

Chemistry 135, First Exam
September 17, 2018

Please only turn this page once instructed to do so by the instructor.

This exam will be worth 15% of your overall grade. Please read all of the instructions/questions carefully and answer the question in the space provided or indicated. There should **10** total pages containing **3** multi-part questions. Be sure to transfer any answers you wish to receive credit for to the space provided. No calculators, phones, electronic devices, etc. may be used during this exam. Good luck!

Questions

1	20 points
2	28 points
3	106 points
Total	150 points total

Remember that whenever you take an exam, you are really taking *two* tests. The first is a test of your knowledge from the class. The second, and more important, is a test of integrity: that the answers you put down represent your answers and not someone else's. Please make sure to pass the more important test!

You will not need any calculators, phones, electronic devices, headphones, etc. to complete this exam (indeed, they will slow you down), so please make sure these are put away.

Pre-Exam Survey: please help us understand the study habits of Chem 135 students and fill out this survey (3 questions) while you wait for the exam to start. There is no incorrect answer – I'm just trying to collect data on how to help students in Chem 135 succeed. ~Prof. Miller

1. I went to the following person's office hours: (circle the choice that most correctly describes you)

Prof. Miller Vanessa Dan Pavel More than one person None

2. I attended the review session held by Prof. Miller: YES NO

3. Did you use the textbook to aid your study preparations? YES NO

The following space is intentionally left blank and may be used as scratch paper, space for a poem, or even an illustration. If you do include work in this area, be sure to transfer any answers you want graded to the provided space in the exam.

1. Please carefully evaluate the following statements. If the statement is correct, please mark it as "True." If the statement is false, please provide the correction(s) that renders the statement true. (4 points each)

For example:

The chemical biology program at Stanford is the best!
This would be marked as "FALSE" and could be corrected in the following way:

The chemical biology program at ~~Stanford~~ *Cal* is the best!

a) _____ Enzymes are special classes of proteins that are able to catalyze chemical reactions. In class we discussed a number of specific ways in which enzymes catalyze reactions. Regardless of the particular details of catalysis, all enzymes lower the ΔG° of reaction to achieve catalysis.

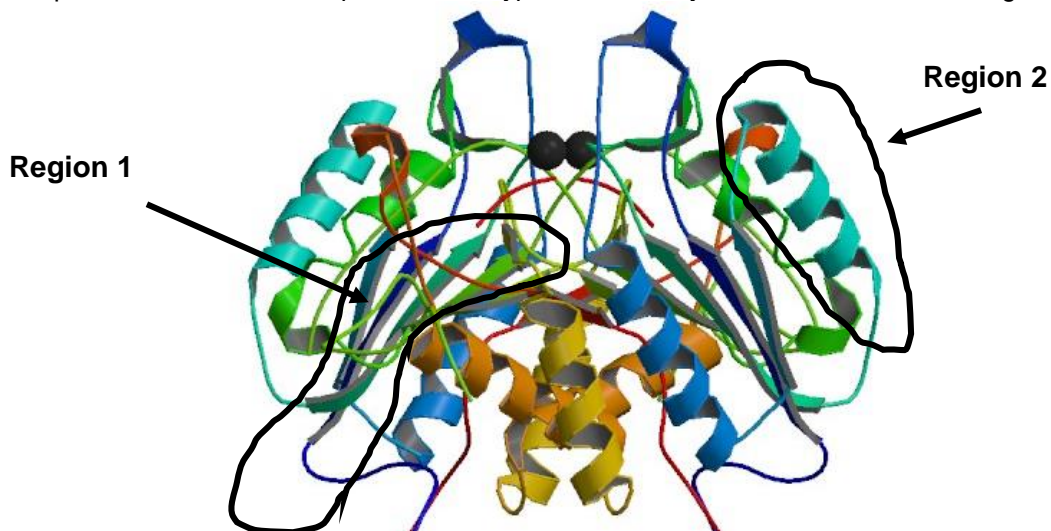
b) _____ The values of pK_a and K_d are thermodynamic parameters describing the equilibria for acids and protein-ligand interactions, respectively. A low pK_a indicates a low affinity for protons and a low numerical K_d value indicate a high affinity for particular ligand.

