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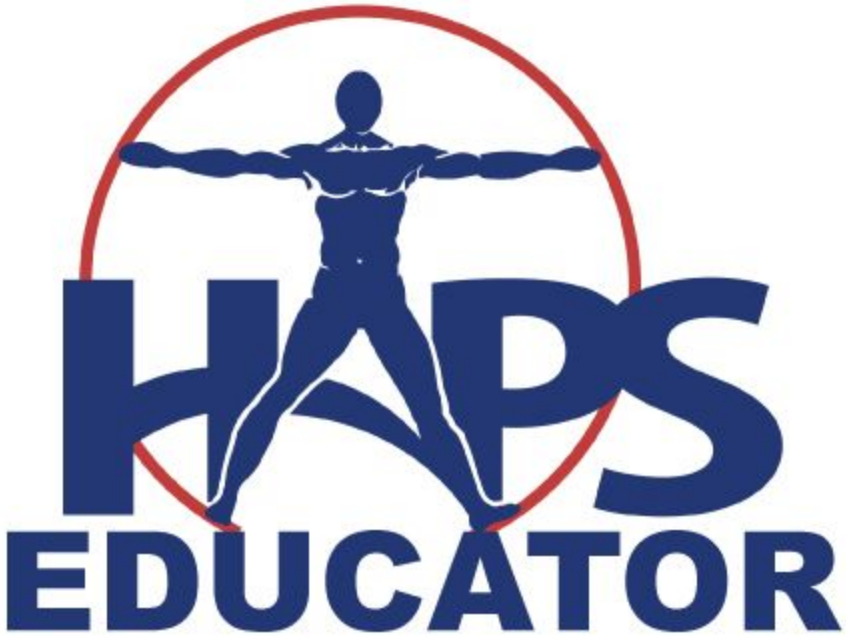
HAPS Educator. Vol 22, No. 1, pp. 73-78. Published April 2018. doi: 10.21692/haps.2018.009



Suchman E.L. et.al. (2018). Modeling Antibody-Epitope Interactions with 3D Printed Kits in Large or Small Lecture Courses. *HAPS Educator* 22 (1): 73-78. doi: 10.21692/haps.2018.009

Modeling Antibody-Epitope Interactions with 3D Printed Kits in Large or Small Lecture Courses

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Abstract
We used 3D printing to manufacture models that allow students to explore antibody-epitope interactions. One of the more difficult concepts for students in general microbiology and immunology courses is visualizing the interactions surrounding antibodies and the multiple epitopes found on antigens. We designed and printed antibodies that recognize different epitopes on the same viral antigen. Students can match different heavy chains and light chains creating 4 possible antigen-binding regions and then attempt to determine if and where on 3 possible viral antigens the antibody would bind. In this article we describe using 3D printing to create kits that can be used to demonstrate difficult concepts to students. The process used to develop these kits will be described as well as how the kits are used in large lecture (up to 150 students) flipped classes to augment student learning. This 3D model is available free for printing at https://www.thingiverse.com/thing:508595. doi: 10.21692/haps.2018.009

Key words: modeling, flipped classroom, team-based learning, active learning, 3D printing

Procedure
Modeling software
The kits were designed by a collaborative pair of honors students as an honors thesis project. One of the students was from Microbiology (Steven Denham) and one was from Computer Science (Dana Shatila). We searched for an open-source 3D modeling program that fit the following specific criteria:

1. An intuitive interface suitable for beginners.
2. Functions to create basic shapes and group them together (for the simple "Y" shape of an antibody or a plausible representation of a virus)
3) Tools that would allow us to make an impression of an object in a second object. In this case, the binding site would be the impression and the epitope the original object).

For those interested in creating their own 3D printed instructional models, there are multiple open-source programs suitable for beginners, such as SketchUp (© Trimble, Inc.), and Tinkercad (© Autodesk, Inc.). We found that Tinkercad, which does not create shapes as faces as SketchUP does, allowed us to create more readily recognizable antigens, epitopes and antibodies. We suggest trying other modeling soft ware if difficulties arise in creating the desired shapes.

