Chemistry 135, First Exam
September 23, 2019
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 **11** total pages containing **4** 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	16 points
2	18 points
3	26 points
4	91 points
Total	**151 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.

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) **TRUE** The unique properties of the amide bond restrict the dihedral angles that can be adopted within a protein. Generally speaking, the phi (Φ) angle is more restricted than the psi (Ψ) angle.

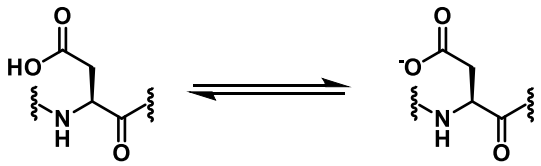
b) **FALSE** The thermodynamic property of ΔG'° refers to the amount of free energy required or released to go from biochemical standard state ~~equilibrium concentrations~~ to equilibrium ~~actual concentration~~.

c) **FALSE** Although many specific strategies for catalysis exist, all enzymes act as catalysts by binding best to the transition states of their substrates.

d) **FALSE** Important aspects of protein structure include the primary structure, or the linear order of amino acid residues in the main chain of the peptide; secondary structure, which depends on the ability to adopt appropriate phi/psi angles and form hydrogen bonds between ~~R-groups~~ main chain N-H and C=O ; and tertiary structure, or the overall folding of the protein, which is driven by favorable entropic ~~enthalpic~~ effects upon liberating free water molecules.

2. For the following equilibria, please provide a value for K_{eq} and for $\Delta G'^{\circ}$ (in units of kcal/mol). Please assume pH = 7. (6 points each)

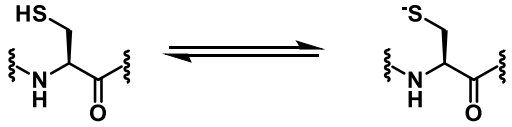
a)



Keq = 1000 (pKa of carboxylic acid = 4; at pH 7, 1000:1 ratio charged:neutral)

$\Delta G'^{\circ}$ = -4.2 kcal / mol (3 x 1.4 kcal)

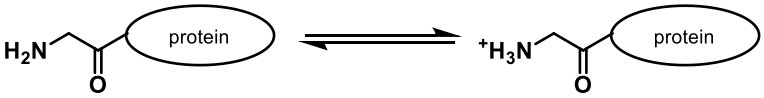
b)



Keq = 1/10 or 0.1 (pKa of thiol = 8; at pH 7, 1:10 ratio charged:neutral)

$\Delta G'^{\circ}$ = +1.4 kcal / mol (1 x 1.4 kcal)

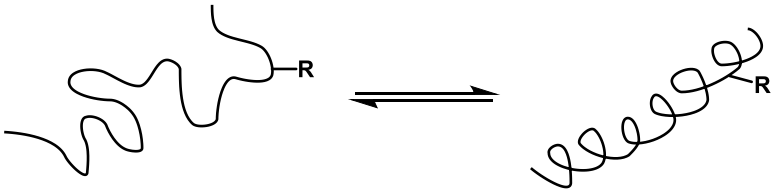
c)



Keq = 10 (pKa of N terminus= 8; at pH 7, 10:1 ratio charged:neutral)

$\Delta G'^{\circ}$ = -1.4 kcal / mol (1 x 1.4 kcal)

3. In class, we examined the likelihood of certain amino acid residues to appear in alpha helical secondary structure. The folding of an unstructured peptide (A) into an alpha helical configuration (B) is depicted below.



A (unstructured peptide) B (alpha-helical peptide)

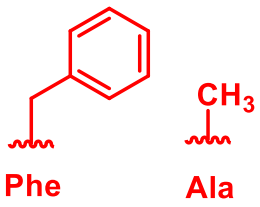
a) In particular, researchers performed the following experiment and measured the ratio of A (unfolded, or unstructured peptide) and B (peptide that had adopted an alpha helical structure). How might one use this information to calculate the ΔG'° for the process above? Explain in 1-2 sentences or with an equation. (4 points)

The ratio of A:B is the K_{eq}. If you know K_{eq}, then you can solve for ΔG'° by $\Delta G'^\circ = -RT \ln (K_{eq})$

b) The researchers calculated the ΔG'° for several different peptides in which the identity of all amino acid residues was kept constant except for the amino acid in position R. If the researchers used every canonical, protein-forming, amino acid at the site indicated by R, how many peptides did they compare? (2 points)

