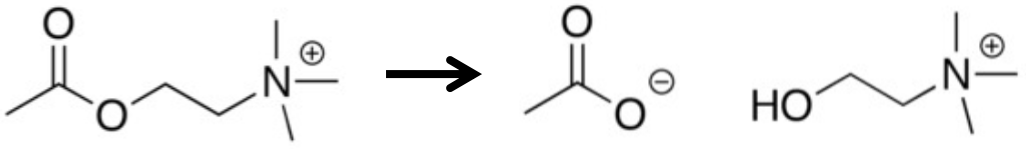
What is an Enzyme?

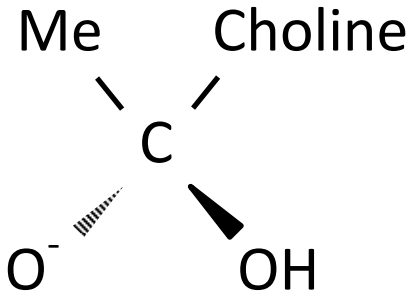
- **Catalyst:** Increases the rate of a reaction without being used up
- **Macromolecule:** Most enzymes are proteins; a few are RNAs
  - Some enzymes require both protein and RNA (e.g., ribosome)
- **Physical topics:** Binding, inhibition, kinetics, transition states

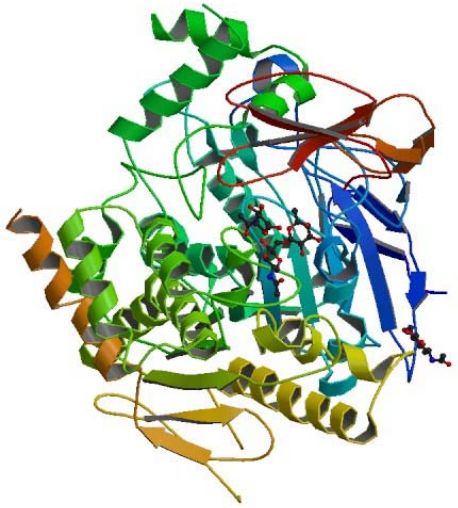
Enzyme Example: Acetylcholinesterase

- Hydrolysis of acetylcholine:



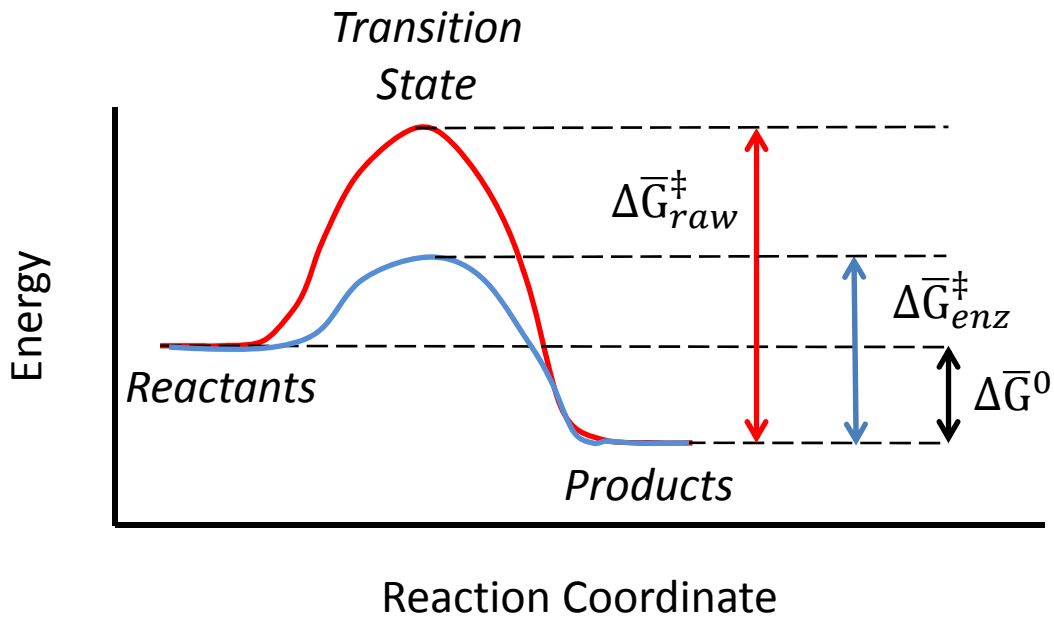
Transition State





"Acetylcholinesterase." *Wikipedia*.

