Intuitive Interactive Software Applications for Enzymology

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Abstract

Some concepts in enzymology can be difficult for students, in part because a deep understanding requires some mathematical sophistication. In many cases, a deeper appreciation of both basic and advanced concepts can be gained by providing intuitive simulations of the dynamic processes occurring in enzyme systems.

Using only the basic elements of JAVA, I have developed interactive software applications that aim to provide students with a more intuitive feel for key concepts in enzymology. The poster and live, in-person, on-line demonstration attempt to show current progress in the development and implementation of this software.

Introduction and Rationale

Many introductory biology courses and all introductory biochemistry courses teach the “Michaelis-Menten” equation and basic, quantitative aspects of enzymology and kinetics, because these fundamental concepts are critical for understanding all areas of biology and biochemistry.

Many students, especially if they lack mathematical sophistication, tend to memorize equations and definitions, thus obtaining a fairly superficial understanding of key, fundamental concepts necessary for understanding more advanced concepts such as metabolic regulation and allosteric control. Interactive and experiential learning approaches can help to provide a deeper and more meaningful introduction to enzyme kinetics and equations that may seem to be “too hard” or “too abstract” for the average student to truly understand and appreciate.

Common Learning Objectives when Teaching Basic Equations of Enzyme Kinetics

1. **What are the meanings of the terms V_o, $[S]_o$, V_{max} and K_m?** (not only how are these key terms defined mathematically, but what does each term represent in a physical sense?)

2. **Under what conditions is the Michaelis-Menten equation valid?** (*e.g.*, initial rate conditions, rapid equilibrium vs. steady-state assumptions, $[S]_o >> [E]_o$)

3. **How can we measure initial reaction rates and how can we plot these data in a practical way to determine the kinetic parameters for an enzyme?** (*e.g.*, direct fitting to the M-M equation using non-linear least squares vs. use of the Eadie-Hofstee and Lineweaver-Burke linear transformations and linear regression)

Common Challenges when Teaching Basic Equations of Enzyme Kinetics

1. "Equation-phobia" (White, 2000)

2. Memorization of definitions and equations provides only a superficial level of understanding to most students.

3. Calculation problems and plotting data using the M-M equation only require students to "plug numbers" into the equation or follow a recipe for plotting data, thus failing to probe for a deeper and more meaningful understanding of concepts.

4. The entire experimental process going from the design of an assay, measurement of initial rates and data analysis is time-consuming, labour-intensive and can be costly to implement even simplified parts of this process for large introductory courses.

5. The whole process of determining kinetic parameters lacks relevance to real life for most people – other than professional enzymologists!

JAVA applets provide an experiential learning environment for exploring the basics of enzymology!!

The following screenshots are taken from a novel JAVA applet which I wrote to allow students the opportunity to see how changes in kinetic parameters (*e.g.*, V_{max}, K_m) affect the initial velocity of enzyme-catalyzed reactions.

