Exploring the immune evasion of SARS-CoV-2 variant harboring E484K by molecular dynamics simulations

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Abstract

Although the current coronavirus disease 2019 (COVID-19) vaccines have been used worldwide to halt spread of the severely acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the emergence of new SARS-CoV-2 variants with E484K mutation shows significant resistance to the neutralization of vaccine sera. To better understand the resistant mechanism, we calculated the binding affinities of 26 antibodies to wild-type (WT) spike protein and to the protein harboring E484K mutation, respectively. The results showed that most antibodies (~85%) have weaker binding affinities to the E484K mutated spike protein than to the WT, indicating the high risk of immune evasion of the mutated virus from most of current antibodies. Binding free energy decomposition revealed that the residue E484 forms attraction with most antibodies, while the K484 has repulsion from most antibodies, which should be the main reason of the weaker binding affinities of E484K mutant to most antibodies. Impressively, a monoclonal antibody (mAb) combination was found to have much stronger binding affinity with E484K mutant than WT, which may work well against the mutated virus. Based on binding free energy decomposition, we predicted that the mutation of four more residues on receptor-binding domain (RBD) of spike protein,
Introduction

The SARS-CoV-2 has already caused over 168 million confirmed cases and over 3 million deaths worldwide by the end of May 2021 [1]. Although there are no specifically effective drugs on market to cure the COVID-19, exceeding 80 vaccines are in clinical development and exceeding 180 vaccines are in pre-clinical development [2]. In fact, vaccines have been widely used to curtail the disease, and mAb therapeutic has attracted great concerns [3, 4].

Current mAbs and vaccines were designed based on the initial SARS-CoV-2 identified at the end of 2019. However, SARS-CoV-2, as an RNA virus, was expected to have a high mutation rate [3]. Since identified in the early phases of the COVID-19 pandemic, considerable variants have been reported, including fast-spreading variants appeared in the UK (B.1.1.7), South Africa (B.1.351) and Brazil (B.1.1.248). Several studies have shown that neutralization by some mAbs and immune sera was diminished against the SARS-CoV-2 variants, particularly for the B.1.351 harboring E484K [5]. For instance, Novavax vaccine (NVX-diminished against the SARS-CoV-2 variants, particularly for the E484K mutant is unlikely caused by its ACE2 binding affinity but its mAb interaction.

Considering that the RBD is a dominant target of neutralizing antibodies and its E484K mutation has led to unsettling immune evasion, it is of great importance to understand the underlying mechanism for better coping with the E484K mutation. In this paper, we calculated the binding affinities of current 26 mAbs to RBDWT and to RBDE484K, respectively, alarming that 22 mAbs have decreased binding affinity to the E484K mutant. Impressively, a mAb combination (52 & 298) was predicted to have enhanced binding affinity. In addition, we predicted other four detrimental mutations that may cause potential immune evasion from most of the currently available mAbs.

Key words: COVID-19; spike; E484K; antibody; MM/GBSA
Results and discussion

Mapping the binding sites of current mAbs on spike protein

Figure 1 shows that the binding sites of current mAbs are largely focused on the S1 fragment of the spike protein, including the N-terminal domain (NTD), the base and the receptor-binding motif (RBM) of RBD, respectively. There are more than ten mAbs targeting NTD that have available crystal structures in PDB, for instance, 4A8 (PDB ID: 7C2L) [25], FC05 (PDB ID: 7CWU) [26] and 2–17 (PDB ID: 7LQW) [27]. Besides, Acharya P. et al. presented a cryo-EM structure of a glycan-dependent mAb (PDB ID: 2G12) targeting N709, N717 and N801 [28], revealing a new S2 epitope on SARS-CoV-2 spike. Although the RBD ‘up’ state is required for the ACE2 binding of spike protein [29], RBD could bind to mAbs in its ‘down’ state. For example, S2E12 (PDB ID: 7K4N) binds to RBD that is in ‘up’ state, while S2M11 (PDB ID: 7K43) binds to RBD that is in ‘down’ state [30]. By structural analysis, we found 26 mAbs bind to RBM and even near the position of E484, indicating that most of the current mAbs are likely influenced by the E484K mutation.

