Enzyme kinetics and inhibition

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Enzyme Kinetic

- Enzyme kinetic is the **quantative study of Enzyme catalysis**.
- Kinetic studies measures rates and affinity of enzyme for substrate and inhibitors.
- Kinetics also provide insight into reaction mechanism.
- The general principles of chemical reaction apply to enzyme catalyzed reaction as well

- To understand enzymes function, we need a kinetic description of their activity
- For many enzymes the rate of catalysis Vo, which is defined as the number of moles of product formed per second, varies with the substrate concentration[S]
- Different theories, Models and plots have been proposed by various scientist to study the kinetics of enzyme

Models to Study Enzymes Kinetics

- Michaelis and Menten equations
- Lineweaver-Burk Plot
- Hanes-woolf plot
- Eadie-Hofstee plot

Study of Kinetics

- To study Kinetics we will analyzing two major plots:
- **Michaelis-Menten** and **Lineweaver-Burk**
- **Michaelis-Menten plot**.
- In order to generate a Michaelis-Menten plot, experimenters use two very specific conditions:
- **Enzyme concentration is held constant**
- **Substrate concentration is increased**

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

v = velocity of reaction

$V_{\max}$ = maximum rate achieved by the system

$[S]$ = concentration of a substrate S

K_M = Michaelis constant

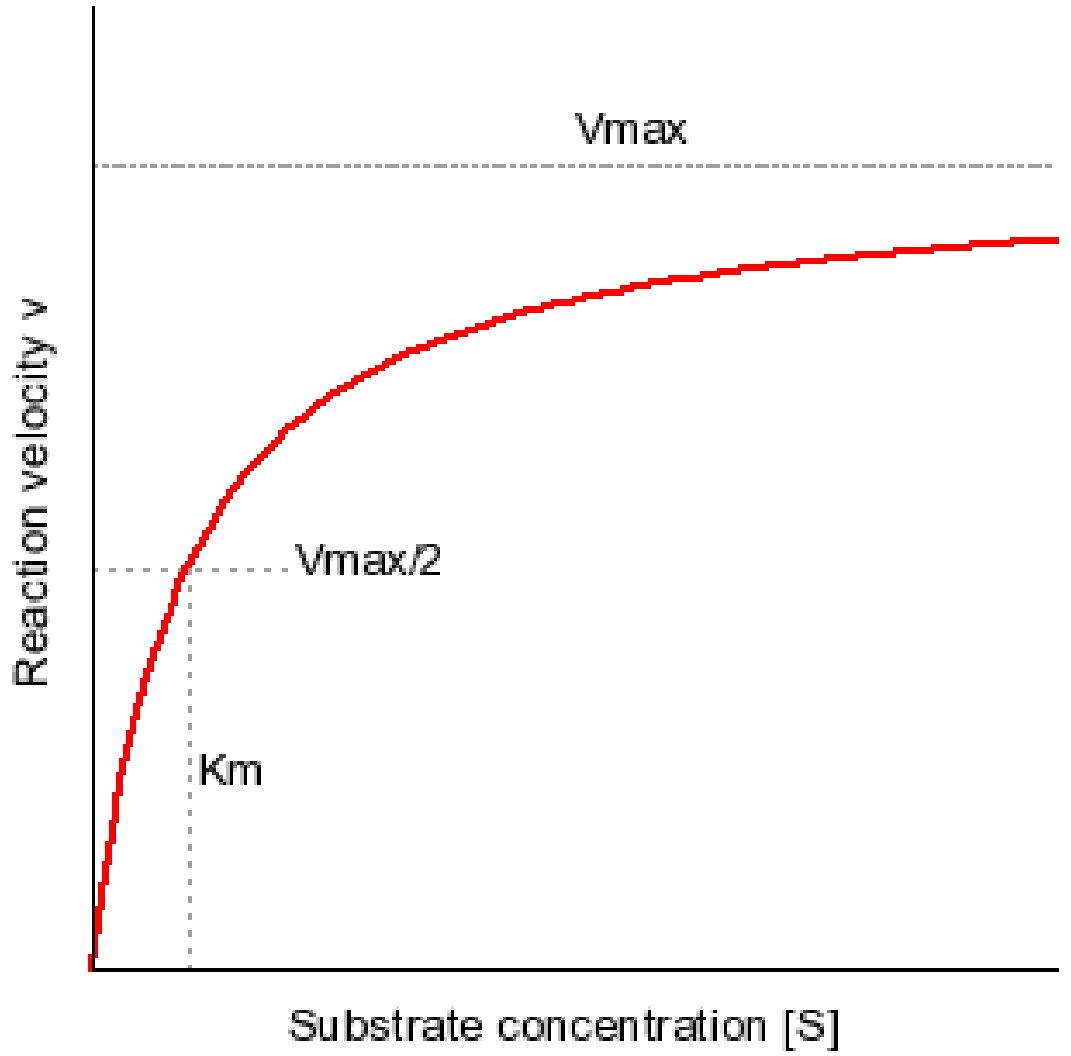
Michaelis Menten Equation

Important Terms

- The two important values that is needed to keep in mind are **V max and Km ,** as substrate concentration increases along the x-axis,
- We define **V max as the maximum speed at which the reaction can proceed** given the current concentration of enzyme and the speed with which the enzymes work.

