

Article

Creating 3D Physical Models to Probe Student Understanding of Macromolecular Structure

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The high degree of complexity of macromolecular structure is extremely difficult for students to process. Students struggle to translate the simplified two-dimensional representations commonly used in biochemistry instruction to three-dimensional aspects crucial in understanding structure–property relationships. We designed four different physical models to address student understanding of electrostatics and noncovalent interactions and their relationship to macromolecular structure. In this study, we have tested these models in classroom settings to determine if these models are effective in engaging students at an appropriate level of difficulty and focusing student attention on the principles of electrostatic attractions. This article describes how to create these

unique models for four targeted areas related to macromolecular structure: protein secondary structure, protein tertiary structure, membrane protein solubility, and DNA structure. We also provide evidence that merits their use in classroom settings based on the analysis of assembled models and a behavioral assessment of students enrolled in an introductory biochemistry course. By providing students with three-dimensional models that can be physically manipulated, barriers to understanding representations of these complex structures can be lowered and the focus shifted to addressing the foundational concepts behind these properties.

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Introduction

One of the most challenging concepts for students studying biochemistry is macromolecular structure [1–3]. Common difficulties students face relate to problematic conceptions about the fundamental forces responsible for the formation and stabilization of these structures [4–7]. Misconceptions about these forces develop at the general chemistry level and persist through biochemistry courses [8–13]. To address these challenges, instructional materials need to be developed that target these areas in novel and engaging ways.

The high complexity of molecular structure in biological macromolecules exacerbates the difficulties students face using two-dimensional (2D) molecular representations [14, 15]. These molecular structures and their properties, including solubility and biological function, are governed by noncovalent interactions. Students struggle to extract this information from 2D representations often used in biochemistry instruction [16–18]. In addition, many of these representations simplify the structural information of these complex molecules which can lead to a fractured understanding of macromolecular structure [19, 20]. One pedagogical response to this issue is to provide students with models that present more realistic details about these molecular structures, however at an appropriate level of difficulty to prevent introducing additional barriers to understanding.

Physical models give students a tangible way to view and manipulate these structures in three dimensions, and have been shown to be preferred by students [21–23]. By using three-dimensional representations, barriers to problems arising from translating between 2D and 3D can be removed [24, 25]. For example, studies that have developed physical models of biological macromolecules have used materials such as pipe cleaners to mimic chromosomes,

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clothespins to represent RNA, binder clips to represent amino acids or transparency cutouts for proteins and DNA [14, 26, 27]. However, while the simplified structures in these physical models are easy to generate, they contain limited structural information [28, 29]. More detailed models that focus student attention on the principles that govern macromolecular structure such as electron distribution are needed to support student understanding of noncovalent interactions in complex molecules [30].

Recent advances in 3D printing have led to an increase in availability and accessibility of this technology [31, 32]. This allows for the creation of detailed and structurally relevant physical models of complex molecules in a cost-effective and user-friendly manner [33–35]. Molecular modeling programs ease the way for designing molecule models by allowing users to save molecule files in a format that is readable by 3D printing software [36, 37]. These programs support the development of detailed models for biochemistry education that are highly tailored to specific instructional goals.

This work focuses on the construction and implementation of four different models designed as hands-on instructional resources for a set of activities aimed at supporting student investigation of noncovalent interactions from the level of small peptide sequences to biological macromolecules. Three of the models were designed to scaffold understanding of the impact of noncovalent interactions on various levels of protein structure: secondary protein structure, tertiary protein structure, and solubility of membrane proteins. The fourth model translates these concepts to the structure of DNA. Each model shows a realistic, accurate representation of the complexity of the molecule and uses electrostatic potential coloring to focus students' attention on relating electron density to noncovalent interactions in simple and complex molecules. We have examined whether students actively engaged with the models with minimal intervention from the instructor and identified conceptions that students struggled with to determine if their attention was directed towards principles of electrostatic interactions. Our findings show that our models effectively target areas that are extremely difficult for the students while providing them with an engaging method to investigate these complex molecules.

