



## **Ozone could be an effective air sanitizer to inactivate Corona virus? - *in silico* docking**

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### **ABSTRACT**

*An indispensable screening for active and non-residual forming virucidal compound destroy the virus in atmosphere by interacting with droplet nuclei is urgent need against COVID19. The paper aimed to predict the interaction of ozone (O<sub>3</sub>) with Cov19 major protein in order to find the efficacy of gas as environmental sanitizing agent to replace chlorine residues. The *in silico* docking with Auto dock was performed and the results reveals that the molecule ozone is an effect interacting agent with viral protein since its is simplest diffusible gas. The results obtained from the *in silico* analysis confirms the strong interactions of ozone against COVID-19 main protease and spike chains were predicted. The docking of ligand with spike protein 2GHV, 6XE1 and 2LU7 protease showed minimum of -12 and maximum of -20 scoring with active hydrogen bond at different torsion angle. The atom have high flexibility and capable to form maximum hydrogen bond. Both spike and protease receptors destabilized by interaction of ozone. The docking scores and experimental data evidenced that the application of ozone have lethal effect towards virus which helps to kill the covid19 in the droplet nuclei itself. Further *in situ* studies are needed to evaluate the ozone therapy of clinical application. Ozone treated water may found to be potent antiviral property because of generation of short live OH radicals. The advantage of application of ozone is simplest, naturally formed gas have target multiple binding domain on spike glycoprotein.*

Keywords: COVID19, Ozone, droplet nuclei, multipurpose, spikes, gliding score

Received 21.06.2021

Revised 19.07.2021

Accepted 12.08.2021

### **INTRODUCTION**

The 2019-nCoV, 32 kb in genome virus, causative agent of severe acute respiratory syndrome coronavirus emerged from Wuhan became pandemic outbreak and thus reflected disastrous effect over more than 105 countries around the world[1-2]. Past Epidemiological data shows that most severe respiratory infections are caused by droplet or microbial aerosol transmission[3]. The uncontrollable spread of respiratory diseases has been core of COVID infection mediated by its stabilized spikes [4]. It is believed that droplet transmission is the principal source of infection of COVID19. Studies on spike proteins stated that interaction with ACE2 [5] to make an entry in the host cell [6]. The spike proteins, and precisely the S B domain, focused as a hot spot for drug screening. Pathogens attach to the droplets and transmit to environment as airborne droplets during breathing, speaking, coughing and sneezing. Studying the droplet transmission may be a common matter but most important to control spreading of infection. Among different size respiratory droplets, those of the large size deposit to the ground in 1 meter or vaporize into droplet nuclei, the other of the relatively small suspend in the air for long time. These smaller droplets may carry more pathogens[7]. high-speed exhaling airflow skims over the mucus on respiratory tract and broken away from mucosal cilia surface, and then form a series of different size of droplets which are evaporated, diffused, deposited or susceptible to individuals. According to droplets of air quality standards formulated by the U.S droplet greater than 10µm almost completely deposit in the nasopharynx, about 10% in the range of 2 to 5µm droplets deposit in the bronchial parts, size[8]. Disinfection and sanitation processes much needed for commonplace and high dense population area. Studies focus on *in vitro* antiviral activity of broad-spectrum antiviral prodrugs and development of Vaccines instead of controlling measures [9]. Therefore we aimed to find out effective agent and selected ozone for inactivate virus environmentally. Ozone naturally occurs at less than 20 µg/m<sup>3</sup> from the earth's surface and capable to disrupts the integrity pathogens through oxidation of the phospholipids and lipoproteins[10]. Tough the contact time is higher than UV, no chemical residue remains after treatment as ozone naturally decomposes to oxygen. Research has shown that ozone are capable of deactivating

enveloped viruses by reacting with plasma membrane fatty acid and its surface proteins. Compare to redox potential of commonly using, residues generating oxidizing agent such as sodium hypochlorite (1.36), ozone have 2.3 redox potential and non residual agent. Ozone will disintegrate as short-living OH-radicals to exhibit stronger oxidation mechanism also used in degradation of antibiotic [11]. Therefore we suggest using the ozone as air disinfectant wherever people work and dense crowded area. Also its need to test drinking of ozonised water may reduce the viral infection also it act as immune stimulant. With this perception this *in silico* study is evaluated to find out the effect of ozone with Spike proteins.

