Understanding how different types of molecules move through cell membranes is a fundamental part of cell biology. To identify and address student misconceptions surrounding molecular movement through cell membranes, we surveyed student understanding on this topic using pre-class questions, in-class clicker questions, and subsequent exam questions in a large introductory biology course. Common misconceptions identified in student responses to the pre-class assessment questions were used to generate distractors for clicker questions. Two-tier diagnostic clicker questions were used to probe incoming common student misconceptions (first tier) and their reasoning (second tier). Two subsequent lectures with assessment clicker questions were used to help students construct a new framework to understand molecular movement through cell membranes. Comparison of pre-assessment and post-assessment (exam) performance showed dramatic improvement in students’ understanding of molecular movement: student answers to exam questions were 74.6% correct with correct reasoning while only 1.3% of the student answers were correct with correct reasoning on the pre-class assessment. Our results show that students’ conceptual understanding of molecular movement through cell membranes progressively increases through discussions of a series of clicker questions and suggest that this clicker-based teaching strategy was highly effective in correcting common student misconceptions on this topic.

INTRODUCTION

One of the major goals of higher education is to improve students’ conceptual understanding. Many students do not develop a complete understanding of fundamental biology concepts and often hold onto alternative conceptions, or misconceptions, despite additional instruction. Such misconceptions can persist even in the face of contradictory evidence, resulting in a lack of deep understanding of those concepts (1). Although there are debates about the differences between alternative conceptions and misconceptions, for simplicity, we will refer to these ideas as misconceptions throughout this paper.

Education researchers have been investigating the nature of student misconceptions for the last several decades. For example, many students believe that photosynthesis is the production of energy for plant growth (2, 3), while others believe that respiration is possible in plant leaves only because of special pores for gas exchange (4). Other misconceptions include such ideas as amino acids being created by the process of translation (5), that a protein spends its entire cellular lifetime in a fully folded native conformation (6), that enzymes work by a lock and key mechanism (suggested by Emil Fisher in 1894) instead of an induced fit model (described by Dan Koshland in 1958, 7), and that individuals rather than populations evolve (8). On the topic of diffusion and osmosis, students often believe that molecules cease to move at equilibrium (9), and that molecules experience directed movement toward lower concentrations rather than random movement (10, 11).

Strategies for helping students overcome misconceptions usually focus on getting students to accept scientific evidence that does not support their original ideas and then using that new knowledge to help them reconstruct a correct framework (12). There are many strategies to help students establish a new conceptual framework including creating “concept maps” (13), using interactive simulated laboratories (14), using in-class demonstrations (15), and using computer-assisted instruction (CAI) (16). Although recent implementations of computer simulations (11) as well as the 5E (engagement, exploration, explanation, extension, and evaluation) learning cycle (17, 18) have been shown to...
help some students construct new frameworks, misconceptions often still persist, indicating a continuing need to create learning strategies and materials that help students construct correct models about these challenging topics.

Our previous study revealed some student misconceptions surrounding the concept of molecular movement (19). In particular, we found that students may choose a correct answer but have incorrect reasoning. This observation led us to develop the current study, designed to dispel these misconceptions. In this paper, we used two-tier in-class pre-assessment questions followed by two-tier diagnostic clicker questions to identify common misconceptions. Two-tier diagnostic clicker questions are similar in format to traditional multiple-choice questions but they contain a second tier of questioning associated with the fundamental concept. The first tier of the question usually pertains to a knowledge statement while the second tier facilitates the testing of the students' learning at higher cognitive levels (4). We further address student common misconceptions using a series of assessment clicker questions.

With the aim of promoting higher levels of thinking and reasoning skills, we examined whether a combination of two-tiered diagnostic and repeated multiple-choice assessment clicker questions provides a method to improve student understanding of a particular biological topic. The basis of our current study is supported by recent literature that shows that repeated testing and retrieval could enhance long-term learning (20–22). We report here that our clicker-based teaching strategy can effectively correct misconceptions on the topic of molecular movement through cell membranes (MMTM).

