proprep

Workbook

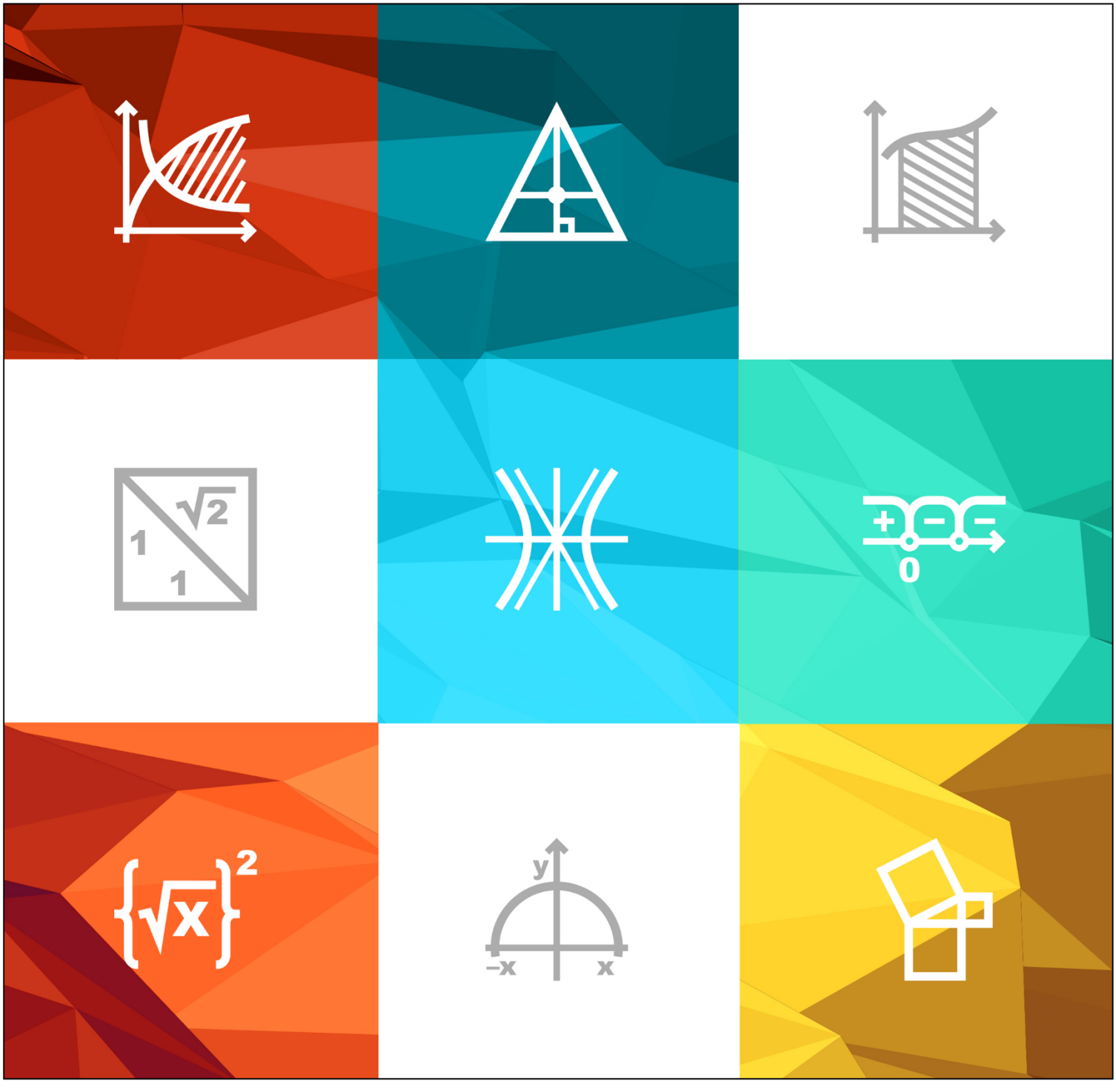

Table of Contents

Enzymes

Introduction to Enzymes

Questions

1) A general and specific outline of the history of discovery of enzymes is described in this lesson, answer the following questions:

a. What was first recognized and described in the late 1700s in studies on the digestion of meat by secretions of the stomach?
b. What did Eduard Buchner discover in 1897 and what was its significance?
c. What did Frederick W. Kühne contribute to the topic?

