Immune escape facilitation by mutations of epitope residues in RdRp of SARS-CoV-2

Author: Aayatti Mallick Gupta¹, SasthiCharan Mandal¹, Sukhendu Mandal² and Jaydeb Chakrabarti¹

Affiliations:

¹ Department of Chemical, Biological & Macro-Molecular Sciences, S. N. Bose National Centre for Basic Sciences, Block-JD, Sector-III, Salt Lake, Kolkata-700 106

² Laboratory of Molecular Bacteriology, Department of Microbiology, University of Calcutta, 35, Ballygunge Circular Road, Kolkata, 700019, India

* To whom correspondence should be addressed:

Corresponding Author: * To whom correspondence should be addressed:
aayattigupta@gmail.com

Corresponding Author: Aayatti Mallick Gupta

Address: Department of Chemical, Biological & Macro-Molecular Sciences, S. N. Bose National Centre for Basic Sciences, Block-JD, Sector-III, Salt Lake, Kolkata-700 106

Email: aayattigupta@gmail.com

ABSTRACT

SARS-CoV-2 has considerably higher mutation rate. SARS-CoV-2 possesses a RNA dependent RNA polymerase (RdRp) which helps to replicate its genome. The mutation P323L in RdRp is associated with the loss of a particular epitope (321-327) from this protein which may influence the pathogenesis of the concern SARS-CoV-2 through the development of antibody escape variants. We consider the effect of mutations in some of the epitope regions including the naturally occurring mutation P323L on the structure of the epitope and their interface with paratope using all-atom molecular dynamics (MD) simulation studies. P323L mutations cause conformational changes in the epitope region by opening up the region associated with increase in the radius of gyration and intramolecular hydrogen bonds, making the region less accessible. Moreover, the fluctuations in the dihedral angles in the epitope:paratope (IgG) interface increase which destabilize the interface. Such mutations may help in escaping antibody mediated immunity of the host.

Keywords: SARS-CoV-2, RdRp, epitope loss, paratope, structural dynamics, dihedrals, thermodynamics

1. Introduction

RNA viruses are prone to mutations, depending on the fidelity of the RNA polymerase. These mutations direct the overall variations in the viral genome in the population [1,2] and are beneficial for viral adaptation [3,4]. SARS-CoV-2 is an enveloped, single and positive stranded RNA virus belonging to the genus beta corona virus [5]. This genus includes two more RNA viruses, like SARS-CoV [6] and MERS-CoV [7], responsible for epidemics, like the Severe Acute Respiratory Syndrome (SARS) and the Middle East Respiratory Syndrome (MERS), respectively. As a common characteristic of RNA viruses, the more the SARS-CoV-2 is spreading across the globe, newer mutational hotspots are emerging in its genome. Lack of pre-existing immunity against a particular variant and its high transmissibility leave us vulnerable to infection that leads the WHO to the announcement of a worldwide public health emergency [8,9]. The microscopic mechanism of immune escape by the mutated variants remains elusive. The emerging SARS-CoV-2 mutant variants include RNA dependent RNA polymerases (RdRp) as one of the mutation hot spots [10]. RdRp is a multi-domain protein that forms key transcription machinery in cooperation with cofactors nsp7 and nsp8 to carry out the proof-reading activity [11-13]. Recurrent P323L mutation of RdRp across the world has been reported [14]. Cryo EM structural study obtained for SARS-CoV-2 RdRp [15] has tempted to speculate the conformation changes due to its mutation. In an earlier work [16] we show that the region 321-327 in RdRp forms an epitope of the antigen that is recognizable by the paratope of the antibody of the human host cell, while P323L mutation is associated with loss in epitope. Such loss of antigenic determinant impairing immune recognition has been found in many viral disease models [17]. So, the question is: Does the loss in epitope in RdRp suffice to cause impairment of the paratope interaction? We address this question in the present work.

The relevant antibody for the epitope region 321-327 is not known. A high concentration of IgG antibody has been observed from week 3 to week 7 after SARS-CoV-2 viral exposure indicating the immune response against the virus [18]. Antigen specific antibody IgM could not be detectable after illness onset suggesting the possibility that most of the antigen specific antibody class switching to IgG occurs within 1 week after infection [19]. The effects of RdRp mutations on the binding of the epitope to IgG paratope is of paramount interest. The immune response involves several mechanisms of molecular recognition and interaction between epitope and paratope. Biomolecular binding typically involves changes in conformation which is associated with free energy and entropy costs [20]. The thermodynamic costs between two conformational (complex versus free states) states can be estimated by comparing the marginal dihedral distributions in the two conformations. The ratio of the peaks in the distributions gives population difference associated with the Boltzmann factor of the underlying free energy, while the entropy is given using the Gibb' s formula [20]. All the dihedral contributions can be added to get the changes in a given residue and overall the residues for the total changes. If the correlations between the binding partners are ignored, one can speak in terms of changes in the thermodynamic quantities of individual components. A negative change in free energy and entropy represent conformational stability and ordering in a given state with respect to the reference state. We base our analysis on changes in conformational thermodynamics, if the changes enhance the stability and order in the epitope-paratope interface.

