بسم الله الرحمن الرحيم

BIOCHEMISTRY

DOPAMINE
SCIENTIFIC TEAM

﴿ وَإِن تَتَوَلَّوْاْ يَسْتَبْدِلْ قَوْمًا غَيْرَكُمْ ثُمَّ لَا يَكُونُوٓاْ أَمْثَٰلَكُم ﴾

اللهم استعملنا لنصرة دينك

Lecture 26

Enzymes II - *Kinetics*

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APPROVED
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Kinetics

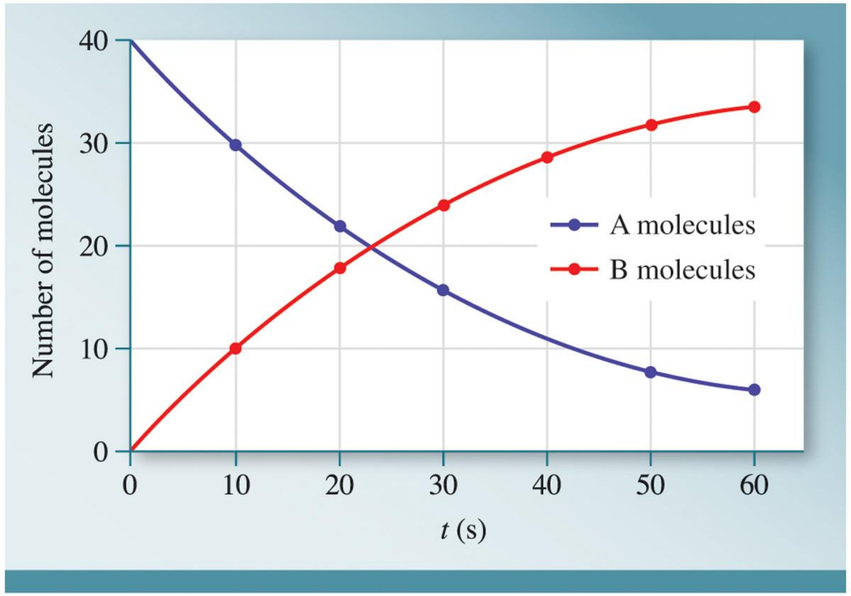
How fast does a chemical reaction take place?

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,

Rate of reaction (velocity or v) = $-\frac{\Delta [A]}{\Delta t}$ *or* $\frac{\Delta [B]}{\Delta t}$ = -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 (time)$^{-1}$, usually sec^{-1}.



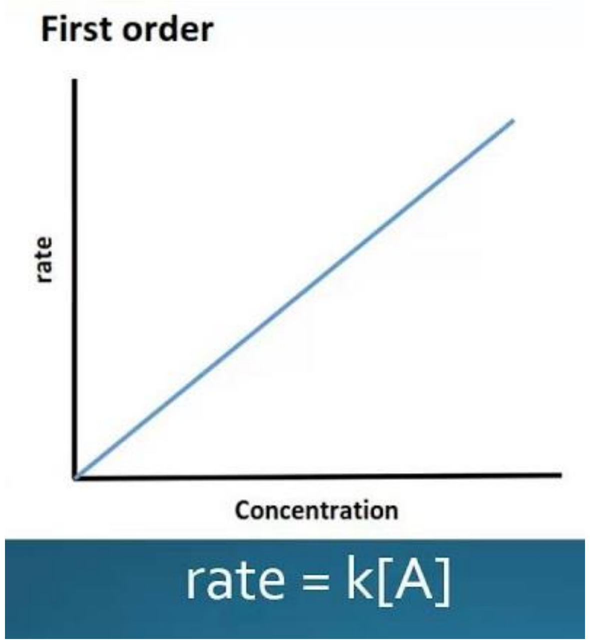
Every chemical reaction has its own rate.

If (A → B) is

A first-order reaction

$$rate = k[A]^1 = k[A]$$

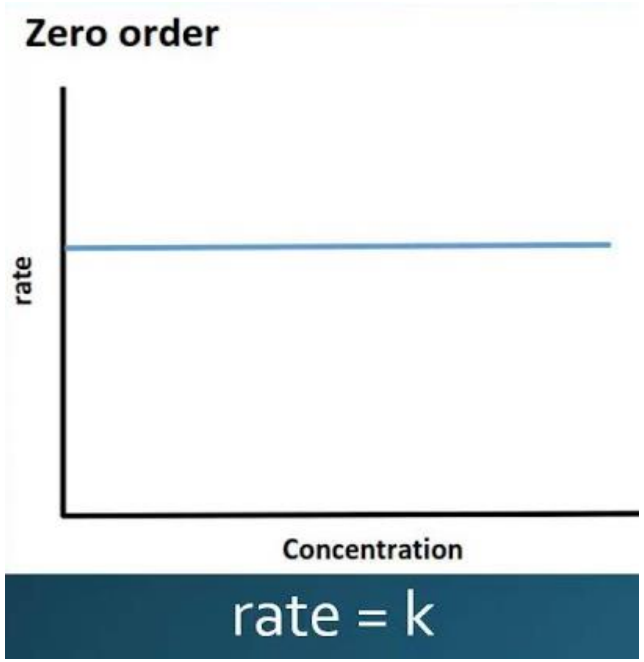
- The rate of a reaction increases linearly with increasing substrate concentration.



A zero-order reaction

$$rate = k[A]^0 = k$$

- The rate of the reaction is independent of substrates.



- If a reaction rate increases linearly with one substrate, and linearly with another substrate, we call this a second-order reaction.
- If the reaction increases quadratically with one substrate, it is also a second-order reaction.

FORGET ABOUT THIS AND FOCUS ON ZERO- AND FIRST-DEGREE REACTIONS.

Rate of reaction (velocity)

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

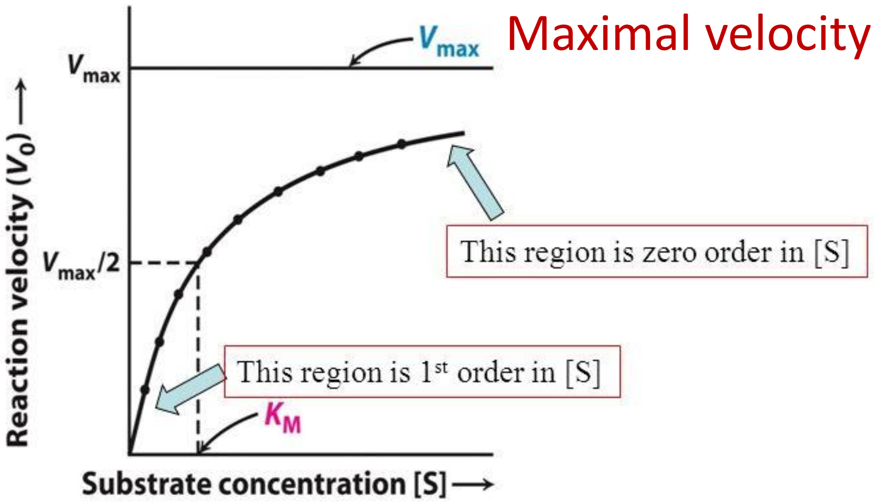
Concentration (M) = mol / vol

Enzyme kinetics

We talk about classical enzymes only; not allosteric ones.

- 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 (Vmax) at very high substrate concentrations.

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



Experimental methods are used to find V_{max} by gradually increasing [S] and observing the trend and then theoretically calculating the rate of the reaction at very high values of [S].

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

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

Why?

