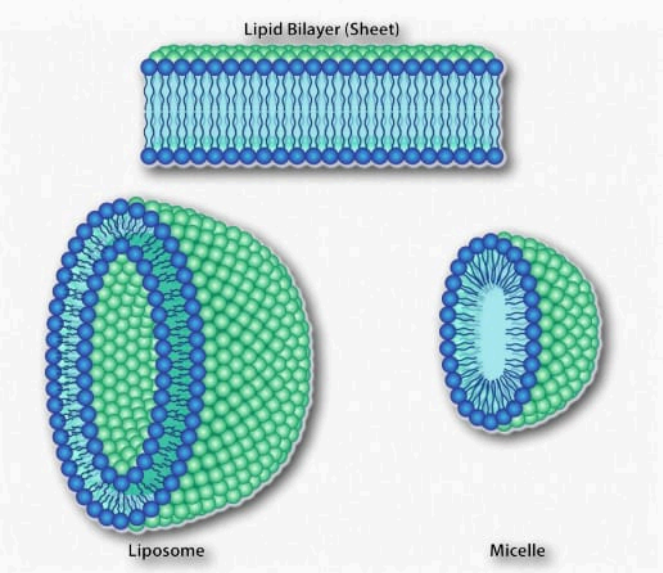


Figure 1.25 - Structures formed by amphiphilic substances in water. Image by Aleia Kim

There is a significant difference, though between surrounding a non-polar molecule with water molecules and surrounding ions (or polar compounds) with water molecules.

The difference is that since non-polar molecules don't really interact with water, the water behaves very differently than it does with ions or molecules that form hydrogen bonds. In fact, around each non-polar molecule, water gets very organized, aligning itself regularly. As any freshman chemistry student probably remembers, entropy is a measure of disorder, so when something becomes ordered, entropy decreases, meaning the ΔS is negative, so the $T\Delta S$ term in the equation is positive (negative of a negative).

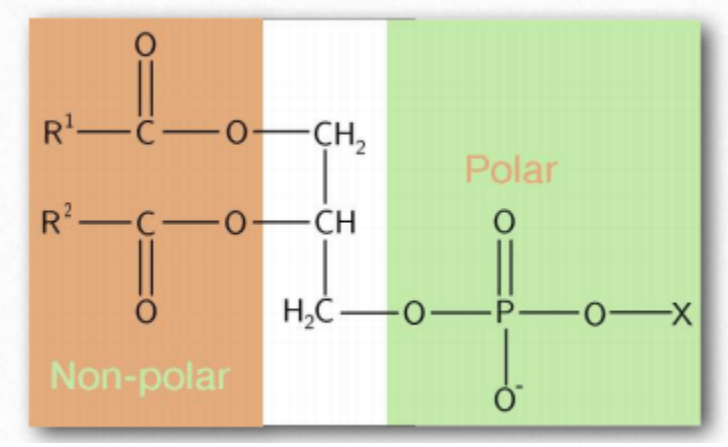


Figure 1.26 - A phospholipid - an amphiphilic substance

Since mixing a non-polar substance with water doesn't generally have any significant heat component, the ΔG is positive. This means, then, that dissolving a non-polar compound in water is not favorable and does not occur to any significant extent. Further,

when the non-polar material associates with itself and not water, then the water molecules are free to mix, without being ordered, resulting in an increase of entropy. Entropy therefore drives the separation of non-polar substances from aqueous solutions.



Figure 1.27 - Vinegar (black) and oil (yellow) A mix of polar and nonpolar compounds Wikipedia

Amphiphilic substances

Next, we consider mixing of an amphiphilic substance, such as a soap, with water (Figure 1.24). The sodium ions attached to the fatty acids in soap readily come off in aqueous solution, leaving behind a negatively charged molecule at one end and a non-polar region at the other end. The ionization of the soap causes in an increase in entropy - two particles instead of one. The non-polar portion of the negatively charged soap ion is problematic - if exposed to water, it will cause water to organize and result in a decrease of entropy and a positive ΔG.

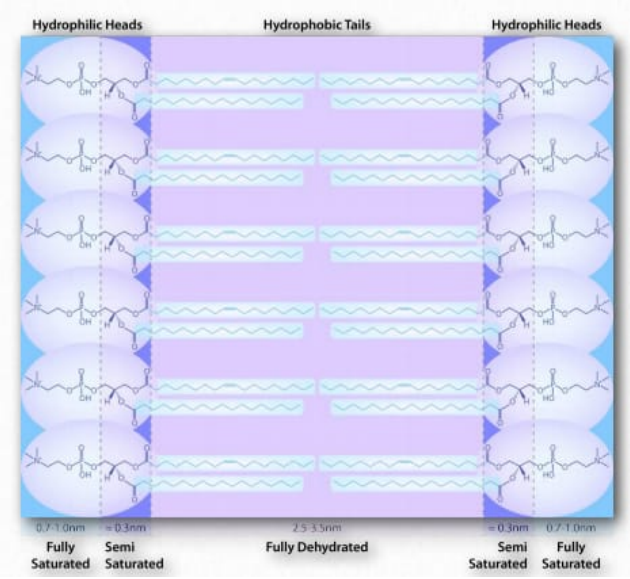


Figure 1.28 - Environment of a lipid bilayer. Water is concentrated away from the hydrophobic center, being saturated on the outside, semi-saturated near the head-tail junction and fully dehydrated in the middle. Image by Aleia Kim

Since we know fatty acids dissolve in water, there must be something else at play. There is. Just like the non-polar molecules in the first example associated with each other and not water, so too do the non-polar portions of the soap ions associate with each other and exclude water. The result is that the soap ions arrange themselves as micelles (Figure 1.25) with the non-polar portions on the interior of the structure away from water and the polar portions on the outside interacting with water.

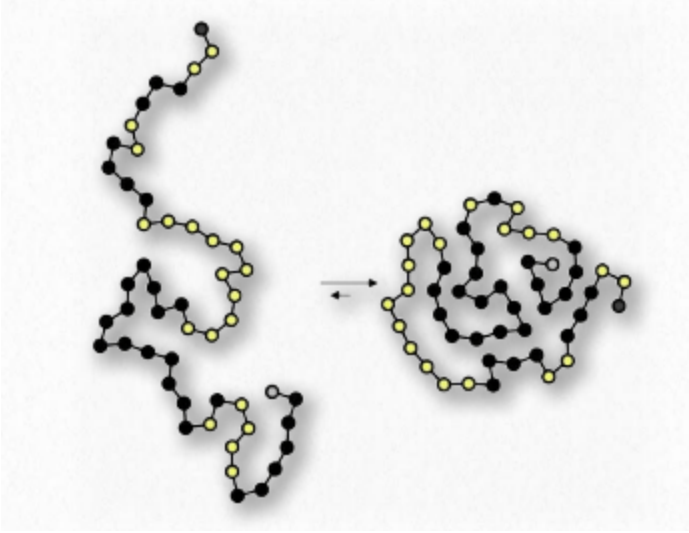


Figure 1.29 - Protein folding arranges hydrophobic amino acids (black dots) inside the protein

The interaction of the polar heads with water returns the water to its more disordered state. This increase in disorder, or entropy, drives the formation of micelles. As will be seen in the discussion of the lipid bilayer, the same forces drive glycerophospholipids and sphingolipids to spontaneously form bilayers where the non-polar portions of the molecules interact with each other to exclude water and the polar portions arrange themselves on the outsides of the bilayer (Figure 1.28).

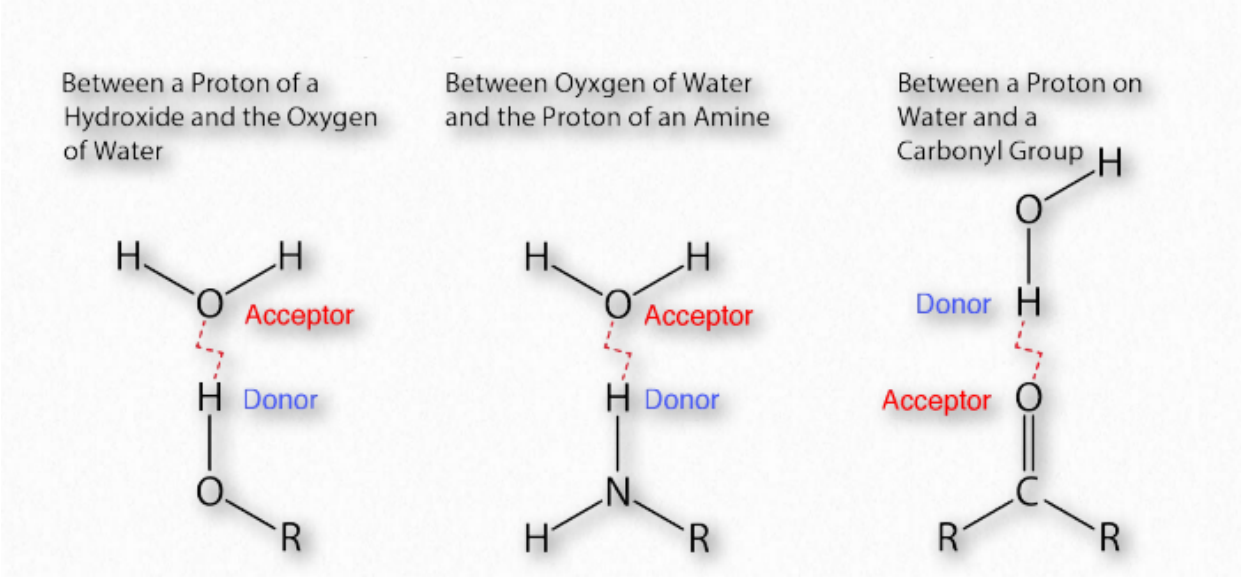


Figure 1.30 - Common hydrogen bonds in biochemistry Image by Aleia Kim

Yet another example is seen in the folding of globular proteins in the cytoplasm. Nonpolar amino acids are found in the interior portion of the protein (water excluded). Interaction of the non-polar amino acids turns out to be a driving force for the folding of proteins as they are being made in an aqueous solution.

Hydrogen bonds

The importance of hydrogen bonds in biochemistry (Figure 1.30) is hard to overstate. Linus Pauling himself said,

" . . . . I believe that as the methods of structural chemistry are further applied to physiological problems it will be found that the significance of the hydrogen bond for physiology is greater than that of any other single structural feature."

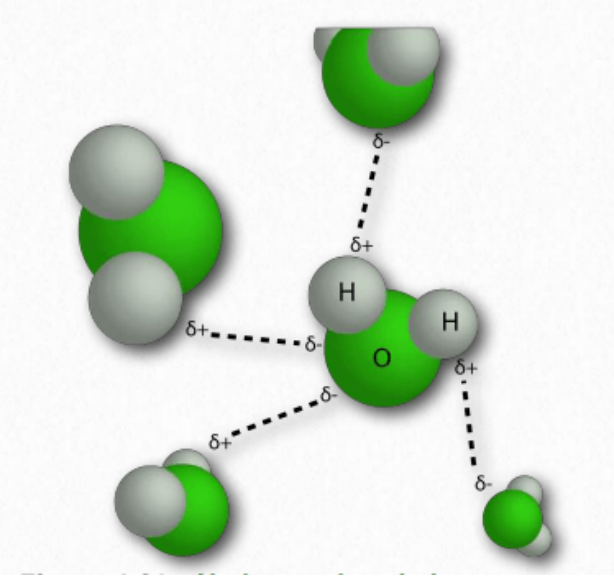


Figure 1.31 - Hydrogen bonds between water molecules Image by Pehr Jacobson

In 2011, an IUPAC task group gave an evidence-based definition of hydrogen bonding that states,

"The hydrogen bond is an attractive interaction between a hydrogen atom from a molecule or a molecular fragment X–H in which X is more electronegative than H, and an atom or a group of atoms in the same or a different molecule, in which there is evidence of bond formation."

Partial Charges

The difference in electronegativity between hydrogen and the molecule to which it is covalently bound give rise to partial charges as described above. These tiny charges (δ+ and δ-) result in formation of hydrogen bonds, which occur when the partial positive charge of a hydrogen atom is attracted to the partial negative of another molecule. In water, that means the hydrogen of one water molecule is attracted to the oxygen of another (Figure 1.31). Since water is an asymmetrical molecule, it means also that the charges are asymmetrical. Such an uneven distribution is what makes a dipole. Dipolar molecules are important for interactions with other dipolar molecules and for dissolving ionic substances (Figure 1.32).

Hydrogen bonds are not exclusive to water. In fact, they are important forces holding together macromolecules that include proteins and nucleic acids. Hydrogen bonds occur within and between macromolecules.

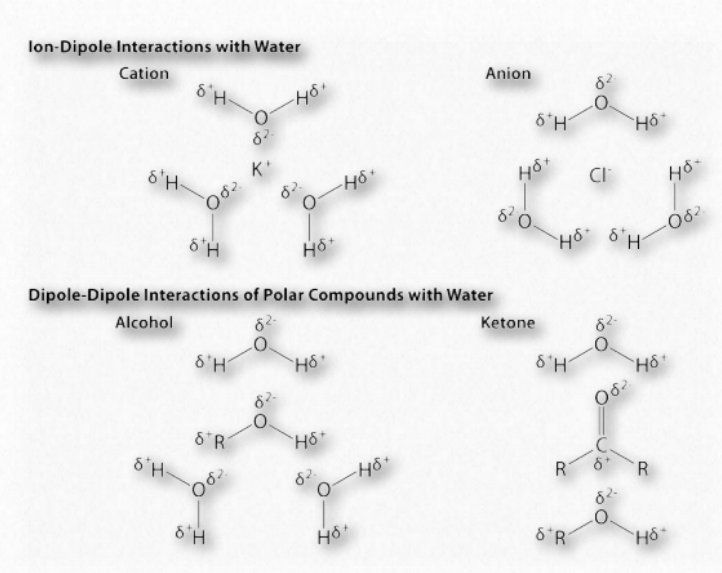


Figure 1.32 - Example dipole interactions in biochemistry Image by Aleia Kim

The complementary pairing that occurs between bases in opposite strands of DNA, for example, is based on hydrogen bonds. Each hydrogen bond is relatively weak (compared to a covalent bond, for example - Table 1.4), but collectively they can be quite strong.

Bond Energies

	Type of Bond	Bond Energy (kJ/mol)
Covalent Bonds	C—H	413
	O—H	460
Noncovalent Bonds	Hydrophobic Interaction	4-12
	Hydrogen Bond	20
	Ion-dipole Interaction	20

Table 1.4 Image by Aleia Kim

Benefits of weak interactions

Their weakness, however, is actually quite beneficial for cells, particularly as regards nucleic acids (Figure 1.33). The strands of DNA, for example, must be separated over short stretches in the processes of replication and the synthesis of RNA. Since only a few base pairs at a time need to be separated, the energy required to do this is small and the enzymes involved in the processes can readily take them apart, as needed. Hydrogen bonds also play roles in binding of substrates to enzymes, catalysis, and protein-protein interaction, as well as other kinds of binding, such as protein-DNA, or antibody-antigen.

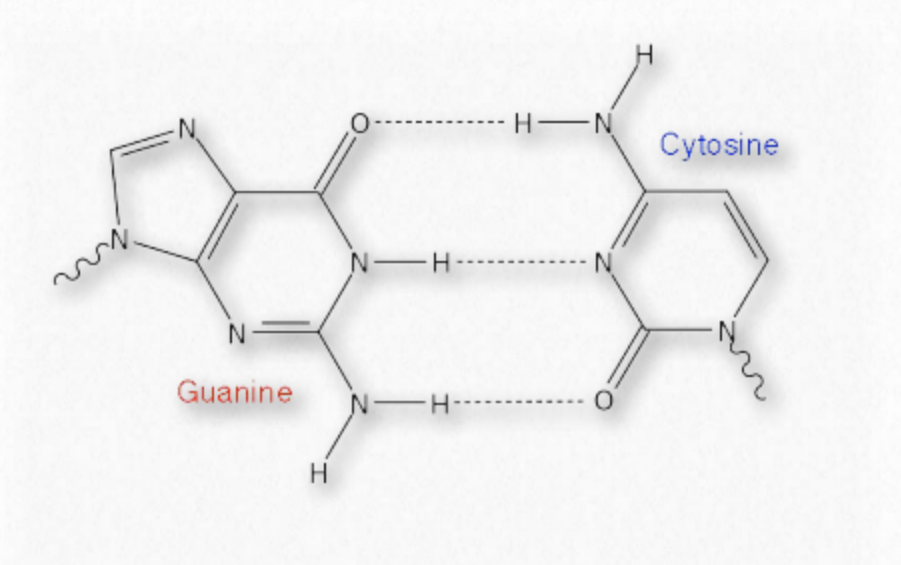


Figure 1.33 - Hydrogen bonds in a base pair of DNA Image by Aleia Kim

As noted, hydrogen bonds are weaker than covalent bonds (Table 1.4) and their strength varies form very weak (1-2 kJ/mol) to fairly strong (29 kJ/mol). Hydrogen bonds only occur over relatively short distances (2.2 to 4.0 Å). The farther apart the hydrogen bond distance is, the weaker the bond is.

The strength of the bond in kJ/mol represents the amount of heat that must be put into the system to break the bond - the larger the number, the greater the strength of the bond. Hydrogen bonds are readily broken using heat. The boiling of water, for example, requires breaking of H-bonds. When a biological structure, such as a protein or a DNA molecule, is stabilized by hydrogen bonds, breaking those bonds destabilizes the structure and can result in denaturation of the substance - loss of structure. It is partly for this reason that most proteins and all DNAs lose their native, or folded, structures when heated to boiling.

Weak Acid pKa Values

Name	Chemical Structure of Acid	Chemical Structure of Salt	pKa
Acetic Acid	CH_3COOH	CH_3COO^-	4.76
Formic Acid	$HCOOH$	$HCOO^-$	3.75
Lactic Acid	$CH_3CHOHCOOH$	$CH_3CH—HCOO^-$	3.86
Pyruvic Acid	$CH_3COCOOH$	$CH_3C—COO^-$	2.50
Oxalic Acid (1)	$HOOC—COOH$	$HOOC—COO^-$	1.23
Oxalic Acid (2)	$HOOC—COO^-$	$OOC—COO^-$	4.19
Carbonic Acid (1)	H_2CO_3	HCO_3^-	6.37
Carbonic Acid (2)	HCO_3^-	CO_3^{2-}	10.20
Malic Acid (1)	$HOOC—CH_2—CHOH—COOH$	$HOOC—CH_2—CHOH—COO^-$	3.40
Malic Acid (2)	$HOOC—CH_2—CHOH—COO^-$	$^-OOC—CH_2—CHOH—COO^-$	5.26
Malonic Acid (1)	$HOOC—CH_2—COOH$	$HOOC—CH_2—COO^-$	2.83
Malonic Acid (2)	$HOOC—CH_2—COO^-$	$^-OOC—CH_2—COO^-$	5.69
Phosphoric Acid (1)	H_3PO_4	$H_2PO_4^-$	2.14
Phosphoric Acid (2)	$H_2PO_4^-$	HPO_4^{2-}	7.20
Phosphoric Acid (3)	HPO_4^{2-}	PO_4^{3-}	12.40
Succinic Acid (1)	$HOOC—CH_2—CH_2—COOH$	$HOOC—CH_2—CH_2—COO^-$	4.21
Succinic Acid (2)	$HOOC—CH_2—CH_2—COO^-$	$^-OOC—CH_2—CH_2—COO^-$	5.63

Image by Aleia Kim Table 1.5

For DNA molecules, denaturation results in complete separation of the strands from each other. For most proteins, this means loss of their characteristic three-dimensional structure and with it, loss of the function they performed. Though a few proteins can readily reassume their original structure when the solution they are in is cooled, most can't. This is one of the reasons that we cook our food. Proteins are essential for life, so denaturation of bacterial proteins results in death of any microorganisms contaminating the food.

The importance of buffers

Water can ionize to a slight extent (10-7 M) to form H+ (proton) and OH- (hydroxide). We measure the proton concentration of a solution with pH, which is the negative log of the proton concentration.

$$pH = -Log[H+]$$

If the proton concentration, [H+]= 10-7 M, then the pH is 7. We could just as easily measure the hydroxide concentration with the pOH by the parallel equation,

$$pOH = -Log[OH^-]$$

In pure water, dissociation of a proton simultaneously creates a hydroxide, so the pOH of pure water is 7, as well. This also means that

$$pH + pOH = 14$$

Now, because protons and hydroxides can combine to form water, a large amount of one will cause there to be a small amount of the other. Why is this the case? In simple terms, if I dump 0.1 moles of H+ into a pure water solution, the high proton concentration will react with the relatively small amount of hydroxides to create water, thus reducing hydroxide concentration. Similarly, if I dump excess hydroxide (as NaOH, for example) into pure water, the proton concentration falls for the same reason.

Acids vs bases

Chemists use the term “acid” to refer to a substance which has protons that can dissociate (come off) when dissolved in water. They use the term “base” to refer to a substance that can absorb protons when dissolved in water. Both acids and bases come in strong and weak forms. (Examples of weak acids are shown in Table 1.5.) Strong acids, such as HCl, dissociate completely in water. If we add 0.1 moles (6.02x1022 molecules) of HCl to a solution to make a liter, it will have 0.1 moles of H+ and 0.1 moles of Cl- or 6.02x1022 molecules of each . There will be no remaining HCl when this happens. A strong base like NaOH also dissociates completely into Na+ and OH- .

Clearing Confusion - Students are often puzzled and expect that

$$[H^+] = [A^-]$$

because the dissociation equation shows one of each from HA. This is, in fact, true ONLY when HA is allowed to dissociate in pure water. Usually the HA is placed into solution that has protons and hydroxides to affect things. Those protons and /or hydroxides change the H^+ and A^- concentration unequally, since A^- can absorb some of the protons and/or HA can release H^+ when influenced by the OH^- in the solution. Therefore, one must calculate the proton concentration from the pH using the Henderson Hasselbalch equation.

$$pH = pK_a + \log([Ac^-]/[HAc])$$

Weak Acids

Weak acids and bases differ from their strong counterparts. When you put one mole of acetic acid (HAc) into pure water, only a tiny percentage of the HAc molecules dissociate into H+ and Ac- . Clearly, weak acids are very different from strong acids. Weak bases behave similarly, except that they accept protons, rather than donate them. Since we can view everything as a form of a weak acid, we will not use the term weak base here.

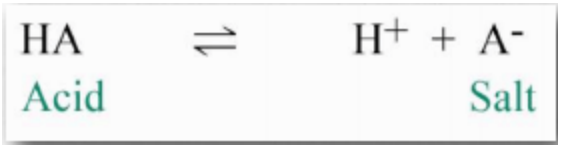


Figure 1.34 - Dissociation of a weak acid Image by Aleia Kim

Students are often puzzled and expect that $[H^+] = [A^-]$ because the dissociation equation shows one of each from HA. This is, in fact, true ONLY when HA is allowed to dissociate in pure water. Usually the HA is placed into solution that has protons and hydroxides to affect things. Those protons and /or hydroxides change the H+ and Aconcentration unequally, since A- can absorb some of the protons and/or HA can release H+ when influenced by the OH- in the solution. Therefore, one must calculate the proton concentration from the pH using the Henderson Hasselbalch equation.

$$pH = pKa + \log ([Ac^-]/[HAc])$$

Salt/Acid Ratio as a Function of pK_as

pH	[Salt]/[Acid]
$pK_a + 3$	1000
$pK_a + 2$	100
$pK_a + 1$	10
pK_a	1
$pK_a - 1$	1/10
$pK_a - 2$	1/100
$pK_a - 3$	1/1000

Image by Aleia Kim Table 1.6

You may wonder why we care about weak acids. You may never have thought much of weak acids when you were in General Chemistry. Your instructor described them as buffers and you probably dutifully memorized the fact that “buffers are substances that resist change in pH” without really learning what Clearing Confusion - this meant. Buffers are much too important to be thought of in this way.

UPS

Weak acids are critical for life because their affinity for protons causes them to behave like a UPS. We’re not referring to the UPS that is the United Parcel Service, but instead, to the encased battery backup systems for computers called Uninterruptible Power Supplies that kick on to keep a computer running during a power failure. The battery in a laptop computer is a UPS, for example.

We can think of weak acids as Uninterruptible Proton Suppliers within certain pH ranges, providing (or absorbing) protons as needed. Weak acids thus help to keep the H+ concentration (and thus the pH) of the solution they are in relatively constant.

Consider the bicarbonate/carbonic acid system. Figure 1.35 shows what happens when H_2CO_3dissociates. Adding hydroxide ions (by adding a strong base like NaOH) to the solution causes the H+ ions to react with OH- ions to make water. Consequently, the concentration of H+ ions would go down and the pH would go up.

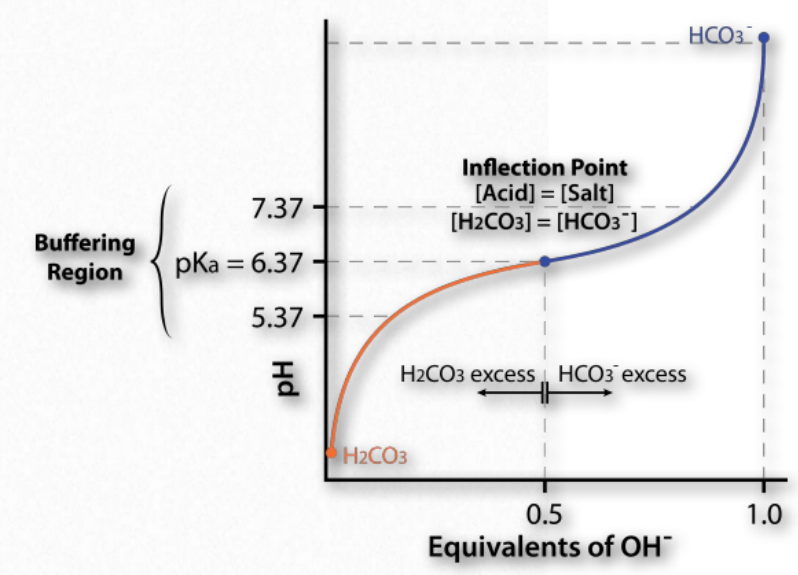


Figure 1.35 - Titration curve for carbonic acid Image by Aleia Kim

However, in contrast to the situation with a solution of pure water, there is a backup source of H+ available in the form of H_2CO_3. Here is where the UPS function kicks in. As protons are taken away by the added hydroxyl ions (making water), they are partly replaced by protons from the H_2CO_3. This is why a weak acid is a buffer. It resists changes in pH by releasing protons to compensate for those “used up” in reacting with the hydroxyl ions.

Why do we care about pH? Because biological molecules can, in some cases, be exquisitely sensitive to changes in it. As the pH of a solution changes, the charges of molecules in the solution can change, as you will see. Changing charges on biological molecules, especially proteins, can drastically affect how they work and even whether they work at all.

Henderson-Hasselbalch

It is useful to be able to predict the response of the H_2CO_3 system to changes in H+ concentration. The Henderson-Hasselbalch equation defines the relationship between pH and the ratio of HCO_3^- and H_2CO_3. It is

$$pH = pKa + \log([HCO_3^-]/[H_2CO_3])$$

This simple equation defines the relationship between the pH of a solution and the ratio of HCO_3^- and H_2CO_3 in it. The new term, called the pKa, is defined as

$$pKa = -\text{Log } K_a,$$

just as

$$pH = -Log\ [H^+].$$

The Ka is the acid dissociation constant and is a measure of the strength of an acid. For a general acid, HA, which dissociates as

$$HA \rightleftarrows H^+ + A^-,\ K_a = [H^+][A^-]/[HA]$$

Thus, the stronger the acid, the more protons that will dissociate from it when added to water and the larger the value its Ka will have. Large values of Ka translate to lower values of pKa. As a result, the lower the pKa value is for a given acid, the stronger the weak acid is.

Constant pKa

Please note that pKa is a constant for a given acid. The pKa for carbonic acid is 6.37. By comparison, the pKa for formic acid is 3.75. Formic acid is therefore a stronger acid than acetic acid. A stronger acid will have more protons dissociated at a given pH than a weaker acid.

Now, how does this translate into stabilizing pH? Figure 1.35 shows a titration curve. In this curve, the titration begins with the conditions at the lower left (very low pH). At this pH, the H_2CO_3 form predominates, but as more and more OH- is added (moving to the 45 Why do we care about pH? Because biological molecules can, in some cases, be exquisitely sensitive to changes in it. As the pH of a solution changes, the charges of molecules in the solution can change, as you will see. Changing charges on biological molecules, especially proteins, can drastically affect how they work and even whether they work at all right), the pH goes up, the amount of HCO3- goes up and (correspondingly), the amount of H_2CO_3 goes down. Notice that the curve “flattens” near the pKa (6.37).

Buffering region

Flattening of the curve tells us is that the pH is not changing much (not going up as fast) as it did earlier when the same amount of hydroxide was added. The system is resisting a change in pH (not stopping the change, but slowing it) in the region of about one pH unit above and one pH unit below the pKa. Thus, the buffering region of the carbonic acid/ bicarbonate buffer is from about 5.37 to 7.37. It is maximally strong at a pH of 6.37.

Now it starts to become apparent how the buffer works. HA can donate protons when extras are needed (such as when OH- is added to the solution by the addition of NaOH). Similarly, A- can accept protons when extra H+ are added to the solution (adding HCl, for example). The maximum ability to donate or accept protons comes when

$$[A^-] = [HA]$$

This is consistent with the Henderson Hasselbalch equation and the titration curve. When $[A^-] = [HA]$, pH = 6.37 + Log(1). Since Log(1) = 0, pH = 6.37 = pKa for carbonic acid. Thus for any buffer, the buffer will have maximum strength and display flattening of its titration curve when $[A^-] = [HA]$ and when pH = pKa. If a buffer has more than one pKa (Figure 1.36), then each pKa region will display the behavior.

Buffered vs non-buffered

To understand how well a buffer protects against changes in pH, consider the effect of adding .01 moles of HCl to 1.0 liter of pure water (no volume change) at pH 7, compared to adding it to 1.0 liter of a 1M acetate buffer at pH 4.76. Since HCl completely dissociates, in 0.01M (10^{-2} M) HCl you will have 0.01M H+. For the pure water, the pH drops from 7.0 down to 2.0 (pH = -log(0.01M)).

By contrast, the acetate buffer’s pH after adding the same amount of HCl is 4.74. Thus, the pure water solution sees its pH fall from 7 to 2 (5 pH units), whereas the buffered solution saw its pH drop from 4.76 to 4.74 (0.02 pH units). Clearly, the buffer minimizes the impact of the added protons compared to the pure water.

Buffer capacity

It is important to note that buffers have capacities limited by their concentration. Let’s imagine that in the previous paragraph, we

had added the 0.01 moles HCl to an acetate buffer that had a concentration of 0.01M and equal amounts of Ac- and HAc. When we try to do the math in parallel to the previous calculation, we see that there are 0.01M protons, but only 0.005M A- to absorb them. We could imagine that 0.005M of the protons would be absorbed, but that would still leave 0.005M of protons unbuffered. Thus, the pH of this solution would be approximately

$$pH = -\log(0.005M) = 2.30$$

Exceeding buffer capacity dropped the pH significantly compared to adding the same amount of protons to a 1M acetate buffer. Consequently, when considering buffers, it is important to recognize that their concentration sets their limits. Another limit is the pH range in which one hopes to control proton concentration.

Multiple ionizable groups

Now, what happens if a molecule has two (or more) ionizable groups? It turns out, not surprisingly, that each group will have its own pKa and, as a consequence, will have multiple regions of buffering.

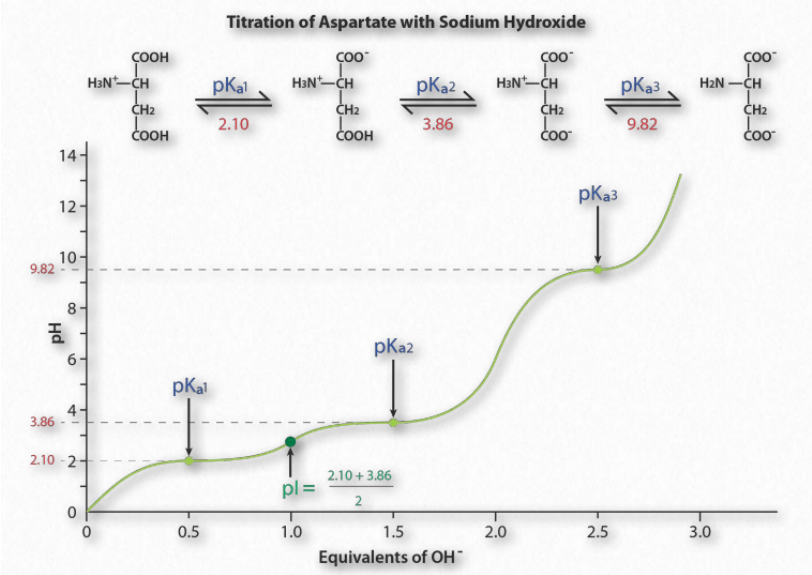


Figure 1.36 - Titration of an acidic amino acid Image by Aleia Kim

Figure 1.36 shows the titration curve for the amino acid aspartic acid. Note that in- stead of a single flattening of the curve, as was seen for acetic acid, aspartic acid's titration curve displays three such regions. These are individual buffering regions, each centered on the respective pKa values for the carboxyl group and the amine group.

Aspartic acid has four possible charges: +1 (α-carboxyl group, α-amino group, and Rgroup carboxyl each has a proton), 0 (α-carboxyl group missing proton, α- amino group has a proton, R-group carboxyl has a proton), -1 (α-carboxyl group and R-group carboxyl each lack a proton, α-amino group retains a proton), -2 (α-carboxyl, R-group carboxyl, and α-amino groups all lack extra proton).

Prediction

How does one predict the charge for an amino acid at a given pH? A good rule of thumb for estimating charge is that if the pH is more than one unit below the pKa for a group (carboxyl or amino), the proton is on. If the pH is more than one unit above the pKa for the group, the proton is off. If the pH is NOT more than one or less than one pH unit from the pKa, this simple assumption will not work.

Further, it is important to recognize that these rules of thumb are estimates only. The pI (pH at which the charge of a molecule is zero) is an exact value calculated as the average of the two pKa values on either side of the zero region. It is calculated at the average of the two pKa values around the point where the charge of the molecule is zero. For aspartic acid, this corresponds to $pK_{a1}$and pK_{a2}.

References

1. http://www.lpi.usra.edu/lunar/missions/apollo/ apollo_12/experiments/surveyor/
2. Arunan, Elangannan; Desiraju, Gautam R.; Klein, Roger A.; Sadlej, Joanna; Scheiner, Steve; Alkorta, Ibon; Clary, David C.; Crabtree, Robert H.; Dannenberg, Joseph J.; Hobza, Pavel; Kjaergaard, Henrik G.; Legon, Anthony C.; Mennucci, Benedetta; Nesbitt, David J. (2011). "Definition of the hydrogen bond". Pure Appl. Chem. 83 (8): 1637–1641. doi:10.1351/PAC-REC-10-01-02

This page titled 2.2: pH and Buffers is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 1.3: Introduction - Water and Buffers by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

2.3: Functional groups and electronegativity

Source: BiochemFFA_1_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

"Organic chemistry is the chemistry of carbon compounds. Biochemistry is the chemistry of carbon compounds that crawl" -Michael Adams.

To understand biochemistry, one must possess at least a basic understanding of organic and general chemistry. In this brief section, we will provide a rapid review of the simple concepts necessary to understand cellular chemistry. Chemistry is chemistry, whether in a cell or outside it, but biological chemistry is a particular subset of organic chemistry that often involves enormous macromolecules, and that happens in the aqueous environment of the cell.



Figure 1.18 shows the various organic functional groups common in biochemistry. You will encounter these functional groups as you study the biosynthetic and breakdown pathways that build and recycle the chemical compounds of which cells are made. In addition to knowing the names and structures of these groups, students need a basic understanding of covalent and ionic bonds.

Class	General Structure	Name	Functional Group
Alkenes	RCH═CHR	Double Bond	C═C
Alcohols	ROH	Hydroxyl	—OH
Ethers	ROR	Ether	—O
Amines	RNH_2 R_2NH R_3N	Amino	—N<
Thiols	RSH	Sulfhydryl	—SH
Aldehydes	R—C(=O)—H	Carbonyl	—C(=O)—
Ketones	R—C(=O)—R	Carbonyl	—C(=O)—
Carboxylic Acids	R—C(=O)—OH	Carboxyl	—C(=O)—OH
Amides	R—C(=O)—NR_2	Amide	—C(=O)—N<
Esters	R—C(=O)—OR'	Ester	—C(=O)—OR
Phosphoric Acid Esters	R—O—P(=O)(OH)—OH	Phosphoric Ester	—O—P(=O)(OH)—OH

Figure 1.18 - Important functional groups in biochemistry Image by Aleia Kim

Covalent bonds, as you know, are the result of sharing of electrons between two atoms. Ionic bonds, by contrast, are formed when one atom donates an electron to another, such as in the formation of sodium chloride. Single covalent bonds can rotate freely, but double bonds cannot. Single bonds around a carbon atom are arranged in a tetrahedron with bond angles of 109.5° relative to each other, with the carbon at the center (Figure 1.19). Double bonded carbons create a planar structure with bond angles typically of about 120°.

Electronegativity

Electronegativity is a measure of the affinity a nucleus has for outer shell electrons (Table 1.2). High electronegativity corresponds to high affinity. Electrons in a covalent bond are held closer to the nucleus with a greater electronegativity compared to a nucleus with lower electronegativity.

Table 1.2

Electronegativities of Various Atoms

Atom	Electronegativity
Oxygen	3.5
Nitrogen	3.0
Sulfur	2.6
Carbon	2.5
Phosphorous	2.2
Hydrogen	2.1

Table 1.2 Image by Aleia Kim

For example, in a molecule of water, with hydrogen covalently bonded to oxygen, the electrons are “pulled” toward the oxygen, which is more electronegative. Because of this, there is a slightly greater negative charge near the oxygen atom of water, compared to the hydrogen (which, correspondingly has a slightly higher positive charge). This unequal charge distribution sets up a dipole, with one side being somewhat negative and the other somewhat positive. Because of this, the molecule is described as polar.

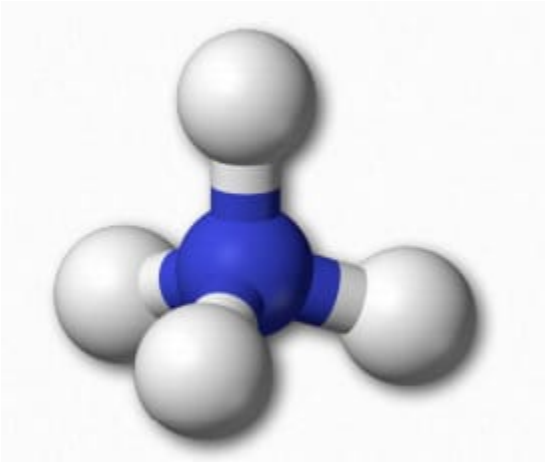


Figure 1.19 - Tetrahedral structure Wikipedia

Hydrogen bonds between water molecules are the result of the attraction of the partial positive and partial negative charges

on different water molecules (Figure 1.20). Hydrogen bonds can also form between hydrogens with a partial positive charge and other strongly electronegative atoms, like nitrogen, with a partial negative charge. It is important to remember that hydrogen bonds are interactions between molecules (or parts of molecules) and are not bonds between atoms, like covalent or ionic bonds. Bonds between hydrogen and carbon do not form significant partial charges because the electronegativities of the two atoms are similar. Consequently, molecules containing many carbon-hydrogen bonds will not form hydrogen bonds and therefore, do not mix well with water. Such molecules are called hydrophobic. Other compounds with the ability to make hydrogen bonds are polar and can dissolve in water. They are called hydrophilic. Molecules possessing both characteristics are called amphiphilic.

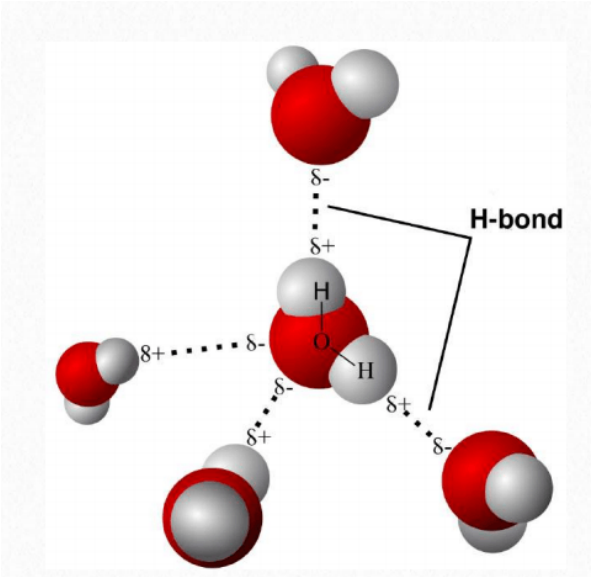


Figure 1.20 Hydrogen bonds (dotted lines) between water molecules Wikipedia

Weak interactions

Hydrogen bonds are one kind of electrostatic (i.e., based on charge) interaction between dipoles. Other forms of electrostatic interactions that are important in biochemistry include weak interactions between a polar molecule and a transient dipole, or between two temporary dipoles. These temporary dipoles result from the movement of electrons in a molecule. As electrons move around, the place where they are, at a given time, becomes temporarily more negatively charged and could now attract a temporary positive charge on another molecule. Since electrons don't stay put, these dipoles are very short-lived. Thus, the attraction that depends on these dipoles fluctuates and is very weak. Weak interactions like these are sometimes called van der Waals forces. Many molecular interactions in cells depend on weak interactions. Although the individual hydrogen bonds or other dipole-dipole interactions are weak, because of their large numbers, they can result in quite strong interactions between molecules.

Oxidation/reduction

Oxidation involves loss of electrons and reduction results in gain of electrons. For every biological oxidation, there is a corresponding reduction - one molecule loses electrons to another molecule. Oxidation reactions tend to release energy and are a source of bioenergy for chemotrophic cells.

Ionization

Ionization of biomolecules, by contrast does not involve oxidation/reduction. In ionization, a hydrogen ion (H+) leaves behind its electron as it exits (leaving behind a negative charge) or joins a group (adding a positive charge). Biological ionizations typically involve carboxyl groups or amines, though phosphates or sulfates can also be ionized. A carboxyl group can have two ionization states - a charge of -1 corresponds to the carboxyl without its proton and a charge of zero corresponds to the charge of the carboxyl with its proton on. An amine also has two ionization states. A charge of zero corresponds to a nitrogen with three covalent bonds (usually in the form of C-NH2) and a charge of +1 corresponds to a nitrogen making four covalent bonds (usually X-NH3 +).

Stereochemistry

A carbon has the ability to make four single bonds (forming a tetrahedral structure) and if it bonds to four different chemical groups, their atoms can be arranged around the carbon in two different ways, giving rise to stereochemical “handedness” (Figure 1.21). Each carbon with such a property is referred to as an asymmetric center. The property of handedness only occurs when a carbon has four different groups bonded to it. Enzymes have very specific 3-D structures, so for biological molecules that can exist in different stereoisomeric forms, an enzyme that synthesizes it would make only one of the possible isomers. By contrast, the same molecules made chemically (not using enzymes) end up with equal amounts of both isomers, called a **racemic mix**.

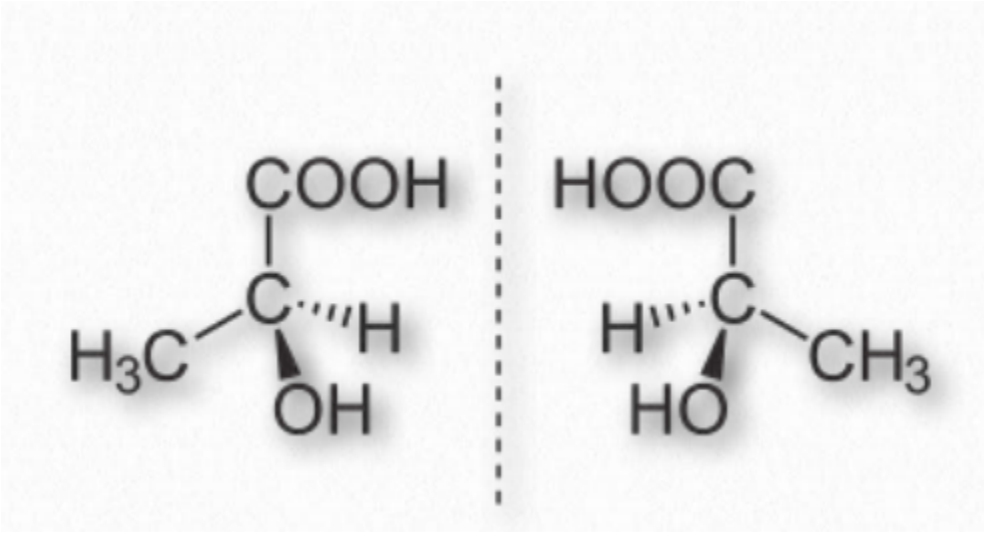


Figure 1.21 - Mirror images of lactic acid

Contributors and Attributions

This page titled 2.3: Functional groups and electronegativity is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 1.2: Introduction - Basic Chemistry by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

2.4: Types of Biological Molecules

The large molecules necessary for life that are built from smaller organic molecules are called biological macromolecules. There are four major classes of biological macromolecules (carbohydrates, lipids, proteins, and nucleic acids), and each is an important component of the cell and performs a wide array of functions. Combined, these molecules make up the majority of a cell's mass. Biological macromolecules are organic, meaning that they contain carbon (with some exceptions, like carbon dioxide). In addition, they may contain hydrogen, oxygen, nitrogen, phosphorus, sulfur, and additional minor elements.

Carbon

It is often said that life is "carbon-based." This means that carbon atoms, bonded to other carbon atoms or other elements, form the fundamental components of many, if not most, of the molecules found uniquely in living things. Other elements play important roles in biological molecules, but carbon certainly qualifies as the "foundation" element for molecules in living things. It is the bonding properties of carbon atoms that are responsible for its important role.

Carbon Bonding

Carbon contains four electrons in its outer shell. Therefore, it can form four covalent bonds with other atoms or molecules. The simplest organic carbon molecule is methane (CH_4), in which four hydrogen atoms bind to a carbon atom (Figure 2.4.1).

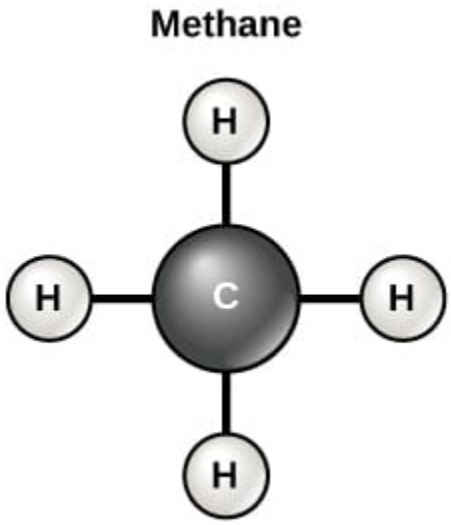


Figure 2.4.1: Carbon can form four covalent bonds to create an organic molecule. The simplest carbon molecule is methane (CH_4), depicted here.

However, structures that are more complex are made using carbon. Any of the hydrogen atoms can be replaced with another carbon atom covalently bonded to the first carbon atom. In this way, long and branching chains of carbon compounds can be made (Figure 2.4.2**a**). The carbon atoms may bond with atoms of other elements, such as nitrogen, oxygen, and phosphorus (Figure 2.4.2**b**). The molecules may also form rings, which themselves can link with other rings (Figure 2.4.2**c**). This diversity of molecular forms accounts for the diversity of functions of the biological macromolecules and is based to a large degree on the ability of carbon to form multiple bonds with itself and other atoms.

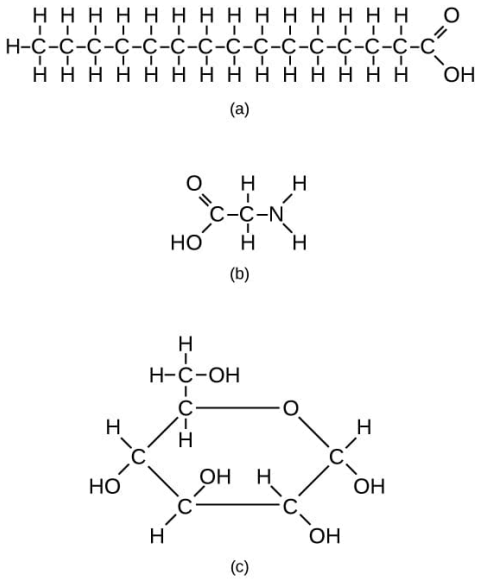


Figure 2.4.2: *These examples show three molecules (found in living organisms) that contain carbon atoms bonded in various ways to other carbon atoms and the atoms of other elements. (a) This molecule of stearic acid has a long chain of carbon atoms. (b) Glycine, a component of proteins, contains carbon, nitrogen, oxygen, and hydrogen atoms. (c) Glucose, a sugar, has a ring of carbon atoms and one oxygen atom.*

Carbohydrates

Carbohydrates are macromolecules with which most consumers are somewhat familiar. To lose weight, some individuals adhere to "low-carb" diets. Athletes, in contrast, often "carb-load" before important competitions to ensure that they have sufficient energy to compete at a high level. Carbohydrates are, in fact, an essential part of our diet; grains, fruits, and vegetables are all natural sources of carbohydrates. Carbohydrates provide energy to the body, particularly through glucose, a simple sugar. Carbohydrates also have other important functions in humans, animals, and plants.

Carbohydrates can be represented by the formula $(CH_2O)_n$, where n is the number of carbon atoms in the molecule. In other words, the ratio of carbon to hydrogen to oxygen is 1:2:1 in carbohydrate molecules. Carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides.

Monosaccharides (mono- = "one"; sacchar- = "sweet") are simple sugars, the most common of which is glucose. In monosaccharides, the number of carbon atoms usually ranges from three to six. Most monosaccharide names end with the suffix -ose. Depending on the number of carbon atoms in the sugar, they may be known as trioses (three carbon atoms), pentoses (five carbon atoms), and hexoses (six carbon atoms).

Monosaccharides may exist as a linear chain or as ring-shaped molecules; in aqueous solutions, they are usually found in the ring form.

The chemical formula for glucose is $C_6H_{12}O_6$. In most living species, glucose is an important source of energy. During cellular respiration, energy is released from glucose, and that energy is used to help make adenosine triphosphate (ATP). Plants synthesize glucose using carbon dioxide and water by the process of photosynthesis, and the glucose, in turn, is used for the energy requirements of the plant. The excess synthesized glucose is often stored as starch that is broken down by other organisms that feed on plants.

Galactose (part of lactose, or milk sugar) and fructose (found in fruit) are other common monosaccharides. Although glucose, galactose, and fructose all have the same chemical formula ($C_6H_{12}O_6$), they differ structurally and chemically (and are known as isomers) because of differing arrangements of atoms in the carbon chain (Figure 2.4.3).

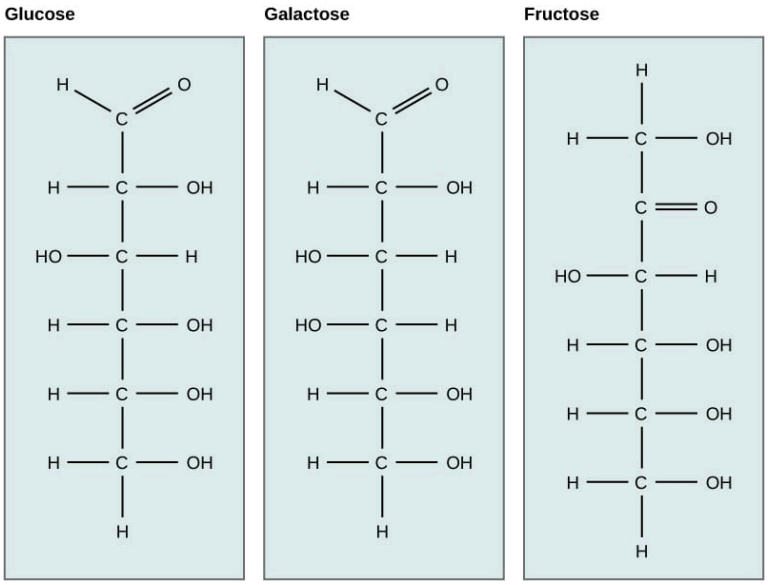


Figure 2.4.3: Glucose, galactose, and fructose are isomeric monosaccharides, meaning that they have the same chemical formula but slightly different structures.

Disaccharides (di- = “two”) form when two monosaccharides undergo a dehydration reaction (a reaction in which the removal of a water molecule occurs). During this process, the hydroxyl group (–OH) of one monosaccharide combines with a hydrogen atom of another monosaccharide, releasing a molecule of water (H_2O) and forming a covalent bond between atoms in the two sugar molecules.

Common disaccharides include lactose, maltose, and sucrose. Lactose is a disaccharide consisting of the monomers glucose and galactose. It is found naturally in milk. Maltose, or malt sugar, is a disaccharide formed from a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose.

A long chain of monosaccharides linked by covalent bonds is known as a polysaccharide (poly- = “many”). The chain may be branched or unbranched, and it may contain different types of monosaccharides. Polysaccharides may be very large molecules. Starch, glycogen, cellulose, and chitin are examples of polysaccharides.

Starch is the stored form of sugars in plants and is made up of amylose and amylopectin (both polymers of glucose). Plants are able to synthesize glucose, and the excess glucose is stored as starch in different plant parts, including roots and seeds. The starch that is consumed by animals is broken down into smaller molecules, such as glucose. The cells can then absorb the glucose.

Glycogen is the storage form of glucose in humans and other vertebrates, and is made up of monomers of glucose. Glycogen is the animal equivalent of starch and is a highly branched molecule usually stored in liver and muscle cells. Whenever glucose levels decrease, glycogen is broken down to release glucose.

Cellulose is one of the most abundant natural biopolymers. The cell walls of plants are mostly made of cellulose, which provides structural support to the cell. Wood and paper are mostly cellulosic in nature. Cellulose is made up of glucose monomers that are linked by bonds between particular carbon atoms in the glucose molecule.

Every other glucose monomer in cellulose is flipped over and packed tightly as extended long chains. This gives cellulose its rigidity and high tensile strength—which is so important to plant cells. Cellulose passing through our digestive system is called dietary fiber. While the glucose-glucose bonds in cellulose cannot be broken down by human digestive enzymes, herbivores such as cows, buffalos, and horses are able to digest grass that is rich in cellulose and use it as a food source. In these animals, certain species of bacteria reside in the rumen (part of the digestive system of herbivores) and secrete the enzyme cellulase. The appendix also contains bacteria that break down cellulose, giving it an important role in the digestive systems of ruminants. Cellulases can break down cellulose into glucose monomers that can be used as an energy source by the animal.

Carbohydrates serve other functions in different animals. Arthropods, such as insects, spiders, and crabs, have an outer skeleton, called the exoskeleton, which protects their internal body parts. This exoskeleton is made of the biological macromolecule chitin,

which is a nitrogenous carbohydrate. It is made of repeating units of a modified sugar containing nitrogen.

Thus, through differences in molecular structure, carbohydrates are able to serve the very different functions of energy storage (starch and glycogen) and structural support and protection (cellulose and chitin) (Figure 2.4.4).

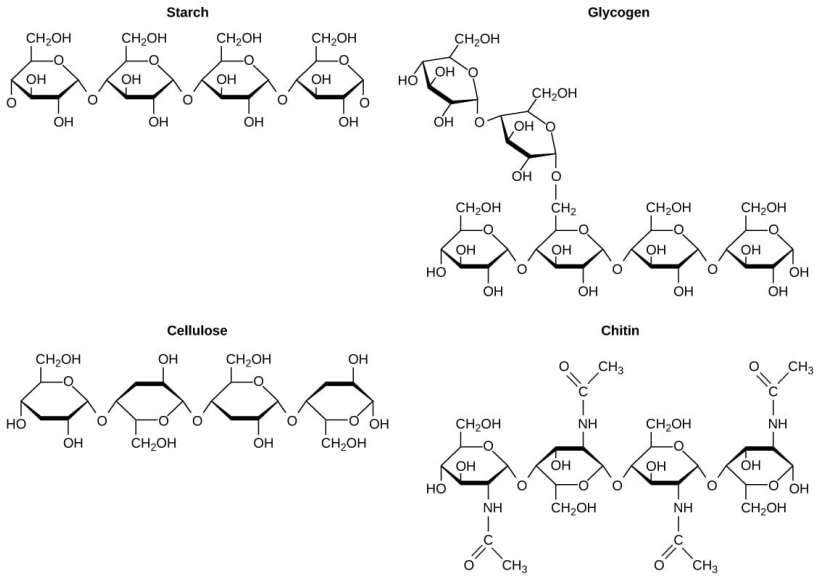


Figure 2.4.4: *Although their structures and functions differ, all polysaccharide carbohydrates are made up of monosaccharides and have the chemical formula $(CH_2O)n$.*

CAREERS IN ACTION: Registered Dietitian

Obesity is a worldwide health concern, and many diseases, such as diabetes and heart disease, are becoming more prevalent because of obesity. This is one of the reasons why registered dietitians are increasingly sought after for advice. Registered dietitians help plan food and nutrition programs for individuals in various settings. They often work with patients in health-care facilities, designing nutrition plans to prevent and treat diseases. For example, dietitians may teach a patient with diabetes how to manage blood-sugar levels by eating the correct types and amounts of carbohydrates. Dietitians may also work in nursing homes, schools, and private practices.

To become a registered dietitian, one needs to earn at least a bachelor's degree in dietetics, nutrition, food technology, or a related field. In addition, registered dietitians must complete a supervised internship program and pass a national exam. Those who pursue careers in dietetics take courses in nutrition, chemistry, biochemistry, biology, microbiology, and human physiology. Dietitians must become experts in the chemistry and functions of food (proteins, carbohydrates, and fats).

Lipids

Lipids include a diverse group of compounds that are united by a common feature. Lipids are hydrophobic ("water-fearing"), or insoluble in water, because they are nonpolar molecules. This is because they are hydrocarbons that include only nonpolar carbon-carbon or carbon-hydrogen bonds. Lipids perform many different functions in a cell. Cells store energy for long-term use in the form of lipids called fats. Lipids also provide insulation from the environment for plants and animals (Figure 2.4.5). For example, they help keep aquatic birds and mammals dry because of their water-repelling nature. Lipids are also the building blocks of many hormones and are an important constituent of the plasma membrane. Lipids include fats, oils, waxes, phospholipids, and steroids.



Figure 2.4.5: Hydrophobic lipids in the fur of aquatic mammals, such as this river otter, protect them from the elements. (credit: Ken Bosma)

A fat molecule, such as a triglyceride, consists of two main components—glycerol and fatty acids. Glycerol is an organic compound with three carbon atoms, five hydrogen atoms, and three hydroxyl (–OH) groups. Fatty acids have a long chain of hydrocarbons to which an acidic carboxyl group is attached, hence the name "fatty acid." The number of carbons in the fatty acid may range from 4 to 36; most common are those containing 12–18 carbons. In a fat molecule, a fatty acid is attached to each of the three oxygen atoms in the –OH groups of the glycerol molecule with a covalent bond (Figure 2.4.6).

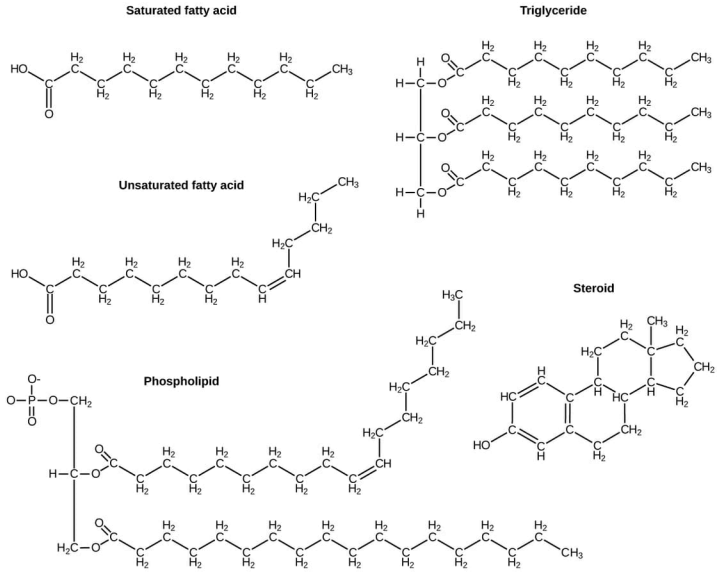


Figure 2.4.6: *Lipids include fats, such as triglycerides, which are made up of fatty acids and glycerol, phospholipids, and steroids.*

During this covalent bond formation, three water molecules are released. The three fatty acids in the fat may be similar or dissimilar. These fats are also called triglycerides because they have three fatty acids. Some fatty acids have common names that specify their origin. For example, palmitic acid, a saturated fatty acid, is derived from the palm tree. Arachidic acid is derived from *Arachis hypogaea*, the scientific name for peanuts.

Fatty acids may be saturated or unsaturated. In a fatty acid chain, if there are only single bonds between neighboring carbons in the hydrocarbon chain, the fatty acid is saturated. Saturated fatty acids are saturated with hydrogen; in other words, the number of hydrogen atoms attached to the carbon skeleton is maximized.

When the hydrocarbon chain contains a double bond, the fatty acid is an unsaturated fatty acid.

Most unsaturated fats are liquid at room temperature and are called oils. If there is one double bond in the molecule, then it is known as a monounsaturated fat (e.g., olive oil), and if there is more than one double bond, then it is known as a polyunsaturated fat (e.g., canola oil).

Saturated fats tend to get packed tightly and are solid at room temperature. Animal fats with stearic acid and palmitic acid contained in meat, and the fat with butyric acid contained in butter, are examples of saturated fats. Mammals store fats in specialized cells called adipocytes, where globules of fat occupy most of the cell. In plants, fat or oil is stored in seeds and is used as a source of energy during embryonic development.

Unsaturated fats or oils are usually of plant origin and contain unsaturated fatty acids. The double bond causes a bend or a "kink" that prevents the fatty acids from packing tightly, keeping them liquid at room temperature. Olive oil, corn oil, canola oil, and cod liver oil are examples of unsaturated fats. Unsaturated fats help to improve blood cholesterol levels, whereas saturated fats contribute to plaque formation in the arteries, which increases the risk of a heart attack.

In the food industry, oils are artificially hydrogenated to make them semi-solid, leading to less spoilage and increased shelf life. Simply speaking, hydrogen gas is bubbled through oils to solidify them. During this hydrogenation process, double bonds of the *cis*-conformation in the hydrocarbon chain may be converted to double bonds in the *trans*-conformation. This forms a *trans*-fat from a *cis*-fat. The orientation of the double bonds affects the chemical properties of the fat (Figure 2.4.7).

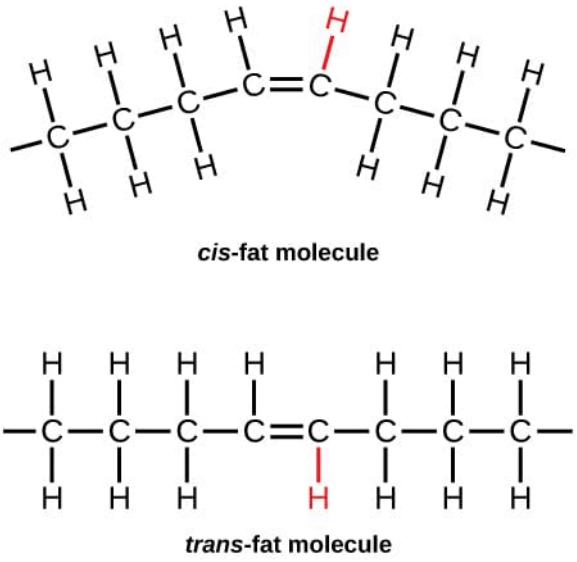


Figure 2.4.7: During the hydrogenation process, the orientation around the double bonds is changed, making a trans-fat from a cis-fat. This changes the chemical properties of the molecule.

Margarine, some types of peanut butter, and shortening are examples of artificially hydrogenated *trans*-fats. Recent studies have shown that an increase in *trans*-fats in the human diet may lead to an increase in levels of low-density lipoprotein (LDL), or "bad" cholesterol, which, in turn, may lead to plaque deposition in the arteries, resulting in heart disease. Many fast food restaurants have recently eliminated the use of *trans*-fats, and U.S. food labels are now required to list their *trans*-fat content.

Essential fatty acids are fatty acids that are required but not synthesized by the human body. Consequently, they must be supplemented through the diet. Omega-3 fatty acids fall into this category and are one of only two known essential fatty acids for humans (the other being omega-6 fatty acids). They are a type of polyunsaturated fat and are called omega-3 fatty acids because the third carbon from the end of the fatty acid participates in a double bond.

Salmon, trout, and tuna are good sources of omega-3 fatty acids. Omega-3 fatty acids are important in brain function and normal growth and development. They may also prevent heart disease and reduce the risk of cancer.

Like carbohydrates, fats have received a lot of bad publicity. It is true that eating an excess of fried foods and other "fatty" foods leads to weight gain. However, fats do have important functions. Fats serve as long-term energy storage. They also provide insulation for the body. Therefore, "healthy" unsaturated fats in moderate amounts should be consumed on a regular basis.

Phospholipids are the major constituent of the plasma membrane. Like fats, they are composed of fatty acid chains attached to a

glycerol or similar backbone. Instead of three fatty acids attached, however, there are two fatty acids and the third carbon of the glycerol backbone is bound to a phosphate group. The phosphate group is modified by the addition of an alcohol.

A phospholipid has both hydrophobic and hydrophilic regions. The fatty acid chains are hydrophobic and exclude themselves from water, whereas the phosphate is hydrophilic and interacts with water.

Cells are surrounded by a membrane, which has a bilayer of phospholipids. The fatty acids of phospholipids face inside, away from water, whereas the phosphate group can face either the outside environment or the inside of the cell, which are both aqueous.

Steroids and Waxes

Unlike the phospholipids and fats discussed earlier, steroids have a ring structure. Although they do not resemble other lipids, they are grouped with them because they are also hydrophobic. All steroids have four, linked carbon rings and several of them, like cholesterol, have a short tail.

Cholesterol is a steroid. Cholesterol is mainly synthesized in the liver and is the precursor of many steroid hormones, such as testosterone and estradiol. It is also the precursor of vitamins E and K. Cholesterol is the precursor of bile salts, which help in the breakdown of fats and their subsequent absorption by cells. Although cholesterol is often spoken of in negative terms, it is necessary for the proper functioning of the body. It is a key component of the plasma membranes of animal cells.

Waxes are made up of a hydrocarbon chain with an alcohol (–OH) group and a fatty acid. Examples of animal waxes include beeswax and lanolin. Plants also have waxes, such as the coating on their leaves, that helps prevent them from drying out.

CONCEPT IN ACTION

For an additional perspective on lipids, explore “Biomolecules: The Lipids” through this interactive animation.

Proteins

Proteins are one of the most abundant organic molecules in living systems and have the most diverse range of functions of all macromolecules. Proteins may be structural, regulatory, contractile, or protective; they may serve in transport, storage, or membranes; or they may be toxins or enzymes. Each cell in a living system may contain thousands of different proteins, each with a unique function. Their structures, like their functions, vary greatly. They are all, however, polymers of amino acids, arranged in a linear sequence.

The functions of proteins are very diverse because there are 20 different chemically distinct amino acids that form long chains, and the amino acids can be in any order. For example, proteins can function as enzymes or hormones. Enzymes, which are produced by living cells, are catalysts in biochemical reactions (like digestion) and are usually proteins. Each enzyme is specific for the substrate (a reactant that binds to an enzyme) upon which it acts. Enzymes can function to break molecular bonds, to rearrange bonds, or to form new bonds. An example of an enzyme is salivary amylase, which breaks down amylose, a component of starch.

Hormones are chemical signaling molecules, usually proteins or steroids, secreted by an endocrine gland or group of endocrine cells that act to control or regulate specific physiological processes, including growth, development, metabolism, and reproduction. For example, insulin is a protein hormone that maintains blood glucose levels.

Proteins have different shapes and molecular weights; some proteins are globular in shape whereas others are fibrous in nature. For example, hemoglobin is a globular protein, but collagen, found in our skin, is a fibrous protein. Protein shape is critical to its function. Changes in temperature, pH, and exposure to chemicals may lead to permanent changes in the shape of the protein, leading to a loss of function or denaturation (to be discussed in more detail later). All proteins are made up of different arrangements of the same 20 kinds of amino acids.

Amino acids are the monomers that make up proteins. Each amino acid has the same fundamental structure, which consists of a central carbon atom bonded to an amino group ($-NH_2$), a carboxyl group (–COOH), and a hydrogen atom. Every amino acid also has another variable atom or group of atoms bonded to the central carbon atom known as the R group. The R group is the only difference in structure between the 20 amino acids; otherwise, the amino acids are identical (Figure 2.4.8).

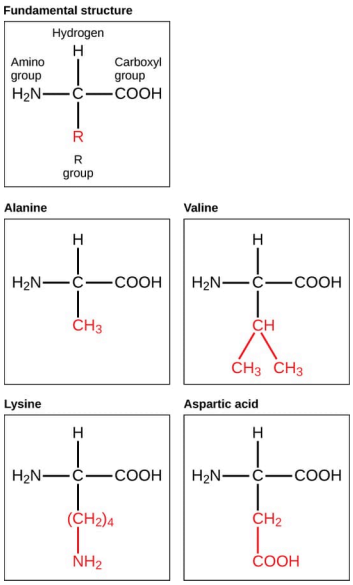


Figure 2.4.8: Amino acids are made up of a central carbon bonded to an amino group ($–NH_2$), a carboxyl group (–COOH), and a hydrogen atom. The central carbon's fourth bond varies among the different amino acids, as seen in these examples of alanine, valine, lysine, and aspartic acid.

The chemical nature of the R group determines the chemical nature of the amino acid within its protein (that is, whether it is acidic, basic, polar, or nonpolar).

The sequence and number of amino acids ultimately determine a protein's shape, size, and function. Each amino acid is attached to another amino acid by a covalent bond, known as a peptide bond, which is formed by a dehydration reaction. The carboxyl group of one amino acid and the amino group of a second amino acid combine, releasing a water molecule. The resulting bond is the peptide bond.

The products formed by such a linkage are called polypeptides. While the terms polypeptide and protein are sometimes used interchangeably, a polypeptide is technically a polymer of amino acids, whereas the term protein is used for a polypeptide or polypeptides that have combined together, have a distinct shape, and have a unique function.

EVOLUTION IN ACTION: The Evolutionary Significance of Cytochrome c

Cytochrome c is an important component of the molecular machinery that harvests energy from glucose. Because this protein's role in producing cellular energy is crucial, it has changed very little over millions of years. Protein sequencing has shown that there is a considerable amount of sequence similarity among cytochrome c molecules of different species; evolutionary relationships can be assessed by measuring the similarities or differences among various species' protein sequences.

For example, scientists have determined that human cytochrome c contains 104 amino acids. For each cytochrome c molecule that has been sequenced to date from different organisms, 37 of these amino acids appear in the same position in each cytochrome c. This indicates that all of these organisms are descended from a common ancestor. On comparing the human and chimpanzee protein sequences, no sequence difference was found. When human and rhesus monkey sequences were compared, a single difference was found in one amino acid. In contrast, human-to-yeast comparisons show a difference in 44 amino acids, suggesting that humans and chimpanzees have a more recent common ancestor than humans and the rhesus monkey, or humans and yeast.

Protein Structure

As discussed earlier, the shape of a protein is critical to its function. To understand how the protein gets its final shape or conformation, we need to understand the four levels of protein structure: primary, secondary, tertiary, and quaternary (Figure 2.4.9).

The unique sequence and number of amino acids in a polypeptide chain is its primary structure. The unique sequence for every protein is ultimately determined by the gene that encodes the protein. Any change in the gene sequence may lead to a different amino acid being added to the polypeptide chain, causing a change in protein structure and function. In sickle cell anemia, the hemoglobin β chain has a single amino acid substitution, causing a change in both the structure and function of the protein. What is most remarkable to consider is that a hemoglobin molecule is made up of two alpha chains and two beta chains that each consist of about 150 amino acids. The molecule, therefore, has about 600 amino acids. The structural difference between a normal hemoglobin molecule and a sickle cell molecule—that dramatically decreases life expectancy in the affected individuals—is a single amino acid of the 600.

Because of this change of one amino acid in the chain, the normally biconcave, or disc-shaped, red blood cells assume a crescent or "sickle" shape, which clogs arteries. This can lead to a myriad of serious health problems, such as breathlessness, dizziness, headaches, and abdominal pain for those who have this disease.

Folding patterns resulting from interactions between the non-R group portions of amino acids give rise to the secondary structure of the protein. The most common are the alpha (α)-helix and beta (β)-pleated sheet structures. Both structures are held in shape by hydrogen bonds. In the alpha helix, the bonds form between every fourth amino acid and cause a twist in the amino acid chain.

In the β-pleated sheet, the "pleats" are formed by hydrogen bonding between atoms on the backbone of the polypeptide chain. The R groups are attached to the carbons, and extend above and below the folds of the pleat. The pleated segments align parallel to each other, and hydrogen bonds form between the same pairs of atoms on each of the aligned amino acids. The α-helix and β-pleated sheet structures are found in many globular and fibrous proteins.

The unique three-dimensional structure of a polypeptide is known as its tertiary structure. This structure is caused by chemical interactions between various amino acids and regions of the polypeptide. Primarily, the interactions among R groups create the complex three-dimensional tertiary structure of a protein. There may be ionic bonds formed between R groups on different amino acids, or hydrogen bonding beyond that involved in the secondary structure. When protein folding takes place, the hydrophobic R groups of nonpolar amino acids lay in the interior of the protein, whereas the hydrophilic R groups lay on the outside. The former types of interactions are also known as hydrophobic interactions.

In nature, some proteins are formed from several polypeptides, also known as subunits, and the interaction of these subunits forms the quaternary structure. Weak interactions between the subunits help to stabilize the overall structure. For example, hemoglobin is a combination of four polypeptide subunits.

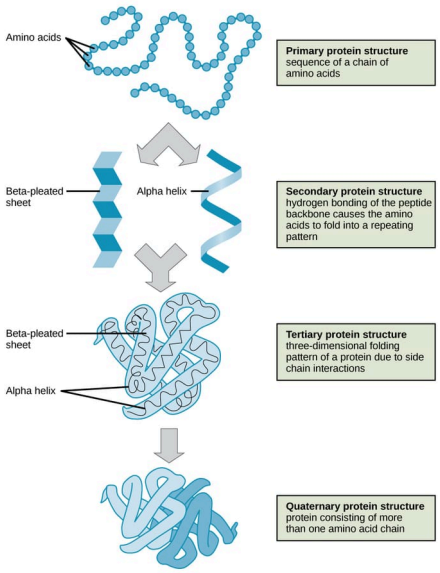


Figure 2.4.9: The four levels of protein structure can be observed in these illustrations. (credit: modification of work by National Human Genome Research Institute)

Each protein has its own unique sequence and shape held together by chemical interactions. If the protein is subject to changes in temperature, pH, or exposure to chemicals, the protein structure may change, losing its shape in what is known as denaturation as discussed earlier. Denaturation is often reversible because the primary structure is preserved if the denaturing agent is removed, allowing the protein to resume its function. Sometimes denaturation is irreversible, leading to a loss of function. One

example of protein denaturation can be seen when an egg is fried or boiled. The albumin protein in the liquid egg white is denatured when placed in a hot pan, changing from a clear substance to an opaque white substance. Not all proteins are denatured at high temperatures; for instance, bacteria that survive in hot springs have proteins that are adapted to function at those temperatures.

CONCEPT IN ACTION

For an additional perspective on proteins, explore “Biomolecules: The Proteins” through this interactive animation.

Nucleic Acids

Nucleic acids are key macromolecules in the continuity of life. They carry the genetic blueprint of a cell and carry instructions for the functioning of the cell.

The two main types of nucleic acids are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). DNA is the genetic material found in all living organisms, ranging from single-celled bacteria to multicellular mammals.

The other type of nucleic acid, RNA, is mostly involved in protein synthesis. The DNA molecules never leave the nucleus, but instead use an RNA intermediary to communicate with the rest of the cell. Other types of RNA are also involved in protein synthesis and its regulation.

DNA and RNA are made up of monomers known as nucleotides. The nucleotides combine with each other to form a polynucleotide, DNA or RNA. Each nucleotide is made up of three components: a nitrogenous base, a pentose (five-carbon) sugar, and a phosphate group (Figure 2.4.10). Each nitrogenous base in a nucleotide is attached to a sugar molecule, which is attached to a phosphate group.

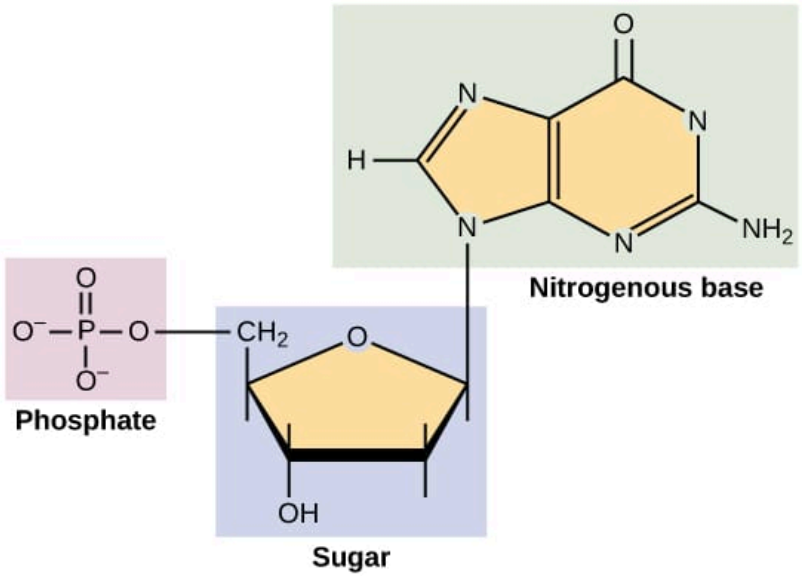


Figure 2.4.10: A nucleotide is made up of three components: a nitrogenous base, a pentose sugar, and a phosphate group.

DNA Double-Helical Structure

DNA has a double-helical structure (Figure 2.4.11). It is composed of two strands, or polymers, of nucleotides. The strands are formed with bonds between phosphate and sugar groups of adjacent nucleotides. The strands are bonded to each other at their bases with hydrogen bonds, and the strands coil about each other along their length, hence the “double helix” description, which means a double spiral.

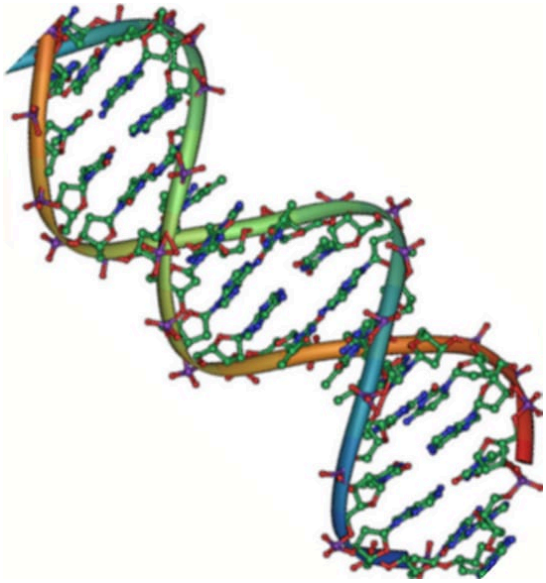


Figure 2.4.11: The double-helix model shows DNA as two parallel strands of intertwining molecules. (credit: Jerome Walker, Dennis Myts)

The alternating sugar and phosphate groups lie on the outside of each strand, forming the backbone of the DNA. The nitrogenous bases are stacked in the interior, like the steps of a staircase, and these bases pair; the pairs are bound to each other by hydrogen bonds. The bases pair in such a way that the distance between the backbones of the two strands is the same all along the molecule.

Summary

Living things are carbon-based because carbon plays such a prominent role in the chemistry of living things. The four covalent bonding positions of the carbon atom can give rise to a wide diversity of compounds with many functions, accounting for the importance of carbon in living things. Carbohydrates are a group of macromolecules that are a vital energy source for the cell, provide structural support to many organisms, and can be found on the surface of the cell as receptors or for cell recognition. Carbohydrates are classified as monosaccharides, disaccharides, and polysaccharides, depending on the number of monomers in the molecule.

Lipids are a class of macromolecules that are nonpolar and hydrophobic in nature. Major types include fats and oils, waxes, phospholipids, and steroids. Fats and oils are a stored form of energy and can include triglycerides. Fats and oils are usually made up of fatty acids and glycerol.

Proteins are a class of macromolecules that can perform a diverse range of functions for the cell. They help in metabolism by providing structural support and by acting as enzymes, carriers or as hormones. The building blocks of proteins are amino acids. Proteins are organized at four levels: primary, secondary, tertiary, and quaternary. Protein shape and function are intricately linked; any change in shape caused by changes in temperature, pH, or chemical exposure may lead to protein denaturation and a loss of function.

Nucleic acids are molecules made up of repeating units of nucleotides that direct cellular activities such as cell division and protein synthesis. Each nucleotide is made up of a pentose sugar, a nitrogenous base, and a phosphate group. There are two types of nucleic acids: DNA and RNA.

Glossary

amino acid
: a monomer of a protein

carbohydrate
: a biological macromolecule in which the ratio of carbon to hydrogen to oxygen is 1:2:1; carbohydrates serve as energy sources and structural support in cells

cellulose
a polysaccharide that makes up the cell walls of plants and provides structural support to the cell

chitin
a type of carbohydrate that forms the outer skeleton of arthropods, such as insects and crustaceans, and the cell walls of fungi

denaturation
the loss of shape in a protein as a result of changes in temperature, pH, or exposure to chemicals

deoxyribonucleic acid (DNA)
a double-stranded polymer of nucleotides that carries the hereditary information of the cell

disaccharide
two sugar monomers that are linked together by a peptide bond

enzyme
a catalyst in a biochemical reaction that is usually a complex or conjugated protein

fat
a lipid molecule composed of three fatty acids and a glycerol (triglyceride) that typically exists in a solid form at room temperature

glycogen
a storage carbohydrate in animals

hormone
a chemical signaling molecule, usually a protein or steroid, secreted by an endocrine gland or group of endocrine cells; acts to control or regulate specific physiological processes

lipids
a class of macromolecules that are nonpolar and insoluble in water

macromolecule
a large molecule, often formed by polymerization of smaller monomers

monosaccharide
a single unit or monomer of carbohydrates

nucleic acid
a biological macromolecule that carries the genetic information of a cell and carries instructions for the functioning of the cell

nucleotide
a monomer of nucleic acids; contains a pentose sugar, a phosphate group, and a nitrogenous base

oil
an unsaturated fat that is a liquid at room temperature

phospholipid
a major constituent of the membranes of cells; composed of two fatty acids and a phosphate group attached to the glycerol backbone

polypeptide
a long chain of amino acids linked by peptide bonds

polysaccharide
a long chain of monosaccharides; may be branched or unbranched

protein
a biological macromolecule composed of one or more chains of amino acids

ribonucleic acid (RNA)
a single-stranded polymer of nucleotides that is involved in protein synthesis

saturated fatty acid
a long-chain hydrocarbon with single covalent bonds in the carbon chain; the number of hydrogen atoms attached to the carbon skeleton is maximized

starch
a storage carbohydrate in plants

steroid
a type of lipid composed of four fused hydrocarbon rings

***trans*-fat**
a form of unsaturated fat with the hydrogen atoms neighboring the double bond across from each other rather than on the same side of the double bond

triglyceride
a fat molecule; consists of three fatty acids linked to a glycerol molecule

unsaturated fatty acid
a long-chain hydrocarbon that has one or more than one double bonds in the hydrocarbon chain

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 2.4: Types of Biological Molecules is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 2.3: Biological Molecules by OpenStax is licensed CC BY 4.0.

2.5: Assembly of Biological Macromolecules

Now that we've discussed the four major classes of biological macromolecules (carbohydrates, lipids, proteins, and nucleic acids), let's talk about macromolecules as a whole. Each is an important cell component and performs a wide array of functions. Combined, these molecules make up the majority of a cell's dry mass (recall that water makes up the majority of its complete mass). Biological macromolecules are organic, meaning they contain carbon. In addition, they may contain hydrogen, oxygen, nitrogen, and additional minor elements.

Comparing the Biological Macromolecules

Macromolecule	Basic Formula, key features	Monomer	Examples	Uses
Proteins		Amino acids	Enzymes, some hormones	Storage; Signals; Structural; Contractile; Defensive; Enzyme; Transport; Receptors
Lipids	C:H:O Greater than 2:1 H:O (carboxyl group)	Fatty acid and glycerol	Butter, oil, cholesterol, beeswax	Energy storage; Protection; Chemical messengers; Repel water
Carbohydrates	C:H:O 1:2:1	Monosaccharides	Glucose, Fructose, Starch, Glycogen, Cellulose	Energy storage; Structure
Nucleic Acids	CHONP pentose, nitrogenous base, phosphate	Nucleotides	DNA, RNA	Genetic information

Dehydration Synthesis

Most macromolecules are made from single subunits, or building blocks, called **monomers**. The monomers combine with each other using covalent bonds to form larger molecules known as **polymers**. In doing so, monomers release water molecules as byproducts. This type of reaction is known as **dehydration synthesis**, which means "to put together while losing water."

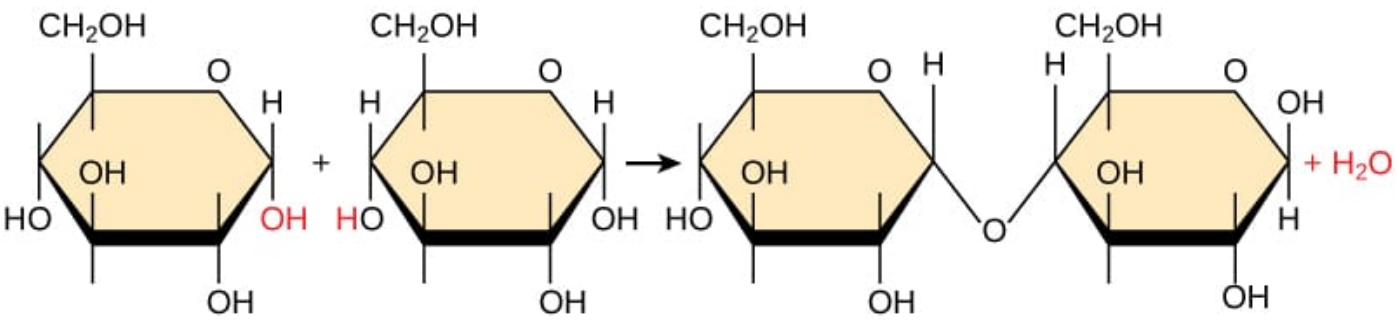


Figure 1. In the dehydration synthesis reaction depicted above, two molecules of glucose are linked together to form the disaccharide maltose. In the process, a water molecule is formed.

In a dehydration synthesis reaction (Figure 1), the hydrogen of one monomer combines with the hydroxyl group of another monomer, releasing a molecule of water. At the same time, the monomers share electrons and form covalent bonds. As additional monomers join, this chain of repeating monomers forms a polymer. Different types of monomers can combine in many configurations, giving rise to a diverse group of macromolecules. Even one kind of monomer can combine in a variety of ways to form several different polymers: for example, glucose monomers are the constituents of starch, glycogen, and cellulose.

Hydrolysis

Polymers are broken down into monomers in a process known as hydrolysis, which means “to split water,” a reaction in which a water molecule is used during the breakdown (Figure 2). During these reactions, the polymer is broken into two components: one part gains a hydrogen atom (H+) and the other gains a hydroxyl molecule (OH–) from a split water molecule.

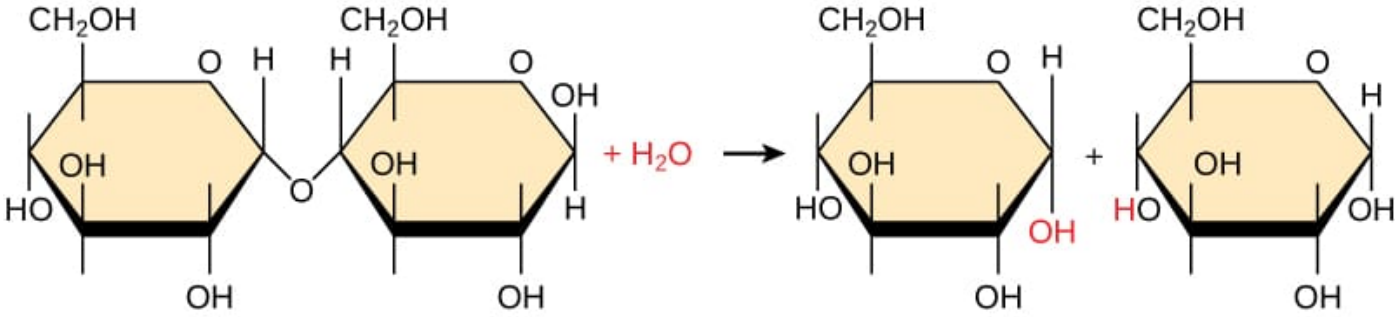


Figure 2. In the hydrolysis reaction shown here, the disaccharide maltose is broken down to form two glucose monomers with the addition of a water molecule. Note that this reaction is the reverse of the synthesis reaction shown in Figure 1.

Dehydration and **hydrolysis reactions** are catalyzed, or “sped up,” by specific enzymes; dehydration reactions involve the formation of new bonds, requiring energy, while hydrolysis reactions break bonds and release energy. These reactions are similar for most macromolecules, but each monomer and polymer reaction is specific for its class. For example, in our bodies, food is hydrolyzed, or broken down, into smaller molecules by catalytic enzymes in the digestive system. This allows for easy absorption of nutrients by cells in the intestine. Each macromolecule is broken down by a specific enzyme. For instance, carbohydrates are broken down by amylase, sucrase, lactase, or maltase. Proteins are broken down by the enzymes pepsin and peptidase, and by hydrochloric acid. Lipids are broken down by lipases. Breakdown of these macromolecules provides energy for cellular activities.

Visit this site to see visual representations of dehydration synthesis and hydrolysis.

Learning Objectives

Proteins, carbohydrates, nucleic acids, and lipids are the four major classes of biological macromolecules—large molecules necessary for life that are built from smaller organic molecules. Macromolecules are made up of single units known as monomers that are joined by covalent bonds to form larger polymers. The polymer is more than the sum of its parts: it acquires new characteristics, and leads to an osmotic pressure that is much lower than that formed by its ingredients; this is an important advantage in the maintenance of cellular osmotic conditions. A monomer joins with another monomer with the release of a water molecule, leading to the formation of a covalent bond. These types of reactions are known as dehydration or condensation reactions. When polymers are broken down into smaller units (monomers), a molecule of water is used for each bond broken by these reactions; such reactions are known as hydrolysis reactions. Dehydration and hydrolysis reactions are similar for all macromolecules, but each monomer and polymer reaction is specific to its class. Dehydration reactions typically require an investment of energy for new bond formation, while hydrolysis reactions typically release energy by breaking bonds.

Contributors and Attributions

CC licensed content, Original

- Revision, adaptation, and original content. **Authored by**: Shelli Carter and Lumen Learning. **Provided by**: Lumen Learning. **License**: *CC BY: Attribution*

CC licensed content, Shared previously

- Biology. **Provided by**: OpenStax CNX. **Located at**: http://cnx.org/contents/185cbf87-c72e-48f5-b51e-f14f21b5eabd@10.8. **License**: *CC BY: Attribution*. **License Terms**: Download for free at http://cnx.org/contents/185cbf87-c72...f21b5eabd@10.8

All rights reserved content

- Biological Molecules - You Are What You Eat: Crash Course Biology #3. **Authored by**: Crash Course. **Located at**: https://youtu.be/H8WJ2KENlK0. **Project**: Crash Course Biology. **License**: *All Rights Reserved*. **License Terms**: Standard YouTube License

---

This page titled 2.5: Assembly of Biological Macromolecules is shared under a license and was authored, remixed, and/or curated by LibreTexts.

- 5.14: Different Types of Biological Macromolecules has no license indicated.

3: AMINO ACIDS

3.2: Amino Acid Charges
3.3: Cysteine Chemistry
3.4: Post Translational Modification of Amino Acids
3.1:Structure and Property of the Naturally-Occurring Amino Acids

This page titled 3: Amino Acids is shared under a license and was authored, remixed, and/or curated by LibreTexts.

3.2: Amino Acid Charges

Monomeric amino acids have an alpha amino group and a carboxyl group, both of which may be protonated or deprotonated, and a R group, some of which may be protonated or deprotonated. When protonated, the amino group has a +1 charge, and the carboxyl group a 0 charge. When deprotonated the amino group has no charge, while the carboxyl group has a -1 charge. The R groups which can be protonated/deprotonated include Lys, Arg and His, which have a + 1 charge when protonated, and Glu and Asp (carboxylic acids), Tyr and Ser (alcohols) and Cys (thiol), which have 0 charge when protonated. Of course, when the amino acids are linked by peptide bonds (amide link), the alpha N and the carboxyl C are in an amide link, and are not charged. However, the amino group of the N -terminal amino acid and the carboxyl group of the C-terminal amino acid of a protein may be charged. The Henderson Hasselbach equation gives us a way to determine the charge state of any ionizable group knowing the pKa of the group. Write each functional group capable of being deprotonated as an acid, HA, and the deprotonated form as A. The charge of HA and A will be determined by the functional group. The Ka for the reaction is:

$$\mathrm{K_a} = \frac{[\mathrm{H_3O^+}][\mathrm{A}]}{[\mathrm{HA}]} \tag{3.2.1}$$

or

$$[\mathrm{H_3O^+}] = \mathrm{K_a}\frac{[\mathrm{HA}]}{[\mathrm{A}]} \tag{3.2.2}$$

$$-\log[\mathrm{H_3O^+}] = -\log\mathrm{K_a} + \log\frac{[\mathrm{A}]}{[\mathrm{HA}]} \tag{3.2.3}$$

or

$$\mathrm{pH} = \mathrm{pK_a} + \log\frac{[\mathrm{A}]}{[\mathrm{HA}]} \tag{3.2.4}$$

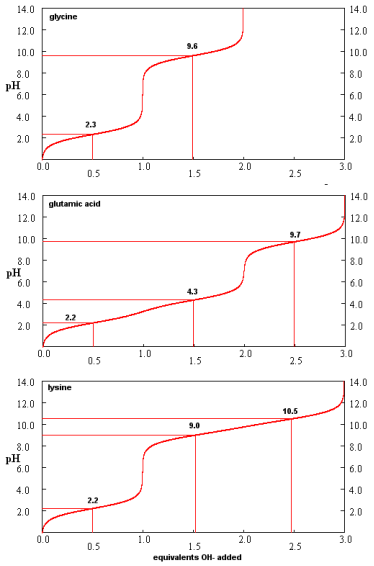
This is the (in)famous Henderson-Hasselbach (HH) equation.

The properties of a protein will be determined partly by whether the side chain functional groups, the N terminal, and the C terminal are charged or not. The HH equation tells us that this will depend on the pH and the pKa of the functional group.

- If the pH is 2 units below the pKa, the HH equation becomes, -2 = log A/HA, or .01 = A/HA. This means that the functional group will be about 99% protonated (with either 0 or +1 charge, depending of the functional group).
- If the pH is 2 units above the pKa, the HH equation becomes 2 = log A/HA, or 100 = A/HA. Therefore the functional group will be 99% deprotonated.
- If the pH = pka, the HH equation becomes 0 = log A/HA, or 1 = A/HA. Therefore the functional group will be 50% deprotonated

From these simple examples, we have derived the +2 rule. This rule is used to quickly determine protonation, and hence charge state, and is extremely important to know (and easy to derive). Titration curves for Gly (no ionizable) side chain, Glu (carboxlic acid side chain) and Lys (amine side chain) are shown below. You should be able to associate various sections of these curves with titration of specific ionizable groups in the amino acids.

Figure: Titration curves for Gly, Glu, and Lys



Buffer Review

The Henderson-Hasselbach equation is also useful in calculating the composition of buffer solutions. Remember that buffer solutions are composed of a weak acid and its conjugate base. Consider the equilibrium for a weak acid, like acetic acid, and its conjugate base, acetate:

$$\mathrm{CH_3CO_2H + H_2O \Leftrightarrow H_3O^+ + [redacted]} \tag{3.2.5}$$

If the buffer solution contains equal concentrations of acetic acid and acetate, the pH of the solution is:

$$\mathrm{pH = pK_a + \log\frac{[A]}{[HA]} = 4.7 + \log\ 1 = 4.7} \tag{3.2.6}$$

A look at the titration curve for the carboxyl group of Gly (see above) shows that when the pH = pKa, the slope of the curve (i.e. the change in pH with addition of base or acid) is at a minimum. As a general rule of thumb, buffer solution can be made for a weak acid/base in the range of +/- 1 pH unit from the pKa of the weak acids. At the pH = pKa, the buffer solution best resists addition of either acid and base, and hence has its greatest buffering ability. The weak acid can react with added strong base to form the weak conjugate base, and the conjugate base can react with added strong acid to form the weak acid (as shown below) so pH changes on addition of strong acid and base are minimized.

- addition of strong base produces weak conjugate base: $\mathrm{CH_3CO_2H + OH^- \rightarrow}$ [redacted] $\mathrm{+ H_2O}$
- addition of strong acid produces weak acid: $\mathrm{H_3O^+ +}$ [redacted] $\mathrm{\rightarrow CH_3CO_2H + H_2O}$

There are two simples ways to make a buffered solution. Consider an acetic acid/acetate buffer solution.

- make equal molar solution of acetic acid and sodium acetate, and mix them, monitoring pH with a pH meter, until the desired pH is reached (+/- 1 unit from the pKa).
- take a solution of acetic acid and add NaOH at substoichiometric amounts until the desired pH is reached (+/- 1 unit from the pKa). In this method you are forming the conjugate base, acetate, on addition of the weak base:

$$\mathrm{CH_3CO_2H + OH^- \rightarrow [redacted] + H_2O} \tag{3.2.7}$$

- EXTERNAL Buffers for pH control: Recipes based on pKas for acids, temperature, and ionic strength

Isoelectric Point

What happens if you have many ionizable groups in a single molecule, as is the case with a polypeptide or protein. Consider a

protein. At a pH of 2, all ionizable groups would be protonated, and the overall charge of the protein would be positive. (Remember, when carboxylic acid side chains are protonated, their net charge is 0.) As the pH is increased, the most acidic groups will start to deprotonate and the net charge will become less positive. At high pH, all the ionizable groups will become deprotonated in the strong base, and the overall charge of the protein will be negative. At some pH, then, the net charge will be 0. This pH is called the isoelectric point (pI). The pI can be determined by averaging the pKa values of the two groups which are closest to and straddle the pI. One of the online problems will address this in more detail

- LIst of pI and MW for proteins derived from 2D gels

Remember that pKa is really a measure of the equilibrium constant for the reaction. And of course, you remember that DGo = -RT ln Keq. Therefore, pKa is independent of concentration, and depends only on the intrinsic stability of reactants with respect to the products. This is true only AT A GIVEN SET OF CONDITIONS, SUCH AS T, P, AND SOLVENT CONDITIONS.

Consider, for example acetic acid, which in aqueous solution has a pKa of about 4.7. It is a weak acid, which dissociates only slightly to form H+ (in water the hydronium ion, H3O+, is formed) and acetate (Ac-). These ions are moderately stable in water, but reassociate readily to form the starting product. The pKa of acetic acid in 80% ethanol is 6.87. This can be accounted for by the decrease in stability of the charged products which are less shielded from each other by the less polar ethanol. Ethanol has a lower dielectric constant than does water. The pKa increases to 10.32 in 100% ethanol, and to a whopping 130 in air!

- A great interactive web site: Amino Acid Acid/Base Titration Curves
- pI calculator for any protein sequence
- Amino Acid Repository: Properties of Amino Acids

Contributors and Attributions

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

This page titled 3.2: Amino Acid Charges is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- A3. Amino Acid Charges by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

3.3: Cysteine Chemistry

Two cysteine side chains can covalently interact in a protein to produce a disulfide. Just as HOOH (hydrogen peroxide) is more oxidized than HOH, RSSR is the oxidized form and RSH is the reduced form of thiols.

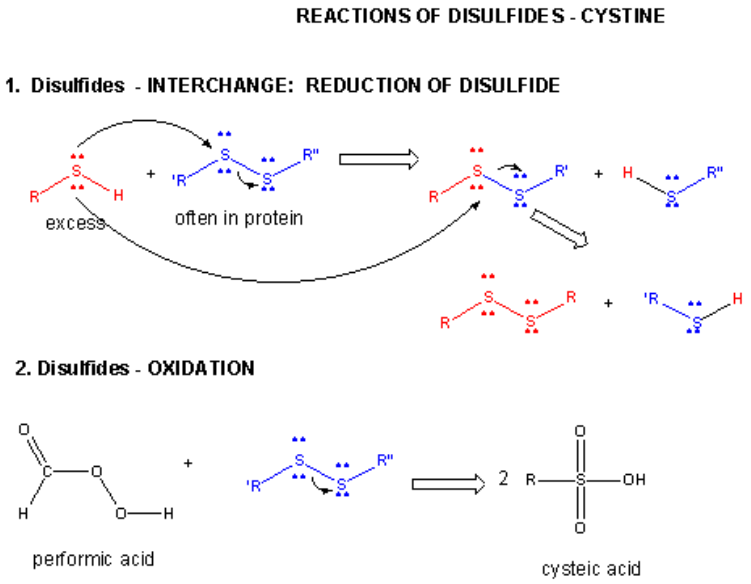


Figure: DISULFIDE - CYSTINE - REACTIONS

When a protein folds, two Cys side chains might approach each other, and form an intrachain disulfide bond. Likewise, two Cys side chains on separate proteins might approach each other and form an interchain disulfide. Such disulfides must be cleaved, and the chains separated before analyzing the sequence of the protein. The disulfide in protein can be cleaved by reducing agents such as beta-mercaptoethanol or dithiothreitol (DTT).

The inside of cells are maintained in a reduced environment by the presence of many "reducing" agents, such as the tripeptide g-glu-cys-gly (glutathione). Hence intracellular proteins usually do not contain disulfides, which are abundant in extracellular proteins (such as those found in blood) or in certain organelles such as the endoplasmic reticulum and mitochondrial intermembrane space where disulfides can be introduced.

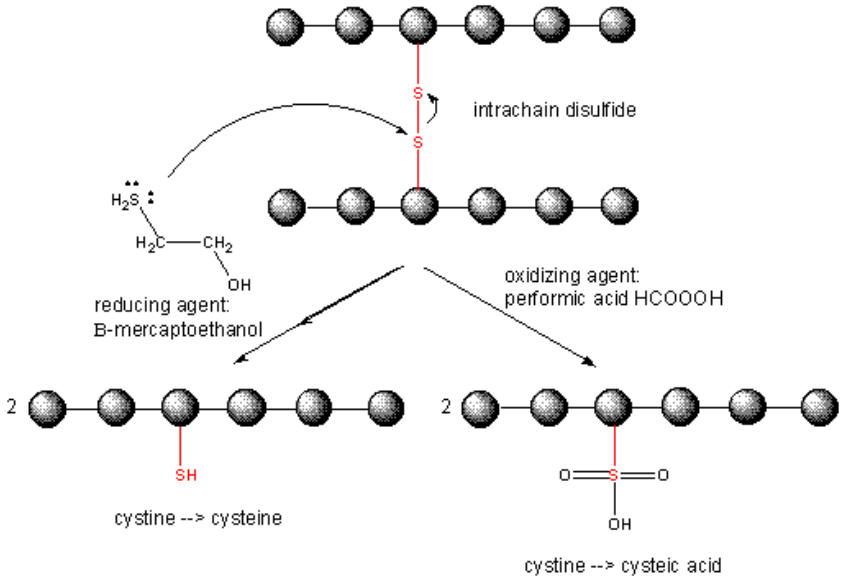


Figure: Cleaving Disulfide Bonds in Proteins

Cysteine Redox Chemistry

The sulfur in cysteine is redox-active and hence can exist in a wide variety of states, depending on the local redox environment and the presence of oxidizing and reducing agents. A potent oxidizing agent that can be made in cells is hydrogen peroxide, which can lead to more drastic and irreversible chemical modifications to the Cys side chains. If a reactive Cys is important to protein function, then the function of the protein can be modulated (sometimes reversibly, sometimes irreversibly) with various oxidizing agents, as shown in the figure below.

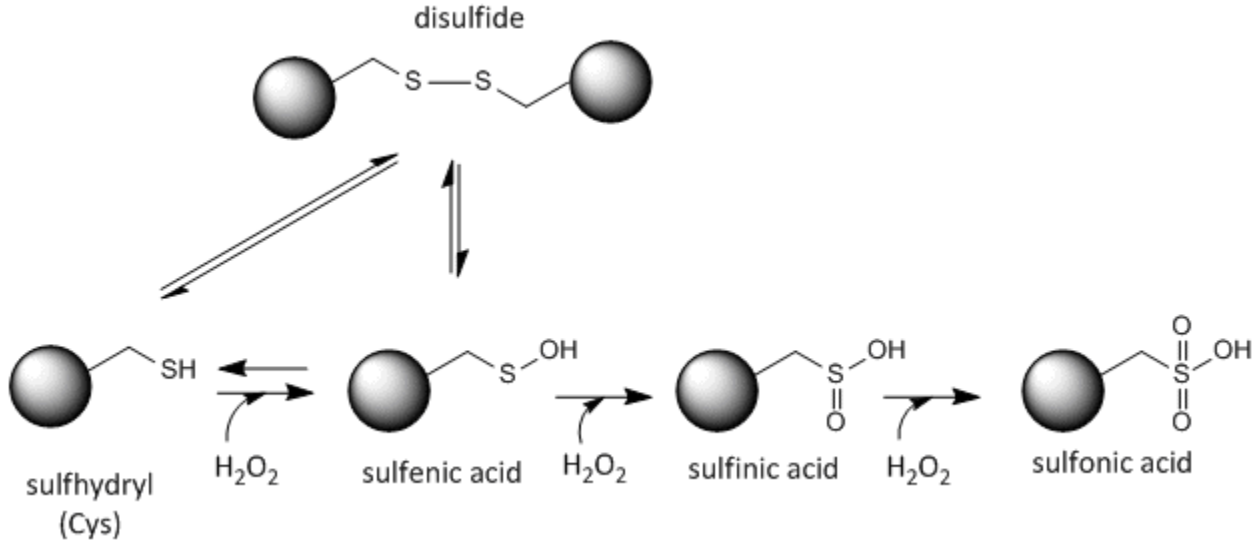


Figure: Redox state of Cysteine

Contributors and Attributions

- ◦ Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

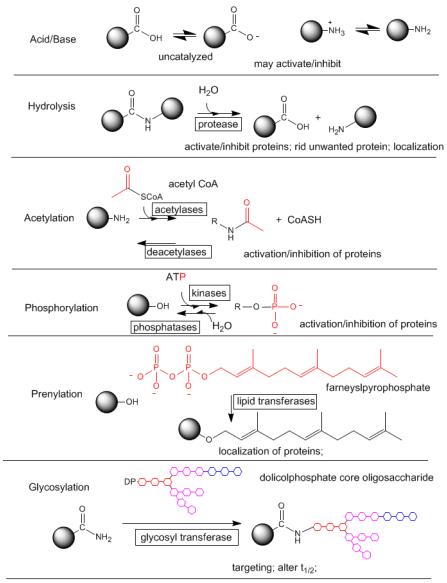
This page titled 3.3: Cysteine Chemistry is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- A7. Cysteine Chemistry by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

3.4: Post Translational Modification of Amino Acids

Amino acids in naturally occurring proteins are also subjected to chemical modification within cells. These modifications alter the properties of the amino acid that is modified, which can alter the structure and function of the protein. Most chemical modifications made to proteins within cells occur after the protein is synthesized in a process called translation. The resulting chemical changes are termed post-translational modifications.

Figure: Post-translational modification of proteins



Here is a list of post-translational modification from the Swiss Institute of Bioinformatics:

- PDOC00001 1 N-glycosylation site
- PDOC00004 1 cAMP- and cGMP-dependent protein kinase phosphorylation site
- PDOC00005 1 Protein kinase C phosphorylation site
- PDOC00006 1 Casein kinase II phosphorylation site
- PDOC00007 1 Tyrosine kinase phosphorylation site
- PDOC00008 1 N-myristoylation site
- PDOC00009 1 Amidation site
- PDOC00010 1 Aspartic acid and asparagine hydroxylation site
- PDOC00012 1 Phosphopantetheine attachment site
- PDOC00013 1 Prokaryotic membrane lipoprotein lipid attachment site
- PDOC00342 1 Prokaryotic N-terminal methylation site
- PDOC00266 1 Prenyl group binding site (CAAX box)
- PDOC00687 2 Intein N- and C-terminal splicing motif profiles

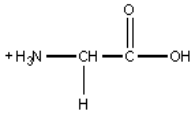
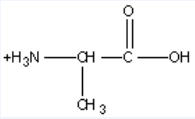
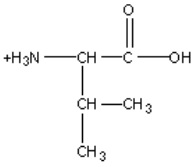
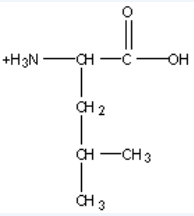
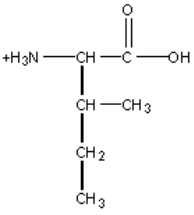
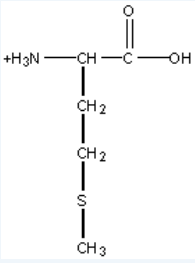
Contributors and Attributions

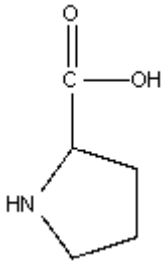
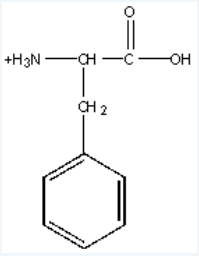
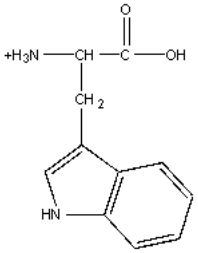
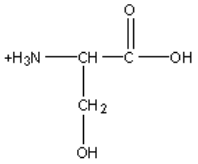
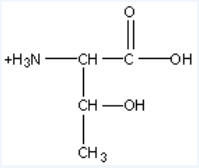
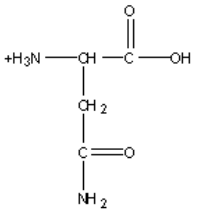
- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

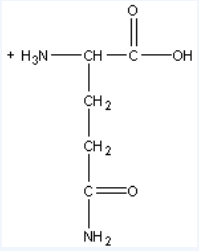
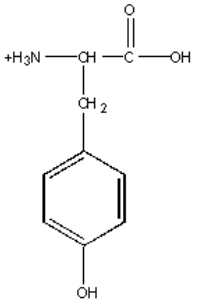
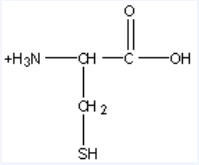
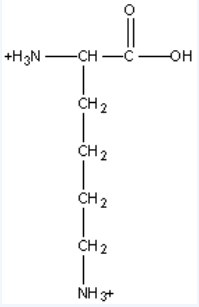
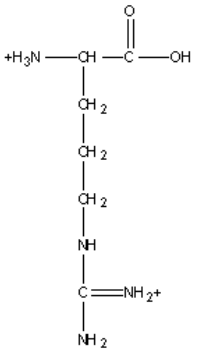
This page titled 3.4: Post Translational Modification of Amino Acids is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

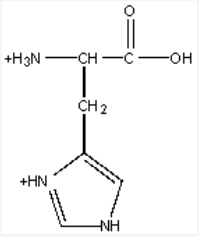
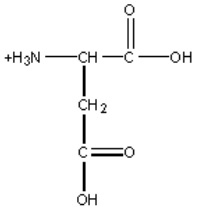
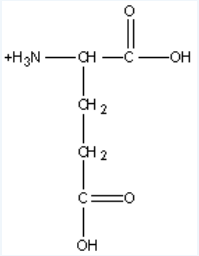
- A9. In Vivo Post Translational Modification of Amino Acids by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

3.1:Structure and Property of the Naturally-Occurring Amino Acids

Amino Acid	3 Letter Code	1 Letter Code	Structure	pK_{a1} (α-carboxyl)	pk_{a2} (α-amino)	pk_{a3} (side chain)
Nonpolar Side Chains						
Glycine	Gly	G		2.35	9.78	.
Alanine	Ala	A		2.35	9.87	.
Valine	Val	V		2.29	9.74	.
Leucine	Leu	L		2.33	9.74	.
Isoleucine	Ile	I		2.32	9.76	.
Methionine	Met	M		2.13	9.28	.

Proline	Pro	P		1.95	10.64	.
Phenylalanine	Phe	F		2.20	9.31	.
Tryptophan	Trp	W		2.46	9.41	.
Uncharged Polar Side Chains						
Serine	Ser	S		2.19	9.21	.
Threonine	Thr	T		2.09	9.10	.
Asparagine	Asn	N		2.14	8.72	.

Glutamine	Gln	Q		2.17	9.13	.
Tyrosine	Tyr	Y		2.20	9.21	10.46
Cysteine	Cys	C		1.92	10.70	8.37
Charged Polar Side Chains10.54						
Lysine	Lys	K		2.16	9.06	10.54
Arginine	Arg	R		1.82	8.99	12.48

Histidine	His	H		1.80	9.33	6.04
Aspartic Acid	Asp	D		1.99	9.90	3.90
Glutamic Acid	Glu	E		2.10	9.47	4.07

Contributors and Attributions

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

This page titled 3.1:Structure and Property of the Naturally-Occurring Amino Acids is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- Structure and Property of the Naturally-Occurring Amino Acids by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

4: PROTEIN STRUCTURE AND FUNCTION

4.1: From Amino Acids to Peptides
4.2: Levels of Protein Structure
4.3: Secondary Structure
4.4: Protein folding
4.5: Protein function, domains and cooperativity
4.6: Protein Aggregation
4.7: Prions and Disease

This page titled 4: Protein structure and function is shared under a license and was authored, remixed, and/or curated by LibreTexts.

4.1: From Amino Acids to Peptides

Proteins are polymers of a bifunctional monomer, the amino acid. The twenty common naturally-occurring amino acids each contain an a-carbon, an a-amino group, an a-carboxylic acid group, and an a-side chain or side group. These side chains (or R groups) may be either nonpolar, polar and uncharged, or charged, depending on the pH and pKa of the ionizable group. Two other amino acids occasionally appear in proteins. One is selenocysteine, which is found in Arachea, eubacteria, and animals. Another just recently found is pyrrolysine, found in Arachea. Shultz et al. have gone one step further. They have engineered bacterial to incorporate two new amino acids, O-methyl-tyrosine and p-aminophenylalanine. More recently, they (Chin et al.) have engineered the yeast strain Saccharomyces cerevisiae to incorporate five new unnatural amino acid (using the TAG nonsense codon and new, modified tRNA and tRNA synthetases) with keto groups that allow chemical modifications to the protein. We will concentrate only on the 20 abundant, naturally-occurring amino acids.

- Structure and Property of the Naturally-Occurring Amino Acids (Too large to include in text: print separately)
- EXTERNAL Learning Amino Acids Structure: YouTube - Part 1 | Part 2

Amino acids form polymers through a nucleophilic attack by the amino group of an amino acid at the electrophilic carbonyl carbon of the carboxyl group of another amino acid. The carboxyl group of the amino acid must first be activated to provide a better leaving group than OH-. (We will discuss this activation by ATP latter in the course.) The resulting link between the amino acids is an amide link which biochemists call a peptide bond. In this reaction, water is released. In a reverse reaction, the peptide bond can be cleaved by water (hydrolysis).

When two amino acids link together to form an amide link, the resulting structure is called a dipeptide. Likewise, we can have tripeptides, tetrapeptides, and other polypeptides. At some point, when the structure is long enough, it is called a protein. The average molecular weight of proteins in yeast is about 50,000 with about 450 amino acids. The large protein might be titin with molecular weight of about 3 million (about 27,0000 amino acids). A new class of very small proteins (30 or fewer amino acids and perhaps better named as polypeptides) called smORFs (small open reading frames) have recently been discovered to have significant biological activity (Science, doi:10.1126/science.1238802, 2013). These are encoded directly in the genome and are produced by the same processes that produce regular proteins (DNA transcription and RNA translation). They are not simply the result of selective cleavage of a larger protein into smaller peptide fragments.

There are many different ways to represent the structure of a polypeptide or protein. each showing differing amounts of information.

Figure: Different Representations of a Polypeptide (heptapeptide)

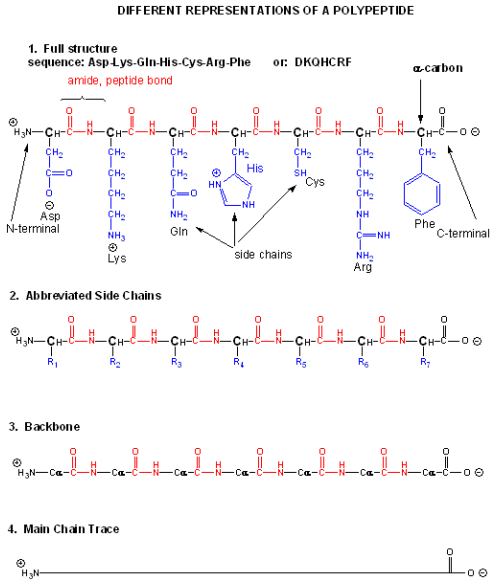
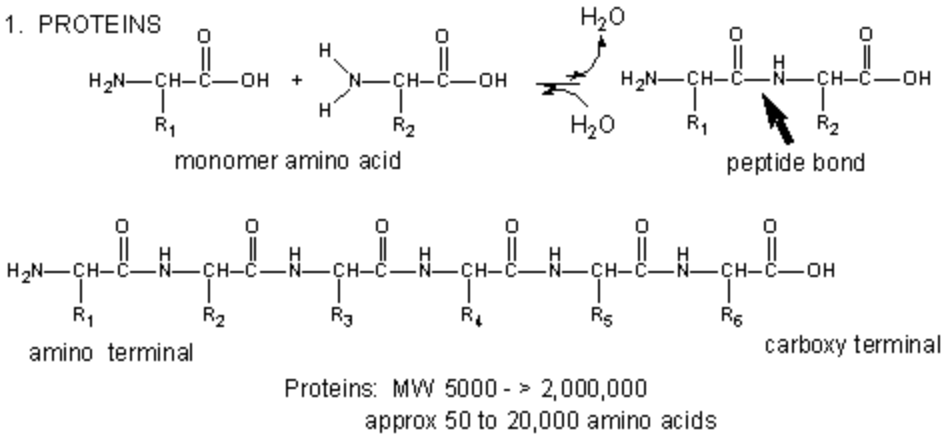


Figure: Amino Acids React to Form Proteins



(Note: above picture represents the amino acid in an unlikely protonation state with the weak acid protonated and the weak base deprotonated for simplicity in showing removal of water on peptide bond formation and the hydrolysis reaction.) Proteins are polymers of twenty naturally occurring amino acids. In contrast, nucleic acids are polymers of just 4 different monomeric nucleotides. Both the sequence of a protein and it's total length differentiate one protein from another. Just for an octapeptide, there are over 25 billion different possible arrangement of amino acids (20^8). Compare this to just 65536 different oligonucleotides (4 different monomeric deoxynucleotides) of 8 monomeric units, an 8mer (4^8). Hence the diversity of possible proteins is enormous.

Please consult the Jmol site below dealing with amino acids. Please learn the 3 letter code for the amino acids.



Jsmol: Amino Acids from Charles S. Gasser, UC Davis Jmol: Amino Acids

Contributors and Attributions

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

This page titled 4.1: From Amino Acids to Peptides is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- A1. Amino Acid Structure by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

4.2: Levels of Protein Structure

Source: BiochemFFA_2_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Proteins are the workhorses of the cell. Virtually everything that goes on inside of cells happens as a result of the actions of proteins. Among other things, protein enzymes catalyze the vast majority of cellular reactions, mediate signaling, give structure both to cells and to multicellular organisms, and exert control over the expression of genes. Life, as we know it, would not exist if there were no proteins. The versatility of proteins arises because of their varied structures.

Proteins are made by linking together amino acids, with each protein having a characteristic and unique amino acid sequence. To get a sense for the diversity of proteins that can be made using 20 different amino acids, consider that the number of different combinations possible with 20 amino acids is 20^n, where n=the number of amino acids in the chain. It becomes apparent that even a dipeptide made of just two amino acids joined together gives us 20^2 = 400 different combinations. If we do the calculation for a short peptide of 10 amino acids, we arrive at an enormous 10,240,000,000,000 combinations. Most proteins are much larger than this, making the possible number of proteins with unique amino acid sequences unimaginably huge.

Levels of Structure

The significance of the unique sequence, or order, of amino acids, known as the protein's primary structure, is that it dictates the 3-D conformation the folded protein will have. This conformation, in turn, will determine the function of the protein. We shall examine protein structure at four distinct levels (Figure 2.17) - 1) how sequence of the amino acids in a protein (primary structure) gives identity and characteristics to a protein (Figure 2.18); 2) how local interactions between one part of the polypeptide backbone and another affect protein shape (secondary structure); 3) how the polypeptide chain of a protein can fold to allow amino acids to interact with each other that are not close in primary structure (tertiary structure); and 4) how different polypeptide chains interact with each other within a multi-subunit protein (quaternary structure).

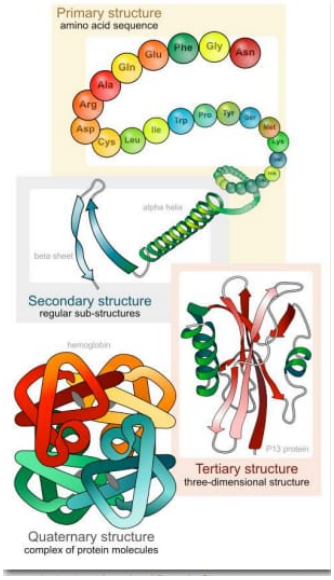


Figure 2.17 - Four Levels of Protein Structure

At this point, we should provide a couple of definitions. We use the term polypeptide to refer to a single polymer of amino acids. It may or may not have folded into its final, functional form. The term protein is sometimes used interchangeably with polypeptide, as in "protein synthesis". It is generally used, however, to refer to a folded, functional molecule that may have one or more subunits (made up of individual polypeptides). Thus, when we use the term protein, we are usually referring to a functional, folded polypeptide or peptides. Structure is essential for function. If you alter the structure, you alter the function - usually, but not always, this means you lose all function. For many proteins, it is not difficult to alter the structure.

Proteins are flexible, not rigidly fixed in structure. As we shall see, it is the flexibility of proteins that allows them to be amazing catalysts and allows them to adapt to, respond to, and pass on signals upon binding of other molecules or proteins. However, proteins are not infinitely flexible. There are constraints on the conformations that proteins can adopt and these constraints govern the conformations that proteins display.

Subtle changes

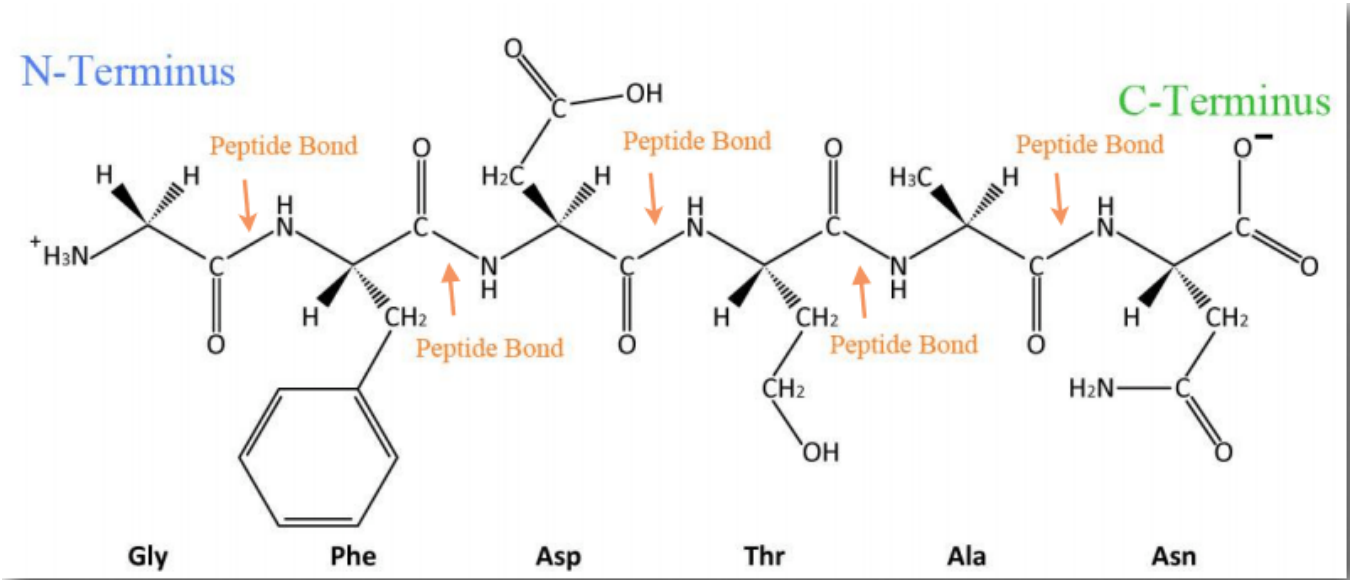


Figure 2.18 - Sequence of a simple polypeptide Wikipedia

Even very tiny, subtle changes in protein structure can give rise to big changes in the behavior of proteins. Hemoglobin, for example, undergoes an incredibly small structural change upon binding of one oxygen molecule, and that simple change causes the remainder of the protein to gain a considerably greater affinity for oxygen that the protein didn't have before the structural change.

Sequence, structure and function

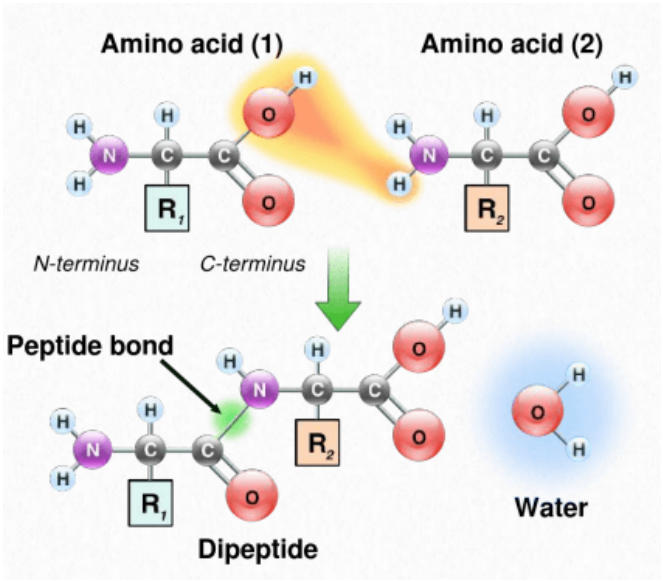


Figure 2.19 Linking of amino acids through peptide bond formation

As discussed earlier, the number of different amino acid sequences possible, even for short peptides, is very large. No two proteins with different amino acid sequences (primary structure) have identical overall structure. The unique amino acid sequence of a protein is reflected in its unique folded structure. This structure, in turn, determines the protein's function. This is why

mutations that alter amino acid sequence can affect the function of a protein.

Protein Synthesis

Synthesis of proteins occurs in the ribosomes and proceeds by joining the carboxyl terminus of the first amino acid to the amino terminus of the next one (Figure 2.19). The end of the protein that has the free α-amino group is referred to as the amino terminus or N-terminus. The other end is called the carboxyl terminus or C-terminus , since it contains the only free α-carboxyl group. All of the other α-amino groups and α-carboxyl groups are tied up in forming peptide Figure 2.19 Linking of amino acids through peptide bond formation bonds that join adjacent amino acids together. Proteins are synthesized starting with the amino terminus and ending at the carboxyl terminus.

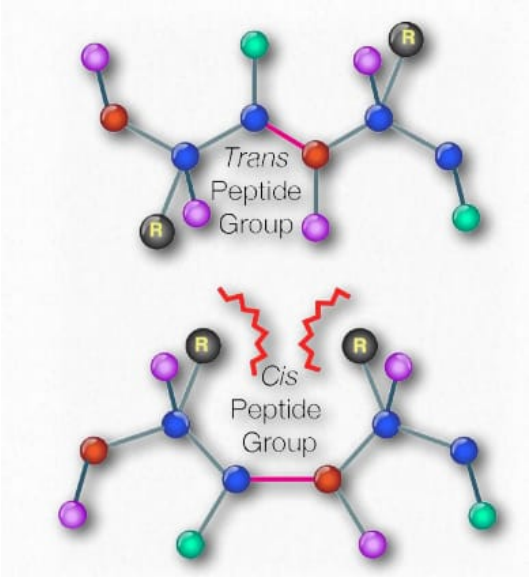


Figure 2.20 - Cis vs trans orientation of R-groups around peptide bond Image by Aleia Kim

Schematically, in Figure 2.18, we can see how sequential R-groups of a protein are arranged in an alternating orientation on either side of the polypeptide chain. Organization of R-groups in this fashion is not random. Steric hindrance can occur when consecutive R-groups are oriented on the same side of a peptide backbone (Figure 2.20)

Primary Structure

Primary structure is the ultimate determinant of the overall conformation of a protein. The primary structure of any protein arrived at its current state as a result of mutation and selection over evolutionary time. Primary structure of proteins is mandated by the sequence of DNA coding for it in the genome. Regions of DNA specifying proteins are known as coding regions (or genes).

The base sequences of these regions directly specify the sequence of amino acids in proteins, with a one-to-one correspondence between the codons (groups of three consecutive bases) in the DNA and the amino acids in the encoded protein. The sequence of codons in DNA, copied into messenger RNA, specifies a sequence of amino acids in a protein. (Figure 2.21).

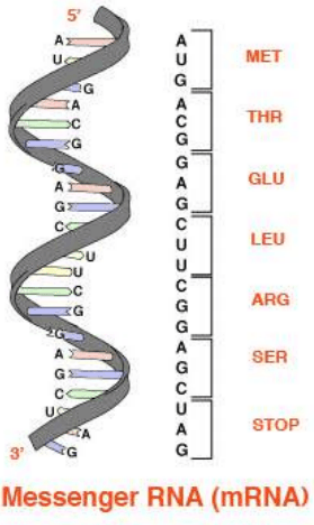


Figure 2.21 - From RNA to amino acids - the genetic code Wikipedia

The order in which the amino acids are joined together in protein synthesis starts defining a set of interactions between amino acids even as the synthesis is occurring. That is, a polypeptide can fold even as it is being made. The order of the R-group structures and resulting interactions are very important because early interactions affect later interactions. This is because interactions start establishing structures - secondary and tertiary. If a helical structure (secondary structure), for example, starts to form, the possibilities for interaction of a particular amino acid Rgroup may be different than if the helix had not formed (Figure 2.22). R-group interactions can also cause bends in a polypeptide sequence (tertiary structure) and these bends can create (in some cases) opportunities for interactions that wouldn't have been possible without the bend or prevent (in other cases) similar interaction possibilities.

Secondary Structure

As protein synthesis progresses, interactions between amino acids close to each other begin to occur, giving rise to local patterns called secondary structure. These secondary structures include the well known α- helix and β-strands. Both were predicted by Linus Pauling, Robert Corey, and Herman Branson in 1951. Each structure has unique features.

α-helix

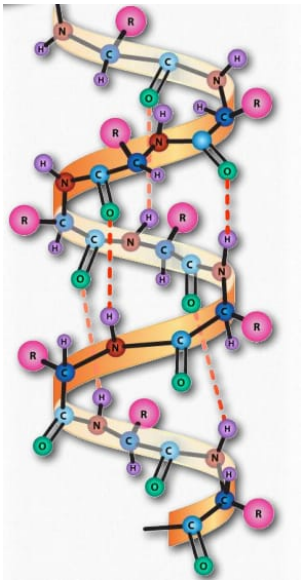


Figure 2.22 - The α-helix. Hydrogen bonds (dotted lines) between the carbonyl oxygen and the amine hydrogen stabilize the structure. Image by Aleia Kim

The α-helix has a coiled structure, with 3.6 amino acids per turn of the helix (5 helical turns = 18 amino acids). Helices are predominantly right handed - only in rare cases, such as in sequences with many glycines can left handed α- helices form. In the α-helix, hydrogen bonds form between C=O groups and N-H groups in the polypeptide backbone that are four amino acids distant. These hydrogen bonds are the primary forces stabilizing the α-helix.

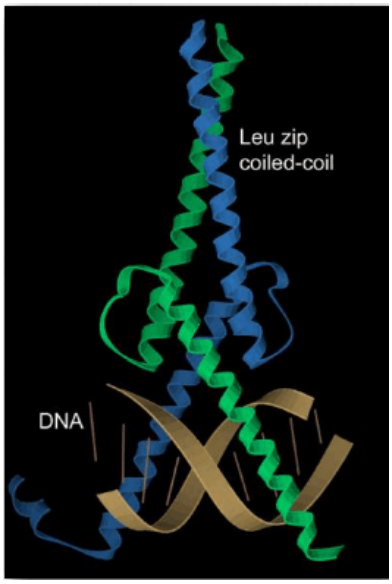


Figure 2.23 - α-helices in a protein with a leucine zipper structural domain. The α-helices are shown in blue and green and are bound to a DNA double helix in brown.

We use the terms rise, repeat, and pitch to describe the parameters of any helix. The repeat is the number of residues in a helix before it begins to repeat itself. For an α-helix, the repeat is 3.6 amino acids per turn of the helix. The rise is the distance the helix elevates with addition of each residue. For an α-helix, this is 0.15 nm per amino acid. The pitch is the distance between complete turns of the helix. For an α-helix, this is 0.54 nm. The stability of an α-helix is enhanced by the presence of the amino acid aspartate.



Figure 2.24 - α-helix sculpture outside Linus Pauling's boyhood home Wikipedia

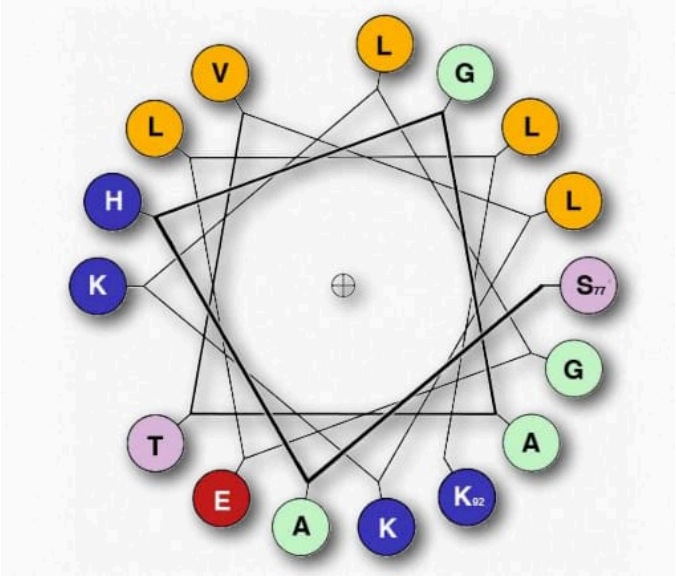


Figure 2.25 - Helical Wheel Representation of an α-Helix. The one letter genetic code is used. The helix starts at Serine #77 at the right and ends at lysine #92 in the lower right. Hydrophobic amino acids are shown in yellow and ionizing amino acids are shown in blue. Hydrophobic amino acids tend to interact with each other and not with ionizing amino acids. Wikipedia

β strand/sheet

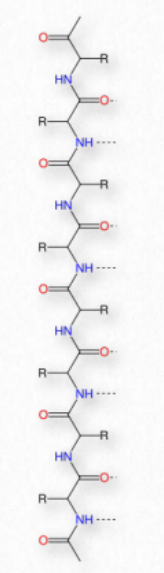


Figure 2.26 - β strand

A helix is, of course, a three-dimensional object. A flattened form of helix in two dimensions is a common description for a β-strand. Rather than coils, β-strands have bends and these are sometimes referred to as pleats, like the pleats in a curtain. β-strands can be organized to form elaborately organized structures, such as sheets, barrels, and other arrangements.

Higher order β-strand structures are sometimes called supersecondary structures), since they involve interactions between amino acids not close in primary sequence. These structures, too, are stabilized by hydrogen bonds between carbonyl oxygen atoms and hydrogens of amine groups in the polypeptide backbone (Figure 2.28). In a higher order structure, strands can be arranged parallel (amino to carboxyl orientations the same) or anti-parallel (amino to carboxyl orientations opposite of each other (in Figure 2.27, the direction of the strand is shown by the arrowhead in the ribbon diagrams).

Turns

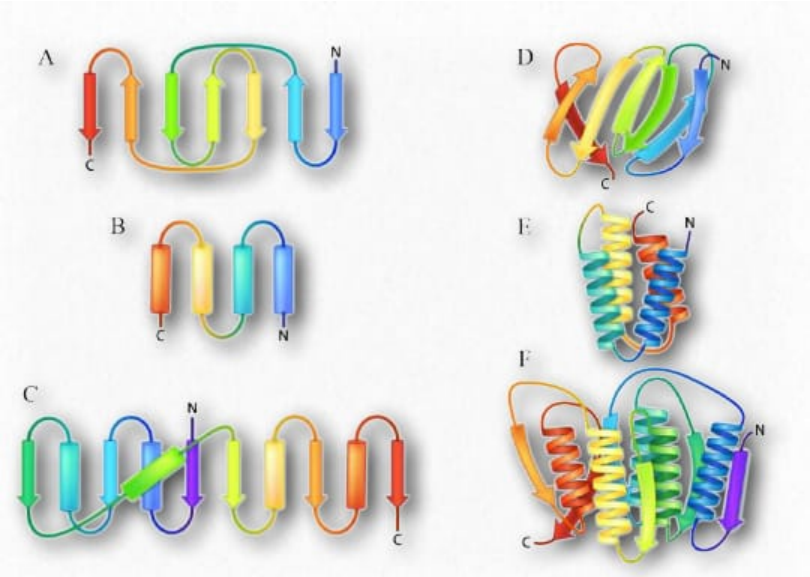


Figure 2.27 - Ribbon depictions of supersecondary β-sheets (A-D) and α-helix arrangements (E-F) Image by Aleia Kim

Turns (sometimes called reverse turns) are a type of secondary structure that, as the name suggests, causes a turn in the structure of a polypeptide chain. Turns give rise to tertiary structure ultimately, causing interruptions in the secondary structures (α- helices and β-strands) and often serve as connecting regions between two regions of secondary structure in a protein. Proline and glycine play common roles in turns, providing less flexibility (starting the turn) and greater flexibility (facilitating the turn), respectively.

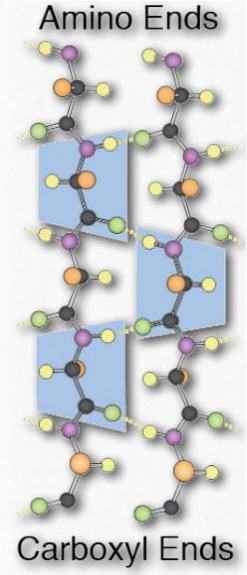


Figure 2.28 - Components of a β-sheet in a parallel arrangement. H-bonds in yellow. Image by Aleia Kim

There are at least five types of turns, with numerous variations of each giving rise to many different turns. The five types of turns are

• δ-turns - end amino acids are separated by one peptide bond

• γ-turns - separation by two peptide bonds

•β-turns - separation by three peptide bonds

•α-turns - separation by four peptide bonds

•π-turns - separation by five bonds

Of these, the β-turns are the most common form and the δ-turns are theoretical, but unlikely, due to steric limitations. Figure 2.29 depicts a β- turn.

3_{10} helices

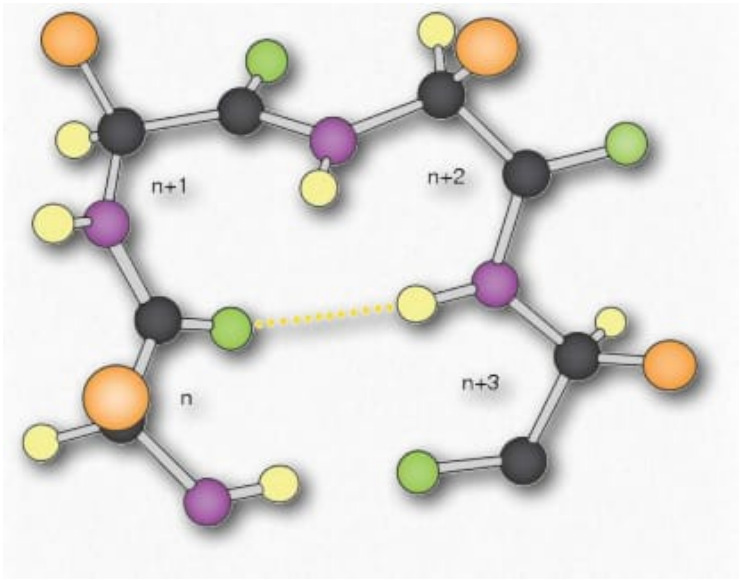


Figure 2.29 - β-turn. R-groups are shown in orange, hydrogens in yellow, carbons in charcoal, nitrogens in purple, and oxygens in green. A stabilizing hydrogen bond is indicated with the dotted line. Image by Aleia Kim

In addition to the α-helix, β-strands, and various turns, other regular, repeating structures are seen in proteins, but occur much less commonly. The 3_{10} helix is the fourth most abundant secondary structure in proteins, constituting about 10-15% of all helices. The helix derives its name from the fact that it contains 10 amino acids in 3 turns. It is right-handed. Hydrogen bonds form between amino acids that are three residues apart. Most commonly, the 3_{10} helix appears at the amine or carboxyl end of an α-helix. Like the α-helix, the 3_{10} helix is stabilized by the presence of aspartate in its sequence.

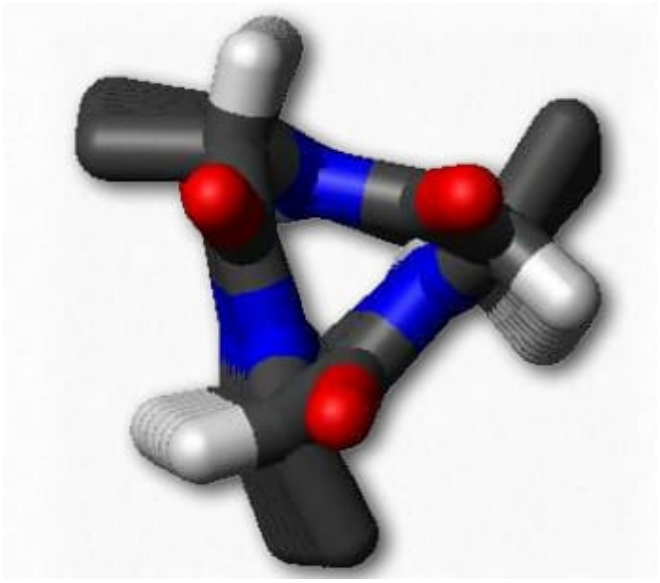


Figure 2.30 - Top view of a 310 Helix. Carbonyl groups are in red and pointed upwards. Note the almost perfect 3-fold symmetry Wikipedia

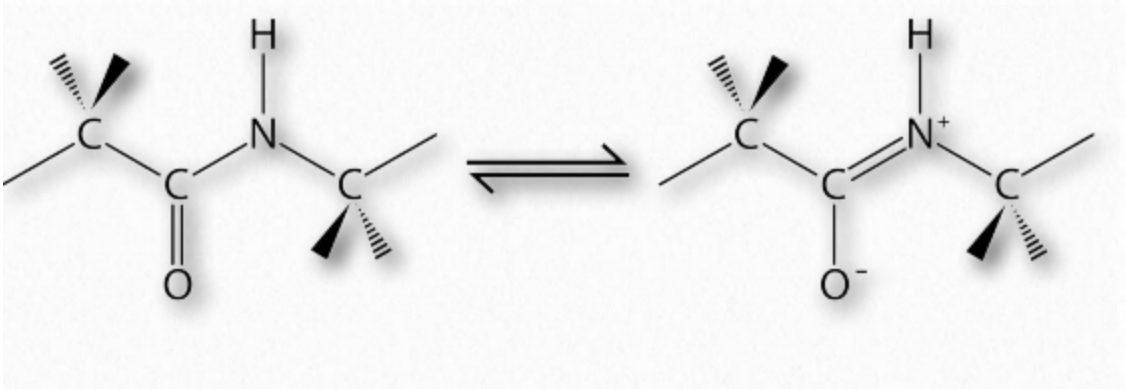


Figure 2.31 - Resonance of the peptide bond Wikipedia

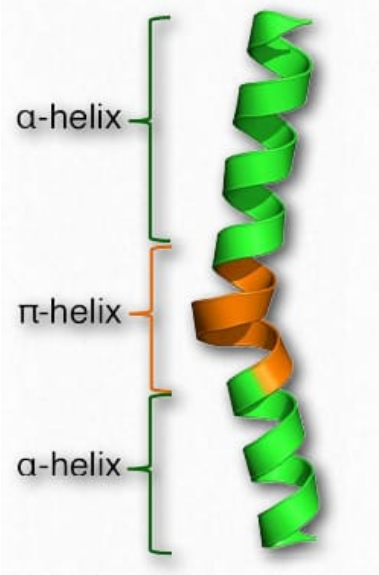


Figure 2.32 - π helix Wikipedia

π-helices

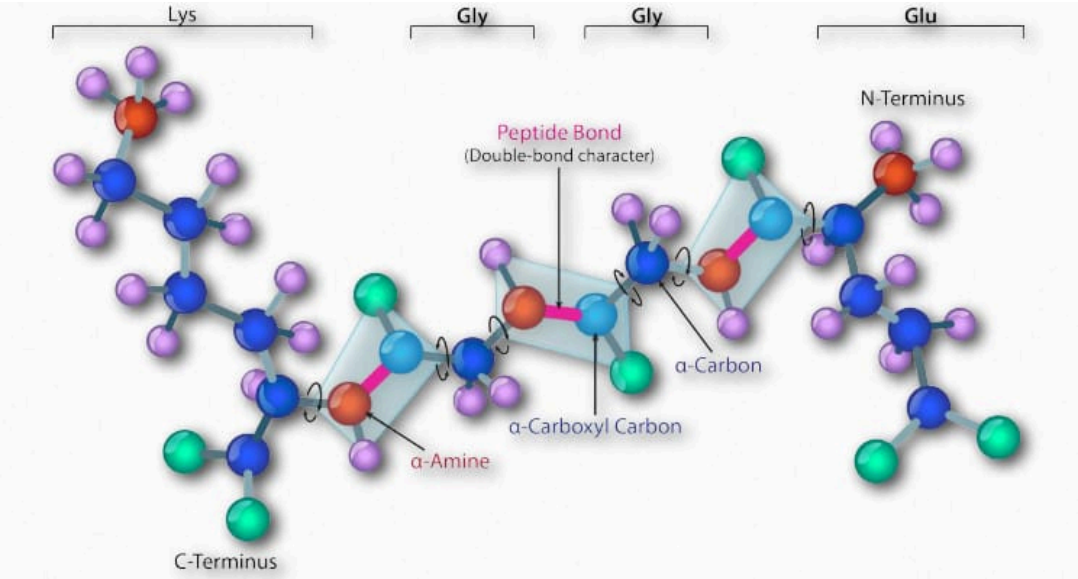


Figure 2.33 - Planes (light blue) defined by the double-bonded character of the peptide bond Image by Aleia Kim

A π-helix may be thought of as a special type of α- helix. Some sources describe it as an α-helix with an extra amino acid stuck in the middle of it (Figure 2.32). π-helices are not exactly rare, occurring at least once in as many as 15% of all proteins. Like the α-helix, the π-helix is right-handed, but where the α-helix has 18 amino acids in 5 turns, the π-helix has 22 amino acids in 5 turns. π-helices typically do not stretch for very long distances. Most are only about 7 amino acids long and the sequence almost always occurs in the middle of an α-helical region.

