nature portfolio

Peer Review File

Structure and dynamics of antibody REGN10987 interaction with Delta and Omicron BA.1 SARS-CoV-2 variants reveal mechanism of action

Corresponding Author: Professor Ekaterina Lyukmanova

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)
The manuscript entitled, “Structure and dynamics of REGN10987 interaction with Delta and Omicron SARS-CoV-2 Variants Revealed Mechanism of the Antibody Action” reports a 2.53 Å cryo-EM structure of full-length Spike of the SARS-CoV2 Delta variant in complex with an analogue of REGN10987. The authors show that the Fab binds the RBDs in up or down conformational states and claim that the structure provides structural insight into the role of all known RBD mutations, including Omicron, in REGN10987 immune evasion.

Cryo-EM was used to determine the structure of full-length trimeric S-protein of the SARS-CoV-2 Delta variant in complex with a recombinant analogue of REGN10987. The authors used 3D variability analysis in cryoSPARC to compute the normal modes associated with changes in relative orientation of Spike domains and also used molecular dynamics simulations to study the impact of the Omicron mutations on the interactions of REGN10987 with the RBD. In addition, umbrella sampling was used to compute a potential of mean force associated with the binding process of REGN10987 and the Delta or Omicron RBDs.

The interaction of multiple mAbs, including REGN10987 with Omicron RBD, was studied by McCallum et al. (Science 375, 864–868 (2022)), showing lack of binding to Omicron by SPR and highlighting the steric clash caused by the G446S Omicron mutation (Fig. 3b). This paper is not cited in the current manuscript.

Since it is experimentally known that REGN10987 does not bind the Omicron RBD, the value of simulating this binding interaction by MD is questionable. A more meaningful approach could be to explore their individual impact by introducing the Omicron mutations one-by-one. While this does not take epistatic effects into account, it will not describe a complex which does not exist (also shown by the authors’ Fig. S11).

Additionally, the technical execution of the MD simulations have not been performed to currently accepted standards in the field.

Major concerns
• “By using 50 sampling windows along this axis, one-dimensional potential of mean force (PMF)”, Do you have plots that show orthogonal degrees of freedom are sampled in a way that reflects an equilibrium distribution?
• “During simulations reaction coordinates were restrained at starting position with harmonic potential with force constant 1000 kJ/mol/nm2”, Did you select this force constant after having verified overlap between adjacent windows? Please show evidence that adjacent windows along the reaction coordinate overlap.
• The structure of REGN10987 bound to the Omicron RBD was generated in PyMol by aligning RBD Omicron to the REGN10987:RBD Delta structure. The authors did not describe how the resulting REGN10987:RBD Omicron interface was relaxed or minimized to relieve unfavorable side chain configurations. The authors did not state that positional restraints were applied during MD equilibration; this combined with an unfavorable interface in the starting structure would lead to pathological trajectories of RBD:REGN10987 unbinding that do not reflect biologically relevant physics.

Minor concerns
• The color scheme in Fig. 1d,e (and Fig. 4 and Fig. 5c,d) may be difficult for some readers who are colorblind. The writing could be improved and the claim, “Our study provides a structural insight into the role of all known to date significant RBD mutations of the Omicron and other SARS-CoV-2 variants in the REGN10987 evasion” may be an overstatement given the

data. Additional citations in the last sentence of the first paragraph of the introduction are needed, and also for the statement "holds promise against the emerging SARS-CoV-2 variants that harbor transmissivity-enhancing mutations within RBM".
• Regarding "Probably, membrane helices solubilized in the LMNG detergent did not adopt a stable conformation", it would be interesting to note whether the conformation/s of this region of the spike have an allosteric impact on the regions of the spike that were resolved–the RMSD of this structure to other structures in which these regions are resolved (e.g. RMSD by domain) would be useful.
• Regarding "Thus, the Fab binding captures the S-protein in the most populated conformation", I would like to see biophysical data like FRET or single-molecule spectroscopy (or a citation to it) to make an assertion like this.
• Regarding "In our structure all three pairs of the 630/FPPR elements are disordered", Do you mean "disordered" or do you mean "unresolved"? If you meant disordered, show that data please (e.g. MD simulation or spectroscopy).
• Regarding "At the same time, a comparison with the 6XDG structure revealed the substantial differences in the loop positions on the RBD/Fab interface (RMSD of 1.4 Å, Supplementary Fig. 6b), that probably is explained by low resolution of the previous structure", Please compute RMSD of the MD using this as the reference to test this hypothesis. If MD spotaneously adopts conformations that look like this, then there is a dynamic equilibrium and that is what the difference in the two structures captures; if the MD never has low RMSD to this, then you can assert your claim.
• "The Delta-RBD/REGN10987-Fab complex showed the slight variability during MD calculations", I would like to see the RMSD vs. time using the experimental structure as the reference. Please plot RMSD of the RBD (using RBD as reference), Fab (using Fab as reference), and RBD:Fab complex (RBD:Fab as reference).
• "The interaction became less tight, and the number of H-bonds...", What algorithm did you use for this? Look at other types of interactions too, please.
• "...revealed that both RBDs showed comparable stability", RMSF does not report on stability (as in free energy, or as in structure)–please clarify. Do you instead mean local flexibility or local dynamics?

