

Investigating the Inhibition of the Conformational Transition of SARS-CoV-2 Spike Protein Using Small Molecules

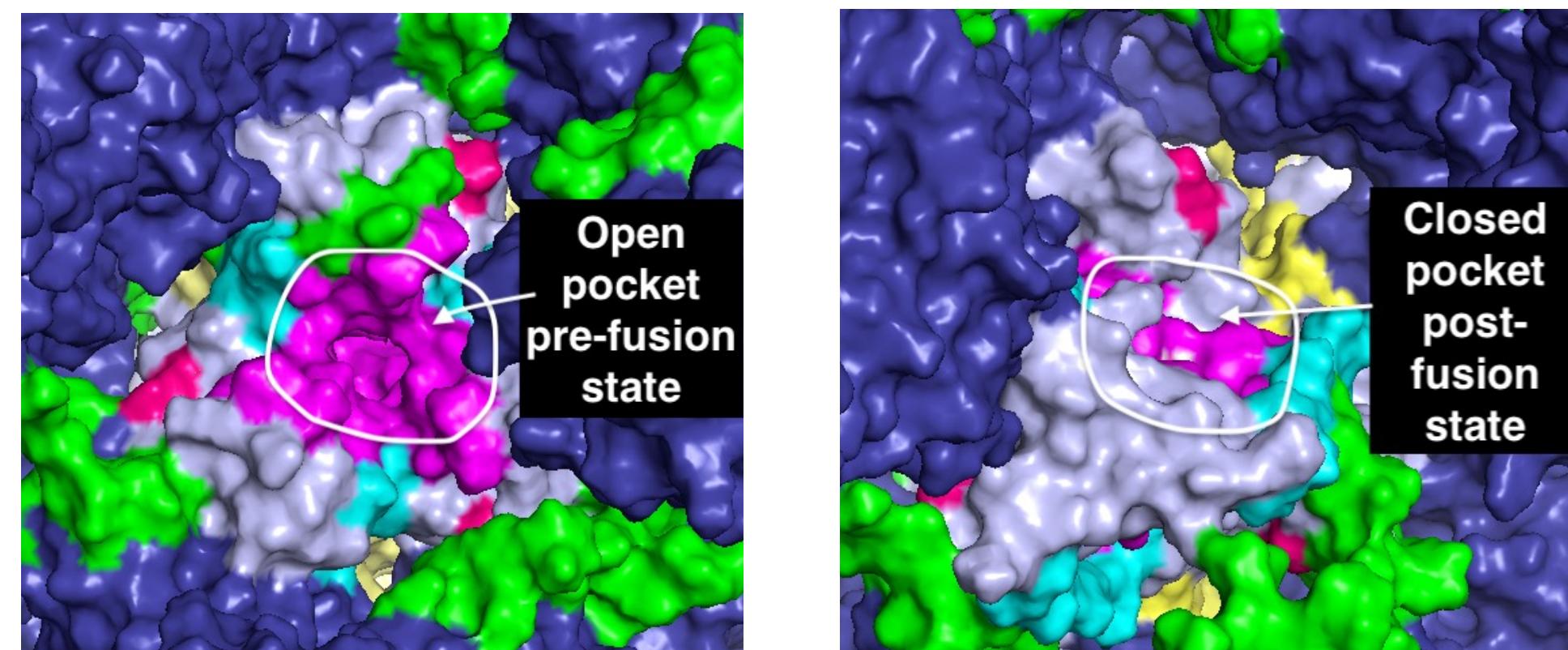


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Introduction

SARS-CoV-2 has infected over 184 million people worldwide as of July 5, 2021. To combat this virus, scientists have developed vaccines that target the spike protein of the virus. These vaccines have effectiveness rates above 90 percent and target the exterior of the spike protein. However, the sequence of the protein exterior is not highly conserved across all coronaviruses and given the rapid rate of mutation of SARS-CoV-2, antibodies cannot recognize the virus promptly. On the other hand, the sequence of the interior of the SARS-CoV-2 spike protein is highly conserved. Therefore, creating drugs to target the interior of the spike protein can be quite effective given the high rate of conservation across all coronaviruses. However, the pockets that are open in the pre-fusion state on the S2 subunit of the SARS-CoV-2 spike protein, which allows access to the protein's interior, become closed when the virus fuses to the host membranes. This decreases the possibility of targeting the interior of the spike protein using drugs. Understanding how we can prevent the spike protein's transition from the pre-fusion to the post-fusion state so that its interior is always open can help create effective drugs to target the interior of the virus.