METHODS

Course background

Introduction to Molecular and Cellular Biology is the first course required of students majoring in several biology and biology-related majors at the University of Colorado Boulder, a public four-year research university. About 350 to 400 students are enrolled in this course each fall, and approximately two-thirds are freshmen. About 70% are biology majors and biology-related majors (e.g., biochemistry and psychology). Student demographics include ~56% female, ~66% white, ~16% Asian, ~10% Hispanic, 2% African American, and 6% other unknown ethnicities. The course meets three times a week for 50 minutes and is co-taught by two instructors (authors JM and NG). In-class clickers are used in every lecture and students practice solving problems in weekly homework assignments.

An initial view of student ideas: in-class pre-assessment

The study design is summarized in Figure 1 and explained in detail here. Student understanding of MMTM was assessed prior to direct instruction through the use of an in-class pre-assessment. Each question was constructed with the goal of documenting student thinking about the MMTM topic. At the beginning of class before lecture began, approximately half of the students (n = 157) were given two questions on MMTM (the other half were given two questions on diffusion, not described in this paper). The first question was a multiple-choice question about the movement of molecules through cell membranes, and the second question included reasons supporting the answer to the first question (Appendix I). Each set of questions was on a single sheet of paper, and distributed so that adjacent students had different questions to minimize the chance of students discussing answers with their neighbors. Students were given about five minutes to answer the two questions, and then papers were collected by teaching assistants. Altogether, the pre-assessment took about ten minutes of class time. Students were informed that their answers would be used to help direct teaching later in the semester but would not be graded.

Use of clicker questions

Diagnostic clicker questions. One week following the pre-assessment, two sets of in-class clicker questions were administered during class, answered (voted on) by a total of 279 students (class 1). Like the pre-assessment, these questions were two-tiered, in that the first question was factual, and the second included reasons for the answer choices to the previous question. The answer choices for the second question were based on student responses on the pre-assessment. Students were given two sets of such questions and instructed to answer each individually (no discussion). The histogram distribution of answers was not revealed to students. After all four questions had been answered, each question set was displayed a second time,
and students discussed their answers with their neighbors. Volunteers were then asked to explain their reasoning, and the instructor ensured that the class heard from at least two students who chose different answers. After discussion the class re-voted on each question set. The instructor then used the student responses to the reasoning questions to discuss the effects of both polarity and size on the ability of a molecule to diffuse through cell membranes. This process occupied the entire 50-minute class period, with no additional lecturing.

Assessment clicker questions. Two clicker questions assessing understanding of the movement of various types of molecules through cell membranes were given at the start of the next class period \((n = 281; \text{ class 2})\). An additional two follow-up clicker questions on the same topic were given in the following class period \((n = 282; \text{ class 3})\). Students only voted once on these clicker questions, which were followed by peer discussion and instructor explanation. The different numbers of participants in classes 1, 2, and 3 was due to variation in student attendance on those days. The complete set of in-class clicker questions is included in Appendix 2.

To summatively assess the effects of the clicker question series, both multiple-choice and short-answer questions were included on the midterm exam \((n = 258; \text{ Appendix 3})\) following these class periods. Seventeen students withdrew from the course after the study was completed, and thus their results were not included in these measures of performance.

Data process and analysis

All students who completed at least some portion of the questions were included in this study. Individual student answers were followed throughout the three class periods. Answers to the relevant exam questions for each student were manually entered into the database and aligned with individuals’ clicker responses. Performance on exam questions from students who participated in only some of the portions of this study was compared with that of the students who participated in all portions of the study. McNemar’s chi-squared test (23) was used for comparing two dichotomous outcomes measured from pairs. In this study, the pair consists of students’ responses before and after discussion for individual clicker questions. Data were analyzed using mcnemar.test function in the statistical software R program. Independent t-tests were used to compare the data from the pre-assessment with the midterm exam questions.

Student survey

We sought student feedback regarding the helpfulness of this approach through an online survey one week after the midterm exam that included questions on MMTM \((n = 237)\). Since the survey was not mandatory, not all students who answered clicker questions participated in the survey \((-85\% \text{ of students participated in the survey})\). The survey was a Likert-type \((1–5)\) survey with an additional open-ended question: “What was the most memorable idea or moment from the past three class periods (addressing misconceptions about MMTM)?”