Reviewer #2

(Remarks to the Author)
In this manuscript titled "Structure and dynamics of REGN10987 interaction with Delta and Omicron SARS-CoV-2 Variants Revealed Mechanism of the Antibody Action" the authors have determined the structure of RBD-directed antibody REGN10987 bound to the full-length, detergent solubilized SARS-CoV-2 Delta spike. Although a structure of REGN10987 with RBD has been determined before, this study adds useful information, including higher resolution definition of the antibody RBD interface, information on the effect of the antibody on spike conformational dynamics, as well as analysis of spike domain motions.

The authors mention "recombinant REGN10987 analogue" in the abstract and text, but it is only upon reviewing Table S2 that it is clarified that the Fab used differs in the light chain constant region. This should be clearly stated in the text. This can be explained in the last paragraph of the introduction when referring to the "recombinant REGN10987 analogue".

In their analysis of the Omicron mutations, the authors state "In the absence of the structures of the REGN10987 complexes with the S-proteins of SARS-CoV-2 variants, including Delta and Omicron, the effects of RBD mutations on the antibody's activity were predicted41,42. For example, it was proposed that the Omicron N440K, G446S, Q498R and N501Y mutations located in RBM can reduce the REGN10987 activity41." and then go on to explain why these predictions do not all agree with the structural data. Instead of relying on predictions, it will be better if the authors revise this section referring to and citing available neutralization data:

Liu, L., Iketani, S., Guo, Y. et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. Nature 602, 676–681 (2022). https://doi.org/10.1038/s41586-021-04388-0

Iketani, S., Liu, L., Guo, Y. et al. Antibody evasion properties of SARS-CoV-2 Omicron sublineages. Nature 604, 553–556 (2022). https://doi.org/10.1038/s41586-022-04594-4

The manuscript should be proof-read for typos, grammar and spelling:

The resolution of the cryo-EM reconstruction is reported at 2.3 A in the abstract and the text, but 2.53 A in Table S1. This needs to be corrected.

"Delta-G446 forms the tight Van-der-Waals contact with N57 of the light REGN10987-Fab chain (Fig. 2ab), and its replacement by serin causes the conformational change of the RBM backbone (Supplementary Fig. 10c) and the steric clashes with Fab." 'serin' should be 'serine'

In the methods section, "As a result, the variable regions of IgG1 were replaced by variable regions of the REGN1098 antibody." 'REGN1098' should be 'REGN10987'.

Reviewer #3

(Remarks to the Author)
The authors report a novel Cryo-EM determined structure of the Spike protein of SARS-CoV-2 Delta variant complexed with monoclonal antibody (mAb) REGN10987 with overall resolution of 2.3 Å. The obtained structure has 2-down Receptor Binding Domains (RBD) and 1-up RBD, all of them bound to one REGN10897 mAb. Cryo-EM maps shows different dynamics for each RBD and the refined structure shows important interactions between the mAb and the RBD, which are crucial for antibody-antigen binding and thus neutralizing the virus attachment to the ACE2 human-cell receptor. Supporting the visualized interactions, Molecular Dynamics (MD) simulations were performed and showed persistence of H-bond, hydrophobic contacts and salt-bridges of important residues in the RBD-mAb interface. MD simulations were also performed for RBD of Omicron variant, showing that the crucial interactions present in Delta variant are absent in Omicron, explaining experimental data of immune evasion of Omicron variant for REGN10987. The work provides structural insights into the role of Delta and Omicron SARS-CoV-2 variants in binding/non-binding to REGN10987 mAb. Mapping crucial mutations in RBD will help in future vaccine formulations and new therapeutic mAbs.

The following minor revision would improve the work discussion quality:

1. According to Barnes et al. (https://doi.org/10.1038/s41586-020-2852-1) REGN10987 is a class III antibody as it binds to an epitope that does not overlap with the hACE2 binding site. Using the 'class III' nomenclature is recommended.

2. In lines 136-137 and Supplementary Figure 7a residues K478 T376 and F374 do not appear to make interactions via side chain. If the contacts are through the main chain, it should be shown in the figure.

3. In lines 145-148, what would be the authors' hypothesis for the 2up/1down conformation to be stabilized?

4. The discussion in lines 210-215 could be improved. It makes no sense to call Y53/K444 N57/G446 interactions hydrophobic since the residues are polar-neutral or polar-charged. Pi-stacking between Y53 and Y449 seems to play a part in stabilization as well. Visually in the Figure 2 Y32/T500, L93/P499, W99/V445 residues appear to be very distant from each other. Improve the figure's angle or revise the contacts in that region.

