

Energetics Based Modeling of Hydroxychloroquine and Azithromycin Binding to the SARS-CoV-2 Spike (S)Protein - ACE2 Complex

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Abstract

The use of hydroxychloroquine to aid in the disruption of the SARS-CoV-2 virus and to cure or at least treat the COVID-19 disease is recently being reviewed in various clinical trials worldwide, but with insufficient examination of the binding of human ACE2 to the viral spike. In order to understand and assess the efficacy of the drug or drug combination, this paper looks at the effect of the pharmaceutical drug hydroxychloroquine, as well as a common co-drug, azithromycin, on the SARS-CoV-2 spike-ACE2 complex by using virtualized quantum mechanical modeling to better characterize binding sites on the complex, assess the binding between these sites and the drug compounds, and enhance community PDB files.

Introduction

The Severe Acute Respiratory Syndrome (SARS) Coronavirus 2 (SARS-CoV-2) that leads to the Coronavirus disease (COVID-19) is an extremely potent virus. It has been frequently demonstrated to attach to the Angiotensin Converting Enzyme II (ACE2) [9], which is critical to the control of blood flow and immune responses; ACE2 is present in endothelial cells, arterial smooth muscle cells, and many other organs, but most abundant in humans epithelia of the lung and small intestine, providing easier routes of entry for SARS-CoV [5]. Similar to SARS-CoV [6], the interaction between the SARS-CoV-2 and the ACE2 exists at the point where the viral spike binds to the edge of the ACE2 receptor; unlike the human-adapted Middle Eastern Respiratory Syndrome Coronavirus (MERS-CoV) [16] spike protein that binds to human dipeptidyl peptidase-4 (DPP4) but not ACE2 [13][24], SARS-CoV-2 does not also bind to DPP4 and seems to only bind to most other animal ACE2 except mouse ACE2 [26].

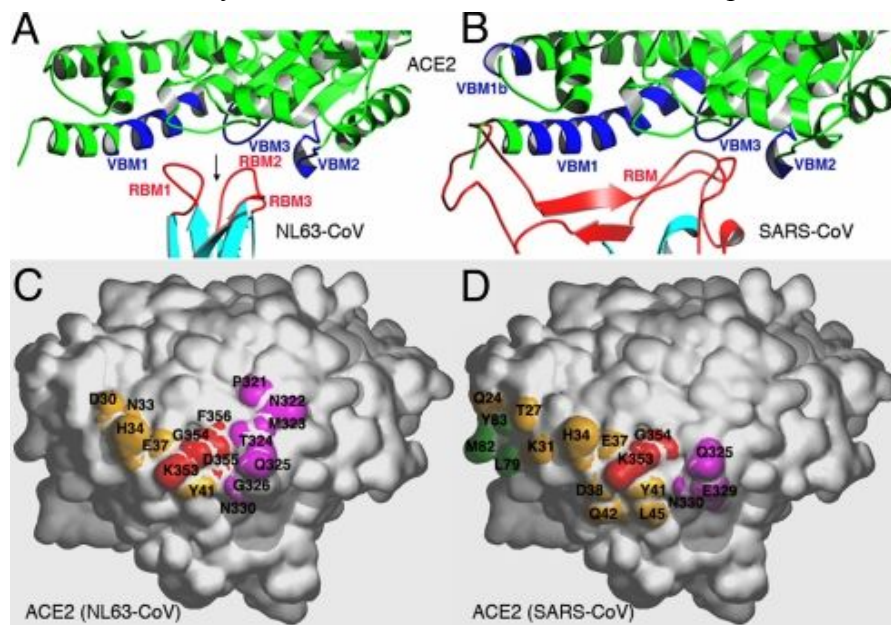


Figure 1: Binding sites on ACE2 of the NL63-CoV and the SARS-CoV, imaged by Wu et al. [11]