Ramachandran plots

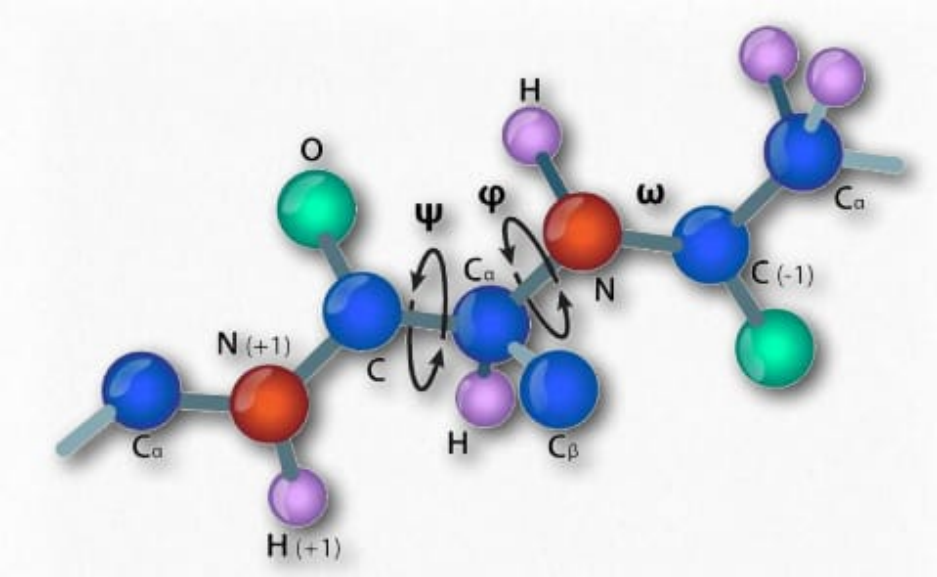


Figure 2.34 - ω, ψ, and φ rotational angles in a peptide Image by Aleia Kim

In 1963, G.N. Ramachandran, C. Ramakrishnan, and V. Sasisekharan described a novel way to describe protein structure. If one considers the backbone of a polypeptide chain, it consists of a repeating set of three bonds. Sequentially (in the amino to carboxyl direction) they are 1) a rotatable bond (ψ) between α-carbon and α-carboxyl preceding the peptide bond (see HERE), 2) a non-rotatable peptide bond (ω) between the α-carboxyl and α-amine groups), and 3) a rotatable bond (φ) between the α-amine and α-carbon following the peptide bond (see HERE). Note in Figures 2.33 and 2.34 that the amino to carboxyl direction is right to left.

The presence of the carbonyl oxygen on the α-carboxyl group allows the peptide bond to exist as a resonant structure, meaning that it behaves some of the time as a double bond. Double bonds cannot, of course, rotate, but the bonds on either side of it have some freedom of rotation. The φ and ψ angles are restricted to certain values, because some angles will result in steric hindrance. In addition, each type of secondary structure has a characteristic range of values for φ and ψ.

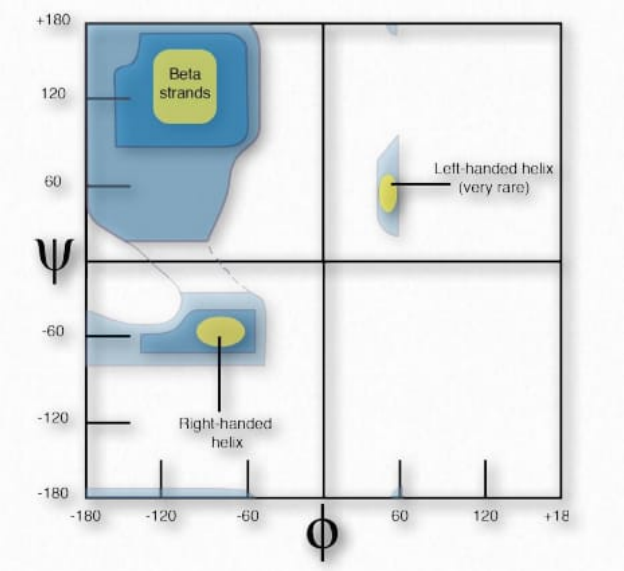


Figure 2.35 - Theoretical Ramachandran plot Image by Penelope Irving

Ramachandran and colleagues made theoretical calculations of the energetic stability of all possible angles from 0° to 360° for each of the φ and ψ angles and plotted the results on a Ramachandran Plot (also called a φ-ψ plot), delineating regions of angles that were theoretically the most stable (Figure 2.35).

Three primary regions of stability were identified, corresponding to φ-ψ angles of β-strands (top left), right handed α- helices (bottom left), and lefthanded α-helices (upper right). The plots of predicted stability are remarkably accurate when compared to φ-ψ angles of actual proteins.

Secondary structure prediction

Amino Acid	α helix	Reverse turn	β sheet
Ala	1.41	0.82	0.72
Arg	1.21	0.90	0.84
Asn	0.76	1.34	0.48
Asp	0.99	1.24	0.39
Cys	0.66	0.54	1.40
Gln	1.27	0.84	0.98
Glu	1.59	1.01	0.52
Gly	0.43	1.77	0.58
His	1.05	0.81	0.80
Ile	1.09	0.47	1.67
Leu	1.34	0.57	1.22
Lys	1.23	1.07	0.69
Met	1.30	0.52	1.14
Phe	1.16	0.59	1.33
Pro	0.34	1.32	0.31
Ser	0.57	1.22	0.96
Thr	0.76	0.96	1.17
Trp	1.02	0.65	1.35
Tyr	0.74	0.76	1.45
Val	0.90	0.41	1.87

Table 2.3 - Relative tendencies of each amino acid to be in a secondary structure. Higher values indicate greater tendency Image by Penelope Irving

By comparing primary structure (amino acid sequences) to known 3D protein structures, one can tally each time an amino acid is found in an α-helix, β-strand/sheet, or a turn. Computer analysis of thousands of these sequences allows one to assign a likelihood of any given amino acid appearing in each of these structures. Using these tendencies, one can, with up to 80% accuracy, predict regions of secondary structure in a protein based solely on amino acid sequence.

This is seen in Table 2.3. Occurrence in primary sequence of three consecutive amino acids with relative tendencies higher than one is an indicator that that region of the polypeptide is in the corresponding secondary structure. An online resource for predicting secondary structures called PSIPRED is available HERE.

Hydrophobicity

Amino Acid Hydropathy Scores

Amino Acid	One Letter Code	Hydropathy Score
Isoleucine	I	4.5
Valine	V	4.2
Leucine	L	3.8
Phenylalanine	F	2.8
Cysteine	C	2.5
Methionine	M	1.9
Alanine	A	1.8
Glycine	G	-0.4
Threonine	T	-0.7
Tryptophan	W	-0.9
Serine	S	-0.8
Tyrosine	Y	-1.3
Proline	P	-1.6
Histidine	H	-3.2
Glutamic acid	E	-3.5
Glutamine	Q	-3.5
Aspartic acid	D	-3.5
Asparagine	N	-3.5
Lysine	K	-3.9
Arginine	R	-4.5

Table 2.4 - Hydropathy Scores

The chemistry of amino acid Rgroups affects the structures they are most commonly found in. Subsets of their chemical properties can give clues to structure and, sometimes, cellular location. A prime example is the hydrophobicity (wateravoiding tendencies) of some Rgroups. Given the aqueous environment of the cell, such R-groups are not likely to be on the outside surface of a folded protein.

However, this rule does not hold for regions of protein that may be embedded within the lipid bilayers of cellular/ organelle membranes. This is because the region of such proteins that form the transmembrane domains are are buried in the hydrophobic environment in the middle of the lipid bilayer.

Not surprisingly, scanning primary sequences for specifically sized/spaced stretches of hydrophobic amino acids can help to identify proteins found in membranes. Table 2.4 shows hydrophobicity values for R-groups of the amino acids. In this set, the scale runs from positive values (hydrophobic) to negative values (hydrophilic). A KyteDoolittle Hydropathy plot for the RET protooncogene membrane protein is shown in Figure 2.36. Two regions of the protein are very hydrophobic as can be seen from the peaks near amino acids 5-10 and 630-640. Such regions might be reasonably expected to be situated either within the interior of the folded protein or to be part of transmembrane domains.

Random coils

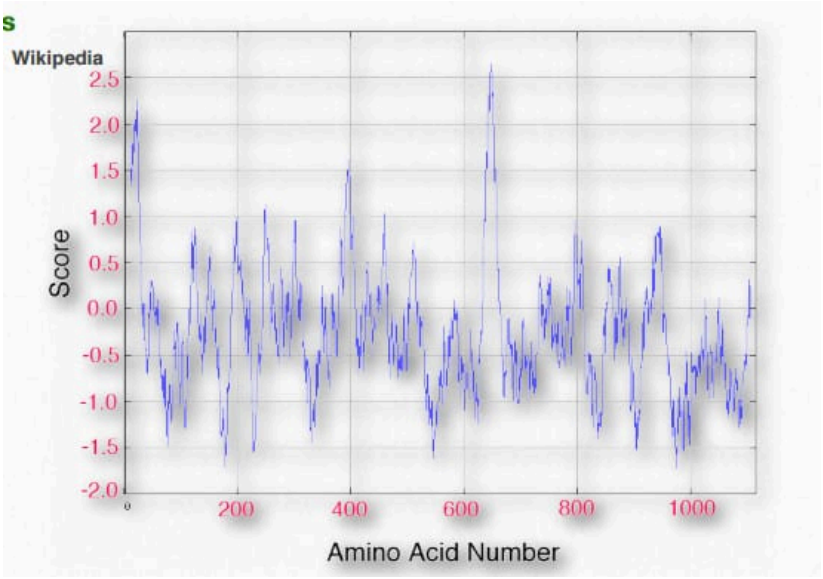


Figure 2.36 Kyte-Doolittle hydropathy plot for the RET protooncogene Wikipedia

Some sections of a protein assume no regular, discernible structure and are sometimes said to lack secondary structure, though they may have hydrogen bonds. Such segments are described as being in random coils and may have fluidity to their structure that results in them having multiple stable forms. Random coils are identifiable with spectroscopic methods, such as circular dichroism Wikipedia and nuclear magnetic resonance (NMR) in which distinctive signals are observed. See also metamorphic proteins (HERE) and intrinsically disordered proteins (HERE).

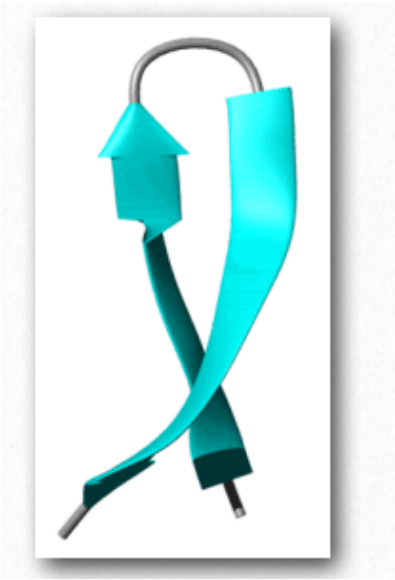


Figure 2.37 - Ribbon depiction of a β-hairpin. Shown are two β strands in turquoise interacting with each other.

Supersecondary structure

Another element of protein structure is harder to categorize because it incorporates elements of secondary and tertiary structure. Dubbed supersecondary structure (or structural motifs), these structures contain multiple nearby secondary structure components arranged in a specific way and that appear in multiple proteins. Since there are many ways of making secondary structures from different primary structures, so too can similar motifs arise from different primary sequences. An example of a structural motif is shown in Figure 2.37.

Tertiary structure

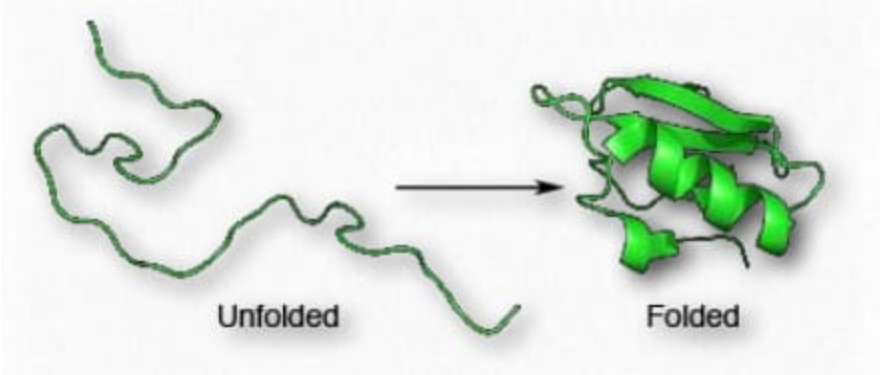


Figure 2.38 - Folding of a polypeptide chain

Proteins are distinguished from each other by the sequence of amino acids comprising them. The sequence of amino acids of a protein determines protein shape, since the chemical properties of each amino acid are forces that give rise to intermolecular interactions to begin to create secondary structures, such as α-helices and β-strands. The sequence also defines turns and random coils that play important roles in the process of protein folding.

Since shape is essential for protein function, the sequence of amino acids gives rise to all of the properties a protein has. As protein synthesis proceeds, individual components of secondary structure start to interact with each other, giving rise to folds that bring amino acids close together that are not near each other in primary structure (Figure 2.38). At the tertiary level of structure, interactions among the R-groups of the amino acids in the protein, as well as between the polypeptide backbone and amino acid side groups play a role in folding.

Globular proteins

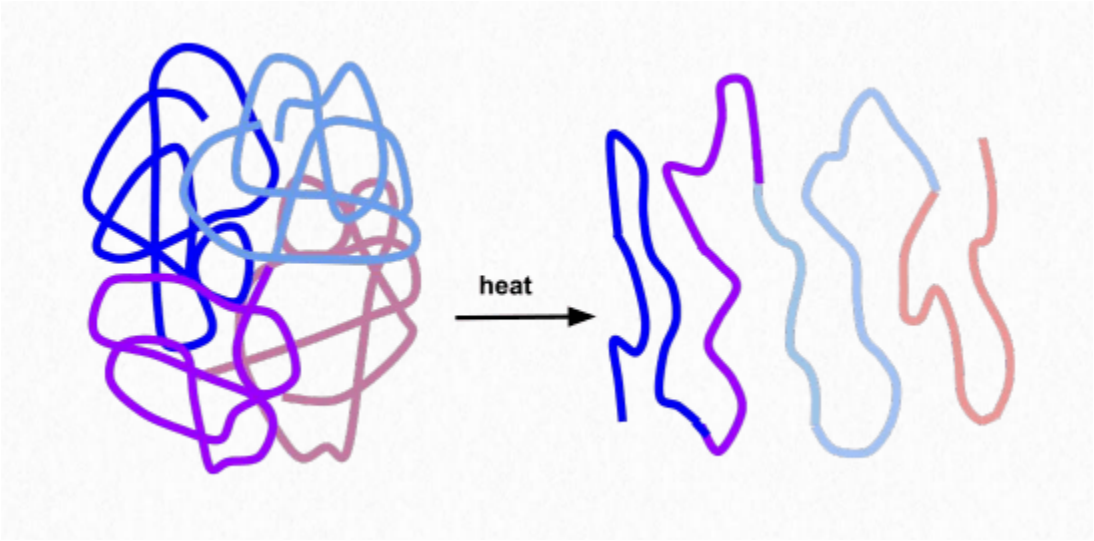


Figure 2.39 - Unfolding (denaturation) of a protein Wikipedia

Folding gives rise to distinct 3-D shapes in proteins that are non-fibrous. These proteins are called globular. A globular protein is stabilized by the same forces that drive its formation. These include ionic interactions, hydrogen bonding, hydrophobic forces, ionic bonds, disulfide bonds and metallic bonds. Treatments such as heat, pH changes, detergents, urea and mercaptoethanol overpower the stabilizing forces and cause a protein to unfold, losing its structure and (usually) its function (Figure 2.39). The ability of heat and detergents to denature proteins is why we cook our food and wash our hands before eating - such treatments denature the proteins in the microorganisms on our hands. Organisms that live in environments of high temperature (over 50°C) have proteins with changes in stabilizing forces - additional hydrogen bonds, additional salt bridges (ionic interactions), and compactness may all play roles in keeping these proteins from unfolding.

Protein stabilizing forces

Before considering the folding process, let us consider some of the forces that help to stabilize proteins.

Hydrogen bonds

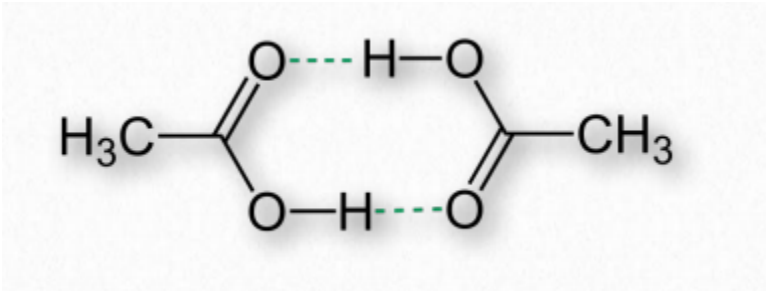


Figure 2.40 - Hydrogen bonds (dotted lines) between two molecules of acetic acid

Hydrogen bonds arise as a result of partially charged hydrogens found in covalent bonds. This occurs when the atom the hydrogen is bonded to has a greater electronegativity than hydrogen itself does, resulting in hydrogen having a partial positive charge because it is not able to hold electrons close to itself (Figure 2.40).

Hydrogen partially charged in this way is attracted to atoms, such as oxygen and nitrogen that have partial negative charges, due to having greater electronegativities and thus holding electrons closer to themselves. The partially positively charged hydrogens are called donors, whereas the partially negative atoms they are attracted to are called acceptors. (See Figure 1.30).

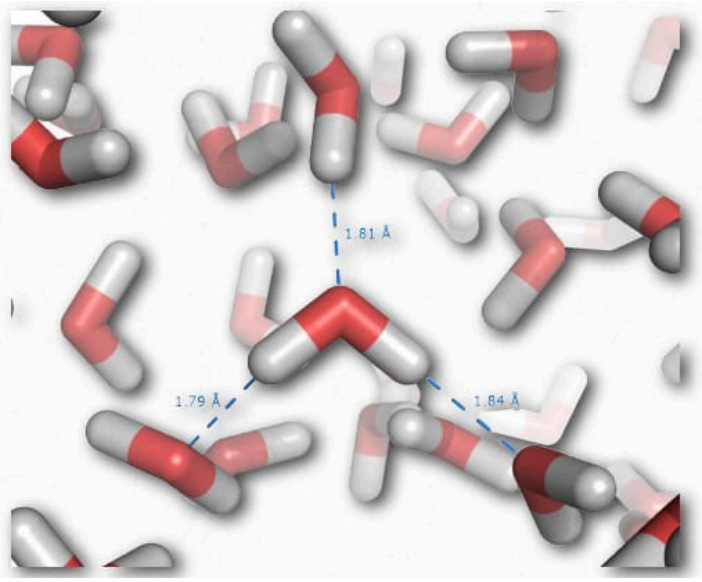


Figure 2.41 - Hydrogen bonding in liquid water Wikipedia

Individual hydrogen bonds are much weaker than a covalent bond, but collectively, they can exert strong forces. Consider liquid water, which contains enormous numbers of hydrogen bonds (Figure 2.41). These forces help water to remain liquid at room temperature. Other molecules lacking hydrogen bonds of equal or greater molecular weight than water, such as methane or carbon dioxide, are gases at the same temperature. Thus, the intermolecular interactions between water molecules help to “hold” water together and remain a liquid. Notably, only by raising the temperature of water to boiling are the forces of hydrogen bonding overcome, allowing water to become fully gaseous.

Hydrogen bonds are important forces in biopolymers that include DNA, proteins, and cellulose. All of these polymers lose their native structures upon boiling. Hydrogen bonds between amino acids that are close to each other in primary structure can give rise to regular repeating structures, such as helices or pleats, in proteins (secondary structure).

Ionic interactions

Ionic interactions are important forces stabilizing protein structure that arise from ionization of R-groups in the amino acids comprising a protein. These include the carboxyl amino acids (HERE), the amine amino acids as well as the sulfhydryl of cysteine and sometimes the hydroxyl of tyrosine.

Hydrophobic forces

Hydrophobic forces stabilize protein structure as a result of interactions that favor the exclusion of water. Non-polar amino acids (commonly found in the interior of proteins) favor associating with each other and this has the effect of excluding water. The excluded water has a higher entropy than water interacting with the hydrophobic side chains. This is because water aligns itself very regularly and in a distinct pattern when interacting with hydrophobic molecules.

When water is prevented from having these kinds of interactions, it is much more disordered that it would be if it could associate with the hydrophobic regions. It is partly for this reason that hydrophobic amino acids are found in protein interiors - so they can exclude water and increase entropy.

Disulfide bonds

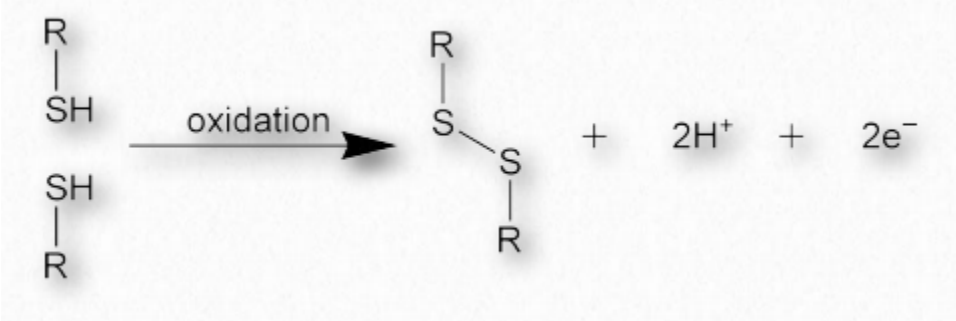


Figure 2.42 - Formation of a disulfide bond

Disulfide bonds, which are made when two sulfhydryl side-chains of cysteine are brought into close proximity, covalently join together different protein regions and can give great strength to the overall structure (Figures 2.42 & 2.43). An Ode to Protein Structure by Kevin Ahern The twenty wee amino A's Define a protein many ways Their order in a peptide chain Determines forms that proteins gain And when they coil, it leaves me merry Cuz that makes structures secondary It's tertiary, I am told That happens when a protein folds But folded chains are downright scary When put together quaternary They're nature's wonders, that's for sure Creating problems, making cures A fool can fashion peptide poems But proteins come from ribosoems These joined residues of cysteine are sometimes referred to as cystine. Disulfide bonds are the strongest of the forces stabilizing protein structure.

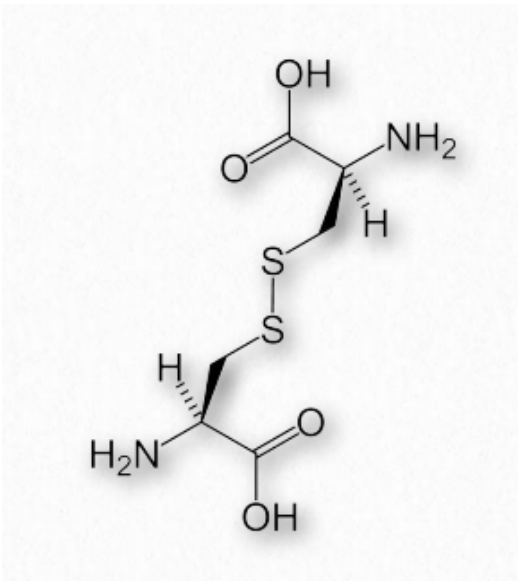


Figure 2.43 - Cystine - Two cysteines joined by a disulfide bond

van der Waals forces

van der Waals forces is a term used to describe various weak interactions, including those caused by attraction between a polar molecule and a transient dipole, or between two temporary dipoles. van der Waals forces are dynamic because of the fluctuating

nature of the attraction, and are generally weak in comparison to covalent bonds, but can, over very short distances, be significant.

Post-translational modifications

Post-translational modifications can result in formation of covalent bonds stabilizing proteins as well. Hydroxylation of lysine and proline in strands of collagen can result in cross-linking of these groups and the resulting covalent bonds help to strengthen and stabilize the collagen.

Folding models

Two popular models of protein folding are currently under investigation. In the first (diffusion collision model), a nucleation event begins the process, followed by secondary structure formation. Collisions between the secondary structures (as in the β-hairpin in Figure 2.37) allow for folding to begin. By contrast, in the nucleation-condensation model, the secondary and tertiary structures form together.

Folding in proteins occurs fairly rapidly (0.1 to 1000 seconds) and can occur during synthesis - the amino terminus of a protein can start to fold before the carboxyl terminus is even made, though that is not always the case.

Folding process

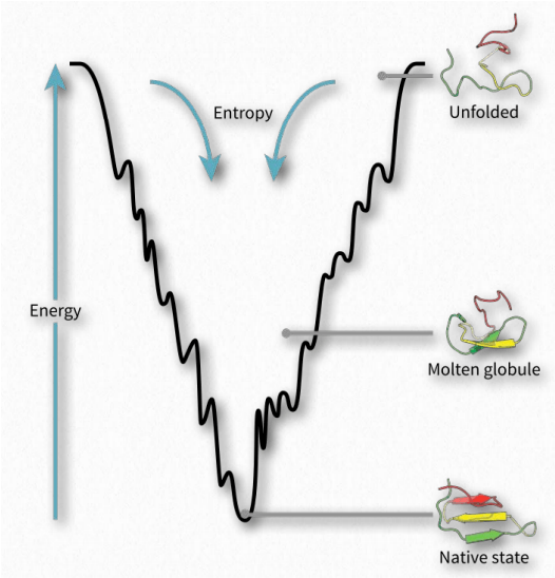


Figure 2.44 Folding funnel energy model of folding Wikipedia

Protein folding is hypothesized to occur in a “folding funnel” energy landscape in which a folded protein’s native state corresponds to the minimal free energy possible in conditions of the medium (usually aqueous solvent) in which the protein is dissolved. As seen in the diagram (Figure 2.44), the energy funnel has numerous local minima (dips) in which a folding protein can become trapped as it moves down the energy plot. Other factors, such as temperature, electric/magnetic fields, and spacial considerations likely play roles.

If external forces affect local energy minima during folding, the process and end-product can be influenced. As the speed of a car going down a road will affect the safety of the journey, so too do energy considerations influence and guide the folding process, resulting in fully functional, properly folded proteins in some cases and misfolded “mistakes” in others.

Getting stuck

As the folding process proceeds towards an energy minimum (bottom of the funnel in Figure 2.44), a protein can get “stuck” in any of the local minima and not reach the final folded state. Though the folded state is, in general, more organized and therefore has reduced entropy than the unfolded state, there are two forces that overcome the entropy decrease and drive the process forward.

The first is the magnitude of the decrease in energy as shown in the graph. Since $\Delta G = \Delta H - T\Delta S$, a decrease in ΔH can

overcome a negative ΔS to make ΔG negative and push the folding process forward. Favorable (decreased) energy conditions arise with formation of ionic bonds, hydrogen bonds, disulfide bonds, and metallic bonds during the folding process. In addition, the hydrophobic effect increases entropy by allowing hydrophobic amino acids in the interior of a folded protein to exclude water, thus countering the impact of the ordering of the protein structure by making the ΔS less negative.

Structure prediction

Computer programs are very good at predicting secondary structure solely based on amino acid sequence, but struggle with determining tertiary structure using the same information. This is partly due to the fact that secondary structures have repeating points of stabilization based on geometry and any regular secondary structure (e.g., α-helix) varies very little from one to another. Folded structures, though, have an enormous number of possible structures as shown by Levinthal's Paradox.

Spectroscopy

Because of our inability to accurately predict tertiary structure based on amino acid sequence, proteins structures are actually determined using techniques of spectroscopy. In these approaches, proteins are subjected to varied forms of electromagnetic radiation and the ways they interact with the radiation allows researchers to determine atomic coordinates at Angstrom resolution from electron densities (see X-ray crystallography) and how nuclei spins interact (see NMR).

Levinthal's paradox

In the late 1960s, Cyrus Levinthal outlined the magnitude of the complexity of the protein folding problem. He pointed out that for a protein with 100 amino acids, it would have 99 peptide bonds and 198 considerations for φ and ψ angles. If each of these had only three conformations, that would result in 3198 different possible foldings or 2.95x1094.

Even allowing a reasonable amount of time (one nanosecond) for each possible fold to occur, it would take longer than the age of the universe to sample all of them, meaning clearly that the process of folding is not occurring by a sequential random sampling and that attempts to determine protein structure by random sampling were doomed to fail. Levinthal, therefore, proposed that folding occurs by a sequential process that begins with a nucleation event that guides the process rapidly and is not unlike the funnel process depicted in Figure 2.44.

Diseases of protein misfolding

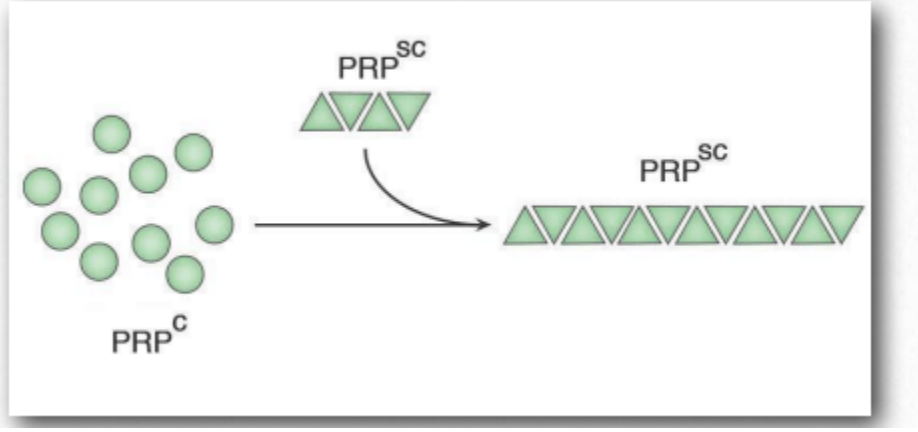


Figure 2.45 - Misfolding of the normal PRPc protein induced by PRPsc Image by Penelope Irving

The proper folding of proteins is essential to their function. It follows then that misfolding of proteins (also called proteopathy) might have consequences. In some cases, this might simply result in an inactive protein. Protein misfolding also plays a role in numerous diseases, such as Mad Cow Disease, Alzheimers, Parkinson's Disease, and CreutzfeldJakob disease. Many, but not all, misfolding diseases affect brain tissue.



Figure 2.46 - Cows with Mad Cow Disease lose their ability to stand

Insoluble deposits

Misfolded proteins will commonly form aggregates called amyloids that are harmful to tissues containing them because they change from being soluble to insoluble in water and form deposits. The process by which misfolding (Figure 2.45) occurs is not completely clear, but in many cases, it has been demonstrated that a "seed" protein which is misfolded can induce the same misfolding in other copies of the same protein. These seed proteins are known as prions and they act as infectious agents, resulting in the spread of disease. The list of human diseases linked to protein misfolding is long and continues to grow. A Wikipedia link is HERE.

Prions

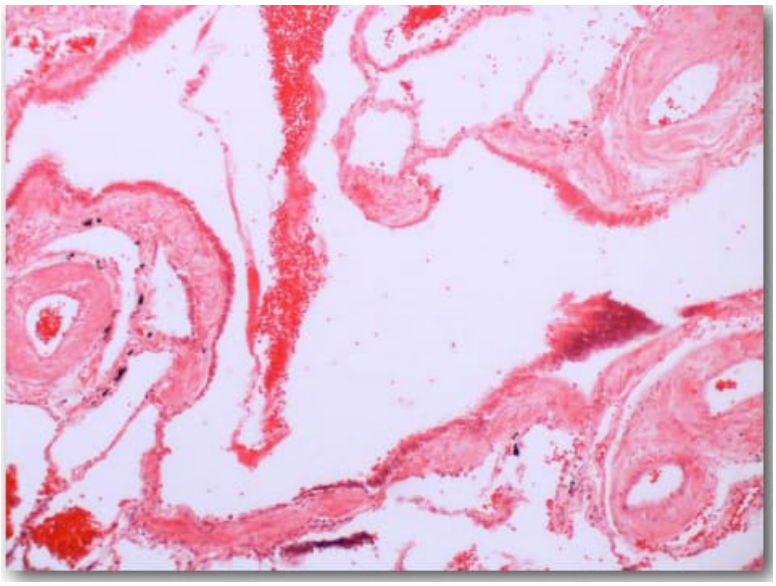


Figure 2.47 - Diffuse amyloidosis in a blood vessel (red dots) Wikipedia

Prions are infectious protein particles that cause transmissible spongiform encephalopathies (TSEs), the best known of which is Mad Cow disease. Other manifestations include the disease, scrapie, in sheep, and human diseases, such as CreutzfeldtJakob disease (CJD), Fatal Familial Insomnia, and kuru. The protein involved in these diseases is a membrane protein called PrP. PrP is encoded in the genome of many organisms and is found in most cells of the body. PrPc is the name given to the structure of PrP that is normal and not associated with disease. PrPSc is the name given to a misfolded form of the same protein, that is associated with the development of disease symptoms (Figure 2.45).

Misfolded

The misfolded PrPSc is associated with the TSE diseases and acts as an infectious particle. A third form of PrP, called PrPres can be found in TSEs, but is not infectious. The 'res' of PrPres indicates it is protease resistant. It is worth noting that all three forms of PrP have the same amino acid sequence and differ from each other only in the ways in which the polypeptide chains are folded. The most dangerously misfolded form of PrP is PrPSc, because of its ability to act like an infectious agent - a seed protein that can induce misfolding of PrPc , thus converting it into PrPSc.

Function

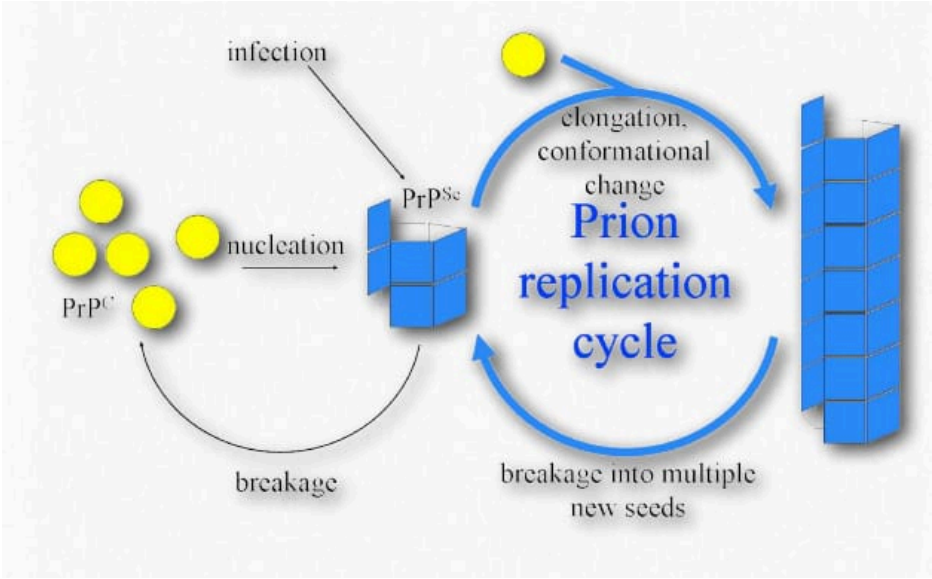


Figure 2.48 - One model of prion propagation Wikipedia

The function of PrPc is unknown. Mice lacking the PrP gene do not have major abnormalities. They do appear to exhibit problems with long term memory, suggesting a function for PrPc . Stanley Prusiner, who discovered prions and coined the term, received the Nobel Prize in Medicine in 1997 for his work. I think that if I chanced to be on A protein making up a prion I'd twist it and for goodness sakes Stop it from making fold mistakes

Amyloids

Amyloids are a collection of improperly folded protein aggregates that are found in the human body. As a consequence of their misfolding, they are insoluble and contribute to some twenty human diseases including important neurological ones involving prions. Diseases include (affected protein in parentheses) - Alzheimer's disease (Amyloid β), Parkinson's disease (α-synuclein), Huntington's disease (huntingtin), rheumatoid arthritis (serum amyloid A), fatal familial insomnia (PrPSc), and others.

Amino acid sequence plays a role in amyloidogenesis. Glutamine-rich polypeptides are common in yeast and human prions. Trinucleotide repeats are important in Huntington's disease. Where sequence is not a factor, hydrophobic association between β-sheets can play a role.

Amyloid β

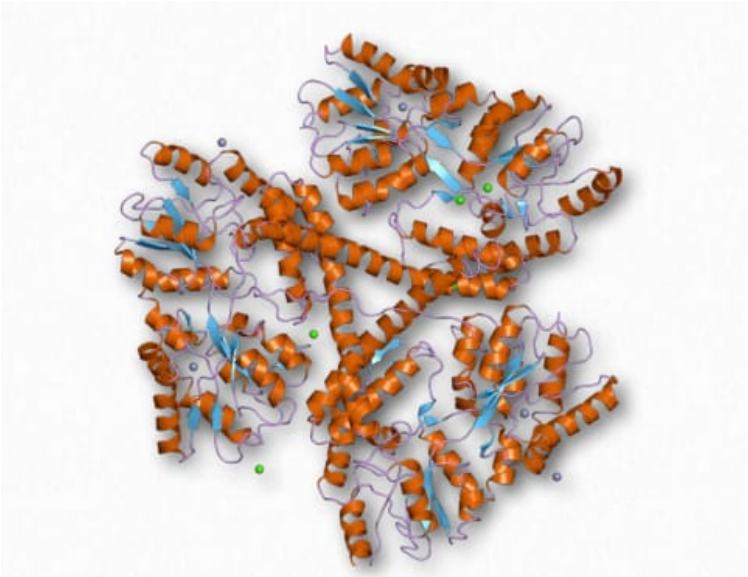


Figure 2.49 - Huntingtin

Amyloid β refers to collections of small proteins (36-43 amino acids) that appear to play a role in Alzheimer's disease. (Tau protein is the other factor.) They are, in fact, the main components of amyloid plaques found in the brains of patients suffering from the disease and arise from proteolytic cleavage of a larger amyloid precursor glycoprotein called Amyloid Precursor Protein, an integral membrane protein of nerve cells whose function is not known. Two proteases, β-secretase and γ- secretase perform this function. Amyloid β proteins are improperly folded and appear to induce other proteins to misfold and thus precipitate and form the amyloid characteristic of the disease. The plaques are toxic to nerve cells and give rise to the dementia characteristic of the disease.

It is thought that aggregation of amyloid β proteins during misfolding leads to generation of reactive oxygen species and that this is the means by which neurons are damaged. It is not known what the actual function of amyloid β is. Autosomal dominant mutations in the protein lead to early onset of the disease, but this occurs in no more than 10% of the cases. Strategies for treating the disease include inhibition of the secretases that generate the peptide fragments from the amyloid precursor protein.

Huntingtin

Huntingtin is the central gene in Huntington's disease. The protein made from it is glutamine rich, with 6-35 such residues in its wild-type form. In Huntington's disease, this gene is mutated, increasing the number of glutamines in the mutant protein to between 36 and 250. The size of the protein varies with the number of glutamines in the mutant protein, but the wild-type protein has over 3100 amino acids and a molecular weight of about 350,000 Da. Its precise function is not known, but huntingtin is found in nerve cells, with the highest level in the brain. It is thought to possibly play roles in transport, signaling, and protection against apoptosis. Huntingtin is also required for early embryonic development. Within the cell, huntingtin is found localized primarily with microtubules and vesicles.

Trinucleotide repeat

The huntingtin gene contains many copies of the sequence CAG (called trinucleotide repeats), which code for the many glutamines in the protein. Huntington's disease arises when extra copies of the CAG sequence are generated when the DNA of the gene is being copied. Expansion of repeated sequences can occur due to slipping of the polymerase relative to the DNA template during replication. As a result, multiple additional copies of the trinucleotide repeat may be made, resulting in proteins with variable numbers of glutamine residues. Up to 35 repeats can be tolerated without problem. The number of repeats can expand over the course of a person's lifetime, however, by the same mechanism. Individuals with 36-40 repeats begin to show signs of the disease and if there are over 40, the disease will be present.

Molecular chaperones

The importance of the proper folding of proteins is highlighted by the diseases associated with misfolded proteins, so it is no surprise, then, that cells expend energy to facilitate the proper folding of proteins. Cells use two classes of proteins known as molecular chaperones, to facilitate such folding in cells. Molecular chaperones are of two kinds, the chaperones, and the

chaperonins. An example of the first category is the Hsp70 class of proteins. Hsp stands for “heat shock protein”, based on the fact that these proteins were first observed in large amounts in cells that had been briefly subjected to high temperatures. Hsps function to assist cells in stresses arising from heat shock and exposure to oxidizing conditions or toxic heavy metals, such as cadmium and mercury. However, they also play an important role in normal conditions, where they assist in the proper folding of polypeptides by preventing aberrant interactions that could lead to misfolding or aggregation. The Hsp70 proteins are found in almost all cells and use ATP hydrolysis to stimulate structural changes in the shape of the chaperone to accommodate binding of substrate proteins. The binding domain of Hsp70s contains a β-barrel structure which wraps around the polypeptide chain of the substrate and has affinity for hydrophobic side chains of amino acids. As shown in Figure 2.50, Hsp70 binds to polypeptides as they emerge from ribosomes during protein synthesis. Binding of substrate stimulates ATP hydrolysis and this is facilitated by another heat shock protein known as Hsp40. The hydrolysis of ATP causes the Hsp70 to taken on a closed conformation that helps shield exposed hydrophobic residues and prevent aggregation or local misfolding.

After protein synthesis is complete, ADP is released and replaced by ATP and this results in release of the substrate protein, which then allows the full length polypeptide to fold correctly.

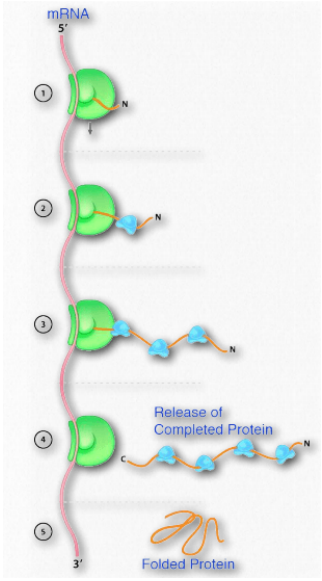


Figure 2.50 - Action of Hsp70 (blue) to facilitate proper folding of a protein (orange) Image by Aleia Kim

In heat shock

In times of heat shock or oxidative stress, Hsp70 proteins bind to unfolded hydrophobic regions of proteins to similarly prevent them from aggregating and allowing them to properly refold. When proteins are damaged, Hsp70 recruits enzymes that ubiquitinate the damaged protein to target them for destruction in proteasomes. Thus, the Hsp70 proteins play an important role in ensuring not only that proteins are properly folded, but that damaged or nonfunctional proteins are removed by degradation in the proteasome.

Chaperonins

A second class of proteins involved in assisting other proteins to fold properly are known as chaperonins. There are two primary categories of chaperonins - Class I (found in bacteria, chloroplasts, and mitochondria) and Class II (found in the cytosol of eukaryotes and archaebacteria). The best studied chaperonins are the GroEL/GroES complex proteins found in bacteria (Figure 2.51).

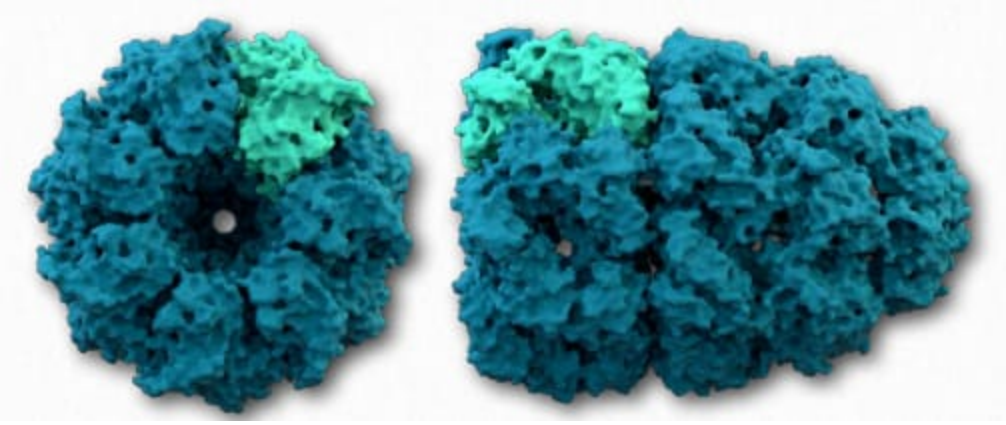


Figure 2.51 - View from bottom of GroEL (left) and GroEL/ GroES complex (right) Wikipedia

GroEL/GroES may not be able to undo aggregated proteins, but by facilitating proper folding, it provides competition for misfolding as a process and can reduce or eliminate problems arising from improper folding. GroEL is a double-ring 14mer with a hydrophobic region that can facilitate folding of substrates 15-60 kDa in size. GroES is a singlering heptamer that binds to GroEL in the presence of ATP and functions as a cover over GroEL. Hydrolysis of ATP by chaperonins induce large conformational changes that affect binding of substrate proteins and their folding. It is not known exactly how chaperonins fold proteins. Passive models postulate the chaperonin complex functioning inertly by preventing unfavorable intermolecular interactions or placing restrictions on spaces available for folding to occur. Active models propose that structural changes in the chaperonin complex induce structural changes in the substrate protein.

Protein breakdown

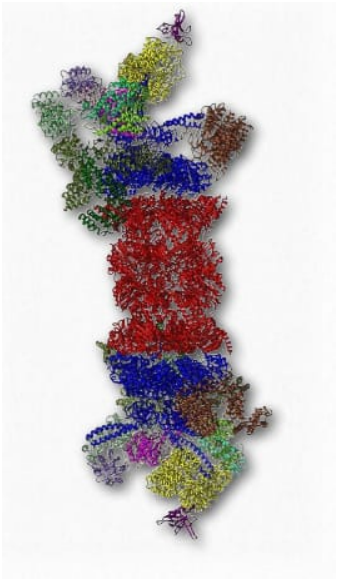


Figure 2.52 - 26S proteasome. Active site shown in red Wikipedia

Another protein complex that has an important function in the lifetime dynamics of proteins is the proteasome (Figure 2.52). Proteasomes, which are found in all eukaryotes and archaeans, as well as some bacteria, function to break down unneeded or damaged proteins by proteolytic degradation. Proteasomes help to regulate the concentration of some proteins and degrade ones that are misfolded. The proteasomal degradation pathway plays an important role in cellular processes that include progression through the cell cycle, modulation of gene expression, and response to oxidative stresses.

Degradation in the proteasome yields short peptides seven to eight amino acids in length. Threonine proteases play important roles. Breakdown of these peptides yields individual amino acids, thus facilitating their recycling in cells. Proteins are targeted for degradation in eukaryotic proteasomes by attachment to multiple copies of a small protein called ubiquitin (8.5 kDa - 76 amino acids). The enzyme catalyzing the reaction is known as ubiquitin ligase. The resulting polyubiquitin chain is bound by the proteasome and degradation begins. Ubiquitin was named due to it ubiquitously being found in eukaryotic cells.

Ubiquitin

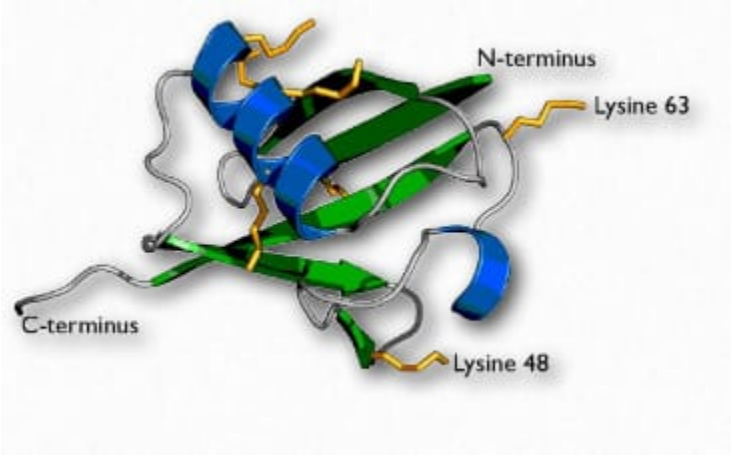


Figure 2.53 - Ubiquitin (lysine side chains shown in yellow) Wikipedia

Ubiquitin (Figure 2.53) is a small (8.5 kDa) multi-functional protein found in eukaryotic cells. It is commonly added to target proteins by action of ubiquitin ligase enzymes (E3 in Figure 2.54). One (ubiquitination) or many (polyubiquitination) ubiquitin molecules may be added. Attachment of the ubiquitin is through the side chain of one of seven different lysine residues in ubiquitin.

The addition of ubiquitin to proteins has many effects, the best known of which is targeting the protein for degradation in the proteasome. Proteasomal targeting is seen when polyubiquitination occurs at lysines #29 and 48. Polyubiquitination or monoubiquitination at other lysines can result in altered cellular location and changed protein-protein interactions. The latter may alter affect inflammation, endocytic trafficking, translation and DNA repair.

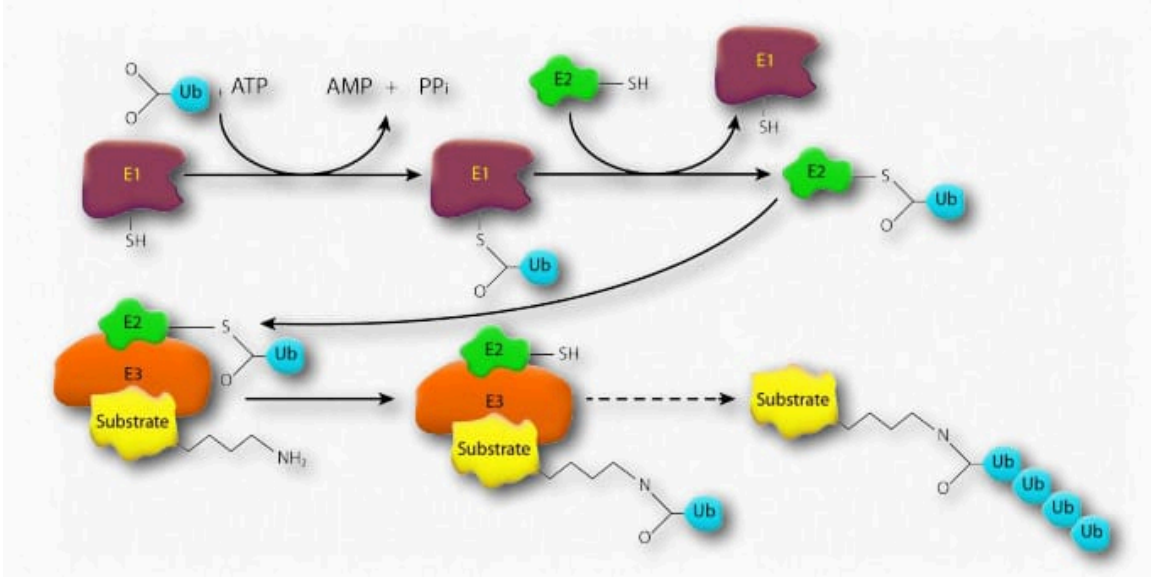


Figure 2.54 - Pathway for ubiquitination of a target substrate protein Image by Pehr Jacobson

Ubiquitin ligase malfunction

Parkin is a Parkinson's disease-related protein that, when mutated, is linked to an inherited form of the disease called autosomal recessive juvenile Parkinson's disease. The function of the protein is not known, but it is a component of the E3 ubiquitin ligase system responsible for transferring ubiquitin from the E2 protein to a lysine side chain on the target protein. It is thought that mutations in parkin lead to proteasomal dysfunction and a consequent inability to break down proteins harmful to dopaminergic neurons. This results in the death or malfunction of these neurons, resulting in Parkinson's disease.

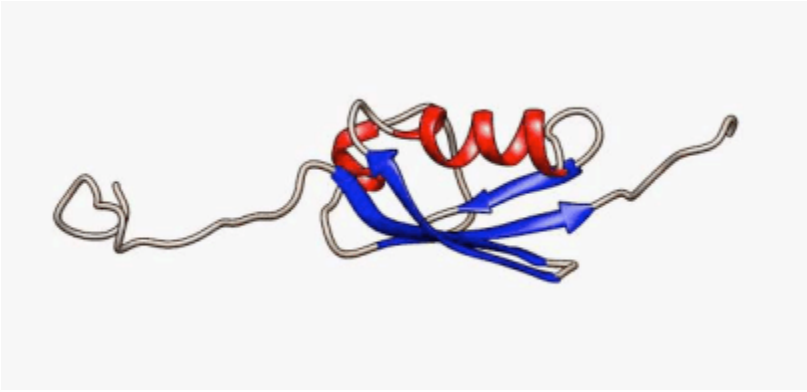
Intrinsically disordered proteins



Movie 2.1 - Dynamic movement of cytochrome C in solution Wikipedia

As is evident from the many examples described elsewhere in the book, the 3-D structure of proteins is important for their function. But, increasingly, it is becoming evident that not all proteins fold into a stable structure. Studies on the so-called intrinsically disordered proteins (IDPs) in the past cou- ple of decades has shown that many proteins are biologically active, even thought they fail to fold into stable structures. Yet other proteins exhibit regions that remain unfolded (IDP regions) even as the rest of the polypeptide folds into a structured form.

Intrinsically disordered proteins and disordered regions within proteins have, in fact, been known for many years, but were regarded as an anomaly. It is only recently, with the realization that IDPs and IDP regions are widespread among eukaryotic proteins, that it has been recognized that the observed disorder is a "feature, not a bug".



Movie 2.2 SUMO-1, a protein with intrinsically disordered sections Wikipedia

Comparison of IDPs shows that they share sequence characteristics that appear to favor their disordered state. That is, just as some amino acid sequences may favor the folding of a polypeptide into a particular structure, the amino acid sequences of IDPs favor their remaining unfolded. IDP regions are seen to be low in hydrophobic residues and unusually rich in polar residues and proline. The presence of a large number of charged amino acids in the IDPs can inhibit folding through charge repulsion, while the lack of hydrophobic residues makes it difficult to form a stable hydrophobic core, and proline discourages the formation of helical structures. The observed differences between amino acid sequences in IDPs and structured proteins have been used to design algorithms to predict whether a given amino acid sequence will be disordered.

What is the significance of intrinsically disordered proteins or regions? The fact that this property is encoded in their amino acid sequences suggests that their disorder may be linked to their function. The flexible, mobile nature of some IDP regions may play a crucial role in their function, permitting a transition to a folded structure upon binding a protein partner or undergoing post-translational modification. Studies on several wellknown proteins with IDP regions suggest some answers. IDP regions may enhance the ability of proteins like the lac repressor to translocate along the DNA to search for specific binding sites. The flexibility of IDPs can also be an asset in protein-protein interactions, especially for proteins that are known to interact with many different protein partners.

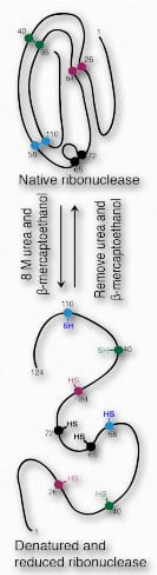


Figure 2.55 - Denaturation and renaturation of ribonuclease Wikipedia

For example, p53 has IDP regions that may allow the protein to interact with a variety of functional partners. Comparison of the known functions of proteins with predictions of disorder in these proteins suggests that IDPs and IDP regions may disproportionately function in signaling and regulation, while more structured proteins skew towards roles in catalysis and transport. Interestingly, many of the proteins found in both ribosomes and spliceosomes are predicted to have IDP regions that may play a part in correct assembly of these complexes. Even though IDPs have not been studied intensively for very long, what little is known of them suggests that they play an important and underestimated role in cells.

Metamorphic proteins

Another group of proteins that have recently changed our thinking about protein structure and function are the so-called metamorphic proteins. These proteins are capable of forming more than one stable, folded state starting with a single amino acid sequence. Although it is true that multiple folded conformations are not ruled out by the laws of physics and chemistry, metamorphic proteins are a relatively new discovery. It was known, of course, that prion proteins were capable of folding into alternative structures, but metamorphic proteins appear to be able to toggle back and forth between two stable structures. While in some cases, the metamorphic protein undergoes this switch in response to binding another molecule, some proteins that can accomplish this transition on their own. An interesting example is the signaling molecule, lymphotactin. Lymphotactin has two biological functions that are carried out by its two conformers- a monomeric form that binds the lymphotactin receptor and a dimeric form that binds heparin. It is possible that this sort of switching is more widespread than has been thought.

Refolding denatured proteins

All information for protein folding is contained in the amino acid sequence of the protein. It may seem curious then that most proteins do not fold into their proper, fully active form after they have been+++ denatured and the denaturant is removed. A few do, in fact. One good example is bovine ribonuclease (Figure 2.55). Its catalytic activity is very resistant to heat and urea and attempts to denature it don't work very well. However, if one treats the enzyme with β-mercaptoethanol (which breaks disulfide bonds) prior to urea treatment and/or heating, activity is lost, indicating that the covalent disulfide bonds help stabilize the overall enzyme structure and when they are broken, denaturation can readily occur. When the mixture cools back down to room temperature, over time some enzyme activity reappears, indicating that ribonuclease re-folded under the new conditions.

Interestingly, renaturation will occur maximally if a tiny amount of β-mercaptoethanol is left in the solution during the process. The reason for this is because β- mercaptoethanol permits reduction (and breaking) of accidental, incorrect disulfide bonds during the folding process. Without it, these disulfide bonds will prevent proper folds from forming.

Irreversible denaturation

Most enzymes, however, do not behave like bovine ribonuclease. Once denatured, their activity cannot be recovered to any significant 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 extent. This may seem to contradict the idea of folding information being

inherent to the sequence of amino acids in the protein. It does not.

Most enzymes don't refold properly after denaturation for two reasons. 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 the process starts.

Chaperonins' role

In other cases, the folding process of some proteins in the cell relied upon action of chaperonin proteins (see HERE). In the absence of chaperonins, interactions that might result in misfolding occur, thus preventing proper folding. Thus, early folding and the assistance of chaperonins eliminate some potential "wrong-folding" interactions that can occur if the entire sequence was present when folding started.

Quaternary structure

A fourth level of protein structure is that of quaternary structure. It refers to structures that arise as a result of interactions between multiple polypeptides. The units can be identical multiple copies or can be different polypeptide chains. Adult hemoglobin is a good example of a protein with quaternary structure, being composed of two identical chains called α and two identical chains called β.

Though the α-chains are very similar to the β- chains, they are not identical. Both of the α- and the β-chains are also related to the single polypeptide chain in the related protein called myoglobin. Both myoglobin and hemoglobin have similarity in binding oxygen, but their behavior towards the molecule differ significantly. Notably, hemoglobin's multiple subunits (with quaternary structure) compared to myoglobin's single subunit (with no quaternary structure) give rise to these differences.

References

1. https://en.Wikipedia.org/wiki/Van_der_W aals_force 105

This page titled 4.2: Levels of Protein Structure is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 2.3: Structure & Function- Proteins I by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

4.3: Secondary Structure

Secondary structures are those repetitive structures involving H bond between amide H and carbonyl O in- the main chain. These include alpha helices, beta strands (sheets) and reverse turns.

Alpha Structure

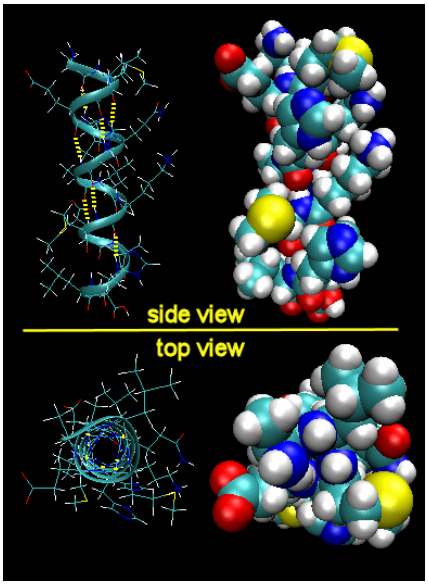


***Figure**: Right Handed Alpha helices - image made with VMD*

These helices are formed when the carbonyl O of the i th amino acid H bonds to the amide H of the i th +4 aa (4 amino acids away). The phi/psi angles for those amino acids in the alpha helix are - 57,-47, which emphasizes the regular repeating nature of the structure. It can also be characterized by n (the number of amino acid units/turn = 3.6) and pitch (the helix rise/turn = 5.4 angstroms). Some facts:

- the alpha helix is more compact than the fully extended polypeptide chain with phi/psi angles of 180o
- in proteins, the average number of amino acids in a helix is 11, which gives 3 turns.
- the left-handed alpha helix, although allowed from inspections of a Ramachandran plot, is never observed, since the side chains are too close to the backbone.
- the core of the helix is packed tightly. There are not holes or pores in the helix.
- All the R-groups extend backward and away from the helix axis.
- Some amino acids are more commonly found in alpha helices than other. Amino acids can be divided into two kinds, those with branches at the beta C and those with none. Consider first those that aren't branched . Gly is too conformationally flexible to be found with high frequency in alpha helices, while Pro is too rigid. The amino acids with side chains that can H-bond (Ser, Asp, and Asn) and aren't too long appear to act as competitors of main chain H bond donor and acceptors, and destabilize alpha helices. The rest with no branches at the beta C can form helices. Those with branches at the beta carbon (Val, Ile) destabilize the alpha helix due to steric interactions of the bulky side chains with the helix backbone. (Remember left-handed alpha helices are not found in nature for similar reasons.) Summary of amino acids propensities for alpha helices (and beta structure as well)
- alpha keratins, the major component of hair, skin, fur, beaks, and fingernails, are almost all alpha helix.


Jmol: Updated An isolated helix from an Antifreeze Protein Jmol14 (Java) | JSMol (HTML5)

helices

There are other kinds of helices that can occur. These include a 310 helix and a \(\pi\) helix, which are stabilized by H-bonds between the amide NH and carbonyl O of residues (i, i+3) and (i, i+5), respectively. Likewise, they have 3 and 4.3 residues/turn, respectively, and a rise per residue of 6 and 4.7 angstrom, respectively. These structures are much rarer than right handed alpha helices.

Helix Type	H bond btw ith and ith+X AA, where X =	Residue/turn	Rise (Angstrom)/turn
310	3	3	6
α	4	3.6	5.4
π	5	4.3	4.7

Beta Structure

Beta Structure: Parallel and antiparallel beta strands are much more extended than alpha helices (phi/psi of -57,-47) but not as extended as a fully extended polypeptide chain (with phi/psi angles of +/- 180). The beta sheets are not quit so extended (parallel -119, +113 ; antiparallel, -139, +135), and can be envisioned as rippled sheets. They can be visualized by laying thin, pleated strips of paper side by side to make a "pleated sheet" of paper. Each strip of paper can be pictured as a single peptide strand in which the peptide backbone makes a zig-zag along the strip, with the alpha carbons lying at the folds of the pleats. Each single strand of the beta-sheet can be pictured as a twofold helix, i.e. a helix with 2 residues/turn. The arrangement of each successive peptide plane is pleated due to the tetrahedral nature of the alpha C. The H bonds are interstrand, not intrastrand as in the alpha helix.

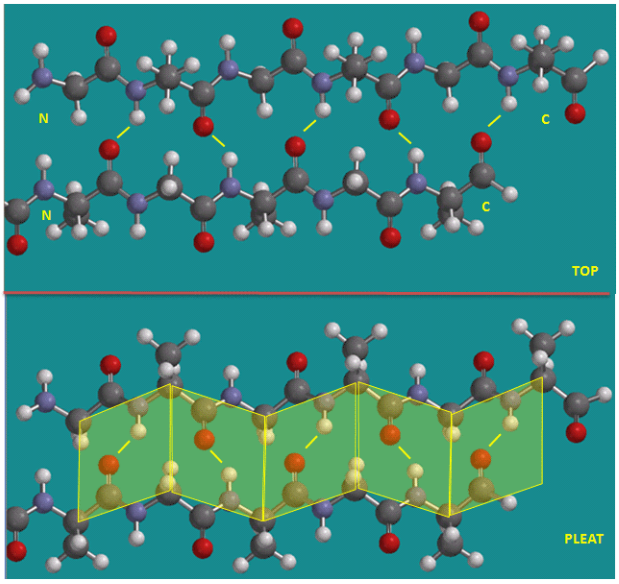


Figure: Parallel beta strands (image made with Spartan)

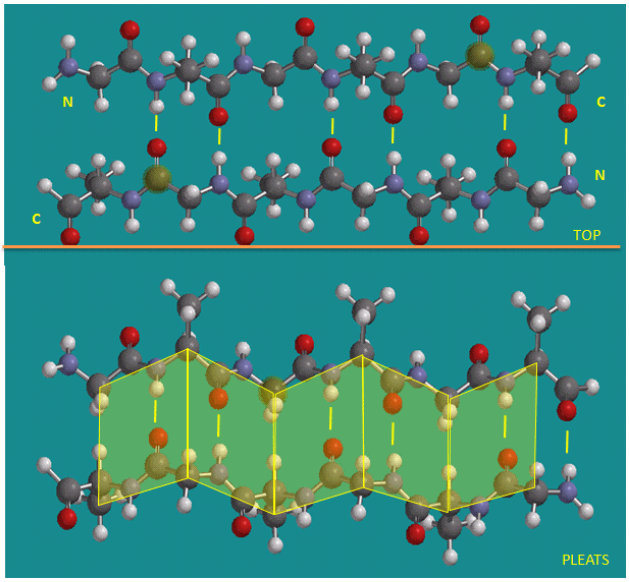


Figure: Antiparallel beta strands (image made with Spartan)

Note

Consider a strand as a continuous and contiguous polypeptide backbone propagating in one direction. Hence, using this definition, a helix consist of a single strand, and all the H-bonds are within the strand (or intrastrand). A beta sheet would then consist of multiple strands, since each "strand" is separated from other "strands" by an intervening contiguous stretch of amino acid which bends within the protein in a way which allows the next section of the peptide backbone, the next "strand", to H-bond with the first "strand". But remember, even in this case, all the H-bonds holding the alpha and beta structure together are intramolecular.

In a parallel beta sheet structure, the optimal H bond pattern leads to a less extended structure (phi/psi of -119, +113) than the optimal arrangement of the H bonds in the antiparallel structure (phi/psi of -139, +135). Also the H bonds in the parallel sheet are bent significantly. (i.e. the carbonyl O on one strand is not exactly opposite the amide H on the adjacent strand, as it is in the antiparallel sheet.) Hence antiparallel beta strands are presumably more stable, even though both are abundantly found in nature. Short parallel beta sheets of 4 strands or less are not common, which might reflect their lower stability.

The side chains in the beta sheet are normal to the plane of the sheet, extending out from the plane on alternating sides. Parallel sheets characteristically distribute hydrophobic side chains on both side of the sheet, while antiparallel sheets are usually arranged with all the hydrophobic residues on one side. This requires an alternation of hydrophilic and hydrophobic side chains in the primary sequence. Antiparallel sheets are found in silk with the sheets running parallel to the silk fibers. The following repeat is found in the primary sequence: (Ser-Gly-Ala-Gly)n), with Gly pointing out from one face, and Ser or Ala from the other.

EXTERNAL Jmol: Silk

Beta strands have a tendency to twist in the right hand direction. This leads to important consequences in how the beta strands are connected. Parallel strands can form twisted sheets or saddles as well as beta barrels.

Figure: Twisted Beta Sheet/Saddle (image made with VMD)

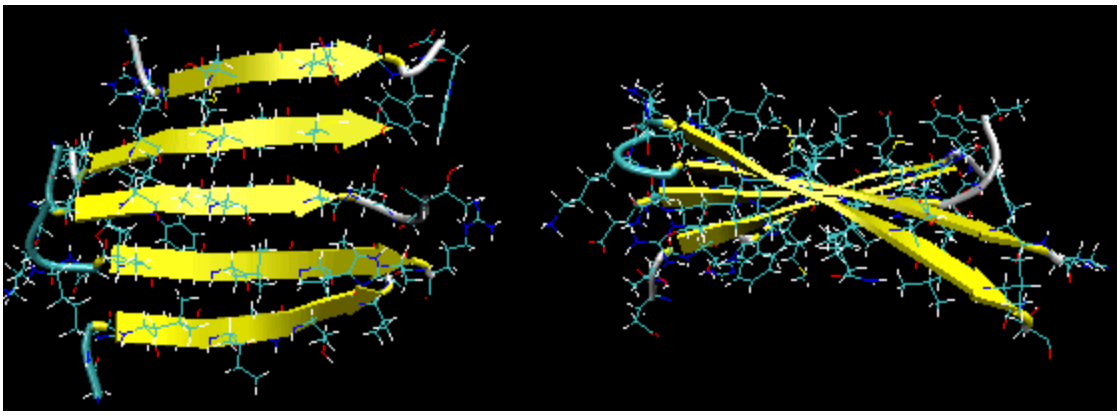
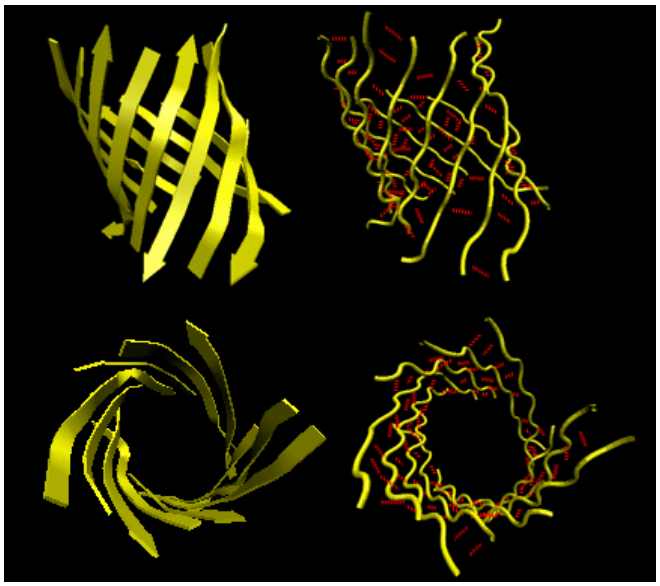


Figure: Beta Barrel (image made with VMD)



- in parallel strands, right handed connectivity is common.
- in a protein with parallel strand in register, and given the inherent twist in the stands, the strands arrange in a way to have the H bonds stretched equally at the ends of the chains, giving rise to a twisted saddle shape (top structure above).



Jmol: Updated Twisted beta sheet from Arabinose Binding Protein Jmol14 (Java) | JSMol (HTML5)

- in a protein with parallel strand out of register, and given the inherent twist in the stands, the strands arrange in a way to have the H bonds stretched equally at the ends of the chains, giving rise to a beta barrel (bottom structure above).

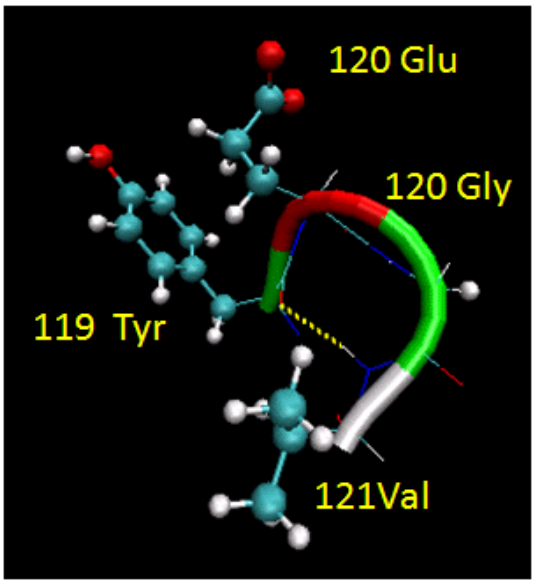


Jmol: Beta barrel from triose phosphate isomerase

Reverse Turns

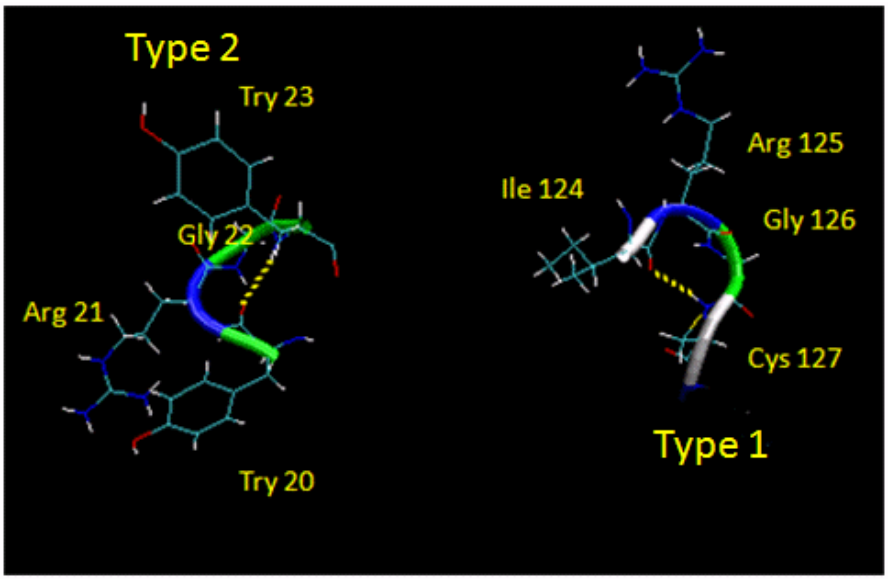
About 50% of the amino acids in a globular protein are in regular secondary structure (alpha or beta). The remaining amino acids are not less ordered, just less regular. An additional example of secondary structures is reverse turns (or beta-bends or beta turns). Reverse turns often connect successive antiparallel beta strands and are then called **beta hairpins**.

Figure: Reverse Turns (image made with VMD)



They are almost always at the surface, and consist of 4 amino acids. There are two types. (I - f2 = -60, y2=-30; f3 = -90, y3 = 0; II - f2 = -60, y2=120; f3 = 90, y3 = 0) Residue 2 of both is often Pro. (Why?) Both have an H bond between the carbonyl O of the i th a.a and the amide H of the i th+3 aa (three amino acids away). In the type 2, the O of residue 2 crowds the beta C of residue 3, so aa2 is usually Gly. Why? Those amino acids which destabilize alpha helices are often found in beta sheets, since the side chains project out of the plan which holds the main chain.

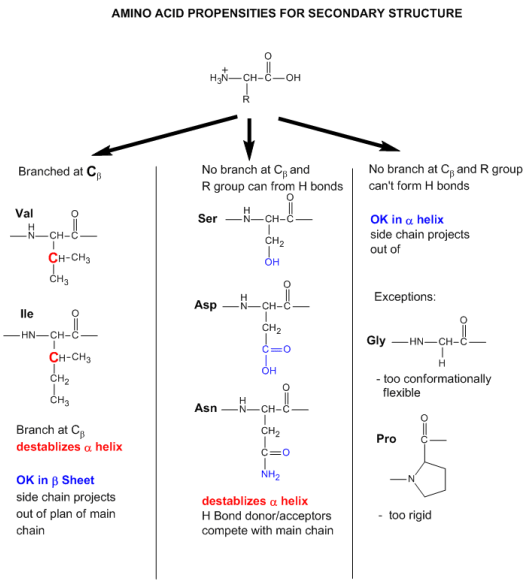
Figure: Type I and Type II Reverse Turns - from hen egg white lysozyme (image make with VMD)



Jmol: Updated Reverse Turn: Trypsin Inhibitor Jmol14 (Java) | JSMol (HTML5)

(Notice the tightness of the reverse turn and the presence of Pro and Gly.)

Figure: Why do amino acid propensities for secondary structure differ?



- EXTERNAL Secondary Structure and Backbone Conformation from ExPASy.(concentrate on second part on secondary structure)

This page titled 4.3: Secondary Structure is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- C2. Secondary Structure by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

4.4: Protein folding

We've seen many static images of lipids and their aggregates as well as proteins. To think about how proteins fold, we have to think dynamically. Luckily we have the tools of molecular dynamics (MD) at our fingertips which helps us imagine how these processes take place and concomitantly how to probe protein folding experimentally. View the following two MD simulations and compare the spontaenously formation of a micelle and the folding of a protein before we delve into the complex topic of protein folding and stability.

- MD simulation of micelle formation
- MD simulation of protein folding

Given the number of possibly nonnative states, it is amazing that proteins fold to the native state at all, let alone in a reasonable time frame. Consider this greatly simplified view of protein folding for a protein containing 100 amino acids. If each amino acid can adopt only 3 possible conformations, the total number of conformations could be $3100 = 5 \times 10^{47}$. Assuming that it would take 10-13s to change each conformation, the time required to "test" all conformations would be 5 x 1034s or 1027 years, longer than the age of the universe (14 x 109 yr). Yet the protein can fold within seconds. This paradox is called the *Levinthal paradox*, after Cyrus Levinthal.

Lubert Stryer (in his classic Biochemistry text), shows a way out of this dilemma by using an analogy of a monkey sitting at a typewriter, and typing this line out of Hamlet: "Me thinks it is like a weasel." Random typing would produce that line after 1040 keystrokes on average, but if the correct letters were maintained, the number of keystrokes would be in the realm of a few thousand. Proteins could fold more quickly if they retain native-like intermediates along the way. Also remember that much of conformation space is already restricted by allowed phi/psi angles. (Remember the blank areas in the Ramachandran plot?)

Before we study the classic experiment of protein folding conducted by Anfinsen, study the simpler analogy below:

Figure: Socks and protein folding

PROBABILITY AND SOCKS: AN ANALOGY FOR PROTEIN FOLDING

You have 4 pairs of socks in your drawer, but they are not pair.
You pull out 1 sock out at a time. What are the chances you will get 4 matched pairs at the end?



The classic experiment of Anfinsen has shown that, at least for some proteins, all the necessary and sufficient information required to direct the folding of a protein into the native state is present in the primary sequence of a protein. Anfinsen studied the *in vitro* (outside the cell, as opposed to *in vivo*, which is inside the cell, tissue, organ) folding of a single chain protein, RNase, which has 4 intrachain disulfide bonds.

Figure: RNase A with 4 Disulfide Bonds in red (image with VMD)



We have previously discussed how chemical agents (such as beta-mercaptoethanol, a disulfide reducing agent) can covalently interact with specific protein functional groups. Other substances can bind through complementary intermolecular forces to the active site or other cavities on the surface. Other reagents, like urea, acting through generalized solvent changes or nonspecific interactions with the protein, can alter protein folding. Anfinsen used two different reagents, 8 M urea and beta-mercaptoethanol, in combination to unfold, or denature, RNase to the nonnative or denatured state. He then removed the bME using dialysis, allowing the disulfides to reform. Next he removed the denaturing reagent, urea. To monitor if the protein was correctly refolded or renatured, he tested the activity of the protein compared to native protein. He found that the "refolded" protein retained only 1% of its initial activity. If, however, he added a catalytic amounts of bME, the protein soon retained 100% of its initial activity. For his work, he was awarded the Nobel Prize in Chemistry in 1972.

Figure: Anfinsen Experiment: Folding of RNase

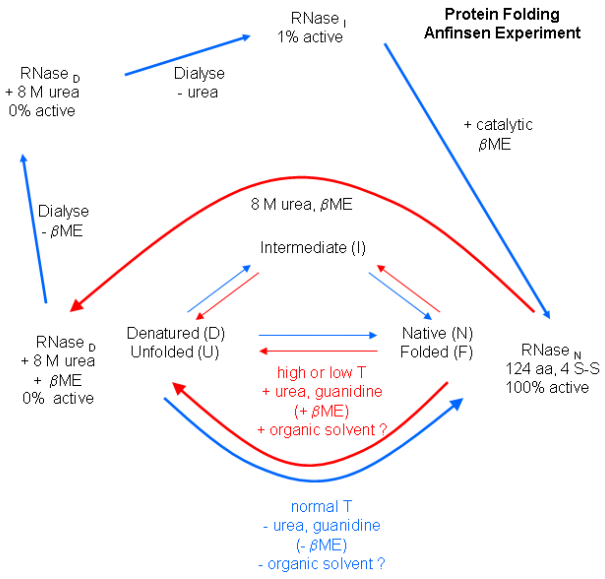
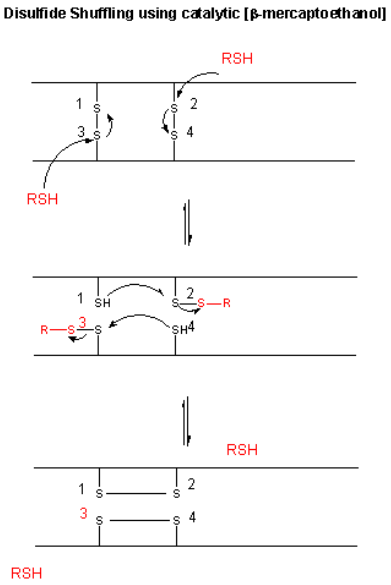


Figure: CATALYTIC SHUFFLING OF DISULFIDES WITH BETA-MERCAPTOETHANOL



Jmol: Updated RNase A Jmol14 (Java) | JSMol (HTML5)

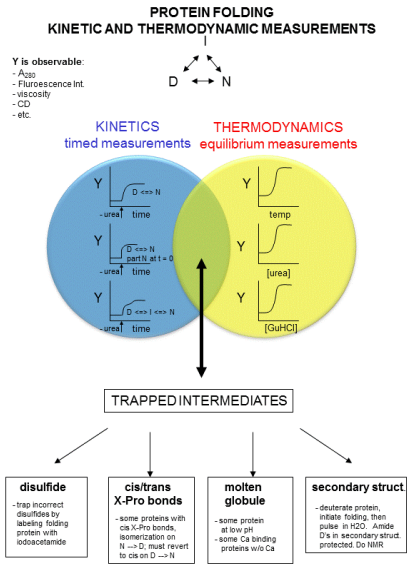
Scientists have investigated the folding of proteins both in vitro and in vivo. In vitro experiments involve denaturing the protein with urea, guanidine hydrochloride, or heat, then refolding the protein by removing the perturbant (denaturing agent), using spectral techniques to follow the process. In vivo experiments involve the study of intracellular proteins that assist folding. The in vitro experiments involve unfolding the native state and then refolding it, while the in vivo ones involve folding of the newly synthesized protein. An understanding of protein folding can not be separated from an understanding of protein stability, and an understanding of the nature of the native and denatured state.

In studying protein folding and stability/structure of the native and denatured states, both equilibrium (thermodynamic) and timed (kinetic) measurements are made. Folding occurs in the ms to second range, which limits the ability to study the presence of intermediates in the process. Some clever methods have been developed to study intermediates in protein folding by trapping specific intermediate structures, and investigating their structure and stability in a "leisurely" fashion. Alternatively, intermediates can be studied as they occur using stop flow kinetics. In this technique, a protein under denaturing conditions is rapidly mixed with a solution containing no denaturant or protein by injecting both solutions into a mixer/cuvette using syringes. The denaturant in the protein solution is now diluted such that renaturation can occur. Spectral measurements can begin at once.

- stop flow Java applet

A diagram summarizing these methods is shown below. Study it in conjunction with the text which follows.

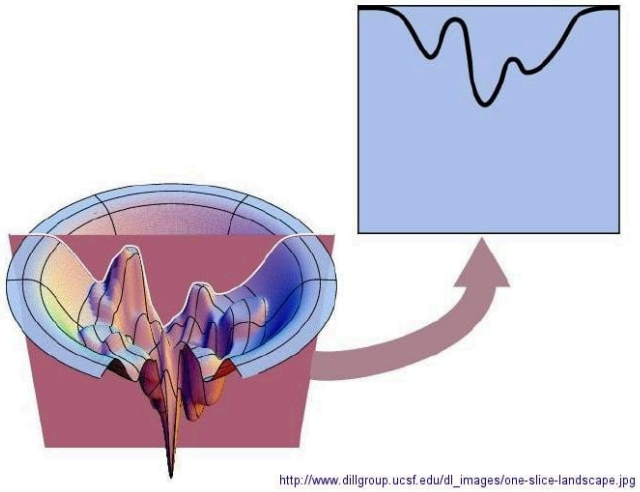
Figure: Kinetic and thermodynamic measurements of proteins stability and folding



In considering the folding pathway, we will consider that the native protein represents the global energy minimum. All other states represent variations of the denatured state. Some, closer in energy to the native state, could be considered intermediates in the folding process. Instead of considering a folding "trajectory", consider protein folding occurring within a large folding landscape of free energy. Folding appears to proceed not by an obligatory pathway but a probabilistic or stochastic search of possible conformation. The free energy landscape must be shaped somewhat like a funnel such that a proteins could adopt a "reasonable" number of conformations which lead to the native state. Evolution has surely selected for sequences that can make it to that state. Localized secondary structure motifs (like a short alpha helix and beta turns) can form quickly (about 1 ms). Small proteins folding occurs, depending on the structure, over a wide time frame (ms to minutes). Mostly likely, a small number of amino acids coalesce into a core which nucleates folding into structures that are similar to the native state. Finally packing interactions collapse the structure into the native state.

In general, the more complex the fold of the backbone, the longer it takes the protein to fold. If complexity requires more interactions among distal regions of the polypeptide change, then the more complex the fold, the less probable that random interactions would lead to quick protein folding. The mechanisms of folding for larger proteins (greater than 100 amino acids) appear to proceed through intermediates, suggesting that different domains of the protein can fold independently.

Figure: Protein Folding Landscape: One View from Ken Dill



Contributors and Attributions

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

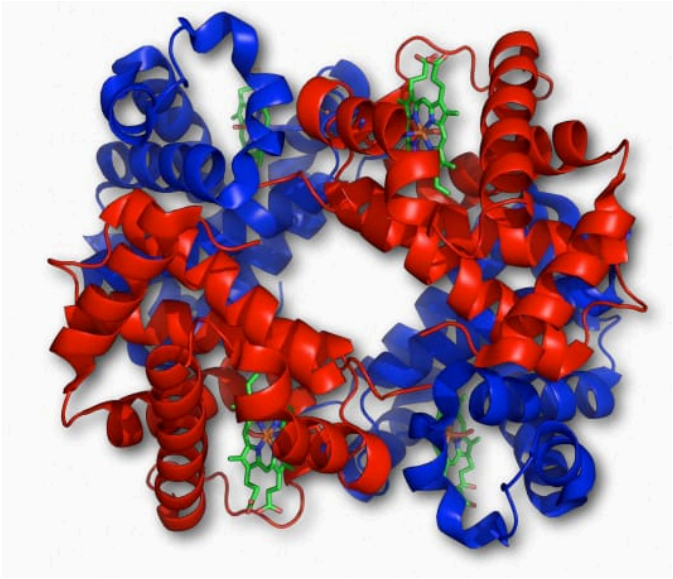
This page titled 4.4: Protein folding is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- D1. Introduction by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

4.5: Protein function, domains and cooperativity

Source: BiochemFFA_2_3.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

In this section, we hope to bring to life the connection between structure and function of proteins. So far, we have described notable features of the four elements (primary, secondary, tertiary, and quaternary) of protein structure and discussed example proteins/motifs exhibiting them. In this section, we will examine from a functional perspective a few proteins/domains whose function relies on secondary, tertiary, or quaternary structure. It is, of course a bit of a narrow focus to ascribe protein function to any one component of structure, but our hope is by presenting these examples, we can bring to life the way in which a protein's secondary, tertiary, and quarternary structure lead to the functions it has.



Hemoglobin Wikipedia

Fibrous proteins - secondary structure

Proteins whose cellular or extracellular roles have a strong structural component are composed primarily of primary and second structure, with little folding of the chains. Thus, they have very little tertiary structure and are fibrous in nature. Proteins exhibiting these traits are commonly insoluble in water and are referred to as fibrous proteins (also called scleroproteins). The examples described in this category are found exclusively in animals where they serve roles in flesh, connective tissues and hardened external structures, such as hair. They also contain the three common fibrous protein structures α -helices (keratins), β-strands/ sheets (fibroin & elastin) and triple helices (collagen). The fibrous proteins have some commonality of amino acid sequence. Each possesses an abundance of repeating sequences of amino acids with small, non-reactive side groups. Many contain short repeats of sequences, often with glycine.

Keratins



Figure 2.56 - The horns of an impala are composed of keratin Wikipedia

The keratins are a family of related animal proteins that take numerous forms. α-keratins are structural components of the outer layer of human skin and are integral to hair, nails, claws, feathers, beaks, scales, and hooves. Keratins provide strength to tissues, such as the tongue, and over 50 different keratins are encoded in the human genome. At a cellular level, keratins comprise the intermediate filaments of the cytoskeleton. α- keratins primarily contain α-helices, but can also have β-strand/sheet structures. Individual α-helices are often intertwined to form coils of coiled structures and these strands can also be further joined together by disulfide bonds, increasing structural strength considerably. This is particularly relevant for α-keratin in hair, which contains about 14% cysteine. The odor of burned hair and that of the chemicals used to curl/uncurl hair (breaking/re-making disulfide bonds) arise from their sulfurous components. β-keratins are comprised of β-sheets, as their name implies.

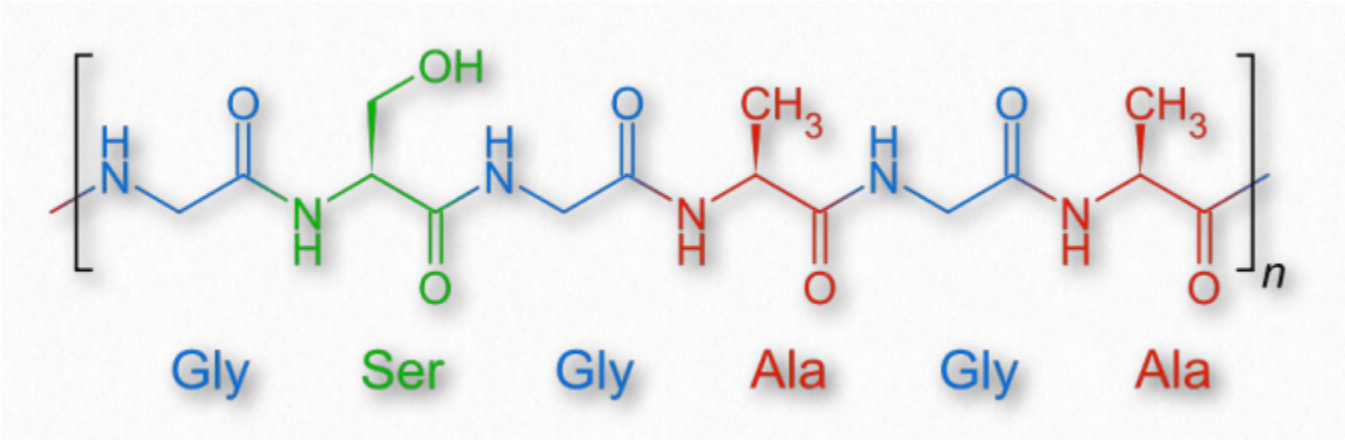


Figure 2.57 - The repeating amino acid sequence of fibroin

Fibroin

An insoluble fibrous protein that is a component of the silk of spiders and the larvae of moths and other insects, fibroin is comprised of antiparallel β-strands tightly packed together to form β- sheets. The primary structure of fibroin is a short repeating sequence with glycine at every other residue (Figure 2.57). The small R-groups of the glycine and alanine in the repeating sequence allows for the tight packing characteristic of the fibers of silk. Wikipedia link HERE Elastin As suggested by its name, elastin is a protein with elastic characteristics that functions in many tissues of the body to allow them to resume their shapes after expanding or contracting. The protein is rich in glycine and proline and can comprise over 50% of the weight of dry, defatted arteries.



Figure 2.58 - Weaving of a silk sari Wikipedia

Elastin

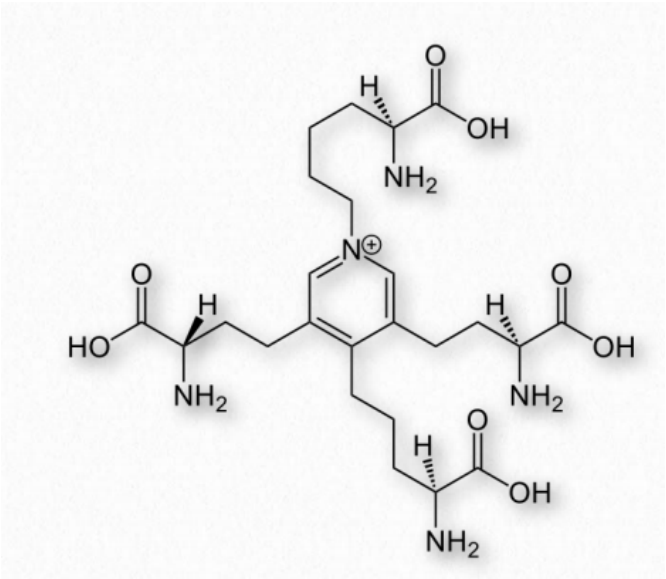


Figure 2.59 - Desmosine Wikipedia

is made by linking tropoelastin proteins together through lysine residues to make a durable complex crosslinked by desmosine. In arteries, elastin helps with pressure wave propagation for facilitating blood flow.

Collagen

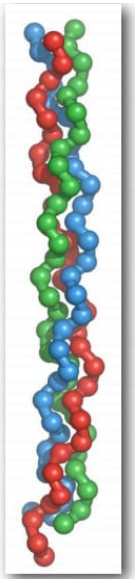


Figure 2.60 - Collagen's triple helix Wikipedia

Collagen is the most abundant protein in mammals, occupying up to a third of the total mass. There are at least 16 types of collagen. Its fibers are a major component of tendons and they are also found abundantly in skin. Collagen is also prominent in cornea, cartilage, bone, blood vessels and the gut.

Collagen's structure is an example of a helix of helices, being composed of three lefthanded helical chains that each are coiled together in a right-handed fashion to make the collagen fiber (Figure 2.60). Each helix is stretched out more than an α-helix, giving it an extended appearance. On the inside of the triple helical structure, only residues of glycine are found, since the side chains of other amino acids are too bulky. Collagen chains have the repeating structure glycinem-n where m is often proline and n is often hydroxyproline (Figure 2.61).

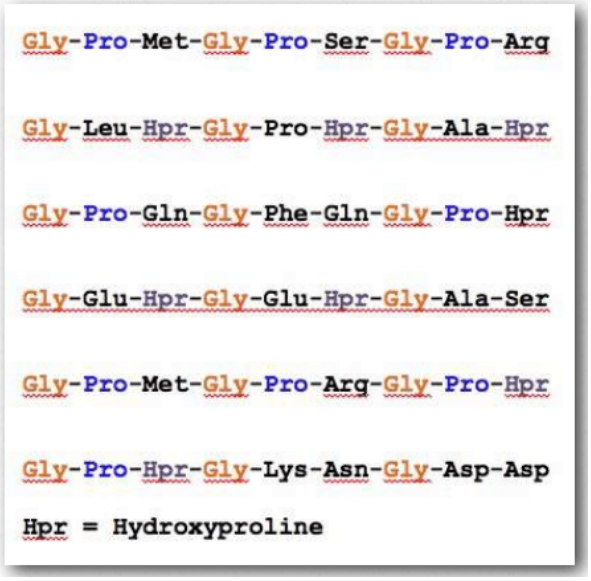


Figure 2.61 - Repeating sequences in collagen

Collagen is synthesized in a pre-procollagen form. Processing of the pre-procollagen in the endoplasmic reticulum results in glycosylation, removal of the 'pre' sequence, and hydroxylation of lysine and proline residues (see below). The hydroxides can form covalent cross-links with each other, strengthening the collagen fibers. As pro-collagen is exported out of the cell, proteases trim it, resulting in a final form of collagen called tropocollagen.

Hydroxylation

Hydroxylation of proline and lysine side chains occurs post-translationally in a reaction catalyzed by prolyl-4-hydroxylase and lysyl-hydroxylase (lysyl oxidase), respectively. The reaction requires vitamin C. Since hydroxylation of these residues is essential for formation of stable triple helices at body temperature, vitamin C deficiency results in weak, unstable collagen and, consequently, weakened connective tissues. It is the cause of the disease known as scurvy. Hydrolyzed collagen is used to make gelatin, which is important in the food industry. collagens. Wikipedia link HERE

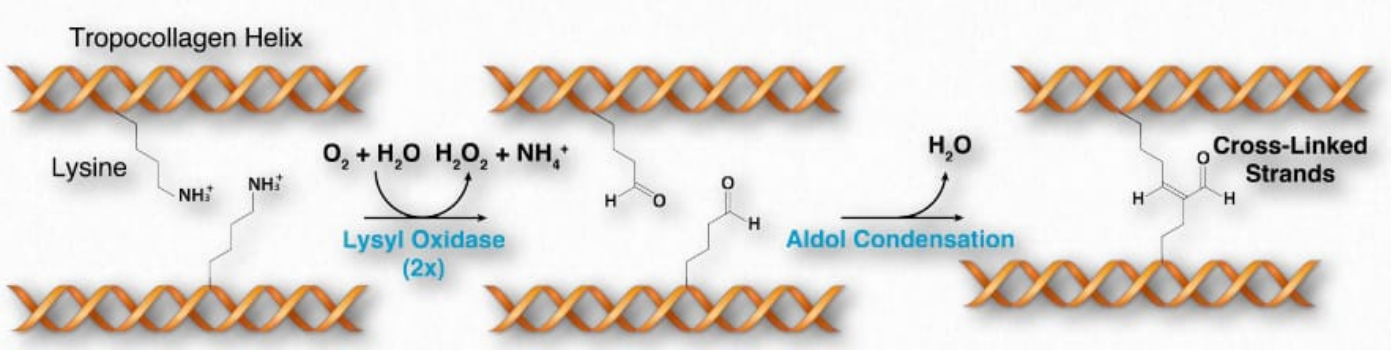


Figure 2.62 - Oxidation and cross-linking of lysine residues in tropocollagen. Only two strands of the triple helix are shown for simplicity Image by Aleia Kim

Lamins

Lamins are fibrous proteins that provide structure in the cell nucleus and play a role in transcription regulation. They are similar to proteins making up the intermediate filaments, but have extra amino acids in one coil of the protein. Lamins help to form the nuclear lamin in the interior of the nuclear envelope and play important roles in assembling and disassembling the latter in the process of mitosis. They also help to position nuclear pores. In the process of mitosis, disassembly of the nuclear envelope is promoted by phosphorylation of lamins by a protein called mitosis promoting factor and assembly is favored by reversing the reaction (dephosphorylation).

Structural domains - tertiary structure

Every globular protein relies on its tertiary structure to perform its function, so rather than trying to find representative proteins for tertiary structure (an almost impossible task!), we focus here on a few elements of tertiary structure that are common to many proteins. These are the structural domains and they differ from the structural motifs of supersecondary structure by being larger (25-500 amino acids), having a conserved amino acid sequence, and a history of evolving and functioning independently of the protein chains they are found in. Structural domains are fundamental units of tertiary structure and are found in more than one protein. A structural domain is selfstabilizing and often folds independently of the rest of the protein chain.

Leucine zipper

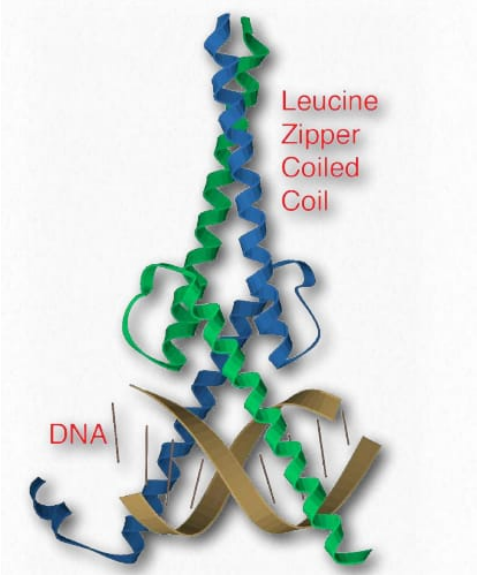


Figure 2.63 - Leucine zipper bound to DNA Wikipedia

A common feature of many eukaryotic DNA binding proteins, leucine zippers are characterized by a repeating set of leucine residues in a protein that interact like a zipper to favor dimerization. Another part of the domain has amino acids (commonly arginine and lysine) that allow it to interact with the DNA double helix (Figure 2.63). Transcription factors that contain leucine zippers include Jun-B, CREB, and AP-1 fos/ jun.

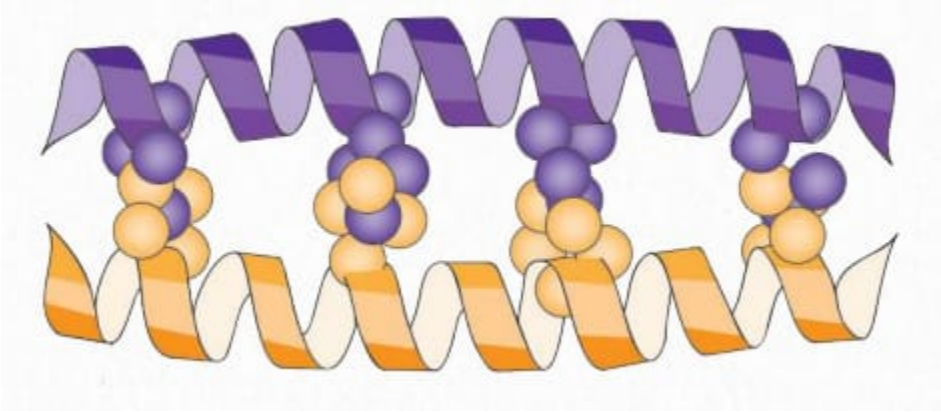


Figure 2.64 - Leucine zipper structure. Leucines are indicated by orange and purple balls. Image by Penelope Irving

Zinc fingers

The shortest structural domains are the zinc fingers, which get their name from the fact that one or more coordinated zinc ions stabilize their finger-like structure. Despite their name, some zinc fingers do not bind zinc. There are many structural domains classified as zinc fingers and these are grouped into different families. Zinc fingers were first identified as components of DNA binding transcription factors, but others are now known to bind RNA, protein, and even lipid structures. Cysteine and histidine side chains commonly play roles in coordinating the zinc.

Src SH_2 domain

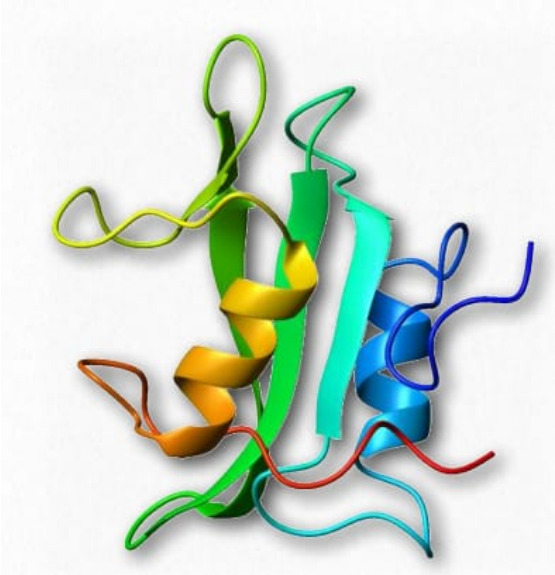


Figure 2.65 - SH2 Domain Wikipedia

The Src oncoprotein contains a conserved SH_2structural domain that recognizes and binds phosphorylated tyrosine side chains in other proteins (Figure 2.65). Phosphorylation is a fundamental activity in signaling and phosphorylation of tyrosine and interaction between proteins carrying signals is critically needed for cellular communication. The SH_2domain is found in over 100 human proteins.

Helix-turn-helix domain



Figure 2.66 - Helix-Turn-Helix Domain of a Protein Bound to DNA Wikipedia

Helix-turn-helix is a common domain found in DNA binding proteins, consisting of two α-helices separated by a small number of amino acids. As seen in Figure 2.66, the helix parts of the structural domain interact with the bases in the major groove of DNA. Individual α-helices in a protein are part of a helix-turn-helix structure, where the turn separates the individual helices.

Pleckstrin homology domain

Pleckstrin Homology (PH) domains are protein domains with important functions in the process of signaling. This arises partly from the affinity for binding phosphorylated inositides, such as PIP2 and PIP3, found in Figure 2.66 - Helix-Turn-Helix Domain of a Protein Bound to DNA Wikipedia Figure 2.65 - SH2 Domain Wikipedia biological membranes. PH domains can also bind to G-proteins and protein kinase C. The domain spans about amino acids and is found in numerous signaling proteins.

These include Akt/Rac Serine/ Threonine Protein Kinases, Btk/ltk/Tec tyrosine protein kinases, insulin receptor substrate (IRS-1), Phosphatidylinositolspecific phospholipase C, and several yeast proteins involved in cell cycle regulation.

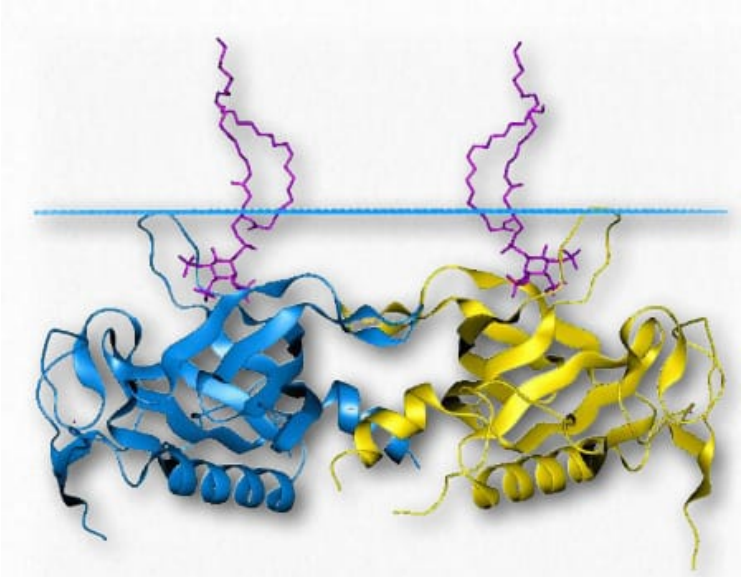


Figure 2.67 - Pleckstrin homology domain of Btk tyrosine protein kinase. The protein is embedded in a membrane (above blue line) Wikipedia

Structural globular proteins

Enzymes catalyze reactions and proteins such as hemoglobin perform important specialized functions. Evolutionary selection has reduced and eliminated waste so that we can be sure every protein in a cell has a function, even though in some cases we may not know what it is. Sometimes the structure of the proFigure 2.68 - Relationship of basement membrane to epithelium, endothelium, and connective tissue tein is its primary function because the structure provides stability, organization, connections other important properties. It is with this in mind that we present the following proteins.

Basement membrane

The basement membrane is a layered extracellular matrix of tissue comprised of protein fibers (type IV collagen) and glycosaminoglycans that separates the epithelium from other tissues (Figure 2.68). More importantly, the basement membrane acts like a glue to hold tissues together. The skin, for example, is anchored to the rest of the body by the basement membrane. Basement membranes provide an interface of interaction between cells and the environment around them, thus facilitating signaling processes. They play roles in differentiation during embryogenesis and also in maintenance of function in adult organisms.

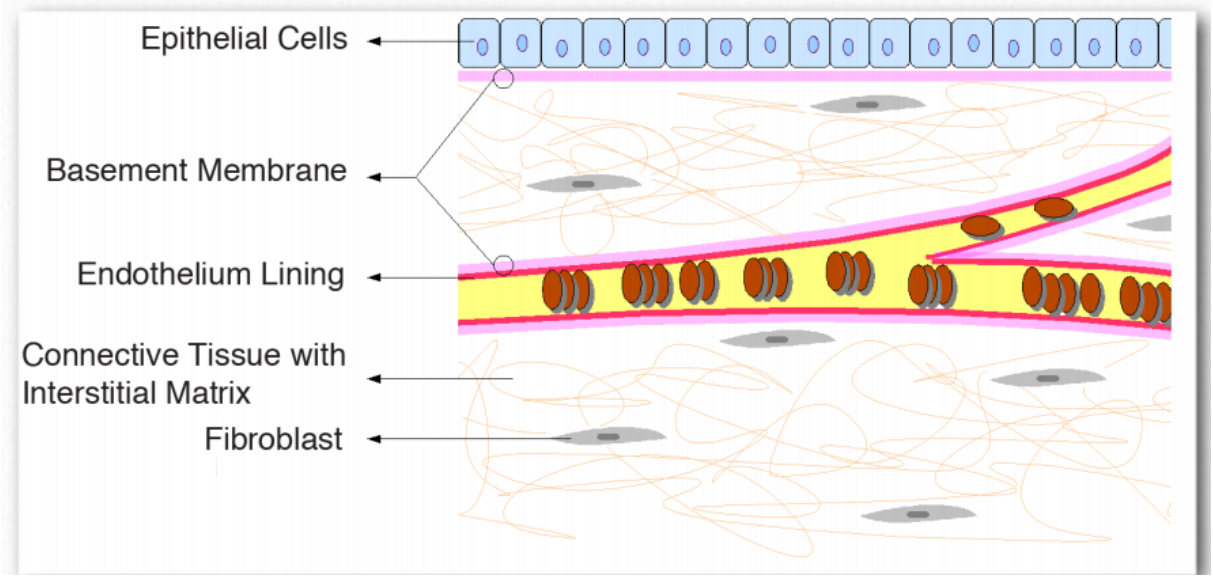


Figure 2.68 - Relationship of basement membrane to epithelium, endothelium, and connective tissue

Actin

Actin is the most abundant globular protein found in most types of eukaryotic cells, comprising as much as 20% of the weight of muscle cells. Similar proteins have been identified in bacteria (MreB) and archaeons (Ta0583). Actin is a monomeric subunit able to polymerize readily into two different types of filaments. Microfilaments are major component of the cytoskeleton and are acted on by myosin in the contraction of muscle cells (See HERE). Actin will be discussed in more detail in the next section HERE.

Intermediate Filaments

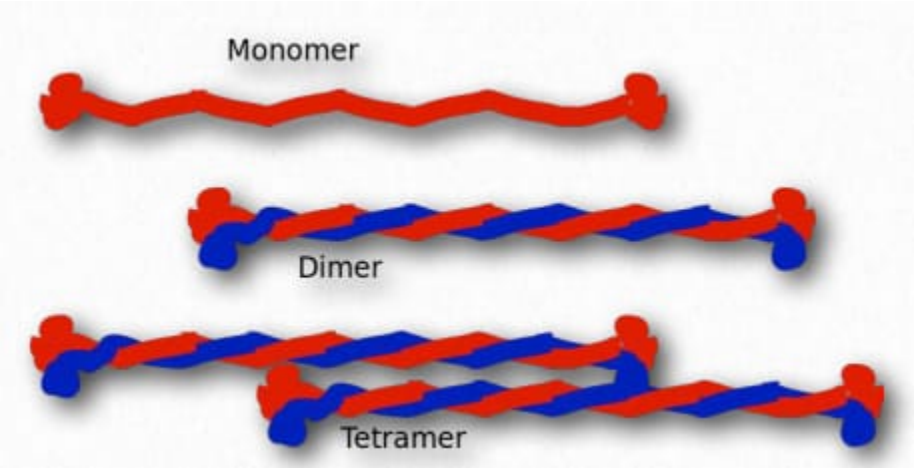


Figure 2.69 - Assembly of intermediate filaments

Intermediate filaments are a part of the cytoskeleton in many animal cells and are comprised of over 70 different proteins. They are called intermediate because their size (average diameter = 10 nm) is between that of the microfilaments (7 nm) and the microtubules (25 nm).

The intermediate filament components include fibrous proteins, such as the keratins and the lamins, which are nuclear, as well as cytoplasmic forms. Intermediate filaments give flexibility to cells because of their own physical properties. They can, for example, be stretched to several times of their original length.

Six types

There are six different types of intermediate filaments. Type I and II are acidic or basic and attract each other to make larger filaments. They include epithelial keratins and trichocytic keratins (hair components). Type III proteins include four structural

proteins - desmin, GFAP (glial fibrillary acidic protein), peripherin, and vimentin. Type IV also is a grouping of three proteins and one multiprotein structure (neurofilaments). The three proteins are α-internexin, synemin, and syncoilin. Type V intermediate filaments encompass the lamins, which give structure to the nucleus. Phosphorylation of lamins leads to their disassembly and this is important in the process of mitosis. The Type VI category includes only a single protein known as nestin.

Tubulin

A third type of filament found in cells is that of the microbutules. Comprised of a polymer of two units of a globular protein called tubulin, microtubules provide “rails” for motor proteins to move organelles and other “cargo” from one part of a cell to another. Microtubules and tubulin are discussed in more detail HERE.

Vimentin

Vimentin (Figure 2.70) is the most widely distributed protein of the intermediate filaments. It is expressed in fibroblasts, leukocytes, and blood vessel endothelial cells. The protein has a significant role maintaining the position of organelles in the cytoplasm, with attachments to the nucleus, mitochondria, and endoplasmic reticulum (Figure 2.70). Vimentin provides elasticity to cells and resilience that does not arise from the microtubules or microfilaments. Wounded mice that lack the vimentin gene survive, but take longer to heal wounds than wild type mice. Vimentin also controls the movement of cholesterol from lysosomes to the site of esterification. The result is a reduction in the amount of cholesterol stored inside of cells and has implications for adrenal cells, which must have esters of cholesterol.

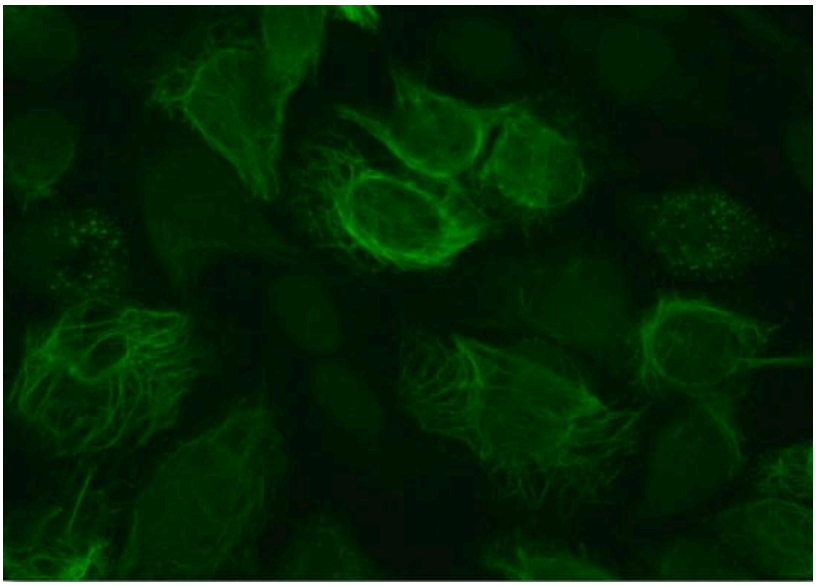


Figure 2.70 - Vimentin in cells Wikipedia

Mucin

Mucins are a group of proteins found in animal epithelial tissue that have many glycosyl residues on them and typically are of high molecular weight (1 to 10 million Da). They are gel-like in their character and are often used for lubrication. Mucus is comprised of mucins. In addition to lubrication, mucins also help to control mineralization, such as bone formation in vertebrate organisms and calcification in echinoderms. They also play roles in the immune system by helping to bind pathogens. Mucins are commonly secreted onto mucosal surfaces (nostrils, eyes, mouth, ears, stomach, genitals, anus) or into fluids, such as saliva. Because of their extensive mucosylation, mucins hold a considerable amount of water (giving them the “slimy” feel) and are resistant to proteolysis.

Vinculin

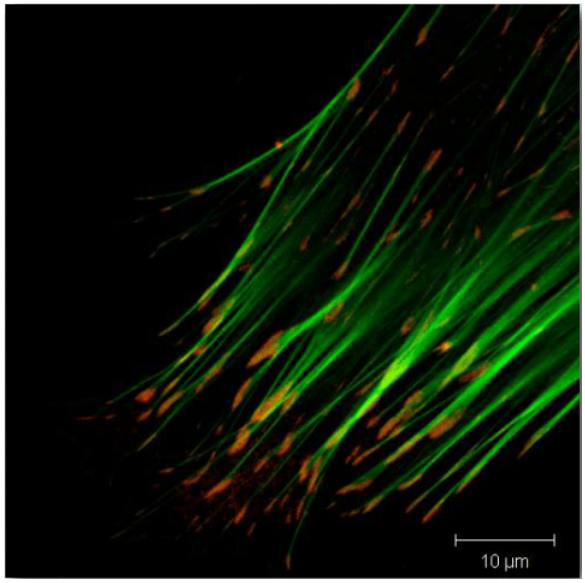


Figure 2.71 - Actin filaments (green) attached to vinculin in focal adhesion (red) Wikipedia

Vinculin (Figure 2.72) is a membrane cytoskeletal protein found in the focal adhesion structures of mammalian cells. It is found at cell-cell and cell-matrix junctions and interacts with integrins, talin, paxillins and F-actin. Vinculin is thought to assist (along with other proteins) in anchoring actin microfilaments to the membrane (Figure 2.71). Binding of vinculin to actin and to talin is regulating by polyphosphoinositides and can be inhibited by acidic phospholipids.

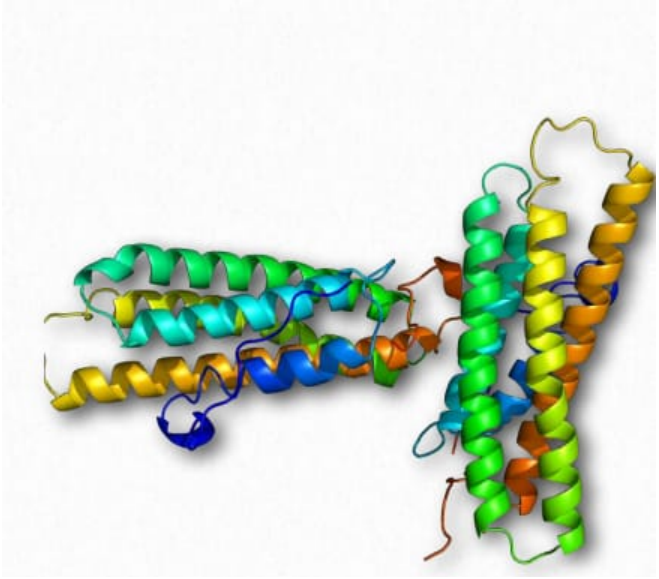


Figure 2.72 - Vinculin Wikipedia

Syndecans

Syndecans are transmembrane proteins that make a single pass with a long amino acid chain (24-25 residues) through plasma membranes and facilitate G proteincoupled receptors' interaction with Figure 2.71 - Actin filaments (green) attached to vinculin in focal adhesion (red) Wikipedia ligands, such as growth factors, fibronectin, collagens (I, III, and IV) and antithrombin-1. Syndecans typically have 3-5 heparan sulfate and chondroitin sulfate chains attached to them.

Heparan sulfate can be cleaved at the site of a wound and stimulate action of fibroblast growth factor in the healing process. The role of syndecans in cell-cell adhesion is shown in mutant cells lacking syndecan I that do not adhere well to each other. Syndecan 4 is also known to adhere to integrin. Syndecans can also inhibit the spread of tumors by the ability of the syndecan 1 ectodomain to suppress growth of tumor cells without affecting normal epithelial cells.

Defensin

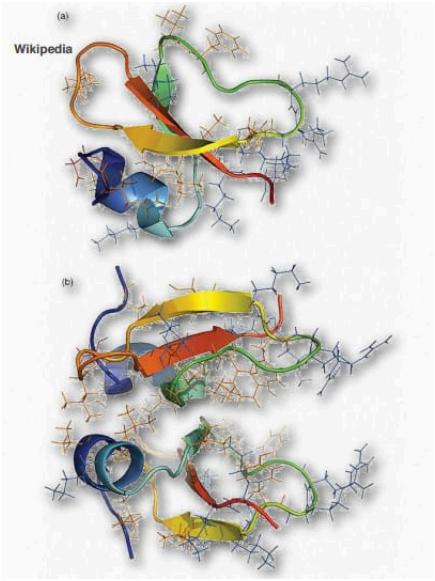


Figure 2.73 - Defensin monomer (top) and dimer (bottom) - Cationic residues in blue, hydrophobic residues in orange, and anionic residues in red Wikipedia

Defensins (Figure 2.73) are a group of small cationic proteins (rich in cysteine residues) that serve as host defense peptides in vertebrate and invertebrate organisms. They protect against infection by various bacteria, fungi, and viruses. Defensins contain between 18 and 45 amino acids with (typically) about 6- 8 cysteine residues. In the immune system, defensins help to kill bacteria engulfed by phagocytosis by epithelial cells and neutrophils. They kill 120 Figure 2.72 - Vinculin Wikipedia bacteria by acting like ionophores - binding the membrane and opening pore-like structures to release ions and nutrients from the cells.

Focal adhesions

In the cell, focal adhesions are structures containing multiple proteins that mechanically link cytoskeletal structures (actin bundles) with the extracellular matrix. They are dynamic, with proteins bringing and leaving with signals regarding the cell cycle, cell motility, and more almost constantly. Focal adhesions serve as anchors and as a signaling hub at cellular locations where integrins bind molecules and where membrane clustering events occur. Over 100 different proteins are found in focal adhesions.

Focal adhesions communicate important messages to cells, acting as sensors to update information about the status of the extracellular matrix, which, in turn, adjusts/ affects their actions. In sedentary cells, they are stabler than in cells in motion because when cells move, focal adhesion contacts are established at the “front” and removed at the rear as motion progresses. This can be very important in white blood cells’ ability to find tissue damage.

Ankyrin

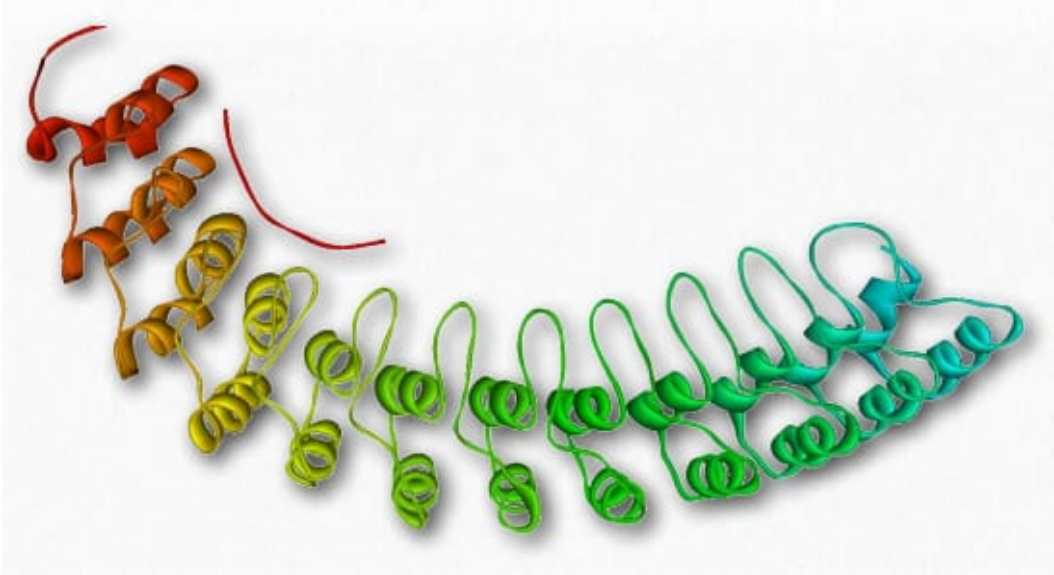


Figure 2.74 - Ankyrin's membrane-binding domain

Ankyrins (Figure 2.74) are a family of membrane adaptor proteins serving as "anchors" to interconnect integral membrane proteins to the spectrin-actin membrane cytoskeleton. Ankyrins are anchored to the plasma membrane by covalently linked palmitoyl-CoA. They bind to the β subunit of spectrin and at least a dozen groups of integral membrane proteins. The ankyrin proteins contain four functional domains: an N-terminal region with 24 tandem ankyrin repeats, a central spectrin-binding domain, a "death domain" interacting with apoptotic proteins, and a C-terminal regulatory domain that is highly varied significantly among different ankyrins.

Spectrin

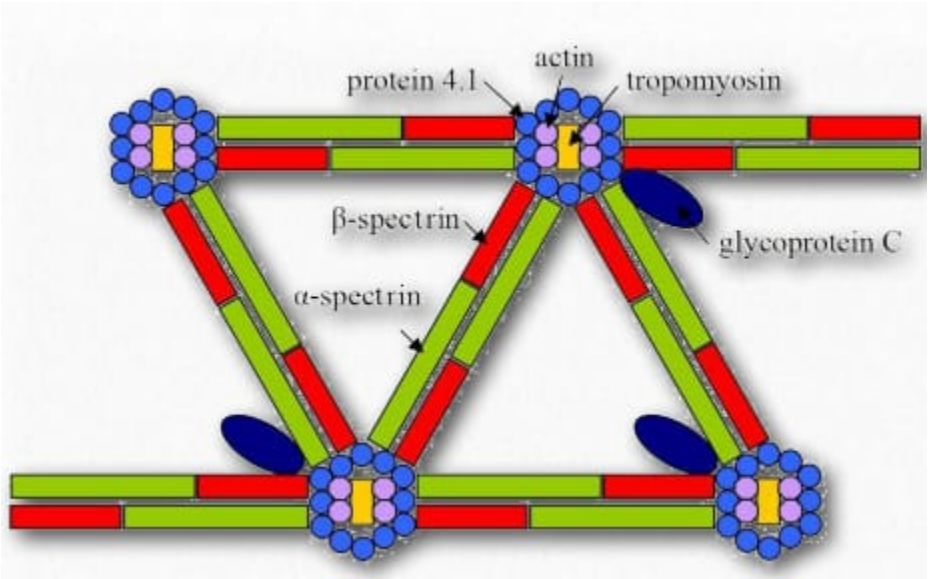


Figure 2.75 - Spectrin and other proteins in the cytoskeleton Wikipedia

Spectrin (Figures 2.75 & 2.76) is a protein of the cellular cytoskeleton that plays an important role in maintaining its structure and the integrity of the plasma membrane. In animals, spectrin gives red blood cells their shape. Spectrin is located inside the inner layer of the eukaryotic plasma membrane where it forms a network of pentagonal or hexagonal arrangements.

Spectrin fibers collect together at junctional complexes of actin and is also attached to ankyrin for stability, as well as numerous integral membrane proteins, such as glycophorin.

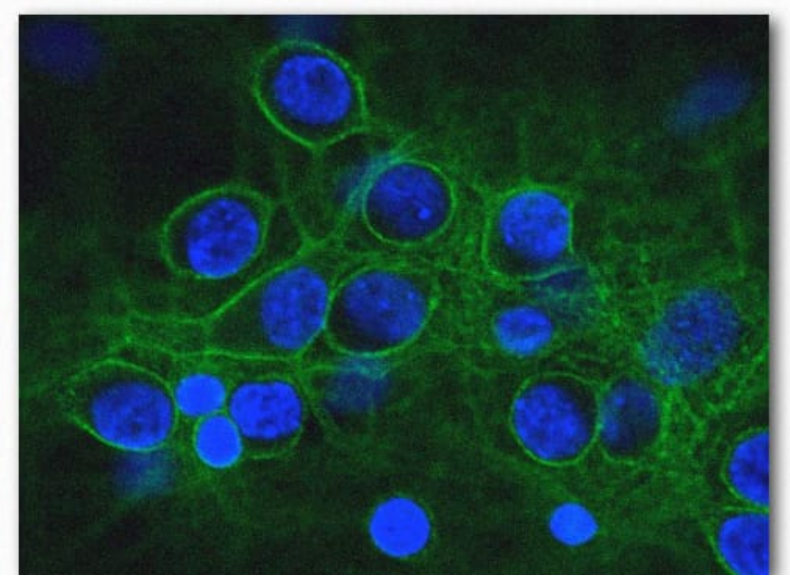


Figure 2.76 - Spectrin (green) and nuclei (Blue) Wikipedia

Integrins

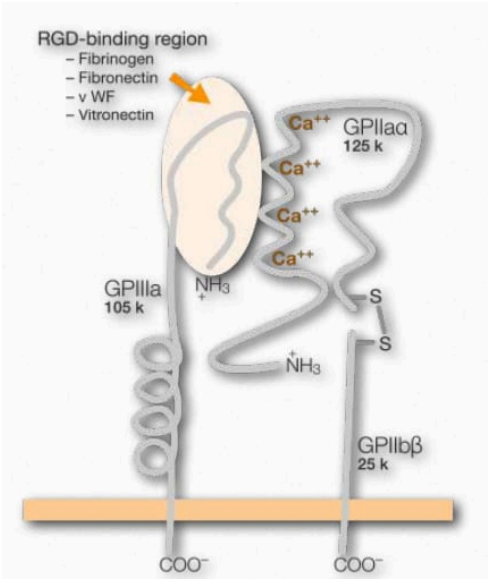


Figure 2.77 - Integrin and its binding site (on top left) Wikipedia

In multicellular organisms, cells need connections, both to each other and to the extracellular matrix. Facilitating these attachments at the cellular end are transmembrane proteins known as integrins (Figure 2.77). Integrins are found in all metazoan cells. Ligands for the integrins include collagen, fibronectin, laminin, and vitronectin. Integrins function not only in attachment, but also in communication, cell migration, virus linkages (adenovirus, for example), and blood clotting. Integrins are able to sense chemical and mechanical signals about the extracellular matrix and move that information to intracellular domains as part of the process of signal transduction. Inside the cells, responses to the signals affect cell shape, regulation of the cell cycle, movement, or changes in other cell receptors in the membrane. The process is dynamic and allows for rapid responses as may be necessary, for example in the process of blood clotting, where the integrin known as GPIbIIIa (on the surface of blood platelets) attaches to fibrin in a clot as it develops.

Integrins work along with other receptors, including immunoglobulins, other cell adhesion molecules, cadherins, selectins, and syndecans. In mammals the proteins have a large number of subunits - 18 α- and 8 β-chains. They are a bridge between its links outside the cell to the extracellular matrix (ECM) and its links inside the cell to the cytoskeleton. Integrins play central role in formation and stability of focal adhesions. These are large molecular complexes arising from clustering of integrin-ECM connections. In the process of cellular movement, integrins at the “front” of the cell (in the direction of the movement), make new

attachments to substrate and release connections to substrate in the back of the cell. These latter integrins are then endocytosed and reused.

Integrins also help to modulate signal transduction through tyrosine kinase receptors in the cell membrane by regulating movement of adapters to the plasma membrane. β1c integrin, for example, recruits the Shp2 phosphatase to the insulin growth factor receptor to cause it to become dephosphorylated, thus turning off the signal it communicates. Integrins can also help to recruit signaling molecules inside of the cell to activated tyrosine kinases to help them to communicate their signals.

Cadherins

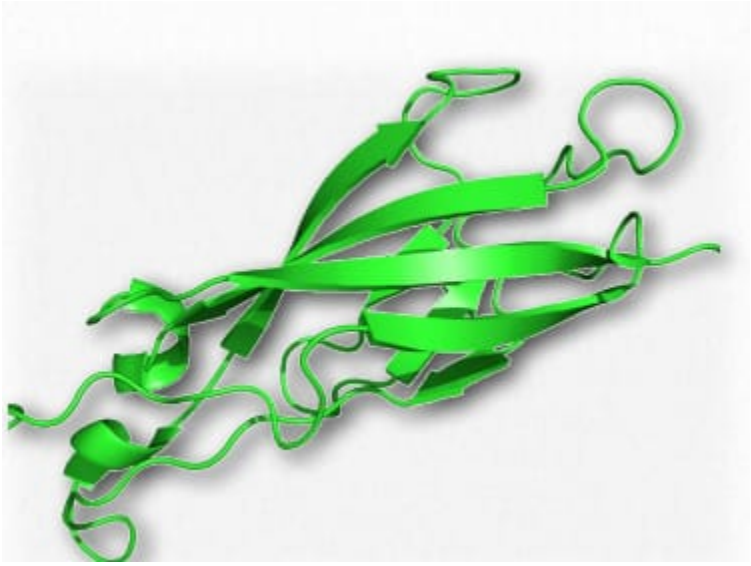


Figure 2.78 - Extracellular ectodomain of a cadherin

Cadherins (Figure 2.78) constitute a type-1 class of transmembrane proteins playing important roles in cell adhesion. They require calcium ions to function, forming adherens junctions that hold tissues together (See Figure 2.69). Cells of a specific cadherin type will preferentially cluster with each other in preference to associating with cells containing a different cadherin type. Caderins are both receptors and places for ligands to attach. They assist in the proper positioning of cells in development, separation of different tissue layers, and cell migration.

Selectins



Figure 2.79 - Selectin bound to a sugar Wikipedia

Selectins (Figure 2.79) are cell adhesion glycoproteins that bind to sugar molecules. As such, they are a type of lectin - proteins that bind sugar polymers (see HERE also). All selectins have an N-terminal calcium-dependent lectin domain, a single transmembrane domain, and an intracellular cytoplasmic tail.

There are three different types of selectins, 1) E-selectin (endothelial); 2) L (lymphocytic; and 3) P (platelets and endothelial cells. Selectins function in lymphocyte homing (adhesion of blood lymphocytes to cells in lymphoid organs), in inflammation processes, and in cancer metastasis. Near the site of inflammation, P-selectin on the surface of blood capillary cells interacts with glycoproteins on leukocyte cell surfaces. This has the effect of slowing the movement of the leukocyte. At the target site of inflammation, E- selectin on the endothelial cells of the blood vessel and L-selectin on the surface of the leukocyte bind to their respective carbohydrates, stopping the leukocyte movement. The leukocyte then crosses the wall of the capillary and begins the immune response. Selectins are involved in the inflammatory processes of asthma, psoriasis, multiple scleroris, and rheumatoid arthritis.

Laminins

Laminins are extracellular matrix glycoproteins that a major components of the basal lamina and affect cell differentiation, migration, and adhesion. They are secreted into the extracellular matrix where they are incorporated and are essential for tissue maintenance and survival. When laminins are defective, muscles may not form properly and give rise to muscular dystrophy.

Laminins are associated with fibronectin, entactin, and perlecan proteins in type IV collagen networks and bind to integrin receptors in the plasma membrane. As a consequence, laminins contribute to cellular attachment, differentiation, shape, and movement. The proteins are trimeric in structure, having one α-chain, a β-chain, and a γ-chain. Fifteen combinations of different chains are known.

Vitronectin

Vitronectin is a glycoprotein (75kDa) found in blood serum (platelets), the extracellular matrix, and in bone. It promotes the process of cell adhesion and spreading and binds to several protease inhibitors (serpins). It is secreted from cells and is believed to play roles in blood clotting and the malignancy of tumors. One domain of vitronectin binds to plasminogen activator inhibitor and acts to stabilize it. Another domain of the protein binds to cellular integrin proteins, such as the vitronectin receptor that anchors cells to the extracellular matrix.

Catenins

Catenins are a family of proteins interacting with cadherin proteins in cell adhesion (Figure 2.69). Four main types of catenins are known, α-, β-, γ-, and δ-catenin. Catenins play roles in cellular organization before development occurs and help to regulate cellular growth. α-catenin and β-catenin are found at adherens junctions with cadherin and help cells to maintain epithelial layers. Cadherins are connected to actin filaments of the cytoskeleton and catenins play the critical role. Catenins are important for the process whereby cellular division is inhibited when cells come in contact with each other (contact inhibition).

When catenin genes are mutated, cadherin cell adhesions can disappear and tumorigenesis may result. Catenins have been found to be associated with colorectal and numerous other forms of cancer.

Glycophorins

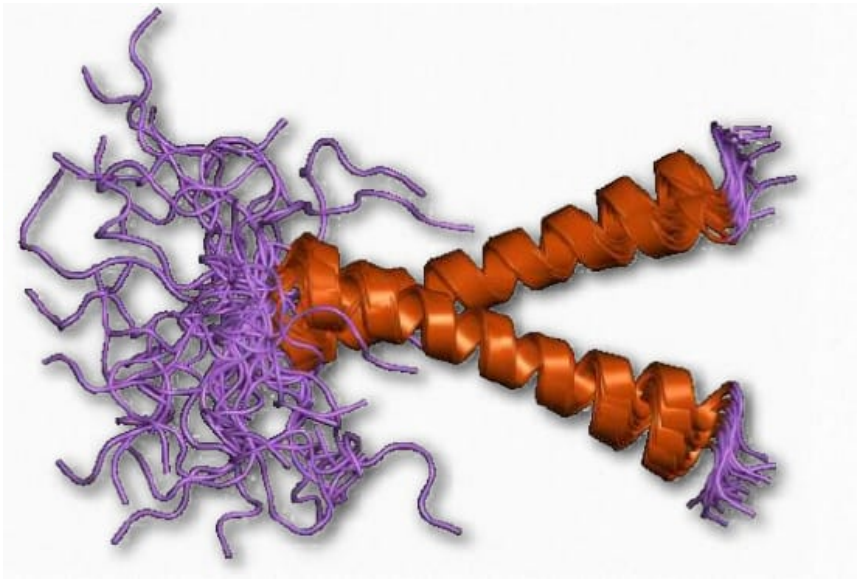


Figure 2.80 - Glycophorin a

All of the membrane proteins described so far are notable for the connections they make to other proteins and cellular structures. Some membrane proteins, though, are designed to reduce cellular connections to proteins of other cells. This is particularly important for blood cells where “stickiness” is undesirable except where clotting is concerned.

Glycophorins (Figure 2.80) are membrane-spanning sialoglycoproteins of red blood cells. They are heavily glycosylated (60%).and rich in sialic acid, giving the cells a very hydrophilic (and negatively charged) coat, which enables them to circulate in the bloodstream without adhering to other cells or the vessel walls.

Five glycophorins have been identified - four (A,B,C,and D) from isolated membranes and a fifth form (E) from coding in the human genome. The proteins are abundant, forming about 2% of the total membrane proteins in these cells. Glycophorins have important roles in regulating RBC membrane mechanical properties and shape. Because some glycophorins can be expressed in various nonerythroid tissues (particularly Glycophorin C), the importance of their interactions with the membrane skeleton may have a considerable biological significance.

Cooperativity and allosterism - quaternary structure

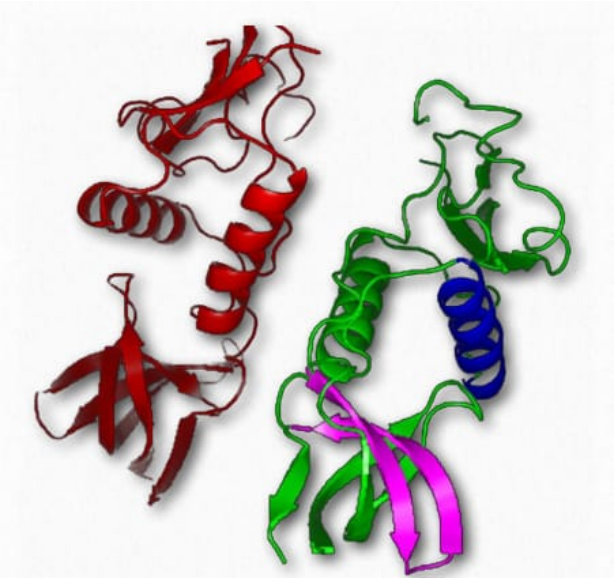


Figure 2.81 - Two polypeptide units of a protein interact in quaternary structure Wikipedia

Quaternary structure, of course describes the interactions of individual subunits of a multi-subunit protein (Figure 2.81). The result

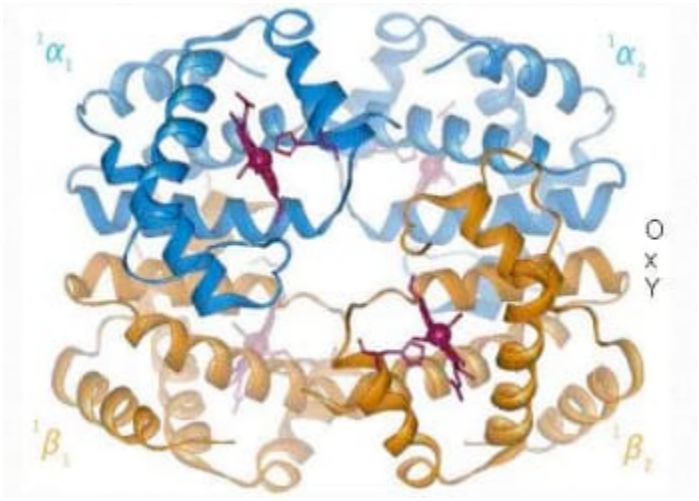
of these interactions can give rise to important biological phenomena, such as cooperative binding of substrates to a protein and allosteric effects on the action of an enzyme.

Allosteric effects can occur by a series of mechanisms, but a common feature is that binding of an effector to an enzyme subunit causes (or locks) the enzyme in either a Tstate (less activity) or an R-state (more activity). Effectors can be enzyme substrates (homotropic effectors) or non-substrates (heterotropic effectors). Allosterism will be covered in more depth in the Catalysis chapter HERE.

We begin our consideration of quaternary structure with a discussion of cooperativity, how it arises in the multi-subunit protein hemoglobin and how its properties contrast with those of the related, single subunit protein myoglobin.

Cooperativity

Cooperativity is defined as the phenomenon where 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).



Movie 2.3 - Hemoglobin's structural changes on binding oxygen Wikipedia

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 Tstate.

CO_2 transport

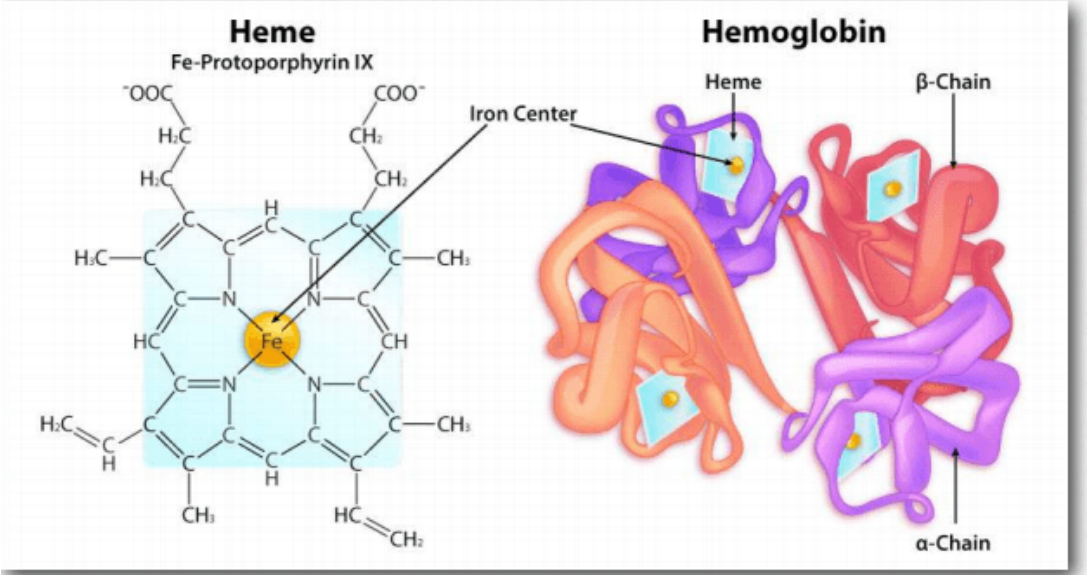


Figure 2.82 - Heme structure within hemoglobin Image by Aleia Kim

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. Like the binding of oxygen to hemoglobin, binding of other molecules to hemoglobin affects its affinity for oxygen. The effect is particularly pronounced when comparing the oxygen binding characteristics of hemoglobin's four subunits with the oxygen binding of the related protein myoglobin's single subunit (Figure 2.83).

