

rate forward = $k_{\text{forward}}[\text{reactants}]$ rate reverse = $k_{\text{reverse}}[\text{products}]$	Carbonic acid $K_a = 1.6 \times 10^{-4}$ $K_{\text{eq}}$ for the reaction of carbon dioxide with water = $1.69 \times 10^{-3}$	Hydrogen carbonate $K_a = 4.68 \times 10^{-11}$
$V_0 = \frac{V_{\text{max}}[S]}{K_M + [S]} \quad k_{\text{cat}} = \frac{kT}{h} * e^{\frac{-\Delta G^\ddagger}{RT}} \quad V_{\text{max}} = k_{\text{cat}} * E_t \quad \Delta G^\ddagger = \text{Energy of activation}$		
$K_{\text{eq}} = [\text{products}]/[\text{reactants}]$ $\Delta G = \Delta H - T\Delta S$ $\Delta G = \Delta G^\circ + RT \ln Q$ $Q = [\text{products}]/[\text{reactants}]$ $\Delta G^\circ = -RT \ln K_{\text{eq}}$	Phosphoric acid $K_a = 7.25 \times 10^{-3}$ Dihydrogen phosphate $K_a = 1.38 \times 10^{-7}$ Monohydrogen phosphate $K_a = 3.98 \times 10^{-13}$ Acetic acid $K_a = 1.78 \times 10^{-5}$ $\Theta = \frac{[PL]}{[P]+[PL]}$	$k = \frac{kT}{h} e^{-\Delta G^\ddagger / RT}$ $k = 1.381 \times 10^{-23} \text{ J/K}$ $h = 6.626 \times 10^{-34} \text{ J*sec}$ $R = 8.315 \text{ J/mol*K}$

**Multiple choice, 3 points each: Circle the correct answers on this test.**

- An enzyme-catalyzed reaction was carried out with the substrate concentration initially a thousand times greater than the  $K_m$  for that substrate. After 3 minutes, 1% of the substrate had been converted to product, and the amount of product formed in the reaction mixture was 9 mmol. If, in a separate experiment, one-fourth (1/4) as much enzyme and three times as much substrate had been combined, how long would it take for the same amount (9 mmol) of product to be formed?
  - 36 min
  - 6 min
  - 12 min
  - 9 min
  - 1 min
  - None of these, the correct answer is \_\_\_\_\_
- What protein connects myosin to the Z disk proteins?
  - Vimentin
  - Troponin
  - Tropomyosin
  - Titan
  - none of these or more than one of these, circle all correct answers.
- Chaperonins \_\_\_\_\_
  - help shape proteins by catalyzing the isomerization of proline
  - protect proteins from denaturing under high temperature conditions
  - help shape proteins by shuffling disulfide linkages
  - are elaborate protein complexes that hydrolyze ATP in the process of folding proteins
  - ensure that bad genes are not replicated accidentally, thereby ruining the future of the organism.
  - none of the above
  - More than one of the above, circle all of the correct answers.
- The steady state of an enzyme-catalyzed reaction is reached when
  - the rate of disappearance of reactant is constant.
  - the concentration of enzyme-substrate complex equals the concentration of product.
  - the rate of enzyme-substrate formation is constant.
  - the concentration of the enzyme-substrate complex is constant over time.
  - none of these

Matching

5) (6 points) Match the following techniques with how they are used to study proteins. Write the number on the blank line.

- A) nuclear magnetic resonance \_\_\_\_\_
- B) circular dichroism spectroscopy \_\_\_\_\_
- C) X-ray crystallography \_\_\_\_\_
- D) SDS PAGE \_\_\_\_\_
- E) mass spectroscopy \_\_\_\_\_

1. determine exact mass of proteins and the sequence of peptides
2. determine protein structure using diffraction patterns and a computer program
3. measure amounts of protein secondary structures
4. determine structure of a protein in solution
5. determine molar mass
6. determine the primary sequence of a protein

**Essay/Problem section: Give specific details in brief answers using complete sentences.**

6) (10 points) Carbon monoxide is a deadly colorless, odorless gas. Everyone should have a CO detector in their home. What makes carbon monoxide so deadly

7) (6 points) A common theme in biochemistry is “shape change leads to function change” describe one step in the power cycle of muscle contraction when a shape change causes the function to change.

