Thermodynamics and Kinetics in Antibody Resistance of the 501Y.V2 SARS-CoV-2 Variant

Son Tung Ngo,^{ab*} Trung Hai Nguyen,^{ab} Duc-Hung Pham,^c and Nguyen Thanh Tung,^{de} Pham Cam Nam,^{f*}

^aLaboratory of Theoretical and Computational Biophysics, Ton Duc Thang University, Ho Chi Minh City 700000, Vietnam

^bFaculty of Applied Sciences, Ton Duc Thang University, Ho Chi Minh City 700000, Vietnam

^cDivision of Immunobiology, Cincinnati Children's Hospital Medical Center, Cincinnati 45229, OH, USA

^dInstitute of Materials Science, Vietnam Academy of Science and Technology, Hanoi 100000, Vietnam

^eGraduate University of Science and Technology, Vietnam Academy of Science and Technology, Hanoi 100000, Vietnam

^fDepartment of Chemical Engineering, The University of Da Nang, University of Science and Technology, Da Nang City 550000, Vietnam

ABSTRACT: Understanding thermodynamics and kinetics of the binding process of antibody to SARS-CoV-2 receptor-binding domain (RBD) of Spike protein is very important for the development of COVID19 vaccines. Especially, it is essential to understand how the binding mechanism may change under the effects of RBD mutations. In this context, we have demonstrated that the South African variant (B.1.351 or 501Y.V2) can resist the neutralizing antibody (NAb). Three substitutions in RBD including K417N, E484K, and N501Y alters the free energy landscape, binding pose, binding free energy, binding kinetics, and unbinding pathway of RBD + NAb complexes. The low binding affinity of NAb to 501Y.V2 RBD confirms the antibody resistance of the South African variant.

TOC Graphic

The novel β -coronavirus, SARS-CoV-2, whose sequence is similar to SARS-CoV-1 and MERS-CoV inducing human respiratory epidemic in the beginning of this century, is the cause of the human respiratory disease (COVID-19) pandemic worldwide.^{1, 2} This virus has been infecting more than 100 million cases and associated with more than 2 million deaths. SARS-CoV-2 is a single-positive-strand RNA virus, whose genome encodes for four main components: spike, envelope, membrane and nucleocapsid.^{3, 4} The spike protein (protein S) of SARS-CoV-2 which is used by the virus to bind to human angiotensin-converting-enzyme 2 (ACE2), has been researched thoroughly. ACE2 is present in different tissues in the body, including the lung, heart and liver,⁵ is employed by SARS-CoV-2 as receptor to bind and infect human cells. The S trimer comprises three copies of S1 and S2 subunits. S1 subunit contains 4 domains: S1A, S1B, S1c and S1D, in which S1B domain is also called receptor-binding domain (RBD), which mediates the attachment of spike protein to target cell via binding to ACE2 receptor.⁶ Once RBD is in the 'up' conformation, it can recognize and bind into ACE2, which leads to the conformational changes of S2 subunit and enables SARS-CoV-2 to fuse with cell membrane and to enter host cells.^{1, 6}

RBD is the main target of neutralizing antibodies (NAbs) which can be isolated from plasma of COVID-19 patients, immunoglobulin libraries, or immunized laboratory animal models¹. These NAbs can be roughly divided into four main classes, of which class 1s' and class 2s' RBD epitopes overlap with the ACE2-binding site, suggesting a neutralization mechanism that involves direct competition with ACE2. Class 1 antibodies, which are encoded by the immunoglobulin V-gene (VH3-53) segment with complementarity-determining regions 1 and 2 (CDRH1 and CDRH2) and a short CDRH3, are mostly elicited by SARS-CoV-2 infection. On the other hand, when class 2 antibodies

also target site I10,15 which is also target epitopes of class 1 antibodies, they bind to RBD in both 'up' and 'down' conformations of S protein.^{1,7} Additionally, class 3 antibodies bind outside ACE2 and recognize both up and down RBD, while class 4 antibodies comprise previously described antibodies that cannot block ACE2 and target only to RBD in 'up' conformation.¹ Besides RBD, the N-terminal domain (NTD) of protein S is also a popular target for NAb and many potent monoclonal antibodies directed against this region show great potential in clinical trials for COVID-19 treatment.⁷ The majority of these antibodies target a single immunodominant site on NTD, including the N1-loop (NTD N-terminus), N3-loop (supersite b-hairpin), and N5 loop (supersite loop). Subsets of these antibodies and NAb in class 1 and class 3 form multi-donor classes, with different set of VH germline restricted mode of spike recognition.⁷

