Enzyme Kinetics

Background/Review:

1. What is the difference between K and k?

2. For the following elementary reaction, explain why $K = \frac{k_1}{k_{-1}}$: A + B ⇌ C

Steady State Approximation: Theory

3. One main assumption of the steady state model of enzyme kinetics is that k_2 is the slowest rate constant. Explain why this is important.

4. Consider the conversion of substrates to products according to the following mechanism. Which of these steps are combined into k_2 in the traditional Michaelis-Menten kinetics model?

$$E + S \rightleftharpoons ES \rightleftharpoons TS \rightleftharpoons EP \rightleftharpoons E + P$$

5. The term used for the overall rate constant for multi-step reactions is **k_{cat}**. Discuss why it makes sense that $k_{cat}=k_2$ in simple Michaelis-Menten reactions.

6. In the Michaelis-Menten model, the units of k_{cat} are s^{-1}. Discuss how the units are consistent with the name "turnover number". If k_{cat} is large, what does that imply about the enzyme?

7. The term efficiency is often used in describing enzymes. What does it mean for an enzyme to be efficient?

8. How is your answer to the previous question consistent with the term for catalytic efficience (k_{cat}/K_M)? Discuss the contributions of k_{cat} and K_M (i.e. do efficient enzymes have large k_{cat}? How about K_M?)

9. Answer the following three questions using the information in the table to the right.

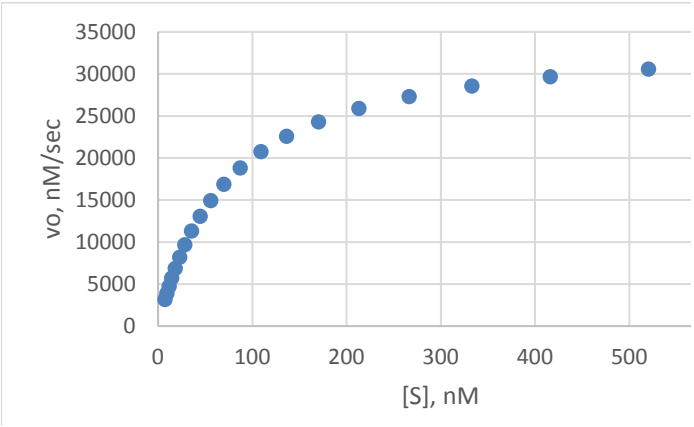
Enzyme	K_M (M)	K_{cat} (s^{-1})
A	9.5×10^{-5}	1.4×10^{4}
B	2.5×10^{-2}	1.0×10^{7}
C	5.0×10^{-6}	8.0×10^{2}

   a. Which enzyme has the highest affinity for the substrate? How do you know?

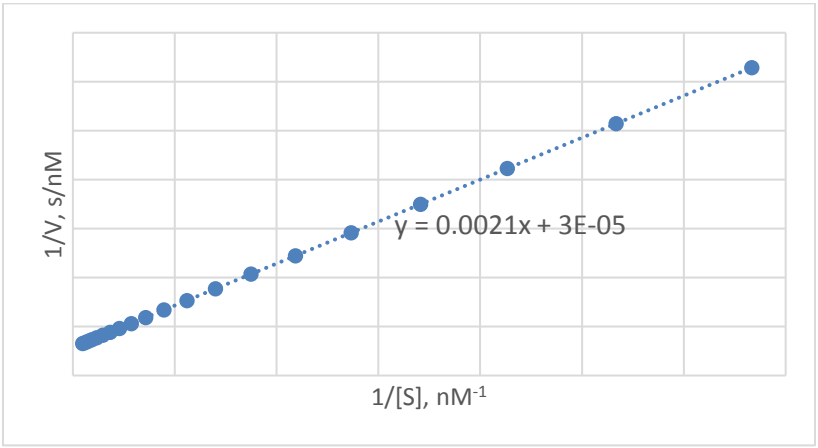
   b. Which enzyme can convert the most substrate to a product in 1 minute? How do you know?

   c. Which enzyme has the highest catalytic efficiency? How do you know?

10. Using the image to the right and E_{tot} = 1 nM, estimate:
    a. V_{max}
    b. K_M
    c. k_{cat}
    d. Catalytic efficiency
    e. The dissociation constant



11. Below is the double reciprocal plot of the data in problem 10. Determine Km and Vmax and compare your estimate from the previous problem.

