Anglo-Chinese Junior College
Class of H3 Chemistry '26

Enzyme Catalysis lecture notes

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Special thanks to H3 chemistry teachers

Intro to enzymes

In catalysis, enzymes act as **specific biological catalysts** in the breakdown or assimilation of substances, speeding up the rate of metabolic reactions.

Nature of enzymes:

- ***Most*** enzymes are **globular (looks round) proteins,** consisting of one or more polypeptides coiled and folded together to form a globular structure.

- Enzymes are **organic catalysts** produced by living cells. They **remain unchanged** at the end of a reaction and do not alter the nature or properties of the end-product(s) of the reaction.

- Enzymes are **highly selective** in their action as the active site has a specific 3D confirmation that complements only targets specific functional groups (**group specificity**).

- Rates of enzymatic reactions are affected by pH, temperature, enzyme concentration and substrate concentration.

- Enzymes have an **active site**, which is the catalytic centre of the enzyme, where the substrate binds for catalysis to take place.

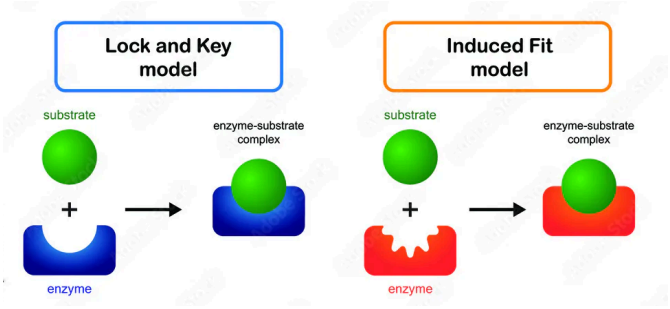
Common Features of Active Site:

1. The **active site** is a three-dimensional cleft formed by R (variable functional) groups of amino acids of the polypeptide
    - The active site has a **specific 3D conformation** that is **complementary** to the substrate, which accounts for the specificity in the types of substrate that it can act on.
    - The specificity of binding depends on the arrangement of amino acids in an active site.

2. The active site takes up a relatively small part of the total volume of an enzyme.
    - Typically consist of 3 – 12 amino acids.
    - These amino acids may be found far apart along the polypeptide chain, but were brought near together through folding.

3. Substrates bind to enzymes at the active site by multiple **weak interactions** such as **Van der Waals forces** and **hydrogen bonds**.

Substrate Binding Mechanisms



Originally, enzymes were thought to be 'locks', through which a substrate 'key' could be fitted. The lock would remain unchanged after each reaction, and they would have to already be conformationally complementary for the interaction to occur. However, more recently, the induced fit model was proposed, which states that enzymes are more like a mould which wraps itself around the substrate, and adjusts its shape accordingly.

Today, the 'lock and key' model and 'induced fit model' are thought to work in conjunction, as two separate mechanisms of enzyme binding.

Mechanisms of substrate binding

Different enzymes favour different modes of binding, depending on their substrate or function. These modes include:

- **Uniform** binding ('lock and key'), where the active site has a high affinity for binding for **both** the substrate (S) and the substrate in transition state ($S^{‡}$).
    - This means that the enzyme **naturally binds** to the substrate by default without needing to change much in conformation.
    - This lowers the energy levels of both the enzyme-substrate complex (ES) and enzyme-substrate transition complex ($ES^{‡}$).

- **Differential** binding ('induced fit'), where the active site has a high affinity for **just the substrate in transition state.**
    - This means that while the substrate and enzyme are not a good 'fit' initially, the enzyme **shifts its conformation** such that it fits well with the transition state.
    - This lowers the energy level of $ES^{‡}$ whilst the energy level of ES remains high.

Enzymes that are usually **saturated** favour **differential** binding. When an enzyme is saturated, all its active sites are occupied by substrate molecules, forming ES(es) which are stable. Thus, the enzyme must overcome the activation energy (E_a) to form $ES^{‡}$ for catalytic action. Saturated enzymes want to be able to favour the formation of $ES^{‡}$ instead of retaining ES. Thus, they favour differential binding.

