Teacher Preparation Notes for
Enzymes Help Us Digest Food¹

Students learn about enzyme function, enzyme specificity and the molecular basis of lactose intolerance through experiments with the enzyme lactase and analysis and discussion questions. Students engage in the scientific practices of designing and carrying out experiments and interpreting data. This activity can be used in an introductory unit on biological molecules and scientific method or later in the course during a discussion of enzymes.

Before beginning this activity, students should have a basic understanding of atoms, molecules, chemical formulae, and chemical reactions. If you want to assess your students’ background knowledge before beginning the activity, you can use the Enzyme Background Quiz on the last page of these Teacher Preparation Notes.

Your students should be able to complete the experiments in one 50-minute laboratory period, if you have your students complete the "Introduction to Sugars and Enzymes" as a pre-lab and page 6 of the Student Handout as a post-lab.

Learning Goals
Specific Learning Goals

• An enzyme is a molecule (usually a protein) that speeds up a chemical reaction. Without the enzyme, the reaction typically occurs extremely slowly or not at all.
• Digestive enzymes break down (digest) larger molecules in our food to smaller molecules that can be absorbed into our blood. For example, lactase breaks down the disaccharide lactose into the monosaccharides glucose and galactose.
• An enzyme acts on substrate(s) to produce product(s). The substrate binds to the active site of the enzyme.
• Each enzyme acts only on a specific substrate (or several chemically similar substrates) because only that specific substrate fits the enzyme’s active site. For example, lactase digests lactose but not sucrose. Because of enzyme specificity, many different enzymes are needed to digest food (e.g. lactase and sucrase).
• An enzyme molecule returns to its original state after acting on the substrate, so each enzyme molecule can be reused over and over again. For example, a single molecule of lactase can break down many many molecules of lactose.
• A person who produces very little lactase can only digest very small amounts of lactose at a time. Consumption of larger amounts of dairy products in a short time period can result in the symptoms of lactose intolerance. This example illustrates that proteins are not just abstract concepts in biology textbooks, but real parts of our bodies that have observable effects on our characteristics and health.

In accord with the Next Generation Science Standards²:

• This activity helps students to prepare for the Performance Expectation, HS-LS1-1, "Construct an explanation based on evidence for how… proteins… carry out the essential functions of life…."

¹ By Dr. Ingrid Waldron, Department of Biology, University of Pennsylvania, © 2016. These Teacher Preparation Notes and the related Student Handouts are available at http://serendipstudio.org/sci_edu/waldron/#enzymes.

² Quotations are from http://www.nextgenscience.org/sites/default/files/HS%20LS%20topics%20combined%206.13.13.pdf
• Students engage in recommended Scientific Practices, including "planning and carrying out investigations", "interpreting data", and "constructing explanations".
• Students learn the Disciplinary Core Idea (LS1.A), "... proteins... carry out most of the work of cells."
• This activity provides the opportunity to discuss the Crosscutting Concepts: "Structure and function" and "Cause and effect: Mechanism and explanation".

Equipment and supplies:
• Lactose solution: 5 g lactose per 100 mL water (20 mL for each group of 3-4 students); lactose is readily available from a variety of suppliers on the web*
• Sucrose solution 5 g sucrose per 100 mL water (10 mL per group)*
• Milk (20 mL per group) *
• Lactase solution: 1 g lactase per 50 mL water (3 mL per group) you can order lactase from Fisher (https://www.fishersci.com/shop/products/mp-biomedicals-lactase-aspergillus-oryzae/p-4605366 ). Store the lactase in the refrigerator. Make the solution on the day your students will use it. When you make the solution you will need to smoosh the lumps and stir a lot.3*
• Beakers†
• 25 mL graduated cylinders to measure lactose solution, sucrose solution, and milk +
• 1 mL transfer pipet per class for lactase solution*
• 15 milliliter test tubes* (3 per group of students in your largest class if they will be able to rinse these between uses; otherwise 5 per group)
• Test tube rack or something else to keep the test tubes upright (1 per group in your largest class)
• Visually readable glucose test strips (a.k.a. urinalysis glucose test strips; 5 per group – 3 for experiment 1 and 2 for experiment 2); glucose test strips are available from http://www.amazon.com/Betachek-Visual-Blood-Glucose-Strips/dp/B00HTVECL6 or http://www.carolina.com/catalog/detail.jsp?prodId=695960&s_cid=ppc_gl_products&gclid=CIWU8MHxr8YCFY09gQodSjUITA or search on the web for urinalysis glucose test strips; you will need to provide your students with instructions for using the particular type of glucose test strip you have ordered and you will want to have the color chart for reading the test strips available for your students
• Gloves (3 per group)
• Permanent marker and tape or labels for labeling test tubes (1 set per group)

