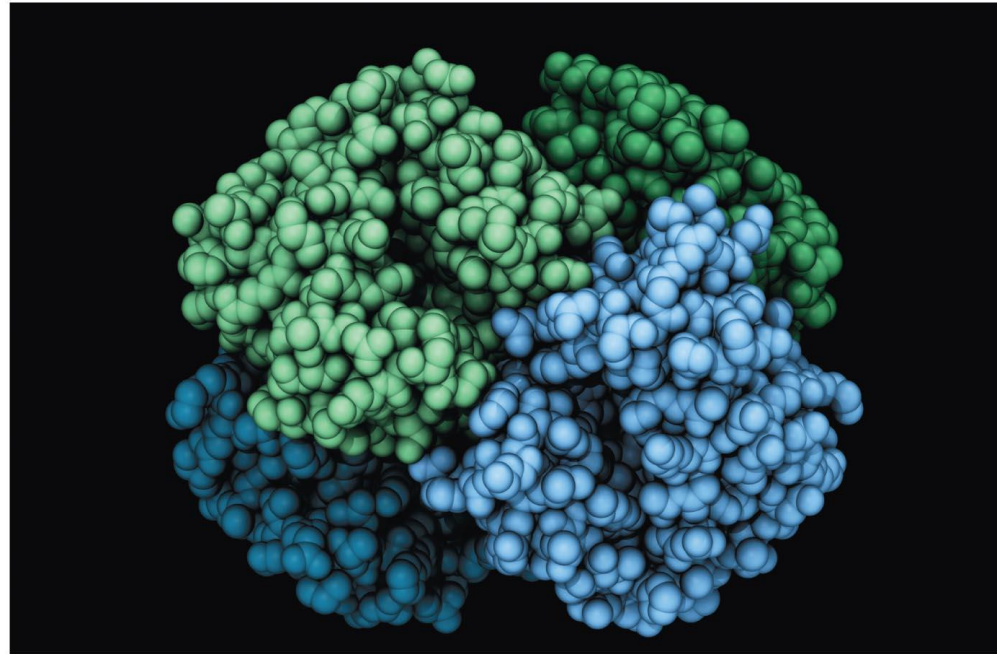


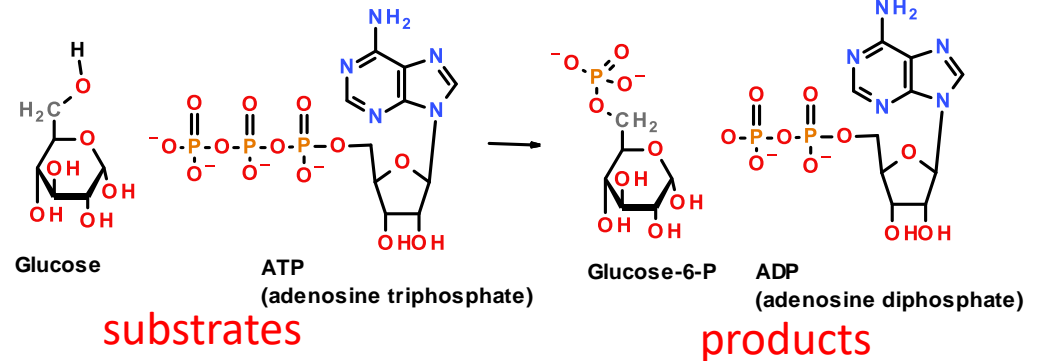
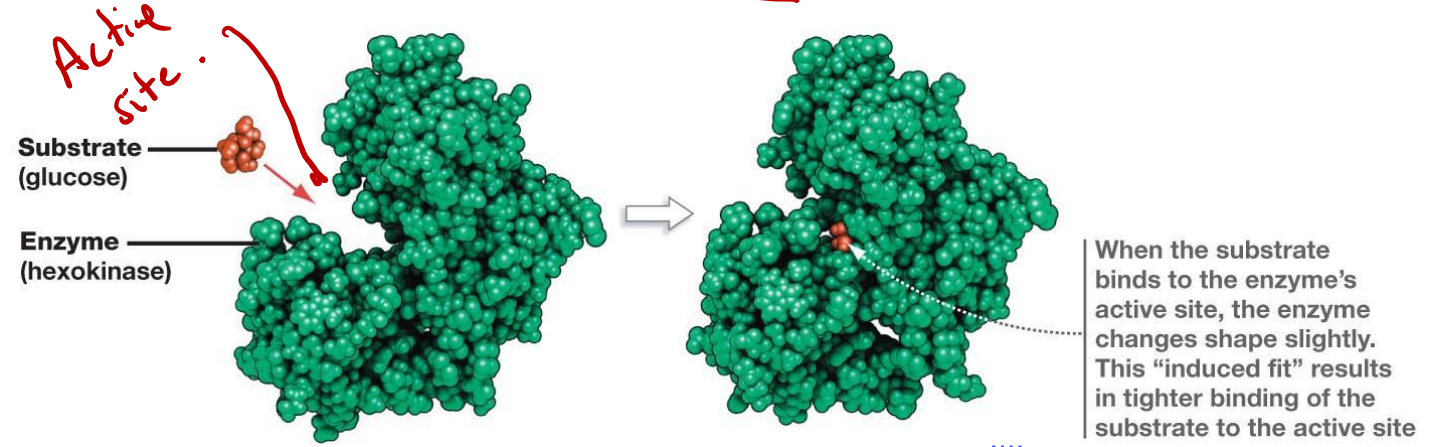
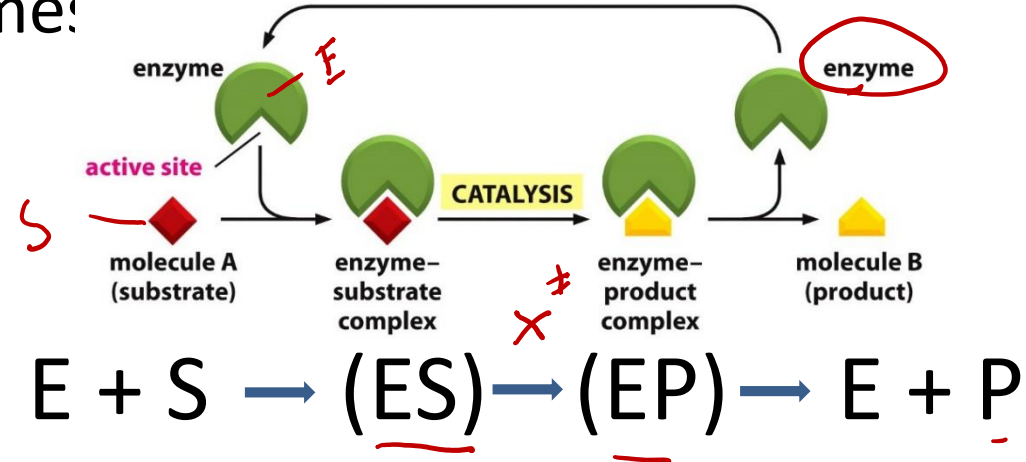
Enzyme Kinetics



