Molecular Antibody Protein Structure Model

BME 200/300
University of Wisconsin - Madison
October 21, 2009

Team:
Andy LaCroix – *BSAC*
Kara Murphy – *BWIG*
Jonathan Mantes – *Team Leader*
Kim Carlson – *Communicator*

Client:
Marge Sutinen, Outreach Specialist
UW School of Medicine and Public Health

Advisor:
Professor Wan-Ju Li
Dept. of Orthopedics and Rehabilitation
Biomedical Engineering

Table of Contents

Abstract

HIV is a serious virus and its impact on the functions of the body are misunderstood and overlooked by many people. The goal of this design project is to create a three-dimensional model that will visually depict the binding of human immunodeficiency virus (HIV) particles to CD4 cells and to demonstrate the irreversibility of the disease. Currently there are no teaching tools that are effective in conveying this message in a simplistic manner. This model will be created to highlight the important aspects of the HIV infection process and allow it to be understood by people with minimal biological knowledge.

Background

HIV causes a disease known as acquired immunodeficiency syndrome (AIDS). Currently, there is no cure for AIDS. As the virus gradually takes over the body, the immune system loses its ability to fight off infection. This causes one to succumb to opportunistic diseases such as pneumonia and tuberculosis that result in death. The virus is spread through contact with infected bodily fluids, most often during sexual contact. The best way to stop AIDS is to prevent the spread of the virus by educating people about methods and personal protection against HIV.

The widespread education about HIV and the resulting condition of AIDS is an ongoing effort of doctors, nurses, and volunteers worldwide. The major problem faced by educators is getting people to grasp the concept that once HIV enters a person's cells there is no way to stop the replication of infected cells and prevent HIV from taking over

all the cells of the body. The use of three-dimensional models is a method used in classes to educate students about the progression of HIV infection.

Problem Statement

Marge Sutinen, of the UW School of Medicine and Public Health, needs a more efficient way to model the simplified process of HIV attachment to CD4 receptors and its subsequent attack on white blood cells. The current model is ineffective to teach a social science class. It is too small to be seen and too detail oriented to be understood by the class. The new model needs to be color coded and clearly visible to a class of 30 students. A simple explanation should accompany the model to explain the interaction of HIV and host cells. Our client is looking to use the model as an aid in demonstrating the irreversibility of contracting HIV to persuade students to use preventative measures to protect themselves.

Motivation

The current model that is being used is manufactured by Merck & Company Inc. It contains a very detailed and complex explanation of the entrance of HIV into a white blood cell. Our client feels that the current model is too focused on the minute biological happenings that take place within the process of infecting a cell. The explanation of the stages a virus undergoes to infect a cell is too advanced for a class studying contemporary issues surrounding HIV, and the model is too small for practical classroom demonstration purposes. By creating a model that is easier to see and conceptualize, more students will be able to understand the destructive implication of HIV infection. This model has a

chance to change many lives by helping students understand the necessity to protect themselves and others from the spread of HIV, especially during sexual activity.

Client Requirements

Our client needs a color-coded, three-dimensional teaching aid that will give an overview of the steps of HIV infection and demonstrate its irreversibility. The model will be used in a class of approximately 30 students, so it must be easily visible from about 20 feet. The students are primarily non-science majors, so the biological details behind HIV infection are of minor concern for our project. The model will focus on three main steps of HIV infection: 1. binding, 2. injection and replication, and 3. budding of new HIV viruses. The progression from step to step will be manually controlled by the client, to give time for explanations between each step.

The goal of the binding step is to model the permanent attachment of HIV to the CD4 receptors on the cell membrane. The attached HIV particle should not fall off when the model is moved. In addition, the CD4 receptor binding sites should differentiate between HIV and other pathogens.

During replication, the mechanisms behind transcription and translation are not important. The main idea behind replication is to show that HIV takes over the host cell's nucleus and controls the production of new HIV particles. These new HIV particles will need to be similar to the first attaching model, but some will be a slightly different color or shape, showing the possibility of mutations.

In budding, the main goal is to show the host cell's DNA reproducing new HIV infected cells instead of carrying out normal cell activities. This step has some room for

creativity and should illustrate a massive release of new (original and mutated) HIV particles from the host cell.

The final model may be used for one lecture per semester so it must be able to withstand long periods of storage between uses. It must function smoothly, even after long periods of time in storage. The model must be less than 5 lbs, and compact enough for it to easily be transported from its storage location to the classroom. The final product must also contain a PDF explanation of each step of the HIV process that is demonstrated by the model.

Existing Devices

There are currently no models that effectively convey the message, in a simple form, that once HIV enters a cell there is no way to stop infection. Models that are available include posters, computer simulations, and a model produced by Merck and Company Inc.

