Effect of the Graphene Nanosheet on Functions of the Spike Protein in Open and Closed States: Comparison between SARS-CoV-2 Wild Type and the Omicron Variant

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ABSTRACT: The spread of coronavirus disease 2019 caused by SARS-CoV-2 and its variants has become a global health crisis. Although there were many attempts to use nanomaterials-based devices to fight against SARS-CoV-2, it still remains elusive as to how the nanomaterials interact with SARS-CoV-2 and affect its biofunctions. Here, taking the graphene nanosheet (GN) as the model nanomaterial, we investigate its interaction with the spike protein in both WT and Omicron by molecular simulations. In the closed state, the GN can insert into the region between the receptor binding domain (RBD) and the N-terminal domain (NTD) in both wild type (WT) and Omicron, which keeps the RBD in the down conformation. In the open state, the GN can hamper the binding of up RBD to ACE2 in WT, but it has little impact on up RBD and, even worse, stimulates the down-to-up transition of down RBDs in Omicron. Moreover, the GN can insert in the vicinity of the fusion peptide in both WT and Omicron and prevents the detachment of S1 from the whole spike protein. The present study reveals the effect of the SARS-CoV-2 variant on the nanomaterial–spike protein interaction, which informs prospective efforts to design functional nanomaterials against SARS-CoV-2.

INTRODUCTION

The global health and economy have been threatened by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) since December 2019. Recently, the Omicron variant has become the latest variant of concern (VOC) by the World Health Organization due to its high transmissibility. More than 6,450,000 deaths and 956 million confirmed cases worldwide have been reported by the World Health Organization (WHO, https://covid19.who.int/). SARS-CoV-2 is a single-stranded RNA-enveloped virus, and its gene fragments encode 4 structural proteins (the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins) and 16 nonstructural proteins (the spike (S), envelope (E), membrane (M), and nucleocapsid (N) proteins) and 16 nonstructural proteins (Nsp1−16).1−5 The trimeric spike protein, a primary subunit in invading host cells, can be divided into two subunits, S1 and S2, which are responsible for binding to the angiotensin-converting enzyme 2 (ACE2) of host cells and cell–virus fusion, respectively.6−15 Notably, Omicron has 30 single-point mutations, 3 deletion mutations, and 1 insertion mutation in the spike protein, which resulted in the different local structure and striking immune evasion from antibodies compared with the wild type (WT).16−20

Previous studies indicated that the spike protein in a viral membrane has two distinct states, namely, the open and closed states.13,14,21,22 When the spike protein exists in a closed state, three receptor binding domains (RBDs) in the S1 subunit are in the down conformation, whereas when the spike protein exists in an open state, one or two of the three RBDs are in the up conformation and have the capability of binding to ACE2.21 After binding to ACE2, the transmembrane protease serine 2 (TMPRSS2) interacts with the S2′ cleavage site and mediates the S1 and S2 detachment.23−27 The S2 conformation rearrangement is triggered after losing the S1 cap: mainly heptad repeat 1 (HR1) turns over to form a continuous stem helix together with the central helix (CH), which causes the fusion peptide (FP) to insert into the host cell membrane and also causes virus–cell fusion.6,22,28−31

Numerous studies reported that the nanotechnology-based strategies could be employed to fight against SARS-CoV-2.32−43 Actually, due to excellent physicochemical properties such as a high surface-to-volume ratio and a quantum size effect, some nanomaterials can be used to detect the surface antigens, antibodies, nucleic acids, cytokines, and the whole virus. For example, researchers developed a gold nanoparticle coated with thiol-modified DNA antisense oligonucleotides, which can specifically detect the N-gene of SARS-CoV-2.32 Poly(amine ester) with carboxyl group (PC)-coated magnetic nanoparticles (pcMNPs) were designed to capture two
different regions of viral RNA, which dramatically reduces operational requirements in detection.33 When the spike protein of SARS-CoV-2 was targeted, the graphene sheets of the field-effect-transistor-based biosensing device coated with a specific antibody also exhibited the highly sensitive immunological diagnostic property.44,45 Furthermore, graphene-based nanomaterials exhibited excellent antiviral properties against COVID-19 by interacting with the spike protein to disrupt infectivity and the viral envelope to inhibit coronavirus replication.44,45 Notably, some studies also indicated that the GN or GN-based nanomaterials of several nanometers can affect the property of the proteins of SARS-CoV-2 and may be used to fight against SARS-CoV-2.45–47 Due to their excellent virucidal effect, the silver nanocluster, silica composite, and graphene were used to produce facial masks.41,42 Despite great progress in nanomaterials in the real applications on SARS-CoV-2, it remains largely unknown how the nanomaterials interact with SARS-CoV-2, especially how their interactions affect the structure and function of SARS-CoV-2 at the molecular level. Moreover, since Omicron has a lot of mutations (compared to SARS-CoV-2 WT), it is also of great importance to reveal whether and how their mutations affect their interaction with the nanomaterials.

In this work, we take the graphene nanosheet (GN) as a typical example of nanomaterials and systematically investigate its interaction with the spike protein of WT and Omicron located on the surface of SARS-CoV-2 by combining the molecular docking and the all-atom molecular dynamics (MD) simulation. As shown below, the GN can insert into the spike protein and subsequently disturb the function of the spike protein no matter whether it is in the closed or open state in WT. In contrast, although the GN can also insert into the Omicron spike protein, it obviously cannot disturb the function of the spike protein, especially when the spike protein is in the open state. The underlying mechanism of their interaction at different states of the spike protein in WT and Omicron is further revealed in detail.

