Assessment of Molecular Construction in Undergraduate Biochemistry

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Today's biochemistry students are well equipped with thick textbooks containing glossy illustrations. These textbooks are typically accompanied by a CD and a Web site containing animations and illustrations of biochemical procedures, processes, and structures. The graphical illustrations of macromolecular structures currently accessible via personal computers are particularly impressive, with a variety of structure-rendering software programs freely available.[1,2] Instructors have used these visualization programs in a variety of ways, from illustrating classroom lectures to guided homework or laboratory exercises to Web tutorials with structure and function themes. Most, if not all, instructors believe that the use of molecular visualization programs enhances student learning, although the literature contains few assessment studies to validate this belief.

The initial published assessments of molecular visualization in biochemistry have focused on surveys of students' attitudes *(1–3).* Weldon and Jones *(1)* examined student impressions of a required visualization project in two biochemistry courses using Kinemage visualizations from a course supplement. Weldon and Jones found that students believed that their understanding of the three-dimensional structure of molecules was greatly improved by this project. Weldon and Jones also found that 61% of the students enjoyed working on the computer project, but also thought it was too time consuming. Similar results were found by Sansom, Waller, and Geddes *(2),* who incorporated three visualization activities into a second-year biochemistry–biophysics course using commercial software from Insight.[3] Surveys indicated that students were well motivated, appeared to understand the content, and remembered material covered in the exercises. Students also indicated that they learned valuable skills during the exercises.

Software preferences were again examined by Weiner et al. *(3).* Student and instructor attitudes were investigated at five Georgia universities where computer-generated three-dimensional images of biological macromolecules that could be manipulated were used for lecture and demonstration purposes. RasMol[1] and Mage[2], both free software programs, were used for the visualizations. Responses to a student survey indicated that the illustrations were most helpful in learning about protein structure and function along with enzyme interactions with substrates, cofactors, and inhibitors. Most of the students surveyed believed that the illustrations helped them better understand biochemistry. Students also indicated a slight preference for the menu-driven program Mage. The instructors indicated that incorporating the molecular visualizations enhanced teaching and learning in their biochemistry courses.

In the first performance assessment of molecular visualization in teaching biochemistry, White et al. *(4)* used a two-question, open-ended test to separately evaluate students' learning of specific biochemical concepts in the general biology lecture and lab. Two lectures were devoted to protein structure using globins, which were followed by one laboratory session exploring the structure of lysozyme using Rasmol.

This study showed that lecture and lab effectively communicated several core concepts in protein biochemistry. In particular, they noted that use of visualization in lecture did not effectively communicate an understanding of the three-dimensional structure of proteins. Rather, the students needed to manipulate the representations themselves to gain a three-dimensional understanding of the molecule.

Science education research shows that student concept construction is a critical part of science learning. The purpose of the current study, therefore, was to build on White's study and investigate what biochemistry students learned from the process of actually constructing annotated graphic images of a biological macromolecule using experimentally solved coordinate sets from the protein data bank along with the related primary literature. The working hypothesis was that the deliberate construction of annotated molecular illustrations required the students to go through a more in-depth learning process than did simple viewing and manipulation of images. This construction, we feel, deeply engages the students' minds and promotes their concept construction. We report here the testing of this hypothesis using assessments of both students' attitudes and students' performances.

Methods

Subjects and Setting

The students involved in this study were junior- and senior-level biochemistry students, in the first semester of a two-semester biochemistry sequence, in nine classes at five universities in the eastern and southeastern U.S. (see Table 1).

Student attitudes were assessed by both quantitative and qualitative analysis methods as outlined in Table 2.

Quantitative data from the Kinemage Attitudinal Survey were collected during the last week of classes from all 235 students. Most classes were assigned as a whole to either treatment or control groups, except for Class 1, which was randomly divided prior to the first day of class into control and treatment groups. Qualitative data were collected from Class 1 as well as from Class 7, a large treatment group. The assessment of student learning, the Performance Assessment Final Exam, was administered only to Class 1.

Software

Students in this study used kinemages (plain-text files containing 3D coordinate information in a hierarchical display list), as well as annotations, color, and so forth. Kinemages are viewed with Mage, a free, open-source graphics program written by David Richardson at Duke University *(5, 6).* In our study the treatment students constructed a kinemage in the same way as the authors in refs *5* and *6,* although the students' experimental data came from the literature and the RCSB Protein Data Bank[4] rather than from original research.

