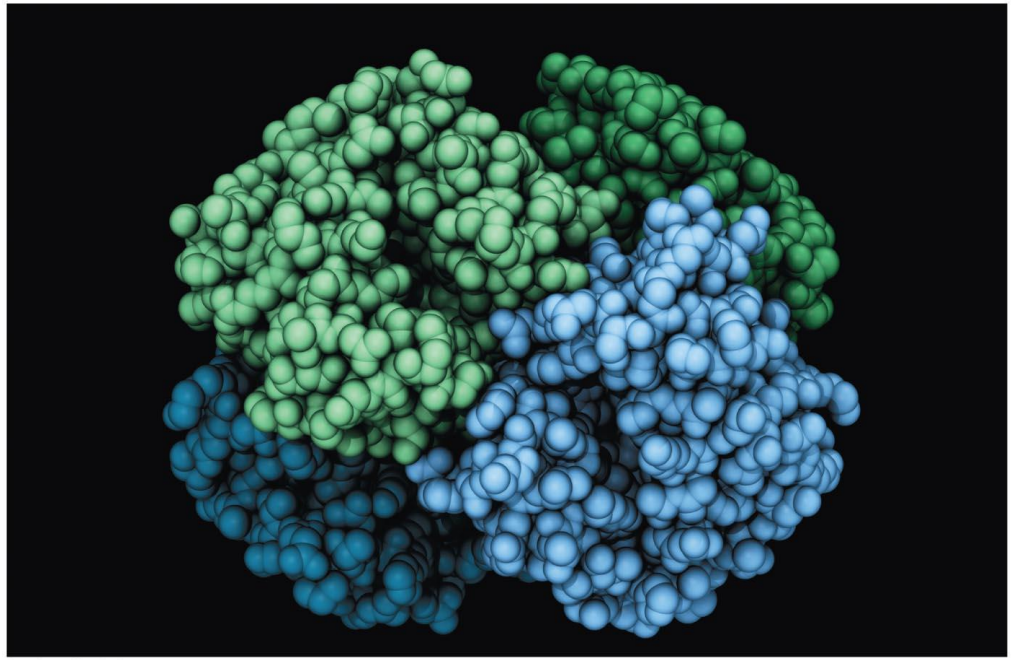
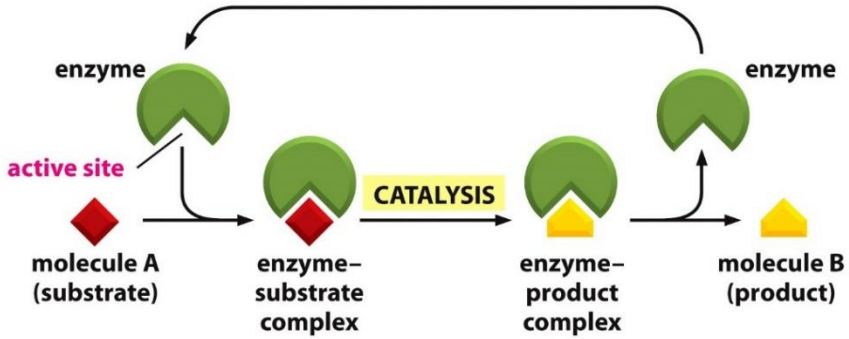
Enzyme Kinetics