2) How did crystallization techniques contribute to the study of enyzmes?

3) Almost all enzymes are proteins, what is the exception to this?

4) A few factors were listed as significant to enzyme catalytic activity. Name a couple of these.

5) Define, explain and give examples of a **cofactor** and a **coenzyme.**

6) Define the following terms relating to enzymes:

a. **Prosthetic group**
b. **Holoenzyme**
c. **Apoprotein**

7) Fill in the blanks to complete the sentences with regard to coenzymes.

I. Coenzymes act as _____ carriers of specific _____ groups.

II. _____ coenzymes are derived from _____ - organic nutrients required in small amounts in the diet.

8) List 3 ways mentioned in the lesson for naming enzymes.

9) What is the system by which biochemists classify and name enzymes, and give an example?

Answer Key

1) a. Biological catalysis was first recognized and described in the late 1700s
- the digestion of meat by secretions of the stomach

b. In 1897 Eduard Buchner discovered that cell-free yeast extracts could ferment sugar to alcohol, proving that fermentation was promoted by molecules that continued to function when removed from cells.
   - This experiment marked the end of vitalistic notions and the beginning of the science of biochemistry.

c. Frederick W. Kühne called these molecules that were detected by Buchner **enzymes** from the Greek word: *Enzymos* = leavened.

2)

- The isolation and crystallization of urease by James Sumner in 1926 provided a breakthrough in early enzyme studies
  - Sumner found that urease crystals consisted entirely of protein, and he postulated that all enzymes are proteins.
- [In the absence of other examples, this idea remained controversial for some time. In the 1930s John Northrop and Moses Kunitz crystallized pepsin, trypsin, and other digestive enzymes and found them also to be proteins.

3) All enzymes are proteins with the exception of a small group of **catalytic RNA molecules**

4)

- Enzyme catalytic activity depends on the integrity of their native protein conformation.
    - If an enzyme is denatured [or dissociated into its subunits,] catalytic activity is usually lost.
    - If an enzyme is broken down into its component amino acids, its catalytic activity is always destroyed.
- The primary, secondary, tertiary, and quaternary structures of protein enzymes are essential to their catalytic activity .
- Some enzymes require only their amino acid residues in order to be active.
- Others require an additional chemical component called a **cofactor** or a **coenzyme**.
- Some enzymes require both a coenzyme and one or more metal ions for activity.

5) Some enzymes require an additional chemical component, this can be 1 of 2:

1. Some enzymes rely on a **cofactor**— which is either one or more inorganic ions.
2. Some enzymes require a complex organic or metalloorganic molecule called a **coenzyme.**
    - Cofactors: Fe^{2+}, Mg^{2+}, Mn^{2+}, or Zn^{2+}
    - Coenzymes:

- Some enzymes require both a coenzyme and one or more metal ions for activity.

6) a. **Prosthetic group** –A coenzyme or metal ion that is very tightly or even covalently bound to the enzyme – protein.

b. **Holoenzyme** - A complete, catalytically active enzyme together with its bound coenzyme and/or metal ions.

C. **Apoprotein** – this is also known as the **apoenzyme**, and it refers to the protein part of a holoenzyme, of an enzyme complex.

