Chapter 13 Lecture Notes: Peptides, Proteins, and Enzymes

Educational Goals

1. Describe the general bonding pattern of **α-amino acids** and understand how amino acids are classified by the ***polarity*** and ***charge of their side-chains***.
2. Given the table of twenty common amino acids, determine the ***total charge*** of the dominant form of an amino acid (at physiological **pH**, at **pH** < 2, and at **pH** > 11).
3. Understand the three-dimensional information contained in the Fischer projection of an amino acid.
4. Given a Fischer projection of an amino acid, determine if it is an ***L****-amino acid* or ***D****-amino acid*.
5. Understand and define the term **peptide**.
6. Given the table of twenty common amino acids, be able to draw the structural formula of the peptide that is obtained when two or more particular amino acids are connected by **peptide bonds**.
7. Given the structural formula of a **peptide** be able to identify the **peptide bonds,** the **C-terminus,** the **N-terminus,** *and* the **peptide groups**.
8. Given the structural formula of a peptide and the table of twenty common amino acids, determine the ***total charge*** of the peptide's dominant form (at physiological **pH**, at **pH** < 2, and at **pH** > 11).
9. Given the structure of a peptide, and the table of twenty common amino acids name a particular peptide using amino acid residue *abbreviations.*
10. Compare and contrast **peptides** and **proteins**.
11. Understand and define **primary, secondary, tertiary, and quaternary protein structure**. Name the noncovalent interactions that are responsible for each level of structure.
12. Explain what is meant by the term **denaturation** and list the ways to denature a protein.
13. Understand the difference between **globular, fibrous, and membrane proteins**.
14. Compare and contrast **simple proteins** and **conjugated proteins**.
15. Understand the terms **cofactor** and **coenzyme**.
16. Understand how **enzymes** work and distinguish between **absolute specificity, relative specificity, and stereospecificity**.
17. Understand and define the terms: **essential amino acid, complete protein, incomplete protein, and complimentary protein**.
18. Understand how changes in **pH** and *temperature* can affect the reaction **rate** of an enzymatically-catalyzed reaction.
19. Understand how enzyme inhibitors and activators control enzymatic reactions, and compare and contrast **reversible** and **irreversible inhibitors**.
20. Understand how organisms regulate metabolic pathways using **feedback inhibition** and **positive feedback**.

Introduction

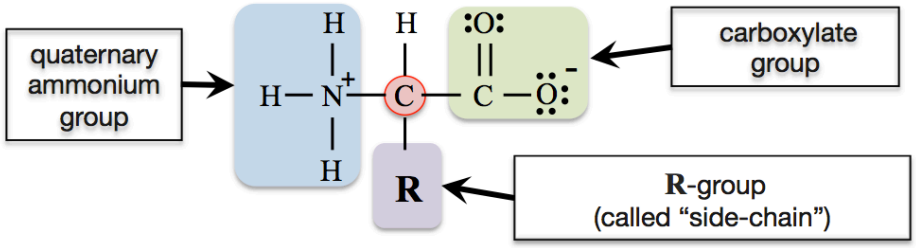
The _____ _____of amino acids in a protein and the chemical nature of the amino acid _____ _____ enable proteins to perform their functions.

- Typical protein functions:
  - Catalyze Reactions (enzymes)
  - Chemical Signaling (hormones)
  - Storage (e.g. myoglobin stores oxygen)
  - Structural (e.g. collagen in skin and tendons)
  - Protective (e.g. antibodies)
  - Contractile (e.g. myosin in muscle)
  - Transport (e.g. hemoglobin)

Amino Acids

Structure of Amino Acids

Amino acids are organic compounds that contain a _____ *and* a _____.

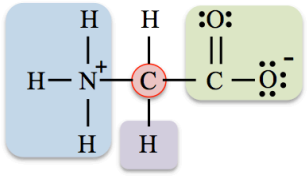


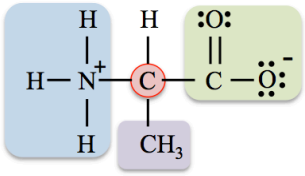
For amino acids, the **R-group** is often called the "**side-chain**" or "**variant group**."

The *side-chain* can be a hydrogen atom, hydrocarbon, or various other groups of bonded atoms.

Amino acids are named based on the identity of their _____.

- For example, if the side-chain is a hydrogen atom (H), then the amino acid is called *glycine*; if the side-chain is a methyl group (CH_3), then the amino acid is called *alanine*.

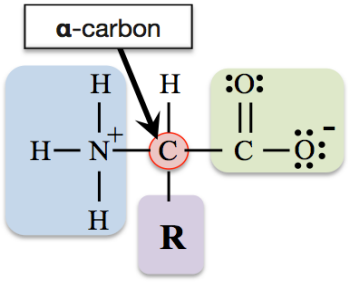

glycine


alanine

There are 23 amino acids that make up the proteins in plants and animals, 20 of them are directly specified by the genetic code in DNA.

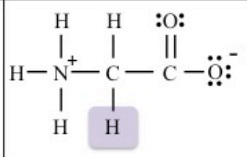
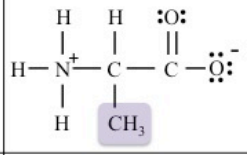
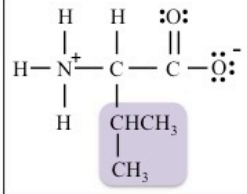
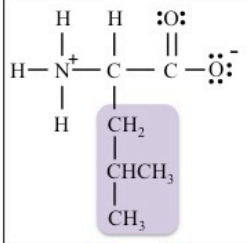
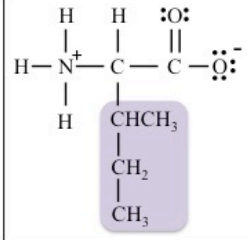
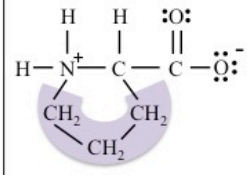
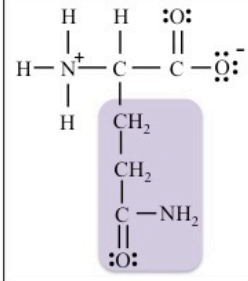
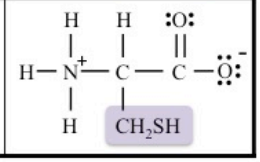
These twenty amino acids are called the _____ *amino acids.*

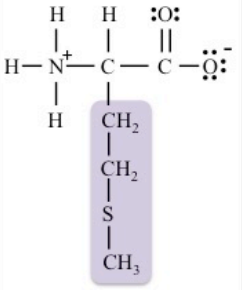
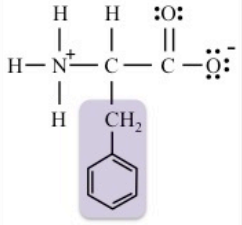
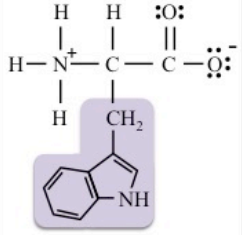
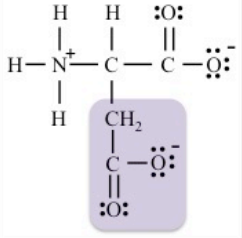
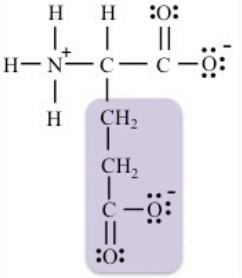
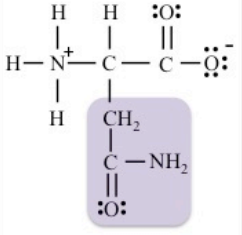
- All twenty *common amino acids* are _____ amino acids.
- They are called ***α***-amino acids because their *side-chains* are attached to ***α***-carbons.

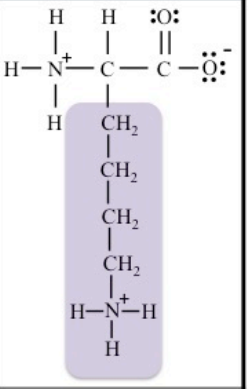
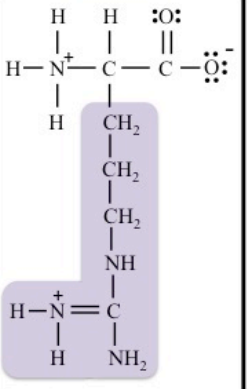
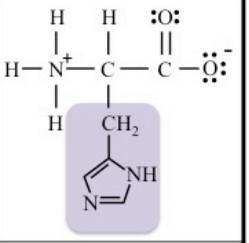
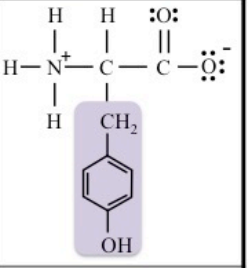
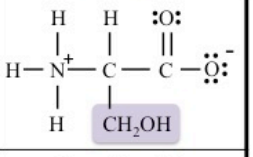
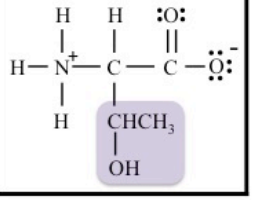


REMINDER: The ***α***-carbon is the carbon that is bonded to the carboxyl group's carbonyl carbon.

The ***twenty common amino acids*** are often referred to using three-letter abbreviations. The structures, names, and abbreviations for the twenty common amino acids are shown below. Note that they are all ***α*-amino acids**.

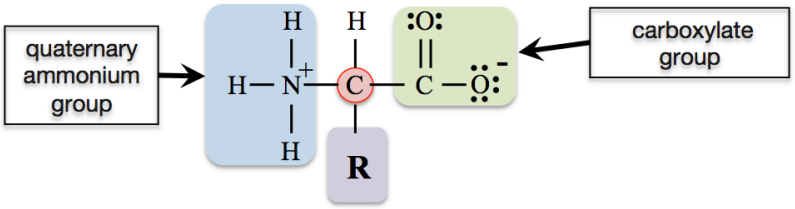
Name (abbreviation)	Structural Formula
Glycine (Gly)	
Alanine (Ala)	
Valine (Val)	
Leucine (Leu)	
Isoleucine (Ile)	
Proline (Pro)	
Glutamine (Gln)	
Cysteine (Cys)	

Name (abbreviation)	Structural Formula
Methionine (Met)	
Phenylalanine (Phe)	
Tryptophan (Trp)	
Aspartic Acid (Asp)	
Glutamic Acid (Glu)	
Asparagine (Asn)	

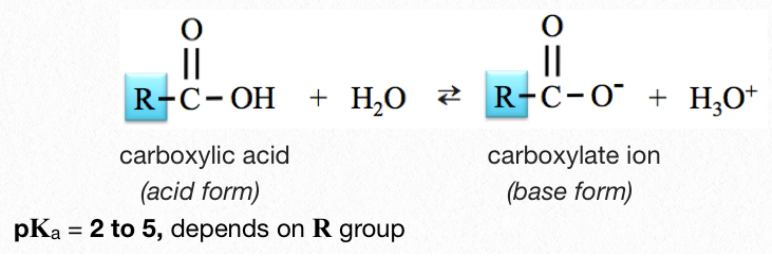
Name (abbreviation)	Structural Formula
Lysine (Lys)	
Arginine (Arg)	
Histidine (His)	
Tyrosine (Tyr)	
Serine (Ser)	
Threonine (Thr)	

Charges on Amino Acids

The structural formulas of the common amino acids all contain at least one ***carboxylate group*** and one ***quaternary ammonium group***.

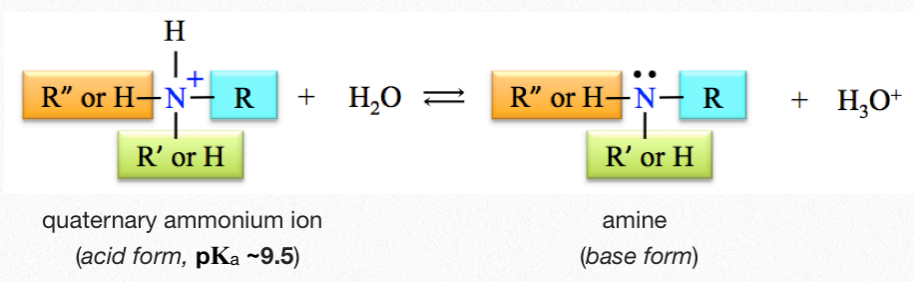


In previous chapters you learned that in aqueous solutions, the ***carboxylate group*** is in equilibrium with its _____, the ***carboxyl group***:



From the _____relation, we know that when the **pH** is less than the **pK**$_a$ of a carboxyl group, then the carboxylic acid form (**R-COOH**) is predominant, and when the **pH** is greater than the **pK**$_a$, then the carboxylate ion form (**R-COO**$^-$) is predominant.

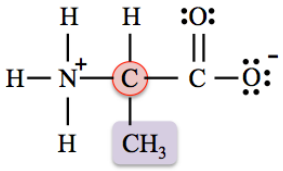
Likewise, the ***quaternary ammonium group*** is in equilibrium with its conjugate, the ***amine group***:



When the **pH** of a solution is less than the **pK**$_a$ **(~ 9.5)**, then the *quaternary ammonium group* (acid form) is predominant, and when the **pH** is greater than the **pK**$_a$, then the amine group (base form) is predominant.

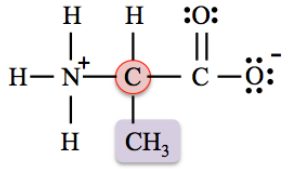
Since amino acids involve the **carboxyl group/carboxylate group conjugate pair** *and* the **quaternary ammonium group/amine group conjugate pair**, then the _____ _____ *of the predominant form of an amino acid* will depend on the _____.

EXAMPLE: Consider the predominant form of *alanine* at physiological **pH** (**pH** ~7.4):



- The **pK**$_a$ values of amino acid carboxyl groups are between **2** and **5** (depending on which amino acid), therefore, at **pH** = **7.4**, the base form (carboxylate ion) is predominant.
- *Quaternary ammonium groups* that are attached to the ***α***-carbons of amino acids have **pK**$_a$ values of about **9.5**, therefore, at **pH** = **7.4**, the quaternary ammonium group (acid form) is predominant.

The predominant form of *alanine* has a negative (1-) formal charge on the *carboxylate group* and a positive (1+) formal charge on the *quaternary ammonium group*, which gives it a *total charge* of _____.



predominant form of *alanine* at **pH** = **7.4**

When an amino acid has a total charge equal to zero, it is called a _____.

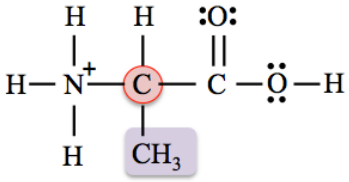
- (*zwitter* is German for *hermaphrodite* or *hybrid*).

The amino acid structures in the table (provided earlier) are the predominant forms at physiological pH.

In sufficiently acidic or basic solutions, the _____ _____ of the predominant form of an amino acid *will change* from its physiological value.

EXAMPLE: Consider the *total charge* of the predominant form of *alanine* in an extremely ***acidic*** solution.

At **pH = 1.0** (an extremely *acidic solution*) the **pH** is _____ than the $\mathbf{pK_a}$ of *both* the carboxyl group and the quaternary ammonium group, therefore both groups exist in their ***acid form***, as shown below.

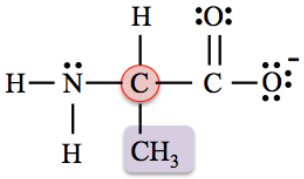


predominant form of *alanine* at **pH** = **1.0**

The predominant form of *alanine at* **pH = 1.0** has an *uncharged* carboxyl group (**COOH**) and has a positive (1+) formal charge on the nitrogen of the quaternary ammonium group, which results in a _____ **(1+)** ***total charge***.

EXAMPLE: Consider the *total charge* of the predominant form of *alanine* in an extremely ***basic*** solution.

At **pH = 12.0** (an extremely *basic solution*) the **pH** is _____ than the $\mathbf{pK_a}$ of *both* the carboxyl group and the quaternary ammonium group, therefore both groups exist in their ***base form***, as shown below.



predominant form of *alanine* at **pH** = **12.0**

The predominant form of *alanine at* **pH = 12.0** has a negative (1-) formal charge on the single-bonded oxygen of the carboxylate group and an *uncharged* nitrogen in the amine group, which results in a _____ **(1-)** *total charge*.

Practice Problems: The amino acid structures in the table provided earlier are the predominant forms at physiological **pH**.

a. Draw the predominant form of valine when the **pH = 7.4**
b. Draw the predominant form of valine when the **pH = 1.0**
c. Draw the predominant form of valine when the **pH = 12.0**
d. What is the *total charge* of the predominant form of valine when the **pH = 7.4?**
e. What is the *total charge* of the predominant form of valine when the **pH = 1.0?**
f. What is the *total charge* of the predominant form of valine when the **pH = 12.0**?

Classification of Amino Acids

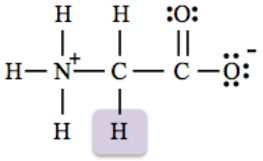
Amino acids are classified by the _____ ***of their side-chain*** and the ***ability of their side-chain to acquire*** _____ _____ (at physiological **pH**).

Amino Acid Class	Side Chain Polarity	Side-Chain Charge at Physiological pH
Nonpolar	nonpolar (hydrophobic side-chain)	zero
Polar neutral	polar (hydrophilic side-chain)	zero
Polar acidic	polar (hydrophilic side-chain)	negative
Polar basic	polar (hydrophilic side-chain)	positive

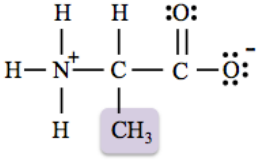
1) Nonpolar Amino Acids

Nonpolar amino acids have ***nonpolar (hydrophobic) side-chains*** and their predominant forms have *uncharged* side-chains at physiological pH.

