The Point Mutations in SARS-CoV-2 Variants Induce Long-Range Dynamical Perturbations in Neutralizing Antibodies

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Abstract

Monoclonal antibodies are emerging as a viable treatment for COVID-19. However, new SARS-CoV-2 variants can reduce the efficacy of currently available antibodies and can diminish vaccine-induced immunity. Here, we demonstrate that the molecular dynamics of neutralizing monoclonal antibodies can be profoundly modified by the amino-acid mutations present in the spike proteins of the SARS-COV-2 variants currently circulating in the world population. The dynamical perturbations within the antibody structure, which can alter the thermodynamics of antigen-antibody binding, are found to be diverse and to depend both on the nature of the antibody and on the spatial location of the spike mutation. The correlation between the motion of the antibody and that of the spike receptor binding domain (RBD) can also be changed, and this, in turn, can additionally modify the binding affinity. Using protein graph connectivity networks, we delineated the mutant-induced modifications in the information-flow along the allosteric pathway throughout the antibody. Changes in the collective dynamics were spatially distributed both locally and across long-range distances within the antibody. On the receptor side, we identified an anchor-like structural element that prevents the detachment of the antibodies; individual mutations there can significantly affect the antibody binding propensity. Our study provides insight into how virus neutralization by monoclonal antibodies can be impacted by local mutations in the epitope via a *dynamical* standpoint. This realization adds a new layer of sophistication to be included in the efforts to rationally design monoclonal antibodies effective against new variants of SARS-CoV2 that take allostery in the antibody in consideration.

Introduction

The coronavirus disease 19 (COVID-19) caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2)[1] has claimed, as of November 2021, more than 5 million lives worldwide, creating a global pandemic and one of the largest public health crises in human

history.[2] To address this urgent problem, scientists across various disciplines are striving to develop drugs, vaccines, and antibodies against the virus.[3–7] The spike proteins, present on the surface of the virus, recognize and bind to the human angiotensin converting enzyme 2 (hACE2) receptor in lung cells to initiate infection.[8,9] The receptor binding domain (RBD) of the spike is a key target for drug development and antibody recognition.[10–16] A large number of monoclonal antibodies (mAb), as well as the natural antibodies (Ab) generated by the immune system block infection by binding to the RBD and prevent it from attaching to the hACE2 receptor. Moreover, many of the currently available vaccines, such as the ones developed by Pfizer,[17] Moderna,[18] and AstraZeneca,[19] use the spike protein as their epitope and induce immunity by generating antibodies and memory cells that can potentially recognize regions of the spike protein, including the RBD.

The process of antigen-antibody recognition is of fundamental importance for developing immune response against invading pathogens. Since their discovery in 1975, monoclonal antibodies (mAb) found a wide range of therapeutic applications, particularly in the treatment of cancer, chronic inflammation and viral infection.[20] Monoclonal antibodies, engineered in the laboratory, have a single sequence, unlike natural antibodies which have varying sequences, and are thereby tailored to treat specific diseases. They are generated by identical B-lymphocyte immune cells cloned from a unique parent white blood cell. The use of engineered antibodies as drugs has become increasingly appealing due to their high binding affinity and antigen specificity, both modulated by the complementarity determining regions (CDR) within the variable heavy (V_H) and variable light (V_L) chains.[21]

Monoclonal antibodies have significant advantages over conventional immunization techniques such as convalescent plasma therapy (CPT), because they reduce the risk of blood-borne diseases, shorten the time needed to generate high-affinity and low-risk antibodies, and allow for variable epitope specificity.[22] A number of monoclonal antibodies have been reported to neutralize the novel coronavirus and are at various stages of clinical trial, with bamlanivimab and REGN-COV2 having received emergency use authorization (EUA) in the

United States of America by the Food and Drug Administration (FDA) as of June 2021.[23] Molecular structures of multiple monoclonal antibodies in complex with the spike protein of SARS-CoV-2 have now been resolved, facilitating a detailed structural -although not as of yet a dynamical- understanding of the antigen recognition process.[24–31]

The major concern for these treatments is the possibility of the appearance of new variants, given mutations in the RBD region which can evade immune response and reduce or nullify the action of neutralizing antibodies. Multiple such variants of SARS-CoV-2 have emerged throughout the course of the pandemic, threatening the effectiveness of current therapeutics against the coronavirus. One of the earliest well-documented variant, with mutations in the RBD, was the B.1.1.7 strain, a.k.a. the *alpha* variant. It emerged in the United Kingdom in the second half of 2020 and spread across the entire world vigorously because of its 70-80% higher transmissibility compared to the wild type.[32] This variant contains a N501Y mutation in the RBD, which has been attributed to its increased immune evasion capability, although no significant increase in reinfection was observed.[33] The next major highly infectious variant was the *beta* variant or the B.1.351 strain, originating in South Africa in October 2020. Apart from N501Y, it has two more mutations in the RBD: K417N and E484K; this mutant reduces the effectiveness of conventional antibody therapy up to 10-fold.[34] Later, a new strain (B.1.617) with two RBD mutations, L452R and E484Q, emerged in India and became officially known as the *kappa* variant. A lineage of that strain, B.1.617.2, is named as the *delta* variant by the World Health Organization (WHO). With 50-67% higher infectivity than the *alpha* strain,[35] the *delta* variant is designated as a *variant of concern* (along with *alpha* and *beta*) because of its potential to make currently available vaccines less effective in preventing serious illness. Evidence of serious decline in vaccine effectiveness against infection (VE-I) has already appeared, along with the proliferation of the *delta* variant.[36,37]

To address this urgent crisis, a number of computational studies have been performed to obtain a molecular level understanding of the effect of mutations in the RBD on its binding

with the hACE2 receptor and antibodies.[38–44] Taken collectively, these studies revealed that the residues which got mutated in the variants of concern can play a key role in antibody binding. In addition to directly computing binding affinities, some of these studies proposed the possibility of allosteric communication between the RBD and the receptor or the antibody,[39,41,44] and showed that dynamical perturbations within the hACE2 receptor can arise due to its binding to the RBD At the same time, simulations have shown that (i) the dynamics of the glycan coat covering the spike affects antibody binding, and (ii) that antibody binding itself affects the motion of the RBD.[45]

Dynamical changes and allosteric effects are key to determining the relative stability of antigen-antibody complexes and can often determine the efficacy of monoclonal antibodies. To understand these allosteric communication mechanisms in greater detail, we performed extensive all-atom molecular dynamics (MD) simulations to gauge the effect of RBD mutations on the stability, dynamics and the unbinding process of monoclonal antibodies targeted to the SARS-CoV-2 spike protein. Apart from the wild type RBD, we studied four mutant strains with the following mutations: B.1.1.7 (*alpha*) (N501Y), B.1.351 (*beta*) (K417N,E484K,N501Y), B.1.617 (*kappa*) (L452R,E484Q) and B.1.617.2 (*delta*) (L452R,T478K). The two antibodies, B38[27] and BD23,[28] were chosen for the current study because their epitopes contain at least two of the four residues involved in the mutations in the studied variants and also because the new mutations in the *beta* variant have been shown to increase the binding affinity for B38 but decrease it for B23.[38] This contrasting property makes it particularly interesting to understand how the molecular interaction, and the allosteric cross-talk with the RBD, differ between these two mAbs.

