

Midterm Exam 1

Please do not open or sign this packet until you are instructed to do so.

Please write all of your answers for this exam in this exam packet. Although you may use as many blue books for scratch work as you would like, the blue books will not be collected at the end of the exam or graded. Answer each question in the space provided if you can, but feel free to continue your answer on the back of the page if you need more room. (Please write a note by your answer pointing us to the continuation if you do this.) You will be given 2 hours total to finish the exam.

This exam contains two problems, which are split into parts. *Do not get stuck* on one part and then assume that you will be unable to answer the rest of the question—move on. In addition, partial credit will be given for incorrect but plausible or consistent answers, so *guess* on problems you cannot answer perfectly.

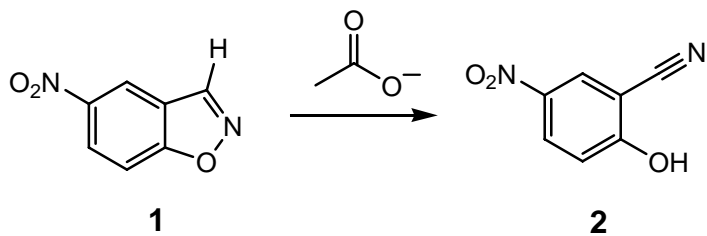
At the end of the 2-hour exam period you will be asked to return your exam to the proctor. (You may, of course, also turn the packet in earlier if you choose.) This exam is *open-resource*—you may use any books, notes, calculator, etc. you have brought with you to the exam. However, you are not allowed to communicate with anyone during the exam, or to bring any materials in or out of the room while you are taking the exam. You are also not allowed to use any devices that could be used to communicate with anyone (laptop computers, cellphones, etc.). Please do not take any part of the exam packet with you when you are done; everything will be returned to you after the exams are graded.

This packet should contain 12 pages, including this one. (Your exam runs from pages 2-11; the last page is a table of standard bond dissociation energies.) Please check to make sure that your packet contains 12 pages before beginning your exam.

Name: _____

Signature: _____

1. The “Kemp elimination” is a base-catalyzed reaction in which a benzisoxazole (**1**) is converted into a hydroxybenzonitrile (**2**).



Kemp originally found acetate ion to be a good catalyst for this reaction.¹

- a. (10 pts) Using electron pushing, draw a two-step mechanism for the conversion of **1** to **2** that involves only the molecules shown above. (In other words, don't involve extra acids, bases or solvent molecules in your electron-pushing.)

Step 1:

Step 2:

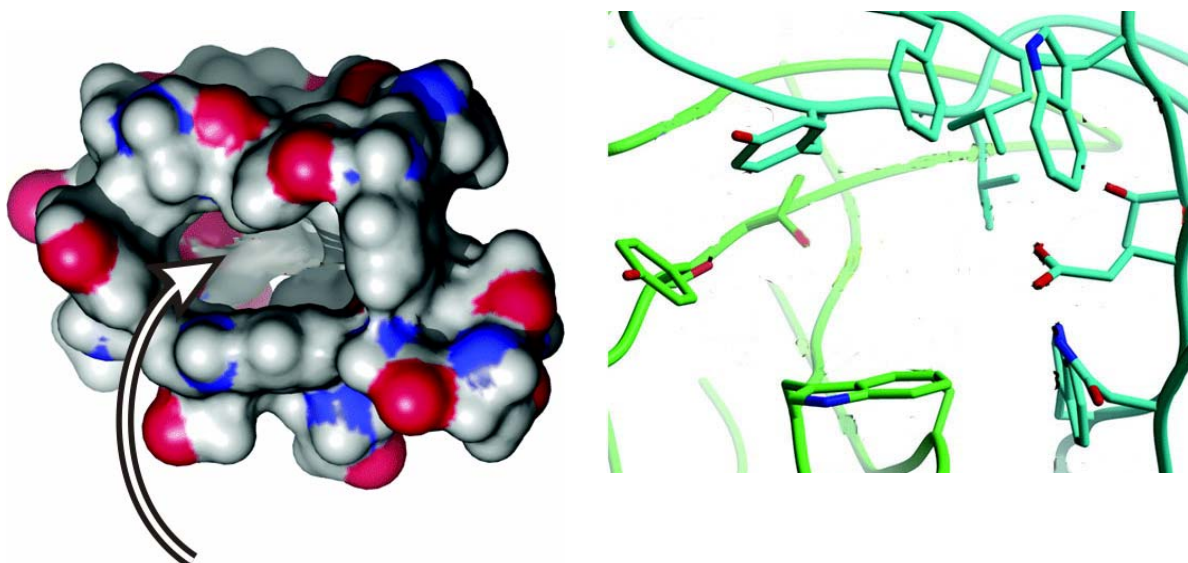
¹ Kemp, D. S.; Paul, K. *J. Am. Chem. Soc.* **1975**, *97*, 7305-7312.