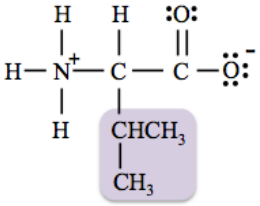
- The *nonpolar amino acids* (their predominant forms at physiological **pH**) are:



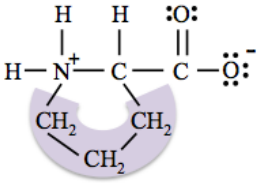
glycine



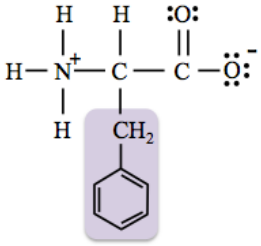
alanine



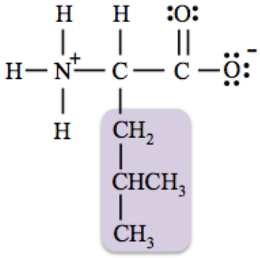
valine



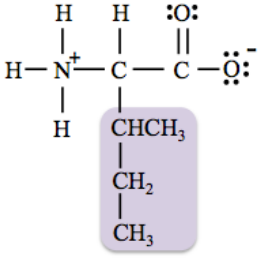
proline



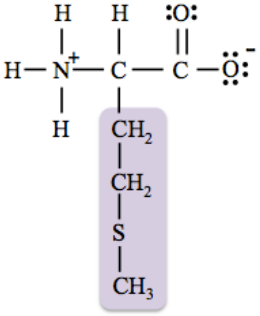
phenylalanine



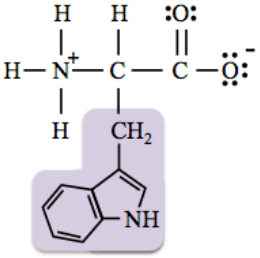
leucine



isoleucine



methionine



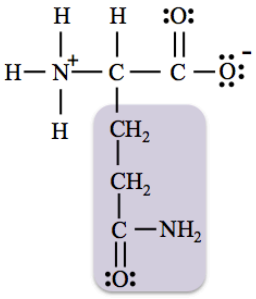
tryptophan

Note that although the side-chain of *tryptophan* contains a few highly-polar bonds, the hydrocarbon part is so large that it dominates the interactions, making the side-chain *hydrophobic*. For this reason, tryptophan is put into the *nonpolar* class.

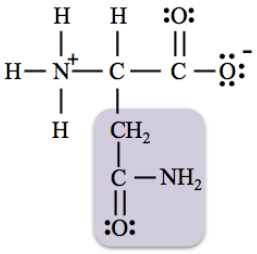
2) Polar Neutral Amino Acids

Polar neutral amino acids have ***polar (hydrophilic) side-chains*** and their predominant forms have *uncharged* side-chains at physiological pH.

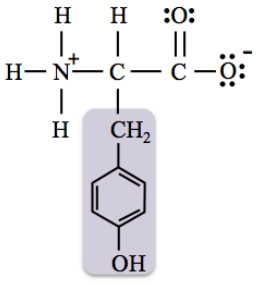
- The *polar neutral amino acids* (their predominant forms at physiological **pH**) are:



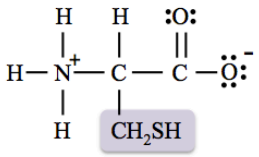
glutamine



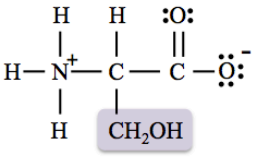
asparagine



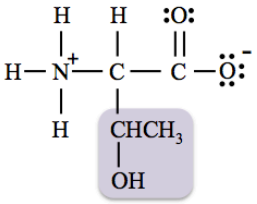
tyrosine



cysteine



serine



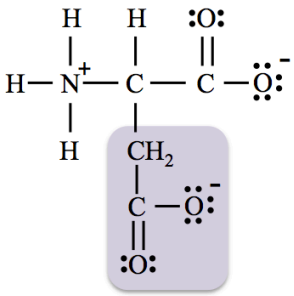
threonine

3) Polar Acidic Amino Acids

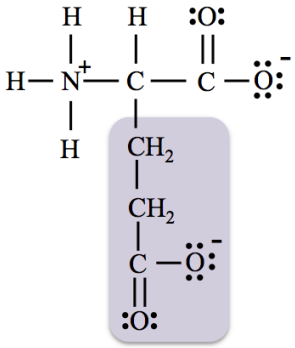
Polar acidic amino acids have ***polar (hydrophilic) side-chains*** and, their predominant forms have side-chains with **negative (1-)** ***formal charge*** at physiological **pH**.

- This formal charge is from a _____ ***group***.

The *polar acidic amino acids* (their predominant forms at physiological **pH**) are:



aspartic acid



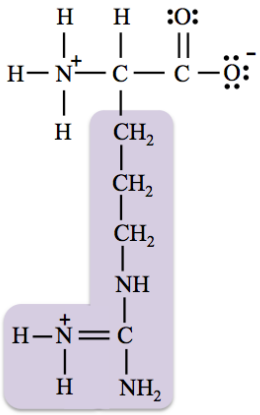
glutamic acid

Polar *acidic* amino acids are given the "*acidic*" term in their classification because their acid forms are stronger acids than those of the polar "*basic*" amino acids (discussed next).

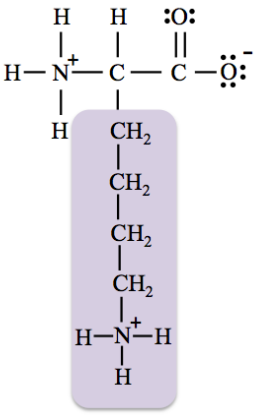
4) Polar Basic Amino Acids

Polar basic amino acids have ***polar (hydrophilic) side-chains*** and, except for *histidine*, their predominant forms have side-chains with **positive (1+)** ***formal charge*** at physiological **pH**.

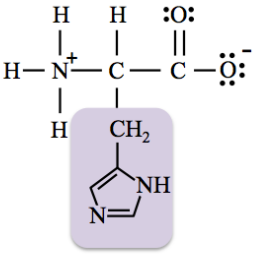
- This formal charge is from a ***quaternary ammonium group***.
- The *polar basic amino acids* (their predominant forms at physiological **pH**) are:



arginine



lysine

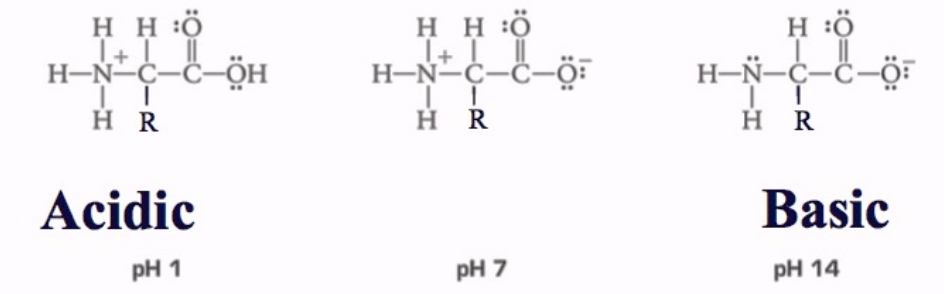


histidine

Properties of Amino Acids

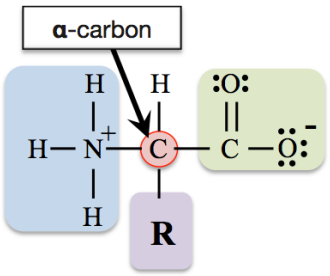
Although some amino acids contain hydrophobic side-chains, overall they are _____ - _____.

All amino acids are _____ due both to the presence of polar covalent bonds that are capable of forming hydrogen bonds with water, and to the fact that they can carry charges ($-COO^-$ and/or $-NH_3^+$).



Stereoisomerism of Amino Acids

With the exception of glycine, all of the α-amino acids are _____ because the α-carbon atom in each is attached to four different groups.



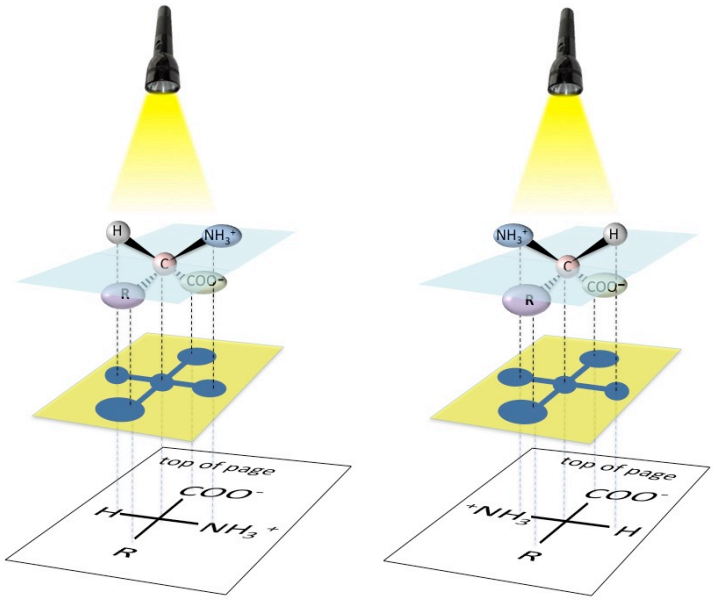
The presence of chiral carbons produces stereoisomers with mirror images: _____.

Fischer projections of amino acids have the *carboxylate group* on **top** and the *side-chain* on the **bottom**.

- **L-amino acids** have the NH_3^+ on the _____.
- **D-amino acids** have the NH_3^+ on the _____.

Organisms use only L-amino acids to produce proteins.

You try one: Draw Fischer projections of the D-isomer and the L-isomer of ***alanine***.



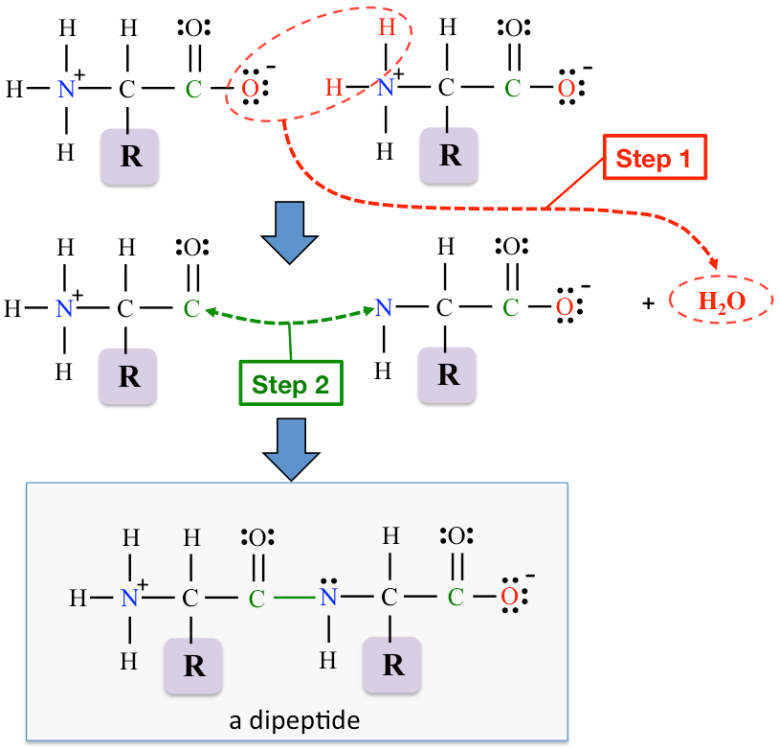
D-amino acid L-amino acid

Peptides and Proteins

The Peptide Bond

Peptides and proteins consist of amino acid residues joined by _____ (amide) bonds.

Formation of a Peptide Bond

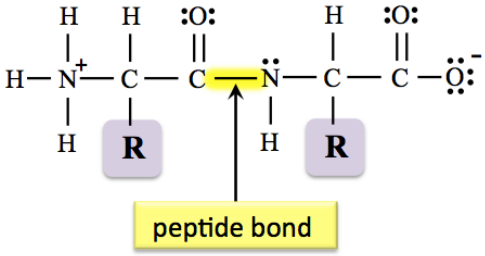


Step 1: The two amino acids are drawn side-by-side. The single-bonded *oxygen atom* is removed from the *carboxylate group* on the *left-most* amino acid. *Two* hydrogen atoms are removed from the *quaternary ammonium group* on the *right-most* amino acid. The oxygen atom and the two hydrogen atoms combine to form a water molecule.

Step 2: A ***new bond*** is made between the carbonyl carbon and the nitrogen.

The peptide formed in this example is called a _____because it contains ***two*** amino acid residues.

The ***new bond*** between the two amino acid residues is called a **peptide bond**.



You try one: Draw the structural formula of the ***dipeptide*** that contains *two* ***valine*** amino acid residues. Label the peptide bond

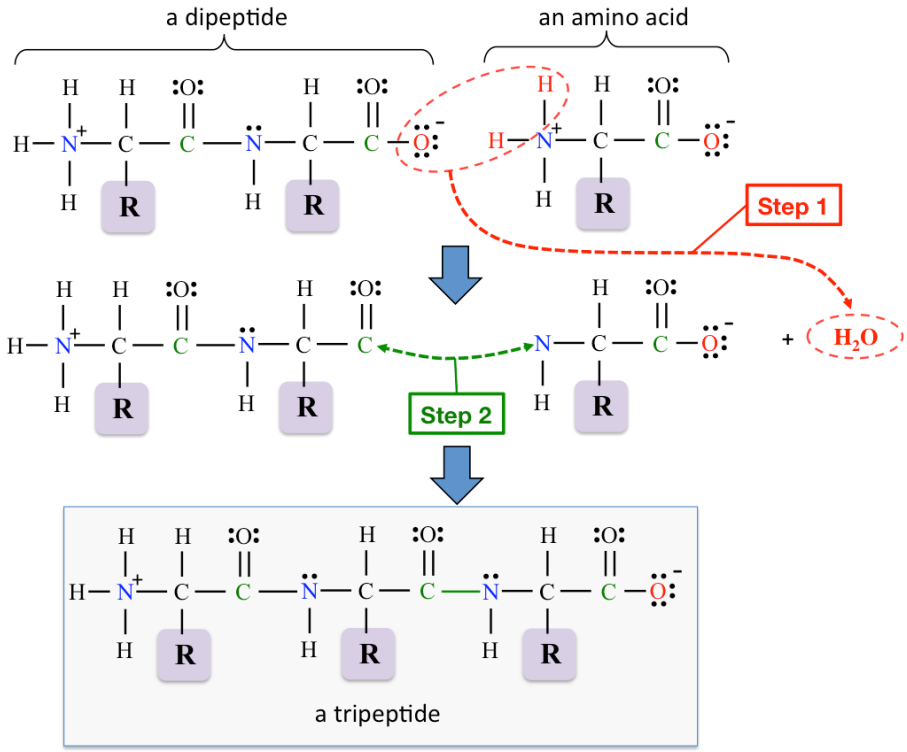
.

Formation of Larger Peptides

Larger peptides are formed by adding more amino acids, one by one, to a growing peptide.

Example: Formation of a Tripeptide

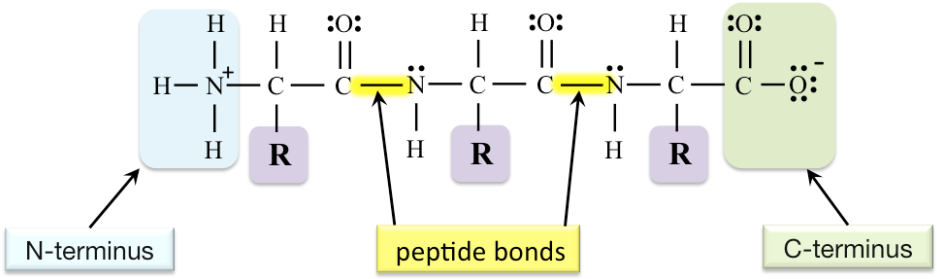
- Begin with the general form of a ***dipeptide*** and then add a *new amino acid residue.*
- The new ***peptide bond*** can be made using the same **two steps** as we used when we made a *dipeptide*.



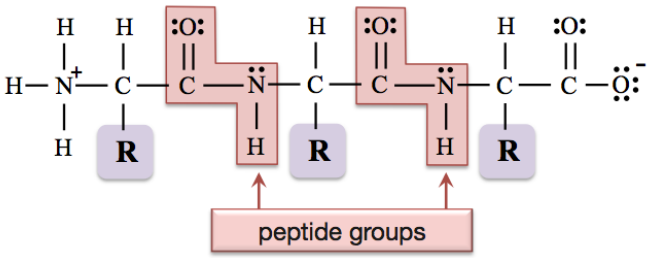
This process can continue and larger peptides can be formed by adding more amino acids, one by one, to a growing peptide.

Peptide Terminology

The ***end*** of the peptide structural formula that has a ***quaternary ammonium group*** is called the ____**-terminus**, and the ***end*** that has a ***carboxylate group*** is called the _____**-terminus**.



