Biochemistry

Thermodynamics, Protein Structure, and Enzyme Kinetics

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Table of Contents

1 Introduction

1.1 Opening Statement

Biochemistry is a captivating blend of biology and chemistry, and certain principles found in biochemistry are needed to fully understand the background of life's most complex biological processes. The laws of energy which govern our universe, the intricate folding processes of the proteins which make cellular life possible, the complex ways in which enzymes allow everyday reactions to be possible, and more essential topics all play their own part in understanding biology and the phenomenon of life.

Although this paper is structured in a way that allows all readers to grasp the main concepts and topics, it may be recommended to read this document after finishing the first biochemistry handout.

2 Bioenergetics

Bioenergetics is defined as the study of energy flow and energy transformations within living organisms. In order to understand how complex life is able to occur, we must understand the primary concepts which dictate how energy is and can be manipulated.

2.1 Energy

Energy, at its simplest definition, is the ability or capacity to do work. Energy can have various forms, all of which have their own definitions and functions in everyday life.

2.1.1 Forms of Energy

Energy associated with motion is known as **kinetic energy**. Kinetic energy can do work through motion, like pushing an object. A motor protein transporting cellular molecules within a human cell has kinetic energy.

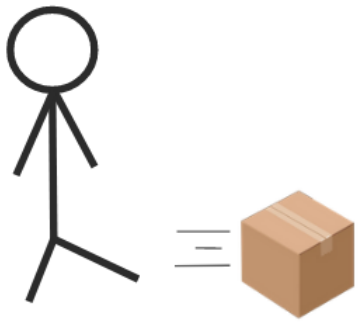


Figure 2.1 A box with kinetic energy. (Source: Andrew Ung)

Thermal energy is a type of kinetic energy. However, thermal energy is associated with the **random movement of atoms** in a collection of matter, whereas kinetic energy is associated with a **directional movement of atoms** in a collection of matter.

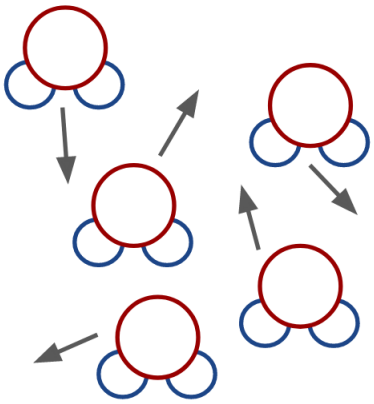


Figure 2.2 A collection of water molecules with thermal energy. Note that, although each water molecule has kinetic energy, their directions of movement are random, thus there is no net movement. (Source: Andrew Ung)

Light energy and **sound energy** are also forms of energy, stemming from electromagnetic radiation and the vibration of matter, respectively. There are many other types of energy not outlined in the document due to irrelevance.

Potential energy is a stored form of energy. Potential energy is a form of energy that is associated with the position, shape, or configuration of an object or system, ready to be converted into other forms of energy and do work when certain conditions are met.

Imagine lifting a box up from the ground and holding it in place in the air. The box is not moving, it has no kinetic energy, but it has a substantial amount of potential energy. By dropping that box, that potential energy is gradually converted into kinetic energy as it speeds up in free fall.

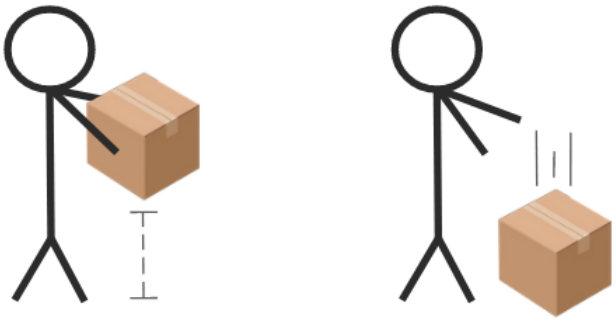


Figure 2.3 A box with potential energy (left). The potential energy gets converted into kinetic energy as the box is dropped (right). (Source: Andrew Ung)

Chemical energy is a type of potential energy stored within the chemical bonds of molecules. Recall that bonds are formed when suitable molecules and atoms approach close enough to share or give and take electrons, forming an attraction between the two.

- In order to form a bond, **energy must be released**. Molecules and atoms associated with large amounts of kinetic or thermal energy move too erratically and frequently to form bonds, meanwhile molecules and atoms associated with small amounts of kinetic or thermal energy move slowly enough to form bonds with other molecules and atoms.

- In order to break chemical bonds, **energy must be added**. Adding kinetic or thermal energy results in movement, resulting in the bond breaking as the molecules or atoms move too far apart to be able to share or give electrons.

Thus, the stronger the bonds within are, the less energy a molecule contains, as it requires a large energy input to break apart. The weaker the bonds within are, the more energy a molecule contains, as it requires less energy to break it apart.

Recall glucose, a carbohydrate molecule important to life as a store of energy. Glucose contains a large amount of chemical potential energy, and when it is broken down, that energy is released. The atoms in glucose are split and rearranged, eventually forming low-energy products such as carbon dioxide (CO_2) and water (H_2O). The bonds between atoms of CO_2 and H_2O are stronger than the bonds between the atoms of glucose, meaning they contain less energy, and thus energy had to have been released when the initial glucose molecule was broken down into those products.

2.2 Laws of Thermodynamics

The laws of thermodynamics are very important to the study of bioenergetics as they are what govern every single energy-related interaction and transformation.

2.2.1 Conservation of Energy

The **first law of thermodynamics** states that energy cannot be created nor destroyed. Energy is thus conserved in a system, and can only be converted into its different forms.

Let's return to the example of a person lifting a box into the air. The box, being lifted into the air, gains potential energy (and some kinetic energy too as it is lifted). This energy doesn't simply appear out of nowhere- another form of energy was converted into that energy.

Chemical energy stored in the bonds of molecules in our cells were converted to the kinetic energy of movement, lifting our arms up in order to move the box. This kinetic energy is then converted into potential energy as the box increases in height. Let's imagine dropping the box—all of this potential energy is converted into kinetic energy as the box falls, and once it hits the ground, into sound energy and kinetic energy as it pushes down on the ground below, making a noise. The sound energy and kinetic don't disappear, however, but are dispersed throughout the floor and throughout the air, eventually becoming negligible.

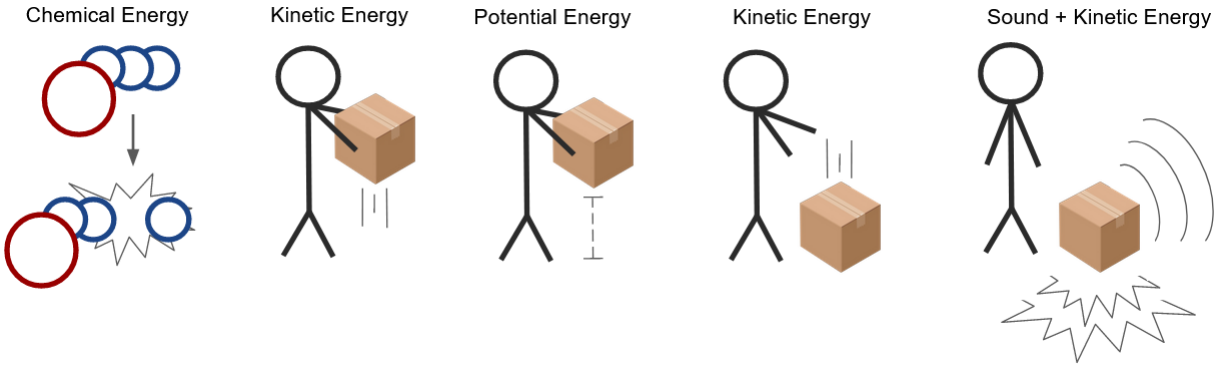


Figure 2.4 A process showing multiple energy transformations and the principle of conservation of energy. (Source: Andrew Ung)

Energy can never be lost or gained, simply transformed. This is known as the law of **conservation of energy**.

2.2.2 Entropy

Entropy is a measure of randomness. Entropy can be found in the arrangements of molecules, the properties of larger collections of matter, energy dispersion, and more.

The **second law of thermodynamics** states that the entropy of a system tends to increase over time. A highly concentrated cloud of gases will eventually disperse over a greater area, a constructed building will eventually crumble and fall to the ground in small pieces, as ordered ice crystals melt, their hexagonal structure fades into random chains of water molecules.

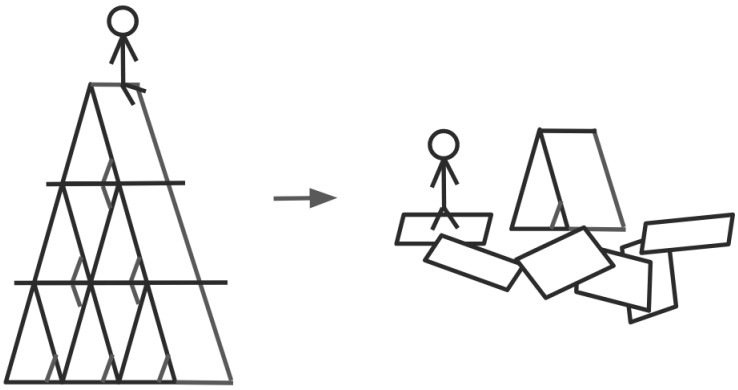


Figure 2.5 The inevitability of entropy. An ordered and complex house of cards will tend to fall into a more stable, simpler arrangement. (Source: Andrew Ung)

In regards to bioenergetics, entropy is increased through every energy transformation or interaction that occurs. Some fraction of energy is always converted to thermal energy and released into the environment. A sliding box with kinetic energy loses some of that energy as thermal energy due to friction.

