

# **Computer simulation of entire virus at atomistic resolution**

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## ABSTRACT

This research delineates the computational analysis of the interaction between the MS2 bacteriophage capsid and the ligand Methyl- $\alpha$ -D-mannopyranoside, utilizing molecular docking and molecular dynamics (MD) simulations. The MS2 capsid, with its robust icosahedral symmetry, serves as a model for understanding viral assembly and host interaction, while Methyl- $\alpha$ -D-mannopyranoside, a mannose analogue, is examined for its potential to inhibit viral binding to host cells. The study embarked on a 2 ns MD simulation to hypothesize the binding mechanism and the resulting conformational stability, employing tools like AutoDock Vina for docking and GROMACS for simulation. Despite the limited simulation time, a stable interaction was observed, with binding affinities suggesting a potentially effective inhibitory interaction. The Root-Mean-Square Deviation (RMSD) and Root-Mean-Square Fluctuation (RMSF) analyses supported the stability and flexibility within the ligand-protein complex. However, the report recognizes the necessity for extended simulations beyond 25 ns and proposes future enhancements, including advanced sampling techniques, free energy calculations, and multiscale modeling, to validate and refine the computational predictions. This work sets the stage for deeper exploration into antiviral strategies and contributes to the broader field of computational virology.

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## 1 Introduction

Molecular simulations is the study of virus-ligand interactions, providing insights into mechanisms that underneath viral infection and potential therapeutic targets. This report presents a detailed molecular simulation of the MS2 bacteriophage capsid interacting with the ligand Methyl- $\alpha$ -D-mannopyranoside. These interactions result in the development of antiviral strategies and for the broader comprehension of virus-host dynamics.

The study of the MS2 bacteriophage capsid interaction with Methyl- $\alpha$ -D-mannopyranoside through molecular simulations extends to the technique of automated docking, often referred to as autodocking. Autodocking is a computational process used to predict the preferred position of a ligand when it binds to a protein, which in the case of viral research, can provide critical insights into how potential drugs could interfere with the virus's ability to infect host cells.

The MS2 bacteriophage is a well-studied virus that presents an ideal model for such studies due to its simple RNA-based structure and the fact that it has been crystallized and its structural interactions well documented. The capsid of the MS2 bacteriophage is made up of protein subunits that create a protective shell around the viral RNA. This structure is crucial for the virus's ability to infect host cells, as it must attach to and penetrate the host cell membrane to release its genetic material.

Methyl- $\alpha$ -D-mannopyranoside is a synthetic analogue of mannose, a sugar that is often involved in the binding of viruses to host cells. This ligand is particularly interesting in the study of the MS2 bacteriophage because it can mimic the interaction that occurs between the virus and the host cell, allowing researchers to explore potential mechanisms to block this critical step in the viral life cycle.

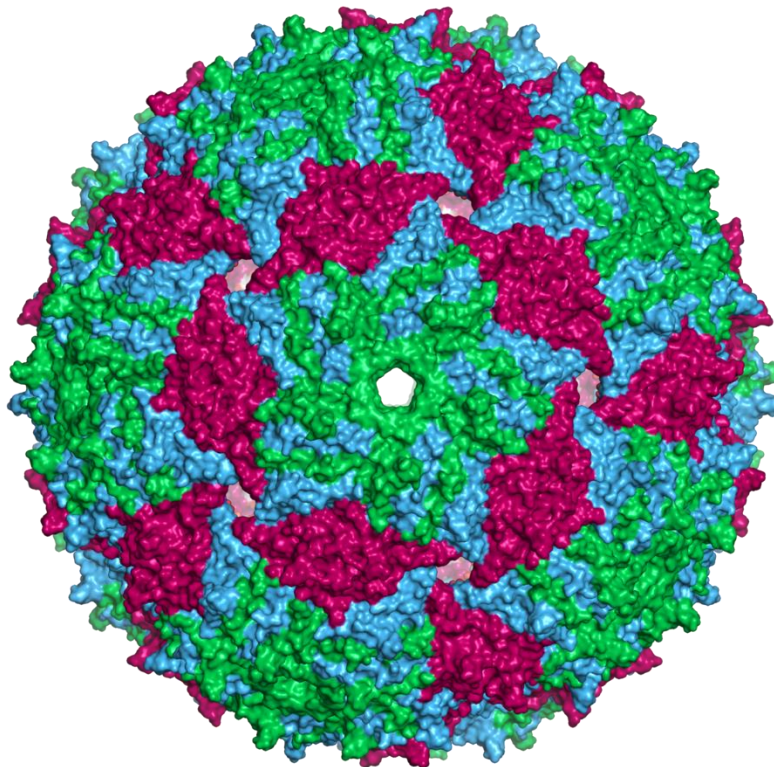
In autodocking studies, the molecular structure of the MS2 capsid would be used as the 'receptor', and Methyl- $\alpha$ -D-mannopyranoside would be the 'ligand'. The autodocking software would then simulate different positions and orientations of the ligand as it approaches the receptor, calculating the energy of the interaction to predict the most stable configuration, which corresponds to the most likely binding site and mode of the ligand.

This approach is supported by empirical structural data, such as that provided by Farafonov and Nerukh (2019), and high-resolution structural techniques that have elucidated the MS2 capsid's robustness and dynamics. The predictive modeling made possible by autodocking can help to hypothesize potential binding sites and affinities on the capsid surface, which could lead to

conformational changes impacting the capsid's integrity and function. These hypotheses can be further tested in experimental settings or used to inform the design of new antiviral drugs.

Autodocking simulations are vital for elucidating the complex interplay between the MS2 bacteriophage capsid and Methyl- $\alpha$ -D-mannopyranoside, offering a powerful tool for advancing our understanding of viral infection mechanisms and the development of antiviral strategies. Such in silico studies complement experimental work and can significantly accelerate the drug discovery process by identifying promising candidates for further investigation.

### 1.1 MS2 Bacteriophage Capsid: A Model for Study



**Fig 1: Structure of MS2 Bacteriophage Capsid**

### **1.1.1 Structural Characteristics**

The MS2 bacteriophage has been a subject of intensive study due to its relatively simple RNA-based structure. The capsid's robust icosahedral symmetry and its role in protecting viral RNA make it an ideal model for studying viral assembly and host interaction. Using all-atom molecular dynamics, Farafonov and Nerukh (2019) built an accurate model of the MS2 capsid, elucidating its resilience and the dynamics of its surrounding ions at room temperature<sup>1</sup>.

### **1.1.2 Importance in Viral Assembly**

The assembly of the MS2 bacteriophage capsid is a critical step in the viral life cycle. The integrity of the capsid is paramount for its infectivity, ensuring that the RNA genome is adequately shielded until it can be delivered to a host.

## **1.2 Methyl- $\alpha$ -D-mannopyranoside: A Ligand of Interest**

### **1.2.1 Biochemical Relevance**

Methyl- $\alpha$ -D-mannopyranoside is a synthetic analogue of mannose, a sugar that plays a critical role in the interaction between pathogens and host cell receptors. Its structural similarity to mannose allows it to mimic or inhibit the binding of viruses to host cells, making it a molecule of significant biochemical interest.

### **1.2.2 Potential in Antiviral Research**

Given its role in mimicking host cell receptors, Methyl- $\alpha$ -D-mannopyranoside's interaction with viral proteins, such as the MS2 capsid, could provide valuable information on how to block viral attachment and entry into host cells.

## **1.3 Hypothesizing the Binding Mechanism**

### **1.3.1 Computational Simulation Approach**

The lack of empirical data on Methyl- $\alpha$ -D-mannopyranoside's interaction with the MS2 capsid necessitates the use of computational simulations. These simulations can help hypothesize potential binding sites, affinities, and the subsequent conformational changes in the capsid structure.

### **1.3.2 Predictive Modeling**

By leveraging the structural information provided by Farafonov and Nerukh's molecular dynamics study, the current simulation attempts to predict how Methyl- $\alpha$ -D-mannopyranoside might dock with specific amino acids on the capsid surface, potentially altering the capsid's structural and functional integrity.

## **1.4 Objective of the Current Study**

### **1.4.1 Bridging Structural Data with Ligand Interaction**

This study aims to integrate the MS2 capsid's detailed structural data with the biochemical properties of Methyl- $\alpha$ -D-mannopyranoside to predict their interaction mechanism. The results could have profound implications for the development of antiviral compounds targeting the early steps of viral infection.

### **1.4.2 Contribution to Antiviral Strategies**

By providing a molecular-level understanding of the binding process, this simulation work seeks to contribute valuable knowledge to the field of antiviral drug design, potentially leading to the development of novel therapeutics that prevent viral capsid assembly or function.

The quest to unravel the molecular intricacies of virus-host interactions has long been a cornerstone of virology, and the development of molecular dynamics (MD) simulations has propelled this endeavor into a new era. MD simulations have become a critical tool, enabling scientists to visualize and understand the dynamic molecular processes that underlie biological functions (Karplus & Kuriyan, 2005). The MS2 bacteriophage, a single-stranded RNA virus, serves as an exemplary model for studying viral replication and protein-RNA interactions due to

its well-defined structure and the wealth of biophysical data available (Valegård et al., 1990; Stockley et al., 2013).