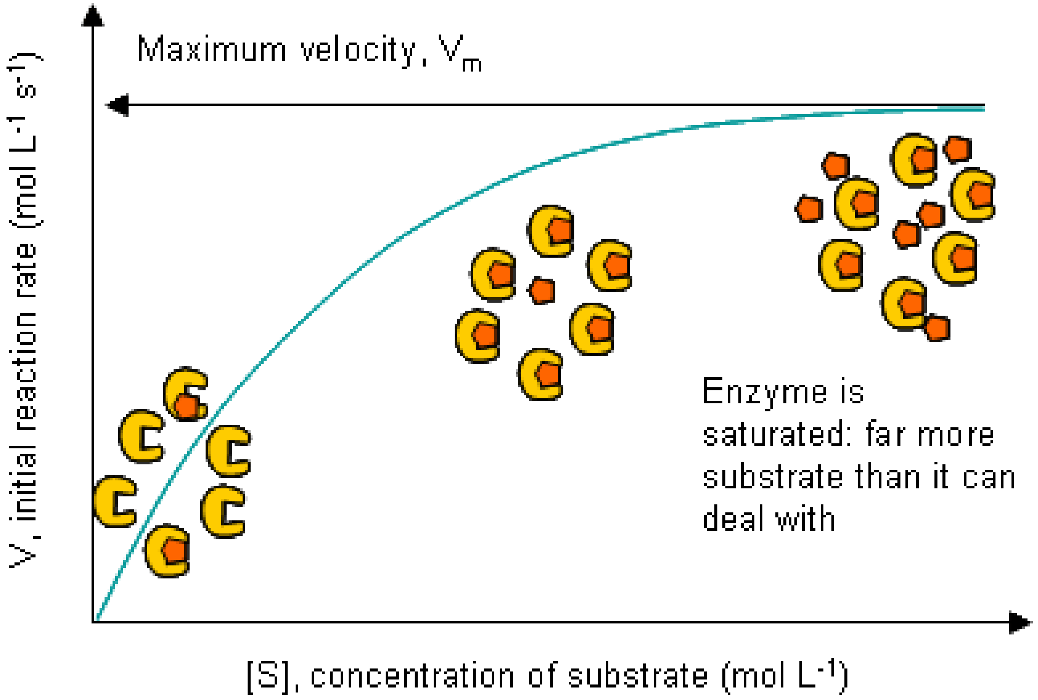
At low [S] → rate depends on [S]
At high [S] → rare depends on the enzyme

- 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.

We can use an analogy to explain the phenomenon of saturation. The velocity of a high-end car on different roads resembles the rate of a reaction.

When the road is bad, the car cannot express its full capacity.

When the road is good, the velocity of the car is its highest possible velocity, governed only by the abilities of the car itself.

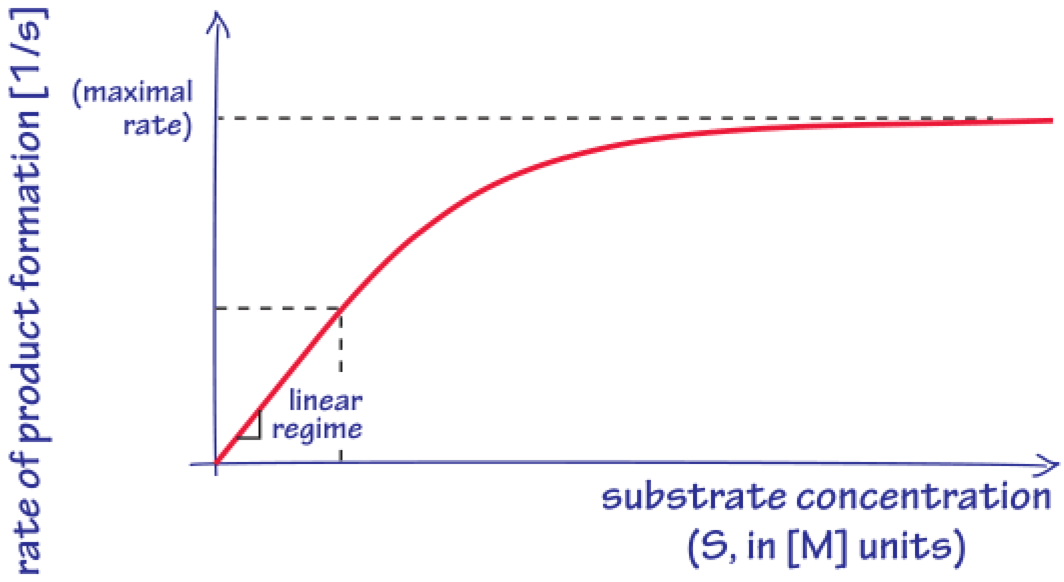


When enzymes become fully saturated, meaning that all active sites are occupied, increasing the concentration of reactants beyond this point won't affect the reaction rate, as it is now solely dependent on the enzyme and its availability, not the substrate concentration.

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 (Vmax).

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

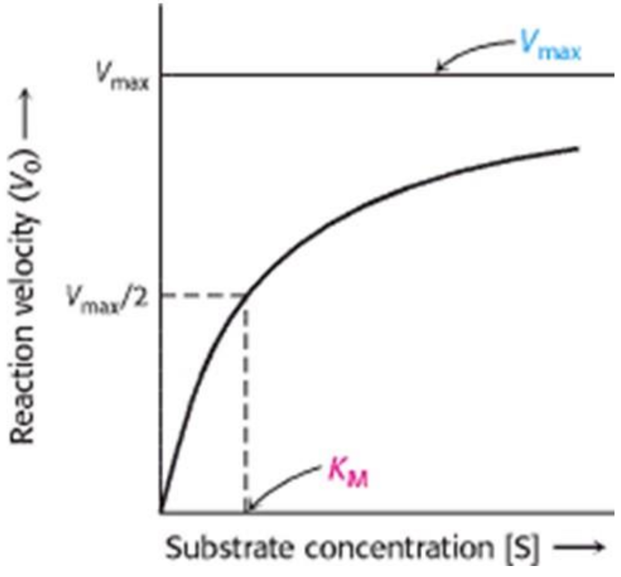
VERY IMPORTANT (MEMORIZE THE EQUATION)

This equation shows why the velocity highly depends on [S] at low levels of [S]; see the table at the bottom.
Note that the velocity does change theoretically even at high [S] levels, but the change is **insignificant**.

The Michaelis constant (K_M)

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

- K_M is the concentration of substrate at which half the active sites are filled.
- When [S] = K_M, then V_o = Vmax/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.



