CONCEPT: INHIBITION CONSTANT

●Recall: every reaction has a _______ constant (k) indicating reaction rate efficiency/probability under set conditions.

□ The *higher* the k, the ________ likely the reaction is __________.

●Reactions that form & breakdown the _____ & ESI complexes also have rate constants:

□ Rate constant for Inhibitor-complex _______________/*association* = k_{EI} or k_{ESI}

□ Rate constant for Inhibitor-complex *breakdown*/________________ = k_{-EI} or k_{-ESI}

Inhibition Constant of Free Enzyme (E)

●__________-state conditions apply to both the ES & _____ complexes (rate of formation of EI = rate of breakdown of EI).

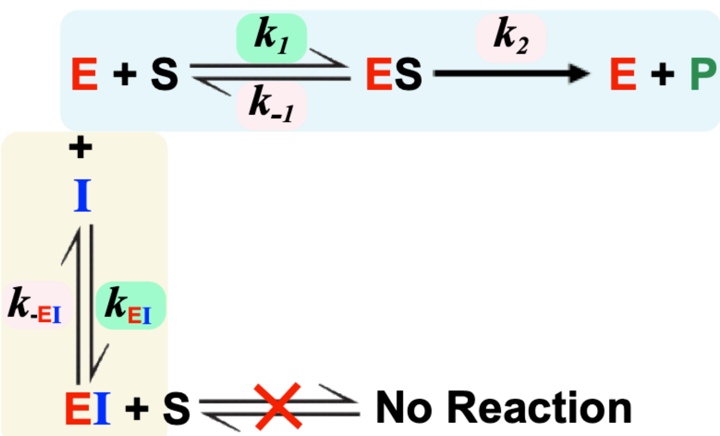
□ Just like we derive the constant _____ from steady-state conditions, we also derive the *inhibition constant* (**K_I**).

●*Inhibition constant* (**K_I**): the dis*sociation* constant for ________-enzyme-inhibitor-complex (EI).

□ Similar to how **K_m** = [**S**] that allows V_0 = ½**V_{max}**, **K_I** = [__] that allows ____ maximum-inhibition.

□ Similar to how **K_m** measures enzyme-*substrate* affinity, **K_I** measures enzyme-____________ *affinity*.

□ The _____ the **K_I**, the _____ the *binding affinity* an enzyme has for that inhibitor.



Review:

$$K_m = \frac{\text{ES dissociation}}{\text{ES association}} = \frac{k_{-1} + k_2}{k_1} = \frac{[E][S]}{[ES]}$$

New:

$$K_I = \frac{\text{EI dissociation}}{\text{EI association}} = \frac{\_\_}{\_\_} = \frac{[\ \ ][\ \ ]}{[\ \ \ ]}$$

EXAMPLE: A) Consider the data in the chart below. Which enzyme has the strongest binding affinity for its *substrate*?

a) Enzyme A. b) Enzyme B. c) Enzyme C.

B) Which enzyme has the strongest binding affinity for its *inhibitor*?

a) Enzyme A. b) Enzyme B. c) Enzyme C.

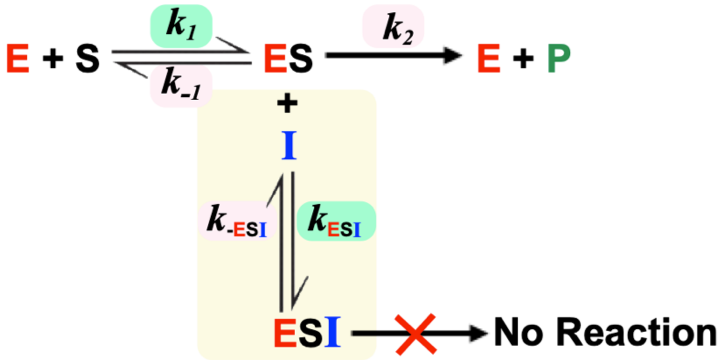
Enzyme	K_m (M)	K_I (M)
Enzyme A	0.031	7.2×10^{-6}
Enzyme B	0.025	8.7×10^{-5}
Enzyme C	0.015	1.4×10^{-3}

CONCEPT: INHIBITION CONSTANT

Inhibition Constant of ES-Complex

● *Inhibition constant* (**K'_I**): the dis*sociation* constant for ___nzyme-___ubstrate-___nhibitor-complex (______).

□ **K'_I** measures ____-complex *affinity* for ____________ to form ESI.



$$K'_I = \frac{\text{ESI dissociation}}{\text{ESI association}} = \frac{k_{-ESI}}{k_{ESI}} = \frac{[\quad][\quad]}{[\quad]}$$

PRACTICE: Use the data in the chart below to determine the answer to the following:

A) Rank the enzymes in order of their binding affinity to their *substrate* (strongest affinity → weakest affinity).

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

Enzyme	k_{cat} (s^{-1})	K_m (M)	$\frac{k_{cat}}{K_m}$ ($M^{-1}s^{-1}$)	K_I (M)
Enzyme A	0.06	0.031	1.94	7.2×10^{-6}
Enzyme B	2.8	0.025	112	8.7×10^{-5}
Enzyme C	0.14	0.015	9.33	1.4×10^{-3}

B) Rank the enzymes in order of their binding affinity to the *inhibitor* (strongest affinity → weakest affinity).

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

C) Which enzyme would you expect the inhibitor to affect the most? Why?

a) Enzyme A. b) Enzyme B. c) Enzyme C.

PRACTICE: Calculate the Michaelis constant (K_m) and the inhibition constant (K_I) given the following information:

[E] = 20 mM. [S] = 15 mM. [ES] = 5 mM. [I] = 8 mM. [EI] = 2 mM.

a) K_m = 7 mM ; K_I = 14 mM.

b) K_m = 10 mM ; K_I = 20 mM.

c) K_m = 20 mM ; K_I = 80 mM.

d) K_m = 60 mM ; K_I = 80 mM.

e) K_m = 60 mM ; K_I = 20 mM.