7)

I. Coenzymes act as transient carriers of specific functional groups

II. Most are derived from vitamins - organic nutrients

8)

1. Many enzymes are named by the addition of the suffix "-ase" to the name of their substrate or to a word or phrase describing their activity:
   - Urease catalyzes hydrolysis of urea
   - DNA polymerase catalyzes the polymerization of nucleotides to form DNA
2. Some enzymes were named by their discoverers for a broad function, before the specific reaction catalyzed was known:
   - An enzyme known to act in the digestion of foods was named pepsin, from the Greek *pepsis* - "digestion"
   - Lysozyme was named for its ability to lyse bacterial cell walls
3. Some enzymes were named for their source:
   - Trypsin – named in part from the Greek *tryein* - "to wear down," was obtained by rubbing pancreatic tissue with glycerin - wearing down the pancreatic tissue
   - Sometimes the same enzyme has two or more names, or two different enzymes have the same name

9) Enzymes Are Classified by the Reactions They Catalyze

- Biochemists, have adopted a system for naming and classifying enzymes in which they are divided into 6 classes, each with subclasses, based on the type of reaction catalyzed.
    - Each enzyme is assigned a 4-part classification number (Enzyme Commission number) and a systematic name, which identifies the reaction it catalyzes.
        - The formal systematic name of the enzyme catalyzing the reaction:

          ATP + D-glucose → ADP + D-glucose 6-phosphate
          is *ATP:D-Hexose 6-phosphotransferase* - which indicates that it catalyzes the transfer of a phosphoryl group from ATP to glucose.
          Its Enzyme Commission number (E.C. number) is 2.7.1.1.

How Enzymes Work

Questions

1) Define the **active site** and the **substrate** and how these play a role in an enzyme catalyzed reaction.

2) Many reactions can take place in the absence of enzymes, yet enzymes' presence play a crucial role in these occurring within living organisms. Explain this concept as presented in the lesson.

3) In the context of enzymes, explain the significance of the phrase below:

$E + S \rightleftharpoons ES \rightleftharpoons EP \rightleftharpoons E + P$

4) Complete the sentence and explain its significance to enzymes:

Enzymes Affect Reaction _____, Not _____.

5) Define and explain the term "ground state".

6) The position and direction of equilibrium are not affected by any catalyst, rather it affects the rate of reaction. How does it do so?

7) What is the transition state?

8) In reference to the ground state and transition state, what is the **activation energy**?

9) Complete the sentence:

The enzymes not only accelerate the reactions, they _____ and _____ them so that the energy released is [recovered in other chemical forms and] made _____ to the cell for other _____.

10) Define the reaction intermediates and the rate-limiting step.

11) From thermodynamics, the relationship between K'_{eq} and $\Delta G'^{\circ}$ can be described by the expression

$$\Delta G'^{\circ} = -RT \ln K'_{eq}$$

Part I:

What do the components of this formula represent?

Part II:

What does this formula signify with regard to enzymatic reactions?

12) Part I:

What is a first-order reaction, and what is the expression that represents it?

Part II:

What does the *k* signify in the expression mentioned in Part I?

Part III:

If a first-order reaction has a rate constant k of 0.03 s^{-1}, what does this mean?

13) What is a **second order** reaction and how is this expressed in the rate equation formual?

Answer Key

1)

- The distinguishing feature of an enzyme-catalyzed reaction is that it takes place within the confines of a pocket on the enzyme called - the **active site**.
- The molecule that is bound in the active site and acted upon by the enzyme is called the **substrate**.

- The surface of the enzyme's active site is lined with amino acid residues with substituent groups that bind the substrate and catalyze its chemical transformation .

    - Frequently, the active site encloses a substrate, sequestering it completely from solution, providing an environment that is conducive to reaction taking place in the enzyme-substrate complex.

2) The enzymatic catalysis of reactions is essential to living systems by increasing their rate and/or probability of occurring.

- Under biologically relevant conditions, reactions tend to be slow—most biological molecules are quite stable in the neutral-pH, mild-temperature, aqueous environment inside cells.
- Many common reactions in biochemistry entail chemical events that are unfavorable or unlikely in the cellular environment, such as the collision of two or more molecules in the precise orientation required for reaction.
- Reactions required to digest food, send nerve signals, or contract a muscle do not occur at a useful rate without catalysis.