Both of these antibodies were first extracted from the plasma of COVID-19 convalescent patients.[27,28] Each is a dimeric protein comprising a heavy and a light chain (labeled as Chain A and B, respectively, in this manuscript) with clearly distinguishable antigen binding domains (Figure 1). The BD23-Fab is smaller (126 and 107 residues in the heavy and light chain respectively) compared to the B38-Fab (222 and 218 residues in the heavy and light

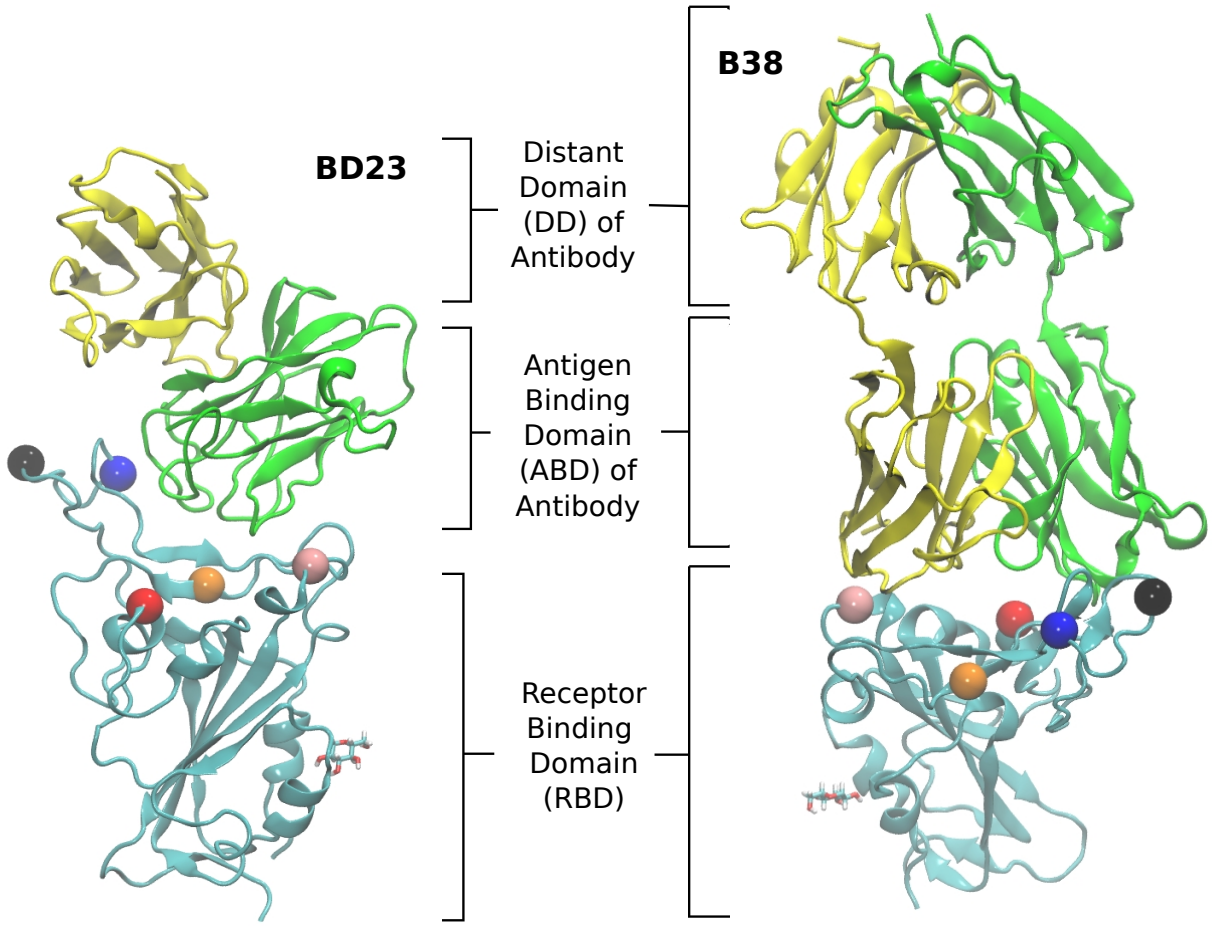

Figure 1: The structure of the RBD in complex with BD23 and B38-Fab, the two neutralizing antibodies studied in this work. Each antibody consists of a heavy (A) and a light chain (B), with chain A in green, chain B in yellow, and the RBD in cyan. The antigen binding domain (ABD) of the Ab is defined as the antibody domain that comes in contact with the RBD; the rest of the antibody is referred to as the distant domain (DD). The glycan moiety on Asn343 is colored in licorice. The residues that are mutated in the studied variants are represented as colored spheres: K417 (red), L452 (orange), T478 (black), E484 (blue) and N501 (pink).

chain respectively). The epitope of the B38 contains the RBD residues 417 and 501 while the residues 452, 484 and 501 are included in the epitope of BD23. The structures of the RBD-antibody complexes as well as the location of the mutations are depicted in Figure 1.

We studied the effect of RBD mutations on the dynamics of the antigen-antibody complex from three different angles. First, we demonstrated the relative flexibility of individual residues across the antibody, and identified the structural changes taking place in the complex when the RBD of the WT coronavirus is replaced with each of the mutants. Next, we quantified the change in the mutual information-based cross-correlation between all pairs of residues and constructed a protein graph connectivity network to decipher how are the allosteric information pathways modulated by the mutations in the RBD. Finally, we studied the change in the dissociation mechanism of the antigen-antibody complex for all combinations of the mutant RBDs using non-equilibrium pulling simulations, and rationalized our finding based on the arguments of enthalpy and hydrogen bonding. From the diverse dynamical perturbations observed in these systems, we delineated a few common themes in how the mutations in the SARS-CoV-2 antigen can impart local and global effects through the antibody structure and potentially impair the neutralizing efficacy of monoclonal antibodies. This can occur by changing interaction energies, as well as, via large-scale motions, the conformational entropy of the binding.

Computational Methods

System Preparation

The coordinates of the antibodies bound to the receptor binding domain (RBD) of the spike protein in wild type SARS-CoV-2, were obtained from the Protein Data Bank (PDB) with the following accession IDs: 7BZ5 (B38)[27] and 7BYR (BD23).[28] Apart from the B38 antibody, the 7BZ5 PDB file contains the RBD domain (only residues 334-526) including one N-acetyl-β-D-glucosamine (NAG) moiety attached to Asn343. This glycan moiety was

preserved in our calculation. The 7BYR PDB file includes the entire spike trimer along with the antibody, so we removed all spike residues except for 334-526 of the RDB bound to the antibody. The bare RBD systems were constructed from the 7BZ5 system by deleting the antibody. The mutations in the RBD were added using CHARMM-GUI input generator.[46] Each system was solvated in a cuboidal water box with 12 Å of padding in each direction, and Na^+ and Cl^- ions were added to neutralize the total charge and maintain an ionic strength of 150 mM. The hydrogen mass repartitioning (HMR) technique[47] was used to allow for 4 fs time-steps facilitating longer simulation runs. The topology files for HMR were prepared using the VMD 1.9.4 alpha 51 software.[48] All systems were modeled using CHARMM36 force field[49] (with CMAP correction for proteins) and TIP3P parameters[?] were used for the water and the ions.

Equilibration and Unbiased Simulation

Each system was minimized using a conjugate gradient algorithm for 10000 steps followed by an equilibration in NPT ensemble for 250 ps (with 2 fs time step) with harmonic restraint on the alpha carbons of all the protein residues. A 10 ns NPT equilibration was performed (with 4 fs time-step) afterwards for every system with no restraint applied on any atom. The temperature was controlled at 303.15 K using a langevin thermostat with damping constant 1 ps^{-1}, and a Langevin piston was used to control the pressure at 1 atm.

Production runs were performed for each RBD-antibody complex for 0.5 μs and for each bare RBD system for 0.3 μs. For all systems, (WT and four variants), the cumulative unbiased simulation time was 6.5 μs. Only the last 0.4 μs of each antibody bound system and 0.25 μs of each bare RBD system were used for further analysis. All simulations were performed using NAMD 2.14 simulation package.[50] The trajectories were saved at an interval of 100 ps.

