1 Enzymes

1.1 What is an Enzyme: Overview and Components

An Enzyme is a macromolecular biological catalysts that extraordinarily accelerate chemical reactions (10^6 fold). Enzymes possess a high degree of specificity. Enzymes are essential to the metabolism by conserving and transforming chemical energy as well as synthesizing biological macromolecules from simple precursors. Approximately 50% of drugs act by binding to enzymes.

Enzymes are either composed of either proteins or catalytically active RNA molecules. They can require additional components such as cofactors or helpers a.k.a. coenzymes.

1.1.1 Cofactors and Coenzymes

Cofactor: A cofactor is a chemical component, often an inorganic ion. **Coenzyme**: A Coenzyme is a complex organic or metallorganic molecules. Vitamins are precursors of coenzymes.

Ions	Enzymes
Cu^{2+}	Cytochrome oxidase
Fe^{2+} or Fe^{3+}	Cytochrome oxidase, catalase, peroxidase
K^{+}	Pyruvate kinase
Mg^{2+}	Hexokinase, glucose 6-phosphatase, pyruvate kinase
Mn^{2+}	Arginase, ribonucleotide reductase
Mo	Dinitrogenase
Ni^{2+}	Urease
Zn^{2+}	Carbonic anhydrase, alcohol dehydrogenase, carboxypeptidases A and B

(a) Cofactors

Coenzyme	Examples of chemical groups transferred	Dietary precursor in mammals
Biocytin	CO_2	Biotin (vitamin B_7)
Coenzyme A	Acyl groups	Pantothenic acid (vitamin B_5) and other compounds
5′-Deoxyadenosylcobalamin (coenzyme B_{12})	H atoms and alkyl groups	Vitamin B_{12}
Flavin adenine dinucleotide	Electrons	Riboflavin (vitamin B_2)
Lipoate	Electrons and acyl groups	Not required in diet
Nicotinamide adenine dinucleotide	Hydride ion ($:H^-$)	Nicotinic acid (niacin, vitamin B_3)
Pyridoxal phosphate	Amino groups	Pyridoxine (vitamin B_6)
Tetrahydrofolate	One-carbon groups	Folate (vitamin B_9)
Thiamine pyrophosphate	Aldehydes	Thiamine (vitamin B_1)

(b) Coenzymes

Figure 1: Examples of Cofactors and Coenzymes

1.1.2 Enzymes Classification

Enzymes are named by adding the suffix "-ase" to the name of their substrate or activity. The more formal version is assigning each enzyme a four-part classification number and a systematic name. **Systematic name**: Includes its precise activity and the substrates it works with (e.g., hexokinase is ATP:glucose phosphotransferase). **Classification number**: Four number code, using again Hexokinase (2.7.1.1) as an example:

i) Class name (2: Transferase)

ii) Subclass name (7: Phosphotransferase)

iii) The acceptor functional group (1: Phosphotransferase with hydroxyl group as acceptor)

iv) The accepting molecule (1: D-Glucose as acceptor molecule)

Class number	Class name	Type of reaction catalyzed
1	Oxidoreductases	Transfer of electrons (hydride ions or H atoms)
2	Transferases	Group transfer
3	Hydrolases	Hydrolysis (transfer of functional groups to water)
4	Lyases	Cleavage of C—C, C—O, C—N, or other bonds by elimination, leaving double bonds or rings, or addition of groups to double bonds
5	Isomerases	Transfer of groups within molecules to yield isomeric forms
6	Ligases	Formation of C—C, C—S, C—O, and C—N bonds

Figure 2: Examples of Cofactors and Coenzymes

1.2 The Thermodynamics and an Enzymes Role

Enzymes reduce the Activation Energy for a given reaction, hence changing the rate of the reaction. They do not influence K_{eq} or ΔG