Transformations regarding chemical energy also are affected by entropy; our common biological processes aren't very energy efficient and lead to the conversion of large amounts of thermal energy. It is estimated that, when transforming chemical energy into kinetic energy in our muscles, around 60 percent of the original energy stored in chemical bonds is lost as heat.

2.3 Gibbs Free Energy

Thermal energy lost through entropy is considered "lost" energy. This isn't in violation of the first law of thermodynamics, but is instead due to the fact that this thermal energy cannot do meaningful work. To perform work, energy must be transferred in a directed and organized manner, rather than randomly. Kinetic energy used to move a box is focused in one direction, leading to work being completed. Thermal energy formed via entropy, being random and disordered, lacks the necessary structure and direction to be harnessed for performing useful work.

This brings us to the topic of what is known as **Gibbs free energy**, hereby shortened to simply **free energy**. Free energy is energy that can be used to perform meaningful work in a system with equal temperature and pressure. This definition thus does not include thermal energy formed from entropy, involved with pressure (thermal energy randomly moving air particles leading to pressure) and temperature. The definition of free energy can thus be simplified to energy present in a system that is able to perform meaningful work.

Free energy becomes useful when isolating specific chemical reactions—it tells us how much meaningful energy is gained or lost, and that information allows us to determine other important properties of the reaction.

The equation for change in free energy of a chemical reaction is as follows:

$$\Delta \mathrm{G} = \Delta \mathrm{H} - \mathrm{T}\Delta \mathrm{S}$$

where ΔG represents change in Gibbs free energy, ΔH represents change in **enthalpy**, or the total energy of a system, T represents temperature in Kelvin, and ΔS represents change in entropy.

The change in free energy value allows us to determine whether a chemical reaction will occur without any energy input, also known as its **spontaneity**, or how **spontaneous** a chemical reaction is.

- A chemical reaction with a **positive ΔG** value is **non-spontaneous**. As the chemical reaction progresses, the amount of energy in the molecules increases, meaning that energy must be inputted, and it does not simply occur on its own. These chemical reactions which use energy are known as **endergonic**.
- A chemical reaction with a **negative ΔG** value is **spontaneous**. As the chemical reaction progresses, the amount of energy in the molecules decreases, meaning that energy is outputted, and it is able to simply occur on its own. These chemical reactions which release energy are known as **exergonic**.

Remember that entropy is inevitable. The reason that spontaneous reactions occur is because they result in greater entropy; by releasing energy through a chemical reaction, more stable and simpler compounds are formed, similarly to how highly concentrated and pressurized molecules disperse, creating stable formation. The more complex a structure is, the more energy it holds, the more energy it is able to release, and the more it is able to contribute to entropy.

2.3.1 Equilibrium

Free energy can be thought of as a system's instability. The more unstable a system is, the more energy it can release as it heads towards a more stable arrangement. This brings us to the topic of **equilibrium**.

Chemical reactions are constantly occurring. Reactants react together to form products in a **forward reaction**, and those products then react and reform back into the original reactants in a **reverse reaction**. A chemical reaction is at **equilibrium** when both the forward reactions and reverse reactions occur at the same rate, or when there is no net change in the concentration of both products and reactants.

When a chemical reaction is at equilibrium, it is most stable, and thus is at its lowest possible free energy value. When is a chemical reaction is not at equilibrium, either the forward reaction rate or the reverse reaction rate increases in order to reach that equilibrium. This causes a meaningful shift in the concentrations towards equilibrium, performing work and affecting free energy in the process.

This is the reason that thermal energy formed from entropy cannot do meaningful work- it is already at its most stable arrangement. It would be different if that thermal energy were concentrated, in which that thermal energy could be harnessed for power, forming the basis of geothermal reactors. Thermal and chemical energy are analogous, a chemical reaction at equilibrium cannot do much work, while a more unbalanced chemical reaction can be used for work.

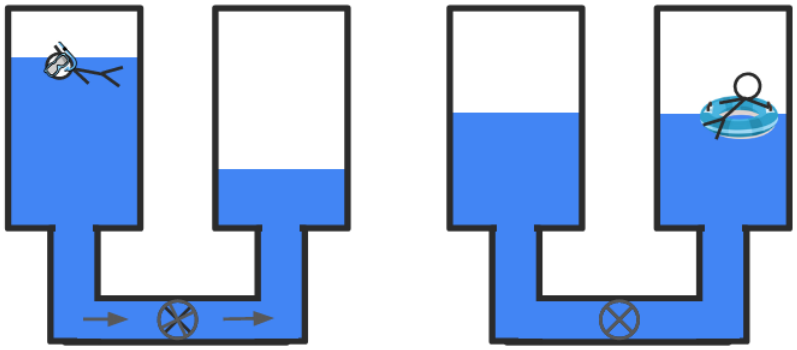


Figure 2.6 Two systems, one that is able to perform work (left), and one that is at equilibrium (right). This system is analogous to those of chemical reactions. (Source: Andrew Ung)

- The collections of water in the system on the left are not in equilibrium, causing a flow of water from the left to the right, which is able to generate power, and thus has a negative ΔG value.
- The collections of water in the system on the right are in equilibrium, and there is no net change occurring, and thus has a ΔG value of zero. Eventually, this is what the system on the left will tend to become, due to the second law of thermodynamics.

3 Proteins

Proteins are important to life as they are what carry out the various functions of the cell. There can be thousands of different proteins in a cell, each complete with their own unique function. The structure and composition of these proteins are what allows them to be so variable, and is what makes them unique to other molecules. Proteins have multiple levels of structure, each focusing on different biochemical properties which all combine and interact to form a finalized, folded protein.

3.1 Primary Structure

The **primary structure** of a protein is simply the sequence of amino acids which make it up. It is very important to know the characteristics of each amino acid, as this **primary structure** determines the secondary, tertiary, and quaternary structures of the proteins.

3.1.1 Amino Acids

Amino acids are the monomers, or **building blocks**, of proteins. They are the smallest subunits of a **polypeptide** chain, which eventually folds to become a protein. Amino acids are made of two chemical groups, namely a **carboxyl group**, an **amine group**, and a variable **R group**, also known as a **side chain**. These chemical groups are all attached to a carbon, which also has an extra hydrogen atom binded to it.

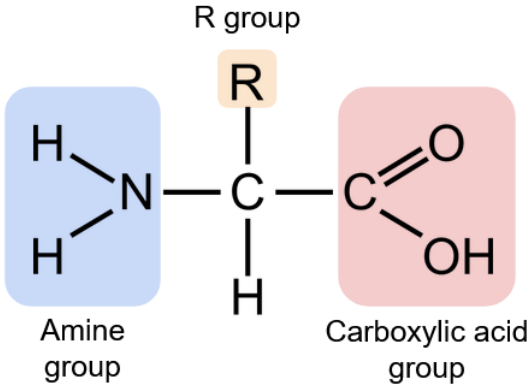
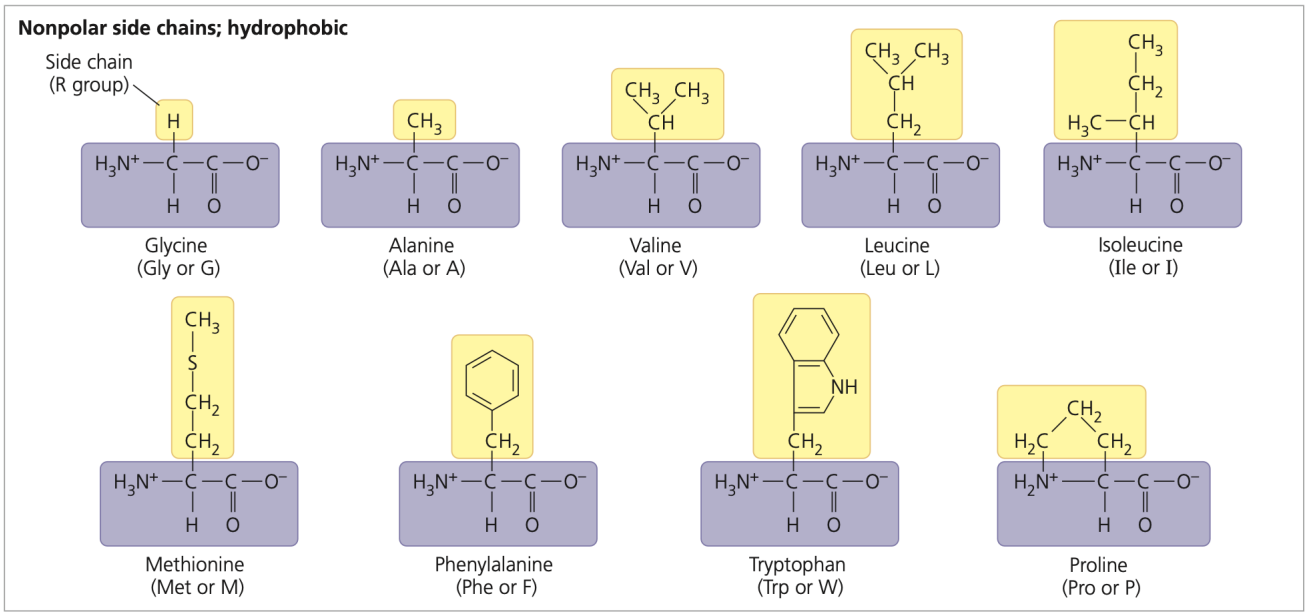


Figure 3.1 The basic structure of an amino acid (left). (Source: Andrew Ung)

The **R groups**, also known as **side chains**, are what determine an amino acid's properties: buffering capabilities, hydrophobic properties, polarity, compressibility, and more. There are twenty unique R groups, and thus twenty unique amino acids, each with their own properties.