Different oxygen binding

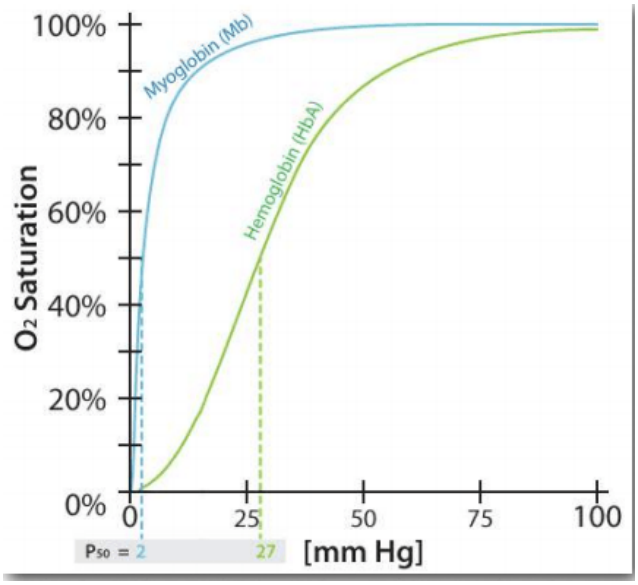


Figure by Aleia Kim

Like hemoglobin, myoglobin contains an iron in a heme group that binds to oxygen. The structure of the globin protein in myoglobin is very similar to the structure of the globins in hemoglobin and hemoglobin is thought to have evolved from myoglobin in evolutionary history. As seen in Figure 2.83, the binding curve of hemoglobin for oxygen is S-shaped (sigmoidal), whereas the binding curve for myoglobin is hyperbolic. What this tells us is that hemoglobin's affinity for oxygen is low at a low concentration oxygen, but increases as the oxygen concentration increases. Since myoglobin very quickly saturates with oxygen, even under low oxygen concentrations, it says that its affinity for oxygen is high and doesn't change.

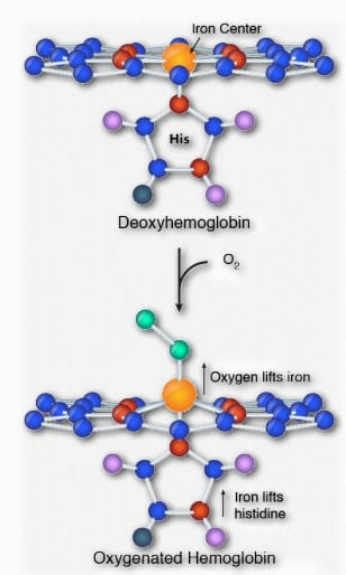
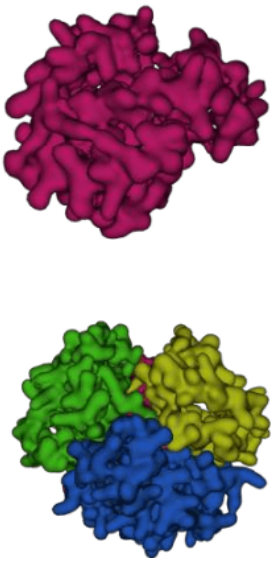


Figure 2.84 - Binding of oxygen at the heme center of hemoglobin Image by Aleia Kim

Because myoglobin has only a single subunit, binding of oxygen by that subunit can't affect any other subunits, since there are no other subunits to affect. Consequently, cooperativity requires more than one subunit. Therefore, hemoglobin can exhibit cooperativity, but myoglobin can't. It is worth noting that simply having multiple subunits does not mean cooperativity will exist. Hemoglobin is one protein that exhibits the characteristic, but many multisubunit proteins do not.



Interactive 2.2 - Hemoglobin in the presence (top) and absence (bottom) of oxygen

Storage vs. delivery

The lack of ability of myoglobin to adjust its affinity for oxygen according to the oxygen concentration (low affinity at low oxygen concentration, such as in tissues and high affinity at high oxygen concentration, such as in the lungs) means it is better suited for storing oxygen than for delivering it according to the varying oxygen needs of and animal body. As we shall see, besides cooperativity, hemoglobin has other structural features that allow it to deliver oxygen precisely where it is needed most in the body.

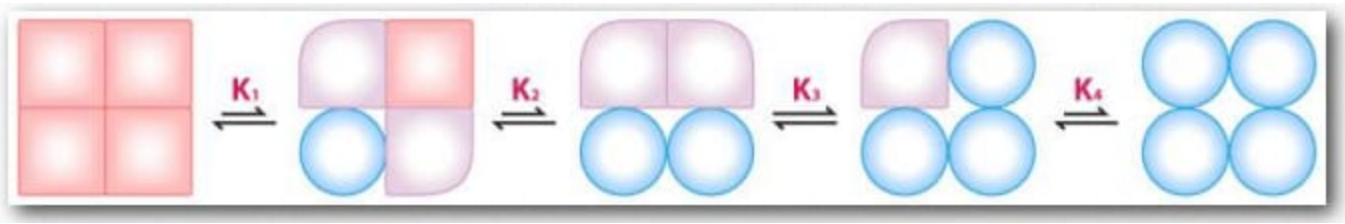


Figure 2.85 - Sequential model of binding. The sequential model is one way to explain hemoglobin's cooperativity. Squares represent no oxygen bound. Circles represent subunits bound with oxygen and rounded subunits correspond to units whose affinity for oxygen increases by interacting with a subunit that has bound oxygen. Image by Aleia Kim 131

Bohr effect

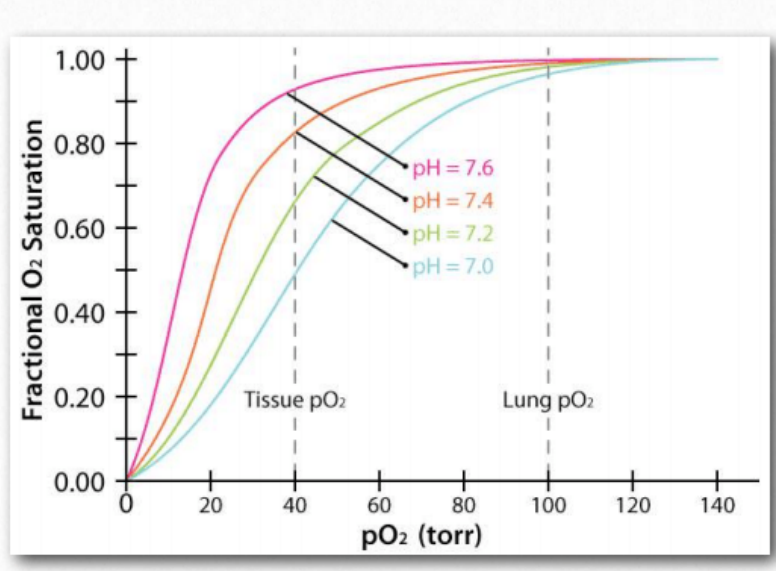


Figure 2.86 - The Bohr effect with respect to pH changes Image by Aleia Kim

The Bohr Effect was first described over 100 years ago by Christian Bohr, father of the famous physicist, Niels Bohr. Shown graphically (Figures 2.86, 2.87, and 2.88), the observed effect is that hemoglobin's affinity for oxygen decreases as the pH decreases and as the concentration of carbon dioxide increases. Binding of the protons and carbon dioxide by amino Figure 2.85 - Sequential model of binding. The sequential model is one way to explain hemoglobin's cooperativity. Squares represent no oxygen bound. Circles represent subunits bound with oxygen and rounded subunits correspond to units whose affinity for oxygen increases by interacting with a subunit that has bound oxygen. Image by Aleia Kim acid side chains in the globin proteins helps to facilitate structural changes in them. Most commonly, the amino acid affected by protons is histidine #146 of the β strands. When this happens, the ionized histidine can form an ionic bond with the side chain of aspartic acid #94, which has the effect of stabilizing the T-state (reduced oxygen binding state) and releasing oxygen. Other histidines and the amine of the amino terminal amino acids in the α-chains are also binding sites for protons.

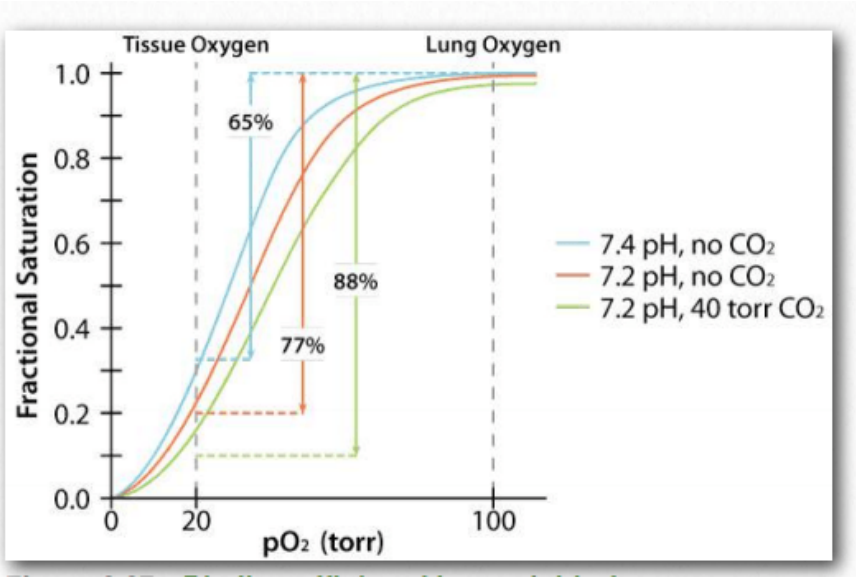


Figure 2.87 - Binding affinity of hemoglobin for oxygen under different conditions Image by Aleia Kim

2,3-BPG

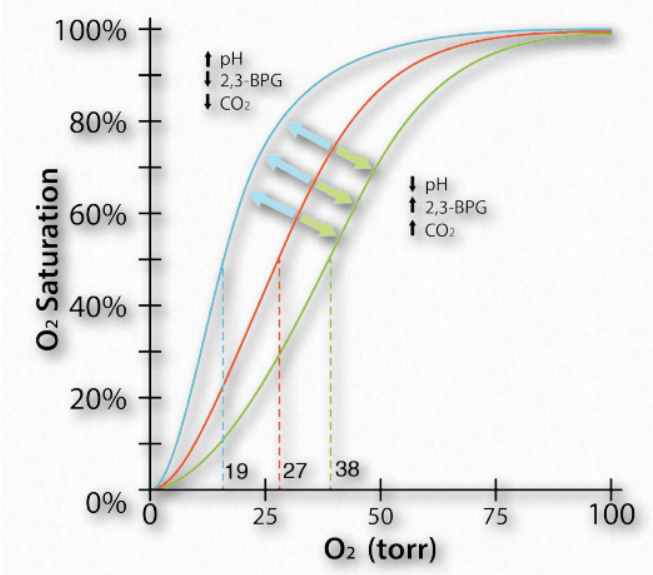


Figure 2.88 - The Bohr effect physiologically - oxygen binding curves for resting muscle (blue), active muscle (green) and reference muscle (orange) with respect to pH, 2,3-BPG, and CO2 Image by Aleia Kim

Another molecule favoring the release of oxygen by hemoglobin is 2,3- bisphosphoglycerate (also called 2,3-BPG or just BPG - Figure 2.89). Like protons and carbon dioxide, 2,3-BPG is produced by actively respiring tissues, as a byproduct of glucose metabolism. The 2,3-BPG mole cule fits into the 'hole of the donut' of adult hemoglobin (Figure 2.89). Such binding of 2,3-BPG favors the T-state (tight - low oxygen binding) of hemoglobin, which has a reduced affinity for oxygen. In the absence of 2,3-BPG, hemoglobin can more easily exist in the R-state (relaxed - higher oxygen binding), which has a high affinity for oxygen.

Smokers

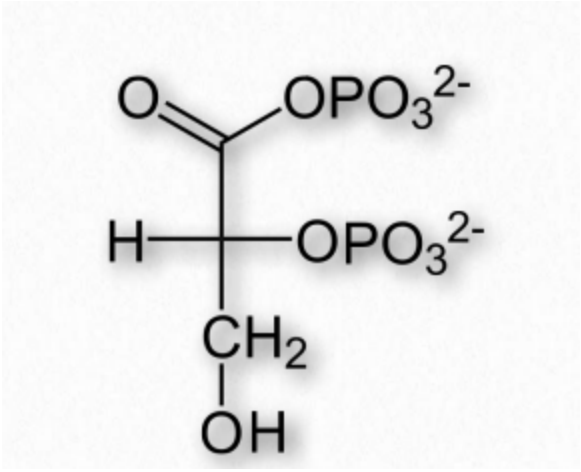


Figure 2.89 - The structure of 2,3 bisphosphoglycerate (2,3-BPG)

Notably, the blood of smokers is higher in the concentration of 2,3-BPG than non-smokers, so more of their hemoglobin remains in the T-state and thus the oxygen carrying capacity of smokers is lower than non-smokers.Another reason why smokers' oxygen carrying capacity is lower than that of non-smokers is that cigarette smoke contains carbon monoxide and this molecule, which has almost identical dimensions to molecular oxygen, effectively outcompetes with oxygen for binding to the iron atom of heme (Figure 2.90). Part of carbon monoxide's toxicity is due to its ability to bind hemoglobin and prevent oxygen from binding.

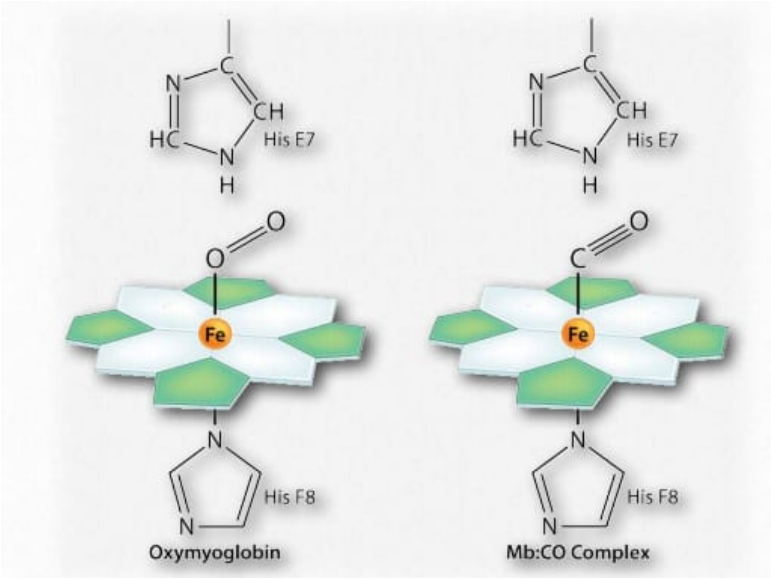


Figure 2.90 - Binding of oxygen (left) and carbon monoxide (right) by a heme group of hemoglobin Image by Aleia Kim

Carbon dioxide

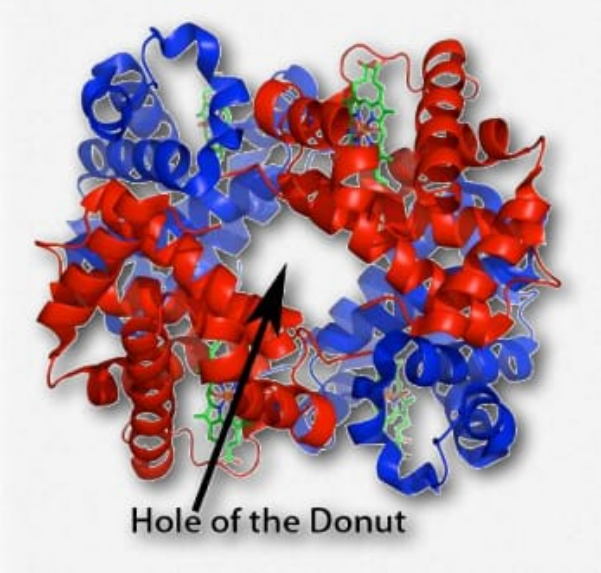


Figure 2.91 - Hemoglobin's hole of the donut for binding 2,3-BPG Wikipedia

Carbon dioxide binds to form a carbamate when binding the α-amine of each globin chain. The process of forming this structure releases a proton, which helps to further enhance the Bohr effect. Physiologically, the binding of CO2 and H+ has 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.

About 40% of the released protons and about 20% of the carbon dioxide are carried back to the lungs by hemoglobin. The remainder travel as part of the bicarbonate buffering system or as dissolved CO2. In the lungs, the process reverses itself. The lungs have a higher pH than respiring tissues, so protons are released from hemoglobin and CO2 too is freed to be exhaled.

Fetal hemoglobin

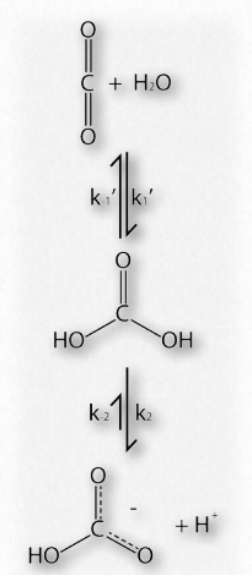


Figure 2.92 - Formation of bicarbonate from CO2 in blood

Adult hemoglobin releases oxygen when it binds 2,3- BPG. This is in contrast to fetal hemoglobin, which has a slightly different configuration (α2γ2) than adult hemoglobin (α2β2). 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. Consequently, fetal hemoglobin remains in the R-state much more than adult hemoglobin and because of this, fetal hemoglobin has greater affinity for oxygen than adult hemoglobin and can take oxygen away from adult hemoglobin. Thus, the fetus can get oxygen from the mother.

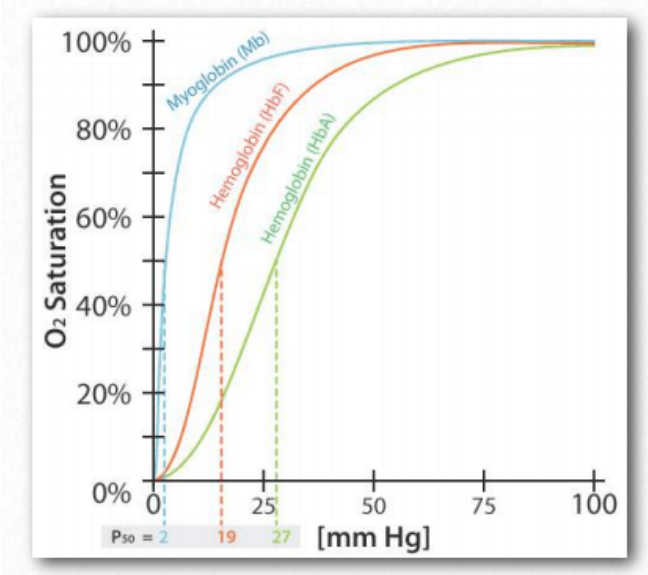


Figure 2.93 - Comparison of oxygen binding of myoglobin (blue), fetal hemoglobin (orange), and adult hemoglobin (green) Image by Aleia Kim

Sickle cell disease



Figure 2.94 - Four normal red blood cells (right) and one sickled red blood cell (left) Wikipedia

Mutations to the globin genes coding for hemoglobin can sometimes have deleterious consequences. Sickle cell disease (also called sickle cell anemia) is a genetically transmitted disease that arises from such mutations. There are different forms of the disease. It is a recessive trait, meaning that to be afflicted with it, an individual must inherit two copies of the mutated gene.

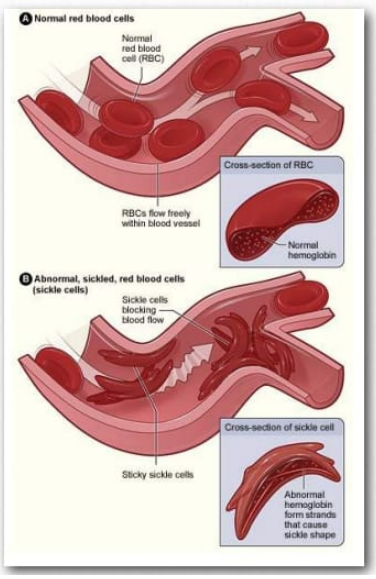


Figure 2.95 - Movement of blood in capillaries. Top - normal red blood cells. Bottom - sickled red blood cells

The predominant form of hemoglobin in adults is hemoglobin A, designated HbA (two α chains and two β chains). The mutant form is known as HbS. The most common mutation is an A to T mutation in the middle of the codon for the seventh amino acid (some counting schemes call it the sixth amino acid) of the β-chain. This results in conversion of a GAG codon to GTG and thus changes the amino acid specified at that position from a glutamic acid to a valine. This minor change places a small hydrophobic patch of amino acids on the surface of the β-globin chains.

Polymerization

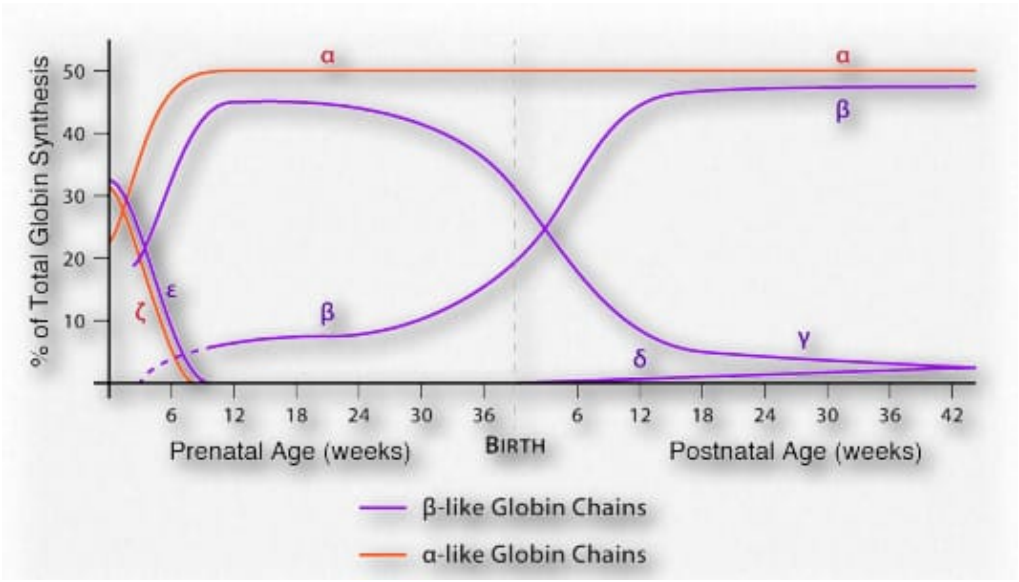


Figure 2.96 - Pattern of expression of six different globins of hemoglobin - α,β,γ,ε,δ, and ζ Image by Aleia Kim

Under conditions of low oxygen, these hydrophobic patches will associate with each other to make long polymers of hemoglobin molecules. The result is that the red blood cells containing them will change shape from being rounded to forming the shape of a sickle (Figure 2.94). Rounded red blood cells readily make it through tiny capillaries, but sickleshaped cells do not.

Worse, they block the flow of other blood cells. Tissues where these blockages occur are already low in oxygen, so stopping the flow of blood through them causes them to go quickly anaerobic, causing pain and, in some cases, death of tissue. In severe circumstances, sickled red blood cells death may result. The disease is referred to as an anemia because the sickling of the red blood cells targets them for removal by the blood monitoring system of the body, so a person with the disease has chronically reduced numbers of red blood cells.

Heterozygote advantage

Interestingly, there appears to be a selective advantage to people who are heterozygous for the disease in areas where malaria is prominent. Heterozygotes do not suffer obvious ill effects of the disease, but their red blood cells appear to be more susceptible to rupture when infected. As a consequence, the parasite gets less of a chance to reproduce and the infected person has a greater chance of survival.

The protective effect of the mutant gene, though, does not extend to people who suffer the full blown disease (homozygotes for the mutant gene). Treatments for the disease include transfusion, pain management, and avoidance of heavy exertion. The drug hydroxyurea has been linked to reduction in number and severity of attacks, as well as an increase in survival time1,2. It appears to work by reactivating expression of the fetal hemoglobin gene, which typically is not synthesized to any significant extent normally after about 6 weeks of age.

Oxygen binding

Animals have needs for oxygen that differ from all other organisms. Oxygen, of course, is the terminal electron acceptor in animals and is necessary for electron transport to work. When electron transport is functioning, ATP generation by cells is many times more efficient than when it is absent. Since abundant ATP is essential for muscular contraction and animals move around a lot - to catch prey, to exercise, to escape danger, etc., having an abundant supply of oxygen is important.

This is particularly a concern deep inside tissues where diffusion of oxygen alone (as occurs in insects) does not deliver sufficient quantities necessary for long term survival. The issue is not a problem for plants since, for the most part, their motions are largely related to growth and thus don't have rapidly changing needs/demands for oxygen that animals have. Unicellular organisms have a variety of mechanisms for obtaining oxygen and surviving without it. Two other important oxygen binding proteins besides hemoglobin are myoglobin and hemocyanin.

Myoglobin

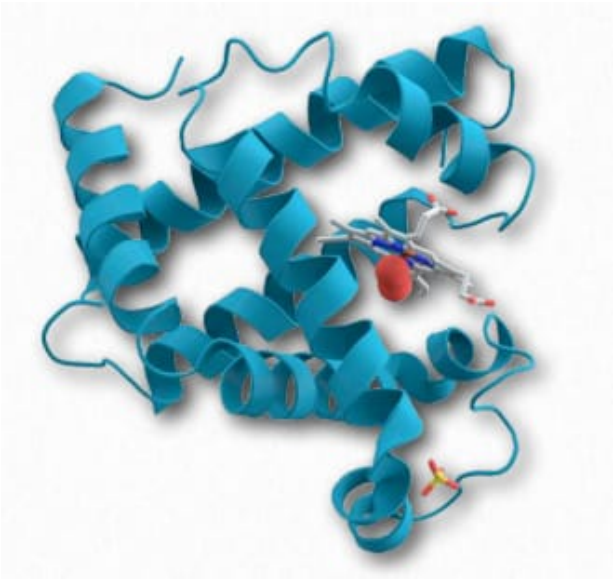


Figure 2.97 - Myoglobin bound to oxygen

Myoglobin is the primary oxygen-storage protein found in animal muscle tissues. In contrast to hemoglobin, which circulates throughout the body, myoglobin protein is only found in muscle tissue and appears in the blood only after injury. Like hemoglobin, myoglobin binds oxygen at a prosthetic heme group it contains.

The red color of meat arises from the heme of myoglobin and the browning of meat by cooking it comes from oxidation of the ferrous (Fe++) ion of myoglobin's heme to the ferric (Fe+++) ion via oxidation in the cooking process. As meat sits in our atmosphere (an oxygen-rich environment), oxidation of Fe++ to Fe+++ occurs, leaving the brown color noted above. If meat is stored in a carbon monoxide (CO) environment, CO binds to the heme group and reduces the amount of oxidation, keeping meat looking red for a longer period of time.

High affinity

Myoglobin (Figure 2.97) displays higher affinity for oxygen at low oxygen concentrations than hemoglobin and is therefore able to absorb oxygen delivered by hemoglobin under these conditions. Myoglobin's high affinity for oxygen makes it better suited for oxygen storage than delivery. The protein exists as a single subunit of globin (in contrast to hemoglobin, which contains four subunits) and is related to the subunits found in hemoglobin. Mammals that dive deeply in the ocean, such as whales and seals, have muscles with particularly high abundance of myoglobin. When oxygen concentration in muscles falls to low levels, myoglobin releases its oxygen, thus functioning as an oxygen "battery" that delivers oxygen fuel when needed and holding onto it under all other conditions. Myoglobin holds the distinction of being the first protein for which the 3D structure was determined by X-ray crystallography by John Kendrew in 1958, an achievement for which he later won the Nobel Prize.

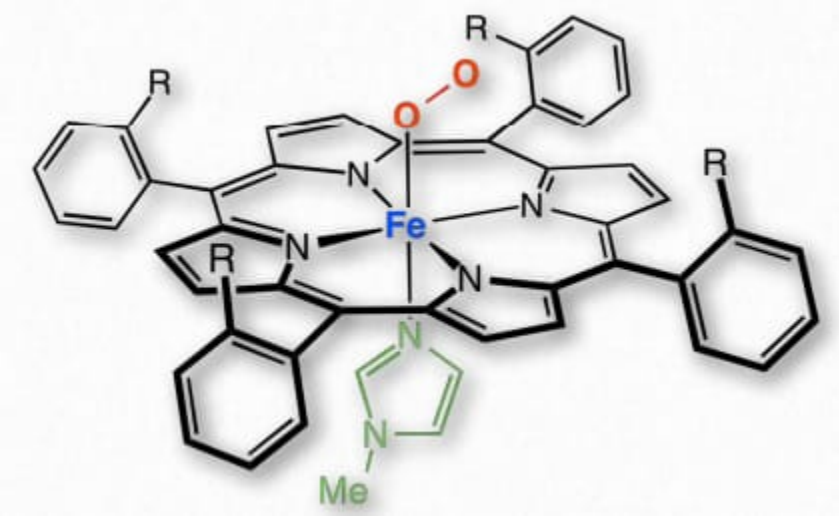


Figure 2.98 - Oxygen bound at heme of myoglobin Wikipedia

Hemocyanin

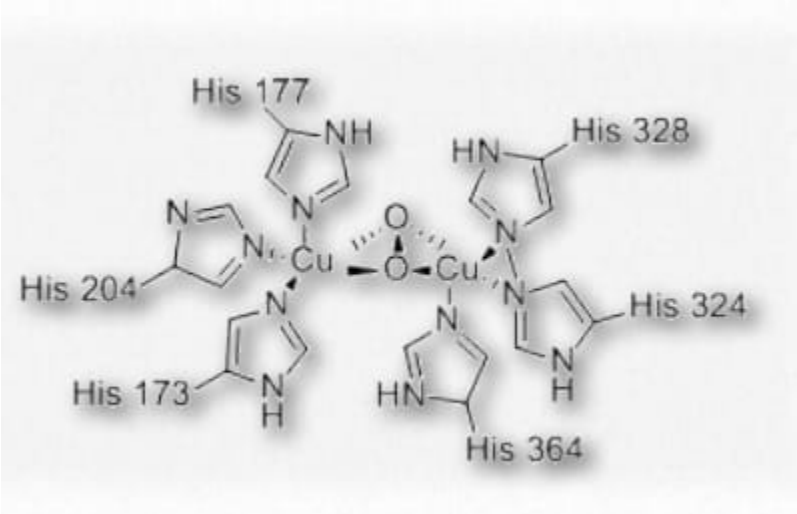


Figure 2.99 - Oxygen binding in hemocyanin Wikipedia

Hemocyanin is the protein transporting oxygen in the bodies of molluscs and arthropods. It is a coppercontaining protein found not within blood cells of these organisms, but rather is suspended in the circulating hemolymph they possess. The oxygen binding site of hemocyanin contains a pair of copper(I) cations directly coordinated to the protein by the imidazole rings of six histidine side chains.



Figure 2.100 - Hemocyanin (purple) in a red rock crab Wikipedia

Most, but not all hemocyanins bind oxygen non-cooperatively and are less efficient than hemoglobin at transporting oxygen. Notably, the hemocyanins of horseshoe crabs and some other arthropods do, in fact, bind oxygen cooperatively. Hemocyanin contains many subunit proteins, each with two copper atoms that can bind one oxygen molecule (O_2). Subunit proteins have atomic masses of about 75 kilodaltons (kDa). These may be arranged in dimers or hexamers depending on species. Superstructures comprised of dimer or hexamer complexes are arranged in chains or clusters and have molecular weights of over 1500 kDa.

This page titled 4.5: Protein function, domains and cooperativity is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 2.4: Structure and Function- Proteins II by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

4.6: Protein Aggregation

We have seen that protein aggregates complicate the lives of people who study protein folding in vitro and who try to express human proteins in prokaryotes like E. Coli in vivo. Instead of viewing these aggregates as junk, some now study them avidly. It turns out that these aggregates are not as non-specific as earlier believed. In addition, an understanding of how and when they form will give us clues into the etiology and treatment of some of the most debilitating and feared diseases. Much of this review is based on the following reference: Taubes, G. Misfolding the Way to Disease, G. Science, 271, 1493-1495 (1996)

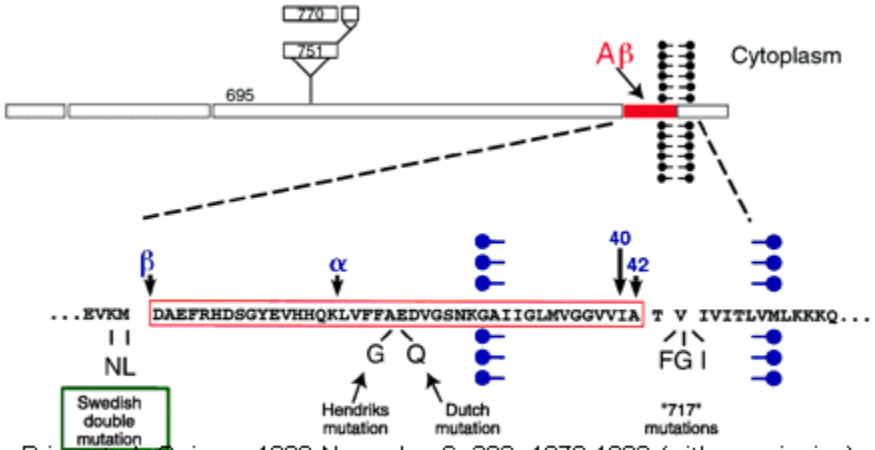
Clues Showing the Specificity of Aggregate Formation

- 1970's: It was shown that chymotrypsinogen could not be folded in vitro without aggregates forming. An intermediate was presumed to have formed that if present in high concentration would aggregate irreversibly instead of fold to the native state. Refolding of tryptophanase showed that it aggregated only with itself, suggesting specificity.
- 1981: King, at MIT, found a single amino acid folding mutant in a viral protein. Both the normal and mutant viral protein unfold at high temperature, but only the mutant would aggregate at high temperature, suggesting that aggregation could be programmed into or out of a gene
- mid 80's: The biotech industry, struggling to express growth hormone, found that a single amino acid change in bovine growth hormone completely prevented aggregation without affecting correct folding.

This knowledge of protein folding and aggregation was soon turn toward understanding several diseases in which protein aggregates were observed which either initiated or were associated with disease. These protein aggregates were termed "amyloid deposits" and seemed to be associated and perhaps causative of several neurodegenerative diseases. The name amyloid was first used by a German pathologist, Rudolf Virchow, who in 1853 described waxy tissue deposits associated with eosinophils (a type of immune cell). These deposits seemed to resemble starch (made of amylose and amylopectin) so he termed them amyloid. All known amyloid deposits are, however, composed of protein, not starch. It now appears that these disease may be caused by improper protein folding and subsequent aggregation. Except in certain rare inherited diseases, the amyloid deposits are composed of normal proteins (often called wild-type proteins) which seems to polymerize into fibrils. Sometimes, in inherited conditions, or when mutations appear in a specific protein, the amyloid protein deposits consist of the mutant protein. The proteins in these deposited fibers are composed predominantly of beta sheets which are perpendicular to the fiber axis, while the polymerized protein usually has little beta sheet structure. Examples are given below:

1. Familial amyloidotic polyneuropathy (FAP) - Affects 1/10,00 to 1/100,000 people. The protein involved is called transthyrein, which normally exists in blood as a tetramer formed by association of 4 identical monomers. In mildly acid condition in vitro, the equilibrium between tetramer and monomer is shifted to monomer, which can aggregate into fibrils. This aggregation could be promoted by possible transition to a molten globule (discussed previously with lactalbumin) like state. This has secondary structure but loosely packed tertiary structure with more exposed hydrophobes. If the concentration is high enough the molten globules aggregate. In people with the disease, mutations in the protein destabilize the tetramer, pushing the equilibrium to the monomer, which presumably increases molten globule formation and aggregation. Specifically, Val30Met and Leu55Pro mutations promote dissociation of the tetramer and formation of aggregates. Conversely, Thr119Met inhibits tetramer dissociation. The aggregates deposit in heart, lungs, kidney, etc, leading to death.
2. Light Chain Amyloidosis; Light Chain Deposition Disease - The light chain protein is a normal component of circulating antibody molecules. Mutants in the light chain cause a destabilization of the native state to state similar to a molten globule, which then aggregates.
3. Lysozyme amyloidosis - This protein, with extensive alpha-helix structure, is usually involved in carbohydrate catabolism. Two mutants, Asp67His and another, Ile56Thr (normal amino acid/number in sequence/mutant amino acid) destabliize the protein structure (as indicated by a decrease in the Tm of about 10 degrees C) to a molten-globule form, which probably aggregates to fibrils characterized by extensive beta structure.
4. Alzheimers-This disease involves a defect in a protein normally found in the membrane of neurons. The protein, called beta-amyloid precursor protein (BAPP), is a transmembrane protein. A slightly truncated, soluble form is also found secreted from cells and is found in extracellular fluid (such as cerebrospinal fluid and blood). The normal function of these BAPP proteins is not yet clear. An endoprotease cleaves a small 40-42 amino acid fragment from this protein, forming the amyloid beta (Ab) protein. It is this protein or a mutant form of it which aggregates to form beta-sheet containing fibrils in Alzheimer's disease. Several mutations in different proteins have been linked to Alzheimer's, but they all seem to increase production or deposition or both of the amyloid beta protein. These deposited plaques are extracellular, and have been shown to cause neuronal damage. The are found in areas of the brain required for memory and cognition. The BAPP gene is found on chromosome 21, the same chromosome which is present in an extra copy (trisomy 21) in Downs Syndrome, whose symptoms

include presenile dementia and amyloid plaques. Aggregate formation appears to be driven by increased expression of BAPP and hence amyloid beta protein. In addition, some mutants may serve to destabilize the amyloid beta protein, increasing its aggregation.



Price et al. *Science* 1998 November 6; 282: 1079-1083 (with permission)
Copyright (1998) American Association for the Advancment of Science

5. Transmissible spongiform encephalopathies (TSEs) - Including scrapie in sheep, bovine spongiform encephalopathy (mad cow disease), and in humans Creutzfeld-Jacob Disease (CJD), Fatal Familial Insomnia (FFI),Gerstman-Straussler-Scheinker Syndrome, and Kuru (associated with cannibalism). In these fatal diseases, the brain, on autopsy, resembles a sponge with holes. In contrast to the diseases above, these diseases can be transmitted from one animal to another, but typically not between species. (However, consider the controversy with mad cow disease.) Also, the infectious agent can self-replicate in vivo. The logical conclusion is that a virus (slow-acting) is the causative agent. However, the infectious agent survives radiation, heat, chemical agents, and enzymes designed to kill viruses and their associated nucleic acids. Mathematical analyses suggested that the infectious agent in such diseases could be nothing more than a protein. Stanley B. Prusiner in the 80's isolated just such a protein which he named a prion, for proteinaceous infectious agent. Since then he and others have amassed substantial evidence to support his contention. In October 1997 he was awarded the Nobel Prize in Medicine.

Contributors and Attributions

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

This page titled 4.6: Protein Aggregation is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- H1. Protein Aggregation by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

4.7: Prions and Disease

The normal cellular form of this protein, PrPc is highly conserved in mammals, and is widely expressed in embryogenesis. Techniques exist to delete or make ineffectual genes in mice. When a mice knockout of the PrPc (i.e. the gene for the protein was deleted in all cells) was made, the mice appeared normal. More recent data suggests however, that these mice had altered circadian rhythms and sleep patterns, which suggest a possible link to Fatal Familial Insomnia. The PrPc is a normal membrane protein in neurons. It is anchored to the membrane through a glycosyl-phosphatidyl inositol link, with the protein chain on the outside of the neuronal plasma membrane. The PrPc (without the PI link) is water soluble, protease sensitive, and consists of 42% alpha helix and 3% beta sheet.


Jmol: Updated Prion Protein, Mad Cow Disease, and Mutations Jmol14 (Java) | JSMol (HTML5)

The problem in the transmissible spongiform encephalopathies (TSE's) is that amyloid-like protein aggregates form which appear to be neurotoxic. The protein found in the plaques (in cases other that those that are inherited) has the same primary sequence as the PrPc but a different secondary and presumably tertiary structure. The protein found in the plaques, called the PrPsc (the scrapie form of the the normal protein) is insoluble in aqueous solution, protease resistant, and has a high beta sheet content (43%) and lower alpha helix content (30%) than the normal version of the protein PrPc.

Figure: Cartoon Models of PrPc and PrPsc



A genetic, inheritable form of disease also exists, in which a mutant form of the PrPc occurs, whose normal structure is destabilized by the mutation. The aggregates caused by the mutant form of the disease are understandable in light of the other diseases which we discussed above. The question is how does the normal PrPc form PrPsc . Evidence shows that if radiolabeled PrP*c from scrapie free cells is added to unlabeled PrPsc from scapie infected cells, the PrP*c is converted to PrP*sc! It appears that the PrPc protein has two forms not that much different in energy, one composed of mostly alpha helix and the other of beta sheet. A dimer of PrP^{c}.PrPsc might form, which destabilizes the PrP*c causing a conformational shift to the PrPsc form, which would then aggregate. Exposure to the PrPsc form would then catalyze the conversion of normal PrPc to PrPsc . Hence, it would be transmissible by contact with just the PrPsc form of the protein. Likewise species specificity could be explained if only dimers of PrP^{c}.PrPsc formed from proteins of the same species could occur. The inherited form of the disease would be explained since the mutant form of the normal protein would more easily form the beta structure found in the aggregate.

It has recently been found that the very same mutation in PrPc, Asp178Asn can cause two different diseases - CJD and FFI. Which disease you get depends on if you have 1 of two naturally occurring, nonharmful variants at amino acid 129 of the normal PrPc

gene. If you have a Met at that position, and acquire the Asp178Asn mutation, you get CJD. If, on the other hand, you have a Val at amino acid 129 and acquire the Asp178Asn mutation, you get FFI. This disease was first observed in 1986 and has been reported in five families in the world. It occurs in the late 50's, equally in men and women. It is characterized by a progressive loss of the ability to sleep and disrupted circadian rhythms. The brain shows neuronal loss. It is known that amino acids 129 and 178 occur at the start of alpha helices, as predicted from propensity calculations. Chronic exposure to micromolar levels of synthetic fragment 106-126 of PrPc kills hippocampal neurons. This peptide also has the greatest tendency to aggregate synthetic PrPc peptides.

A series of recent studies have expanded on our knowledge of prion structure. Nelson et al. have obtained the crystal structure of a fibril aggregate made of a short peptide (7 amino acids) from the yeast prion protein Sup35. As presumably occurs in amyloid fibers, these crystals show beta-sheet structures stacked vertically to produce fibril structures. The unit of stacking appears to be pairs of beta-sheets, with the inner side amino acids of one member of the pair interacting with the inner amino acid side chains of the other member of the pair, in a process which excludes water. Similar studies by Ritter et al, using NMR and fluorescence, found pair of beta sheets to be the motif of the fibril. Using fluorescence, they identified two regions, each 15 amino acids, important in collapse to molten-globule like state for nucleation of fibril formation.

Kuru killed many members of the Fore tribe in New Guinea until the cannibalistic practice of eating dead relatives was stopped. Analysis of the genes for the prion protein in the Fore tribe and other ethnic groups in the world show two version differing by just one amino acid in all people (remember that a single gene is represented in both maternal and paternal chromosomes. That these two forms exist through the world suggest that they have been selected for by evolution and confer some biological advantage. People who have just one form of the protein are more susceptible to the development of prion diseases. Mead and Collinge have shown that about 75% of older Fore women (who had lived through cannibalistic practices) had two different prion genes, compared to about 15% of women from other ethnic groups. This high percentage suggests that these women were protected from the disease, leading through natural selection to a high percentage of heterozygotes in this defined population. The general presence of two forms of the prion gene (which probably offers protection from prion disease) suggests that cannibalism might have been widespread in our early ancestors.

There appears to be one main difference between the formation of amyloid fibers from prion proteins and others such as mutant lysozymes. If you add mutant lysozyme to normal lysozyme, the amyloid fibers contain only the mutant protein. However, if you incubate mutant prion proteins with normal prions, the normal proteins become pathological.

QED - Protein Aggregates are not just test tube artifacts, but rather matters of life and death.

EXTERNAL CDC: Prion Disease

Jsmol: EXTERNAL Protopedia - Prions

Contributors and Attributions

- Prof. Henry Jakubowski (College of St. Benedict/St. John's University)

This page titled 4.7: Prions and Disease is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Henry Jakubowski.

- H2. Prions and Disease by Henry Jakubowski is licensed CC BY-NC-SA 4.0.

5: TECHNIQUES FOR STUDYING PROTEINS

5.1: Cell Disruption
5.2: Fractionation
5.3: Quantification of Protein Concentration
5.4: Electrophoresis
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies
5.6: Western blot / Immunoblot overview
5.7: Steps in Western blotting

This page titled 5: Techniques for studying proteins is shared under a license and was authored, remixed, and/or curated by LibreTexts.

5.1: Cell Disruption

There are several ways to break open cells.

- Lysis methods include lowering the ionic strength of the medium cells are kept in. This can cause cells to swell and burst. Mild surfactants may be used to enhance the efficiency of lysis. Most bacteria, yeast, and plant tissues, which have cell walls, are resistant to such osmotic shocks, however, and stronger disruption techniques are often required.
- Enzymes may be useful in helping to degrade the cell walls. Lysozyme, for example, is very useful for breaking down bacterial walls. Other enzymes commonly employed include cellulase (plants), glycanases, proteases, mannases, and others.
- Mechanical agitation may be employed in the form of tiny beads that are shaken with a suspension of cells. As the beads bombard the cells at high speed, they break them open. *Sonication* (20-50 kHz sound waves) provides an alternative method for lysing cells. The method is noisy, however, and generates heat that can be problematic for heat-sensitive compounds.
- Another means of disrupting cells involves using a “cell bomb". In this method, cells are placed under very high pressure (up to 25,000 psi). When the pressure is released, the rapid pressure change causes dissolved gases in cells to be released as bubbles which, in turn, break open the cells.
- *Cryopulverization* is often employed for samples having a tough extracellular matrix, such as connective tissue or seeds. In this technique, tissues are .ash-frozen using liquid nitrogen and then ground to a fine powder before extraction of cell contents with a buffer.

Whatever method is employed, the crude lysates obtained contain all of the molecules in the cell, and thus, must be further processed to separate the molecules into smaller subsets, or fractions.

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 5.1: Cell Disruption is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 9.1: Cell Disruption by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

5.2: Fractionation

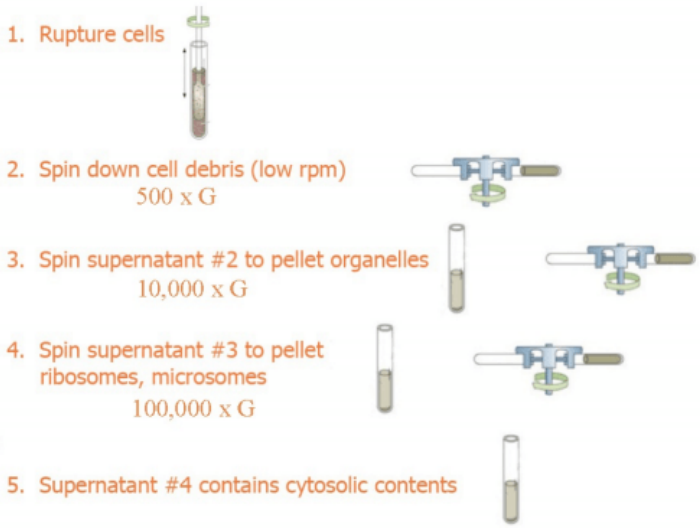


Figure 9.2.1: *Fractionation by centrifugation*

Many chromatographic techniques are performed in “columns." These are tubes containing the material (called the “support") used to perform the separation . Supports are designed to exploit the chemical, or size, differences of the many molecules in a mixture. Columns are “packed" (filled) with the support and a buffer or solvent carries the mixture of compounds to be separated through the support. Molecules in the sample interact differentially with the support and consequently, will travel through it with different speeds.

Contributors and Attributions

Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 5.2: Fractionation is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 9.2: Fractionation by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

5.3: Quantification of Protein Concentration

Review: the Beer-Lambert Law

$$\log(1/T) = \varepsilon c l = A$$
$$T = 1/10^{A}$$

Values for ε and *l*

- Path length ***l*** is usually in units of **cm**. (note: most spectrophotometers are designed to accept 1cm wide cuvettes)
- Molar extinction coefficient **ε** has units of M^{-1} cm^{-1} and is a constant of proportionality that relates the absorption of **molar** solutions
- Mass extinction coefficient $\varepsilon^{1\%}$ refers to the absorbance of a 1% by mass solution. Typically this refers to an aqueous solution that we can take to have a density of 1000g/L. A 1% by mass aqueous solution would therefore refer to the dissolution of 10g/L, or a **10mg/ml** solution of the molecule of interest.
- Since the absorbance of a molecule is a function of the wavelength (i.e. the absorption is not equal for every wavelength) the extinction coefficient must also reference a wavelength. This is typically done using a subscript:

$$\varepsilon^{1\%}_{280nm} = 14.5\ g^{-1}\ L\ cm^{-1}$$

· In this case **a 10mg/ml solution of the molecule will have an absorbance reading of 14.5** (dimensionless units) at l = 280nm (the absorption at other wavelengths may not be known). The units of concentration are g/L, thus e will have dimensions of g^{-1} L cm^{-1}.

Why is it important to be able to quantitate protein concentration in a sample?

An important application of "Biotechnology" is the production of proteins as commercial products. Such products might have pharmaceutical applications (e.g. insulin, human growth hormone, tissue plasminogen activator, erythropoietin, blood clotting factor VIII.), industrial applications (e.g. subtilisin (an enzyme in detergents), 2,5-diketo-D-gluconate reductase (an enzyme in vitamin C production), as materials (e.g. silk protein in textiles, barnacle adhesion protein as a glue). In these cases, there are various aspects of successful production that require quantitation:

- How much of the protein can be produced (i.e. what is the efficiency of production)?
- How pure is the protein that is produced (industrial applications may require 90% pure, pharmaceutical applications may require 99.999% pure)

Such proteins may be isolated from natural sources (e.g. blood clotting factor VIII may be extracted from human blood), or they may be produced recombinantly (e.g. E. coli bacterial cells can be genetically engineered to produce human growth hormone). In both cases, it may be necessary to purify the protein using a series of fractionation steps. We will go into more detail about such fractionation steps in a later lecture, but the general idea is that a heterogeneous mixture of molecules can be fractionated based upon some physical property of the molecules. The following are properties that can be used to fractionate a heterogeneous mixture of biomolecules:

- Molecular mass (i.e. "big" molecules can be separated from "small" molecules)
- pKa (i.e. "acidic" molecules can be separated from "basic" molecules)
- Hydrophobicity (i.e. non-polar molecules can be separated from polar molecules)

For such fractionation steps involving proteins, we need to keep track of how much of the contaminating proteins went into one fraction and how much of our desired protein went into the other fraction. Although the details are somewhat more complicated than this simple description, **it is important to be able to quantitate protein concentration to be able to effectively purify a protein of interest**.

Once a protein is pure, it may be of considerable economic interest to be able to quantify the yield (and, therefore, be able

to determine how much it cost to produce a given mass of protein). For example, the only source for human growth hormone (to treat small stature) used to be to extract it from human pituitary glands harvested from the brains of cadavers. Suffice it to say, this made the protein extremely expensive. Furthermore, the isolation from human tissues meant that the sample could also be potentially contaminated with human pathogens (hepatitis, CJD, AIDS, etc.). With the advent of genetic engineering, the production of human growth hormone by bacterial cells (i.e. E. coli) meant that relative large quantities could be produced far cheaper (and with no threat of human pathogens).

Why not just weigh the protein?

- Most samples are typically quantities of milligrams or even micrograms, not grams, and thus, it is difficult to transfer and measure such small amounts
- Water is present in proteins, and it is extremely difficult to remove all the water (some water molecules hydrogen bond extremely tightly to proteins). Thus, the mass measurement would include some waters, and would increase the apparent mass of the protein

Absorbance spectra of biological molecules

Proteins

Proteins do not absorb in the visible wavelength unless they have a prosthetic group (e.g. Fe^{2+}) or an unnatural amino acid. However, the amino acids tryptophan, tyrosine and cysteine absorb light in the UV wavelength:

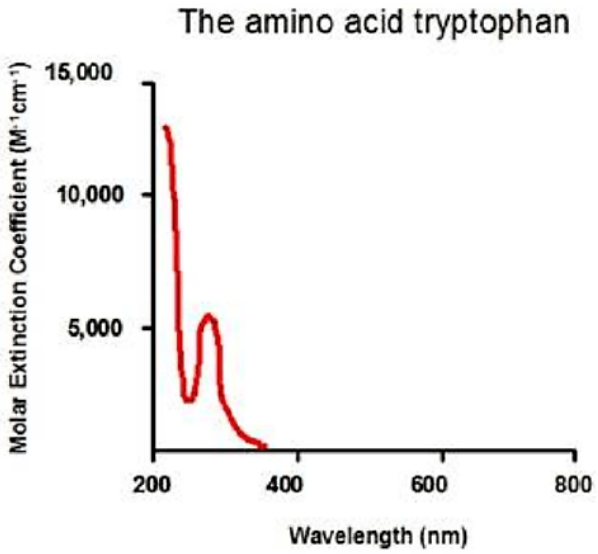


Figure 5.3.1: *Tryptophan absorption*

- Tryptophan has a peak of absorption at 280nm in the UV range
- This is a useful wavelength to quantitate the absorption of tryptophan
- Since the absorption is proportional to concentration, this is a useful way to quantitates protein concentration (for proteins containing Trp)

Nucleic acids

The aromatic rings in the bases of nucleic acids also absorb in the UV range:

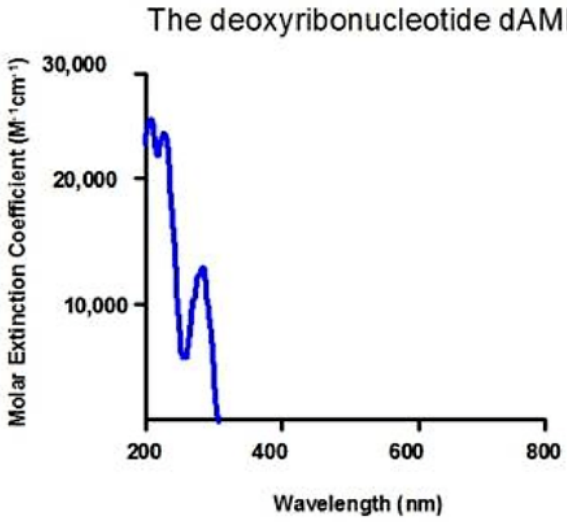


Figure 5.3.2: *dAMP Absorption*

- Each DNA and RNA base has a slightly different absorption spectrum
- 260 or 280nm is a typically useful wavelength to monitor concentration of nucleic acids

Note that samples of nucleic acids and proteins can both absorb at 280nm, therefore, samples of biological molecules should be **pure** in order to quantitate using UV absorption spectroscopy (any contaminating nucleic acids in a protein sample will increase the apparent absorbance, likewise for contaminating proteins in a nucleic acid sample).

Important aspects of quantification of proteins using UV absorbance

- If a protein contains Trp, Tyr or Cys residues it will absorb in the UV. If it does not contain these amino acids, it will not absorb UV light, and we cannot quantify it using this method
- Multiple Trp, Tyr or Cys residues will contribute to the Extinction coefficient for the protein. Thus, we need to know how many of these residues are present in the protein to know the correct extinction coefficient
- Nucleic acids (DNA, RNA) contaminant will also absorb UV light, as will other proteins with Trp, Tyr and Cys residues. Thus, the sample must be PURE to use UV absorption to quantify a protein

Molar extinction coefficients of Trp, Tyr and Cys amino acids:

Amino Acid	E_{280nm} (M^{-1} cm^{-1})
Trp	5690
Tyr	1280
Cys	120

Example 5.3.1: Bovine insulin

Bovine insulin contains 4 Tyr residues, 6 Cys residues and 0 Trp residues. We can determine the expected molar extinction coefficient at 280nm, E_{280nm}, by the following calculation:

$$E_{280nm} = (0)(5690) + (4)(1280) + (6)(120)$$

$$E_{280nm} = 5840\ M^{-1}\ cm^{-1}$$

Thus, a 1.0M solution of pure bovine insulin would give an absorbance of 5,840 at 280nm (obviously, it would have to be diluted considerably to be read accurately).

A useful expression relating the parameters of E, concentration (C) and A are derived from the Beer-Lambert law (assuming 1cm path length):

$$\mathbf{A/E = C}$$

For example, if a sample of bovine insulin was observed to give an absorbance at 280nm of 0.745 we could calculate the concentration to be:

$$0.745/5840\ M^{-1}\ cm^{-1} = C$$

$C = 1.28 \times 10^{-4} M$ (note: cm^{-1} drops out with 1cm pathlength)

It should be noted that a deuterium lamp is required for UV spectrophotometry, as well as quartz cuvettes (since glass absorbs UV light)

Colorimetric (chromogenic) methods of protein concentration determination

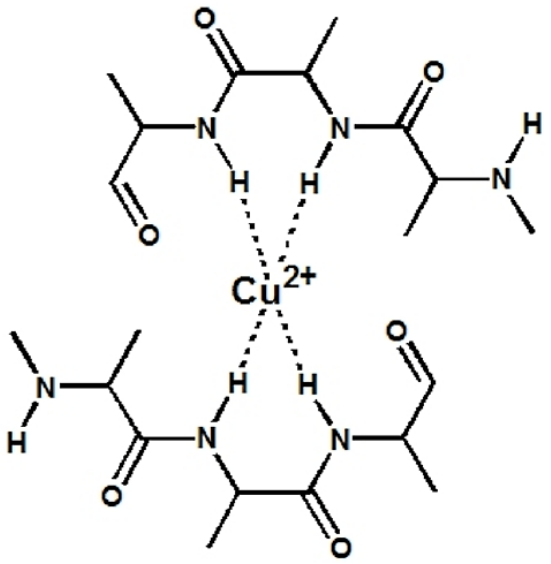
Chromo means color and genesis mean creation, so chromogenic means the "creation of color". Color means color (duh...) and metric means to measure, so colorimetric is to "measure color". Both terms refer to the same sort of thing in the present case - we can modify the protein sample with appropriate reagents so as to produce a color reaction (in visible spectrum) and measure protein concentration using a VIS spectrophotometer. Advantages are:

- Cheap lamp! (tungsten light bulb versus deuterium for UV)
- Cheap cuvette! (cheap glass or plastic versus quartz)
- Not contaminating absorbance from proteins or nucleic acids! (no absorption in VIS spectrum)

We will consider three methods: The **Biuret, Lowry and Bradford** methods of colorimetric determination of proteins.

Biuret

Under high pH (alkaline) conditions the copper II ion (Cu^{2+}) is believed to form a complex with peptide nitrogens of proteins:



Fiugre 5.3.3: *Cu^{2+} complex*

This complex absorbs light at 550nm and has the following useful properties:

- It is dependent upon at least a dipeptide structure (see above), thus, contaminating amino acids will not contribute to the 550nm absorption
- The binding depends upon the peptide backbone nitrogen and not the side chain functional group. Thus, the binding is independent of sequence
- Nucleic acids do not interfere since they don't share the peptide backbone structure
- However, ammonia and certain amines can interfere. Thus, ammonium sulfate salts, and the common biological buffer TRIS (tris hydroxymethyl amino ethane) will provide a false positive and cannot be present in the sample
- Also, the absorbtion is relatively weak, thus, the method is somewhat insensitive and requires a relatively high concentration of protein

Lowry

The Lowry method is a modification of the Biuret method. After treatment with Copper II, the protein is treated with phosphomolybdotungstate mixed acids (acidic compounds of molybdenum and tungsten ions). These acids are known as the **Folin-Ciocalteu (or just Folin) reagent**. The Folin reagent is added under alkaline conditions, and the Folin reagent is subsequently reduced by the the Copper ions as well as Tyr, Trp and polar amino acid side chains. The product of this reaction is **heteropolymolybdenum blue**, which absorbs strongly at 750nm. This assay has the following properties:

- More sensitive than the Biuret assay (can detect lower concentrations of protein)
- Somewhat dependent upon amino acid composition (i.e. relative concentrations of Tyr, Trp and polar amino acids)
- Absobtion reaction is linearly dependent upon protein concentration, but only at low concentrations of protein (i.e. the standard curve and assay must be performed at a low concentration regime).
- Sensitive to contaminants as with the Biuret method, as well as others related to the Folin reagent and redox reactions.
- More critical to timing and precision of person doing the assay

Bradford

A dye known as Coomassie Brilliant Blue was developed by the textile industry. It was noticed to stain skin as well as the textiles. Thus, this dye (which normally absorbs at 465nm) was known to bind to proteins and to absorb strongly at 595nm. The dye forms a wide variety of strong, but non-covalent, interactions including hydrogen bonding donor and acceptor interactions as well as hydrophobic (non-polar) interactions.

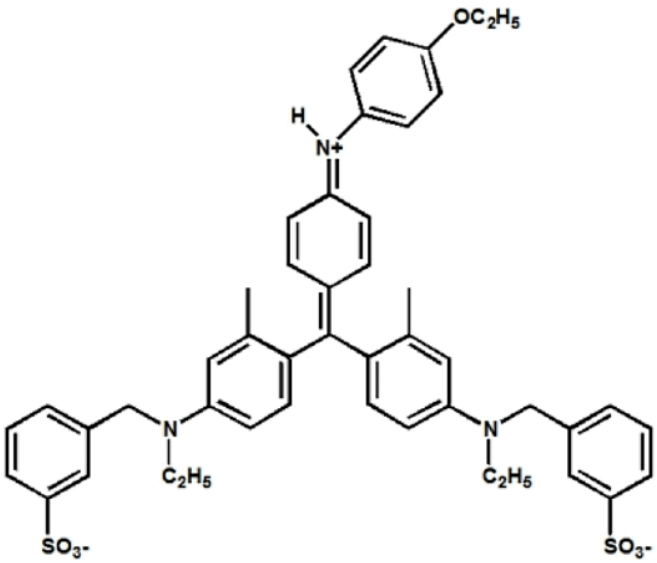


Figure 5.3.4: *Coomassie Brilliant Blue binding*

The method is quite simple: a single step in which the dye is added to the protein solution under acidic conditions, and then the absorbance is read at 595nm. However, the method has the following properties:

- The response is generally independent of the amino acid composition
- The assay is sensitive, but somewhat non-linear. Thus, a standard curve must always be performed (using known concentrations of pure protein)
- The dye stains pretty much everything, including cuvettes, floors, countertops. It can be a real mess if spilled (I know this from personal experience). And since it is a textile dye, if you get it on your clothes, you will need to learn to like blue polka dots.

This page titled 5.3: Quantification of Protein Concentration is shared under a license and was authored, remixed, and/or curated by Michael Blaber.

- 5.3: Quantification of Protein Concentration by Michael Blaber has no license indicated.

5.4: Electrophoresis

Electrophoresis uses an electric field applied across a gel matrix to separate large molecules such as DNA, RNA, and proteins by charge and size. Samples are loaded into the wells of a gel matrix that can separate molecules by size and an electrical field is applied across the gel. This field causes negatively charged molecules to move towards the positive electrode. The gel matrix, itself, acts as a sieve, through which the smallest molecules pass rapidly, while longer molecules are slower-moving.

For DNA and RNA, sorting molecules by size in this way is trivial, because of the uniform negative charge on the phosphate backbone. For proteins, which vary in their charges, a clever trick must be employed to make them mimic nucleic acids - see polyacrylamide gel electrophoresis (PAGE) below. Different kinds of gels have different pore sizes. Like sieves with finer or coarser meshes, some gels do a better job of separating smaller molecules while others work better for larger ones. Gel electrophoresis may be used as a preparative technique (that is, when purifying proteins or nucleic acids), but most often it is used as an analytical tool.

Agarose Gel Electrophoresis

Agarose gel electrophoresis is a technique used to separate nucleic acids primarily by size. Agarose is a polysaccharide obtained from seaweeds (Figure 8.11). It can be dissolved in boiling buffer and poured into a tray, where it sets up as it cools (Figure 8.12) to form a slab. Agarose gels are poured with a comb in place to make wells into which DNA or RNA samples are placed after the gel has solidified. The gel is immersed in a buffer and a current is applied across the slab. Double-stranded DNA has a uniform negative charge that is independent of the sequence composition of the molecule. Therefore, if DNA fragments are placed in an electric field they will migrate from the cathode (-) towards the anode (+). The rate of migration is directly dependent on the ability of each DNA molecule to worm or wiggle its way through the sieving gel. The agarose matrix provides openings for macromolecules to move through. The largest macromolecules have the most difficult time navigating through the gel, whereas the smallest macromolecules slip through it the fastest.

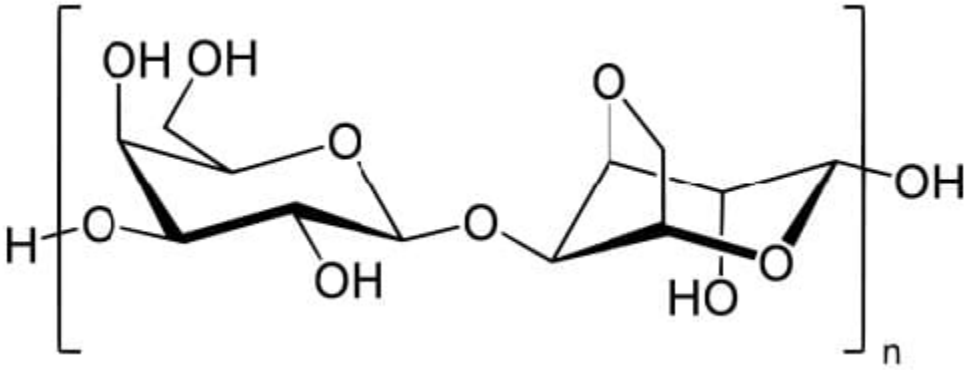


Figure 8.11 - Structure of the agarose polysaccharide. Wikipedia

Because electrophoresis uses an electric current as a force to drive the molecules through the matrix, the molecules being separated must be charged. Since the size to charge ratio for DNA and RNA is constant for all sizes of these nucleic acids, the molecules simply sort on the basis of their size - the smallest move fastest and the largest move slowest.

All fragments of a given size will migrate the same distance on the gel, forming the so-called “bands” on the gel. Visualization of the DNA fragments in the gel is made possible by addition of a dye, such as ethidium bromide, which intercalates between the bases and fluoresces when viewed under ultraviolet light (Figure 8.13) By running reference DNAs of known sizes alongside the samples, it is possible to determine the sizes of the DNA fragments in the sample. It is useful to note that, by convention, DNA fragments are not described by their molecular weights (unlike proteins), but by their length in base-pairs(bp) or kilobases (kb).

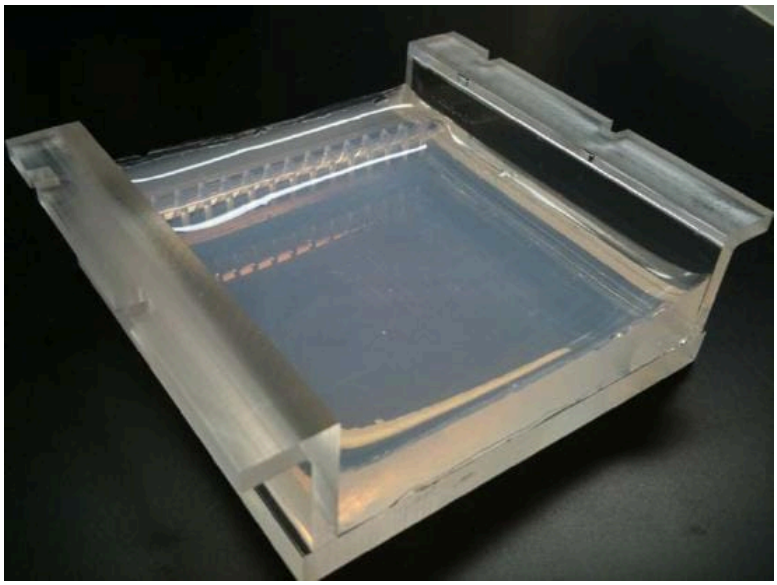


Figure 8.12 - Agarose gel electrophoresis separation of DNA - orange bands are DNA fragments. Wikipedia

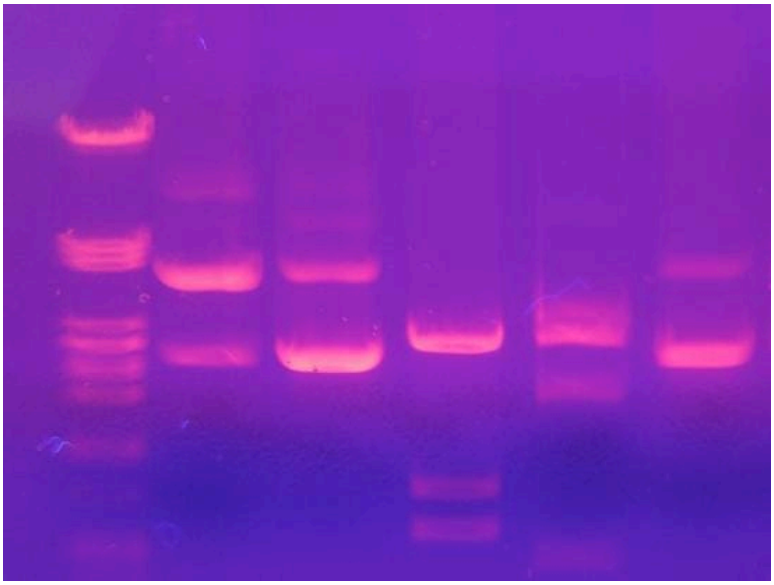


Figure 8.13 - DNA bands visualized with ethidium bromide staining. Wikipedia

Polyacrylamide gel electrophoresis (PAGE)

Like DNA and RNA, proteins are large macromolecules, but unlike nucleic acids, proteins are not necessarily negatively charged. The charge on each protein depends on its unique amino acid sequence. Thus, the proteins in a mixture will not necessarily all move towards the anode.

Additionally, whereas double-stranded DNA is rod-shaped, most proteins are globular (folded). Further, proteins are considerably smaller than nucleic acids, so the openings of the matrix of the agarose gel are simply too large to effectively provide separation. Consequently, unaltered (native) proteins are not very good prospects for electrophoresis on agarose gels. To separate proteins by mass using electrophoresis, one must make several modifications.

Gel matrix

First, a matrix made by polymerizing and cross-linking acrylamide units is employed. A monomeric acrylamide (Figure 8.14) is polymerized and the polymers are cross-linked using N,N'-Methylene-bisacrylamide (Figure 8.15) to create a mesh-like structure.

One can adjust the size of the openings of the matrix/mesh readily by changing the percentage of acrylamide in the reaction. Higher percentages of acrylamide give smaller openings and are more effective for separating smaller molecules, whereas lower percentages of acrylamide are used when resolving mixtures of larger molecules. (Note: polyacrylamide gels are also used to separate small nucleic acid fragments, with some acrylamide gels capable of separating pieces of DNA that differ in length by just one nucleotide.)

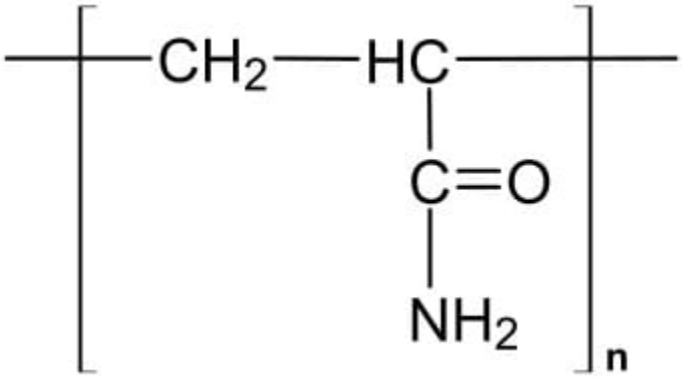


Figure 8.14 - Acrylamide monomer. Wikipedia

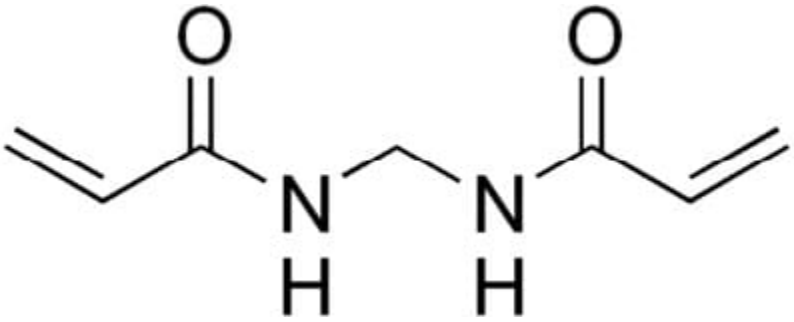


Figure 8.15 - N,N'-Methylenebisacrylamide - acrylamide crosslinking reagent. Wikipedia

Charge alteration by SDS

A second consideration is that proteins must be physically altered to "present" themselves to the matrix like the negatively charged rods of DNA. This is accomplished by treating the proteins with the anionic detergent, SDS (sodium dodecyl sulfate). SDS denatures the proteins so they assume a rod-like shape and the SDS molecules coat the proteins such that the exterior surface is loaded with negative charges, masking the original charges on the proteins and making the charge on the proteins more proportional to their mass, like the backbone of DNA.

Since proteins typically have disulfide bonds that prevent them from completely unfolding in detergent, samples are boiled with mercaptoethanol to break the disulfide bonds and ensure the proteins are as rod-like as possible in the SDS. Reagents like mercaptoethanol (and also dithiothreitol) are sulfhydryl-containing reagents that become oxidized as they reduce disulfide bonds in other molecules (see Figure 8.16)

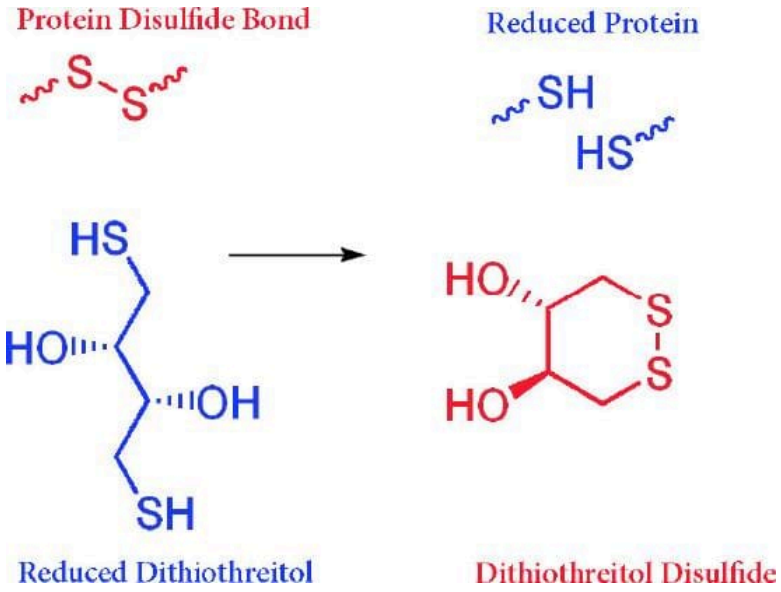


Figure 8.16 - Reduction of disulfide bonds by dithiothreitol. Wikipedia

Stacking Gel

A third consideration is that a “stacking gel” may be employed at the top of a polyacrylamide gel to provide a way of compressing the samples into a tight band before they enter the main polyacrylamide gel (called the resolving gel). Just like DNA fragments in agarose gel electrophoresis get sorted on the basis of size (largest move slowest and smallest move fastest), the proteins migrate through the gel matrix at velocities inversely related to their size. Upon completion of the electrophoresis, proteins may be visualized by staining with compounds that bind to proteins, like Coomassie Brilliant Blue (Figure 8.17) or silver nitrate.

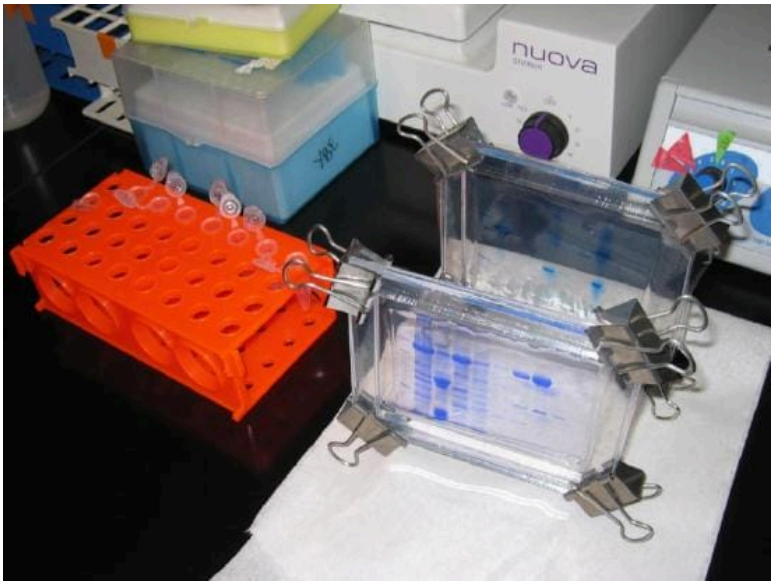


Figure 8.17 - Two SDS-PAGE gels - Proteins are the blue bands (stained with Coomassie Blue). Wikipedia

Non-denaturing gel electrophoresis

The SDS_PAGE technique described above is the commonest method used for electrophoretic separation of proteins. In some situations, however, proteins may be resolved on so-called “native” gels, in the absence of SDS. Under these conditions, the movement of proteins through the gel will be affected not simply by their mass, but by their charge at the pH of the gel, as well. Proteins complexed with other molecules may move as single entity, allowing the isolation of the binding partners of proteins of interest.

Isoelectric focusing

Proteins vary considerably in their charges and, consequently, in their pI values (pH at which their charge is zero). This can be exploited to separate proteins in a mixture. Separating proteins by isoelectric focusing requires establishment of a pH gradient in a tube containing an acrylamide gel matrix. The pore size of the gel is adjusted to be large, to reduce the effect of sieving based on size. Molecules to be separated are applied to the gel containing the pH gradient and an electric field is applied. Under these conditions, proteins will move according to their charge.

Positively charged molecules, for example, move towards the negative electrode, but since they are traveling through a pH gradient, as they pass through it, they reach a region where their charge is zero and, at that point, they stop moving. They are at that point attracted to neither the positive nor the negative electrode and are thus “focused” at their pI (Figure 8.18). Using isoelectric focusing, it is possible to separate proteins whose pI values differ by as little as 0.01 units.

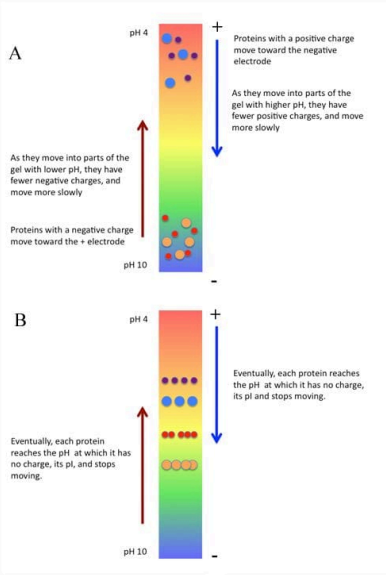


Figure 8.18 - Isoelectric focusing: A. At the start of the run; B. at the end of the run

2D gel electrophoresis

Both SDS-PAGE and isoelectric focusing are powerful techniques, but a clever combination of the two is a powerful tool of proteomics - the science of studying all of the proteins of a cell/tissue simultaneously. In 2-D gel electrophoresis, a lysate is first prepared from the cells of interest. The proteins in the lysate are separated first by their pI, through isoelectric focusing and then by size by SDS-PAGE.

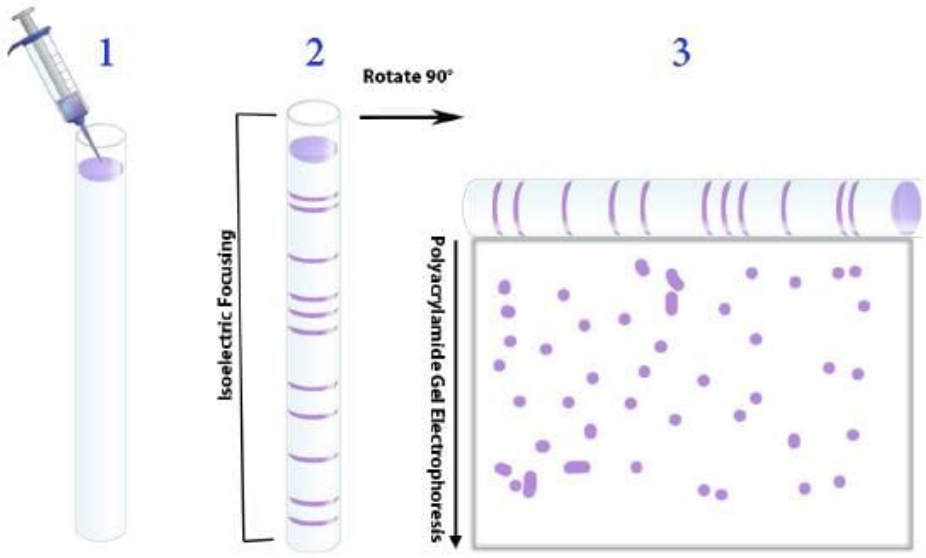


Figure 8.19 - Scheme for performing 2-D gel analysis. Image by Aleia Kim

The mixture of proteins is first applied to a tube or strip (Figure 8.19, Step 1) where isoelectric focusing is performed to separate the proteins by their pI values (Step 2). Next, as shown in the figure, the gel containing the proteins separated by their pIs is turned on its side and applied along the top of a polyacrylamide slab for SDS-PAGE to separate on the basis of size (Step 3). The proteins in the isoelectric focusing matrix are electrophoresed into the polyacrylamide gel and separated on the basis of size. The product of this analysis is a 2-D gel as shown in Figure 8.20.The power of 2-D gel electrophoresis is that virtually every protein in a cell can be separated and appear on the gel as a spot defined by its unique size and pI. In the figure, spots in the upper left correspond to large positively charged proteins, whereas those in the lower right are small negatively charged ones. Every spot on a 2-D gel can be eluted and identified by using high throughput mass spectrometry. This is particularly powerful when one compares protein profiles between different tissues or between control and treated samples of the same tissue.

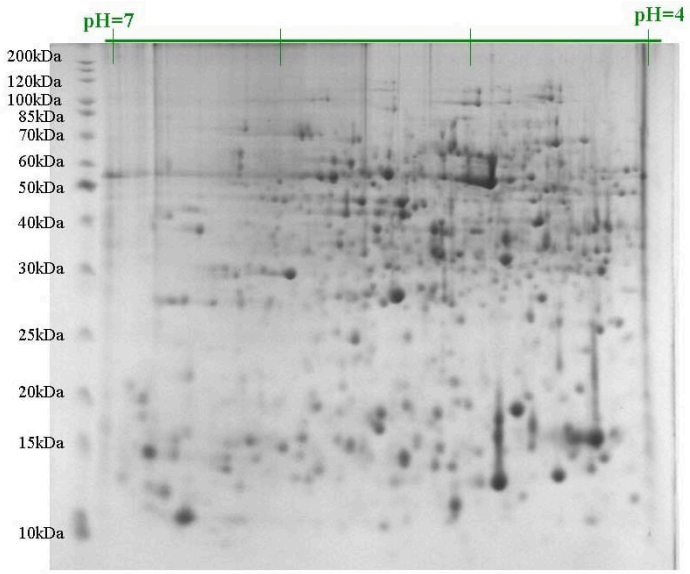


Figure 8.20 - Result of 2-D gel electrophoresis separation. Wikipedia

Protein profiles comparison

Comparison of 2-D gels of proteins from non-cancerous tissue and proteins from a cancerous tissue of the same type provides a quick identification of proteins whose level of expression differs between the two. Information such as this can be useful in designing treatments or in understanding the mechanism(s) by which the cancer develops.

This page titled 5.4: Electrophoresis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 8.3: Electrophoresis by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

Learning Objectives

- Compare the method of development, use, and characteristics of monoclonal and polyclonal antibodies
- Explain the nature of antibody cross-reactivity and why this is less of a problem with monoclonal antibodies

In addition to being crucial for our normal immune response, antibodies provide powerful tools for research and diagnostic purposes. The high specificity of antibodies makes them an excellent tool for detecting and quantifying a broad array of targets, from drugs to serum proteins to microorganisms. With *in vitro* assays, antibodies can be used to precipitate soluble antigens, agglutinate (clump) cells, opsonize and kill bacteria with the assistance of complement, and neutralize drugs, toxins, and viruses.

Measuring Specificity

An antibody's specificity results from the antigen-binding site formed within the variable regions—regions of the antibody that have unique patterns of amino acids that can only bind to target antigens with a molecular sequence that provides complementary charges and noncovalent bonds. There are limitations to antibody specificity, however. Some antigens are so chemically similar that cross-reactivity occurs; in other words, antibodies raised against one antigen bind to a chemically similar but different antigen. Consider an antigen that consists of a single protein with multiple epitopes (Figure 5.5.1). This single protein may stimulate the production of many different antibodies, some of which may bind to chemically identical epitopes on other proteins.

Cross-reactivity is more likely to occur between antibodies and antigens that have low affinity or avidity. Affinity, which can be determined experimentally, is a measure of the binding strength between an antibody's binding site and an epitope, whereas avidity is the total strength of all the interactions in an antibody-antigen complex (which may have more than one bonding site). Avidity is influenced by affinity as well as the structural arrangements of the epitope and the variable regions of the antibody. If an antibody has a high affinity/avidity for a specific antigen, it is less likely to cross-react with an antigen for which it has a lower affinity/avidity.

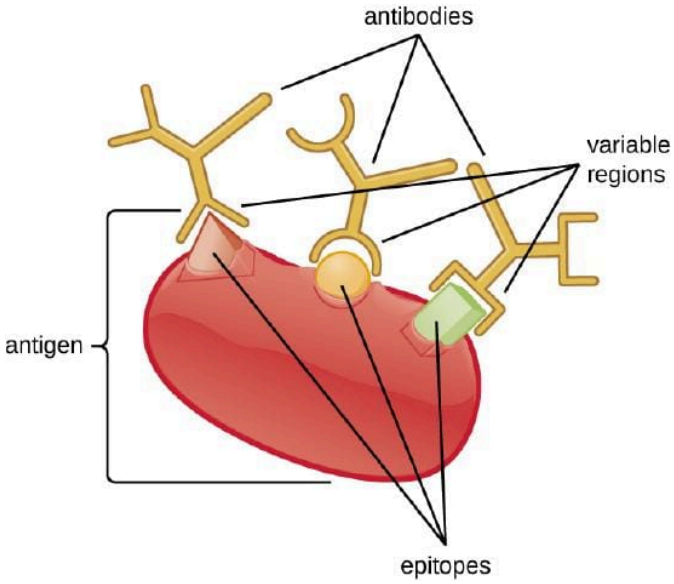


Figure 5.5.1: An antibody binds to a specific region on an antigen called an epitope. A single antigen can have multiple epitopes for different, specific antibodies.

Exercise 5.5.1

1. What property makes antibodies useful for research and clinical diagnosis?
2. What is cross-reactivity and why does it occur?

Producing Polyclonal Antibodies

Antibodies used for research and diagnostic purposes are often obtained by injecting a lab animal such as a rabbit or a goat with a specific antigen. Within a few weeks, the animal's immune system will produce high levels of antibodies specific for the antigen. These antibodies can be harvested in an antiserum, which is whole serum collected from an animal following exposure to an antigen. Because most antigens are complex structures with multiple epitopes, they result in the production of multiple antibodies in the lab animal. This so-called polyclonal antibody response is also typical of the response to infection by the human immune system. Antiserum drawn from an animal will thus contain antibodies from multiple clones of B cells, with each B cell responding to a specific epitope on the antigen (Figure 5.5.2).

Lab animals are usually injected at least twice with antigen when being used to produce antiserum. The second injection will activate memory cells that make class IgG antibodies against the antigen. The memory cells also undergo affinity maturation, resulting in a pool of antibodies with higher average affinity. Affinity maturation occurs because of mutations in the immunoglobulin gene variable regions, resulting in B cells with slightly altered antigen-binding sites. On re-exposure to the antigen, those B cells capable of producing antibody with higher affinity antigen-binding sites will be stimulated to proliferate and produce more antibody than their lower-affinity peers. An adjuvant, which is a chemical that provokes a generalized activation of the immune system that stimulates greater antibody production, is often mixed with the antigen prior to injection.

Antiserum obtained from animals will not only contain antibodies against the antigen artificially introduced in the laboratory, but it will also contain antibodies to any other antigens to which the animal has been exposed during its lifetime. For this reason, antisera must first be "purified" to remove other antibodies before using the antibodies for research or diagnostic assays.

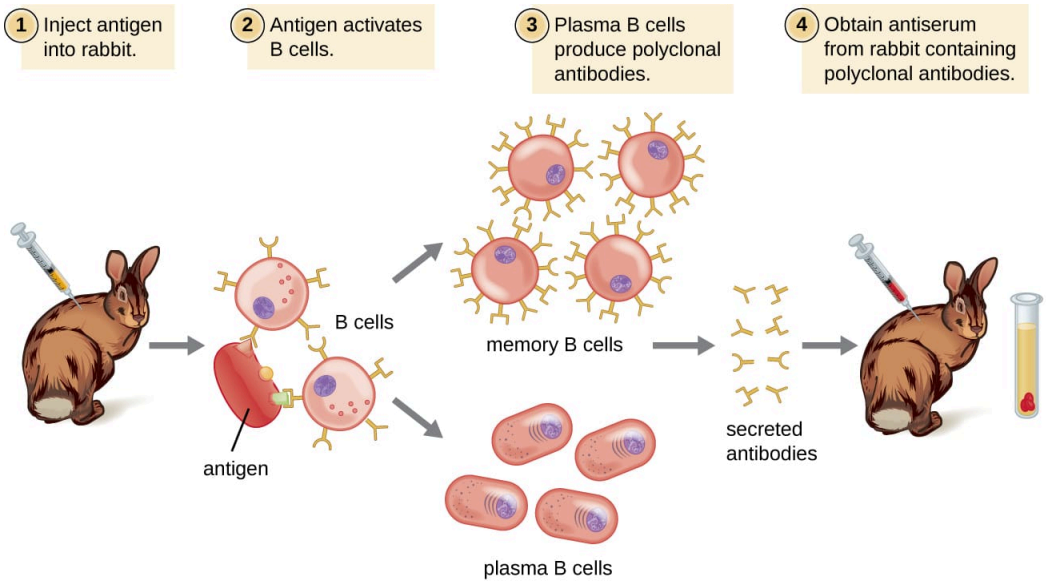


Figure 5.5.2: This diagram illustrates the process for harvesting polyclonal antibodies produced in response to an antigen.

Clinical Uses of Polyclonal Antisera

Polyclonal antisera are used in many clinical tests that are designed to determine whether a patient is producing antibodies in response to a particular pathogen. While these tests are certainly powerful diagnostic tools, they have their limitations, because they are an indirect means of determining whether a particular pathogen is present. Tests based on a polyclonal response can sometimes lead to a false-positive result—in other words, a test that confirms the presence of an antigen that is, in fact, not present. Antibody-based tests can also result in a false-negative result, which occurs when the test fails to detect an antibody that is, in fact, present.

The accuracy of antibody tests can be described in terms of test sensitivity and test specificity. Test sensitivity is the probability of getting a positive test result when the patient is indeed infected. If a test has high sensitivity, the probability of a false negative is low. Test specificity, on the other hand, is the probability of getting a negative test result when the patient is not infected. If a test has high specificity, the probability of a false positive is low.

False positives often occur due to cross-reactivity, which can occur when epitopes from a different pathogen are similar to those found on the pathogen being tested for. For this reason, antibody-based tests are often used only as screening tests; if the results are positive, other confirmatory tests are used to make sure that the results were not a false positive.

For example, a blood sample from a patient suspected of having hepatitis C can be screened for the virus using antibodies that bind to antigens on hepatitis C virus. If the patient is indeed infected with hepatitis C virus, the antibodies will bind to the antigens, yielding a positive test result. If the patient is not infected with hepatitic C virus, the antibodies will generally not bind to anything and the test should be negative; however, a false positive may occur if the patient has been previously infected by any of a variety of pathogens that elicit antibodies that cross-react with the hepatitis C virus antigens. Antibody tests for hepatitis C have high sensitivity (a low probability of a false negative) but low specificity (a high probability of a false positive). Thus, patients who test positive must have a second, confirmatory test to rule out the possibility of a false positive. The confirmatory test is a more expensive and time-consuming test that directly tests for the presence of hepatitis C viral RNA in the blood. Only after the confirmatory test comes back positive can the patient be definitively diagnosed with a hepatitis C infection. Antibody-based tests can result in a false negative if, for any reason, the patient's immune system has not produced detectable levels of antibodies. For some diseases, it may take several weeks following infection before the immune system produces enough antibodies to cross the detection threshold of the assay. In immunocompromised patients, the immune system may not be capable of producing a detectable level of antibodies.

Another limitation of using antibody production as an indicator of disease is that antibodies in the blood will persist long after the infection has been cleared. Depending on the type of infection, antibodies will be present for many months; sometimes, they may be present for the remainder of the patient's life. Thus, a positive antibody-based test only means that the patient was infected at some point in time; it does not prove that the infection is active.

In addition to their role in diagnosis, polyclonal antisera can activate complement, detect the presence of bacteria in clinical and food industry settings, and perform a wide array of precipitation reactions that can detect and quantify serum proteins, viruses, or other antigens. However, with the many specificities of antibody present in a polyclonal antiserum, there is a significant likelihood that the antiserum will cross-react with antigens to which the individual was never exposed. Therefore, we must always account for the possibility of false-positive results when working with a polyclonal antiserum.

Exercise 5.5.2

1. What is a false positive and what are some reasons that false positives occur?
2. What is a false negative and what are some reasons that false positives occur?
3. If a patient tests negative on a highly sensitive test, what is the likelihood that the person is infected with the pathogen?

Producing Monoclonal Antibodies

Some types of assays require better antibody specificity and affinity than can be obtained using a polyclonal antiserum. To attain this high specificity, all of the antibodies must bind with high affinity to a single epitope. This high specificity can be provided by monoclonal antibodies (mAbs). Table 5.5.1 compares some of the important characteristics of monoclonal and polyclonal antibodies.

Unlike polyclonal antibodies, which are produced in live animals, monoclonal antibodies are produced *in vitro* using tissue-culture techniques. mAbs are produced by immunizing an animal, often a mouse, multiple times with a specific antigen. B cells from the spleen of the immunized animal are then removed. Since normal B cells are unable to proliferate forever, they are fused with immortal, cancerous B cells called myeloma cells, to yield hybridoma cells. All of the cells are then placed in a selective medium that allows only the hybridomas to grow; unfused myeloma cells cannot grow, and any unfused B cells die off. The hybridomas, which are capable of growing continuously in culture while producing antibodies, are then screened for the desired mAb. Those producing the desired mAb are grown in tissue culture; the culture medium is harvested periodically and mAbs are purified from the medium. This is a very expensive and time-consuming process. It may take weeks of culturing and many liters of media to provide enough mAbs for an experiment or to treat a single patient. Making mAbs is expensive (Figure 5.5.3).

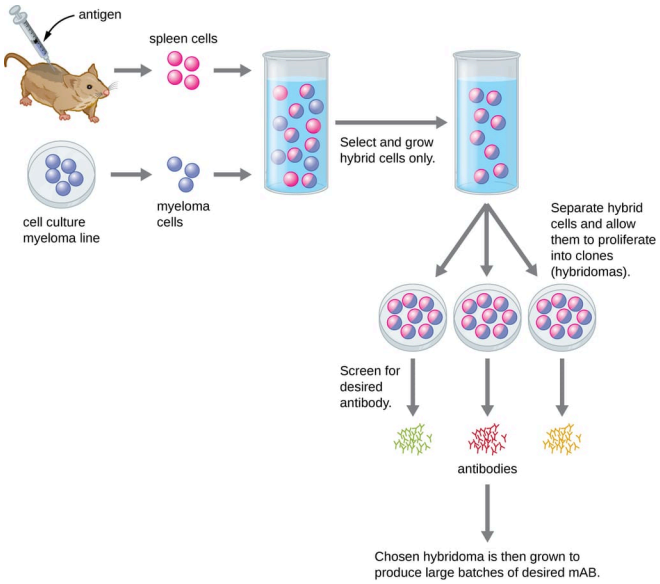


Figure 5.5.3: Monoclonal antibodies (mAbs) are produced by introducing an antigen to a mouse and then fusing polyclonal B cells from the mouse's spleen to myeloma cells. The resulting hybridoma cells are cultured and continue to produce antibodies to the antigen. Hybridomas producing the desired mAb are then grown in large numbers on a selective medium that is periodically harvested to obtain the desired mAbs.

Table* 5.5.1*: Characteristics of Polyclonal and Monoclonal Antibodies

Monoclonal Antibodies	Polyclonal Antibodies
Expensive production	Inexpensive production
Long production time	Rapid production
Large quantities of specific antibodies	Large quantities of nonspecific antibodies
Recognize a single epitope on an antigen	Recognize multiple epitopes on an antigen
Production is continuous and uniform once the hybridoma is made	Different batches vary in composition

Clinical Uses of Monoclonal Antibodies

Since the most common methods for producing monoclonal antibodies use mouse cells, it is necessary to create humanized monoclonal antibodies for human clinical use. Mouse antibodies cannot be injected repeatedly into humans, because the immune system will recognize them as being foreign and will respond to them with neutralizing antibodies. This problem can be minimized by genetically engineering the antibody in the mouse B cell. The variable regions of the mouse light and heavy chain genes are ligated to human constant regions, and the chimeric gene is then transferred into a host cell. This allows production of a mAb that is mostly "human" with only the antigen-binding site being of mouse origin.

Humanized mAbs have been successfully used to treat cancer with minimal side effects. For example, the humanized monoclonal antibody drug Herceptin has been helpful for the treatment of some types of breast cancer. There have also been a few preliminary trials of humanized mAb for the treatment of infectious diseases, but none of these treatments are currently in use. In some cases, mAbs have proven too specific to treat infectious diseases, because they recognize some serovars of a pathogen but not others. Using a cocktail of multiple mAbs that target different strains of the pathogen can address this problem. However, the great cost associated with mAb production is another challenge that has prevented mAbs from becoming practical for use in treating microbial infections.[1]

One promising technology for inexpensive mAbs is the use of genetically engineered plants to produce antibodies (or plantibodies). This technology transforms plant cells into antibody factories rather than relying on tissue culture cells, which are expensive and technically demanding. In some cases, it may even be possible to deliver these antibodies by having patients eat the plants rather than by extracting and injecting the antibodies. For example, in 2013, a research group cloned antibody genes into plants that had the ability to neutralize an important toxin from bacteria that can cause severe gastrointestinal disease.[2]

Eating the plants could potentially deliver the antibodies directly to the toxin.

Exercise 5.5.3

1. How are humanized monoclonal antibodies produced?
2. What does the "monoclonal" of monoclonal antibodies mean?

Using Monoclonal Antibodies to Combat Ebola

During the 2014–2015 Ebola outbreak in West Africa, a few Ebola-infected patients were treated with ZMapp, a drug that had been shown to be effective in trials done in rhesus macaques only a few months before.[3] ZMapp is a combination of three mAbs produced by incorporating the antibody genes into tobacco plants using a viral vector. By using three mAbs, the drug is effective across multiple strains of the virus. Unfortunately, there was only enough ZMapp to treat a tiny number of patients.

While the current technology is not adequate for producing large quantities of ZMapp, it does show that plantibodies—plant-produced mAbs—are feasible for clinical use, potentially cost effective, and worth further development. The last several years have seen an explosion in the number of new mAb-based drugs for the treatment of cancer and infectious diseases; however, the widespread use of such drugs is currently inhibited by their exorbitant cost, especially in underdeveloped parts of the world, where a single dose might cost more than the patient's lifetime income. Developing methods for cloning antibody genes into plants could reduce costs dramatically.

Use of Antibodies in Testing and Identification

Enzyme immunoassays (EIAs) use antibodies to detect the presence of antigens. However, the assays are conducted in microtiter plates or *in vivo*. There are many different types of EIAs, but they all involve an antibody molecule whose constant region binds an enzyme, leaving the variable region free to bind its specific antigen. The addition of a substrate for the enzyme allows the antigen to be visualized or quantified (Figure 5.5.4).

In EIAs, the substrate for the enzyme is most often a chromogen, a colorless molecule that is converted into a colored end product. The most widely used enzymes are alkaline phosphatase and horseradish peroxidase for which appropriate substrates are readily available. In some EIAs, the substrate is a fluorogen, a nonfluorescent molecule that the enzyme converts into a fluorescent form. EIAs that utilize a fluorogen are called fluorescent enzyme immunoassays (FEIAs). Fluorescence can be detected by either a fluorescence microscope or a spectrophotometer.

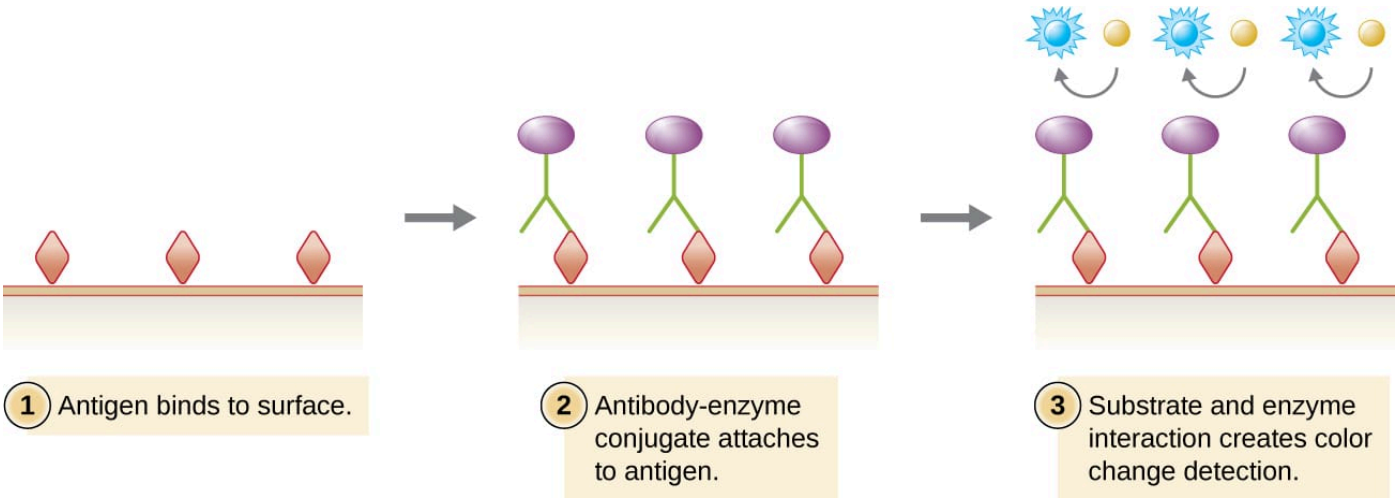


Figure 5.5.4: Enzyme immunoassays, such as the direct ELISA shown here, use an enzyme-antibody conjugate to deliver a detectable substrate to the site of an antigen. The substrate may be a colorless molecule that is converted into a colored end product or an inactive fluorescent molecule that fluoresces after enzyme activation. (credit: modification of work by "Cavitri"/Wikimedia Commons)

The MMR Titer

The MMR vaccine is a combination vaccine that provides protection against measles, mumps, and rubella (German measles). Most people receive the MMR vaccine as children and thus have antibodies against these diseases. However, for various reasons, even vaccinated individuals may become susceptible to these diseases again later in life. For example, some children may receive only one round of the MMR vaccine instead of the recommended two. In addition, the titer of protective antibodies in an individual's body may begin to decline with age or as the result of some medical conditions.

To determine whether the titer of antibody in an individual's bloodstream is sufficient to provide protection, an MMR titer test can be performed. The test is a simple immunoassay that can be done quickly with a blood sample. The results of the test will indicate whether the individual still has immunity or needs another dose of the MMR vaccine.

Submitting to an MMR titer is often a pre-employment requirement for healthcare workers, especially those who will frequently be in contact with young children or immunocompromised patients. Were a healthcare worker to become infected with measles, mumps, or rubella, the individual could easily pass these diseases on to susceptible patients, leading to an outbreak. Depending on the results of the MMR titer, healthcare workers might need to be revaccinated prior to beginning work.

Immunostaining

One powerful use of EIA is immunostaining, in which antibody-enzyme conjugates enhance microscopy. Immunohistochemistry (IHC) is used for examining whole tissues. As seen in Figure 5.5.5, a section of tissue can be stained to visualize the various cell types. In this example, a mAb against CD8 was used to stain CD8 cells in a section of tonsil tissue. It is now possible to count the number of CD8 cells, determine their relative numbers versus the other cell types present, and determine the location of these cells within this tissue. Such data would be useful for studying diseases such as AIDS, in which the normal function of CD8 cells is crucial for slowing disease progression.

Immunocytochemistry (ICC) is another valuable form of immunostaining. While similar to IHC, in ICC, extracellular matrix material is stripped away, and the cell membrane is etched with alcohol to make it permeable to antibodies. This allows antibodies to pass through the cell membrane and bind to specific targets inside the cell. Organelles, cytoskeletal components, and other intracellular structures can be visualized in this way. While some ICC techniques use EIA, the enzyme can be replaced with a fluorescent molecule, making it a fluorescent immunoassay.

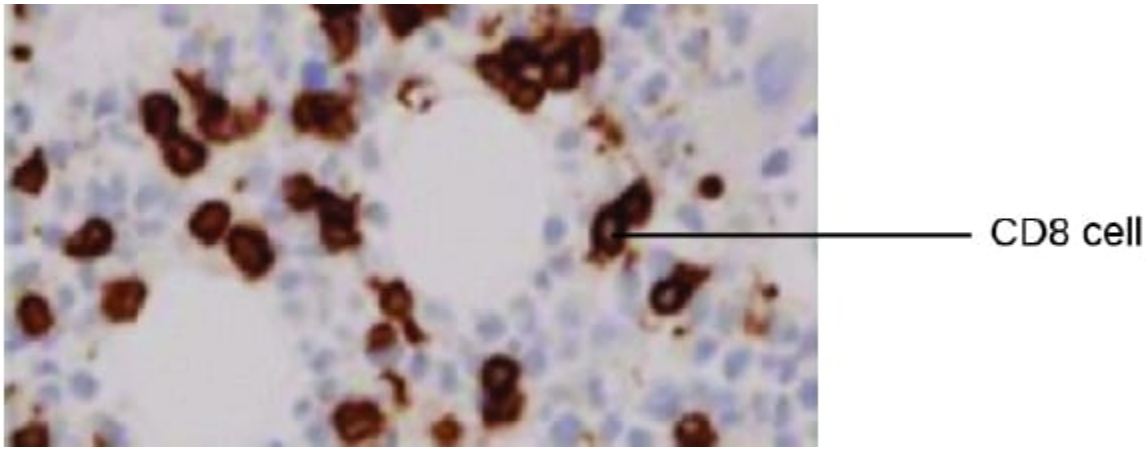


Figure 5.5.5: Enzyme-linked antibodies against CD8 were used to stain the CD8 cells in this preparation of bone marrow using a chromogen. (credit: modification of work by Yamashita M, Fujii Y, Ozaki K, Urano Y, Iwasa M, Nakamura S, Fujii S, Abe M, Sato Y, Yoshino T)

Exercise 5.5.4

1. What is the difference between immunohistochemistry and immunocytochemistry?
2. What must be true of the product of the enzymatic reaction used in immunohistochemistry?

Answer available at the end of this section.

Enzyme-linked Immunosorbent Assays (ELISAs)

The enzyme-linked immunosorbent assays (ELISAs) are widely used EIAs. In the direct ELISA, antigens are immobilized in the well of a microtiter plate. An antibody that is specific for a particular antigen and is conjugated to an enzyme is added to each well. If the antigen is present, then the antibody will bind. After washing to remove any unbound antibodies, a colorless substrate (chromogen) is added. The presence of the enzyme converts the substrate into a colored end product (Figure 5.5.4). While this technique is faster because it only requires the use of one antibody, it has the disadvantage that the signal from a direct ELISA is lower (lower sensitivity).

In a sandwich ELISA, the goal is to use antibodies to precisely quantify specific antigen present in a solution, such as antigen from a pathogen, a serum protein, or a hormone from the blood or urine to list just a few examples. The first step of a sandwich ELISA is to add the primary antibody to all the wells of a microtiter plate (Figure 5.5.6). The antibody sticks to the plastic by hydrophobic interactions. After an appropriate incubation time, any unbound antibody is washed away. Comparable washes are used between each of the subsequent steps to ensure that only specifically bound molecules remain attached to the plate. A blocking protein is then added (e.g., albumin or the milk protein casein) to bind the remaining nonspecific protein-binding sites in the well. Some of the wells will receive known amounts of antigen to allow the construction of a standard curve, and unknown antigen solutions are added to the other wells. The primary antibody captures the antigen and, following a wash, the secondary antibody is added, which is a polyclonal antibody that is conjugated to an enzyme. After a final wash, a colorless substrate (chromogen) is added, and the enzyme converts it into a colored end product. The color intensity of the sample caused by the end product is measured with a spectrophotometer. The amount of color produced (measured as absorbance) is directly proportional to the amount of enzyme, which in turn is directly proportional to the captured antigen. ELISAs are extremely sensitive, allowing antigen to be quantified in the nanogram (10^{-9} g) per mL range.

In an indirect ELISA, we quantify antigen-specific antibody rather than antigen. We can use indirect ELISA to detect antibodies against many types of pathogens, including *Borrelia burgdorferi* (Lyme disease) and HIV. There are three important differences between indirect and direct ELISAs as shown in Figure 5.5.7. Rather than using antibody to capture antigen, the indirect ELISA starts with attaching known antigen (e.g., peptides from HIV) to the bottom of the microtiter plate wells. After blocking the unbound sites on the plate, patient serum is added; if antibodies are present (primary antibody), they will bind the antigen. After washing away any unbound proteins, the secondary antibody with its conjugated enzyme is directed against the primary antibody (e.g., antihuman immunoglobulin). The secondary antibody allows us to quantify how much antigen-specific antibody is present in the patient's serum by the intensity of the color produced from the conjugated enzyme-chromogen reaction.

As with several other tests for antibodies discussed in this chapter, there is always concern about cross-reactivity with antibodies directed against some other antigen, which can lead to false-positive results. Thus, we cannot definitively diagnose an HIV infection (or any other type of infection) based on a single indirect ELISA assay. We must confirm any suspected positive test, which is most often done using either an immunoblot that actually identifies the presence of specific peptides from the pathogen or a test to identify the nucleic acids associated with the pathogen, such as reverse transcriptase PCR (RT-PCR) or a nucleic acid antigen test.

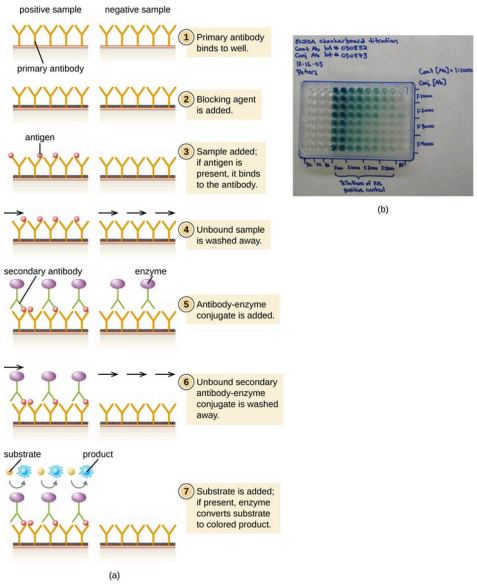


Figure 5.5.6: (a) In a sandwich ELISA, a primary antibody is used to first capture an antigen with the primary antibody. A secondary antibody conjugated to an enzyme that also recognizes epitopes on the antigen is added. After the addition of the chromogen, a spectrophotometer measures the absorbance of end product, which is directly proportional to the amount of captured antigen. (b) An ELISA plate shows dilutions of antibodies (left) and antigens (bottom). Higher concentrations result in a darker final color. (credit b: modification of work by U.S. Fish and Wildlife Service Pacific Region)

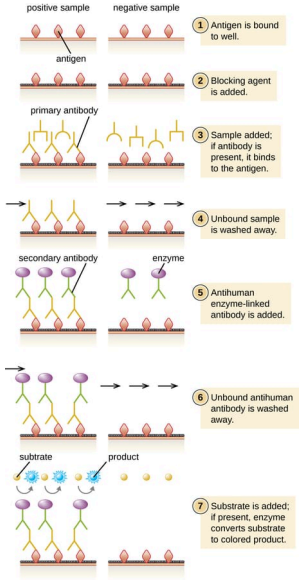


Figure 5.5.7: The indirect ELISA is used to quantify antigen-specific antibodies in patient serum for disease diagnosis. Antigen from the suspected disease agent is attached to microtiter plates. The primary antibody comes from the patient's serum, which is subsequently bound by the enzyme-conjugated secondary antibody. Measuring the production of end product allows us to detect or quantify the amount of antigen-specific antibody present in the patient's serum.

Exercise 5.5.5

1. What is the purpose of the secondary antibody in a direct ELISA?
2. What do the direct and indirect ELISAs quantify?

Key Concepts and Summary

- Antibodies bind with high **specificity** to antigens used to challenge the immune system, but they may also show **cross-reactivity** by binding to other antigens that share chemical properties with the original antigen.

- Injection of an antigen into an animal will result in a **polyclonal antibody** response in which different antibodies are produced that react with the various epitopes on the antigen.
- **Polyclonal antisera** are useful for some types of laboratory assays, but other assays require more specificity. Diagnostic tests that use polyclonal antisera are typically only used for screening because of the possibility of **false-positive** and **false-negative** results.
- **Monoclonal antibodies** provide higher specificity than polyclonal antisera because they bind to a single epitope and usually have high **affinity**.
- Monoclonal antibodies are typically produced by culturing antibody-secreting **hybridomas** derived from mice. mAbs are currently used to treat cancer, but their exorbitant cost has prevented them from being used more widely to treat infectious diseases. Still, their potential for laboratory and clinical use is driving the development of new, cost-effective solutions such as **plantibodies**.
- **Enzyme immunoassays (EIA)** are used to visualize and quantify antigens. They use an antibody conjugated to an enzyme to bind the antigen, and the enzyme converts a substrate into an observable end product. The substrate may be either a chromogen or a fluorogen.
- **Immunostaining** is an EIA technique for visualizing cells in a tissue (**immunohistochemistry**) or examining intracellular structures (**immunocytochemistry**).
- **Direct ELISA** is used to quantify an antigen in solution. The primary antibody captures the antigen, and the secondary antibody delivers an enzyme. Production of end product from the chromogenic substrate is directly proportional to the amount of captured antigen.
- **Indirect ELISA** is used to detect antibodies in patient serum by attaching antigen to the well of a microtiter plate, allowing the patient (primary) antibody to bind the antigen and an enzyme-conjugated secondary antibody to detect the primary antibody.

Footnotes

1. Saylor, Carolyn, Ekaterina Dadachova and Arturo Casadevall, “Monoclonal Antibody-Based Therapies for Microbial Diseases,” *Vaccine* 27 (2009): G38-G46.
2. Nakanishi, Katsuhiro et al., “Production of Hybrid-IgG/IgA Plantibodies with Neutralizing Activity against Shiga Toxin 1,” *PloS One* 8, no. 11 (2013): e80712.
3. Qiu, Xiangguo et al., “Reversion of Advanced Ebola Virus Disease in Nonhuman Primates with ZMapp,” *Nature* 514 (2014): 47–53.

Contributors and Attributions

- Nina Parker, (Shenandoah University), Mark Schneegurt (Wichita State University), Anh-Hue Thi Tu (Georgia Southwestern State University), Philip Lister (Central New Mexico Community College), and Brian M. Forster (Saint Joseph’s University) with many contributing authors. Original content via Openstax (CC BY 4.0; Access for free at https://openstax.org/books/microbiology/pages/1-introduction)

Answers

Exercise 5.5.4

This page titled 5.5: Practical Applications of Monoclonal and Polyclonal Antibodies is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

5.6: Western blot / Immunoblot overview

Immunoblot is a technique for analyzing proteins via antigen-antibody specific reactions.

Learning Objectives

- Describe how Western blotting allows individuals to detect specific solubilized proteins from serum or cell or tissue extracts

Key Points

- In immunoblot techniques such as Western blot analysis, proteins are separated by electrophoresis and transferred onto nitrocellulose sheets, then are identified by their reaction with labeled antibodies.
- Electrophoresis uses an electric current to separate proteins based on their size. Big proteins migrate slower and are represented by the highest bands on the blot, while small proteins migrate faster and are indicated by the lowest bands on the blot.
- Immunoblot assays are usually performed to confirm results obtained by other techniques such as ELISA.

Key Terms

- **sodium dodecyl sulfate**: strong detergent agent used to reduce and unfold native protein.
- **blot**: method of transferring protein, DNA, or RNA onto a carrier membrane.

Immunoblot procedures like protein blotting, or Western blotting, allow individuals to detect specific solubilized proteins from extracts made from cells or tissues, before or after any purification steps.

Immunoblotting Procedures

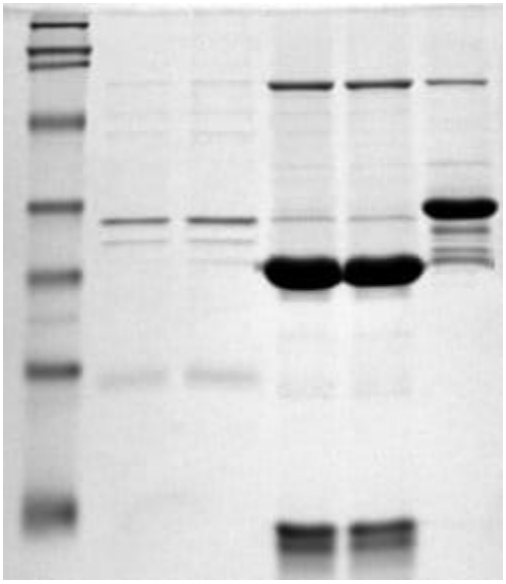


Figure: **Immunoblot**: proteins separated by molecular weight and represented by a dark band on a blot.

This analytic technique proceeds in the following steps. Proteins are generally separated by size using sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. After this, they are transferred to a synthetic membrane via dry, semi-dry, or wet blotting methods. In the electric field generated by a power supply, the proteins coated with negatively charged SDS migrate

toward the positive electrode. As the proteins migrate out of the gel, they are captured on a membrane. Protein binding to the membrane is an irreversible mechanism. Membranes can be of the nitrocellulose, polyvinylidene difluoride (PVDF), or nylon variety. The membrane can then be blocked with serum albumin or milk solution to prevent non-specific antibody binding. This is followed by probing with antibodies specific to the protein being studied on the membrane, a method that is similar to immunohistochemistry, but without a need for fixation. This technique exploits the specificity inherent in antigen-antibody recognition. Detection is typically performed using chromogen or peroxide-linked secondary antibodies to catalyze a chromogenic or chemiluminescent reaction.

Applications of Immunoblotting

Western blotting is a routine molecular biology method that can be used to semi-quantitatively compare protein levels between extracts. The size separation, prior to blotting, allows the protein molecular weight to be gauged, as compared with known molecular weight markers. Immunoblots are most often used in research settings and are usually performed to confirm results from ELISA or other immunoassays. In clinical diagnostic settings, immunoelectrophoresis is applied, which involves the electrophoresis of serum or urine followed by immunodiffusion. The size and position of precipitation bands provides the same type of information about antibody amount as the double immunodiffusion method.

This page titled 5.6: Western blot / Immunoblot overview is shared under a CC BY-SA 4.0 license and was authored, remixed, and/or curated by LibreTexts.

- 12.2J: Immunoblot Procedures is licensed CC BY-SA 4.0.

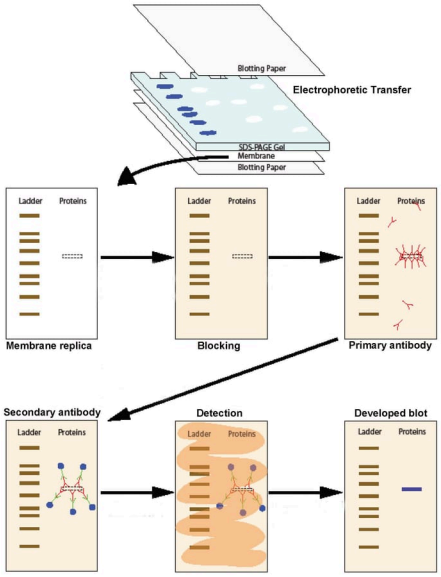
5.7: Steps in Western blotting

In a western blot procedure, proteins are first separated on an SDS-PAGE gel and then transferred to a membrane. This membrane replica is treated with antibodies that specifically recognize a protein or epitope of interest. Additional processing steps generate a signal at the position of the bound antibody. Between the steps, various washes are done to increase the signal-to-noise ratio on the final, developed blot. The major steps in a typical western blot are diagrammed on the following page and discussed in greater detail in sections that follow:

- Electrophoretic transfer of proteins from an SDS-PAGE gel to a membrane
- Blocking of nonspecific protein binding sites on transfer membranes
- Incubation of the membrane with a primary antibody specific for the epitope of interest
- Incubation with a secondary antibody that recognizes primary antibodies
- Visualization of bound antibodies

Electrophoretic transfer of proteins from an SDS-PAGE gel to a membrane

The first step in a western blot is to generate a replica of the SDS-PAGE gel by transferring proteins electrophoretically to a synthetic membrane with a high protein binding capacity. In our experiments, we will use membranes made of polyvinylidine fluoride (PVDF), a kind of plastic. PVDF membranes are hydrophobic and the dry membranes do not wet properly with water. Therefore, PVDF membranes are first wet with methanol, then rinsed with deionized water, and finally rinsed with transfer buffer. They must not be allowed to dry out during the transfer and immunoblot procedures. If they do dry out, they must be re-wet with methanol and rinsed with water before proceeding.



During the transfer process, the gel and membrane are placed directly against each other within a “sandwich” of pre-wet filter papers and foam pads (see the figure below). During the electrophoretic transfer, current should flow evenly across the entire surface area of the gel. It is important, therefore, that air bubbles are not trapped between the gel and membrane. After the electrophoretic transfer, which can be done in a few hours or overnight with reduced voltage, the membrane replica with the transferred proteins can be allowed to dry out and stored for later visualization with antibodies.

Blocking of non-specific protein binding sites on membranes

The transfer membranes used in western blots bind proteins nonspecifically. Before the membranes are incubated with specific (and expensive) antibodies, they must be pretreated with blocking solutions that contain high concentrations of abundant (and cheap) proteins to saturate non-specific binding sites. Think of this step as analogous to an artist priming a canvas with a lower quality paint before the more expensive media is applied. If the transfer membranes are not adequately blocked before the antibody is applied, the nonspecific sites on the membranes will absorb some of the antibodies, reducing the amount of antibody

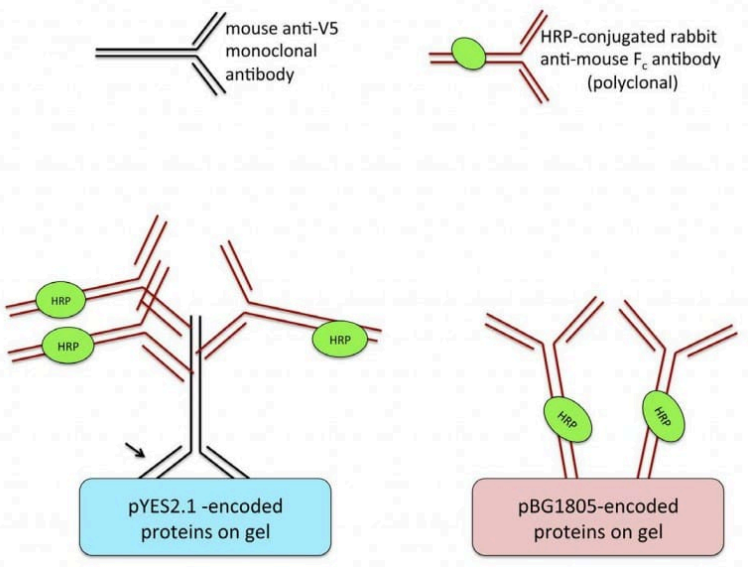
available to bind the
target proteins. In our experiments, we will use casein proteins from milk as blocking reagents. Because our experiments do not require high sensitivity, rehydrated non-fat dry milk (direct from the grocery store!) is an adequate source of caseins.

Primary antibody binding

Either polyclonal or monoclonal antibodies can be used as the primary antibody on western blots. Antibodies can be directed toward a naturally-occurring protein or toward an epitope attached to an overexpressed protein (as we are doing). Increasingly, researchers are using epitope-tagged proteins in their experiments, because antibodies against naturally- occurring proteins are expensive and time-consuming to prepare. In addition, an antibody directed against an epitope can be used to detect many different proteins carrying that same epitope. In our western blots, we will use a mouse monoclonal antibody that binds the V5 epitope on Met and LacZ proteins expressed from the pYES2.1 plasmid.

Secondary antibody binding

The secondary antibodies used in western blots are designed to bind the FC fragments
of primary antibodies, taking advantage of cross-species differences in antibody sequences. Secondary antisera are generally prepared by injecting an animal with FC fragments of IgGs from a second species. The first animal recognizes the FC fragments as foreign antigens and produces antibodies that bind the FC fragments. The secondary antibody in our experiment is a rabbit polyclonal antibody prepared against the FC domains of mouse IgGs. The antibody will bind the FC domains of the mouse anti-V5 antibodies bound to the pYES2.1-encoded proteins.



Visualization of bound antibody

In this final step, the western blot is incubated with substrates for the enzyme that has been conjugated to the secondary antibody. Our secondary antibody has been conjugated to HRP, a hardy enzyme with a high turnover number. (The turnover number is the number of product molecules produced at an enzyme's active site per second.) HRP catalyzes the reaction of hydrogen peroxide and 3,3',5,5' - tetramethylbenzidine (TMB), which generates a dark blue- grey reaction product that precipitates at the reaction site on the western blot. Colored reaction product accumulates with time until the reaction is stopped by washing away unreacted substrate. The reaction should be terminated before nonspecific antibody binding becomes problematic, as evidenced by the appearance of many weakly staining bands.

This page titled 5.7: Steps in Western blotting is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Clare M. O'Connor.

- 15.4: Western blots involve many steps by Clare M. O'Connor is licensed CC BY-NC-SA 4.0. Original source: https://capricorn.bc.edu/bi204/.

6: ENERGETICS AND METABOLISM

6.1: Endergonic and Exergonic Reactions
6.2: Thermodynamics
6.3: Energy in Metabolism

This page titled 6: Energetics and metabolism is shared under a license and was authored, remixed, and/or curated by LibreTexts.

6.1: Endergonic and Exergonic Reactions

Endergonic and exergonic reactions

Any system of molecules that undergoes a physical transformation/reorganization (aka. reaction) will have an associated change in internal energy and entropy. If we examine a single isolated reaction, in which unique reactants convert into unique products the Gibbs energy of the system will be dependent several factors, key among which are (a) the internal energy and entropy differences associated with the molecular rearrangements and (b) the degree to which the reaction is out-of-equilibrium.

If, for the sake of simplicity we begin by considering only the contribution of the molecular transformations in the system on ΔG, we conclude that reactions with ΔG < 0, the products of the reaction have less Gibbs energy than the reactants. Since ΔG is the difference between the enthalpy and temperature-scaled entropy changes in a reaction, a net negative ΔG can arise in through changes largely of enthalpy, entropy or most often both. The left panel of Figure 1 below shows a common graphical representation of an **exergonic** reaction. This graph is called a reaction coordinate diagram. It plots Gibbs energy on the y-axis, and the x-axis in arbitrary units shows the progress of a reaction. With an exergonic reaction, the figure on the left shows two key things: (1) the difference between the free energy of the reactants and products is negative and (2) the progress of the reaction requires some input of free energy (shown as an energy "hill" or barrier). This graph does not tell us how the energy in the system redistributed, only that the difference between enthalpy and temperature-scaled entropy is negative. **Exergonic reactions** are said to occur spontaneously. Understanding which chemical reactions are spontaneous is useful for biologists who are trying to understand whether a reaction is likely to "go" or not.

It is important to note that the term "spontaneous"—in thermodynamics—implies nothing about how fast the reaction proceeds. The change in free energy only describes the difference between beginning and end states, NOT how fast that transition takes place. This is contrary to the everyday use of the term which usually carries the implicit understanding that something happens quickly. As an example, the oxidation/rusting of iron is a spontaneous reaction. However, an iron nail exposed to air does not rust instantly—it may take years.

A chemical reaction with a positive ΔG means that the products of the reaction have a higher free energy than the reactants (see the right panel of Figure 1). These chemical reactions are called **endergonic reactions**, and they are NOT spontaneous. An endergonic reaction will not take place on its own without transferring energy into the reaction or increase of entropy somewhere else.

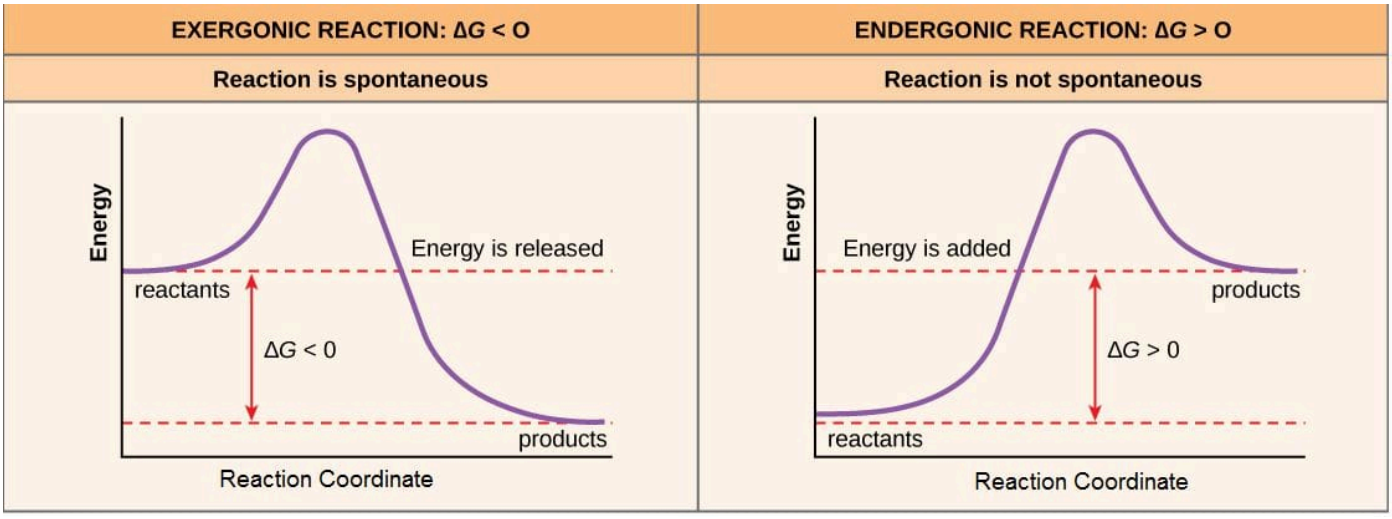


Figure 1. *Reaction coordinate diagrams of exergonic and endergonic reactions. Exergonic and endergonic reactions are characterized by changes in Gibbs energy. In the equilibrium state of an exergonic reaction, the Gibbs energy of the products is lower than that of the reactants. Meanwhile, the equilibrium state of an endergonic reaction in, the Gibbs energy of the products is higher than that of the reactants. Attribution: Marc T. Facciotti (own work)*

The building of complex molecules, such as sugars, from simpler ones is an anabolic process and is endergonic. On the other hand, the catabolic process, such as the breaking down of sugar into simpler molecules, is exergonic. Like the example of rust above, while the breakdown of biomolecules is generally spontaneous, these reactions don't occur need to occur instantaneously (quickly). Remember, the terms endergonic and exergonic only refer to the difference in Gibbs energy between the products and reactants; they don't tell you about the rate of the reaction (how fast it happens). Rate will be discussed in later sections.

An important concept in the study of metabolism and energy is that of chemical equilibrium. Most chemical reactions are reversible. They can proceed in both directions, often transferring energy into their environment in one direction and transferring energy in from the environment in the other direction. The same is true for the chemical reactions involved in cell metabolism, such as the breaking down and building up of proteins into and from individual amino acids, respectively. Reactants within a closed system will undergo chemical reactions in both directions until a state of equilibrium is reached. **Equilibrium** in a chemical reaction is the state in which both reactants and products are present in concentrations that have no further tendency to change with time. Usually, this state results when the forward reaction proceeds at the same rate as the reverse reaction. NOTE THIS LAST STATEMENT! Equilibrium means that the relative concentrations of reactants and products are not changing in time, BUT it does NOT mean that there is no interconversion between substrates and products—it just means that when the reactant(s) are converted to product(s) that product(s) are converted to reactant(s) at an equal rate (see Figure 2). The state of equilibrium is also one of the lowest possible free energy states for the reaction and is a state of maximal entropy.

If a reaction is kept or started far out of equilibrium this state of the system also contributes to the overall Gibbs energy of a reaction. Either a rebalancing of substrate or product concentrations (by adding or removing substrate or product) or a positive change in free energy, typically by the transfer of energy from outside the reaction, can to move a reaction to an out-of-equilibrium state. Note that in a living cell, most chemical reactions do not reach a state of equilibrium—this would require that they reach their lowest free energy state, a state that is almost by definition incompatible with life. Energy is therefore required to keep biological reactions out of their equilibrium state. In this way, living organisms are in a constant, energy-requiring, uphill battle against equilibrium and entropy. This also means that the Gibbs energy of most biological reactions as they occur in the cell must also include a contribution from this out-of-equilibrium state. The Gibbs energy of these reactions, therefore, is often different from that reported under standard conditions.

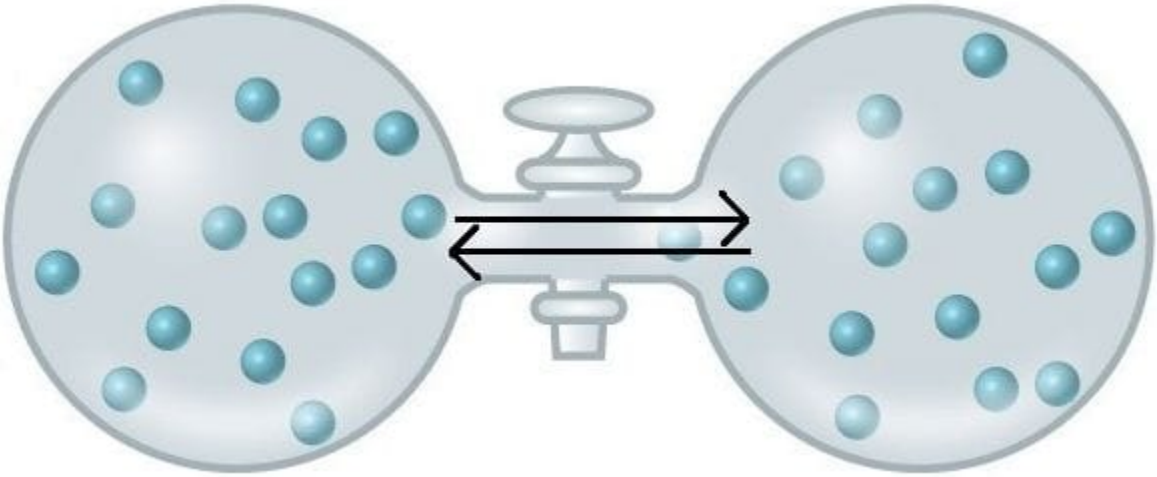


Figure 2. *At equilibrium, do not think of a static, unchanging system. Instead, picture molecules moving in equal amounts from one area to another. Here, at equilibrium, molecules are still moving from left to right and right to left. The net movement, however, is equal. There will still be about 15 molecules in each side of this flask once it reaches equilibrium. Source: https://courses.candelalearning.com/...apter/entropy/*

This page titled 6.1: Endergonic and Exergonic Reactions is shared under a license and was authored, remixed, and/or curated by Marc Facciotti.

6.2: Thermodynamics



Thermodynamics

Thermodynamics is concerned with describing the changes in systems before and after a change. This usually involves a discussion about energy transfers and its dispersion within the system and its surroundings. In nearly all practical cases, these analyses require that the system and its surroundings be completely described. For instance, when discussing the heating of a pot of water on the stove, the system may include the stove, the pot, and the water and the environment or surroundings may include everything else. Biological organisms are what are called open systems; energy is transferred between them and their surroundings.

The First Law of Thermodynamics

The first law of thermodynamics deals with the total amount of energy in the universe. It states that this total amount of energy is constant. In other words, there always has been, and always will be, exactly the same amount of energy in the universe.

According to the first law of thermodynamics, energy may be transferred from place to place, but it cannot be created or destroyed. Energy transfers take place around us all the time. Light bulbs transfer energy from electrical power stations into heat and photons of light. Gas stoves transfer energy stored in the bonds of chemical compounds into heat and light. (Heat, by the way, is the amount of energy transferred from one system to another because of a temperature difference.)

Plants perform one of the most biologically useful energy transfers on earth: they transfer energy in the photons of sunlight into the chemical bonds of organic molecules. In every one of these cases, energy is neither made nor destroyed, and we must try to account for *all* the energy when we examine some of these reactions.

The First Law and the Energy Story

The first law of thermodynamics is deceptively simple. Students often understand that energy cannot be created or destroyed. Yet, when describing an energy story of a process they often make the mistake of saying things such as "energy is produced from the transfer of electrons from atom A to atom B." While most of us will understand the point the student is trying to make, the wrong words are being used. Energy is not made or produced; it is simply transferred. To be consistent with the first law, when telling an energy story, make sure you try to track explicitly all the places that ALL the energy in the system at the start of a process goes by the end of a process.

The Second Law of Thermodynamics

An important concept in physical systems is **entropy**. Entropy relates to how energy can be distributed or dispersed within the particles of a system. The Second Law of Thermodynamics states that entropy is always increasing in a system *and* its surroundings (that is, everything inside and outside the system combined).

This idea helps explain the directionality of natural phenomena. The notion is that the directionality comes from the tendency for energy in a system to move towards a state of maximal dispersion. The Second Law, therefore, implies that in any transformation, we should look for an overall increase in entropy (or dispersion of energy), somewhere. As dispersion of energy in a system or its surroundings increases, the ability of the energy to be directed towards work decreases.

Keep in mind: you will find many examples in which the entropy of a system decreases *locally*. However, according to the Second Law, the entropy of the entire universe can *never* decrease. This must mean that there is an equal or greater increase in entropy somewhere else in the surroundings (most likely in a closely connected system) that compensates for the local decrease.

We associate the four scenarios below with increasing entropy of the system. Try to think of specific examples for when:

a. the system gains energy;
b. a change of state occurs from solid to liquid to gas;
c. a mixing of substances occurs;
d. the number of particles increases during a reaction.

Possible NB Discussion Point

Justify or refute the following statement: "*Biological systems are an exception to the Second Law of Thermodynamics, since cells are known to arrange themselves into very highly organized structures (think: tissues, organs, etc.) rather than into a more disordered state.*" Make sure to check out what your peers are saying -- do you agree or disagree with their position and/or their rationale?

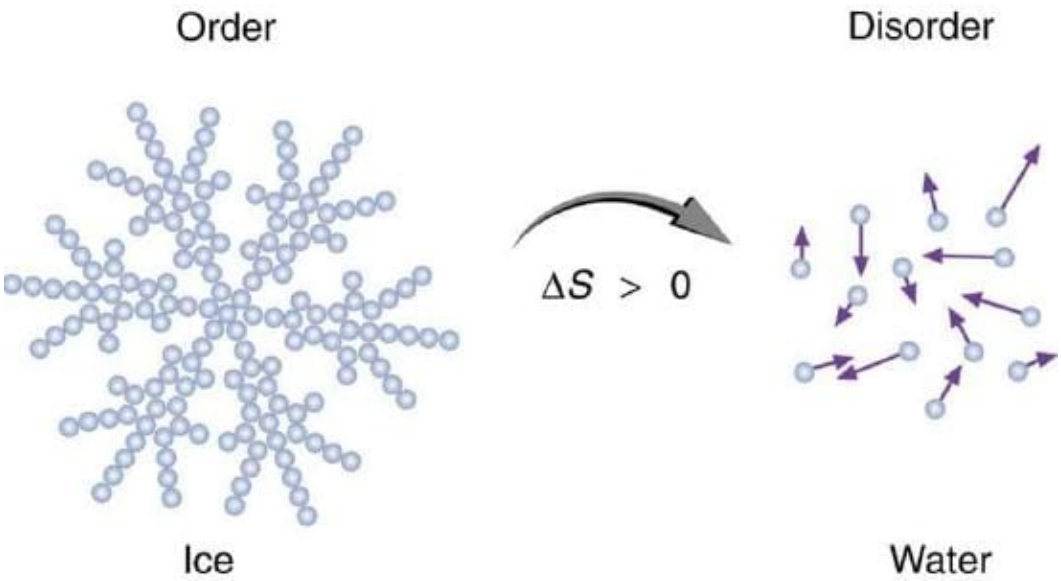


Figure 1. *An increase in disorder can happen in different ways. An ice cube melting on a hot sidewalk is one example. Here, ice is displayed as a snowflake, with organized, structured water molecules forming the snowflake. Over time, the snowflake will melt into a pool of disorganized, freely moving water molecules. It is common to describe entropy as a measure of order as a way to simplify the more concrete description relating entropy to the number of states in which energy can be dispersed in a system. While the idea of measuring order to define entropy has some flaws, it is sometimes a useful, if imperfect, proxy. (Source)*

If we consider the first and second laws together, we come to a useful conclusion. Whenever energy is transferred or redistributed within a system, entropy must increase. This increase in entropy is related to how "useful" the energy is to do work. Recall again that this energy becomes less and less available as entropy increases.

Keep in mind: you will find many examples in which the entropy of a system decreases *locally*. However, according to the Second Law, the entropy of the entire universe can *never* decrease. This must mean that there is an equal or greater increase in entropy somewhere else in the surroundings (most likely in a closely connected system) that compensates for the local decrease.

We conclude that while all the energy must be conserved, if the required change increases entropy, it means that some energy will become distributed in a way that makes it less useful for work. Most times, particularly in biology, some increase in entropy can be chalked up to a transfer of energy to heat in the environment.

This page titled 6.2: Thermodynamics is shared under a license and was authored, remixed, and/or curated by Marc Facciotti.

6.3: Energy in Metabolism

Source: BiochemFFA_5_1.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy



Living organisms are made up of cells, and cells contain a horde of biochemical components. Living cells, though, are not random collections of these molecules. They are extraordinarily organized or "ordered". By contrast, in the nonliving world, there is a universal tendency to increasing disorder. Maintaining and creating order in cells takes the input of energy. Without energy, life is not possible.

Oxidative Energy

The primary mechanism used by non-photosynthetic organisms to obtain energy is oxidation and carbon is the most commonly oxidized energy source. The energy released during the oxidative steps is “captured” in ATP and can be used later for energy coupling. The more reduced a carbon atom is, the more energy can be realized from its oxidation. Fatty acids are highly reduced, whereas carbohydrates are moderately so. Complete oxidation of both leads to carbon dioxide, which has the lowest energy state. Conversely, the more oxidized a carbon atom is, the more energy it takes to reduce it.

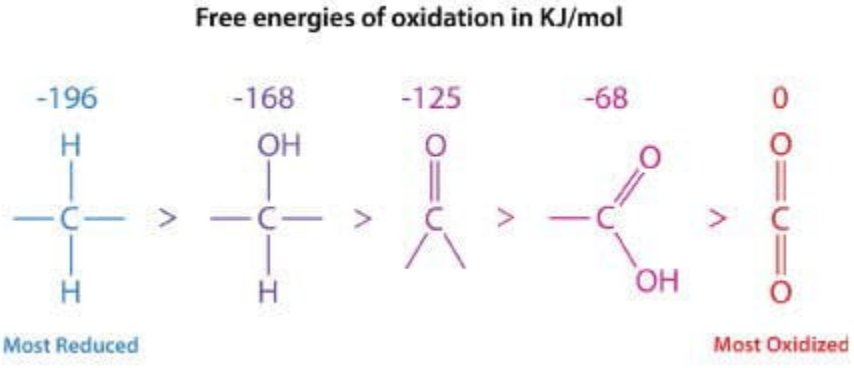


Figure 6.3.1: Five oxidation states of carbon. Image by Pehr Jacobson

In the series shown in Figure 6.3.1, the most reduced form of carbon is on the left. The energy of oxidation of each form is shown above it. The reduction states of fatty acids and carbohydrates can be seen by their formulas.

- Palmitic acid: $C_{16}H_{34}O_2$
- Glucose: $C_6H_{12}O_6$

Palmitic acid only contains two oxygens per sixteen carbons, whereas glucose has six oxygen atoms per six carbons. Consequently, when palmitic acid is fully oxidized, it generates more ATP per carbon (128/16) than glucose (38/6). It is because of this that we use fat (contains fatty acids) as our primary energy storage material.

