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Virtual laboratory lessons in enzymology

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Abstract
The recent COVID-19 pandemic has led to widespread lock-down strategies that force universities to perform all educational activities remotely. In this context, laboratory lessons pose a significant challenge. Here, I present an on-line tool that simulates the kinetics of chemical reactions. Enzymatic mechanisms can be easily modeled and followed through time. In addition, professors can customize the interface to hide the reaction mechanism. This setting will force students to design virtual experiments to uncover the mechanism and obtain the relevant enzymatic parameters. While some of the skills developed in a practical lesson cannot be simulated, this tool can be used to teach students important concepts about data acquisition and processing.

KEYWORDS
computers in research and teaching, enzymes and catalysis, laboratory exercises

1 | INTRODUCTION

On-line tools have become a fixture in educational environments. This is particularly true for higher education, where these on-line methods sustain the concept of *blended learning*, loosely defined as a mix between face-to-face and remote lessons with emphasis on the ability of students to set their own pace with the help of on-line tools.[1] Blended learning shows promise as an enhancer of science education,[2] and has been increasingly adopted in the teaching of health sciences.[3] Furthermore, in European countries, the Bologna Process singles out blended learning as an endpoint in the strategy of harmonization, mobility, lifelong learning and quality assurance in universities.[4]

In addition to these incentives, on-line tools have suddenly become vital in higher education due to the global emergency caused by the COVID-19 pandemic. While professors can provide theoretical lessons and get immediate feedback from students through teleconferencing tools, laboratory work cannot be fully replicated remotely. This is unfortunate, as the diverse skills developed during laboratory lessons are indispensable for the curriculum.[5] Therefore, we need on-line tools that help students to acquire as many practical skills as possible through blended learning if we want to keep universities functioning during this crisis.[6]

In this work, I present the approach we followed in the Enzymology course at the University of Oviedo after lock-down was mandated in Spain. Since laboratory lessons had been scheduled for 2 weeks later, we set out to prepare alternative remote activities. According to the course guide, the relevant skills are:

- Design and implementation of enzymatic assays.
- Use of computer programs to analyze experimental results and determine kinetic parameters and other properties of enzymes.
- Oral and written presentation of information regarding enzymes, their biological importance and their practical applications.

During the course, I had been preparing a kinetics simulator for the students to work on concepts such as reaction mechanisms, mechanistic bottlenecks, and the steady-state approximation. We used this simulator as the basis for three virtual laboratory lessons:

1. Basic characterization of the kinetic parameters of an enzyme under the steady-state model. Students are expected to measure the initial velocity of the reaction (v_0) at a constant concentration of enzyme ($[E]_0$) and different concentrations of substrate ($[S]_0$). The results must then be fitted to the MICHAELIS–MENTEN equation (Equation (1)) and the generalized rate equation (Equation (2)) by different methods to obtain k_{cat} and K_M.

$$v_0 = \frac{k_{cat}[E]_0[S]_0}{K_M + [S]_0} \quad (1)$$

$$v_0 = \frac{([E]_0 + [S]_0 + K_M) - \sqrt{([E]_0 + [S]_0 + K_M)^2 - 4[E]_0[S]_0}}{2} \quad (2)$$

In a second experiment of progress kinetics, students choose values for $[S]_0$ and $[E]_0$ and follow the reaction until substrate depletion is evident. Then, they obtain a table with values of substrate concentration ($[S]$) through time and adjust those values to Equation (3) by variable transformation and linear fit.

$$\frac{1}{t}\log\frac{[S]_0}{[S]_t} = \frac{k_{cat}[E]_0}{K_M} - \frac{1}{K_M}\frac{[S]_0 - [S]_t}{t} \quad (3)$$

2. Basic characterization of two enzyme inhibitors. Students are expected to perform similar measures to the first lesson at different concentrations ($[I]_0$) of each inhibitor separately. From each experiment, they must obtain apparent values for k_{cat} and K_M. From these apparent constants, students infer the mode of inhibition and the inhibitory constant K_i.

