Chapter 8 - An Introduction to Metabolism
Do Now - Introduction to Thermodynamics

1. What is the difference between catabolism and anabolism?

2. Why do nonspontaneous (+ΔG) chemical reactions tend not to occur at STP (0°C/273K, 1 atm pressure)?

3. Draw an energy diagram for an exergonic chemical reaction. Label the positions of the reactants and products, the magnitude of $E_a$, and the magnitude of $\Delta G_{\text{rxn}}$. 
Metabolism
The totality of an organism’s chemical reactions that arises from interactions between molecules

**Catabolism**
Process by which chemical compounds are broken down

Although energy is required to break the bonds holding the molecules together, catabolic processes tend to release energy

Energy released by catabolic reactions can be used to *power* anabolic reactions by coupling one reaction to another

**Anabolism**
Process by which chemical compounds are synthesized

Energy is **absorbed** to produce these new compounds

Energy stored in chemical bonds of synthesized molecules
Endergonic vs. Exergonic Reaction Energy Diagrams

Exergonic Reaction
- Products have less energy than reactants
- Energy released
- Spontaneous
- Entropy increases

Endergonic Reaction
- Products have more energy than reactants
- Energy required
- Not spontaneous
- Entropy decreases
Do Now - Metabolic Energy Conversions

1. Draw the structure of an ATP molecule. You do not have to draw the chemical structure of the nitrogenous base.

2. Which phosphate group is removed from a molecule of ATP during its’ hydrolysis?

3. Would you predict that the hydrolysis of ATP is an exergonic or an endergonic process?

4. Is the regeneration of ATP from ADP and P$_i$ an exergonic or an endergonic process?
1. Cellular respiration uses glucose and oxygen, which have high levels of free energy, and releases CO$_2$ and water, which have low levels of free energy. Is respiration spontaneous or not? Is it exergonic or endergonic? What happens to the energy released from glucose?

2. A key process in metabolism is the transport of hydrogen ions (H$^+$) across a membrane to create a concentration gradient. Other processes can result in an equal concentration of hydrogen ions on each side. Which arrangement of hydrogen ions allows the H$^+$ to perform work in this system?

3. At nighttime celebrations, revelers can sometimes be seen wearing glow-in-the-dark necklaces. The necklaces start glowing once they are “activated,” which usually involves snapping the necklace in a way that allow two chemicals to react and emit light in the form of “chemiluminescence.” Is the chemical reaction exergonic or endergonic? Explain your answer.
Endergonic vs. Exergonic Reaction Energy Diagrams

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ATP Hydrolysis

(a) Adenosine triphosphate (ATP)

(b) Hydrolysis of ATP

\[ \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i + \text{Energy} \]
1. In most cases, how does ATP transfer energy from exergonic to endergonic reactions in the cell?

2. Which of the following combinations has more free energy: glutamic acid + ammonia + ATP, or glutamine + ADP + P<sub>i</sub>? Explain your answer.
How Does Energy Coupling Work within a Biological System?

(a) Glutamic acid + Ammonia $\rightarrow$ Glutamine

$\Delta G = +3.4 \text{ kcal/mol}$

(Endergonic reaction)

Reaction w/o ATP

Unstable (energized) intermediate

(b) Glutamic acid + ATP $\rightarrow$ Glutamine + ADP

$\Delta G = -7.3 \text{ kcal/mol}$

(Exergonic reaction)

Reaction w/ ATP coupling

(c) Glutamic acid + Ammonia $\rightarrow$ Glutamine

$\Delta G = +3.4 \text{ kcal/mol}$

ATP $\rightarrow$ ADP + $P_i$

$\Delta G = -7.3 \text{ kcal/mol}$

Net $\Delta G = -3.9 \text{ kcal/mol}$
1. Using a series of arrows, draw the branched metabolic pathway described by the following statements, then answer the question at the end. Use “minus” signs to indicate inhibition.
   L can form either M or N
   M can from O
   O can form either P or R
   P can form Q
   R can form S
   O inhibits the reaction of L to form M
   Q inhibits the reaction of O to form P
   S inhibits the reaction of O to form R

Which reaction would prevail if both Q and S were present in the cell at high concentrations?
Negative (and Positive) Feedback Pathways

In **feedback inhibition**, the end product of a metabolic pathway shuts or slows down the pathway.

In **feedback activation**, the end product stimulates the metabolic pathway.