Due to many reasons, including high transmissibility, the longevity of the pandemic, and encountering with immunocompromised hosts, SARS-CoV-2 undergoes different rounds of mutations, which has altered the structures of the virus, modulated its infectivity, and changed the antigenicity of the surface proteins.⁸ The variants, including United Kingdom (B.1.1.7) and South African (B.1.351 or 501Y.V2) variants have associated with

increased transmissibility and possibly increased mortality.⁷ Especially, the SARS-CoV-2 lineage in South Africa, included nine mutations in the spike protein, seems to decrease the efficacy of NAb as well as Covid-19 vaccine efficacy of some vaccines currently being used.^{9, 10} The mutations in B.1.351 can be divided into two groups, one concentrates in NTD, including four substitutions and a deletion (L18F, D80A, D215G, Δ 242-244, and R246I), and the other involves three substitutions in RBD (K417N, E484K, and N501Y).¹¹

Evaluating antibody resistance of the 501Y.V2 SARS-CoV-2 variant is greatly attractive to scientists.^{7, 9, 10, 12} Understanding the physical insights into the process probably enhances the vaccine developments, but the knowledge is still limited. Therefore, in this context, atomistic simulations were carried out to reveal the insights at the atomic level of the binding process of NAb and fragment NAb (fNAb) to 501Y.V2 SARS-CoV-2 RBD. Structural changes of the WT/501Y.V2 SARS-CoV-2 RBD + fNAb/NAb complexes were characterized via unbiased MD simulations. Thermodynamics and kinetics of the binding process were then revealed via biased MD simulations. Details of simulations were described in **Figure 1** and the Supplementary (SI) file.

Figure 1. Starting structures of RBD + NAb systems. (A) WT/501Y.V2 RBD + NAb in MD simulations; (B) WT/501Y.V2 RBD + fNAb in MD simulations; (C) + (D) 501Y.V2 RBD + NAb/fNAb in SMD/US simulations; (E) Free energy scheme. The fNAb was mobilized from *bound* to *unbound* states via FPL calculations, then the free energy profile was calculated via US simulations.

Unbiased MD simulations were carried out to understand the structural change at the atomistic level of 501Y.V2 RBD + NAb since the binding affinity of the NAb to 501Y.V2 RBD was altered according to the recent report.^{7, 9, 10, 12} The stabilized conformations of the RBD + NAb complexes were investigated over the equilibrium trajectories (cf. Figure S1 of the SI file). The principal component analysis (PCA)

method was employed to generate the free energy landscape (FEL) of RBD + NAb systems.¹³ The obtained results were described in **Figure 2**. Clearly, the 501Y.V2 variant increases the number of the FEL local minima implying that the 501Y.V2 complex is more flexible than the WT one. It also shows that the binding free energy ΔG_b between 501Y.V2 RBD and NAb is significantly reduced.

Figure 2. Free energy landscape of RBD + antibody was constructed using PCA method. In particular, (A) presents the FEL of the WT RBD + fNAb; (B) mentions the FEL of the WT RBD + NAb; (C) describes the FEL of the 501Y.V2 RBD + fNAb; and (D) denotes the FEL of the 501Y.V2 RBD + NAb.

