Biochemistry I, Fall 2005 Name:_____

Exam II - Face Page

The following equations and constants may be useful:

Ligand Binding: $Y = \frac{[ML]}{[M]+[ML]}$ $Y = \frac{[L]}{K_D + [L]}$ $Y = \frac{K_A[L]}{1 + K_A[L]}$

Scatchard Plot: Y/[L] vs Y
$Y/[L] = -Y/K_D + 1/K_D$
$\nu/[L] = -\nu/K_D + n/K_D$

Hill Plot: log(Y/(1-Y)) vs log[L]
Hill Equation: $\log(Y/(1-Y)) = \log K_{\nu} + n_h \log[L]$

Enzyme Kinetics:

For (E + S <--> ES → E + P)

$V_{MAX} = k_{cat}[E_T]$
$K_M = (k_{-1}+k_{cat})/K_1$

Michaelis-Menton equation: $v = \frac{V_{MAX}[S]}{K_M + [S]}$

Steady State Equation for Enzyme Inhibition: $v = \frac{\frac{V_{MAX}}{\alpha'}[S]}{\frac{\alpha}{\alpha'}K_M + [S]}$

Double Reciprocal Plot: $\frac{1}{v} = \frac{K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$

Competitive Inhibition: $\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{1}{V_{MAX}}$

Noncompetitive Inhibition: $\frac{1}{v} = \frac{\alpha K_M}{V_{MAX}}\frac{1}{[S]} + \frac{\alpha'}{V_{MAX}}$

$\alpha = 1+[I]/K_I$ $\alpha' = 1+[I]/K_I'$
$\alpha' = 1$ for competitive inhibition
$\alpha' > 1$ for noncompetitive inhibition

$\alpha = \frac{slope([I]>0)}{slope([I]=0)}$ $\alpha' = \frac{y\text{-}int([I]>0)}{y\text{-}int([I]=0)}$

General Thermodynamics

T = 300K and pH = 7.0 unless otherwise stated.
R = 8.3 J/mol-K $S = R \ln W$
$\Delta G = \Delta H - T\Delta S$ $\Delta G = -RT \ln K_{eq}$

Section A (24 pts, 3 pts/question). Circle the letter corresponding to the best answer.

1. A ligand that binds a macromolecule more tightly than another ligand probably
    a) has a faster on rate.
    b) has a faster off rate.
    c) has a slower on rate.
    d) has a slower off rate.
2. In the binding of O_2 to hemoglobin, 2,3-bisphosphoglycerate
    a) increases the K_D.
    b) stabilizes the tense state of hemoglobin.
    c) is an allosteric inhibitor.
    d) all of the above.
3. When the Hill coefficient, n_h, is greater than 1,
    a) $K_{D1} < K_{D2}$.
    b) $K_{D1} > K_{D2}$.
    c) the binding of the first ligand competes with the binding of the second.
    d) the binding of the second ligand competes with the binding of the first.
4. The affinity with which a macromolecule binds to its ligand can be determined
    a) only at high ligand concentrations.
    b) using a Hill plot.
    c) using a Scatchard plot.
    d) both b and c.
5. An enzyme increases the rate of a reaction by
    a) lowering the free energy of the product(s).
    b) increasing the free energy of the reactant(s).
    c) lowering the free energy of the transition state.
    d) both a and b.
6. The Michaelis-Menton equation
    a) applies to enzyme catalyzed reactions only when substrate is not saturating.
    b) applies to enzyme catalyzed reactions only when [S] >> Km.
    c) only holds for enzyme catalyzed reactions at steady state.
    d) does not apply in the presence of a noncompetitive inhibitor.
7. During the purification of an enzyme,
    a) the V_{MAX} of the enzyme should increase.
    b) the specific activity should increase.
    c) the fraction with the lowest specific activity is carried through to the next step.
    d) both a and b.
8. The rate of migration of a protein in an SDS-PAGE gel
    a) depends on its charge.
    b) depends on its molecular mass.
    c) depends on its charge to mass ratio.
    d) is unaffected by the presence of disulfide bonds between subunits.

B1. (18 pts) Please answer **three** of the following **five** questions. (6 pts each)

1. Explain how an enzyme stabilizes the transition state. Please use the terms enthalpic and entropic in your explanation.