The root mean squared deviation (RMSD) and the residue-wise root mean squared fluctuations (RMSF) were computed using the `gmx rms` and `gmx rmsf` tools, respectively, in the

GROMACS 2018.1 software.[51] RMSD for all systems were computed with respect to the first frame of the long production run. Trajectories were aligned only with respect to the α-carbons of the RBD using VMD software.[48] RMSD was computed for the RBD, antibody, the antigen binding domain (ABD) of the antibody and the whole system. The antigen binding domain is defined as the one of the two monomers of the BD23 protein which interacts with the RBD and the portions of the two monomers directly interacting with RBD (residue 1-108) for the B38 antibody (Fig. 1 in main text). For computing RMSF, the last 400 ns of each trajectory is split into 4 segments of 100 ns each. Residue wise RMSF was computed for each segment and the mean and standard deviations are reported. The RMSF ratio is then computed for each mutant strains as the ratio of RMSF for each residue and that of the corresponding residue in the WT. Principal component analysis was performed using the PyEMMA 2 package.[52] The non-bonding interaction energy (including the electrostatic energy and van-der Waals energy) between the antibody and the mutated residues of the RBD for different variants were calculated using the NAMD Energy module[53] of VMD.[48] Hydrogen bonds between the RBD and antibodies were also analyzed using VMD. Binding free energy between each RBD-antibody pair was computed using the Molecular Mechanics-Generalized Born Surface Area (MM-GBSA) approach[54] using the last 400 ns of the equilibrium trajectories. The NAMD2.14 package was used to perform the MM-GBSA calculation.[55]

Mutual Information-based Cross-Correlation and Protein Graph Connectivity Network

Linear mutual information (LMI) based cross-correlation matrices were computed for all residue pairs in each system. The theoretical details can be found in Refs. 56–58. Although the LMI-based cross-correlation is primarily used to quantify the allosteric effect of a small molecule ligand on the receptor protein,[59,60] it can also be used to decipher the allosteric regulation within a protein or protein complex.[61]

The linear mutual information (LMI) metric (I_{ij}) is calculated as

$$I_{ij} = H_i + H_j - H_{ij} \tag{1}$$

where H is a Shannon type entropy function:

$$\begin{aligned} H_i &= -\int p(\mathbf{x}_i) \ln p(\mathbf{x}_i) d\mathbf{x}_i \\ H_{ij} &= -\int\int p(\mathbf{x}_i, \mathbf{x}_j) \ln p(\mathbf{x}_i, \mathbf{x}_j) d\mathbf{x}_i d\mathbf{x}_j \end{aligned} \tag{2}$$

$\mathbf{x}_i, \mathbf{x}_j$ are the vectors containing the Cartesian coordinates of the C_α atoms, $p(\mathbf{x}_i)$ and $p(\mathbf{x}_j)$ are their marginal distributions and $p(\mathbf{x}_i, \mathbf{x}_j)$ is their joint distribution.[58,60] A Pearson like correlation coefficient C_{ij} is defined from I_{ij} in the following manner:

$$C_{ij} = (1 - e^{-(2/d)I_{ij}})^{-1/2} \tag{3}$$

where d is the dimensionality of the space. Unlike the conventional correlation coefficient, C_{ij} defined by Eq. 3 can only take values between zero and one.

The residue-wise cross-correlation matrices were averaged over the four 100 ns segments prepared from the last 400 ns of each unbiased production run. The changes of the cross-correlation across the mutants were used to decipher the role of the mutations in the large scale dynamics. We also constructed a protein graph connectivity network model based on the cross-correlation matrices. The α-carbon of each residue is considered as the nodes of the graph and the cross-correlation between residues as the weight of the edges. Two residues were considered connected only if the heavy atoms of those residues were within 5 Å of each other during at least 75 % of the entire production run. The betweenness centrality (BC) of each residue in the network was computed and compared between various mutant strains. The BC quantifies the information flow between nodes or edges of a network. As the α-carbon of each residue is a node in the protein-graph-connectivity network, the value of the

BC denotes the functional relevance of each residue in the dynamics of allosteric information flow through the protein chains. If a node, i, functions as a bridge between two other nodes along the shortest path joining those two, the BC of the node i is

$$BC(i) = \sum_{st} \frac{n_{st}^{i}}{g_{st}}, \tag{4}$$

where g_{st} is the total number of geodesics a.k.a. the shortest paths joining nodes s and t, out of which n_{st}^{i} paths include the node i.[59–61] The change in BC, due to RBD mutations, is used to quantify the dynamical role of mutations in modifying the information flow pathway in the RBD-spike complex. The LMI based cross-correlations were calculated and the protein graph connectivity networks were constructed using the bio3D package.[62]

Steered Molecular Dynamics Simulation

To study the unbinding of the antibodies from the spike RBD, steered molecular dynamics (SMD) simulations[63] were performed. A fictitious particle was attached to the pulling coordinate (center of mass distance between RBD and antibody) with a harmonic spring (1 kcal/mol/Å) to allow for sufficient fluctuations. For SMD simulation, all systems were modeled with the Generalized Born (GB) implicit solvent model[64] with solvent accessible surface area (SASA) correction (GBSA). The ion concentration was set at 0.15 M. Pulling was performed at a constant speed of 4 Å/ns. Five SMD trajectories of 10 ns length were generated for each system. Each trajectory was saved at an interval of 10 ps, and the pulling force and the accumulated work were recorded every 0.1 ps. The starting structures for the SMD trajectories were sampled from the unbiased production runs at the following points: 300 ns, 350 ns, 400 ns, 450 ns and 500 ns. All SMD simulations were started without further equilibration. The colvars[65] module in conjunction with NAMD 2.14[?] was used for SMD simulations.

Results

Modulation of conformational flexibility of RBD-antibody complex

The RBD, with or without the antibody, is a relatively rigid system (RMSD $\sim$ 1-3 Å) with little conformational variability, likely due to the abundance of beta-sheets in the structure. The antibodies, conversely, are highly flexible with large conformational fluctuations, as indicated by their high root-mean-squared deviation (RMSD) values throughout the production runs (see Supporting Information). Unlike the BD23 system where the entire antibody is highly flexible, in the case of B38, the fluctuations are significantly larger in the distant domain (DD), compared to the antigen binding domains for both chains. The RBD-Ab complexes of both the WT and mutants, are also capable of exploring more than one free energy minimum during the 0.4 μs period, as demonstrated by the projection of their dynamics on the first two principal components (Supporting Information). But any discernible difference between the dynamics of the WT and mutant complexes is not apparent from the RMSD and PCA analysis.

However, the RMSF of certain residues of both the RBD and Ab show significant change going from WT to the mutants. To demonstrate this effect, we computed the ratio of RMSF of each residue for every mutant with that of the WT system. Even in the case of the RBD only system, the region comprising residues 460-500 shows increased flexibility in the L452R-E484Q variant and decreased flexibility in the N501Y mutant. Interestingly, the E484Q mutation in the *kappa* variant, and the T478K mutation in *delta* variant are present within this region, which also interacts directly with both the B38 and BD23 antibodies. The change in flexibility, upon mutation, is the highest in the loop formed by residues 470-490, going up or down by a factor of 2-3 in the B.1.617 or the B.1.617.2 strains respectively. The individually mutated residues show a sharp change in RMSF values, particularly in the residue 452, 484 and 501 in the respective variants. This effect is also visible in the antibody bound systems, where the mutated residues show sharp peaks in the RMSF ratio

plot (Figs. 2 and 3). The residue 484 shows a contrasting behavior in the presence of B38 and BD23 antibody. When in complex with B38-Fab, K/Q484 and adjacent residues show a large increase in flexibility (∼2 times that of E484 in WT), but they become almost twice more rigid when bound to BD23. The RBD of *kappa* variant shows the largest increase in rigidity compared to WT, on the average, when bound to either antibody, but becomes the most flexible of all without antibody. The N501Y, the K417N-E484K-N501Y and the L452R-T478K mutants show increase in overall flexibility when in complex with the antibody. But their RMSF remain virtually unchanged with respect to the WT RBD in the absence of any antibody.

