

## **Molecular dynamics simulations of ASC09, ritonavir, lopinavir and darunavir with the COVID-19 protease**

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### **ABSTRACT**

Given the novelty of SARS-CoV-2 infection (COVID-19), and the lack of proven therapies, a wide variety of strategies are being employed to combat COVID-19 pandemic. Many of these emerging strategies rely on repurposing existing drugs and their mechanistic approaches that are effective against either similar viral infections or the serious symptoms that are caused by COVID-19. The recently solved issue of the crystal structure of the COVID-19 protease has made elucidating the structure–activity relationship feasible. The interaction of ASC09, ritonavir, lopinavir and darunavir with COVID-19 protease was simulated using the Site Finder module, molecular docking and molecular dynamics (MD). Analysis of the MD trajectories has provided the ligand/receptor interaction fingerprints, combining information on the crucial receptor residues and frequency of the ligand/residue contacts. The contact frequencies and the contact maps suggest that for all studied antiviral drugs, the interactions with Gln 107, Pro 108, Gln 110 and His 246 are an important factor for drugs affinities toward the COVID-19 protease. However, the leading interactions with Arg 105, Phe 134, Glu 240, Thr 243, Asp 245 or Phe 294 also significantly contribute to the ligand/receptor interplay and, in particular, differentiate their binding affinities toward COVID-19 protease.

*Keywords: COVID-19, Molecular dynamics, ASC09, Ritonavir, Lopinavir, Darunavir*

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## **Introduction**

At the time of this writing, the SARS-CoV-2 infection has sickened more than 11 million people globally and killed more than half a million of them (Worldometer, 2020). No vaccine or direct treatment currently exists (Ahmed et al., 2020; Robson, 2020). The most promising vaccine clinical trials at this moment include an adenovirus-based candidate that is in a phase I/II trial and an mRNA vaccine that is in a phase II trial (Mullard, 2020). As the SARS-CoV-2 infection continues to spread worldwide and more people become critically ill, scientists are racing to find a treatment that will reverse the course (Ren et al., 2020; Robson, 2020; Wang et al., 2020;). Dozens of medicines are in clinical trials in China and the United States to treat the disease, officially named COVID-19 (Harrison 2020; Zhang et al., 2020;). More than 240 clinical trials being conducted involve antiviral drugs that were developed to treat illnesses such as HIV/AIDS, malaria, *etc.* (ClinicalTrials, 2020).

Protease inhibitors are a class of antiviral drugs that are widely used to treat HIV, hepatitis C, SARS-CoV and MERS-CoV (Feng et al., 2019; Pillaiyar et al., 2016; Sheahan et al., 2020). They prevent SARS-CoV replication by selectively binding to viral proteases and blocking proteolytic cleavage of protein precursors that are necessary for the production of infectious viral particles (Haagmans and Osterhaus, 2006). Last five months, at least 50 different trials for SARS-CoV-2 registered in the Clinical Trial Registry (*e.g.*, NCT04251871, NCT04255017, NCT04261270, NCT04261907, NCT04275388, NCT04276688, NCT04286503, NCT04291729, NCT04295551, NCT04303299, NCT04306497, NCT04307693, NCT04315948) proposed the use of protease inhibitors (ASC09, ritonavir, lopinavir and darunavir) in the treatment of COVID-19 (ClinicalTrials, 2020).

A key aspect of the inhibitor discovery process is to determinate the three-dimensional structure of the inhibitor/protein complex. Therefore, elucidating the binding mode of viral protease inhibitors with COVID-19 protease could provide some clues to the design of more promising COVID-19 protease inhibitors. This paper presents and discuss the results of molecular dynamics (MD) simulations of ASC09, ritonavir, lopinavir and darunavir with recently reported, high-resolution crystal structure of COVID-19 protease. The Site Finder module of the Molecular Operating Environment (MOE) software (MOE, 2019), was utilized to define and rank potential ligand-binding sites according to their propensity for ligand binding, which was based on the amino acid composition of the pocket (Soga et al., 2007). Furthermore, molecular docking and molecular dynamics simulations methods were applied as a powerful computational strategy to investigate the detailed interactions of protease inhibitors with COVID-19 protease. The aim is to sketch the mechanism of inducing distinct responses of the COVID-19 protease to the chosen ligands. The study includes independent MD simulations for the top-ranked poses of each ligand and examines frequency of contacts and patterns between viral protease inhibitors and the COVID-19 protease residues.