It is recommended to study and memorize the properties of these amino acids before moving on.



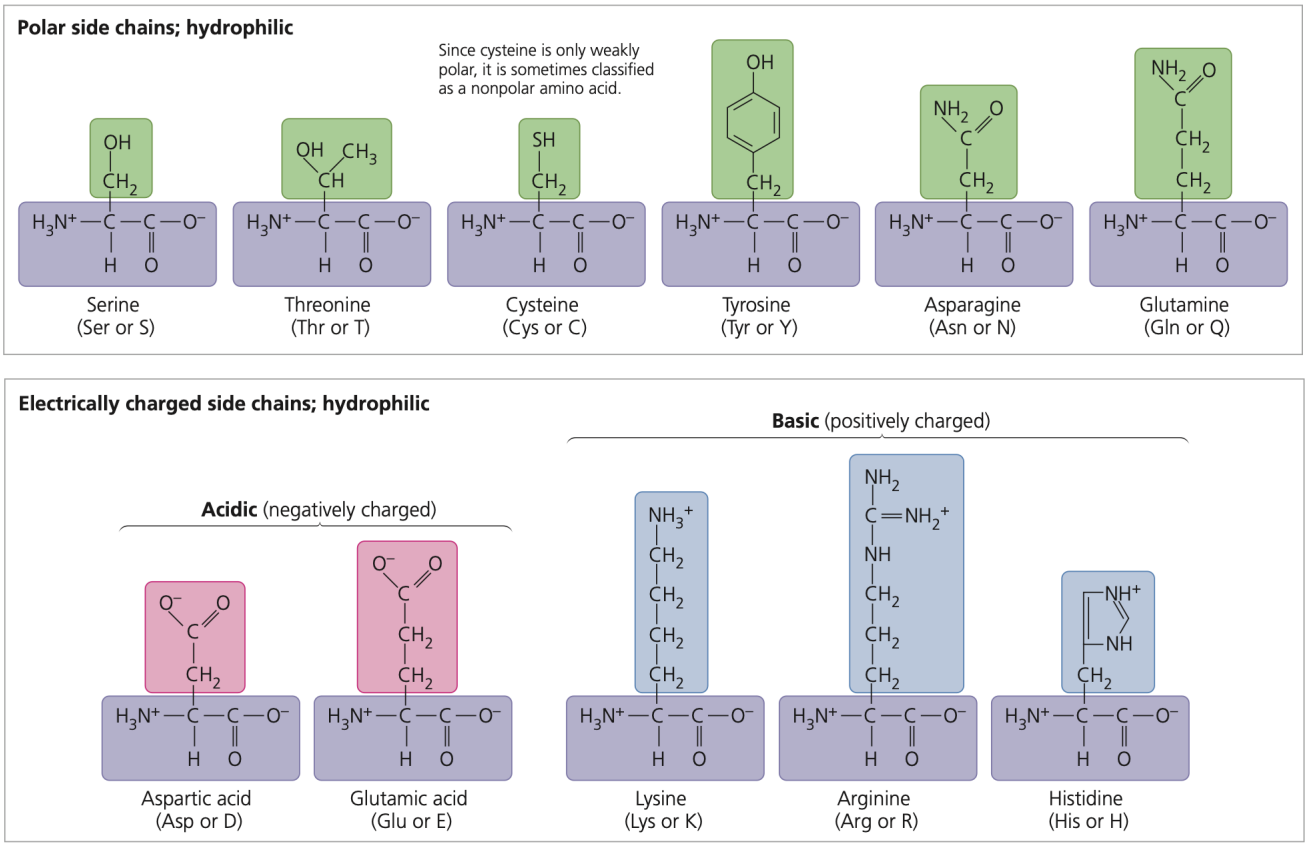


Figure 3.2 The twenty amino acids, divided by some of their properties. (Source: Campbell Biology)

Some other special properties of amino acids relevant to primary structure are as follows:

- **Beta-branched amino acids** include: leucine, isoleucine, and valine. These beta-branched amino acids have unique properties in regards to folding freedom, and can destabilize or stabilize a protein's structure depending on placement.
- **Aromatic amino acids** include: phenylalanine, tyrosine, and tryptophan. These aromatic amino acids act as protein structure stabilizers, and molecular identifier tags.

Polypeptide chains are made of amino acids covalently bounded to each other by their carboxyl groups and amine groups. This means that one end of the polypeptide chain has an unbounded amine group, and the other end has an unbounded carboxyl group. These ends are known as the **N-terminus** and **C-terminus** respectively.

Each amino acid has a three-letter code and a one-letter code. Both are commonly used, and it is recommended to learn these thoroughly using the provided table above. This is useful when listing the amino acids of a polypeptide chain. One example would be the amino acid structure of leucine enkephalin, which can be typed out as Tyr-Gly-Gly-Phe-Leu.

3.2 Secondary Structure

The **secondary structure** of a protein arises from the interactions between all of the chemical groups of an amino acid except for the side chain. This includes the hydrogen atom, the carboxyl group, and the amine group.

In these groups, the hydrogen atoms have a partial positive charge ($\delta+$), and the oxygen atoms (from the carboxyl group) have a partial negative charge (δ-). This leads to hydrogen bonding between different amino acids, which can stabilize the overall protein structure, and even cause new structures to form. Note that the R group does not play a part in secondary structure formation, it is simply the **backbone constituents**, or all the chemical groups excluding the R groups, that are involved in hydrogen bonding.

3.2.1 α Helices

α **helices** are such a structure which can arise from this hydrogen bonding. These helices appear as coils in a polypeptide's conformation. The helix is held in place by bonding between every fourth amino acid's backbone constituents in a chain.

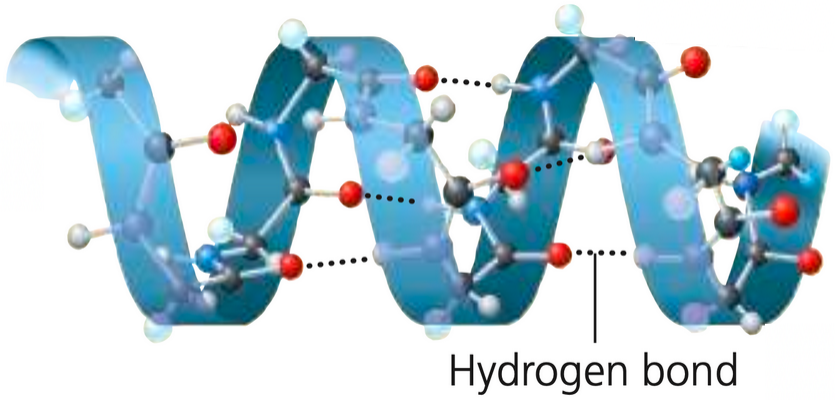


Figure 3.3 Hydrogen bonding between backbone constituents of a polypeptide, causing the formation of an α helix. (Source: Campbell Biology)

Although the R groups of the amino acids in the polypeptide do not play a role in the formation of α helices, there are some exceptions which prevent their formation. The following amino acids are known as **helix breakers**, or **helix disruptors**.

- **Proline** has a unique structure where its R group is incorporated into its backbone chemical groups (technically making it an *imine*), causing it to be unable to bend into the correct conformations to form hydrogen bonds with other amino acids to form an α helix.
- **Glycine** has less of a pronounced effect on α helices when compared to proline, however it can still disrupt its overall structure. Glycine, with a very small R group, has a large amount of conformational freedom and is very flexible. This flexibility causes instability when incorporated in a polypeptide with an α helix.

Example 3.1 (Adapted from USABO Open Exam 2018) You discover a protein that exhibits parts composed of α helices. What is NOT a likely amino acid sequence for some portion of the α helix containing part of the protein? Select all answers that apply.