A starting kinemage for a macromolecule such as a protein is generated with the menu-driven program Prekin from a protein data bank (PDB) coordinate file. Although there are some default scripts, the usual kinemage generation is a true "authoring" because the user is required to make informed decisions about what will be illustrated. Kinemage graphics are deliberately designed to exclude (or to turn off temporarily) most of the nonessential details of the structure and only show the desired features. In this way a kinemage serves as a 3-D communication tool. Further information about instructional uses of kinemages may be found in ref *7.*

A tutorial was designed to teach students in the treatment group how to use the software. This tutorial, sample kinemages, all necessary software, a grading rubric, and helpful hints for instructors and students are available at the Kinemage Authorship Project Home Page at *http://orca.st.usm.edu/~rbateman/kinemage.* A brief description of the construction project itself has been published in this *Journal (8).*

Treatment and Control Groups

Students in both the treatment and control groups visualized interactive molecular images during the lecture by the instructor. Both groups completed several homework assignments that required the manipulation of kinemages obtained as a textbook supplement. These manipulations included such things as rotation and zooming to locate specific structural features, measurements of distances and bond angles, and animation between structural conformations. Treatment group participants were additionally assigned a semester-long project requiring them to create their own annotated images using the free software programs Mage and Prekin. These student-constructed kinemages told a molecular story with primary literature information in the text window and a designed set of views illustrating characteristics of the chosen macromolecular topic. Students were required to use at least one animation and a variety of renderings in their illustrations, with a minimum of three annotated graphic images.

The control groups did visualize interactive molecular images, although they did not construct kinemages. In the one class with both control and treatment group students (the "divided" class), the control group was assigned a term paper in the form of a review article on a structure-based molecular topic that paralleled the research project required of the treatment group. This control group, along with the treatment group in the same class, was used for the performance analysis statistics. In all cases, the fundamental difference between the treatment and control groups was the actual construction of a molecular illustration by the treatment group.

Assessment Methods

Quantitative information was collected from the Kinemage Attitudinal Survey and the Performance Assessment Final Exam. The survey consisted of six demographic questions and twenty-three questions grouped into four general

Table 1. Student Populations Participating in Project

Class Number	Control Group	Treatment Group	Total Participants
1	16	21	37
2	0	6	6
3	20	0	20
4	10	0	10
5	0	17	17
6	27	0	27
7	0	52	52
8	0	37	37
9	0	29	29
Total	73	162	235

Table 2. Assessment Methods and Instruments

Item Assessed	Method Type	Instrument Used	Classes Participating
Attitude	Qualitative	Interview	1, 7
Attitude	Quantitative	Survey	7
Performance	Quantitative	Examination	1

question topics:

- Did molecular construction provide motivation to learn biochemistry?
- Was the authorship project a worthwhile time investment?
- Did students anticipate future benefits from participating in the project?
- Did authorship affect students' perceptions about their understanding of protein structure and the nature of the active site?

The quantitative data from the Kinemage Attitudinal Survey were analyzed using multiple analysis of variance (MANOVA) techniques for all 235 participants. Survey items were associated with the topics by design. Item responses on the survey proved reliable in a pilot study with a Cronbach's α value of 0.9664 *(9).*

Table 3. Summary of Qualitative Assessment Methods

Treament Groups	Assessment Method	Analysis Type	Purpose of Method
Class 1 (*n* = 5)	In-depth interviews	Formative (during semester)	Identify problems and a source for further analysis
Class 7 (*n* = 52)	Web-form questionnaire	Formative	Identify problems and a source for further analysis
Class 7 (*n* = 7)	In-depth interviews	Formative and summative	Project evaluation

Table 4. Kinemage Attitude Survey MANOVA Results[a]

Attitude Topics	Survey Questions[b]	*F* Values (*n* = 235)	*p* Values (α)
Value in learning biochemistry	8, 13, 27, 29	56.2	< 0.001
Learning secondary structure	9, 15, 21, 26	107.7	< 0.001
Learning tertiary structure	10, 22, 28	80.0	< 0.001
Learning quaternary structure	24, 30	66.0	< 0.001
Learning about the active site	17, 20	76.0	< 0.001
Future career benefits	12, 16, 25	2.64	0.106
Motivation to learn biochemistry	7, 14, 19	0.911	0.341
Worthwhile time investment	11, 18, 23	2.55	0.111

[a]See Figure 1 for a summary illustration. [b]Download the survey instrument from *http://orca.st.usm.edu/~rbateman/kinemage/survey.pdf.*

The Performance Assessment Final Exam was administered to the students in Class 1. This written exam incorporated a kinemage half that required the manipulation of the images to answer certain questions. This half of the exam was given in a proctored computer laboratory and consisted of a series of three kinemages (a protein–DNA complex, a piece of secondary structure, and an enzyme–inhibitor complex) that were generated by the instructor immediately prior to the exam. Students had to answer questions that required both an understanding of biochemical concepts (e.g., metal coordinating ligands, inhibitor binding, etc) and application of these concepts in the exploration of an unfamiliar structure (e.g., β-sheet topology, new disulfide bond insertion). The questions required only use of Mage, which was familiar to both treatment and control groups.

