Know your footprint

Teacher's Guide

I. Objectives:

1. To engage AP Biology high school students in STEM by teaching students the biology and chemistry behind our triclosan biosensor
2. To increase awareness and accountability around chemical use and to encourage more prudent usage of chemicals.

II. Relevant Standards: NGSS, AP

Next Generation Science Standards (NGSS)

1. LS: Life Sciences – Earth and Human Activity

HS-LS2-7: **Design, evaluate, and refine a solution for reducing the impacts of human activities on the environment and biodiversity.** *[Clarification Statement: Examples of human activities can include urbanization, building dams, and dissemination of invasive species.]*

In our lesson, we explain how the overarching problem is that we use chemicals without thinking about their consequences. We want to communicate to the students that there are multiple ways to put pressure on this system to reduce our impact including source control at the industry/consumer level. But our project poses just one solution- there are many other pressure points for change, and there is a need for all of us to work together to tackle this complex problem.

2. LS: Life Sciences – From Molecules to Organisms: Structures and Processes

HS-LS1-1: **Construct an explanation based on evidence for how the structure of DNA determines the structure of proteins which carry out the essential functions of life through systems of specialized cells.**

[Assessment Boundary: Assessment does not include identification of specific cell or tissue types, whole body systems, specific protein structures and functions, or the biochemistry of protein synthesis.]

In our lesson, discuss the production and use of a recombinant enzyme. This gives the instructor the opportunity to discuss the relationship between gene structure and protein structure and function.

Advanced Placement (AP) Learning Objectives

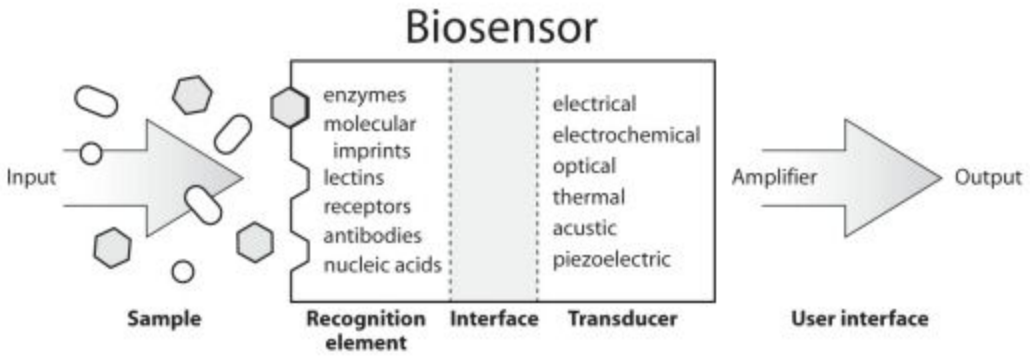
AP Biology Curriculum

Essential Knowledge	4.B.1: Interactions between molecules affect their structure and function	Enzyme inhibition
Science Practice	5.1: The student can analyze data to identify patterns or relationships	Determining rate of reaction from graphing NADH (absorption) over time
Learning Objective	4.17: The student is able to analyze data to identify how molecular interactions affect structure and function	Triclosan, a competitive inhibitor, slows the rate of reaction. Can generate a standard curve to correlate percent inhibition with levels of triclosan in a sample.

The college board recommends 2 labs/big idea. This activity could fulfill one of the lab requirements for "big idea 4," that biological systems interact, and these systems and their interactions possess complex properties.

III. Explanation of our biosensor:*:*

In general, a biosensor consists of a recognition element that is specific to a target analyte and a transducer element that generates a quantifiable signal proportional to the biorecognition event (the recognition element binding to the analyte). Biosensors differ in the type of recognition element and transducer element used.



[1]

For our triclosan biosensor we used:

- an **enzyme** as the **recognition element** and
- an **optical measurement device** (spectrophotometer) as the **transducer element**

Let's start with the recognition element (enzyme)

Triclosan is an effective antimicrobial agent because it prevents bacteria from synthesizing fatty acids, important components of their cell membrane.

The synthesis of fatty acids is a multistep process, but lets just focus on the last step, because that's the step that triclosan inhibits. In this step, the fabi enzyme takes an electron from its cofactor, NADH, and uses this energy to break a double bond on its substrate.

This reaction proceeds at a characteristic rate.

Triclosan is a competitive inhibitor. When it binds to the fabi enzyme, it induces a conformational change that prevents fabi from taking an electron from NADH, thus impeding the rate of reaction.

We are using the fabi enzyme in our biosensor and measuring rates of inhibition to infer levels of triclosan.

We can characterize each reaction by measuring concentrations of NADH periodically over time (in what is called a kinetic read). The slope of the line represents the rate of reaction, with steeper slopes signifying faster rates.

Without triclosan, the reaction proceeds at the fastest rate; thus generating the steepest slope. With increased levels of triclosan, the reaction rate decreases; yielding shallower slopes.

We can then calculate the percent inhibition associated with a certain amount of triclosan by taking the change in slope over the slope of the reaction that takes place without triclosan (what is called the control).

Next, we can plot percent inhibition against concentrations of triclosan.

The idea is for a solution of unknown levels of triclosan, we can measure the concentration of NADH over time, compare these values to a "control", determine % inhibition, and infer levels of triclosan.

Now that we've established that we are determining triclosan by measuring NADH, we need to figure out how we're going to measure NADH (transducer element)

Our device **optically measures how much NADH there is.** We drop our solution into a cuvette, shine some light in, and **measure how much light is absorbed by taking the log of incident light over transmitted light.**

There is a difference in the amount of light that gets transmitted because some of this energy is absorbed by the NADH to bump the electrons up to higher energy levels. The more NADH molecules, the more energy that is absorbed; thus absorption value is proportional to concentration.