8) (10 points) About 20% of the carbon dioxide that is produced in the tissues (from burning fuel) is transported on the hemoglobin molecule.

A. How is carbon dioxide transported on hemoglobin? (Give specific molecular details.)

B. How many carbon dioxide molecules can bind to each hemoglobin molecule?

C. How does the other 80% of carbon dioxide move from the tissues to the lungs?

D. During hyperventilation, too much carbon dioxide leaves the lungs, what effect will this have on the function of hemoglobin? Will the hemoglobin deliver more or less oxygen to the tissues? Explain your answer.

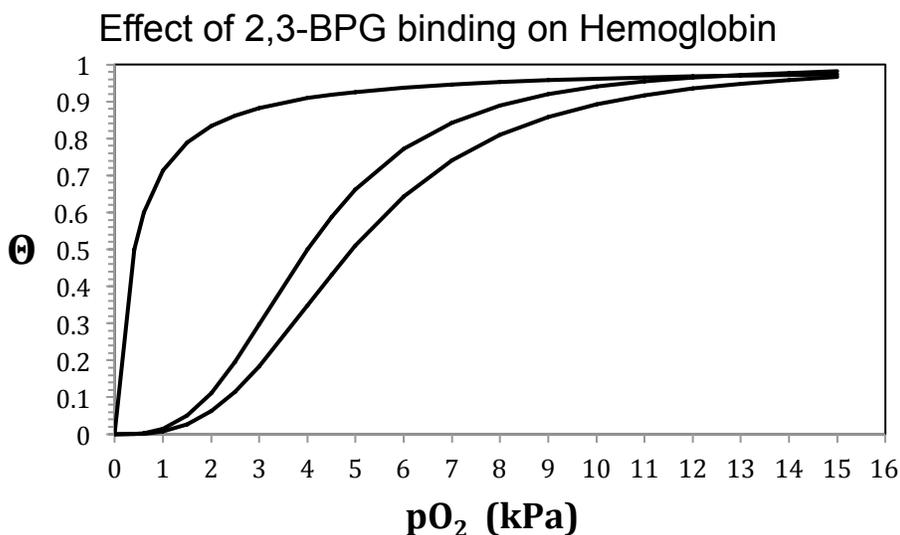
9) (10 points) Using complete sentences, fully explain why the histidine: His<sup>146</sup> has pKa= 8.1 in the tissues. His<sup>146</sup> is located on helix H, 3 residues from the carboxy terminus, (HC3) on the beta subunits. In the lungs, this histidine has its regular pKa (the value that was memorized.)

- 10) (7 points) Both Insulin and Amylin are secreted by the pancreas after a meal has been consumed. Use the following equilibrium dissociation constants, to answer questions about the binding of antibodies to Insulin and to Amylin.

Protein	Antibody 1, $K_d$	Antibody 2, $K_d$	Antibody 3, $K_d$
Insulin	$5 \times 10^{-4} \text{ M}$	$2 \times 10^{-9} \text{ M}$	$2 \times 10^3 \text{ M}$
Amylin	$8 \times 10^{-4} \text{ M}$	$6 \times 10^{-6} \text{ M}$	$3 \times 10^3 \text{ M}$

- Based on these  $K_d$  values, which antibody binds Amylin the tightest?
- For the binding of Antibody 2 to Insulin, what is the amount of Insulin required for  $\Theta = 0.5$
- Choose the best antibody to use in an ELISA that detects Insulin.
- Briefly describe how an ELISA works and the role of the antibody that you selected to detect Insulin.

- 11) (10 points) At high altitudes a person can become light headed, fatigued and nauseous. These effects are exacerbated upon exertion, and can be very dangerous. This effect is due to a lower concentration of oxygen in the air at high altitudes. The body adjusts by making additional red blood cells, so more oxygen can be delivered at the tissues (each hemoglobin delivers about 30% of the oxygen that is bound to it.) But that takes several days, and who wants to wait precious vacation days before having fun on the slopes? The following graph shows the binding curves for three concentrations of 2,3-BPG (2,3-bisphosphoglycerate).



