

Exploring the Potential of *Chromones* as Inhibitors of Novel Coronavirus Infection Based on Molecular Docking and Molecular Dynamics Simulation Studies

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Abstract: The COVID-19 has been declared a global pandemic by the WHO. There are no approved drugs to treat the disease in the present scenario, by which there is an urgent need for best-suited antiviral therapy against COVID-19. Natural compounds (Chromone) have a significant effect on treating COVID-19 and other diseases. Chromone compounds can be used as an adjunctive treatment for SARS-CoV-2 infection. Herein, we have applied a bioinformatics approach to check out the lead molecule's binding capabilities to that of H-Bonding with THE 638 and TYR 612, PI Bonding with VAL622 VAL 610 and PRO 295, that interact with specifically defined residues in substrate binding cavity. Further, molecular simulation studies have been performed that revealed that the ligand's binding made the substrate more fluctuating and dynamic. The study affirms that the Chromone may serve as an effective tool to fight COVID-19 disease having promising binding activities.

Key Words: COVID-19; Chromone; molecular simulation; SARS-Cov-2 infection; inhibitor.

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1. Introduction

As coronavirus infections are approaching about 450,000,000 mark worldwide until August 2021, one must understand how it spreads quickly. SARS-CoV-2 (previously known as 2019-nCoV) possesses a surface "spike" glycoprotein that facilitates the virus's adherence to the host membrane through its specific receptor, resulting in viral invasion into the host posing as a likely target candidate for suppression of infection [1]. The Spike glycoprotein also performs reorganization at structural levels triggered upon the binding of the S1 subunit to that of the host cell receptor. The binding results in the shedding of the S1 subunit and transform the S2 subunit to a steady conformational state. The virus, however, requires a variety of proteins for multiplication and warfare on the host cells [2]. The receptor-binding domain (RBD) has dual conformational states as one becomes the unreachable stable state while the other corresponds to the receptor reachable state. In light of the above information, the S protein seems fit to be a potential target for antibody facilitated blocking or deactivation. The genomic sequence of nCoV S comprises 1-1208 residues (Figure 1a). The domain organization includes

the signal sequence, protease cleavage site (SD1/SD2), fusion peptide, heptad repeat 1, central helix, connector domain, heptad repeat 2, a transmembrane domain, cytoplasmic tail, and other domains [3]. The heptad repeat (HR2) and the transmembrane domain at the C-terminus have not been visualized yet by any research studies. The virus is persistent on a variety of surfaces for a specific amount of time, such as cardboard (1 day), plastic and steel (3 days), wood, copper, and glass (4 days), paper (4-5 days), metal, PVC (5 days), and so on. Some chemicals have also been reported to inactivate the virus, such as ethanol (78-95 percent), 2-propanol (70-100 percent), a mixture of 2-propanol (45 percent) and 1-propanol (30 percent), glutaraldehyde (0.5-2.5 percent), formaldehyde (0.7-1 percent), etc. [4]. Some recent studies have successfully isolated the novel coronavirus from human epithelial cells. Similarly, whole genomic level studies have revealed that the virus (2019-nCoV) was relatively closer to bat-SLCoVZC45 and bat-SLCoVZXC21. Moreover, the 2019-nCoV receptor-binding domain displayed similarity to SARS and SARS-CoV [5]. Also, the ACE2 (angiotensin-convertase enzyme II) was reportedly found as a cellular receptor of nCoV-2019 interacting with the RBD domain of nCoV [6]. Therefore, ACE2 was reported to have a significant role in cellular admittance and entry [7], potentially targeting nCoV-2019 [1, 8]. After the genomic screening of patient samples with viral pneumonia, a novel coronavirus (labeled 2019-nCoV) was reported in Wuhan, China. It belongs to the subgenus Sarbecovirus and shares similarities with bat-derived coronavirus strains, bat-SL-CoVZC45 and bat-SL-CoVZXC21 [9-11]. The average mutation rate for coronavirus was estimated to be 10^{-4} nucleotide substitutions per site per year. It was thought that 2019-nCoV emerged from a single source in a brief period, with the disease outbreak pointing to a hidden virus reservoir in animals and the threat for it to pass over to humans [12, 13] infrequently. In South Africa itself, 42 SARS-CoV-2 lineages or variants have been found throughout eight provinces.