**MODEL AND METHODS**

**Structure Preparation.** In this study, the initial structures for the SARS-CoV-2 trimeric spike protein were obtained from the Protein Data Bank (https://www.rcsb.org/). In the closed state (i.e., all RBDs are in the down conformation), the PDB ID is 6X8S for WT and 7WP9 for Omicron. In the open state (i.e., one RBD is in the up conformation and the other two RBDs are in the down conformation), the PDB ID is 6YVY for WT and 7TGW for Omicron. The missing residues were added and the three-dimensional structures were modeled by using the SWISS-MODEL server49 based on the FASTA sequence. There are four domains in S1, including an N-terminal domain (NTD, residues 27–306), a receptor binding domain (RBD, residues 331–528) and two C-terminal domains (CTD1, residues 529–591, and CTD2, residues 592–686).22 Moreover, S2 contains an S2′ region (residues 817–832), a fusion peptide proximal region (FPPR, residues 833–855), heptad repeat 1 (HR1, residues 910–984), a central helix (CH, residues 985–1035),59,60 and a central helix (CH, residues 985–1035).59,60 The atomistic model of the graphene nanosheet (GN) was constructed by using VMD.81

**Molecular Docking.** After obtaining the structures of the closed and open trimer spike protein and the graphene nanosheet, we performed the docking study using AutoDock Vina (version 1.1.2)52 where the spike protein was treated as the receptor and the GN was treated as the ligand. Previous studies have shown that it is suitable to dock the GN (with a small size) to the proteins using AutoDock Vina.50,51,54 AutoDock Vina can provide the docking score and the root-mean-square deviation (RMSD). The docking score indicates the affinity between the receptor and the ligand, and the RMSD represents the difference in the position of the ligand from the best model. The parameter of exhaustiveness was set to the default value of 8. The ligand search space for docking was the whole protein surface based on the crystal structure. The grid box was set as 12.6 × 12.6 × 12.6 nm³ to accommodate the trimer protein. Finally, 20 binding conformations for each complex were given according to the binding docking scores (Tables S1–S4). We then selected three typical structures for each complex in WT and Omicron in the open and closed states based on the docking scores and the cluster analysis as the initial conformations for the following MD simulation (Figure 1a–d).

**All-Atom Molecular Dynamics (MD) Simulation.** The all-atom molecular dynamics (MD) simulations were performed based on the above selected structures. All the MD simulations were performed using the GROMACS software package (version 2020.4)55 with the CHARMM36 force field56 and TIP3P water model.57 The force-field parameters of graphene were acquired from a previous study.58 The spike protein–graphene complex was initially placed in a cubic box, and the minimum distance from the surface of the box to the complex atoms was set as 1.5 nm. NaCl was then added to neutralize the system, and the salt concentration was set as 0.15 M. LJ interactions were computed using a cutoff of 1.2 nm, and the particle-mesh Ewald summation method was used to calculate the long-range electrostatic interactions.59 The LINCS algorithm was used to constrain the bonds involving hydrogen atoms.60 Periodic boundary conditions were applied in all directions.

The system was first energy-minimized for about 50 000 steps using the steepest-descent method. After energy minimization, the GN and heavy atoms in the protein were restrained to perform a short simulation of 1 ns in the NPT ensemble. The temperature was maintained at 298 K with the velocity-rescale thermostat, and the pressure was kept at 1 bar using Berendsen coupling methods.61 Finally, the restraint of the GN and the protein was removed, and 200 ns free NVT simulations were performed for each system. The integration time step was 2 fs. VMD was used to analyze the trajectory and generate typical snapshots.81 The interface interactions of residues were analyzed using Pymol.
RESULTS AND DISCUSSION

Effect of GN on the Structure and Function of Spike Protein in the Closed State in WT. We first investigated the interaction between the GN and the spike protein in the closed state in WT, where three different binding models were observed (Figure 1a). After a 200 ns MD simulation, the RMSD of the spike protein and the contact surface area/interaction energy between the graphene and the spike protein became relatively stable in the three models (Figure S1). In models 1 and 2, the GN was located in the pocket composed of RBD in chain B (i.e., B_RBD) and NTD and RBD in chain C (i.e., C_NTD, C_RBD). While the GN was in a similar pocket, some differences between the two models were observed. In model 1, the GN was mainly in contact with the residues of C_NTD, accompanied by some edge atoms interacting with B_RBD (Figure 2a). However, in model 2, the
GN not only interacted with the C_NTD with its one corner but also inserted into the interface between two RBDs with its other corner (Figure 2b). Such a binding difference could also be found in the GN–residues contact map (Figure 2c,d).

We then investigated the effect of the GN on the structure of the spike protein in model 1. As shown in Figure S2a, there was no obvious change in the secondary structures of C_NTD and B_RBD, indicating that the internal structure of the spike protein was not affected by the GN. However, as shown in Figure 2e, the contact surface area (CSA) between B_RBD and C_NTD and that between B_RBD and C_RBD was changed after the insertion of the GN. In particular, the CSA between B_RBD and C_RBD was greatly increased from 6.94 to 9.02 nm\(^2\), leading to an obvious increase in their interaction strength. Due to the enhanced interaction with the C_RBD, the B_RBD was in a more stably closed state, which has been proved in previous studies.\(^{62-65}\)

Moreover, we also calculated the residue angle (Figure 3a) and region angle (Figure 3b) in model 1 and found that these angles were both decreased (Figure 3c,d). As illustrated in Figure 3, the decrease of the two angles probably means a tighter packing of RBD to the neighboring regions. Thus, the changes in the above angles demonstrated that the insertion of the GN in model 1 can enhance the down conformation of the RBD in the spike protein.

