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Articles

Physical Models Enhance Molecular Three-dimensional Literacy in an Introductory Biochemistry Course*

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This article reports the results of a recent study to evaluate the usefulness of physical models of molecular structures as a new tool with which to teach concepts of molecular structure and function. Of seven different learning tools used by students in this introductory biochemistry class, the use of the physical models in a laboratory was rated as most useful. These results suggest that physical models can play an important role in capturing the interest of students in the subject of molecular structure and function. These physical models often stimulate more sophisticated questions in the minds of students, which can then be more appropriately explored using computer visualization tools.

Keywords: Physical models, Swiss Protein Data Bank viewer, biochemical education.

The molecular biosciences continue to expand at an ever-increasing rate. Many of the recent advances in this area are based on a deeper understanding of the three-dimensional structures of the macromolecular assemblies that constitute the molecular world. Over 2,000 new protein structures were deposited in the Protein Data Bank (PDB)[1] in 2002. The new field of structural genomics promises to accelerate the rate at which new structures will be determined during the next 10 years. In light of the growing importance of molecular structure in the biological sciences, it is becoming increasingly important that educators develop new ways to introduce students to this discipline, and to prepare them to pursue careers in this field.

A major obstacle faced by educators who teach molecular structure and function is the difficulty that many students have in inferring three-dimensional structure from static, two-dimensional diagrams and photos used in textbooks or projected onto screens in classrooms. In recent years, a variety of computer visualization tools have become available that allow students to explore three-dimensional molecular structure in a computer environment [1–4]. Although the image that is generated on the computer screen is in fact two-dimensional, various shading, depth cueing, and kinetic depth effects can produce an image that takes on three-dimensional character as soon as the user begins to move the molecule about on the screen. Although these computer visualization programs were originally developed for UNIX-based computer workstations, public-domain versions of this freeware (*e.g.* RasMol, Protein Explorer, Swiss PDB Viewer, Mage) can be downloaded and run on any standard desktop computer (PC or Mac). Undergraduate educators have readily adopted this new pedagogical tool. Many outstanding Chime tutorials that present a series of computer-generated images imbedded within a tutorial script have been developed and are widely used.

More recently, the use of rapid prototyping technologies has made it possible to construct accurate physical models of molecular structures, based on the atomic coordinates. These models can now be designed using a modified version of RasMol that automatically generates the data files needed by the rapid prototyping machines (see Table I for further details). As a result, the very same image that can be created in a computer environment using RasMol can now become a tactile object in the hands of a student who is just beginning to explore the molecular world.

Direct access to this technology is currently being provided to undergraduate educators through workshops offered by the Milwaukee School of Engineering (MSOE) Center for BioMolecular Modeling (Table I). In addition, collections of physical models can be borrowed by educators form the MSOE Model Lending Library (Table I). As physical models of molecular structures produced by rapid prototyping technologies are becoming more readily avail-

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[1] The abbreviations used are: PDB, Protein Data Bank; MSOE, Milwaukee School of Engineering; GFP, green fluorescent protein.

TABLE I
Relevant URLs

Topic	Website
Laboratory worksheets	www.depauw.edu/acad/chemistry/jroberts/ModelWks.pdf
Computer tutorial	www.depauw.edu/acad/chemistry/jroberts/ModelExpt1.pdf
Workshops at MSOE	www.rpc.msoe.edu/cbm/sepa/undergraduate.php
Gallery of models	www.rpc.msoe.edu/cbm
Lending library	www.rpc.msoe.edu/lib
Model construction	www.rpc.msoe.edu/sbm/technology.php

able, educators are beginning to explore ways in which these models can enhance the use of computer visualization tools in their classrooms. We report here a study in which the use of physical models in an introductory biochemistry course at DePauw University was found to significantly enhance students' understanding of concepts of molecular structure and function.

