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Exam 2 Example Questions

BIOL/CHEM 3361 Name _____

There will also be some short answer questions on the exam similar to those on the first exam.

1. SDS-PAGE separates proteins on the basis of
    a. charge
    b. size
    c. density
    d. shape
    e. all of the above

2A. In what order will the following proteins elute from a gel filtration (aka gel exclusion) column?
Protein A, M_r 84,000, pI 7.1; protein B, M_r 36,000, pI 3.4; protein C, M_r 14,000, pI 5.6
    a. a, b, c
    b. a, c, b
    c. c, a, b
    d. c, b, a

2B. In what order will the proteins in the above problem elute from a DEAE-cellulose (diethylaminoethyl-cellulose) anion exchange column at pH 7.5?
    a. a, b, c
    b. a, c, b
    c. c, a, b
    d. c, b, a

3. Some non-serine proteases contain the following catalytic moiety
    a. Asp
    b. Cys
    c. Arg
    d. a and b
    e. all of the above

4. The enzyme that catalyzes the following reaction belongs to which enzyme class?

$$^{-}O_2CCH{=}CHCO_2^{-} + H2O \rightarrow {}^{-}O_2CCH(OH)CH_2CO_2^{-}$$

    a. oxidoreductase
    b. transferase
    c. hydrolase
    d. isomerase
    e. lyase

5. The β-α-β super-secondary structure is often used to form
    a. coiled coils
    b. β-hairpins
    c. β-barrels
    d. a & c
    e. b & c

6. The chaperones hsp60 and hsp70
    a. degrade incorrectly folded proteins
    b. serve as templates for protein folding
    c. use the energy from ATP cleavage to catalyze protein folding
    d. only help proteins fold correctly after heat shock

7. Predicting secondary structure from primary structure based only on the probabilities with which amino acids are found in the various secondary structures of known proteins is
    a. accurate most of the time
    b. no better than 50% accurate
    c. almost never accurate
    d. is the best that can be done at present

8. The ψ angle refers to the amount of rotation about which bond(s) in the peptide backbone?
    a. N-C_α
    b. C_α-$C_{carbonyl}$
    c. $C_{carbonyl}$-N
    d. a & b
    e. a & c

9. Parallel beta sheets are found most frequently in the interior of proteins because:
    a. the hydrogen bonds between strands in parallel sheets aren't straight
    b. the side chains of the residues which form parallel sheet have less steric hindrance
    c. not all of the peptide carbonyls in parallel sheets can participate in H-bonding
    d. all of the above

10. Proline is unique among the amino acids because
    a. it is the only amino acid whose alpha carbon is not chiral
    b. it exists naturally in two diastereomeric forms
    c. its ψ angle is fixed
    d. its alpha amino group is a tertiary amine

11. Which of the following factors will influence the native conformation of a protein?
    a. pH of the solution
    b. concentration of salt in solution
    c. sequence of the protein
    d. all of the above
    e. a and c

12. How many more amino acid residues are present in a 5 nm long α-helix than in a β-strand of the same length?
    a. 12
    b. 15
    c. 20
    d. 25

13. Urea and guanidinium chloride denature proteins
    a. irreversibly by reacting with asn residues
    b. reversibly by competing for water of hydration
    c. by disrupting the structure of water and forming hydrogen bonds with the polypeptide
    d. by extensive van der Waal's interactions with the protein
    e. none of the above

14. Consider the oligopeptide, AEFGLKMEP, which is on the surface of a protein. What secondary structure would you predict for this peptide?
    a. α helix
    b. β conformation
    c. γ helix
    d. collagen helix

15. Which of the following is not characteristic of collagen?
    a. a 4.4-fold left-handed helix is the basic structural conformation
    b. about 33% of the amino acid residues are glycine
    c. its secondary structure is a polyproline type
    d. many prolines are modified to hydroxyproline

17. Which of the designations listed below does not correspond to a major class of enzymes as outlined by the International Union of Biochemistry?
    a. hydrolases
    b. transferases
    c. carboxylases
    d. isomerases

