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Letter to the Editor

Host-cell recognition of SARS-CoV-2 spike receptor binding domain from different variants

Dear Editor

Previously in this journal, we predicted that the host cell surface chaperone glucose-regulated protein 78 (Cs-GRP78) could act for SARS-CoV-2 spike recognition.[1] Later on, this was supported by an experimental study by Carlos et al.[2] Further prediction studies reported its enhanced role in recognition of some new variants that emerged in the last two years.[3,4] For Cs-GRP78, the recognition of the spike receptor binding domain (RBD) is reflected in the predicted binding affinity of the GRP78 substrate binding domain β (SBD β) to the C480-C488 region of the spike.

In the current study, we equilibrate the GRP78 and ACE2 systems for 100 ns then we cluster the trajectories. Fig. 1 shows the molecular dynamics simulation (MDS) analysis performed using VMD 1.9.3 software. The two systems are equilibrated after 50 ns with RMSD values of 5 Å and 12 Å for GRP78 and ACE2, respectively (Fig. 1A). The two systems are found stable, as reflected by the RoG, SASA, and H-bonds. The radius of gyration for GRP78 and ACE2 started from 30 Å and 35 Å, respectively, but coincided at 31 Å at the end of the simulation (Fig. 1B). The SASA and the number of H-bonds are also stable during the simulation, with values around 30,000 Å^2 & 40,000 Å^2 and 1000 & 1200 for GRP78 & ACE2, respectively (Fig. 1C and D). The per-residue RMSF for the GRP78 and ACE2 systems are plotted in Fig. 1E and 1F. As reflected from the RMSF, both systems are stable with regions of high fluctuation (RMSF $<$ 5 Å) at the protein terminals, the region (565–590) in GRP78 and (730–760) in ACE2. These two regions are indicated in the structures at the upper part of the figure with dashed-red circles. The region (565–590) is part of the substrate binding domain α (SBDα) of GRP78 and was reported in previous studies to be highly flexible due to its vital role as a lid in covering the SBDβ during the inactivation of the protein (closed conformation). On the other hand, the highly flexible region from ACE2 is the transmembrane domain of the protein, and it is usually stabilized by binding to B(0)AT1 and the membrane.[5] The structures on the top of RMSF show the superposition of the representative conformations of the proteins that will be used to assess their binding affinity against RBDs.

Binding affinities of the GRP78 and ACE2 to different RBDs

Five representative structures for GRP78 and three for ACE2 are used to test their binding affinity to the WT and the mutated spike RBDs. The generated RBDs (WT, alpha, beta or gamma, delta, delta+, C36, lambda, and omicron) are equilibrated and then clustered (Fig. 2A and 2B). All of the RBDs are equilibrated with RMSD values between 2 and 6 Å. The RBDs are found stable during the simulation except for the GRP78 binding region (C480-C488). This region exhibits high fluctuations in the WT, alpha, delta, lambda, and C36 RBD variants, with RMSF $<$ 4 Å. The clusters representatives of each RBDs are docked using HADDOCK V2.4 against GRP78 and ACE2 representative clusters.[6] The active site for docking between the RBDs and ACE2 are F486, Q474, K417, Y453, Q498, N501, & T500 and Q24, M82, Q42, Y41, K353, R357, H34, & D30, respectively. On the other hand, the active site for docking between the RBDs and GRP78 are C480:C488 and T428, V429, V432, T434, F451, S452, V457 & I459, respectively.[7,8] In addition, we predicted the binding affinity using the PRODIGY web server for the docked complexes.[9] Fig. 2C and 2D show the average binding affinity (PRODIGY) and the corresponding HADDOCK scores for each RBD representative cluster conformation. Error bars represent the standard deviation. As reflected in Fig. 2C, the GRP78 has a moderate binding affinity (−8.64 up to −10.50 kcal/mol) against all RBDs, with some variants showing enhanced affinity compared to the WT RBD (beta or gamma and C36). On the other hand, the ACE2 (Fig. 2D) shows almost the same binding affinity against the WT and variant RBDs (−10.02 up to 11.60 kcal/mol), which are higher than that for GRP78. This coincides with the fact that ACE2 is the main recognition element and Cs-GRP78 is an auxiliary recognition site for SARS-CoV-2.[2,10]