Higher Change with change in [S]
V depends on [S]

Lower Change with change in [S]
V "does not depend on [S]"

Suppose K_m = 10

[S]	0	1	2	3	10	100	101	102	103
V_0 / V_{max}	0	0.091	0.167	0.231	0.5000	0.90909	0.90991	0.91071	0.91150

Differences: 0.091, 0.076, 0.064 … 0.00082, 0.00080, 0.00079

The Michaelis constant (K_M)

For the ES complex, there are two possible outcomes: it can either proceed to form the enzyme-product complex (E + Product), or the substrate and enzyme can dissociate, reverting to the separate enzyme (E) and substrate (S)

- For a reaction:

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

Rate of break apart of ES complex

Rate of dissociation

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

Rate of association

Rate of forming ES complex

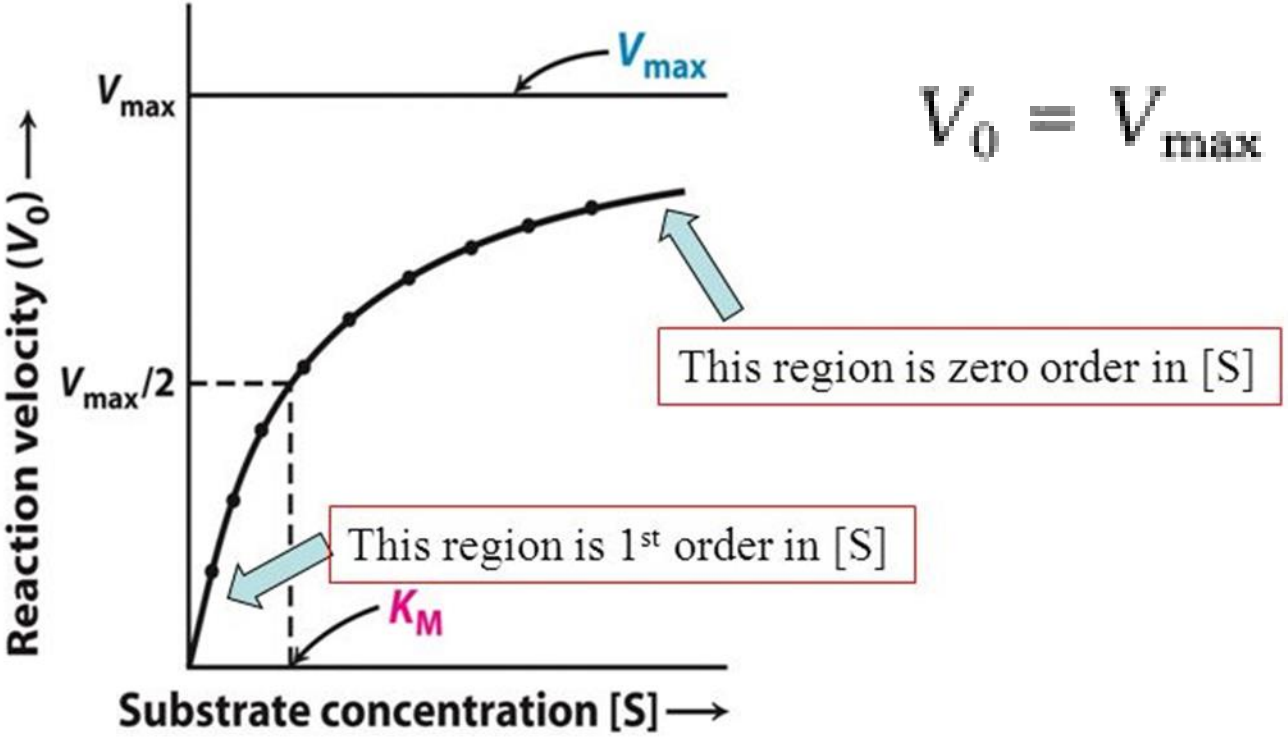
Since ($k_{-1} >> k_2$),
$K_M = k_{-1}/k_1$

- 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 since k_2 is not taken into consideration.

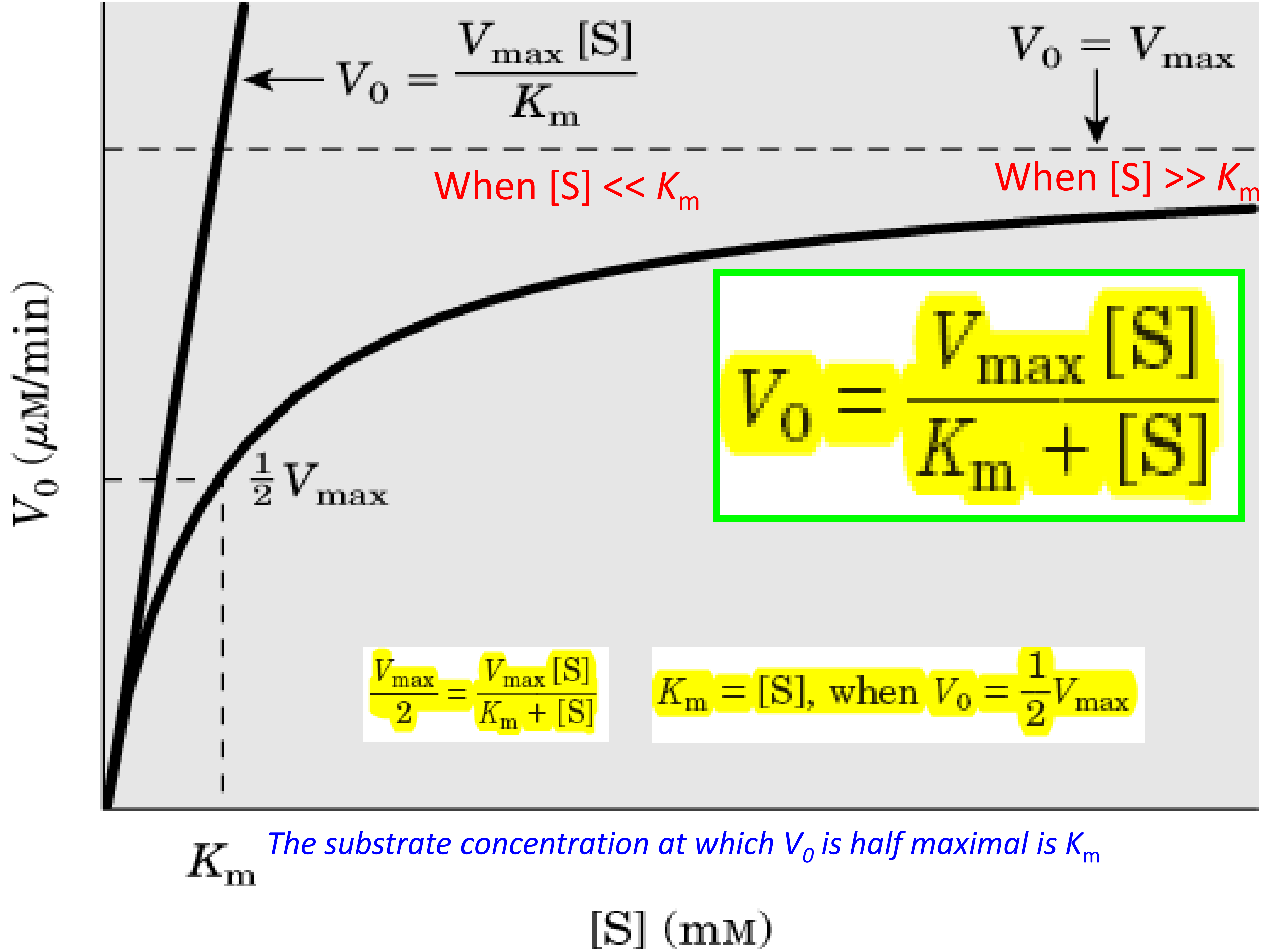
The higher the k_m, the lower the affinity; recall p50.

Reaction order in relation to K_M

- At very low substrate concentration, when [S] is much less than K_M, V_0 = Vmax.[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 high substrate concentration, when [S] is much greater than K_M, V_0 = Vmax; that is, the rate is maximal, independent of substrate concentration or how well an enzyme binds to the substrate.



