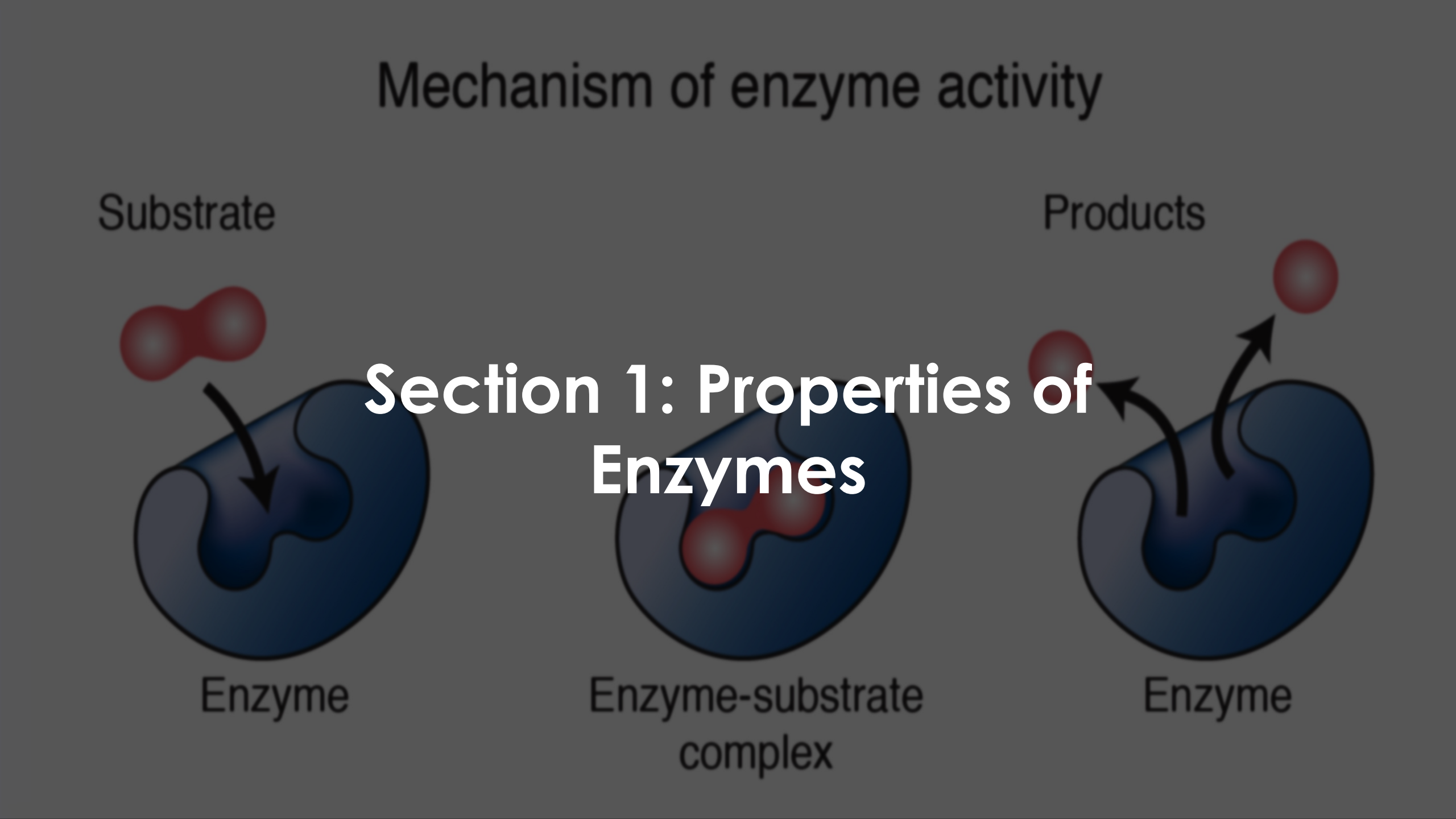
BCHM 270: Module 3

PROTEIN STRUCTURE AND FUNCTION

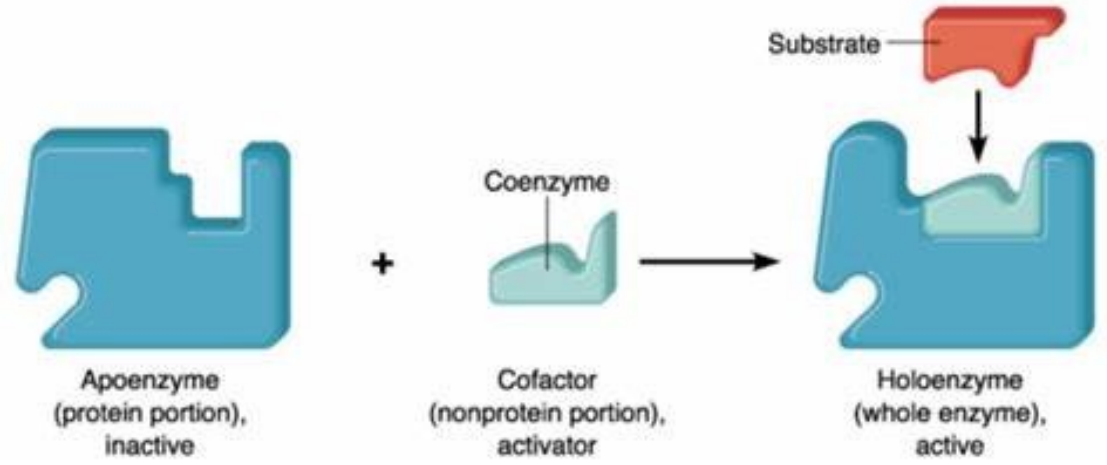
Content Outline



Section 1: Properties of Enzymes

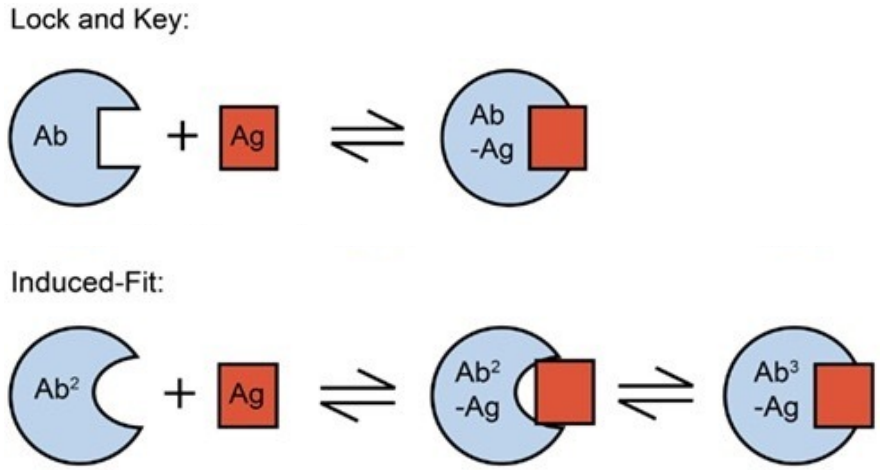
Concept 1.1: Enzyme Complexes

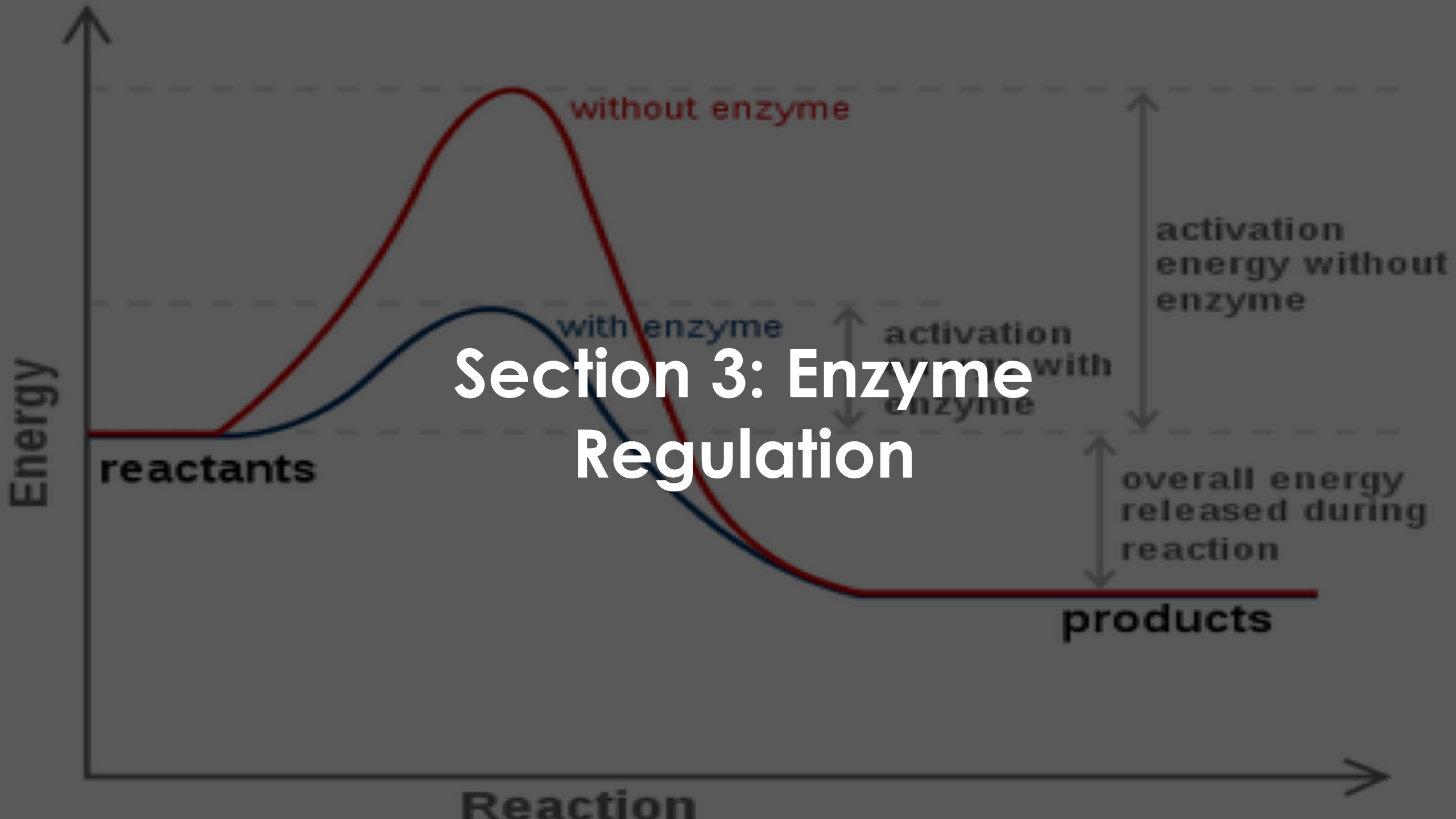
- Enzymes consist of two parts: the protein portion (apoenzyme) and the non-protein portion (cofactor or coenzyme).
- The complete enzyme is called the holoenzyme.
- Coenzymes and cofactors are essential for enzyme activity.
- They facilitate the chemical reaction by binding to the active site of the enzyme.
- Coenzymes are organic molecules and cofactors are usually inorganic ions or molecules.
- They are involved in redox or electron transfer reactions, and stabilizing the protein structure.



