Name Practice Exam Solutions 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.

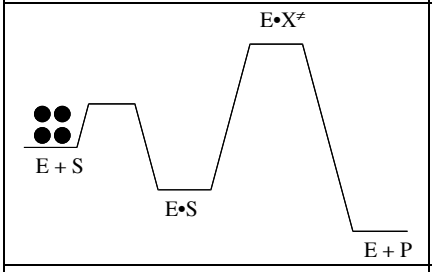
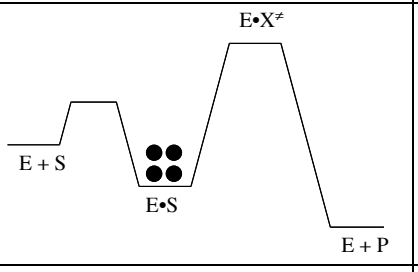
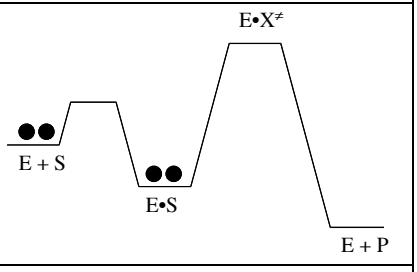
- You must do the work independently. You may not consult with others.

- 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.
  $R = 0.001987\ {}^{kcal}/_{mol \cdot K} = 0.008315\ {}^{kJ}/_{mol \cdot K}$ and
  $F = 96485$ 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,
a. Indicate how the magnitude of [S] compares to K_m, and
b. Give a simplified rate law, based on the Michaelis-Menten equation, for each set of conditions.

i.	ii.	ii.
		
a. **$[S] \ll K_m$**	**a.** **$[S] \gg K_m$**	**a.** **$[S] = K_m$**
b. **$v = (k_{cat}/K_m)[E][S]$**	**b.** **$v = k_{cat}[E]_{tot} = V_{max}$**	**b.** **$v = (1/2)V_{max}$**

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.

The inhibition constant, Ki_i, indicates how tightly the analog binds to the enzyme. The lower the value, the tighter the binding and plausibly the better the mimickry of the TS.

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.

If the metal cation is too strong a Lewis acid, it will stabilize the hydroxide too far and reduce its activity as a nucleophile.

4. (16 pts) Consider an enzyme that requires a protonated residue in its active site for catalysis. Let's call the active enzyme EH, and the inactive enzyme E^-. The following mechanism has been proposed for its function (note that K_a is the acid dissociation constant for the protonated residue).

$$EH + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EHS \xrightarrow{k_{cat}} EH + P$$
$$EH \rightleftharpoons E^- + H^+ \quad (K_a)$$

a. Derive a rate law for the production of P that is dependent on $[E]_{tot}$, [S], and $[H^+]$.

$$\frac{v}{V_{max}} = \frac{k_{cat}[EH \bullet S]}{k_{cat}[E]_{tot}} = \frac{[EH \bullet S]}{[E] + [EH] + [EHS]}$$

$$\frac{v}{V_{max}} = \frac{[E][H^+][S]/K_mK_a}{[E] + [E][H^+]/K_a + [E][H^+][S]/K_mK_a}$$

$$\frac{v}{V_{max}} = \frac{[H^+][S]/K_mK_a}{1 + [H^+]/K_a + [H^+][S]/K_mK_a} = \frac{[S]}{\frac{K_mK_a}{[H^+]} + K_m + [S]}$$