The difference of binding free energy between WT and E484K mutant RBDS to the mAb REGN10933

Recently, Hansen et al. found that the neutralization IC_{50} of REGN10933 on the E484K mutant and SA\Delta9 (B.1.351) variant was 10.5 and 58.8 times lower than that of the WT, respectively [9]. We calculated the binding free energy (\Delta G) of REGN10933 to the WT and E484K mutant, respectively. As shown in Figure 1 (Table S2 for details), the \Delta G to the mutant RBD is \(-24.71 \pm 0.67\), \(-21.13 \pm 0.67\), and \(-29.61 \pm 0.77\) kcal/mol, respectively, in triple runs, while that to the WT is \(-36.19 \pm 0.91\), \(-36.12 \pm 0.79\), and \(-41.61 \pm 0.69\) kcal/mol, respectively, in triple runs. The significantly reduced \Delta G of the SARS-CoV-2 harboring E484K mutation to REGN10933 suggests its weakened neutralization efficacy, which is consistent with the experimentally determined neutralization IC_{50} values (resistance 10.5-fold) [9].

To evaluate whether the E484K mutation affects the ACE2 binding affinity, we calculated the \Delta G of ACE2 to the WT and the E484K mutant, which are \(-36.43 \pm 0.77\) (\Delta G_{WT}) and \(-41.52 \pm 0.69\) kcal/mol (\Delta G_{E484K}), respectively. The slightly increased binding free energies demonstrated that E484K mutation has a little beneficial effect on the binding strength of ACE2 to the RBD, which is consistent with biophysical studies [15]. We decomposed the binding free energy and found that the residue E484 contributed 0.82 kcal/mol to the overall binding strength, indicating that E484 is not a key residue for the RBD-ACE2 interactions. Therefore, the special role of E484K mutation in immune evasion could be attributed to its significantly reduced binding affinity to mAbs.

The diverse impacts of E484K mutation on its binding affinities to 26 mAbs

There are 25 mAb-RBD complex structures found in PDB, including 26 mAbs. To explore the potential effect of the E484K
mutation on its binding to those mAbs, we constructed 25 complex structures of the E484K mutant RBD and the 26 mAbs. Then, the binding free energy calculation was performed for the 50 complexes. After analyzing the $\Delta G$ of seven different lengths of MD simulation time (Table S3), viz., 4–8, 4–12, 4–16, 4–20, 8–20, 12–20 and 16–20 ns, we found that the average $\Delta G$ fluctuation between 4–16 and 4–20 ns is only 1.25 kcal/mol ($P = 0.99$), showed that 20 ns MD simulation is long enough to obtain reliable $\Delta G$, which is consistent with our previous study [13, 31] and other literature reports [11, 32, 33]. To further explore the impact of simulation time on binding free energy, two REGN10933-RBD complexes for WT and E484K mutant, respectively, were run for 500 ns. As shown in Figure S2 and Table S1, the same variation trend of binding free energy change was observed by the two different simulation time.

With 2.00 kcal/mol as criteria, 22 systems show weaker binding affinity of mAb-RBDE484K than mAb-RBDWT (Table S2), indicating that 85% of the RBD-targeting mAbs might exhibit weaker neutralization ability to the E484K mutant virus. Tortorici M. A. et al. showed that S2M11 (PDB ID: 7K43) possessed lower neutralization ability in the E484K mutant [30], and Chen R. E. et al. showed that the neutralization ability of S2E12 (PDB ID: 7K4N) was reduced about 5 folds against the E484K virus [34]. Therefore, our predictions (Figure 2) are in good agreement with these experimental results, indicating the high reliability of the simulation results.