Finally, for each of the docked results, the docked complex with a predicted binding affinity closest to the average binding affinity was used to perform another MDS run for 100 ns to study the stability of the established interactions. Fig. 2E shows the RMSD and RMSF of the GRP78-RBDs (left) and ACE2-RBDs (right) complexes. As reflected from the RMSD curves, the ACE2-RBDs in all the complexes are stable (RMSD 5–7 Å). On the other hand, the GRP78-RBDs complexes show RMSD values ranging from 3 up to 10 Å, except for delta (orange) and delta+ (cyan) variants that show higher values (up to 23 Å). This is also indicated in the RMSF curves (markers at the highly fluctuating RBD and SBDβ, for delta+ variant). The average RMSF at the C480-C488 is also plotted (Fig. 2F) for the different variants where the delta+ variant is not stable (RMSF $<$ 20 Å) at the GRP78-RBD complex compared to the WT and the other variants. While in ACE2-RBDs complexes, all the complexes show an average RMSF $^{480–488}$ around 4 Å.

After clustering the trajectories for the complexes, we calculated the binding energies using the PRODIGY web server (Fig. 2G). For GRP78-RBDs complexes (green), the omicron and lambda variants show enhanced binding affinity compared to WT, while C36 and delta variants show the same affinity as WT. This coincides with our previous results published earlier in this journal.[4] On the other hand, for the ACE2-RBDs complexes (blue), the delta, delta+, lambda, and C36 variants show enhanced affinity compared to WT.

Conclusively, ACE2 and GRP78 can bind to, hence recognize, SARS-CoV-2 spike from different variants, including alpha, beta, delta, delta+, lambda, C36, and omicron. Accordingly, targeting these host-cell receptors would be successful in fighting the pandemic.

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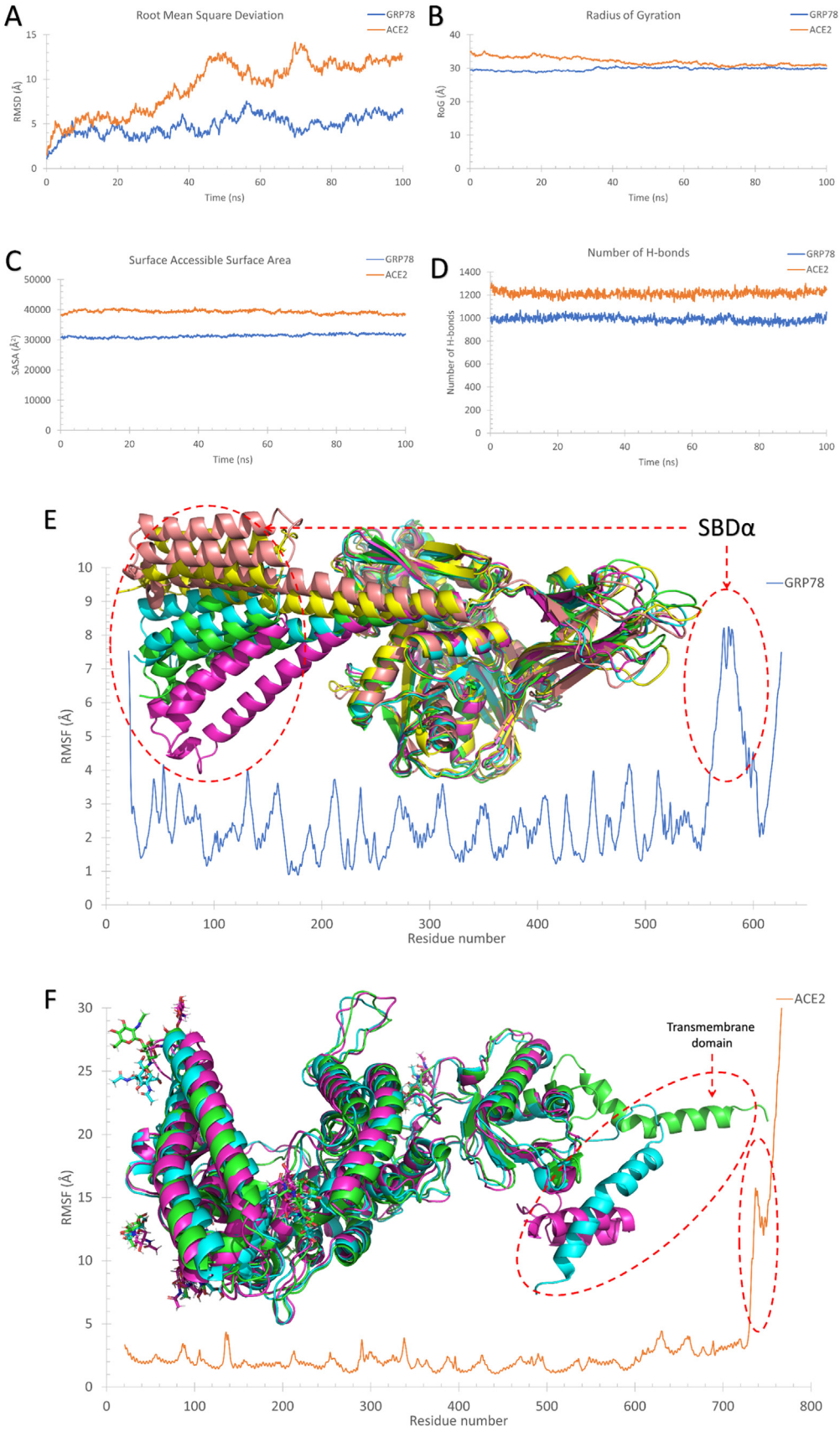

