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Student Understanding of DNA Structure–Function Relationships Improves from Using 3D Learning Modules with Dynamic 3D Printed Models

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Abstract

Understanding the relationship between molecular structure and function represents an important goal of undergraduate life sciences. Although evidence suggests that handling physical models supports gains in student understanding of structure–function relationships, such models have not been widely implemented

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in biochemistry classrooms. Three-dimensional (3D) printing represents an emerging cost-effective means of producing molecular models to help students investigate structure–function concepts. We developed three interactive learning modules with dynamic 3D printed models to help biochemistry students visualize biomolecular structures and address particular misconceptions. These modules targeted specific learning objectives related to DNA and RNA structure, transcription factor-DNA interactions, and DNA supercoiling dynamics. We also designed accompanying assessments to gauge student learning. Students responded favorably to the modules and showed normalized learning gains of 49% with respect to their ability to understand and relate molecular structures to biochemical functions. By incorporating accurate 3D printed structures, these modules represent a novel advance in instructional design for biomolecular visualization. We provide instructors with the materials necessary to incorporate each module in the classroom, including instructions for acquiring and distributing the models, activities, and assessments.

Keywords: DNA, RNA, student misconceptions, 3D printing, model-based learning, nucleic acid structure and function, molecular visualization

Introduction

Understanding the complex interdependence of macromolecular structure and function represents a central goal of undergraduate life science education, particularly within biochemistry [1–3]. However, life science students frequently struggle to visualize and translate between the static two-dimensional (2D) images displayed in textbooks and the dynamic three-dimensional (3D) concepts they represent [4–8]. Hence, many students leave life sciences classrooms with misconceptions about structure–function relationships [8]. One fundamental biological concept with which students struggle is the relationship of DNA structure to its functions. For example, students have misconceptions about the way DNA bases are stacked and accessible to DNA binding proteins, the continuity of and information presented in DNA grooves, the flexibility and dynamic nature of DNA molecules, and the enzymes that cleave and repair DNA [9–12]. For example, students fail to realize that although DNA bases lie between the DNA backbones, they are accessible to proteins [9]. As a result, students do not realize that the presented chemical information varies between the major and minor grooves of a specific DNA segment. Moreover, many students do not realize that transcription factors can interact with a specific DNA segment without breaking the hydrogen bonds between the two complementary strands. In another example, students struggle to recognize and visualize the functional significance between negatively and positively supercoiled DNA for transcription and replication [13].

Multiple studies show that physical models can help students visualize macromolecular structures. Models allow students to engage in higher order concepts [14], answer more advanced application questions [15], and develop more accurate mental scaffolds to translate between 2D and 3D molecular models [5, 10, 16]. Moreover, one study [17] found that female students especially benefit from physical models to master structure–function relationships. Despite these potential benefits, others recognized that instructors lacked a resource to guide visual literacy education. This prompted the development of the Biomolecular Visualization Framework based on the American Society for Biochemistry and Molecular Biology's (ASBMB) foundational learning goals [18]. This framework identifies overarching themes and provides learning goals and objectives that outline core content and competencies for instructing in macromolecular visualization.

Recognizing the potential for physical models to help students understand DNA structure and function, a concept foundational to the field [1–3, 16, 19], educators have developed numerous lessons that incorporate structural representations. Such lessons include cardboard cutouts or computer-based software to distinguish DNA and RNA bases and components of the sugar-phosphate backbone [9, 20], tubing or string to represent supercoiled DNA [13], or laboratory investigations of topoisomerase effects on DNA structure [21]. Unfortunately, these models are neither physical (i.e. software), dynamic (i.e. cutouts), nor atomically correct representations (i.e. cutouts and tubing or string).

The recent dawn of 3D printing has allowed instructors to teach molecular structure–function relationships using more complex physical models [15, 17, 22]. Guided by the Biomolecular Visualization Framework, we leveraged this technology to design three interactive learning modules and assessments that use 3D printed models to target important misunderstandings of DNA structure and function that often stem from visual illiteracy and to help students visualize frequently challenging processes [18, 23]. In Table I, we outline specific learning objectives related to misconceptions or difficult-to-visualize 2D to 3D translations identified from the literature and polling six biochemistry instructors [10–12, 22]. We responded to ASBMB learning goals (Table I, column 1) and the Biomolecular Visualization Framework (column 2) [16] by outlining specific learning objectives for each 3D learning module (column 3) to address specific student misconceptions found in undergraduate majors (column 4). These learning objectives and misconceptions were specifically tested in the assessments (column 5). We designed many of the models from 3D crystallographic data to create structurally accurate 3D representations of DNA and proteins. The cost-effectiveness of 3D printing enables us to print enough models for hands-on activities instead of traditional demonstration.

Table I. Alignment of societal learning goals with the learning objectives, targeted misconceptions, and assessment questions for each 3D learning module

ASBMB learning goal	*Biomolecular visualization learning goal*	*Module's learning objective*	*Targeted misconception*	*Assessment question that targets concept*
Lesson I: DNA versus RNA structure and function				
• Students should be able to discuss the diversity and complexity of various biologically relevant macromolecules and macromolecular assemblies in terms of evolutionary fitness	• MA1: Students can describe macromolecular assemblies • MI1: Students can predict interactions using structural information	1: Distinguish DNA and RNA molecules from each other	Incomplete view of effect of DNA backbone on preventing chemical accessibility/ interaction of macromolecules to DNA bases.	Q1, 2
• Students should be able to describe the basic units of the macromolecules and the types of linkages between them	• MR1: Students can identify monomer units of biological polymers • MI1: Students can predict interactions using structural information • SA1-2: Students can recognize symmetry within macromolecules	2: Distinguish directionality of nucleic acids by counting carbons in the phosphate backbone	Inaccurate perceptions of the DNA polymer direction in DNA replication forks, DNA repair, and transcription factors	Q3, 4, 6a
• Students should be able to discuss the composition, evolutionary change and hence structural diversity of the various types of biological macromolecules found in organisms.	• SA1-2: Students can recognize symmetry within macromolecules	3: Describe the functional significance of the hydroxyl group in RNA		Q5
• Students should be able to recognize the repeating units in biological macromolecules and be able to discuss the structural impacts of the covalent and noncovalent interactions involved • Students should be able to discuss the chemical and physical relationships between composition and structure of macromolecules	• TC1: Students can describe linkages between a macromolecule • MI1: Students can predict interactions using structural information	4: Describe the chemical interactions of nucleotide bases in double stranded DNA and double stranded RNA	Inaccurate view that bases lie flat "like on a page" rather than like stairs; (misconception can lead to misunderstanding of base pairing, stacking, strand stabilization, and interaction energy)	Q6b

