

Natural Science: Biochemistry - Topic: Advanced Enzyme Kinetics

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Standards:

3.1.12.A7: Evaluate metabolic activities using experimental knowledge of enzymes.

3.1.B.A2: Explain the importance of enzymes as catalysts in cell reactions. Identify how factors such as pH and temperature may affect enzyme function

Lesson Objectives:

Students will be able to:

- First and foremost have a prior higher understanding of basic biology and chemistry
- Understand the relationship between enzymes binding to its substrate
- Define catalysts and apply it to chemical reactions
- Use and comprehend the significance of the Michaelis-Menten Equation

Students **will** be only enrolled in AP classes and should be aware of an association between programming and biochemistry topics.

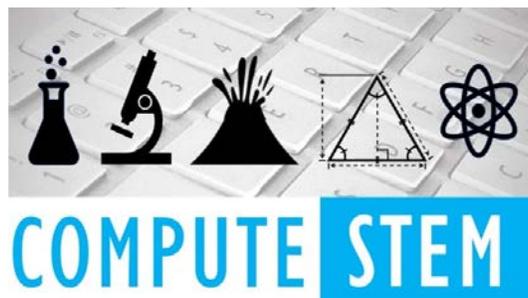
Materials:

- Computers with Python 2.7 or greater
- Small and large Q-tips and timer
- Interactive Graph link

Engage

- In order to put this complex topic into perspectives, the engagement period will entail an activity to represent how enzymes function
- Students will be told to get into groups at the very start of class where there will be ~100 Q-tips at their tables
- They will receive a blank worksheet with instructions that will explain to them how to use their materials and record their data
- Ex: Control- For the first trial, students will break the Q-tips in a 60 second time frame
- They will be given the opportunity to choose different variable (# hands, eyes-open, etc)
- Students will compare data to control and discuss why their data changed
- Students will analyze data in groups and start thinking about the connections of enzyme processes (substrate & catalysis) in [sample worksheet](#)¹
- The significance of the experiment illustrates how enzymes work; speed affecting the product; the role of a catalyst; understanding reaction rates and apply the collected data into code/graph later on

¹ Last page of document

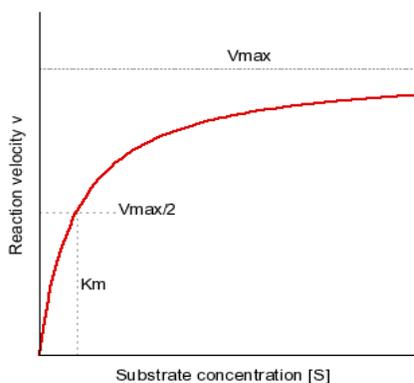


Explore

- The vocabulary that would be emphasized within this lesson plan would include but not limited to: saturation, concentration, catalysis, reaction rate, hyperbolic relationship, V_0 , $[S]$, $[V]$, K_M , Michaelis constant, affinity, transport rate
- The data collected alongside sample data can be applied into the python code² to be introduced and completed by the class
- Students will utilize the equation within the python program using a function
- The opportunity to rearrange the code will be given on paper provided the original equation, and then later executed into the sample code
- They will finish the original function and expected to be able to run the code successfully in calculating different variables of the Michaelis-Menten equation

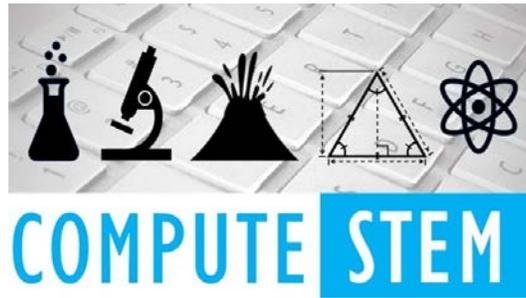
Explain

- Students will be given time to experiment with the [interactive graph](#) online where they will be able to input sample values and observe the trends with some sample problems at the start of class. This should be enough time for them to visualize the relationship (as substrate concentration increases/decreases...)
- Problems will be given on the board where they can use sample variables from, but they will be encouraged to use their own data from the Q-tip experiment
- Using the python code, the students should be able to conduct calculations (sample/in class lab data) to the Michaelis-Menten equation but inputted into the graph online for visualization (trend observations)
- Questions they will be told to think about:
 - What factors affected the reaction?
 - If you change the quantity/ies of the supplies, how would this alter the data?
 - What could possibly limit the rate?