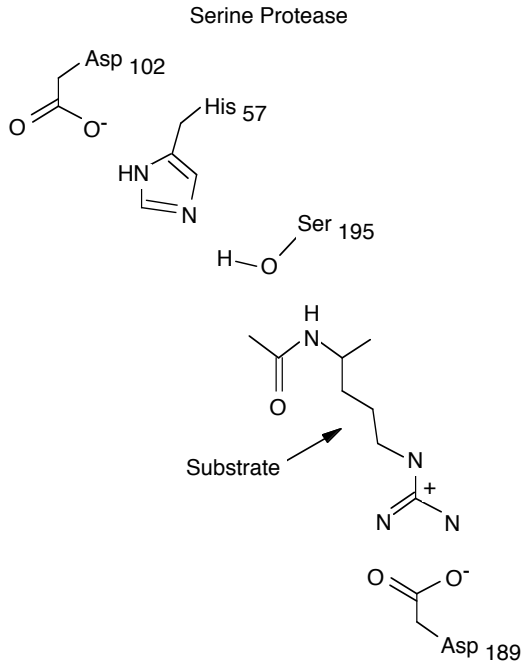
2. If an enzyme lowers the free energy difference between the reactants and the transition state by 30 kJ/mol, what is the rate enhancement provided by the enzyme?

3. Why is it important that an enzyme does not bind its substrate too tightly (ie, that the ES complex is not too stable)?

4. What is the purpose of a Scatchard plot? What is its relationship to a saturation binding curve? Under what assay condition is it imperative to use a Scatchard plot?

5. What property of proteins are utilized in their separation by either anion or cation exchange chromatography? How are the bound proteins eluted from the column?

B2. (16 pts) The figure to the right shows the active site of the serine protease trypsin.



Part A: Select **three** of the **four** residues and indicate its role in peptide bond cleavage. (12 pts)

Asp 102:

His 57:

Ser 195:

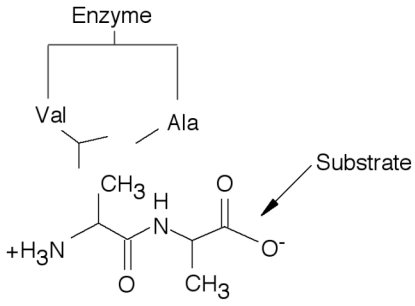
Asp 189:

Part B: Select **one** of the following **two** participants in the mechanism and briefly discuss its role. (4 pts)

Oxyanion hole:

H_2O:

B3. (26 points) Elastase is a serine protease that cleaves peptide bonds following Ala and Gly residues. Shown below is a peptide substrate interacting with the residues (Val and Ala) that line the enzyme's substrate binding pocket.

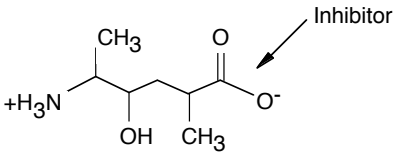


i) Indicate the scissile bond (the bond to be cleaved by the enzyme). (2 pts)

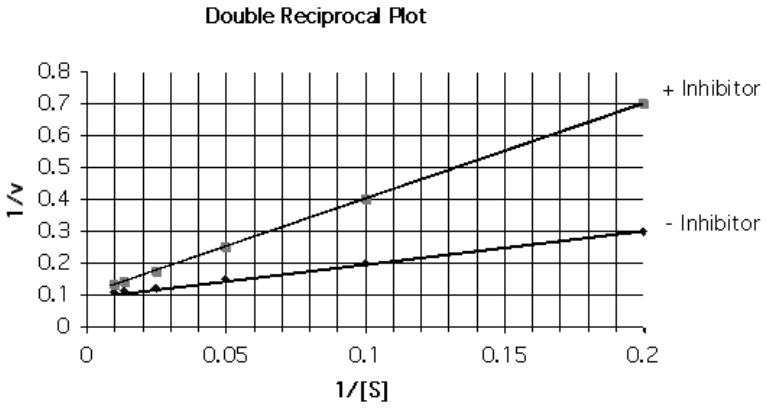
ii) Elastase exhibits a K_m of 10 μM and a k_{cat} of 1000 s^{-1} for the peptide. What is V_{MAX} when the enzyme concentration [E_T] = 10 nM? (2 pts)

iii) A mutant form of the enzyme with a Val to Asp alteration in the substrate binding pocket has been generated. Is this mutation more likely to affect K_m or k_{cat}? *Briefly* justify your answer. (6 pts)

iii) Elastase is inhibited by the compound shown below (labeled inhibitor). Is this inhibitor likely to be a competitive or a noncompetitive inhibitor? Explain your reasoning. (6 pts)



