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Exploring the Dynamics of Biological Macromolecules at Angstrom Scale

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Exploring the Dynamics of Biological Macromolecules at Angstrom Scale

By

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THESIS COMMITTEE APPROVAL

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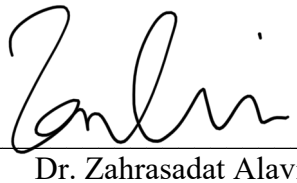
This thesis has been read by each member of the following thesis committee and by majority vote has been found to be satisfactory.



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ABSTRACT

This thesis studied the Guanylate Kinase (GK) enzyme, which catalyzes the reaction of ATP into ADP, using nano-rheological techniques. A unique experimental setup was created in order to observe the conformational dynamics of the biological macromolecules. An oscillatory force was applied by attaching enzymes to a gold-coated surface and gold nanoparticles. An additional gold-coated surface was placed on top of the setup in order to create a parallel plate capacitor configuration. An oscillatory voltage was then applied across the capacitor to drive the gold nanoparticles and exert a force on the enzymes. The setup detected the ensemble averaged movement of the enzymes at the Angstrom scale in order to measure the conformational dynamics. The results of the enzyme deformation revealed that they conformationally change according to a viscoelastic regime. At low frequency oscillations, the enzymes acted like a viscous fluid while at high frequencies, the enzymes were elastic like a stiff spring. Due to the disruption of the COVID-19 pandemic, this thesis transitioned to studying the enzyme computationally using molecular dynamics simulations. The results of this thesis are a better understanding of the conformational dynamics of the enzyme which can lead to improved medicinal applications and more targeted molecular dynamics studies in biochemistry and biophysics. Future work includes experimenting with different chemical solutes in solution with the enzymes to measure how the conformational dynamics change and using the molecular dynamics simulations to tailor the experimental approach.

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ACKNOWLEDGEMENTS

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CHAPTER 1: INTRODUCTION

Biological macromolecules as a class generally includes nucleic acids (DNA and RNA), polysaccharides (glycogen and cellulose), and proteins.[1] These large biological molecules are deformable, which means they can bend and change shape, and their deformation contributes to their functions in cells. In particular, proteins are composed of polypeptide chains which form the primary structure by linking together a unique sequence of amino acids. From this primary structure, the polypeptide chains fold into alpha helices and beta sheets which form the secondary structure. Then, the polypeptide chain's alpha helices and beta sheets fold even further into a complex, usually globular, tertiary structure.[1] This particular shape and composition along with a thin coating of water molecules gives proteins their functionality. Proteins serve numerous purposes in biological systems and are integral to the operations that occur within cells. Within the class of proteins, there are specific biological macromolecules called enzymes which function to catalyze reactions of substrates into products. The catalysis works through enzymes binding to other molecules, lowering the activation energy of the entire complex, and facilitating reactions of substrates into products. The shape of the enzyme must change slightly, called a conformational change, during its binding in order to act as a catalyst.[1] Inside cells, enzymes catalyze many reactions including assembling smaller molecules into larger ones, breaking larger molecules apart, and regulating processes related to growth and replication.

Given the importance of enzyme as biological macromolecules, we studied the deformation and the mechanical properties of the Guanylate Kinase (GK) enzyme, which catalyzes the reaction of ATP into ADP, using nano-rheological techniques and molecular dynamics simulations. The reaction of ATP into ADP supplies energy for cells to function, grow, and replicate, so the GK enzyme plays a vital role in cellular operation. The structure of enzymes is integral to their function, so a deeper understanding of an enzyme's structural properties is beneficial for research and application. Rheology in general is the study of how materials deform, and nano-rheology looks at this deformation at the nano (10^{-9} m) scale in order to analyze molecular deformation. We created a unique experimental setup which has been shown to detect the ensemble averaged movement of a large number of the GK enzymes at the Angstrom (10^{-10} m) scale by applying an oscillatory force and measuring the frequency response.[2, 3, 4, 5] Additionally, we attempted to recreate and analyze the conformational dynamics of the enzyme through molecular dynamics simulations in order to model the experimental results and provide more insight into the GK enzyme's properties.[6]

The dynamics of the enzyme have been investigated by the Zocchi group at UCLA, and we have recreated the experimental setup with the goal of reproducing the group's results.[7] The experimental results show that the enzyme deformation behaves according to a viscoelastic regime. In other words, the protein conformationally changes, or changes shape to function, with viscous and elastic properties. The results indicate that at low frequencies of oscillations, GK behaves like a viscous fluid such as honey, while at higher frequencies, GK behaves like an elastic solid such as a spring. Research from the Zocchi

group has also shown that the hydration shells around enzymes are incredibly important in understanding the function of their dynamics.[2, 8] The group's work has explored how the conformational behavior is affected by various order and disorder inducing agents in solution, but further study of the enzyme will determine more accurate characteristics of GK's molecular dynamics.[5]