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



$V_0 = \frac{V_{max}[S]}{K_m}$
$V_0 = V_{max}$
When [S] << K_m
When [S] >> K_m
$V_0 = \frac{V_{max}[S]}{K_m + [S]}$
$\frac{1}{2} V_{max}$
$\frac{V_{max}}{2} = \frac{V_{max}[S]}{K_m + [S]}$
$K_m = [S]$, when $V_0 = \frac{1}{2} V_{max}$
V_0 (μM/min)
K_m
The substrate concentration at which V_0 is half maximal is K_m
[S] (mM)

Note

Understand
Do not memorize

- 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 **Vmax** is related to the enzyme and is the **same for the same reaction of more than one substrate.**

Example: Hexokinase – enzyme that phophorylates glucose

Glucose + ATP ⟶ Glucose – 6-P + ADP + H^+

table 8–6

K_m for Some Enzymes and Substrates

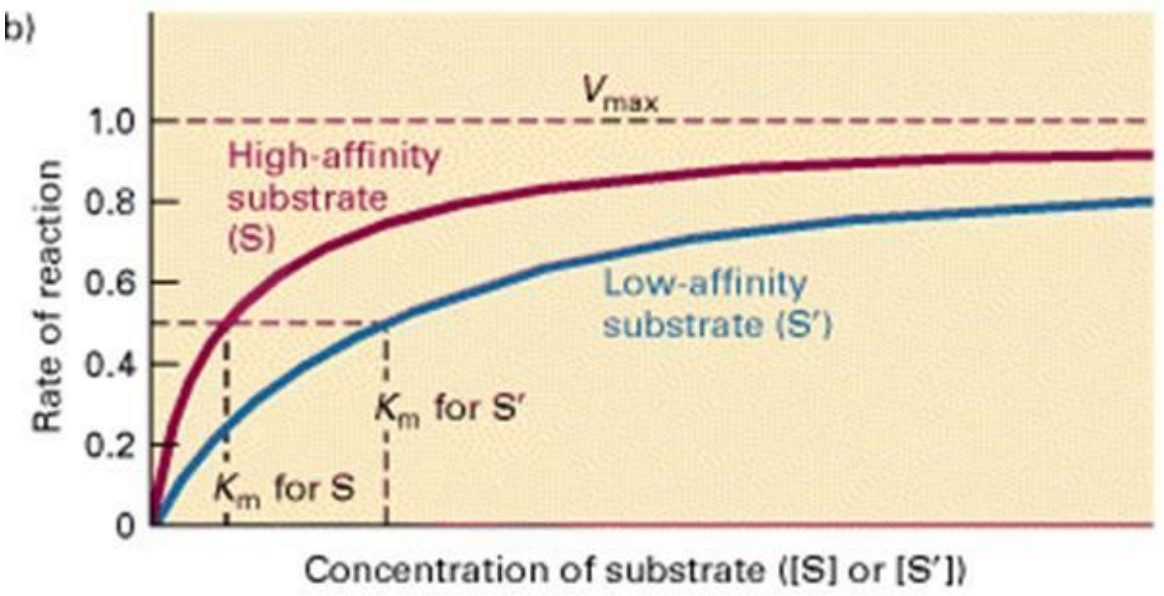
Enzyme	Substrate	K_m (mM)
Catalase	H_2O_2	25
Hexokinase (brain)	ATP	0.4
	D-Glucose	0.05
	D-Fructose	1.5
Carbonic anhydrase	HCO_3^-	26
Chymotrypsin	Glycyltyrosinylglycine	108
	N-Benzoyltyrosinamide	2.5
β-Galactosidase	D-Lactose	4.0
Threonine dehydratase	L-Threonine	5.0

Same enzyme, different substrates, same reaction

Example: Hexokinase – enzyme that phophorylates glucose

Glucose + ATP ⟶ Glucose – 6-P + ADP + H^+

- A reactions 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.



For hexokinase,
Glucose resembles (S)
ATP resembles (S′)

Same enzyme, different substrates, **different reactions**

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

Mannose has the highest affinity of the 3 hexoses for hexokinase while fructose has the lowest affinity.

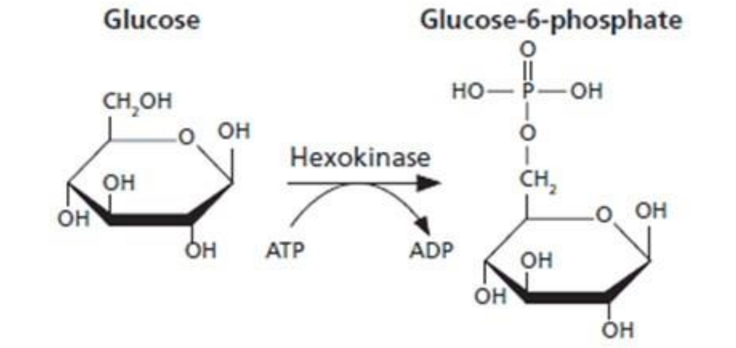
Hexokinase is more efficient in phosphorylating fructose (than the other 2) because of the higher V_{max}.

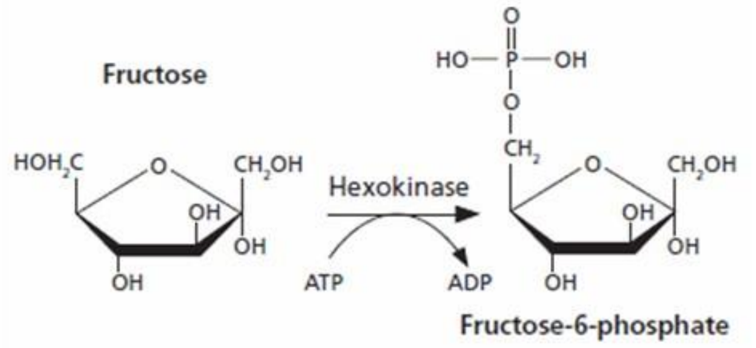
Hexose	K_M (μM)	V_{max} (nmol/(min × mg))
Glucose	59 ± 10	26 ± 2
Mannose	32 ± 2	13 ± 1
Fructose	4436 ± 2275	34 ± 5

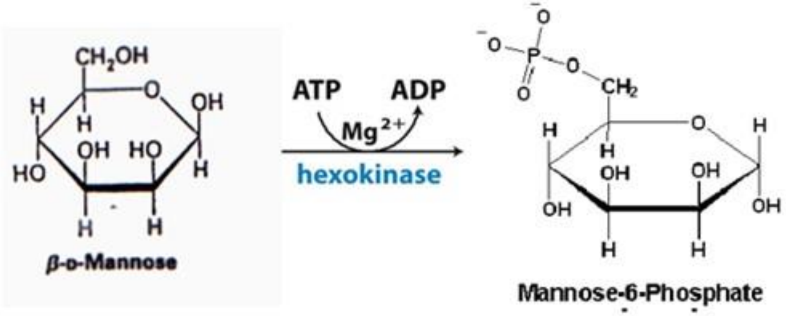
General rule :
Low K_m → High affinity
High affinity → More saturation

