

ENZYME KINETICS

ENZYME CHARACTERISTICS.

- ALL CATALYZE REACTIONS (LOWER ACTIVATION ENERGY OF A REACTION)
- REMAIN UNCHANGED THROUGHOUT REACTION (DO NOT GET USED UP)
- BINDS SUBSTRATES.

ENZYME KINETICS PARAMETERS

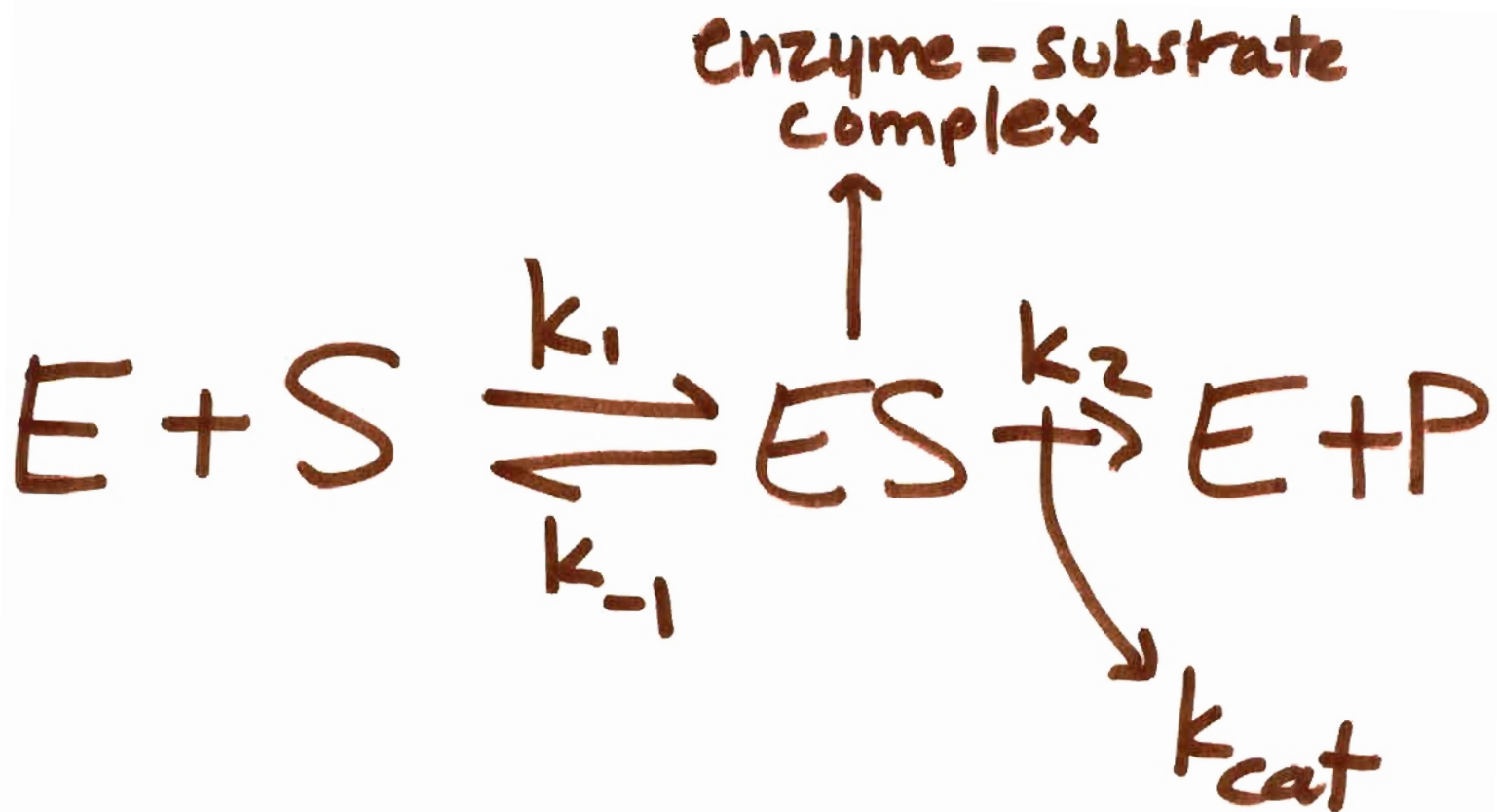
a. MAXIMUM RATE OF
THE REACTION V_{max}

b. AFFINITY OF ENZYME