Table I. Continued (2)

ASBMB learning goal	*Biomolecular visualization learning goal*	*Module's learning objective*	*Targeted misconception*	*Assessment question that targets concept*
Lesson II: DNA-transcription factor binding				
• Students should be able to discuss the interactions between a variety of biological molecules (including proteins, nucleic acids, lipids, carbohydrates and small organics, etc.) and describe how these interactions impact specificity or affinity leading to changes in biological function.	• MI2: Students can evaluate the effect of the local environment on interactions • TC3: Students can explain how a biomolecular interaction site can be made	1: Differentiate between specific and nonspecific interactions between DNA and DNA-binding proteins	Inaccurate distinction of specific and nonspecific DNA to DNA-binding protein interactions, and the function of these on macromolecular scanning and docking to DNA	Q1, 2, 3, 4, 5, 11a, 12a
• Students should be able to discuss the interactions between a variety of biological molecules and describe how these interactions impact specificity or affinity leading to changes in biological function	• TC1-3: Students can follow the chain direction through the molecule, translating between 2D and 3D rendering	2: Compare chemical information presented in the major and minor grooves of DNA	Inaccurate distinction between major and minor grooves, and the effect of the grooves on macromolecular binding	Q6, 7
• Students should be able to discuss the interactions between a variety of biological molecules (including proteins, nucleic acids, lipids, carbohydrates and small organics, etc.) and describe how these interactions impact specificity or affinity leading to changes in biological function	• MA1, MA2: Students can describe and compose renderings of macromolecular assemblies • MI2: Students can evaluate the effect of the local environment on interactions	3: Determine how and what type of protein secondary structures typically interact with DNA		Q8
• Students should be able to discuss the impact of specificity or affinity changes on biological function	• MA1, MA2: Students can describe and compose renderings of macromolecular assemblies • MI2: Students can evaluate the effect of the local environment on interactions	4: Relate the oligomeric state of transcription factors to DNA binding affinity		Q9

Table I. Continued (3)

ASBMB learning goal	*Biomolecular visualization learning goal*	*Module's learning objective*	*Targeted misconception*	*Assessment question that targets concept*
• Students should be able to evaluate chemical and energetic contributions to the appropriate levels of structure of the macromolecule and predict the effects of specific alterations of structure on the dynamic properties of the molecule • Students should be able to predict the effects of either mutation or ligand structural change on the affinity of binding and design appropriate experiments to test their predictions• MA1, MA2: Students can describe and compose renderings of macromolecular assemblies	• MI2: Students can evaluate the effect of the local environment on interactions	5: Determine that structural changes can be induced in DNA upon transcription factor binding		Q10
• Students should be able to compare and contrast the effects of chemical modification of specific amino acids on a three dimensional structure of a protein • Students should be able to predict the biological and chemical effects of either mutation or ligand structural change on the affinity of binding and design appropriate experiments to test their predictions	• MI2: Students can evaluate the effect of the local environment on interactions	6: Connect modifying DNA binding sites in the transcription factor with effects on binding affinity		Q11b-c, Q12b-c

Table I. Continued (4)

ASBMB learning goal	*Biomolecular visualization learning goal*	*Module's learning objective*	*Targeted misconception*	*Assessment question that targets concept*
Lesson III: DNA supercoiling dynamics				
• Students should be able to describe the basic units of the macromolecules and the types of linkages between them	• MD1: Students can describe the impact of dynamic motion of a biomolecule on its function	1: Define the relationship between linking number, writhe, and twists Inaccurate description of physical constraints of supercoiled DNA	Inaccurate description of physical constraints of supercoiled DNA	Q2, 3
• Students should be able to discuss the chemical and physical relationships between composition and structure of macromolecules	• MD1-2: Students can describe the impact of dynamic motion of a biomolecule on its function and predict limits to macromolecular movement	2: Determine how nucleosomes contribute to supercoiling and storage		Q1, 6
• Students should be able to critically discuss the evidence for and against the roles of dynamics in macromolecular function	• MD1: Students can describe the impact of dynamic motion of a biomolecule on its function	3: Differentiate between overwound and underwound DNA, right-handed and left-handed supercoils, and negative and positive supercoiled DNA	Inability to characterize or describe supercoiled DNA	Q4a-b
• Students should be able to discuss the structural basis for the dynamic properties of macromolecules and predict the effects of changes in dynamic properties	• MD1-2: Students can describe the impact of dynamic motion of a biomolecule on its function and predict limits to macromolecular movement	4: Predict what form of supercoiled DNA is more amenable to strand separation (i.e. transcription)	Insufficient understanding of the effect of different forms of supercoiled DNA on DNA transcription, replication, and repair.	Q4c-d, 5
• Students should be able to compare and contrast various mechanisms for regulating the function of a macromolecule	• MA1: Students can describe macromolecular assemblies • MD1: Students can describe the impact of dynamic motion of a biomolecule on its function	5: Differentiate between the actions of Type I and Type II Topoisomerases on supercoiled DNA	Insufficient distinction between enzymes that control DNA supercoiling.	Q7, 8, 9

A detailed interactive set of questions guided students' engagement and manipulations of the models, and facilitated small group discussions relating structure to function [24–26]. Students completed these investigative questions in small groups, and at specific points during the activity, whole-class participation was facilitated through in-class clicker questions with peer-instruction [27, 28].

To facilitate the broader use of these 3D learning modules, we designed each module as a complete, accessible, reproducible, and adaptable package. We have included all the materials and information needed to implement the modules, including instructions for obtaining model sets (Supporting Information Files S7 and S8), the activities (Supporting Information Files S1, S3, and S5), and assessment questions to evaluate performance on learning objectives (Supporting Information Files S2, S4, and S6). The first module (DNA vs. RNA structure and function) compares the general structure and function of DNA and RNA (Supporting Information Files S1 and S2), the second module (DNA transcription factor binding) addresses the role of structure in transcription factor-DNA interactions (Supporting Information Files S3 and S4), and the third module (DNA supercoiling dynamics) addresses the structural dynamics of DNA supercoiling (Supporting Information Files S5 and S6). Here, we show that these three modules facilitate learning DNA structure–function relationships in biochemistry courses, with average learning gains ranging from 43% to 63%.

Methodology

Model and Module Design

In order to address student misconceptions and aid visualization of DNA structure and function, we designed three modules around 3D printed models for integration in upper level biochemistry, molecular biology, or genetics courses. We iteratively designed the models and interactive activities to effectively target specific misunderstandings around 1) the effect of differences in DNA and RNA structure on function, 2) the role of structure in transcription factor-DNA interactions, and 3) the structural dynamics of DNA supercoiling, as outlined in Table I, column 4.