We further investigated the effect of the GN on the structure of the spike protein in model 2. Similarly, the secondary structures of the C_NTD, B_RBD, and C_RBD were not obviously affected by the GN (Figure S2d). Moreover, the CSA between the two RBDs decreased slightly (Figure 2e) due to the occupation of graphene at the interface, but their interaction energy did not change. The CSA between the C_NTD and B_RBD changed little, but due to the closer contact of more residues (Figure S2e,f), their interaction strength was increased a bit (Figure 2e). We also calculated the two angles in model 2, and the same trend was observed as that in model 1 (Figure 3e,f). In general, the binding of the GN close to the B_RBD can enhance the interaction of the B_RBD with its neighboring regions (the C_RBD in model 1 and the C_NTD in model 2), thus the GN can enhance the stability of the closed state of the spike protein, which may prevent the closed-to-open transition of the spike protein and the subsequent binding of the RBD to the ACE2 in host cells.

Apart from the above two models in which the GN was bound to the S1 subunit, a totally distinct model (i.e., model 3) was found, where the GN was occupied at the S2\(^{\prime}\) site and in the FP region of the S2 subunit. FP, composed of many hydrophobic residues, is responsible for embedding in the host cell membrane to mediate membrane fusion.\(^{6,31,68}\) Owing to the hydrophobicity of FP, the GN could be inserted in the vicinity of FP and remained stable during the simulation (Figure 4a,b). Notably, there are many residues in FP or the near-FP region that interact with TMPRSS2 to facilitate the cleavage at the S2\(^{\prime}\) site,\(^{69}\) which may be shielded by the insertion of the GN. As shown in Figure 4c, most of the ΔSASA of the residues was negative in the sites interacting with TMPRSS2, indicating that the solvent-accessible surface area of these residues was smaller than that in the absence of
Meanwhile, nearly all ΔRMSF values were negative, thus the flexibility of TMPRSS2 interaction sites was also weakened (Figure 4d). As a result, the probability of a spike protein interacting with TMPRSS2 became smaller, which can prevent the detachment of S1 from the whole spike protein and the subsequent activation of S2. In this sense, here the GN again exhibits some potential for antivirus infection, although its role is totally different from that in the previous two models.

Effect of GN on the Structure and Function of Spike Protein in the Closed State in Omicron. We then investigated the interaction between the GN and the spike protein in the closed state in Omicron. Similarly, three typical docking models were chosen (Figure 1b), and the binding of the GN to the spike protein became relatively stable within 200 ns in the MD simulations (Figure S3). Since there was little structural difference between the WT spike protein and the Omicron spike protein in the closed state (Figure S4), here the binding sites of the GN were similar to that in WT. However, the binding models was a bit different from that in WT due to some hydrophobic mutations (A67 V and Ins214EPE) in NTD (Figure S5). For example, the GNs were attached to the NTD without contacting the neighboring RBD in model 1 (Figure 5a). Hence, the CSA and the interaction between B_RBD and C_NTD and that between B_RBD and C_RBD were not affected by the GN (Figure S6c). Although the residue angle in model 1 decreased slightly, the region angle remained unchanged compared with that in the free state (Figure 5b,c). Thus, the GN had little impact on the function of the closed spike protein in mode 1 of Omicron.

In model 2, the GN was inserted into the interface between two RBDs (Figure 5d), similar to that in WT. The insertion of the GN resulted in the movement of B_RBD toward C_NTD and the closer contact of more residues (Figure S7). Although the CSA and the interaction between two RBDs was weakened,
the interaction between C_NTD and B_RBD was enhanced significantly due to the insertion of the GN (Figure S6c). Moreover, we also calculated the two angles in model 2 of Omicron, and both angles were decreased obviously (Figure 5e,f). Thus, here the GN can enhance the stability of the closed spike protein in Omicron, which could inhibit the down-to-up transition of RBD and subsequently the recognition by ACE2.

In Omicron, we also found that the GN could occupy the S2' site and the FP region (i.e., model 3; see Figure S8a,b). As shown in Figure S8, the ΔSASA and ΔRMSF of many sites in which the spike protein interacts with TMPRSS2, especially the S2' cleavage site, were negative. Similar to model 3 of WT, these results indicated that many sites were shielded and the flexibility of the residues was decreased due to the attachment of the GN, which would prevent the interaction of spike protein with TMPRSS2.

Effect of GN on the Structure and Function of Spike Protein in the Open State in WT. In this section, we investigated the interaction between the GN and the WT spike protein in the open state. Similarly, three typical models were also observed in this case, and the binding of the GN to the spike protein became relatively stable within 200 ns in the MD simulations (Figure S9). As mentioned above, there was one RBD in the up conformation in the open state, and the up RBD has larger region and residue angles than those in the closed state. For example, the region angle was distributed from 70 to 75°, and the residue angle was distributed from 50 to 57°. Notably, the larger the region and residue angles, the more up the RBD is, which is more favorable for binding to the ACE2.

