Chapter 6

Enzyme Kinetics

Vicki Chandler, Sam Donovan, Will Goodwin, Karen Sprague, and Carl Stiefbold

Department of Biology; University of Oregon
Eugene, OR 97403

Vicki Chandler is a Professor of Biology in the Plant Science Department at the University of Arizona. She was formerly an Associate Professor of Biology and a member of the Institute of Molecular Biology at the University of Oregon. She received a B.A. from the University of California, Berkeley and her Ph.D. from the University of California, San Francisco. Her research is on the control of gene expression in different cell types and at different developmental stages. She studies the regulated expression of the genes required for anthocyanin biosynthesis in maize. Vicki Chandler's teaching experiences include molecular biology for biology majors, advanced molecular genetics, and biochemistry lab for biology majors.

Sam Donovan is pursuing a doctoral degree in Science Education in the Department of Curriculum and Instruction at the University of Wisconsin -Madison. His research interests include the nature of student reasoning and argumentation in evolutionary biology. After receiving a masters degree in biology from the University of Oregon, Sam coordinated the implementation of a Howard Hughes Medical Institute grant for improving undergraduate education in biology.

Will Goodwin, Computer Technology Instructor and Software Developer, received his B.S. in Mathematics and Physics at Western Michigan, his M.S. in Mathematics at the University of Arizona, and another M.S. in Computer and Information Science at the University of Oregon. Since 1992 Will has worked as a Research Assistant in the Biology Software Development Lab funded by a grant from the Howard Hughes Medical Institute. He is involved in the design, development, and implementation of a set of programs which are to be used by undergraduate biology students.

Karen Sprague is a Professor of Biology and a member of the Institute of Molecular Biology at the University of Oregon. She received an AB from Bryn Mawr College in Biology, and a Ph.D. from Yale University, working with G.R. Wyatt on insect metamorphosis. She did post-doctoral work in *Drosophila* genetics with D.F. Poulson, and in protein-nucleic acid interactions with Joan Steitz. Her research at the University of Oregon is on the regulation of transcription by RNA polymerase III -- in particular, the molecular mechanism of tissue-specific tRNA synthesis that accompanies high level silk production in silkworms. The work is supported by grants from the National Institutes of Health. Karen Sprague's teaching interests range from introductory biochemistry to advanced molecular genetics. She has taught at many levels -- including genetics and molecular biology for non-science majors, biochemistry for biology majors, and mechanisms of gene regulation for graduate students. In 1988, she received the UO Biology Department award for outstanding teaching.

Carl Stiefbold (B.S., Portland State University) has been a Biology Teaching Lab Preparator and Lab Instructor at the University of Oregon since 1987. He received the 1996 UO Biology Teaching Recognition Award. Correspondence regarding this laboratory exercise should be addressed to him at the Biology Department, University of Oregon, Eugene, OR 97403; Telephone: (541)346-4537; e-mail: cstiefbo@oregon.uoregon.edu

Contents

Introduction

The laboratory exercise **Enzyme Kinetics** is one in a series that are closely coordinated with lectures in Cellular Biochemistry—a sophomore level core curriculum course required of University of Oregon biology majors. The course is preceded by a course in Genetics and Evolution and a course in Molecular Biology, It is followed by a course in Developmental Biology and Ecology. This set of four courses, each with an associated laboratory course, presents students with the key principles and relationships that underlie all of biology. Prerequisites for enrollment in the courses are completion of General Chemistry and concurrent enrollment in Organic Chemistry. The Cellular Biochemistry laboratory series was created originally by W.R. Sistrom. The lab exercise presented here is new, and was created during a general revision of the Cellular Biochemistry labs as part of a project funded by the Howard Hughes Medical Institute for the Improvement of Undergraduate Life Science Education whose goal was to focus students' intellectual effort on the lab exercise itself, instead of on the lab report. That is, we wanted students to be intellectually engaged while working on the lab—not only while writing the report sometime later. In the case of the Enzyme Kinetics lab, we also wanted to address specific aspects of the mechanism of general enzyme catalysis and one possible laboratory method of investigation. The method of presenting the lab exercises is as important as their content. The exercise is done during a three hour lab period in which 26-30 students, one faculty member and two teaching assistants (one graduate, one undergraduate) participate. The lab work is structured to create a cooperative environment that encourages both group work and questions from individuals. The text of the lab manual is designed to provide continual intellectual challenge, rather than passive data collection.

Materials

Macintosh Computers running System 7.x with 1 Meg RAM
Computer application programs entitled: Enzyme Kinetics and Reaction Rates (available for Macintosh computers from the University of Oregon by contacting Carl Stiefbold, Biology Department, University of Oregon, Eugene, Oregon 97403.)
Spectrophotometers @ 420 nm (1 per pair of students)
13 x 100 mm test tubes for spectrophotometer cuvettes
Reaction Buffer (0.1M pH7 NaPhos + 0.01M KCl)
ONPG (o-nitrophenyl-β-D-galactosidase), Sigma Chemical Co. 1-800-325-5052,
                    Catalogue number N-1127
5 ml pipettes and pipette pumps
P-20 or P-200 pipettors (1 per pair of students)
β-galactosidase (see Appendix A)

Notes for Instructors

The structure of the lab exercise:

Each lab exercise consists of three parts: a pre-lab assignment, a group of lab activities, and a brief report.