Materials and Methodology

Creating the Models

All of the files generated for the creation of models were based off molecular files available on PubChem or the Protein Data Bank. Avogadro Version 1.1.1, an open-source molecular builder and visualization tool, was used to add or delete any atoms that needed to be changed in the files [38]. This program provides a simple interface for altering molecule files. Once saved in Avogadro, files were then converted into the desired format for 3D printing using a free program—UCSF Chimera package from the Computer Graphics Laboratory, University of California, San Francisco

(supported by NIH P41 RR-01081) [39]. A detailed description to generate printable files using these two programs is provided in the Supporting Information. The models were printed on a Lulzbot® Mini printer using the Cura Lulzbot® Edition software and white PLA+ plastic from ESun® or PLA plastic from MeltInc®. Figure 1 shows two example models designed for an activity focused on comparing boiling points of small molecules. A step-by-step description of the printing process and optimized parameters for printing these models are given in the Supporting Information.

Data Collection and Analysis

Participants and Setting

The models were tested in classroom settings and each model was tested with a different set of students enrolled in a single semester introduction to biochemistry course (for nonbiochemistry majors) at a large southeastern university. Students enrolled in the course are typically in their senior year of college, majoring in biological or nutrition sciences, and are predominantly female at a ratio of around three to one. Sixty-one students in sixteen groups completed the secondary structure activity, 34 students in eight groups completed the tertiary structure activity, 36 students in eight groups completed the membrane activity, and 25 students in eight groups completed the DNA activity. Groups ranged in size from two to eight students and were predetermined by random selection by the course instructor at the beginning of the semester. The groups had worked together in several problem sessions for their course before participating in the activities described within. The secondary structure and DNA activities were completed in a single class period while the tertiary structure and membrane protein activities were split across two sessions, a week apart, with the models introduced the second week. All of the models required assembly by the students and these completed models were assessed to see how effectively the students performed the tasks at hand.

Behavioral Observations

A modified version of the Behavioral Observation of Students in Schools® (BOSS®) created by Edward P. Shapiro was used to monitor the level of student engagement during the DNA, membrane protein, and tertiary structure sessions [40]. The original protocol was designed as an assessment tool for monitoring the behavior of an individual student in a classroom and contained the following codes:

- on-task behavior: classified as either actively engaged time (AET) or passively engaged time (PET),
- off-task behavior: classified as off-task motor (OFT-M), off-task verbal (OFT-V), or off-task passive (OFT-P), and
- teacher directed instruction (TDI): one code encompassing all teacher interventions.

In our modification, we adapted this protocol to focus on group engagement with the physical models. We modified the on-task behaviors to include active verbal (AV), active

TABLE I

Definitions and codes for each category used in modified BOSS® assessment

Category	Code	Definition
Active verbal	AV	Actively participating in a discussion
Active model	AM	Actively engaged with the model
Passive	P	Passively writing, not engaged in discussion
Off task	OT	Unrelated discussion or work on unrelated materials
Teacher knowledge	TK	Question to moderator about procedural aspects of activity
Teacher instruction	TI	Question to moderator about content knowledge

model (AM), and passive (P). We combined all off-task behaviors into one off-task (OT) code. Finally, we divided teacher directed instruction into teacher instructional (TI), and teacher knowledge (TK) to differentiate between questions based on procedural issues or conceptual issues respectively. Definitions for each code are given in Table I. Observations were conducted by one observer during the DNA activity and two observers during the tertiary structure and solubility of membrane protein activities.

The original BOSS® protocol includes the observation of the intended subject and brief periods of monitoring student peers for behavioral comparison. The focus for this work was the group of students rather than individuals. Our modified protocol maintained the use of peer groups to enrich the observations. The observer began by selecting a group of students to observe for 100 sec with group behaviors recorded every 10 sec (one interval). During the fifth and tenth interval, the observer monitored instead a peer group next to the current observation group. After the 10 observations were complete, the observer moved to a different group. Each observer monitored different groups at all times of the session. Each group was monitored by each observer for three or four rounds at equally spaced times

so that all groups were observed near the beginning, middle, and end of the activity. The intervals were coded either by the predominant active behavior or as passive if all students were passively writing. The behaviors assigned for each interval are shown in the Supporting Information.

