Received: 1 December 2020 | Revised: 10 June 2021 | Accepted: 10 August 2021

DOI: 10.1002/bmb.21575

ARTICLE

Biochemistry and Molecular Biology Education WILEY

Aiming for the Bullseye: Targeted activities decrease misconceptions related to enzyme function for undergraduate biochemistry students

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Funding information
National Science Foundation, Grant/Award Numbers: 1323414, 1711402, 1725940

Abstract

Biochemistry curricula present a particular challenge to undergraduate students with abstract concepts which can lead to misconceptions that impede learning. In particular, these students have difficulty understanding enzyme structure and function concepts. Targeted learning activities and three-dimensional (3D) physical models are proposed to help students challenge these misconceptions and increase conceptual understanding. Here we assessed such pedagogical tools using the Enzyme-Substrate Interactions Concept Inventory (ESICI) to measure (mis)conceptual changes from Pre- to Post-time points in a single semester undergraduate biochemistry course. A Control group of students engaged with the active learning activities without the 3D physical models and students in the Intervention group utilized these activities with the 3D physical models. At the Post- time point both groups had higher, yet similar ESICI scores of the same magnitude as the highest scoring group from the national sample. Concomitantly, many misconception markers decreased compared to the national sample, although some of these differed between the Control and Intervention groups. Based on this assessment, both pedagogical approaches successfully increased conceptual understanding and targeted many of the misconceptions measured by the ESICI, however, several misconceptions persisted. Surprisingly, the students who used the 3D physical models did not demonstrate a further decrease in the misconception markers. Additionally, psychometric evaluation of the ESICI with our sample recommends the revision of several questions to improve the validity of this assessment. We also offer suggestions to improve instruction and pedagogical tools with further avenues for research on learning.

KEYWOR DS

active learning, assessment and the design of probes for student understanding and learning, assessment of educational activities, concept inventory, enzyme substrate interactions, enzymes and catalysis, molecular visualization, physical models as learning tools

1 | INTRODUCTION

Many studies have investigated the impact of physical and virtual models on student learning in undergraduate biochemistry and molecular biology courses.[1–18] Few have utilized documented misconceptions to design physical models and activities with corresponding assessment(s) on learning.[1,5] This study compares active learning pedagogies with and without the use of 3D physical models that target (mis)conceptual understanding of enzyme structure and function. Enzyme content is a cornerstone of biochemistry courses, as demonstrated by the numerous learning goals related to enzyme structure, function, and regulation listed in the American Society for Biochemistry and Molecular Biology (ASBMB) Foundational Concepts framework.[19] Students begin engaging with enzyme structure and function concepts in high school and continue in introductory undergraduate biology courses. Unfortunately, undergraduate students often enter upper-level biochemistry and molecular biology courses misunderstanding these and related concepts.

In this study, we use the 15-item Enzyme-Substrate Interactions Concept Inventory (ESICI) developed by Bretz and Linenberger with validity evidence for use as a Pre- and Post- measure of students' conceptual and misconceptual understanding of enzyme structure and function.[20] This instrument groups misconceptions into five categories: (1) *Role of shape and charge in selectivity*, (2) *How the enzyme interacts with the substrate*, (3) *Competitive* vs. *noncompetitive inhibition*, (4) *Conformational change*, and (5) *Enzyme and substrate characteristics*.[20] Subsequent work focused on student's understanding of shape and charge in enzyme-substrate interactions.[21,22] Analyses of clinical interviews revealed that students focus on one to two of the three aspects driving enzyme-substrate specificity: complementary electronics, shape, and stereochemistry.[22] Additionally, students struggled to identify that the *allosteric site*, *specificity pocket*, and *active site* are possible regions that a substrate or small molecule could bind to on an enzyme.[21] Based on these studies the researchers suggested that instructors be intentional when choosing representations and to emphasize the features of the representation(s) with a concurrent discussion of the limitations of the representation(s).[21,22] The authors also recommended to "supplement 2D representation with 3D models or computer simulations [22]."

A hypothesis for these robust misconceptions is that inaccurate ideas are implanted from misuse and/or misinterpretation of visual representations.[23–28] Visual literacy, defined by Hortin[29] as "the ability to understand (read) and use (write) images and to think and learn in terms of images, i.e. to think visually." These skills are particularly challenging yet essential in biochemistry courses where a variety of abstract representations convey conceptual information. The use of 3D models to promote conceptual and visual literacy skill development is affirmed in other studies in biochemistry and molecular biology courses.[1–16] These and other studies report that models simulate playful learning,[18,30] increase social engagement,[18,31] can focus attention,[32] and are engaging.[2,9,11,17,31,32] Models help make abstractions visible[33,34] and support knowledge integration.[3] The study of models as kinesthetic thinking tools[8,11,17,18,30,35] supports the Embodied cognition theory wherein interactions of the mind, body and the environment create cognitive processes.[30,36,37] Physical models help students challenge misconceptions,[18] focus attention on foundational concepts,[2] and are thinking tools that aid in understanding higher-order questions.[11] Consequently, better learning gains are reported when students engage with the models compared to observing a model demonstration.[32] While female[4] and lower-achieving students[4,5,32] demonstrate the greatest learning gains, physical models benefit all students.[7,8] Indeed, students report a preference for using physical models over other learning tools.[1,9,11,18]

In this study, we assessed the impact of two pedagogical approaches by comparing students who engaged with the active learning activities versus those who worked with 3D physical models while completing the active learning activities. We hypothesized that the use of 3D models with active learning activities will increase student understanding and decrease misconceptions related to how enzymes' function more than the students who did not use the 3D physical models. We utilized the ESICI as a Pre- and Post-measure of student (mis)conceptual understanding. Additionally, we evaluated the validity of the ESICI with our sample.

