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A teacher-developed inquiry model to teach the molecular basis of hyperbolic kinetics in biological membrane transport

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Marcus L, Plumeri J, Baker GM, Miller JS. A teacher-developed inquiry model to teach the molecular basis of hyperbolic kinetics in biological membrane transport. Adv Physiol Educ 37: 165–175, 2013; doi:10.1152/advan.00010.2013.—A previously published classroom teaching method for helping students visualize and understand Michaelis-Menten kinetics (19) was used as an anticipatory set with high school and middle school science teachers in an Illinois Math and Science Partnership Program. As part of the activity, the teachers were asked to collect data by replicating the method and to analyze and report the data. All concluded that the rate data they had collected were hyperbolic. As part of a guided inquiry plan, teachers were then prompted to reexamine the method and evaluate its efficacy as a teaching strategy for developing specific kinetic concepts. After further data collection and analysis, the teachers discovered that their data trends were not, in fact, hyperbolic, which led to several teacher-developed revisions aimed at obtaining a true hyperbolic outcome. This article outlines the inquiry process that led to these revisions and illustrates their alignment with several key concepts, such as rapid equilibrium kinetics. Instructional decisions were necessary at several key points, and these are discussed.

TEACHING INQUIRY SCIENCE is not always easy. To effectively direct learning experiences that deepen student understanding of science concepts and relationships requires a great deal of preparation and exposure to real-world research activities. Inquiry methods have been identified as one of the best teaching strategies for science (1, 3, 11, 12, 17), and several studies have emphasized the importance of engaging teachers in inquiry activities as part of professional development programs, as this increased the likelihood that teachers would incorporate inquiry activities into their own classrooms (13, 20). Crawford (10) reports that in order for teachers to actively use the inquiry process, several skills must be developed to enhance their ability in creating meaningful science experiences. These abilities include providing a variety of learning activities that allow students to collect and analyze data and draw conclusions, thus modeling the behavior of scientists. Having students gather and analyze their own data sets promotes a better understanding of the process of science and gives students an increased ability to translate this understanding to other scientific disciplines. To model this expectation, we engaged in-service science teachers in a data-gathering discovery process that was both appropriately rigorous and aimed at developing a correct set of molecular concepts and relationships.

Our project began with an innovative inquiry activity published by Runge et al. (19) that involved an easy-to-use, student-centered teaching method that had students use marbles, plastic bins, a blindfold, and a stop watch to collect kinetic data. In this method, a blindfolded student volunteer was instructed to transfer marbles from one plastic bin to another for 10 s. The number of marbles transferred by the volunteer was recorded and graphed against the variable number of marbles initially present in the starting bin, and the data were plotted and fit to a hyperbolic equation. The procedure of Runge et al. was introduced as a teaching method to help students both visualize and understand several important kinetic concepts. Although their report was mostly focused on helping students understand the Michaelis-Menten kinetics of enzyme catalysts, the authors also noted the relevance to biological transport mechanism before the rate-determining step are at equilibrium. This generated some productive discussion on the weaknesses and strengths of the method as a teaching model. The outcome served, in part, to guide the remainder of our project, which ultimately led to a set of teacher-developed revisions to the method of Runge et al. that not only allowed an alternative visualization of a molecular basis for hyperbolic kinetics but also gave important insights into how real transport data are analyzed. This process was not an easy one, as one of the constraints was to not use a blindfold. The teachers tried various revisions to generate hyperbolic outcomes, but the data they collected continued to be fit by two intersecting straight lines. To facilitate a solution, we introduced to them an important concept, called rapid equilibrium kinetics (2, 9), which assumes that all steps in the transport mechanism before the rate-determining step are at equilibrium. This same assumption was made by Michaelis and Menten in 1913, as translated by Johnson and Goody (16), in their landmark study of the enzyme invertase with sucrose as a
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math skills, the relevant equations are included in the DISCUSSION of this article to specifically show the type of math problem the teachers were having and as a point of reference for curricula in experimental physiology and other disciplines. As a validation, we also illustrate how the teacher-developed revisions are aligned with an actual analysis of published kinetic data for a glucose transport protein in erythrocyte membranes. Given the relevance of glucose transport in physiology, this example should also facilitate use of our modeling project in any undergraduate physiology laboratory curriculum. Finally, the broader implications of the present study for science teacher training and professional development programs are discussed.

Project participants and design: guided inquiry stage I. As part of the Illinois Math Science Partnership Program at Northern Illinois University, 4 middle school science teachers and 24 high school science teachers were recruited from the northern Illinois region, representing 16 schools from 9 school districts, to participate in a graduate professional development program aligned with both national and state standards for science teachers. Our role with the cohort was to develop and implement a biochemistry course that incorporated a blend of content and pedagogy. The cohort of teachers took the course during the summer after completion of two semesters of other coursework. Throughout the biochemistry course, teacher participants were organized into classroom teams that worked collaboratively on various modules. One of these, the focus of this report, was aimed at developing key molecular concepts associated with hyperbolic kinetic systems, with an emphasis on biological transport. To introduce this module, teams were each given a copy of the study published by Runge et al. (19) that included the student-centered activity described in the Introduction of this paper. Teams were instructed to simply repeat the method as reported, but without the blindfold, and to evaluate its efficacy as a teaching model for generating hyperbolic kinetic outcomes. Before implementation of this project, we had evaluated the role of the blindfold and concluded, like Runge et al., that it extended the time of transport per marble when the number of marbles in the starting bin became smaller, although our results were variable. Ultimately, however, we felt that the use of the blindfold would be problematic when we introduced the rapid equilibrium condition, which constrains the transport step to always be the slow, or rate-limiting, step. As a result, we developed a scenario where the observed rate would never depend on the “to find” time that Runge et al. referred to when the number of marbles in the starting bin was small (which was the point of the blindfold).