20

c) The researchers examined the propensity of each amino acid to adopt a helical conformation by comparing the ΔG'° for each amino acid. As an example, they found that the ΔΔG'° for Phe compared to Ala was +0.93 kcal/mol. ΔΔG'°, or the difference in ΔG'° values, is defined as $\Delta\Delta G'^\circ = \Delta G'^\circ_{Phe} - \Delta G'^\circ_{Ala}$. In this way, Ala acts as a reference for all of the other amino acids. Draw the structures of the side chains for Phe and Ala, below. (4 points)



d) Based on a ΔΔG'° of +0.93 kcal/mol for Phe vs Ala, is Phe better or worse at existing in an alpha helical structure? Briefly explain your answer (1-2 sentences). (6 points)

Worse. The ΔG'° for Phe to appear in an alpha helix is more positive than that for Alanine. This means that the “A” form, or unstructured form, is more favored/

e) Is a value of ΔΔG'° of +0.93 kcal/mol a significant difference in the ability to form alpha helices? Explain the criteria that you used for answering this question (1-2 sentences). (4 points)

No.

0.93 is less than 1.4 kcal/mol. Since 1.4 kcal/mol is worth a factor of 10/order of magnitude in the K_{eq}, a value of 0.93 will alter K_{eq} by less than a factor of 10.

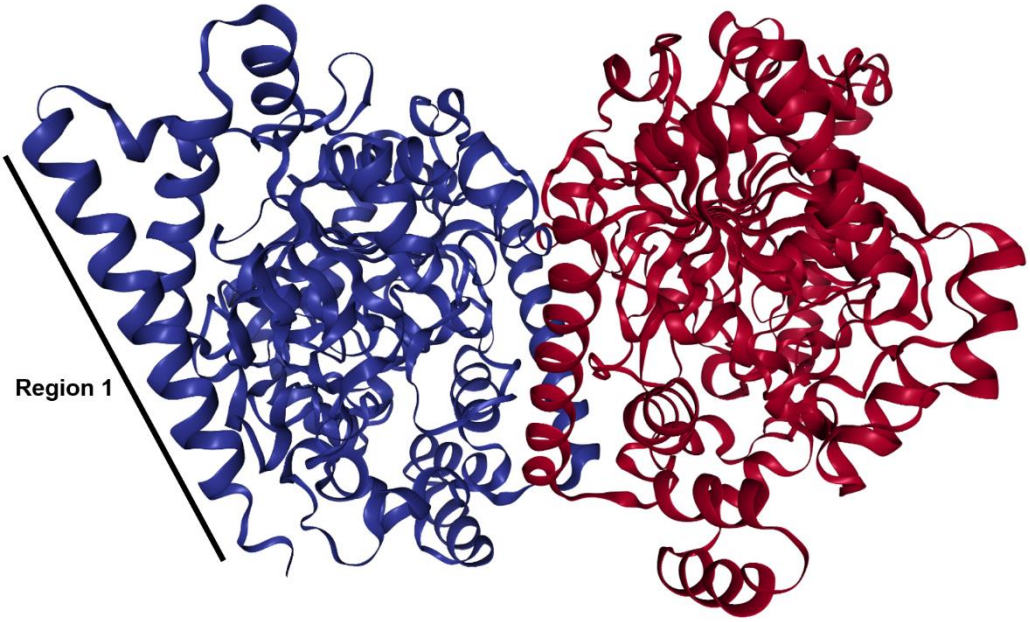
f) Another comparison revealed that Gly has a ΔΔG'° of +1.88 kcal/mol compared to Ala. Draw the structure of the side chain of Gly. Provide an explanation for the large ΔΔG'° for Gly (1-2 sentences). (6 points)

Gly is too flexible; it can adopt multiple phi/psi angles, so there is a thermodynamic penalty for including Gly.

R = H

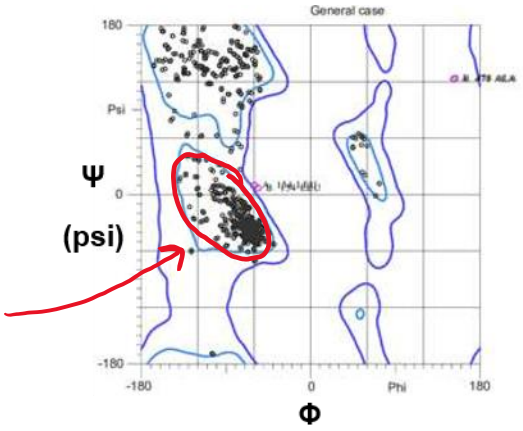
4. Fatty acid amide hydrolase (FAAH) is an important class of serine hydrolase. Its structure is depicted below (from rcsb.org, structure # 4HBP, Kono et al. *Bioorg Med Chem*, **2013**, *21*, 28-41).