5. In the discussion of lines 259-261, this region has mutations from short side chain residues to longer side chain residues, leading to greater conformational entropy. This information could also explains more flexibility and may be added to the discussion.

6. In Supplementary Figure 11 the label of the X axis I believe should be Log of concentration. Also, put the Kd values in the figure for each graph.

Version 1:

Reviewer comments:

Reviewer #2

(Remarks to the Author)
The authors have adequately responded my critiques.

We are very grateful for the reviewers' remarks. Please, find below our point-to-point answers.

Reviewer #1 (Remarks to the Author):

The manuscript entitled, "Structure and dynamics of REGN10987 interaction with Delta and Omicron SARS-CoV-2 Variants Revealed Mechanism of the Antibody Action" reports a 2.53 Å cryo-EM structure of full-length Spike of the SARS-CoV2 Delta variant in complex with an analogue of REGN10987. The authors show that the Fab binds the RBDs in up or down conformational states and claim that the structure provides structural insight into the role of all known RBD mutations, including Omicron, in REGN10987 immune evasion.

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Since it is experimentally known that REGN10987 does not bind the Omicron RBD, the value of simulating this binding interaction by MD is questionable. A more meaningful approach could be to explore their individual impact by introducing the Omicron mutations one-by-one. While this does not take epistatic effects into account, it will not describe a complex which does not exist (also shown by the authors' Fig. S11).

Answer:

We are grateful the reviewer for this valuable remark. According to the reviewer suggestion, we produced the four Delta RBD variants each containing one Omicron mutation and studied their binding to the REGN10987 antibody by Microscale Thermophoresis (MST). Then we performed MD calculations for seven RBD variants (Wuhan, Omicron BA.1, Delta/N440K, Delta/G446S, Delta/Q498R, Delta/N501Y, and Delta/'Other Omicron mutations') in the complex with REGN10987-Fab and calculated free energy of dissociation for these RBD/Fab complexes. Data obtained were included in the revised version of the manuscript.

We also grateful for pointing us to the McCallum et al. publication. Indeed, this publication (see supplementary figure S4 at https://www.science.org/doi/10.1126/science.abn8652) together with (Wang, Q. *et al. Nature* **608**, 603–608 (2022)) are the two works where the weak affinity or neutralization activity of REGN10987 antibody against Omicron BA.1 was observed. Taking in to account our MD data, we propose that REGN10987 is able to interact with Omicron BA.1 RBD/S-protein, but this interaction is weak and was not detected in the majority of prior studies due to insufficient antibody concentration. We argue that, in spite of its weakness, this interaction is probably specific, in other words, it corresponds to the specific structure of the RBD/Fab complex.

Reviewer #1 (Remarks to the Author):

Additionally, the technical execution of the MD simulations have not been performed to currently accepted standards in the field.

Answer:

We performed new MD calculations for each of the seven RBD variants in the complex with Fab. Each MD calculation was done in three replicas. To consider the complexes at equilibrium in each case we calculated 1000 ns of MD, and analyzed the second half (500-1000ns) of the trajectory.

Reviewer #1 (Remarks to the Author), Major concerns:

• "By using 50 sampling windows along this axis, one-dimensional potential of mean force (PMF)", Do you have plots that show orthogonal degrees of freedom are sampled in a way that reflects an equilibrium distribution?

Answer:

We calculated the dispersions of X and Y coordinates of the center of masses of the RBD (i.e. orthogonal degrees of freedom) for two different ξ values (reaction coordinate), took at increasing part of PMF profile, for one of the ΔG calculations for the WT RBD (Fig. R1). Observed decrease in the dispersions profiles with MD time provides evidence that the quasi-equilibrium distribution along these axes was achieved. In the revised paper, we also greatly increase the number of calculated PMF profiles to correctly estimate the ΔG values.