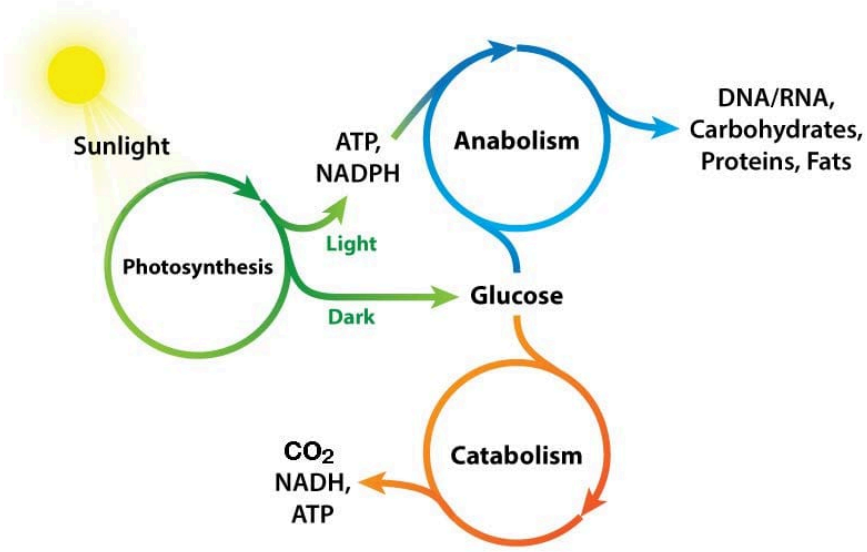


Figure $6.3.2$*: Photosynthesis: The primary source of biological energy. Image by Aleia Kim*

Oxidation vs. Reduction in Metabolism

Biochemical processes that break things down from larger to smaller are called *catabolic processes*. Catabolic processes are often oxidative in nature and energy releasing. Some, but not all, of that energy is captured as ATP. If not all of the energy is captured as ATP, what happens to the rest of it? The answer is simple. It is released as heat and it is for this reason we get hot when we exercise.

By contrast, synthesizing large molecules from smaller ones (for example, making proteins from amino acids) is referred to as anabolism. Anabolic processes are often reductive in nature (Figures 5.3 & 5.4) and require energy input. By themselves, they would not occur, as they are reversing oxidation and decreasing entropy (making many small things into a larger one). To overcome this energy barrier, cells must expend energy. For example, if one wishes to reduce CO_2 to carbohydrate, energy must be used to do so. Plants do this during the dark reactions of photosynthesis (Figure $6.3.3$). The energy source for the reduction is ultimately the sun. The electrons for the reduction come from water, and the CO_2 is removed from the atmosphere and gets incorporated into a sugar.

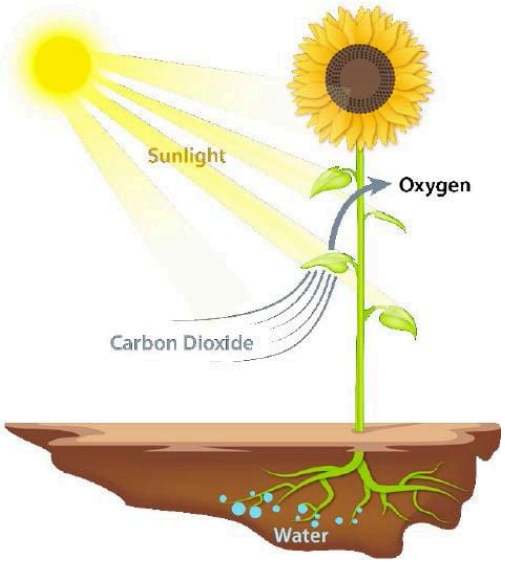


Figure 6.3.3: Movement of biological energy. Image by Aleia Kim

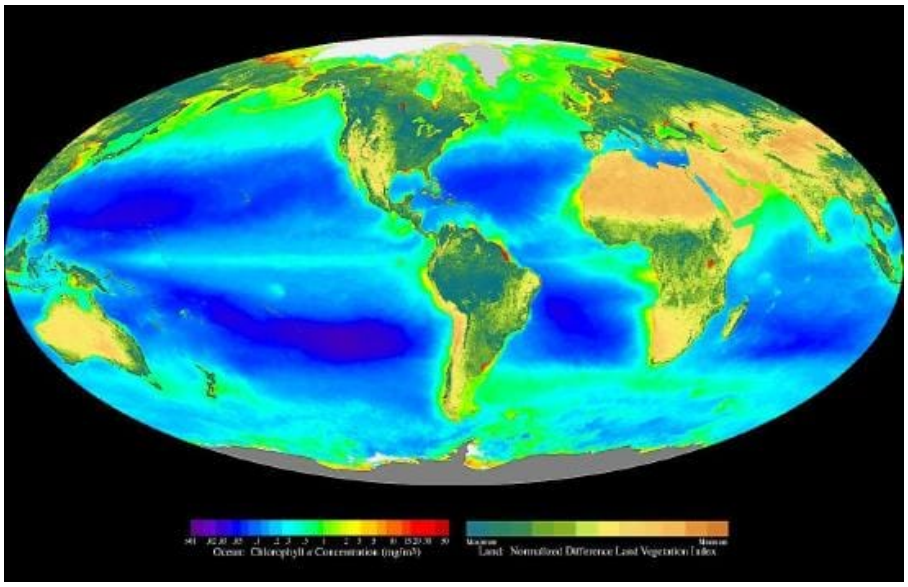


Figure 6.3.4: Photosynthesis as measured by chlorophyll concentration

Energy Coupling

The synthesis of the many molecules needed by cells needs the input of energy to occur. Cells overcome this energy obstacle by using ATP to “drive” the reaction (Figure 6.3.6). The energy needed to drive reactions is harvested in very controlled conditions in enzymes. This involves a process called ‘coupling’. Coupled reactions rely on linking an energetically favorable reaction (i.e., one with a negative $\Delta G^{\circ\prime}$) with the reaction requiring an energy input, which has a positive $\Delta G^{\circ\prime}$. As long as the overall $\Delta G^{\circ\prime}$ of the two reactions together is negative, the reaction can proceed. Hydrolysis of ATP is a very energetically favorable reaction that is commonly linked to many energy requiring reactions in cells. Without the hydrolysis of ATP (or GTP, in some cases), the reaction would not be feasible.

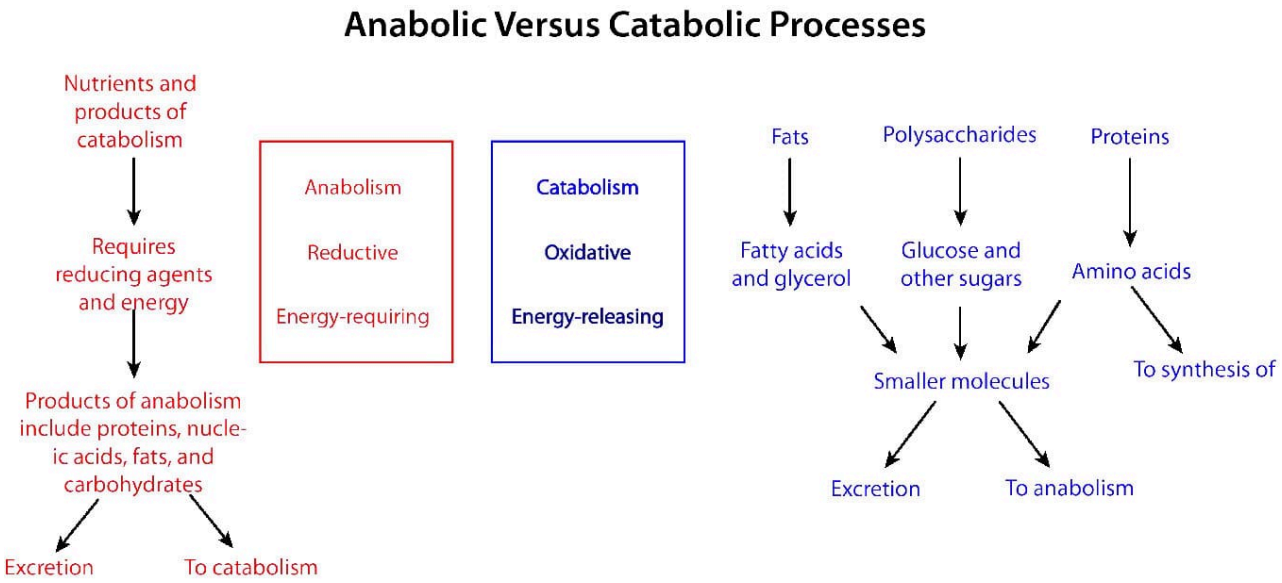


Figure 6.3.5: Synthesis and breakdown pathways in metabolism. Image by Pehr Jacobson

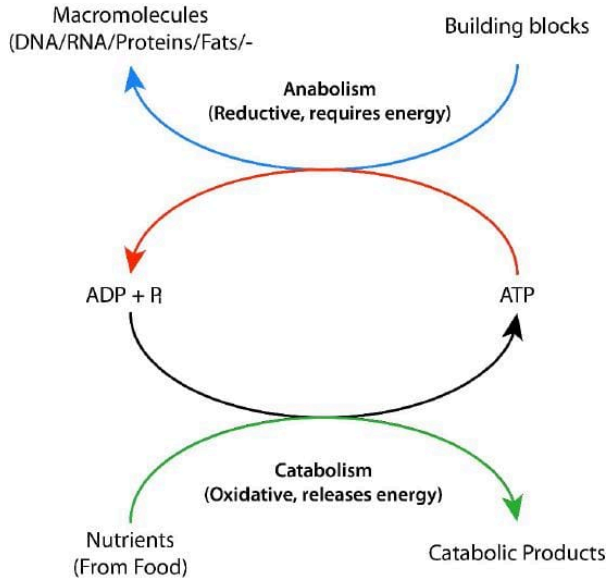


Figure 6.3.6: Cycling of biological energy via ADP and ATP. Image by Pehr Jacobson

Entropy and energy

Most students who have had some chemistry know about the Second Law of Thermodynamics with respect to increasing disorder of a system. Cells are very organized or ordered structures, leading some to mistakenly conclude that life somehow violates the second law. In fact, that notion is incorrect. The second law doesn't say that entropy always increases, just that, left alone, it tends to do so, in an isolated system. Cells are not isolated systems, though, in that they obtain energy, either from the sun, if they are autotrophic, or food, if they are heterotrophic.

To counter the universal tendency towards disorder on a local scale requires energy. As an example, take a fresh deck of cards which is neatly aligned with Ace-King-Queen . . . . 4,3,2 for each suit. Throw the deck into the air, letting the cards scatter. When you pick them up, they will be more disordered than when they started. However, if you spend a few minutes (and expend a bit of energy), you can reorganize the same deck back to its previous, organized state. If entropy always increased everywhere, you could not do this. However, with the input of energy, you overcame the disorder. This illustrates an important concept: the cost of fighting disorder is energy.

Biological energy

There are, of course, other reasons that organisms need energy. Muscular contraction, synthesis of molecules, neurotransmission, signaling, thermoregulation, and subcellular movements are examples. Where does this energy come from? The currencies of energy are generally high-energy phosphate-containing molecules. ATP is the best known and most abundant, but GTP is also an important energy source (energy source for protein synthesis). CTP is involved in synthesis of glycerophospholipids and UTP is used for synthesis of glycogen and other sugar compounds. In each of these cases, the energy is in the form of potential chemical energy stored in the multi-phosphate bonds. Hydrolyzing those bonds releases the energy in them.

Of the triphosphates, ATP is the primary energy source, acting to facilitate the synthesis of the others by action of the enzyme NDPK. ATP is made by three distinct types of phosphorylation – oxidative phosphorylation (in mitochondria), photophosphorylation (in chloroplasts of plants), and substrate level phosphorylation (in enzymatically catalyzed reactions).

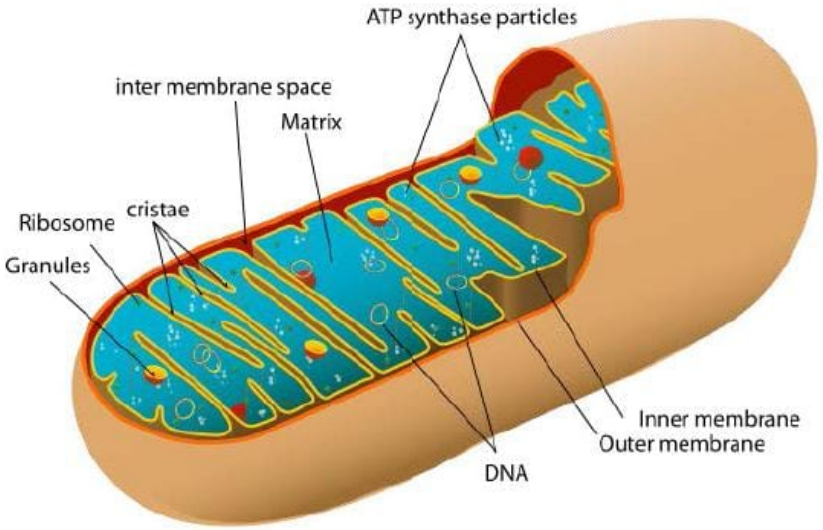


Figure 6.3.7: Mitochondrion

Gibbs free energy in Biology

ATP is generally considered the “storage battery” of cells (See also ‘Molecular Battery Backups for Muscles HERE). In order to understand how energy is captured, we must first understand Gibbs free energy and in doing so, we begin to see the role of energy in determining the directions chemical reactions take.

Gibbs free energy may be thought of as the energy available to do work in a thermodynamic system at constant temperature and pressure. Mathematically, the Gibbs free energy is given as:

$$G = H–TS \tag{6.3.1}$$

where H is the enthalpy, T is the temperature in Kelvin, and S is the entropy. At standard temperature and pressure, every system seeks to achieve a minimum of free energy. Thus, increasing entropy, S, will reduce Gibbs free energy. Similarly, if excess heat is available (reducing the enthalpy, H), the free energy can also be reduced.

Cells must work within the laws of thermodynamics, as noted, so all of their biochemical reactions, too, are ruled by these laws. Now we shall consider energy in the cell. The change in Gibbs free energy (ΔG) for a reaction is crucial, for it, and it alone, determines whether or not a reaction goes forward.

$$\Delta G = \Delta H–T\Delta S. \tag{6.3.2}$$

There are three cases

- ΔG < 0: the reaction proceeds as written

- ΔG = 0: the reaction is at equilibrium
- ΔG > 0: the reaction runs in reverse

For a reaction

$$a\,\mathrm{A} \rightleftharpoons b\,\mathrm{B} \tag{6.3.3}$$

(where 'a' and 'b' are integers and A and B are molecules) at pH 7, ΔG can be determined by the following equation,

$$\Delta G = \Delta G^{\circ\prime} + RT\ln(\frac{[B]^b}{[A]^a}) \tag{6.3.4}$$

For multiple substrate reactions, such as

$$a\,\mathrm{A} + c\,\mathrm{C} \rightleftharpoons b\,\mathrm{B} + d\,\mathrm{D} \tag{6.3.5}$$

$$\Delta G = \Delta G^{\circ\prime} + RT\ln(\frac{[B]^b[D]^d}{[A]^a[C]^c}) \tag{6.3.6}$$

The ΔG°' term is called the change in Standard Gibbs Free energy, which is the change in energy that occurs when all of the products and reactants are at standard conditions and the pH is 7.0. It is a constant for a given reaction.

In simple terms, we can collect all of the terms of the numerator together and call them {Products} and all of the terms of the denominator together and call them {Reactants},

$$\Delta G = \Delta G^{\circ\prime} + RT\ln(\frac{\{\mathrm{Products}\}}{\{\mathrm{Reactants}\}}) \tag{6.3.7}$$

For most biological systems, the temperature, T, is a constant for a given reaction. Since ΔG°' is also a constant for a given reaction, the ΔG is changed almost exclusively as the ratio of {Products}/{Reactants} changes.

Importance of ΔG°'

If one starts out at standard conditions, where everything except protons is at 1M, the RTln({Products}/{Reactants}) term is zero, so the ΔG°' term equals the ΔG, and the ΔG°' determines the direction the reaction will take (only under those conditions). This is why people say that a negative ΔG°' indicates an energetically favorable reaction, whereas a positive ΔG°' corresponds to an unfavorable one.

Increasing the ratio of {Products}/{Reactants} causes the value of the natural log (ln) term to become more positive (less negative), thus making the value of ΔG more positive. Conversely, as the ratio of {Products}/{Reactants} decreases, the value of the natural log term becomes less positive (more negative), thus making the value of ΔG more negative.

System response to stress

Intuitively, this makes sense and is consistent with Le Chatelier's Principle – a system responds to stress by acting to alleviate the stress. If we examine the ΔG for a reaction in a closed system, we see that it will always move to a value of zero (equilibrium), no matter whether it starts with a positive or negative value.

Another type of free energy available to cells is that generated by electrical potential. For example, mitochondria and chloroplasts partly use Coulombic energy (based on charge) from a proton gradient across their membranes to provide the necessary energy for the synthesis of ATP. Similar energies drive the transmission of nerve signals (sodium and potassium gradients) and the movement of some molecules in secondary active transport processes across membranes (e.g., H+ differential driving the movement of lactose). From the Gibbs free energy change equation,

$$\Delta G = \Delta H – T\Delta S \tag{6.3.8}$$

it should be noted that an increase in entropy will help contribute to a decrease in ΔG. This happens, for example when a large molecule is being broken into smaller pieces or when the rearrangement of a molecule increases the disorder of molecules around it. The latter situation arises in the hydrophobic effect, which helps drive the folding of proteins.

Chemical and electrical potential

It is said that absence makes the heart grow fonder. We won't tackle that philosophical issue here, but we will say that separation provides potential energy that cells can and do harvest. The lipid bilayer of cell and (in eukaryotic cells) organelle membranes provide the necessary barrier for separation.

Impermeable to most ions and polar compounds, biological membranes are essential for processes that generate cellular energy. Consider Figure 5.8. A lipid bilayer separates two solutions with different concentrations of a solute. There is a greater concentration of negative ions in the bottom and a greater concentration of positive ions on the top.

Whenever there is a difference in concentration of molecules across a membrane, there is said to be a concentration gradient across it. A difference in concentration of ions across a membrane also creates a charge (or electrical) gradient. Because there is a difference in both the chemical concentration of the ions and in the charge on the two sides of the membrane, this is described as an electrochemical gradient (Figures 5.8 -5.10).

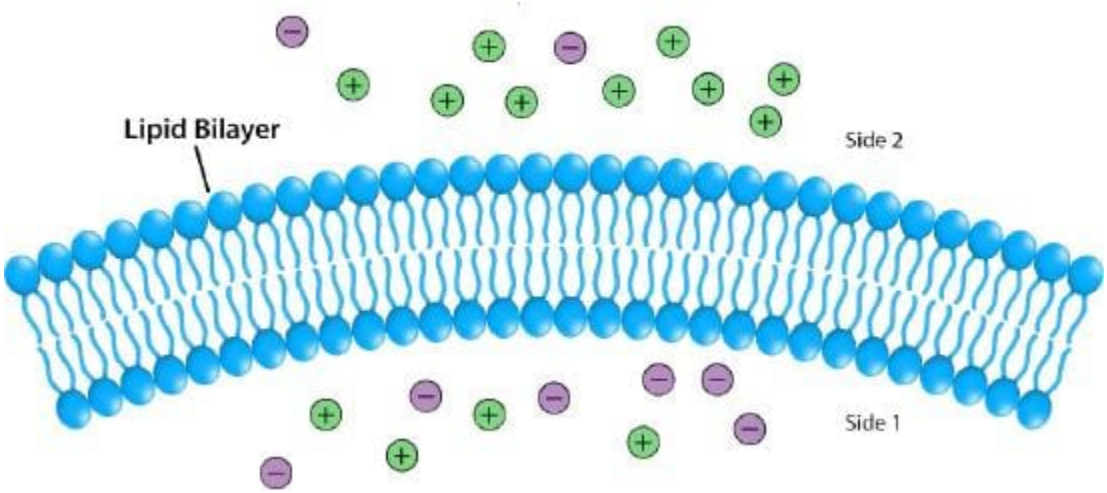


Figure 5.8: Differences in ion concentration across a membrane give rise to chemical and electrical gradients. Image by Pehr Jacobson

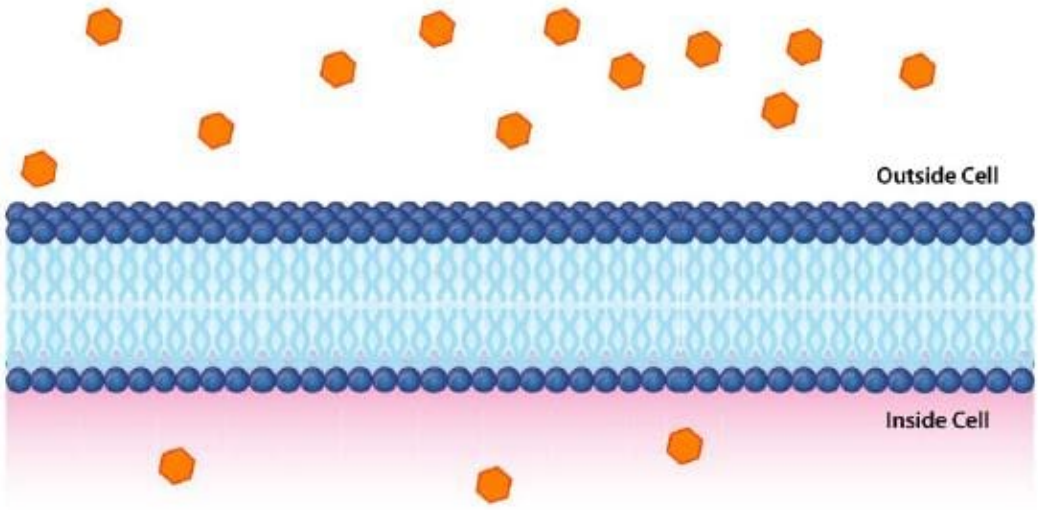


Figure 6.3.9: A chemical gradient. Image by Aleia Kim

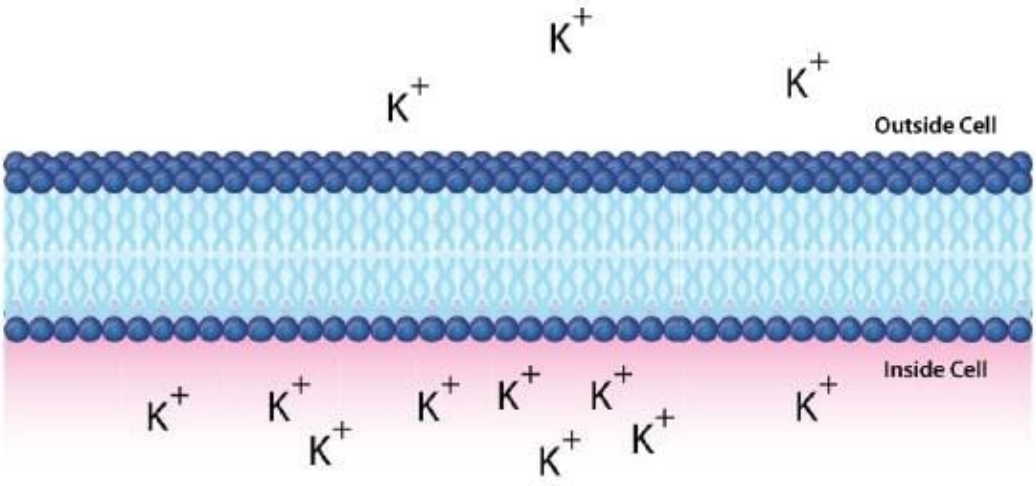
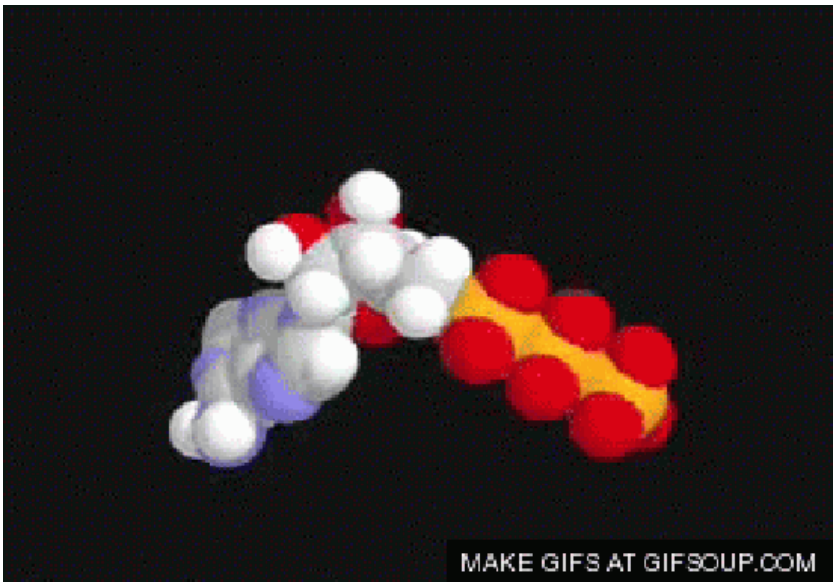


Figure 6.3.10: An electrochemical gradient of potassium ions. Image by Aleia Kim

Energy Storage in Triphosphates



Movie 5.1: ATP: The fuel of the cell

Formation of triphosphates, like ATP, is essential to meeting the cell's energy needs for synthesis, motion, and signaling. In a given day, an average human body makes and breaks down more than its weight in triphosphates. This is especially remarkable considering that there is only about 250 g of the molecule present in the body at any given time. Energy in ATP is released by hydrolysis of a phosphate from the molecule.

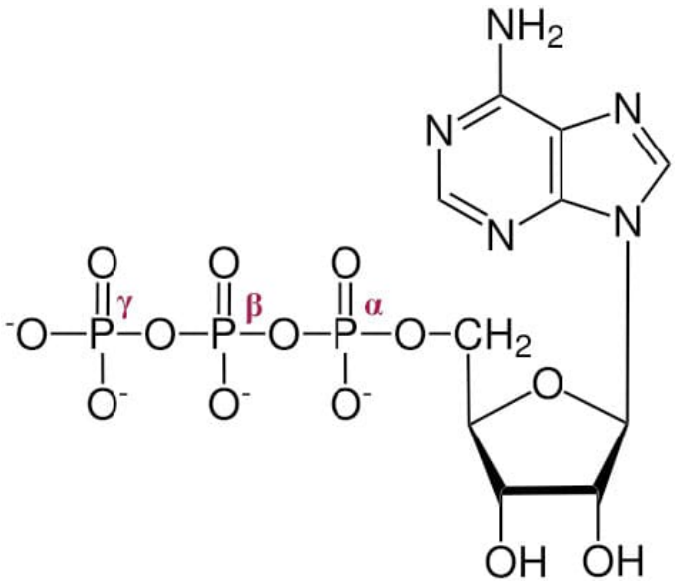


Figure 6.3.12 ATP showing α, β and γ phosphates

The three phosphates, starting with the one closest to the sugar are referred to as α, β, and γ (Figure 6.3.12). It is the γ phosphate that is cleaved in hydrolysis and the product is ADP. In a few reactions, the bond between the α and β is cleaved. When this happens, a pyrophosphate (β linked to γ) is released and AMP is produced. This latter reaction to produce AMP releases more energy (ΔG°' = -45.6 kJ/mol) than the first reaction which produces ADP (ΔG°' = -30.5 kJ/mol).

Since triphosphates are the "currency" that meet immediate needs of the cell, it is important to understand how triphosphates are made. There are three phosphorylation mechanisms – 1) substrate level; 2) oxidative; and 3) photophosphorylation. We consider them here individually.

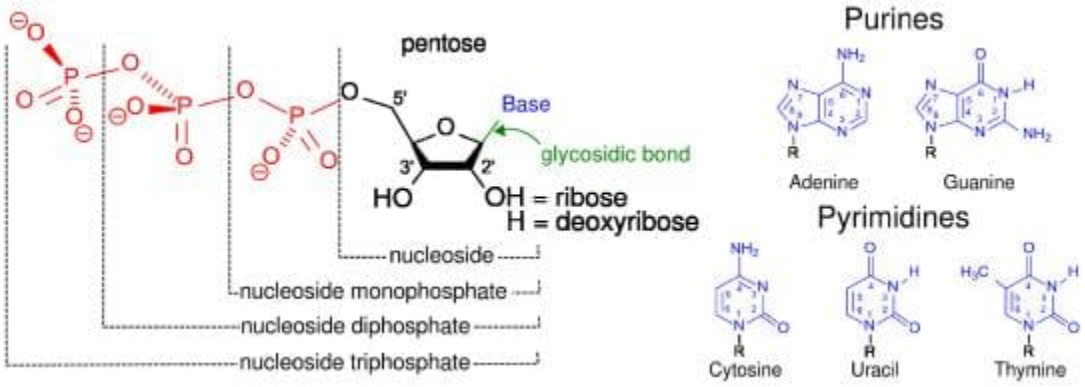


Figure 6.3.13: Nucleotides, nucleosides, and bases

Substrate level phosphorylation

The easiest type of phosphorylation to understand is that which occurs at the substrate level. This type of phosphorylation involves the direct synthesis of ATP from ADP and a high energy intermediate, typically a phosphate-containing molecule. Substrate level phosphorylation is a relatively minor contributor to the total synthesis of triphosphates by cells. An example substrate phosphorylation comes from glycolysis.

Phosphoenolpyruvate (PEP) + ADP ⇌ Pyruvate + ATP

This reaction has a very negative ΔG°' (-31.4 kJ/mol), indicating that the PEP contains more energy than ATP, thus tending to energetically favor ATP's synthesis. Other triphosphates can be made by substrate level phosphorylation, as well. For example, GTP can be synthesized by the following citric acid cycle reaction.

Succinyl-CoA + GDP + Pi ⇌ Succinate + GTP + CoA-SH

Triphosphates can be interchanged readily in substrate level phosphorylations catalyzed by the enzyme Nucleoside Diphosphate Kinase (NDPK). A generalized form of the reactions catalyzed by this enzyme is as follows:

XTP + YDP ⇌ XDP + YTP

where X = adenosine, cytidine, uridine, thymidine, or guanosine and Y can be any of these as well. Further, XTP and YDP can be any of the deoxynucleotides as well.

Last, an unusual way of synthesizing ATP by substrate level phosphorylation is via the reaction catalyzed by adenylate kinase

2 ADP ⇌ ATP + AMP

ATP source

This reaction is an important means of generating ATP when the cell doesn't have other sources of energy. Accumulation of AMP resulting from this reaction activates enzymes, such as phosphofructokinase, of glycolysis, which will catalyze reactions to give the cell additional, needed energy.

It is important to note that enzymes cannot make reactions happen that are energetically unfavorable. Enzymes speed reactions, but do not change their direction. Cells are thus bound by the rules of Gibbs free energy. So, how do energetically unfavorable reactions happen in a cell?

Reaction coupling

Reactions that are energetically unfavorable, can be made favorable by coupling them with the hydrolysis of ATP, a very energetically favorable reaction. There are numerous parallels in the "real world." Movement of automobiles is energetically unfavorable, but coupling movement of the automobile to oxidation of gasoline makes an unfavorable process favorable. Another approach to making an unfavorable reaction favorable is to manipulate the concentration of reactants and products. Consider the reaction below, which occurs in pyrimidine nucleotide metabolism:

orotate + PRPP ⇌ OMP + PP_i

The ΔG°' for this reaction is -0.8 kJ/mol, meaning that if one starts with equal concentrations of reactants and products, at equilibrium, there will be a small excess of products. In the cell, however, this reaction moves strongly to the right (ΔG = very negative). Given that the ΔG°' is very close to zero, a very negative ΔG can only occur if the concentrations of reactants and products are altered, since

$$\Delta G = \Delta G^{\circ\prime} + RT \ln\left(\frac{[\mathrm{OMP}][\mathrm{PP_i}]}{[\mathrm{Orotate}][\mathrm{PRPP}]}\right) \tag{6.3.9}$$

Manipulation is exactly what happens here. The key item whose concentration is adjusted in this reaction is the pyrophosphate (PPi). This is possible because cells contain an enzyme called pyrophosphorylase that catalyzes the following reaction

$PP_i + H_2O$ ⇌ 2 P_i

Hydrolysis of pyrophosphate is very energetically favored, causing the PPi produced in the reaction to be quickly hydrolyzed. As a result, the concentration of PPi in the cell is kept very low. A low concentration of a product (PPi) causes the natural log (ln) term of the orotate equation to become more negative, driving the ΔG term for the overall reaction to become much more negative.

Pushing and pulling

Reactions that yield pyrophosphate as a product are produced in the synthesis of DNA and RNA, as well as many other molecules. As shown in the previous example, this pyrophosphate is rapidly hydrolyzed, causing the overall reaction to move in the direction of pyrophosphate production. When reactants are removed/reduced in a metabolic reaction to decrease the concentration of a product, we say that the reaction is "pulled", to represent the increase in the forward reaction as a result of product depletion.

Pushing happens when reactants in a reaction are added/increased. This too has the effect of reducing the ΔG of a reaction and making it more favorable because the ratio of [Products]/[Reactants] is decreased with increasing [Reactants]. Pushing and pulling of reactions are additional tools for cells to overcome energy barriers, just like coupling energetically favorable processes to energetically unfavorable ones.

This page titled 6.3: Energy in Metabolism is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

7: ENZYMES, CATALYSIS AND KINETICS

7.1: Basic Principles of Catalysis
7.2: Derivation of Michaelis-Menten equation
7.3: Mechanisms of Catalysis
7.4: Control of Enzymatic Activity

This page titled 7: Enzymes, catalysis and kinetics is shared under a license and was authored, remixed, and/or curated by LibreTexts.

7.1: Basic Principles of Catalysis

A printable version of this section is here: BiochemFFA_4_1.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

If there is a magical component to life, an argument can surely be made for it being catalysis. Thanks to catalysis, reactions that can take hundreds of years to complete in the uncatalyzed "real world," occur in seconds in the presence of a catalyst. Chemical catalysts, such as platinum, can speed reactions, but enzymes (which are simply super-catalysts with a "twist," as we shall see) put chemical catalysts to shame (Figure 4.1). To understand enzymatic catalysis, it is necessary first to understand energy. Chemical reactions follow the universal trend of moving towards lower energy, but they often have a barrier in place that must be overcome. The secret to catalytic action is reducing the magnitude of that barrier.

Enzyme	Nonenzymatic Half-Life	Uncatalyzed Rate (k_{un} s^{-1})	Catalyzed Rate (k_{cat} s^{-1})	Rate Enhancement (k_{cat} s^{-1}/k_{un} s^{-1})
OMP decarboxylase	78,000,000 years	28×10^{-16}	39	1.4×10^{17}
Staphylococcal nuclease	130,000 years	1.7×10^{-13}	95	5.6×10^{14}
Carboxypeptidase A	7.3 years	3.0×10^{-9}	578	1.9×10^{11}
Ketosteroid isomerase	7 weeks	1.7×10^{-7}	66,000	3.9×10^{11}
Triose phosphate isomerase	1.9 days	4.3×10^{-6}	4,300	1.0×10^{9}
Chorismate mutase	7.4 hours	2.6×10^{-5}	50	1.9×10^{6}
Carbonic anhydrase	5 seconds	1.3×10^{-1}	1×10^{6}	7.7×10^{6}

Abbreviations:
OMP - Orotine monophosphate
AMP - Adenosine monophosphate

Figure 4.1 - Rate enhancement for several enzymes Image by Aleia Kim

Before discussing enzymes, it is appropriate to pause and discuss an important concept relating to chemical/biochemical reactions. That concept is *equilibrium* and it is very often misunderstood. The "equi" part of the word relates to equal, as one might expect, but it does not relate to absolute concentrations. What happens when a biochemical reaction is at equilibrium is that the concentrations of reactants and products do not change over time. This does not mean that the reactions have stopped. Remember that reactions are reversible, so there is a forward reaction and a reverse reaction: if you had 8 molecules of A, and 4 of B at the beginning, and 2 molecules of A were converted to B, while 2 molecules of B were simultaneously converted back to A, the number of molecules of A and B remain unchanged, i.e., the reaction is at equilibrium. However, you will notice that this does not mean that there are equal numbers of A and B molecules.

Concentration Matters

So, contrary to the perceptions of many students, the concentrations of products and reactants are not equal at equilibrium, unless the ΔG°' for a reaction is zero, because when this is the case,

$$\Delta G = \ln\left(\frac{[\text{Products}]}{[\text{Reactants}]}\right) \tag{7.1.1}$$

since the ΔG°' is zero. Because ΔG itself is zero at equilibrium, then

$$[Products] = [Reactants]. \tag{7.1.2}$$

This is the only circumstance where

$$[Products] = [Reactants] \tag{7.1.3}$$

at equilibrium. Reiterating, at equilibrium, the concentrations of reactant and product do not change over time. That is, for a reaction $A \rightleftharpoons B[A]$ at time zero when equilibrium is reached, $[A]_{T_0}$, will be the same 5 minutes later (assuming A and B are

chemically stable). Thus,

$$[A]_{T_0} = [A]_{T+5} \tag{7.1.4}$$

Similarly,

$$[B]_{T_0} = [B]_{T+5} \tag{7.1.5}$$

For that matter, at any amount of time X after equilibrium has been reached,

$$[A]T0 = [A]T + 5 = [A]TX \tag{7.1.6}$$

and

$$[B]T0 = [B]T + 5 = [B]TX \tag{7.1.7}$$

However, unless ΔG°' = 0, it is wrong to say [A]T0 = [B]T0 As we study biochemical reactions and reaction rates, it is important to remember that 1) reactions do not generally start at equilibrium; 2) all reactions move in the direction of equilibrium; and 3) reactions in cells behave just like those in test tubes - they do not begin at equilibrium, but they move towards it. Dynamic reactions The reactions occurring in cells, though, are very dynamic and complex. In a test tube, they can be studied one at a time. In cells, the product of one reaction is often the substrate for another one. Reactions in cells are interconnected in this way, giving rise to what are called metabolic pathways. There are, in fact, thousands of different interconnected reactions going on continuously in cells.

Attempts to study a single reaction in the chaos of a cell is daunting to say the least. For this reason, biochemists isolate enzymes from cells and study reactions individually. It is with this in mind that we begin our consideration of the phenomenon of catalysis by describing, first, the way in which enzymes work.

Activation energy

Figure 4.2 schematically depicts the energy changes that occur during the progression of a simple reaction. In order for the reaction to proceed, an activation energy must be overcome in order for the reaction to occur.

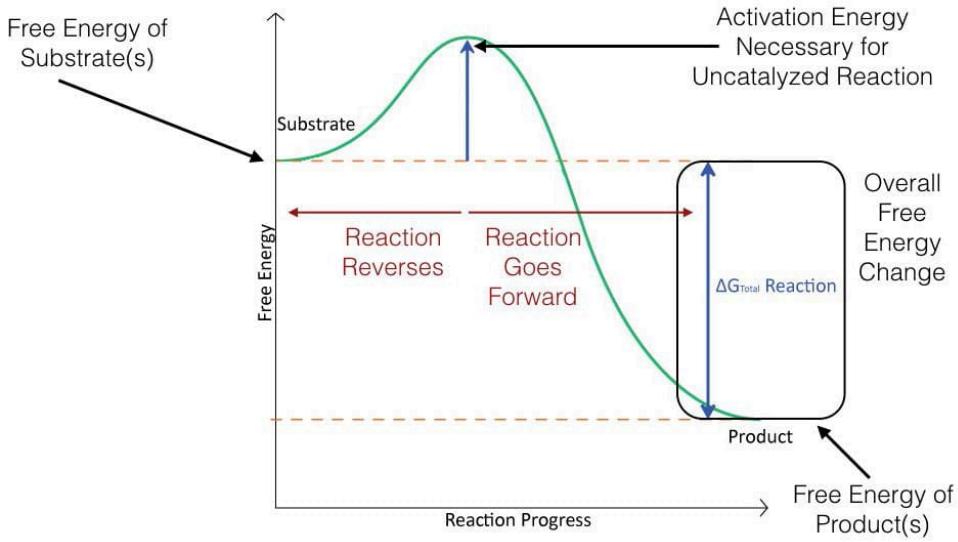


Figure 4.2 - Energy changes during the course of an uncatalyzed reaction. Image by Aleia Kim

In Figure 4.3, the activation energy for a catalyzed reaction is overlaid. As you can see, the reactants start at the same energy level for both catalyzed and uncatalyzed reactions and that the products end at the same energy for both as well. The catalyzed reaction, however, has a lower energy of activation (dotted line) than the uncatalyzed reaction. This is the secret to catalysis - overall ΔG for a reaction does NOT change with catalysis, but the activation energy is lowered.

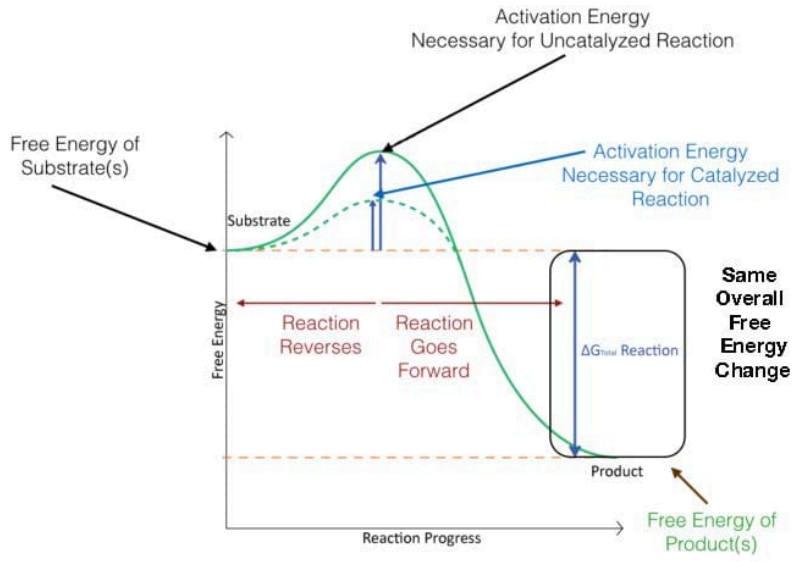


Figure 4.3 - Energy changes during the course of an uncatalyzed reaction (solid green line) and a catalyzed reaction (dotted green line). Image by Aleia Kim

Reversibility

The extent to which reactions will proceed forward is a function of the size of the energy difference between the product and reactant states. The lower the energy of the products compared to the reactants, the larger the percentage of molecules that will be present as products at equilibrium. It is worth noting that since an enzyme lowers the activation energy for a reaction that it can speed the reversal of a reaction just as it speeds a reaction in the forward direction. At equilibrium, of course, no change in concentration of reactants and products occurs. Thus, enzymes speed the time required to reach equilibrium, but do not affect the balance of products and reactants at equilibrium.

Exceptions

The reversibility of enzymatic reactions is an important consideration for equilibrium, the measurement of enzyme kinetics, for Gibbs free energy, for metabolic pathways, and for physiology. There are some minor exceptions to the reversibility of reactions, though. They are related to the disappearance of a substrate or product of a reaction. Consider the first reaction below which is catalyzed by the enzyme carbonic anhydrase:

$$CO_2 + H_2O \rightleftharpoons H_2CO_3 \rightleftharpoons HCO_3^- + H^+ \tag{7.1.8}$$

In the forward direction, carbonic acid is produced from water and carbon dioxide. It can either remain intact in the solution or ionize to produce bicarbonate ion and a proton. In the reverse direction, water and carbon dioxide are produced. Carbon dioxide, of course, is a gas and can leave the solution and escape.

When reaction molecules are removed, as they would be if carbon dioxide escaped, the reaction is pulled in the direction of the molecule being lost and reversal cannot occur unless the missing molecule is replaced. In the second reaction occurring on the right, carbonic acid (H2CO3) is “removed” by ionization. This too would limit the reaction going back to carbon dioxide in water. This last type of “removal” is what occurs in metabolic pathways. In this case, the product of one reaction (carbonic acid) is the substrate for the next (formation of bicarbonate and a proton).

In the metabolic pathway of glycolysis, ten reactions are connected in this manner and reversing the process is much more complicated than if just one reaction was being considered.

General mechanisms of action

As noted above, enzymatically catalyzed reactions are orders of magnitude faster than uncatalyzed and chemical-catalyzed

reactions. The secret of their success lies in a fundamental difference in their mechanisms of action.

Every chemistry student has been taught that a catalyst speeds a reaction without being consumed by it. In other words, the catalyst ends up after a reaction just the way it started so it can catalyze other reactions, as well. Enzymes share this property, but in the middle, during the catalytic action, an enzyme is transiently changed. In fact, it is the ability of an enzyme to change that leads to its incredible efficiency as a catalyst.

Changes

These changes may be subtle electronic ones, more significant covalent modifications, or structural changes arising from the flexibility inherent in enzymes, but not present in chemical catalysts. Flexibility allows movement and movement facilitates alteration of electronic environments necessary for catalysis. Enzymes are, thus, much more efficient than rigid chemical catalysts as a result of their abilities to facilitate the changes necessary to optimize the catalytic process.

Substrate binding

Another important difference between the mechanism of action of an enzyme and a chemical catalyst is that an enzyme has binding sites that not only 'grab' the substrate (molecule involved in the reaction being catalyzed), but also place it in a position to be electronically induced to react, either within itself or with another substrate.

The enzyme itself may play a role in the electronic induction or the induction may occur as a result of substrates being placed in very close proximity to each other. Chemical catalysts have no such ability to bind substrates and are dependent upon them colliding in the right orientation at or near their surfaces.

Active site

Reactions in an enzyme are catalyzed at a specific location within it known as the 'active site'. Substrates bind at the active site and are oriented to provide access for the relevant portion of the molecule to the electronic environment of the enzyme where catalysis occurs.

Enzyme flexibility

As mentioned earlier, a difference between an enzyme and a chemical catalyst is that an enzyme is flexible. Its slight changes in shape (often arising from the binding of the substrate itself) help to optimally position substrates for reaction after they bind.

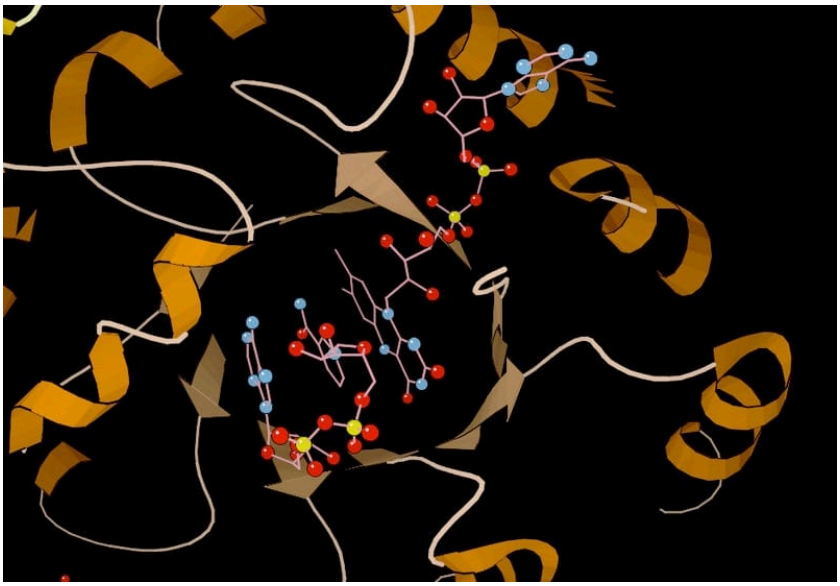


Figure 4.4 - Substrate binding by methylenetetrahydrofolate reductase

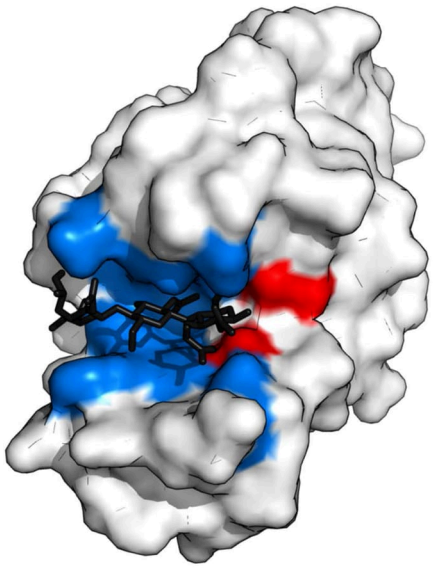


Figure 4.5 - Lysozyme with substrate binding site (blue), active site (red) and bound substrate (black). Wikipedia

Induced fit

These changes in shape are explained, in part, by Koshland's Induced Fit Model of Catalysis (Figure 4.6), which illustrates that not only do enzymes change substrates, but that substrates also transiently change enzyme structure. At the end of the catalysis, the enzyme is returned to its original state. Koshland's model is in contrast to the Fischer Lock and Key model, which says simply that an enzyme has a fixed shape that is perfectly matched for binding its substrate(s). Enzyme flexibility also is important for control of enzyme activity. Enzymes alternate between the T (tight) state, which is a lower activity state and the R (relaxed) state, which has greater activity.

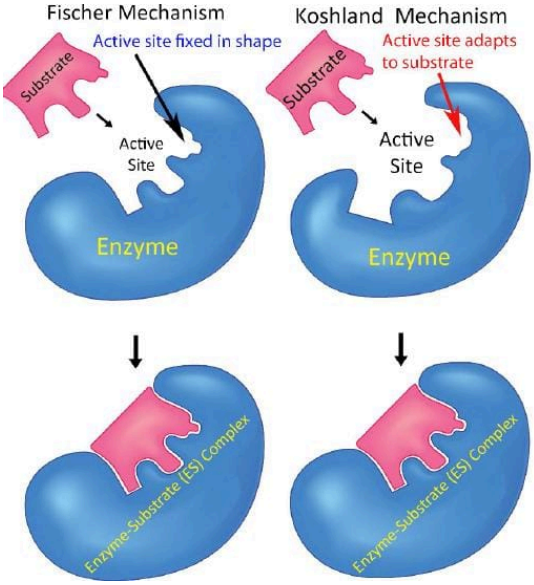


Figure 4.6 - Fischer's lock and key model (left) Vs. Koshland's induced fit model (right). Image by Aleia Kim

Induced Fit

The Koshland Induced Fit model of catalysis postulates that enzymes are flexible and change shape on binding substrate. Changes in shape help to 1) aid binding of additional substrates in reactions involving more than one substrate and/or 2) facilitate formation of an electronic environment in the enzyme that favors catalysis. This model is in contrast to the Fischer Lock and Key Model of catalysis which considers enzymes as having pre-formed substrate binding sites.

Ordered binding

The Koshland model is consistent with multi-substrate binding enzymes that exhibit ordered binding of substrates. For these systems, binding of the first substrate induces structural changes in the enzyme necessary for binding the second substrate.

There is considerable experimental evidence supporting the Koshland model. Hexokinase, for example, is one of many enzymes known to undergo significant structural alteration after binding of substrate. In this case, the two substrates are brought into very close proximity by the induced fit and catalysis is made possible as a result.

Reaction types

Enzymes that catalyze reactions involving more than one substrate, such as

$$A + B \rightleftharpoons C + D \tag{7.1.9}$$

can act in two different ways. Enzymatic reactions can be of several types, as shown in Figure 4.7. In one mechanism, called sequential reactions, at some point in the reaction, both substrates will be bound to the enzyme. There are, in turn, two different ways in which this can occur - random and ordered.

Figure 4.7 - Categories of enzymatic reactions

Types of Reactions	
Single Substrate - Single Product	A ⇄ B
Single Substrate - Multiple Products	A ⇄ B + C
Multiple Substrates - Single Products	A + B ⇄ C
Multiple Substrates - Multiple Products	A + B ⇄ C + D

Consider lactate dehydrogenase, which catalyzes the reaction below:

$$NADH + Pyruvate \rightarrow Lactate + NAD^{+} \tag{7.1.10}$$

This enzyme requires that NADH must bind prior to the binding of pyruvate. As noted earlier, this is consistent with an induced fit model of catalysis. In this case, binding of the NADH changes the enzyme shape/environment so that pyruvate can bind and without binding of NADH, the substrate cannot access the pyruvate binding site. This type of multiple substrate reaction is called **sequential ordered binding**, because the binding of substrates must occur in the right order for the reaction to proceed.

Random binding

A second mechanism of binding/catalysis is exhibited by creatine kinase which catalyzes the following reaction:

$$Creatine + ATP \rightarrow \text{Creatine phosphate} + ADP \tag{7.1.11}$$

For this enzyme, substrates can bind to it in any order. Creatine kinase displays sequential random binding. It is worth mentioning that random binding is not inconsistent with Koshland's induced fit model. Rather, random binding simply means that the enzyme's induced fit doesn't affect substrate binding sites and involves other parts of the enzyme. In summary, sequential binding can occur as ordered binding or as random binding.

Double displacement reactions

Not all enzymes that catalyze multi-substrate reactions, though, bind A and B by the sequential mechanisms above. This other category of enzyme includes those that exhibit what are called "ping-pong" (or double displacement) mechanisms. In these enzymes, the enzyme functions as both a catalyst and a carrier of a group between individually bound substrates. Examples of this type of enzyme include the class of enzymes known as transaminases. A general form of the reactions catalyzed by these enzymes is shown in Figure 4.8.

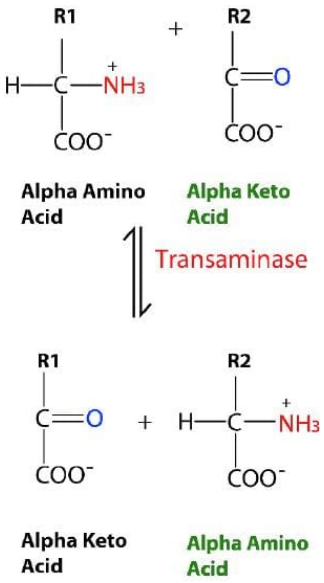


Figure 4.8 - Double displacement reaction mechanism of a transaminase

In reversible transaminase reactions, an oxygen and an amine are swapping between the molecules. It can be represented as follows (where N is the amine and O is the oxygen).

$$A{=}O + C{=}N \rightleftarrows B{-}N + D{=}O$$

This reaction occurs not as one transfer reaction swapping the N and the O, but rather as a set of two half-reactions. In this case, the enzyme is a both donor and a carrier of the group being swapped. The first half-reaction goes as follows

$$A{=}O + Enz{-}N \rightleftarrows B{-}N + Enz{=}O$$

Next a second half-reaction goes as

$$C{-}N + Enz{=}O \rightleftarrows D{=}O + Enz{=}N$$

The sum of these half-reactions then is

$$A{=}O + C{=}N \rightleftarrows B{-}N + D{=}O$$

Note that at no time did the enzyme bind both A and C simultaneously. It is also important to recognize that the enzyme existed in two states - Enz=O and Enz-N. The shuffling of the enzyme between these two states is what gives rise to the ping-pong name of this mechanism - it literally goes back and forth like a ping-pong ball in a table tennis match.

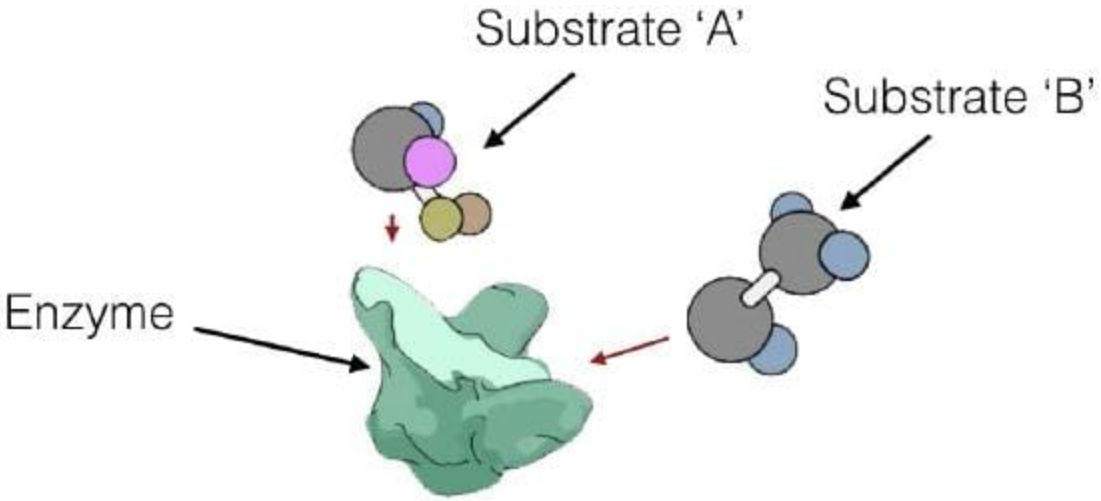


Figure 4.9 - Binding of substrates (A+B) to free enzyme

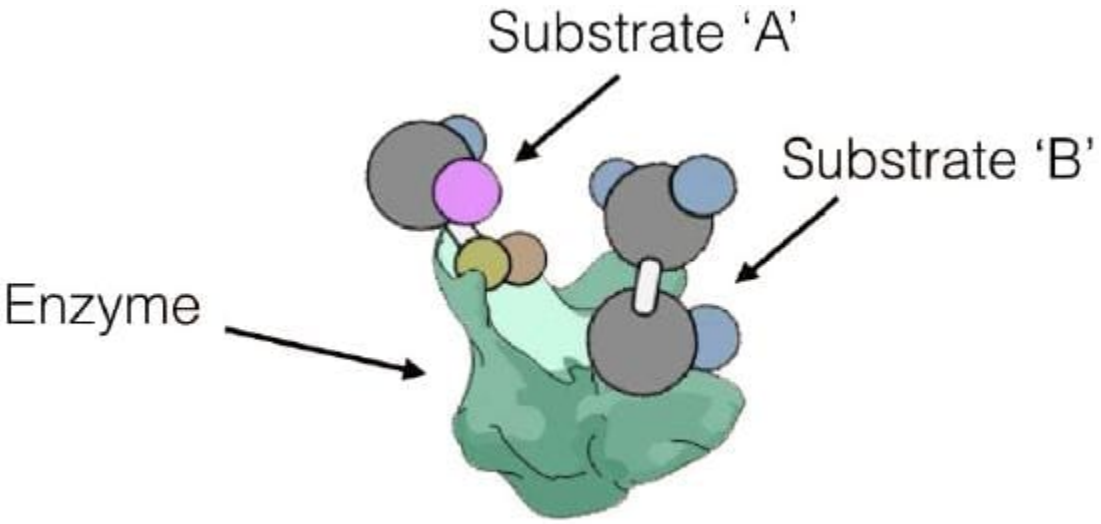


Figure 4.10 ES complex formation

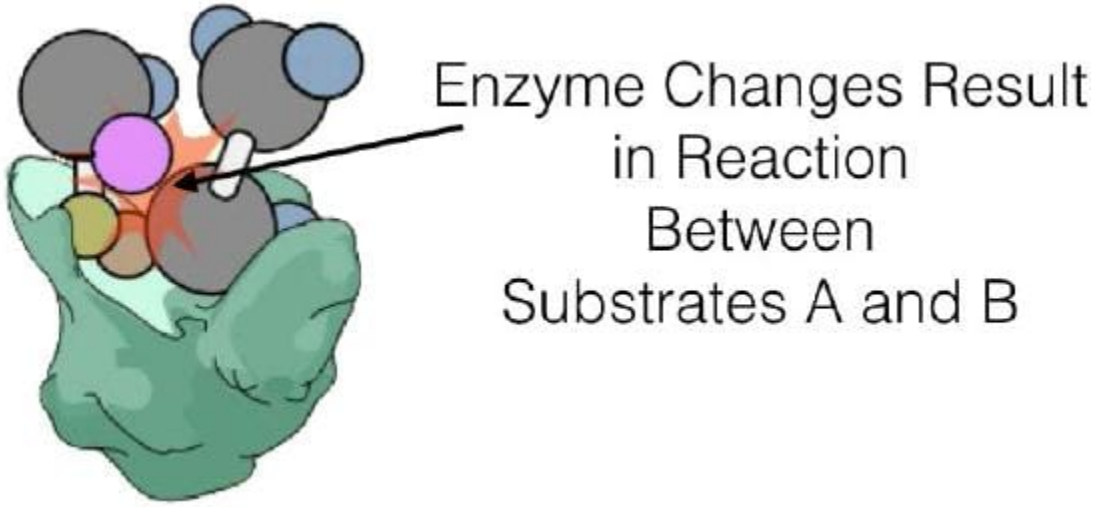


Figure 4.11 - ES* complex - The reaction occurs.

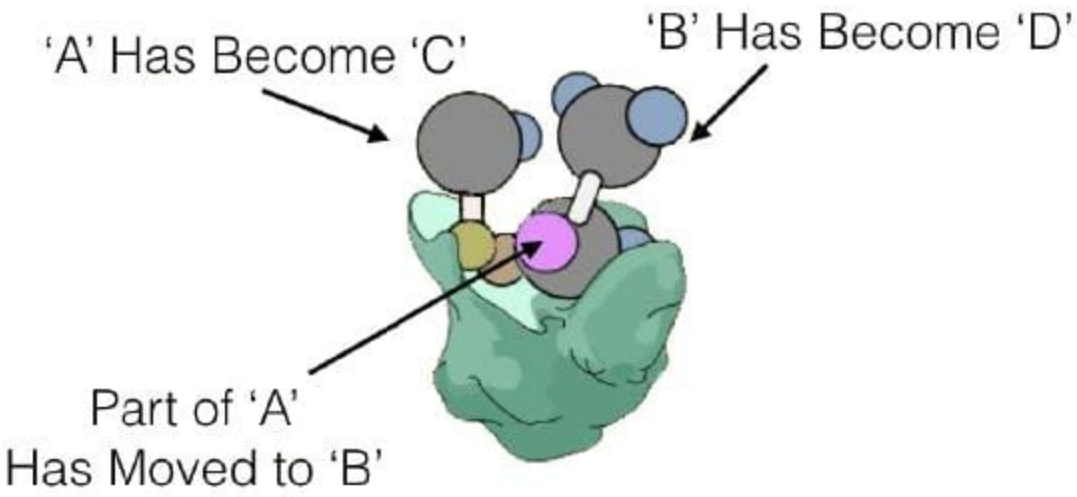


Figure 4.12 - EP complex - the reaction is complete.

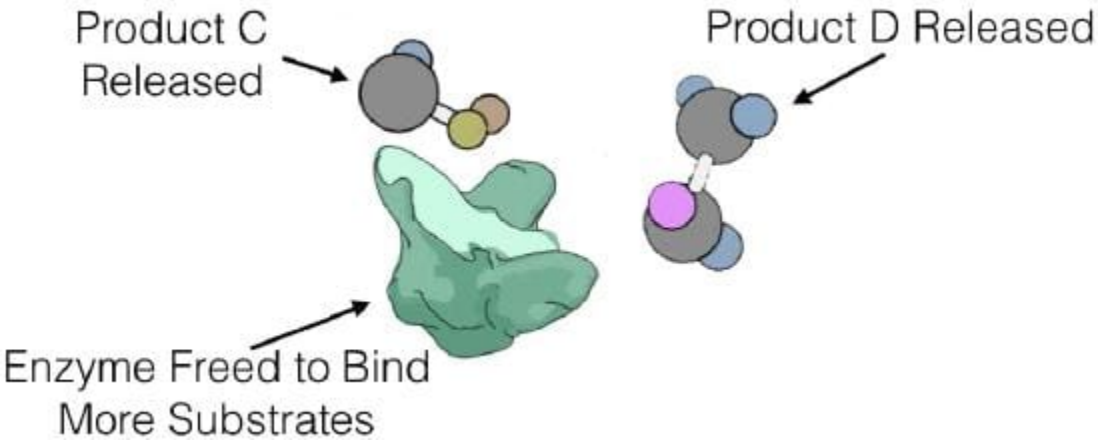


Figure 4.13 - E + P - the products are released

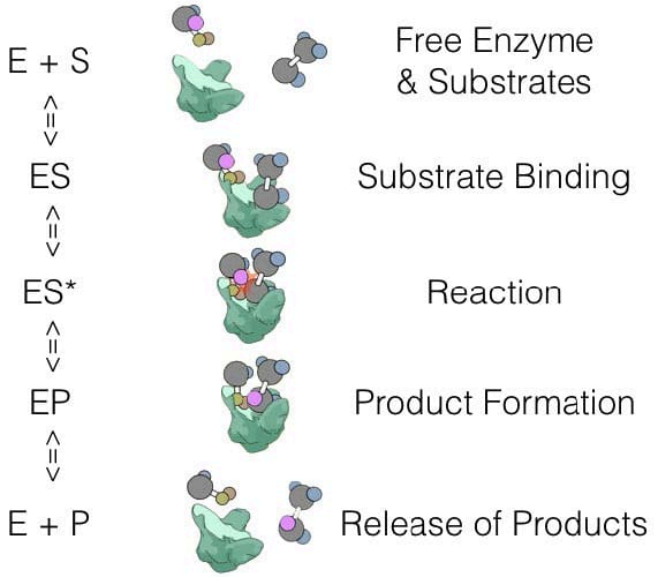


Figure 4.14 - Summary

Enzyme kinetics

To understand how an enzyme enhances the rate of a reaction, we must understand enzyme kinetics. We present a model

here proposed by Leonor Michaelis and Maud Menten. In order to understand the model, it is necessary to understand a few parameters.

First, we describe a reaction in simple terms proceeding as follows

E + S ⇄ ES -> E + P

where E is enzyme, S is substrate, and P is product. In this scheme, ES is the Enzyme-Substrate complex, which is simply the enzyme bound to its substrate.

We could define the ES state a bit further with

E + S ⇄ ES -> ES* -> EP -> E + P

where ES* is the activated state and EP is the enzyme-product complex before release of the product.

The first consideration we have is velocity. The velocity of a reaction is the rate of creation of product over time, measured as the concentration of product per time. The time is a critical consideration when measuring velocity. In a closed system (in which an enzyme operates), all reactions will advance towards equilibrium. Enzymatically catalyzed reactions are no different in the end result from non-enzymatic reactions, except that they get to equilibrium faster.

Equilibrium

At equilibrium, the ratio of product to reactant does not change. That is a property of equilibrium. Since the system is closed, the concentration of product over time will not change. The velocity will thus be zero under these conditions and we will have learned nothing about the reaction if we wait too long to study it.

Velocity

Enzyme	Turnover Number (per second)
Carbonic anhydrase	600,000
3-Ketoesteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymostrypsin	100
DNA Polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Figure 4.22 - Kcat/Km values for perfect enzymes. Image by Aleia Kim

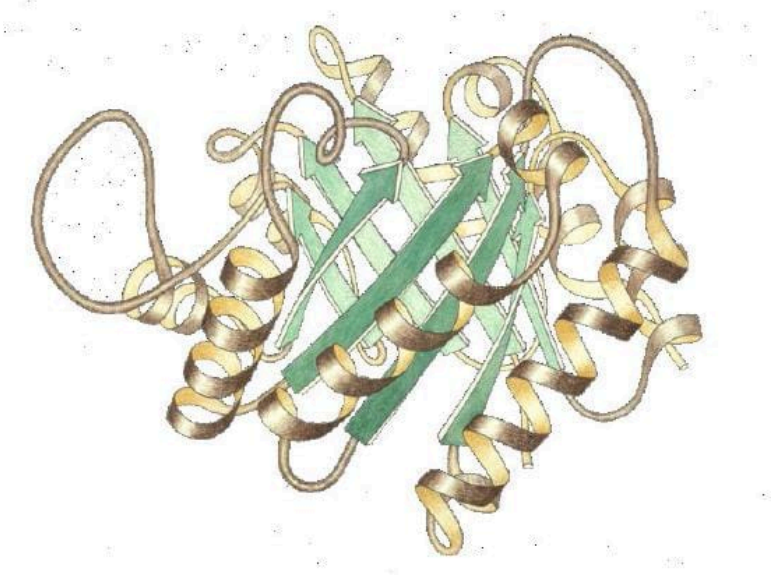


Figure 4.23 - Triose phosphate isomerase - A perfect enzyme. Wikipedia

Consequently, in Michaelis-Menten kinetics, velocity is measured as initial velocity (V0). This is accomplished by measuring the rate of formation of product early in the reaction before equilibrium is established and under these conditions, there is very little if any of the reverse reaction occurring.

The other two assumptions are related. First, we use conditions where there is much more substrate than enzyme. This makes sense. If the substrate is not in great excess, then the enzyme's conversion of substrate to product will occur much faster than the enzyme can bind substrate.

Waiting for substrate

Thus, the enzyme would "wait" for substrate to bind if there were not sufficient amounts of it to bind to the enzyme in a timely fashion (when substrate concentration is low). This would not give an accurate measure of velocity, since the enzyme would be inactive a good deal of the time. Because of this, we assume saturation of the enzyme with substrate will give a maximal velocity of the reaction.

Steady state

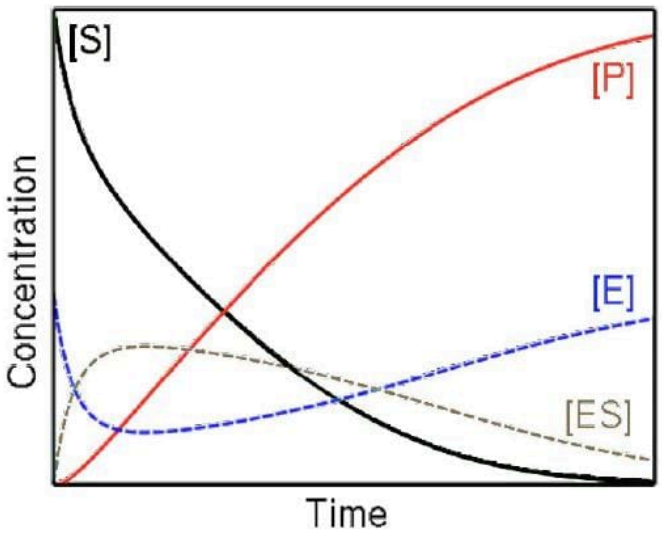


Figure 4.15 - Concentration of product (P), substrate (S), enzyme (E), and enzyme-substrate complex (ES) versus time for an enzymatic reaction

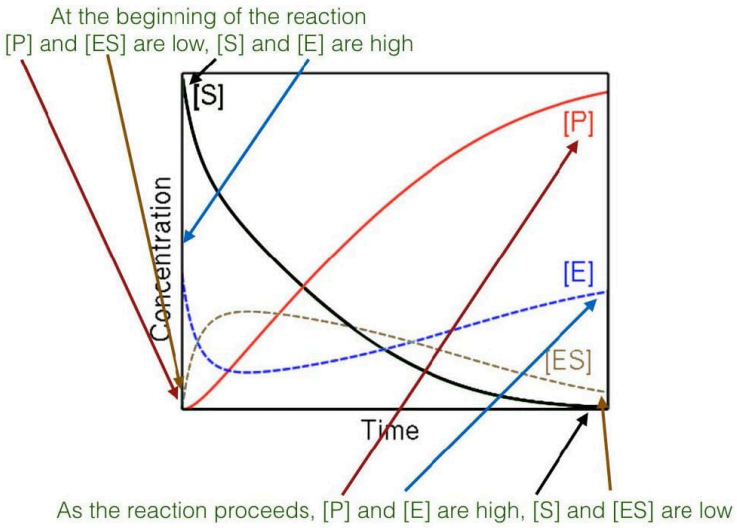


Figure 4.16 - Change in concentration of reaction materials over time

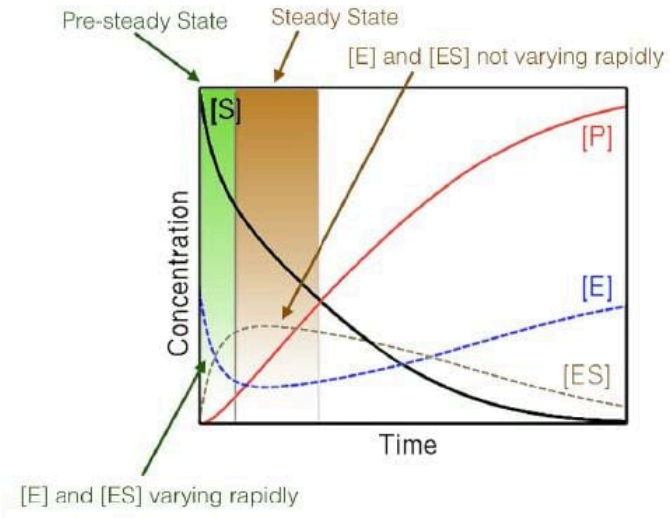


Figure 4.17 - Steady state versus non-steady state conditions

Last, the high concentration of substrate combined with measuring initial conditions results in studying reactions that are under so-called steady state conditions (Figure 4.17). When steady state occurs, the concentration of the ES complex over time is not significantly changing during the period of analysis.

Reiterating, the three assumptions for Michaelis-Menten kinetics are

1. Measurement of initial velocity of a reaction
2. Substrate in great excess compared to enzyme
3. Reaction conditions occurring under steady state

Experimental considerations

Now we turn our attention to how studies of the kinetic properties of an enzyme are conducted. To perform an analysis, one would do the following experiment - 20 different tubes would be set up with enzyme buffer (to keep the enzyme stable), the same amount of enzyme, and then a different amount of substrate in each tube, ranging from tiny amounts in the first tubes to very large amounts in the last tubes. The reaction would be allowed to proceed for a fixed, short amount of time and then the reaction

would be stopped and the amount of product contained in each tube would be determined.

The initial velocity (V0) of the reaction then would be the concentration of product found in each tube divided by the time that the reaction was allowed to run. Data from the experiment would be plotted on a graph using initial velocity (V0) on the Y-axis and the concentration of substrate on the X-axis, each tube, of course having a unique reaction velocity corresponding to a unique substrate concentration.

For an enzyme following Michaelis-Menten kinetics, a curve like that shown in Figure 4.18 or 4.19 would result. At low concentration of substrate, it is limiting and the enzyme converts it into product as soon as it can bind it. Consequently, at low concentrations of substrate, the rate of increase of [P] is almost linear with [S] (Figure 4.19).

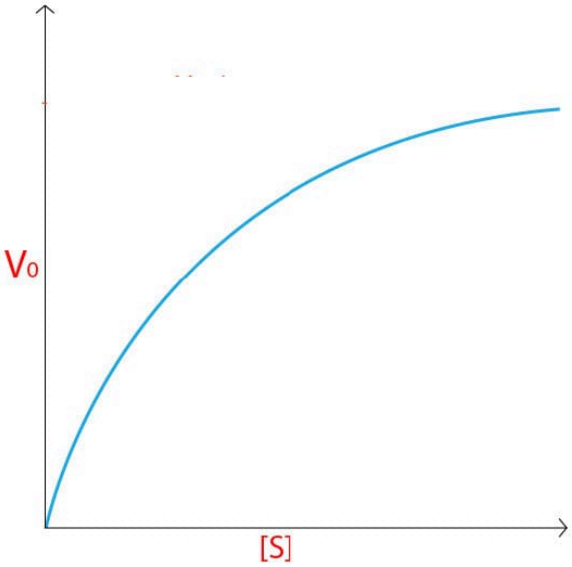


Figure 4.18 - Kinetics of an enzyme obeying Michaelis-Menten kinetics. Image by Aleia Kim

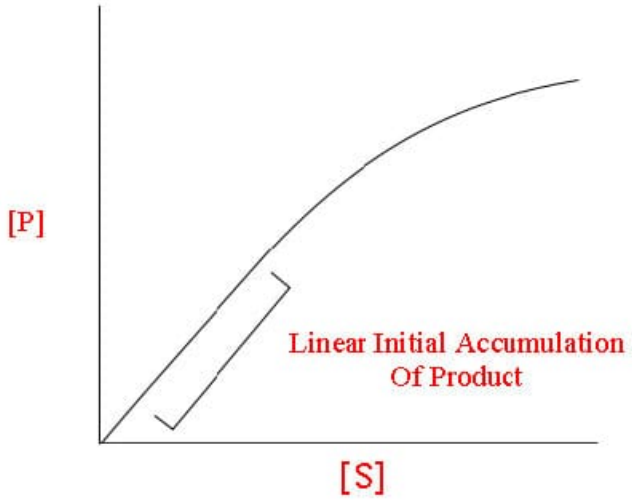


Figure 4.19 - Linear relationship between [P] and [S] at low [S]

Non-linear increase

As the substrate concentration increases, however, the velocity of the reaction in tubes with higher substrate concentration ceases to increase linearly and instead begins to flatten out, indicating that as the substrate concentration gets higher and higher, the enzyme has a harder time keeping up to convert the substrate to product.

Saturation

Not surprisingly, when the enzyme becomes completely saturated with substrate, it will not have to wait for substrate to diffuse to it and will therefore be operating at maximum velocity.

For an enzyme following Michaelis-Menten kinetics will have its velocity (v) at any given substrate concentration given by the following equation:

$$v = \frac{V_{\max}[S]}{K_m + [S]}$$

V_{max}

Two terms in the equation above require explanation. The first is V_{max}. It refers to the maximum velocity of an enzymatic reaction. Maximum velocity for a reaction occurs when an enzyme is saturated with substrate. Saturation is important because it means (per the assumption above) that none of the enzyme molecules are "waiting" for substrate after a product is released. Saturation ensures that another substrate is always instantly available. The unit of V_{max} is concentration of product per time = [P]/time.

On a plot of initial velocity versus substrate concentration (V0 vs. [S]), V_{max} is the value on the Y axis that the curve asymptotically approaches (dotted line in Figure 4.20). It should be noted that the value of V_{max} depends on the amount of enzyme used in a reaction. If you double the amount of enzyme used, you will double the V_{max}. If one wanted to compare the velocities of two different enzymes, it would be necessary to use the same amounts of enzyme in the reaction each one catalyzes.

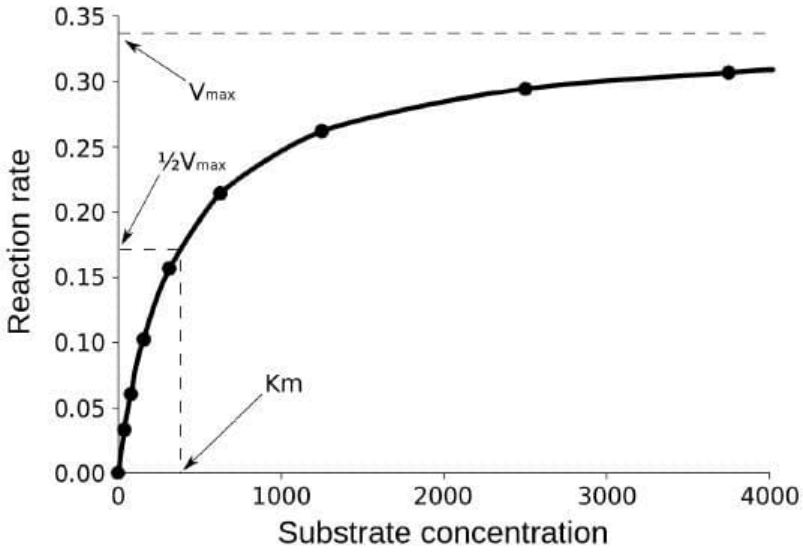


Figure 4.20 - V_{max}, $V_{max}/2$, and Km

Km

The second term is Km (also known as Ks). Referred to as the Michaelis constant, Km is the substrate concentration that causes the enzyme to work at half of maximum velocity (Vmax/2). What it measures, in simple terms, is the affinity an enzyme has for its substrate. The value of Km is inversely related to the affinity of the enzyme for its substrate. Enzymes with a high Km value will have a lower affinity for their substrate (will take more substrate to get to Vmax/2) whereas those with a low Km will have high affinity and take less substrate to get to Vmax/2. The unit of Km is concentration.

Affinities of enzymes for substrates vary considerably, so knowing Km helps us to understand how well an enzyme is suited to the substrate being used. Measurement of Km depends on the measurement of Vmax.

Common mistake

A common mistake students make in describing Vmax is saying that Km = Vmax /2. This is, of course, not true. Km is a substrate concentration and is the amount of substrate it takes for an enzyme to reach Vmax /2. On the other hand Vmax /2 is a velocity and a velocity certainly cannot equal a concentration.

Kcat

It is desirable to have a measure of velocity that is independent of enzyme concentration. Remember that Vmax depended on the amount of enzyme used. For this, we use the Kcat, also known as the turnover number. Kcat is a number that requires one to first determine Vmax for an enzyme and then divide the Vmax by the concentration of enzyme used to determine Vmax. Thus,

Kcat = Vmax /[Enzyme]

Since Vmax has units of concentration per time and [Enzyme] has units of concentration, the units on Kcat are time-1. While that might seem unintuitive, it means that the value of Kcat is the number of molecules of product made by each molecule of enzyme in the time given. So, a Kcat value of 1000/sec means each enzyme molecule in the reaction at Vmax is producing 1000 molecules of product per second. Note that since Kcat is a calculated value, it cannot be read from a V vs [S] graph as Vmax and Km can.

Enzyme	Turnover Number (per second)
Carbonic anhydrase	600,000
3-Ketoesteroid isomerase	280,000
Acetylcholinesterase	25,000
Penicillinase	2,000
Lactate dehydrogenase	1,000
Chymostrypsin	100
DNA Polymerase I	15
Tryptophan synthetase	2
Lysozyme	0.5

Figure 4.21 - Kcat (turnover number) values for several enzymes. Image by Aleia Kim

Amazing Kcat values

A Kcat value of 1000 molecules of product per enzyme per second might seem like a high value, but there are enzymes known (carbonic anhydrase, for example) that have a Kcat value of over 600,000/second (Figure 4.21). This astonishing value illustrates clearly why enzymes seem almost magical in their action. In contrast to V_{max}, which varies with the amount of enzyme used, Kcat is a constant for an enzyme under given conditions.

As seen earlier, enzymes that follow Michaelis-Menten kinetics produce hyperbolic plots of Velocity (V0) versus Substrate Concentration [S] (Figure 4.18). Not all enzymes, though, follow Michaelis-Menten kinetics. Many enzymes have multiple protein subunits and these sometimes interact differently upon binding of a substrate or an external molecule. See ATCase (HERE) for an example.

Perfect enzymes

Now, if we think about what an ideal enzyme might be, it would be one that has a very high velocity and a very high affinity for

its substrate. That is, it wouldn't take much substrate to get to Vmax/2 and the Kcat would be very high. Such enzymes would have values of Kcat / Km that are maximum. Interestingly, there are several enzymes that have this property and their maximal Kcat / Km values are all approximately the same. Such enzymes are referred to as being "perfect" because they have reached the maximum possible value.

Enzyme	K_{Cat} / K_M ($s^{-1}M^{-1}$)
Acetylcholinesterase	1.6×10^8
Carbonic anhydrase	8.3×10^7
Catalase	4.0×10^7
Crotonase	2.8×10^8
Fumarase	1.6×10^8
Triose phosphate isomerase	2.4×10^8
β-Lactamase	1.0×10^8
Superoxide dismutase	7.0×10^9

Figure 4.22 - Kcat/Km values for perfect enzymes. Image by Aleia Kim

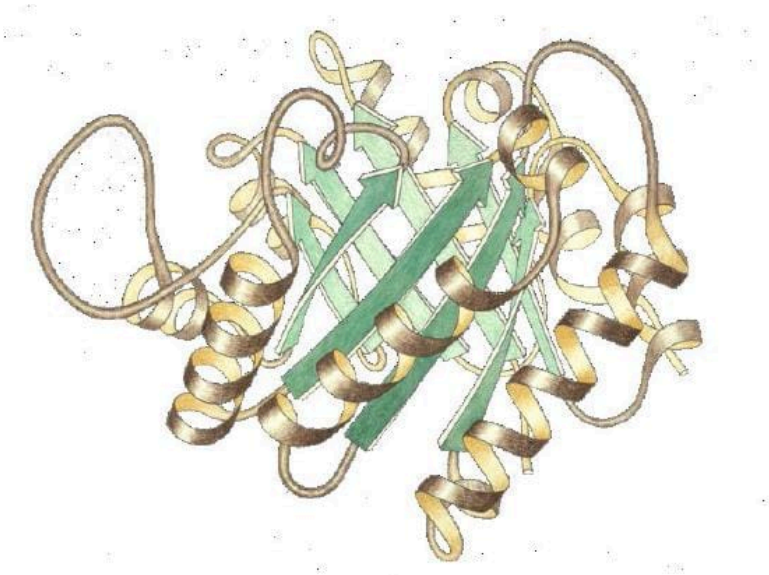


Figure 4.23 - Triose phosphate isomerase - A perfect enzyme. Wikipedia

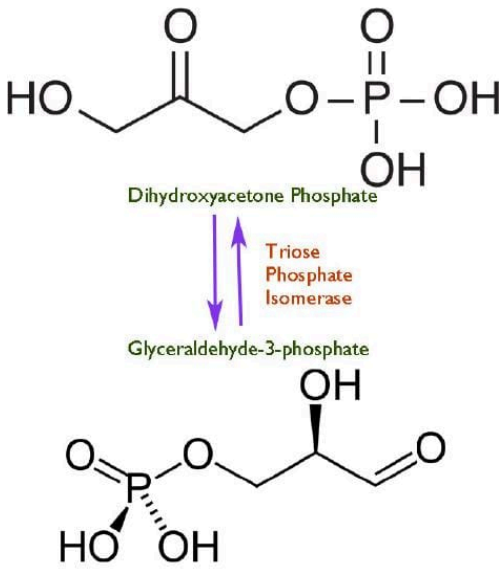


Figure 4.24 - Triose phosphate isomerase-catalyzed reaction

Diffusion limitation

Why should there be a maximum possible value of Kcat / Km? The answer is that movement of substrate to the enzyme becomes the limiting factor for perfect enzymes. Movement of substrate by diffusion in water has a fixed rate at any temperature and that limitation ultimately determines the maximum speed an enzyme can catalyze at. In a macroscopic world analogy, factories can't make products faster than suppliers can deliver materials. It is safe to say for a perfect enzyme that the only speed limit it has is the rate of substrate diffusion in water.

Given the "magic" of enzymes alluded to earlier, it might seem that all enzymes should have evolved to be "perfect." There are very good reasons why most of them have not.

Speed

Speed is a dangerous thing. The faster a reaction proceeds in catalysis by an enzyme, the harder it is to control. As we all know from learning to drive, speeding causes accidents. Just as drivers need to have speed limits for operating automobiles, so too must cells exert some control on the 'throttle' of their enzymes. In view of this, one might wonder then why any cells have evolved any enzymes to perfection. There is no single answer to the question, but a common one is illustrated by triose phosphate isomerase, which catalyzes a reaction in glycolysis shown in Figure 4.24.

The enzyme appears to have evolved this ability because at lower velocities, there is breakdown of an unstable enediol intermediate that then readily forms methyl glyoxal, a cytotoxic compound (Figure 4.25). Speeding up the reaction provides less opportunity for the unstable intermediate to accumulate and fewer undesirable byproducts to be made.

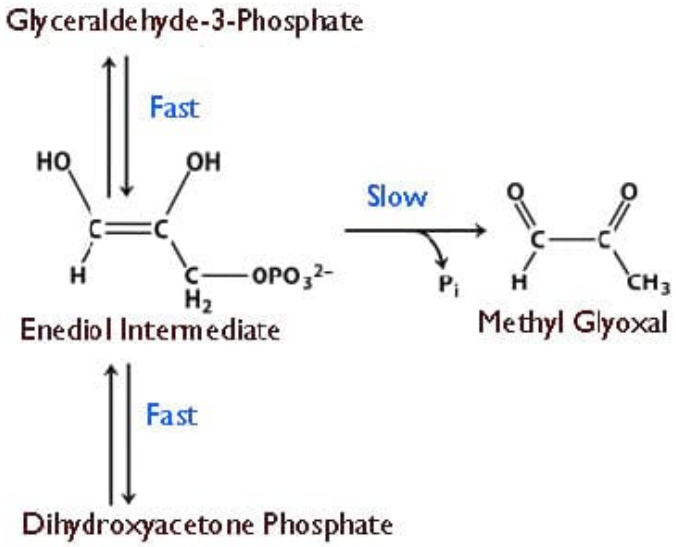


Figure 4.25 - A high speed reaction avoids production of methyl glyoxal. Image by Aleia Kim

Dissociation constant

In studying proteins and ligands, it is important to understand the “tightness” with which a protein (P) “holds onto” a ligand (L). This is measured with the dissociation constant (K_d). The formation of a ligand-protein complex LP occurs as

$$L + P \rightleftharpoons LP \tag{7.1.12}$$

The dissociation of the complex, therefore, is the reverse of this reaction, or

$$LP \rightleftharpoons L + P \tag{7.1.13}$$

so the corresponding dissociation constant is defined as

$$K_d = \frac{[L][P]}{[LP]} \tag{7.1.14}$$

where $[L]$, $[P]$, and $[LP]$ are the molar concentrations of the protein, ligand and the complex when they are joined together.

Smaller values of K_d indicate tight binding, whereas larger values indicate loose binding. The dissociation constant is the inverse of the association constant.

$$K_a = \frac{1}{K_d} \tag{7.1.15}$$

Where multiple molecules bond together, such as

$$J_x K_y \rightleftharpoons xJ + yK \tag{7.1.16}$$

The complex $J_x K_y$ is breaking down into x subunits of J and y subunits of K. The dissociation constant is then defined as

$$K_d = \frac{[J]^x [K]^y}{[J_x K_y]} \tag{7.1.17}$$

where $[J]$, $[K]$, and $[J_x K_y]$ are the concentrations of J, K, and the complex $J_x K_y$, respectively.

Lineweaver-Burk plots

The study of enzyme kinetics is typically the most math intensive component of biochemistry and one of the most daunting

aspects of the subject for many students. Although attempts are made to simplify the mathematical considerations, sometimes they only serve to confuse or frustrate students. Such is the case with modified enzyme plots, such as Lineweaver-Burk (Figure 4.26).

Indeed, when presented by professors as simply another thing to memorize, who can blame students? In reality, both of these plots are aimed at simplifying the determination of parameters, such as K_m and V_{max}. In making either of these modified plots, it is important to recognize that the same data is used as in making a V0 vs. [S] plot. The data are simply manipulated to make the plotting easier.

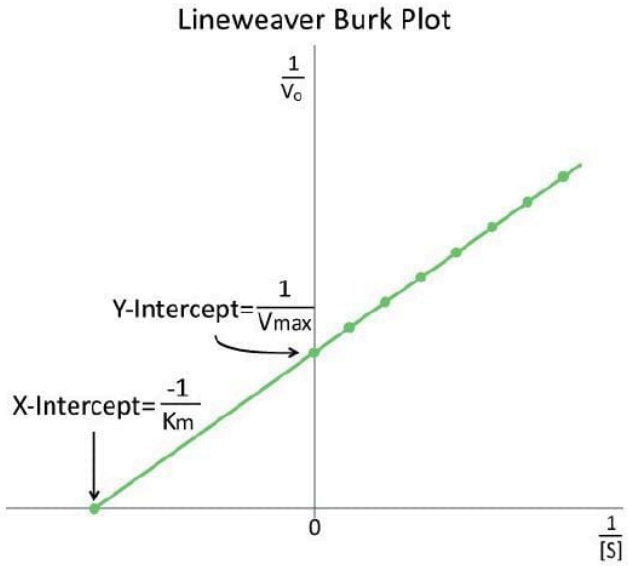


Figure 4.26 - A Lineweaver-Burk plot of $1/V_0$ vs $1/[S]$. Image by Aleia Kim

Double reciprocal

For a LineWeaver-Burk plot, the manipulation is using the reciprocal of the values of both the velocity and the substrate concentration. The inverted values are then plotted on a graph as 1/V0 vs. 1/[S]. Because of these inversions, Lineweaver-Burk plots are commonly referred to as 'double-reciprocal' plots. As can be seen in Figure 4.26, the value of Km on a Lineweaver Burk plot is easily determined as the negative reciprocal of the x-intercept , whereas the Vmax is the inverse of the y-intercept. Other related manipulation of kinetic data include Eadie-Hofstee diagrams, which plots V0 vs V0/[S] and gives Vmax as the Y-axis intercept with the slope of the line being -Km.

Molecularity of reactions

The term molecularity refers to the number of molecules that must come together in order for a reaction to take place. Reactions of the sort of A -> B (where 'A' is the reactant and 'B' is the product) are unimolecular, since A is directly changed into B. The rate of the reaction is related only to the concentration of reactant A. For a bimolecular reaction where A + B ⇄ C the reaction depends on the concentration of both A and B and its rate will be related to the product of the concentration of A and of B.

Coenzymes

Organic molecules that assist enzymes and facilitate catalysis are co-factors called coenzymes. The term co-factor is a broad category usually subdivided into inorganic ions and coenzymes. If the coenzyme is very tightly or covalently bound to the enzyme, it is referred to as a prosthetic group. Enzymes without their co-factors are inactive and referred to as apoenzymes. Enzymes containing all of their co-factors are called holoenzymes.

Cofactor	Enzyme
Coenzyme	
5′-Deoxyadenosyl cobalamin	Methylmalonyl mutase
Biotin	Pyruvate carboxylase
Coenzyme A (CoA)	Acetyl CoA carboxylase
Flavin adenine nucleotide	Monoamine oxidase
Nicotinamide adenine dinucleotide	Lactate dehydrogenase
Pyridoxal phosphate	Glycogen phosphorylase
Tetrahydrofolate	Thymidylate synthase
Thiamine pyrophosphate	Pyruvate dehydrogenase
Metal	
K^+	Propionyl CoA carboxylase
Mg^{2+}	Restriction Endonucleases; Hexokinase
Mn	Superoxide dismutase
Mo	Nitrate reductase
Ni^{2+}	Urease
Se	Glutathione peroxidase
Zn^{2+}	Carbonic anhydrase; Carboxypeptidase

Figure 4.27 - Enzyme cofactors. Image by Aleia Kim

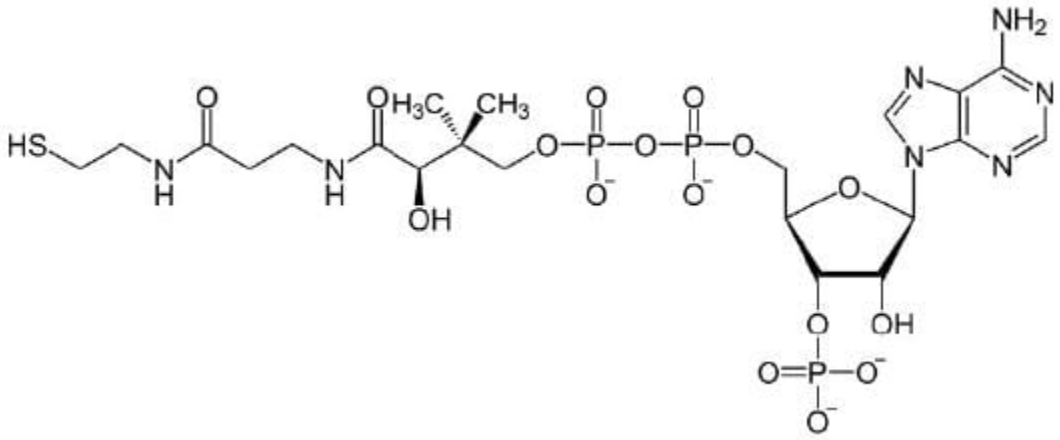


Figure 4.28 - Coenzyme A (CoA)

Pre-steady state kinetic studies

In the study of kinetic rates of enzymatic reactions, time zero is a very critical point. It establishes when the mixing of substrate with enzyme and measurement of formation of product begins. At time zero, there is no product. As shown in Figure 4.29, the appearance of product (on a short time scale) goes through an early burst phase with a steep slope for [product]/time and then changes.

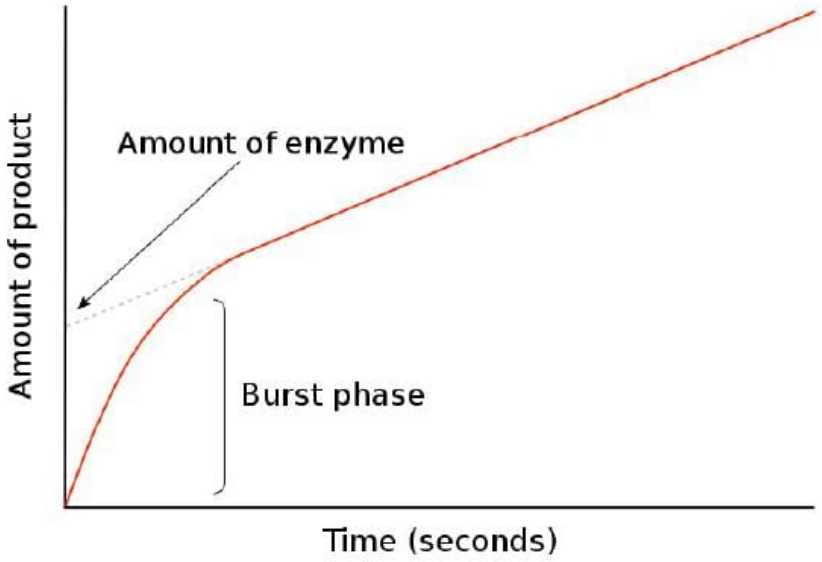


Figure 4.29 - Burst phase of product formation

This change occurs during a critical period in an enzymatic reaction and gives information about the rate of reaction cycles. The duration of the burst phase tells how long a single reaction turnover occurs, whereas the slow of the line post-burst phase tells the amount of “functional” enzyme performing the reaction.

After the burst phase, the slope of the line of the amount of product versus time decreases. This is due to the reaction entering conditions of steady state, used to study Michaelis-Menten kinetics. In steady state conditions, the amount of the enzyme-substrate complex (ES) is relatively constant over time. In simple terms, this occurs when the rate of formation of the ES complex equals the rate of conversion of the substrate to product by the enzyme with release of the product.

Earlier events

Events occurring prior to the conditions of steady state are referred to as pre-steady state. Depending on the enzyme, in as short as a few milliseconds, steady state conditions can be present meaning that if one hopes to study formation of reaction intermediates in pre-steady state, tools for this analysis must work very rapidly. One instrument commonly used for studying pre-steady state kinetics is called a stopped flow instrument.

It loads an enzyme solution and a substrate into separate syringes whose output is pointed into a mixing chamber. The solutions are rapidly mixed and measurements of product concentration begin. With a stopped flow instrument, dead times (time between mixing and detection) can be achieved of as small as 0.3 msec.

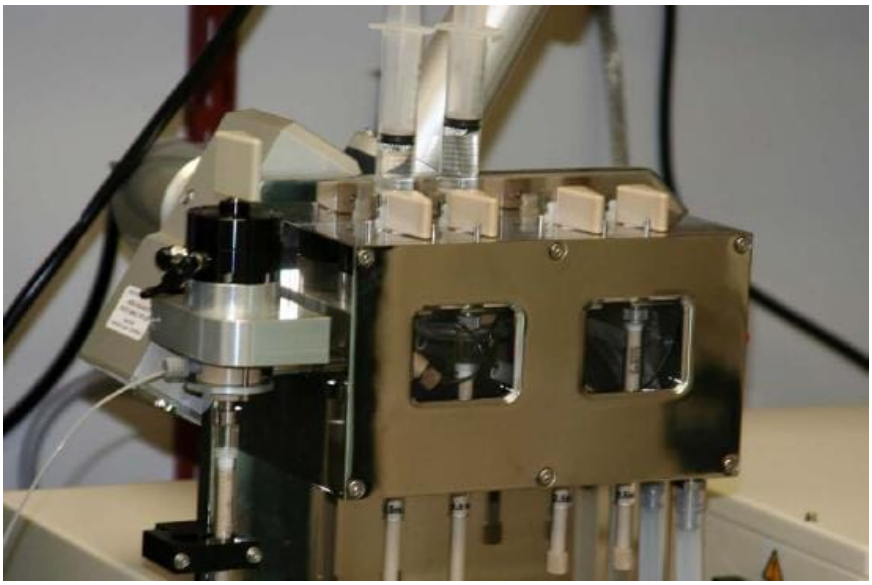
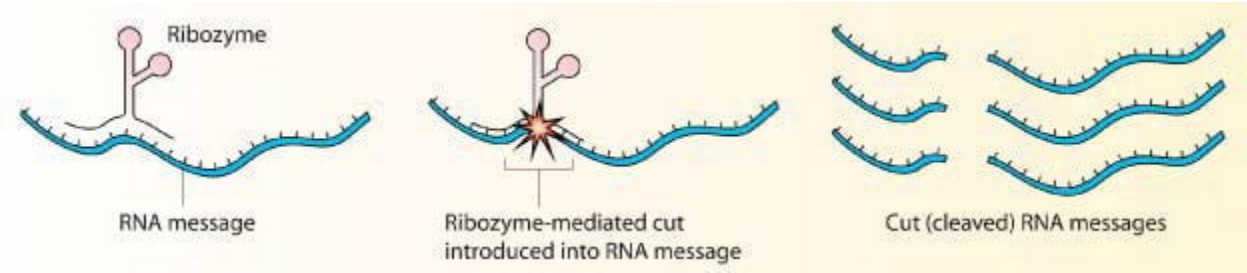


Figure 4.30 - Stopped flow instrument for studying pre-steady state kinetics. Wikipedia

Ribozymes

Proteins do not have a monopoly on acting as biological catalysts. Some RNA molecules are also capable of speeding reactions. The most famous of these molecules was discovered by Tom Cech in the early 1980s Studying excision of an intron in Tetrahymena, Cech was puzzled at his inability to find any proteins catalyzing the process. Ultimately, the catalysis was recognized as coming from the intron itself. It was a self-splicing RNA and since then, many other examples of catalytic RNAs have been found. Catalytic RNA molecules are known as ribozymes.



359

Figure 4.31 - Cleavage of an RNA by a ribozyme. Wikipedia

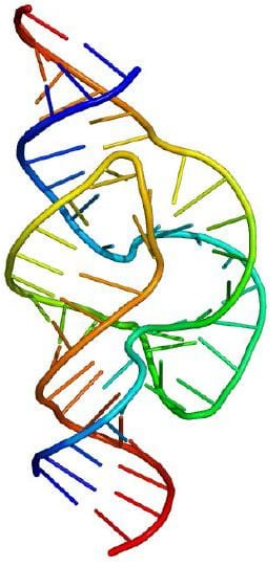


Figure 4.32 - Hammerhead ribozyme. Wikipedia

Not unusual

Ribozymes, however, are not rarities of nature. The protein-making ribosomes of cells are essentially giant ribozymes. The 23S rRNA of the prokaryotic ribosome and the 28S rRNA of the eukaryotic ribosome catalyze the formation of peptide bonds.

Ribozymes are also important in our understanding of the evolution of life on Earth. They have been shown to be capable via selection to evolve self-replication. Indeed, ribozymes actually answer a chicken/egg dilemma - which came first, enzymes that do the work of the cell or nucleic acids that carry the information required to produce the enzymes. As both carriers of genetic information and catalysts, ribozymes are likely both the chicken and the egg in the origin of life.

This page titled 7.1: Basic Principles of Catalysis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 4.1: Basic Principles of Catalysis by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

7.2: Derivation of Michaelis-Menten equation

Enzymes are **protein catalysts,** they influence the **kinetics** but **not the thermodynamics** of a reaction

- Increase the rate of a chemical reaction
- Do not alter the equilibrium

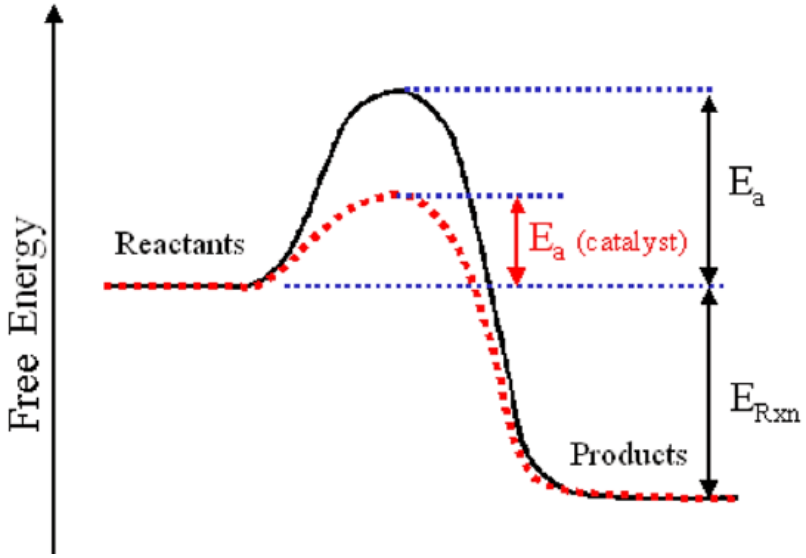
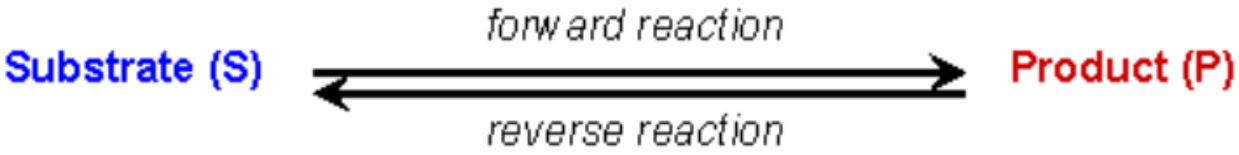


Figure 6.2.1: *Catalyst activity*

- They increase the rate by stabilizing the transition state (i.e. lowering the energy barrier to forming the transition state (they do not affect the energetics of either the reactant(s) or product(s)

Michaelis-Menten derivation for simple steady-state kinetics

The Michaelis-Menten equation is a **mathematical model** that is **used to analyze simple kinetic data**. The model has certain **assumptions**, and as long as these assumptions are correct, it will accurately model your experimental data. The derivation of the model will highlight these assumptions.



In an enzyme catalyzed reaction the substrate initially forms a reversible complex with the enzyme (i.e. the **enzyme and substrate have to interact** for the enzyme to be able to perform its catalytic function). The standard expression to show this is the following:



ASSUMPTION #1:

- There is no product present at the start of the kinetic analysis
- Therefore, as long as we monitor **initial reaction rates** we can **ignore** the reverse reaction of **E+P going to ES**



ASSUMPTION #2:

- During the reaction an **equilibrium** condition is established for the binding and dissociation of the Enzyme and Substrate (Briggs-Haldane assumption)
- Thus, the **rate of formation** of the ES complex is equal to the **rate of dissociation plus breakdown**

ASSUMPTION #3:

- **[E] << [S]**
- The enzyme is a catalyst, it is not destroyed and can be recycled, thus, only small amounts are required
- The amount of S bound to E at any given moment is **small** compared to the amount of free S
- It follows that **[ES] << [S]** and therefore **[S] is constant** during the course of the analysis (NOTE: this assumption requires that the reaction is monitored for a **short period**, so that not much S is consumed and [S] does not effectively change - see next assumption)

ASSUMPTION #4:

- Only the **initial velocity** of the reaction is measured
- [P] = 0 (reverse E + P reaction can be ignored)
- [S] » $[S]_{initial}$

ASSUMPTION #5:

- The enzyme is either present as free enzyme or as the ES complex
- $\mathbf{[E]_{total} = [E] + [ES]}$

Michaelis-Menten derivation using above assumptions:

Rate of ES formation = k_1[E][S] + k_{-2}[E][P]

Assumption #1 says we can ignore the k_{-2} reaction, therefore:

$$\textbf{Rate of ES formation} = k_1[E][S]$$

Assumption #5 says $[E] = [E]_{total} - [ES]$, therefore:

$$\textbf{Rate of ES formation} = k_1([E]_{total} - [ES])[S]$$

The rate of ES breakdown is a combination of the dissociation and the conversion to product:

$$\textbf{Rate of ES breakdown} = k_{-1}[ES] + k_2[ES]$$

$$\textbf{Rate of ES breakdown} = (k_{-1} + k_2)[ES]$$

Assumption #2 says the rate of ES formation equals the rate of breakdown:

$$k_1([E]_{total} - [ES])[S] = (k_{-1} + k_2)[ES]$$

Rearrange to define in terms of rate constants:

$$([E]_{total} - [ES])[S] / [ES] = (k_{-1} + k_2) / k_1$$

$$([E]_{total}\,[S] / [ES]) - [S] = (k_{-1} + k_2) / k_1$$

Define a new constant, $K_m = (k_{-1} + k_2) / k_1$

$$([E]_{total}\,[S] / [ES]) - [S] = K_m$$

Solve for the [ES] term (for reasons that will be given in the next step):

$$[ES] = [E]_{total}\,[S] / (K_m + [S])$$

The actual reaction velocity measured at any given moment is given by:

$$V = k_2[ES]$$

Multiple both sides of the above equation by k_2:

$$k_2[ES] = k_2[E]_{total}\,[S] / (K_m + [S])$$

thus

$$V = k_2[E]_{total}\,[S] / (K_m + [S])$$

The maximum possible velocity (Vmax) occurs when all the enzyme molecules are bound with substrate $[ES] = [E]_{total}$, thus:

$$V_{max} = k_2[E]_{total}$$

Substituting this into the prior expression gives:

$$V = V_{max}\,[S] / (K_m + [S])$$

This is the mathematical expression that is used to model your experimental kinetic data

It is known as the Michaelis-Menten equation

Experimental approach

The general approach is to add a **known concentration of substrate** to the enzyme and to determine the **initial reaction rate**

for that concentration of substrate

- Reaction rates are typically given as moles (or micromole) of product produced per unit of time (sec or min) per mole (or micromole) of enzyme
- The experiment is repeated for a wide range of substrate concentrations
- A table of [S] versus V datapoints are collected
- These datapoints are plotted (V versus S) and should fit a curve that agrees with the Michaelis-Menten equation

The V_{max} and K_m terms are **intrinsic** properties of the particular enzyme/substrate combination that you are studying

- They will be determined from the features of the V versus S plot

V_{max}

There are a limited number of enzyme molecules and they can only perform a single reaction at a time. Thus, at **high [S]** the enzymes can be **saturated**

- Under saturating conditions the reaction is going as fast as it can, and additional increases in [S] do not increase the reaction rate.
- The maximum observable rate is V_{max} and the data will **asymptote to this value** at high [S]
- At low [S] the reaction rate is generally linearly proportional to the [S] (i.e. at low [S] if you double [S] the V will double)

K_m

$$\mathbf{K_m = (k_{-1} + k_2) / k_1 = \text{(rate of breakdown of ES)/(rate of formation of ES)}}$$

- K_m is **similar**, but not exactly equal to, a dissociation constant (K_d) for the ES complex
- If $k_{-1} >> k_2$, then $K_m » K_d$
- Due to this similarity to the expression for K_d, a **low value of K_m** is often interpreted as a **high affinity of the enzyme for the substrate**, and a large value for K_m is often interpreted as a weak affinity of the enzyme for the substrate
- K_m has units of molar concentration (just like the units for [S])

There is a mathematical treatment that allows for the determination of Km from the experimental V versus [S] data

- Consider the situation when the [S] being evaluated results in a value of V that is exactly $^1/_2$ of the maximum reaction velocity:

$$\mathbf{V = V_{max}\,[S] / (K_m + [S])}$$

$$\mathbf{{}^1/_2 V_{max} = V_{max}\,[S] / (K_m + [S])}$$

$$\mathbf{{}^1/_2 = [S] / (K_m + [S])}$$

$$\mathbf{K_m + [S] = 2[S]}$$

$$\mathbf{K_m = [S]}$$

Thus, K_m equals the substrate concentration that results in exactly one half the maximum possible reaction velocity

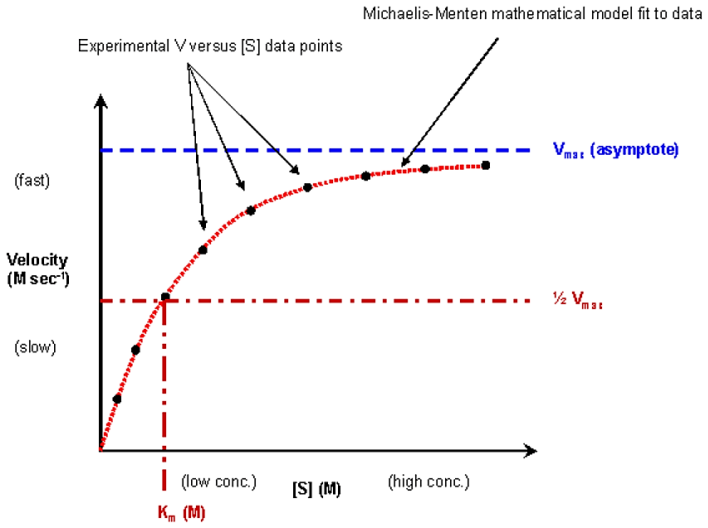


Figure 6.2.2: K_m

Lineweaver-Burke (the "*double reciprocal*" plot)

- The Michaelis-Menten equation can be rearranged by taking the reciprocal, to yield:

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

- If X = 1/[S] and Y=1/***V*** then this is a linear equation with a slope of $K_m/\boldsymbol{V}_{max}$ and a Y intercept of $1/\boldsymbol{V}_{max}$

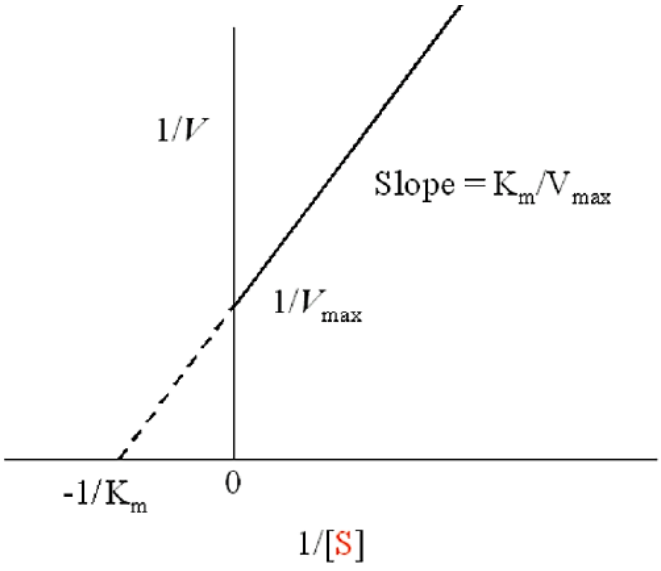


Figure 6.2.3: *1/S and1/V*

- Since the plot of 1/[S] versus 1/v data should be a straight line, it is easier to fit a linear function to the data in this form, and Vmax and K_m can be readily determined from the plot

Reversible Inhibition

There are two major categories of reversible inhibitors: **competitive** reversible inhibitors, and **noncompetitive** reversible inhibitors:

Competitive inhibitors

The inhibitor (**I**) competes with the substrate (**S**) for the enzyme active site (also known as the **S-binding site**). Binding of either of these molecules in the active site is a **mutually exclusive** event

- The substrate and inhibitor share a high degree of structural similarity. However, the inhibitor cannot proceed through the reaction to produce product.
- Increasing the concentration of substrate will **outcompete** the inhibitor for binding to the enzyme active site
- A competitive reversible inhibitor can be identified by its characteristic effects upon kinetic data

The expression for the Michaelis-Menten expression in the presence of a reversible competitive inhibitor is:

$$\mathbf{V = V_{max}\,[S] \,/\, (K_m(1+[I]/K_i) + [S])}$$

Where K_i is the actual EI complex dissociation constant

The effects of the reversible competitive inhibitor on the kinetics are as follows:

- If no inhibitor is present (i.e. if [**I**] = 0) then the equations are the same
- As inhibitor is added, the effect is to modify the apparent value of K_m. In particular, **the apparent K_m will be increased** by a value equal to **$(1 + [I]/K_I)$**. If K_m is increased, **the reaction velocity v will decrease**.
- Note that as [**S**] gets very large the value of the denominator is essentially equal to [**S**] and **v @ v_{max}**. Thus, the reaction velocity can be driven to vmax with a high enough substrate concentration

The diagnostic criteria for reversible competitive inhibition is that while the *apparent* Km is affected by addition of the inhibitor, the value of v_{max} does not change

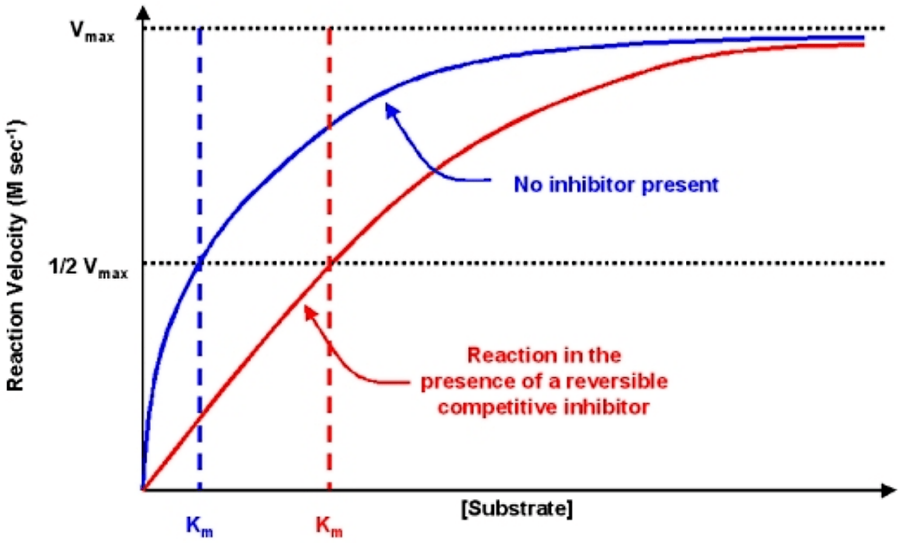


Figure 6.2.4: *Effect of reversible competitive inhibitor*

How is the Lineweaver-Burke double reciprocal plot affected by the presence of a reversible competitive inhibitor?

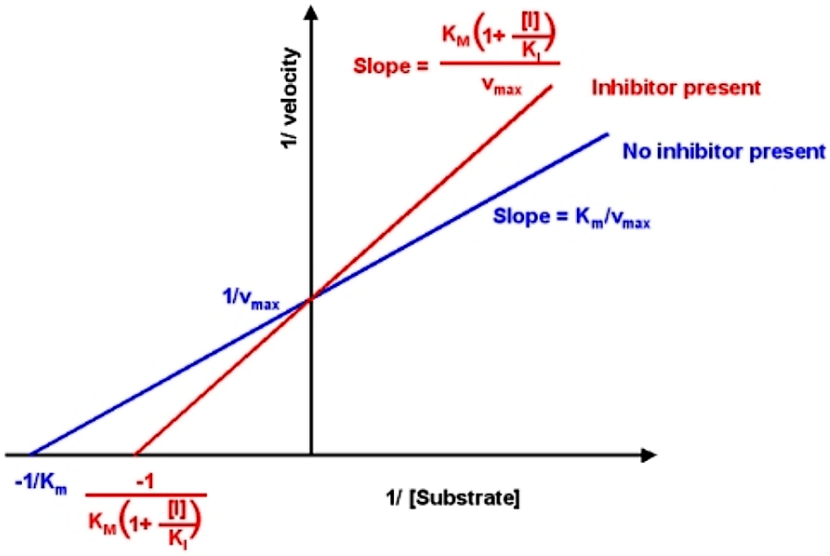


Figure 6.2.5: *Double reciprocal plot with reversible competitive inhibitor*

Noncompetitive Inhibitors

Noncompetitive inhibitors react with both E **and ES** (this is because the noncompetitive inhibitor does not bind at the same site in the enzyme as the substrate)

- Inhibition **cannot** be overcome by increasing the concentration of S
- The effect on kinetics is as if the enzyme were less active (**v_{max} is reduced**), but that the affinity for substrate is unaffected (**K_m remains the same**) since the substrate binding site is not occupied by the noncompetitive inhibitor.

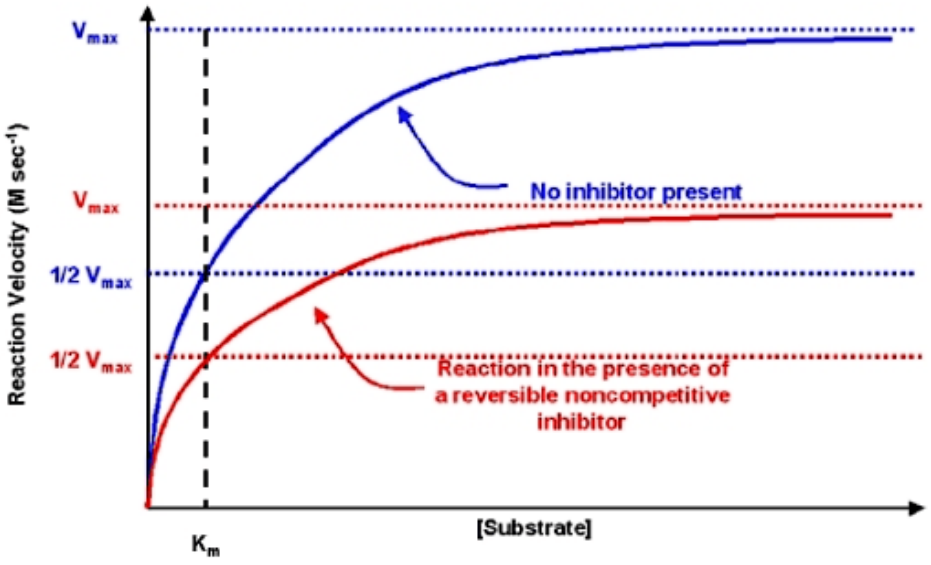


Figure 6.2.6: *Effect of reversible noncompetitive inhibitor*

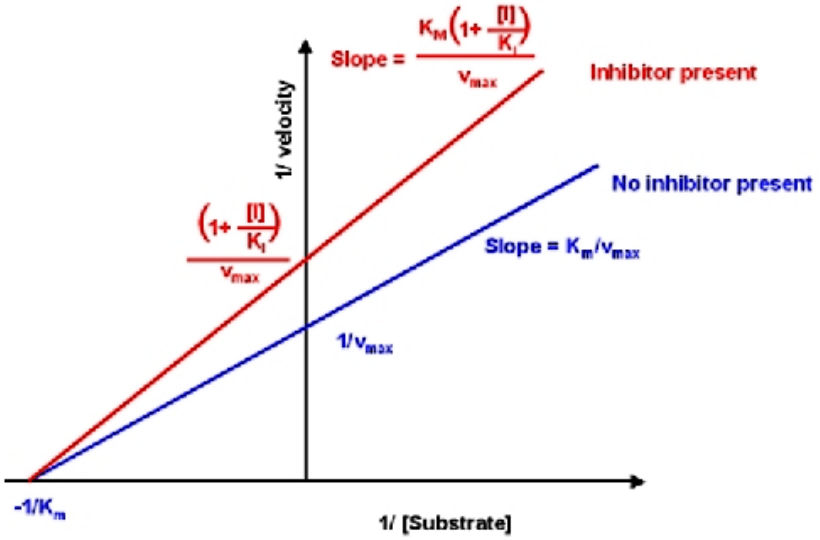


Figure 6.2.7: *Double reciprocal plot with noncompetitive inhibitor*

This page titled 7.2: Derivation of Michaelis-Menten equation is shared under a license and was authored, remixed, and/or curated by Michael Blaber.

- 6.2: Enzyme kinetics by Michael Blaber has no license indicated.

7.3: Mechanisms of Catalysis

A printable version of this section is here: BiochemFFA_4_3.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

The magic of enzymes, as noted, is in their ability to create electronic environments conducive to initiation of a reaction. There are more mechanisms of reaction than we could ever hope to cover in a book like this, and comprehensive discussion of these is not our aim. Instead, we will cite some examples and go into detail on one of them - the mechanism of action of serine proteases.

Chymotrypsin

We will begin with mechanism of action of one enzyme - chymotrypsin. Found in our digestive system, chymotrypsin's catalytic activity is cleaving peptide bonds in proteins and it uses the side chain of a serine in its mechanism of catalysis. Many other protein-cutting enzymes employ a very similar mechanism and they are known collectively as serine proteases (Figure 4.52).

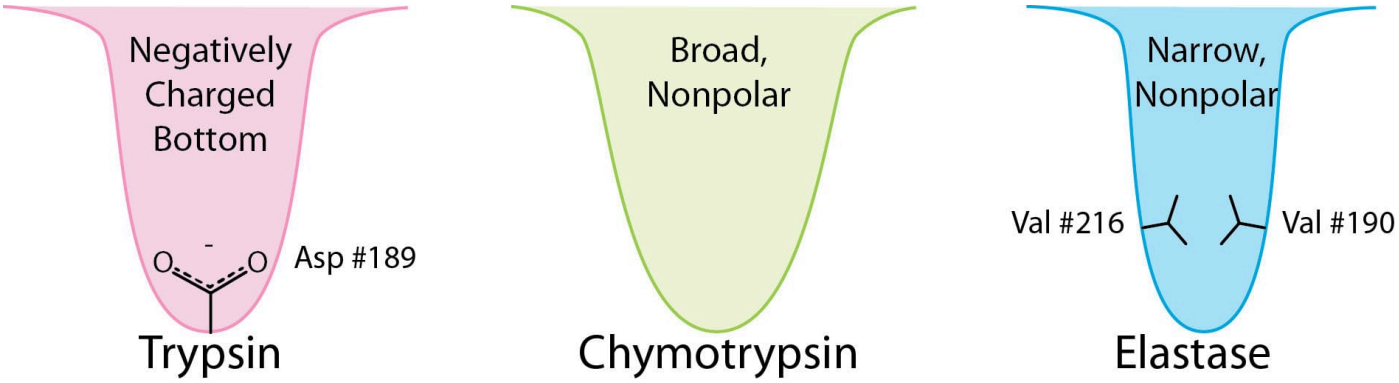


Figure 4.52 - Substrate binding sites (S1 pockets) of three serine proteases. Image by Aleia Kim

These enzymes are found in prokaryotic and eukaryotic cells and all use a common set of three amino acids in the active site called a catalytic triad (Figure 4.53). It consists of aspartic acid, histidine, and serine. The serine is activated in the reaction mechanism to form a nucleophile in these enzymes and gives the class their name. With the exception of the recognition that occurs at the substrate binding site, the mechanism shown here for chymotrypsin would be applicable to any of the serine proteases.

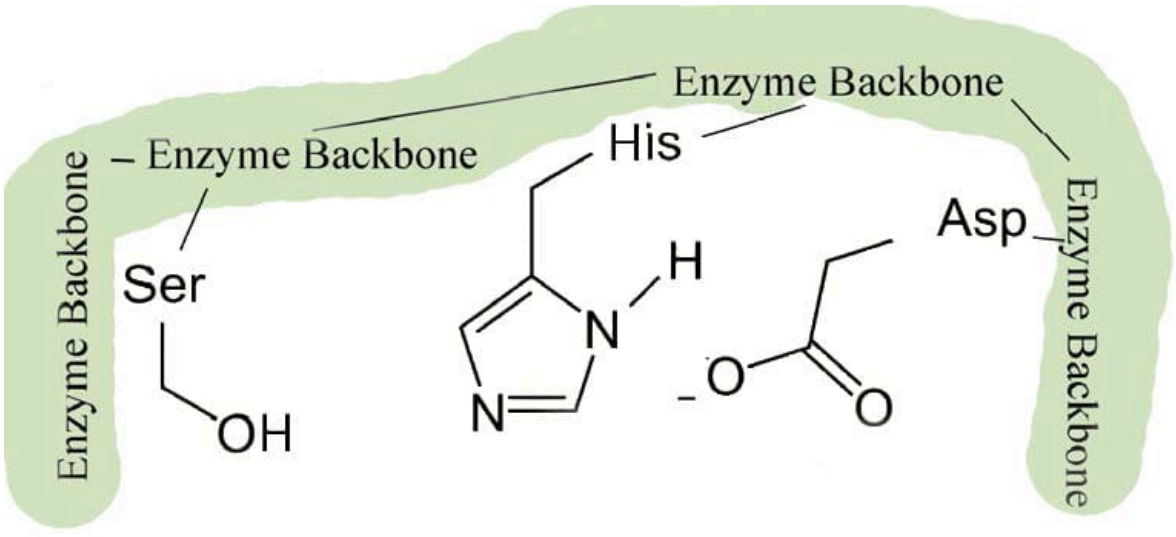


Figure 4.53 - 1. Active site of chymotrypsin showing the catalytic triad of serine - histidine-aspartic acid

Specificity

As a protease, chymotrypsin acts fairly specifically, cutting not all peptide bonds, but only those that are adjacent to relatively non-polar amino acids in the protein. One of the amino acids it cuts adjacent to is phenylalanine. The enzyme's action occurs in two phases – a fast phase that occurs first and a slower phase that follows. The enzyme has a substrate binding site that includes a region of the enzyme known as the S1 pocket. Let us step through the mechanism by which chymotrypsin cuts adjacent to phenylalanine.

Substrate binding

The process starts with the binding of the substrate in the S1 pocket (Figure 4.54). The S1 pocket in chymotrypsin has a hydrophobic hole in which the substrate is bound. Preferred substrates will include amino acid side chains that are bulky and hydrophobic, like phenylalanine. If an ionized side chain, like that of glutamic acid binds in the S1 pocket, it will quickly exit, much like water would avoid an oily interior.

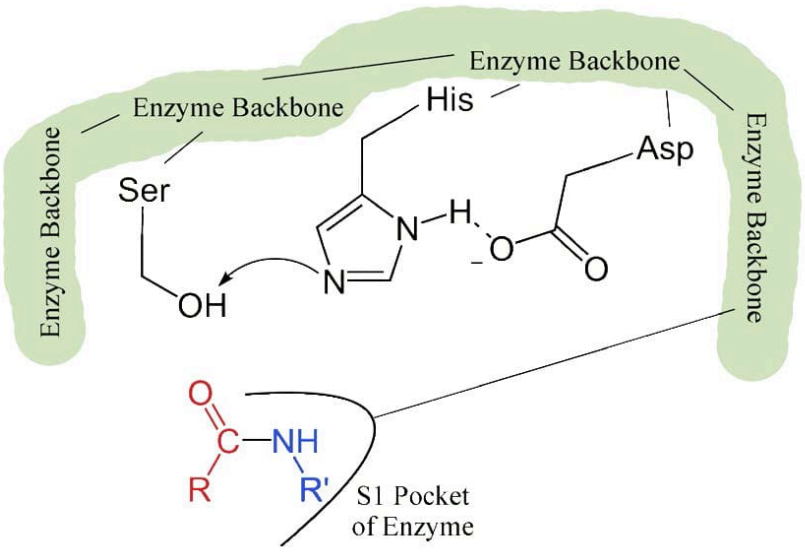


Figure 4.54 - 2. Binding of substrate to S1 pocket in the active site

Shape change on binding

When the proper substrate binds in the S1 pocket, its presence induces an ever so slight change in the shape of the enzyme. This subtle shape change on the binding of the proper substrate starts the steps of the catalysis. Since the catalytic process only starts when the proper substrate binds, this is the reason that the enzyme shows specificity for cutting at specific amino acids in the target protein. Only amino acids with the side chains that interact well with the S1 pocket start the catalytic wheels turning.

The slight changes in shape involve changes in the positioning of three amino acids (aspartic acid, histidine, and serine) in the active site known as the *catalytic triad*.

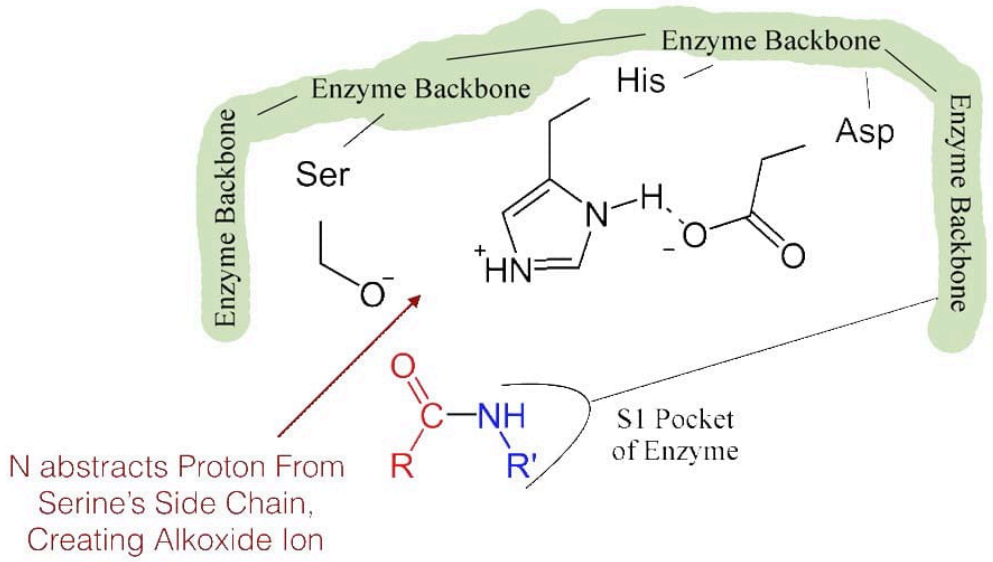


Figure 4.55 - 3. Formation of alkoxide ion

The shift of the negatively charged aspartic acid towards the electron rich histidine ring favors the abstraction of a proton by the histidine from the hydroxyl group on the side chain of serine, resulting in production of a very reactive alkoxide ion in the active site (Figure 4.55).

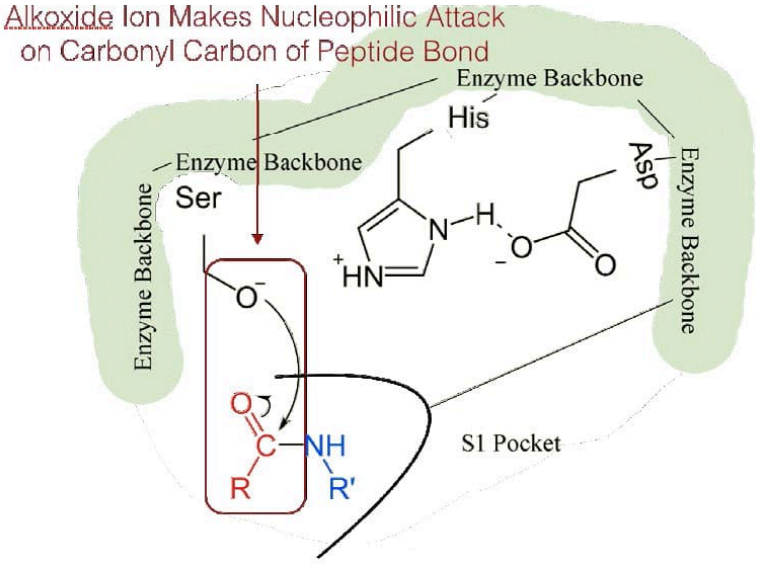


Figure 4.56 - 4. Nucleophilic attack

Since the active site at this point also contains the polypeptide chain positioned with the phenylalanine side chain embedded in the S1 pocket, the alkoxide ion performs a nucleophilic attack on the peptide bond on the carboxyl side of phenylalanine sitting in the S1 pocket (Figure 4.56). This reaction breaks the peptide bond (Figure 4.57) and causes two things to happen.

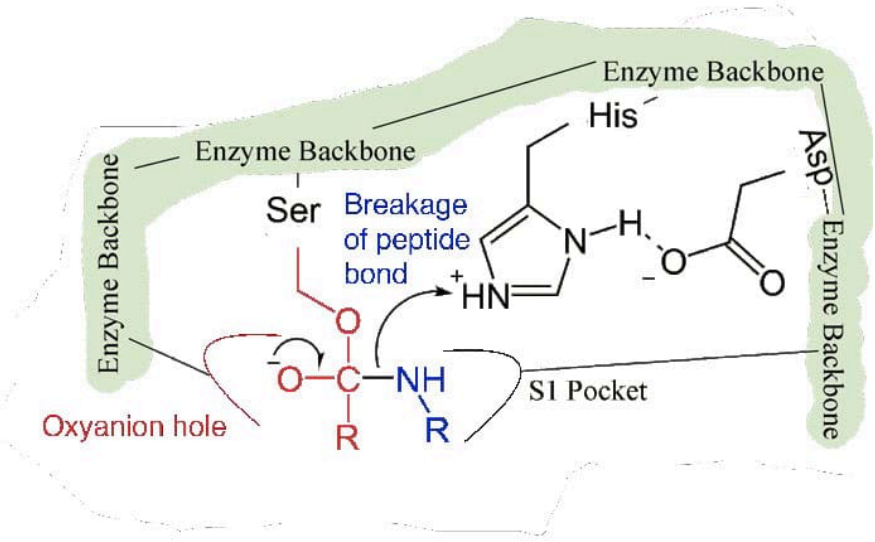


Figure 4.57 - 5. Stabilization by oxyanion hole. Breakage of peptide bond.

First, one end of the original polypeptide is freed and exits the active site (Figure 4.58). The second is that the end containing the phenylalanine is covalently linked to the oxygen of the serine side chain. At this point we have completed the first (fast) phase of the catalysis.

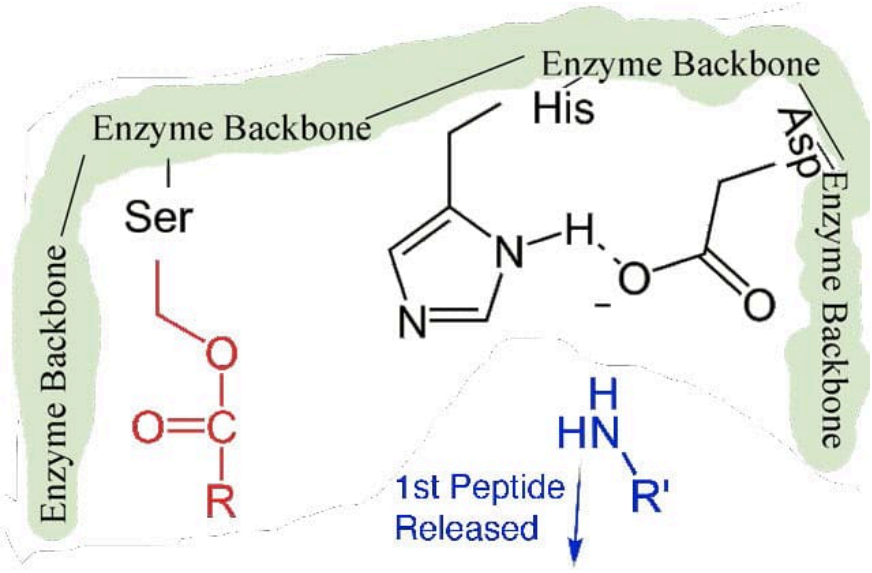


Figure 4.58 - 6. First peptide released. Other half bonded to serine.

Slower second phase

The second phase of the catalysis by chymotrypsin is slower. It requires that the covalent bond between phenylalanine and serine's oxygen be broken so the peptide can be released and the enzyme can return to its original state. The process starts with entry of water into the active site. Water is attacked in a fashion similar to that of the serine side chain in the first phase, creating a reactive hydroxyl group (Figure 4.59) that performs a nucleophilic attack on the phenylalanine-serine bond (Figure 4.60), releasing it and replacing the proton on serine. The second peptide is released in the process and the reaction is complete with the enzyme back in its original state (Figure 4.61).

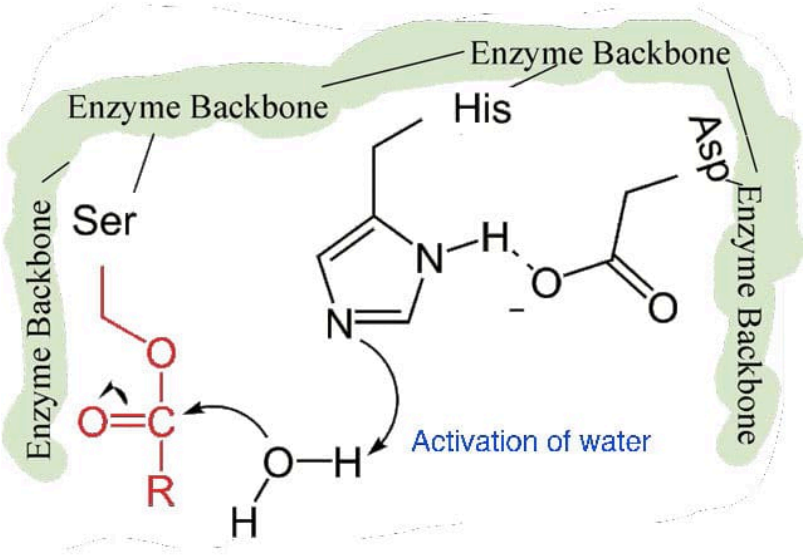


Figure 4.59 - 7. Activation of water by histidine

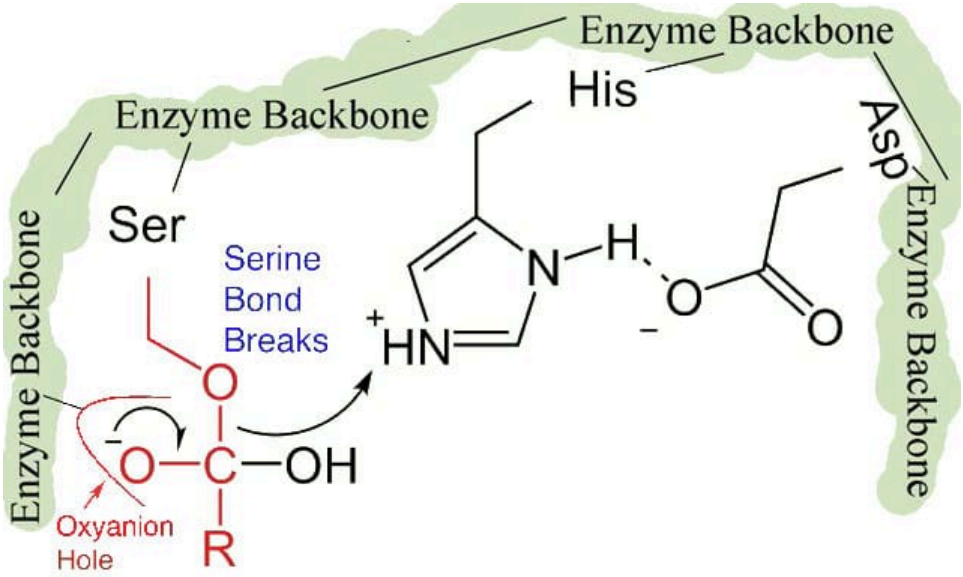


Figure 4.60 - 8. Nucleophile attack by hydroxyl creates tetrahydryl intermediate stabilized by oxyanion hole. Bond to serine breaks.

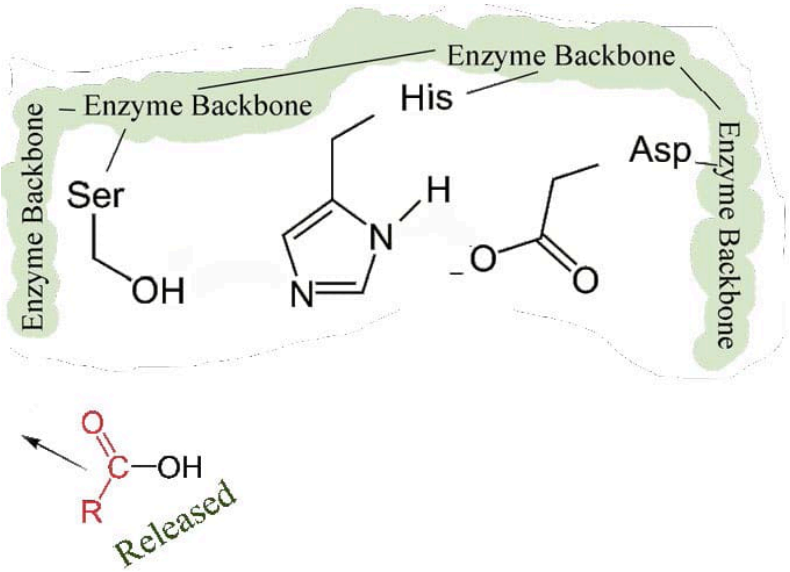


Figure 4.61 - 9. Second half of peptide released. Enzyme active site restored.

Serine proteases

The list of serine proteases is quite long. They are grouped in two broad categories - 1) those that are chymotrypsin-like and 2) those that are subtilisin-like. Though subtilisin-type and chymotrypsin-like enzymes use the same mechanism of action, including the catalytic triad, the enzymes are otherwise not related to each other by sequence and appear to have evolved independently. They are, thus, an example of convergent evolution - a process where evolution of different forms converge on a structure to provide a common function.

The serine protease enzymes cut adjacent to specific amino acids and the specificity is determined by the size/shape/charge of amino acid side chain that fits into the enzyme's S1 binding pocket (Figure 4.62).

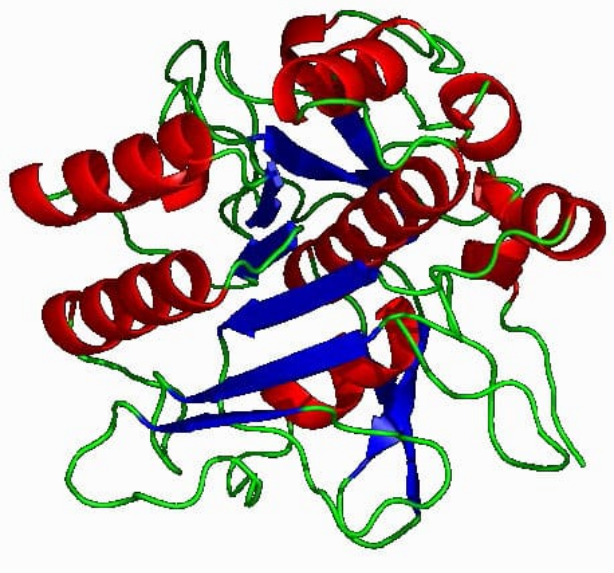


Figure 4.62 - Subtilisin - A serine protease

Examples of serine proteases include trypsin, chymotrypsin, elastase, subtilisin, signal peptidase I, and nucleoporin. Serine proteases participate in many physiological processes, including blood coagulation, digestion, reproduction, and the immune response.

Cysteine proteases

Cysteine proteases (also known as thiol proteases) catalyze the breakdown of proteins by cleaving peptide bonds using a nucleophilic thiol from a cysteine (Figure 4.63). The cysteine is typically found in a catalytic dyad or triad also involving histidine and (sometimes) aspartic acid (very much like serine proteases). The sulfhydryl group of cysteine proteases is more acidic than the hydroxyl of serine proteases, so the aspartic acid of the triad is not always needed.

The mechanism of action is very similar to that of serine proteases. Binding of proper substrate results in activation of the thiol (removal of the proton by the histidine group). The activated thiol acts as a nucleophile, attacking the peptide bond and causing it break. One peptide is released and the other peptide becomes covalently linked to the sulfur. Hydrolysis by water releases the second peptide and completes the cycle. Examples of cysteine proteases include papain, caspases, hedgehog protein, calpain, and cathepsin K.