Concept 1.2: Lock/Key vs. Induced Fit Enzyme Models

- Lock/Key model suggests that the active site of the enzyme is already in the perfect shape for the substrate to bind, like a key fitting into a lock.
- Induced Fit model proposes that the active site of the enzyme is flexible and changes shape to accommodate the substrate when it binds, like a glove molding to fit a hand.
- **Lock/Key** model is more **rigid** and **less adaptable**, with a fixed active site that only allows specific substrates to bind.
- **Induced Fit** model is more **flexible** and **adaptable**, allowing for more specific and efficient enzyme-substrate interactions.





Section 3: Enzyme Regulation

Concept 3.1: Physical Factors that Affect Enzyme Rate

Temperature: Enzyme activity increases as temperature increases

- Until a certain point, where the temperature causes the protein to denature
- Proteins are generally most active from 30-37°c

pH: Enzymes have specific ranges of pH where they function most optimally

- **Changes in pH causes ionization of amino acid side chains**
- **This causes changes in protein structure**
- Extreme pH changes can also affect protein folding and cause denaturation.

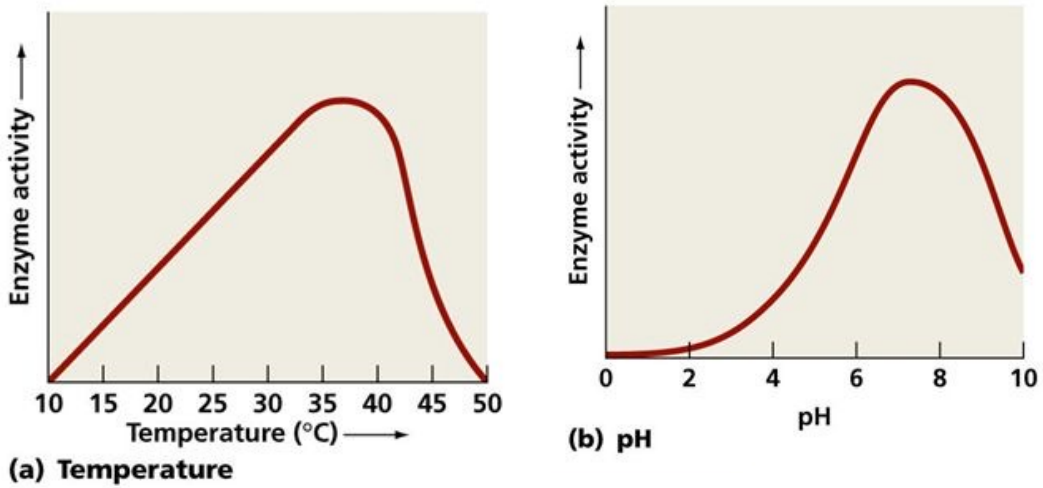


Figure X. (a) Graph displaying enzyme activity vs temperature. (b) Graph displaying enzyme activity vs. pH.

Concept 3.2: Substrate Concentration and Availability

Increases in substrate concentration will directly increase the reaction rate, until maximum velocity Is reached.

In reversible reactions, equilibrium will be reached unless product is removed through other reactions.

Example: Glycogen is broken down to create the substrate for glycolysis (glucose-6-phosphate)

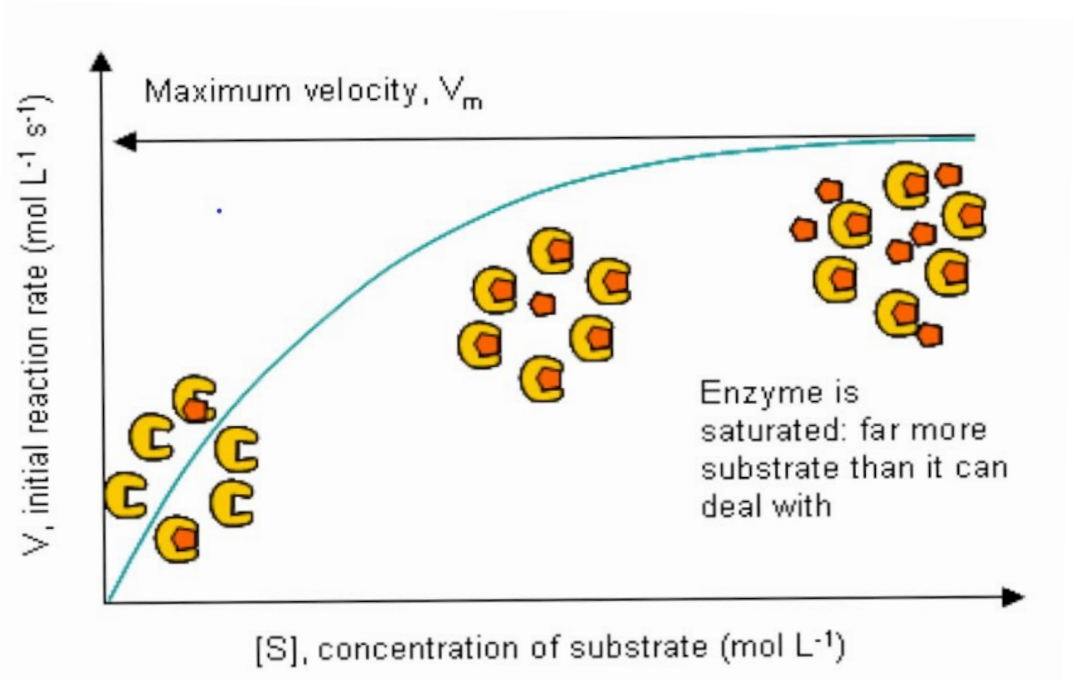


Figure X. Graph of reaction velocity vs. substrate concentration.

Concept 3.2: Product Inhibition

Products can prevent reaction by binding to enzyme and prevent reactants from binding.

Applying Le Chatelier's Principle:

When concentrations of substrates are significantly higher than products, **Forward Reaction is Favoured.**

When concentration of products are significantly higher than reactants, **Reverse Reaction is Favoured.**

Concept 3.3: Covalent Modification

- The addition of certain molecules on enzymes can influence their activity
  - Phosphorylation, glycosylation, etc.
- Phosphate are attached to serine, threonine and tyrsosine amino acids in proteins
  - Can increase/decrease protein activity

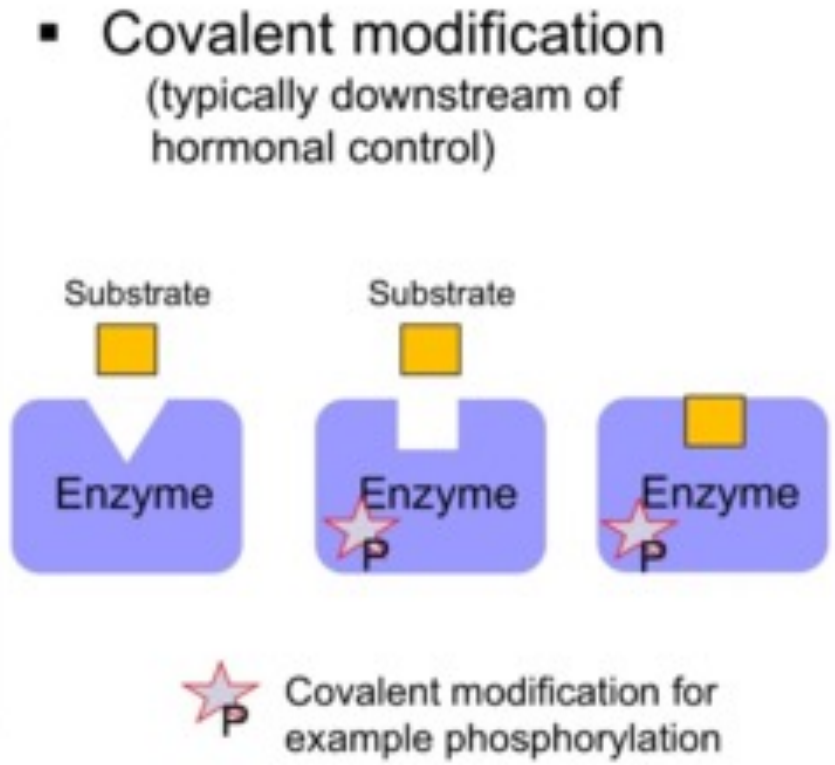


Figure X. Diagram of phosphorylation's effects on enzyme-substrate interactions.

