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Lesson

# It's a Substrate... It's a Protein...No - It's an Enzyme! Teaching Using 3D Serine Protease Physical Modeling Activities to Confront Misconceptions

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## Abstract

Reported misconceptions of enzyme-substrate interactions highlight the necessity for better, targeted instructional tools and assessments. A series of active learning activities with corresponding three-dimensional (3D) physical models were developed to target undergraduate biochemistry students' conceptual understanding of space, electrostatic interactions, and stereochemistry in enzyme-substrate interactions. This lesson includes two activities utilizing physical models of elastase, chymotrypsin, and trypsin. These enzymes are widely taught in undergraduate biochemistry courses and are exceptional examples of a variety of enzyme paradigms. The *Model Exploration* activity guides students in an exploration of these models to connect conceptual and visual content. The *Problem Solving* activity uses two-dimensional representations of the physical models to further build student's understanding of enzyme-substrate interactions. These activities are implemented in two consecutive fifty-minute classes or alternatively combined for a seventy-five-minute class. These lessons are an inclusive, student-centered approach to teaching that enables students to confront misconceptions and promotes mastery of the material.

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**Supporting Materials:** Supporting File S1: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases; S2: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases Answer Key; S3: Enzyme Substrate Interaction - Instructor Guide Video on Teaching using Serine Protease models; S4: Enzyme Substrate Interaction - Model Exploration Activity; S5: Enzyme Substrate Interaction - Problem Solving Activity; S6: Enzyme Substrate Interaction - Model Exploration Activity Answer Key and Rubric; S7: Enzyme Substrate Interaction - Problem Solving Activity Answer Key and Rubric; and S8- Enzyme Substrate Interaction - Sample Exam Questions.

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## Learning Goals

- Students will physically manipulate and explore enzymes in three dimensions.
- Students will understand the importance of shape, electrostatic forces and stereochemistry in enzyme binding and catalysis.
- Students will develop mental models of enzyme binding and catalysis.
- Students will visually compare three serine proteases.

From the Biochemistry and Molecular Biology Learning Framework (1):
- "How are structure and function related?"
- "What is the role of noncovalent intermolecular interactions?"

From Threshold Concepts for Biochemistry (2):
- "The physical basis of interactions: Interactions occur because of the electrostatic properties of molecules. These properties can involve full, partial, and/or momentary charges. Correct understanding of noncovalent interactions is essential in integrating structure and function."

## Learning Objectives

From Schönborn and Anderson Visual Literacy Skills (3):
- Students will be able to "decode the symbolic language composing an External Representation (ER)."
- Students will be able to "interpret and use an ER to solve a problem."
- Students will be able to "spatially manipulate an ER to interpret and explain a concept."
- Students will be able to "translate horizontally across multiple ERs of a concept."

From the authors:
- Students will be able to assess the shape (size) and electrostatic properties (polarity) of the enzyme active site to determine suitability for binding and/or catalysis with a substrate.
- Students will be able to assess a substrate's shape (size), stereochemistry, and electrostatic properties (polarity) to determine suitability for binding and/or catalysis within an enzyme's active site.
- Students will be able to use structural features of active sites to compare binding and/or catalysis.
- Students will be able to compare the residues of the catalytic site and the specificity (binding) pocket within an enzyme and among enzymes.
- Students will be able to predict the product(s) of a serine protease reaction given the substrate and enzyme.

## INTRODUCTION

Our primary aim with this project is to help undergraduate biochemistry students develop mental models for abstract biochemistry concepts. We use *physical models* to promote student conceptual understanding and visual literacy skills, since two dimensional representations are known to propagate learning difficulties (4). In this paper we refer to physical models as any three-dimensional tactile learning aid that is part of the *representation* category that includes all models (image, graph, tables, etc.).

During our literature review we learned about student misconceptions related to how enzymes and substrates interact and the Enzyme Substrate Interaction Concept Inventory (ESICI) that measures these misconceptions (5-7). The nineteen misconceptions measured by the ESICI are grouped into five categories: *Role of shape and charge in selectivity, How the enzyme interacts with the substrate, Competitive versus noncompetitive inhibition, Conformational change, and Enzyme and substrate characteristics* (5). Consistent with our aim, representations of enzymes can be manipulated with molecular modeling software and printed in three dimensions. This generates highly accurate physical models that can be used as learning tools in the classroom. We chose to develop a 3D physical model set of the serine proteases with corresponding activities, because these enzymes are commonly taught in biochemistry courses and the comparison of elastase, trypsin, and chymotrypsin can address several misconceptions documented by Linenberger and Bretz (5). In particular, we designed the activities and physical models to enhance student understanding of how electrostatic properties, stereochemistry and geometry influence enzyme-substrate interactions (Table 1).

While our activities are unique in using physical models designed to address identified misconceptions in biochemistry, others have used physical models to engage students with abstract concepts in the molecular life sciences. Oliver-Hoyo and colleagues presented two studies where students utilized physical models of proteins and small molecules to explore noncovalent interactions in structure-function relationships (8,9). Better learning gains and retention were noted when students engaged in a series of learning activities with the physical models rather than a single activity (9). When exploring how physical models can be used to promote better understanding, students who engaged in a "model-dissecting" activity performed better on the post-test assessment than students who did a "model-building" activity (10). Others have investigated the impact of biomolecular physical models on learning gains with respect to gender. One study using a physical model of the Cdc42-interacting protein 4 (CIP4) reported better learning gains for female students, whereas another study demonstrated learning gains for all genders when students used a set of physical models on the flow of genetic information (11,12). The latter study further reported the highest learning gains for lower achieving students (12). In another study by Newman and colleagues, physical models were particularly beneficial for the deaf/hard of hearing (D/HH) student population within their courses (13). This further demonstrates that physical models can support learning for all students.

