USING GEOGEBRA TO PRESENT KINETIC DATA AND LIGAND BINDING DATA TO A BIOCHEMISTRY CLASS

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Abstract: There are two hyperbolic functions commonly presented in Biochemistry classes. Both of these functions, the Michaelis-Menten enzyme activity curve and the Scatchard ligand binding isotherm, are routinely re-expressed using a linear transform in order to determine the rate and binding constants inherent in each model. We will demonstrate how these transforms, when presented in GeoGebra [1], make useful and interactive [1] [2] [3] teaching tools.

Introduction to the Michaelis-Menten enzyme activity curve

One of the most common mathematical presentations given in a Biochemistry class is that of the simple Michaelis-Menten enzyme activity curve, [2]. This curve is important because it illustrates a mathematical expression of the rate at which enzymes create product from a substrate molecule. This presentation usually derives the following hyperbolic expression from first principles involving a fixed concentration of the enzyme and various concentrations of the substrate:

$$V = \frac{V_{max} s}{K_m + s} \tag{2.1}$$

where V is the velocity of an enzyme converting substrate to product, V_{max} is a constant that describes the maximum rate of this conversion, K_m is the Michaelis-Menten constant specific for the enzyme in question, and s is the substrate concentration. The resulting hyperbolic function is no longer a challenge to modern computer fitting programs, but in the old days it was useful to linearize the hyperbolic Michaelis-Menten curve into the double reciprocal Lineweaver-Burk space, [3]. In that space it is straightforward to determine K_m and V_{max}.

In Figure 2.1, the Lineweaver-Burk plots are the dashed lines, with $1/V_{max}$ as the intercept on the $1/V$ axis and V_{max}/K_m is the slope. Further, the intersection of the line with the $-1/s$ axis in the second quadrant is $-1/K_m$. For the purpose of instruction, it is useful to show the effects on the Lineweaver-Burk plot due to three different types of enzyme inhibition by a second molecule. These three common types of inhibition are:

1. Competitive inhibition, where the second molecule also binds at the enzyme's active site. This produces a Lineweaver-Burk plot with a higher slope but the same V_{max}.

[1] Michaeis Menten: `http://tube.geogebra.org/material/show/id/1491085`
[2] Scatchard: `http://tube.geogebra.org/material/show/id/1491063`
[3] SCATSUM: `http://tube.geogebra.org/material/show/id/1498145`

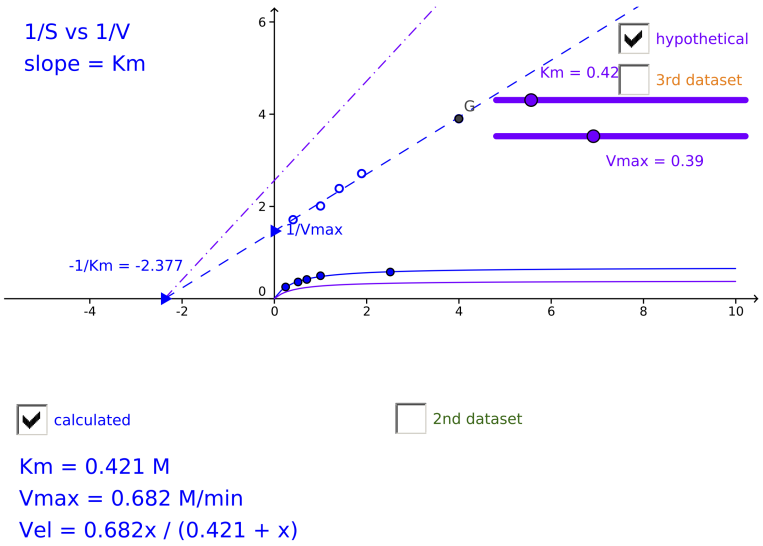

Figure 2.1 Both Michaelis-Menten and Lineweaver-Burk data are plotted in the same GeoGebra space

2. Noncompetitive, where the second molecule binds at a different site on the enzyme but also causes inhibition, which produces a Lineweaver-Burk plot with lower slope but the same K_m.