An enzyme circumvents these problems by providing a specific environment within which a given reaction can occur more rapidly.

3) This is representation of a simple enzymatic reaction, where:

E – enzyme.

S – substrate.

P – product.

ES - is a transient complex of the enzyme with the substrate.

EP – is a transient complex of the enzyme with the product.

4) Enzymes Affect Reaction **Rates,** Not **Equilibria.**

This concept is important to understanding catalysis, which is the role of enzymes in reactions.

- [It is important to grasp] the distinction between reaction equilibria and reaction rates.
- The function of a catalyst is to increase the rate of a reaction.
- Catalysts do not affect reaction equilibria.

5) The starting point for the reaction is the **ground state.**

- The lowest allowed energy state of an atom, molecule, or ion.
    - The ground state represents the most stable configuration.
    - The ground state is considered to have zero-point energy in comparison to other states.
    - ➢ If an electron absorbs energy, it may jump to an excited state and will return to the ground state.

6) The rate of a reaction is dependent on a parameter that enzymes help overcome:

- There is an energy barrier between S [substrate] and P [product]:
    - The energy required [for alignment of reacting groups, bond rearrangements, and other transformations required] for the reaction to proceed in either direction
- For a reaction to proceed, the molecules must overcome this barrier
- *Reaction rate*s can be increased by raising the temperature, thereby increasing the number of molecules with sufficient energy to overcome the energy barrier.
- Alternatively, the *activation energy* can be lowered by adding a catalyst.

Catalysts enhance reaction rates by lowering activation energies

7) The **transition state** is a fleeting molecular moment in which events (such as bond breakage, bond formation, and charge development) have proceeded to the precise point at which decay to either substrate or product is equally likely.

- At the top of the energy hill is a point at which decay to the S or P state is equally probable (it is downhill either way).

8) The difference between the energy levels of the ground state (the starting point for the reaction) and the transition state (the highest point of the energy barrier) is the **activation energy, ΔG‡**

- The rate of a reaction reflects this activation energy: a higher activation energy corresponds to a slower reaction.

9) The enzymes not only accelerate the reactions, they **organize** and **control** them so that the energy released made **available** to the cell for other **tasks**.

10) A reaction intermediate is any species on the reaction pathway that has a finite chemical lifetime (longer than a molecular vibration, ~10^{-13} seconds).

- When the S ⇌ P reaction is catalyzed by an enzyme, the ES and EP complexes can be considered intermediates.

Any reaction may have several steps involving the formation and decay of transient chemical species called **reaction intermediates.**

- Less stable chemical intermediates often exist in the course of an enzyme-catalyzed reaction.
- The interconversion of two sequential reaction intermediates constitutes a reaction step.
- When several steps occur in a reaction, the overall rate is determined by the step with the highest activation energy.
    - This is called the **rate-limiting step.**
    - The rate-limiting step is the highest-energy point in the diagram for interconversion of S and P.
    - The rate-limiting step can vary with reaction conditions.
    - Several steps may have similar activation energies, which means they are all partially rate-limiting.

11) Part I:

- Reaction equilibria are linked to the standard free-energy change for the reaction, ***ΔG***
- An equilibrium such as S ⇌ P is described by an
- Under the standard conditions used to compare biochemical processes, the equilibrium constant **equilibrium constant**, K_{eq}, (K) is denoted K'_{eq} (or K'):
- R is the gas constant, 8.315 J/mol
- T is the absolute temperature, 298 K (25°C)

Part II:

- The equilibrium constant is directly related to the overall standard free-energy change for the reaction.
- A large negative value for $\Delta G°$ reflects a favorable reaction.
- The rate is determined by the concentration of the reactant (or reactants) and by a **rate constant**, usually denoted by k.

12) Part I:

A first-order reaction is a reaction in which the rate depends only on the concentration of S.