We modeled our antibodies and viruses in Tinkercad (https://tinkercad.com/). The in-browser-operated program utilizes two basic functions. The first function is shape creation. Users are initially limited to the shapes Tinkercad has to offer, or those that they can import. The second function is the ability to group shapes in order to create more intricate designs. When grouping, a shape may be designated as a "hole" or a "solid". If overlapping solids are grouped, they will combine to form one shape. If an overlapping hole and solid are grouped, the hole will be subtracted from the new shape, leaving an imprint of its former self on the solid. We used this function to model our antibodies. We pressed antibody cylinder "solids" onto viral antigen "holes" and grouped them, leaving a perfect binding site impression in the antibody. Basic shapes were also arranged and grouped to design a cartoon version of Influenza A virus, which we eventually chose to be our model.

Design
Our complete model consisted of the following three distinct parts:

1. The virus (or virus cross-section).
2. An antibody specific for a distinct viral epitope.
3. A second antibody specific for another epitope from the same viral antigen.

The antibody heavy and light chains were printed separately and combined after inserting magnets at the interface (Figure 1). We considered using accurate viral models reconstructed from X-ray crystallography and Cryo-EM models on the Vipr database to STL (STereoLithography) files for printing (Carrillo-Tripp *et al*. 2009). These models are extremely accurate representations of viral capsids and can serve as excellent teaching tools. However, for the purpose of modeling antibody-epitope interactions, accurate viral prints proved too ambiguous for students (Figures 2). Surface features were not prominent enough for a clear and practical distinction between potential antibody binding sites.

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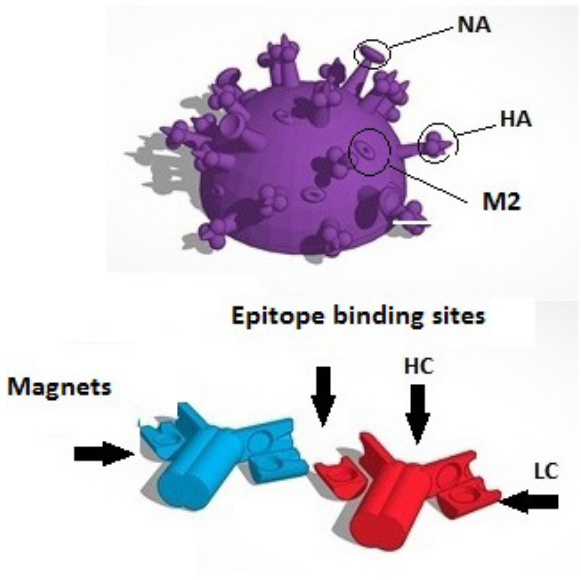

Figure 1: *3D Printed Model Contents. Influenza A cross-section and antibodies. Influenza A virus with hemagglutinin (HA), neuraminidase (NA) and M2 ion channels (M2). Refrigerator 2mm thick, 6 mm diameter magnets are glued (any craft glue) into the circular indents so that the light (LC) and heavy (HC) chains snap together. Note: be sure to glue magnets in such that they attract and do not repel one another.*

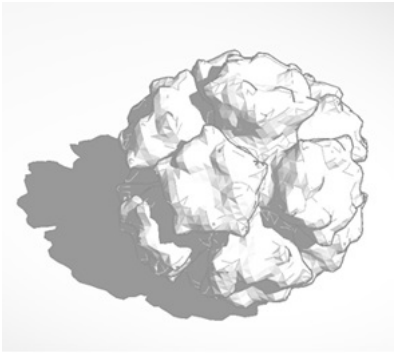

Figure 2. *Adenovirus 3D Model. Adenovirus reconstructed from X-ray crystallography and Cryo-EM models on the Vipr database to STL (STereoLithography) files for printing in Tinkercad.*