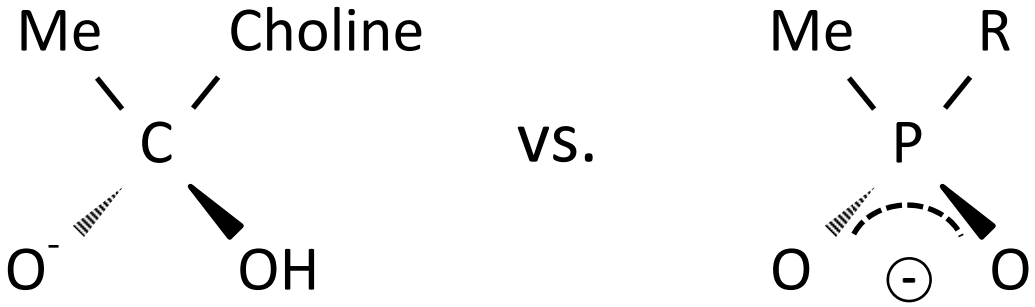
What Does an Enzyme Do?



- Enzymes lower the activation energy by *<u>stabilizing the transition state</u>*

Transition State Analogs

- A good enzyme will bind the transition state:



- A good enzyme will ***<u>not</u>*** bind the reactants or products tightly. Why?

Why Study Enzymes?

- **Interesting:**
  - How does life work?
  - Origins of biochemical processes? (RNA world?)

- **Practical:**
  - Medical applications
  - Engineer better (biologically-based) catalysts
  - Green technology

Enzyme Kinetics

- Decomposition of H_2O_2:

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$

- Observation for constant [catalase]:
  - Low [H_2O_2]: kinetics are first order
  - High [H_2O_2]: kinetics are zero order

- Why? (Discuss)

Enzyme Kinetics

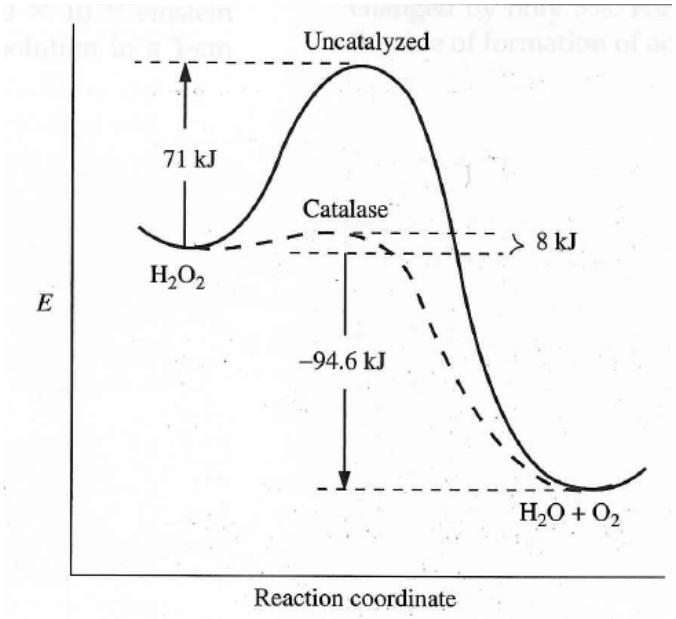


TABLE 8.1 Catalase and H_2O_2 Decomposition: Comparison of Rates and Activation Energies at 25°C*