- For the unimolecular reaction S → P, the rate (or velocity) of the reaction, V—representing the amount of S that reacts per unit time—is expressed by the **rate equation** formula [which is]:

$$V = k[S]$$

Part II:

- The factor *k* is a proportionality constant that reflects the probability of reaction under a given set of conditions (pH, temperature, and so forth)
- Here, [in the case of a first-order reaction] *k* is a first-order rate constant and has units of reciprocal time, such as s^{-1}

Part III:

This may be interpreted (qualitatively) to mean that 3% of the available S will be converted to P in 1 s.

  - If a first-order reaction has a rate constant k of X s^{-1}, it means that X% of the available S (substrate) will be converted to P (product) in 1 second.
  - A reaction with a rate constant of 2,000 s^{-1} will be over in a small fraction of a second.

13) If a reaction rate depends on the concentration of **two different compounds**, or if the reaction is between **two molecules** of the same compound, the reaction is second order and *k* is a second-order rate constant, with units of M^{-1} S^{-1}

- The rate equation then becomes: V = k[S1][S2]

Enzyme Function

Questions

1) Enzymes are highly selective rate enhancement catalysts.

Their ability to do this can be explained with two distinct but interwoven parts. What are these?

2) Complete the sentences regarding enzymes.

Enzymes can increase reaction rates by _____ orders of magnitude, and are specific, such that they effectively differentiate between _____ with similar structures.

3) Which of the following is not true about enzymes:

a. They increase ΔG of reactions.
b. They are usually made of amino acids.
c. They lower the activation energy of chemical reactions.
d. Each one is specific to the particular substrate(s) to which it binds.

4) An allosteric inhibitor does which of the following?

a. Binds to an enzyme away from the active site and changes the conformation of the active site, increasing its affinity for substrate binding.
b. Binds to the active site and blocks it from binding substrate.
c. Binds to an enzyme away from the active site and changes the conformation of the active site, decreasing its affinity for the substrate.
d. Binds directly to the active site and mimics the substrate.

5) Which of the following analogies best describes the induced-fit model of enzyme-substrate binding?

a. a hug between two people**.**
b. a key fitting into a lock.
c. a square peg fitting through the square hole and a round peg fitting through the round hole of a children's toy.
d. the fitting together of two jigsaw puzzle pieces.

6) The modern notion of enzymatic catalysis

Define the modern notion of enzymatic catalysis as first proposed by Michael Polanyi (1921) and Haldane (1930), and later elaborated by Linus Pauling in 1946.

7) Which statement is false and why?

a. weak interactions between substrate and enzyme are formed when the substrate reaches the transition state.
b. The summation of the unfavorable (positive) activation energy $\Delta G^{\ddagger}$ and the favorable (negative) binding energy ΔG_B results in a higher net activation energy.
c. The transition state is not a stable species rather a brief point in.
d. The enzyme-catalyzed reaction is much faster because the hill is much smaller.

8) Part I:

Which statement is true?

a. The free energy (binding energy) released by the formation of weak interactions offsets the energy required to reach the top of the energy hill.
b. The enzyme-catalyzed reaction is much faster than the uncatalyzed process, because the the energy barrier hill is cancelled by enzyme presence.
c. The groups on the substrate that are involved in the weak interactions with the enzyme need to be in close proximity to the bonds that are broken or changed.
d. The requirement for multiple weak interactions to drive catalysis is the reason for the big size of enzymes (and some coenzymes).
e. All of the above

Part II:

Correct the statements that are not true.

a. The free energy (binding energy) released by the formation of weak interactions offsets the energy required to reach the top of the energy hill.
b. The enzyme-catalyzed reaction is much faster than the uncatalyzed process, because the energy barrier hill is cancelled by enzyme presence.
c. The groups on the substrate that are involved in the weak interactions with the enzyme need to be in close proximity to the bonds that are broken or changed.
d. The requirement for multiple weak interactions to drive catalysis is the reason for the big size of enzymes (and some coenzymes).