- b. (15 pts) Based on the energies of bond-making and bond-breaking (ΔH_{BDE}), what would you estimate the reaction enthalpies ΔH to be for each of the two steps you drew? For each step, list bonds broken and bonds made, and give individual ΔH_{BDE} for each. (Ignore other energetic effects such as aromatic conjugation, resonance or entropy; focus only on bond-making and bond-breaking.)

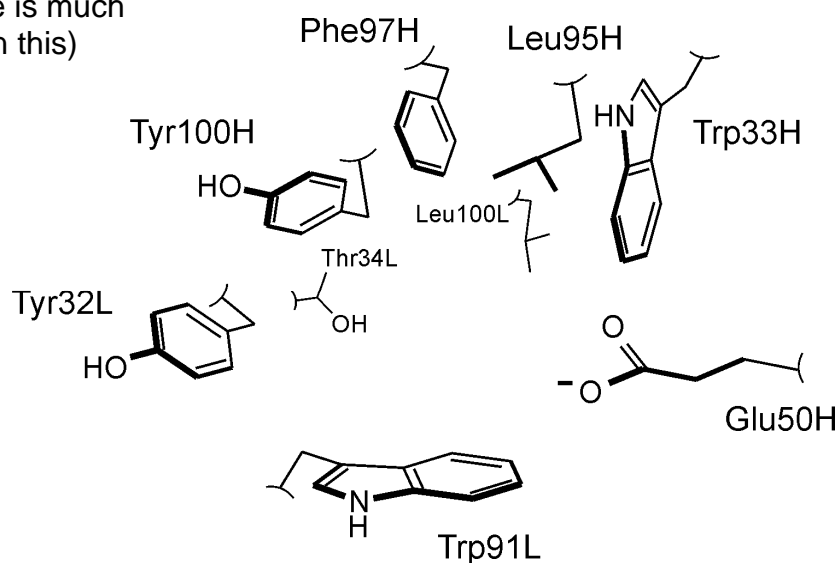
	Bonds made:	ΔH_{BDE} (kcal/mol)	Bonds broken:	ΔH_{BDE} (kcal/mol)
Step 1:				
	Total ΔH for Step 1:			

	Bonds made:	ΔH_{BDE} (kcal/mol)	Bonds broken:	ΔH_{BDE} (kcal/mol)
Step 2:				
	Total ΔH for Step 2:			
	Total ΔH for reaction:			

Hilvert and coworkers recently isolated an enzyme that catalyzes the Kemp elimination.^{2,3} In the presence of this enzyme, no added acetate or other base is required; as a result, Hilvert has proposed that the enzyme itself serves the role of base in the reaction. The Hilvert group has also solved the crystal structure of this enzyme that shows a pocket that might serve as the binding site for the substrate **1**. Some illustrations of this binding pocket, where your point of view is from the surrounding water environment looking into the pocket, are shown below.



binding pocket
(only pocket region shown;
whole enzyme is much
bigger than this)

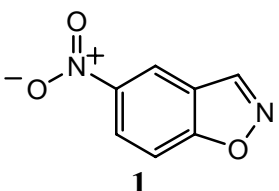
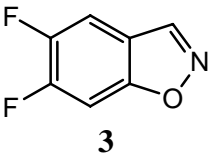
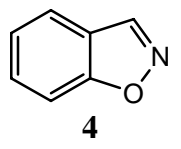


² Debler, E. W.; Ito, S.; Seebeck, F. P.; Heine, A.; Hilvert, D.; Wilson, I. A. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 4984-4989.