2. Methodology

The refined energy models of the surface glycoprotein of Severe acute respiratory syndrome coronavirus 2 (SARS COV-2) Spike protein were engendered employing the Swiss model. The models predicted by the method have been re-evaluated for particular parameters such as stereochemistry checks, the geometry of the structures, and energy distribution using PROCHECK that revealed model 1 as the most stable structure with 85.9% core residues. Overall, model quality analysis (Z-Score) was performed to cross-validate the depicted models and results, which confirmed model 1 as the best fit (Table 1). NCBI Pub-Chem was used for seed molecule search [14, 15]. Searching for Natural ligand 6VSB_A revealed Chromone has the inhibition property, which tends to bind with nCoV protein that best fits the accepted rules of SBDD approach [16-19]. This natural compound has been taken for checking out docking possibilities. For the said purpose, we have run Autodock Vina, which helped in understanding the structures of the molecule which are to be docked along with the specification of the search space [20, 21], including the binding site in the protein structure by using DBQT molecular structure file format (Figure 3). To better understand the conformational dynamics of the bound nCoV complex, an MD simulation study was conducted using GROMACS [22, 23].

The GROMACS 2020.2 package was used to do MD simulations of the nCoV complex employing the CHARMM36 force field [20]. GROMACS' pdb2gmh modules were used to create the protein topology. Separately, PRODRG 2.5, an online server, was used to generate the ligand topology [16, 24]. A dodecahedron box was used for the solvation of protein. Protein was placed 1.0 nm away from the box's edge. The system was checked for electroneutrality,

and required charges were added. Afterward, energy minimization was accomplished. Potential energy (P.E.) was reduced upon a maximum force of 1000.0 KJ/mol/nm in one phase, and energy minimization was done for 50,000 steps. NVT equilibration was done for 100ps. After every 10ps, the complex's coordinates were recorded. Parrinello-Rahman pressure coupling was also used to achieve pressure equilibrium. Finally, the system was put through a 1ns molecular dynamics simulation to see if the nCoV compound was stable. GROMACS' rmsd, rmsf, and gyrate modules were used to do structural analysis (RMSD, RMSF, and Rg), and their graphs were created using xm grace [25, 26].

Table 1. Properties of models predicted.

MODEL	Core Value	G-factors	Z-Score
Model 1	86.9% core	-0.42	-12.59
Model 2	79.3% core	-0.44	-11.67
Model 3	78.9% core	-0.43	-12.18
Model 4	86.4% core	-0.28	-2.36

