Name: KEY
SID:

# Chemistry 135, First Exam

## September 23, 2015

This exam will be worth 15% of your overall grade. Please read all instructions/questions carefully and provide answers in the space provided. There should be **7** total pages containing **3** questions spread out over **5** pages. The first page is this coversheet, and the last page is left intentionally blank. You may use this last page as scratch paper, but be sure to transfer any answer you wish to receive credit for to the space provided. No calculators, phones, electronic devices, etc. may be used during this exam. Good luck!

Be sure to put you name on every page, since we will separate the exam and scan it electronically for grading.

(1) _____ (55 points)

(2) _____ (30 points)

(3) _____ (15 points)

Total _____ (100 points)

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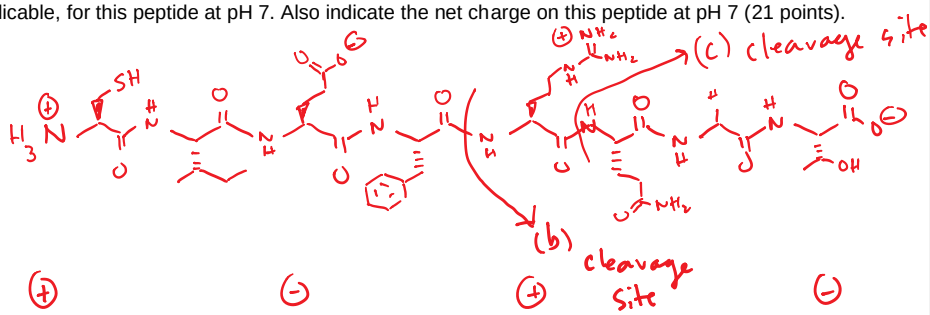
1. As we learned in class, chymotrypsin is a pancreatic serine protease that catalyzes the hydrolysis of peptide bonds, with a rate enhancement on the order of $10^9$! Shown below is the 1-letter amino acid code (N to C terminus, left to right) for a substrate of chymotrypsin.

C-I-E-F-R-Q-G-T

a) Draw out the structure of this peptide, indicating appropriate stereochemistry and charge, where applicable, for this peptide at pH 7. Also indicate the net charge on this peptide at pH 7 (21 points).



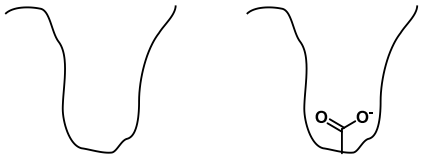

(a) pH = 7 (+) (−) (+) (−)

net charge = 0

b) The so-called "hydrophobic pocket" in chymotrypsin helps to define the specificity of chymotrypsin for its substrate (it cleaves peptide bonds that come after amino acid residues with aromatic side chains). Indicate on your structure where chymotrypsin will cleave this substrate. (3 points)

c) Chymotrypsin's hydrophobic pocket is rather deep, and therefore can accommodate a bulky aromatic side chain. Suppose mutations were made to chymotrypsin within the hydrophobic pocket such that a glutamate side chain now protrudes into the previously empty space (see below). How might this mutation affect the substrate specificity of chymotrypsin? (1-2 sentences) Indicate on your substrate above where the glutamate mutant would cleave the peptide. (6 points)

"Hydrophobic" Pockets of Chymotrypsin and Mutants



Wild-type chymotrypsin Aspartate mutant

This would change the specificity to a protease that cleaves after positively-charged amino acids → to pair w the Asp in the hydrophobic pocket.

d) Would you expect this mutation to have a greater effect on $k_{cat}$ or $K_M$? Briefly explain (1-2 sentences). (6 points)

This mutation would have a greater effect on $K_M$ because the mutation primarily effects binding, not catalysis.