Model design usually began months before the planned class in order to allow time to test multiple approaches to teach the content, evaluate the strengths and weaknesses of a variety of printing materials available, and assess student interactions with the models prior to integration in class. For each iteration, we had to budget time for print and delivery of the models. To test and refine each model, activity, and assessment prior to use, we

conducted think-aloud interviews with 2–5 senior biochemistry students per module. This process helped us develop models that engaged students in the desired thought processes and addressed the desired misconceptions and learning objectives.

Despite being 3D printed in plastic, many models can move and/or respond to neighboring models. For example, in Module I (DNA vs. RNA structure and function), the single-stranded models are flexible to allow students to unwind the nucleic acid strand to differentiate structural components and illustrate different cleaving tendencies in DNA and RNA molecules (Fig. 1A). This module also uses full-color double-stranded DNA and RNA models to compare the effect of RNA's hydroxyl group on helix formation and activity (Fig. 1B). For Module II (DNA-transcription factor binding), we designed a DNA helix and the corresponding DNA binding domain of a bacteriophage λ transcription factor model with magnets so that students could feel and compare the binding strength of the DNA to the transcription factor in different oligomeric and mutant states (Fig. 1C). This module also uses a colored DNA-transcription factor pair to consider the specific versus nonspecific chemical interactions that occur in sequence recognition and binding (Fig. 1D). For Module III (DNA supercoiling dynamics), we 3D printed the DNA model with a flexible material and added magnetic ends to allow students to physically feel and compare the tension that builds up in supercoiled DNA. In this module, students create and characterize DNA writhes, twists, and constraints that occur during supercoiling (Fig. 1E).

We have previously published a guide to design 3D molecular models, including specific directions for the model used in Module III [29]. All of the models are available as structural files that can be adapted to many 3D printers (Supporting Information File S7) or as print-on-demand models through the commercial vendor Shapeways (www.shapeways.com/shops/macromolecules). After models are printed, some must be modified before their intended use (e.g. add magnets). We have provided instructions for these details in Supporting Information File S8. Notably, the models could be used to teach a number of more basic or more advanced concepts by providing alternative activities.

Implementation

Each module's final form follows the same general format (Table II), with module-specific details outlined in the Supporting Information Files S1–S6. When ordering the final iteration of the models, we allowed time to become comfortable with orienting and manipulating the models to demonstrate genetic processes to students. To prepare for class and office hours, the instructor and each teaching assistant completed the activity with the