3. Characterization of an atypical enzymatic reaction. Students are told to perform steady-state measurements on a virtual enzyme like in the first lesson. They are not told that this enzyme is inhibited by substrate. They are expected to infer this mechanism, characterize the relevant constants and assess whether the resulting model (Equation (4)) fits the data.

$$v_0 = \frac{k_{cat}[E]_0[S]_0}{K_M + [S]_0 + \frac{[S]_0^2}{K_s}} \quad (4)$$

2 | METHODS

2.1 | Simulator

The kinetics simulator was coded as a web page. It uses HTML, CSS, and javascript. The CSS code uses Bootstrap and the javascript code includes JQuery. All files are publicly available under the MIT license at https://github.com/vqf/kinetics. An example of use is also included, along with instructions for customization.

Briefly, a model is set as a combination of reactions and kinetic constants. Each species in the model is added to the interface, where users can set its concentration. The professor can hide some of those species programatically with javascript code. When the system is ready, the user presses a button and the simulation starts. The javascript code then generates the differential equations describing the model and offers an approximate solution by the finite difference method with adaptive time.[7] Namely, at each cycle the algorithm looks for impossible situations, such as negative concentrations, and automatically lowers the value of Δt. After several cycles, this value may gradually increase up to the default value of 0.02 s if the simulation is consistent.

Each concentration is shown in real time through a meter and a graph. Once the simulation is stopped, the user clicks on a given species and a table with the corresponding concentration at different times is copied into the system clipboard (Video S1). The starting time and the number of measurements per second in that table are also controlled programmatically. The professor can also set a noise level for the display and the table.

2.2 | Remote lessons

First, I prepared a separate web page for each session containing the corresponding model. Students were provided a script detailing the procedure for each lesson in advance. Sessions were conducted using the *meeting* feature of Microsoft Teams, using a *team* set up by the IT services at the University of Oviedo. At the beginning of each session, I offered a brief explanation and answered questions about the work, using the *share screen* feature to show examples of use.

Then, students worked individually setting and running each simulated experiment. No incompatibilities with any browser were reported. Each student analyzed their results with their tools of choice, except for non-linear fits, where they all used DynaFit v4.08.163.[8] Finally, each student prepared a report with results, analysis, and conclusions and uploaded it to Uniovi Virtual, a web platform maintained by the IT services at the University of Oviedo.

3 | RESULTS

3.1 | Kinetics simulator

The simulator is a web page with a simple interface (Figure 1 and Video S1). Models can be entered as a combination of reversible (with an *equal* sign) and
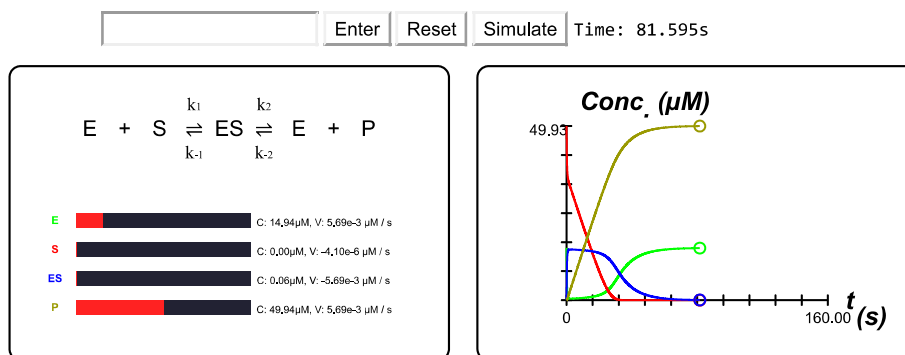
FIGURE 1 Kinetics simulator interface. The top row contains a text box and a button to *Enter* a reaction mechanism, a button to *Reset* the system to the last change and a button to *Simulate* the model. The lower left panel contains a representation of the model at the top, and, for each species, its name (in a different color), a meter showing its concentration (red and gray rectangles) and two labels showing its concentration (*C*) and rate (*V*). The lower right panel contains a graph that shows the concentration of each species during the simulation as a line the same color as its name in the left panel [Color figure can be viewed at wileyonlinelibrary.com]

irreversible (with a *minus* sign) reactions. Different reactions can be separated with a semicolon. For instance, a classic enzyme mechanism might be written as "$E + S = ES - E + P$" or "$E + S = ES$; $ES - E + P$" interchangeably.