c) _____ In the process of protein folding, hydrophobic residues tend to pack on the inside of a protein, away from the surface of the protein, where they would be exposed to the aqueous environment of the cell. This is driven by the hydrophobic effect, meaning the main thermodynamic stabilization comes from favorable enthalpic interaction between non-polar sidechains.

d) _____ We discussed several modes of irreversible enzyme inhibition in class: competitive, uncompetitive, and mixed inhibition. The main difference between competitive and uncompetitive/mixed inhibition is that competitive inhibitors bind to allosteric sites while mixed or uncompetitive inhibitors bind the catalytic site of the enzyme.

2 Caspase is an enzyme involved in programmed cell death, or apoptosis. Shown below is the three-dimensional structure of caspase-3.

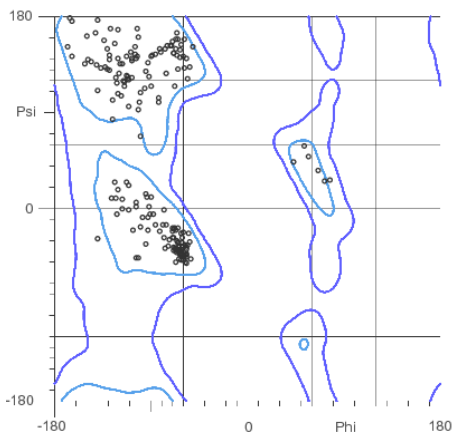
a) In the space below, indicate the predominant type of secondary structure in each of the regions. (4 points)



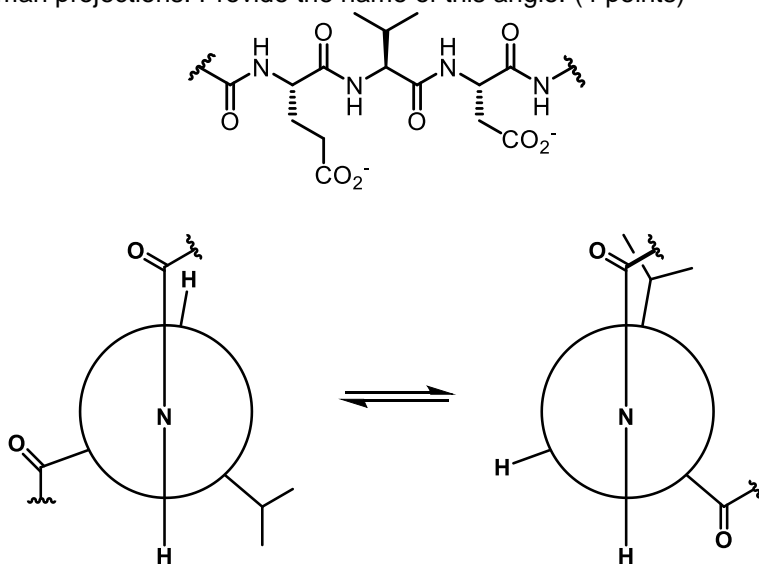
b) Identify the areas on the following Ramachandran plot that correspond to the regions from part (a), above. (4 points)

Region 1

Region 2



c) Shown below is a stretch of amino acid residues in caspase and two Newman projections for a particular amino acid residue in this sequence of amino acids. On the peptide below, indicate which dihedral angle is depicted in the Newman projections. Provide the name of this angle. (4 points)



d) Under each Newman projection above, indicate the value of the dihedral angle you named in part (c). (4 points)

e) For the equilibrium depicted by the two Newman projections, is K_{eq} greater than or less than 1? Please briefly explain your reasoning (1-2 sentences) (4 points)

f) Estimate the standard free energy associated with the interconversion of the two Newman projections, if the ratio of the favored conformation to unfavored conformation is approximately 100:1. Briefly explain how you arrived at your estimation (1-2 sentences/equation). (8 points)