High V_{max} → High efficiency

Understand
Do not memorize





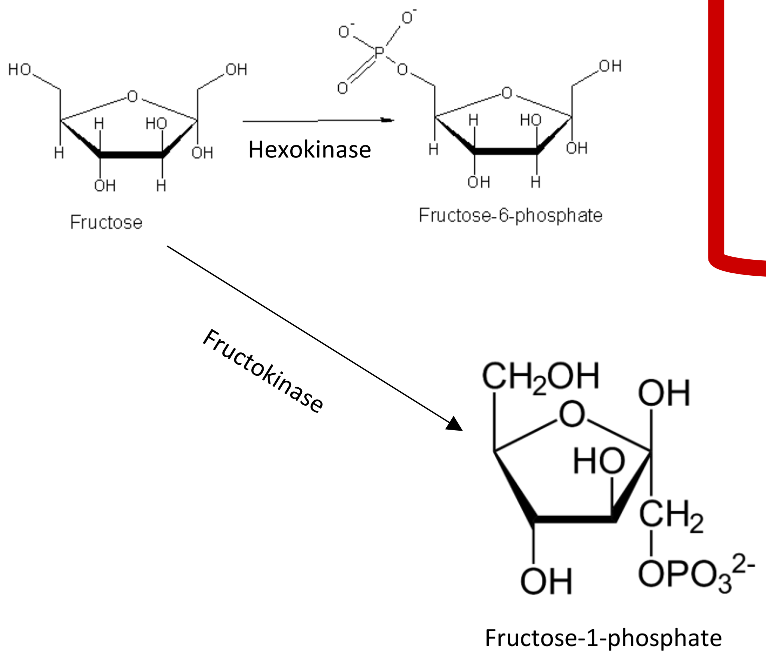


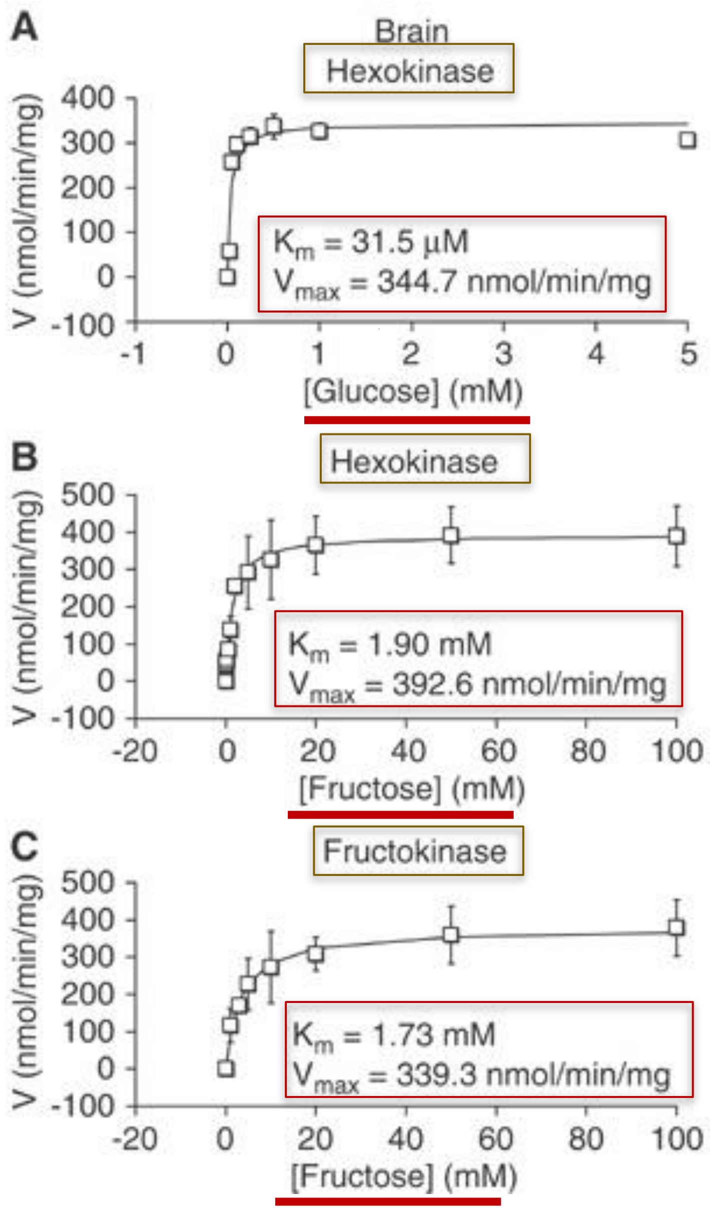
Different enzymes, same substrate, different reactions

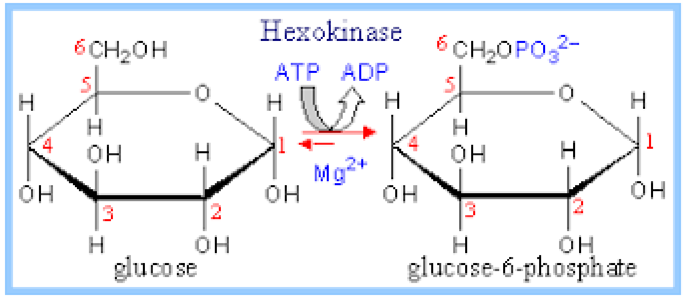
Hexokinase has higher affinity for glucose than for fructose;

Glucose Km = 31.5 x 10^-6 M
Fructose Km = 1.90 x 10^-3 M

Same enzyme, different substrates







Fructokinase has higher affinity for fructose than hexokinase has (see K_m),

but hexokinase has higher efficiency than fructokinase in phosphorylating fructose (See V_{max})

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, Vmax 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 (μmol/min)
1	49
2	96
8	349
50	621
100	676
1000	698
5000	699

Answer is E
Why not B??
Because B present V_{max} 699 and E present 700, its confusing !!
Let's check K_m, E has Km = 8, but B has Km = 500 which is totally wrong because K_m is the concentration of substrate at ½ V_{max}, so whether V_{max} = 700 or 699 when we divide it by 2 its equal approximately 349 or 350 → Km is 8 → Choice E not B.

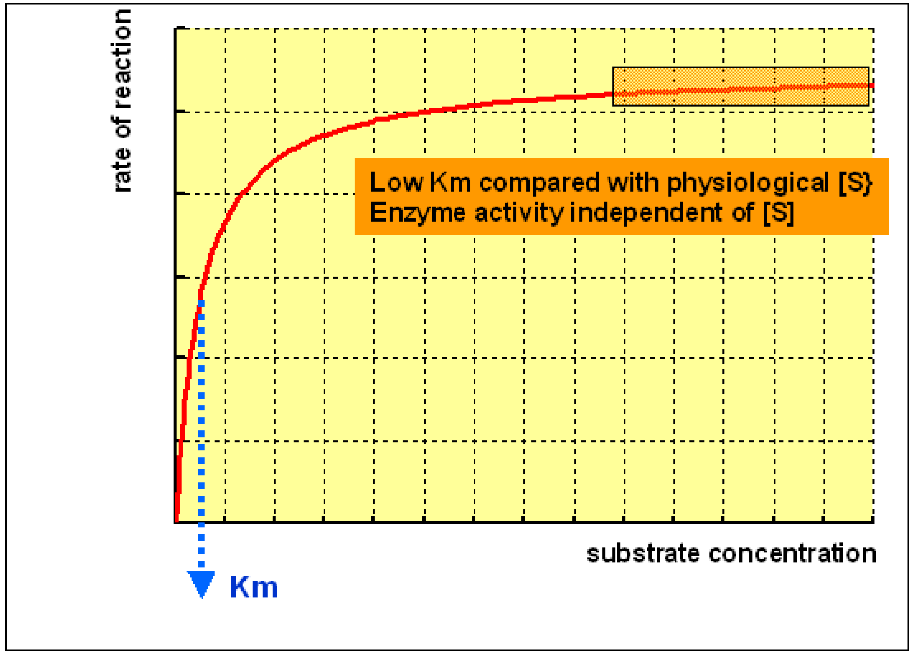
Importance of K_M

It indicate the reaction velocity

If $[S] \gg K_m \rightarrow V_0 = V_{max}$ (depends on the enzyme itself)