Each reversible reaction is assigned two kinetic constants, whose values can be set by directly clicking on them. Irreversible reactions are given one kinetic constant. With this simple arrangement, professors and students can simulate fairly complex mechanisms. The limit to this complexity is given by the client browser, which must run the simulation.

3.2 | Lesson 1

3.2.1 | Steady-state kinetics

I set up a model based on equation "$E + S = ES = E + P$," with $k_{cat} = 20$ s^{-1} and $K_M = 15$ μM. The result contained noise following a quasi-normal distribution centered on the true value with $\sigma = 20$ nM. To show the shortcomings of real experiments, the simulator only gave values separated by 1 s after an initial time of 2 s. This corresponds to a typical experiment with a good spectrophotometer or fluorometer, taking into account the mixing time.

Students measured the initial speed of the reaction at $[E]_0 = 10$ nM and different concentrations of substrate. As intended, they noted the necessity of prior experiments to decide which concentrations to use in the design of the experiment. All students decided to paste the table with $[P]$ versus time into a Microsoft Excel sheet and perform linear regression to obtain v_0, in the understanding that this procedure is less sensitive to error than only computing the final concentration. Then, following my guidelines,

they used v_0 versus $[S]_0$ to calculate k_{cat} and K_M by the Eisenthal–Cornish–Bowden method[9] and a direct fit with a custom tool (data not shown). Students also learned how to fit the results to Equation (2) by non-linear regression (Figure 2).
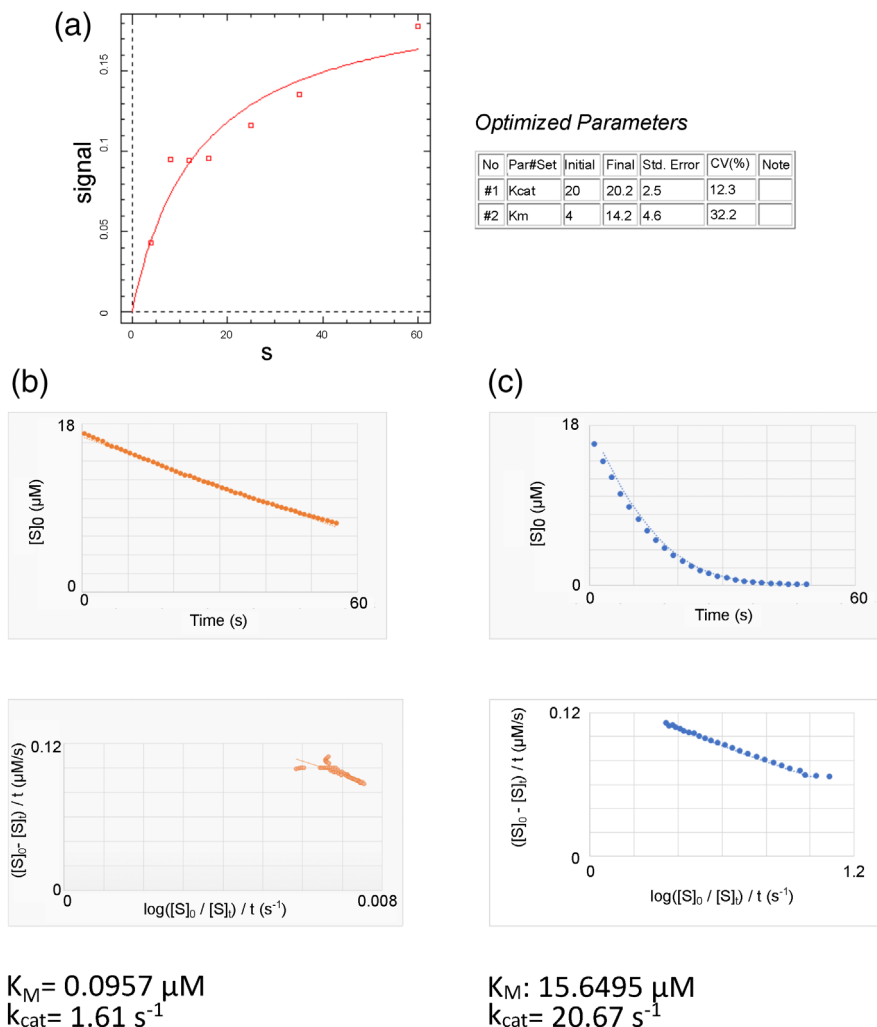
3.2.2 | Progression kinetics