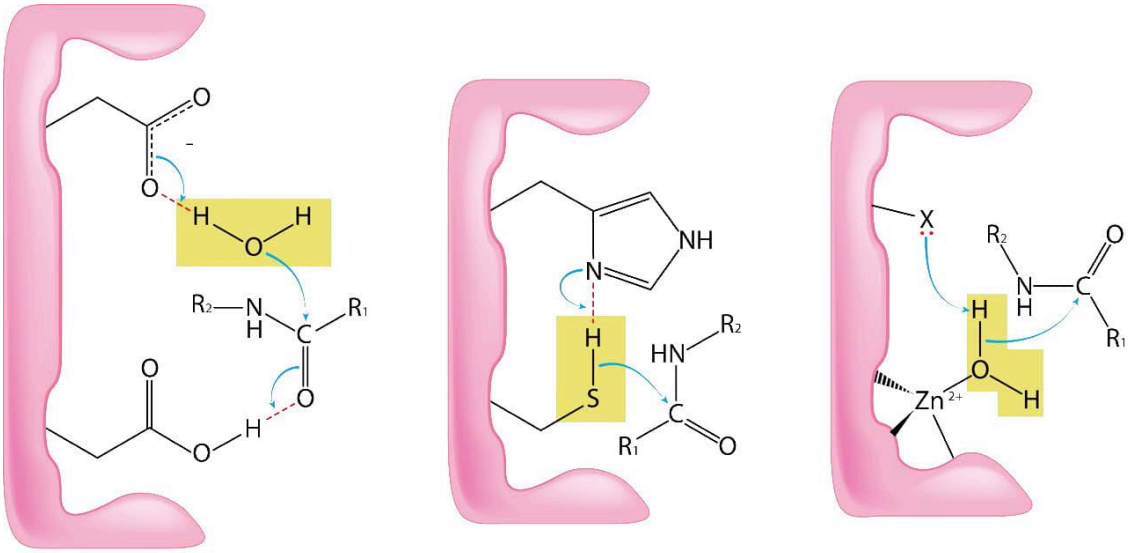


Figure 4.63 - Mechanism of action of proteases. In each case, a nucleophile is created - hydroxyl (aspartyl proteases), thiol (cysteine proteases), and hydroxyl (metalloproteases). Image by Aleia Kim

Caspases

Caspases (Cysteine-ASPartic ProteASEs) are a family of cysteine proteases that play important roles in the body. At the cellular level they function in apoptosis and necrosis and in the body, they are involved in inflammation and the immune system. Maturation of lymphocytes is one such role. They are best known, however, for their role in apoptosis, which has given rise to descriptions of them as “executioner” proteins or “suicide proteases” that dismantle cells in programmed cell death.

There are 12 known human caspases. The enzymes are synthesized as pro-caspase zymogens with a prodomain and two other subunits. The prodomain contains regions that allow it to interact with other molecules that regulate the enzyme’s activity. The caspases come in two forms. The initiator caspases, when activated, activate the effector caspases. The effector caspases cleave other proteins in the cell. Targets for effector caspase cleavage action include the nuclear lamins (fibrous proteins providing structural integrity to the nucleus), ICAD/DFF45 (an inhibitor of DNAse), PARP (flags areas where DNA repair needed), and PAK2 (apoptotic regulation).

The caspase activation cascade can itself be activated by granzyme B (a serine protease secreted by natural killer cells and cytotoxic T-cells), cellular death receptors, and the apoptosome (large protein structure in apoptotic cells stimulated by release of cytochrome C from the mitochondria). Each of these activators is responsible for activating different groups of caspases.

Metalloproteases

Metalloproteases (Figure 4.64) are enzymes whose catalytic mechanism for breaking peptide bonds involves a metal. Most metalloproteases use zinc as their metal, but a few use cobalt, coordinated to the protein by three amino acid residues with a

labile water at the fourth position. A variety of side chains are used - histidine, aspartate, glutamate, arginine, and lysine. The water is the target of action of the metal which, upon binding of the proper substrate, abstracts a proton to create a nucleophilic hydroxyl group that attacks the peptide bond, cleaving it (Figure 4.64). Since the nucleophile here is not attached covalently to the enzyme, neither of the cleaved peptides ends up attached to the enzyme during the catalytic process. Examples of metalloproteases include carboxypeptidases, aminopeptidases, insulinases and thermolysin.



Figure 4.64 - Carboxypeptidase - A metalloprotease

Aspartyl proteases

As the name suggests, aspartyl proteases use aspartic acid in their catalytic mechanism (Figures 4.63 & 4.65). Like the metalloproteases, aspartyl proteases activate a water to create a nucleophile for catalysis (Figure 4.65). The activated water attacks the peptide bond of the bound substrate and releases the two pieces without the need to release a bound intermediate, since water is not covalently attached to the enzyme. Common aspartyl proteases include pepsin, signal peptidase II, and HIV-1 protease.

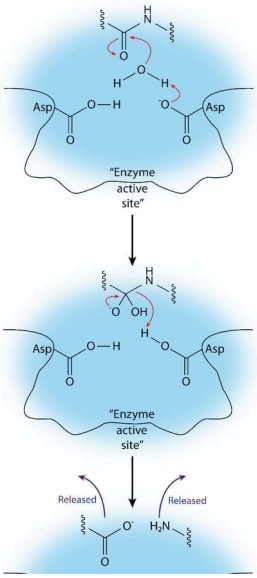


Figure 4.65 - Activation of an aspartyl protease - aspartate side chain abstracts proton (top). Hydroxyl attacks peptide bond (middle). Broken peptide pieces released (bottom). Image by Pehr Jacobson

Threonine proteases

Though threonine has an R-group with a hydroxyl like serine, the mechanism of action of this class of proteases differs somewhat from the serine proteases. There are some similarities. First, the threonine's hydroxyl plays a role in catalysis and that is to act as a nucleophile. The nucleophile is created, however, not by a catalytic triad, but rather as a result of threonine's own α-amine group abstracting a proton.

Because of this, the nucleophilic threonine in a threonine protease must be at the n-terminus of the enzyme. Nucleophilic attack of the peptide bond in the target protease results in breakage of the bond to release one peptide and the other is covalently attached to serine, like the serine proteases. Also, as with the serine proteases, water must come in to release the covalently linked second peptide to conclude the catalytic mechanism.

Examples

Examples of threonine proteases include the catalytic subunits of the proteasome. Some acyl transferases (such as ornithine acyltransferase) have evolved the same catalytic mechanism by convergent evolution. The latter enzymes use ornithine instead of water to break the enzyme-substrate covalent bond, with the result that the acyl-group becomes attached to ornithine, instead of water.

Protease inhibitors

Molecules which inhibit the catalytic action of proteases are known as protease inhibitors. These come in a variety of forms and have biological and medicinal uses. Many biological inhibitors are proteins themselves. Protease inhibitors can act in several ways, including as a suicide inhibitor, a transition state inhibitor, a denaturant, and as a chelating agent. Some work only on specific classes of enzymes. For example, most known aspartyl proteases are inhibited by pepstatin. Metalloproteases are sensitive to anything that removes the metal they require for catalysis. Zinc-containing metalloproteases, for example, are very sensitive to EDTA, which chelates the zinc ion.

One category of proteinaceous protease inhibitors is known as the serpins. Serpins inhibit serine proteases that act like chymotrypsin. 36 of them are known in humans.

Serpins are unusual in acting by binding to a target protease irreversibly and undergoing a conformational change to alter the active site of its target. Other protease inhibitors act as competitive inhibitors that block the active site.

Serpins can be broad in their specificity. Some, for example, can block the activity of cysteine proteases. One of the best known biological serpins is α-1-anti-trypsin (A1AT - Figure 4.66) because of its role in lungs, where it functions to inhibit the elastase protease. Deficiency of A1AT leads to emphysema. This can arise as a result of genetic deficiency or by cigarette smoking. Reactive oxygen species produced by cigarette smoking can oxidize a critical methionine residue (#358 of the processed form) in A1AT, rendering it unable to inhibit elastase. Uninhibited, elastase can attack lung tissue and cause emphysema. Most serpins work extracellularly. In blood, for example, serpins like antithrombin can help to regulate the clotting process.

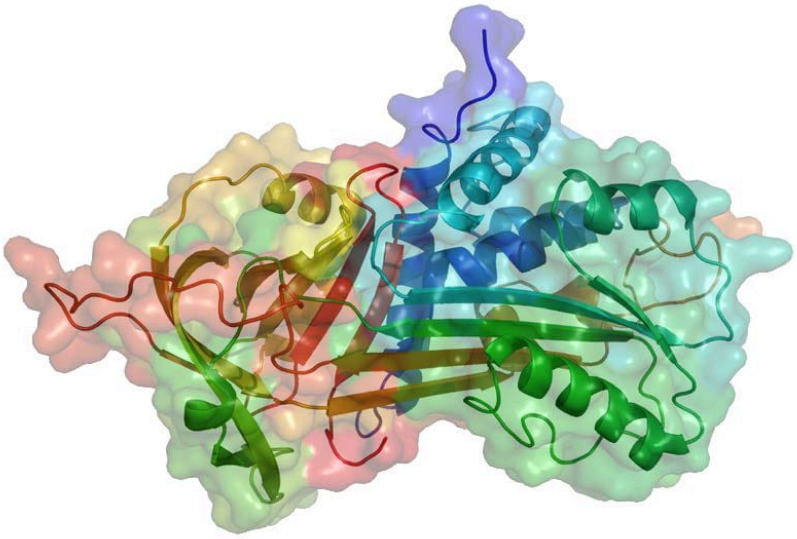


Figure 4.66 - α-1-antitrypsin

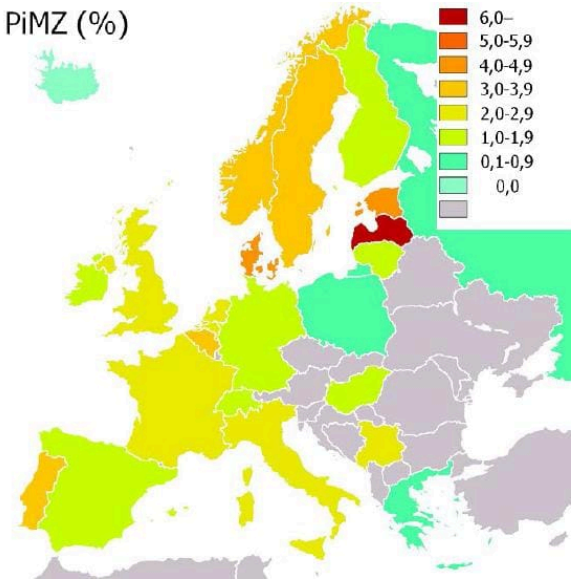


Figure 4.67 - Incidence of α-1-antitrypsin (PiMZ) deficiency in Europe by percent. Wikipedia

Anti-viral Agents

Protease inhibitors are used as anti-viral agents to prohibit maturation of viral proteins - commonly viral coat proteins.

They are part of drug “cocktails” used to inhibit the spread of HIV in the body and are also used to treat other viral infections, including hepatitis C. They have also been investigated for use in treatment of malaria and may have some application in anti-cancer therapies as well.

This page titled 7.3: Mechanisms of Catalysis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 4.3: Mechanisms of Catalysis by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

7.4: Control of Enzymatic Activity

A printable version of this section is here: BiochemFFA_4_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Regulation of enzyme activity

Apart from their ability to greatly speed the rates of chemical reactions in cells, enzymes have another property that makes them valuable. This property is that their activity can be regulated, allowing them to be activated and inactivated, as necessary. This is tremendously important in maintaining homeostasis, permitting cells to respond in controlled ways to changes in both internal and external conditions.

Inhibition of specific enzymes by drugs can also be medically useful. Understanding the mechanisms that control enzyme activity is, therefore, of considerable importance.

Inhibition

We will first discuss four types of enzyme inhibition – competitive, non-competitive, uncompetitive, and suicide inhibition. Of these, the first three types are reversible. The last one, suicide inhibition, is not.

Competitive inhibition

Probably the easiest type of enzyme inhibition to understand is competitive inhibition and it is the one most commonly exploited pharmaceutically. Molecules that are competitive inhibitors of enzymes resemble one of the normal substrates of an enzyme. An example is methotrexate, which resembles the folate substrate of the enzyme dihydrofolate reductase (DHFR). This enzyme normally catalyzes the reduction of folate, an important reaction in the metabolism of nucleotides.

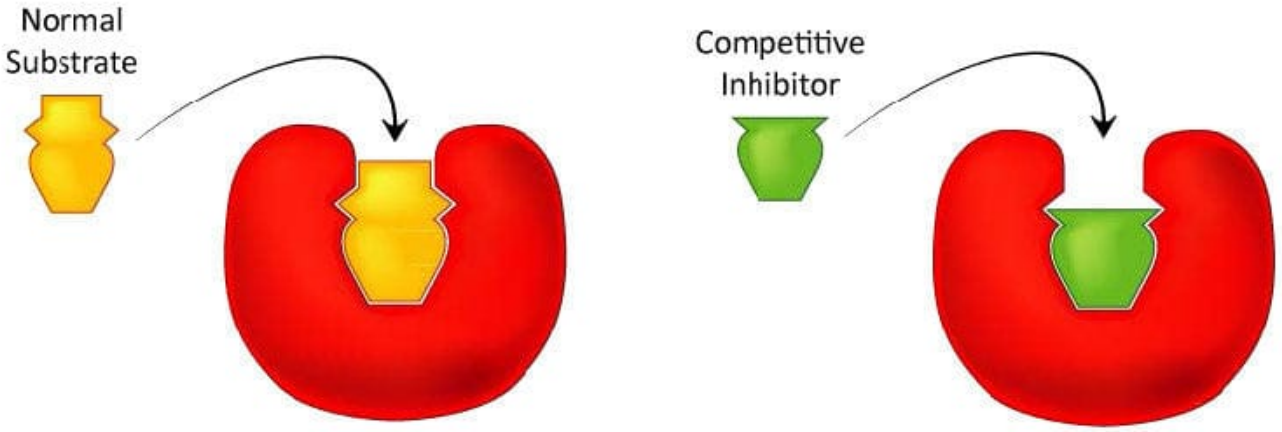


Figure 4.33 - Competitive inhibitors resemble the normal substrate and compete for binding at the active site. Image by Aleia Kim

Inhibitor binding

When the drug methotrexate is present, some of the DHFR enzyme binds to it, instead of to folate, and during the time methotrexate is bound, the enzyme is inactive and unable to bind folate. Thus, the enzyme is inhibited. Notably, the binding site on DHFR for methotrexate is the active site, the same place that folate would normally bind. As a result, methotrexate 'competes' with folate for binding to the enzyme. The more methotrexate there is, the more effectively it competes with folate for the enzyme's active site. Conversely, the more folate there is, the less of an effect methotrexate has on the enzyme because folate outcompetes

it.

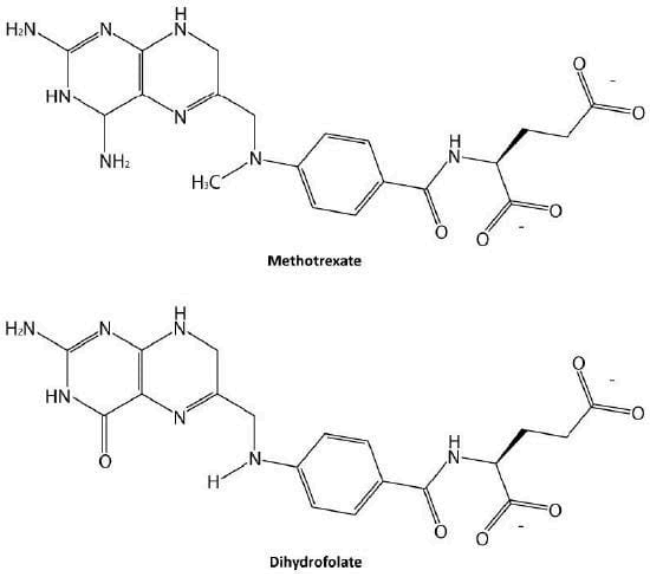


Figure 4.34 - Methotrexate and dihydrofolate. Image by Ben Carson

No effect on Vmax

How do we study competitive inhibition? It is typically done as follows. First, one performs a set of V0 vs. [S] reactions without inhibitor (20 or so tubes, with buffer and constant amounts of enzyme, varying amounts of substrate, equal reaction times). V0 vs. [S] is plotted (Figure 4.35 red line), as well as 1/V0 vs. 1/[S] (Figure 4.36 green line). Next, a second set of reactions is performed in the same manner as before, except that a fixed amount of the methotrexate inhibitor is added to each tube. At low concentrations of substrate, the methotrexate competes for the enzyme effectively, but at high concentrations of substrate, the inhibitor will have a much reduced effect, since the substrate outcompetes it, due to its higher concentration (remember that the inhibitor is at fixed concentration).

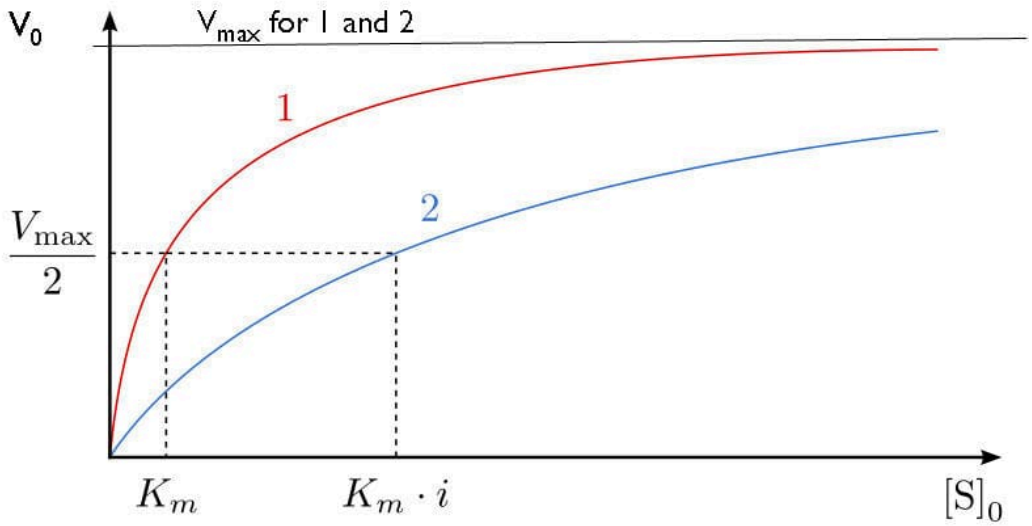


Figure 4.35 V0 vs [S] Plots for uninhibited reactions (red) and competitively inhibited reactions (blue). Both ultimately have same Vmax

Graphically, the results of these inhibitor experiments are shown in Figure 4.35 (blue line) and Figure 4.36 (orange line). Notice that at high substrate concentrations, the competitive inhibitor has essentially no effect, causing the V_{max} for the enzyme to remain unchanged. To reiterate, this is due to the fact that at high substrate concentrations, the inhibitor doesn't compete well. However, at lower substrate concentrations, it does.

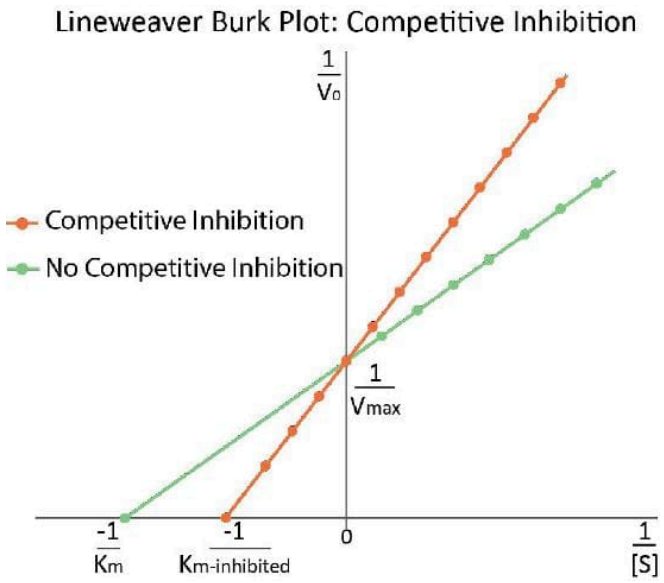


Figure 4.36 - Lineweaver-Burk plots - uninhibited reactions (green). Competitively inhibited reactions (orange). Lines cross on Y-axis at 1/Vmax Since Vmax is the same for both reactions. Image by Aleia Kim

Increased K_m

In competitively inhibited reactions, the apparent Km of the enzyme for the substrate increases ($-1/K_m$ gets closer to zero - red line in Figure 4.36) when the inhibitor is present compared to when the inhibitor is absent, thus illustrating the better competition of the inhibitor at lower substrate concentrations. It may not be obvious why we call the changed Km the apparent Km of the enzyme. The reason is that the inhibitor doesn't actually change the enzyme's affinity for the folate substrate. It only appears to do so. This is because of the way that competitive inhibition works. When the competitive inhibitor binds the enzyme, it is effectively 'taken out of action.' Inactive enzymes have NO affinity for substrate and no activity either. We can't measure Km for an inactive enzyme.

The enzyme molecules that are not bound by methotrexate can, in fact, bind folate and are active. Methotrexate has no effect on them and their Km values are unchanged. Why then, does Km appear higher in the presence of a competitive inhibitor? The reason is that the competitive inhibitor is having a greater effect of reducing the amount of active enzyme at lower concentrations of substrate than it does at higher concentrations of substrate. When the amount of enzyme is reduced, one must have more substrate to supply the reduced amount of enzyme sufficiently to get to Vmax/2.

It is worth noting that in competitive inhibition, the percentage of inactive enzyme changes drastically over the range of [S] values used. To start, at low [S] values, the greatest percentage of the enzyme is inhibited. At high [S], no significant percentage of enzyme is inhibited. This is not always the case, as we shall see in non-competitive inhibition.

Non-competitive inhibition

A second type of inhibition employs inhibitors that do not resemble the substrate and bind not to the active site, but rather to a separate site on the enzyme (Figure 4.37). The effect of binding a non-competitive inhibitor is significantly different from binding a competitive inhibitor because there is no competition. In the case of competitive inhibition, the effect of the inhibitor could be reduced and eventually overwhelmed with increasing amounts of substrate. This was because increasing substrate made increasing percentages of the enzyme active. With non-competitive inhibition, increasing the amount of substrate has no effect on the percentage of enzyme that is active. Indeed, in non-competitive inhibition, the percentage of enzyme inhibited remains the same through all ranges of [S].

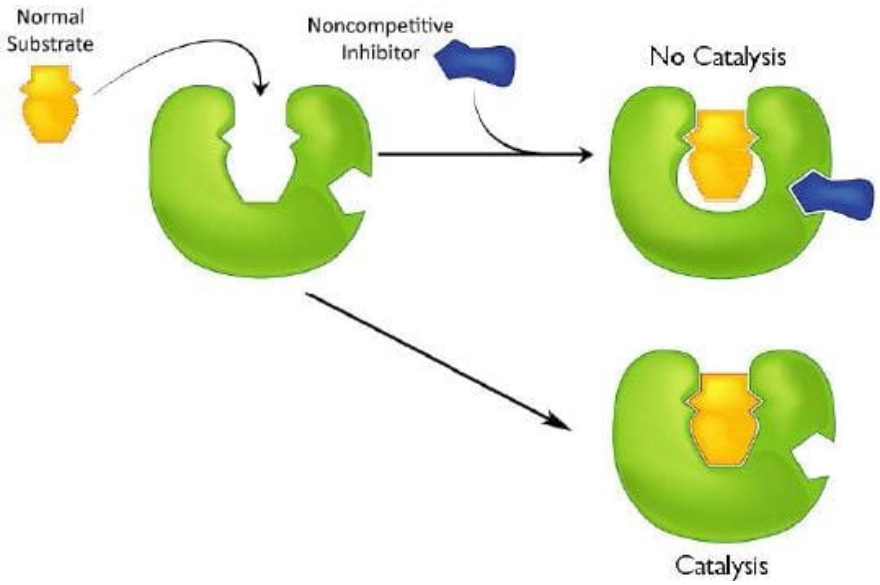


Figure 4.37 - Non-competitive inhibition - inhibitor does not resemble the substrate and binds to a site other than the active site. Image by Aleia Kim

This means, then, that non-competitive inhibition effectively reduces the amount of enzyme by the same fixed amount in a typical experiment at every substrate concentration used The effect of this inhibition is shown in Figure 4.38 & 4.39. As you can see, V_{max} is reduced in non-competitive inhibition compared to uninhibited reactions.

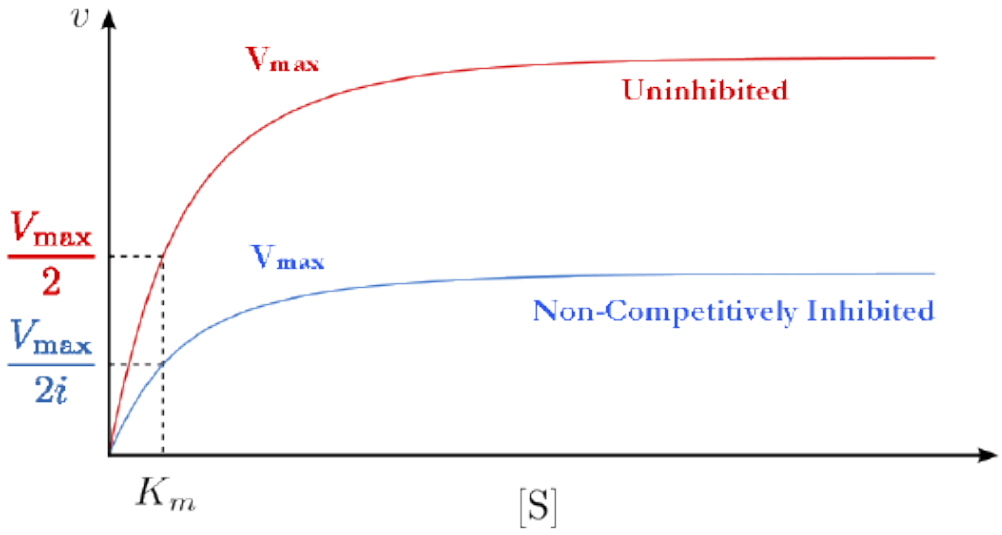


Figure 4.38 - V0 vs [S] plots of uninhibited reactions (red) and non-competitively inhibited reactions (blue). Vmax is reduced, but Km values are unchanged in non-competitively inhibited reactions

This makes sense if we remember that Vmax is dependent on the amount of enzyme present. Reducing the amount of enzyme present reduces V_{max}. In competitive inhibition, this doesn't occur detectably, because at high substrate concentrations, there is essentially 100% of the enzyme active and the V_{max} appears not to change. Additionally, Km for non-competitively inhibited reactions does not change from that of uninhibited reactions. This is because, as noted previously, one can only measure the K_m of active enzymes and K_m is a constant for a given enzyme.

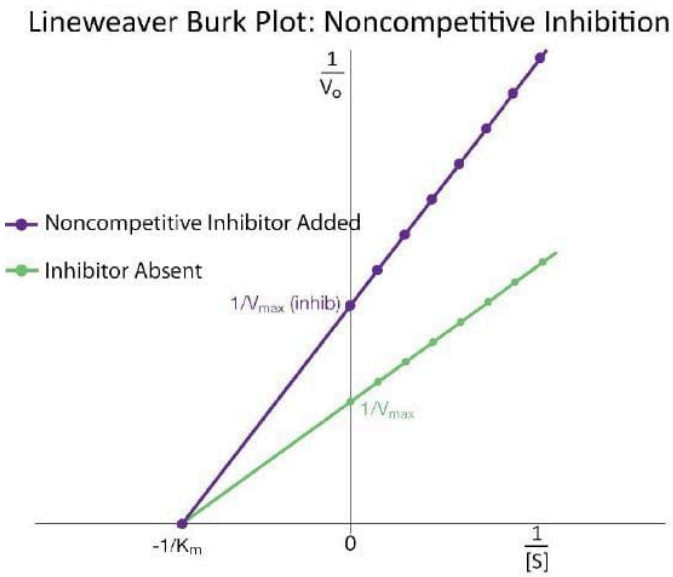


Figure 4.39 - Lineweaver Burk plots of uninhibited reactions (green) and non-competitively inhibited reactions (purple). Image by Aleia Kim

Uncompetitive inhibition

A third type of enzymatic inhibition is that of uncompetitive inhibition, which has the odd property of a reduced Vmax as well as a reduced Km. The explanation for these seemingly odd results is rooted in the fact that the uncompetitive inhibitor binds only to the enzyme-substrate (ES) complex (Figure 4.40). The inhibitor-bound complex forms mostly under concentrations of high substrate and the ES-I complex cannot release product while the inhibitor is bound, thus explaining the reduced V_{max}.

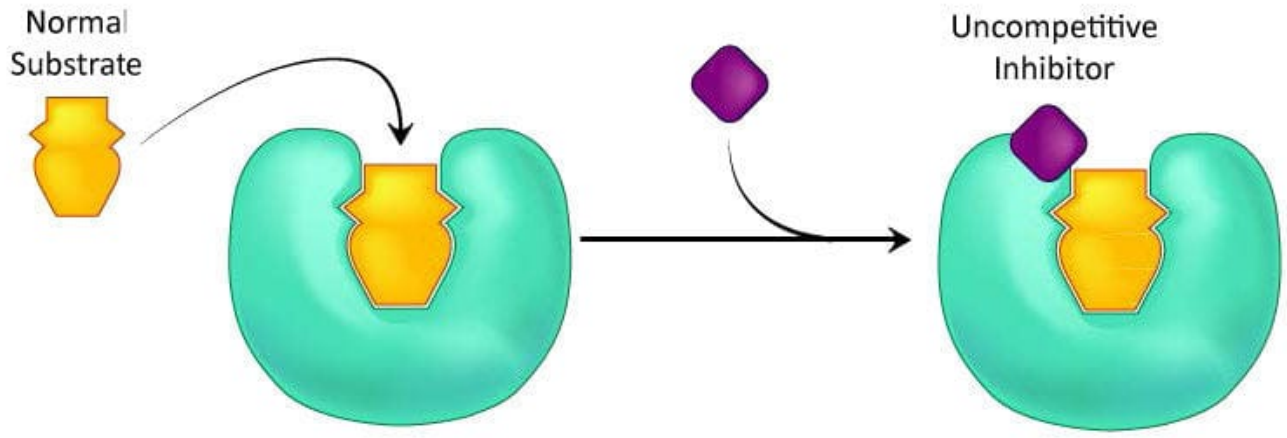


Figure 4.40 - Uncompetitive inhibition. Image by Aleia Kim

The reduced Km is a bit harder to conceptualize. The reason is that the inhibitor-bound complex effectively reduces the concentration of the ES complex. By Le Chatelier's Principle, a shift occurs to form additional ES complex, resulting in less free enzyme and more enzyme in the forms ES and ESI (ES with inhibitor). Decreases in free enzyme correspond to an enzyme with greater affinity for its substrate. Thus, paradoxically, uncompetitive inhibition both decreases V_{max} and increases an enzyme's affinity for its substrate (K_m - Figures 4.41 & 4.42).

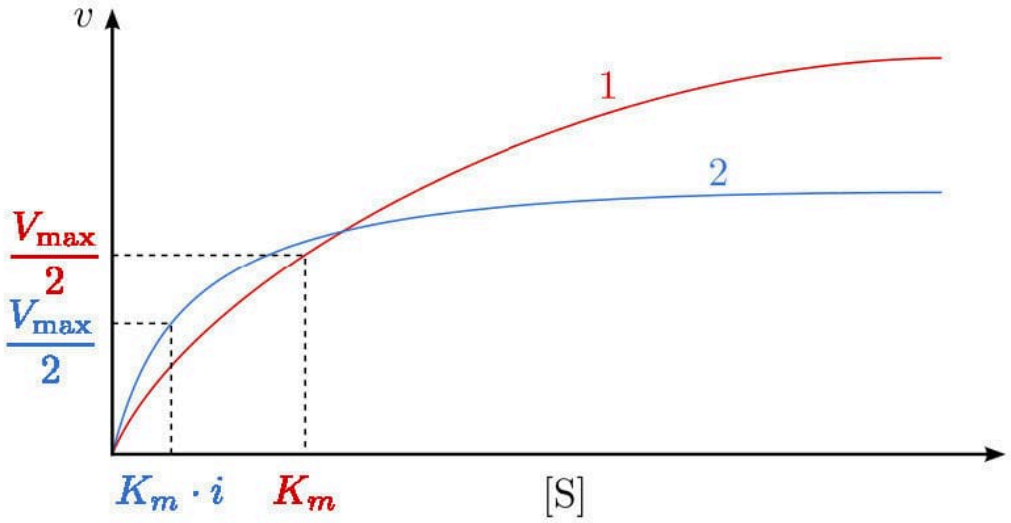


Figure 4.41 - V0 vs [S] plot for uncompetitive inhibition (blue) and uninhibited reactions (red)

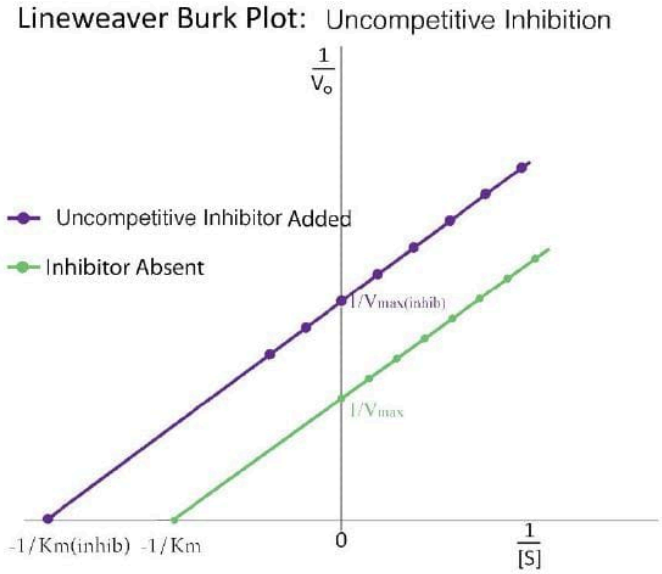


Figure 4.42 - Uncompetitive inhibition (purple) and uninhibited reactions (green). Image by Aleia Kim

Suicide inhibition

In contrast to the first three types of inhibition, which involve reversible binding of the inhibitor to the enzyme, suicide inhibition is irreversible, because the inhibitor becomes covalently bound to the enzyme during the inhibition. Suicide inhibition rather closely resembles competitive inhibition because the inhibitor generally resembles the substrate and binds to the active site of the enzyme. The primary difference is that the suicide inhibitor is chemically reactive in the active site and makes a bond with it that precludes its removal. Such a mechanism is that employed by penicillin (Figure 4.43), which covalently links to the bacterial enzyme, DD transpeptidase and stops it from functioning. Since the normal function of the enzyme is to make a bond necessary for the peptidoglycan complex of the bacterial cell wall, the cell wall cannot properly form and bacteria cannot reproduce.

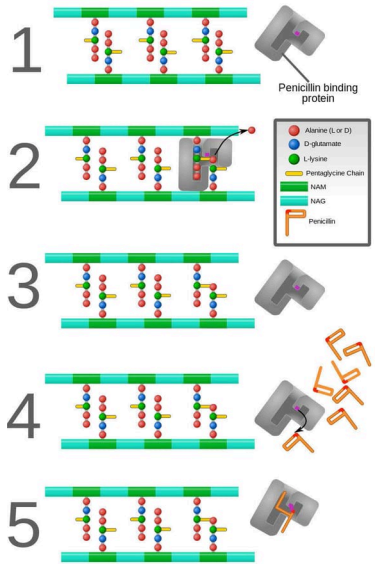


Figure 4.43 - Action of penicillin. DD-transpeptidase builds peptidoglycan layer of bacterial cell wall (1-3). Binding of penicillin by DD-transpeptidase stops peptidoglycan synthesis (4-5). Wikipedia

Control of enzymes

It is appropriate to talk at this point about mechanisms cells use to control enzymes. There are four general methods that are employed:

1. allosterism,
2. covalent modification,
3. access to substrate, and
4. control of enzyme synthesis/breakdown.

Some enzymes are controlled by more than one of these methods.

Allosterism

The term allosterism refers to the fact that the activity of certain enzymes can be affected by the binding of small molecules. Molecules causing allosteric effects come in two classifications. Ones that are substrates for the enzymes they affect are called **homotropic effectors** and those that are not substrates are called heterotropic effectors.

The homotropic effectors usually are activators of the enzymes they bind to and the results of their action can be seen in the conversion of the hyperbolic curve typical of a V0 vs. [S] plot for an enzyme (Figure 4.18), being converted to a sigmoidal plot (Figure 4.44). This is due to the conversion of the enzyme from the T-state to the R-state on binding the substrate/homotropic effector.

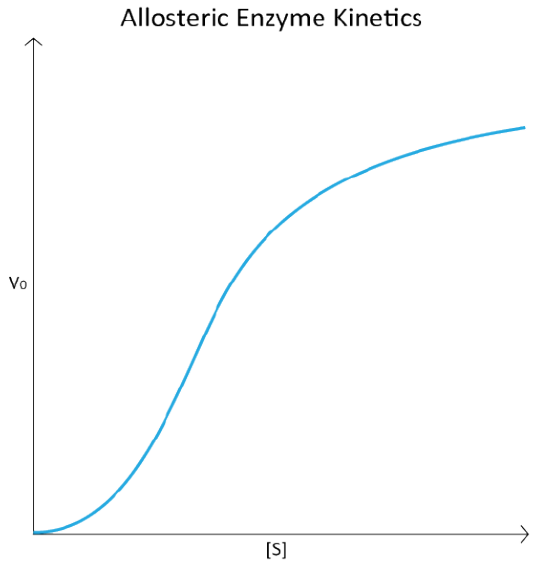


Figure 4.44 - Kinetic profile of an allosteric enzyme whose activity is controlled by a homotropic effector. Image by Aleia Kim

The V0 vs. [S] plot of allosteric enzyme reactions resembles the oxygen binding curve of hemoglobin (see Figure 2.83). Even though hemoglobin is not an enzyme and is thus not catalyzing a reaction, the similarity of the plots is not coincidental. In both cases, the binding of an external molecule is being measured – directly, in the hemoglobin plot, and indirectly by the V0 vs. [S] plot, since substrate binding is a factor in enzyme reaction velocity.

Allosteric inhibition

Allosterically, regulation of these enzymes works by inducing different physical states (shapes, as it were) that affect their ability to bind to substrate. When an enzyme is inhibited by binding an effector, it is converted to the T-state (T=tight), it has a reduced affinity for substrate and it is through this means that the reaction is slowed.

Allosteric activation

On the other hand, when an enzyme is activated by effector binding, it converts to the R-state (R=relaxed) and binds substrate much more readily. When no effector is present, the enzyme may be in a mixture of T- and R-states.

Feedback inhibition

An interesting kind of allosteric control is exhibited by HMG-CoA reductase, which catalyzes an important reaction in the pathway leading to the synthesis of cholesterol. Binding of cholesterol to the enzyme reduces the enzyme's activity significantly. Cholesterol is not a substrate for the enzyme, so it is therefore a heterotropic effector.

Notably, though, cholesterol is the end-product of the pathway that HMG-CoA reductase catalyzes a reaction in. When enzymes are inhibited by an end-product of the pathway in which they participate, they are said to exhibit feedback inhibition.

Feedback inhibition always operates by allosterism and further, provides important and efficient control of an entire pathway. By inhibiting an early enzyme in a pathway, the flow of materials (and ATP hydrolysis required for their processing) for the entire pathway is stopped or reduced, assuming there are not alternate supply methods.

Pathway control

In the cholesterol biosynthesis pathway, stopping this one enzyme has the effect of shutting off (or at least slowing down) the entire pathway. This is significant because after catalysis by HMG-CoA reductase, there are over 20 further reactions necessary to make cholesterol, many of them requiring ATP energy. Shutting down one reactions stops all of them. Another excellent example of allosteric control and feedback inhibition is the enzyme ATCase, discussed below.

ATCase

Another interesting example of allosteric control and feedback inhibition is associated with the enzyme Aspartate

Transcarbamoylase (ATCase). This enzyme, which catalyzes a step in the synthesis of pyrimidine nucleotides, has 12 subunits. These include six identical catalytic subunits and six identical regulatory subunits. The catalytic subunits bind to substrate and catalyze a reaction. The regulatory subunits bind to either ATP or CTP. If they bind to ATP, the enzyme subunits arrange themselves in the R-state.

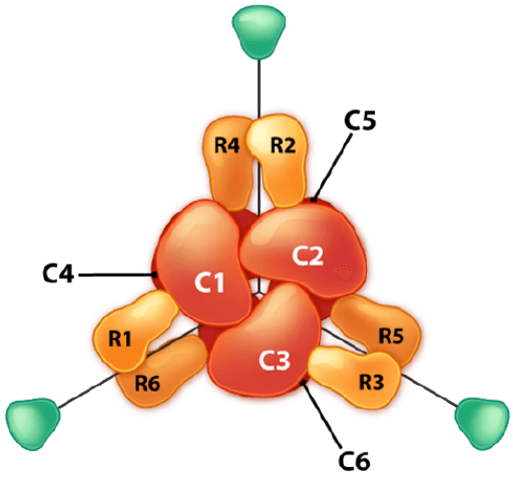


Figure 4.45 - Schematic structure of ATCase. Regulatory Units = R, Catalytic Units = C. Image by Aleia Kim

R-state

The R-state of ATCase allows the substrate to have easier access to the six active sites and the reaction occurs more rapidly. For the same amount of substrate, an enzyme in the R-state will have a higher velocity than the same enzyme that is not in the R-state. By contrast, if the enzyme binds to CTP on one of its regulatory subunits, the subunits will arrange in the T-state and in this form, the substrate will not have easy access to the active sites, resulting in a slower velocity for the same concentration of substrate compared to the R-state. ATCase is interesting in that it can also flip into the R-state when one of the substrates (aspartate) binds to an active site within one of the catalytic subunits.

Aspartate has the effect of activating the catalytic action of the enzyme by favoring the R-state. Thus, aspartate, which is a substrate of the enzyme is a homotropic effector and ATP and CTP, which are not substrates of the enzyme are heterotropic effectors of ATCase.

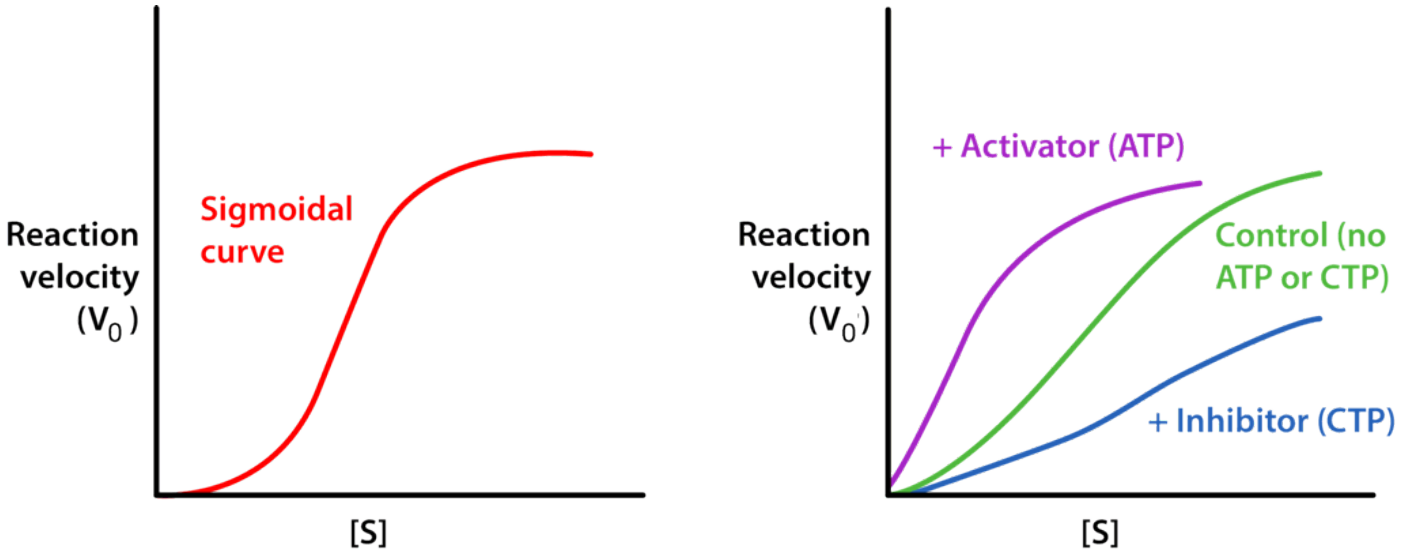


Figure 4.46 - Plots of V0 vs. [S] for ATCase. Left - Allosteric effect of aspartate. Right - Allosteric effects of ATP (activator) and CTP (inhibitor). Image by Pehr Jacobson

Allosteric models

There are three models commonly used to explain how allosterism regulates multi-subunit enzyme activity. They are known as

- the Monod-Wyman-Changeux (MWC) model (also known as the concerted model),
- the sequential model (also known as KNF),
- and the morpheein model.

All models describe a Tense (T) state that is less catalytically active and a Relaxed (R) state that is more catalytically active. The models differ in how the states change.

Sequential model

In the sequential model, binding of an allosteric effector by one subunit causes it to change from T to R state (or vice versa) and that change makes it easier for adjacent subunits to similarly change state. With this model , there is a cause/effect relationship between binding of an effector by one subunit and change of state by an adjacent subunit.

In hemoglobin, for example, binding of one oxygen by one unit of the complex may induce that unit to flip to the R-state and, through interactions with other subunits, cause them to favor adopting the R configuration before they bind to oxygen. In this way, binding of one subunit favors binding of others and cooperativity can be explained by the change in binding affinity as oxygen concentration changes.

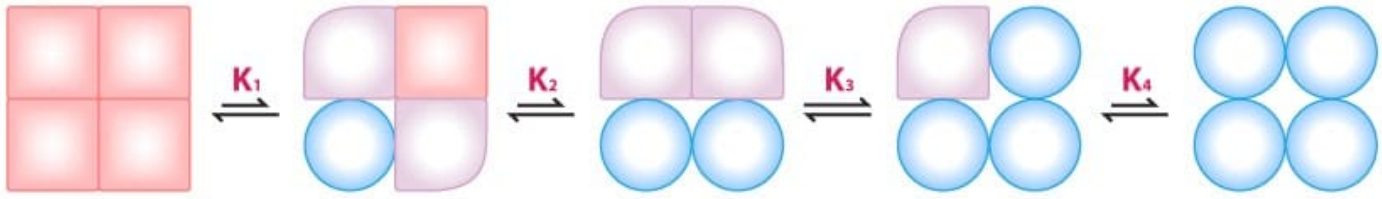


Figure 4.47 - Sequential model of allosteric regulation. Round = R-State. Square = T-state.. Image by Aleia Kim

MWC model

The MWC model is less intuitive. In it, the entire complex changes state from T to R (or vice versa) independently of the binding of effectors. Flipping between T-states and R-states is postulated to be in an equilibrium of states in the absence of effector (for example, a 50 to 1 ratio of T/R. This ratio is referred to as L, so L = T/R). Binding of effector by the enzyme complex has the tendency of “locking” the complex in a state. Binding of inhibitors will increase the ratio of T/R whereas binding of activators will increase R and thus decrease the ration of T/R.

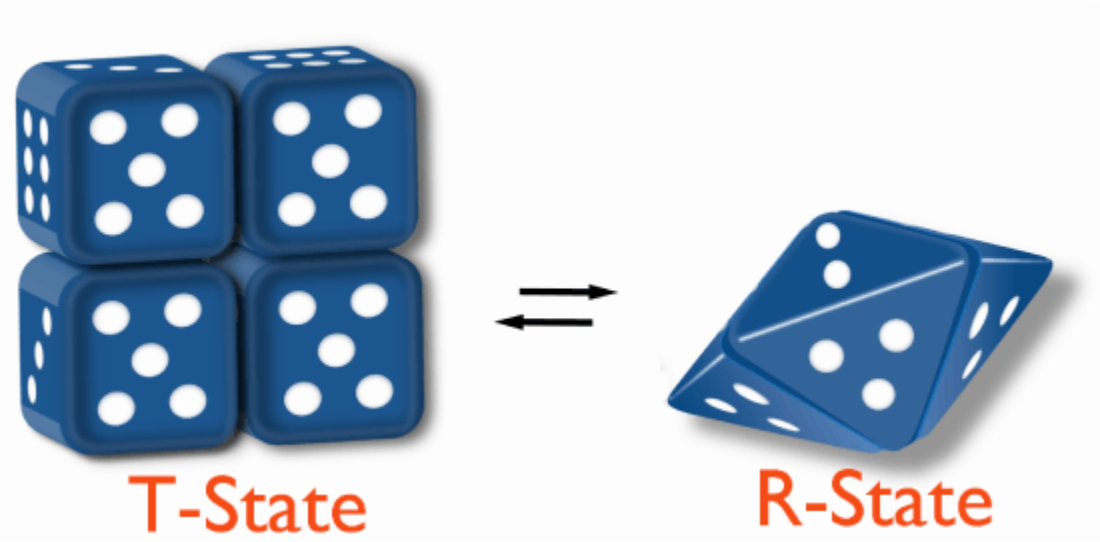


Figure 4.48 - The MWC Model - The multisubunit complex flips as a whole and is in equilibrium. Wikipedia

Morpheein model

The morpheein model is similar to the MWC model, but with an added step of dissociation of the subunits. The MWC model proposes that flipping between R and T states occurs by the complex as a whole and occurs on all units simultaneously. The morpheein model instead proposes that the multi-subunit enzyme breaks down to individual units which can then flip in structure and re-form the complex. In the morpheein model, only identically shaped units (all R, for example) can come together in the complex, thus explaining the “all-R-” or “all-T-” state found in the MWC model.

A large number of enzymes, including prominent ones like citrate synthase, acetyl-CoA carboxylase, glutamate dehydrogenase, ribonucleotide reductase and lactate dehydrogenase have behavior consistent with the morpheein model.

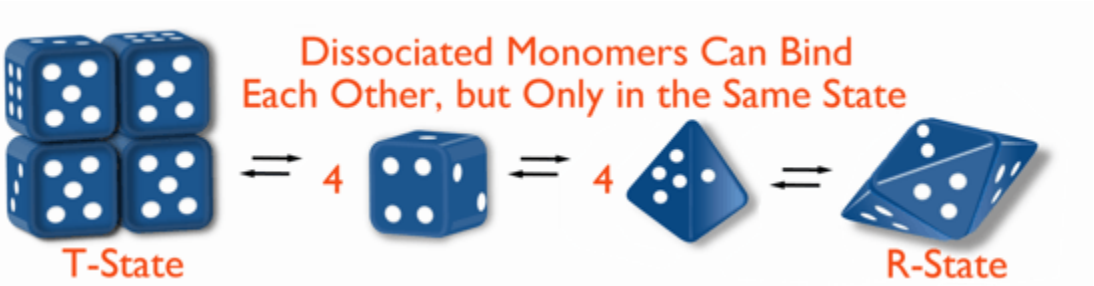


Figure 4.49 - Morpheein model of allosterism. States flip as monomers and aggregate only with same monomer. Wikipedia

Covalent control of enzymes

Some enzymes are synthesized in a completely inactive form and their activation requires covalent bonds in them to be cleaved. Such inactive forms of enzymes are called zymogens. Examples include the proteins involved in blood clotting and proteolytic enzymes of the digestive system, such as trypsin, chymotrypsin, pepsin, and others.

Synthesizing some enzymes in an inactive form makes very good sense when an enzyme’s activity might be harmful to the tissue where it is being made. For example, the painful condition known as pancreatitis arises when digestive enzymes made in the pancreas are activated too soon and end up attacking the pancreas.

Cascades

For both the blood clotting enzymes and the digestive enzymes, the zymogens are activated in a protease cascade. This occurs when activation of one enzyme activates others in a sort of chain reaction. In such a scheme the first enzyme activated proteolytically cleaves the second zymogen, causing it to be activated, which in turn activates a third and this may proceed through several levels of enzymatic action (Figure 4.50).

The advantage of cascades is that they allow a large amount of zymogens to become activated fairly quickly, since there is an amplification of the signal at each level of catalysis.

Zymogens are also abundant in blood. Blood clotting involves polymerization of a protein known as fibrin. Since random formation of fibrin is extremely hazardous because it can block the flow of blood, potentially causing heart attack/stroke, the body synthesizes fibrin as a zymogen (fibrinogen) and its activation results from a “cascade” of activations of proteases that arise when a signal is received from a wound. Similarly, the enzyme catalyzing removal of fibrin clots (plasmin) is also synthesized as a zymogen (plasminogen), since random clot removal would also be hazardous (see below also).

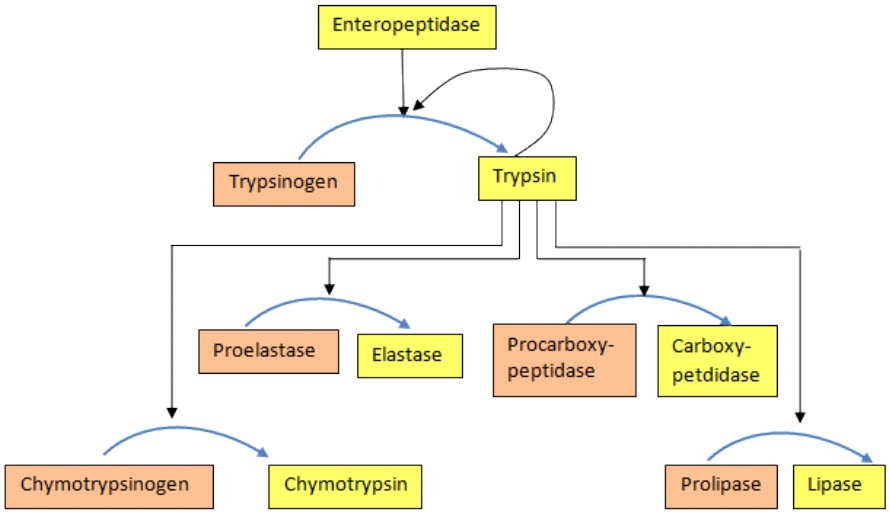


Figure 4.50 - Protease activation scheme. Wikipedia

Phosphorylation/dephosphorylation

Another common mechanism for control of enzyme activity by covalent modification is phosphorylation. The phosphorylation of enzymes (on the side chains of serine, threonine or tyrosine residues) is carried out by protein kinases. Enzymes activated by phosphorylation can be regulated by the addition of phosphate groups by kinases or their removal by phosphatases. Thus, this type of covalent modification is readily reversible, in contrast to proteolytic cleavage.

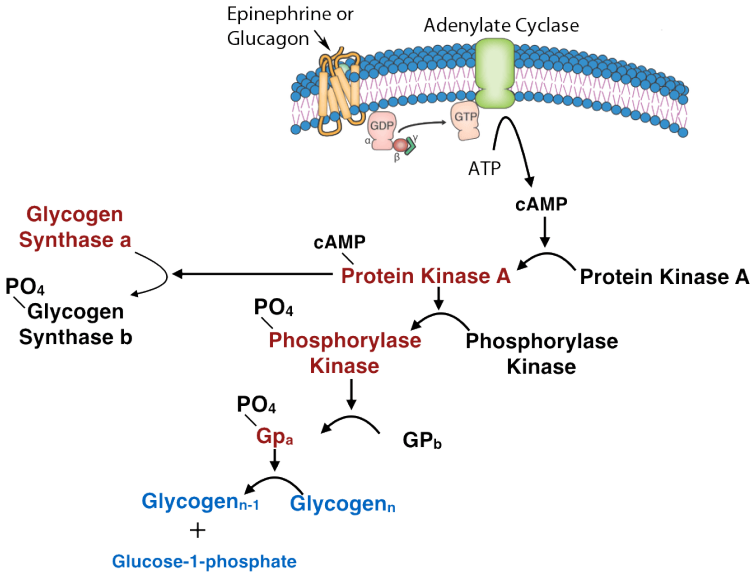


Figure 4.51 - Regulation by covalent modification of glycogen catabolism enzymes

Reduction/oxidation

An interesting covalent control of enzymes using reduction/oxidation is exhibited in photosynthetic plants. In the light phase of photosynthesis, electrons are excited by light and flow through carriers to NADP+, forming NADPH. Thus, in the light, the NADPH concentration is high. When NADPH concentration is high, the concentration of reduced ferredoxin (a molecule donating electrons to NADP+) is also high.

Reduced ferredoxin can transfer electrons to thioredoxin, reducing it. Reduced thioredoxin can, in turn, transfer electrons to proteins to reduce their disulfide bonds. Four enzymes related to the Calvin cycle can receive electrons from thioredoxin and become activated, as a result.

These include sedoheptulose 1,7-bisphosphatase, ribulose-5-phosphate kinase, fructose 1,6-bisphosphatase, and glyceraldehyde 3-phosphate dehydrogenase. Thus, in the light, electrons flow, causing NADPH to accumulate and ferredoxin to push electrons in the direction of these enzymes above, activating them and favoring the Calvin cycle. In the dark, the concentration of reduced NADPH, reduced ferredoxin, and reduced thioredoxin fall, resulting in loss of electrons by the Calvin cycle enzymes (oxidations that re-form disulfide bonds) and the Calvin cycle inactivates.

Other enzyme control mechanisms

Other means of controlling enzymes relate to access to substrate (substrate-level control) and control of enzyme synthesis. Hexokinase is an enzyme that is largely regulated by availability of its substrate, glucose. When glucose concentration is low, the product of the enzyme's catalysis, glucose-6-phosphate, inhibits the enzyme's function.

Regulation of enzymes by controlling their synthesis is covered later in the book in the discussion relating to control of gene expression.

This page titled 7.4: Control of Enzymatic Activity is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 4.2: Control of Enzymatic Activity by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

8: CARBOHYDRATE STRUCTURE AND METABOLISM

8.1: Carbohydrates overview
8.2: Glycolysis
8.3: Gluconeogenesis
8.4: Fermentation
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation
8.6: Citric Acid Cycle and Related Pathways
8.7: Electron Transport and Oxidative Phosphorylation
8.8: Carbohydrate Storage and Breakdown

This page titled 8: Carbohydrate structure and metabolism is shared under a license and was authored, remixed, and/or curated by LibreTexts.

8.1: Carbohydrates overview



Carbohydrates

Carbohydrates are one of the four main classes of macromolecules that make up all cells and are an essential part of our diet; grains, fruits, and vegetables are all natural sources. While we may be most familiar with the role carbohydrates play in nutrition, they also have a variety of other essential functions in humans, animals, plants, and bacteria. In this section, we will discuss and review basic concepts of carbohydrate structure and nomenclature, and a variety of functions they play in cells.

Molecular structures

In their simplest form, **carbohydrates** can be represented by the stoichiometric formula $(CH_2O)_n$, where $_n$ is the number of carbons in the molecule. For simple carbohydrates, the ratio of carbon-to-hydrogen-to-oxygen in the molecule is 1:2:1. This formula also explains the origin of the term “carbohydrate”: the components are carbon (“carbo”) and the components of water (“hydrate”). Simple carbohydrates are classified into three subtypes: monosaccharides, disaccharides, and polysaccharides, which will be discussed below. While simple carbohydrates fall nicely into this 1:2:1 ratio, carbohydrates can also be structurally more complex. For example, many carbohydrates contain functional groups (remember them from our basic discussion about chemistry) besides the obvious hydroxyl. For example, carbohydrates can have phosphates or amino groups substituted at a variety of sites within the molecule. These functional groups can provide additional properties to the molecule and will alter its overall function. However, even with these types of substitutions, the basic overall structure of the carbohydrate is retained and easily identified.

Nomenclature

One issue with carbohydrate chemistry is the nomenclature. Here are a few quick and simple rules:

1. Simple carbohydrates, such as glucose, lactose, or dextrose, end with an "-ose."
2. Simple carbohydrates can be classified based on the number of carbon atoms in the molecule, as with triose (three carbons), pentose (five carbons), or hexose (six carbons).
3. Simple carbohydrates can be classified based on the functional group found in the molecule, i.e ketose (contains a ketone) or aldose (contains an aldehyde).
4. Polysaccharides are often organized by the number of sugar molecules in the chain, such as in a monosaccharide, disaccharide, or trisaccharide.

For a short video on carbohydrate classification, see the 10-minute Khan Academy video by clicking here.

Monosaccharides

Monosaccharides ("mono-" = one; "sacchar-" = sweet) are simple sugars; the most common is glucose. In monosaccharides, the number of carbons usually ranges from three to seven. If the sugar has an aldehyde group (the functional group with the structure R-CHO), it is known as an aldose; if it has a ketone group (the functional group with the structure RC(=O)R'), it is known as a ketose.

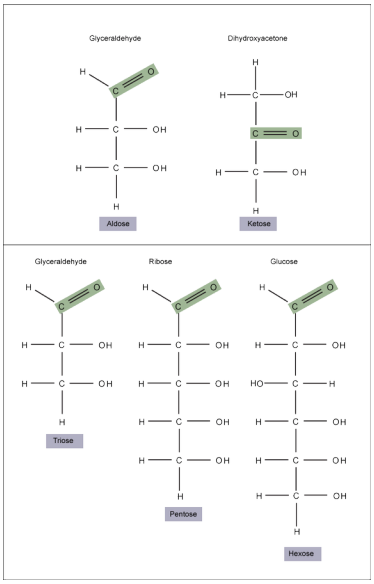


Figure 1. *Monosaccharides are classified based on the position of their carbonyl group and the number of carbons in the backbone. Aldoses have a carbonyl group (indicated in green) at the end of the carbon chain and ketoses have a carbonyl group in the middle of the carbon chain. Trioses, pentoses, and hexoses have three, five, and six carbons in their backbones, respectively. Attribution: Marc T. Facciotti (own work).*

Glucose versus galactose

Galactose (part of lactose, or milk sugar) and **glucose** (found in sucrose, glucose disaccharide) are other common monosaccharides. The chemical formula for glucose and galactose is $C_6H_{12}O_6$; both are hexoses, but the arrangements of the hydrogens and hydroxyl groups are different at position C_4. Because of this small difference, they differ structurally and chemically and are known as chemical isomers because of the different arrangement of functional groups around the asymmetric carbon; both of these monosaccharides have more than one asymmetric carbon (compare the structures in the figure below).

Fructose versus both glucose and galactose

A second comparison can be made when looking at glucose, galactose, and **fructose** (the second carbohydrate that with glucose makes up the disaccharide sucrose and is a common sugar found in fruit). All three are hexoses; however, there is a major structural difference between glucose and galactose versus fructose: the carbon that contains the **carbonyl** (C=O).

In glucose and galactose, the carbonyl group is on the C_1 carbon, forming an **aldehyde** group. In fructose, the carbonyl group is on the C_2 carbon, forming a **ketone** group. The former sugars are called **aldoses** based on the aldehyde group that is formed; the latter is designated as a **ketose** based on the ketone group. Again, this difference gives fructose different chemical and structural properties from those of the aldoses, glucose, and galactose, even though fructose, glucose, and galactose all have the same chemical composition: $C_6H_{12}O_6$.

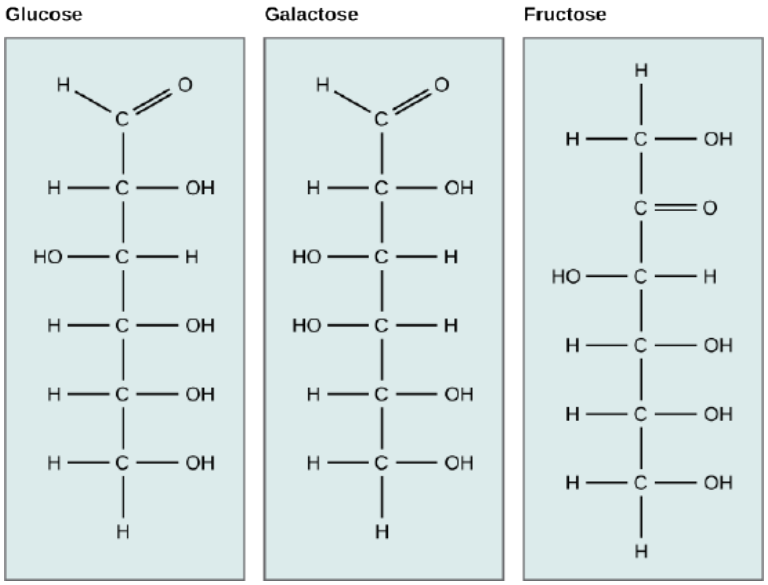


Figure 2. *Glucose, galactose, and fructose are all hexoses. They are structural isomers, meaning they have the same chemical formula ($C_6H_{12}O_6$) but a different arrangement of atoms.*

Linear versus ring form of the monosaccharides

Monosaccharides can exist as a linear chain or as ring-shaped molecules. In aqueous solutions, monosaccharides are usually found in ring form (Figure 3). Glucose in a ring form can have two different arrangements of the hydroxyl group (OH) around the anomeric carbon (C_1 that becomes asymmetric in the process of ring formation). If the hydroxyl group is below C_1 in the sugar, it is said to be in the alpha (α) position, and if it is above C_1 in the sugar, it is said to be in the beta (β) position.

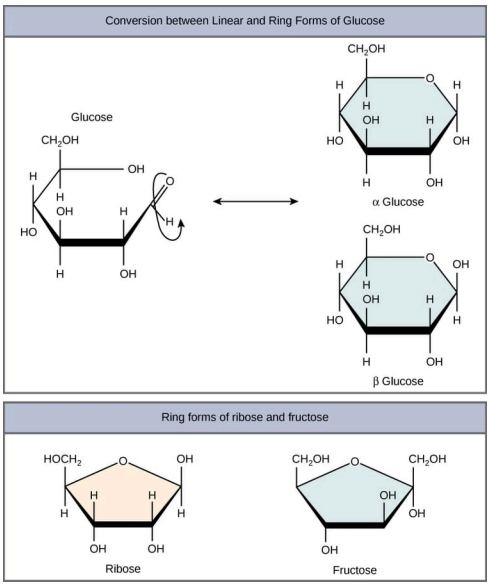


Figure 3. *Five- and six-carbon monosaccharides exist in equilibrium between linear and ring form. When the ring forms, the side chain it closes on is locked into an α or β position. Fructose and ribose also form rings, although they form five-membered rings as opposed to the six-membered ring of glucose.*

Disaccharides

Disaccharides ("di-" = two) form when two monosaccharides undergo a dehydration reaction (also known as a condensation

reaction or dehydration synthesis). During this process, the hydroxyl group of one monosaccharide combines with the hydrogen of another monosaccharide, releasing a molecule of water and forming a covalent bond. A covalent bond formed between a carbohydrate molecule and another molecule (in this case, between two monosaccharides) is known as a **glycosidic** bond. Glycosidic bonds (also called glycosidic linkages) can be of the alpha or the beta type.

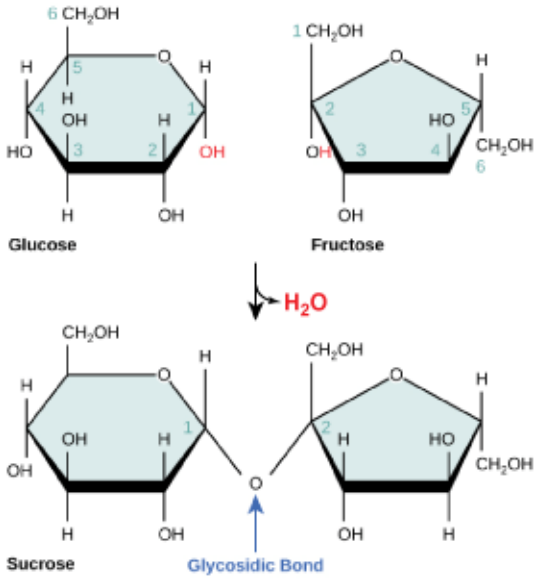


Figure 4. *Sucrose is formed when a monomer of glucose and a monomer of fructose are joined in a dehydration reaction to form a glycosidic bond. In the process, a water molecule is lost. By convention, the carbon atoms in a monosaccharide are numbered from the terminal carbon closest to the carbonyl group. In sucrose, a glycosidic linkage is formed between the C_1 carbon in glucose and the C_2 carbon in fructose.*

Common disaccharides include lactose, maltose, and sucrose (Figure 5). Lactose is a disaccharide consisting of the monomers glucose and galactose. It is found naturally in milk. Maltose, or malt/grain sugar, is a disaccharide formed by a dehydration reaction between two glucose molecules. The most common disaccharide is sucrose, or table sugar, which is composed of the monomers glucose and fructose.

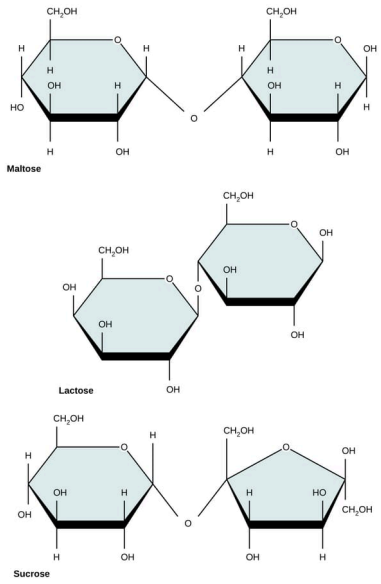


Figure 5. *Common disaccharides include maltose (grain sugar), lactose (milk sugar), and sucrose (table sugar).*

Sucrose	Lactose	Maltose

Polysaccharides

A long chain of monosaccharides linked by glycosidic bonds is known as a **polysaccharide** ("poly-" = many). The chain may be branched or unbranched, and it may contain different types of monosaccharides. The molecular weight may be 100,000 Daltons or more, depending on the number of monomers joined. Starch, glycogen, cellulose, and chitin are primary examples of polysaccharides.

Starch is the stored form of sugars in plants and is made up of a mixture of amylose and amylopectin; both are polymers of glucose. Plants are able to synthesize glucose. Excess glucose, the amount synthesized that is beyond the plant's immediate energy needs, is stored as starch in different plant parts, including roots and seeds. The starch in the seeds provides food for the embryo as it germinates and can also act as a source of food for humans and animals who may eat the seed. Starch that is consumed by humans is broken down by enzymes, such as salivary amylases, into smaller molecules, such as maltose and glucose.

Starch is made up of glucose monomers that are joined by 1-4 or 1-6 glycosidic bonds; the numbers 1-4 and 1-6 refer to the carbon number of the two residues that have joined to form the bond. As illustrated in Figure 6, amylose is starch formed by unbranched chains of glucose monomers (only 1-4 linkages), whereas amylopectin is a branched polysaccharide (1-6 linkages at the branch points).

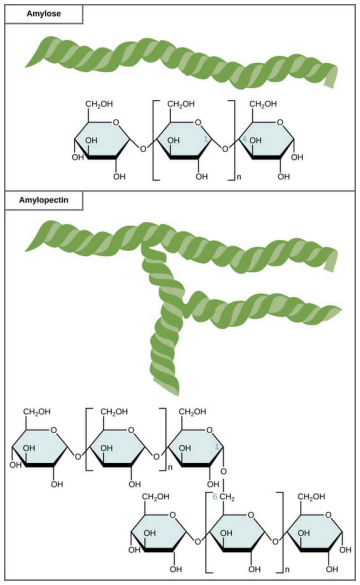


Figure 6. *Amylose and amylopectin are two different forms of starch. Amylose is composed of unbranched chains of glucose monomers connected by 1-4 glycosidic linkages. Amylopectin is composed of branched chains of glucose monomers connected by 1-4 and 1-6 glycosidic linkages. Because of the way the subunits are joined, the glucose chains have a helical structure. Glycogen (not shown) is similar in structure to amylopectin but more highly branched.*

Glycogen

Glycogen is a common stored form of glucose in humans and other vertebrates. Glycogen is the animal equivalent of starch and is a highly branched molecule usually stored in liver and muscle cells. Whenever blood glucose levels decrease, glycogen is broken down to release glucose in a process known as glycogenolysis.

Glycogen

Cellulose

Cellulose is the most abundant natural biopolymer. The cell wall of plants is mostly made of cellulose, which provides structural

support to the cell. Wood and paper are mostly cellulosic in nature. Cellulose is made up of glucose monomers that are linked by β 1-4 glycosidic bonds.

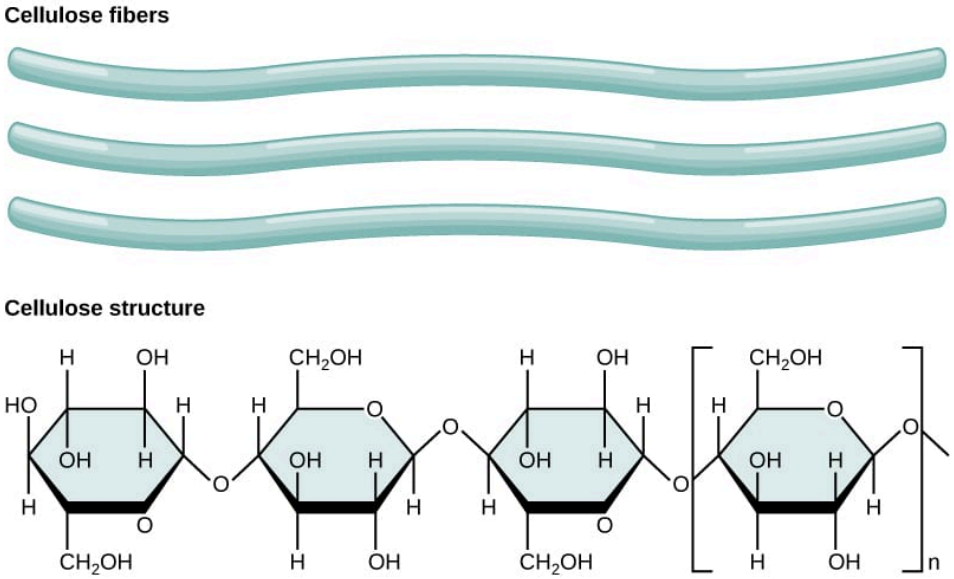


Figure 7. *In cellulose, glucose monomers are linked in unbranched chains by β 1-4 glycosidic linkages. Because of the way the glucose subunits are joined, every glucose monomer is flipped relative to the next one, resulting in a linear, fibrous structure.*

As shown in the figure above, every other glucose monomer in cellulose is flipped over, and the monomers are packed tightly as extended, long chains. This gives cellulose its rigidity and high tensile strength—which is so important to plant cells. While the β 1-4 linkage cannot be broken down by human digestive enzymes, herbivores such as cows, koalas, buffalos, and horses are able, with the help of the specialized flora in their stomach, to digest plant material that is rich in cellulose and use it as a food source. In these animals, certain species of bacteria and protists reside in the rumen (part of the digestive system of herbivores) and secrete the enzyme cellulase. The appendix of grazing animals also contains bacteria that digest cellulose, giving it an important role in the digestive systems of ruminants. Cellulases can break down cellulose into glucose monomers that can be used as an energy source by the animal. Termites are also able to break down cellulose because of the presence of other organisms in their bodies that secrete cellulases.

Interactions with carbohydrates

We have just discussed the various types and structures of carbohydrates found in biology. The next thing to address is how these compounds interact with other compounds. The answer to that is that it depends on the final structure of the carbohydrate. Because carbohydrates have many hydroxyl groups associated with the molecule, they are therefore excellent **H-bond donors** and **acceptors**. Monosaccharides can quickly and easily form H-bonds with water and are readily soluble. All of those H-bonds also make them quite "sticky". This is also true for many disaccharides and many short-chain polymers. Longer polymers may not be readily soluble.

Finally, the ability to form a variety of H-bonds allows polymers of carbohydrates or **polysaccharides** to form strong intramolecular and intermolocular bonds. In a polymer, because there are so many H-bonds, this can provide a lot of strength to the molecule or molecular complex, especially if the polymers interact. Just think of cellulose, a polymer of glucose, if you have any doubts.

Possible NB Discussion  **Point**

Lipids and carbohydrates are not just classes of macromolecules that we discuss in BIS 2A but are also two of the essential macronutrients that we can obtain from eating various foods. Some popularized diet programs (e.g., Atkins, ketogenic) suggest limiting carbohydrates and/or fats. As you learn more about biomolecules and their roles in living systems, are you refining your perspective on foods and diets? What have you learned so far? Do you think there is anything missing in your understanding? Are you able to better understand and evaluate certain diets, such as the aforementioned?

This page titled 8.1: Carbohydrates overview is shared under a license and was authored, remixed, and/or curated by Marc Facciotti.

8.2: Glycolysis

Glycolysis, which literally means “breakdown of sugar," is a catabolic process in which six-carbon sugars (hexoses) are oxidized and broken down into pyruvate molecules. The corresponding anabolic pathway by which glucose is synthesized is termed gluconeogenesis. Both glycolysis and gluconeogenesis are not major oxidative/reductive processes by themselves, with one step in each one involving loss/gain of electrons, but the product of glycolysis, pyruvate, can be completely oxidized to carbon dioxide. Indeed, without production of pyruvate from glucose in glycolysis, a major energy source for the cell is not available. By contrast, gluconeogenesis can synthesize glucose reductively from very simple materials, such as pyruvate and acetyl-CoA/ glyoxylate (at least in plants). For these reasons we include these pathways in the red/ox collection.

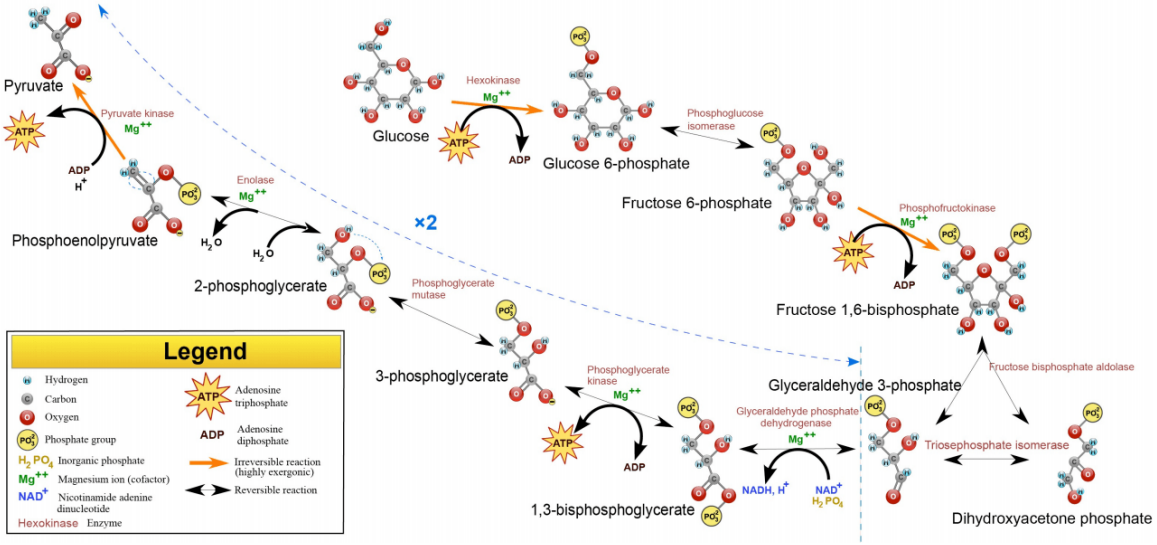


Figure 6.3.1: *The Reactions of Glycolysis*

Glucose is the most abundant hexose in nature and is the one people typically associate with glycolysis, but fructose (in the form of fructose-6-phosphate) is metabolized in the cell and galactose can easily be converted into glucose for catabolism in the pathway as well. The end metabolic products of the pathway are two molecules of ATP, two molecules of NADH and two molecules of pyruvate, which, in turn, can be oxidized further in citric acid cycle.

Intermediates

Glucose and fructose are the sugar ‘funnels’ serving as entry points to the glycolytic pathway. Other sugars must be converted to either of these forms to be directly metabolized. Some pathways, including the Calvin Cycle and the Pentose Phosphate Pathway (PPP, see below) contain intermediates in common with glycolysis, so in that sense, almost any cellular sugar can be metabolized here. Intermediates of glycolysis that are common to other pathways include glucose-6-phosphate (PPP, glycogen metabolism), F6P (PPP), G3P (Calvin, PPP), DHAP (PPP, glycerol metabolism, Calvin), 3PG (Calvin, PPP), PEP (C4 plant metabolism, Calvin), and pyruvate (fermentation, acetyl-CoA genesis, amino acid metabolism).

Reactions

The pathway of glycolysis begins with two inputs of energy. First, glucose gets a phosphate from ATP to make glucose-6-phosphate (G6P) and later fructose-6-phosphate (F6P) gets another phosphate from ATP to make fructose-1,6-bisphosphate (F1,6BP). With the pump thus primed, the pathway proceeds first to split the F1,6BP into two 3-carbon intermediates. Later the only oxidation step in the entire pathway occurs. In that reaction, glyceraldehyde-3-phosphate (G3P) is oxidized and a phosphate is added, creating 1,3-bisphosphoglycerate (1,3 BPG).

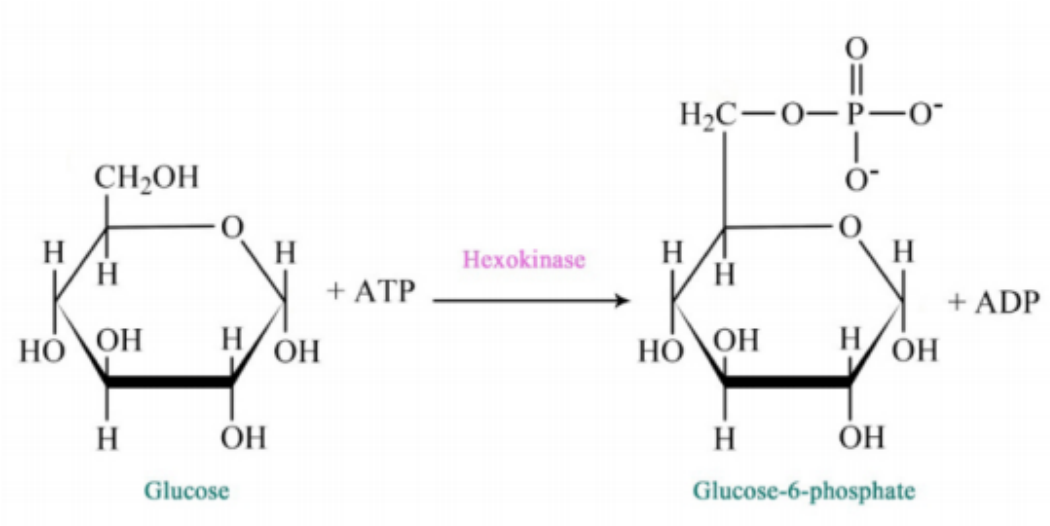


Figure 6.3.2: *Step 1 of Glycolysis*

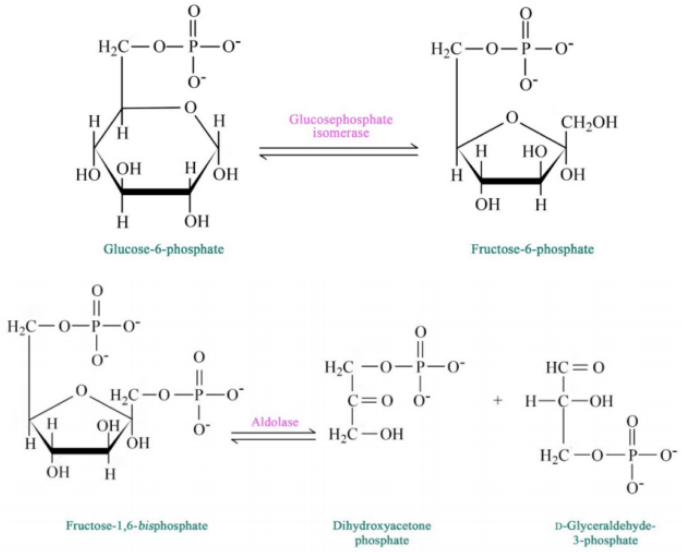


Figure 6.3.3: *Step 2 and 4 of Glycolysis*

The addition of the phosphate sometimes conceals the oxidation that occurred. G3P was an aldehyde. 1,3 BGP is an acid esterified to a phosphate. The two phosphates in the tiny 1,3BPG molecule repel each other and give the molecule high energy. It uses this energy to phosphorylate ADP to make ATP.

Since there are two 1,3 BPGs produced for every glucose, the two ATP produced replenish the two ATPs used to start the cycle.

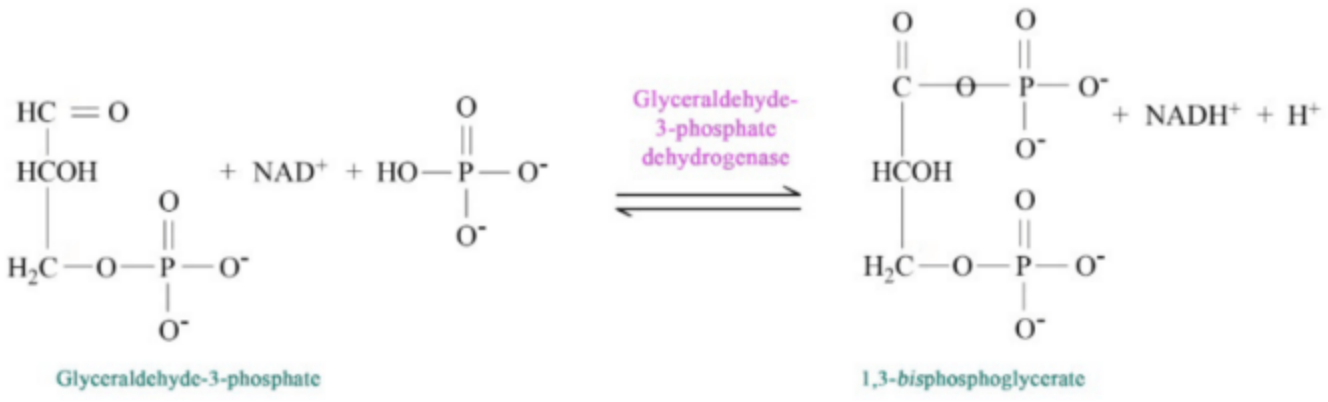


Figure 6.3.4: *Step 5 of Glycolysis*

The synthesis of ATP directly from a metabolic reaction is known as substrate level phosphorylation, though it is not a significant source of ATP. Glycolysis has two reactions during which substrate-level phosphorylation occurs.

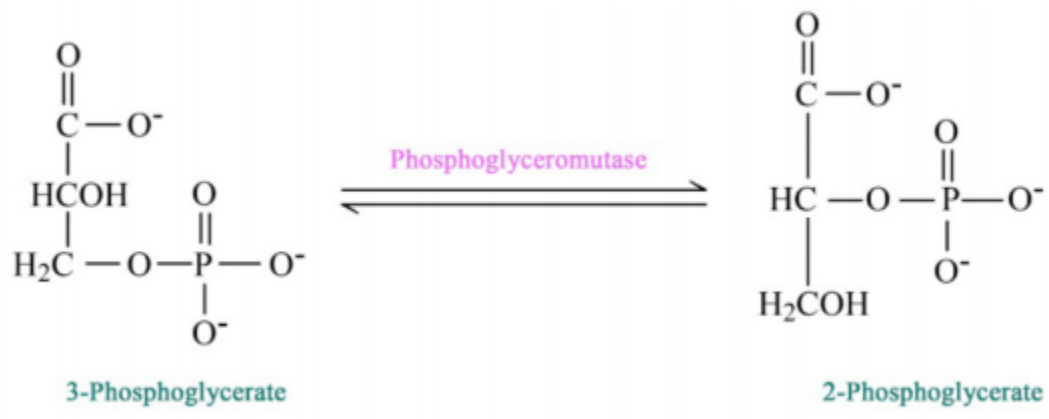


Figure 6.3.5: *Step 8 of Glycolysis*

The transfer of phosphate from 1,3BPG to ATP creates 3-phosphoglycerate (3-PG). Conversion of 3-PG to 2-PG occurs by an important mechanism. An intermediate in the reaction (catalyzed by phosphogly cerate mutase) is 2,3 BPG. This intermediate, which is stable, is released with low frequency by the enzyme instead of being converted to 2-PG. 2,3BPG is important because it binds to hemoglobin and stimulates release of oxygen. Thus, cells which are metabolizing glucose rapidly release more 2,3BPG and, as a result, stimulate release of more oxygen, supporting their needs.

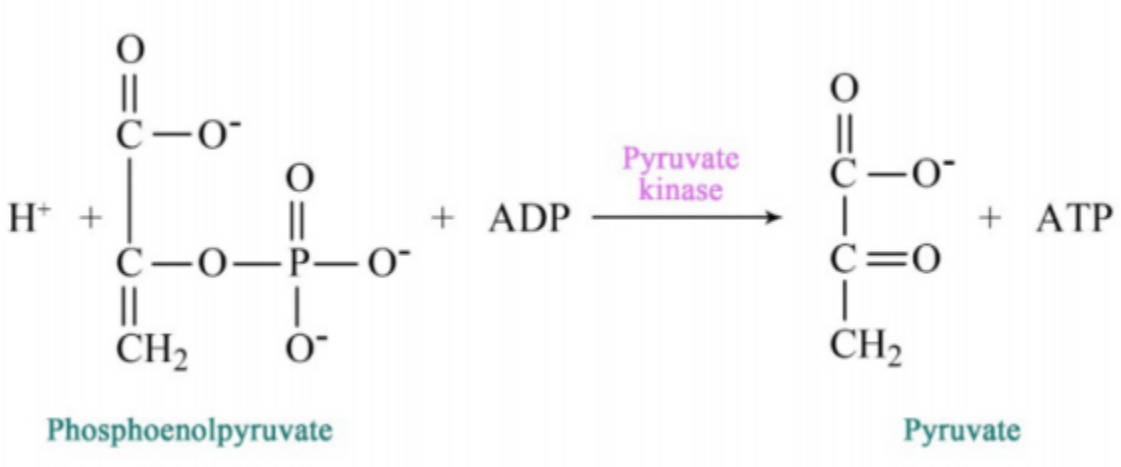


Figure 6.3.6: *Step 10 of Glycolysis*

2-PG is converted to phosphoenolpyyruvate (PEP) by removal of water, creating a very high energy intermediate. Conversion of PEP to pyruvate is the second substrate level phosphorylation of glycolysis, creating ATP. There is almost enough energy in PEP to stimulate production of a second ATP, but it is not used. Consequently, the energy is lost as heat. If you wonder why you get hot when you exercise, the reaction that converts PEP to pyruvate is a prime culprit.

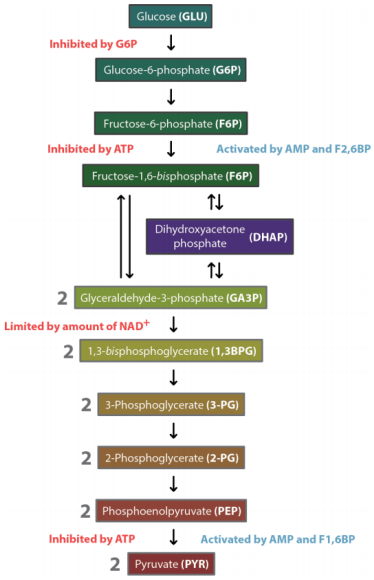


Figure 6.3.7: *Glycolysis Regulation*

Enzymes/Control

Control of glycolysis is unusual for a metabolic pathway, in that regulation occurs at three enzymatic points:

$$\underbrace{\text{Glucose} \rightleftharpoons \text{G}_6\text{P}}_{\text{hexokinase}} \tag{8.2.1}$$

$$\underbrace{\text{F}_6\text{P} \rightleftharpoons \text{F}_1,6\,\text{BP}}_{\text{phosphofructokinase (PFK)}} \tag{8.2.2}$$

and

$$\underbrace{\text{PEP} \rightleftharpoons \text{pyruvate}}_{\text{pyruvate kinase}}. \tag{8.2.3}$$

Glycolysis is regulated in a reciprocal fashion compared to its corresponding anabolic pathway, gluconeogenesis. Reciprocal regulation occurs when the same molecule or treatment (phosphorylation, for example) has opposite effects on catabolic and anabolic pathways. Reciprocal regulation is important when anabolic and corresponding catabolic pathways are occurring in the same cellular location.

As an example, consider regulation of PFK. It is activated by several molecules, most importantly fructose-2,6- bisphosphate (F2,6BP). This molecule has an inhibitory effect on the corresponding gluconeogenesis enzyme, fructose-1,6-bisphosphatase (F1,6BPase).

You might wonder why pyruvate kinase, the last enzyme in the pathway, is regulated. The answer is simple. Pyruvate kinase catalyzes the most energetically rich reaction of glycolysis. The reaction is favored so strongly in the forward direction that cells must do a ‘two-step’ around it in the reverse direction when making glucose. In other words, it takes two enzymes, two reactions, and two triphosphates to go from pyruvate back to PEP in gluconeogenesis. When cells are needing to make glucose, they can’t be sidetracked by having the PEP they have made in gluconeogenesis be converted directly back to pyruvate by pyruvate kinase. Consequently, pyruvate kinase is inhibited during gluconeogenesis, lest a “futile cycle" occur.

Another interesting control mechanism called feedforward activation involves pyruvate kinase. Pyruvate kinase is activated allosterically by F1,6BP. This molecule is a product of the PFK reaction and a substrate for the aldolase reaction. It should be

noted that the aldolase reaction is energetically unfavorable (high +$\Delta G°'$), thus allowing F1,6BP to accumulate. When this happens, some of the excess F1,6BP activates pyruvate kinase, which jump-starts the conversion of PEP to pyruvate. The resulting drop in PEP levels has the effect of “pulling" on the reactions preceding pyruvate kinase. As a consequence, the concentrations of G3P and DHAP fall, helping to move the aldolase reaction forward.

Pyruvate Metabolism

As noted, pyruvate produced in glycolysis can be oxidized to acetyl-CoA, which is itself oxidized in the citric acid cycle to carbon dioxide. That is not the only metabolic fate of pyruvate, though.

Pyruvate is a “starting" point for gluconeogenesis, being converted to oxaloacetate in the mitochondrion in the first step. Pyruvate in animals can also be reduced to lactate when oxygen is limiting. This reaction, which requires NADH produces NAD^+ and is critical for generating the latter molecule to keep the glyceraldehyde-3-phosphate dehydrogenase reaction of glycolysis going when there is no oxygen.

Oxygen is necessary for the electron transport system to operate and this, in turn, is what oxidizes NADH to NAD^+. In the absence of oxygen, thus, an alternative means of making NAD^+ is necessary, or else glycolysis will halt. Bacteria and yeast have NADH requiring reactions that regenerate NAD^+ while producing ethanol from pyruvate under anaerobic conditions, instead of lactic acid. Thus, fermentation of pyruvate is necessary to keep glycolysis operating when oxygen is limiting. It is also for these reasons that brewing of beer (using yeast) involves depletion of oxygen and muscles low in oxygen produce lactic acid (animals).

Pyruvate is a precursor of alanine which can be easily synthesized by transfer of a nitrogen from an amine donor, such as glutamic acid. Pyruvate can also be converted into oxaloacetate by carboxylation in the process of gluconeogenesis (see Figure 6.3.8).

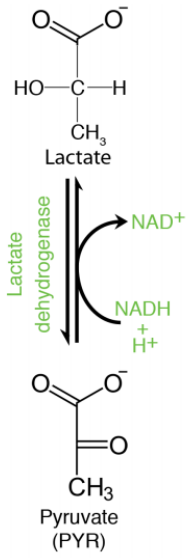


Figure 6.3.8: *Pyruvate Reduction*

The enzymes involved in pyruvate metabolism include pyruvate dehydrogenase (makes acetyl-CoA), lactate dehydrogenase (makes lactate), transaminases (make alanine), an
pyruvate carboxylase (makes oxaloacetate).

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 8.2: Glycolysis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 6.3: Glycolysis by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

8.3: Gluconeogenesis

The anabolic counterpart to glycolysis is gluconeogenesis, which occurs mostly in the cells of the liver and kidney. In seven of the eleven reactions of gluconeogenesis (starting from pyruvate), the same enzymes are used as in glycolysis, but the reaction directions are reversed. Notably, the ΔG values of these reactions in the cell are typically near zero, meaning their direction can be readily controlled by changing substrate and product concentrations.

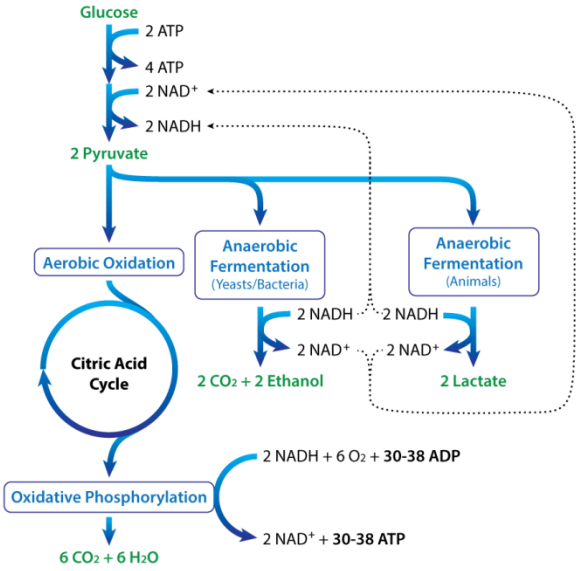


Figure 6.4.1: *Metabolic Redox*

The three regulated enzymes of glycolysis all catalyze reactions whose ΔG values are not close to zero, making manipulation of reaction direction non-trivial. Consequently, cells employ “work-around" reactions catalyzed by four different enzymes to favor gluconeogenesis, when appropriate.

Two of the enzymes (pyruvate carboxylase and PEP carboxykinase -PEPCK) catalyze reactions that bypass pyruvate kinase. F1,6BPase bypasses PFK and G6Pase bypasses hexokinase. Notably, pyruvate carboxylase and G6Pase are found in the mitochondria and endoplasmic reticulum, respectively, whereas the other two are found in the cytoplasm along with all of the enzymes of glycolysis. As a result, all of glycolysis and most of gluconeogenesis occurs in the cytoplasm. Controlling these pathways then becomes of critical importance because cells generally need to minimize the extent to which paired anabolic and catabolic pathways are occurring simultaneously, lest they waste energy and make no tangible product except heat. The mechanisms of controlling these pathways work, in some ways, in opposite fashions, called reciprocal regulation (see above).

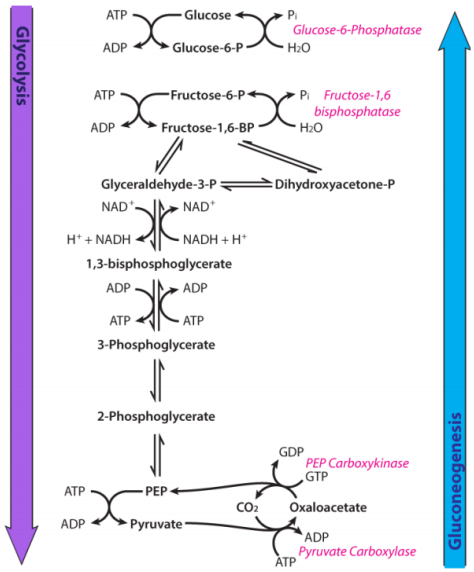


Figure 6.4.2: *Gluconeogensis and Glycolysis*

Besides reciprocal regulation, other mechanisms help control gluconeogenesis. First, PEPCK is controlled largely at the level of synthesis. Overexpression of PEPCK (stimulated by glucagon, glucocorticoids, and cAMP and inhibited by insulin) causes symptoms of diabetes. Pyruvate carboxylase is sequestered in the mitochondrion and is sensitive to acetyl-CoA, which is an allosteric activator. Acetyl-CoA concentrations increase as the citric acid cycle activity decreases. Glucose-6-phosphatase is present in low concentrations in many tissues, but is found most abundantly and importantly in the major gluconeogenic organs – the liver and kidney cortex.

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 8.3: Gluconeogenesis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 6.4: Gluconeogenesis by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

8.4: Fermentation

In aerobic respiration, the final electron acceptor is an oxygen molecule, O_2. If aerobic respiration occurs, then ATP will be produced using the energy of the high-energy electrons carried by NADH or $FADH_2$ to the electron transport chain. If aerobic respiration does not occur, NADH must be reoxidized to NAD^+ for reuse as an electron carrier for glycolysis to continue. How is this done? Some living systems use an organic molecule as the final electron acceptor. Processes that use an organic molecule to regenerate NAD^+ from NADH are collectively referred to as fermentation. In contrast, some living systems use an inorganic molecule (other than oxygen) as a final electron acceptor to regenerate NAD^+; both methods are anaerobic (do not require oxygen) to achieve NAD^+ regeneration and enable organisms to convert energy for their use in the absence of oxygen.

Lactic Acid Fermentation

The fermentation method used by animals and some bacteria like those in yogurt is lactic acid fermentation (Figure 8.4.1). This occurs routinely in mammalian red blood cells and in skeletal muscle that has insufficient oxygen supply to allow aerobic respiration to continue (that is, in muscles used to the point of fatigue). In muscles, lactic acid produced by fermentation must be removed by the blood circulation and brought to the liver for further metabolism. The chemical reaction of lactic acid fermentation is the following:

$$\text{Pyruvic acid} + \text{NADH} \leftrightarrow \text{lactic acid} + \text{NAD}^+$$

The enzyme that catalyzes this reaction is lactate dehydrogenase. The reaction can proceed in either direction, but the left-to-right reaction is inhibited by acidic conditions. This lactic acid build-up causes muscle stiffness and fatigue. Once the lactic acid has been removed from the muscle and is circulated to the liver, it can be converted back to pyruvic acid and further catabolized for energy.

ART CONNECTION

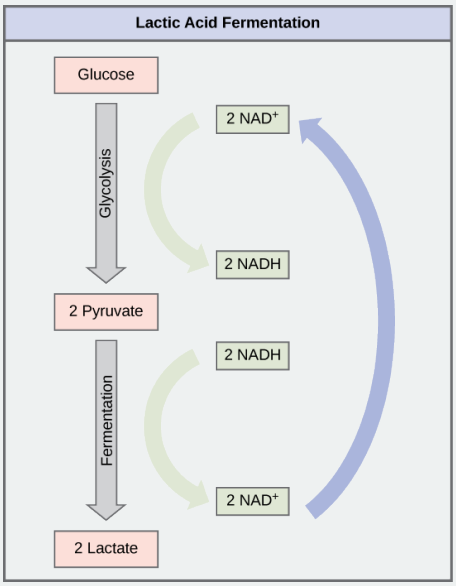


Figure 8.4.1: Lactic acid fermentation is common in muscles that have become exhausted by use.

Tremetol, a metabolic poison found in white snake root plant, prevents the metabolism of lactate. When cows eat this plant, Tremetol is concentrated in the milk. Humans who consume the milk become ill. Symptoms of this disease, which include vomiting, abdominal pain, and tremors, become worse after exercise. Why do you think this is the case?

Alcohol Fermentation

Another familiar fermentation process is alcohol fermentation (Figure 8.4.2), which produces ethanol, an alcohol. The alcohol fermentation reaction is the following:

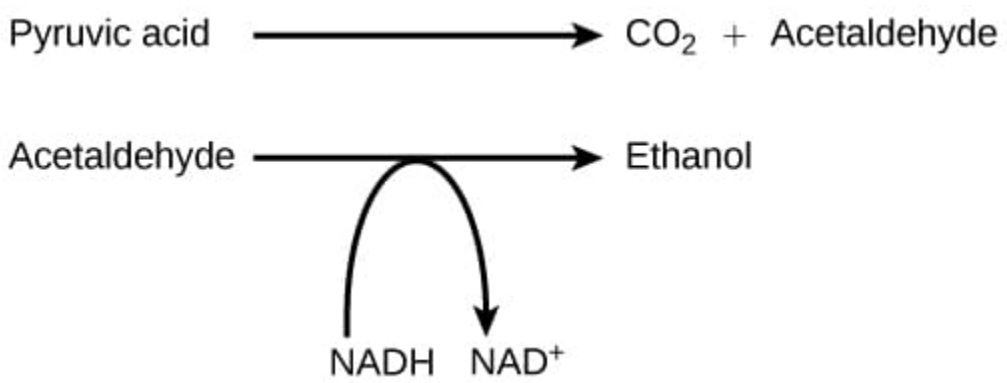


Figure 8.4.2: The reaction resulting in alcohol fermentation is shown.

In the first reaction, a carboxyl group is removed from pyruvic acid, releasing carbon dioxide as a gas. The loss of carbon dioxide reduces the molecule by one carbon atom, making acetaldehyde. The second reaction removes an electron from NADH, forming NAD^+ and producing ethanol from the acetaldehyde, which accepts the electron. The fermentation of pyruvic acid by yeast produces the ethanol found in alcoholic beverages (Figure 8.4.3). If the carbon dioxide produced by the reaction is not vented from the fermentation chamber, for example in beer and sparkling wines, it remains dissolved in the medium until the pressure is released. Ethanol above 12 percent is toxic to yeast, so natural levels of alcohol in wine occur at a maximum of 12 percent.



Figure 8.4.3***:*** *Fermentation of grape juice to make wine produces CO_2 as a byproduct. Fermentation tanks have valves so that pressure inside the tanks can be released.*

Anaerobic Cellular Respiration

Certain prokaryotes, including some species of bacteria and Archaea, use anaerobic respiration. For example, the group of Archaea called methanogens reduces carbon dioxide to methane to oxidize NADH. These microorganisms are found in soil and in the digestive tracts of ruminants, such as cows and sheep. Similarly, sulfate-reducing bacteria and Archaea, most of which are

anaerobic (Figure 8.4.4), reduce sulfate to hydrogen sulfide to regenerate NAD^+ from NADH.

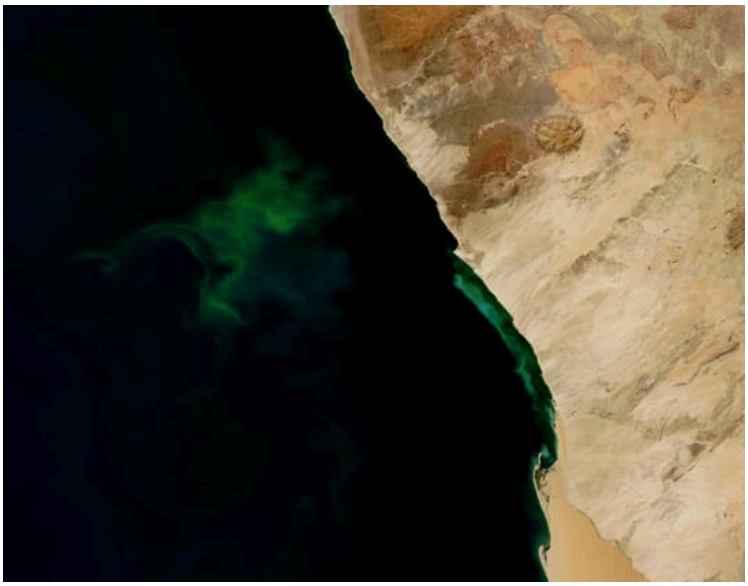


Figure 8.4.4: The green color seen in these coastal waters is from an eruption of hydrogen sulfide. Anaerobic, sulfate-reducing bacteria release hydrogen sulfide gas as they decompose algae in the water. (credit: NASA image courtesy Jeff Schmaltz, MODIS Land Rapid Response Team at NASA GSFC)

CONCEPT IN ACTION

Visit this site to see anaerobic cellular respiration in action.

Other fermentation methods occur in bacteria. Many prokaryotes are facultatively anaerobic. This means that they can switch between aerobic respiration and fermentation, depending on the availability of oxygen. Certain prokaryotes, like *Clostridia* bacteria, are obligate anaerobes. Obligate anaerobes live and grow in the absence of molecular oxygen. Oxygen is a poison to these microorganisms and kills them upon exposure. It should be noted that all forms of fermentation, except lactic acid fermentation, produce gas. The production of particular types of gas is used as an indicator of the fermentation of specific carbohydrates, which plays a role in the laboratory identification of the bacteria. The various methods of fermentation are used by different organisms to ensure an adequate supply of NAD^+ for the sixth step in glycolysis. Without these pathways, that step would not occur, and no ATP would be harvested from the breakdown of glucose.

Section Summary

If NADH cannot be metabolized through aerobic respiration, another electron acceptor is used. Most organisms will use some form of fermentation to accomplish the regeneration of NAD^+, ensuring the continuation of glycolysis. The regeneration of NAD^+ in fermentation is not accompanied by ATP production; therefore, the potential for NADH to produce ATP using an electron transport chain is not utilized.

Art Connections

Figure 8.4.1 Tremetol, a metabolic poison found in white snake root plant, prevents the metabolism of lactate. When cows eat this plant, Tremetol is concentrated in the milk. Humans who consume the milk become ill. Symptoms of this disease, which include vomiting, abdominal pain, and tremors, become worse after exercise. Why do you think this is the case?

The illness is caused by lactic acid build-up. Lactic acid levels rise after exercise, making the symptoms worse. Milk sickness is rare today, but was common in the Midwestern United States in the early 1800s.

Glossary

anaerobic cellular respiration
: the use of an electron acceptor other than oxygen to complete metabolism using electron transport-based chemiosmosis

fermentation
: the steps that follow the partial oxidation of glucose via glycolysis to regenerate NAD^+; occurs in the absence of oxygen and uses an organic compound as the final electron acceptor

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 8.4: Fermentation is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 4.4: Fermentation by OpenStax is licensed CC BY 4.0.

8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

The Citric Acid Cycle

In eukaryotic cells, the pyruvate molecules produced at the end of glycolysis are transported into mitochondria, which are sites of cellular respiration. If oxygen is available, aerobic respiration will go forward. In mitochondria, pyruvate will be transformed into a two-carbon acetyl group (by removing a molecule of carbon dioxide) that will be picked up by a carrier compound called coenzyme A (CoA), which is made from vitamin B_5. The resulting compound is called acetyl CoA. (Figure 8.5.1). Acetyl CoA can be used in a variety of ways by the cell, but its major function is to deliver the acetyl group derived from pyruvate to the next pathway in glucose catabolism.

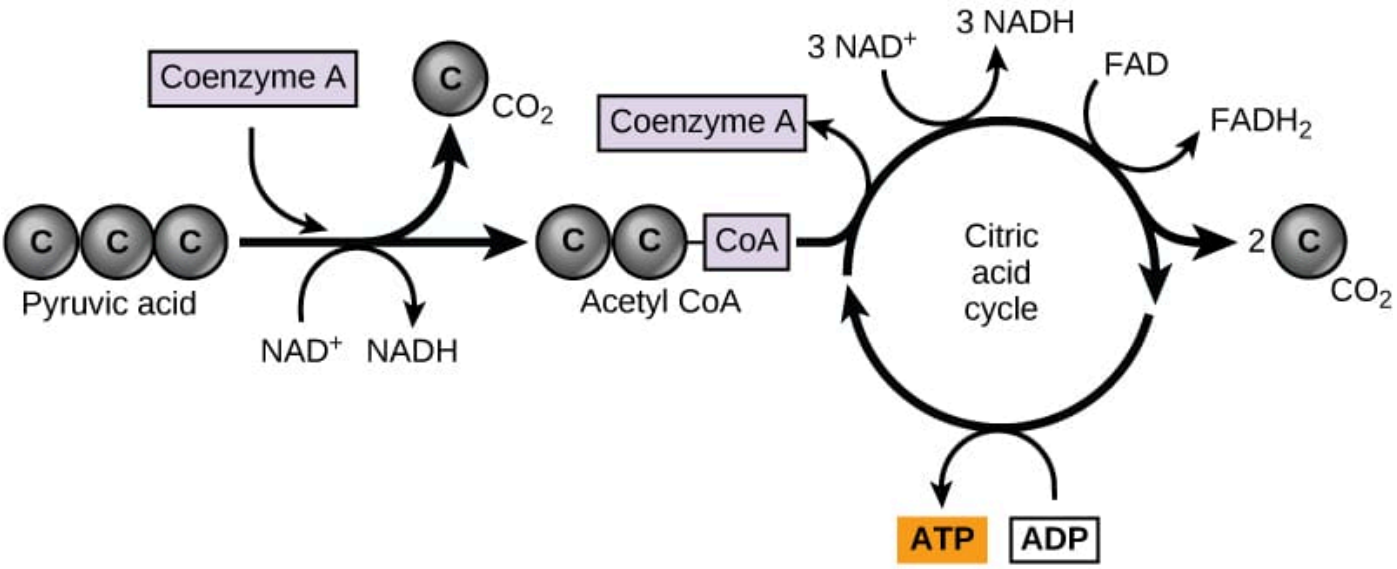


Figure 8.5.1: Pyruvate is converted into acetyl-CoA before entering the citric acid cycle.

Like the conversion of pyruvate to acetyl CoA, the citric acid cycle in eukaryotic cells takes place in the matrix of the mitochondria. Unlike glycolysis, the citric acid cycle is a closed loop: The last part of the pathway regenerates the compound used in the first step. The eight steps of the cycle are a series of chemical reactions that produces two carbon dioxide molecules, one ATP molecule (or an equivalent), and reduced forms (NADH and $FADH_2$) of NAD^+ and FAD^+, important coenzymes in the cell. Part of this is considered an aerobic pathway (oxygen-requiring) because the NADH and $FADH_2$ produced must transfer their electrons to the next pathway in the system, which will use oxygen. If oxygen is not present, this transfer does not occur.

Two carbon atoms come into the citric acid cycle from each acetyl group. Two carbon dioxide molecules are released on each turn of the cycle; however, these do not contain the same carbon atoms contributed by the acetyl group on that turn of the pathway. The two acetyl-carbon atoms will eventually be released on later turns of the cycle; in this way, all six carbon atoms from the original glucose molecule will be eventually released as carbon dioxide. It takes two turns of the cycle to process the equivalent of one glucose molecule. Each turn of the cycle forms three high-energy NADH molecules and one high-energy $FADH_2$ molecule. These high-energy carriers will connect with the last portion of aerobic respiration to produce ATP molecules. One ATP (or an equivalent) is also made in each cycle. Several of the intermediate compounds in the citric acid cycle can be used in synthesizing non-essential amino acids; therefore, the cycle is both anabolic and catabolic.

Oxidative Phosphorylation

You have just read about two pathways in glucose catabolism—glycolysis and the citric acid cycle—that generate ATP. Most of the ATP generated during the aerobic catabolism of glucose, however, is not generated directly from these pathways. Rather, it derives

from a process that begins with passing electrons through a series of chemical reactions to a final electron acceptor, oxygen. These reactions take place in specialized protein complexes located in the inner membrane of the mitochondria of eukaryotic organisms and on the inner part of the cell membrane of prokaryotic organisms. The energy of the electrons is harvested and used to generate a electrochemical gradient across the inner mitochondrial membrane. The potential energy of this gradient is used to generate ATP. The entirety of this process is called oxidative phosphorylation.

The electron transport chain (**Figure** 8.5.2**a**) is the last component of aerobic respiration and is the only part of metabolism that uses atmospheric oxygen. Oxygen continuously diffuses into plants for this purpose. In animals, oxygen enters the body through the respiratory system. Electron transport is a series of chemical reactions that resembles a bucket brigade in that electrons are passed rapidly from one component to the next, to the endpoint of the chain where oxygen is the final electron acceptor and water is produced. There are four complexes composed of proteins, labeled I through IV in Figure 8.5.2**c**, and the aggregation of these four complexes, together with associated mobile, accessory electron carriers, is called the electron transport chain. The electron transport chain is present in multiple copies in the inner mitochondrial membrane of eukaryotes and in the plasma membrane of prokaryotes. In each transfer of an electron through the electron transport chain, the electron loses energy, but with some transfers, the energy is stored as potential energy by using it to pump hydrogen ions across the inner mitochondrial membrane into the intermembrane space, creating an electrochemical gradient.

ART CONNECTION

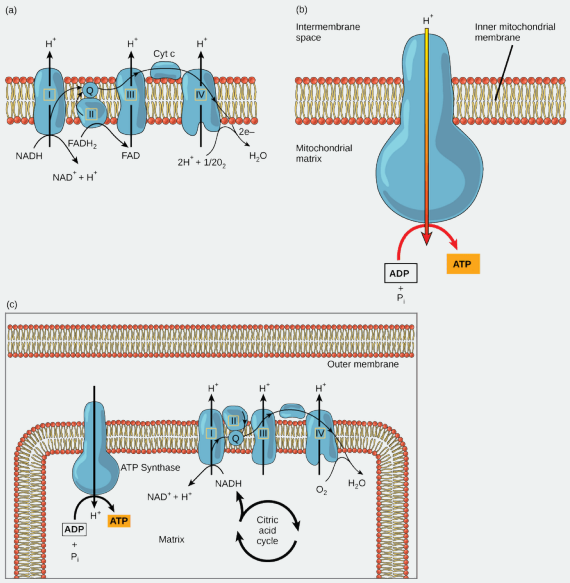


Figure 8.5.2: (a) The electron transport chain is a set of molecules that supports a series of oxidation-reduction reactions. (b) ATP synthase is a complex, molecular machine that uses an H^+ gradient to regenerate ATP from ADP. (c) Chemiosmosis relies on the potential energy provided by the H^+ gradient across the membrane.

Cyanide inhibits cytochrome c oxidase, a component of the electron transport chain. If cyanide poisoning occurs, would you expect the pH of the intermembrane space to increase or decrease? What affect would cyanide have on ATP synthesis?

Electrons from NADH and $FADH_2$ are passed to protein complexes in the electron transport chain. As they are passed from one complex to another (there are a total of four), the electrons lose energy, and some of that energy is used to pump hydrogen ions from the mitochondrial matrix into the intermembrane space. In the fourth protein complex, the electrons are accepted by oxygen, the terminal acceptor. The oxygen with its extra electrons then combines with two hydrogen ions, further enhancing the electrochemical gradient, to form water. If there were no oxygen present in the mitochondrion, the electrons could not be removed from the system, and the entire electron transport chain would back up and stop. The mitochondria would be unable to generate new ATP in this way, and the cell would ultimately die from lack of energy. This is the reason we must breathe to draw in new oxygen.

In the electron transport chain, the free energy from the series of reactions just described is used to pump hydrogen ions across the membrane. The uneven distribution of H^+ ions across the membrane establishes an electrochemical gradient, owing to the H^+ ions' positive charge and their higher concentration on one side of the membrane.

Hydrogen ions diffuse through the inner membrane through an integral membrane protein called ATP synthase (Figure 8.5.2 **b**). This complex protein acts as a tiny generator, turned by the force of the hydrogen ions diffusing through it, down their electrochemical gradient from the intermembrane space, where there are many mutually repelling hydrogen ions to the matrix, where there are few. The turning of the parts of this molecular machine regenerate ATP from ADP. This flow of hydrogen ions across the membrane through ATP synthase is called chemiosmosis.

Chemiosmosis (Figure 8.5.2**c**) is used to generate 90 percent of the ATP made during aerobic glucose catabolism. The result of the reactions is the production of ATP from the energy of the electrons removed from hydrogen atoms. These atoms were originally part of a glucose molecule. At the end of the electron transport system, the electrons are used to reduce an oxygen molecule to oxygen ions. The extra electrons on the oxygen ions attract hydrogen ions (protons) from the surrounding medium, and water is formed. The electron transport chain and the production of ATP through chemiosmosis are collectively called oxidative phosphorylation.

ATP Yield

The number of ATP molecules generated from the catabolism of glucose varies. For example, the number of hydrogen ions that the electron transport chain complexes can pump through the membrane varies between species. Another source of variance stems from the shuttle of electrons across the mitochondrial membrane. The NADH generated from glycolysis cannot easily enter mitochondria. Thus, electrons are picked up on the inside of the mitochondria by either NAD^+ or FAD^+. Fewer ATP molecules are generated when FAD^+ acts as a carrier. NAD^+ is used as the electron transporter in the liver and FAD^+ in the brain, so ATP yield depends on the tissue being considered.

Another factor that affects the yield of ATP molecules generated from glucose is that intermediate compounds in these pathways are used for other purposes. Glucose catabolism connects with the pathways that build or break down all other biochemical compounds in cells, and the result is somewhat messier than the ideal situations described thus far. For example, sugars other than glucose are fed into the glycolytic pathway for energy extraction. Other molecules that would otherwise be used to harvest energy in glycolysis or the citric acid cycle may be removed to form nucleic acids, amino acids, lipids, or other compounds. Overall, in living systems, these pathways of glucose catabolism extract about 34 percent of the energy contained in glucose.

CAREERS IN ACTION

Mitochondrial Disease Physician

What happens when the critical reactions of cellular respiration do not proceed correctly? Mitochondrial diseases are genetic disorders of metabolism. Mitochondrial disorders can arise from mutations in nuclear or mitochondrial DNA, and they result in the production of less energy than is normal in body cells. Symptoms of mitochondrial diseases can include muscle weakness, lack of coordination, stroke-like episodes, and loss of vision and hearing. Most affected people are diagnosed in childhood, although there are some adult-onset diseases. Identifying and treating mitochondrial disorders is a specialized medical field. The educational preparation for this profession requires a college education, followed by medical school with a specialization in medical genetics. Medical geneticists can be board certified by the American Board of Medical Genetics and go on to become associated with professional organizations devoted to the study of mitochondrial disease, such as the Mitochondrial Medicine Society and the Society for Inherited Metabolic Disease.

Summary

The citric acid cycle is a series of chemical reactions that removes high-energy electrons and uses them in the electron transport chain to generate ATP. One molecule of ATP (or an equivalent) is produced per each turn of the cycle.

The electron transport chain is the portion of aerobic respiration that uses free oxygen as the final electron acceptor for electrons removed from the intermediate compounds in glucose catabolism. The electrons are passed through a series of chemical reactions, with a small amount of free energy used at three points to transport hydrogen ions across the membrane. This contributes to the gradient used in chemiosmosis. As the electrons are passed from NADH or $FADH_2$ down the electron transport chain, they lose energy. The products of the electron transport chain are water and ATP. A number of intermediate compounds can be diverted into the anabolism of other biochemical molecules, such as nucleic acids, non-essential amino acids, sugars, and lipids. These same molecules, except nucleic acids, can serve as energy sources for the glucose pathway.

Art Connections

Figure 8.5.2 Cyanide inhibits cytochrome c oxidase, a component of the electron transport chain. If cyanide poisoning occurs, would you expect the pH of the intermembrane space to increase or decrease? What affect would cyanide have on ATP synthesis?

After cyanide poisoning, the electron transport chain can no longer pump electrons into the intermembrane space. The pH of the intermembrane space would increase, and ATP synthesis would stop.

Glossary

acetyl CoA
: the combination of an acetyl group derived from pyruvic acid and coenzyme A which is made from pantothenic acid (a B-group vitamin)

ATP synthase
: a membrane-embedded protein complex that regenerates ATP from ADP with energy from protons diffusing through it

chemiosmosis
: the movement of hydrogen ions down their electrochemical gradient across a membrane through ATP synthase to generate ATP

citric acid cycle
: a series of enzyme-catalyzed chemical reactions of central importance in all living cells that harvests the energy in carbon-carbon bonds of sugar molecules to generate ATP; the citric acid cycle is an aerobic metabolic pathway because it requires oxygen in later reactions to proceed

electron transport chain
: a series of four large, multi-protein complexes embedded in the inner mitochondrial membrane that accepts electrons from donor compounds and harvests energy from a series of chemical reactions to generate a hydrogen ion gradient across the membrane

oxidative phosphorylation
: the production of ATP by the transfer of electrons down the electron transport chain to create a proton gradient that is used by ATP synthase to add phosphate groups to ADP molecules

Contributors and Attributions

- Samantha Fowler (Clayton State University), Rebecca Roush (Sandhills Community College), James Wise (Hampton University). Original content by OpenStax (CC BY 4.0; Access for free at https://cnx.org/contents/b3c1e1d2-83...4-e119a8aafbdd).

This page titled 8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation is shared under a CC BY 4.0 license and was authored, remixed, and/or curated by OpenStax.

- 4.3: Citric Acid Cycle and Oxidative Phosphorylation by OpenStax is licensed CC BY 4.0.

8.6: Citric Acid Cycle and Related Pathways

Source: BiochemFFA_6_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Citric acid cycle

The primary catabolic pathway in the body is the citric acid cycle because it is here that oxidation to carbon dioxide occurs for breakdown products of the cell's major building blocks - sugars, fatty acids, and amino acids. The pathway is cyclic (Figure 6.63) and thus, doesn't really have a starting or ending point. All of the reactions occur in mitochondria, though one enzyme is embedded in the organelle's inner membrane. As needs change, cells may use a subset of the reactions of the cycle to produce a desired molecule rather than to run the entire cycle (see HERE).

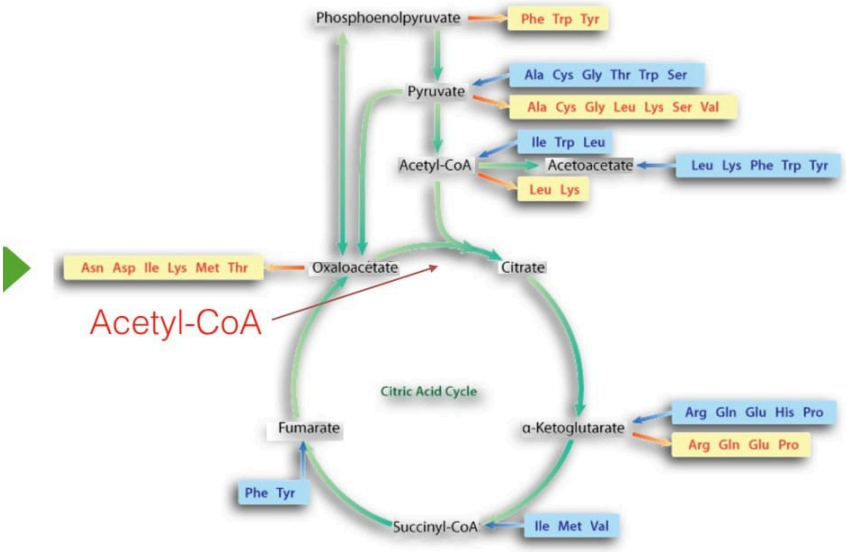


Figure 6.63 - Amino acid metabolism and the citric acid cycle. Amino acids boxed in yellow are made from the indicated intermediate. Amino acids in blue are made into the intermediate in catabolism. Image by Aleia Kim

Acetyl-CoA

The molecule "feeding" the citric acid cycle is acetyl-CoA and it can be obtained from pyruvate (from glycolysis), from fatty acid β-oxidation, from ketone bodies, and from amino acid metabolism. Molecules from other pathways feeding into the citric acid cycle for catabolism make the citric acid cycle 'cataplerotic'. It is worth noting that acetyl-CoA has very different fates, depending on the cell's energy status/needs (see HERE). The description below describes oxidation (catabolism) in citric acid cycle.

Anabolically, acetyl-CoA is also very important for providing building blocks for synthesis of fatty acids, ketone bodies, amino acids and cholesterol. Other citric acid cycle intermediates are also important in amino acid metabolism (Figure 6.63), heme synthesis, electron shuttling, and shuttling of acetyl-CoA across the mitochondrial inner membrane. The ability of the citric acid cycle to supply intermediates to pathways gives rise to the term 'anaplerotic.' It means 'to fill up.' Before discussing the citric acid cycle, it is important to first describe one important enzyme complex that is a major source of acetyl-CoA for the cycle.

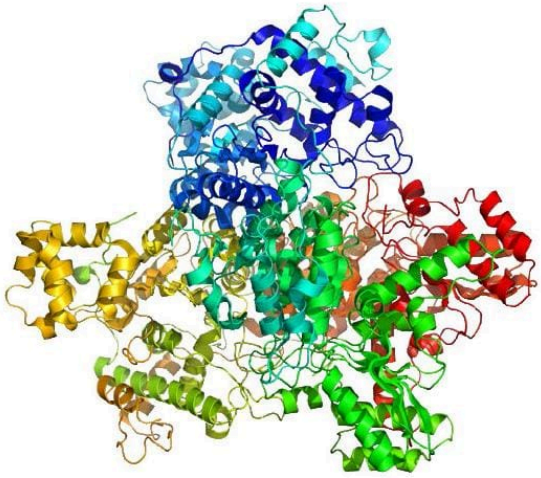


Figure 6.64 - E1 Subunit of Pyruvate Dehydrogenase. Wikipedia

Pyruvate decarboxylation

The pyruvate dehydrogenase enzyme is a complex of multiple copies of three subunits that catalyze the decarboxylation of pyruvate to form acetyl-CoA. The reaction mechanism requires use of five coenzymes. Pyruvate dehydrogenase is an enormous complex in mammals with a size five times greater than ribosomes.

Subunits

The three subunits are designated by E1, E2, and E3. E2 is also referred to as dihydrolipoamide acetyltransferase and E3 is more precisely called dihydrolipoyl dehydrogenase. Confusion arises with the name for E1. Some call it pyruvate dehydrogenase and others give it the name pyruvate decarboxylase. We will use pyruvate decarboxylase solely to refer to E1 and pyruvate dehydrogenase only to refer to the complex of E1, E2, and E3.

The catalytic actions of pyruvate dehydrogenase can be broken down into three steps, each taking place on one of the subunits. The steps, sequentially occurring on E1, E2, and E3, are 1) decarboxylation of pyruvate; 2) oxidation of the decarboxylated product; and 3) transfer of electrons to ultimately form NADH (Figure 6.65).

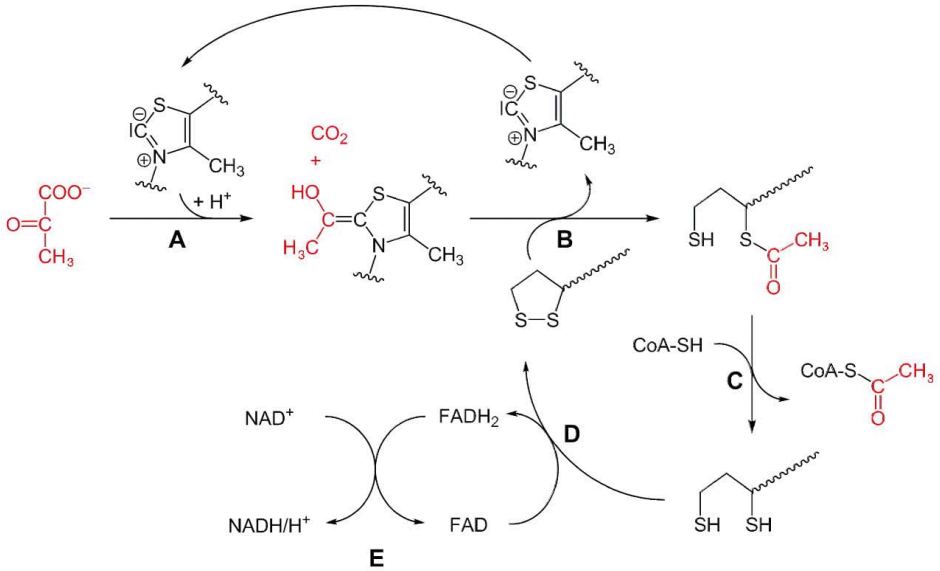


Figure 6.65 - Mechanism of action of pyruvate decarboxylation and oxidation by pyruvate dehydrogenase.

Catalysis

The catalytic process begins after binding of the pyruvate substrate with activation of the thiamine pyrophosphate coenzyme through formation of an ylide intermediate. The nucleophilic carbanion of the ylide attacks the electrophilic ketone carbon on the pyruvate, releasing carbon dioxide and creating an enol that loses a proton on the carbon to become a 1,3 dipole that includes the positively charged nitrogen of the thiamine. The reaction (step A in Figure 6.65) is a non-oxidative decarboxylation. Oxidation of the two carbon hydroxyethyl unit occurs in the transfer to the lipoamide.

Reductive acetylation

Reductive acetylation occurs next (Step B) as the 2-carbon hydroxyethyl unit is transferred to lipoamide on E2. (Lipoamide is the name for a molecule of lipoic acid covalently attached to a lysine side chain in the E2 subunit). In prokaryotes in the absence of oxygen, the hydroxyethyl group is not passed to lipoamide, but instead is released as free acetaldehyde , which can accept electrons from NADH (catalyzed by alcohol dehydrogenase) and become ethanol in the process of fermentation. In the presence of oxygen in almost all aerobic organisms, the process continues with transfer of the hydroxyethyl unit to E2 and continuation of the cycle below.

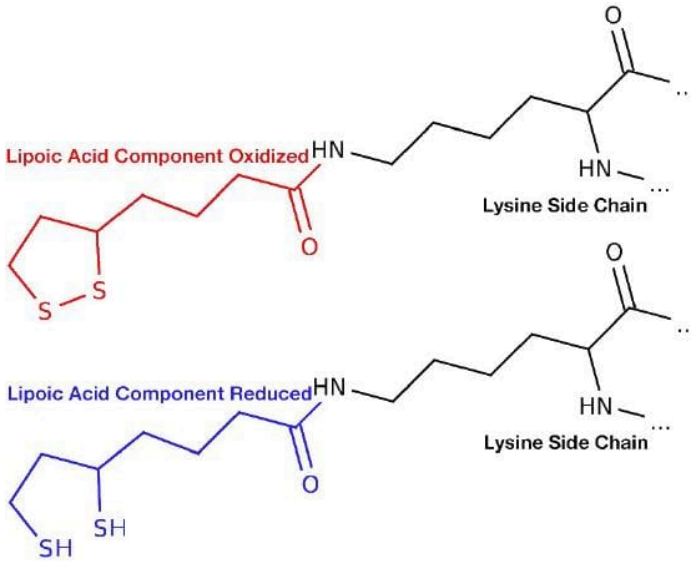


Figure 6.66 - Oxidized and reduced structures of lipoamide (lipoic acid linked to lysine)

Oxidation step

Transfer of the hydroxyethyl group from E1 to the lipoamide coenzyme in E2 is an oxidation, with transfer of electrons from the hydroxyethyl group to lipoamide's disulfide (reducing it) and formation on the lipoamide of an acetyl-thioester (oxidizing it).

The acetyl group is then transferred from lipoamide to coenzyme A in E2 (Step C in Figure 6.65), forming acetyl-CoA, which is released and leaving reduced sulfhydryls on the lipoamide. In order for the enzyme to return to its original state, the disulfide bond on lipoamide must be re-formed. This occurs with transfer of electrons from reduced lipoamide to an FAD covalently bound to E3 (Step D). This reduces FAD to FADH2.

Formation of NADH

In the last step in the process, electrons from FADH2 are transferred to external NAD+, forming NADH (Step E) and completing the overall cycle. Then enzyme can then begin another catalytic round by binding to a pyruvate.

Pyruvate dehydrogenase regulation

Pyruvate deyhdrogenase is regulated both allosterically and by covalent modification - phosphorylation / dephosphorylation. Regulation of pyruvate dehydrogenase, whether by allosteric or covalent mechanisms has the same strategy. Indicators of high energy shut down the enzyme, whereas indicators of low energy stimulate it. For allosteric regulation, the high energy indicators affecting the enzyme are ATP, acetyl-CoA, NADH, and fatty acids, which inhibit it. AMP, Coenzyme A, NAD+, and calcium, on the other hand, stimulate it (Figure 6.67).

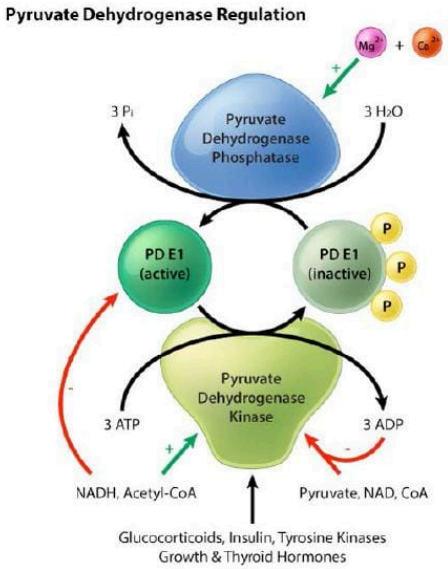


Figure 6.67 - Regulation scheme for pyruvate dehydrogenase (PD). Image by Aleia Kim

Covalent modification

Covalent modification regulation of pyruvate dehydrogenase is a bit more complicated. It occurs as a result of phosphorylation by pyruvate dehydrogenase kinase (PDK - Figure 6.67) or dephosphorylation by pyruvate dehydrogenase phosphatase (PDP).

PDK puts phosphate on any one of three serine residues on the E1 subunit, which causes pyruvate kinase to not be able to perform its first step of catalysis - the decarboxylation of pyruvate. PDP can remove those phosphates. PDK is allosterically activated in the mitochondrial matrix when NADH and acetyl-CoA concentrations rise.

Product inhibition

Thus, the products of the pyruvate dehydrogenase reaction inhibit the production of more products by favoring its phosphorylation by PDK. Pyruvate, a substrate of pyruvate dehydrogenase, inhibits PDK, so increasing concentrations of substrate activate pyruvate dehydrogenase by reducing its phosphorylation by PDK. As concentrations of NADH and acetyl-CoA fall, PDP associates with pyruvate kinase and removes the phosphate on the serine on the E1 subunit.

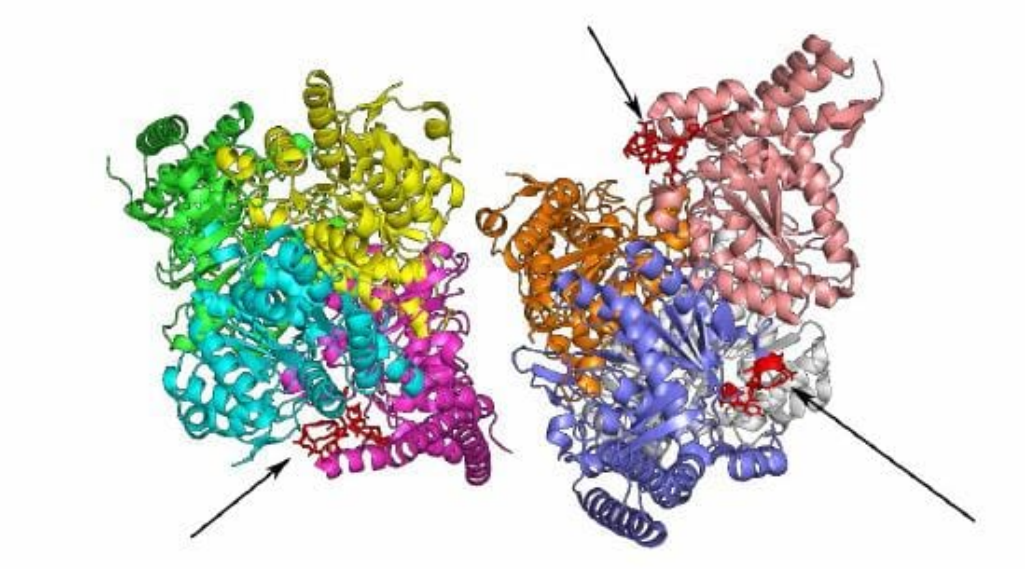


Figure 6.68 - Pyruvate dehydrogenase complex with three phosphorylation sites in red marked by arrows.Wikipedia

Low concentrations of NADH and acetyl-CoA are necessary for PDP to remain on the enzyme. When those concentrations rise, PDP dissociates and PDK gains access to the serine for phosphorylation. Insulin and calcium can also activate the PDP. This is very important in muscle tissue, since calcium is a signal for muscular contraction, which requires energy. Insulin also also activates pyruvate kinase and the glycolysis pathway to use internalized glucose. It should be noted that the cAMP phosphorylation cascade from the β-adrenergic receptor has no effect on pyruvate kinase, though the insulin cascade does, in fact, affect PDP and pyruvate kinase.

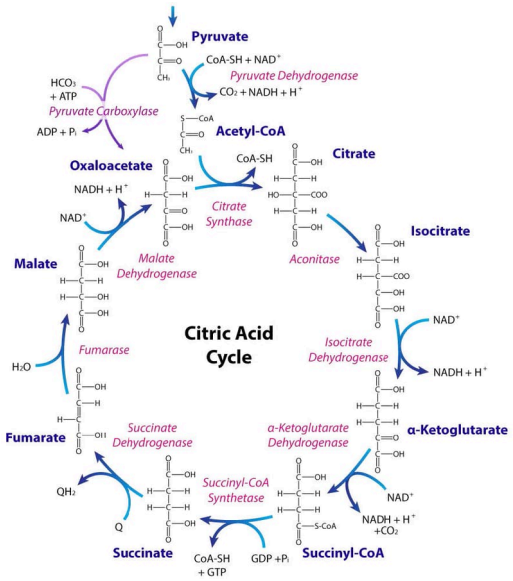


Figure 6.69 - The citric acid cycle. Image by Aleia Kim

Citric acid cycle reactions

Focusing on the pathway itself (Figure 6.69), the usual point to start discussion is addition of acetyl-CoA to oxaloacetate (OAA) to form citrate.

Acetyl-CoA for the pathway can come from a variety of sources. The reaction joining it to OAA is catalyzed by citrate synthase and the ΔG°' is fairly negative. This, in turn, helps to "pull" the malate dehydrogenase reaction preceding it in the cycle.

In the next reaction, citrate is isomerized to isocitrate by action of the enzyme called aconitase.

Isocitrate is a branch point in plants and bacteria for the glyoxylate cycle (see HERE). Oxidative decarboxylation of isocitrate by isocitrate dehydrogenase produces the first NADH and yields α-ketoglutarate.

This five carbon intermediate is a branch point for synthesis of the amino acid glutamate. In addition, glutamate can also be made easily into this intermediate in the reverse reaction. Decarboxylation of α-ketoglutarate produces succinyl-CoA and is catalyzed by α-ketoglutarate dehydrogenase.

The enzyme α-ketoglutarate dehydrogenase is structurally very similar to pyruvate dehydrogenase and employs the same five coenzymes – NAD+, FAD, CoA-SH, thiamine pyrophosphate, and lipoamide.

Regeneration of oxaloacetate

The remainder of the citric acid cycle involves conversion of the four carbon succinyl-CoA into oxaloacetate. Succinyl-CoA is a branch point for the synthesis of heme (see HERE). Succinyl-CoA is converted to succinate in a reaction catalyzed by succinyl-CoA synthetase (named for the reverse reaction) and a GTP is produced, as well – the only substrate level phosphorylation in the cycle.

The energy for the synthesis of the GTP comes from hydrolysis of the high energy thioester bond between succinate and the CoA-SH. Evidence for the high energy of a thioester bond is also evident in the citrate synthase reaction, which is also very energetically favorable. Succinate is also produced by metabolism of odd-chain fatty acids (see HERE).

Succinate Oxidation

Oxidation of succinate occurs in the next step, catalyzed by succinate dehydrogenase. This interesting enzyme both catalyzes this reaction and participates in the electron transport system, funneling electrons from the FADH2 it gains in the reaction to coenzyme Q. The product of the reaction, fumarate, gains a water across its trans double bond in the next reaction, catalyzed by fumarase to form malate.

Fumarate is also a byproduct of nucleotide metabolism and of the urea cycle. Malate is important also for transporting electrons across membranes in the malate-aspartate shuttle (see HERE) and in ferrying carbon dioxide from mesophyll cells to bundle sheath cells in C4 plants (see HERE).

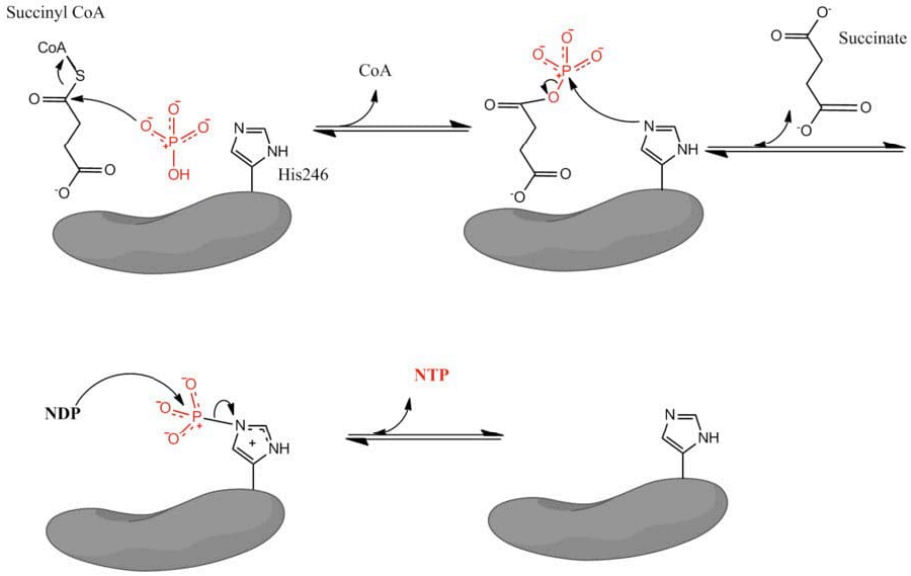


Figure 6.70 - Succinyl-CoA synthetase mechanism

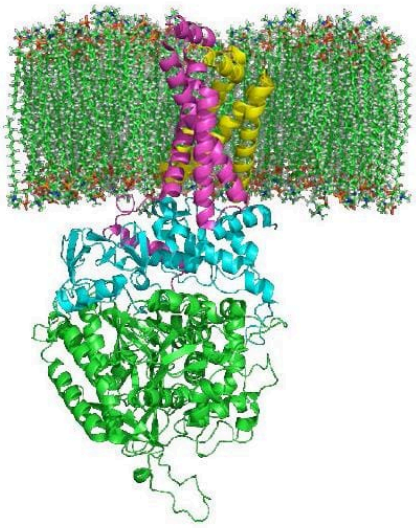


Figure 6.71 - Succinate dehydrogenase embedded in the mitochondrial inner membrane (top). Wikipedia

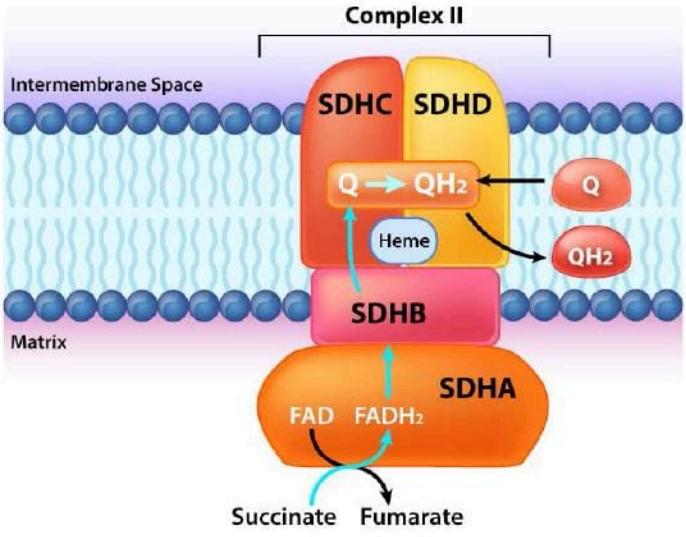


Figure 6.72 - Succinate dehydrogenase reaction. Image by Aleia Kim

Rare oxidation

Conversion of malate to oxaloacetate by malate dehydrogenase is a rare biological oxidation that has a ΔG°' with a positive value (29.7 kJ/mol).