Qualitative data were collected from Classes 1 and 7 using two methods: a Web-form questionnaire, and in-depth interviews. Table 3 summarizes these data.

The open-ended questions on the Web-form questionnaire closely paralleled the Kinemage Attitude Survey and were designed to probe student attitudes and expectations as well as uncover problem areas. For example, students were asked whether the authorship project affected how and what they learned in the course, about their experience and comfort level with computers, and about the current and potential stumbling blocks in their project.

The in-depth interviews were conducted with five treatment-group students in Class 1 and seven treatment-group students in Class 7. Student participants were interviewed several times during the semester, including once before any molecular construction, once during the molecular construction project, and once near the end of the molecular construction project.

Results

Quantitative Data

A summary of the quantitative data concerning student attitudes—collected with the Kinemage Attitudinal Survey—is displayed in Figure 1. More detailed statistics regarding these data are given Table 4. This instrument used a Likert scale where 1 represented the statement "strongly disagree" and 5 represented the statement "strongly agree". The demographic data showed no difference between the treatment and control samples. In both of the groups, most of the students were under 25, female, and senior chemistry majors.

The "value in learning" category was a group of questions that asked students their opinions about how valuable their exposure to kinemages was for learning biochemistry. Students who constructed their own images rated the value of kinemages in learning significantly higher than those who just visualized and manipulated preconstructed images.

Specific topics within protein structure were also examined. Students in the treatment group rated the value of their exposure significantly higher for learning about secondary, tertiary, and quaternary protein structure as well as learning about the nature of a protein's active site.

Standard deviation values were much higher for the treatment group than they were for the control group for all the variables about learning. The qualitative analysis suggests that these large deviations reflect large differences in the way students felt about their experience, and are related to different levels of computer literacy among the students.

In two large treatment classes (8 and 9) kinemages were constructed by students in small, unstructured teams (optimally 2–3 individuals), rather than as individual projects. Figure 2 shows the treatment category from Figure 1 broken out into the students that worked in small teams (left) and as individuals (middle). The students working in teams scored significantly higher in all of the learning categories than students working individually. More data will be required to determine the proper structuring of such collaborative teams.

Qualitative Data

The qualitative analysis revealed both positive and negative aspects of the project. Fortunately, most of the negative findings were regarding how the kinemage project assignment was implemented, and easily correctable. The positive findings dealt more with the inherent, fundamental aspects of authoring as well as student interest and what students felt they learned from this process. Consistent with the results of the attitudinal survey, students generally felt the project helped them understand the various levels of protein structure as well as the structure–function relationship.

A common complaint by student authors was that too much time was spent learning the software, taking time away from studying biochemistry. As one student commented: “It scares you to death at first. It seems overwhelming.” This same student, after completion of the first rough draft, changed her mind stating, “It’s easier than you think, once you learn enough about the programs.” This comment reflected our observation that most students needed more classroom time or structured help getting started with the programs before they felt comfortable working independently on their kinemage project assignments. Many students suggested smaller developmental assignments for the purpose of learning the software.

Performance Assessment

The performance assessment was an examination via kinemage that was administered to the randomly divided class, Class 1, during the fall of 2000. Out of a 50-point total on the kinemage half of the exam, the treatment group scored an average of 29.0 points (SD 8.6; $n = 21$). The control group scored an essentially identical score of 30.3 points (SD 9.8; $n = 18$). Overall final exam scores for the treatment and control groups were 58.2 ± 13.8 and 64.5 ± 16.2, respectively. Similarly, there were no significant differences between the two groups on homework or previous examination scores.

Discussion

Most biochemists use molecular graphics to some extent in their teaching to illustrate fundamental structural concepts and structure–function relationships that are not easily learned via other means. Graphics use in education is a recent phenomenon; we are only now beginning to examine the most effective ways to use this rich, interactive, instructional aid. This study was a first attempt to assess the effectiveness of a semester-long, graphics-based molecular construction project involving the primary literature, electronic databases, and graphics software. It was the students themselves that literally found the pieces of the molecular puzzle and put them together in a way that made a coherent and informative story. The project required considerable work outside of class. While some students complained about this, most took considerable pride in their finished product. We note at this point that, while we used kinemages, any molecular visualization software that allows scripting can be used in similar projects.