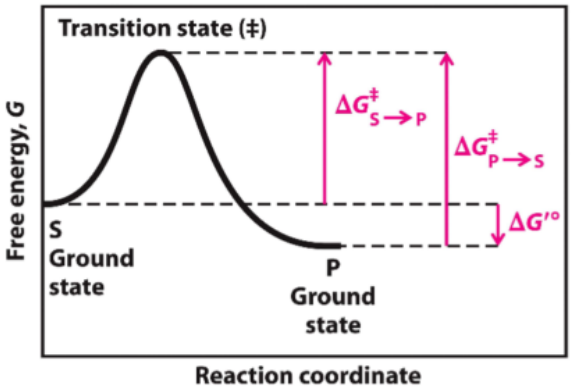


Figure 3: A reaction diagram

1.2.1 Transition State Theory

When plotting the progress of a reaction the Transition State is the very top of the hill. Note, that the **transition state $\neq$ Reaction Intermediate**. It should be understood as the moment in which the reaction is equally likely to progress towards substrate or product.

1.2.2 K_{eq}

The equilibrium constant K_{eq}, or K, is the reaction quotient at chemical equilibrium. K gives is calculated by:

$$K = \frac{[P]}{[S]} \tag{1}$$

An enzyme has no effect on K.

K'_{eq} is the K_{eq} at standard biochemical conditions (298K, pH = 7).

1.2.3 ΔG and $\Delta\ G^{\ddagger}$

The activation energy ($\Delta G^{\ddagger}$) is the difference between the ground state and the transition state.

ΔG° is the standard free energy under standard conditions (T = 298K, 1atm, 1M of each solute), while $\Delta G'^{\circ}$ is also at pH = 7.

1.2.4 The Relationship Between K_{eq} and ΔG

$$\Delta G^{\circ\prime} = -RT \ln K'_{\mathrm{eq}} \tag{2}$$

where, $R = 8.315\,\frac{\mathrm{J}}{\mathrm{mol \cdot K}}$ and $T = 298\,\mathrm{K}$.

Note that large K $\leftrightarrow$ very negative $\Delta G'^{\circ}$

K'_{eq}	$\Delta G'^{\circ}$ (kJ/mol)
10^{-6}	34.2
10^{-5}	28.5
10^{-4}	22.8
10^{-3}	17.1
10^{-2}	11.4
10^{-1}	5.7
1	0.0
10^{1}	−5.7
10^{2}	−11.4
10^{3}	−17.1

Figure 4: Some examples showing the relationship between G and K

1.2.5 Return Rate

The rate of a reaction is determined by the concentration of the reactants and the rate constant k.

For a unimolecular reaction we have that

$$v = k[S] \tag{3}$$

in which the reaction only depends on [S] and k has units s^{-1} and the following formula:

$$k = \frac{k'T}{h} * e^{-\frac{\Delta G^{\ddagger}}{RT}} \tag{4}$$

where k' is the Boltzmann constant and h the Plank constant. This means that the lower $\Delta G^{\ddagger}$ the faster the reaction goes. Since, enzymes lower $\Delta G^{\ddagger}$ it raises k and voila reaction is now speedy Gonzo.

TABLE 6–5 Some Rate Enhancements Produced by Enzymes

Cyclophilin	10^5
Carbonic anhydrase	10^7
Triose phosphate isomerase	10^9
Carboxypeptidase A	10^{11}
Phosphoglucomutase	10^{12}
Succinyl-CoA transferase	10^{13}
Urease	10^{14}
Orotidine monophosphate decarboxylase	10^{17}

Figure 5: Some Rate enhancements Produced by Enzymes

1.3 Enzymes' Role in the Reaction

To piece it all together an enzyme does the following:

- Has no effect on equilibrium related things; it does not influence G or K;
- Lowers the energy of $\Delta G‡$ and with it the activation energy;
- Hence creating a larger k and with that speeding up the rate of the reaction.

