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Making Enzyme Kinetics Dynamic via Simulation Software

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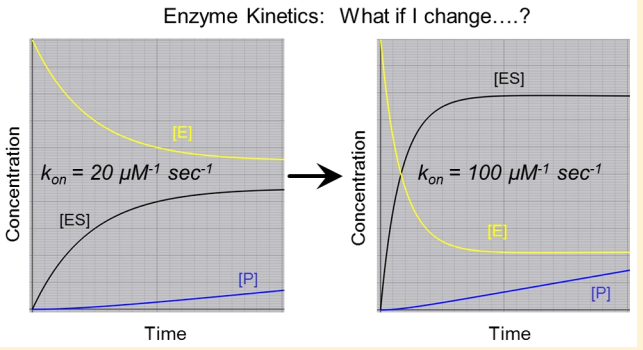
Supporting Information

ABSTRACT: An interactive classroom demonstration that enhances students' knowledge of steady-state and Michaelis−Menten enzyme kinetics is described. The instructor uses a free version of professional-quality KinTek Explorer simulation software and student input to construct dynamic versions of three static hallmark images commonly used to introduce enzyme kinetic concepts. The software, with its ability to change experimental conditions and immediately observe the effects on the kinetics of a system, allows students to be more aware of the experimental conditions that must exist for the assumptions of steady-state and Michaelis−Menten kinetics to be valid. Students report an increased understanding of the interplay between hallmark concentration versus time and rate versus concentration images. After the demonstration, students are prepared for data generation and analysis in a lab experiment focused on the steady-state. Additionally, they can begin independent simulation exercises using the software and are ready for discussions on pre-steady-state kinetics.



KEYWORDS: *Upper-Division Undergraduate, Biochemistry, Demonstrations, Computer-Based Learning, Enzymes, Kinetics*

BACKGROUND

Static Introduction to Enzyme Kinetics

A traditional biochemical lecture on Michaelis−Menten enzyme kinetics relies on the use of static images to introduce students to the ideas of the steady-state, initial reaction rates, and the hyperbolic curve that results from the initial rates following the Michaelis−Menten equation. The Michaelis−Menten equation (eq 1) states that the initial velocity (v) is equal to the product of the maximum initial velocity (v_{max}) and the initial substrate concentration [S] divided by the Michaelis constant, K_M, plus the initial substrate concentration [S].[1]

$$v = \frac{v_{max}[S]}{K_M + [S]} \quad (1)$$

Simulations Make Enzyme Kinetics Dynamic

Simulations represent an advantage over traditional lecture by helping students with recall, comprehension, and transfer of information to novel situations.[2,3] A book detailing the pedagogical role of simulations in chemistry courses has recently been published.[4] Simulations have been developed to help students better understand steady-state kinetics. A hands-on simulation utilizing nuts and bolts provides students with a concrete, real-life process that mimics the physical processes of enzymatic catalysis.[5] A computer simulation allows students to choose substrate values, estimate the beginning of the steady-state, and determine kinetic values using linear regression.[6] A Mathematica-based simulation gives users the freedom to modify parameters and observe how the simulated time traces compare using either the steady-state approximation or numerical analysis.[7] SCILAB is utilized for a reversible enzyme inhibition simulation.[8,9] Herein, a novel demonstration is presented that is designed to enhance students' understanding of Michaelis−Menten kinetics. It utilizes the free unlicensed version of KinTek Explorer[10−12] simulation software that, unlike the aforementioned useful simulations, is capable of creating dynamic versions of three hallmark images used in the traditional kinetics lecture and allows simultaneous viewing and manipulation of two images.