- Label the lines on the graph for 0, 5mM and 8 mM.
- When a person travels from sea level to 11,700 feet, what happens to their 2,3-BPG levels?
- How does 2,3-BPG interact with hemoglobin? Give specific details.

12) (20 points) Chymotrypsin ( $M_r$  21,600) degrades peptides. Under saturation conditions, the substrate glycylyrosinylglycine (GYG), is cleaved at a rate of 4.5 moles/min,  $k_{cat}$  is  $100 \text{ sec}^{-1}$ , and  $K_m$  is 105 mM. The rate of the uncatalyzed reaction is  $1.2 \mu\text{moles/min}$ . Show your work.

a. What is the enzyme concentration?

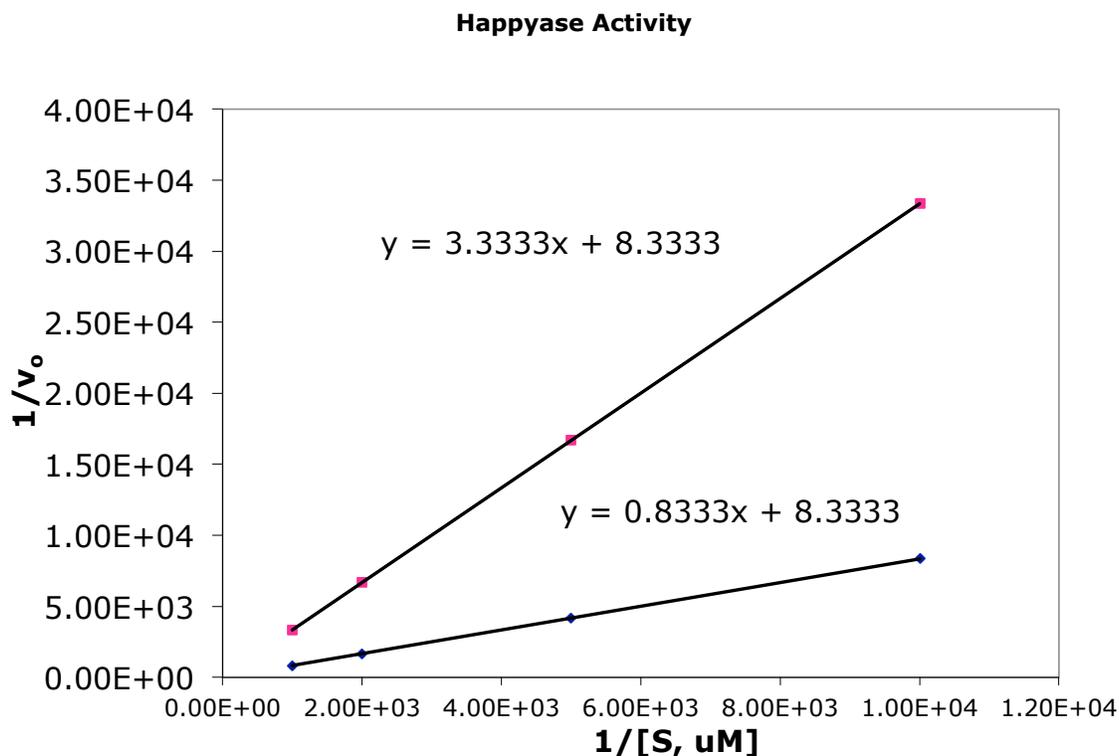
b. What substrate concentration will give a velocity that is  $1/4$  of  $V_{max}$ ?

c. What is the activation energy for the reaction catalyzed by chymotrypsin? [GYG = 0.1M]

d. What is the activation energy for the uncatalyzed reaction? [GYG = 0.1M]

e. What are the products?

- 13) (6 points) Happyase is a naturally occurring enzyme that reacts with sugar to form molecules that act like the active ingredient in chocolate. With Happyase, all is grand. However, Happyase is inhibited by a compound called Nosleepite. An experiment was done to measure the effect of Nosleepite on Happyase and the data is shown in the following graph. (You need to determine which line is for the experiment with Nosleepite.) The velocity of the reaction is measured in mmol/sec, the concentrations of the substrate (Sugar) were micro Molar.



Complete the following table

Experiment	Km	Vmax
Happyase		
Nosleepite + Happyase		

What type of inhibition is this?