Fig. 1. The molecular dynamics simulation (MDS) analysis of the GRP78 and ACE2 systems. The root-mean-square deviation (RMSD) (A) in Å, the radius of gyration (RoG) in Å (B), surface accessible surface area (SASA) in $Å^2$ (C), and the number of H-bonds (D), versus time in ns are plotted for the GRP78 (blue) and ACE2 (orange) systems. The per-residue root-mean-square fluctuations (RMSF) of GRP78 (E) and ACE2 (F) systems are shown (bottom), with the representative cluster members superimposed and depicted in colored cartoons (top).

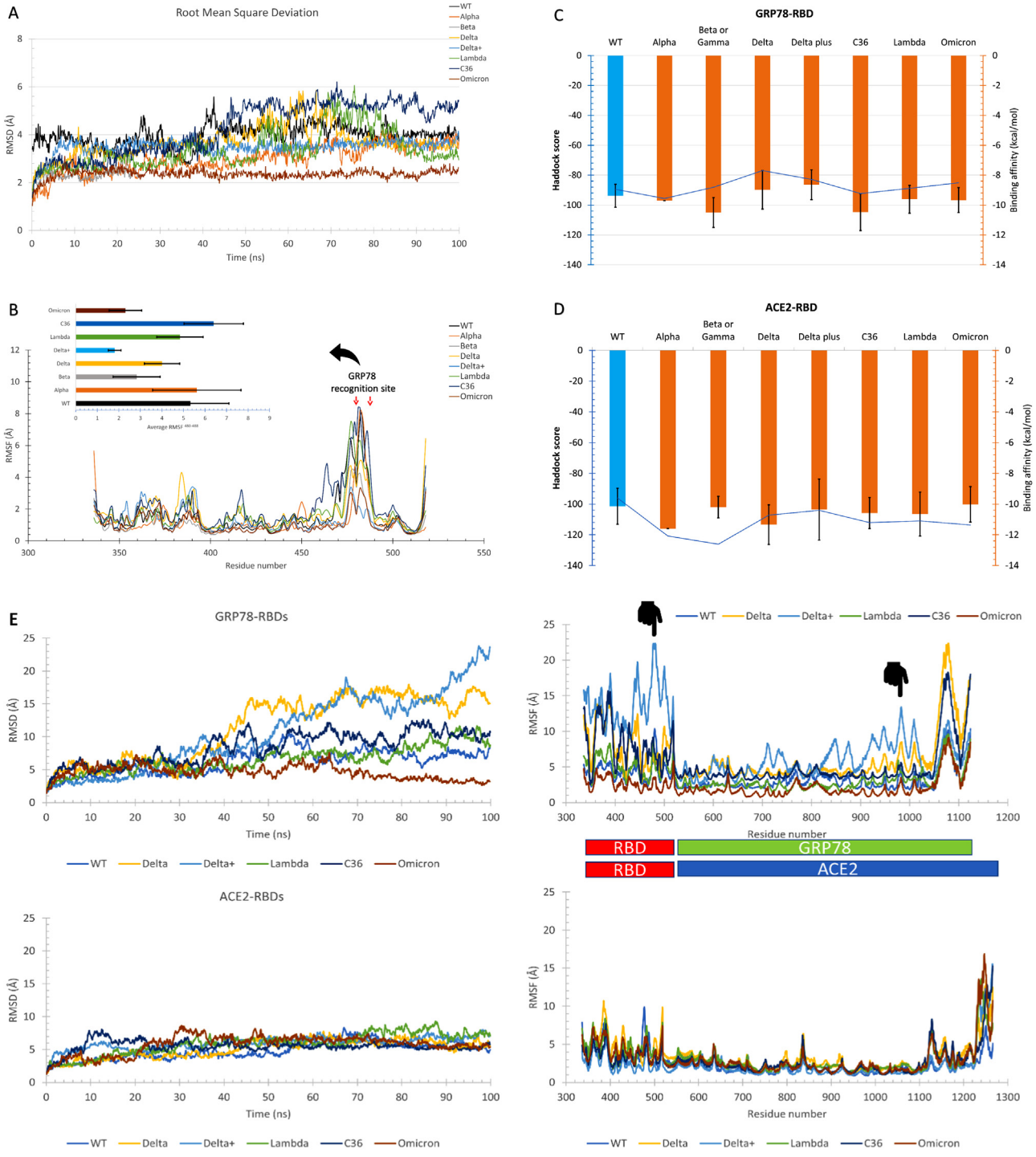

Fig. 2. (A) The root-mean-square deviation (RMSD) of the WT (black) and the mutated variants RBDs (alpha: orange, beta or gamma: gray, delta: yellow, delta+: cyan, lambda: green, C36: blue, and omicron: brown) versus the simulation time in ns. (B) the per-residue root-mean-square