

Design of a
Large-Scale Virus Capsid Model for Educational Use

by

Heather A. Doering

Submitted to the Department of Mechanical Engineering
in Partial Fulfillment of the
Requirements for the Degree of

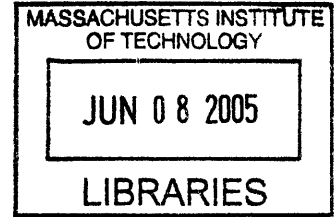
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ARCHIVES

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ABSTRACT

A foot-scale model of an icosahedral virus capsid, as opposed to a helical capsid shape, was modeled in preparation for manufacture for educational use. We chose to model the icosahedral virus capsid for manufacture because understanding the structure of this type of capsid is of biological importance, the icosahedral structure is well-established, and because the icosahedral structure and geometry is representative of many viruses, it is important to virology.

A small icosahedral virus capsid consists of 60 identical proteins. Larger capsids are formed by adding quasi-equivalent proteins, with 60 copies of each unique protein. Our model calls for the creation of an injection mold for one unique protein. 60 parts should snap together with one another after manufacture to create an icosahedron. The protein was modeled using the SolidWorks computer aided drawing (CAD) software. The CAD model could then be converted to a file that enables us to mill a two part mold out of aluminum blocks. This aluminum mold will then be used to injection mold parts made out of polypropylene. The parting lines and sprue of the final parts will be cleaned up and then assembled to complete the model.

This thesis details the specifics of the modeling of this protein, focusing on the design of the attachment method for capsid assembly from a single injection mold.

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Chapter 1

INTRODUCTION

1.1 Motivation

The structure and geometry of protein complexes, such as viruses, are often difficult to visualize and understand from a drawing of the molecule on paper. Not only are one-dimensional representations of virus hard to visualize and understand, but many of the key concepts and structural aspects of viruses are lost. Specifically, the arrangement and attachment of proteins to one another in the virus capsid, or shell, are often difficult to visualize without a three-dimensional representation of the protein, thus students studying these complexes often find them frustrating. A tangible three-dimensional model could be examined and used to aid further discussion of viral structure; this model would be especially useful in a classroom setting for instructors to pass around in order to help students conceptualize protein arrangement and assembly.

There are currently computer models and programs available that allow a rendition of a protein to be virtually manipulated in such a way as to give the appearance of three-dimensionality.ⁱ Although computer animated models permit visual study of the transformation of a protein assembly from procapsid to capsid, it is still difficult to grasp the structure's physical properties.ⁱⁱ Computer models cannot be taken apart, or reassembled by hand.

There is a need for a three-dimensional model of a virus that can be utilized in a classroom to examine structure and virus formation. Viruses cannot function properly if the structure of their outer shell, the capsid, is greatly disrupted or incompletely formed. Therefore finding the key to capsid structure has become increasingly important, and biologists have turned to mathematicians to try and unravel the assembly patterns of virus

capsids.ⁱⁱⁱ Virologists aim to disrupt normal capsid assembly to change the final structure so that the virus will no longer function and cannot cause harm.

A model that shows the overall structure of the capsid and demonstrates the connection between the proteins that form it would be ideal. Such a model would allow students to comprehend the symmetrical nature of the icosahedral viruses and see how 60 identical but irregular subunits can come together to form a regular icosahedron. A series of icosahedral models composed of unique protein sets of 1, 3, 4, and 7 would illustrate both viral growth and quasi-equivalence; this thesis will focus only on the design of a protein capsid model with one unique protein. The additional solid modeling necessary to create the series of capsids is out of the scope of this thesis and is left for future investigation.

Thus, the ultimate goal of this thesis work is to produce a solid model of a protein, such that 60 of the proteins will be able to assemble into an icosahedron, to help students understand the three-dimensional structure and geometry of a virus capsid. Our model is an abstraction of the actual three-dimensional structure and geometry of the proteins, though the representation of the overall capsid is our aim. An irregular two-dimensional contour was chosen to represent a unique protein because it illustrates that the tessellation to create a regular icosahedral capsid does not need to be a regular shape itself; many different protein shapes will tessellate. It is the connection between the proteins that determines whether the result is a successful capsid.ⁱⁱⁱ

1.2 Scope and Organization

This thesis will examine the design and manufacturing processes available to create a foot-scale assembled model of a virus capsid for low volume production. The importance and relevance of the icosahedral virus capsid will be explained and justified. The computer modeling process will be discussed, and this thesis will examine the techniques available for mold and part production. The challenges and trade-offs will be analyzed, and suggestions for future improvements and further investigation will be addressed.

Chapter 2

VIRAL CAPSID

2.1 Biological Importance of the Virus Capsid

Viruses have properties of being both living and non-living as they are neither unicellular nor multicellular organisms. Viruses do not replicate on their own, but require a host cell to provide the enzymes and cell machinery necessary for replication. The virus capsid is the protein shell that encapsulates the genetic material of the virus, protecting it. The capsid is also responsible for choosing a host cell, opening it up and injecting the genetic material of the virus. Virologists work to uncover the formation process of virus capsids in hope that they may discover a way to disrupt the capsid. If the capsid either does not form correctly, or it loses its structure, it will not retain functionality and the virus will be prevented from spreading. A three-dimensional model of a capsid will be an excellent presentation aid for those trying to explain to laymen how the capsids are formed.

2.2 Determining the Structure of the Capsid

There are three main types of capsids: enveloped, helical, and icosahedral (spherical). Enveloped capsids simply take part of their host's membrane with them on their way out of the dying cell. Although the other two types are both fairly common, spherical viruses are more prominent because more animal and human viruses, such as human rhinovirus and HIV, are spherical, therefore we will focus on those. Two landmark papers defined the structure of the spherical virus capsid. In 1956, Watson and Crick^{iv} predicted the virus capsid to be an icosahedral structure made up of identical protein subunits. An icosahedron has 20 equilateral triangular faces and is defined by having 2-, 3- and 5-fold

axis symmetry [Figure 1].^v Using geometry, they found that 60 identical subunits will assemble to make an icosahedron, with three proteins per triangular face.