Michaelis Menten Plot

- It shows that the maximal velocity (Vmax) is approached asymptotically
- (Km)is the substrate concentration yielding a velocity of Vmax/2



- Finally, let's look at Km on the Michaelis-Menten plot, which is defined as the substrate concentration at ½ Vmax.
- We can think of **Km as the binding affinity of the substrate for the enzyme.** Naturally, if the substrate can bind the enzyme with a higher affinity, it is more likely to be "captured" by the enzyme and converted into product. **A small Km indicates a high substrate affinity, and a large Km indicates a low substrate affinity.**
- Catalytic efficiency describes how effective an enzyme is at converting substrate to product, and it is defined as:
- **Catalytic efficiency = kcat/Km**
- **k cat describes the speed of an individual enzyme**, so the higher the speed, the better the efficiency.
- **Km describes the affinity of the enzyme to the substrate, so the lower the Km , the better the efficiency.**

Significance of Km

- Km is the **concentration of substrate at which half the active sites are filled.**
- It provides a measure of the substrate concentration required for significant catalysis to occur
- Consider the following equation $E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \overset{k_2}{\Longrightarrow} E + P$

  Km=k-1+k2/k1

- Consider a case in which k1>>>k2,
- under such circumstances, the ES →E+S much more rapidly than product is formed. So,
- Km=k-1/k1
- When this condition is met, **Km is a measure of the strength of the ES complex:** a **high Km indicates weak binding; a low Km indicates strong binding**

Significance of Vmax

- The maximal rate, Vmax reveals the **turnover number** of an enzyme.
- It is the **number of substrate molecule in a unit time when the enzyme is fully saturated with substrate.**
- The maximal rate, Vmax, reveals the turnover number of an enzyme if the concentration of active site [E]T is known

$$\mathbf{V_{max}=k_2[E]_T}$$

- The enzyme efficiency can be increased as **Kcat has high turnover** and a small number of Km

Turn over number

- It is the **number of substrate molecules converted into product by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.** Represented by Kcat, Unit is S-1.
- At saturating [S]

Kcat=Vmax/Et

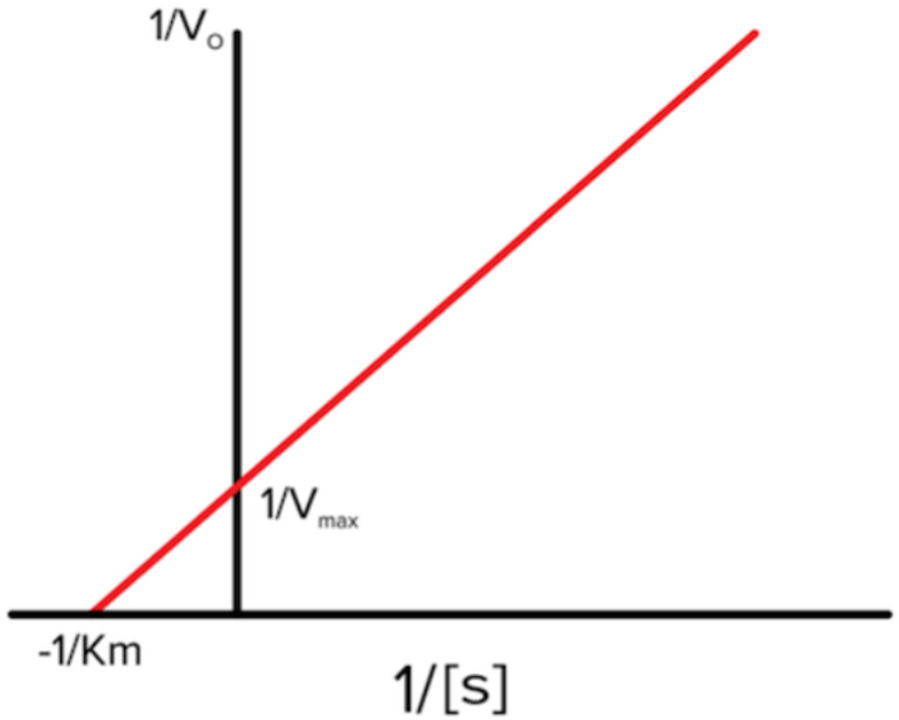
Catalytic efficiency

- **Kcat/Km** ratio called **Specificity constant** is often thought of a measure of **Catalytic efficiency.**
- A comparison of specificity constant for the same enzyme with different substrate is widely used as a measure of catalytic efficiency.

