1. True or False

- Steady-state kinetics should be monitored by the initial, early part of a reaction where S >> P.
- Steady-state kinetics should be monitored after an equilibrium is reached for S and P, S<->P.
- Steady-state kinetics should be monitored after a steady-state is reached for ES ; E+S<-> ES <->E+P
- Steady-state is a true equilibrium state. If not, how different?
- You monitored the amount of product formed over time for a single substrate concentration with a fixed amount of enzyme. Can you obtain both Km and Vmax with this single result? What is the minimum data you need to know Km and Vmax?
- Vmax is independent of [S]T *"A is independent of B"= A should not change however B changes.
- Vmax is independent of [E]Free, [E]T
- kcat is independent of [S]T
- kcat is independent of [E]T
- Km is independent of [S]T
- Km is independent of [E]T
- kcat is independent of the entity of the S. i.e., if you change S to S', you have same kcat?
- kcat is independent of the entity of the E.
- Km is independent of the entity of the S.
- Km is independent of the entity of the E.

2. How does each of the following parameters change by adding an enzyme that catalyzes the reaction?

S $\rightleftharpoons$ P

where K'eq=[P] eq /[S] eq.

(1) K'eq

(2) Activaton energy ΔG^*

(3) Gibbs free energy $\Delta G'^{O}$

(4) Forward rate constant, kf

(5) Reverse rate constant, kf

(6) Time to reach [P]=[P]eq and [S]=[S]eq.

3. Sketch a diagram of Free energy, *G*, of the system versus the reaction coordinate of the uncatalyzed and the enzyme-catalyzed reactions described above and indicate the activation energy of each forward reaction, $dG^{\ddagger}_{f}$ and $dG^{\ddagger}_{f,catalyzed}$. Do the same for reverse reactions.

4. How is the Michaelis constant, K_m described in term of the rate constants? How about the equilibrium constants, K_{dissoc} and K_{assoc}?

5. Express the rate of product formation as a function of the concentration of ES.

6. Know the following about enzyme inhibition mechanisms:
(a) Competitive inhibitors can be competed out when [S] is really large, leaving the Vmax unchanged but Km will be larger by alpha.

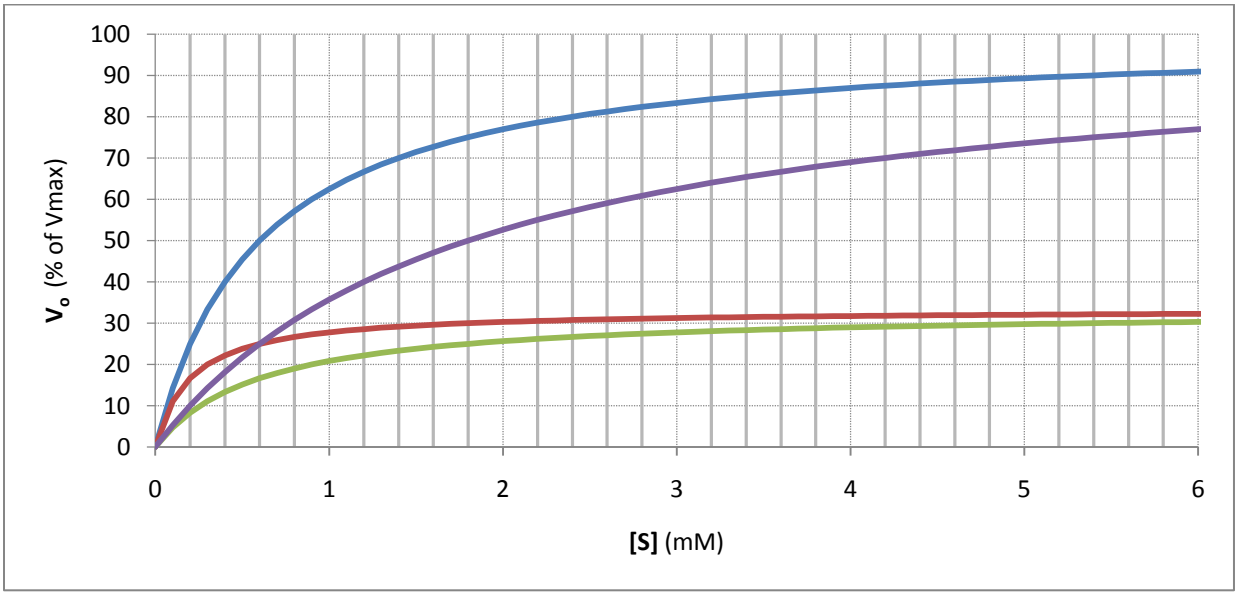
(b) Uncompetitive inhibitors bind to the ES complex but NOT to the free E. Thus, the conc. of inhibited enzyme [ESI] is proportional to [ES] NOT to [E]. These inhibitors lower Vo especially when [S] is really large because ES are major when [S] is large. On the other hand, when [S] is small, the steady state level of [ES] is low. Thus [ESI] is also low. Thus, Vo even in the presence of the inhibitor is not much different from Vo without inhibitor. The overall result shows up as an apparent decrease in Km AND Vmax by alpha'. V max, app=Vmax/alpha' and Km,app=Km/alpha'. When [S] is small, the Vo vs. [S] plot is very similar to that without inhibitor but when [S] is larger, you see a decrease in Vo, especially Vmax.

