Applying Viral Nanoparticles in a Treatment Vector for Alzheimer's Disease Using Molecular Dynamics Simulations

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Abstract

A progressively neurodegenerative disease, Alzheimer's disease presents a serious emotional and physical cost to patients and their families today. In industrialized countries, the increasing overall age of the population creates a large group of people at risk for Alzheimer's disease, so it is imperative that a cure is developed soon. However, new treatments are often too large in size to cross the blood-brain barrier (BBB) and thus do not localize to regions of the brain well. Nanoparticles offer one potential solution to this problem. The extremely small size and high targeting accuracy of nanoparticle vectors allow them to deliver therapeutic molecules to a treatment site without compromising surrounding tissue.

In this project, solvated models of the cowpea mosaic virus (CPMV) capsid were developed to identify its possible therapeutic values in Alzheimer's disease. Using molecular dynamics simulations, the CPMV capsid proteins were shown to interact with a combination of tight-junction protein ZO-2 (a BBB protein) and vimentin (a protein found in Alzheimer's plaques); a combination of tight-junction protein ZO-2, vimentin, occludin, and vinculin (two other BBB proteins); vimentin alone; and a combination of vimentin and beta-amyloid (another protein found in Alzheimer's plaques), without overheating the systems. A Ramachandran plot and contact maps were used respectively to verify the secondary structure of the CPMV capsid proteins and location of interactions within the molecular systems.

The CPMV capsid, based on simulation data, presents one promising drug vector in treating Alzheimer's disease because it could increase the efficacy of established treatment regimens and deliver new, promising drugs to treatment sites. Therefore, it should be explored further through experimental studies in animal models for incorporation into a treatment for Alzheimer's disease.

Section 1 - Personal Connections

My name is Akshata Rudrapatna and I will be entering my freshman year at Case Western Reserve University in the fall of 2016. My intended major is systems biology, born directly out of my experiences with research over the past few years. In a nutshell, my research connects the learning I do in my science and math classes to my desire to innovate for the people of the world. It creates a "hands-on" result that I can explore and dissect. I find myself deeply engrossed in research because I truly enjoy conducting it.

At a young age, I knew I wanted to pursue a career in the sciences, preferably in medicine. The workings of the body fascinated me and captured my attention for hours on end, and it seemed there was always something new I could discover. Knowledge about the body seemed like an endless onion. I peeled back the layers slowly, but I always had more to peel no matter how many layers I removed. Eventually, I realized the brain presented the greatest mystery. I wanted to learn how it functioned, what its purpose was, and which illnesses would affect it. Alzheimer's disease was the natural conclusion to my musings - it became the disease I would focus on throughout my high school career.

My current project came about as a result of a seminar I was fortunate enough to attend. Having volunteered in a lab at Barrow Neurological Institute in Phoenix, Arizona, over the summer before my junior year of high school, I often attended Tuesday lunch seminars where other professors would speak about their work. During one of these seminars, I listened to Dr. Rachael Sirianni speak about her lab's work at Barrow. I was attracted to the idea of using nanoparticles in Alzheimer's disease from her fascinating discourse on the use of polymer nanoparticles to treat brain tumors.

I then conducted further research into nanoparticle vectors for the treatment of Alzheimer's disease, eventually discovering that researchers had identified viral vectors for drug-delivery to specific sites in the body. Because viruses naturally target certain cells and also can be biodegradable, I chose to focus on viral nanoparticles instead of polymer nanoparticles. The original idea behind my project involved designing the actual treatment vector, inoculating mice with Alzheimer's disease with the treatment, and examining the results. It was both impractical and prohibitively expensive.

After talking to Dr. Jeffrey Yarger at Arizona State University (ASU), I decided to change my focus from an experimental to a computational approach using molecular dynamics simulations. I had previous computational experience, having concurrently enrolled in AP Computer Science, but I had to learn the basics of C++ to conduct my research. In addition, since molecular dynamics simulations involve differential equations and spring physics, I began reading about such topics in my math and physics classes. And of course, I read countless articles about Alzheimer's disease, nanoparticles, and drug-delivery methods. Most of my project was conducted on my PC at home though some of the work was conducted with Dr. Stephen Davidowski's help on a larger processor at ASU. As a result, I also learned how to troubleshoot problems I encountered in running simulations.