Permission to use student data

Permission to use clicker data and exam grades was obtained from the University of Colorado Institutional Review Board (exempt status: Protocol no. 0108.9).

RESULTS

Student initial ideas on molecular movement through cell membranes

We asked questions on the in-class pre-assessment with the goal of probing students’ thinking about molecular polarity and charge (question 1) and relative size (question 2) on molecular movement through a membrane. In question 1, the instructor presented a diagram with 11 different molecules (Fig. 2A) and asked students to choose the molecules that are not able to diffuse through a membrane composed only of phospholipids (without proteins, first tier) as well as to explain their reasoning (second tier). In question 2 (Fig. 2B), students were asked which of the three molecules would diffuse fastest through a membrane composed only of phospholipids (without proteins, first tier) and explain their reasoning (second tier). The purpose of this question was to capture the previously described “size-only” misconception (19).

Correct answers, common incorrect answers and sample explanations from the students on the pre-assessment are summarized in Table 1, alongside sample student ideas from the midterm exam questions on this topic, for comparison. On the pre-assessment, only two students \((1.3\%)\) chose the correct molecules for question 1 and provided correct explanations. The most common misconception was that non-polar molecules can’t pass through the polar or charged phospholipid heads of the membrane. A significant number of students \((28.5\%)\) failed to recognize certain polar molecules (e.g., NH₃) or mistaken non-polar molecules for polar molecules (e.g., CO₂). For example, students remarked in response to Q1: “I picked B (propane or C₃H₈) because it is a non-polar molecule and the phospholipid heads are polar and therefore would not interact with it and would not let it go through the membrane.” This student’s answer captured the most common student misconception on this topic, which is that the polar heads of the phospholipids prevent non-polar molecules from diffusing through the membrane. Another student explained, “I picked B because water is polar and can diffuse through the membrane so I figure the polar molecule B can diffuse through the membrane as well.” This student mistook the non-polar molecule C₃H₈ for a polar molecule and also had an incorrect reasoning. Another common misconception was the size-only misconception. Some students thought that because water is a small molecule, it should diffuse through cell membranes easily (without taking into consideration water’s polar nature). Other
misconceptions include stating that circular (e.g., glucose) or linear molecules (e.g., CO$_2$) can’t pass through cell membranes because they can’t bend.

**Using two-tier diagnostic clicker questions to identify student misconceptions**

Two sets of diagnostic clicker questions were used to identify student misconceptions on the topic of MMTM. These clicker questions were targeted at two aspects of the MMTM: the concepts of polarity and relative size. The first question addressed molecular polarity. It asked which of the two molecules (similar in size but different in polarity) was less likely to diffuse through a membrane composed only of phospholipids (without proteins). Sixty-three percent of the students answered correctly, and subsequent peer discussion significantly increased the percentage of correct answers (Fig. 3, Q1 and Q1AD). The second question (Q2) asked the reasoning for Q1 with multiple-choice answers based on the most common responses from students’ pre-assessment. Only 46% of the students had correct reasoning in their individual responses for Q2, and 59% had correct reasons for their responses after discussion for Q2 (Fig. 3, Q2AD). Question 3 was designed to address the issue of relative size. We presented three molecules all with similar polarity but differing in size. Eighty-seven percent of the students answered correctly without any discussion, while peer discussion increased the percentage of correct responses to 97%. Overall, the proportion of students who chose the correct answer in question 3 (Fig. 3, Q3 and Q3AD) and subsequently chose