Objective

To determine if small molecules can bind to the pockets of the S2 subunit of the spike protein that are open in the pre-fusion state and prevent them from closing in the post-fusion state, thus allowing for the creation of drugs that can target the interior of the spike protein.

Method

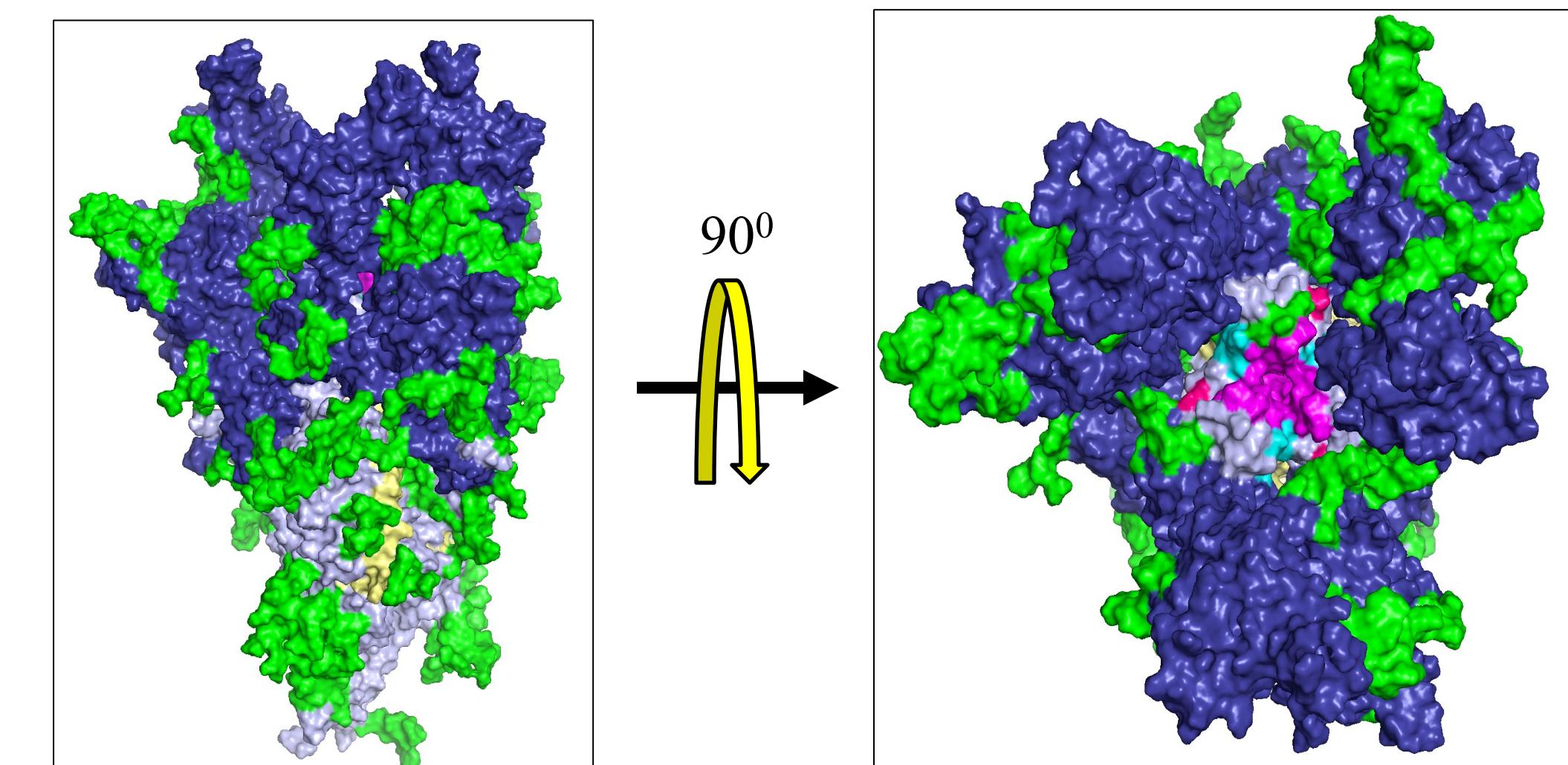
- Took pre-fusion open RBD MD trajectory files based on the 7CAK PDB structure and converted the last frame of the file to a PDB file.
- Loaded the pdb file in PyMOL and using past research identified the S1, S2, SRL, and other regions of importance within the spike protein
- Took trajectory file representing the intermediate state between the pre-fusion and post-fusion states and converted the last frame of the file to a PDB file