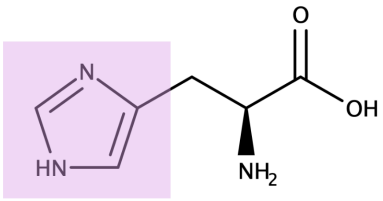
However, some substrates are able to bind to enzymes through either mechanism. Specifically, these substrates must be **small** and **unbound**. Simple, small substrate molecules do not require large conformational change in the enzyme to bind, and can also be held within the active site with just a few bonds. Unbound substrates (i.e. substrates

which are low in concentration) go through uniform binding due to the enzymes' active sites being largely unsaturated.

Types of enzyme interactions

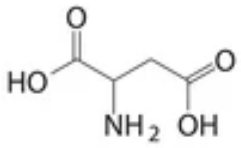
I. Acid-Base Catalysis

Amino acids in the active site can act as acids and bases, to stabilise charges that appear in the substrate's transition state. For example, histidine

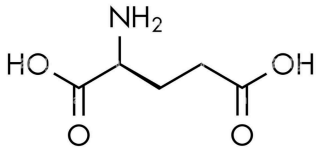


Histidine

has a imidazole (highlighted) side chain which has a pKa of around 6.0, which is close to physiological pH, especially that of the blood, at 7.4. This means that there is a sizable amount of conjugate acid present even at physiological pH, and thus it can act as a kind of buffer. This allows enzymes to protonate and deprotonate substrate molecules in various steps.



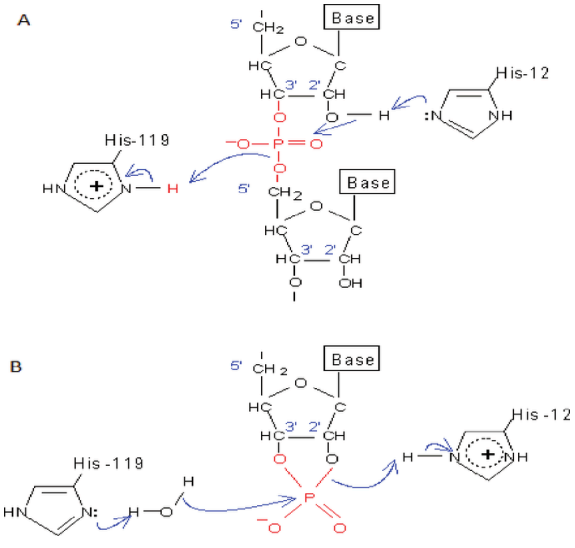
DL-Aspartic acid



glutamic acid

The two carboxylic acid side chains in aspartic and glutamic acid have pKa values of around 4.0, but these can be higher in reality when found in the 3D confirmation of an enzyme. Hence they can be protonated and serve as acids.

In an actual enzyme, acid-base looks as in the example of Ribonuclease A, which hydrolyses an RNA molecule (a polymer of smaller molecules called ribonucleotides):



II. Electrostatic Catalysis

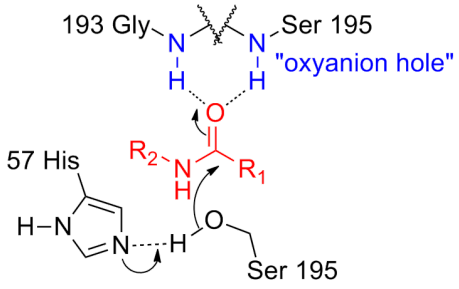
Typically, charges during catalysis can be present for reasons such as:

- Substrate molecules being charged;
- Transition states being charged (deprotonation, bond fission etc.).

Enzymes catalyse reactions involving charges by:

- Surrounding substrate molecules with like charges, therefore destabilising them and driving the reaction towards forming the transition state;
- Placing opposite charges near side chains that are developing a charge in the substrate, therefore stabilising the transition state and lowering the activation energy.

An example of this is observed when the enzyme chymotrypsin catalyses the breakage of peptide bonds.



The enzyme's active site (everything surrounding the peptide substrate $R_2NHC{=}OR_1$) has an active site which contains an 'oxyanion hole'. As the name implies, the 'hole' is meant for a developing oxyanion to be 'inserted'. The perfectly positioned N-H groups have $\delta+$ H atoms and are able to form ion-dipole interactions/hydrogen bonds with the O atom of the substrate, thus stabilising the transition state and allowing nucleophilic attack from the other side of the active site to form a tetrahedral intermediate.

The oxyanion hole is also hydrophobic, and excludes water which could further interfere with the electrostatic forces of attraction between the substrate and the active site.

III. Metal Ion Catalysis

One third of all known enzymes require metal ions to function. Some of these enzymes especially rely on transition metal cations in their active sites, which have vacant d orbitals which allow them to act as Lewis acids and accept electron pairs (Transition Metals stuff).

