Making Enzyme Kinetics Dynamic via Simulation Software

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CONCEPTS ADDRESSED GUIDE

The demonstration has the potential to, but need not, address all of the following concepts. Instructors can decide if their students are ready for or even need some of the finer, more detailed topics. The information below can be used in conjunction with the instructor guide and how-to videos to allow each instructor to effectively implement this demonstration into their unique biochemistry course.

The Simulation Differs from Actual Experiments

The demonstration is performed with knowledge of all the rate constants in the reaction mechanism. Students are alerted to the fact that knowing these values in advance enhances the educational value of the simulation, but that often the goal of kinetic experiments is to determine these very rate constant values.

During the creation of the hallmark 'steady-state image' (Figure 2 main text), students are instructed that it is straightforward to know the initial E and S concentrations. This is because the experimenter added them, but it is much more difficult, and in some cases impossible, to accurately monitor E and ES concentrations during the reaction.[1] Conversely, tracking appearance of P or disappearance of S is straightforward if the product or substrate absorbs UV light or fluoresces. Together, the above statements highlight an important concept. A process or signal that is able to be simulated on the computer does not imply that the process or signal can be monitored in real life. A physical signal must exist that can be monitored.

Identifying the Steady-State Phase

Students are introduced to the unambiguous assignment of the beginning of the steady-state and the vagueness of its conclusion. The steady-state ends when the ES concentration has strayed 'too far' from its maximum such that the value of the ES complex is no longer 'steady'. Students are allowed to debate and wrestle with what it really means that the steady-state occurs when the change in ES concentration per time is approximately zero. How does one define 'approximately' to assign an unequivocal end to the steady-state portion of the reaction?

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Decreasing the Initial Enzyme Concentration Lengthens the Steady-State Phase

In order for all the observables (ES, E, P, S, and ES+E) to be clearly seen on the 'steady-state image' (Figure 2, main text), an initial E concentration was chosen that was only 5 fold less than the initial S concentration. However, steady-state experiments are typically set up with initial E concentrations << initial S concentrations. It is sometimes simply assumed to be the case and students are alerted to this fact. The importance of this assumption is displayed by comparing the duration of the steady-state phase when the initial E concentration is 5 fold, 50 fold, or 500 fold less than initial S (Figure S1). These initial E/S ratios lead to durations of the steady-state phase of 2, 29, and 280 seconds, respectively (as measured using a 10% decrease in the ES value as being approximately no change). With a significantly lower initial E concentration than initial S concentration, the ES concentration during the reaction is drastically lower than the S concentration. Therefore, for a longer period of time [S] + [ES] ≈ the initial S concentration and one can monitor either the initial linear disappearance of S or the initial linear appearance of P to determine the initial rate of reaction for a given initial S concentration. Thus, it is simpler to ensure that data are acquired during the steady-state phase of a reaction if the initial E << initial S because the duration of the steady-state increases.

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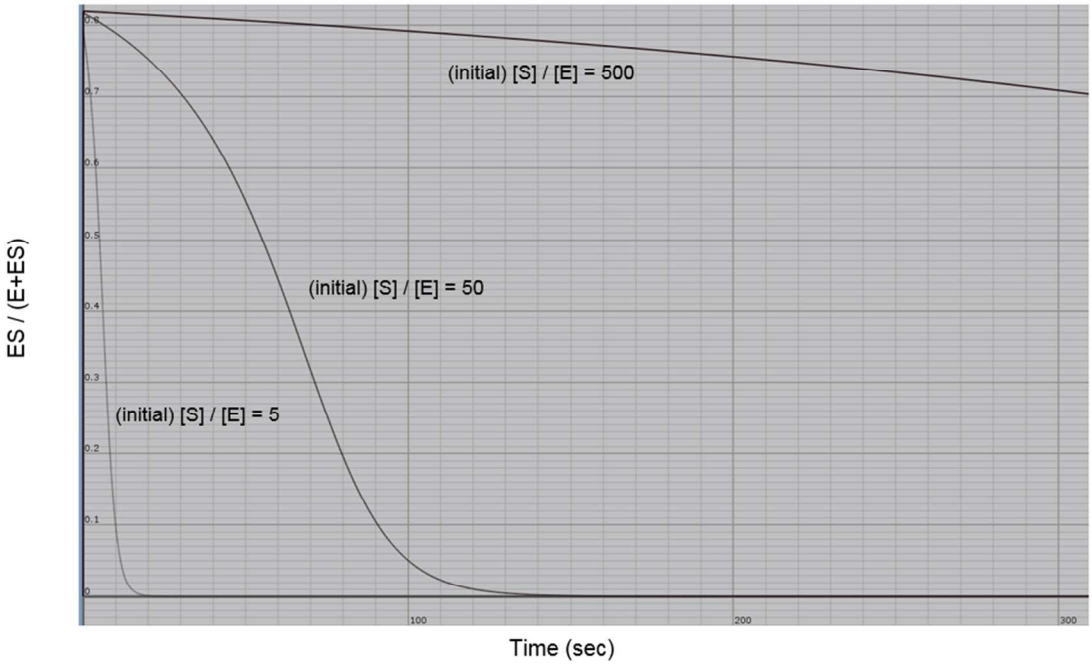