To provide organization, teams were instructed to designate a “transporter” that would transport marbles from one bin to the other and a “time keeper” that would monitor time. The rate data, marbles transferred from bin A to bin B in 10 s (a time set by the Runge et al. method), were graphed against the initial number of marbles in bin A, which ranged from 5 to 40 marbles (Fig. 1). In the original Runge et al. method, a “transfer time” of 1 s/marble was reported, which was separate from a variable “to find” time that increased when the number of marbles in the starting bin was small. By omitting the blindfold, the transfer time could always be assumed to be rate limiting. Thus, the total time needed to transfer a marble was constrained at 1 s, regardless of the number of marbles initially present in the starting bin. In this modeling activity, the transporter represented a membrane carrier protein, the marbles represented the transported solute particles, and the plastic bins represented the aqueous compartments on the two sides of the membrane. At this stage of the guided inquiry, teams were instructed to use whatever criteria they thought were appropriate to evaluate if the graphed data were, in fact, hyperbolic. In addition, each team, before repeating the published method, was given a rubric that would be applied to assess each team at the end of the module (see Table 3).

Tier I revision: guided inquiry stage II. For this revision, teacher participants were organized into seven teams of four participants each with no more than two middle school teachers on a team. This revision was designed to focus on the following: that a rate is different than a

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Fig. 1. Data collection results during guided inquiry stage I. A: initial results after teams repeated the method of Runge et al. (19) without the blindfold. Marbles transferred from bin A to bin B in 10 s were graphed against the initial number of marbles in starting bin A. B: results obtained after teachers were prompted to evaluate possible sources of error. Data sets in A and B were fit to a hyperbolic equation (solid traces) of the following form: \( y = ax/(b + x) \). Insets show residuals after least-squares regression. Other details are given in the text.
Table 1. Recipe for modeling the tier 1 revision

<table>
<thead>
<tr>
<th>Trial</th>
<th>Initial Number of Solutes in Bin A</th>
<th>Number of Transporters</th>
<th>Number of Solutes Transported in 10 s*</th>
<th>Rate of Transport, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>60</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>80</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>100</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>120</td>
<td>4</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

*Values in this column were measured rather than calculated. A rate constant of 1 s⁻¹ was applied to each trial and transporter.

Table 2. Sample recipe for modeling the tier 2 revision for T₀ = 6

<table>
<thead>
<tr>
<th>Trial</th>
<th>Initial Number of Solutes in Bin A*</th>
<th>Number of Solute-Bound Transporters</th>
<th>Number of Dissociators</th>
<th>Number of Solute Particles Transported in 10 s†</th>
<th>“Initial” Rate of Transport, s⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>2</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>200</td>
<td>4</td>
<td>4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>500</td>
<td>5</td>
<td>5</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Undefined‡</td>
<td>6</td>
<td>6</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

*The Excel template shown in Fig. 2 was used to generate the solute number series for a six-membered team for dissociation equilibrium constant (K) = 100. Other values of K and total number of possible transporters (equal to the team size; T₀) were used by different teams. The primary constraints were that T₀ = the team size and that the number of marbles in the starting bin was always larger than T₀. †Values in this column were measured rather than calculated. A rate constant for transport of 0.2 s⁻¹ (1 solute moved from bin A to bin B every 5 s) was applied to each trial and transporter. The dissociation rate constant was 1 s⁻¹. ‡The last trial cannot be modeled since a rectangular hyperbola asymptotically approaches the limiting value (Vₘₐₓ). As a result, it sometimes not possible in practice to approach the saturation condition due to solubility limits or other complications. The saturation rate is therefore obtained by fitting the rate data to a hyperbolic equation or by doing an appropriately weighted linear transform, such as Lineweaver-Burk, Dixon, or Eadie-Hofstee.
The view in column is given by the rate, obtained by extrapolation, and is shown as a horizontal reference line on the graph. Both $K$ and $NH_2$- and COOH- termini of the protein chain are labeled, and the helixes membrane. The protein is postulated to have 12 transmembrane A: view of the transporter showing its approximate orientation in a biological glucose transporter 1 (GLUT1) protein (Protein Data Bank identifier: 1SUK).

Fig. 3. The RasMol-rendered theoretical three-dimensional structure of the glucose transporter 1 (GLUT1) protein (Protein Data Bank identifier: 1SUK). A: view of the transporter showing its approximate orientation in a biological membrane. The protein is postulated to have 12 transmembrane $\alpha$-helixes. The NH$_2$- and COOH-termini of the protein chain are labeled, and the helixes suggested by Salas-Burgos et al. (18) to form the glucose channel (helixes 2, 4, 5, 7, 8, and 10) are shown in red. B: top-down view of the GLUT1 channel. The view in A was rotated toward the reader by 90°.

Table 3

<table>
<thead>
<tr>
<th>S</th>
<th>v$_0$ (s$^{-1}$)</th>
<th>% Bound</th>
<th>T</th>
<th>TS</th>
</tr>
</thead>
<tbody>
<tr>
<td>20</td>
<td>1.00</td>
<td>17%</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>2.00</td>
<td>33%</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>100</td>
<td>3.00</td>
<td>56%</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td>200</td>
<td>4.00</td>
<td>67%</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>500</td>
<td>5.00</td>
<td>83%</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

$V_{max}$ is the saturation rate, obtained by extrapolation, and is shown as a horizontal reference line on the graph. Both $v_0$ and $V_{max}$ were normalized to s$^{-1}$. The percent binding (% Bound) column is given by $(TS/T_{0}) \times 100$ and represents the average percentage of transporter proteins bound with solute for a given solute number. The $S$, $T$, and TS columns show the average numbers of free solute, free transporters, and solute-bound transporters, respectively, calculated for entered values of $T_{0}$ and $K$. The relevant rapid equilibrium equations for performing these calculations were Eqs. 3 and 7. The $S$, $T$, and TS columns also provided the basis for the tier 2 modeling recipes, such as the one shown in Table 3.

