CONCEPT: SPECIFICITY CONSTANT

●Characterizing enzymes in a lab under *saturating* [S] is useful; HOWEVER, [S] are not always *saturating*.

- □ Under ________________ conditions, the [S] _____ K_m.
- □ Also, *saturating* [S] does *not* allow us to account for _____ binding affinity since $[E]_T$ = [ES].
- □ This means that *maximal* catalytic efficiency (k_{cat}) at *saturating* [S] is not always the most *relevant* measure.

Ratio of K_{cat} to K_m Measures Catalytic Efficiency at Low [S]

●______________ *constant* = the ratio of $\frac{K_{cat}}{K_m}$ = an enzyme's "*preference*" for a substrate at ______-saturating or *low* [S].

- □ Substrate "*preference*" is determined by *catalytic efficiency*, but depends on [___] (saturating or non-saturating).
- □ Recall: chymotrypsin has a "*preference*" for which amino acids it recognizes for cleavage.

●Ratio of $\frac{K_{cat}}{K_m}$ is another measure of catalytic _____________ when an enzyme is *not* saturated with substrate.

- □ Ratio accounts for both max catalytic efficiency (k_{cat}) and E ___________ for S (K_m).
- □ *Larger* ratios represent __________ efficient enzymes *and* therefore *higher* preference for S at *low* [S].
- □ Max value of $\frac{K_{cat}}{K_m}$ ≈ 10^9 $M^{-1}s^{-1}$.

Enzyme *preference* for S & ______ catalytic efficiency ***ONLY*** at *saturating* [S]

Enzyme ______________ for S & Catalytic efficiency at ______ [S]

Enzyme	k_{cat} = Turnover Number (s^{-1}) *Under *Saturating* [S]	k_{cat} Speed? ↑Fast or ↓slow?	K_m (M)	ES Affinity ↑Strong or ↓weak?	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)
Urease	10,000		2.5×10^{-2}		4.0×10^5
Penicillinase	2000		5×10^{-5}		4.0×10^7
Chymotrypsin	substrate = F, Y, W _____ Preferred 100		6.6×10^{-4}		1.5×10^5
	substrate = L, M _____ Preferred 0.63		1.1×10^{-4}		5.8×10^3
	substrate = K _____ Preferred 0.02		5.9×10^{-4}		3.4×10^1

Enzyme __________ for substrate.

PRACTICE: Use the data in the chart below to provide answers to the following problems:

A) List the substrates from most preferred to least preferred under physiological conditions.

a) B, A, C. b) C, B, A. c) B, C, A. d) A, C, B.

Substrate	k_{cat} (s^{-1})	K_m (M)
Substrate A	0.36	0.071
Substrate B	2.80	0.025
Substrate C	0.14	0.015

B) List the substrates from most preferred to least preferred under saturating [S].

a) B, A, C. b) C, B, A. c) B, C, A. d) A, C, B.

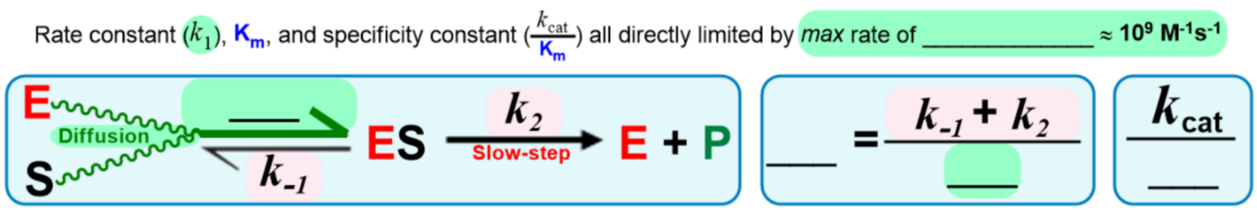
CONCEPT: SPECIFICITY CONSTANT

Diffusion-Controlled Limit of Specificity Constant

●The ______ value of the $\frac{K_{cat}}{K_m}$ ratio is limited by k_1 (E + S ____________ rate constant).