2 | METHODS

During the *Enzymes* module in our biochemistry course, students in the Control semesters engaged in the active learning activities without the 3D physical models and those in the Intervention semesters used the 3D physical models in conjunction with the active learning activities. The same instructor taught both the Control and Intervention groups using highly structured, active learning pedagogies throughout the course. To measure learning changes across the semester, the ESICI was administered during the first week of class and again on the last day of class.

This study was approved by the IRB at the University of Minnesota under STUDY00002273: Seeing and Learning Biochemistry and STUDY0000026: Modeling for the

Enhancement of Learning Chemistry, and per IRB guidelines all identifying information was removed for data analysis and dissemination.

2.1 | Course and enzyme module description

The activities and models described herein were administered in a one-semester undergraduate biochemistry course (Biochemistry I) at a small, primarily undergraduate public university. Our campus serves health science undergraduates with the goal of building skills and knowledge relevant to working in a collaborative healthcare setting. As such, this course uses a variety of high impact and active learning pedagogies. This course also focuses on developing visual literacy skills. This three-credit, upper division course requires a C- or better in general chemistry I and II, organic chemistry I and II, and at least one semester of college-level biology course. The majority of students enrolled in this course are pursuing a Bachelor of Science in Health Science degree. Course content falls broadly into two categories: (1) structure–function relationship of macromolecules (proteins, lipids, and carbohydrates) and (2) eukaryotic heterotrophic metabolism of carbohydrates and lipids and oxidative phosphorylation. Nucleic acid structure and function content is intentionally omitted from the Biochemistry I curriculum as the Molecular Genetics and Integrative Biology courses at UMR cover this and related content on the flow of genetic information (replication, transcription, and translation).

Students in the Intervention semesters watched a lecture content video and completed an assignment prior to attending class (complete flipped classroom), while students in the Control semesters also completed a pre-class assignment but attended the live lecture on the same material in a previous class period (partially flipped classroom). The following briefly describes the activities and models used in this study.

2.2 | Description of the in-class activities and physical models

Six active learning, group-based activities were developed for the *Enzymes* module, of which five were used in both the Control and Intervention groups. These activities correspond to the canonical learning progression in an *Enzyme* module. Table 1 describes the distribution of activities for the Control and Intervention sections. This table also delineates the misconceptions targeted in each activity. The activities also addressed observed, but not validated, student difficulties related to enzyme specificity and catalysis. For the Intervention groups, additional wording was added to the activities as a guide to facilitate learning with the models. The Intervention students also completed individual pre-class assignments before each class session to help prepare students for class. In the Control sections, students worked in groups of 2–3

TABLE 1 Enzyme module activity and misconception distribution

	Misconceptions category targeted in activity[20]	Pre-class activity	In-class activity
Activity 1: Enzyme-Substrate Binding with a Hexokinase Model	(1) Role of shape and charge in selectivity (2) How the enzyme interacts with the substate (5) Enzyme and substrate characteristics	Control Intervention	Control Intervention
Activity 2: Enzyme-Substrate Binding with a Lactate Dehydrogenase Model	(1) Role of shape and charge in selectivity (2) How the enzyme interacts with the substate (5) Enzyme and substrate characteristics	Intervention	Control Intervention
Activity 3: Enzyme-Substrate Catalysis with a Lactate Dehydrogenase Model	(2) How the enzyme interacts with the substate (5) Enzyme and substrate characteristics	Intervention	Control Intervention
Activity 4: Enzyme-Substrate Catalysis and Binding with Serine Proteases Models (aka Model Exploration Activity)	(1) Role of shape and charge in selectivity (2) How the enzyme interacts with the substate (5) Enzyme and substrate characteristics	Intervention	Intervention
Activity 5: Enzyme-Substrate Catalysis and Binding with Serine Proteases Models (aka Problem Solving Activity)	(1) Role of shape and charge in selectivity (2) How the enzyme interacts with the substate (5) Enzyme and substrate characteristics	Neither	Control Intervention
Activity 6: Regulation of Enzyme Activity with the Enzymes in Action Kit© and HIV Protease Models	(1) Role of shape and charge in selectivity (3) Competitive vs. noncompetitive inhibition (4) Conformational change (5) Enzyme and substrate characteristics	Intervention	Control Intervention

persons for each activity. In the Intervention sections, one model set was provided to each group of 2–3 students, with the exception of the serine protease models where two groups of 2–3 students shared one serine protease model set. The groups were kept small to maximize interactions with the activities and model sets. The number of groups for each section ranged from 4 to 12.

We designed the Hexokinase, Lactate Dehydrogenase, and Serine Protease model sets in collaboration with the Milwaukee School of Engineering (MSOE) Center for BioMolecular Modeling (CBM). This is also where the models were printed. The CBM has a set of the serine protease models to borrow through their Lending Library (https://cbm.msoe.edu/lendingLibrary/index.php). We purchased The Enzymes in Action Kit© from 3D Molecular Designs. The virtual model of HIV Protease-1 with tipranavir is explored through a YouTube video produced by Boehringer Ingelheim[38] and two structure-based drug design articles.[39,40]

2.2.1 | Activity 1: Enzyme-substrate binding with a hexokinase model

The first activity in the *Enzymes* models utilized Hexokinase as a model to depict how an enzyme facilitates catalysis. Students started by exploring the enzyme-substrate (ES) complex formation. In doing so, students created a 2D drawing in skeletal structure to represent how Hexokinase brings the substrates, glucose and ATP, in proper orientation and proximity with the enzyme. Students also drew a diagram demonstrating how the enzyme binds the substrates with high selectivity based on a complementary fit of shape and electronics between the substrate and enzyme. Afterward, students explored what is meant for enzymes to 'preferentially bind the transition state' by creating a second drawing that represented the enzyme-transition state complex. Finally, students translated these events into an enzyme-catalyzed reaction coordinate diagram. A goal of this activity was to help students connect the concepts of enzyme-substrate binding and selectivity to representations using a real enzyme as the model.