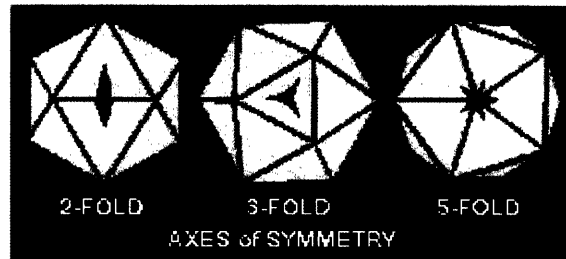


Figure 1: Icosahedral Axes of Symmetry.^{vi} In this representation, the icosahedron is rotated to look straight down on each axis of symmetry. Icosahedrons have 12 vertices, each with 5-fold symmetry, 20 faces with 3-fold geometry and 30 edges with 2-fold geometry. Each set (12,5; 20,3; 30,2) when multiplied gives 60, the number of subunits necessary to completely cover the surface.

In 1962 Caspar and Klug^{vii} wrote a paper confirming the icosahedral nature of the spherical virus capsid which Caspar had successfully crystallized in laboratory. However, capsids are not all the same size, and it was discovered that there were capsids that contained more than one protein, i.e. the proteins were not all identical. Caspar and Klug found that changing the size of the subunit does not greatly affect the overall volume of the icosahedron; a virus would not gain more space for genetic information by using a larger protein to form the shell. However, increasing the number of subunits in the capsid does. Caspar and Klug proposed their theory of “quasi-equivalence,” which stated that viruses used subunits that were *nearly* identical in structure and had *quasi* equivalent bonds between the proteins to allow for larger internal volume. Most viruses fit n subunits into their capsids, where $n = 60T$; T is called the triangulation number of the icosahedron, and is equal to the number of unique subunits. $T = Pf^2$ where P is a value from the series 1, 3, 7, 13, 19... ($= h^2 + hk + k^2$ for all pairs of integers h and k having no common factor) and f is any integer.^{vii} Figure 2 shows different classes of icosahedrons: Class $P=1$, $P=3$ and Skew Classes.

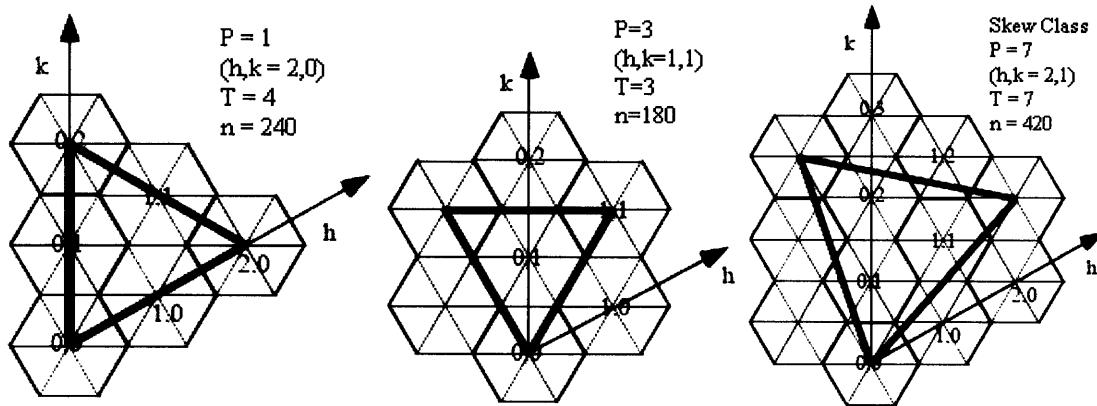


Figure 2: Triangulation Classes. The triangles shown in bold are outlines of a triangular face on an icosahedron.

For example, an icosahedron having three nearly identical subunits ($T = 3$) will have 180 subunits in total ($n = 60T = 180$), significantly increasing the internal volume of the capsid and allowing the virus to carry more genetic material. Figure 3 shows models of the capsid with different triangulation numbers.

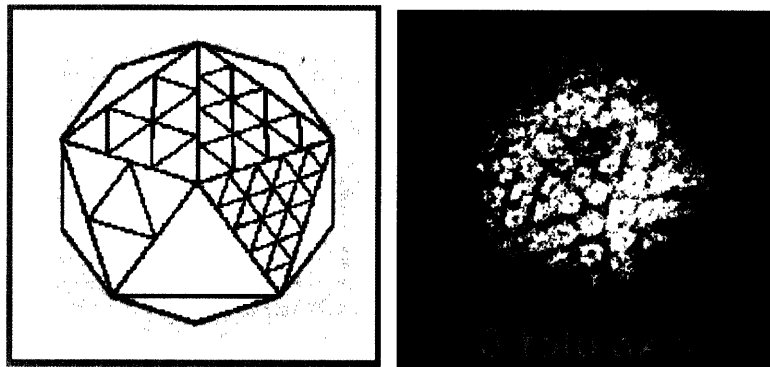


Figure 3: Triangulation on Icosahedrons^{vi}. On the left, a diagram of the first five triangulation numbers having $P=1$; $T= 1, 4, 9, 16,$ and 25 clockwise from the bottom.

On the right is an image of the Herpes Virus, $T=16$, 5-fold symmetry apparent.

Chapter 3

DESIGN CONSIDERATIONS

3.1 Production Processes and Material Selection

Today there are many different production techniques available for creating a three dimensional model. Many techniques have been around for a long time, and others, such as 3D printing have only just become available. Because we are in complete control over the shape of the protein subunit we choose to model, we have virtually the whole range of processes available to us. We would like to have a low-labor, low-cost operation that is easily repeatable. Ideally, the process would have a small production time; to make just three icosahedral capsids we need to produce roughly 200 units. Our goal for total capsid diameter is just shy of a foot, necessitating the protein subunit to be only a few inches across. Therefore, we need a process that can handle small parts precisely so that we can use a snap-fit fastener design as mentioned in 3.2.2.

3.1.1 *Thermoform*

Initially, some design was done with the thermoform process in mind to make our protein. It is a relatively cheap molding process that can be done quickly to get results fast. At first, thermoform seemed promising in some of our initial designs [Figure 44] that looked like the final product could have been made by draping a sheet of material over a mold.