- Referred to as a “feed-forward” pathway.
Negative Feedback Pathways

(a) No end-product inhibition
(b) End-product inhibition
Feedback Inhibition

Initial substrate (threonine)

Threonine in active site

Enzyme 1 (threonine deaminase)

Intermediate A

Enzyme 2

Intermediate B

Enzyme 3

Intermediate C

Enzyme 4

Intermediate D

Enzyme 5

End product (isoleucine)

Figure 8.21
Rate-limiting enzyme

Inhibition of e₂

Rate-limiting enzyme

End product (modulator molecule)
8. A recent revival of the antievolutionary “argument from design” holds that biochemical pathways are too complex to have evolved, because all intermediate steps in a given pathway must be present to produce the final product. Critique this argument. How could you use the diversity of metabolic pathways that produce the same or similar products to support your case?
Energy in Biological Systems

**Energy** - The ability to do work

Cells are the “workhorses” in biology. Cells harness chemical energy in order to perform a particular job.

**Potential energy** - Matter that contains energy due to the arrangement of atoms and/or chemical bonds in a molecule

**Question**: Which type of chemical bond “holds” the greatest amount of energy in a molecule?

**Kinetic energy** - Energy of motion

**Example** - The movement of molecules down a concentration gradient to produce energy
Laws of Thermodynamics

1st Law - Energy can be neither created nor destroyed but it can be transferred from one system to another

2nd Law - Energy transfers tend to increase the disorder (entropy) of the universe

Entropy - Quantitative measure of disorder in the universe
Energy Systems

Closed

Energy in

Energy is “trapped” within the system

Open

Energy in

Energy out

All biological systems are OPEN
**Metabolism**

The totality of an organism’s chemical reactions that arises from interactions between molecules

**Catabolism**

Process by which chemical compounds are broken down

Although energy is required to break the bonds holding the molecules together, catabolic processes tend to *release* energy

Energy *released* by catabolic reactions can be used to “*power*” anabolic reactions by coupling one reaction to another

**Anabolism**

Process by which chemical compounds are synthesized

Energy is *absorbed* to produce these new compounds

Energy stored in chemical bonds of synthesized molecules
A *metabolic pathway* can have many steps that begin with a specific molecule and end with a product.

- Each step of the pathway is catalyzed by a specific enzyme.
Free Energy in Biological Systems

Not all energy can be used to do work. Some will inevitably be lost to the surroundings in the form of heat.

Free energy \((G)\) - The amount of energy in a system that is available to do work.

\[
\Delta G_{\text{biol. rxn.}} = \Delta H - T\Delta S
\]

For our purposes, \(\Delta G_{\text{biol. rxn.}}\) is equivalent to the net difference between the amount of energy that is consumed to break chemical bonds and start a chemical reaction and the amount of energy released from the reaction following the formation of products.

\[
\Delta G_{\text{biol. rxn.}} = [\Delta G_{\text{bonds broken}} - \Delta G_{\text{bonds formed}}]
\]
Free Energy Implications

$\Delta G$ can be calculated for any chemical reaction (You probably did this in Chemistry)

What is important is the value of $\Delta G$ for a biological reaction

$-\Delta G$ ($\Delta G < 0$) = a *spontaneous* reaction (a reaction that *will* occur without a net energy input from the surroundings)

$+\Delta G$ ($\Delta G > 0$) = a *non-spontaneous* reaction (a reaction that *will not* occur without a net energy input from the surroundings)

If $\Delta G = 0$, then the reaction has reached *equilibrium* because there is *no net change* between the energy of the system and the surroundings
**Spontaneous** chemical reactions tend to release energy to the surroundings (i.e. reactions are thermodynamically *favorable*).

**Why** are these reactions favorable?

Due to an overall release of energy by the reactants in the reaction, the reaction is classified as *exergonic*.

![Diagram](image)

**Figure 8.6**  (a) Exergonic reaction: energy released
Non-spontaneous chemical reactions tend to absorb energy from the surroundings (i.e. reactions tend to be thermodynamically unfavorable)

Why are these reactions unfavorable?

Due to an overall absorption of energy by the products of the reaction, the reaction is classified as endergonic.
Endergonic vs. Exergonic Reaction Energy Diagrams

Exergonic Reaction
- Products have less energy than reactants
- Energy released
- Spontaneous
- Entropy increases

Endergonic Reaction
- Products have more energy than reactants
- Energy required
- Not spontaneous
- Entropy decreases
Free Energy and Metabolism

**Endergonic**

- Chemical products store more chemical energy than reactants
- Non-spontaneous reaction ($+\Delta G$)
- $+\Delta G$ is the minimum amount of work required to drive the reaction forward

**Exergonic**

- Chemical products have less free energy than reactants
- Energy is released
- Spontaneous reaction ($-\Delta G$)
- $-\Delta G$ is the maximum amount of work that the reaction can perform
**General Rule** - If a chemical process is exergonic (spontaneous) the **reverse** of that process must be endergonic (nonspontaneous)

\[ A + B \leftrightarrow C + D \]