Lets go back to our sample solution of NADH, Enzyme, Substrate, and triclosan. If we allow the solution to incubate over time, the reaction will proceed, and the NADH in solution changes as a result of the enzyme's action.

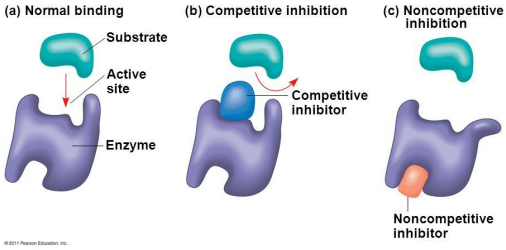
We can take readings of its absorbance over time.. We can then determine the slope, and because different levels of triclosan inhibit at different rates, we can then calculate the

percent inhibition against a control and use our standard curve to determine levels of triclosan.

IV. Vocabulary:

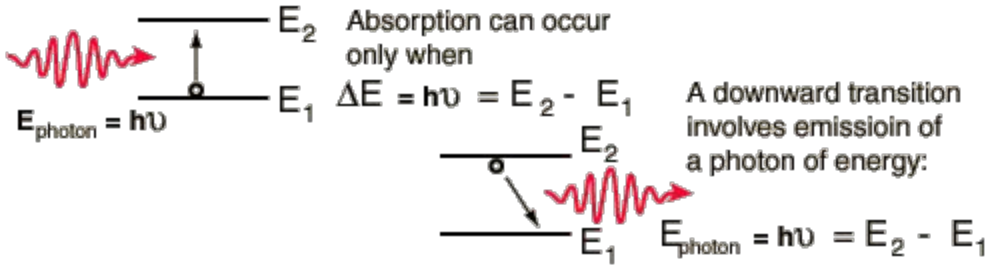
The recognition element of our biosensor is an enzyme. Relevant topics include:

- **Enzyme:** a molecule that catalyzes a reaction by lowering the activation energy required for a reaction, has an active site that is specific to its substrate and cofactor
- **Substrate:** the substance that an enzyme acts on
- **Cofactor:** non-protein chemical compound that is required for the protein's biological activity
- **Competitive Inhibition:** when a molecule prevents an enzyme from binding to its substrate/cofactor, thereby inhibiting the rate of reaction



The transducer element of our biosensor relies on an optical method of detection. Relevant topics includes:

- The concept that energy is quantized
- Photon equation relating energy with wavelength
- How the wavelength of light that is absorbed depends on electron orbitals, and is thus specific to molecules [2]



- Beers-Law relating absorption with concentration *(please refer to video within Teachers Guide video)* [3]
- Standard curve

To expand further: optical methods of detection rely on identifying chemicals through energy emission. Recall, electrons exist in energy levels (orbitals) within an atom. Atomic orbitals are quantized, meaning they exist as defined values instead of being continuous. Electrons may move between orbitals, but in doing so they must absorb or emit energy equal to the energy difference between their atom's specific quantized orbital energy levels. In optical spectroscopy, energy is absorbed to move an electron to a higher energy level. Because each element has a unique number of electrons, an atom will

absorb energy in a pattern unique to its elemental identity and thus will absorb photons in a correspondingly unique pattern. The type of atoms present in a sample, or the amount of atoms present in a sample can be deduced from measuring these changes in light wavelength and light intensity.

Teachers may also wish to spend time explaining how to take reliable measurements: the importance of taking multiple samples, of “controls”

V. References:

[1] J.P.Chambers, B.P. Arulanandam, L.L. Matta, A. Weis, J.J. Valdes, Biosensor recognition elements. Current Issues of Molecular Biology 10 (2
008), 1-12.

[2] Quantum Processes." *Quantum Processes*. Web. 18 Sept. 2015

[3] "How a Simple UV-visible Spectrophotometer Works." *YouTube*. YouTube, n.d. Web. 18 Sept. 2015.

Know your footprint

Lesson plan developed for use in conjunction with the 2015 UC Davis iGEM team's triclosan biosensor

Lesson materials (designed initially for guest instructor)

Preparation

4-5 days in advance, assign students to collect water samples from their local environment (tap water, creek water, etc.) - water samples are also provided if this is not possible

I. Classroom Set-up: students in groups of 4-5

II. Materials (per group of 4-5 students):

- (1) IO Rodeo Colorimeter
- (1) "Know Your Footprint" kit containing:
  - 1 tube labeled "master mix"
  - 1 tube labeled "reaction buffer"
  - 1 tube labeled "water sample"
  - 1 tube labeled "buffer control"
  - 1 tube labeled "triclosan control"
  - 1 tube labeled "enzyme"
  - 1 strip of parafilm
  - 4-5 cuvettes (micro size)

- (2) Micropipettes
  - (1) capable of measuring volumes up to 1000 µl
  - (1) capable of measuring a volume 10 µl
  - pipette tips

III. Delivery of Instruction

To be used with PowerPoint Slides (Provided)

1. Introduction: What are chemicals used for? (5 minutes)

Ask students to name some chemicals and their uses

Highlight that chemicals can often improve our lives, but that there are risks associated with their use.

So how do we manage these risks? We monitor chemical use.

2. How do we monitor/test for chemicals? (5 minutes)

As a primer get students to thinking about the various tests run in lab that differentiate based on size, mass, charge, etc.

- gel electrophoresis
- chromatography
- centrifuge

Another categorization scheme students are likely familiar with is the periodic table, which organizes chemicals based on their valence electrons. Talk about how periodic table groups chemicals based on similar valence electrons because electron configuration determines affinity for bonding, absorption of wavelength that gets absorbed, etc.

Main point from above is that chemicals have "fingerprints" that scientists can use to identify them.

Slide 1-2: Scientists use biosensors, which consist of a receptor element (that is specific to a target analyte) and a transducer element that converts the recognition event (receptor element interacting with target analyte) into a quantifiable signal.

