MOLECULAR DYNAMICS AND SIMULATION OF MYOSIN MOLECULE USING AVOGADRO SOFTWARE

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CERTIFICATION

This is to certify that this project work "MOLECULAR DYNAMICS AND SIMULATION OF MYOSIN MOLECULE USING AVOGADRO SOFTWARE" was carried out by DELE, Nehemiah Oshogwe with matriculation number of PSC1707887 of the Department of Physics, Faculty of Physical Sciences, University of Benin, Benin City, Edo state, Nigeria.

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EXTERNAL EXAMINER DA

DEDICATION

This work is dedicated to my parents, Pastor Dele Joseph and Pastor Helen Dele. Thank you for the support and impact you have made in my life. I will never take this for granted. I love you.

CERTIFICATION OF DISSERTATION ON PLAGIARISM

We the undersigned attest and declare that the dissertation of DELE, Nehemiah Oshogwe titled "MOLECULAR DYNAMICS AND SIMULATION OF MYOSIN MOLECULE USING AVOGADRO SOFTWARE" has successfully passed the anti-plagiarism test and does not violate any copyright regulations.

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Lastly, but not the least, to all biophysicists all over the world, who have discovered and are still discovering new beneficial ideas to humanity. You are great people and I say well done.

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ABSTRACT

Molecular dynamics and simulations are a vital tool used for the description of molecules in terms of their shapes, bonds, length, dihedrals and molecular structure in space. Molecules that exist tends to undergo some discrete changes when subjected to a particular environment. This changes in molecules over the time defines the final or ultimate state in which the molecule can or will exist. One of such molecules which could be simulated is Myosin. Myosin is a rotor protein molecule responsible for muscle contraction of bio-organisms. Considering its aid in kinetic activities, it is therefore a significant molecule to study and investigate under molecular dynamics and simulations. In this study, we are to carry out molecular dynamics simulation on myosin molecule using the Avogadro software, by importing the molecule, visualising, optimising its geometry and carrying out its energy minimisation.

The results show that the minimised energy is the same for any time steps used. Also, the time steps used affects the time taken for the simulation to complete.

Molecular dynamics simulation of myosin molecule is very paramount in aiding scientist on how drug and bio-supplement should be modelled to suite the biological systems they are to be used on.

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND OF STUDY

Molecular Dynamics which is widely recognized as (MD) is described as a computer simulation method for analysing the physical movements of atoms and molecules (Durrant, 2021). It is used to study the behavior of molecules. Molecular dynamics and simulations are often used to study biophysical systems. Molecular dynamics (MD) is basically another simple approach to investigate an atom's location in free space (Polanski, 2009). In this approach, a single point model is replaced by a dynamic model where the nuclear system is forced into motion. Simulation of the motion is achieved by the numerical solution of the classical Newtonian dynamic equations.

Molecular Dynamics (MD) also provides information about the dynamic and thermodynamic properties of the molecules. It helps us to simulate protein structures and refine X-ray structures. To achieve these effectively, the use of certain computer programs or software is employed. Examples of software used in Molecular dynamics and simulations are AVOGADRO, AMBER, CHARMM, GROMACS, GROMOS, NAMD, LAMMPS, QUANTUM etc. These software are capable in Molecular dynamics and simulations. In this study, the software of interest is the AVOGADRO software which is widely accessible.

In the Molecular modelling of molecules (MD), the atoms and molecules are allowed to interact for a fixed period of time, giving a view of the dynamic evolution of the system. The trajectories of atoms and molecules are also determined by numerically solving Newton's equations of motion for a system of interacting particles and their potential energies are often calculated using interatomic potentials or molecular mechanics force fields. This method is applied mostly in Biophysics, chemical physics and material science (Schlick, 1996).

Molecules usually consists of a vast number of particles; thereby molecular systems contain a large number of particles. It is therefore impossible to determine the properties of the molecule (which is complex) analytically. We can't determine the properties with just logical reasoning. Molecular dynamics (MD) settles this problem by using numerical methods.

To model molecules with the Modelling software, a system has been initiated to easily import molecules into the software before modelling and simulations are carried out. All molecules possess a modelling code known as "PDB Number" which is used to import molecules to be modelled into the modelling software for the molecular simulation.

Going further in this study, it is very important to introduce the modelling display software known as VMD (Visual Molecular Dynamics). VMD is a

molecular modelling and visualization computer software. It is not the Molecular Dynamics and Modelling software itself, but it is mainly a tool used to view and analyse the results of molecular dynamics simulations. Visual Molecular Dynamics (VMD) can also be described as a molecular graphics program which is designed for the easy display and analysis of molecular assemblies in a given biopolymer such as protein which will be the area of central focus.

VMD can display any number of structures simultaneously by using a large variety of rendering styles of colouring methods. VMD can load atomic coordinate trajectories from AMBER, NAMD, Charmm, Gromacs, Avogadro and many other simulation software. The data gotten afterwards can be used to animate the molecule being simulated and also to determine the change in molecular properties such as angles, shape and structure, interatomic distances, energies etc. over time.

Therefore, VMD interprets whatever a molecular simulation software has modelled in both graphical forms as in image animation and view and also in numerical form as in the information of the angles, interatomic distances and energies. The Avogadro software also visualises molecules, therefore it can be used in visualising instead of VMD.

1.1.1 JUSTIFICATION OF STUDY

In this study, the molecule of interest is the Myosin molecule. Myosin is a protein molecule which perform the rotor actions in organisms. It is the molecule responsible for muscle contractions and movement in the systems of bio-organisms. Since it possesses a strong binding affinity to bind with other motor biomolecules, it is not out of place to mention that myosin possesses energies in form of ATP which accounts for its binding affinity. Therefore, it is significant to study this molecule and its energy.

1.1.2 AIM OF STUDYTo carry out molecular dynamic and simulations on myosin molecule using Avogadro software.

1.1.3 OBJECTIVES OF STUDY

The objectives of this study are to;

1. import myosin from PDB into Avogadro software.
2. visualise the myosin molecule.
3. carry out the molecular dynamics and simulation of myosin.
4. optimise the geometry of myosin.
5. obtain the energy of myosin molecule at different time steps.

CHAPTER TWO

LITERATURE REVIEW

2.1 BIOMOLECULES

Imagine that an alien lands on Earth, hears about something called a “bicycle,” and wants to understand how it works, how to ride it, and how to fix it when it breaks. Figuring this out given just a picture of a bicycle would be challenging. Watching a movie of someone riding a bicycle would help. Even better, the alien would experiment with an actual bicycle. For example, by turning a pedal and seeing how the wheels respond.

A molecular biologist trying to understand how a protein or other biomolecule works faces a similar challenge. An atomic-level structure is tremendously helpful and typically generates substantial insight about how the biomolecule functions. The atoms in a biomolecule are in constant motion, however, and both molecular function and intermolecular interactions depend on the dynamics of the molecules involved. One would like not just a static snapshot but the ability to watch these biomolecules in action, to perturb them at the atomic level, and to see how they respond. Unfortunately, watching the motions of individual atoms and perturbing them in a desired fashion is difficult. An attractive alternative is to work with an atomic-level computer simulation of the relevant biomolecules.

2.2 MOLECULAR DYNAMICS

Molecular dynamics (MD) simulations predict how every atom in a protein or other molecular system will move over time, based on a general model of the physics governing interatomic interactions (Karplus and Cammon, 2002). These simulations can capture a wide variety of important biomolecular processes, including conformational change, ligand binding, and protein folding, revealing the positions of all the atoms at femtosecond temporal resolution. Importantly, such simulations can also predict how biomolecules will respond at an atomic level to perturbations such as mutation, phosphorylation, protonation, or the addition or removal of a ligand. MD simulations are often used in combination with a wide variety of experimental structural biology techniques, including x-ray crystallography, cryo-electron microscopy (cryo-EM), nuclear magnetic resonance (NMR), electron paramagnetic resonance (EPR), and Forster resonance energy transfer (FRET). Molecular dynamics follows the Newton second law of motion with equation

$$F = ma \tag{2.1}$$

2.2.1 HISTORY OF MOLECULAR DYNAMICS

MD simulations are not new. The first MD simulations of simple gasses were performed in the late 1950s (Alder and Wainwright, 1957). The first MD

simulation of a protein was performed in the late 1970s (McCammon et al., 1977), and the groundwork that enabled these simulations was among the achievements recognized by the 2013 Nobel Prize in Chemistry (Levitt and Lifson, 1969; Lifson and Warshel, 1968). MD simulations have, however, become substantially more popular and visible in recent years, particularly from the perspective of experimental molecular biologists. Simulations have begun to appear frequently in experimental structural biology papers, where they are used both to interpret experimental results and to guide experimental work. This trend is particularly noticeable in neuroscience. Simulations have been used to study proteins critical to neuronal signalling (Dawe *et al*., 2016; Delemotte *et al*., 2011; Jensen *et al*., 2012; Shi *et al*., 2008), to assist in the development of drugs targeting the nervous system (Manglik *et al*., 2016; McCorvy *et al*., 2018), to reveal mechanisms of protein aggregation associated with neurodegenerative disorders (Khandogin and Brooks, 2007; Wu and Shea, 2013), and to provide a foundation for the design of improved optogenetics tools (Takemoto *et al*., 2015; Kato *et al*., 2018).