FOR SUBSTRATE K_m

c. SUBSTRATE
CONCENTRATION $[S]$

d. ENZYME
CONCENTRATION $[E]$

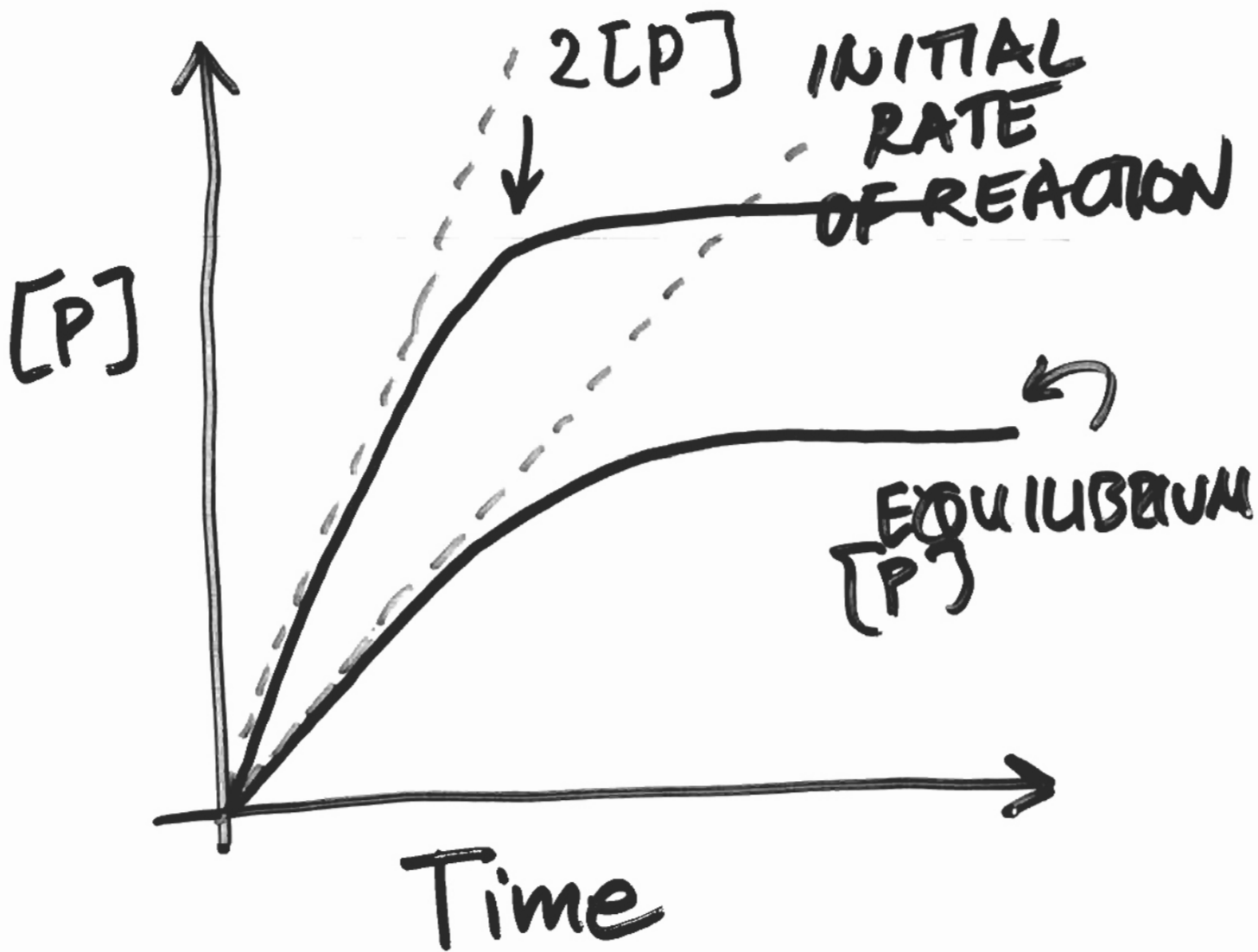
e. k_{cat} TURNOVER RATE
OF THE ENZYME.



k_1 = rate constant
for binding

k_{-1} = rate constant of breakdown
of ES complex to $E + S$

k_{cat} ⇒ turnover rate
often slowest step of rxn.