Based on several prior decades of clinical precedent in broad-spectrum antiviral treatment of malaria, HIV, and other viral infections through immunomodulation using chloroquine, hydroxychloroquine, and their various salts [15][19], several clinical trials are presently under development or running, such as trials of lopinavir/ritonavir or hydroxychloroquine [2]; however, twenty-three clinical trials as of March 10, 2020 from the Chinese Clinical Trial Register were found to be taking place in China to assess the use of chloroquine or hydroxychloroquine in the treatment of COVID-19 [4] but as of March 21 only nine clinical trials were *recruiting* or *not yet recruiting* for clinically testing either chloroquine or hydroxychloroquine internationally and just one at Shanghai Public Health Clinical Center Shanghai in China as recorded by ClinicalTrials.gov [3]. Other researchers have tried to understand the reasoning behind this immunomodulatory and antiviral effect in more depth, with Wang *et al.* establishing a link between the concentration of chloroquine, the resulting increase of the endosomal pH, and interference with the glycosylation of cellular receptors of SARS-CoV [22]. However, the actual binding of these drugs to domains of the SARS-CoV-2 spike protein - ACE2 complex to inhibit the spread of the virus has not been studied sufficiently.

In order to be able to understand the best proteins that could bind to these domains and help stop the spread of the virus, many researchers have begun to use molecular docking techniques to identify proteins that can be used to inhibit the SARS-CoV-2 spike and ACE2 binding and consequent formation of their complex [11]. One such study by Smith and Smith at the Oak Ridge National Laboratory found that the highest binding compound evaluated for potential use in future studies could be Pemirolast, an anti-allergy medication [18]. However, there has yet to be a docking study of hydroxychloroquine and its effect on SARS-CoV-2 spike - ACE2 domains.

Thus, we provide more insight into the study run by Gautret *et al.* [8] of the introduction of hydroxychloroquine and azithromycin, a common antibiotic, into the SARS-CoV-2 spike-ACE2 complex.

Methods

Binding analysis was completed by using Iff Technology's Polar+ tool [14], which was virtualized using Rigetti's Quantum Virtual Machine (QVM) running on the same laptop. Polar+ finds optimal binding sites through simulated quantum electrodynamics interactions. In order to use this tool, a PDB file for the SARS-CoV-2 spike-ACE2 complex was obtained from the Research Collaboratory for Structural Bioinformatics (RCSB) [21]. Running the PDB file through Polar+ created an electrostatics map of the protein using a quantum circuit as an analog, which was output as a list of vectors. In order to allow for analysis to be completed in other software suites for biological modeling, Polar+ also outputs the atoms that are related to the

best binding sites in the form of a PDB file. A PDB file of the atoms related to these binding sites can be found in this short-term repository on Github:

<https://github.com/IffTech/HydroxychloroquineAzithromycinPaperData>

Then, the PDB files for the ligands, in this case hydroxychloroquine and azithromycin, needed to be obtained for further docking analysis, and were obtained on the Canadian Institute of Health's DrugBank [1][10]. Lastly, in order to identify the binding conformations between the ligands and the SARS-CoV-2 spike-ACE2 complex, Autodock Vina [20], one of the most prominent pieces of software used for molecular docking calculations, was utilized on the same computer to calculate the binding affinities of each particular binding site through geometric conformation analysis and charge-to-charge interactions. In order to load the PDB files into Autodock, the files were changed to PDBQT format within the Python Molecule Viewer provided by Autodock Tools, which allows users to save PDB files as PDBQT files.

Results

First, the binding affinities were found using Autodock Vina, and are displayed in tables below. Then, the binding conformations were reviewed within the Python Molecule Viewer, with the top binding conformations for hydroxychloroquine and azithromycin on the SARS-CoV-2 spike-ACE2 complex and the binding sites of both ligands that are virtually modeled as present on the binding point between the SARS-CoV-2 spike and the ACE2 receptors displayed below.

mode	affinity (kcal/mol)	dist from best mode rmsd l.b.	rmsd u.b.
1	-3.7	0.000	0.000
2	-3.6	59.845	63.292
3	-3.6	59.967	63.600
4	-3.6	2.539	3.909
5	-3.5	18.805	22.778
6	-3.4	58.691	60.799
7	-3.4	19.024	23.322
8	-3.4	68.497	70.471
9	-3.4	16.764	21.188

Figure 2: Binding affinities of Hydroxychloroquine to the Spike-ACE2 Complex, with the top binding affinity being the most optimal binding conformation after energetics modeling with Polar+