□ E + S association occurs via ____________ & can only proceed as fast as the *max* rate of diffusion in solvent.

□ Therefore, the *max* values of k_1 and $\frac{K_{cat}}{K_m}$ are equal to the max rate of diffusion in H_2O ≈ _____ $M^{-1}s^{-1}$.



●*Catalytically* ____________ *Enzyme*: an enzyme whose $\frac{K_{cat}}{K_m}$ is equal to this diffusion-controlled max value.

PRACTICE: Which of the following options is correct concerning the turnover number (k_{cat}) and the specificity constant?

a) k_{cat} reveals how well an enzyme works & its preference for S.

b) Specificity constant is defined as (k_{cat})(K_m).

c) A large k_{cat} indicates a less efficient enzyme.

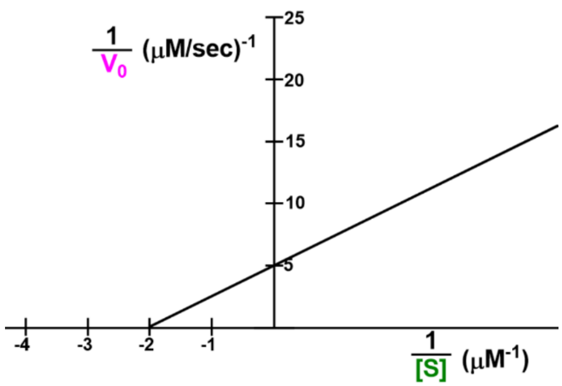
d) k_{cat} = V_{max}/[ES].

e) Specificity constant is defined as K_m / k_{cat}.

f) A small K_m indicates a more efficient enzyme.

PRACTICE: Use the Lineweaver-Burk plot to help you calculate the V_{max}, k_{cat}, K_m and specificity constant for the enzyme. Assume the $[E]_T$ = 2.9 nM. Hint: Pay close attention to units. V_{max} = __________. k_{cat} = __________. K_m = __________.

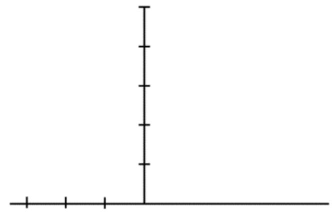
k_{cat}/K_m = __________.



CONCEPT: SPECIFICITY CONSTANT

PRACTICE: Explain the steps you could take to accurately find the K_m, V_{max}, and specificity constant for an enzyme from the following kinetic data, assuming the experiments were all done with $[E]_T$ = 0.1 mM.

[S] (M)	V_0 (M/s)
0.001	5.88
0.002	10.5
0.004	17.4
0.008	25.8
0.016	34
0.032	40.5



Step #1: ______________________________________

Step #2: ______________________________________

Step #3: ______________________________________

Step #4: ______________________________________

Step #5: ______________________________________

PRACTICE: The specificity constant is obtained at low [S] via variable substitution into the Michaelis-Menten equation ($V_{max} = k_{cat}[E]_T$). Considering this about the MM-equation, what is the relationship between changes in [S] & V_0 when the [S] is super small and well below the K_m?

a) The [S] term cancels out completely in this equation, so there is no effect of changing substrate concentration.

b) The [S] term in the numerator is negligible, so there is no impact of changing substrate concentration.

c) Because the enzyme has reached V_{max}, there is no effect of changing substrate concentrations on enzyme velocity.

d) [S] term in the denominator is negligible compared to K_m, so the relationship between [S] & V_0 is directly proportional.

Michaelis-Menten Equation:

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

$$V_0 = \frac{[\quad]_T[S]}{K_m + [S]} \qquad \_\_\_\_ = k_{cat}[E]_T$$

$$V_0 = \frac{k_{cat}[E]_T[S]}{K_m} \qquad \text{*At } \_\_\_\_ \text{ [S]} < K_m$$

$$V_0 = \frac{\_\_\_\_}{\_\_\_\_}[E]_T[S] \qquad \text{*Rearrange.}$$