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Enzymes:



- **Enzymes** are protein or RNA catalysts. They increase the rate of the reaction.
- They bind “substrates” and convert them to “products”. Usually, the substrate undergoes a chemical reaction and is changed in its structure.
- Substrates bind specifically to the enzyme’s **active site**, interacting with amino acid side chains.
- The chemical change caused by the enzyme is catalyzed by additional functional groups in the active site.
- Many enzymes undergo a conformational change when the substrates are bound to the active site; this change is called an **induced fit**.
- **The rate (or velocity) is the number of products produce/unit time.**

$$v = \frac{dP}{dt}$$

Steady-State Enzyme Kinetics & Inhibitors

Goals:

1. Understand steady-state approximation.
2. Experimental parameter (K_M) related to substrate binding.
3. Experimental parameter (k_{CAT}) related to catalytic efficiency, conversion of bound S to P.

Why?

Raw Data
(v , $[S]$)

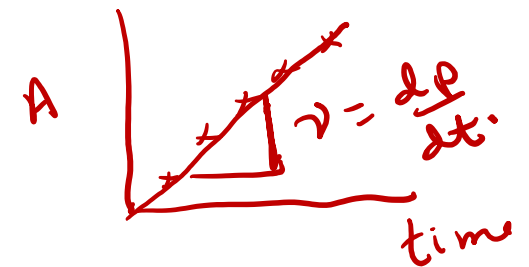
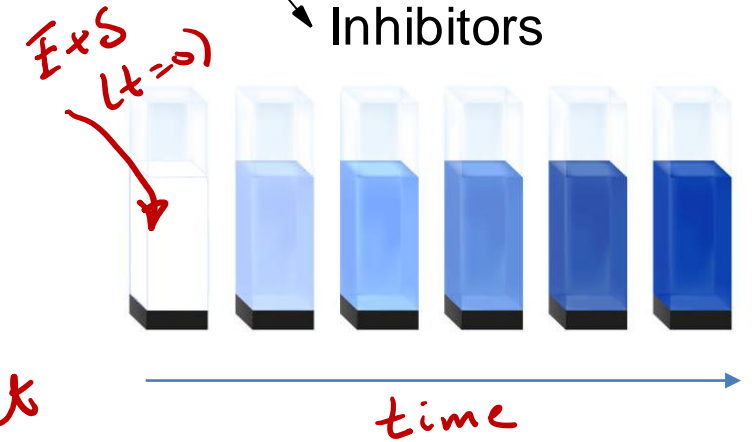
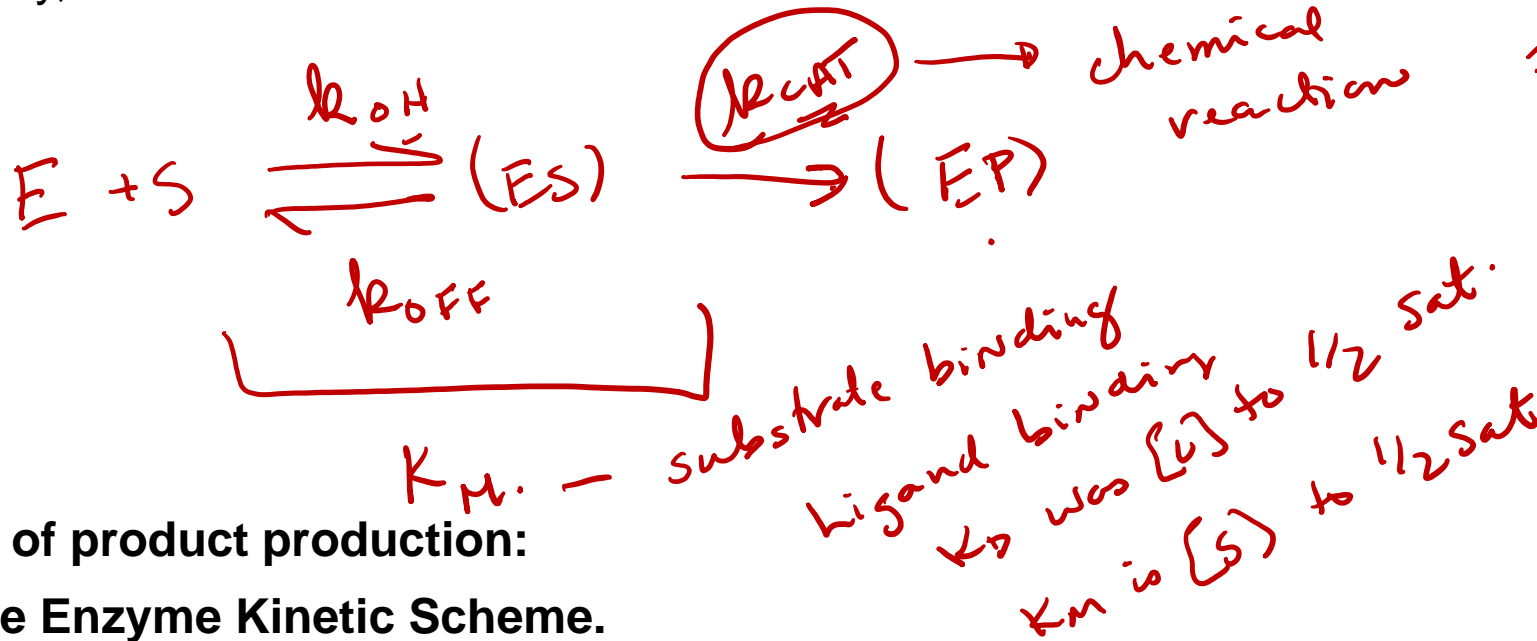
$\rightarrow K_M, k_{CAT}$