| **TABLE 1.** Student ideas about MMTM on the pre-assessment and on the midterm exam. |
|-----------------------------------------------|
| **Student Ideas**                          | **Pre-Assessment** | **Midterm Exam** |
| Molecular polarity and size are both important in determining how fast a molecule can pass through cell membranes; Large polar or charged molecules can’t pass through phospholipid-only membranes. | 1.3% (2 students) chose the correct molecules and provided correct explanations. | 74.6% students chose the correct answer and provided correct explanations. |
| Non-polar molecules can’t pass through the polar or charged phospholipid heads; only the polar or charged molecules can pass through the cell membranes. | 52.8% (28.5% of these students failed to identify polar molecules or mistook polar molecules for non-polar molecules) | 0% (7.2 % students failed to differentiate polar from non-polar molecules) |
| Only size matters: Water is a small molecule and can therefore freely diffuse through cell membranes (without considering polarity feature); molecules comprising O$_2$, H, or OH can pass through cell membranes because they are similar to H$_2$O. | 37.4% | 18.2% |
| Circular (e.g., glucose) or linear molecules (e.g., O$_2$ and CO$_2$) can’t pass through cell membranes because they can’t bend. | 8.5% | n/a |

*The first student idea indicates correct answers, reasoning and the percentage of students who chose the correct answers and correct explanations on the pre-assessment and on the exam. The second, third, and fourth student ideas indicate student misconceptions and the percentage of students who chose these incorrect answers. MMTM = molecular movement through cell membranes; n/a = non applicable.*
the correct reasoning in question 4 (Fig. 3, Q4 and Q4AD) was higher than the proportion of students answering Q1 and Q2 correctly. It is worth noting that the percentages of correct answers for the reasoning questions (Q2 and Q4) were lower than the percentage of correct answers for Q1 and Q3. Student explanations of their ideas in the diagnostic clicker questions were very similar to the incorrect student responses seen in the pre-assessment.

Using clicker questions to address common misconceptions

In the next two classes, assessment clicker questions designed to address both the polarity and size issues were implemented, and students voted individually. The clicker questions were different but tested the same concepts in both class periods. In each class, the first question asked which of the five molecules would diffuse the fastest through a membrane composed only of phospholipids (without proteins). The second question asked which of the same five molecules would diffuse the slowest through a membrane composed only of phospholipids (without proteins). The percentage of correct answers to the first clicker question in class 3 was significantly higher than to the similar first clicker question 1 in class 2. Likewise, the percentage of correct answers to the second clicker question in class 3 was also significantly higher than for the similar second question 2 in class 2 (Fig. 4).

Performance improved significantly from pre-assessment to midterm exam

On the midterm exam following the unit on MMTM, we asked a multiple-choice question (similar to the assessment clicker questions asked in the first and second classes) and asked students to explain their reasoning for their answer choices (Fig. 5).

We compared student answers on the midterm exam to those of the pre-assessment. On pre-assessment question 1, only two students (1.3%) chose the correct molecules and provided correct explanations (Table 1). In contrast, on the midterm exam, 74.6% students chose the correct answer with correct reasoning. The most common misconception, “non-polar molecules can’t pass through the polar charged phospholipid heads,” was completely eliminated in the midterm exam. However, about 7.2% students still had trouble differentiating polar molecules from non-polar molecules. The size-only misconception also persisted for some students on the midterm exam (18.2%).

Although we were not able to directly compare performance on an identical exam question after this intervention to performance in the absence of this intervention, we can report on a comparison of a similar assessment question. In a previous semester of this course, when this topic was taught traditionally, students answered a pre/post assessment question similar to the exam question in this study, and their gain was only 23% (average pretest score: 16.4%; average posttest score 35.2%).
Incorrect answers B and E were chosen respectively by 7.2% and 18.2% of students.

FIGURE 5. Exam question (n = 258). Students were asked to select the molecule that will diffuse the fastest through a pure phospholipid bilayer (without proteins) and explain their reasoning. The correct answer is C (74.6% students chose this answer). Incorrect answers B and E were chosen respectively by 7.2% and 18.2% of students.

**Participation improves performance**

We also compared the midterm exam performance on the MMTM questions between the students who participated in the entire study and those students who participated in only a portion of the clicker series. We found that students who participated in all three classes had a significantly lower percentage of misconceptions about molecular size than students who participated in anything less than all three classes (Table 2). Students who responded to the diagnostic clicker questions and to one of the sets of assessment clicker questions had the lowest percentage of the size-only misconception on the midterm exam (Table 2). Though the sample size was small, these data suggest that both recognition of misconceptions through diagnostic questions (class 1) and an opportunity to apply concepts (class 3) were important in correcting students’ misconceptions.