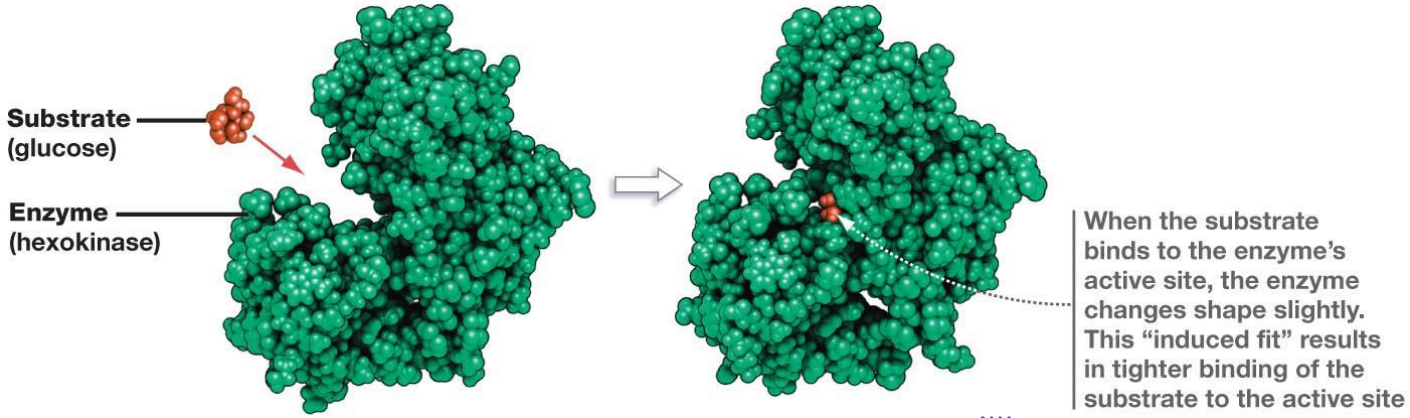


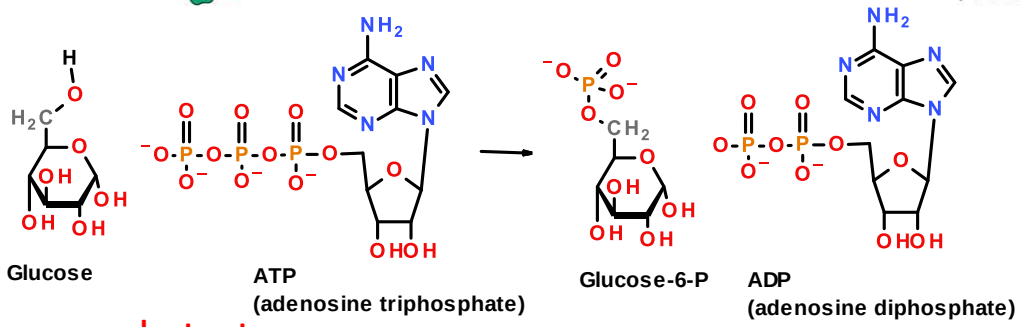
Enzymes

- **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.**



$$E + S \rightarrow (ES) \rightarrow (EP) \rightarrow E + P$$





substrates products

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]) → K_M, k_{CAT} → Compare systems; Mechanism; Predict rates; Inhibitors

The rate of product production:

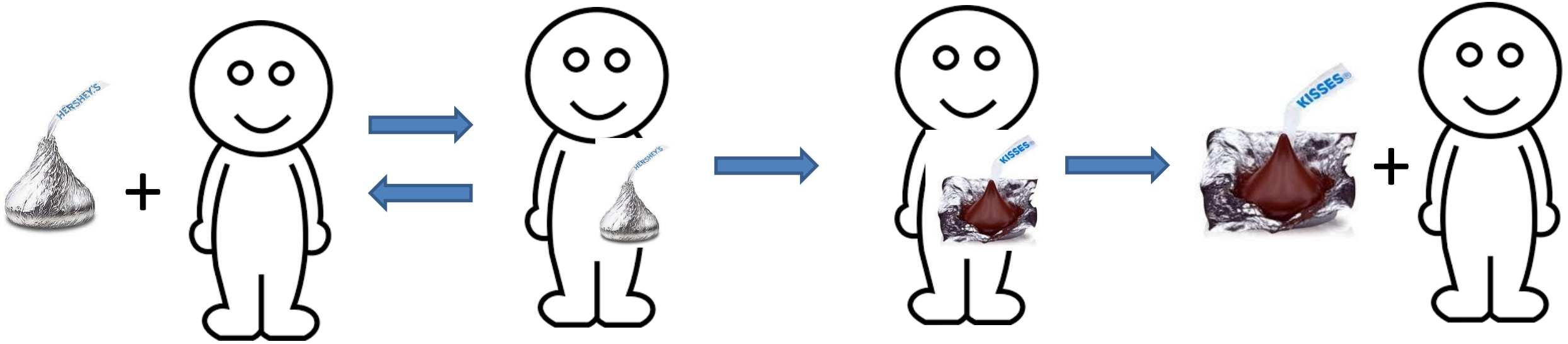
Simple Enzyme Kinetic Scheme.

- 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".

