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

WRITE A MARKOV MATRIX FOR THIS REACTION:

$$\begin{array}{c|cccc} & E & S & E\cdot S & P \\ \hline E & P_{EE} & \emptyset & k_r + k_{cat} & \emptyset \\ S & \emptyset & P_{SS} & k_r & \emptyset \\ E\cdot S & k_f\cdot[S] & k_f[E] & P_{ES/ES} & k_-[E] \\ P & \emptyset & \emptyset & k_{cat} & P_{PP} \end{array} \Bigg\} \text{ NOT SOLVABLE}$$

① SECOND ORDER:

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

[S] CHANGES OVER TIME!

TREAT AS 3D MATRIX...

GO BACK TO RATE LAWS:

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

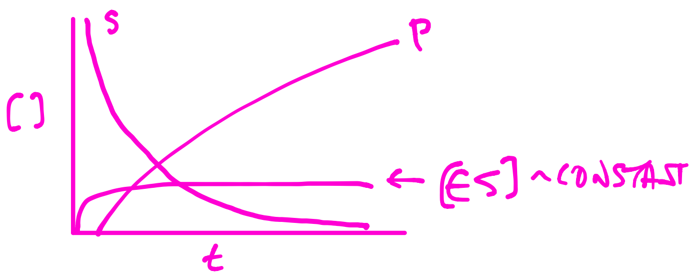
$$\frac{dS}{dt} = -k_f[E][S] + k_r[ES] - k_{cat}[ES]$$

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

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

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

② ASSUME [ES] ~ QUASI STEADY STATE.



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

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

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

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

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

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

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

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

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

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

$$= \frac{[E]_0[S]}{1/\alpha + [S]} \qquad K_M \equiv 1/\alpha = \frac{k_r + k_{cat} \ \leftarrow \text{LEAVING}}{k_f \ \leftarrow \text{COMING IN}} \sim K_D$$

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

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

k_{cat} : RATE OF CATALYSIS (FIRST ORDER!)

K_M : ~BINDING AFFINITY FOR SUBSTRATE

k_{cat}/K_M : CATALYTIC CONSTANT

WHERE DOES k_{cat}/K_M COME FROM? WHAT DOES IT MEASURE?

$$V = k_{cat}[E]\left(\frac{[S]}{[S] + K_m}\right)$$

FOR $[S] \ll K_M$

$$V = \frac{k_{cat}[E][S]}{K_M}$$

COMPARE S VS. S'

$V = \frac{k_{cat}}{K_m}[E][S]$

$V' = \frac{k_{cat}'}{K_m'}[E][S']$

$$\left[\frac{V}{V'} = \frac{k_{cat}/K_m}{k_{cat}'/K_m'}\frac{[S]}{[S']}\right] \Leftarrow$$ ALLOWS YOU TO COMPARE ENZYMES

REVISIT ASSUMPTIONS:

STEADY STATE

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

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

AS $t\to\infty$:

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

AND HOW FAST DOES $e^{-at}\to\emptyset$? DEPENDS ON α!

ONLY IF ENZYME LOADS QUICKLY RELATIVE TO RXN RATE.

WHAT IF WE ALLOW REVERSIBILITY?

ASSUMING STEADY STATE:

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

$k_A = \frac{k_f k_{cat}}{k_r+k_{cat}}$ $\quad k_P = \frac{k_r k_-}{k_r+k_{cat}}$

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