Catalyst	Rate, $-d[H_2O_2]/dt$ ($M\ s^{-1}$)	E_a (kJ mol^{-1})
None	10^{-8}	71
HBr	10^{-4}	50
Fe^{2+}/Fe^{3+}	10^{-3}	42
Hematin or hemoglobin	10^{-1}	—
$Fe(OH)_2TETA^+$	10^{3}	29
Catalase	10^{7}	8

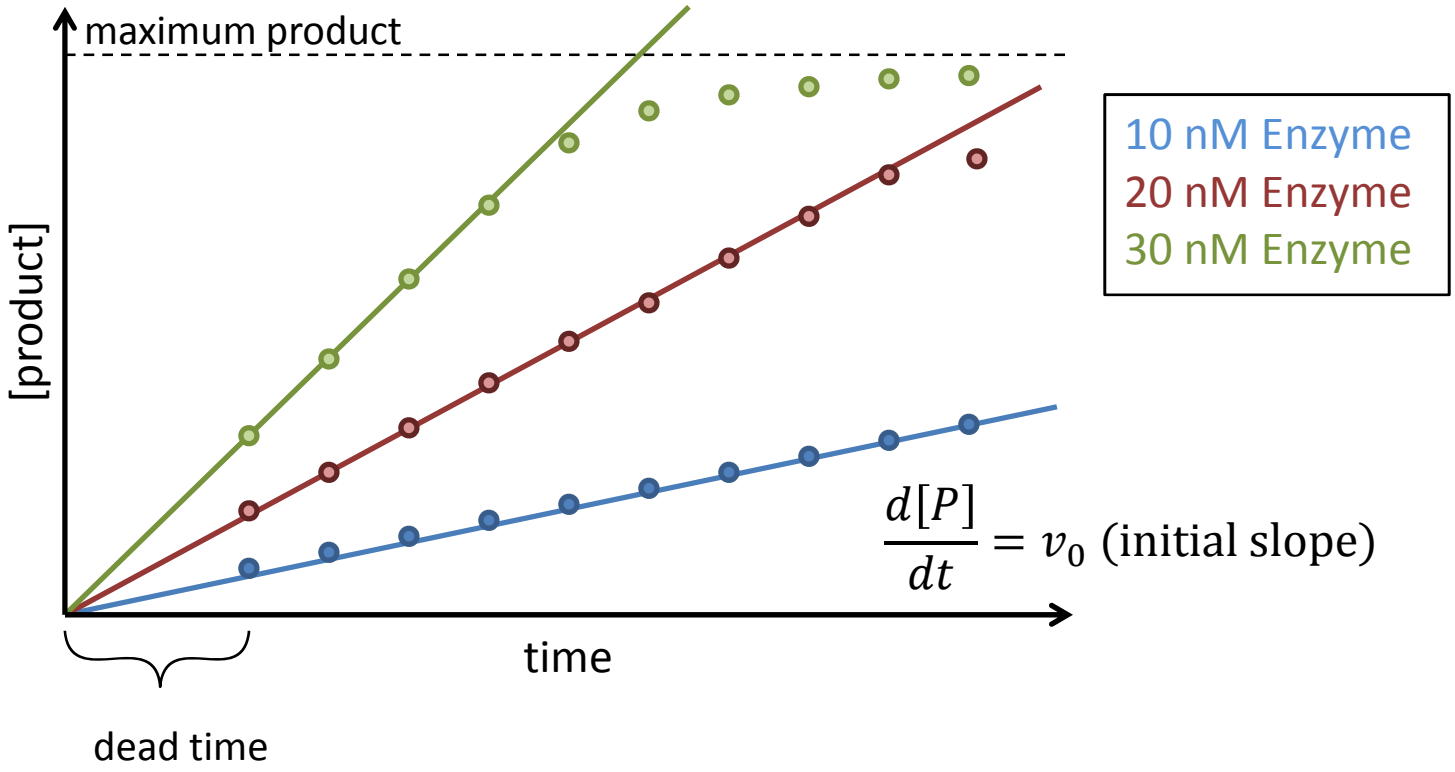
$$-\frac{d[H_2O_2]}{dt} = k[H_2O_2][\text{catalase}]$$

Tinoco, p. 403-4.