Compare systems

Mechanism

Predict rates

Inhibitors



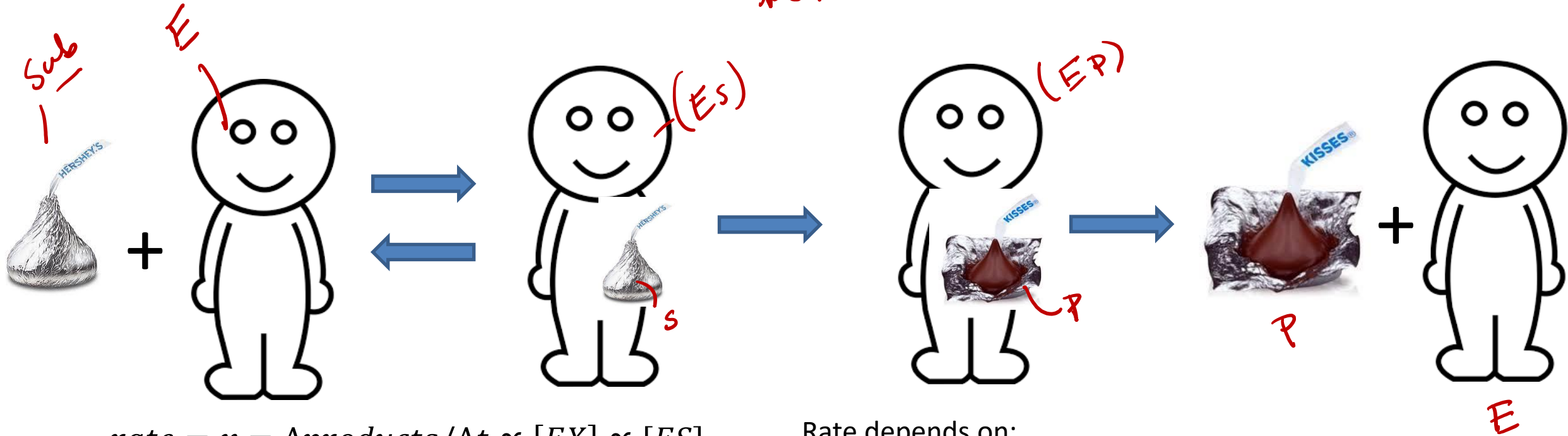
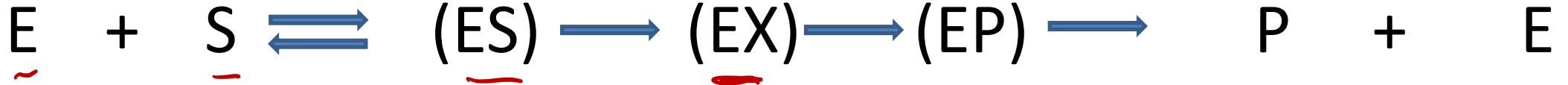
The rate of product production:

Simple Enzyme Kinetic Scheme.

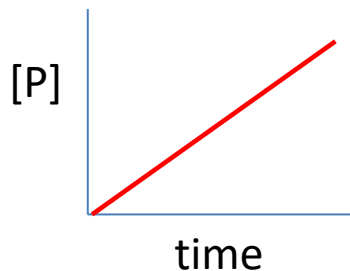
- k_{ON} (also called k_1) is the forward rate constant for substrate binding
- k_{OFF} (also called k_{-1}) is the reverse rate constant for substrate binding
- k_{CAT} (also called k_2) is the catalytic rate constant (containing terms related to the stabilization of the transition state).
- The (ES) complex is also called the "Michaelis complex".

Enzyme Kinetics

Introduction to Enzyme Kinetics



$$\text{rate} = v = \Delta \text{products} / \Delta t \propto [EX] \propto \underline{[ES]}$$



Rate depends on:

- [Substrate] ✓
- [Enzyme] ✓
- pH ✓
- Temperature ✓

A. Empirical Derivation of Rate Law:

Law:

Assume that the rate = $k_{CAT}[ES]$

($k_{CAT} = 1$)