Method (continued)

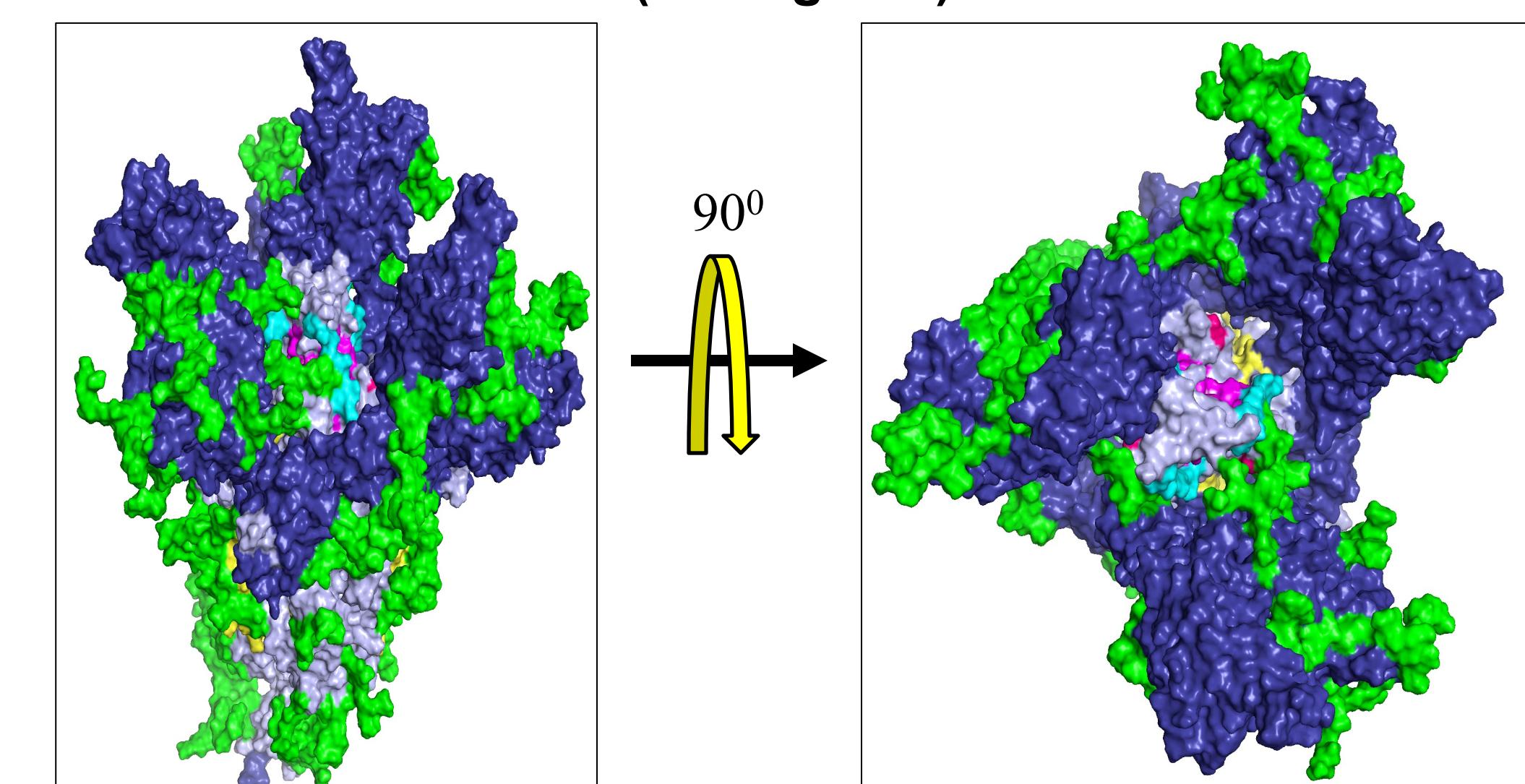
- Located the opening in the CH trimer region by comparing the two PDB files that closes as the protein transitions from the pre-fusion to post-fusion state.
- Discovered binding pockets using fpocket simulations on the CH trimer
- Confirmed and discovered more binding pockets on the CH trimer using DOCK software
- Edited the output of the DOCK simulation to narrow down to the pocket specifically on the CH trimer region that closes as the state changes.
- Used the output above and followed the steps to prepare for docking of small molecules using DOCK software
- Used the VS_5K.mol2 library of over 5000 compounds to perform virtual screening.
- Visualized the results to determine the best small molecules from the virtual screening.