Before this project, I was firmly convinced I would pursue a career in medicine or traditional laboratory research. At the time, my concept of research only incorporated the typical lab-coated scientist painstakingly culturing cells in a cell culture hood. However, my opinion changed dramatically when I entered the world of computational biology research last year. Though the process of conducting simulations presents a steep challenge, it continues to fascinate me everyday. With this project, I have exposed myself to the world of computer programming, along with a desire to learn more computer languages and coding. I have discovered a new way of conducting biological research that I would like to continue in my collegiate career (and perhaps, as a physician-scientist).

My own experiences with research have developed my interests and my passions — which is why I want to encourage all of you who read this article to conduct research as well. Whether you are interested in conducting research the first of its kind, or building on research others have conducted before, you will learn a lot and be able to apply that research. I suggest starting on Wikipedia or another encyclopedia with a broad topic you are interested in and then exploring pages that appeal to you until you encounter an area you want to delve into. Try to form connections between your prior knowledge and new discoveries in research. But above all else, have fun with research!

As you read through my research or have any questions for me, please feel free to contact me at akshata.rudrapatna@gmail.com. I would be happy to respond with advice and answers to your queries.

Good luck in your own research endeavors! I look forward to hearing about your discoveries!

Section 2 - The Research

2.1 Introduction

2.1.1 An Overview

A severe neurodegenerative disease, Alzheimer's disease currently affects about 5.3 million people in the United States. Many drugs for this disease cannot bypass the blood-brain barrier without significant modification, reducing their efficacy. The objective of this research therefore revolves around determining a viral vector capable of interacting with the blood-brain barrier and delivering drug molecules to specific targets in the Alzheimer's brain. By using molecular dynamics simulations, models of the cowpea mosaic virus (CPMV) capsid's interactions with five critical proteins — vimentin, tight-junction protein ZO-2, vinculin, occludin, and beta-amyloid — were developed, and the CPMV capsid was identified as an effective viral vector for treating Alzheimer's disease.

2.1.2 General Information and Facts

A progressively neurodegenerative disease, Alzheimer’s disease currently affects about 5.3 million people in the United States, and, “is the sixth-leading cause of death in the United States” [1]. Currently estimated to cost the United States $226 billion [1], it places a heavy burden on the American healthcare system. Alzheimer’s disease is the most deadly and most common form of dementia, the general term used to describe any disease involving gradual memory loss [2]. It is characterized by loss of cognitive function, increasing difficulty of movement, and progressive catatonia until death occurs [1]. No effective cure for Alzheimer’s disease currently exists.

2.1.3 Pathology of Alzheimer’s Disease

The two major hallmarks of Alzheimer’s disease include beta-amyloid protein plaques and neurofibrillary tau tangles. Beta-amyloid is one subunit of another molecule known as the amyloid precursor protein, which extends through the fatty membrane surrounding neurons. However, when present in larger-than-normal amounts, beta-amyloid can ultimately clump together into an insoluble mass known as a protein plaque [3].

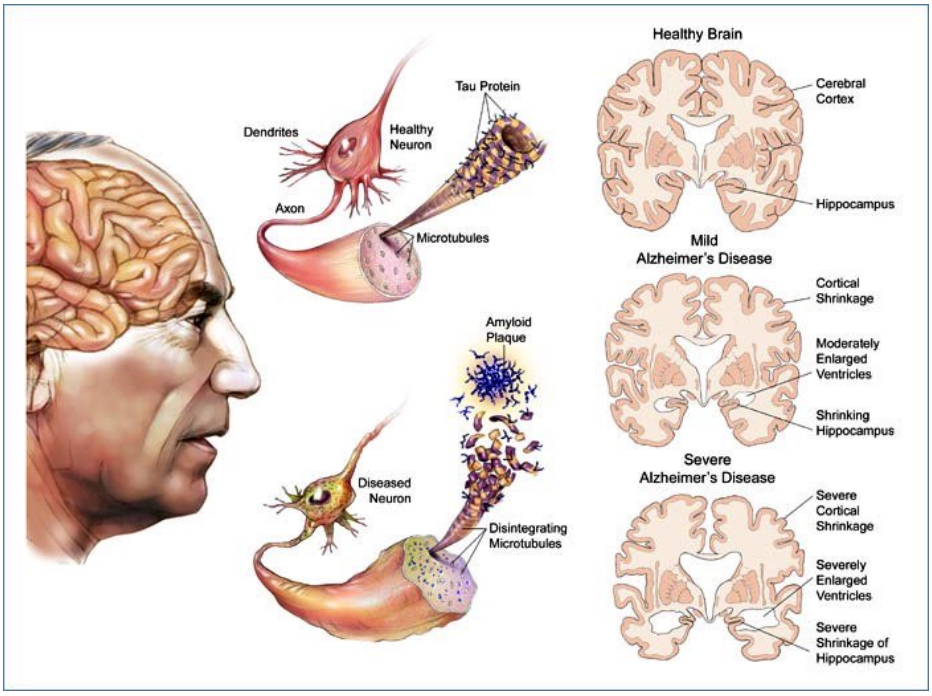

Figure 1 A detailed depiction of the Alzheimer's brain [4].

