

Sample questions on enzymes

1. Penicillin is hydrolyzed and thereby rendered inactive by penicillinase (also known as β -lactamase), an enzyme present in some resistant bacteria. The mass of this enzyme in *Staphylococcus aureus* is 29.6 kDa. The amount of penicillin hydrolyzed in 1 minute in a 10-ml solution containing 10^{-9} g of purified penicillinase was measured as a function of the concentration of penicillin. Assume that the concentration of penicillin does not change appreciably during the assay.

[Penicillin] (μM)	Amount hydrolyzed (nanomoles)
1	0.11
3	0.25
5	0.34
10	0.45
30	0.58
50	0.61

- (a) Plot V_0 versus $[S]$ and $1/V_0$ versus $1/[S]$ for these data. Does penicillinase appear to obey Michaelis-Menten kinetics? If so, what is the value of K_M ?
- (b) What is the value of V_{max} ?
- (c) What is the turnover number of penicillinase under these experimental conditions? Assume one active site per enzyme molecule
2. Suppose that a mutant enzyme binds a substrate 100-fold as tightly as does the native enzyme. What is the effect of this mutation on catalytic rate if the binding of the transition state is unaffected?

3. Your lab partner, who is both systematic and frugal, decides to perform a series of enzyme assays at substrate concentrations of 1, 2, 4 and 8 μM . You argue for doing the experiments at $[S] = 0.1, 0.2, 0.4, 0.6$, and 100 μM . Who had the better idea, and why?
4. Consider the following argument. In subtilisin, mutation of Ser221 to Ala results in a 10^6 -fold decrease in activity. Mutation of His 64 to Ala results in a similar 10^6 -fold decrease. Therefore, simultaneous mutation of Ser 221 to Ala and His 64 to Ala should result in a $10^6 \times 10^6 = 10^{12}$ -fold reduction in activity. Is this correct? Why or why not?
5. In chymotrypsin, a mutant was constructed with Ser189, which is in the bottom of the substrate specificity pocket, changed to Asp. What effect would you predict for this Ser189 \rightarrow Asp189 mutation?
6. Describe the key observation made by Graves, a graduate student in the Krebs lab.
7. Outline in mechanistic detail the hydrolysis of a peptide bond by chymotrypsin.