DESCRIPTION OF DEMONSTRATION

The approximately 30 min demonstration is carried out in an upper-division undergraduate biochemistry classroom with the instructor using KinTek Explorer software (Figure 1) and projecting the simulation for the entire class to view. The instructor asks leading questions and guides the dialogue to create dynamic versions of three hallmark images sequentially (Figures 2−4). The progression of the demonstration facilitates straightforward transitions between image constructions. Specific concepts addressed include how simulations differ from actual experiments, identification and manipulation of the duration of the steady-state reaction phase, the relationship

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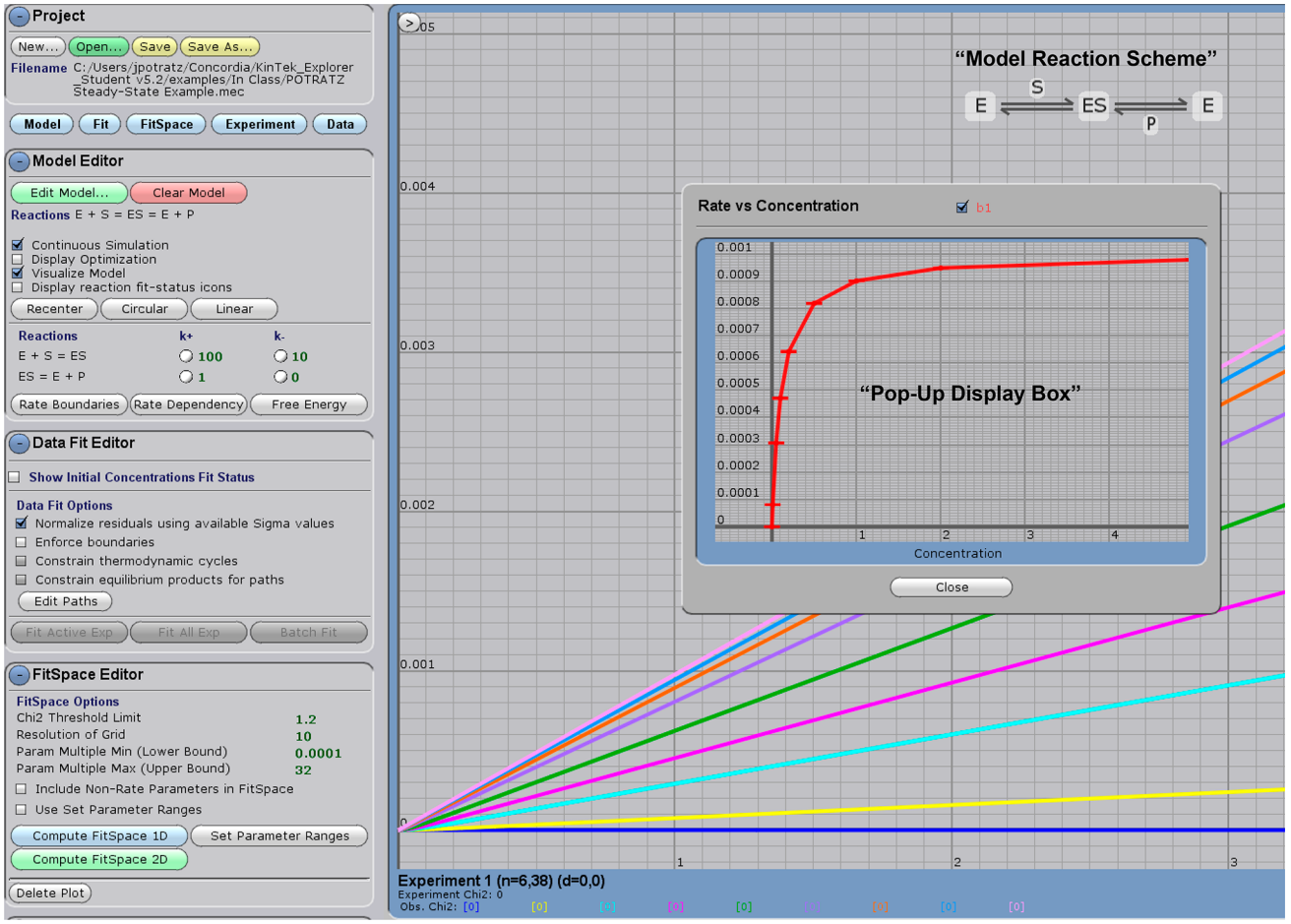