³ Thorn, S. N.; Daniels, R. G.; Auditor, M. T.; Hilvert, D. *Nature* **1996**, *373*, 228-230.

- c. (8 pts) The Hilvert group studied how changing the chemical structure of the substrate affected the binding affinity of the ligand to the pocket. They reported K_d values⁴ for the different substrates shown in the table below, where

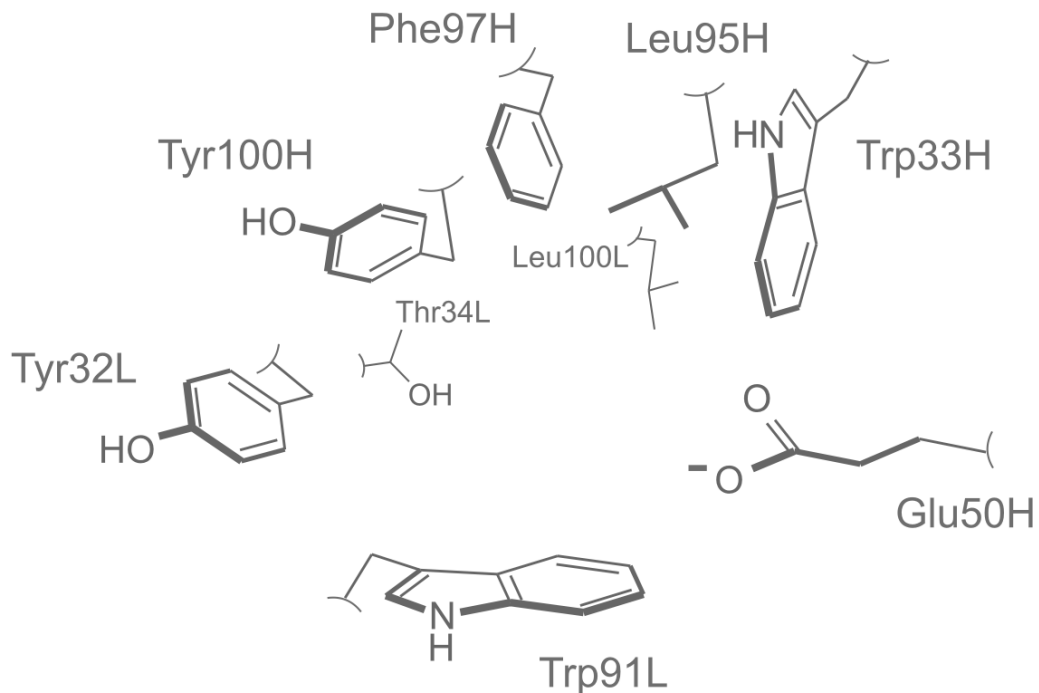
$$K_d = \frac{[\text{enzyme}][\text{substrate}]}{[\text{enzyme} \bullet \text{substrate}]}$$

substrate	K_M		
 <p style="text-align: center;">1</p>	90 μM	binds enzyme stronger or weaker than 1 ?	how much stronger or weaker than 1 , in kcal/mol?
		<i>(circle one answer for each box)</i>	<i>(answer to within 1 kcal/mol)</i>
 <p style="text-align: center;">3</p>	200 μM	stronger <i>or</i> weaker	
 <p style="text-align: center;">4</p>	2 mM	stronger <i>or</i> weaker	

In the third column above, indicate whether **3** and **4** bound the enzyme more or less tightly than **1**. In the fourth column, give $\Delta\Delta G$ values (at room temperature, 298 K) for how much stronger or weaker the binding interaction is for **3** and **4** compared to **1**. (Good to know for this problem: $R = 1.987$ cal/mol K.)