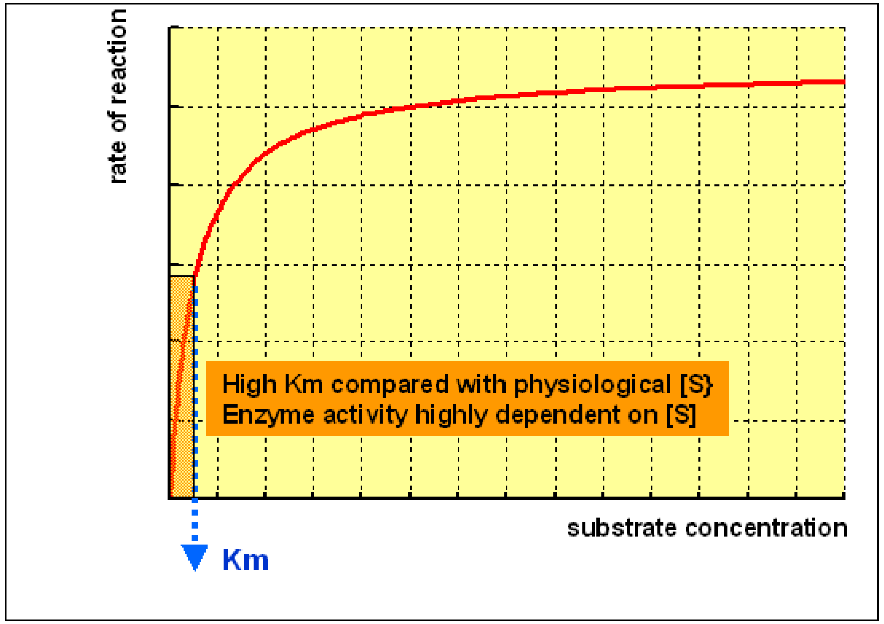
If $[S] \ll K_m \rightarrow V_0$ depends on [S]

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.

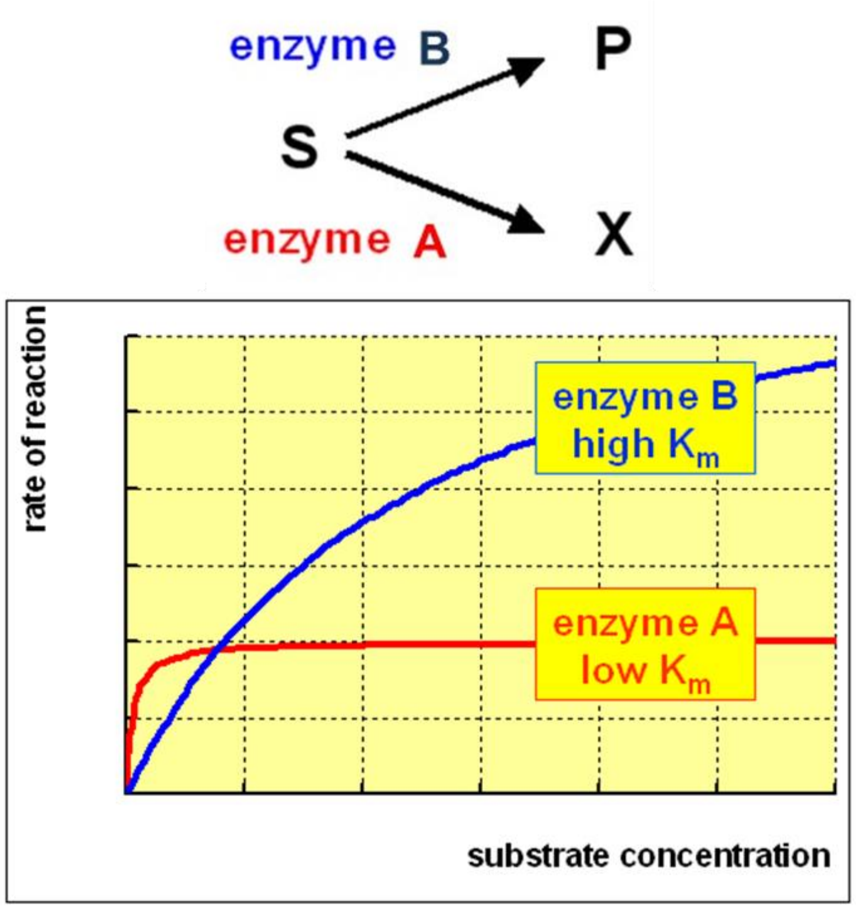
If K_M is higher than physiological concentration of S



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 Vmax 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? A
  - [S] is very high? B



Uses of K_M

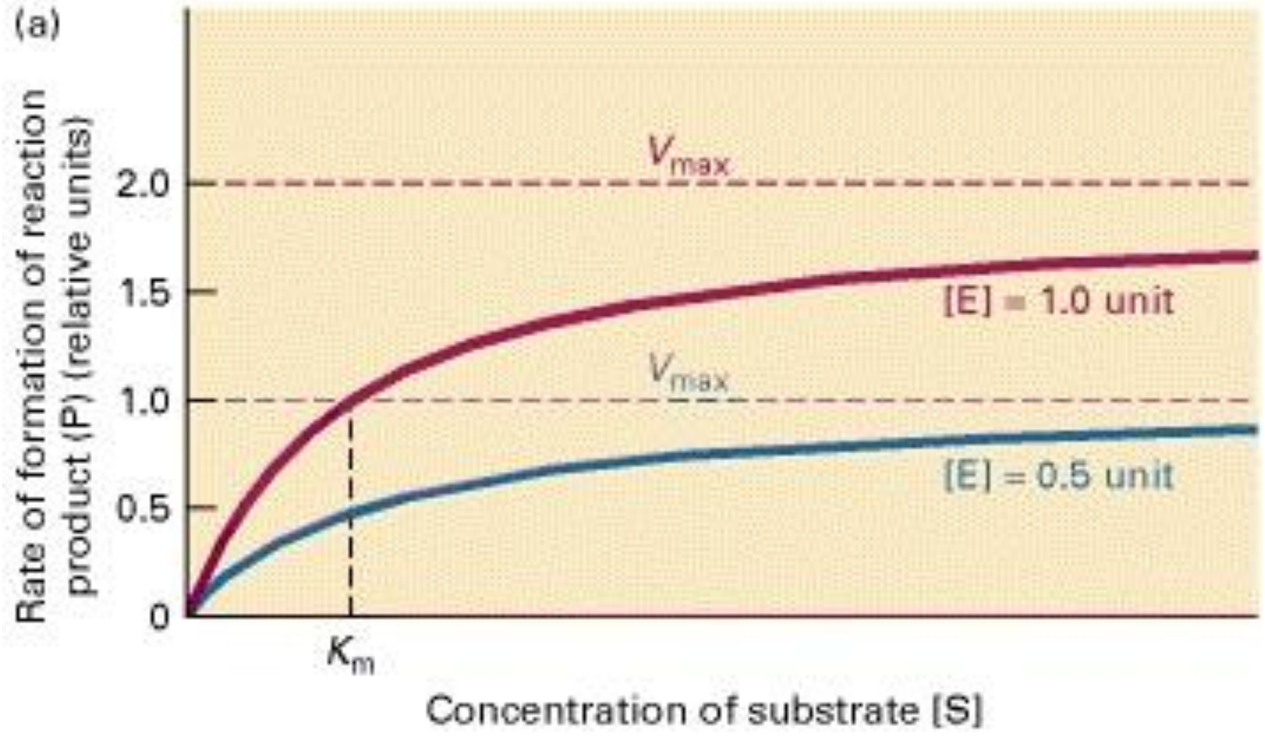
- Determine the substrate preferences of an enzyme.
  - If an enzyme has more than one substrate , the substrate with the lowest K_M is probably the preferred physiological substrate.
- Distinguish isozymes, which are different enzymes catalyzing the same reaction.
  - Isozymes often have different affinities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

