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Article

Evaluation of Software for Introducing Protein Structure

VISUALIZATION AND SIMULATION

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Communicating an understanding of the forces and factors that determine a protein's structure is an important goal of many biology and biochemistry courses at a variety of levels. Many educators use computer software that allows visualization of these complex molecules for this purpose. Although visualization is in wide use and has been associated with student learning, it is quite challenging to develop visualizations that allow students to interactively observe the effects of altered amino acid sequence on protein structure. A software simulation, the protein investigator (PI), has been developed to specifically facilitate this type of exploration. When using the PI, students enter or edit an amino acid sequence; the software then simulates its folding in two dimensions using the major forces involved in protein structure. This study explores freshman undergraduate students' use of visualization and simulation when learning about protein structure. It also evaluates some of the learning outcomes from these two approaches. Our results show that simulation leads to similar learning outcomes as visualization. Because simulation allows a more interactive exploration, a combination of the two approaches may be an effective approach to introducing the basic principles of protein structure.

Keywords: Undergraduate, protein structure, visualization, simulation, evaluation.

Proteins are a major component of all cells and are responsible for carrying out many of the functions of living organisms. A protein's function is determined primarily by its structure, which is largely determined by its amino acid sequence; this process is mediated by a variety of covalent and noncovalent interactions between the amino acids. Mutations can act by causing changes in the amino acid sequence of proteins that can lead to alterations in function, which may have effects on phenotype. An understanding of these issues is an essential part of understanding modern molecular biology and is a key component of biology and biochemistry courses from high school through graduate school, albeit at different levels of precision.

Many methods have been developed to teach students about protein structure and the forces that govern it. These include two-dimensional presentations in textbooks and slides; physical models (for example, [1, 2]); audio feedback [3]; and, quite frequently, computer-aided visualization (for an extensive library of examples, see molviz.org). Visualization typically takes the form of two-dimensional displays, where interactive controls allow the user to explore and experience the three-dimensional structure of the protein under study. A large number of software programs have been developed for this purpose (including [4, 5], and www.jmol.org); these have been received positively by students [3, 4] and their use has been correlated with increased understanding of key elements of protein biochemistry [1, 6].

One important use of these visualizations is to have students develop hypotheses about the interactions they observe and the possible roles these interactions play in forming the protein's overall structure. Having students explore these interactions helps them understand the forces that give proteins the shapes they have. However, although visualization software allows the students to develop hypotheses, it does not easily allow them to test their hypotheses by, for example, determining the structure of a mutant protein where one of the amino acids involved in the hypothesized interaction has been altered. Such tests have been carried out many times by researchers and large datasets of their results are available. However, exercises based on these are challenging to create and limited by the available data.

To facilitate beginning undergraduate students' exploration of the effects of amino acid sequence on protein structure in a classroom situation, we have developed a simulation of protein folding. As with any simulation, it must contain elements of the real world to be pedagogically valuable; however, it must also include elements that are unrealistic but necessary in order for it to be practically useful. We have developed a freely-available open-source simulation of protein folding, the protein investigator (PI); (Software, lab manuals, and source code

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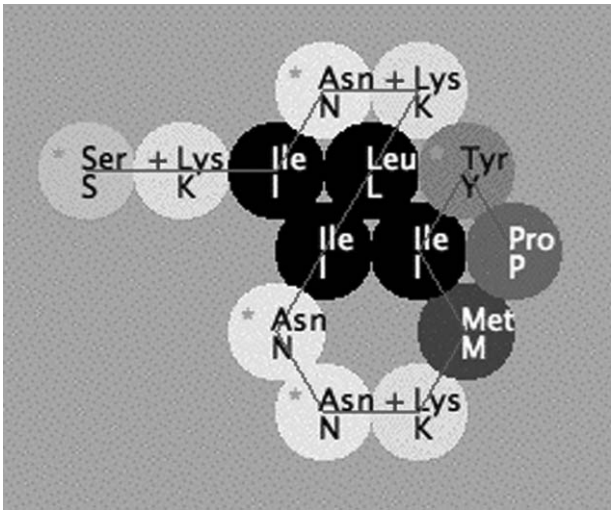

