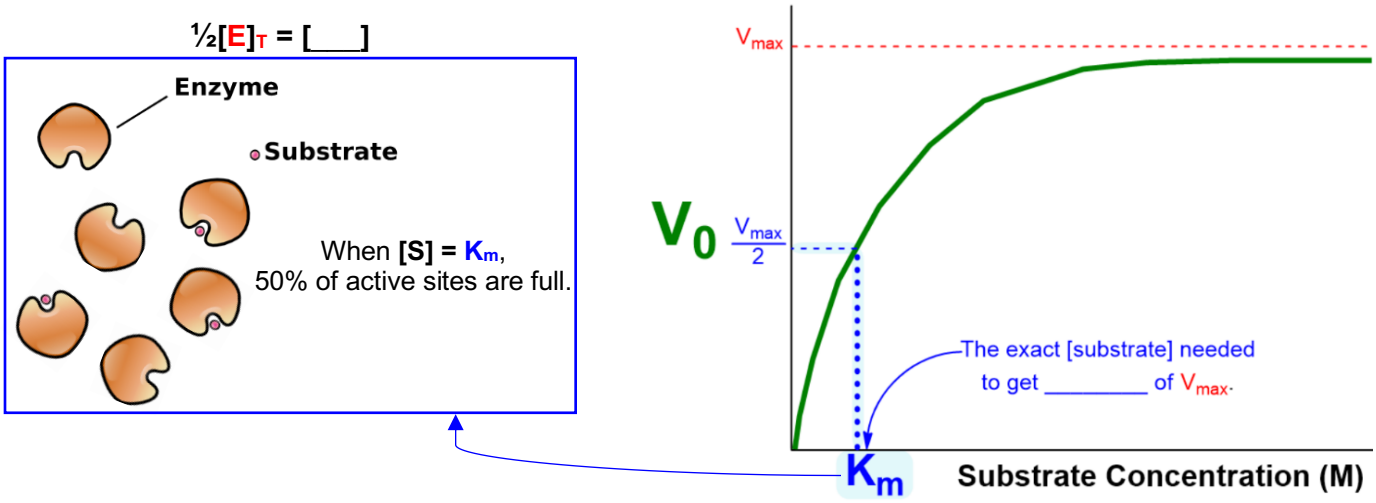
CONCEPT: K_M ENZYME

●Michaelis Constant (_____): exact [substrate] at which V_0 = _____ V_{max}.

□ When [S] = K_m, _____ (½) of all available enzyme active sites are full/occupied with substrate.



●K_m is an *intrinsic* property of an enzyme but can _____ under different conditions (pH, temperature, solvent, etc).

PRACTICE: What is the initial velocity of a reaction when the concentration of substrate is set equal to the K_m?

a) V_0 is equal to V_{max}.
b) V_0 is equal to one-half [S].
c) The [S] is so low that V_0 is negligible.
d) Not enough information to determine V_0.
e) V_0 = one-half V_{max}.

K_m Can Be Expressed with Rate Constants

●Recall: reaction rates (v) can be expressed by *rate laws*, which use rate _____ (k) & there are _____ k *initially*.

□ K_m is defined under *steady-state* conditions as a compilation of these 3 rate constants: $K_m = \frac{K_{-1} + K_2}{K_1}$

V_0 $E + S \underset{K_{-1}}{\overset{K_1}{\rightleftharpoons}} ES \xrightarrow{K_2} E + P$

K_m = ES dissociation / ES association = ___ + ___ / ___

Units = ____

PRACTICE: Select the best description of the K_m.

a) Equal to the product concentration at initial reaction conditions.
b) Equal to the substrate concentration when the reaction rate is half its maximal value.
c) Equal to the ratio of the sum of the ES dissociation rate constants over the ES association rate constant.
d) More than one of the above are true.

CONCEPT: K_M ENZYME

K_m Measures an Enzyme's Affinity for its Substrate

●**K_m** also defined as ratio of [E][S] over the [ES], which measures an enzyme's binding _____ for its substrate.

□ The _____ the **K_m**, the _____ the *binding affinity* an enzyme has for that substrate.

□ **K_m** only indicates *affinity* but does not necessarily indicate the "*preference*" an enzyme has for its substrate.