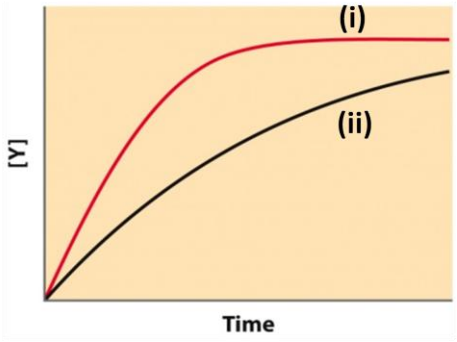
(c) Mixed type inhibitors can bind to both free E and ES. So, it results in a change in Km and Vmax that combines both Competitive and Uncompetitive inhibition. Vmax=Vmax/alpha'. Km=Km*alpha/alpha'.

(c') Noncompetitive inhibitors are a special type of mixed type inhibitors which happen to have the same Ki for both E and ES and thus their alpha and alpha' are the same. Thus, for these inhibitors, Vmax=Vmax/alpha'. Km=Km.

(1) Can you distinguish different inhibition mechanisms for the following curves? Assume blue is a curve obtained without any inhibitor. Which one can you estimate from the curves? Alpha's, Ki's, or [I]'s?For those you can estimate, what are the values? *Note the y axis is showing % of Vmax rather than any absolute value of concentration per time.

(2) Can you sketch a Lineweaver-Burke plot of these curves and vice versa?





7. An enzyme (E) catalyzes the conversion of substrate X to product Y. The plot shows the concentrations of Y ([Y]) versus time of reactions. Can the following change in the reaction condition shift the curve from (i) to (ii)? Answer Yes(Y) or No(N). If no, why?

(1) Use less enzyme..

(2) Add a mixed typ inhibitor.

(3) Replace the substrate X with X' that shows a higher K_m. (No –why?)

(4) Replace the substrate X with X' that shows a lower kcat. (No –why?)

(5) Decrease the total reaction volume without changing the concentrations of reaction components.

(6) Decrease reaction temperature.

8. If the shift in the curve from (i) to (ii) happened at a fixed [X] at 100*Km, which of the following conditions is consistent with the shift?

(a) Add a competitive inhibitor [I]=Ki.

(b) Add a uncompetitive inhibitor [I]=Ki.

9. If the curves (i) and (ii) differ only by the enzymes that catalyzed the reaction, what can you say about their Km and kcat? What if [X]>> Km(i) and Km(ii)? What if [X] << Km(i) and Km(ii)?

E.g., If the curves (i) and (ii) differ only by the enzymes that catalyzed the reaction (E(i) and E(ii), respectively), what can you conclude about the following parameters? The concentrations of the enzymes were the same and the reactions were carried out with a saturating of X for both enzymes.

(1) K_m of E(i) is (lower, higher, the same, cannot be determined –*Circle one*) than that of E(ii).

(2) k_{cat} of E(i) is (lower, higher, the same, cannot be determined –*Circle one*) than that of E(ii).

10. Which of the followings can you determine from the two curves shown above? Assume you can monitor the reaction for much longer than shown in the plot and also assume reaction mixture initially had only the enzyme at a fixed concentration at two different concentrations of X. Also assume the reaction can convert almost all X to Y at the end.Contribution from uncatalyzed reaction can also be ignored.

(a) Vo

(b) kcat

(c) Vmax

(d) Approximate total concentrations of X

(e) Approximate total concentration of enzyme

11. At what substrate concentration would an M-M enzyme work at one quarter of its maximum initial velocity? Ho w about 3/4 or 1/8 of Vmax?

12. Taken from your textbook…

(1) Carbonic anhydrase (MW =30,000 Da) catalyzes the reversible hydration of CO2:

If 15.0 ug of pure carbonic anhydrase catalyzes the hydration of 0.44 g of CO_2 (MW=44 Da) in 1 min at 37°C at V_{max}. what is the turnover number of carbonic anhydrase (in unit of min^{-1})?

(2) Assuming that the catalytic efficiency of the carbonic anhydrase is only limited by diffusion rates of molecules in an aqueous solution, what is the K_m? Take the diffusion-limited bimolecular rate constant as $1x10^8$ M^{-1} s^{-1}.

13. Many drugs in the market are in fact inhibitors of certain enzymes. Thus in pharmaceutical industry, much effort is put into synthesizing more effective inhibitors. Can you sketch a Vo vs. [S] or a Lineweaver-Burk plot of an enzyme reaction with and without a given type of inhibitor (i.e, Competitive, Uncompetitive and Noncompetitive or Mixed) and indicate the changes you expect as the potency of the inhibitor increases?

14. Study the catalytic mechanism of chymotrypsin. Can you recognize the catalytic triad and explain their roles when given a 3D structure depiction like that you saw in Exam2? *Terms that you should know: Catalytic triad, oxyanion hole, transition state, substrate-enzyme complimentarity and transition-state-enzyme complimentarity.