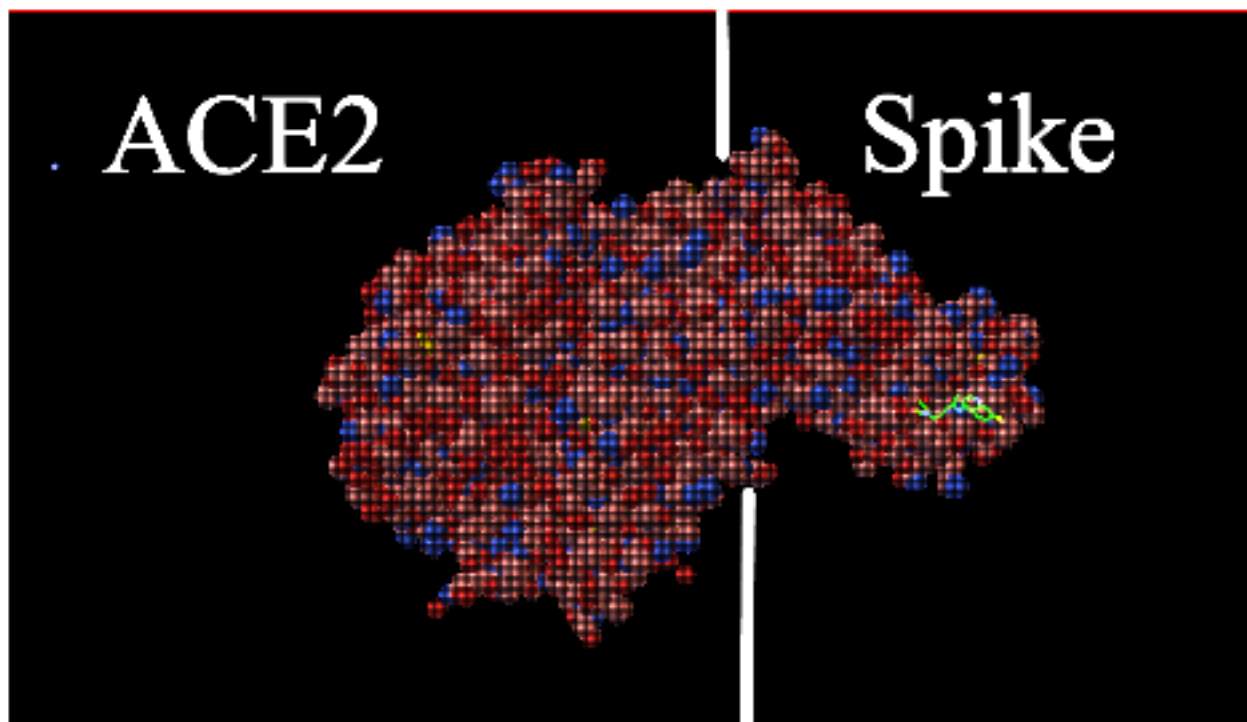


Figure 3: Highest conformation binding site of Hydroxychloroquine to the Spike-ACE2 complex

mode	affinity (kcal/mol)	dist from best mode	
		rmsd l.b.	rmsd u.b.
1	-5.2	0.000	0.000
2	-4.9	21.295	23.032
3	-4.9	54.091	55.797
4	-4.7	33.615	36.683
5	-4.4	16.242	22.056
6	-4.3	24.410	29.637
7	-4.3	16.301	19.531
8	-4.3	43.162	48.006
9	-4.3	23.376	28.815

Figure 4: Binding affinities of Azithromycin to the Spike-ACE2 Complex, with the top binding affinity being the most optimal binding conformation after energetics modeling with Polar+