3. Transition from chemicals in general to a specific example: triclosan

We monitor some chemicals but not all**.** One of the chemicals that we don't monitor regularly is triclosan. Triclosan is an antimicrobial agent found in a range of consumer and industrial product. It has undergone scrutiny in recent years because of its negative impacts on the environment and human healthy.

- Suggested question -

> Q: Knowing where triclosan is found have students hypothesize about levels of triclosan in densely populated areas vs. more rural areas

Today we are going to use a triclosan biosensor to determine the amount of triclosan in the water sample you brought in!

Slide 3-4: Biosensors differ in the type of recognition element or transducer element they use.

Slide 5-6: The triclosan biosensor the 2015 UC Davis iGEM team developed uses an enzyme as the recognition element and an optical device (spectrophotometer) as the transducer element

Slide 7: Let's start with the recognition element

Slide 8: Triclosan is an effective antimicrobial agent because it prevents bacteria from synthesizing fatty acids, important components of their cell membrane.

Slide 9: The synthesis of fatty acids is a multistep process, but let's just focus on the last step, because that's the step that triclosan inhibits. In this step, the fabi enzyme takes an electron from its cofactor, NADH, and uses this energy to break a double bond on its substrate.

Slide 10: This reaction proceeds at a characteristic rate.

Slide 11: Triclosan is a competitive inhibitor. When it binds to the FabI enzyme, it induces a conformational change that prevents fabi from taking an electron from NADH, thus impeding the rate of reaction.

- Suggested question -

Slide 13 -14

Q: Given two solutions (one with triclosan and one without) predict and compare the final concentrations of NADH.

A: In the solution on the left, the reaction proceeds at the maximum rate, resulting in the lowest levels of final NADH. In the solution on the right, however, triclosan inhibits the rate of reaction so less NADH is used up by the enzyme.

If we keep starting levels of enzyme, NADH, and experimental conditions constant, the difference in final NADH levels is due to triclosan.

The UC Davis iGEM team's triclosan biosensor uses the fabi enzyme as the recognition element and infers levels of triclosan by measuring the rate of NADH oxidation (a proxy for the rate of reaction)

- Suggested question -

Slide 16- 17:
Q: Predict what happens with different levels of triclosan

A: The more triclosan (inhibitor) the slower the rate of reaction

Additional reference: http://www.kscience.co.uk/animations/model.swf (animation to show how different levels of enzyme affect rate of reaction)

Slide 19 - 20: Summary slide with main points

- there is a difference in final concentrations of NADH due to triclosan's inhibition of the enzyme
- the more triclosan (inhibitor) the slower the rate of reaction

Now that we've established that we can determine triclosan by measuring NADH, we need to figure out how we're going to measure NADH (transducer element)

4. Explaining how to take measurements with the spectrophotometer
Slide 23: Explanation of how device works (additional reference on **slide 37**)

Slide 24: There is a difference in the amount of light that gets transmitted because some of this energy is absorbed by the NADH to bump the electrons up to higher energy levels.

- Suggested questions -
Slide 26 - 28:
Q: Given these two solutions (the top with more molecules of NADH) which solution will let more light through?

A: A solution with more NADH will let less light through because some of this energy is absorbed by the NADH.

Slide 30 - 31:

Q: Given these three solutions with varying levels of NADH predict absorption values

A: The more molecules, the more light that gets absorbed, the higher the absorbance

5. Synthesis and Application

Back to our sample solution of NADH, Enzyme, Substrate, and triclosan. If we allow the solution to incubate over time, the reaction will proceed, and the NADH in solution changes as a result of the enzyme's action.

- Suggested questions -

Slide 33 - 35: How might we observe this reaction and represent the data graphically?

We can characterize each reaction by measuring concentrations of NADH periodically over time (in what is called a kinetic read). The slope of the line represents the rate of reaction, with steeper slopes signifying faster rates.

Without triclosan, the reaction proceeds at the fastest rate; thus generating the steepest slope. With increased levels of triclosan, the reaction rate decreases; yielding shallower slopes.

We can then calculate the percent inhibition associated with a certain amount of triclosan by taking the change in slope over the slope of the reaction that takes place without triclosan (what is called the control).

Next, we can plot percent inhibition against concentrations of triclosan.

The idea is for a solution of unknown levels of triclosan, we can measure the concentration of NADH over time, compare these values to a "control", determine % inhibition, and infer levels of triclosan.

Q: What is the significance of the slope? (Rate of reaction) What does a steeper slope mean? (Faster rate of reaction)

Here students may draw graphs of absorbance over time and show how different levels of triclosan will affect the slope.

6. Demonstrating how to use the device

Here teachers can also mention the importance of accuracy, precision, running triplicates in taking measurements

Q: What is the importance of running samples in triplicate?

Q: Might there be interfering components in the water sample? How to account for this?

7. Activity

Have students count off "1, 2, 3, 4" and break up into groups of 4-5.

After a demonstration of how to use the device, students will use the device to measure levels of triclosan in their water samples and:

1. Record absorption values.
2. Graph absorption vs. time
3. Calculate the slope (rate of product formation),
4. Calculate the % inhibition against a control,
5. Use a standard curve to determine levels of triclosan.

8. Post Activity Discussion

- Ask students how they think triclosan ends up in these bodies of water (answer: mainly through consumer products)
- Highlight the fact that triclosan doesn't even need to be in these consumer products - it provides little added benefit, yet incurs substantial harm to aquatic organism health
- Inform students that there are resources to find products that do not have triclosan (Skin Deep, Good Guide), and highlight that it's not particularly hard to find these products, students just need to be aware and actively avoid - not contribute to its wasteful overuse.
- Next, emphasize how today we just measured one of these chemicals. But the fact is we release many, many more chemicals into our surroundings, and we ought to be more aware of our chemical footprint!