A few studies have looked at the impact of combining virtual and physical models in the classroom. Students not only prefer the physical models but also rate them as the better tool for aiding in conceptual understanding, compared to other active learning tools (14-16). In one of these studies, the control and intervention groups performed similarly on course assessments; however, when a subset of students from both groups were interviewed, the intervention students answered the "higher order" questions better than the control students (14).

The *Enzymes* module of our biochemistry course follows the canonical flow of content in undergraduate biochemistry courses (See Figures 1 and 2). The module begins with how enzymes bind substrates with specificity and how enzymes lower the transition state by making optimal shape and electronic interactions with the transition state molecule. Following this, we cover enzyme kinetics, with an emphasis on Michaelis-Menten kinetics. This leads to the serine protease lessons. We use these enzymes at this point in the module, because they are excellent examples

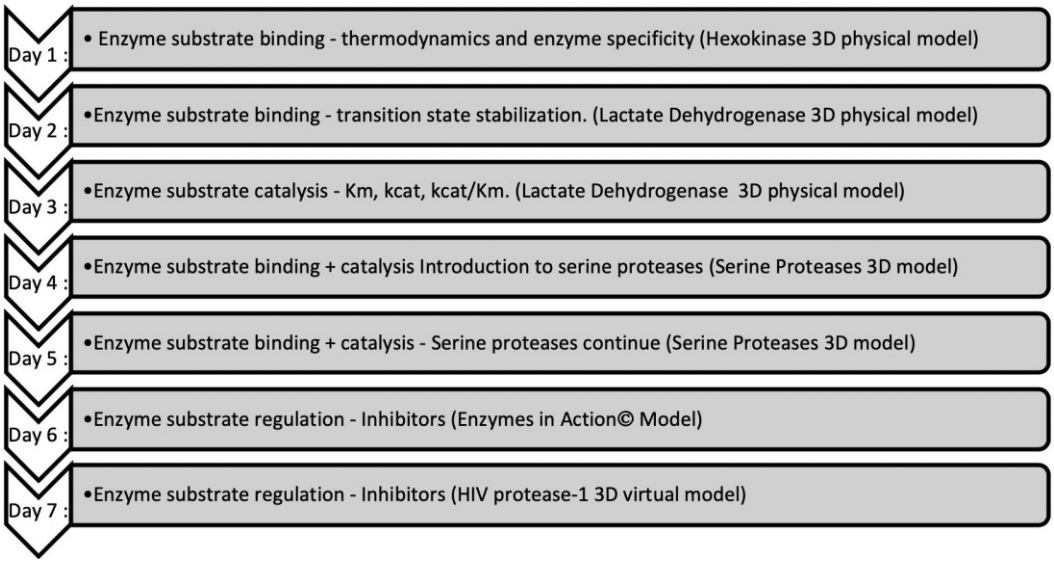


Figure 1. Sequence of content in our three days a week biochemistry course. The flow of content in our Enzymes module in our biochemistry course that meets three times per week for 50 minutes each session. This course follows the flipped-classroom model and uses active learning methodologies during class time. Each classroom session uses 3D physical models designed to accompany the contents listed.

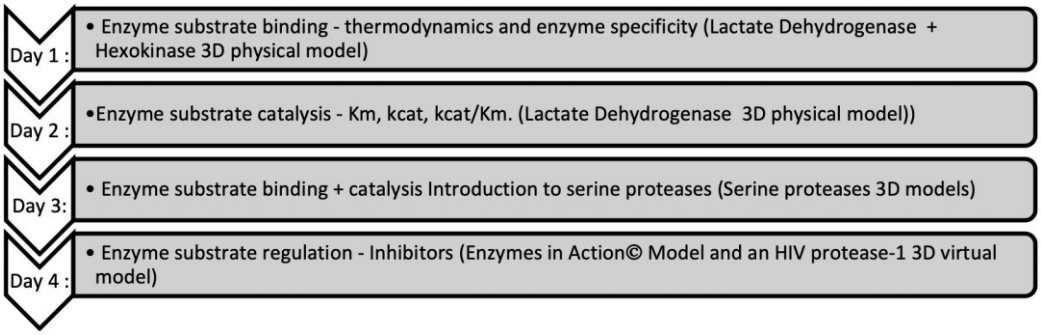


Figure 2. Sequence of content in our two days a week biochemistry course. The flow of the Enzymes module in a biochemistry course that meets two times per week for 75 minutes each session. This course follows the flipped-classroom model and uses active learning methodologies during class time. Each classroom session uses 3D physical models designed to accompany the contents listed.

of the content covered in the prior enzyme binding and catalysis lessons. We end the module with enzyme regulation content. For our *Enzymes* module each lesson includes an active learning activity with accompanying physical or virtual model (See Figures 1 and 2).