Impressively, one mAb combination system (PDB ID: 7K9Z) with two mAbs (52 & 298) has stronger binding affinity to E484K mutant than to WT ($\Delta G = −15.87 ± 1.13$ kcal/mol), which may work well to fight against the mutated virus. Two systems, viz., 6XE1 (CV30) [35] and 7CDJ (P2C-1A3) [36], have similar binding free energies between WT and E484K mutant ($\Delta G < 2.00$ kcal/mol, Table S2), indicating that the neutralization of these mAbs is insensitive to the E484K mutation. In summary, the E484K mutation might cause an obviously weakened binding affinity of about 85% mAbs to the spike protein of SARS-CoV-2.

**Effect of protein glycosylation on the calculated binding free energy**

Protein glycosylation plays a crucial role in modulating the conformational dynamics of spike’s RBD. Specifically, N-glycans at N165 and N234 potentially affect RBD binding to ACE2 [37]. As representatives of RBD-up and RBD-down states, we built two glycosylated SARS-CoV-2 spike models at GLYCAMP-Web (www.glycam.org) based on the structures of 7K43 (RBD-down, Figure S3) and 7K4N (RBD-up, Figure S4). In total, 51 N-linked glycans (Table S4) were added to the spike protein, and the calculated binding free energy of mAb-spike (glycan) was summarized in Table 1.

For the RBD-down state (7K43), the sum of N-glycan contribution to the overall binding free energy is $-1.06 ± 0.16$ kcal/mol.
Table 1. The predicted mAb-spike binding free energies of the systems 7K43 and 7K4N (kcal/mol)

| PDB ID  | System-Type   | $\Delta G_{WT}$ | $\Delta G_{E484K}$ | $\Delta G$  | $|\Delta G|/\Delta G_{WT}|$ |
|---------|---------------|-----------------|-------------------|-------------|-----------------|
| 7K43 (S2M11) Glyco-trimer | $-60.41 \pm 0.96$ | $-37.90 \pm 1.10$ | $22.51 \pm 1.03$ | 37.26%         |
| 7K43 (S2M11) Trimer       | $-52.39 \pm 0.64$ | $-34.86 \pm 0.92$ | $17.53 \pm 0.78$ | 33.46%         |
| 7K43 (S2M11) RBD          | $-42.02 \pm 0.53$ | $-25.67 \pm 0.56$ | $16.34 \pm 0.55$ | 38.90%         |
| 7K4N (S2E12) Glyco-trimer | $-36.96 \pm 0.48$ | $-28.22 \pm 0.62$ | $8.74 \pm 0.55$  | 23.65%         |
| 7K4N (S2E12) Trimer       | $-47.13 \pm 0.60$ | $-24.79 \pm 0.48$ | $22.34 \pm 0.54$ | 47.40%         |
| 7K4N (S2E12) RBD          | $-47.93 \pm 0.73$ | $-39.70 \pm 0.49$ | $8.23 \pm 0.61$  | 17.17%         |

Table 2. The predicted binding free energies of 7 mAbs to spike trimer or to RBD (kcal/mol)