2.2.2 SIGNIFICANCE OF MOLECULAR DYNAMICS

The basic idea behind an MD simulation is straightforward. Given the positions of all the atoms in a biomolecular system (e.g., a protein surrounded by water and perhaps a lipid bilayer), one can calculate the force exerted on each atom by all the other atoms. One can thus use Newton's laws of motion to predict the

spatial position of each atom as a function of time. In particular, one steps through time, repeatedly calculating the forces on each atom and then using those forces to update the position and velocity of each atom. The resulting trajectory is, in essence, a three-dimensional movie that describes the atomic-level configuration of the system at every point during the simulated time interval.

These simulations are powerful for several reasons. First, they capture the position and motion of every atom at every point in time, which is very difficult with any experimental technique. Second, the simulation conditions are precisely known and can be carefully controlled: the initial conformation of a protein, which ligands are bound to it, whether it has any mutations or post-translational modifications, which other molecules are present in its environment, its protonation state, the temperature, the voltage across a membrane, and so on. By comparing simulations performed under different conditions, one can identify the effects of a wide variety of molecular perturbations.

The forces in an MD simulation are calculated using a model known as a molecular mechanics' force field, which is fit to the results of quantum mechanical calculations and, typically, to certain experimental measurements. For example, a typical force field incorporates terms that capture electrostatic (Coulombic) interactions between atoms, spring-like terms that model the

preferred length of each covalent bond, and terms capturing several other types of interatomic interactions. Such force fields are inherently approximate. Comparison of simulations to a variety of experimental data indicates that force fields have improved substantially over the past decade (Lindorff-Larsen *et al*., 2012), but they remain imperfect, and the uncertainty introduced by these approximations should be considered when analysing simulation results. Moreover, in a classical MD simulation, no covalent bonds form or break. Quantum mechanics/molecular mechanics (QM/MM) simulations, in which a small part of the system is modelled using quantum mechanical calculations and the remainder by MD simulation, are frequently employed to study reactions that involve changes to covalent bonds or are driven by the absorption of light (Senn and Thiel, 2009).

To ensure numerical stability, the time steps in an MD simulation must be short, typically only a few femtoseconds (10–15 s) each. Most of the events of biochemical interest. For example, functionally important structural changes in proteins take place on timescales of nanoseconds, microseconds, or longer. A typical simulation thus involves millions or billions of time steps. This fact, combined with the millions of interatomic interactions typically evaluated during a single time step, causes simulations to be very computationally demanding.

Over the past several decades, improvements in computing hardware and in the algorithms and software used for MD have allowed longer and cheaper simulations. Recent improvements have been particularly remarkable. Highly specialized hardware (Shaw *et al*., 2008; Shaw *et al*., 2014) has led to a major increase in maximum achievable speed, allowing certain simulations to reach millisecond timescales. Perhaps more importantly, GPUs have allowed simulations running on one or two inexpensive computer chips to outperform those previously performed on most supercomputers (Salomon-Ferrer *et al*., 2013). These GPUs have made simulations on biologically meaningful timescales accessible to far more researchers than ever before.

Indeed, performing simulations is now relatively straightforward (see the practical considerations section of this review), and the computational resources to perform useful amounts of simulation are increasingly widely accessible. What requires expertise is figuring out what questions can be addressed by simulations, designing simulations to address these questions, and interpreting the simulation results. Interpreting simulation results gaining biological insight from a large amount of trajectory data describing a mass of jiggling atoms can be particularly challenging. In addition, a wide variety of advanced simulation techniques are available to address questions that are intractable by simple "brute force" simulation.

Some simulation software that are widely known are AVOGADRO, AMBER, CHARMM, GROMACS, GROMOS, NAMD, LAMMPS, QUANTUM etc. Also, MD simulations can be used to simulate variety of molecules, especially protein molecules. Myosin is one of the numerous molecules which can be modelled using MD simulations.

2.3 MYOSINS

Myosin belongs to a family of motor proteins in muscles to enable muscle contraction. They may also be present in other cells such as amoebae and macrophages as a motor protein involved in different motility processes. Their fundamental properties include capability to bind with actin and ATPase enzyme activity. Most of them are comprised of the head, the neck, and the tail domains. The head of the myosin is that part that binds with Actin as in the Fig 2.1 below.