Know your footprint

Lesson plan developed for use in conjunction with the 2015 UC Davis iGEM team's triclosan biosensor

Student Materials

Materials

Make sure that you have the following at your workstation

- (1) IO Rodeo Colorimeter
- (1) "Know Your Footprint" kit containing:
  - 1 tube labeled "master mix"
  - 1 tube labeled "water sample"
  - 1 tube labeled "reaction buffer"
  - 1 tube labeled "triclosan dilute stock"
  - 1 tube labeled "triclosan concentrated stock"
  - 1 tube labeled "enzyme"
  - 1 strip of parafilm
  - 4-5 cuvettes (micro size)

- (2) Micropipettes
  - (1) capable of measuring volumes up to 1000 µl
  - (1) capable of measuring a volume 10 µl
  - pipette tips
  - Sharpie pen
  - timer (cell phone)

Measurement Protocol

1) Label 5 of your cuvettes as follows:

    a) Sample
    b) Negative control
    c) Positive control 1
    d) Positive control 2
    e) Blank

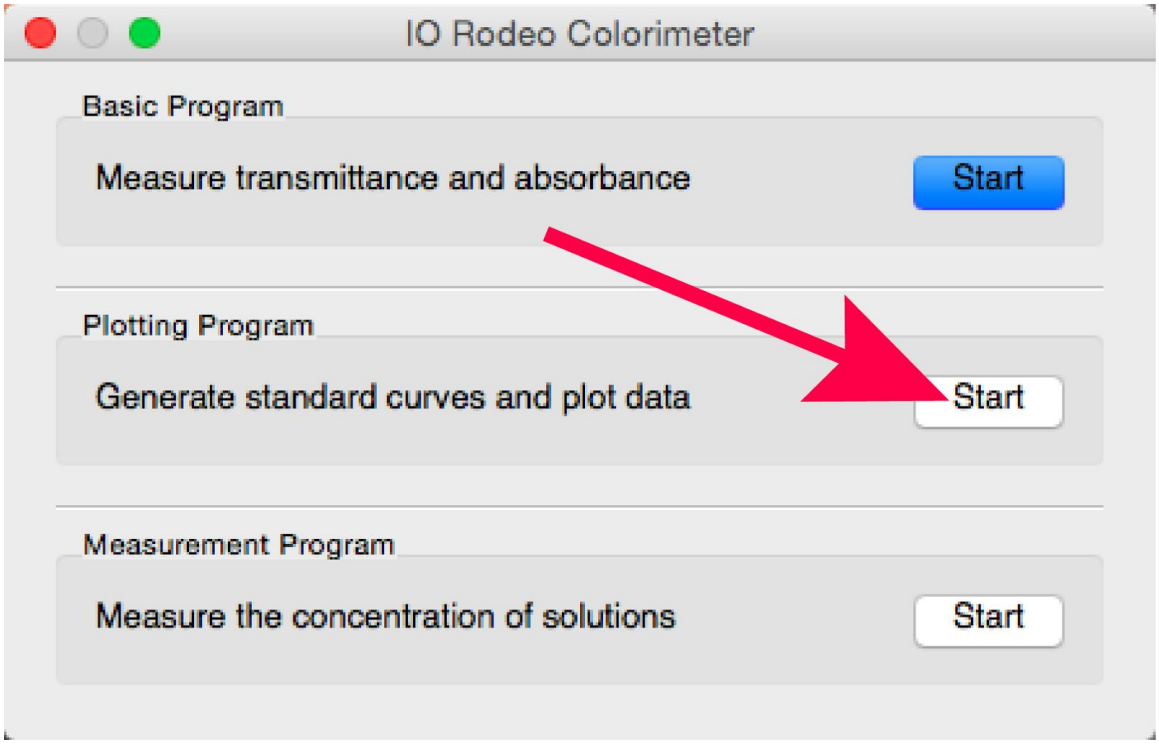
2) Mix the following in each cuvette

Cuvette a: Sample	**Cuvette b: Neg. control**	**Cuvette c: Pos. control 1**	**Cuvette d: Pos. control 2**	**Cuvette e: Blank**
770 µl wastewater 10 µl tap water 210 ul master mix	780 µl reaction buffer 0 µl triclosan 210 ul master mix	770 µl wastewater 10 µl dilute triclosan stock 210 ul master mix	770 µl wastewater 10 µl concentrated triclosan stock 210 ul master mix	1000 µl reaction buffer