FIG. 1. **Sample protein folded using the protein investigator simulation.** The protein is folded on a two-dimensional hexagonal lattice. Darker shaded amino acids are more hydrophobic; *'s indicate hydrogen bond capability; + and − indicate charge; the thin line connecting the amino acids indicates the polypeptide backbone.

are available at http://intro.bio.umb.edu/PI/) [7], which folds proteins on a two-dimensional hexagonal lattice using forces that are discussed in most introductory biology courses: ionic bonds, hydrogen bonds, disulfide bonds, and hydrophobic interactions. Using this program, a student types in an amino acid sequence of a short polypeptide (typically not more than 20 amino acids), clicks the "Fold" button, and the two-dimensional structure is determined using a simple energy minimization algorithm in about 0.5 seconds [7]. A sample folded protein is shown in Fig. 1.

This rapid response allows the students to explore the role of amino acid sequence in protein structure within the time, material, and equipment constraints of a typical undergraduate teaching lab. The range of sequences, folded shapes, and mutations is only limited by the student's imagination and the speed of the folding algorithm.

Others have developed interactive simulations of protein folding that show the results of student-generated mutations; one example is the molecular workbench [8] created by the Concord Consortium. In this simulation, amino acids are shown as circles of differing sizes on a two-dimensional field. The motions of the amino acids are shown as the simulated protein folds based on the hydrophobic effect. Students can alter individual amino acids and observe the effects on the structure of the resulting protein [9].

Even with their inherent simplifications, research suggests that simulations can be particularly productive educational tools. For example, de Jong [10] states that, "Computer simulations enhance inquiry-based learning—in which students actively discover information—by allowing scientific discovery within a realistic setting." (p. 532). In their 1998 review of research on use of simulations for scientific discovery learning, de Jong and van Joolingen [11] conclude that, when used in a context that supports inquiry learning, simulations can promote deeper understanding of concepts than other teaching methods. In a study that parallels the differences between our uses of visualization and simulation, Windschitl and Andre [12] found that using simulations in a 'constructivist learning situation'—where college students devised and tested hypotheses with a simulation—led to improved content learning about the cardiovascular system when compared to using the simulation as simply a demonstration.

Preliminary evaluation of the PI has shown that students enjoy using the software, and they can be observed applying their understanding of protein structure when using it [13]. Although the students enjoyed using the PI and many preferred it to lab exercises using visualization, a more rigorous comparison of the two approaches was clearly required; this is the goal of the studies described in this article. Specifically, we compared the impact of using the PI simulation to that of using visualization (RasMol or Jmol) on students' understanding of protein structure using an open-ended survey [6]. Next, we explored the protein-related vocabulary, students used when working with the two programs. We then used a multiple-choice survey to assess the extent to which the simulation left students with the misconception that proteins are two-dimensional. Finally, we asked students to compare the simulation with the visualization: which they enjoyed using more; and which they felt had taught them more about protein structure.

SUBJECTS AND METHODS

Our results are based on data collected from four different studies of students enrolled in General Biology I (Bio 111) at the University of Massachusetts, Boston. UMass Boston is a large public university located in an urban area; General Biology I is the first semester course for Biology majors. It covers introductory Genetics, Cell Biology, Biochemistry, and Molecular Biology; its typical enrollment varies from 250 to 350 students. It is taught as three 50-minute lectures and one three-hour lab per week; the lectures are given by BW, and the labs are taught by graduate teaching assistants (TAs). The TAs are provided with an extensive set of lesson plans for the lab sessions [14].