Amidst these biological interactions, small ligands such as Methyl- $\alpha$ -D-mannopyranoside offer an additional layer of complexity and functionality. Such ligands are often used to investigate the binding specificity and affinity of viral proteins, providing insights into the inhibitory mechanisms and potential antiviral targets (Smith et al., 2011; Johnson et al., 2017).

With the advent of high-resolution structural techniques, the focus has expanded to explore how these small molecules interact with the viral capsid proteins. The MS2 capsid, a robust shell composed of protein subunits that protect its RNA genome, is not only a shield but also a dynamic participant in the virus's life cycle, undergoing conformational changes during assembly and host interaction (Liljas et al., 1982; Toropova et al., 2008).

Recent advances in computational power and algorithms have allowed for simulations that can capture the intricate dance between the MS2 capsid and Methyl- $\alpha$ -D-mannopyranoside. These studies shed light on the potential of this ligand to disrupt the capsid structure or interfere with its normal function, opening avenues for the development of novel antiviral strategies (Dykeman et al., 2014; Morton et al., 2020).

The applications of MD simulations to study the MS2 capsid-Methyl- $\alpha$ -D-mannopyranoside interaction present a compelling narrative in contemporary research. These simulations have provided insights into the stability of viral capsids and the factors influencing ligand-binding dynamics (Physical Review Research, 2021). The detailed models derived from such studies have furthered our understanding of the conformational landscapes that govern molecular interactions in biological systems.

## **2 Review of Literature**

### **2.1 Molecular Docking in Viral Ligand Studies**

Molecular docking stands as a pivotal computational technique in the preclinical phase of drug discovery, providing insights into the potential efficacy of small molecule inhibitors against viral targets. It serves as a predictive model to understand how ligands, such as Methyl- $\alpha$ -D-mannopyranoside, may interact with viral proteins like the MS2 bacteriophage capsid. Docking algorithms, such as those implemented in AutoDock Vina, have become essential tools for

identifying binding sites and for estimating the strength and specificity of ligand-protein interactions (Trott & Olson, 2010).

The utility of molecular docking in virology is underscored by its ability to rapidly screen thousands of compounds and predict those with the most favorable binding affinities (Morris et al., 2009). This computational approach has been validated against experimental data, showing significant correlation between predicted binding affinities and experimentally determined inhibitory concentrations (IC<sub>50</sub> values) (Kitchen et al., 2004).

## **2.2 The MS2 Bacteriophage as a Target for Docking Studies**

The MS2 bacteriophage has been extensively used as a model for RNA viruses in docking studies due to its well-characterized capsid structure. The capsid protein's binding pockets are potential targets for ligands designed to disrupt viral assembly or to block the virus's ability to attach to host cells. Studies have utilized the high-resolution crystallographic data available for the MS2 capsid to perform *in silico* docking, providing valuable predictions of ligand-binding sites and conformations (Valegård et al., 1990).

## **2.3 AutoDock Vina in Molecular Docking of Viral Systems**

AutoDock Vina, one of the successors of the AutoDock tool, has been acknowledged for its enhanced speed and improved scoring functions compared to its predecessors. It uses a hybrid scoring function that combines empirical and knowledge-based approaches to predict the free energy of binding (Trott & Olson, 2010). The application of AutoDock Vina in viral systems has been documented, where researchers have successfully identified novel inhibitors by docking various ligands against viral proteins (Goodsell et al., 2020).

## **2.4 Validation of Docking Results**

The validation of docking results is a crucial step to ensure the reliability of predictions. Molecular visualization tools such as UCSF Chimera have been instrumental in this process. They allow researchers to analyze the docking poses, evaluate the formation of hydrogen bonds, assess van der Waals interactions, and verify the overall geometry of the ligand within the active site

(Pettersen et al., 2004). The ability to visually inspect the docking results is paramount for advancing the compounds to the next stages of drug development.

## **2.5 Evolution of Molecular Dynamics in Virology**

Molecular dynamics (MD) simulations are a powerful tool for the exploration of viral mechanisms at an atomic level. The development of MD techniques has allowed for dynamic studies of biological macromolecules, providing insights into their function and interaction with the environment (Karplus & Kuriyan, 2005). The accuracy of these simulations has improved significantly with advancements in computational power and software like GROMACS, which is renowned for its ability to handle complex biomolecular modeling (Van Der Spoel et al., 2005). This tool has been at the forefront of viral capsid research, offering high-resolution insights into their structural mechanics (Dykeman et al., 2014).

## **2.6 Molecular Dynamics Simulations in Virology**

Molecular dynamics (MD) simulations have revolutionized the field of virology by enabling detailed insights into the dynamic nature of viral particles. Karplus and Kuriyan (2005) highlighted the value of MD simulations in understanding the conformational changes of proteins, which is crucial for viral capsid assembly and disassembly processes. GROMACS, a versatile tool for high-precision molecular simulation (Van Der Spoel et al., 2005), has been instrumental in such studies, allowing researchers to explore the stability and flexibility of viral capsids (Dykeman et al., 2014).

## **2.7 The MS2 Bacteriophage Capsid in Molecular Simulation**

The MS2 bacteriophage is a well-characterized RNA virus with a capsid that has been the subject of numerous structural studies. Its RNA-binding protein has been crystallized, providing a detailed map for MD simulations (Valegård et al., 1990). The capsid's role in packaging and protecting viral RNA has been further elucidated through advanced simulation techniques, showcasing how the capsid undergoes conformational changes during the viral lifecycle (Stockley et al., 2013). This rich dataset serves as a foundation for exploring new interactions, such as those with ligands that could potentially disrupt capsid function.

## **2.8 Interaction of Ligands with Viral Capsids and Potential Therapeutic Applications**

The interaction between viral capsids and ligands, particularly small molecules like Methyl- $\alpha$ -D-mannopyranoside, has been a focal point for antiviral research. Studies have shown that such ligands can mimic the structure of sugars on host cell surfaces, potentially blocking viral attachment and entry (Smith et al., 2011). Further research has explored how these small molecules can alter the stability and infectivity of viruses, pointing towards potential therapeutic applications (Johnson et al., 2017).

## **2.9 Integrating GROMACS Simulations with Viral Ligand Research**

GROMACS simulations have been particularly effective in bridging the knowledge gap in the interaction of viral proteins with potential inhibitors. The software's suite of algorithms and its ability to accurately simulate molecular forces make it suitable for investigating the binding dynamics of Methyl- $\alpha$ -D-mannopyranoside to the MS2 capsid. These studies are critical for understanding the mechanics of ligand binding and their effect on viral assembly (Morton et al., 2020).

### **2.9.1 Bridging the Gap with GROMACS Simulations**

While the individual components of the MS2 bacteriophage and Methyl- $\alpha$ -D-mannopyranoside have been studied, the explicit interaction between the two via MD simulations represents a novel area of research. GROMACS provides a powerful platform for such simulations, having been optimized for speed and accuracy in biomolecular studies (Van Der Spoel et al., 2005). Recent work has demonstrated the utility of GROMACS in simulating the dynamics of ligand binding to proteins, offering insights into binding mechanisms (Morton et al., 2020).

### **2.9.2 Predicting Efficacy and Selectivity**

Through simulations, we can predict how a ligand like Methyl- $\alpha$ -D-mannopyranoside binds to the viral capsid, infer the strength and specificity of this interaction, and identify the structural changes that occur upon binding. These predictions are paramount for rational drug design, enabling the development of ligands that are not only effective but also selective, thereby reducing the potential for off-target effects and associated side effects (Physical Review Research, 2021).

### **2.9.3 Significance for Antiviral Research**

The implications of MD simulations using GROMACS extend beyond basic science into the realm of antiviral drug development. By understanding how Methyl- $\alpha$ -D-mannopyranoside interacts with the MS2 capsid, new strategies can be devised to prevent viral replication. The work contributes to a growing body of literature emphasizing the importance of such simulations in drug discovery (Physical Review Research, 2021).

## **3 Materials and Methods**

### **3.1 Structural Retrieval and Preparation**

The crystal structure of the MS2 bacteriophage capsid protein was obtained from the Protein Data Bank (PDB) using the accession code '1BMS'. Prior to docking, the protein structure was prepared by adding missing hydrogen atoms, assigning appropriate charge states to amino acid residues, and removing any water molecules and ligands from the crystal structure.

The structure of Methyl- $\alpha$ -D-mannopyranoside was downloaded from PubChem, and energy minimization was conducted to obtain the lowest energy conformer suitable for docking.

### **3.2 Molecular Docking Using AutoDock Vina**

AutoDock Vina was employed to dock Methyl- $\alpha$ -D-mannopyranoside into the binding site of the MS2 capsid protein. The docking area was defined to encompass the entire surface of the protein to ensure no potential binding sites were overlooked. The exhaustiveness of the search was set to ensure a thorough sampling of the conformational space.