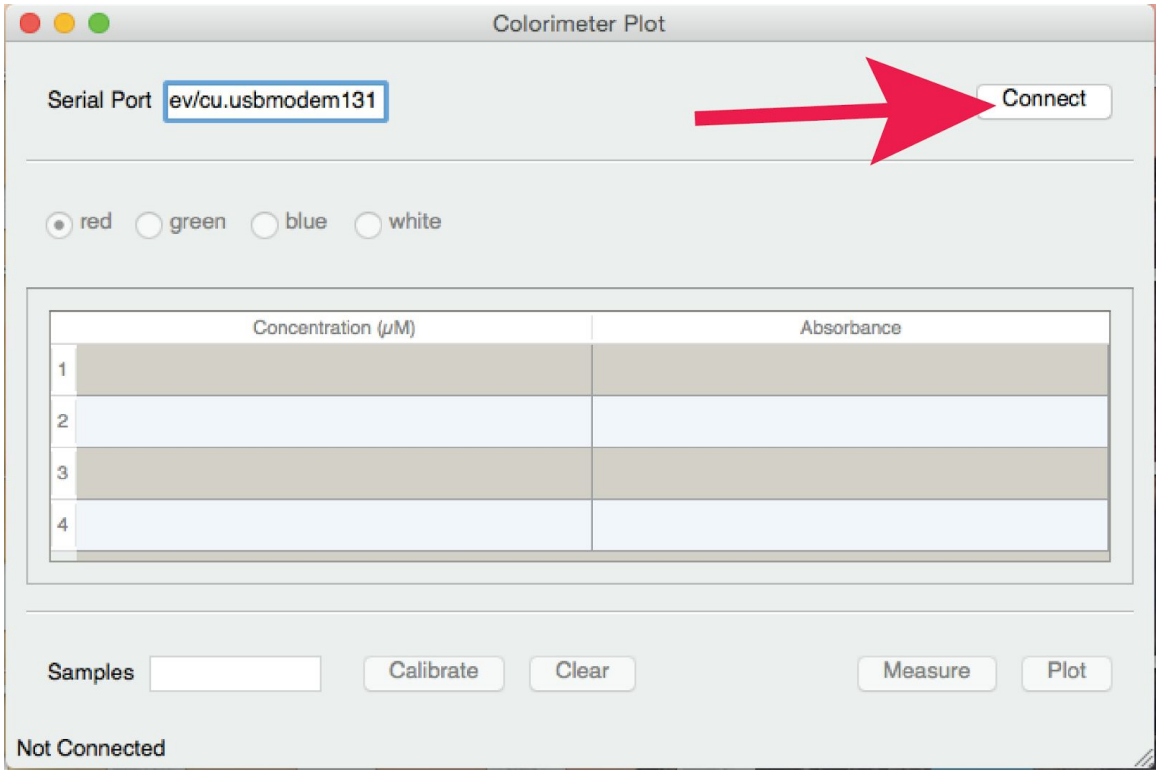
3) Mix each sample by placing the parafilm strip on top of the cuvette and inverting it 4-5 times

4) Open the colorimeter-4 application on your computer and connect the spectrophotometer through the USB dongle.

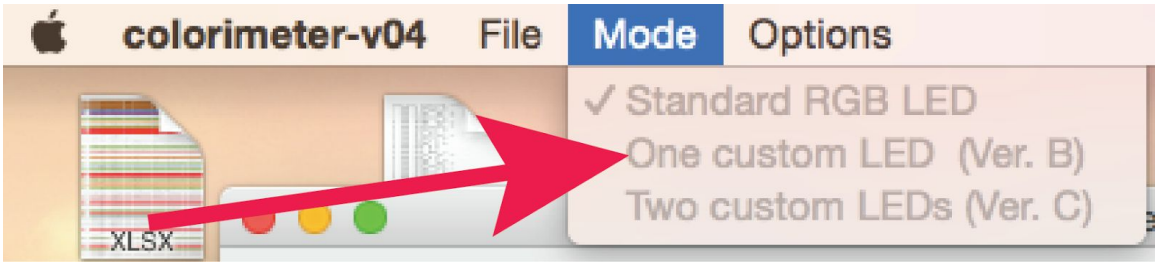
5) Select the second "Start" button to "Generate standard curves and plot data"



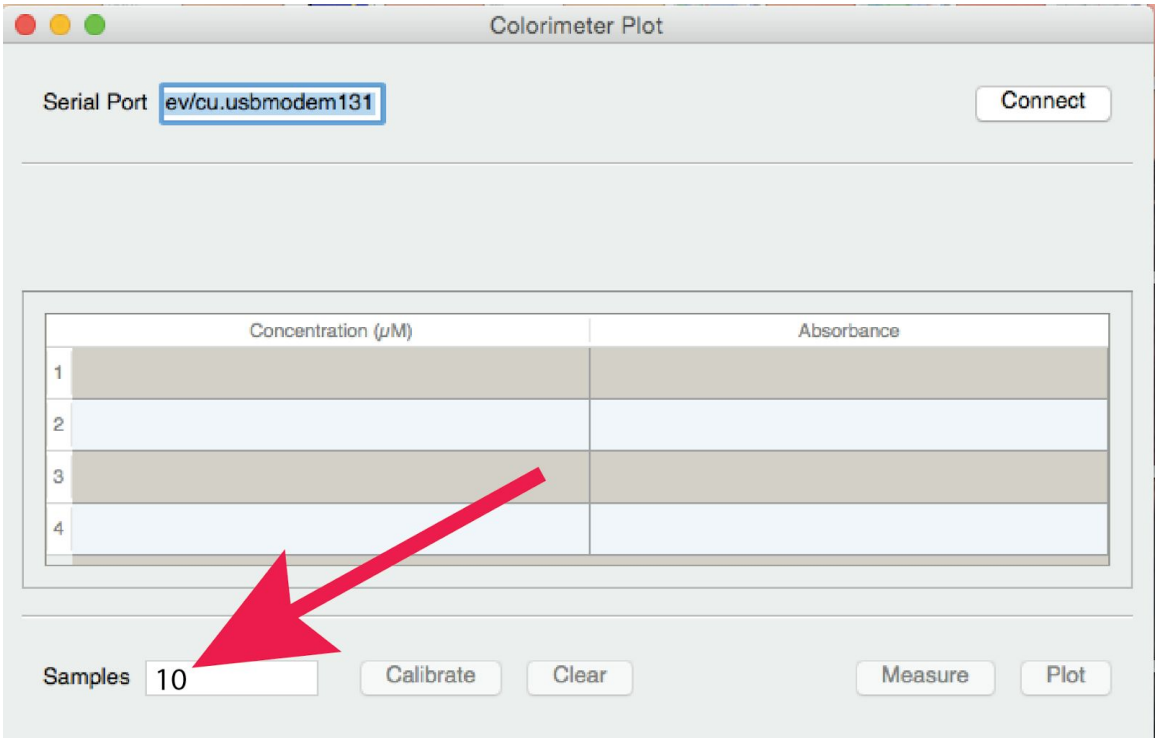
6) Press "Connect" to connect the IORodeo spectrophotometer. (If the spectrophotometer does not connect, please ask your instructor for assistance).