Results and Discussion

Spike protein pre-fusion open state (s1 subunit in navy blue and s2 subunit in light blue), side view(left), top view(right). Opening seen in the CH Trimer area (in magenta) in top view

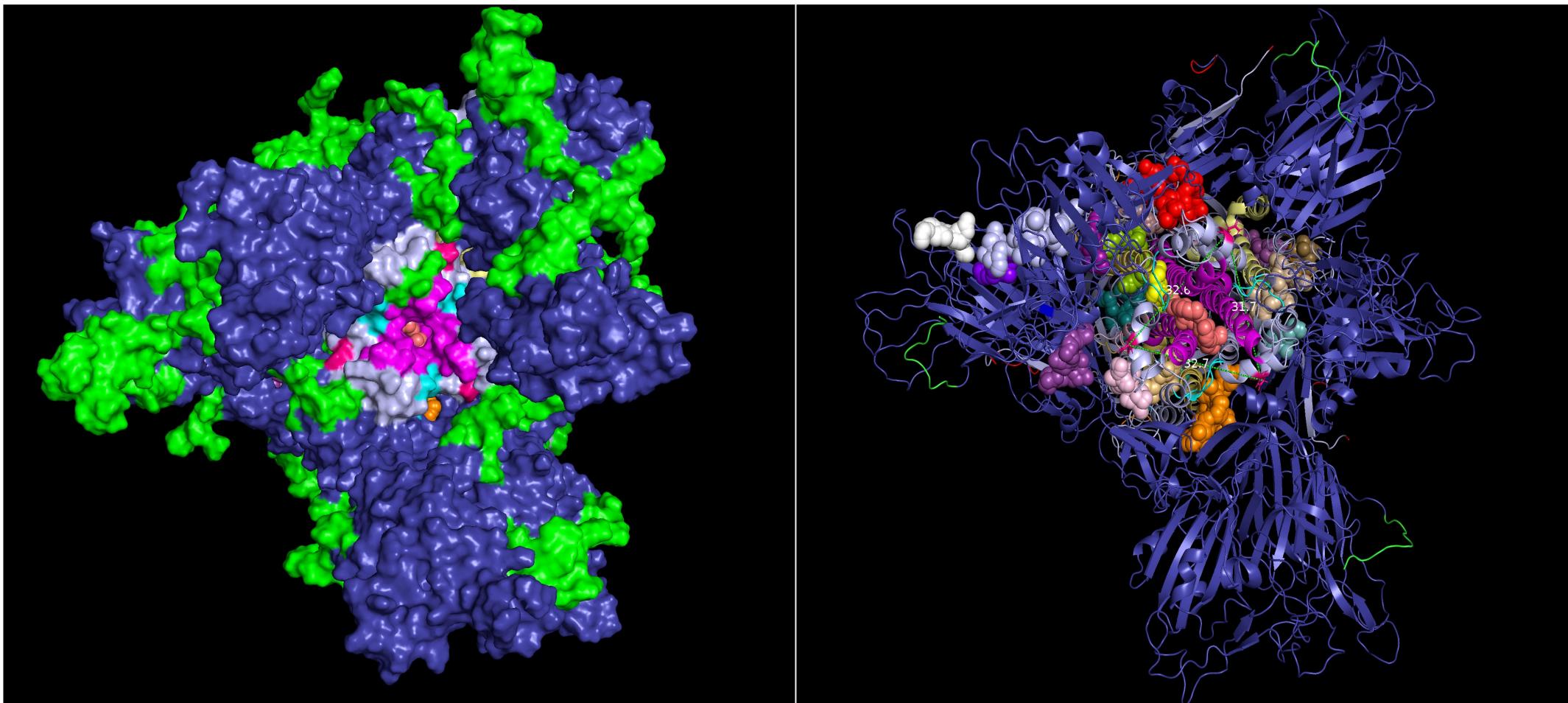


Spike protein intermediate state (between pre-fusion and post-fusion states), side view (left), top view(right). CH Trimer area (in magenta) is closed