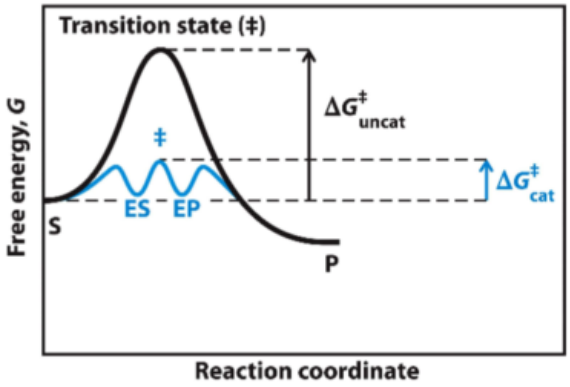


Figure 6: The Enzyme introduces a couple of new transition states (ES and EP), each of which is more stable than the original transition state

1.3.1 Catalytic Power and Mechanism

An enzyme has two ways to make a reaction faster:

i) Providing a **lower-energy reaction path**.

ii) Releasing energy through the **non-covalent binding** between the substrate and the enzyme. That energy is referred to as $\Delta \mathbf{G}_B$. This energy gain is a major driving force for the reaction to even happen

PSA: Your friendly reminder that the enzyme will remain unchanged when comparing beginning and end of reaction!

1.3.2 Active Site

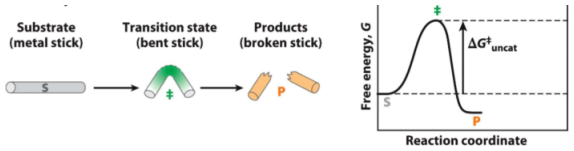
The active site is the place of the reaction. This site is lined with amino acids residue, which possess qualities to bind to the substrate and catalyze its transformation. Often times an enzymes will envelop a substrate separating it from the solution.

This binding is highly specific. This is where the lock and key principal comes into play. However it is slightly misleading as then the transition state would have to be more unfavorable than the substrate. Hence, it is more precise to see the **enzyme as very specific to the transition state** (which is till similar to the substrate).

1.3.3 Example for Transition State Complementarity and Rate Enhancement

Using the example of stickase we show the importance of the transition state complementarity, the fault of lock and key, and how that leads to rate enhancement.

No enzyme:



Enyzme which is complementary to the substrate:

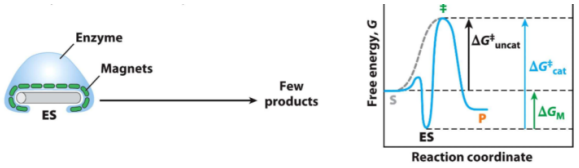


Figure 7: because the enzyme is complementary to the substrate it will stabilize the stick, making the ES state the most stable one and the required ΔG‡ larger than without an enzyme.

Enzyme which is complementary to the transition state:

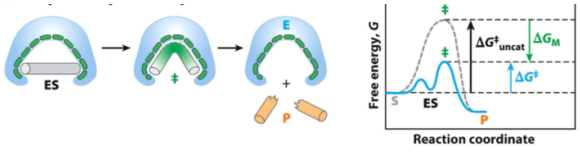


Figure 8: With the enzyme being complementary to the TS, the ES-state would still be more favorable, but not too favorable. More importantly the ΔG‡ would be lowered as the TS is significantly more stable.

1.3.4 Binding and Specificity

There are numerous not favorable physical and thermodynamical factors which contribute to ΔG‡, which need to be overcome:

i) **freedom through entropy**: The motion of molecules which reduces the possibility of proximity and hence reaction;

ii) **Solvation of the water shell**

iii) **Distortion of substrates**

iv) **proper alignment** of the catalytic functional groups on the enzyme

All these factors are overcome by the binding energy, which is released once ES enter the transition state. The need for this binding energy further enhances specificity. Here is how exactly the binding energy comes to be and is favorable:

i) **Entropy Reduction**: Through the binding of substrates to the enzyme, the freedom of motion of the substrates is significantly limited. This leads to the probability that they collide to react skyrocketing.

ii) **Desolvation**: Due to the binding of enzyme and substrate water molecules are are removed. While locally that means a slight decrease in entropy, overall it leads to an increase, making it energetically favorable.

iii) **Substrate Distortion**: The binding energy we get later on formed through the transition state and enzyme help compensate for any initial distortion, especially electronic redistribution.

iv) **Catalytic Group alignment**: As the substrate binds to the enzyme, the enzyme undergoes a so called induced fit, meaning it envelops the substrate in such a way that its functional groups can properly catalyze at the right position, as well as put the reaction sites of the substrates in the right position.

