Enhanced Understanding of Triacylglyceride Digestion and Saponification using Physical and Computer Modeling

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Visualization activities such as molecular modeling can allow students to easily grasp complex biochemical subjects and can provide a fun and rewarding experience that motivates them to learn. However, modeling activities for large classes and laboratories can also be frustrating for students because the instructor may not be able to offer personalized guidance on how to construct or manipulate the models. This exercise remedies this problem by providing animated presentations that show in a step-by-step fashion how to build triacylglycerides and how to perform a "hand held" saponification reaction. It also reviews the reaction mechanisms of saponification and the lipase enzyme. The accompanying presentation's detailed visual cues can make modeling complex molecular changes easier. During the exercise, students work in groups with molecular model kits and then view the lipase active site using the PyMol® molecular modeling software.

Keywords: molecular modeling, saponification, lipase, soap

Link to Supplemental Materials: **https://doi.org/10.37590/able.v41.sup31**

Introduction

Molecular modeling activities can enhance chemistry and biochemistry learning, making complex organic chemistry and biochemistry topics accessible, even for very young students (Fried et. al., 2019, Fried et. al., 2019). Modeling can help students with low confidence or anxiety about learning science by offering a fresh learning experience that is both visual and tactile. All students can benefit from modeling, as it allows them to experience aspects of chemistry such as molecular geometry, flexibility, and connectivity, that cannot readily be appreciated by reading a textbook or even by watching a video (Kali, et. al., 2008). Molecular modeling sets like those manufactured by Molymod® provide students with the opportunity to assemble organic structures that have realistic bond geometry, and provide the opportunity for "hand held chemical reactions" in which students break and form bonds to simulate a chemical reaction. Despite the benefits of modeling, large class sections, like those common in colleges and universities, can make implementing modeling activities difficult. Not all students easily connect standard written chemical nomenclature to the concept of a physical molecular structure, so they may

not be able to easily follow the cues for the model building activities. These students may require extensive support during the exercises to build and manipulate the structures, which may not be feasible for the instructor to provide. Students who build incorrect structures during the activities do not benefit from the activity and may even become further confused. Finding ways to keep all students on track with complex model building activities, with minimal direct intervention of the instructor, is key.

This exercise demonstrates how classroom modeling activities can be enhanced through visual presentations. The goal is to guide and support students through the building activities so that complex structures and reactions can be modeled by all students, even those with a weak understanding of organic chemistry or who have difficulty interpreting organic chemistry drawings.

The activity uses saponification and the lipase enzyme mechanism to demonstrate the concept of "modeling the model". Students follow along with the model building activity through animated presentation slides with figures generated in PyMol®. The 3D images rendered with PyMol® allow students to easily build a triacylglyceride and to hydrolyze it into fatty acid salts. The saponification reaction, modeled during the activity, is

connected both to soap making as well as the enzymatic digestion of fats and oils by lipase enzymes. By providing very clear visual cues, the class can progress more quickly and more efficiently through the session. Student anxiety about whether they are "building this right" can be replaced by a more thoughtful focus on the science, and can lead students to begin asking questions about chemistry, work cooperatively as a group, or build improvised structures to test a hypothesis.

Chemical Background

Soap is generally made from triacylglycerides, hydrophobic molecules constructed from three fatty acids linked through ester bonds to a glycerol molecule. Triacylglycerides are often a component of stains, but by hydrolyzing the ester bonds with a strong base, such as sodium hydroxide, soap molecules are generated. The glycerol component of the molecule can remain in the soap solution, but does not have any cleansing activity on its own. The fatty acid salts, released by the saponification reaction, are amphipathic, and are responsible for the cleansing ability of soap by binding to lipophilic molecules. Soapy water consists of spherical micelles, with the charged, fatty acid salts oriented into the aqueous phase and the non-polar lipophilic carbon chains oriented inward. When the soap micelles encounter hydrophobic molecules, such as those in stains, their hydrophobic component binds to the stain molecules, causing them to be more easily washed away.

Lipase enzymes catalyze a process analogous to saponification—they hydrolyze the ester bonds of triacylglycerides, but do so, not with hydroxide as in saponification, but with the nucleophilic hydroxyl group of an active site serine, part of a catalytic triad. The lipase mechanism will be familiar to students of biochemistry because lipases are related to serine protease enzymes, which are covered in depth by most biochemistry text books. The mechanism for a lipase is the same mechanism for serine protease enzymes such as chymotrypsin, trypsin, and elastase. Instead of hydrolyzing a peptide bond, as do the serine proteases, lipases hydrolyze the esters, releasing fatty acids in the form of their carboxylates.

