

Name Practice for 2018

Final Exam
Chemistry 391 – Structural Biochemistry
Fall 2016

Do not open the exam until ready to begin!

Rules of the Game:

This is a take-home Exam. The exam is due on Thursday, December 15th at 5 PM. Otherwise, you may hand it in to me or to Kayla Johnston personally. DO NOT LEAVE IT IN MY MAILBOX OR SLIDE IT UNDER MY DOOR.

- You have **three** consecutive hours in which to take the exam.
- This is a closed book/notes exam. You may only use a calculator and a pencil. If you feel I have omitted some important information, please let me know immediately - or note in the exam that I was unavailable, and that you believe a certain piece of data was essential.
- You must do the work independently. You may not consult with others.
- You may contact me during the 3-hour exam period, my office number is 503-517-7679, and my home number is 503-239-4042. Do NOT call my home after 9 PM. I can't promise to be available except during office hours, but if you call me before starting the exam, I can tell you whether or not I'll be around.
- Unless stated otherwise, the temperature is 298 K.
 $R = 0.001987 \text{ kcal/mol}\cdot\text{K} = 0.008315 \text{ kJ/mol}\cdot\text{K}$ and
 $F = 96485 \text{ C/mol e}^-$. $E = E^\circ - (RT/nF)\ln Q$; $\Delta G = -nFE$

Note that there are 7 pages to this exam, including this front page.
11 Questions, total of 150 pts.

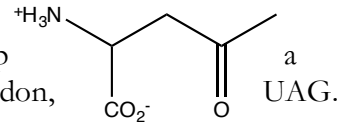
1. (18 points) Consider the following reaction profiles, which represent the Michaelis-Menten reaction mechanism. The dots represent the relative proportion of enzyme in two possible states. For each diagram,
- Indicate how the magnitude of $[S]$ compares to K_m , and
 - Give a simplified rate law, based on the Michaelis-Menten equation, for each set of conditions.

i.	ii.	ii.
a.	a.	a.
b.	b.	b.

2. (6 pts) A transition state analog is prepared for an enzyme-catalyzed reaction. What kinetic parameter(s) would you like to obtain to determine how well the analog is designed? Explain briefly how it/they would be valuable.

3. (6 pts) An enzyme functions with a zinc-stabilized hydroxide as an active site nucleophile. When the Zn^{2+} ion is substituted with Co^{2+} , the enzyme remains active, though the pK_a of the metal bound water (conjugate acid of the hydroxide) is somewhat higher. When the Zn^{2+} is substituted with Fe^{3+} , the pK_a of the metal-bound water is lowered, but the enzyme is inactive. Suggest a reason why that might happen.

6. (24 pts) There are a couple of obvious functional groups missing among the 20 regular amino acids, including ketones. I would like to develop strain of *E. coli* that encodes the amino acid at right using the amber codon,



a. First I will need an aminoacyl tRNA synthetase/tRNA pair for this project.

i. Suggest a starting point— what native aaRS would you choose to work with?

ii. It would be ideal if the enzyme does not have an editing site. Do you think the native enzyme would need hydrolytic editing to achieve specificity? Why or why not?

iii. The pair must be “orthogonal” to those in the host organism. Briefly suggest experiments to assure that your chosen tRNA is orthogonal to tRNA’s in *E. coli*.

iv. How would you propose going about altering the activity of the native synthetase to handle the above substrate?

b. Assuming I am able to obtain the aaRS and tRNA I need, how can I convince reviewers that I have succeeded? Suggest two experiments. If one of them is Mass Spec, take care, because it might be trickier than you think. You will need to show some imagination. But if you have none, feel free to at least explain why it’s tricky.

Exp. #1

Exp. #2

7. (12 pts) Rubredoxin is a small electron transport protein that contains an Fe^{3+} ion bound by four cysteine residues. The reduction potential of Fe^{3+} in water is +0.77 V. The reduction potential of the bound iron(III) in rubredoxin is -0.077 V.

