



Enzymes II

Kinetics

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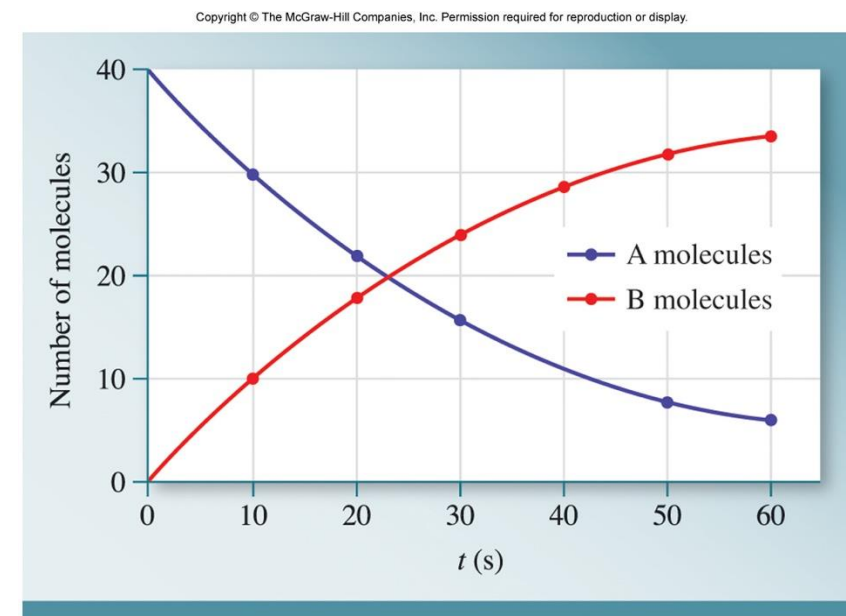
Kinetics

Understand
Do not memorize

- Kinetics deals with the rates of chemical reactions.
- Enzyme kinetics is the study of the rates of enzymatic reactions.
- For the reaction ($A \rightarrow B$), velocity (v) or rate of reaction is the amount of B formed (or the amount of A consumed) per unit time, t . That is,

$$\text{Rate of reaction (velocity or } v) = -\frac{\Delta [A]}{\Delta t} \quad \text{or} \quad \frac{\Delta [B]}{\Delta t} = -k[A] = k[B]$$

- This is known as the rate law, which describes how concentrations of reactants affect the rate of the reaction during a certain period.
- Note: the rate is proportional to the concentration of A, and k is the rate constant.
 - k has the units of $(\text{time})^{-1}$, usually sec^{-1} .





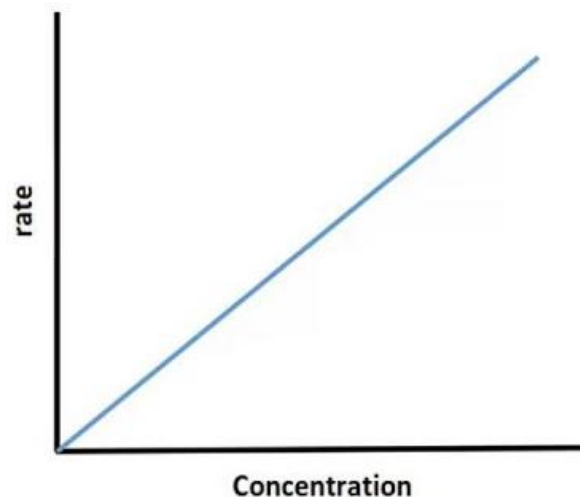
If $(A \rightarrow B)$ is

A first-order reaction

$$\text{rate} = k[A]$$

- The rate of a reaction increases linearly with increasing substrate concentration.

First order



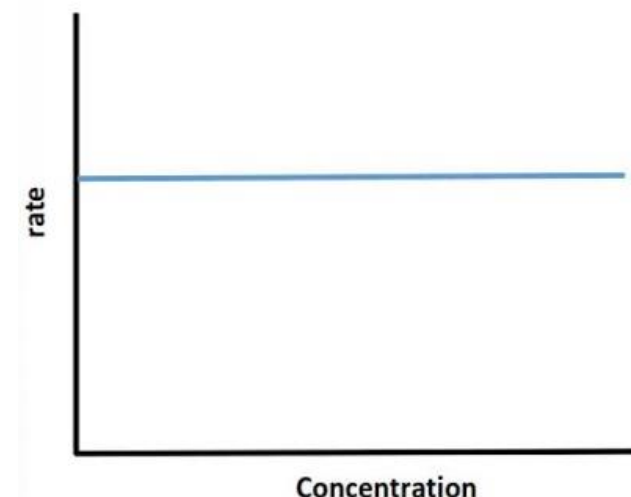
$$\text{rate} = k[A]$$

A zero-order reaction

$$\text{rate} = k[A]^0 = k$$

- The rate of the reaction is independent of substrates.

Zero order



$$\text{rate} = k$$



Rate of reaction (velocity)

- Rate of reaction is calculated as concentration of substrate disappearing (or concentration of product appearing) per unit time ($\text{mol L}^{-1} \cdot \text{sec}^{-1}$ or $\text{M} \cdot \text{sec}^{-1}$).

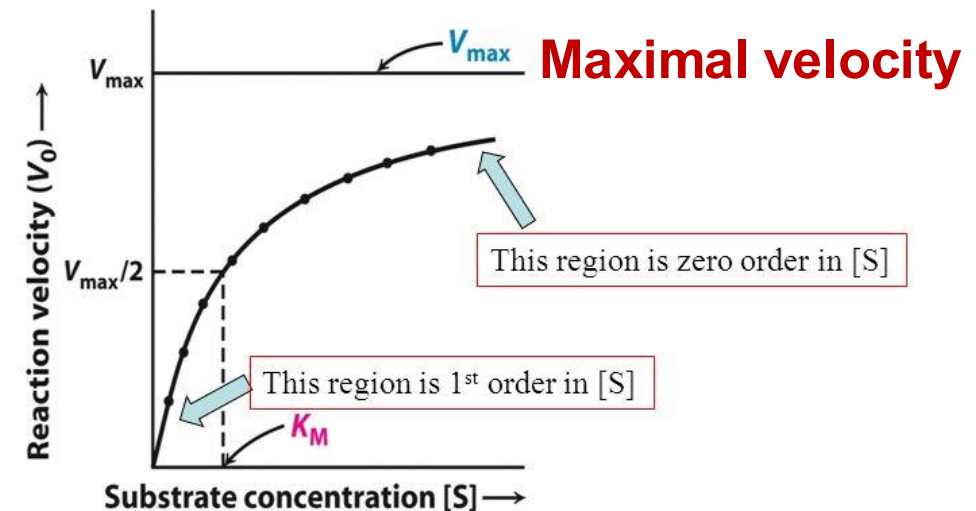
Remember: Concentration (M) = mol / vol



Enzyme kinetics

- Enzyme-catalyzed reactions have hyperbolic plots.
- Initial velocity (V_0) varies with the substrate concentration $[S]$ where the rate of catalysis rises linearly as the substrate concentration increases and then levels off and approaches a constant, maximal velocity (V_{max}) at very high substrate concentrations.