The first study was conducted in 2000 and compared students' ($n = 161$; 67% of the students enrolled in the class) performance on an open-response survey targeting key concepts in protein structure. The survey was administered before and after a RasMol-based protein visualization lecture and lab sequence. The survey and scoring methods have been described previously [6]. Briefly, the survey consists of two questions; first, how two proteins, collagen and albumin, can be made of the same material but have different shapes; and, second, how a protein can be present but nonfunctional. A student's two answers are scored as correct/incorrect and further scored for their use of key ideas in protein structure (genetics, chemical interactions, protein structure, amino acid sequence) in clear and reasonable statements, for the presence of key misconceptions (teleol-

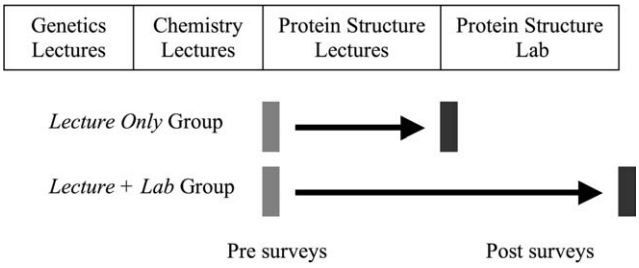

FIG. 2. **Survey administration timeline.**

ogy), or as miscellaneous uncategorizable responses. All surveys were scored blind and independently by two reviewers; the scorers showed better than 90% agreement. The scores on the two questions were then combined into an overall numerical score by awarding one point for each correct answer (max of 2) and 0.5 points for each key idea included in either answer (max of 2) yielding scores that ranged from 0 to 4.

In the 2000 study, we used a modified prepost survey administration design that allows measurement of the effect on students' learning of the lab portion of the class. In this design, shown in Fig. 2, all students fill out a presurvey as a take-home exercise following lectures on genetics and basic chemistry. Lectures on protein structure using three-dimensional CHIME or Jmol-based visualization follow. Then, half of the lab sections (the Lecture Only group) complete the post survey in lab at the beginning of the target lab and the other half (the Lecture + Lab group) complete the post survey in lab at the end of the target lab. Only students from whom we collected both a pre- and a post-survey were included in the analysis. By comparing the learning gains of the two groups, it is possible to assess the effect of the lab activities themselves. These data were published previously [6]; in this study, we present these data again, this time analyzed using the summary score described above for comparison with our new data.

The next study was conducted in 2006 and compared students' use of protein-related terminology when using simulation or visualization. Here, we videotaped groups of two to three students, two groups using Jmol and two groups using PI, and transcribed their conversations during the lab. We then counted the number of utterances that referred to key concepts in protein structure as defined previously [6]. Although discussion of specific atoms had not been part of the previous study, we noticed that atoms were frequently mentioned by name in the transcripts. As they are relevant to the protein's structure, we therefore also counted the number of times students mentioned particular atoms by name. All transcripts were scored independently by two reviewers who then reached a consensus scoring that was used in our analyses.

The third study was conducted in 2007 and used the same survey, scoring, and administration scheme as the 2000 study. Here, we compared students' ($n = 316$; 86% of the students enrolled in the class) understanding of protein structure before and after visualization in lecture and use of PI in lab. All surveys were scored independently by two investigators; their agreement was 95%. In addition, this survey contained a multiple-choice question designed to assess the extent to which PI left students with the misconception that proteins are two-dimensional. This question asked which of the images in Fig. 4 was the most realistic representation of the structure of a small protein. This question was administered on the presurvey, both post surveys, and to all students as a question on the final exam. Only students for whom we were able to collect a presurvey, a postsurvey, and a final exam were included in our analyzes.

The final study was conducted in 2008, where the protein structure lab exercises used both simulation (PI) and visualization (Jmol). Students ($n = 276$; 84% of students enrolled) were asked to report which of the two activities they enjoyed using more and which they felt that they had taught them more about protein structure on a 5-level scale on a survey administered at the end of the combined lab exercise.

Statistical analyses were performed using R version (2.9.0) and Microsoft Excel.