- d. (5 pts) Hilvert hypothesized that this enzyme works by positioning the substrate in exactly the right place to undergo the Kemp elimination. In the cartoon *on the next page*, draw the benzisoxazole **1** in the binding pocket the way it is oriented right before it undergoes elimination. Sketch your structure in three dimensions to illustrate noncovalent interactions between the substrate and enzyme.

⁴ The authors actually reported K_M values. Don't worry about this distinction; you will learn more about it later.



- e. (12 pts) Describe two noncovalent interactions that reinforce the orientation of the substrate in this way. As well as you can, label them “A” and “B” on your cartoon above, and then describe them in words below. Be as specific as you can be—if your interaction involves a specific amino acid, name that amino acid. Feel free to include a drawing in your answer if appropriate.

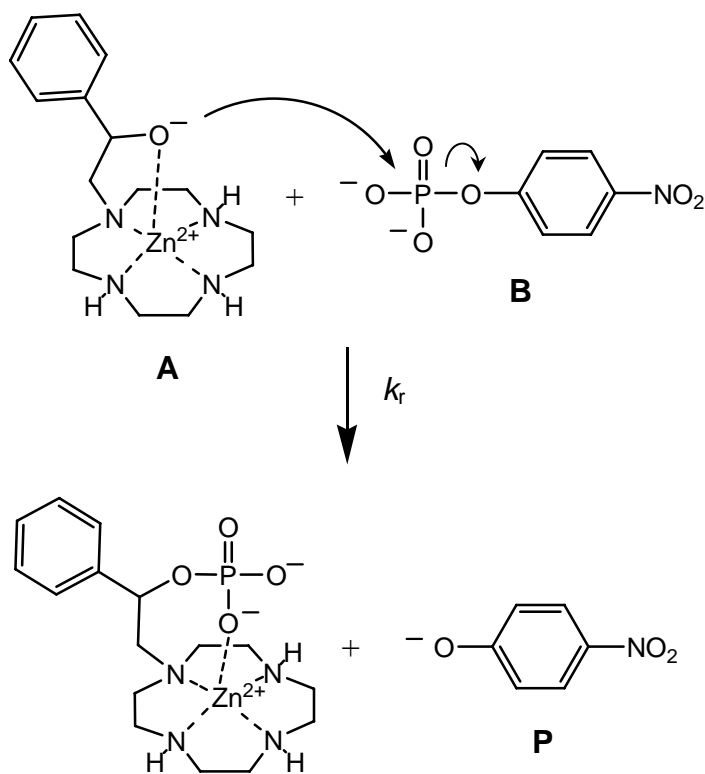
<i>interaction A</i>	<i>interaction B</i>

- f. (10 pts) Explain the differences and similarities between the binding affinities of **1**, **3** and **4** in terms of the weak interactions involved in the enzyme•ligand complex.