fluctuations (RMSF) of the WT and the mutated RBDs. The enlarged panel shows the average RMSF for the GRP78 binding region (C480-C488). The average binding affinity (kcal/mol) was predicted using PRODIGY for the docking of GRP78 (C) and ACE2 (D) against the WT (blue) and the different variants (orange) of SARS-CoV-2 spike RBDs. (E) shows the RMSD and RMSF of GRP78-RBDs (top) and ACE2-RBDs (bottom) complexes simulated for 100 ns. (F) The RMSF of the RBD C480-C488 region for the GRP78-RBDs (left) and ACE2-RBDs (right) systems. (G) The calculated average binding energies of the different systems of RBDs bound to GRP78 (green) and ACE2 (blue), with error bars, represent the standard deviation.

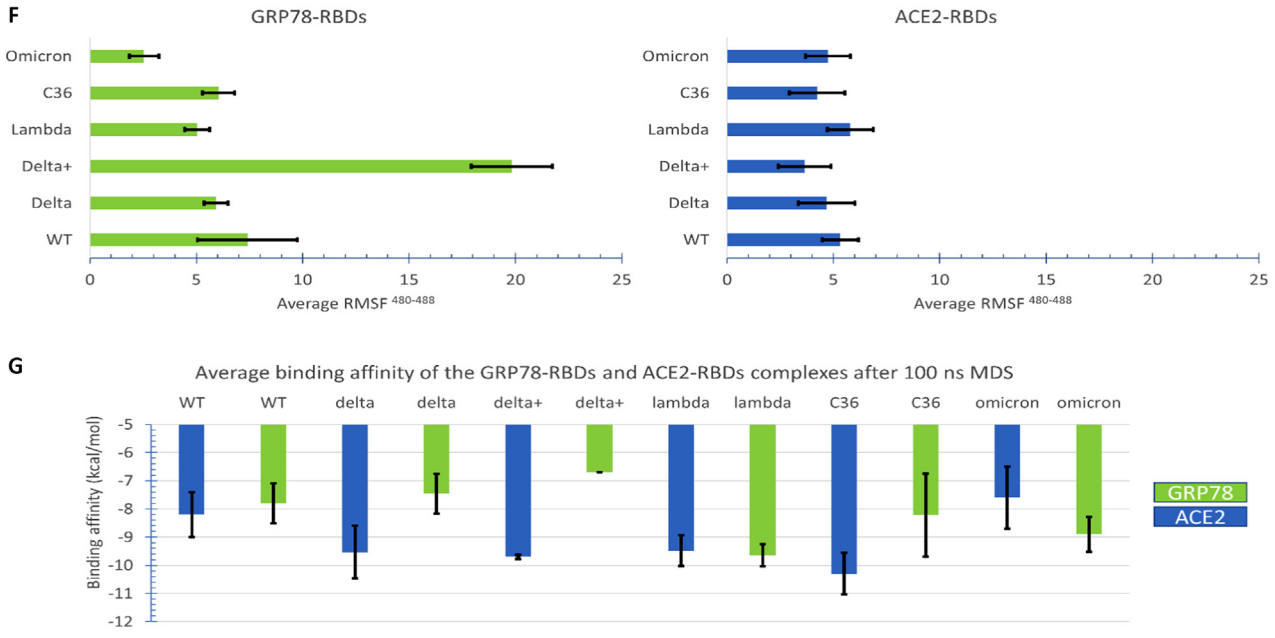

Fig. 2. Continued

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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