Since saponification and lipase activity are so closely related, this exercise helps to reinforce both processes, providing a mechanistic explanation for how soap is made and how organisms digest fats and oils.

Model Building Experience

This exercise can be used as a dry laboratory experience or active learning lecture activity to reinforce a unit on lipids or a unit on enzymatic activity. The accompanying presentation slides are used throughout the activity to guide student building and to reinforce scientific understanding. First, students review the covalent bonding behavior of the atoms which comprise triacylglycerides, as well as the standard color coding for these elements. The

organic structure nomenclature used in the activity is also reviewed. Students view and draw the structures of glycerol, fatty acids, and the triacylglyceride, and are reminded that by convention, hydrogen atoms and their bonds are omitted from the drawings, but are not actually missing from the structures. If students wish to, they can draw in the hydrogen atoms, adding the correct number of bonds to each carbon.

Students then view PyMol®-rendered images of the same molecules. The instructor can help struggling students by connecting the organic chemistry drawings to the 3D, computer-generated images of the molecules. In small groups, students then begin working to build the glycerol and the fatty acids, and then to combine them together through ester linkages. The instructor should emphasize that in creating the ester linkage, a water molecule is generated, classifying the reaction as a dehydration reaction. Although students do not simulate the precise enzymatic reaction that creates these ester linkages in cells, highlighting the dehydration reaction is beneficial for new students since similar reactions are found throughout biology. An animation showing how three water molecules are produced as the triacylglyceride forms helps students to quickly build the correct structure.

If enough model kits are available, a trilaurin molecule (a fat found in coconut oil) can be built by each group. Smaller or larger triacylglycerides could also be constructed, depending on class size. The resulting fats or oils are then subjected to a "hand held saponification reaction". Students first view an animated mechanism, reviewing the electron pushing and the formation of reaction intermediates, as previously learned in organic chemistry courses. They then watch a PyMol®-rendered version of the same mechanism showing how an electron lone pair from hydroxide attacks the ester carbonyl carbons. The role of the sodium in generating fatty acid salts is also reinforced in the animation. Again, showing a 3D, computer-generated version of the reaction can prepare students for the hand-held reaction activity. As a group, students build hydroxide ions and use a free bond (or lone pair electron piece, if available) to simulate the attack at the carbonyl, formation of the tetrahedral intermediate, and release of the carboxylate. If available, a purple atom piece can be used to portray the sodium salt of the fatty acid.

The final use of the models involves laying out the fatty acid salts on the floor to simulate a soap micelle. Animate lecture slides help show students how to do this. Once the micelle is created, a hydrophobic molecule made beforehand by the instructor, can be placed inside the micelle. Another triacylglyceride could be used for this, but a heme, or chlorophyll model could also be used, simulating a blood or grass stain. Students can now see how a hydrophobic molecule can be washed away by the micelle.

Mechanism Review and Conclusion

This exercise finishes with an interactive lesson about lipases. First, images of a lipase and its active site are shown (Yapoudjian et al., 2002). If possible, students should render and view the structure themselves with PyMol®, allowing them to explore the active site. The action of the catalytic triad of a lipase is shown in the presentation, helping students appreciate the similarity to the saponification mechanism they have just studied.

Suggested assessment questions and activities include the following. 1) Save images of the PyMol®-rendered lipase structure and its active site with bound fatty acid. 2) Redraw the mechanism for saponification and/or the lipase enzyme. 3) Answer questions about the lipase active site, found in part G of the lab, and reflect on the use of PyMol® and model building in understanding enzyme structure and function.

Student Outline
Understanding Saponification and Lipase Mechanisms Using Physical and Computer Modeling

Objectives
- Work as a group of three to build a model of a triacylglyceride.
- Perform a "hand-held chemical reaction" to demonstrate saponification, and use models to show how soap works.
- Use PyMol® molecular modeling software to view the active site of a lipase enzyme.
- Reflect on your use of molecular models.