All these barriers and conditions to resolve them, make an enzyme very specific in which molecules it can catalyze. Those that work however, it is then able to create a huge rate enhancement.

1.4 Other Contributions to Enzyme Catalysis

Intermediates can often be very unstable, making them very unfavorable to get to (high $\Delta G‡$). Here are some ways enzymatic complexes overcome this and hence enhance reaction rates:

i) **Acid-Base Catalysis**: Some intermediates will be charged, which can lead to great instability. In order to stabilize them protonating/deprotonating can create a much more stable intermediate. Since water is rather weak, it will often be catalyzed with an amino acid residue which has acid or base properties.

ii) **Covalent Catalysis**: An intermediate is formed in which a bond is formed between a substrate and the enzyme. This only happens, if the new pathway has lower $\Delta G‡$. A further condition is the nucelophilic properties of the enzyme, which several amino acid residues and some cofactors possess. Of course they then proceed to go further reaction freeing them back up.

iii) **Metal Ion Catalysis**: Metals can participate in catalysis in numerous ways. Ionic interactions can stabilize or orient charged reaction transition states. Its effects are similar to the enzyme-substrate binding energy of above. Metals can also mediate redox reaction through reversible changes in their oxidation state. Nearly a third of all enzymes require metal ions.

1.5 Enzyme Kinetics

We want to understand the role of enzyme mechanisms rate of a reaction. In particular how it changes in respect to changes in experimental parameters. This is called enzyme kinetics. The main factor affecting the rate is the substrate [S].

Studying the effects of [S] is pretty tough because it is constantly changing. Instead an easier approach is to study the initial velocity (V0).

1.5.1 v_0

To find V0 Because we take a look at the beginning of the reaction we can assume that the amount of product is negligible.

V0 increases nearly linearly with an increase in [S], at low [S] concentrations:

$$[S]_t = [S]_0 - [P]_t V_0 = k[S]_0 \tag{5}$$

However, as [S] increases the change in V0 becomes smaller and smaller until we reach a point where it starts to plateau. That plateau it approaches is V_{max}.

The intuitive explanation: It's all about **Enzyme Saturation**; intitally when the enzymes aren't saturated every new [S] can immediately be turned into product, leading to the linear increase in V0. However, once the system starts to be saturated that effect diminishes, all the way until where the enzymes are fully saturated which leads us to approach V_{max}.

1.5.2 ES Complex and v_0

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\rightarrow} E + P \tag{6}$$

Looking at this equation, we can see that if the step from [ES] to [E] + [P] is rate limiting than that is what determines V0. So this gives us the equation:

$$V0 = k_2[ES] \tag{7}$$

1.5.3 Michaelis-Menten - Derivation and Conclusions

The Michaelis-Menten equation:

$$V0 = \frac{V_{\max}[S]}{K_m + [S]}$$

Before getting into the derivation here are the core things to take away:

i) **Steady state assumption**: We assume that V0 reflects a condition where [ES] is constant, that means that [ES] produced = [ES] breakdown. If we don't do this Michaelis-Menten falls apart, basically this is assuming that enzymes will immediately take up a new substrate when they leave the [ES] complex.

ii) S « $K_M \rightarrow$ V0 = k[S]

iii) S » $K_M \rightarrow$ V0 = V_{Max}

iv) V0 = $\frac{V_{Max}}{2} \rightarrow K_M$ = [S]