Description of Activities and Findings

Secondary Structure

The first model based on protein structure was designed for students to explore the secondary structure of an eight amino acid peptide segment of an alpha helical protein (PDB ID 2H95) shown in Fig. 2A [41]. Each amino acid from the peptide was printed individually to expose details. Each amino acid was painted with electronegative regions in red and electropositive regions in blue based on electrostatic potential maps generated in Avogadro. The amino acid sequence selected (LVVAASII) contains primarily hydrocarbon side chains with one serine containing an additional hydroxyl group. Therefore, the blue and red coloring indicated the backbone of the molecule and identified the one polar side chain. The amino acid models were strung onto a lanyard in order of the peptide sequence. This linear sequence was given to the students and they were asked about the secondary structure of the models.

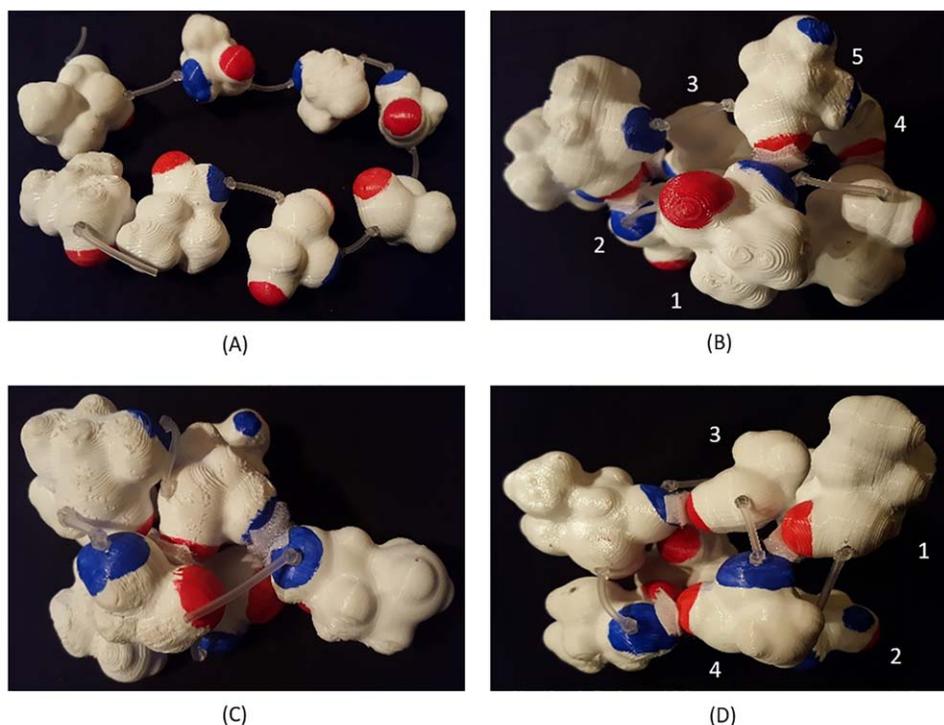
All of the students correctly identified the lanyard as representing the backbone and the bulbous white portions of the models as the side chains and recognized that the sequence of the models on the lanyard matched the sequence they were given in the activity. When asked how they made this identification, 27 of the 61 students stated that they used the unique serine side chain to match with the sequence or matched the side chains of repeating amino acids. This indicates that the model clearly represented composition and structure.

After reviewing the models, students were prompted to assemble the model into the correct secondary structure using Velcro®. Examples of correctly and incorrectly assembled models and the accuracy of each group's model



FIG 1

Methanol (left) and methanethiol (right) models used in the small molecule activity. [Color figure can be viewed at wileyonlinelibrary.com]



Group	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Electropositive to Electronegative	✓	✓	✓	✓	✓	✓	✓	✓	x	✓	✓	✓	✓	✓	✓	✓
n+4 Rule	✓	✓	x	✓	x	x	✓	✓	x	✓	✓	x	✓	x	✓	x