3. In class, we discussed the mechanism of action of chymotrypsin, a serine protease which catalyzes the hydrolysis of amide bonds. Caspases are also a family of proteases, but use a catalytic cysteine to perform catalysis. Caspases (cysteine-dependent, aspartate-directed proteases) are an integral part of a cell's programmed cell death, or apoptosis, pathway. Since this pathway often goes awry in cancer, caspases have been widely studied to understand their substrate specificity, mode of catalysis, and regulation. Shown below is the 1-letter amino acid code for a consensus substrate of caspase-3. (N to C terminus, left to right).

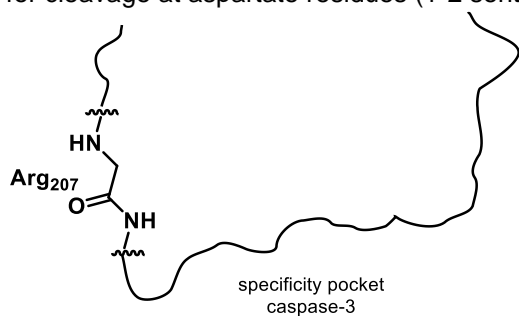
D – E – V – D – G

a) Draw out the structure of this peptide, N to C terminus, left to right, indicating the correct stereochemistry at the α carbons and charge, where applicable, for this peptide at pH 7. Please also calculate the net charge on this peptide at pH 7. In addition, estimate the relative ratio of charged to neutral forms for any ONE of the groups you indicated is charged at pH 7. (16 points)

b) Caspase-3 cleaves peptides between aspartate and glycine. Indicate, on the substrate you drew above, where caspase-3 would cleave this peptide. (2 points).

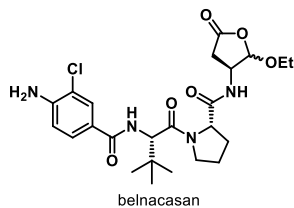
c) The research group of Jim Wells at UCSF showed that the aspartate immediately before the glycine in the caspase-3 consensus sequence confers substrate specificity. The Wells group demonstrated that some replacements at this aspartate were tolerated. Provide the name of an amino acid you think could replace the aspartate with minimal effect on the ability of caspase-3 to cleave the substrate. Provide a brief (1-2 sentence) answer. (6 points)

d) Much like chymotrypsin's "hydrophobic pocket," caspase-3 contains a specificity pocket (shown below) that helps direct the protease to cleave at aspartate residues. Inside the specificity pocket, Arg₂₀₇ provides a key interaction to confer substrate specificity. Draw in the missing side-chain of Arg₂₀₇ below, being sure to indicate the correct stereochemistry at the alpha carbon. Briefly explain why Arg helps to define selectivity for cleavage at aspartate residues (1-2 sentences). (6 points)

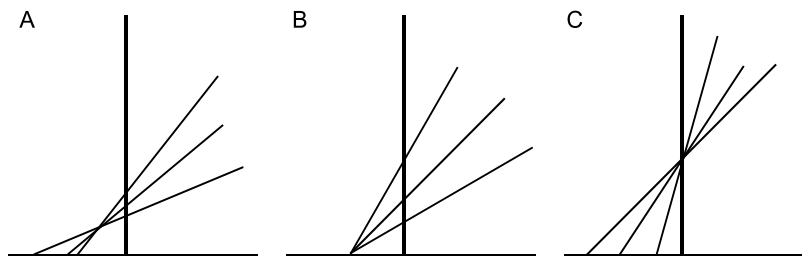


e) Suppose you measure the rate of reaction between wild-type caspase-3 (i.e. no mutations) and the substrate from part (a). Sketch a plot of what you would expect reaction velocity vs. substrate concentration would look like, label the axes, and indicate graphically where V_{max} and K_M would appear on this plot. What additional information would you need to solve for k_{cat} ? (10 points)