## **Experimental**

Examined viral protease inhibitors have been generated using the builder panel in the MOE software. Using the MOE LigX module, partial atomic charges were ascribed and possible ionization states were generated at a pH of 7.0. The MMFF94x force field was used for optimization and the resulting structures were used for modeling studies (Halgren, 1996). Conformational search was carried out by MOE LowModelMD method which performs molecular dynamic perturbations along with low frequency vibrational modes with energy window of 7 kcal mol<sup>-1</sup>, and conformational limits of 1000.

Several X-ray crystallographic structures of COVID-19 protease (PDB: 6Y84, 6Y2E, 5R84, 6Y2F, 6M03, 6LU7 and 6Y2G) have been recently published (Protein Data Bank, 2020). Considering the receptor resolution (King, 1958), the crystallographic structure (PDB: 6Y84) was elected as the top structure for further molecular modeling (Owen et al., 2020). The inaccuracy of COVID-19 protease was corrected by the Structure Preparation process in MOE. After the correction, hydrogens were added and partial charges (Gasteiger methodology) were calculated. Energy minimization (AMBER14:EHT, RMS gradient: 0.100) was performed.

The Site Finder module of the MOE was used to identify possible ligand-binding sites within the optimized structure of COVID-19 protease. Hydrophobic or hydrophilic alpha spheres served as probes denoting zones of tight atom packing. These alpha spheres were utilized to define and rank potential ligand-binding sites according to their propensity for ligand binding (PLB) score, which was based on the amino acid composition of the pocket (Soga et al., 2007).

The molecular docking study was performed using the MOE to understand the ligand protein interactions in detail. The default Triangle Matcher placement method was used for the induced fit docking (MOE, 2019). GBVI/WSA dG scoring function which estimates the free energy of binding of the ligand from a given pose was used to rank the final poses (Corbeil et al., 2012). Each ligand/protein complex with lowest relative binding free energy ( $\Delta G$ ) score was selected.

The molecular dynamics simulation of selected viral protease inhibitors on COVID-19 protease was carried out using the Desmond (Desmond, 2018). The structure of the added water was based on the simple point charge (SPC) solvent model. The system was neutralized with Na<sup>+</sup> ions to balance the net charge of the whole simulation box to neutral. The final system contained approximately 36800 atoms. The system was passed through a 6-step relaxation protocol before molecular dynamics simulations. The relaxed system was simulated for 10 ns, using a normal pressure temperature (NPT) ensemble with a Nosé–Hoover thermostat at 300 K and Martyna–Tobias–Klein barostat at 1.01325 bar pressure. Atomic coordinate data and system energies were recorded every 1 ps. The root mean square deviation (RMSD) and root mean square fluctuation (RMSF) of the inhibitor/COVID-19 protease complexes were analyzed with respect to simulation time.

## Results and Discussion

The binding site residues in the COVID-19 protease have been identified using the Site Finder module implemented in the MOE software. The results from the analysis highlighted that amino acid residues like Phe 3, Arg 4, Lys 5, Met 6, Ala 7, Phe 8, Pro 9, Lys 102, Val 104, Arg 105, Ile 106, Gln 107, Pro 108, Gly 109, Gln 110, Thr 111, Gln 127, Pro 132, Phe 134, Phe 150, Asn 151, Ile 152, Asp 153, Tyr 154, Cys 156, Val 157, Ser 158, Phe 159, Cys 160, Gly 183, Ile 200, Thr 201, Val 202, Asn 203, Glu 240, Pro 241, Thr 243, Asp 245, His 246, Ile 249, Phe 291, Thr 292, Pro 293, Phe 294, Asp 295, Val 296, Arg 298, Gln 299, Gly 302, Val 303 and Thr 304 constituted the top binding pocket of the COVID-19 protease (Table 1).

The intermolecular contacts between four representative viral protease inhibitors and COVID-19 protease were analyzed using the ligand interaction diagram of MOE suite. According to binding free energy, it was predicted that ASC09 (-7.83 kcal mol<sup>-1</sup>) could inhibit COVID-19 protease better than ritonavir (-7.42 kcal mol<sup>-1</sup>), lopinavir (-6.98 kcal mol<sup>-1</sup>) and darunavir (-6.56 kcal mol<sup>-1</sup>).