1. Pre-lab assignment   The pre-lab assignment is used to focus students' thinking on the important concepts connected with the lab exercise. Instead of emphasizing technical issues, pre-lab assignments require students to construct a framework for understanding before coming to lab. Pre-lab exercises are due at the beginning of the lab period, and are checked and returned during the period. This procedure allows instructors to discover quickly what students don't understand, and to address those problems during the lab session.

2. Lab activities   The lab exercise is divided into several distinct activities, each of which focuses on a particular concept. As part of each activity, students must answer questions that require specific predictions or novel applications of concepts. These questions are an important tool for monitoring understanding. Teaching assistants can use them to engage individual students in discussions of the lab material, and also to assess the progress of the group as a whole.

3. Lab report   The lab report is short. It focuses on applications of concepts learned during the lab. If there is time, students are encouraged to complete the lab report during the lab period. This often leads to group interactions and problem solving that can be monitored by the teaching assistants.

The role of the lab instructors:

It is important to recognize that this lab format demands very active participation by the lab instructors. We spend a great deal of time making sure that the teaching assistants understand the material, and training them to interact effectively with the students. The teaching assistants must be sufficiently confident to elicit questions from students and to probe the students' comprehension. This involves circulating through the lab room and engaging individual students in specific discussions that focus on key ideas. These discussions should be encouraging, but they should also be very clear -- so

that students can recognize misconceptions, and instructors can identify common problems that should be discussed by the group as a whole.

Student Outline

Enzyme Kinetics: Pre-lab Assignment

- Read the lab exercise, focusing on the Overview section, and the introductory material for each activity.
- Answer the questions in Part I below using the Enzyme Kinetics software in the Biology Mac Lab.
- Work the problems and answer the questions in Part II.

Part I:

Using the Software:
        For this pre-lab you will be using a piece of simulation software called Enzyme Kinetics. It is available on the computers in the Biology Macintosh Lab. It is also available on disk from the Mac Lab if you have a Macintosh computer at home and would prefer to use it there. Please bring a disk to the Mac Lab if you are planning to use the software at home.

Do not wait until the day of your lab to begin this pre-lab. Inability to access a computer is not a valid excuse for not completing this assignment on time.

Getting Started:

- Double-click on the application titled "Enzyme Kinetics Pre-lab".
- Work through the following questions.

Questions:
        The first screen you see will contain a graphic representing a beaker with several types of molecules in solution. Click the "Run" button to observe a simulation of the chemical reaction occurring in the beaker.

1. What are the different types of molecules represented? Please list the molecules, including their colors. Select the "Screen 2" button. Now you have a new representation of the reaction you were just watching. To make it easier to track the events in the field of view, only one enzyme molecule is shown and it is stationary. There are 2 beakers, side-by-side. You will be comparing what happens in them. Start the displays by clicking the "Run" button. Observe the 2 situations and then answer the following questions.
2. What is the fundamental difference between these two reactions?
3. Is the molecule on the right an enzyme? Why or why not?
4. Write a symbolic representation for the reaction you are observing.
5. List the steps in one complete catalytic cycle. What does the light green icon represent?
6. How could you determine the rate of the reaction you are observing? Determine the rate showing the data you used. Now select "screen 3". The left side of the screen is the same enzyme but the molecule in the right hand beaker has been replaced with a different molecule.
7. What is the rate of the reaction for the new molecule in the right-hand beaker?
8. What part of the cycle makes the two enzyme cycling rates different?

Part II:

1. Suppose that glucose can be converted to another substance by a certain amount of an enzyme at the following rates

Glucose Concentration (mM)	mM glucose converted per minute
1×10^{-5}	0.15
2×10^{-5}	0.25
1×10^{-4}	0.60
3×10^{-4}	0.77
5×10^{-4}	0.81

Plot these points on the following axes, indicating the appropriate numerical values for the divisions, and the units for reaction velocity and substrate concentration.