Figure 1 is an example of a current poster model that depicts the HIV lifecycle. It was produced by the National Institute of Allergy and Infectious Diseases. It has very small captions that are difficult to see and the material is hard to understand without prior knowledge of cell biology. It also does not show the permanency of HIV taking over a cell.

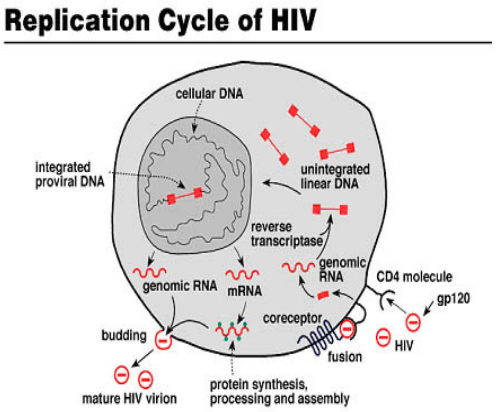


Figure 1 Photo courtesy of NIAID

Computer simulations are widely available on the Internet. Figure 2 is taken from a computer simulation video clip that shows HIV entering a white blood cell. These videos are simulations of HIV entering the host cell. They are very detailed and anatomically correct, but do not include explanations.

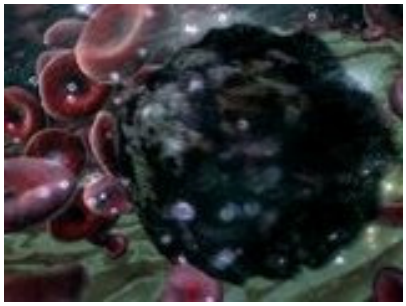


Figure 2: Photo courtesy of gettyimages.com

Computer models are very beneficial if one can identify all of the model's parts and be able to distinguish what is represented in the model. Computer simulations are hard to manually control in order to add additional explanations if students do not fully understand a specific point.

The most pertinent model to our current project is the model manufactured by Merck and Company Inc. It is a three-dimensional model that displays the process of HIV entering a host cell. The host cell is able to be split in half and contains three-dimensional interactive moving parts, diagrams and an accompanying explanation on a CD-ROM. The model itself is hard to see in the back of the classroom because the compact size that makes the model portable. The text is difficult to see from far away and the detailed steps of diagrams and processes are hard to distinguish. Also the descriptions of the process are too in depth.

Figure 3: Model manufactured by Merck and Company Inc,

All three of these devices provide an explanation of how HIV functions and how it affects the cells that it attacks. However, they are overly scientific and for students without a lot of science knowledge they can be overwhelming.

Ethics

HIV/AIDS is a controversial subject and should be discussed a professional manor. Because the students in this class are from very diverse backgrounds, the description of HIV infection must be scientific. The final product will be purely under the control of the client, so the misuse of the model is not a concern.

Ergonomics

During the attachment step, the torque required to twist on the HIV attachment cell should be relatively minor. Also, when the HIV particle attaches, it should not stick to the host cell and it should be able to be unscrewed and removed with ease. In the replication step, the force required to push the fluid from the syringe through the tubes should be reasonable. During budding, the door which releases the new viral HIV cells should be secure so that it is able to store the modeled HIV particles, but not too strong that it is difficult to open. Also, a simple loading mechanism for the placement of model HIV particles should be incorporated into the design, such that the particles do not fall out of the door before it can be closed.

Design Proposal Overview:

The entire process of the binding of HIV particles to CD4 cells and its subsequent infection is very long and complex. The purpose of this model is to condense the process in such a way that students who do not have full knowledge of the specific cell parts and processes can grasp it. In order to simplify this process, we broke it down into three steps: the binding of the HIV particle to receptors on the CD4 cell, injection of the viral capsid into the cell's cytoplasm, and the integration of the viral RNA into the host cell DNA which causes replication of the virus.