On the other hand, neurofibrillary tangles consist of another molecule called tau, which maintains the stability of neuronal microtubules. When tau is phosphorylated at certain neuronal regions (such as at the Ser262 site), it causes microtubule dysfunction in neurons, creating protein tangles [5]. These two factors eventually decimate neurons throughout the brain. Figure 1 depicts the adverse effect of neurofibrillary tangles and amyloid plaques on the Alzheimer's brain.

2.1.4 The Blood-Brain Barrier in Alzheimer's Disease

The blood-brain barrier (BBB) normally works to prevent the passage of pathogens and extraneous material from the blood into the brain [6]. However, the BBB has been found to be damaged in the Alzheimer's brain [7]. Many Alzheimer's treatments, unfortunately, still fail before localizing to damaged areas in the brain partly because these drugs cannot cross even the damaged BBB with full efficacy [8].

2.1.5 Nanomedicine and Drug-Delivery Mechanisms

Because of their nontoxicity, ability to degrade in the human body, and ability to target specific types of cells via the use of peptide ligands [9], nanoparticle vectors created from plant and/or

bacterial viruses are considered some of the most advanced forms of nanoparticles that can be successfully used in the body [10]. However, viral nanoparticles have not been fully investigated as treatment vectors for Alzheimer's disease, offering an attractive idea for improving Alzheimer's drug-delivery mechanisms.

2.1.6 The Cowpea Mosaic Virus

The cowpea mosaic virus (CPMV), a small, icosahedral plant virus, is 30 nanometers in diameter. Its nanochemical properties have been well-characterized by researchers, and it can be cultivated quickly and easily in the black-eyed pea plant [11]. Its surface can be modified with fluorescent dye particles, metal particles, and peptide ligands for a variety of uses in the body. CPMV targets and binds to a mammalian protein called vimentin [12]. This protein is found in the cytoskeletons of several types of cells, most importantly in certain astrocytes in the central nervous system [13]. In Alzheimer's disease, astrocytes with high concentrations of vimentin are found at the edges of protein plaques. And in many cases, the astrocytes extend into the plaques themselves [14].

Though CPMV infects plants [14], it has been found to be to be non-toxic at extremely high doses to animals, with few symptoms as a result of the administration of CPMV. In addition, it aggregates in inflamed regions in the brain [15], important since the Alzheimer's brain contains inflammation [8]. Since the virus also clears from the bloodstream rapidly without side effects [12], there is a low risk of toxicity to surrounding tissues.

2.1.7 An Overview of Molecular Dynamics

Molecular dynamics, first developed by Alder and Wainwright in the mid-twentieth century, was initially used to examine the "interactions of hard spheres" [16]. Currently utilized in computational disciplines to study molecules in theoretical environments, molecular dynamics provides a wealth of information on molecules at a microscopic level. This method is used today to derive protein structures from methods such as nuclear magnetic resonance (NMR) experimentation and X-ray crystallography, as well as "study…complex, dynamic processes that occur in biological systems" [16].

2.2 Research Questions

Can molecular-dynamics simulations be used to develop models of interactions between blood-brain barrier proteins, Alzheimer's-related proteins, and viral capsid proteins? Can these be used to compose a targeted drug-delivery system suitable for treating Alzheimer's disease?

2.3 Objectives

The objectives of this project are to:

1. Target the blood-brain barrier without damaging or compromising any other part of the structure.
2. Target Alzheimer's plaques without inducing cellular death or dysfunction in surrounding tissue.
3. Demonstrate the viability of a viral drug vector for the treatment of Alzheimer's disease.

2.4 Rationale/Importance of Work

Alzheimer's disease is currently incurable and progressively neurodegenerative. With the increasing age of the baby-boomer population factoring in as a prime risk factor for Alzheimer's disease, a cure must be found as soon as possible. As well, the classification of Alzheimer's disease as both a tauopathy and an amyloidosis places it in the same category as Parkinson's disease, amyotrophic lateral sclerosis (ALS), and Huntington's disease. This treatment model therefore offers a possible new method of treating these diseases.