When one RBD is up, the neighboring down RBD could adjust its configuration, and the space between the down RBD and the neighboring NTD becomes larger. Thus, the GN can insert into the fissure of B_NTD and A_RBD (i.e., model 1; see Figure 6a). Since the GN did not directly interact with the up RBD (i.e., B_RBD), the epitope in RBD (that binds to ACE2) cannot be occupied. Actually, there was no obvious decrease in the SASA of the key residues in the epitope (Figure S10a). Notably, the distance between B_NTD and A_RBD increased after the GN insertion (Figure S10c). This local rearrangement led to the adjustment of B_RBD. Thus, the CSA and the interaction strength between A_RBD and B_RBD increased a bit after the insertion of the GN (Figure S11c) in model 1, while the CSA and the interaction strength between the up RBD (i.e., B_RBD) and C_NTD decreased significantly (Figure S11c). This indicates that the up RBD preferred to attach to the neighboring down RBD rather than the neighboring C_NTD due to the insertion of GN, which is quite different from that in the free system (Figure S12). Notably, Li et al found that the up RBD is intrinsically inclined to tilt toward the more stable downward orientation and the NTD could block this motion as a wedge. In fact, as shown in Figure 6b,c, both the region angle and the residue angle were decreased in the presence of the GN, indicating that the “wedge” effect was impaired by the GN. Thus, here the more favorable conformation for the up RBD binding to ACE2 was prevented by the GN indirectly.

We also observed a model (i.e., model 2) in which the GN was bound to the S1 subunit of the spike protein. But different from model 1, the GN was inserted in the region between C_CTD1 and C_NTD, which was even in contact with the central axial of the spike protein (Figure 6d). There was also no obvious decrease of the SASA of the key residues in the epitope of RBD (Figure S10b). More importantly, the distance between C_NTD and C_CTD1 decreased after the GN insertion (Figure S10d), causing the movement of B_RBD. Therefore, there was an increase in the CSA and a decrease in the interaction energy between B_RBD and the A_RBD (Figure S11c), while the CSA and the interaction strength between the B_RBD and the C_NTD were reduced (Figure S11c). As a result, the “wedge” effect (on the up RBD) by the neighboring NTD was also impaired in this case. As shown in Figure 6e,f, although the residue angle did not change obviously, the region angle shifted toward the left, indicating that the tilt toward the down state was enhanced by the GN indirectly.
Figure 8. (a) CSA and the vdW and Coul interaction energy between the inserted domains. Frequency of the down RBD (C_RBD) residue angle and region angle in Omicron model 1 (b, c) and model 3 (d, e) in the open state.

Figure 9. (a) Final snapshot illustrating the interaction of the GN with the spike protein in the open state in Omicron model 2. (b) Time evolutions of the GN residues contact map. (c, d) ΔSASA and ΔRMSF of residues that mediate cleavage at the S2′ site.
Similar to the closed state, here we found that the GN can also be inserted into the S2 subunit in the open state (i.e., model 3). Due to the loose structure of the spike protein in the up conformation, the GN was mainly inserted into the region between S1 (NTD and CTD2) and S2 (FP, FPPR and HR1), which was different from the previous finding for the closed state. However, similar to the closed state, many residues interacting with TMPRSS2 were also hindered by the GN (Figure S13c). Meanwhile, in the presence of GN, the movement of the contact region was constrained, and the flexibility of the residues decreased a bit (all of the ΔRMSF values were negative, Figure S13d). Hence, the GN still weakened the interaction of the key residues at the S2′ site and in the FP region with TMPRSS2.

Effect of GN on the Structure and Function of Spike Protein in the Open State in Omicron. Similarly, three typical models were chosen to investigate the effect of the GN on the structure of the Omicron spike protein in the open state, and the interaction of the GN with the spike protein became stable within 200 ns in the MD simulations (Figures S14 and S15). Notably, there was an obviously structural difference between the Omicron spike protein and WT protein in the open state, and more importantly, the spike protein packed more tightly in the case of Omicron (Figure S4). As a result, the binding sites of GN here were quite different from that in WT. In more detail, the GN is attached to A_NTD with an edge interacting with the down RBD (C_RBD) instead of the up RBD (B_RBD) in models 1 and 3 of Omicron (Figure 7a,d). As a result, here the effects of the GN on the up RBD cannot be observed (Figure 7b,c,e,f). More importantly, the interaction of the GN with the down C_RBD constrained the movement of C_RBD, resulting in a more closed contact and a stronger interaction strength between them than in the free state (Figures 8a and S16), although the interaction between two RBDs was weakened slightly (Figure 8a). Moreover, both the residue and region angle of C_RBD were increased (Figure 8b–e) in the two models, indicating that the down B_RBD was prone to translate to the up conformation after the insertion of GN. Notably, a recent study found that the Omicron spike protein was predominantly in the open conformation, and when two or more RBDs were in the up conformation, the binding of the spike protein to the ACE2 was greatly enhanced (rather than in the case of one up RBD), which may further increase the infection ability of SARS-CoV-2. In general, in both models 1 and 3, the GN cannot decrease the probability of the binding of the up RBD to the ACE2 and could even simulate the down-to-up transition of the down RBD, which is certainly harmful.