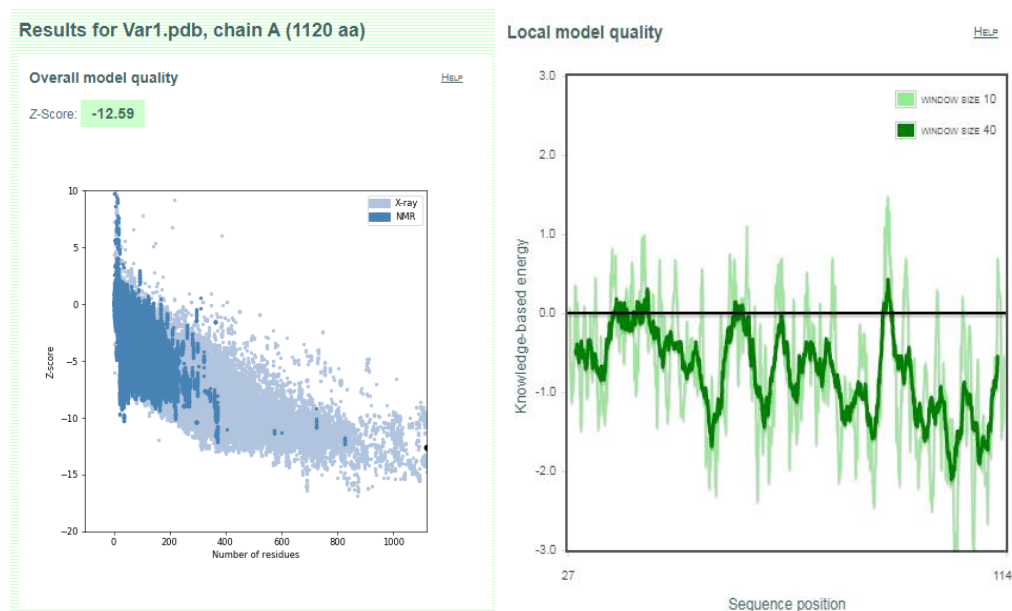
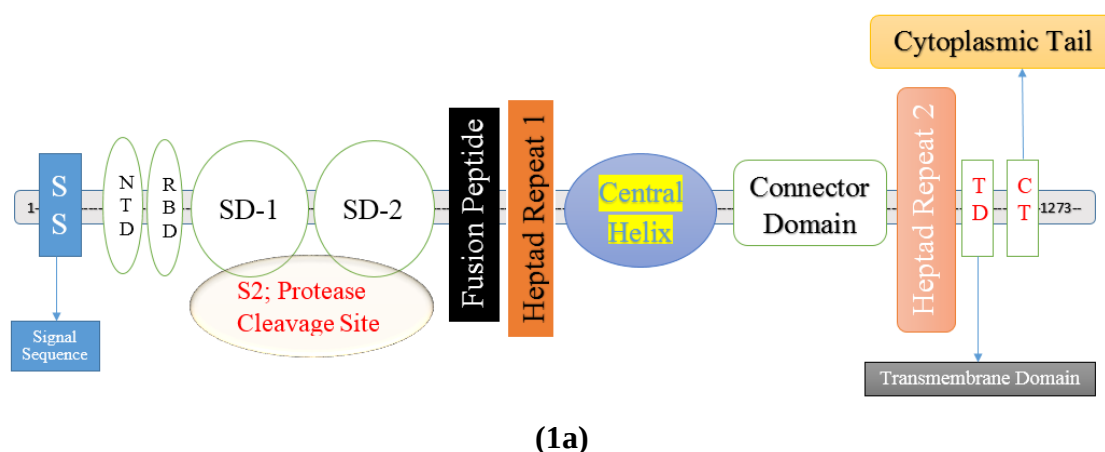


Figure 1. (a) Domain structure of novel coronavirus disease, (b) Residue energy plots of Spike glycoprotein & Superimposition of template and model protein.