We perform all-atom molecular dynamics (MD) simulations on the wild type (wt) and mutated type (mt) protein in their free and antibody bound states using theGROMOS96 53a6 force-field in the GROMACS 2018.6 package. We study the structural changes associated with the mutation and compute the corresponding thermodynamic changes. This is done by comparing the dihedral

distributions in the mutated RdRp conformation for the wild type RdRp conformation. We further consider both mutated and wild type RdRp in presence of IgG antibody to study the relative conformational stability and order the complexes. Our observations are as follows: (1) We consider a few mutations, namely, the epitope residues P323L, F324L and T326L. We observe that the epitope region undergoes conformational disorder due to the mutations. (2) The wild type RdRp shares a conformationally stable interface with the antibody, whereas mutated RdRp does not. Thus, the mutated RdRp cannot form a stable interface with IgG compared to the wild type protein. Antigen binding to the antibody is vital in immune response and has several biomedical applications including vaccines and immune-therapeutics [21,22].

2. Materials and methods

2.1 System preparation

We have considered the cryo-EM structure of SARS-CoV-2 RNA-dependent RNA polymerase (PDB id: 6M71, chain A) [15] to study the effect of mutation on structural stability. The mutations are obtained from the cryo-EM structure. The epitope is identified using a 5-fold cross-validation approach considering computed volume, hydrophobicity, polarity, together with the relative surface accessibility and secondary structure [23]. IgG antibody (PDB id: 2DD8, chain H and L) [24] is used for paratope prediction using RdRp as antigen with antibody i-patch program [25]. Antibody i-Patch uses antibody-specific statistics to annotate residues with a score indicating how likely they are to be in contact with the supplied antigen. Antibody i-patch Scores range from 0 to 12, with residues higher values corresponding to greater binding likelihood with antigen. Thus, the complementarity determining region (CDR) according to Immunogenetics Information System (IMGT) [26, 27] definition can be located.

2.2 Docking studies

Antigen-antibody docking studies have been carried out using the Antibody Mode of the ClusPro server (www.cluspro.bu.edu) [28]. CDR residues of an antibody are defined while the rest of the residues that does not fall in the CDR are masked. We have performed docking separately for IgG antibody with wildtype and mutated RdRp protein respectively. Vina1.1.2 [29] has been utilized to perform molecular docking with the drug molecule and RdRp to predict the effect of the later on drug susceptibility due to P323L mutation. The docking boxes have been set at the hydrophobic cleft close to P323L mutation site. Two separate dockings have been carried out using (i) wild type RdRp and Tegobuvir (drug molecule) and (ii) mutated RdRp and Tegobuvir. The search exhaustiveness is set as 32, and the number of binding modes is set as 9.

2.3. MD simulation

We perform molecular dynamics simulations in explicit water with counter-ions using GROMOS96 53a6 force-field [30]. spc216 (simple point charge) water molecule [31] is used for solvation under a cubic box with a minimum distance between the protein and the box 1.0 Å. Chlorine and sodium counter ions are added as required replacing the water molecules to neutralize the positive or negative charge. Particle mesh Ewald (PME) sum method has been used for treating the long-range electrostatic interactions [32]. The energy minimization has been performed until the maximum force of the system became < 100 kJ/mol/nm to relieve steric clashes and inappropriate geometry. For equilibration of the systems, a short NVT equilibration phase with the heavier atoms fixed and then follows a longer NPT runs. The systems are subjected to isothermal (300K) and isobaric (1 atm) conditions under periodic boundary conditions and 2 fs time-step. The total number of particles (N= 254640) including water, pressure and temperature have been fixed for each of the systems to make the simulated ensembles equivalent. The simulation trajectories are calculated up to 500 ns. The equilibrations

of the simulated structures are assessed from the root-mean square deviation (RMSD). RMSD determines the structural changes over time. The RMSD data (Supplementary information, SI Figs. S1(A)-(E)) show that the systems are equilibrated beyond 300ns. We analyze the data from the last 200 ns trajectory. Various analyses are carried out with tools in GROMACS to examine the system properties. Conformational thermodynamics changes for (a) P323L mutation in RdRp and (b) epitope-paratope interface are estimated from equilibrium fluctuations of the dihedral angles using the Histogram Based Method (HBM) [20]. The histograms of the dihedrals are computed considering all the structures from the equilibrated trajectory (between 300-500ns). 100 samples each with 1000 conformations are generated from the equilibrated 300-500 ns trajectory for each wildtype and mutated system. The final structure of each of the systems given by a snapshot is also shown as SI Fig. S2.

3. Results and discussions

The epitope regions of RdRp include residues F321, P322, P323, T324, S325, F326 and G327 [16]. For the given epitope residues(Figs. 1(a) and (b)), the paratope residues are identified using the standard immuno-informatics approach [see methods for details]. The paratope region of IgG as an antibody includes S31, Y32 and T33 at H1 region and H2 region consist of L55, I57, N59 and Y60. R94, D95, T96, G99, D101 and V102 are typically present in the H3 region(Fig. 1c). In the light chain, K31 and S32 comprise the L1 region, Y49-P55 forms the L2 region and L3 region consist of W91-Y97. Docking using the crystal structures of wild type (wt-) RdRp and IgG, biased by the identified epitope and paratope residues yields the paratope-epitope interface within 0.5 to 1 nm distance in the wt-RdRp-IgG complexes shown in Table 1. The interface predominantly consists of the epitope residues P323, F324 and T326. We consider point mutations, like the reported one P323L, and additional mutations F324L and T326L. We

denote the mutated systems by mt(P323L)-RdRp, mt(T324L)-RdRp and mt(F326L)-RdRp respectively. In all the cases, we have replaced the residue with hydrophobic residue L, which has a less preference to be epitope residue [33]. The MD simulation studies are carried out in two stages: First, we consider the changes in the epitope conformation under mutation and thermodynamics quantities associated with these changes. Next, we consider the changes in the epitope-paratope region due to mutation.