(A) ALQQMMDSILDY
(B) ALILALMWWLLF
(C) FPPALGGLPFAMG
(D) LMQKPMDSLPDY
(E) LKMELAKMILLA

Solution: The question is asking which amino acid sequences would most likely not make up an α helix containing part of a protein. Recall that glycine (G) and proline (P) are helix breakers. Both sequences FPPALGGLPFAMG and LMQKPMDSLPDY contain either glycine or proline, thus the answers are C and D.

3.2.2 β Pleated Sheets

β **pleated sheets** are the second major type of secondary structure. β pleated sheets form when parallel parts of a polypeptide chain form hydrogen bonds between each other. The parts of a polypeptide which line up to form β pleated sheets are known as β **strands**. Note that β strands refer to parts of a single polypeptide, not separate polypeptides.

These sheets, when in large concentrations, can be very strong—the fibers found in silk clothing are made of proteins primarily composed of β pleated sheets.

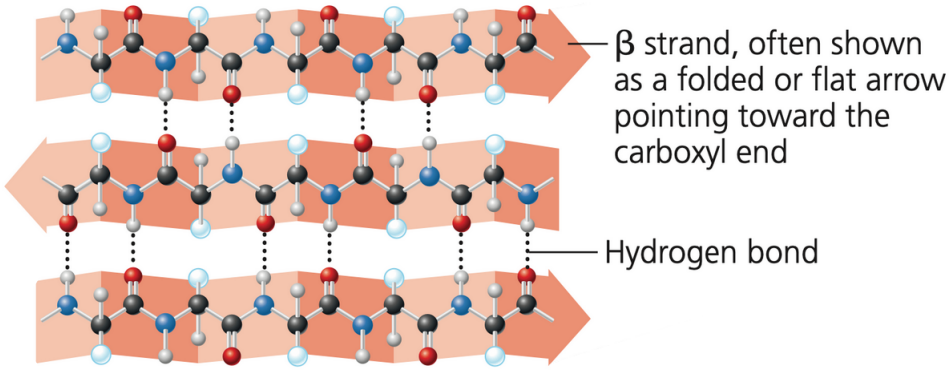


Figure 3.4 Hydrogen bonding between parallel β strands, forming a β pleated sheet. (Source: Campbell Biology)

3.3 Tertiary Structure

The **tertiary structure** of a protein arises from interactions between the R groups of amino acids in a polypeptide. Many types of bonding and intermolecular forces come together to form the overall shape of a protein. Note that tertiary structure does not necessarily come after secondary structure, they both affect the protein at the same time and are **superimposed** upon each other.

Hydrophobic interactions are the interactions caused by nonpolar and polar R groups interacting with the environment around them.

- Polar amino acids have R groups with partial charges which can interact and bond with the water molecules in the aqueous solution surrounding and inside a cell, making them hydrophilic.
- Nonpolar amino acids have R groups without partial charges and cannot interact or bond with the water water molecules in the aqueous solution surrounding and inside a cell, making them hydrophobic.

Nonpolar amino acids in a polypeptide chain thus tend to aggregate in groups, beneath polar amino acids, similarly to how phospholipids in a cell membrane cluster their hydrophobic tails inwards and their hydrophilic heads outwards.

Intermolecular forces between R groups of amino acids cause attraction and shape changes in the resulting protein. Hydrogen bonding and other **van der Waals forces** keep R groups of different polarities together.

Ionic bonding and **covalent bonding** can also occur, leading to shape changes.

- Ionic bonding is affected by the **pH** of the surrounding solution. At different pH levels, amino acids can have different forms, losing or gaining protons which affect their availability to form ionic bonds with other amino acids.
- Covalent bonding is present in **disulfide bridges**, made from two **cysteine** amino acids interacting with each other. Cysteine is unique in which it has a sulfhydryl chemical group making up part of its R group, which can form covalent bonds with other sulfhydryl groups to create disulfide bridges.

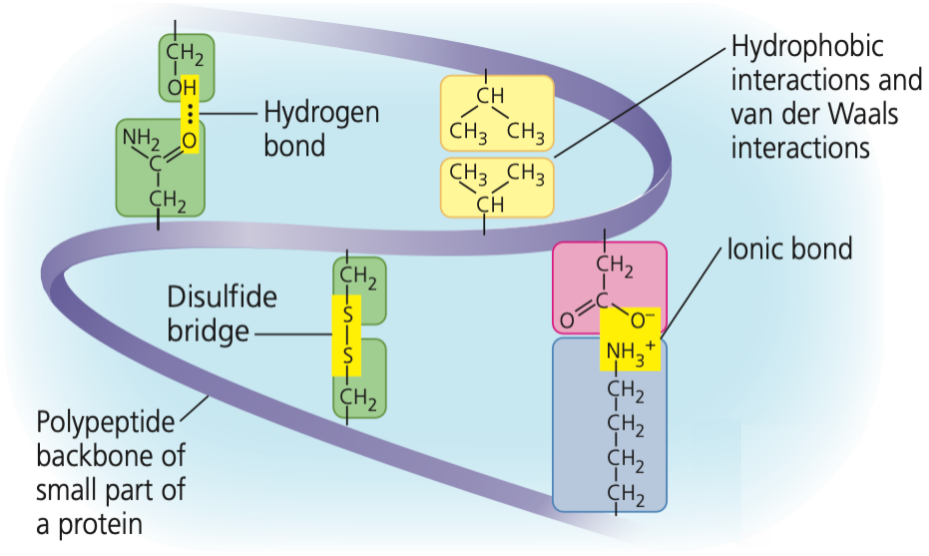


Figure 3.5 The various interactions which make up the tertiary structure of a protein. (Source: Campbell Biology)

Amino acids can also be chemically modified. The three amino acids **serine**, **threonine**, and **tyrosine** can all be modified through the process of **phosphorylation**, or the adding of the chemical group **phosphate**. Phosphate groups are hydrophilic, and thus phosphorylations of these amino acids can play a role in the tertiary structure of a protein.

3.4 Quaternary Structure

The **quaternary structure** of a protein arises when multiple polypeptides interact with each other to form one aggregate protein. Not all proteins have a quaternary structure.

One example of a protein with a quaternary structure is **collagen**. Collagen is made of three polypeptide chains coiled around each other in a triple helix, creating one strong protein fiber, and used extensively in animal organisms for its strength in tissue.

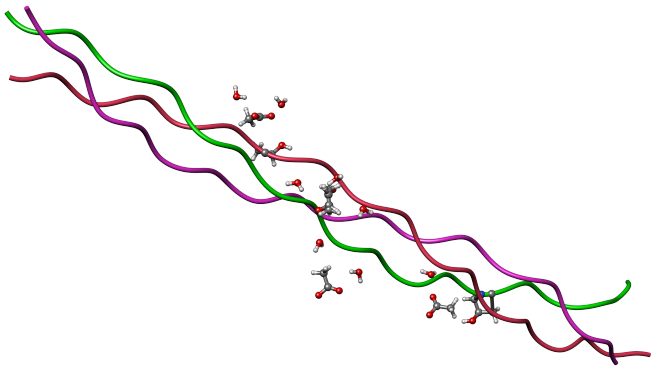


Figure 3.6 Collagen, a protein which exhibits a quaternary structure. Each differently-colored strand is a separate polypeptide chain.

Polypeptides involved in quaternary structure do not necessarily have to be the same polypeptide. The protein **hemoglobin**, which transports oxygen and is found in red blood cells, exhibits quaternary structure and has two different polypeptide **subunits**. Hemoglobin is made of two α and two β subunits, which interact to form a singular hemoglobin protein.

4 Enzymes

Enzymes are a type of protein which allow many chemical reactions to occur in the cell to be possible. Enzymes catalyze reactions, speeding them up and sometimes being the sole reason they occur. Without enzymes, many cellular processes simply could not happen.

4.1 Enzyme Structure

The molecules which enzymes act upon and bind to are known as **substrates**. Most enzymes are very specific to their substrate and can only accept one type of molecule.

Each enzyme has a specific region, known as the **active site**, which chemically binds to and interacts with the substrate. This active site needs to be shaped specifically for the substrate it's binding to in order for it to properly function. The resulting bound enzyme and substrate are known as an **enzyme-substrate complex**.