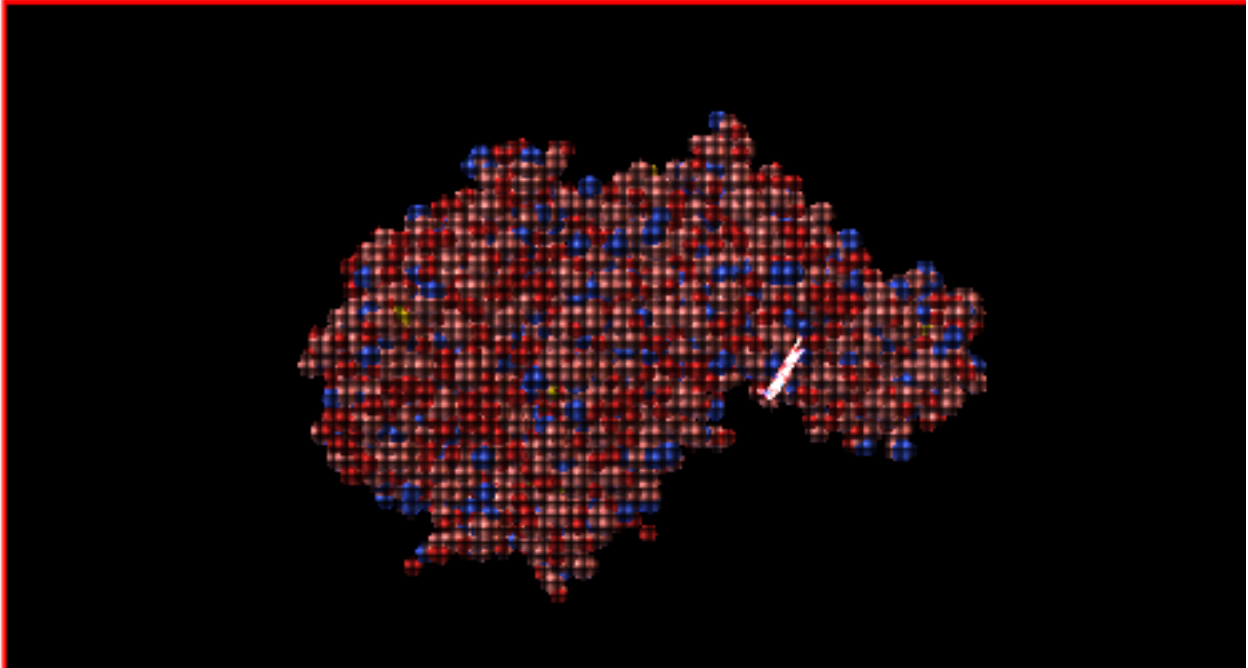


Figure 5: Highest conformation binding site of Azithromycin to the Spike-ACE2 complex