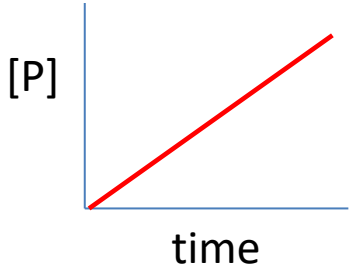
Enzyme Kinetics

Introduction to Enzyme Kinetics

$$E + S \rightleftharpoons (ES) \longrightarrow (EX) \longrightarrow (EP) \longrightarrow P + E$$



$$rate = v = \Delta products/\Delta t \propto [EX] \propto [ES]$$



Rate depends on:

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

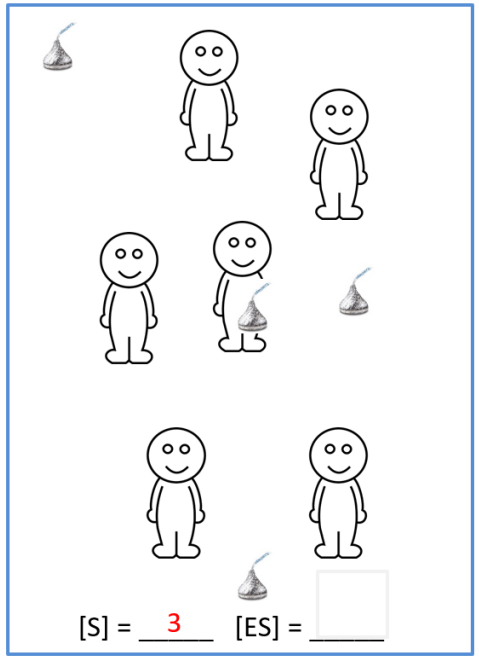
A. Empirical Derivation of Rate Law:

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

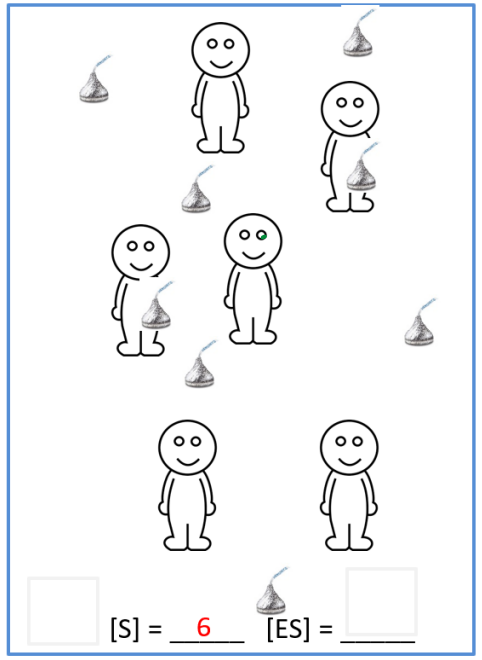
($k_{CAT} = 1$)

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

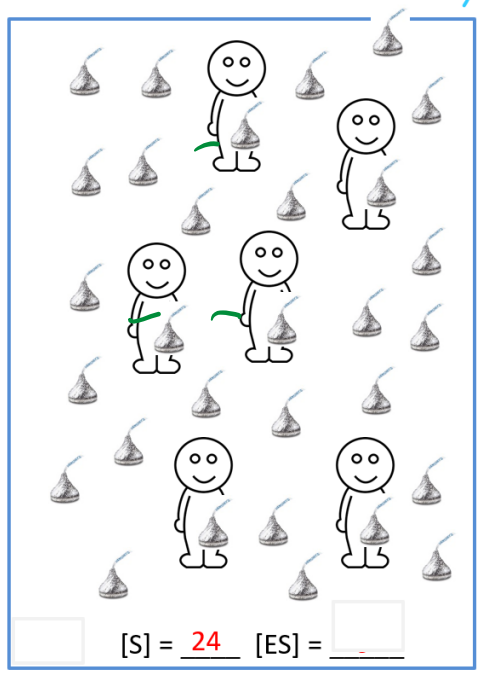
low [S]:



V = ____



V = ____



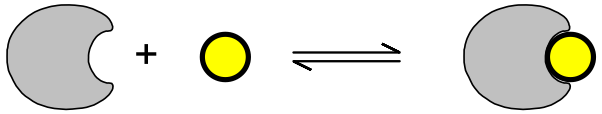
V = ____

high [S]:

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

B. Empirical Derivation of Rate Law:

A. Ligand Binding

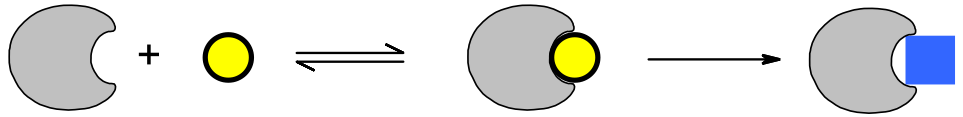


$$Y = \frac{[L]}{K_D + [L]} = \frac{[ML]}{[M] + [ML]}$$

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:

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

When (E) is saturated (Y=1), the rate is 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_{Total} = [E] + [ES]

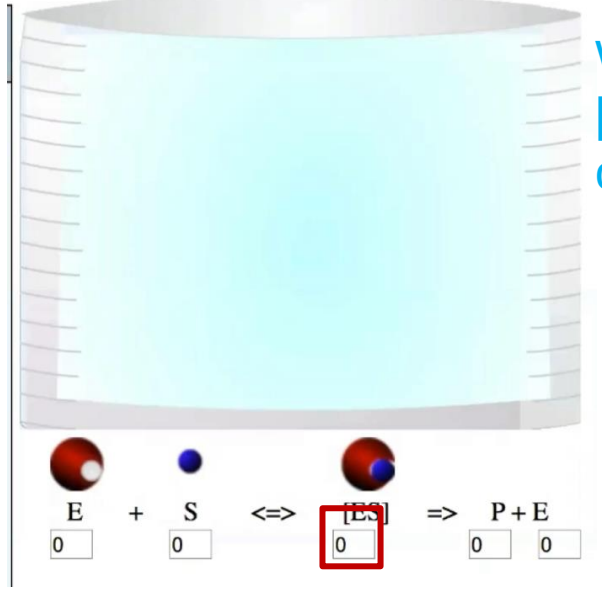
ii) the concentration of substrate: [S]

iii) the measured velocity (v = k_{CAT} [ES])

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

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

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?

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

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

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

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

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

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

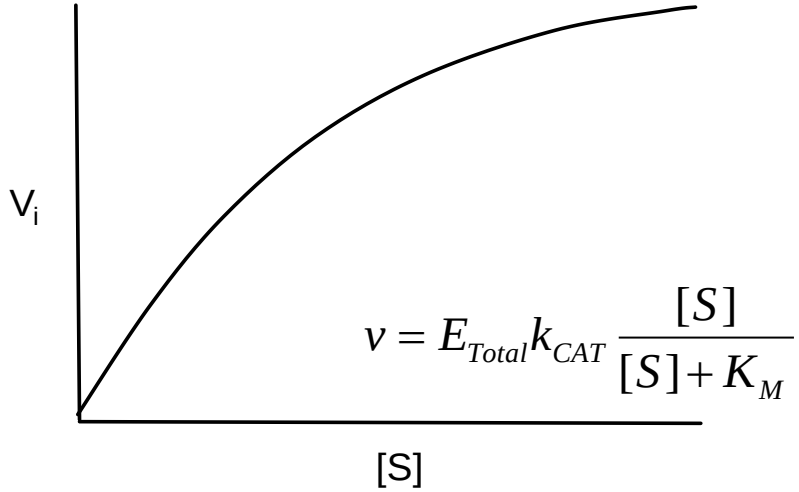
Summary of Kinetic Parameters

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.*

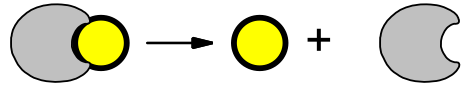
When [S]=K_M the enzyme is ½ saturated with substrate: v = ½ V_{Max}

ii) V_{MAX} = $k_{CAT}[E_T]$: This is the highest rate of product production possible. It is obtained at high substrate levels ([S]>>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: $k_{CAT}=V_{MAX}/[E_T]$.