More interesting is the change in RMSF in the antibody, as it indicates that mutations in the antigenic epitope can influence the microscopic dynamics of the antibody. The change in the residue-wise RMSF of both the antibodies are, as expected, less dramatic, compared to that in the RBD, as these changes come due to a secondary perturbation caused by mutations outside the protein domain via non-covalent interactions. Figs. 2 and 3 show the ratio of the RMSF of the antibodies in complex with the mutant RBDs with respect to their wild type counterparts. For B38-Fab, the deviation is small (RMSF ratio within 0.5-1.5), except for specific residues in the distant domain, when bound to the L452R-E484Q mutant. However, the changes in flexibility are much more prominent for the BD23 antibody, for which RMSF increases on average in response to a N501Y mutation in the *alpha* variant, but the effect is largely compensated by the K417N and E484K mutations in the *beta* variant, bringing the RMSF down to a level comparable to a WT complex. The L452R and E484Q mutation in the B.1.617 strain causes an increase in overall rigidity of the antibody structure, particularly in the distant domain. The change of antibody RMSF for the *delta* variant case is almost identical to the *kappa* variant, except the antigen binding domain for BD23 shows a slight increase of flexibility in comparison to the *kappa* variant.

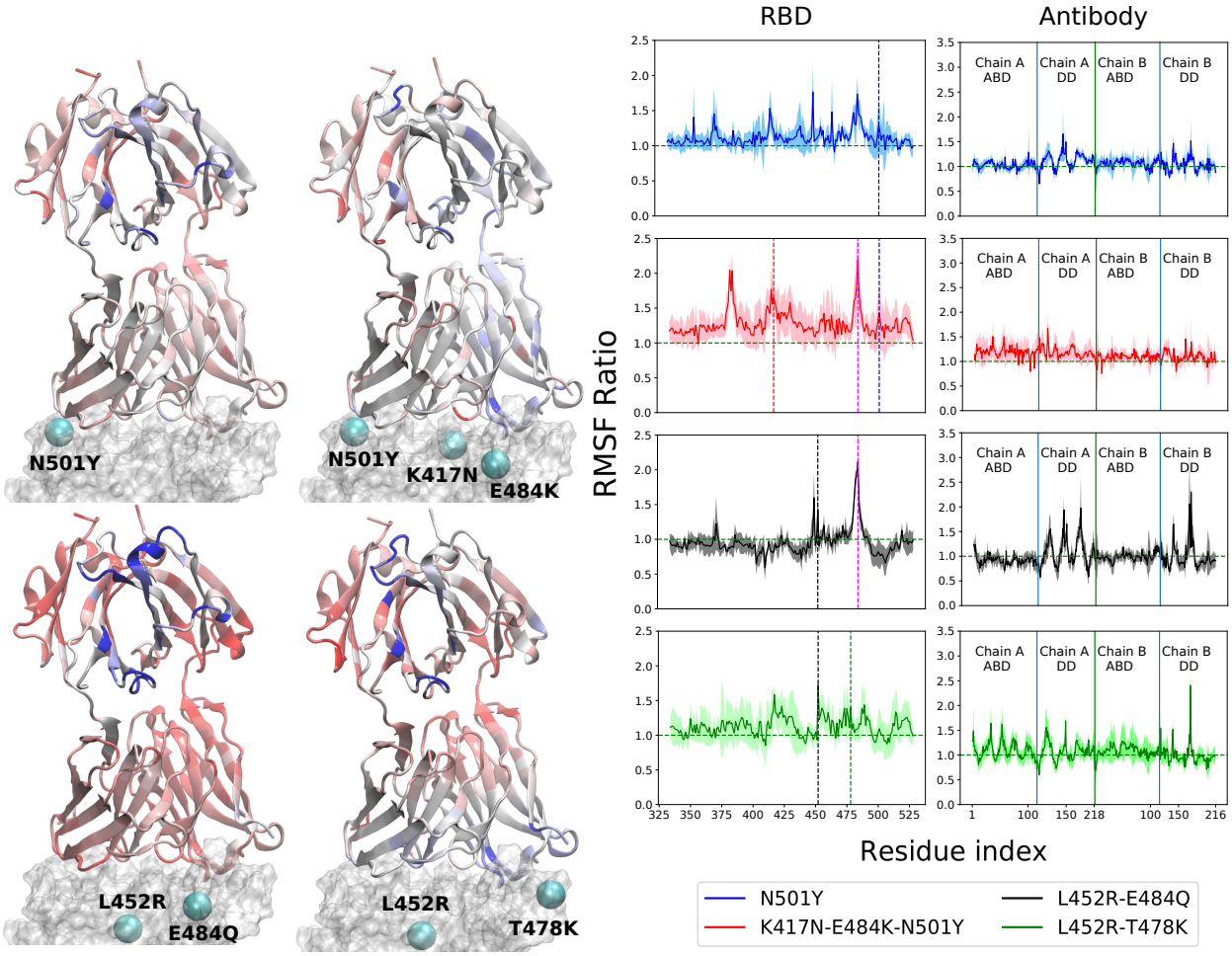

Figure 2: Change of root mean squared fluctuation (RMSF) of the RBD-B38 Fab complex due to the mutations in the variants. The RBD is represented by a transparent surface. The mutated residues are shown as cyan spheres. The antibody residues are colored according to the ratio of their RMSF when bound to a mutant RBD with that of the WT case. Blue color indicates increase in RMSF and red color indicates decrease in RMSF. The residue RMSF ratio is projected on the structure of the B38-Ab complex for *alpha* (N501Y), *beta* (K417N-E484K-N501Y), *kappa* (L452R-E484Q) and the *delta* (L452R-T478K) variants. The RMSF ratio for the RBD and the antibody are shown separately in the right column. See text for the definition of the RMSF Ratio. In the RBD RMSF ratio figures, the location of the mutations are shown as vertical dashed lines.