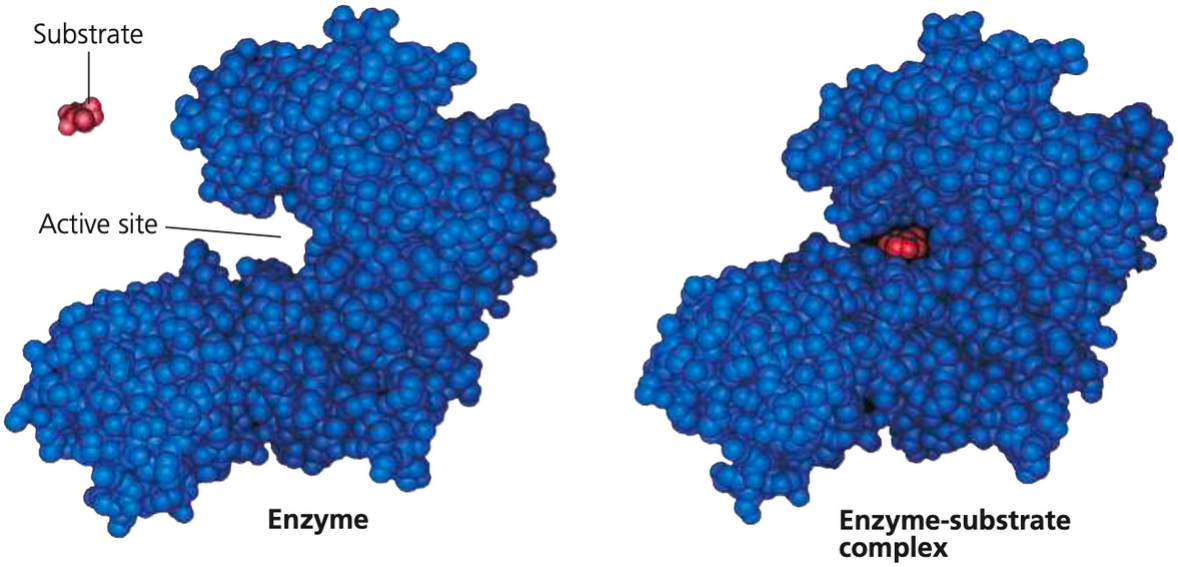


Figure 4.1 An image depicting the binding of a substrate to an enzyme's active site, forming an enzyme-substrate complex. (Source: Campbell Biology)

There are currently two popular models which depict enzyme-substrate binding.

- In the **Lock-and-Key hypothesis**, an enzyme is structurally rigid and contains the correct groove that only its substrate can fit in. The enzyme thus acts as a lock, and the substrate as a key.
- In the **Induced Fit hypothesis**, an enzyme is not structurally rigid. When a substrate is bound to the enzyme, interactions between the chemical groups of the enzyme and the substrate cause the enzyme to undergo changes in its conformation, binding and shifting to hold on tighter to its bound substrate.

Both models are generally accepted as correct and are complementary to each other.

The polypeptides which make up an enzyme are very complex and specific enough that when placed in a suitable environment, they would fold into an enzyme protein. This protein has an almost perfectly-shaped active site which promotes the bonding of a specific substrate. After the initial binding of a substrate, further interactions result in even stronger binding.

The complexity of protein folding is so vast that, if a given polypeptide were to shift conformations every picosecond, it would take longer than the age of the universe to fold in every possible way. This is the thought experiment known as **Levinthal's paradox**. The reason that polypeptides are able to fold into their biologically correct conformations so quickly, rather than in the timeline of a universe, is due to various local interactions between the amino acid monomers of a polypeptide and also help from other proteins. **Chaperonins** are a group of proteins which aid in the polypeptide folding process.

4.2 Catalysis

Catalysis is defined as the acceleration of a chemical reaction by a **catalyst**. Enzymes are the primary biological catalyst found in cells, and they perform their roles fantastically well.

4.2.1 Energy of Activation

Recall that chemical reactions have a ΔG value, or a change in Gibbs free energy. A negative ΔG value represents energy being released, and a positive ΔG value represents energy being inputted. Recall that, through the second law of thermodynamics, entropy is inevitable and reactions and changes that increase entropy are favored; a house of cards tends to fall.

In isolation, both of these principles make sense. However, when combining the two, some inaccuracies appear to arise. For example, why does paper, made of cellulose built from glucose molecules, not instantly break down into smaller molecules? The breakdown of glucose results in energy being released, and ΔG is negative, thus the reaction is spontaneous. Why doesn't everything simply break down?

This is due to something called **activation energy**, $\mathbf{E}_a$. Although the breakdown of paper is energetically favored and spontaneous, it doesn't simply occur without some form of external input. Spontaneity doesn't signify the speed of a reaction, simply whether it is energetically favorable or not. There first needs to be an input of energy, the E_a, in order for this reaction to proceed. When put to a flame, the paper burns, releasing energy in the form of heat and light, and breaks down into its basic constituents. The flame would act as the energy input to fulfill the necessary E_a, allowing the rest of the paper to burn and release energy. Similarly, a house of cards will not fall until it is pushed, and the same goes for chemical reactions at the molecular scale.

The reason for the existence of E_a is that there are pre-existing bonds between the atoms of a molecule. Recall that, in order to break a bond, energy must be inputted to separate individual atoms until their bonds break. This required input of energy is what forms the basis of E_a. Before a glucose molecule can break down, energy must be added to separate the bonds. Note that, although an input of energy is required, the released energy is always greater than the E_a, so the ΔG value is always negative for a spontaneous reaction.

Although theoretically, spontaneous chemical reactions will always proceed, sometimes they will not occur on its own, or rather will occur too slowly for practical purposes, unless in the correct environment. Heat is a source of energy to fulfill E_a, however is impractical in many cases, especially in the cell. Constantly using energy as heat for chemical reactions would be too inefficient and would lead to many other problems. Heat, in addition to breaking the bonds of the desired molecule for reacting, will also interact with the bonds of other cellular molecules, like proteins, leading to denaturing and dysfunction.

Example 4.1 (USABO Open Exam 2010) When the temperature increases, which of the following statements is not true?

(A) Dissolved oxygen decreases at higher temperatures and higher salinity.
(B) Many corals die when the temperature exceeds 86°F.
(C) Metabolic reactions are less likely to achieve their activation energy.
(D) The amount of carbon dioxide that can be absorbed by the ocean decreases.
(E) Zooxanthellae are released from the coral and thrive when the temperature exceeds 86°F.

Solution: Immediately, by looking at the provided answers, the correct choice should be obvious, even if one doesn't know whether the other answers are true or not. As temperature increases, there is more thermal energy free to break and weaken bonds, thus allowing metabolic reactions to be more likely to reach their activation energy. The answer is thus C.

The impracticality of constantly increasing cellular temperature is where **enzyme catalysis** comes in. Enzymes and other catalysts decrease the E_a, lowering the amount of energy necessary to start a chemical reaction. Sometimes, they lower it enough that chemical reactions can simply occur in the cellular environment, through thermal energy already present. This also speeds up chemical reactions, as the less E_a required, the faster a reaction can proceed.

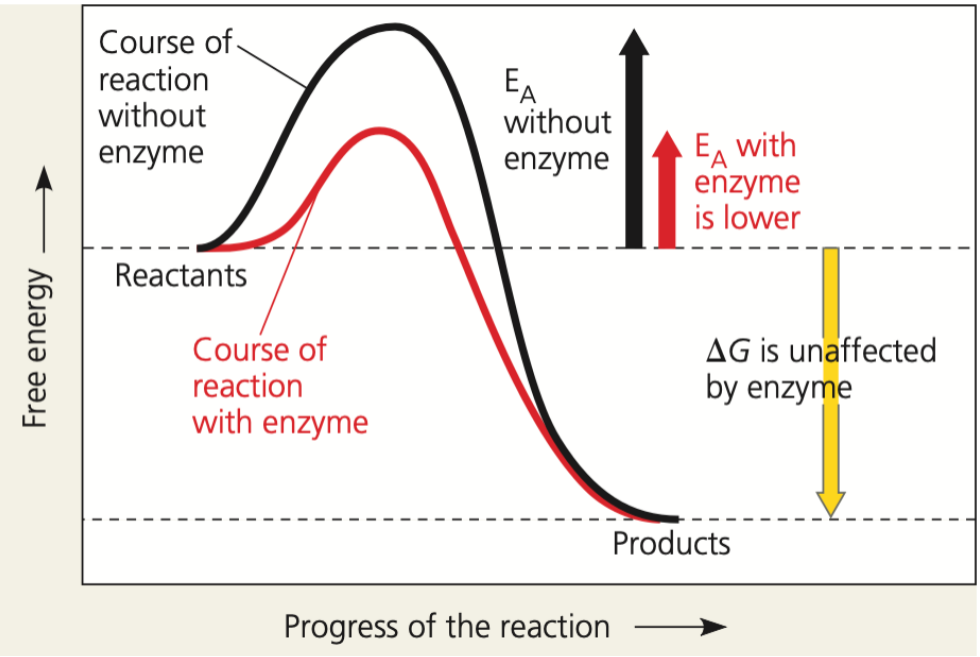


Figure 4.2 A graph showing an enzyme's effect on the E_a of a chemical reaction. Note that ΔG remains unchanged with or without an enzyme. (Source: Campbell Biology)

Enzymes lower E_a through placing mechanical stress on the its bound substrates. Energy is only required to excite atoms and separate them enough that bonds break. Enzymes interact with and bond with the substrate in such a way that it is mechanically pulled, putting strain on the bonds and pushing atoms away from each other, decreasing the amount of energy needed to fully separate them.

4.3 Enzymatic Regulation

In the case that a chemical reaction need be slowed or quickened, enzymatic regulation processes in the cell can alter the overall effect of enzymes.

4.3.1 Types of Inhibition

Recall that enzymes, although they are very specific to their substrate, are able to bind to other molecules. This phenomenon is what allows the **inhibition** of enzymatic activity.