9) The requirement for multiple weak interactions to drive catalysis is one reason why enzymes are so large, and they must provide these things for the interaction to successfully occur:

- _____ for ionic, hydrogen-bond, and other interactions.
- Position these groups so that _____ is optimized in the _____ state.
- Adequate binding is accomplished most readily by positioning a _____ in a cavity - the _____ site, where it is effectively removed from water.

10) Part I:

What can be said about the binding energy of an enzyme?

Part II:

How does binding energy contribute to the functions mentioned in Part I of the question?

11) Part I:

Which statement is false:

a. More than 80% of the enzymatic rate acceleration has been traced to enzyme-substrate interactions involving the phosphate group on carbon 3 of the substrate.
b. A given enzyme might incorporate several types of mechanisms, which are not mutually exclusive, that yield the reaction rate acceleration in its overall mechanism of action.
c. For most enzymes, It is difficult to quantify the contribution of any one catalytic mechanism to the rate and/or specificity of a particular enzyme-catalyzed reaction.
d. Binding energy makes an important, yet mostly small and lesser, contribution to catalysis.

Part II:

Correct the false statement from Part I:

12) 4 specific barriers were mentioned as needed to be overcome in order for a reaction to occur. State at least 2 of these.

13) Explain the concept of **induced fit.**

Answer Key

1)

1. The first lies in the rearrangements of covalent bonds during an enzyme-catalyzed reaction.
- Catalytic functional groups on an enzyme may form a transient covalent bond with a substrate and activate it for reaction, or a group may be transiently transferred from the substrate to the enzyme
- Covalent interactions between enzymes and substrates lower the activation energy and thereby accelerate the reaction by providing an alternative, lower-energy reaction path

2. The second part of the explanation lies in the *non-covalent* interactions between enzyme and substrate
- Much of the energy required to lower activation energies is derived from weak, noncovalent interactions between substrate and enzyme
- The interaction between substrate and enzyme in the ES complex is mediated by [the same forces that stabilize protein structure, including] hydrogen bonds and hydrophobic and ionic.
- Formation of each weak interaction is accompanied by release of a small amount of free energy that provides a degree of stability to the interaction.

2) Enzymes can increase reaction rates by **5 to 17** orders of magnitude, and are specific, such that they effectively differentiate between **substrates** with similar structures.

3) a

4) c

5) a

6) In order to catalyze reactions, an enzyme must be complementary to the **reaction transition state.** This means that optimal interactions between substrate and enzyme occur in the transition state.

7) **b-** The summation of the unfavorable (positive) activation energy $\Delta G^{\ddagger}$ and the favorable (negative) binding energy ΔG_B results in a lower net activation energy.

8) Part I:

a- The free energy (binding energy) released by the formation of weak interactions offsets the energy required to reach the top of the energy hill

Part II:

a. **Is True, so will leave it as is**
b. The enzyme-catalyzed reaction is much faster because the energy barrier hill is **much smaller.**
c. The groups on the substrate that are involved in these weak interactions **can be at some distance** from the bonds that are broken or changed.
d. The requirement for multiple weak interactions to drive catalysis is **one reason** for the big size of enzymes (and some coenzymes).

9) An enzyme must provide:

- **Functional groups** for ionic, hydrogen-bond, and other interactions.
- Position these groups so that **binding energy** is optimized in the **transition** state.
- Adequate binding is accomplished most readily by positioning a substrate in a cavity - the **active** site, where it is effectively removed from water.

  - The size of proteins reflects the need for superstructure to keep interacting groups properly positioned and to keep the cavity from collapsing.

10) Part I:

The same binding energy that provides energy for catalysis also gives an enzyme its specificity, the ability to discriminate between a substrate and a competing molecule.

Part II:

As for binding energy providing energy for catalysis, [this was explained in the previous lesson, and expanded in this lesson to detail that):

- The energy available from formation of a single weak interaction is generally estimated to be 4-30 kJ/mol.
- The overall energy available from several such interactions is sufficient to lower activation energies by the 60-100 kJ/mol required to explain the rate enhancements observed for enzymes.