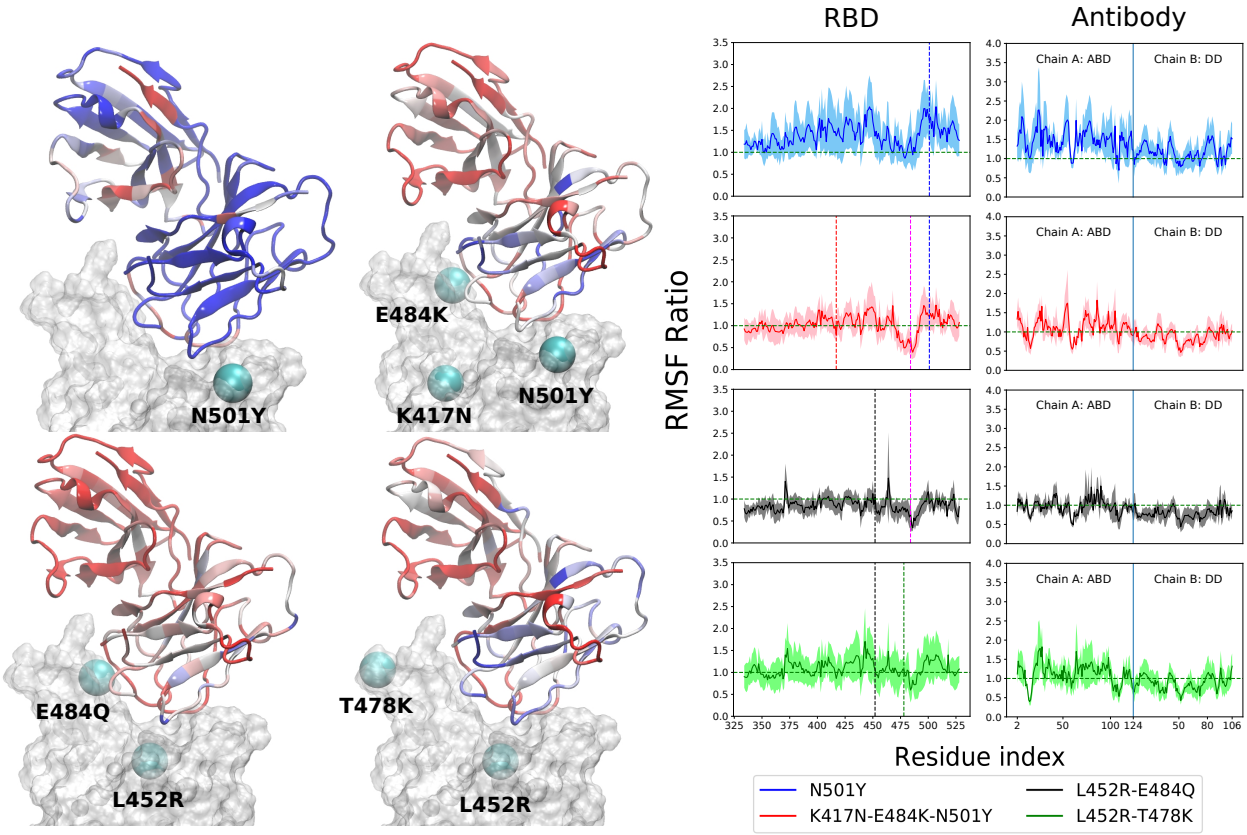

Figure 3: Same as Fig. 2 but for the RBD-BD23 Fab complex.

Change in correlated motion and allosteric information flow

Significant changes in the residue-wise LMI-based cross-correlation can be observed throughout the complex (both RBD and antibody) in presence of the RBD mutations (see Supporting information). We particularly focus here on the cross-correlation coefficient between the RBD and the antibody residues, as this can provide a direct quantitative measure of the degree of dynamical coupling between them. In Fig. 4 we depict the relative RDB-antibody cross-correlation for each mutant system, with respect to the WT complex. One of the key features of the B38-Fab is that Ab residues show large change in correlation, particularly with the mutated RBD residues and the residues adjacent to the mutation. For example, in the N501Y variant, many residues of B38 Ab show a decrease in correlation, by 0.1-0.2, with the residue 501 and its nearby RBD residues. More interestingly, this effect is propagated over a long distance, even to the distant domains of the antibody, indicating that single point mutations in RBD can have a strong long range perturbative effect on the dynamics of the antibody bound to the RBD. A similar phenomenon is observed for N417 and its neighboring residues (410-420) in the K417N-E484K-N501Y mutant, the Q484 residue in the case of the L452R-E484Q mutant, and the R452 and its nearby residues (440-455) in the L452R-E484Q and L452R-T478K mutant. Although this effect is much less pronounced in the BD23-Fab, the change in cross-correlation coefficient with the mutated RBD residues is indeed observed when, for example, in complex with the K417N-E484K-N501Y mutant. Unlike B38, this effect is much less uniform within the antibody, where certain groups of residues (e.g. near residue 50, 70 and 100 in the ABD), show large change of correlation with the mutated residues as well as for the whole RBD. This can be visualized as vertical patches of red or blue color, as opposed to horizontal patches for B38.

Moreover, there is, on average, an increase in the cross-correlation between the RBD and the B38 antibody, going from the *alpha* variant to the *beta* variant and vice versa in BD23. Previous computational studies have established that B38 binds more weakly with the spike of the *alpha* variant compared to the *beta* variant, but BD23 binds at least twice

as strongly to *alpha* variant than to the *beta* variant.[38,40] This observation indicates the mutual information based cross-correlation and the binding affinity between the monoclonal antibodies and the spike epitopes follow a similar trend.

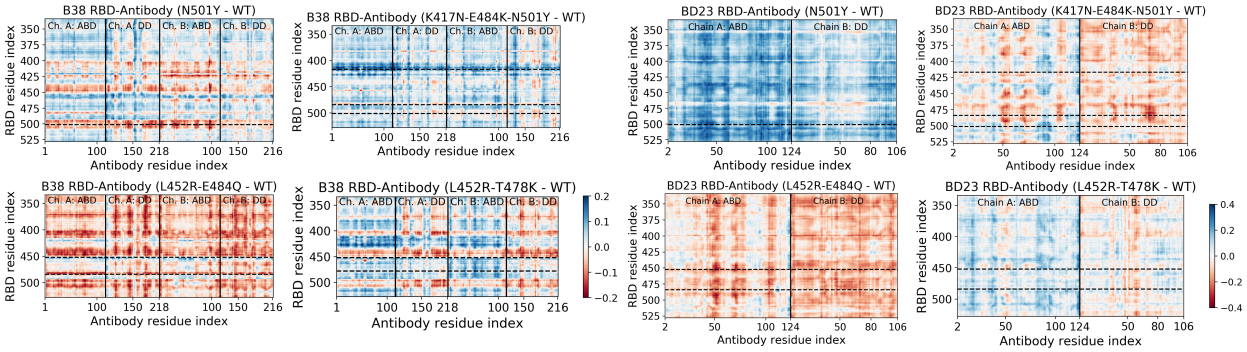

Figure 4: Change in the mutual information based cross-correlation between the antibody and the RBD residues, due to the mutations in the variants. The differences are reported as the cross-correlations of the WT species subtracted from that of the mutant species. The locations of specific mutations are labelled as dashed horizontal lines. The continuous vertical lines separate different domains and chains of the protein (for details see figure 1).

This contrasting effect in the correlated motion and the binding affinity suggests that there are allosteric communication paths between the RBD and antibody that follow different routes in the two antibodies. To probe these pathways, we built a protein graph-connectivity network model, treating each residue as the node and the negative logarithm of the cross-correlation between the residues as the weights of the edges of the graph. We computed the betweenness centrality (BC) measure, a metric which computes the relative importance of each node in the information flow pathway. We then computed the residue wise difference of BC in each mutant relative to the WT system. Changes in BC point us to the residues which either gain or lose importance in the allosteric information flow pathway in the protein. In B38-Fab, significant changes in the BC metric are primarily limited to the residues of the antigen binding domain, showcasing a situation where local correlation propagates through the amino acid sequences, leading to long range dynamical changes. Particularly, we see a large increase in the BC value for a few consecutive residues in the region of residues 92-98 in the chain A, and a large decrease in the BC for residues 23-30 in chain B, for all the

mutant species. This imbalance is primarily caused by the differential interaction of the 501 residue in its WT (N501) compared to its mutated form (Y501). The change in BC in chain B is reduced by a significant amount when the N501Y mutation is removed, for example in the L452R-E484Q variant. This effect can be a result of the reduced interaction energy of Y501 with the B38 mAb as well as the loss of the hydrogen bond between the RDB N501 residue and the S30 residue of chain B of B38, although, there is an overall increase in the hydrogen bond occupancy for Y501 as the lost bond is replaced by a hydrogen bond with S67. In essence, the allosteric information flow through the B38 antibody follows a re-wiring mechanism. The mutations switch off the information flow pathway along the residues 23-30 in chain B and increases the information traffic along residues 92-98 in chain A. This effect is highly asymmetric and location specific, and leads to a drop or rise of the cross-correlation of all antibody residues with particular mutated residues in RBD.