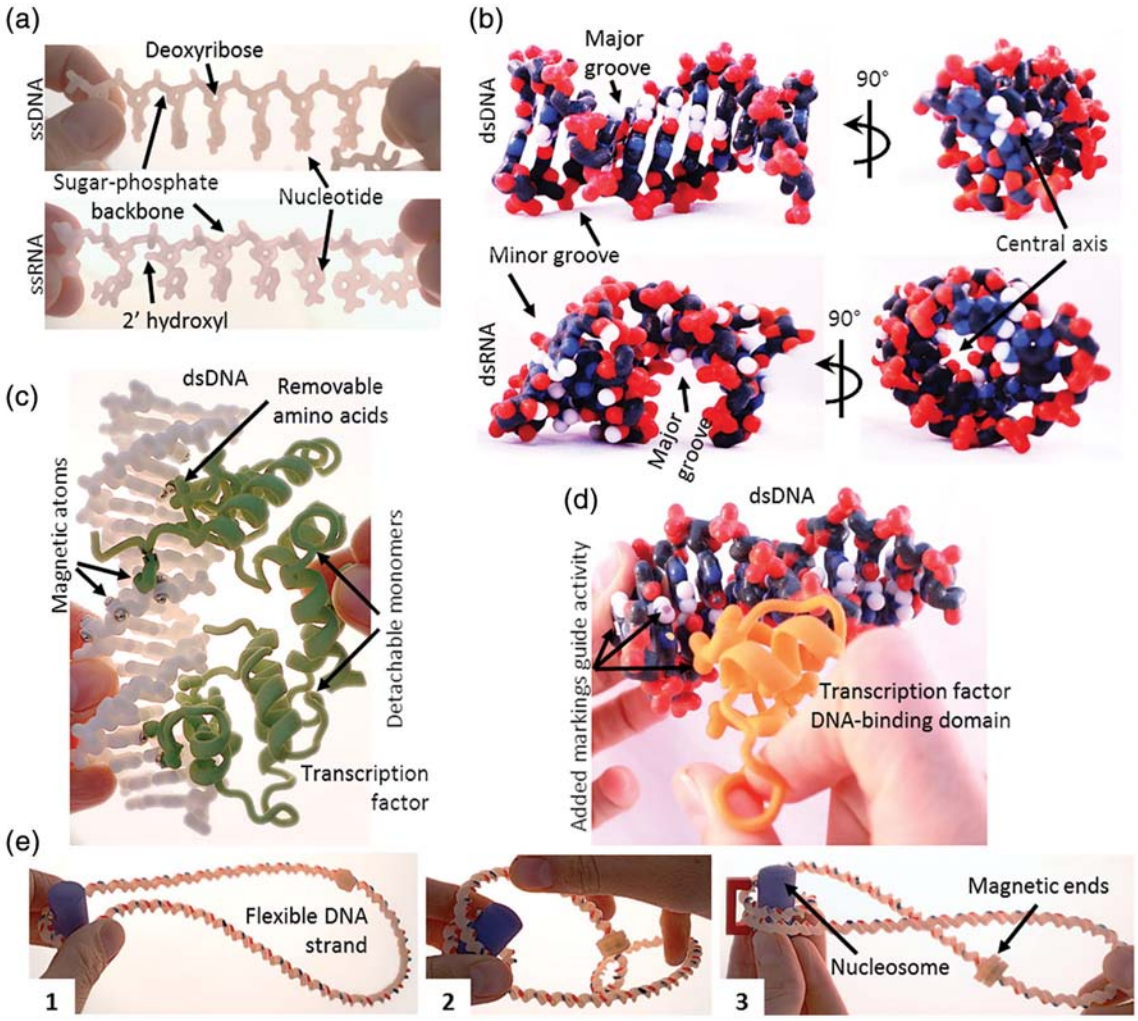

Figure 1. 3D printed models to target common student misconceptions and hard-to-visualize DNA structures and functions. The models designed illustrate key concepts of molecular structure implicating biochemical function of DNA for undergraduate students to learn. (A) In Module I, flexible models of single-stranded DNA and RNA allow students to unwind the molecules to identify the sugar-phosphate backbone and unique bases, compare structural variations, and predict functional differences between the two molecules. (B) In Module I, students also compare atomic-colored double-stranded DNA and RNA helices to measure distinguishing features (height, width, center axis, etc.) between the two molecules. (C) In Module II, we designed a DNA helix and a dimer of the corresponding DNA-binding domain of the bacteriophage λ transcription factor with magnets so that students could feel the effect of complementary chemical interactions and predict the impact of altering the oligomeric state or introducing mutations. (D) Module II also uses the atomic-colored DNA helix from Module I, as well as a portion of bacteriophage λ's DNA-binding domain to consider sequence specificity and recognition, the information presented and accessible to binding proteins in the major and minor grooves, and specific versus nonspecific interactions. (E) The long, flexible DNA strands designed for Module III allow students to mimic the dynamics of DNA supercoiling and count or calculate the writhes, twists, and linking number in supercoiled DNA, feeling and comparing the tension created between underwound and overwound supercoiled DNA. In the sample exercise displayed, students wrap the DNA twice around a blue histone octamer model in Steps 1 and 2. In Step 3, after holding the DNA in place with the addition of an H1 protein mimic, students characterize the handedness of the toroidal and interwound supercoils.

Table II. Overview of typical learning module structure

Task	*Description*	*Timeline*	*Responsible individual*
Pre-class preparation	Order, print, package models	3–4 weeks before deployment	Instructor or teaching assistant
Preassessment	6–12 MC/MTF content quiz (online)	3–7 d prior to deployment	Student (completed independently)
Pre-class activity to conserve class time (optional)	Time-intensive model-based interactive content assignment	2–3 d prior to in-class deployment	Student (completed independently or in groups)
In-class activity	Model-based interactive content assignment	Deployment class period (50–75 min)	Student (completed in group of 3)
Postassessment (identical to the preassessment to assess learning gains)	6–12 MC/MTF content quiz (online)	Upon completion of in-class activity (allow up to 1 wk to complete)	Student (completed independently)

models. For easy distribution in class, we prepackaged the models in plastic containers. Before integrating the modules in class, students had up to 1 week to individually complete a 6 (Module I), 12 (Module II), or 9 (Module III) closed-ended question preassessment online to evaluate their initial understanding (Supporting Information Files S2, S4, and S6). For each module, we expected students to have prerequisite content knowledge in order to find the module effective (Table III). After completing the in-class module, students had 1 week to individually complete the same questions as an online postassessment to evaluate learning gains.

Our implementation of the modules progressively improved across each subsequent module based on student feedback. In the first module (DNA vs. RNA structure and function) in the large-enrollment course, students worked in groups of 4–5 per model set. We observed many students waiting to interact with the models during the activity. Moreover, students expressed concern regarding the limited time they had to interact with the models because of the group size. Thus, we needed a higher model-to-student ratio to reach 100% model engagement and improve the peer-learning environment. For the later modules, students worked in groups of three per model set, maintaining group-learning benefits and cost-effective use of the models. At first, each group member submitted his/her own responses to the in-class activity. However, by structuring the groups such that a designated note-taker submitted responses on behalf of the group, we enhanced group efficiency while at the same time prompting peer discussion. The note-taker also engaged with the models so as not to be disadvantaged. Although groups submitted the activities in an electronic Qualtrics-based

Table III. Prerequisite content knowledge for effective student engagement of the modules

3D learning module	*Broad expectation*	*Specific examples (if applicable)*
Module I: DNA versus RNA structure and function	General understanding of the chemical composition of DNA and RNA	1. DNA uses the base thymine, whereas RNA uses uracil 2. the sugar of DNA lacks the 20 hydroxyl found in the ribose of RNA 3. cellular DNA typically consists of two long strands of complementary polynucleotides coiled around each other into a B-form helix, while RNA is usually a single-stranded polynucleotide that can take on a variety of secondary and tertiary structures
	Ability to recognize the basic structures that DNA and RNA adopt	i.e. helices
Module II: DNA-transcription factor binding	Basic conceptual understanding of gene expression	1. the central dogma of molecular biology 2. the difference between constitutive and regulated gene expression 3. the need for specific DNA-protein interactions for regulation of transcription 4. the secondary structure of proteins, including a basic recognition of the properties of amino acids 5. weak interactions that affect binding affinity
Module III: DNA supercoiling dynamics	Foundational understanding of supercoiling	1. the role of DNA supercoiling in genome packing and storage 2. how supercoiling affects DNA accessibility to replication and transcription machinery 3. the basic classifications of supercoiled DNA 4. the roles of topoisomerase enzymes.

format, paper versions were used as a reference during the class. Using the paper versions allowed students to learn by translating between 2D and 3D. Although we taught the material with 2D and 3D, we tested only with 2D. Ultimately, 2D has a functional primacy because students will generally encounter 2D representations during their careers. Finally, in the first module, the students worked through the material exclusively with their groups, with the instructor and teaching assistants providing guidance to individual

groups. However, after students requested more guidance and confirmation, we integrated formative assessment clicker questions followed by brief whole-class discussions at specific points to assess understanding, identify and resolve student misunderstandings, and to keep the module streamlined [27, 28, 30]. We provided additional application questions and readings for faster groups, and the paper version enabled students to proceed through the material beyond the checkpoints at their own pace instead of waiting for the rest of the class.

Data Collection and Analysis

We integrated all three learning modules in a large-enrollment (n = 130) junior/senior-level undergraduate biochemistry course for majors with a large prehealth population. Although the entire class completed the modules, we only used the data from consenting students who completed both the pre- and postassessments (for paired analysis), with n = 109, n = 81, and n = 110 for Modules I, II, and III, respectively. We also integrated the first module in a small-enrollment (n = 22) junior-level undergraduate biochemistry course for majors and only used the data from consenting students who completed both pre- and postassessments (n = 21). For comparison, we had students in another section of the junior/senior-level undergraduate biochemistry course complete the pre- and postassessment for Module II without completing the module. The control was taught by a different professor at the same university as the large-enrollment course. We only used data from consenting students who completed both assessments (n = 22). Each module was taught in a lecture-format course, and each portion of the module (preassessment, activity, and postassessment) was graded for completion.