A commonly observed behavior for enzyme catalyzed reactions showing the change in V_0 as $[S]$ is changed



This behavior can be described mathematically by the Michaelis-Menten equation

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



Why?

- The hyperbolic plot is known as a saturation plot because the enzyme becomes "saturated" with the substrate, i.e., each enzyme molecule has a substrate molecule associated with it.

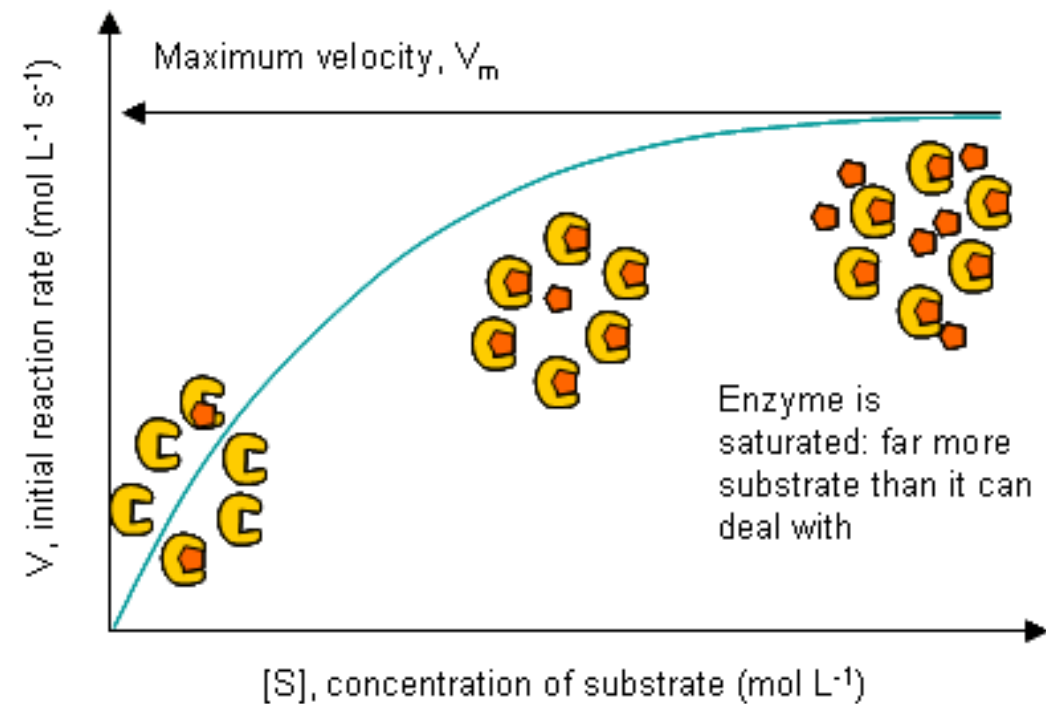
What is the limiting factor of the speed of a car? The car or the road?



When the road (substrate) is smooth, the speed of the car (enzyme) depends on the car, not the road.



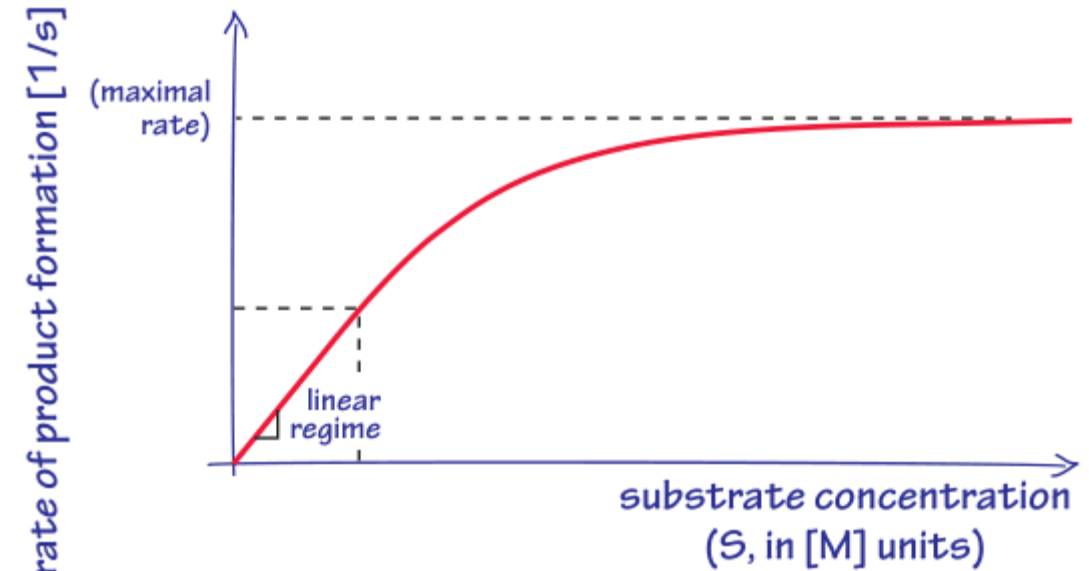
When the road (substrate) is rocky, the speed of the car (enzyme) depends on the road, not the car.





More explanation

- At a fixed concentration of enzyme, V_o is almost linearly proportional to $[S]$ when $[S]$ is small.
- However, V_o is nearly independent of $[S]$ when $[S]$ is large.
- The maximal rate, V_{max} , is achieved when the catalytic sites on the enzyme are saturated with substrate.
- V_{max} reveals the **turnover number** of an enzyme.
 - The number of substrate molecules converted into products by an enzyme molecule in a unit time when the enzyme is fully saturated with substrate.