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

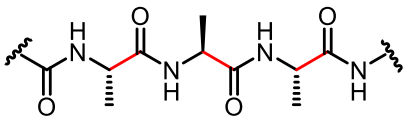


alpha helix

b) The Ramachandran plot below catalogs all of the phi (Φ) and psi (Ψ) angles adopted by the amino acid residues in FAAH. Indicate which region of Ramachandran space corresponds to the secondary structure you identified above. (4 points)

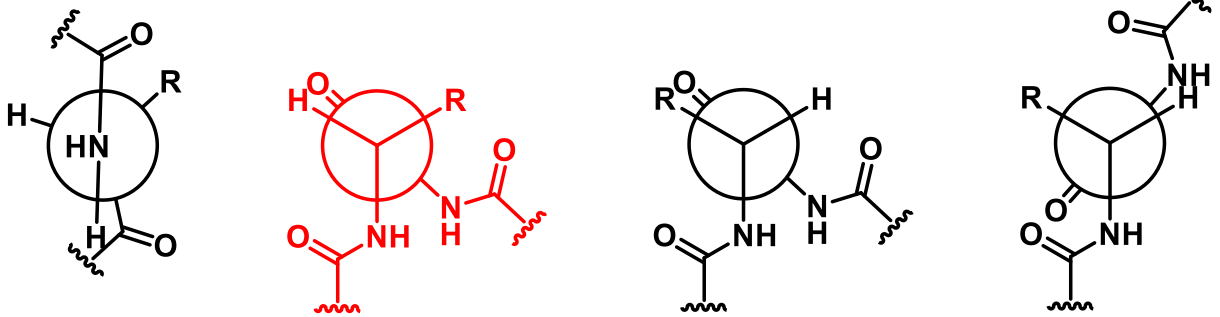


c) A particular range of psi (Ψ) are required to form this type of secondary structure you identified above. The peptide sequence shown below is located within the region of Ramachandran space you identified above. In the stretch of peptide below, indicate one dihedral angle corresponding to psi (Ψ). (4 points)



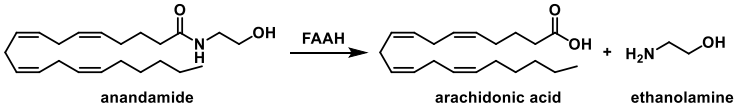
Any of the bonds in red represent the dihedral angle of psi.

d) From the following Newman projections, select the Newman projection that most accurately describes the psi (Ψ) angles required to form the structures you identified in FAAH. Briefly explain your choice (1-2 sentences). (6 points)

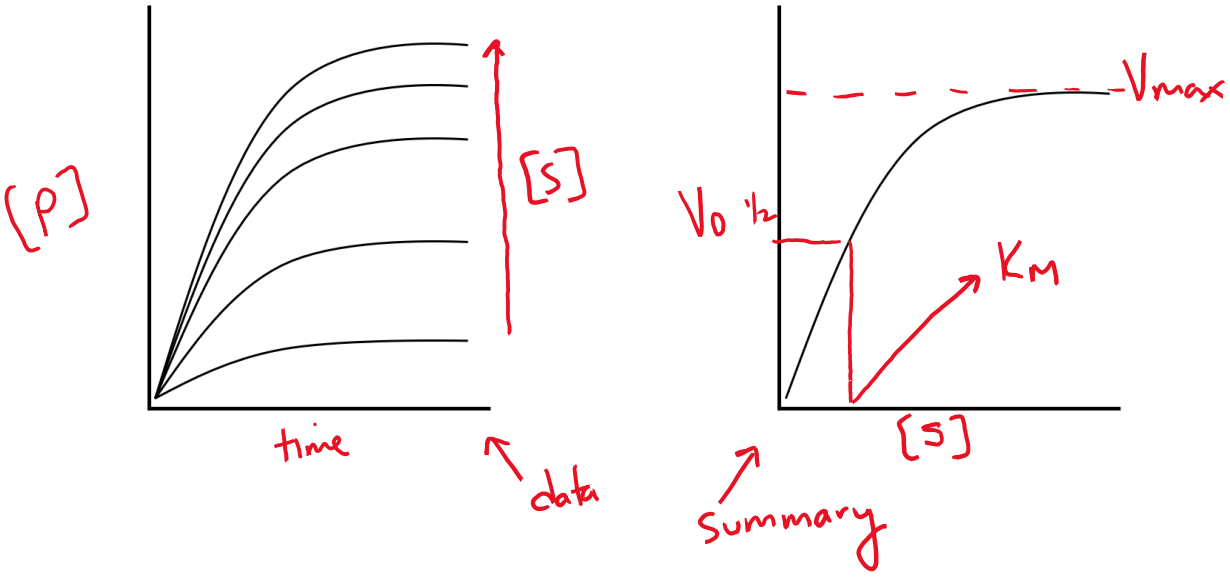


This projection has the correct angle (-60°) that matches the psi angle for an alpha helix. There are no eclipsing interactions with R.