FIG 2

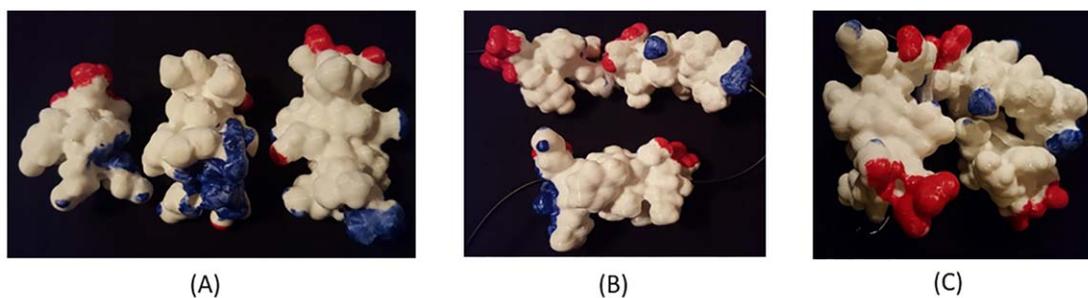
Secondary structure models. (A) Linear model presented to students. (B) Constructed model pairing electropositive to electronegative and correct amino acid n (1) connected to amino acid $n + 4$ (5). (C) Constructed model incorrectly pairing like electron densities to one another and amino acid n (1) to amino acid $n + 1$ (2). (D) Constructed model pairing electropositive to electronegative, but amino acid n (1) connected to amino acid $n + 3$ (4). Table shows correct (✓) or incorrect (x) responses by category for each group. [Color figure can be viewed at wileyonlinelibrary.com]

are shown in Fig. 2. Fifteen of the sixteen groups correctly identified the regions that correlated to the areas that participate in hydrogen bonding and paired these areas using the Velcro® as shown in Fig. 2B. Only one group incorrectly paired areas of the same charge together as shown in Fig. 2C. The students struggled more with pairing the correct amino acids together, and seven of the sixteen groups incorrectly paired each amino acid with the fourth amino acid in the sequence instead of the fifth as the $n + 4$ rule stipulates. Multiple groups attempted to connect each amino acid to the amino acids next to it. The course instructor intervened prompting them to reconsider their assembly. Even after instructor intervention, six of the groups submitted their models with amino acids $n + 3$ apart paired as shown in Fig. 2D, demonstrating that their understanding of the number of amino acids per turn was not accurate. While 15 of the 16 groups were able to correlate the electrostatic attractions of the molecule to the models coloring, the misunderstanding of the 3.6 residues per turn supports the need to give students a means to visualize these characteristics of protein structure.

Tertiary Structure

The next model based on protein structure was designed for students to explore tertiary structure using three helical segments based on the surface of a three helix bundle protein (PDB ID 1BDD) shown in Fig. 3A [42]. Only the helical portions of the protein were printed to maintain flexibility in the model. Each segment was painted using the red and blue color scheme based on electrostatic potential maps calculated in Swiss PDB Viewer (www.expasy.org/spdbv), which can generate electrostatic potential maps for proteins [43]. Each helical segment was printed with a tunnel running through the center for a lanyard to be strung through.

Students received a model of each helical segment with a lanyard and were asked to string the models onto the lanyard in the correct order. Eight groups participated in the activity and six groups of students completed the model assembly. All groups completing the assembly were able to identify the shortest helix as Helix 1, but four of the groups struggled to differentiate between the two longer helices and placed Helix 3 in the center. Only two groups correctly



Group	1	2	3	4	5	6	7	8
Order of Helices	x	x	✓	x	✓	Did Not Finish	x	Did Not Finish
Hydrophobic Collapse	x	x	x	x	No Velcro		No Velcro	

FIG 3

Tertiary structure models. (A) Three helix models given to students. (B) Model with Helix 3 in middle and Velcro® attaching end on end. (C) Model with partially correct tertiary structure. Table shows correct (✓) or incorrect (x) responses by category for each group. No Velcro® indicates group used lanyard, but did not join models with Velcro® so not scored for hydrophobic collapse. [Color figure can be viewed at wileyonlinelibrary.com]

ordered the helices. Figure 3 shows examples of correctly and incorrectly assembled models and the accuracy of each group's model.