In the Intervention sections, the activity prompts remained the same, however, each group of 2–3 students received a 3D physical model of ATP, alpha-D-glucose, and Mg^{2+} rendered in spheres with CPK coloring of the atoms (Figure 1). In these sections, students manipulated the model in three dimensions to satisfy the prompt requirements, then they drew a 2D skeletal representation of their placement. It is important to note that the physical models used for this activity were not flexible but did have functional groups attached with magnets that could be moved around to create the product molecules. This model set did not include the enzyme. In both the Control and Intervention section, the class focused first on the substrates and created a hypothetical active site that would satisfy the criteria in the prompts. Students were asked to predict and draw an active site with sidechains around the substrates that would demonstrate enzyme-substrate (transition state) selectivity.

2.2.2 | Activity 2: Enzyme-substrate binding with a lactate dehydrogenase (LDH) model

In the second activity, the learning objectives remained the same as the first activity but introduced a second enzyme model. The rationale here was three-fold. First, we wanted to reinforce the complex content introduced in the first activity with further practice. Second, this activity focuses on the active site and mechanism of lactate dehydrogenase (LDH), instead of a hypothetical active site. Third, the LDH model can also be used for the third activity on Michaelis-Menton kinetics. Ideally, this would allow students to better connect the binding and catalytic events. It is important to note that the second and the third activity in this series were adapted from Lehninger Principles of Biochemistry, 6th ed., Chapter 6: Enzymes end of chapter problem: *Exploring and Engineering Lactate Dehydrogenase*.[41]

The progression through the second activity was similar to the first one, but here student groups started with a 2D skeletal rendering of the LDH active site. They began by drawing the substrates in the active site using skeletal structure in optimal orientation and proximity. They repeated this for the transition state molecule. Using the two drawings, students evaluated how LDH facilitates catalysis by preferentially binding the transition state. In the final section, students draw the products in the active site and evaluate whether or not LDH is regenerated during the reaction and could be considered a biological catalyst.

The 3D physical model set accompanying this activity contained the enzyme sidechains in the 2D representation, the substrates, and products (Figure 1). This model had magnets placed to allow manipulation of the enzyme mechanism and to aid student understanding of how the enzyme participates in the binding and catalytic conversion of the substrate(s) to product(s), and the subsequent release of the product(s). The sidechains were printed in spheres with CPK coloring to help students visualize how shape and electronics impact geometric and charge complementary interactions between the enzyme and substrates. In class, students worked in groups of 2–3 and placed the sidechains on top of the 2D active site