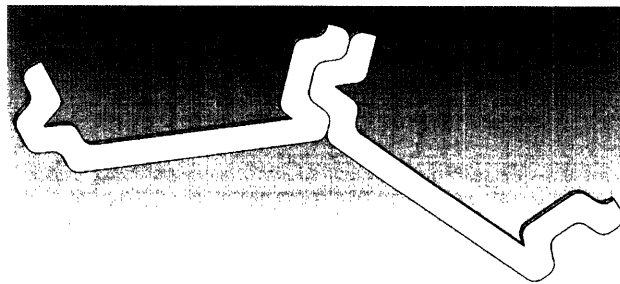


Figure 4: Cross-Sectional Profile of Two Subunits.

However, the size of the subunit is very small and would be difficult to make on the equipment available to us. Secondly, the thermoform mold would have to be a multi-piece mold and would therefore take a long time to set up and increase the process time. The process time for a simple single-body thermoform part is already quite long, on the order of minutes, and we would like to complete three full models. The time required to thermoform 200 small parts, with a multi-piece mold, is too great. Additionally, thermoform is not repeatable enough on this scale to guarantee 60 identical parts.

3.1.2 Injection Molding

The injection molding process is more fine-tuned than the thermoform processes available to us. Injection molding can be used to create small precision parts to a very high level of detail. It is also one of the fastest manufacturing processes—small cycle time, with repeatable results. We need to produce 60 identical parts for each model assembled; therefore Statistical Process Control (SPC) analysis of our run parts will be done. Following recommendations drawn from SPC analysis will lead to the production of consistently identical units.

3.1.3 Materials

Our model scale is comparable to the yo-yo pieces designed and made every semester in the Manufacturing and Design II class. As the resources of this class are readily available to us, the same materials will be used: an aluminum block die set for the mold, and polypropylene (PPE) for the injection material; their properties are found in table 1.

Table 1: Properties of Aluminum and PPE

Properties	Aluminum	Polypropylene
Young's modulus (E), GPa	70	2
Yield strength (Y), MPa	33-500	30
Brinell Hardness (HB), MPa	120	96 (Rockwell R)
Density (ρ), kg / m^3	2700	937
Melting point (T_m), $^{\circ}C$	660	160
Specific heat (C_p), $J / kg-K$	900	2000
Thermal conductivity (k), $W / m-K$	220	0.11

3.2 Protein Interface Attachment

We will model the simple case of $T=1$; in such a model there is only one unique protein that makes up the capsid. This one protein must then be able to attach to all of its identical neighbors to assemble into the icosahedral capsid. Because one of the main goals in creating this model is to allow users to interact with it, to both assemble and disassemble the model at will, permanent methods of attachment cannot be used and will not be investigated.

3.2.1 Mechanical Fasteners

To have a model that can be assembled and disassembled made of identical subunits requires a complex unit that can snap into itself, one that would be quite difficult to design. We explored the idea of a simpler protein model that allows for the subunits to just barely touch, using a separate additional connecting piece to fasten them together. A nut and bolt fastening through overlapping holes was one possible design. A sort of double pin that would join adjacent planes, as seen in Figure 3, was another. Although the protein subunit was easier to design, the manufacture of an additional mold and many, many fasteners and subsequent complicated assembly led us away from separate mechanical fasteners.

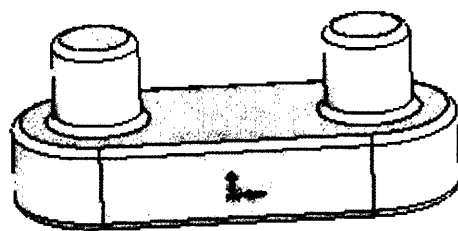


Figure 5: Pegged fastener.

3.2.2 Fastening Elements

To avoid complicated assembly, the interface at each connecting point of the protein subunit must be unique and visually identifiable; the junctures must be an integral part of the protein design. We strove to connect the interfacing points in unique ways such that picking up two units a student could correctly connect them without guidance or

instruction. The order of assembly was planned into the design of the fasteners; three units can be assembled to create a roughly triangular plane that is one of the 20 faces of the icosahedron. Figure 6 shows the five protruding points on the subunit model, A-E.