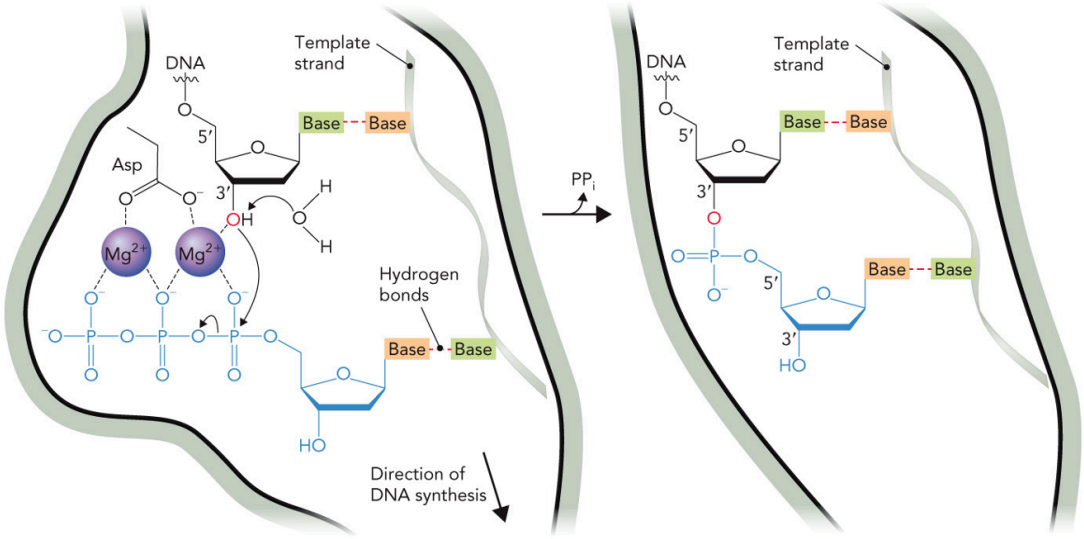
Hence, metal ions can help enzymes in a number of ways:

- Facilitating the binding of enzyme to substrate;
- Neutralising substrate charges to facilitate catalysis;
- Enhancing nucleophiles (like water)

Firstly, from the enzymes' active sites, metal ions can **coordinate with substrate molecules**, forcing the substrate into a position where it can be acted on by catalytic amino acids (like histidine, aspartic acid and glutamic acid).

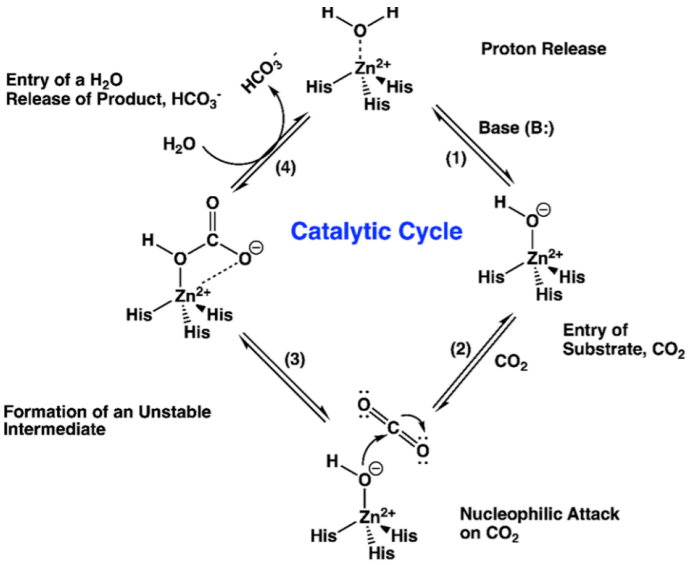
Secondly, some substrate molecules tend to be negatively charged, such as those from RNA, DNA or ATP, due to the presence of negatively charged phosphate groups in their structure. This repels nucleophiles like OH^- which can be important in catalysis. Metal ions counter this by coordinating with the phosphate groups, pulling electrons away from them and reducing repulsion such that nucleophilic attack can occur.

For example, DNA polymerase (an enzyme which joins DNA monomers together to form DNA strands) contains Mg^{2+} ions which coordinate with the phosphate groups of the DNA and neutralise their charge. This allows for nucleophilic attack by the OH group of the monomer above.