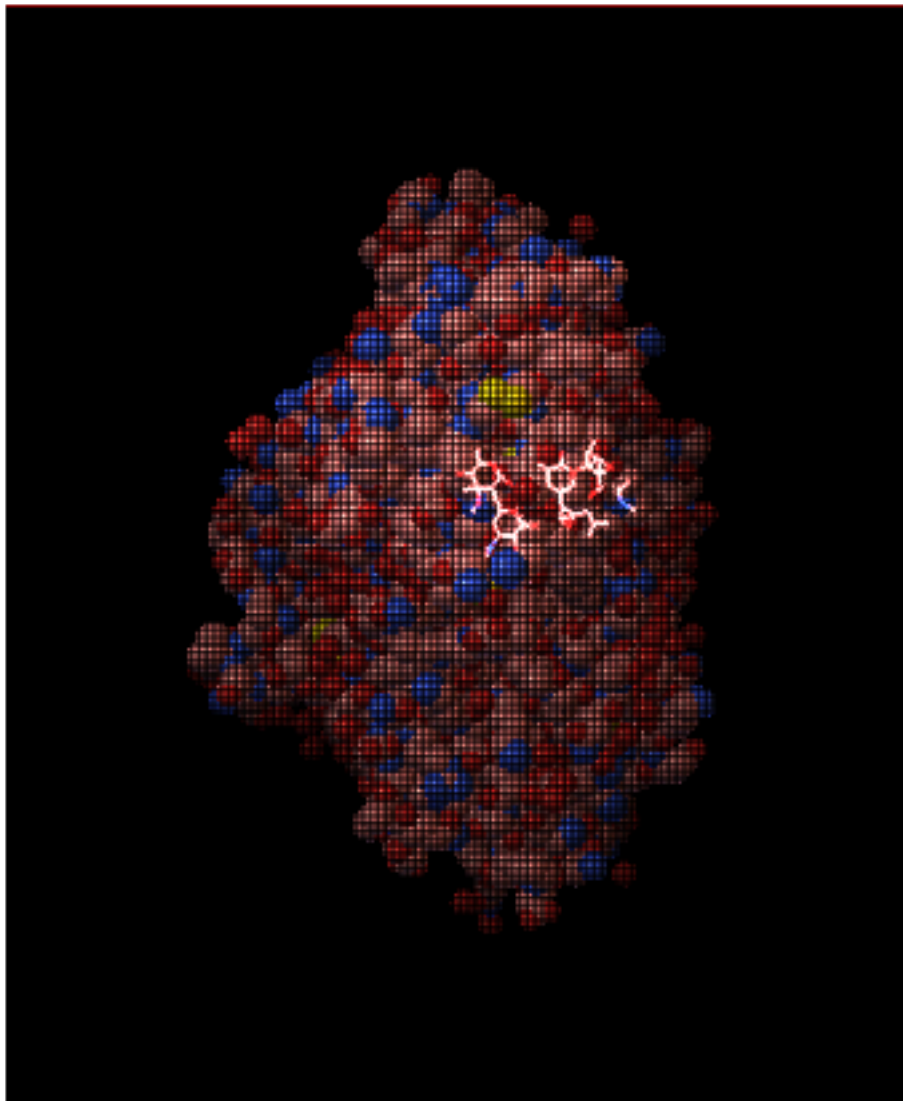


Figure 6: Side view of the highest conformation binding site between Azithromycin and Spike-ACE2

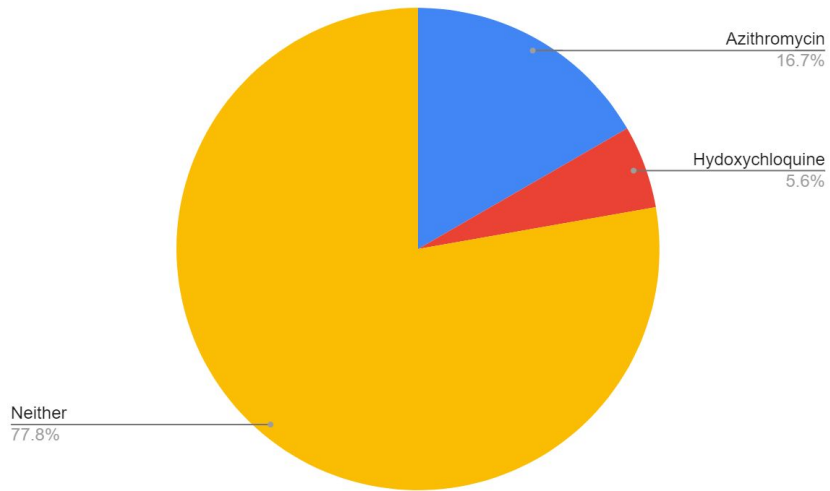


Figure 7: Binding conformations that are within the interaction region between the spike and ACE2