Section 3 Quiz: Multiple Choice

Which fundamental principle surrounding enzymes explains why transition state analogues are able to act as potent inhibitors of naturally occurring enzymes?

a. The induced fit theory
b. Allosteric inhibition
c. Enzyme specificity
d. Proximity and orientation effects

Section 3 Quiz: Multiple Choice

Answer: A

Remember that substrates and enzymes do not fit together immediately upon binding. Enzymes and substrates will change their structure slightly upon binding to increase the affinity between the two during the reaction's transition state. The induced fit theory states that binding between enzymes and substrates is strongest at the corresponding reaction's transition state.

Section 4: Enzyme Kinetics

Concept 4.1: The Enzyme-Substrate Complex and Reaction Rate

- Enzyme-substrate complex
  - Formation of this complex is rate-limiting step of reaction
  - Overall reaction = proportional to concentration of this complex

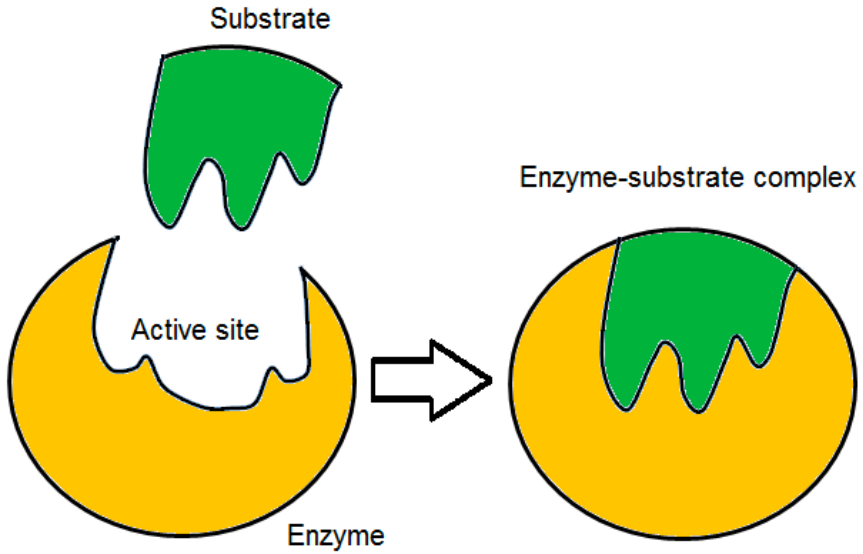


Figure 1. Enzyme-substrate complex

Concept 4.2: Michaelis-Menten Equation

V0 = (Vmax[S])/(Km + [S])

- V0 = initial reaction velocity
- Vmax = maximum reaction rate
- [S] = initial substrate concentration
- Km = Michaelis-Menten constant
  - Specific to each enzyme
  - Reflective of enzyme's affinity for substrate
  - Low Km --> low [S] needed to reach 50% saturation
  - High Km --> high [S[needed to reach 50% saturation

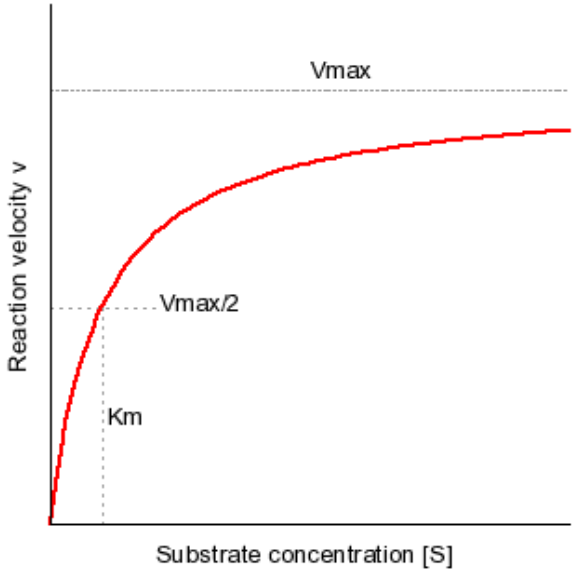


Figure 2. Michaelis-Menten equation graphed

Concept 4.3: Enzyme-Substrate Assumptions

- Measurements must be conducted when substrate concentration is significantly higher than enzyme concentration and significantly below maximum velocity
- Measurements are conducted immediately after mixing of enzyme and substrate
- Reactions are performed at one particular enzyme concentration

Concept 4.4: Michaelis-Menten vs. Lineweaver-Burk Plots

- Lineweaver-Burk plot
  - Linear plot
  - Reciprocal of each side of Michaelis Menten equation
  - Equation: (1/V0) = (Km/(Vmax*[S]) + (1/Vmax)
  - X-intercept = -1/Km
  - Slope = Km/Vmax
  - Y-Intercept = 1/VMax

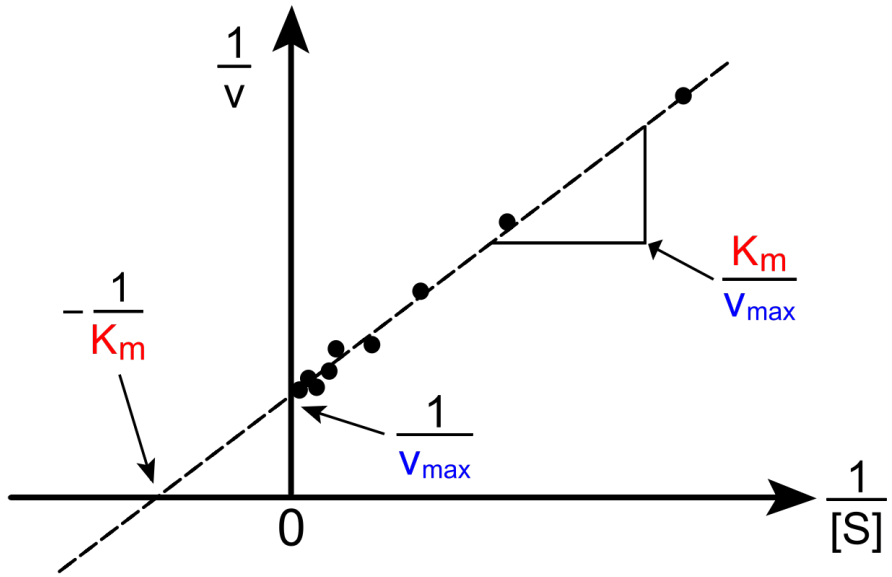


Figure 2. Lineweaver-Burk Plot Graphed

Concept 4.5: Regulation of Allosteric Enzymes

Hill equation

- For allosteric enzymes
- Sigmoidal curve
- N = degree of cooperativity
  - N > 1 --> positive cooperativity (binding of effector increases enzyme activity)
  - N < 1 --> negative cooperativity (binding of effector decreases enzyme activity)
- K1/2 = affinity constant (same as Km)

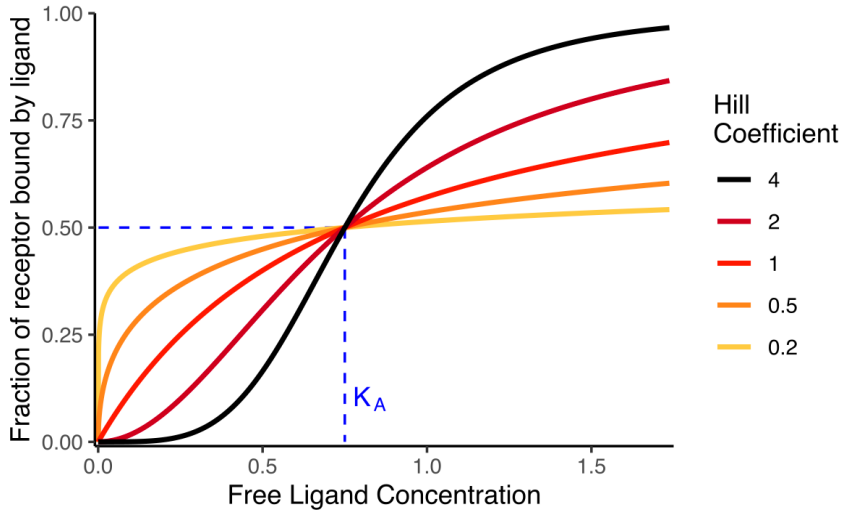
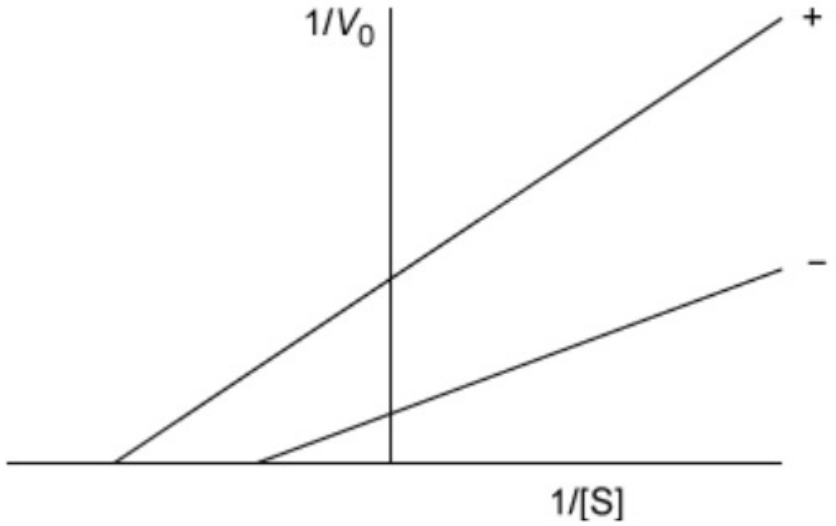