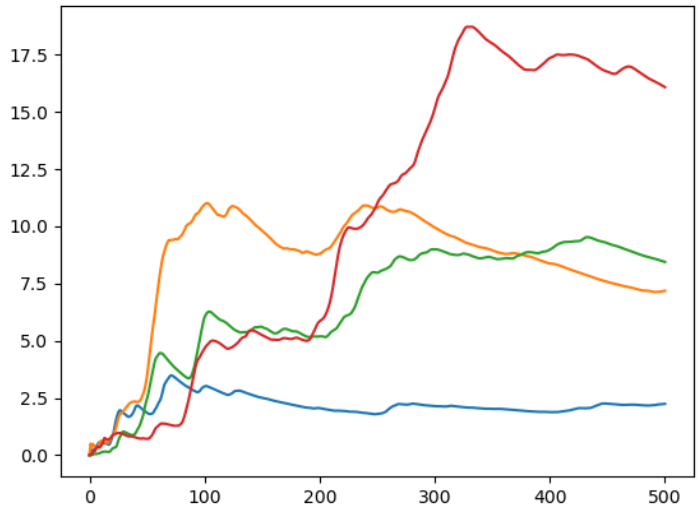


Fig. R1. Dispersion (Å^2) of X and Y coordinates (orthogonal degrees of freedom) of the RBD center of masses calculated for two reaction coordinates (ξ=4.3 nm, green and blue lines, and ξ=5.3 nm, red and orange lines) from the MD length (in 10 ps) used for umbrella sampling. The 5 ns of MD is sufficient to achieve equilibrium distribution along the X and Y axes. The reaction coordinate (ξ) corresponds to the distance between centers of masses of the RBD and Fab's *N*-terminal domain along Z axis.

Reviewer #1 (Remarks to the Author):

• "During simulations reaction coordinates were restrained at starting position with harmonic potential with force constant 1000 kJ/mol/nm2", Did you select this force constant after having verified overlap between adjacent windows? Please show evidence that adjacent windows along the reaction coordinate overlap.

Answer:

Yes, we verified that adjacent windows overlap on histogram plot (Fig. R2). If calculated PMF profile had spikes, or gromacs 'wham' routine pointed to a lack of sampling around particular ξ values, additional conformations were added to the analysis.

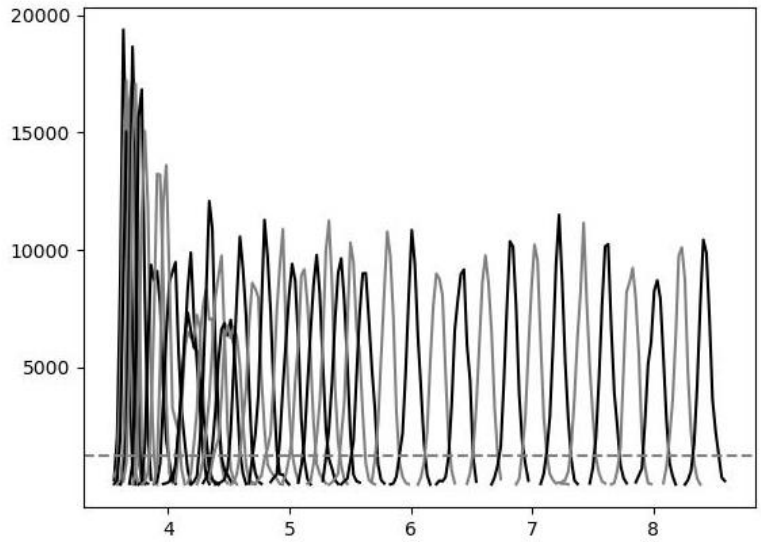


Fig. R2. The histograms of the configurations within the umbrella sampling windows. The number of configurations (counts) versus reaction coordinate (ξ) are shown for each of the umbrella sampling run for one of the ΔG calculations for the WT RBD.

Reviewer #1 (Remarks to the Author):

• The structure of REGN10987 bound to the Omicron RBD was generated in PyMol by aligning RBD Omicron to the REGN10987:RBD Delta structure. The authors did not describe how the resulting REGN10987:RBD Omicron interface was relaxed or minimized to relieve unfavorable side chain configurations. The authors did not state that positional restraints were applied during MD equilibration; this combined with an unfavorable interface in the starting structure would lead to pathological trajectories of RBD:REGN10987 unbinding that do not reflect biologically relevant physics.

Answer:

Positional restraints were applied during the heating stage just before MD equilibration. We checked RMSD from the starting structure during MD equilibration (Fig. R3). RMSD from the starting structure for the Omicron RBD variant was less than for the Wuhan (WT) RBD variant.

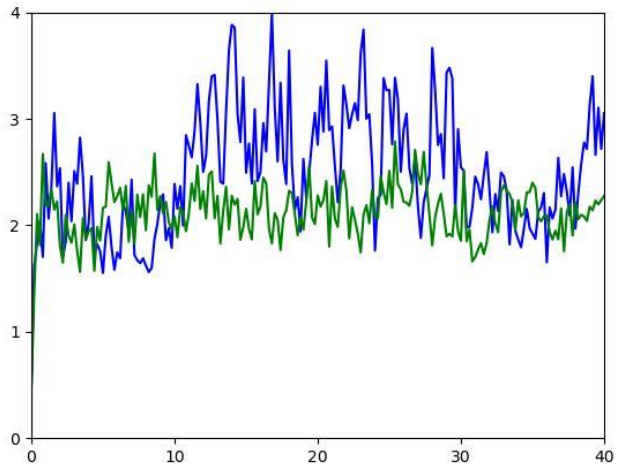


Fig. R3. RMSD (Å) from the starting structure during MD equilibration (MD time, ns) for the Wuhan (blue line) and Omicron (green line) RBD variants. RMSD was calculated for backbone atoms of the RBD and backbone atoms of the residues 3-111 and 3-121 of light and heavy chains of Fab, respectively.