## MATERIAL AND METHODS

### Receptor selection and preparation

All the docking experiments were performed by using SWISS DOCK and chimera UCSF 1.4 [12]. The receptor model of the COVID-19 main protease was downloaded from the Protein Data Bank (www.rcsb.org). The crystal structure of the COVID-19 main protease complex with N3 inhibitor (PDB ID: 6LU7, chain A) and two spike proteins such as 2GHV –chain E and 6XE1 Chain L were selected as target. All the three protein structures were fetched from protein data bank and the ligands were removed by dockprep using UCSF chimera 1.4.

### Multiple Sequence Alignment

Above three protein chain amino acids are retrieved and prepared in FASTA format. Global protein sequence end to end alignment of the full-length corona virus spike proteins and main protease was performed by Clustal omega at EMBL. Percentage of similarity and guide tree is predicted.

### Ligand preparation

SMILES are retrieved from pubchem and structure was build using UCSF Chimera 1.4 and all parameters are used to minimize structure. Charges and hydrogen's are added and saved as mol file.

### Molecular docking

In order to differentiate highly active atoms interact with ligands to form from weak or pseudo bonds, multiple docked poses is tried. Swisdock software was utilized in all the docking experiments, with the optimized model as the docking target. UCSF chimera 1.4 was used to predict hydrogen bond and energy calculation.

## RESULTS AND DISCUSSION

### Multiple Sequence Alignment

Sequence of 2GHV, 6XE1 and 6XLU7 were retrieved after processing of specific chains and pairwise alignment performed using CLUSTAL Omega. 2GHV assigned as Cov1, 6XE1 as Cov2 and 6LU7 as cov3. These protein are strong vital target for inactivate viral agent. We have generated multiple sequence alignments and phylogenetic trees for representative spike proteins in order to analyze the specificity relatedness causing infection in humans. Our results show in figure 1a indicates that main protease 6LU7 with 306 amino acid 23% identity to 2GHV and 6XE1 spike sequence. Sequence of 2GHV and 6XE1 showed 71% sequence identity each other. The pair wise sequence search result given in figure 1b. Figure 2 reveals the phylogram of guide tree is calculated based on the distance matrix that is generated from the pair wise score 0.15 for spike proteins and 0.4 for main protease. Homology modeling and sequence alignment are key tool for confirming a 3D structure of any proteins. Qamar *et al* [13] reported 100% similarity 3CL<sup>pro</sup> Multiple sequence alignment results of SAARSCoV 2.

### Docking of COVID with Ozone

In this study we applied Swiss dock and AutoDockvina for molecular docking to identify multipose binding in three already demonstrated receptors to exhibit different binding modes of ozone in their respective predicted protein structures. Results of 2GHV scoring was given in table 1. Nearly 37 poses were predicted and of which six pockets found to be form maximum of 4 to 5-H bonds. the interaction analysis of docking results between ozone and binding sites of 2GHV are given in Fig. 2a. Ozone found to interact at 37 regions (V0 to V37) and formed active at hydrophobic region and pseudo hydrogen bonds and ionic interaction with hydrophilic surfaces. Region V0, V15, V23, V25, V32 and V36 are found to produce maximum 4 to 5 hydrogen bonding. Of which V15 presence of 4 different amino acid such as PRO459, SER461, GLY464 and LYS465. Similarly V32 contain CYS323 and GLY326 showed 4 hydrogen bonding formation with ozone. V1, V7, V18, V21, V22, V27, V29, V30, V33 and V35 are found to be formation of pseudohydrogen bond and stearic interaction. GLY, TYR, ASN, GLU are most frequently interacted amino acids in this study. The following residues such as GLN, MET, PRO are take part only once in entire interaction. The shortest hydrogen bond distance is 1.714/1.715 Å° respectively by ASN409/LEU412. The S protein plays potential role in viral entry inside the host [14] is found to interact with ozone and many residues are took part in interaction for formation of hydrogen bond. Mothay and

Ramesh [15] used AUTODOCK tools and found drug interacting residues are gln, ala, arg, thr and his with 2.5-3 Å hydrogen bonding.