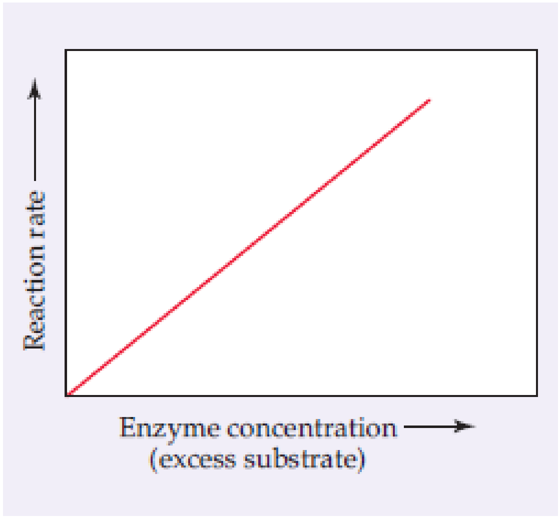
  If the K_m for a given enzyme changes, this can indicate an abnormality in the enzyme.

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.

Reaction rate depends mainly on the probability of collision between the enzyme and the substrate, so increasing concentration of the enzyme will increase the probability of collision which means that the enzyme is going to find the substrate much faster → velocity increases.





V_{max} & k_{cat}

(a measure of enzyme efficiency)

In other words
The efficiency of an enzyme means: how good the enzyme is at converting the substrate to the product before the substrate detaches from the enzyme.

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

- The maximal rate, V_{max}, is equal to the product of k_2, also known as kcat, and the total concentration of the enzyme. $V_{max} = k_2\,[E]_T$ *OR* $k_{cat} = V_{max} / [E]_T$

K_2 is constant, so efficiency is also constant, but V_{max} is not constant and depends on the concentration of the enzyme.

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

K_{cat}: catalytic efficiency

k_{cat} is a constant for any given enzyme

If the enzyme acts on another substrate, it can have a different kcat

Understand
Do not memorize

K_{cat}

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

$$k_{cat} = V_{max}/ [E]_T$$

- 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.

40,000,000 molecules of H_2O_2 are converted to H_2O and O_2 by ONE catalase molecule within one 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)

Understand
Do not memorize

Table 6.2

Turnover Numbers and Km for Some Typical Enzymes

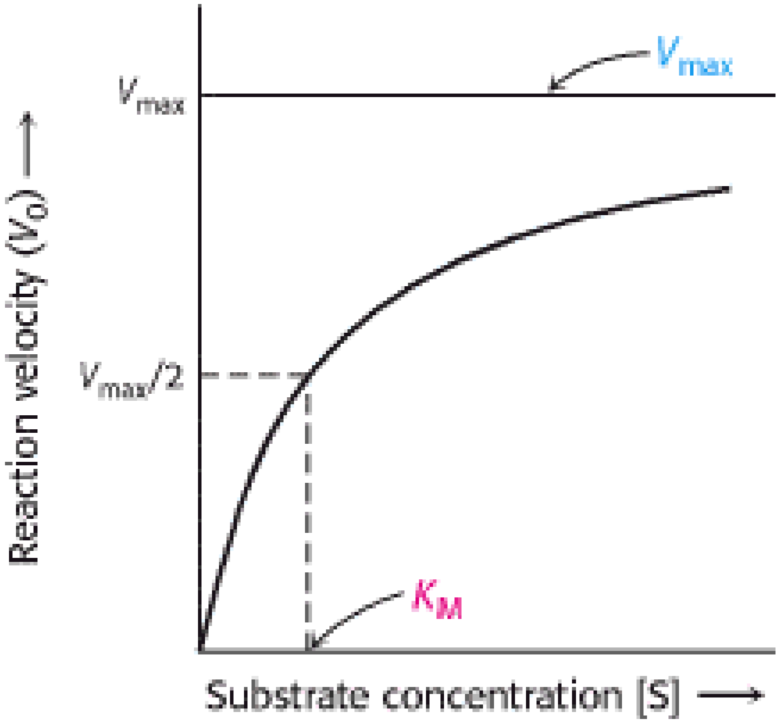
Enzyme	Function	k_{cat} = Turnover Number*	K_M**
Catalase	Conversion of H_2O_2 to H_20 and O_2	4×10^7	25
Carbonic Anhydrase	Hydration of CO_2	1×10^6	12
Acetylcholinesterase	Regenerates acetylcholine, an important substance in transmission of nerve impulses, from acetate and choline	1.4×10^4	9.5×10^{-2}
Chymotrypsin	Proteolytic enzyme	1.9×10^2	6.6×10^{-1}
Lysozyme	Degrades bacterial cell-wall polysaccharides	0.5	6×10^{-3}

Catalytic efficiency of enzymes = k_{cat} / K_M

The most efficient enzyme is the one that has a fast turnover and high affinity to its substrate.
K_m is in the denominator because it is inversely related to the affinity.

A disadvantage of the Michaelis-Menten equation

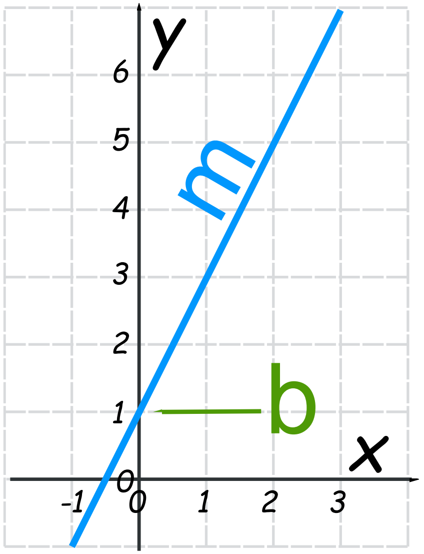
- Determination of K_M from hyperbolic plots is not accurate since a large amount of substrate is required in order to reach V_{max}.
- This prevents the calculation of both V_{max} and K_M.



Solution

We use algebraic manipulations to convert into a slop-intercept form (y=mx+b)

$y = mx + b$	*m* is the slope *b* is the *y*-intercept



The Lineweaver-Burke or double-reciprocal plot

- A plot of 1/ V_o versus 1/[S], called a Lineweaver-Burke or double-reciprocal plot, yields a straight line with an intercept of $1/V_{max}$ and a slope of K_M / V_{max}.
- The intercept on the x-axis is $-1/K_M$.

