Test-Prep
Exam 2
10/20/25

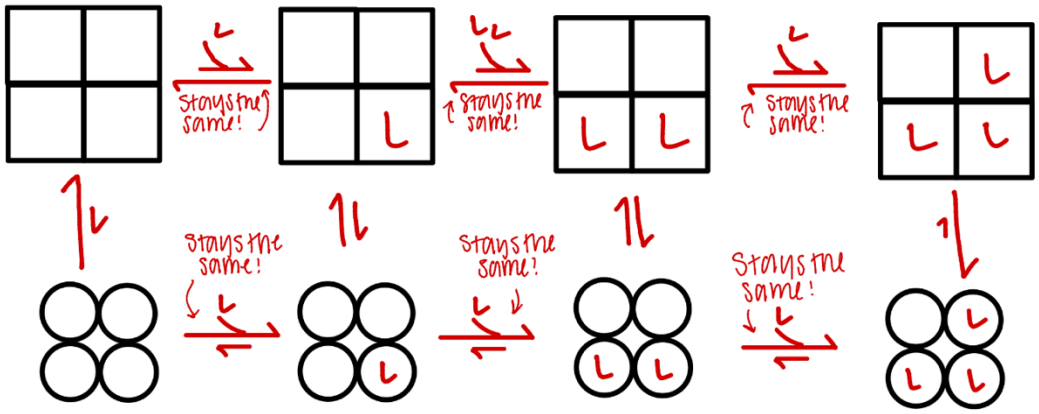
- Define Allostery
  The binding of a ligand to a protein that causes an effect at a spatially distinct site on the same protein.
  Ligand: A molecule that binds to a specific site on a larger molecule—The ligand is what causes conformational change!

- Define binding site and active site
  Active site: implies enzymatic activity or chemical change. This is where the substrate binds. Only enzymes have these active sites.
  Binding site: Site where a ligand binds to a protein or enzyme. There is NO chemical change present at this site.

- What are the general traits of allosteric interactions?
  1. Proteins must be at least a dimer
  2. Each subunit must be identical
  3. All subunits must exist in two conformations R(relaxed) or T (Taut)

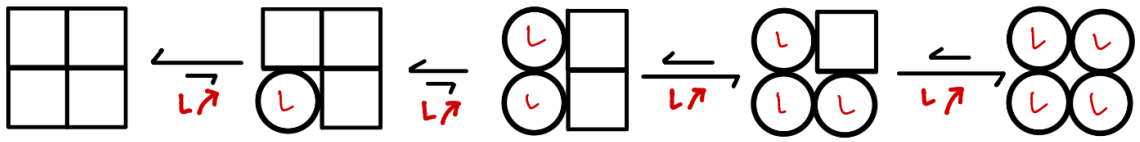
- What are the concerted assumptions?
  1. All subunits must be either R or T confirmation
  2. Ligand binds to R with HIGH affinity and Ligand binds to T with LOW affinity.
  3.The binding of the ligand shifts the equilibrium towards the R confirmation

- Draw the concerted model



- What are the sequential model assumptions?
  1. Each subunit can exist in either R or T confirmation.
  2. Binding of ligand to a subunit in T confirmation changes ONLY that subunit to R confirmation
  3. Binding of a ligand increases the affinity of other subunits for the binding of that ligand.

- Draw the sequential model



- What are the subunits and quantities of hemoglobin?

  $\alpha_2\beta_2$

  alpha = 2, beta = 2 } so essentially $\alpha = \beta$

- Where does oxygen bind to hemoglobin?