V_{max} = represents the maximum velocity achieved by the system at maximum(saturated) substrate concentration
 $V_{max}/2$ = is where the concentration of substrate at which the reaction velocity is 50% of the V_{max} it is also known as K_m

² Second to last page of document



Elaborate

- The lecture will be more discussion based since a heavy onset of terminology will be introduced- focusing on “relationship between the rate of substrate conversion by an enzyme (V) and the concentration of the substrate $[S]$ ”
- The topic is a little complex, but it will be explained only on the basis of the students’ observations. Questions that are discussion based:
(ex: What trends did you see in the graphs? → Topic introduction: The substrate concentration is only introduced gradually to the enzyme until it reaches equilibrium under the axis of time. This is seen in all those graphs etc.)
 - Plugging in different values, what differences do you see when you increase K_m , V_{max} ?
 - How would an inhibitor change up the results?
 - If you were to continue this earlier lab and break more qtips for ~300 seconds, what would happen to the rate of reaction? Why?
 - Why can the equation be helpful when certain parts are known/unknown?
- As a class, we will take a look at the different components of graphs, breaking them down into simpler terms and understanding the relationship of each variable of the following Michaelis equation $V_0 = V_{max}[S] / K_m + [S]$
- The S is the amount of Substance we are using, V_{max} is the maximum value or time a substance would take. K_m is simple the value at which the substance or enzyme is 50% complete, meaning that it is in a equilibrium state meaning that have the substrate broken down and half is not.
 - A K_m of 25 vs a K_m value of 15 would change the graph. The graph would show that the value with the lower K_m value would have a lower rate of transfer at 50% or a lower V_0 value then the higher K_m due to the fact that the lower value has a higher affinity which that the affinity of that substrate being higher.

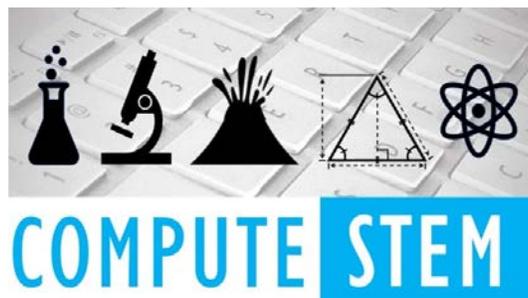


Evaluate

- Students will be given a worksheet to turn in by the end of the lesson plan to see if they understand the content
- This will be handed in with their data sheet to be graded collectively
- It would contain a set of multiple choice, T/F as well as calculation-based questions

Sample:

1. Which of the following is true about the Michaelis constant for any given enzyme?
 - a. It is equal to $V_{\max}/2$
 - b. It is dependent of the type of substrate
 - c. It is independent of the type of substrate
 - d. It increases as the enzyme's affinity for the substrate decrease.
2. What is the ratio of V_0/V_{\max} when $[S] = 5K_M$? You can use sample data or QTipase values/your data. You can use your python calculator/code:
3. As ___ increase ___ decreases
 - a. Substrate concentration, affinity
 - b. Affinity, K_m
 - c. Substrate concentration, K_m value decrease
 - d. K_m , affinity
4. Which of the following will increase the reaction rate of an enzymatic reaction/
 - I. Adding a competitive inhibitor
 - II. Increase the substrate concentration
 - III. Decreasing the affinity of substrate to the enzyme
 - a. I and II
 - b. I only
 - c. I and III
 - d. I,II,III
 - e. None of them
5. True or False. If you increase the substrate concentration your K_m value would increase which in turn would increase the V_{\max}



ENGAGE WORKSHEET:

One person's fingers are the enzyme *Qtipase* (Molarity provided: 4.0×10^{-3})

The Q-tips are the SUBSTRATE

Qtipase is a DIGESTIVE ENZYME meaning it breaks down into several units.

In order to hydrolyse the enzyme, place a Qtip between the thumb and the first finger of each hand. Break into pieces.

Instructions: Assign students in groups of three (breaks qtips, keeps time, record data).

The student breaking the Qtips will start as soon as the time keeper gives them the signal to start. The breaker will use one hand to pick up qtips and break them in half, then place them back with the rest, without looking they will pick up another to do the same. This will happen for a period of 10 seconds. The recorder will record the amount of Qtips that were broken in half. Then the students will take that same bowl of qtips and repeat the process this time for a 20 second interval. They will repeat this 2 more times at 30 and 60 second intervals.

