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)

ΔG° = - RT ln K = - 8.31*300*ln 300 = - 14.2 kJ/mol

From $k_{uncat} = (k_B T/h)\exp(-\Delta G^{‡}/RT)$,

$\Delta G^{‡} = RT \ln (k_b T/hk_{uncat}) = 8.31*300*\ln((300/1.5*10^{-4})*2.08*10^{10}) = 95.3$ kJ/mol

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

ΔG° = - RT ln K = - 8.31*300*ln 300 = - 14.2 kJ/mol

$\Delta G^{‡}$ = 95.3 - 30 = 65.3 kJ/mol

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

$k_{cat} = (k_B T/h)\exp(-\Delta G^{‡}/RT) = 300*2.08*10^{10}*\exp(-65300/(8.31*300)) = 26.3\ s^{-1}$

$k_{cat}/k_{uncat} = 26.3/(1.50*10^{-4}) = 1.75 * 10^{5}$

(D) In a study of unfolding of a small protein, the free energy of unfolding is linearly dependent on urea concentration according to $\Delta G = \Delta G_{H2O} - \mathrm{m}[Urea]$, 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 $\Delta G^{‡} = (\Delta G^{H2O})^{‡} - m^{‡}[Urea]$. 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 ($m^{‡}/m$) 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

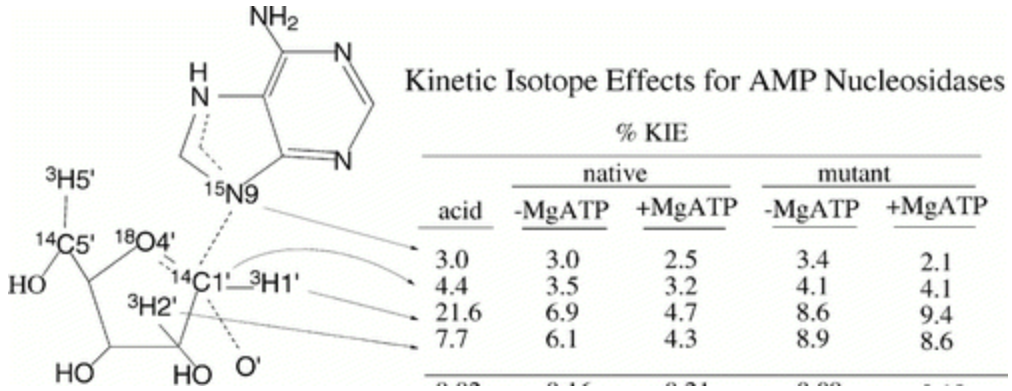
Using the relation $\Delta G^{\ddagger} = -RT \ln(kh/k_B T)$, we can convert k into $\Delta G^{\ddagger}$ values at different urea concentrations.

[Urea] (M)	$k\ (s^{-1})$	$\Delta G^{\ddagger}$ (kJ/mol)
3	3.16	70.1
4	5.01	69.0
7	12.0	66.8
8	15.8	66.1

Plotting $\Delta G^{\ddagger}$ as a function of [Urea] and fitting to $\Delta G^{\ddagger} = (\Delta G^{H2O})^{\ddagger} - m^{\ddagger}[Urea]$ gives $m^{\ddagger} = 0.78$. The fractional increase in solvent exposure in the transition state is therefore $m^{\ddagger}/m = 0.78/3.2 = 0.24$. The small fractional increase in solvent exposure in the transition state compared to the unfolded structure suggests that the transition state of folding/unfolding remains globally folded, in which most of the protein is largely protected from solvent.

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)

A primary KIE is found when a bond to the isotope-labeled atom is directly being formed or broken, and a secondary KIE is found when other bonds indirectly related to isotope-labeled atom (but not the bonds to the isotope-labeled atom) is being formed or broken. Primary KIEs are values from C1' and N9; secondary KIEs can be measured for H1' and H2'.

(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)

All observed KIE values for the acid-catalyzed hydrolysis indicates an S_N1-like transition state. Although the nature of enzyme-catalyzed transition state might be obscure, it is likely to be an S_N1-like transition state based on the KIE of C1' and N9. The enzyme-catalyzed AMP hydrolysis might go through an S_N1-like transition state with the out-of-plane bending of H1' hindered by active site interaction.