Figure S1. Importance of the assumption that the reaction conditions contain initial E << initial S for steady-state experiments. The black curves show the different ES concentration time traces that have been normalized by the total enzyme concentration (E + ES). Note that the steady-state duration increases as enzyme concentration decreases. The duration of the steady-state for the traces for the two higher initial E values are not able to be resolved on the time-scale shown in the image, but were quantified when observing smaller time-scales.

The 'Hyperbolic Curve Image' is Produced from the 'Initial Rates Image' Data

From experience, students tend to lack an understanding of the number of kinetic traces that are required to produce the hyperbolic Michaelis-Menten graph. This may stem from a general lack of attention given to axis labels on graphs. As multiple initial S concentrations are input into the software, it is stressed that each one of these values corresponds to a distinct experiment in which that unique S concentration is mixed with a fixed initial E concentration. Multiple product formation time traces are visualized and initial rates obtained from a linear fit taken during the steady-state phase. The numerical value of one initial rate is highlighted and the corresponding data point on the Michaelis-Menten graph is identified.

After creating the 'initial rates image' and the 'hyperbolic curve image', the connection between the two images is apparent to some students. However, the direct relationship between these two graphs may still be unclear to some and the connection of the graphs often takes some time to sink in. The simulation can emphasize and reinforce this connection as both of the 'dynamic images' are displayed on the software simultaneously (Figure S2, Panel A

or B and Figure 1, main text). Therefore, the effects of changing a reaction variable (eg. the initial E concentration or a rate constant) can be seen nearly instantaneously on both graphs, ensuring that students witness striking evidence that the graphs are interrelated and are merely different representations of the same data.

