In this study we measured the learning outcomes resulting from using molecular visualization software in lecture and in the teaching laboratory of a large introductory-level undergraduate biology majors’ course. The study was initially carried out in the Fall semester of 1999; the results of this study were used to devise an expanded laboratory component that was evaluated in a second study carried out in the Fall of 2000. In both studies, students (n = 175 and 161) attended two 50-min lectures that used molecular visualization software to explain protein structure and function and the gene-protein connection. Students also used this software during one 3-h laboratory session as a tool for exploring these topics. Students completed open-ended pre- and post-surveys that involved a related but unfamiliar task. Survey responses were scored for correctness, as well as by the type(s) of explanations used in the response. We found the following eight types of responses that students employed to explain protein structure and function: genetics, protein structure, chemical interactions, amino acid sequence, purpose/teleology, extrinsic factors, miscellaneous, and none. In both studies, the frequencies of correct answers, as well as the frequencies of each response type, showed significant changes as a result of lecture and/or lab. The effects of lecture were highly similar in both studies. The changes in the expanded lab resulted in significant changes in outcome. Overall, the curriculum effectively communicated several core concepts in protein biochemistry and expanded the conceptual “toolkit” that students applied to problems of protein structure and function. Lecture increased students’ understanding of the role of amino acid sequence, whereas lab tended to increase their understanding of three-dimensional structure and the gene-protein connection. Our results demonstrate that exposure to molecular visualization, even for a relatively brief time, can improve students’ understanding of protein structure and function. In addition, we demonstrate the differing and largely non-overlapping effects of lecture and lab, suggesting that effective use of molecular visualization should involve both types of activities.

Keywords: Evaluation, molecular visualization, protein structure.
more authentic “pseudo-three-dimensional” view of protein structures than is possible with static two-dimensional representations found in textbooks. This molecular visualization software is available freely to students as a stand-alone application (RasMol) and a web-browser plug-in (Chime), thus facilitating classroom, laboratory, and home use [5]. Several articles have been published that recommend the use of this technology for teaching biology and chemistry [1, 4, 6–8]. Although this technology is relatively new, there have been several reports evaluating its use in classrooms. Weiner et al. [4] found that students felt visualizations of protein molecules helped them to learn the material. Khoo and Koh [9] showed that students self-reported ability scores increased following exercises based on visualization of simple molecules. Others have shown that the ability of students to visualize the rotation of simple molecules in three dimensions increased after watching a videotape of molecular visualization [10, 11]. In a very detailed series of studies [12] Wu et al. found significant gains in high school students’ understanding of representations of simple molecules after using molecular visualization software. In the studies described in this paper, we examined the changes in students’ concepts of more complex molecules, proteins, resulting from a relatively brief exposure to molecular visualizations. We also examined the effects of expanding the use of molecular visualization in the lab exercise.

General Biology I is a first-semester course for undergraduate biology majors. In this course, we use molecular visualization presentations in lecture and molecular visualization exercises in the teaching laboratory to communicate an understanding of protein structure and function. We measured the effects of these activities on students’ concepts of protein structure and function using an open-ended survey that examined their understanding of concepts 2, 3, 4, and 5. This study was conducted twice, once in the Fall of 1999 and again in the Fall of 2000 after revising and expanding the lab. Our results show that lecture and lab had significant and differing effects on students’ conceptual understanding and that changes to the lab curriculum had measurable, if mixed, effects on its outcome. Based on our findings, molecular visualization can be an effective means for communicating these important concepts.

**METHODS**

*Subjects and Curriculum*—The subjects of these studies were the students enrolled in General Biology I in the Fall semesters of 1999 and 2000. There were roughly 250 students enrolled in the course per semester; their average age was 22.3, they were 76% female, and they were 42% non-white. The course consists of three weekly lectures plus a weekly lab and serves as an introductory course for Biology majors.