Finally, metal ions can enhance nucleophiles - especially observed with water, which is turned into a more powerful OH^- in the example of carbonic anhydrase. Carbonic anhydrase is responsible for catalysing this reaction that occurs within the blood buffer system:

$$CO_2 + H_2O \leftrightharpoons H_2CO_3$$



The Zn^{2+} ion coordinates with a water molecule, drawing electrons away from it, which allows the base (another histidine molecule in the active site) to deprotonate H_2O to form OH^- (acid-base catalysis). This creates a strong OH- nucleophile which can attack the CO_2 substrate, eventually resulting in HCO_3^- being released.

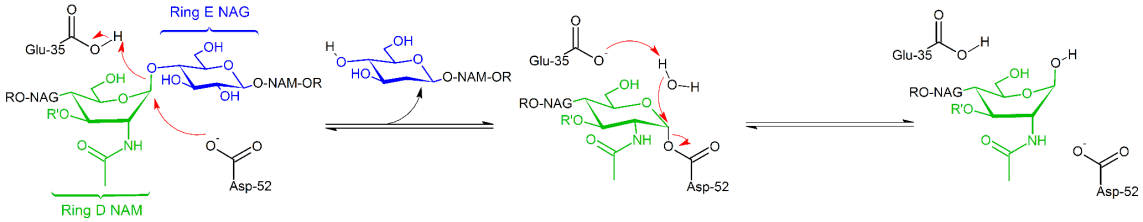
IV. Bond Strain

Due to the affinity of the enzyme to the transition state being greater than to the substrate itself, the enzyme preferentially binds and stabilises the transition via differential binding. This induces structural rearrangements that strain substrate bonds into a position closer to the conformation of the transition state. This lowers the energy difference between the substrate and transition state, catalysing the reaction.

Binding forces the substrate into a less favourable conformation by:

- stretching bonds
- compressing bond angles
- twisting bonds
- forcing eclipsed conformations
- distorting ring structures

An example of bond strain is observed when lysozyme (an enzyme that protects against bacterial infection by breaking down the bacterial cell wall) hydrolyses the polysaccharide peptidoglycan cell wall of bacteria.



R = cell wall oligosaccharide chain
R' = cell wall peptide side chain

The polysaccharide binds to the active site of lysozyme via multiple weak interactions such as hydrogen bonds and hydrophobic interactions. Since the active site is **more complementary to the transition state than to the substrate in its ground-state conformation**, one of the sugar rings (typically the NAM residue at the D subsite) is forced to adopt a **distorted half-chair conformation** instead of its stable chair conformation.

The distorted sugar now closely resembles the **transition state** for glycosidic bond cleavage.

As the reaction proceeds, lysozyme forms even stronger interactions with the developing transition state than with the original substrate through differential binding.

V. Covalent Catalysis

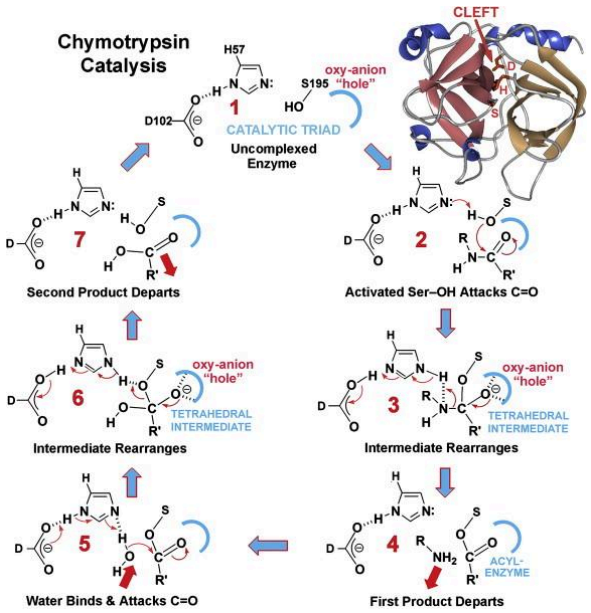
Covalent catalysis is the mechanism by which an enzyme forms a temporary covalent bond with the substrate, creating a reactive intermediate that provides an alternative pathway with lower activation energy.

Substrate binds to the enzyme's active site, where a nucleophilic residue (RCOO-, RNH, ROH) on the enzyme's active site can attack the electrophilic centre (acyl, phosphoryl, glycosyl groups) of the substrate. A temporary covalent bond is formed between the enzyme and substrate, producing a covalent intermediate.