+2 for $K_M$, +4 for explanation

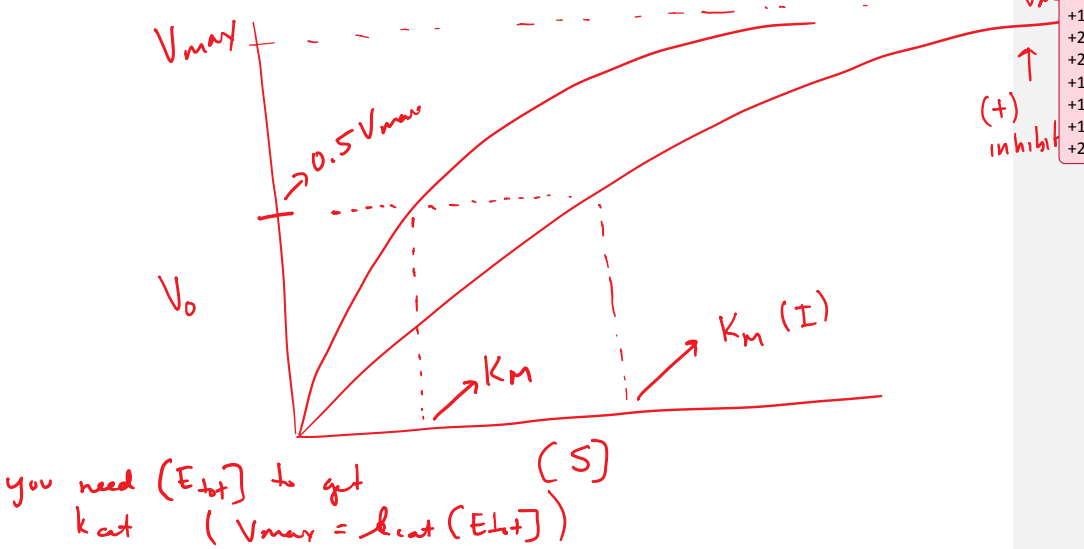
**Commented [EWM1]:** +2 pts for each correct amino acid sidechain (16 pts total)
+4 pts for correct charges at pH 7
+1 for net charge

**Commented [EWM2]:** +3 correct cleavage site

**Commented [EWM3]:** +3 for explanation because of pairing with negatively charged Asp
+3 for cleavage after the Arg

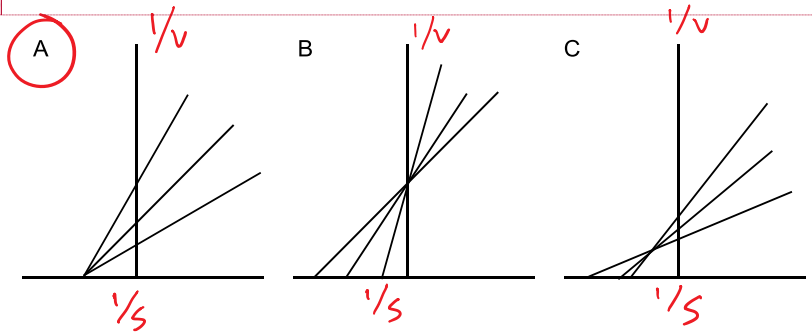
**Commented [EWM4]:** +2 for Km
+4 for explanation

e) Suppose you measure the rate of reaction between wild-type chymotrypsin (i.e. no mutations) and your substrate from part (a). Sketch a plot of what you would expect reaction velocity vs. substrate concentration would look like and indicate graphically where $V_{max}$ and $K_M$ would appear on this plot. What additional information would you need to solve for $k_{cat}$? How would the addition of a competitive chymotrypsin inhibitor change the plot of reaction velocity vs. substrate concentration? Graph this on the same plot and indicate where $K_M$ and $V_{max}$ would be for the case of inhibition. (11 points)




**Commented [EWM5]:** +1 for Y axis
+1 for X axis
+2 for Vmax of substrate without inhibitor/curve
+2 for Km without inhibitor
+1 for curve with inhibitor
+1 for Km + inhibitor
+1 for same Vmax
+2 for needing Enzyme total for kcat

f) Separately, you also determined double reciprocal plots for the substrate in part (a) with different concentrations of chymotrypsin (see below). Which of the following Lineweaver-Burk plots below would you expect to see? Please add labels to the axes on the plots below. Explain your answer (1-2 sentences). (8 points)




**Commented [EWM6]:** +1 for y axis
+1 for x axis (on at least one graph)
+2 for picking A
+4 for explanation

(A) Is the answer, because changing $[E_{total}]$ alters the $V_{max}$ without changing the $K_M$, which is the situation depicted in (A).

