

Midterm Exam
Closed Book and Closed Notes
One 8.5 x 11 in. page of notes allowed

Section 1. Short Answers

1. Name four interactions involved in maintaining the tertiary structure of proteins.

Hydrogen bond

Hydrophobic Interactions

Ionic Interactions (electrostatic)

Van der Waals

Disulfide bonds

2. Define the Damkohler number for an immobilized enzyme reaction and explain its physical meaning.

$$Da = \frac{V_{\max}}{K_s S_o} \quad Da = \frac{\text{Maximum Reaction Rate}}{\text{Maximum Transport Rate}};$$

3. Explain why, in physical terms, $\eta_I \ll 1$ when $\phi \gg 1$. What is the controlling resistance in such a case?

$$f = \left(\frac{R}{3} \right) \sqrt{\frac{V_{\max}}{K_m D_{\text{eff}}}}$$

If $\phi \gg 1$ then $V_{\max} \gg K_m D_{\text{eff}}$, hence the system must be transport-limited. Reactant molecules are consumed before they diffuse very far, and the reaction is limited to a thin region near the periphery of the particle. Thus, the observed reaction rate is much smaller than the rate in the absence of an internal concentration gradient ($\eta_I \ll 1$)

4. What are three of the major differences between RNA and DNA that we discussed in class?

- RNA has a hydroxyl group at the 2' carbon of its sugar
- RNA uses uracil as one of its base, while DNA uses thymine as one of its base
- The extra hydroxyl group of RNA prevents it from forming a stable double stranded structure, hence RNA exists as a single stranded molecule.
- Some regions of RNA can form double helix (hairpin loops) (i.e. secondary structure)
- RNA can form many different tertiary structures, or stable three-dimensional structures

5. In site-directed mutagenesis, a *neutral mutation* has no effect on the properties of the mutated protein. Indicate whether the following would likely be neutral or non-neutral mutations, and *briefly* explain why. (Ignore possible effects on protein secondary structure.)

i. glutamate changed to aspartate

Neutral – similar size and pKa's.

ii. leucine changed to valine

Neutral – both branched-chain hydrophobics of similar size.

iii. valine changed to glycine

Not neutral – G is very small, allows significant free rotation around peptide bonds.

iv. cysteine changed to alanine

Not neutral – A is hydrophobic, C is polar and can even dissociate.

v. serine changed to threonine

Neutral – both contain –OH in side chains and similar size.

vi. tryptophan changed to phenylalanine

Neutral – both are hydrophobic aromatics of similar size.

vii. arginine changed to lysine

Neutral – both are large and basic.

viii. histidine changed to alanine

Not neutral – H is polar and ionizes with a pKa around neutrality, A is non-polar.

6. What is the two-stage model for enzyme inactivation, and what is the corresponding expression for k_{obs} , the observed rate constant for inactivation?

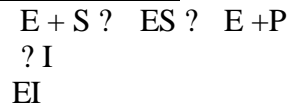
The two-stage model of enzyme inactivation is: $N \xrightleftharpoons{K} D \xrightarrow{k} I$

The corresponding expression for k_{obs} is:

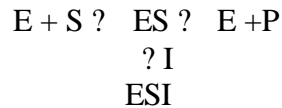
$$k_{obs} = \frac{k}{1 + K}, \text{ where } K = \frac{[N]}{[D]}$$

7. a) What are the general reaction schemes for competitive and uncompetitive enzyme inhibition?

Competitive Inhibition: Inhibitor competes with substrate for enzyme active site

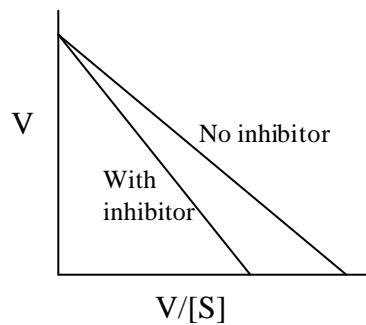


Uncompetitive Inhibition: Inhibitor binds to enzyme-substrate complex instead of enzyme active site

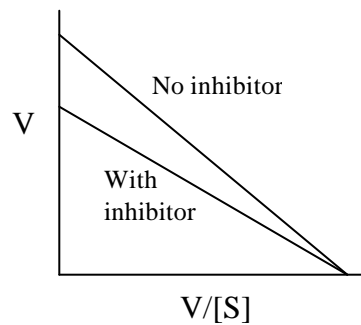


b) Sketch an Eadie-Hofstee plot for each type of inhibition in part (a). On each plot, include lines for results obtained in the presence and in the absence of the inhibitor. Be sure to label each line and each axis.