The Michaelis-Menten equation

- The Michaelis-Menten equation is a quantitative description of the relationship between the rate of an enzyme catalyzed reaction (V_o), substrate concentration $[S]$, a rate constant (K_M) and maximal velocity (V_{max}).

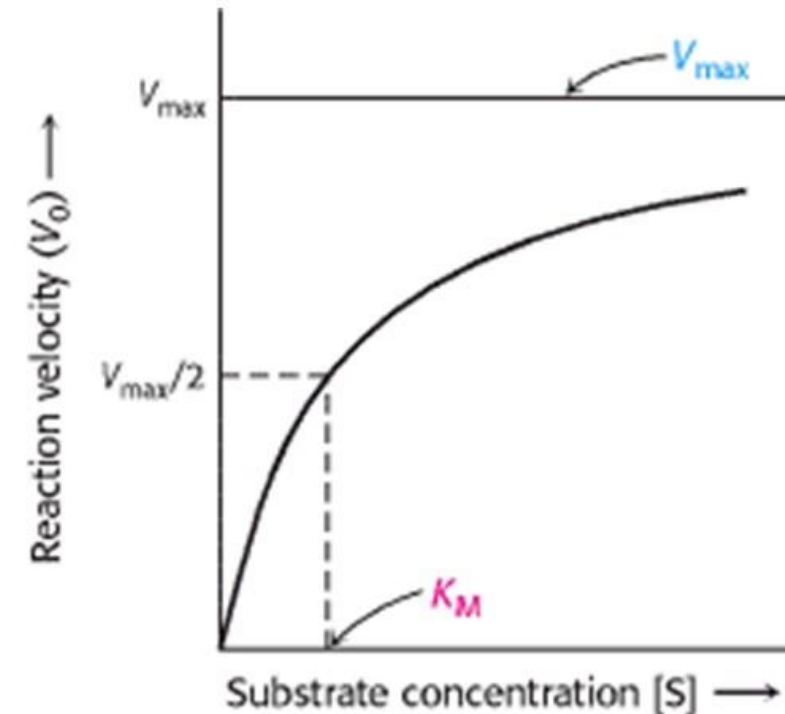
$$V_o = V_{max} \frac{[S]}{[S] + K_M}$$



The Michaelis constant (K_M)

- K_M is the concentration of substrate at which half the active sites are filled or half the maximal velocity is reached.
- When $[S] = K_M$, then $V_o = V_{max}/2$
- Therefore, it provides a measure of enzyme affinity towards a substrate.
 - It is not a true measure of affinity, though.
- The lower the K_M of an enzyme towards a substrate is, the higher its affinity to the same substrate is.

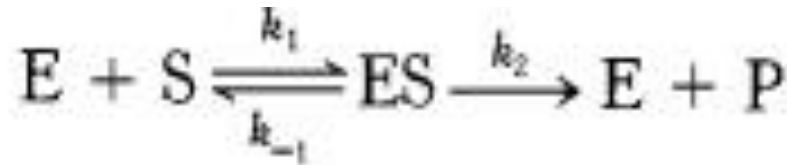
$$V_o = V_{max} \frac{[S]}{[S] + K_M}$$





The Michaelis constant (K_M)

- For a reaction:



Rate of dissociation

Rate of association

$$K_M = \frac{k_{-1} + k_2}{k_1}$$

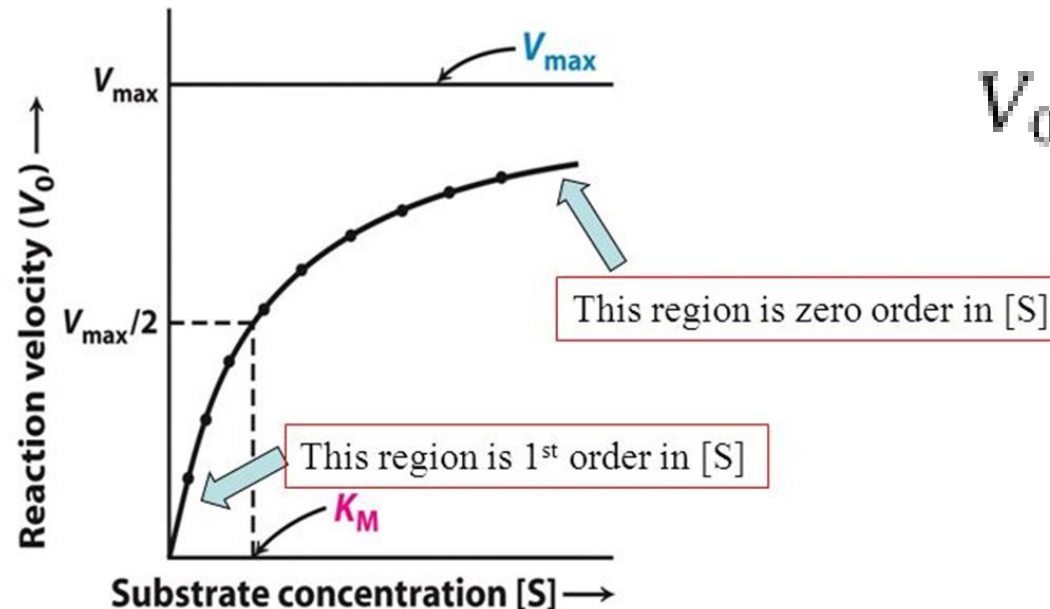
Assume $k_{-1} \gg k_2$,
 $K_M = k_{-1}/k_1$

- K_M is related to the rate of dissociation of a substrate from the enzyme to the rate of enzyme-substrate association.
- K_M *describes* the affinity of an enzyme for its substrate but is NOT an accurate measure of affinity.