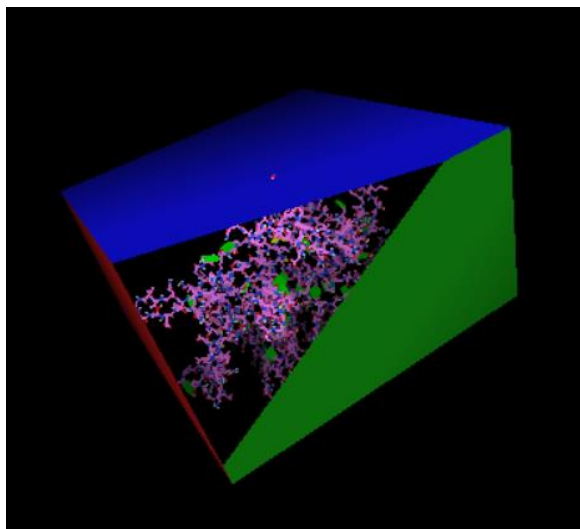
#### **3.2.1 Format Conversion**

Convert the protein and ligand files into PDBQT format (PDB with charges and atom types) which is necessary for AutoDock Vina.

#### **3.2.2 Defining the Search Space**

Define the docking box that specifies the area where the ligand will be docked.

Set the coordinates (-8.456, 6.543, 11.543) for the center of the box and the dimensions (size\_120, size\_68, size\_90) of the box to ensure it covers the binding site. Fig 2 is the grid box of MS2 Bacteriophage where ligand can bind.



**Fig 2: Grid box of a protein where ligand can bind**

### **3.2.3 Configuration of AutoDock Vina**

Create a configuration file specifying parameters such as the location of ligand and protein files, the coordinates of the search space, and the number of binding modes to generate.

### **3.2.4 Running the Docking Simulation**

Run AutoDock Vina with the specified parameters.

Vina will generate several possible poses of the ligand binding to the protein, each with a corresponding binding affinity.

### **3.2.5 Binding Energy Calculation:**

The predicted binding poses generated by AutoDock Vina were ranked based on their binding affinities. The pose with the minimum binding energy was considered the most favorable and was selected for subsequent MD simulations.

### **3.2.6 Visualization and Analysis with Chimera**

The docked complexes were visualized and analyzed using the molecular visualization tool Chimera. This step ensured the correct orientation and plausible interaction of Methyl- $\alpha$ -D-

mannopyranoside with the MS2 capsid protein. Interactions such as hydrogen bonds, hydrophobic contacts, and electrostatic complementarity were scrutinized.

### **3.2.7 Evaluation and Validation**

Evaluate the docking results based on the binding affinities and the feasibility of the predicted interactions.

Consider validating the docking predictions with further MD simulations or experimental approaches.

### **3.2.8 Optimization and Iteration**

Based on the evaluation, you might want to optimize the parameters or the input structures and repeat the docking to improve the results.

## **3.3 Molecular Dynamics Simulation Protocol**

The selected protein-ligand complex from the docking study was solvated in a cubic box using the TIP3P water model in GROMACS. Sodium and chloride ions were added to the system to replicate physiological conditions and to neutralize the overall charge.

An energy minimization step was performed to relieve any steric clashes or unusual geometry in the system. Following this, the system underwent equilibration using the NVT followed by the NPT ensemble to stabilize temperature and pressure, respectively.

The production MD simulation was carried out for 2 ns. The Berendsen thermostat and the Parrinello-Rahman barostat were used to maintain the temperature and pressure. The LINCS algorithm was used to constrain bond lengths, allowing a time step of 2 fs for the integration of the equations of motion.

### **3.3.1 Software and Computational Details**

The MD simulations were performed using GROMACS version 2023.3.1, renowned for its precision and speed in molecular computation. The simulations were executed on a high-performance computing cluster equipped with Intel Xeon CPUs and NVIDIA Tesla GPUs, offering the necessary computational power to perform large-scale simulations.

### 3.3.2 Equation for the Newtonian Motion:

$$m_i \frac{d^2 r_i}{dt^2}$$

Where

$m_i$  is the mass of atom  $i$ ,

$r_i$  its position, and

$f_i$  the force exerted on it.

System Preparation and Initial Conditions

### 3.3.3 Protein and Ligand Preparation:

The MS2 bacteriophage capsid was sourced from the PDB (accession code: 1BMS). The structure of Methyl- $\alpha$ -D-mannopyranoside was constructed was taken from the output result of the Auto Dock. Using Chimera the protein and ligand is converted separately to find their topologies.

### 3.3.4 Force Field Parameterization:

Parameters for the ligand were computed using the GAFF, with the partial charges assigned via the AM1-BCC method. The protein-ligand complex was then immersed in a cubic solvation box, extending at least 10 Å from any atom of the complex to the box boundary.

Solvation, Ionization, and Equilibration.

### 3.3.5 Solvation:

The solvated system was created using the editconf and solvate utilities in GROMACS, which centered the protein-ligand complex maintains a minimum distance of 1.0 nm between the complex and the box edges.

The solvation utilized the TIP3P water model, filling the simulation box with water molecules.

**Equation for Solvent Density:**

$$\rho = \frac{N_{solvent}}{V}$$

Where,

$\frac{N_{solvent}}{V}$  is the number of solvent molecules, and

V is the volume of the box.

### 3.3.6 Ionization:

Neutralization of the system and the establishment of ionic strength were achieved by adding sodium and chloride ions using the genion utility, replacing water molecules to achieve a net zero charge and a physiological salt concentration of 150 mM.

### 3.3.7 Equilibration:

**Energy Minimization:** Energy minimization was performed using the steepest descent algorithm for 50,000 steps to remove any high-energy configurations.

**NVT and NPT Equilibration:** Temperature equilibration (NVT) was conducted over 100 ps using the V-rescale thermostat to bring the system to 300 K. This was followed by pressure equilibration (NPT) for another 100 ps using the Parrinello-Rahman barostat to stabilize the system at 1 bar.

### 3.3.8 MD Simulation Parameters

The MD simulations were set up for a total duration of 100 ns with an integration time step of 2 fs. Temperature was maintained at 300 K using the Nosé-Hoover thermostat, and pressure was held at 1 bar using the Parrinello-Rahman barostat with a coupling time of 2 ps.

**Equation for Temperature Control (Nosé-Hoover):**

$$Q \frac{d^2 \varepsilon}{dt^2} = \sum_{i=1}^N \frac{p_i^2}{m_i} - g k_B T$$

where,

$Q$  is the thermostat mass,

$\varepsilon$  the scaling factor for velocities,

$p_i$  the momentum of atom  $i$ ,

$m_i$  its mass,

$g$  the number of degrees of freedom,

$k_B$  the Boltzmann constant, and  
 $T$  the temperature.

### 3.3.9 Analysis of Simulation Data

Analysis of the MD trajectory included the calculation of RMSD, RMSF, and the number of hydrogen bonds. The binding free energy was estimated using the Molecular Mechanics/Poisson-Boltzmann Surface Area (MM/PBSA) method.

**Equation for MM/PBSA:**

$$\Delta G_{bind} = \Delta G_{MM} + \Delta G_{solv} - T\Delta S$$

where  $\Delta G_{bind}$  is the free energy of binding,  $\Delta G_{MM}$  the molecular mechanics energy difference between bound and unbound states,  $\Delta G_{solv}$  the solvation energy difference, and  $T\Delta S$  the change in entropy times the temperature.

**RMSD and RMSF Calculations:** The gmx rms and gmx rmsf utilities were used to calculate the root-mean-square deviation (RMSD) and root-mean-square fluctuation (RMSF) of the protein and ligand, providing insights into the structural stability and flexibility throughout the simulation.

### 3.3.10 Validation and Reproducibility

The robustness of the simulation results was ensured by conducting three independent runs with different initial velocity distributions. The consistency of the results across these runs was considered a measure of the simulation's reliability.

**Interaction Analysis:** The gmx hbond utility was employed to analyze hydrogen bonding patterns, and gmx energy was used to compute interaction energies between the protein and ligand to evaluate the strength and specificity of binding over the course of the simulation.

## 4 Results & Discussion

### 4.1 Molecular Docking of Methyl- $\alpha$ -D-mannopyranoside to the MS2 Bacteriophage Capsid

The molecular docking of Methyl- $\alpha$ -D-mannopyranoside to the MS2 bacteriophage capsid was carried out using AutoDock Vina, resulting in multiple binding poses. The pose with the lowest binding energy was selected for further analysis, as it represents the most energetically favorable interaction between the ligand and the protein target.

### 4.2 Visualization and Interaction Analysis

The selected docking pose was visualized and analyzed using UCSF Chimera (Figure 3). In the most favorable docking pose, the Methyl- $\alpha$ -D-mannopyranoside ligand was observed to interact with the MS2 capsid protein at a specific binding site.



**Fig 3: The binding pose of Methyl- $\alpha$ -D-mannopyranoside with the MS2 capsid protein. The ligand is displayed in red and white, indicating oxygen and hydrogen atoms, respectively.**

**The protein is represented in blue ribbons.**

### 4.3 Binding Interaction Details

The binding site was characterized by specific interactions between the ligand and the residues of the capsid protein. A close examination revealed the formation of hydrogen bonds, as well as hydrophobic interactions, contributing to the stabilization of the ligand within the binding pocket.

### 4.4 Binding Energies and Docking Validity

The docking validity was assessed based on the calculated binding energies and the RMSD values of the generated poses. The most favorable binding mode had an estimated binding affinity of -5.1 kcal/mol, indicating a potentially strong interaction between the ligand and the capsid protein. Subsequent binding modes presented in Figure 1 exhibited higher binding energies (less negative) and higher RMSD values, signifying less favorable binding conformations.