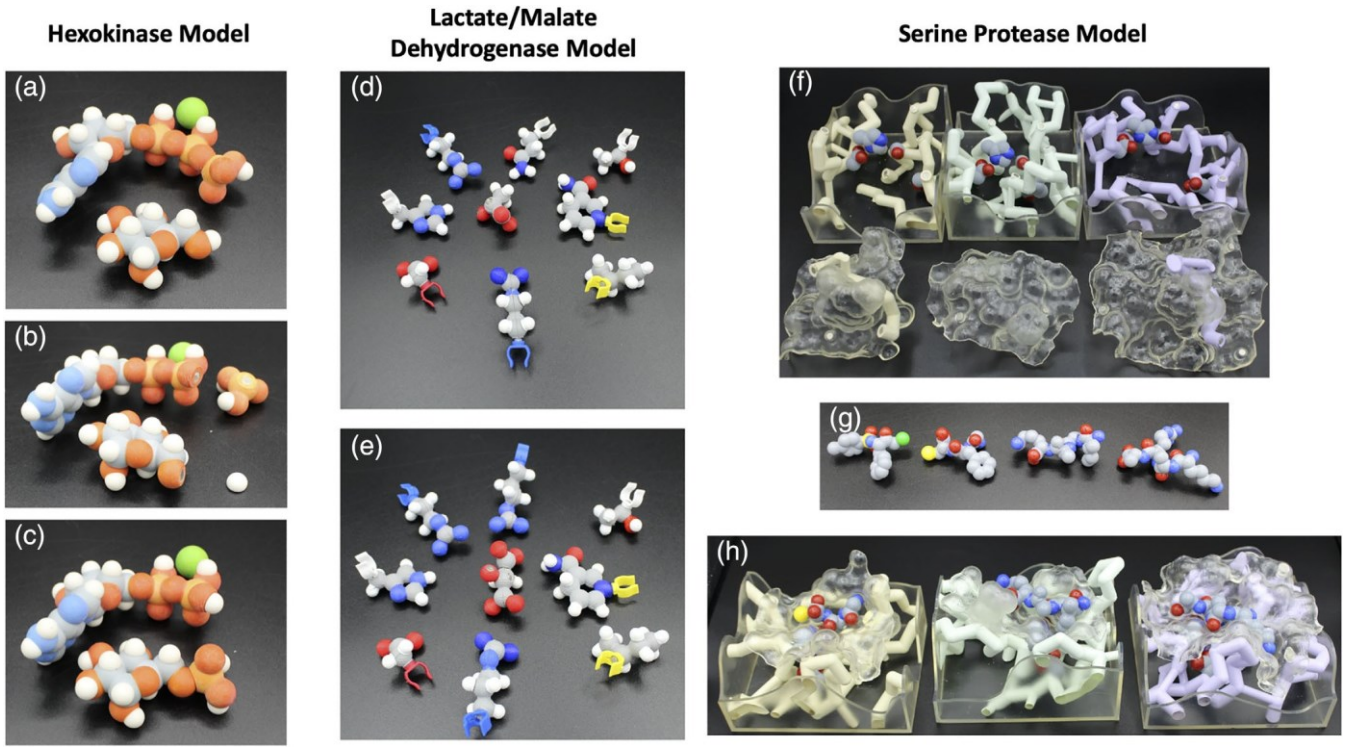

FIGURE 1 3D physical models used by the intervention groups. (a–c) ATP, alpha-D-glucose, and Mg^{2+} models for the reaction catalyzed by hexokinase. The atoms involved in the mechanism are attached via magnets and can be manipulated to represent the substrates and products. (d, e) The lactate dehydrogenase model set (d) includes active site residues, substrates, and products. There are additional residues to model point mutations that help distinguish binding and catalytic residues (not shown). This model can be altered to represent the active site and reaction catalyzed by malate dehydrogenase (e). The atoms involved in the mechanism are attached via magnets and can be manipulated to represent both enzyme-catalyzed reactions. (f–g) The complete serine protease set includes a backbone model, surface plate, and one substrate for chymotrypsin, elastase, and trypsin. The set also includes an epimer of the chymotrypsin substrate (not shown) and a competitive inhibitor for chymotrypsin. When combined (h) each enzyme has one surface plate and one substrate that fit optimally with the backbone model. *Note the Enzymes in Action Kit© from 3DMD is not shown

representations, moved the substrate models around to meet the criteria of each question, and finally drew the 2D skeletal structure of their manipulation of the model.

2.2.3 | Activity 3: Enzyme-substrate catalysis with a lactate dehydrogenase model

The LDH enzyme was used again in the third activity as an application of Michaelis–Menten kinetics concepts. Students investigated the impact of point mutations in the LDH active site to have a structural understanding of kinetic constants: k_{cat}, K_m and k_{cat}/K_m. Again, this activity was adapted from Lehninger Principles of Biochemistry, 6th ed., Chapter 6 Enzymes end of chapter problem: *Exploring and Engineering Lactate Dehydrogenase*.[41] The 3D LDH model kit (Figure 1) included these point mutations in CPK coloring to give students a better visual understanding of shape and electronic contributions to enzyme-substrate interactions. The final question in this activity challenged students to assess how a point mutation (Gln109Arg) in LDH enables a larger substrate (malate) to bind. The 3D modeling kit included this point mutation and the ability to alter the substrate/product from pyruvate/lactate to malate/oxaloacetate (Figure 1). Again, we wanted the 3D physical model to give students a physical representation of the space and electronics of these active site alterations, beyond the 2D skeletal diagram in the activity. Analyses of student responses to LDH activities with corresponding Exam 2 performance are forthcoming.

2.2.4 | Activities 4 and 5: Enzyme-substrate catalysis and binding with serine proteases models

The fourth and fifth activities, also referred to as the Model Exploration Activity and Problem Solving activities, respectively, apply the enzyme binding and catalysis concepts using three serine proteases: trypsin, chymotrypsin, and elastase. These activities and corresponding 3D

physical models are described elsewhere.[42] In brief, the Model Exploration activity guided Intervention students through the representations used in the serine protease model set. The Problem Solving activity used 2D representations and assessed Control and Intervention students' understanding of how geometric complementary, stereochemistry, and electronics impact interactions between the proteases and their substrates. The serine protease 3D physical model set included a backbone active site box, surface plate, and substrate for each of the proteases (Figure 1). In addition, an epimer of the chymotrypsin substrate and an inhibitor for chymotrypsin were part of this model set. Student groups in the Intervention sections worked with the serine protease model set for two consecutive class sessions while completing the fourth and fifth activities in this series. In the Control sections, two additional questions were included in the activities. In the Intervention sections, however, these questions were offered as practice problems as there was no time for Intervention groups to finish the activity and have a class-wide discussion.

2.2.5 | Activity 6: Regulation of enzyme activity with the enzymes in action kit© and HIV protease models

The last activity in the *Enzymes* module was the same for the Control and Treatment students. The Enzymes in Action Kit© from 3D Molecular Designs was used along with a virtual model of HIV protease. Students explored different modes of enzymes inhibition. First, students in groups of 2–3 used the Enzymes in Action Kit© to gain a better understanding of how enzymes interact with inhibitors. This physical model foam kit contained concept-based parts that were not specific to any one enzyme. Then, as a class, we employed an HIV protease virtual model with the Tipranavir inhibitor as an example of enzyme inhibition. This class session was also used to synthesize prior material by asking students to gather all the information a researcher would need in order to rationally design an inhibitor for an enzyme.

2.3 | Sample

The final sample of 125 students consisted of 87 Intervention students and 38 Control students with ESICI response at Pre and Post (Table 2). The Intervention condition had a descriptively higher proportion of female students and fewer total credits taken, though neither difference was statistically significant. Intervention students did have a significantly higher average cumulative GPA ESICI scores from both groups at Pre were descriptively similar and statistically nonsignificant (Table 2), indicating the groups had comparable baseline conceptual understanding.