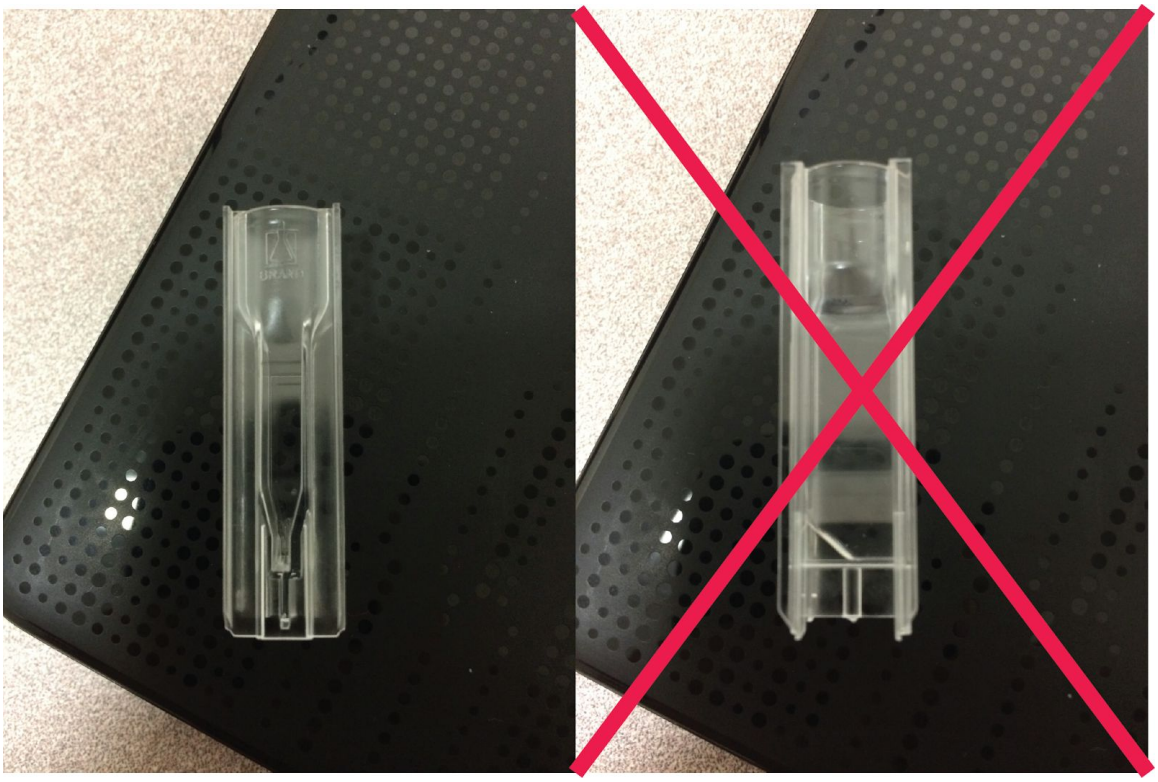
7) In the menu bar, select Mode -> One custom LED (Ver. B).



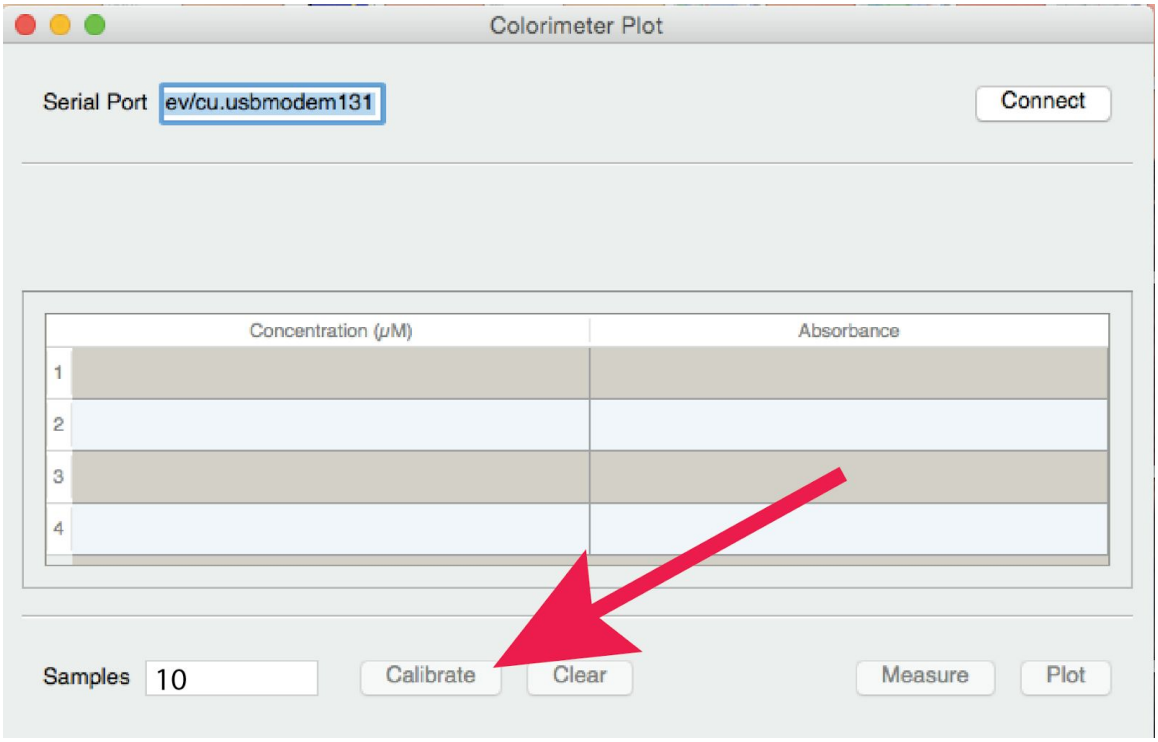
8) Enter "10" in the cell labeled Samples - this is the number of times the spectrophotometer will measure each sample.



