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Laboratory Experiment

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3D Printing of Protein Models in an Undergraduate Laboratory: Leucine Zippers

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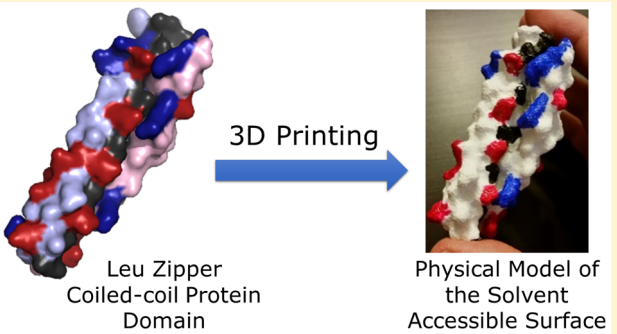
Supporting Information

ABSTRACT: An upper-division undergraduate laboratory experiment is described that explores the structure/function relationship of protein domains, namely leucine zippers, through a molecular graphics computer program and physical models fabricated by 3D printing. By generating solvent accessible surfaces and color-coding hydrophobic, basic, and acidic amino acid residues, students are able to visualize noncovalent interactions that are important in protein folding and protein−protein interactions.



KEYWORDS: *Upper-Division Undergraduate, Biochemistry, Computer-Based Learning, Hands-On Learning/Manipulatives, Proteins/Peptides, Laboratory Computing/Interfacing*

INTRODUCTION

The connection between a biological molecule's structure and its function is one of the most important concepts in biochemistry. It has been shown that students who have access to both molecular imaging programs on the computer and physical models of biological molecules are better able to answer questions about structure/function relationships than other students.[1] Due to the complex structures of proteins and other biological molecules, it has been challenging in the past to generate physical models for students to use when studying structure/function relationships in biochemistry.[2−4] However, 3D printing (additive manufacturing) has recently become much more accessible for students. 3D printing, along with high quality molecular imaging software, now provide students with powerful visualization tools to help them understand the interactions of complex biological molecules, especially proteins.

The utility of 3D printers as a pedagogical tool has recently become evident, particularly in the fabrication of physical models used to enhance student learning.[5−8] In an effort to integrate this emerging technology into the biochemistry curriculum, a lab was developed that utilizes printed 3D models to explore protein folding and protein−protein interactions with a blog post by Jessica Polka[9] as a starting point. The lab experiment connects a common molecular graphics system, PyMOL,[8,10] with 3D printed models to visualize the structure and fold of several coiled-coil leucine zipper protein domains[11] (Figure 1). By representing the solvent accessible surfaces of each helix of the coiled-coil domain, students are able to visualize how the two helices interact by literally fitting the two models together, allowing them to explore how different amino acid residues interact in a very detailed, yet easily understood, system.

Leucine zippers were chosen for this project because of their ubiquity in nature[13] and their utility in designed systems,[14] but also because of their potential for the demonstration of important noncovalent interactions that drive protein folding. Leucine zippers were first proposed in 1988 as a motif in several DNA binding proteins. Their name is derived from the presence of heptad repeats (Figure 2A) in which leucine residues are arranged at the solvent-excluded coiled-coil interface in an interdigitated fashion, reminiscent of a zipper (Figure 2B).[11] The geometry of the helices allows amino acid residues on opposite strands to interact. In particular, acidic and basic residues can participate in electrostatic interactions, stabilizing the dimer (Figure 2C). The hydrophobic and electrostatic interactions that stabilize the coiled-coil are also important in the folding of globular proteins. The solvent excluded surface or "ridge" of the coiled-coil domain is analogous to the hydrophobic core present in most globular proteins, while the electrostatic interactions are common in both intra- and intermolecular interactions of proteins. By modeling leucine zipper domains with these interactions highlighted, both in the computer graphics system and as 3D printed models, students can gain a better understanding of those interactions and, hence, protein folding more generally. Previous work by others[1] has shown that the combination of computer models and physical models enhances student understanding of biological molecules. In this project, the concepts covered can be extended from leucine zipper domains