After placing the helices on the lanyard, students were asked to use Velcro® to mimic the tertiary interactions of the protein. Of the six groups completing the model, only one group attempted to show the hydrophobic center by placing a Velcro® interaction between the white faces of two of the helical segments as shown in Fig. 3B. The same group also showed an interaction between a red and blue face, placing polar side chains in the hydrophobic center as well. Two of the groups did not use the Velcro®, suggesting no understanding of the tertiary structure of the models. The remaining three groups used Velcro® to connect the ends of each helix together as seen in Fig. 3C. All groups focused on either pairing hydrophobic surfaces together or electron rich and electron poor surfaces together, indicating that their primary considerations in assembling the models were electrostatic interactions.

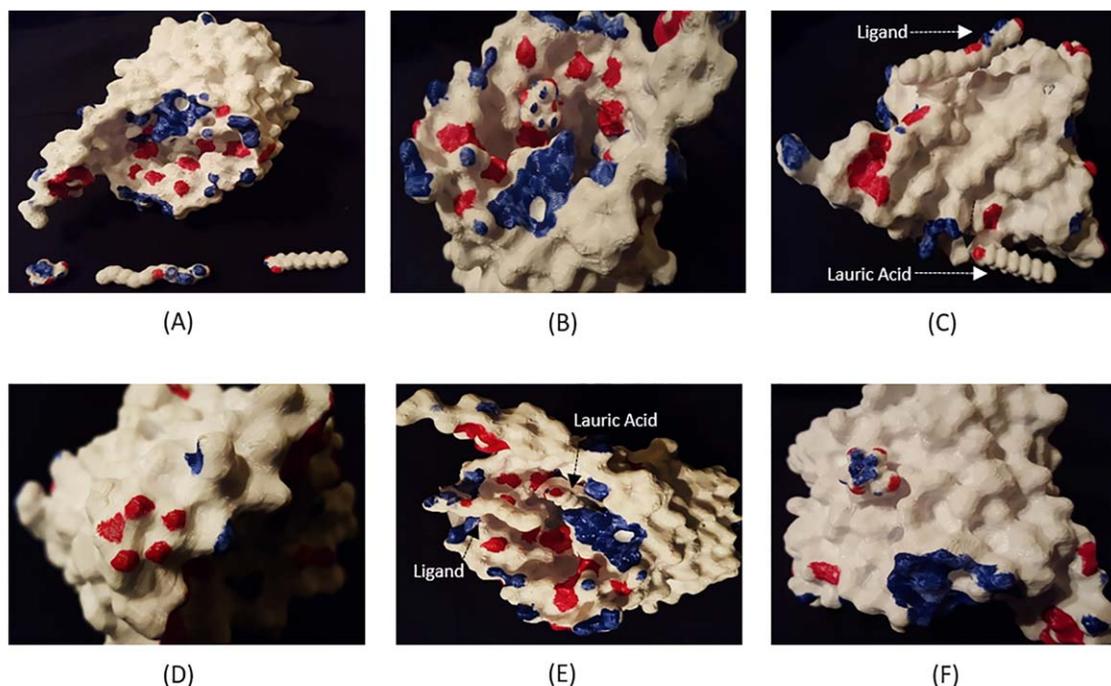
The tertiary structure model results showcase the difficulties students have with translating concepts of hydrophobicity and electron density to large macromolecules. The problems the students exhibited in correctly matching the helical segments indicate that they need further guidance on characteristics of the 3D shape of proteins. These difficulties build upon the needs identified in the secondary structure models to address student understanding of the principles that govern protein structure.

Membrane Protein

The final model in the protein series was designed for investigating the principles of noncovalent interactions regarding the solubility of a membrane protein. The

constructed models included a glucose transport porin membrane protein (PDB ID 4GF4), glucose (the transport molecule), lauric acid (a membrane mimic), and (hydroxyethyl)oxytri(ethyloxy)octane (ligand found on the outer surface of the protein). These models are shown in Fig. 4A [44]. The small molecules were printed in scale with the protein to accurately show comparative sizes. All four models were painted with the red and blue color scheme based on the electrostatic potential maps generated in Swiss PDB Viewer for the protein and Avogadro for the small molecules. A written prompt asked the students to describe where they were placing each of the small molecules on the models to document their reasoning.