With regard to enzyme specificity:

- An enzyme active site has functional groups arranged optimally to form a variety of weak interactions with a particular substrate; in the transition state, the enzyme will not be able to interact to the same degree with any other molecule.
  - If the substrate has a hydroxyl group that forms a hydrogen bond with a specific Glu residue on the enzyme, any molecule lacking a hydroxyl group at that particular position will be a poorer substrate for the enzyme.
  - In addition, any molecule with an extra functional group for which the enzyme has no pocket or binding site is likely to be excluded from the enzyme.

- Specificity is derived from the formation of many weak interactions between the enzyme and its specific substrate molecule.

11) Part I:

d

Part II:

d.Binding energy makes an important, and sometimes **the dominant**, contribution to catalysis.

12) Prominent physical and thermodynamic factors contributing to ΔG‡ , the barrier to reaction, might include:

1. a reduction in entropy, in the form of decreased freedom of motion of two molecules in solution.

2. the solvation shell of hydrogen-bonded water that surrounds and helps to stabilize most biomolecules in aqueous solution.

3. the distortion of substrates that must occur in many reactions (primarily electron redistribution, that the substrate must undergo to react.).

4. The need for proper alignment of catalytic functional groups on the enzyme.

- Binding energy can be used to overcome all these barriers.

13) Induced fit describes the change in conformation an enzyme undergoes when its substrate binds.

- A mechanism postulated by Daniel Koshland in 1958.
- This fit is induced by multiple weak interactions with the substrate.
- It serves to bring specific functional groups on the enzyme into the proper position to catalyze the reaction.
- The conformational change also permits formation of additional weak bonding interactions in the transition state.
- The new enzyme conformation has enhanced catalytic properties.
    - A common feature of the reversible binding of ligands to proteins.
    - Is important in the interaction of almost every enzyme with its substrate.

Enzyme Kinetics

Questions

1) What is the **Initial Rate** (or **initial velocity**) of a reaction, and what is **maximum velocity**?

2) Describe the **Theory of enzyme action** (partially by filling in the blanks)**.**

- The enzyme first combines ___ with its __ to form an ____ complex:

  $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$

- The ___ complex then breaks down in a slower second step to yield :

  $ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$

- Because the __ second reaction ___ the __ of the overall reaction, the overall [of the reaction] is proportional to the _____ of the species that reacts in the second step, that is - ____.

- At any given instant in an enzyme-catalyzed reaction, the enzyme exists in ____: _____.
- At low [S], most of the _____ is in the uncombined form E.
  - Here, the rate is _____ to [S] [because the equilibrium is pushed toward formation of more ____ as [S] increases. $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$
  - The maximum initial rate of the catalyzed reaction (Vmax) is observed when virtually all the enzyme is present as _____ and ____ is vanishingly small.
  - Under these conditions, the enzyme is _____ with its substrate, so that further ____in [S] have no effect on _____.

- After the ES complex breaks down to yield the ___, the enzyme is ____to catalyze reaction of another molecule of substrate.

3) Define and explain **steady-state kinetics**, and what the pre-steady state is.

4) Explain the rate equation for a one-substrate enzyme-catalyzed reaction.

5) Write and explain the Michaelis-Menten equation.

6) Explain the steady state and maximum rate and how these relate.

7) What is the Michaelis constant?

8) What is The Michaelis-Menten equation, and what relationship does it depict?

9) Which statement is false?

a. Michaelis-Menten kinetics is also called steady-state kinetics.
b. K_m and V_{max} have different meanings for different enzymes.
c. The limiting rate of an enzyme-catalyzed reaction at saturation is described by the constant k_{cat}, the turnover number.
d. Every enzyme has an optimum pH (or pH range) at which it has maximal activity.
e. All of the above

10) Explain enzyme inhibition and the various types mentioned in the lesson.

