BiochemAR: An Augmented Reality Educational Tool for Teaching Macromolecular Structure and Function
Rou-Jia Sung, Andrew T. Wilson, Stanley M. Lo, Logan M. Crowl, Joseph Nardi, Katie St. Clair, and Jane M. Liu

ABSTRACT: Using 2-dimensional (2D) images in order to teach about 3-dimensional (3D) molecules can limit the ability of students to grasp key visuospatial elements of 3D macromolecular structures, including depth perception and a sense of scale. The lack of simple, accessible, and easy-to-use teaching tools for visualizing and interacting with (i.e., rotating, translating, zooming) 3D virtual representations of macromolecular structure also continues to be a limitation for biochemistry instructors interested in incorporating more 3D visualizations in their classrooms. To address this current gap in available instructional tools, a novel app utilizing augmented reality (AR) technology to visualize macromolecular structures is described. The app allows users to easily visualize and manipulate the 3D structure of the potassium channel and is accompanied by a set of curricular materials to facilitate app implementation during a discussion on membrane transport. The app is free to download on both iOS and Android systems, is suitable for use on mobile and tablet systems, and is aimed for target audiences ranging from introductory to advanced undergraduate levels.

KEYWORDS: Second-Year Undergraduate, Upper-Division Undergraduate, Biochemistry, Computer-Based Learning, Hands-On Learning/Manipulatives, Multimedia-Based Learning, Molecular Properties/Structure, Molecular Recognition, Noncovalent Interactions, Proteins/Peptides

INTRODUCTION
A core concept in biochemistry is understanding the relationship between macromolecular structure and function. The ability to visualize the complex 3D arrangement of amino acids or nucleotides in space to form a functional biological macromolecule is critical for facilitating student learning of this concept. In addition to simply viewing the visualization, the ability of students to learn about complex 3D objects is also closely tied to their ability to directly manipulate and to “control” the visualization itself. Currently, multiple software packages (such as PyMOL, Chimera, J Mol, Rasmol, VMD, etc.) exist that create a digital environment for users to open and manipulate (rotate, translate, and zoom) a virtual 3D representation of experimentally determined macromolecular structures. The pedagogical benefits of these programs are highlighted by demonstrated gains in visuospatial learning and retention following the incorporation of computer models in class. These gains often require the instructor to set aside significant class time (either in lecture or lab, if that is an option) in order for students to gain proficiency in and practice actually using these programs. These programs also vary widely with regards to hardware requirements, the degree of control over the types of representations that can be seen/manipulated, and ease of use (Table 1). For programs that require a computer, logistical and equity issues can arise in trying to ensure all students have access to the necessary technical resources to use the program (either at the student or institutional level). Not all students may have access to a personal laptop; in order to take advantage of institutionally provided computer lab spaces, the instructor must typically first obtain permission from and coordinate with Information Technology Services at their institution for installation of specialized software programs in academic computer lab spaces, and program installation is also usually limited to 1 or 2 computer laboratories. Many of the successful pedagogical interventions utilizing these programs involve instructor...
guidance in real time when students are beginning to learn how to use the software; however, most computer lab spaces simply cannot accommodate an entire lecture class in one sitting (for example, the largest computer lab space at the University of Minnesota, Minneapolis houses 64 terminals for an average biochemistry class of 86 students; the largest computer lab space at Carleton College has 26 terminals for an average biochemistry class of 43 students), limiting application of these interventions using institutional resources to courses that can have smaller sections (such as lab sections) that can fit in these spaces. From the student perspective, limited operating hours for computer lab spaces can also be an additional barrier for resource accessibility, particularly for student schedules constrained with commitments to student employment hours, athletics practices, and/or other extracurricular activities. Consequently, even with the potential of the existing technology, accessibility to that technology can keep students and instructors from building understanding of and engagement with macromolecular structure and function.

Augmented reality (AR) technology has significant potential as a simple tool that can be easily used by students and instructors of all levels to visualize and interact with 3D macromolecular structures in a time-efficient manner. As shown in Figure 1, AR is an interactive system in which computer-generated virtual 3D objects are integrated and superimposed onto the real environment. The interactive nature of the system lies in the ability of users to interact with the virtual 3D object by directly manipulating their point of view (translate, rotate, zoom) around the object in the real world. As indicated in Table 1, these manipulations in the AR system require significantly simpler “commands” compared to current software systems.