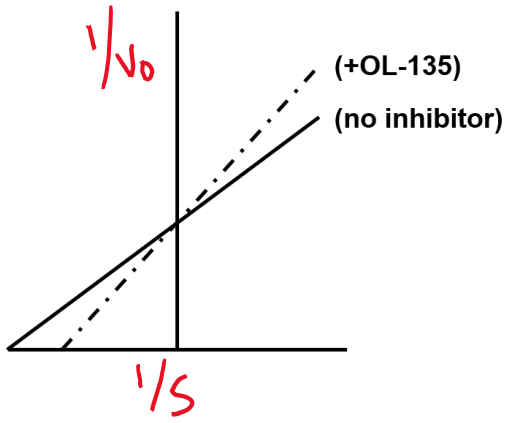
e) Below is an example of a reaction catalyzed by FAAH: the hydrolysis of anandamide to arachidonic acid.



If you were to study the kinetics of this reaction in the lab, you might generate plots that looks something like the ones shown below. Based on our discussion of enzyme kinetics in class, label the axes of the two plots below. For the plot on the left, label, or indicate what is different for each of the 5 lines in the plot. Which of the two plots represents data that you collect in the lab? Which plot represents a summary of the data you collected? Indicate how you would use one of these plots to graphically determine K_M (8 points).



f) Inhibitors of FAAH are attractive targets, because anandamide, the natural substrate for FAAH, is an endocannabinoid and can bind to the central cannabinoid receptor to mediate a number of neurobiological processes, including pain, feeding, and cognition. One such inhibitor is OL-135 (*Bioorg Med Chem Lett*, **2011**, *21*, 4674). Below is a Lineweaver-Burk (or double-reciprocal) plot depicting the hydrolysis of anandamide by FAAH in the presence and absence of OL-135. Label the axes. Based on this data, is OL-135 a competitive or uncompetitive inhibitor of FAAH? Explain your answer (1-2 sentences). (6 points)



This is competitive binding, because there is no change to Vmax.

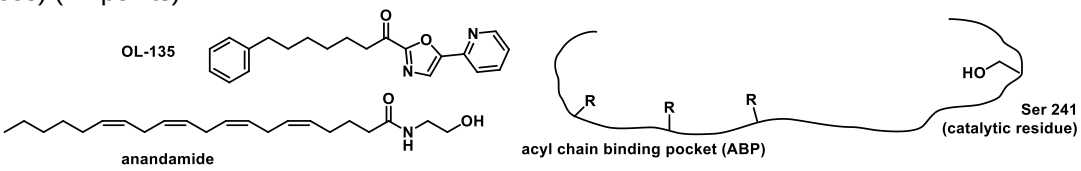
g) The inhibitor OL-135 has a K_d of 4.7 nM for FAAH. If OL-135 is present at a concentration of 4.7 μM, please estimate the ratio of inhibitor-bound to free FAAH. Show how you would estimate this ratio, using an equilibrium expression and equation. (6 points)

$PL \rightleftharpoons P + L$

$K_d = [P][L] / [PL]$ $[P] / [PL] = K_d / [L]$ $[P] / [PL] = 4.7\ nM / 4.7\ \mu M = 1 / 1000$

Note: you could also use "fraction bound", or theta, but would need to convert to a ratio

h) Similar to chymotrypsin's hydrophobic pocket, the enzyme FAAH contains an acyl chain binding pocket (ABP) which helps determine the specificity for binding to substrates like anandamide, or inhibitors like OL-135. Provide the names or abbreviations of three different amino acids that you think are likely to exist in the ABP. Please draw the side chains for these amino acids. Briefly explain why you picked these (1-2 sentences) (12 points)



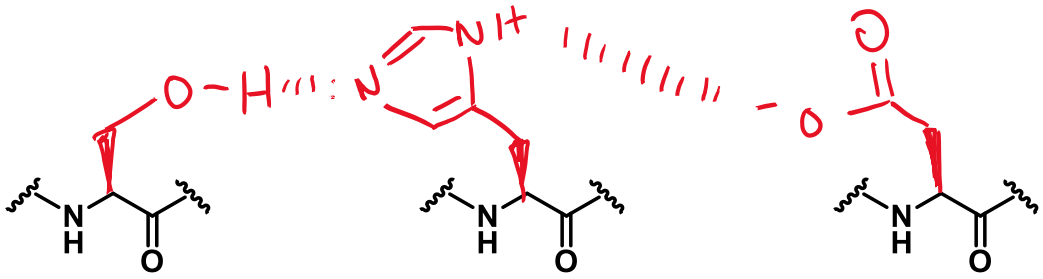
amino acid 1 amino acid 2 amino acid 3

Any three hydrophobic amino acids would be appropriate (Ala, Val, Ile, Leu, Trp, Phe, Met). Hydrophobic amino acids would interact favorably with the greasy tails of anandamide or OL-135.