The WT RBD + fNAb only formed one minimum noted as **w1** in **Figure 2A**, which is located at (CV1; CV2) coordinates of (0.40; 0.40). In particular, the antibody adopted HBs to 4 residues of the WT RBD including G447, Y449, N450, and E484 (cf. **Figure 3**). These results suggest that a mutation E484K will be significantly altered the binding affinity/mechanism of the RBD + fNAb. Two minima were observed in FEL of 501Y.V2 RBD + fNAb, which are located at (CV1; CV2) coordinates of (1.60; -1.40) and (-3.60; -1.00) denoted as **m1** and **m2**, respectively. Analyzing the representative structure **m1**, the antibody was found to be

able to form HBs to the residues K444, G447, Y449, and N450 of the 501Y.V2 RBD. The corresponding residues of **m2**, which formed HBs to RBD 2-4, are G447, Y449, N450, and K484 (cf. **Figure 3**). The observed structural changes imply that the binding affinity and kinetics between RBD and fNAb probably change. A similar story of RBD + NAb, which is detailedly mentioned in the SI file, was obtained confirming the results.

Figure 3. The representative structures of WT and 501Y.V2 RBD + fNAb in different perspective. The structures corresponds to the minima **w1**, **m1**, and **m2**.

As discussed above, the RBD + fNAb structure is more flexible when the 501Y.V2 variant was induced. The binding affinity/mechanism of the complex are thus altered. In this work, a combination of steered-molecular dynamics (SMD)/umbrella sampling (US) simulations were carried out to probe the change in RBD + NAb association. The SMD was used to generate US windows (cf. the SI file). The free energy profile was then calculated using the WHAM.¹⁴ The binding free energy ΔG_b between RBD and NAb is able to calculate via PMF curve as mentioned in **Figure 1E**.^{15, 16} Moreover, the free energy barriers ΔG_{on}^{++} and ΔG_{off}^{++} , which were associated with the binding kinetic rate constant k_{on} and the unbinding kinetic rate constant k_{off} can be also estimated, respectively.

The calculated results for free energy barriers (cf. Table S2) indicated that the NAb will bind to 501Y.V2 RBD more difficult than WT one because of the larger ΔG_{on}^{++} . NAb is much easier to bind to than to unbind from RBD, because the ΔG_{off}^{++} is larger than the ΔG_{on}^{++} . However, in the **M3** case, the $\Delta G_{off}^{++} = 0.14 \pm 0.18$ kcal mol⁻¹ is significantly smaller than the $\Delta G_{on}^{++} = 2.83 \pm 0.65$ kcal mol⁻¹ indicating that it takes more time for NAb to bind to 501Y.V2 RBD for them unbind. Moreover, the observations were also confirmed due to the binding free energy, ΔG_b , calculations, in which the thermodynamic metric corresponding to the association

between NAb and RBD is significantly decreased when the 501Y.V2 variant was induced (Table S2). The NAb is thus resisted to bind to 501Y.V2 RBD. Therefore, it may be argued that the 501Y.V2 variant could reduce the vaccine efficiency. The observation is in good agreement with the experimental data.^{7, 9, 10, 12}

The collective-variable FEL,¹⁷ was constructed by number of contacts between two proteins within 0.45 nm and the displacement of the antibody, revealed the unbinding pathway of NAb. The obtained FEL was shown in **Figure 4** and Figure S3 of the SI file. The representative structures of the complexes within a backbone RMSD of 0.2 nm were then estimated using clustering method.¹³ The unbinding pathways were significantly altered under effects of the 501Y.V2 variant. A larger number of transition states of the WT RBD + fNAb complex implies that it is hard to unbind the antibody from WT system than 501Y.V2 variant. Moreover, the representative structures **B**, **b**, and **b'** correspond to the binding model of the RBD + fNAb complexes. The structures **D7**, **d6**, and **d4'** respond to the minima where the fNAb completely detached from RBD. The other conformations correspond to dissociated structures along unbinding pathways. The similar picture was also observed when the RBD + NAb complexes were investigated (Figure S3).

Figure 4. The collective-variable FEL revealed the unbinding pathways of fNAb from the binding mode with WT/501Y.V2 RBD. The representative structures of complexes were also estimated.