3.1. *Conformation changes in epitope due to point mutations*

Let us consider in detail the conformations of wt-RdRp and mt(P323L)-RdRp. We characterize epitope conformation using the cosine of the angle θ between the vectors joining C_{α} atoms of F321 and T324 and G327. We show the distribution of $\cos\theta$, $H(\cos\theta)$ over the equilibrium trajectories for both the wild type and the mutated proteins in Fig. 2a. The wt-RdRp reveals a sharp peak at $\theta = 90^\circ$. In mt(P323L)-RdRp, there is a shift to $\theta = 180^\circ$. This represents the opening of the epitope region due to P323L mutation. The changes of conformation are manifested in the other quantities as well. Radius of gyration (R_g), a measure for the compactness of the structure has been calculated using the standard method as given at Gromacs manual [30]. The distribution $H(R_g)$, shown in Fig. 2b, has a peak for larger R_g in mt(P323L)-RdRp than wt-RdRp, depicting lack of compactness associated with loss of epitope region due to P323L mutation. Solvent accessible surface area, S is given by the area of the surface of RdRp with radius $r_{vdW} + r_{sol}$, where the center of a spherical solvent molecule or probe (r_{sol} in contact with the atomic van der Waals sphere r_{vdW}). The histogram distribution of solvent accessible area (SASA), $H(SASA)$ (Fig. 2c) shows that the SASA in mt(P323L)-RdRp is less than that in wt-RdRp. Hydrogen bond analysis gives the number of hydrogen bonds at a distance of less than 3.5 Å between all possible donors D and acceptors A with a D-H-A angle of 180° to 30° . The

distribution of number of hydrogen bond in the epitope region, $H(hb)$ is higher in mt(P323L)-RdRp than in wt-RdRp (Fig. 2d). We have used the DSSP [34] to assign the secondary structural elements to the residues. Secondary structural changes due to P323L mutation of RdRp from the simulated trajectory reveal the predominance of 5-helix over beta bridge (Figs. 2e and 2f). The change of secondary structural elements in the epitope residue is consistent to epitope loss due to P323L mutation as epitope residues are typically rich in loops and depleted of strands and helices [33,35].

We consider changes in the equilibrium distributions of the dihedral angles, ϕ , ψ , and χ_1 . We denote the distribution of a dihedral angle θ of the i -th residue in wt-RdRp by $H_i^{wt}(\theta)$, and mt(P323L)-RdRp by $H_i^{mt}(\theta)$. The distributions $H_{T324}^{wt}(\phi)$ and $H_{T324}^{mt}(\phi)$, shown in Fig. 3a, are both unimodal but peaks at different locations indicating isomeric transition. This is observed also for $H_{T324}^{wt}(\psi)$ and $H_{T324}^{mt}(\psi)$ (Fig. 3b) and the side chain dihedrals, $H_{T324}^{wt}(\chi_1)$ and $H_{T324}^{mt}(\chi_1)$ (Fig. 3c). More pronounced changes are observed for F326. $H_{F326}^{wt}(\phi)$ is unimodal whereas $H_{F326}^{mt}(\phi)$ shows bimodal distribution conferring an increase in flexibility at this degree of freedom due to mutation (Fig. 3d). A bimodal distribution is found in $H_{F326}^{wt}(\psi)$, while $H_{F326}^{mt}(\psi)$ is quite flat with multiple peaks (Fig. 3e). $H_{F326}^{mt}(\chi_1)$ is widened more than $H_{F326}^{wt}(\chi_1)$ (Fig. 3f). The cases of the dihedral angles of the other residues of the epitope region are shown in SI Figs. S3 and S4. Overall, the distribution of dihedral angles shows a decrease in flexibility due to P323L mutation.

The changes in the conformational free energy (ΔG) and entropy ($T\Delta S$) are computed from comparing the histogram of mt(P323L)-RdRp with respect to wt-RdRp[20]. Careful examination of the epitope region shows that the residues like S325, F326 and G327 undergo enhanced disorder, the maximum being in F326 (Table S1). This is consistent with enhanced flexibilities of

the dihedral angles of these residues. On the other hand, there is an increase in order in F321, P322 and T324. The maximum ordering is in T324. The overall change in entropy is 2.53 KJ/mol where the major changes are in the backbone dihedrals. The changes in the stability are marginal for all the cases. Thus, the epitope region undergoes conformational disorder due to P323L mutation of RdRp mainly via enhanced flexibility of the backbone dihedrals.

In case of other point mutations, mt(T324L)-RdRp the epitope conformation is defined by the angle θ between the vectors joining C_{α} atoms of F321 and L324 and G327. $H(\cos(\theta))$ here is bimodal with peaks at $\theta = 110^{\circ}$ and $\theta = 130^{\circ}$. Thus, the epitope prefers more open conformations than in wt-RdRp. In case of mt(F326L)-RdRp, the angle θ between the vectors joining C_{α} atoms of F321 and P324 and G327, shows a single sharp peak at $\theta = 100^{\circ}$. The mutation here also opens the protein conformation compared to the wild type protein (SI Fig. S5). The conformational free energy and entropy in both cases show destabilization and disorder compared to the wild type. In mt(T342L)-RdRp, the free energy change is 3.30 KJ/mol and the entropy change is noted as 15.86 KJ/mol. The free energy change and entropy change for mt(F326L)-RdRp is 4.71 KJ/mol and 9.24 KJ/mol respectively. Thus, the mutations in the epitope region tend to result in the open conformation of the epitope region and destabilization and disorder compared to the wild type protein.

