**BMB178 – Fall 2018 – Problem Set 1**

**Due: 10/26/2018, noon**

**Office hour: 10/25/2018, SFL GSR218 7 – 9 pm**

## Problem 1. Transition state theory (20 points):

Consider a unimolecular reaction where a substrate **S** is converted to a product **P** at 300 K, with the equilibrium constant $K_{eq}$ = 300. When the reaction is not catalyzed, the rate constant is $k_{uncat}$ = 1.50 x $10^{-4}$ $s^{-1}$. An enzyme **E** is known to stabilize the transition state by 30.0 kJ/mol, and the rate constant of the **E**-catalyzed reaction is $k_{cat}$. The reaction catalyzed by **E** does not involve detectable intermediates.

(A) Calculate ΔG° and ΔG‡ for the uncatalyzed reaction. (4 points)

(B) Calculate ΔG° and ΔG‡ for the reaction catalyzed by **E**. (4 points)

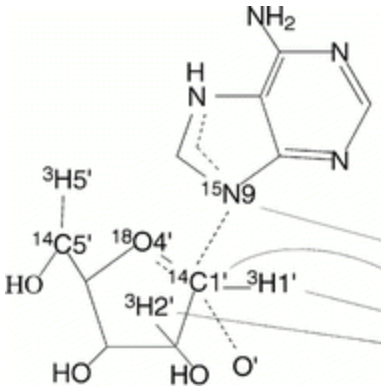
(C) Calculate $k_{cat}/k_{uncat}$ of the enzyme **E**. (4 points)

(D) In a study of unfolding of a small protein, the free energy of unfolding is linearly dependent on urea concentration according to $\Delta G = \Delta G_{H2O} - \mathrm{m}[\mathrm{U}rea]$, where m is a proportionality constant that measures the degree of solvent exposure. A similar equation relates the urea concentration and the activation energy of unfolding, as $\Delta G^{\ddagger} = (\Delta G^{H2O})^{\ddagger} - m^{\ddagger}[Urea]$. The value of $m$ from equilibrium unfolding experiments is 3.2. The data for the kinetics of unfolding are shown in the table below, where k is the rate constant of unfolding. Calculate the fractional increase in solvent exposure ($m^{\ddagger}/m$) for the protein in the transition state of unfolding. What can you conclude about the structure of the transition state, i. e., is the transition state 'late', resembles the folded protein of 'early', or resembles the unfolded state? (8 points)

| [Urea] (M) | $k\ (s^{-1})$ |
|---|---|
| 3 | 3.16 |
| 4 | 5.01 |
| 7 | 12.0 |
| 8 | 15.8 |

## Problem 2. Transition state and kinetic isotope effect (20 points):

AMP nucleosidase is a prokaryotic enzyme that catalyzes AMP hydrolysis to generate adenine and ribose-5-phosphate. MgATP functions is an allosteric activator that stabilizes the active conformation of the enzyme (V. L. Schramm, *J. Biol. Chem*, **1976**, *251*, 3417–24). Kinetic isotope effects for AMP hydrolysis (C1'–N9 bond cleavage) were measured in the presence and absence of MgATP for wildtype AMP nucleosidase ('native') and a mutant of this enzyme ('mutant' columns) and shown in the figure below. The KIE for the same reaction catalyzed by acid ('acid' column) was measured and compared to those of the enzyme-catalyzed reaction.




Kinetic Isotope Effects for AMP Nucleosidases

| | % KIE | | | |
|---|---|---|---|---|
| | native | | mutant | |
| acid | -MgATP | +MgATP | -MgATP | +MgATP |
| 3.0 | 3.0 | 2.5 | 3.4 | 2.1 |
| 4.4 | 3.5 | 3.2 | 4.1 | 4.1 |
| 21.6 | 6.9 | 4.7 | 8.6 | 9.4 |
| 7.7 | 6.1 | 4.3 | 8.9 | 8.6 |

(A) Locate the atoms where heavy atom substitutions report on primary KIE and secondary KIE. (5 points)

(B) AMP can be hydrolyzed through either $S_N1$ (in which bond dissociation to leaving group precedes nucleophilic attack) or $S_N2$-like (in which bond formation and breakage occur simultaneously) mechanism, and these two mechanisms generally have different % KIE. The expected KIE for $S_N1$ and $S_N2$-like mechanisms are on the table below. (The stark difference between the expected KIE for H1 is due to an out-of-plane bending of H1 in the $S_N1$-like transition state.)