The two activities presented here use the serine protease model set and occur in the middle of our *Enzymes* module (See Figures 1 and 2). Students complete the *Model Exploration* activity first and the *Problem Solving* activity second. Depending on the structure of the course, these activities happen either on consecutive days or are combined into one day. In either case, our biochemistry course is conducted in a flipped-classroom model, with students watching the content video and completing an individual pre-class activity prior to class. At the beginning of class, we begin with student-posed questions from the video and/or pre-class activity. Also, we typically practice the content and representations with recall question(s) from the instructor. After this, students work through the activity in groups using the physical models while the instructor moves through the room answering questions. Each group shares one serine protease physical model set (Figure 3). Groups are guided through the activity with a paper worksheet or learning management system (LMS) quiz. Each student receives a copy of the activity and submits individual answers. A class-wide discussion can occur during the lesson as needed and always at the end. This is a time to go over any questions that students struggle with, pose thought-provoking questions, bring all the concepts and skills together, and allow for reflection.

### *Intended Audience*

The intended audience for these activities is third- and fourth-year undergraduate students enrolled in an upper-level biochemistry course. Our students are pursuing a Bachelor of Science in Health Sciences degree at a small, public, primarily undergraduate institution. Prior to enrollment in our biochemistry course, students have completed four semesters of college-level chemistry (general chemistry I and II, organic chemistry I and II), at least one semester of college-level biology, and at least pre-calculus, with a minimum grade of C- in all prerequisite coursework.

### *Required Learning Time*

The *Model Exploration* and *Problem Solving* activities can be completed in two consecutive 50 minute course periods. Alternatively, they can be combined for longer class times or laboratory times. These models could also be used in the laboratory setting for more in-depth discussion and to offer students more time to build their visual literacy skills. Prior to class, students individually watch a content video (~37 minutes) and complete a pre-class assignment (~20 minutes).

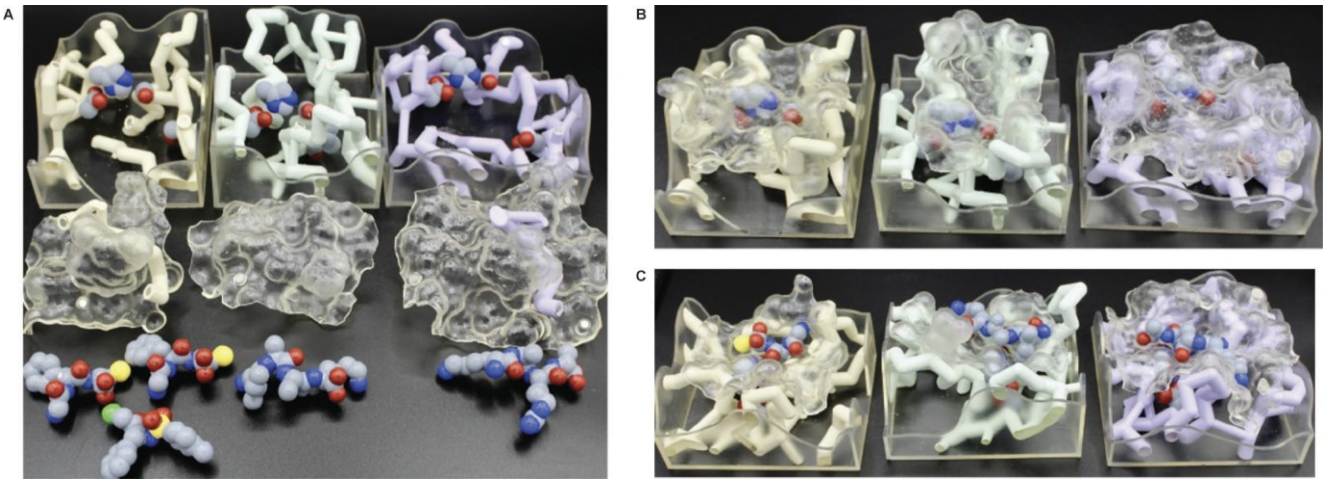


Figure 3 The serine protease model set. (A) Complete set with an active site box (top), surface plate (middle) and associated small molecules (bottom) for chymotrypsin, elastase and trypsin (left to right). The chymotrypsin set includes substrate, epimer and inhibitor; elastase and trypsin have one substrate each. (B) Surface plates snapped onto active site boxes using magnets. Each surface plate will attach only to the correct active site box. (C) Substrates docked into the surface plate-active site box set. These small molecules sit on top in the active site and only one substrate molecule will fit into the active site space. The physical models were designed at the University of Minnesota, Rochester in collaboration with the Center for Biomolecular Modeling (CBM) and printed at the CBM.

### *Prerequisite Student Knowledge*

Prior to the *Enzymes* module, students complete the *Introduction to Biochemistry and Protein Structure and Function* modules. Students should also be familiar with CPK (Corey, Pauling, and Koltun) colors and the various renderings of macromolecules (e.g., ball and stick, surface, ribbon diagram, etc.). Our students are required to memorize the one and three letter amino acid codes, along with biologically relevant functional groups. Also, they can draw these functional groups and amino acids in skeletal and Lewis structure form at any pH between 0 – 14. Our students are also able to draw and identify non-covalent interactions between functional groups.

### *Prerequisite Teacher Knowledge*

The instructor needs knowledge of elastase, trypsin and chymotrypsin with respect to enzyme specificity and catalytic mechanism. We created an instructor's guide to using the models (Supporting File S3: Enzyme Substrate Interaction - Instructor Guide Video on Teaching using Serine Protease models) and an accompanying description of the timeline in Table 2.

## SCIENTIFIC TEACHING THEMES

### *Active Learning*

Activities outside of class:

- Students watch a content video prior to class.
- Students complete a pre-class activity prior to class.