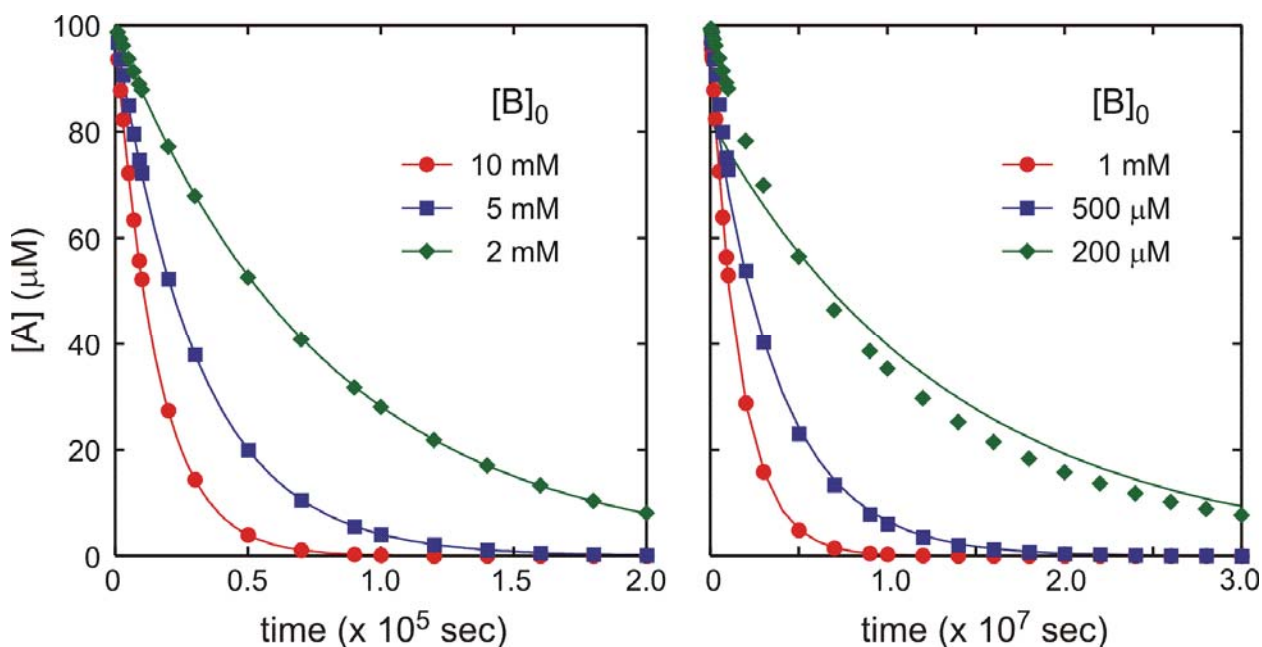
compare enzyme•**3** to enzyme•**1**

compare enzyme•**4** to enzyme•**1**

2. Kimura and coworkers have studied the reaction of zinc alkoxides with *p*-nitrophenyl phosphoesters, with the hope of developing catalysts for cleaving phosphodiesters (like DNA and RNA). For example, the Kimura group has characterized the kinetics of the reaction between the (cyclen)Zn alkoxide **A** and *p*-nitrophenylphosphate **B** in water.⁵ Because the observed rate depended on both **[A]** and **[B]**, they proposed the single-step mechanism shown at right. The kinetic data below shows the results of six kinetic runs, performed with different starting concentrations **[B]₀** in buffered water, in which the disappearance of **A** was monitored over time. In all runs, **[A]₀** = 100 μM, and the data for each run has been fitted to the expression



$$[A]_t = [A]_0 e^{-Ct}$$



⁵ Kimura, E.; Kodama, Y.; Koike, T.; Shiro, M. *J. Am. Chem. Soc.* **1995**, *117*, 8304-8311.

Note that, while the vertical axes of the two graphs are the same, the horizontal axes have different scales. The fitted values for C (in the equation $[A]_t = [A]_0 e^{-Ct}$) are given in the chart below.

$[B]_0$	C
10 mM	6.4×10^{-6} /sec
5 mM	3.2×10^{-6} /sec
2 mM	1.3×10^{-6} /sec
1 mM	5.9×10^{-7} /sec
500 μ M	2.6×10^{-7} /sec
200 μ M	7.3×10^{-8} /sec

- a. (10 pts) Assuming that the reaction rate was dependent only on $[A]$ and $[B]$, for the equation

$$-\frac{\partial[A]}{\partial t} = k_r [A]^x [B]^y,$$

what are the values of k_r , x and y ?