$S = \text{ONPG}$
 $E = \beta\text{-gal}$
 $P = \text{ONP}$

A

Typical Michaelis-Menten Curve

substrate limiting → enzyme limiting
→ observed rate → observed rate
is slower than fastest possible → maximum possible
→ rate $\propto [S]$ → rate $\propto [E]$

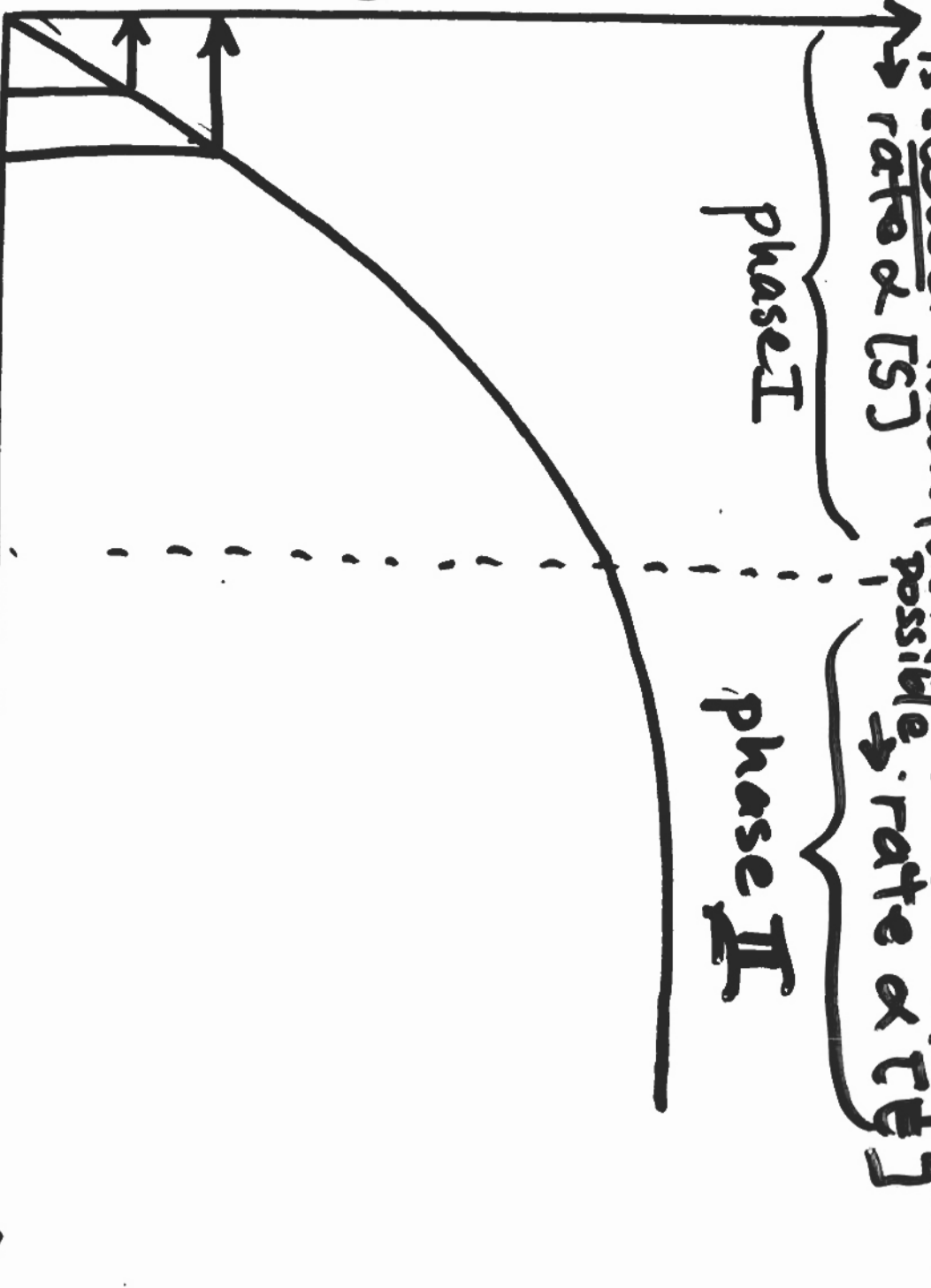
Phase I

Phase II

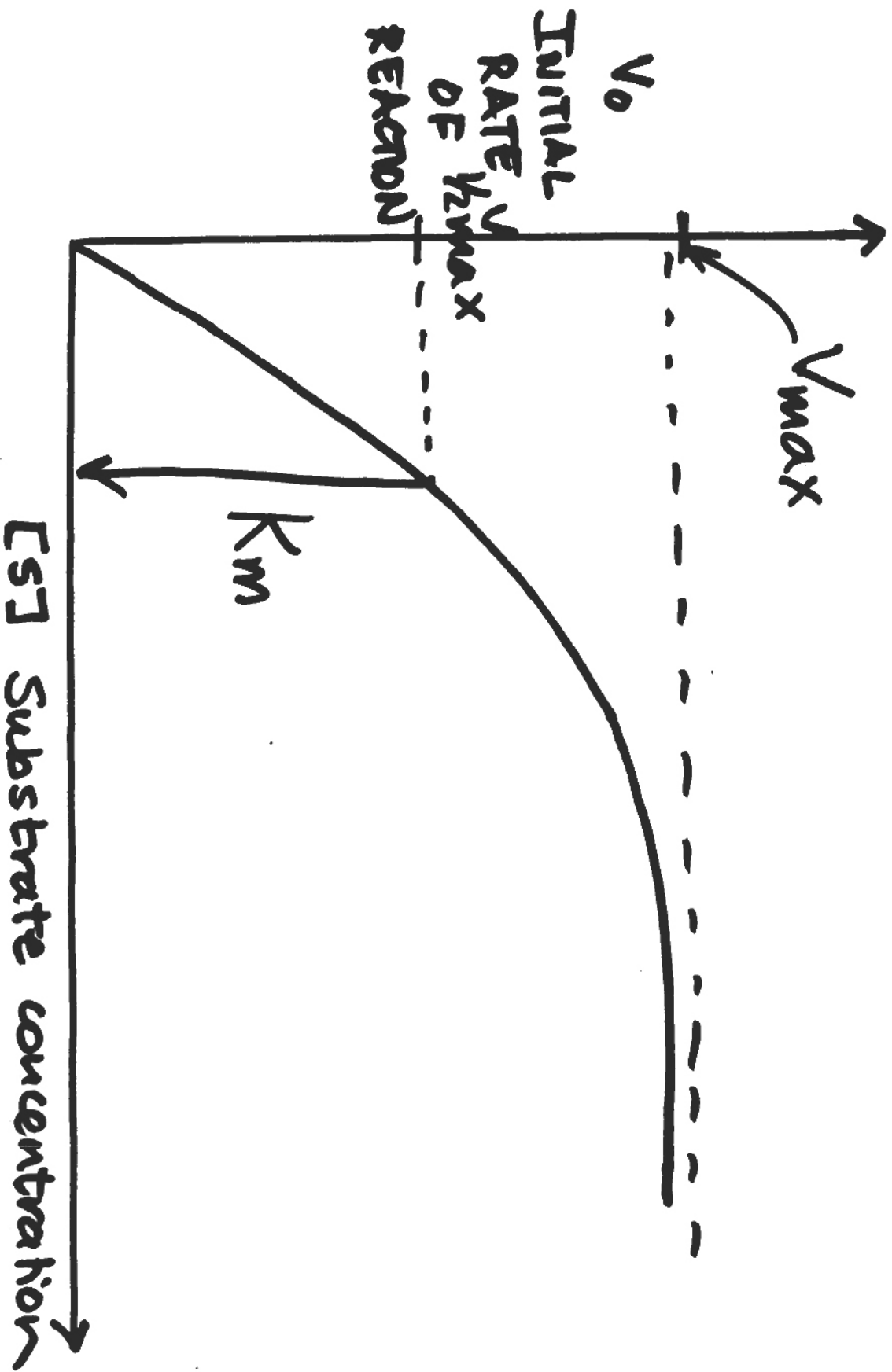
V_0
INITIAL
RATE
OF