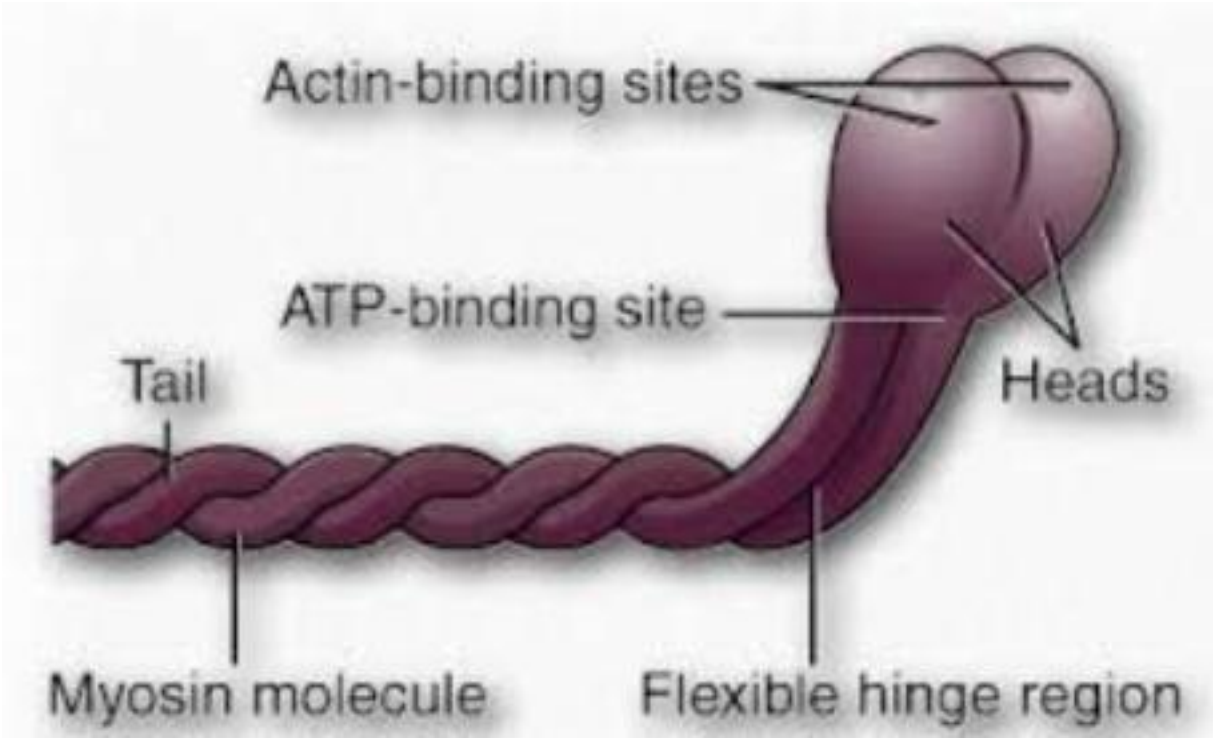


FIG 2.1 Myosin and Actin binding structure

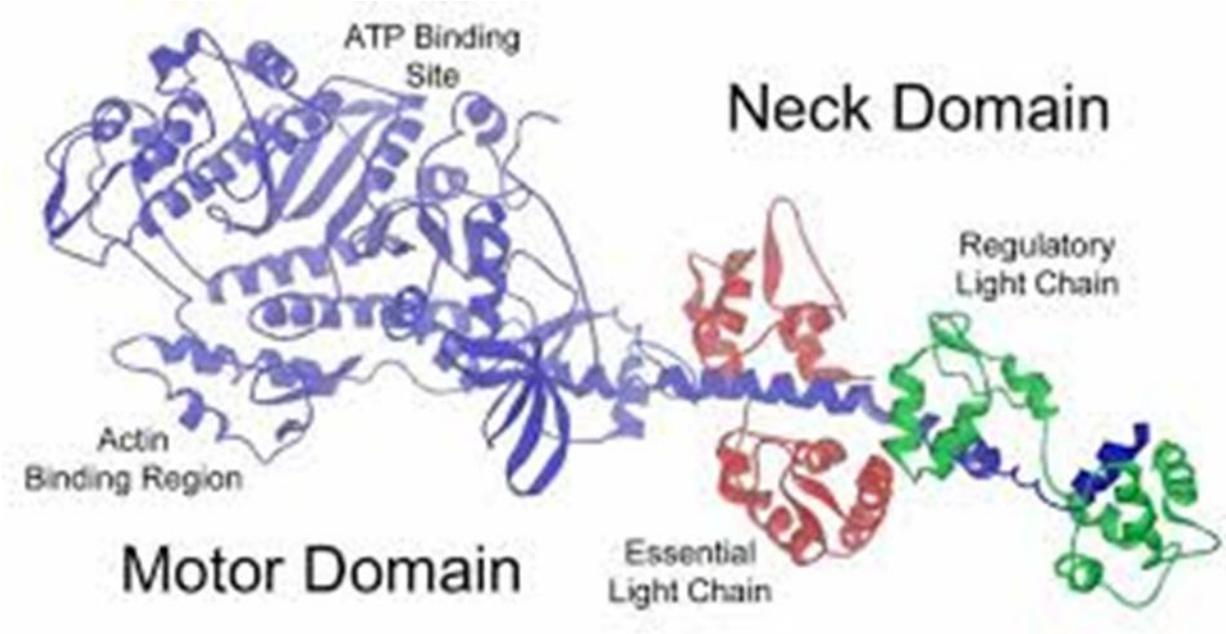


FIG 2.2 Structure of myosin molecule

Actin is a protein that binds together with myosin to form the contractile filaments of cells found in muscles, and is also involved in motion. Myosin is not Actin. The difference between them is that Myosin forms the thick contractile filaments within muscles and Actin forms the thin contractile filaments within muscles. Myosin molecule exist in numerous isoforms ranging from variety of organisms. Fig 2.3 below is the structure of a how myosin binds with actin.

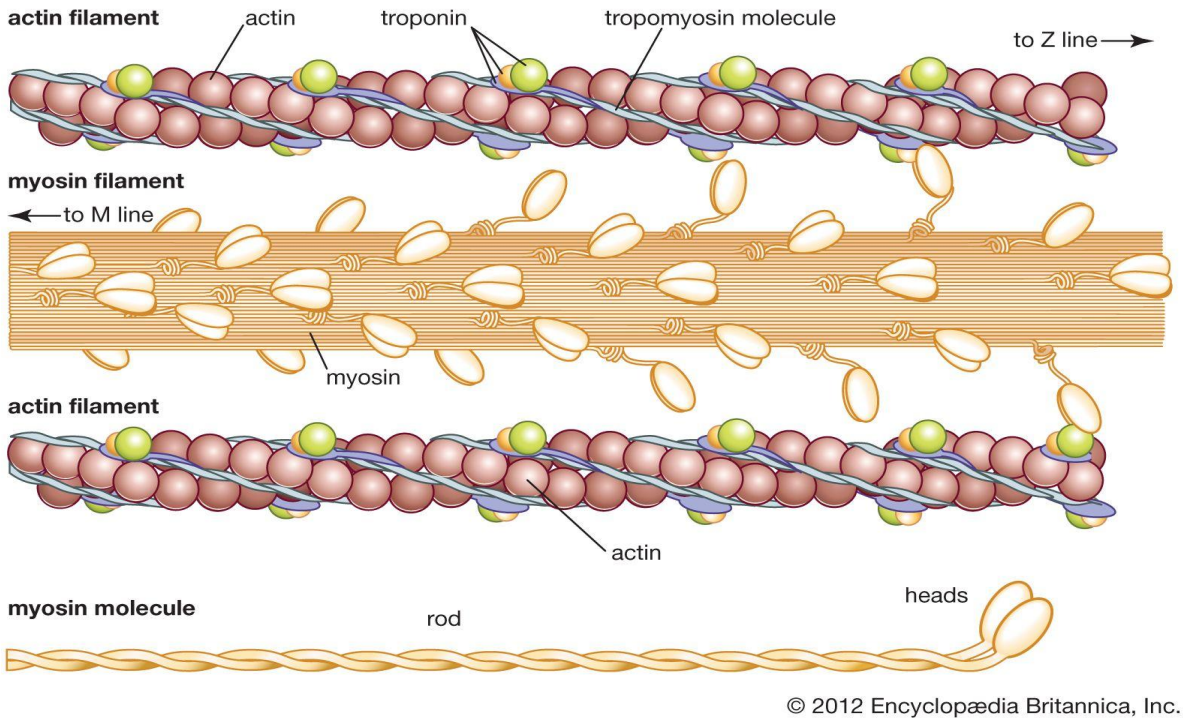


FIG 2.3 Myosin and Actin structure on muscular filaments.

Since myosin possess a motor attribute, thereby making it to exerts energy, it is therefore an outstanding molecule which researchers performs simulations on.

2.3.1 MOLECULAR DYNAMICS OF MYOSIN

Minghui Li and Wenjun Zheng carried out and all atom molecular dynamics and simulations of actin-myosin interactions in 2013 that myosins are motor proteins that move along filamentous actin powered by adenosine triphosphate hydrolysis that are involved in many key biophysical functions ranging from muscle contraction to intracellular transportation. They are at least 20 classes of myosin.

The actin-myosin interaction is highly dynamic involving various flexible loops at the actomyosin interface. Therefore, it is essential to probe the actomyosin dynamics with high spatial and temporal resolutions. To this end, computer molecular dynamics (MD) and simulations have proven to be useful in complementing experimental studies of myosin functions.

In the method used, a homology model of fast skeletal muscle of a chicken myosin motor domain was being used. Chimera was being used to model and describe the chicken fast skeletal muscle myosin. On the basis of the chicken fast skeletal muscle myosin, MODELLER was used to build three homology models for the motor domains of three myosin isoforms, human Cβ myosin, human Cα myosin and rabbit fast skeletal muscle myosin. The hydrogen atoms were added with VMD also were all parameters stationed as proposed to carry out the necessary MD.

Hydrogen bond analysis, salt bridge analysis and calculation of the actin-myosin binding free energy was obtained. Obtaining all this fully denoted their success in carrying out molecular dynamics and simulations of Actin-myosin molecule. (Minghui and Wenjun 2013).

Likewise, Hiraku Oshima, Tomohiko Hayashi and Masahiro Kinoshita (2016) performed statistical thermodynamics for Actin-myosin Binding (the crucial importance of hydration effect). Actomyosin consisting of actin and myosin is one of the most extensively studied molecular motors. It drives muscle contraction by the interaction between the actin and myosin which is coupled with the ATP hydrolysis cycle.

The method employed involved the binding of actin and myosin of Dictyostelium discoideum which forms actomyosin in the rigor state. The MD simulation for actomyosin was carried out using gromacs version 5.0.4 combined with Amber99SB force field. The various temperatures at which the material was to be modelled were determined and MD carried out afterwards. Their initial structures are prepared simply by separating the initial structure of the actomyosin. The strategy for each MD simulation is the same. Snapshots structures are stored every 100ps in the 10-ns production run yielding 100 structures for the calculation of the thermodynamic quantities.

The binding free energy and its physically insightful components were obtained where,

$$E_{total} = E_{intra.B} + (E_{intra\ I.J} + E_{hyd\ I.J}) + (E_{intra,ele} + E_{hyd,ele}) \quad (2.2)$$

Where E_{total} is the system energy, ($E_{intra\ I.J}$ + $E_{hyd\ I.J}$) and ($E_{intra,ele}$ + $E_{hyd,ele}$) are the electrostatic components respectively. This experiment was carried out in two phases or processes.

Table 2.1 Free-Energy Functions and Their Energetic and Entropic Components for Actomyosin in Process 1.