The reaction is 'pulled' by the energetically favorable conversion of oxaloacetate to citrate in the citrate synthase reaction described above. Oxaloacetate intersects other important pathways, including amino acid metabolism (readily converted to aspartic acid), transamination (nitrogen movement) and gluconeogenesis.

It is worth noting that reversal of the citric acid cycle theoretically provides a mechanism for assimilating CO2. In fact, this reversal has been noted in both anaerobic and microaerobic bacteria, where it is called the Arnon-Buchanan cycle (Figure 6.73).

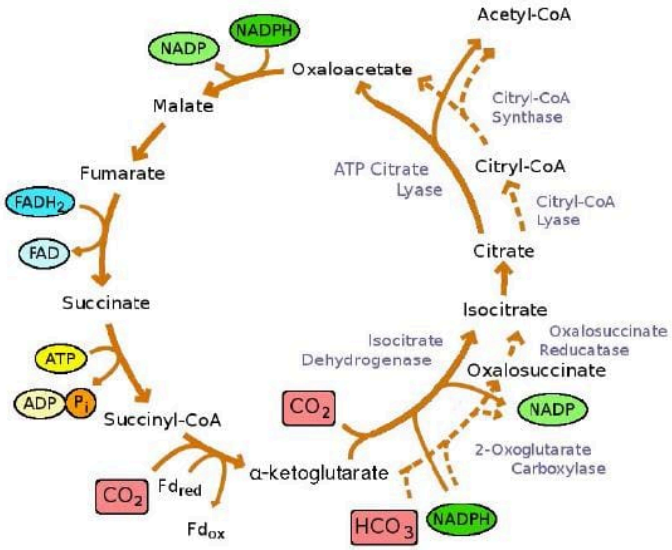


Figure 6.73 - Arnon-Buchanon cycle. Alternative enzymes shown on right in lavender. Fd = ferredoxin. Wikipedia

Regulation of the citric acid cycle

Allosteric regulation of the citric acid cycle is pretty straightforward. The molecules involved are all substrates/products of the pathway or molecules involved in energy transfer. Substrates/products that regulate or affect the pathway include acetyl-CoA and succinyl-CoA .

Inhibitors and activators

High energy molecular indicators, such as ATP and NADH will tend to inhibit the cycle and low energy indicators (NAD+, AMP, and ADP) will tend to activate the cycle. Pyruvate dehydrogenase, which catalyzes formation of acetyl-CoA for entry into the cycle is allosterically inhibited by its product (acetyl-CoA), as well as by NADH and ATP.

Regulated enzymes

Regulated enzymes in the cycle include citrate synthase (inhibited by NADH, ATP, and succinyl-CoA), isocitrate dehydrogenase (inhibited by ATP, activated by ADP and NAD+), and α-ketoglutarate dehydrogenase (inhibited by NADH and succinyl-CoA and activated by AMP).

Anaplerotic/cataplerotic pathway

The citric acid cycle is an important catabolic pathway oxidizing acetyl-CoA into CO2 and generating ATP, but it is also an important source of molecules needed by cells and a mechanism for extracting energy from amino acids in protein breakdown and other breakdown products. This ability of the citric acid cycle to supply molecules as needed and to absorb metabolic byproducts gives great flexibility to cells. When citric acid cycle intermediates are taken from the pathway to make other molecules, the term used to describe this is cataplerotic, whereas when molecules are added to the pathway, the process is described as anaplerotic.

Cataplerotic molecules

The citric acid cycle's primary cataplerotic molecules include α-ketoglutarate, succinyl-CoA, and oxaloacetate. Transamination of α-ketoglutarate and oxaloacetate produces the amino acids glutamate and aspartic acid, respectively. Oxaloacetate is important for the production of glucose in gluconeogenesis.

Glutamate plays a very important role in the movement of nitrogen through cells via glutamine and other molecules and is also needed for purine synthesis. Aspartate is a precursor of other amino acids and for production of pyrimidine nucleotides. Succinyl-CoA is necessary for the synthesis of porphyrins, such as the heme groups in hemoglobin, myoglobin and cytochromes.

Citrate is an important source of acetyl-CoA for making fatty acids. When the citrate concentration is high (as when the citric acid cycle is moving slowly or is stopped), it gets shuttled across the mitochondrial membrane into the cytoplasm and broken down by the enzyme citrate lyase to oxaloacetate and acetyl-CoA. The latter is a precursor for fatty acid synthesis in the cytoplasm.

Anaplerotic molecules

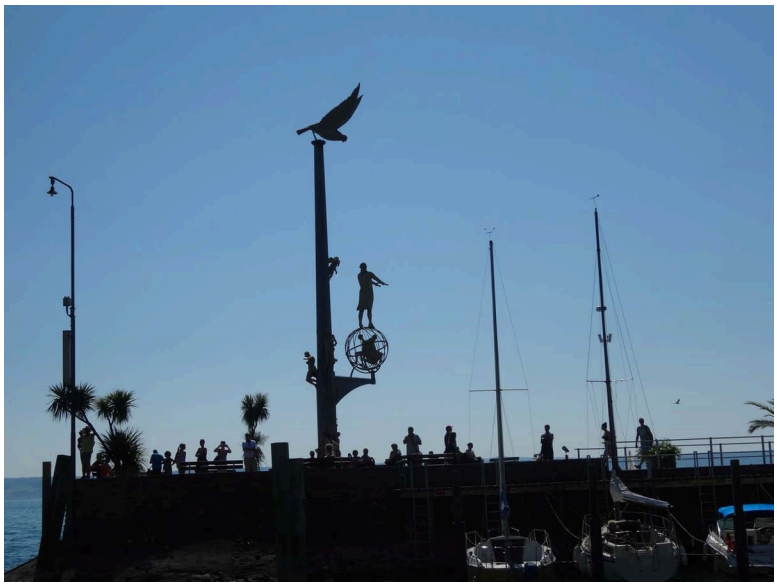
Anaplerotic molecules replenishing citric acid cycle intermediates include acetyl-CoA (made in many pathways, including fatty acid oxidation, pyruvate decarboxylation, amino acid catabolism, and breakdown of ketone bodies), α-ketoglutarate (from amino acid metabolism), succinyl-CoA (from propionic acid metabolism), fumarate (from the urea cycle and purine metabolism), malate (carboxylation of PEP in plants), and oxaloacetate (many sources, including amino acid catabolism and pyruvate carboxylase action on pyruvate in gluconeogenesis.

This page titled 8.6: Citric Acid Cycle and Related Pathways is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 6.2: Citric Acid Cycle & Related Pathways by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

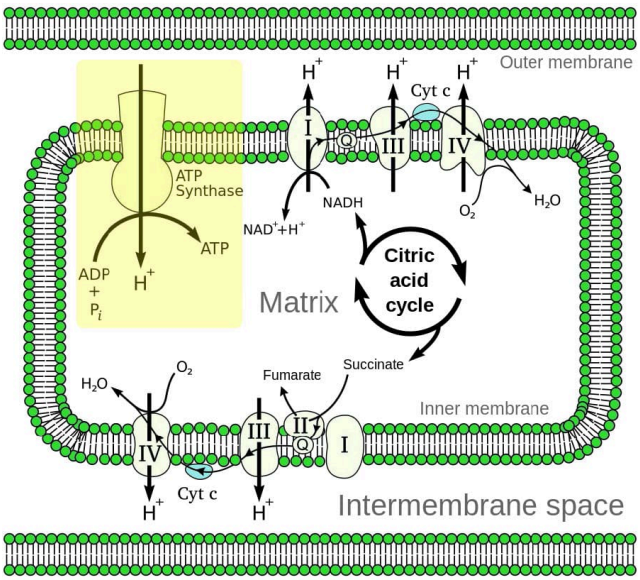
8.7: Electron Transport and Oxidative Phosphorylation

Source: BiochemFFA_5_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy



In eukaryotic cells, the vast majority of ATP synthesis occurs in the mitochondria in a process called oxidative phosphorylation. Even plants, which generate ATP by photophosphorylation in chloroplasts, contain mitochondria for the synthesis of ATP through oxidative phosphorylation.

Oxidative phosphorylation is linked to a process known as electron transport (Figure 5.14). The electron transport system, located in the inner mitochondrial membrane, transfers electrons donated by the reduced electron carriers NADH and FADH2 (obtained from glycolysis, the citric acid cycle or fatty acid oxidation) through a series of electrons acceptors, to oxygen. As we shall see, movement of electrons through complexes of the electron transport system essentially “charges” a battery that is used to make ATP in oxidative phosphorylation. In this way, the oxidation of sugars and fatty acids is coupled to the synthesis of ATP, effectively extracting energy from food.



Chemiosmotic model

Dr. Peter Mitchell introduced a radical proposal in 1961 to explain the mechanism by which mitochondria make ATP. It is known as the chemiosmotic hypothesis and has been shown over the years to be correct. Mitchell proposed that synthesis of ATP in mitochondria depends on an electrochemical gradient, across the mitochondrial inner membrane, that arises ultimately from the energy of reduced electron carriers, NADH and FADH2.

Electron transport

Further, the proposal states that the gradient is created when NADH and FADH2 transfer their electrons to an electron transport system (ETS) located in the inner mitochondrial membrane. Movement of electrons through a series of of electron carriers is coupled to the pumping of protons out of the mitochondrial matrix across the inner mitochondrial membrane into the space between the inner and outer membranes. The result is creation of a gradient of protons whose potential energy can be used to make ATP. Electrons combine with oxygen and protons at the end of the ETS to make water.

ATP synthase

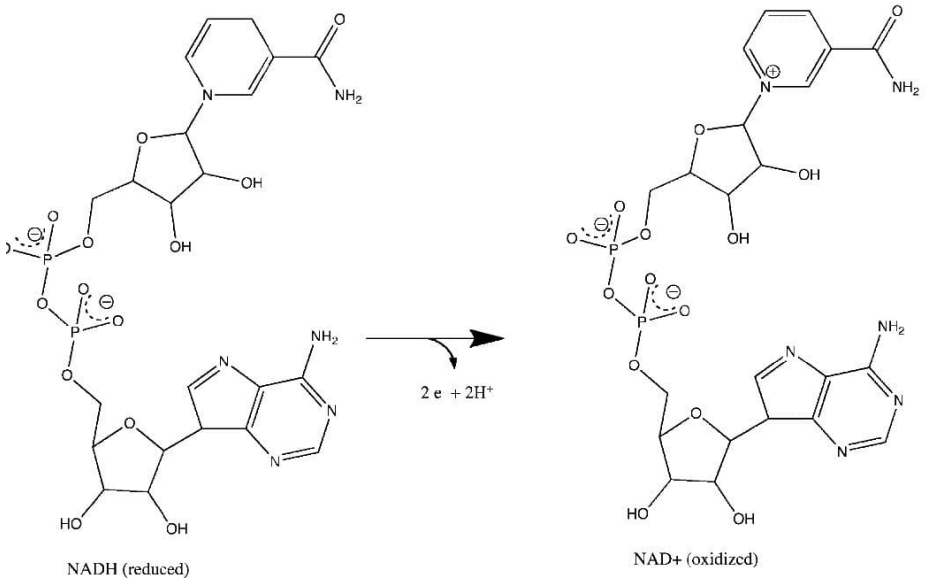
In oxidative phosphorylation, ATP synthesis is accomplished as a result of protons re-entering the mitochondrial matrix via the transmembrane ATP synthase complex, which combines ADP with inorganic phosphate to make ATP. Central to the proper functioning of mitochondria through this process is the presence of an intact mitochondrial inner membrane impermeable to protons.

Tight coupling

When this is the case, tight coupling is said to exist between electron transport and the synthesis of ATP (called oxidative phosphorylation). Chemicals which permeabilize the inner mitochondrial membrane to protons cause uncoupling, that is, they allow the protons to leak back into the mitochondrial matrix, rather than through the ATP synthase, so that the movement of electrons through the ETS is no longer linked to the synthesis of ATP.

Power plants

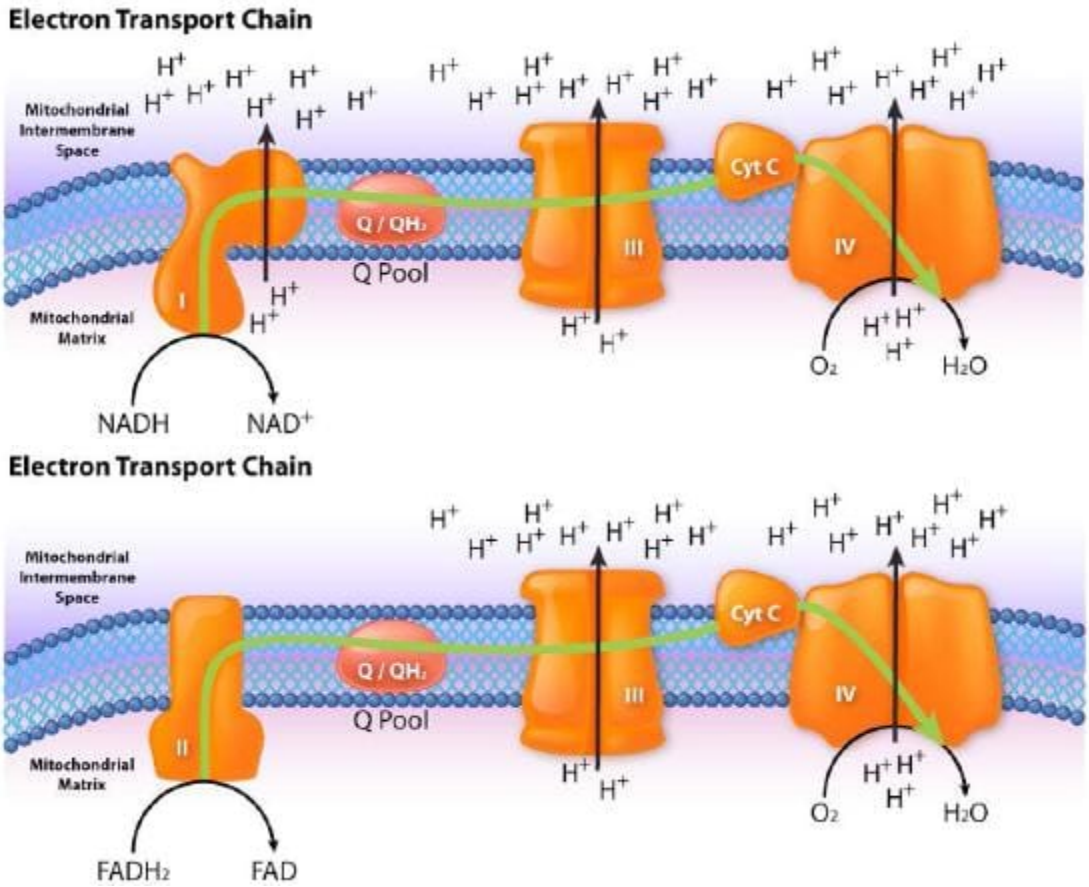
Mitochondria are called the power plants of the cell because most of a cell's ATP is produced there in the process of oxidative phosphorylation. The mechanism by which ATP is made in oxidative phosphorylation is one of the most interesting in all of biology.



NADH (reduced) NAD+ (oxidized)

Considerations

The process has three primary considerations. The first is electrical – electrons from reduced electron carriers, such as NADH and FADH2, enter the electron transport system via Complex I and II, respectively. As seen in Figure 5.16 and Figure 5.17, electrons move from one complex to the next, not unlike the way they move through an electrical circuit. Such movement occurs a a result of a set of reduction-oxidation (redox) reactions with electrons moving from a more negative reduction potential to a more positive one.



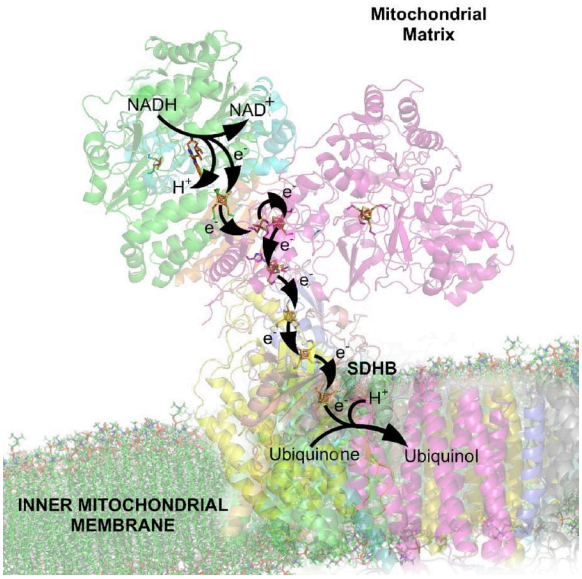
One can think of this occurring as a process where carriers “take” electrons away from complexes with lower reduction potential, much the way a bully takes lunch money from a smaller child. In this scheme, the biggest “bully” is oxygen in Complex IV. Electrons gained by a carrier cause it to be reduced, whereas the carrier giving up the electrons is oxidized.

Entry of electrons to system

Movement of electrons through the chain begins either by 1) transfer from NADH to Complex I (Figure 5.16) or 2) movement of electrons through a covalently bound FADH2 (Figure 5.17) in the membrane-bound succinate dehydrogenase (Complex II). (An alternate entry point for electrons from FADH2 is the Electron Transferring Flavoprotein via the electron-transferring-flavoprotein dehydrogenase, not shown).

Traffic cop

Both Complex I and II pass electrons to the inner membrane’s coenzyme Q (CoQ - Figures 5.18 & 5.19). In each case, coenzyme Q accepts electrons in pairs and passes them off to Complex III (CoQH2-cytochrome c reductase) singly. Coenzyme Q thus acts as a traffic cop, regulating the flow of electrons through the ETS.



Docking station

Complex III is a docking station or interchange for the incoming electron carrier (coenzyme Q) and the outgoing carrier (cytochrome c). Movement of electrons from Coenzyme Q to Complex III and then to cytochrome C occurs as a result of what is referred to as the Q-cycle (see below).

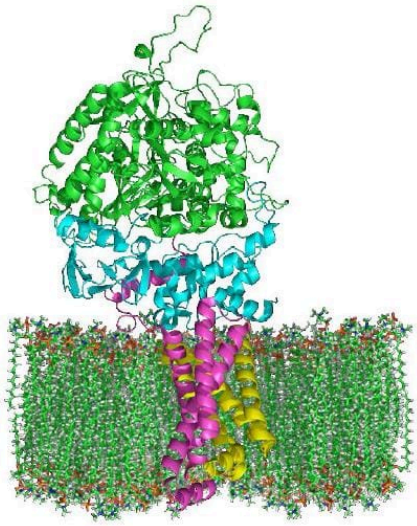
Complex III acts to ferry electrons from CoQ to cytochrome c. Cytochrome c takes one electron from Complex III and passes it to Complex IV (cytochrome oxidase). Complex IV is the final protein recipient of the electrons. It passes them to molecular oxygen (O2) to make two molecules of water. Making two water molecules requires four electrons, so Complex IV must accept, handle, and pass to molecular oxygen four separate electrons, causing the oxidation state of oxygen to be sequentially changed with addition of each electron.

Proton pumping

As electrons pass through complexes I, III, and IV, there is a release of a small amount of energy at each step, which is used to pump protons from the mitochondrial matrix (inside of mitochondrion) and deposit them in the intermembrane space (between the inner and outer membranes of the mitochondrion). The effect of this redistribution is to increase the electrical and chemical potential across the membrane.

Potential energy

As discussed earlier, electrochemical gradients have potential energy. Students may think of the process as “charging the battery.” Just like a charged battery, the potential arising from the proton differential across the membrane can be used to do things. In the mitochondrion, what the proton gradient does is facilitate the production of ATP from ADP and Pi. This process is known as oxidative phosphorylation, because the phosphorylation of ADP to ATP is dependent on the oxidative reactions occurring in the mitochondria.



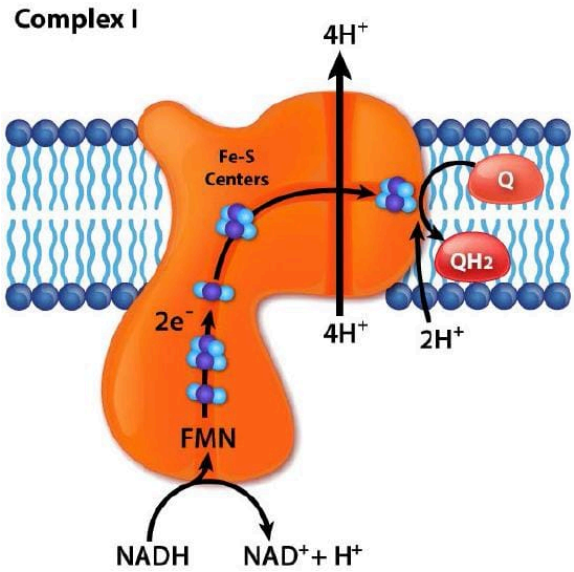
Having understood the overall picture of the synthesis of ATP linked to the movement of electrons through the ETS, we will take a closer look at the individual components of the ETS.

Complex I

Complex I (also called NADH:ubiquinone oxidoreductase or NADH dehydrogenase (ubiquinone)) is the electron acceptor from NADH in the electron transport chain and the largest complex found in it.

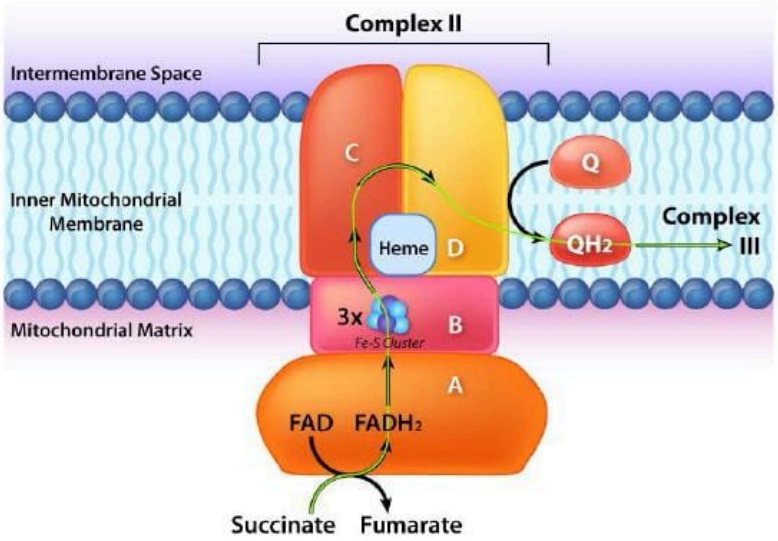
Complex I contains 44 individual polypeptide chains, numerous iron-sulfur centers, a molecule of flavin mononucleotide (FMN) and has an L shape with about 60 transmembrane domains. In the process of electron transport through it, four protons are pumped across the inner membrane into the intermembrane space and electrons move from NADH to coenzyme Q, converting it from ubiquinone (no electrons) to ubiquinol (gain of two electrons). An intermediate form, ubisemiquinone (gain of one electron), is found in the Q-cycle.

Electrons travel through the complex via seven primary iron sulfur centers. The best known inhibitor of the complex, rotenone, works by binding to the CoQ binding site. Other inhibitors include ADP-ribose (binds to the NADH site) and piericidin A (rotenone analog). The process of electron transfer through complex I is reversible and when this occurs, superoxide (a reactive oxygen species) may be readily generated.



Complex II

Complex II (also called succinate dehydrogenase or succinate-coenzyme Q reductase) is a membrane bound enzyme of the citric acid cycle that plays a role in the electron transport process, transferring electrons from its covalently bound FADH2 to coenzyme Q. The process occurs, as shown in Figure 5.20 and Figure 5.21, with transfer of electrons from succinate to FAD to form FADH2 and fumarate. FADH2, in turn, donates electrons to a relay system of iron-sulfur groups and they ultimately reduce ubiquinone (CoQ) along with two protons from the matrix to ubiquinol. The role of the heme group in the process is not clear. Inhibitors of the process include carboxin, malonate, malate, and oxaloacetate. The role of citric acid cycle intermediates as inhibitors is thought to be due to inhibition of the reversal of the transfer process which can produce superoxide.

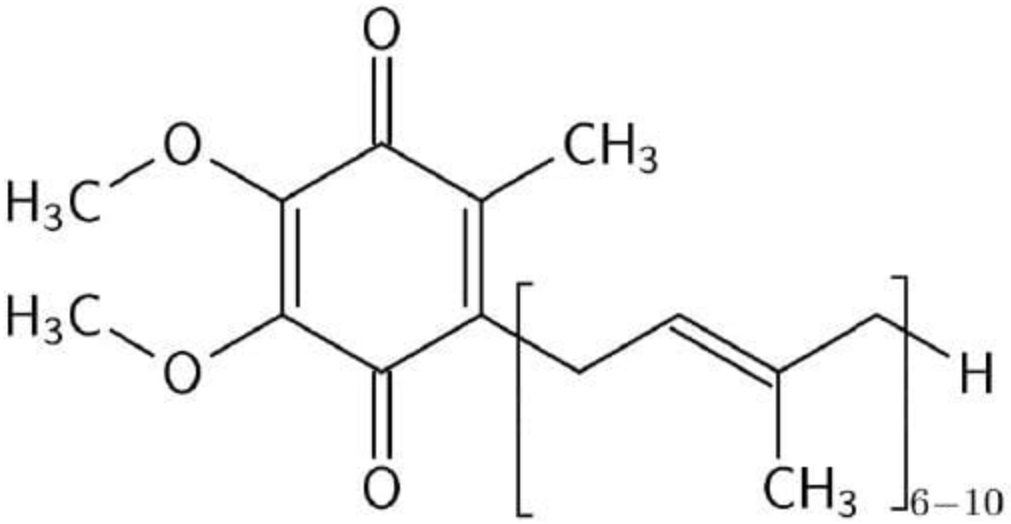


Coenzyme Q

Coenzyme Q (Figure 5.23) is a 1,4 benzoquinone whose name is often given as Coenzyme Q10, CoQ, or Q10. The 10 in the name refers to the number of isoprenyl units it contains that anchor it to the mitochondrial inner membrane. CoQ is a vitamin-like lipid substance found in most eukaryotic cells as a component of the electron transport system. The requirement for CoQ increases with increasing energy needs of cells, so the highest concentrations of CoQ in the body are found in tissues that are the most metabolically active - heart, liver, and kidney.

Three forms

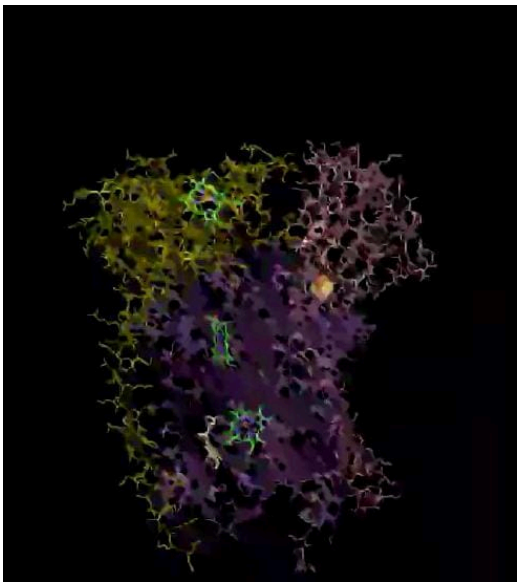
CoQ is useful because of its ability to carry and donate electrons and particularly because it can exist in forms with two extra electrons (fully reduced - ubiquinol), one extra electron (semi-reduced - ubisemiquinone), or no extra electrons (fully oxidized - ubiquinone). This ability allows CoQ to provide transition between the first part of the electron transport system that moves electrons in pairs and the last part of the system that moves electrons one at a time.



Complex III

Complex III (also known as coenzyme Q : cytochrome c — oxidoreductase or the cytochrome bc1 complex - Figure 5.24) is the third electron accepting complex of the electron transport system. It is a transmembrane protein with multiple subunits present in the mitochondria of all aerobic eukaryotic organisms and and the cell membrane of almost all bacteria. The complex contains 11 subunits, a 2-iron ferredoxin, cytochromes b and c1 and belongs to the family of oxidoreductase enzymes.

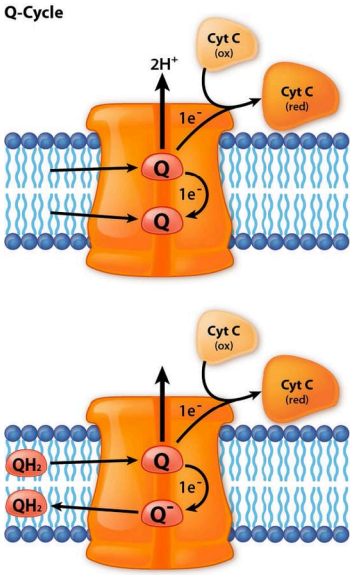
It accepts electrons from coenzyme Q in electron transport and passes them off to cytochrome c. In this cycle, known as the Q cycle, electrons arrive from CoQ in pairs, but get passed to cytochrome c individually. In the overall process, two protons are consumed from the matrix and four protons are pumped into the intermembrane space. Movement of electrons through the complex can be inhibited by antimycin A, myxothiazol, and stigmatellin. Complex III is also implicated in creation of superoxide (a reactive oxygen species) when electrons from it leak out of the chain of transfer. The phenomenon is more pronounced when antimycin A is present.





Q-cycle

In the Q-cycle, electrons are passed from ubiquinol (QH2) to cytochrome c using Complex III as an intermediary docking station for the transfer. Two pair of electrons enter from QH2 and one pair is returned to another CoQ to re-make QH2. The other pair is donated singly to two different cytochrome c molecules.



Step one

The Q-cycle happens in a two step process. First, a ubiquinol (CoQH2) and a ubiquinone (CoQ) dock at Complex III. Ubiquinol transfers two electrons to Complex III. One electron goes to a docked cytochrome c, reducing it and it exits (replaced by an oxidized cytochrome c). The other goes to the docked uniquinone to create the semi-reduced semiubiquinone (CoQ.-) and leaving behind a ubiquinone, which exits. This is the end of step 1.

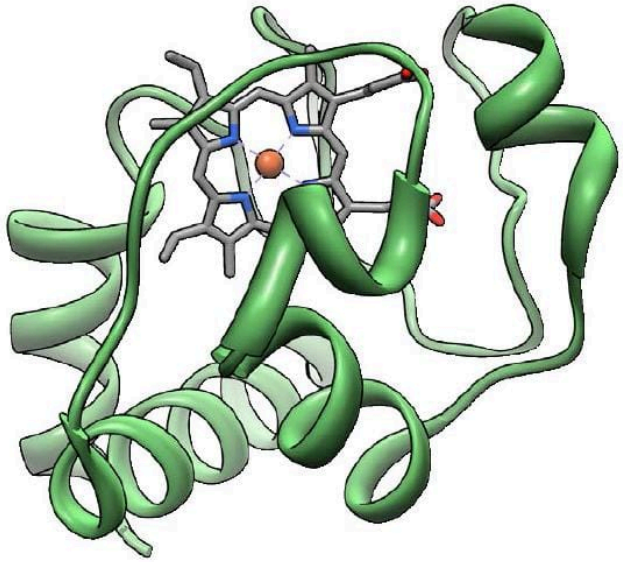
Step two

The gap left behind by the ubiquinone (Q) that departed is replaced by another ubiquinol (QH2). It too donates two electrons to Complex III, which splits them. One goes to the newly docked oxidized cytochrome c, which is reduced and exits. The other goes to the ubisemiquinone. Two protons from the matrix combine with it to make another ubiquinol. It and the ubiquinone created by

the electron donation exit Complex III and the process starts again. In the overall process, two protons are consumed from the matrix and four protons are pumped into the intermembrane space.

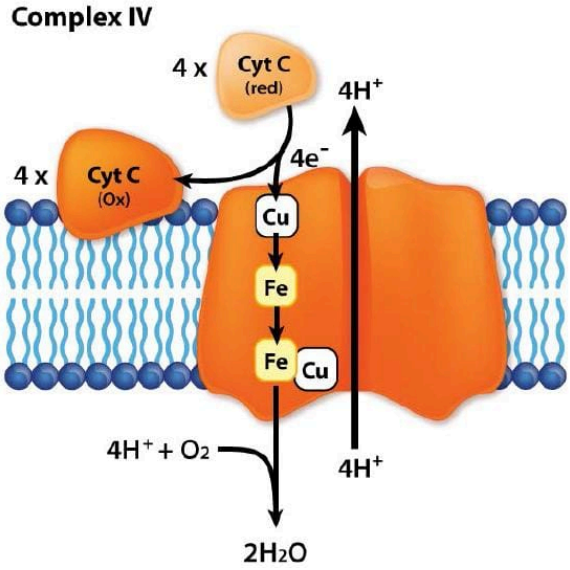
Cytochrome c

Cytochrome c (Figure 5.26) is a small (12,000 Daltons), highly conserved protein, from unicellular species to animals, that is loosely associated with the inner mitochondrial membrane where it functions in electron transport. It contains a heme group which is used to carry a single electron from Complex III to Complex IV. Cytochrome c also plays an important role in apoptosis in higher organisms. Damage to the mitochondrion that results in release of cytochrome c can stimulate assembly of the apoptosome and activation of the caspase cascade that leads to programmed cell death.



Complex IV

Complex IV, also known as cytochrome c oxidase is a 14 subunit integral membrane protein at the end of the electron transport chain (Figure 5.27). It is responsible for accepting one electron each from four cytochrome c proteins and adding them to molecular oxygen (O2) along with four protons from the mitochondrial matrix to make two molecules of water. Four protons from the matrix are also pumped into the intermembrane space in the process. The complex has two molecules of heme, two cytochromes (a and a3), and two copper centers (called CuA ad CuB). Cytochrome c docks near the CuA and donates an electron to it. The reduced CuA passes the electron to cytochrome a, which turns it over to the a3-CuB center where the oxygen is reduced. The four electrons are thought to pass through the complex rapidly resulting in complete reduction of the oxygen-oxygen molecule without formation of a peroxide intermediate or superoxide, in contrast to previous predictions.

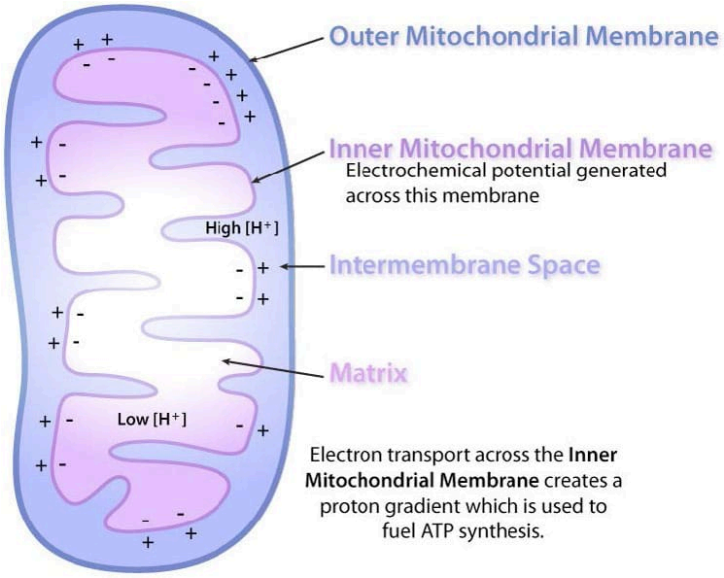


Respirasome

There has been speculation for many years that a supercomplex of electron carriers in the inner membrane of the mitochondrion may exist in cells with individual carriers making physical contact with each other. This would make for more efficient transfer reactions, minimize the production of reactive oxygen species and be similar to metabolons of metabolic pathway enzymes, for which there is some evidence. Now, evidence appears to be accumulating that complexes I, III, and IV form a supercomplex, which has been dubbed the respirasome1.

Oxidative phosphorylation

The process of oxidative phosphorylation uses the energy of the proton gradient established by the electron transport system as a means of phosphorylating ADP to make ATP. The establishment of the proton gradient is dependent upon electron transport. If electron transport stops or if the inner mitochondrial membrane's impermeability to protons is compromised, oxidative phosphorylation will not occur because without the proton gradient to drive the ATP synthase, there will be no synthesis of ATP.



ATP synthase

The protein complex harvesting energy from the proton gradient and using it to make ATP from ADP is an enzyme that has several names - Complex V, PTAS (Proton Translocating ATP Synthase), and ATP synthase (Figure 5.29). Central to its function is the movement of protons through it (from the intermembrane space back into the matrix). Protons will only provide energy to make ATP if their concentration is greater in the intermembrane space than in the matrix and if ADP is available.

It is possible, in some cases, for the concentration of protons to be greater inside the matrix than outside of it. When this happens, the ATP synthase can run backwards, with protons moving from inside to out, accompanied by conversion of ATP to ADP + Pi. This is usually not a desirable circumstance and there are some controls to reduce its occurrence.

Normally, ATP concentration will be higher inside of the mitochondrion and ADP concentration be higher outside the mitochondrion. However, when the rate of ATP synthesis exceeds the rate of ATP usage, then ATP concentrations rise outside the mitochondrion and ADP concentrations fall everywhere.

This may happen, for example, during periods of rest. It has the overall effect of reducing transport and thus lowering the concentration of ADP inside the matrix. Reducing ADP concentration in the matrix reduces oxidative phosphorylation and has effects on respiratory control (see HERE).

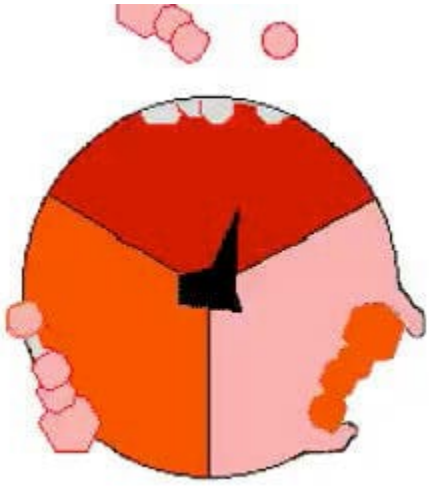
Another important consideration is that when ATP is made in oxidative phosphorylation, it is released into the mitochondrial matrix, but must be transported into the cytosol to meet the energy needs of the rest of the cell. This is accomplished by action of the adenine nucleotide translocase, an antiport that moves ATP out of the matrix in exchange for ADP moving into the matrix. This transport system is driven by the concentrations of ADP and ATP and ensures that levels of ADP are maintained within the mitochondrion, permitting continued ATP synthesis.

One last requirement for synthesis of ATP from ADP is that phosphate must also be imported into the matrix. This is accomplished by action of the phosphate translocase, which is a symport that moves phosphate into the mitochondrial matrix along with a proton.

There is evidence that the two translocases and ATP synthase may exist in a complex, which has been dubbed the ATP synthasome.

In summary, the electron transport system charges the battery for oxidative phosphorylation by pumping protons out of the mitochondrion. The intact inner membrane of the mitochondrion keeps the protons out, except for those that re-enter through ATP Synthase. The ATP Synthase allows protons to re-enter the mitochondrial matrix and harvests their energy to make ATP.

ATP synthase mechanism



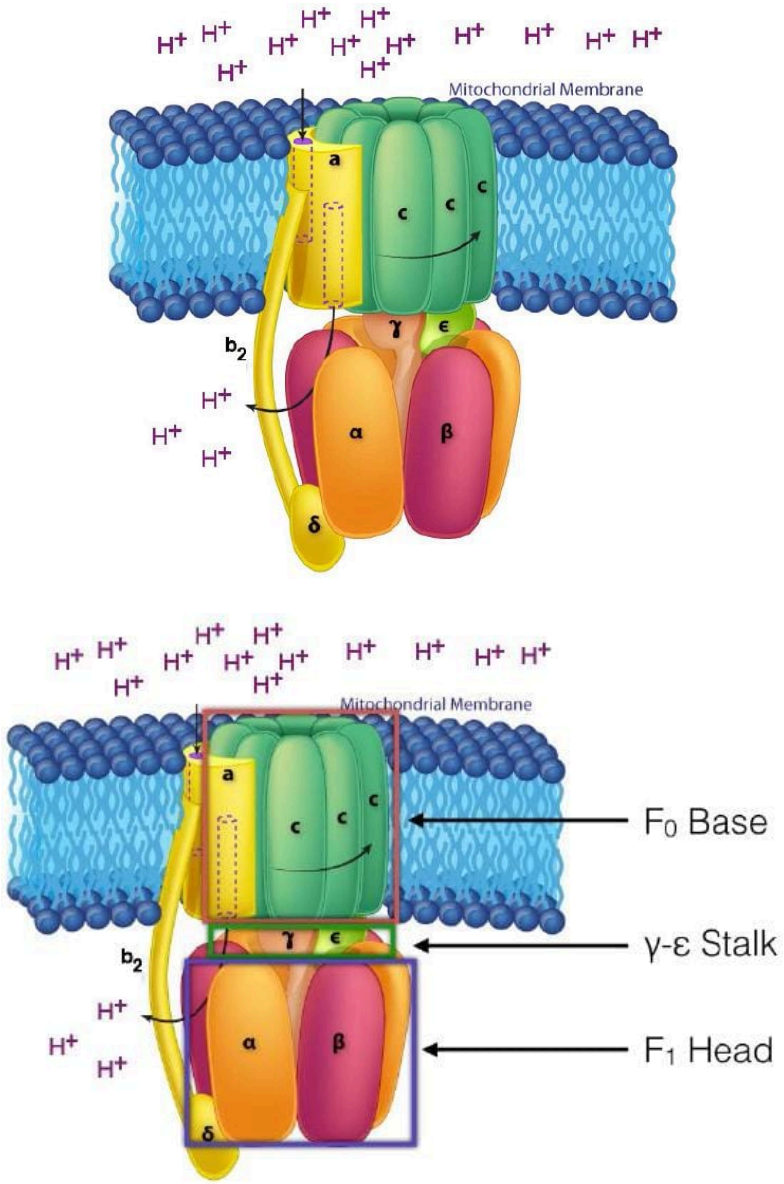


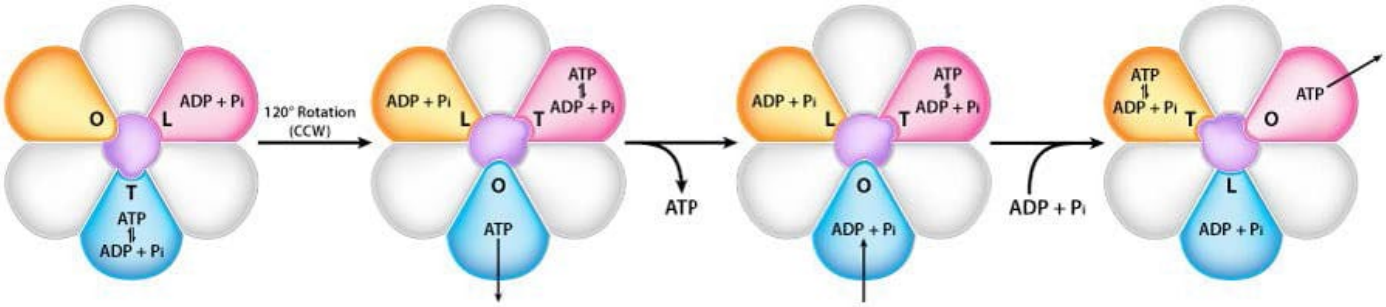
Figure 5.32 illustrate the multi-subunit nature of this membrane protein, which acts like a turbine at a hydroelectric dam. The movement of protons through the ATP Synthase c-ring causes it and the γ-ε stalk attached to it to turn. It is this action that is necessary for making ATP.

In ATP Synthase, the spinning components, or rotor, are the membrane portion (c ring) of the FO base and the γ-ε stalk, which is connected to it. The γ-ε stalk projects into the F1 head of the mushroom structure. The F1 head contains the catalytic ability to make ATP. The F1 head is hexameric in structure with paired α and β proteins arranged in a trimer of dimers. ATP synthesis occurs within the β subunits.

Rotation of γ unit

Turning of the γ shaft (caused by proton flow) inside the α-β trimer of the F1 head causes each set of β proteins to change structure slightly into three different forms called Loose, Tight, and Open (L,T,O - Figure 5.31). Each of these forms has a function.

The Loose form binds ADP + Pi. The Tight form “squeezes” them together to form the ATP. The Open form releases the ATP into the mitochondrial matrix. Thus, as a result of the proton flow through the ATP synthase, from the intermembrane space into the matrix, ATP is made from ADP and Pi.



Respiratory control

When a mitochondrion has an intact inner membrane and protons can only return to the matrix by passing through the ATP synthase, the processes of electron transport and oxidative phosphorylation are said to be tightly coupled.

Interdependence

In simple terms, tight coupling means that the processes of electron transport and oxidative phosphorylation are interdependent. Without electron transport going on in the cell, oxidative phosphorylation will soon stop.

The reverse is also true, because if oxidative phosphorylation stops, the proton gradient will not be dissipated as it is being built by the electron transport system and will grow larger and larger. The greater the gradient, the greater the energy needed to pump protons out of the mitochondrion. Eventually, if nothing relieves the gradient, it becomes too large and the energy of electron transport is insufficient to perform the pumping. When pumping stops, so too does electron transport.

ADP dependence

Another relevant point is that ATP synthase is totally dependent upon a supply of ADP. In the absence of ADP, the ATP synthase stops functioning and when it stops, so too does movement of protons back into the mitochondrion. With this information, it is possible to understand the link between energy usage and metabolism. The root of this, as noted, is respiratory control.

At rest

To illustrate these links, let us first consider a person, initially at rest, who then suddenly jumps up and runs away. At first, the person's ATP levels are high and ADP levels are low (no exercise to burn ATP), so little oxidative phosphorylation is occurring and thus the proton gradient is high. Electron transport is moving slowly, if at all, so it is not using oxygen and the person's breathing is slow, as a result.

Exercise

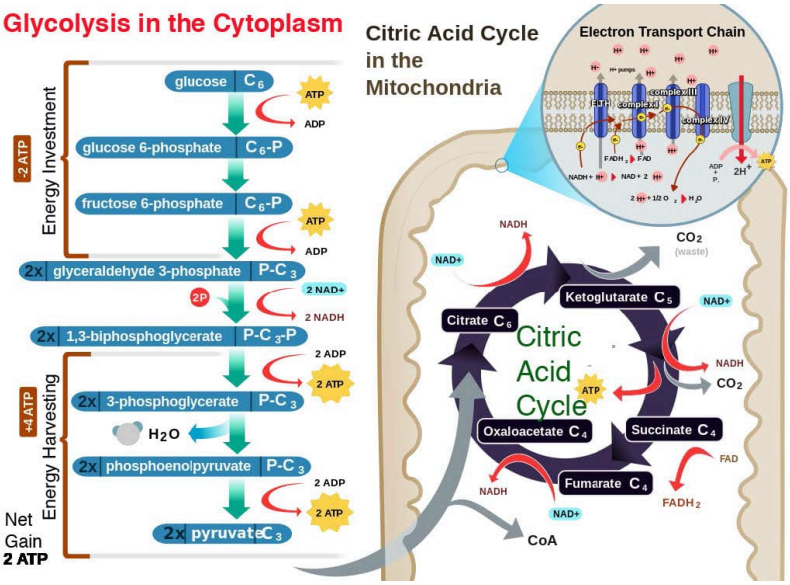
When running starts, muscular contraction, which uses energy, causes ATP to be converted to ADP. Increasing ADP in muscle cells favors oxidative phosphorylation to attempt to make up for the ATP being burned. ATP synthase begins working and protons begin to come back into the mitochondrial matrix. The proton gradient decreases, so electron transport re-starts.

Electron transport needs an electron acceptor, so oxygen use increases and when oxygen use increases, the person starts breathing more heavily to supply it. When the person stops running, ATP concentrations get rebuilt by ATP synthase. Eventually, when ATP levels are completely restored, ADP levels fall and ATP synthase stops or slows considerably. With little or no proton movement, electron transport stops because the proton gradient is too large. When electron transport stops, oxygen use decreases and the rate of breathing slows down.

Electron transport critical

The really interesting links to metabolism occur relative to whether or not electron transport is occurring. From the examples, we can see that electron transport will be relatively slowed when not exercising and more rapid when exercise (or other ATP usage) is occurring. Remember that electron transport is the way in which reduced electron carriers, NADH and FADH2, donate their electrons to the ETS , becoming oxidized to NAD+ and FAD, respectively.

Oxidized carriers, such as NAD+ and FAD are needed by catabolic pathways, like glycolysis, the citric acid cycle, and fatty acid oxidation. Anabolic pathways, such as fatty acid/fat synthesis and gluconeogenesis rely on reduced electron carriers, such as FADH2, NADH, and the related carrier, NADPH.



Links to exercise

High levels of NADH and FADH2 prevent catabolic pathways from operating, since NAD+ and FAD levels will be low and these are needed to accept the electrons released during catabolism by the oxidative processes.

Thanks to respiratory control, when one is exercising, NAD+ and FAD levels increase (because electron transport is running), so catabolic pathways that need NAD+ and FAD can function. The electrons lost in the oxidation reactions of catabolism are captured by NAD+ and FAD to yield NADH and FADH2, which then supply electrons to the electron transport system and oxidative phosphorylation to make more needed ATP.

Thus, during exercise, cells move to a mode of quickly cycling between reduced electron carriers (NADH/FADH2) and oxidized electron carriers (NAD+/FAD). This allows rapidly metabolizing tissues to transfer electrons to NAD+/FAD and it allows the reduced electron carriers to rapidly become oxidized, allowing the cell to produce ATP.

Rest

When exercise stops, NADH and FADH2 levels rise (because electron transport is slowing) causing catabolic pathways to slow/stop. If one does not have the proper amount of exercise, reduced carriers remain high in concentration for long periods of time. This means we have an excess of energy and then anabolic pathways, particularly fatty acid synthesis, are favored, so we get fatter.

Altering respiratory control

One might suspect that altering respiratory control could have some very dire consequences and that would be correct. Alterations can take the form of either inhibiting electron transport/oxidative phosphorylation or uncoupling the two . These alterations can be achieved using compounds with specific effects on particular components of the system.

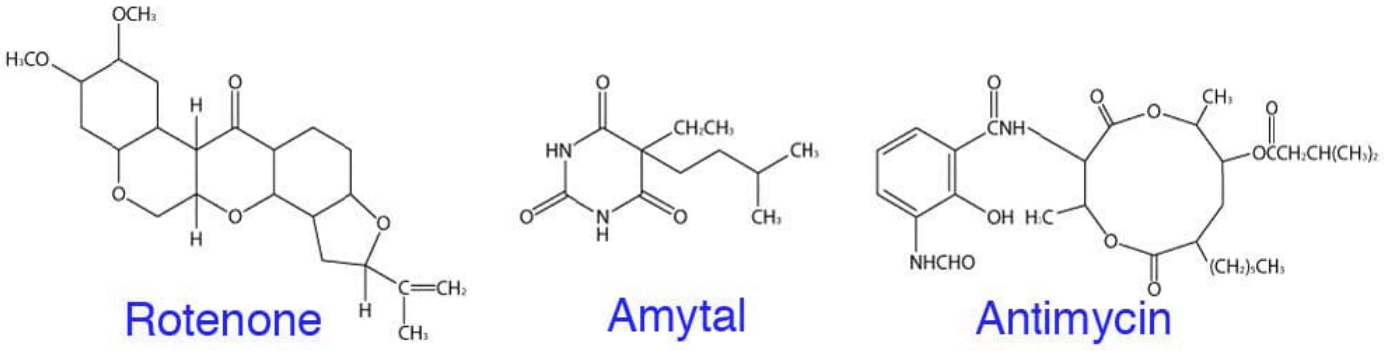
All of the chemicals described here are laboratory tools and should never be used by people. The first group for discussion are the inhibitors. In tightly coupled mitochondria, inhibiting either electron transport or oxidative phosphorylation has the effect of inhibiting the other one as well.

Electron transport inhibitors

Common inhibitors of electron transport include rotenone and amytal, which stop movement of electrons past Complex I, malonate, malate, and oxaloacetate, which inhibit movement of electrons through Complex II, antimycin A which stops movement of electrons past Complex III, and cyanide, carbon monoxide, azide, and hydrogen sulfide, which inhibit electron movement through Complex IV (Figure 5.33). All of these compounds can stop electron transport directly (no movement of electrons) and oxidative phosphorylation indirectly (proton gradient will dissipate). While some of these compounds are not commonly known, almost everyone is aware of the hazards of carbon monoxide and cyanide, both of which can be lethal.

ATP synthase inhibitor

It is also possible to use an inhibitor of ATP synthase to stop oxidative phosphorylation directly (no ATP production) and electron transport indirectly (proton gradient not relieved so it becomes increasingly difficult to pump protons out of matrix). Oligomycin A (Figure 5.34) is an inhibitor of ATP synthase.



Rotenone

Rotenone, which is a plant product, is used as a natural insecticide that is permitted for organic farming. When mitochondria are treated with this, electron transport will stop at Complex I and so, too, will the pumping of protons out of the matrix. When this occurs, the proton gradient rapidly dissipates, stopping oxidative phosphorylation as a consequence. There are other entry points for electrons than Complex I, so this type of inhibition is not as serious as using inhibitors of Complex IV, since no alternative route for electrons is available. It is for this reason that cyanide, for example, is so poisonous.

2,4-DNP

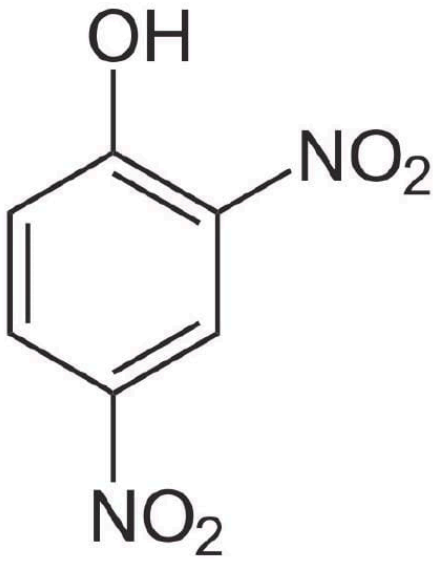


Figure 5.35). Treatment of mitochondria with 2,4 DNP makes the mitochondrial inner membrane “leaky” to protons. This has the effect of providing an alternate route for protons to reenter the matrix besides going through ATP synthase, and uncouples oxidative phosphorylation from electron transport.

Imagine a dam holding back water with a turbine generating electricity through which water must flow. When all water flows through the turbine, the maximum amount of electricity can be generated. If one pokes a hole in the dam, though, water will flow through the hole and less electricity will be created. The generation of electricity will thus be uncoupled from the flow of water. If the hole is big enough, the water will all drain out through the hole and no electricity will be made.

Bypassing ATP synthase

Imagine, now, that the proton gradient is the equivalent of the water, the inner membrane is the equivalent of the dam and the ATP synthase is the turbine. When protons have an alternate route, little or no ATP will be made because protons will pass through the membrane's holes instead of spinning the turbine of ATP synthase.

It is important to recognize, though, that uncoupling by 2,4 DNP works differently from the electron transport inhibitors or the ATP synthase inhibitor. In those situations, stopping oxidative phosphorylation resulted in indirectly stopping electron transport, since the two processes were coupled and the inhibitors did not uncouple them. Similarly, stopping electron transport indirectly stopped oxidative phosphorylation for the same reason.

Such is not the case with 2,4 DNP. Stopping oxidative phosphorylation by destroying the proton gradient allows electron transport to continue unabated (it actually stimulates it), since the proton gradient cannot build no matter how much electron transport runs. Consequently, electron transport runs like crazy but oxidative phosphorylation stops. When that happens, NAD+ and FAD levels rise, and catabolic pathways run unabated with abundant supplies of these electron acceptors. The reason such a scenario is dangerous is because the body is using all of its nutrient resources, but no ATP is being made. Lack of ATP leads to cellular (and organismal) death. In addition, the large amounts of heat generated can raise the temperature of the body to unsafe levels.



Thermogenin

One of the byproducts of uncoupling electron transport is the production of heat. The faster metabolic pathways run, the more heat is generated as a byproduct. Since 2,4 DNP causes metabolism to speed up, a considerable amount of heat can be produced. Controlled uncoupling is actually used by the body in special tissues called brown fat. In this case, brown fat cells use the heat created to help thermoregulate the temperature of newborn children.

Permeabilization of the inner membrane is accomplished in brown fat by the synthesis of a protein called thermogenin (also known as uncoupling protein). Thermogenin binds to the inner membrane and allows protons to pass through it, thus bypassing the ATP synthase. As noted for 2,4 DNP, this results in activation of catabolic pathways and the more catabolism occurs, the more heat is generated.

Dangerous drug

In uncoupling, whether through the action of an endogenous uncoupling protein or DNP, the energy that would have normally been captured in ATP is lost as heat. In the case of uncoupling by thermogenin, this serves the important purpose of keeping

newborn infants warm. But in adults, uncoupling merely wastes the energy that would have been harvested as ATP. In other words, it mimics starvation, even though there is plenty of food, because the energy is dissipated as heat.

This fact, and the associated increase in metabolic rate, led to DNP being used as a weight loss drug in the 1930s. Touted as an effortless way to lose weight without having to eat less or exercise more, it was hailed as a magic weight loss pill. It quickly became apparent, however, that this was very dangerous. Many people died from using this drug before laws were passed to ban the use of DNP as a weight loss aid.

Energy efficiency

Cells are not 100% efficient in energy use. Nothing we know is. Consequently, cells do not get as much energy out of catabolic processes as they put into anabolic processes. A good example is the synthesis and breakdown of glucose, something liver cells are frequently doing. The complete conversion of glucose to pyruvate in glycolysis (catabolism) yields two pyruvates plus 2 NADH plus 2 ATPs. Conversely, the complete conversion of two pyruvates into glucose by gluconeogenesis (anabolism) requires 4 ATPs, 2 NADH, and 2 GTPs. Since the energy of GTP is essentially equal to that of ATP, gluconeogenesis requires a net of 4 ATPs more than glycolysis yields. This difference must be made up in order for the organism to meet its energy needs. It is for this reason that we eat. In addition, the inefficiency of our capture of energy in reactions results in the production of heat and helps to keep us warm, as noted. You can read more about glycolysis (HERE) and gluconeogenesis (HERE).

Metabolic controls of energy

It is also noteworthy that cells do not usually have both catabolic and anabolic processes for the same molecules occurring simultaneously inside of them (for example, breakdown of glucose and synthesis of glucose) because the cell would see no net production of anything but heat and a loss of ATPs with each turn of the cycle. Such cycles are called futile cycles and cells have controls in place to limit the extent to which they occur. Since futile cycles can, in fact, yield heat, they are used as sources of heat in some types of tissue. Brown adipose tissue of mammals uses this strategy, as described earlier.

Summary

In summary, energy is needed for cells to perform the functions that they must carry out in order to stay alive. At its most basic level, this means fighting a continual battle with entropy, but it is not the only need for energy that cells have.

References

1. Winge, D.R., Mol Cell Biol. 2012 Jul; 32(14): 2647–2652. doi: 10.1128/MCB.00573-12

Energy: Electron Transport & Oxidative Phosphorylation

429

YouTube Lectures

by Kevin

HERE & HERE

YouTube Lectures

by Kevin

HERE & HERE

430

Figure 5.14 - Overview of electron transport (bottom left and top right) and oxidative phosphorylation (top left - yellow box) in the mitochondrion

431

Figure 5.15 - Loss of electrons by NADH to form NAD+. Relevant reactions occur in the top ring of the molecule.

432

Figure 5.16 - Flow of electrons from NADH into the electron transport system. Entry is through complex I

Image by Aleia Kim

Figure 5.17 - Flow of electrons from FADH2 into the electron transport chain. Entry is through complex II.

Image by Aleia Kim

Interactive Learning

Module

HERE

433

Figure 5.18 - Complex I embedded in the inner mitochondrial membrane. The mitochondrial matrix at at the top

Wikipedia

434

Figure 5.19 - Complex II embedded in inner mitochondrial membrane. Matrix is up.

Wikipedia

YouTube Lectures

by Kevin

HERE & HERE

435

Figure 5.20 - Movement of electrons through complex I from NADH to coenzyme Q. The mitochondrial matrix is at the bottom

Image by Aleia Kim

Figure 5.21 - Movement of electrons from succinate through complex II (A->B->C->D->Q). Mitochondrial matrix on bottom.

Image by Aleia Kim

436

Figure 5.22 - Complex II in inner mitochondrial membrane showing electron flow. Matrix is up.

Wikipedia

Figure 5.23 - Coenzyme Q

437

Movie 5.2 - The Q-cycle

Wikipedia
Figure 5.24 - The Q-Cycle Image by Aleia Kim

Figure 5.24 - Complex III

Wikipedia

438

YouTube Lectures

by Kevin

HERE & HERE

Figure 5.25 - The Q-cycle. Matrix is down.

Image by Aleia Kim

439

Figure 5.26 - Movement of electrons and protons through complex IV. Matrix is down

Image by Aleia Kim

Figure 5.25 - Cytochrome c with bound heme Group

Wikipedia

440

Figure 5.27 - Mitochondrial anatomy. Electron transport complexes and ATP synthase are embedded in the inner mitochondrial membrane

Image by Aleia Kim

441

Figure 5.28 - ATP synthase. Protons pass from intermembrane space (top) through the complex and exit in the matrix (bottom).

Image by Aleia Kim

Interactive Learning

Module

HERE

442

Movie 5.3 - ATP Synthase - ADP + Pi (pink) and ATP (red). The view is end-on from the cytoplasmic side viewing the β subunits
Movie 5.3 - ATP Synthase - ADP + Pi (pink) and ATP (red). The view is end-on from the cytoplasmic side viewing the β subunits

443

Figure 5.29 - Important structural features of the ATP synthase

Image by Aleia Kim

444

Figure 5.30 - Loose (L), Tight (T), and Open (O) structures of the F1 head of ATP synthase. Change of structure occurs by rotation of γ-protein (purple) in center as a result of proton movement. Individual α and β units do not rotate

Image by Aleia Kim

445

Figure 5.31 - Respiration overview in eukaryotic cells

Wikipedia

YouTube Lectures

by Kevin

HERE & HERE

446

Rest

ATP High / ADP Low

Oxidative Phosphorylation Low

Electron Transport Low

Oxygen Use Low

NADH High / NAD+ Low

Citric Acid Cycle Slow

Exercise

ATP Low / ADP High

Oxidative Phosphorylation High

Electron Transport High

Oxygen Use High

NADH Low / NAD+ High

Citric Acid Cycle Fast

Interactive Learning

Module

HERE

447

Figure 5.32 - Three inhibitors of electron transport

Image by Aleia Kim

448

Figure 5.33 - Oligomycin A - An inhibitor of ATP synthase

Figure 5.34 - 2,4 DNP - an uncoupler of respiratory control

449

In Cells With Tight Coupling

O2 use depends on metabolism
NAD+ levels vary with exercise
Proton gradient high with no exercise
Catabolism depends on energy needs
ETS runs when OxPhos runs and vice versa

In Cells That Are Uncoupled

O2 use high
NAD+ Levels high
Little or no proton gradient
Catabolism high
OxPhos does not run, but ETS runs rapidly

YouTube Lectures

by Kevin

HERE & HERE

450

451

Figure 5.35 - Alternative oxidase (AOX) of fungi, plants, and protozoa bypasses part of electron transport by taking electrons from CoQ and passing them to oxygen.

452

Figure 5.36 - Structure of an oxygen free radical

Wikipedia

NADPH + 2O2

NADP+ + 2O2– + H+

Figure 5.37 - Three sources of reactive oxygen species (ROS) in cells

Wikipedia

453

454

YouTube Lectures

by Kevin

HERE & HERE

Figure 5.38 A hydroxyl radical

Wikipedia

455

Reduced Glutathione (GSH) + H2O2

Oxidized Glutathione (GSSG) + H2O

Figure 5.40 - Detoxifying reactive oxygen species

Figure 5.39 - Catalase

456

1. O2- + Enzyme-Cu++

O2 + Enzyme-Cu+

2. O2- + Enzyme-Cu+ + 2H+

H2O2 + Enzyme-Cu++

Figure 5.41 - SOD2 of humans

Figure 5.42 3 - Peroxynitrite Ion

Figure 5.44 - SOD1 of humans

Wikipedia

Figure 5.45 - SOD3 of humans

457

Figure 5.43 - Peroxynitrite's effects on cells lead to necrosis or apoptosis

Wikipedia

458

RH + O2 + NADPH + H+

ROH + H2O + NADP+

459

Figure 5.46 - Cytochrome c with its heme group

460

YouTube Lectures

by Kevin

HERE & HERE

Figure 5.47 - Fe2S2 Cluster

Figure 5.48 - Redox reactions for Fe4S4 clusters

461

Figure 5.49 - Tyramine

Figure 5.50 - Phenethylamine

462

Figure 5.51 - Guanine and 8-oxo-guanine

Figure 5.52 - Adenine-8-oxo-guanine base pair. dR = deoxyribose

463

Figure 5.53 - Good antioxidant sources

464

Figure 5.55 - Oxidized glutathiones (GSSG) joined by a disulfide bond

Wikipedia

Figure 5.54 - Structure of reduced glutathione (GSH)

465

Figure 5.56 - Resveratrol

YouTube Lectures

by Kevin

HERE & HERE

466

Graphic images in this book were products of the work of several talented students. Links to their Web pages are below

Click HERE for

Martha Baker's

Web Page

Click HERE for

Pehr Jacobson's

Web Page

Click HERE for

Aleia Kim's

Web Page

Click HERE for

Penelope Irving's

Web Page

Problem set related to this section HERE

Point by Point summary of this section HERE

To get a certificate for mastering this section of the book, click HERE

Kevin Ahern's free iTunes U Courses - Basic / Med School / Advanced

Biochemistry Free & Easy (our other book) HERE / Facebook Page

Kevin and Indira's Guide to Getting into Medical School - iTunes U Course / Book

To see Kevin Ahern's OSU ecampus courses - BB 350 / BB 450 / BB 451

To register for Kevin Ahern's OSU ecampus courses - BB 350 / BB 450 / BB 451

Biochemistry Free For All Facebook Page (please like us)

Kevin Ahern's Web Page / Facebook Page / Taralyn Tan's Web Page

Kevin Ahern's free downloads HERE

OSU's Biochemistry/Biophysics program HERE

OSU's College of Science HERE

Oregon State University HERE

Email Kevin Ahern / Indira Rajagopal / Taralyn Tan

I'm a little mitochondrion
Who gives you energy
I use my proton gradient
To make the ATPs

He's a little mitochondrion
Who gives us energy
He uses proton gradients
To make some ATPs

Electrons flow through Complex II
To traffic cop Co-Q
Whenever they arrive there in
An FADH-two

Electrons flow through Complex II
To traffic cop Co-Q
Whenever they arrive there in
An FADH-two

Tightly coupled is my state
Unless I get a hole
Created in my membrane by
Some di-ni-tro-phe-nol

Yes tightly coupled is his state
Unless he gets a hole
Created in his membrane by
Some di-ni-tro-phenol

Both rotenone and cyanide
Stop my electron flow
And halt the calculation of
My "P" to "O" ratio

Recording by Tim Karplus

Lyrics by Kevin Ahern
Recording by Tim Karplus Lyrics by Kevin Ahern

I'm a Little Mitochondrion

To the tune of "I'm a Lumberjack"

Metabolic Melodies Website HERE

In the catabolic pathways that our cells employ
Oxidations help create the ATP
While they lower Gibbs free energy
Thanks to enthalpy

If a substrate is converted from an alcohol
To an aldehyde or ketone it is clear
Those electrons do not disappear
They just rearrange – very strange

N-A-D is in my ears and in my eyes
Help-ing mol-e-cules get oxidized
Making N-A-D-H then

And the latter is a problem anaerobically
'Cuz accumulations of it muscles hate
They respond by using pyruvate
To produce lactate

Catalyzing is essential for the cells to live
So the enzymes grab their substrates eagerly
If they bind with high affinity
Low Km you see, just as me

N-A-D is in my ears and in my eyes
Help-ing mol-e-cules get oxidized
Making N-A-D-H then

N-A-D

To the tune of "Penny Lane"

Metabolic Melodies Website HERE

Recorded by Tim Karplus

Lyrics by Kevin Ahern
Recorded by Tim Karplus Lyrics by Kevin Ahern

When oxygen's electrons all are in the balanced state

There's twelve of them for oh-two. The molecule is great

But problems sometimes happen on the route to complex IV

Making reactive species that the cell cannot ignore

Oh superoxide dismutase is super catalytic

Keeping cells from getting very peroxynitritic

Faster than a radical, its actions are terrific

Superoxide dismutase is super catalytic

Enzyme, enzyme deep inside

Blocking all the bad oxides

The enzyme's main advantage is it doesn't have to wait

By binding superoxide in a near-transition state

It turns it to an oxygen in mechanism one

Producing "h two oh two" when the cycle is all done

Oh superoxide dismutase you're faster than all them

You've got the highest ratio of kcat over KM

This means that superoxide cannot cause too much mayhem

Superoxide dismutase is faster than all them

Superoxide dismutase

Stopping superoxide's ways

The enzyme's like a ping-pong ball that mechanistic-ly

Bounces between two copper states, plus one and two you see

So S-O-D behaves just like an anti-oxidant

Giving as much protection as a cell could ever want

Oh superoxide dismutase, the cell's in love with you

Because you let electron transport do what it must do

Without accumulation of a radical oh two

Superoxide dismutase - that's why a cell loves you

Superoxide Dismutase

To the tune of "Supercalifragilistiexpialidocious"

Metabolic Melodies Website HERE

Lyrics by Kevin Ahern

No Recording Yet For This Song

This page titled 8.7: Electron Transport and Oxidative Phosphorylation is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 5.2: Electron Transport and Oxidative Phosphorylation by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

8.8: Carbohydrate Storage and Breakdown

Carbohydrates are important cellular energy sources. They provide energy quickly through glycolysis and passing of intermediates to pathways, such as the citric acid cycle, amino acid metabolism (indirectly), and the pentose phosphate pathway. It is important, therefore, to understand how these important molecules are made.

Plants are notable in storing glucose for energy in the form of amylose and amylopectin (see and for structural integrity in the form of cellulose. These structures differ in that cellulose contains glucoses solely joined by beta-1,4 bonds, whereas amylose has only alpha1,4 bonds and amylopectin has alpha 1,4 and alpha 1,6 bonds.Animals store glucose primary in liver and muscle in the form of a compound related to amylopectin known as glycogen. The structural differences between glycogen and amylopectin are solely due to the frequency of the alpha 1,6 branches of glucoses. In glycogen they occur about every 10 residues instead of every 30-50, as in amylopectin.

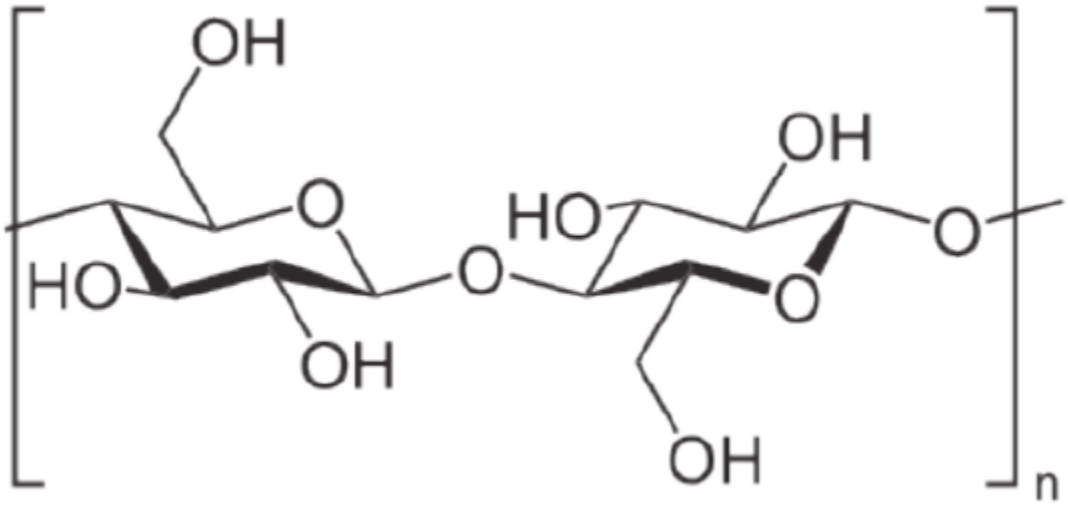


Figure 7.1.1: *The Repeating Structure of Cellulose*

Glycogen provides an additional source of glucose besides that produced via gluconeogenesis. Because glycogen contains so many glucoses, it acts like a battery backup for the body, providing a quick source of glucose when needed and providing a place to store excess glucose when glucose concentrations in the blood rise. The branching of glycogen is an important feature of the molecule metabolically as well. Since glycogen is broken down from the "ends" of the molecule, more branches translate to more ends, and more glucose that can be released at once. Breakdown of glycogen involves

1. release of glucose-1- phosphate (G1P),
2. rearranging the remaining glycogen (as necessary) to permit continued breakdown, and
3. conversion of G1P to G6P for further metabolism. G6P can be 1) broken down in glycolysis, 2) converted to glucose by gluconeogenesis, and 3) oxidized in the pentose phosphate pathway.

Just as in gluconeogenesis, the cell has a separate mechanism for glycogen synthesis that is distinct from glycogen breakdown. As noted previously, this allows the cell to separately control the reactions, avoiding futile cycles, and enabling a process to occur efficiently (synthesis of glycogen) that would not occur if it were simply the reversal of glycogen breakdown.

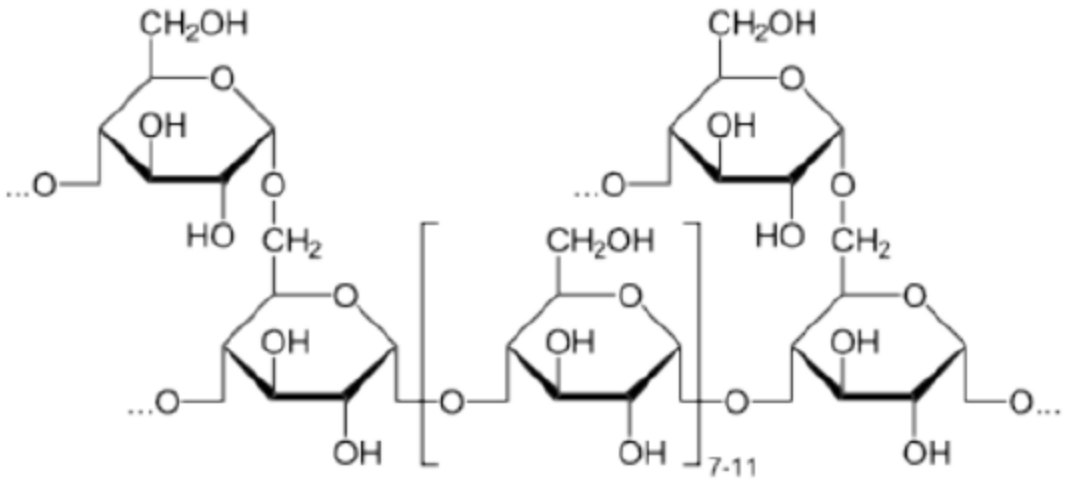


Figure 7.1.2: *The Repeating Unit of Glycogen*

Synthesis of glycogen starts with G1P, which is converted to an 'activated' intermediate, UDP-glucose. This activated intermediate is what 'adds' the glucose to the growing glycogen chain in a reaction catalyzed by the enzyme known as glycogen synthase. Once the glucose is added to glycogen, the glycogen molecule may need to have branches inserted in it by the enzyme known as branching enzyme.

Glycogen Breakdown

Glycogen phosphorylase (sometimes simply called phosphorylase) catalyzes breakdown of glycogen into Glucose-1-Phosphate (G1P). The reaction, (see above right) that produces G1P from glycogen is a phosphorolysis, not a hydrolysis reaction. The distinction is that hydrolysis reactions use water to cleave bigger molecules into smaller ones, but phosphorolysis reactions use phosphate instead for the same purpose. Note that the phosphate is just that - it does NOT come from ATP. Since ATP is not used to put phosphate on G1P, the reaction saves the cell energy.

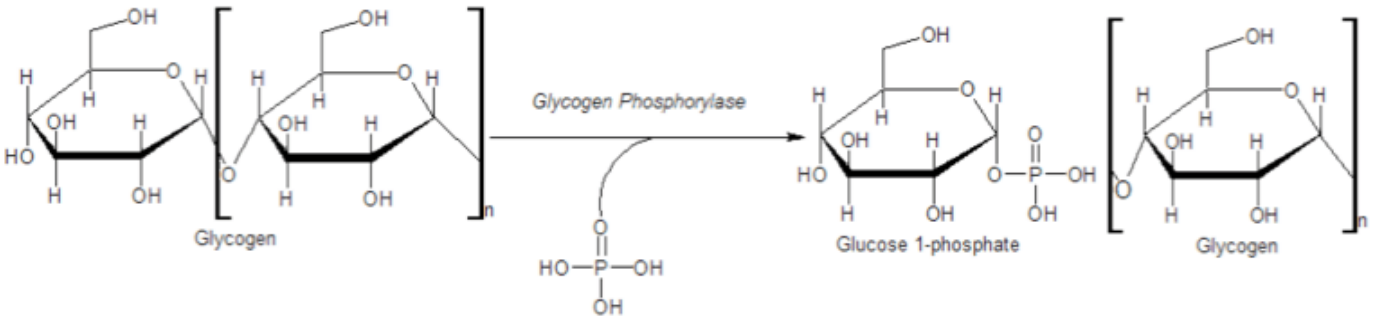


Figure 7.1.3: *Phosphorolysis of Glycogen*

Glycogen phosphorylase will only act on non-reducing ends of a glycogen chain that are at least 5 glucoses away from a branch point. A second enzyme, Glycogen Debranching Enzyme (GDE), is therefore needed to convert alpha(1-6) branches to alpha(1-4) branches. GDE acts on glycogen branches that have reached their limit of hydrolysis with glycogen phosphorylase. GDE acts to transfer a trisaccharide from a 1,6 branch onto an adjacent 1,4 branch, leaving a single glucose at the 1,6 branch. Note that the enzyme also catalyzes the hydrolysis of the remaining glucose at the 1,6 branch point. Thus, the breakdown products from glycogen are G1P and glucose (mostly G1P, however). Glucose can, of course, be converted to Glucose-6-Phosphate (G6P) as the first step in glycolysis by either hexokinase or glucokinase. G1P can be converted to G6P by action of an enzyme called phosphoglucomutase. This reaction is readily reversible, allowing G6P and G1P to be interconverted as the concentration of one or the other increases. This is important, because phosphoglucomutase is needed to form G1P for glycogen biosynthesis.

Regulation of Glycogen Metabolism

Regulation of glycogen metabolism is complex, occurring both allosterically and via hormone-receptor controlled events that result in protein phosphorylation or dephosphorylation. In order to avoid a futile cycle of glycogen synthesis and breakdown simultaneously, cells have evolved an elaborate set of controls that ensure only one pathway is primarily active at a time.

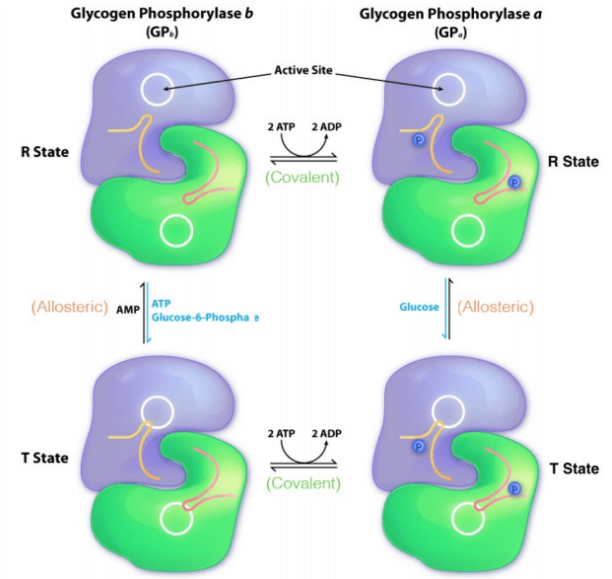


Figure 7.1.4: *Regulation of Glycogen Phosphorylase*

Regulation of glycogen metabolism is managed by the enzymes glycogen phosphorylase and glycogen synthase. Glycogen phosphorylase is regulated by both allosteric factors (ATP, G6P, AMP, and glucose) and by covalent modification (phosphorylation/dephosphorylation). Its regulation is consistent with the energy needs of the cell. High energy substrates (ATP, G6P, glucose) allosterically inhibit GP, while low energy substrates (AMP, others) allosterically activate it.

GPa/GPb Allosteric Regulation

Glycogen phosphorylase exists in two different covalent forms – one form with phosphate (called GPa here) and one form lacking phosphate (GPb here). GPb is converted to GPa by phosphorylation by an enzyme known as phosphorylase kinase. GPa and GPb can each exist in an 'R' state and a 'T' state. For both GPa and GPb, the R state is the more active form of the enzyme. GPa's negative allosteric effector (glucose) is usually not abundant in cells, so GPa does not .ip into the T state often. There is no positive allosteric effector of GPa, so when glucose is absent, GPa automatically flips into the R (more active) state.

GPb can convert from the T state to the GPb R state by binding AMP. Unless a cell is low in energy, AMP concentration is low. Thus GPb is not converted to the R state very often. On the other hand, ATP and/or G6P are usually present at high enough concentration in cells that GPb is readily flipped into the T state.

GPa/GPb Covalent Conversion

Because the relative amounts of GPa and GPb largely govern the overall process of glycogen breakdown, it is important to understand the controls on the enzymes that interconvert GPa and GPb. This is accomplished by the enzyme Phosphorylase Kinase, which transfers phosphates from 2 ATPs to GPb to form GPa. Phosphorylase kinase has two covalent forms – phosphorylated (active) and dephosphorylated (inactive). It is phosphorylated by the enzyme Protein Kinase A (PKA). Another way to activate the enzyme is with calcium. Phosphorylase kinase is dephosphorylated by the same enzyme, phosphoprotein phosphatase, that removes phosphate from GPa.

PKA is activated by cAMP, which is, in turn produced by adenylate cyclase after activation by a G-protein. G-proteins are activated ultimately by binding of ligands to specific 7-TM receptors, also known as G-protein coupled receptors. These are discussed in greater detail in Chapter 8. Common ligands for these receptors include epinephrine (binds beta-adrenergic receptor) and glucagon (binds glucagon receptor). Epinephrine exerts it greatest effects on muscle and glucagon works preferentially on the

liver.

Turning Off Glycogen Breakdown

Turning OFF signals is as important, if not more so, than turning them ON. The steps in the glycogen breakdown regulatory pathway can be reversed at several levels. First, the ligand can leave the receptor. Second, the G-proteins have an inherent GTPase activity that serves to turn them off over time. Third, cells have phosphodiesterase (inhibited by caffeine) for breaking down cAMP. Fourth, an enzyme known as phosphoprotein phosphatase can remove phosphates from phosphorylase kinase (inactivating it) AND from GPa, converting it to the much less active GPb.

Glycogen Synthesis

The anabolic pathway contrasting with glycogen breakdown is that of glycogen synthesis. Just as cells reciprocally regulate glycolysis and gluconeogenesis to prevent a futile cycle, so too do cells use reciprocal schemes to regulate glycogen breakdown and synthesis. Let us first consider the steps in glycogen synthesis. 1) Glycogen synthesis from glucose involves phosphorylation to form G6P, and isomerization to form G1P (using phosphoglucomutase common to glycogen breakdown). G1P is reacted with UTP to form UDP-glucose in a reaction catalyzed by UDP-glucose pyrophosphorylase. Glycogen synthase catalyzes synthesis of glycogen by joining carbon #1 of the UDPG-derived glucose onto the carbon #4 of the non-reducing end of a glycogen chain. to form the familiar alpha(1,4) glycogen links. Another product of the reaction is UDP.

It is also worth noting in passing that glycogen synthase will only add glucose units from UDPG onto a preexisting glycogen chain that has at least four glucose residues. Linkage of the first few glucose units to form the minimal "primer" needed for glycogen synthase recognition is catalyzed by a protein called glycogenin, which attaches to the first glucose and catalyzes linkage of the first eight glucoses by alpha(1,4) bonds. 3) The characteristic alpha(1,6) branches of glycogen are the products of an enzyme known as Branching Enzyme. Branching Enzyme breaks alpha(1,4) chains and carries the broken chain to the carbon #6 and forms an alpha(1,6) linkage.

Regulation of Glycogen Synthesis

The regulation of glycogen biosynthesis is reciprocal to that of glycogen breakdown. It also has a cascading covalent modification system similar to the glycogen breakdown system described above. In fact, part of the system is identical to glycogen breakdown. Epinephrine or glucagon signaling can stimulate adenylate cyclase to make cAMP, which activates PKA, which activates phosphorylase kinase.

In glycogen breakdown, phosphorylase kinase phosphorylates GPb to the more active form, GPa. In glycogen synthesis, protein kinase A phosphorylates the active form of glycogen synthase (GSa), and converts it into the usually inactive b form (called GSb). Note the conventions for glycogen synthase and glycogen phosphorylase. For both enzymes, the more active forms are called the 'a' forms (GPa and GSa) and the less active forms are called the 'b' forms (GPb and GSb). The major difference, however, is that GPa has a phosphate, but GSa does not and GPb has no phosphate, but GSb does. Thus phosphorylation and dephosphorylation have opposite effects on the enzymes of glycogen metabolism. This is the hallmark of reciprocal regulation. It is of note that the less active glycogen synthase form, GSb, can be activated by G6P. Recall that G6P had the exactly opposite effect on GPb.

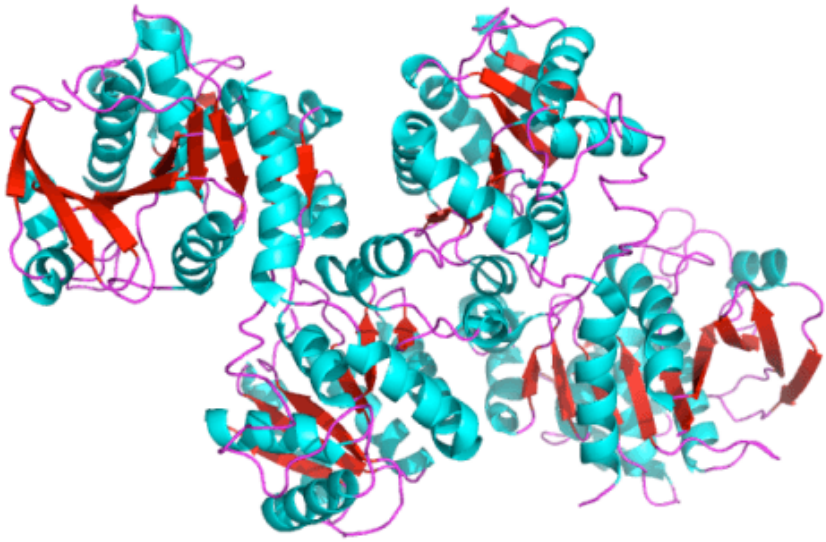


Figure 7.1.5: *Glycogen Synthase*

Glycogen synthase, glycogen phosphorylase (and phosphorylase kinase) can be dephosphorylated by several enzymes called phosphatases. One of these is called Protein Phosphatase and it is activated when insulin binds to a receptor in the cell membrane. It causes PP to be activated, stimulating dephosphorylation, and thus activating glycogen synthesis and inhibiting glycogen breakdown. Again, there is reciprocal regulation of glycogen synthesis and degradation.

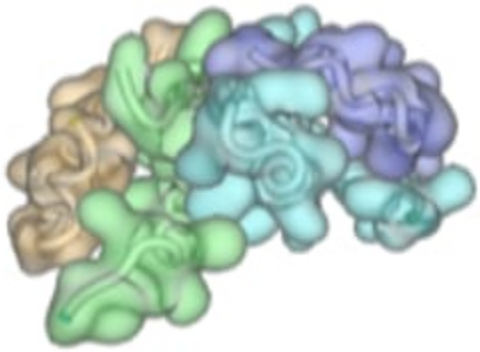


Figure 7.1.6: *The 3D structure of Insulin*

Maintaining Blood Glucose Levels

After a meal, blood glucose levels rise and insulin is released. It simultaneously stimulates uptake of glucose by cells and incorporation of it into glycogen by activation of glycogen synthase and inactivation of glycogen phosphorylase. When blood glucose levels fall, GPa gets activated (stimulating glycogen breakdown to raise blood glucose) and GSb is formed (stopping glycogen synthesis).

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 8.8: Carbohydrate Storage and Breakdown is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 7.1: Carbohydrate Storage and Breakdown by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

9: LIPIDS AND MEMBRANES

9.1: Structure and Function - Lipids and Membranes
9.2: Basic Concepts in Membranes
9.3: Transport in Membranes
9.4: Metabolism of Fat
9.5: Fatty Acid Oxidation
9.6: Ketone body production and acidosis

This page titled 9: Lipids and membranes is shared under a license and was authored, remixed, and/or curated by LibreTexts.

9.1: Structure and Function - Lipids and Membranes

Source: BiochemFFA_2_7.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy



Lipids are a diverse group of molecules that all share the characteristic that at least a portion of them is hydrophobic. Lipids play many roles in cells, including serving as energy storage (fats/oils), constituents of membranes (glycerophospholipids, sphingolipids, cholesterol), hormones (steroids), vitamins (fat soluble), oxygen/ electron carriers (heme), among others. For lipids that are very hydrophobic, such as fats/ oils, movement and storage in the aqueous environment of the body requires special structures. Other, amphipathic lipids, such as glycerophospholipids and sphingolipids spontaneously organize themselves into lipid bilayers when placed in water. Interestingly, major parts of many lipids can be derived from acetyl-CoA.

Fatty acids

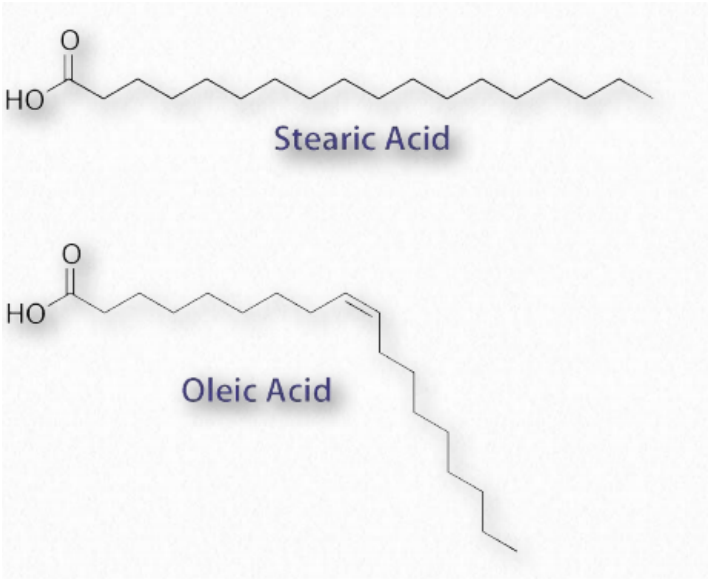


Figure 2.19Ø - Saturated fatty acid (stearic acid) and unsaturated fatty acid (oleic acid)

The most ubiquitous lipids in cells are the fatty acids. Found in fats, glycerophospholipids, sphingolipids and serving as as

membrane anchors for proteins and other biomolecules, fatty acids are important for energy storage, membrane structure, and as precursors of most classes of lipids. Fatty acids, as can be seen from Figure 2.190 are characterized by a polar head group and a long hydrocarbon tail. Fatty acids with hydrocarbon tails that lack any double bonds are described as saturated, while those with one or more double bonds in their tails are known as unsaturated fatty acids. The effect of double bonds on the fatty acid tail is to introduce a kink, or bend, in the tail, as shown for oleic acid.

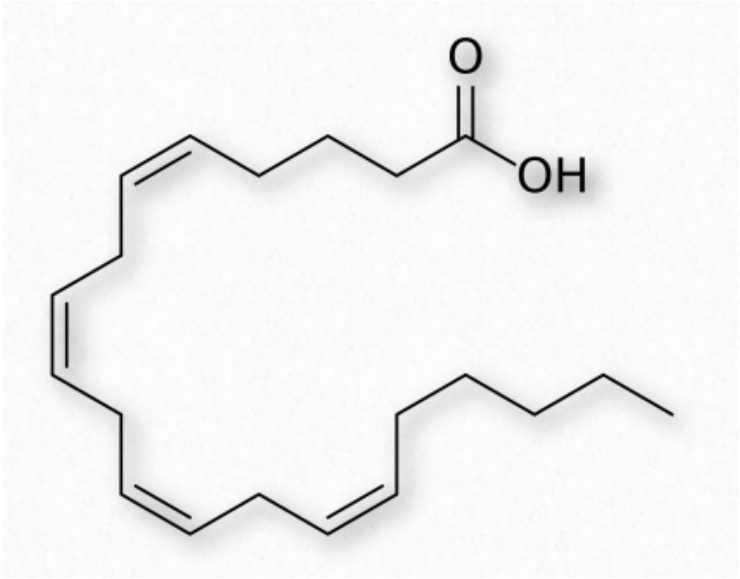


Figure 2.191 - Arachidonic acid - A polyunsaturated fatty acid Wikipedia

Stearic acid, a saturated fatty acid, by contrast has a straight hydrocarbon tail. Figures 2.190-2.194 show the most common saturated and unsaturated fatty acids. Fatty acids with unsaturated tails have a lower melting temperature than those with saturated tails of the same length. Shorter tails also decrease melting temperature. These properties carry over to the fats/oils containing them.

Common name	Chemical structure	
Caprylic acid	$CH_3(CH_2)_6COOH$	8
Capric acid	$CH_3(CH_2)_8COOH$	10
Lauric acid	$CH_3(CH_2)_{10}COOH$	12
Myristic acid	$CH_3(CH_2)_{12}COOH$	14
Palmitic acid	$CH_3(CH_2)_{14}COOH$	16
Stearic acid	$CH_3(CH_2)_{16}COOH$	18
Arachidic acid	$CH_3(CH_2)_{18}COOH$	20
Behenic acid	$CH_3(CH_2)_{20}COOH$	22
Lignoceric acid	$CH_3(CH_2)_{22}COOH$	24
Cerotic acid	$CH_3(CH_2)_{24}COOH$	26

Figure 2.192 - Saturated fatty acids. Number of carbons in right column Wikipedia

Fatty acids with more than one double bond are called polyunsaturated. Plants are excellent sources of unsaturated and polyunsaturated fatty acids. The position of the double bond(s) in fatty acids has important considerations both for their synthesis and for their actions in the body. 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.

Name	Double Bond Info	
Myristoleic acid	*cis*-Δ^9	14:1
Palmitoleic acid	*cis*-Δ^9	16:1
Sapienic acid	*cis*-Δ^6	16:1
Oleic acid	*cis*-Δ^9	18:1
Elaidic acid	*trans*-Δ^9	18:1
Vaccenic acid	*trans*-Δ^{11}	18:1
Linoleic acid	*cis,cis*-Δ^9,Δ^{12}	18:2
Linoelaidic acid	*trans,trans*-Δ^9,Δ^{12}	18:2
α-Linolenic acid	*cis,cis,cis*-$\Delta^9,\Delta^{12},\Delta^{15}$	18:3
Arachidonic acid	*cis,cis,cis,cis*-$\Delta^5\Delta^8,\Delta^{11},\Delta^{14}$	20:4
Eicosapentaenoic acid	*cis,cis,cis,cis,cis*-$\Delta^5,\Delta^8,\Delta^{11},\Delta^{14},\Delta^{17}$	20:5
Erucic acid	*cis*-Δ^{13}	22:1
Docosahexaenoic acid	*cis,cis,cis,cis,cis,cis*-$\Delta^4,\Delta^7,\Delta^{10},\Delta^{13},\Delta^{16},\Delta^{19}$	22:6

Figure 2.193 - Unsaturated fatty acids. Right column Indicates number of carbons and double bonds Wikipedia

In humans, consumption of trans fat 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 Figure 2.194 - Fatty acid models. Carboxyl end labeled in red Wikipedia common fatty acids in our body include palmitate, stearate, oleate, linolenate, linoleate, and arachidonate. Two notable shorter fatty acids are nonanoic (9 carbons) and decanoic acid (10 carbons), both of which appear to have anti-seizure effects. Decanoic acid directly inhibits excitatory neurotransmission in the brain and may contribute to the anticonvulsant effect of the ketogenic diet.

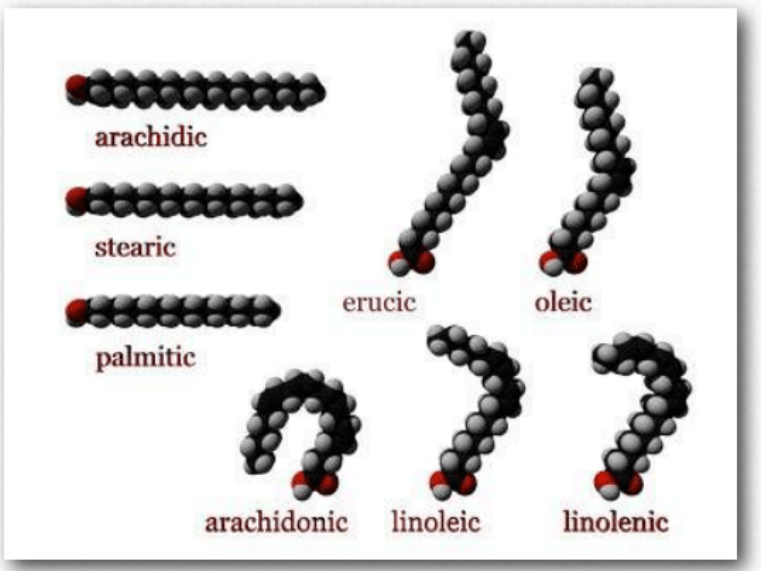


Figure 2.194 - Fatty acid models. Carboxyl end labeled in red Wikipedia

Numbering

Figure 2.195 shows two different systems for locating double bonds in a fatty acid. The ω system counts carbons starting with the methyl end (shown in red) while the Δ system counts from the carboxyl end (shown in blue). For example, an ω-3 (omega 3) fatty acid would have a double bond at the third carbon from the methyl end. In the Δ system, a fatty acid that has a cis double bond at carbon 6, counting from the carboxyl end, would be written as cis-Δ6.

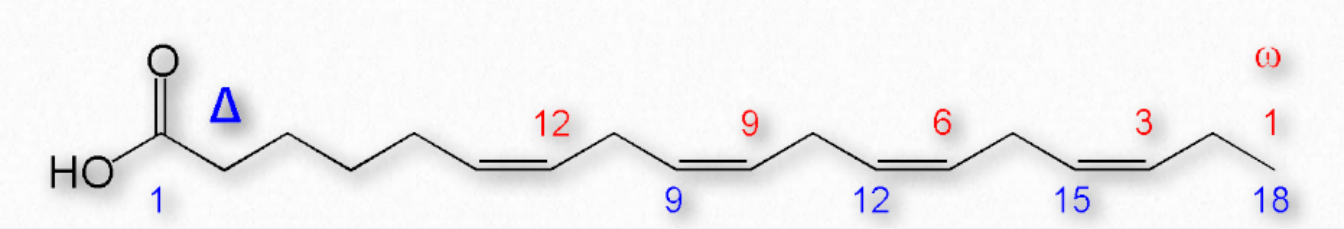


Figure 2.195 - Δ and ω numbering systems for fatty acids Image by Pehr Jacobson

Fatty acids are described as essential fatty acids if they must be in the diet (can't be synthesized by the organism) and nonessential fatty acids if the organism can synthesize them. Humans and other animals lack the desaturase enzymes necessary to make double bonds at positions greater than Δ-9, so fatty acids with double bonds beyond this position must be obtained in the diet. Linoleic acid and linolenic acid, both fall in this category. Related unsaturated fatty acids can be made from these fatty acids, so the presence of linoleic and linolenic acids in the diet eliminates the need to have all unsaturated fatty acids in the diet. Both linoleic and linolenic acid contain 18 carbons, but linoleic acid is an ω-6 fatty acid, whereas linolenic acid is an ω-3 fatty acid. Notably, ω-6 fatty acids tend to be proinflammatory, whereas ω-3 fatty acids are lesser so.

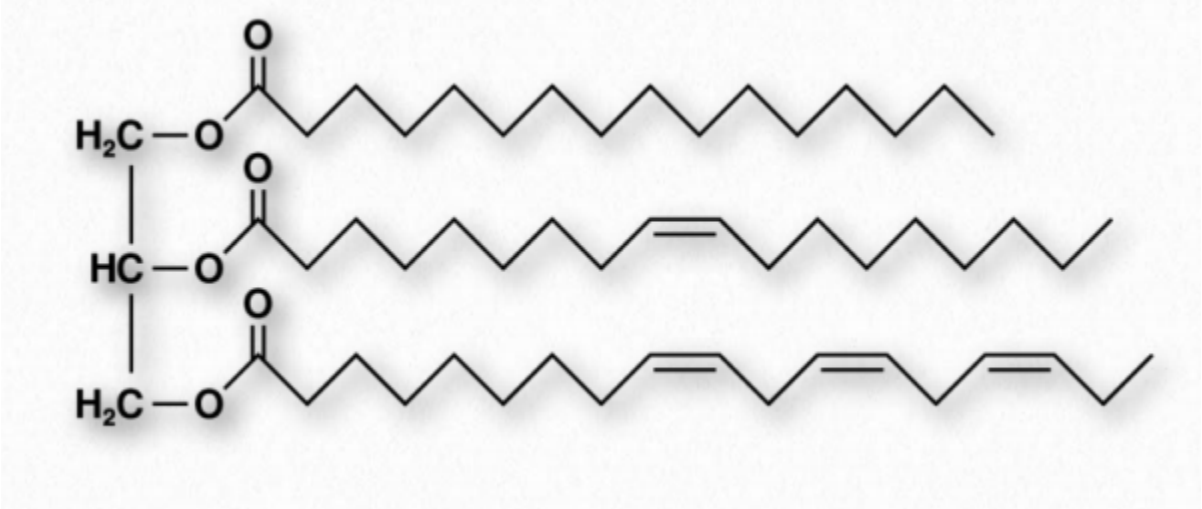


Figure 2.196 - Structure of a fat/oil

Fats/oils

Fats and oils are the primary energy storage forms of animals and are also known as triacylglycerols and triglycerides, since they consist of a glycerol molecule linked via ester bonds to three fatty acids (Figure 2.196). Fats and oils have the same basic structure. We give the name fat to those compounds that are solid at room temperature and the name oil to those that are liquid at room temperature. Note that biological oils are not the same as petroleum oils.

Increasing the number of unsaturated fatty acids (and the amount of unsaturation in a given fatty acid) in a fat decreases the melting temperature of it. Organisms like fish, which live in cool environments, have fats with more unsaturation and this is why fish oil contains polyunsaturated fatty acids.

Adipocytes

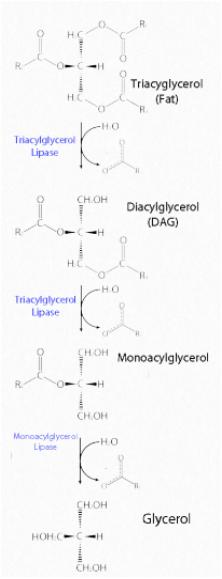


Figure 2.197 - Lipase action on a fat Image by Aleia Kim

Fats are stored in the body in specialized cells known as adipocytes. Enzymes known as lipases release fatty acids from fats by hydrolysis reactions (Figure 2.197). Triacylglycercol lipase (pancreatic - Figure 2.198) is able to cleave the first two fatty acids from the fat. A second enzyme, monoacylglycerol lipase, cleaves the last fatty acid. Fats can be synthesized by replacing the phosphate on phosphatidic acid with a fatty acid.



Figure 2.198 - Pancreatic lipase

Glycerophospholipids

Glycerophospholipids (phosphoglycerides) are important components of the lipid bilayer of cellular membranes. Phosphoglycerides are structurally related to fats, as both are derived from phosphatidic acid (Figure 2.199). 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 (Figure 2.200) to the phosphate of phosphatidic acid. All of these compounds form lipid bilayers in aqueous solution , due to their amphiphilic nature.

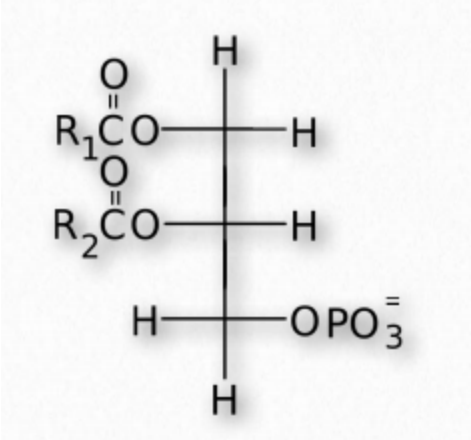


Figure 2.199 - Structure of phosphatidic acid. R1 and R2 are alkyl groups of fatty acids.

Phosphatidylethanolamines

Since all glycerolipids can have a variety of fatty acids at positions 1 and 2 on the glycerol, they all are families of compounds. The phosphatidylethanolamines are found in all living cells and are one of the most common phosphatides, making up about 25% of them. They are common constituents of brain tissue and in the spinal cord, making up as much as 45% of the total phospholipids. Phosphatidylethanolamines are asymmetrically distributed across membranes, being preferentially located on the inner leaflet (closest to the cytoplasm) of the plasma membrane. Metabolically, phosphatidylethanloamines are precursors of phosphatidylcholines. Phosphatidylserines Phosphatidylserines are another group of phosphatidyl compounds that are preferentially distributed across the lipid bilayer of the plasma membrane. Like the phosphatidylethanolamines, phosphatidylserines are preferentially located on the inner leaflet of the plasma membrane. When apoptosis (cell suicide) occurs, the preferential distribution is lost and the phosphatidylserines appear on the outer leaflet where they serve as a signal to macrophages to bind and destroy the cell.

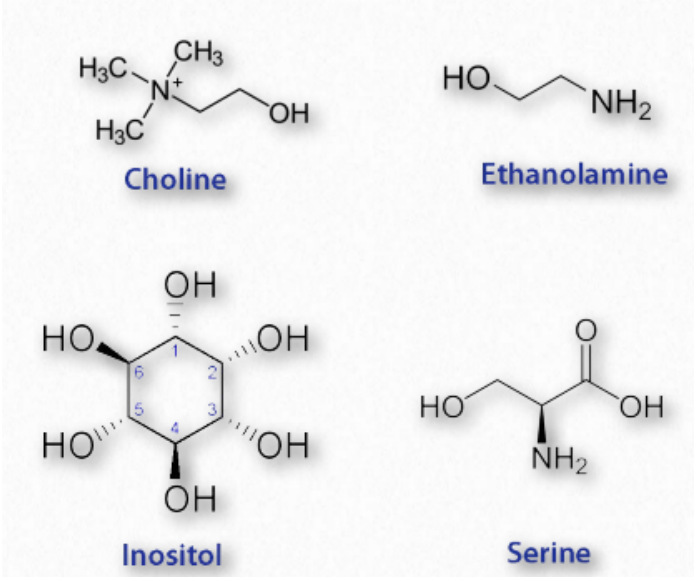


Figure 2.200 - Four common components of phosphatides Wikipedia

Phosphatidylcholines

Phosphatidylcholines (Figure 2.201) are another group of important membrane components. They tend to be found more commonly on the outer leaflet of the plasma membrane. Nutritionally, the compounds are readily obtained from eggs and soybeans. Phosphatidylcholines are moved across membranes by Phosphatidylcholine transfer protein (PCTP). This protein, which is sensitive to the levels of phosphatidylcholines, acts to stimulate the activity of a thioesterase (breaks thioester bonds, such as acyl-CoAs) and activates PAX3 transcription factors.

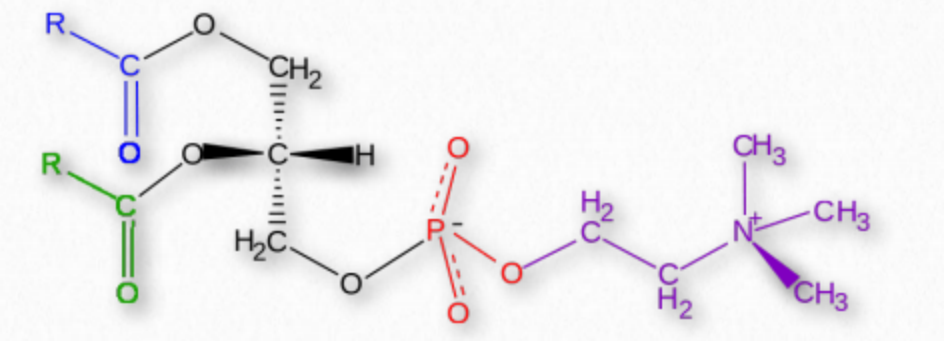


Figure 2.201 - Phosphatidylcholine

Cardiolipins

Cardiolipins are an unusual set of glycerophospholipids in containing two diacylglycerol backbones joined in the middle by a diphosphoglycerol (Figure 2.202). It is an important membrane lipid, constituting about 20% of the inner mitochondrial membrane and is found in organisms from bacteria to humans. In both plants and animals, it is found almost totally in the inner mitochondrial membrane.

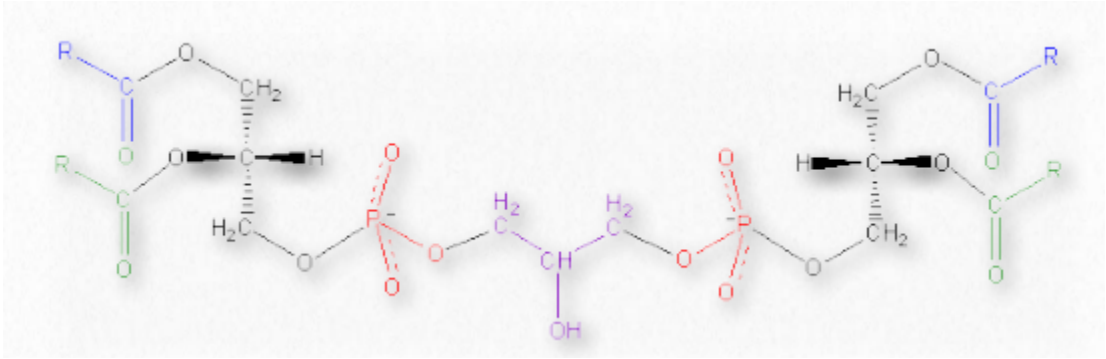


Figure 2.202 - Cardiolipin

The molecules appear to be required for both Complex IV and Complex III of the electron transport chain to maintain its structure. The ATP synthase enzyme (Complex V) of the oxidative phosphorylation system also binds four molecules of cardiolipin. It has been proposed that cardiolipin functions as a proton trap in the process of proton pumping by Complex IV.

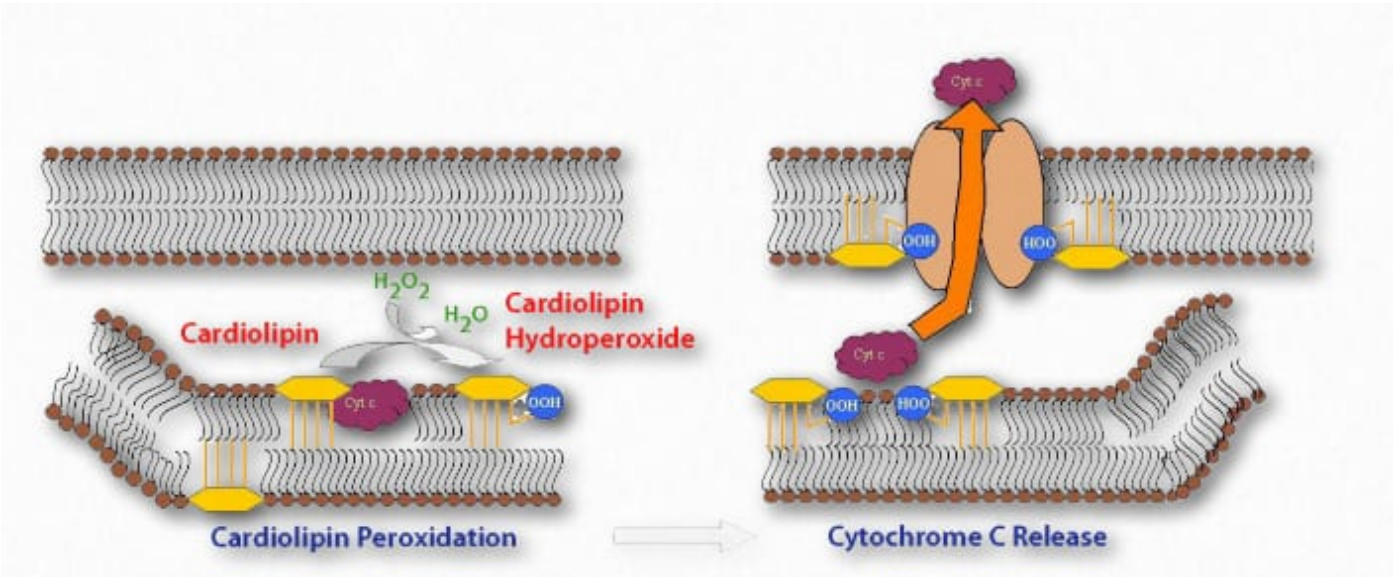


Figure 2.203 - Cardiolipin oxidation and the release of cytochrome C in apoptosis

Cardiolipin also plays a role in apoptosis. As shown in Figure 2.203, oxidation of cardiolipin by a cardiolipin-specific oxygenase causes cardiolipin to move from the inner mitochondrial membrane to the outer one, helping to form a permeable pore and facilitating the transport of cytochrome c out of the intermembrane space and into the cytoplasm - a step in the process of apoptosis.

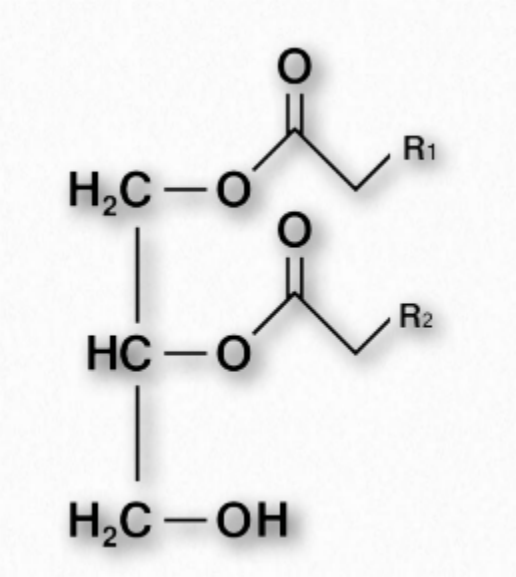


Figure 2.204 - Diacylglycerol

Diacylglycerol

Diacylglycerol (also called diglyceride and DAG - Figure 2.204) is an important intermediate in metabolic pathways. It is produced, for example, in the first step of the hydrolysis of fat and is also produced when membrane lipids, such as PIP2 (phosphatidylinositol-4,5-bisphosphate) are hydrolyzed by phospholipase C in a signaling cascade.

DAG is itself a signaling compound, binding to protein kinase C to activate it to phosphorylate substrates. Synthesis of DAG begins with glycerol-3-phosphate, which gains two fatty acids from two acyl-CoAs to form phosphatidic acid. Dephosphorylation of phosphatidic acid produces DAG. DAG can also be rephosphorylated by DAG kinase to re-make phosphatidic acid or another fatty acid can be added to make fat.

Inositol

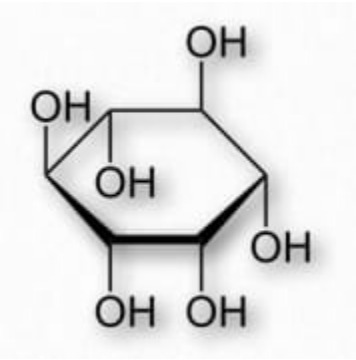


Figure 2.205 - Inositol

Though technically not a lipid itself, inositol is found in many lipids. Inositol is a derivative of cyclohexane containing six hydroxyl groups - one on each carbon (Figure 2.205. It has nine different stereoisomers of which one, cis-1,2,3,5-trans-4,6-cyclohexanehexol (called myo-inositol) is the most common. It has a sweet taste (half that of sucrose).

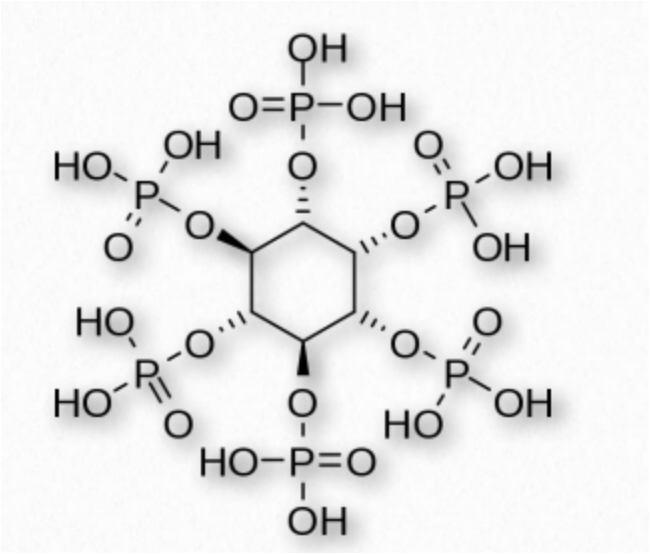


Figure 2.206 - Phytic acid

Numerous phosphorylated forms of the compound exist, from a single phosphate to six (one on each carbon). Phytic acid, for example, in plants, has six phosphates (Figure 2.206) that it uses to store phosphate. Inositol is produced from glucose and was once considered vitamin B8, but is made by the body in adequate amounts, so it is not now considered a vitamin. Phosphorylated forms of inositol are found in phosphoinositides, such as PIP2 and PIP3, both of which are important in signaling processes. Some of these include insulin signaling, fat catabolism, calcium regulation, and assembly of the cytoskeleton.

Phosphoinositides

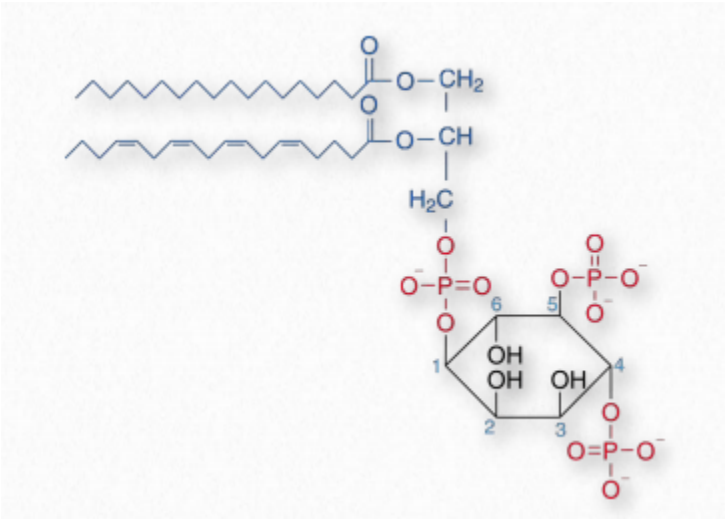


Figure 2.207 - Structure of PIP_2

Compounds based on phosphatidylinositol (PI) are often called phosphoinositides. These compounds have important roles in signaling and membrane trafficking. Hydroxyls on carbons 3,4, and 5 of the inositol ring are targets for phosphorylation by a variety of kinases. Seven different combinations are used. Steric hindrance inhibits phosphorylation of carbons 2 or 6. Naming of these phosphorylated compounds follows generally as PI(#P)P, PI(#P, #P)P, or PI(#P, #P, #P)P where #P refers to the number of the carbon where a phosphate is located. For example, PI(3)P refers to a phosphatidyl compound with a phosphate added to carbons 3 of the inositol ring, whereas PI(3,4,5)P is a phosphatidyl compound with a phosphate added to carbons 3,4,and 5.

Phosphatidylinositol-4,5- bisphosphate

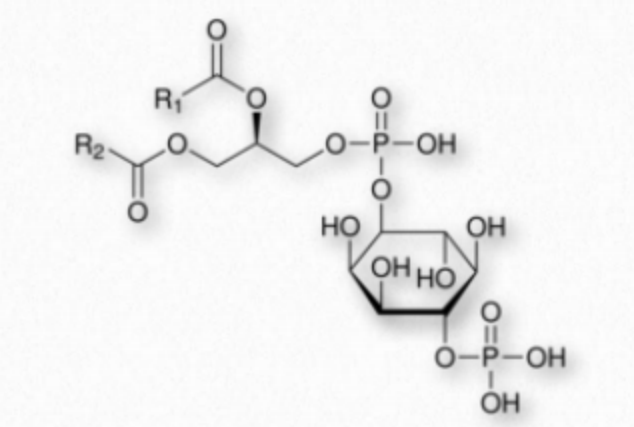


Figure 2.208 - Phosphatidylinositol-4- phosphate

Phosphatidylinositol-4,5-bisphosphate (PIP2 - Figure 2.207) is a phospholipid of plasma membranes that functions in the phospholipase C signaling cascade. In this signaling pathway, hydrolysis catalyzed by phospholipase C releases inositol-1,4,5-trisphosphate (IP3) and diacylglycerol. Synthesis of PIP2 begins with phosphatidylinositol, which is phosphorylated at position 4 followed by phosphorylation at position 5 by specific kinases.

PIP2 can be phosphorylated to form the signaling molecule known as phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Along with PIP3, PIP2 serves as a docking phospholipid for the recruitment of proteins that play roles in signaling cascades. Binding of PIP2 is also required by inwardly directed potassium channels.

Phosphatidylinositol (3,4,5)- trisphosphate

Phosphatidylinositol (3,4,5)-trisphosphate (PIP3) is an important molecule for the activation of signaling proteins, such as AKT, which activates anabolic signaling pathways related to growth and survival. PIP3 can be dephosphorylated by phosphatase PTEN to yield PIP2 and can be synthesized from PIP2 by kinase action of Class I PI 3- kinases. Kinase activity to synthesize PIP3 results in movement of PIP3-binding proteins to the plasma membrane. They include Akt/ PKB, PDK1, Btk1, and ARNO and each is activated by binding to PIP3.

Plasmalogens

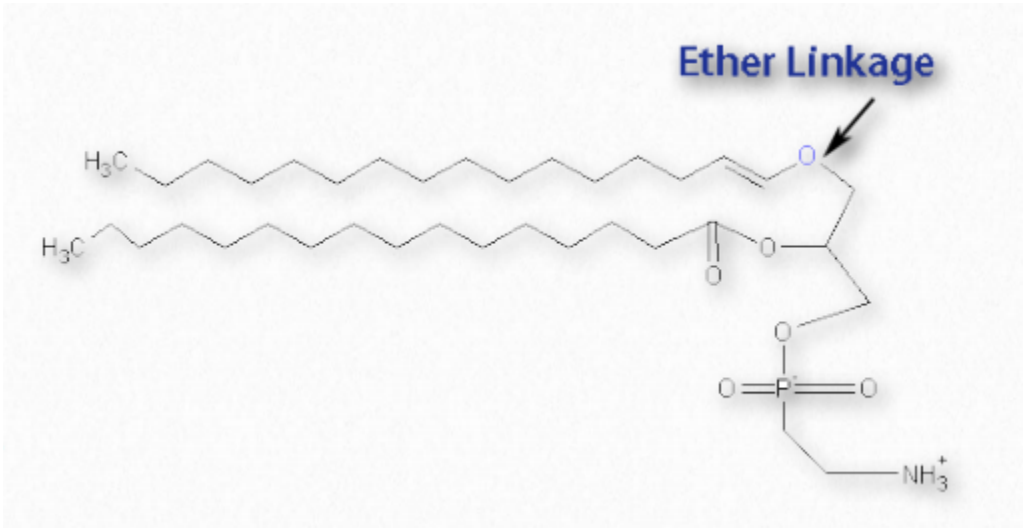


Figure 2.209 - Plasmalogen - A vinyl ether lipid Wikipedia

A special class of the glycerophospholipids are the plasmalogens (Figure 2.209). They differ in containing a vinyl ether linkage at position 1 of glycerol, in contrast to other glycerophopsholipids, which have an ester linkage at this position. Position 2 of each is an ester. The precursor for the ether linkage is typically a 16 or 18 carbon saturated alcohol or an 18 carbon unsaturated alcohol.

At the phosphate tail, the most commonly attached groups are ethanolamine or choline. Plasmalogens are found abundantly in

humans in heart (30-40% of choline phospholipids). 30% of the glycerophospholipids in brain are plasmalogens and 70% of the ethanolamine lipids of the myelin sheath of nerve cells are plasmalogens.

Though their function is not understood, it is believed that plasmalogens may provide some protection against reactive oxygen species and have roles in signaling.

Lecithin

Lecithin is a generic term for a combination of lipid substances that includes phosphoric acid, glycerol, glycolipids, triglycerides, and phospholipids. Lecithin is a wetting agent helpful with emulsification and encapsulation and is even used as an anti-sludge additive in motor lubricants. Lecithin is used in candy bars to keep cocoa and cocoa butter from separating. Though considered safe as a food ingredient, lecithin can be converted by gut bacteria to trimethylamine-N-oxide which may contribute to cholesterol deposition and atherosclerosis.

Sphingolipids

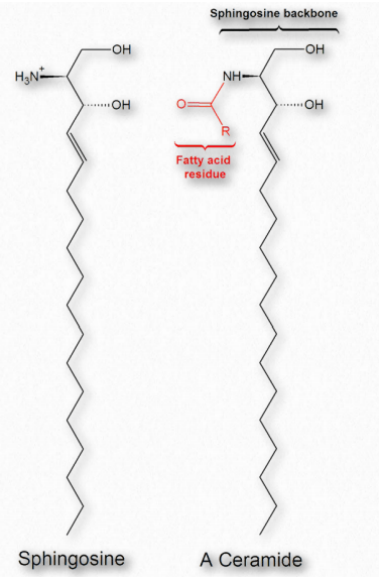


Figure 2.210 - Sphingosine and a ceramide made from it Wikipedia

Fatty acids are also components of a broad class of molecules called sphingolipids. Sphingolipids are structurally similar to glycerophospholipids, though they are synthesized completely independently of them starting with palmitic acid and the amino acid serine. Sphingolipids are named for the amino alcohol known as sphingosine (Figure 2.210), though they are not directly synthesized from it. Figure 2.211 shows the generalized structure of sphingolipids.

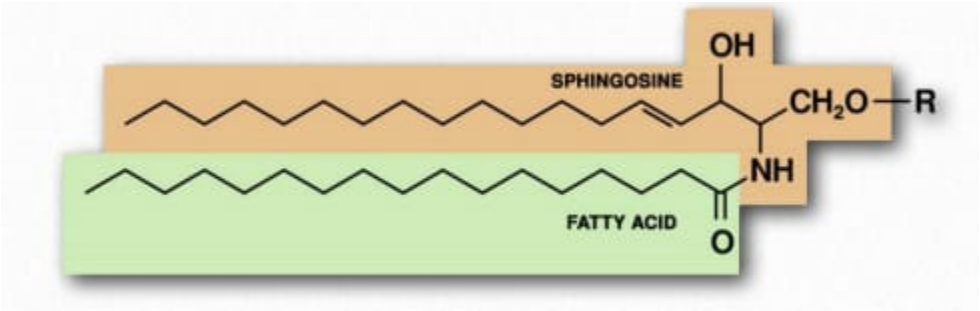


Figure 2.211 Schematic structure of a sphingolipid

If the R-group is a hydrogen, the molecule is called a ceramide. When the R-group is phosphoethanolamine the resulting molecule is sphingomyelin, an important component of the myelin sheath and lipid membranes. If a single, simple sugar is instead added, a cerebroside is created (Figure 2.212). Addition of a complex oligosaccharide creates a ganglioside.

Complex sphingolipids may play roles in cellular recognition and signaling. Sphingolipids are found most abundantly in plasma

membrane and are almost completely absent from mitochondrial and endoplasmic reticulum membranes. In animals, dietary sphingolipids have been linked to reduced colon cancer, reductions in LDLs, and increases in HDLs. Like the glycerophospholipids, sphingolipids are amphiphilic. Most sphingolipids except sphingomyelin do not contain phosphate.

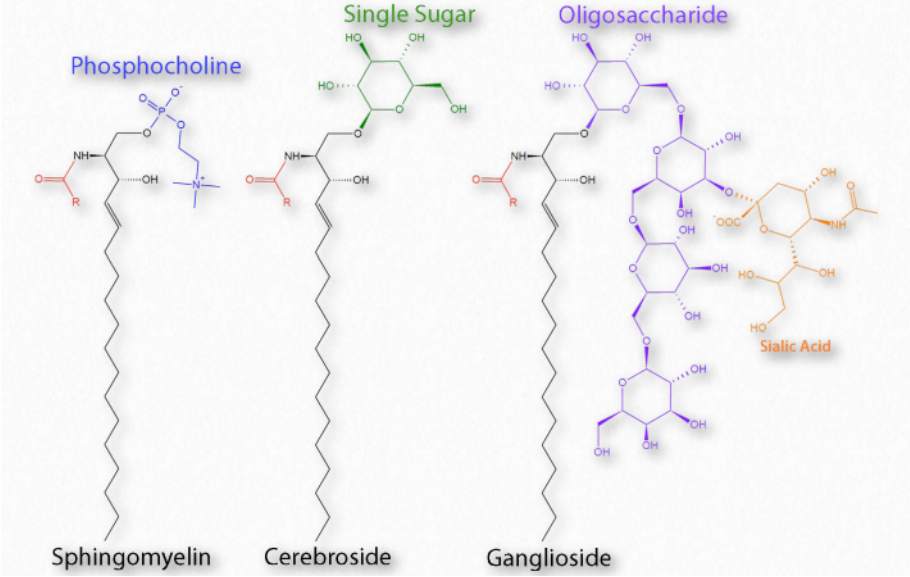


Figure 2.212 - Categories of Sphingolipid Wikipedia

Eicosanoids

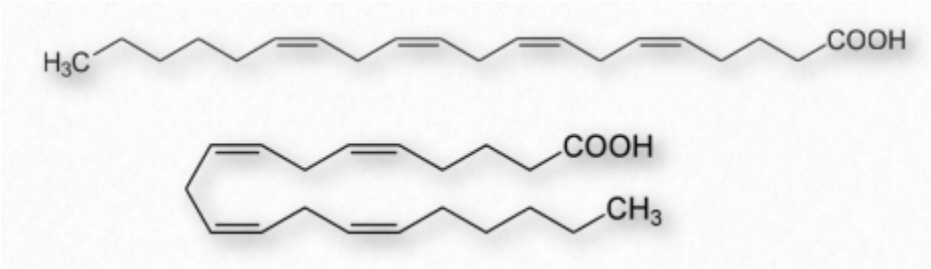


Figure 2.213 - Arachidonic acid drawn as straight (top) and bent (bottom)

Fatty acids made from omega-6 and omega-3 fatty acids include three important fatty acids containing 20 carbons. They include arachidonic acid (an ω-6 fatty acid with four double bonds (Δ-5,8,11,14) - Figure 2.213), eicosapentaenoic acid (an ω-3 fatty acid with five double bonds, and dihomo-γ-linolenic acid (an ω-6 fatty acid with three double bonds). The class of compounds known as eicosanoids is made by oxidation of these compounds. Subclasses include include prostaglandins, prostacyclins, thromboxanes, lipoxins, leukotrienes, and endocannabinoids (Figures 2.214-2.219). Eicosanoids play important roles affecting inflammation, immunity, mood, and behavior.

Prostaglandins

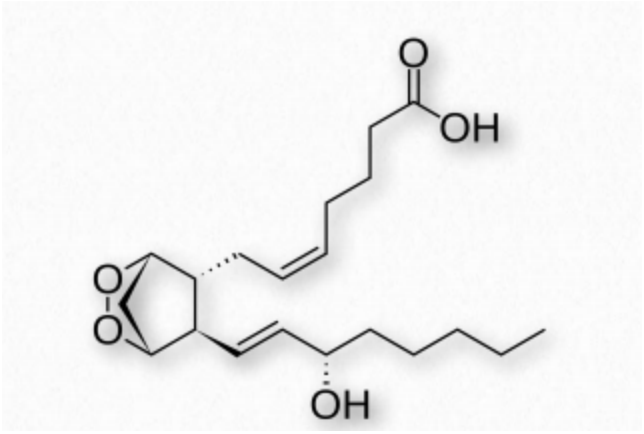


Figure 2.214 - Prostaglandin PGH_2

A collection of molecules acting like hormones, prostaglandins are derived from arachidonic acid and have many differing (even conflicting) physiological effects. These include constriction or dilation of vascular smooth muscle cells, induction of labor, regulation of inflammation, and action on the thermoregulatory center of the hypothalamus to induce fever, among others.

Prostaglandins are grouped with the thromboxanes (below) and prostacyclins (below), as prostanoids. The prostanoids, which all contain 20 carbons are a subclass of the eicosanoids. Prostaglandins are found in most tissues of higher organisms. They are autocrine or paracrine compounds produced from essential fatty acids. The primary precursor of the prostaglandins is the fatty acid known as arachidonic acid and the prostaglandin made from it is known as PGH2 (Figure 2.214), which, in turn is a precursor of other prostaglandins, as well as the prostacyclins and thromboxanes.

Interesting prostaglandins

PGD_2 - inhibits hair follicle growth, vasodilator, causes bronchial constriction, higher in lungs of asthmatics than others.

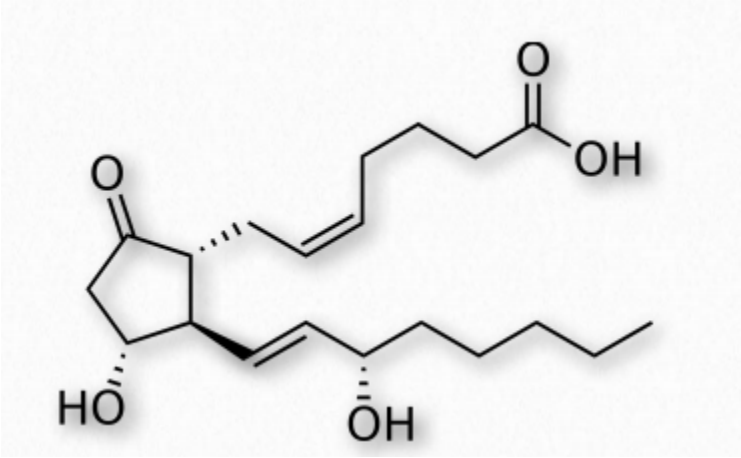


Figure 2.215 Prostaglandin E

PGE_2 (Figure 2.215) - exerts effects in labor (soften cervix, uterine contraction), stimulates bone resorption by osteoclasts, induces fever, suppresses T-cell receptor signaling, vasodilator, inhibits release of noradrenalin from sympathetic nerve terminals. It is a potent activator of the Wnt signaling pathway.

A prostaglandin can have opposite effects, depending on which receptor it binds to. Binding of PGE2 to the EP1 receptor causes bronchoconstriction and smooth muscle contraction, whereas binding of the same molecule to the EP2 receptor causes bronchodilation and smooth muscle relaxation.

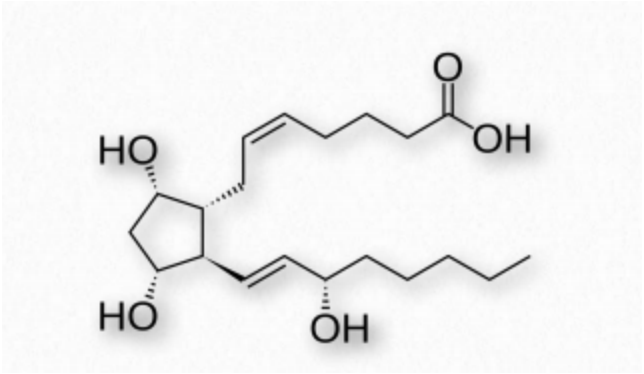


Figure 2.216 - Prostaglandin $F_{2\alpha}$

$PGF_{2\alpha}$ (Figure 2.216)- uterine contractions, induces labor, bronchoconstriction.

PGI_2 - vasodilation, bronchodilation, inhibition of platelet aggregation.

Thromboxanes

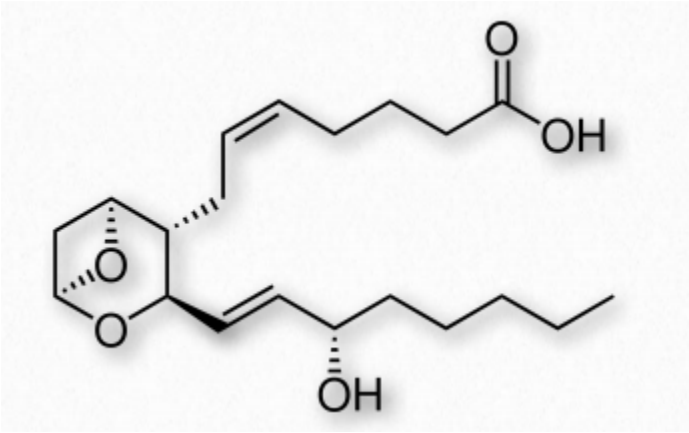


Figure 2.217 Thromboxane $A2_2$

Thromboxanes play roles in clot formation and named for their role in thrombosis. They are potent vasoconstrictors and facilitate platelet aggregation. They are synthesized in platelets, as well. The anti-clotting effects of aspirin have their roots in the inhibition of synthesis of PGH2, which is the precursor of the thromboxanes. The most common thromboxanes are A2 (Figure 2.217) and B2.

Prostacyclin

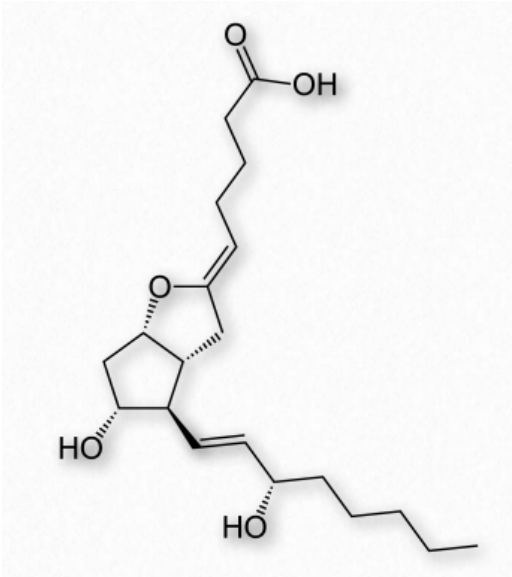


Figure 2.218 - Prostacyclin

Prostacyclin (also known as prostaglandin I2 or PGI2 - Figure 2.218) counters the effects of thromboxanes, inhibiting platelet activation and acting as vasodilators. It is produced from PGH2 by action of the enzyme prostacyclin synthase.

Leukotrienes

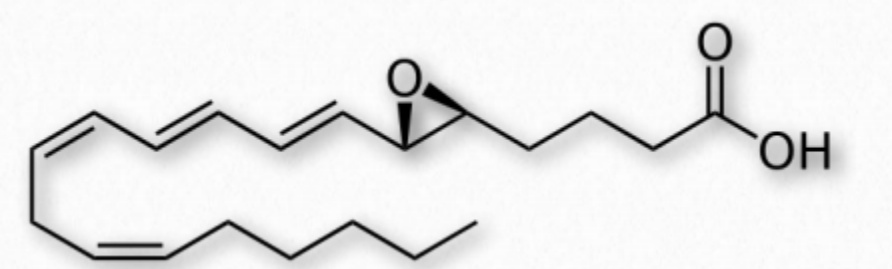


Figure 2.219 - Leukotriene A_4 (LTA_4)

Another group of eicosanoid compounds are the leukotrienes (Figure 2.219). Like prostaglandins, leukotrienes are made from arachidonic acid. The enzyme catalyzing their formation is a dioxygenase known as arachidonate 5-lipoxygenase. Leukotrienes are involved in regulating immune responses. They are found in leukocytes and other immunocompetent cells, such as neutrophils, monocytes, mast cells, eosinophils, and basophils. Leukotrienes are associated with production of histamines and prostaglandins, which act as mediators of inflammation. Leukotrienes also trigger contractions in the smooth muscles of the bronchioles. When overproduced, they may pay a role in asthma and allergic reactions. Some treatments for asthma aim at inhibiting production or action of leukotrienes.

Cholesterol

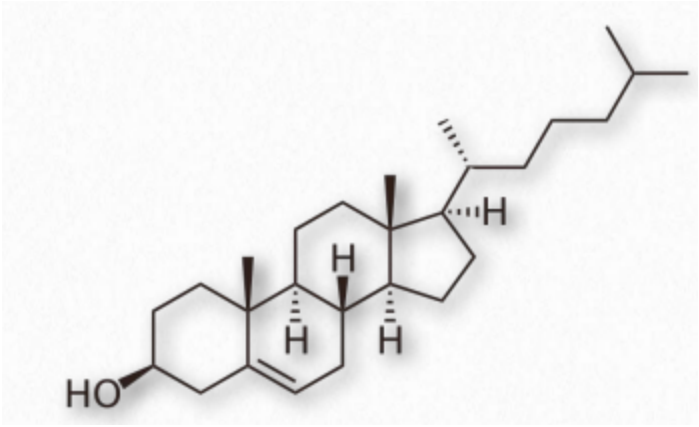


Figure 2.220 - Structure of cholesterol

Arguably, no single biomolecule has generated as much discussion and interest as has cholesterol (Figure 2.220). Certainly, from the perspective of the Nobel Prize committee, no small molecule even comes close, with 13 people having been awarded prizes for work on it. Evidence for cholesterol's importance comes from the study of brain tissue where it comprises 10-15% of the dry mass.

Membrane flexibility

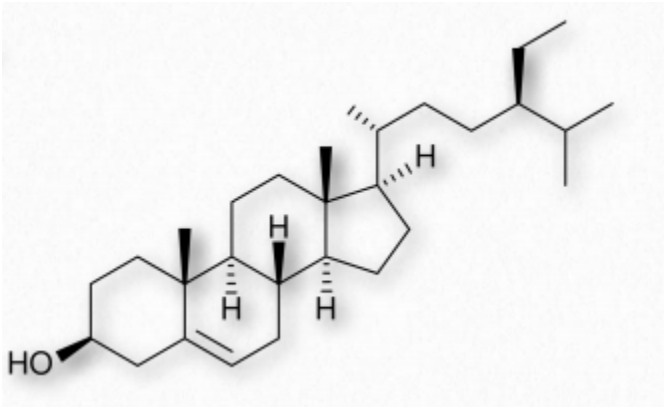


Figure 2.221 - Sitosterol - A phytosterol

In animal cells, cholesterol provides for membrane flexibility that allows for cellular movement that is in contrast to plant and bacterial cells with fixed structures. Cholesterol is made in many cells of the body, with the liver making the greatest amount. The anabolic pathway leading to synthesis of cholesterol is known as the isoprenoid pathway and branches of it lead to other molecules including other fat-soluble vitamins.



Figure 2.222 - Margarine - A common source of trans fat Wikipedia

Cholesterol is only rarely found in prokaryotes (Mycoplasma, which requires it for growth, is an exception) and is found in only trace amounts in plants. Instead, plants synthesize similar compounds called phytosterols (Figure 2.221). On average, the body of a 150 pound adult male makes about 1 gram of cholesterol per day, with a total content of about 35 grams.

Packaging

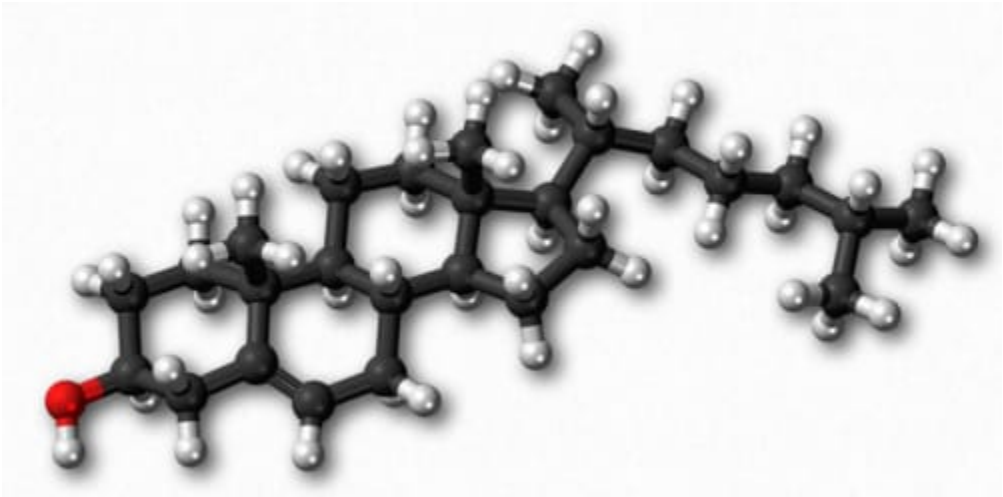


Figure 2.223 - Cholesterol - Ball and stick model

Cholesterol's (and other lipids') hydrophobicity requires special packaging into lipoprotein complexes (called chylomicrons, VLDLs, IDLs, LDLs, and HDLs) for movement in the lymph system and bloodstream. Though cholesterol can be made by cells, they also take it up from the blood supply by absorbing cholesterol-containing LDLs directly in a process called receptor-mediated endocytosis.