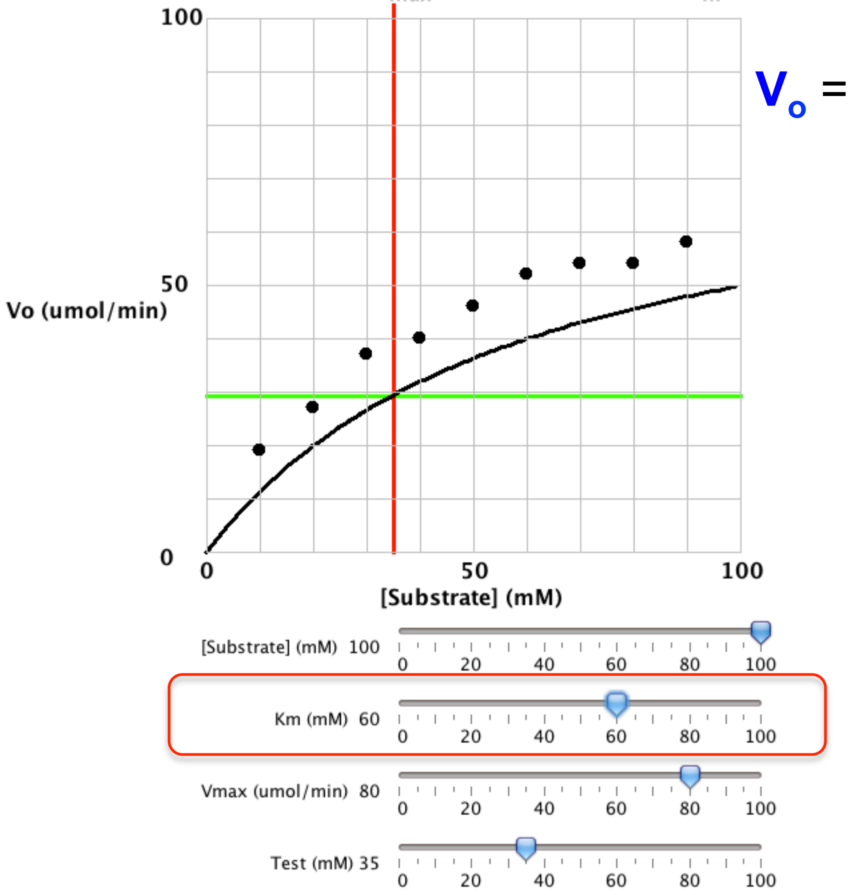
The sliders allow the user to vary the progress of the reaction, as well as the kinetic parameters. It is hoped that the simplicity and intuitive nature of the user interface will make it easy for students to interact with the applet to see how kinetic curves change as a function of changes to the kinetic parameters.

The best way to see how this works is to go to my website and try out the applets for yourself:

http://people.ucalgary.ca/~ngk/enzyme/able2013.html

Michaelis-Menten Applet Exercise 1: How do changes in K_m affect V_o?