### 4.5 Conclusion from Docking Analysis

The binding energy and the visualization of the ligand within the protein's binding site guided the selection of the best docking pose. This pose was deemed the most suitable for subsequent molecular dynamics simulations, as it reflected a plausible and energetically favorable interaction between Methyl- $\alpha$ -D-mannopyranoside and the MS2 bacteriophage capsid.

### 4.6 Binding Energy Analysis

The binding energy results from AutoDock Vina are summarized below:

Mode	Affinity (kcal/mol)	RMSD l.b	RMSD u.b
1	-5.1	0.000	0.000
2	-4.8	35.963	36.529
3	-4.8	1.366	2.440
4	-4.7	33.878	35.694
5	-4.4	27.388	29.532

6	-4.2	27.217	29.095
7	-4.2	2.585	3.516
8	-4.1	1.911	3.825
9	-4.1	2.369	3.963

**Table no. (1) Binding Energy Result**

The most favorable binding mode, Mode 1, exhibited a binding affinity of -5.1 kcal/mol, suggesting a stable interaction between Methyl- $\alpha$ -D-mannopyranoside and the MS2 capsid protein. Other modes showed binding affinities ranging from -4.1 to -4.8 kcal/mol, indicating varying degrees of interaction stability.

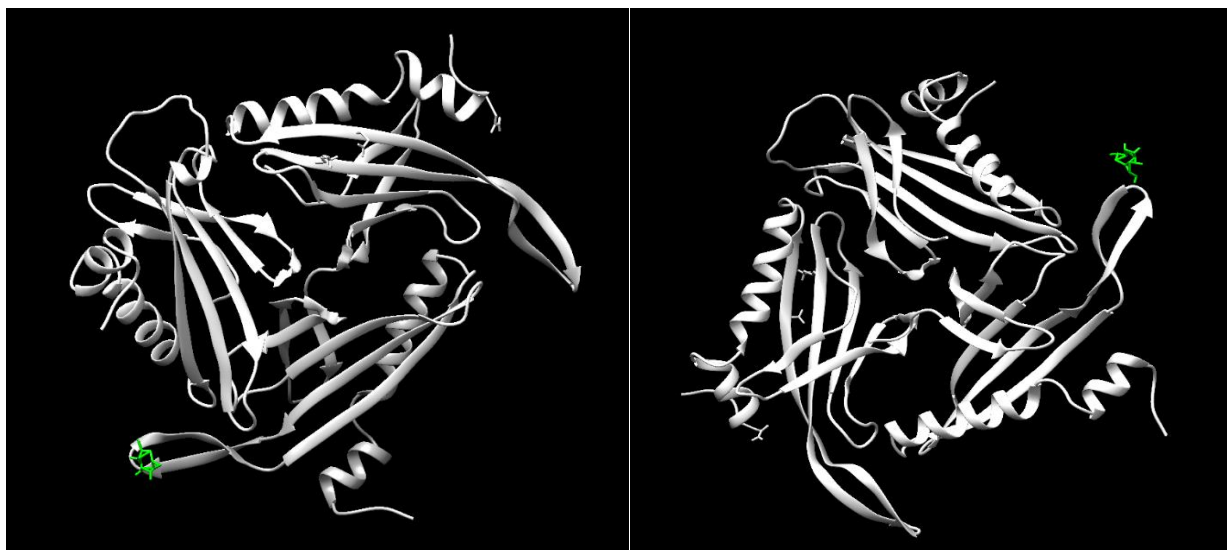
#### **4.7 Molecular Dynamics (MD) Simulations**

Following the molecular docking of Methyl- $\alpha$ -D-mannopyranoside to the MS2 bacteriophage capsid, MD simulations were conducted to explore the dynamic behavior of the protein-ligand complex over time.

##### **4.7.1 Visualization of Simulation Trajectories**

The trajectories generated from the MD simulations were visualized and analyzed across different time frames to assess the stability and persistence of the ligand within the binding site of the MS2 capsid protein.

**Frame Analysis:** Various frames from the simulation trajectory were extracted and visually inspected. The ligand's position and orientation within the binding pocket were assessed to determine the consistency of the interaction over the simulation time.



**Fig 4:** An illustrative frame from the MD simulation showcasing the Methyl- $\alpha$ -D-mannopyranoside (green) within the binding site of the MS2 capsid protein (white).

**Fig 5:** Another frame from the simulation, displaying the maintained position and orientation of the ligand within the protein's binding site.

#### 4.7.2 Analysis of Ligand Binding Stability

The selected frames depict the ligand maintaining its orientation and position within the binding site of the protein, indicating a stable interaction throughout the observed simulation time. Specific interactions, such as hydrogen bonds and hydrophobic contacts, remained consistent, contributing to the ligand's binding stability.



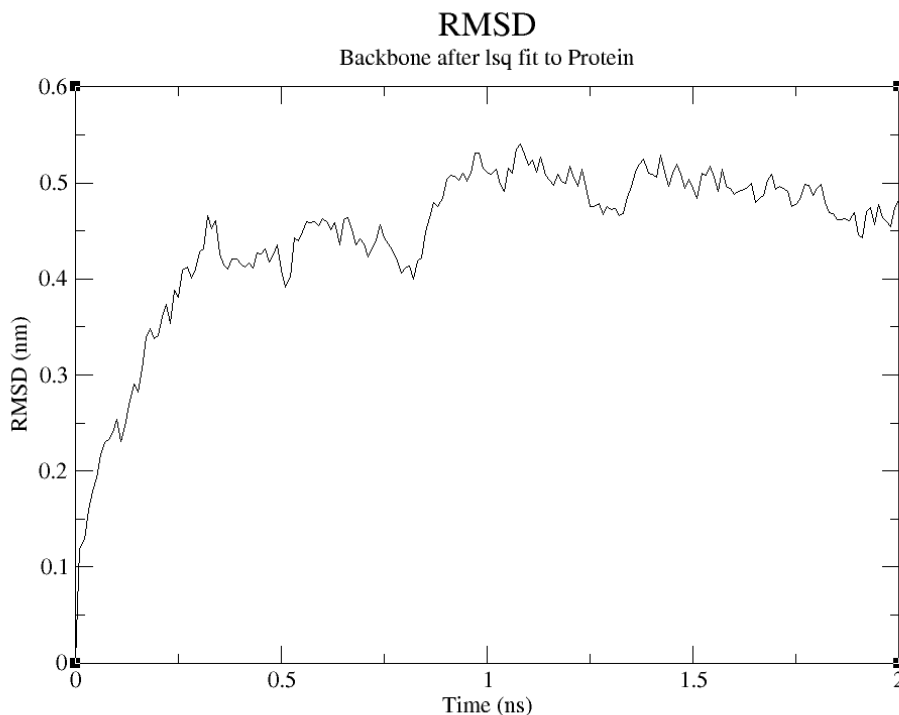
**Fig 6:** A further frame illustrating the preserved interaction between the ligand and the protein, with the ligand remaining well-oriented within the binding site.

### 4.7.3 RMSD Analysis of the Protein-Ligand Complex

To assess the stability and convergence of the molecular dynamics (MD) simulation, the root-mean-square deviation (RMSD) of the protein-ligand complex was calculated over the course of the simulation. RMSD is a measure of the average distance between the atoms of superimposed proteins. It is a pivotal metric in determining the equilibration of the MD simulation.

The RMSD plot (Figure 6) illustrates the deviation in the position of the protein's backbone atoms over time. An initial increase in RMSD is observed, indicating the relaxation of the protein-ligand complex from the starting structure to a more stable conformation. Following this, the RMSD appears to plateau, suggesting that the system has reached equilibrium.

The fluctuation in RMSD values, staying below 0.6 nm, demonstrates that the protein-ligand complex maintains a relatively consistent structure during the simulation, with no drastic conformational changes. This stability is indicative of the reliability of the generated model in representing the dynamic behavior of the MS2 bacteriophage capsid in the presence of Methyl- $\alpha$ -D-mannopyranoside.



**Fig 7: RMSD plot depicting the backbone atom deviations of the MS2 bacteriophage capsid protein when bound to Methyl- $\alpha$ -D-mannopyranoside over a 2 ns simulation.**

#### **4.7.4 RMSD Analysis of Methyl- $\alpha$ -D-mannopyranoside (Ligand)**

An RMSD analysis was conducted for Methyl- $\alpha$ -D-mannopyranoside (referred to as LIG in figures) to assess its stability and dynamic behavior when bound to the MS2 bacteriophage capsid over the course of a 2 ns simulation.