LAB EXERCISES

Visualization Activities with Jmol

In these lab exercises, groups of two or three students explored the three-dimensional structure of a sample protein—lysozyme—using the molecular visualization program RasMol or Jmol. They began with a detailed examination of the protein. Each group was assigned an 8-amino-acid segment of lysozyme; they then characterized their eight amino acids as hydrophobic or hydrophilic and determined their location within the protein (interior, exterior, or substrate binding pocket) as well as their secondary structure. The class then pooled their results and discussed how the secondary structure related to the overall structure, how the hydrophobic effect influences which amino acids were on the surface or interior of the protein, and how folding can result in amino acid that are very distant in the primary structure being in close proximity in the fully-folded structure. In the next series of exercises, they were given pairs or small groups of amino acids and asked to use information from the three-dimensional structure to propose plausible noncovalent interactions between them; they carried out a similar analysis of two enzyme-substrate interactions. In their lab reports, they describe their findings and design a hypothetical enzyme's binding site for a substrate of their choice.

Simulation Activities with PI

In these lab exercises, students built and modified two-dimensional hypothetical proteins using the PI. These exercises began by familiarizing students with the user interface and the forces that govern folding in the simulation; students predicted the structure of simple peptides, folded them, and then determined if the folded shape matched their prediction. The next task demonstrated the effects of mutations. Students explored the effects of mutations by building a protein and making

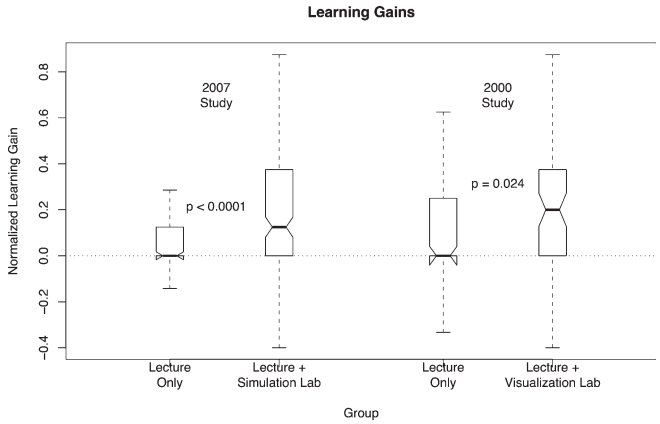

FIG. 3. **Normalized learning gains with visualization lectures followed by simulation or visualization labs.** In the 2007 study, students used simulation; in the 2000 study, visualization; lectures in both studies used visualization exclusively. Boxplots indicate medians with a thick horizontal line; the limits of the box indicate the interquartile range; the 'whiskers' indicate the extremes; the notches approximate the 95% confidence limits of the medians ([17] or see websites like [18]). Differences between the lecture only and lecture + Lab groups in each study are significant using the Wilcoxon rank sum test (*p* values shown on figure).

several mutant versions of it. Some mutations have little effect on the structure while others have a dramatic effect; students were asked to explain one mutation of each type. Students were then asked to demonstrate the roles of hydrogen and ionic bonds by building a protein that required each of these bonds for its structure and using mutation to show that these interactions were required. Finally, students were asked to design three different proteins, each matching a particular target shape. There was no lab report for this exercise.

The lab manual and other materials for both activities are available upon request from the first author.

RESULTS

Part I: Comparison of Learning Gains with Visualization and Simulation Lab Activities

Figure 3 compares the normalized learning gains ([Post–Pre]/[Max–Pre]; calculated for each student) for Lecture Only and Lecture + Lab groups from the 2000 study, where the lab used RasMol for visualization, and the 2007 study, where the lab used the PI for simulation. These data showed significant deviations from normality ($p << 0.05$ using a Shapiro-Welk test); as a result, we used a nonparametric test (Wilcoxon rank sum test) for comparing the different samples. In both cases, the Lecture + Lab group showed a significantly higher normalized learning gains than the Lecture Only group, indicating that both versions of the lab led to significantly increased performance on the survey. This conclusion was also found if we reanalyzed the data using non-normalized learning gains (Post–Pre) or a modification of the scoring scheme that gave more weight to correctly answering the more challenging second survey question (data not shown).