Table 1. Interaction of ozone with 2GHV spike protein

POSE	ENERGY SCORE kcal/mol	NUMBER OF H2 BOND	AMINOACID	DISTANCE Å°
V0	-20.1479	2	GLU 502	1.923/1.833
	16.4787	4		2.499/2.782
V1	-15.7731	3	PHE 501	2.156/ 2.146
V2	-17.8853	3	ALA398/LEU412 TYR410	1.849/2.068 2.020
V3	-14.027	2	GLY368	1.803
V4	-17.902	3	VAL397/ILE397	2.196/1.911
V5	-16.4955	3	CYS 323/GLY326 ASP351	1.949/2.550 1.984
V6	-15.2374	2	GLU502	1.905/1.958
V7	-16.7405	2	ASP415	2.027
V8	-17.066	3	GLU502	1.799
V9	-14.9698	3	ASN381/GLU502	2.477 2.012/2.221
V10	-16.2896	2	MET417	1.960/2.325
V12	-14.9531	2	ILE428	2.024
V13	-15.2773	1/1	VAL458/ LEU443	2.375/2.099
V14	-17.2164	2	ASN381	1.887/2.335
V15	-15.2646	4	PRO459/SER461 GLY464/LYS465	2.20/2.120 2.027/1.918
V16	-16.583	2	GLY482/TYR491	1.990/2.158
V19	-14.9165	2	GLU327	2.707
V20	-14.9419	3	TYR352/SER353/LEU355	1.969/2.358/2.163
V23	-14.5191	4	PHE334/ASN437	2.513/1.979
V24/V26	-14.9679	1	PHE329 /ASN424	1.904/1.910
V25	-14.3658	5	TYR338/ASN409 ASP454	2.938/1.714 1.810/1.932
V28	-13.959	3	GLY482/TYR484/TYR491	1.843/2.220/2.642
V31	-14.4925	2	TYR352/TYR356	2.069/2.015
V32	-15.4484	4	CYS323 GLY326	2.344/2.081 2.468/2.278
V34	-13.9243	2	HSD445	1.949/2.269
V36	-9.75748	5	LEU412 GLN396 ALA398	1.715/2.010 2.436 2.046
V37	-10.9441	1	GLY368	1.877

Table 2. Interaction of Ozone with 6XLU7 Spike Protein

POSE	ENERGY SCORE	NUMBER OF H <sub>2</sub> BOND	AMINOACID	DISTANCE Å°
V0	-19.6823	4	LEU4	1.881/1.943
			GLY143/LEU141	2.273/2.022
V2/V3	-17/-18	2	VAL202 /LEU141	2.367/2.022
V4/V6	-15.59/16.62	2	PRO108/ THR111	2.146/2.397
V5	-17.986	3	THR 304/PHE 305	1.935/1.954
			GLN 256	2.003
	-16.1913		THR257	2.414
V7	-16.2414	3	CYS22	1.875/2.362
	-15.5938	2	VAL42/THR24	2.877/2.479
	-14.22	2	ILE43/CYS44	2.519/2.140
V10	-15.0449	2	LEU4/GLN189	1.869/2.057
V11	-17.3299	2	MET6	1.960/2.409
	-12.8458	2	MET6/ARG4	1.826/3.003
V13	-14.9619	2	ASP153	1.880
V14	-14.5673	2	GLU55	2.078
V15	-14.9658	4	PHE219	2.035/2.303
			LEU220	2.693
V16	-13.5988	2	LEU287	2.007
V17	-16.4662	2	GLY15/MET17	1.931/1.947
V18	-17.3919	2	LEU4/PHE140	1.886/2.764