screenshot of curve with optimized V_{max} but suboptimal K_m



[Substrate] (mM) 100

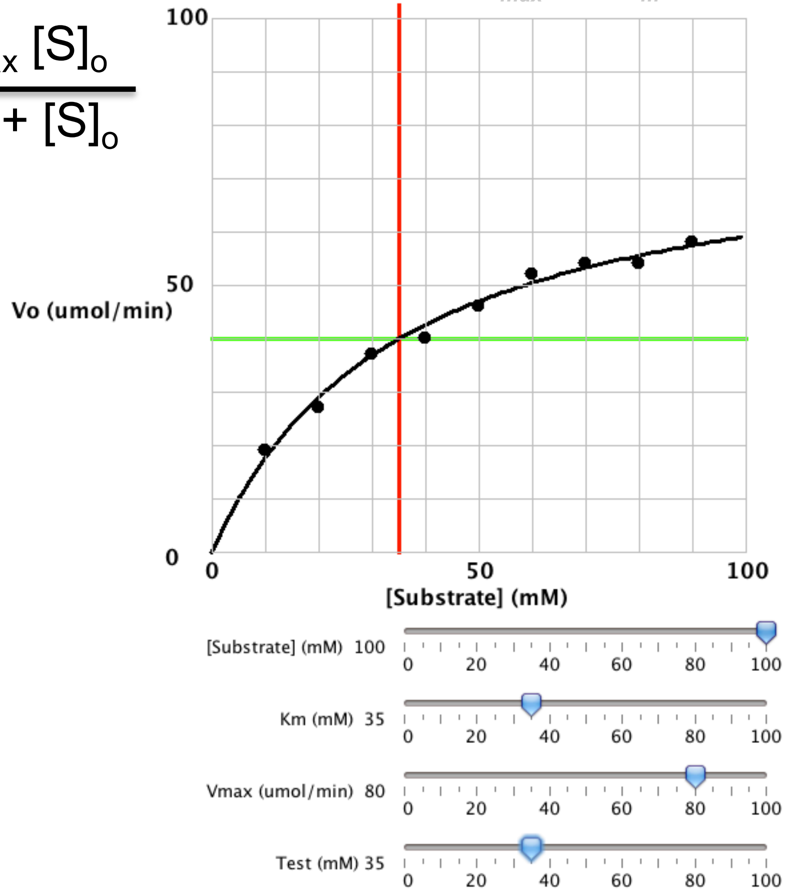
Km (mM) 60

Vmax (umol/min) 80

Test (mM) 35

$$V_o = \frac{V_{max}\,[S]_o}{K_m + [S]_o}$$

screenshot of curve with optimized V_{max} and K_m



[Substrate] (mM) 100

Km (mM) 35

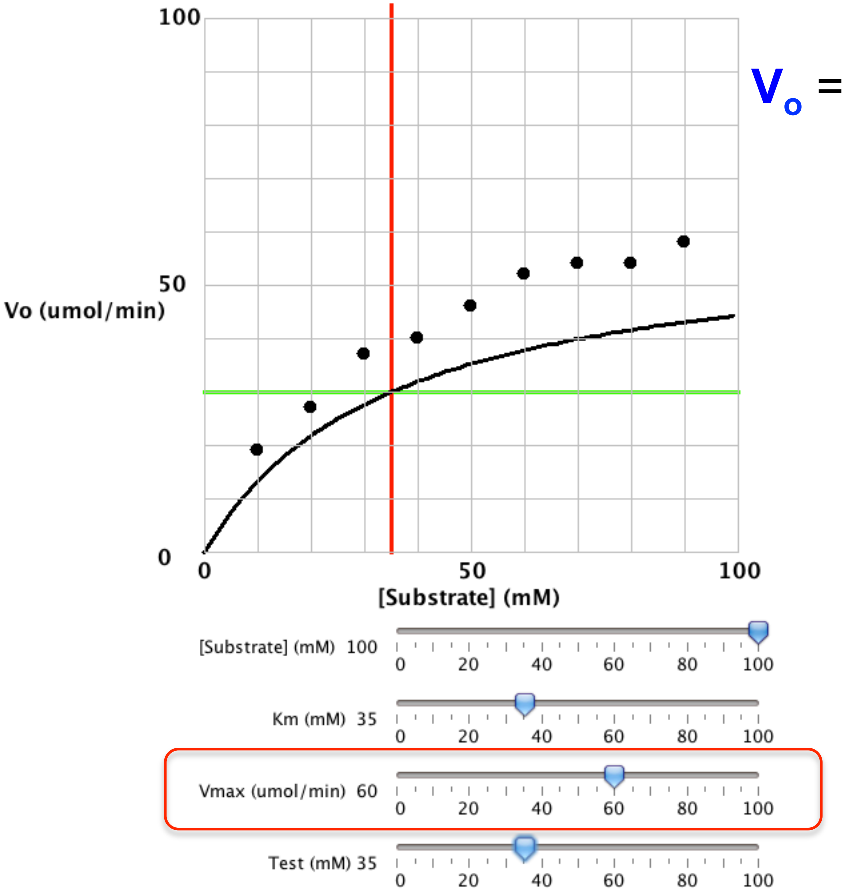
Vmax (umol/min) 80

Test (mM) 35

Students are asked to adjust the "K_m" slider to see how, for example, raising K_m causes the curve to become flatter in shape, as V_o decreases for any given [Substrate].

Michaelis-Menten Applet Exercise 2: How do changes in V_{max} affect V_o?