  Interacts with the iron within the heme group

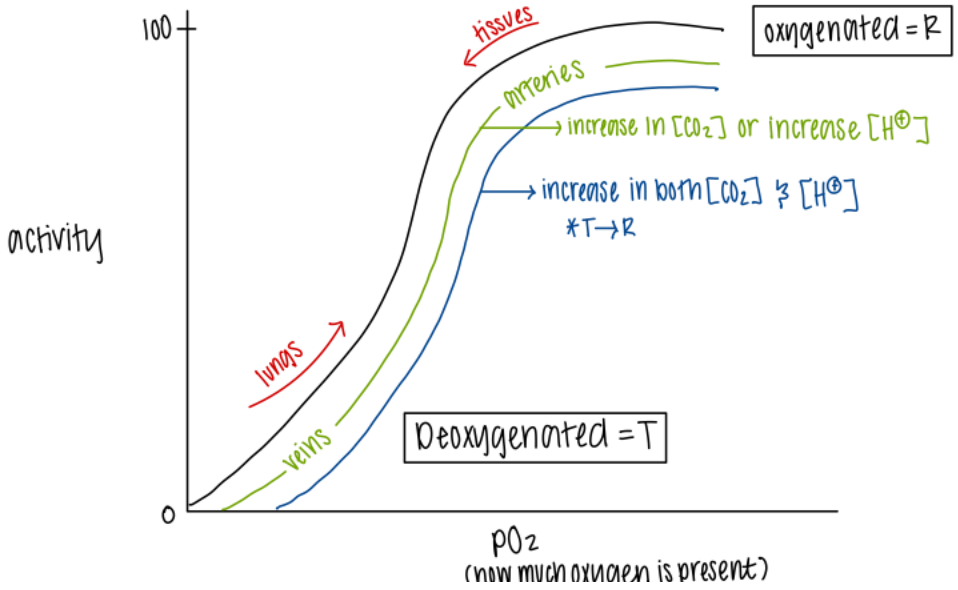
  Four oxygen molecules can bind to each hemoglobin (this is because of the four subunits). Each subunit has a heme group.

- What is the importance of Histidine? Why is this amino acid special in regard to the binding of Hb?

  H^+ binds to the Histidine molecule. Histidine has a pka of approximately 6. Because of its neutrality, this allows for histidine to be readily available for protonation and deprotonation.

  The protonation of histidine is pH dependent. Histidine will carry a positive charge in more acidic conditions and will be neutral in more basic conditions.

- Draw the graph associated with hemoglobin. Label the location of arteries, veins, lungs, and tissues along the curve. Draw the additional curves representing the addition of ____ and ___ concentrations.



- Lungs pO_2 = High
- Tissues pO_2 = Low

- What causes oxygen to dissociate from Hb? Where is this occurring?
  Low PH and CO_2 production. Tissues / Muscle cells

- Why is oxygen concentration relatively low in tissues/muscle cells?
  Oxygen is being used during cellular respiration.

- Where does CO_2 bind to Hb? Write out the chemical equations for the binding of CO_2 relative to their location in the body.
  Specifically binds to N-Terminus of a subunit
  Tissues
  CO_2 + NH_3^+-Subunit → COO^-NH-Subunit
  Lungs
  CO_2 + NH_3^+-Subunit ← COO^-NH-Subunit

- Where is Mb found?
  In muscle cells (cardiac, skeletal, etc.)

- How many subunits make up Mb?
  One

- The muscles of deep diving mammals such as whales contain exceptionally large amounts of Mb. How does this circumstance contribute to prolonged dives?
  Mammals such as whales do not have the same respiratory rate that humans/other animals do. Compared to humans, their respiratory rate can be as little as 1.25 breaths per MINUTE. They must have high amounts of Mb in order to maintain a relatively stable concentration of O2 in muscles/tissues. Without high amounts of Mb (an oxygen reservoir), whales would be in a constant hypoxic state.

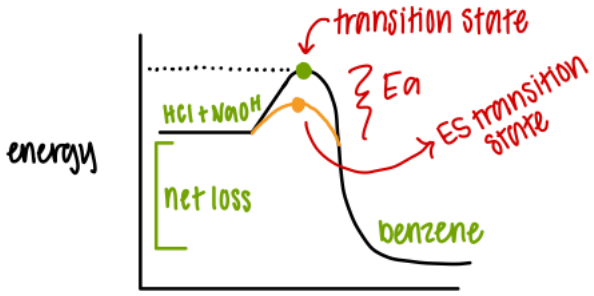
- All enzymes are? Name the exception.
  Proteins

  Ribozymes are the exception, they are made of RNA

- What is the function of an enzyme?
  To catalyze/speed up reactions (and are not consumed)

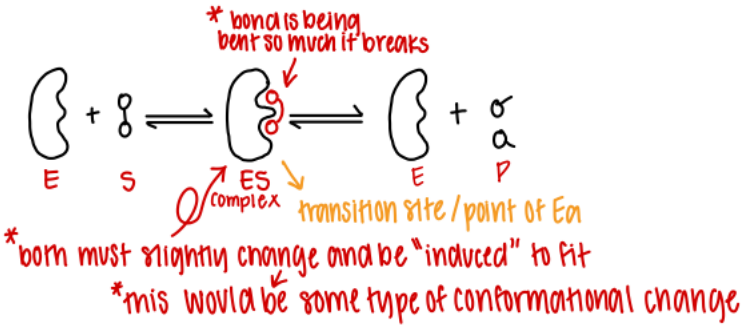
- Define activation energy
  The energy needed to complete a reaction. This energy comes from the breaking or formation of bonds.

