

**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 50 minutes 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.

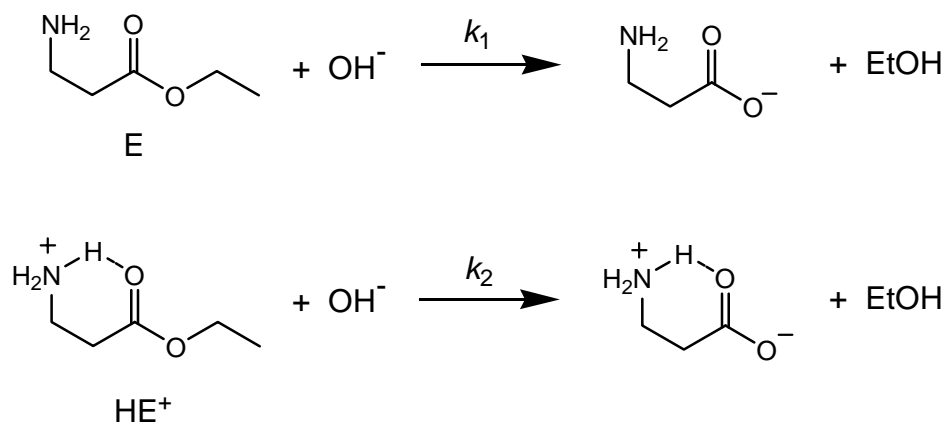
At the end of the 50-minute exam period you will be asked to return your exam to the proctor. (You may, of course, also turn the packet in earlier if you choose.) This exam is *open-resource*—you may use any books, notes, calculator, etc. you have brought with you to the exam. However, you are not allowed 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 9 pages, including this one. Please check to make sure that your packet contains 9 pages before beginning your exam.

**Name:** \_\_\_\_\_

**Signature:** \_\_\_\_\_

1. The cleavage of  $\gamma$ -amino esters in aqueous base can take place both through the unprotonated (E) and protonated ( $\text{HE}^+$ ) form of the ester.



Both of these are elementary reactions, and rates of each relate to the rate constants and reactant concentrations in the usual way. As you might imagine, the concentration of  $\text{HE}^+$  is low under basic conditions; for the reactants shown above, the equilibrium constant

$$K_b = \frac{[\text{HE}^+][\text{OH}^-]}{[\text{E}]} = 5.01 \times 10^{-7} \text{ M}.$$

As a result,  $[\text{E}] \gg [\text{HE}^+]$ . However, if  $k_2 \gg k_1$ , then the second mechanism could still predominate.