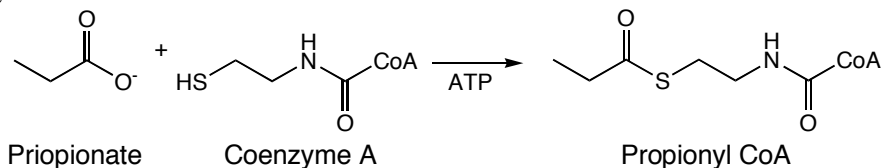
a. Provide a brief explanation, with drawing if appropriate, for the difference in the reduction potential between free and rubredoxin-bound Fe^{3+} .

b. One of the cysteine side chains interacts with a backbone amide from Val44. When residue 44 is mutated, the distance between the amide nitrogen and the sulfur decreases, and the following changes in reduction potential are observed. Suggest a reason for these experimental observations. Again, feel free to provide a sketch if it assists in your explanation.

protein	E°_{red} (V)	N-S dist (Å)
Wild-type	-0.077	2.87
Val44Ile	-0.053	2.75
Val44Ala	-0.024	2.55
Val44Gly	-0.000	2.36

8. (12 pts) Build an enzyme! Design an enzyme active site that will catalyze hydrolysis of the phosphodiester linkage in a DNA dinucleotide, leaving the phosphate group on the 5' oxygen of one of the two products. Be specific with respect to the amino acid side chains that you would use. Draw an arrow pushing mechanism and show how catalytic amino acids positioned within your active site will promote the desired reaction, and will not leave the phosphate on the 3' oxygen of a product nucleotide.

9. (20 pts) Propionyl-Coenzyme A (CoA) synthetase is an ATP-dependent enzyme that catalyzes the formation of propionyl-CoA as shown below (CoA is a large molecule whose salient feature is a primary thiol). Thioesters, like propionyl-CoA, are unstable to hydrolysis and do not form spontaneously from carboxylates and thiols.



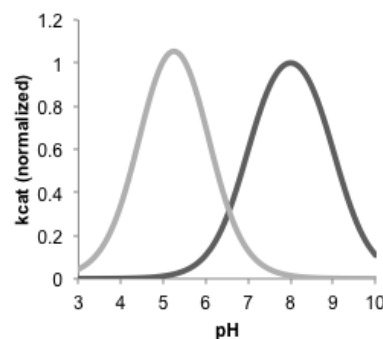
a. Suggest a mechanism by which the synthesis of propionyl-CoA is made thermodynamically favorable. Feel free to note any important compounds involved.

b. It is found that the enzyme is selective for propionate over related compounds. Given the kinetic data below, what is the specificity for propionate over acetate and over butyrate in thioester production.

Substrate	K_m (μM)	k_{cat} (s^{-1})
Propionate ($\text{C}_3\text{H}_5\text{O}_2^-$)	20	40
Acetate ($\text{C}_2\text{H}_3\text{O}_2^-$)	2000	40
Butyrate ($\text{C}_4\text{H}_7\text{O}_2^-$)	8000	8

c. Given the kinetic data in “b”, do you believe it is likely that kinetic proof-reading is used in this enzyme for either acetate or butyrate? Explain briefly for both substrates, and then describe an experiment that would allow you to detect the presence of a proof-reading step.

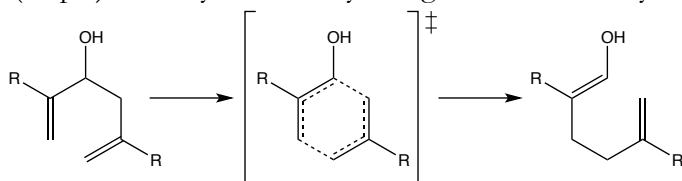
10. (12 pts) Sedolisins are a class of “acidic” serine proteases that function at a lower pH than the digestive proteases like trypsin which function at slightly basic pH. For example the following plot shows pH activity profiles for a sedolisin (light curve) vs. chymotrypsin (dark curve). The significant change in the active site is that the His of the catalytic triad in chymotrypsin is replaced with Glu in sedolisin.



a. Draw the catalytic triad of chymotrypsin and explain why an enzyme employing that triad would lose function at low pH.

b. Draw the catalytic triad with the His→Glu substitution in sedolisin. Why is it able to be active at low pH's, but loses activity as pH climbs above 6?

11. (14 pts) A catalytic antibody was generated to catalyze the oxy-Cope rearrangement below:



Where the “R” groups are phenyls.

a. In the space above right, **draw** a transition state analog that might be used to generate an antibody capable of catalyzing this reaction.

b. The antibody-catalyzed reaction has a ΔS^\ddagger of -28 cal/molK, while the uncatalyzed reaction has a ΔS^\ddagger of -4 cal/molK. Suggest a reason for this difference.

c. Suggest how the enzyme might enhance catalysis by enthalpic means.