Introduction
Saponification is the process by which a fat or oil (triacylglyceride, also known as a triglyceride) is broken down by a base, such as sodium hydroxide, into three fatty acid salts and glycerol. The saponification mechanism is used to make soap, but is also analogous to the action of lipase enzymes, which break down dietary fats so that they can be utilized by the organism. We will review the reaction mechanisms using electron-pushing drawings, and them will perform the saponification reaction on a hand-built model of a fat. The class will work together to construct a model of a micelle, simulating the activity of soap. We will then explore the active site of a lipase enzyme. The catalytic triad of lipase allow hydrolysis of the fat's ester bonds to proceed at physiological conditions, while soap making (no catalyst) requires harsh treatment of oils with a strong base.

During the session, pay special attention to the accompanying presentation slides; they will show you in a step-by-step manner how to build and manipulate the models.

Methods

Part A: Build a Triacylglyceride

Obtain molecular model pieces and build a fatty acid such as lauric acid (dodecanoic acid) as shown in the center of the scheme below. As a reminder of organic chemistry nomenclature, carbons are shown as black dots and are represented by black atom model pieces. Remember to use hydrogen (white) atoms to give each carbon four bonds. Use flexible bonds to create the carbonyl bonds to one of the oxygen (red) atoms.

If model pieces are in short supply, build a shorter-chain fatty acid such as caproic acid (pentanoic acid.) Once you have built your fatty acid, join a group of two other students. Each group should now build one glycerol molecule as shown to the left in the scheme below. Next, we will construct a triacylglyceride, as shown to the right in the scheme below (Figure 1). In nature, this is a complex enzymatic reaction and is more involved than the scheme shown below, but for the purposes of this exercise, consider it to be a dehydration reaction. Please view the animation in the presentation to see how to remove the atoms of a water molecule to properly create each ester bond in the triacylglyceride. The molecule below is trilaurin, the triple ester of lauric acid molecules and one glycerol molecule.
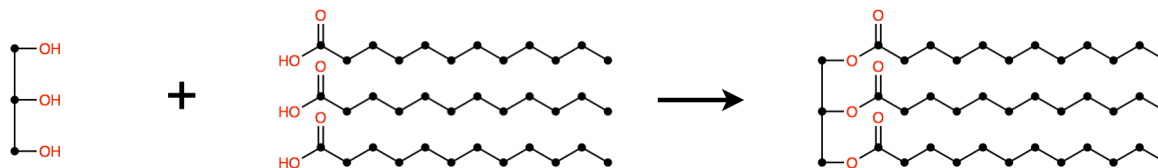

Figure 1. Assembly of a triacylglyceride. Three water molecules are also liberated in the process.

Part B: Review the Electron Pushing for the Saponification Mechanism.

Follow along with the arrows in the animated presentation to review how hydroxide attacks the carbonyl carbons of the triacylglyceride to first produce a tetrahedral intermediate and then to cleave the fatty acid from the glycerol. Draw the final products in the box on the following page (Figure 2).
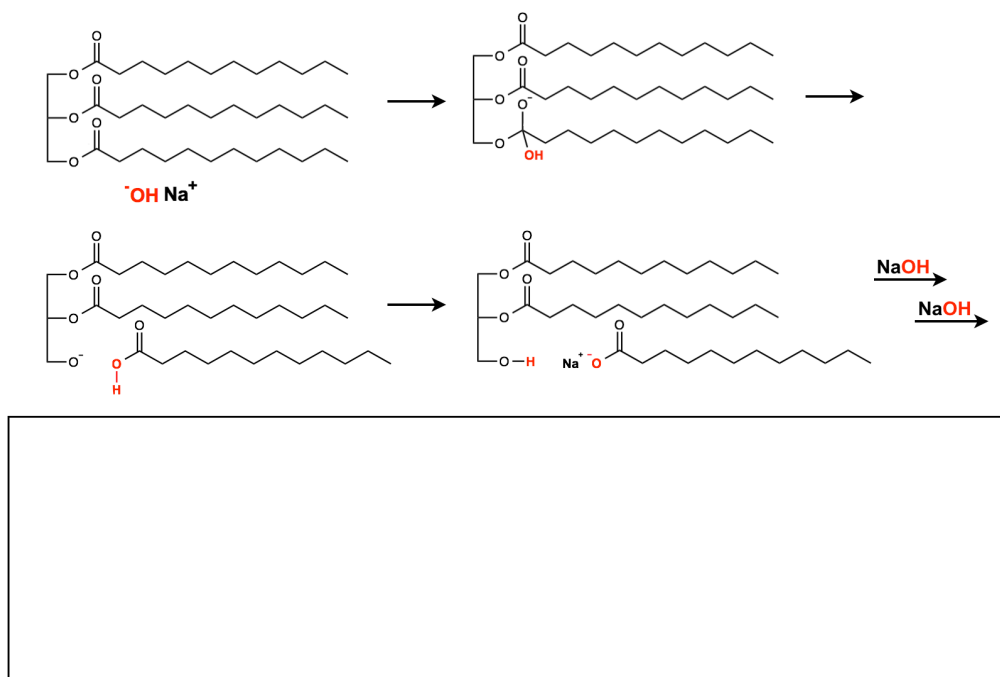