MATERIALS AND METHODS

"Structure and Function of Biomolecules" (Chem 240) is the new introductory biochemistry course that is part of a four-course introductory core in the Department of Chemistry at DePauw University. This sophomore-level course is designed to introduce students to biochemistry by building a solid foundation in structure and function of the four major biomolecules: proteins, carbohydrates, lipids, and nucleic acids. The goal of the course is to provide students with an understanding that each biomolecule has a unique shape, and that this shape dictates the function (chemistry) of the molecule. Many students struggle with the structural aspects of these biomolecules and develop many misconceptions and misunderstandings regarding the link between structure and function. As two examples, many students do not realize that an amino acid can be far apart in the primary sequence but when folded can be close in three-dimensional space. Many also do not realize the size difference between an enzyme and its substrate; they think the substrate is as large as the enzyme. In these cases and many others, the models provide a context to clear up these misconceptions.

The first section of this course (5 wk) focuses on concepts of protein structure and function. The specific goals for this section of the course are for students to understand that:

1. The shape of a protein helps to determine not only its function but also potential interactions with other molecules.
2. The shape of a protein is determined by the sequence of amino acids that make up the primary structure.
3. A protein folds and maintains its shape based on noncovalent chemical interactions.
4. A small change in a protein (resulting from a mutation in its gene) can lead to a larger change in its shape and consequently its function.
5. There is a difference between the linear sequence and the spatial interactions of amino acids.

The course format consists of three lectures and one 3-h laboratory period each week. Five weeks are spent on the protein structure and function section of this course. During this time, students are introduced to these concepts via traditional "blackboard-based" lectures, complete with overheads and handouts. In addition to these traditional pedagogical tools, physical models of proteins are brought into the lecture on a daily basis (see Table I for a link to a gallery of models). Because this is a class with a maximum of 24 students, the instructor can hold the model up to demonstrate certain aspects of protein structure or function. The model is then passed around to each student. Therefore, each student has an opportunity to interact with the models as they are discussed in lecture. In a large lecture, however, the use of physical models as props might be less effective, as the students would be unable to either see the models or interact with them in lecture. Instead the models would need to be utilized in smaller discussions or in laboratory sections.

In addition to the models, the computer visualization program Swiss PDB viewer is used on two different days. This computer visualization tool is first used in lecture to project interactive images of protein structures and to demonstrate how the figures in the textbook, the physical models, and the computer images are complementary representations of molecular structures. This exposure to the computer visualization program provides students with some familiarity with the tool before they begin their self-guided tutorial using it as part of the laboratory experience.

Concurrent with the introduction of these concepts in lecture, the students are also involved in a 3-wk laboratory experience involving different physical models combined with a computer tutorial. This laboratory takes the students to an entirely different level of interaction with and exploration of the physical models as they work individually to answer questions related to models at four different stations (Fig. 1). Station 1 introduces the students to α-helices, β-sheets, recognizing different R groups, and the concept of a peptide bond. Station 2 has a series of C_2H_2 zinc finger models (an important DNA-binding element). Each model represents this common protein motif in a different format, ranging from a simple α-carbon backbone model, to an all-atoms model in a "sticks" format, to a surface model. Station 3 provides students with an opportunity to construct both an α-helix and a β-sheet using magnetic amino acid backbone units with ϕ/ψ angles preset to one of these common secondary structural elements. The final station contains models of three different proteins. β-Globin is used as an example of a protein composed of α-helices; green fluorescent protein (GFP) is used as an example of a protein with a prominent β-sheet; and lysozyme is used as an example of a protein composed of both of these structural elements. At each station, students work individually with the models to answer questions on the worksheets (see Table I for a link to these worksheets).

At the same time the students are working with the physical models at the four stations, they are also completing a computer tutorial using the visualization program Swiss PDB viewer (see Table I for a link to the tutorial). This guided tutorial takes them step-by-step through the different features of the program by asking different questions about the protein lysozyme. Once they have gained operational familiarity with the program, the students begin to examine the structure in more detail. For example, students examine hydrogen bonds, details of how substrate binds to the enzyme active site, and the consequences of mutating different amino acids in the active site.