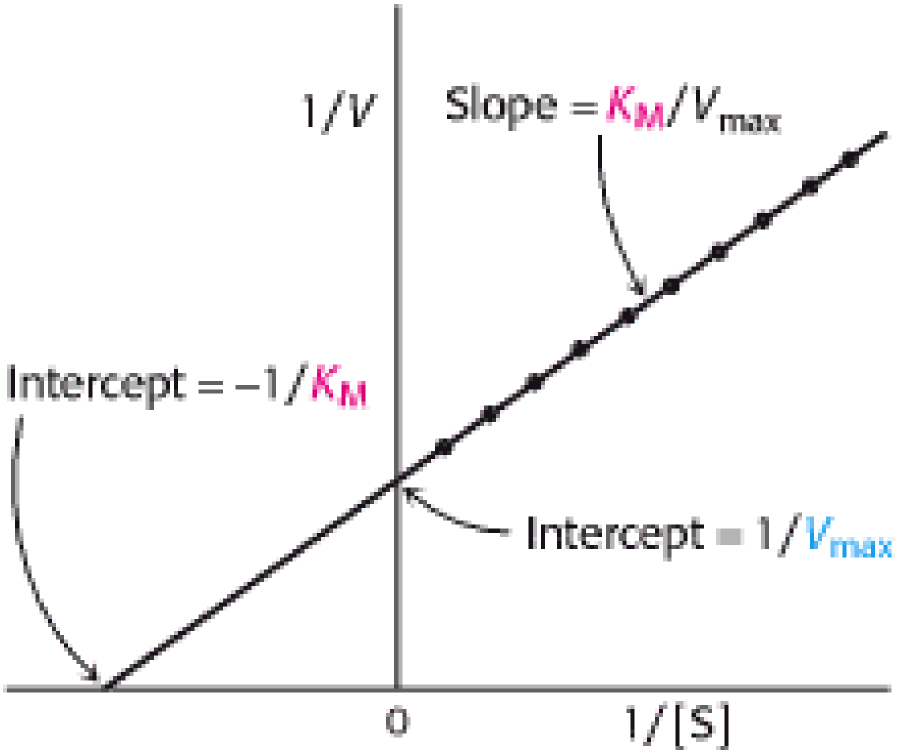
$$V_0 = V_{max} \frac{[S]}{[S] + K_M}$$

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

VERY IMPORTANT (MEMORIZE THE EQUATION)

This equation is purely mathematical, and it can yield some values which cannot be obtained from the original form in slide 8.

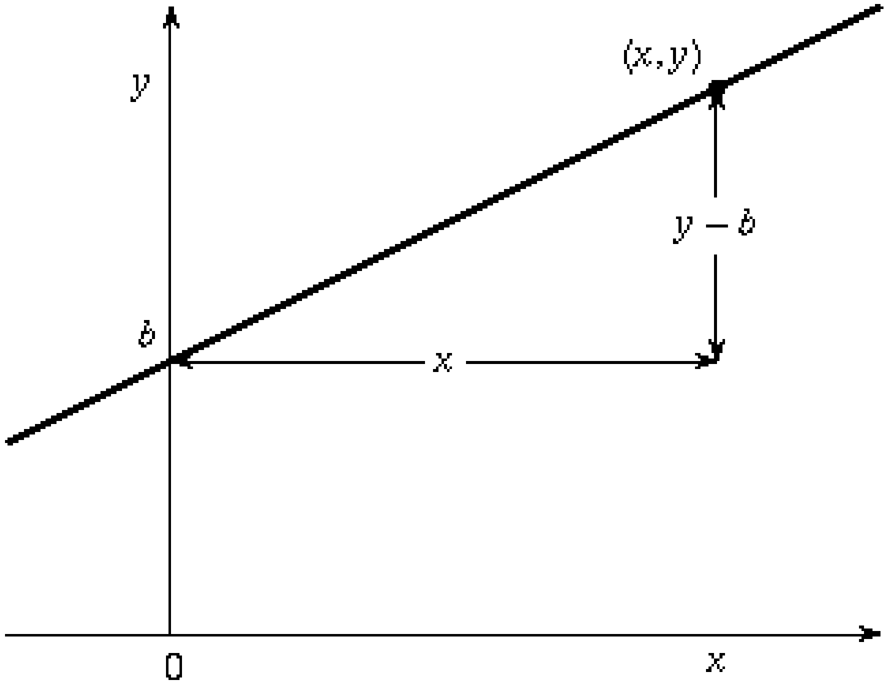
Some values can be negative or nonintuitive, but believe it or not, this can help us reach to the value of V_{max} and k_m (see next slides).



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

$$y = b + mx$$

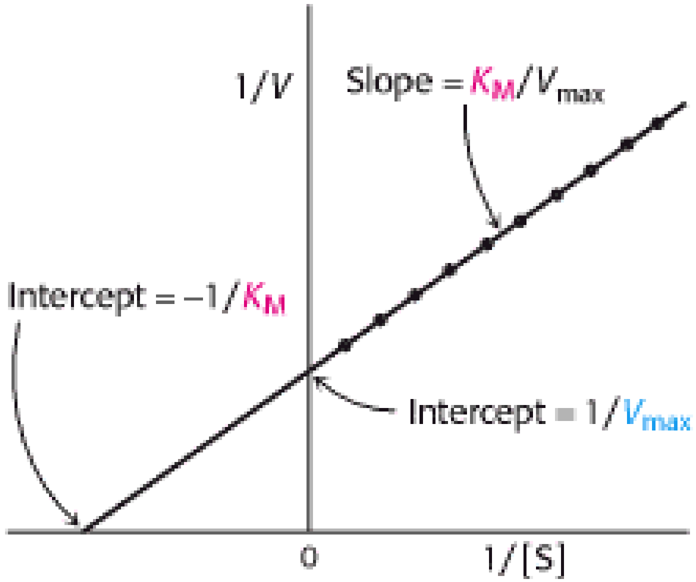
- y is y-axis = 1/ V_o
- x is x-axis = 1/[S]
- m is slope = K_M/ Vmax
- B is 1/Vmax



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

$$y = b + mx$$

- If x = 0, then y = b (x-axis is 0, then y-intercept = 1/Vmax)



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

$$y = b + mx$$

If y = 0, then mx = -b (y-axis is 0, then x-intercept = $-1/K_M$)

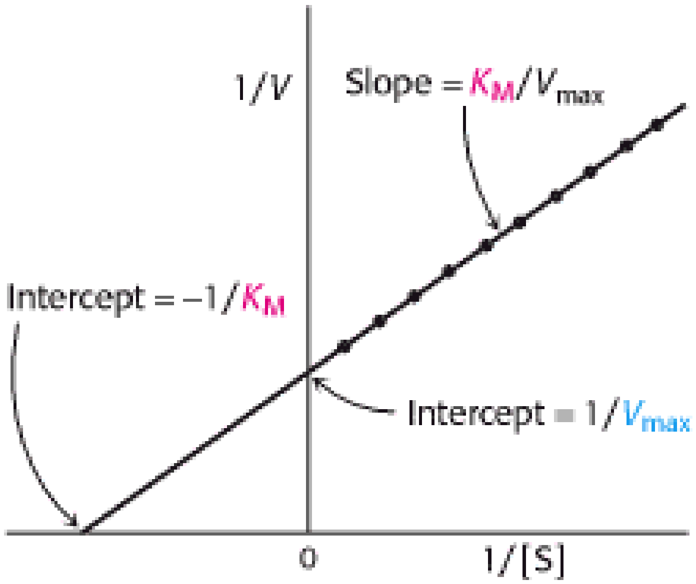
How?

$0 = 1/V_{max} + (K_M/V_{max}) . (1/[S])$

$-1/V_{max} = (K_M/V_{max}) . (1/[S])$

$-1 = K_M . (1/[S])$

$-1/ K_M = 1/[S]$



For any feedback, scan the code or click on it.

Corrections from previous versions:

Versions	Slide # and Place of Error	Before Correction	After Correction
V1 → V2			
V2 → V3			

Additional Resources Used: رسالة من الفريق العلمي:

العلم زينٌ فكن للعلم مكتسباً ... وكن له طالبا ما عشت مقتبساً