Lineweaver-Burk plot

- Lineweaver-Burk plots show the **exact same data as Michaelis-Menten plots,** but they are simply graphed in a different way by taking the reciprocal of each axis on the Michaelis-Menten plot.
- The x-axis changes from [S] on the Michaelis-Menten plot to 1/[S] on the Lineweaver-Burk plot, and the y-axis changes from V0 on the Michaelis-Menten plot to 1/V0 on the Lineweaver-Burk plot.

Lineweaver Burk Plot



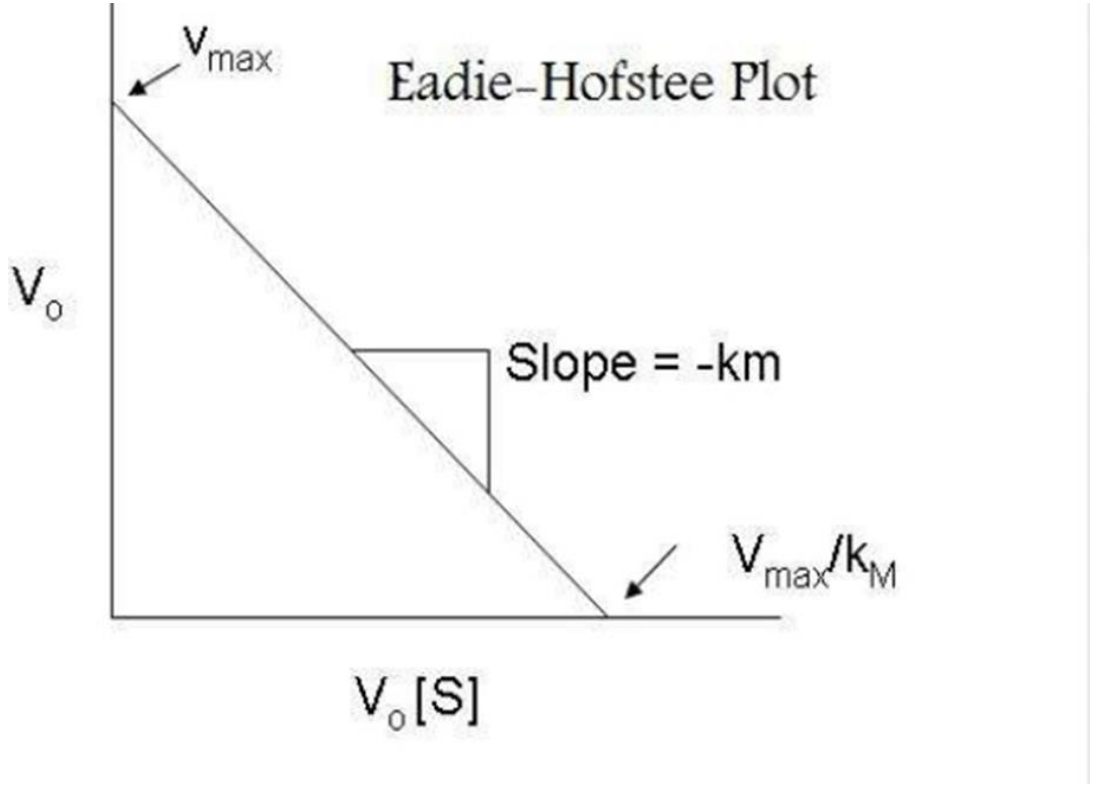
LINEWEAVER-BURK PLOT

Significance of Line weaver Burk Plot

- For determining inhibition

Eadie-Hofstee plot

- The Eadie-Hofstee plot is a more accurate **linear plotting method with v is plotted against v/[S].**
- A plot of v against v/[S] will hence yield Vmax as the y-intercept, Vmax/Km as the x-intercept, and Km as the negative slope



Hanes-Woolf plot

- The Hanes-Woolf plot is another method that linearizes the Michaelis-Menten equation, plotting [S] (x-axis) against [S]/v (y-axis).
- The Hanes-Woolf plot is thought to be **more accurate than Lineweaver-Burk** for the determination of kinetic parameters.