Figure 2. Add arrows to show the mechanism for saponification.

Part C: Perform a "Hand-Held Chemical Reaction" to Saponify your Triacylglyceride Model with NaOH

View the accompanying presentation to see an animation of saponification. Notice that the hydroxide ions are shown with three lone pairs of electrons, depicted by the three gray projections. If you have access to tetravalent red oxygen atom pieces, you can use three bond pieces or three lone pair electron pieces to represent the lone pairs. If you have access to divalent red oxygen atom pieces, use the one available bond to represent the electron pair involved in the nucleophilic attack.

Each of the three students in the group should get a chance to use a hydroxide ion as a nucleophile to attack the carbonyl carbons of the built triacylglyceride. As you exchange the glycerol for each hydroxide, imagine the formation of the tetrahedral intermediate. When the hydroxide attacks the carbon, you must break one of the double bonds. Then reform the double bond and break the bond with the glycerol, as shown in the presentation. Once all three students have saponified the three ester bonds, you should be left with three carboxylates and glycerol. Use a colored atom piece to represent the sodium atom of the fatty acid salts. Purple atom pieces would be best for this, but other atom pieces can be used as well. You have now simulated soap making!

Part D: Demonstrate the Activity of Soap on A Hydrophobic Molecule

Soap is highly amphipathic, containing a charged head group (carboxylate) and a hydrophobic alkyl tail. The longer the carbon chain, the more lipophilic this part of the soap is. Soap molecules' duel nature (hydrophilic and hydrophobic) is the key to how soap washes away stains and dirt. Hydrophobic stain molecules, which cannot easily be washed off a surface with water alone, can be made soluble when treated with soap. In soapy water, soap molecules arrange into spherical micelles with their polar, charged head groups interacting with the surrounding water, while the lipophilic tails pack into a hydrophobic core. This hydrophobic area of the micelle associates with water-insoluble molecules, allowing greasy stains to be washed away. View the animated presentation showing a simplified model for the treatment of blood stains (heme) and grass staines (chlorophyll) with soap.

After watching the animation, work together as a class to create a soap micelle on a tabletop or floor. Create a circle with your sodium laurate structures making sure their charged head groups face outward. Use the water molecules, released earlier, to simulate the aqueous phase that surrounds the micelle. Simulate the hydrophobic association of the core of the micelle with a model of a hydrophobic stain molecule. Use an un-saponified trilaurin fat molecule, or a model of a heme or chlorophyll prepared by your instructor. Imagine the stain being carried away as the micelle binds to it.

Part E: Review the Electron Pushing for the Lipase Mechanism

Lipases (enzymes that digest triacylglycerides) perform a saponification-like reaction to digest dietary fats and oils. Instead of using hydroxide as the nucleophile, they use a nucleophilic, deprotonated serine residue inside the enzyme's active site. The unusual deprotonation of serine's hydroxy group is accomplished by a famous active site amino acid residue arrangement called the catalytic triad. The mechanism for the hydrolysis of triacylglycerides is reviewed in the animated presentation. Follow along and fill in the electron pushing arrows on the scheme on the following page. The mechanism is a two-step process; the first step involves the formation of an enzyme-substrate complex through a tetrahedral intermediate, and the second step involves attack by water to release the free enzyme.

Part F: Explore the Active Site of a Lipase Enzyme in a 3D Molecular Modeling Program

Lipases are enzymes that digest triacylglycerides in the gut and perform a saponification reaction to digest dietary fats and oils. This allows fatty acid salts to be liberated and absorbed by the organism. We will now view the enzyme structure of a lipase from *Thermomyces lanuginosus*, a common soil fungus. Open PyMol® on your computer and view the PyMol® session file provided by your instructor, or create one yourself from the Protein Data Bank, PDB entry 1GT6 (Yapoudjian et al., 2002).