Pre- and Post-Test to Evaluate Learning—To investigate the impact of both the physical models and the computer visualization tool on the students' learning, a pre- and post-test was designed to explore student understanding related to the five goals of this section of the course. Three open-ended questions patterned after those reported by White *et al.* [5] were presented to students as a pre-test. The post-test consisted of the same three questions, plus three additional attitudinal questions: 1) elaborating on their confidence in answering the questions after completing the learning experiences (open-ended question); 2) rating the learning value (on a 5 point Likert scale) of a variety of course materials (text, handout, physical models, software,

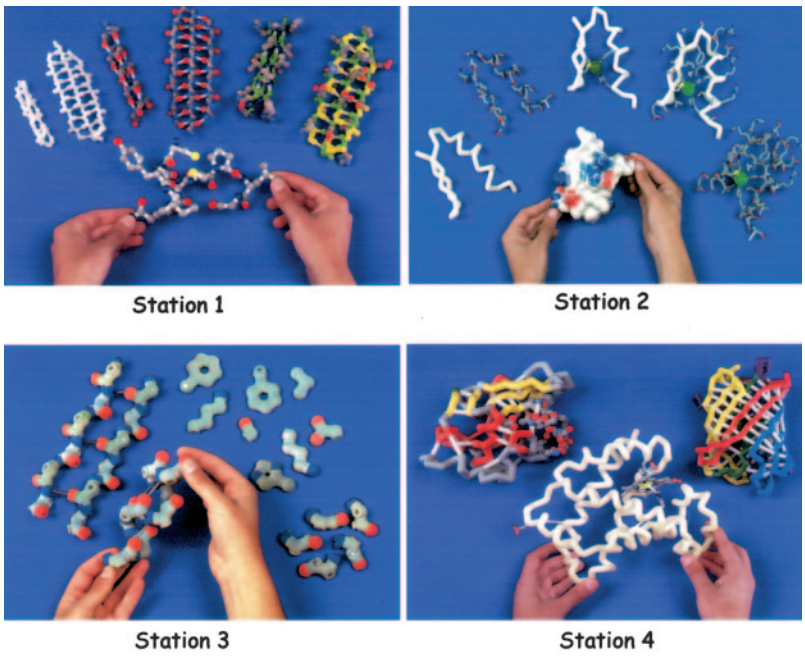

FIG. 1. **Photo of the physical models found at each of the four student workstations.** *Station 1*, (from *top*, *left* to *right*) α-helix and β-sheet backbone models, uncolored; α-helix and β-sheet backbone models, cpk coloring scheme; α-helix and β-sheet models with side chains; and (*bottom*) a *ball-and-stick* model of oxytocin, a nine-amino acid peptide hormone. *Station 2*, the classic C_2H_2 zinc finger in six different formats, (clockwise from the *left*) α-carbon backbone model; "sticks" format showing all backbone atoms; α-carbon backbone model with selected side chains and zinc; α-carbon backbone model with all side chains and zinc; "sticks" model showing all atoms; and a surface model with electrostatic potential coloring scheme. *Station 3*, the α-helix construction kit, consisting of individual backbone units (*left*) with preset ϕ and ψ angles of an α-helix that are joined by magnets and stabilized by hydrogen bonds (metal posts). Individual side chains (*right*) are then added via magnets to the α-carbon of each backbone unit. *Station 4*, (clockwise from the *bottom*) α-carbon backbone models of β-globin, lysozyme, and GFP.

etc.); and 3) asking whether students would use physical models, computer software, or both to solve an additional provided question (forced choice - one out of three). See Appendix A for copies of the three open ended questions and the additional questions found on the post-test.