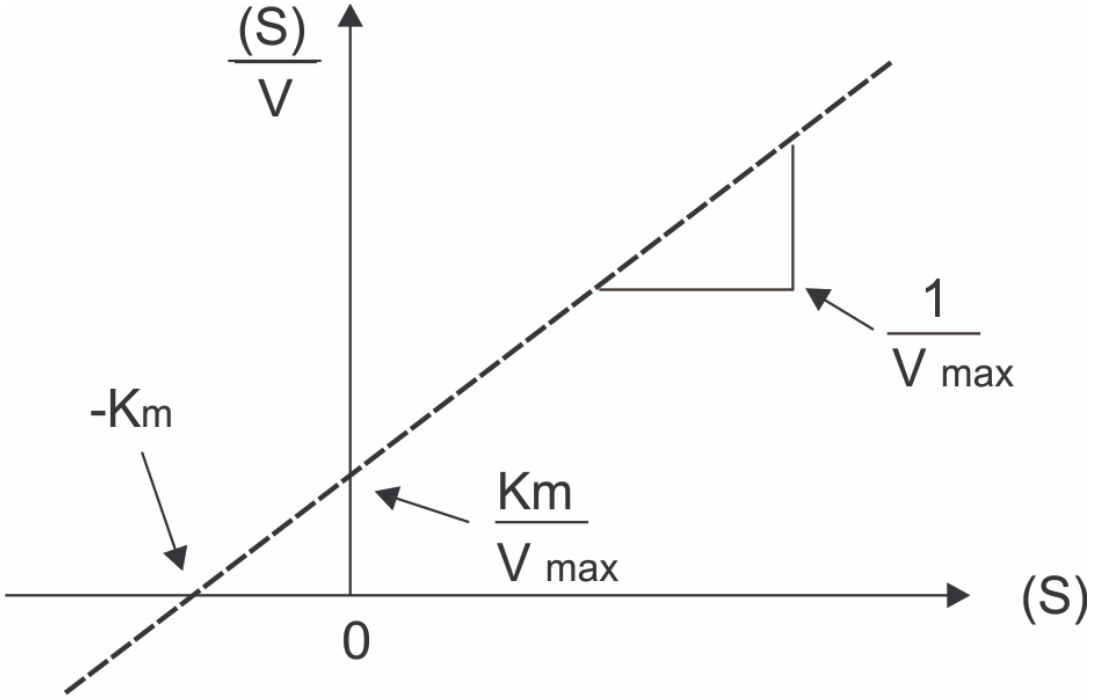


Fig. : Hanes Woalf plot

Enzyme Inhibition

- Certain compounds **inhibit enzymes** i.e., **decrease the rate of their catalysis**
- Inhibition can be **reversible or irreversible**
- Three types of **reversible inhibitors**
    i. **Competitive inhibitors**
    ii. Non-Competitive inhibitors
    iii. Un-competitive inhibitors
- **Irreversible inhibition**: suicide inhibitors
- The various types of inhibitors can be distinguished by the kinetics of their inhibition

Competitive inhibition

- In competitive inhibition, **an inhibitor binds to the enzyme's active site, which prevents the substrate from binding.** As the amount of substrate is increased, however, the effect of the inhibitor decreases, and Vmax can be reached.
- If the inhibitor is potentially blocking the binding site, the substrate will not be able to bind as often. Therefore, the binding affinity will become worse, so Km will increase.
- Finally, the **Lineweaver-Burk plot will look like the following in the presence of a competitive inhibitor**