| PDB ID  | System type | $\Delta G_{WT}$ | $\Delta G_{E484K}$ | $\Delta G$  | $|\Delta G|/\Delta G_{WT}|$ |
|---------|-------------|-----------------|-------------------|-------------|-----------------|
| 7K43 (S2M11) Trimer | $-52.39 \pm 0.64$ | $-34.86 \pm 0.92$ | $17.53 \pm 0.78$ | 33.46%         |
| 7K43 (S2M11) RBD    | $-42.02 \pm 0.53$ | $-25.67 \pm 0.56$ | $16.34 \pm 0.55$ | 38.90%         |
| 7K4N (S2E12) Trimer | $-47.13 \pm 0.60$ | $-24.79 \pm 0.48$ | $22.34 \pm 0.54$ | 47.40%         |
| 7K4N (S2E12) RBD    | $-47.93 \pm 0.73$ | $-39.70 \pm 0.49$ | $8.23 \pm 0.61$  | 17.17%         |
| 7K8T (C002) Trimer  | $-45.25 \pm 1.06$ | $-13.37 \pm 0.85$ | $31.89 \pm 0.97$ | 70.46%         |
| 7K8T (C002) RBD     | $-48.90 \pm 1.11$ | $-18.39 \pm 1.03$ | $30.51 \pm 1.03$ | 62.39%         |
| 7DK4 (2H2) Trimer   | $-60.54 \pm 1.16$ | $-18.89 \pm 1.03$ | $41.65 \pm 1.03$ | 68.46%         |
| 7DK4 (2H2) RBD      | $-60.29 \pm 0.72$ | $-17.37 \pm 0.78$ | $42.92 \pm 0.78$ | 69.46%         |
| 7K8X (C121) Trimer  | $-49.03 \pm 1.08$ | $-14.98 \pm 1.22$ | $34.05 \pm 1.22$ | 69.46%         |
| 7K8X (C121) RBD     | $-77.42 \pm 0.78$ | $-25.92 \pm 0.78$ | $51.50 \pm 0.78$ | 66.52%         |
| 7K90 (C144) Trimer  | $-37.67 \pm 0.86$ | $-18.34 \pm 1.14$ | $19.33 \pm 1.14$ | 51.32%         |
| 7K90 (C144) RBD     | $-37.67 \pm 0.86$ | $-18.34 \pm 1.14$ | $19.33 \pm 1.14$ | 51.32%         |

Figure 3. The contribution of E484 or K484 to overall binding free energy. Per residue energy decomposition is based on 4–20 ns MD trajectories of mAb-RBD systems.

(WT) and $2.20 \pm 0.06$ kcal/mol (E484K), respectively. The $\Delta G$ between the WT and E484K mutant are $22.51 \pm 1.03$, $17.53 \pm 0.78$ and $16.34 \pm 0.55$ kcal/mol for glycosylated spike trimer, the spike trimer and the RBD, respectively (Table 1). The results showed that the glycosylation of spike protein may change the specific values of the calculated free energy, but does not alter the variation tendency of the E484K mutation effect.

For the RBD-up state (7K4N), similar results were observed, the sum of N-glycan contribution in the predicted binding free energy is also very small, which is $0.06 \pm 0.00$ kcal/mol (WT) and $0.97 \pm 0.04$ kcal/mol (E484K), respectively. Additionally, we compared seven pairs of mAb-spike (trimer) and mAb-RBD (Table 2), the same effect tendency of E484K mutation on binding free energy was observed by using trimer structure or RBD only.

Hence, we postulated that the binding free energy of mAb-RBD can reflect the overall change tendency of mAb-spike binding free energy.

The role of E484 and K484

To further explore the mechanism of the E484K mutation affecting the neutralization ability of mAbs, we compared energy contributions of E484 and K484 to the overall binding free energy (Figure 3, Table S2). In most of mAb-RBD systems (72%), E484 have obvious greater contribution ($\gtrsim 1.00$ kcal/mol) to the binding free energy than that of K484. To further look into different binding modes, 3 mAb-RBD complexes are taken as representative examples, whose neutralization ability is decreased (PDB ID:
7CWO), unchanged (PDB ID: 6XE1) and enhanced (PDB ID: 7K9Z) after the E484K mutation, respectively.

The energy contribution of E484 in 7CWO (P17) is $-16.80 \pm 2.35$ kcal/mol (a strong attraction), while the energy contribution of K484 is $10.24 \pm 3.08$ kcal/mol (a strong repulsion). By structural analyses, we found that the negatively charged E484 could form a strong electrostatic interaction with mAb, while the positively charged K484 should be repulsive to the positively charged binding site of the mAb (Figure 4A and B). For the two mAb-RBD systems (6XE1 and 7CDJ) that share similar binding affinities between K484 mutant and WT, the difference in the residue contribution to overall binding free energy is also slight. By analyzing the crystal structure, e.g., 6XE1, we found that the position of E484 is 7.7 Å away from the mAb-RBD interface (Figure 4C and D); therefore, the E484K mutation should have little effect on the overall binding free energy. However, the binding site for E484 in 7K9Z is negatively charged (Figure 4E); therefore, the E484K mutation should enhance the binding of RBD to mAbs (Table S2). By decomposing the binding free energy of 7K9Z complexes, four residues in the K484 mutant RBD (Q474, T478, E465 and R466) have stronger binding strength with 7K9Z than WT ($\leq -1.00$ kcal/mol for each of the residues, Figure 4F).
Furthermore, by structural analyses, we found that the E484K mutation causes the four residues closer to the mAb, enhancing their interactions with the mAb.