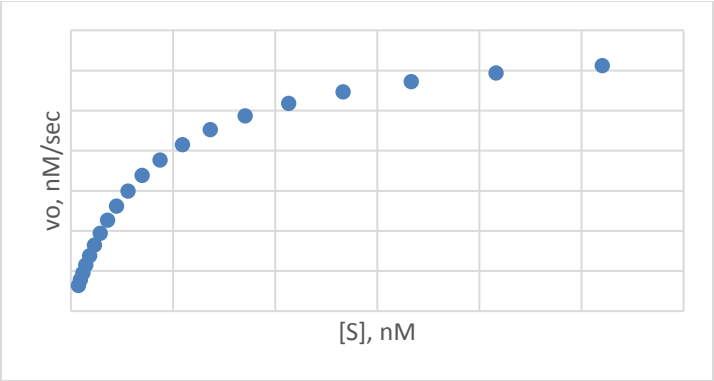


Enzyme Inhibition

12. For each type of inhibition, determine how K_M and Vmax will change (increase or decrease) upon addition of an inhibitor. On each graph, sketch what you would expect the plot to look like when an inhibitor is present.

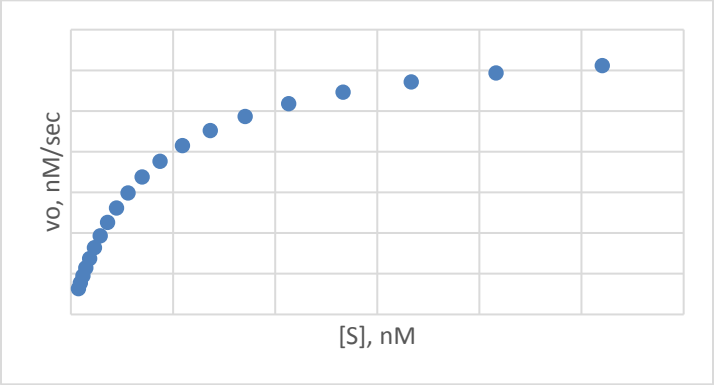
Competitive:

K_M	Increase	Decrease
Vmax	Increase	Decrease



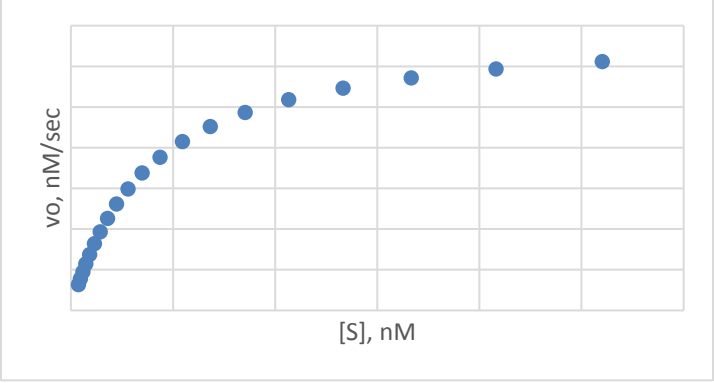
Uncompetitive:

K_M	Increase	Decrease
Vmax	Increase	Decrease



Mixed:

K_M	Increase	Decrease
Vmax	Increase	Decrease



13. Recall that K_M is the ratio rate constants: $\frac{ES_{decomposition}}{ES_{formation}} = \frac{k_2+k_{-1}}{k_1}$. Based on this, what would the K_M expression be if a competitive inhibitor was present?

14. K_I is the dissociation constant for an inhibitor. Which of the following would be better competitive inhibitor of an enzyme? Explain your answer.

A: K_I = 100 nM B: K_I = 10 μM C: K_I = 10 nM

15. When a competitive inhibitor is added to a reaction mixture, the apparent K_M' is related to the uninhibited KM according to the following equation. If KM is determined to be 100 nM without and inhibitor present and 250 nM with 5 nM inhibitor, what is K_I?

$$K'_M = K_M\left(1 + \frac{[I]}{K_I}\right)$$

16. For uncompetitive inhibition, V_{max} and K_M are both changed by a factor of $\frac{1}{1+\frac{[I]}{K_I}}$. If K_M is normally 10 nM but is determined to be 8 nM in the presence of 50 nM inhibitor, determine:
    a. K_I.

    b. The uninhibited Vmax if the inhibited Vmax (Vmax') is determined to be 108 nM min^{-1}.