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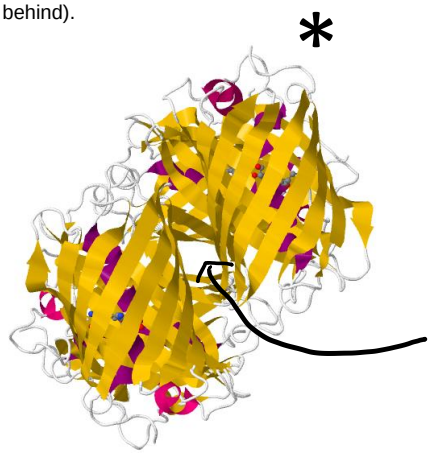
2. The green fluorescent protein, GFP, and its multicolored cousins have been invaluable tools in cell biology because they allow researchers to “spy” on proteins in their native environment. GFP and related proteins were isolated originally from jellyfish and later from various corals. The structure of a red fluorescent protein (dsRed) is shown below (four monomers, or separate polypeptides, two in the front, two behind).




Part (e)

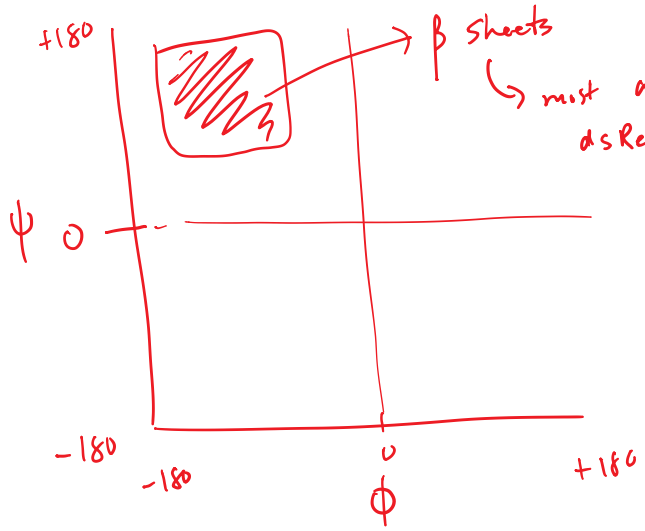
a) What element of secondary structure dominates the overall fold of the dsRed monomers (oftentimes called the “soda can” fold)? (4 points)

**Commented [EWM7]:** +4 for beta sheet
+1 if parallel beta sheet

beta sheet (anti-parallel)

b) Based on this answer, sketch a Ramachandran plot and indicate approximately where the majority of amino acid residues in dsRed would exist. (8 points)

**Commented [EWM8]:** +1 for phi
+1 for psi
+2 for angles listed on x and y axis
+4 for correct approximate positioning of beta sheet angles



c) What two amino acids would you expect to find in the unstructured loop areas (indicated by an asterisk) of dsRed. Briefly explain your answer (1-2 sentences). (8 points)

Proline: It is restricted in the conformations it can adopt and cannot provide an H-bond donor on account of its tertiary amide. Coformational flexibility and H-bond donation and acceptance are required for secondary structure.

Glycine: While glycine can provide both H-bond donors and acceptors, it is too flexible in its conformation about the phi and psi angles, so it doesn't "stay put" in secondary structure.

**Commented [EWM9]:** +2 for proline
+1 for restricted rotation
+1 for no H bond donation
+2 for glycine
+2 for flexibility

d) Wild-type, or native, dsRed exists as a dimer or tetramer, that is, two or four individual polypeptide chains folded into globular proteins and existing together as a single functional unit (see below). This is an example of what level of protein structure? (4 points)