2.4 | Analysis

We were interested in comparing students' responses to the ESICI across time (i.e., from Pre to Post) and by condition (i.e., Intervention and Control). First, for each ESICI item, we compared the proportion of students responding correctly by time and condition. This provided a summary of students' conceptual accuracy. Furthermore, we reviewed the item response frequencies by time and condition to discern any patterns in the types of misconceptions students held.

2.5 | Psychometric evidence for scale score interpretation

We were also interested in making comparisons based on a total scale score derived from the sum of correct item responses. Appropriate interpretation of scores requires supporting validity evidence.[43] Using the dichotomously scored (correct/incorrect) items to create the total score,

TABLE 2 Proportion or mean (SD) of background characteristics by condition

Demographic	Control	Intervention	Total
n	38	87	125
Female	53%	69%	64%
SOC	34%	30%	31%
Total credits	78.47 (24.35)	68.88 (21.36)	74.01 (23.35)
Term credits	12.42 (2.92)	13.18 (2.38)	12.77 (2.69)
ACT math	25.71 (2.86)	25.78 (2.77)	25.76 (2.79)
Cumulative GPA	3.17 (0.38)	3.36 (0.35)*	3.31 (0.37)

Note: Independent sample *z*-tests for proportion differences and t-tests for mean differences were run to examine the comparability of the Intervention and Control groups. *$p < 0.05$.

initial evidence was provided by Bretz and Linenberger[20] in their article documenting the development of the ESICI. Nonetheless, validation is an ongoing process to which we aimed to contribute. All analyses used the psych package (v. 1.8.12)[44] in R (v. 3.6.1).[45]

We conducted an item analysis grounded in classical test theory and also estimated score reliability with α and ω.[46,47] One assumption of α and the interpretation of a total scale score is the approximate unidimensionality of the ESICI. In other words, all of the items on the ESICI should be measuring one common construct rather than multiple constructs. To investigate the dimensionality of the ESICI, we first calculated the Kaiser–Meyer–Olkin (KMO) Measure of Sampling Adequacy.[48] KMO measures the proportion of variance in item responses that is common variance. Values >0.80 indicate adequate common variance to model in a factor analytic model. We then created scree plots and ran a parallel analysis to aid in selecting the number of factors underlying the ESICI responses.[49] Finally, we compared the fit of exploratory factor analysis (EFA) models with either 1 or 2 factors extracted. The EFA models were run using ordinary least squares and an oblimin rotation. Given the dichotomized item responses, the polychoric correlation matrix, as opposed to the Person correlation matrix, was used to estimate α, ω, KMO, and the EFA models.[50] The item analysis and EFA models were run separately on the Pre and Post responses to determine if items functioned consistently across time.

3 | RESULTS

3.1 | Total score performance

ESICI total score performance was descriptively similar for Control and Intervention groups (Table 3) and statistically nonsignificant based on an independent samples t-test at both Pre ($t[64] = 0.25$; $p = 0.80$) and Post ($t[69] = 1.66$; $p = 0.10$). Both groups' had higher total scores at Post than Pre. When comparing the total scores to previously published data parsed out by major, the Control and Intervention groups (all of whom were Health Science majors) had higher scores than the reported majors except Chemistry and Biochemistry/Molecular Biology (Table 3).[51] Here the Control and Intervention performed similarly to students in these majors.

3.2 | Item performance

Overall, the Intervention and Control students performed similarly across the 15 ESICI at both Pre and Post (Figure 2). On average, students from both conditions improved from Pre to Post with the exception of items Q1 and Q3 where performance was flat. Conspicuously, performance on Q15 dropped 2 percentage points for the Intervention conditional while the Control condition increased 26 percentage points (Table S1). Items Q4, Q9, Q11, and Q13 are also notable in that the rate of improvement from Pre to Post differed somewhat between the Intervention and Control conditions.

In addition to examining overall rates of correct responses, analyzing the response frequency of incorrect responses provides information about which types of misconceptions persist (Table 4).

In Table 4, we compare our Post data for the Control and Intervention groups, to national data published by Bretz and Linenberger.[20] For the 22 distractors, the Control and Intervention performed better than the national average (i.e., had a smaller percent) on over half of the distractors (14 and 12 items, respectively). Of these, nine markers were the same misconception. The Control and Intervention groups had decreased misconception scores in every category except *Conformational change*. Similarly, the Control and Intervention groups scored the same or worse on 8 and 11 misconception markers, respectively, compared to the national sample. Again, of these five were of the same misconception markers and are represented in every category except *Competitive* versus *noncompetitive inhibition*. Other than these observations, there is no clear pattern in these data. Each category shows at least two of the three scenarios, wherein students in either Control or Intervention performed better than, worse than, or about the same as the national average. When looking at the questions that had representations (Q3, Q7, Q9, Q11, Q14, and Q15) there are, again, no clear patterns in these data. In some cases, the Control performed better than the Intervention, while the opposite is true in other cases.

3.3 | Psychometric evidence for scale score interpretation

Item difficulty is the proportion of students who responded correctly to the item (Table 5). The item difficulties at Pre and Post were highly correlated with each other ($r = 0.90$) and with the item difficulties found by Bretz and Linenberger: $r = 0.91$ for both Pre and Post. This suggests that across administrations the rank order of items from most to least difficult was consistent. Conversely, the magnitude of the difficulties differed. At Pre, the items unsurprisingly tended to be more difficult than at Post or in Bretz and Linenberger.[20] Item discrimination is the correlation between item response and total scale score. Discrimination values <0.30 indicate response to the item is a poor indicator of the student's total score. At Pre, 9 of the 15 items had low

discrimination. In Bretz and Linenberger[20] and at Post, only three and two items, respectively, were low. Item Q1 had low discrimination across all three administrations while Q3 and Q11 were low on two of the three.