Figure 4. Hill equation graphed

Section 3 Quiz: 1 MC

An enzyme was assayed in the absence (-) or presence (+) of an allosteric effector, and the following Lineweaver-Burke plot was obtained.



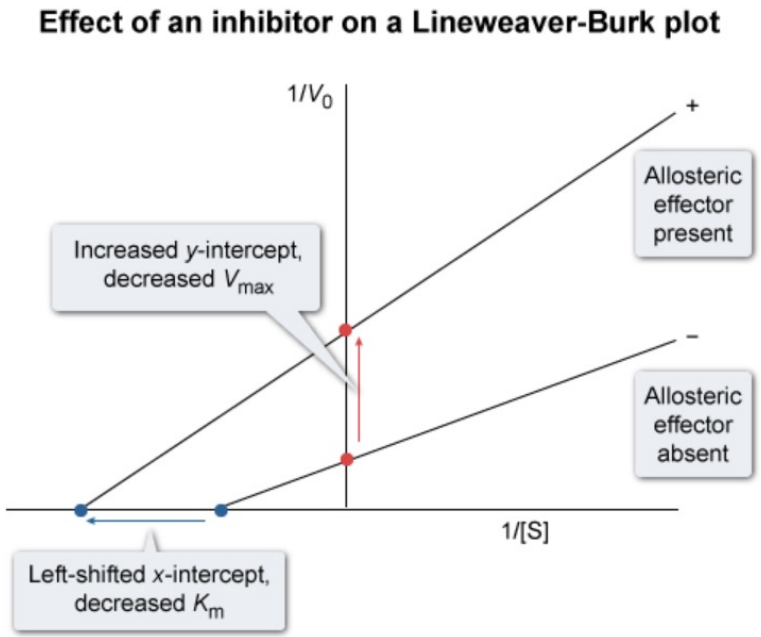
Which statement about the allosteric effector is correct?

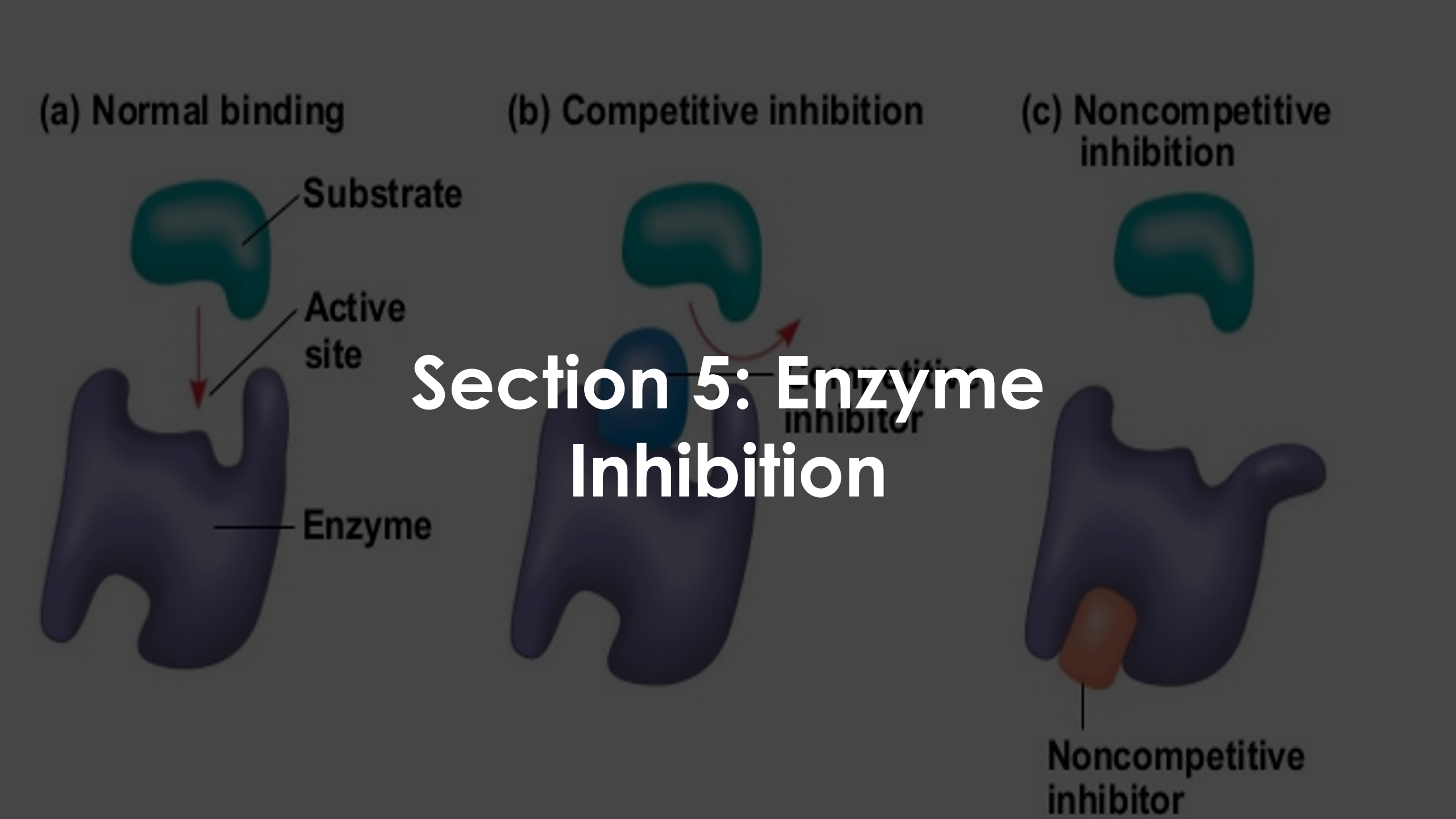
a. The effector is an activator that increases both Vmax and Km
b. The effector is an activator that increases Vmax but decreases Km
c. The effector is an inhibitor that decreases both Km and Vmax
d. The effector is an inhibitor that increases Km but decreases Vmax

Section 3 Quiz: 1 MC

Answer: C

The effector is an inhibitor that decreases both Km and Vmax





Section 5: Enzyme Inhibition

Concept 5.1: Reversible vs. irreversible inhibitors

Reversible inhibitors

- Can bind and unbind to the enzyme
- Has 4 different classes

Irreversible inhibitors

- A substrate analogue forms a permanent covalent bond to the enzyme's active site
- Creates a stable enzyme-inhibitor complex

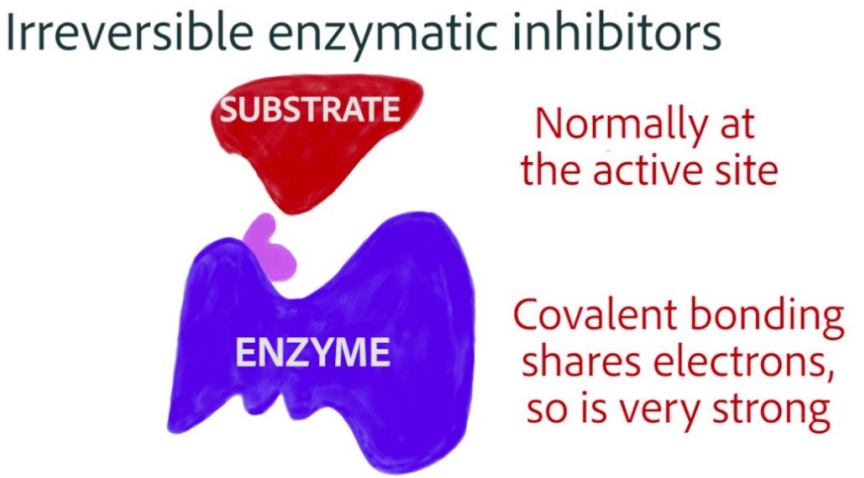


Figure 1. Diagram of how an irreversible inhibitor blocks enzyme binding.