screenshot of curve with optimized K_m but suboptimal V_{max}



[Substrate] (mM) 100

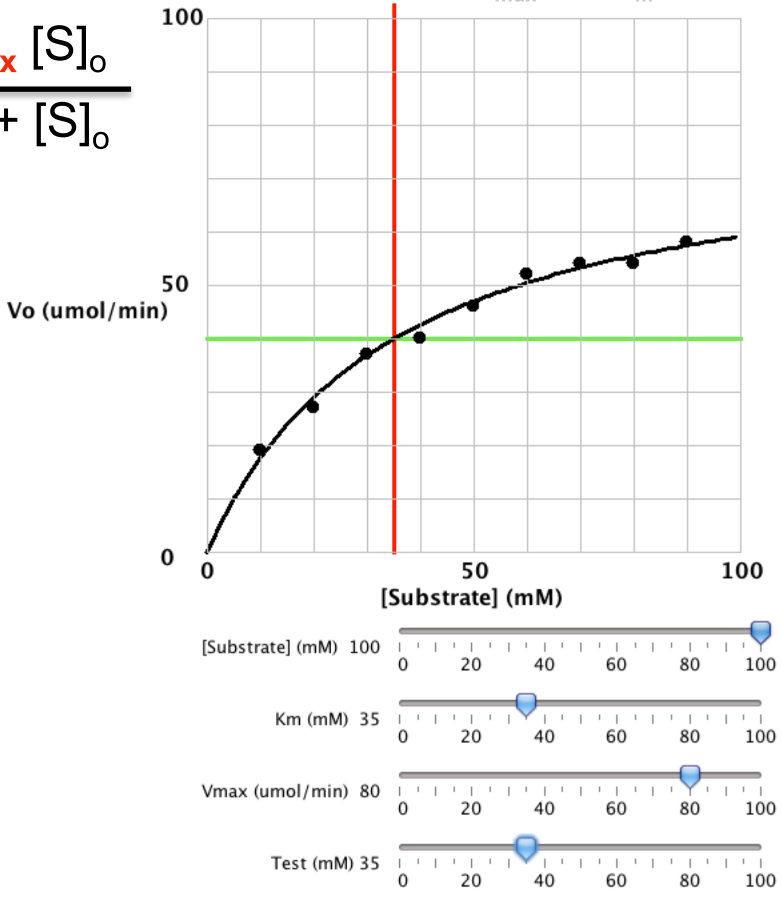
Km (mM) 35

Vmax (umol/min) 60

Test (mM) 35

$$V_o = \frac{V_{max}\,[S]_o}{K_m + [S]_o}$$

screenshot of curve with optimized V_{max} and K_m



[Substrate] (mM) 100

Km (mM) 35

Vmax (umol/min) 80

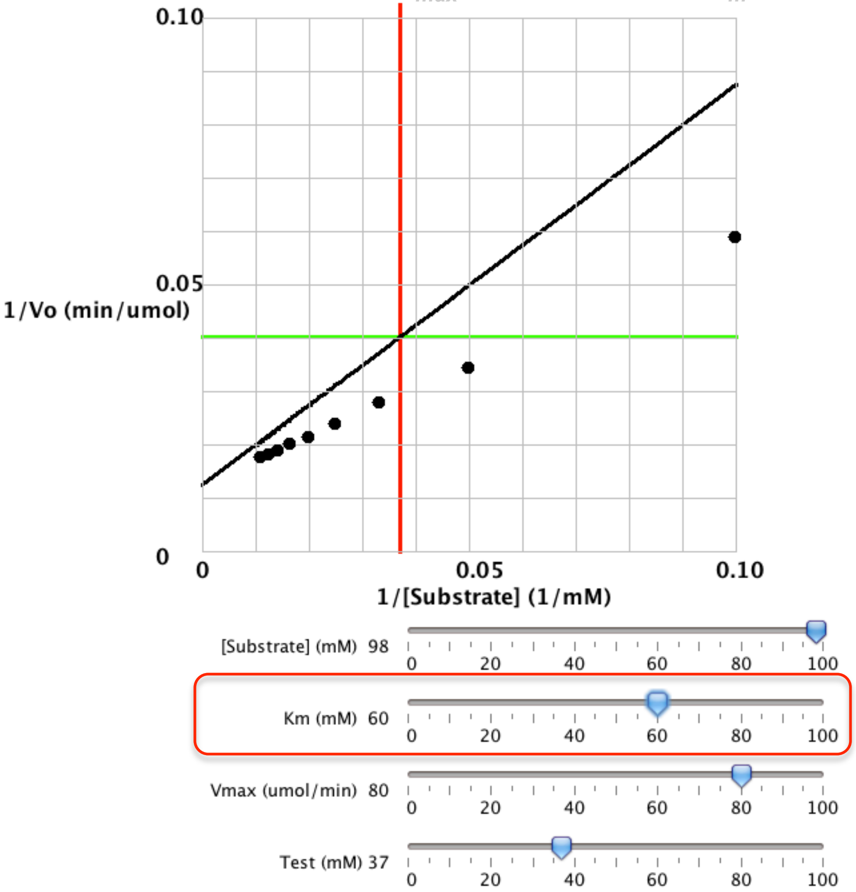
Test (mM) 35

Students are asked to adjust the "V_{max}" slider to see how, for example, lowering V_{max} causes the curve to become flatter in shape, as V_o decreases for any given [Substrate].