$v = k_{CAT}[ES]$
 $= 1$

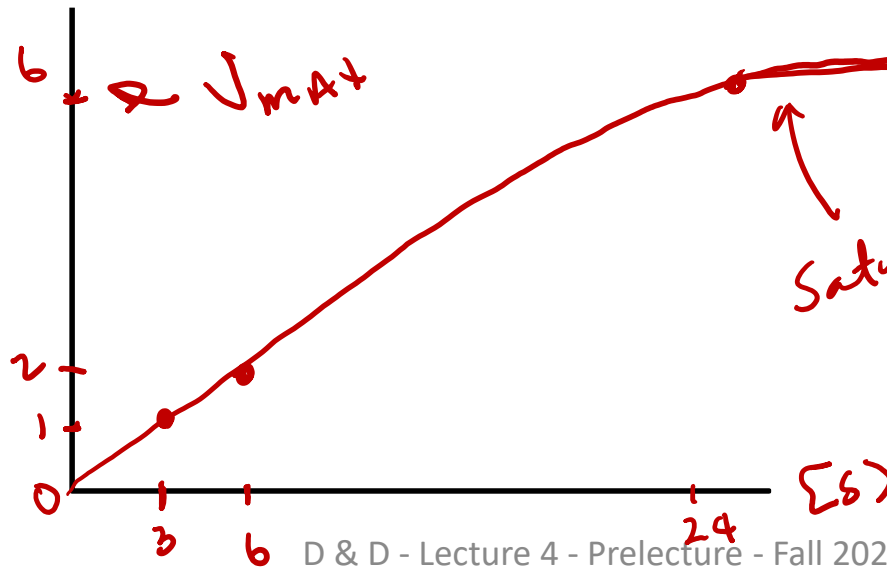
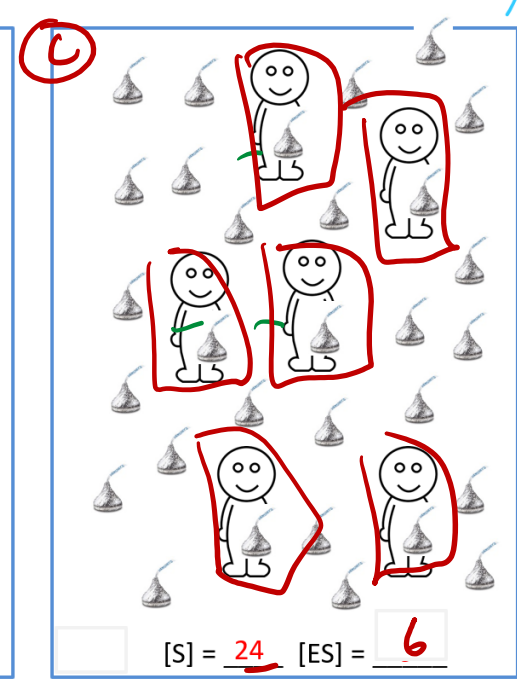
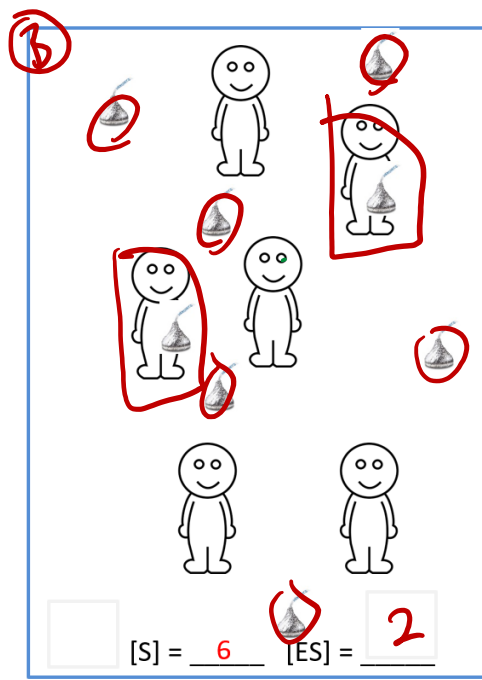
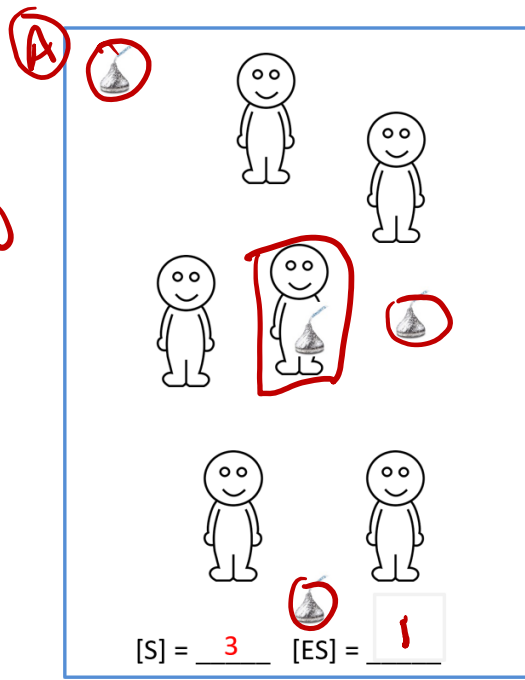
i) How does the rate depend on the substrate concentration, $[S]$?

low $[S]$:

$v \propto [S]$

high $[S]$:

fixed at a maximum value
 all E have bound 100% (ES) form

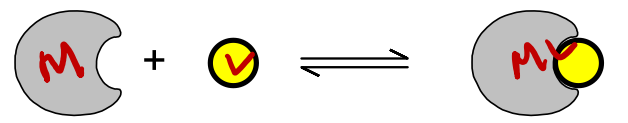


ii) How would you expect the rate to depend on the total amount of enzyme, $[E_{TOT}]$? For example, what happens if you double the amount of enzyme?

Saturated.
 $v \propto [E_T]$
 $[E_{TOT}] = 6$
 $v = 6$
 $2 \times [E_{TOT}] = 12$
 $v = 12$