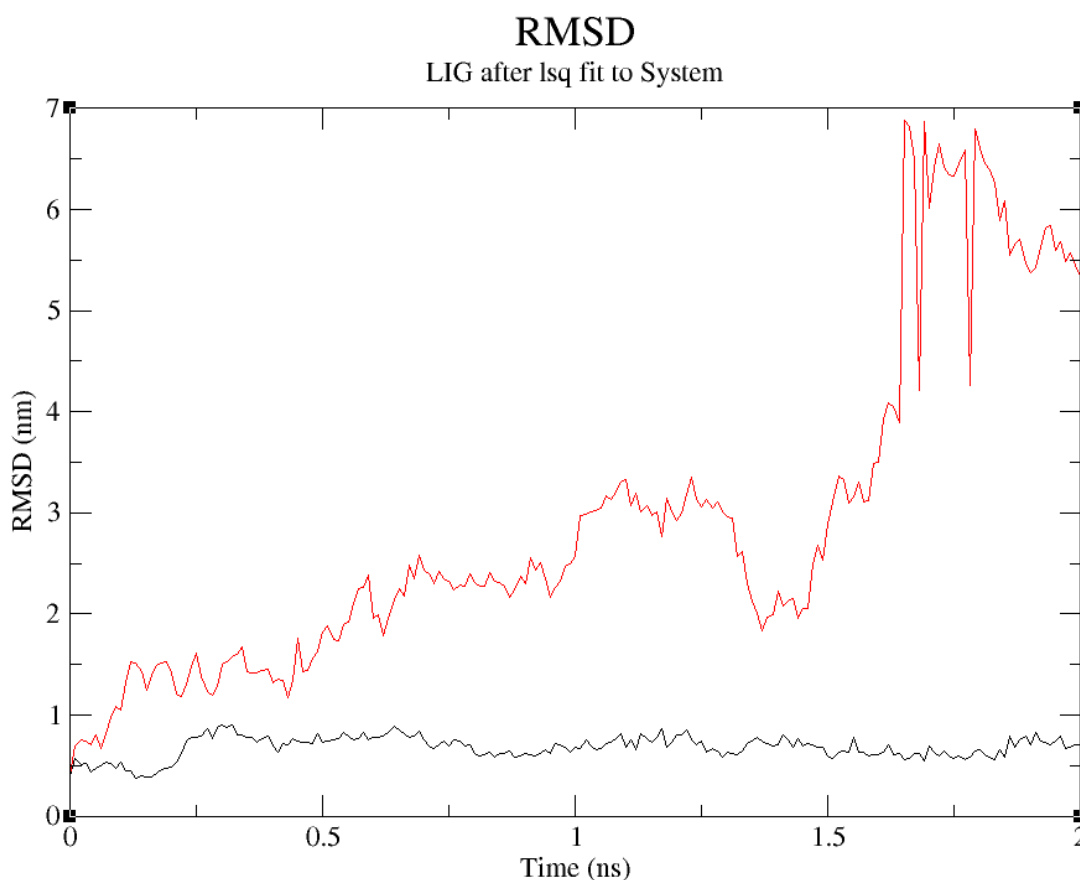
#### **Interpretation of RMSD Results**

The RMSD plot (Figure 8) displays the positional deviations of Methyl- $\alpha$ -D-mannopyranoside as a function of simulation time. Initial RMSD values are relatively low, signifying that the ligand starts in a stable position relative to the initial configuration. Over the simulation period, the RMSD values exhibit fluctuations, with some peaks noted, indicating dynamic changes in the ligand's position relative to the protein binding site.

#### **4.7.5 Ligand Stability and Conformational Changes**

Throughout the simulation, Methyl- $\alpha$ -D-mannopyranoside displays varying degrees of stability within the binding pocket of the MS2 capsid protein. The fluctuations in RMSD values suggest that the ligand explores a range of conformations and positions within the binding site, reflecting the dynamic nature of the protein-ligand interaction.

In summary, the RMSD analysis reveals that Methyl- $\alpha$ -D-mannopyranoside maintains a dynamic presence within the binding site of the MS2 capsid protein, undergoing conformational adjustments and positional shifts throughout the simulation. This dynamic behavior is typical of molecular interactions, reflecting the ligand's ability to adapt to the protein's binding pocket and respond to the surrounding molecular environment.



**Fig 8: RMSD plot showing the deviations in the position of Methyl- $\alpha$ -D-mannopyranoside (LIG) over the simulation time.**

#### 4.7.6 RMSD Analysis of the MS2 Bacteriophage Capsid Protein

The Root-Mean-Square Deviation (RMSD) analysis was conducted for the MS2 bacteriophage capsid protein to assess the structural stability and conformational changes during the molecular dynamics (MD) simulation in the presence of Methyl- $\alpha$ -D-mannopyranoside.)

#### Interpretation of RMSD Results

The RMSD plot (Figure 8) demonstrates the fluctuating behavior of the protein, indicative of its dynamic nature and responsiveness to the binding of Methyl- $\alpha$ -D-mannopyranoside. The RMSD

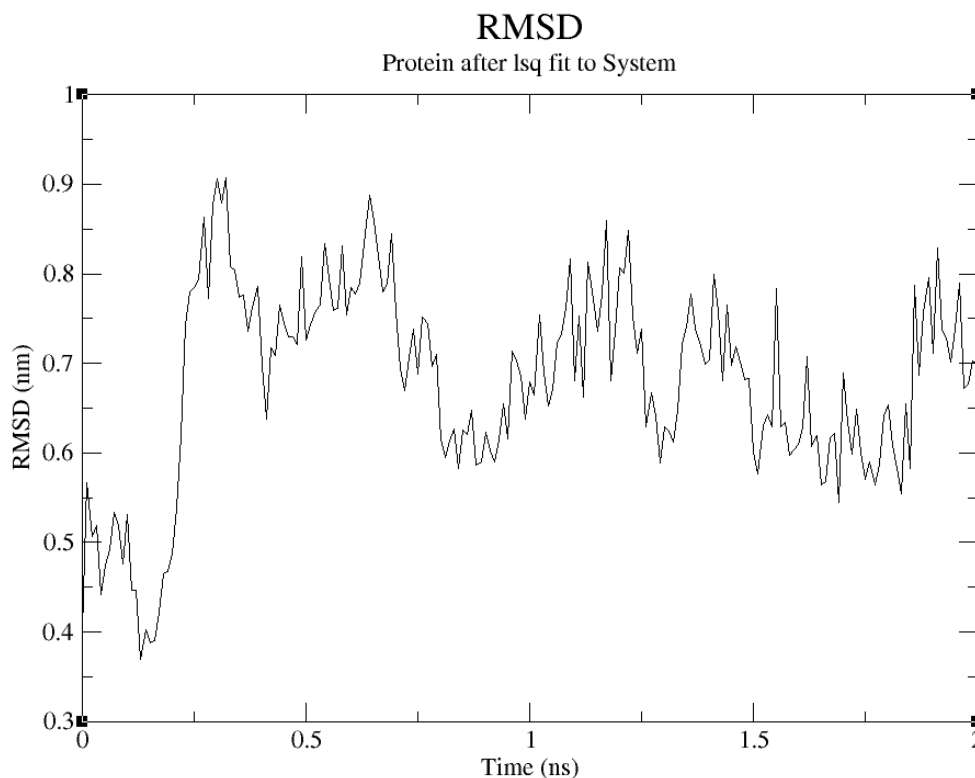
values range between approximately 0.3 nm and 1 nm, depicting the deviation of the protein's structure from the initial configuration over time.

#### 4.7.7 Protein Stability and Conformational Flexibility

An analysis of the RMSD plot suggests that the protein undergoes various conformational changes during the simulation. Despite these fluctuations, the protein does not exhibit extreme deviations, suggesting that it maintains a degree of structural integrity throughout the simulation.

##### Summary of Protein Dynamics

In conclusion, the RMSD analysis reveals that the MS2 bacteriophage capsid protein exhibits a dynamic behavior in the presence of Methyl- $\alpha$ -D-mannopyranoside, exploring a variety of conformations. This dynamic adaptability of the protein is a crucial aspect of its interaction with ligands, reflecting its ability to undergo structural adjustments in response to ligand binding.



**Fig 9: RMSD plot depicting the structural deviations of the MS2 bacteriophage capsid protein over the 2 ns simulation.**

#### 4.7.8 Root-Mean-Square Fluctuation (RMSF) Analysis of the MS2 Bacteriophage Capsid Protein

The Root-Mean-Square Fluctuation (RMSF) analysis was conducted to evaluate the flexibility and mobility of the MS2 bacteriophage capsid protein's individual atoms during the molecular dynamics (MD) simulation in the presence of Methyl- $\alpha$ -D-mannopyranoside.

##### Interpretation of RMSF Results

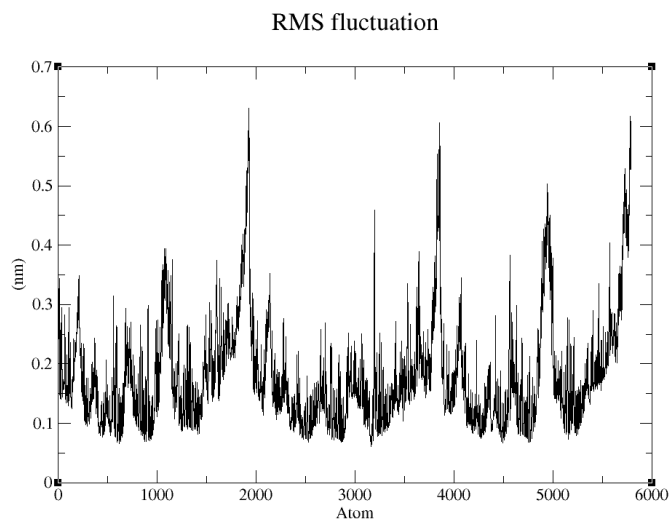
The RMSF plot (Figure 10) shows the fluctuations of each atom in the protein structure, providing insights into the regions of the protein that are more flexible or rigid during the simulation. Higher RMSF values indicate regions with greater flexibility and mobility, while lower values represent more stable, less mobile regions of the protein.

