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Combining 3D-Printed Models and Open-Source Molecular Modeling of p53 To Engage Students with Concepts in Cell Biology†

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INTRODUCTION

Students learn best when engaged, for example with student-centered learning strategies that allow them to experience concepts through multiple modes, including kinesthetically (1–4). In STEM fields, easy access to tools such as 3D printers and open-source molecular structure viewers has provided affordable and practical alternatives to engage students by allowing them to model biologically relevant entities—including molecules and electron orbitals—and their functions (5–15).

In this report, we describe the combined use of 3D-printed macromolecular models and Jmol (an open-source Java 3D molecular structure viewer) to facilitate students' engagement with molecular concepts in cancer biology. While the literature has demonstrated the individual utility of 3D-printed models and Jmol exercises in the undergraduate classroom, we chose to combine these complementary activities to deepen students' understanding of the relationship between structure and function of macromolecules through multimodal learning (16–18). This report's modules focus on the biology, structure, and function of the human p53 protein, a tumor suppressor molecule famous for coordinating DNA repair, senescence, and apoptosis and for its frequent mutation in many different types of human cancers (19). The role of p53 as a transcription factor that binds DNA and activates target gene expression exemplifies the close relationship between macromolecular structure and cellular function. Student engagement is also encouraged by the relevance and relatability of the content, including the importance of p53 to human disease states that have touched many of their lives at a personal level.

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We share two modules: one that was implemented at the undergraduate level and the other at the graduate level. To complete these modules, students used a 3D-printed model of p53 bound to DNA, its corresponding Jmol files, and database and literature searches to answer a series of questions. In the process of doing this work, students had the opportunity to review and integrate previously learned content that helped them hit the ground running with course material.

PROCEDURE

Materials

Students require access to an instructional handout (Appendix 1 or 2; answer keys for instructors are available from the authors upon request), a white 3D-printed model of p53 bound to DNA (see Appendix 3 for detailed instructions and tips), and different color paints and a brush to paint the model as indicated. Students receive their own 3D-printed p53 bound to DNA model kit (Fig. 1). Students also need a computer for access to relevant Jmol sites.

Below we describe not only the two contexts in which we have implemented these modules but additional alternatives for course implementation.

Upper-Level Undergraduate Course: Cell Biology

Our module (Appendix 1) was used as a beginning-of-the-semester take-home assignment so that students could review concepts they had learned previously through foundational biology courses, setting the stage for subsequent discussions of new cell biology course content. The worksheet was introduced and assigned during the very first class period and covered macromolecules, protein and DNA structure, and basic cellular functions. During the 2-week period provided to complete it, students reached out to the instructor for assistance as needed, kicking off the semester with open communication and conversations between instructor and students. Students ultimately turned in their assignments through the online course management

system and orally presented their findings to the class (see Appendix 1 for presentation details and options). At the end of the activity, students were encouraged to keep their 3D model to use it as a study aid for future discussions and content. We referred back to p53 several times during the course to help segue between topics or units, for example, to transition from basic macromolecular structure to how different macromolecules (e.g., proteins, DNA) can interact with one another to generate new functionalities in cellular processes, such as transcription. Other content segue examples that benefitted from this exercise included transitions from transcription to control of gene expression, from intracellular compartments to systems like nuclear protein transport, and from cell cycle and cell death to cancer. Students' engagement in the exercise provided them with a wealth of information to refer to as they strived to make connections between new content and fundamental principles of macromolecular structure and function. Some of these details are reflected in the student comments provided in Appendix 4.

Graduate Biology Course: Molecular Mechanisms of Cancer

This is a graduate-level course modeled after a published Course-Based Undergraduate Research Experience (CURE) (20). In brief, students worked in pairs to analyze different p53 missense mutations identified in one or more human tumors but not yet fully characterized. This activity (Appendix 2) was implemented in the third week of the course, after students were introduced to the molecular mechanism of p53 function. The introduction period focused on p53 regulatory elements required for transactivation and provided training in qualitative and quantitative assays (growth fitness, beta-galactosidase filter assays) to assess the ability of each p53 mutant to transactivate expression of reporter genes. The mutants worked on by the students presented different transactivation defects, enabling the students to develop hypotheses for why their mutant was defective. During the activity, students conducted DNA sequence alignments on their mutant versus normal p53 and determined the amino acid identity of their missense mutation. They then used online molecular modeling software (Jmol) to view the published structure of p53 binding to DNA. As part of this exercise, each student received their own 3D-printed p53 bound to DNA model kit (Fig. 1) and located their mutation on the painted models.

