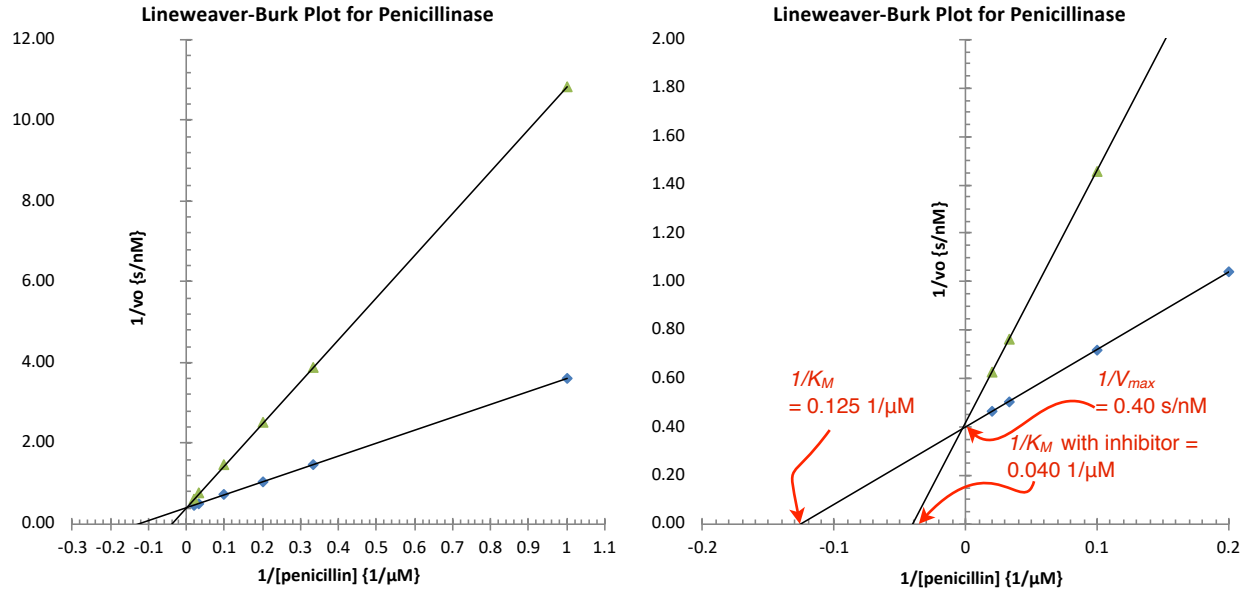
Name _____ Key _____

Chem 352 - Fall 2018
Quiz 2

$R = 8.314\ J/(mol \cdot K) = 0.08206\ (L \cdot atm)/(mol \cdot K)$

1. Penicillin-resistant bacteria produce an enzyme called *penicillinase*, which is able to chemically inactivate the antibiotic *amoxicillin*, a member of the penicillin class of antibiotics. When bacterial infections are treated with amoxicillin, the drug is often augmented with an inhibitor of penicillinase called *clavulanic* acid. Below are graphs showing kinetics data for the penicillinase reaction in the presence and absence of 10 µM clavulanic acid. These data were collected using a penicillinase concentration of 50 pM. The graph on the right is an expansion of the one shown on the left.



a. Is the affinity of the penicillinase enzyme for its substrate penicillin affected by the presence of the clavulanic acid (Y/N)? Y

Describe your evidence for this claim: The Michaelis-Menten constant, K_M, is a measure of the affinity of an enzyme for its substrate; the higher the K_M value the lower the affinity. From a Lineweaver-Burk plot, the K_M, is given by the -1/x-intercept. Since the x-intercept for the the Lineweaver-Burk plot is decreasing in the presence of inhibitor, the affinity is lowered by the presence of the clavulanic acid. In this experiment, the K_M increased from $K_M = \frac{1}{0.125\ \frac{1}{\mu M}} = 8.0\ \mu M$ to $K_M = \frac{1}{0.040\ \frac{1}{\mu M}} = 25\ \mu M$

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b. On average, how many reactions does each penicilinase enzyme molecule catalyze each second when saturated with the substrate in the absence of clavulanic acid? (Assume that each enzyme molecule has only one active site.) This question is asking for the the *turnover number*, which is equal to the k_{cat}. k_{cat} can be determined from the V_{max} and the total enzyme concentration, ($k_{cat} = V_{max}/[E]_{total}$). V_{max} is determined from the *y*-intercept on the Lineweaver - Burke plot, and $[E]_{total}$ was given to us. This question is asking for the the *turnover number*, which is equal to the k_{cat}. k_{cat} can be determined from the V_{max} and the total enzyme concentration, ($k_{cat} = V_{max}/[E]_{total}$). V_{max} is determined from the *y*-intercept on the Lineweaver - Burke plot, and $[E]_{total}$ was given to us.

$V_{max} = \frac{1}{0.40\ \frac{1}{nM}} = 2.5\ \frac{nM}{s} = 2.5 \times 10^{-9}\ \frac{M}{s}$; $[E]_{total} = 50\ pM = 50 \times 10^{-12}\ M$

$k_{cat} = \frac{V_{max}}{[E]_{total}} = \frac{2.5 \times 10^{-9}\ \frac{M}{s}}{50 \times 10^{-12}\ M} = 50\ \frac{1}{s}$

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c. Is the number you calculated for part b. affected by the presence of clavulanic acid (Y/N)? N
Describe your evidence for this claim:

4 Since k_{cat} is determined from V_{max} and $[E]_{total}$, and neither of these is affected by the clavulanic acid, k_{cat} is, therefore, not affected by the inhibitor.

d. Is it possible for the penicillin-resistant bacterium, which produced the penicillinase that was studied above, to improve on the enzyme's efficiency (Y/N) Y?
Describe your evidence for this claim: This question is asking if the catalytic efficiency ($=k_{cat}/K_M$) is greater than 10^8 1/M•s. If it is, the rate of the reaction is limited by the rate at

8 which substrate diffuses into the active site of the enzyme, so there is nothing more that can be done to the enzyme to speed up the reaction.

$$V_{max} = \frac{1}{0.40\ \frac{s}{nM}} = 2.5\ \frac{nM}{s} = 2.5 \times 10^{-9}\ \frac{M}{s};\ [E]_{total} = 50\ pM = 50 \times 10^{-12}\ M$$

$$k_{cat} = 50\ \frac{1}{s};\ K_M = 8.0\ \mu M = 8 \times 10^{-6}\ M$$

$$\frac{k_{cat}}{K_M} = \frac{50\ \frac{1}{s}}{8 \times 10^{-6}\ M} = 6.25 \times 10^{6}\ \frac{1}{M \cdot s}$$

Which is 1 to 2 orders of magnitude less than 10^8 1/M•s, therefore this enzyme is not exhibiting "catalytic perfection" and so could be tweaked to improve its efficiency.

e. The follow reaction equation is used to describe enzyme-catalyzed reactions that adhere to the Michaelis-Menton model for enzyme kinetics. Using the letter "I" to represent the inhibitor clavulanic acid, modify this reaction equation to show what happens when clavulanic acid is

3 present. When an inhibitor, such as clavulanic acid, affects the observed K_M value without affecting the V_{max} value, it is an indication that the inhibitor is a competitive inhibitor that binds reversibly to the same site on the enzyme as the substrate. So the enzyme can either bind substate (S) or inhibitor (I), but not both at the same time:

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

$$+$$
$$I$$
$$\Updownarrow$$
$$EI$$

25/25