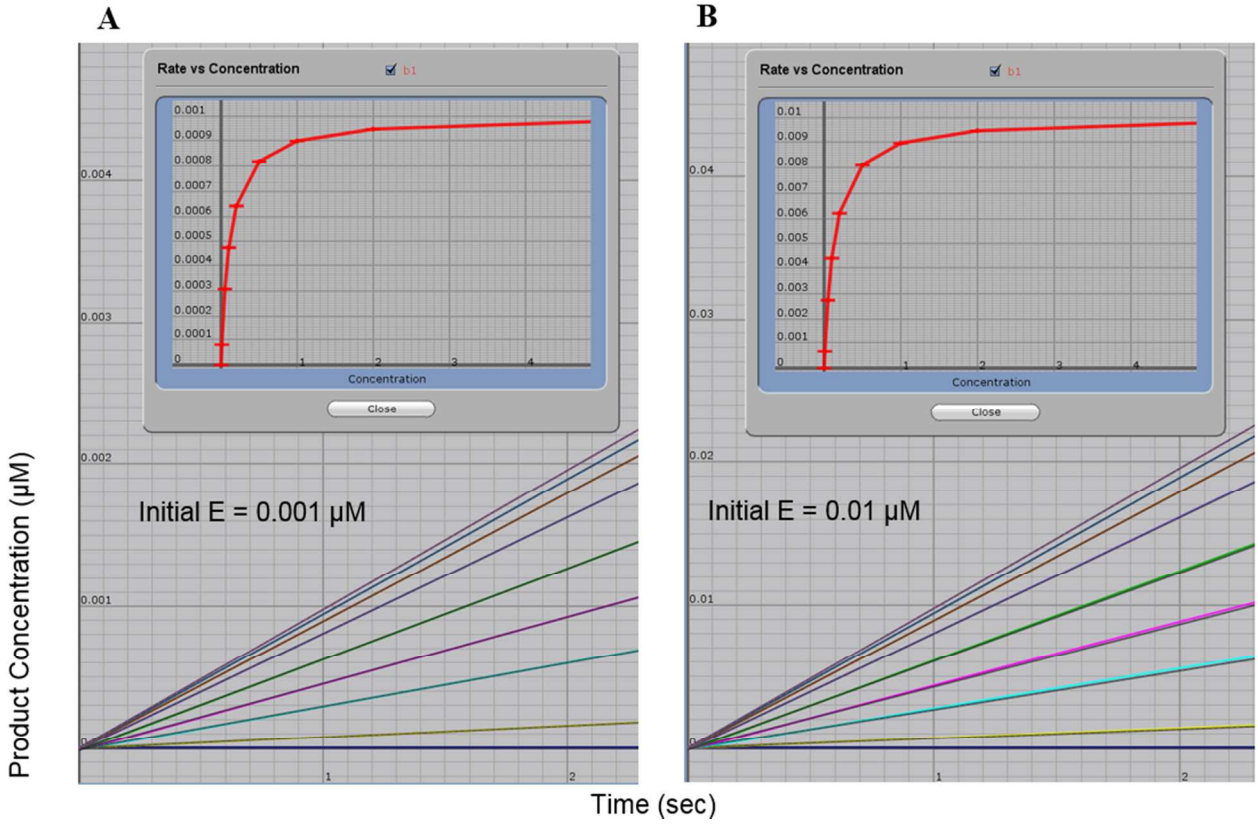


Figure S2. The software displays the 'initial rates image' data and the 'hyperbolic curve image' data at the same time (shown in both Panel A and B). This feature allows the instructor to change a variable and have the effect on both of the graphs be readily apparent to the students, thus providing a clear example of the relationship of the two graphs. Panel A shows data for an initial E value of 0.001 µM while Panel B shows data after changing the initial E value to 0.01 µM. Close inspection of the y-axis values will reveal that this change in initial E value produces an order of magnitude change in both the concentration vs time and rate vs concentration displays. Note the difference in v_{max} values between A and B, but also how, once normalized by the appropriate total enzyme concentration, both sets of data give rise to the same k_{cat} value of 1 sec^{-1}.

Normalizing v_{max} Yields k_{cat}

Changing the initial E concentration and directly observing the effects on both the 'initial rates' and 'hyperbolic curve' images naturally leads to a discussion of normalizing the initial rates by the total enzyme concentration. Calculating the same k_{cat} value from different hyperbolic rate versus concentration curves produced with different initial E concentrations highlights that k_{cat}

values, unlike υ_{max} values that depend on the initial E value used, do not change based on the initial E value (Figure S2, Panels A and B).

Non-Linear Curve Fitting of Hyperbolas

The 'hyperbola' that is shown via this demonstration is simply a result of the software 'connecting the dots' between the data points and is not a true hyperbolic fit as can be acquired if the data are plotted and fitted using non-linear regression to the Michaelis-Menten equation. The KinTek Explorer software is certainly capable of performing non-linear data fitting (and much more); however, the quick *Rate vs Conc* feature (Figure S2, Panel A and B) simply 'connects the dots' and is sufficient for the purposes of this demonstration. After completing the demonstration using the values given in Table 1 of the main text, the "experimentally" determined values of k_{cat} and K_M can be compared to the expected values of k_{cat} and K_M. To arrive at the "experimental" k_{cat} value, simply normalize the visually-estimated υ_{max} value by the total enzyme concentration. Referencing Figure 4 in the main text will show that the υ_{max} value can be visually approximated as 0.001 μM sec^{-1}. Dividing by the 0.001 μM total enzyme concentration returns an approximate value of 1 sec^{-1} for k_{cat}, the catalysis rate constant in the reaction shown in Equation 2 in the main text. Not surprisingly, this value of approximately 1 sec^{-1} agrees quite well with the 1 sec^{-1} value that was used as the rate constant to simulate the reaction. Additionally, plotting and fitting the data in Figure 4 in the main text to the Michaelis-Menten equation using KaleidaGraph[2] (to arrive at a k_{cat} value that was not visually estimated but is generated via a true hyperbolic data fit) shows less than a 0.2% difference from the 1 sec^{-1} value. (Compare 1 sec^{-1} to the m1 value, 1.0017 sec^{-1}, in Figure S3.) To arrive at the "experimental" K_M value, determine the initial substrate concentration when the velocity is at half its maximal value. Referencing Figure 4 in the main text will show that the visually-estimated "experimental" K_M value is approximately 0.10 μM. The mathematical equation for the K_M value for a reaction as in Equation 2 of the main text is given by Equation S1.