3.2. *Changes in epitope-paratope binding interface due to mutation*

We carry out docking of the paratope to the epitope with various mutations to ascertain their effects on epitope-paratope binding. The docked complexes are shown in SI Fig. S6. The docking results are shown in Table S2. The binding free energy ΔG and the dissociation constant (K_d) at 25°C are reported from the docking studies. Both ΔG and K_d are less in wt-RdRp-IgG than that in the mutated complexes. Thus, the mutations tend to decrease the binding affinity

219 of IgG. The paratope residues G99 of H1 and W91 of L3 forms van der Waals interactions with
220 the epitope residues T324 and F326 respectively in mt(P323L)-RdRp-IgG. In mt(T324L)-RdRp-
221 IgG complex a single van der Waal interaction is noticed between the epitope residue F326 and
222 L55 of H2 region of paratope. In mt(F326L)-RdRp-IgG complex, we find three hydrogen bonds
223 and one van der Waal interaction in the epitope:paratope interface (Table S3). Thus, the
224 mutations in the paratope region lead to the loss of epitope which impairs the paratope binding.
225 We carry out MD simulations to the microscopic details of impaired paratope binding due to
226 mutation of the paratope residues in the naturally occurring mutation, namely, mt(P323L)-RdRp-
227 IgG and compare the conformational stability with respect to wt-RdRp-IgG. First, we
228 characterize the interface and interactions as revealed by the simulated trajectories. The
229 interfacial residues and remain largely unchanged. We also noted the changes in the nature of
230 interactions between the epitope and paratope interface, listed in Table1. First, we consider the
231 wt-RdRp-IgG complex. A carbon hydrogen bond is formed between P323 (CD:H donor) of wt-
232 RdRp and S32 (O:H acceptor) from L1 region of IgG. The second conventional H-bond
233 interaction is found between L1-S32 (HG: H donor) of paratope and F326 (O: H acceptor) of
234 epitope. The polar epitope residue T324 of wt-RdRp when interacts with charged group H3-D95.
235 The polar epitope residue T324 when interacts with polar paratope residue is responsible for
236 polar interactions (Table 1). The van der Waals interaction energy is about -2.0 KJ/mol. The loss
237 of epitope due to P323L mutation of RdRp disrupts the paratope binding as manifested by a less
238 number of interactions at the mt(P323L)-RdRp-IgG interface (Table1). The paratope residues
239 G99 of H1 and W91 of L3 form van der Waals interactions with the epitope residues T324 and
240 F326 respectively in mt(P323L)-RdRp-IgG. The rest of the CDR from IgG and the associated
241 epitope residues are not found to interact with the interfacial residues of mt(P323L)-RdRp-IgG.

242 We show the changes in dihedral distributions in dihedral angles in epitope residues in the
 243 epitope-paratope complex in Fig. 4. We consider T324 and F326 which participate in binding in
 244 both the wild type and mutated complexes. We compare the distributions of the interface
 245 residues in the wild type ($H_i^{wt-C}(\theta)$, solid line) to those in the mutated RdRp ($H_i^{mt-C}(\theta)$, dotted
 246 lines). The backbone dihedral ϕ in both $H_{T324}^{wt-C}(\phi)$ and $H_{T324}^{mt-C}(\phi)$ are similar (Fig. 4a).
 247 $H_{T324}^{wt-C}(\psi)$ has a tail and is broader than $H_{T324}^{mt-C}(\psi)$ (Fig. 4b). The side chain dihedral (χ_1) also
 248 depicts uniformity in H_{T324}^{wt-C} and H_{T324}^{mt-C} (Fig. 4c). $H_{F326}^{wt-C}(\phi)$ and $H_{F326}^{mt-C}(\phi)$ are similar (Fig.
 249 4d). $H_{F326}^{wt-C}(\psi)$ is a unimodal distribution, whereas $H_{F326}^{mt-C}(\psi)$ is a multimodal, responsible for the
 250 increase in flexibility for this degree of freedom in the epitope:paratope binding interface due to
 251 mutation (Fig. 4e). $H_{F326}^{wt-C}(\chi_1)$ is wider than $H_{F326}^{mt-C}(\chi_1)$ (Fig. 4f). The distributions for additional
 252 epitope residues due to mutation are described in SI Figs. S7 and S8. The changes in the dihedral
 253 distribution indicate that the epitope residues due to P323L mutation become more flexible.

254 Some of the changes in distributions of the paratope dihedral angles are shown in Fig. 5. We
 255 show distributions: (a) IgG in free state ($H_i^{f,p}(\theta)$, solid line), (b) in bound state of wt-RdRp-IgG
 256 ($H_i^{wt,p}(\theta)$, dotted line) and (c) that in mt(P323L)-RdRp-IgG ($H_i^{mt,p}(\theta)$, dashed
 257 line). $H_{HY32}^{f,p}(\phi)$ shows a broader distribution than $H_{HY32}^{wt,p}(\phi)$ which is sharper, while $H_{HY32}^{mt,p}(\phi)$
 258 becomes almost as broad as in the free case (Fig. 5a). Thus, the complex in the wild type
 259 paratope reduces the flexibility of ϕ of the residue but the mutation renders it as flexible as in the
 260 free paratope. Similarly, $H_{HY32}^{f,p}(\psi)$ is flatter than $H_{HY32}^{wt,p}(\psi)$, whereas, $H_{HY32}^{mt,p}(\psi)$ shows a wider
 261 distribution similar to $H_{HY32}^{f,p}(\psi)$ (Fig. 5b). In the case of side-chain dihedral χ_1 , $H_{HY32}^{f,p}(\chi_1)$,
 262 $H_{HY32}^{wt,p}(\chi_1)$ and $H_{HY32}^{mt,p}(\chi_1)$ are unimodal with sharp peaks and do not show significant changes
 263 (Fig. 5c). From the L chain, $H_{LS32}^{f,p}(\phi)$ is multimodal but $H_{LS32}^{wt,p}(\phi)$ is unimodal, but $H_{LS32}^{mt,p}(\phi)$ is