| Atom | % KIE for transition state with $S_N1$ character | % KIE for transition state with $S_N2$ character |
|---|---|---|
| C1’ | 0 - 5 | 9 – 14 |
| N9 | 2 - 4 | 1 – 2 |
| H1’ | 10 - 40 | 0 - 10 |

Explain the difference between the expected KIE for N9, for transition state with $S_N1$ and with $S_N2$ characters. The greater detachment of leaving group N9 from the oxycarbonium ion in $S_N1$-like mechanism causes the KIE of N9 to be greater in $S_N1$-like transition state. Looking at the observed KIE values, which mechanism does the acid-catalyzed hydrolysis likely proceed through? Which mechanism does the native enzyme-catalyzed hydrolysis reaction proceed through? (6 points)

(C) Given this information, can you rationalize how acidic conditions may catalyze the hydrolysis reaction? How may the enzyme active site catalyze the reaction? (5 points)

(D) In general, a reduced KIE suggests better stabilization of the reacting group in the transition state. Compare the KIE values for the native enzyme with and without MgATP. How does MgATP change stabilizing transition state interactions at C1’ and N9 in the enzyme? Does the mutation change the effectiveness of MgATP, and if so, how? (5 points)

**Problem 3. Covalent catalysis (30 points):**

Serine proteases cleave peptide bonds, in which serine is the nucleophilic amino acid at the protease active site. Subtilisin-like serine protease, a category of serine proteases, have a conserved active site with a ‘catalytic triad’ including a serine, a histidine, and an aspartic acid.

(A) Download the crystal structure of porcine pancreatic elastase complexed with human beta-casomorphin-7 (PDB: 1QIX). Create an image using PyMOL that shows the active site

interactions that assist in the breakdown of the acyl-enzyme intermediate. Start by showing and labeling all the residues in the catalytic triad (Ser195, His57, and Asp102) and the substrate (VEPI). Label the nucleophilic water and measure the distance between: (1) the nucleophilic water and the electrophilic carbonyl group in the substrate; and (2) the nucleophilic water and the general base that facilitate proton transfer. Lastly, suggest and label a reasonable proton donor that can help regenerate the hydroxyl group on Ser195 during the breakdown of the acyl-enzyme intermediate. (8 points)

(B) The breakdown of the acyl-enzyme intermediate is energetically taxing due to charge accumulation on the reactive carbonyl of the substrate (forming an oxyanion). Elastase and other serine proteases circumvent this issue by placing two "stabilizing" residues in close proximity. Locate these residues using PyMOL and create an image showing the interactions. Briefly explain how the interactions aid in stabilization. (5 points)

(C) Steady-state kinetics of the wild type and mutants are as follows.

| Protein | $k_{cat}$ ($s^{-1}$) | $K_m$ (μM) |
|---|---|---|
| Wild type | $(2.0 \pm 0.1) \times 10^{1}$ | 400 ± 25 |
| D102V | $(5.5 \pm 0.3) \times 10^{-2}$ | 1250 ± 25 |
| H57V | $(5.5 \pm 0.2) \times 10^{-4}$ | 900 ± 25 |
| S195V | $(2.3 \pm 0.1) \times 10^{-5}$ | 700 ± 25 |
| D102E | $(5.7 \pm 0.2) \times 10^{-1}$ | 410 ± 35 |
| S195T | $(2.5 \pm 0.1) \times 10^{-1}$ | 590 ± 44 |

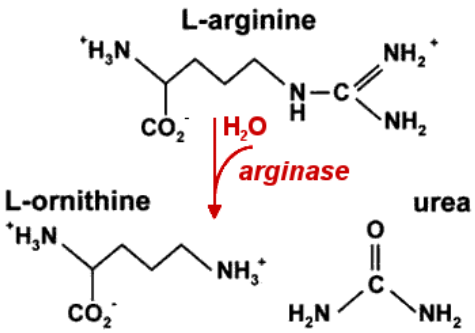
Consider the $k_{cat}$ and $K_m$ for the residues in the catalytic triad and assume the values of $K_m$ approximates the binding affinity for the substrate tested. What can you conclude about their roles in substrate binding and catalysis? (5 points)

(D) Consider the mutations D102E and S195T. Why do these mutations cause a modest defect compared to the corresponding D102V and S195V mutations? What is likely to be the primary source of defect associated with the mutations D102E and S195T? (6 points)