By utilizing viral nanoparticles in this project, it is possible to explore their range of uses in the body, especially in the brain, where viral nanoparticles have not been extensively applied. Further development of the field of viral nanomedicine, which is a relatively new area of study, offers many possibilities for the future of drug-delivery methods.

2.5 Materials and Methods

2.5.1 Software

The software programs Nanoscale Molecular Dynamics (NAMD) [17] and Visual Molecular Dynamics (VMD) [18] were downloaded open-source from the Theoretical and Computational Biophysics Group website of the University of Illinois at Urbana-Champaign. "NAMD was

developed by the Theoretical Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign" [17]. "VMD was developed by the Theoretical and Computational Biophysics Group in the Beckman Institute for Advanced Science and Technology at the University of Illinois at Urbana-Champaign" [18]. The latest versions (Version 2.10 of NAMD and Version 1.9.2 of VMD) were used. VMD and NAMD example simulations (found in tutorial documents) were run on a Windows 8 PC with an AMD A6-5200 APU Processor with 2.0 GHz processing speed and four cores to understand the mechanics of the software programs and prepare them for use. Certain simulations were run on a Power Max with a 2 x 3 (3.0) GHz Quad-Core Intel Xeon Processor with eight cores and 16 GB of RAM at the ASU Magnetic Resonance Research Center.

2.5.2 Protein Files

Protein Data Bank (PDB) files for the combined CPMV capsid proteins — the large and small subunits (PDB ID: 1NY7) [19] - vimentin (PDB ID: 1GK4) [20], tight-junction protein ZO-2 (PDB ID: 2OSG) [21], occludin (PDB ID: 1WPA) [22], vinculin (PDB ID: 4PR9) [23], and beta-amyloid (PDB ID: 2MXU) [24] were downloaded from the Protein Data Bank website. Files were evaluated for their completeness and length of protein residues. The CPMV large and small subunit file contained 4407 atoms, the vimentin file contained 4169 atoms, the tight-junction protein ZO-2 contained 2674 atoms, the occludin file contained 1012 atoms, the vinculin file contained 8174 atoms, and the beta-amyloid file contained 5712 atoms.

Proteins were first color-coded with the ColorID function on VMD Representations tab; interactions and/or bonds were identified and noted within the structures. Protein Structure Files (PSF) for each protein were generated by the AutoPSF function in VMD and/or typed commands into the Tk Console. Proteins were merged together when necessary for simulations using the Merge Protein Structures function in VMD. The PDB files were then solvated into a water box using the Tk Console in VMD. Solvated PDB files were created of: a) CPMV and vimentin together, c) CPMV, vimentin, and tight junction protein ZO-2 together, d) CPMV, vimentin, tight-junction protein ZO-2, occludin, and vinculin together, and e) CPMV, vimentin, and beta-amyloid together.

2.5.3 Configuration Files

Configuration files in the general format of NAMD configuration files were generated for use in simulations for all four systems described above. All default values were kept except for the following: a) the timestep was modified to 2 femtoseconds to decrease the amount of memory needed for the simulation log files. Periodic boundary conditions were also modified by measuring and analyzing the size of each system. Analysis of the system's size was conducted using Tk Console commands to determine the minimum boundary, maximum boundary, and center of the solvated system. The file paths in the configuration file were changed to reflect the locations of the PSF, PDB, and topology files on the operating system.

After solvation, no patches were applied to the system. An appropriate simulation parameter file - CHARMM22 All-Hydrogen Parameter File for Proteins and Lipids – and an appropriate molecular topology file - CHARMM22 All-Hydrogen Topology File for Proteins and Lipids - were identified for use in the simulation [25].

2.5.4 Energy Minimization and Analysis

Energy minimization trials were run on the Windows 8 operating system. Minimization for the vimentin and CPMV system; the vimentin, CPMV, and tight junction protein ZO-2 system; and the vimentin, CPMV, tight-junction protein ZO-2, occludin, and vinculin system was followed using the generated simulation logs and evaluated for regular temperature and total energy values. Bad contacts between atoms and line minimizer gradient values were also monitored. After minimization was complete, contact maps were developed for each set of proteins and zoomed in to specific amino acids displaying the relevant residues involved in the interactions between the proteins. These contact maps were developed using the Contact Map function in VMD.