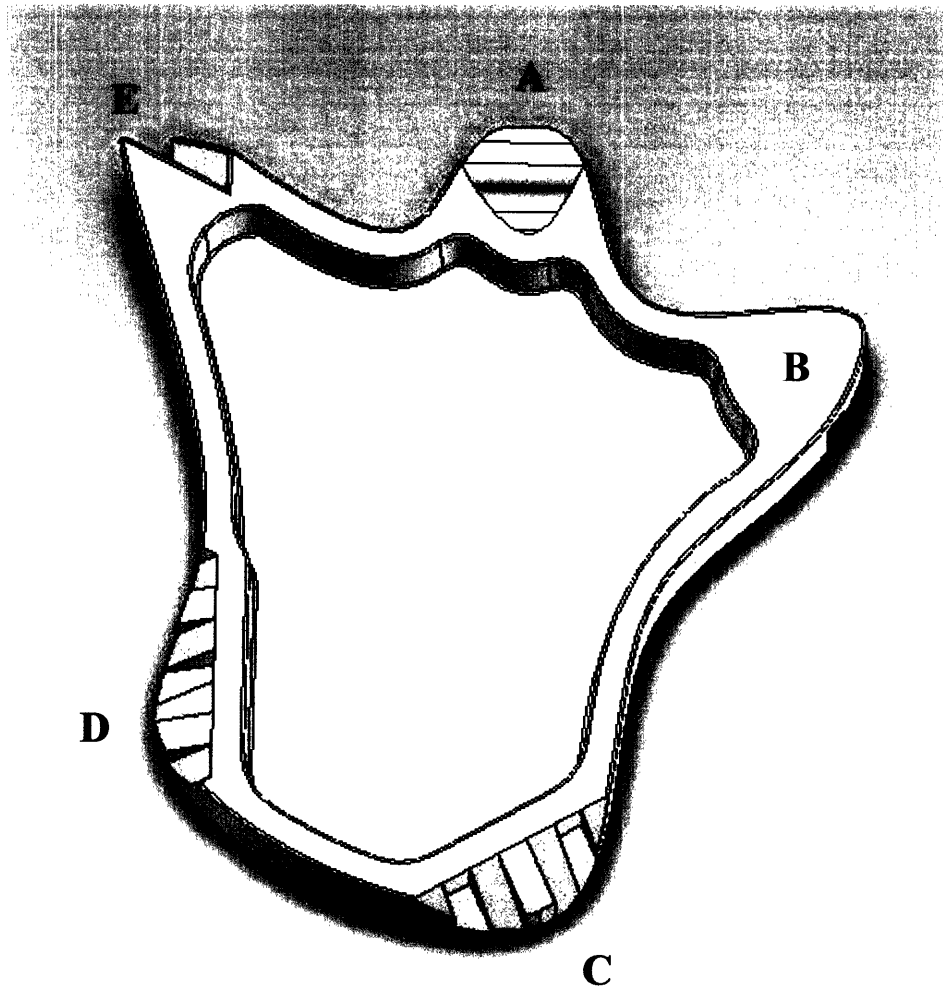


Figure 6: Protein Subunit. Joints are A—B, C—D, and E—E.

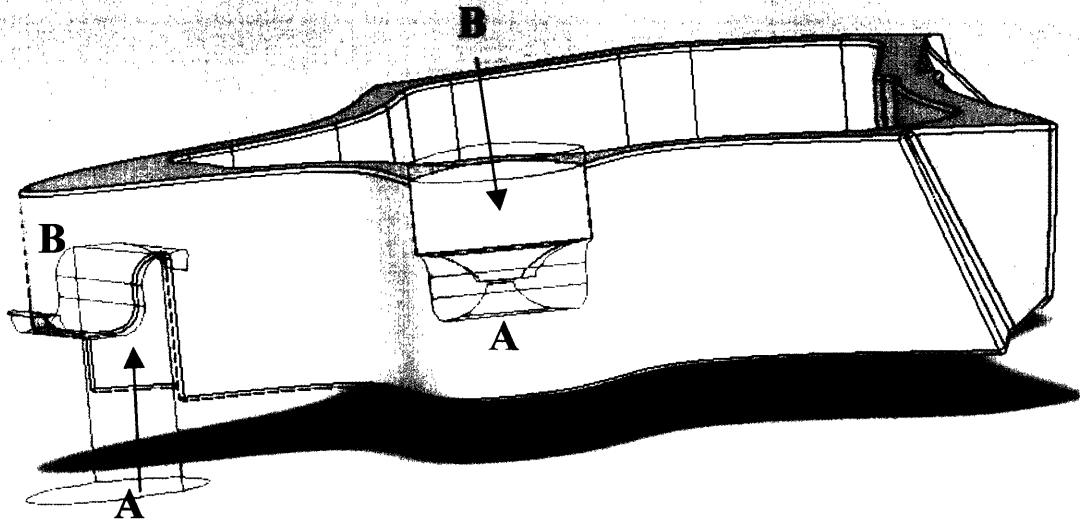


Figure 7: Joint A-B Solid model of hook mechanism.

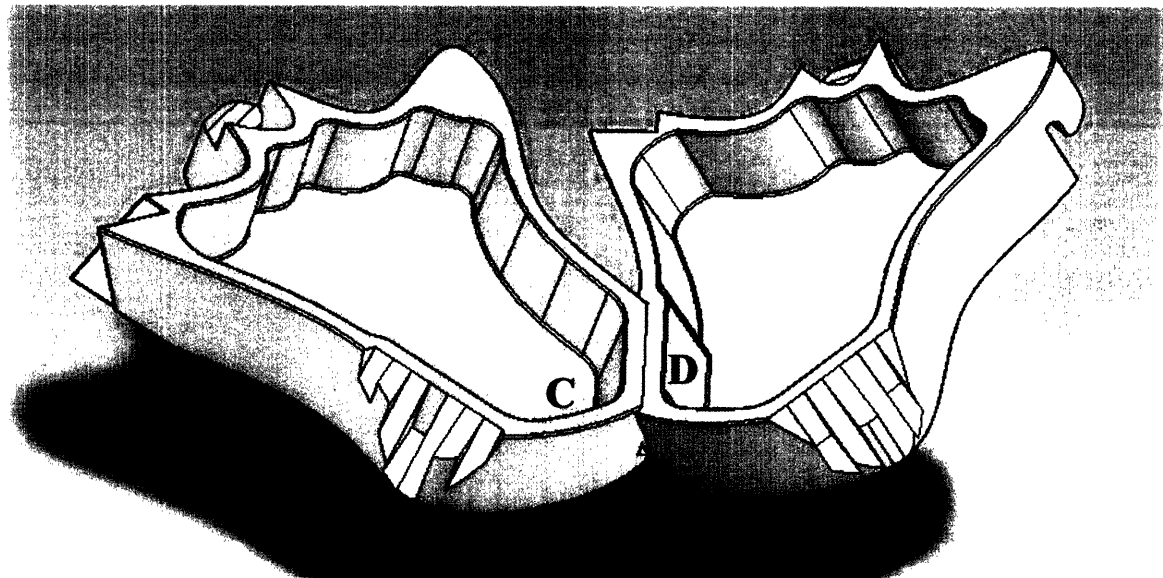
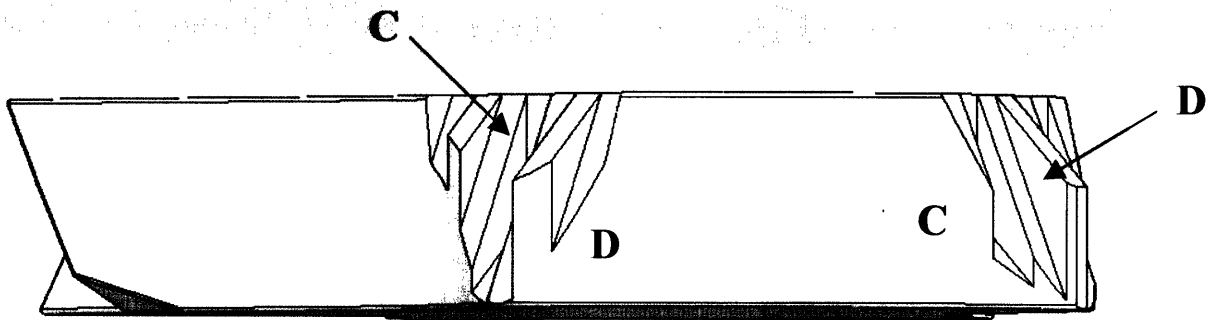


Figure 8: Joint C-D is based on insertion of tabs.