Table 1: Qtipase Activity Data (V_{max})

Interval Time(s)	Time Elapsed (s)	Total # Qtips Broken
10	10	
20	30	
30	60	
60	120	

Table 2: Rate of Qtipase Activity (V in Equation)

Time Interval (s)	Change in Time (s)	Rate of Activity (# Qtips broken/sec)
0-10	10	
10-30		
30-60		

Formula: $\text{Total \# of Qtips Broken} / \text{Change in Time} = \text{Reaction Rate (V in Equation)}$

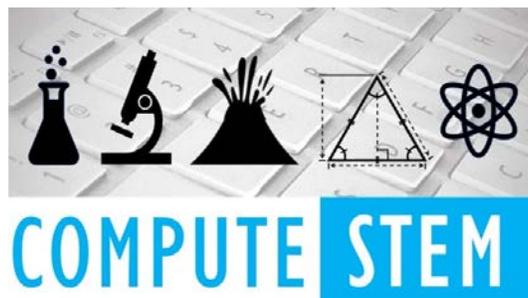


Table 3

Time Interval (s)	# of small Qtips broken	# of larger Qtips broken
0-10		
10-30		
30-60		

Table 4

Alternative Table

Chosen Variable: _____

Time Interval (s)	_____	_____
0-10		
10-30		
30-60		

This portion of the “lab” is upto the students but it must be relevant to the Michaelis-Menten equation. They can think of different ways enzymes are affected to figure out a variable.



Code: <https://repl.it/@aimen1/UtilizedDarkorangeScreenscraper>

```
#Fractional Saturation Calculator via Michaelis-Menten Equation
#COMPLETED VERSION#
#Do not change anything from here, fill in the code using provided data. For
reference it is also listed as comments within this code:
#Enzyme name           Km(M)           kcat (1/2)      kcat/Km
#chymotrypsin          1.5*10e-2      0.14            9.3
#pepsin                 3.0*10e-4      0.50            1.7*10e3
#ribonuclaease          7.9*10e-3      7.9*10e2        1.0*10e5
#carbonic anhydrase     2.6*10e-2      4.0*10e5        1.5*10e7
#fumarase                5.0*10e-6      8.0*10e         1.6*10e8

#These variables are dependent on enzyme. Additionally the reaction rate from
your experimental data can be used as a sample calculation too. Complete the
code.

def RegMMEquation():
    Vmax = input("Enter a value for Vmax using the interactive graph!")
    S = 0.5#mmol SAMPLE VALUE
    Km = input("Enter a value for Km!")
    #V = Vmax [S] / [S] + Km
    V = (Vmax*S)/(S+Km)
    return V

def SaturationMMEquation(ES,S):#students create this equation if they'd like to
use the MM equation to calculate saturation
    KM = 1.5*10e-2#mmol SAMPLE VALUE
    Saturation = (ES)/(KM+S) #students create this equation
    return Saturation

print("")
print("")
print("")
```



```
print("")
print("Hello and welcome to our mini biochemistry python calculator. Our
functions maybe a little limited but let's have some BIOCHEM FUN! For this sample
code, you can see there are different ways to rearrange the amazing Michaelis-
Menten Equation. To begin let's see if you want to calculate V or KM!")
print ("")
print ("")
rearra= raw_input("Do you want to rearrange the Michaelis-Menten Equation?"+"
"For this code you can try finding saturations from the sample/class data, or the
Reaction Rate(V)! Enter 'yes' for saturation or 'no' for Reaction Rate(KM).")

print ("")

if rearra == "no":
print (str(RegMMEquation())+"is your calculated value! Remember the equation can
be used in a variety of ways and derived seperately.")
print ("")
print ("")
print ("")
elif rearra == "yes":
ES = input ("Enter your value: ")
S = input ("Enter your value: ")
print (str(SaturationMMEquation(ES,S))+"is your calculated value! Remember the
equation can be used in a variety of ways and derived seperately.")
print ("")
print ("")
print ("")
else:
print ("Only enter 'yes' or 'no'")

#Reference variable/formulas
#V0 = kcat[ES]
#Vmax = kcat[E]t
#V0/Vmax = [ES]/[E]t
```



$$#V_0 = V_{max}[S] / K_m + [S]$$

$$#\text{or it can be written: } V_0/V_{max} = [S] / K_m + [S]$$

$$#\text{Combined} > V_0/V_{max} = [ES]/[E]t = [S] / K_m + [S]$$