REACTION

V_{02}
 V_{01}

S_1, S_2 [S] Substrate concentration



A Typical Michaelis-Menten Curve



$$V_0 = \frac{V_{\max} [S]}{K_m + [S]}$$

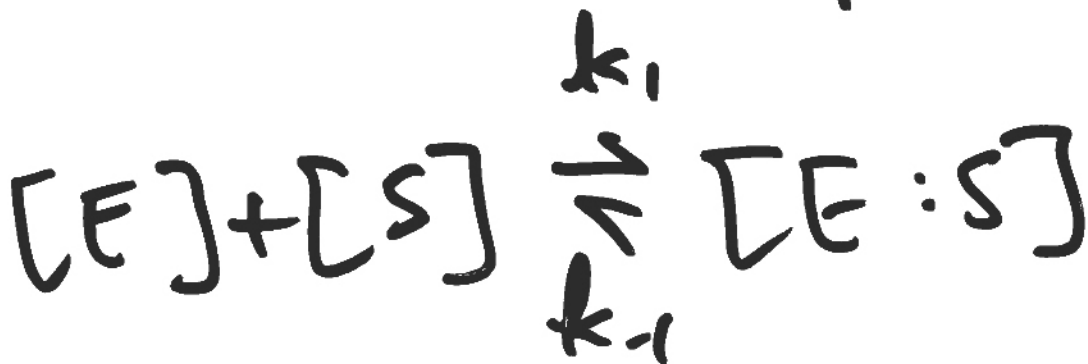
Michaelis-Menten Eqⁿ

$$V_{\max} = k_{\text{cat}} [E]_{\text{total}}$$

$$K_m = \frac{k_{\text{cat}} + k_{-1}}{k_1}$$

$$K_m = \frac{k_{cat} + k_{-1}}{k_1}$$

$$K_m \approx \frac{k_{-1}}{k_1} = K_D$$