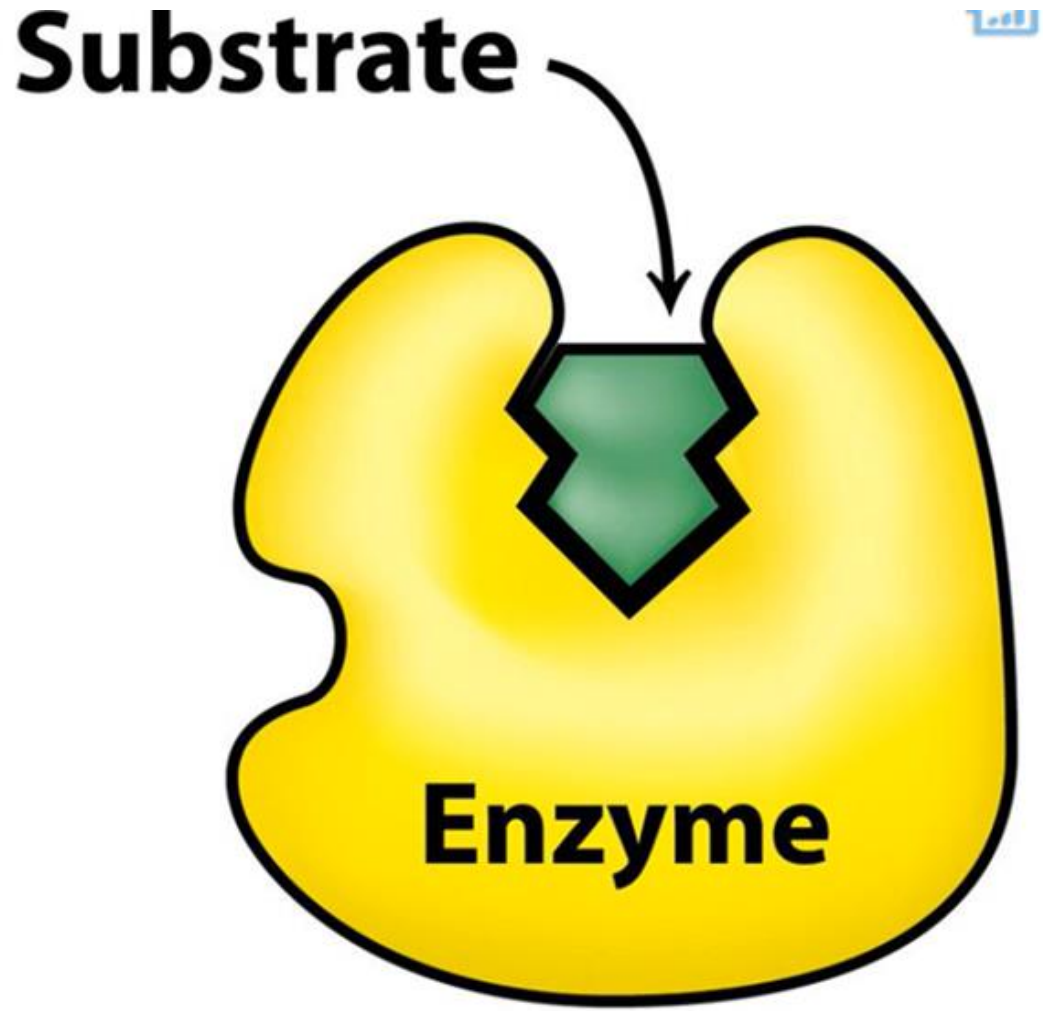


Figure 8-15a
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