3. Uncompetitive; where the second molecules only binds to the enzyme after the substrate binds to the active site, which produces a Lineweaver-Burk plot parallel to the original plot with changes in both V_{max} and K_m.

The changes in both the Michaelis-Menten and the Lineweaver-Burk lines are readily apparent on the GeoGebra plot when used in the interactive mode. The two hyperbolic Michaelis-Menten curves seen at the bottom of Figure 2.1 appear only slightly different. When these are transformed into the Lineweaver-Burk space (dashed lines) the difference in the two K_m and V_{max} values are much more readily apparent. Note that Figure 2.1 shows an example of Competitive inhibition since K_m is different in the two dashed lines but V_{max} is the same.

Method 1

How the GeoGebra charts were made: Given a set of data in the spreadsheet, we can use a GeoGebra command to create a list of points. This list will have the property that the points will update when we change values in the spreadsheet. The traditional biochemistry technique for the data is to plot the point that has absicssa equal to the reciprocal of the x-value and ordinate equal to the y-value. In GeoGebra this is done through the `Sequence` command to create a new list of points:

`list2=Sequence[(1/x(Element[list1,i]),1/y(Element[list1,i])),i,1,Length[list1]]`.

From that list, we can use the `FitLine` command to do the least squares regression.

Mathematically, this is interesting for a few reasons. First, it is an excellent real world application of rational functions, which seem to be few and far between. This even has the bonus of a contextually significant horizontal asymptote. The asymptote is the maximum velocity of the enzyme's ability to convert substrate to product at the given conditions of temperature and pH. Also, the traditional biochemist's technique of linear transformation is a nice bit of algebra. If $y = ax/(b+x)$ then $1/y = (b+x)/(ax) = (b/a)(1/x) + (1/a)$. where $1/y$ is linear with respect to $1/x$. Then we can see the y-intercept of this line is the reciprocal of the maximum velocity, and the slope of the line lets us derive the K_m parameter from knowing the maximum velocity.

Introduction to the Scatchard binding isotherm

The second type of hyperbolic function concerns the binding interactions of macromolecules and smaller ligand molecules and is a branch of biochemical kinetics. This type of kinetics is often treated in advanced undergraduate biochemistry courses or in qualifying graduate courses. The goal of these kinetic studies is to determine both the binding affinity of the smaller ligand molecule for the macromolecule and the number of potential binding sites available to the ligand often using radiolabelled ligands. The binding kinetics are described mathematically as

$$B = \frac{nFK_a}{1 + FK_a} \tag{2.2}$$

where
B is the amount of ligand bound to the macromolecule,
n is a constant that describes the maximum number of ligand binding sites on the macromolecule,
Ka is the association constant specific for the ligand in question, and
F is the free substrate concentration.

The resulting hyperbolic binding function is also no longer a challenge to modern computer fitting programs, but in the old days it was useful to linearize the binding function into the well-studied Scatchard binding isotherm [4] by plotting Bound/Free on the y-axis and Bound on the x-axis.

$$B/F = nKa - BKa \tag{2.3}$$

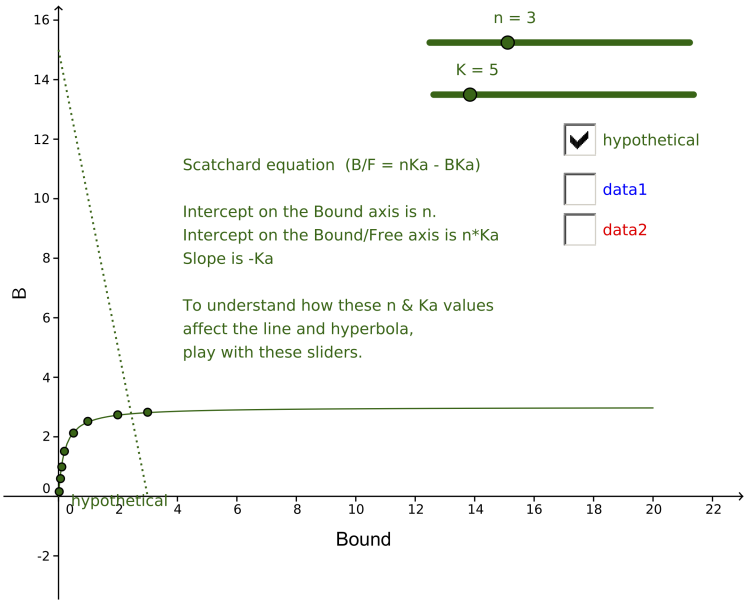

Figure 2.2 Example of a linear Scatchard plot with 1 class of equivalent binding sites