Fig. 2. Screen capture of the interactive Excel template used by teachers to explore the rapid equilibrium kinetics concept. The template allowed input of $k$, $T_{0}$, and $K$, which correspond to the first-order rate constant for transport (in units of s$^{-1}$), the total number of possible transporters (equal to the team size), and the dissociation equilibrium constant for the $TS \rightleftharpoons T + S$ reaction [where $T$ is the transporter ($T$), $S$ is the solute, and $TS$ is the solute-bound transporter complex], respectively. Changing one or more input values automatically updated the table entries and the initial rate of transport ($v_0$) versus $S$ graph. $V_{max}$ is the saturation rate, obtained by extrapolation, and is shown as a horizontal reference line on the graph. Both $v_0$ and $V_{max}$ were normalized to s$^{-1}$. The percent binding (% Bound) column is given by $(TS/T_{0}) \times 100$ and represents the average percentage of transporter proteins bound with solute for a given solute number. The $S$, $T$, and TS columns show the average numbers of free solute, free transporters, and solute-bound transporters, respectively, calculated for entered values of $T_{0}$ and $K$. The relevant rapid equilibrium equations for performing these calculations were Eqs. 3 and 7. The $S$, $T$, and TS columns also provided the basis for the tier 2 modeling recipes, such as the one shown in Table 3.

point about the role that bias can have in data analysis and the need to identify and eliminate it. Visual bias, in particular, can skew interpretation and create misconceptions. A more robust determination of goodness of fit was then presented to the teams and is shown in the insets in Fig. 1, A and B, where the residuals (the difference between the actual data and the fitted line) are shown as a set of drop-down lines, or spikes. The meaning of residuals was unknown to most teachers and time was taken to explain it. The main point was that the oscillatory pattern of large positive and negative residuals is diagnostic of a nonoptimal fit (4). Comparison of the residual patterns in Fig. 1, A and B, showed the same systematic deviations (and similar magnitudes of deviations) in both data sets, despite very different visual impressions of the raw data fits.

An informal survey administered at this stage of the inquiry indicated that most teachers were not concerned that the data-gathering method did not produce a hyperbolic outcome. The more important considerations, and despite our lesson on visual bias, were “use of inexpensive materials” and “an easy-to-implement method” that was “student friendly.” In addition, the majority of teachers argued that their data (Fig. 1B) showed that the rate of transport depended on the initial number of “solutes” and that the data showed a saturation effect. The failure to replicate a hyperbolic shape was considered mostly irrelevant at this stage.

Modeling as a strategy. Modeling is an important process in science (14). Many practitioners attempt to model data as a way of better understanding cause-and-effect relationships or to make forecasts. This practice is not unique to the science, technology, engineering, and mathematics fields but is also common to areas such as economy and sociology. But modeling is also a way of knowing and is an important component of inquiry. Given that biological transport data are often observed to be hyperbolic, we felt it necessary to refocus the teacher teams on the importance of developing a model that would generate hyperbolic data and correctly illustrate a molecular basis for hyperbolic transport. In addition, several
loose-end questions remained, such as the following: Did a limiting rate arise because we limited the time of data acquisition to 10 s? Could the rate of transport for a single transporter be measured directly? Our primary objective at this stage was to promote more critical reflection among the teachers and to engage them in developing revisions to their data-gathering method that could actually model a hyperbolic transport system and teach about a possible molecular basis.

**Guided inquiry stage II: implementing the tier 1 revision.**

The teacher teams at this stage of the inquiry were prompted to critically review their method. One prompt asked about the reason for a single student volunteer. In particular, in this single-student method, the transporter is always transporting, regardless of the number of solute particles in the starting bin. Although relevant for some systems, our intent was to transition the teachers to a model in which transport rates depended only on the number of transporters bound with solute and not on other steps, such as the bimolecular event that forms the solute-bound complex. When asked about the single-student method, several teams suggested a revision that used several students as transporters. After some discussion, several teams noted that increases in transport rate could be achieved by using more transporters but that more solute would likely be required to do this. The multistudent model also suggested a cause for the saturation effect: that a \( V_{\text{max}} \) would occur because of a limited number of transporters. The teams formed the consensus that a multistudent method could accurately model molecular events in biological transport.

This consensus decision by the teams to model biological transport using multiple team members was an essential conceptual step because it could now lead to a more accurate, student-centered visualization of how the saturation effect arises in real systems. It also bridged into a clear illustration of why it is common practice to measure \( V_{\text{max}} \), where essentially all transporter proteins are bound with solute. This measured rate is used, in practice, to calculate the intrinsic efficiency of a single transporter, the so-called rate constant (an important kinetic parameter that cannot be measured directly and that is often called a “turnover number” in enzyme catalysis). An illustration of this important approach is presented in the Discussion using published data for the GLUT1 protein in erythrocyte membranes.