264 similar to $H_{LS32}^{f,p}(\phi)$ (Fig. 5d). $H_{LS32}^{wt,p}(\psi)$ has sharp peak compared to broader peaks in $H_{LS32}^{mt,p}(\psi)$ and
 265 $H_{LS32}^{f,p}(\psi)$ (Fig. 5e). A sharp peak is found in $H_{LS32}^{wt,p}(\chi_1)$, whereas $H_{LS32}^{mt,p}(\chi_1)$ and $H_{LS32}^{f,p}(\chi_1)$ are
 266 multimodal in nature (Fig. 5f). The rest of the paratope residues are shown in SI Figs. S9 and
 267 S10. Therefore, the flexibility of the free IgG reduces due to epitope: paratope association of the
 268 wildtype complex which again increases and become comparable to the free paratope. due to
 269 mutation (Fig. 5f). Overall, the changes in dihedral distribution due to the paratope residues
 270 indicate that the free unbound paratope loses its flexibility when binding with the epitope region
 271 of wt-RdRp.

272 The majority of the residues at the epitope region were found to get ordered and stabilized in wt-
 273 RdRp-IgG complex compared to the free state of the epitope in wt-RdRp (Table S4). It is found
 274 that F326 is responsible for the largest increase in order. The side chain dihedral (χ_1) causes a
 275 major increase in order. For the change in free energy, the maximum stability is due to T324.
 276 The backbone dihedral (ψ) contributes most to the increase in stability. Thus, the epitope
 277 residues of wt-RdRp are observed to attain stability (-12.93 KJ/mol) and order (-4.04 KJ/mol)
 278 due to the paratope binding. On the other hand, binding of mt(P323L)-RdRp with IgG leads to
 279 disorder and destabilization in most of the residues compared to those in wt-RdRp (Table S5).
 280 The backbone dihedrals are chiefly responsive to the decrease in order. In the case of free energy
 281 change due to mt(P323L)-RdRp-IgG complex formation than in the free state of the epitope in
 282 wt-RdRp, the residues are marginally destabilized. The backbone dihedrals can be held
 283 responsive to the decrease in stability of the region. Thus, the epitope residues in mt(P323L)-
 284 RdRp-IgG get destabilized (2.89 KJ/mol) and disordered (4.61 KJ/mol) over wt-RdRp.

285 We examine the conformational contributions to the free-energy and entropy changes in the
 286 paratope residues from the dihedral distributions. First, let us consider wt-RdRp-IgG case (Table

S6), in which most of the paratope residues in the complex get ordered and stabilized in the complex compared to their free states. At the paratope region, the backbone dihedral (ψ) contributes for the maximum ordering in the complex compared to their free states. Likewise, with respect to free energy change, the maximum stability is imparted by (ϕ) dihedral. The backbone dihedrals contribute to the stability of the paratope in wild type complex than the free state. The paratope IgG is found to get ordered (-20.38 KJ/mol) and stabilized (-6.62 KJ/mol) in the wt-RdRp-IgG complex compared to their free states. In the case of mt(P323L)-RdRp-IgG, most of the paratope residues gets disordered and destabilized in the complex than in its free state (Table S7). The mt(P323L)-RdRp-IgG shows the disorder most in all the paratope residues except Y32 and R94 of H chain and S93 of L chain. Excepting Y32 of H chain and W91 and S94 of L chain, all are found to get destabilized due to mt(P323L)-RdRp-IgG complex formation than in its free IgG state. The backbone dihedrals (ψ) have a major contribution for the disorder (15.34 KJ/mol) and ϕ dihedrals lead to decrease in stability (14.92 KJ/mol) of the mutated complex than in its free state.

4. Discussion

The detailed conformational thermodynamics data yield the following microscopic picture of the loss in epitope by a mutation in the epitope region of RdRp. In the mutated protein, the epitope residues are having disordered conformation compared to the wild type protein mostly via the backbone dihedrals, where residues S325 to G327 are primarily affected. The changes in the backbone fluctuations affect the interface with the paratope of IgG. While the stability of the backbone helps to build up a stable interface with the paratope, the enhanced backbone fluctuations in the wild type RdRp disrupt the interactions. The gain in order and stability in the wild type epitope-paratope interface show up in the epitope and paratope residues together both

through the backbone and side chain fluctuations. Note that the changes take place all over the epitope-paratope interface, indicating cooperative binding. This is further evident in the wild type case where the epitope-paratope entire region gets disordered and destabilized via both backbone and dihedral fluctuations. Clearly, the mutated RdRp can escape the immune response of the system by altering the backbone fluctuations. Since the conformational disorder can be directly measured by NMR experiments [36,37], the mechanism for immune-escape can be established experimentally.