With the circumstances surrounding the COVID-19 pandemic, we were not able to work with the enzyme in the lab to study its conformational dynamics. At that point, the research transitioned into working remotely to study the enzyme. Various computational software was considered including AMBER (Assisted Model Building with Energy Refinement), LAMMPS (Large-scale Atomic/Molecular Massively Parallel Simulator), and NAMD (Nanoscale Molecular Dynamics). Ultimately, we chose to use VMD (Visual Molecular Dynamics) to visualize the molecules, and GROMACS (derived from Groningen Machine for Chemical Simulations) to process the GK enzyme's molecular dynamics.[9, 10] Using these tools, the position and charge of every atom in the enzyme is fixed inside a box filled with water molecules, and GROMACS simulates what forces the enzyme experiences. The output files can then be analyzed by looking at the raw data and by uploading to VMD to see a rendering of the system. The simulations require lots of processing power and time to execute, so doing the molecular dynamics simulations is time consuming. The results to date have shown the GK enzyme experiencing normal fluctuations over time and prove the software's ability to output viable simulations. Whether from the experimental setup in person or the computer simulations, the results of this research can be extrapolated to more classes of proteins in the human body and provide important insight into their structure and

function. A better understanding of these proteins can lead to improved medicinal applications and more targeted molecular dynamics studies in biochemistry and biophysics.

1.1 METHODOLOGY: EXPERIMENTAL AND COMPUTATIONAL

First, the experimental setup in the laboratory requires production of the enzyme. To study the motion of the GK enzyme, the protein's amino acid chain had to be altered to include additional Cysteines that bind to gold.[7] The enzyme was synthesized by inserting genetically modified plasmids in E. coli bacteria and promoting bacterial growth. Then, protein expression in the bacteria was induced using IPTG.[7] The cells were lysed, and the extracted solution was purified to isolate the protein. The altered enzymes could then be tethered to gold nanoparticles and to a gold surface. With an additional gold-coated surface placed on top of the setup, a parallel plate capacitor configuration could be achieved. An oscillatory AC electric field across the capacitor then could drive the negatively charged gold nanoparticles (GNPs). The mechanical response of the enzyme could be quantified by measuring the amplitude of oscillation of these particles using evanescent wave microscopy. The oscillating GNPs could scatter an evanescent wave created by a laser beam. Intensity of the scattered beam could be detected by a microscope and converted to an electrical signal by a photomultiplier tube (PMT). The signal could then be sent to a lock-in amplifier to be processed. Due to the COVID-19 pandemic, we assembled the experimental setup and synthesized the protein but were unable to execute tests and obtain results.

Fortunately, the GROMACS software was easy to download, install, and use. The software package requires a C or C++ compiler in addition to the program CMake. For the compiler, the GNU Compiler Collection (GCC) was installed, and CMake is a standalone program to install. The program Homebrew is a package management system that, once installed in MacOS, accesses many other programs to install, and Homebrew was used to install GCC, CMake, and GROMACS. In order to utilize GROMACS, files from the Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB) were downloaded. The GK enzyme file used was the "1ZNW Crystal Structure Of Unliganded Form Of Mycobacterium tuberculosis Guanylate Kinase" which was downloaded in the PDB format as 1znw.pdb and was compatible with GROMACS and VMD.[11] Tutorials were used to understand how to use the GROMACS functionality courtesy of Dr. Justin A. Lemkul from Virginia Tech Department of Biochemistry.[12] This computational methodology has provided results for this thesis unlike the experimental methodology.

1.2 INTRODUCTION TO MOLECULAR DYNAMICS SIMULATIONS

We simulated the GK enzyme using the GROMACS software with follows typical molecular dynamics simulation methods.[10] First, the position of every atom in the system is determined, then a force field is applied to the atoms, and equations of motion are solved for over time from the Lennard-Jones potential in order to find new atomic positions. In order to simulate a complex system such as a protein via computational software, the movement of every atom in the system must be understood and represented. Using a classical formulation, the individual equations of motion for a particle are given in the following:[13]

$$m_i \ddot{r}_i = f_i \quad f_i = -\frac{\partial}{\partial r_i} \mathcal{U} \tag{1}$$

Here, f is the force acting on the particle, r is the atomic coordinates, and U is the potential energy. For non-bonded interactions, including the movement of water molecules, the potential energy is the sum of the potential fields surrounding individual particles which is the following:[13]

$$\mathcal{U}_{\text{non-bonded}}(r^N) = \sum_i u(r_i) + \sum_i \sum_{j>i} v(r_i, r_j) + \dots \tag{2}$$

The potential between two particles is typically governed by the Lennard Jones potential which is characterized by σ, the diameter of the potential well, and ε, the well depth:

$$v^{\text{LJ}}(r) = 4\varepsilon \left[\left(\frac{\sigma}{r}\right)^{12} - \left(\frac{\sigma}{r}\right)^{6} \right] \tag{3}$$