Interestingly, mutations that mapped directly to the p53/DNA interface were easily interpreted by the students as clear DNA binding defects, using either visualization software or 3D-printed models. On the other hand, mutations merely in close proximity to the DNA interface proved difficult to interpret using visualization software alone, and students only recognized the potential of these mutations to indirectly influence DNA binding upon visualizing them on their 3D-printed models. Additionally, students used their models throughout the semester to discuss their results during mock lab meetings as well as during their final poster presentations. Students found their models particularly useful during poster presentations, because they provided an easier platform for discussing their findings (Appendix 4).

FIGURE 1. 3D-printed p53 bound to DNA model kit. Each undergraduate and graduate student received the kit to work with as part of the activity described. They were encouraged to keep their model throughout the semester to use as a learning tool as needed.

Alternative Applications

This activity could benefit students enrolled in introductory courses. It could be implemented as an in-class lab.

Safety Issues

None

CONCLUSION

Coupling macromolecule 3D-printed models and online molecular viewing provides students with two learning tools that immediately engage them in questions of structure and function in a visual, memorable, and kinesthetic way while also providing downstream benefits following the activity. We found that the two components complemented one another well, providing opportunities for students to recall prior knowledge and apply it to novel situations.

SUPPLEMENTAL MATERIALS

Appendix 1: Instructional handout for undergraduate students
Appendix 2: Instructional handout for graduate students
Appendix 3: Step-by-step guide
Appendix 4: Table S1, student feedback from two pilot institutions

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Appendix 1: Instructional Handout for Undergraduate Students

Using 3D-printed macromolecular models as a review tool in the Cell Biology classroom—p53 subunit bound to DNA

INTRODUCTION:

Welcome to our Cell Biology course! The general Biology and Chemistry courses you have taken so far have prepared you well to hit the ground running in our study of Cell Biology. You may already be familiar with the key concepts of macromolecular structure. For example, What is a protein? What is an amino acid? What is DNA and what does it look like at the molecular level? We will not have time to review these basic concepts in detail, but will build on this previously learned material and delve into additional layers of detail related to the inner workings of cells. To facilitate our reactivating knowledge that we have already acquired, we will have review activities throughout the semester. This is our first review activity and it focuses on concepts related to macromolecular structure.

Using this first assignment as a tool, you will have the opportunity to do some directed review of important concepts related to protein and DNA structure. You will also be able to think about how the function of these macromolecules is influenced by their molecular shape or structure. Lastly, you will be able to demonstrate your knowledge in these areas using the written and spoken word. As scientists-in-training, not only do you have to master specific content, but you need to demonstrate mastery of this content though writing and speaking—showing your full grasp of technical vocabulary and ideas.

After engaging in this activity, students will be able to:
-Discuss and explain, in their own words and in front of an audience, the meaning and interconnectedness of the following concepts:

- Macromolecule
- Polymer
- Monomer
- Proteins
- Protein primary, secondary, tertiary, and quaternary structure
- Protein subunit
- Protein Domain
- Nucleic Acid
- DNA
- DNA structure
- DNA Major groove
- DNA Minor groove
- Amino acids
- Nucleotides
- Covalent bond
- Peptide bond
- Phosphodiester bond
- Non-covalent interactions
- Hydrogen bonds
- Base pairs
- Mutation
- Binding

INSTRUCTIONS:

1. *Before you begin:*
During class time, you will take the 15-multiple choice question pre-assessment on macromolecular structure.

2. Alongside this worksheet you will receive a 3D-printed p53 model kit, a box containing the following: a macromolecular model of a p53 subunit bound to DNA, a paint set, and a brush.

3. As a take-home assignment, generate a document/write-up that answers the questions and completes the prompts that are listed below. While some parts of the document direct you to answer questions, others require that you paint parts of your 3D model certain colors and refer to online resources to formulate hypotheses about macromolecular function. When citing references used, if applicable, please use the American Medical Association (AMA) style of reference and in-text citations (http://guides.highpoint.edu/ama). The AMA format requires that you add a superscript number at the point in the paper where you are citing a resource(s). At the end of the document, list references numerically in the order by which they were cited in the text.