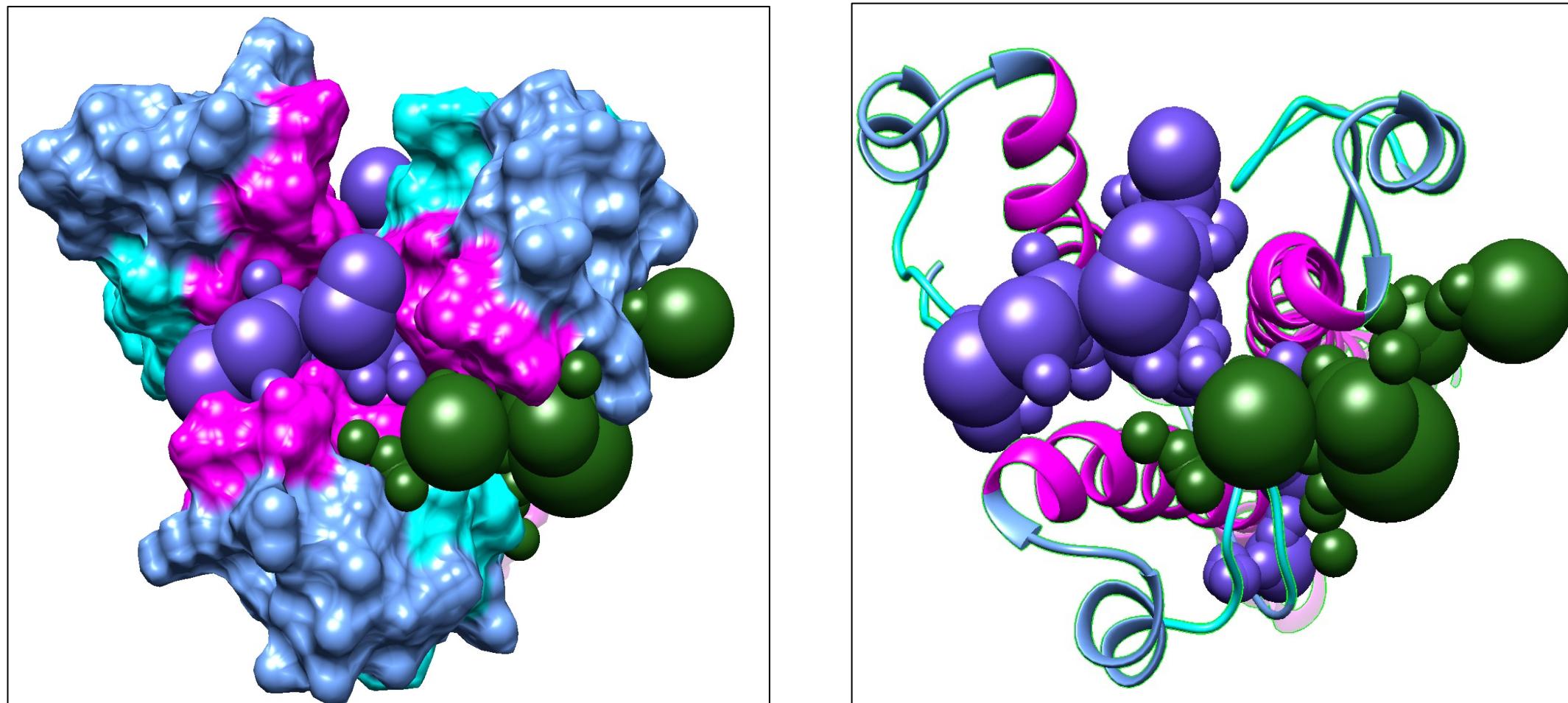


Results and Discussion

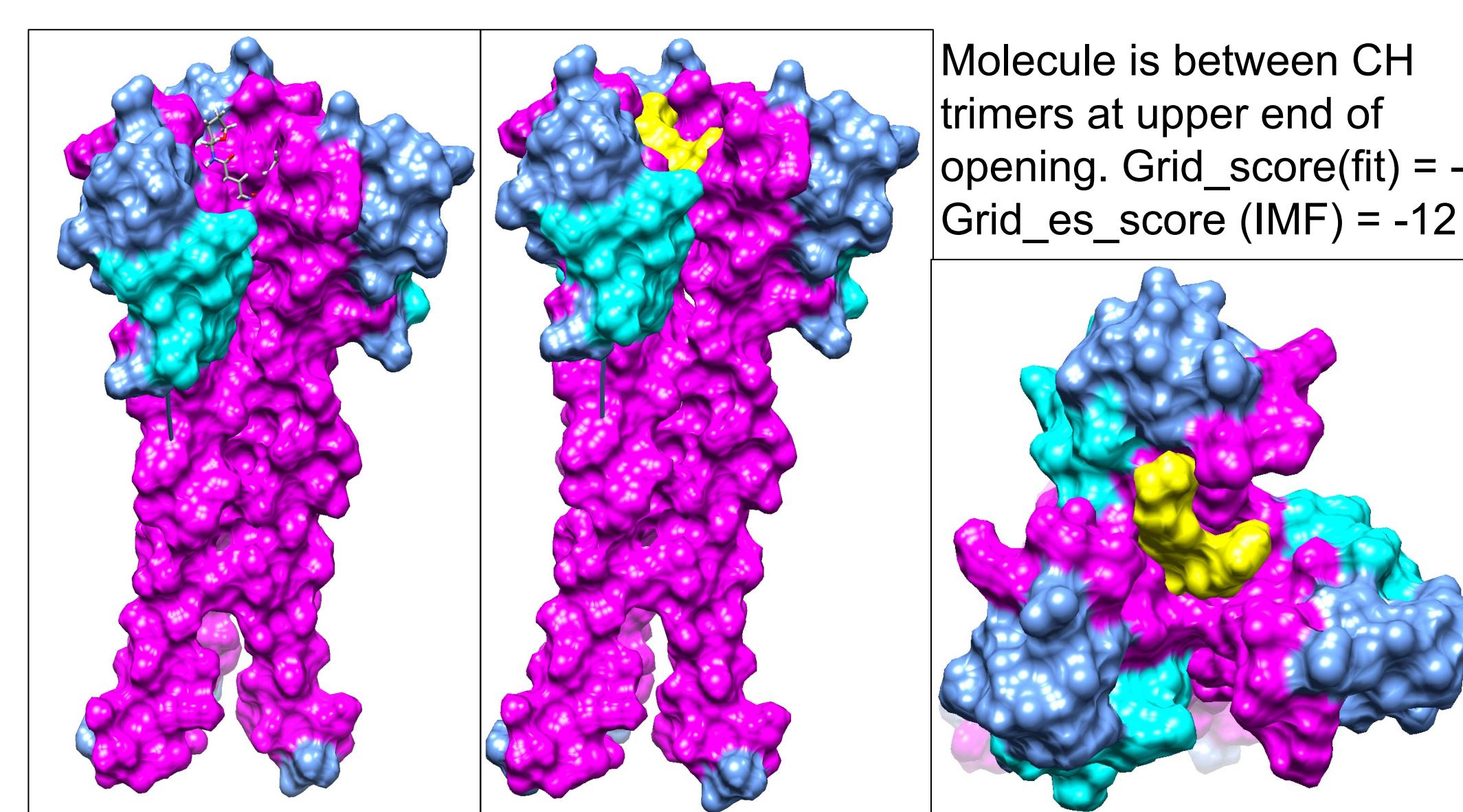
Fpocket simulations found pockets in the pre-fusion open state near the CH Trimer area opening but it was not close to the top of the opening (pocket shown as orange spheres)