Executing proper molecular simulations, however, also requires force fields to calculate potentials and interactions, and the explanation of these fields is beyond the scope of this research. For the simulations, the all-atom Optimized Potential for Liquid Simulations (OPLS- AA) was used.[14]

1.3 TIMELINE

Beginning collaboration in the Spring of 2019, we started work by researching the topic of nano-rheology on biological macromolecules which consisted of reading papers and discussion meetings to elucidate the methodology. Background understanding of the enzyme under study, the attachment mechanism, and the underlying physics of the evanescent wave microscopy were all necessary. During this introductory work, we began assembling the experimental tools needed for the laboratory including lock-in amplifier, photomultiplier tube, pipettes, glass slides, and more. The process of preparing the samples of the protein for experiments began over Summer of 2019 and into the Fall semester. Protein synthesis required lab space, materials, and protocols which were accomplished with the gracious help of the Zocchi group at UCLA. The necessary components for the experimental setup were assembled by the beginning of 2020, and we were testing samples of the purified protein for experimental use when the COVID-19 pandemic began in the Spring of 2020. Since then, the project has transitioned to working remotely with the same goal of understanding the GK enzyme except through molecular dynamics simulations on computational software. The computational research has been ongoing from the Spring of 2020 and will continue until completion of the thesis.

CHAPTER 2: EXPERIMENTAL METHODS

We have studied and used two distinct methods to approach research into the Guanylate Kinase enzyme: nano-rheology with an experimental setup, and molecular dynamics simulations with computational software. While the experimental setup was unable to be used to produce data or any results, the framework and mechanisms were studied in depth, and the information remains relevant to this thesis which will review the experimental methods. The molecular dynamics simulations, on the other hand, have yielded useful data. Given that the research with these methods into the GK enzyme hasn't been undertaken before, the technical details will be carefully examined and explained.

2.1 EXPERIMENTAL SETUP

As discussed in section 1.1, the experimental setup involves attaching slightly modified GK enzymes to a gold coated slide and to gold nanoparticles (GNPs) and then using an electric field to force the GNPs and stretch and compress the enzymes. A diagram (not to scale) of the enzyme's attachment is shown in Figure 1 below. In order to acquire the GK enzyme, the methods of protein synthesis and purification have already been discussed and are shown in Figure 2 below. The voltage to the slides is provided by the lock-in amplifier which also allows for manipulation of the supply. Beneath the gold slides sits a glass prism, and a calibrated helium neon laser is targeted to hit one end of the prism and escape through

the other end. While inside the prism, the laser light is refracted and scattered through the thin gold coating. This scattering produces an evanescent wave that is deflected by the GNPs as it travels upward. The scattered laser light is received by the photomultiplier tube (PMT) which transmits the signal to the lock-in amplifier (Lock-in). This entire process is captured by Figure 3 below.

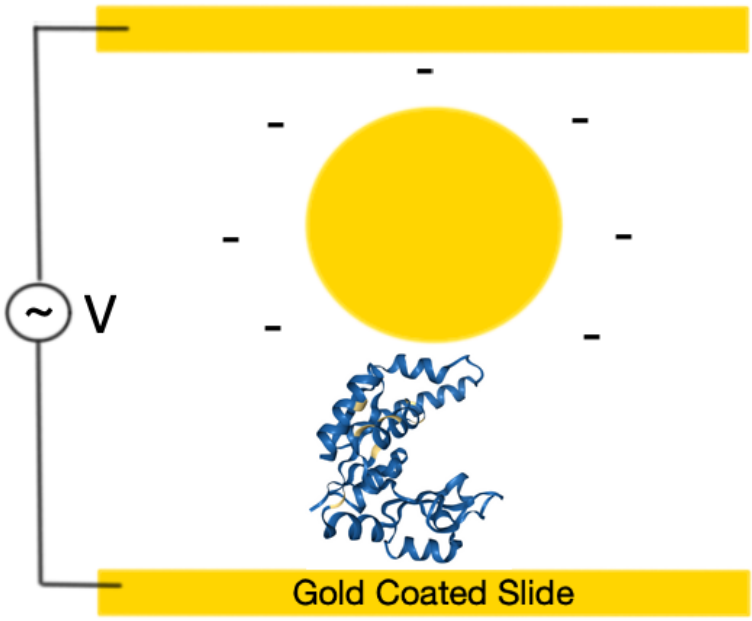

Figure 1: The enzyme attaches on the GNP and the gold coated slide with an oscillatory voltage applied across the parallel plate capacitor.