4. Upload your written work to the portal that can be found on our online course management system.

5. Bring your finished 3D model to class, where you will have 5 minutes to present your model and present the highlights of what you learned or reviewed through this activity. You are encouraged to practice your presentation so that you use all of the time available to you and do not go over your allotted time.

3D MODEL: p53 SUBUNIT BOUND TO DNA

I. **Read these three short articles to learn about the discovery and function of p53, as well as about the scientist who helped discover and characterize it: https://pdb101.rcsb.org/motm/31**
http://www.bioinformatics.org/p53/introduction.html
https://www.molbiolcell.org/doi/10.1091/mbc.E18-06-0396

Answer the following questions. **You may also use your textbook to help you answer them.**

1-What is an oncogene?
2-What is a tumor suppressor gene?
3-Is the gene coding for p53 considered an oncogene or a tumor suppressor gene? Why? Briefly explain.
4-How did Dr. Lozano ultimately test her hypothesis that p53 functions as a transcription factor?
5-What kind of polymer/macromolecule is p53?
6-How many and what kind of monomers is p53 composed of?
7-What type of bond is responsible for joining together these monomers into a functional complex?
8-What is the location of the p53 gene in the human genome?
9-What is the size of the p53 gene (in base pairs)?
10-Can DNA be considered a polymer? Briefly explain.
11-Name the type of bond responsible for joining monomers together in a large DNA molecule.

II. **Paint your model using the following color code:**
-Paint the DNA double helix **Green**
-Paint protein alpha-helices **Blue**
-Paint protein beta-sheets **Red**
-Paint protein unstructured regions **Yellow**

Answer the following questions. **You may also use your textbook to help you answer them.**

1-Which of the 4 levels of protein structure are present in your 3D model? Briefly explain.

2-What kind of diagram was used to highlight the structure of p53 in your model (i.e. ribbon, space-filling, backbone, or ball-and-stick)? What are the advantages of this type of representation and what element(s) of p53 structure does it highlight?
3-Are unstructured regions important for p53 function? Briefly explain and cite any additional sources of information used.
4- Is the presence of hydrogen bonds implied in your model? Where? Briefly explain.
5- Any other non-covalent interactions whose presence is implied in your model? (Hint: Zn^{2+})

III. Use the webpage links below, including the PDB files (click on "*3D View*") 1tup and 1olg to explore p53 structures. Also, answer the questions below. You may also use your textbook, primary literature and other reputable sources to help you answer them.

https://www.rcsb.org/structure/1tup
https://www.rcsb.org/structure/1olg

1-Is one p53 subunit sufficient to yield a functional transcription factor? Briefly explain how you reached this answer and provide additional references used, if applicable.
2-Does p53 oligomerize? Briefly explain how you reached this answer and provide additional references used, if applicable.
3-Does p53 depend on a ligand for functionality and/or structure? Briefly explain how you reached this answer and provide additional references used, if applicable.
4-How many domains can be found in the wild type p53 protein? Briefly explain how you reached this answer and provide additional references used, if applicable.
5-What is the function of each of these domains? Briefly explain how you reached this answer and provide additional references used, if applicable.
6-p53 mutations that are associated with human cancers often map to one of these domains. Which one? Briefly explain how you reached this answer and provide additional references used, if applicable.
7-If possible, find this highly mutated region in your model and hypothesize the effects these mutations might have on p53 binding of DNA.
8-Are all p53 domains present in your 3D printed model? Identify the domains present in your model. Briefly explain how you reached this answer.
9-Locate the N- and C-terminus of p53 in your 3D model (Hint: Using the appropriate PDB file and 3D View linked above will help)
10-How does p53 recognize and bind the DNA regions of interest? Briefly explain how you reached this answer and provide additional references used, if applicable.
11-Have scientists identified the consensus DNA sequence that p53 binds? Briefly explain how you reached this answer and provide additional references used, if applicable.
12-What kind of chemical bond and/or interaction is involved in this binding? Briefly explain how you reached this answer and provide additional references used, if applicable.

IV. Prepare for your p53 3D model presentation in class.

You will have 5 minutes to walk our class through your 3D model and salient p53 features. You have two options for your presentation:

Option 1: In your presentation discuss the following (You have already tackled all of these questions above, now you just have to tell us about what you know/learned in your own words):

Hint: The points to discuss during your presentation have been listed in decreasing level of scope—from most general and overarching to most specific and detailed. It would be a good idea to stick to this general structure.