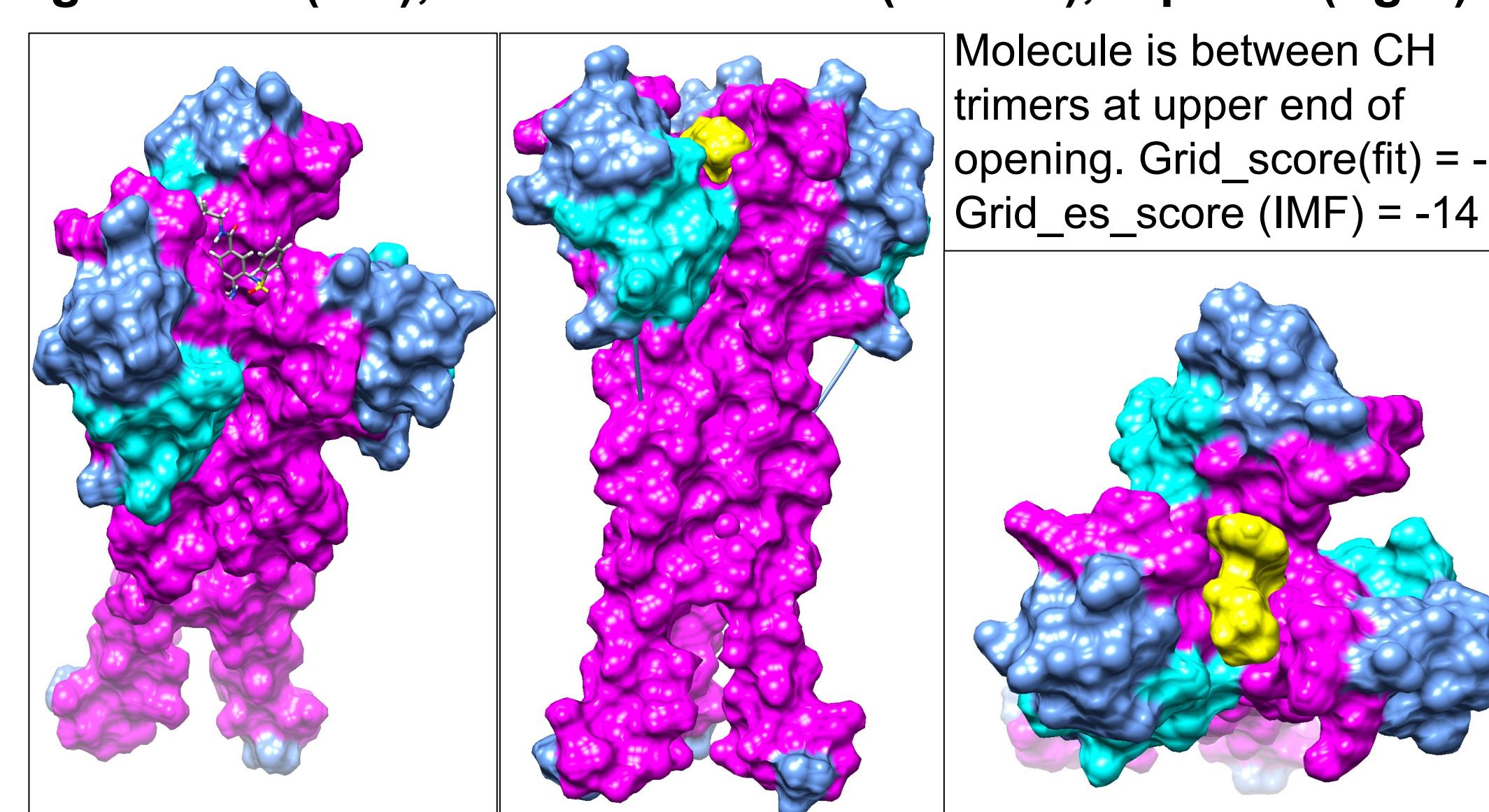
DOCK simulations found pockets in the pre-fusion open state near the CH Trimer area opening and it was close to the top of the opening (pocket shown as purple spheres)



Docked small molecule ZN33033712 (yellow surface) ligand view(left), surface side view(middle), top view(right)