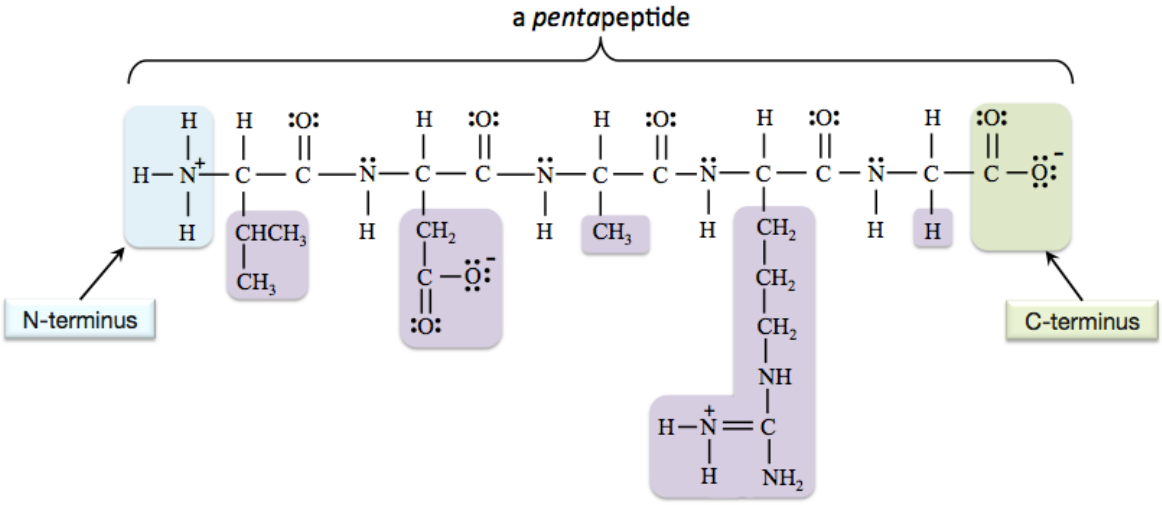
The bonding pattern around a *peptide bond* is called the **peptide** _____.



Note that ***nitrogen in a peptide group does not*** have a (1+) formal charge, as does the *nitrogen in the quaternary ammonium group at the N-terminus.*

Peptides are identified by the use of a common name ***or***, by listing its amino acid residues' *three-letter abbreviations* in order from N-terminus to C-terminus.

Example of identifying a peptide from its amino acid residue's *abbreviations*: **Val-Asp-Ala-Arg-Gly**.



Val-Asp-Ala-Arg-Gly

I drew this pentapeptide by forming peptide bonds between the predominant forms of the amino acids at physiological **pH**, therefore the resulting pentapeptide is also in the form that is predominant at physiological **pH**. Note that *two* of the *side-chains* in this peptide carry a formal charge. This peptide has a total charge equal to ***zero*** because the *two negative charges* and *two positive charges* add up to ***zero***.

You try one:

a. Draw the *structural formula* for the predominant form of Gly-Lys-Tyr-Ala at physiological **pH**.
b. Label the **peptide bonds** and *circle* the **peptide groups**.

NOTE: If you correctly connect the amino acid structural formulas from the amino acid table, then the peptide that you draw will be the predominant form at physiological **pH**.

Also: What is the ***total charge*** of the peptide that you drew for in the previous problem?

Examples of Biologically-Relevant Peptides

A **protein** consists of one or more ***large peptides*** and has ***a specific biological function***. Although shorter peptide chains (less than about *fifty* amino acid residues) have specific biological functions, they are generally not classified as proteins. Short peptide chains function as chemical signaling compounds; over one hundred of them have been identified.

Endorphins are examples of chemical signaling peptides. They are natural painkillers that are produced in the body. They interact with receptors in the brain to inhibit the transmission of pain signals. Five endorphins have been found (so far). An example of an endorphin peptide is ***α-endorphin***. It contains sixteen amino acid residues, which are connected in the sequence (N-terminus to C-terminus) shown below:

Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr

Another example of a peptide is ***oxytocin***. It is produced by the pituitary gland, and stimulates uterine contractions in labor. *Oxytocin* contains *nine* amino acid residue, which are connected in the sequence shown below:

Cys-Tyr-Ile-Gln-Asn-Cys-Pro-Leu-Gly

Oxytocin was the first naturally-occurring hormone to be produced in a laboratory.

Protein Structure

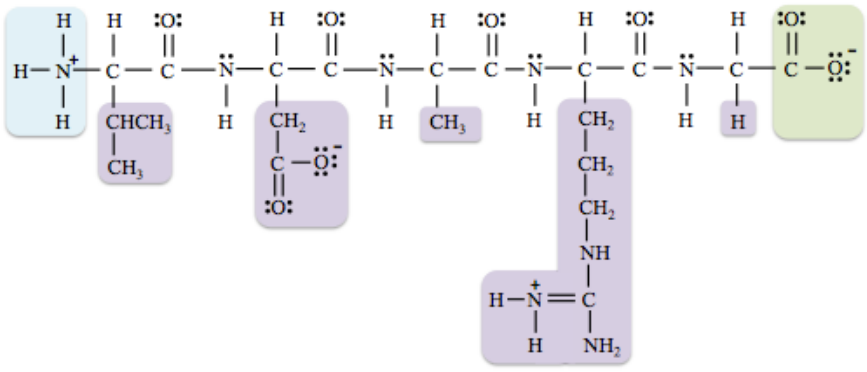
The structure of proteins is understood in terms of four levels of organization:

1) _____
2) _____
3) _____
4) _____

Primary Protein Structure

The _____ of amino acid residues in a peptide or protein is referred to as its ***primary structure***.

- Example: The primary structure of the pentapeptide is shown below:



Primary Structure:

valine asparagine alanine arginine glycine

(Val-Asp-Ala-Arg-Gly)

The primary structure of peptides and proteins is analogous to the arrangement of letters in a word.

edit ≠ diet ≠ tide ≠ tied

Primary structure of a protein is the linear sequence of amino acids connected by peptide bonds.

- Different proteins typically contain from about 40 to over 4000 amino acids
- There are 400 distinct dipeptides (20^2).
- There are 8000 distinct tripeptides (20^3).
- When there are 100 amino acids in the chain, there are $20^{100} = 1.27 \times 10^{130}$ distinct peptides!

Understanding Check: Write the names (using the three letter abbreviation method) of all of the tripeptides that can be made by combining one glycine (gly), one alanine (ala), and one aspartic acid (asp) residue. For example, one of the tripeptides is gly-ala-asp.

Secondary Protein Structure

The properties of proteins depend not only on their sequence of amino acid residues, but also on how they are folded, twisted, and bent.

Secondary protein structure describes the geometric patterns that occur when individual peptide chains "fold" back on themselves.

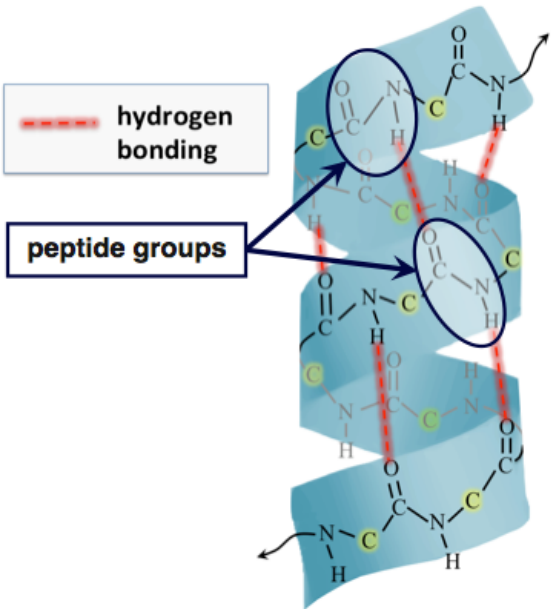
Secondary structure results from _____ _____ between ***peptide groups*** within an individual peptide.

There are ***two common types*** of secondary structures, the _____ _____ **(*α* helix)** and the _____ _____ **(β sheet)**.

The Alpha Helix

The *alpha helix* geometric pattern resembles a

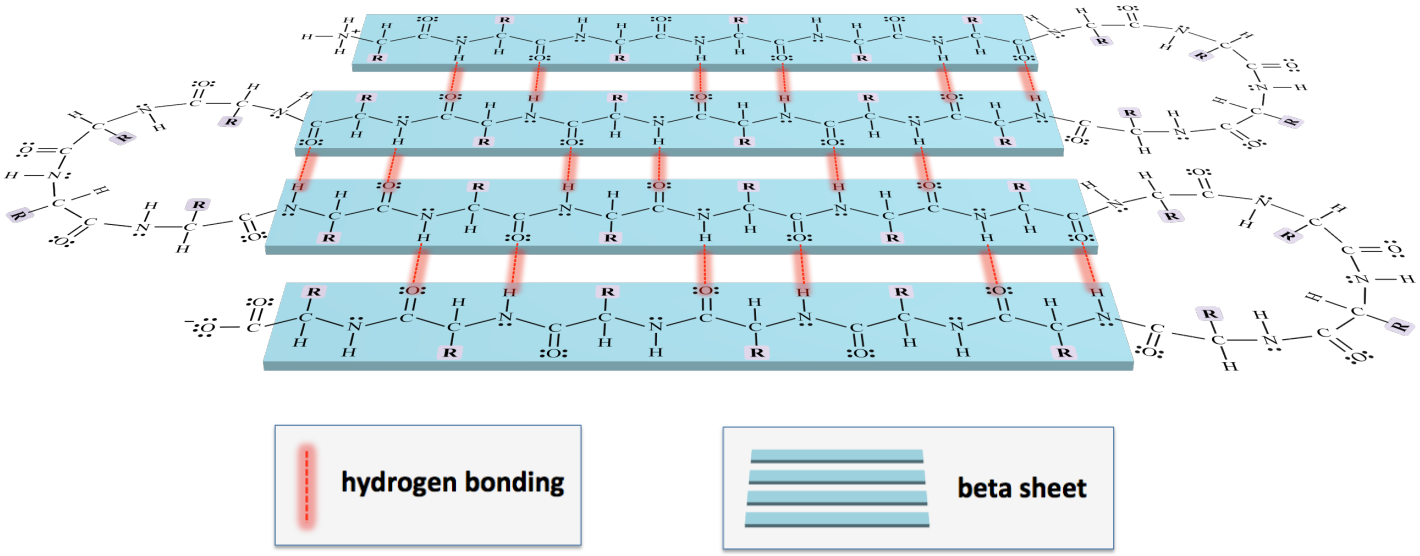
_____.



The Beta Sheet

The **beta sheet** geometry occurs when a peptide folds back on itself in a _____ arrangement.

Illustrative Model of a Beta Sheet



In addition to *alpha helices* and *beta sheets*, there are a few other, much less frequently seen geometries that are also categorized as secondary structures. Since these other secondary structures are relatively rare, I will not discuss their particularities.

A key feature of *secondary protein structure* is that it **only** involves ***hydrogen bonding between peptide groups within an individual peptide chain***.

Tertiary Protein Structure

Alpha helices and/or beta sheets, along with the *unorganized sections* of a peptide chain, "***fold***" into a more compact shape.

- The _____ shape of a peptide is called the **tertiary structure**.

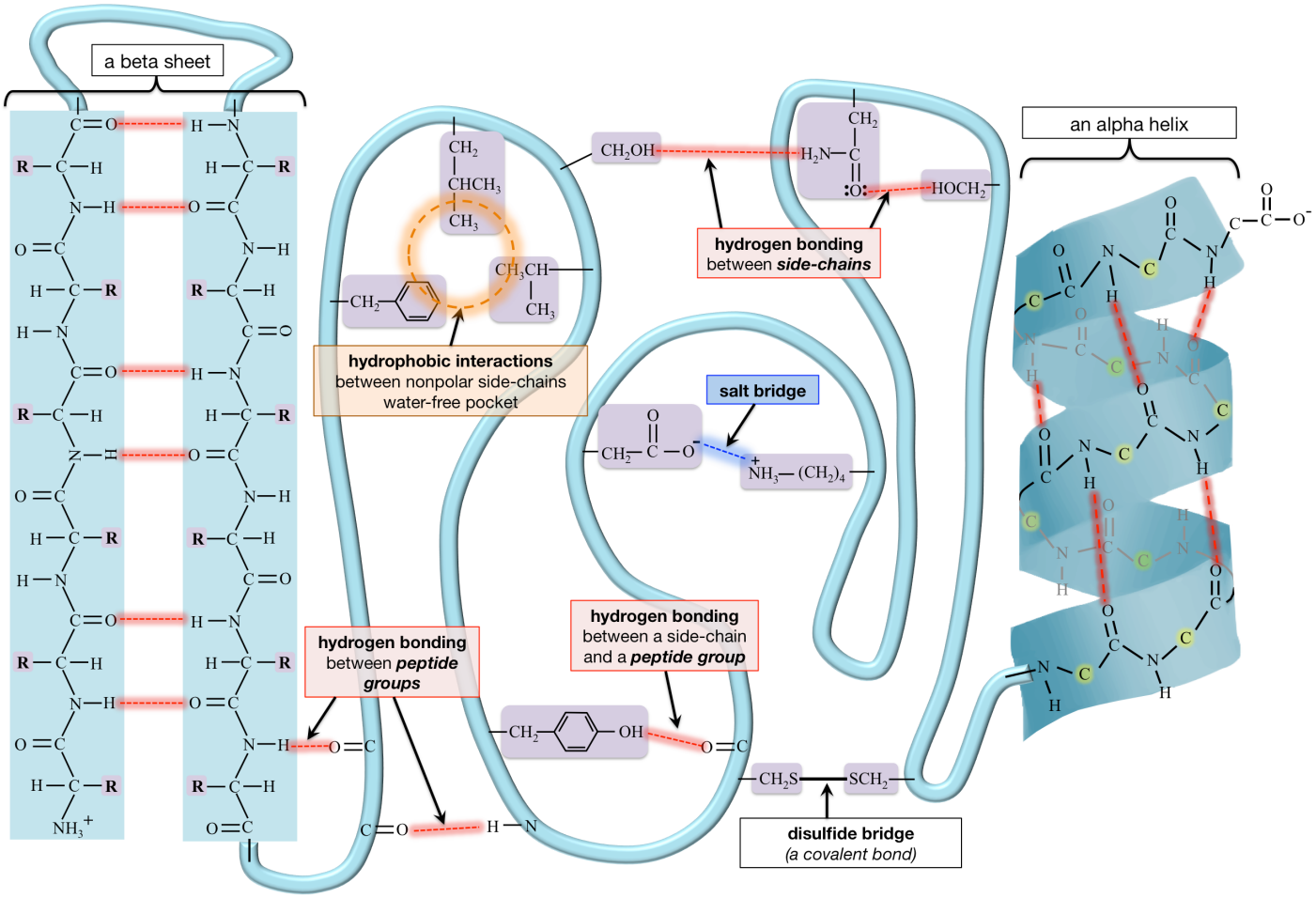
"***Ribbon models***" are often used in order to visualize tertiary protein structure. These illustrative models use ribbon-like shapes to represent the geometry of secondary structures. The spring-like ribbons represent alpha helices and the flat side-by side ribbons represent beta sheets. Sometimes arrows are used at the ends of ribbons to indicate the direction (from N-terminus to C-terminus). Lines or thin tubes are used for *unorganized sections* of a peptide chain. The ribbon model for *ribonuclease A* protein (RNase A), an enzyme used to break down RNA, is shown on the right.

Ribbon Model of an *RNase A* Protein

Of the many folding patterns (conformations) possible for a protein, there is usually only one that leads to a _____(biologically active) molecule.

The sequence of amino acids (primary structure) ultimately determines which folding pattern is selected, so both secondary and tertiary structure _____on primary structure.

Some of the interactions that are involved in **tertiary structure** are illustrated below.



Description of Tertiary Structure Interactions:

1) Hydrophobic Interactions

Nonpolar side-chains are attracted to ***other*** *nonpolar side-chains* through *London forces,* and form "**water-free pockets**" in the interior region of the folded and compacted peptide (see the illustration above).

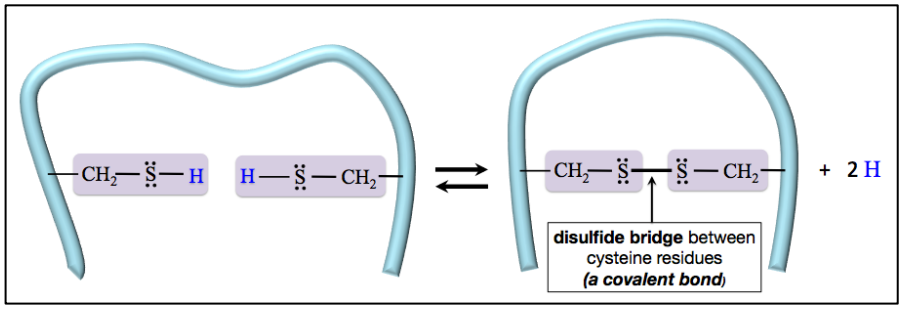
2) Hydrogen Bonding

Hydrogen bonding in *tertiary structures* can occur between polar side chains (that contain the features necessary for hydrogen bonding) and/or peptide groups. See the illustration above.

3) Salt Bridges

I introduced ***salt bridges*** to you, in chapter 4, as one of the *five noncovalent interactions*. A *salt bridge* is an attractive force between the ***positive*** formal charge on *polar basic* amino acid residue and a ***negative*** formal charge on a *polar acidic* residue (see the example in the illustration above).

4) Disulfide Bridges



In a previous chapter, you learned that *disulfide (covalent) bonds* can be formed by the oxidation of two thiol (**SH**) groups. *Disulfide bonds* in proteins are called ***disulfide bridges***. Each *cysteine* residue contains a thiol group in its side-chain that is capable of forming a disulfide bridge with *another* cysteine residue**,** as shown above.

5) Dipole-Dipole and Ion-Dipole Forces

Dipole-dipole attractive forces can occur between polar side-chains and/or peptide groups. These interactions are not included in the illustration on the previous page. If needed, you can review dipole-dipole and ion-dipole interactions in section 6 of chapter 4.