18. Phosphofructokinase, which catalyzes the reaction below, is classified as a

$$\text{fructose-6-PO}_4 + \text{ATP} \rightarrow \text{fructose-1,6-bisPO}_4 + \text{ADP}$$

    a. ligase
    b. transferase
    c. isomerase
    d. hydrolase
    e. carboxylase

20. A competitive inhibitor ($K_I = 1 \times 10^{-5}$ M) binds to an enzyme that has a true $K_m = 1 \times 10^{-6}$ M for its substrate and a V_{max} of 1×10^{-4} moles/min. Calculate the apparent K_m value in the presence of 1×10^{-3} M inhibitor.
    a. 1×10^{-7} M
    b. 1×10^{-6} M
    c. 1×10^{-5} M
    d. 1×10^{-4} M
    e. 1×10^{-3} M

21. What is the maximum velocity that could be observed in the presence of the competitive inhibitor in the previous problem?
    a. 1×10^{-7} mol/min
    b. 1×10^{-6} mol/min
    c. 1×10^{-5} mol/min
    d. 1×10^{-4} mol/min
    e. 1×10^{-3} mol/min

22. Assume the inhibitor in the question above is a classic noncompetitive inhibitor. What is the apparent K_m value in the presence of 1×10^{-3} M inhibitor?
    a. 1×10^{-7} M
    b. 1×10^{-6} M
    c. 1×10^{-5} M
    d. 1×10^{-4} M
    e. 1×10^{-3} M

23. If V_{max} = 140 µmol/min and v_o = 70 µmol/min at 70 µM substrate for an enzyme that obeys Michaelis-Menten kinetics, what is its K_m?
    a. 50 µM
    b. 70 µM
    c. 140 µM
    d. 175 µM

24. For another enzyme that obeys Michaelis-Menten kinetics, what is the V_{max} value in µmoles/min if v = 70 µmoles/min when [S] = 0.5 K_m?
    a. 25 µmol/min
    b. 70 µmol/min
    c. 140 µmol/min
    d. 210 µmol/min

25. Calculate the ratio $[S]/K_m$ when the velocity of an enzyme catalyzed (no inhibitor) reaction is 10% of V_{max}.
    a. 1/6
    b. 1/3
    c. 1/9
    d. 8/9

26. Given a turnover number of 1×10^{3} s^{-1} and K_m of 2×10^{-3} M for an enzyme, how much less efficient would the enzyme be than the best known enzymes, i.e., perfected enzymes?
    a. 10 times
    b. 10^{2} times
    c. 10^{5} times
    d. 10^{7} times

27. A ping pong bisubstrate reaction is
    a. a single dislacement reaction
    b. a double displacements reaction
    c. not easily distinguished by its kinetics
    d. a and b
    e. b and c

28. Which of the following statements is true about Michaelis-Menten enzymes?
    a. They never have more than one subunit
    b. They always follow rapid equilibrium kinetics
    c. They never have allosteric effectors
    d. a and c
    e. all of the above

29. The Briggs and Haldane steady state assumption rests on the premise that
    a. the concentration of enzyme-substrate complex does not change
    b. the product concentration is insignificant
    c. the substrate concentration is large and does not change significantly
    d. the free enzyme concentration is always in great excess to the concentration of the enzyme-substrate complex

30. Reversible inhibitors of enzyme-catalyzed reactions can be characterized by examining double reciprocal plots of reaction kinetics. In the case of mixed-type noncompetitive inhibition, the presence of the inhibitor yields a curve that
    a. crosses the 1/v axis at the same intercept as in the absence of the inhibitor
    b. crosses the 1/[S] axis at the same intercept as in the absence of the inhibitor
    c. crosses the 1/[S] axis at a point different than that in the absence of the inhibitor
    d. is parallel to the curve determined in the absence of the inhibitor