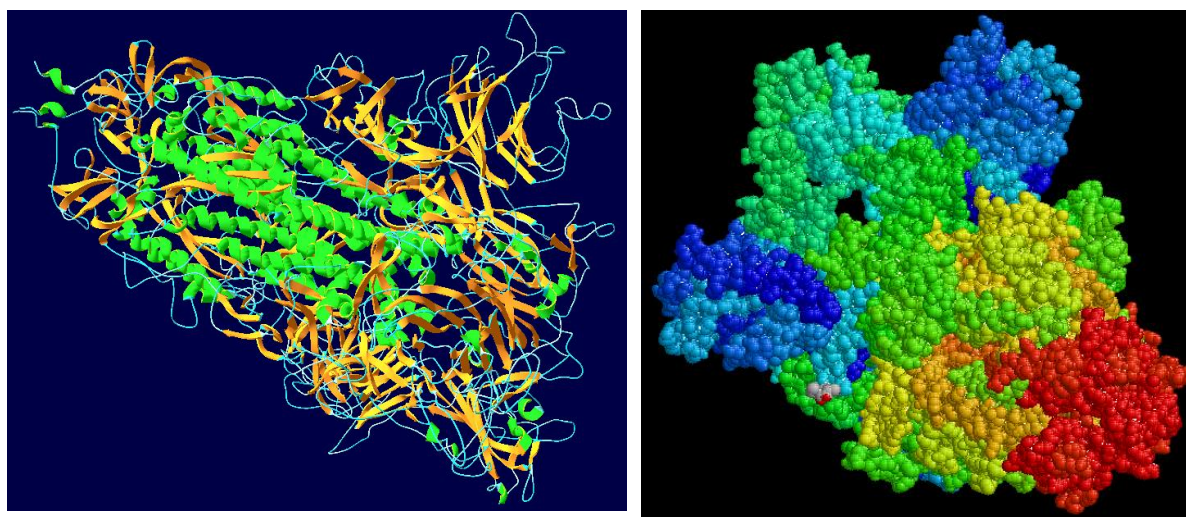


Figure 2. Finally selected 3D Structure of Protein (Ribbon & Surface Structure).