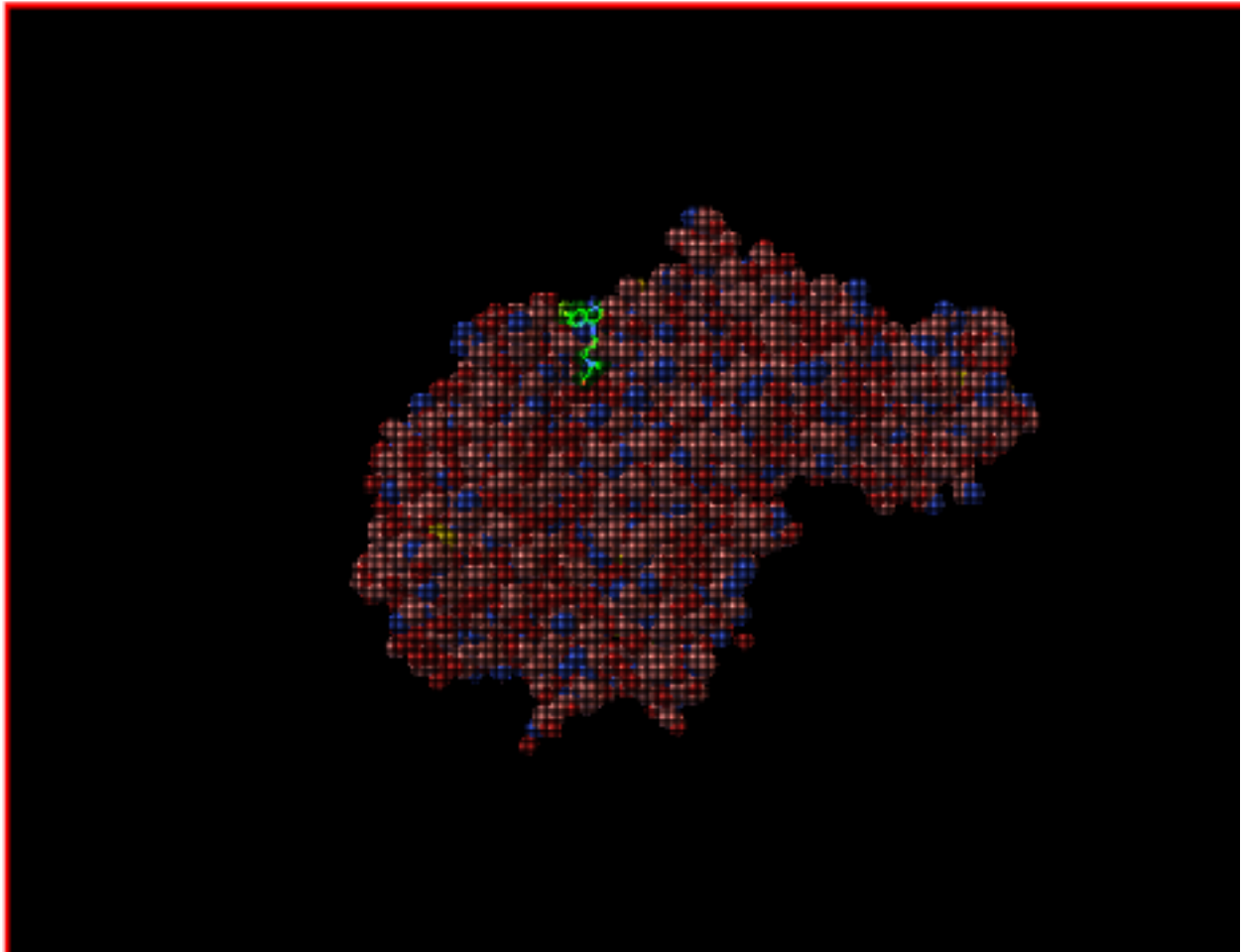


Figure 8: Binding conformation of hydroxychloroquine that touches the interaction points between the Spike and ACE2. This binding conformation is referenced as the 6th mode in Figure 1



Figure 9: Binding conformation of Azithromycin that touches the interaction points between the Spike and ACE2. This binding conformation is referenced as the 4th mode in Figure 4

Discussion

Based on the binding results provided above, hydroxychloroquine by itself appears to be ineffective in directly inhibiting the SARS-CoV-2 spike-ACE2 interaction. Instead, it seems to serve to increase the acidity of the ACE2 system in the interaction between the ACE2 and SARS-CoV-2 spike that could in many cases result in the degradation of the spike, and potentially the discontinuation of the virus' ability to spread further.

On the other hand, azithromycin provides high binding affinity when adjusted for energetics and has a much better ability at directly targeting the binding interaction point between the SARS-CoV-2 spike and ACE2. Much of this has to do with azithromycin's nearly symmetric design: this allows the small molecule to effectively handle binding with ligands between both the spike and the ACE2, which could possibly create an energetics barrier between the two proteins. Dinos *et al.* was one of the few groups that looked deeper into the pharmacokinetics of azithromycin, and found that the interaction between azithromycin and other small molecules leads to the competition for binding sites, which the azithromycin wins due to its ability to isomerize rapidly [7]. This, coupled with its ability to increase pH, allows for it to, as Gautret *et al.* had identified, decrease the viral effects of COVID-19 [8].