Figure 1. Screenshot of the KinTek Explorer simulation software. The column on the left is a scrollable interface where the parameters of the simulation are entered. The concentrations of the observed species as a function of time are displayed on the right. Also visible are the model reaction scheme being simulated and the pop-up rate vs concentration display box. This pop-up box can be opened and closed, moved around the screen, and zoomed for easier viewing. The user can change parameters of the simulation and observe the effects on the concentration vs time plot as well as the rate vs concentration plot nearly instantaneously. Note: The black, bolded text in quotations has been added, and the screenshot does not show the entire right side.

between the "initial rates" (Figure 3) and "hyperbolic curve" (Figure 4) images, normalization of v_{max}, nonlinear curve fits, and the importance of obtaining data during the steady-state phase when using the steady-state assumption. See the concepts addressed guide in the Supporting Information for a detailed explanation of how these topics are considered.

The demonstration is used after students have already been briefly introduced to Michaelis–Menten kinetics via the traditional static images and can identify K_M on a graph. In this way, students have a baseline understanding of steady-state kinetics and are ready for an in-depth exploration that promotes an awareness of the experimental conditions that must be met for the assumptions of steady-state and Michaelis–Menten kinetics to be valid. The demonstration is utilized before a corresponding lab in which students collect and fit data to the Michaelis–Menten equation using nonlinear curve fitting. The demonstration helps students comprehend the data gathering and analysis process and serves as a de facto prelab exercise. In addition, the demonstration sets up a natural flow toward discussions on pre-steady-state kinetics.

BENEFITS

Having access to "dynamic images" that allow students to manipulate variables and view real-time changes in the data is certainly helpful when learning a topic as dynamic as enzyme kinetics. This demonstration not only produces "dynamic images", but also relies on student interaction to construct them. Therefore, students are more aware of the reaction conditions and assumptions that were present in order to produce the hallmark images as they had "behind the scenes access" during construction. In student critiques, variable manipulation and corresponding changes in the kinetic traces have overwhelmingly been cited as major contributing factors to their increased understanding of how chemical species change over time and how the "hyperbolic curve image" (Figure 4) is produced using data from the "initial rates image" (Figure 3). During the demonstration students may ask questions like, "What happens if we use a ridiculously high substrate concentration?" The software and demonstration provide a wonderful opportunity to respond with, "Let's make an educated guess, try it, and see!"

PROCEDURE

Good Practices and Model Enzymatic Reaction

Ideally, the static hallmark images themselves are in plain sight during the demonstration. The instructor can simply encourage students to open up their textbooks to the appropriate figures.

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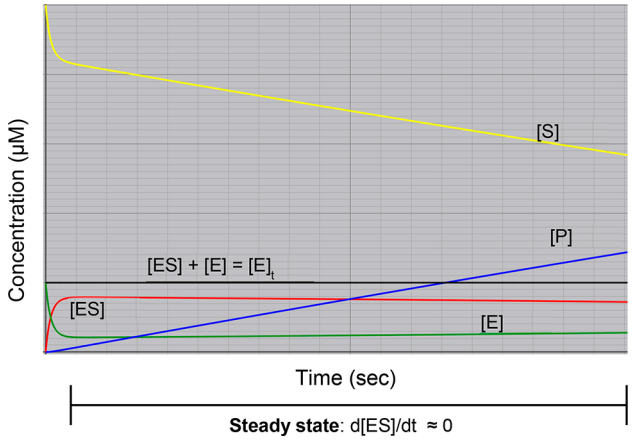

Figure 2. Screenshot of the dynamic hallmark "steady-state image" produced using the software. The portion of the reaction time course where the concentration of the enzyme–substrate complex (ES, red line) is essentially unchanged or steady is highlighted. Other species shown are substrate (S, yellow line), product (P, blue line), free enzyme (E, green line), and total enzyme (E_t, black line). The portion denoted as the steady-state contains a 10% decrease in ES concentration. Note: The axis labels and species labels have been added.