Concept 5.2: Types of Reversible Enzyme Inhibitors

Competitive inhibitors

- Bind to enzyme active site to block the binding of substrates
- Increases Km but does not change Vmax

Uncompetitive inhibitors

- Bind to enzyme when substrate binds to active site
  - Binds to allosteric site and prevents enzyme from being active
- Decreases Km and Vmax

Noncompetitive inhibitors

- Binds the enzyme or enzyme-substrate complex at allosteric site
- Decreases Vmax but does not change Km

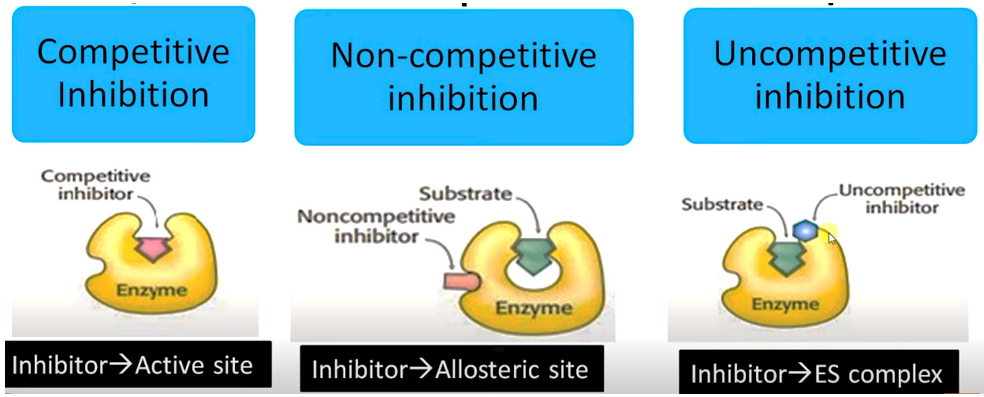


Figure 2. A comparison between the three types of reversible inhibitors.

Section 5 Quiz: MC

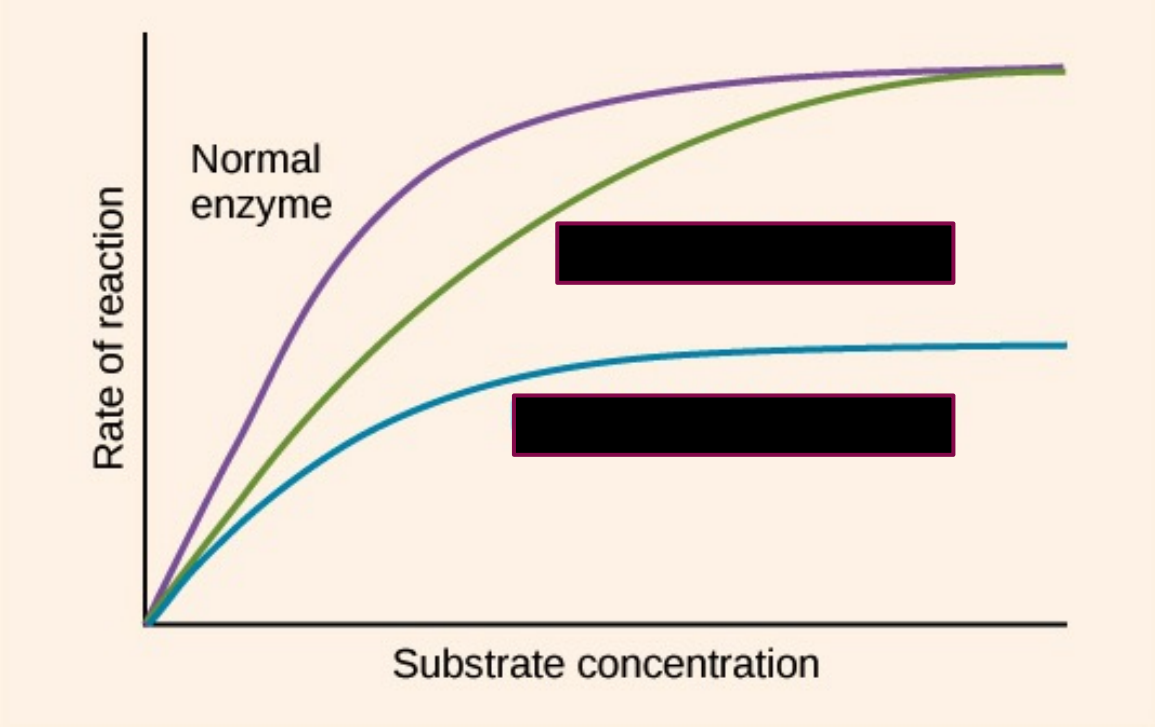
What type of inhibitor is the green line?

a. Uncompetitive

b. Competitive

c. Noncompetitive

Answer: b



What type of inhibitor is the blue line?

a. Uncompetitive

b. Competitive

c. Noncompetitive

Answer: c

Section 5 Quiz: MC

What type of inhibitor is the green line?

a. Uncompetitive

b. Competitive

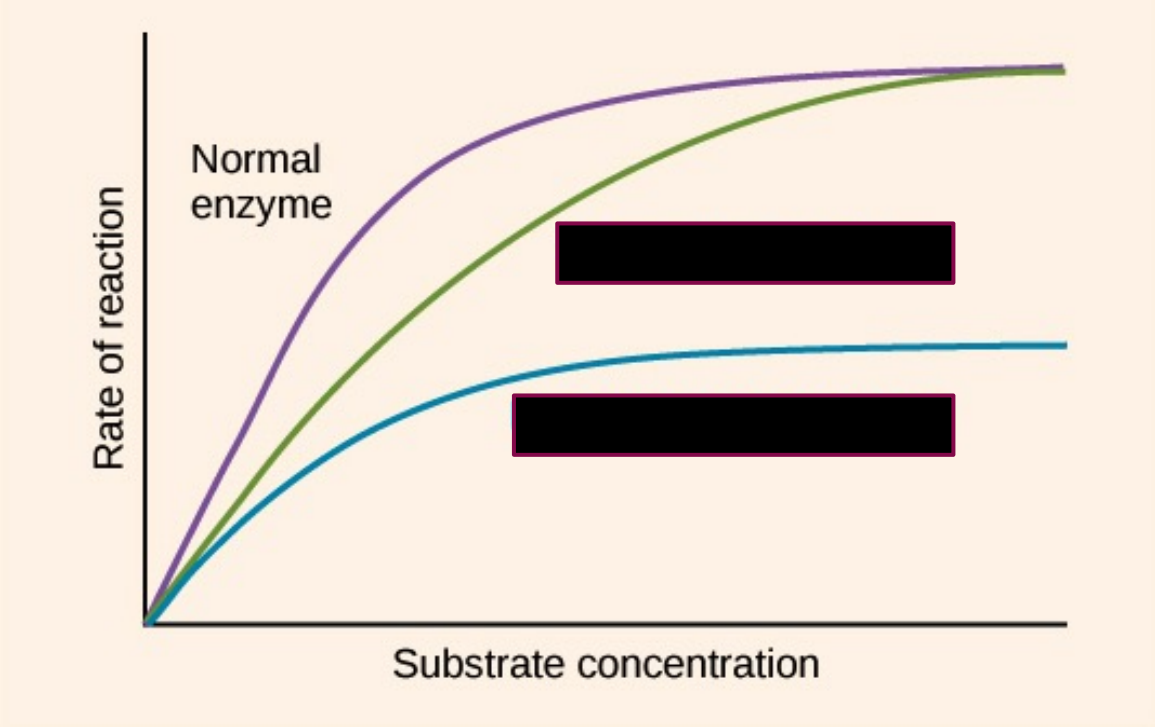
c. Noncompetitive

What type of inhibitor is the blue line?