Oxidative damage to LDLs can lead to formation of atherosclerotic plaques and this is why cholesterol has gotten such a negative image in the public eye. The liver excretes cholesterol through the bile for elimination into the digestive system, but the compound is recycled there.

Reducing cholesterol levels

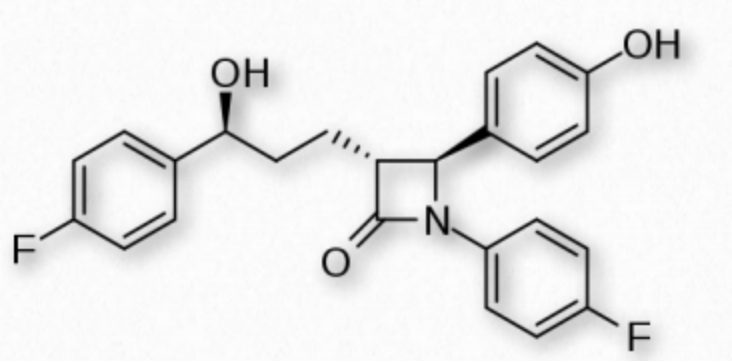


Figure 2.224 - Ezetimibe - An inhibitor of cholesterol absorption

Strategies for reducing cholesterol in the body focus primarily on three areas - reducing consumption, reducing endogenous synthesis, and reducing the recycling. Dietary considerations, such as saturated fat versus unsaturated fat consumption are currently debated. Dietary trans fats, though, correlate with incidence of coronary heart disease. Consumption of vegetables may provide some assistance with reducing levels of cholesterol recycled in the digestive system, because plant phytosterols compete with cholesterol for reabsorption and when this happens, a greater percentage of cholesterol exits the body in the feces. Drugs related to penicillin are also used to inhibit cholesterol recycling. One of these is ezetimibe, shown in Figure 2.224.

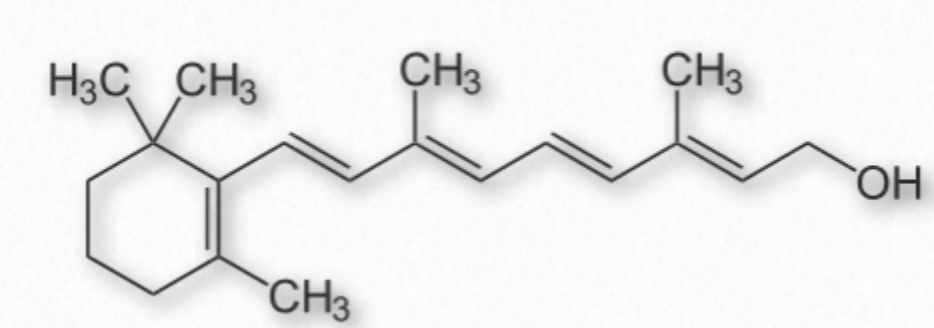


Figure 2.225 - All-trans retinol

Genetic defects in the cholesterol movement system are a cause of the rare disease known as familial hypercholesterolemia in which the blood of afflicted individuals contains dangerously high levels of LDLs. Left untreated, the disease is often fatal in the first 10-20 years of life. While LDLs have received (and deserve) a bad rap, another group of lipoprotein complexes known as the HDLs (high density lipoprotein complexes) are known as “good cholesterol” because their levels correlate with removal of debris (including cholesterol) from arteries and reduce inflammation.

Membrane function

In membranes, cholesterol is important as an insulator for the transmission of signals in nerve tissue and it helps to manage fluidity of membranes over a wide range of temperatures. Stacked in the lipid bilayer, cholesterol decreases a membrane’s fluidity and its permeability to neutral compounds, as well as protons and sodium ions. Cholesterol may play a role in signaling by helping with construction of lipid rafts within the cell membrane.

Vitamin A

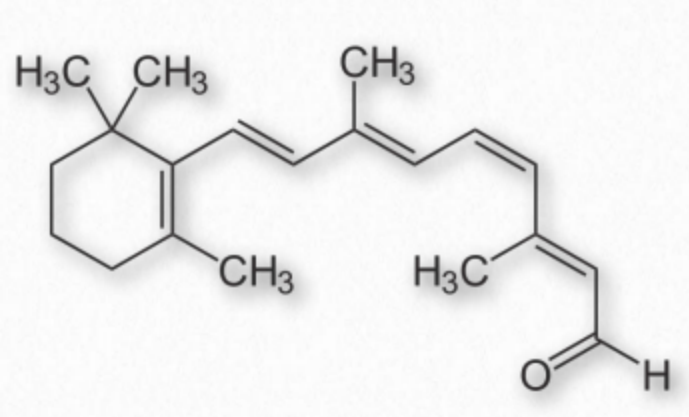


Figure 2.226 - 11-cis retinal

Vitamin A comes in three primary chemical forms, retinol (storage in liver - Figure 2.225), retinal (role in vision - Figure 2.226), and retinoic acid (roles in growth and development). All vitamin A forms are diterpenoids and differ only in the chemical form of the terminal group. Retinol is mostly used as the storage form of the vitamin.

Retinol is commonly esterified to a fatty acid and kept in the liver. In high levels, the compound is toxic. Retinol is obtained in the body by hydrolysis of the ester or by reduction of retinal. Importantly, neither retinal nor retinol can be made from retinoic acid. Retinoic acid is important for healthy skin and teeth, as well as bone growth. It acts in differentiation of stem cells through a specific cellular retinoic acid receptor.

Sources

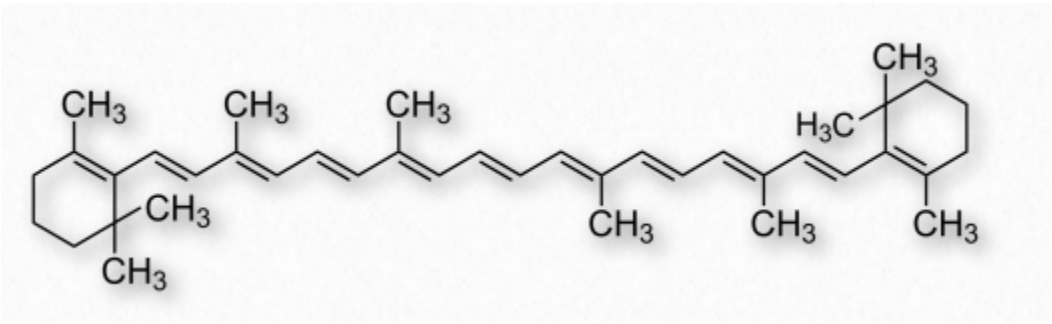


Figure 2.227 - β-Carotene

Good sources of vitamin A are liver and eggs, as well as many plants, including carrots, which have a precursor, β-carotene (Figure 2.227) from which retinol may be made by action of a dioxygenase.

Light sensitivity The conjugated double bond system in the side chain of vitamin A is sensitive to light and can flip between cis and trans forms on exposure to it. It is this response to light that makes it possible for retinal to have a role in vision in the rods and cones of the eyes. Here, the aldehyde form (retinal) is bound to the protein rhodopsin in the membranes of rod and cone cells.

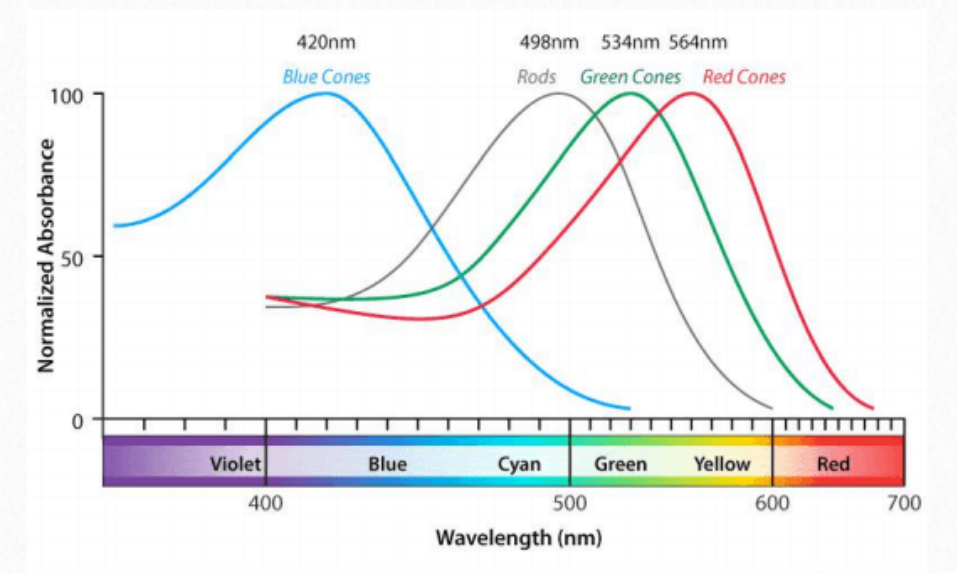


Figure 2.228 - Color sensitivity for cones and rods Image by Aleia Kim

When exposed to light of a particular wavelength, the “tail” of the retinal molecule will flip back and forth from cis to trans at the double bond at position 11 of the molecule. When this happens, a nerve signal is generated that signals the brain of exposure to light. Slightly different forms of rhodopsin have different maximum absorption maxima allowing the brain to perceive red, green and blue specifically and to assemble those into the images we see (Figure 2.228). Cones are the cells responsible for color vision, whereas rods are mostly involved in light detection in low light circumstances.

Deficiency and surplus

Deficiency of vitamin A is common in developing countries and was inspiration for the design and synthesis of the geneticallymodified golden rice, which is used as a source of vitamin A to help prevent blindness in children. Overdose of vitamin A, called hypervitaminosis A is dangerous and can be fatal. Excess vitamin A is also suspected to be linked to osteoporosis. In smokers, excess vitamin A is linked to an increased rate of lung cancer, but non-smokers have a reduced rate.

Vitamin D

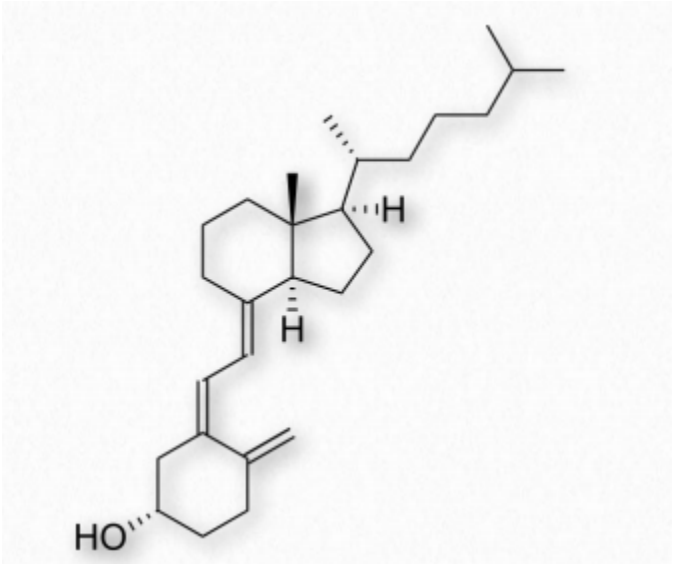


Figure 2.229 - Cholecalciferol - Vitamin D3

The active form of vitamin D plays important roles in the intestinal absorption of calcium and phosphate and thus in healthy bones. Technically, vitamin D isn’t even a vitamin, as it is a compound made by the body. Rather, it behaves more like a hormone.

Derived from ultimately from cholesterol, vitamin D can be made in a reaction catalyzed by ultraviolet light. In the reaction, the intermediate 7-dehydrocholesterol is converted to cholecalciferol (vitamin D3) by the uv light (Figure 2.229). The reaction occurs most readily in the bottom two layers of the skin shown in Figure 2.230.

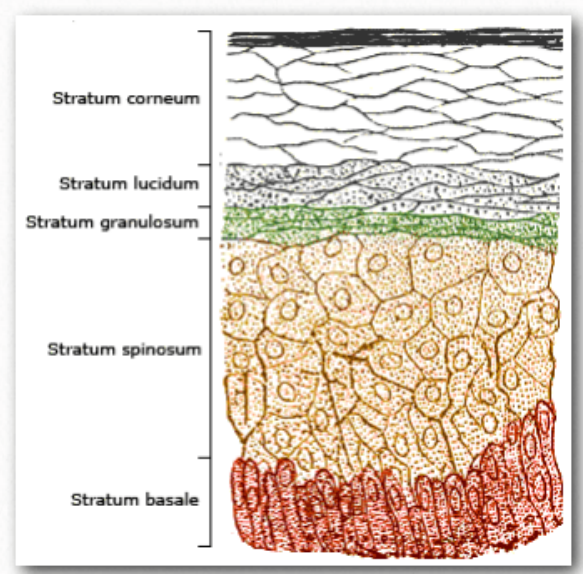


Figure 2.230 - Layers of the skin. Outside is at top.

Forms of vitamin D

Five different compounds are referred to as vitamin D. They are

Vitamin D1 - A mixture of ergocalciferol and lumisterol

Vitamin D2 - Ergocalciferol

Vitamin D3 - Cholecalciferol Vitamin

D4 - 22-Dihydroergocalciferol Vitamin

D5 - Sitocalciferol

Vitamin D3 is the most common form used in vitamin supplements and it and vitamin D2 are commonly obtained in the diet, as well. The active form of vitamin D, calcitriol (Figure 2.231), is made in the body in controlled amounts. This proceeds through two steps from cholecalciferol. First, a hydroxylation in the liver produces calcidiol and a second hydroxylation in the kidney produces calcitriol. Monocyte macrophages can also synthesize vitamin D and they use is as a cytokine to stimulate the innate immune system.

Mechanism of action

Calcitriol moves in the body bound to a vitamin D binding protein, which delivers it to target organs. Calcitriol inside of cells acts by binding a vitamin D receptor (VDR), which results in most of the vitamin's physiological effects. After binding calcitriol, the VDR migrates to the nucleus where it acts as a transcription factor to control levels of expression of calcium transport proteins (for example) in the intestine. Most tissues respond to VDR bound to calcitriol and the result is moderation of calcium and phosphate levels in cells.

Deficiency/excess

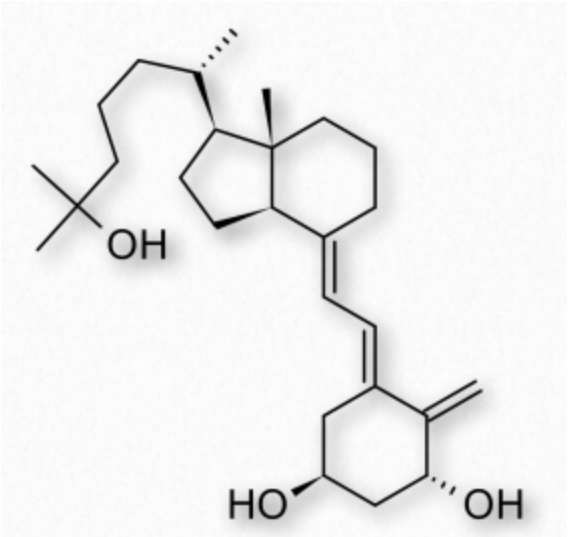


Figure 2.231 - Calcitriol - Active form of vitamin D

Deficiency of vitamin D is a cause of the disease known as rickets, which is characterized by soft, weak bones and most commonly is found in children. It is not common in the developed world, but elsewhere is of increasing concern.

Excess of vitamin D is rare, but has toxic effects, including hypercalcemia, which results in painful calcium deposits in major organs. Indications of vitamin D toxicity are increased urination and thirst. Vitamin D toxicity can lead to mental retardation and many other serious health problems.

Vitamin E

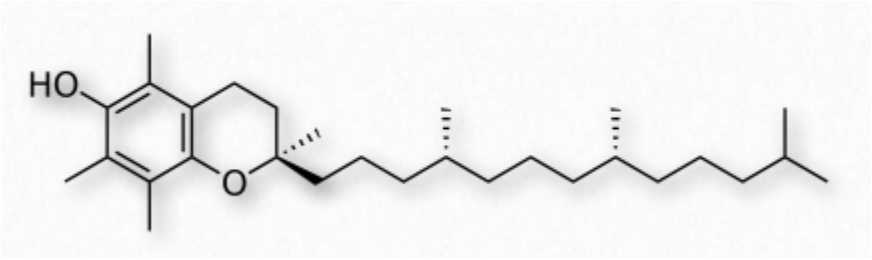


Figure 2.232 α-tocopherol - The most biologically active form of vitamin E Figure

Vitamin E comprises a group of two compounds (tocopherols and tocotrienols - Figure 2.232) and stereoisomers of each. It is commonly found in plant oils. The compounds act in cells as fat-soluble antioxidants. α-tocopherol (Figure 2.233), the most active form of the vitamin, works 1) through the glutathione peroxidase protective system and 2) in membranes to interrupt lipid peroxidation chain reactions. In both actions, vitamin E reduces levels of reactive oxygen species in cells.

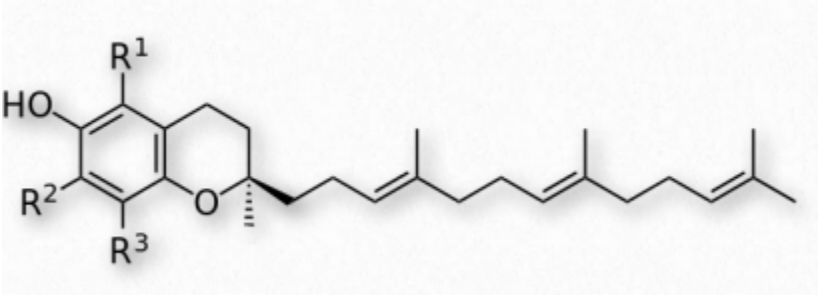


Figure 2.233 - Structure of tocotrienols

Action

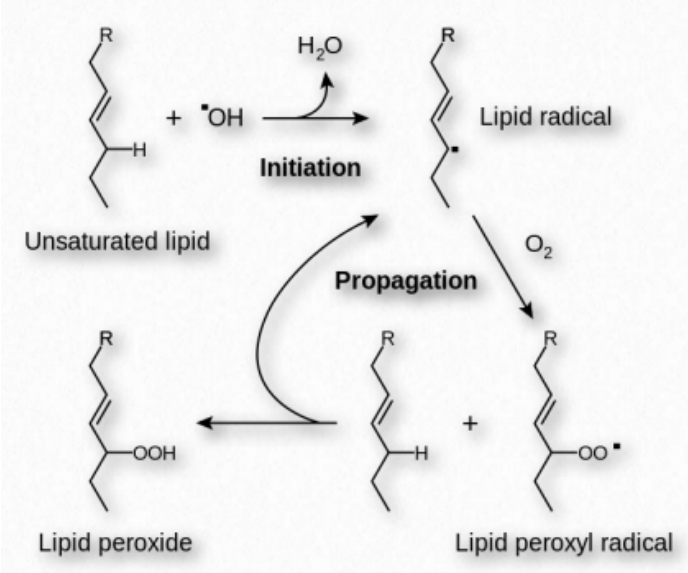


Figure 2.234 - Lipid peroxidation reactions

Vitamin E scavenges oxygen radicals (possessing unpaired electrons) by reacting with them to produce a tocopheryl radical. This vitamin E radical can be converted back to its original form by a hydrogen donor. Vitamin C is one such donor. Acting in this way, Vitamin E helps reduce oxidation of easily oxidized compounds in the lipid peroxidation reactions (Figure 2.234).

Vitamin E also can affect enzyme activity. The compound can inhibit action of protein kinase C in smooth muscle and simultaneously activate catalysis of protein phosphatase 2A to remove phosphates, stopping smooth muscle growth.

Deficiency/excess

Deficiency of vitamin E can lead to poor conduction of nerve signals and other issues arising from nerve problems. Low levels of the vitamin may be a factor in low birth weights and premature deliveries. Deficiency, however, is rare, and not usually associated with diet.

Excess Vitamin E reduces vitamin K levels, thus reducing the ability to clot blood. Hypervitaminosis of vitamin E in conjunction with aspirin can be life threatening. At lower levels, vitamin E may serve a preventative role with respect to atherosclerosis by reducing oxidation of LDLs, a step in plaque formation.

Vitamin K

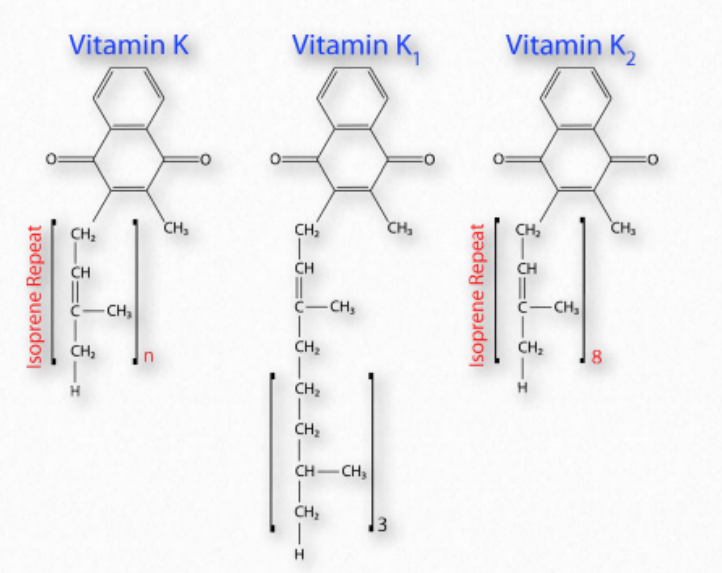


Figure 2.235 - Forms of vitamin K Image by Pehr Jacobson

Like the other fat-soluble vitamins, Vitamin K comes in multiple forms (Figure 2.235) and is stored in fat tissue in the body. There are two primary forms of the vitamin - K1 and K2 and the latter has multiple sub-forms . Vitamins K3, K4, and K5 are made synthetically, not biologically.

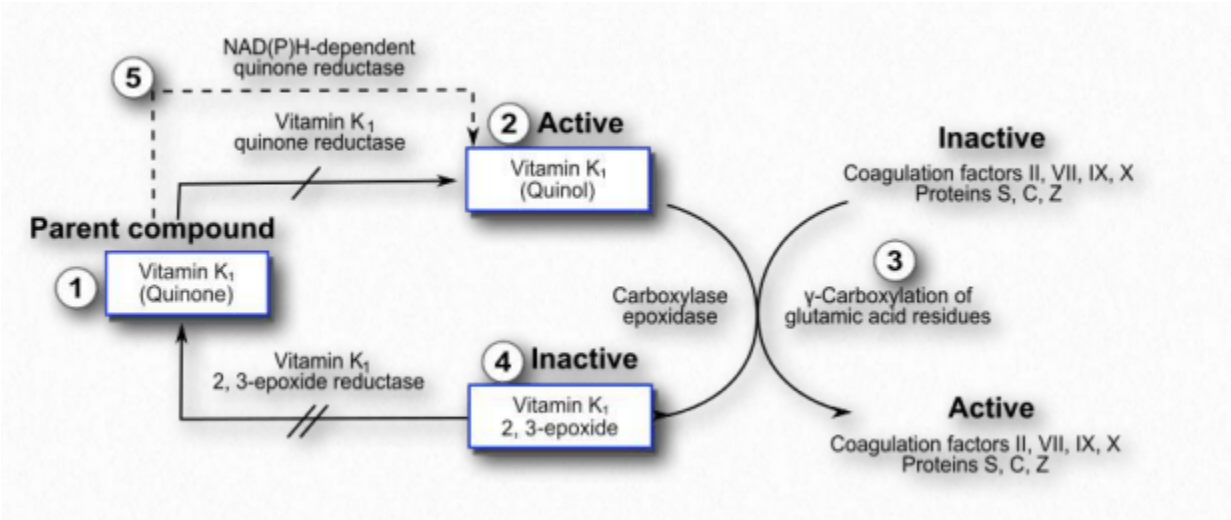


Figure 2.236 - Recycling of vitamin K Wikipedia

Action

Vitamin K is used as a co-factor for enzymes that add carboxyl groups to glutamate side chains of proteins to increase their affinity for calcium. Sixteen such proteins are known in humans. They include proteins involved in blood clotting (prothrombin (called Factor II), Factors VII, IX, and X), bone metabolism (osteocalcin, also called bone Gla protein (BGP), matrix Gla protein (MGP), and periostin) and others.

Modification of prothrombin is an important step in the process of blood clotting (see HERE). Reduced levels of vitamin K result in less blood clotting, a phenomenon sometimes referred to as blood thinning. Drugs that block recycling of vitamin K (Figure 2.236) by inhibiting the vitamin K epoxide reductase, produce lower levels of the vitamin and are employed in treatments for people prone to excessive clotting. Warfarin (coumadin) is one such compound that acts in this way and is used therapeutically. Individuals respond to the drug differentially, requiring them to periodically be tested for levels of clotting they possess, lest too much or too little occur.

Sources

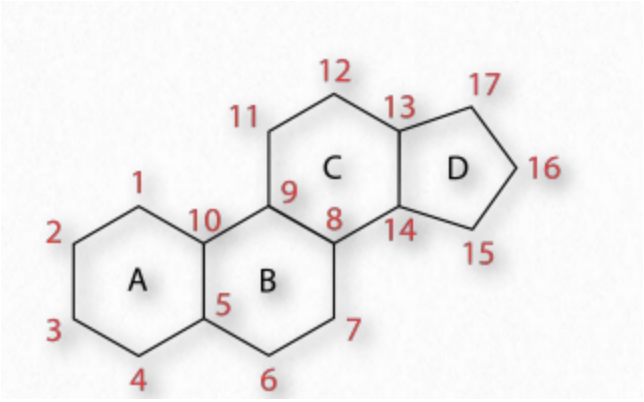


Figure 2.237 - Steroid numbering scheme Image by Pehr Jacobson

Vitamin K1 is a stereoisomer of the plant photosystem I electron receptor known as phylloquinone and is found abundantly in green, leafy vegetables. Phylloquinone is one source of vitamin K, but the compound binds tightly to thylakoid membranes and tends to have low bioavailability. Vitamin K2 is produced by microbes in the gut and is a primary source of the vitamin. Infants in the first few days before they establish their gut flora and people taking broad spectrum antibiotics may have reduced levels, as a result. Dietary deficiency is rare in the absence of damage to the small bowel. Others at risk of deficiency include people with chronic kidney disease and anyone suffering from a vitamin D deficiency. Deficiencies produce symptoms of easy bruising, heavy menstrual bleeding, anemia, and nosebleeds.

Steroids

Steroids, such as cholesterol are found in membranes and act as signaling hormones in traveling through the body.

Steroid hormones are all made from cholesterol and are grouped into five categories - mineralocorticoids (21 carbons), glucocorticoids (21 carbons), progestagens (21 carbons), androgens (19 carbons), and estrogens (18 carbons).

Mineralocorticoids

Mineralocorticoids are steroid hormones that influence water and electrolyte balances. Aldosterone (Figure 2.238) is the primary mineralocorticoid hormone, though other steroid hormones (including progesterone) have some functions like it. Aldosterone stimulates kidneys to reabsorb sodium, secrete potassium, and passively reabsorb water. These actions have the effect of increasing blood pressure and blood volume. Mineralocorticoids are produced by the zona glomerulosa of the cortex of the adrenal gland.

Glucocorticoids

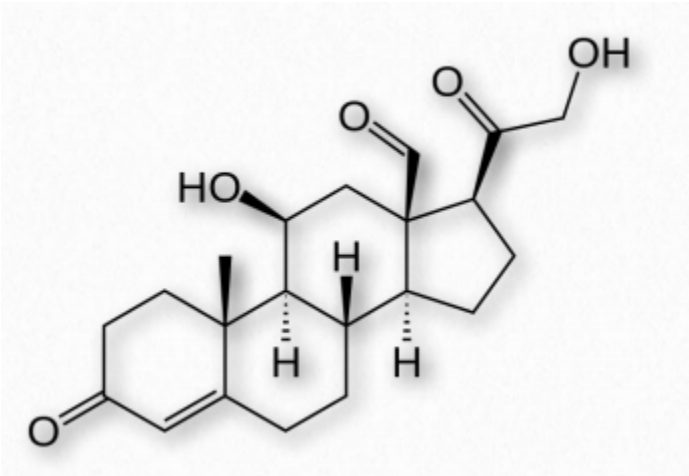


Figure 2.238 - Aldosterone - A mineralocorticoid

Glucocorticoids (GCs) bind to glucocorticoid receptors found in almost every vertebrate animal cell and act in a feedback

mechanism in the immune system to reduce its activity. GCs are used to treat diseases associated with overactive immune systems. These include allergies, asthma, and autoimmune dis- Figure 2.237 - Steroid numbering scheme Image by Pehr Jacobson eases. Cortisol (Figure 2.239) is an important glucocorticoid with cardiovascular, metabolic, and immunologic functions. The synthetic glucocorticoid known as dexamethasone has medical applications for treating rheumatoid arthritis, bronchospasms (in asthma), and inflammation due to its increased potency (25-fold) compared to cortisol. Glucocorticoids are produced primarily in the zona fasciculata of the adrenal cortex.

Progestagens

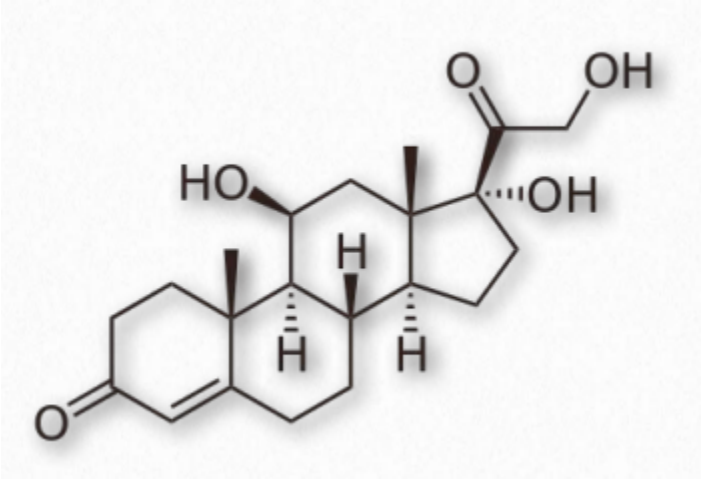


Figure 2.239 - Cortisol - A glucocorticoid

Progestagens (also called gestagens) are steroid hormones that work to activate the progesterone receptor upon binding to it. Synthetic progestagens are referred to as progestins. The most common progestagen is progesterone (also called P4 - Figure 2.240) and it has functions in maintaining pregnancy. Progesterone is produced primarily in the diestrus phase of the estrous cycle by the corpus luteum of mammalian ovaries. In pregnancy, the placenta takes over most progesterone production.

Androgens

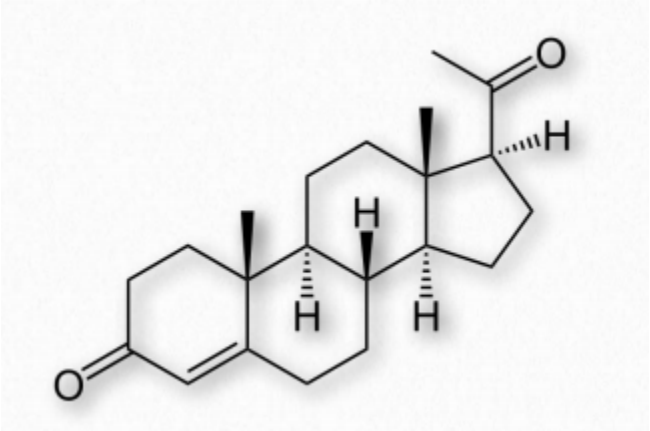


Figure 2.240 Progesterone - A progestagen

Androgens are steroid hormones that act by binding androgen receptors to stimulate development and maintenance of male characteristics in vertebrates. Androgens are precursors of estrogens (see below). The primary androgen is testosterone (Figure 2.241). Other important androgens include dihydrotestosterone (stimulates differentiation of penis, scrotum, and prostate in embryo) and androstenedione (common precursor of male and female hormones).

Estrogens

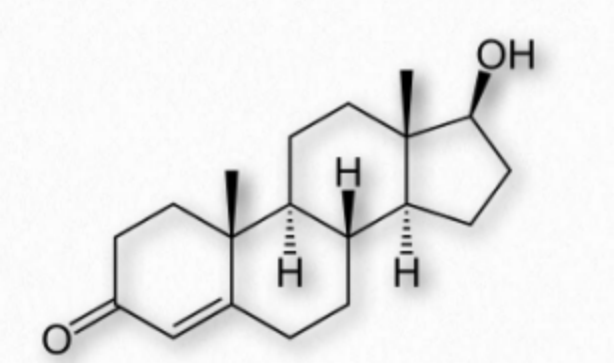


Figure 2.241 - Testosterone - An androgen

The estrogen steroid hormones are a class of compounds with important roles in menstrual and estrous cycles. They are the most important female sex hormones. Estrogens act by activating estrogen receptors inside of cells. These receptors, in turn, affect expression of many genes. The major estrogens in women include estrone (E1), estradiol (E2 - Figure 2.242), and estriol (E3). In the reproductive years, estradiol predominates. During pregnancy, estriol predominates and during menopause, estrone is the major estrogen.

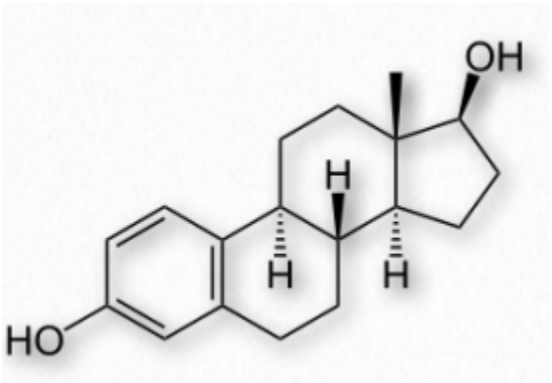


Figure 2.242 - Estradiol - An estrogen

Estrogens are made from the androgen hormones testosterone and androstenedione in a reaction catalyzed by the enzyme known as aromatase. Inhibition of this enzyme with aromatase inhibitors, such as exemestane, is a strategy for stopping estrogen production. This may be part of a chemotherapeutic treatment when estrogenresponsive tumors are present.

Cannabinoids

Cannabinoids are a group of chemicals that bind to and have effects on brain receptors (cannabinoid receptors), repressing neurotransmitter release. Classes of these compounds include endocannabinoids (made in the body), phytocannabinoids (made in plants, such as marijuana), and synthetic cannabinoids (man-made).

Endocannabinoids are natural molecules derived from arachidonic acid. Cannabinoid receptors are very abundant, comprising the largest number of G-protein- 247 Figure 2.243 - Tetrahydrocannabinol - Active ingredient in marijuana coupled receptors found in brain. The best known phytocannabinoid is Δ-9- tetrahydrocannabinol (THC), the primary psychoactive ingredient (of the 85 cannabinoids) of marijuana (Figure 2.243).

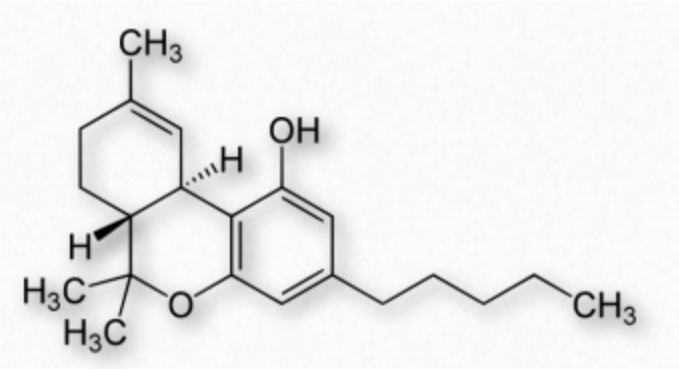


Figure 2.243 - Tetrahydrocannabinol - Active ingredient in marijuana

Anandamide

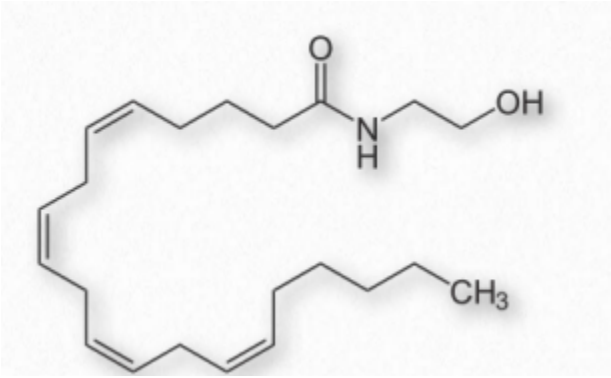


Figure 2.244 - Anandamide - An endocannabinoid

Anandamide (N-arachidonoylethanolamine - Figure 2.244) is an endocannabinoid neurotransmitter derived from arachidonic acid. It exerts its actions primarily through the CB1 and CB2 cannabinoid receptors, the same ones bound by the active ingredient in marijuana, Δ9-tetrahydrocannabinol. Anandamide has roles in stimulating eating/appetite and affecting motivation and pleasure. It has been proposed to play a role in "runners high," an analgesic effect experienced from exertion, especially among runners. Anandamide appears to impair memory function in rats.

Anandamide has been found in chocolate and two compounds that mimic its effects (N-oleoylethanolamine and Nlinoleoylethanolamine) are present as well. The enzyme fatty acid amide hydrolase (FAAH) breaks down anandamide into free arachidonic acid and ethanolamine.

Lipoxins

Lipoxins (Figure 2.245) are eicosanoid compounds involved in modulating immune responses and they have anti-inflammatory effects. When lipoxins appear in inflammation it begins the end of the process. Lipoxins act to attract macrophages to apoptotic cells at the site of inflammation and they are engulfed. Lipoxins further act to start the resolution phase of the inflammation process.

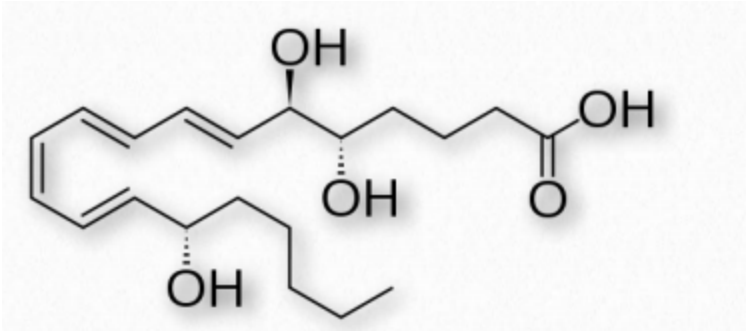


Figure 2.245 - Lipoxin A4

At least one lipoxin (aspirin-triggered LX4) has its synthesis stimulated by aspirin. This occurs as a byproduct of aspirin's acetylation of COX-2. When this occurs, the enzyme's catalytic activity is re-directed to synthesis of 15R-hydroxyeicosatetraenoic acid (HETE) instead of prostaglandins. 15R-HETE is a procursor of 15-epimer lipoxins, including aspirin-triggered LX4.

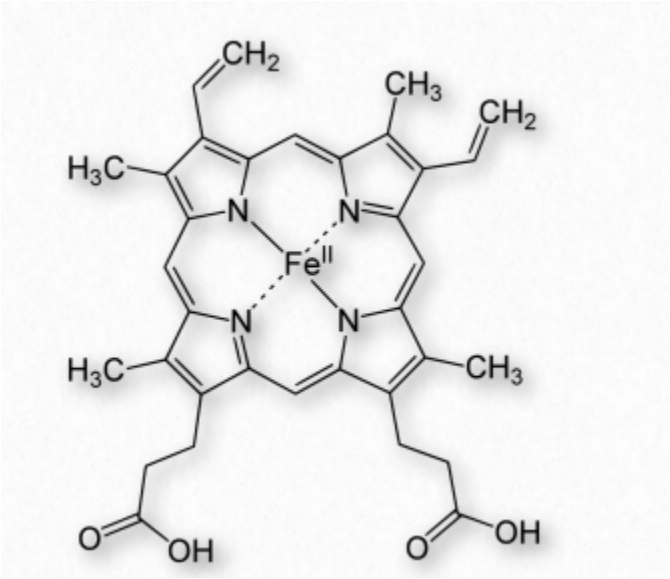


Figure 2.246 - Structure of heme B

Heme

Heme groups are a collection of protein/ enzyme cofactors containing a large heterocyclic aromatic ring known as a porphyrin ring with a ferrous (Fe++) ion in the middle. An example porphyrin ring with an iron (found in Heme B of hemoglobin), is shown in Figure 2.246. When contained in a protein, these are known collectively as hemoproteins (Figure 2.247).

Heme, of course, is a primary component of hemoglobin, but it is also found in other proteins, such as myoglobin, cytochromes, and the enzymes catalase and succinate dehydrogenase. Hemoproteins function in oxygen transport, catalysis, and electron transport. Heme is synthesized in the liver and bone marrow in a pathway that is conserved across a wide range of biology.

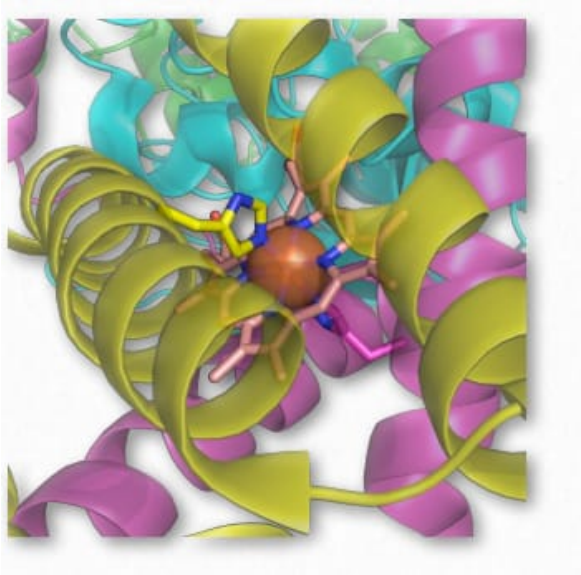


Figure 2.247 - Heme embedded in the succinate dehydrogenase hemoprotein Wikipedia

Porphobilinogen

Porphobilinogen (Figure 2.248) is a pyrrole molecule involved in porphyrin metabolism. It is produced from aminolevulinate by action of the enzyme known as ALA dehydratase. Porphobilinogen is acted upon by the enzyme porphobilinogen deaminase. Deficiency of the latter enzyme (and others in porphyrin metabolism) can result in a condition known as porphyria, which results in accumulation of porphobilinogen in the cytoplasm of cells.

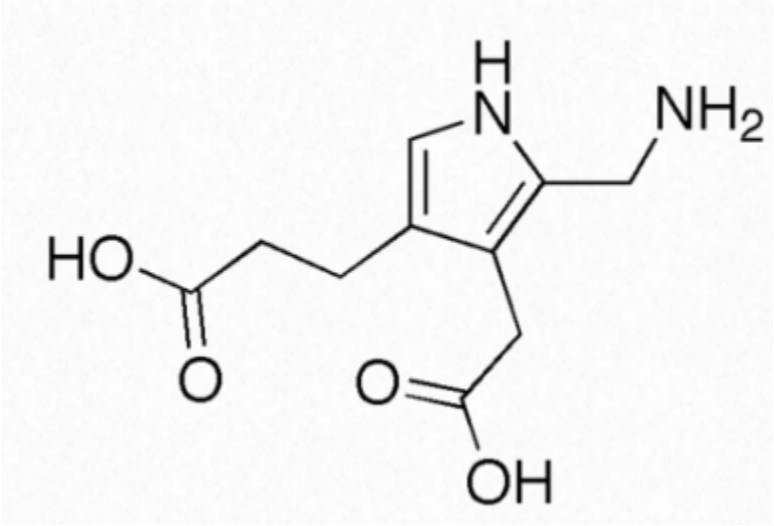


Figure 2.248 - Porphobilinogen

The disease can manifest itself with acute abdominal pain and numerous psychiatric issues. Both Vincent van Gogh and King ` George III are suspected to have suffered from porphyria, perhaps causing the “madness of King George III.” Porphyria is also considered by some to be the impetus for the legend of vampires seeking blood from victims, since the color of the skin in non-acute forms of the disease can be miscolored, leading some to perceive that as a deficiency of hemoglobin and hence the “thirst” for blood imagined for vampires.

Dolichols

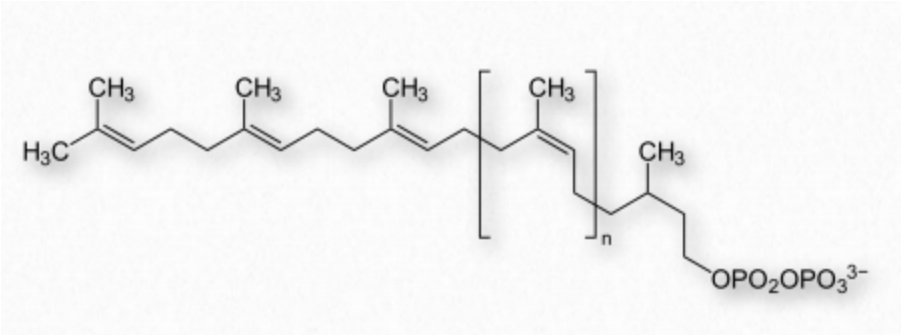


Figure 2.249 - Structure of dolichol pyrophosphate

Dolichol is a name for a group of non-polar molecules made by combining isoprene units together. Phosphorylated forms of dolichols play central roles in the N-glycosylation of proteins. This process, which occurs in the endoplasmic reticulum of eukaryotic cells, begins with a membrane-embedded dolichol pyrophosphate (Figure 2.249) to which an oligosaccharide is attached (also see HERE). This oligosaccharide contains three molecules of glucose, nine molecules of mannose and two molecules of N-acetylglucosamine.

Interestingly, the sugars are attached to the dolichol pyrophosphate with the pyrophosphate pointing outwards (away from) the endoplasmic reticulum, but after attachment, the dolichol complex flips so that the sugar portion is situated on the inside of the endoplasmic reticulum. There, the entire sugar complex is transferred to the amide of an asparagine side chain of a target protein.

The only asparagine side chains to which the attachment can be made are in proteins where the sequences Asn-X-Ser or Asn-X-Thr occur. Sugars can be removed/added after the transfer to the protein. Levels of dolichol in the human brain increase with age, but in neurodegenerative diseases, they decrease.

Terpenes



Figure 2.250 - Pine tree resin - A source of terpenes Wikipedia

Terpenes are members of a class of nonpolar molecules made from isoprene units. Terpenes are produced primarily by plants and by some insects. Terpenoids are a related group of molecules that contain functional groups lacking in terpenes.

Terpenes have a variety of functions. In plants, they often play a defensive role protecting from insects. The name of terpene comes from turpentine, which has an odor like some of the terpenes. Terpenes are common components of plant resins (think pine) and they are widely used in medicines and as fragrances. Hops, for example, gain some of their distinctive aroma and flavor from terpenes. Not all terpenes, however have significant odor.

Synthesis

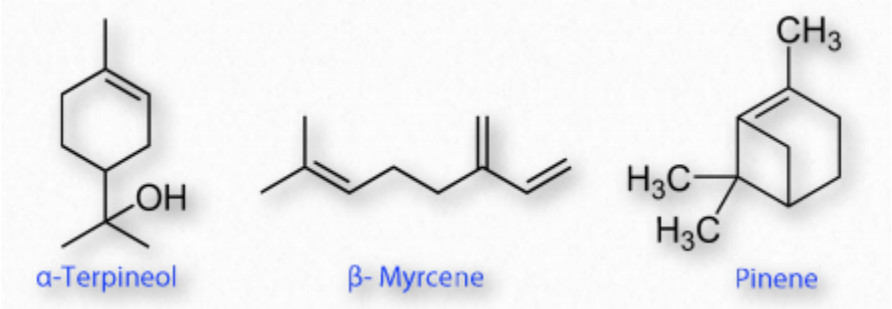


Figure 2.251 - Three monoterpenes

Terpenes, like steroids, are synthesized starting with simple building blocks known as isoprenes. There are two of them - dimethylallyl pyrophosphate and the related isopentenyl pyrophosphate and (Figures 2.252 and 2.253) which combine 1-2 units at a time to make higher order structures. Terpene synthesis overlaps and includes steroid synthesis.

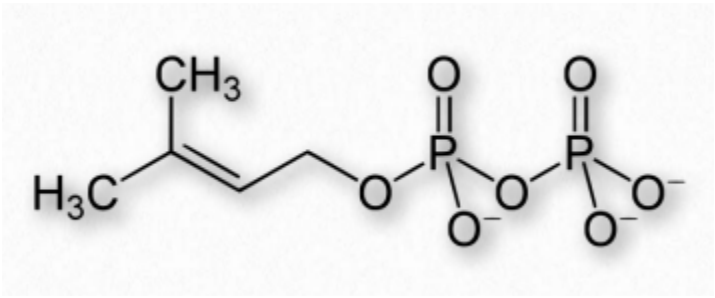


Figure 2.252 - Dimethylallyl pyrophosphate

Terpenes and terpenoids are classified according to how many isoprene units they contain. They include hemiterpenes (one unit), monoterpenes (two units), sesquiterpenes (three units), diterpenes (four units), sesterterpenes (five units), triterpenes (six units), sesquarterpenes (seven units), tetraterpenes (eight units), polyterpenes (many units). Another class of terpene-containing molecules, the norisoterpenoids arise from peroxidase-catalyzed reactions on terpene molecules.

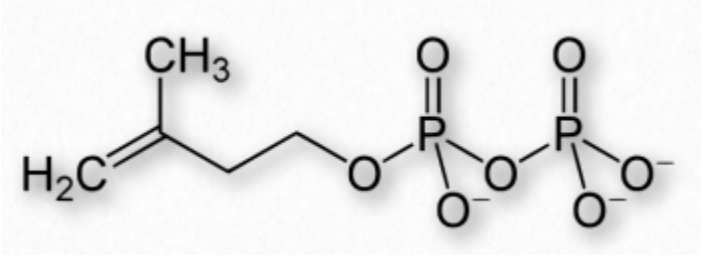


Figure 2.253 - Isopentenyl pyrophosphate

Examples

Common terpenes include monoterpenes of terpineol (lilacs), limonene (citrus), myrcene (hops), linalool (lavender), and pinene (pine). Higher order terpenes include taxadiene (diterpene precursor of taxol), lycopene (tetraterpenes), carotenes (tetraterpenes), and natural rubber (polyterpenes).

Steroid precursors geranyl pyrophosphate (monoterpene derivative), farnesyl pyrophosphate (sesquiterpene derivative), and squalene (triterpene) are all terpenes or derivatives of them. Vitamin A and phytol are derived from diterpenes.

Caffeine

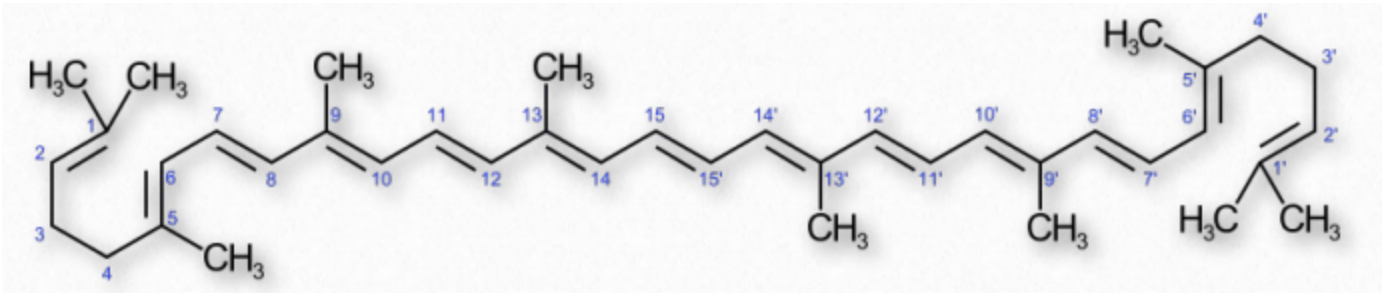


Figure 2.254 - Lycopene - A tetraterpene

Caffeine is the world's most actively consumed psychoactive drug (Figure 2.255). A methylxanthine alkaloid, caffeine is closely related to adenine and guanine and this is responsible for many effects on the body. Caffeine blocks the binding of adenosine on its receptor and consequently prevents the onset of drowsiness induced by adenosine. Caffeine readily crosses the blood-brain barrier and stimulates release of neurotransmitters. Caffeine stimulates portions of the autonomic nervous system and inhibits the activity of phosphodiesterase. The latter has the result of raising cAMP levels in cells, which activates protein kinase A and activates glycogen breakdown, inhibits TNF-α and leukotriene synthesis, which results in reduction of inflammation and innate immunity.

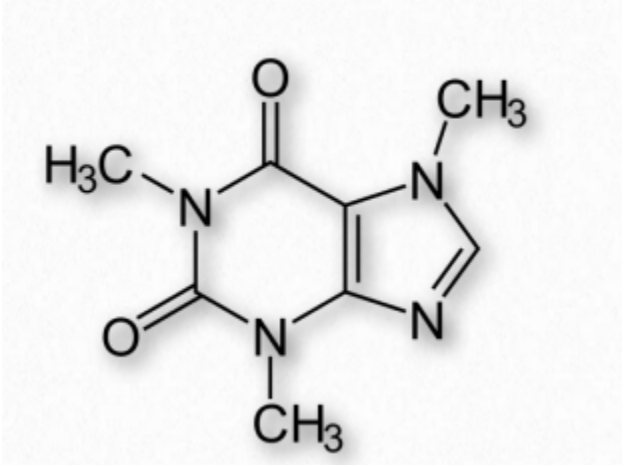


Figure 2.255 - Caffeine

Caffeine also has effects on the cholinergic system (acetylcholinesterase inhibitor), is an inositol triphosphate receptor 1 antagonist, and is a voltage independent activator of ryanodin receptors (a group of calcium channels found in skeletal muscle, smooth muscle, and heart muscle cells).

The half-life of caffeine in the body varies considerably. In healthy adults, it has a half-life of about 3-7 hours. Nicotine decreases the half-life and contraceptives and pregnancy can double it. The liver metabolizes caffeine, so the health of the liver is a factor in the halflife. CYP1A2 of the cytochrome P450 oxidase enzyme is primarily responsible. Caffeine is a natural pesticide in plants, paralyzing predator bugs.

Lipoprotein complexes and lipid movement in the body

Lipoprotein complexes are combinations of apolipoproteins and lipids bound to them that solubilize fats and other non-polar molecules, such as cholesterol, so they can travel in the bloodstream between various tissues of the body. The apolipoproteins provide the emulsification necessary for this. Lipoprotein complexes are formed in tiny "balls" with the water soluble apolipoproteins on the outside and non-polar lipids, such as fats, cholesteryl esters, and fat soluble vitamins on the inside.

They are categorized by their densities. These include (from highest density to the lowest) high density lipoproteins (HDLs), Low Density Lipoproteins (LDLs), Intermediate Density Lipoproteins (IDLs), Very Low Density Lipoproteins (VLDLs) and the chylomicrons. These particles are synthesized in the liver and small intestines.

Apolipoproteins

Name	Lipoprotein Complex(es)	Function
ApoA-I	HDL	Promotes Fat Movement to Liver
ApoA-II	HDL	Inhibit LCAT
ApoA-IV	Chylomicrons / HDL	Activate LCAT
AboB-48	Chylomicrons	Cholesterol Transport
AboB-100	VLDL / LDL	Bind LDL Receptor
ApoC-I	VLDL / LDL	Unknown
ApoC-II	All	Activate Lipoprotein Lipase
ApoC-III	All	Inhibit Lipoprotein Lipase
ApoD	HDL	Unknown
ApoE	VLDL / Chylomicrons / HDL	Clearance of Chylomicrons Remnants and VLDLs

Figure 2.256 - Apolipoproteins

Each lipoprotein complex contain a characteristic set of apolipoproteins, as shown in Figure 2.256. ApoC-II and ApoC-III are notable for their presence in all the lipoprotein complexes and the roles they play in activating (ApoC-II) or inactivating (ApoC-III) lipoprotein lipase. Lipoprotein lipase is a cellular enzyme that catalyzes the breakdown of fat from the complexes. ApoE (see below) is useful for helping the predict the likelihood of the occurrence of Alzheimer's disease in a patient.

Gene editing



Figure 2.257 - ApoA-I

ApoB-48 and ApoB-100 are interesting in being coded by the same gene, but a unique mRNA sequence editing event occurs that converts one into the other. ApoB-100 is made in the liver, but ApoB-48 is made in the small intestine. The small intestine contains an enzyme that deaminates the cytidine at nucleotide #2153 of the common mRNA. This changes it to a uridine and changes the codon it is in from CAA (codes for glutamine) to UAA (stop codon). The liver does not contain this enzyme and does not make the change in the mRNA. Consequently, a shorter protein is synthesized in the intestine (ApoB-48) than the one that is made in the liver (ApoB-100) using the same gene sequence in the DNA.

Movement

The movement of fats in the body is important because they are not stored in all cells. Only specialized cells called adipocytes

store fat. There are three relevant pathways in the body for moving lipids. As described below, they are 1) the exogenous pathway; 2) the endogenous pathway, and 3) the reverse transport pathway.

Exogenous pathway

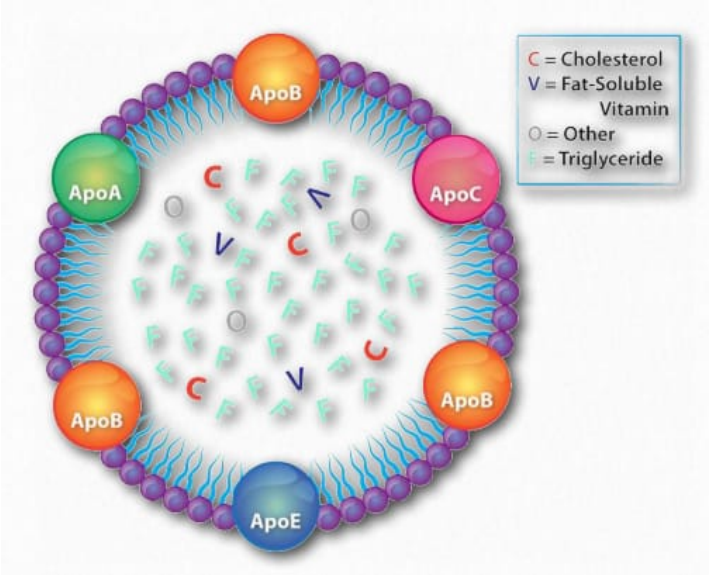


Figure 2.258 - Schematic diagram of a chylomicron Image by Aleia Kim

Dietary fat entering the body from the intestinal system must be transported, as appropriate, to places needing it or storing it. This is the function of the exogenous pathway of lipid movement in the body. All dietary lipids (fats, cholesterol, fat soluble vitamins, and other lipids) are moved by it. In the case of dietary fat, it begins its journey after ingestion first by being solubilized by bile acids in the intestinal tract. After passing through the stomach, pancreatic lipases clip two fatty acids from the fat, leaving a monoacyl glycerol. The fatty acids and monoacyl glycerol are absorbed by intestinal cells (enterocytes) and reassembled back into a fat, and then this is mixed with phospholipids, cholesterol esters, and apolipoprotein B-48 and processed to form chylomicrons (Figures 2.258 & 2.259) in the Golgi apparatus and smooth endoplasmic reticulum.

Exocytosis

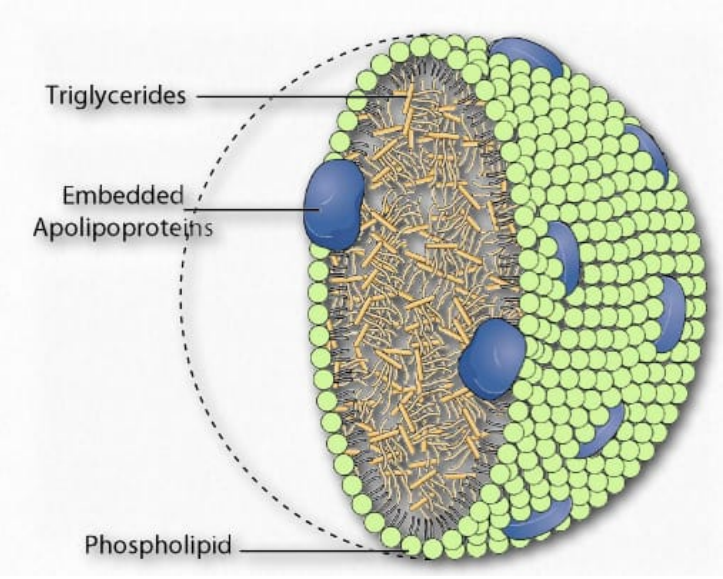


Figure 2.259 - Another perspective of a chylomicron WIkipedia

These are exocytosed from the cell into lymph capillaries called lacteals. The chylomicrons pass through the lacteals and enter the bloodstream via the left subclavian vein. Within the bloodstream, lipoprotein lipase breaks down the fats causing the chylomicron

to shrink and become what is known as a chylomicron remnant. It retains its cholesterol and other lipid molecules.

The chylomicron remnants travel to the liver where they are absorbed (Figure 2.260). This is accomplished by receptors in the liver that recognize and bind to the ApoE of the chylomicrons. The bound complexes are then internalized by endocytosis, degraded in the lysosomes, and the cholesterol is disbursed in liver cells.

Endogenous pathway

The liver plays a central role in managing the body's needs for lipids. When lipids are needed by the body or when the capacity of the liver to contain more lipids than is supplied by the diet, the liver packages up fats and cholesteryl esters into Very Low Density Lipoprotein (VLDL) complexes and exports them via the endogenous pathway. VLDL complexes contain ApoB-100, ApoC-I, ApoC-II, ApoC-III, and ApoE apolipoproteins. VLDLs enter the blood and travel to muscles and adipose tissue where lipoprotein lipase is activated by ApoC-II. In the muscle cells, the released fatty acids are taken up and oxidized. By contrast, in the adipoctyes, the fatty acids are taken up and reassembled back into triacylglycerides (fats) and stored in fat droplets. Removal of fat from the VLDLs causes them to shrink, first to Intermediate Density Lipoprotein (IDL) complexes (also called VLDL remnants) and then to Low Density Lipoprotein (LDL) complexes.

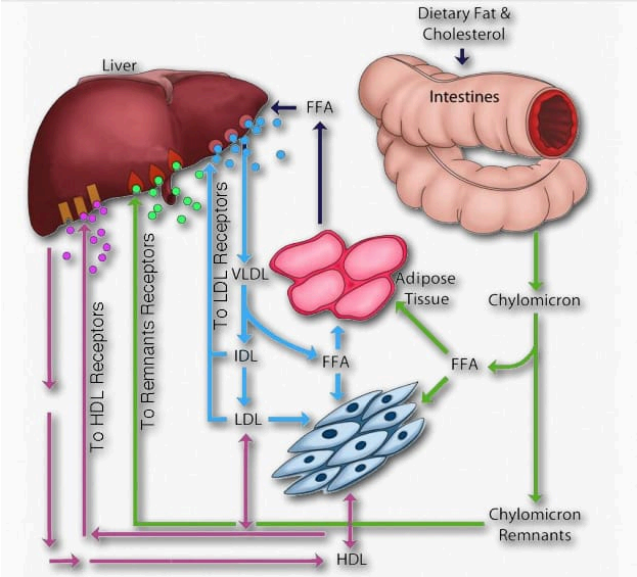


Figure 2.260 - Movement of lipids in the body - Green = exogenous pathway; Blue = endogenous pathway; Purple = reverse transport pathway
Image by Aleia Kim

Shrinking of VLDLs is accompanied by loss of apolipoproteins so that LDLs are comprised primarily of ApoB-100. This lipoprotein complex is important because cells have receptors for it to bind and internalize it by receptor-mediated endocytosis (Figure 2.261). Up until this point, cholesterol and cholesteryl esters have traveled in chylomicrons, VLDLs, and IDLs as fat has been stripped stripped away. For cholesterol compounds to get into the cell from the lipoprotein complexes, they must be internalized by cells and that is the job of receptormediated endocytosis.

Reverse transport pathway

Another important consideration of the movement of lipids in the body is the reverse transport pathway (Figure 2.260). It is also called the reverse cholesterol transport pathway, since cholesterol is the primary molecule involved. This pathway involves the last class of lipoprotein complexes known as the High Density Lipoproteins (HDLs). In contrast to the LDLs, which are commonly referred to as "bad cholesterol" (see below also), the HDLs are known as "good cholesterol."

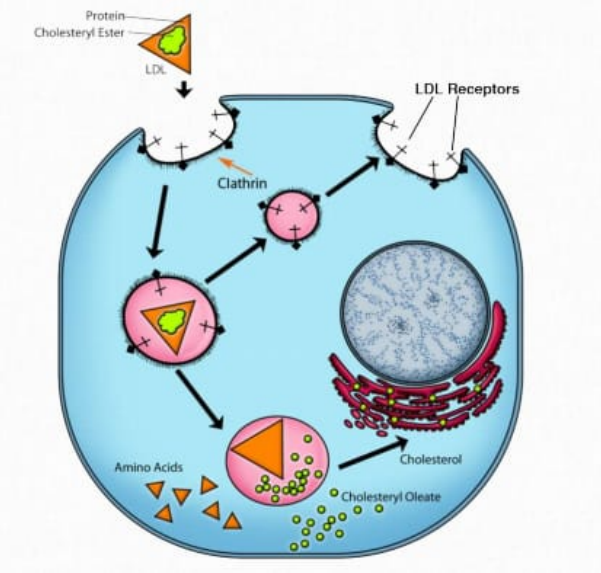


Figure 2.261 - The process of receptor-mediated endocytosis Image by Aleia Kim

HDLs are synthesized in the liver and small intestine. They contain little or no lipid when made (called depleted HDLs), but serve the role of “scavenger” for cholesterol in the blood and from remnants of other (damaged) lipoprotein complexes in the blood. To perform its task, HDLs carry the enzyme known as lecithincholesterol acyl transferase (LCAT), which they use to form cholesteryl esters using fatty acids from lecithin (phosphatidylcholine) and then they internalize them.

The cholesterol used for this purpose comes from the bloodstream, from macrophages, and from foam cells (macrophage-LDL complexes - Figure 2.262). Addition of cholesteryl esters causes the HDL to swell and Figure 2.261 - The process of receptor-mediated endocytosis Image by Aleia Kim when it is mature, it returns its load of cholesterol back to the liver or, alternatively, to LDL molecules for endocytosis. HDLs have the effect of lowering levels of cholesterol and it is for that reason they are described as “good cholesterol.”

Regulation of lipid transfer

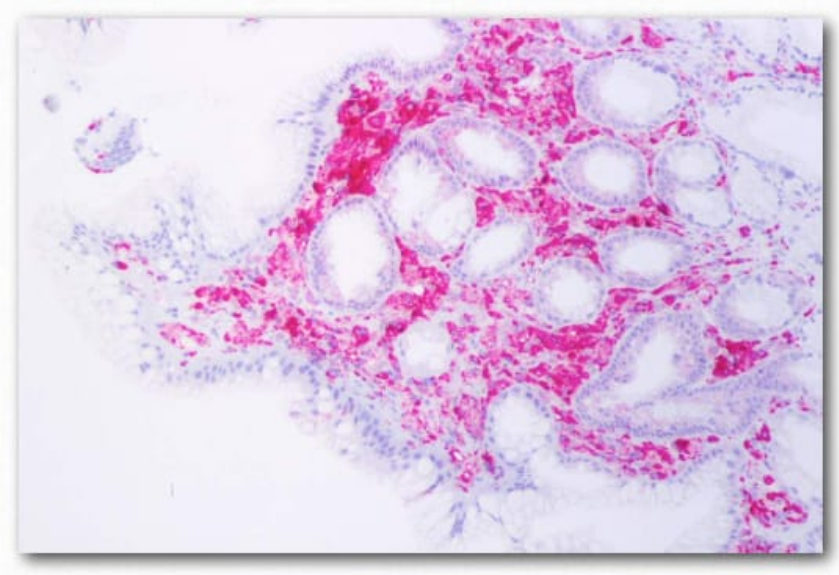


Figure 2.262 - Foam cell aggregate Wikipedia

It is important that cells get food when they need it so some control of the movement of nutrients is critical. The liver, which plays the central role in modulating blood glucose levels, is also important for performing the same role for lipids. It accomplishes this task the use of specialized LDL receptors on its surface. Liver LDL receptors bind LDLs that were not taken up by other cells in their path through the bloodstream. High levels of LDLs are a signal to the liver to reduce the creation of VLDLs for release.

People with the genetic disease known as familial hypercholesterolemia, which manifests with dangerously high levels of LDLs, lack properly functioning LDL receptors on their liver cells.Figure 2.263 - Progression of atherosclerosis Wikipedia Figure 2.263 - Progression of atherosclerosis Wikipedia Figure 2.263 - Progression of atherosclerosis Wikipedia

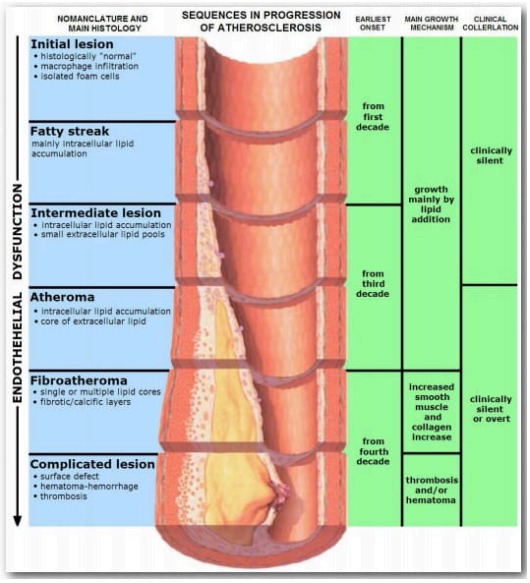


Figure 2.263 - Progression of atherosclerosis Wikipedia

In sufferers of this disease, the liver never gets the signal that the LDL levels are high. In fact, to the liver, it appears that all VLDLs and LDLs are being taken up by peripheral tissues, so it creates more VLDLs to attempt to boost levels. Untreated, the disease used to be fatal early, but newer drugs like the statins have significantly increased life spans of patients. Cellular needs for the contents of LDLs are directly linked to the levels of synthesis of LDL receptors on their membranes. As cells are needing more cholesterol, their synthesis of components for receptors goes up and it decreases as need diminishes.

Good cholesterol / bad cholesterol

It is commonly accepted that “high cholesterol” levels are not healthy. This is due, at least indirectly, to the primary carriers of cholesterol, the LDLs. A primary function of the LDLs is to deliver cholesterol and other lipids directly into cells by receptor mediated endocytosis (Figure 2.237). High levels of LDLs, though, are correlated with formation of atherosclerotic plaques (Figure 2.263 & 2.264) and incidence of atherosclerosis, leading to the description of them as “bad cholesterol.” This is because when LDL levels are very high, plaque formation begins. It is thought that reactive oxygen species (higher in the blood of smokers) causes partial oxidation of fatty acid groups in the LDLs. When levels are high, they tend to accumulate in the extracellular matrix of the epithelial cells on the inside of the arteries. Macrophages of the immune system take up the damaged LDLs (including the cholesterol).

Since macrophages can’t control the amount of cholesterol they take up, cholesterol begins to accumulate in them and they take on appearance that leads to their being described as “foam cells.” With too much cholesterol, the foam cells, however, are doomed to die by the process of programmed cell death (apoptosis). Accumulation of these, along with scar tissue from inflammation result in formation of a plaque. Plaques can grow and block the flow of blood or pieces of them can break loose and plug smaller openings in the blood supply, ultimately leading to heart attack or stroke.

Good cholesterol

On the other hand, high levels of HDL are inversely correlated with atherosclerosis and arterial disease. Depleted HDLs are able to remove cholesterol from foam cells. This occurs as a result of contact between the ApoA-I protein of the HDL and a transport protein on the foam cell (ABC-G1). Another transport protein in the foam cell, ABCA-1 transports extra cholesterol from inside the cell to the plasma membrane where it is taken up into the HDL and returned to the liver or to LDLs by the reverse transport cholesterol pathway.



Figure 2.264 - Actual carotid artery plaque Wikipedia

Deficiency of the ABCA-1 gene leads to Tangier disease. In this condition, HDLs are almost totally absent because they remain empty as a result of not being able to take up cholesterol from foam cells, so they are destroyed by the body.

ApoE and Alzheimer's disease

ApoE is a component of the chylomicrons and is also found in brain, macrophages, kidneys, and the spleen. In humans, it is found in three different alleles, E2, E3, and E4. The E4 allele (present at about 14% of the population) is associated with increased likelihood of contracting Alzheimer's disease. People heterozygous for the allele are 3 times as likely to contract the disease and those homozygous for it are 15 times as likely to do so. It is not known why this gene or allele is linked to the disease. The three alleles differ only slightly in amino acid sequence, but the changes do cause notable structural differences. The E4 allele is associated with increased calcium ion levels and apoptosis after injury. Alzheimer's disease is associated with accumulation of aggregates of the β- amyloid peptide. ApoE does enhance the proteolytic breakdown of it and the E4 isoform is not as efficient in these reactions as the other isoforms.

This page titled 9.1: Structure and Function - Lipids and Membranes is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 2.8: Structure and Function - Lipids and Membranes by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

9.2: Basic Concepts in Membranes

Source: BiochemFFA_3_1.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Lipid bilayers

The protective membrane around cells contains many components, including cholesterol, proteins, glycolipids, glycerophospholipids, and sphingolipids. The last two of these will, when mixed vigorously with water, spontaneously form what is called a lipid bilayer (Figure 3.1), which serves as a protective 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 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).

Facilitated Diffusion

In other cases, no external energy is required and they move by diffusion through specific cellular channels. This is referred to as facilitated diffusion. Before we discuss movement of materials across membranes, it is appropriate we discuss the composition of cellular membranes. Plasma membranes differ from cell walls both in the materials comprising them and in their flexibility. Cell walls will be covered near the end of this chapter.

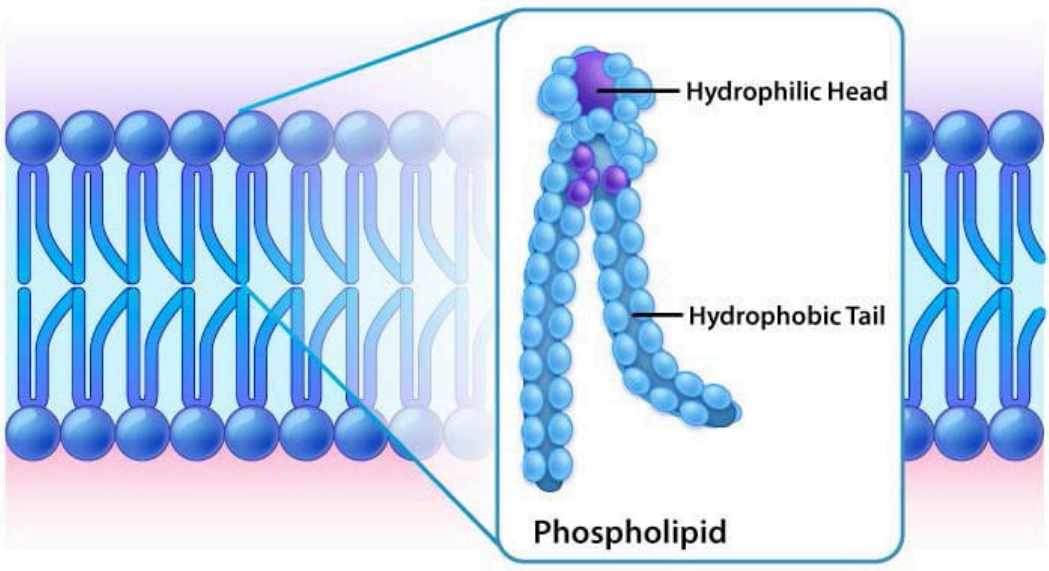


Figure 3.1: Lipid bilayer closeup Image by Aleia Kim

Though some cells do not have cell walls (animal cells) and others do (bacteria, fungi, and plants), there is commonality among cells in that they all possess plasma membranes. There is also commonality in the components of the membranes, though the relative amount of constituents varies. Figures 3.1 and 3.2 illustrate the structure and environments of plasma membranes. All plasma membranes contain a significant amount of amphiphilic substances linked to fatty acids. These include the glycerophospholipids and the sphingolipids. The fatty acid(s) are labeled as hydrophobic tails in the figures.

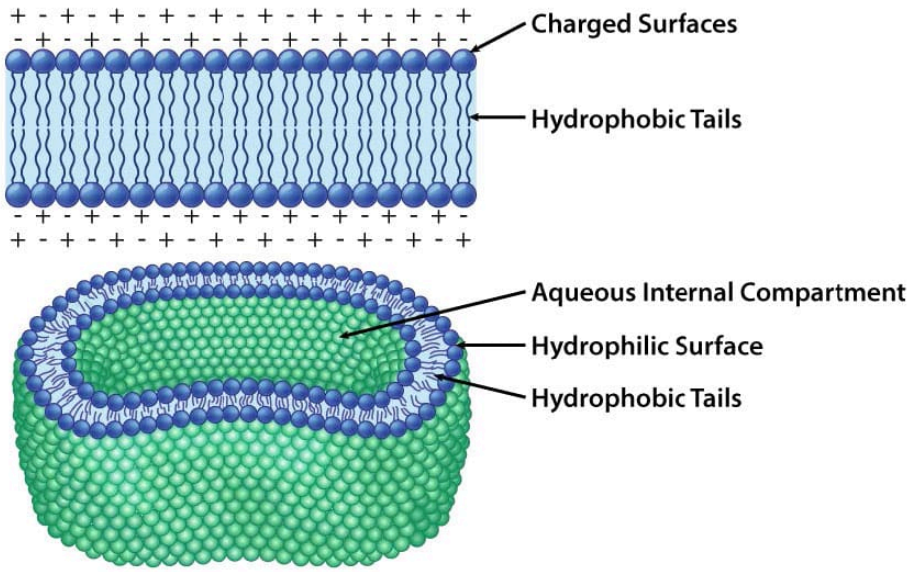


Figure 3.2: Organization of the lipid bilayer Image by Aleia Kim

Hydrophilic heads

The composition of the hydrophilic heads varies considerably. In glycerophospholipids, a phosphate is always present, of course, and it is often esterified to another substance to make a phosphatide (Figure 3.3). Common compounds linked to the phosphate (at the X position) include serine, ethanolamine, and choline. These vary in the their charges so in this way, the charge on the external or internal surface can be controlled. Cells tend to have more negative charges on the exterior half of the lipid bilayer (called the outer leaflet) and more positive charges on the interior half (inner leaflet).

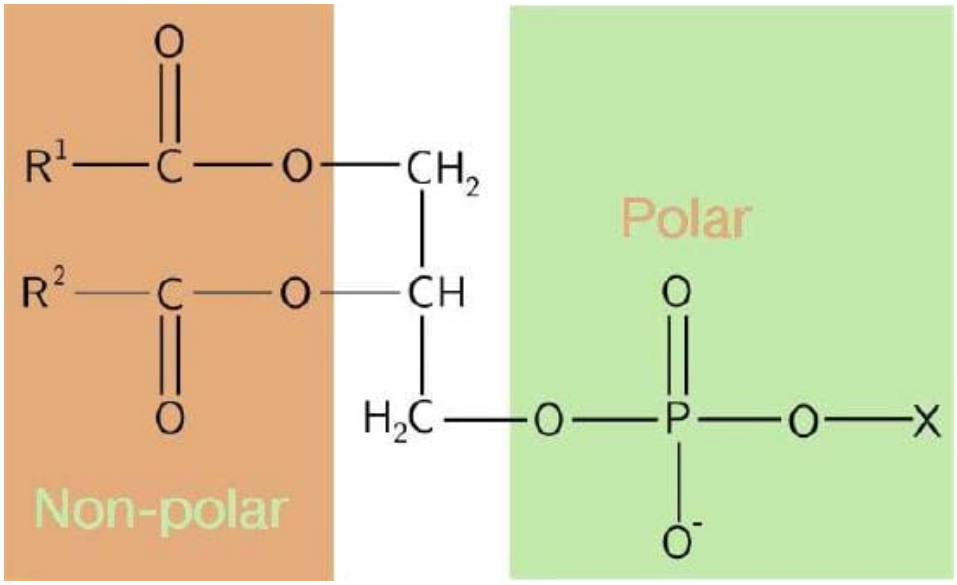


Figure 3.3 - Schematic diagram of a phosphatide

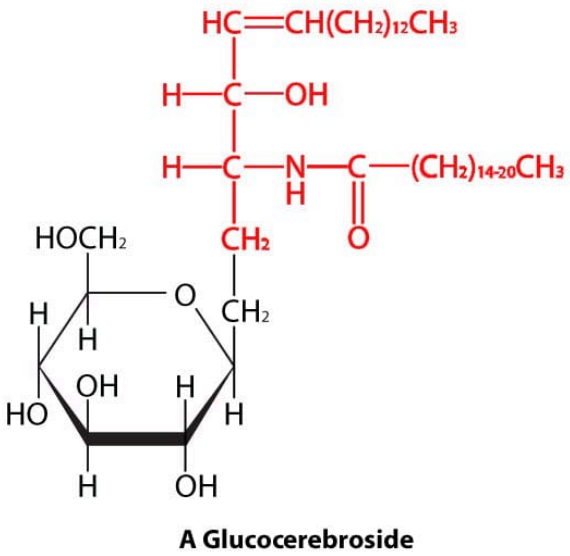


Figure 3.4: A sphingolipid. Polar head in black. Nonpolar tail in red Image by Aleia Kim

Sphingolipids

In sphingolipids (Figure 3.4), the hydrophilic head can contain a phosphate linked to ethanolamine or choline and this describes the structure of sphingomyelin, an important component of neural membranes. Most sphingolipids lack the phosphate and have instead a hydrophilic head of a single sugar (cerebrosides) or a complex oligosaccharide (gangliosides).

Water exclusion

In each case, the glycerophospholipid or sphingolipid has one end that is polar and one end that is non-polar. As we saw in the organization of amino acids with hydrophobic side chains occurring preferentially on the inside of a folded protein to exclude water, so too do the non-polar portions of these amphiphilic molecules arrange themselves so as to exclude water. Remember that the cytoplasm of a cell is mostly water and the exterior of the cell is usually bathed in an aqueous layer. It therefore makes perfect sense that the polar portions of the membrane molecules arrange themselves as they do - polar parts outside interacting with water and non-polar parts in the middle of the bilayer avoiding/excluding water.

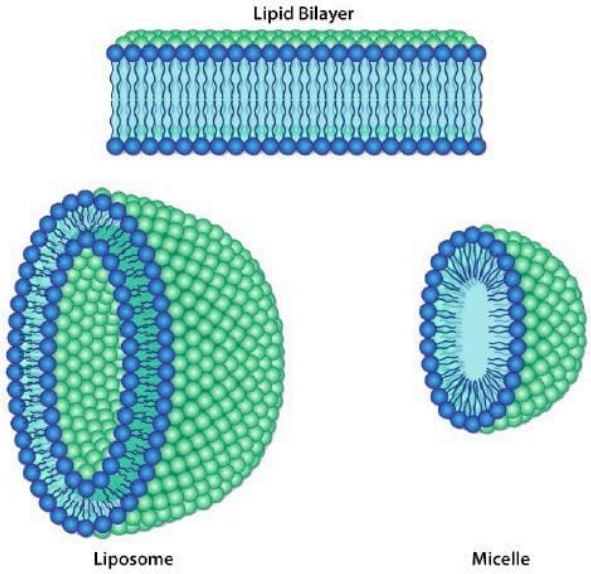


Figure 3.6 - Different lipid bilayer structures Image by Aleia Kim

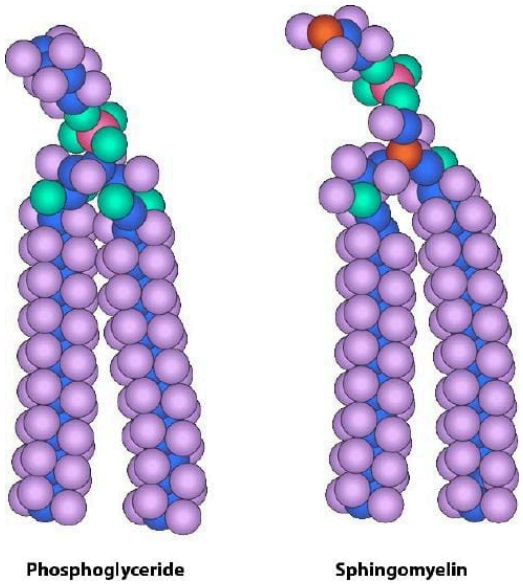


Figure 3.5 - Similarity of form between a phosphoglyceride and sphingomyelin Image by Aleia Kim

Composition Bias

The plasma membrane has distinct biases of composition relative to its inside and the outside (Figure 3.7). First, glycosylation (of lipids and proteins) has the sugar groups located almost exclusively on the outside of the cell, away from the cytoplasm (Figure 3.8). Among the membrane lipids, sphingolipids are much more commonly glycosylated than glycerophospholipids. In addition, some of the glyerophospholipids are found preferentially on one side or the other (Figure 3.7). Phosphatidylserine and phosphatidylethanolamine are found preferentially within the inner leaflet of the plasma membrane, whereas phosphatidylcholine tends to be located on the outer leaflet. In the process of apoptosis, the phosphatidylserines appear on the outer leaflet where they serve as a signal to macrophages to bind and destroy the cell. Sphingolipids are found preferentially in the plasma membrane and are almost completely absent from mitochondrial and endoplasmic reticulum membranes (Figure 3.9).

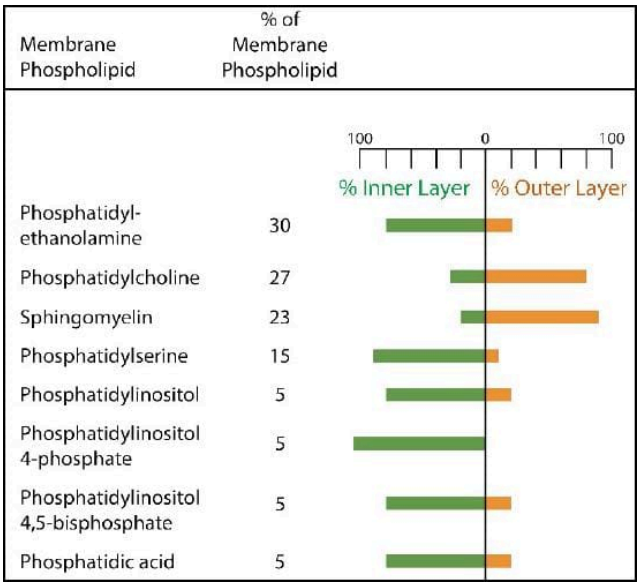


Figure 3.7: Differential distribution of membrane lipids by inner and outer leaflet Image by Pehr Jacobson

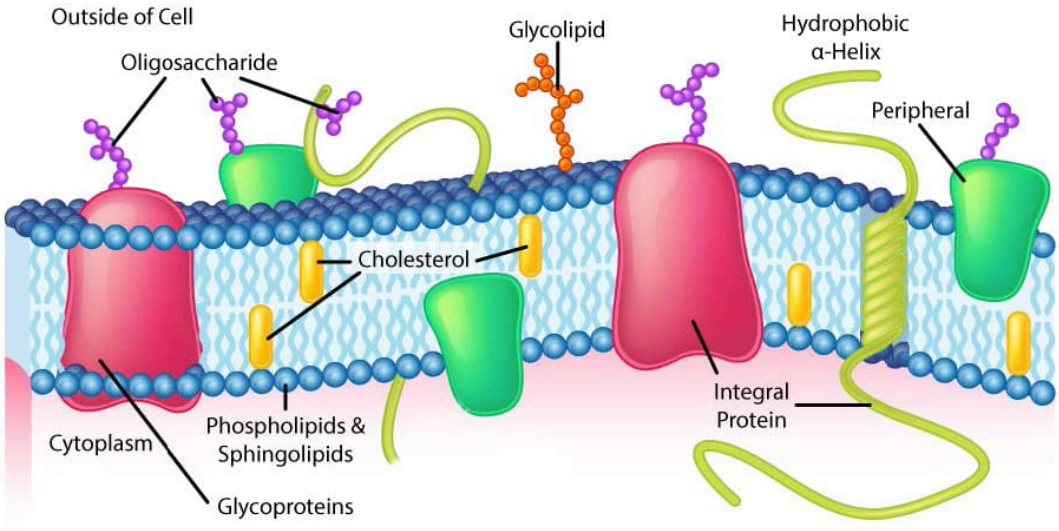


Figure 3.8 - Molecular organization of the lipid bilayer Image by Aleia Kim

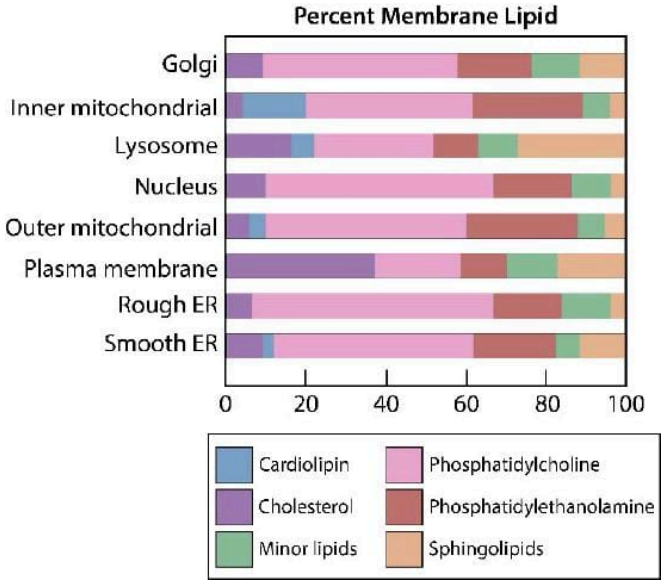


Figure 3.9 - Distribution of lipids in organelle membranes Image by Pehr Jacobson

Organelle membranes

Bias of lipid composition also exists with respect to organelle membranes. The unusual diphosphodiglycerolipid known as cardiolipin, for example, is almost only found in mitochondrial membranes (see HERE) and like phosphatidylserine, its movement is an important step in apoptosis. In signaling, phosphatidylinositols play important roles providing second messengers upon being cleaved (see HERE).

Lateral Diffusion

Movement of lipids within each leaflet of the lipid bilayer occurs readily and rapidly due to membrane fluidity. This type of movement is called lateral diffusion and can be measured by the technique called FRAP (Figure 3.10, see HERE also). In this method, a laser strikes and stains a section of the lipid bilayer of a cell, leaving a spot as shown in B. Over time, the stain diffuses out ultimately across the entire lipid bilayer, much like a drop of ink will diffuse throughout when added to a glass of water. A measurement of the rate of diffusion gives an indication of the fluidity of a membrane.

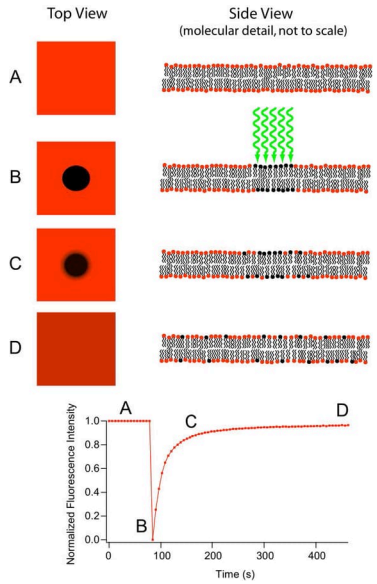


Figure 3.10 - Fluorescence recovery after photobleaching (FRAP)

Transverse Diffusion

While the movement in lateral diffusion occurs rapidly, movement of molecules from one leaflet over to the other leaflet occurs much more slowly. This type of molecular movement is called transverse diffusion and is almost nonexistent in the absence of enzyme action. Remember that there is a bias of distribution of molecules between leaflets of the membrane, which means that something must be moving them.

There are three enzymes that catalyze movement of compounds in transverse diffusion. Flippases move membrane glycerophospholipids/ sphingolipids from outer leaflet to inner leaflet (cytoplasmic side) of cell. Floppases move membrane lipids in the opposite direction. Scramblases move in either direction.

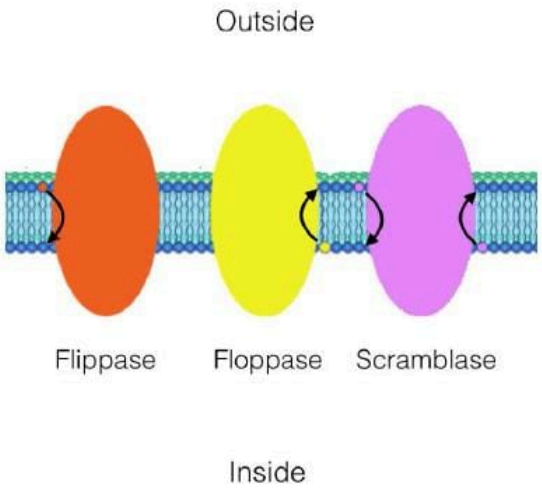


Figure 3.11 - Catalytic action of a flippase, a floppase, and a scramblase

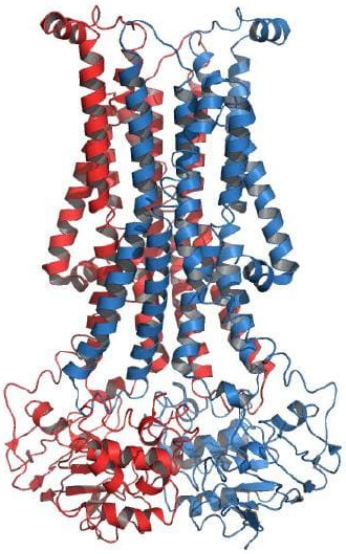


Figure 3.12 - Structure of a flippase Wikipedia

Other components of lipid bilayer

Besides glycerophospholipids and sphingolipids, there are other materials commonly found in lipid bilayers of cellular membranes. Two important prominent ones are cholesterol (Figure 3.13) and proteins. Besides serving as a metabolic precursor of steroid hormones and the bile acids, cholesterol's main role in cells is in the membranes. The flatness and hydrophobicity of the sterol rings allow cholesterol to interact with the nonpolar portions of the lipid bilayer while the hydroxyl group on the end can interact with the hydrophilic part.

Membrane fluidity

Cholesterol's function in the lipid bilayer is complex (Figure 3.13). It influences membrane fluidity. Figure 3.14 shows the phase transition for a membrane as it is heated, moving 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. It is for this reason that fish, which live in cool environments, have a higher level of unsaturated fatty acids in them - to use to make membrane lipids that will remain fluid at ocean temperatures. Interestingly, cholesterol does not change the Tm value, but instead widens the transition range between frozen and fluid forms of the membrane, allowing it to have a wider range of fluidity.

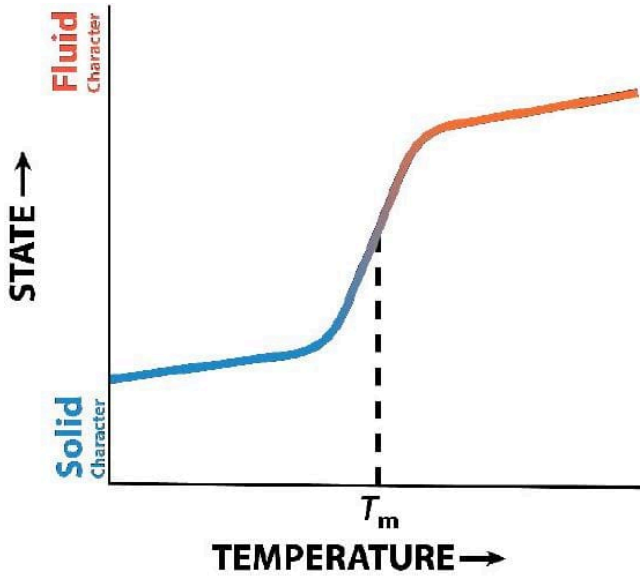


Figure 3.14 - Membrane transition temperature Image by Aleia Kim

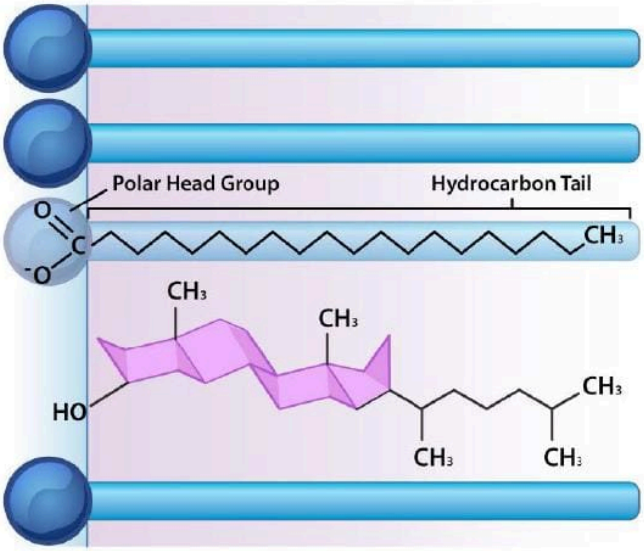


Figure 3.13 - Cholesterol in the lipid bilayer Image by Aleia Kim

Lipid Rafts

Cholesterol is also abundantly found in membrane structures called lipid rafts. Depicted in Figure 3.15, lipid rafts are organized structures within the membrane typically containing signaling molecules and other integral membrane proteins. Lipid rafts affect membrane fluidity, neurotransmission, and trafficking of receptors and membrane proteins.

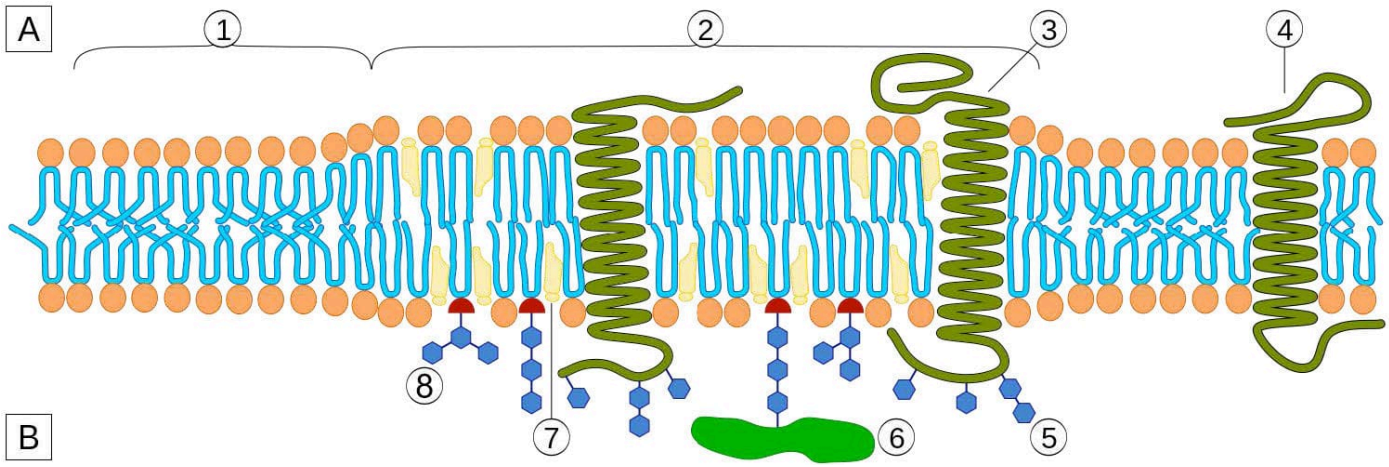


Figure 3.15 - A lipid raft - 1 = Non-raft membrane / 2 = Lipid raft / 3 = Lipid raft associated transmembrane protein / 4 = Non-raft membrane protein / 5 = Glycosylation modifications (on glycoproteins and glycolipids) / 6 = GPIanchored protein / 7 = Cholesterol / 8 = Glycolipid

Features

Distinguishing features of the rafts is that they are more ordered than the bilayers surrounding them, containing more saturated fatty acids (tighter packing and less disorganization, as a result) and up to 5 times as much cholesterol. They also are relatively rich in sphingolipids, with as much as 50% greater quantities of sphingomyelin than surrounding areas of the bilayer. The higher concentration of cholesterol in the rafts may be due to its greater ability to associate with sphingolipids (Figure 3.16). Some groups, such as prenylated proteins, like RAS, may be excluded from lipid rafts.

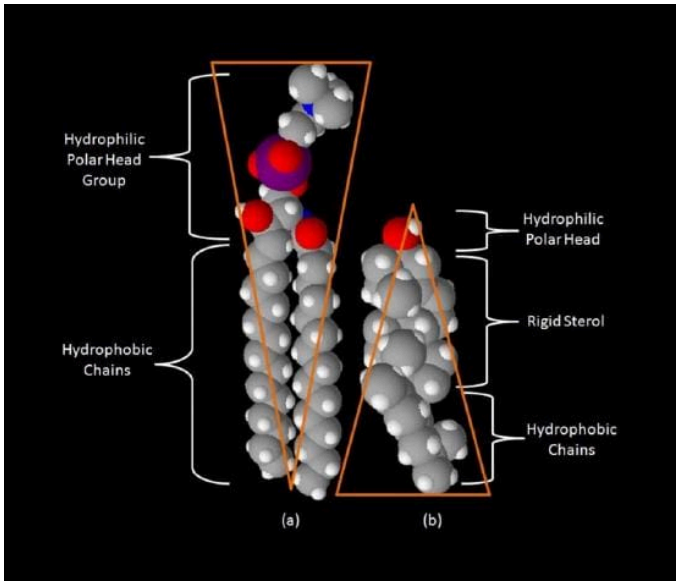


Figure 3.16 - A sphingolipid (left) associating with cholesterol (right)

Lipid rafts may provide concentrating platforms after individual protein receptors bind to ligands in signaling. After receptor activation takes place at a lipid raft, the signaling complex would provide protection from nonraft enzymes that could inactivate the signal. For example, a common feature of signaling systems is phosphorylation, so lipid rafts might provide protection against dephosphorylation by enzymes called phosphatases. Lipid rafts appear to be involved in many signal transduction processes, such as T cell antigen receptor signaling, B cell antigen receptor signaling, EGF receptor signaling, immunoglobulin E signaling, insulin receptor signaling and others. For more on signaling, see HERE.

Barrier

Transport of materials across membranes is essential for a cell to exist. The lipid bilayer is an effective barrier to the entry of most molecules and without a means of allowing food molecules to enter a cell, it would die. The primary molecules that move freely

across the lipid bilayer are small, uncharged ones, such as H2O, CO2, CO, and O2, so larger molecules, like glucose, that the cell needs for energy, would be effectively excluded if there were not proteins to facilitate its movement across the membrane.

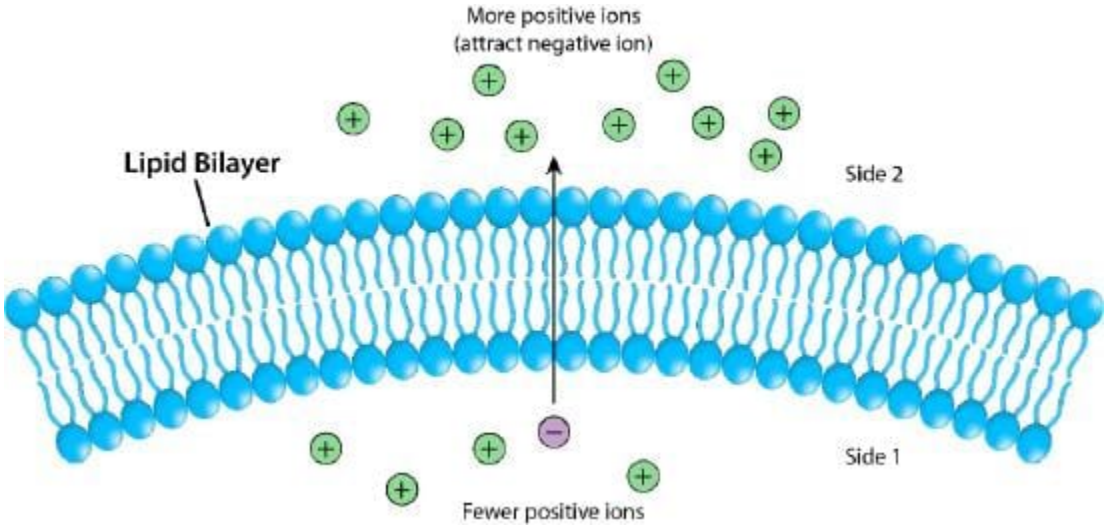


Figure 3.17 - The lipid bilayer as a barrier Image by Pehr Jacobson

Figure 3.17 depicts the barrier that the lipid bilayer provides to movement across it and the pressures (ionic attraction, in this case) that can affect movement. Potential energy from charge and concentration differences are harvested by cells for purposes that include synthesis of ATP, and moving materials against a concentration gradient in a process called active transport.

Membrane proteins

Proteins in a lipid bilayer can vary in quantity enormously, depending on the membrane. Protein content by weight of various membranes typically ranges between 30 and 75% by weight. Some mitochondrial membranes can have up to 90% protein. Proteins linked to and associated with membranes come in several types.

Transmembrane proteins

Transmembrane proteins are integral membrane proteins that completely span from one side of a biological membrane to the other and are firmly embedded in the membrane (Figure 3.18). Transmembrane proteins can function as docking sites for attachment (to the extracellular matrix, for example), as receptors in the cellular signaling system, or facilitate the specific transport of molecules into or out of the cell.

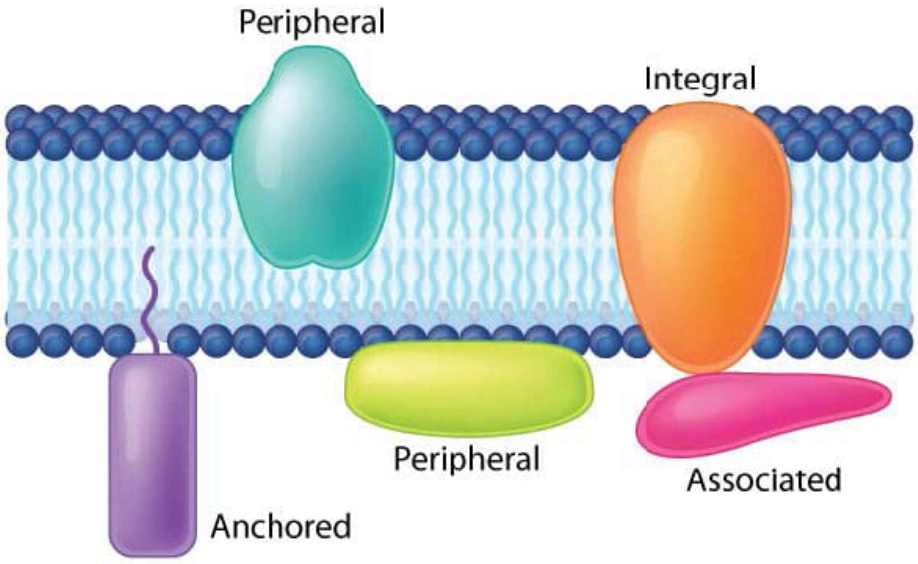


Figure 3.18 - Membrane protein types Image by Aleia Kim

Example of integrated/ transmembrane proteins include those involved in transport (e.g., Na+/K+ ATPase), ion channels (e.g., potassium channel of nerve cells) and signal transduction across the lipid bilayer (e.g., GProtein Coupled Receptors).

Peripheral membrane proteins interact with part of the bilayer (usually does not involve hydrophobic interactions), but do not project through it. A good example is phospholipase A2, which cleaves fatty acids from glycerophospholipids in membranes. Associated membrane proteins typically do not have external hydrophobic regions, so they cannot embed in a portion of the lipid bilayer, but are found near them. Such association may arise as a result of interaction with other proteins or molecules in the lipid bilayer. A good example is ribonuclease.

Anchored membrane proteins

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 (Figure 3.19). The oncogene family of proteins known as ras are good examples. These proteins are anchored to the lipid bilayer by attachment to non-polar farnesyl groups catalyzed by the enzyme farnesyltransferase.

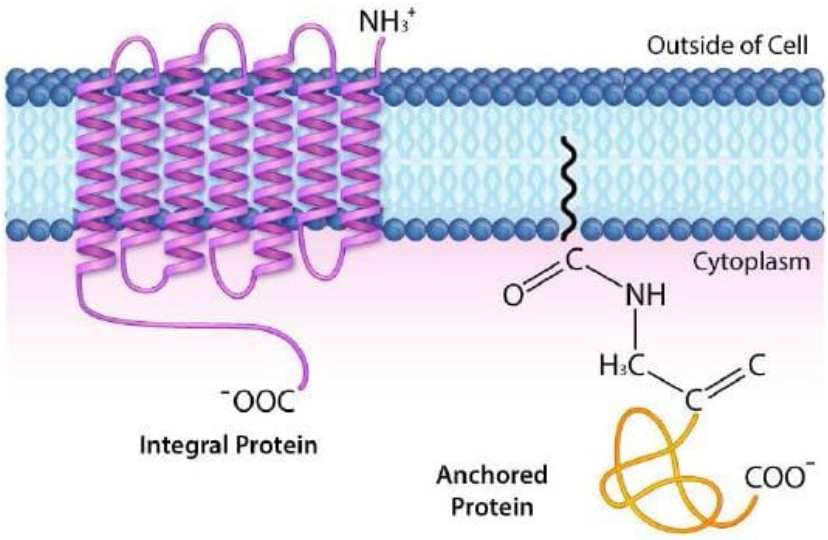


Figure 3.19 - Integral and anchored proteins Image by Aleia Kim

Finer classification

A more detailed classification scheme further categorizes the integral and anchored proteins into six different types (Figure 3.20). Type I and Type II have only one portion of the protein pass through the membrane. They differ in the orientation of the amine and carboxyl end with respect to inside/outside. Type I transmembrane proteins have the amino terminus on the outside and carboxy terminus on the inside, whereas Type II proteins have this reversed. Type III proteins are a single polypeptide chain that has multiple regions of it cross back and forth across the membrane, often to form a channel. Type IV is a multi-polypeptide protein which has multiple crossings of the membrane. Type V transmembrane proteins do not have a part of them that crosses the membrane, but they are anchored to the membrane by a lipid (such as a fatty acid) embedded in the lipid bilayer. Type VI transmembrane proteins both have a portion of them that crosses the membrane and they are attached to a lipid embedded in the lipid bilayer.

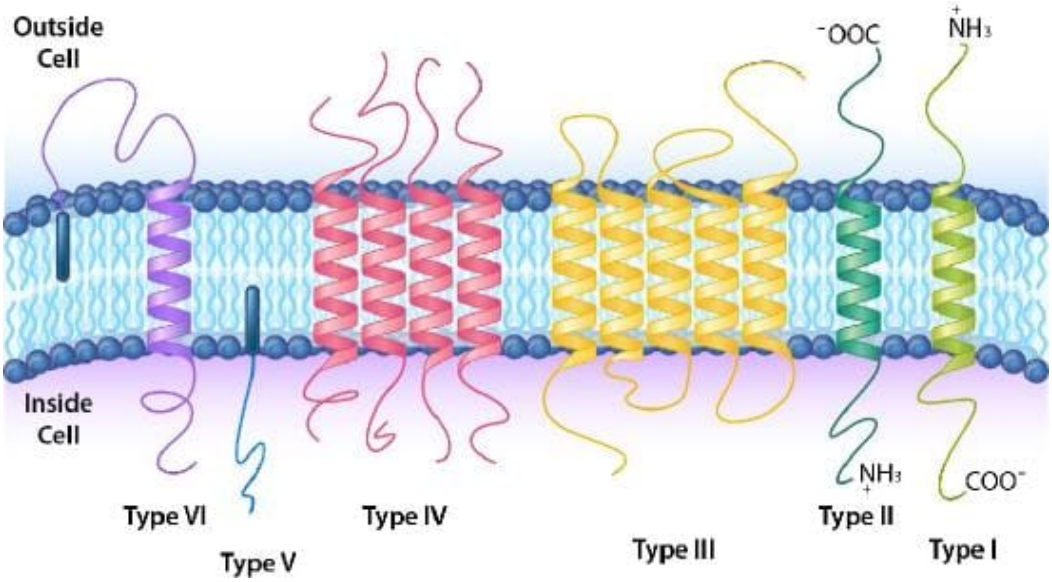


Figure 3.20 Types of integral and anchored membrane proteins Image by Aleia Kim

Blood Types

Cells have hundreds-thousands of membrane proteins and the protein composition of a membrane varies with its function and location. Glycoproteins embedded in membranes 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 (Figure 3.21).

	Group A	Group B	Group AB	Group O
Red blood cell type	A	B	AB	O
Antibodies in Plasma	Anti-B	Anti-A	None	Anti-A and Anti-B
Antigens in Red Blood Cell	A antigen	B antigen	A and B antigens	None

Figure 3.21

Osmotic Pressure

Membranes provide barriers/boundaries for most molecules, but the permeability of water across a lipid bilayer creates a variable that must be considered. The variable here is osmotic pressure. Osmotic pressure (loosely) refers to the tendency of a solution to take in water by the process of osmosis. In Figure 3.22, one can see a visual representation of the concept of the pressure.

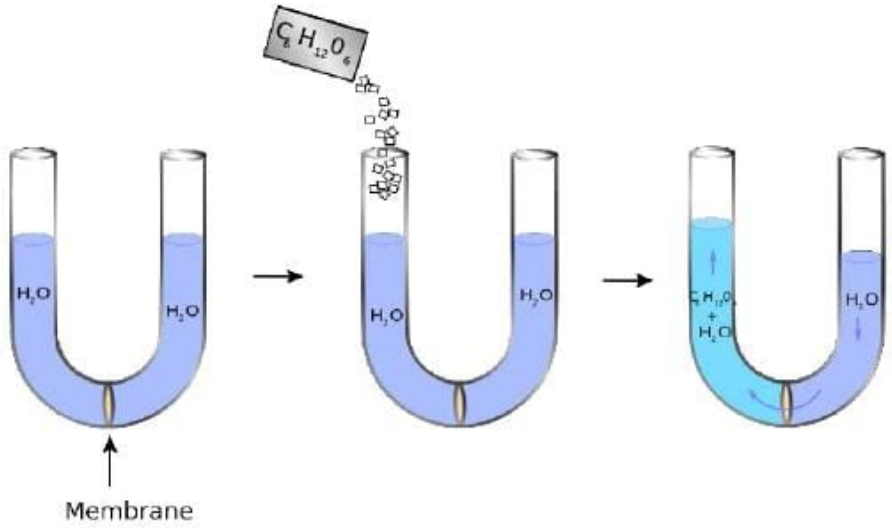


Figure 3.22

A U-shaped tube has at its bottom a semipermeable membrane. Water can pass through the membrane, but sugar molecules (C6H12O6) cannot. On the left side, sugar is added creating a concentration difference between the right and left chambers. Water diffuses across the membrane from right to left in an attempt to equalize the concentrations, causing the level of the right side to decrease and the left side to increase. The pressure resulting from the differences in height is felt at the membrane.

Equalizing concentrations

The liquid on the right does not completely move to the left, though, as might be expected if the only force involved is equalizing the concentration of sugar across the membrane (no sugar on right = no water). Instead, an equilibrium of sorts of water levels is reached even though the concentrations don't equal out. The pressure existing at the membrane then from the differences in level corresponds to the osmotic pressure of the mixture. The osmotic pressure of a solution is the pressure difference needed to halt the flow of solvent across a semipermeable membrane. Osmotic pressure can also be thought of as the pressure required to counter osmosis. The osmotic pres- Figure 3.21 - Blood types arise from cell surface glycoproteins Figure 3.22 - Osmotic pressure. Water diffuses leftwards to try to equalize the solute concentration. The pressure realized at the membrane in the right figure is the osmotic pressure sure of a dilute solution mathematically behaves like the ideal gas law

$$P_{osmotic} = \frac{nRT}{V} \tag{9.2.1}$$

where n is the number of moles, R is the gas constant, T is the temperature in Kelvin, and V is the volume.

It is more convenient in solutions to work with molarity, so

$$P_{osmotic} = MR^*T \tag{9.2.2}$$

where M is the molarity of the dissolved molecules, R* is the gas constant expressed in (L atm)/(K mol), and T is the temperature. The Greek letter Π is used to refer to the $P_{osmotic}$term, so

$$\Pi = MR^*T \tag{9.2.3}$$

Remember when calculating the molarity to include the molarity of each particle. For example, when one dissolves sucrose in solution, it does not split into smaller particles, so

$$Molarity_{Particles} = Molarity_{Sucrose} \tag{9.2.4}$$

However, for salts, like KOH, which forms two ions in solution (K+ and OH-),

$Molarity_{Particles}$= 2* $Molarity_{KOH}$.

Significant consideration

Osmotic pressure is a significant consideration for cells. Consider the fact that water can move freely across cellular membranes, but most of the contents of the cell, such as proteins, DNA, ions, sugars, etc., cannot. Second, the concentration of these compounds inside the cell is different than the concentration of them outside of the cell. Third, since water can move through the lipid bilayer, it will tend to move in the direction that will tend to equalize solute.

Hypotonic, hypertonic, isotonic

We consider three situations (Figure 3.23). First, if the concentration of solutes is greater inside the cell than outside, water will tend to move into the cell, causing the cell to swell. This circumstance is called hypotonic. Conversely, if the solute concentration is greater outside the cell than inside of it, water will exit the cell and the cell with shrink. This is a hypertonic situation. Last, if the concentrations of solutes into and outside of the cell is equal, this is called an isotonic solution. Here, no movement of water occurs across the cell membrane and the cell retains its size.

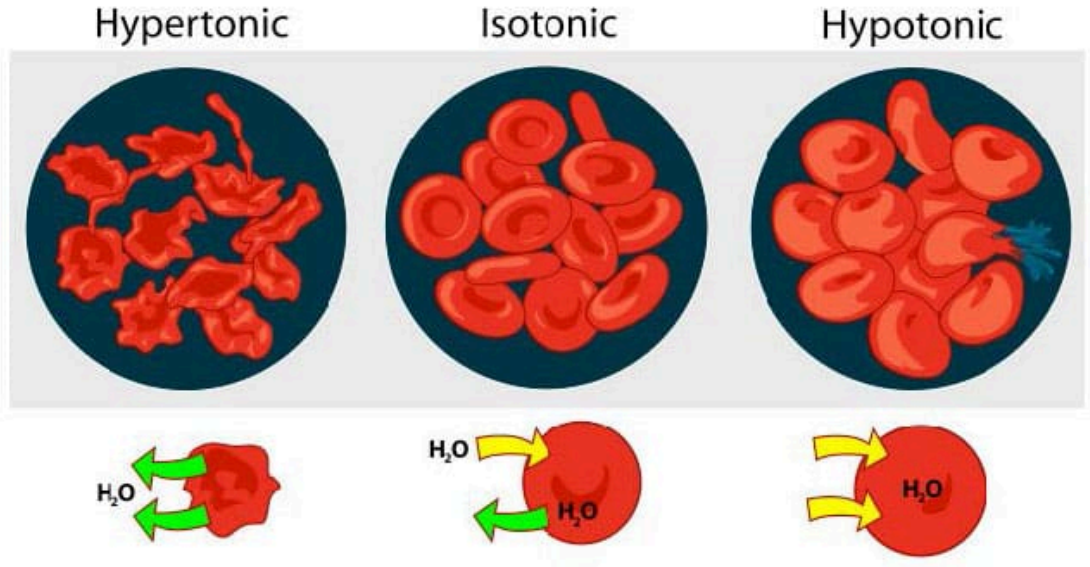
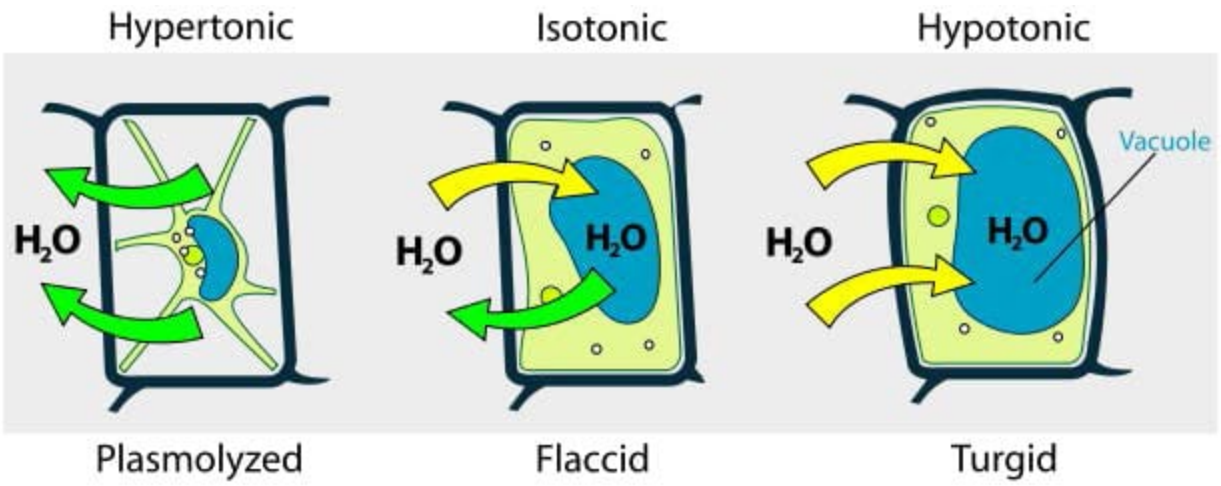


Figure 3.23 - The effect of three different osmotic conditions on red blood cells concentrations on either side of the membrane

If the osmotic pressure is greater than the forces holding together a cellular membrane, the cell will rupture. Because of this, some cells have built in defenses to prevent problems. Plant cells, for example have a fairly rigid cell wall that resists expansion in hypotonic solutions (Figure 3.24). Bacteria also have a cell wall that provides protection.



This page titled 9.2: Basic Concepts in Membranes is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 3.1: Basic Concepts in Membranes by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

9.3: Transport in Membranes

Source: BiochemFFA_3_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Movement of materials across membranes

As noted earlier, it is essential for cells to be able to uptake nutrients. This function along with movement of ions and other substances is provided by proteins/protein complexes that are highly specific for the compounds they move.

Selective movement of ions by membrane proteins and the ions' extremely low permeability across the lipid bilayer are important for helping to maintain the osmotic balance of the cell and also for providing for the most important mechanism for it to make ATP - the process of oxidative phosphorylation.

Terminology

A protein involved in moving only one molecule across a membrane is called a uniport (Figure 3.25). Proteins that move two molecules in the same direction across the membrane are called symports (also called synporters, synports, or symporters). If two molecules are moved in opposite directions across the bilayer, the protein is called an antiport. Proteins involved in moving ions are called ionophores.

If the action of a protein in moving ions across a membrane results in a net change in charge, the protein is described as electrogenic and if there is no change in charge the protein is described as electroneutral (Figure 3.26). When the driving force for movement through the membrane protein is simply diffusion, the process is called facilitated diffusion or passive transport and when the process requires other energy input, the process is called active transport.

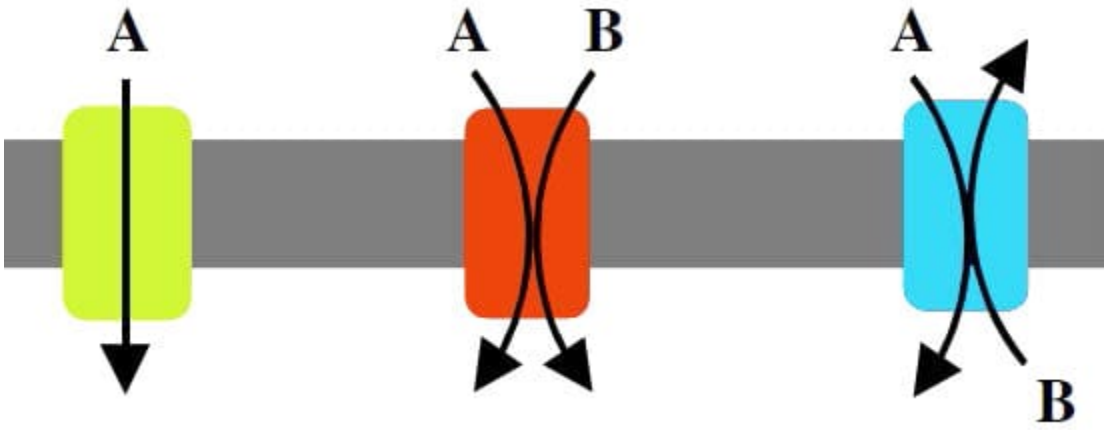


Figure 3.25 - A uniport, a symport, and an antiport

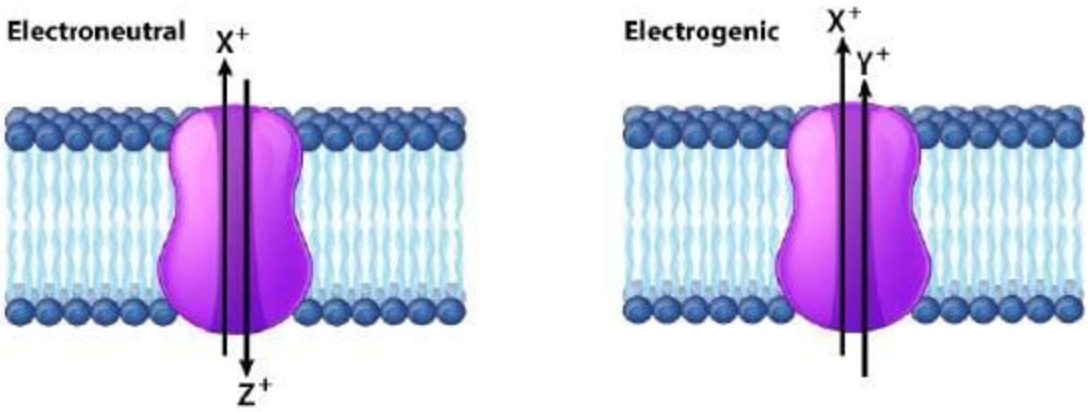


Figure 3.26 - Electroneutral and electrogenic transporters - Image by Aleia Kim

Channels and transporters

With respect to movement of materials through membrane proteins, there is a difference between channels (sometimes called pores) and transporters. Channels largely provide openings with some specificity and molecules pass through them at close to the rate of diffusion. They usually involve movement of water or ions. Examples would be the sodium or potassium channels of nerve cells. Transporters have high specificity and transfer rates that are orders of magnitude slower. Transport proteins include the sodium-potassium pump, the sodium-calcium exchanger, and lactose permease, amongst many others).

Facilitated diffusion

As noted, the driving force for facilitated diffusion is concentration, meaning that in facilitated diffusion, materials will only move from a higher concentration to a lower concentration and that at the end of the process, the concentration of materials on each side of a bilayer will be equal (Figure 3.28). This may work well in many cases.

For example, the blood concentration of glucose is sufficiently high that red blood cells can use facilitated diffusion as a means of acquiring glucose. Other cells, further removed from the blood supply where the glucose concentration is lower, must use active transport mechanisms because there is not a sufficient concentration of glucose to provide cells with the glucose they need.

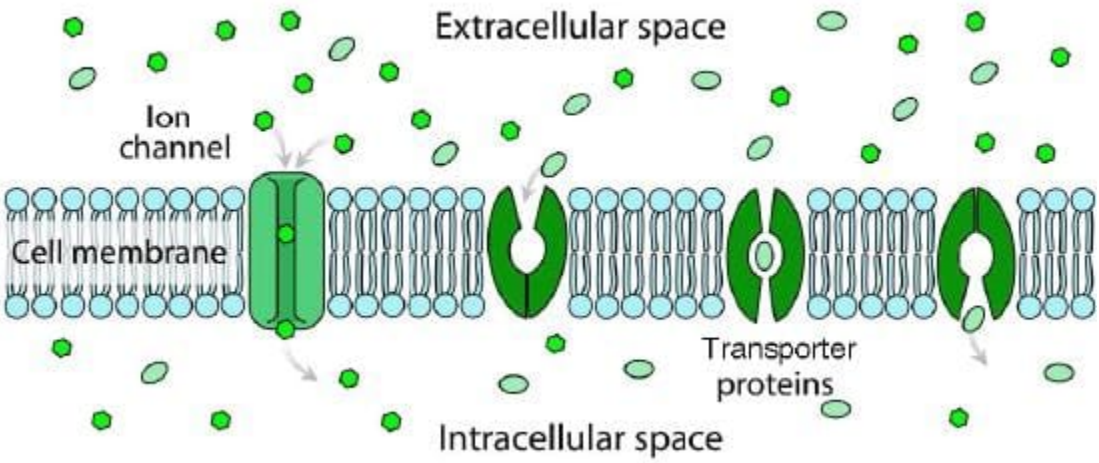


Figure 3.27 - Ion channel and transporter proteins

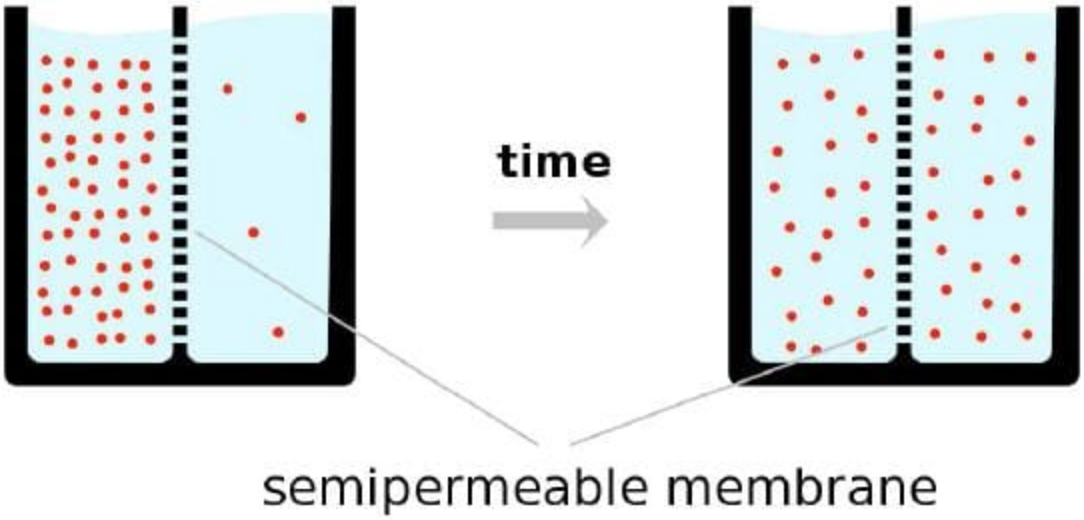


Figure 3.28 - In diffusion, solutes move from high concentration to lower concentration and eventually equalize

Ion channels

Ion channels are pore-forming membrane proteins in the membranes of all cells that regulate movement of selected ions across a membrane (Figures 3.29 & 3.30). They help to establish the resting membrane potential and to affect action potentials and other electrical signals. They are very important in the process of nerve transmission. Ion channels control the flow of ions across secretory and epithelial cells, and consequently help to regulate cell volume by affecting osmotic pressure.

Ion channels are essential features of almost all cells, functioning as selective “tunnels” that restrict movement through them to ions with specific characteristics (typically size). The size of the opening is very narrow (usually one or two atoms wide) and is able to select even against ions that are too small.

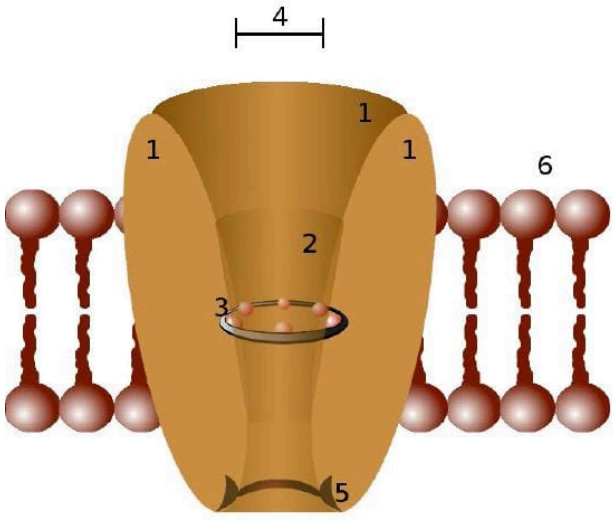


Figure 3.29 - Schematic structure of an ion channel protein - 1 = General structure / 2 = Entrance / 3 = Selectivity filter / 4 = Effective diameter / 5 = Controllable opening

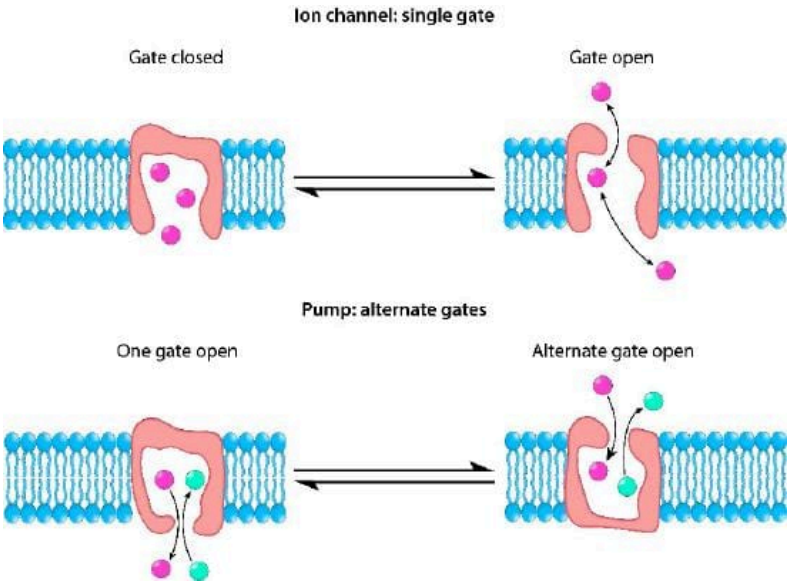


Figure 3.30 - Two types of ion channel - single and double gates - Image by Pehr Jacobson

Control mechanisms

Ion channels are controlled by mechanisms that include voltage, ligands, light, temperature, and mechanical deformation (stretch activated). Ligand-gated ion channels (LGICs) are transmembrane proteins which open to selectively allow ions such as Na+, K+, Ca++, or Cl– to pass through the membrane in response to the binding of a ligand messenger.

Sound waves cause mechanical deformation of hair cells in the ear. This results in the opening of ion channels and initiation of a nerve signal to the brain.

Sodium ion channels in the tongue for sugar receptors open in response to binding of sucrose, allowing sodium concentration in the nerve cell to increase and initiate a nerve signal to the brain. In this case, the default for the gate is to be closed and it opens in response to binding of a ligand (sucrose).

In light sensing cells of the eye, calcium gates are open by default, but stimulation by light causes them to close, triggering a series of events that result in a signal being sent the brain about the perception of light. Thus, in this case, the stimulus (light) causes an open channel to close.

Moving the other direction, nerve signals originating in the brain travel to muscle tissue and through a complicated set of exchanges, result in the opening of calcium gates of muscle cells, increasing the concentration of calcium and stimulating muscular contraction (see HERE).

Voltage gated channels are essential for transmission of nerve signals, a process discussed in more depth HERE.

Ion movement through channels

The ability of ion channels to select against ions too large is intuitive - the size of the opening in the ion channel simply isn't big enough for a larger ion to fit through the opening. Potassium, for example, passes through sodium channels rarely because the opening is too small.

Potassium channels that are selective for potassium ions must be big enough to allow potassium to enter, but if size were the only selection means, then sodium ions would also readily pass through potassium channels, since sodium ions (0.95 Å) are smaller than potassium ions (1.33 Å). In order for potassium channels to select against sodium ions and favor potassium ions, other considerations come into play.

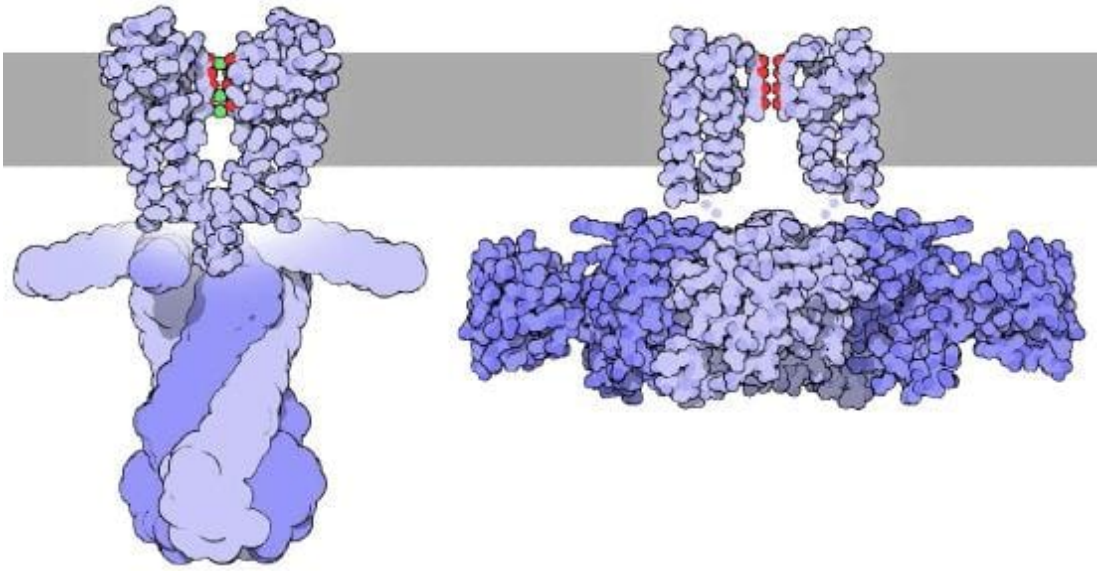


Figure 3.31 - A potassium channel closed (left) and open (right) - David Goodsell - "Molecule of the Month: Potassium Channels". (from Wikipedia)

Hydration shell

To understand this unique selectivity, it is important to understand how ions move through channels. Before an ion can pass through a channel, it must first be dissociated from (stripped of) the water molecules in its hydration shell - water molecules surrounding ions in aqueous solutions (Figure 3.32). This process requires an input of energy. The initial energy required to strip the water molecules from the hydration shell has been compared to the activation energy of an enzymatic reaction.

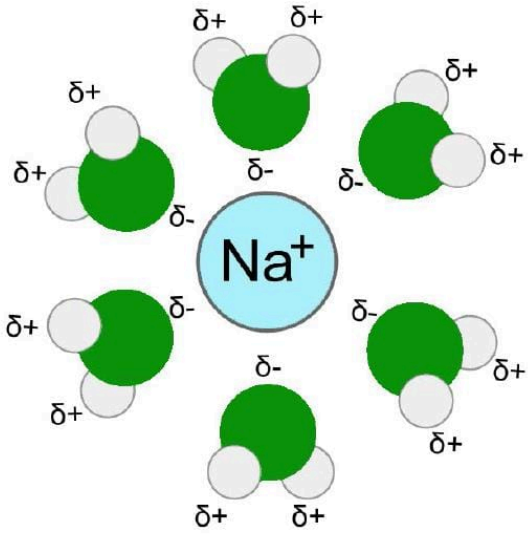


Figure 3.32 - Sodium surrounded by water molecules in a hydration shell

Comparable to enzymes

Just as enzymes lower the activation energy of enzymatic reactions and thus allow them to more readily occur, so too do channel proteins lower the energy requirements for a molecule to traverse a lipid bilayer. In the absence of the channel protein, the dehydration energy is mostly prohibitive for most polar molecules to occur, so very few make it across the lipid bilayer without the channel protein. This is why ion channel/transport proteins are so important to the cell.

After the water has been stripped, the ion can pass through the channel and when it arrives at the other side of the channel, the diffusing ion becomes rehydrated, thus regaining the energy that was required initially to strip away the water molecules from the

ion.

Selectivity of the potassium channel

The potassium channel (Figure 3.33) uses the dimensions of the potassium ion precisely to shepherd it through the channel. The sodium ion, which has different dimensions has a more difficult time making it through the channel despite its smaller size. The reason this is rooted in the energy required for dehydration.

For potassium ions, after the water has been stripped off, precisely positioned carbonyl groups along the channel help to stabilize the ion as it moves. The sodium ion, on the other hand is too small and does not make efficient connections with carbonyl groups and thus has a more difficult path. Because of this, the energy difference between dehydration and rehydration of a sodium ion in a potassium channel is energetically unfavorable (requires net input of energy) but the same process for a potassium ion is energetically favorable (results in a net gain of energy).

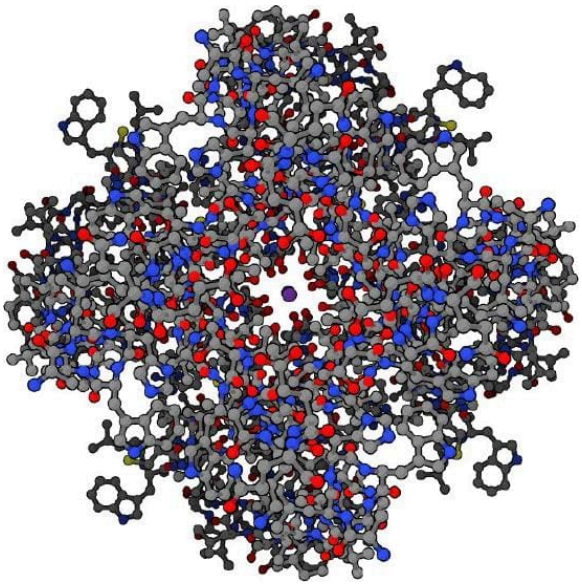
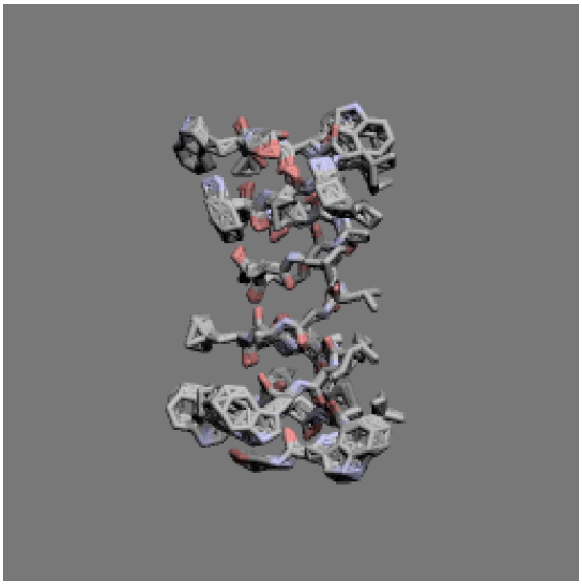


Figure 3.33 - A potassium ion (center) transiting the potassium channel - David Goodsell



Movie 3.1 - Gramicidin A Wikipedia (animated gif, download to view)

Energy factor

Thus the selection in favor of potassium and against sodium ions in a potassium channel is based on energy, not physical size, whereas in the selection of sodium ions over potassium ions in a sodium channel, size is the primary consideration.

Ion balance

The movement of ions across a lipid bilayer is tightly regulated, and with good reason. Maintaining a proper balance of ions inside and outside of cells is important for maintaining osmotic balance. It is also important inside and outside of organelles like the mitochondria and chloroplasts for energy generation. If the ionic balance of a cell is sufficiently disturbed by an uncontrolled ionophore, a cell may die.

Gramicidin

Gramicidins (Movie 3.1) are antibiotic polypeptides synthesized by the soil bacterium known as Bacillus brevis. These small pentadecapeptides (15 amino acids) are synthesized by the bacterium to kill other bacteria.

When released by the Bacillus brevis, the gramicidins insert themselves in the membranes of Gram positive bacteria and allow the movement of sodium ions into the target cells, ultimately killing them. Gramicidins can also cause hemolysis in humans so they cannot be used internally, but instead are used topically.

Aquaporins

Aquaporins are pore-containing integral membrane proteins that selectively permit passage of water molecules in and out of the cell, while preventing ions and other solutes from moving (Figures 3.34 & 3.35). Some aquaporins called aquaglyceroporins, also transport other small uncharged entities, such as glycerol, ammonia, urea, and CO2, across the membrane,. The water pores are completely impermeable to charged molecules, such as protons, which is important for the preserving the membrane's electrochemical potential difference.

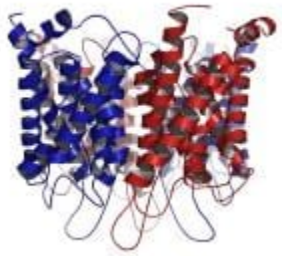


Figure 3.34 - View of aquaporin from the side - Wikipedia

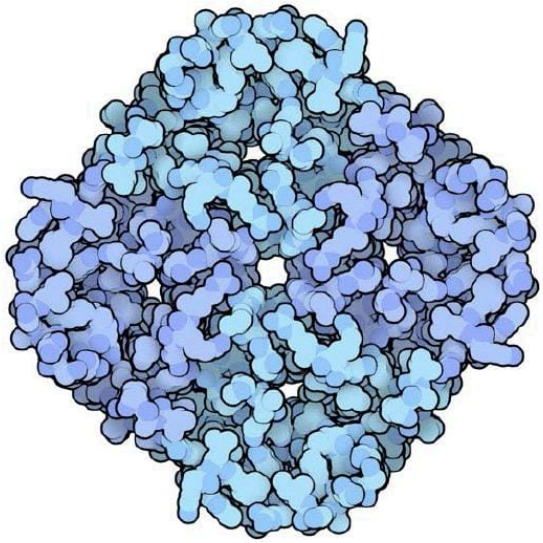


Figure 3.35 - View of aquaporin from the top - David Goodsell

Porins

Porins are proteins containing a β-barrel structure that crosses the cell membrane/wall and acts as a pore/channel through which specific molecules diffuse. Porins are found in the outer membrane of Gram-negative bacteria and some Gram-positive bacteria, mitochondria, and chloroplasts.