**Other potential mutations with high risk of immune evasion**

By binding free energy decomposition (Table S5), we systematically investigated the detailed roles of the key residues playing in the RBD-mAb interaction with $\Delta G \leq -1.00$ kcal/mol (Figure 5). Among the 25 complexes, E484 was found to be the key interaction residue of 17 systems, accounting for 68% of the mAbs. In addition to E484, we found 10 residues (Figure 5A) that are mostly involved in the mAb binding with occupancy $>30\%$, which are Y489 (84%), Y449 (80%), F490 (80%), F486 (68%), Q493 (56%), V483 (52%), G485 (44%), S494 (40%), F456 (36%) and N487 (32%). Recently, Starr et al. found that a lysine mutation at residue 486 (F486K) of the RBD helps SARS-CoV-2 escape neutralization by REGN10933 with slightly decreased binding affinity to ACE2. However, the F486K was not accessible via a single-nucleotide change [38].

The major difference of the interaction between the RBD-ACE2 and RBD-mAb is the key binding residues (Table S6). In details, Y489, Y449, F486, Q493, F456 and N487 can form stable interactions with ACE2 [13, 39], their mutations may obviously affect the infection efficacy of the SARS-CoV-2, which may lead to less risk. Differently, the rest of four residues, viz., F490, V483, G485 and S494, without strong binding to ACE2, could form stable interaction with most mAbs. Therefore, the mutations in F490, V483, G485 and S494 may impair the binding of the new RBD mutants to mAbs, causing immune evasion. Although there is no evidence showing the immune evasion caused by F490 mutation [38], the high occupancy (80%) of F490 among 26 mAbs indicates its dangerous potential to escape vaccine-induced immunity. Therefore, close attention should be paid to the four residues in developing new mAbs against the RBM of spike protein. In addition, it is not surprising that the residues of mAb (Figure 5B) that are high frequently observed on the interface of RBD-mAb could match well the residues of RBD, to form strong binding interaction. For instance, tyrosine and serine on mAb appear most frequently on the interface of RBD-mAb, which can form hydrogen bonds with residues on RBD (Y489, Y449, E484, Q493, etc.), and the four residues on RBD (Y489, Y449, F490 and F486)
with the highest occupancy can form π-π stacking with tyrosine on mAb.

**Conclusion**

The rapid development and use of vaccines and mAbs have made people see the dawn of success in the fight against the COVID-19 pandemic. However, the immune evasion caused by the mutation of SARS-CoV-2 spike protein, such as the B.1.351 harboring E484K, may reinflect the survivors, weaken or even invalidate the efficacy of mAbs and vaccines. To understand the underlined mechanism, we calculated and analyzed the binding affinities of 26 mAbs to WT spike protein and to the E484K mutant, respectively. The results reveal that the E484K mutation impairs most of mAbs (~85%) to bind to the spike RBD. Further calculations on the glycosylated and trimer spike proteins show the same change tendency as that based on the mAb-RBD. In addition, we predicted that the mutation of the four residues in the RBD, viz., F490, V483, G485 and S494, are also likely to cause immune evasion. Overall, this work sheds light on the impact of the E484K mutation on mAb efficacy and predicts other potential residue mutations with risk of immune evasion, which may serve as an alarm for future mAb and vaccine development.