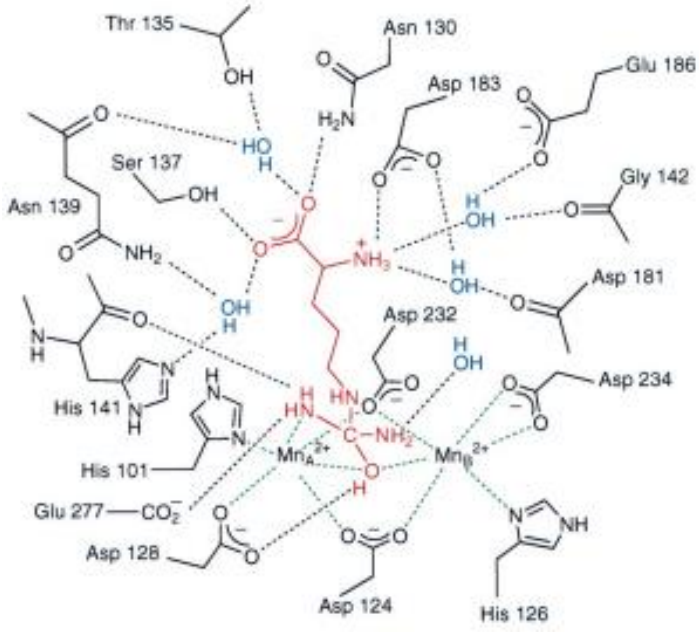
(E) Imagine that a new student wants to engineer a mutation to destabilize the oxyanion hole during breakdown of the covalent intermediate. The student suggests that G193 be mutated to an alanine. Is this a good suggestion? Explain why or why not. If not, suggest an alternate amino acid to which you would mutate G193. (6 points)

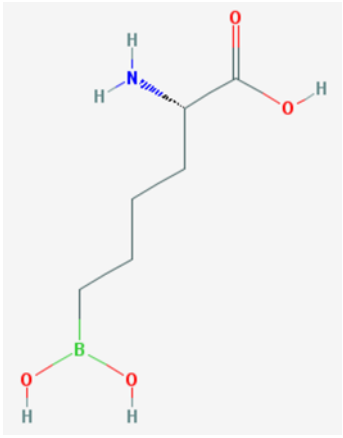
**Problem 4. Acid/base catalysis and metal ions (30 points):**

Arginase is an enzyme containing two $Mn^{2+}$ coordinated to a nucleophilic hydroxide ion which catalyzes the following reaction: L-Arginine + $H_2O$ -> L-ornithine + urea.




(A) The active site interaction of recombinant wild type rat liver arginase and the tetrahedral reaction intermediate can be summarized in the left panel of the following figure. ("Tetrahedral" refers to the geometry of the active site guanidium carbon)



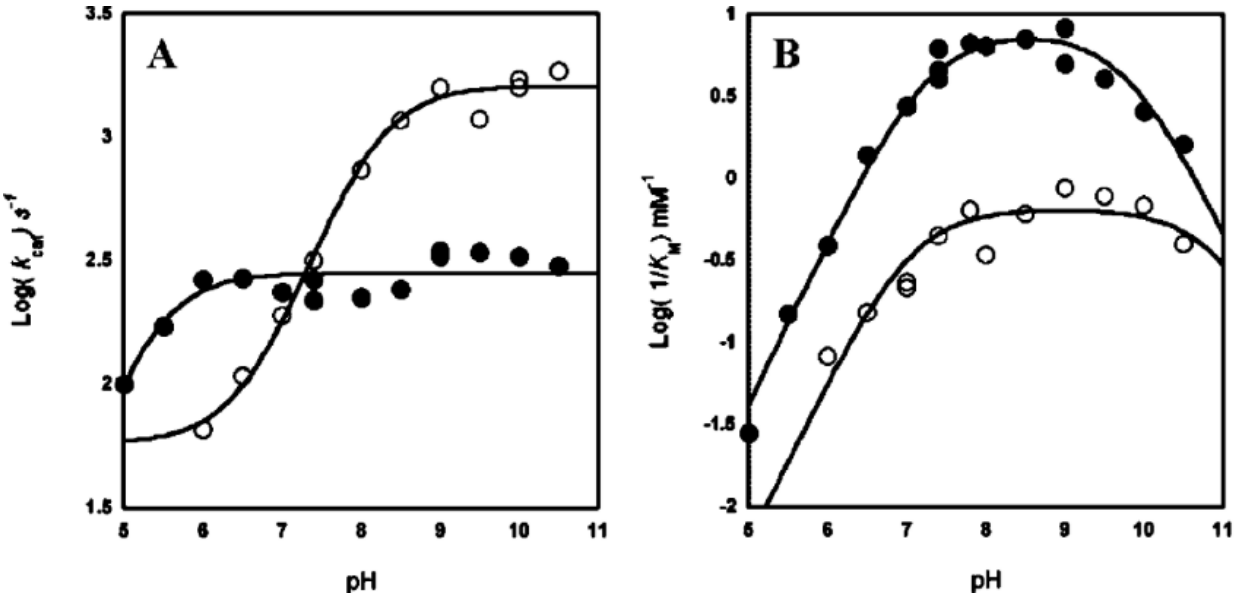
(ABH)