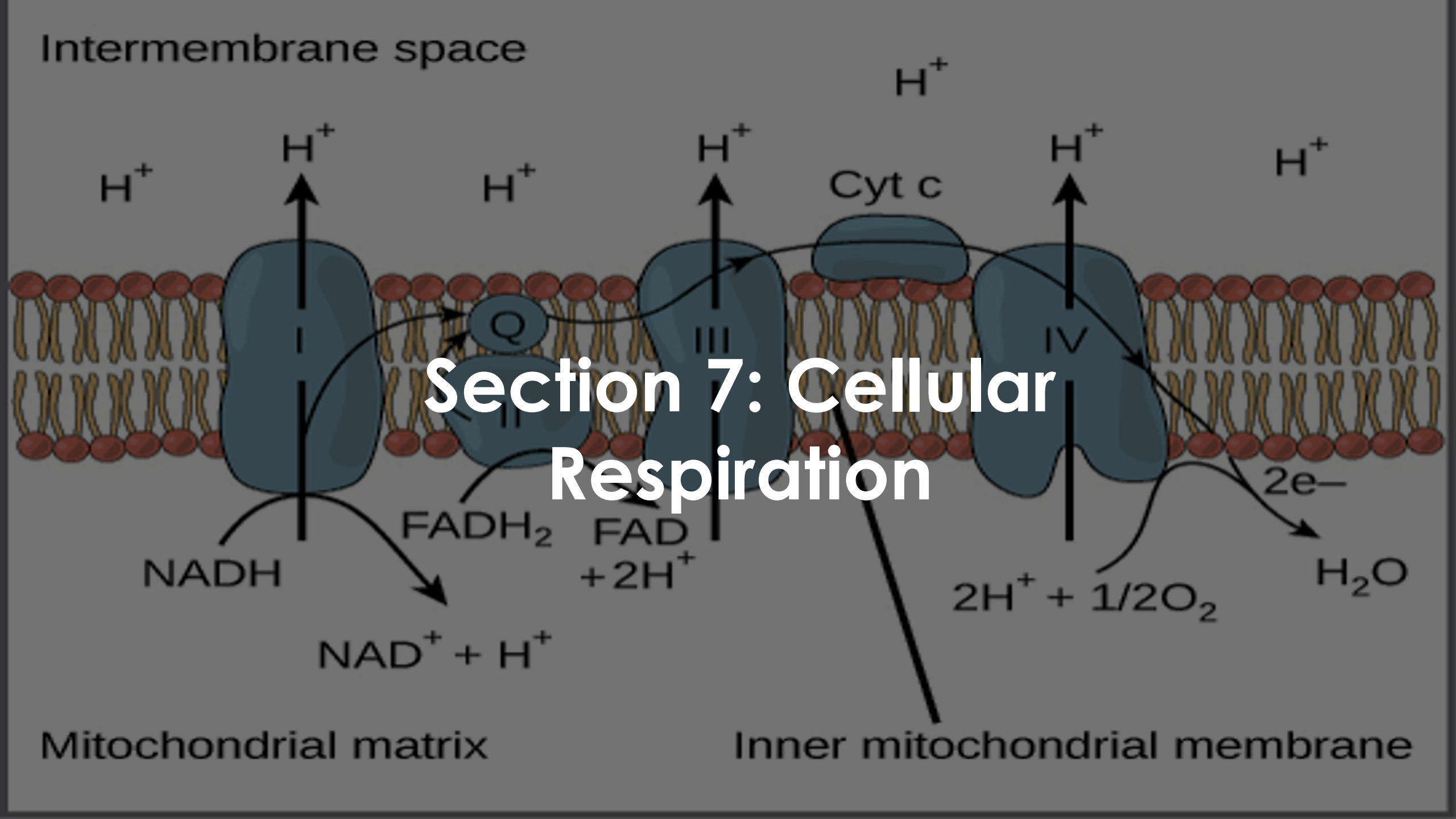
a. Uncompetitive

b. Competitive

c. Noncompetitive

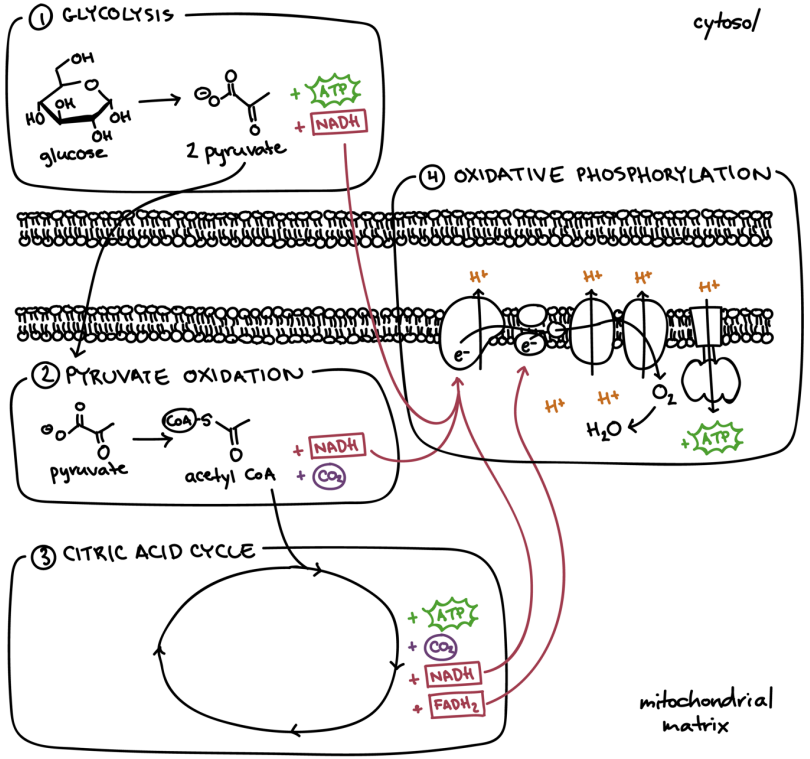


Section 7: Cellular Respiration



Concept 7.1: Overview of Cellular Respiration

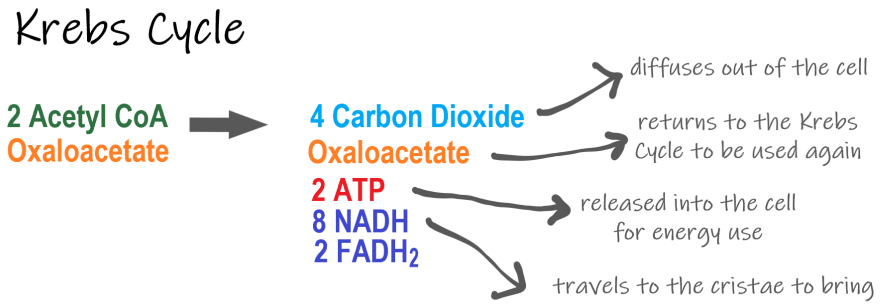
- TCA cycle is the 1st stage of cellular respiration, that removes high-energy electrons from carbon fuels like acetyl-CoA.
- These electrons reduce oxygen to generate a proton gradient, along the inner mitochondrial membrane, used to synthesize ATP
- Oxidative phosphorylation (OXPHOS) is the linkage of reduction of oxygen to the synthesis of ATP

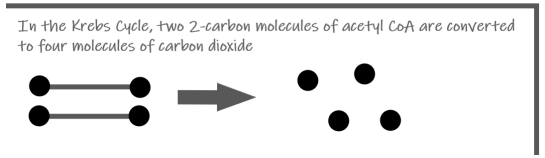


Steps of Cellular respiration.

Concept 7.2: The TCA Cycle

- Reaction converts Acetyl CoA into 2 CO_2 and generates high-energy e^-
- Oxaloacetate is combined with acetyl group of Acetyl-CoA, and oxaloacetate is formed as the cycle ends
- TCA cycle does not result in the gain or loss of cycle intermediates
- Intermediates produced from degradation of AA, and conversion of pyruvate to oxaloacetate.
  - Intermediates can exit cycle to be used for biosynthesis of AA and
    formation of glucose from AA by gluconeogenesis

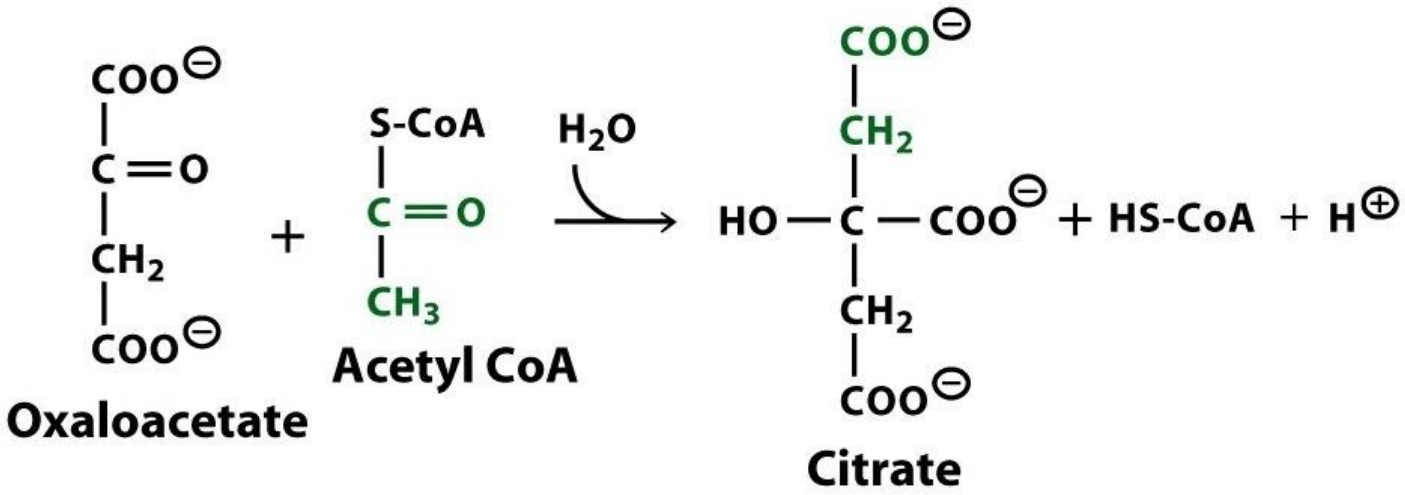




TCA Cycle Equation Overview.