* In order to conserve materials and thus reduce the cost of purchasing lactase, you can use smaller test tubes and correspondingly smaller amounts of each solution. If you do this, you will need to modify the instructions in the Student Handout.
+ If you keep the solutions at your desk, you will need four beakers (for each solution and the milk). You can have students measure the amounts they need with three graduated cylinders and one 1 mL transfer pipette or with four 1 mL transfer pipettes.

3 A cheaper alternative is to purchase lactase capsules or pills from a web supplier or your pharmacy. One teacher has reported success with Webber Naturals Lactase Enzymes Capsules Extra Strength. Another teacher has reported success with the following procedure for lactase pills. Use a mortar and pestle to crush four tablets. Suspend the crushed tablets in 50 mL of water and keep the mixture/solution on a stir plate during the lab. Have students use 3 mL (instead of 1 mL) of this solution. (12,000-18,000 lactose units is roughly equivalent to 1 g of lactase, but, if you use this approach, the experiment will require more lactase because of the poor solubility of the lactase pills.) If you try this, please let us know how it works for you (iwaldron@upenn.edu). Thank you.
Instructional Suggestions and Background Information
To maximize student participation and learning, I recommend that you have students work on groups of related questions individually or in pairs before having a class discussion of their answers.

In the Student Handout, numbers in bold indicate questions for the students to answer and ➢ indicates a step in the experimental procedure for the students to do.

If you use the Word version of the Student Handout to make changes for your students, please check the PDF version to make sure that the figures and formatting in the Word version are displaying correctly on your computer.

A key is available upon request to Ingrid Waldron (iwaldron@sas.upenn.edu). The following pages provide instructional suggestions and additional background information – some for inclusion in your class discussions and some to provide you with relevant background that may be useful for your understanding and/or for responding to student questions.

Introduction to Sugars and Enzymes
Glucose, galactose and fructose all have the chemical formula C₆H₁₂O₆, but the atoms are arranged differently so these three sugars are isomers. Galactose is a stereoisomer of glucose since the identical chemical groups are bonded to the same carbon atoms, but with one difference in orientation. Fructose is a structural isomer of glucose since it contains the same chemical groups, but some of these are bonded to different carbon atoms.

Sucrose is familiar to students as "sugar". Common sources of sucrose in our diet are sugar cane, sugar beets, and fruits. Fruits also contain the monosaccharides, glucose and fructose.

If your students are not entirely comfortable with molecular diagrams, you may want to insert the following question:

1b. Use the diagrams of the monosaccharides to add to the sucrose diagram all the carbon and hydrogen atoms that are not explicitly shown.

In discussing the definition of an enzyme, you may want to point out that we say that a chemical reaction requires an enzyme when the rate of reaction without an enzyme is so slow that no
meaningful amount of product molecules is produced in the short timeframe of cellular metabolism. You may find the following figure useful if you want to explain to your students that enzymes speed up biological processes by reducing the activation energy required to get to the transition state in chemical reactions. An enzyme can lower the required activation energy by stressing particular chemical bonds of a substrate or bringing two substrates together in the correct orientation to react with each other. Without an enzyme, the activation energy typically is so high that very few molecules have sufficient thermal energy to undergo the reaction. In contrast, with an enzyme, many more molecules will have sufficient thermal energy to meet the lower activation energy requirement and the reaction will proceed at a biologically useful rate.

![Figure](image.png)

The paragraph after question 4 on page 2 of the Student Handout explains how an enzyme molecule is active repeatedly, so a single enzyme molecule catalyzes the reaction for many substrate molecules. To reinforce this concept, we recommend that you show the animation provided at [http://www.biotopics.co.uk/other/anenz.html](http://www.biotopics.co.uk/other/anenz.html).