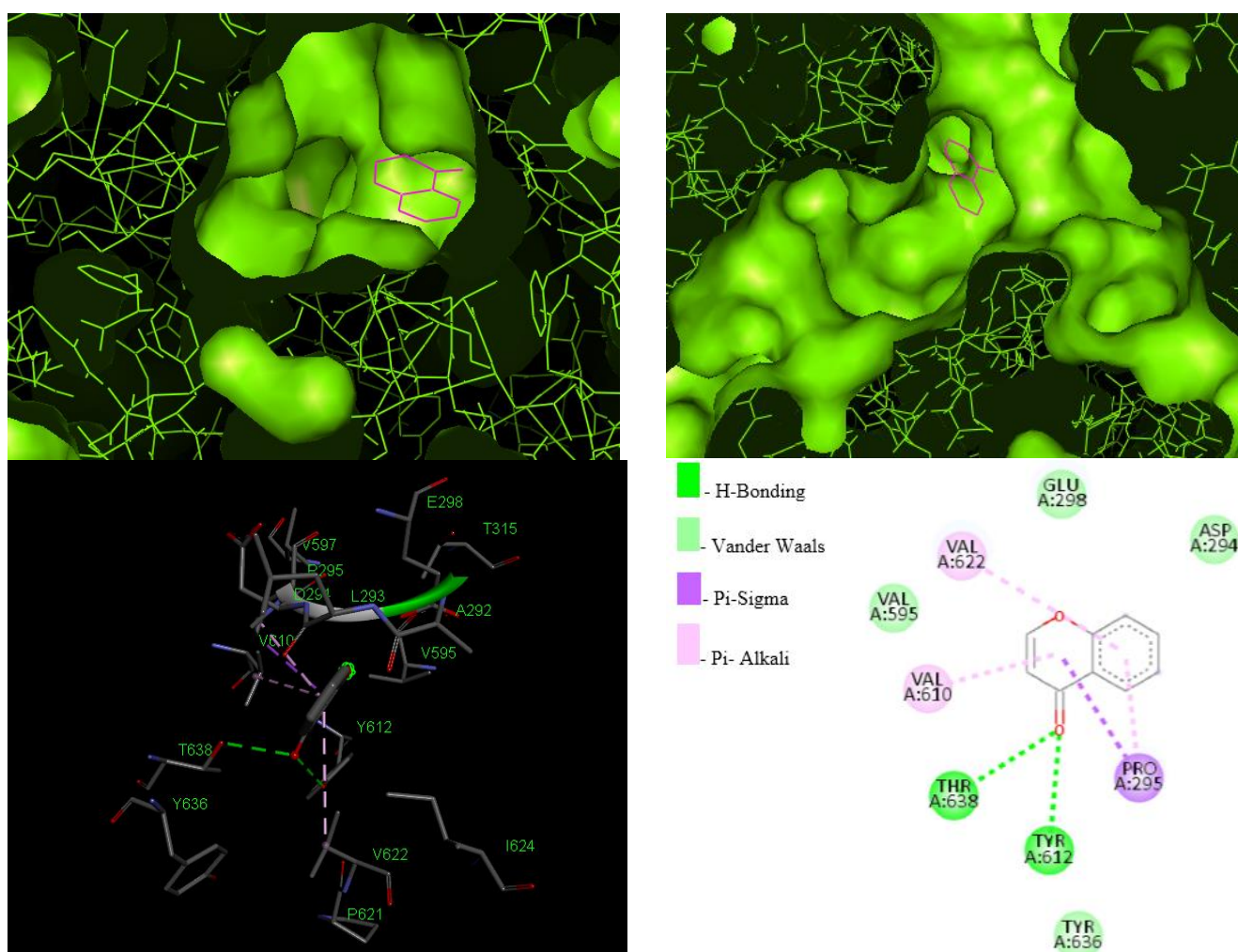


Figure 3. The best mode of docking, selective mode of docking for Simulation studies.

3. Results and Discussion

In the current study using a structure-based drug design approach, we have proposed a potent ligand molecule targeting nCOV S comprising 1208 residues. SARS-Cov-2 was used as a query, and it was aligned for similarity search against the RCSB PDB database using BLAST, which showed the best similar structures, which were then used in the Swiss model run. We did homology modeling and built four protein structure models using the query and the

template. These generated structures were validated using PROCHECK & PROSA tools. Based on the validated observations, we evaluated and selected the best model (Model1) with an 86.9% core value, along with a significant G factor (-0.42) and z score (-12.59) (Table 1). Other active site searches were performed as well.

Further, we also surfed for seed molecule over NCBI Pub-chem and subsequently explored on Drug database [27]. Chromone molecules have been observed with the best possible inhibition properties. De-novo generation of the probable molecule was performed using ACD Labs freeware (Chemsketch) [28]. Using the seed molecule and template PDB result as an input, we have run AutoDock Vina (Figure 3) [29]. The flexibility of active residues has also been examined. Residue THE 638 and TYR 612, PI Bonding with VAL622, VAL 610, and PRO 295 can play a crucial role in stable binding to accommodate the ligand. During MD simulations, the varying rmsd values indicate the system's fluctuating nature, and high peaks of RMSF indicate the dynamic nature of the system.

Solvation of the nCoV protein system was achieved using the standard Gromacs solvate tool, and two sodium ions were added post hoc to make it electroneutral. The system was then equilibrated by a 100 ps NVT run to thermalize the system, followed by another 100 ps NPT equilibration run to stabilize the volume. The system's volume was found 8450.24 nm³ and density was measured 1004.23 (g/l). Post hoc NVT and NPT, the system went under Potential energy minimization. The system's potential energy displayed a decline in the first few ps, but got stabilized later on. System's potential energy minimization was achieved at 2911 E.M. steps (Figure 4). System temperature fluctuated between 299 K to 300.5 K with constant pressure (Figure 5; Figure 6). The system's density fluctuated from 1002 to 1005 kg/m³ throughout the simulation, although the average was closer to 1004.23 kg/m³. The system's density remained consistent over time, showing that it was well-equilibrated in pressure and density (Figure 7). After all necessary equilibrations, the system was put to MD Simulations for 1ns [30].

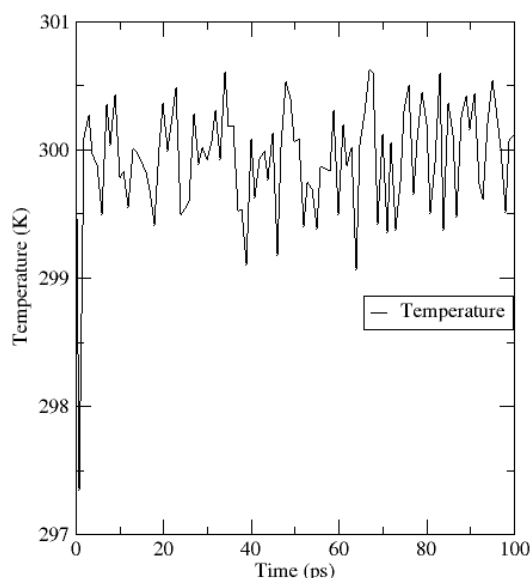


Figure 4. Temperature accounted during NVT equilibration.