**Cell Respiration vs. Photosynthesis**

\[ C_6H_{12}O_6 + O_2 \rightarrow CO_2 + H_2O + \text{ENERGY} \]

\[ CO_2 + H_2O + \text{ENERGY} \rightarrow C_6H_{12}O_6 + O_2 \]
At maximum stability, the system is at \textit{equilibrium}

- More free energy (higher \(\Delta G\))
- Less stable
- Greater work capacity

- Less free energy (lower \(\Delta G\))
- More stable
- Less work capacity

In a \textbf{spontaneous change}
- The free energy of the system decreases (\(\Delta G<0\))
- The system becomes more stable
- The released free energy can be harnessed to do work

\begin{itemize}
\item \textbf{Gravitational motion.} Objects move spontaneously from a higher altitude to a lower one.
\item \textbf{Diffusion.} Molecules in a drop of dye diffuse until they are randomly dispersed.
\item \textbf{Chemical reaction.} In a cell, a sugar molecule is broken down into simpler molecules.
\end{itemize}

Figure 8.5
What would happen if $\Delta G$ reached zero in a biological system?

The process/reaction will be at equilibrium but...

**For living organisms, Equilibrium = Death**

To prevent a reaction from ever reaching equilibrium, products from one reaction are pulled away and used as reactants in a subsequent reaction as part of a metabolic pathway OR simply leave the system as waste products.

To prevent a reaction from ever reaching equilibrium, products from one reaction are pulled away and used as reactants in a subsequent reaction as part of a metabolic pathway OR simply leave the system as waste products.

This process guarantees that the rate of the reverse reaction will never equal the rate of the forward reaction (i.e. equilibrium)
Do Now - Free Energy Relationships

1) Briefly explain why endergonic reactions are thermodynamically unfavorable but exergonic reactions are thermodynamically favorable.

2) Write the equation for calculating a change in Gibbs’ free energy.

3) What would likely happen to the value of $\Delta G_{\text{rxn}}$ if the temperature of the surroundings were suddenly increased? Would this reaction more likely be spontaneous or nonspontaneous? (Assume $\Delta H$ and $\Delta S$ remain the same)

4) How would a decrease in $\Delta H$ and an increase in $\Delta S$ affect $\Delta G_{\text{rxn}}$? (Assume $\Delta S$ is positive and $\Delta H$ is negative)

5) Which phosphate group is lost during ATP hydrolysis? Can you explain why there is such a large amount of potential energy stored in this chemical bond?
A Summary of Metabolic Processes Inside of the Cell

METABOLISM:
CHANGES IN FREE ENERGY IN A CELL

RESPIRATION
Glucose $\rightarrow$ CO$_2$ + H$_2$O

SYNTHESIS
Monomers $\rightarrow$ Macromolecules

[Enzymes catalyze steps]

Decrease in FREE ENERGY
$- \Delta G$

Increase in FREE ENERGY
$+ \Delta G$

EXERGONIC

ENERGONIC

Energy Shuttle
ATP

Anabolic Reactions

Catabolic Reactions
The Importance of Adenoside Triphosphate (ATP)

ATP powers cellular work by coupling **exergonic** reactions to **endergonic** reactions.

A cell does three main kinds of work:

- Mechanical
- Transport
- Chemical

![Diagram of ATP's role in cellular work](Figure 8.11)

- **Mechanical work**: ATP phosphorylates motor proteins.
- **Transport work**: ATP phosphorylates transport proteins.
- **Chemical work**: ATP phosphorylates key reactants.
The Structure and Function of ATP

**ATP** (adenosine triphosphate) is the primary source of cellular energy as well as the energy coupling agent for exergonic and endergonic reactions.
The Structure and Function of ATP

Unstable bonds between phosphate groups can be hydrolyzed to release energy

\[ \text{ATP} + \text{H}_2\text{O} \rightarrow \text{ADP} + \text{P}_i + \text{ENERGY} \] (-31 kJ/mole)

78% more energy is released upon the hydrolysis of ATP inside of a cell than can be measured under standard conditions in the laboratory - Cells are amazing little machines!

ATP can be regenerated from ADP and P\(_i\) with the input of energy

\[ \text{ADP} + \text{P}_i + \text{ENERGY} \rightarrow \text{ATP} + \text{H}_2\text{O} \] (+31 kJ/mole)

10\(^7\) ATP molecules are regenerated every second inside of a single cell
ATP---> ADP Cycle
Catabolic pathways drive the regeneration of ATP from ADP and phosphate

ATP synthesis from ADP + $P_i$ requires energy

ATP hydrolysis to ADP + $P_i$ yields energy

Energy from catabolism (exergonic, energy yielding processes)

Energy for cellular work (endergonic, energy-consuming processes)

Figure 8.12
ATP Hydrolysis

(a) Adenosine triphosphate (ATP)

(b) Hydrolysis of ATP

\(-31 \text{ kJ/mole}\)
Chemical structure of ATP

ATP + H₂O → ADP + Pᵢ + H⁺ + 7 kcal/mol
How Does Energy Coupling Work within a Biological System?