9) Place Cuvette E into the spectrophotometer, making sure that the cuvette is positioned so that light will properly go through the cuvette.



10) Cover the spectrophotometer and press "Calibrate"



IMPORTANT: Do not go to step 11 (adding enzyme) until you are ready to start your measurements. You will be taking readings every 10 minutes from each tube a total of 4 times. BE PREPARED.

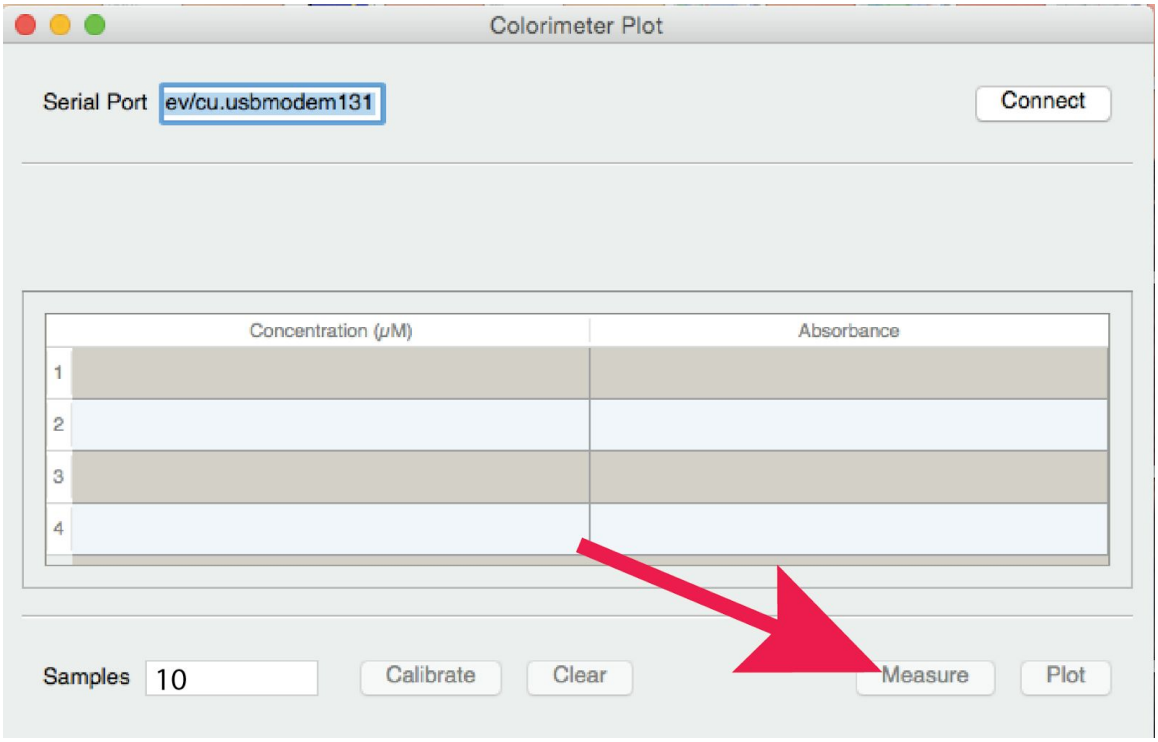
- Make sure your IORodeo spectrophotometer is ON, the colorimeter-4 application is open on the computer, the instrument is connected, and you have calibrated your device (Steps 4-10).
- You have your datasheet ready to record
- You have divided measurement and data recording tasks among members of the groups

11) Add 20 µl of enzyme solution to tubes #1, #3, and #4

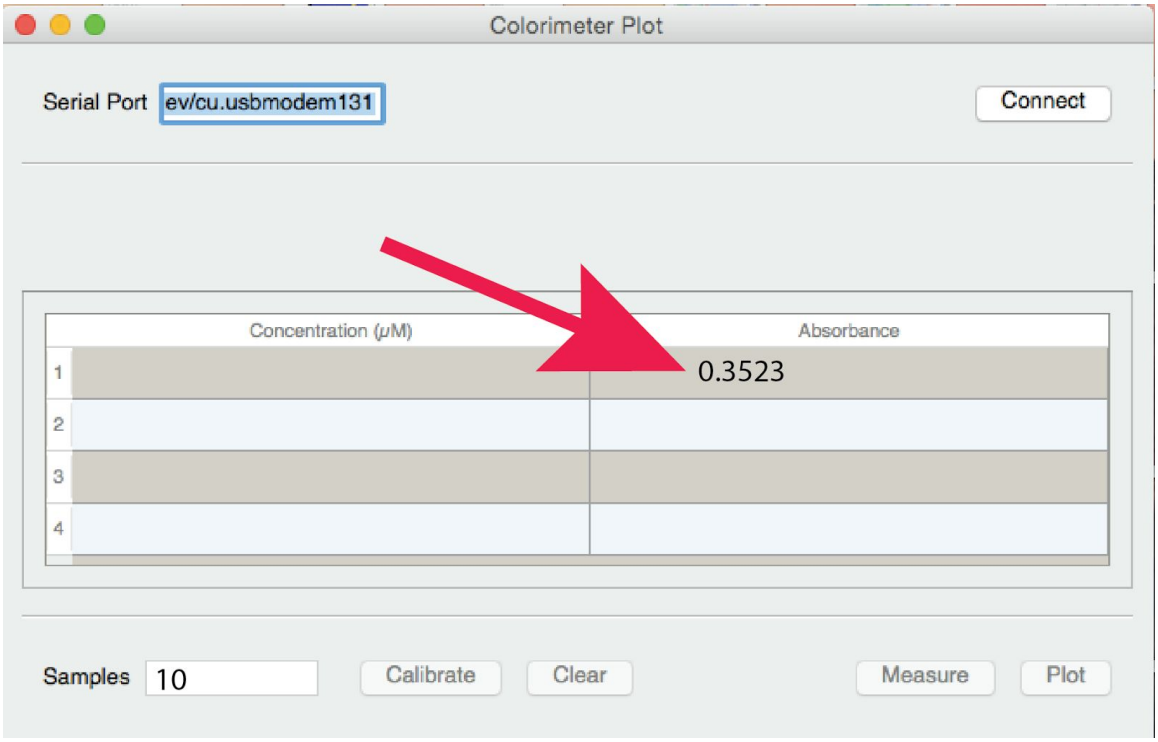
Cuvette a: Sample	**Cuvette b: Neg. control**	**Cuvette c: Pos. control 1**	**Cuvette d: Pos. control 2**
ADD 20 µl ENZYME	**NO ENZYME - ADD 20 µl OF WATER INSTEAD**	ADD 20 µl ENZYME	ADD 20 µl ENZYME