In this work, the NAb resistance of 501Y.V2 variant was investigated using atomistic simulations. In particular, the binding pose of NAb/fNAb to WT/501Y.V2 RBD was revealed using atomistic simulations. Increasing FEL minima of 501Y.V2 RBD + NAb/fNAb in comparison with the WT RBD systems infer that the complex 501Y.V2 RBD + NAb/fNAb is more unstable than the WT one. Thermodynamics and kinetics of the binding process between RBD and NAb were also determined using SMD/US simulations. Interestingly, the binding free energy ΔG_b of WT RBD + NAb/fNAb is significantly smaller than that of 501Y.V2 RBD + NAb/fNAb. It is consistent with results of the binding kinetic rate constant k_{on} and the unbinding kinetic rate constant k_{off} . Poorly binding affinity of NAb/fNAb to 501Y.V2 RBD confirm the antibody resistance of the South African variant.^{7, 9, 10, 12}

ASSOCIATED CONTENT

Supporting Information

Supporting Information Available: The material is available free of charge via the Internet at <http://pubs.acs.org>.

AUTHOR INFORMATION

Corresponding Author

*Email: ngosontung@tdtu.edu.vn and pcnam@dut.udn.vn

Author Contributions

All authors contributed to doing computations and preparing the manuscript.

Notes

The authors declare no competing financial interests.

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REFERENCES

1. Barnes, C. O.; West, A. P.; Huey-Tubman, K. E.; Hoffmann, M. A. G.; Sharaf, N. G.; Hoffman, P. R.; Koranda, N.; Gristick, H. B.; Gaebler, C.; Muecksch, F.; Lorenzi, J. C. C.; Fink, S.; Hägglöf, T.; Hurley, A.; Millard, K. G.; Weisblum, Y.; Schmidt, F.; Hatzioannou, T.; Bieniasz, P. D.; Caskey, M.; Robbani, D. F.; Nussenzweig, M. C.; Bjorkman, P. J., Structures of Human Antibodies Bound to SARS-CoV-2 Spike Reveal Common Epitopes and Recurrent Features of Antibodies. *Cell* **2020**, *182* (4), 828-842.e16.