Empty Cell	**Actomyosin**	**Actin**	**Myosin**	**Difference**
F	−13,110.09 ± 14.24	−7036.99 ± 11.39	−6000.51 ± 9.64	−72.60 ± 1.46
Etotal	−54,736.25 ± 11.72	−31,624.86 ± 9.82	−23142.78 ± 7.84	31.40 ± 0.85
−TShyd	41,626.15 ± 7.31	24,587.87 ± 5.37	17,142.28 ± 4.34	−103.99 ± 1.21
Eintra	−17,355.75 ± 29.71	−45,81.27 ± 19.55	−12,535.86 ± 16.48	−238.62 ± 10.47
Eintra,B	40,283.00 ± 12.28	23,810.60 ± 10.33	16,472.40 ± 8.25	0.00 ± 0.00
Eintra,LJ	−8715.39 ± 7.27	−5146.22 ± 5.01	−3412.59 ± 4.71	−156.58 ± 0.71
Eintra,ele	−48,923.36 ± 25.98	−23,245.65 ± 16.34	−25,595.67 ± 14.81	−82.04 ± 10.44
Ehyd	−37,380.50 ± 25.20	−27,043.59 ± 17.12	−10,606.93 ± 14.21	270.02 ± 10.41
Ehyd,LJ	−603.77 ± 2.00	−440.75 ± 1.52	−298.33 ± 1.40	135.31 ± 0.90
Ehyd,ele	−36,776.73 ± 24.90	−26,602.84 ± 16.89	−10,308.59 ± 13.91	134.70 ± 10.01
Eintra,LJ+Ehyd,LJ	−9319.16 ± 7.10	−5586.97 ± 4.87	−3710.92 ± 4.67	−21.27 ± 0.89
Eintra,ele+Ehyd,ele	−85,700.09 ± 7.45	−49,848.49 ± 5.51	−35,904.26 ± 4.45	52.66 ± 1.26
−TShyd,EV	43,409.99 ± 6.87	25,802.96 ± 4.86	17,934.10 ± 3.77	−327.07 ± 1.48
−TShyd,WAS	−1783.84 ± 4.41	−1215.09 ± 3.46	−791.82 ± 3.07	223.08 ± 1.23

Table 2.2 Free-Energy Functions and Their Energetic and Entropic Components for Actomyosin, Actin, and Myosin in Process 2.

Empty Cell	**Actomyosin**	**Actin**	**Myosin**	**Difference**

Empty Cell	**Actomyosin**	**Actin**	**Myosin**	**Difference**
F	−13110.09 ± 14.24	−6970.31 ± 12.31	−6075.67 ± 8.88	−64.12 ± 20.81
Etotal	−54736.25 ± 11.72	−31680.23 ± 9.88	−23126.54 ± 8.19	70.52 ± 17.38
−TShyd	41626.15 ± 7.31	24709.92 ± 5.90	17050.87 ± 4.58	−134.64 ± 10.45
Eintra	−17355.75 ± 29.71	−4421.09 ± 17.51	−12621.84 ± 16.24	−312.82 ± 38.12
Eintra,B	40283.00 ± 12.28	23821.07 ± 10.22	16503.09 ± 8.20	−41.15 ± 17.96
Eintra,LJ	−8715.39 ± 7.27	−5069.90 ± 5.14	−3472.79 ± 4.10	−172.71 ± 9.81
Eintra,ele	−48923.36 ± 25.98	−23172.26 ± 15.86	−25652.14 ± 15.42	−98.96 ± 34.12
Ehyd	−37380.50 ± 25.20	−27259.14 ± 15.65	−10504.70 ± 14.39	383.34 ± 32.97
Ehyd,LJ	−603.77 ± 2.00	−494.13 ± 1.72	−289.97 ± 1.22	180.33 ± 2.90
Ehyd,ele	−36776.73 ± 24.90	−26765.01 ± 15.13	−10214.73 ± 13.92	203.01 ± 32.29
Eintra,LJ+Ehyd,LJ	−9319.16 ± 7.10	−5564.03 ± 5.08	−3762.75 ± 3.99	7.63 ± 9.60
Eintra,ele+Ehyd,ele	−85700.09 ± 7.45	−49937.27 ± 5.58	−35866.87 ± 4.07	104.05 ± 10.16
−TShyd,EV	43409.99 ± 6.87	25922.86 ± 5.75	17877.40 ± 3.52	−390.27 ± 9.63
−TShyd,WAS	−1783.84 ± 4.41	−1212.94 ± 3.22	−826.53 ± 3.04	255.63 ± 6.25

All values are expressed as the mean ± SE in units of kcal/mol.

They investigated the binding of an actin trimer and myosin S1. The complex is in the rigor state. Changes in thermodynamic quantities upon binding are calculated using a hybrid of statistical-mechanical theories. They calculate the hydration energy by the 3D-RISM theory and the hydration entropy by the ADIET combined with the MA. This hybrid theory possesses the following merits. The binding free energy can be decomposed into physically insightful components, and thereby, the factors driving or opposing the binding can be identified. Since only advantageous aspects of each of the 3D-RISM, ADIET, and MA are utilized, the computational load is not heavy at all, even for very

large biomolecules, and the results are quantitatively reliable. They began to use the hybrid just about two years ago, and it has not yet been employed by any other group. Also, it will become a popular method for analysing a variety of self-assembly processes (Hiraku, Tomohiko and Masahiro 2016).

2.4 LIMITATIONS OF MOLECULAR DYNAMICS

2.4.1 CLASSICAL APPROXIMATION

Molecular mechanics is based on classical methods to represent the system (using the force field) and propagate it forward in time (using Newton's equations of motion). We usually don't consider the dynamicity of electron densities and therefore can't fully account for processes such as hydrogen bonding interactions, bond making and breaking, and excited states (Anders, 2017).

2.4.2 FORCE FIELDS

When writing lab reports for lab classes in undergrad, we had to include a discussion of our results and explain why they might be as expected. The typical go-to answer was "human error." (I've observed this as a TA, too, when grading post-lab assignments of gen chem students.) Then you give a laundry list of reasons of things you possibly did wrong — "I put in water instead of

ethanol", "my yield was low because I spilled a lot", etc. The apparent go-to answer in a paper that uses MD simulations is "force field accuracy." That's not to say it's not a valid reason! The accuracy of the simulation depends on the accuracy of the force field comprising those simulations. And, force fields are inherently approximations of true reality.

There's not one standard way to develop force fields, so then you also have variations in results if comparing different force fields. Which one is correct? Hard to say. We don't even know how the uncertainty in the force field to adequately know the uncertainty in our results. Many in the field would say that we need better force fields to yield more reliable simulations (Anders, 2017).

2.4.3 FOOTNOTES

The force field represents a potential energy surface which is typically the ground state, and we're usually not interested in excited states in MD simulations anyway. This means you wouldn't use classical MD to study things like chemical reactions that involve bond breaking/making and multiple electronic states (Anders, 2017).

Other limitations include Sampling and Ergodic Hypothesis (Anders, 2017).

CHAPTER THREE

MATERIALS AND METHODS

3.1 MATERIALS

In molecular dynamics and simulation of the myosin molecule, some materials were needed to carry out this experiment smoothly. These materials ranges from;

3.1.1 THE PROTEIN DATA BANK (PDB)

The protein data bank is a colony of proteins in their 3D structures. It is a database for which all 3-dimensional structure of large biomolecules such as nucleic acids and proteins are listed in codes in order for easy download and import into the molecular dynamics software to carry out necessary experiment.

3.1.2 THE AVOGADRO SOFTWARE

The Avogadro software is a MD simulation software for carrying out molecular dynamics and simulations of biomolecules. Inside it are various tools that enables the success of the MD simulations such as the force fields, integration algorithm, the time steps, sol etc. This software is designed for molecule editing and visualizing. The version of the software is version 1.1.0

3.1.3 COMPUTER OPERATING SYSTEM

In molecular dynamics and simulations, computer systems are vital material being used. The higher the processor of the computer, the better and definite the simulation process. It is then advisable to use computers with high processor speed. In this study, the computer used was the HP Pavilion 15 which is a very suitable computer to carry out this kind of MD simulation.

3.1.4 THE MYOSIN MOLECULE

As biophysicists, we know that some molecules exist in different isoform or class such as myosin, therefore the most suitable class of myosin which could be simulated on the Avogadro software and also with the HP Pavilion 15 was used. This molecule is the class V Myosin with a PDB number of IM45.pdb. Its chemical formula is $C_{864}H_{2066}N_{228}O_{537}S_{2.}$ It is a complex molecule.