$$v = \frac{V_{max}[S]}{K_m\left(1 + {}^{K_a}/_{[H^+]}\right) + [S]}$$

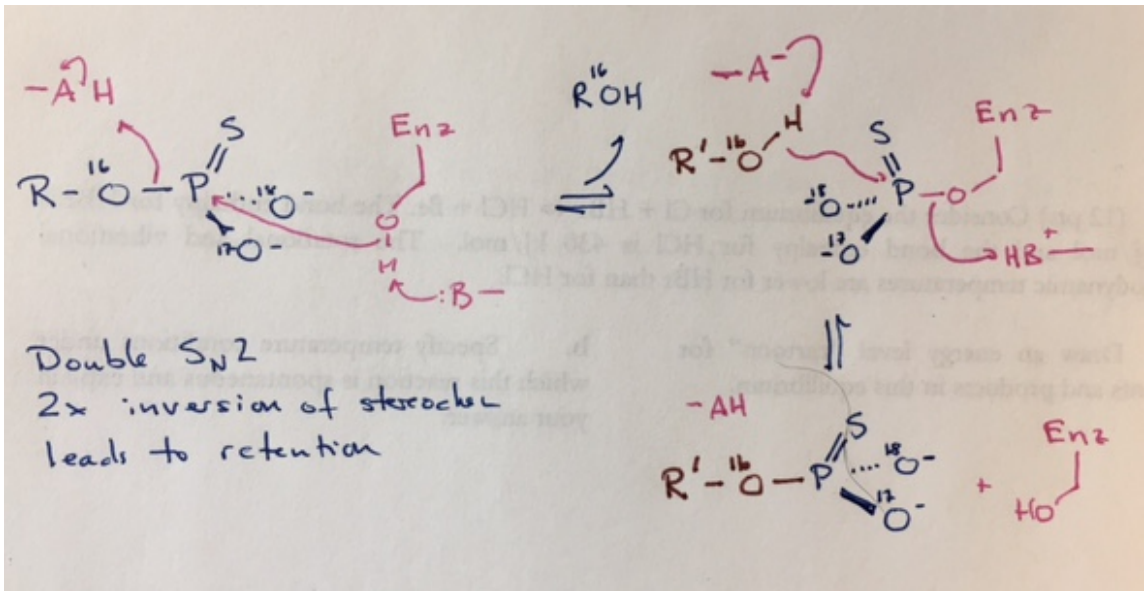
b. How would high pH affect the maximal velocity of the enzyme-catalyzed reaction?

At saturating [S] (>> $K_m(1 + K_a/[H^+])$) v will tend to V_{max}.

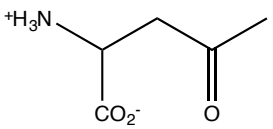
c. How would high pH affect the apparent K_m of the enzyme for its substrate?

At high pH, the term $(1 + K_a/[H^+])$ will become large and the apparent K_m will therefore become large.

5. (10 pts) An enzyme-catalyzed phosphoryl transfer reaction is shown to proceed by retention of configuration of a chiral phosphate, created through the use of ^{16}O, ^{17}O, ^{18}O and S. Suggest a mechanism that would lead to this observed stereochemical outcome. Assume that phosphoryl transfers proceed similarly to S_N2 reactions.



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 a strain of E. coli that encodes the amino acid at right using the amber codon, UAG.



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?

I'd go with either LeuRS or AsnRS. Both sidechains occupy roughly the same volume as the new aa above.

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?

LeuRS could potentially get by without one by excluding β-branched aa's, though Cys could still cause problems. AsnRS can avoid Asp by having 2 HBond acceptors for amide NH_2.

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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.*

Negative selection: Create suppressor from tRNA that has CUA anticodon. It should not suppress mutation in lethal gene when placed in regular E. coli.

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

Create a bunch of alanine mutations around side chain binding region of aaRS active site, then use random mutagenesis to generate library of variants. Test until suppression is achieved with (and only with) the above amino acid.

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- Mass spec is tricky because Leu both weighs the same as the above. But, fix that by using CD_3 on side chain of new aa to create unique mass, heavier than Leu (or Asn or Asp). Another suggestion is to use with ^3H label.

Exp. #2 – Demonstrate that gene containing UGA codon is suppressed with the above amino acid, and none others, and leads to expression of protein detectable by protein gel (Western blot)?

Exp. #3 – Perform kinetic analysis showing selectivity strongly in favor of the newAA.

Exp. #4 – protein NMR. NewAA has unique methyl group. Place in protein with known structure, look for expected cross-peak. (Xtallography will fail – electron density map won't be clear in distinguishing newAA from Asp or Asn).

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+}.