Unlike the B38, in BD23-Fab, a large fraction of the RBD-mAb interface, (specifically the region containing residues 97-107 of the ABD) loses centrality, along with some isolated distant domain residues; this theme is common across all mutants. Reduction of BC is also observed for residues 40-50 in the ABD and residues 80-90 in the DD, indicating the possibility of long distance propagation of allosteric signal. But the dynamical effect is different from that in the B38, as the perturbation is not localized in the vicinity of any specific mutation. The same set of residues experiences a decrease in centrality measure, irrespective of the specific location and the nature of the mutation (except for the *alpha* and *delta* variants, where some residues show a gain in BC, particularly the ABD 45-50 and DD 85-90). This explains qualitatively the observation of the mutation-specific long distance correlation changes in the case of B38 (horizontal correlation changes in Fig. 4). Contrarily, in BD23, specific residues lose or gain correlation with the majority of the RBD residues and not particularly with the mutated ones (vertical correlation changes in Fig. 4).

Taken together, these findings provide fundamental insight into the molecular mechanism of how mutations in the spike protein of SARS-CoV-2 variants impact antibody recognition

and the stability of the antigen-antibody complex. Not only do these observations bolster the necessity to develop vaccines against mutated or alternative epitopes, but also focus attention on specific regions of the antibodies that can be mutated or modified to engineer a more robust neutralizing antibody, capable to act against potential future variants of the virus.

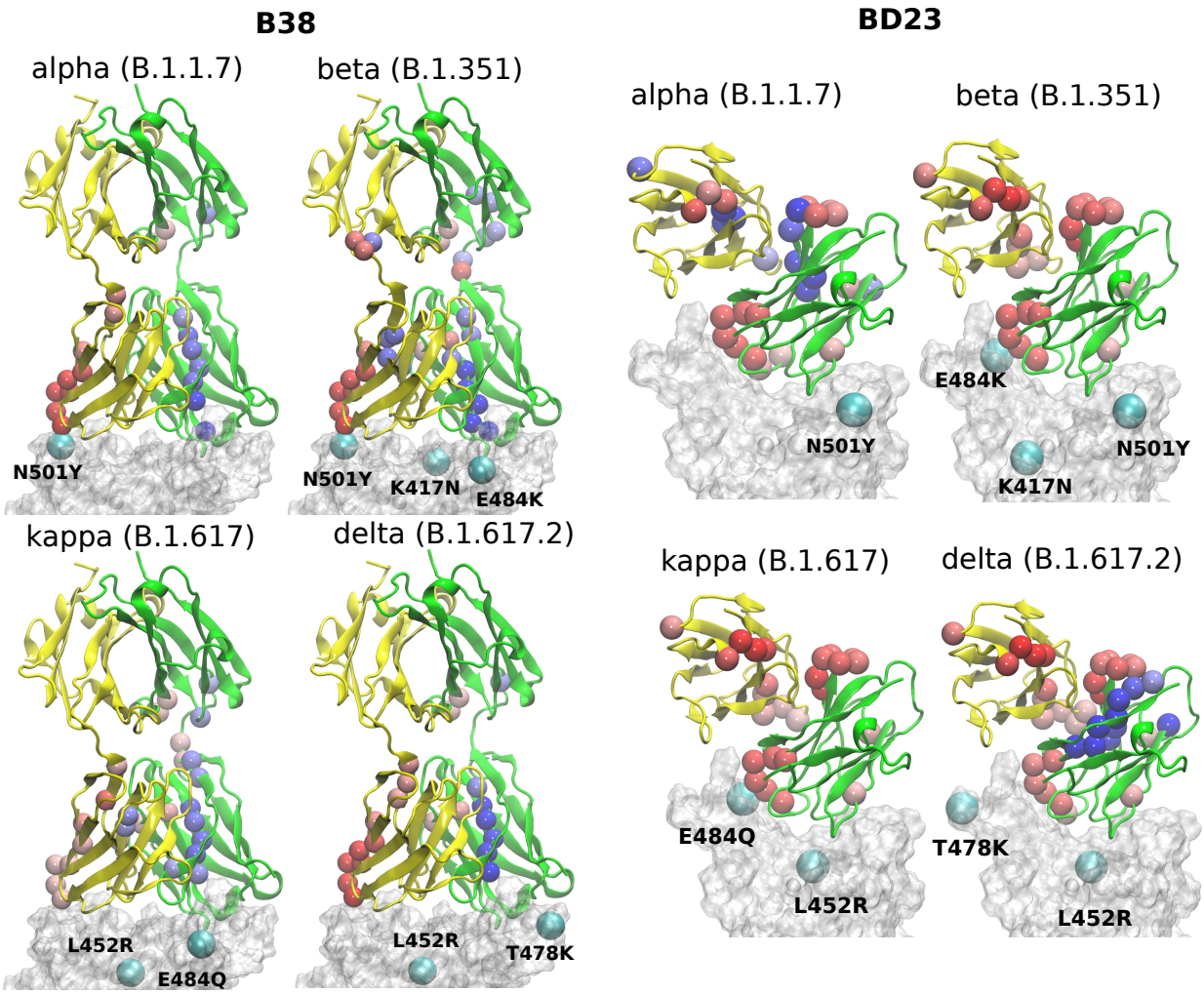

Figure 5: Change of betweenness centrality (BC) of each residue of the RBD-Ab complex due to the mutations in the variants. The RBD is represented by a transparent surface. The mutated residues are shown as cyan spheres. Only residues with a change in BC of greater than 0.005 is shown in colored sphere. Blue color indicates increase in BC and red color indicates decrease in BC.

Dissociation dynamics and interactions at the RBD-Antibody interface

From multiple steered molecular dynamics simulations (SMD), we elucidated the unbinding mechanism of the monoclonal antibodies from the spike RBD. For detailed inspection, we picked the pathway that corresponds to the lowest amount of non-equilibrium work for each RBD variant and mAb combination, as it would contribute the most to the real unbinding process. For all variants and both the antibodies, the loop consisting of residues 470-490 is found to play an important role in the unbinding process. It is the last point of contact between the RBD and the antibody in the lowest work dissociation pathway for every system, and it functions like an anchor to adhere to the antibody before a complete detachment occurs. A recent computational study revealed that this loop region also functions like an anchor while binding to the hACE2 receptor.[66] Unsurprisingly, one of the key mutating residue (E484) is situated in this region of the RBD, and, when not mutated, it is often the last RBD residue that forms a hydrogen bond with the dissociating antibody. But in the K417N-E484K-N501Y and L452R-E484Q variants, this residue is mutated and no longer forms the "last" hydrogen bond to anchor the departing antibody. Instead, other residues from the same loop, primarily S477, which has a hydroxyl side-chain, performs the role. We also observed A475 to form the "last" hydrogen bond for the L452R-E484Q RBD with B38 antibody, with its main chain hydrogen bond donor group (See supporting information). Although the 484 residue is not mutated in the *delta* variant, the last hydrogen bonds between the *delta* RBD and the departing antibody is formed by the A475, S477 and E486 residues.

We computed the average non-equilibrium work required to detach the antibody for all systems, as $-\ln\langle\exp(-W_i)\rangle$, where W_i denotes the accumulated work in the ith SMD run, and $\langle\rangle$ indicates arithmetic mean. The average work is heavily dominated by the dissociation path that requires the lowest amount of work. In the limit of infinite number of pulling trajectories or in the limit of infinitely slow pulling, the average non-equilibrium work will

become equal to the unbinding free energy. Provided E484 is the key anchoring residue, we expect the non-equilibrium work will depend on how strongly this particular residue or its mutants interact with the antibody. From the non-bonded energy calculation on the equilibrium trajectory it is apparent that the three mutated residues: E484, K484 and Q484, have little difference in their interaction energy with the B38-Fab antibody, primarily because this residue is not in direct contact with the antibody interface (Fig. 6b). Consequently, the four mutants do not show any discernible difference in the work required for the dissociation of the RBD-mAb complex (Fig. 6c). But, the *alpha* and *beta* mutants require the least amount of work for antibody detachment in comparison to the WT, an effect that can partially be attributed to the N501Y mutation. From non-bonded energy calculation, we see that the N501Y mutation destabilizes the interaction between RBD and B38 by lowering (less negative) the interaction energy (see Supporting information).