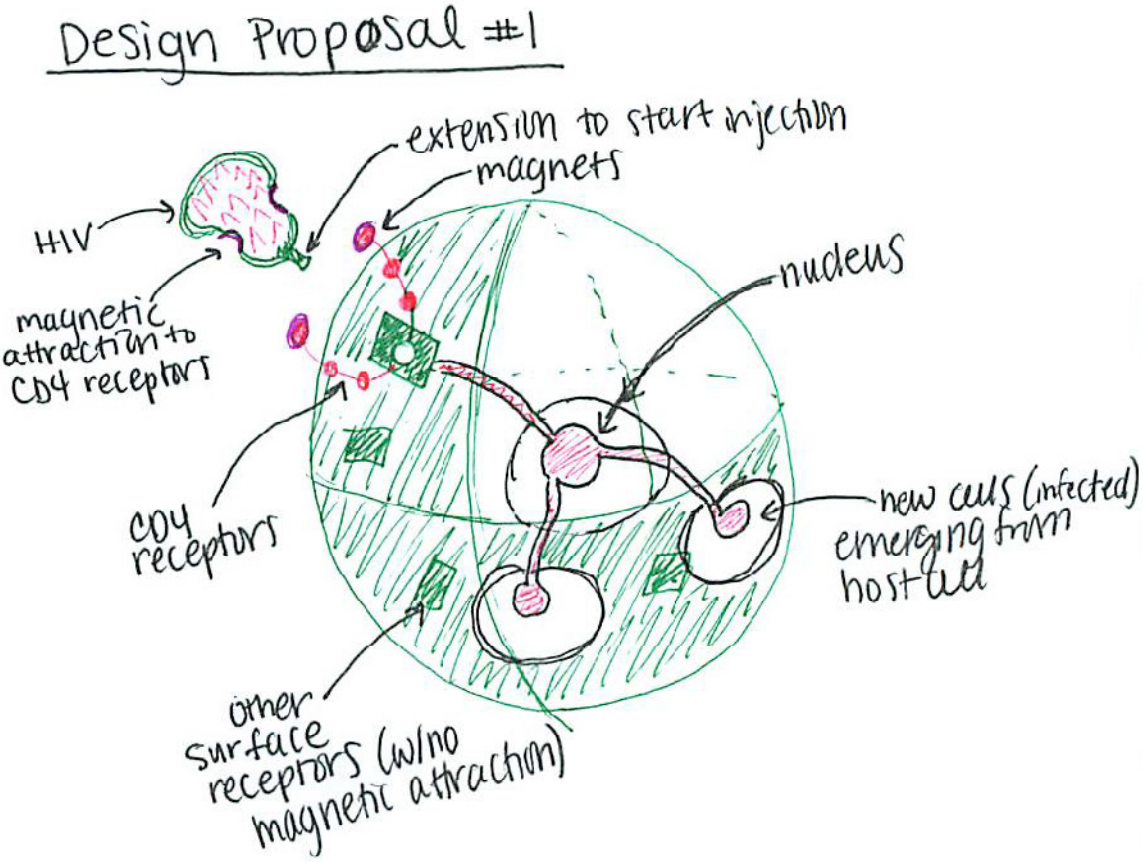
When developing design ideas, instead of looking at the model as a whole each of the steps was looked at individually. We came up with three ideas for each stage and chose the best idea from each to come up with our final design.

Design Proposal 1:

The first design proposal is an automatic model where each step progresses continuously after the binding of HIV to the host cell. This would be the most convenient for the user to operate since it only requires activation of the entire system by binding, instead of each individual step of the process. However, with this method, the process would be complete within a relatively short time frame and would be hard to repeat multiple times in a lecture without cleaning out the model and starting all over. It would not be a very efficient teaching tool for a lecture because the user would not be able to explain each step of the process while it is occurring on the model.

This model consists of two separate structures, the HIV particle and a white blood cell with a CD4 receptor. The white blood cell will be composed of a sphere with a

quarter of it being transparent in order to see the process inside the cell once the binding occurs.



The Binding Stage: The white blood cell will contain various protein receptors on its surface. One of the receptors will be different in shape and color from the others to portray the CD4 receptor, which will consist of 2 extension chains with magnets at the end. The HIV particle will be a sphere with two indentations in its surface on opposite sides that are an exact match for the ends of the CD4 receptors. These indentations will be magnetic such that when the HIV particle is brought near the white blood cell the chains of the CD4 receptor will magnetically bind to the indentations on the HIV surface.

The bottom of the HIV particle will consist of a cylindrical extension that will fit into an opening on the surface of the white blood cell between the CD4 receptor chains. Thus as soon as the magnets bind the HIV to the cell, this extension will enter an opening where it will apply the required pressure for the injection process to precede.