- Draw the Ea graph of HCl + NaOH → Benzene with and without the presence of an enzyme.



- What determines the specificity of the substrates?
  The shape (which is caused by arrangements of R groups of enzymes).

- Draw the induced fit model. What is occurring here?

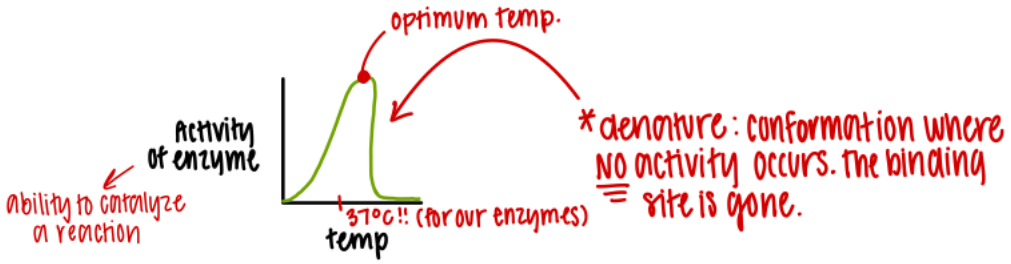


- What are the rules of a chemical reaction?
  1. Substrates must be close together
  2. Substrates must be correctly oriented towards each other
  3. Bonds must be broken and/or formed.

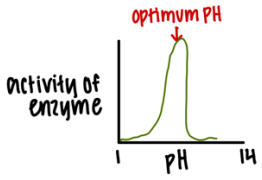
- How are enzymes regulated?
  1. Allostery: The binding of a ligand to a site other than the active site causes the enzyme to go from an inactive to active conformation.

  2. Binding of a cofactor, prosthetic group, coenzyme, metal ion (anything other than an amino acid): commonly needed for enzymatic activity.

  3. Temperature: optimal temperature that an enzyme functions at



4. pH: Optimal pH that enzyme functions at



5. Zymogens: Enzymes is synthesized in an inactive state and must be proteolytically cleaved to become active. Timing is important here! EXAMPLE: ability to form blood clots at the correct time!! (when its needed)
Trypsinogen (inactive) vs trypsin (active)

6. Inhibition: molecules that bind to enzymes to reduce activity of enzyme

7. Genetic: Regulators within the cell that can limit production of enzymes in transcription and translation.
- Constitutively expressed: genes that are always turned on
- Induced expression: forced to make mRNA (which will increase enzymes/protein production). These genes cannot be turned on or off.

- Name the classes of enzymes

Class 1: Oxidoreductase
In redox reactions; loss or gain of electrons (transfer)

Class 2: Transferase
A molecular group is being transferred from one molecule to another

Class 3: Hydrolase
Water is being used to cleave a covalent bond

Class 4: Lyase
Formation of a double bond by cleaving C-O, C-N, or C-C bond. C=C bond is usually formed

Class 5: Isomerase

Enzyme rearranges a molecule and creates an isomer

Class 6: Ligase (only class that requires energy)
Formation of covalent bond between two molecules using energy

- What is the steady state assumption?
  [ES]/time = 0
  [ES] is a constant!
  The amount of enzyme present (in the ES complex) remains the same throughout the reaction.
  Formation [ES] = Breakdown [ES]

- Define velocity in an enzymatic sense
  V = [product] / Δtime
  -Amount of product produced over a certain amount of time
  -Rate of product formation

- Define initial velocity
  No active sites (on enzymes!) are occupied by substrates. This is at t = 0. If there are no active sites bound by substrates, we have no product (yet).

- Draw the simplest enzymatic reaction

- From the reaction above, how would we derive the equation for velocity?

  V = [ES] x K3

- Derive the equation for Vmax from the above reaction. What does Vmax represent?
  Vmax: all active sites are bound by substrates. This is the point of the fastest rate of product formation.