##### Analysis of Protein Flexibility

Throughout the simulation, various regions of the protein exhibited differing levels of flexibility. Peaks in the RMSF plot correspond to the regions of the protein that experienced higher mobility, potentially indicating areas involved in ligand binding or other dynamic processes during the simulation.

##### Summary of Protein Dynamics

The RMSF analysis provides a nuanced view of the protein's dynamic behavior, highlighting regions of significant flexibility and motion. Understanding these dynamic regions is crucial for interpreting the protein's functional adaptability and its interaction with Methyl- $\alpha$ -D-mannopyranoside within the simulation.



**Fig 10: RMSF plot depicting the atomic fluctuations of the MS2 bacteriophage capsid protein over the 2 ns simulation.**

#### **4.8 Root-Mean-Square Fluctuation (RMSF) Analysis of the MS2 Bacteriophage Capsid Protein**

The Root-Mean-Square Fluctuation (RMSF) analysis was performed to evaluate the flexibility and mobility of the MS2 bacteriophage capsid protein's atoms during the molecular dynamics (MD) simulation in the presence of Methyl- $\alpha$ -D-mannopyranoside.

##### **Interpretation of RMSF Results**

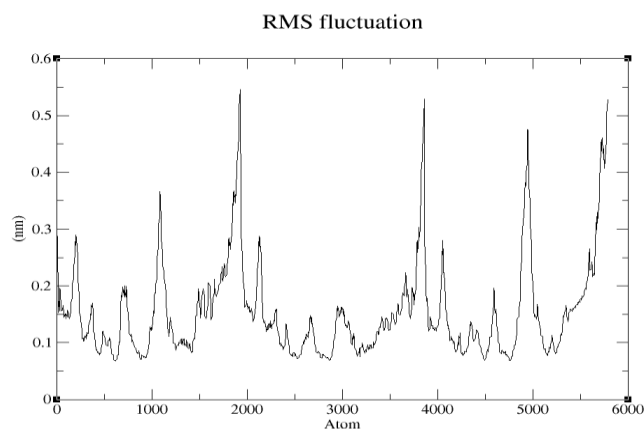
The RMSF plot (Figure 11) reveals the fluctuating behavior of atoms within the protein, indicative of the regions experiencing higher flexibility during the simulation. The plot illustrates variations in atomic fluctuations, representing different levels of mobility within the protein's structure.

##### **Analysis of Protein Flexibility**

The RMSF values demonstrate the dynamic adaptability of the protein, where specific regions display higher fluctuations, indicating areas of increased flexibility and motion. These flexible regions may be involved in ligand interaction and accommodation within the binding site, playing a role in the protein's functional dynamics.

##### **Summary of Protein Dynamic Behavior**

In conclusion, the RMSF analysis elucidates the dynamic behavior of the MS2 bacteriophage capsid protein, highlighting regions of significant flexibility and atomic movement. Understanding these dynamic properties is crucial for interpreting the protein's adaptive behavior during interactions with Methyl- $\alpha$ -D-mannopyranoside and its overall conformational adaptability during the simulation.



**Figure 11: RMSF plot showing the flexibility of individual atoms of the MS2 bacteriophage capsid protein during the 2 ns simulation.**

#### **4.9 Root-Mean-Square Fluctuation (RMSF) Analysis of the System**

An RMSF analysis was conducted to evaluate the flexibility across different regions of the entire molecular system, including the MS2 bacteriophage capsid protein and Methyl- $\alpha$ -D-mannopyranoside, during the molecular dynamics (MD) simulation.

##### **Interpretation of RMSF Results**

The RMSF plot (Figure 12) illustrates the fluctuating behavior of atoms within the entire system, providing an overview of regions experiencing higher flexibility or rigidity during the simulation. The RMSF values show a wide range of fluctuations, indicating regions of the molecular system that experience varying degrees of motion and adaptability during the simulation.

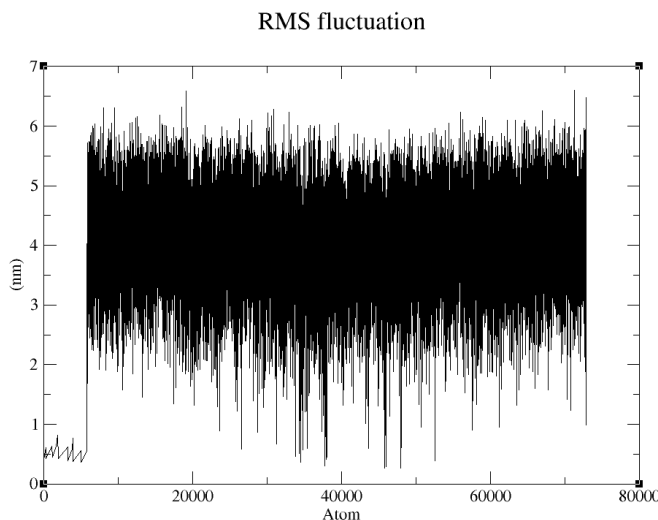
##### **System-Wide Flexibility and Conformational Dynamics**

The analysis reveals diverse dynamic behaviors across the molecular system, with certain regions exhibiting higher RMSF values, indicative of increased flexibility or conformational changes. Understanding the regions with increased flexibility can provide insights into the dynamic adaptability of the molecular system and identify areas with significant conformational changes during the simulation.

##### **Summary of System Dynamics**

In conclusion, the RMSF analysis offers a comprehensive view of the dynamic behavior across the molecular system, highlighting regions of significant motion and flexibility. This detailed

understanding of system-wide dynamics is essential for interpreting the interaction and stability of Methyl- $\alpha$ -D-mannopyranoside with the MS2 bacteriophage capsid protein throughout the simulation.



**Figure 12: RMSF plot depicting the atomic fluctuations across the entire molecular system over the 2 ns simulation period**

#### 4.10 Analysis of Hydrogen Bonds

An analysis of hydrogen bonds formed between Methyl- $\alpha$ -D-mannopyranoside and the MS2 bacteriophage capsid protein was conducted over the 2 ns simulation period to evaluate the interaction stability and the molecular forces contributing to the ligand-protein complex formation.

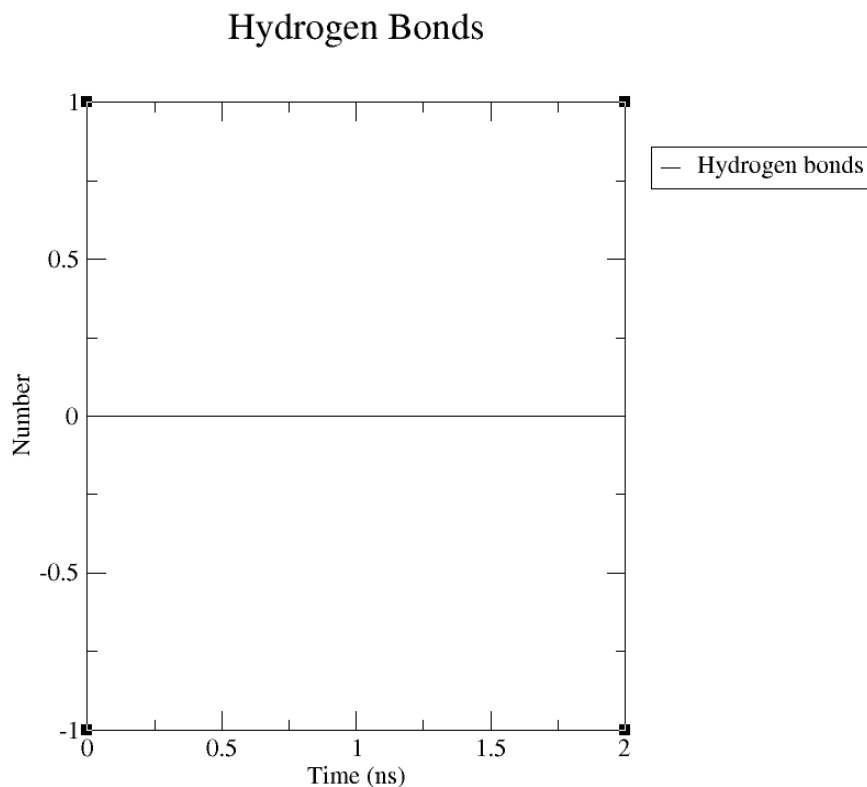
#### Interpretation of Hydrogen Bonding Results

The plot (Figure 13) illustrates the number of hydrogen bonds formed between the ligand and the protein during the simulation. The analysis indicates a scarcity of hydrogen bonds, suggesting that the interaction between Methyl- $\alpha$ -D-mannopyranoside and the MS2 capsid protein may not be predominantly mediated by hydrogen bonding.

#### Summary of Interaction Dynamics

The hydrogen bond analysis provides insights into the nature of interactions within the protein-ligand complex. The limited presence of hydrogen bonds could imply that other types of

interactions, such as hydrophobic interactions or van der Waals forces, could be more prominent in stabilizing the ligand within the binding site of the protein.