One important variation of the MM equation is the following where we substitute Vmax by kcat (basically the max turnover rate) and the total enzyme concentration.

$$V_{\max} = k_{\text{cat}} \cdot [E]_T \tag{8}$$

$$V_0 = \frac{k_{\text{cat}}[E]_T[S]}{K_m + [S]} \tag{9}$$

Now, the actual derivation:

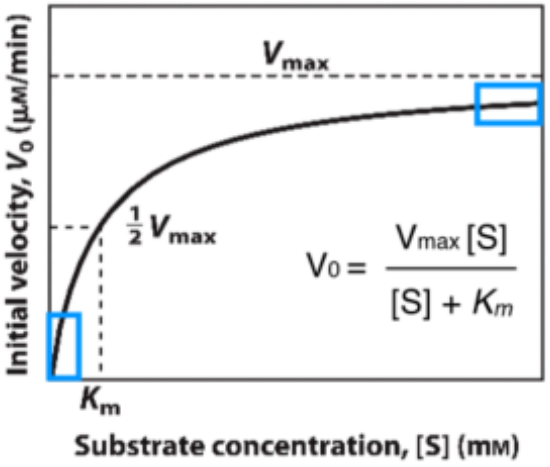


Figure 9: The blue box bottom left is when S « K_M, while the top right is when S » K_M

$$\mathrm{E} + \mathrm{S} \rightleftharpoons \mathrm{ES} \xrightarrow{k_2} \mathrm{E} + \mathrm{P}$$

$$\text{Rate of formation of [ES]} = k_1([E]_{\text{tot}} - [ES])[S]$$

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

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

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

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

$$[ES] = \frac{k_1[E]_{\text{tot}}[S]}{k_1[S] + k_{-1} + k_2}$$

$$\textbf{Define } K_m = \frac{k_{-1} + k_2}{k_1} \Rightarrow [ES] = \frac{[E]_{\text{tot}}[S]}{[S] + K_m}$$

$$V_0 = k_2[ES] = \frac{k_2[E]_{\text{tot}}[S]}{[S] + K_m}$$

$$V_{\max} = k_2[E]_{\text{tot}} \Rightarrow V_0 = \frac{V_{\max}[S]}{[S] + K_m}$$

1.5.4 Lineweaver-Burk

Due to the asymptotic behavior of V0, it's difficult to determine K_M and V_{max} from it. Hence we create a double-reciprocal plot, which is linear, called the Lineweaver-Burk equation:

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}} \tag{10}$$

where:

- $m = \frac{K_m}{V_{\max}}$ is the slope

- $b = \frac{1}{V_{\max}}$ is the y-intercept
- $c = -\dfrac{1}{K_m}$ is the x-intercept

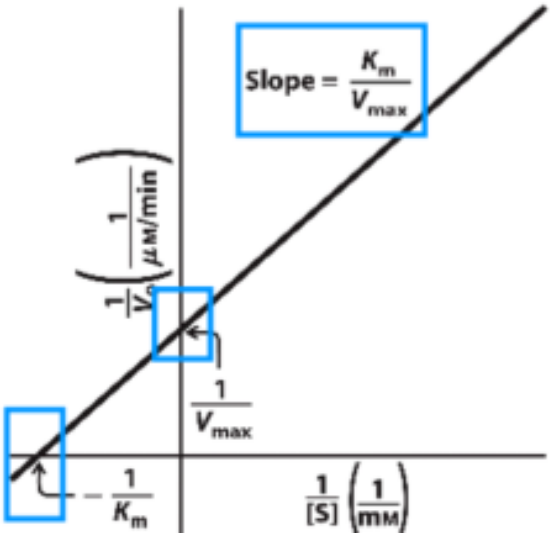


Figure 10: The blue boxes highlight the slope, y-intercept, and x-intercept of the Lineweaver-Burk equation. Those spots give are what make it so easy to find K_M and V_{max}