**Diagram (a)**

1. **Unstable (energized) intermediate**
   - Reaction w/o ATP
   - Glutamic acid + Ammonia $\rightarrow$ Glutamine
   - $\Delta G = +3.4$ kcal/mol (Endergonic reaction)

**Diagram (b)**

1. **Unstable (energized) intermediate**
   - Reaction w/ ATP coupling
   - Glutamic acid + ATP $\rightarrow$ Glutamine + ADP
   - $\Delta G = +3.4$ kcal/mol
   - ATP $\rightarrow$ ADP + $P_i$
   - $\Delta G = -7.3$ kcal/mol
   - Net $\Delta G = -3.9$ kcal/mol

**Diagram (c)**

- Glutamic acid + Ammonia $\rightarrow$ Glutamine
- ATP $\rightarrow$ ADP + $P_i$
- $\Delta G = +3.4$ kcal/mol
- $\Delta G = -7.3$ kcal/mol
- Net $\Delta G = -3.9$ kcal/mol
**Do Now** - Work with a partner and a whiteboard to outline the steps of an enzymatic reaction. Draw a diagram that shows the steps of enzyme catalysis. Be sure that your diagram is labeled correctly.
Structure and Function of Enzymes

**Enzyme** - catalytic molecules that speed up a chemical reaction by lowering the activation energy for the reaction

Every enzyme (E) has at least one substrate (S) on which it will act upon

```
E + S ⇔ [ES*] ⇔ E + P
```

(transition state)

Enzymes are not consumed or permanently altered during chemical reactions; they can be re-used over and over again

Enzymes **do not determine whether or not a reaction will take place**; they simply increase the rate of a particular chemical reaction
Most reactions (enzymatic or not) require an energy input to get started.

- This energy quantity is known as the activation energy ($E_a$) for the reaction.

Heat (thermal energy) can be used to overcome $E_a$ in some situations (usually when $E_a$ is very small).

However, increased temperature can cause cellular damage.

$\Delta G$ is constant

Unstable intermediate (cannot be isolated)
Enzymes do not convert non-spontaneous reactions into spontaneous reactions or vice-versa ($\Delta G_{\text{rxn}}$ remains constant)
Activation Energy and Catalysis

Activation Energy

Reactant → Product

Catalysis

Uncatalyzed → Catalyzed

Activation energy

Energy supplied

Energy released
Enzyme Structure Lends to Its Function

**Active site** - groove, pocket, cleft, crevasse, or other region of the enzyme that binds to substrate molecule/s

The amino acid side groups positioned within an active site may function either as catalytic groups or may provide binding stabilization for the substrate (examples on subsequent slides).

Active site is not a static structure (*"Induced Fit"* model)
Substrate binding to the active site induces a conformational change in the 3-D shape of the enzyme

**Simplified example of “induced-fit” model of enzyme action**

**Induced Fit Model of Enzyme Action**
Amino acids
Intermolecular and Intramolecular Forces Influence Substrate Binding to the Active Site

Predict the chemical properties of a substrate molecule that might bind to the active site of this enzyme.
Ligand and protein binding site

Substrate = Ligand

Bound complex
**Ligand**: Common term used to describe generic substrate molecule

A particular protein may be able to bind to several different types of ligands.

Most enzymes are specific for only one type or class of substrate molecule.
Putting it All Together...

- The active site can lower an $E_A$ barrier by
  - Orienting substrates correctly
  - Straining substrate bonds
  - Providing a favorable microenvironment
  - Covalently bonding to the substrate
**Increasing affinity of enzyme**

**Enzyme affinity** - The overall “stickiness” or attractive force between an enzyme and its substrate.

![Graph showing increasing affinity of enzyme](image-url)
The binding sites

Ligand

Protein 1: High-affinity binding site

Protein 2: Intermediate-affinity binding site

Protein 3: Low-affinity binding site
Each enzyme has an **optimal temperature** and **pH** at which it can function.

What is happening within the structure of the protein that alters its ability to bind to substrate and catalyze the reaction?

Why does the rate of enzymatic reactions slow down at lower temperatures?
Oxyhemoglobin Dissociation Curve

As partial pressure of $O_2$ increases, more and more hemoglobin molecules become saturated with oxygen.
Oxyhemoglobin Dissociation Curve Under Various Conditions

(Bohr Shift)

Lower affinity for $O_2$
How Do pH and Temperature Affect Hemoglobin’s Affinity for Oxygen?