$$K_M = \frac{k_{-1} + k_2}{k_1} = \frac{10\ sec^{-1} + 1\ sec^{-1}}{100\ \mu M^{-1}\ sec^{-1}} = 0.11\ \mu M \qquad \text{(Equation S1)}$$

Therefore, there is less than a 10% difference between the visually-estimated and the mathematically calculated K_M value. Additionally, plotting and fitting the data in Figure 4 in the main text to the Michaelis-Menten equation using KaleidaGraph (to arrive at a K_M value that was not visually estimated but is generated via a true hyperbolic data fit) shows less than a 4% difference from the 0.11 μM value that was mathematically calculated. (Compare 0.11 μM to the m2 value, 0.11399 μM, in Figure S3.)

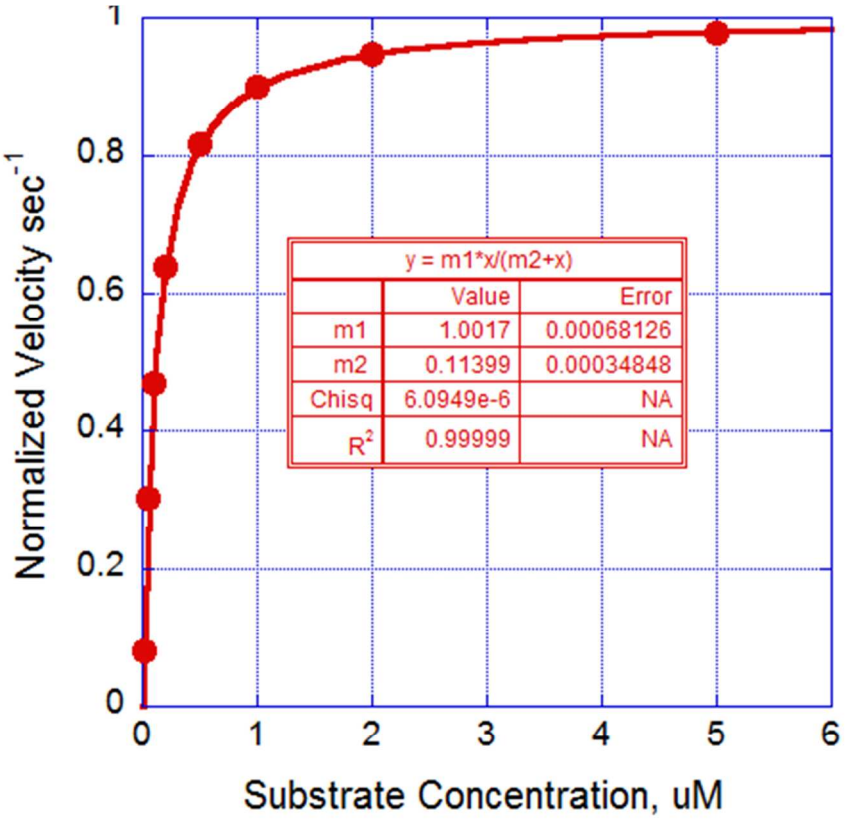
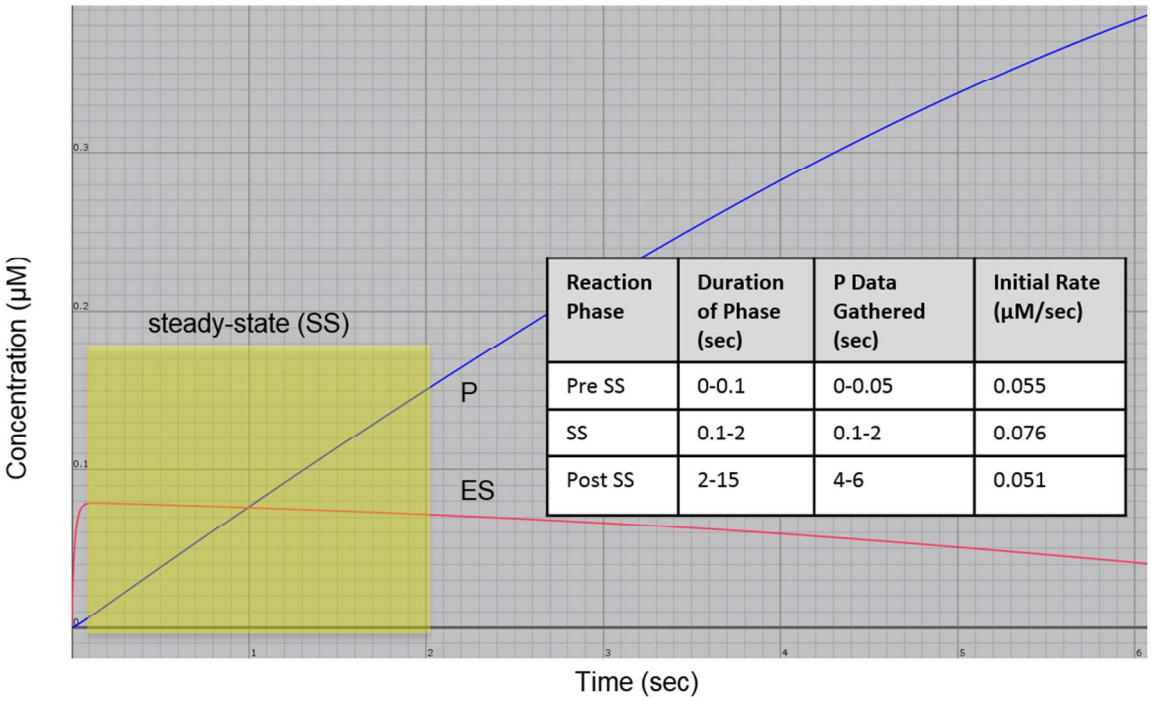


