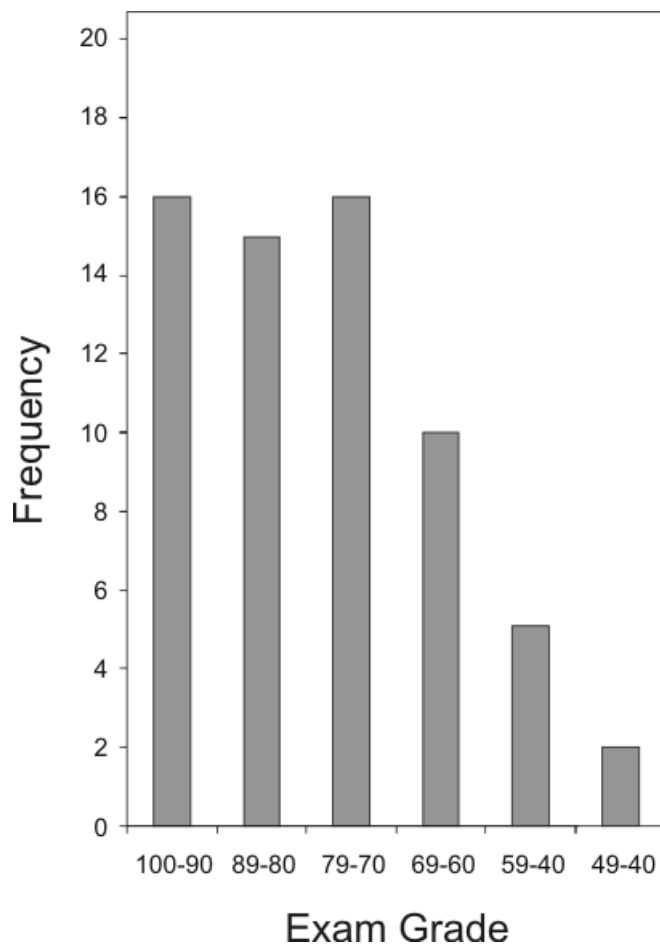
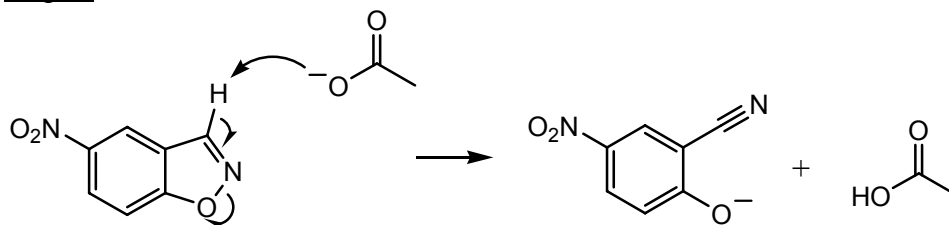
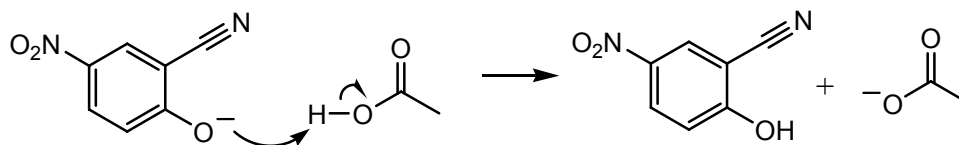


**Exam 1
Answer Key**

Exam 1 Mean: 78
Exam 1 Median: 79
Exam 1 St. Dev.: 17



Exam 1 Solutions**1. a.**Step 1:Step 2:*Rubric:*

Step 1: 2 points – proton comes off
 2 points – arrow pushing and charges logical (one step)
 1 point – intermediate correct

Step 2: 2 points – proton addition
 2 points – arrow pushing and charges logical (one step)
 1 point – get to product

b.