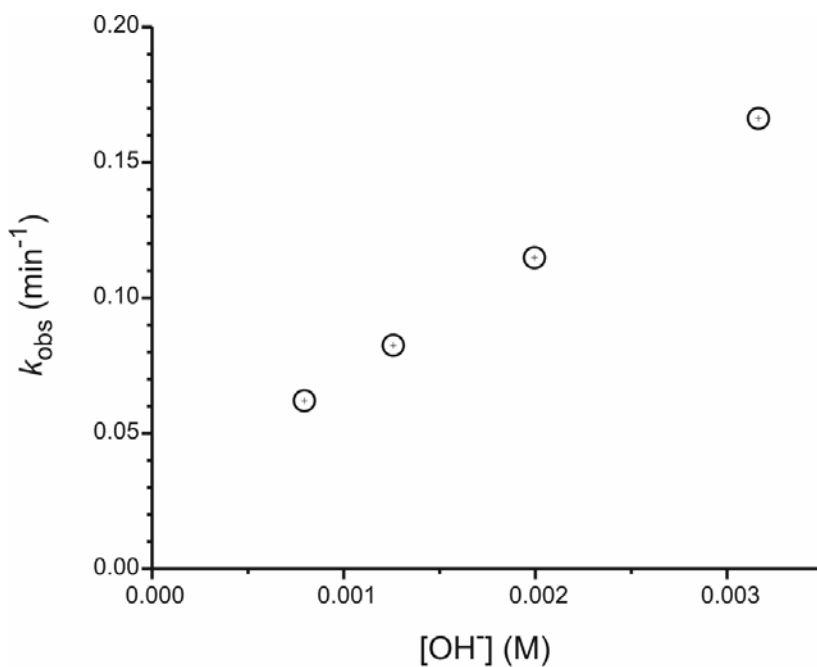
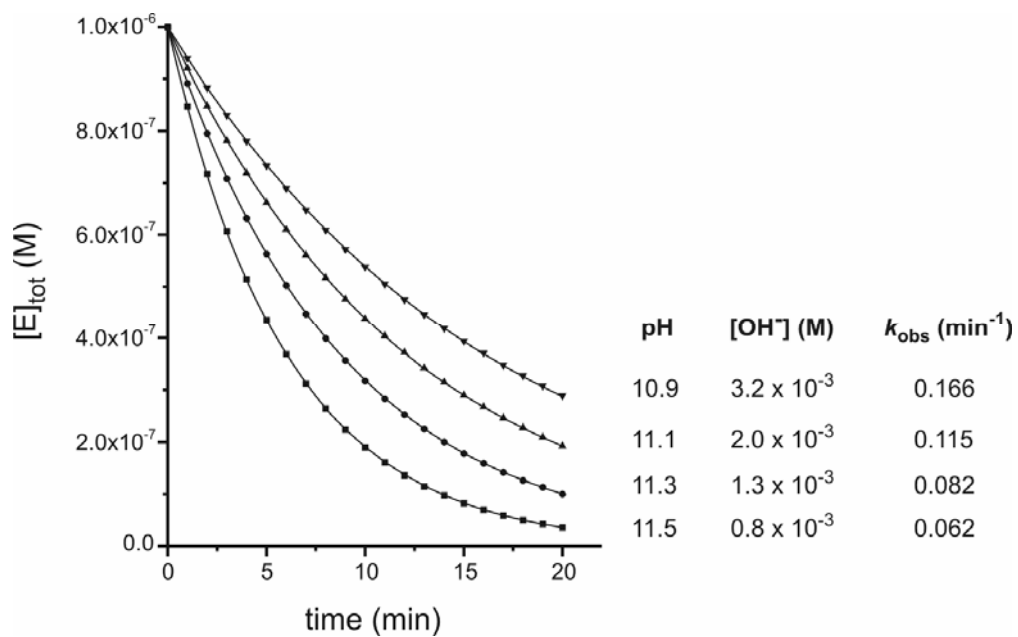
- a. (25 pts) Kinetics experiments show that, as long as  $\text{OH}^-$  is in vast excess (which is usually true under basic conditions), the disappearance of E is pseudo-first order in the total concentration of E:

$$-\frac{\partial[\text{E}]_{\text{tot}}}{\partial t} = k_{\text{obs}}[\text{E}]_{\text{tot}},$$

where  $[\text{E}]_{\text{tot}} = [\text{E}] + [\text{HE}^+] \approx [\text{E}]$ . (This assumption must be made because neither  $[\text{E}]$  nor  $[\text{HE}^+]$  can be measured directly.) **Derive a rate expression for  $-\partial[\text{E}]_{\text{tot}}/\partial t$  in terms of measurable concentrations, known constants and the individual rate constants  $k_1$  and  $k_2$ .** Do not use  $[\text{E}]$  or  $[\text{HE}^+]$  in your expression, and make sure your answer is consistent with the pseudo-first-order kinetics described above. (Write your answer on the next page.)

(answer, part a)

- b. (10 pts) A series of measurements were made in which pseudo-first-order rate constants  $k_{\text{obs}}$  were evaluated at different pH's (and thus different concentrations  $[\text{OH}^-]$ ). The decays are shown in the graph below, and the evaluated  $k_{\text{obs}}$  values are plotted vs.  $[\text{OH}^-]$  in the following graph.



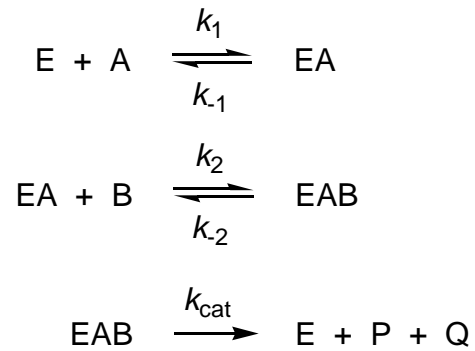
**From these graphs, estimate  $k_1$  and  $k_2$ .** Include units in your answer. You might solve this problem by drawing a function onto the plotted points, or by setting up your rate expression for two of the points as a set of simultaneous equations.

$k_1 =$

$k_2 =$

*(put any work you'd like us to see below, or on graphs on previous page)*

2. Glycerol kinase couples the phosphorylation of 1,2-diols into phosphono-1,2-diols with the dephosphorylation of MgATP into MgADP. The enzyme, in other words, converts two substrates into two products. Like many other enzymes that do this, glycerol kinase binds the two substrates in a specific order: one (A) binds first, and then the other (B) binds next.