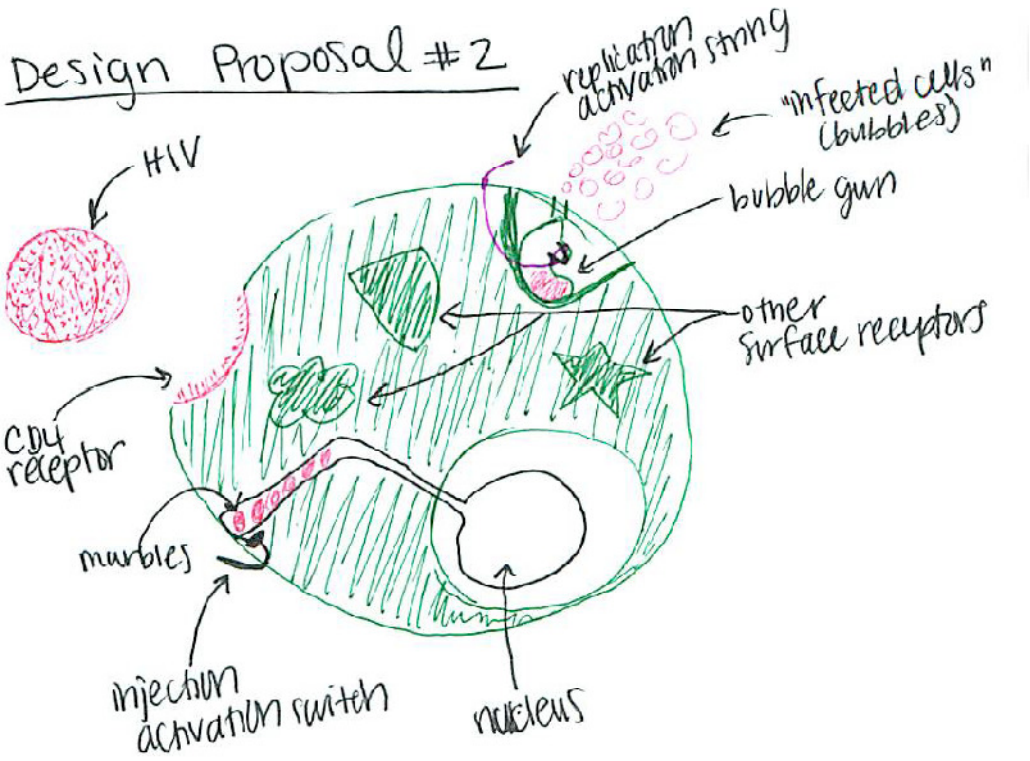
*The Injection Stage***:** The inside of the white blood cell will consist of a clear tube that will flow from the area of binding to the nucleus. This will be visible through the opening of the cell. Directly under the binding area there will be a liquid filled sac placed on a secure surface. As soon as the HIV particle binds, the extension will enter into the cell and provide enough pressure to cause the liquid to flow from the binding area to the nucleus through the tube. The fluid sac will be stored under the non-transparent part of the cell so it cannot be seen from the outside, but the fluid flowing into and filling the nucleus will be visible.

*The Replication Stage***:** In order to portray the replication of HIV in the nucleus and its release out of the cell we will have two tubes extending out of the nucleus so that after the nucleus fills with liquid, it will extend to two other cells that will be partially budding from the white blood cell. These new budding cells will be previously empty and transparent so as to not draw attention to them until they are filled with liquid portraying the HIV infected DNA.

Design Proposal 2:

The second design proposal is a manual system. It requires the user to activate each of the steps individually. This will allow the user to add explanations about each step before continuing on to the next step. This gives the user much more flexibility for

the length of time that the model can be used in a lecture. This model consists of two separate structures, the HIV particle and a white blood cell with a CD4 receptor, just like the first design proposal; however, the materials and actions at each stage differ from our first design. The cell will have a covered surface with a transparent window in order to see the nucleus.



The Binding Stage: **T**he white blood cell will contain various protein receptors on its surface. The CD4 receptor will be a vacant hemisphere nested in the surface of the cell. The HIV particle will be a sphere that is the same size as the CD4 receptor and will be able to fit directly into the receptor opening. We will show exclusive binding by

having all the other surface receptors be different shapes and sizes so that the HIV can only bind to the CD4 receptor. They will also be the same color to emphasize exclusive binding. The material used for this design will be Velcro and will work in a similar way to the "Stick-Ums Velcro Catch" game in the figure to the right. The HIV particle will be a sphere with a fuzzy outer covering and the CD4 receptor will be the materials like those of the catch pads. Thus the user will be able to bind the HIV cell to the CD4 receptor where it will stick.

Figure 4: "Stick-Ums Velcro Catch" game

This is a way to show permanency of binding. However, there is a risk that the Velcro may wear over time and not be consistent for binding. Unlike the magnets that are attracted to the HIV when it is only brought into the vicinity of the CD4 receptors, the user must directly place the HIV particle into the CD4 receptor for binding.

The Injection Stage: To show the injection of HIV into the host cell, marbles will be used instead of a liquid. This will be easier to manufacture because the tubing will not have to be removed to be cleaned between uses. Since this design is a manual system, the injection process is independent of the binding. In order to show injection, the user is going to have to initiate the process by pushing down on a latch that will slightly extend out of the side of the cell. The marbles are going to be stored in tubing in such a way that they will not flow into the nucleus without this latch pushing the tubing up to allow the marbles to flow down into the nucleus. The marbles will be the same color as the HIV particle to show that this is RNA from the HIV entering into the host cell nucleus and infecting it.