The study was further extended to assess the stability of inhibitor/COVID-19 protease complexes through the molecular dynamics simulations. The RMSD and RMSF plots for COVID-19 protease showed that docking complexes were stable during entire simulation period (Figure 1). The RMSD and RMSF values for C $\alpha$ , side chains and heavy atoms remained within the limit of 2 Å (Figure 1). The obtained results indicated small structural rearrangements, less conformational changes and confirmed stability of inhibitor/COVID-19 protease complexes (Liu and Kokubo, 2017). The interactions observed during 10 ns molecular simulation confirmed the importance of Arg 105, Gln 107, Pro 108, Gln 110, Phe 134, Glu 240, Thr 243, Asp 245, His 246 and Phe 294 in the formation of inhibitor/COVID-19 protease complexes (Figures 2 and 3).

**Table 1.** Summary of the possible inhibitor-binding sites in the COVID-19 protease.

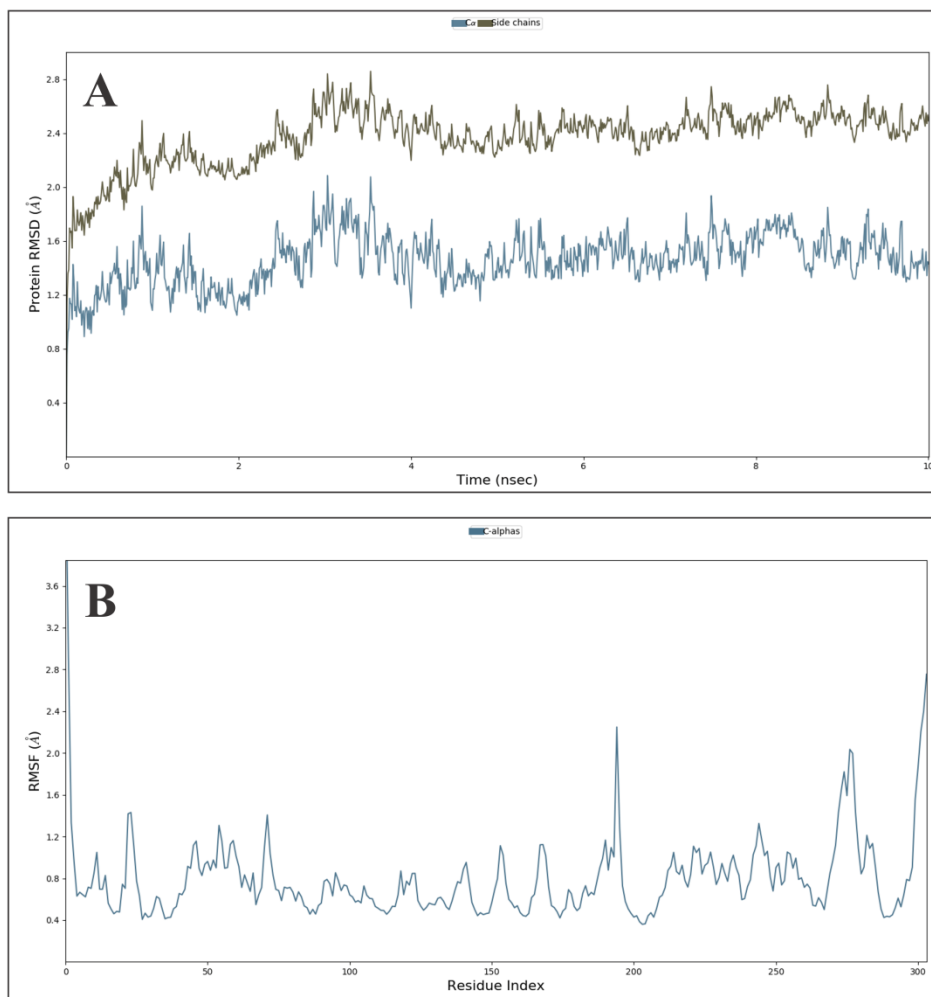
Site	Size	PLB	Hyd	Side	Residues
1	193	4.5	61	112	Phe 3, Arg 4, Lys 5, Met 6, Ala 7, Phe 8, Pro 9, Lys 102, Val 104, Arg 105, Ile 106, Gln 107, Pro 108, Gly 109, Gln 110, Thr 111, Gln 127, Pro 132, Phe 134, Phe 150, Asn 151, Ile 152, Asp 153, Tyr 154, Cys 156, Val 157, Ser 158, Phe 159, Cys 160, Gly 183, Ile 200, Thr 201, Val 202, Asn 203, Glu 240, Pro 241, Thr 243, Asp 245, His 246, Ile 249, Phe 291, Thr 292, Pro 293, Phe 294, Asp 295, Val 296, Arg 298, Gln 299, Gly 302, Val 303, Thr 304
2	21	0.68	14	22	Trp 218, Phe 219, Leu 220, Asn 221, Phe 223, Ser 267, Glu 270, Leu 271, Asn 274, Gly 275, Met 276, Asn 277
3	18	0.33	11	18	Leu 220, Asn 221, Phe 223, Ile 259, Asp 263, Met 264, Ser 267
4	54	0.33	13	34	His 41, Met 49, Asn 142, His 164, Met 165, Glu 166, Leu 167, Pro 168, Thr 169, Gly 170, Arg 188, Gln 189, Thr 190, Ala 191, Gln192