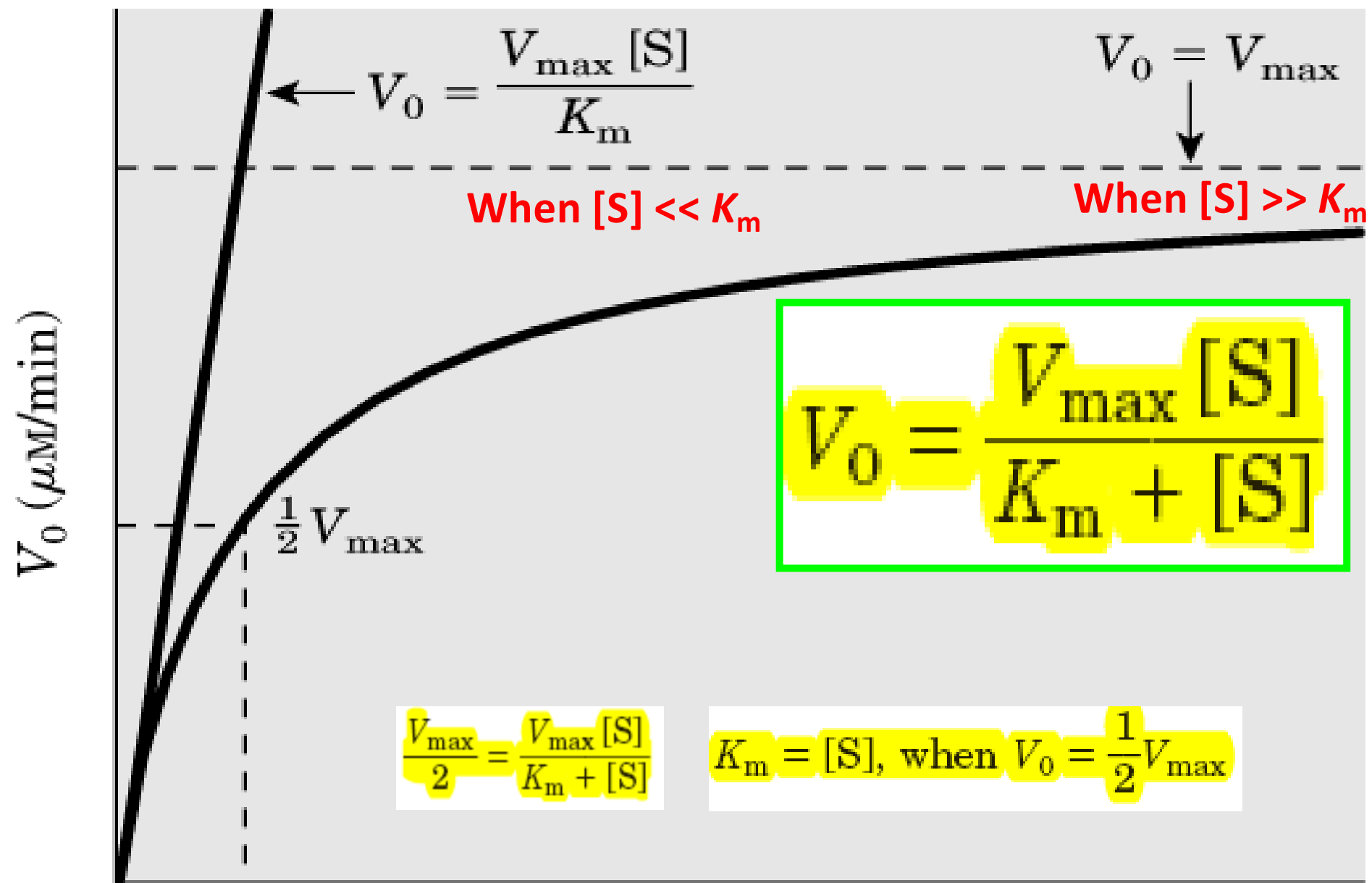


Reaction order in relation to K_M

- At very low substrate concentration, when $[S]$ is **much less than K_M** , $V_0 = V_{\max} \cdot [S] / (K_M + [S])$; that is, the rate is directly proportional to the substrate concentration and is affected by how well a substrate binds to an enzyme.
- At a high substrate concentration, when $[S]$ is **much greater than K_M** , $V_0 = V_{\max}$; that is, the rate is maximal, independent of substrate concentration or how well an enzyme binds to the substrate.



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



K_m The substrate concentration at which V_0 is half maximal is K_m

$[S] (\text{mM})$



Note

- The K_M values of enzymes range widely (mostly, 10^{-7} to 10^{-1}).
- Each substrate has a unique K_M for a given enzyme, but V_{max} is related to the enzyme and is the same for the same reaction of more than one substrate.

Understand
Do not memorize

Example: Hexokinase – enzyme that phosphorylates glucose



table 8–6

K_m for Some Enzymes and Substrates		
Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
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

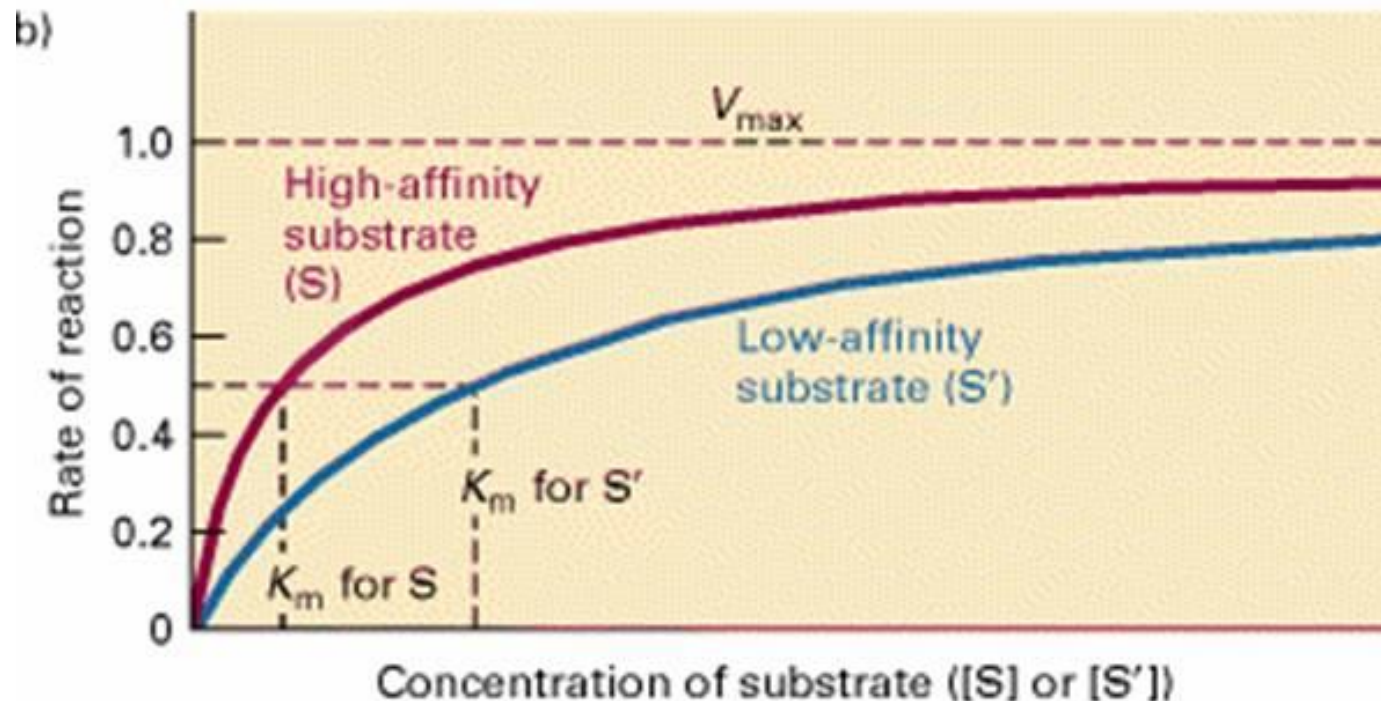
Same enzyme, different substrates, same reaction