Similar to WT model 3 in the open state, the GN was also inserted into the region between S1 and S2 in model 2 of Omicron, which is near the TMPRESS2 interaction sites (Figure 9a,b). Although the ΔSASA and ΔRMSF of some residues were negative (Figure 9c,d), almost all residues, involving the interactions between the spike protein and TMPRSS2, were not hindered by the GN (Figure 9b). Hence, the solvent-accessible surface area and the flexibility of these sites were not decreased by the GN, indicating that the interaction of the spike protein of Omicron with TMPRSS2 would not be affected by the GN in this case. In other words, here the insertion of GN cannot effectively prevent the detachment of S1 from the whole spike protein.

### CONCLUSIONS

We have systematically compared the interaction of a graphene nanosheet with the spike protein between the SARS-CoV-2 WT and Omicron in the closed and open states by using the molecular simulations. It is found that the GN can insert into the pocket composed of NTD and two RBDs in the closed spike protein of both WT and Omicron, which leads to a decrease in the residue/region angles and preserves the RBD in the down conformation. Moreover, although the GN cannot directly block the interaction of the up RBD with ACE2 in the open state in WT, the GN can impair the “wedge” effect of the NTD on the up RBD, which may decrease the probability of the binding of the up RBD to the ACE2 indirectly. Nevertheless, the GN cannot decrease the probability of the binding of the up RBD to the ACE2 and could even stimulate the down-to-up transition of the down RBD in the open state in Omicron, which may increase the infection ability of Omicron. Furthermore, the GN can prevent the interaction of FP with TMPRSS2 and inhibit the detachment of S1 from the whole spike protein in both closed and open states of WT, while such a result is found only in the close state of Omicron but disappears in the open state of Omicron.

Although this study sheds some light on the underlying mechanism of the GN–spike protein interaction in both WT and Omicron, it does have some limitations or unsolved problems. First, previous studies indicated that the size of the GN could be an important factor in the GN–protein interactions, thus the effect of the size of the GN deserves to be systematically investigated. However, since here we mainly focused on the effects of the state and the mutation of the spike protein on the result and the simulation system is quite large (there are more than 700,000 atoms in the simulation box), we did not conduct a systematic investigation of the size effect but gave some inference based on the docking results. As shown in Figure S17, when the size of the GN is smaller (1.0 × 1.0 nm²), the GN can bind everywhere in the spike protein, and due to its small size, the effect of the GN on the function of the spike protein could be negligible. When the size of the GN is greater (5.0 × 5.0 nm²), the typical models are similar to that in the case of 3.0 × 3.0 nm² GN (Figure S18), indicating that the slight increase in the size has little impact on the result. With further increases in the size of the GN, the docking result may be invalid since the GN can no longer be treated as a ligand, which is beyond the scope of the present study.

Second, the spike protein of SARS-CoV-2 is highly glycosylated, thereby the glycan on the surface of the spike protein could affect its interaction with nanomaterials. However, in this work, the initial structures for the MD simulations were based on the docking results. Thus, the accuracy of the docking result has a great impact on the MD result. Unfortunately, although the AutoDock Vina was successfully used in the docking of the ligand or small GN to the proteins, the docking accuracy of the GN to the glycosylated protein has not been validated since the glycan is highly flexible, and the optimal parameters that can well describe the CH−π interaction and torsional glycosidic linkage are still lacking in the present docking methods. On the other hand, if we did not use the docking result as the initial structure (for the MD simulation), we should place the GN randomly in the simulation box. Nevertheless, due to the large system modeled here and, more importantly, the very long
time (∼ns) needed for the GN to search and bind to the protein pocket, it is impossible to run such a simulation in the enhanced stage. One possible way to solve this problem is to use an enhanced sampling method such as replica exchange molecular dynamics (REMD) or Gaussian accelerated molecular dynamics (GaMD). But due to the large number of atoms (the number of replicas is usually proportional to the number of atoms), the computational cost could also be very expensive, and it is still a challenging problem to simulate such a system even using the enhanced sampling method.