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V19	-14.5482	3	GLY2/GLN299/SER1	1.976/1.881/2.131
V21	-13.1125	1	MET17	1.933
V22	-15.3983	2	ASP289	2.265
	-14.6868	2	LEU287	2.090
V23	-14.6574	1	GLY275	1.997
V24	-15.31	1	ILE249/PRO293	1.845/1.985
V25	-16.1555	1	LEU4	1.948
	-15.8616	2	THR26/GLY43	2.360/2.513
	-12.8831	1	HSD41	2.204
V26	-14.0631	1	TYR239	1.893
V28	-14.2277	2	ASN133/GLY195	1.887/2.130
V29	-14.5541	2	LYS100	2.272
V30	-16.7849	3	GLY71/GLY120/ASN119	2.378/2.055/2.343
	-16.5724		ASN19/GLY20/	2.121/2.065/
	-15.4485		GLY71/ASN119	2.414/1.991
V31	-15.311	2	GLY15	1.993
V33	-14.9798	3	MET17/GLY120	2.137/2.675/2.008
			GLY143	2.215
	-13.751	2	THR26/ASN119	2.784/2.573
V34	-13.9883	1	LEU4	1.833
V35	-14.755	1	PRO108	1.915
V36	-13.7967	1	TRP218	2.102

Table 3. Interaction of Selected Ligand with 6XE1 Spike Protein

POSE	ENERGY SCORE	NUMBER OF H2 BOND	AMINOACID	DISTANCE
V0	-18.8301	5	GLN409/ALA411/LEU425/TYR423	1.987/1.732/2.295/2.011
		4	ALA411/LEU425	1.786/2.247/2.470
V1			STEARIC INTERACTION	
V2	-16.6151	3	GLY496/TYR505	2.068/2.474/2.098
V3	-16.051	3	VAL407/ILE410	2.096/1.868
V4	-16.8238	1	GLU471	1.928
	-15.7154	2	GLU471	2.037/2.671
V5	-16.0744	1	ILE472	2.216
V6	-16.7469	2	PRO330/GLY526	1.898/2.371
V7	-15.9272	1	LEU441	1.937
	-15.4825	1	THR345	2.104
V8			STEARIC INTERACTION	
V9	-15.8871	1	ASP428	1.931
V10			IONIC	
V11	-15.0683	2	ASN460	1.900
V12	-16.117	3	ILE472/CYS480	2.047/2.218
V13	-15.8613	3	TYR369/PHE374/	2.077/2.375
	-14.7529	3	PHE374/ TYR369	2.333/2.383/2.411
V14			STEARIC INTERACTION	
V15	-14.7457	2	CYS336/ASP364	1.906/2.489
V16	-15.3993	2	THR430	2.349/1.994
	-14.9164	1	PHE515	2.048
V17	-14.6955	1	PHE377/CYS379	1.974/2.330
V19	-14.8122	1	PHE342/ALA344	1.969/2.497
V20	-15.2486	1	ALA397	2.116
V21	-13.8758	4	TYR421/ASN422/ASP467	1.754/1.722/1.858
	-13.5639	4	TYR351/ TYR421/ ASP467/ASP422	1.920/1.802/2.253/1.748
V23	-14.465	3	VAL341/ARG346	2.423/2.041
V25	-13.8121	2	ASP427	2.419
V26	-15.3653	3	PHE490	2.233
V27	-13.2741	1	LEU455	1.873
V28	-13.5209	1	PHE374	1.916
V29	-14.2383	2	THR345/LEU441	1.978/1.979