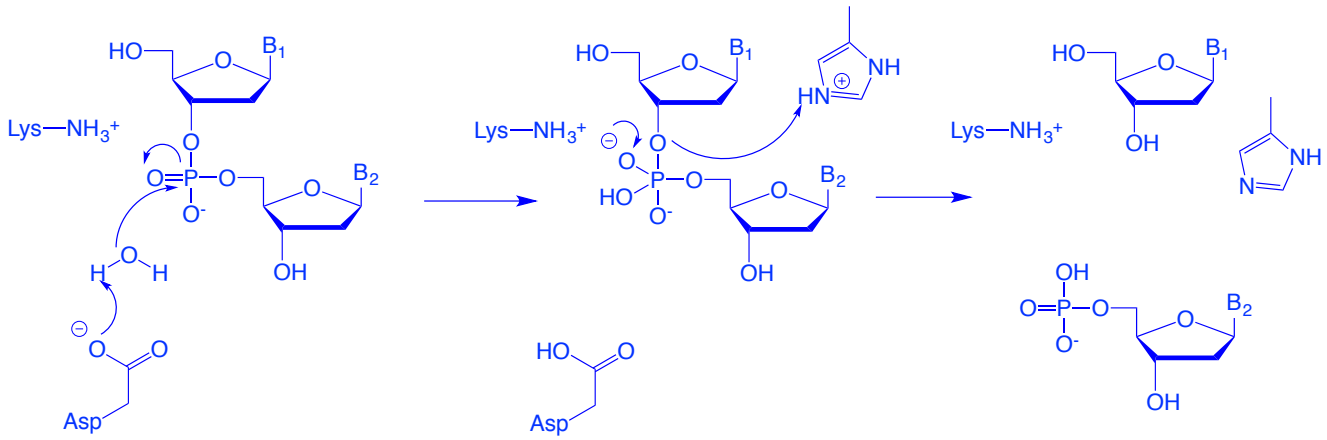
The anionic cysteine residues (thiolate side chains) donate more electron density to the Fe^{3+} than the neutral water molecules. Hence, the Fe^{3+} receives greater stabilization in rubredoxin and is a weaker oxidant.

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.

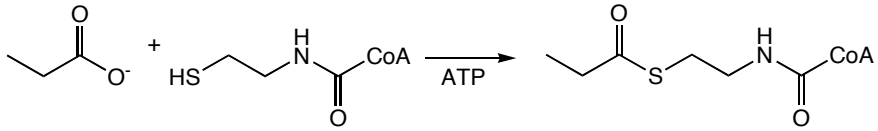
protein	E°_{red} (V)	N-S dist (Å)
Wild-type	-0.077	2.87
Val44Ile	-0.053	2.75
Val44Ala	-0.024	2.55
Val44Gly	-0.000	2.36

The N-H donates the partial positive charge of the amide hydrogen to the thiolate. That reduces electron density on the sulfur, and likewise the ability of the thiolate to donate electron density to the Fe^{3+}. That, in turn, destabilizes the Fe^{3+} and as the amide N-H grows closer, the destabilization increases and so does the reduction potential.

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.

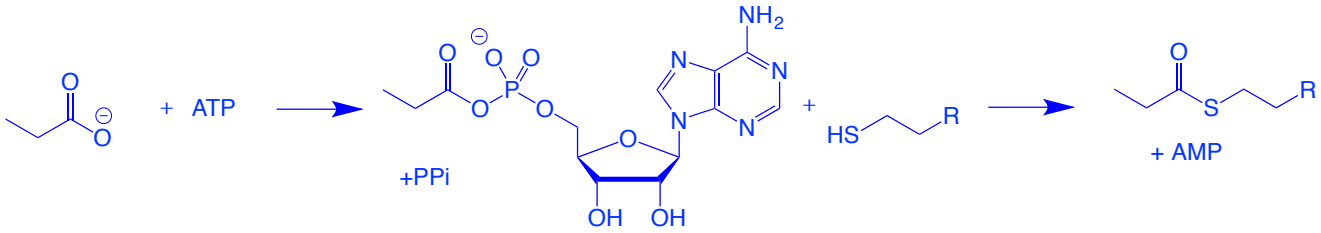


9. (20 pts) Propionyl-Coenzyme A (CoA) synthetase is an ATP-dependent enzyme that catalyzes the formation of propionyl-CoA as shown below (CoA is a large molecule whose salient feature is a primary thiol). Thioesters, like propionyl-CoA, are unstable to hydrolysis and do not form spontaneously from carboxylates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



Priopionate Coenzyme A Propionyl CoA

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



Propionate is activated by ATP to make a mixed acid anhydride. That activates the carboxylate to react with the thiol to generate the thioester. The formation of the thioester is accompanied by hydrolysis of a phosphate anhydride in ATP to make AMP and inorganic pyrophosphate (PP_i).