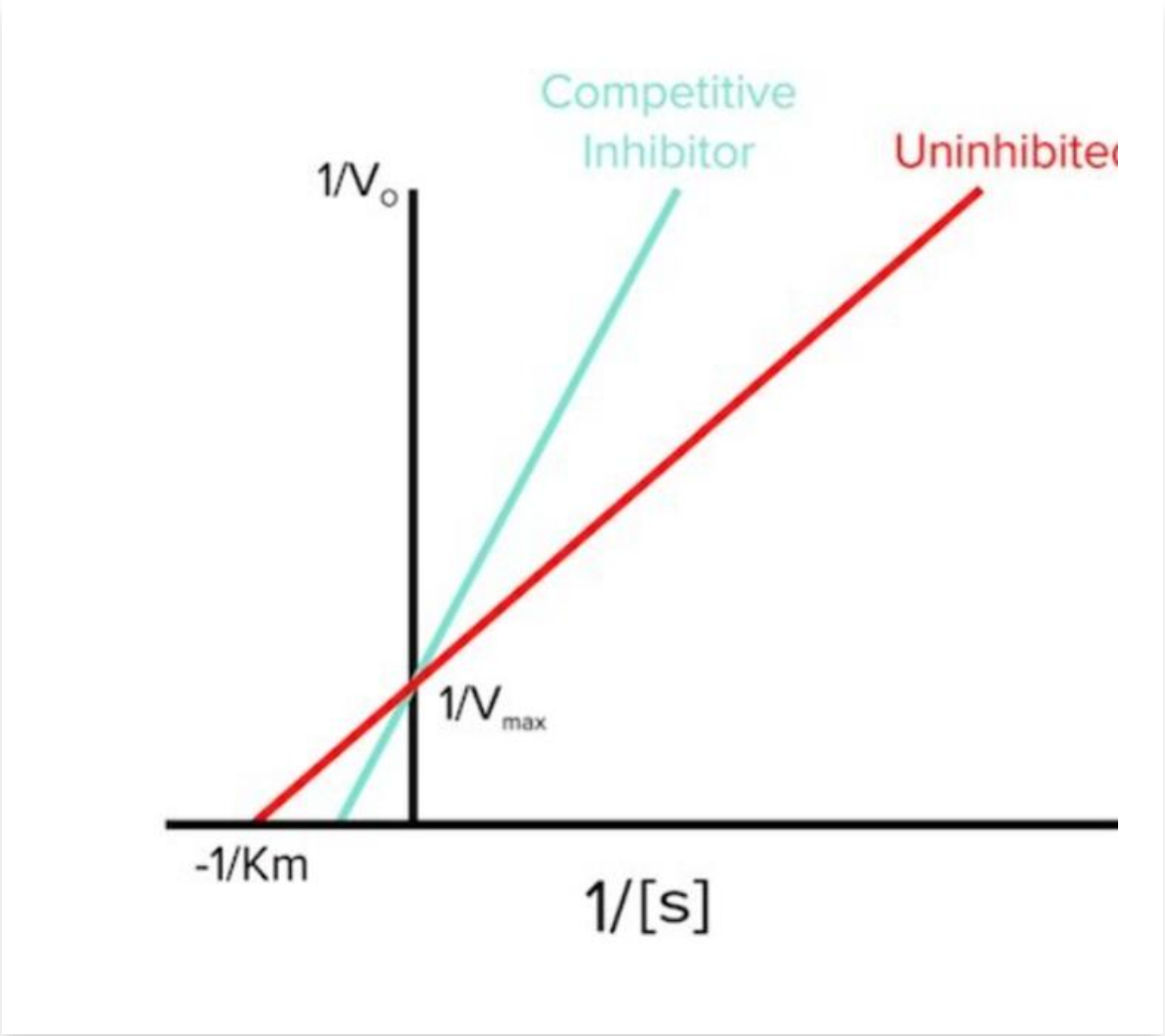
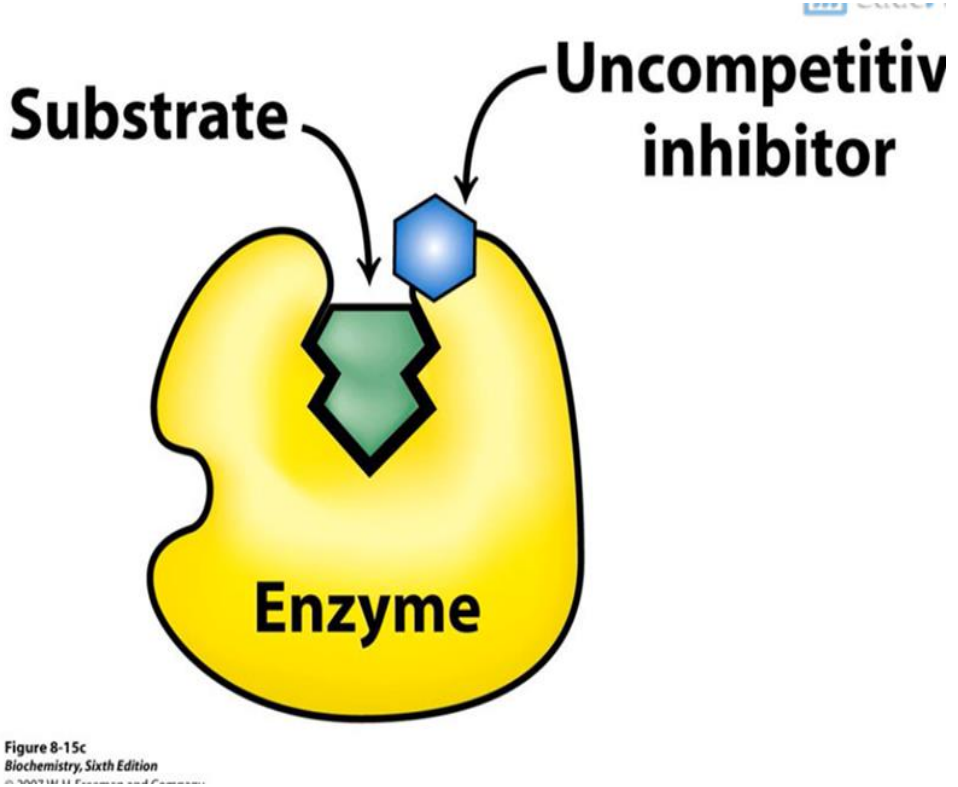
Plot for Competitive inhibitor