What is the coordination geometry of each metal ion in this state? Identify where the one equivalent of water participating in the reaction is located. What is likely to be the role of these metal ions in the reaction based on the intermediate shown in the figure? (6 points)

(B) Refer to the structure of 2(S)-amino-6-boronohexanoic acid (ABH), a boronic acid analog of L-arginine, in the right panel of the figure above. How does ABH inhibit arginase? (4 points)

(C) While ABH closely mimic the structure of Arginine, the interaction of ABH with arginase active site is different from that of arginine. Which residues or cofactors in rat liver arginase might experience different hydrogen bonding interactions with ABH, and why? (4 points)

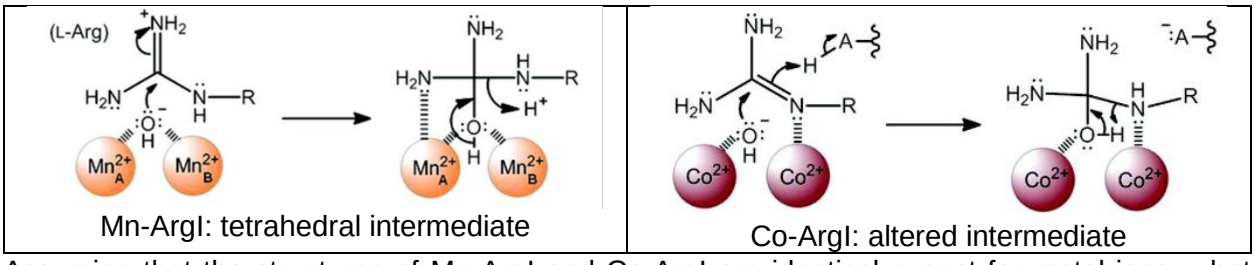
(D) Arginase I with $Mn^{2+}$ ions (Mn-ArgI; open circles) has highly alkaline pH optimum (8.5~9) compared to physiologically relevant pH. To improve arginase activity, the $Mn^{2+}$ ions in Arginase were substituted with $Co^{2+}$ ions (Co-ArgI; filled circles). The pH dependence of Mn-ArgI and Co-ArgI is described in the following graphs with log base 10.




Refer to the pH dependence of log $k_{cat}$ in panel A and estimate the apparent pKa of the two arginases. Next, refer to the pH dependence of log ($1/K_m$) in panel B, and approximate the apparent pKa in the ascending limb for the two arginases. (4 points)

(E) Note the region of physiologically relevant pH (7~7.4) in graphs A and B. Estimate and compare the $k_{cat}$ and $K_m$ values for Mn-ArgI and Co-ArgI. The catalytic proficiency of enzymes is often expressed by $k_{cat}/K_m$. Roughly how much does Co-ArgI function better than Mn-ArgI around pH 7 in terms of $k_{cat}/K_m$? (6 points)

(F) Curiously, arginine decomposition by Co-ArgI is proposed to proceed through a different mechanism than that by Mn-ArgI. The Nε atom (nitrogen of the secondary amine in the guanidinium ion) is proposed to coordinate directly to a $Co^{2+}$ ion in the Co-ArgI reaction, whereas the guanidine $NH_2$ rather than Nε coordinates a $Mn^{2+}$ ion in the Mn-ArgI reaction (Figure below). In addition, the bidentate interactions of $Mn^{2+}$ ion with the nucleophilic water were not maintained in Co-ArgI.




Assuming that the structures of Mn-ArgI and Co-ArgI are identical except for metal ions, what properties of Mn and Co ions may explain these differences in active site metal ion interactions? How do these altered interactions affect the catalytic efficiency of the enzyme? (6 points)