(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)

Acid can stabilize the leaving group NH and therefore promote an S_N1-like mechanism. Acidic solutions will also be able to stabilize the increased negative charge on C1' in the S_N1-like mechanism in the transition state. Enzyme active sites can stabilize a similar transition state by general acid catalysis at the leaving group N9, and by providing basic residues or hydrogen bond donating groups to stabilize negative charge accumulation on C1'.

(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)

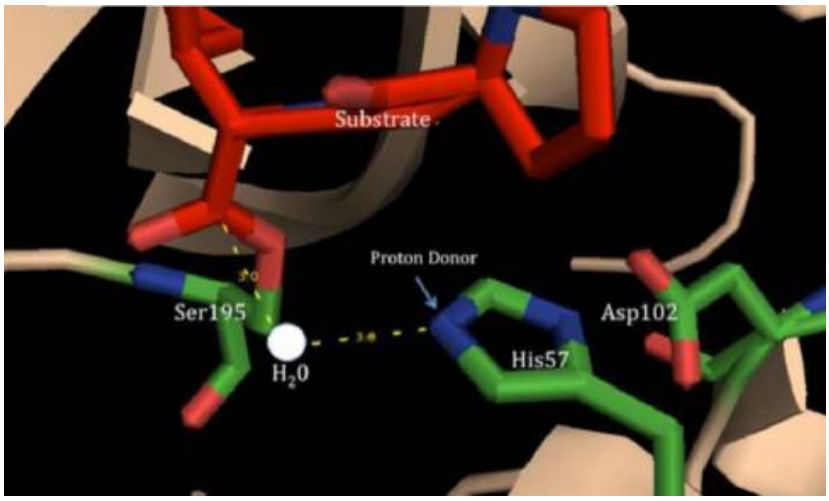
All KIE values decrease with MgATP in the wildtype enzyme. MgATP helps the native enzyme to stabilize the ribooxocarbenium ion at C1' and to protonate the leaving group at N9. While the mutation increases the effectiveness of MgATP in helping leaving group protonation, the mutation makes MgATP not effective in helping to stabilize the ribooxocarbenium ion.

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.

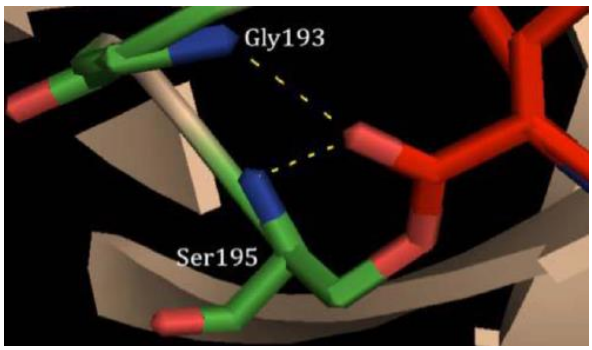
(A) Download the crystal structure of porcine pancreatic elastase complexed with human beta-casomorphin-7 (PDB: 1QIX). Create an image using PyMOL that shows the active site interactions that assist in the breakdown of the acyl-enzyme intermediate. Start by showing and

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)

Figure: 

His57 extracts a proton from the water molecule to generate the nucleophilic hydroxide ion. His57 is then poised to donate it back to S195 for regeneration.

(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)

Figure: 

Stabilization occurs because Gly193 and Ser195 use their main chain amido nitrogen groups to hydrogen bond with the oxyanion. The distances between the oxygen and nitrogen atoms are approximately 2.7-2.9 Å.

(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)

The values for K_m, which relay information about substrate binding, vary by at most 4 fold amongst the mutants. The values for k_{cat}, which relay information about the chemistry step, are drastically decreased by the mutation. Therefore, the catalytic triad residues are not required for substrate binding and contribute mainly to catalysis.

(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)

S195T is less deleterious because it preserves the nucleophilic nature of the hydroxyl group. Ser195 does not participate in stabilizing interactions for substrate binding, so the value of K_m did not change. The precise positioning of the nucleophile relative to the substrate is disrupted, however, resulting in a modest reduction in k_{cat}.