i) If one of the amino acids in the ABP of FAAH is mutated to Arg, what effect do you think this would have on the K_d of OL-135. Briefly explain your answer (1-2 sentences). (4 points)

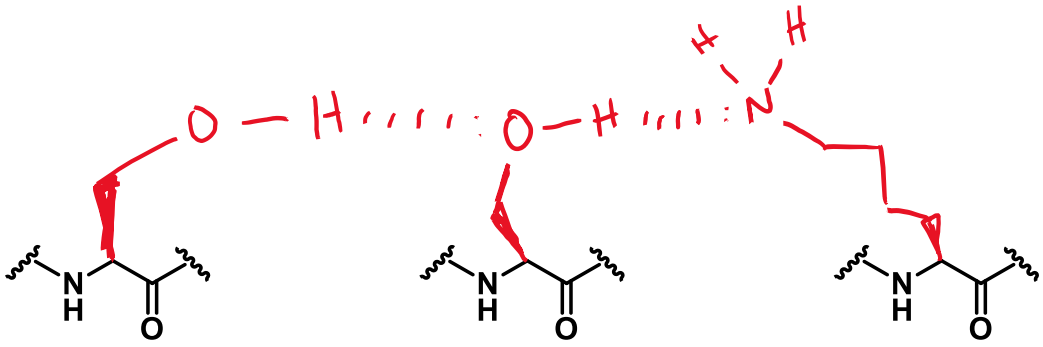
This would raise the K_d. OL-135 would not bind as well, because the greasy tail would clash with the charged Arg residue.

j) FAAH, like chymotrypsin, is a member of a class of enzymes called serine hydrolases, which means that they use serine as a catalytic residue to facilitate the hydrolysis of their substrates. Based on your understanding of chymotrypsin, propose the identity of the catalytic triad in FAAH that helps to facilitate the hydrolysis of the substrate. Draw in the side chains below, indicating the appropriate stereochemistry at the alpha carbon. Please also indicate how interactions between these residues might enhance the nucleophilic character of the catalytic residue. (9 points)



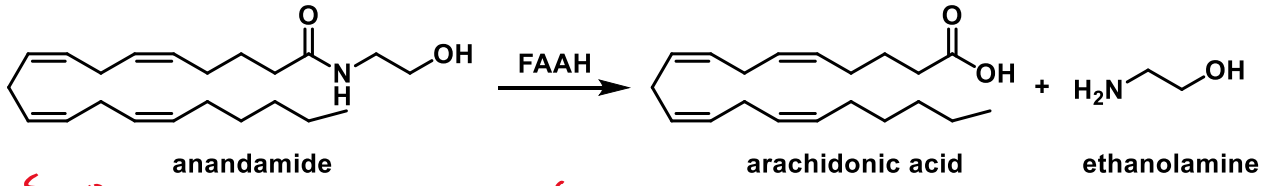
amino acid 1 (catalytic residue) amino acid 2 amino acid 3

k) It turns out that FAAH does not use the same catalytic triad as chymotrypsin. Although the catalytic residue is identical, amino acid 2 (in the scheme above) is replaced by a Ser residue (Ser 217, indicating the location in the primary sequence of FAAH), and amino acid 3 is replaced by Lys (Lys 142), which is in its neutral form in the active site of FAAH. Draw in the side chains for the actual catalytic triad of FAAH, below, again indicating the stereochemistry at the alpha carbon and the key interactions that enhance the nucleophilic character of the catalytic residue. (12 points)



Ser 241 (catalytic residue) Ser 217 Lys 142

I) Based on your understanding of the mode of action of chymotrypsin, coupled with this new knowledge about the identity of the amino acids in the catalytic triad of FAAH, propose an arrow-pushing mechanism for the FAAH-catalyzed hydrolysis of the anandamide. You can abbreviate the lipid tail of anandamide and arachidonic acid as "R", if you wish. Please make sure your mechanism indicates how the catalytic triad of FAAH helps to improve the activity of FAAH. (20 points)



anandamide arachidonic acid ethanolamine