Quaternary Structure

A large number of native proteins are a combination of

_____ _____ _____ polypeptide chain.

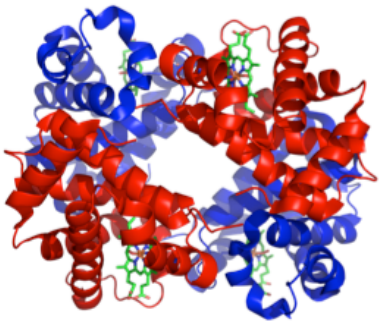


Image source: Wikimedia Commons, Author: Richard Wheeler, CC-BY-SA, http://creativecommons.org/licenses/by-sa/3.0/legalcode

- **Example:** *Hemoglobin*

Quaternary protein structure is the overall shape that occurs when **two or more** _____ *peptide chains* assemble to make a protein.

In proteins composed of ***two or more*** peptide chains, the individual peptide chains are referred to as "**subunits**."

The quaternary structures of large proteins are sometimes depicted using *space-filling models*. In these models, the various subunits are often shaded with different colors or grey-scale tones.

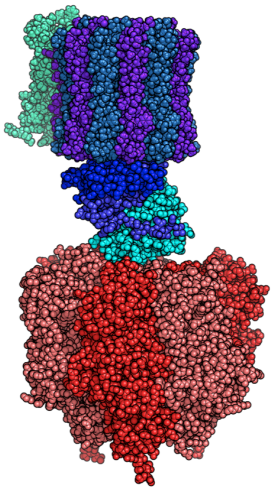


Image source: Wikimedia Commons, Author: Alex.X CC-BY-SA, http://creativecommons.org/licenses/by-sa/3.0/legalcod

- **Example:** *ATP synthase*

The forces that hold the subunits together in *quaternary structures* are **the same** as those involved in *tertiary structures*.

Understanding Check: In which of the following levels of protein structure can hydrogen bonding play a role?

a) primary structure
b) secondary structure
c) tertiary structure
d) quaternary structure

Globular, Fibrous, and Membrane Proteins

Proteins generally fall into one of three categories:

1) _____ proteins
2) _____ proteins
3) _____ proteins

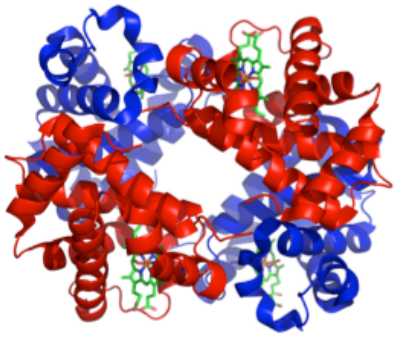
Globular Proteins

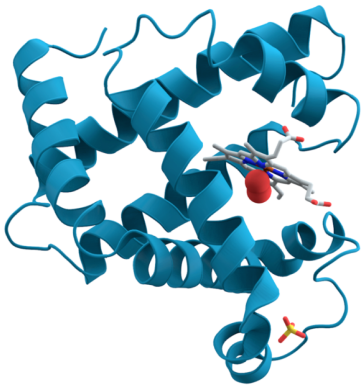
Globular proteins have a highly- _____ and compact shape.

- The overall shapes of these proteins are more "sphere-like" than "string-like."
- The globular shape allows for *hydrophobic* side-chains to be directed to the protein's interior (forming "water-free pockets"), while *polar* side-chains are oriented outward to form a *hydrophilic* exterior. The hydrophilic exterior allows globular proteins to be more easily dispersed in solutions (intercellular and extracellular).

Globular proteins function as enzymes, chemical signaling compounds, transporters of other compounds, and antibodies.

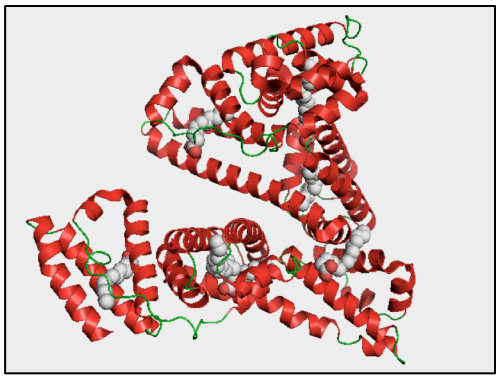
Hemoglobin (shown on the right) is an example of a globular protein





Myoglobin (shown on the left) is another example of a globular protein. It is used to store oxygen (O_2) in muscle tissue, thereby allowing organisms to function while holding their breath. *Myoglobin* is responsible for the red color of meat. It is found in especially high concentration in diving animals, such as seals and whales. It is composed of just one peptide chain. Human myoglobin contains 153 amino acid residues and eight alpha helices. It contains a *heme* prosthetic group (shown in grey) that binds oxygen (shown as red spheres next to the heme group).

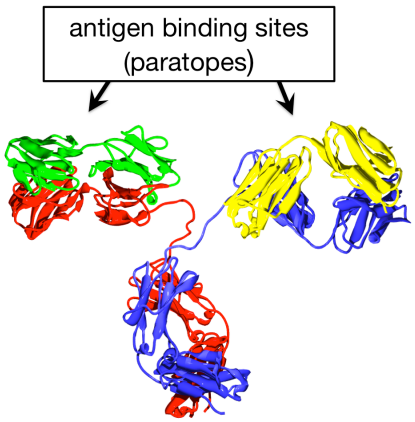
Albumin is another example of a globular protein.



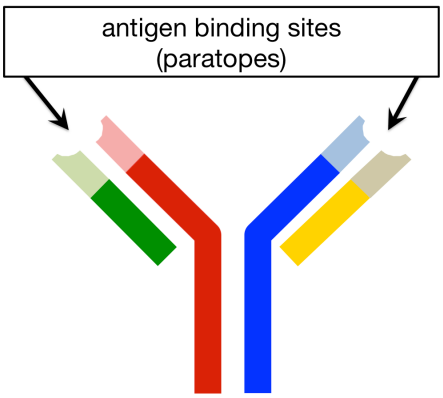
Human *albumin* is the most abundant protein in human blood plasma. Its biological functions include transporting hormones, fatty acids, and other compounds, acting as a buffer, and maintaining osmotic pressure.

Antibodies, also referred to as **immunoglobulins** (Ig), are *globular proteins*. Antibodies are able to act as protective agents by binding to specific, usually harmful, objects - called *antigens*. Antigens are often foreign (nonself) objects such as harmful bacteria or viruses. When an antibody binds to an antigen, it either directly neutralizes the antigen, or marks it so that the antigen can be subsequently neutralized by other components of the immune system.

The smallest antibodies are composed of a *single* immunoglobulin unit, called an **immunoglobulin monomer**. An immunoglobulin monomer is shaped a bit like the letter "Y," and is often illustrated using a Y-shape, as shown on the *right-hand side* of the illustration.



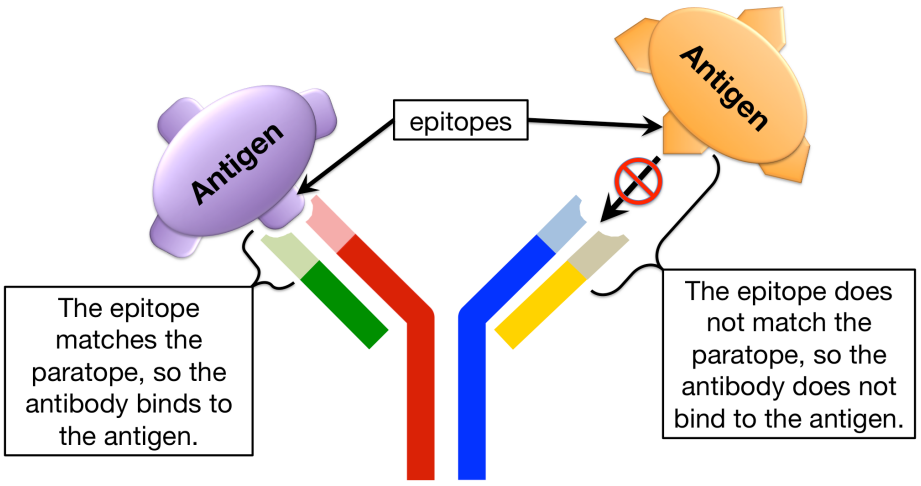
Ribbon model of an immunoglobulin monomer



"Y-shape" model of of an immunoglobulin monomer

An antigen binding site, called the **paratope**, binds to a particular part of an antigen called the **epitope**.

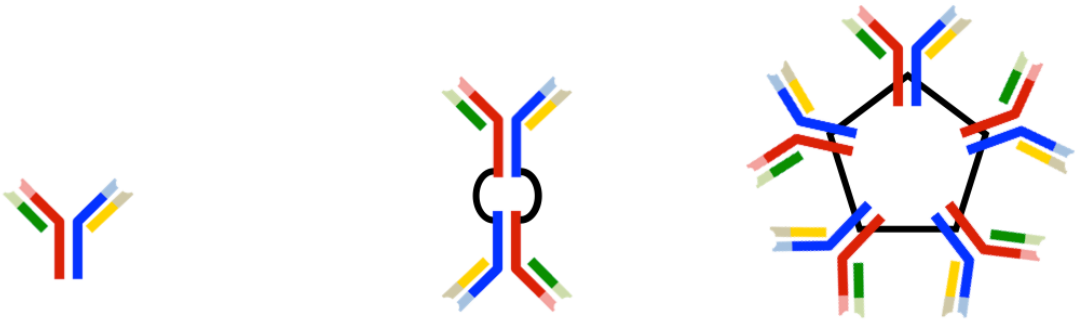
- The binding between antigen and antibody occurs because of ***noncovalent attractive forces****, which are maximized due to the* _____ *shapes of the paratope and epitope.*



Note that in the Y-shape antibody model shown on the left, the *paratope* will only bind with one of the two antigens - the antigen that has a complementary epitope. We say that the paratope (or antibody binding site) is "_____" for a particular epitope.

The immune system can produce an almost infinite variety of paratope shapes by varying the paratope region's amino acid sequence (and therefore its shape). By doing so, antibodies are produced to be *specific* for one particular antigen, much like a lock is *specific* for one key.

Some antibodies contain ***more than one*** *immunoglobulin unit.* Placental mammals, which includes humans, have immunoglobulin monomer, immunoglobulin dimer, and immunoglobulin pentamer antibodies. Immunoglobulin *dimers* are made from ***two*** immunoglobulin monomers, and immunoglobulin *pentamers* are made from ***five*** immunoglobulin monomers. These three types of antibody structures are illustrated below.



immunoglobulin monomer
(single immunoglobulin unit)

immunoglobulin dimer

immunoglobulin pentamer

One last note on antibodies:

Antibodies have _____ (oligosaccharides) that are covalently bound to ***some*** of their amino acid residue *side-chains*.

- Proteins, such as antibodies, that contain carbohydrates are called _____.

Fibrous Proteins

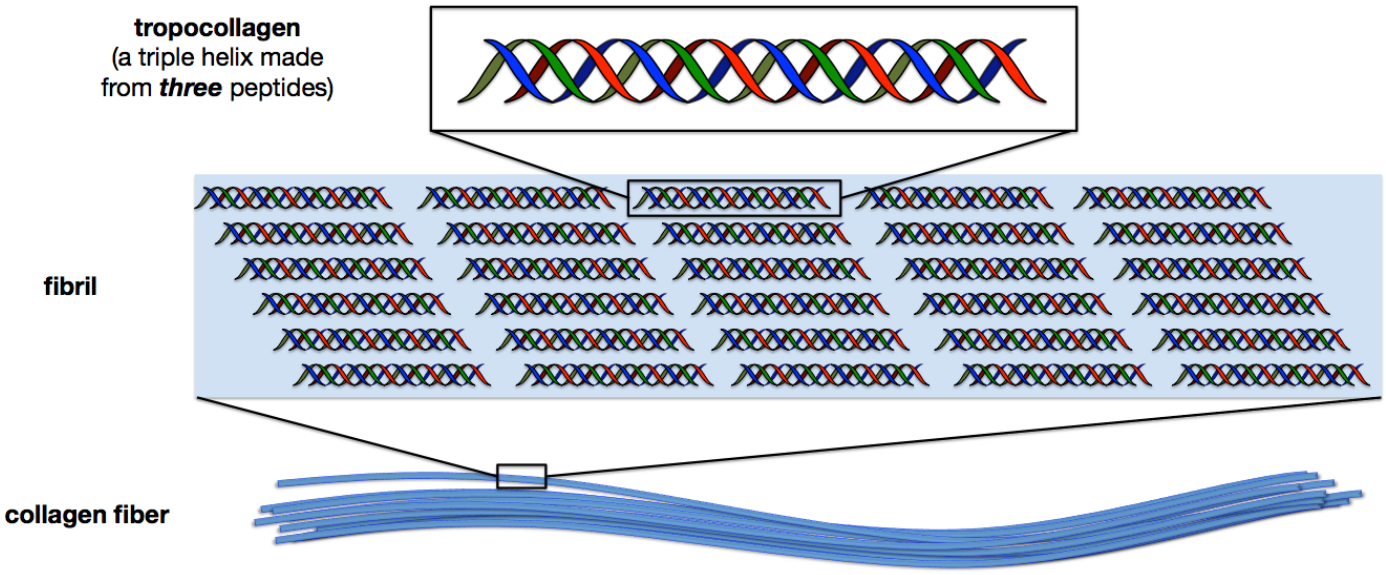
Fibrous proteins have long and narrow "_____ -like" shapes.

- They are *much less compact* than *globular* proteins.

The narrower shape makes it difficult for hydrophobic side-chains to be oriented toward the interior region of a fibrous protein, and results in a *hydrophobic* exterior. For this reason, fibrous proteins tend to be water-insoluble.

Fibrous proteins play important roles in providing structural rigidity and in contractile movement (muscles).

An example of a *fibrous protein* is ***collagen***. *Collagen* is the most abundant protein in the body. Its function is to provide structural rigidity and stiffness. It is found in skin, ligaments, tendons, and other parts of the body. An illustration of the components of *collagen* are shown below.

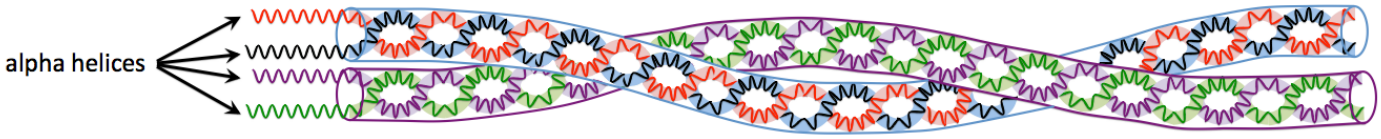


Other examples of fibrous proteins are **keratins**. Their primary role is to provide structural rigidity and stiffness. *Keratins* are some of the strongest natural materials.

- K*eratins can be* classified as **alpha-keratins** or **beta-keratins**.

Alpha-keratins are found in places such as hair, wool, horns, hooves, claws, and nails.

In hair, *two* peptide ***double*** helices are twisted around each other to form a **protofibril**, as shown below.

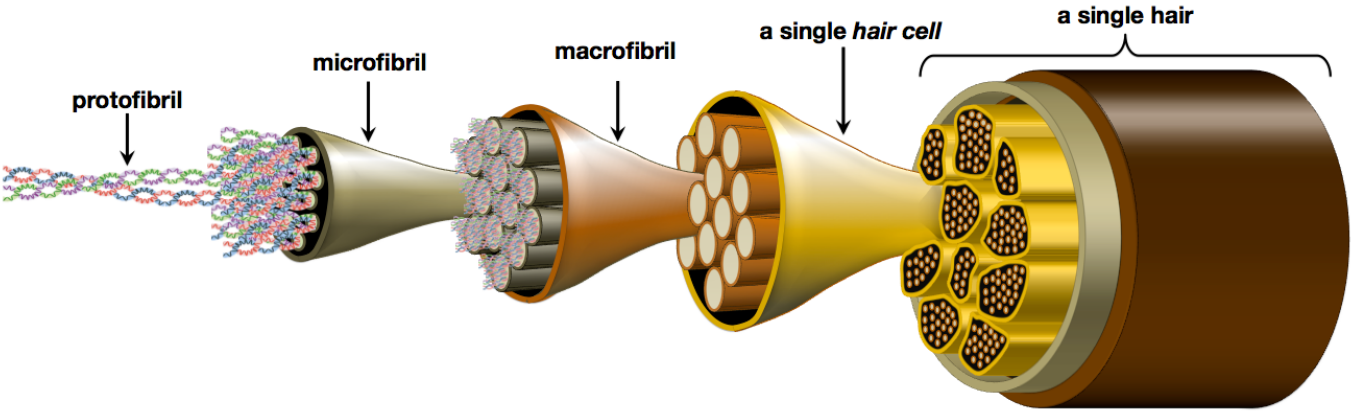


a protofibril

two peptide ***double helices*** that are twisted around each other

Protofibrils bundle together to form **microfibrils**. *Microfibrils* bundle together to form **macrofibrils**. Each ***hair cell*** is primarily composed of bundled *macrofibrils*.

A **single hair** consists of bundled hair _____.