Figure 1. multiple sequence pair wise alignment

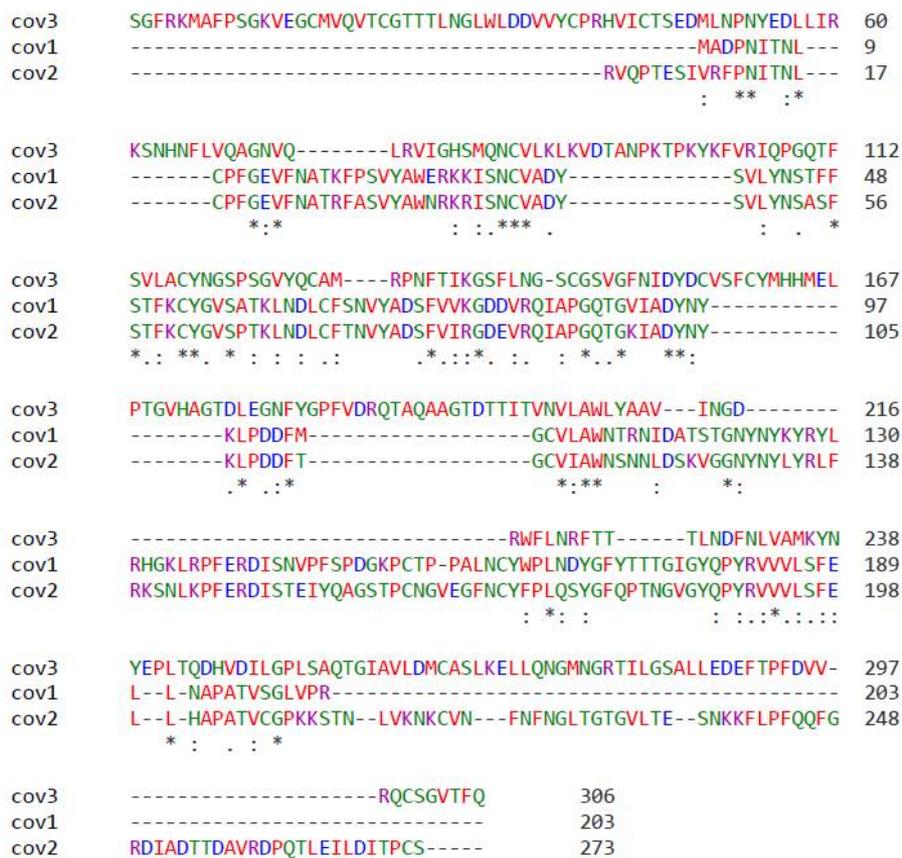


Figure 2. PHYLOGRAM of distance matrix

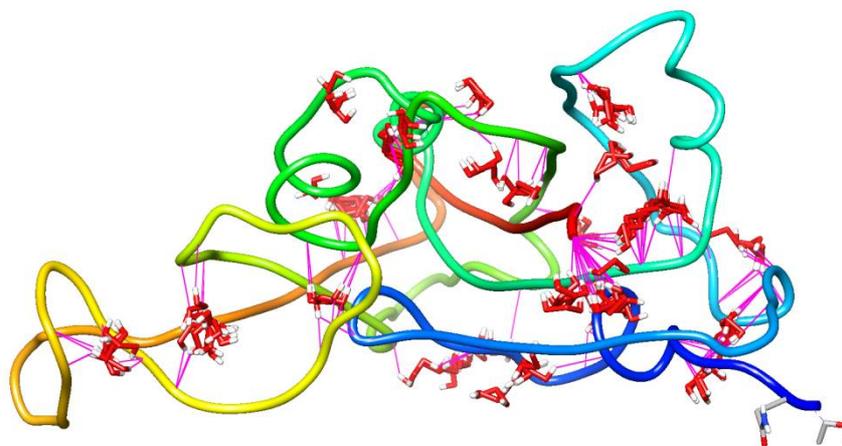
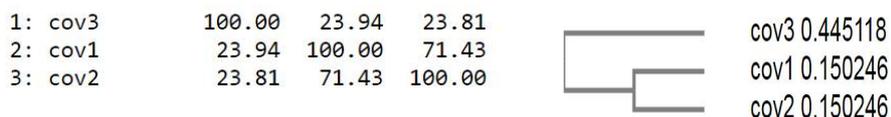