Moreover, the minimal hardware necessary is a video camera and a display screen, technologies that are embedded in smartphones and/or tablet computers, two tools that are much more tractable for the larger class sizes found in STEM classrooms (Figure 1, steps 3–4).

In practice, this means that students and instructors can readily utilize the AR system to display 3D macromolecular structures directly in classrooms using either personal smartphones or tablet devices, eliminating the need for computer lab time/space or personal laptops. Multiple users can observe the same virtual 3D object using a single, shared screen display, a useful feature of the system for facilitating peer-to-peer interactions within a classroom environment. The increasing availability of AR-based teaching tools (such as Augmented Chemistry and Augmented Reality Metabolic Pathways (ARMET)) underscores the potential for AR technology to engage students with visually demanding content. The goal of this project was to design a simple, easy-to-use AR-based teaching tool and assess the usability of this tool for novice users to begin easily engaging with 3D structures and/or incorporate more examples of 3D structures into their instructional materials. Given the previously demonstrated pedagogical gains in visuospatial learning and retention associated with student use of virtual 3D models, this project aims to supplement the current software toolbox for molecular visualization by providing an additional option for the incorporation of virtual 3D models into the classroom. Herein, the development and implementation of BiochemAR is described, a novel AR application with a learning module that uses the structure of the KscA potassium channel (PDB ID: 1BL8) to illustrate the role of amino acid functional groups and protein secondary structure in facilitating ion transport across a lipid bilayer channel.

### COURSE CONTEXT AND ACTIVITY DESIGN

BiochemAR was implemented into an upper-level undergraduate biochemistry course in Fall 2017 (47 enrolled) and Fall 2018 (32 enrolled), with approximately equal numbers of female and male students in both years. The course curriculum is covered in 33 lecture hours and includes the chemical and thermodynamic basis for macromolecular structure and function, binding and reaction equilibria, membrane transport, signal transduction, and metabolism. Course prerequisites are one term of general chemistry, two terms of organic chemistry, and two terms of introductory biology. Two weeks prior to

![Figure 1. How an Augmented Reality experience works. Technical setup for an AR system involves (1) designing the virtual 3D object, (2) association of the virtual 3D object with an image marker in the real world, (3) recognition of the image marker by a camera on mobile or tablet device, and (4) superposition of virtual 3D object onto the real world environment by app and display onto screen. Step 1 is done by application developer; steps 2–4 are done by user.](https://doi.org/10.1021/acs.jchemed.8b00691)

<table>
<thead>
<tr>
<th>Software</th>
<th>Rotate</th>
<th>Translate</th>
<th>Zoom</th>
</tr>
</thead>
<tbody>
<tr>
<td>PyMOL, UCSF</td>
<td>Left mouse button, drag mouse</td>
<td>Middle mouse button, drag mouse</td>
<td>Right mouse button, drag mouse up/down</td>
</tr>
<tr>
<td>Chimera</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>VMD</td>
<td>Set “Mouse” to “Rotation” mode using main menu</td>
<td>Set “Mouse” to “Translate” mode using main menu</td>
<td>Set “Mouse” to “Scale” mode using main menu</td>
</tr>
<tr>
<td>X,Y: Left mouse button, drag</td>
<td>Left mouse button, drag</td>
<td>Left mouse button, drag</td>
<td></td>
</tr>
<tr>
<td>Z: Right mouse button, drag</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Jmol</td>
<td>X,Y: Left mouse button, drag mouse up/down</td>
<td>Shift key, double click left mouse button, drag mouse</td>
<td>Shift key, Left mouse button, drag mouse up/down</td>
</tr>
<tr>
<td>Z: Left mouse button, drag mouse left/right</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BiochemAR</td>
<td>Physical rotation of screen or image marker</td>
<td>Physical translation of screen or image marker</td>
<td>Physically bringing the screen closer to the image marker</td>
</tr>
</tbody>
</table>

Table 1. Commands To Manipulate Virtual 3D Objects
using BiochemAR, students used PyMOL for a full class period (70 min) to manipulate virtual 3D models of protein−nucleic acid complexes during the nucleic acids unit. For the majority of the students in the course, this was their first exposure to using molecular visualization software.