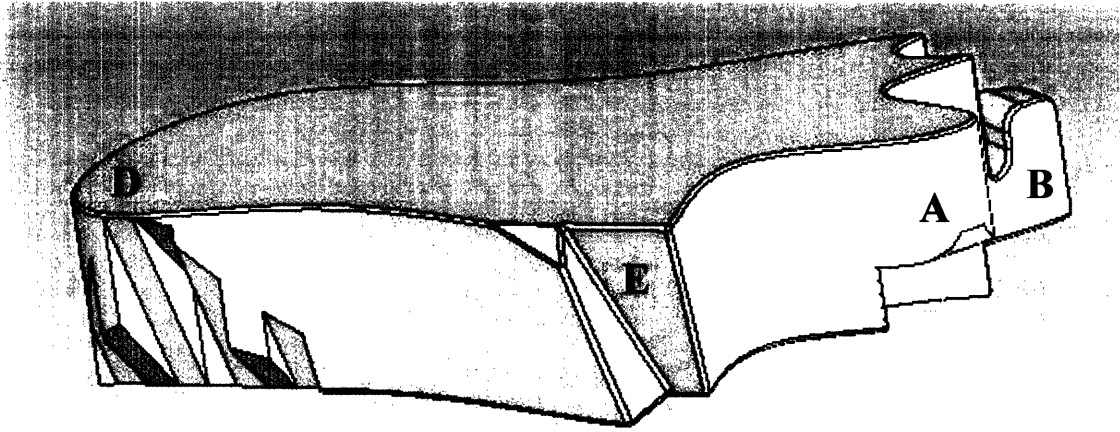


Figure 9: Closer look at D and E.

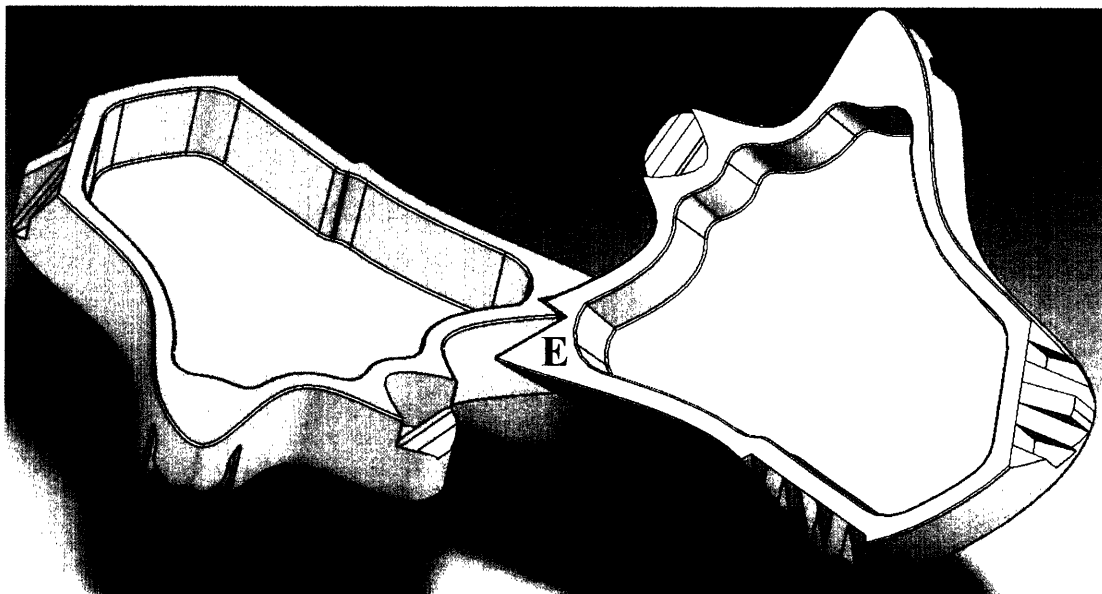
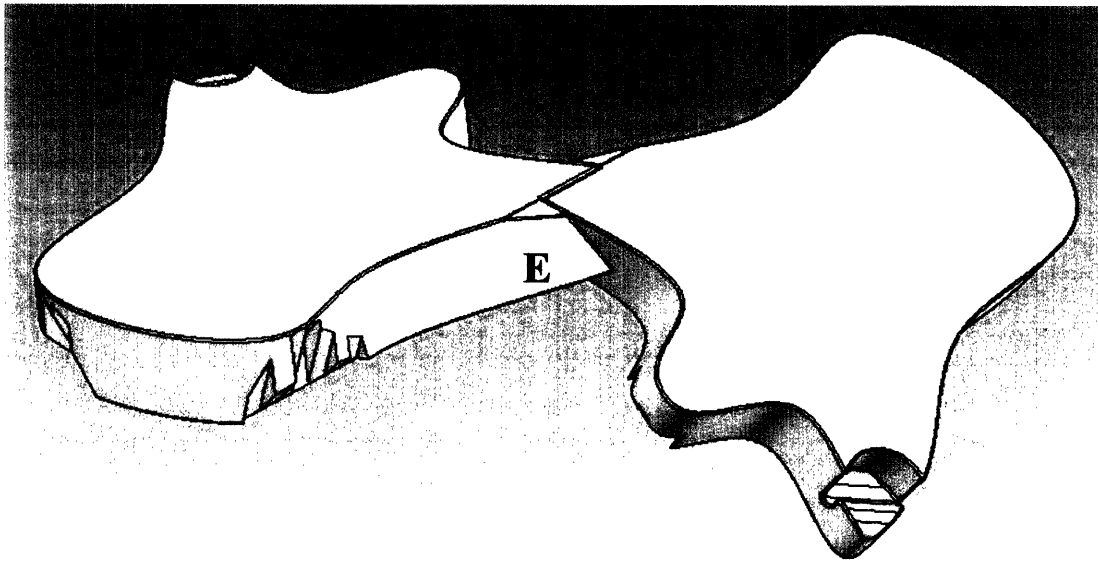


Figure 10: Joint E stabilizes the others but does not, in fact, lock into itself.