Our pre-post assessments were designed to assess learning of the targeted concepts, with an emphasis on how these concepts occur in 3D space. These assessments included multiple-choice as well as multiple-true-false questions, which require students to evaluate multiple options on a single topic and help diagnose misconceptions [31, 32]. Table I provides an alignment of assessment questions with learning goals and misconceptions. We assigned equal weight to multiple-choice questions and individual multiple-true-false statements and calculated normalized learning gains for the whole class by first calculating the pre- and postassessment scores for each student, and then averaging these scores across the class. We then divided the raw class gain (average postassessment score − average preassessment score) by the gain possible (100% − average preassessment score) to give the whole class normalized learning gains, and performed a paired Student's t-test on individual student performance. To determine student learning gains related to the specific misconceptions targeted, we calculated the pre- and postassessment scores for each student for each learning objective, and

then performed a paired Student's t-test analysis of the pre- and postassessments. To compare class performance on individual assessment items, we plotted the class average pre- and postassessment scores in a scatter plot. We designed the assessment items to test concepts in the context of unfamiliar systems, preventing students from simply memorizing the system used in the activity. Specific items also provided opportunities for students to do 2D-3D translation. Together, these approaches shed light on student visualization skills.

Halfway through the semester, the large-enrollment students completed a 6-question Qualtrics survey on their experiences with the 3D model-based learning modules (Fig. 6A; Supporting Information File S9-A). We administered this survey after the DNA supercoiling postassessment, but responses also reflect the DNA versus RNA module. The small-enrollment students completed a similar survey on their experiences with the 3D learning module after completing the DNA versus RNA postassessment (Fig. 6B–6D; Supporting Information File S9-B).

After each module in the large-enrollment course, an external evaluator reached out to the class to recruit willing students to participate in focus group interviews to discuss their experiences with a specific module. From this pool, we selected 3–5 students at random who were interviewed by a non-instructor. We assured the students that their names and feedback would not be shared with their instructor during the semester and would have no impact on their grades.

Results

Model-Based Activities Improved Student Performance on Content Assessments

To determine the impact of the 3D modules, we analyzed the pre-post normalized learning gain for each assessment. Our data show that student performance increased (Fig. 2A, first three bars) after each 3D module was used in a large enrollment biochemistry course, with average normalized gains of 51%. Moreover, student learning gains were also observed when one of the modules was integrated in a small biochemistry course (Fig. 2A, fourth bar), suggesting that these 3D learning modules can be impactful in a variety of teaching environments. Thus, these improvements were large for students who used the module, compared to the no-module control for Module II's assessment that showed gains of only 9.0% (Fig. 2A, fifth bar).

The improvements observed for all three modules with the large-enrollment course were independent of students' course performance ($p = 0.32$, 0.43, and 0.16 from an analysis of variance comparing normalized gains for

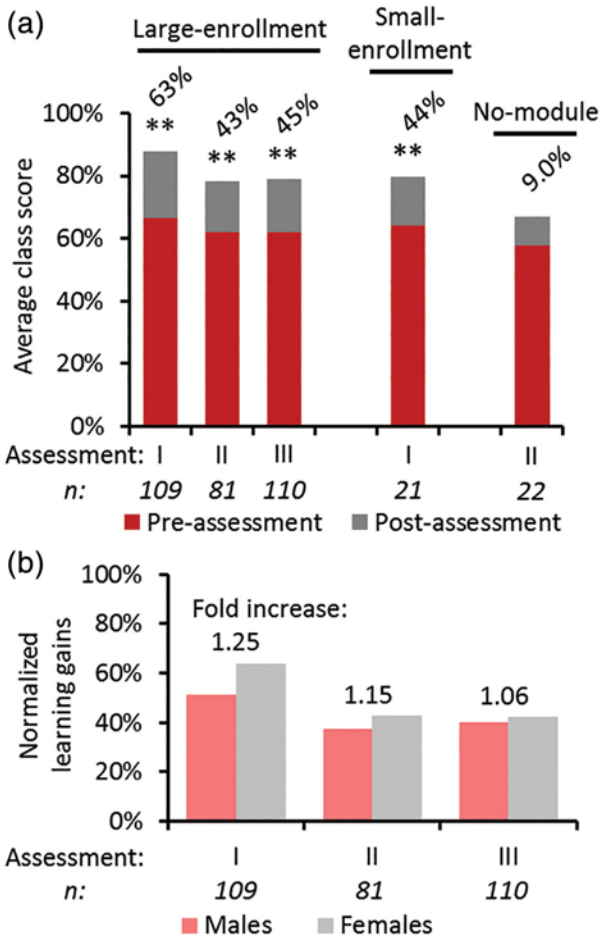

Figure 2. Consenting student performance for 3D learning modules. (A) Average class values of the preassessment scores (red) and postassessment scores (gray) were compared from DNA-content assessments. The normalized learning gain is shown above for each assessment. Data for Modules I, II, and III were collected in a large-enrollment undergraduate biochemistry class (n = 109, 81, and 110, respectively). To test alternate learning environments, data from Module I were collected in a small-enrollment undergraduate biochemistry class (n = 21). For a proof-of-concept no-module control, data from Module II assessment were also collected. The no-module control data were from a class with a different professor at the same university as the large-enrollment course. (B) Normalized learning gains for males (pink) and females (light gray) were compared from DNA-content assessments for each 3D learning module in the large-enrollment undergraduate biochemistry course (n = 109, 81, and 110, respectively). The fold increase in the female population is indicated, with p values greater than 0.056, 0.414, and 0.655, for each module, respectively. Student's paired two-tailed t-tests were used to measure significance; **p < 0.001.

students in four quartiles based on course performance). However, while we found a trend of female students benefiting more from the model-based modules compared to their male peers (1.25-, 1.15-, and 1.06-fold better on the assessments), these values were not significant (p > 0.056, 0.414, and 0.655, for each module, respectively; Fig. 2B).

Model-Based Activities Improved Student Performance on Specific Learning Objectives

To assess student performance on content areas that directly target the misconceptions discussed, we measured student gains for the specific learning objectives outlined in Table I (Fig. 3). The data show an increase in student performance for each of the tested learning objectives, indicating significant gains in nearly all of the objectives. Thus, our data support that these 3D learning modules help address misconceptions held by undergraduate biochemistry students (Table I).

Model-Based Activities Improved Student Performance on Assessment Items

To evaluate overall student achievement of the tested learning goals, we measured the average of the class' performance on each assessment item. We plotted the percent correct on each item for the pre- compared to the postassessment. Analysis of pre- and post-performance on each assessment item revealed that the students collectively improved on nearly all of the tested concepts when using the modules, independent of class size (Fig. 4).