Example: Hexokinase – enzyme that phosphorylates glucose



- A reaction is catalyzed by an enzyme with substrate S (high affinity) and with substrate S' (low affinity).
- $V_{\max}$ is the same with both substrates, but K_M is higher for S', the low-affinity substrate.



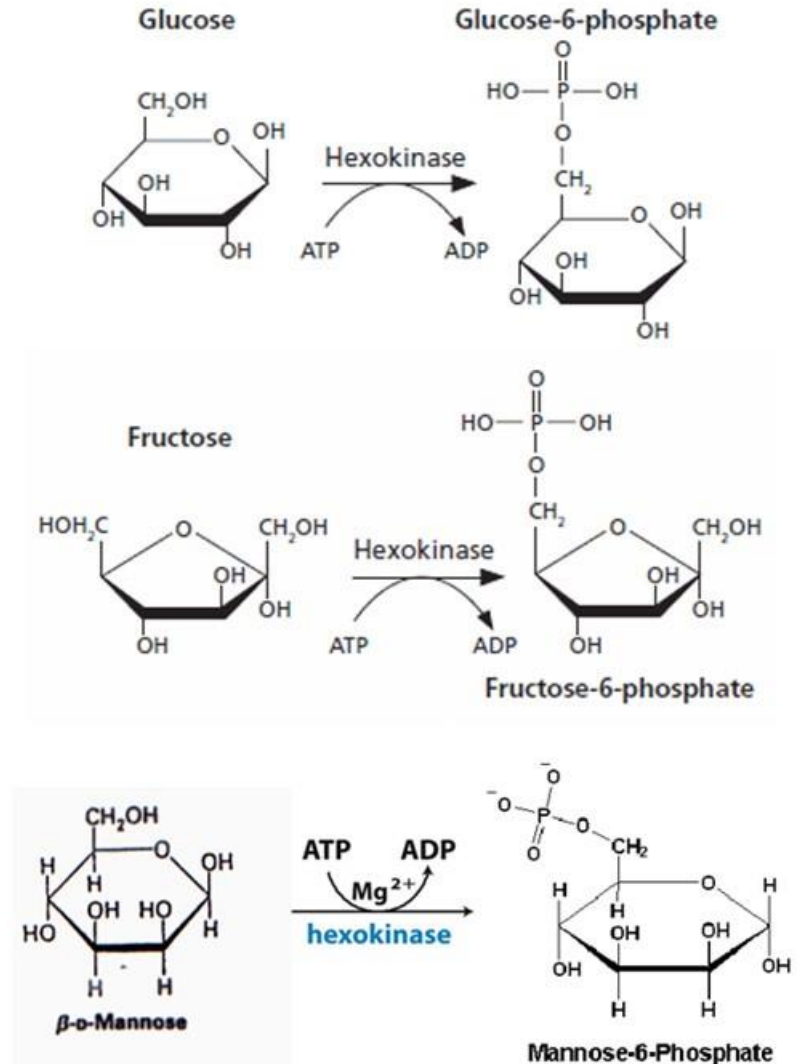
Same enzyme, different substrates, different reactions



- If an enzyme binds to another substrate generating different product(s), then $V_{\max}$ will be different.
 - For example, hexokinase phosphorylates glucose, fructose, and mannose at different $V_{\max}$ values.

Hexose	K_M (μM)	$V_{\max}$ $\text{mol L}^{-1} \cdot \text{sec}^{-1}$
Glucose	59 ± 10	26 ± 2
Mannose	32 ± 2	13 ± 1
Fructose	4436 ± 2275	34 ± 5