2. de Wit, E.; van Doremalen, N.; Falzarano, D.; Munster, V. J., SARS and MERS: recent insights into emerging coronaviruses. *Nature Reviews Microbiology* **2016**, *14* (8), 523-534.
3. Ngo, S. T.; Quynh Anh Pham, N.; Thi Le, L.; Pham, D.-H.; Vu, V. V., Computational Determination of Potential Inhibitors of SARS-CoV-2 Main Protease. *Journal of Chemical Information and Modeling* **2020**, *60* (12), 5771-5780.
4. Schoeman, D.; Fielding, B. C., Coronavirus envelope protein: current knowledge. *Virology Journal* **2019**, *16* (1), 69.
5. Hoffmann, M.; Kleine-Weber, H.; Schroeder, S.; Kruger, N.; Herrler, T.; Erichsen, S.; Schiergens, T. S.; Herrler, G.; Wu, N. H.; Nitsche, A.; Muller, M. A.; Drosten, C.; Pohlmann, S., SARS-CoV-2 Cell Entry Depends on ACE2 and TMPRSS2 and Is Blocked by a Clinically Proven Protease Inhibitor. *Cell* **2020**.
6. Lan, J.; Ge, J.; Yu, J.; Shan, S.; Zhou, H.; Fan, S.; Zhang, Q.; Shi, X.; Wang, Q.; Zhang, L.; Wang, X., Structure of the SARS-CoV-2 spike receptor-binding domain bound to the ACE2 receptor. *Nature* **2020**, *581* (7807), 215-220.
7. Wibmer, C. K.; Ayres, F.; Hermanus, T.; Madzivhandila, M.; Kgagudi, P.; Oosthuysen, B.; Lambson, B. E.; de Oliveira, T.; Vermeulen, M.; van der Berg, K.; Rossouw, T.; Boswell, M.; Ueckermann, V.; Meiring, S.; von Gottberg, A.; Cohen, C.; Morris, L.; Bhiman, J. N.; Moore, P. L., SARS-CoV-2 501Y.V2 escapes neutralization by South African COVID-19 donor plasma. *Nature Medicine* **2021**.
8. WHO, Coronavirus disease 2019 (COVID-19) Situation Report – 52. **2020**.
9. Wang, P.; Nair, M. S.; Liu, L.; Iketani, S.; Luo, Y.; Guo, Y.; Wang, M.; Yu, J.; Zhang, B.; Kwong, P. D.; Graham, B. S.; Mascola, J. R.; Chang, J. Y.; Yin, M. T.; Sobieszczyk, M.; Kyratsous, C. A.; Shapiro, L.; Sheng, Z.; Huang, Y.; Ho, D. D., Antibody Resistance of SARS-CoV-2 Variants B.1.351 and B.1.1.7. *Nature* **2021**.
10. Hoffmann, M.; Arora, P.; Groß, R.; Seidel, A.; Hörnich, B. F.; Hahn, A. S.; Krüger, N.; Graichen, L.; Hofmann-Winkler, H.; Kempf, A.; Winkler, M. S.; Schulz, S.; Jäck, H.-M.; Jahrsdörfer, B.; Schrezenmeier, H.; Müller, M.; Kleger, A.; Münch, J.; Pöhlmann, S., SARS-CoV-2 Variants B.1.351 and P.1 Escape from Neutralizing Antibodies. *Cell* **2021**.
11. Weisblum, Y.; Schmidt, F.; Zhang, F.; DaSilva, J.; Poston, D.; Lorenzi, J. C. C.; Muecksch, F.; Rutkowska, M.; Hoffmann, H.-H.; Michailidis, E.; Gaebler, C.; Agudelo, M.; Cho, A.; Wang, Z.; Gazumyan, A.; Cipolla, M.; Luchsinger, L.; Hillyer, C. D.; Caskey, M.; Robbiani, D. F.; Rice, C. M.; Nussenzweig, M. C.; Hatzioannou, T.; Bieniasz, P. D., Escape from neutralizing antibodies by SARS-CoV-2 spike protein variants. *eLife* **2020**, *9*, e61312.
12. Cele, S.; Gazy, I.; Jackson, L.; Hwa, S.-H.; Tegally, H.; Lustig, G.; Giandhari, J.; Pillay, S.; Wilkinson, E.; Naidoo, Y.; Karim, F.; Ganga, Y.; Khan, K.; Bernstein, M.; Balazs, A. B.; Gosnell, B. I.; Hanekom, W.; Moosa, M.-Y. S.; Lessells, R. J.; de Oliveira, T.; Sigal, A.; Ngs, S. A.; Team, C.-K., Escape of SARS-CoV-2 501Y.V2 from Neutralization by Convalescent Plasma. *Nature* **2021**.
13. Papaleo, E.; Mereghetti, P.; Fantucci, P.; Grandori, R.; De Gioia, L., Free-Energy Landscape, Principal Component Analysis, and Structural Clustering to Identify Representative Conformations from Molecular Dynamics Simulations: The Myoglobin Case. *J Mol Graph Model* **2009**, *27* (8), 889-899.
14. Hub, J. S.; de Groot, B. L.; van der Spoel, D., g_wham—A Free Weighted Histogram Analysis Implementation Including Robust Error and Autocorrelation Estimates. *J. Chem. Theory Comput.* **2010**, *6* (12), 3713-3720.
15. Ngo, S. T., Estimating the ligand-binding affinity via λ -dependent umbrella sampling simulations. *J. Comput Chem* **2021**, *42*, 117-123.
16. Ngo, S. T.; Vu, K. B.; Bui, L. M.; Vu, V. V., Effective Estimation of Ligand-Binding Affinity Using Biased Sampling Method. *ACS Omega* **2019**, *4* (2), 3887-3893.
17. Nguyen, T. H.; Rossetti, G.; Arnesano, F.; Ippoliti, E.; Natile, G.; Carloni, P., Molecular Recognition of Platinated DNA from Chromosomal HMGB1. *J. Chem. Theor. Comput* **2014**, *10* (8), 3578-3584.