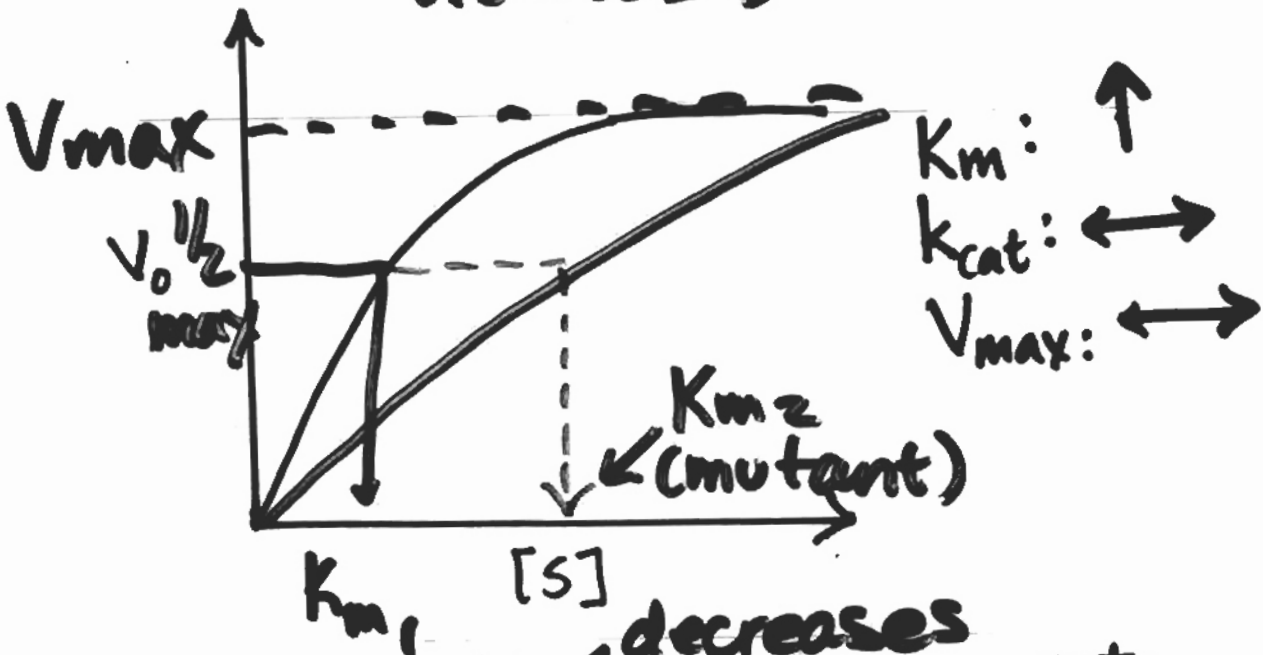
$$K_D = \frac{[E][S]}{[E-S]}$$

if K_m is LARGE, then affinity of enzyme for substrate is LOW.
 if K_m is SMALL, then affinity of enzyme for substrate is high



Effects of mutations on K_m & V_{max}

1. Mutant that affects binding affinity **decreases** for S



2. Mutant that affects turnover rate **decreases** (assume binding not affected)