Answer Key

1) **Initial Rate** (or **initial velocity**) of reaction - is designated ***V*$_0$**
   - V_0 varies by different [S] (substrate concentrations), even when the enzyme concentration is held constant [as can be seen in the figure.
   - At relatively low concentrations of substrate, V_0 increases almost linearly with an increase in [S].
   - At higher substrate concentrations, V_0 increases by smaller [and smaller] amounts in response to increases in [S].
   - Finally, a point is reached [beyond] where increases in V_0 are small as [S] increases.
     - This plateau-like V_0 region is close to the **maximum velocity**, **Vmax**.

2) This theory was an expansion of known ideas by Leonor **Michaelis** and Maud **Menten** in 1913.

- The enzyme first combines reversibly with its substrate to form an enzyme-substrate complex:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

- The ___ complex then breaks down in a slower second step to yield the free enzyme and the reaction product P:

$$ES \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E + P$$

- Because the slower second reaction limits the rate of the overall reaction, the overall rate is proportional to the concentration of the species that reacts in the second step, that is - ES

- At any given instant in an enzyme-catalyzed reaction, the enzyme exists in two forms: the free form E, and the combined form ES.
- At low [S], most of the enzyme is in the uncombined form E.
    - Here, the rate is proportional to [S] [because the equilibrium [of the Equation:] is pushed toward formation of more ES as [S] increases.

      $$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES$$

    - The maximum initial rate of the catalyzed reaction (Vmax) is observed when virtually all the enzyme is present as the ES complex and [E] is vanishingly small.
    - Under these conditions, the enzyme is "saturated" with its substrate, so that further increases in [S] have no effect on rate.

- After the ES complex breaks down to yield the product P, the enzyme is free to catalyze reaction of another molecule of substrate.

3) When the enzyme is first mixed with a large excess of substrate, there is an initial period, the **pre–steady state** – during which the concentration of ES builds up.

- This period is microseconds.
- The reaction quickly achieves a steady state in which [ES] remains approximately constant.
- The concept of a steady state was introduced by G. E. Briggs and Haldane in 1925.
- The measured V_0 generally reflects the steady state, analysis of these initial rates is referred to as **steady-state kinetics**.

4) The **rate equation** for a one-substrate enzyme-catalyzed reaction is the **Michaelis-Menten equation.**

- It is a statement of the quantitative relationship between the :
  - initial velocity V_0,
  - maximum velocity Vmax,
  - initial substrate concentration [S],
  - Michaelis constant K_m.

5) The equation is: $V_0 = \frac{V_{max}[S]}{K_m + [S]}$

This equation is a quantitative expression of the relationship between Substrate Concentration and Reaction Rate.

- The curve expressing the relationship between [S] and V_0 [Fig. 6–11] has the same general shape for most enzymes .
- It is based on the hypothesis that the rate-limiting step in enzymatic reactions is the breakdown of the ES complex to product and free enzyme.

6) When substrate is added to an enzyme, the reaction rapidly achieves a steady state in which the rate at which the ES complex forms balances the rate at which it reacts.

As [S] increases, the steady-state activity of a fixed concentration of enzyme increases in a hyperbolic fashion to approach a characteristic maximum rate, V_{max}, at which essentially all the enzyme has formed a complex with substrate.

7) The substrate concentration that results in a reaction rate equal to one-half V_{max} is the Michaelis constant Km, which is characteristic for each enzyme acting on a given substrate.

8) The Michaelis-Menten equation relates initial velocity to [S] and V_{max} through the constant K_m.

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

9) e

10)

1. Reversible inhibition of an enzyme is competitive, uncompetitive, or mixed.
   a. Competitive inhibitors compete with substrate by binding reversibly to the active site, but they are not transformed by the enzyme.
   b. Uncompetitive inhibitors bind only to the ES complex, at a site distinct from the active site.
   c. Mixed inhibitors bind to either E or ES, again at a site distinct from the active site.
2. In irreversible inhibition an inhibitor binds permanently to an active site by forming a covalent bond or a very stable noncovalent interaction.