Competitive Inhibition
(same y-intercept, steeper slope with I)



Uncompetitive Inhibition
(same x-intercept, more shallow slope with I)



Competitive inhibition:

$$V_{max} \text{ is unchanged, } K_m^{app} = K_m \left(1 + \frac{[I]}{K_I} \right)$$

Uncompetitive inhibition:

$$V_{max}^{app} = \frac{V_{max}}{\left(1 + \frac{[I]}{K_I} \right)}, \quad K_{max}^{app} = \frac{K_{max}}{\left(1 + \frac{[I]}{K_I} \right)},$$

Section 2. Short Problems

1. Enzyme that is immobilized on a nonporous support obeys the following reaction scheme:



You are also given the following information:

$$\begin{array}{lll} k_p = 5 \times 10^{-3} \text{ cm/sec} & K_m = 0.5 \text{ mM} & v'_{\max} = 100 \mu\text{M cm}^{-2} \text{ min}^{-1} \\ S_o = 1 \text{ mM} & \eta_E = 0.40 & \end{array}$$

- a) Write an expression that relates the flux of product from the surface to the rate of substrate consumption at the surface. Please define any parameters in your expression that are not given above.

$$k_p (P^* - P_o) = \frac{2 v'_{\max} S^*}{K_m + S^*} \quad \underline{\text{OR}} \quad k_p (P^* - P_o) = \frac{2 \eta_E v'_{\max} S_o}{K_m + S_o}$$

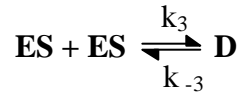
P^* = concentration of product at surface

S^* = concentration of substrate at surface

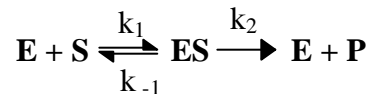
- b) Calculate the measured rate of appearance of product in the bulk per unit area.

$$k_p (P^* - P_o) = \frac{2 \eta_E v'_{\max} S_o}{K_m + S_o} = \frac{2 |0.40| |100 \mu\text{M}| |1 \text{ mM}|}{| \text{cm}^2 \text{ min} | |0.5 \text{ mM} + 1 \text{ mM}|} = 53.3 \mu\text{M cm}^{-2} \text{ min}^{-1}$$

2. A biochemical engineer, who was attempting to enhance the rate of a particular enzyme by manipulating its structure via site-directed mutagenesis, produced a mutant that exhibited rather unusual behavior. The enzyme-substrate complex was found to reversibly aggregate into an inactive dimer, according to the following reaction:



However, the mutant enzyme still follows general enzyme kinetics:



Use these definitions in answering the following questions:

$$K_3 = \frac{k_{-3}}{k_3}; \quad K_m = \frac{k_{-1} + k_2}{k_1}$$

- a) Assuming the reaction occurs in a closed system, begin by writing mass balances for the enzyme complexes (ES and D):

$$\begin{aligned} \frac{d[\text{ES}]}{dt} &= k_1[\text{E}][\text{S}] - k_{-1}[\text{ES}] - k_2[\text{ES}] - 2k_3[\text{ES}]^2 + 2k_{-3}[\text{D}] \\ \frac{d[\text{D}]}{dt} &= k_3[\text{ES}]^2 - k_{-3}[\text{D}] \end{aligned}$$