CO₂ produced during aerobic activity combines with H₂O to form H₂CO₃ (carbonic acid)

Carbonic acid lowers blood pH decreasing hemoglobin’s affinity for oxygen

Why might this be important in terms of normal human physiology?

Interestingly, elevated blood pH increases the affinity of hemoglobin for oxygen

Why might this be important in terms of normal human physiology?
How Enzymes Work

Manipulating the Conditions of an Enzymatic Reaction
How Does Enzyme Catalysis Work?

1. Substrates enter active site; enzyme changes shape so its active site embraces the substrates (induced fit).

2. Substrates held in active site by weak interactions, such as hydrogen bonds and ionic bonds.

3. Active site (and R groups of its amino acids) can lower $E_A$ and speed up a reaction by:
   - acting as a template for substrate orientation,
   - stressing the substrates and stabilizing the transition state,
   - providing a favorable microenvironment,
   - participating directly in the catalytic reaction.

4. Substrates are converted into Products.

5. Products are Released.

6. Active site is available for two new substrate molecules.

Figure 8.17

Hydrolysis of Sucrose
Factors in rate of enzyme-mediated reactions

Enzyme concentration (enzyme synthesis, enzyme breakdown)

Enzyme activity (allosteric activation or inhibition, covalent activation or inhibition)

Substrate (substrate concentration)

Product (product concentration)

(rate)
Effects of Physical and Chemical Environment on Enzyme Activity

Variables that can affect enzyme activity include: enzyme concentration, substrate concentration, temperature, pH, salinity, and presence of various cellular metabolites.

1) Enzyme concentration

![Graph showing the relationship between enzyme concentration and reaction rate.](image)

(b) Large excess of substrate present

2) Substrate (ligand) concentration

![Graph showing the Michaelis-Menten kinetics.](image)

(a) Constant enzyme concentration
Types of Enzyme Inhibition

1) Competitive Inhibition

Structure of inhibitor is very similar to structure of substrate

The competitive inhibitor is able to fit at the active site. When it is there, the substrate is unable to bind to the enzyme and the desired reaction does not occur. The inhibitor and substrate both compete for the active site!
Tokyo Sarin Attack: March 20, 1995

Members of Aum Shinrikyo placed five disguised canisters on busy Tokyo Subway. Toxic vapor later identified as dilute sarin was released. Targeted Kasumigaseki Station. Impacted Hibiya, Marunouchi, Chiyoda lines.
Tokyo Sarin Attack, cont’d

Five railcars directly affected
Victims at 15 stations
12 died (3 at scene, 9 in hospital)
5,000 - 6,000 exposed
(most minor or perceived)
**Irreversible Competitive Inhibition**

Inhibitor molecules react with amino acids in the enzyme’s active site and effectively “destroy” the enzyme.

**Example** - Sarin gas (organofluorphosphate)

Sarin covently bonds with a serine (amino acid) residue which is located within the active site of **acetylcholinesterase**

Acetylcholinesterase (AChE) breaks down acetylcholine present in the synapse --> sarin prevents AChE from breaking down acetylcholine

Consequently, [ACh] increases in the synapse causing the paralysis of muscles that control breathing, heart rate, etc.
An Example of a Biologically Important Enzyme

Acetylcholinesterase and nerve transmission

This enzyme is needed to transmit a nerve signal at a neuromuscular junction.

- Arrival of a nerve signal causes $\text{Ca}^{2+}$ levels to increase.
- This causes acetylcholine containing vesicles to move to end of the nerve cell and is released.
- Acetylcholine then diffuses across synapse to pass the signal to the muscle.
- Acetylcholinesterase then destroys the acetylcholine to stop the signal.
Acetylcholinesterase and nerve transmission

Presence of acetylcholine at receptor causes a flow of sodium and potassium ions. This causes a muscle contraction.

Nerve Impulse

Ca^{2+}

K^{+} \leftrightarrow Na^{+}

acetylcholine receptor protein

acetylcholinesterase - destroys excess acetylcholine

synaptic cleft
Acetylcholinesterase and nerve transmission

Without the enzyme, muscles would continue to contract causing spasms.

Acetylcholinesterase inhibitors are used as drugs and poisons.

**Organo fluorophosphates**
- bind to the enzyme. Death can occur.
Another Example of Irreversible Competitive Inhibition

Aspirin acetylates serine 530 of cyclooxygenase 2 (COX2)
2) Non-Competitive Inhibition (A Form of Allosterism)

3) Uncompetitive Inhibition

Inhibitor binds to [ES] complex

Few examples of this phenomenon --> Be aware that it exists
A Summary of Enzymatic Inhibition

(a) ES complex (no inhibitor)

(b) Competitive

(c) Noncompetitive

"I" can bind to E or ES.