12) Mix each sample by placing the parafilm strip on top of the cuvette and inverting it 4-5 times
13) Set a timer for 10 minutes. Working quickly, place cuvette a in the correct orientation in the spectrophotometer. Cover the spectrophotometer and

press “Measure”.



14) Record the Absorbance measurement given by the device in your datasheet.



15) Repeat step 13 for cuvettes b, c, and d.

16) After 10 minutes repeat Step 14 for all 4 cuvettes. Record each Absorbance measurement in your datasheet (do not forget to set another 10 minute timer).
17) After 10 more minutes repeat Step 14 for all 4 cuvettes. Record each Absorbance measurement in your datasheet (do not forget to set another 10 minute timer).
18) After 10 more minutes repeat Step 14 for all 4 cuvettes. Record each Absorbance measurement in your datasheet (do not forget to set another 10 minute timer). Now it is time to process your data!

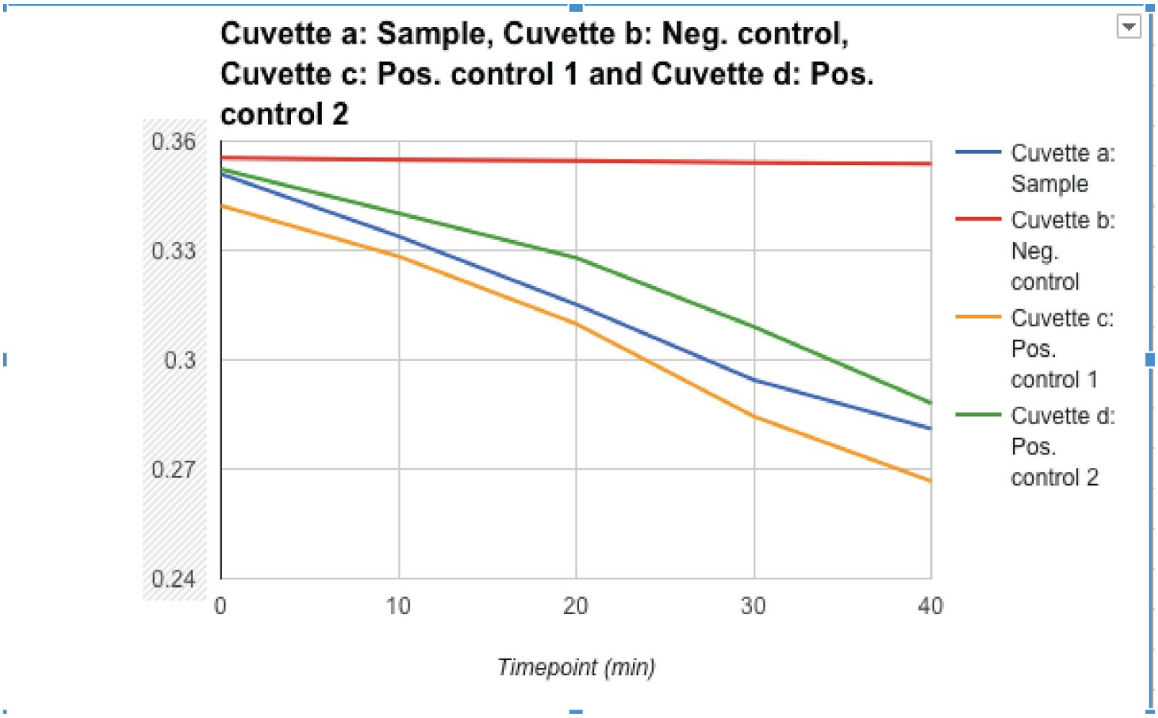
Data Processing Protocol

1) After you complete data collection - your datasheet should be filled out (your values could be much different than the example - that is okay!).

Timepoint (min)	Cuvette a: Sample	Cuvette b: Neg. control	Cuvette c: Pos. control 1	Cuvette d: Pos. control 2
0	0.351	0.3554	0.3423	0.3523
10	0.3339	0.3549	0.3284	0.3402
20	0.3152	0.3546	0.3099	0.328
30	0.2945	0.3541	0.2845	0.3091
40	0.2811	0.3538	0.2667	0.2881

2) Either on a sheet of graph paper or by transferring your data in Microsoft Excel, plot your data where the x-axis is time in minutes and your y-axis is

absorbance. Your plot might look like this.



3) Use the graph to look for a linear region in each curve. Some curves could be completely linear, some could be only linear between a subset of samples.
4) Calculate the slope of the linear region for each curve. Remember the equation for slope (y2-y1)/(x2-x1). You can also use Excel to make a linear fit to your curve.
5) Compare the slopes of each respective sample - this is the rate, or velocity of the reaction.

6) Plot each slope as a bar plot either by hand on graph paper or in Excel. Your data might look like this.