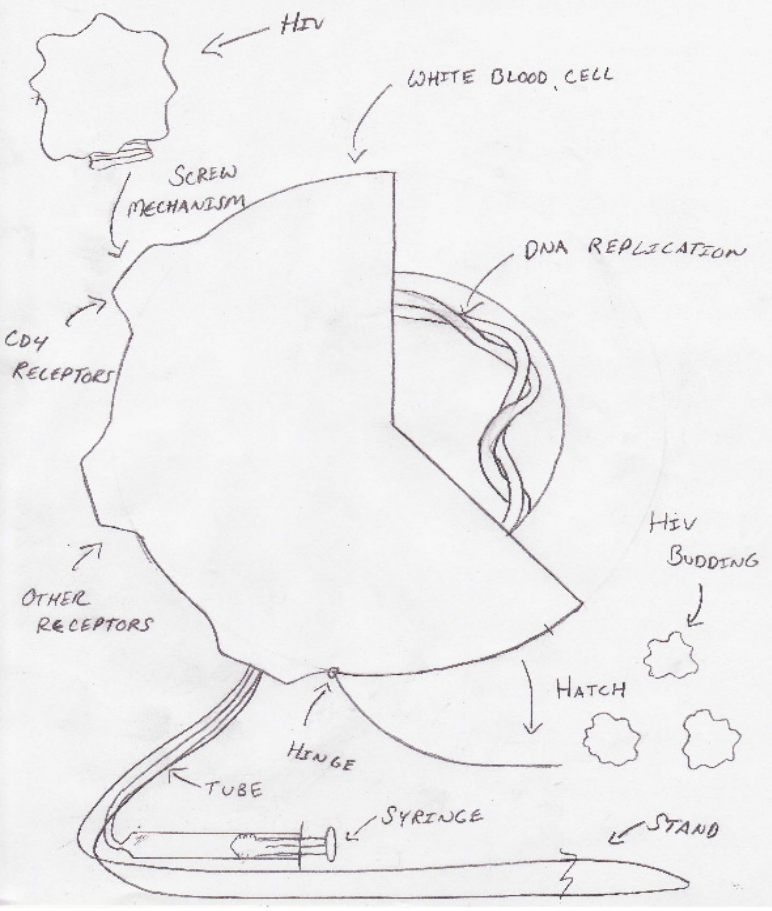
The Replication/Budding Stage: As soon as the marbles enter the nucleus, the user will have as much time as is needed to lecture about that stage before moving on to the budding stage. In order to show the replication of infected cells and their budding from the host cell, we are going to use colored bubbles. Inside the cell we are going to place a bubble gun, like one shown in the figure on the right. A string will be wrapped around the trigger. This string will hang outside of the cell, so in order to activate the budding stage the user must pull on this string in order for the bubbles to be released from the cell. This design must be manufactured so the cell opens and the bubble gun can be accessed and removed by the user easily so that it can be refilled when necessary.

Figure 5: Bubble gun

Final Design Proposal:

Similar to the second model, the third design is a manually operated model. This allows the user to speak about each step as the model progresses. The final design consists of two main parts: the initial HIV cell and a white blood cell with CD4 receptors. The model also consists of smaller color-coded HIV particles that show replication and budding of the virus. The white blood cell will be constructed of an acrylic sphere approximately 30 cm in diameter. The cell's surface will be covered in mold clay to add the texture and appearance of a white blood cell with CD4 receptors. The acrylic ball will only be two-thirds of a sphere, therefore exposing an area to see the nucleus. The nucleus will be constructed out of a smaller acrylic ball approximately 8 cm in diameter.

The nucleus will be supported by the outer shell. The entire model will rest on a stand for easy viewing from all dimensions.



Binding Stage: The HIV virus will be approximately 5 cm in diameter and it will be constructed of clay. On the surface of the white blood cell there will be colored CD4 receptors that are also constructed out of clay. There will also be other colored receptors on the white blood cell to simulate improper binding sites. The HIV cell will be attached to the correct CD4 receptor via a screw-like mechanism similar to that of a bottle cap. This screw-like mechanism will simulate the permanent attachment of the HIV virus. This will prevent the HIV from falling off if the model is moved.

Injection Stage: After the HIV is attached to the CD4 receptor on the white blood cell, the next stage in the HIV replication process is injection. For this model, there will be two twisted tubes in the nucleus to symbolize the DNA double helix strands. One tube will already be colored and the other will be transparent. A syringe containing a colored liquid at the base of the model will be connected to the clear tube in the nucleus. Once the syringe is pressed, the liquid will fill the tube and intertwine between the colored tubes, simulating viral DNA integration. To reset the model, the syringe is simply pulled back and the release of pressure will cause the liquid to be released.