We then looked in detail at the particular vocabulary students used in their survey responses. The results of both the 2000 (Visualization lab) and 2007 (Simulation lab) studies were the same: on the presurveys, the most frequent response categories were "Misc"—noncategorizable nonbiological responses; following lecture and lab, the most popular category in both studies was "Chemical Interaction"—explanations that included ionic bonds, hydrogen bonds, hydrophobic effect, etc. The results of both analyses indicate that both versions of the lab, simulation and visualization, have similar learning effects as measured by this survey.

The speed and flexibility of the folding algorithm used by the PI depend on simplifying the folding to two-dimensions; thus, one important concern is that using the PI will leave students with the misconception that proteins are two-dimensional. Although Bio 111 contains many examples of three-dimensional proteins both in lecture and other lab exercises, it is possible that students would find the simplified two-dimensional representation used in PI more compelling than the correct three-dimensional representation. As part of the surveys in the 2007 study and the final exam, students were asked which of the three images in Fig. 4 was the most realistic representation of a small protein. Figure 4 shows the fraction of students choosing each of the representations at different time points. In these samples, 5–12% chose the incorrect PI-like representation; there are no significant differences among the samples. Thus, use of PI does not significantly increase the fraction of students choosing a clearly two-dimensional image of protein structure. This suggests that PI does not leave a significant number of students with the misconception that proteins are two-dimensional.

Part 2: Analysis of Videotape of Students Using Visualization and Simulation

As a preliminary examination of students' work during the two activities, we videotaped groups of students

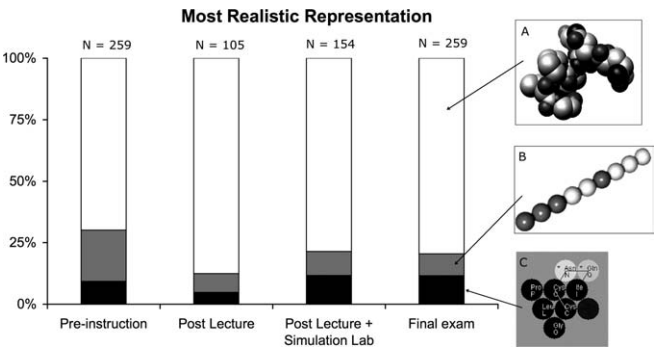

FIG. 4. **Students' selections of the most realistic representation of a small protein following visualization-based lectures and a simulation-based lab exercise.** Bars indicate fraction of students in each sample who chose each of the three representations (A = correct 3-dimensional; B = incorrect 3-dimensional; C = incorrect 2-dimensional). Differences in the fraction of students choosing C between successive samples were nonsignificant (χ^2 test).

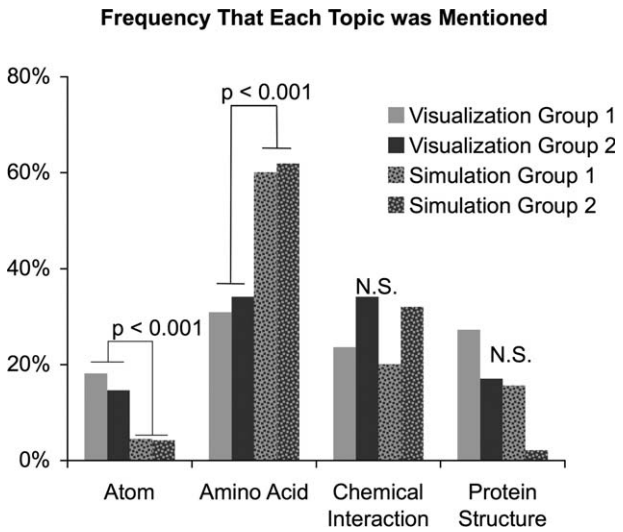

FIG. 5. **Frequency of Mention of Key Terms during Videotaped Lab Exercises.** Four groups of students were recorded as they completed either a simulation- or visualization-based lab activity. Bars indicate the percentage of relevant utterances that fell into each of the categories. Where shown, results from the two groups using the same software were pooled for significance testing (χ^2 test). N.S. = nonsignificant.