In this problem, you will determine which of “A” or “B” corresponds to which of 1,2-diol or MgATP.

- a. (10 pts) *For this part only*, assume that EAB is a steady-state intermediate. **Write an expression that relates  $[EAB]_{\text{ss}}$  to any other species in the kinetic scheme above.**

$[EAB]_{\text{ss}} =$

- b. (25 pts) *For the rest of this problem*, assume instead that the first two steps of the mechanism are in pre-equilibrium, with  $K_A = k_{-1}/k_1$  and  $K_{AB} = k_{-2}/k_2$ . (These equilibrium constants are the inverse of what you might ordinarily express from the reactions as written.) Using the additional simplification  $[E]_{\text{tot}} = [E] + [EA] + [EAB]$ , **derive an expression for the initial rate  $\partial[P]/\partial t$  in terms of  $[E]_{\text{tot}}$ ,  $[A]$ ,  $[B]$ , and constants.**

- c. (10 pts) Your answer to part (b) should not have been commutative; in other words, if you switched the variables [A] and [B] in your rate equation, you would not get the same equation back. To put it another way, the order of addition of A and B to the enzyme active site matters in this case.

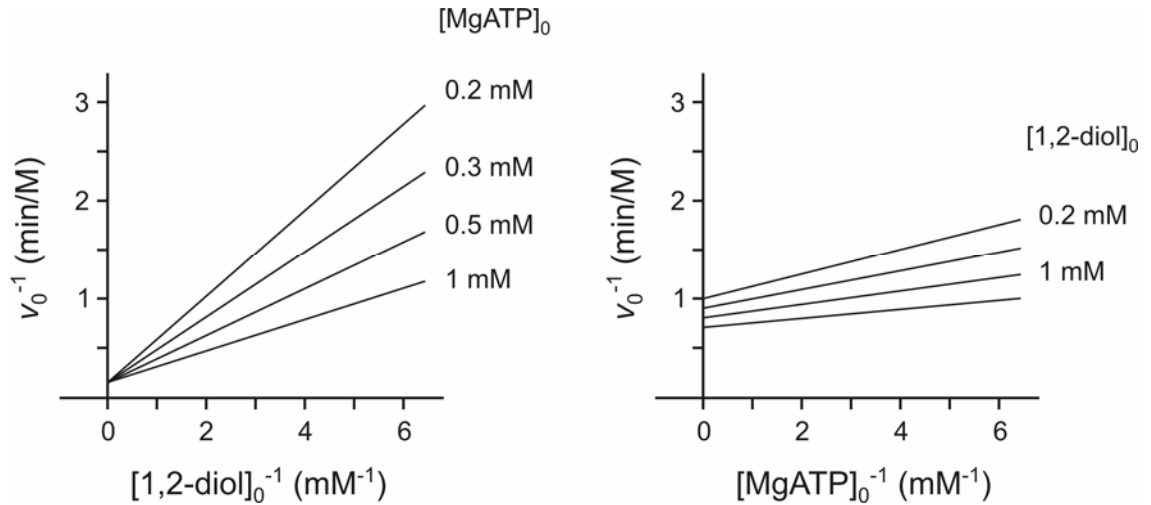
One consequence of this is that the dependence of rate on [A] and [B] are different. **Re-write your answer to part (b) as two different Lineweaver-Burke expressions: one as  $1/\text{RATE}_0$  vs.  $1/[\text{A}]_0$  and the other as  $1/\text{RATE}_0$  vs.  $1/[\text{B}]_0$ .** Use the substitution  $V_{\text{max}} = k_{\text{cat}}[\text{E}]_{\text{tot}}$ .

$1/\text{RATE}_0$  vs.  $1/[\text{A}]_0$

$1/\text{RATE}_0$  vs.  $1/[\text{B}]_0$



- d. (20 pts) Initial reaction rates were measured for a variety of concentrations [1,2-diol] and [MgATP]. The data could be plotted either as  $1/\text{RATE}_0$  vs.  $1/[1,2\text{-diol}]$  or as  $1/\text{RATE}_0$  vs.  $1/[\text{MgATP}]$ :



Based on the relative values of  $(1/V_{\max})_{\text{app}}$  and  $(K_m/V_{\max})_{\text{app}}$  that might be measured from these graphs, **which starting material is A and which is B** according to the reaction scheme at the beginning of the problem? **Why?**

1,2-diol:      **A**   or   **B**   ?   (*circle one*)

MgATP:      **A**   or   **B**   ?   (*circle one*)

Explain.