Figure S3. Values extracted from true hyperbola and 'connect-the-dots' hyperbola agree. The true hyperbolic fit of the initial rate data from the simulation normalized by the enzyme concentration is shown. The m1 value is the "experimentally" determined k_{cat} and m2 is the "experimentally" determined K_M.

Gathering Initial Rate Data Only During the Steady-State Phase

Proper care is not always given to ensure that initial rates are acquired only during the steady-state phase, as it may simply be assumed to be the case. The time interval of the simulation can be modified to observe initial reaction rates at areas outside the bounds of the steady-state. One way to demonstrate the outcomes of this ill-fated practice is shown in Figure S4. Initial rate values are compared that were acquired prior, during, or after the steady-state

portion of the reaction. Discrepancies in the data appear that increase as the data are acquired further from the steady-state. This technique requires more advanced knowledge of the software and the 'extended footage 2' in supporting information contains a video how-to of this step.



Reaction Phase	Duration of Phase (sec)	P Data Gathered (sec)	Initial Rate (µM/sec)
Pre SS	0-0.1	0-0.05	0.055
SS	0.1-2	0.1-2	0.076
Post SS	2-15	4-6	0.051

Figure S4. Initial rates depend on reaction phase. Data from pre, post, and during the steady-state portion of the reaction are shown. The yellow box highlights the steady-state portion of the reaction, the product time trace (P) is shown in blue, and the enzyme-substrate (ES) complex time trace is shown in red. The initial substrate concentration was 0.5 µM and initial enzyme concentration was 0.1 µM, but both 0.01 µM and 0.001 µM initial E values produced similar results when acquiring data outside of their respective steady-state ranges. Note how the initial rate value can vary by over 30% if the initial rate is not taken during the steady-state phase. This variance can drastically increase (+90%) if the portion of the P time trace used deviates even farther from the steady-state portion (10-15 sec) of the reaction (data not shown).

References

1. Mathews, C. K. *Biochemistry,* 4th ed.; Pearson Education, Inc.: Upper Saddle River, New Jersey, 2013; pp 431–433.
2. Synergy Software: An Overview of KaleidaGraph. http://www.synergy.com/wordpress_650164087/kaleidagraph/ (accessed August 2017)