Activities used during class:

- Problem-based learning within the activity.
- Group discussion of the activity questions.
- Group use of the physical models.
- Group and individual reflection.
- Class-wide discussion.

### *Assessment*

Learning was measured using these methods:

- Assessment of student responses to the activity questions, using either rubric analysis or scores generated from the LMS.
- Pre/post analysis of the Enzyme Substrate-Interaction Concept Inventory.
- Assessment of student responses to exam questions pertaining to the serine proteases.
- Overall student performance on exam 2 (pertains to the Enzyme and Lipids modules).
- Students self-evaluate learning by reviewing answer keys that correspond to pre-class and in-class activities. Students also engage with practice problems and study guide questions related to the materials. Additionally, each class session ends with a reflection.

### *Inclusive Teaching*

These activities use all five strategies for developing a culture of inclusivity and equity in the classroom described by Tanner (17): (1) we know our students' names, which helps facilitate the conversations that arise during the lesson; (2) relevant examples of treatments of peptidic ulcers and acid reflux using protease inhibitors are incorporated into the class discussion; (3) the majority of class time is spent with students collaborating in small groups; (4) the lessons use various active learning strategies (i.e., physical models, collaborative work, reflective writing, interactive lecture, pre/post questions); and (5) we explicitly talk about how the physical models and activities promote access and equity for all students. In class, we talk about how spatial abilities are typically lower in female students due in part to the toys and games females are introduced to as children. This slow development of spatial ability has been linked to lower retention and success in STEM (18, 19). Ideally these models will help those with low spatial ability train their brains to build a more robust mental model and therefore increase their success and retention in STEM programs. Additionally, the models are in CPK colors which should be accessible to those with color blindness.

## LESSON PLAN

### *Classroom Seating Arrangement*

The University of Minnesota Rochester classrooms are called "Learning Labs;" with tables arranged in a circle around the room or on wheels that can be moved at the instructor's discretion. Students sit at tables with up to six classmates. For these activities, students work in groups of six, each sharing one model set. Students are assigned to groups randomly using the course LMS.

### *Pre-Semester Preparation*

While we have our own set of models, the Milwaukee School of Engineering (MSOE) Center for BioMolecular Modeling has a set to borrow through their Lending Library. We administer the Enzyme-Substrate Concept Inventory (ESICI) as a method of assessment at the beginning and end of the semester. For access and permission, contact Stacy Lowery-Bretz (bretzsl@miamioh.edu).

### *Preparation for Serine Protease Model Exploration Class Session*

Prior to the Model Exploration in-class activity, students watch a content video and complete the corresponding pre-class assignment (Supporting File S1: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases). The content video, available on YouTube or by request, compares the active sites of three serine proteases: chymotrypsin, trypsin, and elastase. The role of each catalytic triad residue and the role of key binding pocket residues are described. Additionally, the arrow-pushing serine protease catalyzed mechanism for chymotrypsin and a hypothetical substrate is drawn and talked through in detail. The video also ties in prior knowledge from the previous activities in the Enzyme module. The pre-class assignment is due at the beginning of class and the answer key (Supporting File S2: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases Answer Key) is provided to students after the due date. Students complete the pre-class activity worksheet individually and by hand, so the assignment is posted as a Word and PDF document for accessibility. The pre-class activity and content video are available to students at least one week prior to the Model Exploration in-class activity.

Also, a week prior to the in-class session we acquire the stickers, check over the models and prepare a cart for ease of transport (see Table 3. Lesson Plan for the *Model Exploration* and *Problem Solving* Activities). The in-class activity worksheet (Supporting File S4: Enzyme Substrate - Model Exploration Activity) is either printed so that each student has a copy or created in our LMS for individual students to complete.

As indicated in Table 3, we created an instructor guide video that highlights the features of the models and how we use these models with the students (Supporting File S3: Enzyme Substrate Interaction - Instructor Guide Video on Teaching using Serine Protease models). Watching this video can help prepare instructors for this set of lessons. Additionally, we describe the video timeline in Table2.

Figure 4. Layout of the models prior to class. Each model set is disassembled prior to class and placed on a cart for ease of transportation. The gem and CPK colored dot stickers we used are also shown here.

### *Serine Protease Model Exploration Activity In-Class Session*

Prior to the start of class, the model kits are placed on the tables with all of the pieces separate. Class begins with an instructor-led recall discussion (see Table 3. Lesson Plan for the *Model Exploration* and *Problem Solving* Activities for script) using a whiteboard drawing of a serine protease active site (see Figure 5). We then orient students to the models (see Table 3. Lesson Plan for the *Model Exploration* and *Problem Solving* Activities for script) and follow that by either passing out the hard copy of the activity worksheet or directing students to the online LMS version. Since many students want to play with the models and ignore the activity, we remind the students to work through the questions in order. Although each student has a copy of the in-class activity, they are encouraged to work together. Our students readily interact with one another on this assignment, in part because they must share the model set. As students work though the questions, we wander through the room and answer questions. We frequently help re-orient students with the models; like showing them how to find the N-terminus of each peptide substrate, asking them where the catalytic versus binding residues are located, and/or showing how to find the best placement of the substrate in the active site. Often, we notice that the female students are reluctant to work with the models, whereas the male students are not, so we give gentle reminders to share and be equitable with the models. In the last ten to fifteen minutes, we lead a class-wide discussion of the activity, models, commonly missed concepts, and reflection (see Table 3. Lesson Plan for the Model Exploration and Problem Solving Activities for script). The activity worksheets are collected or submitted online, and the entire activity is graded on correctness (See Supporting File S6: Enzyme Substrate - Model Exploration Activity Answer Key and Rubric). Prior to leaving the classroom, students help place the models back on the cart.