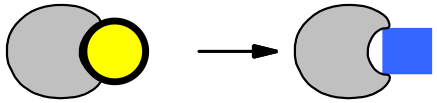
iii) k_{CAT} is also called the turn-over number – *how many products are produced/sec by a **single** enzyme molecule.*



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



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



k_{cat}:

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

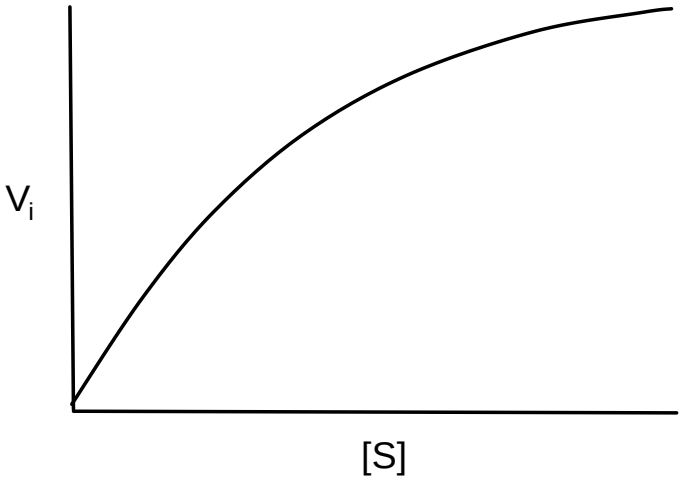
K_m:

- 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 ($M^{-1}\ sec^{-1}$)
Acetylcholinesterase	Acetylcholine	1.4×10^4	9×10^{-5}	1.6×10^8
Carbonic anhydrase	CO_2	1×10^6	0.012	8.3×10^7
	HCO_3^-	4×10^5	0.026	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	Fumarate	800	5×10^{-6}	1.6×10^8
	Malate	900	2.5×10^{-5}	3.6×10^7

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].*

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



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})

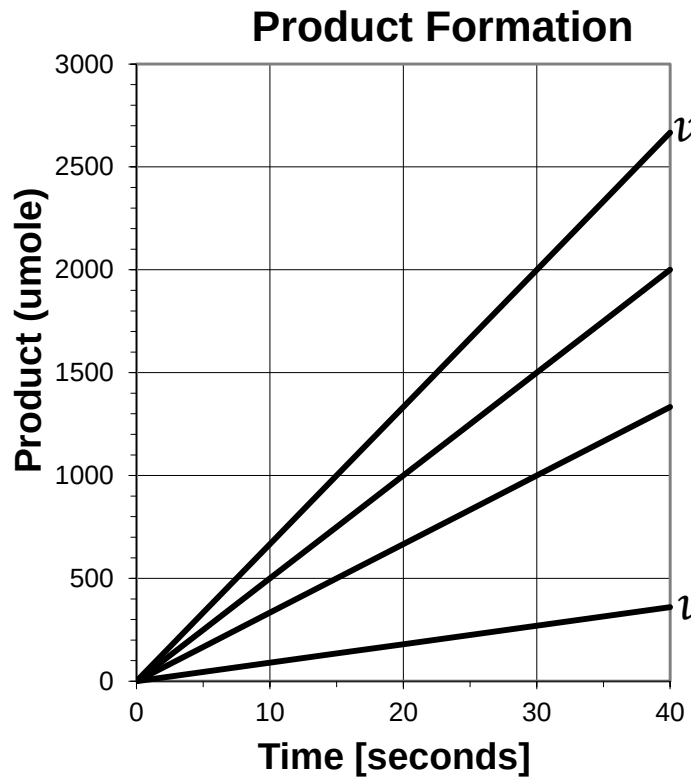
Step A: Measure the *initial* velocity at different substrate concentrations, usually keeping the enzyme concentration *constant*.