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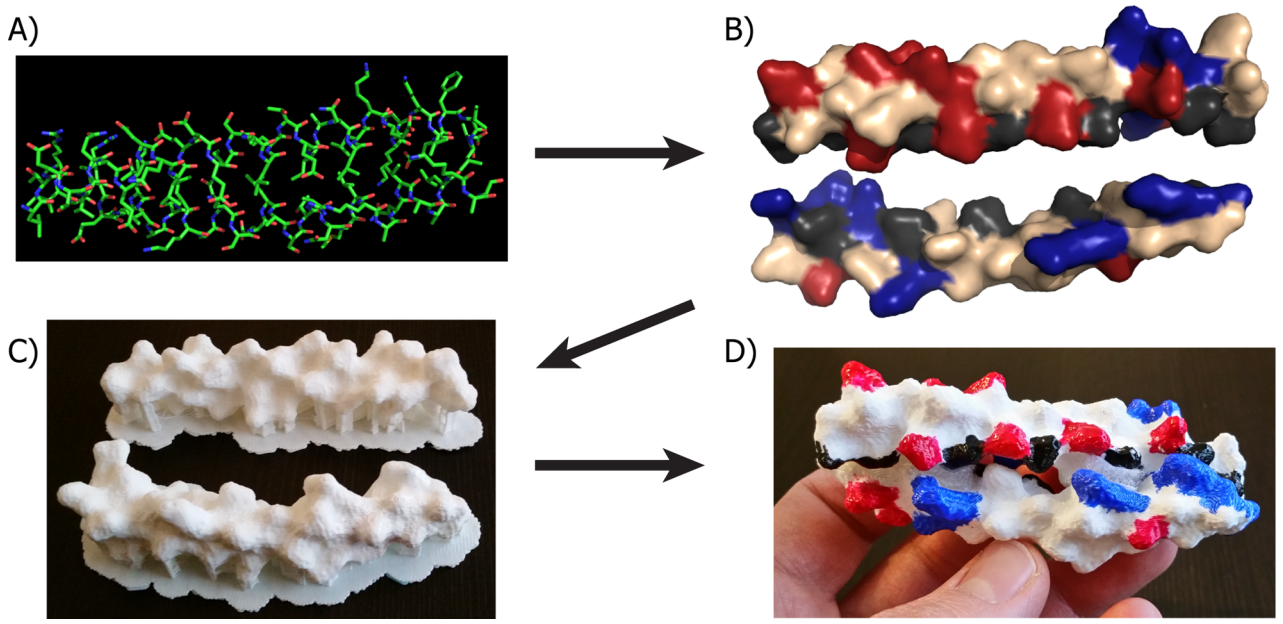

Figure 1. (A) The stick model of the Fos−Jun leucine zipper protein domain (PDB ID: 1FOS)[12] is shown with the default display settings using PyMOL molecular graphics software. (B) The solvent accessible surface for the separate Fos and Jun helices generated using PyMOL. (C) The rough models of the separate helices as fabricated by a 3D printer. (D) The finished physical model of the Fos−Jun leucine zipper.

(as important as they are) to globular protein folds more generally.

PEDAGOGICAL GOALS

The pedagogic goals for the experiment presented here include the following:

- Introduction to the PyMOL Molecular Graphics System and manipulation of different 3D graphical representations of proteins.
- Introduction to 3D printing (additive manufacturing).
- Increased understanding of 3D structures of proteins, noncovalent interactions of proteins, protein folding, and leucine zipper coiled-coil domains.

EXPERIMENTAL OVERVIEW

The lab consists of two class meetings of 3 h each. In the first class meeting, students are given a prelab lecture reviewing noncovalent interactions in proteins and other concepts pertaining to protein folding. Students then familiarize themselves with the PyMOL software and manipulate their assigned leucine zipper computer models. At the end of the first class meeting, students begin the process of fabricating the physical 3D models of their leucine zipper domains. In the second class meeting, students complete the printing of their leucine zipper models, color-code selected amino acid residues, and explore the interactions of the two helices of the coiled-coil.