In order to reduce ambiguity concerning potential viral epitopes, we chose to model our own representation of a virus (Figure 1). We used Influenza A based on its prominent antigens hemagglutinin (HA) and neuraminidase (NA). HA is a glycoprotein that binds sialic acid on host cell membranes and mediates viral entry. NA is an enzyme that cleaves sialic acid when progeny virions bud from host cells, allowing them to spread to new cells. Both HA and NA are essential for efficient Influenza A replication. They are also highly immunogenic and form the basis for influenza vaccine development, which provides a translational component to the exercise. We did not replicate actual viral structure in our model, but rather intended to create a clear representation of the virus and its antigens. The result is more of a cartoon representation Influenza A that can be printed as a cross section or as a complete virus. HA is represented as a spike with three spheres attached and NA is a cylinder with a bowl at the end (Figures 1). We designed two antibodies that would both bind epitopes of HA. One antibody binds to M2 ion channels the HA spheres and the other binds to the HA spike (Figure 1). HA and NA were modeled at an accurate ratio of 4:1 (HA:NA) on the virus cross-section (Kilbourne *et al*, 2013). M2 ion channels (oval holes), which maintain pH across the viral envelope during endocytosis into the cell, were added to the viral surface for additional detail, but are not intended to be bound by our antibodies (Figure 1). The antibodies and virus are not modeled to scale. For accurate scaling, antibody size would need to be reduced by 75%. Antibodies are about 15-20nm (Alzari *et al*. 1988) in length, while Influenza A virus particles are 80-120nm in diameter (Norkin 2009).

Printers

We received valuable consultation and support from Colorado State University's Idea2Product (I2P) lab, which supports a wide range of creative projects by providing CSU affiliates and the local community with access to their 3D printing services. We printed our models (STL file format) from Afinia© printers using polylactic acid plastic. Multiple printer types and materials are available with varying costs and suitability for projects. Multiple printers may need to be explored to find the best printer and material for a specific print job.

Before In-Class Activity

Students are asked to watch a mini-lecture video on the immune system and antibodies at home before coming to the flipped class period. The mini-lecture video is 13 minutes long. The video was created by the instructor using lecture capture software and then posted to the University's learning management system. To ensure that students watch the video, they are required to take a five-point, five-question, online pre-quiz before coming to class on the day of the activity.

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25-Minute In-Class Activity
Learning outcomes for this activity in a flipped classroom
After this activity, students should be able to:

1. Describe how antigens and epitopes are related.
2. Explain why some antibodies that do not bind to epitopes are produced.
3. Discuss which regions on the heavy and light chains come together to bind to specific epitopes.
4. Identify the region on the antibody that determines its class or isotype.

Students are given the following:

1. A student directions handout (Appendix 1).
2. A kit containing two heavy chains (disulfide bonded together), one yellow and one red; and four light chains, two blue and two green.

To maximize time, the instructor gives the students brief directions while undergraduate learning assistants (ULAs) hand out the materials (five minutes).
Once all the materials are handed out, the instructor spends about 15 minutes going through a worksheet (Appendix 1) and an ungraded PowerPoint clicker quiz (Appendix 2) in which students are asked to predict the epitope binding of given pairs of heavy and light chains.
In total, they will work with four different combinations, two of which will bind an epitope on the same antigen on the virus, and two of which will not have specificity for the virus. This allows students to understand that not all antibodies will be specific for an epitope on an infecting microbe. The remaining few minutes of activity are used to collect the kits. The second half of the 50-minute class is devoted to lecturing about vaccination.

Discussion

On average over four semesters 91% of students were able to correctly identify the epitope to which an antibody would bind using these kits (Figure 3). Interestingly, when the combination of heavy and light chains did not bind to any epitopes on the virus only 63% of students answered that the antibodies were not specific for any epitope. This could indicate either that students do not understand that not all of the randomly created antibodies will have specificity for a given infection, or they are not confident enough to answer "none of these". However, after seeing the first antibody that was not specific for any epitopes and discussing how this was possible, when they were given a second antibody that was not specific for the virus 91% answered "none of these", and 96% correctly identified the epitope binding site of the second antibody that had viral specificity (Figure 3).