In those biochemistry lectures, it is usually pointed out that a linear Scatchard plot results when binding involves a single class of equivalent and independent sites. In that case it is straightforward to determine Ka and n, since $-Ka$ is the slope of the line, nKa is the intercept on the B/F axis and n is the intercept on the B axis, as shown in Figure 2.2. Often examples are given to illustrate the effects of variation in Ka and n on the Scatchard line shape.

In more advanced classes, examples of ligand binding that produce non-linear Scatchard plots are examined. In contrast to the simple linear plot, a non-linear Scatchard plot is produced when two or more non-equivalent binding sites exist on the macromolecule for the same ligand. The non-linear Scatchard plot is the vector summation of a number of straight line Scatchard plots[5]. Each line corresponds to an appropriate Equation 2.3. The total concentration of bound ligand (B) is the sum of concentrations of all the species of bound ligand, where each kind of bound ligand is characterized by its intrinsic binding constant to its own binding site.

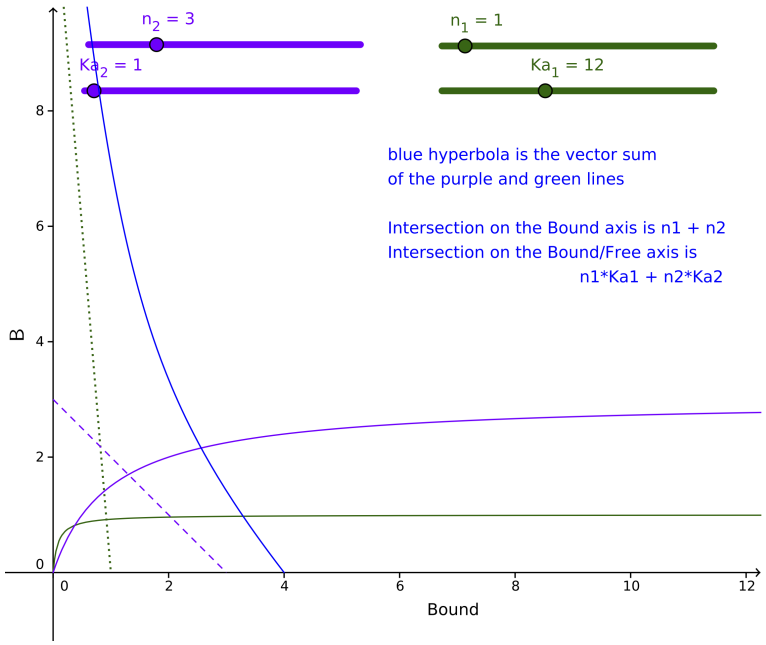

Figure 2.3 Example of a non-linear Scatchard plot with 2 classes of equivalent binding