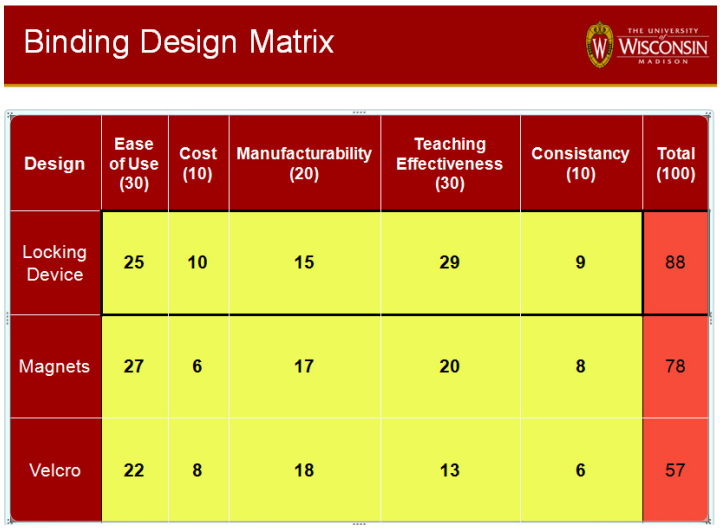
Budding Stage: Once the viral RNA is replicated and integrated into the host cell DNA, the cell will release the newly copied DNA to other cells to continue viral reproduction. A hatch on the bottom of the model will accomplish this. Inside the hatch will be colored cells including HIV cells and other mutated cells. Pressing a button will open the hatch and the cells will spill out on the stand of the model. This allows for easy pick up the marble-like cells.

Design Evaluation

In order to choose the proper design for the model, we created a design matrix for each of the three stages of HIV transmission: binding, injection/replication, and budding. The criteria we chose to judge each model on were: ease of use, cost, manufacturability, teaching effectiveness, and consistency. Ease of use is fairly important and we judged it out of 30 points. The model should be easy for our client to effectively demonstrate the infection process to a class of approximately 30 students. We scored cost out of 10 points because our budget is rather small and therefore we must keep in mind the cost of each

design. Manufacturability determines how well the model can be built. Each design was evaluated out of 20 points for manufacturability. Teaching effectiveness is a very important criterion. The model must be effective in displaying the message of HIV transmission and its effect on the body; therefore it was scored out of 30 points. Consistency or repeatability is a judgment of how well the model will continuously work each time that it is activated. This category was judged out of 10 points. Taking into account all of these criteria, each stage of each design was evaluated out of a maximum of 100 points.

The binding stage needs to demonstrate the permanent attachment of HIV to CD4 receptors. The three options were the locking device from design proposal three, the magnets from design proposal one and the Velcro from design proposal two (see Design Matrix 1 below). For the category ease of use, magnets scored the highest because of their easy attachment and the locking device was a close second. The cost of the locking device was scored the highest because a soda bottle top will be sufficient in creating this device. As for manufacturability, the locking device was hardest because it would need to be molded into the HIV and CD4 receptor. Magnets and Velcro would be easy to manufacture because of their attachment to the surface of each part. For teaching effectiveness, the locking device scored well above the other two options because it simulates something more permanent whereas magnets and Velcro can be pulled off. Consistency was also high for the locking device because it will not fail easily whereas magnets and Velcro may not provide such reliably attachment for every use. Overall the locking device proved to be the best design with a total of 88 points out of 100.

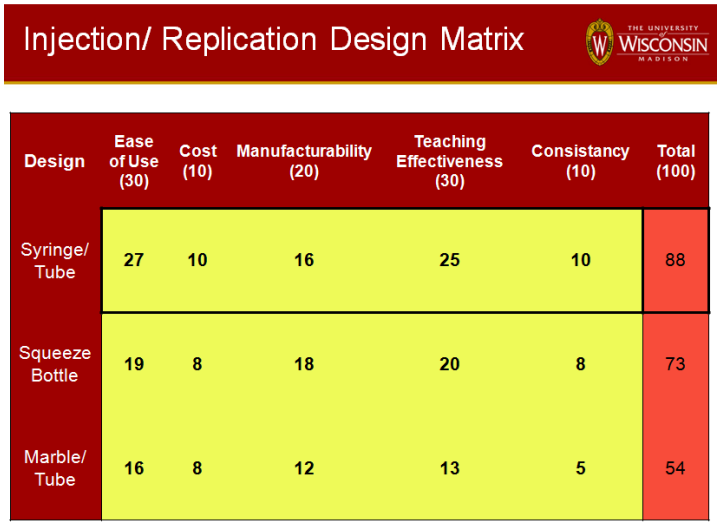


Design	Ease of Use (30)	Cost (10)	Manufacturability (20)	Teaching Effectiveness (30)	Consistancy (10)	Total (100)
Locking Device	25	10	15	29	9	88
Magnets	27	6	17	20	8	78
Velcro	22	8	18	13	6	57