Uncompetitive Inhibition

- In uncompetitive inhibition, the **inhibitor binds selectively to the enzyme-substrate (ES) complex**. Now, even if we add more substrate, the enzyme will not be able to work as fast because the enzyme-substrate complex has been bound by the inhibitor. As a result, Vmax will decrease.
- As inhibitors bind the enzyme-substrate complex, the amount of uninhibited enzyme-substrate complex decreases. This decrease in enzyme-substrate complex causes an increase in the affinity between the substrate and the enzyme as they now need to replenish the lost enzyme-substrate complex (according to Le Chatelier's principle)! So, the increase in affinity means that Km will decrease.
- The Lineweaver-Burk plot will look like the following in the presence of an uncompetitive inhibitor



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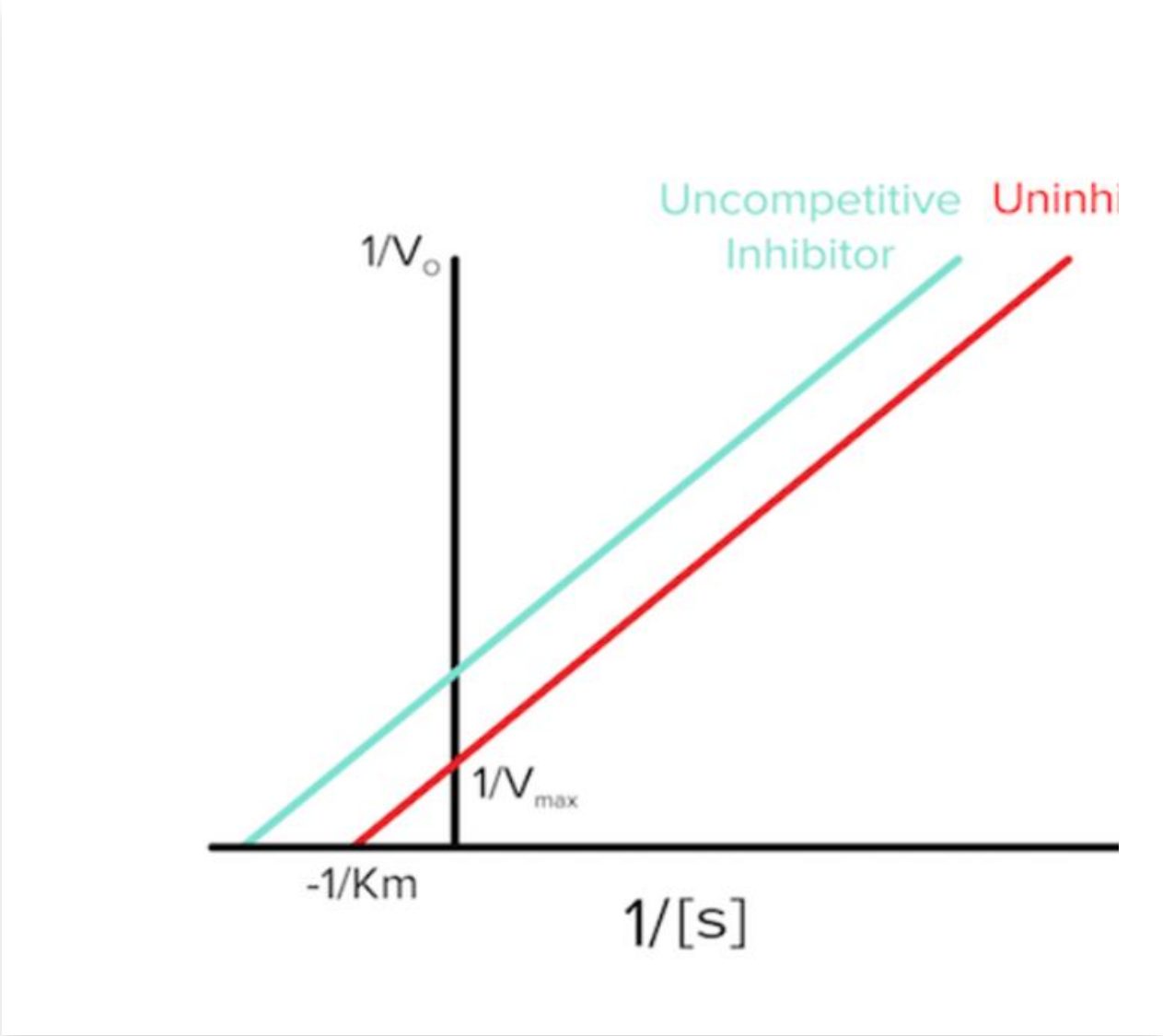
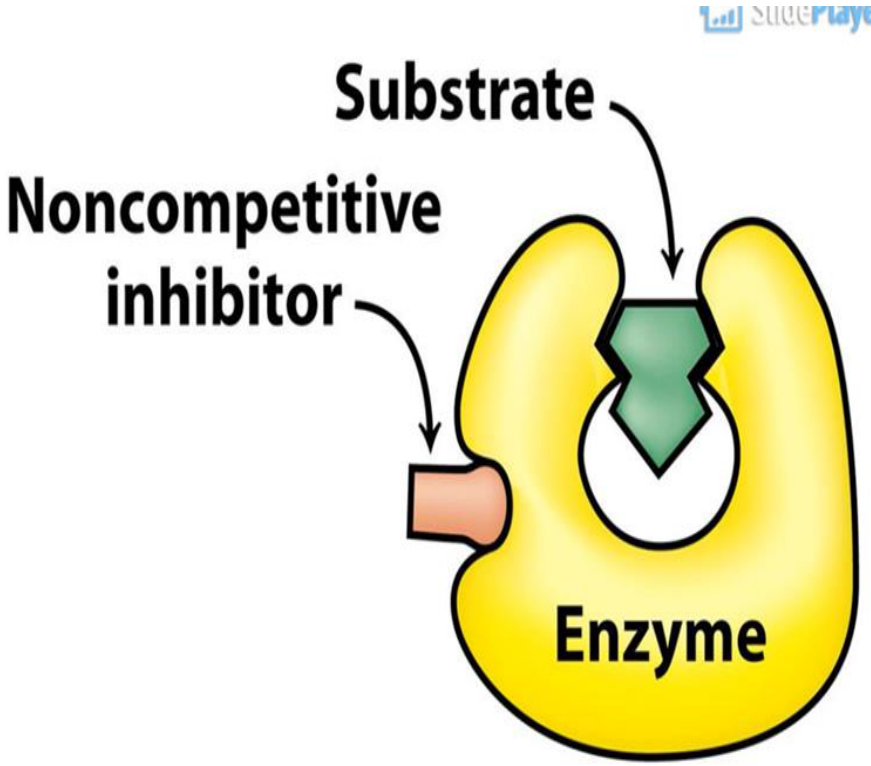
Uncompetitive Inhibition



Mixed and noncompetitive inhibition



- In mixed inhibition, the **inhibitor binds to an allosteric site, or a non-active site regulatory pocket**, on both the free enzyme and the enzyme-substrate complex. Many times, however, this binding is biased towards one or the other. For example, 70% of the inhibitors might bind the enzyme alone and 30% might bind the enzyme-substrate complex. As a result, mixed inhibition can be quite complicated.
- There is a special case, though, in which 50% of the inhibitors bind the enzyme alone and 50% bind the enzyme-substrate complex, and this is known as noncompetitive inhibition. Again, the inhibitor is binding an allosteric site on the enzyme as with all mixed inhibitors.
- Since the enzyme-substrate complex is being bound by the inhibitor, Vmax must decrease as we previously proved for uncompetitive inhibition. Interestingly, since both the enzyme alone and the enzyme-substrate complex are bound with equal affinity by the inhibitor, Km will stay the same.
- The Lineweaver-Burk plot for noncompetitive inhibition will look like the following