In the non-linear type of Scatchard plot as shown in Figure 2.3, the intercept on the Bound (B) axis is the sum of the individual ligand binding numbers, n values, ($n_1 + n_2 + \ldots + n_j$) and the intercept on the bound/free (B/F) axis is the sum of the individual nKa products ($n_1Ka_1 + n_2Ka_2 + \cdots + n_jKa$). More directly, in Figure 2.3, the blue hyperbolic line represents the experimentally derived, non-linear Scatchard line. The intercept on the Bound axis is 4 and the intercept on the bound/free axis is 15. Since this curve is non-linear, we know it is formed by two or more straight lines; in this case two straight lines. By definition, the simple Scatchard plot is linear with a slope of $-Ka$, a Bound intercept of n and a bound/free intercept of nKa, as in Figure 2.2. So the program allows the user to define the two component Scatchard lines and then does the vector addition to draw the non-linear composite line which would be found experimentally given the conditions of the two component lines. In Figure 2.3 the first simple Scatchard line is defined by $n_1 = 1, Ka_1 = 12$. The second simple Scatchard line is defined by , $n_2 = 3$, and $Ka_2 = 1$. This represents in nature the idea that the macromolecule has two types (classes) of response to the same binding ligand. The first class is a strong binding response with a Ka value of 12. This response is specific with an n_1 value of 1 and usually is employed when ligand availability is low. The second class of response is weaker with a Ka_2 value of 1 and is non-specific with an n_2 value of 3. This weak class of response is employed when ligand availability is high. These conditions are often seen in circulating blood proteins when there is a single, high affinity binding site represented by the $n_1 = 1$ line and also a number of low affinity, non-specific sites represented by the $n_1 = 3$ line. Note that in the hyperbolic summation line, the intercept on the Bound axis is $n_1 + n_2 = 4$ and the intercept on the bound/free axis is $n_2Ka_1 + n_2Ka_2 = 15$ as would be expected by theory.

Second Method

The simplest example of a non-linear Scatchard plot involves the case of two classes of independent sites. To produce this type of plot precisely, the sheet, SCATSUM, was written in GeoGebra. SCATSUM uses the curve peeling method of Rosenthal [5] as a model to construct the resultant Scatchard curve from two straight Scatchard lines. This task is achieved by vector addition of points on each straight line. Rosenthal's original method offers a full explanation of deconvoluting a curved Scatchard plot into its straight line components. Here, we are doing the reverse, starting with the known, n_1, Ka_1, n_2 and Ka_2 values that form straight lines and creating the resulting curvilinear Scatchard line.

Biochemists making a multiple variable Scatchard plot decompose the data into lines fit to data in (Bound, Bound/Free) form. These are the decomposition of a hyperbola that is the vector sum of these lines. This is a very unusual construction in mathematics and quite interesting! In order to do this in the GeoGebra sketch, the lines were converted to a common parametric form, $y = ax + b$ to $(b/(t-a), bt/(t-a))$ where $t = 0$ corresponds to the x-intercept. This allows us to easily add the lines. Parametric curves are graphed in GeoGebra using the Curve function. For example, the curve in the SCATSUM sketch is

```
Curve[Ka_1 n_1/(t+Ka_1)+Ka_2 n_2/(t+Ka_2),t(Ka_1 n_1/(t+Ka_1)+Ka_2 n_2/(t+Ka_2)),t,0,100]
```

Remember, in the Scatchard context, Ka is the opposite of the slope, and n is the x-intercept. So the y-intercept of the Scatchard lines is nKa.

In SCATSUM, the sliders allow the user to create their own n_1, Ka_1, n_2 and Ka_2 values. These values are used to create the two linear Scatchard lines, then these lines are summed by vector addition to form the resultant non-linear curve. SCATSUM output utilizes the Ka and n values as well as a column of fixed Free ligand values, column A, in the spreadsheet portion. Column B shows the list of generated values on the Bound axis and column C shows the generated values on the Bound/Free axis associated with one set of n and Ka values. Columns D and E respectively show the Bound and Bound/Free values associated with the other set of n and Ka constants. Because SCATSUM will produce precise Bound and Bound/Free values for the curvilinear Scatchard plot, the student can use those values and a different curve fitting program to determine the constants using the summation equation

$$B = \frac{n_1 F K a_1 + n_2 F K a_2}{(1 + K a_1 F)(1 + K a_2 F)}. \tag{2.4}$$

Discussion

In GeoGebra, the `Function` command can be used to restrict the domain of a function.
`Function[<Expression>,<Parameter Variable 1>,<Start Value>,<End Value>]`.
This is nice in modeling contexts, to have the start value and the end value be physically reasonable limits. Using this function and the Sliders in GeoGebra, one can quickly and dramatically present the effect of changing parameters on changes in line shapes. We hope to further develop a suite of line transformations that would be useful to biochemistry students such as irreversible/covalent inhibitors, cooperativity and Hill coefficients and mixed inhibitors.

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