Twenty-one students took a three question pre-test. Twenty of these students took the same three question post-test, along with the additional questions. The pre-test was administered on the third day of class, before any information on proteins had been presented. The post-test was administered after the last material on protein function had been discussed, and after the worksheets and questions from the computer tutorial had been turned in from lab. Each student was assigned a number so we could follow the progress from the pre- to the post-test.

Analysis of Pre- and Post-Tests—Student responses to questions 1–3 of the pre- and post-test were scored by the instructor in two different ways. First, the responses were scored for correctness on a scale of 0–3 as follows: a "0" for a completely inappropriate response or a response of "I do not know"; a "1" for an appropriate, but not entirely complete or accurate response; a "2" for an accurate response; and a "3" for a strong response that was clear, accurate, and detailed. Second, the student responses were assigned to one of the following seven categories [5], reflecting the kind of information that was contained in the answer:

1. Genetics—Utilization of genetic terms (DNA, RNA, mutations, genes, etc.).
2. Protein Structure—Utilization of terms describing protein structure (primary structure, folding, shape, secondary structure, etc.).
3. Chemical Interactions—Utilization of specific terms describing chemical interactions (bonds, noncovalent forces, hydrogen bonds, hydrophobic interactions, ionic bonds, etc.).
4. Amino Acid Sequence—Utilization of terms describing the amino acid sequence (amino acids, peptide bonds, side-chains, etc.).
5. Purpose—Utilizing terms described by Tamir and Zohar [6] when students use teleological arguments to explain chemical or biological phenomena. In other words, the benefit from a particular function is enough explanation, and there is not any need to further explore further mechanisms.
6. Extrinsic Factors—Utilizing chemical terms such as pH or inhibitors to explain protein structure and function. The appropriateness of these terms was evaluated based on the context of the answer.
7. Miscellaneous—These terms did not fit into other categories.
8. None—"I don't know."

Except for "I don't know," each student answer could contain multiple categories in the response. Two different evaluators scored the surveys separately. This second scoring of the student responses follows the procedure reported by White *et al.* [5] to determine if the sophistication of the student responses improved between the pre- and post-tests.

RESULTS

The students' ability to correctly answer questions 1–3 on the post-test showed a dramatic improvement over their responses on the pre-test. The total number of responses that received the highest possible rating (a "3") increased from 8% on the pre-test to 67% on the post-test. In addition, while a total of 22% of the responses on the pre-test were rated "completely inappropriate" or "I don't know" ("0"), no responses were rated in this category on the post-test. The mean scores for each question, standard deviations, and standard errors of the means

were calculated (Table II). The mean gain for "student learning" in each question is 0.90 for question 1, 1.30 for question 2, and 1.65 for question 3 (Fig. 2). The Shapiro-Wilk test [7] of normality indicated that the scores on each of the three questions on the pre- and post-test significantly differed from the normal distribution, so Wilcoxon signed ranks tests [8] were used to determine whether significant improvements had occurred on each question from pre- to post.

How students improved, as organized by pre-test scores and post-test scores, was determined. Of the seven students who received a 0 or a 1 on the pre-test score for question 1, all improved to either a 2 or a 3 on the post-test. Of the 15 students who scored a 0 or 1 on question 2, two students did not improve from a score of 1, and the remaining 13 students all improved to a 2 or a 3 on the post-test. On question 3, 15 students scored a 0 or a 1 on the pretest. Fourteen of these students improved to a 2 or a 3, with only one student improving from 0 to 1 on the post-test. All the students who received a 2 on any pre-test question improved to a 3 on their post-test. All the students who received a 3 on any pre-test question maintained a 3 on their post-test.

Categories of Responses to Open-Ended Questions—The responses to questions 1–3 were also assigned to one of the eight categories described in the "Materials and Methods." Comparison of pre-test and post-test responses relative to this categorization also revealed an increase in the level of sophistication at the end of this instructional unit. In general, the students either did not know the answer (category 8) or just tossed out random chemical terms in an effort to answer the pre-test questions. On the other hand, students were much more focused and used correct responses when answering the post-test questions.