1-Give a one sentence introduction to p53, its function and what your 3D model is showing in terms of macromolecular structures/domains.

2-Discuss how the 3D model at hand displays a particular set of protein structure levels using a particular schematic type. Remember to use your colors as a tool to indicate to your audience what you are referring to during your presentation.
3-Discuss how non-covalent interactions contribute to both protein and DNA structure by sharing one example that applies to p53.
4-Discuss what type of chemical interactions allow for binding of p53 to DNA.
5-Discuss what type of mutation can lead to human disease and their likely location in your model.

Option 2: Select 5 discussion points or interesting facts related to p53 (learned through your work above) and share those with the group during your presentation.

It is important that you practice this presentation if you want to fluently engage with your audience and not go over the allotted time. You have 5 aspects to discuss and 5 minutes, 1 minute per discussion point, approximately.

Appendix 2: Instructional Handout for Graduate Students

Objectives

Experiment 1: Computer Analysis, 3D printed models of p53 DNA and Protein Structure

In this exercise, you will learn how to use online computer programs to analyze, align and translate DNA and protein sequences. You will also learn how to manipulate a 3-dimensional structure of p53 bound to DNA and to identify the location of particular amino acid residues within this structure. You will use these skills in your Post-lab assignment to identify the amino acid change produced by your p53 mutation and to identify the location of this altered amino acid within the p53 structure. You will also study 3D printed models to understand macromolecular structure. **This worksheet was adapted from Hekmat-Scafe DS, et al. *Biochemistry and Molecular Biology Education* (2017) 45(2):161-78.**

MATERIALS

Computer with internet connection and 3D modeling Set

Procedure

PART 1: DNA SEQUENCE COMPARISON AND ALIGNMENT

To analyze a DNA sequence and compare it to other DNA sequences, we will use a web-based program called Basic Local Alignment Search Tool (BLAST). The most commonly used BLAST server is at the National Center for Biotechnology Information (NCBI).

We will use the nucleotide BLAST (blastn) program to compare DNA sequences.

1. Access blastn here: http://goo.gl/i2yvR
2. Copy the sequence below and paste it into the box at the top of the window labeled: "Enter accession number(s), gi(s), or FASTA sequence(s)". Click on the blue "BLAST" button (bottom left) to initiate the search for similar sequences.

ATGGAGGAGCCGCAGTCAGATCCTAGCGTCGAGCCCCCTCTGAGTCAGGAAACATTTTCAGACCTATGGAA
CTACTTCCTGAAAACAACGTTCTGTCCCCCTTGCCGTCCCAAGCAATGGATGATTTGATGCTGTCCCCGGAC
GATATTGAACAATGGTTCACTGAAGACCCAGGTCCAGATGAAGCTCCCAGAATGCCAGAGGCTGCTCCCCCC
GTGGCCCCTGCACCAGCAGCTCCTACACCGGCGGCCCCTGCACCAGCCCCCTCCTGGCCCCTGTCATCTTCTG
TCCCTTCCCAGAAAACCTACCAGGGCAGCTACGGTTTCCGTCTGGGCTTCTTGCATTCTGGGACAGCCAAGT
CTGTGACTTGCACGTACTCCCCTGCCCTCAACAAGATGTTTTGCCAACTGGCCAAGACCTGCCCTGTGCAGC
TGTGGGTTGATTCCACACCCCCGCCCGGCACCCGCGTCCGCGCCATGGCCATCTACAAGCAGTCACAGCACA
GACGGAGGTTGTGAGGCGCTGCCCCCACCATGAGCGCTGCTCAGATAGCGATGGTCTGGCCCCTCCTCAGCA
TCTTATCCGAGTGGAAGGAAATTTGCGTGTGGAGTATTTGGATGACAGAAACACTTTTCGACATAGTGTGG
TGGTGCCCTATGAGCCGCCTGAGGTTGGCTCTGACTGTACCACCATCCACTACAACTACATGTGTAACAGTT
CCTGCATGGGCGGCATGAACCGGAGGCCCATCCTCACCATCATCACACTGGAAGACTCCAGTGGTAATCTAC
TGGGACGGAACAGCTTTGAGGTGCGTGTTTGTGCCTGTCCTGGGAGAGACCGGCGCACAGAGGAAGAGAAT
CTCCGCAAGAAAGGGGAGCCTCACCACGAGCTGCCCCCAGGGAGCACTAAGCGAGCACTGNCCAACAACAC
AGCTCCTCTCCCCAGCCAAAGAAGAAACCACTGGATGGAGAATATTTCACCCTTCAGATCCGTGGGCGTGA
CGCTTCGAGATGTTCCGAGAGCTGAATGAGGCCTTGGAACTCAAGGATGCCCAGGCTGGGAAGGAGCCAGG