2.5.5 Equilibration and Analysis

Equilibration was run immediately after energy minimization, utilizing the same configuration files and same conditions as in minimization. Equilibration trajectories (velocity and time values) were recorded to the DCD file. Simulation logs were evaluated for normal temperature values as equilibration progressed. After completion of the simulations, the simulation results of the CPMV and vimentin system were plotted in Ramachandran space, both as a two-dimensional map

and a three-dimensional plot. These were created with the RamaPlot plugin in VMD. The Ramachandran map was compared to that of the Ramachandran map found in the online PDB entry for the CPMV capsid subunits to demonstrate the stability of their structures [18].

2.6 Discussion of Results

2.6.1 Stability of Systems and Ramachandran Plots

The stability of the systems throughout simulations after energy minimization and equilibration was demonstrated. The temperature of the system at the end of the simulation was 310.424 K in the vimentin and CPMV system, 310.656 K in the vimentin, CPMV, and tight-junction protein ZO-2 system, and 309.382 K in the vimentin, CPMV, occludin, vinculin, and tight-junction protein ZO-2 system. This indicates that they did not overheat and equilibrated properly. The Ramachandran plot for the CPMV capsid subunits and vimentin system after minimization and equilibration mostly matched that of crystallographic data [18], indicating that the capsid proteins are at their most natural configuration and underwent minimization properly. Figure 2 depicts the aforementioned Ramachandran plot as a 2-D map of residues. In the 2-D map, the majority of the protein residues (yellow boxes) are within the most allowed (blue) and partially allowed regions (green), which also speaks to the validity of the system's structure.

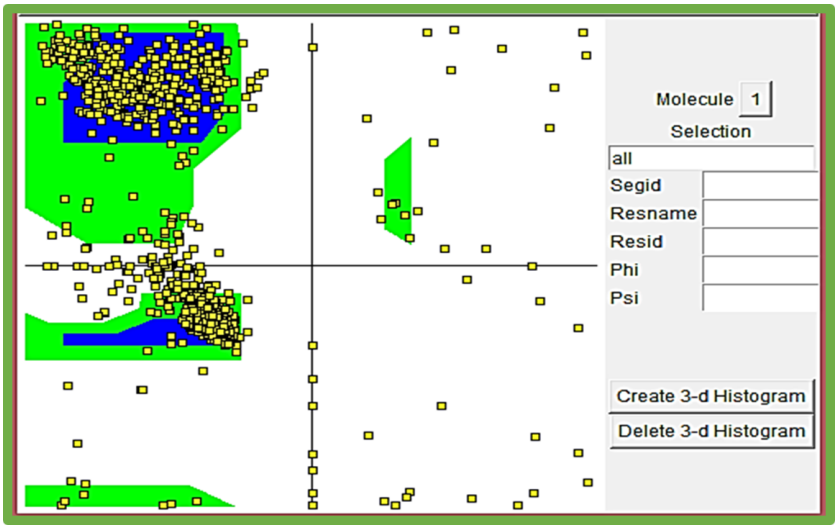

Figure 2 The Ramachandran plot for the CPMV and vimentin system.

2.6.2 Interactions between CPMV, Vimentin, and Tight-Junction Protein ZO-2

CPMV demonstrated interactions with vimentin and tight-junction protein ZO-2. The solvated system contained 203,809 atoms. The line minimizer gradient (using the method of conjugate gradients) restarted fewer than twenty times during minimization of the CPMV, vimentin, and tight-junction protein ZO-2 system, indicating fewer contacts that needed to be resolved between the atoms. Therefore, there were few overlaps in atoms between the molecules before minimization was concluded. Figure 3 demonstrates the regions and amount of interactions between the residues of the molecules at a specific location in a contact map. The closest points of interactions between vimentin and CPMV are highlighted with black, with additional, more distant, contact points highlighted in gray.