Reviewer #1 (Remarks to the Author): Minor concerns:

• The color scheme in Fig. 1d,e (and Fig. 4 and Fig. 5c,d) may be difficult for some readers who are colorblind.

Answer:

We changed the colors on all of the figures in the manuscript according to this remark. Thank you.

Reviewer #1 (Remarks to the Author):

The writing could be improved and the claim, "Our study provides a structural insight into the role of all known to date significant RBD mutations of the Omicron and other SARS-CoV-2 variants in the REGN10987 evasion" may be an overstatement given the data. Additional citations in the last sentence of the first paragraph of the introduction are needed, and also for the statement "holds promise against the emerging SARS-CoV-2 variants that harbor transmissivity-enhancing mutations within RBM".

Answer:

We thank you for this comment. In the revised version of the manuscript, we have added new experimental data and significantly reduced the claims of the study. We have also revised the citations used and added new references. The manuscript abstract now reads, "Our study explains the influence of the known-to-date SARS-CoV-2 RBD mutations on REGN10987 recognition and highlights the importance of considering data on dynamics beyond the static structure of the RBD/Fab complex." This, in our opinion, reflects the results obtained.

Reviewer #1 (Remarks to the Author):

• Regarding "Probably, membrane helices solubilized in the LMNG detergent did not adopt a stable conformation", it would be interesting to note whether the conformation/s of this region of the spike have an allosteric impact on the regions of the spike that were resolved–the RMSD of this structure to other structures in which these regions are resolved (e.g. RMSD by domain) would be useful.

Answer:

There is a problem to observe the membrane helices of the S-protein using cryo-EM. Currently, there are no the S-protein structures showing both ectodomain and transmembrane domain simultaneously. The structure of the membrane domain has been only determined by NMR (Fu and Chou, JACS, 2021). We decided not to discuss this issue in the manuscript and deleted the sentence about "membrane helices solubilized in the LMNG detergent".

Reviewer #1 (Remarks to the Author):

• Regarding "Thus, the Fab binding captures the S-protein in the most populated conformation", I would like to see biophysical data like FRET or single-molecule spectroscopy (or a citation to it) to make an assertion like this.

Answer:

We provided the references to the two smFRET study (Lu, M. *et al. Cell Host Microbe* **28**, 2020; Díaz-Salinas, *et al. eLife* **11**, 2022), which proves that the three-RBD-down S-closed conformation and one-RBD-up S-open conformation are simultaneously presented in the WT apo-S-protein and S-protein containing D614G mutation (like the presently studied Delta variant). According to these data, the one-RBD-up S-open conformation is the most populated state of apo-S-protein containing D614G mutation.

Reviewer #1 (Remarks to the Author):

• Regarding "In our structure all three pairs of the 630/FPPR elements are disordered", Do you mean "disordered" or do you mean "unresolved"? If you meant disordered, show that data please (e.g. MD simulation or spectroscopy).

Answer:

We agree with this remark. These fragments of structure were non observed in our cryo-EM map. We rewrote the corresponding sentence.

Reviewer #1 (Remarks to the Author):

• Regarding "At the same time, a comparison with the 6XDG structure revealed the substantial differences in the loop positions on the RBD/Fab interface (RMSD of 1.4 Å, Supplementary Fig. 6b), that probably is explained by low resolution of the previous structure", Please compute RMSD of the MD using this as the reference to test this hypothesis. If MD spotaneously adopts conformations that look like this, then there is a dynamic equilibrium and that is what the difference in the two structures captures; if the MD never has low RMSD to this, then you can assert your claim.

Answer:

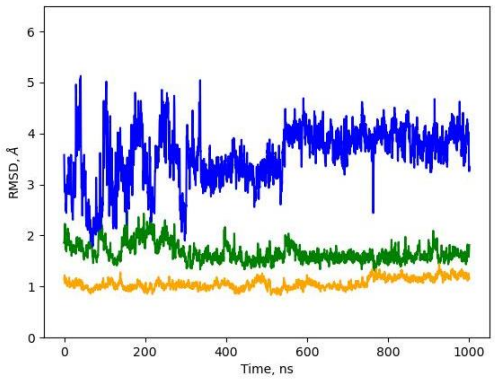
In the revised paper, we calculated MD for the complex of WT-RBD/Fab. This corresponds to the previous cryo-EM structure, where the WT RBD was used. (The Delta RBD variant used in our cryo-EM study differ by two mutations and both of them are not on the RBD region which contact Fab). We calculated RMSD of this 3*1000 ns MD with our structure of Delta-RBD1/Fab1 and previous WT-RBD/Fab complexes (see revised supplementary figure 6). RMSD was calculated over the two RBD loops (N437-N450 and Q498-Y508) which contact with the Fab. The comparison revealed significantly lower RMSD value to our structure (1.15 ± 0.27 Å, mean ± S.D., minimal value ~0.57 Å) than to the previous structure (1.44 ± 0.26 Å, minimal value ~0.79 Å).