(d) Uncompetitive

"I" can bind to ES only.
Allosteric Regulation

- Describes a condition when a protein’s function at one site is affected by binding of a regulatory molecule at another site

Virtually synonymous with noncompetitive inhibition

Can *positively activate* or *negatively inhibit* enzyme activity
How Allosteric Enzymes Work

**ALLOSTERIC ENZYME REGULATION**

1. **Repression**
   - Enzyme active
   - Substrate
   - Products

2. **Activation**
   - Enzyme inactive
   - Substrate
   - Repressor

3. **Activation**
   - Enzyme inactive
   - Substrate
   - Activator
   - Products

- Enzyme active
Allosteric Regulation

- Proteins change shape when regulatory molecules bind to specific sites, affecting function

(a) Allosteric activators and inhibitors. In the cell, activators and inhibitors dissociate when at low concentrations. The enzyme can then oscillate again.
(a) Allosteric modulation
(b) Covalent modulation
Multiple sites can modulate enzyme activity

- Site of covalent activation
- Sites of allosteric activation
- Sites of allosteric inhibition
- Site of covalent inhibition
Enzyme Kinetics – Michaelis/Menten Equilibrium

\[ E + S \xrightleftharpoons[{k_{-1}}]{{k_1}} ES \xrightarrow{k_2} E + P \]

To simplify, we will ignore the back reaction that would regenerate enzyme and substrate

\( V_0 \) is the rate of the forward reaction when the concentration of products is low (Think of it as \( V_{\text{initial}} \))

\( V_0 \) can be determined for a given substrate concentration by measuring the rate of product formation at early times before \( P \) accumulates to a significant extent

We can measure \( V_0 \) experimentally

Michaelis Menten Kinetics

Michaelis-Menten Summary
Enzyme Kinetics – Michaelis/Menten Equilibrium

Figure A illustrates reaction progression under initial (pre-steady state) conditions

- Substrate concentration ↑, free enzyme [E] concentration ↓ (compared to [S])
Enzyme Kinetics – Michaelis/Menten Equilibrium

Figure B illustrates reaction progression after *equilibrium* or a *steady-state* has been reached

- Steady-state refers to the condition where the rate of formation and breakdown of [ES] is **constant**
- Enzyme molecules bound w/ substrate - [ES] complex
- Reaction has **not** stopped but product is produced at a maximum rate \((V_{\text{max}})\) - All enzyme molecules are utilized
Enzyme Kinetics – Michaelis/Menten Equilibrium

We can examine the effect of increasing [substrate] on the reaction rate by performing an enzymatic reaction at various substrate concentrations.
Enzyme Kinetics – Michaelis/Menten Equilibrium

Michaelis-Menten Equation

\[ V_0 = V_{\text{max}} \frac{[S]}{[S] + K_M} \]

Michaelis constant \((K_M)\)
\((K_m\) is measured in units of concentration)\)

At very low substrate concentrations, when \([S]\) is much less than \(K_M\),
\[ V_0 = (V_{\text{max}}/K_M)[S] \]
The rate is directly proportional to the substrate concentration.

At high substrate concentrations, when \([S]\) is much greater than \(K_M\),
\[ V_0 = V_{\text{max}} \]
The rate is maximal and is independent of substrate concentration.

\[ K_M = \frac{k_{-1} + k_2}{k_1} \]
Enzyme Kinetics – Michaelis/Menten Equilibrium

Michaelis-Menten Equation

\[ V_0 = V_{\text{max}} \frac{[S]}{[S] + K_M} \]

What does \( K_M \) represent?

When \([S] = K_M\), then \( V_0 = V_{\text{max}}/2\). Thus, \( K_M \) is equal to the substrate concentration at which the reaction rate is half its maximal value.

\( K_M \) is an important characteristic of an enzyme-catalyzed reaction and is significant for its biological function.

\( K_M \) is a measurement of an enzyme’s **affinity** for its substrate

\( \downarrow K_M = \text{High affinity} \)

\( \uparrow K_M = \text{Low affinity} \)
Do Now - Enzyme Kinetics

For the following problem, I would like you to plot two graphs using Excel, LoggerPro, or a graphing calculator.