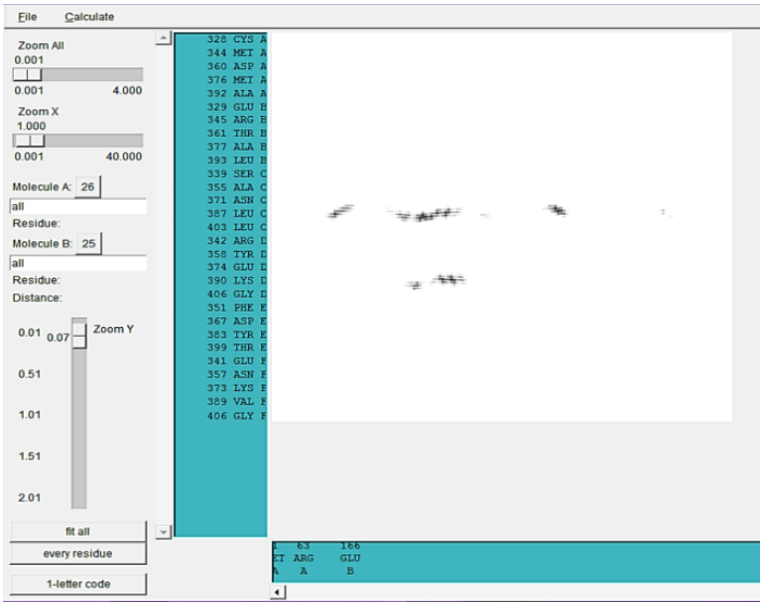

Figure 3 The contact map for the CPMV, vimentin, and tight-junction protein ZO-2 system.

Periodic boundary analysis produced the following boundary matrix for the solvated system:

cellBasisVector1	124.4	0.0	0.0
cellBasisVector2	0.0	139.4	0.0
cellBasisVector3	0.0	0.0	206.7
cellOrigin	-10.23	22.21	56.02

The minimum boundary for the system is {-60.898, -35.892, -33.945} and the maximum boundary is {40.472, 80.451, 147.996}, indicating a large system for molecular dynamics simulations in NAMD.

2.6.3 Interactions between CPMV, Vimentin, Tight-Junction Protein ZO-2, Occludin, and Vinculin

CPMV demonstrated interactions with vimentin, tight-junction protein ZO-2, occludin, and vimentin. The solvated system contained 481,314 atoms. The line minimizer gradient restarted more than twenty times during minimization of the system, indicating that the molecules had many contacts that needed to be resolved between their respective atoms. Therefore, the five molecules overlapped to some extent before minimization was complete.

Periodic boundary analysis produced the following boundary matrix for the solvated system:

cellBasisVector1	163.4	0.	0.0
cellBasisVector2	0.0	148.7	0.0
cellBasisVector3	0.0	0	206.5
cellOrigin	11.2	52.3	54.0

The minimum boundary for the system is {-17.289, 19.504, 14.402} and the maximum boundary is {83.021, 116.443, 147.531}, indicating a large system for molecular dynamics simulations in NAMD.

2.6.4 Interactions between CPMV and Vimentin

As well, CPMV also demonstrated interactions with vimentin alone. Here, the size of the solvated system was identified as 259,829 atoms. The line minimizer gradient restarted more than twenty times, revealing that the molecules had many contacts that needed to be resolved between their respective atoms. Therefore, the three molecules overlapped to some extent before minimization was complete. Figure 4 reveals the extent of interactions between the residues of CPMV and vimentin at a specific location in a contact map. The closest points of interactions between vimentin

and CPMV are highlighted with the black line, with additional, more distant, contact points highlighted in gray.

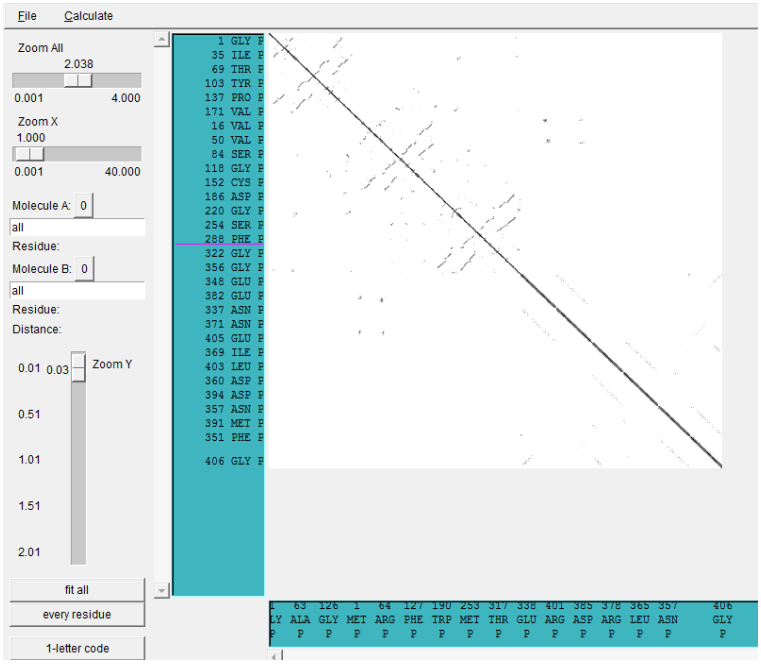

Figure 4 The contact map for the CPMV and vimentin system.