Lineweaver-Burke Applet I

$$\frac{1}{V_o} = \frac{1}{[S]_o}\left(\frac{K_m}{V_{max}}\right) + \frac{1}{V_{max}}$$

screenshot of curve with optimized V_{max} but suboptimal K_m



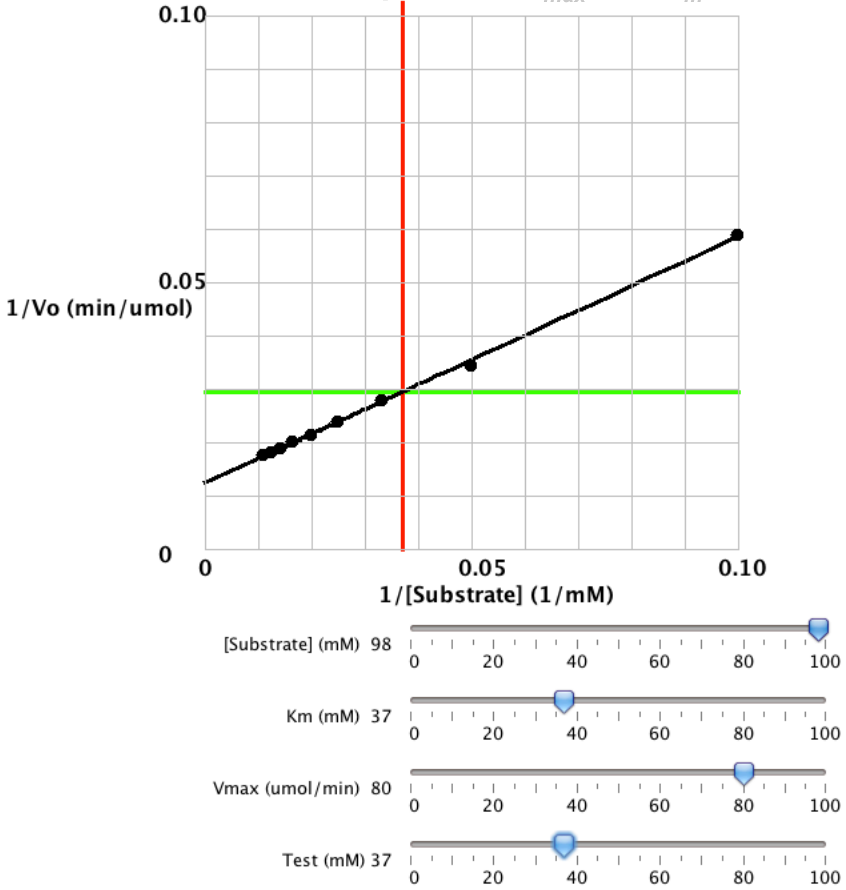
[Substrate] (mM) 98

Km (mM) 60

Vmax (umol/min) 80

Test (mM) 37

screenshot of curve with optimized V_{max} and K_m



[Substrate] (mM) 98

Km (mM) 37

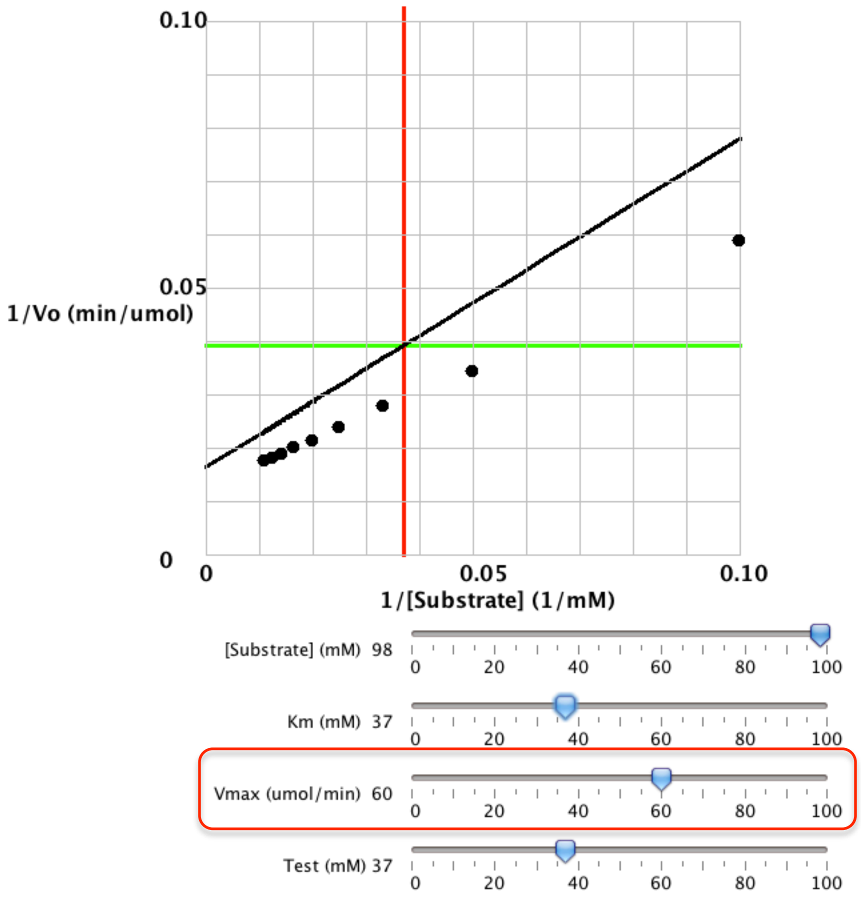
Vmax (umol/min) 80

Test (mM) 37