3.1.5 FORCE FIELDS

Force fields in molecular modelling is a computational method which is being used to determine or estimate the forces that acts between atoms that are within a given biomolecule and also between biomolecules. This force field is denoted or defined by the parameter below;

$$F = \Sigma k_b (b - b_0)^2 + \Sigma k_\theta (\theta - \theta_0)^2 + \Sigma V_n/2 [1+ \cos (n\phi - \delta)] + \Sigma k_w (\acute{\omega} - \acute{\omega}_0)^2 + + \Sigma k_u (r_{1,3} - r_{1,3,0})^2 \quad (3.1)$$

Where the first to last term denotes bonds, angles, dihedrals, impropers and Urey-Bradley oscillations respectively.

There are various force fields, depending the molecule being optimized.

However, the default force field used in Avogadro is the Universal Force Field (UFF) which is the force field used in this study. The other force fields may be better suited to optimize other biomolecules.

3.1.6 INTEGRATION ALGORITHM

It is the driver of the MD Simulation program solving the equations of motion of the interacting atoms and following their trajectories respectively.

3.2 METHODOLOGY

in carrying out MD of the myosin molecule, some steps were taken to ensure that the simulation was run successfully ranging from the importation of the molecule file from PDB to the recording of the energy values after energy minimization. These steps are;

3.2.1 IMPORTATION OF MYOSIN INTO AVOGADRO

Myosin was imported into the Avogadro software for MD simulation. This was achieved by first connecting the computer to the internet, afterwards log on to www.pdb.org . After accessing this site, search for myosin on the search column. All available classes of myosin present in PDB will pop out. Choose the class V

myosin which is best suitable for MD simulation of this manner. Molecules on PDB appears on the Avogadro space with a solvation box which represents the parameters at which the molecule exist in space. The molecule file was downloaded and saved to the computer after which the Avogadro software was launched and the import tool was used to import the molecule into the software space for further experiment as shown in the Fig. 3.1 below

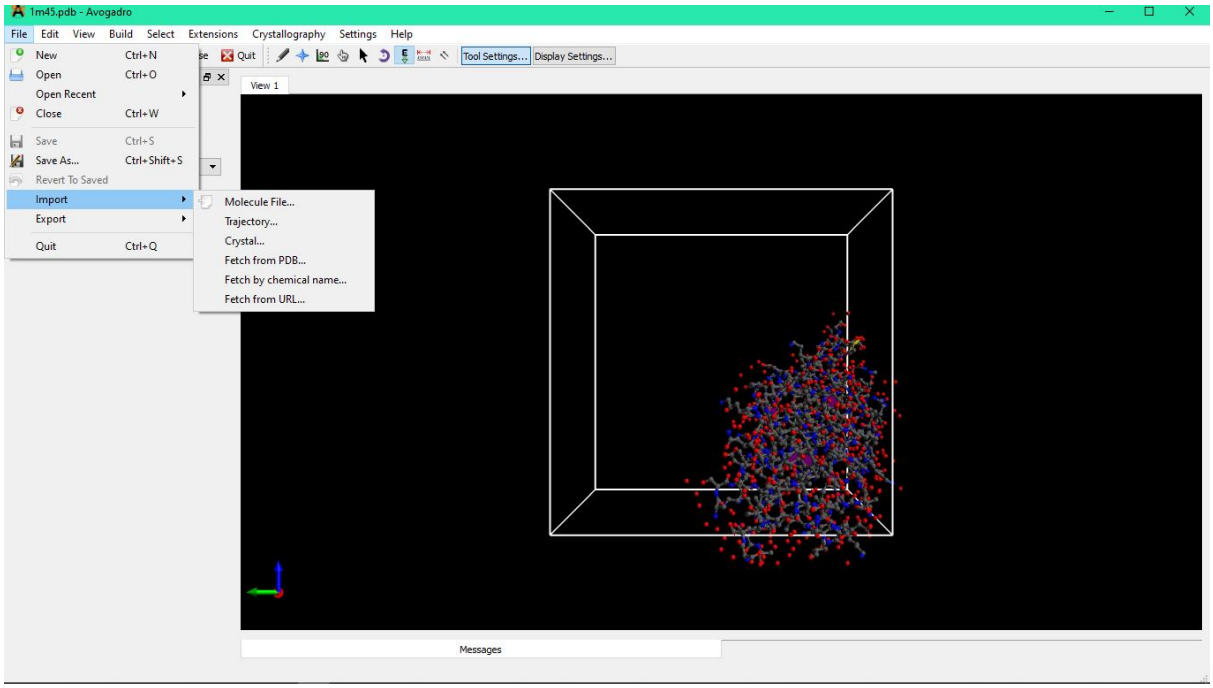


FIG 3.1 Pictorial view of Avogadro import tool

3.2.2 ENERGY MINIMISATION

At the right top of the Avogadro space, there is a symbol denoted by letter “E” which represents the energy minimisation. it was clicked on and then the various parameters were set.

3.2.3 SETTING THE FORCE FIELD

In this experiment, to suit the molecule after it has been imported into the Avogadro space, the force field was set to Default which is the Universal Force Field (UFF).

3.2.4 STEPS PER UPDATE

Steps per update also known as time steps is a function of time. It determines the rate at which the molecule will be simulated. Since the molecule is myosin class V molecule, the time steps were set to 1,2,3 and 4 respectively in order to get record a concise result. The larger and complex the molecule, the smaller the time step that is required for use. Myosin is a large and complex molecule; therefore, the least 4-time steps were used to carry out the energy minimisation respectively.

3.2.5 DETERMINING INTEGRATION ALGORITHM

The integration algorithm was set to steepest descent being that the molecule is complex and the computer used is not a super computer. The least integration algorithm which is steepest descent was used.

3.2.6 RUNNING THE SIMULATION

The start button below the integration algorithm was clicked on after all parameters were set to begin the simulation. The energy values keep changing

until dE equals to 0. The energy values are recorded afterwards. This was done for time step 1 to time step 4 respectively.

CHAPTER FOUR

RESULTS AND DISCUSSIONS

4.1 RESULTS

After carrying out simulations on the myosin molecule using Avogadro, results were obtained which includes visualisation of the myosin molecule, optimized geometry of the myosin molecule as well as the energy minimisation at time step 1,2,3 and 4.

4.1.1 VISUALISING THE MYOSIN MOLECULE

After importing the myosin class V molecule into Avogadro, the 3D image of the molecule could be seen which accounts for how the molecule looks like and its physical features as seen in Fig. 5 below.

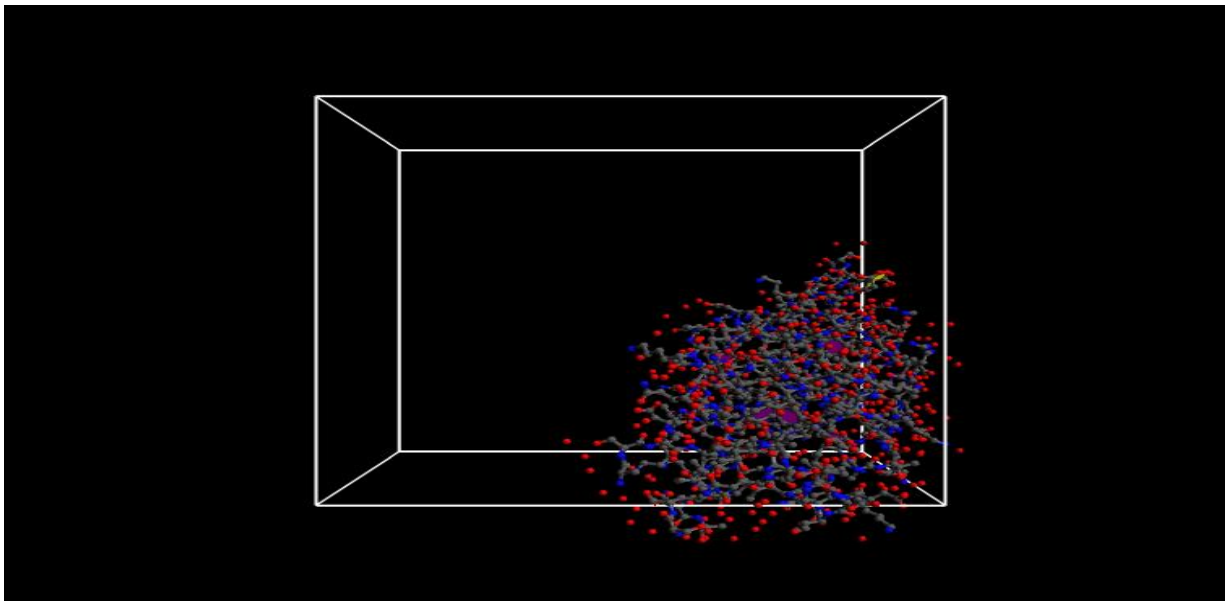


FIG 4.1 Visualised 3D structure of Myosin Class V molecule.