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References

1. Kim, D.; Lee, J. Y.; Yang, J. S.; Kim, J. W.; Kim, V. N.; Chang, H. The architecture of SARS-CoV-2 transcriptome. Cell 2020, 181, 914–921.
2. Chan, J. F.-W.; Kok, K. H.; Zhu, Z.; Chu, H.; To, K. K.-W.; Yuan, S.; Yuen, K.-Y. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. Emerg. Microbes Infect. 2020, 9, 221–236.
3. Zhou, P.; Yang, X. L.; Wang, X. G.; Hu, B.; Zhang, L.; Zhang, W.; Si, H. R.; Zhu, Y.; Li, B.; Huang, C. L. A pneumonia outbreak with 20 novel coronavirus of probable bat origin. Nature 2020, 579, 270–273.
4. Shin, M. D.; Shukla, S.; Chung, Y. H.; Beiss, V.; Chan, S. K.; Ortega-Rivera, O. A.; Wirth, D. M.; Chen, A.; Sark, M.; Pokorski, J. K. COVID-19 vaccine development and a potential nanomaterial path forward. Nat. Nanotechnol. 2020, 15, 646–655.
5. Jungreis, I.; Sealfon, R.; Kellis, M. SARS-CoV-2 gene content and COVID-19 mutation impact by comparing 44 Sarbecovirus genomes. Nat. Commun. 2021, 12, 2642.
6. Tang, T.; Bidon, M.; James, J. A.; Whittaker, G. R.; Daniel, S. Coronavirus membrane fusion mechanism offers a potential target for antiviral development. Antivir. Res. 2020, 178, 104792.
7. Shang, J.; Ye, G.; Shi, K.; Wan, Y.; Luo, C.; Ahara, H.; Geng, Q.; Auerbach, A.; Li, F. Structural basis of receptor recognition by SARS-CoV-2. Nature 2020, 581, 221–224.
8. Shang, J.; Wan, Y.; Luo, C.; Ye, G.; Geng, Q.; Auerbach, A.; Li, F. Cell entry mechanisms of SARS-CoV-2. Proc. Natl. Acad. Sci. U.S.A. 2020, 117, 11727–11734.
9. Yan, R.; Zhang, Y.; Li, Y.; Xia, L.; Guo, Y.; Zhou, Q. Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science 2020, 367, 1444–1448.
10. Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020, 581, 215–220.
11. Letko, M.; Marzi, A.; Munster, V. Functional assessment of cell entry and receptor usage for SARS-CoV-2 and other lineage B betacoronaviruses. Nature microbiology 2020, 5, 562–569.
12. Ding, H. M.; Yin, Y. W.; Sheng, Y. J.; Ma, Y. Q. Accurate evaluation on the interactions of SARS-CoV-2 with its receptor ACE2 and antibodies CR3022/BC6. Chin. Phys. Lett. 2021, 38, No. 018701.
13. Walls, A. C.; Park, Y. J.; Tortorici, M. A.; Wall, A.; McGuire, A. T.; Veesler, D. Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein. Cell 2020, 181, 281–292.
14. Wrapp, D.; Wang, N.; Corbett, K. S.; Goldsmith, J. A.; Hsieh, C.-L.; Abiona, O.; Graham, B. S.; McLellan, J. S. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020, 367, 1260–1263.
15. Zhang, Y.; Kutateladze, T. G. Molecular structure analyses suggest strategies to therapeutically target SARS-CoV-2. Nat. Commun. 2020, 11, 2920.
16. Cameroni, E.; Bowen, J. E.; Rosen, L. E.; Saliba, C.; Zepeda, S. K.; Culp, K.; Pinto, D.; VanBlargan, L. A.; De Marco, A.; di Iulio, J. Broadly neutralizing antibodies overcome SARS-CoV-2 Omicron antigenic shift. Nature 2022, 602, 664–670.
17. Cao, Y.; Wang, J.; Jian, F.; Xiao, T.; Song, W.; Yisimayi, A.; Huang, W.; Li, Q.; Wang, P.; An, R. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. Nature 2022, 602, 657–663.
(25) Breit, D.; Heindl, M. R.; Limburg, H.; Pilgram, O.; Moulton, H.; Stein, D. A.; Hardes, K.; Eickmann, M.; Dolnik, O.; Rohde, C.; Hoffmann, M.; Kleine Weber, H.; Pöhlmann, S. A multibasic cleavage site in the spike protein of SARS-CoV-2 is essential for virus entry and its immune cross-reactivity with SARS-CoV. Acta Pharmacol. Sin. 2020, 41, 1141–1149.

(26) Mounier, B.; Alfaee, M.; Dighe, K.; Freshman, M. B.; Pan, D.; Nitsche, A. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020, 181, 271–280.

(27) Maunsbach, R. A.; Chakrabarty, S.; Nguy, K.; Montefiori, D. C.; Korber, B.; Gnann, L. S. The SARS-CoV-2 spike variant D614G favors an open conformational state. Science advances 2021, 7, eabf3671.

(28) Huang, Y.; Yang, C.; Xu, X. X.; Xu, W.; Liu, S. W. Structural and functional properties of SARS-CoV-2 spike protein: potential antiviral drug development for COVID-19. Acta Pharmacol. Sin. 2020, 41, 1141–1149.

(29) Fan, X.; Cao, D.; Kong, L.; Zhang, X. Cryo-EM analysis of the post-fusion structure of the SARS-CoV-2 spike glycoprotein. Nat. Commun. 2020, 11, 3618.

(30) Xia, S.; Zhu, Y.; Liu, M.; Lan, Q.; Xu, W.; Wu, Y.; Ying, T.; Liu, S.; Shi, Z.; Jiang, S. Fusion mechanism of 2019-nCoV and fusion inhibitors targeting HR1 domain in spike protein. Cell. Mol. Immunol. 2020, 17, 765–767.

(31) Koppinetti, R. K.; Fulcher, Y. G.; Van Doren, S. R. Fusion peptide of SARS-CoV-2 spike rearranges into a wedge inserted in bilayer micelles. J. Am. Chem. Soc. 2021, 143, 13205–13211.

(32) Miotra, F.; Alaeef, M.; Dighe, K.; Freshman, M. B.; Pan, D. Selective naked-eye detection of SARS-CoV-2 mediated by N gene targeted antisense oligonucleotide capped plasmic nanoparticles. ACS Nano 2020, 14, 7617–7627.

(33) Zhao, Z.; Cui, H.; Song, W.; Xu, Y.; Zhou, W.; Yu, X. A simple magnetic nanoparticle-based viral RNA extraction method for efficient detection of SARS-CoV-2. biosens. bioelectron 2020, DOI: 10.1011/2020.02.22.961268.

(34) Seo, G.; Lee, G.; Kim, M. J.; Baek, S. H.; Choi, M.; Ku, K. B.; Lee, C. S.; Jun, S.; Park, D.; Kim, H. G. Rapid detection of COVID-19 causative virus (SARS-CoV-2) in human nasopharyngeal swab specimens using field-effect transistor-based biosensor. ACS Nano 2020, 14, 5135–5142.