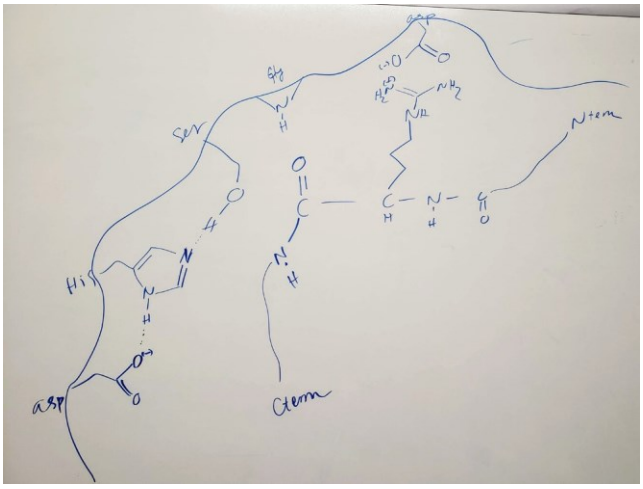


Figure 5. Instructor drawn representation of the active site of trypsin bound to a peptide. This image is drawn on the whiteboard prior to the start of class and is used for a short discussion at the beginning of class time.

### *Preparation for Serine Protease Problem Solving Class Session*

A day prior to the second in-class session we check over the models and prepare a cart for ease of transport (See Table 3. Lesson Plan for the *Model Exploration* and *Problem Solving* Activities). The in-class activity worksheet (Supporting File S5: Enzyme Substrate -Problem Solving Activity) is either printed so that each student has a copy or created in our LMS (we use Canvas, so this activity is set up as a "Quiz").

### *Serine Protease Problem Solving Activity In-Class Session*

Prior to the start of class, the model kits are placed on the tables with all of the pieces separate. At the beginning of class, the instructor covers any questions posed by the students. Typically, there are few, if any, questions at this time. This shorter introduction to class enables a longer end-of-class discussion. We then hand out the printed activities or direct students to open the LMS activity. Again, we move through the room and answer questions as students work through the activity. At the end of class, or throughout, we cover a series of questions as a class (See Table 3. Lesson Plan for the *Model Exploration* and *Problem Solving* Activities for script). We have whiteboards along every wall in our classroom, so one student from each group will be at the whiteboard answering a question. Groups rotate through students for each question. At the end of the class the activity worksheets are collected or submitted online. For this activity we grade the entire activity on correctness (See Supporting File S8: Problem Solving Activity Answer Key and Rubric - the answer key and rubric to the Problem Solving activity). Before leaving the classroom, students help store the models.

## TEACHING DISCUSSION

The previously documented research about misconceptions of enzyme-substrate interaction expressly points to a need for better teaching tools, particularly those that are three-dimensional (5-7). We developed two such lessons with 3D physical models of serine proteases. These lessons have several benefits. First, by having all three serine protease models, students are able to physically manipulate and see their similarities and differences. This enables a physical understanding of the role of size,

stereochemistry, and electrostatic forces in enzyme-substrate interactions. In doing so, students also engage in four visual literacy skills (3), and while we cannot directly measure the development of mental models, we perceive this is happening based on discussions with students and from their responses to the activity questions. Physically manipulating the three enzymes also helps students with the misconception that a "specificity pocket is not a binding site." Additionally, the models make it easy for the instructor to address other misconceptions, such as "the 'key' image represents the enzyme" and "the enzyme will bind most tightly to the active site." For example, asking students to hold up a substrate, then to hold up an enzyme, and then to point to an active site helps to address the "active site is on the substrate" misconception. Furthermore, asking students to hold up the molecules with a peptide bond addresses the "enzyme is a protein and therefore a protein cannot be a substrate" misconception. Additionally, these activities facilitate robust discussions that can help students build better shared mental models of enzyme-substrate interactions. These discussions help students teach each other, confront misconceptions, and gain further mastery of the material in a low-stakes environment. The formative activities also give the students further practice to master the material. In addition to the small group collaborations and varied active learning strategies, these lessons incorporate relevant examples and promote development of spatial reasoning that facilitate an inclusive teaching environment.

### *Improvements and Adaptions*

These lessons were employed by sections of Biochemistry I with one instructor and up to 33 students in a section. Due to the amount of interaction between the instructor and student groups scaling this up to larger section sizes would likely require additional teaching help, from either teaching assistants or instructional colleagues. Additionally, these activities could be adapted to a laboratory setting. Here the instructor could expand the activities to include enzyme inhibition.

In general, students needed more time with the in-class activity when the physical models were present, compared to the students who used the activity without the models. Models must be introduced in a systematic way, and students need time to explore both the meaning and the use of models (20). Our observations of students interacting with the physical models affirm this finding. There is more to process and connect when transitioning from 2D representations to the 3D physical models. As such, the beginning of class time is spent orienting students to the models around the meaning of colors, orientation of the catalytic site, what the surface plate represents, which pieces represent the enzyme versus the substrate, and the intended purpose of providing the models to the students. This initial orientation to the models helps creates a shared mental model facilitating discussion that enriches student learning.