- b) Using the pseudo-steady-state-hypothesis for each enzyme complex, derive an expression for [ES] in terms of E_0 (and other parameters, including constants, but no other enzyme complexes):

$$\frac{d[\text{D}]}{dt} = 0 = k_3[\text{ES}]^2 - k_{-3}[\text{D}] \quad \Rightarrow \quad [\text{D}] = \frac{k_3}{k_{-3}}[\text{ES}]^2 = \frac{[\text{ES}]^2}{K_3}$$

also, since $k_3[\text{ES}]^2 = k_{-3}[\text{D}]$:

$$\begin{aligned} \frac{d[\text{ES}]}{dt} = 0 &= k_1[\text{E}][\text{S}] - k_{-1}[\text{ES}] - k_2[\text{ES}] - 2k_3[\text{ES}]^2 + 2k_{-3}[\text{D}] \\ [\text{E}] &= \frac{(k_{-1} + k_2)[\text{ES}]}{k_1} = K_m \frac{[\text{ES}]}{[\text{S}]} \end{aligned}$$

$$[E_o] = [E] + [ES] + 2 [D]$$

$$[E_o] = K_m \frac{[ES]}{[S]} + [ES] + 2 \frac{[ES]^2}{K_3}$$

$$\left(\frac{2}{K_3}\right) [ES]^2 + \left(\frac{K_m}{[S]} + 1\right) [ES] - [E_o] = 0$$

This can be solved using the quadratic equation:

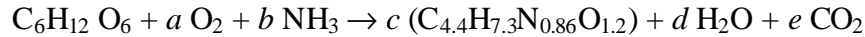
$$[ES] = \frac{-\left(\frac{K_m}{[S]} + 1\right) + \sqrt{\left(\frac{K_m}{[S]} + 1\right)^2 + 4\left(\frac{2}{K_3}\right)[E_o]}}{4/K_3}$$

- c) Use your expression from part (b) and the general definition of the reaction rate to obtain an expression for the rate, v , that contains K_m and K_3 :

$$v = \frac{d[P]}{dt} = k_2 [ES] = \frac{k_2 \left[\sqrt{\left(\frac{K_m}{[S]} + 1\right)^2 + 4\left(\frac{2}{K_3}\right)[E_o]} - \left(\frac{K_m}{[S]} + 1\right) \right]}{4/K_3}$$

$$v = \frac{k_2 K_3}{4} \left[\sqrt{\left(\frac{K_m}{[S]} + 1\right)^2 + 4\left(\frac{2}{K_3}\right)[E_o]} - \left(\frac{K_m}{[S]} + 1\right) \right]$$

3. Consider our “oil-eating” organism discussed in class. In addition to hexadecane, the same organism can also grow on glucose according to the following biological reaction:



Assume that the cells can convert 2/3 (wt/wt) of the substrate carbon to biomass.

a) Calculate each of the stoichiometric coefficients, a , b , c , d , and e .

Amount of C per mol of glucose: 72g; $2/3 * (72\text{g}) = 48\text{g}$ converted to biomass

Biomass balance:	$48 \text{ g/mol} = (4.4)(c)12\text{g/mol}$	<u>$c = 0.909$</u>
N balance:	$14b = (0.86)(0.909)14$	<u>$b = 0.782$</u>
H balance:	$12 + 3(0.782) = (0.909)(7.3) + 2d$	<u>$d = 3.86$</u>
C balance:	$6(12) = 0.909(12)(4.4) + 12e$	<u>$e = 2$</u>
O balance:	$6(16) + 2(16)a = (0.909)(1.2)(16) + (3.86)(16) + 2.16e$	<u>$a = 1.475$</u>

b) Calculate the yield coefficient $Y_{X/S}$ (g dw cell/g substrate).

$$Y_{X/S} = (0.909)(\text{MW biomass})/(\text{MW glucose}) = (0.909)(91.34)/180$$

$$Y_{X/S} = 0.461 \text{ g biomass/ g glucose}$$

c) How would you expect this coefficient to compare to $Y_{X/S}$ for hexadecane (i.e., larger or smaller)? Why?

It should be lower because hexadecane is more reduced than glucose (i.e. $Y_{X/S}^{\text{Hex}} > Y_{X/S}^{\text{Glu}}$). In other words, hexadecane has a greater degree of reductance, so it has more electrons/energy to form biomass.