

Name: _____

Lab Day: _____

Biology 3515/Chemistry 3515
Biological Chemistry Laboratory
Spring Semester 2017
Quiz 2 - 9 March 2017

Please write your name on each page.

Be sure to show your work and include correct units in all of your answers!

25 points total.

1. β -lactamases are enzymes that hydrolyze an amide bond within a four-member lactam ring found in many antibiotics, including members of the penicillin and cephalosporin families. These enzymes are found in many bacteria and enable these bacteria to escape the effects β -lactam antibiotics, thus contributing to the very important problem of antibiotic-resistance. After graduating from the U, you have been hired by the giant pharmaceutical company, Proteins-R-Us, and have been asked to characterize the β -lactamase from a newly discovered antibiotic-resistant bacterial strain.

(a) (6 pts.) After spending a few weeks isolating the enzyme, you have enough pure enzyme to do some kinetic experiments, using a chromogenic substrate. The product produced by the enzyme from this substrate has an extinction coefficient of $20,500 \text{ cm}^{-1}\text{M}^{-1}$ at 486 nm, whereas the substrate does not absorb light significantly at this wavelength. For these experiments, you have used an enzyme concentration of $1.75 \mu\text{g}/\text{mL}$ and monitored the change of absorbance at 486 nm, using cuvettes with a path length of 1 cm. The initial results are summarized in the table below:

[S] (μM)	$\Delta A/\text{min}$
0	0.0
50	0.020
100	0.028
250	0.036
500	0.040
750	0.042
1000	0.043

From the data above, estimate the value of K_m , in μM units, and V_{max} , in $\mu\text{M}/\text{min}$. You should not have to do any graphing, curve-fitting or extensive calculations to estimate these parameters! (Space is provided on the next page.)

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- (b) (5 pts.) Further studies show that the β -lactamase you are studying has a molar mass of 187,000 g/mole. With this new information, calculate the value of k_{cat} , with appropriate units.

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2. (6 pts.) For your next experiments, you would like to use a substrate concentration of $300\ \mu\text{M}$, and you would like the reaction velocity to be $10\ \mu\text{M}/\text{min}$. What enzyme concentration should you use for this experiment?

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3. The bosses at Proteins-R-Us want to develop a reversible inhibitor of β -lactamases that can be administered together with penicillin, so that the degradation of the antibiotic can be slowed down.

(a) (4 pts.) Because the concentrations of penicillin at different locations in a patient might be quite varied, this inhibitor should be effective at both high and low penicillin concentrations (relative to the K_m). With this goal in mind, should the inhibitor be designed to bind to the free enzyme, the enzyme-substrate or to both? Briefly explain your reasoning.

(b) (4 pts.) The organic chemists at Proteins-R-Us have synthesized a compound designed to mimic the structure of penicillin, but not undergo the reaction with β -lactamase. Do you think that this compound will have the desired properties outlined above? Again, briefly explain your reasoning.