5	13	0.16	8	11	Asn 63, His 64, Phe 66, Leu 67, Val 68, Gln 74, Leu 75, Arg 76, Val 77
6	13	0.15	8	19	Arg 40, Asn 84, Cys 85, Arg 105, Gly 179, Asn 180, Phe 181, Gly 183, Phe 185, Val 186, Asp 187
7	25	0.11	4	17	Phe 140, Leu 141, Asn 142, Gly 143, Ser 144, Cys 145, His 163, Met 165, Glu 166
8	28	-0.01	12	18	Thr 199, Tyr 237, Tyr 239, Leu 271, Leu 272, Gly 275, Met 276, Asn 277, Gly 278, Arg 279, Ala 285, Leu 286, Leu 287
9	15	-0.01	6	8	Glu 14, Gly 15, Cys 16, Met 17, Val 18, Trp 31, Ala 70, Gly 71, Gly 120, Pro 122
10	31	-0.07	7	24	Lys 5, Arg 131, Lys 137, Asp 197, Thr 198, Thr 199, Leu 286, Leu 287, Glu 288, Asp 289, Glu 290

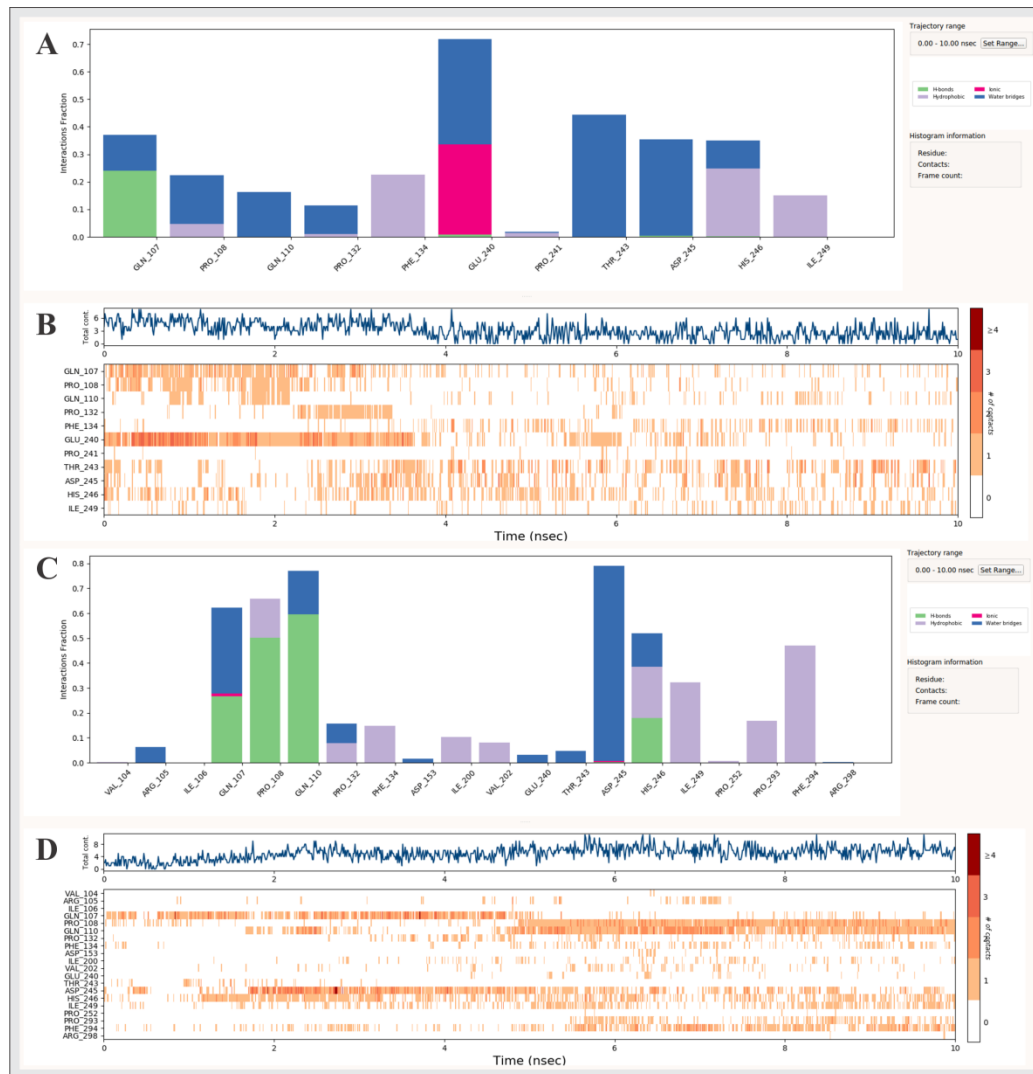
Examined inhibitor/COVID-19 protease complexes, throughout the molecular dynamics simulation exhibited four types of interactions: hydrophobic, ionic, water-bridged and hydrogen bonds (Figures 2 and 3). The molecular dynamics simulations of ASC09/COVID-19 protease (Figure 2A and 2B), revealed firm ionic/water-bridged interactions with Glu 240 (72 % of the simulation time) and water-bridged interactions with Thr 243 (44 % of the simulation time), Asp 245 (35 % of the simulation time) and Gln 110 (16 % of the simulation time). In addition, the ASC09/COVID-19 protease complex uncovered H-bonding/water-bridged interactions with Gln 107 (37 % of the simulation time), as well as hydrophobic/water-bridged interactions with His 246 (35 % of the simulation time) and Pro 108 (22 % of the simulation time). The interaction profile of ritonavir/COVID-19 protease revealed slightly different results (Figure 2C and 2D). The molecular dynamics showed stable water-bridged interactions with Asp 245 (78 % of the simulation time) as well as forceful H-bonding/water-bridged interactions with Gln 110 (76 % of the simulation time) and Gln 107 (62 % of the simulation time). Furthermore, examined complex showed substantial H-bonding/hydrophobic interactions with Pro 108 (65 % of the simulation time) and His 246 (52 % of the simulation time) (Figure 2C and 2D).