EXPERIMENT

Simplified PDB files are prepared for students by isolating the leucine zipper domains from seven different structures downloaded from the Protein Data Bank (PDB) Web site.[17,18] The structures are chosen solely based on the presence of leucine zipper domains and are given to students without further analysis or manipulation by the instructor. The structures used are the following: the GCN4 homodimer (PDB ID: 2ZTA),[19] the Fos−Jun heterodimer (PDB ID: 1FOS),[12] the cGMP dependent protein kinase I beta (PDB ID: 3NMD),[20] the c-Myc-Max heterodimer (PDB ID: 1A93),[21] the MafA homodimer (PDB ID: 4EOT),[22] the HY5 homodimer from *Arabidopsis thaliana* (PDB ID: 2OQQ), and a synthetically designed leucine zipper (PDB ID: 1U2U)[23] (see Supporting Information for Instructor Notes and the simplified PDB files).

Prior to the first class meeting, students download three pieces of software onto their laptops: PyMOL[24,25] (a molecular visualization program), MeshLab[26,27] (a 3D mesh processing and editing program), and MakerBot Desktop[28,29] (a 3D printing control program). All three of these programs are currently available free of charge for students.

During the first class meeting, a short prelab lecture is given on protein folding, after which groups of two students choose a leucine zipper domain from the list, download their simplified leucine zipper PDB files, and carry out several manipulations of the structures in PyMOL to acquaint themselves with the program, while at the same time highlighting various aspects of the leucine zipper structures. After students become comfortable with the basics of PyMOL, the groups separate their coiled-coils into two different PDB files, each containing one helix of the leucine zipper. Each student then continues the procedure individually with his or her own helix from the pair.

Using the new PDB files with the isolated helices, students color all leucine and isoleucine residues black, all of the acidic residues (aspartate and glutamate) red, and all of the basic residues (arginine, histidine, and lysine) blue, regardless of their position within the amino acid sequence. Isoleucine residues are included because they sometimes, though rarely, are found in the position of the heptad repeat normally occupied by leucine. Instructors might wish to exclude isoleucine residues for the sake of simplicity. Once the pertinent residues are color-coded, students use PyMOL to calculate the solvent accessible surface of their helix. Next, students prepare a 3D mesh file (VRML 2 format) of the resulting structure. Currently, the only 3D mesh file format available in PyMOL is not compatible with

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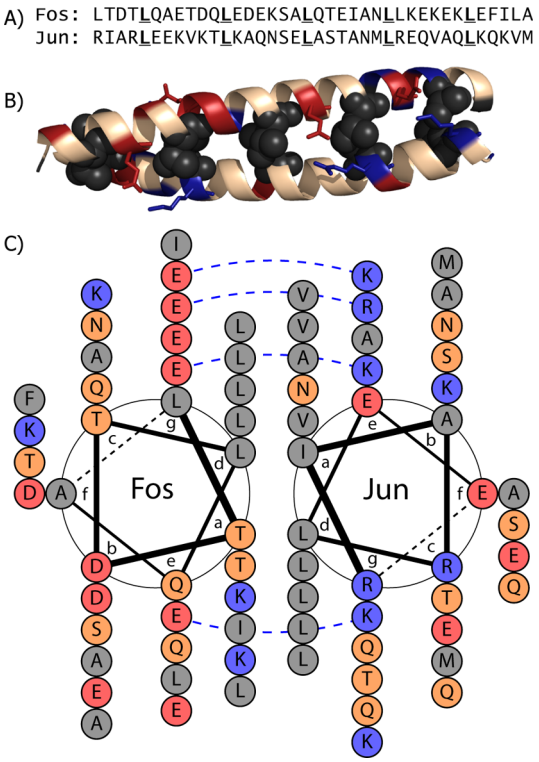