Anecdotally, we observed some student difficulties during the lessons. Some students struggle to identify the catalytic triad and the binding pocket. They seem to have trouble reconciling that these are unique, geographical locations in the active site of serine proteases. Additionally, some students refer to the surface plate and backbone as '*separate molecules*' and talk about the amino acid residues shown on the backbone as *molecules* - instead of as part of the protein. Further refinement of the activity and/or corollary class wide discussions with the instructor modeling what they 'see' when looking at this physical model set could improve student understanding. Also, many students will treat the model set as a puzzle-solving experience and ignore the activity, so it's important to remind students early that there is an activity with questions that are meant to be followed in order.

In order to better help students prepare for the lesson, the pre-class activity could be altered so that students create backbone and surface renderings of these enzymes using a virtual modeling program, like UCSF Chimera or PyMOL. There is some evidence that combining physical and virtual models leads to better learning gains (14). The pre-class activity could also have students label the catalytic triad and binding pocket. Additionally, conceptual physical models could be used for students to gain a general understanding of the geometric, electronic, and stereochemical aspects of enzyme-substrate interactions prior to introducing the serine protease kit (for example *Enzymes in Action* and the *Substrate Specificity* kits from 3D Molecular Designs). These types of conceptual models could be introduced in first- and second-year biology courses, like introductory and cellular biology, to better prepare students to use the serine protease set in biochemistry. These alterations to the curriculum may decrease the perceived cognitive load of these models for the students and lead to deeper learning.

## SUPPORTING MATERIALS

- Supporting File S1: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases. An activity for students to complete after watching/learning about serine proteases and before attending class.
- Supporting File S2: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases Answer Key. The answer key for the pre-class activity.
- Supporting File S3: Enzyme Substrate Interaction - Instructor Guide Video on Teaching using Serine Protease models. A video for instructors demonstrating how to use the serine protease model set.
- Supporting File S4: Enzyme Substrate Interaction - Model Exploration Activity. The first activity for students that accompanies the model kit.
- Supporting File S5: Enzyme Substrate Interaction - Problem Solving Activity. The second activity for students that accompanies the model kit.
- Supporting File S6: Enzyme Substrate Interaction - Model Exploration Activity Answer Key and Rubric. The answer key and rubric to the Model Exploration activity.
- Supporting File S7: Enzyme Substrate Interaction - Problem Solving Activity Answer Key and Rubric. The answer key and rubric to the Problem Solving activity.
- Supporting File S8- Enzyme Substrate Interaction - Sample Exam Questions.

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Table 1. Enzyme-substrate Misconceptions Targeted with the Model Exploration and Problem Solving Activities.

| Misconception (5) | Model Exploration Activity | Problem Solving Activity |
|---|---|---|
| **Role of shape and charge in selectivity** | | |
| Charged amino acid interacts with OH regardless of sterics | ☑ | ☑ |
| Students consider charge but not shape | ☑ | ☑ |
| Similar amino acid will bind in pocket | ☑ | ☑ |
| **How the enzyme interacts with the substrate** | | |
| Disregard of relationship between scissile bond and specificity interaction | ☑ | ☑ |
| Enzyme will bind most tightly to the substrate | | |
| Active site is the only place of interaction | | |
| Allosteric site is not a binding site | | |
| Binding only occurs at transition state | | |
| Active site is on the substrate | ☑ | ☑ |
| Specificity pocket is not a binding site | ☑ | ☑ |
| Enzyme will bind most tightly to the active site | ☑ | ☑ |
| **Competitive vs. noncompetitive inhibition** | | |
| Inhibitors can bind to the substrate | | |
| Inhibitors interact only via competitive inhibition | | |
| **Conformational change** | | |
| Allosteric effecter must change enzyme conformation | | |
| An enzyme must change conformation prior to interacting with substrate | | |
| **Enzyme and substrate characteristics** | | |
| Solvent cannot be a substrate | | |
| Enzyme is a protein therefore a protein cannot be a substrate | ☑ | ☑ |
| The ''key'' images represent the enzyme | ☑ | ☑ |
| Nucleotides cannot be substrates | | |

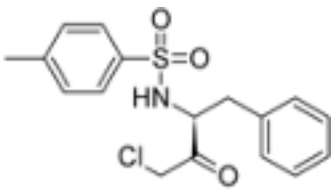
Table 2. Descriptive Timeline of Topics in the Instructor Guide Video.