For the BD23-Fab, an almost opposite effect is observed. The residue E484, being situated in the RBD-mAb interface, plays a key stabilizing role for the complex via hydrogen bond to the S103 and S104 residues of the Ab. Once mutated, the hydrogen bond propensity of residue 484 diminishes, leading to almost 80 kcal/mol decrease (less negative) in the non-bonded interaction energy with the mAb, in agreement with a recent computational study.[40] The E484 forms hydrogen bonds with the S104 (26.37% with backbone and 54.19% with side-chain) and S103 (3.85% with backbone and 8.62% with side-chain) residues of BD23 with high occupancy in the WT and with G102 (31.9%), S103 (4.15%), Y32(10.7%), Y110 (3.95%) and Y111 (2.73%) residues of the antibody in the N501Y mutant. In the K417N-E484K-N501Y variant, K484 forms hydrogen bonds only with N92 (6.86%) and Y110 (1.18%) with relatively low occupancy. This effect is clearly reflected in the non-equilibrium work profile, where, going from N501Y mutant to K417N-E484K-N501Y and L452R-E484Q variants, we see a significant drop in the work required for the detachment of the antibody. Conversely, for the *delta* (L452R-T478K) variant, where the 484 residue is not mutated, the E484 forms hydrogen bonds with >50% occupancy with the S103 and Y109 residue of the BD23 antibody.

This increases the amount of non-equilibrium work required for the dissociation of the RBD antibody complex to a strikingly high level. This is reflected also in the high binding free energy of the BD23 antibody with the *delta* variant RBD.

The N501Y mutant, contrarily, binds more strongly with the BD23-Fab (Figure 6f) compared to the WT, because the non-bonding interaction energy of Y501 is higher (more negative) than that of the N501 residue (Supporting info). This effect can be attributed to the increased probability of hydrogen bonding between the RBD and the antibody by the Y501 residue compared to the N501. In the WT system, N501 participates in one hydrogen bond with the D73 residue of the BD23-Fab with negligible occupancy (0.62%). But in the N501Y mutant, the Y501 participates in two hydrogen bonds with the L72 and the Y80 residues of the antibody with significantly higher occupancy (5.77% and 6.27% respectively). The rupture forces of the spike antibody complexes follow a similar trend as the average non-equilibrium work for the WT and the mutants (see Supporting Information).

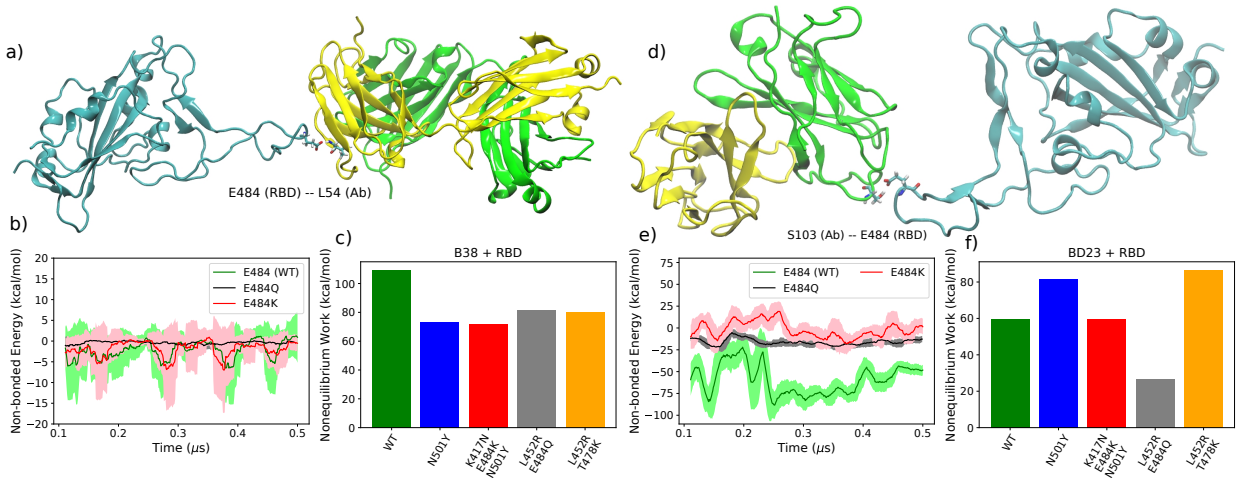

Figure 6: (a) A representative intermediate structure for the B38 antibody unbinding from the RBD from the SMD simulation. Intermediates for all variants are shown in supporting information. (b) The non-bonded interaction energy between the RBD residue 484 and the B38 antibody from the unbiased simulation. (c) The average non-equilibrium work performed to dissociate the B38 antibody from all variants of the RBD using SMD simulation. Figure (d-f) are identical as (a-c) but for BD23 antibody.

The relative ordering of the binding free energies between each RBD antibody pair is also consistent with the average non-equilibrium work necessary for the dissociation of respective

complexes (Fig 7). In agreement with the earlier work,[38] the binding affinity of the BD23 with the *beta* variant was observed to be weaker than the *alpha* strain, and vice versa for the B38 Fab monoclonal antibody. It indicates that the arguments presented above, in terms of non-bonded interaction energy and hydrogen bonding propensity, also holds true for the binding free energy of the antigen-antibody complexes. The practical implication of these findings is that antibodies, such as B23, that uses the RBD 470-490 loop as an epitope, are more likely to have significantly different affinity against mutated versions of the virus. Alternatively, fairly consistent neutralization of most variants is possible if antibodies or vaccines are generated using an alternative region of the receptor binding domain as the epitope.

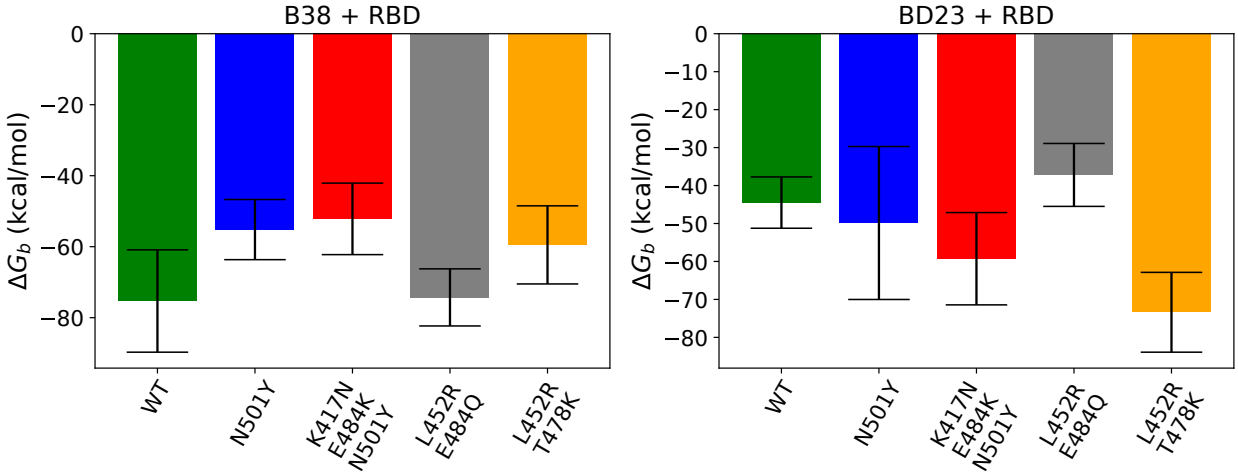

Figure 7: Binding free energy of the spike protein variants with the B38 and BD23 antibody.