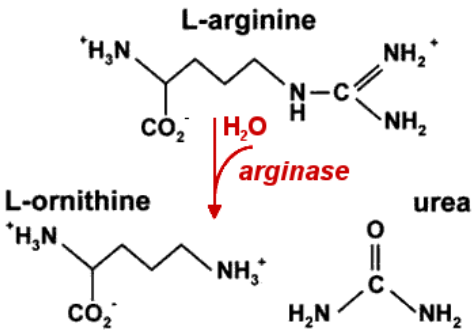
D102E is not very deleterious because it preserves the carboxylate group for hydrogen bonding with and stabilization of the imidazole in H57. The modest reduction in k_{cat} likely results from less precise positioning of the carboxylate group to H57 in the catalytic triad.

(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)

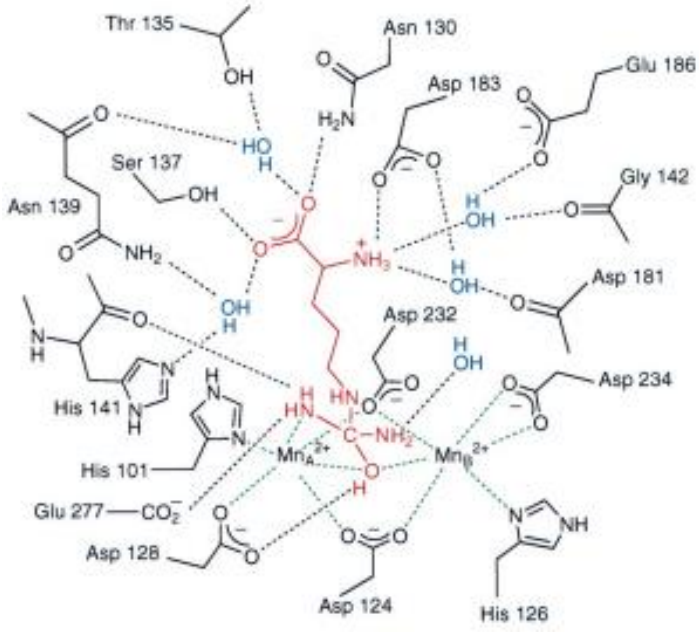
G193A is not a good mutation because the hydrogen bonding between the carbonyl oxygen in the intermediate and the residue backbone NH is unlikely to be affected by small side-chain mutations. A better mutation would be G193P, because the proline backbone does not provide a hydrogen bonding group.

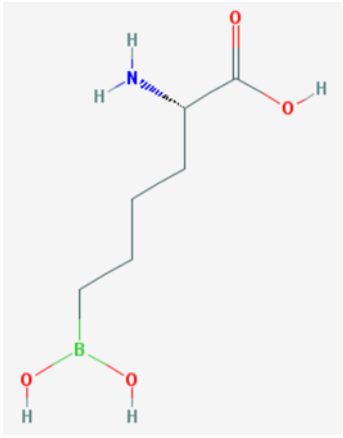
Problem 4. Acid/base catalysis and metal ions (30 points):

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)

Both manganese ions are taking octahedral geometry. The water molecule is bonded to the active site guanidium carbon. The manganese ions likely coordinated with water to orient the water molecule close enough to the arginine so that water can act effectively as a nucleophile and attack L-arginine.