carrying out visualization or simulation labs. Figure 5 shows the fraction of relevant utterances in each of four major categories of protein-related terminology made by members of two groups of students ($N = 3, 2$) using Jmol for visualization and two groups of students ($N = 3, 3$) using PI as a simulation. When the frequencies for the two visualization groups were pooled, they showed some significant differences from the two pooled simulation groups. Visualization groups mentioned atoms significantly more often than simulation groups; this is not surprising as individual atoms are shown only in the visualization. Simulation groups mentioned individual amino acids more often than visualization groups; this is likely the result of the design tasks in the lab, which require students to enter many amino acid sequences and thus discuss particular amino acids by name. Interestingly, both groups mentioned chemical interactions at roughly equal frequencies; this is one of the core principles that both labs are designed to emphasize. The intergroup variation in the "protein structure" category was too high for a meaningful analysis. These results suggest that the students we observed were applying relevant knowledge of protein structure in a manner consistent with the different approaches of the two lab exercises.

Part 3: Student Opinions of Simulation and Visualization

In Fall 2008, students used PI and then Jmol in a single three-hour lab session. At the end of this session, they were asked to rate which part of the lab they "liked doing more" on the following scale: "Strongly preferred PI" (−2); "Somewhat preferred PI" (−1), "Preferred Both Equally" (0); "Somewhat preferred Jmol" (1); or "Strongly preferred Jmol" (2). Overall, students 'somewhat' preferred the simulation; this was statistically significant (Wilcoxon signed rank test; $p < 0.0001$; pseudo median $= -1.00$). In addition, they were asked which part of the lab "taught you more about protein structure" on a similar scale from "PI definitely taught me more" (−2) to "Jmol definitely taught me more" (+2), where 0 corresponds to "Both taught me equally." Overall, the students felt that the simulation taught them slightly more than the visualization; this effect was very small but statistically significant (Wilcoxon signed rank test; $p = 0.0027$; pseudo median $= -0.00006$).

DISCUSSION

Molecular visualization has been used to teach protein structure for nearly two decades and is widely-used, highly-recommended, and effective [1, 6, 15]. Simulated protein folding, where the students can enter and edit amino acid sequences for folding, is an alternative method for teaching this material. In our studies, we have shown that our simulation activity is as effective as our visualization activity for teaching basic principles of protein structure to beginning-level undergraduates as measured by our survey and videotape studies. When embedded in a curriculum that emphasizes the three-dimensional nature of proteins, the simulation does not appear to significantly increase the fraction of students with the misconception that proteins are two-dimensional hexagonal structures. Furthermore, students prefer to work with the simulation and feel that it teaches them more about protein structure.

In addition, the simulation affords several types of exploration that are either impossible or impractical when using visualization; many of these have been shown to be effective teaching methods. The first of these is that simulation allows students to practice inquiry. Using PI, students can develop and test hypotheses about interactions between amino acids and the role of these interactions in protein structure. Students explore the effects of mutations that they have chosen to develop an understanding of the different properties of the 20 amino acids and their role in the formation of a protein's structure. Previous work has shown that this type of exploration can be highly successful [11].

The second type of exploration facilitated by simulation is design. In our exercise, students design proteins to match particular target shapes—a task where they both learn the properties of the different amino acids and apply this knowledge in a concrete task. Roth, *et al*. [16] suggest that, "...the act of designing focuses student attention on doing something rather than knowing something, which changes the school learning context to a more natural condition that resembles learning situations outside schools, learning on a "need-to-know" basis (p. 27). Design involves learning along the way in the process of pursuing goals..." In our original study of PI [7], we found that students were using detailed knowledge of protein structure when carrying out the design portions of the lab. It is therefore likely that the combination of visualization and simulation will lead to deeper learning than visualization alone; further study is required to

explore this hypothesis. Finally, as real proteins have significantly more complex structures than those shown in the PI, these simulation exercises at the introductory level should only serve as a foundation for further, in depth, work with native 3-dimensional protein structures.

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