Concept 7.3: Formation of Citrate

- Acetyl-CoA and Oxaloacetate are joined together by citrate synthase, which forms citrate and CoA



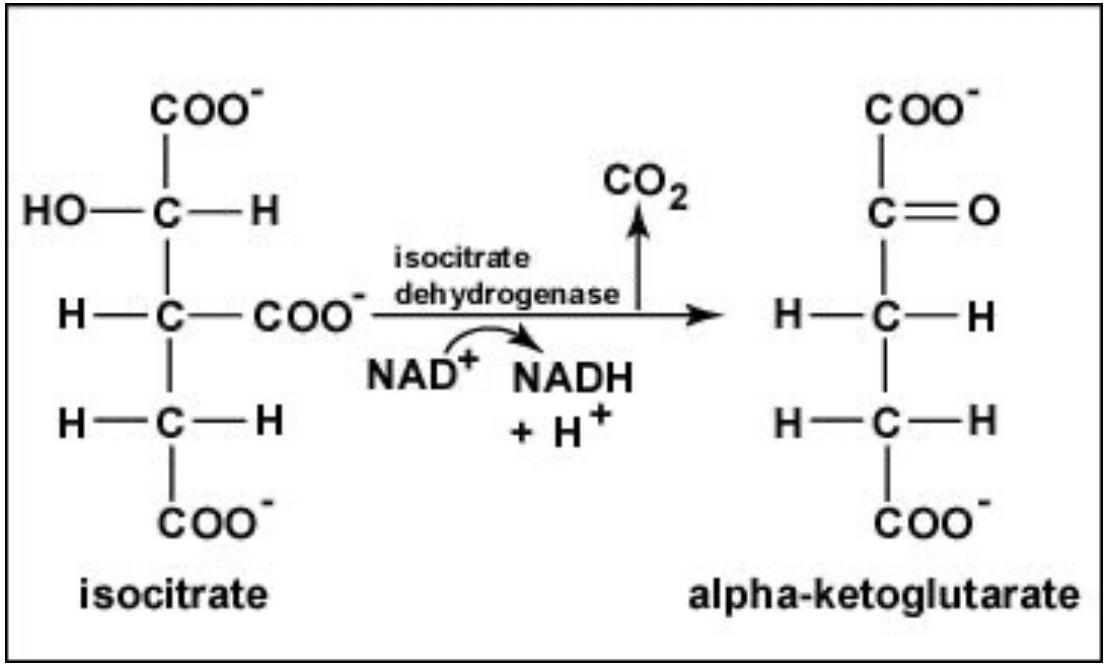
- Citrate synthase regulated by product inhibition
  - Inhibited by citrate

Citrate Synthesis.

Concept 7.4: Formation of α-ketoglutarate

- Isocitrate is decarboxylated (loses CO2) by isocitrate dehydrogenase, generating the 1st NADH and CO2 (from acetyl group)

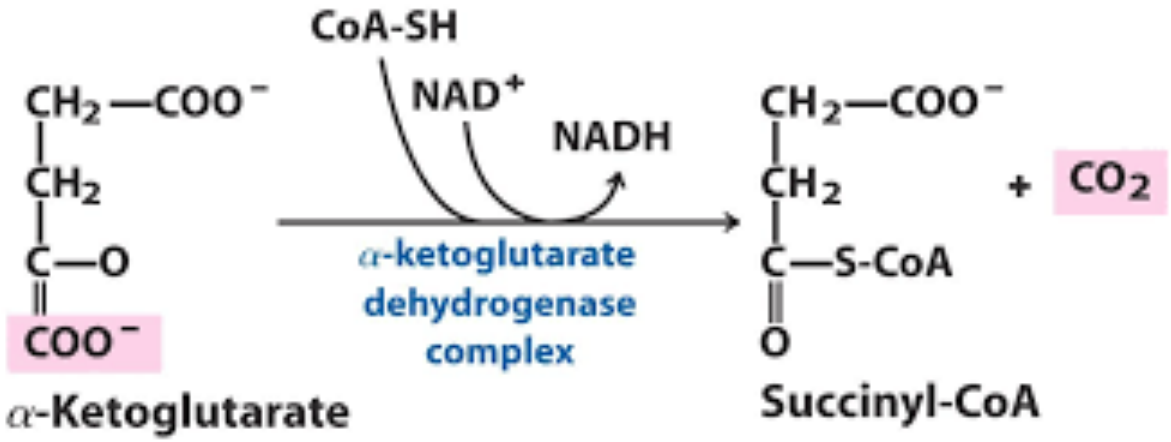
- * Catalysis of Isocitrate dehydrogenase is the rate-limiting step
  - Inhibited by ATP and NADH
  - Activated by ADP and Ca^{2+}



Formation of of α-ketoglutarate.

Concept 7.5: Oxidative Decarboxylation of α-ketoglutarate

- CoA is added to a-ketoglutarate to form succinyl CoA releasing 2nd CO_2 and NADH
- Reaction catalyzed by a-- ketoglutarate dehydrogenase complex
  - Inhibited by NADH, Succinyl CoA
  - Activated by Ca^{2+}



$\Delta G'^{\circ} = -33.5$ kJ/mol

Oxidative Decarboxylation of α-ketoglutarate.

Concept 7.6: Products of the TCA Cycle

NADH/FADH2:

- 4 pairs of electrons transferred to ETC: 3 pairs from 3NADH, 1 pair from FADH2
- 4 reduced coenzymes per acetyl-CoA oxidized to CO2

GTP:

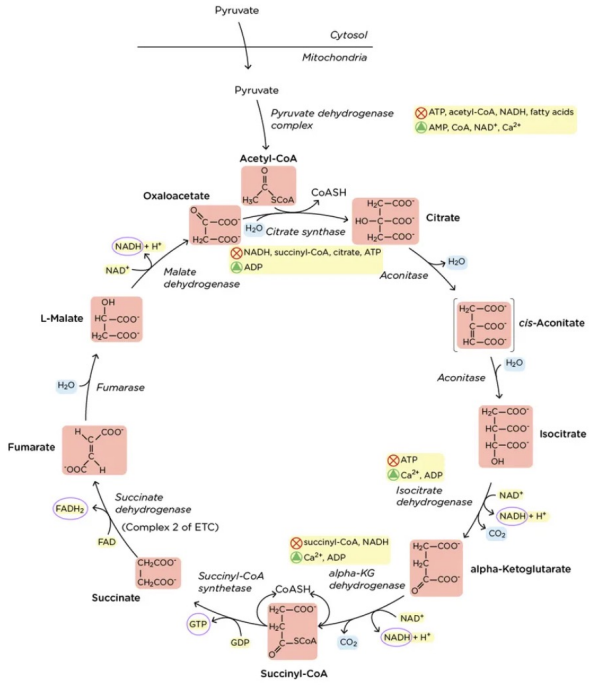
- 1 GTP produced
- GTP is essentially an equivalent energy source to ATP

CO2:

- 2 CO2 produced
- 2 C from Acetyl-CoA leave as CO2 (also a reduced coenzyme)

TCA Net Equation:

Acetyl-CoA + 3 NAD^+ + FAD + GDP + P_i + 2 H_2O 2 CO_2 + 3 NADH + $FADH_2$ + GTP + 3 H^+ + CoA

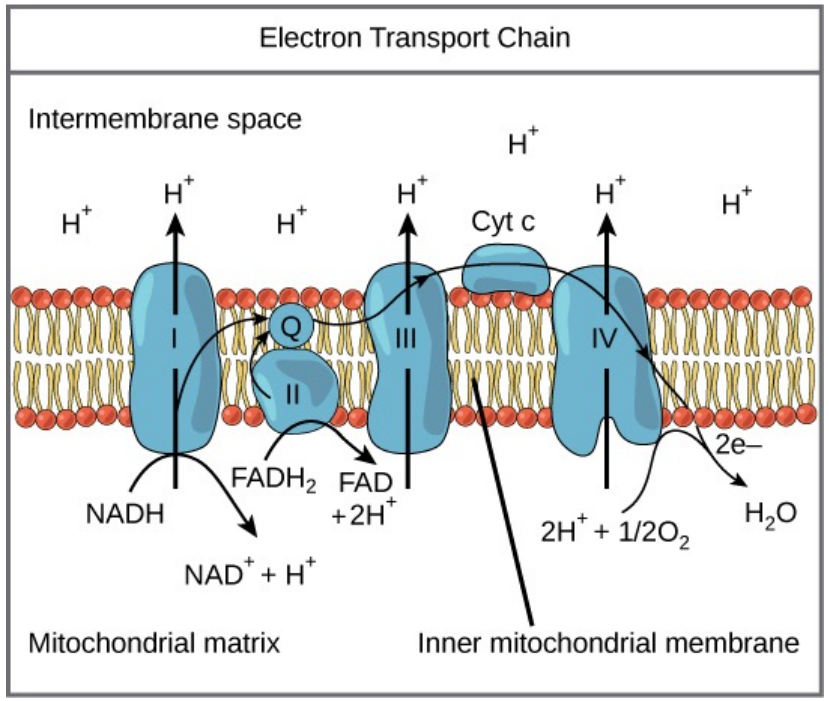


One cycle yields 7 products: GTP, 3 NADH (2.5 ATP per), 1 FADH2 (1.5 ATP per), 2 CO2

[Overview of TCA Cycle.]