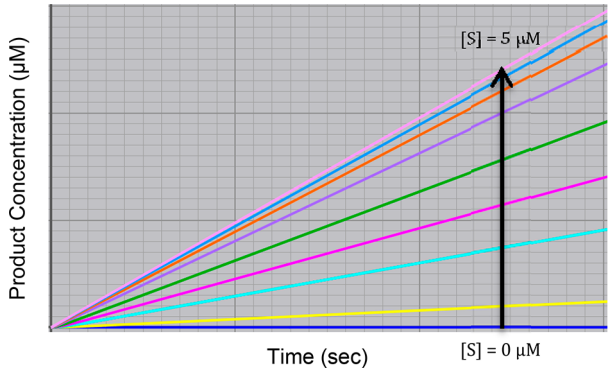

Figure 3. Screenshot of the dynamic hallmark "initial rates image". Incrementally increasing the initial substrate concentration from 0 to 5 μM in the simulated reaction produces different product formation time traces (colored lines) that can be fit to a linear function to give increasing initial rates of product formation. Note: The axis labels, species labels, and arrow have been added.

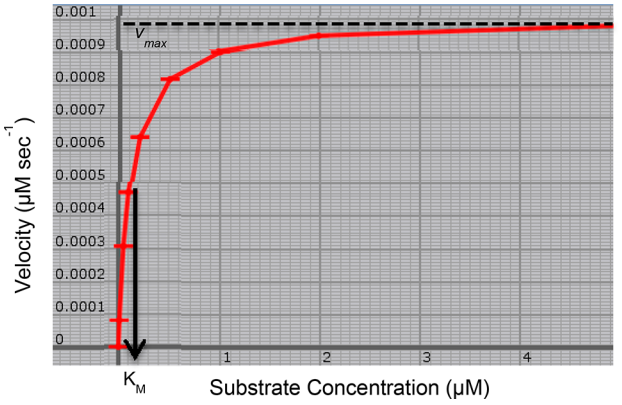

Figure 4. Screenshot of the dynamic hallmark "hyperbolic curve image". The simulated reaction follows Michaelis–Menten kinetics as initial reaction velocity is plotted as a function of substrate concentration (red line) to arrive at v_{max} and K_M. This curve is drawn in a "connect-the-dots" fashion and is not a true hyperbola. See the concepts addressed guide in the Supporting Information for proof that this does not change the estimated v_{max} or K_M values obtained if the fit was hyperbolic. Note: The axis labels, v_{max}, K_M, arrow, and dashed line have been added.

Scheme 1

Model Enzymatic Reaction

$$E + S \underset{10\ s^{-1}}{\overset{100\ \mu M^{-1}\ s^{-1}}{\rightleftharpoons}} ES \xrightarrow{1\ s^{-1}} E + P$$

Initial Concentrations: [E] = 0.1 μM [S] = 0.5 μM

Table 1. List of Experimental Conditions for Simulation

E (μM)	S (μM)	k (rate constants)
0.1[a]	0.5[a]	Formation of ES = 100 μM^{-1} s^{-1}
0.001	0, 0.01, 0.05, 0.1, 0.2, 0.5, 1, 2, 5	Dissociation of ES = 10 s^{-1}
		Catalysis and product release = 1 s^{-1}
		ES formation from E + P = 0 s^{-1}

[a]Values used to create hallmark "steady-state image" (Figure 2).

This allows students to keep in mind the "destinations" of the demonstration. Having the static hallmark images and the model enzymatic reaction information readily visible at all times reduces the extraneous load burden as it relates to Cognitive Load Theory (CLT).[2,13,14] Students are introduced to the model enzymatic reaction (Scheme 1), including rate constants and initial concentrations, when the instructor writes the information on the whiteboard to begin the demonstration. Refer to Table 1 for a complete list of experimental conditions used during the simulation.

Introducing the Simulation Software

Calling on students to help with the rudimentary task of transferring the rate constant and initial concentration information present on the whiteboard into the simulation software is a great way to familiarize them with the software interface. Additionally, this practice ensures that they remain engaged and allows the instructor to detect and correct any early signs of misunderstanding. Clearly, instructors will need to download and become familiar with the software to utilize it effectively during the demonstration. Anyone can download and use the unlicensed version of the software that is used for this demonstration at no cost on a Mac or PC.[10] Simply download the appropriate version, uncompress the file, and double click the application to open and begin using.[15]

Instructors will quickly ascertain that units are not included explicitly on the software interface, either when entering the model enzymatic reaction parameters or while viewing the simulated results. This is not to imply that the values are unitless, as the values entered and simulated certainly have associated units. Soliciting student input to reinforce the units of an axis is a great way to highlight their importance. The software inherently displays no units so that the user can choose to enter the simulation parameters in units of their choice (e.g., μM and seconds or M and minutes). The user simply has to stay consistent and cognizant of unit choice.