The two class periods prior to using BiochemAR were focused on lipids, membranes, and mechanisms of membrane transport. This provided the necessary foundational knowledge for the primary literature discussion topic (see below).

**Topic Selection**

A discussion of how transmembrane macromolecules mediate solute transport across the membrane can provide valuable opportunities for students to apply and integrate a wide knowledge base ranging from thermodynamics, binding equilibria, amino acid chemistry, and the role of conformational changes in regulating protein function. Thus, the KscA channel structure was deliberately selected as a seminal, illustrative example of fundamental concepts in membrane transport that could also be broadly applicable for a wide range of course content. Specifically, the following two learning objectives were addressed in the module: (1) Explain the thermodynamic parameters that accompany transport of solutes across a lipid bilayer, and (2) describe the molecular mechanism underlying ion selectivity in the potassium channel.

**Learning Module Design**

The learning module includes the (1) learning objectives, (2) a virtual 3D object associated with each learning objective, and (3) guiding questions to facilitate student interpretation of the

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**Table 2. Learning Module Design**

<table>
<thead>
<tr>
<th>Learning objectives</th>
<th>AR visual</th>
<th>Guiding question(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explain the thermodynamic parameters that accompany transport of solutes across a lipid bilayer.</td>
<td>Full potassium channel structure shown in cartoon representation (gray), accompanied by K⁺ ions shown as spheres (pink), as well as pore lining alpha helices (blue). Amino acids lining central cavity shown as yellow sticks (see right).</td>
<td>Q1: Would you expect the following molecules or molecular structures to stabilize or destabilize a K⁺ ion in the center of a membrane bilayer? Briefly explain your answers. a. C-terminus of an alpha helix b. N-terminus of an alpha helix c. Water molecules d. Hydrophobic molecules e. Basic amino acid side chains (like Lys, Arg) f. Acidic amino acid side chains (like Glu, Asp)</td>
</tr>
<tr>
<td>Describe the molecular mechanism underlying ion selectivity in the potassium channel.</td>
<td>A zoomed in view of the selectivity filter (shown below), in which the pore helices are shown as cartoon (gray), and the selectivity filter is highlighted as yellow sticks. K⁺ ions are still present (pink spheres).</td>
<td>Q3: After leaving the cavity, the internal pore constricts such that any ion entering the selectivity filter must completely dehydrate. a. The selectivity filter is lined, on all 4 sides, with the conserved sequence TVGYG. Would you expect that the side chains of these amino acids could help compensate for ion dehydration? How about the main chain atoms? b. The selectivity filter is structured such that the TVGYG motifs form a rigid cage with a defined diameter in the middle. Put together a model for why the K⁺ channel cannot accommodate Na⁺ ions.</td>
</tr>
</tbody>
</table>

**Figure 2.** Screen captures of views through BiochemAR app. (A) Main page of app showing “Potassium channel” learning module. (B) User view after opening (via tapping) “Potassium channel” button from main page shows app interface without image marker, showing real world (desk and wall) and touch-sensitive "buttons" (top right corner) that can be used to switch between different virtual objects. (C) View of the virtual 3D object associated with “Model 1” button. (D) View of virtual 3D object associated with “Model 2” button. Vuforia logo is blacked out.
structure (Table 2; questions numbered as on in-class worksheet, see Supporting Information). The guiding questions were integrated within a longer in-class worksheet (with additional questions). The colors and representations for each virtual 3D object were deliberately chosen by the application developers (using PyMOL) to highlight key features of the channel.27

Using BiochemAR

BiochemAR is freely available for both Android and iOS operating systems.28,29 Navigation through the app is based on simple on-screen touch-sensitive buttons. Upon starting the app, users can begin the activity by selecting the "Potassium channel" button from the main screen (Figure 2A). As shown in Figure 2, the virtual 3D object associated with the first learning objective appears when the "Model 1" button is tapped, while the virtual 3D object associated with the second learning objective appears when the "Model 2" button is tapped. To simplify the user experience, both virtual 3D objects were associated with one QR code.