**Experiment 1 – Is an enzyme needed to digest lactose?**

**Can the same enzyme digest lactose and sucrose?**

The Student Handout asks whether an **enzyme is needed** to digest the sugar lactose. The experiment that the students carry out shows that without the enzyme lactase the digestion of lactose is so exceedingly slow that no detectable glucose is produced in three minutes. In general, when we say that an enzyme is needed for a chemical reaction, we mean that without the enzyme the reaction does not occur at any biologically meaningful rate, whereas with the enzyme the reaction occurs at a much more rapid, biologically useful rate. (Of course, the student experiment does not demonstrate that no other catalyst would work to speed up the digestion of lactose.)
Questions 5 and 6 in the Student Handout should help your students to understand why testing for glucose is a reasonable method for evaluating whether the enzyme lactase digests the sugars lactose and sucrose.

Glucose test strips are used by people with diabetes to test for glucose in their urine or blood; when glucose is present in the urine this indicates that blood glucose levels are too high, which can be harmful to health. Note that glucose test strips do not react with glucose when the glucose is part of the disaccharides lactose or sucrose. The glucose test strip only reacts with the monosaccharide glucose.

While your students are waiting for the lactase to act, you may want to introduce the concept that enzymes play an important role in the synthesis of molecules, as well as digestion. For this purpose, you could use this question.

Some enzymes play a role in the synthesis of molecules. For example, in plant cells an enzyme is needed to make sucrose from glucose and fructose.

7a. Which part of this figure shows the synthesis of sucrose in plant cells?

7b. Which part of this figure shows the digestion of sucrose in plant cells or the human digestive system?

To calculate the number of lactose molecules per lactase molecule in question 10 of the Student Handout, we used the amount of lactose and lactase solutions added to the test tube, the concentrations of lactose and lactase in the solutions, and the molecular weight of lactose (342) and lactase (approximately 150,000-300,000). If your students have trouble answering question 10, you may want to re-show the animation at http://www.biotopics.co.uk/other/anenz.html.
The figure in question 14b can be described as illustrating the lock and key model of enzyme specificity. However, it should be noted that enzymes are not rigid and static like a lock on a door. Instead, enzymes are flexible and dynamic. For many enzymes, the active site changes shape when the substrate binds to the active site. This change in shape is called an induced fit.

If you want to show your students an animation of enzyme specificity, you might want to use [http://www.phschool.com/science/biology_place/labbench/lab2/binding.html](http://www.phschool.com/science/biology_place/labbench/lab2/binding.html).

Some enzymes (e.g. lactase) act on only a single substrate. Other enzymes act on a specific type of chemical bond flanked by specific chemical structures (e.g. pepsin; [http://osp.mans.edu.eg/medbiochem_mi/Cources/Biochemistry/1st_year_medicine/Enzymes/files/Lecture_02.pdf](http://osp.mans.edu.eg/medbiochem_mi/Cources/Biochemistry/1st_year_medicine/Enzymes/files/Lecture_02.pdf)).

You may want to ask your students to suggest improvements in the design of Experiment 1. For example, it might be useful to add 1 mL of water to Tube 1 for greater comparability to Tube 2.

**Experiment 2 –Does the enzyme lactase digest the sugar in milk?**

Answering question 17 should help students understand why they need to test for glucose both in 10 mL of milk without enzyme and in 10 mL of milk with 1 mL of lactase solution.

Students can use the procedure provided for Experiment 1 to guide them in designing the procedure for Experiment 2.
The Digestive System

The first paragraph on page 6 of the Student Handout mentions the extreme differences in size between molecules and a human body. You may want to link these extreme size differences to the:

- very large numbers of molecules in each cell (roughly 20 billion protein molecules per human cell and trillions of water molecules)
- many many cells in each human body (roughly 40 trillion cells).

If you want to reinforce student understanding of size differences and different levels of organization in biology, you may want to have your students do the Card Sort Activity – From Coffee to Carbon (available at http://teach.genetics.utah.edu/content/cells/CoffeetoCarbon.pdf). This activity has students sort the cards (each with a molecule, organelle or cell) according to size. To use this activity to reinforce student understanding of different levels of organization, I recommend that you begin by having your students sort the cards into four categories: molecules, organelles, cells, and other. After you have discussed this initial card sort, then have your students organize the cards from smallest to largest. (Depending on your students, you may want to omit some cards such as adenine, influenza virus, baker's yeast.) After students have completed the card sort by size, discuss the results and show the animation which illustrates the relative sizes (available at http://learn.genetics.utah.edu/content/begin/cells/scale/).
If you would like to expand the discussion of levels of organization in biological organisms and relative sizes at these different levels of organization, the following resources and figure may be helpful. Brief introductions to levels of organization are available at:

- http://wps.pearsoncustom.com/wps/media/objects/3014/3087289/Web_Tutorials/01_A02.swf

Lactose Intolerance
Lactose intolerance is a result of decreased production of lactase as a child grows toward adulthood. (Lactose intolerance in infancy is very rare – less than 1 in 60,000 newborns.) The decrease in production of lactase as a person gets older is called lactase nonpersistence.
The alleles for the gene for lactase differ in the nucleotide sequence in the regulatory DNA; this difference influences the age trend in the rate of transcription of the coding DNA for the protein, lactase, and thus influences the rate of production of lactase.

- **Lactase persistence alleles** result in substantial production of lactase throughout life.
- **The lactase nonpersistence allele** results in substantial production of lactase by infants, but very low levels of lactase in adults which can result in lactose intolerance.

For virtually all infants and for adults with **lactase persistence**:  
- in the small intestine:
  
  \[
  \text{lactase} 
  \xrightarrow{\text{glucose + galactose}} 
  \text{lactose} 
  \]

For the roughly two-thirds of adults worldwide who have **lactase nonpersistence**:  
- in the small intestine, most lactose is not digested due to low levels of lactase  
- so, in the colon of the large intestine, lactose is fermented by anaerobic bacteria:

  \[
  \text{fermentation} 
  \xrightarrow{\text{short-chain fatty acids + gases (e.g. CO}_2\text{)}} 
  \text{lactose} 
  \xrightarrow{\text{osmotic influx of water}} 
  \xrightarrow{\text{diarrhea}} 
  \xrightarrow{\text{flatulence and discomfort}} 
  \xrightarrow{\text{lactose intolerance}} 
  \]

Dairy products are an important source of calcium, as well as protein and some vitamins. People with lactose intolerance can continue to consume dairy products but **minimize symptoms** by:

- using lactase supplements
- consuming dairy products with reduced lactose due to treatment with lactase (e.g. lactose-free milk) or fermentation by bacteria (e.g. traditionally made cheese or yogurt)
- consuming small amounts of dairy products at multiple times during the day
- adaptation of bacteria in the colon by gradually increasing regular consumption of modest amounts of dairy products

The lactase persistence allele provides an example of **natural selection in humans**:

- Lactase nonpersistence alleles are nearly universal in mammals and were nearly universal in early humans.
- When some groups of humans began raising dairy animals, natural selection favored lactase persistence alleles which became more common in these groups.
- Different lactase persistence alleles are observed in European and African herding groups. This illustrates how similar characteristics can evolve independently in different populations (convergent evolution).

Lactose intolerance is different from a **milk allergy** which happens when the body's immune system reacts to proteins in milk. (A good summary of milk allergy is available at [http://www.mayoclinic.org/diseases-conditions/milk-allergy/basics/definition/con-20032147](http://www.mayoclinic.org/diseases-conditions/milk-allergy/basics/definition/con-20032147).)

**Related Activities**

Most of these activities are aligned with the Next Generation Science Standards⁴, as indicated by NGSS at the end of the description.

"Enzyme Investigation" is presented on pages 12-13 of these Teacher Preparation Notes. This inquiry activity can be used as an extension activity.

Students can expand their understanding of enzymes in the bioengineering design challenge included in "Alcoholic Fermentation in Yeast – A Bioengineering Design Challenge", available at http://serendipstudio.org/sci_edu/waldron/#fermentation. This multi-part minds-on, hands-on activity helps students to understand both alcoholic fermentation and the engineering design process. In the first two parts of this activity, students learn about alcoholic fermentation and test for alcoholic fermentation by assessing CO₂ production by live yeast cells in sugar water vs. two controls. The third part of this activity presents the bioengineering design challenge where students work to find the optimum sucrose concentration and temperature to maximize rapid CO₂ production. Structured questions guide the students through the basic engineering steps of applying the relevant scientific background to the design problem, developing and systematically testing proposed design solutions, and then using initial results to develop and test improved design solutions. (NGSS)