Score reliability was good at Post ($\alpha = 0.73$; $\omega = 0.74$) and higher than what Bretz and Linenberger[20] found ($\alpha = 0.53$). At Pre, however, the score reliability was extremely low ($\alpha = 0.37$; $\omega = 0.41$), suggesting responses across items had little in common. A similar conclusion emerges from KMO with unacceptable sampling adequacy at Pre (0.19) and Post (0.17).[47] The scree plots (Figure S1) also suggest that the unidimensionality of the ESICI is tenable at best. At Pre and Post, the parallel analysis recommends seven factors. Although 7 is probably an overestimate,[48] the location of the "elbow" suggests at least two factors at Post and is ambiguous at Pre.

The results of the one and two factor EFA models also favor a multidimensional interpretation of the ESICI. A χ^2 difference test between the one and two factor models demonstrated that the two-factor model fit the data better at both Pre (χ^2 [14] = 69.01; $p < 0.01$) and at Post (χ^2 [14] = 159.90; $p < 0.01$). In order for a total scale score to have a meaningful interpretation there needs to be evidence of a single strong common factor. Such evidence would include a one-factor EFA model accounting for the preponderance of total variance with most items loading strongly onto the single factor. The factor loadings and variance accounted for by the factors, however, suggest that even the two-factor model was insufficient for accounting for the preponderance of total variance (Table S2). The one-factor model only accounted for 10% of the total variance at Pre and 18% at Post. Although adding a second factor substantially increased the explained variance, the two-factor model still only accounted for 20% and 29% of the variance at Pre and Post, respectively. Lastly, most items at Pre had weak factor loadings in both the one- and two-factor models. Although factor loadings were stronger at Post, 6 of the 15 items still did not load strongly onto a factor in either model.

In summary, the psychometric evidence suggests the items on the ESICI measure multiple constructs rather than a single common construct. This makes interpretation of total scale score rather difficult. The item discriminations suggest items Q1, Q3, and Q11 are good candidates for revision or dropping entirely to improve the unidimensionality of the ESICI. Q2 and Q8 may also be good candidates based on the low factor loadings across both time and model type.

4 | DISCUSSION

Previously, Linenberger and Bretz provided evidence of student misconceptions related to how enzymes and

TABLE 3 ESICI scores by academic major

	Nutrition exercise science[a]	Pre-health[a]	Biology[a]	Other[a]	Chemistry[a]	Biochemistry molecular biology[a]	*Health science (Co. pre)*	*Health science (Co. post)*	*Health science (In. pre)*	*Health science (In. post)*
n	100	142	224	41	61	139	*38*	*38*	*87*	*87*
Median	7.00	8.00	8.00	9.00	10.00	10.00	*7.00*	*10.00*	*6.00*	*9.00*
Mean	6.74	6.64	8.24	8.51	9.48	9.57	*6.74*	*9.89*	*6.64*	*9.07*
SD	2.12	2.23	2.46	2.47	2.34	2.31	*1.98*	*2.58*	*1.78*	*2.51*

Note: Mean differences between control and intervention groups were statistically nonsignificant at pre and post based on an independent samples *t*-test with $\alpha = 0.05$. Italics indicate student performance in our study. Co. and In. refer to Control and Intervention groups, respectively.

[a]Data from Reference 51.