To promote the transition to a multistudent model, the seven teacher teams were each given the recipe shown in Table 1 and instructed to complete the last two columns in the table. The objective at this stage was not to model a hyperbolic outcome (that would come later) but to instead apply the multistudent model to illustrate a correct conceptual difference between a variable first-order rate and rate constant and to visualize why a saturation effect occurs. During open discussion, the question was “how do we know how many marbles to transfer in the 10-s data collection period?” A suggestion of using a 1 marble/s transfer rate was accepted by all the teams. We made clear, however, that this number could not vary from one trial to another, nor could it vary during the 10-s data acquisition period for any given team member with a transporter role. A metronome was introduced to enforce this constraint. The teams now found it intuitive to define 1 s\(^{-1}\) as a rate constant, a number that was intrinsic to (and thus a property of) each identical transporter, which would not vary. The set numbers of solute particles (see Table 1) that would activate increasing numbers of transporters was, however, largely arbitrary at this stage. Numbers, however, were set large enough to ensure that marbles would not be depleted during the 10-s data collection period for any transport condition. Apart from this, the solute numbers largely stemmed from the trend shown in Fig. 1B: that doubling the amount of solute should also double the rate, until saturation is reached. Again, the objective at this stage was not to model a hyperbolic outcome. That would require introducing the rapid equilibrium condition (tier 2).

Even though the rate column shown in Table 1 could easily be calculated, given a rate constant of 1 s\(^{-1}\), teams were still instructed to simulate or build a membrane transport system using inexpensive materials and to collect their rate data by applying lessons learned from stage I, such as obtaining replicates, making measurements at smaller x-intervals, and so on. The collected data were then compared against theoretical outcomes. For example, given a rate constant of 1 s\(^{-1}\), two transporters could transport 20 marbles in 10 s, which gives a normalized rate of 2 marbles/s; three transporters would give a rate of 3 marbles/s, and four transporters, the saturation condition, would give a limiting rate of 4 marbles/s. The multistudent model allowed each team to visualize that the rate was a measured, but variable, quantity that depended on the number of teachers actually transporting solute (symbolically, \( V_{\text{max}} \approx TS \), where TS is the number of transporters bound with solute) and that the rate constant (the intrinsic rate for a single transporter) was invariant at 1 s\(^{-1}\). From this, a more accurate visualization of the saturation effect was now possible: that transport rate is limited by the number of transporters. Add more transporters and \( V_{\text{max}} \) will increase; add more solute to a system that is already saturated, and it will have no further effect on rate. The multistudent model now provided a correct molecular visualization of the saturation effect observed with many real transport systems and allowed easy visualization of the difference between a simple first-order rate and first-order rate constant. Again, there are other kinetic scenarios, but we always centered on our eventual introduction of the rapid equilibrium condition. The tier I revision was a guided inquiry step toward that goal, where the measured rate of transport was always proportional to TS and therefore always a first-order process. A simple assessment at this point had teams illustrate how a rate constant could be calculated from the maximal (or saturation) rate, as is done in practice for real transport systems. The majority of teams correctly deduced that the rate constant could be calculated by dividing the observed rate by the number of actual transporters. For example, the \( V_{\text{max}} \) predicted by the recipe shown in Table 1 when all four team members are transporting is 4 s\(^{-1}\) (the saturation condition). Dividing this value by four gave a rate constant of 1 s\(^{-1}\).

**Rationale for a two-tier strategy.** The tier I revision met several objectives. It retained the student-centered approach and use of inexpensive materials in the Runge et al. method (19). Additionally, it engaged teachers in making changes to the Runge et al. method that they felt helped them to better visualize and understand the difference between a rate and a rate constant and why there is a \( V_{\text{max}} \). However, the recipe shown in Table 1 still did not produce a hyperbolic outcome. To resolve this and to provide an alternative approach to the Runge et al. method, we chose to introduce the rapid equilibrium kinetics concept (2, 9). Our initial approach was algebra based and relied on symbolically solving one equation and sub-
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stitting into another (see the Discussion) to show that the average fraction of transporters bound with solute is a hyperbolic function of solute number. In effect, this final derived equation provided a recipe for calculating the solute numbers that would “activate” increasing numbers of team members as transporters and would eliminate the need for the arbitrary set of solute numbers shown in Table 1. However, frustration became a factor with most of the teams, as they were unable to work through the necessary math. Primarily because of this, we chose to differentiate our guided inquiry plan into tier 1 and 2 revisions. The challenge for us at the curricular level was to continue the project flow by having the teachers correctly model a hyperbolic outcome while revealing a correct molecular basis for it.

Guided inquiry stage III: implementing the tier 2 revision. To achieve this curricular goal, we used an Excel-based approach, similar to that used by Sinex and Gage (21), as a math transparency tool. This tool, which we built for this project, was an Excel template we called a “magic number generator” to elicit interest. A screenshot of the template is shown in Fig. 2 for a team composed of six teachers. The team size was entered into the T0 cell, as this represented the maximum number of transporters (T0 = TS = 6). However, the actual number of transporters would be less than this if less than saturating amounts of solute were present. The template applied the rapid equilibrium condition to automatically calculate a series of solute numbers (the so-called “magic numbers”) needed for TS values less than six. For example, as shown in Fig. 2, 50 marbles (solute) in bin A means that two team members (TS = 2) can transport marbles into bin B. Three team members engaged in transport (TS = 3) would need 100 marbles to be initially present in bin A, and so on. The solute numbers correspond to a hyperbolic series. Our instructional decision at this stage was to have teachers focused on revisions to the Runge et al. method that would allow them to correctly visualize the underlying rapid equilibrium kinetics concept and to defer the relevant equations until later. To begin this phase of the inquiry, we had the teams revisit the tier 1 activity to reflect on their outcomes. In particular, when the teams were collecting data to provide the missing entries in Table 1, we observed that there were team members (transporters) that never interacted with solute. For example, in trial 1, it was always the same teacher that transported the marbles from bin A to bin B. To initiate discussion, we asked the teams if it was reasonable to assume that some transporters (all identical and all with the same rate constant) would never bind a solute particle during that 10-s data collection period. Most teams acknowledged that this constraint was unlikely and that the system was more likely to have a “random behavior.” We then focused the discussion on whether a transport event would occur every time a solute particle “landed” on a transporter. What is the probability of a transport event for a solute particle? For example, as shown in Fig. 2, for the k = 1 case, the transporters could bind T0 = 6 marbles. The k = 2 case, the transporters could bind T0 = 18 marbles. The above-noted relationships were essential to advancing our project, and the Excel template provided an effective interactive tool for helping the teachers visualize the interplay among k, K, and T0.