For question 1 on the pre-test, seven categories were utilized (no student said "I do not know") for answers, suggesting that students were randomly providing responses to this question. On the post-survey for question 1, only three categories (2-protein structure, 4-amino acid sequence, 5-purpose) were used. Over 90% of the answers focused on categories 2 and 4, which were the responses necessary to answer this question correctly.

All eight categories were represented in the responses to question 2 on the pre-survey (five students said "I do not know"). On the post-survey, only four categories were represented (no students responded with "I do not know") with 80% of the responses focusing on two different categories: 1-genetics and 6-extrinsic factors. As with question 1, we saw the student responses narrow to more specific categories. The responses that utilized category 1 (50%) were more appropriate than those that utilized category 6 (30%).

The responses to question 3 on the pre-survey covered six different categories (five students said "I do not know"). The two unused categories were 1-genetics and 7-miscellaneous. Only three categories were utilized to respond to the post-survey question. Over 75% of the students responded with category 3-chemical interaction, which is the appropriate response to this question. The other two categories were 2-protein structure and 4-amino acid sequence and though not as correct, were utilized in an appropriate manner.

Learning Tool Preferences—To relate student learning with a particular learning tool, we asked each student to rate the value of the seven different learning tools used in this instructional unit (question 5 on post-survey). The rating scale ranged from "1" (was of no help) up to "5" (helped a great deal). The mean score and confidence intervals based on twice the standard error of the mean given to each learning tool are provided in Fig. 3.

Of the seven learning tools rated in this question, the "Textbook" and "Overheads" received the lowest rating by the students. In contrast, the "Models in Lab" (mean = 4.81, S.D. = 0.402) and the "Models in Lecture" (mean = 4.52, S.D. = 0.512) received the highest ratings. These two ratings were statistically significantly higher than the next highest rating, "Swiss PDB-Lab" (mean = 4.10, S.D. = 0.768), as determined by Wilcoxon signed ranks tests. The "Models in Lab" differed from "Swiss PDB-Lab" at the $p = 0.000$ level, and the "Models in Lecture" differed from the "Swiss PDB-Lab" at the $p = 0.045$ level. In addition, the students' rating of "Models in Lab" showed the lowest standard deviation (0.402) of all the learning tools, indicating a great deal of consistency in responses; 17 students rated "Models in Lab" as having "helped a great deal" (a

TABLE II

The mean scores, standard deviations, and standard errors of the mean were calculated for questions 1, 2, and 3 of both the pre- and post-tests on a scale of 0–3

Question	Mean	*N*	S.D.	S.E.M.
Pre-test 1	1.75	20	0.786	0.176
Post-test 1	2.65	20	0.587	0.131
Pre-test 2	1.15	20	0.933	0.209
Post-test 2	2.45	20	0.686	0.153
Pre-test 3	1.05	20	0.577	0.126
Post-test 3	2.70	20	0.561	0.122

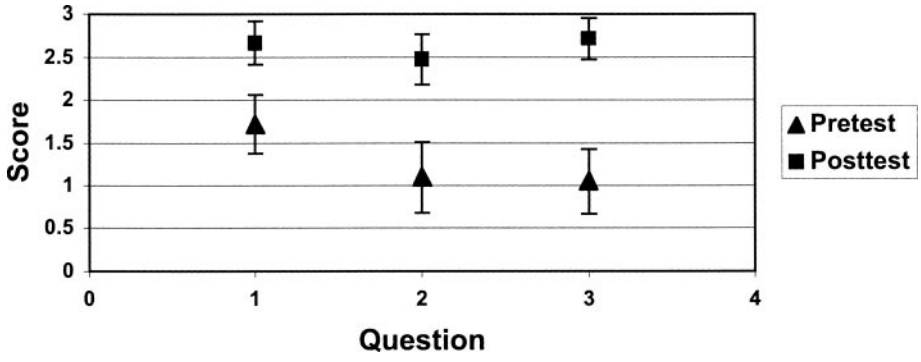

FIG. 2. **The mean scores and confidence intervals (twice the standard errors of measurement) were calculated for student scores on questions 1, 2, and 3 of the pre- and post-tests.**