Reaction
Velocity
(   )
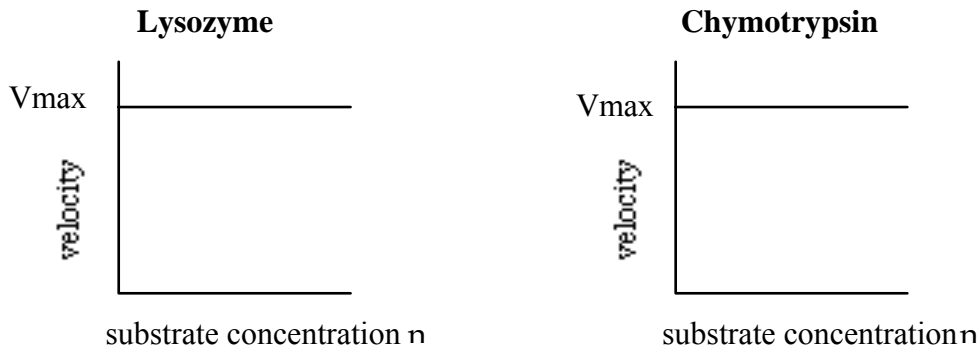
Substrate Concentration
(   )
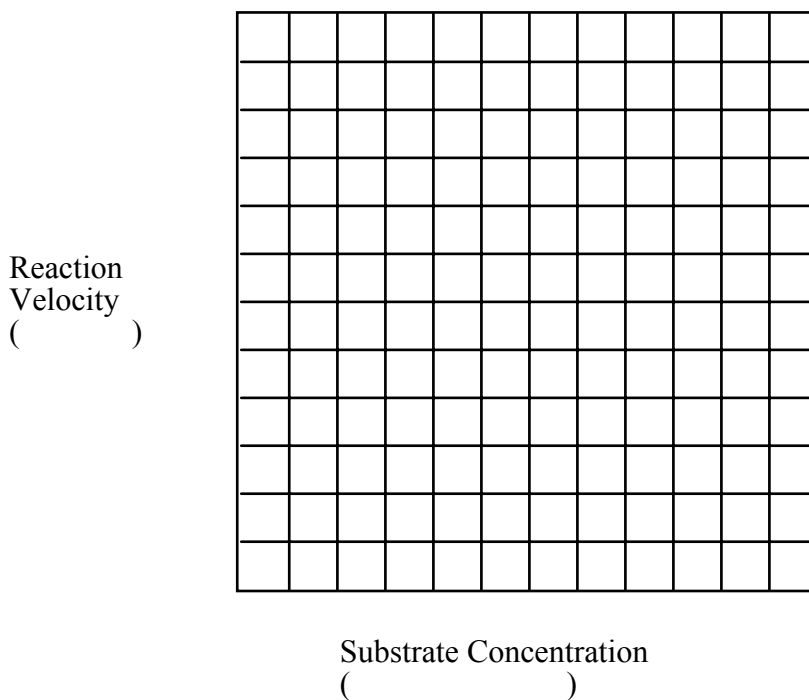
Does the shape of this curve suggest that the reaction will reach a maximal rate? By inspection of the curve, estimate the maximal rate, including units.
Draw a horizontal line on the graph to indicate your estimate for the maximal rate of this reaction (Vmax).
Using your estimated Vmax, indicate **on the graph** how you would estimate the Km value for this enzyme. Record your estimate here, including units.

2. Lysozyme is an enzyme that breaks down bacterial cell walls. Its K_m is 6 mM. Chymotrypsin is a digestive enzyme that breaks peptide bonds, and its K_m is 5000 mM. Sketch the curves below, inserting numbers to indicate the appropriate substrate scale.

Lysozyme   **Chymotrypsin**

Vmax   Vmax

velocity   velocity

substrate concentration n   substrate concentration n

Which of these two enzymes has the higher affinity for its substrate?
Which enzyme(s) [lysozyme only (L), chymotrypsin only (C), both (B) or neither (N)] will be operating at maximum efficiency at the following concentrations of substrate:

1 mM _____   100 mM _____

6 mM _____   5000 mM _____

50,000 mM _____

3. If the number of enzyme molecules available to catalyze a reaction were to increase, what would happen to the value of:

Vmax _____

Enzyme Kinetics

Objectives:
• Understand how a spectrophotometer works.
• Understand why the rate of an enzyme-catalyzed reaction reaches a maximum.
• Understand how the affinity of an enzyme for its substrate is measured.

Overview:
        In this lab exercise you will explore two important parameters of enzyme-catalyzed reactions: maximum rate (**Vmax**) and affinity for substrate (**Km**).

Vmax:

A single enzyme molecule can convert molecules of substrate into product multiple times during a given time period. The maximum rate for a group of molecules is called Vmax. Imagine that the concentration of substrate is so high that there is essentially no time when an enzyme molecule is not bound to substrate. That is, as soon as a bound substrate molecule reacts to form product, it leaves the enzyme and is instantly replaced by a fresh substrate molecule. This is what we call a "saturating" concentration of substrate—because at this concentration, the active site is always filled (saturated) with substrate. Under such conditions, the rate at which an individual enzyme molecule can turn substrate into product is the highest it can possibly be. This maximum cycling rate *per* molecule is called the turnover number (TON). A group of 100 enzyme molecules, each able to convert substrate to product at the same maximum cycling rate, would produce an observed Vmax that was 100-fold higher than the turnover number.

Km:

The Michaelis constant (Km) of an enzyme is a measure of the affinity of the enzyme for its substrate. The value of Km for a particular enzyme is defined as the substrate concentration at which half of the enzyme molecules are complexed with substrate. Under these conditions, at any instant, half of the total enzyme molecules are capable of catalysis. Therefore, the observed reaction rate is half of the maximum possible rate—the rate achieved when all the enzyme molecules are complexed with substrate, Vmax.