Figure 2. (A) The amino acid sequence of the leucine zipper portion of the Fos−Jun dimer. The Leu residues of the heptad repeat are shown in bold underline. (B) A PyMOL rendered cartoon model of the Fos−Jun leucine zipper coiled-coil domain with Leu residues shown in black, basic residues (Arg, His, Lys) colored blue, and acidic residues (Asp, Glu) colored red (PDB ID: 1FOS).[12] The Leu residues of the heptad repeat are shown as spheres, while acidic and basic residues predicted to participate in electrostatic interactions (based on the helical wheel diagram below) are shown as sticks. (C) A helical wheel diagram of the Fos/Jun interaction emphasizing the heptad repeat. Predicted electrostatic interactions are each highlighted as a dotted blue line. The helical wheel was produced using the DrawCoil 1.0 program.[15,16]

the software provided with the MakerBot 3D printers. However, several freely available programs exist that can convert 3D mesh files to different formats. The program used in this experiment, MeshLab, is reliable and available free of charge to students.[27] Once students convert their files to the OBJ (Alias Wavefront Object) format, they import them into the MakerBot Desktop software.

To maximize the efficiency of the print, students manipulate their helices in the MakerBot Desktop software and lay them as flat as possible on the build platform of the 3D printer without disrupting their original shape. Students also use the software to generate scaffolding and rafts, as well as scaling the models to 150% of the original size. The models are printed on a 3D printer using ABS (Acrylonitrile Butadiene Styrene) filament (Figure 1C). Please see Supporting Information for detailed experimental procedures and potential issues with the use of the 3D printers.

During the second class meeting, the printed models are completed. The scaffolding and rafts on the printed models are removed, and black, red, and blue nail polish is used to paint the models according to the coloring scheme previously implemented in PyMOL. Mistakes can be covered using white correction fluid or white nail polish. Nail polish remover dissolves the ABS polymer and must be avoided. After the nail polish is dry, students join with their partners and orient the two helices of their assigned leucine zipper with the help of the PyMOL files that they had previously generated. Once the correct orientation is established, students couple the two helices via two small patches of Velcro, which form a sturdy but reversible connection for the coiled-coil dimer. Students use the completed models to explore the leucine zipper structure and to answer questions for their lab reports.

HAZARDS

The 3D printers extrude hot ABS polymer; therefore, safety instructions from the manufacturer should be followed. The extruders and the build plate of the 3D printers are heated and thus represent a burn risk. The nail polish and correcting fluid do not pose a significant safety hazard; however, care should be taken to avoid spills and drips in the lab.

RESULTS AND DISCUSSION

The experiment was completed one time by 10 students divided into two sections of five students each. In one section, two groups of two students and a student working individually completed the experiment. In the second section, one group of two students and another group of three students completed the experiment. While groups of two seemed to function best, all of the teams completed the assignments without undue difficulty.

Seven PDB files were provided to students. While the procedure could easily be expanded to include the retrieval of leucine zipper coordinate files from the Protein Data Bank, it was felt that the lack of familiarity of the students with the database would prove to be an impediment to the main thrust of the experiment. Students were able to acquaint themselves with the leucine zipper structure using PyMOL (Figure 3A) and to color-code the pertinent residues (Figure 3B). When the models were printed, the students scaled their structures to 150% of the default size exported from PyMOL. This size provided a good compromise between length of the print

