webZyme – A Web-Based Enzyme Simulation System for Education and Research

Rebecca L. Fagan, Brian Haber, and Bruce A. Palfey
Department of Biological Chemistry, University of Michigan Medical School, Ann Arbor, MI USA

Introduction

Kinetics is critical for discovering the mechanism of enzyme action and many other important processes in biochemistry. Such studies clearly form the basis for the detailed understanding we have of flavoenzymes. Unfortunately, students have trouble learning this vital subject. The mathematics intimidates many students and they fail to grasp its relation to experimental observables. Furthermore, the reasoning typical in classrooms and textbooks flows from postulated schemes to predicted results; this is opposite to what occurs in the laboratory, where the kineticist constructs reaction schemes based on experiments and designs new experiments to test the evolving model. Thus, traditional classes in biochemical kinetics often fail to convey the power of this vital subject. Extensive lab work could fill this gap, but is not generally practical. Kinetics, especially transient kinetics, can require expensive instruments, biochemicals, and enzymes and often requires long hours in the lab. Computer simulations could serve as a practical alternative to lab exercises. Many computer programs are available to solve the differential equations describing enzyme reactions, with some explicitly designed for biochemistry. However, these simulate mechanisms that are known *a priori* by the student – there is no discovery, which is the essence of a scientific investigation.

Our approach is different. We are developing a system which we call webZyme that simulates enzymes on a web-server and is used through a browser. The enzyme to be simulated is a "black-box" created by the instructor but unknown to the student. The behavior of the experiments are controlled by differential equations, rate constants, and other parameters defined by the instructor, and by the concentrations, wavelengths, etc., chosen by the student. Experimental data with realistic noise are returned to the student for analysis. Students can hypothesize mechanisms based on their results and test their ideas with new experiments that they design. Exercises can be created for beginning students that are as simple as demonstrating the Michaelis-Menten behavior of a single-substrate enzyme, or, for graduate students, as complex as determining the spectra of transient intermediates in stopped-flow experiments.

Materials and Methods

webZyme is a system of webMathematica (Wolfram, Inc.) packages and JavaScript-based web-pages which simulates experiments from web-browsers. The computational engines are Mathematica packages which communicate through Apache Tomcat. Instructors and students interact with webZyme through web-pages driven by JavaScript. Data collected from web-pages are checked for errors and delivered to appropriate Mathematica packages. Data collected from the instructor define the reaction mechanism. Rate constants, realistic absorbance spectra (approximated as sums of Gaussians), and the noise-level on data also come from the instructor. The Mathematica package parses this input, creates directories related to the exercise, and writes files defining the mechanism and absorbance spectra. Exercises may be recalled, modified, and saved for other students.

Students use webZyme by entering reactant concentrations, data-collection time-ranges, and the wavelength of data collection on a web-page driven by JavaScript. After initial error-checking, these parameters are sent to a webMathematica package, which also reads the files created by the instructor defining the exercise. Mathematica uses this information to numerically solve the systems of differential equations for the concentrations as a function of time, which are used to calculate the absorbance trace. Realistic noise is added, the trace is sampled at intervals specified by the student, and data are returned both as a simple text file to be downloaded from the server and analyzed with other software, and as gif images displayed on the web page.

Results

A prototype of webZyme was used with great success at the University of Michigan in the Winter semesters of 2007 and 2008 in Biological Chemistry 673, a graduate course on enzyme mechanisms and kinetics. Students became adept at using webZyme after their first assignment, and their opinion of it in course evaluations was generally positive and often enthusiastic. Unique enzymes were routinely created for a class of three-dozen students without difficulty. As of this writing, the capabilities of webZyme are limited to stopped-flow experiments, but it is clear that webZyme can be developed in a straight-forward way into a broadly useful tool. The flexibility of webZyme allowed students to conduct virtual experiments of any type normally performed in a stopped-flow instrument, including: burst analyses, single turnovers, partial reactions, binding reactions, competition experiments, and enzyme-monitored turnover. The use of webZyme is illustrated here with an example similar to those from Biological Chemistry 673.