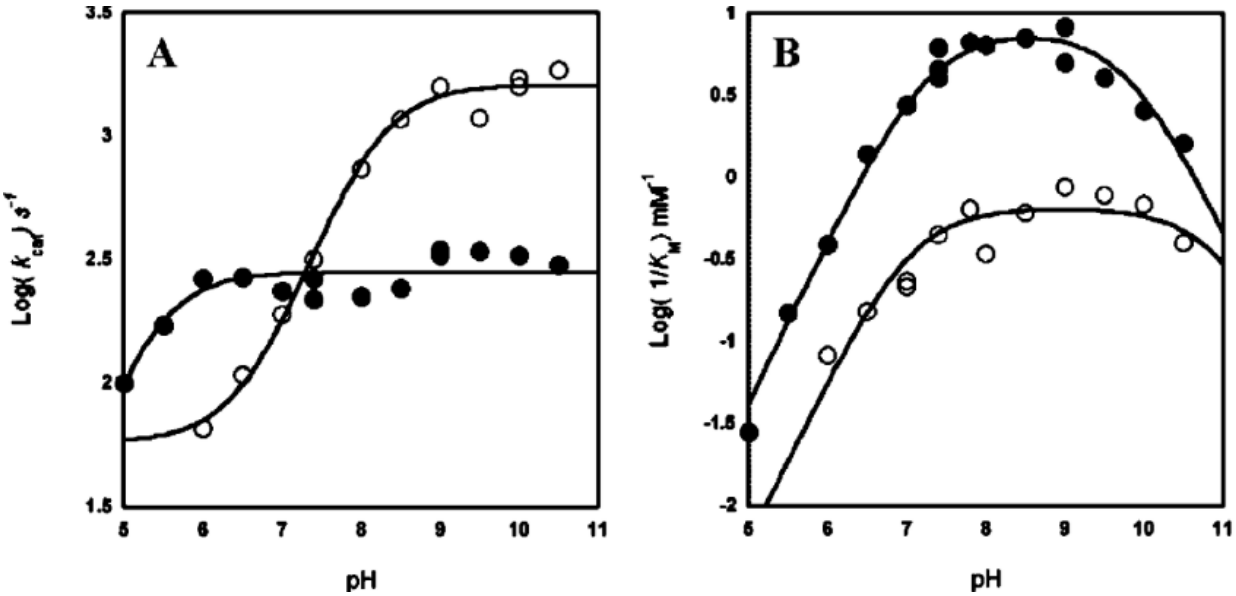
(B) Refer to the structure of 2(S)-amino-6-boronohexanoic acid (ABH), a boronic acid analog of L-arginine, in the right panel of the figure above. How does ABH inhibit arginase? (4 points)

ABH is a stable boronic acid that mimics the structure of arginine. The boron atom in ABH is more electron deficient than a carbon atom, and readily accepts nucleophiles such as water. Attacked by the water molecule, ABH binds as the tetrahedral boronate anion to the active site and mimics the structure of the native intermediate.

(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)

The tetrahedral boronate anion does not have any amine group; instead, the boron atom is bonded to only hydroxyl groups. Thus, His141 and the Mn_A^{2+} cofactor – originally hydrogen bonded to amine N-H – are likely to experience different hydrogen bonding interactions.

(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)

Graph A: Mn-ArgI has an apparent pKa of ~8. Co-ArgI has an apparent pKa of ~5.5.

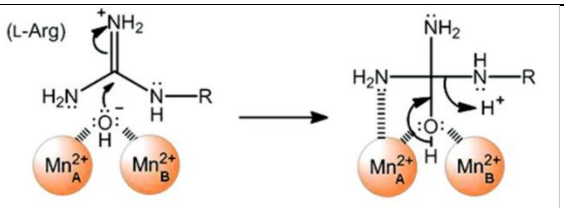
Graph B: Mn-ArgI has ascending pKa of ~7. Co-ArgI has ascending pKa of ~7.5.

(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)

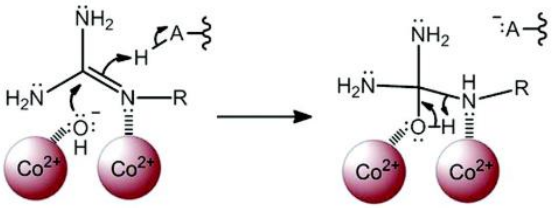
The k_{cat} values in the physiologically relevant pH for both arginases is ~ $10^{2.4}$ = 250 s^{-1}. Mn-ArgI has a K_m of ~$10^{0.35}$ = 2.2 mM, and Co-ArgI has a K_m of ~$10^{-0.75}$ = 0.17 mM. Co-ArgI (k_{cat}/K_m ~ 110 $mM^{-1}s^{-1}$) performs an order of magnitude better than Mn-ArgI (k_{cat}/K_m ~ 1470 $mM^{-1}s^{-1}$).

(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.



Mn-ArgI: tetrahedral intermediate



Co-ArgI: altered intermediate

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)

The larger size and lower coordination flexibility of Cobalt makes bidentate coordination of water hard. As a result, precise positioning of nucleophilic water is harder. Cobalt is also generally better at deprotonating chemical groups, and would deprotonate the guanidinium ion. This reduces the effectiveness of guanidino group at absorbing negative charge accumulation in the transition state. (Helps but not necessary: because of the changes in active site coordination, Co-ArgI have to resort to alternative functional groups at the active site for catalysis, but these alternative groups are not evolutionarily optimized.)