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

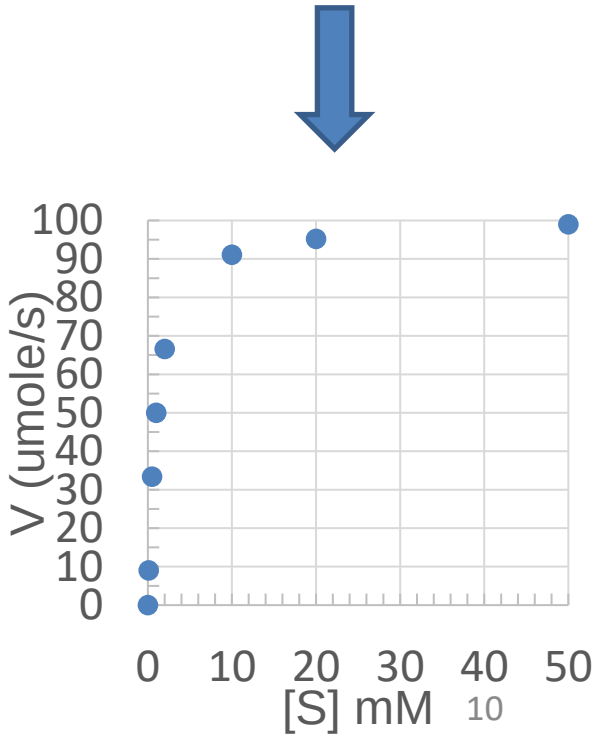
T=0

T=40s

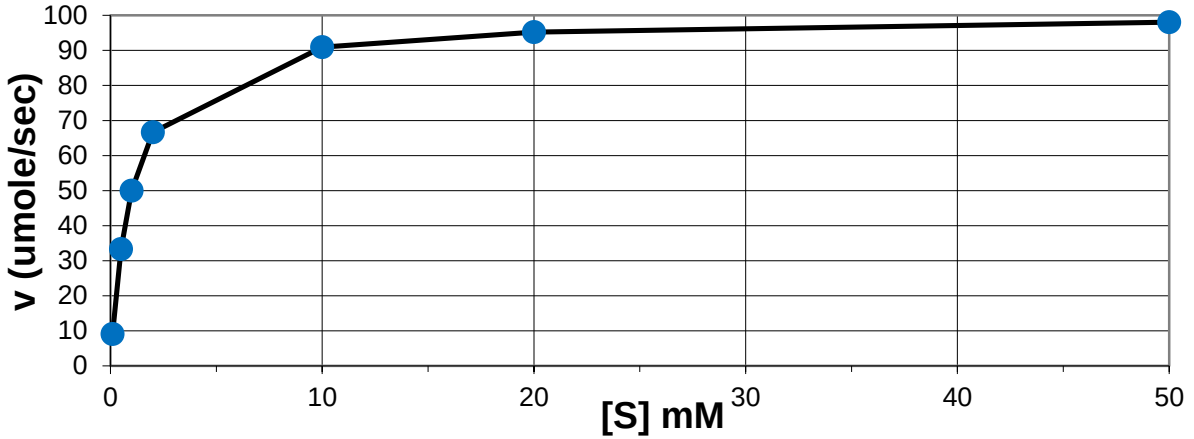


$$v_{(S=2)} = \frac{\Delta P}{\Delta t} = \frac{2664}{40} = 66.6$$

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



Step B: Analyze data



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].

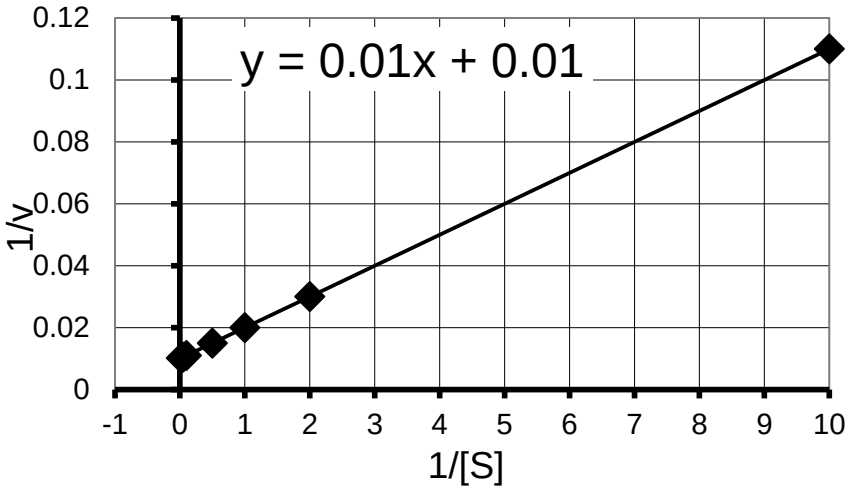
  ii) Plot 1/v versus 1/[S]

  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 = $1/V_{MAX}$ V_{MAX} =

$k_{CAT} = V_{MAX}/E_{tot}$ =

($[E]_{Tot}$=1 nmol)

Slope = K_M/V_{MAX} K_M = slope × V_{MAX} =