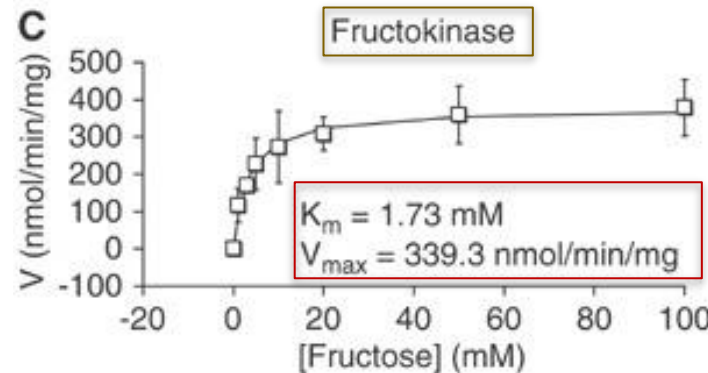
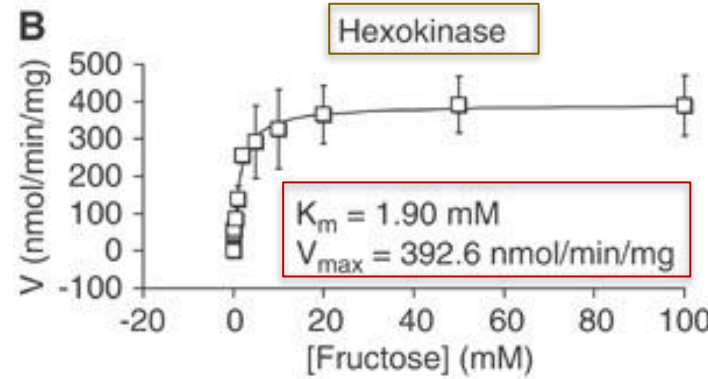
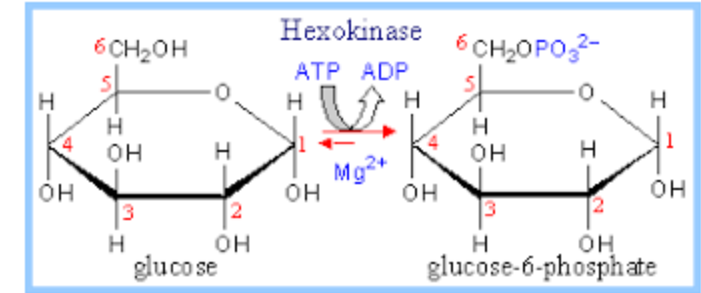
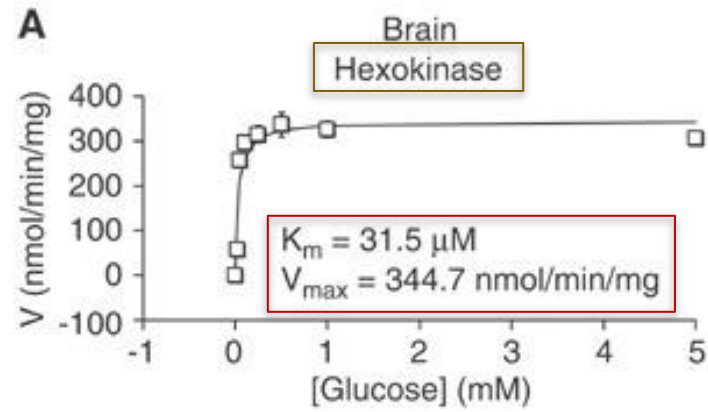
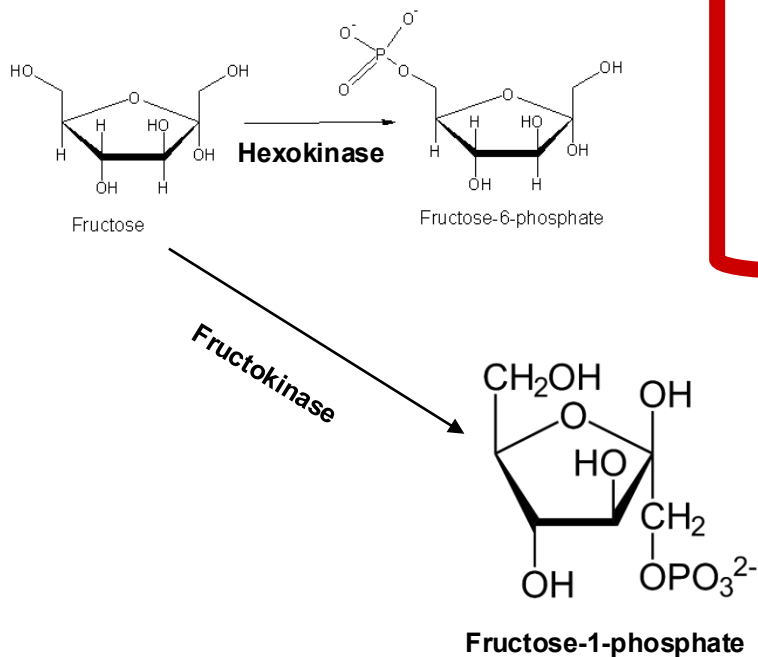
Understand
Do not memorize



Different enzymes, same substrate, different reactions



**Same enzyme,
different substrates**



**Different enzymes,
same substrate**

**Understand
Do not memorize**



Example

- A biochemist obtains the following set of data for an enzyme that is known to follow Michaelis-Menten kinetics. Approximately, V_{max} of this enzyme is ... & K_M is ...?

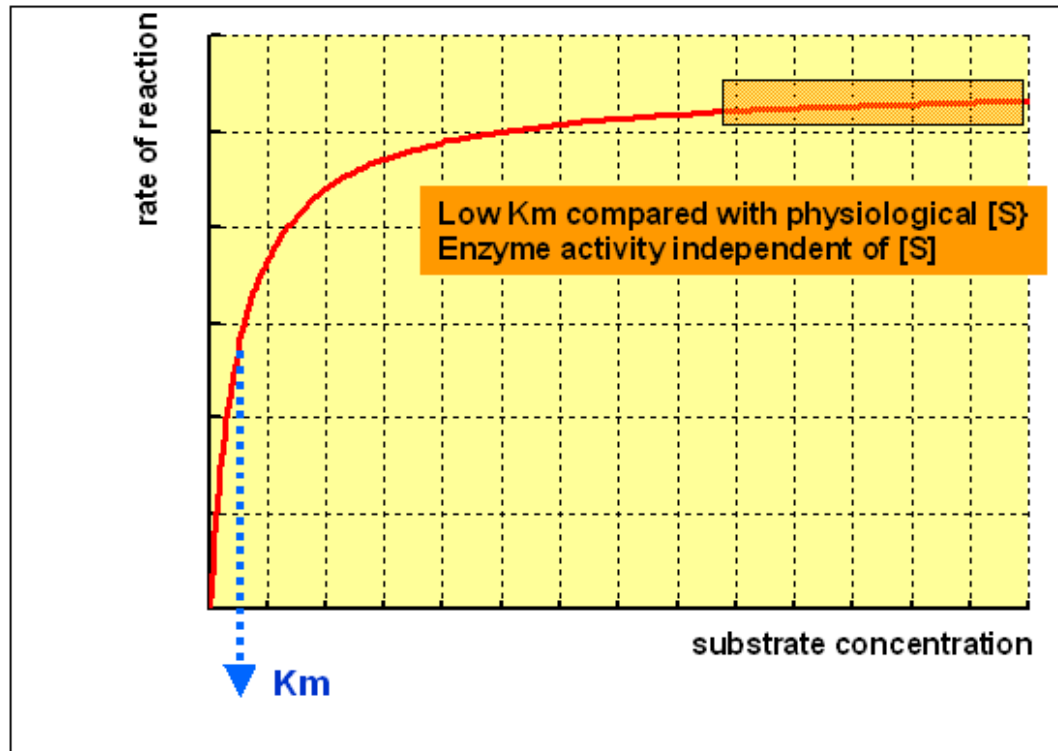
- A. 5000 & 699
- B. 699 & 5000
- C. 621 & 50
- D. 94 & 1
- E. 700 & 8