**Student survey**

We gave a midterm survey to students one week after this study was conducted to gather attitudes about this introductory biology class. Table 3 shows the student responses to survey questions regarding the teaching strategy reported in this paper. All survey questions used a Likert scale of 1 to 5 (1 = strongly disagree; 5 = strongly agree). Student responses were in the range of 3.4 to 4.1 for all questions. The lowest-rating statement (3.4) was in regard to hearing explanations from other students for their answers. Students also answered a free response question: “What was the most memorable idea or moment from the past three class periods (addressing misconceptions about MMTM)?” Examples of student responses to this question are: “using the clicker questions, going over the clicker questions, then explaining why the answers were right and the exact processes were very helpful and I feel that doing it in that order helped it to stick better.” “I can’t imagine too many people, had they attended class, would still have any misconceptions.” About sixty percent of students voluntarily mentioned that MMTM is easier after this approach and report wanting to use this approach to learn other topics. However, about 3% of students noted that too much time had been spent on this topic despite feeling the approach was helpful.

**DISCUSSION**

Using different types of in-class clicker questions over several class periods, we showed that introductory biology students can overcome their misconceptions about a fundamental biology topic, the movement of molecules through membranes. The most common misconceptions from an in-class pre-assessment were used to generate distracters for in-class diagnostic clicker questions. Discussing these questions and being subsequently assessed on this topic led to a significant increase in student understanding of molecular movement through cell membranes.

**Summary of student misconceptions and possible causes**

Carefully crafting questions on a pre-assessment is the key to successfully identifying students’ misconceptions. As students’ written explanations often include both correct and incorrect ideas, these ideas can be collected to construct questions that capture student thinking. To identify such misconceptions, we asked questions that involved hypothetical cell membranes, which were composed only of phospholipids (without proteins). Completely correct ideas about the effects of molecular polarity and size on MMTM
require students to be able to distinguish polar molecules from non-polar molecules based on their molecular features. In addition, they have to know why non-polar molecules can pass through the hydrophilic heads of the phospholipids, and why polar or charged molecules can’t pass through the interior of phospholipid membranes. Students with partially correct ideas understood the interaction between non-polar molecules and membranes composed only of phospholipids, but these same students failed to identify the polarity of a given molecule. Alternatively, students could identify the polarity of a given molecule but could not understand how non-polar molecules moved through membranes.

A random sample of approximately half of the class answered the pre-assessment questions on MMTM. In using the two-tier diagnostic clicker questions developed from the student misconceptions revealed in the pre-assessment questions, we were able to gauge whether these misconceptions existed among all students in the course. Given prior evidence that peer discussion improves student performance on clicker questions (24), we were not surprised to see this trend confirmed in our study. However, we also found that a smaller number of students exhibited incorrect reasoning despite having answered the question correctly after peer discussion. This suggests that answering a clicker question correctly does not necessarily mean students have a complete understanding of the concept being tested.

In dissecting the common incorrect ideas about MMTM, we were able to address two separate components, polarity and size. By separating student reasoning on these two components, we gained insight into where student misconceptions may originate. Students frequently thought that non-polar molecules do not interact with the hydrophilic heads of the membrane and are therefore unable to pass through the lipid bilayer. In reality, the long portion of the hydrophobic tails, rather than the short hydrophilic heads, determines whether a molecule can pass through a membrane composed only of phospholipids (25). Student misconceptions about the impact of size on MMTM can reflect an incomplete understanding of either molecular polarity or charge. For example, although H2O is the smallest molecule in the question students answered, it is polar and therefore passes through membranes composed of phospholipids more slowly than non-polar molecules such as CH4. In this case, students had trouble accounting for both polarity and size. One reason students may not understand polarity well could be that they have just learned that the structure of membranes consists of hydrophilic heads and hydrophobic tails; this may lead them to attribute too much importance to the hydrophilic part of the membrane. Another possible source of student misconceptions is likely the idea that molecular size is the primary determinant for movement through a membrane, thus leading students to ignore molecular polarity.