31. The K_m/K_I ratio for a transition state analog that is an effective reversible inhibitor will be
    a. less than 1
    b. equal to 1
    c. greater than 1
    d. a, b, or c depending upon whether the enzyme has rapid equilibrium kinetics

32. Lineweaver-Burk plots are
    a. semi-log plots used to determine Km values
    b. used determine the number of substrate binding sites n distinguish between single and double displacement reaction mechanisms
    c. used to distinguish ordered from random single displacement bisubstrate reactions
    d. used to evaluate $\Delta G^{\ddagger}$
    e. double reciprocal plots used to determine V_{max}

33. Four competitive inhibitors of an enzyme were found to exhibit the following K_I values. Which is the best inhibitor?
    a. $K_I = 1 \times 10^{-2}$ M
    b. $K_I = 7 \times 10^{-11}$ M
    a. $K_I = 5 \times 10^{-9}$ M
    b. $K_I = 3 \times 10^{-5}$ M

34. The cellular concentration of the substrate of an enzyme is very often found to be
    a. much greater than its K_m value
    b. much less than its K_m value
    c. approximately equal to its K_m value
    d. equal to k_{cat}/K_m

36. The organophosphorus nerve gases, such as sarin, and insectides, such as malathion,
    a. irreversibly inactivate acetylcholine esterase by forming a stable covalent bond with serine
    b. inhibit acetylcholine esterase by transferring a phosphate group to the protein
    c. are strong competitive inhibitors of acetylcholine esterase
    d. must first be hydrolyzed in order to be active

37. Anti-freeze is toxic because alcohol dehydrogenase participates in the conversion of ethylene glycol in the anti-freeze to oxalic acid, which precipitates in the kidneys. The same enzyme is responsible for the toxicity of methanol by converting methanol to
    a. cyanide
    b. formaldehyde
    c. formic acid
    d. dimethyl ketone

38. The catalytic rate constant k_{cat} is
    a. the rate at which substrate binds to an enzyme
    b. a measure of the affinity of an enzyme for substrate
    c. a constant evaluated by a Scatchard plot
    d. the forward rate constant for the rate limiting step of an enzyme

39. The unstable covalent intermediate in the chymotrypsin-catalyzed reaction contains a bond formed between
    a. serine and the carbonyl carbon in the peptide backbone
    b. serine and the nitrogen in the peptide backbone
    c. histidine and the carbonyl carbon in the peptide backbone
    d. histidine and the nitrogen in the peptide backbone

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Problem Set #2: Due Friday 10/14 at 5:00PM in FO 3.602 or turn in class or at workshop
Exam #2 Review: Sunday 10/16 from 1:00-3:00PM in SLC 1.102
Exam #2: Monday 10/17 at 10:00AM in normal classroom

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1. SDS-PAGE separates proteins based on size. When running a SDS-PAGE, the SDS adds negative charges to every 2 residues (making proteins of different charges the same, only size dependent). Reagents like DTT can also be added to denature proteins, therefore eliminating any shape and density dependency. Thus, only size (molecular weight) matters. Thus, **B is the correct answer**.

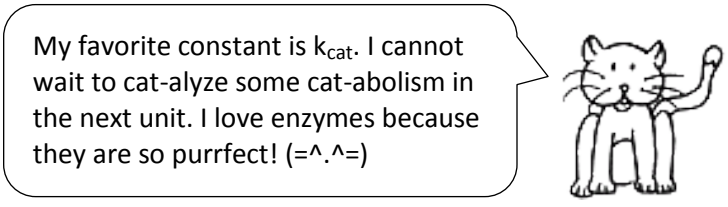
2A. Gel filtration filters based on molecular weight in which an aqueous solution is used to transport the sample through the column resin. It can be combined with other techniques to further separate by acidity, basicity, charge, and affinity for certain compounds. Smaller molecules enter the network of pores and move more slowly. Bigger molecules (ones larger than the pores) do not enter the pores and move around to quickly exit through the bottom of the column. Thus, the larger proteins are eluted before the smaller ones. In order of decreasing size (molecular weight): A > B > C. Thus, **A is the correct answer**.