GGGGAGCAGGGCTCACTCCAGCCACCTGAAGTCCAAAAAGGGTCAGTCTACCTCCCGCCATAAAAAACTCAT
GTTCAAGACAGAAGGGCCTGACTCAGACTGA

When the search result is ready, you will see a page with a box of horizontal red lines.

3. Scroll down the page to see the "Alignments"

   a) The Alignments are ordered with the best match to your sequence displayed first. What is
   the closest match to your sequence?

   b) Note the closest match sequence indicates **"Identities = 1181/1182 (99%)".** Find the one position where the sequences do not match. (**Note**: each vertical line indicates a nucleotide that is identical between your input DNA ("Query") and the homologous DNA Subject ("Sbjct") identified by the BLAST search.)

   c) Copy the p53-wt sequence above and paste it into a word processing document. Based on your answer to part (b), type in the correct nucleotide for the p53-wt DNA sequence, so that the ambiguous position ("N") now has the correct nucleotide.

PART 2: TRANSLATION OF DNA SEQUENCE INTO PROTEIN SEQUENCE

To analyze a p53 protein sequence, we will first use online tools to find the "open reading frame" (ORF) in the DNA sequence and translate it into the protein sequence of wild-type p53.

4. To identify the ORF in your DNA sequence, use ORF Finder:
   http://www.ncbi.nlm.nih.gov/gorf/gorf.htmL
   This program scans your sequence in all six possible reading frames for start and stop codons. Each ORF reflects the sequence extending from a start codon to a stop codon. Usually, the longest ORF is the protein-coding sequence.

   a) What is a codon?

b) What is a reading frame?

c) What is an open reading frame?

5. Enter your corrected p53-wt sequence in the box labeled "or sequence in FASTA format." Click the "OrfFind" button. In the following results window, click on the longest ORF (shown in blue/ turquoise). Your translated sequence will appear on the bottom. Click "Accept." Your ORF will appear in green. Now click "View" (top left).

   a) What do the letters in this translated sequence represent?

   b) List the two main processes that would have occurred in the cell to convert a DNA sequence into the sequence shown.

6. Copy your translated p53-wt protein sequence.

7. Now we will use protein BLAST (blastp) to compare protein sequences: http://goo.gl/Sfghm
Paste the p53-wt protein sequence you copied into the "Enter Query Sequence" box.

Leave "Database" at the default "non-redundant protein sequences (nr)."

Click on the blue "BLAST" button (bottom left).

a) What is the name of the protein that comes up first (the closest match)?

b) Will two proteins with the exact same amino acid sequences always have the same DNA sequences? Why or why not?

PART 3: VIEWING PROTEIN STRUCTURE

Note:

The Jmol applet you will use to view 3D structures requires a web browser that supports Java applets. Hence, before beginning this portion of the exercise, you will need to determine whether or not your browser supports Java applets by going to http://jmol.sourceforge.net/browsercheck/. Java downloads are available at: http://tinyurl.com/3ezf5gw. Please note that if you are using a **Mac**, you should use **Google Chrome** for Jmol.

To view p53 protein structure, we will use the Research Collaborative for Structural Bioinformatics (**RCSB**) Protein Data Bank. The three-dimensional shape, or structure, of proteins is most often determined by the method of X-ray crystallography. A high quality "crystal structure" can reveal the position of all of the atoms in a protein in three-dimensional space.

We will examine the structures of DNA (not a protein!) and, then, part of p53 bound to DNA.

8. Structure of B-form DNA: http://goo.gl/dLNFE
   a) Click on "3D View" in the biological assembly box. What are the building blocks of DNA called? What do the polygon shapes in the structure represent?

   b) Click on “Custom View” (to the right of the structure), select "Style," and change from Cartoon to Backbone or Ball and Stick, then back to again to Cartoon.

   c) Use your mouse to rotate the structure and identify the major and minor grooves. You may find it helpful to set “Surface” to “Cavities.” How do the major and minor groove differ?