The substrate concentration required to drive half of the enzyme molecules into enzyme-substrate (ES) complexes depends on the ability of the enzyme to bind its substrate. Thus, an enzyme with a high affinity for its substrate will have a low K_m. That is, 50% of the enzyme molecules will have bound substrate at a relatively low concentration of substrate. In contrast, an enzyme with a low affinity for its substrate has a high Km value—because a relatively high concentration of substrate is required to drive 50% of the enzyme molecules into complexes with substrate.

β-galactosidase:

In today's lab, you will be introduced to another enzyme and another way to assay enzyme activity. β-galactosidase is used by bacteria to split lactose, a disaccharide, into the monomers galactose and glucose, which can then be utilized by the cell as energy sources.

Lactose   **β-galactosidase**   **Galactose and Glucose**
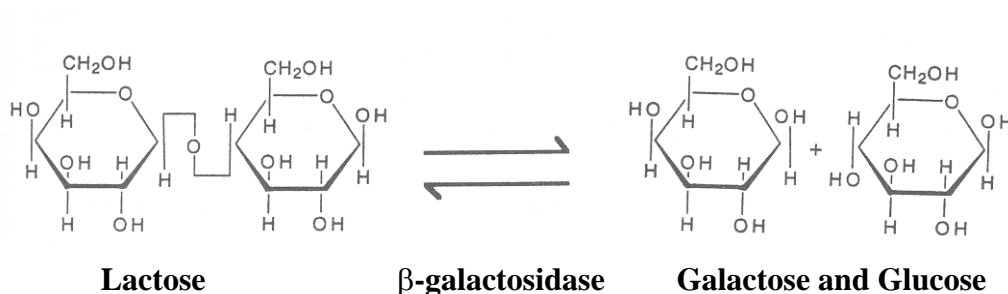
Today you will measure the enzyme's action with a substitute substrate, o-nitrophenyl galactoside. β-galactosidase speeds up the conversion of o-nitrophenyl galactoside (ONPG) to o-nitrophenol (ONP) in much the same way it acts on lactose.
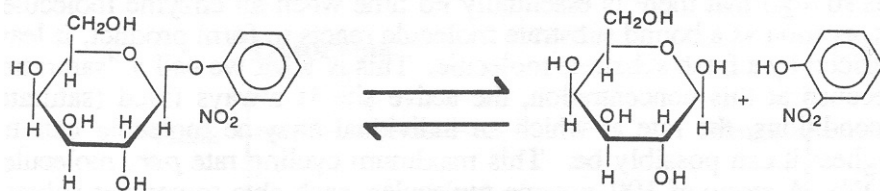
ONPG (colorless)   **Galactose and   ONP (yellow)**

ONPG is useful as an assay material because, although it is colorless, the product of its hydrolysis is yellow. Thus, we can measure the rate at which the product accumulates by measuring the appearance of yellow color.

Activity 1: Using the Spectrophotometer:

You have probably already used the spectrophotometer, but we will take a few minutes to review how it works. This instrument will be our main measurement tool for the next several labs.

The spectrophotometer is used to measure the concentration of compounds or particles in a solution based on the amount of light that passes through the solution.

What is inside a spectrophotometer?

At a station in the back of the lab room, you will find a partially disassembled spectrophotometer. This is for you to examine. Look closely at its inner workings. Try to identify the following functional components (or their analogous parts) in the disassembled spectrophotometer :

• Light source - This is an incandescent lamp that produces white light (a mixture of visible wave lengths).

• Slit - This controls the amount of light being passed through the sample.

• Wavelength selector - The white light is split into its component wave lengths by a prism, and monochromatic light (a single wavelength) is then selected by rotating the prism so that the desired wavelength of light passes through the sample.

• Sample holder - This keeps the sample you are measuring in the proper position.

• Light detector - This is a photosensitive cell that measures the light that gets through the sample.

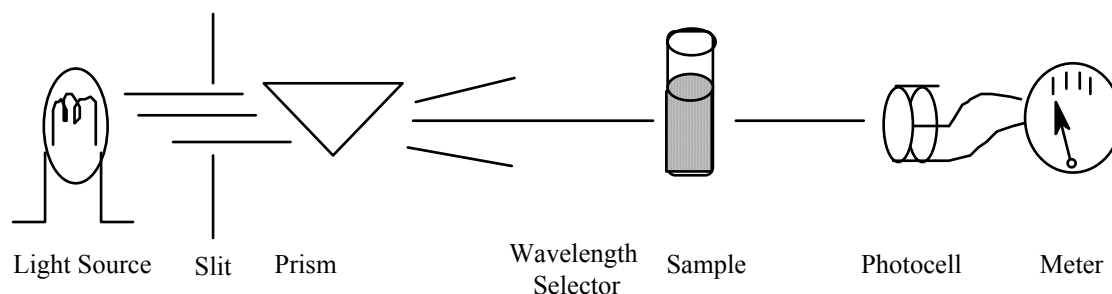• Meter - This gives the readout from the photocell.