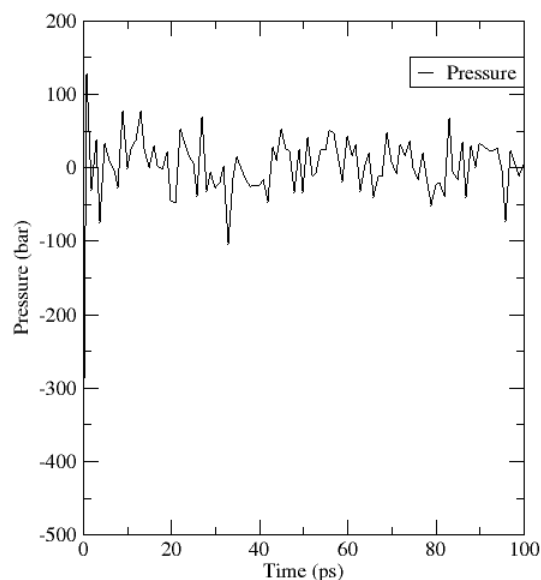


Figure 5. Pressure accounted during NPT equilibration.

The RMSD graph demonstrates how the 3D structure of proteins fluctuated during the simulation. A slight decrease in RMSD of the equilibrated structure was observed compared to the crystal structure (Figure 8). During simulation, the red line depicts the protein backbone of the equilibrated structure, which remained below an acceptable value in terms of crystal

structure (Blackline). The radius of gyration was computed to determine the degree of protein compactness during simulation. A steady value of 4.7 Rg was obtained throughout 1000 ps, indicating that the protein remained in its stable folded state during simulations. (Figure 9). The RMSF of each residue was computed, which illustrates Amino Acid residues between 450-480 undergoing maximum fluctuation over simulation (Figure 10).

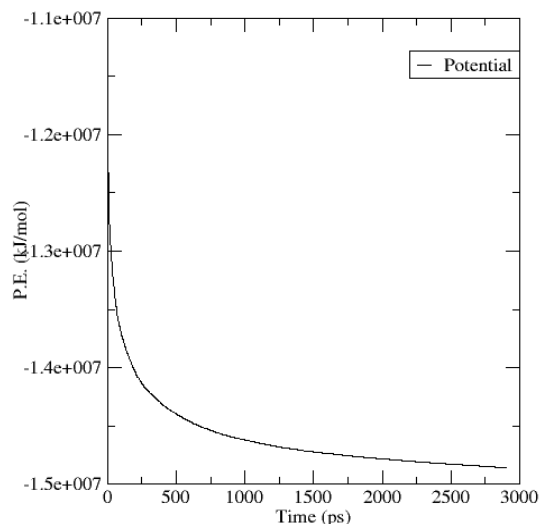


Figure 6. P.E. equilibration of system.

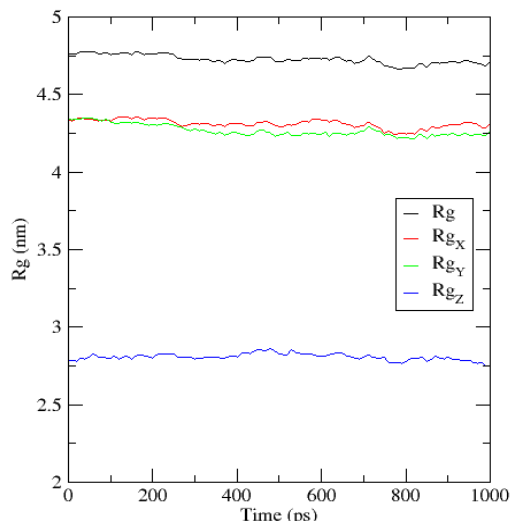


Figure 7. The radius of gyration of system.

