11/20/19 - SECOND-ORDER KINETICS.

$$E + S \underset{k_r}{\overset{k_f}{\rightleftharpoons}} E{\cdot}S \underset{k_-}{\overset{k_{cat}}{\rightleftharpoons}} E + P$$

WRITE A MARKOV MATRIX FOR THIS REACTION:

	E	S	E·S	P
E	P_{ee}	∅	$k_r + k_{cat}$	∅
S	∅	P_{ss}	k_r	∅
E·S	$k_f \cdot [S]$	$k_f [E]$	$P_{es/es}$	$k_-[E]$
P	∅	∅	k_{cat}	P_{PP}

NOT SOLVABLE

① SECOND ORDER:

$$\frac{1.0}{dt} = P_{ee} + k_f[S]$$

↑ [S] CHANGES OVER TIME!

↗ TREAT AS 3D MATRIX...

TO SOLVE: MAKE ASSUMPTIONS TO PRETEND IT'S FIRST-ORDER.

GO BACK TO RATE LAWS:

$$\frac{dE}{dt} = -k_f[E][S] + k_r[ES] + k_{cat}[ES] - k_-[P][E]$$

$$\frac{dS}{dt} = -k_f[E][S] + k_r[ES]$$

$$\frac{dES}{dt} = k_f[E][S] - k_r[ES] - k_{cat}[ES] + k_-[P][E]$$

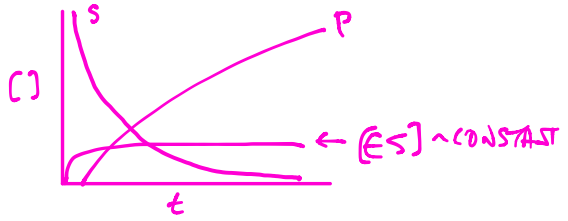
$$\frac{dP}{dt} = k_{cat}[ES] - k_-[P][E]$$

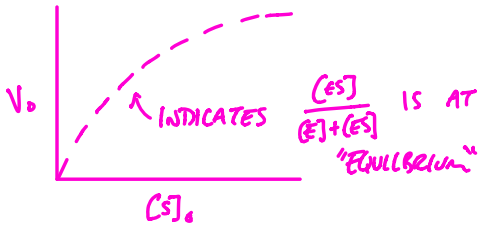
① ASSUME $k_{cat} >> k_-$ (IRREVERSIBLE)

↓

TRUE @ $[S]_0$ WHEN $[P] = \emptyset$

② ASSUME $[ES]$ ~ QUASI STEADY STATE ← TRUE @ $[E] << [S]$





$$\frac{d[ES]}{dt} \sim \emptyset = k_f[E][S] - k_r[ES] - k_{cat}[ES]$$

$$\emptyset = k_f [E][S] - (k_r + k_{cat})[ES]$$

$$(k_r + k_{cat})[ES] = k_f [E][S]$$

$$\frac{[ES]}{[E][S]} = \frac{k_f}{k_r + k_{cat}} \equiv \alpha$$

$$[E]_0 = [E] + [ES]$$

$$\frac{[ES]}{([E]_0 - [ES])[S]} = \alpha$$

$$[ES] = \alpha [E]_0 [S] - \alpha [ES][S]$$

$$[ES] + [ES]\alpha [S] = \alpha [E]_0 [S]$$

$$[ES](1 + \alpha [S]) = \alpha [E]_0 [S]$$

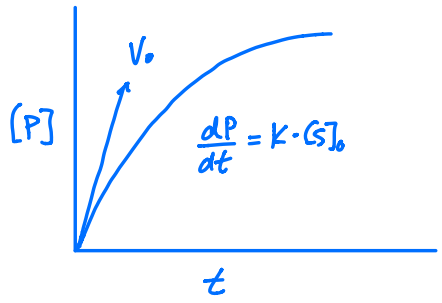
$$[ES] = \frac{\alpha [E]_0 [S]}{1 + \alpha [S]}$$

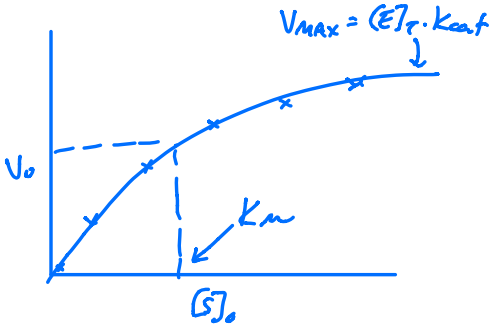
$$= \frac{[E]_0 [S]}{1/\alpha + [S]} \qquad K_M \equiv 1/\alpha = \frac{k_r + k_{cat}}{k_f} \leftarrow \begin{matrix} \text{AFFINITY} \\ \text{COMING APART} \end{matrix} \sim K_D$$

$$[ES] = \frac{[E]_0 [S]}{K_M + [S]}$$

$$\frac{dP}{dt} = k_{cat}[ES] = k_{cat}[E]\left(\frac{[S]}{K_M + [S]}\right)$$

k_{cat} : RATE OF CATALYSIS (FIRST ORDER!)
K_M : ~BINDING AFFINITY FOR SUBSTRATE





STRATEGIES FOR DISSECTING COMPLICATED RXN:

1) FIND REACTIONS YOU CAN IGNORE (P + E → ES, FOR EXAMPLE)

2) FIND SIMPLIFYING CONDITIONS: (STEADY STATE, INITIAL, EQUILIBRIUM ETC.) AND SOLVE THERE.

3) SIMULATE RATHER THAN SOLVE

KEY POINT: KNOW WHAT APPROXIMATIONS YOU MADE AND THINK ABOUT HOW THIS WILL ALTER YOUR CONCLUSIONS.

NOTE: YOU CAN SOLVE WITH RELAXED ASSUMPTIONS, BUT IT GETS GNARLY.

REVISIT ASSUMPTIONS:

STEADY STATE

ONE CAN SHOW (ASSUMING $[S] = [S]_0$ AND IRREVERSIBLE)

$$V = \frac{k_{cat} \cdot [E]_0 [S]_0 \left(1 - e^{-(k_f [S]_0 + k_r + k_{cat})t}\right)}{K_M + [S]_0}$$

← WHEN DOES STEADY STATE APPLY?

AS $t \to \infty$:

$$V = \frac{k_{cat} [E]_0 [S]_0 (1-\alpha)}{K_M + [S]}$$

AND HOW FAST DOES $e^{-\alpha t} \to 0$? DEPENDS ON α!

ONLY IF ENZYME LOADS QUICKLY RELATIVE TO RXN RATE.

WHAT IF WE ALLOW REVERSIBILITY?

ASSUMING STEADY STATE:

$$V = \frac{K_A [E]_0 [S] - k_P [E]_0 [P]}{1 + \frac{[S]}{K_{M,A}} + \frac{[P]}{K_{M,P}}}$$

$$K_A = \frac{k_f k_{cat}}{k_r + k_{cat}} \qquad k_P = \frac{k_r k_-}{k_r + k_{cat}}$$

$$K_{M,A} = \frac{k_r + k_{cat}}{k_f} \qquad K_{M,P} = \frac{k_r + k_{cat}}{k_-}$$