Periodic boundary conditions revealed the following boundary matrix:

cellBasisVector1	120.4	0.	0.0
cellBasisVector2	0.0	112.2	0.0
cellBasisVector3	0.0	0	201.33
cellOrigin	-10.36	34.06	56.53

The minimum boundary for this system is {-70.398, -22.241, -43.642} and the maximum boundary is {49.974, 89.951, 157.691}, indicating a large system for molecular dynamics simulations in NAMD.

2.6.5 PROJECTED Interactions between CPMV, Vimentin, and Beta-Amyloid

CPMV also demonstrated interactions with vimentin and beta-amyloid. The size of the solvated system here was identified as 298,473 atoms. Based on the results of the earlier simulation conducted between CPMV and vimentin, it was projected that the interactions within the CPMV,

vimentin, and beta-amyloid system would result in a similar number of line minimizer gradient restarts, as well as in a similar contact map.

2.6.6 Ribbon Models of Solvated Systems

All systems were additionally remapped in VMD to determine the exact areas of contact between the molecules. Figure 5 offers a ribbon model of the CPMV (pink), vimentin (purple), tight-junction protein ZO-2 (red), occludin (blue), and vinculin (gray) system, which demonstrates areas of interaction within the greater blood-brain barrier.

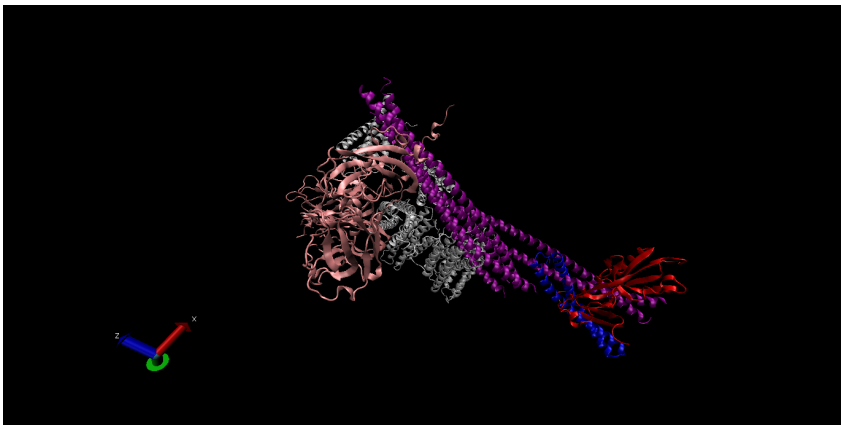

Figure 5 A ribbon model of the CPMV, vimentin, tight-junction protein ZO-2, occludin, and vinculin system.

Figure 6 offers a ribbon model of the CPMV (red), vimentin (green), and beta-amyloid (blue) system, which offers more insight into how Alzheimer's plaques could be treated with the CPMV capsid.

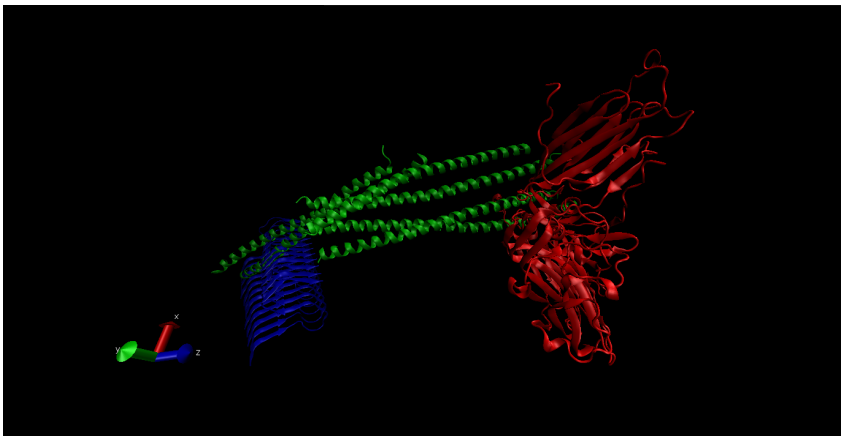

Figure 6 A ribbon model of the CPMV, vimentin, and beta-amyloid system.