Substrate Concentration (μM)	Initial velocity ($\mu\text{mol/min}$)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699



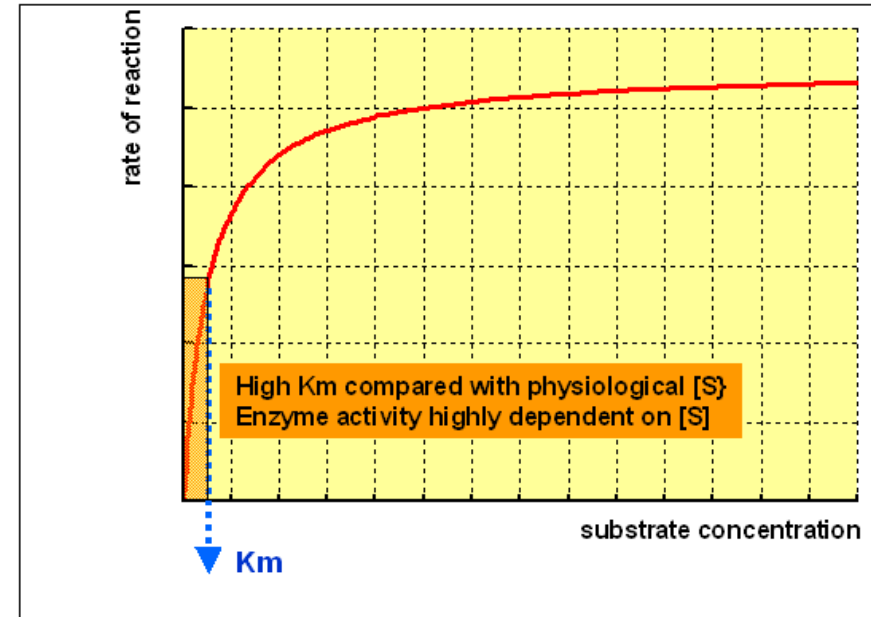
Importance of K_M

If K_M is lower than physiological concentration of S



The enzyme is normally saturated with substrate and will act at a constant rate, regardless of variations in the concentration of substrate.

If K_M is higher than physiological concentration of S

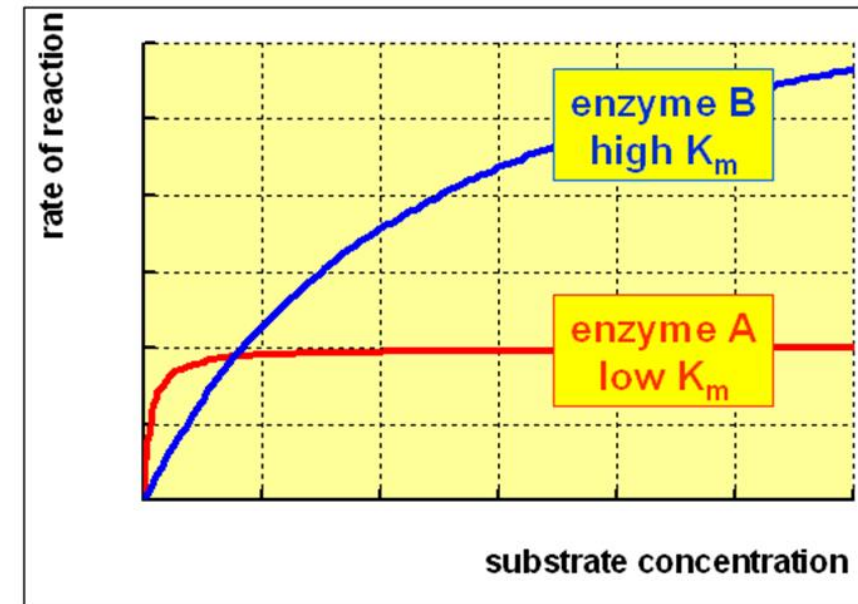
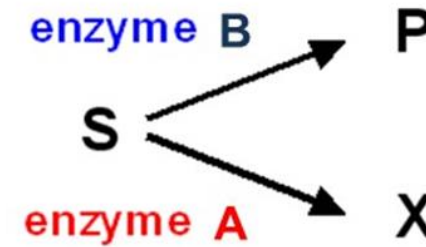


The enzyme is not saturated with substrate and its activity will vary as the concentration of substrate varies and the rate of formation of product will depend on the availability of substrate.



Metabolic pathways

- If two enzymes, in different pathways, compete for the same substrate, then knowing the values of K_M and V_{max} for both enzymes permits prediction of the metabolic fate of the substrate and the relative amount that will flow through each pathway under various conditions.
- Which reaction is favorable when:
 - $[S]$ is very low?
 - $[S]$ is very high?