| Timeline of Topics in Instructor Guide Video (S3) | Time in video |
|---|---|
| Introduction and considerations for what to say at beginning of class time, how to orient the students with the models, whether to use these in-class or in-lab. | 0 – 3:32 min |
| Discussion as to whether or not students learn better with the serine protease models. | 3:32 – 4:17 min |
| Re-capping the pieces of the model, the renderings, and the meanings of each piece. | 4:17 – 5:02 min |
| Role-playing the first task in the Model Exploration Activity – matching the surface plates to the backbone models. | 5:02 – 6:20 min |
| Role-playing the second task in the Model Exploration Activity – make observations and identifying which model is which protease. Discussion points to have with students for this task. | 6:20 – 9:14 min |
| Role-playing the third task in the Model Exploration Activity – comparing the catalytic and binding site of the enzymes | 9:14 – 10:44 min |
| Classroom management and common student behaviors to notice. | 10:44 – 11:12 min |
| Role-playing the fourth task in the Model Exploration Activity – placing the CPK stickers on the surface plate, making observations and common student struggles with this task. | 11:12 – 13:36 min |
| Role-playing the fifth task in the Model Exploration Activity – matching the substrate to the enzyme, making observations and common student struggles with this task. | 13:36 – 21:10 min |
| Discussion points to have with students at this point in the Model Exploration Activity | 21:10 – 21:50 min |
| Role-playing the sixth task in the Model Exploration Activity – placing gem stickers on catalytic site and correct peptide bond to help students understand the specificity of hydrolysis. | 21:50 – 23:14 min |
| Role-playing the seventh task in the Model Exploration Activity – observing the impact of stereochemistry on binding and/or catalysis. Using the epimer substrates. | 23:14 – 24:40 min |
| Discussion around the inhibitor model and how it can be used in class, either in addition to this activity or in the enzyme regulation class session. | 24:40 – 25:37 min |
| Common student misconceptions, struggles, and classroom management | 25:37 – 28:10 min |

Table 3. Lesson Plan for the *Model Exploration* and *Problem Solving* Activities.

| Activity | Description | Estimated Time | Notes |
|---|---|---|---|
| **Preparation for Serine Protease Model Exploration Class Session** | | | |
| Request the physical models | Send request to the MSOE Lending Library for a set of the serine protease physical models | 10-15 minutes to fill out the request form. | Milwaukee School of Engineering (MSOE) Center for BioMolecular Modeling Lending Library |
| Administer the Concept Inventory (Optional) | 1. Acquire permission to use the ESICI<br>2. Administer the concept inventory to students | 20 minutes to administer the ESICI | • For access to the Enzyme-Substrate Concept Inventory (ESICI), email Stacy Lowery-Bretz (bretzsl@miamioh.edu).<br>• We administer the ESICI on the first day of the semester and again on the last day. |
| Pre-class preparation (At least two – three days prior class periods) | 1. Cover topics related to enzyme binding and catalysis with an introduction into serine proteases.<br>2. Watch the instructor's guide to using the serine protease models. | Several class periods to cover prerequisite enzyme content.<br>About 20 minutes to set-up the LMS activity. | • The pre-class assignment is in Supporting File S1: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases.<br>• The answer key for the pre-class activity is in Supporting File S2: Enzyme Substrate Interaction - Pre-class Activity Serine Proteases Answer Key. |
| Pre-class preparation (At least one day prior to class) | 1. Acquire stickers that are easily removable from the model surface. We use rhinestone face gems (found in Halloween section at Walmart). You will need two-six gem stickers per group. Also need a sheet of red and blue dot stickers. (Figure 4)<br>2. EITHER (A) Print in-class activity so that each student has a copy. OR (B) Create the assignment in your LMS (we use Canvas, so this activity is set up as a "Quiz").<br>3. Check over all the models and super glue any broken pieces.<br>4. Lay out the models on a cart so that set-up in-class is quick and easy (see Figure 4). | About 15 minutes to prepare 12 sets.<br>About 30 minutes to set-up the LMS activity. | • Activity to print or add to your LMS is in Supporting File S4: Enzyme Substrate Interaction - Model Exploration Activity.<br>• Answer key and rubric for Model Exploration Activity is in Supporting File S6: Enzyme Substrate Interaction - Model Exploration Activity Answer Key and Rubric.<br>• Here is a list of materials in each model kit (NOTE the stickers are not included in the kit from CBM):<br>  • The three backbone models – (labeled A, B and C)<br>  • The three surface plates (these are subtly labeled but you will be able to tell the difference based on pocket size. Each group should have one of each)<br>  • 5 small molecules (labeled 1, 2, 3, 4 and 5)<br>  • Sheets of "CPK" stickers<br>  • Sheets of gem stickers (or used the other stickers from the CPK sheet that are neither red nor blue) |
| Pre-class preparation (10-15 minutes prior to class) | For each group, lay out the models<br>Note that we have groups of 6-9 students share one model set. | 10-15 minutes prior to class | Lay out all pieces individually:<br>• The three backbone models (labeled A, B and C)<br>• The three surface plates (these are subtly labeled A-C but you will be able to tell the difference based on pocket size). Each group should have one of each.<br>• 5 small molecules (labeled 1, 2, 3, 4 and 5)<br>• Sheet for "CPK" stickers<br>• Sheet of gem stickers |