3D printing is a powerful tool and will assume an influential role in years to come. Our project provided an example of how it can be used to create a valuable teaching kit. Though the basic concept is the same for most all printers, the material capabilities and precision is extremely varied. Before embarking on a 3D printing project, it is essential to research and choose the right printer and modeling software for the job.

Our efforts yielded cartoon antibody and virus models, which interact in order to demonstrate some key concepts in antibody-epitope binding. Though designed in order to show how a single antigen may have multiple epitopes, students can also explore concepts such as agglutination, crosslinking, neutralization and isotypes (with the addition of antibodies with longer heavy chains to the instructor's kit). Many students excel at various types of learning, and having a physical model in front of them can be a beneficial addition to textbooks and lectures.

About the Authors

Dr. Erica Suchman, professor and Dr. Jennifer McLean, assistant professor Department of Microbiology, Immunology and Pathology, worked with honor students Steven T Denham (microbiology) and Dana Shatila (Computer Science) in collaboration with Dr David Prowel Assistant Research Professor Department of Mechanical Engineering to develop 3D printed models for teaching.

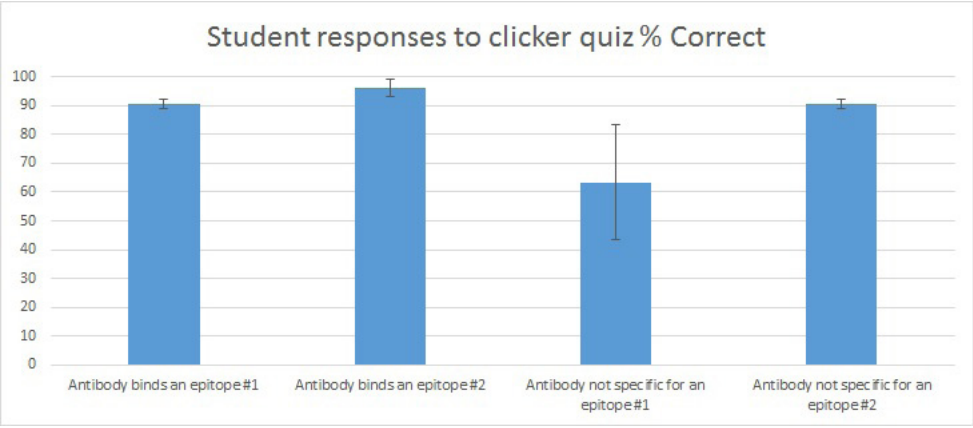

Figure 3. *Student responses in percentage during antibody epitope flipped classroom activity with classroom response system in 3 classes in 3 different semesters. n=174*

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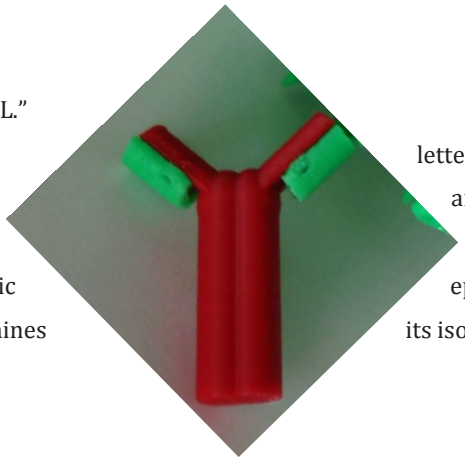
Supplemental Materials:

Appendix 1: Student Directions Handout worksheet to be filled out during class, and key

Antibody-Antigen-Epitope
3D Model Flipped Classroom Activity



Before you begin…

1. Label the light chains of this antibody with the letter "L."
2. Label the heavy chains of this antibody with the letter "H."
3. Draw rectangles around the variable regions on this antibody.
4. Circle the constant region(s) on this antibody.
5. Draw an arrow to the region that may bind to a specific epitope.
6. Draw a star on the region of this antibody that determines its isotype/class.