Figure 3 a. Interaction of Ozone atom with 2GHV E Chain of SPIKE PROTEIN

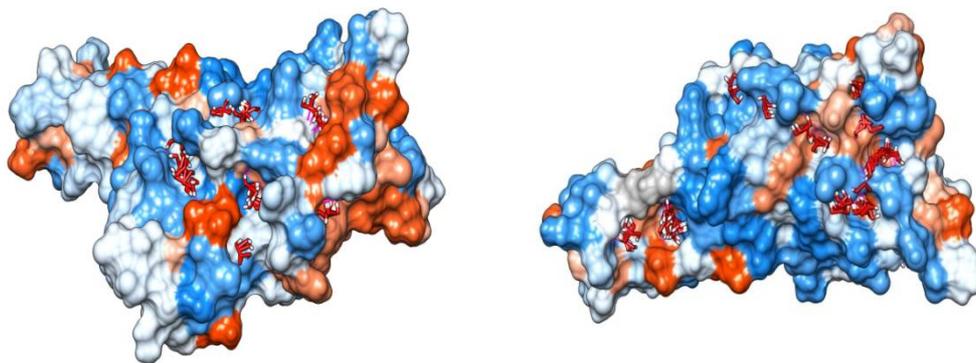


Figure 3 b. surface binding analysis of Ozone atom with 2GHV SPIKE PROTEIN

Figure 4 a . Interaction of Ozone atom with 6XLU7 A chain of SPIKE PROTEIN

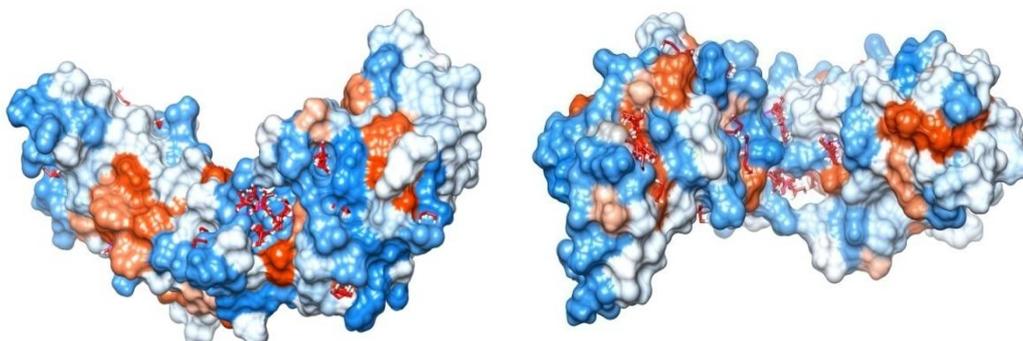
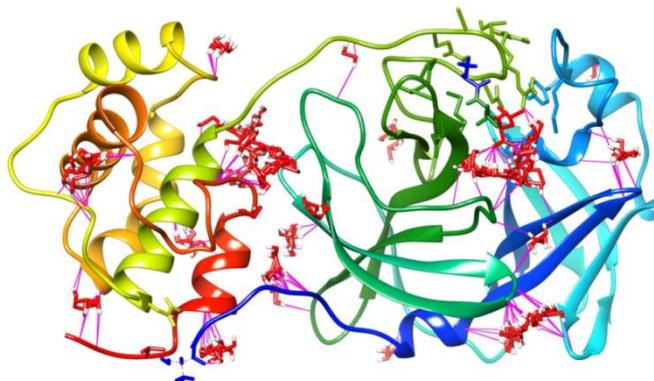