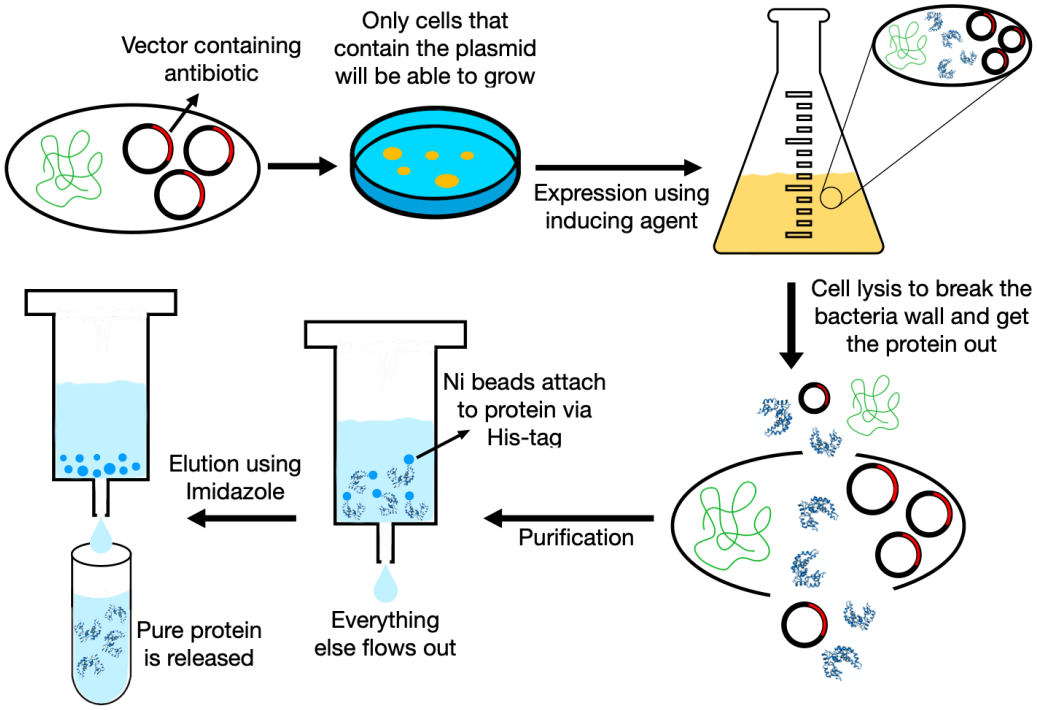

Figure 2: The enzyme is first synthesized using bacteria, and then the cells are broken open, and the enzyme is purified from the surrounding mixture.

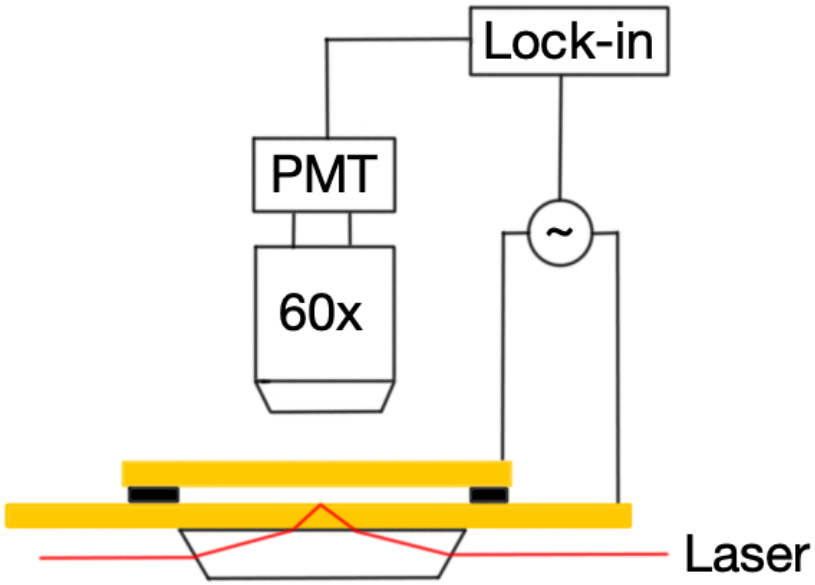

Figure 3: The laser light is refracted, scattered by the gold, and eventually received by the PMT which relays the information to the Lock-in. The Lock-in also supplies the voltage.

The experimental setup took many months to assemble, and the sample required a three-day expression protocol that followed biology lab procedures. Specific details of the expression protocol can be found in Dr. Zahrasadat Alavi's PhD thesis dissertation under Guanylate Kinase Expression, but a summary of the methods will be described here.[7] In the first day, a plasmid containing the sequence of the GK enzyme and antibiotic resistance is inserted into *E. coli* bacteria, and bacterial growth in promoted in agar plates containing antibiotic. In the second day, the growing bacterial colonies have taken up the plasmid, and their growth is further encouraged via mixtures with broth to feed the bacteria. Finally, the last day involves centrifuging and filtering the mixture in order to obtain more concentrated solution of cells and then purifying to obtain pure GK protein. After obtaining solution of the enzyme, we used gel electrophoresis in order to check the weight of the enzyme to confirm that the expression protocol was successful.