(35) Chen, Z.; Zhang, Z.; Zhai, X.; Li, Y.; Lin, L.; Zhao, H.; Bian, L.; Li, P.; Yu, L.; Wu, Y. Rapid and sensitive detection of anti-SARS-CoV-2 IgG using lanthanide-doped nanoparticles-based lateral flow immunoassay. Anal. Chem. 2020, 92, 7226–7231.

(36) Qiu, G.; Gai, Z.; Tao, Y.; Schmitt, J.; Kullak Ulbig, G. A.; Wang, J. Dual-functional plasmic photothermal biosensors for highly accurate severe acute respiratory syndrome coronavirus 2 detection. ACS Nano 2020, 14, 5268–5277.

(37) Sun, R.; Chu, K. Y.; Shen, A. Q. Detection of antibodies against SARS-CoV-2 spike protein by gold nanospikes in an opto- microfluidic chip. Biosens. Bioelectron. 2020, 115, 112578.

(38) Kumar, S.; Karmacharya, M.; Joshi, S. R.; Gulenko, O.; Park, J.; Kim, G. H.; Cho, Y. K. Photocative antiviral face mask with self-sterilization and reusability. Nano Lett. 2021, 21, 337–343.

(39) Chen, M.; Rosenberg, J.; Cai, X.; Lee, A. C. H.; Shi, J.; Nguyen, M.; Wignakumar, T.; Mirle, V.; Edobor, A. J.; Fung, J. Nanotrays for the containment and clearance of SARS-CoV-2. Matter 2021, 4, 2059–2082.

(40) Lee, Y.; Kang, B. H.; Kang, M.; Chung, D. R.; Yi, G. S.; Lee, L. P.; Jeong, K. H. Nanoplasmonic on-chip PCR for rapid precision molecular diagnostics. ACS Appl. Mater. Interfaces 2020, 12, 12533–12540.

(41) Balagna, C.; Perero, S.; Percivalle, E.; Nepita, E. V.; Ferraris, M. Virucidal effect against coronavirus SARS-CoV-2 of a silver nanocolloidal/silica composite sputtered coating. Open Ceramics 2020, 1, 100006.

(42) De Maio, F.; Palmieri, V.; Babini, G.; Augello, A.; Palucci, I.; Perini, G.; Salustri, A.; Spilman, P.; De Spirito, M.; Sanguinetti, M. Graphene nanoplatelet and Graphene oxide functionalization of face mask materials inhibits infectivity of trapped SARS-CoV-2. Sciencenews 2021, 24, 102788.

(43) Yin, Y. W.; Sheng, Y. J.; Wang, M.; Ma, Y. Q.; Ding, H. M. Interaction of serum proteins with SARS-CoV-2 RBD. Nanoscale 2021, 13, 12865–12873.

(44) Donskii, I. S.; Nie, C.; Ludwig, K.; Trimpert, J.; Ahmed, R.; Qauss, E.; Achazi, K.; Radnik, J.; Adeli, M.; Haag, R. Graphene sheets with defined dual functionalities for the strong SARS-CoV-2 interactions. Small 2021, 17, 2007091.

(45) Unal, M. A.; Bayrakdar, F.; Nazir, H.; Besbinar, O.; Gurcan, C.; Lozano, N.; Arellano, L. M.; Yalcin, S.; Panatli, O.; Celik, D. Graphene Oxide Nanosheets Interact and Interfere with SARS-CoV-2 Surface Proteins and Cell Receptors to Inhibit Infectivity. Small 2021, 17, 2101483.

(46) Pramanik, A.; Sharma, P. C.; Patibandla, S.; Gao, Y.; Ruppa-Kasani, V.; Goli, J.; Kumar, A.; Chatterjee, A.; Sinha, S. S.; Bates, J. T. Blocking SARS-CoV-2 Delta Variant (B. 1.617. 2) Spike Protein Receptor-Binding Domain Binding with the ACE2 Receptor of the Host Cell and Inhibiting Virus Infections Using Human Host Defense Peptide-Conjugated Graphene Quantum Dots. ACS omega 2022, 7, 8150–8157.

(47) Du, J.; Yang, C.; Ma, X.; Li, Q. Insights into the conformation changes of SARS-CoV-2 spike receptor-binding domain on graphene. Appl. Surf. Sci. 2022, 578, 151934.

(48) Yin, W.; Xu, Y.; Xu, P.; Cao, X.; Wu, C.; Gu, C.; He, X.; Wang, X.; Huang, S.; Yuan, Q. Structures of the Omicron Spike trimer with ACE2 and an anti-Omicron antibody. Science 2022, 375, 1048–1053.

(49) Waterhouse, A.; Berto, M.; Biennert, S.; Studer, G.; Tauriello, G.; Gumienery, R.; Heer, F. T.; de Beer, T. A. P.; Rempfer, C.; Bordoli, L. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 2018, 46, W296–W303.

(50) Xiong, X.; Qu, K.; Ciazynska, K. A.; Hosmillo, M.; Carter, A. P.; Ebrahimi, S.; Ke, Z.; Scheres, S. H.; Bergamaschi, L.; Grice, G. L. A thermostable, closed SARS-CoV-2 spike protein trimer. Nat. Struct. Mol. Biol. 2020, 27, 934–941.

(51) Humphrey, W.; Dalke, A.; Schulten, K. VMD: visual molecular dynamics. J. Mol. Graph. 1996, 14, 33–38.