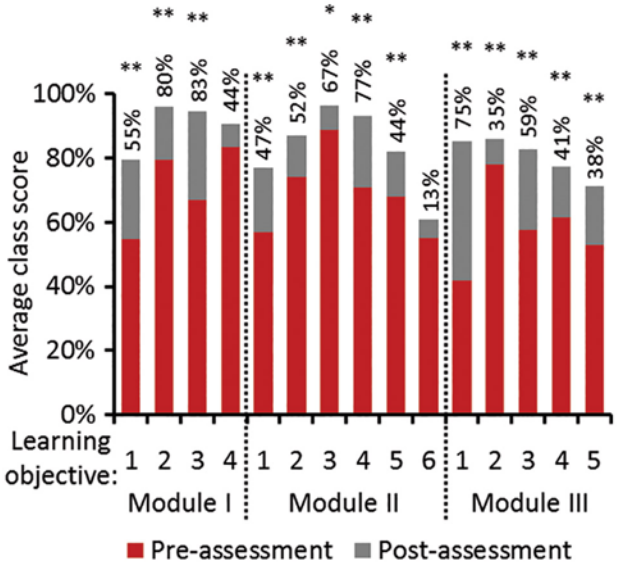

Figure 3. Consenting class performance for tested learning objectives. Student average values for each learning objective tested by the DNA-content assessments are plotted. Preassessment scores (red) and postassessment scores (gray) were compared for each learning objective. The normalized learning gain is shown for each learning objective. Learning objectives are given in Table I, column 3. Data plotted are from a large-enrollment undergraduate biochemistry class (n = 109, 81, or 110 for Modules I, II, or III, respectively). Student's paired two-tailed t-tests were used to measure significance; **$p < 0.001$; *$p < 0.05$.

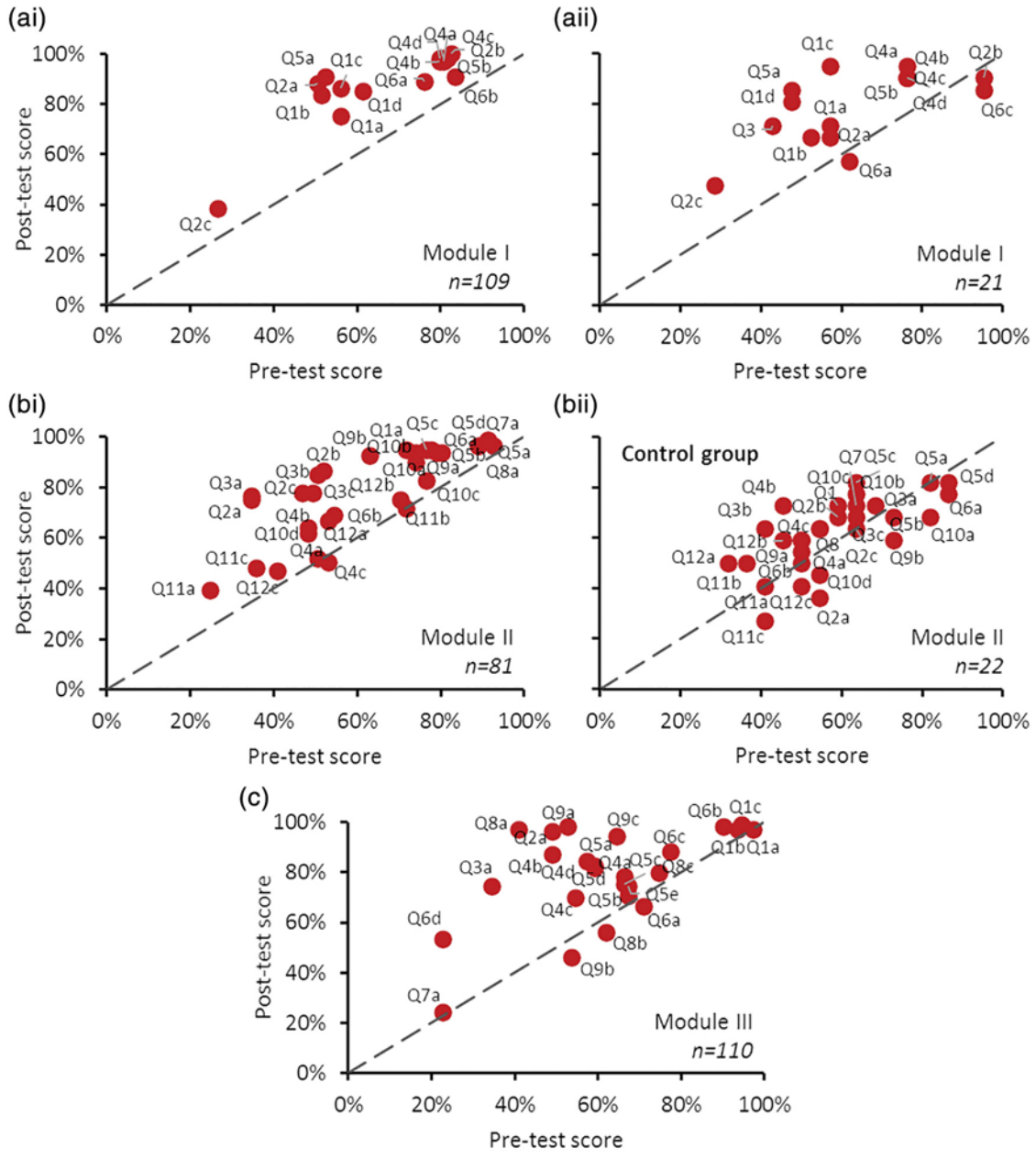

Figure 4. Consenting class performance on individual assessment items for each 3D learning module. Class average performance on each assessment item for the pre- and postassessments for each module. Data are shown for assessments from A(i) Module I in large-enrollment class (n = 109), A(ii) Module I in small-enrollment class (n = 21), B(i) Module II in large-enrollment class (n = 81), B(ii) Module II in no-module control (n = 22), and (C) Module III in large-enrollment class (n = 110).

Students Applied Learned Concepts to Different Systems and Had Opportunities to Translate between 2D and 3D

To assess students' ability to apply learned content to unfamiliar systems, we designed some of the assessment questions to test the concepts in different systems than discussed in class. For example, while Module II focused on the bacteriophage λ transcription factor, Question 9 of the corresponding assessment asks students to apply what they learned about the effect of oligomeric state on binding affinity to the Factor-for- Inversion Stimulation protein transcription factor (Fig. 5A). Students exhibited 71% and 80% normalized learning gains on the items in this question (Fig. 5C).