The biochemistry section of the course follows a section on genetics and an introduction to basic chemistry and places particular emphasis on protein structure and function. Following a blackboard-based introduction to macromolecules and proteins,
students were shown views of the molecule hemoglobin. Two 50-min presentations used molecular visualization to highlight the structural features of hemoglobin, describe how these features were related to its amino acid sequence, and detail the effects of two mutations on its structure and function. The presentations are described in detail in Table I and Table II. Fig. 1 shows several sample molecular visualizations.

The lectures were followed by one 2-h lab where students explored the structure of the enzyme lysozyme, an anti-microbial protein that degrades the cell walls of certain bacteria. Students worked in groups of three using molecular visualization software running on Macintosh computer workstations as a tool for answering questions about protein biochemistry. Groups were responsible for characterizing a specific series of eight amino acids with respect to secondary structure, location within the protein, and hydrophobicity of the side chains. As a class, they pooled their data and discussed any patterns they had found. Finally, students were asked to rationalize the effects of two different mutations, based on the locations of the altered amino acids within the structure of the protein. In the Fall of 2000, the lab was expanded to 3 h by the addition of an examination of six specific amino acids and their interactions with other parts of the protein, as well as the substrate. This is shown in Table III; sample visualizations can be found in Fig. 1. The complete lecture and lab curriculum is available for download from intro.bio.umb.edu/downloads/.

Surveys and Survey Administration—To explore the cognitive changes associated with these activities, the students in General Biology I completed pre- and post-surveys targeted to four of the five core concepts described in the Introduction. The surveys consisted of two open-ended questions about protein structure involving proteins that had not been mentioned in lecture or lab; a copy of the survey can be found under “Appendix.” The first question assessed students’ understanding of concepts 3 and 4, the factors that govern protein structure, by asking the students to explain how two particular proteins could have different shapes even though they are made of the same material (polypeptide). Question two examined students’ understanding of concepts 2 and 5, the gene-protein connection, by asking how a protein could be present but non-functional.

We employed the modified pre-/post-survey administration protocol diagrammed in Fig. 2. Pre-instruction surveys were administered to the entire class as a take-home assignment following lectures covering genetics and basic chemistry. The students then attended the lectures on protein structure, which included molecular visualization of hemoglobin. Students then performed a lab exercise that introduced them to molecular visualization using small molecules of a few atoms. During the week when the students performed the lab exercise involving the molecular visualization of lysozyme, half of the students completed an identical survey at the beginning of lab, and the other half completed it at the end of lab. This resulted in the following two groups of students (Fig. 2). 1) Lecture only; these students completed the pre-survey before the lectures (Pre-Lecture scores) and completed the post-survey at the beginning of lab (Post-Lecture scores). 2) Lecture and lab; these students completed the pre-survey before the lectures (Pre-Lecture & Lab) scores and completed the post-survey at the end of lab (Post-Lecture & Lab) scores.

In the Fall of 1999, there were 260 students enrolled in General Biology I; from 175 of these students (67%) we recovered both a pre- and a post-survey; surveys from the remaining students were discarded. Of these 175 students, 91 completed the post-survey at the start of the molecular visualization lab; the remaining 84 completed it immediately following the lab. In the Fall of 2000, there were 242 students in the course; we recovered pre- and post-surveys from 161 (67%). Of these 161 students, 92 completed the post-survey at the start of the molecular visualization lab; the remaining 69 completed it immediately following the lab.

Analysis of Survey Responses—We analyzed the students’ responses at two levels. At the most basic level, we scored their

![Sample molecular visualizations of hemoglobin.](image)

**Fig. 1.** Sample molecular visualizations of hemoglobin. A, deoxy-hemoglobin; space-fill view. B, oxy-hemoglobin; protein backbone shown, heme groups in ball and stick view, oxygen in space-fill view. C, close up of β-globin subunit; two side chains in ball and stick view, oxygen bound indicated with dotted line. D, β-globin protein; protein backbone shown, heme group in ball and stick view, side chain of amino acid altered in HbChirstchurch shown in space-fill view.