Students received the four models and were asked to attach the small molecules to an area of the protein based on the electron distribution shown on the models using Velcro® strips. Examples of correctly and incorrectly assembled models and the number of groups that fit each category are shown in Fig. 4. Eight groups of students participated in the activity and six groups assembled the models, but only two groups accurately attached the glucose models to the aqueous pore of the protein model as shown in Fig. 4B. Based on the written responses, Group 3 correctly identified that the glucose molecule would be "within protein b/c interactions" while Group 8 incorrectly identified their reasoning as "inside - hydrophobic." Interestingly, Groups 1 and 2 identified a specific area of the protein to attach the glucose to, located on the outer surface of the protein. This location has a distinctive pattern of negative electron density that resembles a set of points in a circle as shown in Fig. 4D. Students from Group 1 indicated that they selected this area since "[glucose is] hydrophilic so we attached it to the outside of the protein" and one student stated that "[glucose] is hydrophilic so it attaches on



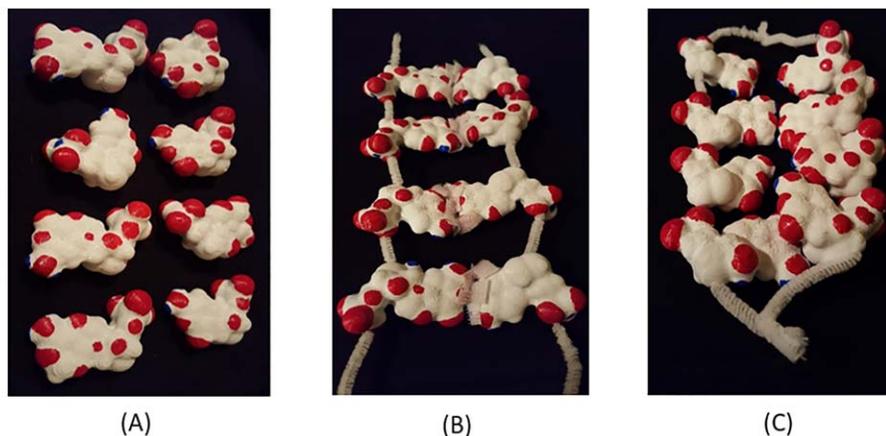
Group	1	2	3	4	5	6	7	8
Ligand Attachment	x	x	✓	x	Did Not Finish	x	Did Not Finish	x
Lauric Acid Attachment	x	x	✓	x		x		x
Glucose Attachment	x	x	✓	x		x		✓

FIG 4

Membrane protein models. (A) Models given to students of membrane protein (top), glucose (bottom left), ligand (bottom middle), and lauric acid (bottom right). (B) Model with glucose correctly placed on inside of protein pore. (C) Model with ligand and lauric acid correctly placed on outside surface of protein. (D) Model showing circular electron density pattern identified as glucose binding site by some groups. (E) Model with ligand and lauric acid on inside of protein pore. (F) Model with glucose incorrectly placed on outside surface of protein. Table shows correct (✓) or (x) incorrect responses by category for each group. [Color figure can be viewed at wileyonlinelibrary.com]

the outside to red b/c it is red” indicating that they were unable to recognize that opposite charges would interact. Students from Group 2 stated that they placed the glucose on this region “since there is both red and blue on the molecule it will bind to an area w/red and blue near the outside” showing an understanding of the attraction of opposing charges, but incorrectly identifying the hydrophilic region of the membrane protein. The remaining groups placed the glucose on the hydrophobic exterior of the protein as shown in Fig. 1F. Students from Groups 4 and 5 described their reasoning as “outside, because it’s polar,” but students from Group 6 interestingly claimed that the glucose would bind “outside on white region since white can bind to positive or negative.” While the reasoning varied between the groups that placed the glucose on the outside, it is apparent that all of the students in these groups struggled with relating electron densities to polarity of the surfaces of the membrane protein.