Toward a Model for Enzyme Kinetics

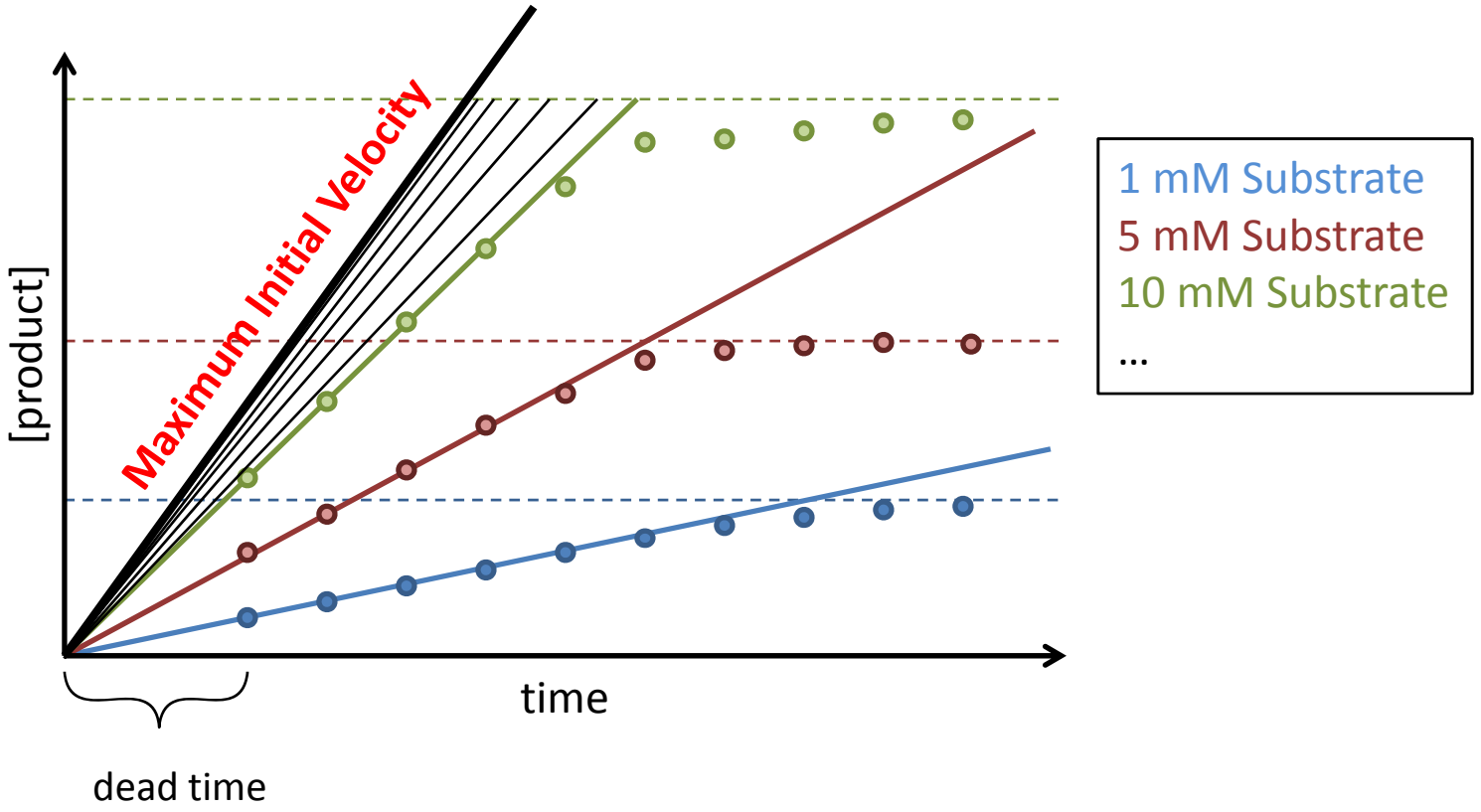
- **Make some observations:**
  - How does rate change with $[H_2O_2]$, enzyme?
- **Propose/change the model:** The hardest part
- **Test the model:**
  - Does it agree with the observations?
- **Try to disprove the model:**
  - Collect more data!