### Key Points
- The recently identified SARS-CoV-2 variants harboring E484K mutation was found to have great resistance to numerous mAbs.
- We calculated the binding free energy of current 26 mAbs to WT spike protein and its E484K mutant, respectively. Twenty-two mAbs (85% of all the studied mAbs) have significantly decreased binding affinity to the E484K mutant, indicating that E484K mutant is likely resistant to most mAbs.
- In most of mAb-RBD complexes (72%), E484 has obvious greater contribution to the binding strength of the RBD to the mAb (~1.00 kcal/mol) than K484 does.
- The mutations of F490, V483, G485 and S494 are predicted to have immune evasion risk, as these residues are involved in the binding of most mAbs.

### Supplementary Data

Supplementary data are available online at https://academic.oup.com/bib.

### Data Availability

The data that support the findings of this study are available from the corresponding authors upon reasonable request.

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### References
1. WHO. Coronavirus disease (COVID-19) situation reports. https://www.who.int/emergencies/diseases/novel-coronavirus-2019/situation-reports/ (28 May 2021, date last accessed), 2021.
2. WHO. Draft landscape and tracker of COVID-19 candidate vaccines. https://www.who.int/publications/m/item/draft-landscape-of-COVID-19-candidate-vaccines 2020.
3. Forni G, Mantovani A. COVID-19 vaccines: where we stand and challenges ahead. Cell Death Differ 2021; 28:626–39.
4. Sempowski GD, Saunders KO, Acharya P, et al. Pandemic preparedness: developing vaccines and therapeutic antibodies for COVID-19. Cell 2020; 181:1458–63.
5. Mahase E. Covid-19: Novavax vaccine efficacy is 86% against UK variant and 60% against south African variant. Br Med J 2021; 372:n296.
6. Shinde V, Bhikha S, Hoosain Z, et al. Efficacy of NVX-CoV2373 Covid-19 Vaccine against the B.1.351 Variant. N Engl J Med 2021; 384:1899–1909.
7. Andreano E, Piccini G, Licastro D, et al. SARS-CoV-2 escape from a highly neutralizing COVID-19 convalescent plasma. Proc Natl Acad Sci U S A 2021; 118(36):e2103154118.
8. Ferrareze PAG, Franceschi VB, Mayer AM, et al. E484K as an innovative phylogenetic event for viral evolution: Genomic analysis of the E484K spike mutation in SARS-CoV-2 lineages from Brazil. Infect Genet Evol 2021;93:104941.
9. Wang F, Nair MS, Liu L, et al. Antibody resistance of SARS-CoV-2 variants B.1.351 and B.1.1.7. Nature 2021; 593(7857):130–135.
10. Lan J, Ge J, Yu J, et al. Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. Nature 2020; 581:215–20.
11. Adhikari P, Li N, Shin M, et al. Intra- and intermolecular atomic-scale interactions in the receptor binding domain of SARS-CoV-2 spike protein: implication for ACE2 receptor binding. Phys Chem Chem Phys 2020; 22:18272–83.
12. Amin M, Sorour MK, Kasry A. Comparing the binding interactions in the receptor binding domains of SARS-CoV-2 and SARS-CoV. J Phys Chem Lett 2020; 11:4897–900.
13. Peng C, Zhu Z, Shi Y, et al. 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; 11:10482–8.
14. Wang Y, Liu M, Gao J. Enhanced receptor binding of SARS-CoV-2 through networks of hydrogen-bonding and hydrophobic interactions. Proc Natl Acad Sci U S A 2020a;117:13967–74.
15. Starr TN, Greaney AJ, Hilton SK, et al. Deep mutational scanning of SARS-CoV-2 receptor binding domain reveals constraints on folding and ACE2 binding. Cell 2020;182:1295–310 e1220.
16. Biasini M, Bienert S, Waterhouse A, et al. SWISS-MODEL: modelling protein tertiary and quaternary structure using evolutionary information. Nucleic Acids Res 2014;42:W252–8.
17. Waterhouse A, Bertoni M, Bienert S, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46:W296–303.