We also designed questions to enable students to translate between 2D and 3D. For example, Question 10 of this same assessment demonstrates students' skill in translating between 2D and 3D, as they need to wrap a segment of DNA around a DNA-binding protein in their mind and predict the effect that this action would have on the binding activity (Fig. 5B). Students exhibited 62%, 76%, 26%, and 26% normalized learning gains on items 1–4 in this question (Fig. 5C).

Students Valued the Model-Based Activities

When surveyed anonymously about their experiences with the 3D learning modules, many students agreed that the models were beneficial to their learning. While students in the large-enrollment course completed all of the modules, students in the small-enrollment course only completed the first module but received a more detailed survey on their experiences. Of students in the large-enrollment class, nearly 60% stated that overall, the physical models made it easier to learn the material taught. Of students from the small-enrollment class, 81% stated that Module I helped them understand nucleic acid structure and function, and 91% requested similar models continue to be used in their class and future classes (Fig. 6).

In interviews, students reflected on challenges they experienced in using the 3D learning modules. After the first deployment of Module I, concerns included that 1) there was insufficient introduction of the models and concepts targeted, 2) groups were too large, limiting some students' interactions with the models, and 3) there was insufficient feedback and regrouping during the class. We were able to rectify these challenges for future modules, including a second deployment of the first module in the small-enrollment class. We did this by 1) spending 10–15 minutes orienting students with the models, major themes, and key background concepts for each subsequent module, 2) forming groups of three students per model set to increase contact with the models, and 3) providing more written and

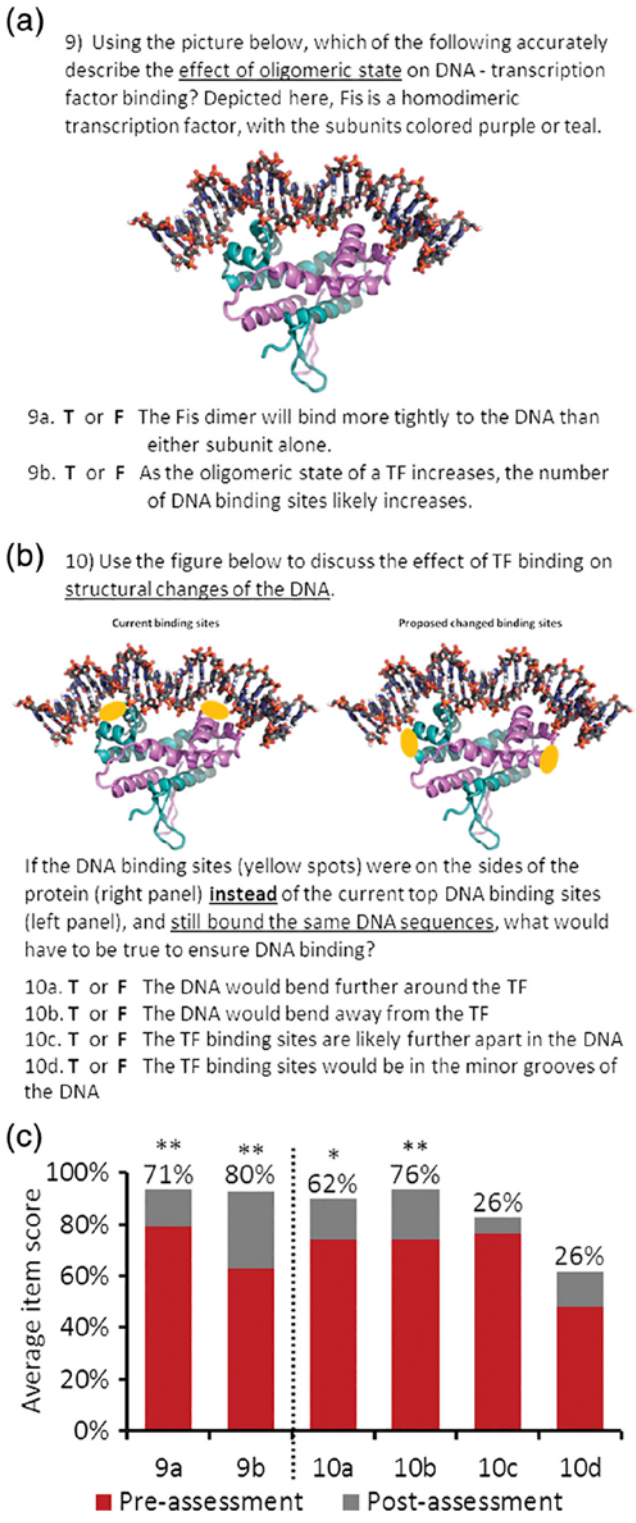

Figure 5. Sample questions test student ability to apply content to unfamiliar systems and between 2D and 3D. Sample questions from the Module II (DNA-transcription factor binding) assessment illustrate skills in (A) applying learned concepts to new systems and (B) translating between 2D and 3D. (C) Student average values for each item in Questions 9 and 10 are plotted. Preassessment scores (red) and post-assessment scores (gray) were compared for each item, with the normalized learning gain shown. Data plotted are from a large-enrollment undergraduate biochemistry class (n = 81). Student's paired two-tailed t-tests were used to measure significance; **p < 0.001; *p < 0.01.

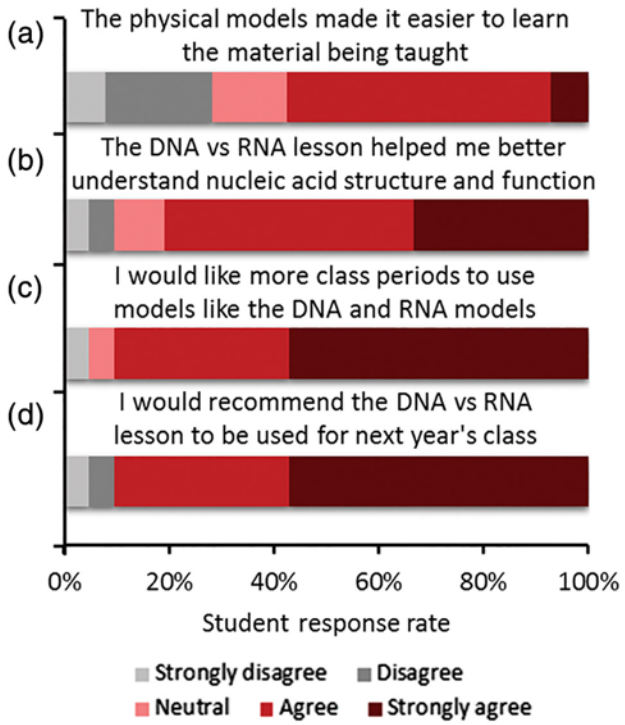

Figure 6. Student perceptions of using 3D printed models and learning modules in the classroom. Students in the large-enrollment (bar A, n = 110) and small-enrollment (bars B–D, n = 21) classes were surveyed regarding their experiences with the 3D learning modules. Survey questions had a 5-point Likert scale ranging from "Strongly disagree" to "Strongly agree."

oral feedback with summary slides and designated clicker-question checkpoints. After implementing these changes, students expressed fewer concerns about model contact time.