Enzyme Kinetic Data



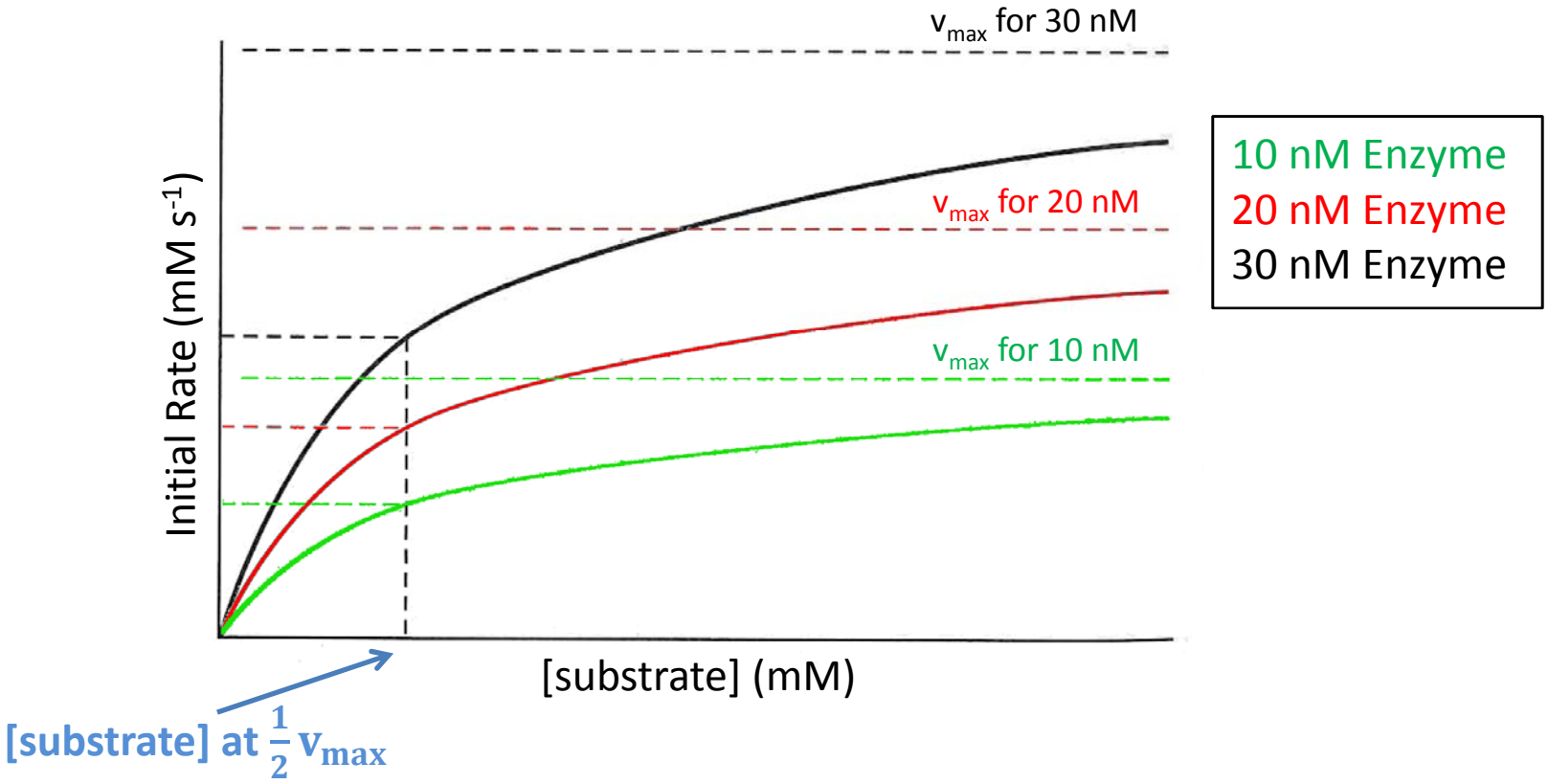
- **Experiment Set #1:** Start with constant concentration of substrate (e.g., $[H_2O_2]$), vary [enzyme]