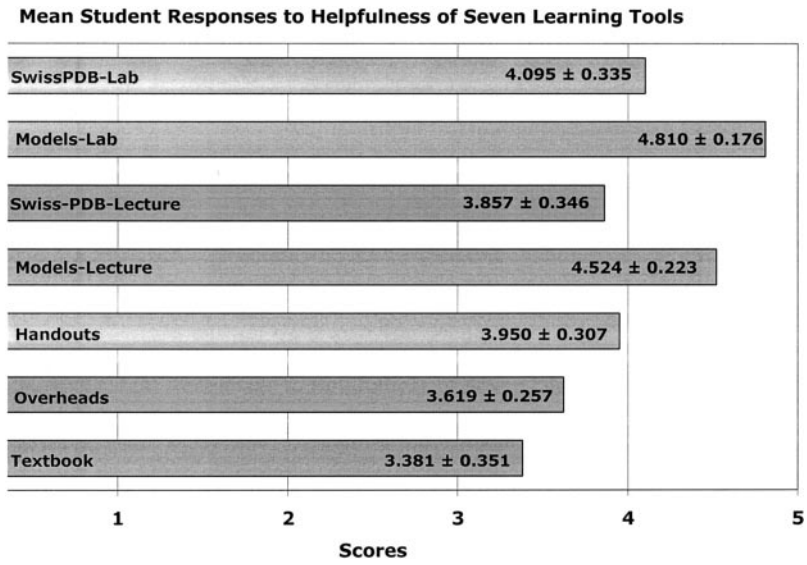

FIG. 3. **The mean score and confidence intervals of twice the standard error of measurement were calculated for the student ratings of the seven different learning tools as described in question 5 on the post-survey (Appendix A).**

"5") while the remaining four students rated "Models in Lab" as having "helped a good deal" (a "4").

DISCUSSION

The instructional strategies used in this study were clearly effective in increasing students' understanding of basic concepts of molecular structure and function. This learning is documented by multiple criteria including the improvement in students' responses to questions 1–3 of the post-test and by their more focused answers that used appropriate language and reasoning to respond to these questions at the end of this instructional unit. To quote one student's response to question 4 of the post-survey—"The protein models are a very valuable tool in learning not only structure, but structural significance of proteins. Also, learning with models is much easier than trying to look at a 2D picture." Students who scored well on the pre-test (2's or 3's) scored well on the post-test, but more significantly those who scored poorly on the pre-test (0's and 1's) showed improvement on the post-test.

When students were asked to rate seven different learning tools that were employed in this course, the use of physical models in both a lecture and in a laboratory setting received the two highest ratings. These interactive learning tools were rated more highly than traditional but static learning tools such as the textbook, handouts, and overheads. It is also notable that the students' preference for using physical models in laboratories was more uniform than their rating for all of the other tools. Of the 21 students who rated the helpfulness of the models in laboratory, 17 students rated these models in the category "helped a great deal" while the remaining four students indicated the models "helped a good deal." Other learning tools, such as Swiss PDB Viewer in lecture, were given ratings of 2-"helped a little" up to ratings of 5-"helped a great deal." Therefore, while the more varied ratings of the other tools most likely reflect the different learning styles of students, all students seemed to find the physical models useful.