B. Empirical Derivation of Rate Law:

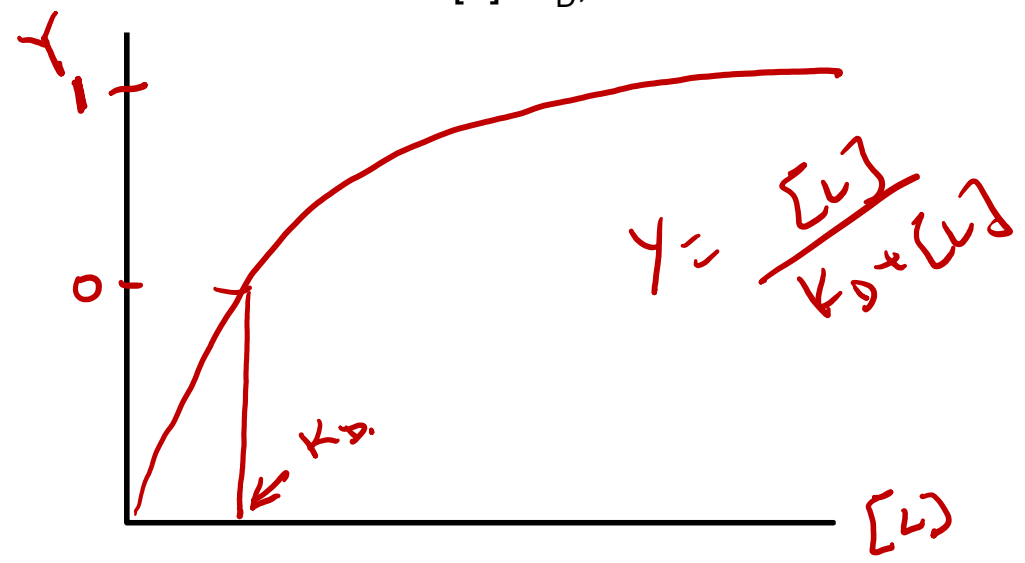
A. Ligand Binding



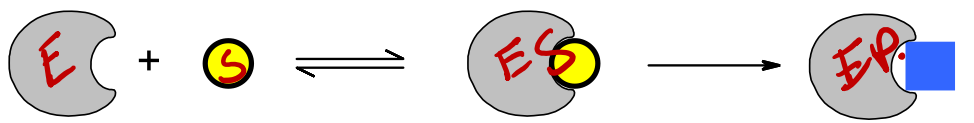
$$Y = \frac{[L]}{K_D + [L]} = \frac{[ML]}{[M] + [ML]}$$

Y is the **fraction** of proteins with substrate bound:

When $[L]=K_D$, $Y=0.5$



B. Enzyme Kinetics



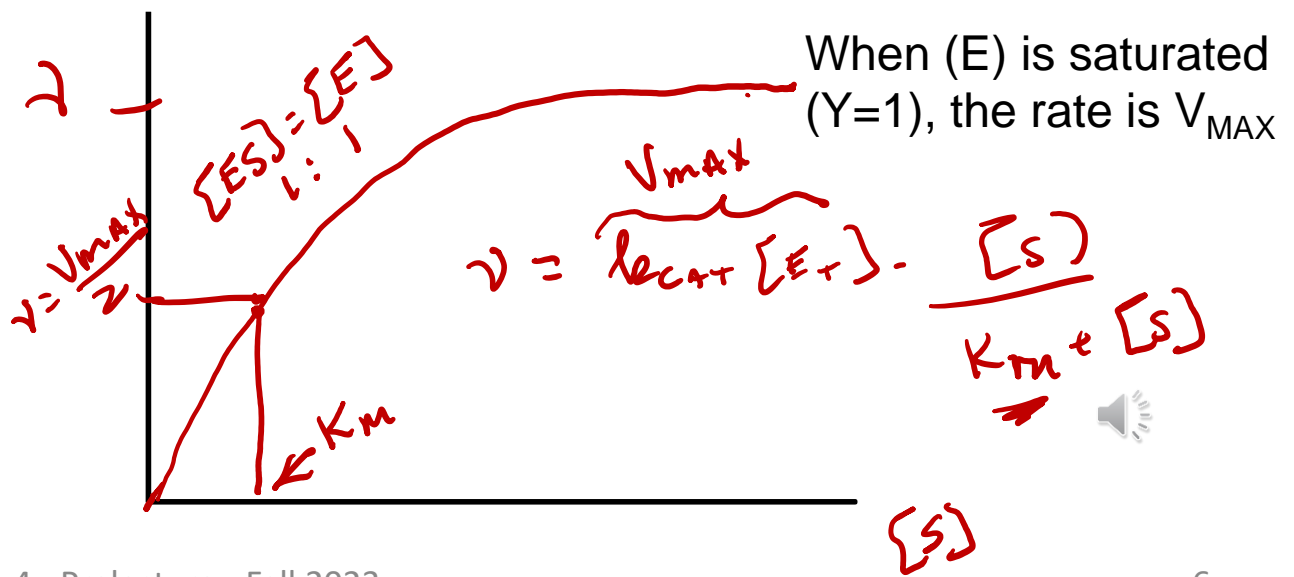
- a. The **total** number of enzymes with substrate bound is:
- b. The rate that each of those converts S to P is k_{CAT} .
- c. The overall rate is:

$$[E_{TOT}] \cdot Y = [ES]$$

$$v = k_{CAT} [ES]$$

$$= k_{CAT} [E_{TOT}] \cdot \frac{[S]}{K_M + [S]}$$

When $[S]=K_{1/2}$, $v=0.5 V_{MAX}$



Analytical Derivation of Rate Law - Steady-State Assumption

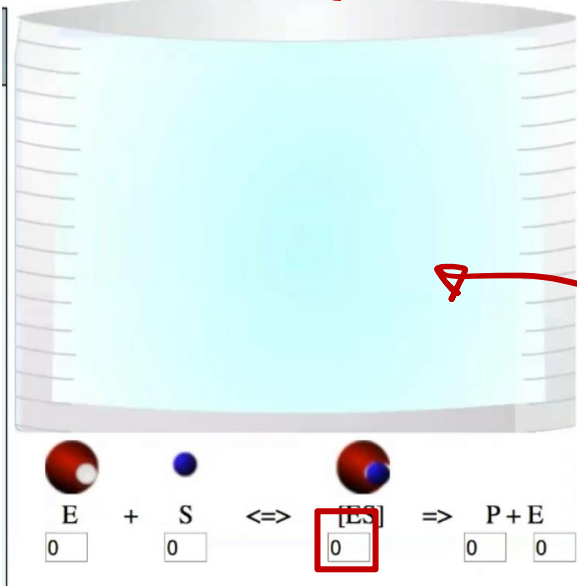
The goal is to relate the kinetic measurements to *readily measurable* experimental parameters:

- i) The total amount of enzyme: $E_{\text{Total}} = [E] + [ES]$ ✓
- ii) the concentration of substrate: $[S]$ ✓
- iii) the measured velocity ($v = k_{\text{CAT}} [ES]$)

$[ES](t)$ can be obtained from the following differential eq.

$$\frac{d[ES]}{dt} = +k_{\text{ON}}[E][S] - k_{\text{OFF}}[ES] - k_{\text{CAT}}[ES]$$

If we make the assumption that we are working under steady-state conditions: $d[ES]/dt = 0$.