Reaction pathway:
Substrate -> Transition state 1 -> covalent intermediate -> transition state 2 -> product

Each step has a lower-energy transition state than the single transition state of the uncatalysed reaction.

An example can be seen in chymotrypsin (a digestive enzyme that catalyses the hydrolysis of peptide bonds in proteins, breaking them down into smaller peptides)



The polypeptide substrate binds specifically to the active site of chymotrypsin.

Asp102 helps orient and stabilise His57, while His57 acts as a general base to activate Ser195. His57 abstracts the proton from the hydroxyl group of Ser195, which generates a serine alkoxide ion (Ser–O^-), which is a much stronger nucleophile.

The activated Ser195 attacks the **electrophilic carbonyl carbon** of the peptide bond. This nucleophilic attack forms a **temporary covalent bond** between Ser195 and the substrate.

A short-lived **tetrahedral intermediate** (NOT the covalent intermediate) is formed first. The

peptide bond of the tetrahedral intermediate breaks, and an acyl-enzyme intermediate remains. The **acyl–enzyme intermediate** is the **covalent enzyme–substrate intermediate**.

A water molecule enters the active site. His57 removes a proton from water, producing hydroxide (OH^-), which acts as a strong nucleophile.

The hydroxide attacks the carbonyl carbon of the acyl–enzyme intermediate. Another tetrahedral intermediate forms and then collapses. The covalent bond between Ser195 and the substrate is broken.

Transition State Theory

Transition state theory states that chemical reactions proceed through a transition state, which is the highest-energy, most unstable arrangement of atoms along the reaction pathway. Enzymes accelerate reactions by stabilising the transition state more than the ground-state substrate, thereby lowering the activation energy required to reach the transition state. This is the basis of **differential binding.**

Transition state is defined as the state corresponding to the highest potential energy along a reaction coordinate.

- Least stable state
- Characterised by partially broken and partially formed bonds
- Cannot be isolated because it exists for an extremely short period of time

The transition state represents the **energy barrier** that must be overcome before products can form. The energy required to reach it is the **activation energy (ΔG‡)**.

Eyring Equation:

$$k = \frac{k_B T}{h} e^{-\Delta G^{\ddagger}/RT}$$

k = rate constant

k_B = Boltzmann constant

T = absolute temperature (K)

h = Planck's constant

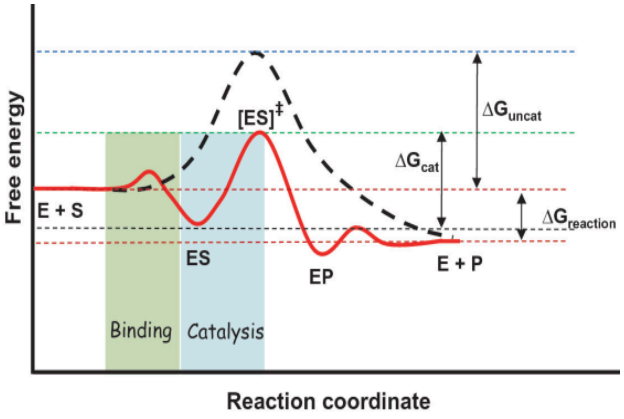
R = gas constant

$\Delta G^{\ddagger}$ = Gibbs free energy of activation

$$k \propto e^{-\Delta G^{\ddagger}/RT}$$

Enzyme kinetics

An enzyme, often written "E" in reactions, does catalysis through binding of one or more other molecules, its substrate (S), to form the desired product. The substrate binds to the active site of the enzyme to produce an enzyme-substrate complex (ES), and is transformed into an enzyme-product complex (EP) and from there to the product or products (P), via a transition state (ES‡).



- This series of steps is known as the mechanism:
  E + S ⇄ ES ⇄ [ES]‡ ⇄ EP ⇄ E + P
  - For simplicity we will only cover
    E + S ⇄ ES → E + P (stable-state assumption)

The Michaelis constant (K_m)

K_m is defined as the **substrate concentration** required to achieve V= **half of the maximum velocity** ($0.5V_{max}$) of an enzyme-catalyzed reaction, this also reflects the affinity of the enzyme for the substrate.