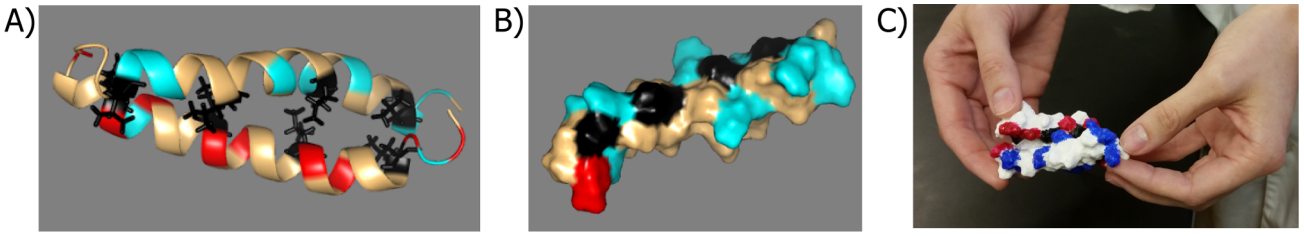

Figure 3. Student-generated data for a synthetically designed *acidic/basic* leucine zipper (PDB ID: 1U2U).[23] (A) The cartoon of leucine zipper dimer. (B) The solvent accessible surface for the isolated *basic* helix. (C) The completed leucine zipper printed and color-coded by the students.

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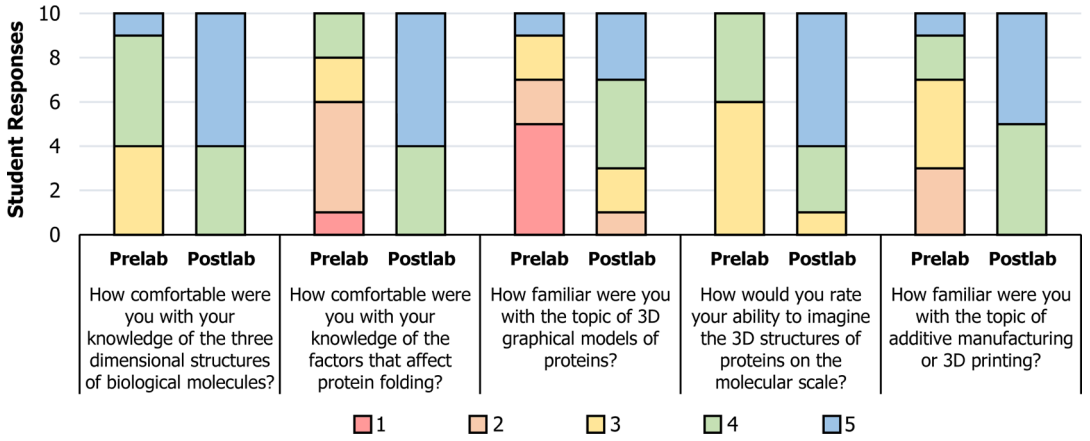

Figure 4. Prelab and postlab survey results. Students answered the questions indicated on a scale of 1−5, where 1 represented the lowest confidence level and 5 represented the highest. The surveys were given before running the experiment (Prelab) and after the experiment was completed (Postlab).

(larger objects take longer to print) and detail of the resulting models. Several students made errors in the scaling of the models, resulting in helices that were not of the same size and, thus, did not fit together in the final analysis. The erroneous models were rescaled and reprinted. Properly scaled models ranged from 75 to 100 mm as measured along the length of the helix. At that scale, the individual helices took approximately 1 h to print and consumed roughly 10 g of filament. Because there were more students than 3D printers available (5 students per section, 2 printers), several of the models were printed outside of class time. Those students who were not able to print their models before the second class meeting were assisted in doing so at that time. A completed leucine zipper is shown in Figure 3C.

Student feedback for the lab was overwhelmingly positive. Each student demonstrated progress on the pedagogical goals. The goals of learning about PyMOL and 3D printing were assessed by the students' ability to produce high quality images and 3D models of their proteins, respectively. Every student submitted good quality images of the leucine zipper and successfully produced a 3D printed model. The goals of increasing student understanding of 3D structures of proteins, noncovalent interactions of proteins, protein folding, and leucine zipper coiled-coil domains were assessed by pre- and postlab surveys, as well as questions included in their lab reports (see Instructor's Notes in Supporting Information for answers to questions included in the lab reports). In the surveys, students assessed their comfort level, using a 5 point Likert scale, with their knowledge of the three-dimensional structures of biological molecules and the factors that affect protein folding. The results showed an increase in comfort level for both of these questions (Figure 4), although the sample size was small. Through the survey, students also expressed an increased familiarity with 3D graphical models of proteins and 3D printing, as well as a better ability to imagine the 3D structures of proteins on the molecular scale.