4.1.2 OPTIMISED GEOMETRY

The geometry of the myosin class V molecule changed after simulation. Due to the complexity of this molecule, it has numerous bonds, but a special bond was considered in this study which is the OCN bond. The OCN bond which is angle 6 had a value of 123.2376º before simulation as shown in Fig. 4.2 below.

FIG. 4.2 Geometry of OCN bond of myosin class V before simulation.

After simulating this molecule, the OCN bond angle value which is angle 6 changed from 123.2376º to 120.6198º as shown in Fig. 4.3 below.

FIG 4.3 Geometry of OCN bond of myosin class V molecule after simulation.

Taking good observation at fig. 6 and 7, it is observed that there were changes in other bonds apart from the OCN bond which is used as case study. This changes in bond angle value results in an optimized geometry of the molecule.

4.1.3 ENERGY MINIMISATION

After carrying out the molecular dynamics and simulation process, the energy values at time steps 1,2,3 and 4 were obtained in KJ/mol and time taken for simulation to complete was recorded as well, as shown in Fig. 4.4,4.5,4.6 and 4.7 respectively.

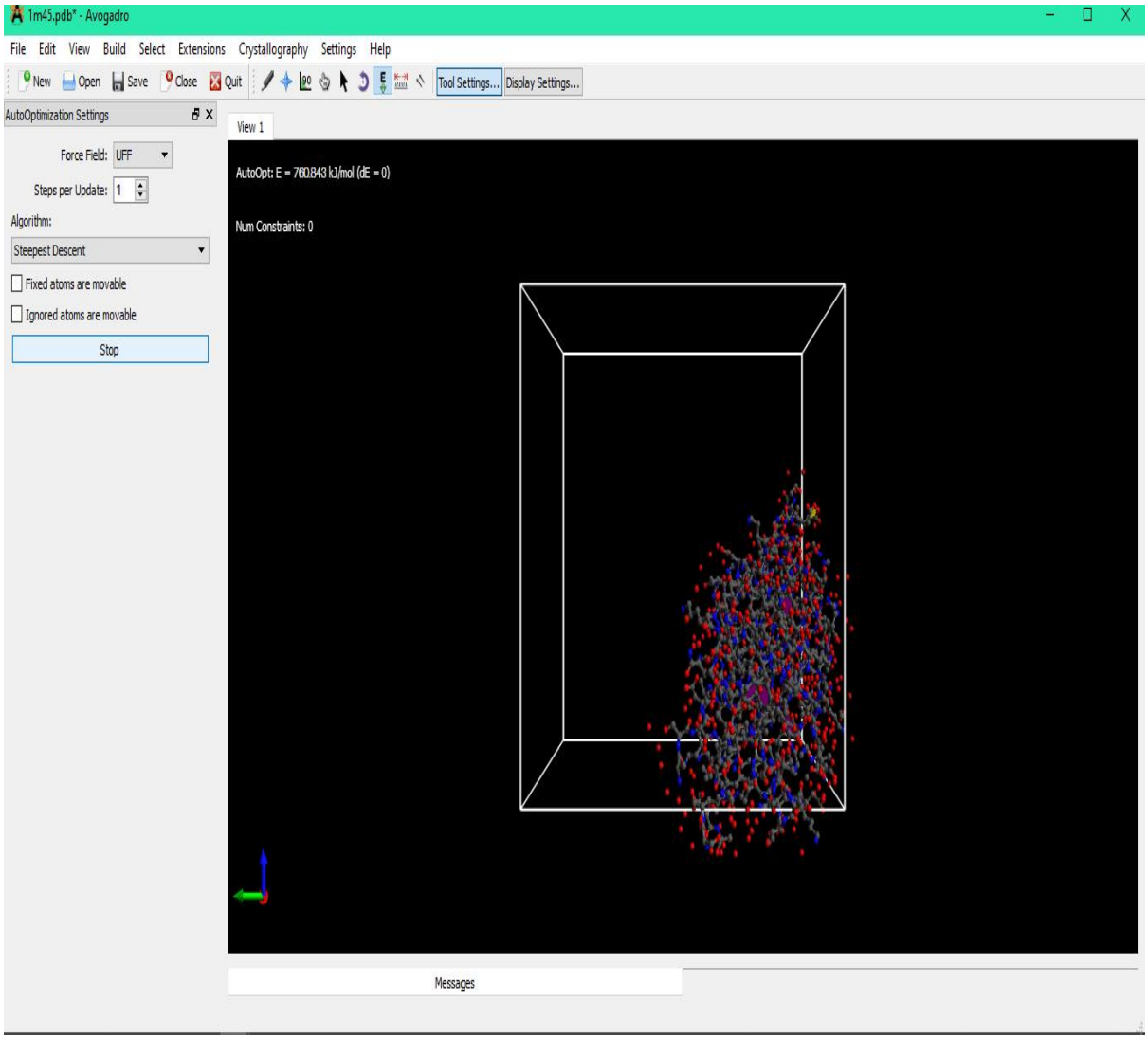


FIG 4.4 Energy minimisation of myosin class V molecule at time step 1

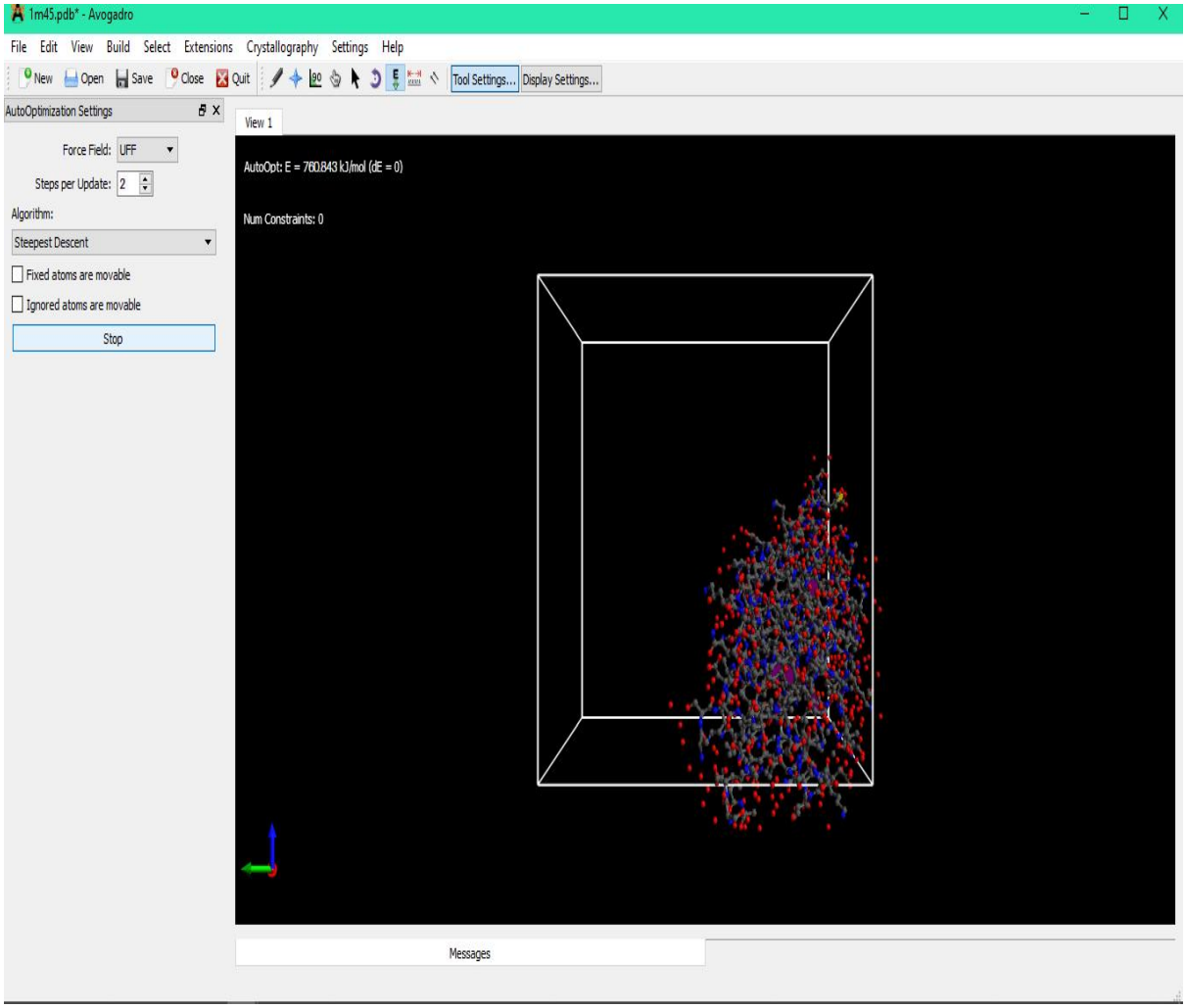


FIG 4.5 Energy minimisation of myosin class V molecule at time step 2

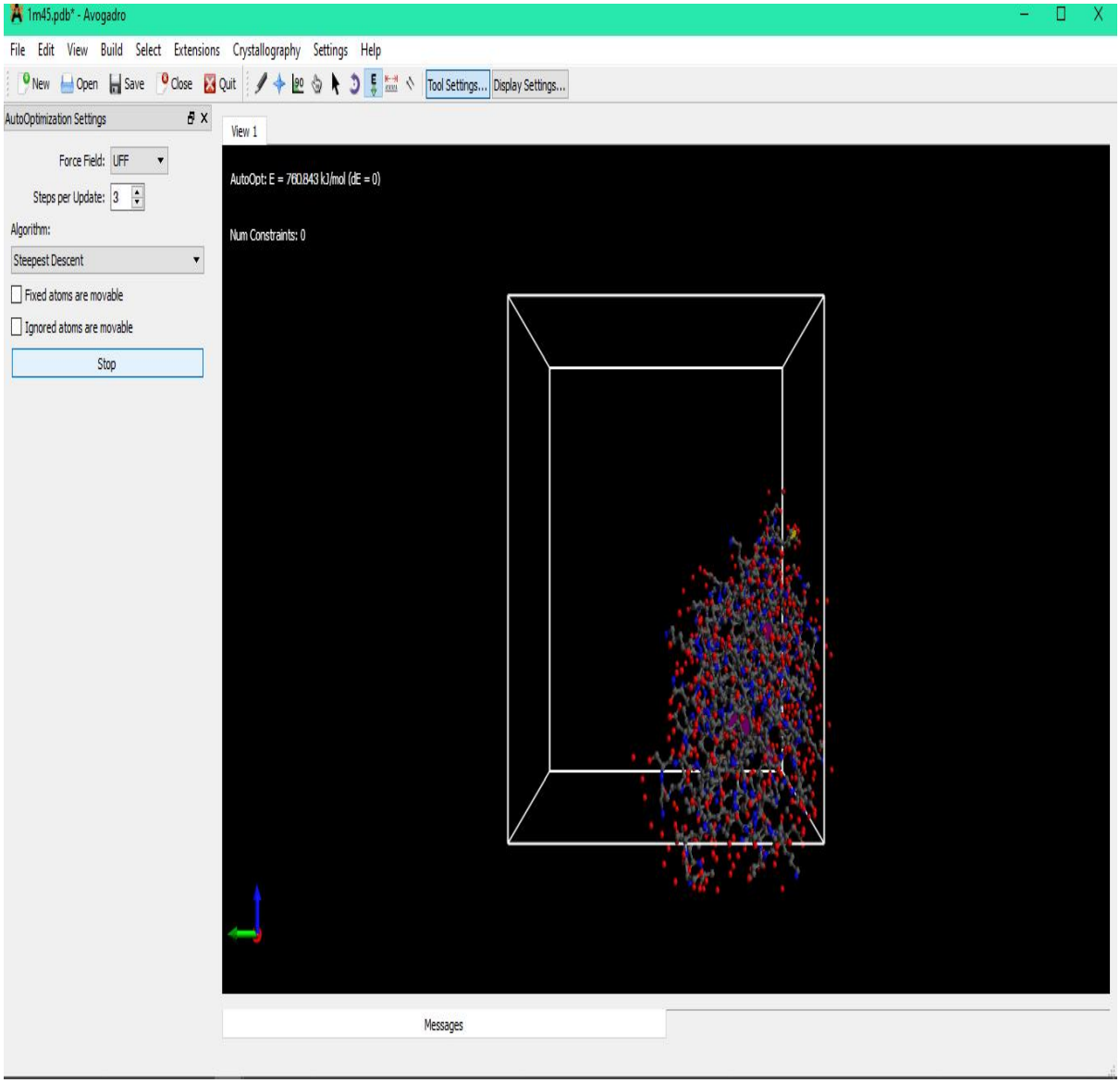


FIG 4.6 Energy minimisation of myosin class V molecule at time step 3

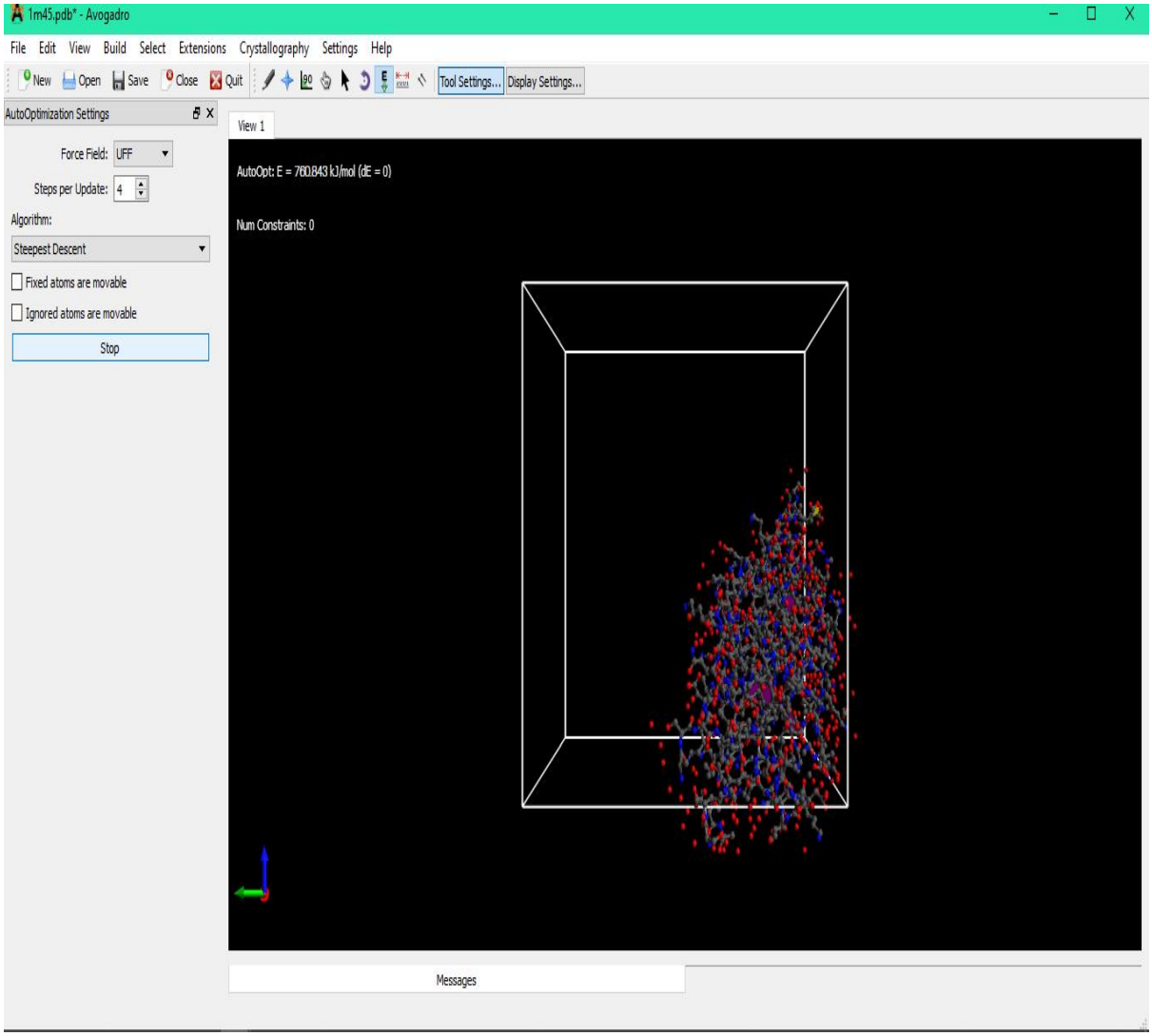


FIG 4.7 Energy minimisation of myosin class V molecule at time step 4

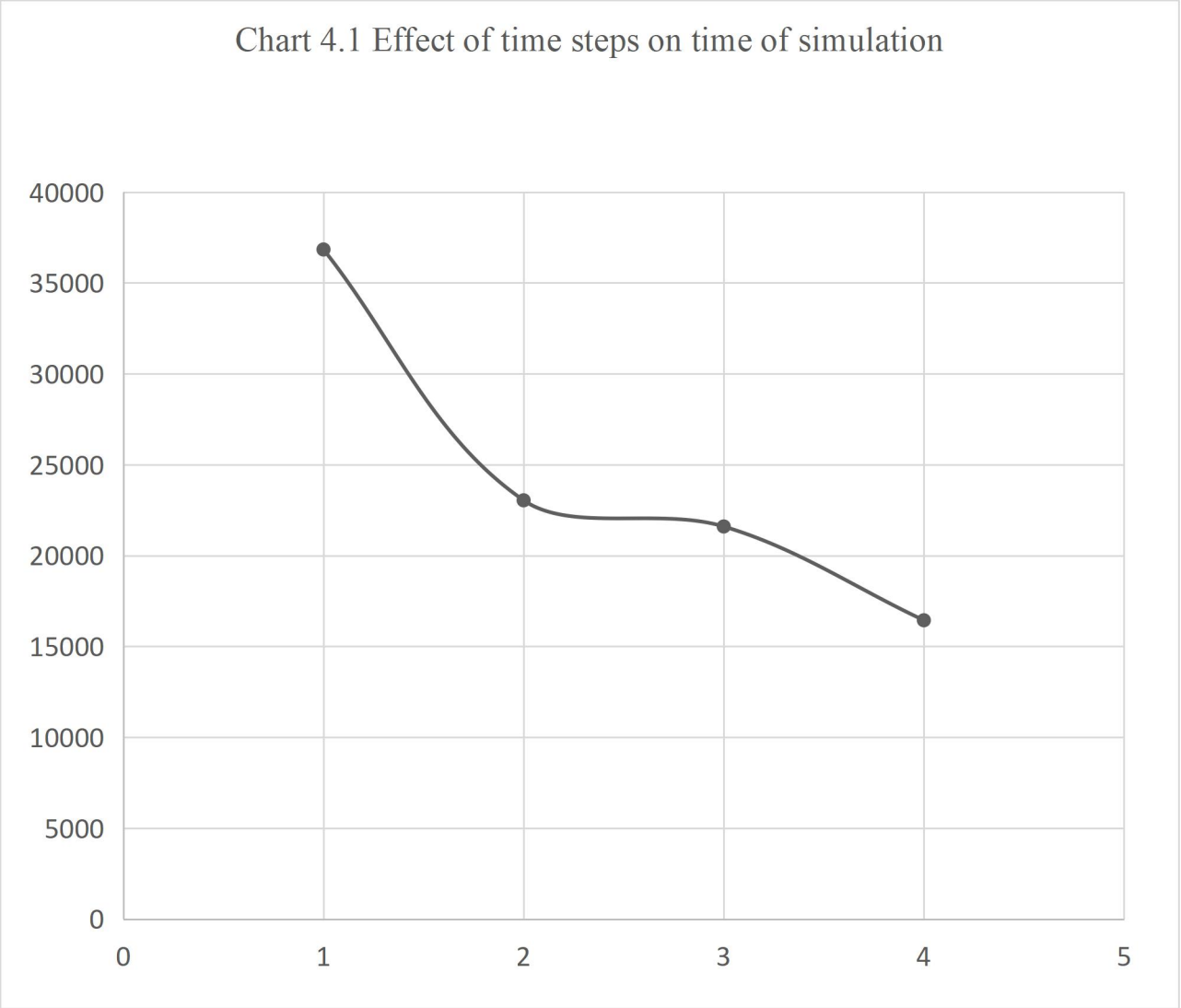
After simulation, the energy minimisation value was gotten to be 760.843KJ/mol.

The computation of the time steps and time taken for the energy minimisation was done as shown in the Table 4.1

S/N	TIME STEPS	ENERGY (KJ/MOL)	TIME (S)
1.	1	760.843	36840
2.	2	760.843	23040
3.	3	760.843	21600
4.	4	760.843	16440

Table 4.1 Recorded values of time taken against time steps.

A graph of the effect of steps per update on the x-axis against simulation time on the y-axis was plotted as shown in chart 4.1



The result from this graph is interpreted therefore as the higher the time step used in simulation, the faster the time taken for simulation to occur and vice versa.