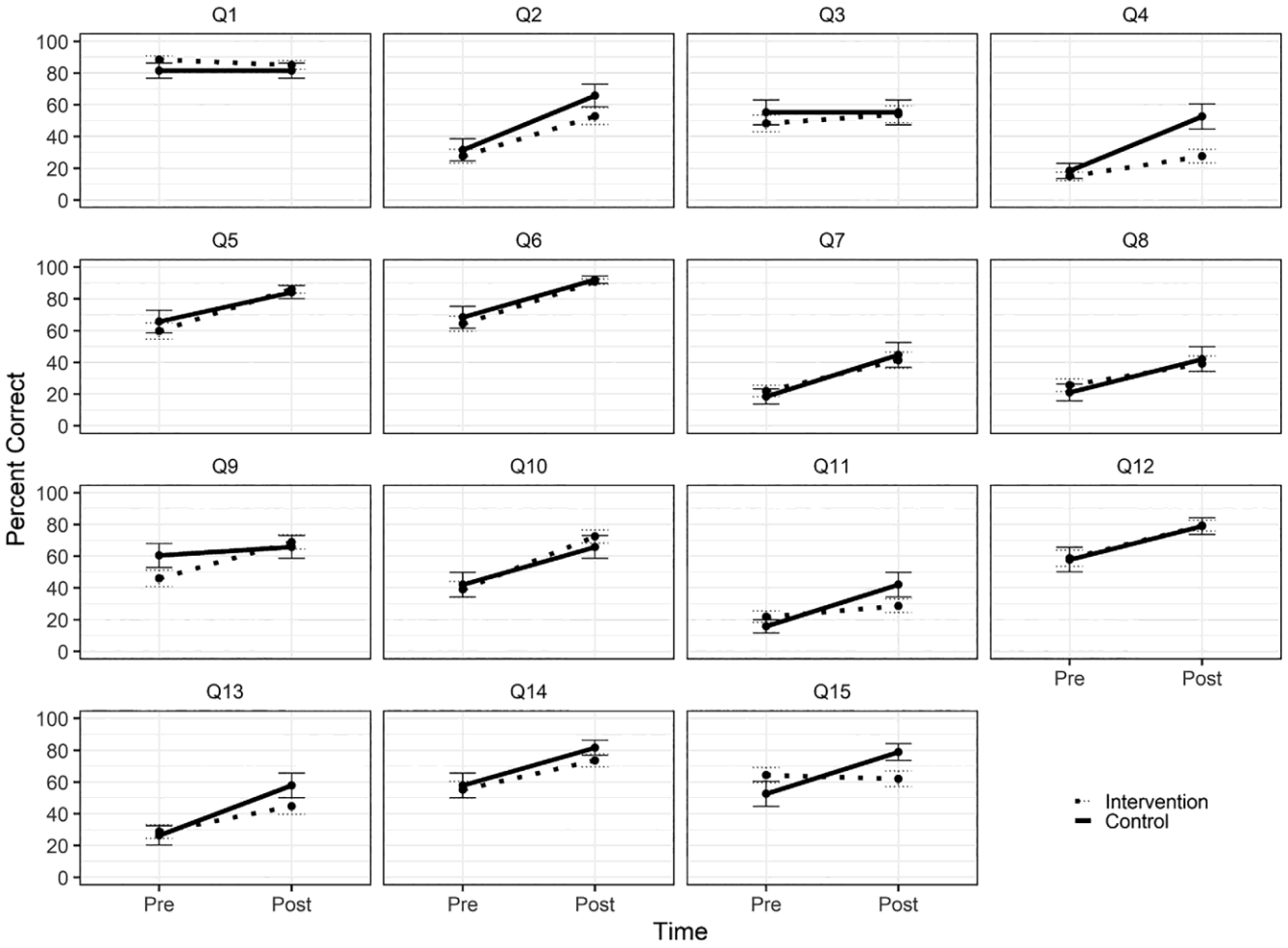

FIGURE 2 ESICI item analysis for control and intervention groups. Percent correct and 95% confidence interval for each ESICI item by time (pre/post) and condition (intervention/control)

substrates interact.[20–22] These researchers suggested that better learning tools were needed to help students overcome these misconceptions, citing the use of 3D physicals models as one such tool. Here we present the results of our study on the implementation of a series of six active learning activities wherein the Intervention group engaged with physical models during the activities and the Control group did not. With the exception of the sixth activity, wherein both groups used the same model with the same activity. Both the activities and physical models were designed to target the identified misconceptions. In this paper, we used the ESICI as the assessment of learning.*

Evaluation of the total ESICI score suggests that the Control and Intervention groups were equally successful, as there was an increase in the total score for both groups with no significant difference in total ESICI performance between the groups at the Post time point. Moreover, the Control and Intervention groups, performed similarly to the highest-scoring students in the national sample seeking degrees in Chemistry and Biochemistry/Molecular Biology. This is important, because our students are Health Science majors and would represent the lowest-scoring groups nationally. These data indicate that both pedagogical approaches helped students better understand the enzyme content.

That being said, the psychometric evaluation in our sample calls into question the use of a total ESICI score as a measure of learning. Specific items (Q1, Q3, and Q11) were found to have a low correlation with the total score (i.e., low discrimination) or a low correlation (i.e., factor loading) with an underlying common factor (Q2, Q8). Revising these items so they contribute information to the total score will enhance the interpretability of the score. Furthermore, the EFA results suggested the ESICI is a multidimensional, rather than unidimensional, measure. This is not a surprise, however, as the ESICI measures many misconceptions. Based on the psychometric evidence, it is more appropriate to glean information from each item separately rather than through a total score across all ESICI items in the present sample.

TABLE 4 Misconceptions detected by ESICI on post-test

Misconception (item distractor)	Percent of students		
	National[a]	Co.	In.
Role of shape and charge in selectivity			
Charged amino acid interacts with OH regardless of sterics (7a, 7d, 3d)	50.9, 41.5	37, 26	29, 31
Students consider charge but not shape (9c)	26.6	26	24
Similar amino acid will bind in pocket (3b)	12.2	11	10
How the enzyme interacts with the substrate			
Disregard of relationship between scissile bond and specificity interaction (11c)	44.6	45	*49*
Enzyme will bind most tightly to the substrate (4a)	37.9	26	31
Active site is the only place of interaction (11a, 12d)	27.7, 13.3	11, 3	14, *17*
Allosteric site is not a binding site (2b, 2d)	27.2	19	*29*
Binding only occurs at transition state (4d)	20.8	16	*30*
Active site is on the substrate (12a)	15.7	11	3
Specificity pocket is not a binding site (2a, 2d)	14.4	*19*	*25*
Enzyme will bind most tightly to the active site (4b)	13.6	5	11
Competitive vs. noncompetitive inhibition			
Inhibitors can bind to the substrate (10d)	33.5	26	10
Inhibitors interact only via competitive inhibition (10a, 6a)	18.8, 15.3	8, *18*	15, 6
Conformational change			
Allosteric effecter must change enzyme conformation (15c)	28.7	21	*34*
An enzyme must change conformation prior to interacting with substrate (14b)	12.3	13	*15*
Enzyme and substrate characteristics			
Solvent cannot be a substrate (8c)	27.3	18	13
Enzyme is a protein therefore a protein cannot be a substrate (8d)	18.8	*26*	*25*
The "key" images represent the enzyme (5b)	17.7	16	9
Nucleotides cannot be substrates (8b)	16.8	13	*23*

Note: Bold indicates group performed better than national average, italics indicate group performed worse than national average. Co. and In. refer to Control and Intervention groups, respectively.
[a]Data from Reference 20.