Use the data table on the right to:

1) Plot \([S] \text{ vs. } V_0\)

2) Plot \(1/[S] \text{ vs } 1/V_0\) to determine \(K_m\) and \(V_{\text{max}}\)

<table>
<thead>
<tr>
<th>[Substrate] (mM)</th>
<th>Initial Velocity ((\mu)moles/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25</td>
<td>1.7</td>
</tr>
<tr>
<td>0.5</td>
<td>3.0</td>
</tr>
<tr>
<td>1.0</td>
<td>5.0</td>
</tr>
<tr>
<td>2.0</td>
<td>7.3</td>
</tr>
<tr>
<td>3.0</td>
<td>8.6</td>
</tr>
<tr>
<td>5.0</td>
<td>10.1</td>
</tr>
<tr>
<td>10.0</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Do these kinetic data obey the Michaelis-Menten equation? What is the principle limitation to using this equation?
Physiological Consequence of $K_M$

Certain individuals are sensitive to ethanol. Ingestion of small quantities of ethanol can cause facial flushing and rapid heart rate (i.e. tachycardia).

In the liver, alcohol dehydrogenase converts ethanol to acetylaldehyde:

$$\text{CH}_3\text{CH}_2\text{OH} + \text{NAD}^+ \overset{\text{Alcohol dehydrogenase}}{\longrightarrow} \text{CH}_3\text{CHO} + \text{H}^+ + \text{NADH}$$

Normally, acetylaldehyde is converted to acetate by acetylaldehyde dehydrogenase:

$$\text{CH}_3\text{CHO} + \text{NAD}^+ \overset{\text{Acetaldehyde dehydrogenase}}{\longrightarrow} \text{CH}_3\text{COO}^- + \text{NADH} + 2 \text{H}^+$$
Physiological Consequence of $K_M$

Most people have two forms of the acetylaldehyde dehydrogenase enzyme, a low $K_M$ mitochondrial form and a high $K_M$ cytosolic form.

Individuals who are sensitive to ethanol have a mutation that inactivates the mitochondrial form of the enzyme and acetylaldehyde can only be metabolized by the high $K_M$ cytosolic form of the enzyme.

As a result, acetylaldehyde builds up in the body causing the signs and symptoms of intoxication.
A Simpler Way to Calculate $V_{\text{max}}$ and $K_m$ - The Lineweaver-Burk Plot

A Lineweaver-Burk plot is a double reciprocal plot of the Michaelis-Menten equation

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

$$\frac{1}{V_0} = \frac{K_m}{V_{\text{max}}} \cdot \frac{1}{[S]} + \frac{1}{V_{\text{max}}}$$

Slope: $\frac{K_m}{V_{\text{max}}}$
Y-intercept: $\frac{1}{V_{\text{max}}}$

A plot of $1/V_0$ vs $1/[S]$ should produce a straight line with a slope equal to $K_m/V_{\text{max}}$, an x-intercept equal to $-1/K_m$ and a y-intercept equal to $1/V_{\text{max}}$.

Lineweaver Burk Applet
Enzyme Kinetics Practice Problem

Fumarase catalyzes the hydration of fumurate to L-malate.

\[
\text{HO} - \text{C} - \text{H} \quad \text{CH}_2 \quad \text{COO}^- \\
\text{H} - \text{C} \quad \text{+} \quad \text{H}_2\text{O} \quad \text{H}_2\text{O} \quad \text{H} - \text{C} - \text{H} \\
\text{COO}^- \quad \text{COO}^- \\
\text{Fumarate} \quad \text{L-Malate}
\]

The enzyme is composed of four identical subunits. The data in the table on the next slide were obtained when fumurate was used as the substrate, and initial rates of hydration were measured at pH 5.7 and 25°C with an enzyme concentration of 2 x 10^{-6} M.

Plot the data in Lineweaver-Burk (double-reciprocal) form and determine \( V_{\text{max}} \) and \( K_M \) for fumarase under these conditions.
### Enzyme Kinetics Practice Problem

<table>
<thead>
<tr>
<th>Fumarate (mM)</th>
<th>Rate of product formation (mmol L&lt;sup&gt;-1&lt;/sup&gt; min&lt;sup&gt;-1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Substrate</strong></td>
<td></td>
</tr>
<tr>
<td>2.0</td>
<td>2.5</td>
</tr>
<tr>
<td>3.3</td>
<td>3.1</td>
</tr>
<tr>
<td>5.0</td>
<td>3.6</td>
</tr>
<tr>
<td>10.0</td>
<td>4.2</td>
</tr>
<tr>
<td>13.0</td>
<td>4.3</td>
</tr>
<tr>
<td>15</td>
<td>4.4</td>
</tr>
</tbody>
</table>
Effect of Substrate Concentration on the Initial Rate of an Enzymatic Reaction

![Graph showing the effect of substrate concentration on the initial rate of an enzymatic reaction. The graph plots the initial rate of reaction (mmol/L s) against the substrate concentration (mM). The data points are connected by a curve, indicating a positive relationship between substrate concentration and initial reaction rate.]
Effect of Fumarate Concentration on the Enzymatic Activity of Fumarase

\[ y = 0.4062x + 0.1976 \]

\[ R^2 = 0.9996 \]
Making Connections - How Can the Lineweaver-Burk Plot Be Used to Investigate the Actions of Various Enzymatic Inhibitors?
**Do Now - Enzyme Kinetics (Now with Inhibitors!)**

The kinetics of an enzyme were analyzed in the absence and presence of inhibitors A and B.