<table>
<thead>
<tr>
<th>Image</th>
<th>Description</th>
<th>Task</th>
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</table>
| 1       | Similar to Fig. 1D Wire-frame view of enzyme; substrate and individual amino acid in space-fill view | • For each of eight amino acids, determine location (interior, exterior, or substrate binding pocket)  
• For each of eight amino acids, determine hydrophobic/hydrophilic nature of side chain  
• Class-wide discussion of regularities in structure  
• Explanation of effects of two mutations  
The lab ended here in Fall 1999; the remaining material was added in Fall 2000 |
| 2       | Similar to Fig. 1D Wire-frame view of enzyme; substrate and pair of amino acids in space-fill view | • Examination of sample ionic bond, hydrogen bond, and hydrophobic interaction between side chains within protein  
• Examination of sample hydrogen bond and hydrophobic interaction between side chains of protein and substrate  
• Lab report; design a binding site for a molecule of your choice and give an example how a mutation could alter the binding |
| 3       | Similar to Fig. 1D Wire-frame view of enzyme; substrate and individual amino acid in space-fill view | • For each of eight amino acids, determine location (interior, exterior, or substrate binding pocket)  
• For each of eight amino acids, determine hydrophobic/hydrophilic nature of side chain  
• Class-wide discussion of regularities in structure  
• Explanation of effects of two mutations  
The lab ended here in Fall 1999; the remaining material was added in Fall 2000 |

TABLE III

Protein structure lab curriculum in Fall 1999 and Fall 2000
results of their answers. The categories were as follows:

- Genetics: These students' explanations included genetic terms, for example “DNA,” “RNA,” “mutations,” “genes,” “alleles,” “genetic code,” etc. This corresponds to concepts 2 and 5.
- Protein Structure: These students' explanations included descriptions of protein structure, for example “shape,” “lock and key,” “1° structure,” etc. This is one major component of concept 3.
- Chemical Interactions: These students' explanations made specific reference to particular chemical interactions, for example: “bonds,” “hydrogen bonds,” “ionic bonds,” “hydrophobic interactions,” “dehydration synthesis,” etc. This is the other major component of concept 3.
- Amino Acid Sequence: These students' explanations included ideas related to the amino acid sequence of the protein, for example “sequence,” “order,” “peptide bonds,” “side-chains,” etc. This corresponds to concept 4.
- Purpose: These students explanations involved teleological arguments, for example “it is structured to do its particular job.” This is a misconception that is frequently found in a variety of biological contexts [13].
- Extrinsic Factors: These students' explanations involved factors other than the amino acid sequence and chemical interactions, for example “pH,” “inhibitors,” “denaturation,” “heat,” etc. Although this is a correct response category, the lectures and lab de-emphasized the influence of these factors on protein structure. Thus, in this context, it is a logical but irrelevant response.
- Miscellaneous: These answers were not categorizable.
- None: For example “I have no idea,” blank answers, etc.

With the exception of “None,” the survey of any given student could contain more than one type of response. Subjects were also required to write their names on their survey responses, which allowed us to track conceptual changes of individual students. All surveys were scored separately by two of the investigators; after comparing their scorings and eliminating trivial errors, the inter-rater reliability was greater than 90%. The results of the Fall 1999 and Fall 2000 studies were scored by the same pair of investigators.

### RESULTS

The objectives of these studies were 2-fold: first, to measure the effects of molecular visualizations on students' understanding of protein structure and function; second, to measure the changes in outcome resulting from the expanded emphasis on amino acids and their interactions, which was included in the Fall 2000 lab. We examined these effects at the following four levels of resolution: the most frequently used response categories, the complexity of the answers of individual students, the frequency of correct answers to the survey questions, and the frequencies of each of the eight response categories.