9. Structure of p53 tetramer bound to DNA: http://goo.gl/TQz22
   a) Click on "3D View" in the biological assembly box. If the picture is cut off, right click in the Jmol structure box, choose “Zoom” then “Zoom out.”

   This structure only shows part of the whole p53 protein, the DNA binding domain. What other domains does p53 protein have that are not shown here?

   b) What is a tetramer? How many individual p53 molecules are shown in this structure?

   c) Use your mouse to rotate the structure. The yellow arrows represent secondary structures called □-sheets, and the pink helices represent □-helices. What properties of the protein dictate the formation of secondary structure?

   d) What type(s) of secondary structure interacts with the DNA? Do they recognize the major or minor groove? Why do you think that p53 binds specifically to the DNA-binding domain?

e) Under Display Mode (to the right of the structure), click on “Subunit” to see the 4 monomers in different colors.

10. Now, we will use the Jmol viewer to highlight one of the amino acid residues that is most commonly mutated in cancerous tumors. In a normal p53 protein, amino acid number 273 is an arginine (designated R273). In the mutated p53 found in some cancer cells, amino acid 273 is not arginine, but a different amino acid, histidine. This change is designated R273H.

    a) What specific difference from normal cells would cause tumor cells to produce p53 carrying the amino acid substitution R273H?

    b) Right click in the Jmol structure box. Choose "Select" and check "Selection Halos". The whole structure should appear yellow. Right-click "Select" and click on "None.” The yellow should disappear.

    c) Select and highlight all the atoms of residue Arginine 273 (R273) on all 4 monomers of p53 as follows: click on “Scripting Options” (bottom), type **select [arg]273** in the input box, then click on “Submit.”

    d) What effect would you predict the mutation, R273H, would have on p53 function?

11. Based on your results from the experiments today, in what domain of p53 (TAD, DBD, or OD) do you think your mutation will be located?

Now Paint your model using the following color code:

-Paint the DNA double helix **Green**
-Paint protein alpha-helices **Blue**
-Paint protein beta-sheets **Red**
-Paint protein unstructured regions **Yellow**
-Indicate the approximate location of the point mutation on your model using **Black**

12. Based on your results from your model and where your point mutation is located, how do you think this mutation will affect p53 functionality?

Appendix 3: A Step-by-Step Guide to Integrating 3D Printed Macromolecule Models and Open Source Molecular Modeling to Generate Inquiry-based Instructional Experiences

Step 1: Identify learning goals to be addressed through the activity

Our Example:
Target learning goals for our "p53 bound to DNA" activity:

1. Compare and contrast the structure and function of macromolecules
2. Discuss the potential effects of a mutation on DNA sequence, protein expression and/or function, phenotype, and cellular homeostasis

Step 2: Identify 3-D printable macromolecular structure(s) of interest to work with that is (are) aligned with course learning goals

1. Search the Protein Database Bank (PDB) http://www.rcsb.org/ for the macromolecular structure(s) of interest (you can download PDB files here if needed; make a note of database accession code). For users with little 3D printing experience, we suggest choosing smaller proteins with 2-3 secondary structures (beta sheet, alpha-helices).

2. Search https://3dprint.nih.gov/ for 3D-print files of interest using the database accession code from Step 1. If there are no 3D-print files of interest already available for download, you can enter your database accession code from Step 1 to generate a 3D-printable file. You can also modify a file to fit your needs (3).

3. Check that relevant coordinates/files are accessible in Jmol (1) or other open-source molecular viewer such as iCn3D (2).

Step 3: Download the newly generated 3D-printing file (STL) of interest as needed (3).

Step 4: Print models and assemble the kits (3).

Notes on performing 3D printing: Typically, we scale our models >150X and manually generate supports as needed. We also prefer using 3D printers with heated beds so that the printing surface is warm; makes the printing easier. Prints are usually optimized for speed and quality. For example, p53 bound to DNA model was modified to include a single p53 monomer. Print time for a single model was approximately 3 hours and required 15 mins of post-processing (support removal, trimming, sanding). Typically, we would printed six models overnight without technical difficulties. Printing filament, brushes, and cardboard boxes for the kits were purchased from Amazon. Each kit (as pictured to the left) cost us approximately $3.00 to assemble.