The template demonstrated that the solute numbers were no longer arbitrary, as shown in Table 1, but were now a predicted outcome of the rapid equilibrium model. The conclusion we wanted the teams to make at this point was that the hyperbolic outcome was a specific consequence of the rapid equilibrium condition, which could be modeled by introducing an intrinsic inefficiency into the TS complex that would allow for rapid dissociation into T + S rather than an obligatory transport of S across the membrane. Accordingly, the k, T, and TS columns shown in Fig. 2 were used to build the sample recipe shown in Table 2, where T and TS refer, respectively, to the numbers of

| T + S ⇄ T → transport |

It was now time to introduce the rapid equilibrium condition into the discussion. As noted above, a TS complex, once it forms, has a choice. If the rate constant (a measure of probability) for dissociation (TS → T + S) is much greater than the rate constant for transport (TS → transport), then a so-called rapid equilibrium condition occurs. In other words, as soon as solute is added, a rapid equilibrium is established between T, S, and TS, followed by a much slower transport of solute across the membrane. Adding more solute will quickly “push” this equilibrium toward more TS, but the greater the rate constant for dissociation (compared with that for transport), the more and more solute will be needed to generate the same number of TS complexes. It made sense to the teams that a new parameter was needed to quantify this dissociation. But rather than use a dissociation rate constant for this, we introduced a related parameter that is often used in practice to characterize the dissociative behavior in real transport systems: the dissociation equilibrium constant (shown as “K” in Fig. 2, although it may have other designations, such as K1/2 or K0). Because of the way it is defined, referring specifically to the equilibrium written as TS ⇄ T + S, rather than T + S ⇄ TS, a larger value for K generally implies a larger rate constant for dissociation and, therefore, less affinity of T for S. Empirically, the dissociation equilibrium constant is defined as the number (or concentration) of solute particles needed to bind, on average, 50% of the transporters. In Fig. 2, for example, the K value is set to 100, which matches the number of solute particles needed to bind, on average, three of the six transport proteins. The Excel template proved to be an important interactive tool to help the teachers visualize the meaning of K and how it differs from the rate constant for transport (k), as they could vary each to quickly assess that the equilibrium constant only affected solute numbers, whereas the rate constant only affected Vmax. To design their experiment, the teachers were free to vary T0 to match their team size, which was either six or eight; the equilibrium constant could be varied within the range of 10–100, and k could be changed (see below).

The importance of the rapid equilibrium condition in this project must again be emphasized. This condition implies that the measured rate of transport will always be directly proportional to the number of TS complexes, regardless of the initial amount of solute. This is consistent with the results shown in Fig. 2, which shows k = 1 (in units of s−1). Accordingly, one transporter showed a rate of 1 s−1 in the v0 column; two transporters showed a rate of 2 s−1, and so on, until the system saturated with a limiting rate of 6 s−1. (A solute number is not listed for this as it rigorously requires an infinite number of solute particles due to an asymptotic approach to Vmax.) The rapid equilibrium condition is the reason here that the rate dependence becomes hyperbolic (as shown by the inset in Fig. 2). The above-noted relationships were essential to advancing our project, and the Excel template provided an effective interactive tool for helping the teachers visualize the interplay among k, K, and T0.
dissociators and transporters. To implement this recipe, it was now necessary to assign six members of a team (T₀ = 6) with either a transporter or dissociator role, as reflected by the column headings in Table 2. The transporters would move a solute from bin A to bin B, as in the tier 1 revision, but the dissociators would model the intrinsic inefficiency implied by the rapid equilibrium model by moving a solute from bin A back to bin A, rather than transporting it. Each team also assigned additional roles of timer and recorder. The tier 2 revision challenge was for the teams to revise the tier 1 activity to model the rapid equilibrium condition, collect the rate data by following the recipe shown in Table 2, graph the results, and evaluate if the outcome was hyperbolic (perhaps by examining residuals, as shown in the insets in Fig. 1, A and B).

Building the tier 2 revision: a team challenge. Several teams quickly realized that they had a logistical problem to solve. The rapid equilibrium condition requires that the rate constant for dissociation (returning the solute to bin A) be greater than the rate constant for transport (moving the solute from bin A to bin B). We suggested a fivefold condition to address their concern of “how much greater.” All teams realized that the rate constant for transport of 1 s⁻¹, used in their earlier tier 1 activity, was too fast for the dissociators to achieve a fivefold condition, since one dissociator would need to consecutively return five marbles to bin A in 1 s. To fix this, the teams decided to use a smaller rate constant for transport. By consensus, k was set to 0.2 s⁻¹ in the Excel template (corresponding to 1 solute transported every 5 s). Changing this rate constant affected the initial rates (column v₀ in Fig. 2) but not the solute numbers (which are determined by K and T₀). The rate constant for dissociation was set to 1 s⁻¹ (which is fivefold the rate constant for transport), thus approximating a rapid equilibrium condition. For different trials, transporter and dissociator roles were swapped to introduce some randomness into the modeling. A time keeper ensured that every dissociator returned a solute to bin A with a rate constant of 1 s⁻¹ and that every transporter moved a solute from bin A to bin B with a rate constant of 0.2 s⁻¹ (1 per 5 s). The required rate data in Table 2 were collected and graphed in Excel against S using the graphing template distributed to the teams during the tier 1 activity. As expected, the results graphed as a true hyperbola and not as two intersecting straight lines (as in Fig. 1B). An outcome for T₀ = 8, K = 80, and k = 0.2 is shown in Fig. 4.