RdRp is an important therapeutic target to various drug inhibitors [38-44]. Some of the drug molecules are predicted to have binding moiety in a hydrophobic cleft close to the epitope [45]. Thus, it is important to know drug resistance on viral phenotypes due to such mutation. To see the drug resistance at a qualitative level, we dock one drug molecule, Tegobuvir to wt-RdRp and mt(P323L)-RdRp complexes with the grid box center set to (X: 63.99 Å, Y: 91.88 Å, Z: 75.98 Å) and (X: 64.34 Å, Y: 89.74 Å, Z: 74.63 Å) respectively (SI Fig. S11). We find that the binding affinity of Tegobuvir is better for wt-RdRp ($\Delta G = -9.1$ Kcal/mol) than that of mt-RdRp ($\Delta G = -7.9$ Kcal/mol). Thus, P323L mutation of RdRp alters interactions with Tegobuvir.

To summarize we study here the effect of antibody binding by RdRp due to mutations in the epitope region from MD simulation. We observe that mutations of the epitope residues may cause immune evasion by escaping the antibody-mediated neutralization and producing increased pathogenicity. Albeit the availability of WHO approved emergency vaccines, the impact of SARS-CoV-2 variants of interest and variants of concern on transmissibility, disease severity, risk of reinfection and vaccine performance is a big concern. Thus, such study upon the immune escape variants is a continuous need to understand their epidemiological and clinical

impacts. Further vigorous study on the effect of drug molecules on the mutation hot spot of RdRp is essential in future.

Author contributions

AMG curate analyzed and interpreted the data and wrote the manuscript. SCM helped in writing the codes. SM reviewed and edited the manuscript. JC interpreted the data, reviewed and edited the manuscript.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Acknowledgements

AMG is thankful to the Technology Research Centre, S.N. Bose National Centre for Basic Sciences, Kolkata for the computational facilities and the Council for Scientific and Industrial Research for financial support through Research Associateship.

Disclosure statement: The authors wish to declare that they do not have any conflict of interest.

References

- [1] Domingo E. Viruses at the edge of adaptation. *Virol* 2000; 270:251-253.
- [2] Domingo E, Holland JJ. RNA virus mutations and fitness for survival. *Annu Rev Microbiol* 1997; 51:151-178.
- [3] Dufy S. Why are RNA virus mutation rates so damn high? *PloS Biol.* 2018;16: e3000003.
- [4] Wang C, Liu Z, Chen Z, Huang X, Xu M, He T, Zhang Z. The establishment of reference sequence for SARS-CoV-2 and variation analysis. *J Med Virol* 2020;92:667-674.
- [5] Wu F, Zhao S, Yu B, Chen YM, Wang W, Song ZG, et al. A new coronavirus associated with human respiratory disease in China. *Nature* 2020;579 265-269.

- 355 [6] . Peiris JSM, Lai ST, Poon LL, Guan Y, Yam LYC, Lim W, et al. Coronavirus as a
356 possible cause of severe acute respiratory syndrome. *Lancet* 2003;361:1319–1325.
- 357 [7] Zaki AM, Boheemen SV, Bestebroer TM, Osterhaus AD, Fouchier RA. Isolation of a
358 novel coronavirus from a man with pneumonia in Saudi Arabia. *N Engl J Med* 2012;367:1814–
359 1820.
- 360 [8] Chan JFW, Yuan S, Kok KH, To KKW, Chu H, Yang J, et al. A familial cluster of
361 pneumonia associated with the 2019 novel coronavirus indicating person-to person transmission:
362 A study of a family cluster. *Lancet* 2020;395:514–523.
- 363 [9] Cucinotta D, Vanelli M. WHO declares COVID-19 a pandemic. *Acta Biomed.*
364 2020;91:157-160.
- 365 [10] Pachetti M, Marini B. Benedetti F, Giudici F, Mauro E, Storici P, et al. Emerging SARS-
366 CoV-2 mutation hot spots include a novel RNA-dependent-RNA polymerase variant. *J Transl*
367 *Med.* 2020;18:179.
- 368 [11]. Bruenn JA. A structural and primary sequence comparison of the viral RNA-dependent
369 RNA polymerases. *Nucleic Acids Res.* 2003;31:1821-1829.
- 370 [12]. Venkataraman S, Prasad BVLS, Selvarajan R. RNA dependent RNA polymerases: insights
371 from structure. *Function Evol Viruses.* 2018;10:76.
- 372 [13]. Jia H, Gong P. A structure-function diversity survey of the RNA-dependent RNA
373 polymerases from the positive-strand RNA viruses. *Front Microbiol.* 2019;10:1945.
- 374 [14] Mercatelli D, Giorgi FM. Geographic and genomic distribution of SARS-CoV-2 mutations,
375 *Front Microbiol.* 2020;11:1800.
- 376 [15] Gao Y, Yan L, Huang Y, Liu F, Zhao Y, Cao L. Structure of the RNA-dependent RNA
377 polymerase from COVID-19 virus, *Science* 2020;368:779-782.