Chapter 4

COMPUTER SOLID MODEL

4.1 SolidWorks

SolidWorks is a computer aided drawing program that allows for the creation of 3D solid models. It is a useful engineering tool that helps with visualization of design ideas, and can also be a useful design tool, allowing for assembly of designed parts before they are fabricated. SolidWorks also makes it easy to fabricate a part from the file, allowing users to save the part in many different file types. To this end, an IGES file created was created from our solid model in preparation for transfer to the mold modeling program.

The creation of the solid model was the main work of this thesis and went through many rebuilding and several redesigning steps.

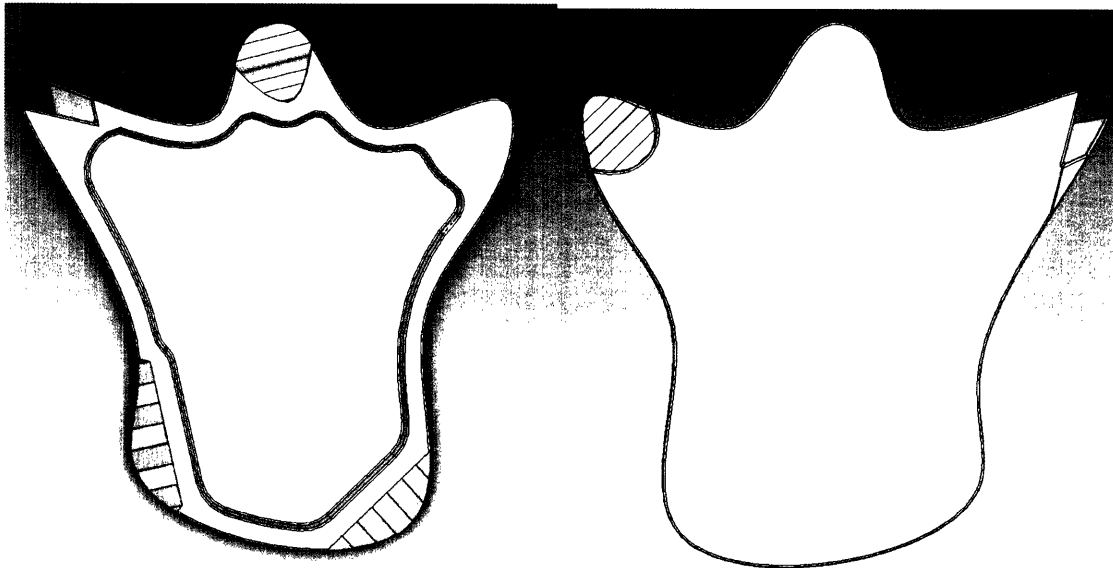


Figure 11: Inner and Outer View. On the left is a view of one protein subunit from the inside of the capsid. On the right is the view of one from the outside of the capsid.