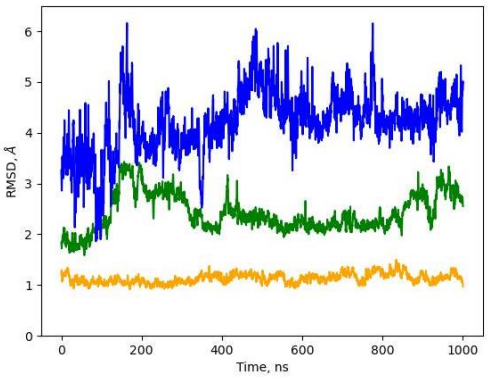
Reviewer #1 (Remarks to the Author):

• "The Delta-RBD/REGN10987-Fab complex showed the slight variability during MD calculations", I would like to see the RMSD vs. time using the experimental structure as the reference. Please plot RMSD of the RBD (using RBD as reference), Fab (using Fab as reference), and RBD:Fab complex (RBD:Fab as reference).

Answer:

The revised version of the manuscript does not contain the MD of Delta-RBD/Fab complex, but we compared traces obtained for WT-RBD with our Delta-RBD1/Fab1 structure (Fig. R4. below shows the required graphs for the three replicas). We see not very large differences between the cryo-EM structure and MD traces. Therefore, we think that such graphs are not needed in the main manuscript.





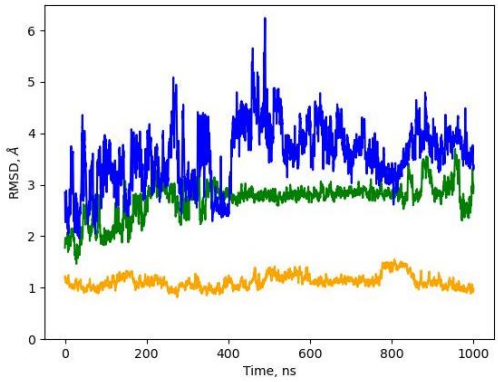


Fig. R4. RMSD of MD traces of the WT-RBD/Fab complex (three replicas) to the cryo-EM structure of the Delta-RBD1/Fab1 complex.
Orange – Fab
Green – RBD
Blue – RBD/Fab complex

Reviewer #1 (Remarks to the Author):

• "The interaction became less tight, and the number of H-bonds...", What algorithm did you use for this? Look at other types of interactions too, please.

Answer:

In the revised version of the manuscript all this section was overwritten. Now, there are no comparison of H-bonds. Indeed, we compared the total number of interactions (Ionic+H-bonds+Stacking+Pi-cation, see revised supplementary figure 9). The detailed lists of the contacts observed in each MD replica with their lifetime are collected in the supplementary tables S5-S11. For the identification of intermolecular interactions, we used inhouse software IMPULSE.

Reviewer #1 (Remarks to the Author):

• "...revealed that both RBDs showed comparable stability", RMSF does not report on stability (as in free energy, or as in structure)–please clarify. Do you instead mean local flexibility or local dynamics?

Answer:

In the revised version of the manuscript, the RMSF data are collected in the revised supplementary figures 12 and 13. The text of the manuscript war rewritten "However, root-mean-square fluctuation (RMSF) analysis revealed the highest mobility for the N440K and G446S RBD variants in the complex with Fab, both across the entire RBD and the antibody binding site"

Reviewer #2 (Remarks to the Author):

In this manuscript titled "Structure and dynamics of REGN10987 interaction with Delta and Omicron SARS-CoV-2 Variants Revealed Mechanism of the Antibody Action" the authors have

determined the structure of RBD-directed antibody REGN10987 bound to the full-length, detergent solubilized SARS-CoV-2 Delta spike. Although a structure of REGN10987 with RBD has been determined before, this study adds useful information, including higher resolution definition of the antibody RBD interface, information on the effect of the antibody on spike conformational dynamics, as well as analysis of spike domain motions.

The authors mention "recombinant REGN10987 analogue" in the abstract and text, but it is only upon reviewing Table S2 that it is clarified that the Fab used differs in the light chain constant region. This should be clearly stated in the text. This can be explained in the last paragraph of the introduction when referring to the "recombinant REGN10987 analogue".

Answer:

Thank you for this comment. We added description of our recombinant Fab analogue to the introduction section. "This fragment, hereafter referred to as REGN10987-Fab, differs from the original antibody in the light chain constant region (Supplementary Table 1)."