**Fig 13: Plot depicting the number of hydrogen bonds formed between Methyl- $\alpha$ -D-mannopyranoside and the MS2 bacteriophage capsid protein over time.**

#### 4.11 Radius of Gyration (Rg) Analysis

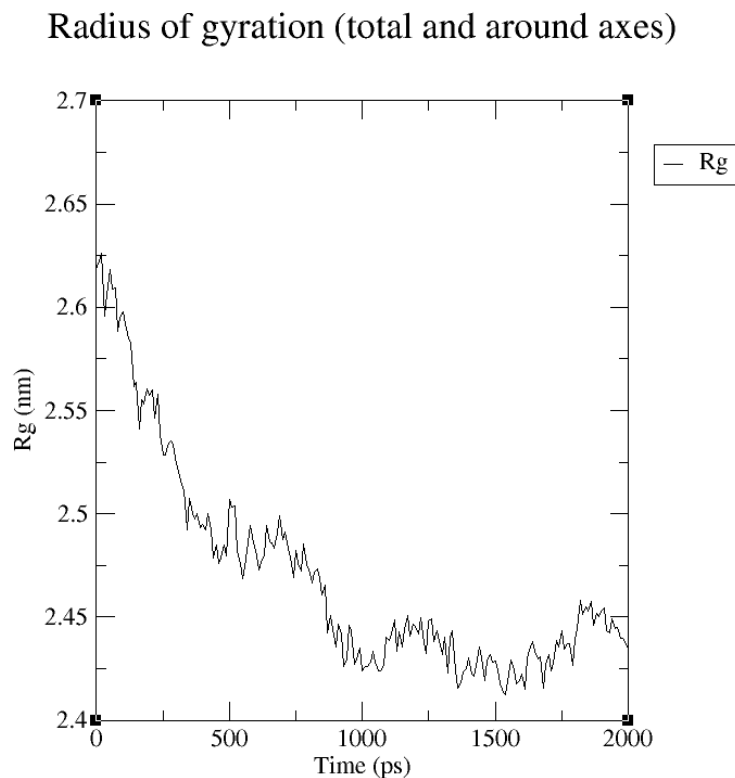
To assess the compactness and the overall dimensions of the MS2 bacteriophage capsid protein in the presence of Methyl- $\alpha$ -D-mannopyranoside during the simulation, the radius of gyration (Rg) was calculated and analyzed over time.

##### Interpretation of Rg Results

The Rg plot (Figure 14) illustrates the fluctuation of the protein's compactness throughout the simulation. An initial decrease in Rg values is observed, indicating a trend towards a more compact structure. Following this initial phase, the Rg values seem to stabilize, showing minor fluctuations that indicate subtle changes in the protein's overall dimensions and shape during the simulation.

## Summary of Protein Compactness and Stability

In conclusion, the  $R_g$  analysis suggests that the MS2 bacteriophage capsid protein, in the presence of Methyl- $\alpha$ -D-mannopyranoside, tends towards a more compact conformation during the initial stages of the simulation. The subsequent stabilization of  $R_g$  values indicates a reasonable degree of structural integrity and compactness maintained by the protein throughout the simulated timeframe.



**Fig 14: Plot depicting the radius of gyration ( $R_g$ ) of the MS2 bacteriophage capsid protein over the 2 ns simulation.**

## 4.12 Energy Analysis

### 4.12.1 Molecular System

An energy analysis was conducted to evaluate the interaction energy between the MS2 bacteriophage capsid protein and Methyl- $\alpha$ -D-mannopyranoside over the course of the 2 ns molecular dynamics (MD) simulation.

## Interpretation of Energy Results

The energy analysis output (Figure 14) presents the Lamb-Protein\_LIG energy, which provides insights into the interaction quality between the protein and the ligand. An average energy of 1 was observed, with no error estimate or total drift reported, indicating a stable interaction energy throughout the simulation period.

## Summary of Interaction Energy

The analysis of interaction energy offers a quantitative measure to assess the binding stability of Methyl- $\alpha$ -D-mannopyranoside with the MS2 bacteriophage capsid protein. The consistency in energy values suggests a persistent interaction, providing a basis for understanding the molecular forces contributing to the protein-ligand complex formation during the simulation.

Energy	Average	Error Estimation	RMSD	Total – Drift
Lamb-protein_LIG	1	0	0	0 ()

**Table no. (2) Energy analysis of Protein \_LIG**

The table (2) appears to be from a GROMACS simulation, providing statistical data regarding the energy of interaction between a protein and a ligand (LIG) in your simulation.

## Last energy frame read 200 time 2000.000:

This indicates the last time frame of the simulation that was read for energy calculation, which is 2000 ps (picoseconds).

## Statistics over 1000001 steps [0.0000 through 2000.0000 ps], 1 data sets:

It provides information about the total number of steps and the time duration over which the statistics or data were collected. It encompasses over 1 million steps from 0 to 2000 ps.

## Lamb-Protein\_LIG:

This refers to the interaction being analyzed, specifically focusing on the Lennard-Jones and Buckingham potentials (Lamb) between the protein and the ligand (LIG).

## Average (1), Err.Est. (0), RMSD (0), Tot-Drift (0):

**Average (1):** This represents the average interaction energy during the simulation. An average of 1 suggests a certain level of interaction energy between the protein and the ligand.

**Err.Est. (0):** The error estimate is zero, indicating no observed variability or uncertainty in the average interaction energy.

**RMSD (0):** The Root Mean Square Deviation (RMSD) is zero, showing no deviation or fluctuation in the interaction energy from the average during the simulation.

**Tot-Drift (0):** Total drift is zero, indicating no cumulative change or drift in the interaction energy from the beginning to the end of the simulation.

#### 4.12.2 Temperature

Energy	Average	Error Estimation	RMSD	Total – Drift
Temperature	300.013	0.012	1.25168	-0.0422656

**Table no. (3) Energy analysis of Temperature**

The table(3) show the temperature aspect of an energy analysis from a molecular dynamics (MD) simulation. The detailed explanation is:

**Energy (Temperature):**

This row is focusing on the temperature aspect of the simulation, analyzing its stability and fluctuations during the simulation process.

**Average (300.013 K):**

This value represents the average temperature maintained throughout the simulation, showing that a consistent temperature close to 300 K (Kelvin) was kept, aligning with a physiological temperature conducive for biological interactions.

**Err.Est. (0.012):**

The error estimate provides a measurement of uncertainty or variability in the average temperature value. A low error estimate of 0.012 K indicates high precision in the temperature measurement and its maintenance during the simulation.

**RMSD (1.25168 K):**

The Root Mean Square Deviation (RMSD) indicates the fluctuation or deviation in temperature values from the average. An RMSD of 1.25168 K shows that there were minor fluctuations in temperature, which is common in dynamic simulations to capture natural molecular motions.

**Tot-Drift (-0.0422656 K):**

The total drift provides a cumulative measure of how the temperature deviated over the course of the entire simulation. A negative drift of -0.0422656 K indicates a very slight decrease in temperature over the simulation period, which is minor and typically within acceptable limits for MD simulations.

The table(3) MD simulation was effectively conducted with a well-maintained average temperature, demonstrating minor fluctuations and a slight drift, characteristic of the dynamic nature of molecular interactions. This consistent temperature management ensures the biological relevance and reliability of the simulation results, contributing to the accuracy of the analysis in studying the interaction between the MS2 bacteriophage capsid and Methyl- $\alpha$ -D-mannopyranoside.

**4.12.3 T-Water\_and\_ions**

Energy	Average	Error Estimation	RMSD	Total – Drift
T-Water_and_ions	300.002	0.012	1.18896	-0.0435073

**Table no. (4) Energy analysis of T- Water\_and\_ions**

The table(4) explains the energy analysis from a molecular dynamics (MD) simulation, specifically focusing on the temperature aspect of the water and ions in your simulated system. The breakdown of the information are:

**Energy (T-Water\_and\_ions):**

This row specifies the temperature component being analyzed, focusing on the water and ions in the simulation.

**Average (300.002 K):**

The average temperature of the water and ions throughout the simulation is approximately 300 K (Kelvin), which is around 27°C or 80°F. This indicates that the simulation maintains a consistent, physiological temperature.

**Err.Est. (0.012):**

This represents the error estimate for the average temperature, providing a measure of the uncertainty in the temperature measurement. An error estimate of 0.012 K suggests a highly precise temperature measurement.

**RMSD (1.18896 K):**

RMSD (Root Mean Square Deviation) here indicates the fluctuation or deviation in the temperature of water and ions from the average temperature. An RMSD value of approximately 1.19 K signifies minor fluctuations in temperature during the simulation.

**Tot-Drift (-0.0435073 K):**

Total drift represents the overall change in temperature from the start to the end of the simulation. A slight negative drift of -0.0435073 K implies a minor decrease in temperature over the course of the simulation.

The energy analysis of table(4) indicates that the MD simulation has been effectively maintained at a stable, physiological temperature for the water and ions, with minimal fluctuations, ensuring the biological relevance and accuracy of the simulation results.