While the potential benefit of using computer visualization tools to explore molecular structure has been previously demonstrated, several limitations of this technology have been noted. As demonstrated by our findings, if students are only allowed to passively view images projected by the instructor in a lecture setting, they don't perceive as much of a benefit to their understanding. Richardson and Richardson [9] addressed this point in their seminal article in which they point out that computer visualization cannot be a passive tool if it is to make a unique and important contribution to student learning of molecular structure and function. Rather, the use of these programs should be interactive, allowing students to individually work to develop analytical skills related to three-dimensional visualization and to learn the subject matter. This approach has been recognized and used by others as well [5, 10–14].

One goal of this study was to examine the way in which the use of physical models can complement the use of computer visualization tools to explore molecular structure. Our initial hypothesis was that the immediacy and the tactile nature of the physical models would make them more useful than computer visualization tools in understanding the basic elements of protein structure. However, once the basic concepts of protein structure were understood, then computer visualization tools would become more useful as students began to consider more sophisticated questions related to the functional consequences of molecular structure. This hypothesis seems to be supported by the results of this study. When students were presented with a more difficult question at the end of this instructional unit and asked to choose from a list of tools they could use to answer the question, 16 of 21 students chose to use the combination of both physical models and computer visualization tools. Only five chose to use physical models alone, and no students chose computer visualization alone.

We have noted in this and other related field-tests of the use of physical models that these tools have a positive impact on both students and teachers alike. From the perspective of the teacher, the physical models provide an alternative to the use of computer visualization technology. Just as students are recognized as having different learning styles, teachers have different teaching styles. A collection of physical models now makes it possible to teach the basic elements of protein structure and function with-

out relying primarily on the use of computer visualization. And because the engaging, interactive nature of the physical models readily captures the interest and enthusiasm of the students, teachers report that their students begin to ask more and better questions. This ready engagement of the students allows the instructor to introduce even more sophisticated concepts into the ensuing discussion and elevate the level of the material presented in the course. Finally, with the addition of this multi-week laboratory, less time is needed in lecture to introduce this material, because the students learn most of the more complex and difficult concepts as they explore the models at the individual stations. Because the students are actively learning this material, they are able to ask and answer even more challenging questions during discussion and on exams.

One obvious limitation to the widespread use of physical models in courses dealing with molecular structure is the high cost and limited availability of the models. This current problem will diminish with time as new technology makes it possible to mass-produce these models at lower costs. In the meantime, a model lending library has recently been established from which educators can borrow collections of physical models for use in their classrooms (Table I).

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APPENDIX A

Open-Ended Questions on Both Pre- and Post-Survey

1. Collagen and myoglobin are both human proteins. Collagen is a long rope-like structure while myoglobin is a rounded blob. Both of these are made up of protein material. How can these both be made of the same basic material but be shaped so differently? Explain your answer in words.
2. How can a nonfunctional protein be present but unable to work properly in a biological system? Explain your answer in words.
3. How do proteins maintain their shape? Explain your answer in words.

The Remaining Questions Were Only on the Post-Survey

4. Do you feel more confident answering these questions after completing this section of the course? Explain your answer.
5.

Rate how the following tools helped facilitate your learning of the course materials.	Was of No Help	Helped a Little	Helped Some	Helped a Good Deal	Helped a Great Deal
	1	2	3	4	5
a. The textbook	1	2	3	4	5
b. Overheads as a tool used in lecture.	1	2	3	4	5
c. Handouts as a tool from lecture.	1	2	3	4	5
d. Physical models as a tool in lecture.	1	2	3	4	5
e. Swiss PDB Viewer in lecture	1	2	3	4	5
f. Four Stations of Physical models in lab	1	2	3	4	5
g. Swiss PDB tutorial in lab.	1	2	3	4	5

6. Identify the amino acids that make up the interior region around the heme group in hemoglobin. What are general properties of these amino acids? What side chains are interacting directly with the heme group? Which materials would you most likely use to answer these questions? (choose only one): i) the file for hemoglobin displayed on Swiss PDB Viewer computer program; ii) a physical model of hemoglobin with all the side chains displayed; or iii) a combination of both Swiss PDB Viewer and the physical model.