2B. DEAE-cellulose anion exchange column has a positively charged stationary resin, meaning more negatively charged proteins will be retained. More positively charged proteins will elute first. Remember that pI represents the pH at which a protein has a net neutral charge. At a pH of 7.5, proteins A, B, and C will carry a net negative charge. Protein B will elute last because it is the most negatively charged (lowest pI) at a pH of 7.5. In order of increasing negatively charged: A < C < B. **B is the correct answer**.

3. Moiety means a half or divisions of something. Your two options of non-Ser proteases are the Cys analogs which have a AspHisCys catalytic triad and are similar to the Ser version and the Asp proteases which have two Asp residues. Of the answers, Arg does not match either of these. Thus, **D is the correct answer**.

4. Lyases are the enzymes that catalyze addition to double bonds so **E is the only logical answer**. Don't feel fooled by the addition of water. Hydrolysis, as the name implies, requires the cleavage of something. Your molecule is <u>not</u> cleaved here. Also, although the double bond is being reduced, there isn't an explicit transfer of electrons between molecules (as in NAD^+/FAD^+, etc.).

Number	Classification	Biochemical Properties
1	Oxidoreductases	Act on many chemical groupings to add or remove hydrogen atoms. $A^- + B \leftrightarrow A + B^-$
2	Transferases	Transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. $A\text{-}B + C \leftrightarrow A + B\text{-}C$
3	Hydrolases	Add water across a bond, hydrolyzing it. $A\text{-}B + H_2O \leftrightarrow A\text{-}H + B\text{-}OH$
4	Lyases	Add water, ammonia or carbon dioxide across double bonds, or remove these elements to produce double bonds. $\begin{matrix} X & Y \\ \vert & \vert \\ A\text{-}B \end{matrix} \leftrightarrow A{=}B + X\text{-}Y$
5	Isomerases	Carry out many kinds of isomerization: L to D isomerizations, mutase reactions (shifts of chemical groups) and others. $\begin{matrix} X & Y \\ \vert & \vert \\ A\text{-}B \end{matrix} \leftrightarrow \begin{matrix} Y & X \\ \vert & \vert \\ A\text{-}B \end{matrix}$
6	Ligases	Catalyze reactions in which two chemical groups are joined (or ligated) with the use of energy from ATP. $A + B \leftrightarrow AB$



5. A is wrong because coiled coils (ex. leucine zippers) are made of alpha helices (as the name implies). Beta hairpins are made of only beta sheets and are used to change peptide direction. Thus only **C, beta barrels, is the correct answer**. Beta-alpha-beta (β-α-β) is an important supersecondary motif and beta barrels are their most common domain form.

6. The principal chaperones are heat-shock proteins (HSPs). Hsp60 and Hsp70 are chaperonins that use ATP hydrolysis to drive conformational changes via facilitation of folding. **C is the correct answer**.

7. Amino acids exhibit preferences for certain types of secondary structure, but final secondary structure is no more than 50% accurate to the many different stable conformations a peptide can have. In other words, we can determine a protein sequence given a protein structure but we can predict at best 50% accurately the native structure of a protein from its amino acid sequence. Thus, **B is the correct answer**.

8. The ψ (psi) angle refers to the rotation between the carbonyl and α-carbon. The ϕ (phi) angle refers to the rotation between the α-carbon and C-N. **B is the correct answer**. Please don't get this question wrong.

9. Antiparallel beta sheets are often found at the surface of a protein, while parallel beta sheets are found in the interior of proteins. Parallel beta sheets are defined by their skewed H-bonding. For answer choice B, you would expect skewed bonding would lead to more steric hindrance. **A is the correct answer**.

10. The review list tells you to know that proline has a fixed ϕ (phi) angle. What that angle is can be debated, but its fixed nature due to proline's unique cyclic side chain is important. The only amino acid whose α-carbon is not chiral is glycine. **C is the correct answer**.