Concept 7.7: Overview of the Electron Transport Chain

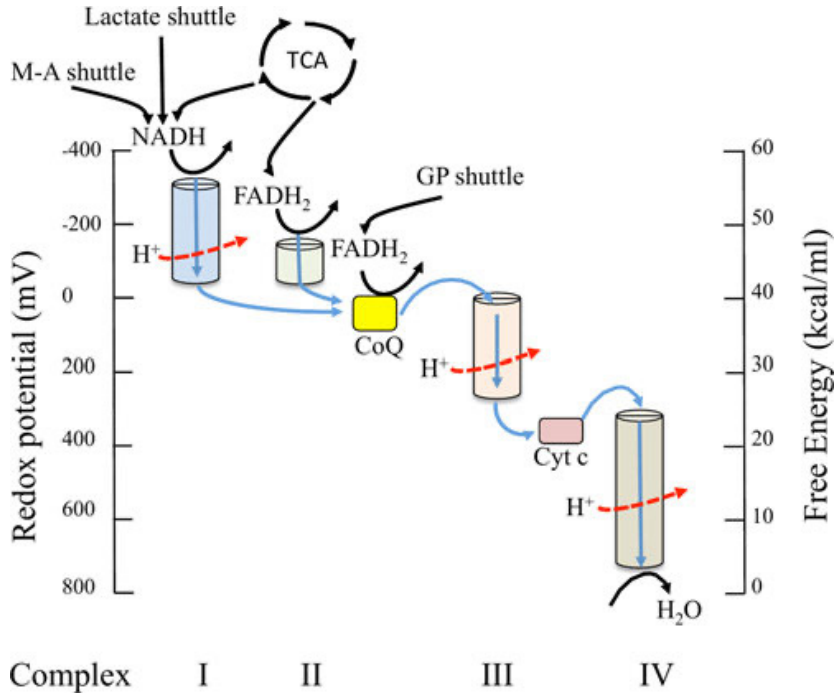
- First step of OXPHOS
- Protein complexes found on mitochondrial inner membrane (IM)
- Uses energy from electron carriers (NADH, $FADH_2$) generated from catabolic pathways (glycolysis, TC cycle) to pump protons into IM space to eventually create ATP



Overview of the 4 complexes in the Electron Transport Chain.

Concept 7.8: The ETC and Free Energy

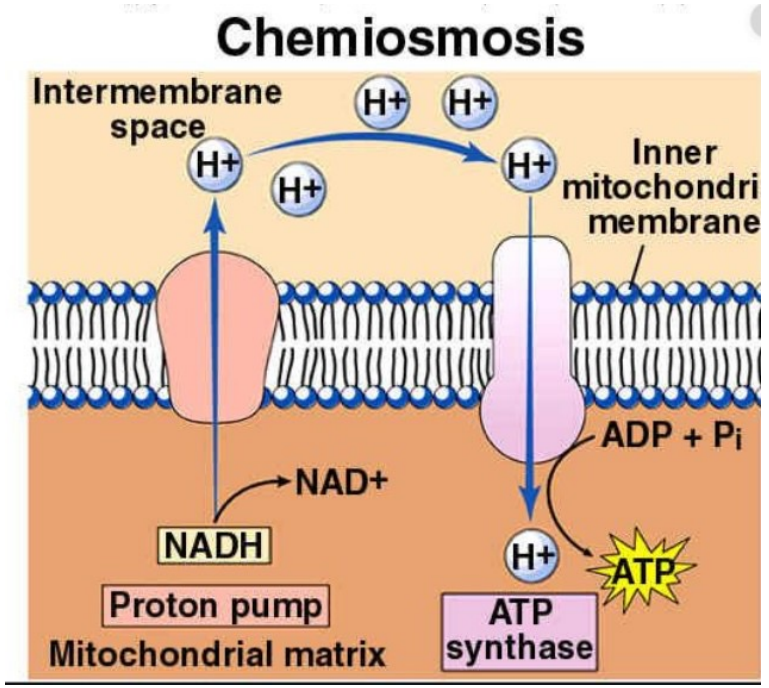
- ETC complex is determined by the affinity of different electron carriers (redox potential & free energy)
  - Redox potential: Tendency for a molecule to acquire electrons by becoming reduced
- NADH and FADH2 readily pass their e- to the ETC (low redox potential)
- In ETC, e- flows from low redox potentials to higher redox potential compounds (higher to lower free energy)
- 3. Oxygen final acceptor for e- in the ETC (high redox potential).
  - Produces H2O



Free Energy of ETC. Top (High energy) and bottomm (low energy).

Concept 7.9: The Chemiosmotic Gradient

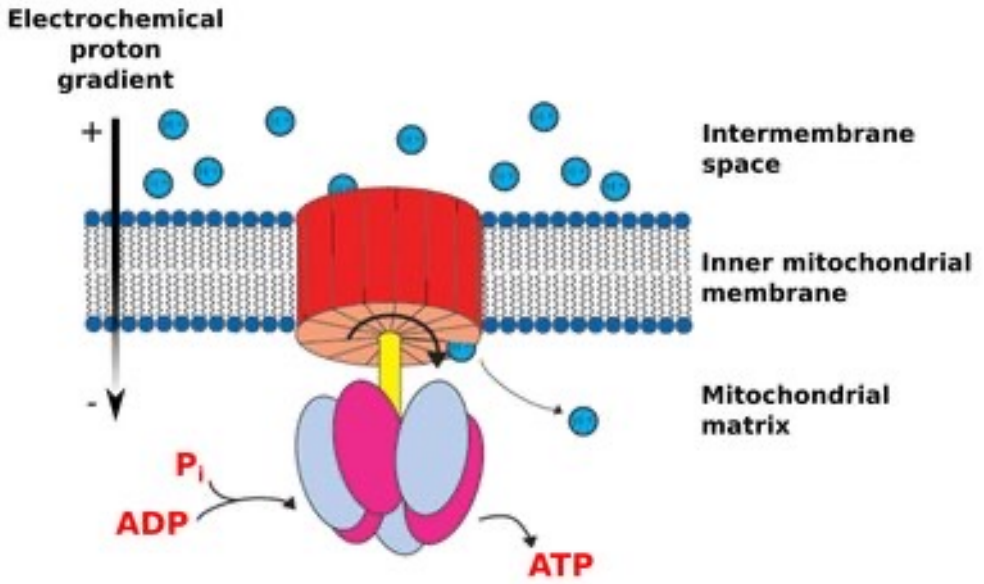
- Gradient occurs due to differences in ion across semipermeable membrane
  - Created when e- passes down ETC, free energy can be used to move H^+ from the matrix into the inter-membrane space
  - 10-fold gradient across inner membrane, equivalent to 1 pH unit difference
- Membrane potential created across inner mitochondrial membrane (IMM) due to + charge from H^+ in IMM space and the net - charge in the matrix
- Proton motive force (PMF) is a pH gradient and membrane potential drives H^+ across the inner membrane back to the matrix, driving ATP synthesis by ATP synthase



Chemiosmosis.

Concept 7.10: ATP Synthase

- Chemiosmotic gradient and resulting PMF causes the beta-subunits to cycle between 3 conformational states to bind ADP to Pi, creating
- ATP synthase required for OXPHOS but not part of ETC
- Proton motive force causes the -subunits to cycle between 3 conformational states to bind ADP to Pi, creating



ATP synthase

Spotlight on Disease: Uncouplers of Oxidative Phosphorylation

Uncoupling proteins

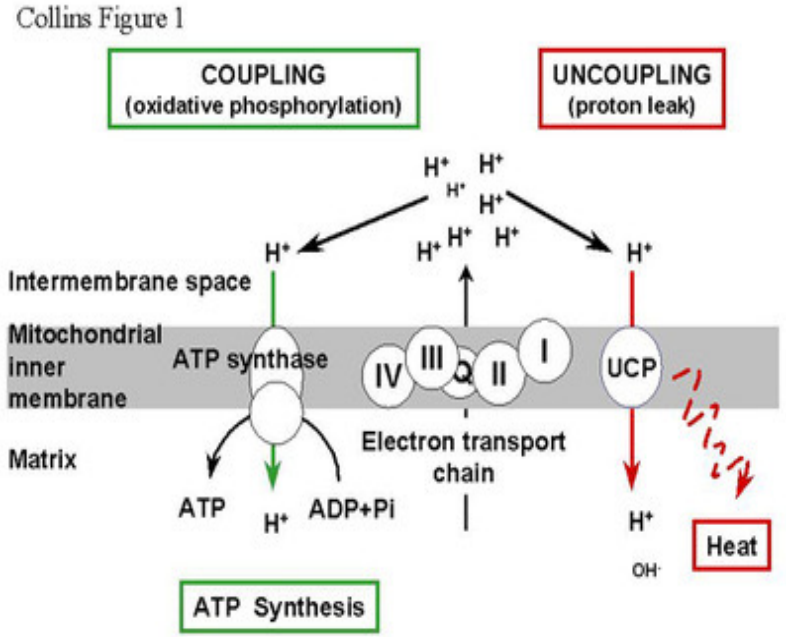
- Brown fat generates heat (infants and hibernating animals) by uncoupling proteins in the ETC, which bypass ATP synthase, allowing H+ to renter the matrix without generating ATP but heat

Oligomycin

- Drug that binds the FO domain of ATP synthase (the part in IMM), and blocks proton channel
- Gradient across IMM become too great that ETC eventually halts

Synthetic uncouplers

- Uncouple OXPHOS
- (ex. 2, 4-dinitrophenol) diffuses through the membrane and allows e- in ETC to move through the membrane without pumping protons
- Heat is produced



Mechanism of Uncouplers of OXPHOS.

Section 7 Quiz: 1 MC

1.Which molecule has the highest redox potential (lowest free energy)?

A.FADH2
B.NADH
C.Oxygen
D.Hydrogen

Answer: C

Section 7 Quiz: 1 SA

2. Explain the process of OXPHOS

I. ETC: Protein complexes found on mitochondrial IM that use energy from electron carriers generated from catabolic pathways to pump protons into IM space to eventually create ATP

II. Chemiosmotic gradient: Created when e- passes down ETC, free energy can be used to move H+ from the matrix into the inter-membrane space to fuel ATP synthase