Derivation of the Lineweaver-Burk equation:
We start with the Michaelis–Menten equation:

$$v = \frac{V_{\max}[S]}{K_m + [S]} \tag{11}$$

To linearize this, we take the reciprocal of both sides:

$$\frac{1}{v} = \frac{1}{\frac{V_{\max}[S]}{K_m + [S]}} \tag{12}$$

By inverting the fraction on the right-hand side:

$$\frac{1}{v} = \frac{K_m + [S]}{V_{\max}[S]} \tag{13}$$

Now split the numerator:

$$\frac{1}{v} = \frac{K_m}{V_{\max}[S]} + \frac{[S]}{V_{\max}[S]} \tag{14}$$

Which leaves us with the Lineweaver-Burk equation:

$$\frac{1}{v} = \frac{K_m}{V_{\max}} \cdot \frac{1}{[S]} + \frac{1}{V_{\max}} \tag{15}$$

1.5.5 K_m and k_{cat}

The meaning of K_m can vary greatly between different enzymes and even substrates.

One possible meaning for K_m can be the following: if $k_2 \ll k_{-1}$ then K_m essentially boils down to the following:

$$K_m = \frac{k_{-1}}{k_1} = K_D \tag{16}$$

So, for the case of $k_2 \ll k_{-1}$ K_m tells us the affinity of a certain enzyme for its substrate. Note, that this is however only true in this one special case!

k_{cat} **is the rate constant at the rate-limiting step**. This will often be k_2, however that is not always the case (especially if we have more than two reaction steps).

1.5.6 Determining Enzyme Efficiency

The best way to determine the efficiency of an enzyme is by determining the following ratio:

$$\frac{k_{\text{cat}}}{K_M} \tag{17}$$

There is an upper limit to this ratio, set by the rate at which E and S can diffuse together. The **diffusion-controlled limit is** 10^8 **to** $10^9 M^{-1}s^{-1}$. Enzyme which have values close to this have achieved catalytic perfection.

PSA: this seems overkill from the lecture but there's a whole slide of it, so here is how we get the units by starting with the MM equation:

$$v = \frac{k_{\text{cat}}[E]_T[S]}{K_m + [S]} \tag{18}$$

When $[S] \ll K_m$, the equation simplifies to:

$$v \approx \frac{k_{\text{cat}}}{K_m}[E]_T[S] \tag{19}$$

This resembles a second-order rate law: first order in both enzyme and substrate concentrations.

Units:

- k_{cat}: has units of s^{-1} (per second)
- K_m: has units of M (molar, i.e., mol/L)

Thus, the specificity constant has units:

$$\frac{k_{\text{cat}}}{K_m} = \frac{1\,\text{s}^{-1}}{1\,\text{M}} = \text{M}^{-1}\text{s}^{-1} \tag{20}$$

1.5.7 V_{max}

V_{max} is the maximum initial velocity, and with that also the maximum velocity, that is attainable for a certain enzyme/substrate duo.

The equation for V_{max} is the following:

$$V_{\text{max}} = k_{\text{cat}} \cdot [E]_T \tag{21}$$

This equation comes from the fact we take the rate-limiting's step rate constant. Multiplying that with the enzyme concentration gives us the speed the enzymes can convert the substrate with at the choke point.

TABLE 6–6 K_m for Some Enzymes and Substrates

Enzyme	Substrate	K_m (mM)
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

(a) Km

TABLE 6–7 Turnover Number, k_{cat}, of Some Enzyme

Enzyme	Substrate	k_{cat} (s^{-1})
Catalase	H_2O_2	40,000,000
Carbonic anhydrase	HCO_3^-	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.5

(b) kcat

TABLE 6–8 Enzymes for Which k_{cat}/K_m Is Close to the Diffusion-Controlled Limit (10^8 to 10^9 $M^{-1}s^{-1}$)