11. The pH (which affects protonated states of your amino acids), the salt concentration (which can affect pH), and the sequence of the peptide (the most obvious) affect the native conformation of a protein. Thus, **D is the correct answer**.

12. The number of residues in a 5 nm alpha helix is (5 nm/0.54 nm per turn)(3.6 residues per turn)=33.3 residues. For a 5 nm beta sheet, it's (5 nm/0.695 nm per repeat)(2 residues per repeat)=14.4 residues, roughly 20 less than the alpha helix of the same length. **C is the correct answer**.

13. Neither the book nor the powerpoint are really clear on this. Urea is a biological compound that handles the disposal of excess nitrogen in the body and acts as an agent in the denaturation of proteins. These denaturants unravel the tertiary structure of proteins by destabilizing internal, non-covalent bonds between atoms. One method involves direct interaction via hydrogen bonding to polarized areas of charge. Urea can also denature proteins indirectly. Anyways, **C is a common theme of denaturants and is the correct answer here**.

14. The actual sequence is Ala-Glu-Phe-Gly-Leu-Lys-Met-Glu-Pro. It's an alternation of hydrophobic and hydrophilic residues, which matches that of anti-parallel beta sheets, something stated on the review sheet. **B is the correct answer**.

15. **A is the correct answer** because collagen is a 3-fold left-handed helix.

17. The six major classes of E.C. enzymes are oxidoreductases, transferases, hydrolases, lyases, isomerases, ligases (look at problem 4 for table of the six classes). Carboxylases are a subclass of ligases and are not class of enzyme by themselves. Thus, **C is the correct answer**.

18. From the reaction we can see the *transfer* of phosphate from the ATP to the sugar, so a transferase would catalyze that reaction. Transferases transfer functional groups between donor and acceptor molecules. Kinases are specialized transferases that regulate metabolism by transferring phosphate from ATP to other molecules. **B is the correct answer**.

20. Using the formula $K_{(m)}=K_m(1+[I]/K_I)$, we get $K_{(m)}=(1e\text{-}6)(1+1e\text{-}3/1e\text{-}5)$ to get 1e-4 M or **answer choice D**.

TABLE 13.6 The Effect of Various Types of Inhibitors on Apparent K_m and Apparent V_{max}

Inhibition Type	Apparent K_m	Apparent V_{max}
None	K_m	V_{max}
Competitive	$K_m(1 + [I]/K_I)$	V_{max}
Noncompetitive	K_m	$V_{max}/(1 + [I]/K_I)$
Mixed	$K_m(1 + [I]/K_I)/(1 + [I]/\alpha K_I)$	$V_{max}/(1 + [I]/\alpha K_I)$
Uncompetitive	$K_m/(1 + [I]/K_I)$	$V_{max}/(1 + [I]/K_I)$

21. Competitive inhibitors do not affect V_{max} since with enough substrate, the inhibition can be overcome. Thus, $V_{max} = V_{(max)}$ and the V_{max} remains as **choice D**.

22. The Michaelis constant is the substrate concentration at which the initial velocity is half of its V_{max}. Classic noncompetitive inhibitors do not affect K_m. Thus, $K_m = K_{(m)}$ and the original K_m is the correct answer. **B is the correct answer**.

23. If V_{max} is 140 µmol/min and our current velocity is 70 µmol/min, we are at $0.5V_{max}$. Our substrate concentration is thus the K_m. Remember that the Michaelis constant is the substrate concentration at which the initial velocity is half of its V_{max}. Thus, the K_m is 70 µM. **B is the correct answer**.

24. The Michaelis-Menten equation is $v = (V_{max}*[S])/(K_m+[S])$. Plugging in $0.5K_m$ wherever we have [S] is in the MM equation, we get 70 µmol/min = $(V_{max}*0.5K_m)/(K_m+0.5K_m)$, which simplifies to 70 µmol/min = $V_{max}/3$. This solves to V_{max} being 210 µmol/min. **D is the correct answer**.