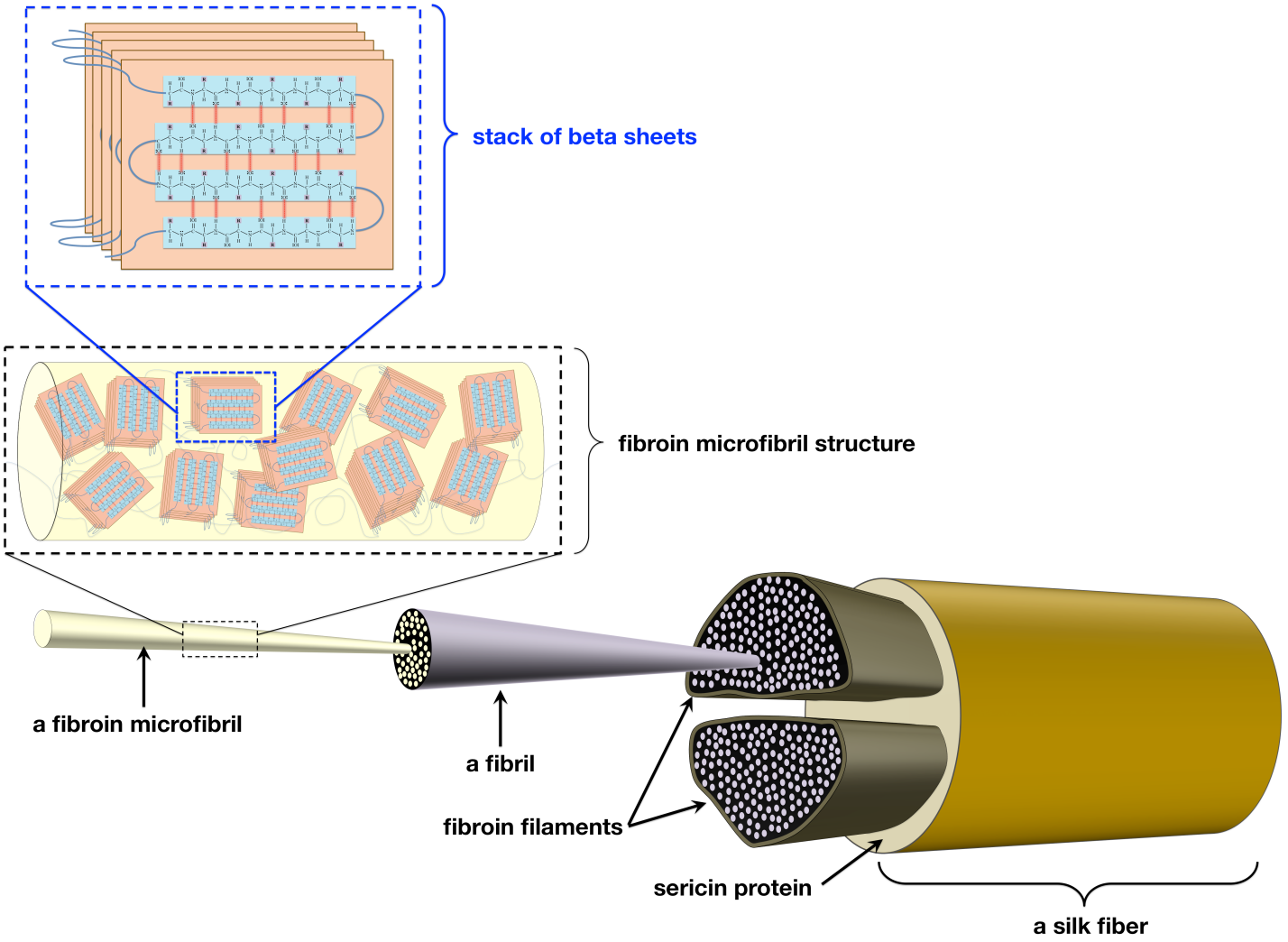


Beta-keratins

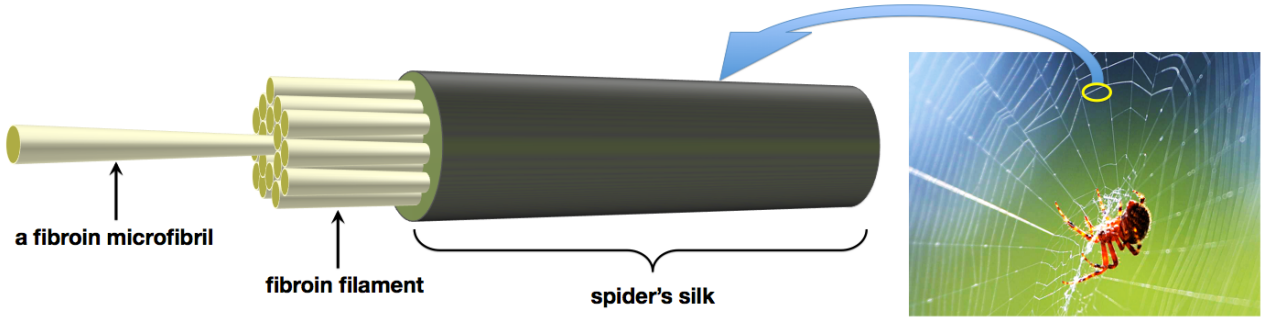
Beta-keratins, which are also fibrous proteins, are found in places such as reptilian skin, the outer layer of human skin, bird feathers and beaks, turtle shells, silk, and the tongue.

- Beta-keratins are composed of fibers that primarily contain ***beta sheet*** *secondary* structures.
- The *beta sheets* are stacked in _____ *tertiary* structures.

An example of a beta-keratin structure can be seen in **silk**. The stacked beta sheets, which are held together by disulfide bridges and noncovalent interactions, entwine to form a **fibroin microfibril**. *Fibroin microfibrils* assemble to form **fibrils.** *Fibrils* assemble to form **fibroin filaments**. Two *fibroin filaments* are held together by *sericin protein*, which acts like a glue to hold the two fibroin filaments together in a single silk fiber, as shown below.



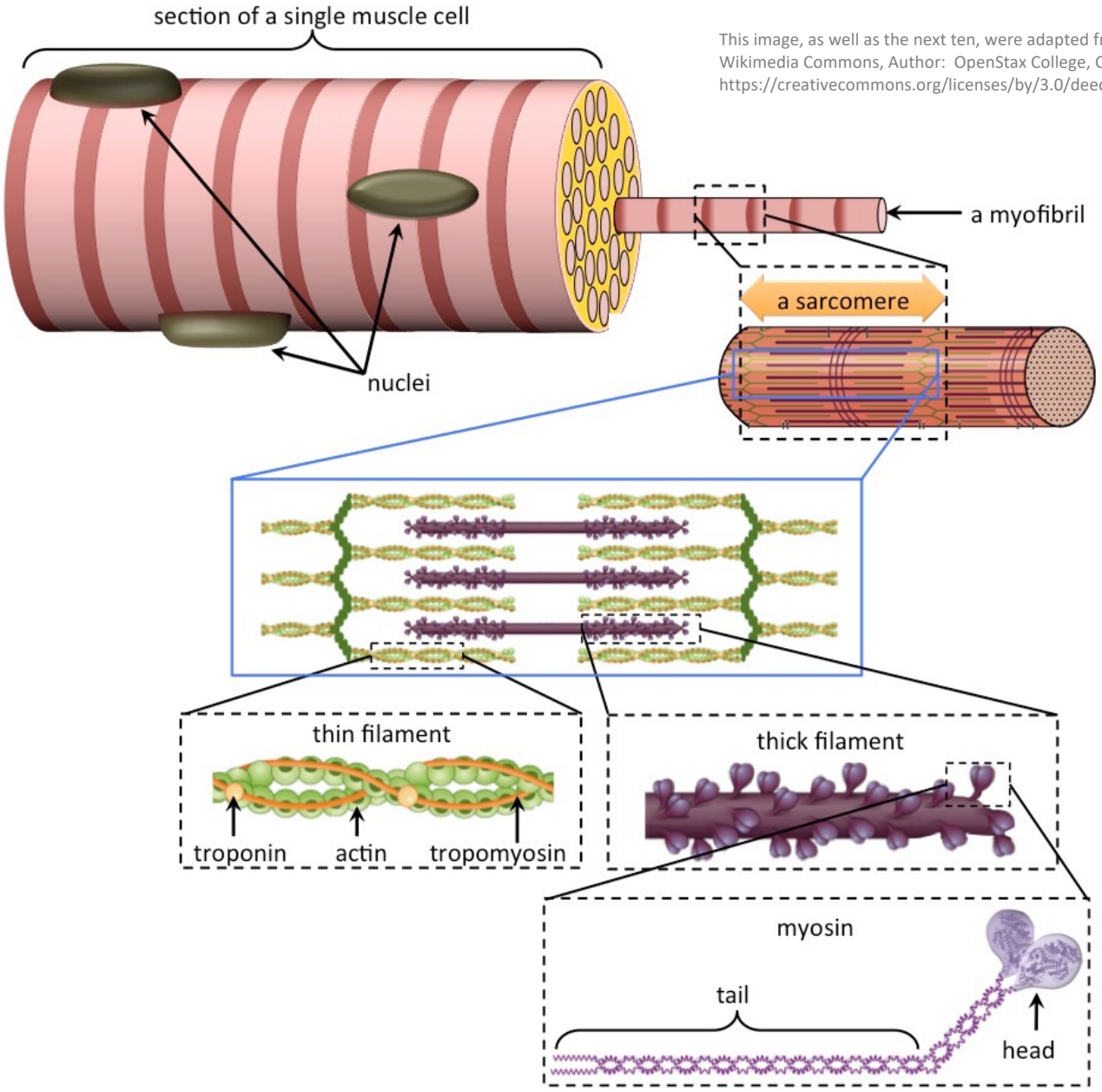
Fibroin microfibril keratin fibers are also found in spider webs. The structure of *spider's silk* is illustrated below.



Fibrous Proteins in Muscles

Muscle contraction involves the interaction of *fibrous proteins*.

Muscles are composed of bundled muscle_____.



A muscle cell is a polynuclear (many nuclei) cell that contains long protein fibers called **myofibrils**.

Myofibrils are composed of individual contractile units called **sarcomeres**.

Sarcomeres contain fibrous proteins called "_____ **filaments**" and "_____ **filaments**."

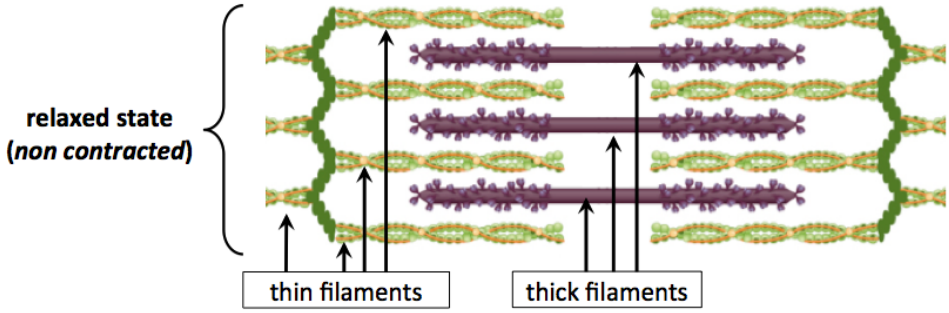
Thick filaments are composed of **myosin** fibrous protein.

- The ***myosin tail region*** is composed of two alpha helices that are twisted around each other.
- In the ***myosin head region***, the individual alpha helices split apart from each other and fold into more compacted tertiary structures.

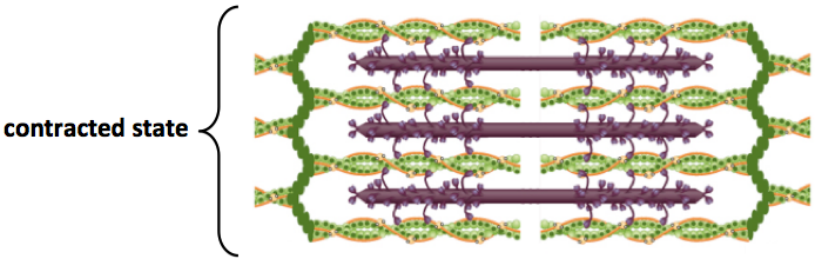
Thin filaments are composed of three proteins: actin, troponin, and tropomyosin.

How Muscles Work: The Sliding Filament Model

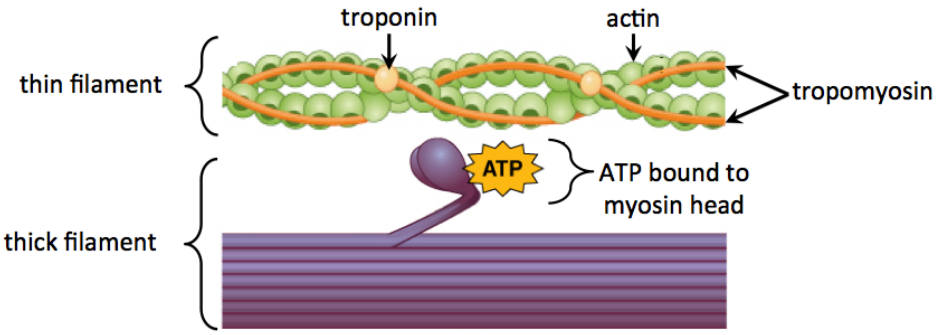
Before contraction, the thick and thin filaments are in a relaxed state (non contracted), as shown in the top of the figure on the right.



During muscle contraction, the *thick filaments* and *thin filaments* "slide" past each other to shorten (contract) the overall structure, as shown in the contracted state in the bottom of the figure.



Let's now consider how and when the filaments "slide" past each other. We will begin with a small section of a thick and a thin filament in the state illustrated below.



In this initial state, adenosine triphosphate (ATP) is attached to the head region of myosin.

The chemical energy stored in _____ is used to make the muscle contract.

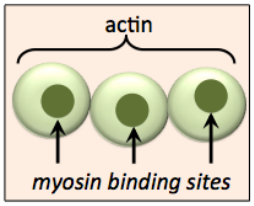
- The hydrolysis of ATP reaction is capable of releasing energy:

$$ATP\ (aq)\ +\ H_2O\ (l)\ \rightleftarrows\ ADP\ (aq)\ +\ P_i\ (aq)\quad \mathbf{\Delta G = -7{,}300}\ \text{Joules per mole of ATP}$$

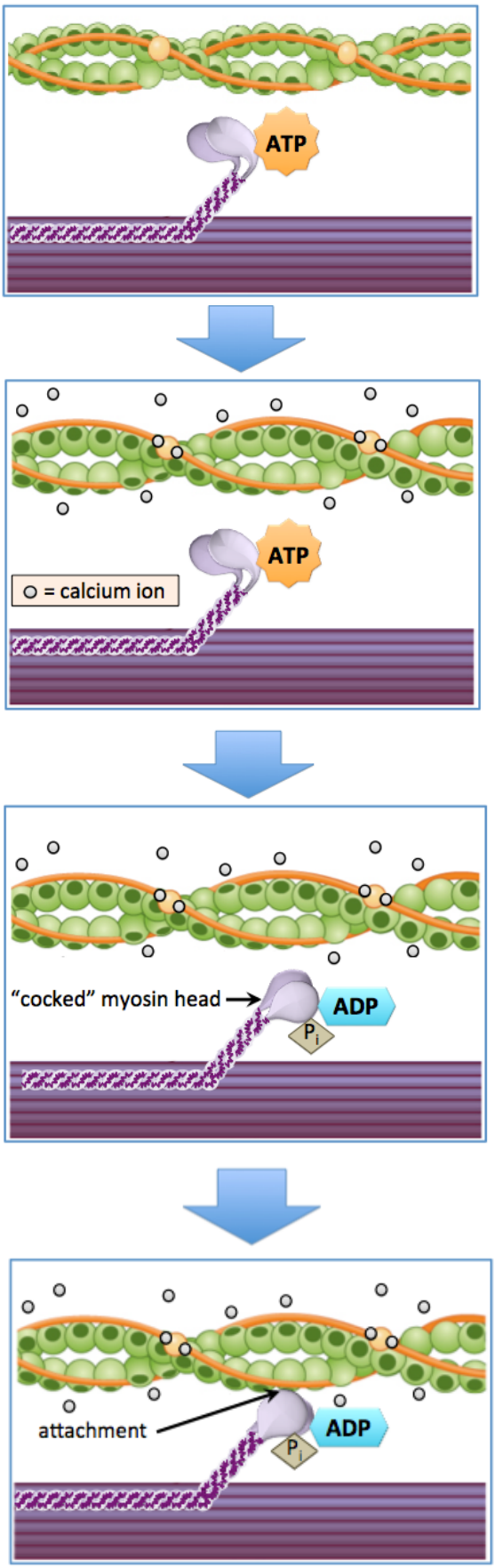
P_i is an abbreviation for a phosphate group, and ADP is ***a****denosine* ***di****phosphate*.

The energy released by this reaction can be used to slide the thin and thick filaments past each other.

1) *Actin* contains sites to which *myosin* heads can bind.



In our initial state, *tropomyosin fibers* block *actin's* myosin binding sites so that the myosin heads are unable to attach to the *thin filament,* as shown in the illustration on the right.



2) Muscle contraction begins in response to an action potential (nerve impulse) that originates in the central nervous system.

   - The electrical signal is transferred to a particular muscle and causes an organelle called the sarcoplasmic reticulum to release calcium ions.
   - When calcium ions are released, they bind to *troponin*, which causes the *tropomyosin fibers* to move and thereby exposes the *myosin binding sites*.

3) ATP is hydrolyzed to ADP and P_i. Energy released from the hydrolysis of ATP reaction ***is used to change the conformation (shape) of myosin***. This results in a "cocked" myosin head.