Enzyme	Substrate	K_{cat} (s^{-1})	K_m (M)	K_{cat}/K_m ($M^{-1}s^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	1.2×10^{-2}	8.3×10^7
	HCO_3^-	4×10^5	2.6×10^{-2}	1.5×10^7
Catalase	H_2O_2	4×10^7	1.1×10^0	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-6}	2.8×10^8
Fumarase	Fumarate	8×10^2	5×10^{-6}	1.6×10^8
	Malate	9×10^3	2.5×10^{-4}	3.6×10^7
β-Lactamase	Benzylpenicillin	2.0×10^3	2×10^{-6}	1×10^9

Source: Fersht, A. (1999) *Structure and Mechanism in Protein Science*, p. 166, W. H. Freeman and Company, New York.

(c) limit

Figure 11: Examples of rate for K_m, k_{cat}, and their ratio determining the enzyme's efficiency.

1.6 Inhibition

Inhibitors are molecules that interfere with catalysis, decreasing or halting enzyme activity. They are critical for drug design, as nearly 50% of all pharmaceutical agents are enzyme inhibitors. Inhibitors are classified into two main types:

- Reversible Inhibitors: Interact transiently with the enzyme.
- Irreversible Inhibitors: Form a covalent bond or permanently alter the enzyme.

1.6.1 Reversible Inhibition

Reversible inhibitors can act by competitive, non-competitive and uncompetitive mechanisms. The inhibition constant K_I quantities the strength of inhibition in blocking the activity of an enzyme. To take this into account in kinetics, the factor α and α' are used, where α is derived from K_I (binding to enzyme) and α' from K_I' (binding to enzyme-substrate complex).

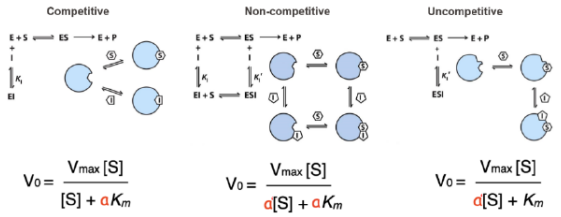


Figure 12: Types of Reversible Inhibition

- **Competitive Inhibitor**: The inhibitor competes with the substrate for the enzyme's active site. This increases K_m but does not affect V_{max}.

- **Non-competitive Inhibitor**: The inhibitor binds to a site other than the active site, reducing the concentration of the active enzyme-substrate complex. This decreases V_{max}, but does not affect K_m.
- **Uncompetitive Inhibitor**: The inhibitor binds only to the enzyme-substrate complex, reducing the concentration of the active enzyme-substrate complex. Both V_{max} and K_m decrease.

Figure 13: Impact of different Reversible Inhibition Types

1.6.2 Irreversible Inhibition

Irreversible inhibitors form covalent bonds with the enzyme, **permanently inactivating** it. Here are two types of irreversible inhibitors, their function, and usage:

- **Suicide Inhibition**: Also known as mechanism-based inactivators, these molecules are not reactive until they reach the active site, where they then go through the first steps of the reaction. At some point the inactivator becomes so reactive in a transition state it reacts irreversibly with the enzyme. Because these compounds are so highly specifiic, and passive otherwise, they often have little side effects.
- **Transition-State Analogs**: Molecules which are very similar to a transition-state. They bind significantly better to the enzyme and its active state than the substrate, making them an irreversible inhibitor.

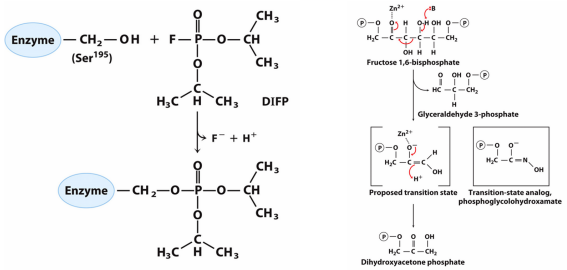


Figure 14: Mechanism of different types of inhibition; on the left is a suicide mechanism and on the right a transition-state analog.