Noncompetetive Inhibitor

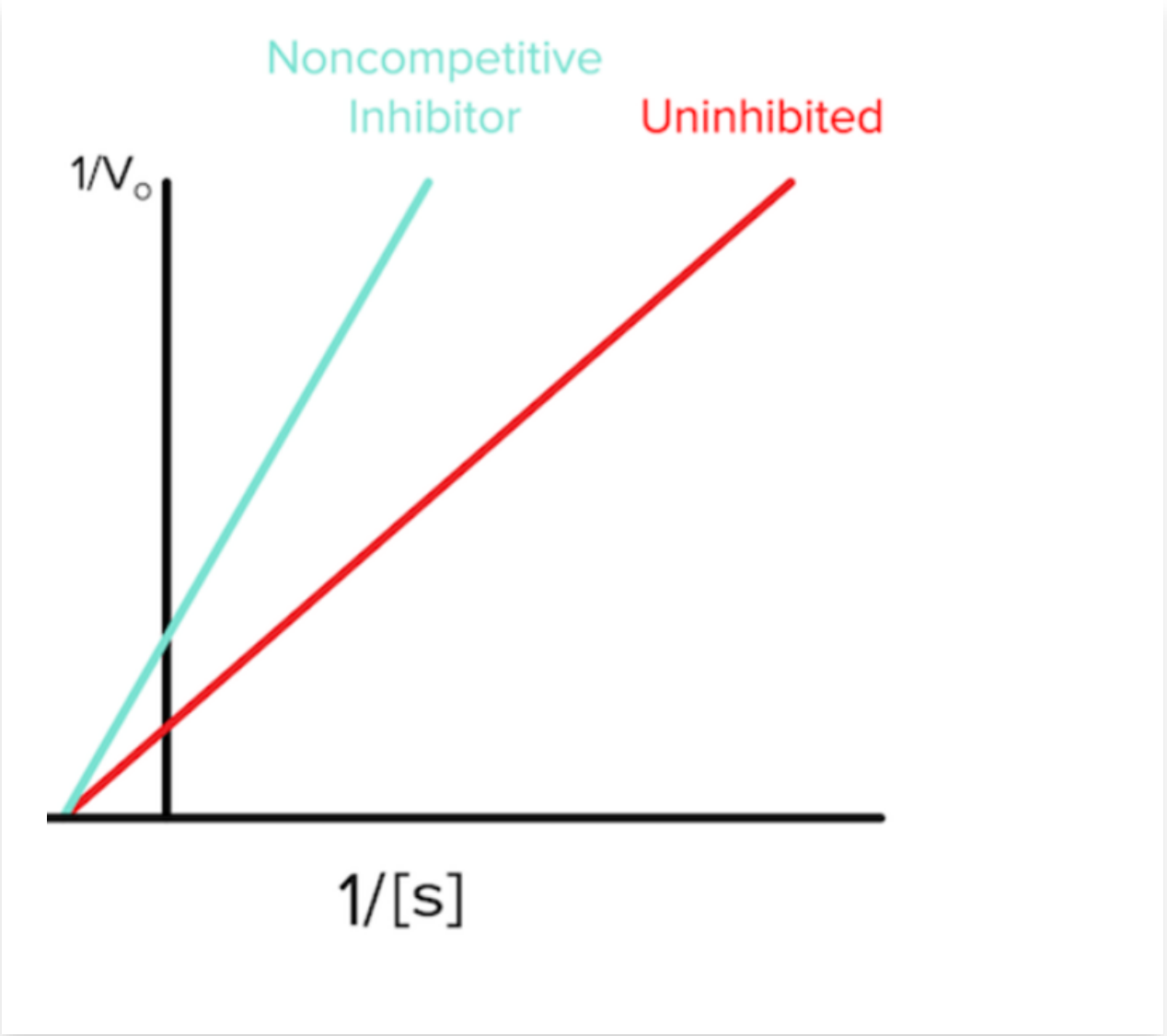


Table to summarize Enzyme Inhibition

	V_{max}	K_m	Binding site
Competitive	Same	Increases	Active
Uncompetitive	Decreases	Decreases	Allosteric
Mixed	Decreases	Increases or decreases	Allosteric
Noncompetitive	Decreases	Same	Allosteric

References

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- Voet, D Voet, J.G. and Pratt, C.W.2013. Fundamentals of Biochemistry: Life at molecular level
- Berg, J.M., Tymocozko, J.L.and Stryer, L., 2012. Biochemistry

Thank You