Creating enzymes

The instructor creates an enzyme for each student by entering information into the Professor's Page (Figure 1). In this example, the catalytic cycle of a simple oxidase

Figure 1. Reaction entry table of the Professor's Page. Chemical reactions are written in a simple syntax in boxes on the left, and rate constants for these steps are entered in the boxes on the right.

is entered. The problem is identified by the student's name or ID number and the problem-number (an integer). Reactions are created by entering simple, chemically intuitive syntax into a table, and entering values for rate constants in the appropriate boxes (Figure 1). The allowed wavelength range is entered, and the spectra of selected species are defined as sums of Gaussians whose parameters are entered in another table (Figure 2). The instructor also enters a list of the species that will be available to the student to load into the syringes for stopped-flow experiments. The noise-level to be used on absorbance data is also entered. Hitting the Create button sends the parameters to a Mathematica package, which creates a number of directories and files that define the exercise, and a web-page that the student uses to run the exercise. This example is available at: http://brupalf.googlepages.com/flavinista

Performing experiments
Students investigate the mechanism of their enzyme by performing simulated stopped-flow experiments on the web-page created by the instructor (Figure 3). The reagents available to the student are listed on the web-page. The student loads the syringes of their stopped-flow instrument by filling the tables with the names and concentrations of the reactants. Students are free to load any combination of reactants and concentrations into either syringe. Before simulating the reaction, webZyme will first calculate the equilibrium composition of each syringe. This

Wavelength Range [nm]:

300 to 600

Enter data in this form:

$$mu * e^{\left(\dfrac{-(Lambda - omega)^2}{sigma}\right)}$$
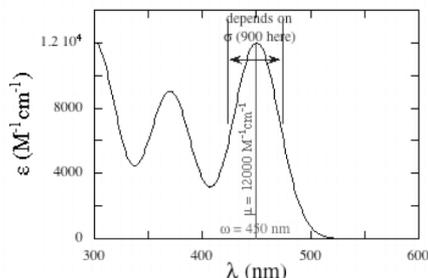
Enter Species	Peak #1			Peak #2			Peak #3			Peak #4		
	mu	omega	sigma	mu	omega	sigma	mu	omega	sigma	mu	omega	sigma
Eox	11800	450	800	9050	380	500	25000	280	2000	900	465	20
Ered	1000	380	6000	3500	370	600	25000	280	2000	0	0	1
EoxS	11500	455	800	9000	380	500	25000	280	2000	900	470	20
EredP	2000	400	3000	3600	370	600	25000	280	2000	1700	455	1000
	0	0	0	0	0	0	0	0	0	0	0	0

Figure 2. Spectrum entry table of the Professor's Page. Spectra are approximated as sums of Gaussians, defined by the peak-position (ω), height (μ), and width (σ). The instructor also enters the allowed wavelength range.

allows users to make preformed enzyme-ligand complexes to react with another reagent. Students also enter a schedule for data collection, defined by up to four time-blocks, and the wavelength of observation. The experiment is initiated by clicking SHOOT. The results are returned to the student as five pictures of the reaction – one of the whole trace on a logarithmic timescale, and four scaled according to the time-blocks in the data collection schedule. This immediate graphical feedback allows students to adjust their experiments interactively. The data can also be downloaded as a space-delimited text file for further analysis in a fitting program. The flexibility of webZyme allows a student to perform many types of experiments, such as reductive half-reactions and enzyme-monitored turnover (Figure 4).

Students can explore particular aspects of the chemistry in detail and draw conclusions about the mechanism. As an example, the oxidative half-reaction was performed using five concentrations of O_2 (Figure 5). Data were saved from the server and fit to a single exponential using Kaleidagraph. A plot of k_{obs} as a function of O_2 concentration gave a straight line with a slope of 1.47×10^5 $M^{-1}s^{-1}$, reproducing the value entered by the instructor (Figure 1).

*Remember - Mixing dilutes by a factor of 2!

Syringe 1		
Species	Concentration [M]	
Eox	4e-5	M
		M
		M
		M

Syringe 2		
Species	Concentration [M]	
S	2e-3	M
		M
		M
		M

Available Reagents
Eox, Ered, S, O2

| Time Intervals			
Time Interval	tMin [sec]	tMax [sec]	Sample Points
#1	.001 s	.01 s	100
#2	.01 s	.1 s	100
#3	.1 s	1 s	100
#4	1 s	20 s	100