Reviewer #2 (Remarks to the Author):

In their analysis of the Omicron mutations, the authors state "In the absence of the structures of the REGN10987 complexes with the S-proteins of SARS-CoV-2 variants, including Delta and Omicron, the effects of RBD mutations on the antibody's activity were predicted41,42. For example, it was proposed that the Omicron N440K, G446S, Q498R and N501Y mutations located in RBM can reduce the REGN10987 activity41." and then go on to explain why these predictions do not all agree with the structural data. Instead of relying on predictions, it will be better if the authors revise this section referring to and citing available neutralization data:

Liu, L., Iketani, S., Guo, Y. et al. Striking antibody evasion manifested by the Omicron variant of SARS-CoV-2. Nature 602, 676–681 (2022). https://doi.org/10.1038/s41586-021-04388-0

Iketani, S., Liu, L., Guo, Y. et al. Antibody evasion properties of SARS-CoV-2 Omicron sublineages. Nature 604, 553–556 (2022). https://doi.org/10.1038/s41586-022-04594-4

Answer:

Thank you for pointing our attention to these really important publications. In the revised version of the manuscript, the Discussion section was completely rewritten using the data from these publications also. The corresponding references were cited.

Reviewer #2 (Remarks to the Author):

The manuscript should be proof-read for typos, grammar and spelling:

Answer:

We carefully proof read the manuscript. We hope that our revised version will be found acceptable for publication.

Reviewer #2 (Remarks to the Author):

The resolution of the cryo-EM reconstruction is reported at 2.3 A in the abstract and the text, but 2.53 A in Table S1. This needs to be corrected.

Answer:

Thank you for pointing us on this mistake. 2.3 A is the resolution of intermediate reconstruction with C3 symmetry. We corrected the abstract. 2.5 A is a correct number for the final C1 reconstruction.

Reviewer #2 (Remarks to the Author):

"Delta-G446 forms the tight Van-der-Waals contact with N57 of the light REGN10987-Fab chain (Fig. 2ab), and its replacement by serin causes the conformational change of the RBM backbone (Supplementary Fig. 10c) and the steric clashes with Fab." 'serin' should be 'serine'

Answer:

This and other typos were corrected.

Reviewer #2 (Remarks to the Author):

In the methods section, "As a result, the variable regions of IgG1 were replaced by variable regions of the REGN1098 antibody." 'REGN1098' should be 'REGN10987'.

Answer:

This and other typos were corrected.

Reviewer #3 (Remarks to the Author):

The authors report a novel Cryo-EM determined structure of the Spike protein of SARS-CoV-2 Delta variant complexed with monoclonal antibody (mAb) REGN10987 with overall resolution of 2.3 Å. The obtained structure has 2-down Receptor Binding Domains (RBD) and 1-up RBD, all of them bound to one REGN10897 mAb. Cryo-EM maps shows different dynamics for each RBD and the refined structure shows important interactions between the mAb and the RBD, which are crucial for antibody-antigen binding and thus neutralizing the virus attachment to the ACE2 human-cell receptor. Supporting the visualized interactions, Molecular Dynamics (MD) simulations were performed and showed persistence of H-bond, hydrophobic contacts and salt-bridges of important residues in the RBD-mAb interface. MD simulations were also performed for RBD of Omicron variant, showing that the crucial interactions present in Delta variant are absent in Omicron, explaining experimental data of immune evasion of Omicron variant for REGN10987. The work provides structural insights into the role of Delta and Omicron SARS-CoV-2 variants in binding/non-binding to REGN10987 mAb. Mapping crucial mutations in RBD will help in future vaccine formulations and new therapeutic mAbs.

The following minor revision would improve the work discussion quality:

1. According to Barnes et al. (https://doi.org/10.1038/s41586-020-2852-1) REGN10987 is a class III antibody as it binds to an epitope that does not overlap with the hACE2 binding site. Using the 'class III' nomenclature is recommended.

Answer:

Thank you for pointing our attention to this very important publication. We added information about antibody classification to the introduction section. "According to the classification proposed by Barnes et al[21], REGN10987 belongs to the nAbs from Class 3, which bind outside the ACE2 binding site and recognize both the 'up' and 'down' RBDs". The corresponding reference was cited.

Reviewer #3 (Remarks to the Author):

2. In lines 136-137 and Supplementary Figure 7a residues K478 T376 and F374 do not appear to make interactions via side chain. If the contacts are through the main chain, it should be shown in the figure.

Answer:

Thank you to pointing us on this mistake. The figure S7 was redrawn to show the sidechain-mainchain interactions also. The corresponding text was rewritten.