2.2 MOLECULAR DYNAMICS SIMULATIONS

While the necessary equipment for the experimental setup was successfully acquired and prepared, we were unable to run tests using the setup. During the COVID-19 pandemic, Loyola Marymount University's Westchester campus has been closed to students, and access to facilities, including the lab in the physics department, has been restricted. However, in order to progress with research, the decision was made to transition the focus to methods that could be achieved computationally. The purpose of the computational work has been to simulate the molecular dynamics of the Guanylate Kinase enzyme in order to understand its behavior better before returning to the lab environment. While we will not be able to return to campus to run tests, future work will be conducted on this topic, and

this thesis will assist that work. In order to simulate the enzyme's molecular dynamics, a background in coding and computer science was required. First, we took virtual courses called "Python for Everybody" via the online learning provider Coursera to learn introductory Python coding. Then, the aforementioned programs AMBER, LAMMPS, and NAMD were researched to determine their utility in doing molecular dynamics simulations. The software GROMACS along with the tutorial for using GROMACS to conduct the simulations proved to be the most accessible and useful. The software VMD accepts numerous file types including .gro which it visualizes as shown in the Figures 4 and 5 below, and .xtc which loads into the .gro file and creates a movie that can be played.

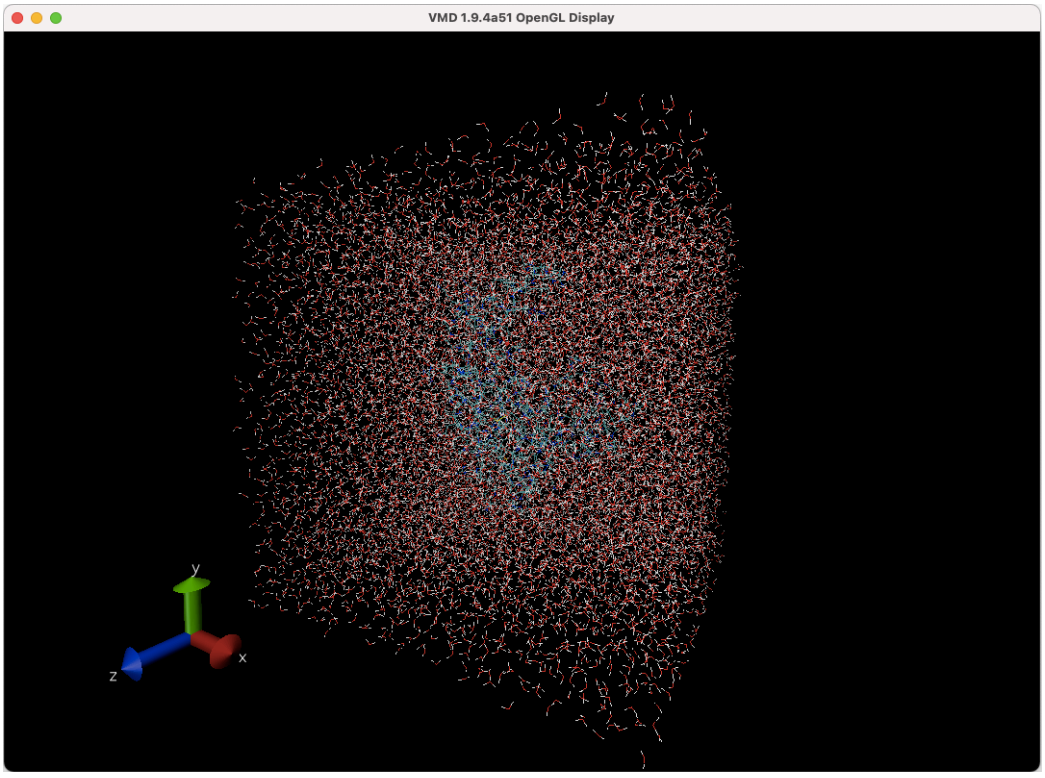

Figure 4: The GK enzyme (in blue) surrounded by a box of water molecules (red and white) in VMD's display with Graphics->Representations set to "Lines."