As seen in Figure 1, the survey data clearly showed a positive effect on student attitudes about learning various aspects of protein structure, especially higher-order structural concepts such as tertiary and quaternary structure, conforma-

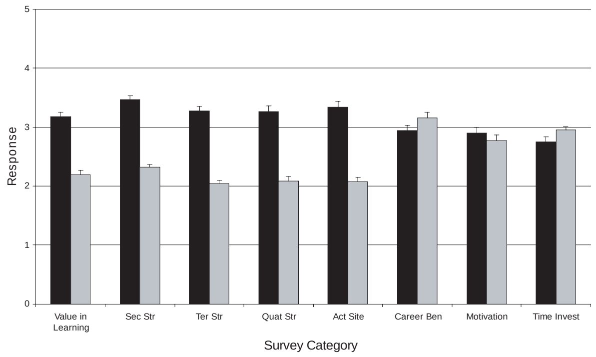

Figure 1. Results of Kinemage attitudinal survey. Columns represent Likert score mean values plus standard error of the mean for treatment (black bars) and control (gray bars) groups. Each treatment–control pair represents the average of 2–4 related questions on the survey. From left to right, pairs are: 1, Value in learning biochemistry; 2, Learning about secondary structure; 3, Tertiary structure; 4, Quaternary structure; 5, Active site; 6, Future career benefits; 7, Motivation to learn biochemistry; 8, Time investment. Topics 1–5 show a clearly significant difference in favor of the treatment group; topics 6–8 show no significant difference.

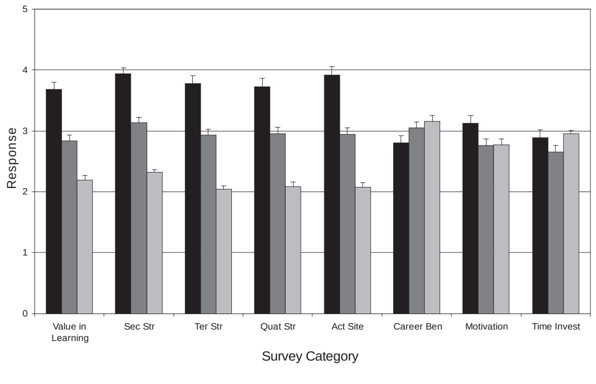

Figure 2. Comparison of attitudes towards team versus individual projects. Categories and data are the same as in Figure 1 except that the composite treatment group from Figure 1 is broken out into individual student construction projects (middle column, classes 1, 2, 5, and 7 in Table 1) and team construction projects (left column, classes 8 and 9 in Table 1). The right column (solid bar) is the same control group as in Figure 1. Topics 1–5 show a clearly significant difference between treatment groups in favor of the team projects.

tional changes, and structure–function relationships. Another positive aspect of this construction project that came to light in formal interviews is that it helped the students make a better connection between the literature and the three dimensional image. Many of the stronger students liked the project because it was open-ended enough to allow them to explore and construct at the level of their own ability. The project also seemed to appeal to students with different learning styles, particularly visual learners and students who favor manipulation and construction. Most students enjoyed the project once they got started, perhaps because the images are colorful and interactive as well as informative. Even when projects were individual assignments, students were encouraged to talk to each other and help each other with software questions. At one school where the project was done in sequential years, there was some evidence of carryover from one year to the next: student "experts" from the previous year could help the next class with the project.

As mentioned in the Results section, a number of difficulties came to light in the course of the project and in the formal interviews. Although these were mainly procedural or technical problems, the major difficulty that surfaced in both the survey and interviews was the time commitment required of the students. Students did not quantify this time commitment; therefore, it is not clear that the project took significantly more time than a well-done term paper. Also, students failed to differentiate between the time spent on literature search, retrieval, and analysis, and the time spent for the actual kinemage construction. Because the construction project builds over the course of the semester, it is very unforgiving of procrastination.

A few words of advice are appropriate for instructors planning to use construction of kinemages or any other annotated molecular graphics in their classes. The instructor must know the software well and be willing to help students. Mage can be used with no difficulty because it can utilize pre-made kinemages, but it is important to get students started on Prekin as early as possible, because it requires the user to make decisions on what information to select from a PDB file. We suggest that instructors recruit individuals who have previously completed the project to act as tutors to help students learn the unfamiliar software.

Our initial attempt at a kinemage-based performance assessment as a portion of the final exam in a randomly divided class showed no difference in performance between kinemage authors (the treatment group) and kinemage users (the control group). This may be because the control group wrote a term paper on the same type of structure topics as the kinemage authors, so they received heavy exposure to the same topics. In our opinion, however, a true, valid, performance assessment of molecular three-dimensional literacy measures something different and less content-specific than standard topics in a biochemistry exam, and therefore presents a challenge and opportunity for instructors to design.

In summary, this study set out to examine the question of whether construction of three-dimensional molecular illustrations was a more effective learning tool than student use of pre-made images. Student authors clearly viewed construction in a more favorable light than did the control group (Table 4). In our opinion, this was due both to the active learning process as well as the fact that the graphics project was implemented over the course of the semester in an extensive scaffold of primary literature, electronic databases, textbook graphic supplements, and appropriate course content. Effective learning with molecular visualization, therefore, requires effort on the part of the students and clear connections with other course content.

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Notes

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