Porins typically transport only one group of molecules or, in some cases, one specific molecule. Antibiotics, such as β-lactam and fluoroquinolone pass through porins to reach the cytosol of Gram negative bacteria. Bacteria may develop resistance to these antibiotics when a mutation occurs to the porin involved that results in exclusion of the antibiotics that would otherwise pass through.

Transporter proteins

Not all facilitated transport occurs through ion channel proteins. Transporter proteins, as noted earlier (HERE and Figure 3.27) facilitate movement of materials across a lipid bilayer, but are slower than ion channels. Figure 3.36 illustrates a transporter protein in action. As can be seen, transporter proteins rely on a specific receptor site for proper recognition of the molecule to be moved.

Binding of the proper molecule causes a conformational change in the shape of the protein (an eversion) which results in a flipping of the open side of the protein to the other side of the lipid bilayer. In this way, the molecule is moved. Like ion channels, transporter proteins facilitate movement of materials in either direction, driven only by the concentration difference between one side and the other.

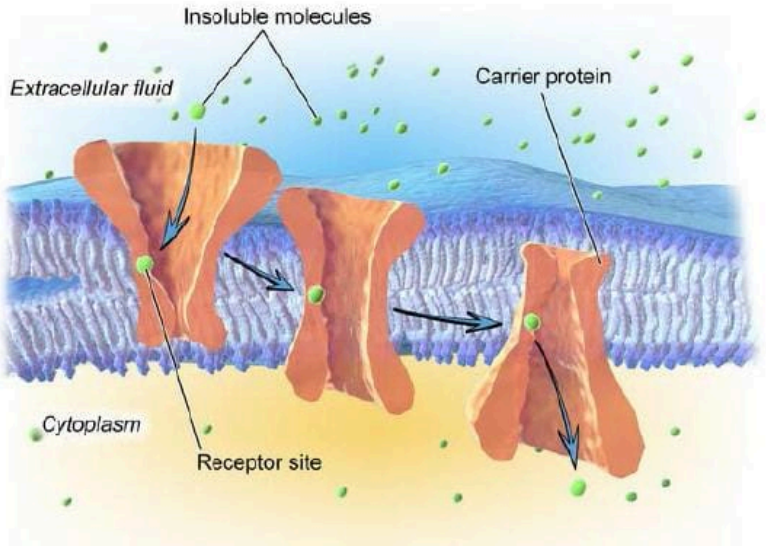


Figure 3.36 - Facilitated diffusion with a transporter protein - Wikipedia

Active transport

All of the transport mechanisms described so far are driven solely by a concentration gradient - moving from higher concentrations in the direction of lower concentrations. These movements can occur in either direction and, as noted, result in equal concentrations on either side of the bilayer, if allowed to go to completion. Many times, however, cells must move materials against a concentration gradient and when this occurs, another source of energy is required. This process is known as active transport.

A good definition of active transport is that in active transport, at least one molecule is being moved against a concentration gradient. A common, but not exclusive, energy source is ATP (see Na+/K+ ATPase), but other energy sources are also 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.

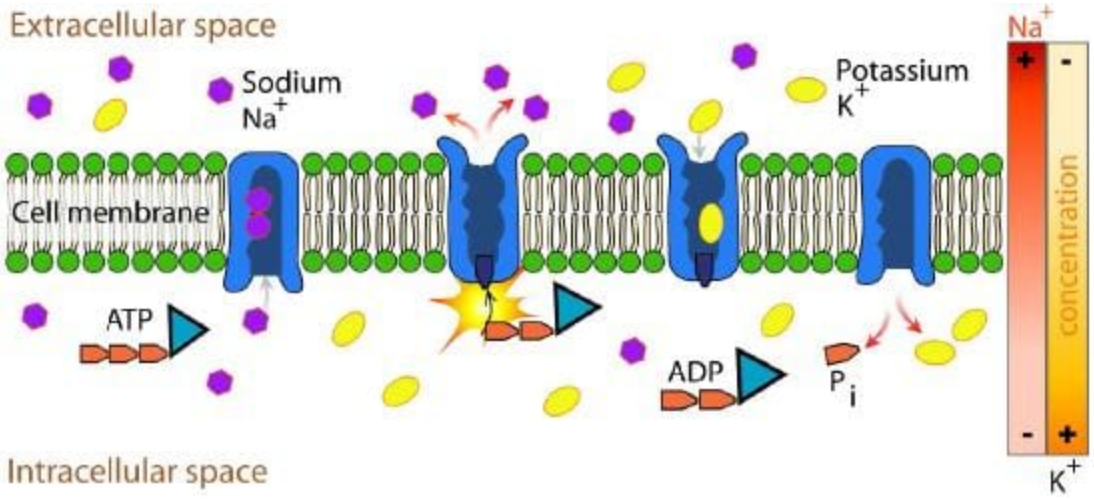


Figure 3.37 - An overview of active transport by the Na+K+ ATPase

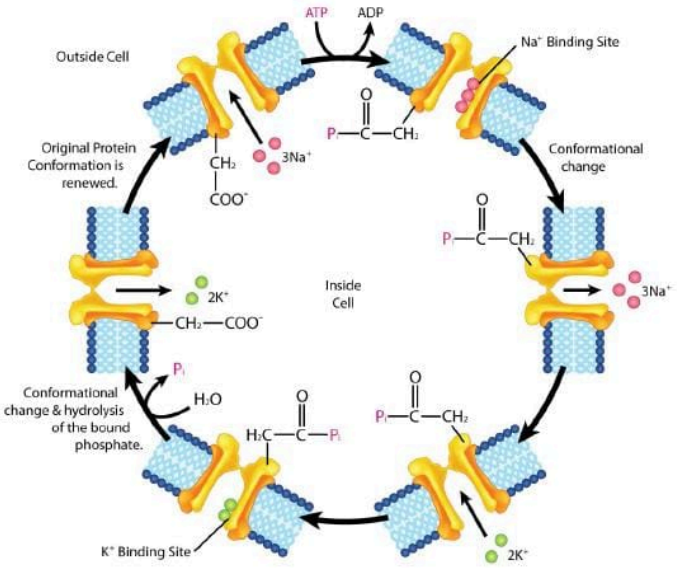


Figure 3.38 - Sequential steps in the active transport of ions by theNa+K+ ATPase - Wikipedia

Na+/K+ ATPase

An important integral membrane transport protein is the Na+/K+ ATPase antiport (Figures 3.37 and 3.38), which moves three sodium ions out of the cell and two potassium ions into the cell with each cycle of action. In each case, the movement of ions is against the concentration gradient. Since three positive charges are moved out for each two positive charges moved in, the system is electrogenic.

The protein 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 sodium and potassium gradients necessary for signal transmission. Failure of the system to function results in swelling of the cell due to movement of water into the cell through osmotic pressure. The transporter expends about one fifth of the ATP energy of animal cells. The cycle of action occurs as follows:

1. Pump binds ATP followed by binding of 3 Na+ ions from cytoplasm of cell
2. ATP hydrolysis results in phosphorylation of aspartate residue of pump. ADP is released
3. Phosphorylated pump undergoes conformational change to expose Na+ ions to exterior of cell. Na+ ions are released.
4. Pump binds 2 extracellular K+ ions.
5. Pump dephosphorylates causing it to expose K+ ions to cytoplasm as pump returns to original shape.
6. Pump binds 3 Na+ ions, binds ATP and releases 2 K+ ions to restart process

The Na+/K+ ATPase is classified as a P-type ATPase. This category of pump is notable for having a phosphorylated aspartate intermediate and is present across the biological kingdoms - bacteria, archaeans, and eukaryotes.

ATPase types

ATPases have roles in either the synthesis or hydrolysis of ATP and come in several different forms.

- F-ATPases (F1FO-ATPases) are present in mitochondria, chloroplasts and bacterial plasma membranes and are the prime ATP synthesizers for these systems. Each uses a proton gradient as its energy source for ATP production. Complex V of the mitochondrion is an F-type ATPase.
- V-ATPases (V1VO-ATPases) are mostly found in vacuoles of eukaryotes . They utilize energy from ATP hydrolysis to transport solutes and protons into vacuoles and lysosomes, thus lowering their pH values.

The V-type and F-type ATPases are very similar in structure. The V-type (Figure 3.39) uses ATP to pump protons into vacuoles and lysosomes, whereas F-types use proton gradients of the mitochondria and chloroplasts to make ATP.

- A-ATPases (A1AO-ATPases) are found in archaeans and are similar to F-ATPases in function.
- P-ATPases (E1E2-ATPases) are in bacteria, fungi and in eukaryotic plasma membranes and organelles. They transport a diversity of ions across membranes. Each has a common mechanism of action which include autophosphorylation of a conserved aspartic acid side chain within it. Examples of P-type ATPases include the Na+/K+ ATPase and the calcium pump.
- E-ATPases are enzymes found on the cell surface. They hydrolyze a range of extracellular nucleoside triphosphates, including ATP.

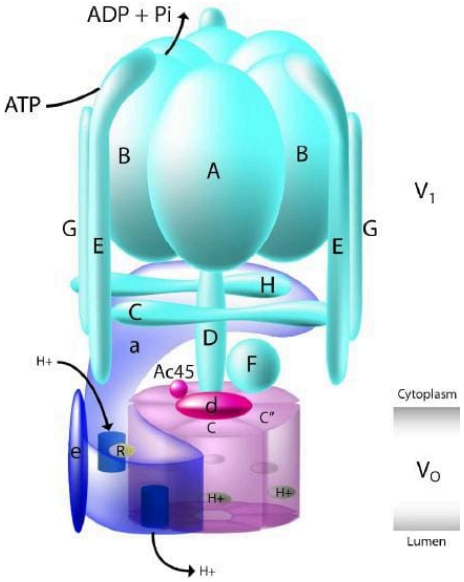


Figure 3.39 - Schematic structure of V-ATPases- Wikipedia

Nerve transmission

Now that you have seen how the Na+/K+ ATPase functions, it is appropriate to discuss how nerve cells use ion gradients created with it to generate and transmit nerve signals. Neurons are cells of the nervous system that use chemical and electrical signals to rapidly transmit information across the body (Figure 3.40). The sensory nerve system links receptors for vision, hearing, touch, taste, and smell to the brain for perception. Motor neurons run from the spinal cord to muscle cells. These neurons have a cell body and a very long, thin extension called an axon, that stretches from the cell body in the spinal cord all the way to the muscles they control. Nerve impulses travel down the axon to stimulate muscle contraction.

Signals travel through neurons, ultimately arriving at junctions with other nerve cells or target cells such as muscle cells. Note that neurons do not make physical contact with each other or with muscle cells. The tiny space between two neurons or between a neuron and a muscle cell is called the synaptic cleft. At the synaptic cleft, the neuron releases neurotransmitters that exit the nerve cell and travel across the junction to a recipient cell where a response is generated. That response may be creating another nerve signal, if the adjacent cell is a nerve cell or it may be a muscular contraction if the recipient is a muscle cell (Figure 3.41).

In considering information movement via nerve cells, then, we will discuss two steps - 1) creation and propagation of a signal in a nerve cell and 2) action of neurotransmitters exiting a nerve cell and transiting a synaptic junction.

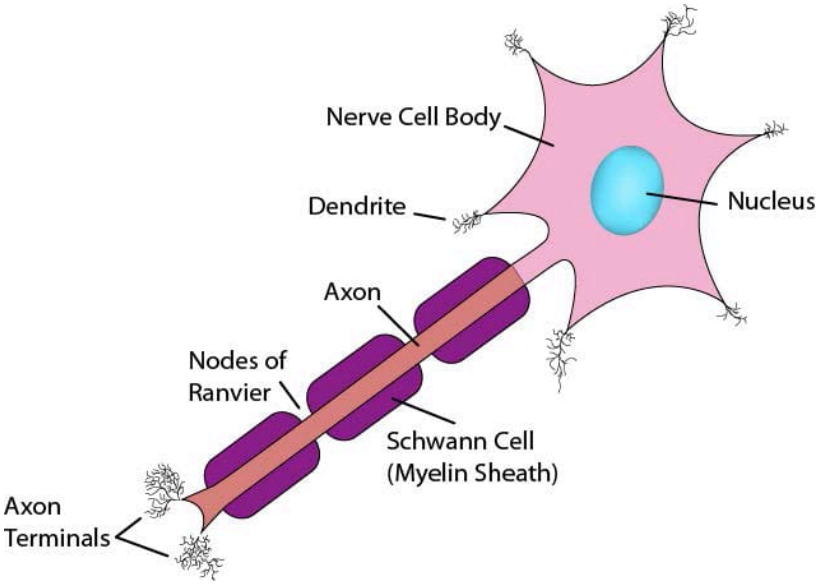


Figure 3.40 - Anatomy of a nerve cell - Image by Pehr Jacobson

Signal source

Creation of a nerve signal begins with a stimulus to the nerve cell. In the case of muscle contraction, the motor cortex of the brain sends signals to the appropriate motor neurons, stimulating them to generate a nerve impulse. How is such an impulse generated?

Resting potential

In the unstimulated state, all cells, including nerve cells, have a small voltage difference (called the resting potential) across the plasma membrane, arising from unequal pumping of ions across the membrane. The Na+/K+ ATPase, for example, pumps sodium ions out of the cell and potassium ions into cells. Since three sodium ions get pumped out for every two potassium ions pumped in, a charge and chemical gradient is created. It is the charge gradient that gives rise to the resting potential.

Altering the gradients of ions across membranes provide the driving force for nerve signals. This happens as a result of opening and closing of gated ion channels. Opening of gates to allow ions to pass through the membrane swiftly changes the ionic balance across the membrane resulting in a new voltage difference called the action potential. It is the action potential that is the impetus of nerve transmission.

Initiation of signal

The signal generated by a motor neuron begins with opening of sodium channels in the membrane of the nerve cell body causing a rapid influx of sodium ions into the nerve cell. This step, called depolarization (Figure 3.42), triggers an electrochemical signal - the action potential. Remember that the Na+/K+ ATPase has created a large sodium gradient, so sodium ions rush into the cell when sodium channels open. After the initial depolarization, potassium channel gates, responding to the depolarization, open, allowing potassium ions to rapidly diffuse out of the cell (remember K+ ions are more abundant inside of the cell). This phase is called the repolarization phase and during it, the sodium gates close.

The rapid exit of potassium ions causes the voltage difference to “overshoot” the resting potential and potassium gates close. This followed by the so-called refractory period, when the Na+/K+ ATPase begins its work to re-establish the original conditions by pumping sodium ions out and potassium ions into the nerve cell. Eventually, the system recovers and the resting potential is re-established. The initiating end of the nerve cell is then ready for another signal.

Propagation of action potential

What we have described here is only the initiation of the nerve signal in one part of the nerve cell. For the signal to be received,

the action potential must travel the entirety of the length of the nerve cell (the axon) and cause a chemical signal to be released into the synaptic cleft to get to its target. Propagating the nerve signal (action potential) in the original nerve cell is the function of all of the rest of the gated ion channels (Figure 3.43) positioned on the sides of the nerve cell. The sodium and potassium gates involved in propagation of the signal all act in response to voltage changes created by the electrochemical gradient moving down the nerve cell (Figure 3.44). Remember that opening of the initial gates at initiation of the signal created an influx of sodium ions and an efflux of potassium ions.

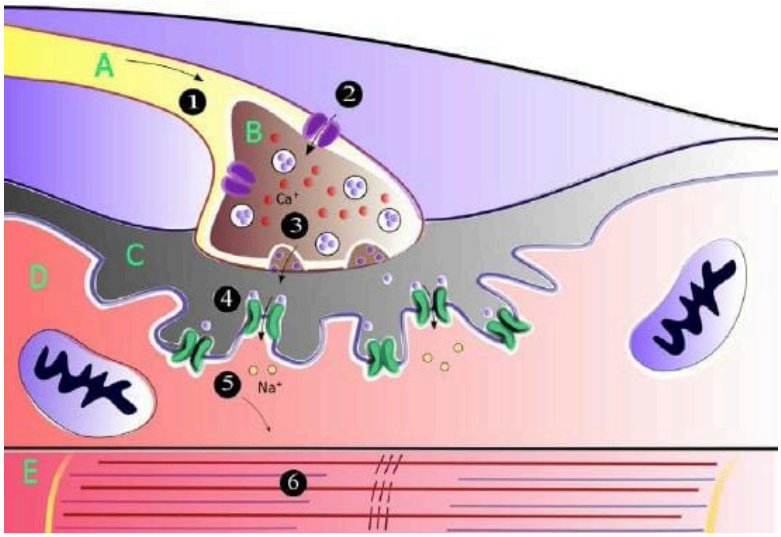


Figure 3.41 - (1) The action potential reaches the axon terminal; (2) voltage-dependent calcium gates open - calcium enters the axon terminal; (3) neurotransmitter vesicles fuse with the presynaptic membrane and release acetylcholine (ACh); (4) ACh binds to postsynaptic receptors on the sarcolemma; (5) ion channels open in response and allow sodium ions to flow into the muscle cell; (6) The flow of sodium ions into the muscle cell generates an action potential which travels to the myofibril and results in muscle contraction. - Wikipedia

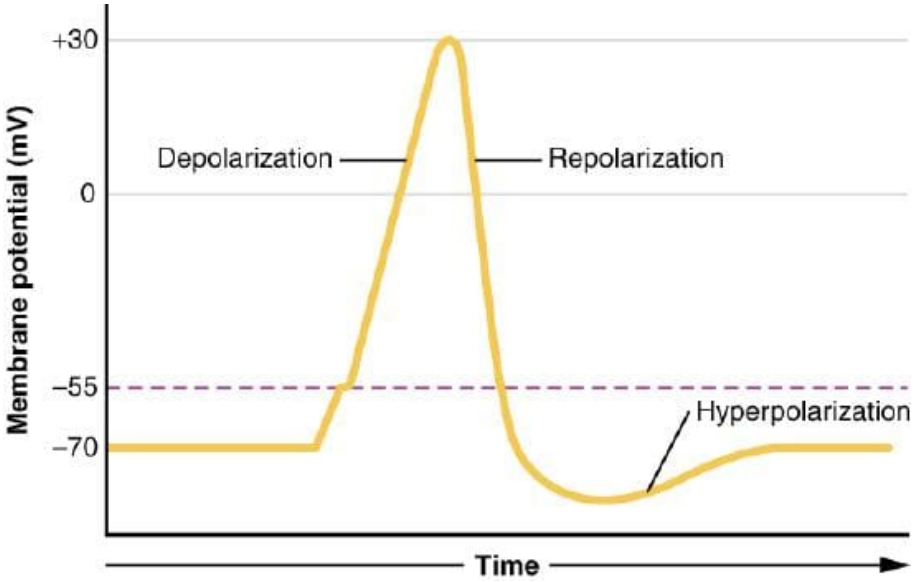


Figure 3.42 - Depolarization and repolarization of a nerve cell - Wikipedia

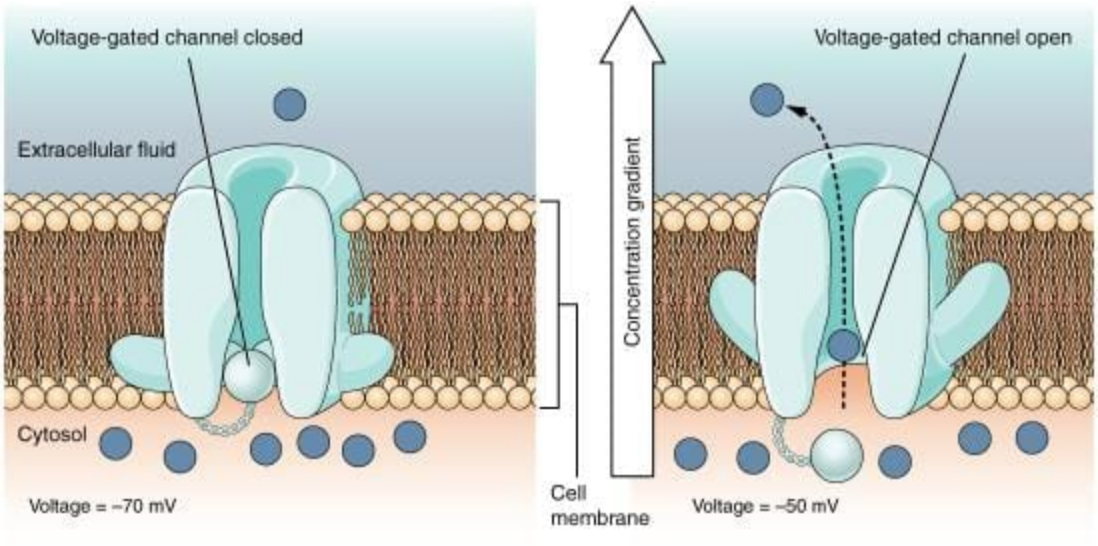


Figure 3.43 - Voltage gated ion channels - Wikipedia

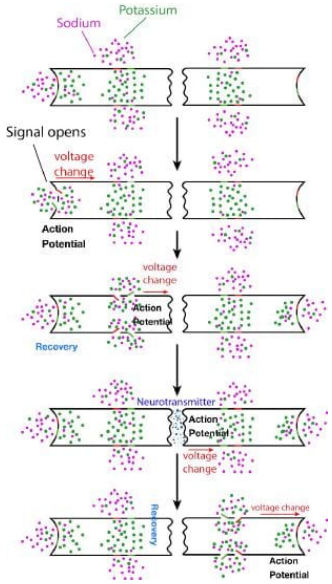


Figure 3.44 - Action potential moving across a synaptic junction of two nerve cells - Image by Pehr Jacobson

Moving signal

This chemical and electrical change that creates the action potential leaves the end of the nerve cell where it started and travels down the axon towards the other end of the nerve cell. Along the way, it encounters more sodium and potassium gated channels. In each case, these respond simply to the voltage change of the action potential and open and close, exactly in the same way the gates opened to start the signal. Thus, a rapid wave of increasing sodium ions and decreasing potassium ions moves along the nerve cell, propagated (and amplified) by gates opening and closing as the ions and charges move down the nerve cell. Eventually, the ionic tidal wave reaches the end of the nerve cell (axon terminal) facing the synaptic cleft.

Crossing the synaptic cleft

For the signal to be received by the intended target (postsynaptic cell) from the originating neuron (presynaptic neuron), it must cross the synaptic cleft and stimulate the neighboring cell (Figure 3.45). Communicating information across a synaptic cleft is the job of neurotransmitters. These are small molecules synthesized in nerve cells that are packaged in membrane vesicles called synaptic vesicles in the nerve cell. Neurotransmitters come in all shapes and chemical forms, from small chemicals like acetylcholine to peptides like neuropeptide Y. The most abundant neurotransmitter is glutamate, which acts at over 90% of the synapses in the human brain.

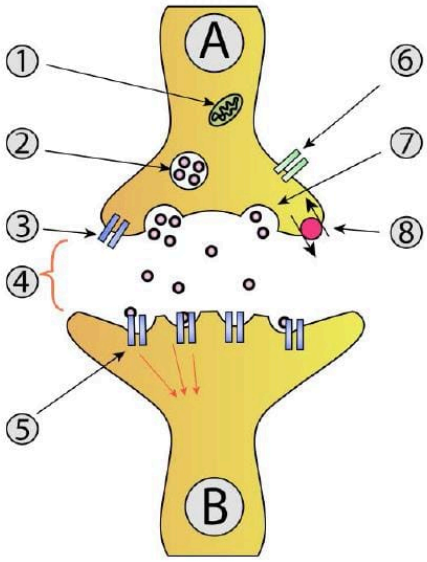


Figure 3.45 - A) Pre-synaptic neuron; B) post-synaptic neuron; 1) Mitochondria; 2) synaptic vesicle with neurotransmitters; 3) autoreceptor; 4) synapse with neurotransmitter released (serotonin); 5) postsynaptic receptors activated by neurotransmitter; 6) calcium channel; 7) exocytosis of a vesicle; 8) recaptured neurotransmitter. Wikipedia

Movie 3.2 - Movement of an action potential down a nerve cell - Wikipedia

Into the cleft

As the action potential in the presynaptic neuron approaches the axon terminus, synaptic vesicles begin to fuse with the membrane and their neurotransmitter contents spill into the synaptic cleft. Once in the cleft, the neurotransmitters diffuse, some of them reaching receptors on the postsynaptic cell. Binding of the neurotransmitter to the receptors on the membrane of the postsynaptic cell stimulates a response.

For motor neurons, the postsynaptic cell will be a muscle cell, and the response will be muscle contraction/relaxation. At this point, the originating nerve cell has done its job and communicated its information to its immediate target. If the postsynaptic cell is a nerve cell, the process repeats in that cell until it gets to its destination.

Na+/glucose transporter

Absorbing nutrients from the digestive system is necessary for animal life. The sodium/glucose transport protein is an electrogenic symporter that moves glucose into intestinal cells. It is found in the intestinal mucosa and the proximal tubule of the nephron of the kidney. The sodium/glucose transport system functions in the latter to promote reabsorption of glucose.

The pump works in conjunction with the Na+/K+ transport system. The gradient of sodium ions built up by the Na+/K+ pump is used as an energy source to drive movement of glucose into cells (see Figure 3.38). Use of an ion gradient established by a separate pump is known as secondary active transport. For intestinal mucosa, the pump transports glucose out of the gut and into gut cells. Later, the glucose is exported out the other side of the gut cells to the interstitial space for use in the body.

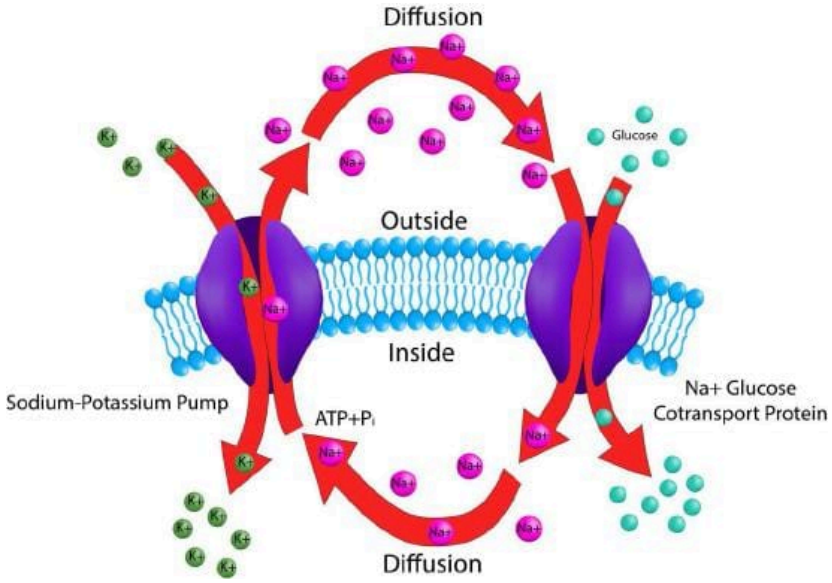


Figure 3.46 - The sodium/glucose pump (right) and the Na+/K+ ATPase (left). The sodium gradient generated by the Na+/K+ ATPase is used by the sodium/glucose pump to import glucose into the cell. Wikipedia

Calcium pumps

Calcium ions are necessary for muscular contraction and play important roles as signaling molecules within cells. In addition, when calcium concentrations rise too high, DNA in chromosomes can precipitate. Calcium concentration in cells is therefore managed carefully. It is kept very low in the cytoplasm as a result of action of pumps, both in the plasma membrane, which pump calcium outwards from the cytoplasm and in organelles, such as the endoplasmic reticulum (sarcoplasmic reticulum of muscle cells), which pump calcium out of the cytoplasm and into these organelles.

Opening of calcium channels, then, increases calcium concentration quickly in the cytoplasm resulting in a quick response, whether the intention is signaling or contraction of a muscle. After the response is generated, the calcium is pumped back out of the cytoplasm by the respective calcium pumps.

Some calcium pumps use ATP as an energy source to move calcium and others use ion gradients, such as sodium for the same purpose.

Na+/Ca++ transporter

One calcium pump of interest uses the sodium gradient as an energy source. It is the sodium/calcium pump. This electrogenic antiport system uses sodium's movement into the cell as a driving force to move calcium out of the cell, although its direction can reverse in some circumstances. The pump is a high capacity system to move a lot of calcium quickly, moving up to 5000 calcium ions per second and is found in many tissues with many functions.

Digitalis

One important function of the Na+/Ca++ pump occurs in heart cells. Ca++ is important for contraction of heart muscle. Calcium efflux from the cells is the normal operation of the pump, however, during the upstroke of the cycle, there is a large movement of sodium ions into the heart cell. When this occurs, the pump reverses and pumps Na+ out and Ca++ in briefly. Since calcium helps stimulate contraction of cardiac muscle, this can help make the heart beat stronger and is the focus of the use of digitalis to treat congestive heart failure.

Digitalis blocks the sodium-potassium ATPase and interferes with the sodium ion gradient. As noted above, when the Na+ gradient is oriented in the wrong direction, calcium is pumped in. Digitalis is therefore used to treat congestive heart failure because it increases the concentration of calcium in the heart cells, favoring more forceful beats.

ABC transporters

ABC transporters are another class of transmembrane proteins that use ATP energy to transport things against concentration gradients (Figures 3.47 & 3.48). This protein superfamily includes hundreds of proteins (48 in humans alone) and spans all extant phyla from prokaryotes to humans. These proteins function not only in membrane transport, but also in processes that include DNA repair and the process of translation.

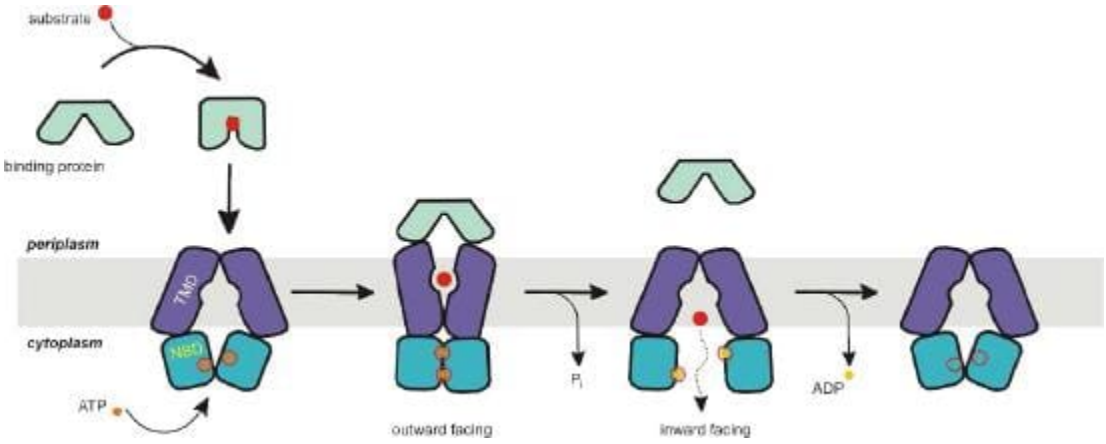


Figure 3.47 - Mechanism of an ABC importer

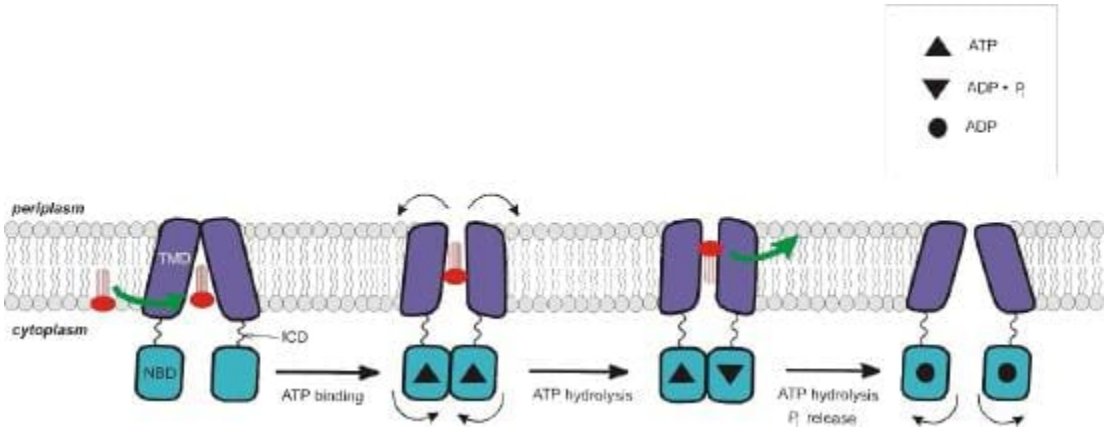


Figure 3.48 - Another view of an ABC exporter. NBD = Nucleotide Binding Domain

Transport

Substances that ABC transporters move across membranes include metabolic products, lipids, sterols, and drugs. ABC transporters function in multi-drug resistance of many cells, and provide resistance to antibiotics in bacteria as well as resistance to chemotherapy in higher cells by exporting drugs used to treat both of these types of cells.

ABC transporters are divided into three main groups - 1) importers (prokaryotes only); 2) exporters (prokaryotes and eukaryotes), and 3) non-transporters with roles in DNA repair and translation. All ABC transport proteins have four protein domains - two that are cytoplasmic and two that are membrane bound. They are alternately open to the cytoplasmic or extracellular (or periplasmic) regions and this is controlled by hydrolysis of ATP.

Disease

ABC transporters have roles in cystic fibrosis and other inherited human diseases. They are very involved in development of resistance to multiple drugs by a diverse group of cells. ABC transporters provide multi-drug resistance by expelling drug(s) from cells. ABCB1 protein, for example, pumps tumor suppression drugs out of the cell. Another ABC transporter known as Pgp transports organic cationic or neutral compounds.

Cystic fibrosis

Cystic fibrosis (CF) is a an autosomal recessive genetic disorder arising from mutations in both copies of the gene for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. This ABC transporter system, which moves chloride and thiocyanate ions across epithelial tissue membranes exerts its effect mostly in the lungs but the pancreas, liver, kidneys, and intestine are all also affected by it.

Function

CFTR has roles in the production of sweat, mucus, and digestive fluids. Manifestations of the disease include breathing difficulty and overproduction of mucus in the lungs. When CFTR is functional, these fluids are normally thin, but when the gene is non-functional, they become much thicker and are points of infection.

CFTR contains two ATP-hydrolyzing domains and two cell membrane-crossing domains with 6 α-helices each. It can be activated by phosphorylation by a cAMP-dependent protein kinase. The carboxyl end of CFTR is linked to the cytoskeleton by a PDZ domain.

Lactose permease

Another integral membrane protein performing active transport is lactose permease. It facilitates the movement of the sugar lactose across the lipid bilayer of the cell membrane (Figures 3.49- 3.51). The transport mechanism is classified as a secondary active transport since it exploits the inwardly directed H+ electrochemical gradient as an energy source. When lactose is transported into cells, it is broken down into its substituent monosaccharide sugars - glucose and galactose - for energy creation.

The enzyme catalyzing this reaction is known as lactase and deficiency of it in humans leads to lactose intolerance (see HERE).

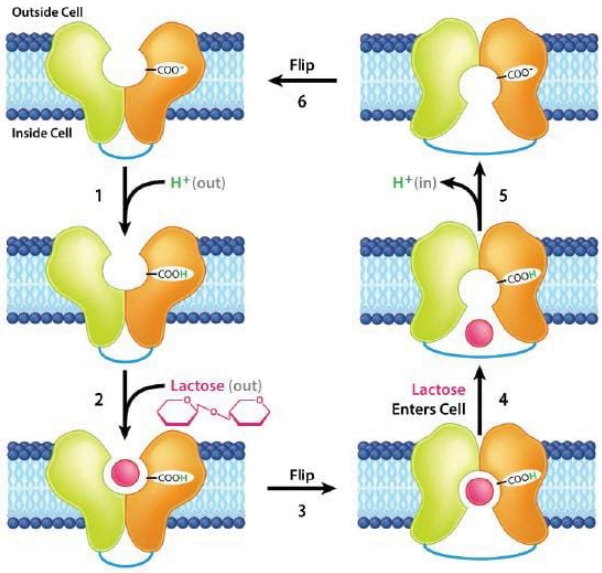


Figure 3.49 - Mechanism of action of lactose permease - Note that the proton acts by altering the charge of a carboxyl group - Image by Aleia Kim

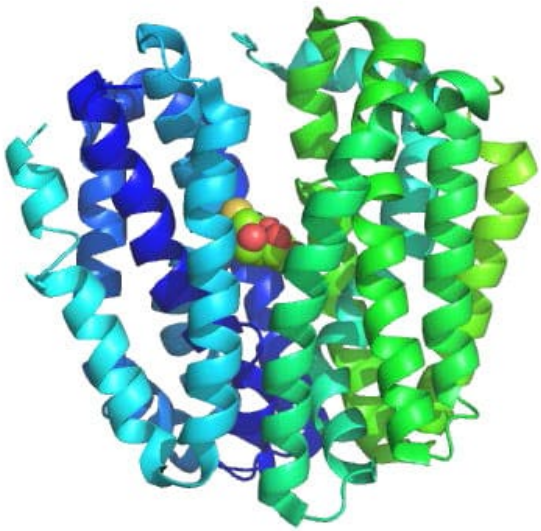


Figure 3.50 - Structure of lactose permease - Wikipedia

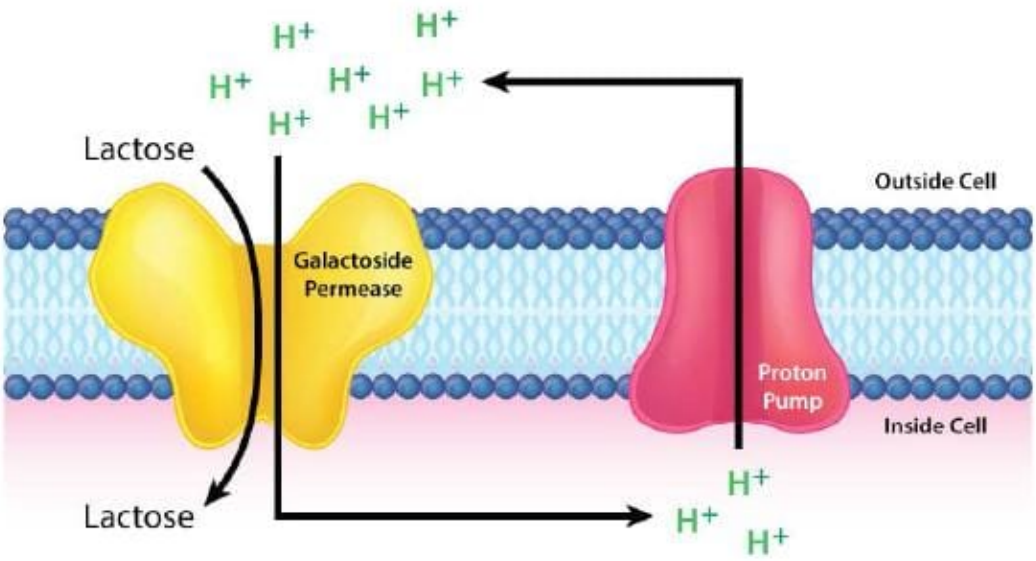


Figure 3.51 - Lactose (galactoside) permease is a secondary transporter, using a proton gradient as an energy source to pump lactose into the cell - Image by Aleia Kim

GLUTs

GLUTs (GLUcose Transport proteins) are uniport, type III integral membrane proteins that participate in the transport of glucose across membranes into cells. GLUTs are found in all phyla and are abundant in humans, with 12 GLUT genes. GLUT1, in erythrocytes is well-studied. Through GLUT 1, glucose enters and passes through it via facilitated diffusion at a rate that is 50,000 higher than in its absence. GLUTs of various types are found in different cells of the body. The one in red blood cells is known as GLUT 1 and has 12 membrane-spanning hydrophobic helices.

Though the structure of GLUT 1 is not known, it is speculated that the 12 helices form a chamber able to form hydrophilic bonds with glucose to facilitate its passage.

GLUT 1 levels in erythrocytes go up as glucose levels decrease and decrease when glucose levels go down. GLUT 1 can also transport ascorbate (vitamin C) in addition to glucose in mammals (such as humans) that do not produce their own vitamin C.

Glut 4

GLUT 4 is regulated by insulin and is found primarily in adipose and striated muscle tissue. Insulin alters intracellular trafficking pathways in response to increases in blood sugar to favor movement of various GLUT proteins (including GLUT 4) from intracellular vesicles to the cell membrane, thus stimulating uptake of the glucose. GLUT 4 is also found in the hippocampus where, if trafficking is disrupted, the result can be depressive behavior and cognitive dysfunction.

For all of the GLUT proteins, a key to keeping the glucose in the cell is phosphorylation of it by the glycolysis enzyme, hexokinase, in the cytoplasm. Phosphorylated molecules cannot enter GLUTs and don't have an easy means of exiting the cell.

Glucose Transporters

Name	Tissue	K_M (μM)	Comments
GLUT1	All mammalian tissues	1	Basal glucose uptake
GLUT2	Liver and pancreatic β cells	15-20	Participates in insulin regulation in the pancreas; removes excess glucose from the blood in the liver
GLUT3	All mammalian tissues	1	Basal glucose uptake
GLUT4	Muscle and fat cells	5	Concentration in plasma membrane of muscles rises with endurance training
GLUT5	Small intestine	---	Fructose transport

Figure 3.52 - GLUTs found in cells - Image by Aleia Kim

This page titled 9.3: Transport in Membranes is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 3.2: Transport in Membranes by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

9.4: Metabolism of Fat

Breakdown of fat in adipocytes requires catalytic action of three enzymes, hormone sensitive triacylglycerol lipase (called LIPE) to remove the first fatty acid from the fat, diglyceride lipase to remove the second one and monoglyceride lipase to remove the third. Of these, only LIPE is regulated and it appears to be the rate limiting reaction. Synthesis of fat starting with glycerol-3-phosphate requires action of acyl transferase enzymes, such as glycerol-3-phosphate acyl transferase, which catalyze addition of fatty acids to the glycerol backbone.

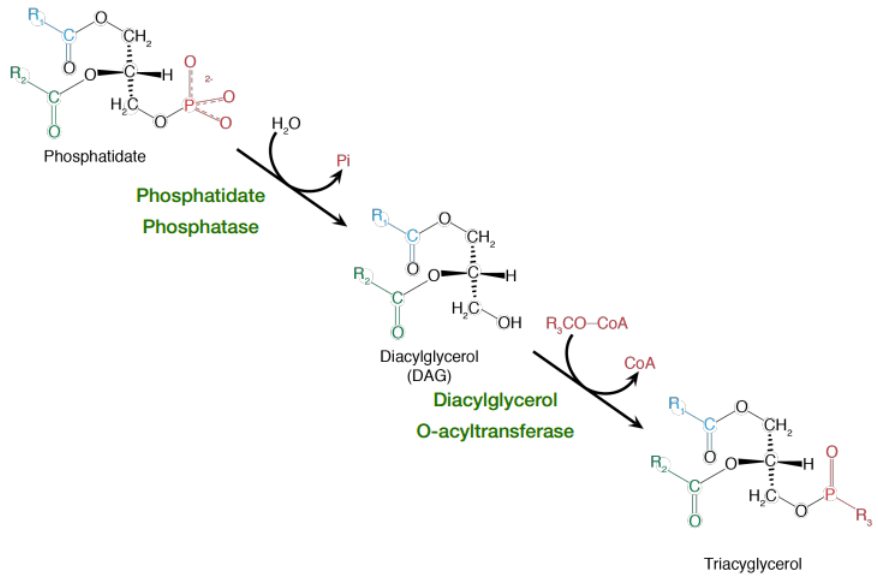


Figure 6.13.1: *Synthesis of Fat*

Interestingly, there appear to be few controls of the metabolism of fatty acids. The primary control of their oxidation is availability. One way to control that is by control of the breakdown of fat. This process, which can be stimulated by the epinephrine kinase cascade, is controlled through LIPE, found in adipocytes (fat-containing cells). Breakdown of fat in apidocytes requires action of three enzymes, each hydrolyzing one fatty acid from the glycerol backbone. As noted earlier, only HSTL, which catalyzes the first hydrolysis, is regulated.

Synthesis of fat requires glycerol-3-phosphate (or DHAP) and three fatty acids. In the first reaction, glycerol-3-phosphate is esterified at position 1 with a fatty acid, followed by a duplicate reaction at position 2 to make phosphatidic acid. This molecule, which is an intermediate in the synthesis of both fats and phosphoglycerides, gets dephosphorylated to form diacylglycerol before the third esterification to make a fat.

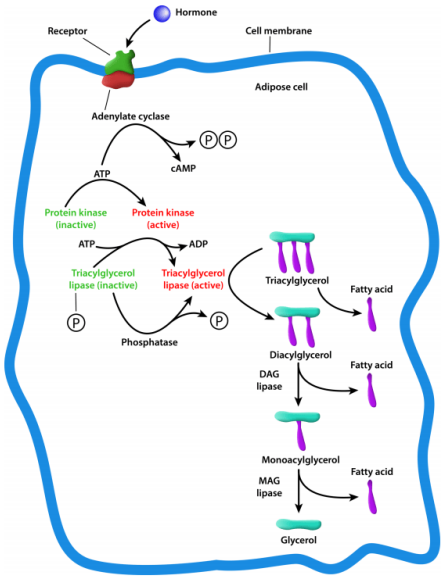


Figure 6.13.2: *Activation of Fat Hydrolysis*

Glycerophospholipid Metabolism

Phosphatidic acid, as noted above, is an important intermediate in the metabolism of glycerophospholipids. These compounds, which are important membrane constituents, can be synthesized in several ways.

Contributors and Attributions

Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 9.4: Metabolism of Fat is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 6.13: Metabolism of Fat by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

9.5: Fatty Acid Oxidation

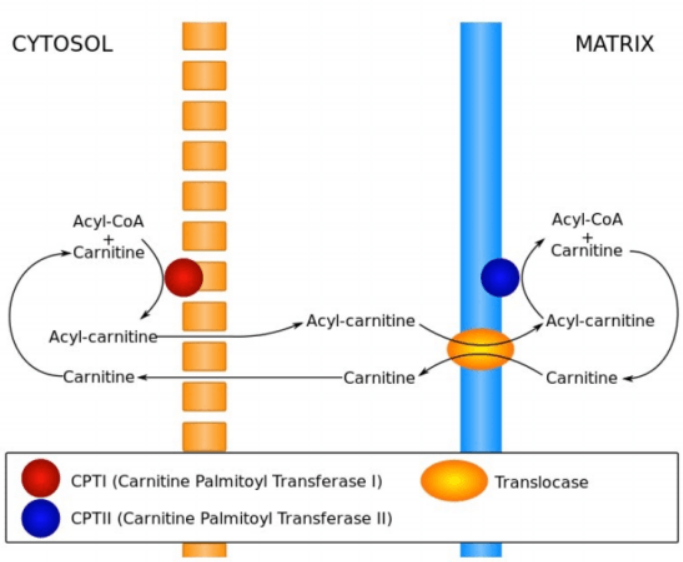


Figure 6.11.1: *Movement of Acyl-CoAs into the Mitochondrial Matrix*

The process of fatty acid oxidation, called beta oxidation, is fairly simple. The reactions all occur between carbons 2 and 3 (with #1 being the one linked to the CoA) and sequentially include the following:

1. dehydrogenation to create $\mathrm{FADH_2}$ and a fatty acyl group with a double bond in the trans configuration;
2. hydration across the double bond to put a hydroxyl group on carbon 3 in the L configuration;
3. oxidation of the hydroxyl group to make a ketone; and
4. thiolytic cleavage to release acetyl-CoA and a fatty acid two carbons shorter than the starting one.

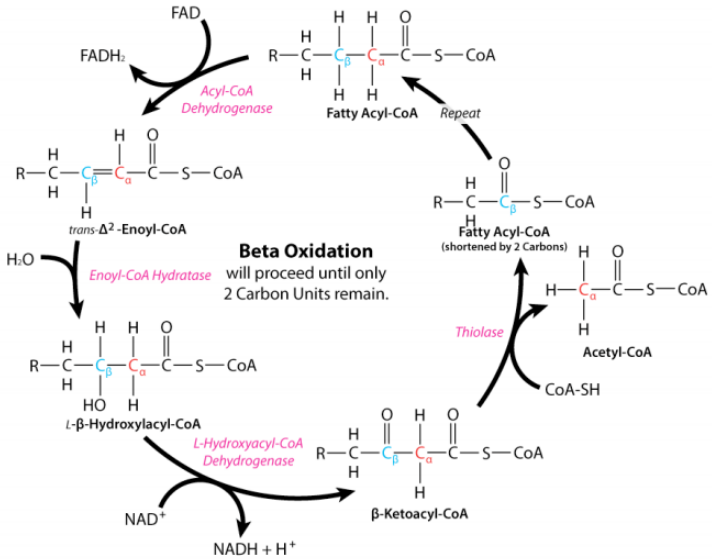


Figure 6.11.2: *Beta Oxidation of Fatty Acids*

Reactions two and three in beta oxidation are catalyzed by enoyl-CoA hydratase and 3-hydroxyacyl-CoA dehydrogenase, respectively. The latter reaction yields an NADH. The final enzyme of beta oxidation is thiolase and this enzyme is notable in not only catalyzing the formation of acetyl-CoAs in beta oxidation, but also catalyzing the joining of two acetyl-CoAs (essentially the reversal of the last step of beta oxidation) to form acetoacetyl-CoA– essential for the pathways of ketone body synthesis and

cholesterol biosynthesis.

Oxidation of Odd-Chain Fatty Acids

Though most fatty acids of biological origin have even numbers of carbons, not all of them do. Oxidation of fatty acids with odd numbers of carbons ultimately produces an intermediate with three carbons called propionyl-CoA, which cannot be oxidized further in the beta-oxidation pathway. Metabolism of this intermediate is odd. Sequentially, the following steps occur:

1. carboxylation to make D-methylmalonyl-CoA;
2. isomerization to L-methylmalonyl-CoA;
3. rearrangement to form succinyl-CoA. The last step of the process utilizes the enzyme methylmalonyl-CoA mutase, which uses the B_12 coenzyme in its catalytic cycle. Succinyl-CoA can then be metabolized in the citric acid cycle.

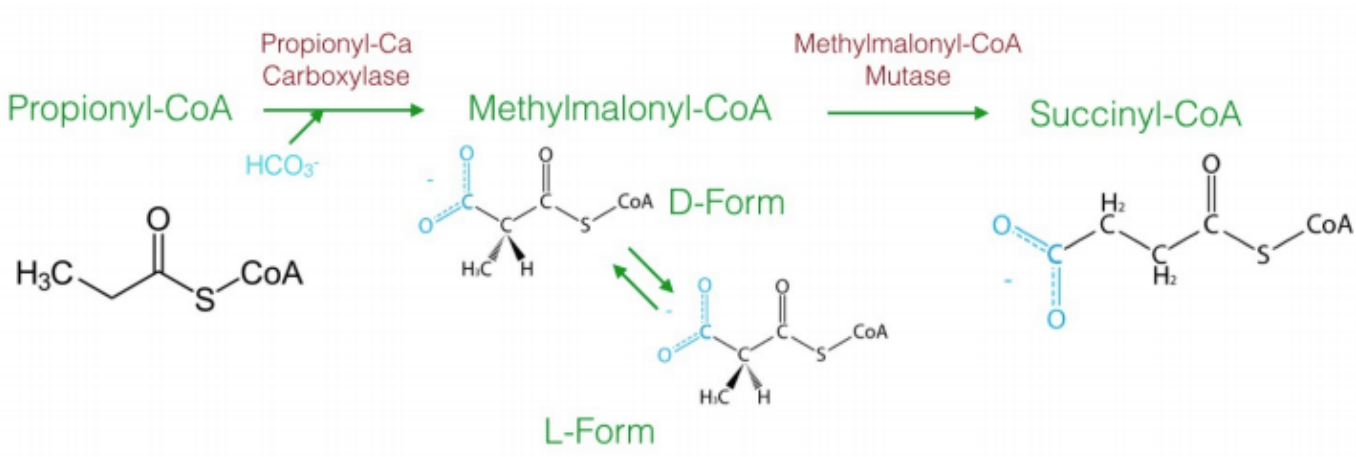


Figure 6.11.4: *Unsaturated Fatty Acid Oxidation*

On the other hand, if beta oxidation produces an intermediate with a cis double bond between carbons four and five, the first step of beta oxidation (dehydrogenation between carbons two and three) occurs to produce an intermediate with a trans double bond between carbons two and three and a cis double bond between carbons four and five. The enzyme 2,4 dienoyl CoA reductase reduces this intermediate (using NADPH) to one with a single cis bond between carbons three and four. This intermediate is then identical to the one acted on by cis-Δ3-Enoyl-CoA Isomerase above, which converts it into a regular beta oxidation intermediate, as noted above.

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 9.5: Fatty Acid Oxidation is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 6.11: Fatty Acid Oxidation by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

9.6: Ketone body production and acidosis

Source: BiochemFFA_6_2.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

Ketone
body metabolism

Ketone bodies are molecules made when the blood levels of glucose fall very low. Ketone bodies can be converted to acetyl-CoA by reversing the reaction of the pathway that makes them (Figure 6.78). Acetyl CoA, of course, can be used for ATP synthesis via the citric acid cycle. People who are very hypoglycemic (including some diabetics) will produce ketone bodies (Figure 6.79) and these are often first detected by the smell of acetone on their breath.

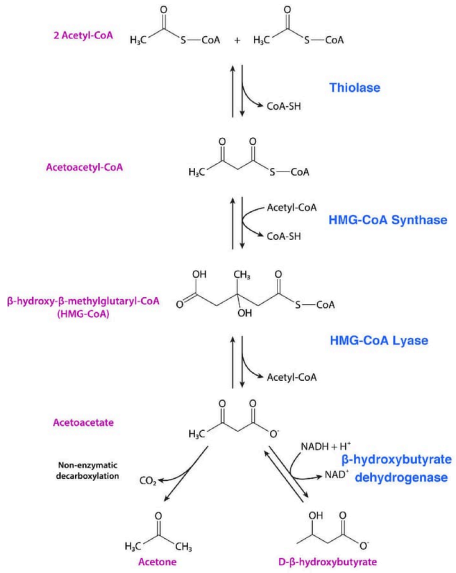


Figure 6.78 Ketone body metabolism. Image by Pehr Jacobson

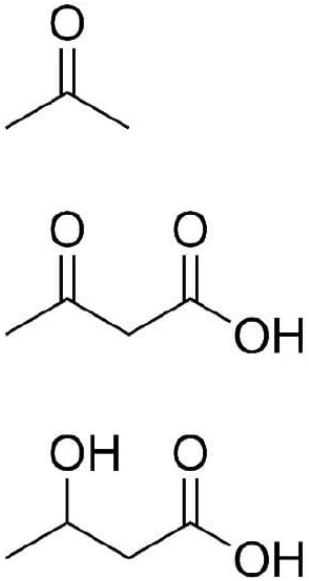


Figure 6.79 - Three ketone bodies - acetone (top), acetoacetic acid (middle), and β-hydroxybutyrate (bottom)

Overlapping pathways

The pathways for ketone body synthesis and cholesterol biosynthesis (Figure 6.80 and see HERE) overlap at the beginning. Each of these starts by combining two acetyl-CoAs together to make acetoacetyl-CoA. Not coincidentally, that is the next to last product of β-oxidation of fatty acids with even numbers of carbons (see HERE for fatty acid oxidation). In fact, the enzyme that catalyzes the joining is the same as the one that catalyzes its breakage in fatty acid oxidation – thiolase. Thus, these pathways start by reversing the last step of the last round of fatty acid oxidation.

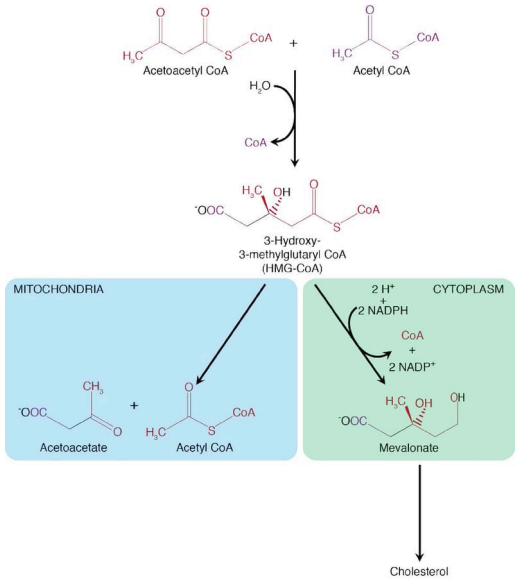


Figure 6.80 - Diverging biosynthetic pathways for ketone bodies (left) and cholesterol biosynthesis (right). Image by Penelope Irving

HMG-CoA formation

Both pathways also include addition of two more carbons to acetoacetyl-CoA from a third acetyl-CoA to form hydroxy-methyl-glutaryl-CoA, or HMG-CoA, as it is more commonly known. It is at this point that the two pathways diverge. HMG-CoA is a branch point between the two pathway and can either go on to become cholesterol or ketone bodies. In the latter pathway, HMG-CoA is broken down into acetyl-CoA and acetoacetate.

Acetoacetate is itself a ketone body and can be reduced to form another one, D-β-hydroxybutyrate (not actually a ketone, though). Alternatively, acetoacetate can be converted into acetone. This latter reaction can occur either spontaneously or via catalysis by acetoacetate decarboxylase. Acetone can be converted into pyruvate and pyruvate can be made into glucose.

D-β-hydroxybutyrate travels readily in the blood and crosses the blood-brain barrier. It can be oxidized back to acetoacetate, converted to acetoacetyl-CoA, and then broken down to two molecules of acetyl-CoA for oxidation in the citric acid cycle.

Ketosis

When a body is producing ketone bodies for its energy, this state in the body is known as ketosis. Formation of ketone bodies in the liver is critical. Normally glucose is the body's primary energy source. It comes from the diet, from the breakdown of storage carbohydrates, such as glycogen, or from glucose synthesis (gluconeogenesis). Since the primary stores of glycogen are in muscles and liver and since gluconeogenesis occurs only in liver, kidney, and gametes, when the supply of glucose is interrupted for any reason, the liver must supply an alternate energy source.

From fatty acid breakdown

In contrast to glucose, ketone bodies can be made in animals from the breakdown of fat/fatty acids. Most cells of the body can use ketone bodies as energy sources. Ketosis may arise from fasting, a very low carbohydrate diet or, in some cases, diabetes.

Acidosis

The term acidosis refers to conditions in the body where the pH of arterial blood drops below 7.35. It is the opposite of the condition of alkalosis, where the pH of the arterial blood rises above 7.45. Normally, the pH of the blood stays in this narrow pH range. pH values of the blood lower than 6.8 or higher than 7.8 can cause irreversible damage and may be fatal. Acidosis may have roots in metabolism (metabolic acidosis) or in respiration (respiratory acidosis).

There are several causes of acidosis. In metabolic acidosis, production of excess lactic acid or failure of the kidneys to excrete acid can cause blood pH to drop. Lactic acid is produced in the body when oxygen is limiting, so anything that interferes with oxygen delivery may create conditions favoring production of excess lactic acid. These may include restrictions in the movement of blood to target tissues, resulting in hypoxia (low oxygen conditions) or decreases in blood volume. Issues with blood movement can result from heart problems, low blood pressure, or hemorrhaging.

Strenuous exercise can also result in production of lactic acid due to the inability of the blood supply to deliver oxygen as fast as tissues require it (hypovolemic shock). At the end of the exercise, though, the oxygen supply via the blood system quickly catches up.

Respiratory acidosis arises from accumulation of carbon dioxide in the blood. Causes include hypoventilation, pulmonary problems, emphysema, asthma, and severe pneumonia.

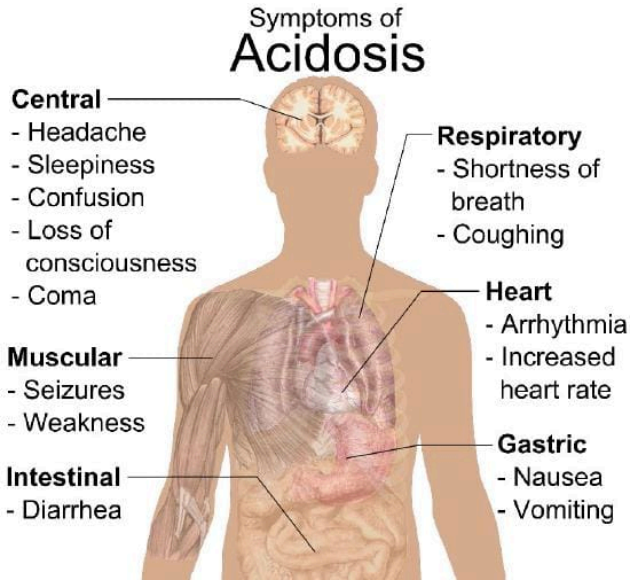


Figure 6.81 - Symptoms of acidosis

This page titled 9.6: Ketone body production and acidosis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 6.2: Citric Acid Cycle & Related Pathways by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

10: NITROGEN METABOLISM AND THE UREA CYCLE

10.1: Amino Acid Metabolism
10.2: Amino Acid Catabolism
10.3: Urea Cycle

This page titled 10: Nitrogen metabolism and the urea cycle is shared under a license and was authored, remixed, and/or curated by LibreTexts.

10.1: Amino Acid Metabolism

The pathways for the synthesis and degradation of amino acids used in proteins are the most varied among the reactions synthesizing biological building blocks. We start with some terms. First, not all organisms can synthesize all the amino acids they need. Amino acids that an organism cannot synthesize (and therefore must have in their diets) are called *essential amino acids*. The remaining amino acids that the body can synthesize are called non-essential.

Glucogenic and Ketogenic Amino Acids

Glucogenic	Both Glucogenic and Ketogenic	Ketogenic
Aspartate	Isoleucine	Leucine
Asparagine	Phenylalanine	Lysine
Alanine	Tryptophan	
Glycine	Tyrosine	
Serine		
Threonine		
Cysteine		
Glutamate		
Glutamine		
Arginine		
Proline		
Histidine		
Valine		
Methionine		

Figure 7.7.1: *Glucogenic and Ketogenic Amino Acids*

Amino acids are also divided according to the pathways involved in their degradation; there are three general categories. Ones that yield intermediates in the glycolysis pathway are called *glucogenic* and those that yield intermediates of acetyl-CoA or acetoacetate are called *ketogenic*. Those that involve both are called glucogenic and ketogenic.

An important general consideration in amino acid metabolism is that of transamination. In this process, an exchange of amine and oxygen between an amino acid and an alpha-ketoacid occurs (see below)

$$\text{Alpha-ketoacid} + \text{amino acid} \leftrightarrow \text{amino acid} + \text{alpha-ketoacid} \tag{10.1.1}$$

An example reaction follows

$$\text{Pyruvate} + \text{Aspartic acid} \leftrightarrow \text{Alanine} + \text{Oxaloacetate} \tag{10.1.2}$$

This reaction is catalyzed by an enzyme known as a transaminase. Amino acids, such as glutamate, can also gain nitrogen directly from ammonium ion, as shown below

$$\text{Alpha-ketoglutarate} + \text{NH}_4^+ \leftrightarrow \text{Glutamate} \tag{10.1.3}$$

This reaction can occur, for example, in nitrifying bacteria, and in places where ammonia waste is produced. Many amino acids can be synthesized from citric acid cycle intermediates. For example, synthesis of the non-essential amino acids occurs as follows: aspartic acid can be made by transamination of oxaloacetate. Glutamate comes from transamination of alpha-ketoglutarate. Pyruvate, as noted, is a precursor of alanine (via transamination). Amino acids that can be made from glutamate include glutamine (by addition of an additional ammonium ion), proline, and arginine, Asparagine is made from aspartate by addition of ammonium ion also. Serine is formed from 3-phosphoglycerate and is itself the precursor of both glycine and cysteine. Cysteine and serine are also made from methionine. Tyrosine is made by hydroxylation of phenylalanine.

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 10.1: Amino Acid Metabolism is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 7.7: Amino Acid Metabolism by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

10.2: Amino Acid Catabolism

Breakdown of glutamine by glutaminase is a source of ammonium ion in the cell. The other product is glutamate. Glutamate, of course, can be converted by a transamination reaction to alpha-ketoglutarate, which can be oxidized in the citric acid cycle.

- Asparagine can similarly be broken to ammonium and aspartate by asparaginase and aspartate can be converted by transamination to oxaloacetate for oxidation in the citric acid cycle.
- Alanine is converted to pyruvate in a transamination reaction, making it glucogenic.
- Arginine is hydrolzyed in the urea cycle to yield urea and ornithine.
- Proline is catabolized to glutamate in a reversal of its synthesis pathway.
- Serine donates a carbon to form a folate and the other product of the reaction is glycine, which is itself oxidized to carbon dioxide and ammonia. Glycine can also be converted back to serine, which can also be converted back to 3-phosphoglycerate or pyruvate.
- Threonine can be broken down in three pathways, though only two are relevant for humans. One pathway leads to acetyl-CoA and glycine. The other leads to pyruvate.
- Cysteine can be broken down in several ways. The simplest occurs in the liver, where a desulfurase can act on it to yield hydrogen sulfide and pyruvate.
- Methionine can be converted to cysteine for further metabolism. It can be converted to succinyl-CoA for oxidation in the citric acid cycle. It can also be converted to S-Adenosyl-Methionine (SAM), a carbon donor.
- Isoleucine and valine can also be converted to succinyl-CoA after conversion first to propionyl-CoA. Since conversion of propionyl-CoA to succinyl-CoA requires vitamin B_{12}, catabolism of these amino acids also requires the vitamin.
- Phenylalanine is converted during catabolism to tyrosine, which is degraded ultimately to fumarate and acetoacetate. Thus, both of these amino acids are glucogenic and ketogenic. Tyrosine can also be converted to dopamine, norepinephrine, and epinephrine.
- Leucine and lysine can be catabolized to acetoacetate and acetyl-CoA. Lysine is also an important precursor of carnitine.
- Histidine can be catabolized by bacteria in intestines to histamine, which causes construction or dilation of various blood vessels when in excess.
- Tryptophan's catabolism is complex, but can proceed through alanine, acetoacetate and acetyl-CoA

In summary, the following are metabolized to pyruvate – alanine, cysteine, glycine, serine, and threonine

- Oxaloacetate is produced from aspartate and asparagine
- Succinyl-CoA is produced from isoleucine, valine, and methionine
- Alpha-ketoglutarate is produced from arginine, glutamate, glutamine, histidine and proline.
- Phenylalanine and tyrosine are broken down to fumarate and acetoacetate
- Leucine and lysine yield acetoacetate and acetyl-CoA.
- Tryptophan leads to alanine, acetoacetate and acetyl-CoA.

Last, amino acids, besides being incorporated into proteins, serve as precursors of important compounds, including serotonin (from tryptophan), porphyrin heme (from glycine), nitric oxide (from arginine), and nucleotides (from aspartate, glycine, and glutamine).

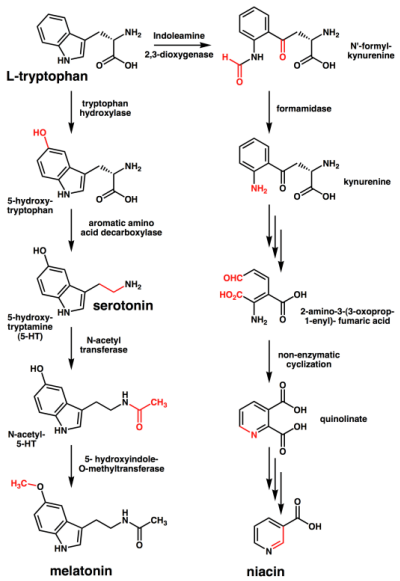


Figure 7.8.1: *Conversion of L-Tryptophan into Serotonin, Melatonin, and Niacin*

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 10.2: Amino Acid Catabolism is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 7.8: Amino Acid Catabolism by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

10.3: Urea Cycle

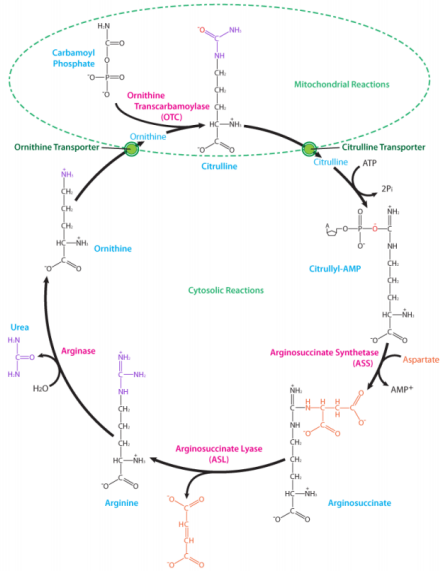


Figure 7.5.1: *The Urea Cycle*

The cycle scavenges free ammonia (as ammonium ion) which is toxic if it accumulates. The capture reaction also requires ATP, and bicarbonate, and the product is carbamoyl phosphate. This molecule is combined with the non-protein amino acid known as ornithine to make another non-protein amino acid known as citrulline. Addition of aspartate to citrulline creates argninosuccinate, which splits off a fumarate, creating arginine (a source of arginine). If arginine is not needed, it can be hydrolyzed to yield urea (excreted) an
ornithine, thus completing the cycle.

The first two reactions described here occur in the mitochondrion and the remaining ones occur in the cytoplasm. Molecules of the urea cycle intersecting other pathways include fumarate (citric acid cycle), aspartate (amino acid metabolism), arginine (amino acid metabolism), and ammonia (amino acid metabolism).

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 10.3: Urea Cycle is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 7.5: Urea Cycle by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

11: NUCLEOTIDE AND NUCLEIC ACID STRUCTURE AND METABOLISM

11.1: Structure and Function - Nucleic Acids
11.2: Pyrimidine de novo Biosynthesis
11.3: Deoxyribonucleotide de novo Biosynthesis
11.4: Epigenetic Control- Regulating Access to Genes within the Chromosome

This page titled 11: Nucleotide and nucleic acid structure and metabolism is shared under a license and was authored, remixed, and/or curated by LibreTexts.

11.1: Structure and Function - Nucleic Acids

Source: BiochemFFA_2_5.pdf. The entire textbook is available for free from the authors at http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy

The nucleic acids, DNA and RNA, may be thought of as the information molecules of the cell. In this section, we will examine the structures of DNA and RNA, and how these structures are related to the functions these molecules perform.



We will begin with DNA, which is the hereditary information in every cell, that is copied and passed on from generation to generation. The race to elucidate the structure of DNA was one of the greatest stories of 20th century science. Discovered in 1869 by Friedrich Miescher, DNA was identified as the genetic material in experiments in the 1940s led by Oswald Avery, Colin MacLeod, and Maclyn McCarty. X-ray diffraction work of Rosalind Franklin and the observations of Erwin Chargaff were combined by James Watson and Francis Crick to form a model of DNA that we are familiar with today. Their famous paper, in the April 25, 1953 issue of Nature, opened the modern era of molecular biology. Arguably, that one-page paper has had more scientific impact per word than any other research article ever published. Today, every high school biology student is familiar with the double helical structure of DNA and knows that G pairs with C and A with T.

The double helix, made up of a pair of DNA strands, has at its core, bases joined by hydrogen bonds to form base pairs - adenine always paired with thymine, and guanine invariably paired with cytosine. Two hydrogen bonds are formed between adenine and thymine, but three hydrogen bonds hold together guanine and cytosine (Figure 2.127).

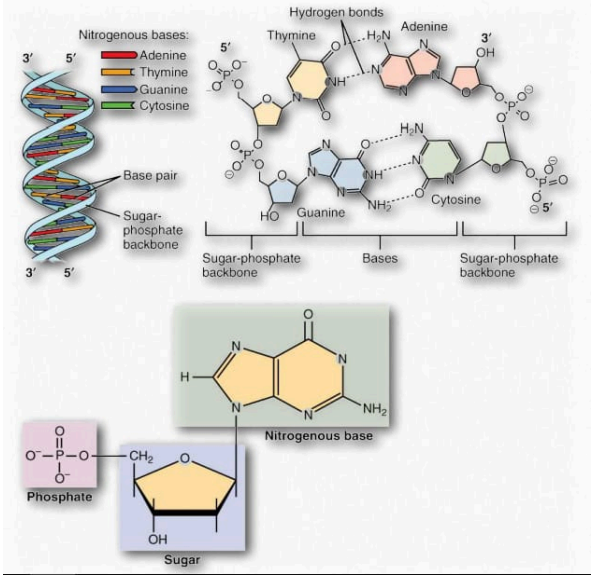


Figure 2.127 - A DNA duplex with base pairs, a closeup of base pairing, and a closeup of a nucleotide Wikipedia

The complementary structure immediately suggested to Watson and Crick how DNA might be (and in fact, is) replicated and it further explains how information is DNA is transmitted to RNA for the synthesis of proteins. In addition to the hydrogen bonds between bases of each strand, the double helix is held together by hydrophobic interactions of the stacked, non-polar bases. Crucially, the sequence of the bases in DNA carry the information for making proteins. Read in groups of three, the sequence of the bases directly specifies the sequence of the amino acids in the encoded protein.

Structure

A DNA strand is a polymer of nucleoside monophosphates held together by phosphodiester bonds. Two such paired strands make up the DNA molecule, which is then twisted into a helix. In the most common Bform, the DNA helix has a repeat of 10.5 base pairs per turn, with sugars and phosphate forming the covalent phosphodiester “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.

Building blocks

The term nucleotide refers to the building blocks of both DNA (deoxyribonucleoside triphosphates, dNTPs) and RNA (ribonucleoside triphosphates, NTPs). In order to discuss this important group of molecules, it is necessary to define some terms.

Nucleotides contain three primary structural components. These are a nitrogenous base, a pentose sugar, and at least one phosphate. Molecules that contain only a sugar and a nitrogenous base (no phosphate) are called nucleosides. The nitrogenous bases found in nucleic acids include adenine and guanine (called purines) and cytosine, uracil, or thymine (called pyrimidines). There are two sugars found in nucleotides - deoxyribose and ribose (Figure 2.128). By convention, the carbons on these sugars are labeled 1’ to 5’. (This is to distinguish the carbons on the sugars from those on the bases, which have their carbons simply labeled as 1, 2, 3, etc.) Deoxyribose differs from ribose at the 2’ position, with ribose having an OH group, where deoxyribose has H.

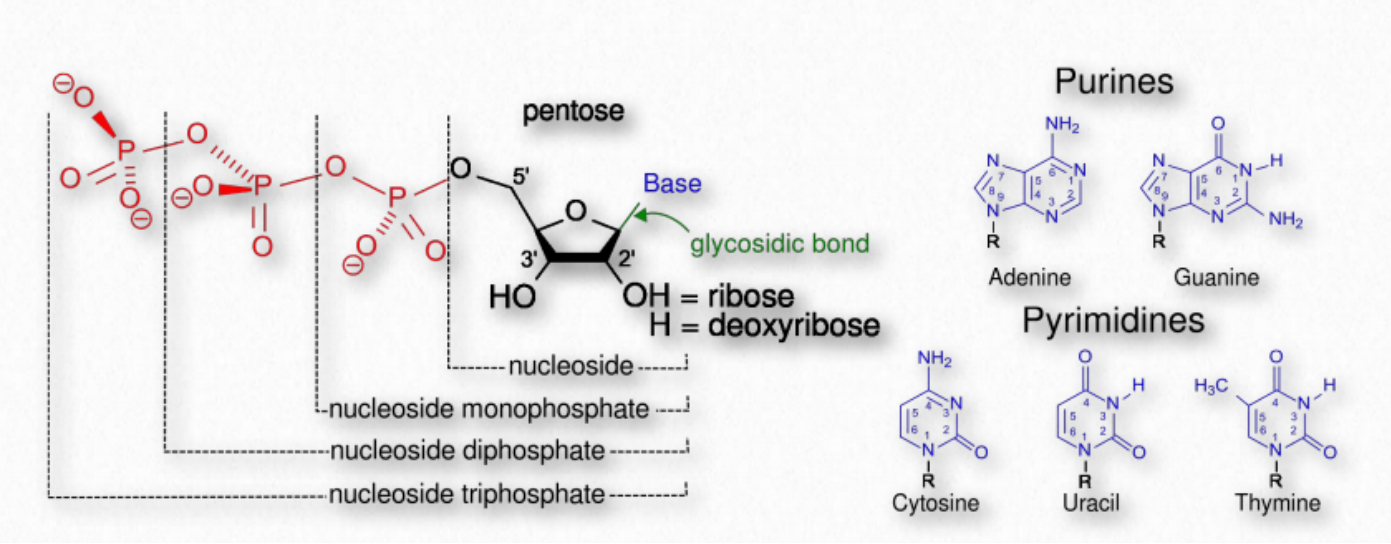


Figure 2.128 - Nucleotides, nucleosides, and bases

Nucleotides containing deoxyribose are called deoxyribonucleotides and are the forms found in DNA. Nucleotides containing ribose are called ribonucleotides and are found in RNA. Both DNA and RNA contain nucleotides with adenine, guanine, and cytosine, but with very minor exceptions, RNA contains uracil nucleotides, whereas DNA contains thymine nucleotides. When a base is attached to a sugar, the product, a nucleoside, gains a new name.

- **uracil-containing** = uridine (attached to ribose) / deoxyuridine (attached to deoxyribose)
- **thymine-containing** = ribothymidine (attached to ribose) / thymidine (attached to deoxyribose)
- **cytosine-containing** = cytidine (attached to ribose - Figure 2.129) / deoxycytidine (attached to deoxyribose)
- **guanine-containing** = guanosine (attached to ribose) / deoxyguanosine (attached to deoxyribose)
- **adenine-containing** = adenosine (attached to ribose) / deoxyadenosine (attached to deoxyribose)

Of these, deoxyuridine and ribothymidine are the least common. The addition of one or more phosphates to a nucleoside makes it a nucleotide. Nucleotides are often referred to as nucleoside phosphates, for this reason. The number of phosphates in the nucleotide is indicated by the appropriate prefixes (mono, di or tri).

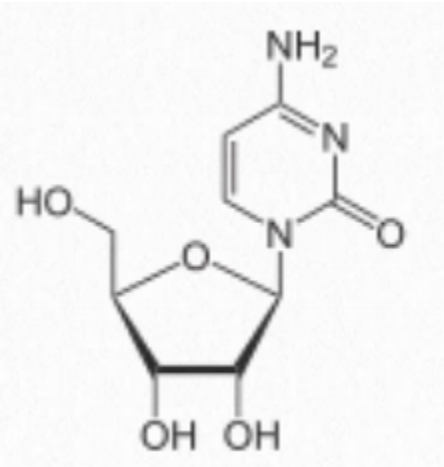


Figure 2.129 Cytidine

Thus, cytidine, for example, refers to a nucleoside (no phosphate), but cytidine monophosphate refers to a nucleotide (with one phosphate). Addition of second and third phosphates to a nucleoside monophosphate requires energy, due to the repulsion of negatively charged phosphates and this chemical energy is the basis of the high energy triphosphate nucleotides (such as ATP) that fuel cells.

Note: Ribonucleotides as Energy Sources

Though ATP is the most common and best known cellular energy source, each of the four ribonucleotides plays important roles in providing energy. GTP, for example, is the energy source for protein synthesis (translation) as well as for a handful of metabolic reactions. A bond between UDP and glucose makes UDP-glucose, the building block for making glycogen. CDP is similarly linked to several different molecular building blocks important for glycerophospholipid synthesis (such as CDP-diacylglycerol).

The bulk of ATP made in cells is not from directly coupled biochemical metabolism, but rather by the combined processes of electron transport and oxidative phosphorylation in mitochondria and/or photophosphorylation that occurs in the chloroplasts of photosynthetic organisms. Triphosphate energy in ATP is transferred to the other nucleosides/nucleotides by action of enzymes called kinases. For example, nucleoside diphosphokinase (NDPK) catalyzes the following reaction

$$\mathrm{ATP} + \mathrm{NDP} \longleftrightarrow \mathrm{ADP} + \mathrm{NTP} \tag{11.1.1}$$

where 'N' of "NDP" and "NTP corresponds to any base. Other kinases can put single phosphates onto nucleosides or onto nucleoside monophosphates using energy from ATP.

Deoxyribonucleotides

Individual deoxyribonucleotides are derived from corresponding ribonucleoside diphosphates via catalysis by the enzyme known as ribonucleotide reductase (RNR). The deoxyribonucleoside diphosphates are then converted to the corresponding triphosphates (dNTPs) by the addition of a phosphate group. Synthesis of nucleotides containing thymine is distinct from synthesis of all of the other nucleotides and will be discussed later.

Building DNA strands

Each DNA strand is built from dNTPs by the formation of a phosphodiester bond, catalyzed by DNA polymerase, between the 3'OH of one nucleotide and the 5' phosphate of the next. The result of this directional growth of the strand is that the one end of the strand has a free 5' phosphate and the other a free 3' hydroxyl group (Figure 2.130). These are designated as the 5' and 3' ends of the strand.

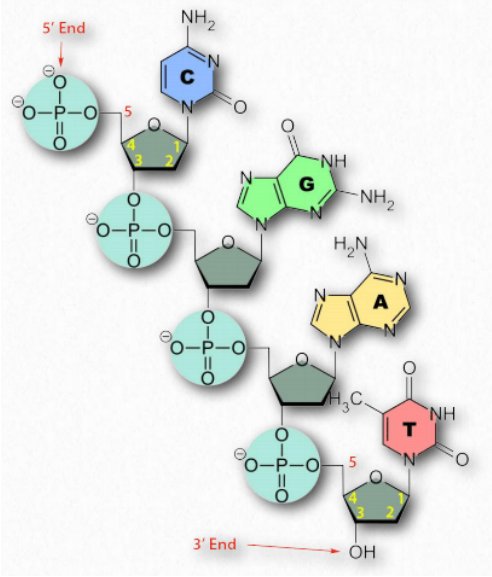


Figure 2.130 - 5'-3' Polarity of a DNA strand

Figure 2.131 shows two strands of DNA (left and right). The strand on the left, from 5' to 3' reads T-C-G-A, whereas the strand on the right, reading from 5' to 3' is T-C-G-A. The strands in a double-stranded DNA are arranged in an anti-parallel fashion with the 5' end of one strand across from the 3' end of the other.

Hydrogen bonds

Hydrogen bonds between the base pairs hold a nucleic acid duplex together, with two hydrogen bonds per A-T pair (or per A-U pair in RNA) and three hydrogen bonds per G-C pair. The B-form of DNA has a prominent major groove and a minor groove tracing the path of the helix (Figure 2.132). Proteins, such as transcription factors bind in these grooves and access the hydrogen bonds of the base pairs to “read” the sequence therein.

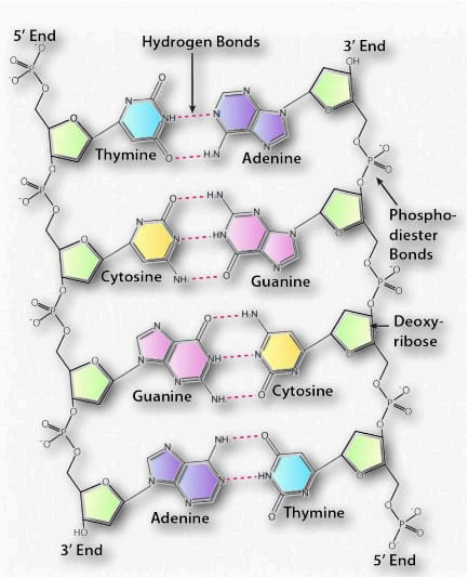


Figure 2.131 - Anti-parallel orientation of a DNA duplex, phosphodiester backbone, and base pairing Image by Aleia Kim

Other forms of DNA besides the B-form (Movie 2.5) are known (Figure 2.133). One of these, the A-form, was identified by Rosalind Franklin in the same issue of Nature as Watson and Crick’s paper. Though the A-form 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.

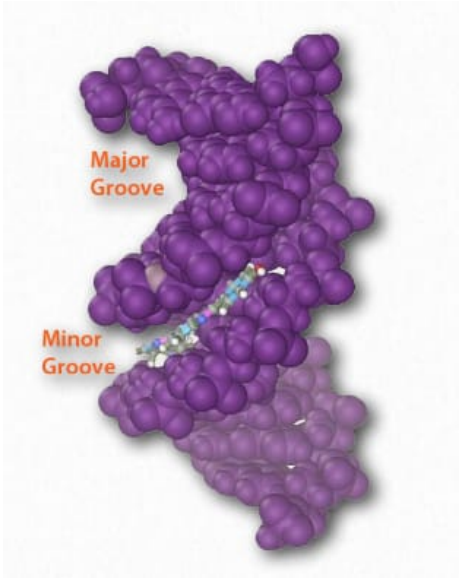
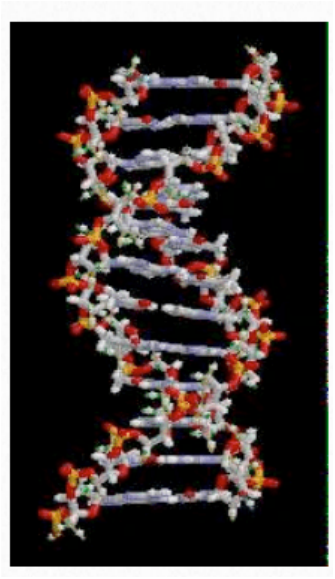


Figure 2.132 - Major and minor grooves of DNA. The minor groove has been bound by a dye Wikipedia



Movie 2.5 - B-form DNA duplex rotating in space Wikipedia

Z-DNA

The A-form and the B-form stand in contrast to another form of DNA, known as the Z-form. ZDNA, 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 (Figure 2.133). The Z-form has a sort of zig-zag shape, giving 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 topological 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 Bform DNA into other forms. ZDNA forms are favored by long stretches of alternating Gs and Cs. Superhelical tension is discussed below.

Superhelicity

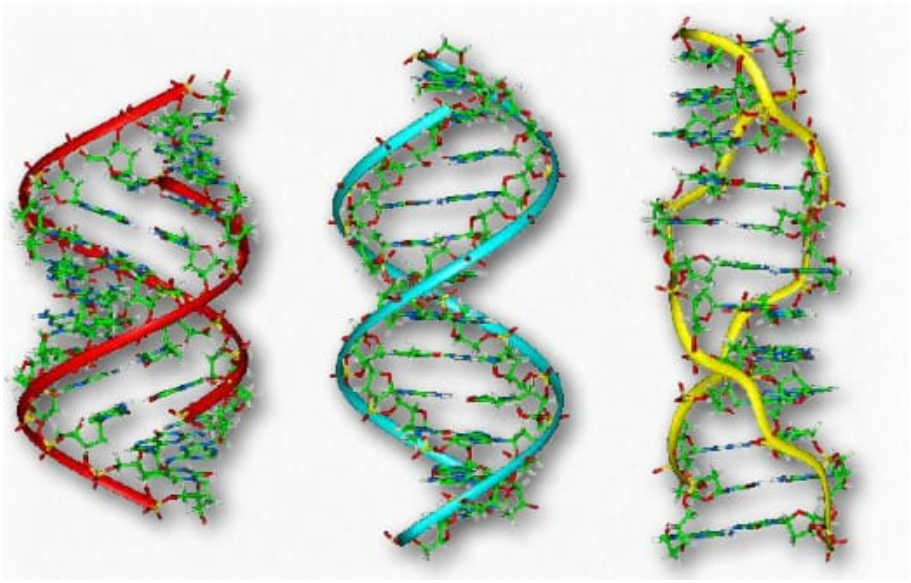


Figure 2.133 - From left to right, the A-, B-, and Zforms of DNA

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.

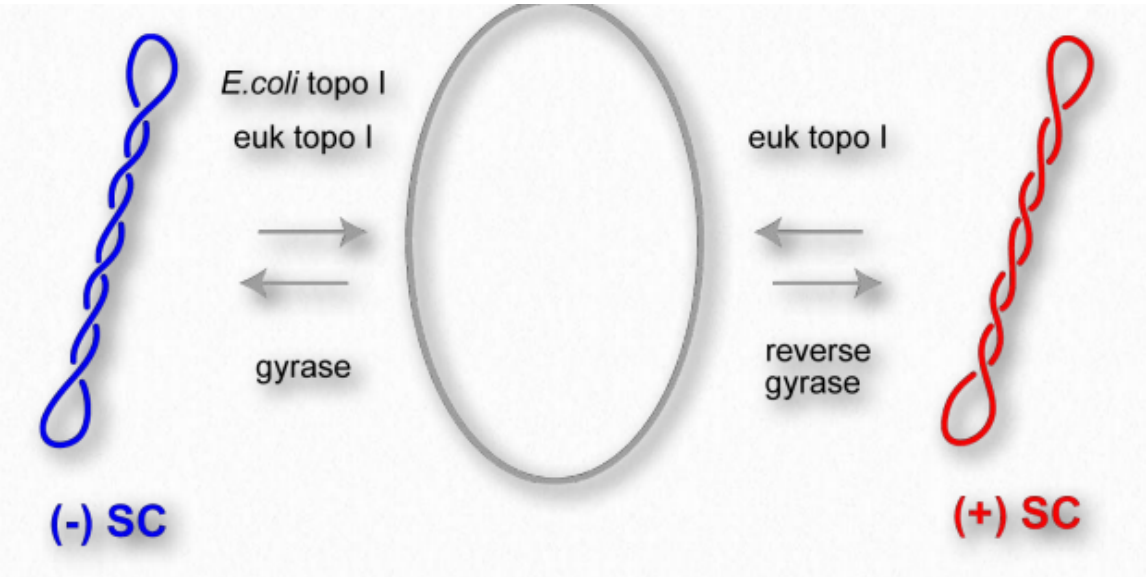


Figure 2.134 - Negatively supercoiled, relaxed circular, and positively supercoiled DNA interconverted by topoisomerase enzymes Wikipedia

Such adjustments can 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 (Figures 2.134 & 2.135). In this method, the duplex crosses over itself 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.

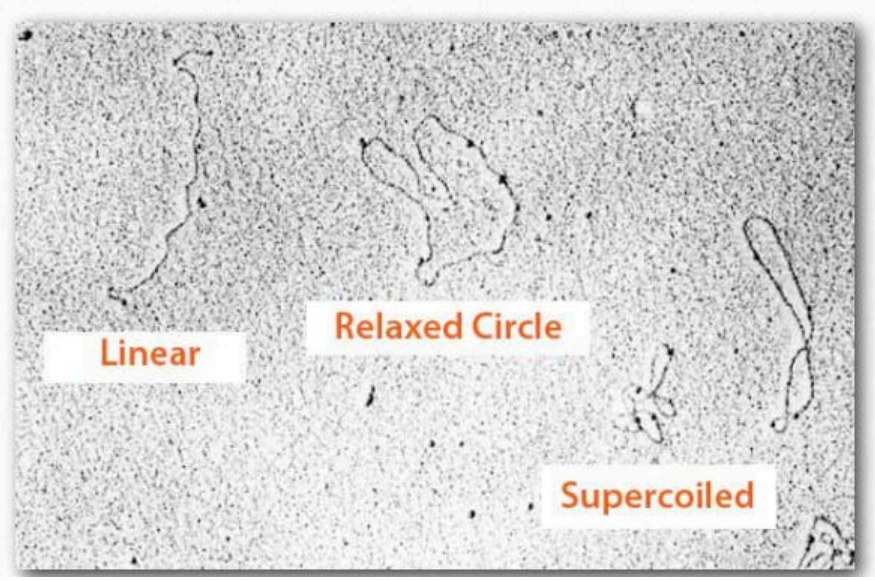


Figure 2.135 - Three topological forms of DNA Wikipedia

Topological isomers

As noted, so-called "relaxed" DNA has 10.5 base pairs per turn. Each turn corresponds to one twist of the DNA. Using enzymes, it is possible to change the number of base pairs per turn. In either the case of increasing or decreasing the twists per turn, tension is introduced into the DNA structure. If the tension cannot be relieved, the DNA duplex will act to relieve the strain, as noted. This is most easily visualized for circular DNA, though long linear DNA (such as found in eukaryotic chromosomes) or DNAs

constrained in other ways will exhibit the same behavior.

Parameters

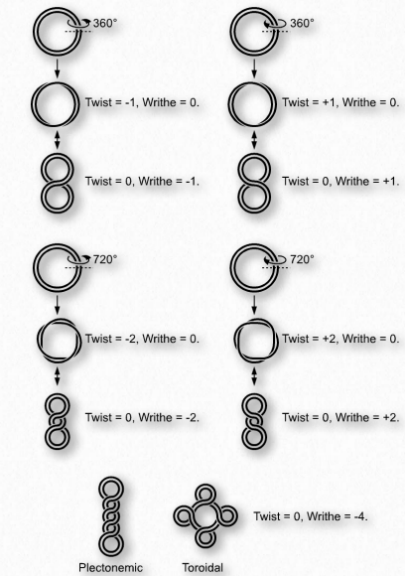


Figure 2.136 - Twists and writhes - DNA duplex coiling omitted for simplicity Wikipedia

To understand topologies, we introduce the concepts of 'writhe' and 'linking number'. First, imagine either opening a closed circle of DNA and either removing one twist or adding one twist and then re-forming the circle. Since the strands have no free ends, they cannot relieve the induced tension by re-adding or removing the twists at their ends, respectively. Instead, the tension is relieved by "superhelices" that form with crossing of the double strands over each other (figure 8 structures in Figure 2.136). Though it is not apparent to visualize, each crossing of the double strands in this way allows twists to be increased or decreased correspondingly. Thus, superhelicity allows the double helix to reassume 10.5 base pairs per turn by adding or subtracting twists as necessary and replacing them with writhes.

We write the equation L= T + W where T is the number of twists in a DNA, W is the number of writhes, and L is the linking number. The linking number is therefore the sum of the twists and writhes. Interestingly, inside of cells, DNAs typically are in a supercoiled form. Supercoiling affects the size of the DNA (compacts it) and also the expression of genes within the DNA, some having enhanced expression and some having reduced expression when supercoiling is present. Enzymes called topoisomerases alter the superhelical density of DNAs and play roles in DNA replication, transcription, and control of gene expression. They work by making cuts in one strand (Type I topoisomerases) or both strands (Type II topoisomerases) and then add or subtract twists as appropriate to the target DNA. After that process is complete, the topoisomerase re-ligates the nick/cut it had made in the DNA in the first step.

Topoisomerases may be the targets of antibiotics. The family of antibiotics known as fluoroquinolones work by interfering with the action of bacterial type II topoisomerases. Ciprofloxacin also preferentially targets bacterial type II topoisomerases. Other topoisomerase inhibitors target eukaryotic topoisomerases and are used in anti-cancer treatments.

RNA

The structure of RNA (Figure 2.137) is very similar to that of a single strand of DNA. Built of ribonucleotides, joined together by the same sort of phosphodiester bonds as in DNA, RNA uses uracil in place of thymine. In cells, RNA is assembled by RNA polymerases, which copy a DNA template in the much same way that DNA polymerases replicate a parental strand. During the synthesis of RNA, uracil is used across from an adenine in the DNA template. The building of messenger RNAs by copying a DNA template is a crucial step in the transfer of the information in DNA to a form that directs the synthesis of protein. Additionally, ribosomal and transfer RNAs serve important roles in "reading" the information in the mRNA codons and in polypeptide synthesis. RNAs are also known to play important roles in the regulation of gene expression.

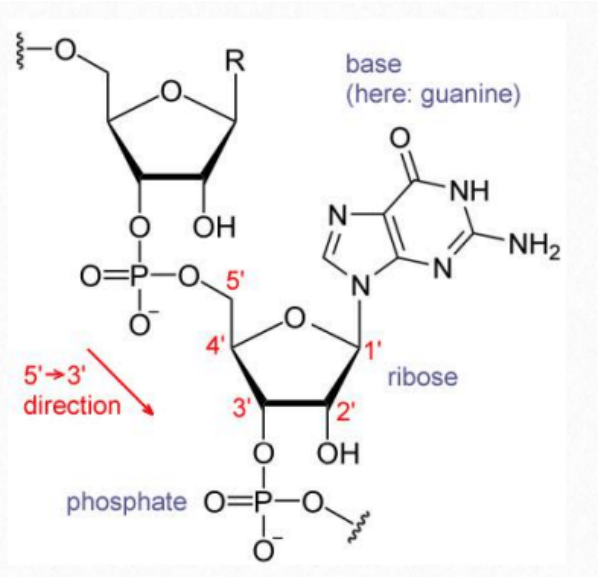


Figure 2.137 A section of an RNA molecule Wikipedia

RNA world

The discovery, in 1990, that RNAs could play a role in catalysis, a function once thought to be solely the domain of proteins, was followed by the discovery of many more so-called ribozymes- RNAs that functioned as enzymes. This suggested the answer to a long-standing chicken or egg puzzle - if DNA encodes proteins, but the replication of DNA requires proteins, how did a replicating system come into being? This problem could be solved if the first replicator was RNA, a molecule that can both encode information and carry out catalysis. This idea, called the “RNA world” hypothesis, suggests that DNA as genetic material and proteins as catalysts arose later, and eventually prevailed because of the advantages they offer. The lack of a 2’OH on deoxyribose makes DNA more stable than RNA. The double-stranded structure of DNA also provides an elegant way to easily replicate it. RNA catalysts, however, remain, as remnants of that early world. In fact, the formation of peptide bonds, essential for the synthesis of proteins, is catalyzed by RNA.

Secondary structure



Figure 2.138 - tRNA Images - 3D projection (left) and 2D projection (inset) Wikipedia

With respect to structure, RNAs are more varied than their DNA cousin. Created by copying regions of DNA, cellular RNAs are synthesized as single strands, but they often have self-complementary regions leading to “foldbacks” containing duplex regions. These are most easily visualized in the ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) (Figure 2.138), though other

RNAs, including messenger RNAs (mRNAs), small nuclear RNAs (snRNAs), microRNAs (Figure 2.139), and small interfering RNAs (siRNAs) may each have double helical regions as well.



Figure 2.139 - MicroRNA stem loops. Wikipedia

Base pairing

Base pairing in RNA is slightly different than DNA. This is due to the presence of the base uracil in RNA in place of thymine in DNA. Like thymine, uracil forms base pairs with adenine, but unlike thymine, uracil can, to a limited extent, also base pair with guanine, giving rise to many more possibilities for pairing within a single strand of RNA.

These additional base pairing possibilities mean that RNA has many ways it can fold upon itself that single-stranded DNA cannot. Folding, of course, is critical for protein function, and we now know that, like proteins, some RNAs in their folded form can catalyze reactions just like enzymes. Such RNAs are referred to as ribozymes. It is for this reason scientists think that RNA was the first genetic material, because it could not only carry information, but also catalyze reactions. Such a scheme might allow certain RNAs to make copies of themselves, which would, in turn, make more copies of themselves, providing a positive selection.

Stability

RNA is less chemically stable than DNA. The presence of the 2' hydroxyl on ribose makes RNA much more prone to hydrolysis than DNA, which has a hydrogen instead of a hydroxyl. Further, RNA has uracil instead of thymine. It turns out that cytosine is the least chemically stable base in nucleic acids. It can spontaneously deaminate and in turn is converted to a uracil. This reaction occurs in both DNA and RNA, but since DNA normally has thymine instead of uracil, the presence of uracil in DNA indicates that deamination of cytosine has occurred and that the uracil needs to be replaced with a cytosine. Such an event occurring in RNA would be essentially undetectable, since uracil is a normal component of RNA. Mutations in RNA have much fewer consequences than mutations in DNA because they are not passed between cells in division.

Catalysis

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 in ribosomes (Figure 2.114). 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 necessary for enzymes that attach amino acids to them to do so properly. Further, 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 foldback 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.

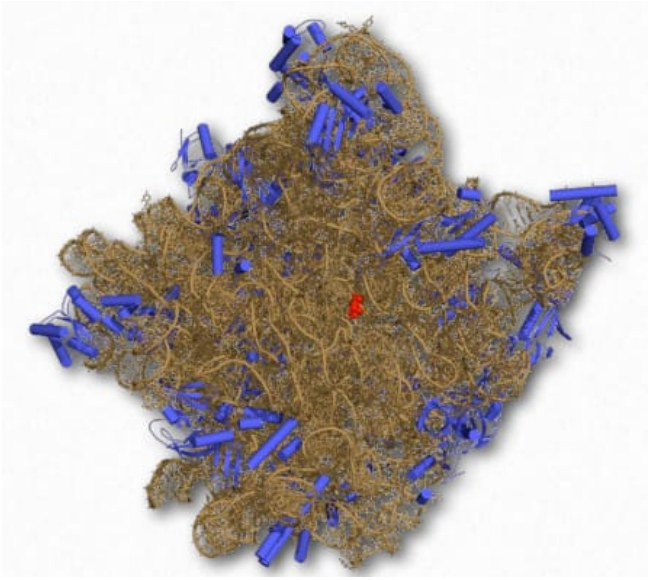


Figure 2.140 - Structure of the 50S ribosomal subunit. rRNA shown in brown. Active site in red Wikipedia

Denaturing nucleic acids

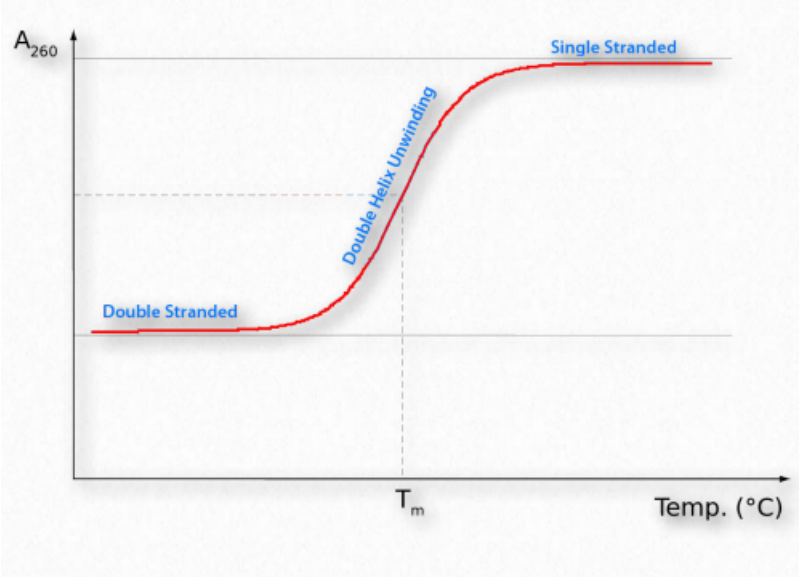


Figure 2.141 - The hyperchromic effect Wikipedia

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. This is known as the hyperchromic effect (Figure 2.141)and is a consequence of the disruption of interactions among the stacked bases. The changes in absorbance allow one to easily follow the course of DNA denaturation. Denatured duplexes can readily renature when the temperature is lowered below the “melting temperature” or Tm, the temperature at which half of the DNA strands are in duplex form. Under such conditions, the two strands can re-form hydrogen bonds between the complementary sequences, returning the duplex to its original state. For DNA, strand separation and rehybridization are important for 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 single stranded primers to “find” matching sequences on the template DNA and form a duplex. Considerations for efficient hybridization (also called annealing) include temperature, salt concentration, strand concentration, and magnesium ion levels (for more on PCR, see HERE).

DNA packaging

DNA is easily the largest macromolecule in a cell. The single chromosome in small bacterial cells, for example, can have a molecular weight of over 1 billion Daltons. If one were to take all of the DNA of human chromosomes from a single cell and lay them end to end, they would be over 7 feet long. Such an enormous molecule demands careful packaging to fit within the confines of a nucleus (eukaryotes) or a tiny cell (bacteria). The chromatin system of eukaryotes is the best known, but bacteria, too, have a system for compacting DNA.

DNA in Bacteria

In bacteria, there is no nucleus for the DNA. Instead, DNA is contained in a structure called a nucleoid (Figure 2.142). It contains about 60% DNA with much of the remainder comprised of RNAs and transcription factors. Bacteria do not have histone proteins that DNA wrap around, but they do have proteins that help organize the DNA in the cell - mostly by making looping structures.

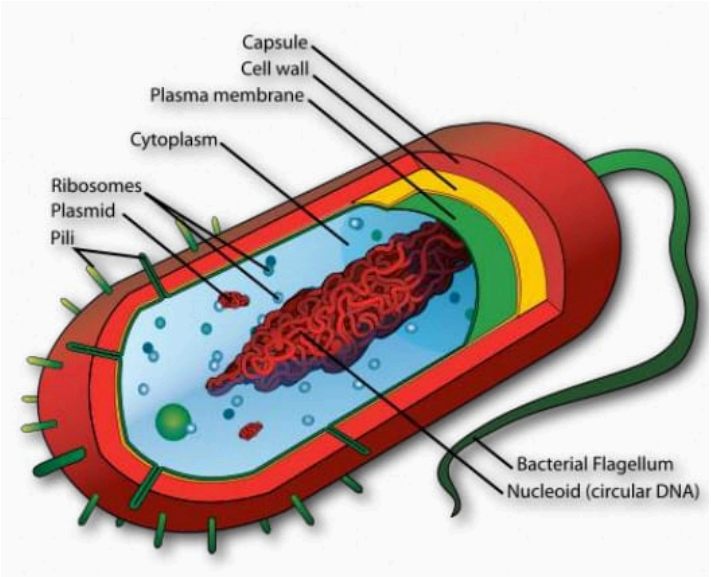


Figure 2.142 - Anatomy of a bacterial cell.

These proteins are known as Nucleoid Associated Proteins and include ones named HU, H-NS, Fis, CbpA, and Dps. Of these, HU most resembles eukaryotic histone H2B and binds to DNA non-specifically. The proteins associate with the DNA and can also cluster, which may be the origin of the loops. It is likely these proteins play a role in helping to regulate transcription and respond to DNA damage. They may also be involved in recombination.

Eukaryotes

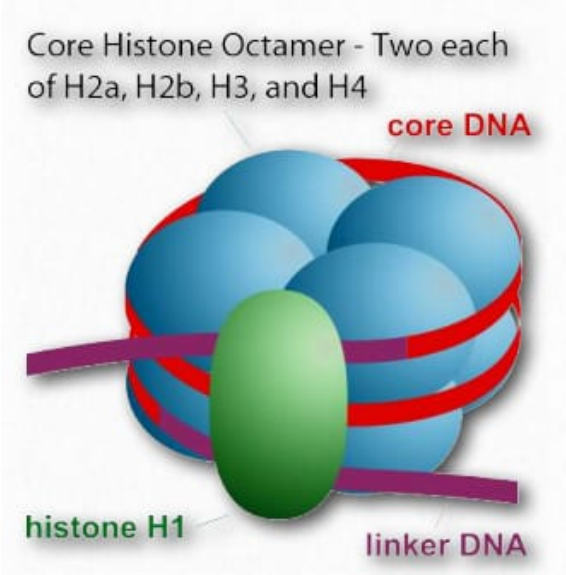


Figure 2.143 - Structure of a nucleosome Wikipedia

The method eukaryotes use for compacting DNA in the nucleus is considerably different, and with good reason - eukaryotic DNAs are typically much larger than prokaryotic DNAs, but must fit into a nucleus that is not much bigger than a prokaryotic cell. Human DNA, for example, is about 1000 times longer than c DNA. The strategy employed in eukaryotic cells is that of spooling - DNA is coiled around positively charged proteins called histones. These proteins, whose sequence is very similar in cells as diverse as yeast and humans, come in four types, dubbed H1, H2a, H2b, H3, and H4. A sixth type, referred to as H5 is actually an isoform of H1 and is rare. Two each of H2a, H2b, H3, and H4 are found in the core structure of what is called the fundamental unit of chromatin - the nucleosome (Figure 2.143).

Octamer

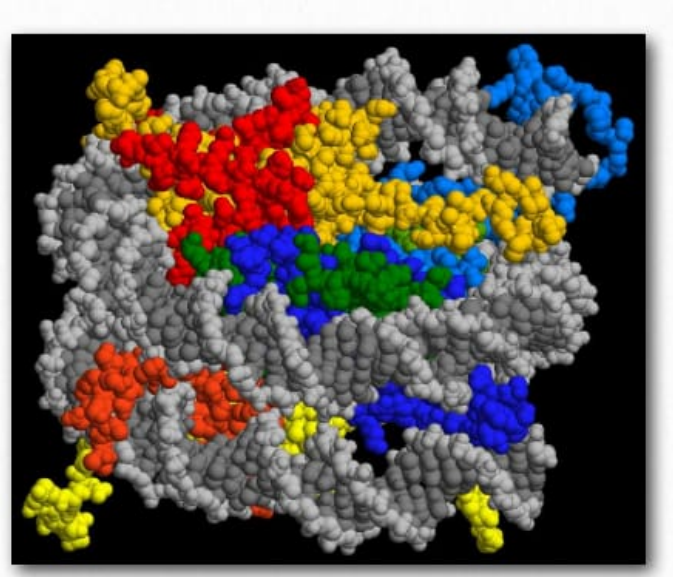


Figure 2.144 - Detailed core particle structure - DNA in gray, histones in red, yellow, green, blue Wikipedia

The core of 8 proteins is called an octamer. The stretch of DNA wrapped around the octamer totals about 147 base pairs and makes 1 2/3 turns around it. This complex is referred to as a core particle (Figure 2.144). A linker region of about 50-80 base pairs separate core particles. The term nucleosome then refers to a a core particle plus a linker region (Figure 2.143). Histone H1 sits near the junction of the incoming DNA and the histone core. It is often referred to as the linker histone. In the absence of H1, non-condensed nucleosomes resemble “beads on a string” when viewed in an electron microscope.

Histones



Figure 2.145 - Amino acid sequence (1- letter code) of histone H3 of S. cerevisiae. Arginines (R) and lysines (K) shown in red.

Histone proteins are similar in structure and are rich in basic amino acids, such as lysine and arginine (Figure 2.145). These amino acids are positively charged at physiological pH, with enables them to form tight ionic bonds with the negatively charged phosphate backbone of DNA.

For DNA, compression comes at different levels (Figure 2.146). The first level is at the nucleosomal level. Nucleosomes are stacked and coiled into higher order structures. 10 nm fibers are the simplest higher order structure (beads on a string) and they grow in complexity. 30 nm fibers consist of stacked nucleosomes and they are packed tightly. Higher level packing produces the metaphase chromosome found in meiosis and mitosis.

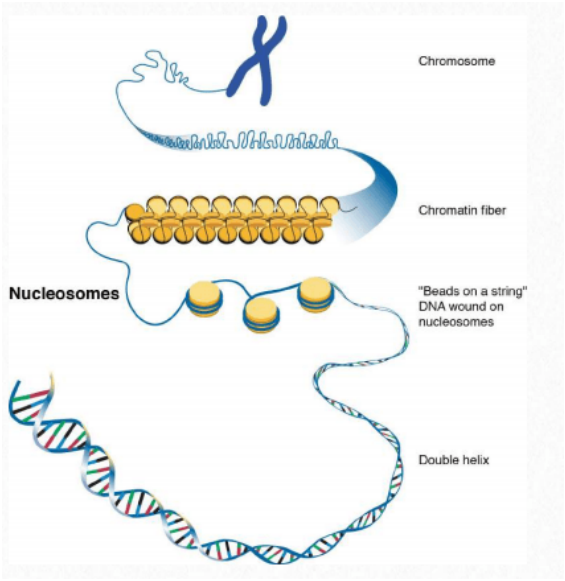


Figure 2.146 Packaging of DNA into a chromosome

The chromatin complex is a logistical concern for the processes of DNA replication and (particularly) gene expression where specific regions of DNA must be transcribed. Altering chromatin structure is therefore an essential function for transcriptional activation in eukaryotes. One strategy involves adding acetyl groups to the positively charged lysine side chains to “loosen their grip” on the negatively charged DNA, thus allowing greater access of proteins involved in activating transcription to gain access to the DNA.

This page titled 11.1: Structure and Function - Nucleic Acids is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern, Indira Rajagopal, and Taralyn Tan via source content that was edited to the style and standards of the LibreTexts platform.

- 2.6: Structure and Function - Nucleic Acids by Kevin Ahern, Indira Rajagopal, and Taralyn Tan is licensed CC BY-NC-SA 4.0. Original source: http://biochem.science.oregonstate.edu/content/biochemistry-free-and-easy.

11.2: Pyrimidine de novo Biosynthesis

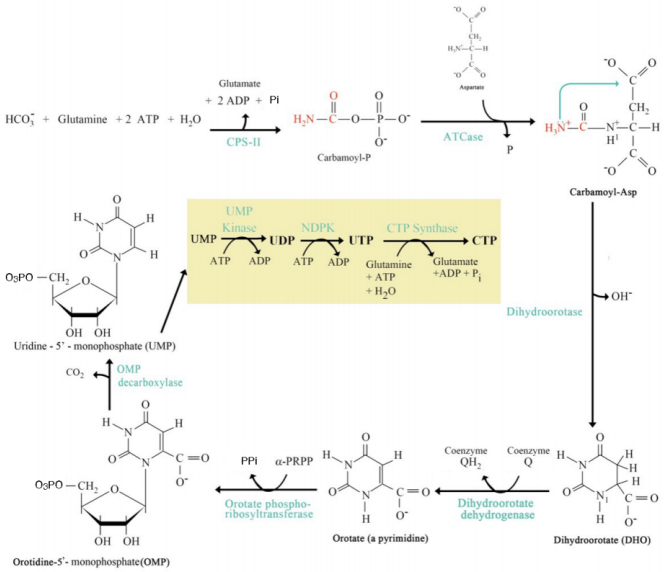


Figure 7.10.1: *De Novo Synthesis of Pyrimidine Nucleotides*

ATCase is regulated by three compounds. One of these (aspartate) is a substrate and it activates the enzyme by binding to the catalytic site and favoring the enzyme's R state. The other two regulators bind to regulatory subunits of the enzyme and either inhibit (CTP) or activate (ATP) the enzyme.

The reaction product, carbamoyl aspartate, is transformed in two reactions to orotic acid, which is, in turn combined with phosphoribosylpyrophosphate PRPP). The product of that reaction, orotidyl monophosphate (OMP) is decarboxylated to form the first pyrimidine nucleotide, UMP. Conversion of UMP to UDP is catalyzed by nucleoside monophosphate kinases (NMPs) and UDP is converted to UTP by nucleoside diphosphokinase (NDPK).

UDP (like all of the nucleoside diphosphates) is a branch point to deoxyribonucleoside diphosphates, catalyzed by ribonucleotide reductases, which are discussed later. UTP is converted to CTP by CTP synthase. This enzyme, which uses an amino group from glutamine for the reaction, serves to balance the relative amounts of CTP and UTP, thanks to inhibition by excess CTP.

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 11.2: Pyrimidine de novo Biosynthesis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 7.10: Pyrimidine de novo Biosynthesis by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

11.3: Deoxyribonucleotide de novo Biosynthesis

Synthesis of deoxyribonucleotides de novo requires an interesting enzyme called ribonucleotide reductase (RNR). RNR catalyzes the formation of deoxyribonucleotides from ribonucleotides. The most common form of RNR is the Type I enzyme, whose substrates are ribonucleoside diphosphates (ADP, GDP, CDP, or UDP) and the products are deoxyribonucleoside diphosphates (dADP, dGDP, dCDP, or dUDP). Thymidine nucleotides are synthesized from dUDP. RNR has two pairs of two identical subunits - R1 (large subunit) and R2 (small subunit). R1 has two allosteric binding sites and an active site. R2 forms a tyrosine radical necessary for the reaction mechanism of the enzyme.

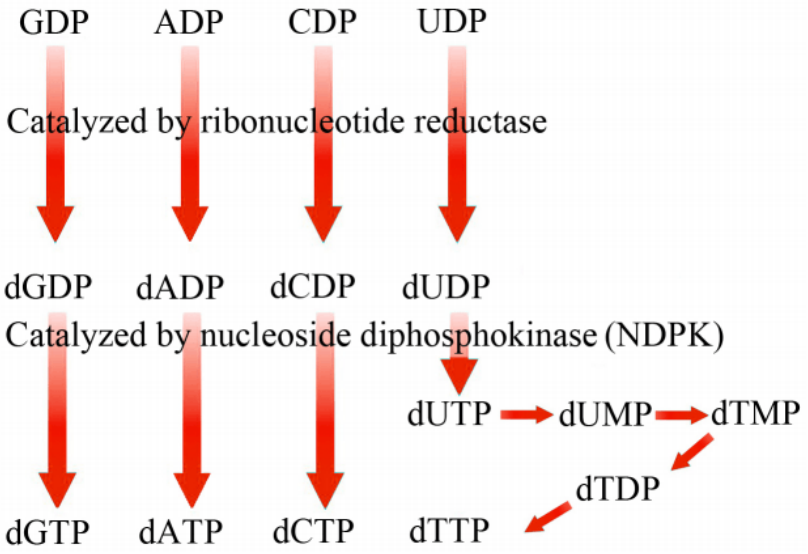


Figure 7.12.1: *dNTP de novo Synthesis*

Because a single enzyme, RNR, is responsible for the synthesis of all four deoxyribonucleotides, it is necessary to have mechanisms to ensure that the enzyme produces the correct amounts of each dNDP. This means that the enzyme must be responsive to the levels of the each deoxynucleotide, selectively making more of those that are in short supply, and preventing synthesis of those that are abundant. These demands are met by having two separate control mechanisms, one that determines which substrate will be acted on, and another that controls the enzyme's catalytic activity.

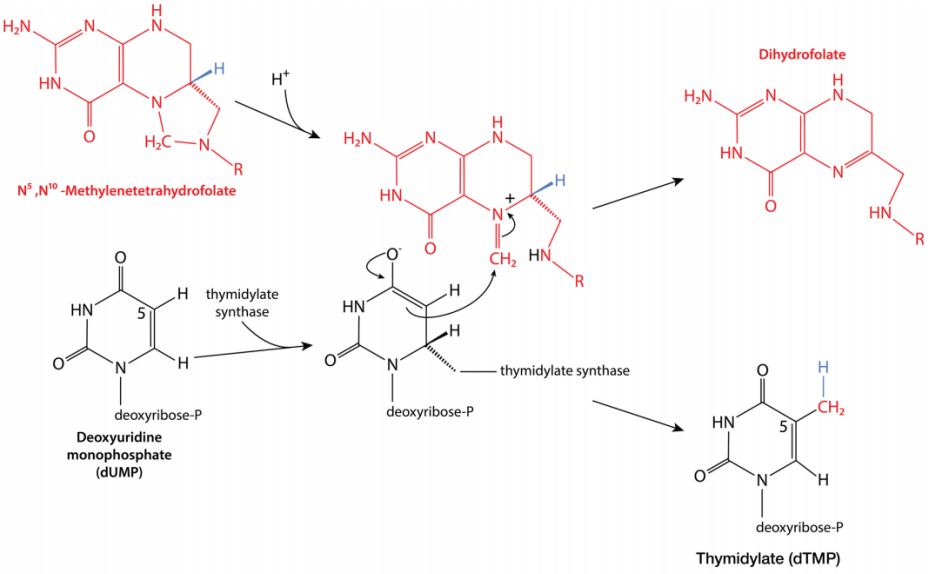


Figure 7.12.2: *Thymidylate (dTMP) Synthesis*

Ribonucleotide reductase is allosterically regulated via two binding sites - a specificity binding site (binds dNTPs and controls which substrates the enzyme binds and thus, which deoxyribonucleotides are made) and an activity binding site (controls whether or not enzyme is active - ATP activates, dATP inactivates).

When a deoxypyrimidine triphosphate, dTTP is abundant, it binds to the specificity site and inhibits binding and reduction of pyrimidine diphosphates (CDP and UDP) but stimulates binding and reduction of GDP by the enzyme. Conversely, binding of the deoxypurine triphosphate, ATP stimulates reduction of pyrimidine diphosphates, CDP and UDP.

Students sometimes confuse the active site of RNR with the activity site. The active site is where the reaction is catalyzed, and could also be called the catalytic site, whereas the activity site is the allosteric binding site for ATP or dATP that controls whether the enzyme is active.

Synthesis of dTTP by the de novo pathway takes a convoluted pathway from dUDP to dUTP to dUMP to dTMP, then dTDP, and finally dTTP. Conversion of dUMP to dTMP, requires a tetrahydrofolate derivative and the enyzme thymidylate synthase. In the process, dihydrofolate is produced and must be converted back to tetrahyrdolate in order to keep nucleotide synthesis occurring. The enzyme involved in the conversion of dihydrofolate to tetrahydrofolate, dihydrofolate reductase (DHFR), is a target of anticancer drugs like methotrexate or aminopterin, which inhibit the enzyme.

Contributors and Attributions

- Dr. Kevin Ahern and Dr. Indira Rajagopal (Oregon State University)

This page titled 11.3: Deoxyribonucleotide de novo Biosynthesis is shared under a CC BY-NC-SA 4.0 license and was authored, remixed, and/or curated by Kevin Ahern and Indira Rajagopal via source content that was edited to the style and standards of the LibreTexts platform.

- 7.12: Deoxyribonucleotide de novo Biosynthesis by Kevin Ahern and Indira Rajagopal is licensed CC BY-NC-SA 4.0. Original source: https://biochem.oregonstate.edu/undergraduate/educational-resources.

11.4: Epigenetic Control- Regulating Access to Genes within the Chromosome

Both the packaging of DNA around histone proteins, as well as chemical modifications to the DNA or proteins, can alter gene expression.

Learning Objectives

- Discuss how eukaryotic gene regulation occurs at the epigenetic level and the various epigenetic changes that can be made to DNA

Key Points

- DNA is packaged by wrapping around histone proteins into structures called nucleosomes, which resemble beads on a string.
- When DNA is to be transcribed, the nucleosomes can slide away from that region of DNA, opening it up to the transcription machinery of the cell.
- Chemical modifications to either the histone proteins or the DNA itself signals whether or not a particular region of the genome should be “open” or “closed” to the transcription machinery.
- Modifications such as acetylation or methylation of the histones can alter how tightly DNA is wrapped around them, while methylation of DNA changes how the DNA interacts with proteins, including the histone proteins that control access to the region.
- This type of genetic regulation is called epigenetic regulation (“above genetics”) as it does not change the nucleotide sequence of the DNA.

Key Terms

- **nucleosome**: any of the subunits that repeat in chromatin; a coil of DNA surrounding a histone core
- **epigenetics**: the study of heritable changes caused by the activation and deactivation of genes without any change in DNA sequence
- **histone**: any of various simple water-soluble proteins that are rich in the basic amino acids lysine and arginine and are complexed with DNA in the nucleosomes of eukaryotic chromatin

Epigenetic Control: Regulating Access to Genes within the Chromosome

The human genome encodes over 20,000 genes; each of the 23 pairs of human chromosomes encodes thousands of genes. The DNA in the nucleus is precisely wound, folded, and compacted into chromosomes so that it will fit into the nucleus. It is also organized so that specific segments can be accessed as needed by a specific cell type.

The first level of organization, or packing, is the winding of DNA strands around histone proteins. Histones package and order DNA into structural units called nucleosome complexes, which can control the access of proteins to the DNA regions. Under the electron microscope, this winding of DNA around histone proteins to form nucleosomes looks like small beads on a string. These beads (histone proteins) can move along the string (DNA) and change the structure of the molecule.

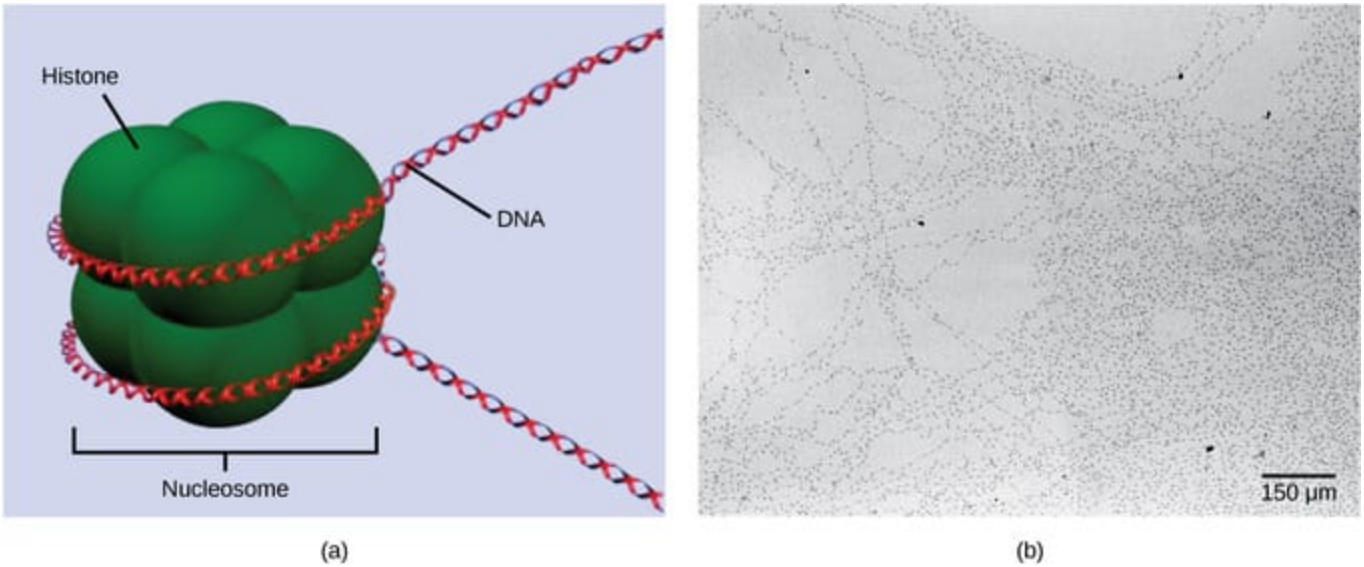


Figure 11.4.1: **DNA Packaging**: DNA is folded around histone proteins to create (a) nucleosome complexes. These nucleosomes control the access of proteins to the underlying DNA. When viewed through an electron microscope (b), the nucleosomes look like beads on a string.

If DNA encoding a specific gene is to be transcribed into RNA, the nucleosomes surrounding that region of DNA can slide down the DNA to open that specific chromosomal region and allow for the transcriptional machinery (RNA polymerase) to initiate transcription. Nucleosomes can move to open the chromosome structure to expose a segment of DNA, but do so in a very controlled manner.

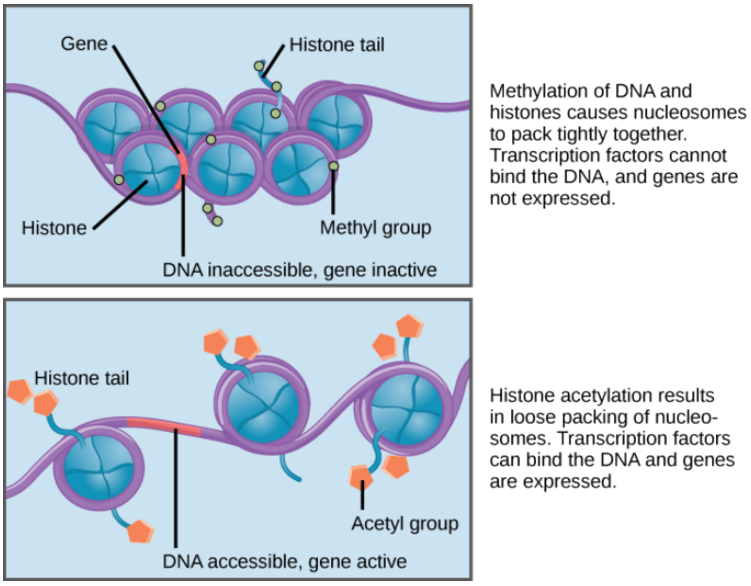


Figure 11.4.1: **Nucleosomes can change position to allow transcription of genes**: Nucleosomes can slide along DNA. When nucleosomes are spaced closely together (top), transcription factors cannot bind and gene expression is turned off. When the nucleosomes are spaced far apart (bottom), the DNA is exposed. Transcription factors can bind, allowing gene expression to occur. Modifications to the histones and DNA affect nucleosome spacing.

How the histone proteins move is dependent on signals found on both the histone proteins and on the DNA. These signals are tags, or modifications, added to histone proteins and DNA that tell the histones if a chromosomal region should be open or closed. These tags are not permanent, but may be added or removed as needed. They are chemical modifications (phosphate, methyl, or acetyl groups) that are attached to specific amino acids in the protein or to the nucleotides of the DNA. The tags do not alter the DNA base sequence, but they do alter how tightly wound the DNA is around the histone proteins. DNA is a negatively-charged molecule; therefore, changes in the charge of the histone will change how tightly wound the DNA molecule will be. When unmodified, the histone proteins have a large positive charge; by adding chemical modifications, such as acetyl groups, the charge becomes less positive.

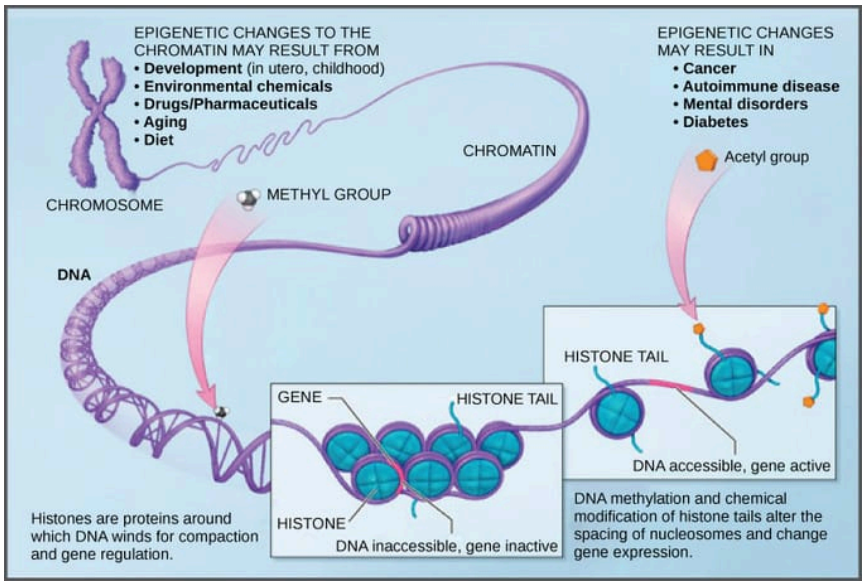


Figure 11.4.1: **Modifications to histones and DNA can alter gene expression**: Histone proteins and DNA nucleotides can be modified chemically. Modifications affect nucleosome spacing and gene expression.

The DNA molecule itself can also be modified. This occurs within very specific regions called CpG islands. These are stretches with a high frequency of cytosine and guanine dinucleotide DNA pairs (CG) found in the promoter regions of genes. When this configuration exists, the cytosine member of the pair can be methylated (a methyl group is added). This modification changes how the DNA interacts with proteins, including the histone proteins that control access to the region. Highly-methylated (hypermethylated) DNA regions with deacetylated histones are tightly coiled and transcriptionally inactive. These changes to DNA are inherited from parent to offspring, such that while the DNA sequence is not altered, the pattern of gene expression is passed to the next generation.

This type of gene regulation is called epigenetic regulation. Epigenetics means “above genetics.” The changes that occur to the histone proteins and DNA do not alter the nucleotide sequence and are not permanent. Instead, these changes are temporary (although they often persist through multiple rounds of cell division) and alter the chromosomal structure (open or closed) as needed. A gene can be turned on or off depending upon the location and modifications to the histone proteins and DNA. If a gene is to be transcribed, the histone proteins and DNA are modified surrounding the chromosomal region encoding that gene. This opens the chromosomal region to allow access for RNA polymerase and other proteins, called transcription factors, to bind to the promoter region, located just upstream of the gene, and initiate transcription. If a gene is to remain turned off, or silenced, the histone proteins and DNA have different modifications that signal a closed chromosomal configuration. In this closed configuration, the RNA polymerase and transcription factors do not have access to the DNA and transcription cannot occur.

This page titled 11.4: Epigenetic Control- Regulating Access to Genes within the Chromosome is shared under a license and was authored, remixed, and/or curated by Boundless (now LumenLearning).

INDEX

A

acetyl CoA
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

acid
2.1: Water

adhesion
2.1: Water

affinity
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

alpha helix
4.3: Secondary Structure

amino acid
2.4: Types of Biological Molecules

amino acid catabolism
10.2: Amino Acid Catabolism

amino acid metabolism
10.1: Amino Acid Metabolism

anaerobic cellular respiration
8.4: Fermentation

anion
1.6: The Building Blocks of Molecules

ATCase
11.2: Pyrimidine de novo Biosynthesis

atomic number
1.6: The Building Blocks of Molecules

ATP synthase
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

B

base
2.1: Water

beta hairpins
4.3: Secondary Structure

beta strands
4.3: Secondary Structure

buffer
2.1: Water

C

carbohydrate
2.4: Types of Biological Molecules

Cardiolipins
9.1: Structure and Function - Lipids and Membranes

carnitine
9.5: Fatty Acid Oxidation

cation
1.6: The Building Blocks of Molecules

Cell Disruption
5.1: Cell Disruption

cell wall
1.4: Eukaryotic Cells

cellulose
2.4: Types of Biological Molecules

central dogma
1.1: Transcription

central vacuole
1.4: Eukaryotic Cells

centrosome
1.4: Eukaryotic Cells

chemical bond
1.6: The Building Blocks of Molecules

chemiosmosis
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

Chemiosmotic model
8.7: Electron Transport and Oxidative Phosphorylation

chitin
2.4: Types of Biological Molecules

chlorophyll
1.4: Eukaryotic Cells

chloroplast
1.4: Eukaryotic Cells

chromatin
1.4: Eukaryotic Cells

chromosome
1.4: Eukaryotic Cells

Chymotrypsin
7.3: Mechanisms of Catalysis

citric acid cycle
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation
8.6: Citric Acid Cycle and Related Pathways
9.6: Ketone body production and acidosis

codon
1.2: Translation

cohesion
2.1: Water

collagen
4.5: Protein function, domains and cooperativity

covalent bond
1.6: The Building Blocks of Molecules

Cysteine Redox Chemistry
3.3: Cysteine Chemistry

cytoplasm
1.4: Eukaryotic Cells

cytosol
1.4: Eukaryotic Cells

D

denaturation
2.4: Types of Biological Molecules

Deoxyribonucleic acid (DNA)
2.4: Types of Biological Molecules

Deoxyribonucleotides
11.3: Deoxyribonucleotide de novo Biosynthesis

disaccharide
2.4: Types of Biological Molecules

DNA (Z Form)
11.1: Structure and Function - Nucleic Acids

E

electron
1.6: The Building Blocks of Molecules

electron transfer
1.6: The Building Blocks of Molecules

electron transport chain
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

electrophoresis
5.4: Electrophoresis

element
1.6: The Building Blocks of Molecules

enzyme
2.4: Types of Biological Molecules

essential amino acids
10.1: Amino Acid Metabolism

eukaryotic cell
1.4: Eukaryotic Cells

evaporation
2.1: Water

exon
1.1: Transcription

F

fat
2.4: Types of Biological Molecules

Fatty Acid Oxidation
9.5: Fatty Acid Oxidation

fermentation
8.4: Fermentation

Fractionation
5.2: Fractionation

G

genetic code
1.2: Translation

glmol:yes
8.1: Carbohydrates overview

gluconeogenesis
8.3: Gluconeogenesis

glycogen
2.4: Types of Biological Molecules

glycolysis
8.2: Glycolysis

gsmol:yes
8.1: Carbohydrates overview

H

hormone
2.4: Types of Biological Molecules

hybridomas
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

hydrogen bond
1.6: The Building Blocks of Molecules

hydrophilic
2.1: Water

hydrophobic
2.1: Water

I

intron
1.1: Transcription

ion
1.6: The Building Blocks of Molecules

ionic bond
1.6: The Building Blocks of Molecules

isoelectric focusing
5.4: Electrophoresis

isotope
1.6: The Building Blocks of Molecules

J

jsmol:yes
8.1: Carbohydrates overview

K

keratins
4.5: Protein function, domains and cooperativity

L

Levinthal paradox
4.4: Protein folding

lipids
2.4: Types of Biological Molecules

litmus paper
2.1: Water

lysis
5.1: Cell Disruption

lysosome
1.4: Eukaryotic Cells

M

macromolecule
2.4: Types of Biological Molecules

mass number
1.6: The Building Blocks of Molecules

matter
1.6: The Building Blocks of Molecules

Mitochondria
1.4: Eukaryotic Cells

monoclonal antibodies
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

Monosaccharide
2.4: Types of Biological Molecules

Morpheein model
7.4: Control of Enzymatic Activity

mRNA
1.1: Transcription

N

neutron
1.6: The Building Blocks of Molecules

nonpolar covalent bond
1.6: The Building Blocks of Molecules

nontemplate strand
1.1: Transcription

notebene:no
6.1: Endergonic and Exergonic Reactions
8.1: Carbohydrates overview

nuclear envelope
1.4: Eukaryotic Cells

nucleic acid
2.4: Types of Biological Molecules

nucleic acids
11.1: Structure and Function - Nucleic Acids

nucleoid
1.3: Prokaryotic Cells

nucleolus
1.4: Eukaryotic Cells

nucleoplasm
1.4: Eukaryotic Cells

nucleotide
2.4: Types of Biological Molecules

nucleus
1.4: Eukaryotic Cells
1.6: The Building Blocks of Molecules

O

octet rule
1.6: The Building Blocks of Molecules

oil
2.4: Types of Biological Molecules

organelle
1.4: Eukaryotic Cells

oxidative phosphorylation
8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation

P

PEPCK
8.3: Gluconeogenesis

periodic table of elements
1.6: The Building Blocks of Molecules

peroxisome
1.4: Eukaryotic Cells

pH scale
2.1: Water

phospholipid
2.4: Types of Biological Molecules

pi helix
4.3: Secondary Structure

plantibodies
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

plasma membrane
1.4: Eukaryotic Cells

polar covalent bond
1.6: The Building Blocks of Molecules

Polyacrylamide gel electrophoresis
5.4: Electrophoresis

polyclonal antibody
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

Polyclonal antisera
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

polypeptide
2.4: Types of Biological Molecules

polysaccharide
2.4: Types of Biological Molecules

prions
4.7: Prions and Disease

prokaryote
1.3: Prokaryotic Cells

promoter
1.1: Transcription

protein
2.4: Types of Biological Molecules

proton
1.6: The Building Blocks of Molecules

pyrimidine
11.2: Pyrimidine de novo Biosynthesis

pyrimidine biosynthesis
11.2: Pyrimidine de novo Biosynthesis

R

radioactive isotope
1.6: The Building Blocks of Molecules

Ribonucleic Acid (RNA)
2.4: Types of Biological Molecules

ribonucleotide reductase (RNR)
11.3: Deoxyribonucleotide de novo Biosynthesis

ribosome
1.4: Eukaryotic Cells

RNA polymerase
1.1: Transcription

rRNA
1.2: Translation

S

Saturated fatty acid
2.4: Types of Biological Molecules

secondary structure
4.5: Protein function, domains and cooperativity

secondary structure
4.3: Secondary Structure

serine proteases
7.3: Mechanisms of Catalysis

solvent
2.1: Water

Sonication
5.1: Cell Disruption

specificity
5.5: Practical Applications of Monoclonal and Polyclonal Antibodies

splicing
1.1: Transcription

starch
2.4: Types of Biological Molecules

start codon
1.2: Translation

steroid
2.4: Types of Biological Molecules

stop codon
1.2: Translation

subtilisin
7.3: Mechanisms of Catalysis

surface tension
2.1: Water

T

temperature
2.1: Water

template strand
1.1: Transcription

tertiary structure
4.5: Protein function, domains and cooperativity

transcription bubble
1.1: Transcription

transmissible spongiform encephalopathies
4.7: Prions and Disease
triglyceride
2.4: Types of Biological Molecules
tRNA
1.2: Translation

U

unsaturated fatty acid
2.4: Types of Biological Molecules

V

vacuole
1.4: Eukaryotic Cells
van der Waals interaction
1.6: The Building Blocks of Molecules
Vesicle
1.4: Eukaryotic Cells

This page titled Index is shared under a license and was authored, remixed, and/or curated by LibreTexts.

GLOSSARY

S

Sample Word 1 | Sample Definition 1

This page titled Glossary is shared under a license and was authored, remixed, and/or curated by LibreTexts.

DETAILED LICENSING

Overview

Title: Principles of Biochemistry

Webpages: 78

Applicable Restrictions: Noncommercial

All licenses found:

- CC BY-NC-SA 4.0: 48.7% (38 pages)
- Undeclared: 37.2% (29 pages)
- CC BY 4.0: 12.8% (10 pages)
- CC BY-SA 4.0: 1.3% (1 page)

By Page

- Principles of Biochemistry - *Undeclared*
  - Front Matter - *Undeclared*
    - Principles of Biochemistry - *Undeclared*
    - InfoPage - *Undeclared*
    - Table of Contents - *Undeclared*
    - Licensing - *Undeclared*
  - 1: Biology and Chemistry Review - *Undeclared*
    - 1.1: Transcription - *CC BY 4.0*
    - 1.2: Translation - *CC BY 4.0*
    - 1.3: Prokaryotic Cells - *CC BY 4.0*
    - 1.4: Eukaryotic Cells - *CC BY 4.0*
    - 1.5: Essential elements for life - *Undeclared*
    - 1.6: The Building Blocks of Molecules - *CC BY 4.0*
  - 2: Introduction to Biochemistry - *Undeclared*
    - 2.1: Water - *CC BY 4.0*
    - 2.2: pH and Buffers - *CC BY-NC-SA 4.0*
    - 2.3: Functional groups and electronegativity - *CC BY-NC-SA 4.0*
    - 2.4: Types of Biological Molecules - *CC BY 4.0*
    - 2.5: Assembly of Biological Macromolecules - *Undeclared*
  - 3: Amino Acids - *Undeclared*
    - 3.1:Structure and Property of the Naturally-Occurring Amino Acids - *CC BY-NC-SA 4.0*
    - 3.2: Amino Acid Charges - *CC BY-NC-SA 4.0*
    - 3.3: Cysteine Chemistry - *CC BY-NC-SA 4.0*
    - 3.4: Post Translational Modification of Amino Acids - *CC BY-NC-SA 4.0*
  - 4: Protein structure and function - *Undeclared*
    - 4.1: From Amino Acids to Peptides - *CC BY-NC-SA 4.0*
    - 4.2: Levels of Protein Structure - *CC BY-NC-SA 4.0*
    - 4.3: Secondary Structure - *CC BY-NC-SA 4.0*
    - 4.4: Protein folding - *CC BY-NC-SA 4.0*
    - 4.5: Protein function, domains and cooperativity - *CC BY-NC-SA 4.0*
    - 4.6: Protein Aggregation - *CC BY-NC-SA 4.0*
    - 4.7: Prions and Disease - *CC BY-NC-SA 4.0*
  - 5: Techniques for studying proteins - *Undeclared*
    - 5.1: Cell Disruption - *CC BY-NC-SA 4.0*
    - 5.2: Fractionation - *CC BY-NC-SA 4.0*
    - 5.3: Quantification of Protein Concentration - *Undeclared*
    - 5.4: Electrophoresis - *CC BY-NC-SA 4.0*
    - 5.5: Practical Applications of Monoclonal and Polyclonal Antibodies - *CC BY 4.0*
    - 5.6: Western blot / Immunoblot overview - *CC BY-SA 4.0*
    - 5.7: Steps in Western blotting - *CC BY-NC-SA 4.0*
  - 6: Energetics and metabolism - *Undeclared*
    - 6.1: Endergonic and Exergonic Reactions - *Undeclared*
    - 6.2: Thermodynamics - *Undeclared*
    - 6.3: Energy in Metabolism - *CC BY-NC-SA 4.0*
  - 7: Enzymes, catalysis and kinetics - *Undeclared*
    - 7.1: Basic Principles of Catalysis - *CC BY-NC-SA 4.0*
    - 7.2: Derivation of Michaelis-Menten equation - *Undeclared*
    - 7.3: Mechanisms of Catalysis - *CC BY-NC-SA 4.0*
    - 7.4: Control of Enzymatic Activity - *CC BY-NC-SA 4.0*
  - 8: Carbohydrate structure and metabolism - *Undeclared*
    - 8.1: Carbohydrates overview - *Undeclared*
    - 8.2: Glycolysis - *CC BY-NC-SA 4.0*
    - 8.3: Gluconeogenesis - *CC BY-NC-SA 4.0*
    - 8.4: Fermentation - *CC BY 4.0*

- 8.5: Overview of Citric Acid Cycle and Oxidative Phosphorylation - *CC BY 4.0*
- 8.6: Citric Acid Cycle and Related Pathways - *CC BY-NC-SA 4.0*
- 8.7: Electron Transport and Oxidative Phosphorylation - *CC BY-NC-SA 4.0*
- 8.8: Carbohydrate Storage and Breakdown - *CC BY-NC-SA 4.0*

◦ 9: Lipids and membranes - *Undeclared*
- 9.1: Structure and Function - Lipids and Membranes - *CC BY-NC-SA 4.0*
- 9.2: Basic Concepts in Membranes - *CC BY-NC-SA 4.0*
- 9.3: Transport in Membranes - *CC BY-NC-SA 4.0*
- 9.4: Metabolism of Fat - *CC BY-NC-SA 4.0*
- 9.5: Fatty Acid Oxidation - *CC BY-NC-SA 4.0*
- 9.6: Ketone body production and acidosis - *CC BY-NC-SA 4.0*

◦ 10: Nitrogen metabolism and the urea cycle - *Undeclared*
- 10.1: Amino Acid Metabolism - *CC BY-NC-SA 4.0*
- 10.2: Amino Acid Catabolism - *CC BY-NC-SA 4.0*
- 10.3: Urea Cycle - *CC BY-NC-SA 4.0*

◦ 11: Nucleotide and nucleic acid structure and metabolism - *Undeclared*
- 11.1: Structure and Function - Nucleic Acids - *CC BY-NC-SA 4.0*
- 11.2: Pyrimidine de novo Biosynthesis - *CC BY-NC-SA 4.0*
- 11.3: Deoxyribonucleotide de novo Biosynthesis - *CC BY-NC-SA 4.0*
- 11.4: Epigenetic Control- Regulating Access to Genes within the Chromosome - *Undeclared*

◦ Back Matter - *Undeclared*
- Index - *Undeclared*
- Glossary - *Undeclared*
- Detailed Licensing - *Undeclared*

This page titled Principles of Biochemistry is shared under a license and was authored, remixed, and/or curated by LibreTexts.