	Bonds made:	ΔH_{BDE} (kcal/mol)	Bonds broken:	ΔH_{BDE} (kcal/mol)
Step 1:	C≡N (from C=N)	(213 - 147) = 66	C-H	99
	O-H	111	N-O	55

Total ΔH for Step 1: (kcal/mol)

	Bonds made:	ΔH_{BDE} (kcal/mol)	Bonds broken:	ΔH_{BDE} (kcal/mol)
Step 2:	O-H	111	O-H	111

Total ΔH for Step 2: (kcal/mol)

Total ΔH for reaction: (kcal/mol)

Rubric:

Step 1: 5 points – bonds made and broken
2 points – ΔH (correct value and sign)

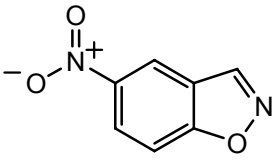
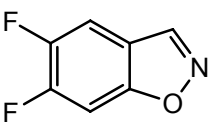
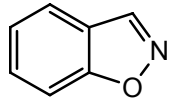
Step 2: 5 points – bonds made and broken
1 point – ΔH (correct value)

2 points – correct ΔH reaction

* Full credit for answers consistent with part a) (in which the reactant logically goes to product)

c. The equation for K_M shows that the higher the value of K_M , the less substrate is bound; so both **3** and **4** must bind the enzyme more weakly than **1**.

$$\Delta\Delta G = -RT\ln(K_n/K_1) = -(0.592 \text{ kcal/mol})\ln(K_n/K_1)$$

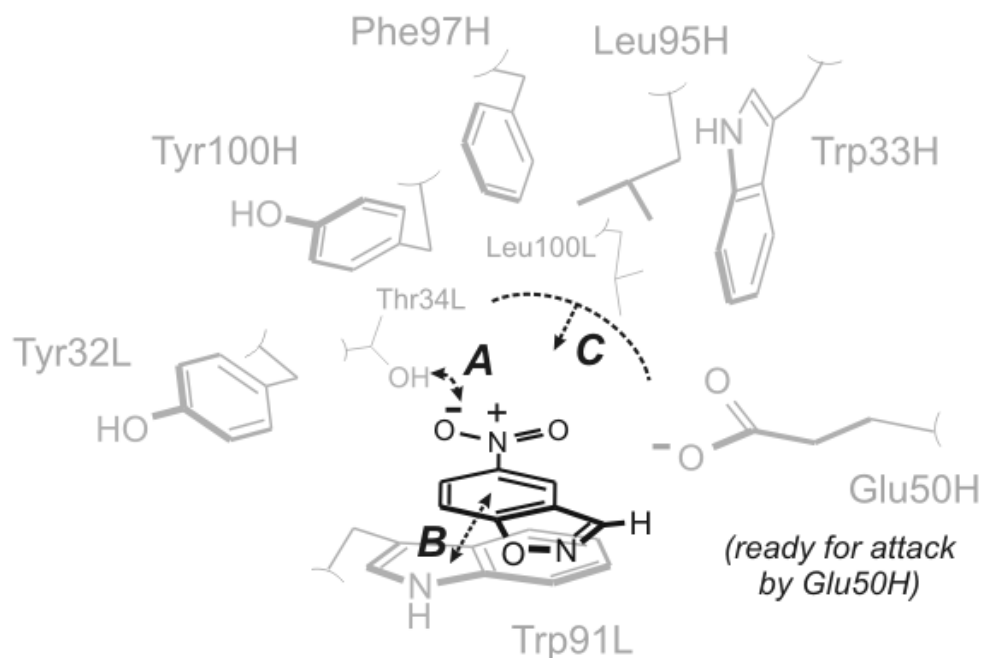
substrate	K_M		
 <p>1</p>	90 μM	binds enzyme stronger or weaker than 1 ? <i>(circle one answer for each box)</i>	how much stronger or weaker than 1 , in kcal/mol? <i>(answer to within 1 kcal/mol)</i>
 <p>3</p>	200 μM	stronger <i>or</i> weaker	0.47 kcal/mol
 <p>4</p>	2 mM	stronger <i>or</i> weaker	1.8 kcal/mol

Though I calculated these $\Delta\Delta G$ values, you could have easily estimated them; $K_M(\mathbf{3})$ is much less than an order of magnitude higher than $K_M(\mathbf{1})$, so $\Delta\Delta G$ must be much less than 1.4 kcal/mol (guess 0.5), and $K_M(\mathbf{4})$ is between one and two orders of magnitude higher than $K_M(\mathbf{1})$, so $\Delta\Delta G$ must be between 1.4 and 2.8 kcal/mol (guess 2).

Rubric:

2 points for each blank

d & e.