Group 3 was the only group to correctly attach the lauric acid model and the predominantly nonpolar ligand model to the outer hydrophobic protein surface as shown in Fig. 4C and the students described their model placement as “outside” without further explanation. Group 8 placed the glucose model correctly, but added the lauric acid and ligand to the polar pore. The students described placing the lauric acid “inside/outside, [lauric acid is] hydrophilic–hydrophobic amp[h]lipathic.” The remaining four groups could not apply polarity concepts to the protein and attached the ligand and lauric acid models to the polar region of the model as shown in Fig. 4E. Groups 1, 4, and 6 reasoned that the lauric acid would be “inside of the protein b/c it is hydrophobic.” Group 2 similarly placed the lauric acid inside the protein, but their responses show that a consensus was not reached. Of the five students in Group 2, three stated that lauric acid “is nonpolar so placed inside of protein” while two stated that lauric acid is “nonpolar



Group	1	2	3	4	5	6	7	8
Hydrogen Bonding	✓	Did Not Finish	✓	✓	No Velcro	✓	No Velcro	No Velcro

FIG 5

DNA structure models. (A) Nucleic acid models given to students. (B) Assembled model with Velcro® used to show hydrogen bonding interactions. (C) Assembled model with pipe cleaners used to pair base pairs together. Table shows correct (✓) or incorrect (x) responses for each group. No Velcro® indicates group used pipe cleaners to tie ends to join chains instead of pairing models with Velcro® (to mimic hydrogen bonding) as seen in C. [Color figure can be viewed at wileyonlinelibrary.com]

and [would bind] outside.” The 28 of the 36 participants struggled to relate the nonpolar characteristic of lauric acid to the nonpolar exterior of the protein surface, but eight were able to make this connection.

The four groups that struggled to correctly place the lauric acid also had difficulties with the ligand. Group 1 attached their ligand to a polar region at the top of the pore, but reasoned that the ligand “looks hydrophobic, but from previous knowledge we know that ligands attach to receptors on the outside of proteins so we placed it outside” and stated that the ligand would have an “outside binding site of protein b/c it changes proteins confirmation when bound” indicating that they were relying on their previous knowledge of ligand protein interactions, but were not applying the concepts of partial charges and noncovalent interactions to resolve this understanding. One student of Group 2 stated that the ligand would bind in the “white region” while the others did not answer or put a question mark indicating they were unable to determine how the ligand would interact with the protein. Groups 4 and 6 struggled with the polarity of the ligand stating that they placed it at the opening of the pore because it would be “located on the outside, because it’s polar.” The reasoning for the placement of the ligand shows that the majority of the students were unable to correctly match the neutral ligand to the neutral outer surface of the protein, again struggling with basic concepts of polarity and charge distribution.

In reviewing the students’ written responses, 20 of the 36 students correctly identified glucose as polar and the ligand and lauric acid as nonpolar, but attributed the

incorrect polarities to the faces of the protein. Although the same color scheme was used for all the models, it seems the students could more easily connect the electron distribution with molecule polarity for the small molecules they attached to the membrane protein than the macromolecule. This supports what was observed with the tertiary models, demonstrating once again the need for instructional support for students in connecting ideas from secondary structure through higher levels of protein structure.

DNA

The final set of models were designed to relate a second class of biological macromolecules to the concepts of electrostatic attraction, noncovalent interactions, and structure using a short segment of DNA (PDB ID 5L06) [45]. The DNA models consisted of four sets of base pairs, eight nucleic acids, with a hole drilled through the backbone section of each nucleic acid model (shown in Fig. 5A). The blue and red coloring scheme used was based upon electrostatic potential maps of each individual nucleic acid generated in Avogadro. Students were asked to assemble the DNA sequence based on two-dimensional representations of the DNA and the electrostatic potential maps of each nucleic acid. The students were given pipe cleaners to string the models on and Velcro® to represent the noncovalent interactions.