- 378 [16]Gupta AM, Chakrabarti J, Mandal S. Non-synonymous mutations of SARS-CoV-2 leads
379 epitope loss and segregates its variants, *Microbes Infect.* 2020;22:30182-30189.
- 380 [17] Francica JR, Rohena AV, Medvec A, Plesa G, Riley JL, Bates P. Steric shielding of
381 surface epitopes and impaired immune recognition induced by the ebola virus glycoprotein.
382 *PLoS Pathog* 2010;6:e1001098.
- 383 [18] J. Zhao, Q. Yuan, H. Wang, W. Liu, X. Liao, Y. Su, et al. Antibody responses to
384 SARSCoV-2 in patients of novel coronavirus disease 2019, *Clin Infect Dis.* 2020;71:2027-2034.
- 385 [19] Xiao AT, Gao C, Zhang S. Profile of specific antibodies to SARS-CoV-2: the first report. *J*
386 *Infect.* 2020;81:147e78.
- 387 [20] Das A, Chakrabarti J, Ghosh M. Conformational contribution to thermodynamics of
388 binding in protein-peptide complexes through microscopic simulation. *Biophys J.*
389 2013;104:1274-84.
- 390 [21] Gershoni JM, Roitburd-Berman A, Siman-Tov DD, Freund NT, Weiss Y. Epitope mapping:
391 the first step in developing epitope-based vaccines. *BioDrugs.* 2007;21:145–156.
- 392 [22] Irving MB, Pan O, Scott JK. Random-peptide libraries and antigen-fragment libraries for
393 epitope mapping and the development of vaccines and diagnostics, *Curr Opin Chem Biol.*
394 2001;5:314–324.
- 395 [23] Zhang Q, Wang P, Kim Y, Haste-Andersen P, Beaver J, Bourne PE. et al. Immune
396 epitope database analysis resource (IEDB-AR). *Nucleic Acids Res.* 2008;36:W513e8.
- 397 [24] Prabakaran P, Gan J, Feng Y, Zhu Z, Choudhry V, Xiao X, Ji X, Dimitrov DS. Structure
398 of severe acute respiratory syndrome coronavirus receptor-binding domain complexed with
399 neutralizing antibody, *J Biol Chem.* 2006;281:15829-15836.

- 400 [25] Baker KT, Shi J, Deane CM. Antibody i-Patch prediction of the antibody binding site
401 improves rigid local antibody-antigen docking. *Protein Eng Des Sel.* 2013;26:621-629.
- 402 [26] Lefranc MP. IMGT unique numbering for the variable (V), constant (C), and groove (G)
403 domains of IG, TR, MH, IgSF, and MhSF. *Cold Spring Harb. Protoc.* 2011;6:633–642.
- 404 [27] Lefranc MP, Pommie' C, Ruiz M, Giudicelli V, Foulquier E, Truong L. et al. IMGT
405 unique numbering for immunoglobulin and T cell receptor variable domains and Ig superfamily
406 V-like domains. *Dev. Comp. Immunol.* 2003;27:55– 77.
- 407 [28] Brenke R, Hall DR, Chuang GY, Comeau SR, Bohnuud T, Beglov D, Schueler-Furman
408 O, Vajda S, Kozakov D. Application of asymmetric statistical potentials to antibody-protein
409 docking, *Bioinformatics* 2012;28:2608-2614
- 410 [29] Trott O, Olson AJ. AutoDock Vina: improving the speed and accuracy of docking with a
411 new scoring function, efficient optimization and multithreading, *Journal of Computational*
412 *Chemistry* 2010;31:455-461
- 413 [30] Oostenbrink C, Villa A, Mark AE, van Gunsteren WF. A biomolecular force field based
414 on the free enthalpy of hydration and solvation: the GROMOS force-field parameter sets 53A5
415 and 53A6, *J Comput Chem* 2004;25:1656-1676.
- 416 [31] Berendsen HJC, Postma JPM, van Gunsteren WF, Hermans J. Interaction models for
417 water in relation to protein hydration, *Nature* 1969;224:175–177.
- 418 [32] Darden D, York L. Pedersen Particle mesh Ewald: an N^2 ; $\log(N)$ method for Ewald sums
419 in large systems, *J Chem Phy.* 1993;98:10089-10092.
- 420 [33] Rubinstein ND, Mayrose I, Halperin D, Yekutieli D, Gershoni JM, Pupko T.
421 Computational characterization of B-cell epitopes. *Mol Immuno.* 2008;45:3477–3489.

- 422 [34] Kabsch W, Sander C. Dictionary of protein secondary structure: pattern recognition of
423 hydrogen-bonded and geometrical features, *Biopolymers* 1983;22:2577-2637.
- 424 [35] Sun J, Xu T, Wang S, Li G, Wu D, Cao Z. Does difference exist between epitope and
425 non-epitope residues? Analysis of the physicochemical and structural properties on
426 conformational epitopes from B-cell protein antigens. *Immunol Res* 2011;7:1e11.
- 427 [36] Frederick KK, Marlow MS, Valentine KG, Wand AJ. Conformational entropy in
428 molecular recognition by proteins, *Nature*.2007;448:325–329.
- 429 [37] Lee AL, Kinnear SA, Wand AJ. Redistribution and loss of side chain entropy upon
430 formation of a calmodulin-peptide complex. *Nat. Struct. Biol.* 2000;7:72–77.
- 431 [38] Furuta Y, Gowen BB, Takahashi K, Shiraki K, Smee DF, Barnard DL. Favipiravir (T-
432 705), a novel viral RNA polymerase inhibitor. *Antiviral Res.* 2013;100:446-454.
- 433 [39] Agostini MK, Andre EL, Sims AC, Graham RL, Sheahan TP, Lu X, et al. Coronavirus
434 susceptibility to the antiviral remdesivir (GS-5734) is mediated by the viral polymerase and the
435 proofreading exoribonuclease. *MBio.* 2018;9:e00221-18.
- 436 [40] Lim SY, Osuna C, Lakritz J, Chen E, Yoon G, Taylor R, et al. Galidesivir, a direct-acting
437 antiviral drug, Abrogates Viremia in Rhesus Macaques challenged with zika virus. *Open Forum*
438 *Infect Dis.* 2017;4:S55.
- 439 [41] Morgenstern B, Michaelis M, Baer PC, Doerr HW, Cinatl JJ. Ribavirin and interferon- β
440 synergistically inhibit SARS-associated coronavirus replication in animal and human cell lines,
441 *Biochem Biophys Res Commun.* 2005;326:905-908.
- 442 [42] Manli W, Ruiyuan C, Leike Z, Yang X, Liu J, M. Xu M, et al. Remdesivir and
443 chloroquine efectively inhibit the recently emerged novel coronavirus (2019-nCoV) in vitro. *Cell*
444 *Res.* 2020;30:269-271.