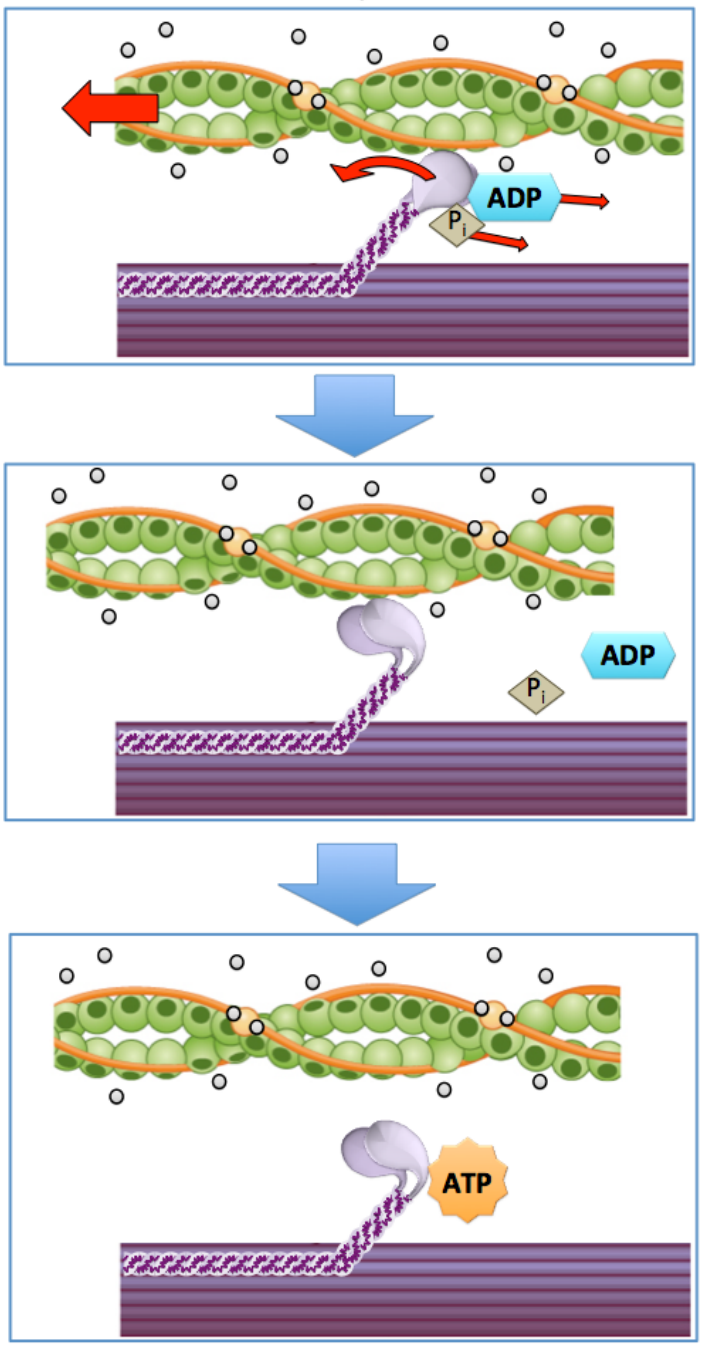
   - This is analogous to "cocking the hammer" of a pistol, or pulling back on the string of a bow-and-arrow. In this step, the ADP and Pi that are produced remain attached to the "cocked" myosin head, as shown on the right.

4) The "cocked" myosin head attaches to a myosin binding site on the thin filament. This attachment is a noncovalent interaction.

5) ADP and P_i are released from the myosin head. This allows the myosin to bend back to its original "un-cocked" position.

- In our "cocked" pistol analogy, this step represents what happens when the trigger of a pistol is pulled: the pistol's "hammer" springs forward (to strike the bullet's cartridge).
- In our *bow-and-arrow analogy*, this step represents what happens when the string is released: it moves forward and accelerates the arrow.

Because the myosin head is attached to the thin filament, as the myosin bends, the thin filament "slides" past the thick filament.



6) ATP binds to the myosin head, which causes the head to detach from the thin filament. ***This completes the cycle***; the system is now back to its original configuration and the cycle can repeat so long as calcium and ATP are present. As this cycle repeats, the muscle can continue to shorten. Since calcium ions are constantly being transported ***back*** *into the sarcoplasmic reticulum*, their release must be continuously induced by central nervous system impulses in order for muscle contraction to continue.

If ATP is not present, the myosin remains bound to the thin filament. This state is observed after death, since ATP is no longer produced, and is called *rigor mortis*.

Membrane Proteins

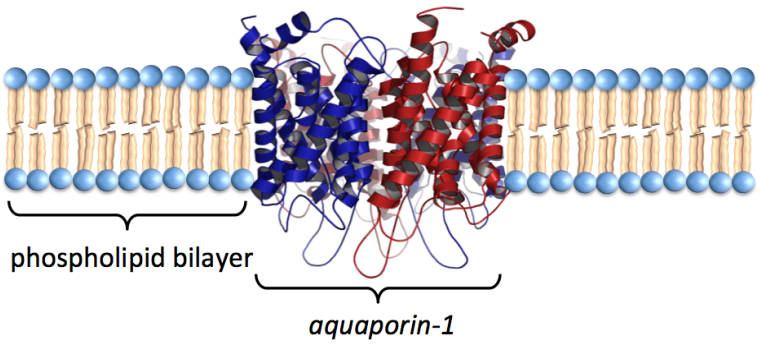
Membrane proteins are proteins that are _____ to biological membranes.

Membrane proteins function as enzymes, cell recognition markers, receptors (allowing chemical signals to be relayed between the interior and exterior of cells), and transporters of compounds in and out of cells.

Some membrane proteins extend through the _____ membrane and are called **transmembrane proteins**.

Examples of ***transmembrane proteins*** include the *aquaporins*.

- *Aquaporins* function as *transporter proteins;* they facilitate the transport of water molecules (only) in and out of cells. There are several types of aquaporins, one of them, *aquaporin-1*, is illustrated on the right.



Source: The protein structure is from Wikimedia Commons, Author: Vossman
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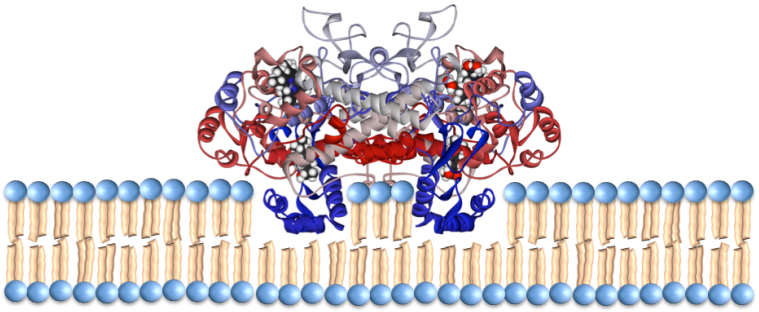
Some membrane proteins ***do not*** *completely extend through* the membrane; these are called

_____ **proteins**.

An example of a ***monotopic protein*** is *cyclooxygenase-2*.

- *Cyclooxygenase-2* is responsible for converting eicosanoic acid into prostoglandins, prostoscyclin, and thromboxane (you learned about this enzyme and these reactions in a previous chapter). An illustration of cyclooxygenase-2 attached to a membrane is shown on the right.



Understanding Check: Globular vs. Fibrous vs. Membrane Proteins

Do a bit of online research to determine if *succinate dehydrogenase* is a ***globular***, ***fibrous***, or ***membrane*** protein.

Prosthetic Groups: Simple vs. Conjugated Proteins

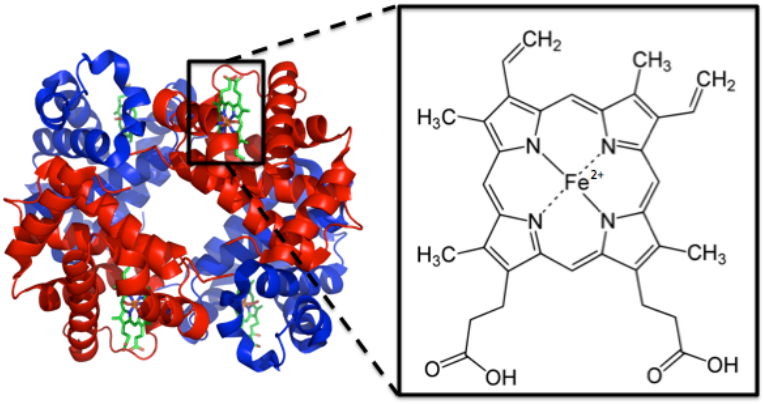
Some proteins contain only amino acid residues, these are called _____ **proteins**.

Other proteins contain amino acid residues ***and*** _____ amino acid components.

Proteins that contain non amino acid components are called _____ **proteins**.

- The *non* amino acid components of these proteins are called _____ **groups**.
- An example of a *prosthetic group* is the *heme* group, which is present in *hemoglobin*. The main role of *hemoglobin* is to transport oxygen (O_2) molecules. Heme groups contain an iron ion, to which an oxygen molecule can be quite strongly attached.

A ribbon model of human hemoglobin, with a magnification insert showing the bonding pattern in one of its four heme groups, is shown below.



Hemoglobin contains *four* heme groups (shaded green in the figure above). Each heme group is capable of binding one oxygen molecule. Heme prosthetic groups are also found in *myoglobin*, *catalase*, and other proteins.

Denaturation of Proteins

The shape of a protein is the key factor in its ability to perform its biological role. ***Protein shape is maintained by the attractive forces involved in secondary, tertiary, and quaternary structures***. When these attractive forces are disrupted, the native shape of proteins can be changed enough that a partial or complete loss of bioactivity (function) occurs.

When a protein loses some or all of its biological activity in such a manner, this is referred to as

"**protein** _____."

In most cases, unless the shape change is very minor, the denaturation is ***irreversible***.

Any mechanical or chemical agent that causes the denaturation of a protein is called a _____ _____.

Some of the most common ***denaturing agents*** are listed and described below:

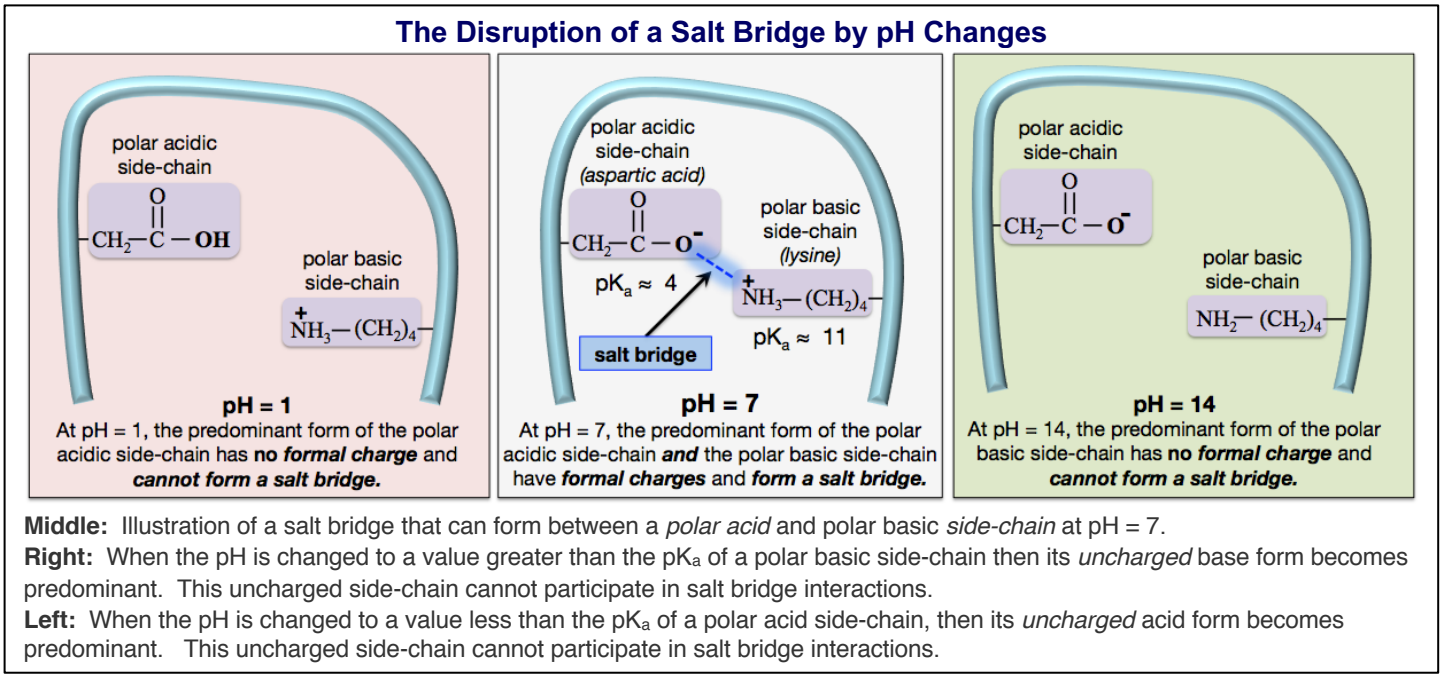
1) _____ The noncovalent attractive forces involved in ***secondary, tertiary, and quaternary structures*** in proteins are easily disrupted by heating. The cooking of an egg is an example of heat denaturation.

2) _____ _____ , which can disrupt the noncovalent attractive forces involved in secondary, tertiary, and quaternary structures. An example of denaturation of protein by mechanical agitation is the foaming that occurs during beating of raw egg (yolks removed). Chefs use this process to make *meringue*.

3) _____ _____ Some amphipathic compounds can cause denaturation by inserting their nonpolar ends into an association of hydrophobic side chains and thereby displacing some of the side chains. For example, *detergents* are capable of denaturing proteins.

4) _____ _____ Some polar solvents, such as acetone or ethanol, can interfere with hydrogen bonding, dipole-dipole, and ion-dipole interactions by competing for a protein's existing interactions.

5) _____ _____**,** which can disrupt a protein's ***salt bridges*** and ***ion-dipole interactions.*** Near physiological pH, the predominant form of *polar acidic* side-chains and *polar basic* side-chains (except for histidine) have a *formal charge*. These charged side-chains help proteins maintain their tertiary and/or quaternary structure because they participate in *salt bridges* and *ion-dipole* interactions.



Middle: Illustration of a salt bridge that can form between a *polar acid* and polar basic *side-chain* at pH = 7.
Right: When the pH is changed to a value greater than the pK_a of a polar basic side-chain then its *uncharged* base form becomes predominant. This uncharged side-chain cannot participate in salt bridge interactions.
Left: When the pH is changed to a value less than the pK_a of a polar acid side-chain, then its *uncharged* acid form becomes predominant. This uncharged side-chain cannot participate in salt bridge interactions.

- An example of the denaturation of proteins by a pH change is the use of citric acid in the marination (soaking) of fish and shellfish in a dish called ceviche. The citric acid comes from citric juices such as lemon, lime, orange, or grapefruit juice.

Essential Amino Acids: Complete, Incomplete, and Complementary Proteins

Organisms produce (synthesize) protein from dietary amino acids.

Our bodies are capable of producing _____ of the twenty common amino acids (from other amino acids or certain other compounds.

- Therefore we do not necessarily need to obtain these eleven amino acids in our diet.

The other _____ amino acids *can only be obtained by eating proteins that contain them*.

- These nine amino acids are called _____ **amino acids**, and are listed below.

Histidine (His)	Methionine (Met)	Lysine (Lys)
Leucine (Leu)	Threonine (Thr)	Valine (Val)
Isoleucine (Ile)	Tryptophan (Typ)	Phenylalanine (Phe)

Foods that contain ***all*** of the essential amino acids are called _____ **proteins.**

- Most animal products are *complete proteins*. Examples: eggs, meat, milk, fish, and poultry.

Foods that contain proteins but do not contain *all of the essential amino acids* are called _____ **proteins**.

- These include most plant proteins.
    - Examples of *incomplete proteins* and their missing essential amino acids are listed in on the right:

Food	Amino Acid Deficiency
rice, wheat, oats	lysine
beans	methionine, tryptophan
peas	methionine
soy	low in methionine
corn	lysine, tryptophan
almonds, walnuts	lysine, tryptophan

Combining of two or more *incomplete proteins* that are deficient in *different* amino acids is a dietary strategy used to ensure the intake of all nine essential amino acids.

- For example, if you eat beans and rice, you obtain all of the essential amino acids since rice contains the amino acids that beans lack, and vice versa.
- When proteins are combined in this way, they are called _____ **proteins**.

Understanding Check

Which two foods (from the table above) could ***each*** be eaten with *corn* as a ***complementary protein***?

Enzymes

Catalysts are substances that increase the rates of chemical reactions. Life requires that many chemical reactions occur within organisms. The human body employs over a thousand chemical reactions. Many of these reactions would occur ***too slowly*** to be useful in the absence of a catalyst. Nature provides humans and other biological organisms with proteins that are capable of catalyzing reactions.

Protein catalysts are called _____.

- Among all plants and animal species, over 5,000 chemical reactions are catalyzed by enzymes.
- Enzymes are capable of increasing the rate of a chemical reaction by up to a factor of one thousand.

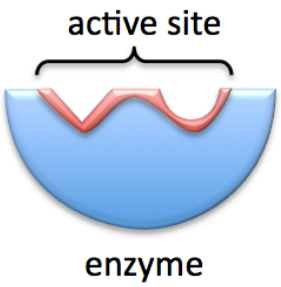
Scientists who specialize in studying enzymes are called *enzymologists*.

Enzymologists refer to the *reactants* of catalyzed reactions as _____.

- Most enzymes are composed of hundreds or thousands of amino acid residues, however only a small region of the enzyme makes contact with the *substrates*.

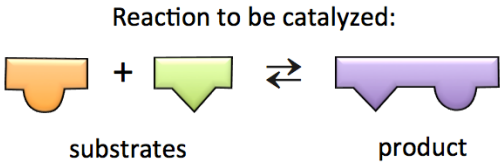
Let's take a look at a model that describes *enzymatic catalysis*.

The part of the enzyme that makes contact with substrates is called the "_____ _____."