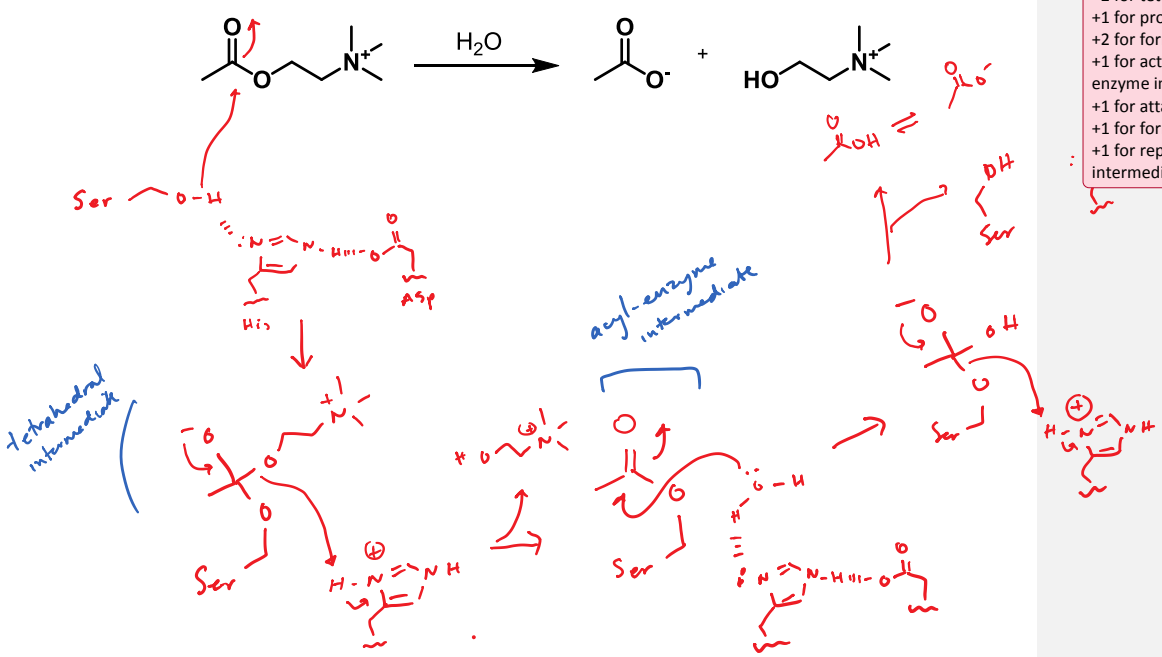
quaternary structure

e) A key step in the "domestication" of dsRed for use in the lab was breaking their need to exist as dimers and tetramers. Native dsRed have amino acids like Leucine that project their "R" groups into the interface between the individual protein monomers (arrow, above). What mutation could you make that might promote monomerization of dsRed? Briefly explain your answer. (6 points)

Substituted Leucines for a positively charged amino acid (Asp, Glu, Lys, Arg) might cause monomerization. The formerly buried hydrophobic residues would now be hydrophilic and would be able to face the bulk solvent (water) in order to participate in H-bonding. Also, putting several Args together in a tight space would cause unfavorable charge repulsion. (Mutation to Arg is actually how this was done in real life).

**Commented [EWM10]:** +2 for charged amino acids
+2 for charge repulsion
+2 for increased stability if solvent exposed vs buried

3. Acetylcholine esterase (AChE) is an enzyme that catalyzes the hydrolysis of acetylcholine (ACh) into acetate and choline (shown below) with a $k_{cat}/K_M$ that approaches the diffusion rate. AChE possesses an active-site Serine that is responsible for catalysis, and experimental studies show that His and Asp are also critical for achieving high catalytic efficiency. Finally, mechanistic studies point to the generation of an acyl-enzyme intermediate in the reaction pathway. Draw an arrow-pushing mechanism for the action of AChE on the substrate that accounts for these observations. Be sure to include the key amino acid side chain residues that are involved. (15 points)




**Commented [EWM11]:** +2 for serine attack on carbonyl
+3 for structures of ser, his, aspartate (1 each)
+1 for a H-bonding network to increase serine nucleophilicity
+2 for tetrahedral intermediate #1
+1 for protonation of choline leaving group from His
+2 for formation of acyl enzyme intermediate
+1 for activation of H2O by His for attack on the acyl enzyme intermediate
+1 for attack on carbonyl of acyl enzyme
+1 for formation of tetrahedral intermediate #2
+1 for reprotonation of ser as it leaves from the acyl enzyme intermediate.

This last page is intentionally left blank as “scratch paper” or as a space for a poem or illustration. Be sure to transfer any work you wish to receive credit for to the corresponding sections of the exam.