Table 3. Sample Student Responses Pre/Post Using BiochemAR

<table>
<thead>
<tr>
<th>Guiding Question</th>
<th>Original Response Prior to Using BiochemAR Model</th>
<th>Additional Revisions to Response after Using BiochemAR Model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Q1 (Would you expect the following molecules to stabilize or destabilize a K⁺ ion...)</td>
<td>Group 1, FA17: “C-terminus of α helix stabilize because of the polarity of the carbonyl group. N-terminus destabilize because of the repulsion between the charge of NH₁ and K⁺.”</td>
<td>Group 1, FA17: “The alpha helices are pointing from the N-terminus/towards the extracellular to the C-terminus (inside the center of the membrane) such that the C-terminus are pointing towards K⁺.”</td>
</tr>
<tr>
<td>Q2 (After leaving the cavity, the internal pore constricts such that any ion entering the selectivity filter...)</td>
<td>Group 2, FA18: “C-terminus would stabilize because the negative charge at the carboxy group in the end would stabilize the positive charge.”</td>
<td>Group 2, FA18: “Holes are oriented with their negative end point to the cavity.”</td>
</tr>
<tr>
<td>Q3 (Would you expect the side chains of these amino acids to compensate for the ion dehydration because we expect them to part of the larger structure of the channel?)</td>
<td>Group 3, FA17: “The side chains are not particularly polar or charged, so will not have a large effect on the stabilization. The main chain atoms, however, have partial negative charges, which allows them to form favorable charge–charge interactions and stabilize the dehydrated K⁺ ion.”</td>
<td>Group 3, FA17: “The side chains are oriented away from the K⁺ ions and the oxygen atoms in the backbone are oriented towards the K⁺.”</td>
</tr>
<tr>
<td>Q4 (We don't expect the side chains of these amino acids to compensate for the ion dehydration because we expect them to part of the larger structure of the channel)</td>
<td>Group 4, FA18: “We saw how the bulky tyrosines were faced away from the ion, positioning the carbonyl grooves closer to the ion.”</td>
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</tr>
</tbody>
</table>

Supporting Information

Q3 (After leaving the cavity, the internal pore constricts such that any ion entering the selectivity filter...)

The side chains are oriented away from the K⁺ ions and the oxygen atoms in the backbone are oriented towards the K⁺.

Q4 (We don't expect the side chains of these amino acids to compensate for the ion dehydration because we expect them to part of the larger structure of the channel)

We saw how the bulky tyrosines were faced away from the ion, positioning the carbonyl grooves closer to the ion.

Activity Impact and Student Feedback

From both years of implementation, analysis of student responses to the follow-up questions on the in-class worksheet showed unanimous reporting that the use of BiochemAR was helpful. As indicated by the quotes below, students found the app helpful as a general visualization tool:

"The interactions and spacing made more clear."

"It shows the precision of the contact between K⁺ and carbonyl."
Table 4. Student Self-Efficacy

<table>
<thead>
<tr>
<th>Item (N = 27)</th>
<th>Pre</th>
<th>Post</th>
<th>Effect Size</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I am confident in my ability to imagine the 3D structures of proteins at the molecular scale.</td>
<td>3.89 ± 1.22</td>
<td>4.65 ± 0.85</td>
<td>0.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>I am confident with my knowledge of three-dimensional structures of biological molecules.</td>
<td>3.89 ± 1.15</td>
<td>4.59 ± 0.97</td>
<td>0.61</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>I am confident with my knowledge of factors that affect transport of nonpermeable solutes across a lipid bilayer.</td>
<td>4.41 ± 0.89</td>
<td>4.80 ± 0.74</td>
<td>0.44</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>I am confident with my understanding of the thermodynamic parameters involved in the transport of solutes across lipid bilayer.</td>
<td>4.30 ± 0.95</td>
<td>4.63 ± 0.88</td>
<td>0.35</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

Table 5. Usability of Molecular Visualization Software

<table>
<thead>
<tr>
<th>Item (N = 27)</th>
<th>PyMOL</th>
<th>Augmented Reality</th>
<th>Effect Size</th>
<th>p-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>I would use... again in the future.</td>
<td>3.92 ± 1.41</td>
<td>5.29 ± 0.75</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>It was easy to identify molecular interactions (intra or inter) using...</td>
<td>3.50 ± 1.29</td>
<td>4.75 ± 1.15</td>
<td>0.97</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>It was easy to learn to manipulate (rotate, translate, zoom) molecules using...</td>
<td>3.98 ± 1.40</td>
<td>5.04 ± 1.26</td>
<td>0.76</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