The molecular dynamics simulations of lopinavir and darunavir with COVID-19 protease (Figure 3), revealed prevalent H-bonding/water-bridged interactions with Gln 107 (exceeding 116 % of the simulation time). In addition, lopinavir and darunavir exhibited modest interactions with previously discussed Pro 108, Gln 110 and His 246 (Figure 3). The contact frequencies and the contact maps suggest that for all studied antiviral drugs, the interactions with Gln 107, Pro 108, Gln 110 and His 246 are an important factor for drugs affinities toward the COVID-19 protease. Highlighted residues of COVID-19 protease are comparable with structural features and catalytic residues of SARS-CoV 3CL<sup>pro</sup> which inhibition by peptidomimetics and small molecule inhibitors is well documented (Pillaiyar et al., 2016). Of note, the importance of Gln (as Gln 189 or Gln 192) and His (as His 41) in the anti-SARS-CoV 3CL<sup>pro</sup> chemotherapies is clearly established in Pillaiyar et al. (2016) and references therein. However, the

molecular dynamics simulations of examined complexes reveal that leading interactions with Arg 105, Phe 134, Glu 240, Thr 243, Asp 245 or Phe 294 also significantly contribute to the ligand/receptor interplay and, in particular, differentiate their binding affinities toward COVID-19 protease (Figures 2 and 3).