- A **low value** of K_m is often interpreted as a **high affinity** of the enzyme for the substrate, and vice versa.
- When $[S] = K_m$, half of all available active sites are occupied with substrate
- K_m is an *intrinsic* property of an enzyme, but can be altered under different conditions (e.g. pH, temperature, solvent, etc.)
- K_m has units $moldm^{-3}$

Consider the following reaction: $\mathrm{E + S} \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} \mathrm{ES} \xrightarrow{k_2} \mathrm{E + P}$

- $K_m = \frac{ES\ dissociation}{ES\ association} = \frac{(k_{-1} + k_2)}{k_1}$

  Due to steady state assumption (Refer to annex A for assumption)

Example 1:
According to the chart, which one of the following enzymes has the strongest affinity for its substrate? Take M = $moldm^{-3}$

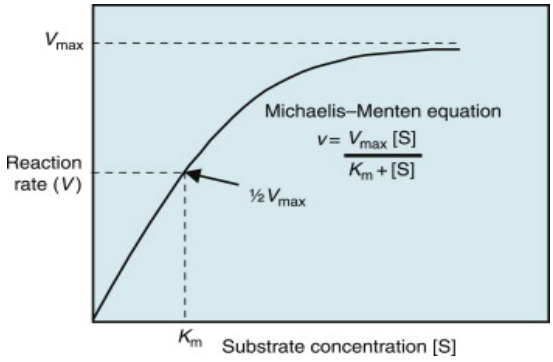
Enzyme	Substrate	K_m (μM)
Lysozyme	Hexa-N-actylhlucosamine	6
Penicillinase	Benzylpenicillin	50
β-Galactosidase	Lactose	4000
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Carbonic anhydrase	Carbon dioxide (CO_2)	8000

A. Lysozyme.
B. Penicillinase.
C. β-Galactosidase.
D. Chymotrypsin.
E. Carbonic anhydrase. ( )

Michaelis–Menten kinetics

In biochemistry, Michaelis–Menten kinetics is the simplest case of enzyme kinetics, applied to enzyme-catalysed reactions involving the transformation of one substrate into one product.

The **Michaelis–Menten equation** describes the **initial reaction rate** (V_0) as a function of the **initial substrate concentration** [S]. By restricting the relationship to the initial rate where product concentration is effectively zero ($p \approx 0$)



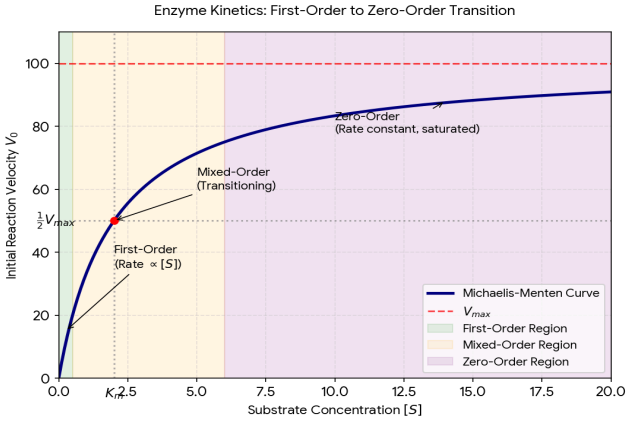
Below is the Michaelis–Menten equation:

$$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$ (Derivation in annex A)

Biochemical reactions involving a **single substrate** are often assumed to follow Michaelis–Menten kinetics, without regard to the model's underlying assumptions. Only a small proportion of enzyme-catalysed reactions have just one substrate, but the equation still often **applies if only one substrate concentration is varied**. (recall pseudo-first-order rxn) Common units for V are Ms^{-1} ($moldm^{-1}s^{-1}$) or Us^{-1} ($\mu moldm^{-1}s^{-1}$).

Notice how initially, the rate increases proportionally with respect to [S] (i.e. first order). This can be seen in the Michaelis-Menten equation, where when [S] << K_m, K_m + [S] ≈ K_m. Thus we observe that V_0 = (V_{max} / K_m) [S].

Additionally, notice at high substrate concentrations, we can take it as though the velocity is relatively constant despite [S] is increasing (i.e. zeroth order) due to all of the enzyme active sites being saturated and no more active sites can be utilised on the enzyme.



Practice one:

Calculate the initial reaction velocity for an enzyme with Vmax of 150 μmol/min, Km of 15 mM, and substrate concentration of 10 mM.

[60 μmol/min]

Enzyme regulation - inhibition

The activity of enzymes can also be regulated by specific inhibitors that slow or stop catalysis (besides pH and temperature which inhibits all enzymes).