What happens to $[ES]$ as function of time?

watch videos



$d[ES]/dt = 0$

$$0 = +k_{\text{ON}}[E][S] - k_{\text{OFF}}[ES] - k_{\text{CAT}}[ES] \text{ and } v = k_{\text{CAT}}[ES] \text{ gives:}$$

$$v = k_{\text{CAT}}[E]_T \frac{[S]}{[S] + \frac{k_{\text{OFF}} + k_{\text{CAT}}}{k_{\text{ON}}}}$$

$$K_M = \frac{k_{\text{off}} + k_{\text{cat}}}{k_{\text{on}}}$$

$$= k_{\text{CAT}} E_{\text{Total}} \frac{[S]}{[S] + K_M}$$

$$= V_{\text{MAX}} \frac{[S]}{[S] + K_M}$$

The last equation is called the **Michaelis-Menton** equation

$$K_{1/2} = K_M = (k_{\text{off}} + k_{\text{cat}}) / k_{\text{on}}$$

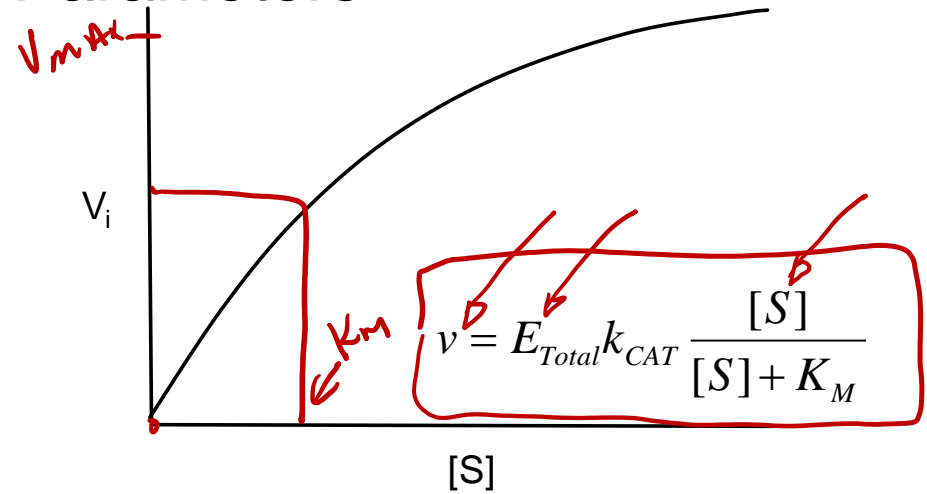
Summary of Kinetic Parameters

i) **The K_M or Michaelis constant:** This is *almost* the same as the $K_D (= k_{off}/k_{on})$, the dissociation constant, except for the presence of the k_{CAT} term. Therefore, it is related to the affinity of a substrate to an enzyme. *It is a constant for any particular enzyme-substrate pair. Substrates with slow off-rates (k_{off}) bind more tightly, and possess a smaller K_M .*

When $[S]=K_M$ the enzyme is $\frac{1}{2}$ saturated with substrate: $v = \frac{1}{2} V_{MAX}$

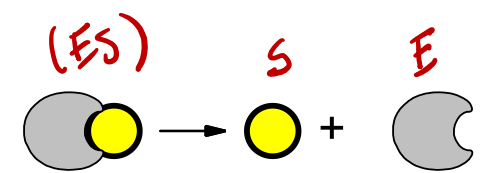
ii) **$V_{MAX} = k_{CAT}[E_T]$:** This is the highest rate of product production possible. It is obtained at high substrate levels ($[S] \gg K_M$). Under these conditions *all* of the enzyme is in the $[ES]$ form (i.e. $[ES]=[E_T]$), the enzyme is **saturated** with substrate. k_{CAT} is obtained from V_{MAX} since the total amount of enzyme is known: $k_{CAT} = V_{MAX}/[E_T]$.

iii) **k_{CAT} is also called the turn-over number** – how many products are produced/sec by a **single enzyme molecule**.



$$K_M = \frac{k_{off} + k_{CAT}}{k_{on}}$$

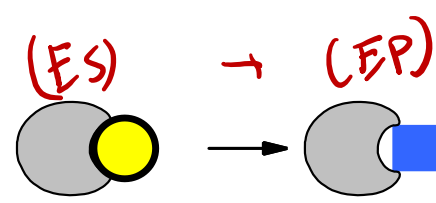
high affinity
low K_M



$K_D = \frac{k_{off}}{k_{on}}$
high affinity
low K_D

$$v = V_{MAX} \frac{[S]}{[S] + K_M} = E_{TOT} k_{CAT} \frac{[S]}{[S] + K_M}$$

$k_{CAT} = 1000 s^{-1}$



$k_{CAT} = \frac{V_{MAX}}{E_{TOT}}$
one enzyme (ES)

k_{cat}

- depends on the enzyme **and** its substrate.
- Wide range of catalytic efficiencies.

K_m

- depends on the enzyme **and** its substrate.
- Wide range of binding affinities

Enzyme	Substrate	k_{cat} (sec^{-1})	K_m (M)	k_{cat}/K_m ($M^{-1} \text{sec}^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2 HCO_3^-	1×10^6 4×10^5	0.012 0.026	8.3×10^7 1.5×10^7
Catalase	H_2O_2	4×10^7	1.1	4×10^7
Crotonase	Crotonyl-CoA	5.7×10^3	2×10^{-5}	2.8×10^8
Fumarase	1 Fumarate	800	5×10^{-6}	1.6×10^8
	2 Malate	900	2.5×10^{-5}	3.6×10^7

$(E+S) \rightarrow (ES)$ capture substrate.