Eight groups of students worked on the activity and 7 groups finished the model, all of which identified the nucleic acids and assemble them in the correct order. Four of the groups used the Velcro® to show the correct



TABLE II

Percentages of observation codes for each activity by observer

	DNA structure	Tertiary structure		Membrane protein	
		Observer 1	Observer 2	Observer 1	Observer 2
AV	28.7%	33.0%	37.7%	39.7%	39.4%
AM	51.9%	23.9%	32.9%	11.4%	25.2%
P	18.6%	20.2%	22.4%	28.4%	23.9%
OT	0.0%	17.4%	7.1%	8.5%	7.1%
TI	2.3%	1.8%	0.0%	2.1%	1.3%
TK	0.0%	0.0%	0.0%	0.0%	0.0%

hydrogen bonding between the base pairs, and two groups joined the ends of the pipe cleaners together to similarly show the base pair interactions as seen in Figs. 5B and 5C, respectively. Two of the groups used multiple pieces of Velcro® on each base to differentiate the two hydrogen bonding interactions between the adenine and thymine models and the three between the cytosine and guanine models as shown in Fig. 5B. Students easily translated between the electron density maps and the structure of the models indicating that these models could be useful early in the sequence to support student familiarity with these concepts before the more complex protein structure models, depending on when in the classroom sequence the concept of DNA structure is introduced.

Behavioral Observations

The modified version of the BOSS® observation protocol was used to record the types of behaviors students were engaged in while completing activities and using the models. Table II shows the results for each behavior by activity and observer. Very little involvement from the investigator was necessary with only <2.5% of the intervals showing teacher instructional and 0% teacher knowledge codes throughout all activities. This was one of our main goals in the design of the models and activities. Students also remained predominantly on task during the DNA and membrane protein activities with <10% of intervals corresponding to off task during the membrane protein activity and 0% during the DNA activity. A larger proportion of the intervals were off task for the groups completing the tertiary structure model specifically for Observer 1. The relatively low percentages of off-task intervals for most of the observations indicate that the activities were of an appropriate difficulty to keep students engaged.

Active verbal, active model, and passive were the most commonly observed behaviors. For most of the observations, active verbal and active model were more commonly

observed than passive indicating that students were spending most of their time discussing or working with the models. Active verbal accounted for a larger percentage of the observations than model manipulation and building as seen in Table II for the observations during the tertiary structure and membrane protein activities. This is unsurprising since the models only pertained to half of the activity. Active model codes accounted for a considerable portion of the observations in all cases, showing that the models were engaging and challenging enough for students to spend a significant portion of their time manipulating and building the models.

Conclusions

Physical models have shown to lower barriers when translating between representations [46–48]. Our physical models provide students with a hands-on three-dimensional representation of the physical characteristics of complex molecules. These model sets target the complexities of biological macromolecules by focusing student attention on electrostatic attractions. The interactive aspect of the models introduced with model assembly was at an appropriate level of difficulty as it kept students engaged with minimal instructor assistance.

We were able to observe/record different difficulties that arise during instructional sessions. The students who participated in the DNA and secondary structure activities seemed to struggle the least with the electron density depictions and relating these to the interactions between the molecules. These topics are commonly the main focus when addressing hydrogen bonding and the effects of molecule polarity. However, the topics that are typically taught with less of a focus on these issues, tertiary and quaternary structure, proved much more challenging. The participants in these activities struggled to understand how to apply principles of polarity to the larger areas related to

hydrophobic collapse and protein solubility. This set of models targets these difficult areas and can potentially be used to scaffold this understanding by introducing these concepts at the small molecule level and continuing as a sequence to complex macromolecules.

Novel materials that support student understanding of the principles governing the structure of macromolecules are needed to address this prominent area in biochemistry education. Our findings show that this set of models directly targets these issues, and may be used in sequence to address some of the common problematic conceptions. These activities introduce pertinent concepts at the protein secondary structure level and expand this knowledge to higher levels of macromolecular structure in an engaging and unique manner. The use of these 3D models uncovered students' difficulties with translating their understanding and provide a potential means to strengthen student comprehension of macromolecular structure.

Limitations

Even though the created models and activities were designed to target specific problematic conceptions about noncovalent interactions in biologically relevant macromolecules, this study did not focus on learning gains rather on the effective use of these models to engage students. Future studies will investigate whether learning gains are reached by using the set of models in sequence. Also, the models are intended to show a realistic spatial representation of each molecule, but the inflexibility of 3D printed materials required the use of lanyards and pipe cleaners in model assembly. These gaps in the volume of the molecule models and the inclusion of tunnels in the model backbones should be explicitly addressed so that students visualize these as being a filled volume in molecules when using the models during instruction.

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