The catalytic triad, analogous to those found in proteases such as trypsin, chymotrypsin, and elastase, is highlighted in yellow. These are serine[146] histidine[258] and aspartate[201]. Follow the animation in the presentation to see how the electron-pushing arrows are drawn on the 3D structure. The crystalized structure does not contain the triacylglyceride structure, but instead shows the fatty acid product, highlighted in orange. The mechanism shown in the presentation is therefore only to demonstrate how the catalytic triad generally attacks a carbonyl carbon on a substrate such as a fat molecule—it would not attack the fatty acid *product* as shown. In the presentation, three amino acid residues are highlighted in green to show part of the hydrophobic pocket that allows the fatty acid alkyl chain to bind to the active site. Tumble and zoom into the structure in PyMol® to explore its three-dimensional geometry. Fill in arrows in the box for Figure 3.

Part G: Explore the Active Site of a Lipase Enzyme in a 3D Molecular Modeling Program

While exploring the structures: How does the fatty acid fit into the binding pocket? How could the binding pocket be altered to accommodate other substrates? Why is the fatty acid folded into a "tuning fork" conformation, rather than extended? Turn the surface transparency back on. How could the enzyme be disabled? What would happen if the yellow or green residues were mutated? What other substrates could this enzyme accommodate? Before you close PyMol®, take a screen shot of the lipase active site showing the important amino acid residues and bound fatty acid. Attach this image to your activity write-up. Reflect on the use of PyMol® and model building in understanding enzyme structure and function. How did this experience change how you might approach your future learning? Are you more likely to investigate a protein on your own using the PDB? Might you try building a chemical structure on your own to learn more about how it works or how it reacts?
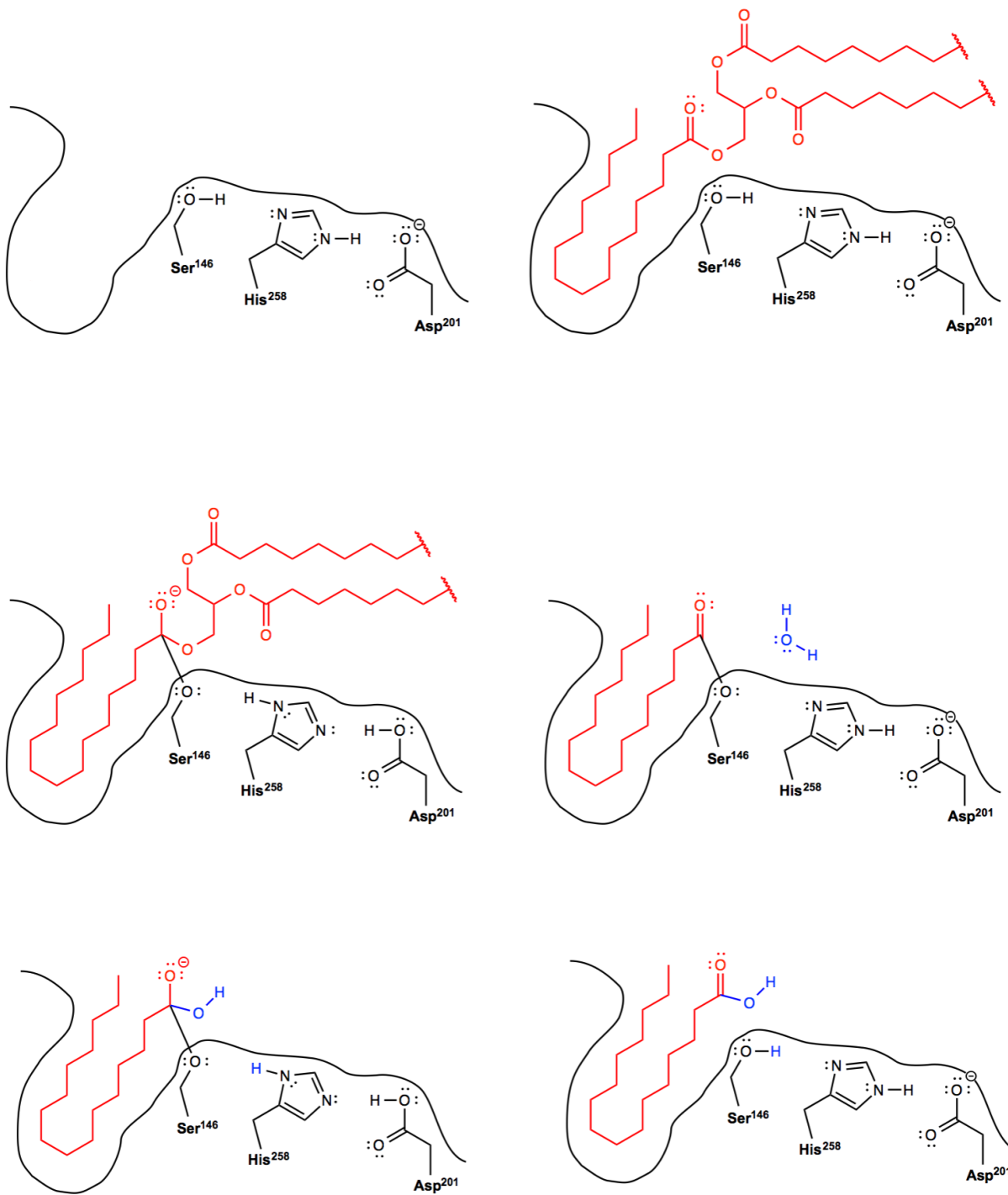
Figure 3. Add arrows to show the mechanism for the lipase enzyme.