Lots of possible answers here. We accepted many potential interactions.

- A:** Based on the binding data (discussed below in (f)), it seems likely that there is some kind of stabilizing hydrogen bonding going on, possibly between a –NO₂ lone-pair and the Thr34L proton. This is ideally positioned to force the C-H to be broken near the mouth of the pocket, where the carboxylate base is.
- B:** The substrate is an electron-poor aromatic (because of the electron-withdrawing character of the –NO₂ group), and should stack well on the large aromatic face of Trp91L. You could potentially draw arene-arene interactions with many of the surrounding aromatic residues.
- C:** Actually, simple hydrophobic effects probably also play a role in allowing the enzyme to capture substrate. The hydrophobic pocket (with lots of hydrophobic amino acids) is right at the water's edge, and would much rather interact with **1** than with water molecules.

Some things we were more skeptical about:

H-bonding to Glu50H: C-H is not an H-bond donor.

H-bonding to Tyr32L, Tyr100H, Trp33H, Trp91L: These are pointed out of the pocket towards water environment. Will not be as available for H-bonding. Difficult to imagine how molecule could be oriented into pocket by one of these outward-facing contacts.

Rubric:

- d) 4 points – placement of molecule in pocket
1 point – molecule placed with realistic interactions

- e) 4 points each – interaction discussed
2 points each – interaction logical

f. Referring to the letters I used in parts (d) & (e):

Enzyme•**3** should still exhibit the hydrophobic interactions (**C**) shown above in part (d). In addition, fluorine atoms are electronegative, and so the substrate ring should still be electron-poor and arene stacking (**B**) should still occur for **3** just as it did for **1**. However, molecule **3** lacks an electron-pair donor, and so it should not participate in H-bonding (**A**) with Thr34L. The binding energy in enzyme•**3** should be weaker than enzyme•**1** by one H-bond (0.5-1.5 kcal/mol).

Enzyme•**4**, again, should show the same hydrophobic interactions (**C**) with the pocket. However, the ring in **4** doesn't have any electron-withdrawing groups or H-bond acceptors. So, binding energy in enzyme•**4** should be weaker than in enzyme•**1** by an H-bond and an arene-arene interaction (though some edge-to-face arene interactions probably still take place).

Rubric:

2 points each – differences

2 points each – similarities

2 points when answer logically makes sense with d) and e)

2. a. The first few kinetic runs ($[\mathbf{B}]_0 = 10 \text{ mM}, 5 \text{ mM}, 2 \text{ mM}$) were all performed under pseudo-first order conditions, with $[\mathbf{B}]_0 \gg [\mathbf{A}]_0$, such that the concentration of \mathbf{B} effectively did not change over the course of the reaction. For pseudo-first order kinetics, it is possible to write a rate expression

$$\frac{-\partial[\mathbf{A}]}{\partial t} = k_r [\mathbf{A}]^x [\mathbf{B}]^y = k_{\text{obs}} [\mathbf{A}], \text{ where } k_{\text{obs}} = k_r [\mathbf{B}].$$

So, $x = 1, y = 1$. The integrated rate law for this expression would be

$$[\mathbf{A}]_t = [\mathbf{A}]_0 e^{-k_{\text{obs}} t},$$

and so the exponent C determined from each of the curve fits is actually a k_{obs} . The variation of k_{obs} with $[\mathbf{B}]$ gives k_r :

$$k_r = \frac{\Delta k_{\text{obs}}}{\Delta [\mathbf{B}]} = \frac{6.4 \times 10^{-6} / \text{sec} - 3.2 \times 10^{-6} / \text{sec}}{10 \text{ mM} - 5 \text{ mM}}$$

$$k_r = 6.4 \times 10^{-4} \text{ M}^{-1} \text{ sec}^{-1}.$$

Rubric:

2 points each – correct x and y

4 points – correct k_r

2 points – correct units

b. For these last couple of runs, $[\mathbf{B}]_0$ was not that much larger than $[\mathbf{A}]_0$, and as a result the reaction would not have followed pseudo-first order kinetics. Under these conditions, the reaction would be more likely to follow normal second-order kinetics, where

$$[\mathbf{A}]_t = \frac{\Delta_0 [\mathbf{A}]_0}{[\mathbf{B}]_0 e^{\Delta_0 k_r t} - [\mathbf{A}]_0}, \quad \Delta_0 = [\mathbf{B}]_0 - [\mathbf{A}]_0.$$

We derived this equation in lecture for a second-order process with reactants **A** and **B**.