- 445 [43] Warren TK, Wells J, Panchal RG, Stuthman KS, Garza NL, van Tongeren SA, et al.
 446 Protection against flavivirus diseases by a novel broad-spectrum nucleoside analogue BCX4430.
 447 Nature. 2014;508:402-405.
- 448 [44] Najjar M, Suebsuwong C, Ray SS, Thapa RJ, Maki JL, Nogusa S, et al. Structure guided
 449 design of potent and selective ponatinib-based hybrid inhibitors for RIPK1. Cell Rep.
 450 2015;10:1850-1860.
- 451 [45] Ruan Z, Liu C, Guo Y, He Z, Huang X, Jia X, et al. Potential inhibitors targeting RNA-
 452 dependent RNA polymerase activity (NSP12) of SARS-CoV-2. J Med Virol. 2021;93:389-400.
 453

454 Table1: The interacting residues at the epitope:paratope interface

System	Epitope	Paratope residue	Nature of interaction
wt-RdRp-IgG	T324	H-Y32	Polar
	P323	H-G99	Van der Waals
	T324	H-D95	Polar-charged
	T324	H-Y96	Polar
	P323	L-S32	Hydrogen
	T324	L-S32	Polar
	F326	L-S32	Hydrogen
	P323	L-Y49	Van der Waals
	P323	L-D50	Van der Waals
	P323	L-W91	Van der Waals
	T324	L-W91	Polar
	T324	L-Y96	Polar
	F326	L-W91	Van der Waals
	F326	L-S93	Van der Waals
	F326	L-S94	Van der Waals
mt(P323L)-RdRp-IgG	T324	H-G99	Van der Waals
	F326	L-W91	Van der Waals

455

456

457 Figs. 1

458

459 **Figs. 1:** (a) RdRp protein (red) with the epitope region highlighted in yellow.(b) Close up view
460 of the epitope region (ball and stick model) (c) IgG with H chain (dark blue) and L chain (light
461 blue). The paratope regions are shown in yellow.

462

463 Figs. 2

464

465 **Figs. 2:** Comparative structural analysis of epitope region in wt-RdRp(solid line) and
466 mt(P323L)-RdRp(dotted line): (a) histogram of $\cos\theta$, $H(\cos\theta)$ where θ is the angle between the
467 vectors joining C_α atoms of F321 and T324 and G327. (b) Histogram $H(R_g)$ of the radius of
468 gyration. (c) Histogram $H(SASA)$ of accessible surface area. (d) Histogram $H(hb)$ of the number
469 of hydrogen bond due to intramolecular interactions. Secondary structure with time plot: (e) wt-
470 RdRp and (f) mt(P323L)-RdRp.

471

Figs. 3

Figs. 3: Representative histograms of dihedral angles of wt-RdRp (solid line) and mt(P323L)-RdRp (dotted line) in the epitope. (a) $H_{T324}^{wt}(\phi)$ and $H_{T324}^{mt}(\phi)$. (b) $H_{T324}^{wt}(\psi)$ and $H_{T324}^{mt}(\psi)$. (c) $H_{T324}^{wt}(\chi_1)$ and $H_{T324}^{mt}(\chi_1)$. (d) $H_{F326}^{wt}(\phi)$ and $H_{F326}^{mt}(\phi)$. (e) $H_{F326}^{wt}(\psi)$ and $H_{F326}^{mt}(\psi)$. (f) $H_{F326}^{wt}(\chi_1)$ and $H_{F326}^{mt}(\chi_1)$.

Figs. 4

Figs. 4: Representative histograms of epitope dihedral angles of the complexes, wt-RdRp-IgG (solid line) and mt(P323L)-RdRp-IgG (dotted line) : (a) $H_{T324}^{wt-C}(\phi)$ and $H_{T324}^{mt-C}(\phi)$. (b) $H_{T324}^{wt-C}(\psi)$ and $H_{T324}^{mt-C}(\psi)$. (c) $H_{T324}^{wt-C}(\chi_1)$ and $H_{T324}^{mt-C}(\chi_1)$. (d) $H_{F326}^{wt-C}(\phi)$ and $H_{F326}^{mt-C}(\phi)$. (e) $H_{F326}^{wt-C}(\psi)$ and $H_{F326}^{mt-C}(\psi)$. (f) $H_{F326}^{wt-C}(\chi_1)$ and $H_{F326}^{mt-C}(\chi_1)$.

Figs. 5

Figs. 5: Representative histograms of the paratope dihedral angles IgG in free state ($H_i^{f,p}(\theta)$, solid line) and in bound state of wt-RdRp-IgG ($H_i^{wt,p}(\theta)$, dotted line) and mt(P323L)-RdRp-IgG ($H_i^{mt,p}(\theta)$, dashed line). (a) ϕ , (b) ψ and (c) χ_1 of Y32 of H chain. (d) ϕ , (e) ψ and (f) χ_1 of S32 of L chain.