Assessment

1. Turn in a PyMol® screen shot showing the lipase active site and any other views of the enzyme you find interesting.

2. Redraw the completed saponification mechanism and attach the completed lipase mechanism page.

3. Answer the questions in part G.

Cited References

Yapoudjian S, Ivanova M, Brzozowski A, Patkar S, Vind J, Svendsen A, Verger R. 2002. Binding of *Thermomyces (Humicola) lanuginosa* lipase to the mixed micelles of cis-parinaric acid/NaTDC, European Journal of Biochemistry. 269:1613-1621.

Materials

Access to computers with the PyMOL[®] molecular modeling program installed (the free educational version is available from **http://pymol.org/educational/**). The program is suggested for the students, if including part F and G, but is required for the instructor.

Model kits from Molymod[®] or other similar manufactures are needed. The exercises can be performed with a small number of model kits, but work better when large organic chemistry kits, rich in carbon and hydrogen atoms, are available. It is recommended that instructors provide one student-sized kit per 1-2 students (Molymod[®] MMS-008) or one instructor-sized kit per 3-4 students (Molymod[®] MMS-003).

Instructors also need to load the accompanying PowerPoint presentation which includes the animations and visualizations.

Notes for the Instructor

If enough models are available, the instructor should construct a hydrophobic stain molecule, such as a heme or a chlorophyll. This will be used at the conclusion of part D. If models are in short supply, use an un-saponified triacylglyceride molecule, a prop, or printout of a molecule as the stain.

If students do not have much experience with PyMol[®], the instructor may wish to provide students with a PyMol® session file for the lipase structure. Show the enzyme's secondary structure cartoon, its surface with some transparency, and show as sticks the catalytic triad, serine[146] histidine[258] and aspartate[201]. Finally, show the fatty acid ligand as sticks, depicted as OLA in the sequence. If the class has experience using the PDB and PyMol[®], the instructor may choose to allow students to generate the worked-up PyMol[®] structure themselves from the PDB entry 1GT6.

Cited References

Fried D, Pablo T, Gubi A, Gaffney J. 2019. Enhancing elementary science learning through organic chemistry modeling and visualization. European Journal of Science and Mathematics Education 7:73-82.

Fried D, Pablo T, Paneque D, Hughes A. 2019. Elementary student achievement and teacher perception of advanced chemistry curriculum. European Journal of Science and Mathematics Education 7:137-148.

Kali Y, Linn M. 2008. Designing effective visualizations for elementary school science. The Elementary School Journal, 109:181-198.

PyMOL The PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC.

Yapoudjian S, Ivanova M, Brzozowski A, Patkar S, Vind J, Svendsen A, Verger R. 2002. Binding of *Thermomyces (Humicola) lanuginosa* lipase to the mixed micelles of cis-parinaric acid/NaTDC. European Journal of Biochemistry. 269: 1613-1621.

Acknowledgments

About the Author

Daniel Fried has been a professor at Saint Peter's University since 2016. He teaches General Chemistry, Organic Chemistry, Biochemistry, and Medicinal Chemistry. Dr. Fried also creates biochemistry curricula, specifically designed for elementary school students.

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