CHAPTER 5

FINDINGS, CONCLUSION AND SUGGESTIONS FOR FURTHER STUDIES

5.1 FINDINGS

The following are the findings of this work;

1. Myosin class V molecule was imported with PDB number IM45 into the Avogadro software. Larger or heavy mutations of myosin takes a while to appear on the Avogadro space.
2. The molecule was visualised after importing into Avogadro as shown in Fig 4.1.
3. Simulations were run at time step 1,2,3 and 4 respectively to obtain optimised geometry and energy minimisation.
4. Bond length changed after simulation. Taking the OCN bond as case study, it changed from 123.2376º to 120.6198º as shown in Fig 4.2 and 4.3 thereby resulting in geometry being optimised.
5. Energy levels at time steps 1,2,3 and 4 were obtained to be at 760.843KJ/mol.

5.2 CONCLUSION

Molecular dynamics and simulation are a vital tool to understand how to model molecules, it gives precise description of molecules than lab experiment. Simulations have always proven to be more accurate that laboratory experiment since it is done with the use and help of a computer which express little of no error. With MD, scientist can understand how drugs, bio-supplements can be made to suite biophysical systems. Molecular dynamics and simulations play a vital role in the making a modelling of drugs and bio-supplements in order not to pose a negative threat on the host that receives it.

MD on very complex molecules is very cost effective as it requires a workstation of supercomputers, steady power, and high internet access to run simulation. Sometimes these simulations may take up to 9 months to complete. In this study, the estimated total time taken for all simulations to complete was about 27.2 hours. It took this relatively short time because myosin class V is not a very complex class of myosin compared to human myosin and the likes of it. The steps per update used during simulation can speedy the rate of the simulation. It is advisable to use a higher time step during simulation when working with a high performing computer in order to save some time taken for simulation to complete. But in the case where there an average performing computer, it is advisable to use a lower time step for simulation in order for the