Finally, students used the integrated form of the Michaelis–Menten equation (Equation (3)) to obtain the same parameters. They chose a value for $[S]_0$ close to the K_M value they had calculated and set up the reaction with a suitable $[E]_0$. They followed $[S]$ through time and transformed the variables to represent Equation (3) as a straight line. Interestingly, for this equation to yield meaningful values of the parameters, one needs to follow $[S]$ until the rate of loss is no longer linear. The results obtained by the students allowed me to emphasize this point (Figure 2).

3.3 | Lesson 2

3.3.1 | Competitive inhibition

I set up a model based on equations "$E + S = ES = E + P$; $EI = E + I$," with $k_{cat} = 20$ s^{-1}, $K_M = 15$ μM, and $K_i = 2$ μM. Students calculated k_{cat}^{app} and K_M^{app} by steady-state kinetics at different values of $[I]_0$. Since the mechanism was unknown to them, they first represented both parameters and their reciprocals as functions of $[I]_0$. They all realized that k_{cat}^{app} remained constant, whereas K_M^{app} increased linearly with $[I]_0$, and identified the mode of inhibition as competitive. I also asked them to

FIGURE 2 Determination of kinetic parameters. (a) example of direct non-lineal fit for Michaelis–Menten kinetics using DynaFit. (b) Fit for linearized progression kinetics where the reaction did not proceed long enough. (c) Same as in B, but the reaction proceeded long enough. Notice how the values for K_M and k_{cat} are more similar to those shown in panel (a) than those of panel (b). All panels were extracted and adapted from student reports [Color figure can be viewed at wileyonlinelibrary.com]

(a)

Optimized Parameters

No	Par#Set	Initial	Final	Std. Error	CV(%)	Note
#1	Kcat	20	20.2	2.5	12.3	
#2	Km	4	14.2	4.6	32.2	

(b)

K_M= 0.0957 µM
k_{cat}= 1.61 s^{-1}

(c)

K_M: 15.6495 µM
k_{cat}= 20.67 s^{-1}

represent a Dixon plot[10] and show that there was a point where all lines converged and which allowed a rough estimation of K_i (Figure 3).

3.3.2 | Uncompetitive inhibition

I set up a model based on equations "$E + S = ES = E + P$; $ESI = ES + I$," with $k_{cat} = 20$ s^{-1}, $K_M = 15$ µM, and $K_i = 6$ µM. Students proceeded like in the previous section and observed that both $1/K_M{}^{app}$ and $1/k_{cat}{}^{app}$ linearly depended on $[I]_0$. With both representations, they calculated similar values of K_i and correctly identified the mode of inhibition as uncompetitive. A Dixon plot showed parallel lines, as expected in this mode of inhibition (Figure 3).

3.4 | Lesson 3

I set up a model based on equations "$E + S = ES = E + P$; $ESS = ES + S$," with $k_{cat} = 10$ s^{-1}, $K_M = 4$ µM, and

$K_s = 20$ µM for the second reaction. Students characterized the mechanism by steady-state kinetics and immediately identified the enzyme as inhibited by substrate. They then used DynaFit to fit the data to Equation (4) and obtain the relevant parameters (Figure 4).