25. Plugging in $0.1V_{max}$ for the V_0 gives us an MM-equation of $0.1V_{max} = (V_{max}*[S])/(K_m+[S])$. The V_{max}es cancel out, and then you solve for the ratio of [S] to K_m (not hard algebraically) and will get a ratio of 1/9, or **answer choice C**.

26. Efficiency is K_{cat}/K_m, so our efficiency is 500,000 $M^{-1}s^{-1}$. I personally don't like this question since the "perfect enzyme's" efficiency is debatable (the notes say 10^9), but **B is the answer choice** that's most within the reasonable range of how much more efficient a perfect enzyme is compared to the given enzyme. 10 or 10,000 times more efficiency are both too extreme of answers to be correct no matter the efficiency of a perfect enzyme.

27. Ping-pong reactions are double displacement reactions, hence the ping-pong analogy. Choice D doesn't make sense since it can't be both at once and choice C (and thus E) is wrong because we have our diagnostic Lineweaver-Burk plots to distinguish bisubstrate reactions. **B is the correct answer**.

28. Choice A is wrong since MM enzymes can have more than one subunit (and more than one active site). Choice B is wrong since they can follow steady state kinetics as well. **C is the correct answer**. MM enzymes can have allosteric inhibitors (noncompetitive inhibitors), but not allosteric activators (effectors); these will be covered with Dr. Spiro's first lecture next Wednesday.

29. **A is the stated assumption and thus the correct answer**.

30. Mixed noncompetitive inhibitors lower the V_{max} and change the K_m (up or down depends on the inhibitor). Thus, the Lineweaver-Burk line should change at both intercepts, eliminating both A and B. **C is the correct answer**.

31. The K_m/K_I ratio is a measure of relative affinity for inhibitor to substrate. The higher the ratio, the higher the affinity for inhibitor (because K_I is small, thus increasing the fraction). Remember that the lowest K_I (like the lowest K_m) is the best affinity because it represents the concentration of inhibitor to effectively inactivate an enzyme. We would expect effective inhibitors to have more affinity for the enzyme than the substrate so K_I should be less than K_m, leading to a K_m/K_I ratio that's greater than 1. **C is the correct answer**.

32. Choice A is wrong; Lineweaver-Burk plots are not semi-log plots. Choice B refers to the Scatchard plot. Choice C would be nice if it was true, but it's not sadly. Choice D refers to energy diagrams. Thus, **E is the correct answer**.

33. The lowest K_I (like the lowest K_m) is the best affinity because it represents the concentration of inhibitor to effectively inactivate an enzyme. **B is the correct answer**.

34. **C is the correct answer**. Just a factoid from the powerpoint slides that when $v_o = 1/2V_{max}$ then Km = [S]. Also, it is the reason we use K_m so often. It describes both affinity and estimates physiological concentration.

36. Nerve gases and organophosphorus insecticides are common examples of irreversible inhibitors which form stable covalent bonds at the active site, permanently inhibiting catalytic activity. **A is the correct answer**.

37. Alcohol dehydrogenase (ADH) oxidizes methanol (an alcohol) to an aldehyde, which is the toxic formaldehyde in this case. **B is the correct answer**.

38. K_{cat} is another name for K_2, which is the forward rate constant of conversion of ES to E+P, or the rate-limiting step. **D is the correct answer**. Choice A is K_1 (the rate at which substrate binds to an enzyme), choice B is K_m (a measure of the affinity of an enzyme for substrate, $K_m = (k_{-1}+k_2)/k_1$), and choice C is K_S (a constant evaluated by a Scatchard plot, also a measure of the affinity of an enzyme for substrate, $K_S = k_{-1}/k_1$).

39. Chymotrypsin is a serine protease and has the catalytic triad AspHisSer at its active site. Ser-195 forms a covalent bond with the peptide. Serine's oxygen performs a nucleophilic attack on the carbonyl carbon of the peptide backbone, forming the unstable covalent intermediate. This is shown in stage b of figure 14.21. **A is the correct answer**.