Detailed data from the Fall 1999 study are presented in Fig. 3; the results of both studies are summarized in Table IV. The effects of lecture can be found by comparing the Pre-Lecture and Post-Lecture groups; any significant differences here are indicated by one or more asterisks (*) (McNemar’s test). The effects of the combination of lecture and lab can be found by comparing the Pre-(Lecture & Lab) and Post-(Lecture & Lab) groups; any significant differences here are indicated by one or more asterisks (*) (McNemar’s test). The incremental effects of lab can be found by comparing the Post-Lecture and Post-(Lecture & Lab) groups; any significant differences are indicated by one or more crosses (+) (chi-square test). Note that this last comparison assumes that the two pre-instruction groups are not significantly different; this was found to be true in all cases (chi-squared test).

**Overall Pattern of Responses**—When the overall pattern of responses was examined, the most frequent type of response to the pre-instruction surveys in both studies was “Miscellaneous.” After lecture and lab in Fall 1999, the most frequent type was “Amino Acid Sequence,” followed closely by “Chemical Interactions”; in Fall 2000, the categories were the same but reversed in rank. Notably, the emphasis on chemical interactions in the Fall 2000 lab was reflected in the very high percentage of students using this response category. In addition, the complexity of students' response, as measured by the average number of catego-
of 1999, lecture significantly increased responses in one desired category. Simultaneously, lecture significantly reduced the undesired response categories.

In the Fall of 2000, it is likely that this difference results from random variation in the student population. Fall 1999 and Fall 2000 results compared—The effects of lecture are very similar in both the Fall 1999 and Fall 2000 studies. In six of eight categories, the effects were the same. The one remaining category “Chemical Interaction,” the results are very similar. In Fall 1999, both lecture and lab were required for a significant increase whereas in Fall 2000, a significant increase was observed with lecture and again with lab. This is not surprising, given that the lectures were virtually identical in both years. Interestingly, the increase and decrease of “Extrinsic Factors” was only observed in Fall 1999; in the Fall 2000 study this category was unchanged throughout the course of the study.

Expanding the portion of the lab curriculum that explored amino acid side-chain interactions changed the effects of lab. However the changes observed are not entirely positive. In three of eight categories, the effects were the same with either version of the lab. The expanded lab used in Fall 2000 improved students’ responses in two categories. The added work on side-chain interaction in the Fall 2000 lab resulted in an increased effect on the “Chemical Interaction” category. Surprisingly, the ex-

<table>
<thead>
<tr>
<th>Category</th>
<th>Lecture only</th>
<th>Lab only</th>
<th>Both lecture and lab</th>
</tr>
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<tbody>
<tr>
<td>Genetics</td>
<td>0</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>Purpose/Teology</td>
<td>-</td>
<td>0</td>
<td>-</td>
</tr>
<tr>
<td>Structure</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Chemical Interaction</td>
<td>0</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Amino Acid</td>
<td>+</td>
<td>+</td>
<td>0</td>
</tr>
<tr>
<td>Extrinsic Factors</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>0</td>
<td>0</td>
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</table>

+ , increased significantly; 0, no significant change; −, decreased significantly.

![Fig. 3](image-url)
discussions, the effects of the combination of lecture and lab were similar in Fall 1999 and Fall 2000. There were significant increases in desired response categories ("Chemical Interaction" and "Amino Acid"), significant increases in the rate of correct answers to the survey questions, and significant decreases in undesired response categories ("Purpose," "Miscellaneous," and "None"). The Fall 1999 curriculum also increased the two other desired response categories, "Genetics" and "Structure." The Fall 2000 curriculum increased "Chemical Interaction" to a greater extent than Fall 1999 but did not increase "Genetics" or "Structure"; the fraction of students with correct answers to the survey questions was also lower in Fall 2000.