Additional activities to help students understand the functions of proteins are presented in "Understanding the Functions of Proteins and DNA", available at http://serendipstudio.org/exchange/bioactivities. This overview provides a sequence of learning activities to help students understand that proteins and DNA are not just abstract concepts in biology textbooks, but rather crucial components of our bodies that affect functions and characteristics that students are familiar with. Students learn about how proteins contribute to the digestion of food and to characteristics such as albinism, sickle cell anemia and hemophilia. Then, students learn about the relationship between the genetic information in DNA and the different versions of these proteins. The discussion, web-based, and hands-on learning activities presented are appropriate for an introductory unit on biological molecules or as an introduction to a unit on molecular biology. (NGSS)

A hands-on activity, "A Scientific Investigation – What types of food contains starch and protein?" is available at http://serendipstudio.org/sci_edu/waldron/#starch. In this activity, students first learn about the structure and functions of starch and protein and the role of glucose in the synthesis of starch and amino acids. Then, students learn about scientific investigation by carrying out key components of the scientific method, including developing experimental methods, generating hypotheses, designing and carrying out experiments to test these hypotheses and, if appropriate, using experimental results to revise the hypotheses. Students design and carry

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out two experiments which test whether starch and protein are found in some or all foods derived from animals or plants or both. (NGSS)

An analysis and discussion activity that will help students to understand levels of organization in biology and the function of the digestive system is "Structure and Function of Cells, Organs and Organ Systems", available at http://serendipstudio.org/exchange/bioactivities/SFCellOrgan. In this activity, students learn how the structure of cells, organs and organ systems is related to their functions. Students analyze multiple examples of the relationships between structure and function in diverse eukaryotic cells and in the digestive system. Students also learn that cells are dynamic structures with constant activity, and they learn how body systems interact to accomplish important functions. (NGSS)
Your task is to identify and name the unknown enzyme that is in the numbered bottle. Use your previous knowledge to plan and carry out an investigation to identify your enzyme. Then, complete a standard lab report with your findings.

You have previously observed that glucose test strips change color when glucose (as a monosaccharide) is present. Any color change indicates at least trace amounts of glucose and bigger color changes indicate more glucose.

Here are the possible enzymes.

**Enzyme Sucrase, which catalyzes the reaction:**

Sucrose → Glucose + Fructose

**Enzyme Lactase, which catalyzes the reaction:**

Lactose → Glucose + Galactose

There are also bottles with no enzymes, so you have three possibilities.

Here is a **partial** list of materials to help you get started:

- One Numbered Bottle containing sucrase or lactase or no enzyme.
- Test Tubes
- Test Tube Rack
- Glucose test strips
- YOU COMPLETE THE LIST

Your lab report will be in the standard format. Please make sure to identify the unknown and explain why you came to that conclusion. Also, include possible sources of error.

Have fun.
This lab is intended as an extension activity for the Enzymes Help Us Digest Food activity. It may be used independently, depending on student readiness and their understanding of enzyme specificity.

Refer to the Teacher Preparation Notes for Enzymes Help Us Digest Food for solution concentrations and amounts.

Prepare one bottle for each student group:
- one third of the bottles with lactase enzyme (labeled Bottle #1)
- one third of the bottles with sucrase enzyme (available as invertase from Carolina Biological Supply) (labeled Bottle #2)
- one third of the bottles with water (Add some baking soda or other solute to make it look like Bottles #1 and #2.) (labeled Bottle #3)

The students should select the sucrose solution and either the lactose solution or milk to test their enzyme. They may also use a glucose solution and/or water as controls.
Enzyme Background Quiz

Name ________________________________

1a. Draw a circle around each molecule in the figure below.

1b. Which symbols in this figure represent atoms?

1c. Draw a rectangle around one of the symbols that represents a bond that connects two atoms.

2a. Circle the products produced by this chemical reaction.

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 6 \text{O}_2 \rightarrow 6 \text{CO}_2 + 6 \text{H}_2\text{O}
\]

2b. Will this reaction result in an increase ____ or decrease ____ in the amount of C\(_6\)H\(_{12}\)O\(_6\)?

2c. If the rate of reaction increased, then
   a. less H\(_2\)O would be produced in a minute.
   b. more C\(_6\)H\(_{12}\)O\(_6\) would be produced in a minute.
   c. more CO\(_2\) would be produced in a minute.
   d. more O\(_2\) would be produced in a minute.

3. What is an enzyme?