7. What is/are the antigen(s) in this picture?
8. Where are the potential epitopes?

After this activity, you should be able to:

1. Describe how antigens and epitopes are related.
2. Explain why some antibodies get made that do not bind to any epitopes.
3. Discuss which regions on the heavy and light chains come together to bind to a specific epitope.
4. Identify the region on the antibody that determines its class or isotype.

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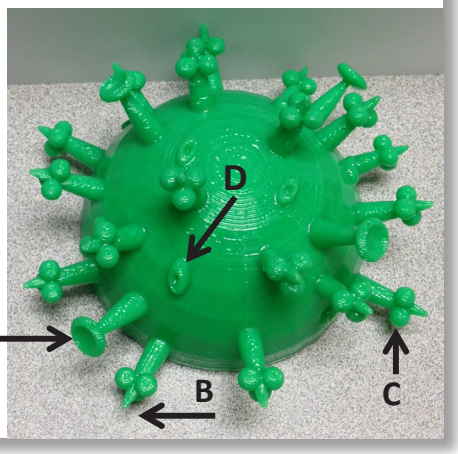
Appendix 2: In class clicker quiz PowerPoint

What epitope does the combination of Heavy chain yellow and light chain blue bind to?

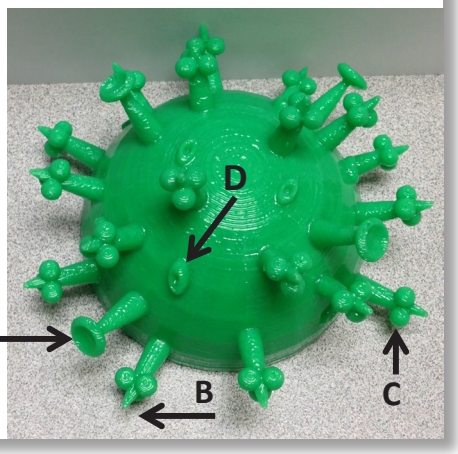
A. A
B. B
C. C
D. D
E. None of these



Heavy chain Yellow and light chain blue binds epitope C

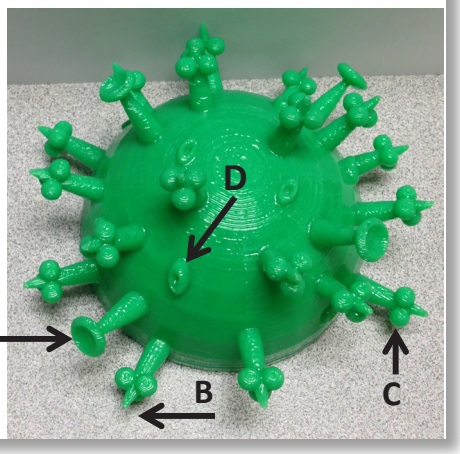
What epitope does the combination of Heavy chain yellow and light chain green bind to?

A. A
B. B
C. C
D. D
E. None of these



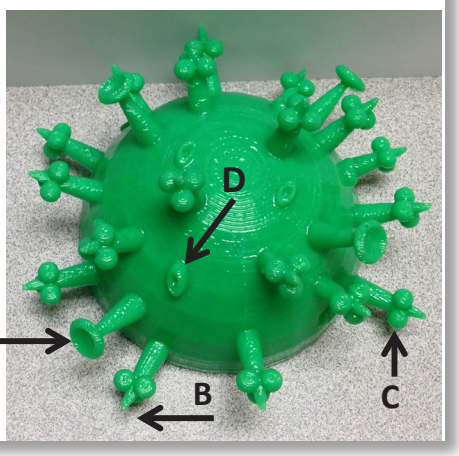
What epitope does the combination of Heavy chain Red and light chain blue bind to?

A. A
B. B
C. C
D. D
E. None of these



What epitope does the combination of Heavy chain Red and light chain Green bind to?

A. A
B. B
C. C
D. D
E. None of these



Heavy chain Red and light chain Green bind B

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Software:

Tinkercad: https://tinkercad.com/

Back to TOC