Design Matrix 1

The injection/replication stage is important to show how HIV is reproduced by the white blood cell. The three options for demonstrating injection and replication are: using a syringe and tube combination from design proposal three, squeeze bottle from design proposal one and a marble and tube combination from design proposal two (see Design Matrix 2 below). For ease of use, the syringe and tube combination scored the highest because this method required no clean up and no refilling of liquids. This method also scored the highest in cost because syringes and tubes are fairly cheap. The squeeze bottle scored the highest in manufacturability because it only consists of a tube to fill the nucleus while the other methods have more parts. The syringe and tube combination proved to be the most effective teaching device as it shows viral reproduction by simulating infusion of viral RNA into the host cell DNA. The other two options will not show the replication of DNA as effectively. Consistency was highest for the syringe as well because everything will be contained in the system which makes it easier to use and

less chances for error. Overall, the syringe and tube combination scored an 88 out of a possible 100 points, making that the desired choice.

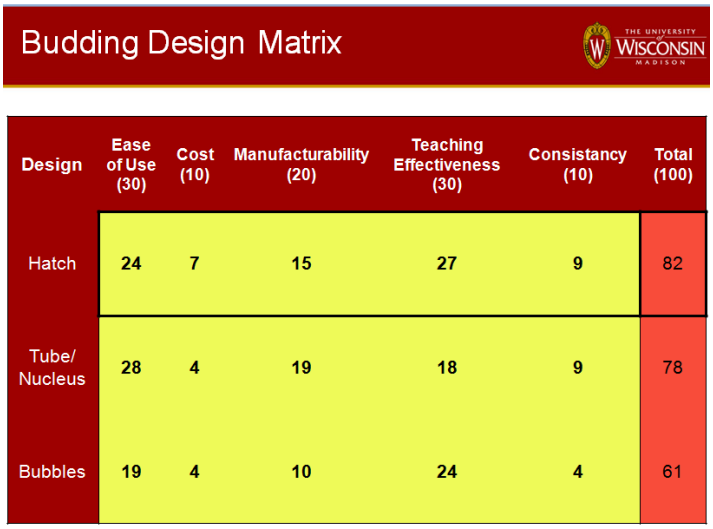


Design	Ease of Use (30)	Cost (10)	Manufacturability (20)	Teaching Effectiveness (30)	Consistancy (10)	Total (100)
Syringe/ Tube	27	10	16	25	10	88
Squeeze Bottle	19	8	18	20	8	73
Marble/ Tube	16	8	12	13	5	54

Design Matrix 2

The final stage of HIV transmission is the budding stage. The three options for showing the budding stage include: the hatch from design option three, the tube and nucleus combination from design option one and the bubble gun from design option two (see Design Matrix 3 below).The tube and nucleus combination was the easiest to use because it incorporated the filling of the nucleus from the injection/ replication stage. The hatch was the lowest cost because only a hinge and small cells are needed whereas bubbles need constant refilling and the tube and nucleus combination needs more white blood cells. The easiest the manufacture was the tube and nucleus for the same reason as the cost, but the hatch design would also be fairly reasonable to manufacture. Teaching effectiveness for the hatch was best because it shows the new cells being released from the initial host cell while the other two do not demonstrate this as effectively. The hatch was given a nine on consistency because it is fairly easy to set up and will work every

time. Overall the hatch design scored a 82 out of 100 and proved to be the best design to shown HIV budding.



Design	Ease of Use (30)	Cost (10)	Manufacturability (20)	Teaching Effectiveness (30)	Consistancy (10)	Total (100)
Hatch	24	7	15	27	9	82
Tube/ Nucleus	28	4	19	18	9	78
Bubbles	19	4	10	24	4	61

Design Matrix 3

Taking into account all of the design options for each stage in HIV transmission, design proposal three proved to be the best design and the design which we will pursue to construct for the remainder of the semester. Design proposal three incorporates the three highest scoring options for each stage: the locking mechanism for binding, syringe and tube combination for injection/ replication, and the hatch for budding. This design will be most effective in teaching and will display the transmission of HIV most effectively.

Future Work

During the second half of the semester, there are more steps that need to be completed in order for this design project to be a success. It will be necessary to decide on an effective loading mechanism for the budding HIV particles. Also, a support structure for the nucleus needs to be designed to hold it inside the cell. Research of

materials will continue, and these materials will be tested in order to determine which is the most cost effective and durable. After the design has been finalized and the materials have been selected, the client will be asked for final approval of the design and construction of the final prototype will begin. When construction is complete, testing will be conducted on the final prototype for factors such as ergonomic ease of use, durability, and overall weight.