$V = [ES] \times k_3$

$[E_t] = [ES] + [E]$ 0 bc no free enzyme at Vmax

$[E_t] = [ES]$

$V_{max} = [E_t] \times k_3$

- What is the Michaelis-Menten constant (Km)? What does it represent?
  Km = 1/2Vmax concentration
  (Half of the active sites are bound by substrate)
  It tells us the affinity for a substrate binding to an enzyme.
  Smaller km = higher affinity

- Derive the Michaelis-Menten equation

1. $[E][S]k_1 = [ES]k_2 + [ES]k_3$
2. $[ES] = \frac{[E][S]k_1}{k_2 + k_3}$
3. $km = \frac{k_2 + k_3}{k_1}$
4. $[ES] = \frac{[E][S]}{km}$
5. $[E_T] = [E] + [ES]$
6. $[ES] = \frac{([E_T] - [ES])(S)}{km}$
7. $[ES] = \frac{[E_T][S]}{km + [S]}$
8. $V = k_3[ES]$
9. $\frac{V}{k_3} = \frac{[E_T][S]}{km + [S]}$
10. $V_{max} = k_3[E_T]$
11. $V = \frac{V_{max}[S]}{km + [S]}$

- What are the advantages and disadvantages of the MM equation?
- Advantages
  Easier to read

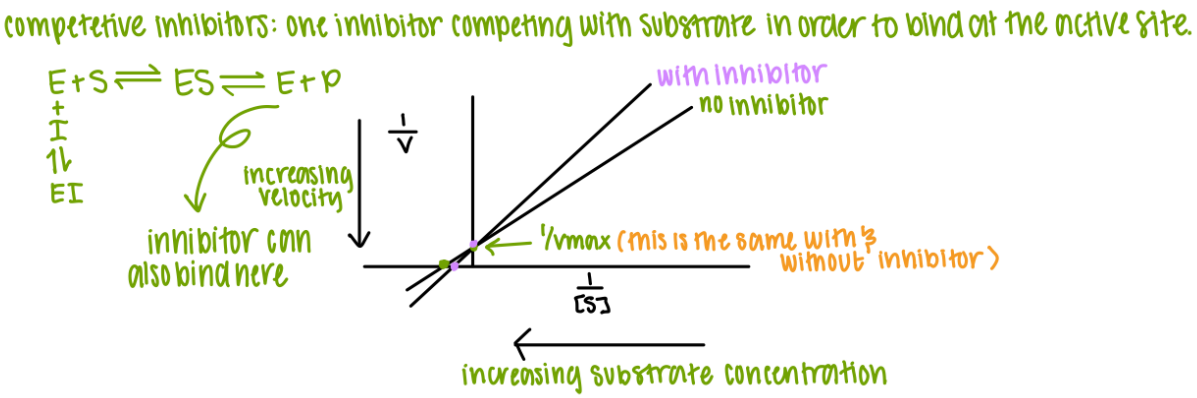
- Disadvantages
  Lacks consistency in estimating Vmax and Km
  Difficult to see inhibition type

- What are the advantages and disadvantages of the LWB equation?
- Advantages
  Ability to get the value of Vmax and Km consistently
  Easier to see the inhibition types

- Disadvantages
  Must solve for Km and Vmax

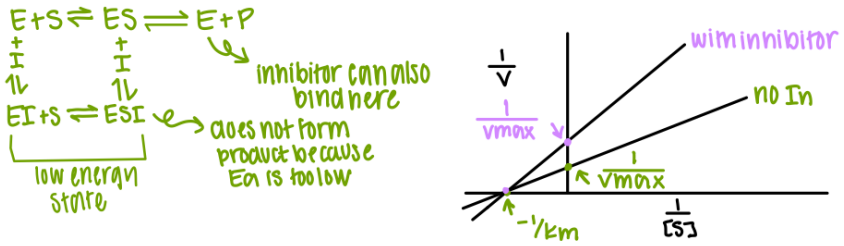
- Bigger Km = LOWER affinity

- Define competitive inhibition and draw the reaction. Give both the written chemical equation and associated graph.