18. Duan Y, Wu C, Chowdhury S, et al. A point-charge force field for molecular mechanics simulations of proteins based on condensed-phase quantum mechanical calculations. J Comput Chem 2003;24:1999–2012.
19. Kirschner KN, Yongye AB, Tschampel SM, et al. GLYCAM06: a generalizable biomolecular force field. Carbohydrates, J Computational Chemistry 2008;29:622–55.
20. Case DA, Ben-Shalom YI, Brozell SR, et al. AMBER 2018. San Francisco: University of California, 2018.
21. Ryckaert JPC, Berendsen, H. J. C. Numerical integration of the cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes. J Comput Phys 1977;23:327–41.
22. Essmann U, Perera L, Berkowitz ML, et al. A smooth particle mesh Ewald method. J Chem Phys 1995;103:8577–93.
23. Hansen J, Baum A, Pascal KE, et al. Studies in humanized mice and convalescent humans yield a SARS-CoV-2 antibody cocktail. Science 2020;369(6506):1010–1014.
24. Kollman PA, Massova I, Reyes C, et al. Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models. Acc Chem Res 2000;33:889–97.
25. Chi X, Yan R, Zhang J, et al. A neutralizing human antibody binds to the N-terminal domain of the spike protein of SARS-CoV-2. Science 2020;369:650–5.
26. Wang N, Sun Y, Feng R, et al. Structure-based development of human antibody cocktails against SARS-CoV-2. Cell Res 2021b;31:101–3.
27. Cerutti G, Guo Y, Zhou T, et al. Potent SARS-CoV-2 neutralizing antibodies directed against spike N-terminal domain target a single supersite. Cell Host Microbe 2021;29(5):819–833.
28. Acharya P, Williams W, Henderson R, et al. A glycan cluster on the SARS-CoV-2 spike ectodomain is recognized by Fab-dimerized glycan-reactive antibodies. bioRxiv 2020:doi:10.1101/2020.1106.1130.178897. preprint: not peer reviewed
29. Wrapp D, Wang N, Corbett KS, et al. Cryo-EM structure of the 2019-nCoV spike in the prefusion conformation. Science 2020;367:1260–3.
30. Tortorici MA, Beltramello M, Lempp FA, et al. Ultrapotent human antibodies protect against SARS-CoV-2 challenge via multiple mechanisms. Science 2020;370:950–7.
31. Peng C, Wang J, Yu Y, et al. Improving the accuracy of predicting protein–ligand binding-free energy with semiempirical quantum chemistry charge. Future Med Chem 2019;11:303–21.
32. Hou T, Wang J, Li Y, et al. Assessing the performance of the MM/PBSA and MM/GBSA methods. 1. The accuracy of binding free energy calculations based on molecular dynamics simulations. J Chem Inf Model 2011;51:69–82.
33. Xu L, Sun H, Li Y, et al. Assessing the performance of MM/PBSA and MM/GBSA methods. 3. The impact of force fields and ligand charge models. J Phys Chem B 2013;117:8408–21.
34. Chen RE, Zhang X, Case JB, et al. Resistance of SARS-CoV-2 variants to neutralization by monoclonal and serum-derived polyclonal antibodies. Nat Med 2021;27:717–26.
35. Hurlburt NK, Seydoux E, Wan YH, et al. Structural basis for potent neutralization of SARS-CoV-2 and role of antibody affinity maturation. Nat Commun 2020;11(1):5413.
36. Ge J, Wang R, Ju B, et al. Antibody neutralization of SARS-CoV-2 through ACE2 receptor mimicry. Nat Commun 2021;12(1):250.
37. Casalino L, Gaieb Z, Goldsmith JA, et al. Beyond shielding: the roles of Glycans in the SARS-CoV-2 spike protein. ACS Central Science 2020;6:1722–34.
38. Starr TN, Greaney AJ, Addetia A, et al. Prospective mapping of viral mutations that escape antibodies used to treat COVID-19. Science 2021;371:850–4.
39. Shang E, Axelsen PH. The potential for SARS-CoV-2 to evade both natural and vaccine-induced immunity. bioRxiv 2020. 13 December 2020, doi 10.1101/2020.12.13.422567, Arxiv bioxrv;2020.12.13.422567v1, preprint: not peer reviewed