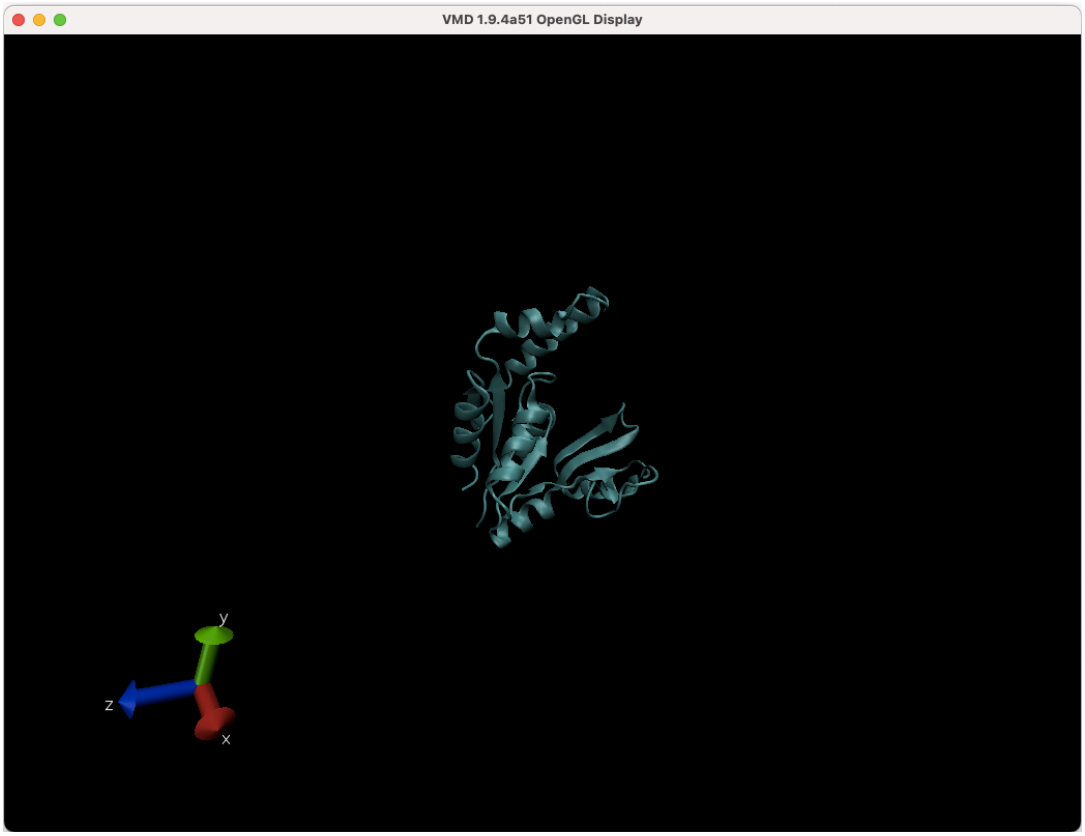

Figure 5: The same GK enzyme as Fig. 4 but with the Representations set to "NewCartoon" to show the alpha helices and beta sheets in the molecule.

Given the medium of this thesis, the actual movie of the enzyme deforming in the box of water cannot be shown, but it can be viewed through the following YouTube link: youtu.be/xHXmizyBeS0.[15] The short video shows the GK enzyme in the "NewCartoon" Representation moving in the box of water molecules over 22 frames which constitutes 20 picoseconds of simulation. The duration of the video is small compared to the timescale on which the enzyme functions (at the nanosecond scale), but one major difficulty in executing the molecular dynamics simulations is the amount of processing power and computational time the simulations require. In order to obtain the 20-picosecond movie, a MacBook Air with a 1.8 GHz Dual-Core Intel Core i5 processor, 8 GB 1600 MHz DDR3 memory, and Intel HD Graphics 6000 1536 MB graphics was used, and the simulation took around 6

hours to complete. From experimenting with other simulations, shorter movies on the order of 1-10 picoseconds take on the order of 1-10 hours, and longer movies of 100 picoseconds take closer to at least 24 hours of continuous running to process (meaning the computer cannot turn off at all). A sample of the code run is shown in Figure 6 below.

```
grep -v HOH 1ZNW.pdb > 1ZNW_clean.pdb

gmx pdb2gmx -f 1ZNW.pdb -ignh -ter -o complex.gro
gmx pdb2gmx -f 1ZNW_clean.pdb -o 1ZNW_processed.gro -water spce
     15 enter

gmx editconf -f 1ZNW_processed.gro -o 1ZNW_newbox.gro -c -d 1.0
-bt cubic

gmx solvate -cp 1ZNW_newbox.gro -cs spc216.gro -o 1ZNW_solv.gro
-p topol.top

gmx grompp -f ions.mdp -c 1ZNW_solv.gro -p topol.top -o ions.tpr

gmx genion -s ions.tpr -o 1ZNW_solv_ions.gro -p topol.top -pname
NA -nname CL -neutral

gmx grompp -f minim.mdp -c 1ZNW_solv_ions.gro -p topol.top -o
em.tpr

gmx energy -f em.edr -o potential.xvg
     10 0 enter
```

```
gmx grompp -f nvt.mdp -c em.gro -r em.gro -p topol.top -o
nvt.tpr

gmx mdrun -deffnm nvt

gmx energy -f nvt.edr -o temperature.xvg
     16 0 enter

gmx grompp -f npt.mdp -c nvt.gro -r nvt.gro -t nvt.cpt -p
topol.top -o npt.tpr

gmx mdrun -deffnm npt

gmx energy -f npt.edr -o pressure.xvg
     18 0 enter

gmx energy -f npt.edr -o density.xvg
     24 0 enter

gmx grompp -f md.mdp -c npt.gro -t npt.cpt -p topol.top -o
md_0_1.tpr

gmx mdrun -deffnm md_0_1
```

Figure 6: Instructions to create movie of GK enzyme (1ZNW.pdb) in the order in which they are run in the terminal. For lines that prompt a response, numbers are given to specify the correct choice.