DISCUSSION

The combined application of molecular visualization in lecture and hands-on lab was successful in communicating the key ideas for which the curriculum was designed. In both studies, the fraction of correct answers to the two survey questions increased significantly. Thus, the students learned concepts that they were able to apply correctly in a novel situation. Prior to instruction, the most common responses were categorized as "Miscellaneous"; the curriculum changed these to more biologically reasonable explanations. Furthermore, this curriculum increased students' vocabulary of ideas related to protein structure, as measured by the complexity of their answers.

When examined in detail, the lecture presentation had specific and highly reproducible effects on students' understanding of protein structure and function. The lectures eliminated or significantly reduced the fraction of students who had no idea how to approach the question, as well as significantly reducing teleological explanations. These were replaced by explanations that involved particular details of protein structure, amino acids and their interactions. This is consistent with the observed increase in correct answers to Question 1 and indicate an improved understanding of the factors that govern protein structure. Additionally, the same series of lectures delivered in Fall 1999 and Fall 2000 had highly similar effects, thus demonstrating the reliability of our measurement technique.

In general, lab helped the students gain an understanding of the three-dimensional nature of proteins and the gene-protein connection. The expanded lab used in Fall 2000 involved substantially more work with amino acid side chains and their interactions. This expanded treatment resulted in substantial gains in students' understanding; after lecture and lab 70% of the students answers included "Chemical Interactions" in Fall 2000 as compared with only 40% in Fall 1999. Thus, the changes in the lab curriculum were reflected in changes in outcome.

However, in terms of "Genetics" and "Protein Structure," students performing the expanded lab showed less improvement than those performing the original lab. In principle, these lower gains could result from poorer understanding of these concepts. Alternatively, the students in the expanded lab may have an adequate understanding of genetics and protein structure but may have chosen explanations involving chemical interactions, because that theme was particularly emphasized in the revised lab. Given the limitations of our measurements, it is not possible to distinguish between these alternatives. A detailed analysis of the actions of the students in lab would help to resolve this.

From these data it is clear that the specific effects of lecture and lab were mostly non-overlapping; that is, most response categories were effected by lecture or lab alone. Lecture gave some biologically reasonable ideas to those students who had no idea how to answer the question initially. Lecture also increased the students' understanding of the role of amino acids in determining the shape and function of the protein (concept 4). Lecture did not effectively communicate an understanding of the three-dimensional structure of proteins (concept 3) or the gene-protein connection (concepts 2 and 5); understanding these required hands-on lab activity where the software was used as a tool for answering questions about protein biochemistry, at least in the Fall 1999 study. These results indicate that the students needed to manipulate the representations themselves to get a three-dimensional picture of the molecule. Interestingly, although the gene-protein connection was emphasized more in lecture, these ideas were more effectively communicated by the Fall 1999 lab. Overall, our results clearly demonstrate that this combination of visualization lecture and lab was an effective way to communicate this material.

APPENDIX (SURVEY INSTRUMENT)

Notes:

- You will receive full credit (10 points) for whatever you write in the spaces below; there is no need to consult outside sources when working out your answer. The more you write, the better I can teach the course.
- This is intended to help me in setting up my lectures on proteins and as a warm-up for the material we will be dealing with in the next section of the course.
- This is also intended to help me in evaluating the lectures and lab (I will ask the same question after the relevant lectures and lab).

The Problem:

Although we have not talked about what proteins look like and how they work, most of you may have heard at least a little about these topics elsewhere. I am interested in what you know before we cover it in Bio 111. I am not interested in the correct answer, I am interested in your answer. Try your best, but if you do not know, say so. To the best of your ability, and based on only what you know now, answer the following questions.

a) Collagen and albumin are both proteins found in the human body. Collagen molecules are long and thin whereas albumin molecules are rounded blobs. This is shown below.

    collagen molecule:  albumin molecule: ●

Both collagen and albumin are made of the same material, protein. How is it that they can be made of the same
material but be shaped so differently? Explain in words and/or draw a picture.

b) How can a protein be “broken,” present but unable to function? Explain in words and/or draw a picture.

REFERENCES