There are two main types of enzyme inhibition:

- **Noncompetitive inhibition**, where a molecule which acts as an inhibitor binds to a region outside of the active site of the enzyme. This binding induces a shape change in the enzyme, leading to decreased or stopped enzymatic activity. The inhibitor decreases enzymatic activity by altering the enzyme itself.
- **Competitive inhibition**, where a molecule which acts as an inhibitor binds to the active site of an enzyme. The inhibitor decreases enzymatic activity by competing with the substrate for catalysis.

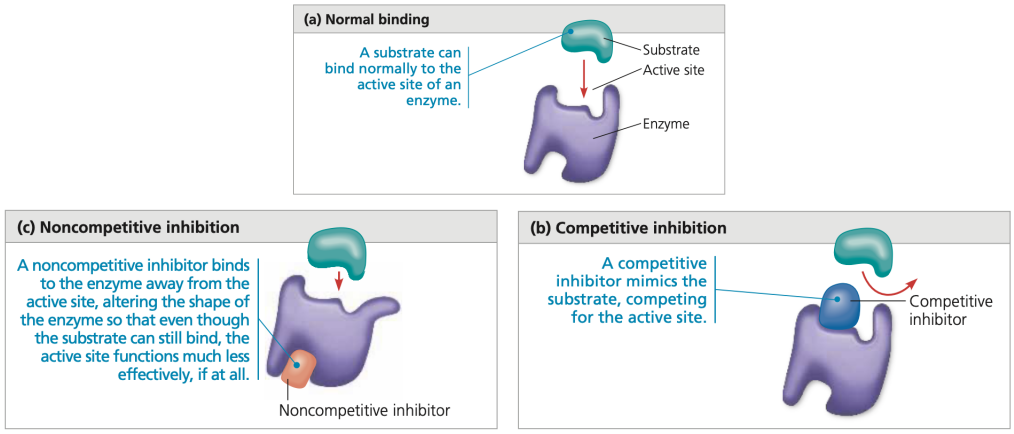


Figure 4.3 Models depicting normal enzymatic activity (top), noncompetitive inhibition (bottom left), and competitive inhibition (bottom right). (Source: Campbell Biology)

These types of inhibition can also be **irreversible** or **reversible**.

- In **reversible inhibition**, the inhibitor binds weakly to the enzyme through intermolecular forces. A **reversible competitive inhibitor** may bind to the active site, then release after a short period of time. A **reversible noncompetitive inhibitor** may induce a temporary shape change in an enzyme which eventually returns back to normal when released.
- In **irreversible inhibition**, the inhibitor binds strongly to the enzyme through covalent bonds. An **irreversible competitive inhibitor** may bind to the active site, then remain permanently, preventing any more enzymatic activity from occuring. Typically, there are no irreversible noncompetitive inhibitors due to the nature of noncompetitive inhibition.

4.3.2 Allosteric Regulation

The control of enzymatic activity through the binding of a molecule at a location other than the active site is known as **allosteric regulation**. The term allosteric regulation includes both stimulation and inhibition of enzymatic activity. Molecules which increase enzymatic activity are known as **activators**. All forms of noncompetitive inhibition are classified under allosteric regulation.

Allosteric regulation is typically always reversible due to the constantly fluctuating needs of the cell. Although there are some forms of irreversible allosteric regulation, they are few and far between due to the lack of necessity and danger of a permanent change in enzymatic activity.

Enzymes are not entirely static; they constantly oscillate and dance between multiple possible configurations. This phenomenon is referred to as **conformational dynamics**. These configurations have different levels of enzymatic activity.

- The configuration where a substrate is most easily able to bind to an enzyme and catalysis is most prominent is known as the **active form**.
- The configuration where a substrate is least easily able to bind to an enzyme and catalysis is least prominent is known as the **inactive form**.

The sites at which allosteric regulator molecules bind to an enzyme are known as **allosteric sites**.

- **Allosteric activators** bind to the allosteric site on an enzyme and increase the likelihood that an enzyme is in its active form. This leads to an increase in overall enzymatic activity.
- **Allosteric inhibitors** bind to the allosteric site on an enzyme and increase the likelihood that an enzyme is in its inactive form. this leads to a decrease in overall enzymatic activity.

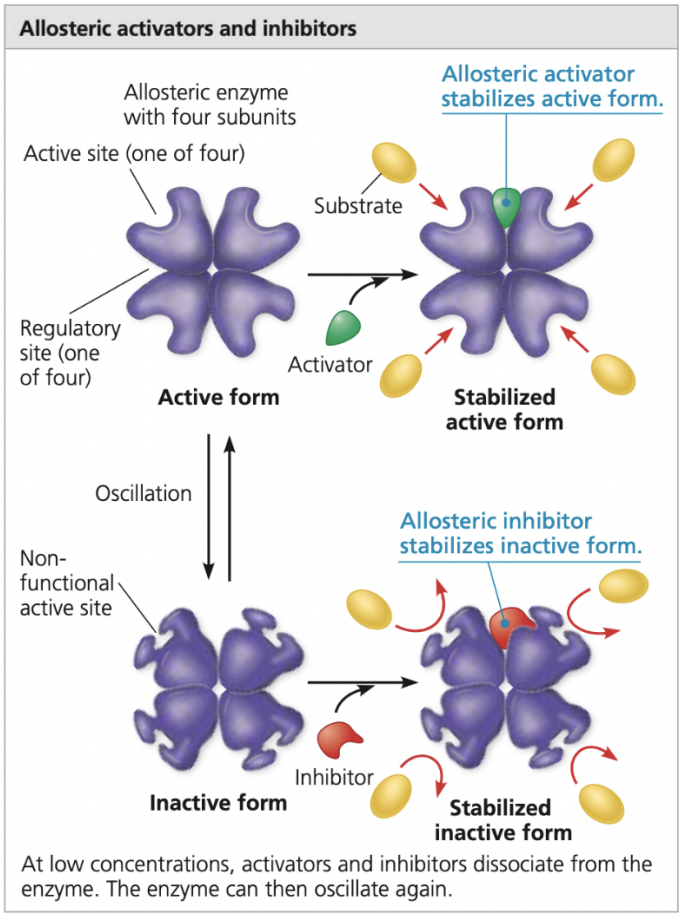


Figure 4.4 A chart depicting the different types of allosteric regulation. Note the conformational dynamics of the shown enzyme, and the oscillation between its active and inactive forms.(Source: Campbell Biology)

4.3.3 Cooperativity

Many enzymes which undergo allosteric regulation have multiple **subunits**. Recall the quaternary structure present in certain proteins and their multiple polypeptide chains. In such enzymes, each subunit is made of a separate polypeptide chain, and has its own active site and allosteric site. During allosteric regulation, the inhibition or activation at one allosteric site is reflected on the rest of the enzyme.

Sometimes, a substrate is able to act as an allosteric regulator. This phenomenon is known as **cooperativity**. Although it binds to the active site rather than the allosteric site, it has effects on the other active sites on the other subunits of an enzyme and is thus classified under allosteric regulation.

There are two types of cooperativity which can increase or decrease activity throughout an enzyme.

- **Positive cooperativity** is a form of cooperativity which increases the likelihood of substrate binding in an enzyme. A substrate is bound, which then promotes further substrate binding to other active sites.
- **Negative cooperativity** is a form of cooperativity which decreases the likelihood of substrate binding an an enzyme. A substrate is bound, which then decreases further substrate binding to other active sites. Forms of negative cooperativity are less common in cellular processes.

An example of positive cooperativity can be found in the enzyme **ATP synthase**. When a H^+ ion binds to an active site on a subunit of the enzyme, a conformational change occurs, leading to greater H^+ ion affinity in the other subunits.

An example of negativity cooperativity can be found in the protein **hemoglobin**, found in our red blood cells. Although not an enzyme, as it doesn't catalyze any reactions, hemoglobin exhibits negative cooperativity in substrate binding. When an O_2 molecule binds to the active site of one of the subunits in hemoglobin, the affinity for CO_2 molecules in the rest of the protein is decreased.

Substrates and proteins can have different levels of cooperativity. These **degrees of cooperativity** are quantified by a value known as the **Hill coefficient**, or $\mathbf{n}_{Hill}$.

- Molecules that exhibit positive cooperativity have $n_{Hill} > 1$.
- Molecules that exhibit negative cooperativity have $n_{Hill} < 1$.
- Molecules that exhibit no cooperativity have n_{Hill} of 1.

n_{Hill} can range from 0-∞. Molecules which do not exhibit any binding at all have a n_{Hill} value of 0. In practice, molecules typically have a hill coefficient of $0.5 < n_{Hill} < 3$. Molecules with n_{Hill} values outside of this range are rarely observed.