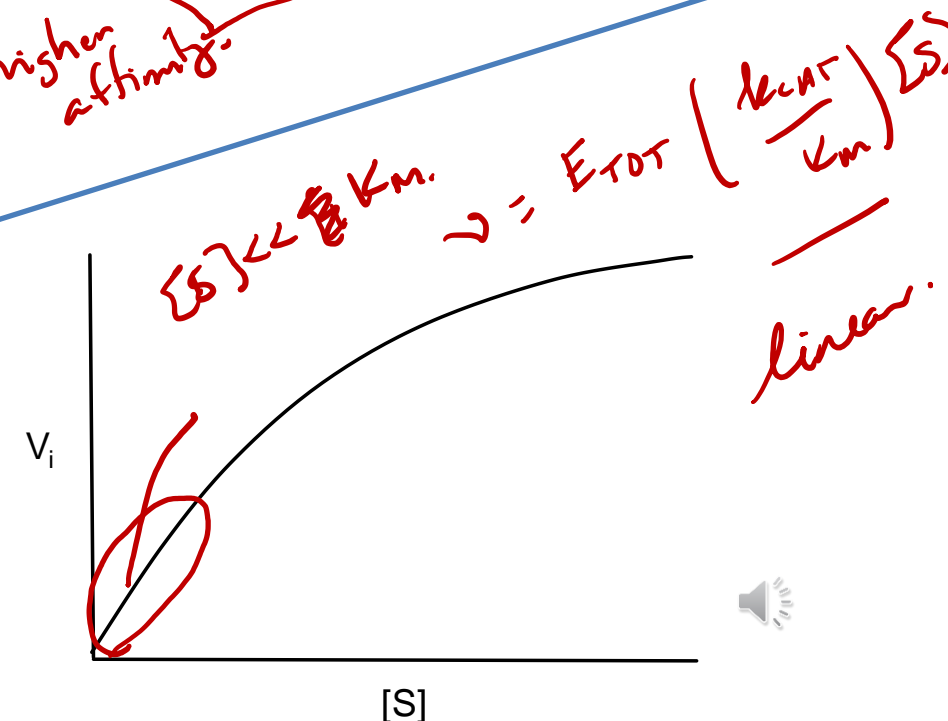
higher affinity

Specificity constant: k_{CAT}/K_M = rate at low substrate. This combines information on the catalytic efficiency (k_{CAT}), and substrate specificity. Useful to predict rates at low $[S]$.

$$v = E_{Total} k_{CAT} \frac{[S]}{[S] + K_M} \rightarrow E_{Total} \left(\frac{k_{CAT}}{K_M} \right) [S]$$

rate constant

Note: k_{CAT}/K_M is often used to compare one enzyme to another, this is usually misleading because it does not separate catalytic efficiency from binding, it is best to compare k_{cat} and K_M separately – they are apples and oranges!

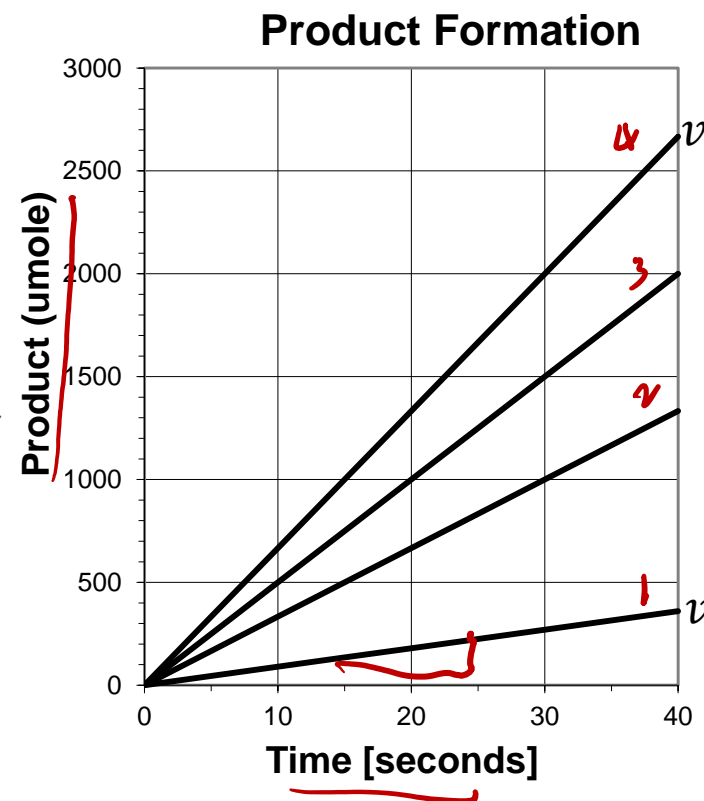
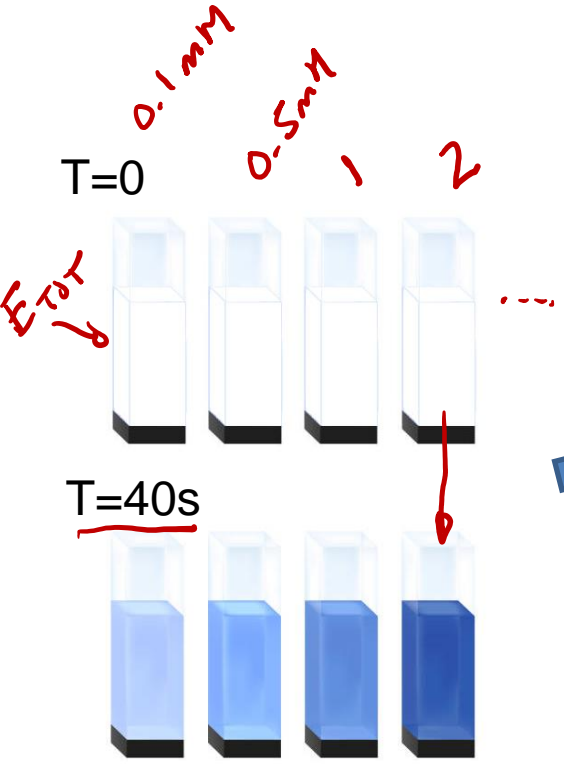


Measuring K_M and k_{CAT} (V_{MAX})

Step A: Measure the *initial* velocity at different substrate concentrations, usually keeping the enzyme concentration *constant*.