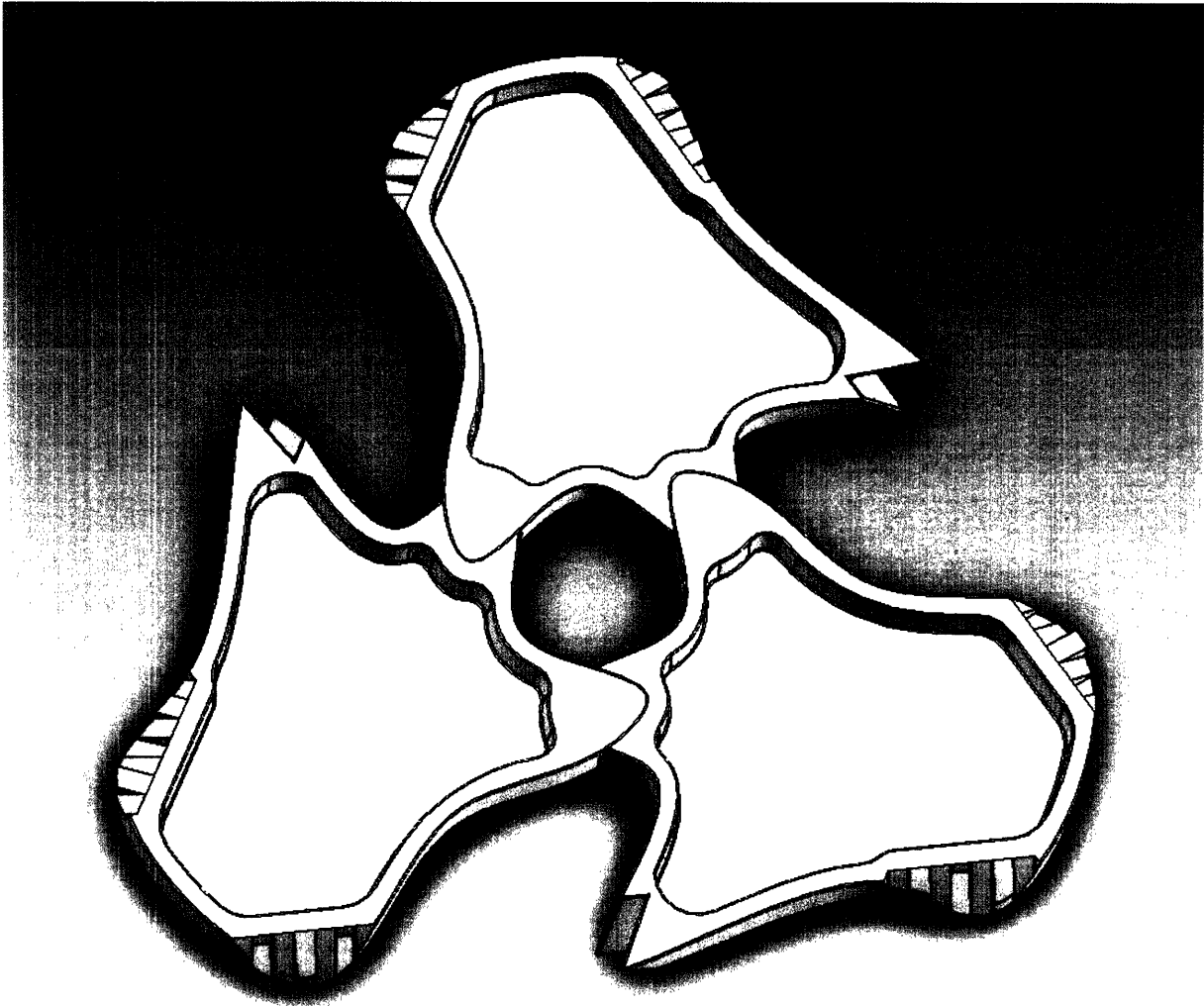


Figure 12: Icosahedra Inner Face. Three proteins snap together to form one face of the icosahedron.

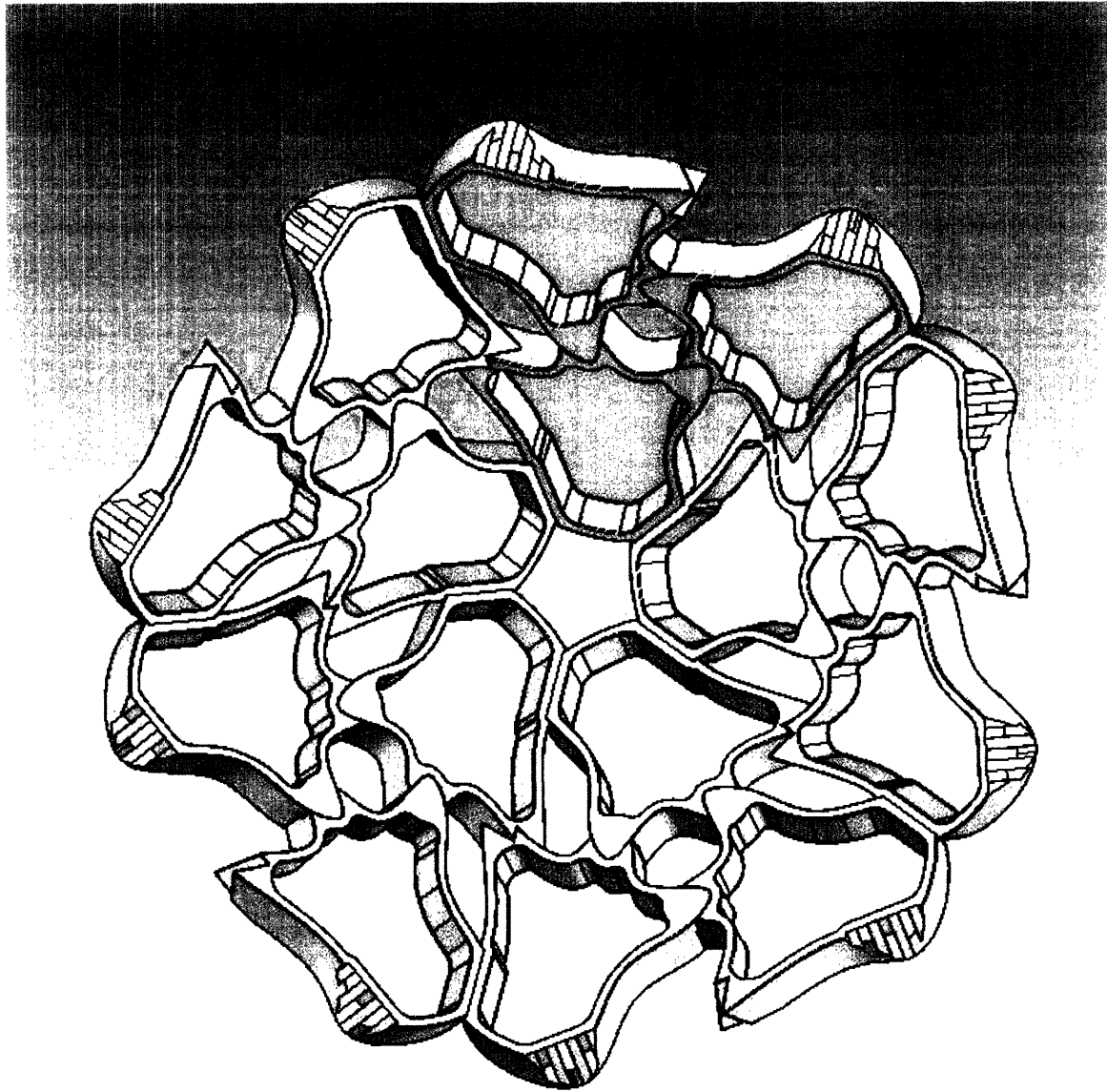


Figure 13: Five-fold Symmetry.

15 subunits and 5 faces make up this view, shown from the “inside” for clear viewing of the joints.