Figure 4 b. surface binding analysis of Ozone atom with 6XLU7 SPIKE PROTEIN

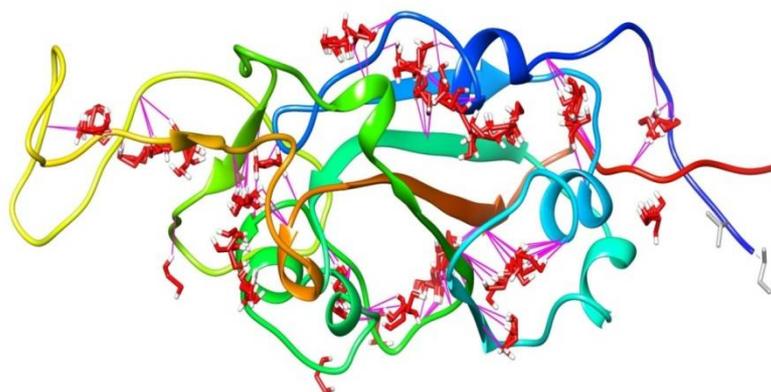


Figure 5 a .interaction of Ozone atom with 6XE1 L chain SPIKE PROTEIN

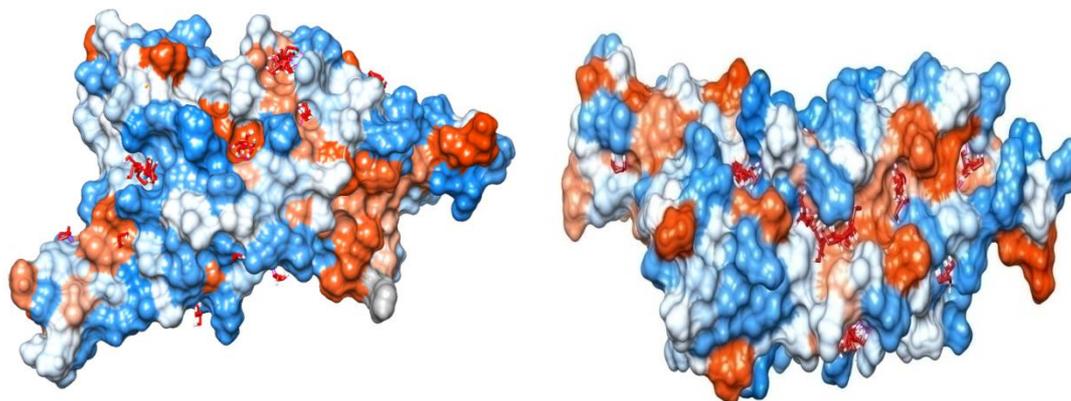


Figure 5 b. surface binding analysis of Ozone atom with 6XE1 SPIKE PROTEIN

Multipose interaction of ozone with main protease 6XLU7 A given in table 2 shows formation of active live hydrogen bonds. The pockets V0 to V36 were individually analyzed for hydrogen bond formation. The minimum score was found as -12 at V and maximum of -19 at V0. Out 36 pose, V1, V8, V9, V2, V20 and V32 are showed mainly steric interactions. Pocket V0 showed maximum score -19.6823 and maximum of 4 hydrogen bond with LEU4, GLY143 and LEU141. Aminoacids at V15 PHE219 and LEU220 also showed formation of 4 hydrogen bonds and the free energy is -14.9658. pocket V7 showed different kind of amino acid residual interaction (CYS22, VAL42, THR24, ILE43, CYS44). Figure 2b shows formation of active hydrogen bonds. Figure 2b reveals maximum hydrogen bonding under hydrophobic region. Glycine and Leucin are most frequently repeated aminoacid take part in maximum hydrogen bond formation and proline and cystein are rarely formed hydrogen bond. The short distanced hydrogen bond 1.880 Å formed by ASP153 and 2.877 Å is longest distance formed by VAL42. According to Anand et al [16] the main protease with highly conserved catalytic domain found to be an ideal choice for drug development against corona.

Table 3 data of 6XE1 L chain with ozone showed 29 different poses (V0 to V29). V1, V8, V14 do not showed any stable live hydrogen formation. Maximum score is -18.8301 with 5 hydrogen bonding. four amino acid in this region GLN409/ALA411/LEU425/TYR423 were acted as main interacting residues. Followed by V0 the pose V 21 contains TYR351/ TYR421/ ASP467/ASP422 interact with O<sub>3</sub> molecule and formed 4 hydrogen bonding. The calculated score is -13.5639. v2, v12, v13, v23 and v26 showed max 3 hydrogen bonding of which two are live and one is pseudo hydrogen bonding. ASN422 showed short distanced hydrogen bonding formation and the distance is 1.722 Å. ASP, TYR, LEU and PHE are frequently interacted residues where as GLU, GLN, PRO, VAL are less frequently take part. No methionine residues were found unlike other studied chains. Figure 2c shows molecular interaction of ozone with 6XE1. Figure 5b shows formation of numerous hydrogen bonds in hydrophobic region. Structure of protein ozone interaction shows loops are predominantly formed hydrogen bond and thus it play a major role in the instability of the protein structure. Balasco et al., [17] stated loops are major structure in stability of spike and hence a molecule binding to loop is a potential affecter of stability.