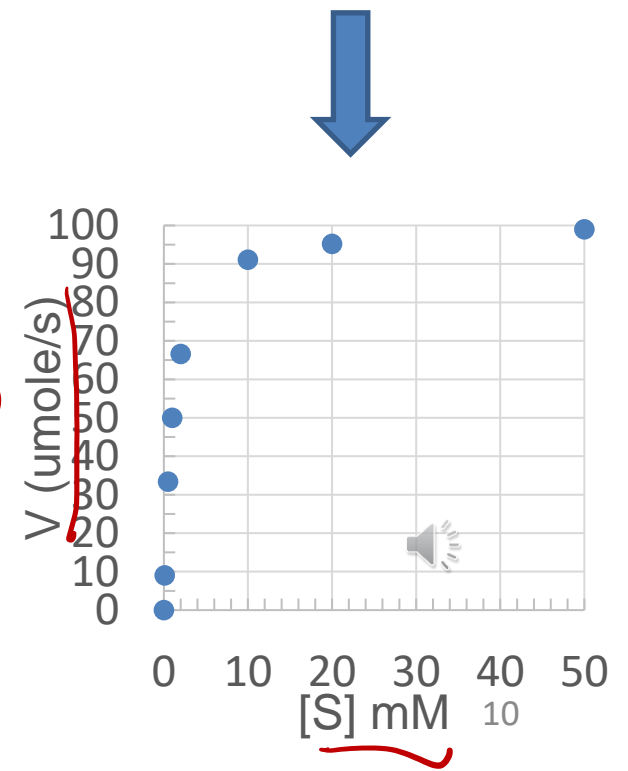
Example: The following velocity data was obtained for a number of substrate concentrations at a fixed enzyme concentration ($[E]_{Tot}=1$ nmoles). Note different units for (S, mM) and (P, umole).

Exp. #	[S] (mM)	v (umoles/s)
1	0.1	9.0
2	0.5	33.4
3	1.0	50.0
4	2.0	66.6
5	10.0	91.1
6	20.0	95.2
7	50.0	99.0



$$v_{(s=2)} = \frac{\Delta P}{\Delta t} = \frac{2664}{40} = 66.6$$

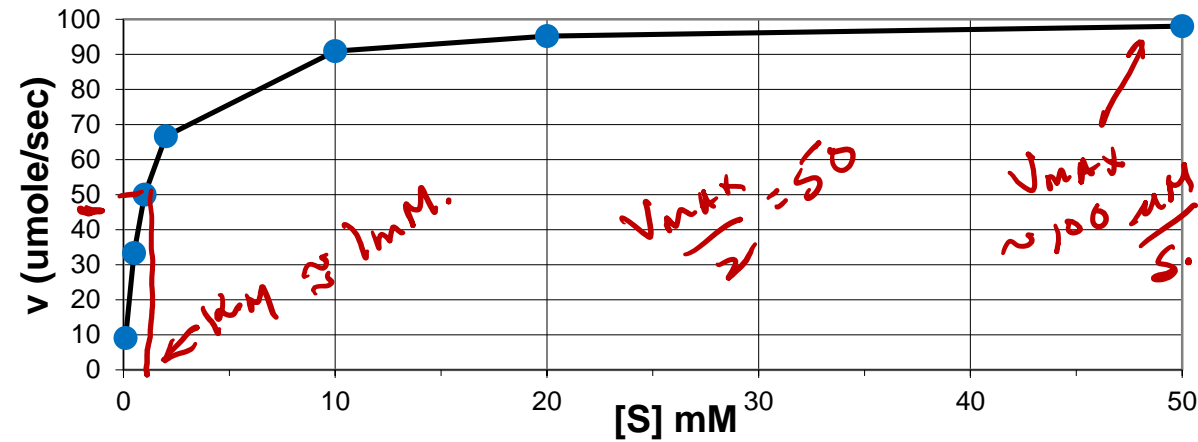
$$v_{(s=0.1)} = \frac{\Delta P}{\Delta t} = \frac{360}{40} = 9$$



Step B: Analyze data → k_m, V_{max}

B1. [S] not limiting - Velocity Curve (Least accurate):

- i) Plot v_{OBS} versus $[S]$.
- ii) Obtain V_{MAX} from v at very high $[S]$ (~ saturated).
- iii) K_M is the substrate concentration at gives $v = V_{MAX}/2$



B2. Double reciprocal plot (Lineweaver-Burk Plot):

Useful graphical tool to identify type of inhibitor and to provide more accurate values for K_M and V_{MAX}

- $[S]$ is extrapolated to ∞ ($1/[S] = 0$)
- i) Take inverse of velocity and $[S]$.
- ii) Plot $1/v$ versus $1/[S]$
- iii) Analysis of double-reciprocal plot:

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

$$\frac{1}{v} = \frac{K_M + [S]}{V_{MAX} [S]}$$

$$\frac{1}{v} = \frac{K_M}{V_{MAX}} \frac{1}{[S]} + \frac{1}{V_{MAX}}$$

y-intercept = $1/V_{MAX}$ 0.01 $V_{MAX} = 100 \frac{\mu\text{mol}}{\text{sec}}$

$k_{CAT} = V_{MAX}/E_{tot} =$
 $([E]_{Tot} = 1 \text{ nmol})$

$k_{CAT} = \frac{100 \times 10^{-6}}{10^{-9}} = 100 \times 10^3 \text{ sec}^{-1} = 10^5 \text{ sec}^{-1}$

Slope = K_M/V_{MAX} $K_M = \text{slope} \times V_{MAX} = 0.01 \times 100 = 1 \text{ mM}$