Reviewer #3 (Remarks to the Author):

3. In lines 145-148, what would be the authors' hypothesis for the 2up/1down conformation to be stabilized?

Answer:

In our experimental structure, we observed only the 2down/1up conformation (RBDs 1 and 2 were ‘down’ and RBD3 was ‘up’). “Attempts” of RBD2 to move from the ‘down’ to the ‘up’ conformation were evident from the results of 3DVA analysis (Fig. 4, mode #1). At the same time, we do not have enough structural data to describe a possible structure with RBD2 in the ‘up’ conformation. It is likely that this conformation is also present in the collected set of cryo-EM images, but the number of the corresponding particles is very small and the 3D classification did not distinguish this class from the major 3D class with the 2down/1up conformation. In this case, structural characterization of the 2up/1down conformation would require an enormous increase in the number of particles collected (increasing the experiment time). Since our data do not describe this possible 2up/1down conformation, we decided not to make assumptions about stabilizing interactions in this case.

Reviewer #3 (Remarks to the Author):

4. The discussion in lines 210-215 could be improved. It makes no sense to call Y53/K444 N57/G446 interactions hydrophobic since the residues are polar-neutral or polar-charged. Pi-stacking between Y53 and Y449 seems to play a part in stabilization as well. Visually in the Figure 2 Y32/T500, L93/P499, W99/V445 residues appear to be very distant from each other. Improve the figure's angle or revise the contacts in that region.

Answer:

We thank you for this comment. The corresponding part of the manuscript has been rewritten in the revised version. The figures have also been redrawn. All hydrophobic contacts observed in the cryo-EM structure and MD simulations are listed in supplemental Tables S4-S11. These contacts correspond to the side chains where the C-C spacing was less than 5 Å.

Reviewer #3 (Remarks to the Author):

5. In the discussion of lines 259-261, this region has mutations from short side chain residues to longer side chain residues, leading to greater conformational entropy. This information could also explains more flexibility and may be added to the discussion.

Answer:

We thank you for this comment. The revised version of the manuscript contains an entirely new section on MD simulation. This discussion is no longer cited in the text of the manuscript.

Reviewer #3 (Remarks to the Author):

6. In Supplementary Figure 11 the label of the X axis I believe should be Log of concentration. Also, put the Kd values in the figure for each graph.

Answer:

We fully agree with this remark. In the revised version, this figure contains additional data on mutant variants of RBD, and we decided to move it to the main body of the manuscript. It is now Figure 5. Concentrations are now given in nM, and the resulting Kd values along with their uncertainties are given in each panel of the figure.

Reviewer #1/Editorial Board Member (Remarks to the Author):

"I don't have other issues, except the way they compute the dissociation free energy is quite puzzling. To compute the protein-ligand/protein dissociation PMF, a one-dimensional reaction coordinate won't be sufficient because, as the distance increases, the phase space also increases (see the seminal work by Woo and Roux: PNAS). However, there is no mention of using geometric restraints during umbrella sampling. Please have the authors clarify or justify their free energy calculation."

Answer:

We are grateful the Editorial Board Member for this remark. Indeed, we used the standard weighted histogram analysis method (WHAM) implemented in the Gromacs software. This procedure includes all the necessary corrections to calculate the free energy change (ΔG) using the umbrella sampling algorithm and the one-dimensional PMF approach. At the same time, this procedure is slightly different from the classical procedure proposed previously (Woo and Roux: PNAS, the reference have been added). For example, Gromacs adds a $k_B \cdot T \cdot \ln(4\pi \cdot \xi^2)$ term to the PMF to eliminate the entropic decrease in the PMF due to the increase in the number of configurations on a sphere of radius ξ.

In our calculations, we did not constrain the Fab orientation and conformation during the sampling, whereas in the RBD the position of the secondary structure elements was fixed. Thus, the calculated PMF profiles contained all ΔG terms except the free energy changes due to the change in the RBD conformation upon Fab dissociation.

Despite initial enthusiasm for the use of one-dimensional PMF, recent publications revealed a strong dependence of the calculated ΔG values on the initial conformation of the complex [You, et al, Potential Mean Force from Umbrella Sampling Simulations: What Can We Learn and What Is Missed? *J. Chem. Theory Comput.* **15**, 2433–2443 (2019)] and from the dissociation pathway of the molecules [Aho, et al, Do All Paths Lead to Rome? How Reliable is Umbrella Sampling Along a Single Path? *J. Chem. Theory Comput.* **20**, 6674–6686 (2024)]. To evaluate these factors, we repeated ΔG calculations for the different initial structures and indeed observed a very large scatter in the calculated values. The simplest way we found to solve this problem is to average the results of several calculations, taking into account the Boltzmann weights. The resulting weighted averages are in agreement with the trend in the experimental data. Of course, the results of this approach may strongly depend on whether or not we obtained the sufficient sampling of possible energy values during ΔG calculations.

Changes made to the main text of the manuscript in response to the above question are highlighted with a yellow background.