2.7 Conclusions and Future Work

All three listed objectives of this project were met by the simulation results. For the first objective, the blood-brain barrier needed to be targeted without resulting in additional damage to the barrier. This was accomplished by simulating the system containing the CPMV capsid subunits, vimentin, and tight-junction protein ZO-2, as well as the system containing the CPMV capsid subunits, vimentin, tight-junction protein ZO-2, occludin, and vinculin, and observing their interactions. For the second objective, Alzheimer's plaques had to be targeted without damaging surrounding tissue. This was represented by simulating the system containing the CPMV capsid subunits and vimentin and observing their interactions, in addition to projecting the simulation for the system containing the CPMV capsid subunits, vimentin, and beta-amyloid. Finally, for the third objective, the viability of a viral vector for treating Alzheimer's disease needed to be demonstrated. The simulations did not overheat the system and yielded stable normal temperature values. Therefore, the capsid presents viability as a treatment vector in the body.

This project represents the innovative convergence between inorganic nanoparticles used for the diagnosis of Alzheimer's disease and viral nanoparticles used as vaccines or as treatment vectors for other illnesses including cancer. It offers a method of treating Alzheimer's disease that precisely delivers drugs to the appropriate locations without increasing the risk of toxicity to other tissues in the brain. In addition, it further develops the field of viral nanomedicine by demonstrating the possibility of using viral nanoparticles in the central nervous system, especially in the brain.

These results also support prior work conducted in-vitro and in-vivo with CPMV to determine its ability to target specific proteins and/or regions of the body. Most importantly, they further back up the ability of CPMV to target vimentin [12] and localize to regions of the brain and blood-brain barrier [15].

Currently, drug development for Alzheimer's disease is hindered, among other factors, by a need to bypass the blood-brain barrier. By using nanoparticles to encapsulate medication, unmodified drug molecules can be transported directly to a treatment site, increasing their efficacy and reducing the amount needed to treat a patient. In addition, the usage of computer simulations and software can reduce the amount of time and money spent on drug development, increasing the affordability of the

process and identifying the most suitable molecules for further study. As shown with this project, molecular dynamics simulations offer a valid way of determining interactions between molecules of interest. In the end, this ultimately benefits patients and their families, as well as researchers involved in drug development.

In the future, this research can be expanded in many directions. On the computational side, larger-scale studies (restart simulations) with these systems can be conducted with VMD and NAMD. This can be used to offer further data for the development of a physical nanoparticle from the CPMV capsid, as well as provide more information about molecular interactions. A detailed model of CPMV can be designed using VMD and NAMD to identify targets on the capsid for ligand decoration.

However, because this project is computational and theoretical in nature, its results should be justified with wet-lab methods. The rate of degradation, efficacy of encapsulation, and ability to deliver treatment to Alzheimer's plaques can be identified with *in-vivo* testing to validate the conclusions of this project. Wet-lab experimentation would also provide a method of studying the results gained from computer simulations in the context of a living system, which could help improve computational biology techniques in nanomedicine and increase the validity of any results gained from such studies.

The major questions that can be explored next include the following. "How does time impact interactions between molecules in a molecular dynamics study (if at all)?" "How well do computational simulations mimic what occurs in actual biological systems?" "How can we improve simulation software programs such that simulations can mimic actual systems with a high degree of accuracy?"

2.8 Acknowledgements

I would like to thank the following people for their influence on and help with my project. Dr. Rachael Sirianni inspired me to work with nanoparticles in the first place. Her laboratory's work on polymer nanoparticles for the treatment of brain tumors inspired me to look into similar treatments for Alzheimer's disease. Dr. Jeffrey Yarger helped me identify the software programs used in the project as a cost-effective alternative to conducting the experiment *in-vivo*. Dr. Stephen

Davidowski showed me how to use the different aspects of the software. He also helped me run longer simulations (those with upwards of 100000 timesteps) on an ASU Magnetic Resonance Research Center computer.

The teachers of my Honors Science Research course - Mrs. Debbie Nipar, Mr. Herb Tilley, and Mrs. Raxha Bhagdev - aided me in catching any errors in logic or reasoning as I developed my project. They all offered me encouragement as I continued my project. The VMD and NAMD online communities, tutorials, and user guides helped me resolve software issues I was experiencing and answered my questions about specific aspects of the software. In addition, my family provided moral support as I conducted my project, and my father helped me troubleshoot problems I encountered while running simulations and using the software.

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Additional Resources

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