Remediating student misconceptions through in-class questioning

The diagnostic clicker questions in this study were used both to diagnose and help correct common misconceptions. The combination of in-class diagnostic clicker questions and assessment clicker questions led to dramatic improvement in student understanding the principles of MMTM.

Since misconceptions are commonly persistent despite instruction (26), we did not expect that we could correct student misconceptions in a single class period. Accordingly, our intervention included repeated testing using both formative (assessment clicker questions) and summative (exam) assessments. Our results showed that students did best on the clicker questions asked in the third class (compared with the first and second class), and also performed better on the exam. Our findings are consistent with previous findings that testing and repeated testing enhances learning and retention (20–22).

Our findings also indicate that students who participated in the diagnostic clicker questions and one or two subsequent classes with assessment clicker questions understood the role of relative size in the context of polarity better than students who did not participate in the initial diagnostic clicker questions. This observation raises the importance of the initial diagnostic questions and is consistent with the idea that students need to realize their original idea is not supported by scientific evidence.

### TABLE 3.
Student attitude survey.

| Survey Question                                                                 | Average Response (± SEM) |
|-------------------------------------------------------------------------------|--------------------------|
| 1. Answering clicker questions targeted at the incorrect ideas on MMTM helped me learn. | 3.8 (± 0.07)             |
| 2. Listening to the explanations of clicker questions from other students helped me learn. | 3.4 (± 0.07)             |
| 3. Listening to my instructor’s explanations of concepts covered by clicker questions helped me learn. | 4.1 (± 0.05)             |
| 4. As a result of the past three class periods (addressing misconceptions about membrane permeability), I now have a strong understanding of this material. | 3.9 (± 0.05)             |

*aLikert scale 1 – 5 (1: strongly disagree; 5: strongly agree), n = 237. MMTM = molecular movement through cell membranes; SEM = standard error of the mean.*
in order to change this idea (12, 26). Through the use of two sets of diagnostic clicker questions in which the first question reveals a student misconception and the second question reveals possible incorrect reasoning, students can begin to reconstruct a correct framework once they realize their reasoning does not make sense. The follow-up assessment clicker questions in subsequent class periods then encourage students to use both size and polarity features to determine how molecules are able to pass through phospholipid-only membranes.

**Time spent on clicker questions**

We devoted a 50-minute lecture period plus 14 minutes at the beginning of the subsequent two classes (a total of 64 minutes) to reinforce the correct concepts about MMTM. In fall 2009, the same instructor spent 35 minutes on the topic of MMTM, a difference of 29 minutes. Thus, it is possible that simply spending more time on the topic in class was responsible for the higher student performance in 2010. However, the amount of instructional time may not be as important as how that time is spent (27). Although students spent more time on task in this study than in previous years, they also had the opportunity to be frequently tested, a strategy shown to be helpful for learning and long-term retention (22). The testing effect described by Karpicke and Roediger is not simply a result of students gaining re-exposure to the material during testing, because re-studying allowed students additional exposure to the material but produced poor long-term retention. Consistent with those studies, our data support the idea that repeated testing is powerful for learning and retention, likely having a more pronounced impact that lecturing on that topic for the same additional amount of time.

**Student survey**

Overall, the midterm student survey indicates that students find the MMTM topic easy to learn when it is presented in this format. This is compelling, given that over 90% of students had some kind of misconceptions about the topic prior to the study. The only neutral response (3.4) from the students was in regard to hearing explanations from other students for their answers. Students may not find peer explanations as satisfying as instructor explanations, despite evidence that student explanations are as valuable for learning (28). Ultimately, student-reported confidence in their understanding of this topic could reflect a shift from naïve to more expert-like thinking, brought about by confronting their misconceptions.

**SUPPLEMENTAL MATERIALS**

Appendix 1: Pre-assessment on MMTM  
Appendix 2: Diagnostic and assessment clicker questions  
Appendix 3: Midterm exam questions on MMTM

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