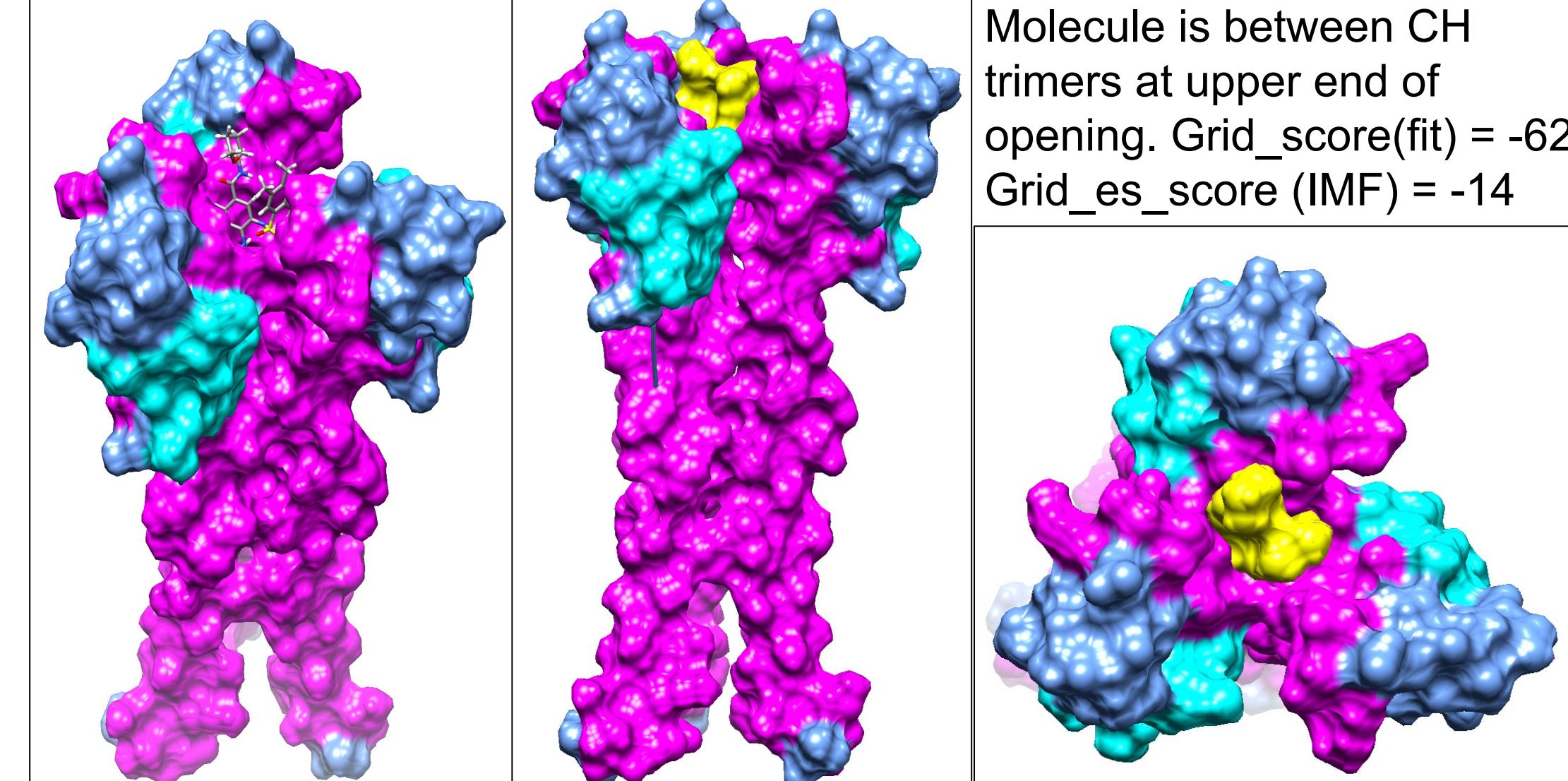
Docked small molecule ZN33033707 (yellow surface) ligand view(left), surface side view(middle), top view(right)



Results and Discussion

Docked small molecule ZN33033722 (yellow surface) ligand view(left), surface side view(middle), top view(right)

Molecule is between CH trimers at upper end of opening. Grid_score(fit) = -62 Grid_es_score (IMF) = -14



Docked molecules were shortlisted based on the location, how well the ligand fit in the pocket, and the electrostatic forces of attraction between the pocket and the ligand.

Conclusions/Future Directions

By looking at the surfaces of the pre-fusion state and the intermediate states we were able to confirm the results of a previous study that the opening at the top of the CH trimer area in the intermediate state is buried and therefore inaccessible from the surface of the spike protein. We were able to find pockets lying in the CH trimer region in the pre-fusion state through fpocket simulations and DOCK simulations. With DOCK simulations, we were able to find two better pockets very close to the open area at the top of the CH trimer region whereas fpocket did not yield this pocket. Then, by running DOCK simulations using the VS_5K.mol2 library, we were able to find 3 small zinc compounds that docked to the top of the CH trimer region. These bindings can prevent the transition from the pre-fusion to the post-fusion states in the S2 subunit of the spike protein and keep the interior of the spike in an open state which would allow drugs to effectively target the interior of SARS-CoV-2.

Our docking simulations were able to only filter ~1000 small molecules from the VS_5K.mol2 library due to time limitations. In the future, all molecules in the library could be analyzed. Also, Molecular Dynamics simulations could be performed on the docked complex to verify its stability.

References and Acknowledgements

- (1) Wang, Yuzhang, et al. "Receptor Binding May Directly Activate the Fusion Machinery in Coronavirus Spike Glycoproteins." *BioRxiv*, Cold Spring Harbor Laboratory, 1 Jan. 2021, doi.org/10.1101/2021.05.10.443496.

Many Thanks to Dr. Simmerling for his mentorship. Special thanks to Lucy Fallon and Christopher Corbo for help with the trajectory files and the software.