Students are asked to adjust the "K_m" slider to see how, for example, raising K_m causes an increase in the slope of the line, as $1/V_o$ increases for any given [Substrate], except when saturating (Y-intercept).

Lineweaver-Burke Applet II

$$\frac{1}{V_o} = \frac{1}{[S]_o}\left(\frac{K_m}{V_{max}}\right) + \frac{1}{V_{max}}$$



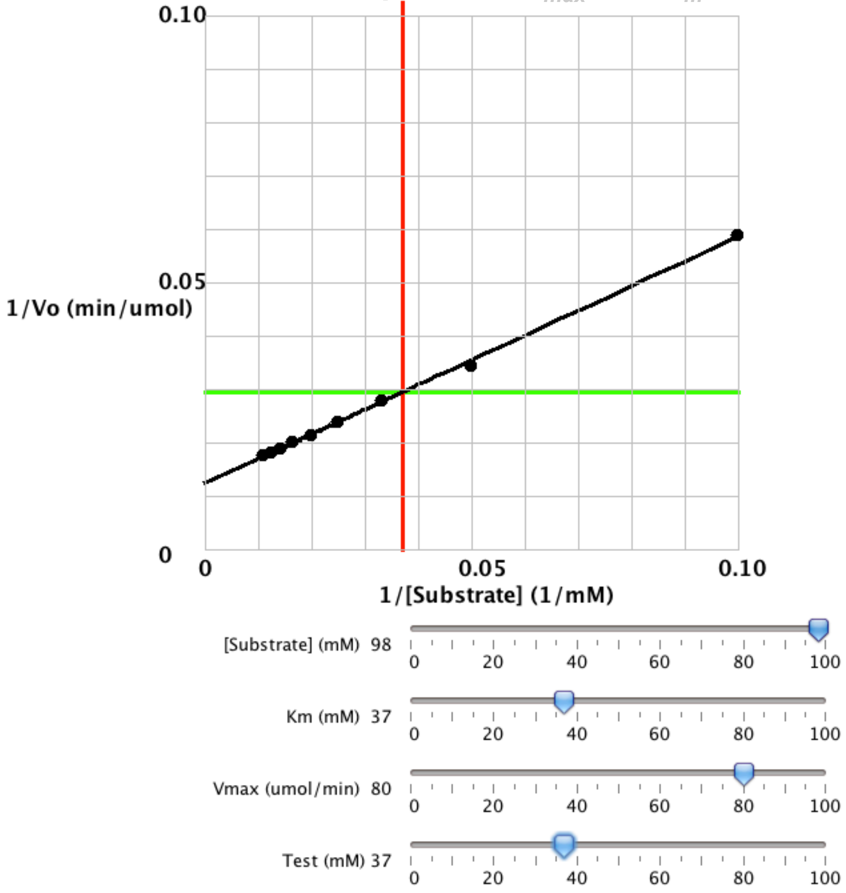
screenshot of curve with optimized K_m but suboptimal V_{max}