Enzyme inhibition can either be reversible or irreversible. In reversible inhibition, the inhibitor can bind (usually non-covalently) and dissociate, allowing enzyme activity to return back to its original, uninhibited level. Irreversible inhibitors bind to the enzyme permanently and thus permanently inhibit enzyme activity.

Reversible inhibition

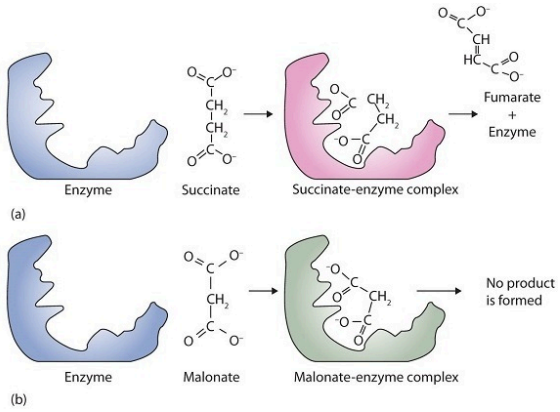
Reversible inhibition can be *competitive*, *uncompetitive*, or *noncompetitive*, depending on where the inhibitor binds to the enzyme, substrate, or enzyme-substrate complex.

Competitive inhibition:

- The inhibitor binds reversibly to an enzyme at the enzyme active site, competing with the substrate for binding.
- The inhibitor must be **structurally similar to the substrate**, allowing it to interact with the active sites of the enzyme using similar non-covalent interactions.
    - However, as a reversible inhibitor, it can disassociate from the enzyme eventually allowing for the correct substrate to bind and the catalysis to occur. Because the inhibitor and substrate are in competition for the same active site, **inhibition is concentration-dependent** (le chatelier's principle).
- Modifies the Michaelis–Menten equation to:

$$v_i = \frac{V_{\max}[S]}{K_{\mathrm{m}}^{\mathrm{app}} + [S]} \text{ where: } K_{\mathrm{m}}^{\mathrm{app}} = K_{\mathrm{m}}\left(1 + \frac{[I]}{K_{\mathrm{i}}}\right)$$

A classic example of competitive inhibition is the effect of malonate on the enzyme activity of succinate dehydrogenase (**Krebs cycle**). Malonate and succinate are the anions of dicarboxylic acids and contain three and four carbon atoms, respectively. The malonate molecule binds to the active site because the spacing of its carboxyl groups is not greatly different from that of succinate. However, no catalytic reaction occurs because malonate does not have a CH2CH2 group to convert to CH=CH.



Uncompetitive inhibition:

- The inhibitor can only bind to the enzyme when the substrate is already bound
  - (i.e. it binds to ES complex rather than E)
- Maximum reaction rate is lowered, and cannot be increased by adding more substrate
- This is most commonly seen when the enzyme reaction involves two substrates
- As long as the concentration of the inhibitor remains constant, the maximum reaction rate does not change.
- Modifies the Michaelis–Menten equation to:

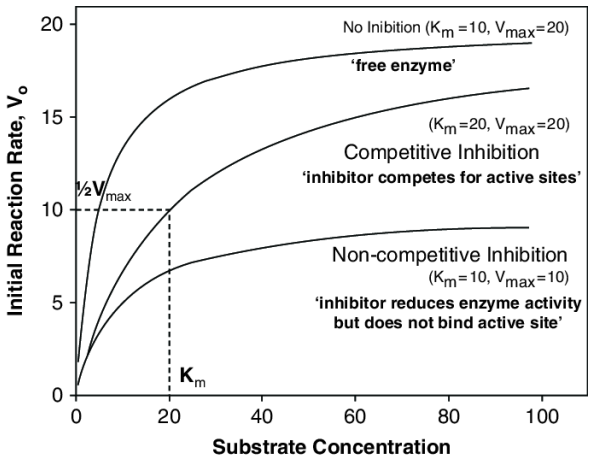
$$v_i = \frac{V_{\max}^{\text{app}}[S]}{K_{\text{m}}^{\text{app}} + [S]} \quad \text{where: } K_{\text{m}}^{\text{app}} = \frac{K_{\text{m}}}{\left(1 + \frac{[I]}{K_{\text{i}}}\right)} \text{ and } V_{\max}^{\text{app}} = \frac{V_{\max}}{\left(1 + \frac{[I]}{K_{\text{i}}}\right)}$$