4 | DISCUSSION

The tools and activities described here allowed us to attain some of the practical aims described in the course guide under lock-down conditions. Thus, students got acquainted with the design of enzymatic assays and with the importance of prior tentative experiments. They also used both traditional and modern, computer-based methods to calculate kinetic parameters. The second and third lessons showed students how researchers need to identify the putative mechanism underlying their results and decide which one provides the best fit. Importantly, these lessons made them understand that this is not always easy, as several models can explain a given set of data, especially if these data are noisy.
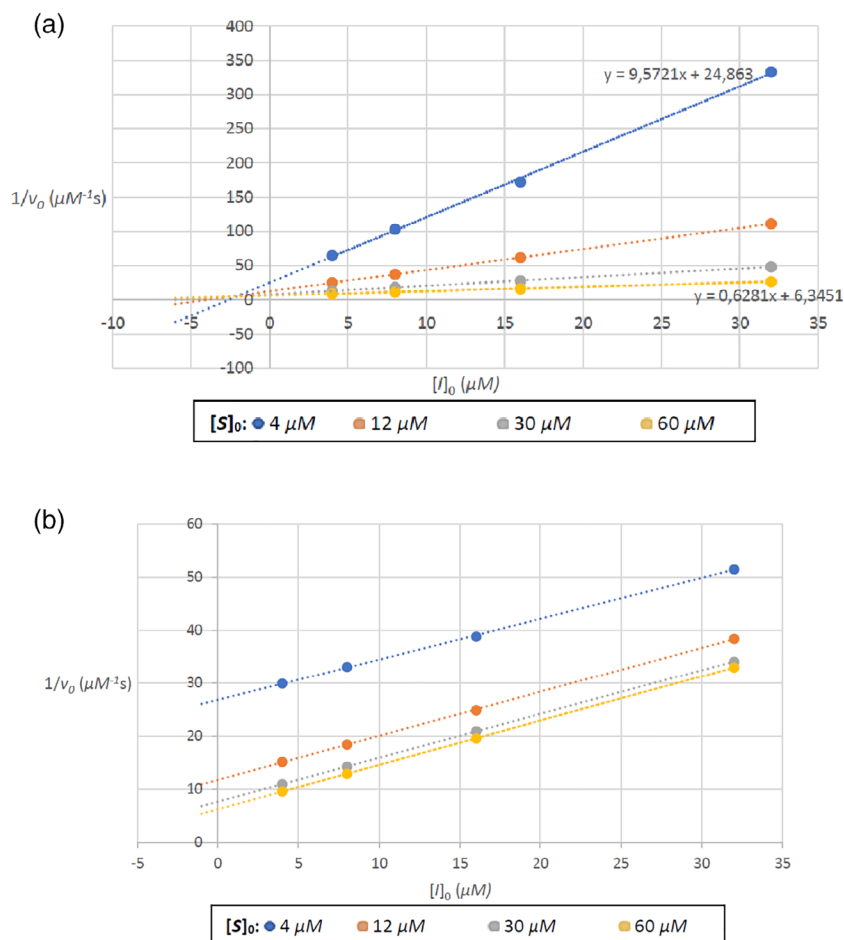
(a)

(b)

FIGURE 3 Dixon plots of inhibited reactions. (a) Competitive inhibitor. (b) Uncompetitive inhibitor. Both panels were extracted from student reports [Color figure can be viewed at wileyonlinelibrary.com]
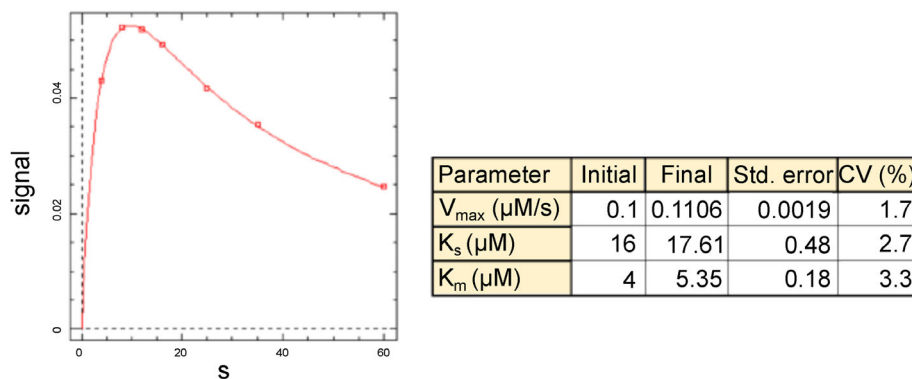