Light Source   Slit   Prism   Wavelength Selector   Sample   Photocell   Meter

Figure 6.1. The basic components of a spectrophotometer.

Observing different wavelengths:

Turn on your spectrophotometer and allow it to warm up (about 5 minutes). You can leave it on for the rest of the lab period. Place an empty tube in the sample holder and slide a strip of white paper into the tube.   Then remove the filter and turn up the light intensity (by adjusting the 100% transmittance knob fully clockwise). As you look down the tube, you should see light from the light source reflecting off the paper.   Adjust the wave lengths, starting at 650 nm, filters, and the "% Transmission" control, and observe the changes in the light.

Fill in the following table:

Wavelength (in nanometers)	Color
450	
500	
550	
600	
650	

The choice of wave length for measuring a particular substance is based on the color of light that is absorbed by that substance.

Why is ONP yellow?

What color(s) of light does ONP absorb?

What wave length(s) might you choose to measure ONP? _____nm

What wave length(s) would you not choose to measure ONP? _____nm

Why?

Measuring absorbance accurately with a spectrophotometer:

In order to use the spectrophotometer to measure the concentration of a substance (ONP, for example), you will take advantage of the fact that the amount of light absorbed is linearly proportional to the concentration of the substance. Therefore, you will be concerned with something called "Optical Density" (OD) -- a measure of how effective the substance is at blocking (being dense to) light. High optical density corresponds to strong absorbance -- and strong absorbance is the consequence of high concentration.

In order to make accurate measurements with a spectrophotometer, there are two main factors to consider:

1. You must set the limits of possible measurements:

   a. One limit corresponds to **infinite absorbance**. That is, none of the light is transmitted. You will use a mechanical shutter inside the spectrophotometer to block all of the light, and thus simulate infinite absorbance. This is done with the "0 Set" button ("0" in this case corresponds to 0% transmittance of light). With the "0 Set" button depressed, set the absorbance to infinity (% transmittance = 0).
   b. The other limit corresponds to **no absorbance**. That is, all of the light is transmitted. You will use a solution that contains everything except the substance you want to measure (a so-called "blank") to set the baseline absorbance to 0. Your blank should contain 3 ml of buffer solution and one aliquot of enzyme. Because there is no substrate present, no product will be produced. By setting absorbance to 0 for the blank, only the newly produced material will be sensed by the machine when you do a real experiment, with substrate present.

2. You must minimize extraneous influences on light readings

   To do this, you should take the following precautions: mark the tubes so that they are always read in the same orientation, wipe the tubes before placing them in the spectrophotometer, and always close the lid of the sample chamber when taking a reading. These steps will reduce variation due to unevenness of the glass or dirt on the tubes. Also, use the mirror next to the scale to aid you in reading the meter needle accurately (to avoid parallax).

Practice reading the Optical Density of several of the vials provided. First, estimate the relative ODs by using your eyes as a spectrophotometer. You may be surprised at how good they are! Rank the lettered vials (A-F) according to decreasing "eyeball OD".

Highest eyeball OD --> Lowest eyeball OD

Now, use the spectrophotometer to measure the Optical Density of the same set of vials. Based on the absorption spectra for ONP, 420 nm is an appropriate wavelength to use.

Highest OD --> Lowest OD

Are your spectrophotometer results reproducible?
Do the measurements made with the spectrophotometer agree with your eyeball measurements?

Activity 2: Assaying the Activity of β-galactosidase

By monitoring the appearance of product, ONP (yellow color), you will examine the time course of the enzyme catalyzed conversion of ONPG to ONP.

Qualitative measurement:

1. First prepare a tube containing some substrate (3 ml of 2 mM ONPG). Add an aliquot of enzyme. (We'll suggest an appropriate amount of β-galactosidase to use. The enzyme aliquot size varies from enzyme prep to enzyme prep, but it is usually about 50 μl.). In this section we are using a concentrated solution of substrate to make the color change easier to see. Use a fingercot to cover the tube and invert the tube to mix the solution. Watch the tube closely over several minutes. Holding the tube up against a white piece of paper may help make the color more obvious.
2. When was the yellow color first visible? Compare the tube to your blank.
3. Approximately how long did the mixture continue to become more yellow?

Quantitative measurement:

1. Now we will make the same observations more quantitatively by using the spectrophotometer. This exercise is designed to help you understand how the rates of reactions are determined using spectrophotometric data.
2. Based on your initial qualitative analysis, construct a data table below that will allow you to record the activity of β-galactosidase over a 10 minute time period. Run your analysis twice using 3 ml of 0.2 mM ONPG mixed with enzyme. Make any adjustments you feel are necessary to make the second trial more accurate.

Table of data for OD versus time.
(Space should be provided in the student handout so that students can construct their own data table.)