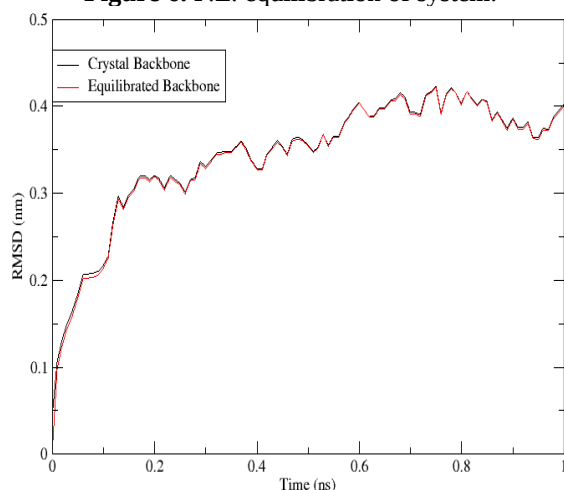


Figure 8. System RMSD.

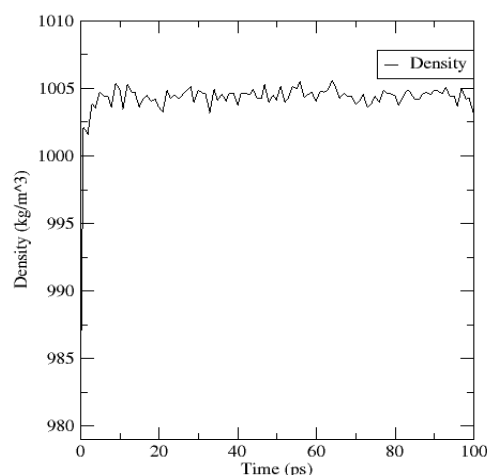


Figure 9. System density.

nCoV System RMSF

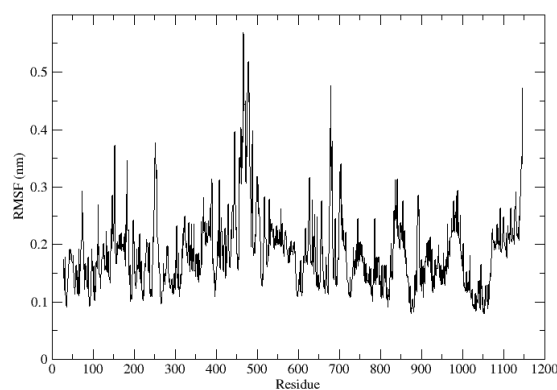


Figure 10. RMSF of System.

Figures 4-10. MD observations of nCoV system: (4) Temperature graph during NVT equilibration, (5) Pressure graph during NPT equilibration, (6) Potential Energy minimisation, (7) Radius of gyration of system whole () x coordinate (), y coordinate (), z coordinate (), (8) Crystal Backbone () and Equilibrated Backbone () system RMSD, (9) System density and (10) System RMSF.

4. Conclusions

The world faces a catastrophic health crisis, which needs an effective solution to combat COVID-19. Designing or finding the natural drugs or inhibitors for the disease is another challenging task due to the high mutation rate of SARS-CoV-2. In this study, we have tried to find out the best possible interactions of Chromone, a natural compound, with that of the spike protein, which reveals that our molecular docking analysis is stable and safe concerning Chromone. The Chromone acts as the potential inhibitor for SARS-CoV-2, as evident from the best scores of binding affinities to the mentioned target protein. Our study accentuated the chromone derivative, which displayed prominent antiviral activity against coronavirus. Knowledge gained from the study might stimulate rapid and practical clinical research on the probable Chromone derivatives repositioning against COVID-19.

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Conflict of Interest

There exists no conflict of interest amongst authors regarding the publication of this manuscript.

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