Parameter	Initial	Final	Std. error	CV (%)
V_{max} (μM/s)	0.1	0.1106	0.0019	1.7
K_s (μM)	16	17.61	0.48	2.7
K_m (μM)	4	5.35	0.18	3.3

FIGURE 4 Inhibition by substrate. Non-linear fit of data from lesson 3 to a model of inhibition by substrate, with calculation of K_s and other parameters. Extracted and adapted from student reports [Color figure can be viewed at wileyonlinelibrary.com]

Obviously, some of the necessary practical skills cannot be easily simulated. For instance, students did not practice preparing, diluting and mixing stock solutions to start a reaction. While it would be possible to provide a more realistic simulation of these processes, the tools presented here are not intended as a substitute for laboratory lessons. Rather, they should be used primarily by the students to understand the concepts showed in the classroom at their own pace. In our experience, they are also useful to illustrate concepts like steady-state kinetics and kinetic modeling in practical classroom lessons. Easy improvements to the simple examples presented here include adding a nonenzymatic reaction so that students need to subtract it. This would be as simple as using a mechanism of the type "$E + S = ES = E + P$; $S = P$," and it would illustrate the need for a control reaction.

The design of the simulator as a web page allowed all students to run the mechanisms with their computers and tablets (it should also work on cell-phone browsers). However, in this remote setting professors need to adapt to

uneven computer and network resources. For instance, two of the students had problems to run DynaFit on their computers. We solved this problem by using the *share desktop* and *take control* Microsoft Teams features. This way, both students could use my computer to run DynaFit on their own data.

In summary, we have followed the principles of blended learning to develop some of the practical skills involved in enzymology in a remote environment. This allowed us to cope with the emergency measures forced by the COVID-19 pandemic. Hopefully, once we are able to restart laboratory lessons, we will keep using these tools in their intended settings, namely practical lessons in the classroom and self-paced work by the students.

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REFERENCES

1. Margolis AR, Porter AL, Pitterle ME. Best practices for use of blended learning. Am J Pharm Educ. 2017;81:49.
2. Stockwell BR, Stockwell MS, Cennamo M, Jiang E. Blended learning improves science education. Cell. 2015;162:933–936.
3. Liu Q, Peng W, Zhang F, Hu R, Li Y, Yan W. The effectiveness of blended learning in health professions: Systematic review and meta-analysis. J Med Internet Res. 2016;18:e2.
4. Commission/EACEA/Eurydice E. The European Higher Education Area in 2018: Bologna Process Implementation Report. Luxembourg: Publications Office of the European Union, 2018.
5. Anwar YAS, Senam S, Laksono EW. The use of orientation/decision/do/discuss/reflect (OD3R) method to increase critical thinking skill and practical skill in biochemistry learning. Biochem Mol Biol Educ. 2018;46:107–113.
6. Procko K, Bell JK, Benore MA, et al. Moving biochemistry and molecular biology courses online in times of disruption: Recommended practices and resources—A collaboration with the faculty community and ASBMB. Biochem Mol Biol Educ. 2020. https://doi.org/10.1002/bmb.21354.
7. Perez-Silva JG, Araujo-Voces M, Quesada V. nVenn: generalized, quasiproportional Venn and Euler diagrams. Bioinformatics. 2018;34:2322–2324.
8. Kuzmic P. DynaFit—A software package for enzymology. Methods Enzymol. 2009;467:247–280.
9. Eisenthal R, Cornish-Bowden A. The direct linear plot. A new graphical procedure for estimating enzyme kinetic parameters. Biochem J. 1974;139:715–720.
10. Dixon M. The determination of enzyme inhibitor constants. Biochem J. 1953;55:170–171.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.