Students were also given an opportunity to express their opinions about the lab and suggest improvements. The comments were overwhelmingly positive, both with regard to use of PyMOL and to 3D printing. However, there were a few areas of concern for students. The most common negative comment from students was that the actual 3D printing of the models took too long. The major reasons for the long downtime during the printing were that 10 students across two sections were using only two printers, and the printers needed to be monitored constantly. Both of these concerns can be addressed by purchasing new 3D printers (see discussion below). Also, the time waiting for the printers to complete the model could be filled by asking students to complete more activities with PyMOL. Extra work with PyMOL could have an added benefit of increasing student understanding of this multifaceted program.

An area of concern for students who were unfamiliar with the use of nail polish was difficulty in painting the models. Students familiar with nail polish did not share this concern. Colored permanent markers were explored as a substitute for nail polish, although the quality of the resulting models suffered (see Supporting Information for further discussion).

One potential barrier to integrating 3D printing into laboratory curricula is the cost of the printers. Like many emerging technologies, the costs of 3D printers were initially high, but have decreased rapidly. Several companies now offer 3D printers with varying levels of cost and features. MakerBot Industries, LLC produced the *MakerBot Replicator 2X* printers used in the experiment described here, but a cheaper printer, the MakerBot *Replicator Mini* ($1,375)[30] could be used without significant changes to the lab procedure. An added benefit of these lower-cost printers is that they, like other newer models, feature an onboard camera that allows for remote, real-time monitoring of the print jobs. Purchasing several printers with onboard cameras should address the aforementioned student concern about the time intensive monitoring of the printing. Due to the decreasing cost and increasing features of 3D printers, this technology will likely be accessible for many institutions, especially if the costs can be defrayed by interdepartmental collaboration and sharing of the devices. The lab could also be run concurrently with other experiments on a rotating basis so that fewer students would need to use the 3D printers in any particular week.

As 3D printers mature as a technology, they will inevitably emerge as powerful tools for education. The experiment described here can be expanded to encompass protein domains other than leucine zippers. For instance, students could model interesting aspects of globular proteins, including protein−ligand interactions, protein−protein interactions, and conformational change. In addition to allowing for the fabrication of models, 3D printers also provide the opportunity for students to design and produce custom lab equipment.

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Furthermore, applications in other fields such as physics, engineering, math, and art could make 3D printers ubiquitous in higher education. It is reasonable to believe that as 3D printing becomes commonplace, it will eventually be considered an essential part of many curricula.

CONCLUSION

In the lab presented here, students used the buried hydrophobic residues and electrostatic interactions found in leucine zippers as a simplified system for the study of protein folding in general. Like leucine zippers, globular proteins fold in a way that excludes water from the hydrophobic core. Furthermore, the folds of globular proteins are stabilized by noncovalent interactions like the hydrophobic and electrostatic interactions highlighted on the models generated in this lab. Through the use of the molecular graphics software and the physical models, the hydrophobic and ionic interactions that are ubiquitous in folded globular proteins were displayed in an easily understood format in the guise of leucine zippers. The separate chains and simple structures of the leucine zipper models lay bare these important folding interactions that are usually buried in globular proteins. Student feedback indicated that the lab was informative and engaging.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available on the ACS Publications website at DOI: 10.1021/acs.jchemed.5b00207.

Notes for instructors (PDF, DOCX)
Laboratory procedure hand-out for students (PDF, DOCX)
Prelab and postlab surveys and quizzes (PDF, DOCX)
Acidic-basic peptides (PDB)
c-Myc−Max heterodimer (PDB)
Fos−Jun heterodimer (PDB)
GCN4 homodimer (PDB)
HY5 homodimer (PDB)
MafA homodimer (PDB)
PKG Leu zip (PDB)

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Notes

The authors declare no competing financial interest.

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