(52) Trot, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J. Comput. Chem. 2010, 31, 455–461.
(53) Raval, B.; Srivastav, A. K.; Gupta, S. K.; Kumar, U.; Mahapatra, S.; Gajar, P.; Banerjee, I. Synthesis of exfoliated multilayer graphene and its putative interactions with SARS-CoV-2 virus investigated through computational studies. *J. Biolom. Struct. Dyn.* 2022, 40, 712–721.

(54) Patel, V.; Shah, J.; Gupta, A. K. Design and In-silico study of bioimaging fluorescence Graphene quantum dot-Bovine serum albumin complex synthesized by diimide-activated amidation. *Comput. Biol. Chem.* 2021, 93, 107543.

(55) Abraham, M. J.; Murtola, T.; Schulz, R.; Páll, S.; Smith, J. C.; Hess, B.; Lindahl, E. GROMACS: High performance molecular simulations through multi-level parallelism from laptops to supercomputers. *SoftwareX* 2015, 1, 19–25.

(56) Huang, J.; MacKerell, A. D., Jr CHARMm36 all-atom additive protein force field: Validation based on comparison to NMR data. *J. Comput. Chem.* 2013, 34, 2135–2145.

(57) Jorgensen, W. L.; Madura, J. D. Quantum and statistical mechanical studies of liquids. 25. Solvation and conformation of methanol in water. *J. Am. Chem. Soc.* 1983, 105, 1407–1413.

(58) Chong, Y.; Ge, C.; Yang, Z.; Garase, J. A.; Gu, Z.; Weber, J. K.; Liu, J.; Zhou, R. Reduced cytotoxicity of graphene nanosheets mediated by blood-protein coating. *ACS Nano* 2015, 9, 5713–5724.

(59) Eismann, U.; Pererà, L.; Berkowitz, M. L.; Darden, T.; Lee, H.; Pedersen, L. G. A smooth particle mesh Ewald method. *J. Chem. Phys.* 1995, 103, 8577–8593.

(60) Hess, B.; Bekker, H.; Berendsen, H. J.; Fraaije, J. G. LINCS: a linear constraint solver for molecular simulations. *J. Comput. Chem.* 1997, 18, 1463–1472.

(61) Berendsen, H. J.; Postma, J. v.; Van Gunsteren, W. F.; DiNola, A.; Haak, J. R. Molecular dynamics with coupling to an external bath. *J. Chem. Phys.* 1984, 81, 3684–3690.

(62) Toelzer, C.; Gupta, K.; Yadav, S. K.; Borucu, U.; Davidson, A. D.; Williamson, M. K.; Shoemark, D. K.; Garzoni, F.; Stauffer, O.; Milligan, R. Free fatty acid binding pocket in the locked structure of SARS-CoV-2 spike protein. *Science* 2020, 370, 725–730.

(63) Mori, T.; Jung, J.; Kobayashi, C.; Dokainish, H. M.; Re, S.; Sugita, Y. Elucidation of interactions regulating conformational stability and dynamics of SARS-CoV-2 S-protein. *Biophys. J.* 2021, 120, 1060–1071.

(64) Bangaru, S.; Ozoowski, G.; Turner, H. L.; Antanasijevic, A.; Huang, D.; Wang, X.; Torres, J. L.; Diedrich, J. K.; Tian, J.-H.; Portnoff, A. D. Structural analysis of full-length SARS-CoV-2 spike protein from an advanced vaccine candidate. *Science* 2020, 370, 1089–1094.

(65) Wrobel, A. G.; Benton, D. J.; Xu, P.; Roustan, C.; Martin, S. R.; Rosenthal, P. B.; Skehel, J. J.; Gamblin, S. J. SARS-CoV-2 and bat RaTG13 spike glycoprotein structures inform on virus evolution and furin-cleavage effects. *Nat. Struct. Mol. Biol.* 2020, 27, 763–767.

(66) Peng, C.; Zhu, Z.; Shi, Y.; Wang, X.; Mu, K.; Yang, Y.; Zhang, X.; Xu, Z.; Zhu, W. Computational insights into the conformational accessibility and binding strength of SARS-CoV-2 spike protein to human angiotensin-converting enzyme 2. *J. Phys. Chem. Lett.* 2020, 11, 10482–10488.

(67) Li, Y.; Wang, T.; Zhang, J.; Shao, B.; Gong, H.; Wang, Y.; He, X.; Liu, S.; Liu, T. Y. Exploring the Regulatory Function of the N-terminal Domain of SARS-CoV-2 Spike Protein through Molecular Dynamics Simulation. *Adv. Theory Simul.* 2021, 4, 2100152.

(68) Gorgun, D.; Lihan, M.; Kapoor, K.; Tjahkhorshid, E. Binding mode of SARS-CoV-2 fusion peptide to human cellular membrane. *Biophys. J.* 2021, 120, 2914–2926.

(69) Hussain, M.; Jabeen, N.; Amanullah, A.; Baig, A. A.; Aziz, B.; Shabbir, S.; Raza, F.; Uddin, N. Molecular docking between human TMPRSS2 and SARS-CoV-2 spike protein: conformation and intermolecular interactions. *AIMS microbiology* 2020, 6, 350–360.

(70) Pak, A. J.; Yu, A.; Ke, Z.; Briggs, J. A.; Voth, G. A. Cooperative multivalent receptor binding promotes exposure of the SARS-CoV-2 fusion machinery core. *Nat. Commun.* 2022, 13, 1002.