“Helped to visualize C-termini in the pore, and to get an understanding of the relative size of the ions and pore.” Students also found using the app helpful for verifying or clarifying their thinking to the guiding questions: “Helped reinforce our ideas, made it easier to understand structure of channel.” “It helped us visualize the structure and reinforced our conclusion that the C-terminus stabilizes the K⁺ ion in the central cavity.” “The displayed model helps change our thinking because we see the carbonyl oxygens interacting and stabilizing effects with the side chains facing out.”

In addition, students could choose to revise or include additional thoughts for their original answers to the guiding questions after each use of the app. As shown in Table 3, sample responses of the original answers for both guiding questions focused largely on the role of chemical properties in dictating function. Interestingly, the revised answers for both guiding questions indicated a new appreciation for the role of spatial arrangements of the relevant molecules for function, reflected in the use of terms such as “pointing, orientation, positioning.”

Analysis of student responses on the pre/post surveys (Table 4; Supporting Information pp S7–S10) was done using data from the Fall 2018 implementation, which consisted of 27 matched pairs between pre/post responses. The results indicate that use of the AR app resulted in increases in student self-efficacy in their knowledge of 3D structures of biological macromolecules (pre = 3.89 ± 1.15; post = 4.59 ± 0.97; Cohen’s d = 0.61 [medium]; p < 0.01) and transport across lipid bilayers (pre = 4.41 ± 0.89; post = 4.80 ± 0.74; Cohen’s d = 0.44 [small]; p < 0.05). These results are consistent with pilot data collected during the Fall 2017 implementation (Supporting Information pp S2–S6).

In addition, students were asked about usability of PyMOL in the presurvey and augmented reality in the postsurvey (Table 5). Students indicated that, compared to PyMOL, they found augmented reality easier to learn how to use for both general manipulations (PyMOL = 3.98 ± 1.40; augmented reality = 5.04 ± 1.26; Cohen’s d = 0.76 [medium]; p < 0.05) as well as specifically identifying molecular interactions (PyMOL = 3.50 ± 1.29; augmented reality = 4.75 ± 1.15; Cohen’s d = 0.97 [large]; p < 0.001).

Incorporation of the AR app into the class discussion also involved significantly less preparation for the instructor, compared to extensive preparation necessary for the PyMOL-based class discussion that took place 2 weeks prior in the same course. Since computer lab space is limited, students with personal laptops were asked to download and install PyMOL before class while the instructor also arranged to borrow extra laptops for those without computers, as well as a set of three-button mice (to facilitate manipulation using PyMOL, Table 1), from Information Technology Services. In order to streamline use of class time, the instructor (an advanced PyMOL user with over 10 years of experience) also created a set of PyMOL session files containing customized virtual 3D models with specific features highlighted using different colors and/or representations for students to download and use in class. During class, students received a worksheet with instructions for how to manipulate the molecules in the session files and questions to guide their exploration of the molecules. Those instructions were also supplemented by 15 min of instructor-led in-class guidance for how to use PyMOL. In contrast, students were able to intuitively learn how to use the AR app with minimal instructions, conserving valuable class time for viewing and interacting with the structure. Moreover, now students can simply download the app to their personal smartphones, relieving the additional burden to identify resources to borrow the necessary hardware.

CONCLUSIONS

The goal of this project was to design a simple, easy-to-use teaching tool to facilitate interactions with virtual 3D objects in the classroom. The data collected from two consecutive years of implementation in a Biochemistry course suggest that this goal was largely achieved. Not only did students find BioChemAR easy to learn how to use (Table 5), but app use also improved spatial awareness of students in their understanding of the mechanisms of K⁺ channel function and selectivity (Table 3, student quotes). Advanced users may prefer the variety of customizable controls that are present in currently available software programs (such as PyMOL). Given the reduced hardware requirements and simplicity of use, however, BioChemAR has the potential to be broadly utilized in a variety of classroom environments (with respect to class size, course curriculum, and institution-type).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available at https://pubs.acs.org/doi/10.1021/acs.jchemed.8b00691.
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