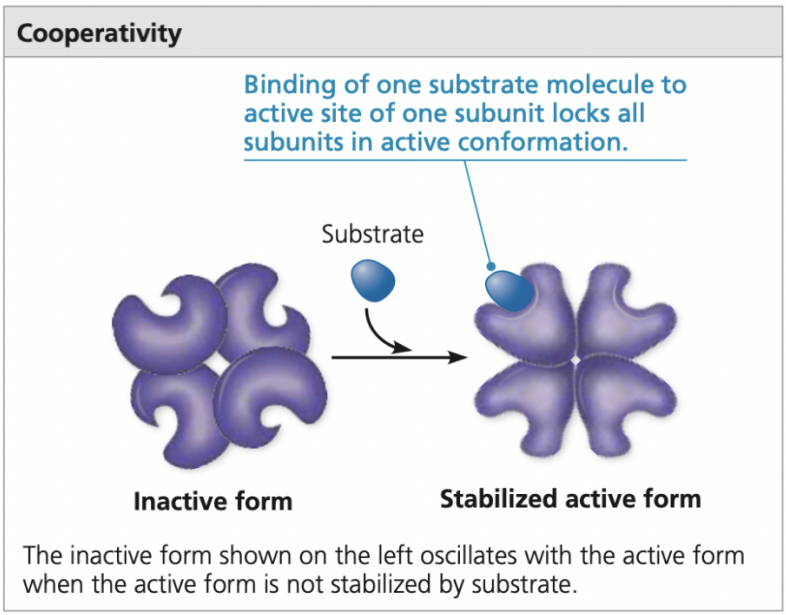


Figure 4.5 A diagram depicting positive cooperativity in an enzyme. (Source: Campbell Biology)

4.3.4 Feedback Inhibition

One interesting phenomenon found in many cellular processes involving enzymes is **feedback inhibition**. The products of an enzyme-controlled reaction eventually go back and inhibit its creation.

One example of feedback inhibition can be found in the process of cellular respiration. **Pyruvate dehydrogenase** is an enzyme which catalyzes the formation of **Acetyl-CoA** in the cell. The molecule **Acetyl-CoA** then inhibits **pyruvate dehydrogenase**. This may seem counter-intuitive at first, however it leads to overall regulation of Acetyl-CoA levels; when too much of the molecule is present, the enzyme involved in its creation is inhibited, and when too little of the molecule is present, the enzyme is uninhibited and free to catalyze more reactions.

Feedback inhibition can also reach back many steps in a chemical pathway. **ATP**, the source of energy for the cell and one of the final products of cellular respiration, is able to inhibit an enzyme involved in the very beginning steps of the entire process.

All of these types of enzyme control play a part in maintaining the homeostasis of a cell. Through allosteric regulation, competitive and noncompetitive inhibitors and activators, feedback inhibition and activation, cooperativity, and more, the multitudes of processes in a cell are catalyzed and regulated.

4.4 Enzyme Kinetics

Enzyme kinetics is the study of the rate of catalyzed reactions, and how different factors can affect the impact an enzyme has on a particular chemical reaction.

4.4.1 Michaelis-Menten Kinetics

The Michaelis-Menten equation is an important tool used by biologists to determine how quickly an enzyme-catalyzed reaction will proceed, and is as follows:

$$v = \frac{V_{max} a}{K_M + a}$$

where v represents the velocity of the reaction, V_{max} represents the maximum rate of the reaction, a represents the concentration of the substrate, and K_M represents the Michaelis constant.

An enzyme catalyzes a reaction very quickly—substrates enter the active site, a reaction is catalyzed, and a product leaves the enzyme—up to millions of times a second. Most of the time, an enzyme is idly waiting for a substrate to bind to its active site. By increasing the concentration of a substrate a in an environment, this wait time is decreased, and the enzyme can thus catalyze reactions quicker. At some point, increasing a will have no effect on the rate of a reaction, as the enzyme will have no time between individual catalyses. This is V_{max}. K_M is a constant which measures the affinity between the enzyme and its substrate. It is the substrate concentration at which the reaction rate is half of V_{max}.

It is important to note that there are other ways of increasing reaction rate than increasing a. Simply increasing the amount of enzymes will lead to a greater total reaction velocity. This is reflected in the Michaelis-Menten equation, where since enzyme concentration is increased, V_{max} is increased. Enzymes also have optimal temperature and pH levels where they work best, also reflected in the Michaelis-Menten equation by increasing V_{max} as conditions become closer to ideal.

The Michaelis-Menten equation for a chemical reaction can be graphed, creating a **Michaelis-Menten curve**, allowing biologists to visualize the change in v against a.

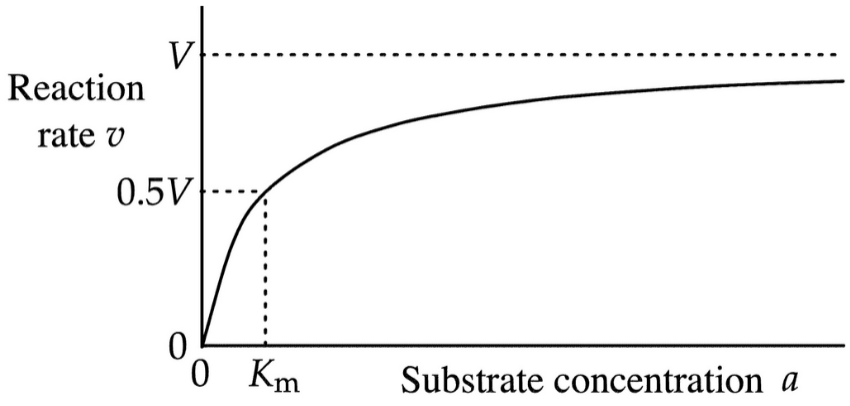
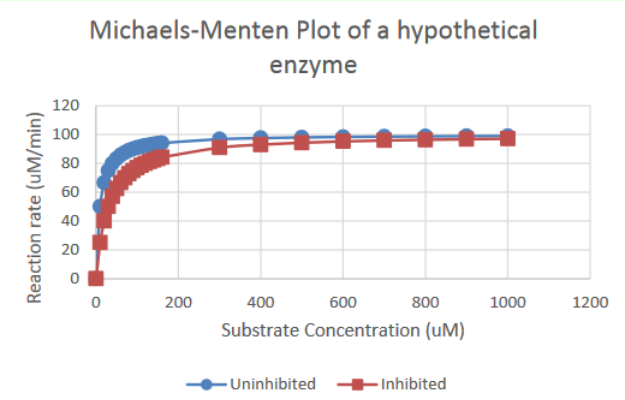
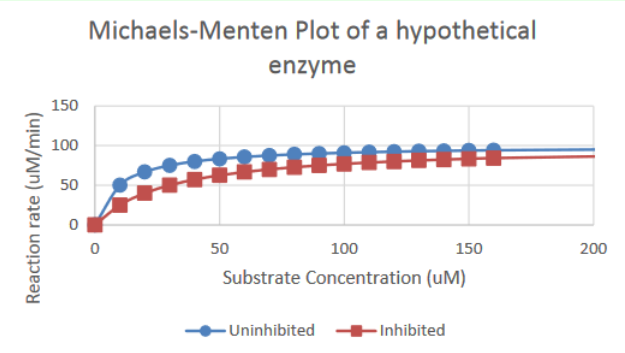


Figure 4.6 A Michaelis-Menten curve. (Source: Athel Cb)

Example 4.2 (USABO Semifinal Exam 2015) Hypothetical data for an enzyme on its reaction kinetics were collected in the presence and absence of an inhibitor. Assuming that the enzyme is properly modeled using Michaelis-Menten kinetics, Use the two plots below

to answer the following questions.





Note that the plots show the same data, however, the horizontal axes are scaled differently.

What is the maximum reaction rate possible for the uninhibited enzyme under these conditions?

(A) 10 uM/min
(B) 40 uM/min
(C) 80 uM /min
(D) 100 uM /min
(E) 120 uM /min

Solution: In this question, we are being asked to find the V_{max} of the uninhibited enzyme. We can thus ignore the red line representing the kinetics of the inhibited enzyme, and focus solely on the blue one. Recall that the Michaelis-Menten equation is:

$$v = \frac{V_{max} a}{K_M + a}$$

In the case of this graph, only substrate concentration is being increased. As a approaches infinity, v eventually approaches V_{max}, forming a horizontal asymptote at $v = V_{max}$. When looking at the graph, there is a clear horizontal asymptote at $v = 100$ uM /min. The answer is thus D.

4.4.2 Lineweaver-Burk Plot

The **Lineweaver-Burk plot**, also known as the **double reciprocal plot**, is a transformation of the Michaelis-Menten equation. From its name, the reciprocals of both sides of the equation are taken and plotted. The transformation equation is as follows:

$$\frac{1}{v} = \frac{K_M}{V_{max}} \cdot \frac{1}{a} + \frac{1}{V_{max}}$$

Note its resemblance to the general linear equation $y = mx + b$. When plotting a Lineweaver-Burk plot with $1/V_{max}$ over 1/a, the result is a linear output with a slope of K_M/V_{max} and y-intercept of $1/V_{max}$.

Lineweaver-Burk plots are used especially commonly to show the effects of enzyme inhibition.