Students also described the perceived benefits of the 3D learning modules. Cited benefits included 1) the hands-on advantage, 2) having to answer questions and justify your own answers, rather than having to memorize the answers from the instructor, and 3) self-paced learning with a group. One student summarized his/her experiences with the models, "[Using the 3D models] can only help you. To read something doesn't really process, and then to hear it in lecture you kind of get a feeling for it, but physically seeing it makes something abstract very real, and like I feel like I got a lot more out of the physical modules." In response to the first two modules, another student agreed that this method of learning "was valuable; the whole 'doing' thing. .. I keep it in my mind better if I'm physically doing it."

Even one student who "strongly disagreed" that the physical models made it easier to learn the material being taught (Fig. 6A) volunteered the following about the third module in the free response portion of the survey:

"Getting a chance to see the right and left-handed DNA from a 3D perspective was much more advantageous than looking at it on a piece of paper." Furthermore, there was no significant difference in assessment performance between dissenting students and approving students ($p > 0.35$ between students who "strongly disagreed" or "disagreed" and those who "strongly agreed" or "agreed" that the physical models made it easier to learn the material being taught). This apparent disconnect is worth noting, but it also reflects the benefits that students experience with physically interacting with the 3D models, even when resistant to using them.

Discussion

Summary

Responding to calls from the biology, biochemistry, and molecular biology societies to improve instruction and combat widespread difficulty that students have to visualize how molecular structure affects biological function [1, 2, 4, 23], we designed and implemented three unique 3D learning modules that require students to interact with physical models. Because out-of-field spatial training does not enhance in-field visualization [33, 34], our study tested dynamic, physical, subject-specific models and modules as a way to teach 3D biochemical visualization.

We designed these modules to target key learning objectives and undergraduate student misconceptions regarding structure–function relationships in nucleic acids. Student performance on the pre- and postassessments for each module (Figs. 2–5) reveal that the model-based modules improve student mastery of the content taught, with collective normalized learning gains of 49% compared to 9.0% in a no-module control, effectively targeting specific misconceptions. The fact that a different professor taught the no-module control could contribute to the observed difference in performance. Modules I and II (DNA vs. RNA structure and function and DNA-transcription factor binding, respectively) led to a decrease in the prevalence of student misconceptions related to base stacking, orientation to the backbone, and differentiation between the major and minor grooves, including how macromolecules access and interact with the DNA bases (Figs. 2 and 4). Module III (DNA supercoiling dynamics) helped students visualize DNA supercoiling to classify different forms of supercoiled DNA and determine the implications of the different forms of supercoiled DNA on physiological mechanisms (Figs. 2 and 4).

The learning modules add to the instructional resources available to life science educators. As biochemists seek tools to instruct and assess

macromolecular visualization [34–36], these resources can be used to reveal individual item responses for formative purposes. After years of relying on static, 2D models to teach DNA molecular structure and biochemical function [13, 20, 21], our modules provide life science instructors access to relatively low-cost, dynamic, and atomically representative models of DNA, RNA, and associated proteins with which students can interact.

Moreover, our general observations of student engagement with the models as well as student interviews and surveys demonstrate that students valued the learning modules and benefitted from them (Fig. 6). First, the models used in these activities are tangible and dynamic, a trait that helps students solidify knowledge and create a deeper understanding of the content. Regarding their experiences with the DNA and RNA models, one student reflected, "All of the information was given in the book/lecture, so I already knew it all but the models made it easier to understand the information and to see it. I think I would be able to explain the structure of DNA/RNA to someone now versus just being able to recite some facts from the book." After engaging with the models, this student described an increased depth of understanding that enabled them to teach the material. Second, the models designed for these modules provide students with a more thorough perspective as they translate biochemistry between 2D and 3D (Fig. 5). Students can then apply skills learned in these individual modules to novel concepts, making it easier for them to translate between these dimensions.

In considering student performance in light of their experiences with the 3D learning modules, it is important to compare the students in the small- and large-enrollment classes. While the students in the small-enrollment class were accustomed to inquiry-based instruction, a pedagogical approach used throughout their undergraduate career, the large-enrollment class did not employ this method extensively. However, even though students in the large-enrollment class reflected some reluctance with using the models and learning modules (Fig. 6A), these students still showed improved learning gains. In fact, their gains on the same module were higher than those for the small-enrollment class (Fig. 2).

Recommendations for Incorporation

Individual instructors can decide how many 3D learning modules to incorporate and how to use the modules in the classroom. We provide the final version of each module in the Supporting Information (Module I interactive, S1; and assessment, S2; Module II interactive, S3; and assessment, S4; Module III interactive, S5; and assessment, S6; model-specific resources, S7; instructions for model preparation, S8; and surveys for student experiences,

S9). The interactive portions of each module are designed to fit into one class period with actual times ranging from 50 to 75 minutes depending on how much discussion the instructor integrates into the class.

When deciding order in the course, we recommend that instructors begin with the DNA versus RNA structure and function module, but that the other modules can occur in any order. Moreover, instructors can customize the module by adjusting which pieces or how much of the activities are deployed and how often to regroup students during the class. Although shown to be effective in lecture courses, these 3D learning modules can also be used in recitations or small-group tutoring environments.

For instructors planning to implement these modules in their class, we suggest considering motivations for delivering a pre- and postassessment. For the pilot studies, we used identical pre- and postassessments to measure learning gains. However, instructors might give a preassessment covering background material or knowledge from pre-class readings and a higher-level postassessment that tests understanding of the content taught. Alternatively, an instructor might eliminate the preassessment entirely, or might integrate the postassessment with the course's exams in order to test on concepts covered with the models and activities.

Conclusion

Through interaction with these 3D learning modules, students gained skills in relating molecular structure to biochemical function, evaluating molecular dynamics in light of structure–function relationships, and translating between the 2D and 3D. Moreover, instructors can employ these modules in any context or course for which the content is relevant, including lecture, flipped-classrooms, recitation, or small group tutoring. Finally, we provided complete instructor guidelines for each module in the Supporting Information.

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Conflict of Interest — The authors declare that there are no conflict of interests.

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