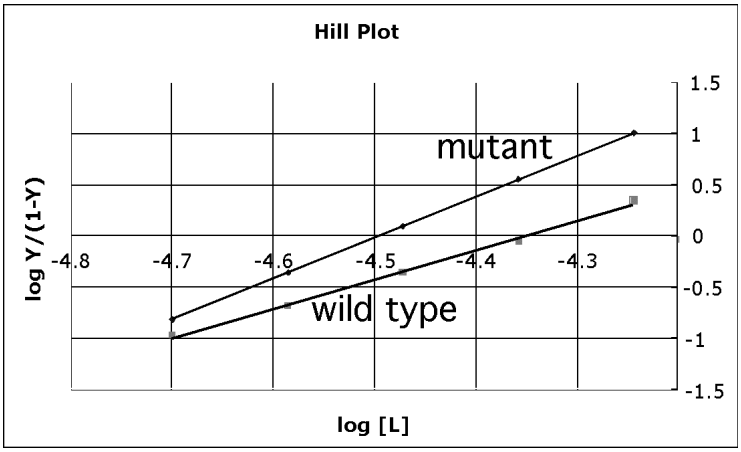
iv) Shown to the right is a double reciprocal plot for elastase in the absence or presence of 10 μM of the above inhibitor. What is the affinity of the inhibitor for the enzyme? (6 pts)



v) Does the inhibitor bind to the ES complex? *Briefly* justify your answer. (4 pts)

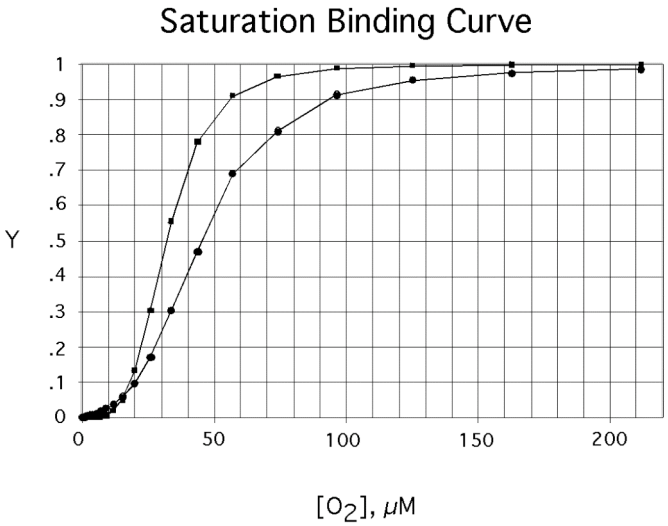
B4. (16 pts) Below is a graph depicting the Hill plot of normal hemoglobin (wild type) and a mutant form of hemoglobin. Both wild type and mutant forms consist of four subunits; each subunit binds one O_2.

i) Which hemoglobin binds O_2 with a higher degree of cooperatively? *Briefly* justify your answer. (4 pts)

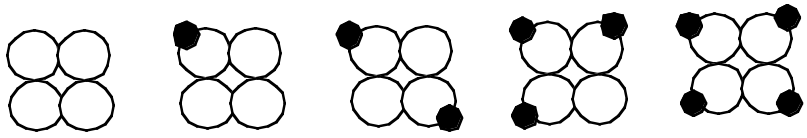


ii) Which hemoglobin has a higher overall affinity for O_2? *Briefly* justify your answer. (4 pts)

iii) To the right is a graph depicting two saturation binding curves. Based on the information provided above, indicate which curve represents the wild type protein and which curve represents the mutant protein.
(4 pts)



iv) Below are five possible liganded forms of hemoglobin. Circle the forms likely to be present when a mutant form of hemoglobin with a Hill coefficient, n_h, of 4 is half saturated with O_2. (4 pts)



A:_____/24
B1:_____/18
B2:_____/16
B3:_____/26
B4:_____/16
Total:_____/100