The full GK enzyme contains thousands of atoms, and during the simulations, the position of each atom must be logged, a force must be applied, and the equations of motions must be solved in order to determine how the particle reacts. This granular approach gives accurate representations of the molecule but results in enormous amounts of computational time to complete the simulations. In order to combat this issue, we also experimented with a portion of the GK enzyme from amino acid 168 to acid 179, and the short piece can be seen below in Figure 7.

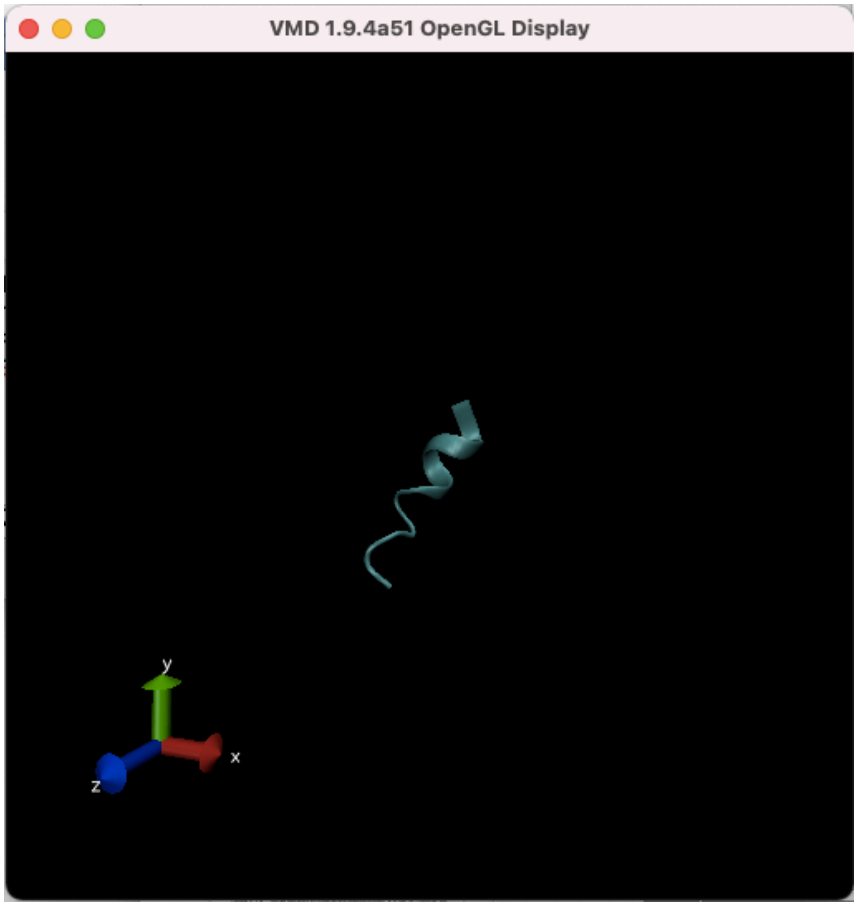

Figure 7: The short piece of the GK enzyme from 168-179 in "NewCartoon" representation to show the alpha helix shape.

With the shorter piece of the enzyme, computation time was drastically reduced as the entire system has less than a few hundred atoms. Using the pulling code on this short piece also produced more clear results for the deformation of the protein. The movie of the short piece being pulled can be viewed at the following YouTube link: youtu.be/QToyi7bd6X0.[16] Similar to the previous simulation, this movie consists of 22 frames and 20 picoseconds of movement. The same settings were used in the simulation, so given the smaller size of the protein, the pulling of the two ends of the short piece can be clearly seen in the movie.

CHAPTER 3: RESULTS AND ANALYSIS

After the simulations ran, the code produced numerous GROMACS output files that had different uses to analyze behavior. The file types that actually contained information on the atoms and their position were GROMACS files with ".gro" endings. When loading molecules into VMD, the software detects the file type and determines how to open it. The ".gro" files load into VMD as a single frame with a specified number of atoms in the system. The display window shows the simplest representation of the atoms in the system which uses lines to represent atoms (shown in Figure 4). The representation graphics can be changed to give a different perspective on the system. In order to view the actual molecular dynamics simulation, the ".gro" file needs more data to make it into a movie. By right-clicking on the molecule in the VMD Main window, there is an option to load data into the molecule. From there, the associated ".xtc" file to the molecule can be selected which is a GROMACS XTC Compressed Trajectory file. This information adds frames that represent movement and changes to the single frame ".gro" file.