Works Cited

Anonymous,. "The Human Immune System:Our Defense Mechanism." Davidson College. 5 October 2009. http://www.bio.davidson.edu/Courses/Molbio/MolStudents/spring2003/Cobain/geneprotein.html

Cichocki, M. "The HIV Life Cycle" about.com. Medical Review Board. 5 October 2009. <http://aids.about.com/od/newlydiagnosed/ss/livecycle.htm>

"Computer Generated Model of HIV in Bloodstream." Getty Images. 5 October 2009. <cache.gettyimages.com>

Noble, Rob. "HIV Structure and Life Style." Avert. 18 September 2009.<http://www.avert.org/hiv-virus.htm>

Poeschla, E. M. "The HIV-1 Life Cycle." Mayo Clinic. 5 October 2009. http://mayoresearch.mayo.edu/mayo/research/poeschla/life_cycle.cfm

"Replication Cycle of HIV." National Institute of Allergies and Infectious Diseases. 5 October 2009.<www3.niaid.nih.gov/topics/HIVAIDS/Understanding/How+HIV+Causes+AIDS/howhiv.htm>

APPENDIX A

Molecular Antibody Protein Structure Model
Product Design Specification Report

Team Members
Andy LaCroix – *BSAC*
Kara Murphy – *BWIG*
Jonathan Mantes – *Team Leader*
Kim Carlson – *Communicator*

Problem Statement
HIV is a virus that progressively leads to the development of acquired immunodeficiency syndrome (AIDS), a deadly condition in which the immune system collapses and fails to protect against infection. Our client, Marge Sutinen of the UW School of Medicine and Public Health, works to educate students on the permanent effects of HIV in her Contemporary Issues in HIV/AIDS Prevention course. She has asked our team to develop a 3D model that captivates the attention of a class of undergraduates and visually illustrates the HIV attachment to CD4 cells and the permanent effect it has on the body. This model will be used as an aid in demonstrating the irreversibility of contracting the HIV virus to persuade students to use preventative measures to protect themselves.

Client Requirements
- The model must be able to be easily transported from a car to a classroom by our client. It should weight no more than 5 lbs and be less than 1 ft^3
- The parts must be large enough so that all of the stages are visible for a class of 30 students to see from a maximum distance of 20 ft.
- The model must be 3 dimensional.
- The model must be color-coded in such a way that it is obvious to the students what receptors the HIV binds to as well as determining the difference between the host cell DNA and the infected HIV RNA.
- The model should be directed towards college undergraduate students with minimal scientific knowledge
- The number of separate pieces and need for assembly should be minimized.

Design Requirements

Physical and Operational Requirements
a) *Performance Requirements* – The device will only be used for one lecture every semester. It should be durable enough for handling and viewing in the classroom and be able to be passed around by the students. It should also be functional after being in storage for a long time between uses. It should have a smooth texture to allow it to be sterilized and cleaned easily.

b) *Safety* – The model should have an absence of sharp edges and an abrasive surface. There must be no parts that provide a safety hazard to our client during transportation or operation. It must be able to be handled often.
c) *Accuracy and Reliability* – It does not need to be biologically proportional to an actual cell. It is not intended for exact structure and scientific use. It only needs to be a general representation of the structure, emphasizing the parts involved in the process. The parts should not deform once the model is disassembled and the parts should fit back together for easy assembly.
d) *Life in Service* – The parts should not wear over time. The model will be a teaching tool for undergraduate students during its lifetime.
e) *Shelf Life* – The model will spend the majority of its time in service as it will only be used for one lecture every semester. Thus the model should not be composed of any materials that will degrade while in storage.
f) *Operating Environment* – The model will be displayed and operated as a teaching tool for students. It also must be able to be passed around a classroom for students to see. Model will be operated at room temperature and pressure.
g) *Ergonomics* – Model must not injure or cause harm to user. Parts should be large enough to handle easily. The torque required for screwing on and off and releasing parts should be reasonable. Our client should not have to strain herself the slightest bit during operation of the model. It should run smoothly and efficiently during each run.
h) *Size* – The model should be no larger than 1.5ft x 1.5ft x 1.5ft.
i) *Weight* – The model should weigh no more than 5 lbs.
j) *Materials* – All materials need to be non-radioactive, non-flammable, and non-corrosive.
k) *Aesthetics* – The model should be pleasant to touch, comfortable to hold, and have soothing colors.

Product Characteristics

a) *Quantity* – One prototype
b) *Target Product Cost* – Must be under $100 to produce model.

Miscellaneous

a) *Standards and Specifications* – N/A
b) *Customer* – The model must be easy to view and be understandable by undergraduate students with no scientific background.
c) *Patient-related concerns* – The model should be able to be sterilized to prevent contact transmission of viruses such as H1N1.
d) *Competition* – There are similar items on the market but none that are marketed towards our target audience and effectively demonstrate the severity of HIV.