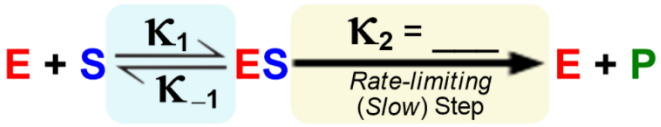
CONCEPT: K_{CAT}

●Catalytic Constant (k_{cat}): _____ constant for the *rate-limiting*-step (_____-step) of an enzyme-catalyzed reaction.

- □ A reaction can't go any faster than its *slowest* step, so k_{cat} dictates _____, which only occurs at *saturating* [**S**].
- □ For simple enzyme-catalyzed reactions, $k_{cat} = k_2$, (k_2 = _____ formation rate constant).

EXAMPLE: Determine the rate law that uses k_{cat}.



Rate Law: When enzyme is ***saturated*** with substrate: $\frac{\Delta[P]}{\Delta t} = V_0 \approx V_{max} = \_\_\_ [E]_T$

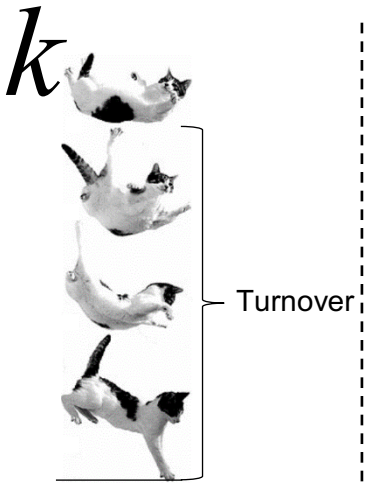
$\frac{V_{max}}{[E]_T} = \_\_\_$

Calculating & Interpreting K_{cat}

●Both $[E]_T$ & V_{max} must be known to _____ k_{cat}.

●k_{cat}: max amount of _____ *converted* to product per second by one single enzyme molecule under *saturating* [**S**].

- □ k_{cat} is also called the _____ number & has units of seconds^{-1} (s^{-1}).
- □ The _____ of k_{cat} ($1/k_{cat}$): has units of seconds & represents *time* required for _____ catalytic event.
- □ k_{cat} alone is used to measure _____ *catalytic efficiency* only under *saturating* [**S**].



_____ Number = ___ = $\frac{V_{max}}{[E]_T}$ = _____ of catalysis.

Enzyme	Function	k_{cat} = Turnover Number (s^{-1}) *Under *Saturating* [S]	$\frac{1}{k_{cat}}$ (s)
Catalase	Converts H_2O_2 to H_2O & O_2	40,000,000	2.5×10^{-8}
Acetylcholinesterase	Regenerates acetycholine from acetate and choline.	14,000	7.1×10^{-5}
Chymotrypsin	Peptidase: Cleaves c-terminal peptide bond of F, Y & W	100	0.01
DNA Polymerase I	DNA Replication	15	0.07

EXAMPLE: What is the turnover number for carbonic anhydrase if V_{max} = 60,000 M/s and $[E]_T$ = 0.1 M?

a) 1.67×10^{-6} s^{-1} b) 600,000 s^{-1} c) 0.08 s^{-1} d) 6,000 s^{-1}

PRACTICE: To calculate the turnover number of an enzyme, you need to know:

a) Total enzyme concentration $[E]_T$.
b) V_0 of the catalyzed reaction when [S] >> K_m.
c) V_0 of the catalyzed reaction at low [S].
d) K_m of the enzyme.
e) Both a & b.

CONCEPT: K_{CAT}

PRACTICE: If 10 µg of an enzyme (MW = 50,000 g/mol) is added to a solution containing a [substrate] 100 times greater than the K_m, it catalyzes the conversion of 75 µmol of substrate into product in 3 min. What is the enzyme's turnover #?

a) 1.25×10^5 min^{-1}. b) 2.5×10^4 min^{-1}. c) 1.5×10^2 min^{-1}. d) 3.5×10^6 min^{-1}.

K_{cat} vs K_m

●Recall: k_{cat} is a measure of the *maximum* _____ *efficiency* of an enzyme under _____ [S].

□ K_m is a measure of the binding _____ an enzyme has for its substrate.

□ Enzyme _____to its substrate and enzyme _____ are two completely *separate* events.

PRACTICE: Studies with mutated forms of an enzyme show that changing some active-site amino acids decrease the enzyme's turnover number (k_{cat}) but do not affect the K_m of the reaction. What is the best interpretation of these results?

a) The K_m of the enzyme for the substrate does not depend on amino acid side chains found in the active site.
b) The two terms, K_m and turnover number, are inversely proportional.
c) The transition state for this reaction is formed prior to the formation of the ES complex.
d) Amino acids involved in stabilizing transition-state complexes can be different than those affecting the ES-complex.

PRACTICE: The turnover number for an enzyme is known to be 5000 min^{-1}. From the following set of data, determine both the K_m and the total amount of enzyme E_T.

[Substrate], mM	Initial Velocity, µmol/min
1	167
2	250
4	334
6	376
100	498
1,000	499

A) What is the K_m of the enzyme?

a) 1 mM. b) 2 mM. c) 4 mM. d) 1000 mM.

B) What is the total amount of enzyme?

a) 0.001 µM. b) 0.01 µM. c) 0.1 µM. d) 10 µM.