Rubric:

6 points – correct equation

1 point – define Δ_0

- c. Writing the rate expression $-\partial[B]/\partial t$ as the sum of the two processes,

$$\frac{-\partial[B]}{\partial t} = k_r[B][A] + k_H[B] = [B](k_r[A] + k_H)$$

As we did in class for simple second-order kinetics, we need to express $[A]$ in terms of $[B]$. The number of molecules of A consumed during the reaction is exactly equal to the number of molecules of B consumed. So,

$$[A]_0 - [A]_t = [B]_0 - [B]_t$$

$$[A] = [A]_0 - [B]_0 + [B] = \Delta_0 + [B], \text{ where } \Delta_0 = [A]_0 - [B]_0.$$

Note that this is actually the opposite of how we defined Δ_0 in class (as $[B]_0 - [A]_0$), because in lecture we were putting everything in terms of $[A]$ rather than $[B]$. Substituting this expression for $[A]$ into the rate equation,

$$\frac{-\partial[B]}{\partial t} = [B](k_r(\Delta_0 + [B]) + k_H) = [B](k_r\Delta_0 + k_H + k_r[B])$$

Rubric:

1 point – half-expression containing $k_H[B]$

1 point – half-expression containing $k_r[B][A]$

1 point – substituting for $[A]$

5 points – correct answer

(-1 point only for trivial math errors)

- d. Clearly the hardest question on the exam. Rearranging and integrating the answer to (c),

$$\frac{-\partial[B]}{[B](k_r\Delta_0 + k_H + k_r[B])} = \partial t$$

$$\int_{[B]_0}^{[B]_t} \frac{\partial[B]}{[B](k_r\Delta_0 + k_H + k_r[B])} = -\int_0^t \partial t$$

For this, we can use the standard integral form in the problem,

$$\int \frac{dx}{x(a+bx)} = -\frac{1}{a} \ln \frac{a+bx}{x}, \quad \text{where } x = [B], \quad a = (k_r\Delta_0 + k_H), \quad b = k_r;$$

$$-\frac{1}{k_r\Delta_0 + k_H} \ln \left(\frac{k_r\Delta_0 + k_H + k_r[B]}{[B]} \right) \Bigg|_{[B]_0}^{[B]_t} = -t$$

$$\ln \left(\frac{k_r\Delta_0 + k_H + k_r[B]_t}{[B]_t} \right) - \ln \left(\frac{k_r\Delta_0 + k_H + k_r[B]_0}{[B]_0} \right) = (k_r\Delta_0 + k_H)t$$

Now our goal is to solve for $[B]_t$. Making each side a power of e ,

$$\frac{[B]_0(k_r\Delta_0 + k_H + k_r[B]_t)}{[B]_t(k_r\Delta_0 + k_H + k_r[B]_0)} = e^{(k_r\Delta_0 + k_H)t}$$

$$[B]_0(k_r\Delta_0 + k_H + k_r[B]_t) = e^{(k_r\Delta_0 + k_H)t} [B]_t(k_r\Delta_0 + k_H + k_r[B]_0)$$

$$[B]_0(k_r\Delta_0 + k_H) = e^{(k_r\Delta_0 + k_H)t} [B]_t(k_r\Delta_0 + k_H + k_r[B]_0) - k_r[B]_t[B]_0$$

$$[B]_0(k_r\Delta_0 + k_H) = [B]_t \left(e^{(k_r\Delta_0 + k_H)t} (k_r\Delta_0 + k_H + k_r[B]_0) - k_r[B]_0 \right)$$

$$[B]_t = \frac{[B]_0(k_r\Delta_0 + k_H)}{e^{(k_r\Delta_0 + k_H)t} (k_r\Delta_0 + k_H + k_r[B]_0) - k_r[B]_0}$$

Rubric for 2(d):

5 points – Setting up integral from part (c), with [B] on one side, t on other

3 points – Integrating over $[B]_t \rightarrow [B]_0$, $t \rightarrow 0$

3 points – Solving for $[B]_t$

4 points – Correct answer