$k_r =$

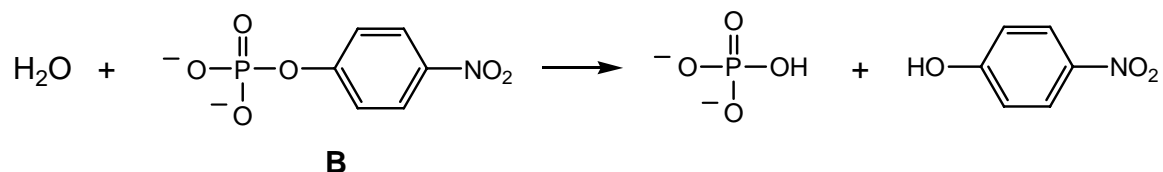
(make sure to include units)

$x =$ $y =$

- b. (7 pts) Although most of the curve fits look good, the fits for $[B]_0 = 500 \mu\text{M}$ and $200 \mu\text{M}$ are substantially worse than the others. What integrated rate expression would have been more appropriate for fitting these kinetic runs? To match the data in the graphs, write your answer *on the next page* in the form “ $[A]_t = \dots$ ”. If you create any new variables or constants that aren't discussed in this problem, make sure to define them (in the box).

$$[A]_t =$$

- c. (8 pts) In addition to reacting with the zinc alkoxide **A**, *p*-nitrophenylphosphate **B** in water can also be consumed by hydrolysis:



Because the effective concentration of water is so high, the kinetics of this hydrolysis reaction can be described by a pseudo-first order rate law where

$$\frac{-\partial[\mathbf{B}]}{\partial t} = k_h[\mathbf{B}].$$

In principle, both this hydrolysis and the reaction of **B** with **A** could occur simultaneously. Write a unified rate law for $-\partial[\mathbf{B}]/\partial t$ (with contributions from both reactions) in terms of the variable $[\mathbf{B}]$ and any constants. Your expression should not include the variable $[\mathbf{A}]$. Again, define any new variables or constants that you create. Do not use the pseudo-first order approximation for the reaction of **B** with **A**.

$$\frac{-\partial[\mathbf{B}]}{\partial t} =$$

- d. (15 pts) Derive an integrated rate law from your expression in (c) in the form “[**B**]_t = ...”. This problem will be graded both for your derivation and for your answer, so show your work (so that we can give you any partial credit that you’ve earned). Use additional pages if you need to. You may need the integral form

$$\int \frac{dx}{x(a+bx)} = -\frac{1}{a} \ln \frac{a+bx}{x}.$$

Standard Bond Dissociation Energies (BDEs)

Bond Type	BDE (kcal/mol)
H-H	104.2
C-C	83
N-N	38.4
O-O	35
F-F	36.6
Si-Si	52
P-P	50
S-S	54
Cl-Cl	58
Br-Br	46
I-I	36
H-C	99
H-N	93
H-O	111
H-F	135
H-Cl	103

Bond Type	BDE (kcal/mol)
H-Br	87.5
H-I	71
H-B	90
H-S	81
H-Si	75
H-P	77
B-F	150
B-O	125
C-N	73
N-CO	86
C-O	85.5
O-CO	110
C-S	65
C-F	116
C-Cl	81
C-Br	68

Bond Type	BDE (kcal/mol)
C-I	51
C-B	90
C-Si	83
C-P	70
N-O	55
S-O	87
Si-F	135
Si-Cl	90
Si-O	110
P-Cl	79
P-Br	65
P-O	90
C=C	146
N=N	109
O=O	119
C=N	147

Bond Type	BDE (kcal/mol)
C=O (CO ₂)	192
C=O (aldehyde)	177
C=O (ketone)	178
C=O (ester)	179
C=O (amide)	179
C=O (halide)	177
C=S (CS ₂)	138
N=O (HONO ₂)	143
P=O (POCl ₃)	110
P=S (PSCl ₃)	70
S=O (SO ₂)	128
S=O (DMSO)	93
P=P	84
P≡P	117
C≡O	258
C≡C	200
N≡N	226
C≡N	213

From <http://www.cem.msu.edu/~reusch/OrgPage/bndenrgy.htm>.