3. Plot your results on the axes below.
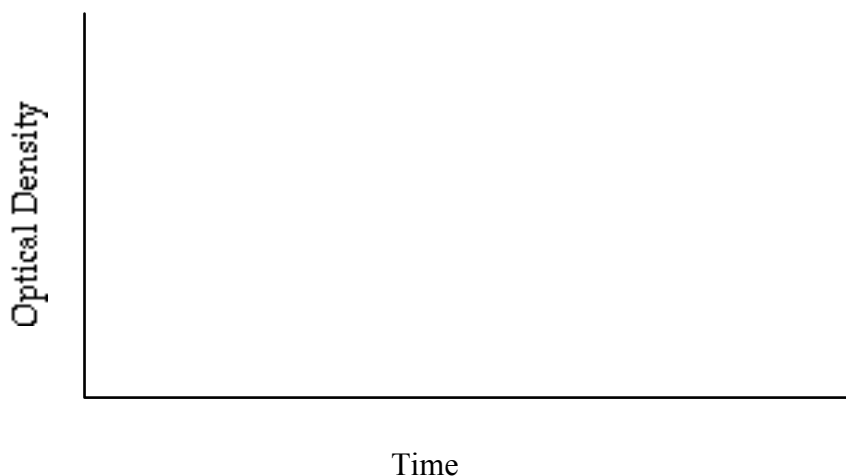
4. Label the part of the curve where the ***rate*** of the reaction is highest.

5. Label the part of your curve where the ***rate*** of the reaction is lowest.

6. The highest rate is _____ (estimate value, include units)

7. The lowest rate is _____ (estimate value, include units)

8. Now, re-analyze your data—calculating the ***rate*** of the reaction for each 1 minute interval.

Table of data for rate over time.
(Space should be provided in the student handout so that students can construct their own data table.)

9. Plot the ***rate*** of the reaction as a function of time, using the axes below:

Time (minutes)
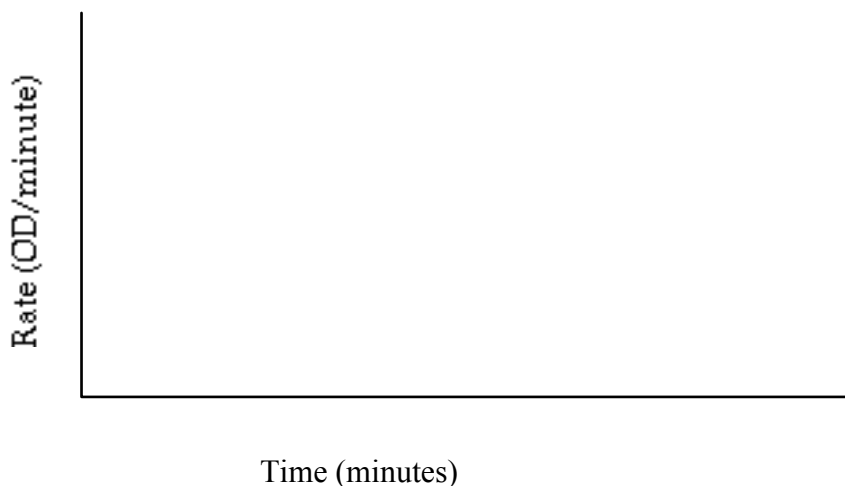
10.   What do you conclude from this experiment?   Do all time intervals provide equally reliable measurements of the reaction rate at 0.2 mM ONPG? Why or why not?

Activity 3: Effect of Substrate Concentration on the Reaction Rate

For this section, you will be measuring the activity of β-galactosidase at several different substrate concentrations. You need to decide how to make these measurements accurately.

1. What is a potential problem in determining rates at low substrate concentrations? (Answer: The substrate concentration changes rapidly.)

2. How will you get around this problem? (Answer: Measure the initial rate.)

You will measure the rates of the enzyme-catalyzed conversion of ONPG to ONP at four different concentrations of ONPG.

3. Write the protocol that you will follow to measure the reaction rates. Exactly what time intervals will you use to determine the rate of the reaction?
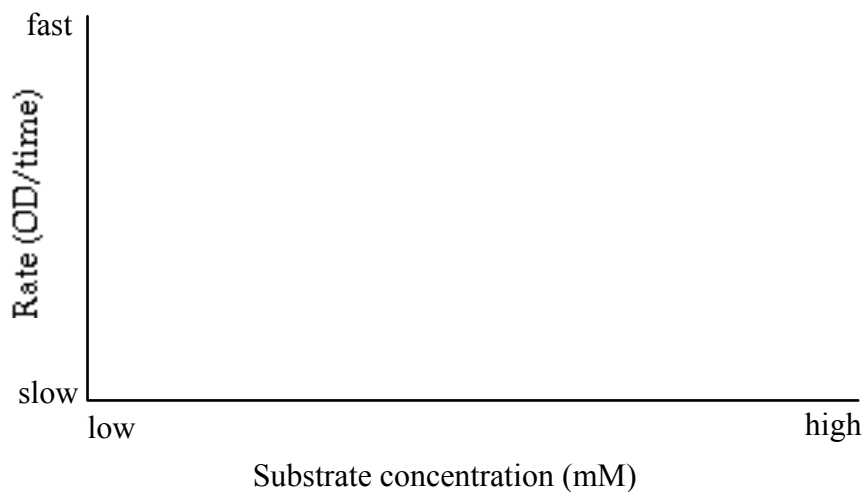
4. Set up 4 reaction mixtures as follows:

	Rxn 1	Rxn 2	Rxn 3	Rxn 4
2 mM ONPG	3.0 ml	.5 ml	--	--
0.2 mM ONPG	--	--	3.0 ml	1.5 ml
Buffer	--	1.5 ml	--	1.5 ml

What is the initial substrate concentration in each of the reaction mixtures?

|	Rxn 1	Rxn 2	Rxn 3	Rxn 4
initial [ONPG]:				

5. Before you actually do the experiment, predict what will happen by plotting points that you expect on the axes below.

6. Make a data table to record your results.
(Space should be provided in the student handout so that students can construct their own data table.)

7. Run the assays. Plot your results on the axes provided. Add the appropriate units to the axes.

Substrate concentration
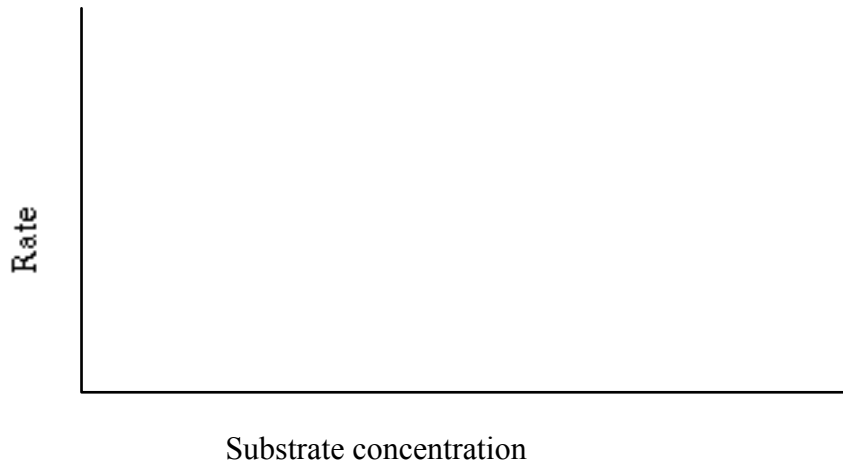
Questions to consider:

1. If you think that your data are unreliable, you may alter your protocol and make the measurements again.
2. Label the parts of your curve where the reaction rates are highest and lowest.
3. Is the reaction rate equally sensitive to all changes in substrate concentration? Why or why not?
4. Where the rate is highest, estimate the fraction of enzyme molecules that are in complexes with substrate?
5. Does this rate approximate V_{max}? Why or why not?
6. Assuming that the maximum rate you have measured does equal V_{max}, explain in words and symbols how you would calculate the turnover number for β-galactosidase.
7. Calculate the TON for β-galactosidase. There are some reference numbers (which you may or may not find useful) available below.

Molecular weight of β-galactosidase = 500,000

Number of enzyme molecules in 1 aliquot = 1.2×10^{12} (The enzyme aliquot size used in the reactions is chosen to give a $\Delta OD420 +\sim 0.5$/min when [S] =2 mM. We estimated the number of enzyme molecules in an aliquot sufficient to produce that rate based on published activities for β-galactosidase.)

Extinction coefficient of ONP: a 2.5×10^{-3} M solution of ONP has an optical density (OD) of 1.0

Design an Experiment to Measure K_m:

A characteristic, other than TON, that distinguishes one enzyme from another is affinity for substrate. This property is reflected by the value of the Michaelis constant (K_m) for the enzyme.

Your job is to design a protocol that will allow you to determine the K_m value for β-galactosidase. Don't be dismayed if your ideal experiment seems to involve a lot of work. You will let the computer be your lab tech, and make the actual measurements. You must be the Principal Investigator, though, and tell the computer what data you would like to have collected. The actual collection of these data, using the computer program "Enzyme Kinetics" will be part of your lab report.

In the space below, be specific about what you want to measure. Write down the substrate and enzyme concentrations you will require and how you will analyze the results.

Your protocol for K$_m$ measurement:

Measuring K$_m$:

Use the program *Reaction Rates* in the Mac Lab to complete the following questions. You will use your "protocol for K$_m$ measurement" and perform three experiments, collecting data using the program. You will determine the V$_{max}$ and K$_m$ parameters for the wild type enzyme first. After those have been determined, click the mutation box to change the enzyme from wild type to mutant. Perform the analyses on two mutants. Open the mutant box twice. Each time it is opened a new mutant will appear. Be sure to include the appropriate units when answering the questions below.

	Wild Type	Mutant 1	Mutant 2
Vmax	_____	_____	_____
Km	_____	_____	_____

Sketch a composite graph of the wild type and mutated enzymes. Label the graph clearly, including units.