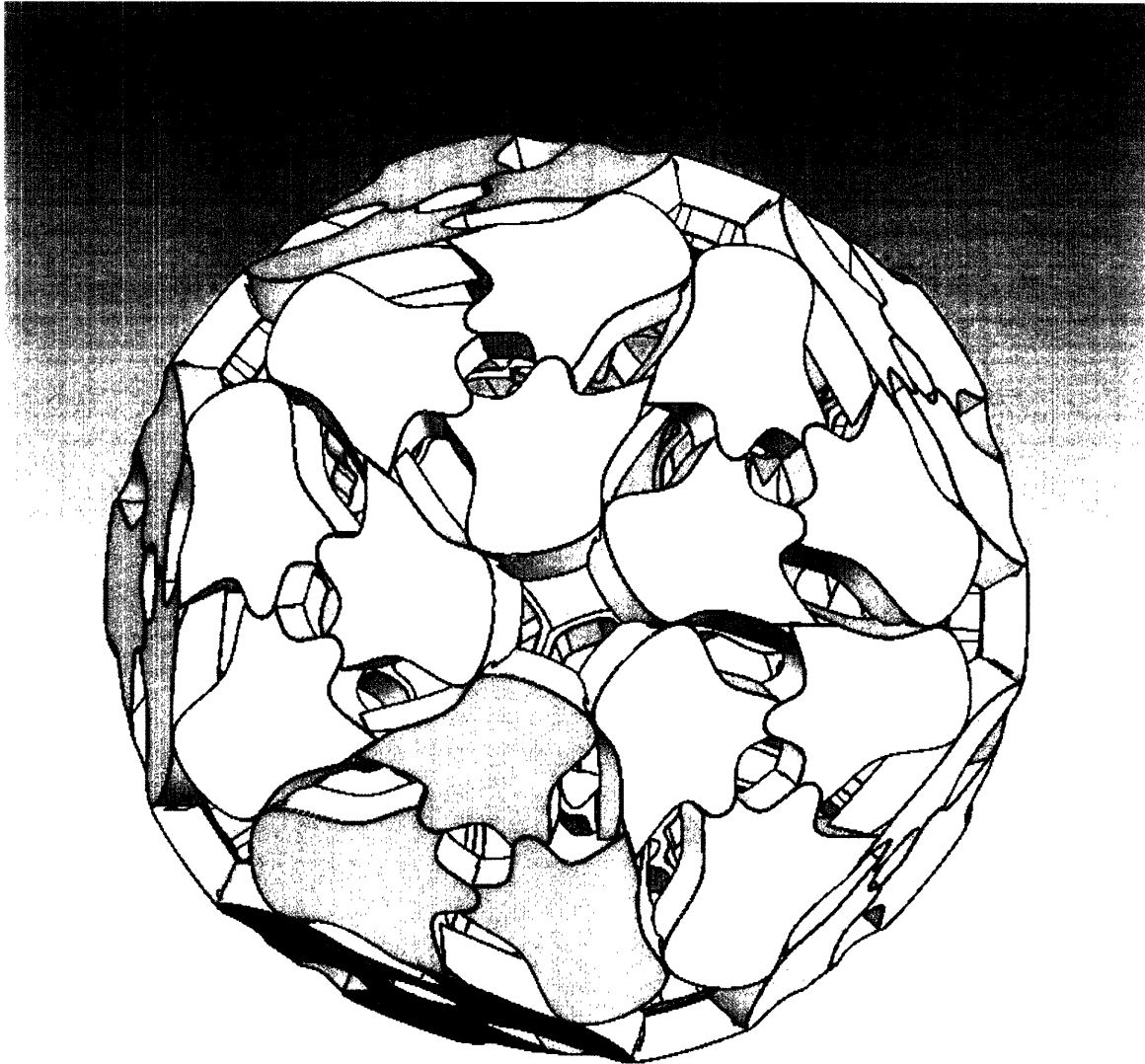


Figure 14: Virus Capsid. This is a view of a fully assembled capsid model made with 60 parts.

4.2 Mastercam

Mastercam is a modeling program that allows for the planning of Computer Numerical Control Milling; users can generate a mill path and code automatically, allowing for a smooth transition from solid model to mold fabrication. Mastercam was able to import the IGES file created in SolidWorks so the two halves of the injection mold could be designed. Unfortunately, due to time constraints, the mold design is at this point incomplete.

Chapter 5

CONCLUSIONS AND RECOMMENDATIONS

A foot-scale model of an icosahedral virus capsid was designed and prepared for manufacture for educational use. The solid model of the protein subunit was modeled in SolidWorks, a computer aided drawing program, and an IGES file created. The IGES file allowed the part to be transferred into Mastercam so the two halves of the injection mold could be designed. The resulting Mastercam file for the molds will allow for automatic CNC Mill control and the cutting of the aluminum mold blocks during the manufacturing process. The aluminum mold can then be used in the injection-molding machine to create the protein subunit that was originally modeled in Solidworks. After sprue removal and finishing, parts will snap together to form the complete virus capsid model.

Much consideration was given to different processes for the protein subunit part production. Research was done to determine the best mold making process and technique. Materials requirements were determined and different materials were considered for the different molding processes. Ultimately, polypropylene was found to be the most suitable material for its crystalline properties and ease of use with an aluminum injection mold. However, the scope of this project was narrowed to the design of the solid model, and so we do not yet have the injection molded protein subunits.

Unfortunately, the change in scope and intention of this project means that we are still two steps away from successfully manufacturing a functional educational tool. Future investigators may wish to utilize the solid model created to complete a die set for injection molding. The mold designed in this process should be usable for such a project. Much attention would need to be given to the cooling time in the mold and the packing of PPE, to ensure a good mating and assembly of subunits. If injection molding proves

unsatisfactory, three-dimensional printing or another rapid prototyping process may be useful technologies to consider and explore.

The solid model detailed in this thesis would be of use not only in the classroom, for better instruction and understanding, but potentially for pharmaceutical and other companies examining viruses. Companies could use the model to illustrate important concepts to sales representatives and costumers.

Much has been learned while working on this project and it is our hope that the work presented in this thesis will be of use to future investigators working on similar design and molding projects.

Chapter 6

REFERENCES

-
- ⁱ **VIPER: V**irus **P**article **E**xplore**R** <http://mmtsب.scripps.edu/viper/util.php>
- ⁱⁱ Modeling Complex Structures and Viruses
<http://www.pegacat.com/cbetts/phd/virus/virus.html>
- ⁱⁱⁱ **Peterson, Ivars.** Mathematics and Computers: A surprising view of how a virus shell assembles itself. *Science News Online*. March 25, 1995.
http://www.sciencenews.org/pages/sn_edpik/mc_1.htm
- ^{iv} **Watson J. D. & Crick F. H. C.** Structure of Small Viruses. *Nature* 177:473-475, 1956.
- ^v The Icosahedron: Geometric proof and definition.
<http://kjmaclean.com/Geometry/Icosahedron.html>
- ^{vi} **Stannard, Linda M.** Virus Ultra Structure: Principals of Virus Architecture 1995.
<http://web.uct.ac.za/depts/mmi/stannard/virarch.html>
- ^{vii} **Caspar D L D & Klug A.** Physical principles in the construction of regular viruses. *Cold Spring Harbor Symp.* 27:1-24, 1962.