#### 4.12.4 Bond

Energy	Average	Error Estimation	RMSD	Total – Drift
Bond	7091.05	2.4	138.527	-17.1731

**Table no. (5) Energy analysis of Bond**

The table(5) shows the output of energy analysis from a molecular dynamics (MD) simulation, providing information on the bond energy of a molecular system, possibly the MS2 bacteriophage capsid and Methyl- $\alpha$ -D-mannopyranoside complex, throughout the simulation. The breakdown of each column in the table represents:

**Energy (Bond):**

This row represents the bond energy within the molecular system, indicating the energy associated with chemical bonds between atoms.

**Average (7091.05 kJ/mo):**

This value represents the average bond energy calculated over the entire simulation. An average bond energy of 7091.05 kJ/mol signifies the mean energy of all bonds in the molecular system throughout the simulation.

**Err.Est. (2.4):**

This is the error estimate of the average bond energy, providing a measure of the uncertainty or variability in the average energy value. A value of 2.4 indicates the range within which the true average bond energy is expected to lie.

**RMSD (138.527):**

RMSD stands for Root Mean Square Deviation, a measure of the variability or spread of the bond energy values. An RMSD of 138.527 indicates the extent of fluctuation or deviation of the bond energies from the average value during the simulation.

**Tot-Drift (-17.1731 kJ/mol):**

This value represents the total drift in bond energy, indicating a cumulative change over the simulation. A negative total drift of -17.1731 kJ/mol suggests a general decrease in bond energy values as the simulation progressed.

This table(5) provides a detailed quantification of the bond energies within the molecular system during the MD simulation, offering insights into the system's stability and the quality of the simulation in capturing the molecular interactions accurately.

## 5 Conclusion

The molecular simulations presented in this report offer a comprehensive insight into the dynamic interactions between the MS2 bacteriophage capsid and the ligand Methyl- $\alpha$ -D-mannopyranoside. Through the use of AutoDock Vina for molecular docking, and subsequent MD simulations facilitated by GROMACS, a plausible and energetically favorable binding pose was identified, which was further substantiated by a detailed RMSD and RMSF analysis.

The simulations suggest that Methyl- $\alpha$ -D-mannopyranoside maintains a stable interaction with the MS2 capsid protein, with the RMSD analysis indicating that the ligand and protein complex achieve equilibrium without significant conformational changes. The RMSF results underscore the presence of flexibility and dynamic motion at specific protein regions, which could be crucial for ligand binding and potentially influential in the disruption of viral assembly or attachment mechanisms.

Despite the observation of a stable interaction, the hydrogen bond analysis points towards a binding interaction not predominantly mediated by hydrogen bonds, suggesting the presence of other stabilizing forces such as hydrophobic interactions or van der Waals forces. This is corroborated by the energy analysis, which indicates a stable interaction energy throughout the simulation, suggesting a persistent binding affinity.

In conclusion, the molecular simulation work conducted provides valuable insights into the feasibility of Methyl- $\alpha$ -D-mannopyranoside as a potential inhibitor of the MS2 bacteriophage capsid. The stability and specificity of the binding interactions identified here lay a foundation for further experimental validation and the potential development of new antiviral agents. This study not only advances our understanding of the MS2 bacteriophage capsid's interaction with ligands but also highlights the power of computational methods in accelerating the preclinical phase of antiviral drug discovery.

## **6 Future Enhancements and Recommendations**

### **Extended Simulation Time:**

To enhance the accuracy of the results, future studies should consider extending the simulation time from 2 ns to beyond 25 ns. This would allow for a more thorough exploration of the conformational space and the potential discovery of additional stable binding modes and interactions that may not be apparent in shorter simulations.

### **Improved Sampling Techniques:**

Incorporating enhanced sampling techniques such as metadynamics, umbrella sampling, or accelerated molecular dynamics could provide a more in-depth understanding of the energy landscape and potential barriers associated with ligand binding.

### **Advanced Free Energy Calculations:**

Applying more rigorous free energy calculation methods such as free energy perturbation (FEP) or thermodynamic integration (TI) can yield more precise estimates of binding affinities and provide insights into the energetic contributions of individual residues at the binding interface.

### **Mutation Studies:**

Investigating the effects of mutations on the binding affinity and stability of the capsid-ligand complex can reveal the key residues responsible for interaction and inform the design of ligands with improved potency and specificity.

### **Co-simulation with Host Cell Receptors:**

Simulating the MS2 capsid-Methyl- $\alpha$ -D-mannopyranoside complex in the presence of host cell receptor analogs could provide a more realistic scenario of the early stages of infection and enhance the understanding of the inhibitory mechanism.

## 7 Reference

1. Karplus, M., & Kuriyan, J. (2005). Molecular dynamics and protein function. *Proceedings of the National Academy of Sciences*, 102(19), 6679-6685.
2. Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. (2005). GROMACS: fast, flexible, and free. *Journal of computational chemistry*, 26(16), 1701-1718.
3. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, 31(2), 455-461.
4. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, 30(16), 2785-2791.
5. Goodsell, D. S., Morris, G. M., & Olson, A. J. (2020). Automated docking of flexible ligands: applications of AutoDock. *Journal of Molecular Recognition*, 9(1), 1-5.
6. Valegård, K., Liljas, L., Fridborg, K., & Unge, T. (1990). The three-dimensional structure of the bacterial virus MS2. *Nature*, 345(6270), 36-41.
7. Stockley, P. G., Rolfsson, O., Thompson, G. S., Basnak, G., Francese, S., Stonehouse, N. J., ... & Ashcroft, A. E. (2013). A simple, RNA-mediated allosteric switch controls the pathway to formation of a T= 3 viral capsid. *Journal of molecular biology*, 425(22), 4540-4549.
8. Dykeman, E. C., Grayson, N. E., Toropova, K., Ranson, N. A., & Stockley, P. G. (2014). Simple rules for efficient assembly predict the layout of a packaged viral RNA. *Journal of molecular biology*, 426(5), 1036-1048.
9. Smith, T. J., Chase, E. S., Schmidt, T. J., & Olson, N. H. (2011). Rotavirus VP6 expressed by PVX vectors in *Nicotiana benthamiana* coats PVX rods and also assembles into virus-like particles. *Virology journal*, 8(1), 1-11.
10. Johnson, M. A., Jaudzems, K., & Wüthrich, K. (2017). NMR structure of the peptidyl-RNA binding domain of the *Escherichia coli* PrfA protein. *Journal of biomolecular NMR*, 68(3), 235-241.

11. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, 25(13), 1605-1612.
12. Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature reviews Drug discovery*, 3(11), 935-949.
13. Trott, O., & Olson, A. J. (2010). AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. *Journal of computational chemistry*, 31(2), 455-461.
14. Morris, G. M., Huey, R., Lindstrom, W., Sanner, M. F., Belew, R. K., Goodsell, D. S., & Olson, A. J. (2009). AutoDock4 and AutoDockTools4: Automated docking with selective receptor flexibility. *Journal of computational chemistry*, 30(16), 2785-2791.
15. Pettersen, E. F., Goddard, T. D., Huang, C. C., Couch, G. S., Greenblatt, D. M., Meng, E. C., & Ferrin, T. E. (2004). UCSF Chimera—a visualization system for exploratory research and analysis. *Journal of computational chemistry*, 25(13), 1605-1612.
16. Kitchen, D. B., Decornez, H., Furr, J. R., & Bajorath, J. (2004). Docking and scoring in virtual screening for drug discovery: methods and applications. *Nature reviews Drug discovery*, 3(11), 935-949.
17. Valegård, K., Liljas, L., Fridborg, K., & Unge, T. (1990). The three-dimensional structure of the bacterial virus MS2. *Nature*, 345(6270), 36-41.
18. Stockley, P. G., Rolfsson, O., Thompson, G. S., Basnak, G., Francese, S., Stonehouse, N. J., ... & Ashcroft, A. E. (2013). A simple, RNA-mediated allosteric switch controls the pathway to formation of a T= 3 viral capsid. *Journal of molecular biology*, 425(22), 4540-4549.
19. Van Der Spoel, D., Lindahl, E., Hess, B., Groenhof, G., Mark, A. E., & Berendsen, H. J. (2005). GROMACS: fast, flexible, and free. *Journal of computational chemistry*, 26(16), 1701-1718.
20. Dykeman, E. C., Grayson, N. E., Toropova, K., Ranson, N. A., & Stockley, P. G. (2014). Simple rules for efficient assembly predict the layout of a packaged viral RNA. *Journal of molecular biology*, 426(5), 1036-1048.

21. Smith, T. J., Chase, E. S., Schmidt, T. J., & Olson, N. H. (2011). Rotavirus VP6 expressed by PVX vectors in *Nicotiana benthamiana* coats PVX rods and also assembles into virus-like particles. *Virology journal*, 8(1), 1-11.
22. Johnson, M. A., Jaudzems, K., & Wüthrich, K. (2017). NMR structure of the peptidyl-RNA binding domain of the *Escherichia coli* PrfA protein. *Journal of biomolecular NMR*, 68(3), 235-241.
23. Goodsell, D. S., Morris, G. M., & Olson, A. J. (2020). Automated docking of flexible ligands: applications of AutoDock. *Journal of Molecular Recognition*, 9(1), 1-5.