EXAMPLE: Which enzyme has the stronger affinity for its substrate? a) Enzyme A. b) Enzyme B

$$K_m = \frac{\text{ES dissociation}}{\text{ES association}} = \frac{k_{-1} + k_2}{k_1} = \frac{[\ ][\ ]}{[\ ]}$$

↑ K_m = Dissociation / Association = _____ affinity

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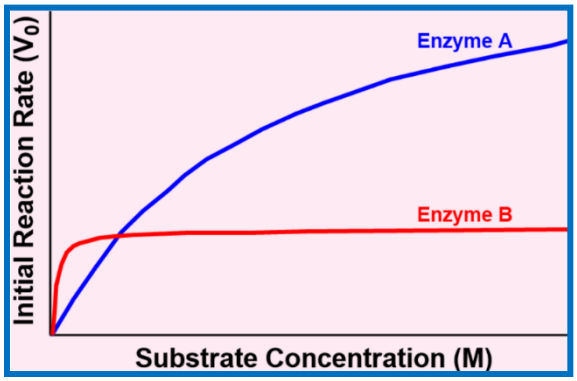


PRACTICE: According to the chart below, which one of the following enzymes has the strongest affinity for its substrate?

a) Lysozyme.
b) Penicillinase.
c) β-Galactosidase.
d) Chymotrypsin.
e) Carbonic anhydrase.

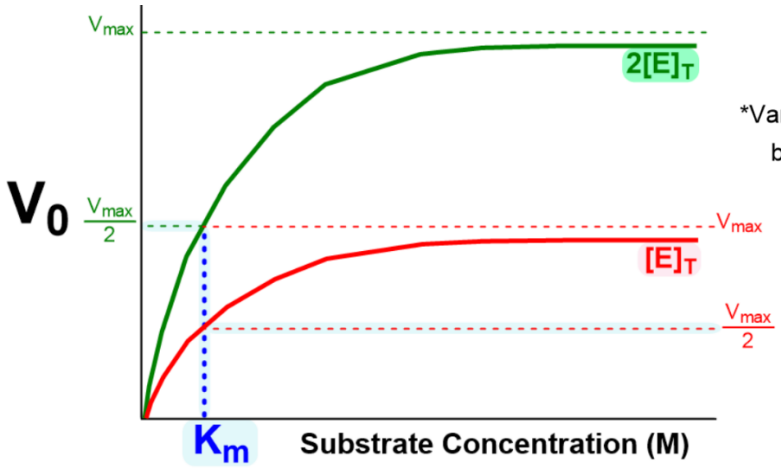
Enzyme	Substrate	K_m (μM)
Lysozyme	Hexa-N-actylhlucosamine	6
Penicillinase	Benzylpenicillin	50
β-Galactosidase	Lactose	4000
Chymotrypsin	Acetyl-L-tryptophanamide	5000
Carbonic anhydrase	Carbon dioxide (CO_2)	8000

$[E]_T$ Does *Not* Affect the K_m

●The Michaelis constant (**K_m**) is an *intrinsic* _____ of an enzyme that is *independent* of the [enzyme].

●When [S] = **K_m**, _____ (½) of all available enzyme active sites are _____, *regardless* of $[E]_T$.

□ Recall: $[E]_T$ _____ both V_0 and V_{max}; *HOWEVER*, altering the $[E]_T$ does *not* affect the _____.



*Varying **$[E]_T$** *changes* **V_0** & **V_{max}**, but does _____ change **K_m**.

CONCEPT: K_M ENZYME

PRACTICE: Indicate which region of the Enzyme Kinetics plot below best corresponds to each statement.

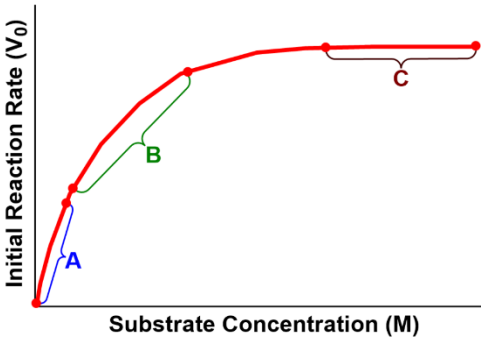
A) Initial reaction velocity is limited mainly by the [S] present: _____

B) Initial reaction velocity limited mainly by the [E] present: _____

C) The active site of an enzyme is most likely free/unoccupied: _____

D) The active site of an enzyme is most likely occupied by substrate: _____

E) This region includes the points corresponding to K_m & ½V_{max}: _____



PRACTICE: Use the data in the following chart to determine the K_m of the enzyme.

a) 1 mM.

b) 1,000 mM.

c) 2 mM.

d) 4 mM.

[Substrate] (mM)	Initial Reaction Rate, V_0 (µmol/min)
0.7	216
2.1	324
4	435
6.1	489
1000	648