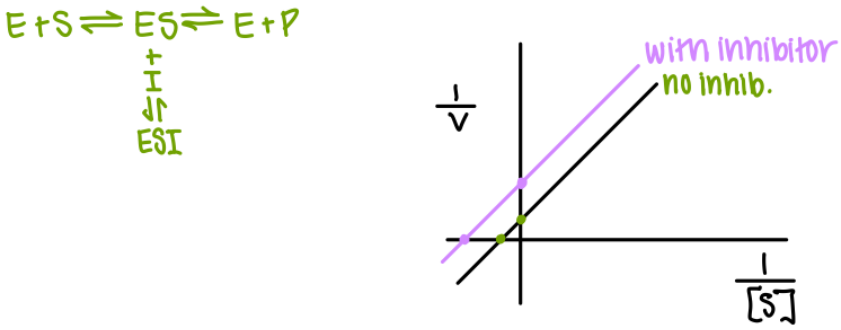
- Define noncompetetive inhibition and draw the reaction. Give both the written chemical equation and associated graph.

noncompetetive: inhibitor can bind to E or ES on active site or another site (inhibitory site)

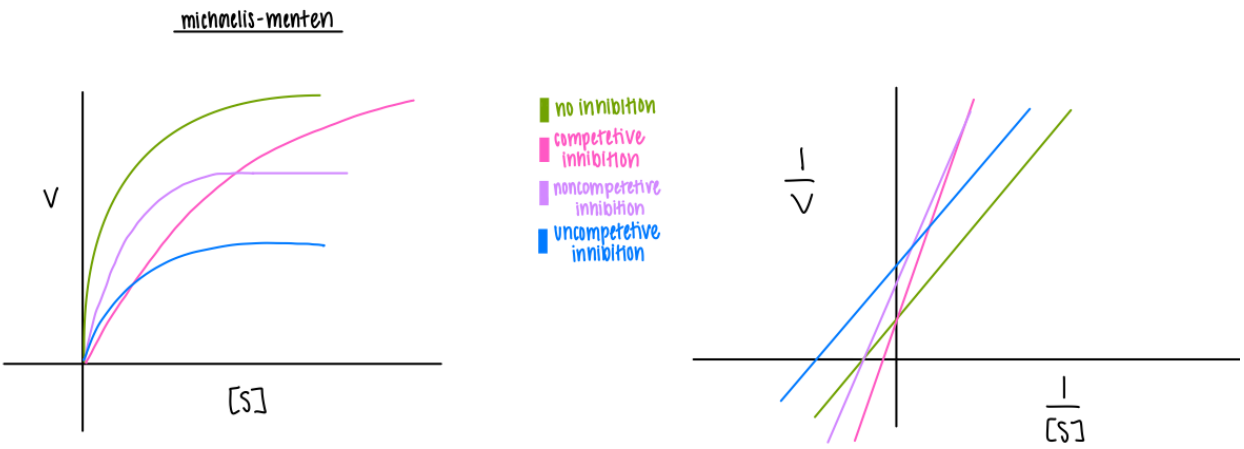


- Define uncompetetive inhibition and draw the reaction. Give both the written chemical equation and associated graph.

uncompetetive: conformational change allows for a new site to form in the ES complex

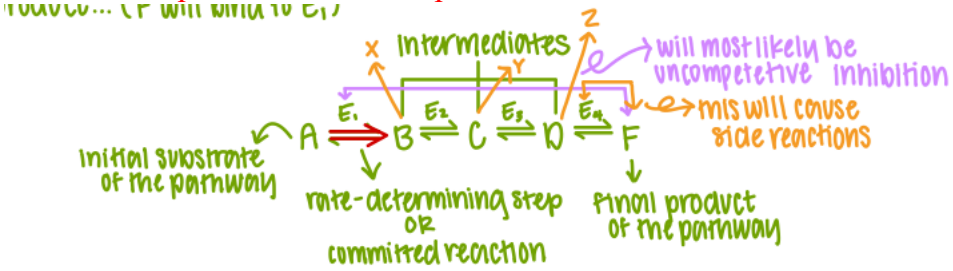


- Draw a LWB and MM graph illustrating competitive, noncompetetive, and uncompetitive inhibition.



- Assume you have a LWB plot and determine the x-intercept is approximately -8. What is the corresponding Km value?
  -1/Km = X-intercept
  -1/Km = -8
  Km = 0.125

- Define feedback inhibition and draw the reaction
  Formation of product inhibits the production of itself.



- Differentiate between a random mechanism and an ordered mechanism.
  Random: It does NOT matter which substrate binds first because product will form either way.

  Ordered: It DOES matter which substrate binds first because product will only form under correct circumstances.

- Draw and explain what is happening during a ping pong mechanism.
  Substrate binds to Enzyme, (enzyme changes) then the 1st product is released. 2nd Substrate binds to e, (enzyme changes back to original state), then the 2nd product is released.