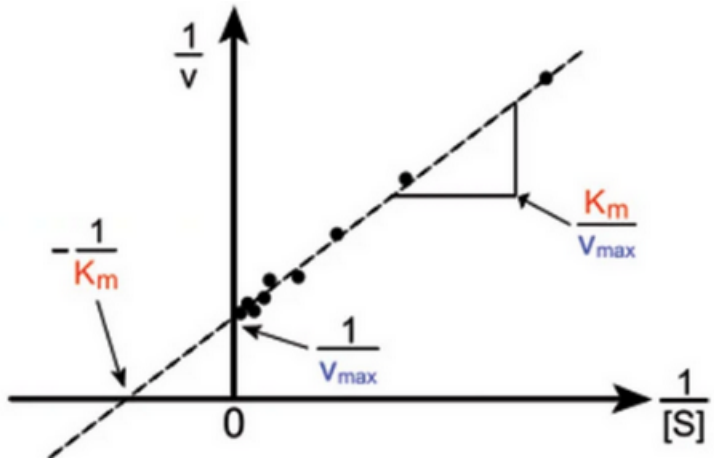


Figure 4.7 A Lineweaver-Burk plot. Note that substrate concentration is represented by [S] in this depiction. (Source: PhD Nest)

Example 4.3 (IMDO Exam 2022) In the Lineweaver Burk plot, what is the x-intercept?

(A) V_{max}
(B) K_M
(C) $1/V_{max}$
(D) $-1/K_M$

Solution: Recall the transformed Michaelis-Menten equation used for a Lineweaver-Burk double reciprocal plot:

$$\frac{1}{v} = \frac{K_M}{V_{max}} \cdot \frac{1}{a} + \frac{1}{V_{max}}$$

Substituting y for $\frac{1}{v}$ and x for $\frac{1}{a}$ leaves us with the new equation:

$$y = \frac{K_M}{V_{max}} x + \frac{1}{V_{max}}$$

This equation is similar in form to the general straight line equation $y = mx + b$. The

x-intercept of a graph is the point at which $y = 0$, so setting $y = 0$ and solving for x will lead to the x-intercept.

$$0 = \frac{K_M}{V_{max}}x + \frac{1}{V_{max}}$$

$$\frac{K_M}{V_{max}} = -\frac{1}{V_{max}}$$

$$x = -\frac{1}{K_M}$$

The x-intercept of a Lineweaver-Burk plot is $-\frac{1}{K_M}$, and thus the answer is D.

4.4.3 Effects of Inhibition

Inhibition affecets the K_M and V_{max} of an enzyme-catalyzed reaction, which can then be portrayed effectively using Michaelis-Menten curves or Lineweaver-Burk plots.

Competitive inhibition leads to **increased K_M** and has **no effect on V_{max}**.

- In competitive inhibition, an inhibitor molecule binds to the active site of an enzyme, effectively reducing the total amount of active enzymes available for binding by the substrate. A higher substrate concentration is required to achieve half of the maximum reaction rate, so K_M is increased.
- Since the competitive inhibitor does not affect the catalytic properties of an enzyme, V_{max} is unchanged.

Noncompetitive inhibition has **no effect on K_M** and leads to **decreased V_{max}**.

- In noncompetitive inhibition, an inhibitor molecule binds to the allosteric site of an enzyme, reducing its catalytic efficiency. since it does not affect the affinity of the enzyme for its substrate, K_M is unchanged.
- Since the noncompetitive inhibitor affects the catalytic properties of an enzyme, V_{max} is decreased.

It is also important to note the existence of **uncompetitive inhibition**, which leads to both **decreased K_M** and **decreased V_{max}**.

- In uncompetitive inhibition, the inhibitor molecule is only able to bind to enzyme-substrate complexes. When bound, it increases the tightness of the grip between the enzyme and the substrate, essentially trapping the substrate to the enzyme. This leads to a decreased K_M, increasing the affinity of the substrate to the enzyme.
- When inhibiting an enzyme-substrate complex, the substrate is trapped and cannot release as easily. This decreases the catalytic efficiency of the enzyme, and thus V_{max} is decreased.

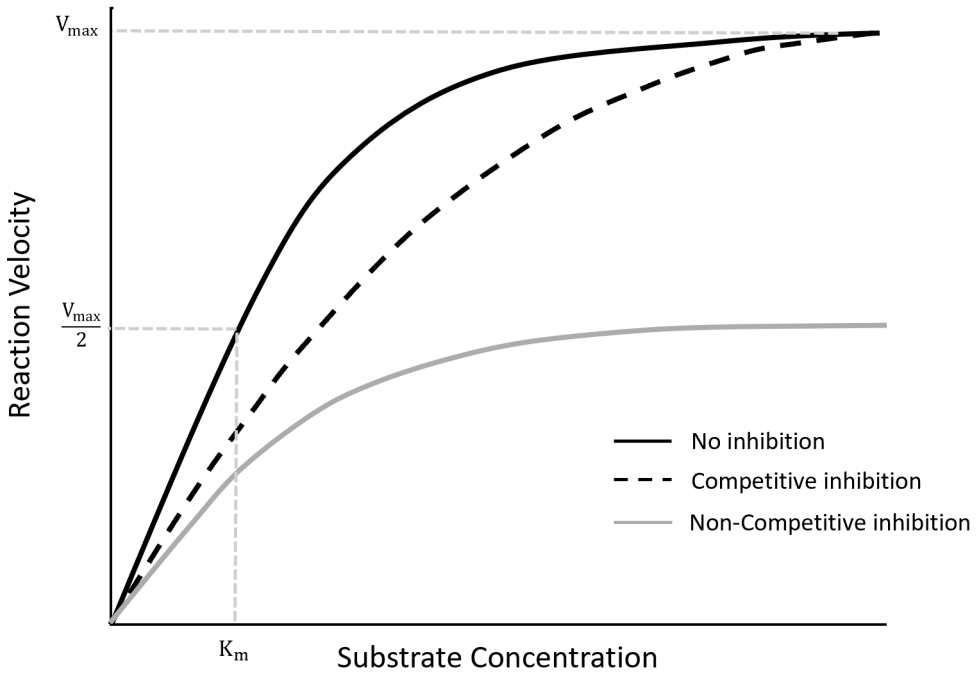


Figure 4.8 A Michaelis-Menten curve of an enzyme-catalyzed reaction under normal conditions, competitive inhibition, and noncompetitive inhibition. (Source: Hansehan)

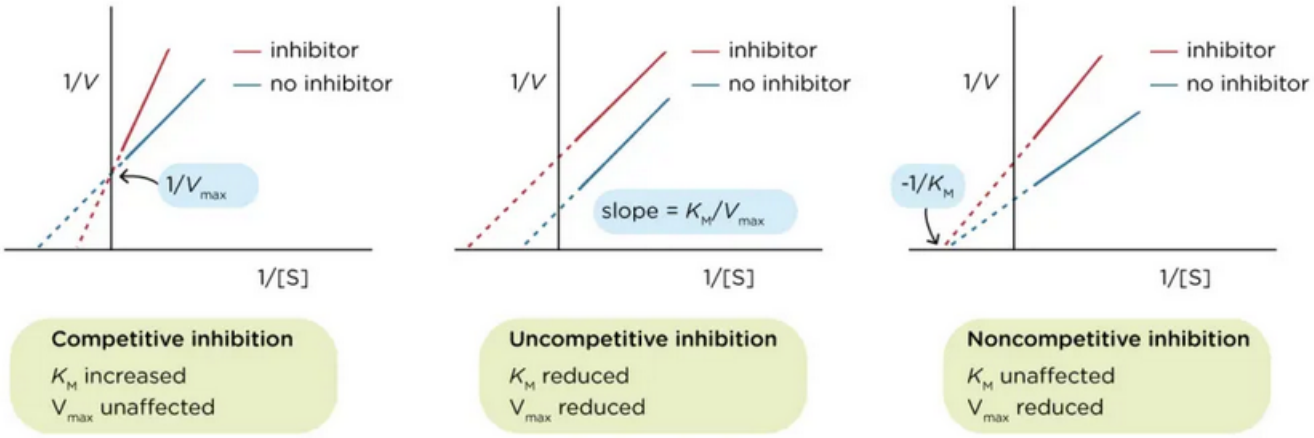


Figure 4.9 Multiple Lineweaver-Burk plots depicting the effects of competitive, uncompetitive, and noncompetitive inhibition on an enzyme-catalyzed reaction. (Source: Jack Westin)

Example 4.4 (Adapted from IMDO Exam 2022) Which of the following is found in non-competitive inhibition of an enzyme?

(A) Increased V_{max}
(B) Unaffected V_{max}
(C) Increased K_M
(D) Unaffected K_M

Solution: It may be helpful to think about how each type of inhibition affects an enzyme and deduce its effects on K_M and V_{max} from there. For example, remember that noncompetitive inhibition is a form of allosteric regulation where the inhibitor molecule does not bind to the active site of an enzyme. Because of this, noncompetitive inhibition has no effects on K_M, as it doesn't affect the affinity of the enzyme to its substrate. The answer is thus D.

5 Conclusion

5.1 Closing Statement

The concepts which have been outlined in this paper have hopefully been enlightening to a new reader who has not yet learned the laws of thermodynamics and the universe, and opened their eyes to the depth of biology and how far life has come. As we study physics and make observations of the world around us, we realize the pure, unbreakable laws which govern our lives, of entropy and energy. As we study biology, we see the miraculous ways our cells have evolved in order to overcome and bypass such laws. We exist in a world constantly breaking apart, and eventually the entropic heat death of the universe will occur, but our cells and life as we know it have seemed to temporarily overcome entropy and have built our house of cards.