Next Steps

In order to understand the effects of azithromycin and hydroxychloroquine on the SARS-CoV-2, further pharmacokinetics analysis needs to be completed to characterize the effects of different dosages of each drug on the entire delivery pathway of the drug combination. This study assumes

that both the azithromycin and the hydroxychloroquine are in equal concentrations and are not acting on another when binding to the complex; further analysis adjusted for each drug's dosage would provide better understanding of the counterbalance that each provides. More specifically, an analysis of the entire onset of pharmacokinetics at every stage of the onset, as characterized by Siddiqi *et al.* [17], would provide greater insight into, more specifically, the impact of the small molecules at each stage of the process.

Moreover, there needs to be more docking analysis for ligands whose highest conformation binds exactly to the interaction point and has a majority conformation binding rate to the interaction domain. Doing so would provide invaluable insight into not only pharmaceuticals that could lead to SARS-CoV-2 suppression, but also into nutraceuticals and foods that could be extremely valuable in ensuring that those with mild symptoms are sufficiently enabled to prevent and/or inhibit viral distribution [25].

Lastly, there needs to be more focus on docking to the entire envelope, not just the interaction domain. The SARS-CoV-2 envelope should have immense importance in inflammation, especially inflammation related to death, as found for SARS-CoV by DeDiego *et al.* in 2014 [5]. With the envelope model for SARS-CoV-2 made available only on the afternoon of March 19 in low quality, Iff Technology has only completed a binding site analysis of the protein, with the top binding atoms available in PDB form temporarily here: <https://github.com/IffTech/CovidEnvelopeBinding>