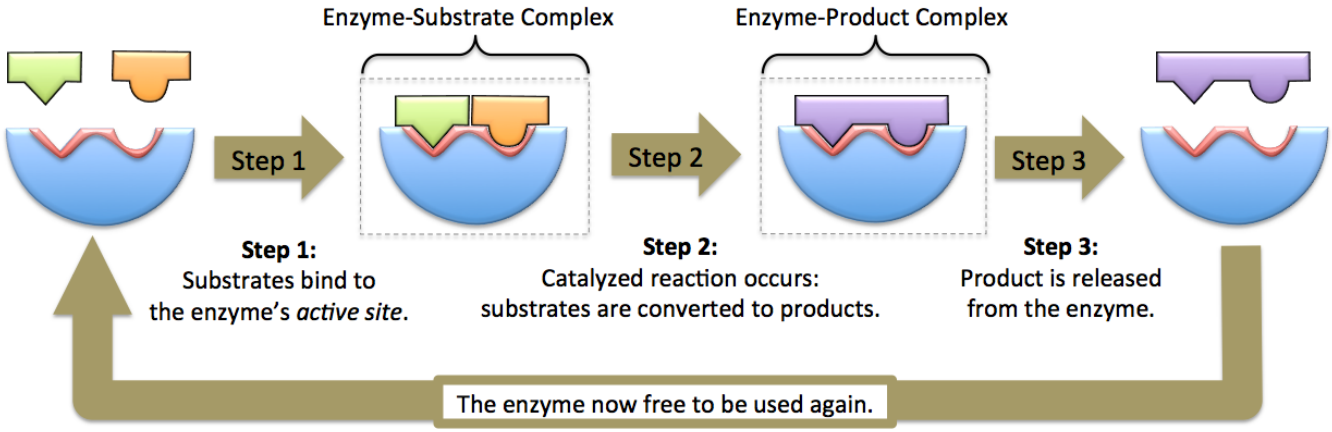
In this model, we will represent an enzyme and its active site as illustrated on the right.

We will consider a reaction where ***two*** substrates (reactants) are converted to ***one*** product, as illustrated below.



In this example, *two* substrates react to form *one* product, however this model *will also apply to other cases* such as *one* substrate compound forming *two* products, or *two* substrate compounds forming *two* products.

The *enzymatic catalysis model* is illustrated below.



In **Step 1**, the substrates bind to the ***active site*** of an enzyme.

- The substrates are held tightly in the active site by ***noncovalent attractive forces****, which are maximized due to the complementary shapes of the substrates and active site*.
- The particle that is formed when the *substrates* are bound to the enzyme is called the **enzyme-substrate complex**.

In **Step 2**, the chemical reaction occurs.

- Substrates are converted to products when covalent bonds within the substrates are broken **and/or** new bonds are made.
- The particle that is formed when the *product* is bound to the enzyme is called the **enzyme-product complex**.

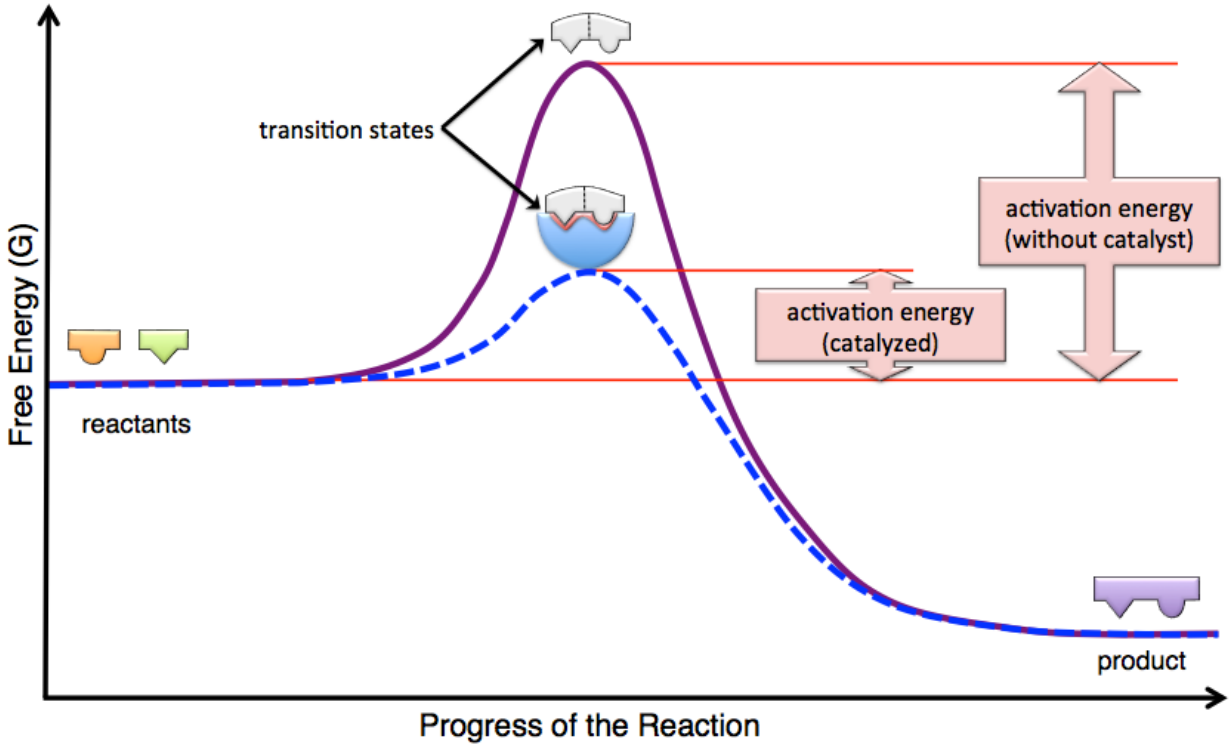
In **Step 3**, the newly formed product is released.

- Note that after products are released from enzymes, the enzymes are free to accept new substrates and the cycle can repeat.

Enzymes ____ _____ ***affect*** the *equilibrium concentrations* of products and reactants (substrates), they only increase the reaction rates, and therefore equilibrium is reached more quickly.

All catalysts, including enzymes, _____ the ***activation energy*** of a reaction and thereby *increase the reaction rate*.

Compare the reaction energy diagram of an ***enzymatically catalyzed*** reaction with that of an ***un-catalyzed*** reaction:



The *un-catalyzed* reaction is represented by the solid curve and the *enzymatically catalyzed* reaction is represented by the dashed curve. In catalyzed *and* un-catalyzed reactions, one or more of a reactant's covalent bonds and/or several noncovalent attractive interactions involving reactants are disrupted or completely broken. This process *requires* energy. At some point in the progress of the reaction, the energy reaches a *maximum value*. This state is a *temporary*, short-lived configuration of atoms called the ***transition state***. In chapter 6 you learned that amount of *energy* required to reach the *transition state* is called the ***activation energy***. As the reaction progresses, *new* covalent bonds and/or noncovalent attractive interactions form and generate the product; these processes *release* energy. The formation of the new covalent bonds and/or noncovalent attractive interactions is responsible for the *decrease in energy* that is seen in the diagram as the transition state changes to product.

How does an enzyme *increase the rate of a reaction*? When substrates bind to an enzyme's active site, ***interactions with the enzyme*** change the shape of the substrates (and enzyme) to a configuration that lowers the energy of the transition state (relative to an un-catalyzed reaction).

Understanding Check:

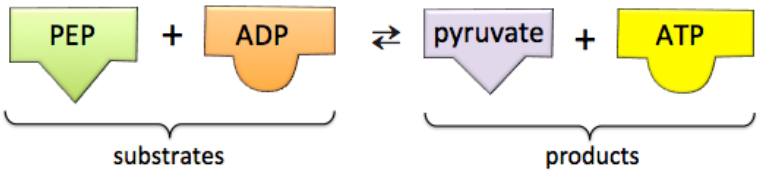
Determine whether each of the following statements are true or false.

a. A catalyzed reaction has a lower activation energy than an un-catalyzed reaction.
b. The greater the activation energy, the faster the reaction rate.
c. At equilibrium, a catalyzed reaction will result in a greater amount of products than would an un-catalyzed reaction.

Example of Enzymatic Catalysis

As an example of enzymatic catalysis, let's consider an enzyme called *pyruvate kinase*. Kinases are a class of enzyme that catalyze reactions in which a phosphate group is transferred from one compound to another.

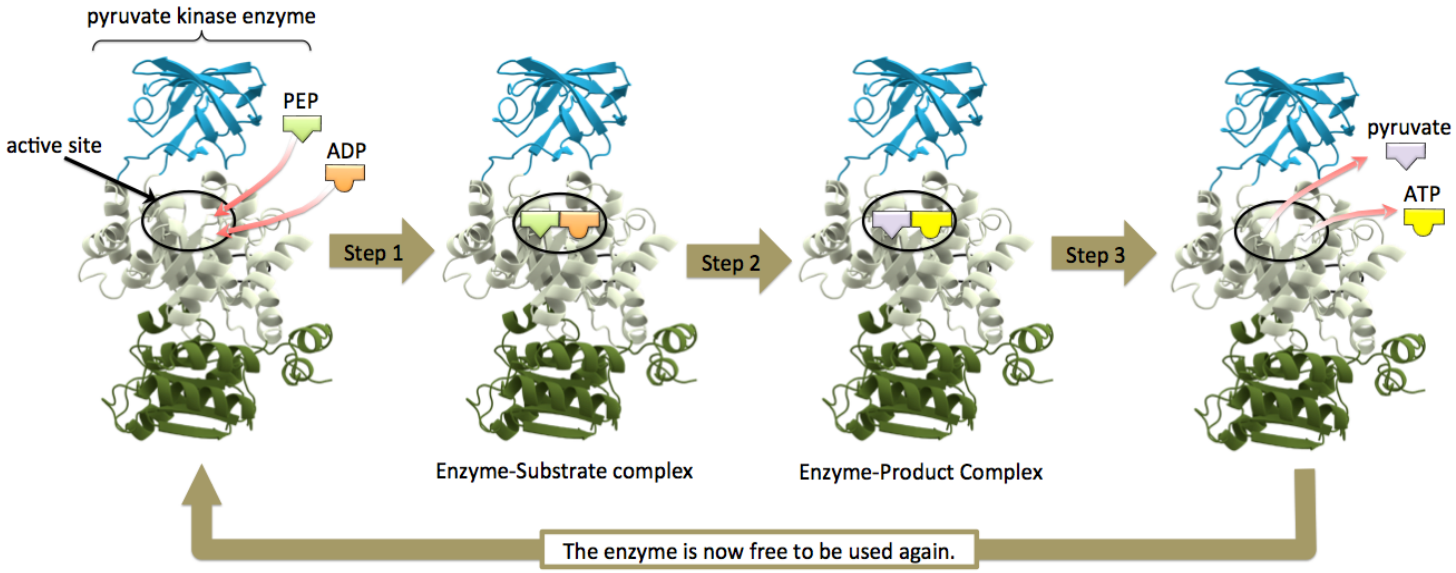
- Pyruvate kinase catalyzes the transfer of a phosphate group from ***p**hospho**e**nol**p**yruvate* (**PEP**) to **ADP**, thereby forming *pyruvate* and **ATP**:



- This is the last reaction that occurs in a series of reactions called *glycolysis*.

A detailed knowledge of the bonding patterns involved in this reaction *is not important* at this time. One of the goals of the glycolysis process is to transfer potential energy stored within glucose (from our diet) to potential energy in the form of **ATP**. In chapter 15, I will discuss the details of how this is done, in part, by the reaction shown above.

The illustration below depicts the three steps of our enzymatic process model for the case of catalysis.



Enzyme image from: Wikimedia Commons, Author: Thomas Splettstoesser, CC https://creativecommons.org/licenses/by/3.0/deed.en

In **Step 1**, the substrates, PEP and ADP, bind to the ***active site*** of the *pyruvate kinase* enzyme.

In **Step 2**, the chemical reaction occurs.

- A phosphate group is transferred from PEP to ADP to form pyruvate and ATP.

In **Step 3**, the products, pyruvate and ATP, are released.

- The enzyme is free to accept new substrates so that the cycle can repeat.

Enzyme Specificity

Enzymes are _____ for particular substrates *or* groups of substrates.

- Their specificity is due to both the selective geometry of their active site and their ability to lower activation energy for particular substrates.

Some enzymes will only catalyze the reaction of a particular substrate; this is called _____ ***specificity***.

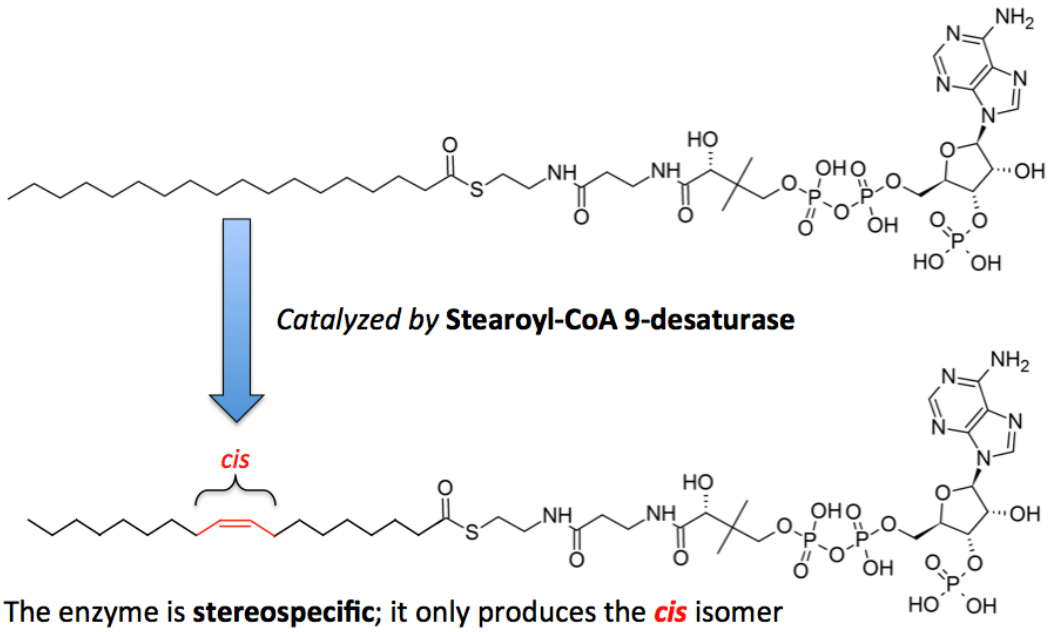
- An example of an enzyme with *absolute specificity* is *urease. Urease* is used by some plants, fungi, and bacteria to catalyze a reaction in which *urea* (only) is converted to ammonium and bicarbonate.

Some enzymes are *less specific*, and will catalyzed reactions for a particular family of substrates; this is called _____ ***specificity***.

- Examples of enzymes with r*elative specificity* include *proteases,* which catalyze the hydrolysis of various proteins.

Many enzymes will only catalyze the reaction of, or production of *one particular stereoisomer*; this is called _____.

- An example of an enzyme with stereospecificity is *stearoyl-CoA 9-desaturase, an enzyme that is involved in fatty acid metabolism and is present in every cell in the body.*
- *Stearoyl-CoA 9-desaturase* only catalyzes the production the *cis* stereoisomer, as shown below.



Almost all enzyme names use the "_____" suffix.

Enzymes are named and categorized based on their _____ *and/or* the _____ that they catalyze.

The table below lists some of the classes of enzymes, the reactions they catalyze, and some examples.

Enzyme Class	Reaction Involved	Examples
Isomerases	Catalyze rearrangement reactions: The reactant and the product contain the same atoms, only the bonding pattern changes.	Cis-trans isomerases - Convert *cis* to *trans* or vice versa.
Ligases	Use energy from ATP to form chemical bonds between substrates	DNA ligase - forms bonds between two DNA fragments.
Hydrolases	Catalyze hydrolysis reactions	• Lipases - hydrolyze ester bonds in lipids • Proteases - hydrolyze peptide bonds • Phosphatases - hydrolyze phosphoester bonds • Nucleases - hydrolyze bonds in DNA and RNA • Carbohydrases - hydrolyze glycosidic bonds
Oxidoreductases	Catalyze oxidation-reduction reactions	• Oxidases - oxidize a substrate • Reductases - reduce a substrate • Dehydrogenases - remove two hydrogens from neighboring carbons and form a double bond
Transferases	Catalyze the transfers of a group of atoms	• Kinases - transfer a phosphoryl group (PO_3^-) • Transaminases - transfer an amino group

Understanding Check: Enzyme Specificity

Choose one of the enzyme classes (from the table above) that would catalyze each of the following reactions.

a. The conversion of a *cis* double bond to a *trans* double bond.
b. The digestion of fat.
c. The conversion of starch to D-glucose.
d. The conversion of a dipeptide into two amino acids.
e. The hydrolysis of ATP to form ADP and P_i.

Cofactors

A _____ is defined as a non protein compound that must be permanently *or* temporarily bound to an enzyme in order for the enzyme to function.

- Example: A nickel ion (Ni^{2+}) must be bound to a *urease* enzyme in order for the enzyme to catalyze the conversion of urea to ammonium and bicarbonate.

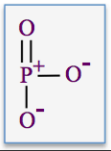
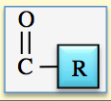
Cofactors are either ***inorganic ions*** or ***organic compounds***.

- When *cofactors* are *organic compounds*, they are often referred to as _____.

In most cases, coenzymes are actually one of the _____ in the catalyzed reaction.

- The reason that certain substrates are also referred to as *coenzymes* is that they are ***common substrates to many different enzymatic reactions*** in which they the ***donate atoms or groups of atoms*** to other substrates ***or*** accept ***atoms or groups of atoms*** from other substrates.
    - For example, ATP and ADP are classified as coenzymes because they are involved in the *transfer* of phosphoryl groups ($-PO_3^-$) ***in many different enzymatically catalyzed reactions***.
    - Many *coenzymes* are derived from dietary _____.

Some of the atom/group-transfer *substrates* that are also classified as *coenzymes,* and their dietary sources are listed below.