simulation process not to be terminated due the computer crashing or what have you.

It can be observed that myosin possesses a relatively high energy from the energy value obtained. This accounts for part if the reason it is responsible for kinetic activities in the body of bio-organisms.

5.3 SUGGESTIONS FOR FURTHER STUDIES

The following are the suggestions for further studies;

1. In this study, the MD simulation was carried out only on myosin without combining with another molecule. Studies have shown that myosin combines with actin to form actomyosin in muscles. Researchers who wish to carry out MD simulations on myosin can as well go further to simulate actomyosin and obtain the binding energy which accounts for why they bind together in muscles. This study can be achieved using a relatively high performing computer as in super computers and a more advanced simulation software such as Chimera and GROMOS, GROMACS, NAMD etc.
2. Other complex isoforms of myosin molecule can also be modelled and simulated. It is appreciable to perform molecular dynamics and simulations on these isoforms using other simulation software.

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APPENDIX (ACRONYM AND ABBREVIATIONS)

ADIET……. Angle Dependent Integral Equation Theory

AMBER……...…… Assisted Model Building with Energy Refinement

ATP…….....….. Adenosine Triphosphate

CHARMM……...Chemistry at HARvard Macromolecular Mechanics

Cryo-EM…….Cryo- Electron Microscopy

EPR……....….Electron Paramagnetic Resonance

FRET……...…...…Forster Resonance Energy Transfer

GROMACS……..….... GROnigen Machine for Chemical Simulations

GROMOS……..……GROnigen Molecular Simulation

GPU……..…… Graphics Processing Unit

LAMMPS……...…Large-scale Atomic/Molecular Massively Parallel Simulator

MA……..……Morphometric Approach

MD……...……..…….. Molecular Dynamics

MM…….……. Molecular Mechanics

NAMD…….……... Nanoscale Molecular Dynamics

NMR……...……..... Nuclear Magnetic Resonance

OCN……...……Oxygen-Carbon-Nitrogen

PDB……...……Protein Data Bank

TA……...…….…… Telomere Associated

QM……...……. Quantum Mechanics

UFF……...……..…… Universal Force Field

VMD……...…….. Visual Molecular Dynamics

3D……..……. 3-Dimensional

3D-RISM……...…. 3-Dimensional Reference Interaction Site Model