## CONCLUSION

Due to the lack of clinical experimental data and less explored research, as well as the severity of spread of infection of deadly coronaviruses, we evaluated an alternate compound to control the virus viability in atmosphere. The tested ozone was known to be potent lethal viral agent capable to control the spread of virus due to its high affinity towards COVID19.

## CONFLICT OF INTEREST

The authors don't have any conflict of interest

## ACKNOWLEDGEMENT

Authors acknowledge and thank to the management of DhanalakshmiSrinivasan College of arts and science for Women(A), Perambalur, Tamilnadu for providing software facilities.

## REFERENCES

1. Ji W, Wang W, Zhao X, Zai J, Li X. (2020) Cross-species transmission of the newly identified coronavirus 2019-nCoV. *J Med Virol*; 92: 433-440
2. Schoeman D, Fielding BC. (2019) Coronavirus envelope protein: current knowledge. *Virology*; 16:69

3. Sun J.L, Liu H, Hu J, Xu L.X. (2005) Study of SARS transmission via liquid droplets in air, *Journal of Biomechanical Engineering*;127: 32- 38.
4. Kirchdoerfer RN, Wang N, Pallesen J(2018) Stabilized coronavirus spikes are resistant to conformational changes induced by receptor recognition or proteolysis. *Sci Rep*;8:15701.
5. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X, Zhong W, Hao P.(2020) Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. *Sci China Life Sci*; 63(3): 457–460.
6. Walls AC, Park YJ, Tortorici MA, Wall A, McGuire AT, Veesler D. (2020) Structure, Function, and Antigenicity of the SARS-CoV-2 Spike Glycoprotein. *Cell* ; 181(2):281-292.
7. N. Q. Chen. Severe acute respiratory syndrome prevention and control, China, Beijing: China science and technology press, 2004: 1-2
8. Li F, Liu J, Pei J, Lin C.H, Chen Q. (2014) Experimental study of gaseous and particulate contaminants distribution in an aircraft cabin. *Atmospheric Environment* ;85: 223-233.
9. Sheahan TP, Sims AC, Graham RL, Menachery VD, Gralinski LE, Case JB, Leist SR, Pirc K, Feng JY, Trantcheva I, Bannister R. (2017) Broad-spectrum antiviral GS-5734 inhibits both epidemic and zoonotic coronaviruses. *SciTransl Med*;9:396.
10. Di Paolo N, Bocci V, Gaggioti E. (2004) Ozone therapy editorial review. *Int J Artificial Organs*; 27:168–75
11. Yargeau V, Leclair C, YargeauV,Leclair, C. (2008) Impact of Operating Conditions on Decomposition of Antibiotics During Ozonation: A Review Impact of Operating Conditions on Decomposition of Antibiotics During Ozonation: A Review. *Ozone Sci. Eng*; 30, 175–188.
12. 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 Inc. *J ComputChem*; 25: 1605–1612,
13. Qamar MT, Alqahtani SM, Alamri MA, Chen LL. (2020) Structural basis of SARS-CoV-2 3CLpro and anti-COVID-19 drug discovery from medicinal plants. *J Pharm Anal*;10(4):313-319
14. Li F. (2016) Structure, Function, and Evolution of Coronavirus Spike Proteins. *Annu Rev Virol*; 3:237–61.
15. Mothay D, Ramesh K.V. (2020) Binding site analysis of potential protease inhibitors of COVID-19 using AutoDock. *VirusDis*; 31: 194–199
16. Anand K, Ziebuhr J, Wadhwani P, Mesters J.R, Hilgenfeld R. (2003) Coronavirus main proteinase (3CLpro) structure: basis for design of anti-SARS drugs. *Science*; 300(5626):1763–1767.
17. Balasco N, Esposito L, De Simone A.D and Vitagliano L. (2013) Role of loops connecting secondary structure elements in the stabilization of proteins isolated from thermophilic organisms. *Protein Sci*; 22: 1016-1023.

#### CITATION OF THIS ARTICLE

A.Raja and P.Gajalakshmi. Ozone could be an effective air sanitizer to inactivate Corona virus? - *In Silico* docking. *Bull. Env.Pharmacol. Life Sci.*, Vol10[9] August 2021 : 147-154