Coenzyme	Species that is Transferred	Dietary Source
ATP/ADP	phosphoryl group = 	meat and sugars
$NAD^+/NADH$	hydride ion ($H:^-$) **or** electrons	niacin (vitamin B_3)
$FAD/FADH_2$	hydride ion ($H:^-$) **or** electrons	riboflavin (vitamin B_2)
coenzyme A	acyl group = 	pantothenic acid (vitamin B_5)

Effect of Temperature on the Rates of Enzymatically Catalyzed Reactions

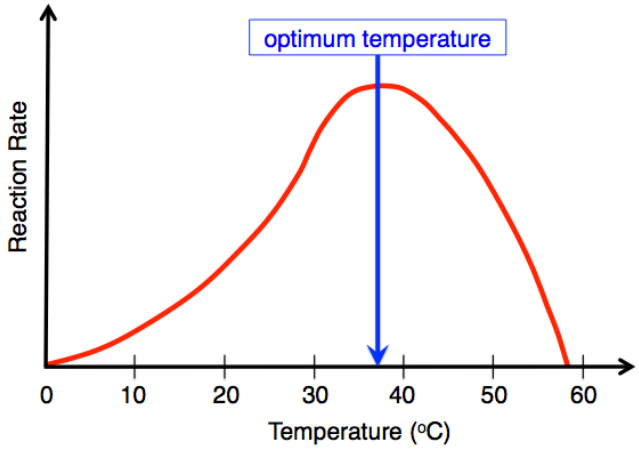
A typical graph of the rate of an enzymatically catalyzed reaction vs. temperature is shown below.

The temperature at which the rate of the reaction is greatest is called the enzyme's _____ ***temperature***.

The reason that the reaction rate does not continue to increase after reaching the *optimum temperature* is that the enzyme begins to *denature* at the higher temperature.

An enzyme's optimum temperature is usually very close to the normal temperature of the organism in which it exists.

- For example, the optimum temperature of most human enzymes is at normal body temperature (~37 °C), as depicted in the graph.



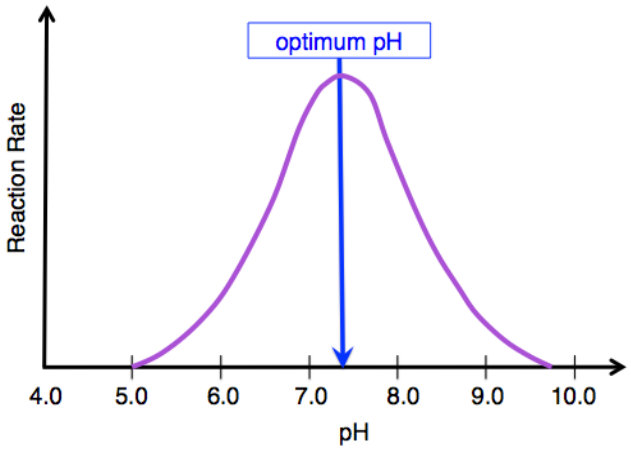
Effect of pH on the Rates of Enzymatically Catalyzed Reactions

A typical graph of the rate of an enzymatically catalyzed reaction vs. pH is shown below.

The pH at which the rate of an enzymatically catalyzed reaction is greatest is called the enzyme's _____ **pH**.

An enzyme's *optimum* pH is usually very close to the normal pH of the region of an organism in which the enzyme exists.

- For example, the normal pH in most regions of the body is about 7.4 (physiological pH), so the *optimum* pH for enzymes found in these regions is also near 7.4 (as depicted in the graph).



As the pH increases *or* decreases from its *optimum* pH value, the reaction rate decreases because of enzyme denaturation.

Not all parts of the body have a normal pH near 7.4. The stomach has a normal pH range of 1 to 3. It is not surprising that the digestive enzyme called *pepsin,* which functions in the stomach, has an optimum pH of 2.

Control of Enzymatic Reactions

All life forms employ reaction regulation mechanisms that involve *controlling* enzymatically-catalyzed reactions by processes called ***enzyme*** _____ and ***enzyme*** _____.

The body uses chemical feedback systems that can increase or decrease an enzyme's ability to catalyze a reaction.

The amount of substrate that an enzyme converts to product (per second) is referred to as the enzyme's "_____."

1) Enzyme Inhibition

When a particular molecule (or ion) forms a covalent or noncovalent bond with an enzyme, it can result in a decrease in the enzyme's *activity*.

A species that decreases a particular enzyme's activity is called an _____.

- Unlike temperature, pH, and denaturing agents, which affect all types of enzymes, inhibitors will only affect specific enzymes.

Enzyme inhibition can be classified as _____ **inhibition** or _____ **inhibition**.

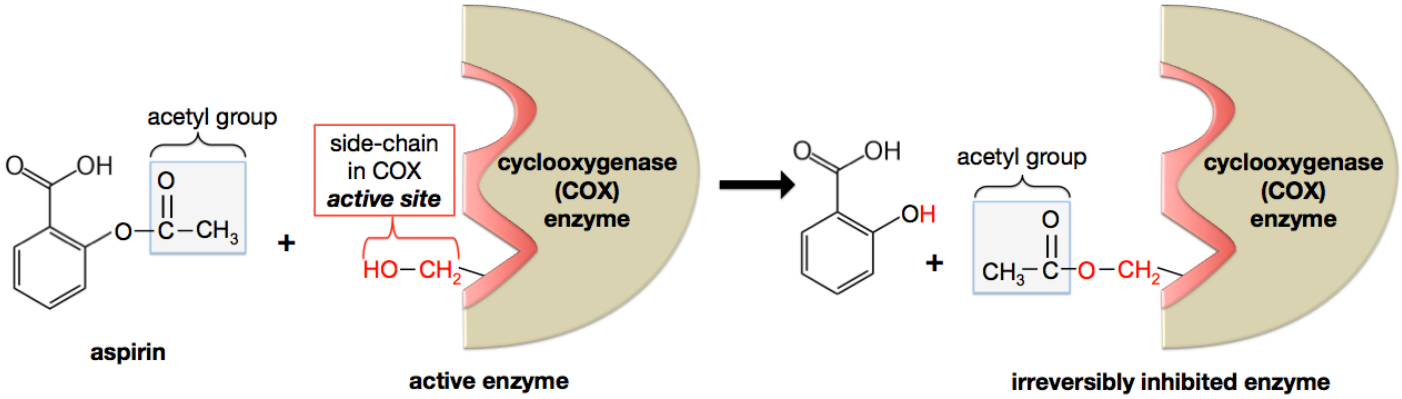
i) Irreversible inhibition

Irreversible inhibition occurs when an *inhibitor* ***reacts*** with an enzyme, forming a ***new*** and _____ ***covalent bond*** *to* the enzyme.

- In almost all cases of irreversible inhibition, the new bond is made to the enzyme's *active site*, which results in *complete and permanent loss* of the enzyme's activity.
- In order to re-initiate catalysis, an organism must produce *new enzymes* (in the absence of the inhibitor).

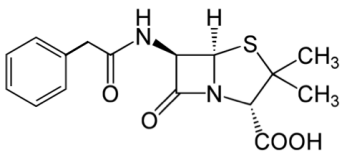
An example of *irreversible inhibition* is ***aspirin's*** **mode of operation**. Aspirin *irreversibly inhibits* the *cyclooxygenase* (COX) enzyme, which catalyzes one of the reactions involved in prostaglandin production. *Prostaglandins* have a wide range of biological effects, including causing pain, inflammation, and fever. In order to *prevent* pain, inflammation, and fever, we use *aspirin* (or other **n**on**s**teroidal **a**nti **i**nflammatory **d**rugs (NSAIDs)).

Irreversible inhibition occurs when aspirin reacts with an amino acid side-chain in the COX enzyme's *active site,* as illustrated below.

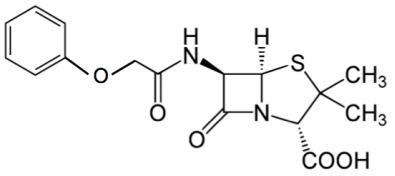


In this reaction, an *acetyl group* from **aspirin** is exchanged for a hydrogen atom (**H**) from a particular side-chain in the COX enzyme's active site. When an acetyl group is bonded to the enzyme's active site, it is no longer possible for *substrates* to bind to the enzyme, and therefore the enzyme is permanently inactivated.

Another example of *irreversible inhibition* can be seen in the mode of action of the antibiotic drug called ***penicillin***. The structural formulas of the intravenously-administered *penicillin G* and the orally-administered *penicillin V* are shown below.



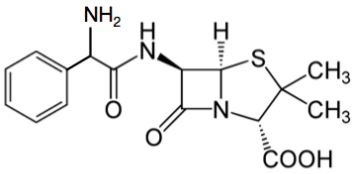
penicillin G
(given intravenously)



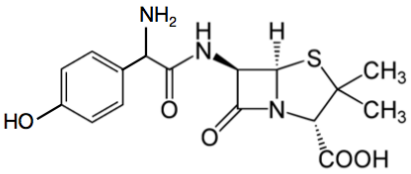
penicillin V
(given orally)

Penicillins are a class of antibiotics that work by *irreversibly inhibiting* a bacterial enzyme called DD-*transpetidase*. This enzyme is involved in constructing cell walls in some types of bacteria. If the cell wall cannot be correctly assembled, then the bacteria cannot divide (reproduce), and therefore do not persist. *Penicillin is* ***specific*** for the DD-*transpetidase* enzyme's active site because the shapes of the *penicillins* are quite similar to the shape of the substrate that is normally catalyzed by the enzyme. Once a penicillin is in the active site of this enzyme, a chemical reaction occurs in which *penicillin forms a* ***covalent bond*** to a particular side-chain in the active site. The reaction is *irreversible* and results in the complete loss of the enzyme's activity.

Some bacteria develop a resistance to *penicillin* G and V. In response, medical researchers have developed penicillin derivatives to which most bacteria have not yet developed a resistance. The structural formulas for a couple of these penicillin derivatives, *ampicillin* and *amoxicillin*, are shown below.



ampicillin



amoxicillin

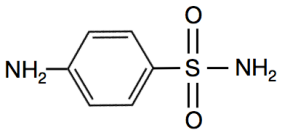
ii) **Reversible Inhibition**

_____ ***inhibition*** occurs when an inhibitor is weakly bound to an enzyme and decreases its activity.

- This type of inhibitor is called a **reversible inhibitor**.

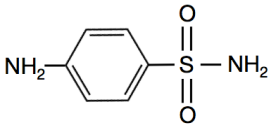
Reversible inhibitors do not form covalent bonds to the enzyme, therefore they repeatedly attach to and detach from enzymes. When a reversible inhibitor detaches from an enzyme, the enzyme's activity is completely restored. The greater concentration of the inhibitor, the more frequently it will attach to enzymes, and the greater the reduction in enzyme activity. Reversible inhibitors decrease an enzyme's activity by attaching to either an enzyme's *active site* **<u>or</u>** to a region of an enzyme *other than the active site*.

An example of a *reversible inhibitor* is the antibacterial drug called *sulfanilamide*. Sulfanilamide, like most reversible inhibitors, reversibly binds to an enzyme's active site, forcing the enzyme's substrate to *compete* for access. Binding of reversible inhibitors to the active sites of enzymes can occur because the inhibitors' shapes often resemble the shapes of substrates, and are therefore complementary to the active sites. *Sulfanilamide* inhibits a bacterial enzyme that catalyzes the production of a compound that is essential for bacterial growth.

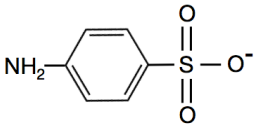


sulfanilamide
(antibacterial drug)

As shown below, *sulfanilamide* very closely resembles *p*-aminobenzoate ion, which is the bacterial enzyme's substrate.



sulfanilamide
(antibacterial drug)



p-aminobenzoate ion
(substrate)

Sulfanilamide was first used extensively as an antibacterial agent in World War II. Since then, many other antibacterial agents have been synthesized by exchanging the amino group (that is bound to the sulfur) with other organic groups. These *sulfanilamide analogs* are called **antibacterial sulfa drugs**.

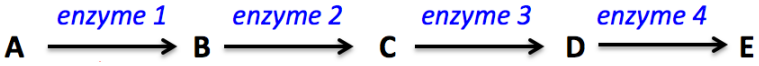
Organisms often use several reactions in series (one after another) in order to carry out the chemical changes they require to meet their physiological needs.

- These reaction series are referred to as _____ _____.
- Examples: *Photosynthesis*, *glycolysis,* and the *citric acid cycle*.

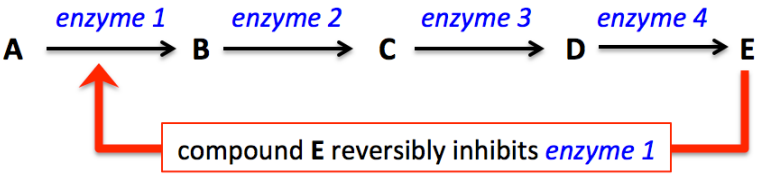
Many of the reactions in metabolic pathways require enzymes; therefore organisms can regulate (slow down) a metabolic process, according to their needs, by _____ one (or more) of the enzymes involved in the metabolic pathway.

The _____ of a reaction in a metabolic pathway often acts as a *reversible inhibitor* for one (or more) of the reactions in the pathway.

This biological strategy makes sense because when there is a relatively high concentration of a product in a metabolic pathway, it would be inefficient, and in some cases harmful, to continue its production. For example, consider the following hypothetical metabolic pathway that involves *four enzymatically-catalyzed reactions* and eventually converts compound "**A**" into compound "**E**."



When the concentration of compound **E** is high enough to meet the organism's needs, it would be inefficient, and in some cases dangerous, for the metabolic pathway to proceed. For this reason, compound **E** acts as a r*eversible inhibitor* of ***enzyme 1***:



If compound **E** is not present, or is present in relatively low concentration, the reactions proceed, and therefore compound **E** is produced at a significant rate.

If compound **E** is produced at a greater rate than the organism uses it, then excess **E** is available to bind to and inhibit, ***enzyme 1***.

- This decreases the production of **B**, **C**, **D**, and, ultimately, a decrease in the production of **E**.

Until the organism uses or breaks down a significant amount of compound **E**, compound **E** will continue to *inhibit its own production*.

The *regulation* of a metabolic pathway by *the inhibition of an enzyme* is called _____ **inhibition**.

An example of *feedback inhibition* is the regulation of *glycolysis*. Glycolysis is a metabolic pathway that involves a series of ten chemical reactions. It is used by organisms to convert glucose to ATP, NADH, and pyruvate. If the concentrations of these products are sufficient, it would be inefficient and potentially harmful to continue to produce them. Each of the ten reactions in the glycolysis pathway requires an enzyme. Three of the enzymes in the pathway are regulated by inhibitors in the *feedback inhibition* process.

2) Enzyme Activation

Enzyme activation can occur when an "**activator"** binds to an enzyme and _____ its activity.

Binding of the *activator* species to an enzyme induces changes in the active site that increases the enzyme's activity.

Just like the substrates and enzyme inhibitors, ***enzyme activators*** are specific for a particular enzyme or group of enzymes.

Enzyme activators can regulate metabolic pathways by activating one or more of the pathway's enzymes.

- Increasing the production of a metabolic pathway by an enzyme activator is called _____ **feedback**.
- An example of *positive feedback* is the activation of *pyruvate kinase*, an enzyme used in one of the *glycolysis* reactions. *Pyruvate kinase* is ***activated*** by PEP, which is also one of its own substrates (PEP).

One last note **on the** ***control of enzyme activity*****:** In this section, I discussed how enzyme activity could be decreased or increased by the binding of inhibitors or activators (respectively). Nature employs additional strategies in order to increase or decrease enzyme activity. In some cases, one enzyme catalyzes the breaking of chemical bonds, or the formation of new bonds, in a ***second enzyme*** in order to activate or deactivate the second enzyme. The details of these processes are beyond the scope of this course, however, you should know that this type of activation and deactivation is commonly employed by organisms to regulate metabolic pathways.

Examples of the Involvement of Enzymes in Disease

The underproduction or overproduction of enzymes, or the inability of an organism to control enzymes can lead to _____. When such diseases result from a defect (mutation) in a gene that is responsible for the production of a particular enzyme, they are categorized as **genetic diseases**. In the next chapter, you will learn details of how the information in DNA is used to produce proteins (including enzymes). There are thousands of different types of enzymes that are produced in the body, and the inability to correctly produce or control just one type of enzyme could result in death. You may recall that I discussed ***Tay-sach's*** and other ***sphingolipidosis*** genetic diseases that result from a deficiency of the enzymes responsible for the breakdown of sphingolipids. Although some forms of *sphingolipidosis* are treatable with **enzyme replacement therapy**, most sphingolipidosis cases result in death before five years of age. *Enzyme replacement therapy* is also used to treat other enzyme deficiencies, such as *lactose intolerance* (deficient lactase enzyme activity) and *exocrine pancreatic deficiency* (insufficient pancreatic production of digestive enzymes).

Another example of disease that is related to enzymes occurs when DNA is replicated. Before a cell divides, a duplicate copy of its DNA must be made. The new DNA is "proofread" for errors and then repaired. ***DNA repair enzymes*** catalyze the repair of mistakes made in the DNA replication process. If an individual's *DNA repair enzymes* are not functional, this results in an accumulation of new mutations, and leads to various types cancer.

Phenylketonuria (PKU) is an enzyme-related disease that can be controlled by a special diet. PKU is caused by deficient activity of the *phenylalanine hydroxylase* enzyme (PAH). This enzyme is responsible for breaking down excess phenylalanine (an amino acid). When the PAH enzyme is not fully functional, high levels of phenylalanine result, which affects brain development and causes intellectual disabilities, seizures, and other medical issues. If PKU is diagnosed and treated early, the damaging effects can be minimized and normal mental development can occur. For individuals with PKU, the consumption of foods rich in phenylalanine residues, such as meats and nuts, can be poisonous. Treatment of PKU is a strict life-time diet that *restricts* phenylalanine-containing foods, and *includes* dietary supplementation of the non phenylalanine amino acids, and other nutrients. Individuals with PKU must be careful not to consume the artificial sweetener called *aspartame* (NutraSweet) because phenylalanine is produced when aspartame is broken down in the body.