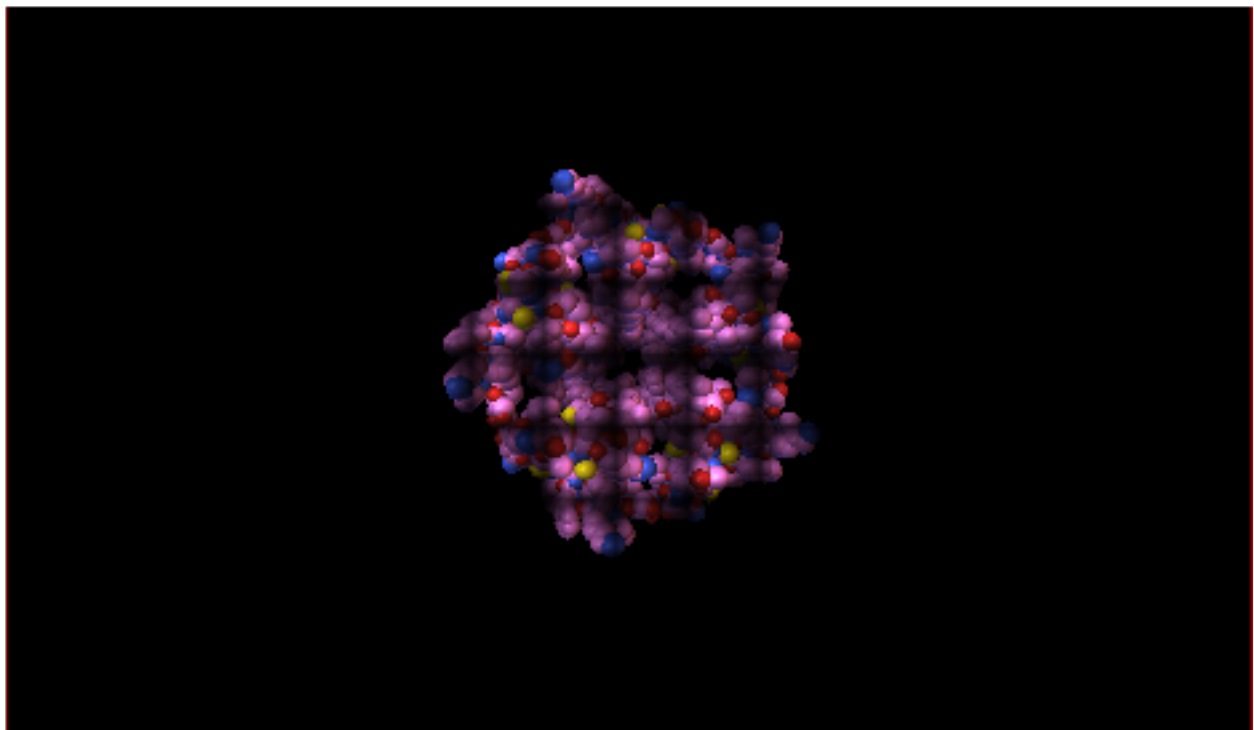


Figure 11: SARS-CoV-2 Envelope Protein Model provided by SwissModels as “Envelope small membrane protein (E protein) | YP_009724392.1 | P0DTC4”

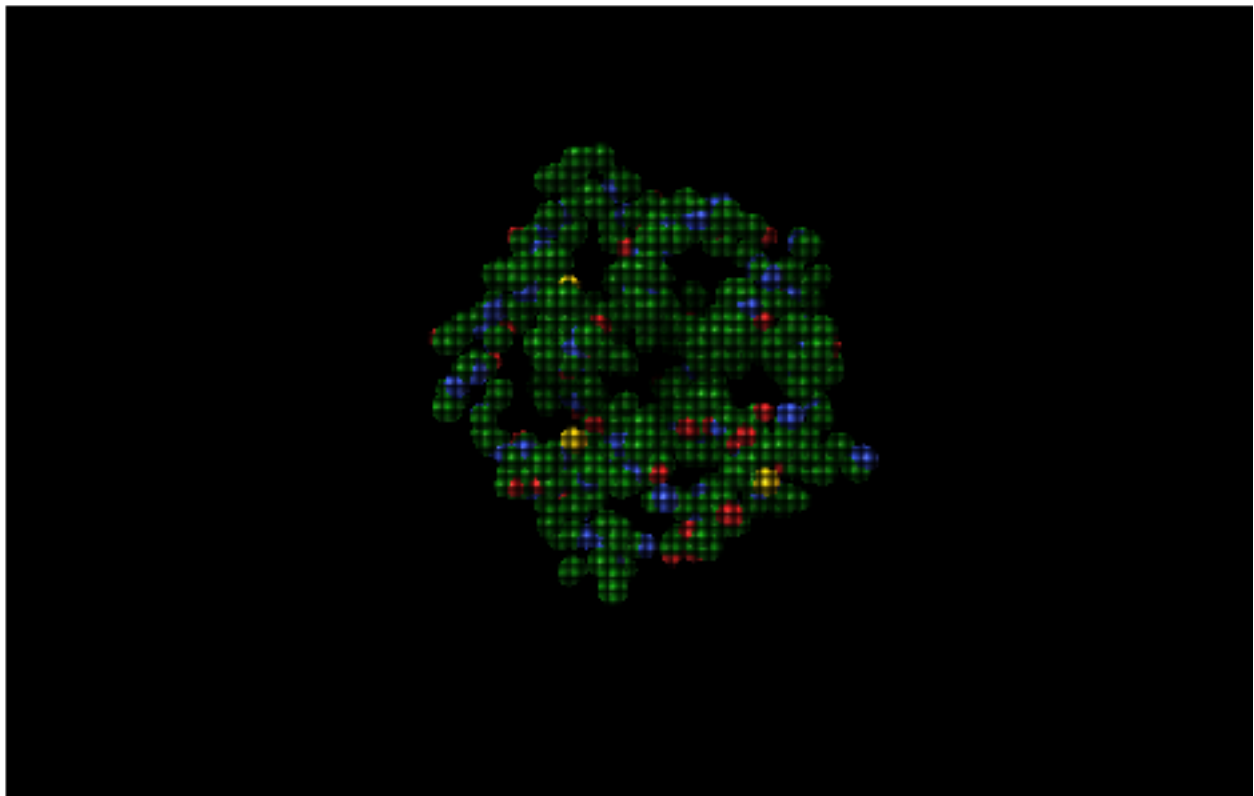


Figure 12: SARS-COV-2 Envelope Protein Model’s top binding atoms, identified using Polar+ , with PDB file available in the supporting materials

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