With these steps, the molecular dynamics simulations for the Guanylate Kinase enzyme and the smaller piece of that enzyme could be watched as animated movies. The movies, which were given earlier as YouTube links, show that the proteins jiggle and shake during the simulation, and the smaller piece of enzyme actually pulls slightly over time. In order

to find the amount of distance that the proteins were pulled, a trajectory conversion command in GROMACS was used and then a Bash shell script was able to output the summary of pulling distances. The script used to do this process are shown below in Figure 8.

```
gmx trjconv -s pull.tpr -f pull.xtc -o conf.gro -sep


sh get_distances.sh

     echo 0 | gmx trjconv -s pull.tpr -f pull.xtc -o conf.gro
-sep

     for (( i=0; i<21; i++ ))
     do
          gmx distance -s pull.tpr -f conf${i}.gro -n index.ndx
-select 'com of     group "r_168" plus com of group "r_179"'
-oall dist${i}.xvg
     done

     touch summary_distances.dat
     for (( i=0; i<21; i++ ))
     do
          d=`tail -n 1 dist${i}.xvg | awk '{print $2}'`
          echo "${i} ${d}" >> summary_distances.dat
          rm dist${i}.xvg
     done

     exit;
```

Figure 8: The first two lines are run in the terminal, and below the second line, the instructions must be saved as a separate file called get_distances.sh in order to run. The group names can be changed for different simulations, and this example is from a short piece simulation.

With this code, the results of the pulling in the simulation are given in a summary distance data file ".dat" which shows the distance between the two groups that were pulled on in the simulation. The results of both simulations with the GK enzyme and with the short piece of the enzyme are given below in Tables 1 and 2.

0 4.363	0 1.961
1 4.364	1 1.992
2 4.391	2 1.961
3 4.407	3 1.954
4 4.407	4 1.977
5 4.424	5 1.969
6 4.430	6 1.979
7 4.467	7 1.924
8 4.461	8 2.014
9 4.466	9 2.035
10 4.506	10 1.988
11 4.523	11 1.973
12 4.544	12 1.992
13 4.525	13 2.001
14 4.567	14 2.056
15 4.555	15 2.025
16 4.556	16 2.049
17 4.576	17 2.076
18 4.599	18 2.057
19 4.620	19 2.104
20 4.627	20 2.132

Table 1 (Left): The summary of pulling distances for GK enzyme in nanometers.
Table 2 (Right): The summary of pulling distances for short piece of GK enzyme in nanometers.

The results for the GK enzyme show that the two ends being pulled on the enzyme moved 0.264 nanometers. Overall, the enzyme only deformed by about 6% of its original size. The two ends of the short piece of the enzyme, though, moved 0.171 nanometers and deformed by about 9% of its original size. This change for the short piece was more easily viewed in the movie compared to the whole enzyme's movement. The pulling did not change the enzyme much, but there are numerous reasons for constraints on the simulation. In order to make the simulation run for 20 picoseconds, the processing time required for the entire GK enzyme was over five hours, and even for the smaller piece of the enzyme, the code

ran for almost an hour and a half. In order to run longer movies, the processing time continues to get longer, and when the computation long is too long, the simulations are not feasible. The short time scale of the simulations can only show a small amount of change in the system.

CHAPTER 4: CONCLUSIONS AND FUTURE WORK

The original aim of our research was to study the deformation and the mechanical properties of the Guanylate Kinase (GK) enzyme using nano-rheological techniques. Due to the COVID-19 pandemic, in-person work in the lab was limited, and the research focus shifted to modeling the deformation of the enzyme using molecular dynamics simulations. From the simulations, we obtained movies showing the behavior of the GK enzyme in the simulated environment over small timescales. When applying force to both ends of the protein, the macromolecule was deformed in a similar manner to the nano-rheological techniques that would have been performed in-person. The results confirm previous research that the enzyme follows a viscoelastic regime of deformation, and the movies display what this deformation looks like in a simulated environment. Further work with the simulations includes changing the surrounding hydration layer of the enzyme in the simulation and determining how the deformation is affected. Additionally, when in-person work in the lab is possible again, these simulations can be used concurrently with the nano-rheology experiments to gain a new perspective on the process.

While the circumstances were not ideal, we tried to be flexible, like the enzyme, in approaching the main research question: how do the structure and behavior of the enzyme affect its function? The answer that we have arrived upon confirms previous work that the

enzyme follows a particular regime of viscoelastic behavior. Viscoelastic behavior means that the enzyme is viscous at low frequency of applied force and elastic at high frequency. At the high frequency of applied force in the simulation, the enzyme pulled like an elastic spring. The results of the GK enzyme can be applied to other types of proteins and provide insight into their structure and function. With these results, further work can be undertaken in the medical field, and more targeted studies can be done in biochemistry and biophysics.

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