| Activity | Description | Estimated Time | Notes |
|---|---|---|---|
| **Serine Protease Model Exploration Activity Class Session** | | | |
| At beginning of class, instructor-led Q&A | 1. Ask if students have questions/comments from the video (or prior class material).<br>2. We usually draw the active site of a serine protease on the board with a substrate bound and ask a series of questions. | ~ 10 minutes | These are some questions we cover using the white board drawing (see Figure 5):<br>• Circle the catalytic triad and ask them, "What region of the active site is this?" Draw the mechanism to create oxyanion.<br>• Circle the Gly backbone and ask them, "What region of the active site is this?" Talk about how enzymes preferentially bind the transition state.<br>• Circle the binding pocket residue and ask them, "What region of the active site is this?" Then ask, "Is this a representation of trypsin, chymotrypsin or elastase?"<br>• Circle the two peptide bonds on each side residue in the binding pocket and ask, "Which peptide bond will get cleaved – both, neither?" (Many students assume the protease will cut all peptide bonds, but only the one closest to the catalytic triad will get cut; then the products leave so a new peptide substrate can enter.)<br>• Go through each of the proteases to remind them what each one is selective for.<br>• Ask if there are any questions related to the content.<br>Then orient the students to the models as such:<br>• "These are backbone models (point to them) – they represent the backbone of the enzyme active site. The rest of the enzyme has been cut away for simplicity. A few amino acid side chains are shown in sphere rendering."<br>• "These are surface plate models – if all the atoms of the active site were shown and a blanket draped over the top – you would get the surface of the enzyme. It shows the topology/geography of the surface of the enzyme."<br>• "There are 5 small molecules that you will explore throughout this activity."<br>• "This is a good time to review CPK colors (red is oxygen, blue is nitrogen, gray is carbon, yellow is sulfur, etc)."<br>• "The model does not show double bonds or hydrogens." |
| Students begin the activity in small groups | Hand out the printed group activities (one per person) OR open the LMS activity. Remind students to share and to use soft hands. The models are breakable. | ~ 20-40 minutes | |
| Class-wide discussion | Time to wrap- up class with a reflection. | ~5-10 minutes | "Which model (surface plate, backbone, small molecule) best represents electrostatic features? Which model (surface plate, backbone, small molecule) best represents geometric complementarity, and which model (surface plate, backbone, small molecule) best represents stereochemistry?" |
| After class | 1. Collect group activities (if doing paper activities)<br>2. Ask students to help you place models on the cart. You will need them for the next session so no need to put them away. | | |

| Activity | Description | Estimated Time | Notes |
|---|---|---|---|
| **Preparation for Serine Protease Problem Solving Class Session** | | | |
| Pre-class preparation (At least one day prior to class) | 1. EITHER (A) Print in-class activity so that each student has a copy. (Each group will only turn in one.) OR (B) Create the assignment in your LMS (we use Canvas, so this activity is set up as a "Quiz").<br>2. Check over all the models and super glue any broken pieces.<br>3. Lay out the models on a cart set-up in-class. | 10-15 minutes prior to class | • Activity to print or add to your LMS is in Supporting File S5: Enzyme Substrate Interaction - Problem Solving Activity.<br>• Answer key and rubric for the Problem Solving activity is in Supporting File S7: Enzyme Substrate Interaction - Problem Solving Activity Answer Key and Rubric. |
| **Serine Protease Problem Solving Activity Class Session** | | | |
| At beginning of class, instructor-led Q&A | Ask if students have questions/ comments. | ~5 minutes | |
| Students begin activity in small groups | Hand out the printed group activities OR open the LMS activity. Remind students to share and to use soft hands. The models are breakable. | ~20-30 minutes | |
| Class wide discussion | Go over questions. | ~20 minutes | Also pose the following questions for the class (we usually draw on the white board):<br>• "Draw the complete chemical structures of the expected products when chymotrypsin, elastase and trypsin catalyze the hydrolysis of a peptide with the sequence R-L-A-T-F (change up the peptide: WEAR, YEAR, etc). Include any charges that are on the products, assuming the reaction takes place in aqueous solution at pH 7."<br>• Answer for WEAR: WEAR → (chymotrypsin) W + EAR → (trypsin) W + EAR → (elastase) W + EA + R<br>Overall answer: W + EA + R<br>(Our students have their one and three letter codes memorized along with the structures of all the amino acids and can draw them at any pH between 0 – 14.)<br>• Talk about the purpose of the proteases in the digestions of dietary proteins and how uncontrolled protease activity would be bad.<br>• This leads to a discussion about protease inhibitors – which is where the physical models come back in: "Tosyl phenylalanyl chloromethyl ketone (TPCK) (shown below) successfully inhibits chymotrypsin but has NO effect on elastase or trypsin's catalytic activity. Briefly explain why."<br>  |

| Activity | Description | Estimated Time | Notes |
|---|---|---|---|
| | | | • Answer: The phenyl groups on TPCK has the right shape and electrostatic properties to bind to the pocket of chymotrypsin (because it is selective for large aryl groups), but they won't fit in the binding pockets of trypsin or elastase.<br>• Students can test this using the model kit. TPCK is the inhibitor in the serine protease physical model kit.<br>• Also, this molecule is a "peptide analog" - discuss which part is analogous to the peptide.<br>• Challenge students to work out the arrow pushing mechanism of this inhibition outside of class.<br>• We usually do this question on the board next: "In the following scenario: The amino acids Histidine, Serine, and Aspartate are mixed with the tripeptide R-Y-A in a beaker. What will be in the mixture after 5 minutes? (i.e., What are the products?)"<br>• Answer: Nothing would happen, because there is no enzyme.<br>• "If catalytic Ser was mutated to Ala, would this alter Km, kcat, both, or neither? Increase or decrease? Which enzyme(s) would this impact (chymotrypsin, elastase, trypsin, all, none,or some combination)<br>• Answer: kcat would decrease. All the proteases have this residue, so any of the proteases could be impacted by this mutation.<br>• "If binding pocket Ser (for chymotrypsin) was mutated to Ala - would this alter Km, kcat, both neither? Increase or decrease? Which enzyme(s) would this impact (chymotrypsin, elastase, trypsin, all, none, some combination)?"<br>• Answer: Km would increase, only chymotrypsin would be impacted because it's the only protease of the three that has a Ser in the binding pocket. |
| After class ends - clean-up | 1. Collect group activities.<br>2. Ask students to help you wrap up the models. | 10-15 minutes | |