Wavelength

450 nm

Figure 3. Student's Page. The reactants available to the student are listed in the upper-right. Students fill the syringes of their stopped-flow instrument with the upper tables, designate a data-collection schedule in the lower table, and a wavelength for data collection in the box below. A reaction is initiated by clicking SHOOT.
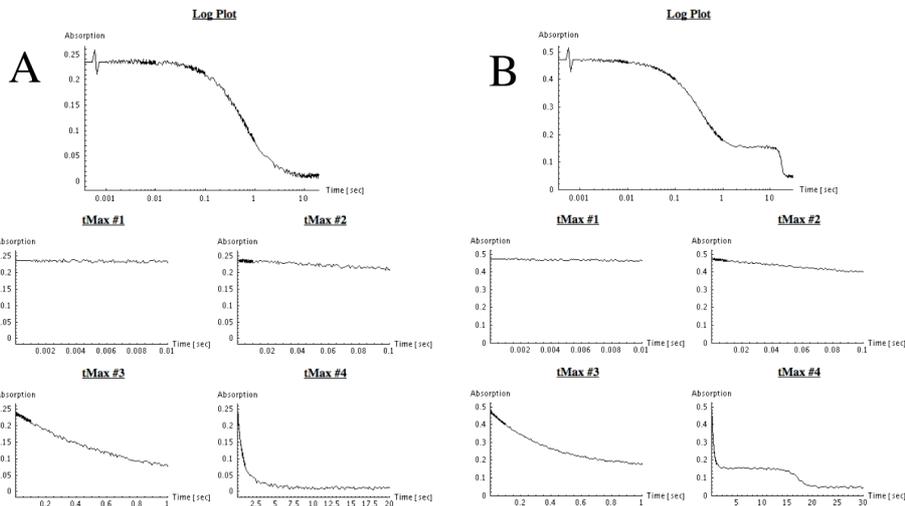
Figure 4. Sample graphical output. webZyme returns a picture of the reaction trace on a logarithmic timescale, and four pictures on linear timescales defined in the data-collection schedule. **A** shows a reductive half-reaction, while **B** shows enzyme-monitored turnover with limiting O_2.

Discussion

webZyme engages students in the process of discovery and model-building used by enzymologists. A web-based approach is much cheaper than actual lab exercises and is much more versatile. Because the simulations are run through a web-browser, students need only rudimentary computer skills, allowing them to focus on the science. Delivering exercises by the web avoids the need to install software on large numbers of computers and allows students to work wherever it is convenient. Instructors can create reaction mechanisms of arbitrary complexity without struggling with practical problems such as enzyme availability or stability.
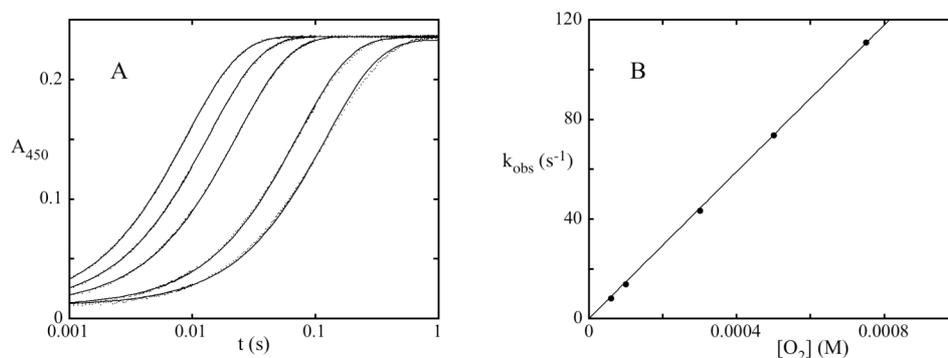
Figure 5. Analysis of the oxidative half-reaction. Reactions were simulated with webZyme using five O_2 concentrations maintaining pseudo-first-order conditions (**A**). Note the logarithmic timescale. Traces were fit to single exponentials, giving the concentration dependence shown in **B**.

The design of webZyme has been guided by a balance between realism and convenience. Key features that enhance the realism include normally distributed noise on reaction traces, an upper absorbance limit of 1.5, a dead-time of 1 ms, and the calculation of the equilibrium composition of each syringe prior to mixing. Taken together, the realism of webZyme allows students to design good or bad experiments, and fosters the development of good judgment in experimental design.

Potential as a research tool

The easy user interface and its emphasis on realistically representing experiments could make webZyme a useful simulation tool for research with unique advantages over other simulation packages currently in use. Its web-based design could allow users at distant locations to work collaboratively on simulating a kinetic model; each user could modify the kinetic model through the Professor's Page and run the simulation through the Student's page. Facilities for importing real experimental data and for fitting are planned, in order to enhance its utility for research.

Distribution

webZyme will be made available in two ways to all interested academic users. For those who wish to run webZyme on a server at their institution, our code for webZyme, consisting of JavaScript web-pages and webMathematica package files, will be supplied at no charge. Such users would need to obtain and install webMathematica independently. Alternatively, academic users will be allowed to create webMathematica exercises on a server in the Department of Biological Chemistry at Michigan for no charge in the foreseeable future. Those interested in using webZyme should contact Bruce Palfey by e-mail (brupalf@umich.edu).