Chapter 8 - Enzyme Kinetics - Catalysis

\[ k = Ae^{-E_a/RT} \]

**Reactants**

**Products**

**Slow**

**FAST**

**THERMO IS IDENTICAL!**

Forward rate increases,

\[ \Rightarrow A \text{ Catalyst lowers the energy of the transition state.} \]

**Q:** Can a catalyst speed up the forward reaction, but not the reverse?

**A:** No. Thermo does not change

Enzyme is a type of catalyst. Period.

Enzymes/catalysts cannot change thermodynamics.

**N.B.**
Enzymes can speed reactions by $10^n$ or more.

Wow!

**Michaelis-Menten Kinetics**

**HOLD THE BOAT! LET'S UNDERSTAND**

**Kinetic Mechanism**

$$E + S \overset{k_1}{\rightleftharpoons} ES \overset{k_2}{\rightarrow} E + P$$

- Substrate must bind first
- Reaction occurs and product is released.

At very early time, no $P$ is produced.

$[ES]$ increases. As it does, some gets converted to $E + P$. The more $[ES]$, the faster $P$ is produced.

Soon production of $ES$ = Consumption of $ES$

**STEADY STATE**

$$\frac{d[ES]}{dt} = 0 = k_1[E][S] - k_2[ES] - k_1[ES] + k_2[ES]$$
Leads to: \[ \frac{k_1 + k_2}{k_1} = \frac{[E][S]}{[ES]} \]

Looks like an equilibrium, but it's not. It's steady state.

Note that if \( k_1 \) and \( k_1 \gg k_2 \), then
\[ \frac{k_1}{k_1} = \frac{[E][S]}{[ES]} = k_d \quad \text{EQUILIBRIUM} \]

In any case,
\[ \frac{dP}{dt} = k_2[ES] \quad \text{so we need} \quad [ES]. \]

How to solve? \([E], [S], [ES]\) are changing.

Assume that \underline{MASS BALANCE}:

\[ [E]_0 = [E] + [ES] \quad \Rightarrow \quad [E] = [E]_0 - [ES] \]

\[ [S]_0 = [S] + [ES] \quad \Rightarrow \quad [S] = [S]_0 - [ES] \]

\[ \frac{k_1 + k_2}{k_1} = K_M = \left( \frac{[E]_0 - [ES]}{[ES]} \right) \left( \frac{[S]_0 - [ES]}{[S]} \right) \]

Solve for \( x \):
\[ = \frac{(E_0 - \alpha x)(S_0 - x)}{x} \]
Traditional treatment:

If $[S]$ is in large excess

Then $[S] \approx [S]_o$ \quad ([ES] \ll [S]_o)

\[\therefore \quad K_M \approx \frac{(E_o-x) \cdot [S]}{x} \quad \text{Solve for } x\]

\[x = [ES] = \frac{1}{1 + \frac{K_M \cdot [E_o]}{[S]}}\]

\[\therefore \quad \nu_o = \frac{dP}{dt} = k_2 [ES] = \frac{k_2 [E]_o}{1 + \frac{K_M}{[S]}}\]

$k_2[E_o]$ is the velocity you would expect if ALL "E" were bound by substrate.

ie. $[E]_o$ is the maximum value of $[ES]$

So

$\nu_o = \frac{V_{MAX}}{1 + \frac{K_M}{S}}$

\text{CLASSIC EQUATION}

\text{ONLY VALID WHILE } [S] \text{ in excess over } [ES]$
RULER BEST-FIT APPROACHES

Re-arrange to yield:

\[ \frac{1}{V_0} = \frac{1}{V_{\text{MAX}}} + \frac{K_m}{V_{\text{MAX}}} \frac{1}{S} \]

\[ y = b + mx \]

Line-Weaver-Burk

\[ \frac{S}{V_0} = \frac{S}{V_{\text{MAX}}} + \frac{K_m}{V_{\text{MAX}}} \]

Dixon

\[ V_0 = -K_m \frac{V_0}{S} + V_{\text{MAX}} \]

Edlin-Hofstee

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\[ E + S \xrightarrow{k_1} ES \xrightarrow{k_2} ES' \xrightarrow{k_3} ES'' \xrightarrow{k_4} E + P \]

More complex, but still has same form.

\[ k \text{ and } K_m \text{ are new complex functions of } k_1, k_2, k_3, k_4 \]

BE AWARE!!!
\[
\frac{k_{\text{cat}}}{K_M} \Rightarrow \text{specificity constant}
\]

relates rate to \([E] \rightarrow \text{free enzyme}\)

\[
V_0 = \left(\frac{k_{\text{cat}}}{K_M}\right)[E][S]
\]

Free enzyme, not total

:: Comparing two substrates A and B

\[
\frac{V_A}{V_B} = \frac{(\frac{k_{\text{cat}}}{K_M})_A [A][S]}{(\frac{k_{\text{cat}}}{K_M})_B [B][S]}
\]

For \([A] = [B]\)

\[
\frac{V_A}{V_B} = \frac{\left(\frac{k_{\text{cat}}}{K_M}\right)_A}{\left(\frac{k_{\text{cat}}}{K_M}\right)_B}
\]

INHIBITION - SEE TEXT