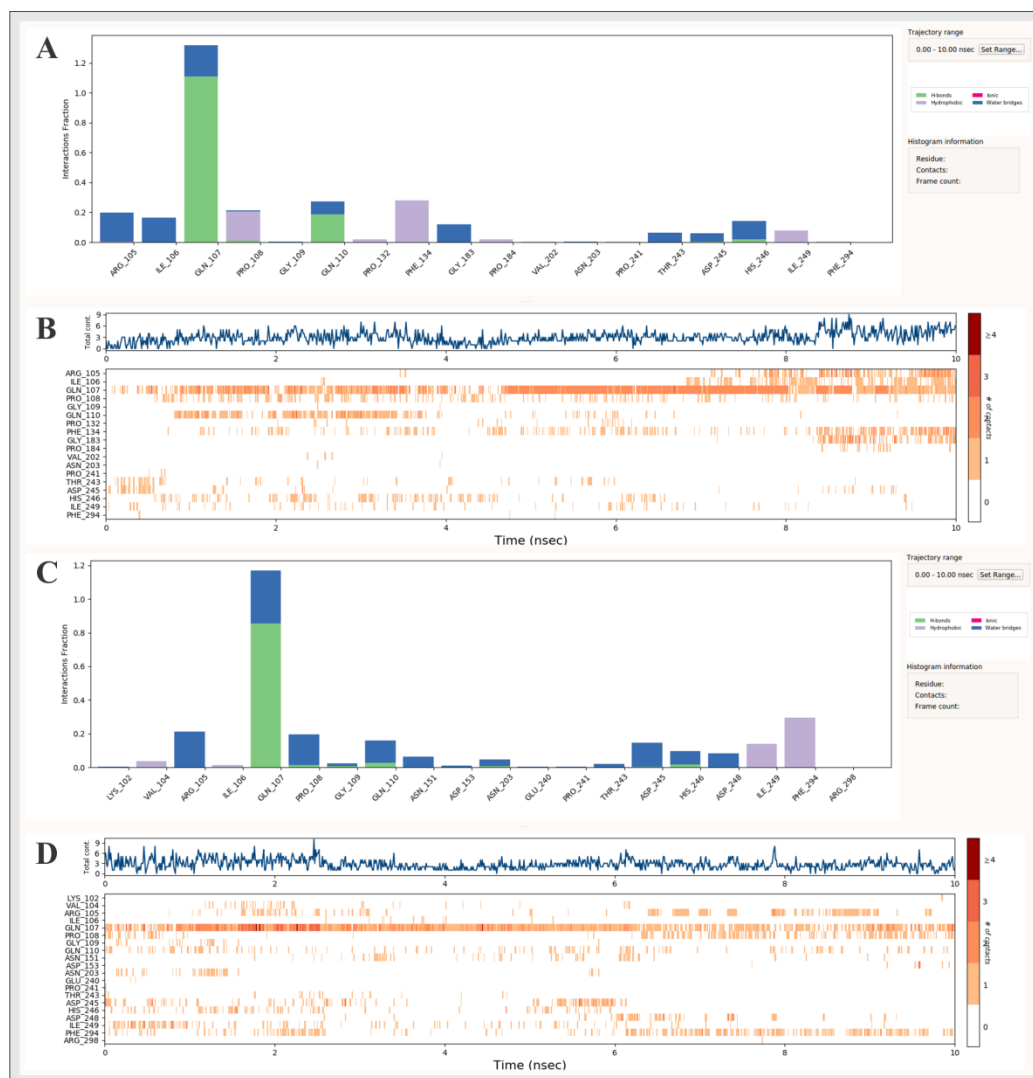


**Figure 1.** RMSD (A) and RMSF (B) plot of COVID-19 protease, during the course of 10 ns molecular dynamics simulation.



**Figure 2.** Normalized stacked bar chart representation and timeline representation of interactions and contacts between COVID-19 protease and antiviral drugs ASC09 (A, B) and ritonavir (C, D), during the course of 10 ns molecular dynamics simulation.





**Figure 3.** Normalized stacked bar chart representation and timeline representation of interactions and contacts between COVID-19 protease and antiviral drugs lopinavir (A, B) and darunavir (C, D), during the course of 10 ns molecular dynamics simulation.

## Conclusion

In summary, given the novelty of SARS-CoV-2 infection, and the lack of proven therapies, a wide variety of strategies are being employed to combat COVID-19 pandemic. The results presented herein provide dynamic insight into the binding of ASC09, ritonavir, lopinavir and darunavir with COVID-19 protease through the computational modeling approaches. Analysis of the observed intermolecular contact frequencies together with the contact maps indicates that the interactions with Gln 107, Pro 108, Gln 110 and His 246, retained by all of the studied ligands, are a contributing factor to the affinity of the ligand/COVID-19 protease interaction. However, the leading interactions with Arg 105, Phe 134, Glu 240,

Thr 243, Asp 245 or Phe 294 also significantly contribute to the ligand/receptor interplay and, in particular, differentiate their binding affinities toward COVID-19 protease. I anticipate that the results presented herein will open the way for a deeper understanding of inhibitor/COVID-19 protease interactions, especially for peptidomimetics that have recently been included in the treatment of COVID-19.

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### **Conflict-of-Interest Statement**

Declarations of interest: none.

### **Informed Consent Statement**

Informed consent was obtained from all individual participants included in the study.

### **Human and Animal Rights Statement**

This article does not contain any studies with human participants or animals performed by any of the authors.

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