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})**	**k_{cat}/K_m ($\mu M^{-1}s^{-1}$)**
Propionate ($C_3H_5O_2^-$)	20	40	2
Acetate ($C_2H_3O_2^-$)	2000	40	0.02
Butyrate ($C_4H_7O_2^-$)	8000	8	0.001

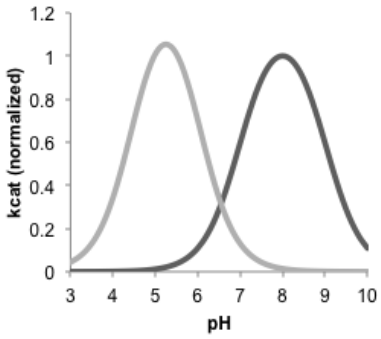
Comparing k_{cat}/K_m values, we see that the enzyme has 100x specificity for propionate vs. acetate and 2000x specificity for propionate vs. butyrate.

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.

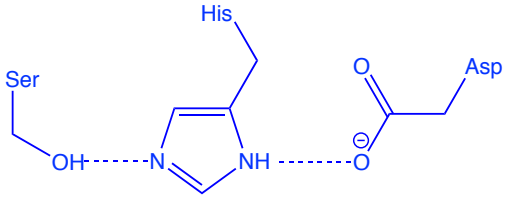
No. These specificities are reasonable given a single binding site. If hydrolytic editing were taking place, I'd expect specificity of greater than 10,000 to 1. The 100x and 2000x specificity fall short of that.

If hydrolytic editing were taking place, you could look for it by seeing an excess of AMP being produced for every molecule of the thioester that forms with either acetate or butyrate.

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.



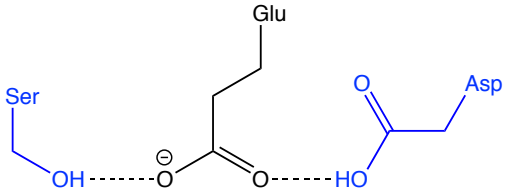
a. Draw the catalytic triad of chymotrypsin and explain why an enzyme employing that triad would lose function at low pH.



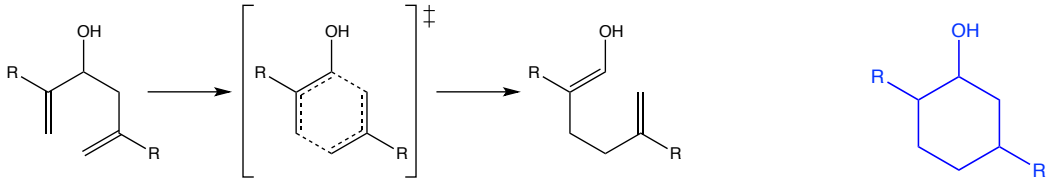
At low pH the His residue will be protonated and no longer able to act as a general base in catalysis.

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?

The loss of activity above pH 6 is likely due to deprotonation of the Asp residue, which must be protonated in order to H-bond to the glutamate, which now performs the role of general base at pH's lower than those that His can accommodate.



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



Where the "R" groups are phenyls.

a. <u>In the space above right</u>, **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 $\Delta S^{\ddagger}$ of -28 cal/molK, while the uncatalyzed reaction has a $\Delta S^{\ddagger}$ of -4 cal/molK. Suggest a reason for this difference.

The antibody likely becomes more rigid as the reaction proceeds, and the loss of conformational flexibility is reflected in a more negative $\Delta S^{\ddagger}$ than an enzyme would normally accept.

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

Catalysis by strain makes the best sense, since the carbon-only reaction is unlikely to have significant partial charges.