Rate

Substrate concentration
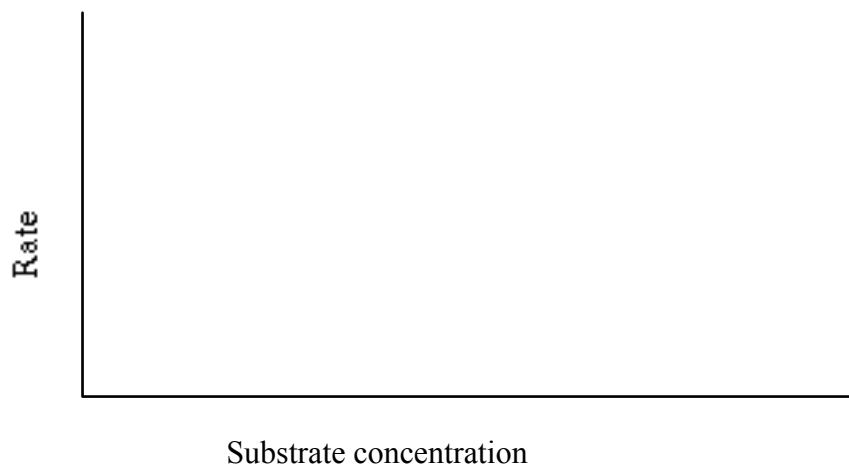
Discuss how the kinetics of the mutant enzymes are different from wildtype. What types of defects might these mutant enzymes have?

Enzyme Kinetics Lab Report

1. Is the reaction rate equally sensitive to all changes in substrate concentration? Why or why not?

2. Does the highest rate that you determined in lab approximate V_{max}? Why or why not?

3. Using the maximum rate that you determined for β-galactosidase using the computer program, calculate the TON for β-galactosidase. Show your work and units.

4. In your own words describe the protocol you used to determine the K_m measurement.

5. **Analysis of mutant enzymes**. On the axes below, sketch a graph of "Rate versus Substrate Concentration" for the wild type and the two mutant enzymes. Label the graph clearly, including units.

Rate

Substrate concentration
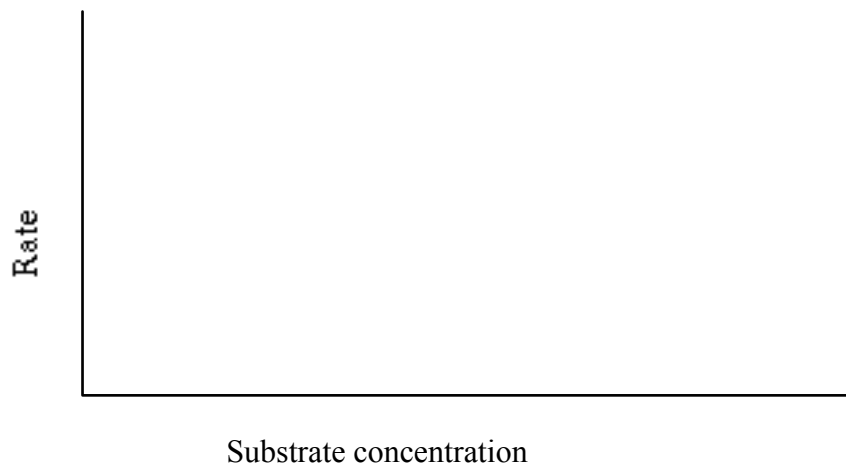
6. Discuss how the kinetics of the mutant enzymes are different from wild type. What does this tell you about the mutant enzymes? Do they bind substrate better, worse, or the same, relative to wild type? Do they catalyze the chemical reaction, faster, slower, or the same, relative to wild type?

Appendix A
Preparation of β-galactosidase

Grow a 500 ml overnight culture of a *lac$^+$I$^-$ E. coli* strain (CSH36) in LBH broth (or grow an inducible strain in the presence of the inducer IPTG). Record the OD_{500} of the culture. Harvest cells by centrifugation. Discard the supernatant solution. Wash the cells by resuspending them in Reaction Buffer. Centrifuge the cells again; discard the supernatant solution. Resuspend the cells in 25 ml Reaction Buffer. To rupture the cells, sonicate the cells in a 50 ml stainless steel beaker kept on ice. (We use a Bronwill Biosonik III sonicator with 3x1 minute pulses at 85% power with the large probe.) **Caution: Use ear protection when sonicating!** Recheck the OD_{500} of the sonicated cell suspension to determine the extent of cellular destruction. Centrifuge the suspension at 27,000 x g for 15 minutes to remove cellular debris. Transfer 1.0 ml aliquots of the supernate to microcentrifuge tubes for storage at -20°C. [In the assays, use an aliquot size (with a substrate concentration of 2 mM ONPG) to give a ΔOD_{420} = ~0.5/2 minutes.]

E. coli strain CSH36 is available from Carl Stiefbold upon request.