Enzyme Kinetic Data



- **Experiment Set #2:** Hold [enzyme] constant, vary initial concentration of substrate: *maximum initial velocity*

Collected Enzyme Kinetic Data



- **Note the change in axes!!!**
- Each point on the curves represent a *different experiment*!

Summary of Observations

- Initial velocity is proportional to [S] at low [S]

$$v_0 = k[S]$$

- At high [S], initial velocity approaches a maximum

$$v_0 = v_{max}$$

- Maximum initial velocity is proportional to total enzyme concentration ($[E_0]$)

$$v_{max} = k'[E_0]$$

Michaelis-Menten Kinetic Model

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

- **Irreversible:** applies for initial formation of product

- **Rate equations:**

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$
$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_{cat}[ES]$$
$$\frac{d[P]}{dt} = k_{cat}[ES]$$

Michaelis-Menten Kinetic Model

- **Goal:** Find an expression for v_0 vs. [S], $[E_0]$, see if it agrees with our observations

- **Approximations:**
  - ES complex is at steady-state: $\frac{d[ES]}{dt} = 0$
  - Concentration of complex is small compared to substrate:

$$[S_0] = [S] + [ES] \approx [S]$$

- You *cannot* make the same assumption for $[E_0]$!

$$[E_0] = [E] + [ES]$$

Derivation of MM Kinetics

- **Rate equations:**

$$\frac{d[S]}{dt} = -k_1[E][S] + k_{-1}[ES]$$

$$\frac{d[ES]}{dt} = k_1[E][S] - k_{-1}[ES] - k_{cat}[ES]$$

$$\frac{d[P]}{dt} = k_{cat}[ES]$$

Derivation of MM Kinetics

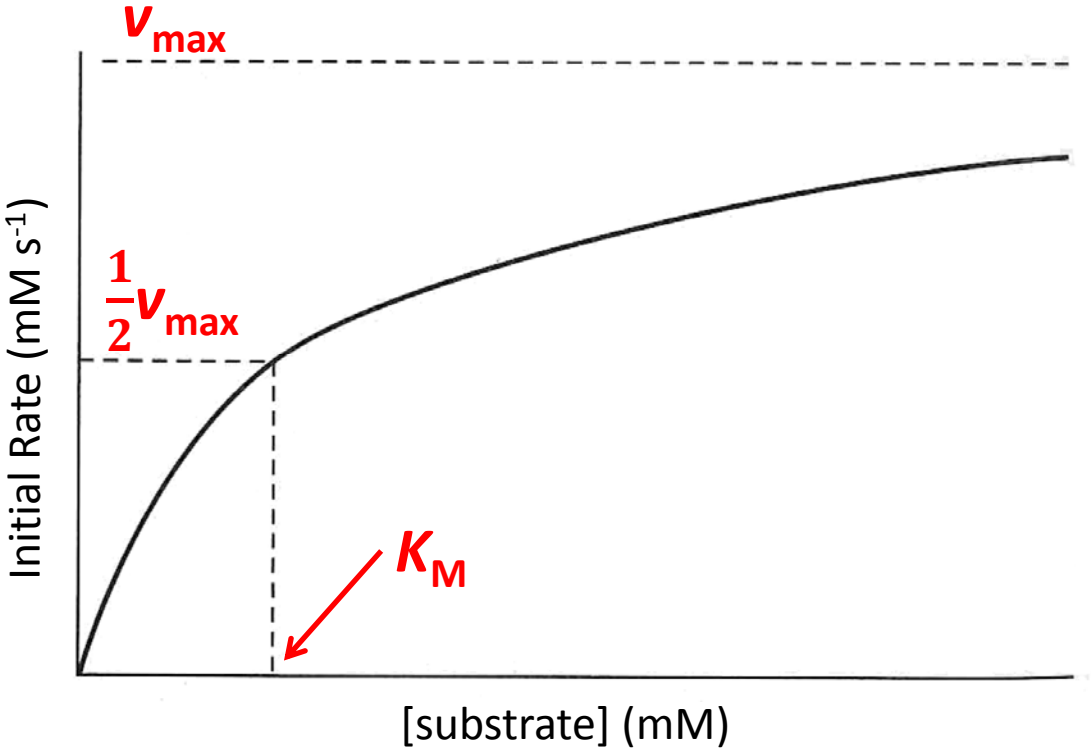
- **Result:**