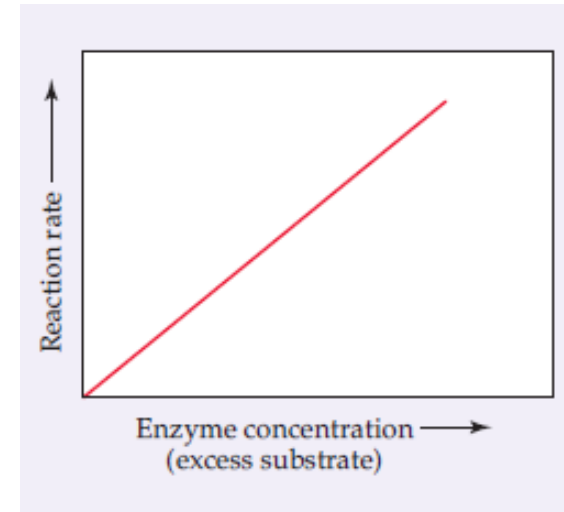
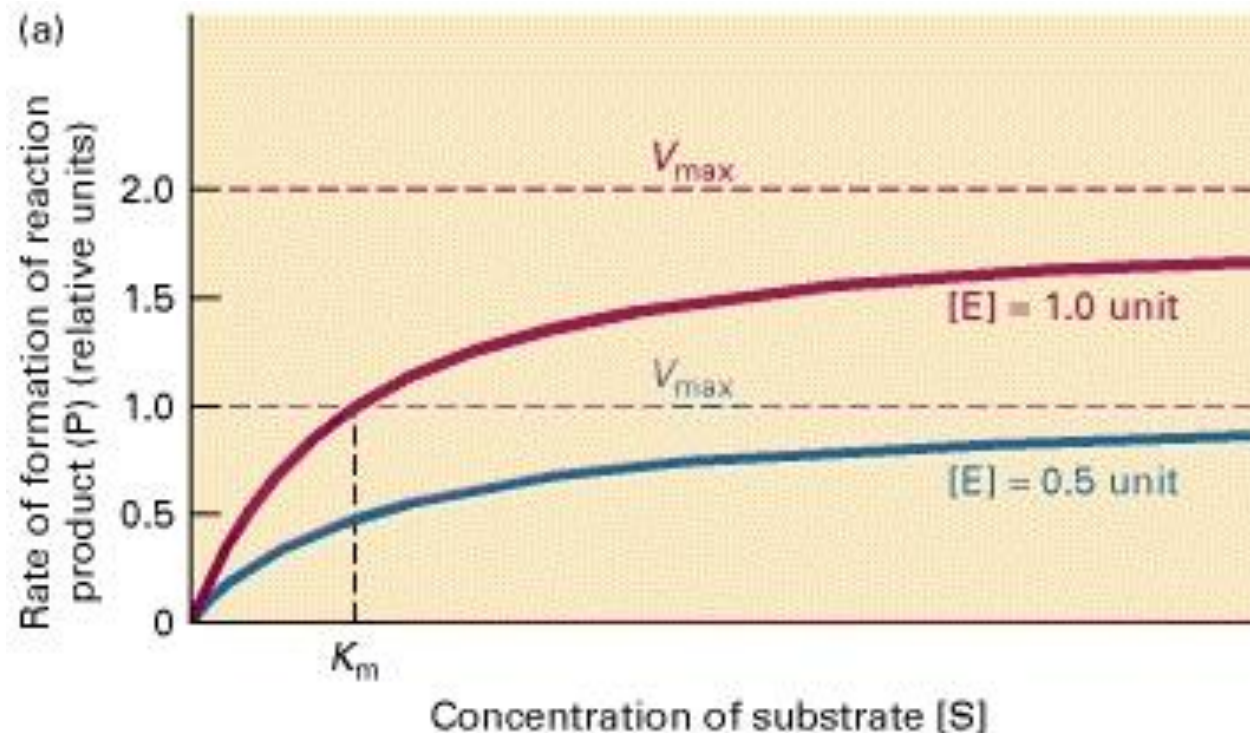
Uses of K_M

- Determine the substrate preferences of an enzyme.
 - If an enzyme has more than one substrate, the substrate with the lowest K_M is probably the preferred physiological substrate.
- Distinguish isozymes, which are different enzymes catalyzing the same reaction.
 - Isozymes often have different affinities for the same substrate.
- Check for abnormalities in an enzyme.



$V_{\max}$ and enzyme concentration

- Doubling the concentration of enzyme causes a proportional increase in the reaction rate, so that the maximal velocity $V_{\max}$ is doubled; the K_M , however, is unaltered.





V_{max} & k_{cat}

(a measure of enzyme efficiency)

- For the enzymatic reaction
$$E + S \xrightleftharpoons[k_{-1}]{k_1} ES \xrightarrow{k_2} E + P$$
- The maximal rate, V_{max}, is equal to the product of k₂, also known as k_{cat}, and the total concentration of the enzyme.

$$V_{\max} = k_2 [E]_T \text{ OR } k_{\text{cat}} = V_{\max} / [E]_T$$

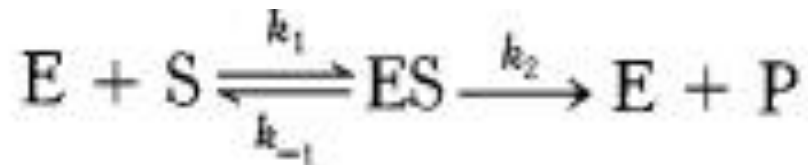
Turnover Numbers (k _{cat}) of Some Enzymes		
Enzyme	Substrate	k _{cat} (s ⁻¹)
Catalase	H ₂ O ₂	40,000,000
Carbonic anhydrase	HCO ₃ ⁻	400,000
Acetylcholinesterase	Acetylcholine	14,000
β-Lactamase	Benzylpenicillin	2,000
Fumarase	Fumarate	800
RecA protein (an ATPase)	ATP	0.4

k_{cat} is a constant for any given enzyme.

Understand
Do not memorize



K_{cat}



$$V_{\max} = k_2 [E]_T \text{ OR } k_{\text{cat}} = V_{\max} / [E]_T$$



- k_{cat}, turnover number, is the concentration (or moles) of substrate molecules converted into product per unit time per concentration (or moles) of enzyme, **when fully saturated**.
- It describes how quickly an enzyme acts, i.e., how fast the ES complex proceeds to E + P.
- In other words, the maximal rate, V_{max}, reveals the turnover number of an enzyme if the total concentration of active sites [E]_T is known.



Example

- You are working on the enzyme “Medicine” which has a molecular weight of 50,000 g/mol. You have used 10 μg of the enzyme in an experiment and the results show that the enzyme at best converts 9.6 μmol of the substrate per min at 25°C. The turnover number (kcat) for the enzyme is:

$$\text{mol} = \text{g}/\text{MW}$$

A. 9.6 s^{-1}

B. 48 s^{-1}

C. 800 s^{-1}

D. 960 s^{-1}

E. 1920 s^{-1}



- MW = 50,000 g/mol
- Weight = 10 μg
- V_{max} = 9.6 μmol of the substrate per min

$$\begin{aligned} K_{\text{cat}} &= (9.6/60)/(10 \mu\text{g} / 50,000) \\ &= 800 \text{ s}^{-1} \end{aligned}$$



Number of reactions
per second

Understand
Do not memorize

table 8-7

Turnover Numbers (k_{cat}) of Some Enzymes

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	0.4



Catalytic efficiency (k_{cat} vs. K_M)

Table 6.2

Turnover Numbers and K_M for Some Typical Enzymes

Enzyme	Function	k_{cat} = Turnover Number*	K_M **
Catalase	Conversion of H_2O_2 to H_2O and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

Understand
Do not memorize

Catalytic efficiency of enzymes = k_{cat} / K_M