Non-competitive inhibitor:

- It can bind to either the free enzyme or the enzyme-substrate complex because its binding site on the enzyme is distinct from the active site. (i.e. it **need not** be structurally similar to the substrate)
- Binding of this kind of inhibitor **alters the three-dimensional conformation** of the enzyme, c**hanging the configuration of the active site** with one of two results.
  - Either [ES] complex does not form at a normal rate
    Or once [ES] complex is formed, it does not yield P at the normal rate
- Because the inhibitor does not structurally resemble the substrate, nor is it competing with the substrate for the active site, the addition of excess substrate does not reverse the inhibitory effect.
- Non-competitive inhibition has no effect on substrate binding, thus Km is unchanged. **This inhibitor type exclusively decreases Vmax.**
- Modifies V_{max} to:

$$V'max = \frac{Vmax}{1+\frac{[I]}{Ki}}$$

Overview of how inhibition affects the initial rate of reaction for all types of reversible inhibitions.



Practice two:
It is known that Memantine is a non-competative inhibitor of N-methyl-D-aspartate receptors found in the brain. Given that the Ki of Memantine $2.5mM$ and $Vmax = 0.25moldm^{-3}s^{-1}$ and that $[I] = 1.0\,mM$, find actual $Vmax$. [1.25 Ms^{-1}]

Irreversible inhibition

An irreversible inhibitor inactivates an enzyme by **bonding covalently** to a particular group at the active site. When the inhibitor is bound, the enzyme active site is blocked, the substrate does not bind, and catalysis cannot occur, similar to competitive inhibition. The difference here is that the inhibition is irreversible, meaning that the inhibitor remains bound and does not dissociate from the enzyme because the **enzyme-inhibitor covalent bonds are not easily broken**. In the presence of an irreversible inhibitor, the substrate cannot bind the active site at all, nor can high substrate concentrations outcompete the inhibitor, hence the enzyme is completely inactivated. Many of the known irreversible inhibitors are poisons because they inactivate an enzyme completely.

- Alters the Michaelis-Menten equation to:

$$V = \frac{V'max\,[S]}{Km + [S]}\,;\, V'max = Vmax\,(1 - \frac{[I]}{[E]i})$$

Practice three:
It is known that acetylsalicylic acid (aspirin) is an irreversible inhibitor of the enzyme Cyclooxygenase (COX). Given that initially, $Vmax = 0.02Ms^{-1}$, $[E] = 0.2M$, $[I] = 0.1M$, find $V'max$ [$V = 0.01Ms^{-1}$]

Annex A: Derivation of Michaelis–Menten equation

Consider the initial reaction:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

Assumptions:

1. **No product exists initially** (ignores ES ← E + P and its k_{-2})
2. Assume [ES] is constant. (Briggs-Haldane assumption i.e. steady state)
    - Thus **rate of formation = rate of breakdown**
3. [E] << [S], thus **[ES] << [S]** and we can use pseudo-first-order kinetics (w.r.t E)
4. Only the **initial velocity (rate)** of the reaction is measured
5. **$[E]_{total}$ = [E] + [ES]**

Derivation:

Rate of ES formation = $k_1[E][S] = k_1([E]_{total} - [ES])[S]$ {Due to assumption 5}

Rate of ES breakdown = $k_{-1}[ES] + k_2[ES] = (k_{-1} + k_2)[ES]$

Due to Assumption 2:
$k_1([E]_{total} - [ES])[S] = (k_{-1} + k_2)[ES]$

$$\frac{([E]_{total} - [ES])[S]}{[ES]} = \frac{(k_{-1} + k_2)}{k_1}$$

$$\frac{[E]_{total}[S]}{[ES]} - [S] = \frac{(k_{-1} + k_2)}{k_1} \text{ ; Let } K_m = \frac{(k_{-1} + k_2)}{k_1}$$

$$\frac{[E]_{total}[S]}{[ES]} = K_m + [S]$$

Actual velocity measured at any time: $V = k_2[ES]$ (conversion to product is slow step)
Maximum velocity measured at any time: $V_{max} = k_2[E]_{total}$ (all enzymes are bound)

Subbing in relationships:

$$\frac{\frac{Vmax[S]}{k_2}}{\frac{V}{k_2}} = K_m + [S]$$

$$V = \frac{Vmax[S]}{Km + [S]}$$ (proven :p lets go guys)