Assessment of teacher outcomes. As noted earlier, a rubric (Table 3) was developed and distributed to teachers at the beginning of the project. The initial replication of the Runge et al. (19) method, without the blindfold, and implementation of the tier 1 revisions (components A–C of the rubric) involved seven teams, each composed of four teachers. The tier 2 revisions required that these teams collaborate to have the minimum six members needed for transporter or dissociator roles. To collect the data needed for the tier 2 revisions, the teachers organized into two eight-membered teams and two six-membered teams. After their data collection, these teams collapsed into their original four-membered teams to develop their unit plans (component D). Application of the scoring rubric to each team’s final report gave the aggregated results shown in Table 4. Indicator B did not assess the teacher’s ability to derive the hyperbolic equation that follows from the rapid equilibrium condition, but they were required to know the general mathematical form of both straight line and hyperbolic functions.

DISCUSSION

The original method of Runge et al. (19) provided a valuable starting point for this report, with its easy-to-implement, student-centered method of collecting rate data using inexpensive materials. In their method, an approximate hyperbolic outcome was obtained through use of a blindfold, which slowed the transporter’s ability to find a marble in the starting bin when initial numbers were small, thereby delaying transport (a diffusion effect). For the present project, we engaged teams of middle and high school science teachers in developing a set of teacher-generated revisions to the Runge et al. method to illustrate an alternative way that hyperbolic kinetics can arise. In particular, teams collected rate data and applied a statistical criterion (residuals) to examine if a hyperbolic fit of their data was, in fact, a good fit, thus illustrating a facet of data analysis in which bias can affect interpretation (cf. Fig. 1, A and B). This method of assessing “goodness of fit” has been discussed by Baker and Weng (4) and was introduced into this project to illustrate the importance of applying sufficient rigor when collecting and analyzing data. Additionally, we also had the teachers assess the efficacy of the Runge et al. method as a teaching method for illustrating the concepts of rate, rate constant, and saturation (the tier 1 revision). Finally, we engaged the teachers in a revision challenge that had them model the rapid equilibrium condition as a basis for understanding how hyperbolic kinetics can arise in real molecular systems (the tier 2 revision). The differentiated approach of separating the revisions into tier 1 and tier 2 as well as the use of interactive Excel templates to make the associated graphic display and equations transparent were identified by the teachers as effective formative strategies. In particular, the use of the magic number generator (Fig. 2) was viewed positively by the teachers as an instructional decision for moving the project forward when we observed that the majority of teachers were unable to derive the relevant hyperbolic equation (discussed further below). Activities to reactivate the necessary math were planned as a tier 3 revision, but project time constraints prevented their implementation.

An important goal of this inquiry project was to develop molecular insights into hyperbolic transport systems using...
engaging strategies, but we also wanted the tier 1 and tier 2 revisions to aid understanding of how real kinetic data are analyzed. Below, the relevant equations are presented, and the alignment between our proposed tier 1 and tier 2 revisions and a practical analysis of real kinetic data is illustrated.

Mathematic foundation. Applying the rapid equilibrium condition to the transport model in Eq. 1, \( v_0 \) becomes proportional to the average number, or concentration, of TS complexes, as follows:

\[
v_0 = k \times TS
\]  
(2)

where \( k \) (Fig. 2) is the proportionality constant and the first-order rate constant for transport. In the saturation limit, all transporter proteins are assumed to be bound with solute, and TS approaches \( T_0 \) and \( v_0 \) approaches \( V_{\text{max}} \), the saturation rate. Equation 2 can now be written as follows:

\[
V_{\text{max}} = k \times T_0
\]  
(3)

According to Eq. 3, the rate constant for transport can be calculated by dividing \( V_{\text{max}} \) by the total number of transporters, a quantity that can often be measured or at least estimated. This relationship between rate and rate constant is illustrated by the tier 1 revision.

The transport model in Eq. 1 includes an equilibrium step. According to this model, a transporter protein can only be in one of two states, free or bound. A mass balance expression for \( T_0 \) is therefore given by \( T_0 = TS \). Accordingly, the fraction \( f \) of transporters bound with \( S \) is given by the following:

\[
f = \frac{TS}{T + TS}
\]  
(4)

The rapid equilibrium condition (introduced as the tier 2 revision) means that the relative concentrations of \( T, S \), and TS can be expressed by an equilibrium constant, or, more specifically, a dissociation equilibrium constant (\( K; \) Fig. 2), that is specifically defined for the equilibrium, written as \( TS \rightleftharpoons T + S \), as follows:

<table>
<thead>
<tr>
<th>Measure</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average score</td>
<td>95</td>
</tr>
<tr>
<td>Median score</td>
<td>96</td>
</tr>
<tr>
<td>Minimum</td>
<td>84</td>
</tr>
<tr>
<td>Maximum</td>
<td>100</td>
</tr>
<tr>
<td>SD</td>
<td>5.9</td>
</tr>
</tbody>
</table>
study by Brahm (6) of GLUT1 in erythrocyte membranes were solved for $T_0$ from Eqs. 4 and 5 was the specific hurdle that the majority of teachers in this project were unable to solve. Equation 6 shows that the fraction of bound transporter is a hyperbolic function of solute number. Since Eq. 2 shows that $v_0$ is proportional to this fraction, it follows that $v_0$ must also be a hyperbolic function of $S$ and that $v_0$ would be a linear function of $S$ when $S \ll K$ (the dilution effect). Equation 6 can be solved for $S$ to give the following:

$$ S = \frac{f}{1-f} K $$

Equation 7 was embedded into the magic number generator (Fig. 2) to generate the hyperbolic series of solute numbers (so-called magic numbers) for different user-entered values of $K$ and $T_0$. The empirical meaning of $K$ can also be derived from this equation since it reduces to $S = K$ when $f = 0.5$, corresponding to 50% binding, on average. One other important implication of the rapid equilibrium condition is that the relative concentrations of $T$, $S$, and $TS$ do not change much during the initial rate measurement. This is reasonable, in practice, if $S \gg T_0$. This condition is reasonably maintained during the marble transfer period for all solute numbers shown in Table 2 except trial 1.

An illustration of how tier 1 and tier 2 concepts can be applied to real transport data for GLUT1 in erythrocyte membranes. To validate the relevance of the concepts illustrated by the tier 1 and tier 2 revisions, data from a published study by Brahm (6) of GLUT1 in erythrocyte membranes were examined. In those experiments, red blood cells equilibrated with various concentrations of $[^{14}C]glucose$ were packed by centrifugation and resuspended in glucose-free buffer to create a concentration gradient across the cell membrane. Initial rates of glucose efflux were then measured and graphed against measured initial amounts of intracellular glucose. The resulting hyperbolic data were then analyzed to determine the maximal initial rate ($V_{\text{max}}$) and the Michaelis constant ($K_m$), which equaled the glucose concentration needed for $V_{\text{max}}/2$ (Brahm denoted this constant as $K^{1/2}$). The reported values of $V_{\text{max}}$ and $K_m$ were $6.3 \times 10^{-10}$ mol glucose cm$^{-2}$ s$^{-1}$ and 8.2 mM, respectively (pH 7.2 and 38°C). To give perspective to the rate when essentially all GLUT1s are bound (saturated) with glucose solute. It is the quantity that, in practice, is used to calculate the rate constant for the first-order transport event, which, in this case, is a measure of the intrinsic ability of a single GLUT1 protein to transport glucose across the membrane. For the GLUT1 system, units for $V_{\text{max}}$ are expressed as moles per second per square centimeter of membrane surface. In the analysis that follows, dimensional analysis will be applied to calculate the rate constant for transport from $V_{\text{max}}$, a concept modeled by the tier 1 revision.

The relevant equation for this analysis is Eq. 3, where $T_0$ is the total number of GLUT1s. According to this equation, $k = V_{\text{max}}/T_0$. A dimensionally appropriate value for $T_0$ can be estimated from the total surface area of a human red blood cell, which is $\sim 1.4 \times 10^{-6}$ cm$^2$, and the total number of GLUT1 proteins per cell, which can approach $3.0 \times 10^{5}$ (7). Thus, the total number of GLUT1 proteins (expressed as mol/cm$^2$ of membrane surface) can be estimated as follows:

$$ \frac{3.0 \times 10^{5} \text{GLUT1}}{\text{cell}} \times \frac{1 \text{ mol GLUT1}}{1.4 \times 10^{-6} \text{ cm}^2} \times \frac{6.0 \times 10^{3} \text{ GLUT1}}{6.0 \times 10^{-13} \text{ mol GLUT1/cm}^2} $$

$$ = \frac{3.6 \times 10^{-11} \text{ mol GLUT1/cm}^2}{6.0 \times 10^{-13} \text{ mol GLUT1/cm}^2} $$

$$ = 1.8 \times 10^3 \text{ s}^{-1} $$

Thus, each GLUT1 molecule is able to facilitate transport of $\sim 1,800$ molecules of glucose every second. The rapid equilibrium assumption would imply that $v_0$ is directly proportional to the average number of GLUT1-glucose complexes. For example, a $v_0$ that is 50% of $V_{\text{max}}$ would mean that 50% of GLUT1s are bound, on average, with glucose and that the number (or concentration) of glucose molecules that give this condition would be the dissociation equilibrium constant for the GLUT1-glucose $\Rightarrow$ GLUT1 + glucose reaction. An understanding of these relationships is facilitated by both tier 1 and tier 2 revisions and the use of the magic number generator.

**Interpretation of $V_{\text{max}}$.** The $V_{\text{max}}$ observed by Brahm (6) was a parameter that corresponded empirically to the glucose concentration that gave $V_{\text{max}}/2$. A further interpretation requires that we assign rate constants to the individual steps in the transport model shown by Eq. 1, as follows:

$$ T + S \quad k_a \quad k \quad k_d \quad TS \quad \text{transport} $$

where rate constants $k_a$ and $k_d$ are the second-order and first-order rate constants for formation and dissociation of the TS complex, respectively. In the most general case where this model applies, the Michaelis constant is given by Eq. 10, as follows:

$$ K_m = \frac{k_a + k_d}{k_a} $$

The rapid equilibrium condition implies $k_d \gg k$ (the probability of dissociation is much greater than that for transport), and $K_m$ can be approximated as follows:
Thus, the rapid equilibrium condition means that the measured Michaelis constant can now be interpreted as a true dissociation equilibrium constant for the TS $\rightleftharpoons$ T + S reaction. In the tier 2 revision, the $k_d$ >> $k_a$ condition was modeled as a fivefold condition, where a dissociator returned a marble to bin A five times faster than a transporter moved a marble to bin B. In practice, the observed difference should be at least an order of magnitude. For example, from Eq. 9, the rate constant for glucose transport is $1.8 \times 10^3$ s$^{-1}$. For the rapid equilibrium condition to apply in practice, $k_d$ should be at least 10-fold larger than this, or at least $1.8 \times 10^2$ s$^{-1}$. The fivefold difference used in the tier 2 revision was simply easier for the teachers to model than a 10-fold condition, and it still made the point. The $K_m$ in Eq. 11 now becomes the $K$ referenced in Eqs. 5–7 and in the Excel template shown in Fig. 2. Larger values of $K$ are interpreted to mean a lower affinity of solute for transporter. Thus, as $K$ increases, a larger amount, or concentration, of solute will be required to achieve a given equilibrium number of TS complexes. The tier 2 revision is valid for those kinetic systems where the rapid equilibrium assumption applies. Thus, by having the teachers model this condition, it reinforced the idea that if a rapid equilibrium is established on addition of solute, the kinetically determined Michaelis constant can be interpreted as a true (thermodynamic) equilibrium constant, and the dependence of $v_0$ on solute number will be hyperbolic, as implied by Eq. 6.

We can now apply this background to interpret the $K_m$ value of 8.2 mM. If the measured Michaelis constant is, in fact, a dissociation equilibrium constant, then 8.2 mM becomes the concentration of glucose needed to bind, on average, 50% of GLUT1s. We can therefore alternatively express $K_m$ as the number of glucose molecules per cell needed to bind 50% of GLUT1s, as follows:

$$K_m \sim \frac{k_d}{k_a}$$

(11)

If the number of GLUT1s per cell can approach $3.0 \times 10^5$ (7), then a 2,100-fold excess of glucose is needed to achieve 50% binding of GLUT1 [(3.2 $\times 10^8$ glucose/cell)/(1.5 $\times 10^5$ GLUT1/cell)] and thus give an initial rate of $V_{max}/2$. This result illustrates the inefficiency condition that we discussed with the teachers after introducing the transport model in Eq. 1, where we suggested that a solute bound to a transporter protein could dissociate without being transported. We had made the point with the teachers that this inefficiency can be quantified by introducing $K$ into the model (Fig. 2) and that larger values of $K$ implied that larger amounts of solute would be needed to achieve the same average percentage of binding. A calculated 2,100-fold excess of glucose to bind 50% of GLUT1s is consistent with a rapid equilibrium condition as the molecular basis of hyperbolic transport in the GLUT1 system.

Implications for science teacher certificate programs and professional development. In the present study, teacher participants developed a set of revisions to the original method of Runge et al. (19) that would give students an alternative visualization of a molecular basis for hyperbolic transport kinetics. However, a majority concern of the teachers was that it did not give them something they could bring back to their classrooms, given that the analysis of hyperbolic kinetic systems was not part of their curricula. As noted above, most said that the largest value was in the method of Runge et al. that they were asked to repeat at the beginning of this inquiry project. It did not matter that the method, without the blindfold, did not actually generate a hyperbolic outcome or that the method did not necessarily model the concepts or relationships that we viewed as important. The use of the blindfold would perhaps have been the easier extension to obtain hyperbolic rate data, but the teachers nonetheless agreed that the tier 1 and tier 2 revisions they had developed would be more effective in visualizing those kinetic concepts we stated were important (i.e., the difference between a simple rate and rate constant, or the rapid equilibrium kinetic condition and its implications for interpreting $K_m$). A concern we have is the potential misuse of inquiry in the classroom where students are engaged in data-gathering activities that do not necessarily address errors or expose students to different models and their respective limitations. For example, a useful extension of the present study would be a compare and contrast activity where groups of students examined the Runge et al. model and the model presented in this study as two separate engaging ways of generating hyperbolic data. Each may bring something important and useful to a whole class discussion of enzyme or transport kinetics. In fact, a comprehensive analysis by Coburn et al. (8) examined 138 studies of inquiry-based science instruction in K–12 grades and found that most had significant design flaws. A comparison of inquiry and direct instruction in their study revealed little difference in promoting science learning. Instead, the most important factor was engaging the students in meaningful activities that were appropriately rigorous and aligned with the practices of science. The method of Runge et al. and the activities described in the present study both provide meaningful and correct ways of engaging students in understanding an important biological problem.

Despite the curricular concern, informal conversations between teacher participants and different faculty associated with the Illinois Math and Science Partnership Program indicated that the teachers had a more favorable attitude about inquiry as a result of actually being immersed in inquiry activities across the program and felt that they were more confident with the idea of implementing inquiry in their own classrooms. We hope that teachers will be encouraged to design inquiry that focuses on possible sources of error while also illustrating the importance of using different models to explore a biological problem. The incorporation of this and similar projects in a methods course in science teacher training programs could also be potentially effective for helping preserve teachers build a reform-minded professional identity for engaging diverse students in the process of learning science.

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No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTOR CONTRIBUTIONS

Author contributions: L.M., J.P., G.M.B., and J.S.M. analyzed data; L.M., J.P., and G.M.B. prepared figures; L.M., J.P., G.M.B., and J.S.M. drafted manuscript; G.M.B. conceived and designed of research; G.M.B. and J.S.M. edited and revised manuscript; G.M.B. and J.S.M. approved final version of manuscript.

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