<table>
<thead>
<tr>
<th>[S] (mM)</th>
<th>$V_0$ (nmoles/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No Inhibitor</td>
</tr>
<tr>
<td>1.0</td>
<td>43</td>
</tr>
<tr>
<td>2.0</td>
<td>68</td>
</tr>
<tr>
<td>5.0</td>
<td>105</td>
</tr>
<tr>
<td>10.0</td>
<td>128</td>
</tr>
<tr>
<td>20.0</td>
<td>144</td>
</tr>
</tbody>
</table>

Again, I would like you to plot two graphs using Excel, LoggerPro, or a graphing calculator.

What types of inhibitors are A and B? Competitive or non-competitive?
Making Connections - How Can the Lineweaver-Burk Plot Be Used to Investigate the Actions of Various Enzymatic Inhibitors?
Cofactors and Coenzymes

**Cofactor** - General term for an ion or non-protein molecule that “assists” the enzyme during a reaction; often inorganic

Active sites of many enzymes contain metal ions (Zn$^{+2}$, Fe$^{+2}$, Mg$^{+2}$, Cu$^{+2}$, Mn$^{+2}$, or Ca$^{+2}$.) that draw electrons away from substrate molecules making bonds less stable (i.e. easier to break)

**Example** - Carbonic anhydrase
Catalyzes reaction btw CO$_2$ and H$_2$O to form H$_2$CO$_3$

$$
\text{CO}_2 + \text{H}_2\text{O} \leftrightarrow \text{H}_2\text{CO}_3 \leftrightarrow \text{H}^+ + \text{HCO}_3^-
$$

Mechanism for carbonic anhydrase activity
Coenzyme - Cofactor that is a non-protein, organic molecule

One of the most common (and biologically important) coenzymes is NAD$^+$ (oxidized form)/NADH (reduced form)

NADH = nicotinamide Dinucleotide (reduced form)

NAD$^+$ “picks” up high energy electrons [and protons (H$^+$)] from an enzyme/substrate complex and transports them to another molecule that can be reduced

Energy is efficiently “shuttled” from one molecule to the next in a biochemical pathway through NADH
The coenzymes NAD+ and FAD

Reaction 1: $R_1 - 2H$ to $R_1$

Reaction 2: $R_2 - 2H$ to $R_2$

Reaction 3: $R_3 - 2H$ to $R_3$

Reaction 4: $R_4 - 2H$ to $R_4$
Negative (and Positive) Feedback Pathways

In *feedback inhibition*, the end product of a metabolic pathway shuts or slows down the pathway.

In *feedback activation*, the end product stimulates the metabolic pathway.
- Referred to as a “feed-forward” pathway.
Negative Feedback Pathways

(a) No end-product inhibition

(b) End-product inhibition
Feedback Inhibition

Isoleucine used up by cell

Initial substrate (threonine)

Active site available

Threonine in active site

Enzyme 1 (threonine deaminase)

Intermediate A

Enzyme 2

Intermediate B

Enzyme 3

Intermediate C

Enzyme 4

Intermediate D

Enzyme 5

End product (isoleucine)

Isoleucine binds to allosteric site

Active site of enzyme 1 no longer binds threonine; pathway is switched off

Figure 8.21
Rate-limiting enzyme

Inhibition of $e_2$

Rate-limiting enzyme

End product (modulator molecule)
Not All Enzymes Are Proteins...

Catalytic RNA

Since their discovery, it was believed that all enzymes were proteins.

In 1981–1982, two research group reported results on catalytic RNA.

In 1989, the Nobel Prize in Chemistry was awarded to Sidney Altman (Yale) and Thomas Cech (University of Colorado - Boulder) for their discovery.

The term *ribozyme* is now used for RNA enzymes.
Ribonuclease P

This was the first type of catalytic RNA discovered and is present in all organisms.

- Substrates are at least 60 inactive, precursor forms of tRNA.
- Ribonuclease P acts to remove a segment of the ribonucleotide, producing mature tRNA.
- The enzyme consists of a small protein subunit with a molecular weight of 14,000 and an RNA component of 377 nucleotides.
RNase P functions by hydrolytic cleavage of the phosphodiester bond.
Significance of ribozymes

Other forms of catalytic RNA continue to be discovered.

- Small ribozymes have been found as components of plant RNA viruses.
- The active region of this RNA consists of only 19 - 30 nucleotides.
- Because of their characteristic shape and action, they are called “hammerhead” ribozymes.