f) Belnacasan (shown below) is an inhibitor of caspases that was developed by the pharmaceutical company, Vertex, and underwent a clinical trial for the treatment of epilepsy (the drug was ultimately withdrawn). If belnacasan is a competitive inhibitor of caspases, sketch a plot of reaction velocity vs. substrate in the presence of belnacasan on the same plot as in part (e). Indicate on this same plot where K_M and V_{max} would be for the case of inhibition. (6 points)

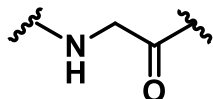


g) In a separate set of experiments, you also determined double reciprocal plots for the substrate in part **(a)** with different concentrations of wild-type caspase (see below). Which of the following Lineweaver-Burk plots below would you expect to see? Please add labels to the axes on the plot you select, and indicate where you could graphically determine K_M and V_{max} . Explain your answer (1-2 sentences). (10 points)

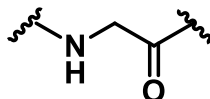


h) Caspases are proteases, like chymotrypsin. Chymotrypsin makes use of a “catalytic triad” to hydrolyze proteins. Please draw out the structures of the 3 residues involved in the catalytic triad of chymotrypsin (please indicate appropriate stereochemistry at the alpha carbon). Indicate how interactions between these residues enhances the reactivity of chymotrypsin’s catalytic serine. (8 points)

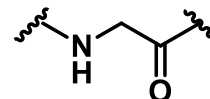
chymotrypsin



amino acid 1
(catalytic residue)



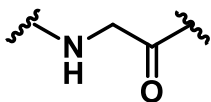
amino acid 2



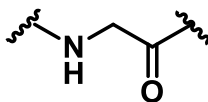
amino acid 3

i) Caspases use a similar, yet distinct strategy for enhancing the potency of its catalytic nucleophile. Unlike chymotrypsin, caspases use a catalytic cysteine residue instead of serine to achieve catalysis. Additionally, the interaction provided by the third amino acid in chymotrypsin's catalytic triad is replaced in caspase by the carbonyl of the amide in the main protein chain, rather than the functionality of the "R" group ("Thr", in the case of caspase). In the space below, please indicate, with the appropriate stereochemistry, the structure of the side chains in caspase's "triad". Please also indicate which interactions enhance the nucleophilic character of the catalytic cysteine. (Amino acid 2 is the same for both chymotrypsin and caspase). (8 points)

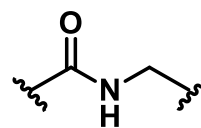
caspase



amino acid 1
(catalytic residue)



amino acid 2



amino acid 3

j) Mutation of amino acid 2 in the catalytic "triad" of caspase to alanine results in a significant decrease in the overall efficiency of caspase to process its substrate. What is a convenient, generalizable metric used to assess the efficiency of an enzyme? Is there an upper limit to this efficiency rating? If so, please provide a value estimate for the upper limit. Briefly explain your answer (1-2 sentences). (8 points)

k) When all of the amino acids in the "catalytic triad" of caspase are mutated to alanine, the enzyme caspase still shows a small rate enhancement for substrate hydrolysis compared to the uncatalyzed reaction. Provide an explanation for this observation (2-3 sentences). (8 points)

l) Provide an arrow-pushing mechanism for the caspase-catalyzed cleavage of a protein. Experimental evidence points to the formation of an acyl-enzyme intermediate in the catalytic mechanism of caspase. Please make sure your reaction mechanism reflects this fact and indicates how the catalytic "triad" you identified in part (j) helps to improve the catalytic activity of caspase. (20 points)