On a question-to-question basis, the Control and Intervention groups performed similarly on all ESICI questions, except Q4 and Q15 where the Intervention performed worse than Control at the Post time point. Again, this indicates that the two pedagogical approaches yield similar results. When looking at the misconception markers that the Control and Intervention groups scored better than the national sample, nine of these were the same marker and spanned all of the categories, except *Conformational change*. This is not surprising considering that none of the 2D representations on the activities illustrated this concept and only one physical model could demonstrate conformation change. Interestingly both the Control and Intervention used this model. This observation, in addition to the misconceptions that the Control and Intervention scored either the same or worse than the national sample, indicates areas for activity and model refinement. Misconceptions related to

TABLE 5 Item statistics for the 15 ESICI items at pre and post

	Difficulty		Discrimination	
Item	Pre	Post	Pre	Post
Q1	0.864	0.840	0.235	0.244
Q2	0.288	0.568	0.230	*0.403*
Q3	0.504	0.544	0.058	*0.312*
Q4	0.160	0.352	0.030	*0.428*
Q5	0.616	0.856	*0.424*	*0.393*
Q6	0.656	0.912	*0.302*	*0.384*
Q7	0.208	0.424	0.296	*0.491*
Q8	0.242	0.400	*0.313*	0.225
Q9	0.504	0.680	*0.320*	*0.377*
Q10	0.400	0.704	0.173	*0.413*
Q11	0.200	0.200	0.166	*0.382*
Q12	0.584	0.792	0.292	*0.367*
Q13	0.280	0.488	*0.394*	*0.381*
Q14	0.560	0.760	*0.475*	*0.396*
Q15	0.608	0.672	0.277	*0.504*

Note: Italics indicate adequate discrimination (>0.30).

Conformational change may be particularly robust as developing a mental model would require an expert-like synthesis of many cognitive elements. Here introducing an animated virtual model that correlates with the physical model and 2D representation may help students better visualize how conformation changes arise in response to substrate and/or effector molecule binding. Additionally, two other misconception markers that the Control and Intervention scored worse than the national sample on (*Specificity pocket is not a binding site* and *Disregard of relationship between scissile bond and specificity interaction*) used vocabulary words that Linenberger and Bretz previously noted as troublesome (i.e., *specificity pocket, active site, and allosteric site*),[21] and we anecdotally observed the same with our students. The activities could be altered to include better descriptions of these terms, the pre-class activities can be scaffolded to include practice associating these terms with representations and the instructor can review these terms in discussion with the class. The similarities in item analysis between the groups further indicate that both pedagogical approaches offer students targeted practice with the concepts and the ability to confront many misconceptions. What is more, instructors without access to physical models can create learning experiences that address misconceptions.

We were surprised that the use of the 3D physical models did not have an additional benefit with respect to the results of the ESICI and propose ideas as to why. Biochemistry is a visual discipline that requires students to understand a variety of representations that contain diverse levels of abstraction to portray information. If students are trying to overcome prior misconceptions and assimilate new knowledge, adding in new tools, such as the 3D physical models, may add to the high cognitive load in biochemistry. What we thought were concise, appropriately challenging 3D physical models that should break down the barriers between misconceptions and facilitate a more realistic experience, may have ended up creating another cognitive barrier for students to overcome. This sentiment is echoed in a paper by Villafañe and Loertscher, wherein the authors suggest that single semester efforts in biochemistry often produce modest results and prior coursework should incorporate aligned active learning to better challenge student (mis)conceptions.[52] Thus, to try and phase out these misconceptions before new information is learned, and ultimately decrease cognitive load, 3D physical models could be introduced during introductory chemistry and biology courses.

While the 3D physical models did not have the proposed impact of further decreasing misconceptions related to how enzymes function, these results spur further questions and ideas for refinement and assessment. First, we are unsure how much time a student needs to orient to and understand highly accurate 3D physical models, especially those using multiple representations.[33] Better learning gains for students could arise from using 3D physical models in laboratory sessions and conceptual demonstrative models during lecture sections to increase the time needed to understand the model. Other groups have shown that combining physical models and virtual activities is efficacious for student learning.[9–11] Virtual-modeling exercises could accompany the pre-class activity and also be used during the in-class session. However, models with multiple representations can hinder learning if design improperly or if students are unable to make connections across the representations.[33] If this is the case, we can employ the four-level framework from Grayson, Anderson, and Crossley[53] cycle to uncover student difficulties with respect to the models and reveal areas for pedagogical improvement. Also, worth exploring are other types of assessment to probe students' conceptual understanding of how enzymes function and the development of mental models. Analysis of student performance on activity and exam items may provide evidence of expert-like thinking.

ACKNOWLEDGMENTS

This work was partially supported by the National Science Foundation under award number 1711402 to the University of Minnesota, Rochester and award numbers 1323414 and 1725940 to the Milwaukee School of

Engineering. We thank Jeffery Ewertz, Olivia Stiller, Sarah Styer, Cassandra Kersten, Thor Wirth, Naima Yusuf for helping design the 3D physical models used in this study. We thank Michael Warden for final model design and construction of the physical models. Finally, we thank all of the students who participated in this study.

ENDNOTE

* Note that the findings presented herein are based on data collected at one institution with a relatively small sample of students.

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SUPPORTING INFORMATION

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How to cite this article: Terrell CR, Ekstrom T, Nguyen B, Nickodem K. Aiming for the Bullseye: Targeted activities decrease misconceptions related to enzyme function for undergraduate biochemistry students. Biochem Mol Biol Educ. 2021;49:904–16. https://doi.org/10.1002/bmb.21575