Discussions and Conclusions

Atomistic simulation of interaction between the receptor binding domain of the SARS-CoV-2 spike protein and the neutralizing monoclonal antibodies reveals the functional role of point mutations in the new variants in the process of immune evasion, via perturbing the microscopic dynamics of the antibodies and destabilizing the antigen-antibody complexes. Some key findings are the following. First, the structures of the antibodies are largely flexible

and can undergo significant conformational transitions over a timescale of several hundreds of nanoseconds. However, the flexibility of certain residues in the antigen-antibody complex are modified disproportionately when the WT RBD is replaced with one from the mutant strains. The mutated RBD residues (and the residues in proximity with the mutations) show the largest change in flexibility. Particularly, in the absence of an antibody, the loop involving residues 470-490 becomes flexible when a Glu to Lys (or Gln) mutation at 484 position makes it incapable of forming a stabilizing hydrogen bonding interaction. While local motions in the antibody occurred next to the mutation site in the spike, there were also significant large-scale collective motions throughout the antibody structure, which, due to entropic considerations, we speculate, can modulate the free energy of binding. This can also come from the overall change of stability of the antigen-antibody complex arising from the noticeable changes in rigidity which could be observed not only in the antigen binding domain but also in the distant regions. The effect is more prominent for the smaller antibody, BD23 Fab, where the N501Y mutation drastically increases the dynamic motion across both the domains in the antibody structure. The K417N and E484K mutations reverse this effect to a large extent, and in the B.1.617 variant, both the antibodies become structurally more rigid compared to the WT complex. As the dynamical perturbations in the antibody results from mutations in the antigen, this serves as a striking example of inter-protein allosteric information propagation, which potentially takes effect in the evolutionary forces helping the virus to adapt against therapeutic interventions.

The linear mutual information-based cross-correlation between the antigen-antibody residue pairs is a useful metric that can capture quantitative information about the allosteric information flow. Cross-correlation values, for both the RBD and the antibody, can change significantly due to mutations in RBD, but there is a stark contrast in the manifestation of this change in correlated dynamics in the two antibodies. Unlike B38-Fab, where correlations with specific mutated RBD residues are modified across the entire antibody, such effects in BD23 Fab are fairly localized albeit universal. Certain groups of residues in the

BD23-Fab, gain or lose correlation with the entire RBD and not just with the mutated residues. The antibody residues impacted by the mutation are conserved for all mutants, but whether they show a positive or negative change in cross-correlation can depend on the nature of the mutation. We previously observed such an effect within the spike protein,[61] but now we see that the allosteric effect can be propagated into the antibodies that interact with the spike only via non-covalent interactions. One key observation is that the gain or loss in cross-correlation follows the trend of binding affinity fairly well. This phenomenon behooves one to investigate further, for wide range of protein-protein complexes, the relationship that inter-residue cross-correlations has with, on one hand binding free energies. On the other hand, simulation-based correlation paths can put in relationship with experimental measures of allosteric signals transduced within and between proteins. For example, using cryo-EM, Frank and collaborators have delineated a way to retrieve functional pathways of biomolecules from single-particle snapshots.[67]

A protein graph connectivity network provides a deeper understanding of the information flow pathway inside the antibody. With the aid of graph theoretical measures like betweenness or eigenvector centrality, one can quantify the importance of individual resides in the allosteric information propagation. We specifically looked at the change in betweenness centrality for the antibody residues induced by the mutations in the spike antigen. While a group of amino acids in the B38 light chain (proximal to RBD residue 501) experience centrality reduction, it increases in virtually the opposite end of the heavy chain in the RBD-antibody interface. Absence of the N501Y mutation reduces this effect, revealing a antigen variant induced re-wiring mechanism that decreases the traffic of allosteric communication inside antibody in the vicinity of the N501Y mutation and increases near the K417N and E484K/Q mutations. This effect being particularly specific to the location of the mutation in the epitope, it explains the mutation specific collective change in the cross-correlation throughout the B38 antibody. In contrast, all the BD23 residues near the RBD binding interface experience a reduced centrality, irrespective of the specific location of the mutations. Some distant

residues, following the trend of interfacial residues, show a change in BC value without the regard of the location of the mutation, explaining the specific RBD-BD23 cross-correlation pattern. These contradictory features point to inherent differences in the microscopic dynamics of the antibody, depending on the nature of mutations in the antigen and on the nature of the antibody. They have repercussions beyond the current pandemic, controlling the fundamental process of antigen-antibody recognition at a molecular level.

The process of unbinding of the antibodies from the spike proteins of different variants is also of significant interest as it provides a more mechanistic insight into the mutation induced modification of the antibody binding propensity. Irrespective of the variant or the antibody, the RBD loop, consisting of residues 470-490, works like an anchor and is always the last region to interact with the antibody in the lowest energy dissociation pathways. The E484K/Q mutation in this region reduces the anchoring capability with the BD23 antibody by the loss of key hydrogen binding interactions. So, the N501Y, K417N-E484K-N501Y, and L452R-E484Q mutants show a decreasing trend in the non-equilibrium work required to dissociate the BD23 antibody, and the recovery of the E484 residue in the *delta* variant regains the binding affinity. B38, in contrast, does not interact with residues 484 or 452 in the bound state, and mutations in those residues have little to no impact on the interaction energy of B38 with the spike protein. So, all the RBD variants in this study have almost identical binding affinity with the B38 antibody as apparent from the steered MD results. But the mutants have lower "binding strength" than the WT because of the loss of stabilizing hydrogen bonds as a result of the N501Y mutation. It is interesting to note that the newly emerging highly contagious *delta* (L452R-T478K) and *delta plus* variants (K417N-L452R-T478K) also gained a different mutation in this important loop region that is vital in preventing the dissociation of the antibody.

In summary, we quantified how the microscopic dynamics of the antigen-antibody complex is perturbed by the mutations in the antigen and the nature of the antibody, with a particular focus on the SARS-CoV-2 spike protein, relevant to the recent pandemic. Al-

though there is significant diversity in the dynamical perturbations observed in the molecular level, there are some common underlying themes: modulation of inter-residue correlations, allosteric information propagation, and destabilization of non-bonded interactions dictate the stability of the antigen-antibody complex which in turn determines the efficacy of monoclonal antibody therapies. Taken together, this study explores the molecular signatures of the antigen induced allosteric effects pronounced within the antibody, with a particular focus on coronavirus to address the challenges of the ongoing pandemic. But the scope of our findings is much broader, and future studies in this direction can lead to a deeper understanding of the immune evasion mechanism of mutating viruses to help in designing adaptive therapeutic strategies to immunize the population against new viral strains via monoclonal antibodies and vaccination.

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Data Availability Statement

All the trajectory data are available from the Zenodo Repository: `https://doi.org/10.5281/zenodo.5676393`. The codes and analysis scripts are available from the GitHub repository: `https://github.com/dhimanray/COVID-variant-antibody.git`

Author Contributions

DR designed research with inputs from IA. DR and RNQ performed unbiased molecular dynamics simulations and analyzed results. DR performed SMD simulations and analyzed results. DR constructed the protein-graph connectivity network model using mutual information based cross correlation. DR and IA wrote the paper. All authors contributed to discussing the results and reviewing the manuscript.

Supporting Information Available

Table S1: List of all simulations performed in this study

Figure S1-S22: Additional figures.

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