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Creating the Dynamic Versions of the Hallmark Images

Refer to the Supporting Information for a screen-capture, how-to video demonstrating the use of the software to create dynamic versions of the three hallmark images (Figures 2-4). The video is 8 min long and contains step-by-step instructions for all the "mouse clicks" needed. A link to two other screen-capture, how-to videos and an instructor guide containing a written and pictorial how-to, a suggested list of leading questions to use, and helpful notes are found in the Supporting Information and provide ample details concerning the effective implementation of the demonstration.

IMPLEMENTATION AND ADAPTIBILITY

The demonstration has been employed for four semesters at two institutions with a total of approximately 120 upper-division undergraduate students. Anecdotal and formal student and professor feedback have been solicited to continually improve the demonstration. See the Supporting Information for specific survey questions and responses. Students suggested that providing an explicit list of assumptions and a protocol on a handout may benefit the demonstration. Therefore, the Supporting Information contains a student handout that lists the model reaction, lists assumptions for steady-state and Michaelis−Menten kinetics, and includes an overview of the demonstration.

The demonstration can effectively be exploited as an isolated exercise and the lone instance KinTek Explorer software is used. Therefore, it has been designed so instructors need only learn the bare minimum about the software. The demonstration was first utilized as a stand-alone appearance of the software. However, for the latest iterations, students were familiar with the software before the demonstration as simulations involving buffers and ligand binding were employed earlier in the semester. After this demonstration and subsequent lectures, students completed a homework assignment utilizing the software to simulate steady-state kinetics, reversible inhibition kinetics, and pre-steady-state kinetics. Students had access to tutorial videos showing how to use the software for simulations while they completed the assignment. Seventy-eight students have completed the assignment with an average score of 82%. See the Supporting Information for the homework assignment. The assignment was a prelude to a take-home exam problem. The problem required higher-order thinking as students created and simulated a realistic model enzymatic system to extract kinetic values. See the Supporting Information for the exam problem. Seventy-seven students have attempted the problem with an average result earning 82% of the points possible.

The demonstration is quite adaptable. The instructor can choose to spend more or less time on a concept depending on the topics on which the instructor would like to focus, the student responses to leading questions, the instructor's perceived student comprehension, and/or any questions students may pose. Additionally, this demonstration could be converted into a self-guided handout given to students to work on at individual computers. This self-guided process would likely take longer than 30 min and would require the instructor to help individual students as they have questions. This adaptation may be better suited to take place in a laboratory setting.

CONCLUSION

The importance of engaging students in active learning experiences and the value of simulations make this interactive demonstration a valuable addition to an upper-division biochemistry course. Students have overwhelmingly found the dynamic hallmark image construction a worthwhile exercise and report a much clearer understanding of the interplay between the "initial rates" and "hyperbolic curve" images. Exhaustive video, pictorial, and written Supporting Information should answer any questions an instructor may have during implementation. Additionally, this demonstration showcases only the tip of the iceberg of the capabilities and usefulness of KinTek Explorer simulation software in the undergraduate curriculum. Even the unlicensed version (previously referred to as a "student version"), on which the demonstration is performed, is professional-quality software that the instructor will not outgrow for educational purposes. The software undergoes constant upkeep and will remain relevant well into the future. Another instructor has posted an online video showing how the software can be used in a biochemistry course to globally fit kinetic data instead of simply simulating it.[16]

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.7b00350.

Concepts addressed guide (PDF, DOCX)
Homework using the software (PDF, DOCX)
Student handout (PDF, DOCX)
Instructor guide (PDF, DOCX)
Links to videos (PDF, DOCX)
Survey questions and responses (PDF, DOCX)
Exam problem (PDF, DOCX)
Screen-capture, how-to video (AVI)

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Notes

The author declares no competing financial interest.

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