Notes about on campus or online resources that might offer free or low-cost 3D printing: Keep in mind that Media, Printing Centers, and/or Maker Spaces on campus will likely have 3D printers available for your use for free or at low cost. These centers or spaces will likely also have staff members that will be able to help you print the desired models. Alternatively, if none of these are available to you, there are online sites that specialize in offering affordable 3D printing services which would likely raise price per kit to $10. **Step 5:** Design the worksheet to help guide students through inquiry-based instructional experience. You can use our worksheet as a guide, keeping in mind what elements you would like your instructional experience to have.

Step 6: Implement the activity in the course/laboratory of interest (4)

Sample Implementation Scenario	**Description**	**Implementation Tips**
Classroom Review Technique	Implement in upper-level courses to review macromolecule structure/function basics.	-Assign to individuals or groups as a take home assignment -Assign to groups to work on together during class time -Have students/groups present the highlights of their findings at the end using their printed model as a tool.
Laboratory Review Technique; Introduction to p53 CURE experience (5)	Implement in laboratory courses to lay the foundation for research question comprehension and hypothesis generation.	
Classroom Segue Technique	Implement in upper-level courses to highlight relationship between macromolecule structure/function and cellular processes. First implement at the beginning of the semester as a review technique and then throughout the semester to segue between different units.	

Appendix 4

Supplementary Table 1: Student Feedback from Two Pilot Institutions

(Data collection complied with all relevant federal guidelines and institutional policies.)

What did you like about this activity?
Undergraduate Student Feedback • I thought it was a great start to the semester, if we keep the models, we can use them throughout the semester. • Painting the model. • It really helped me understand p53, which was helpful the entire semester as we discussed related content. *Graduate Student Feedback* • Having a physical representation of the protein we were working with all semester. • I liked that this activity allowed me to see the structure of the p53 protein bound to DNA and helped gain a better visual understanding. • 3D models are just cool in general, so adding this makes it more engaging and it's definitely easier to gain a conceptual understanding of 3D protein structure in this more physical format than simply using some software alone
What did you dislike about this activity?
Undergraduate Student Feedback • It was time consuming, not your fault though, depending on the individual and getting into every corner of the model to make it perfect. • For the first assignment questions seemed to difficult at first • Painting of the molecule • Nothing *Graduate Student Feedback* • Nothing. • The online software that was used to determine where the mutation was located was not super reliable. It was not supported by certain browsers and proved difficult to get it to function properly. • Painting the model is tedious and not particularly effective in more deeply analyzing the protein structure. There is maybe 20 to 30 seconds of making some mental division of the protein into its domains of interest and then a half hour to 45 minutes of mindlessly painting.
What did you find difficult about this activity?
Undergraduate Student Feedback • Nothing was difficult about the activity. • Some of the questions. • The part where we had to find sources (…) was very difficult. • Painting the molecule *Graduate Student Feedback* • Reaching some areas of the model with paint was somewhat difficult. • Identifying with confidence the relative location of our group's point mutation was probably the most difficult part of this activity.
What did you find easy about this activity?

Undergraduate Student Feedback • Coloring was the easiest and (…) relaxing part, not often do we get tasked with coloring, so having this as an assignment was enjoyable! • Some of the questions were ones that I already knew the answers to from genetics, so those were easy. *Graduate Student Feedback* • Recognizing different motifs of the protein. • Painting the different parts was fairly easy to determine, but was also super helpful in visualizing what was going on in the structure. • Conceptualization is much easier. Understanding what is meant by tertiary structure is simple to get as it relates purely to its definition, but generating a visual is sometimes more difficult. This activity allows more senses, both sight and touch, to be stimulated in order to assist in generating a more accurate view of the p53 protein.
What would you change about this activity to make it better?
Undergraduate Student Feedback • The distinguishing between the alpha-helices, beta-sheets was quite difficult, (…) it is hard to decide where one stopped and the other started. • Make it shorter. • More instructions about how to carry out literature research would help *Graduate Student Feedback* • Nothing. • I think going over how to determine where the mutation is on the structure in class or going over an example could have been useful! • I would eliminate the painting and focus only on marking the divisions and recognizing each domain and its function. I would incorporate virtual reality and also attempt to make sure that each group is working with mutations in more distant regions from each other so that something could be gained by explaining each model within the context of their mutation to each other.

Instructors collected student feedback provided in the table above. When student comments were similar to each other, one representative comment was chosen and presented in the interest of brevity.