$$v_0 = \frac{d[P]}{dt} = \frac{k_{cat}[E_0]}{\frac{K_M}{[S]}+1} = \frac{k_{cat}[E_0][S]}{K_M+[S]}$$

- As [S] $\rightarrow \infty$, $v_0 \rightarrow k_{cat}[E_0] = v_{max}$
  - There is a limit to the velocity (check)
  - The limit is proportional to the enzyme concentration (check)

- As [S] $\rightarrow 0$, $v_0 \approx \frac{v_{max}}{K_M}[S]$
  - Rate is proportional to [S] at low [S] (check)

How to Determine K_M, v_{max}?



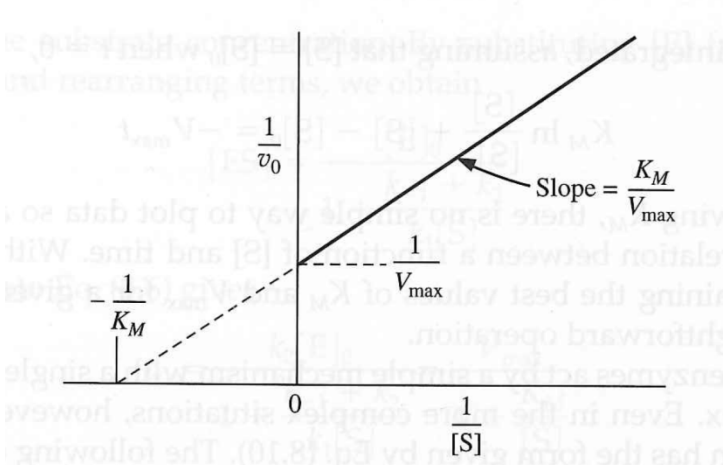
- When [S] = K_M, $v_0 = \frac{1}{2} v_{max}$
- But the asymptote may be hard to determine!

How to Determine K_M, v_{max}?

- **By eye:** This is not usually reliable!
- **Nonlinear fitting:** The best method
  - Requires a computer to optimize parameters
- **Linearize the data:** Good for undergraduates
  - Get K_M, v_{max} from slope, intercept of a line

Linearizing Michaelis-Menten

- Lineweaver-Burk Plot
  - Plot $\frac{1}{v_0}$ vs. $\frac{1}{[S]}$
  - Slope: $\frac{K_M}{v_{max}}$
  - Intercept: $\frac{1}{v_{max}}$



- Mathematical form:

$$\frac{1}{v_0} = \frac{1}{v_{max}} + \frac{K_M}{v_{max}} \cdot \frac{1}{[S]}$$

Tinoco, p. 410.

Linearizing Michaelis-Menten

- Eadie-Hofstee
  - Plot v_0 vs. $\frac{v_0}{[S]}$
  - Slope: $-K_M$
  - Intercept: v_{max}

- Mathematical form:

$$v_0 = -K_M \frac{v_0}{[S]} + v_{max}$$

Complications to Enzyme Kinetics

- Many models, with different parameters, can result in the same functional form as Michaelis-Menten

- Can only detect slowest step

- Forward and reverse reactions can help, but intermediates may complicate interpretation