[Substrate] (mM) 98

Km (mM) 37

Vmax (umol/min) 60

Test (mM) 37



screenshot of curve with optimized V_{max} and K_m

[Substrate] (mM) 98

Km (mM) 37

Vmax (umol/min) 80

Test (mM) 37

Students are asked to adjust the “V_{max}” slider to see how, for example, lowering V_{max} causes an increase in the slope of the line and an increase in the value of the Y-intercept, as $1/V_o$ increases for any given [Substrate].

Example Exercise Using the M-M JAVA Applet

Goal: Use the **M-M applet** to find the parameters of K_m and V_{max} which give the best fit to the experimental V_o measurements (black dots).

1. Drag the top slider to see the values of V_o predicted given the starting values of K_m and V_{max} set by default in the applet.

2. Does the curve fit the data? Can you adjust the parameters of K_m and V_{max} to improve the fit to the data?

3. You can see that this is a 2-dimensional search in which K_m changes the **steepness of the curve**, but not the saturating value, whereas V_{max} changes the **saturating value** but not the steepness.

4. Can you find **the combination of K_m and V_{max}** that gives the best fit to the data? How sure are you that you have determined the **best fit**?

Followup Exercise Using the L-B Applet

Goal: Use the **L-B applet** to explore how changing the parameters of K_m and V_{max} give lines with better or worse fits to the experimental V_o measurements (black dots).

1. Drag the top slider to see the values of **$1/V_o$** predicted given the starting values of K_m and V_{max} set by default in the applet.

2. **Does the line fit the data?** Can you adjust the parameters of K_m and V_{max} to improve the fit to the data?

3. While this is still a 2-dimensional search, it is a simpler search in which K_m changes the **slope of the line**, but not the Y-intercept (*i.e.*, saturating value), whereas V_{max} changes **both the Y-intercept and the slope.**

4. Can you find **the combination of K_m and V_{max}** that gives the best fit to the data? How sure are you that you have determined the **best fit**?

5. Is the determination of optimal K_m and V_{max} **harder or easier** to do than with the M-M equation directly? Why is it harder or easier?

Conclusions

Interactive JAVA applets can provide an interactive and pseudo-experiential learning environment for novice and advanced students to explore some of the more abstract quantitative concepts and equations central to enzymology.

Computer-based exercises provide a complement to more traditional wet labs that require a significant investment of time, expertise and cost to obtain enough high-quality quantitative results for students to appreciate the fundamental principles enzyme kinetics.

Interactive computer-based exercises can be used in combination with more traditional labs to engage students in a deeper learning experience in which students “play” with the applet to better understand the meaning of somewhat complex equations.

Future Work

The basic programming framework used to provide an interactive interface for students to "play" with the M-M and L-B equations shown here can be further developed to help students explore other equations in enzyme kinetics (*e.g.*, first-order and second-order transient-state kinetics, multisubstrate kinetics, enzyme inhibition, cooperativity and enzyme regulation).

The programming approach can be used to provide a simple, interactive interface for students to explore other equations in biology, chemistry and physics.

The applets can be further refined and integrated into specific exercises or components of laboratory exercises that would ideally involve a wet-lab, experimental component to place the theoretical components in context.

References

Chandler, V., S. Donovan, W. Goodwin, K. Sprague, and C. Stiefbold. 1998. Enzyme kinetics. *ABLE Proceedings* 19: 81-97.

González-Cruz, J., R. Rodríguez-Sotres, M. Rodríguez-Penagos. 2003. On the convenience of using a computer simulation to teach enzyme kinetics to undergraduate students with biological chemistry-related curricula. *Biochem Mol Biol Educ*. 31:93-101.

Runge, S.W., B.J.F. Hill and W.M. Moran. 2006. A simple classroom teaching technique to help students understand Michaelis-Menten Kinetics. *CBE Life Sci Educ* 5:348-352.

White, H.B. (2000) Why teach enzyme kinetics. *Biochem Mol Biol Educ*. 28:330-331.