

A computational method for anti-SARS-CoV-2 antibodies neutralization power: A blueprint with monoclonal antibody Sotrovimab

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Abstract

Background: The rapid evolution of SARS-CoV-2 imposed a huge challenge on disease control. Immune escape caused by genetic variations of SARS-CoV-2 S protein immunogenic epitopes affects the efficiency of monoclonal antibody-based therapy of COVID-19. Therefore, a rapid method is needed to evaluate the efficacy of the available monoclonal antibodies against the new emerging variants or potential novel variants.

Objective: The aim of this study is to develop a rapid computational method to evaluate the neutralization power of SARS-CoV-2 monoclonal antibodies against new SARS-CoV-2 variants and other potential new mutations.

Methods: The amino acid sequence of the extracellular domain of SARS-CoV (YP_009825051.1) and SARS-CoV-2 (YP_009724390.1) spike proteins were used to create computational 3D models for the native spike proteins. Specific mutations were introduced to the collected sequence to generate the different variant spike models. Neutralization potential of S309 against these variants was evaluated based on the molecular interactions and binding energy (?G) in comparison to a reference model after molecular replacement of the reference RBD with the variant's RBD.

Results: The results showed a loss in binding affinity of the neutralizing antibody S309 with both SARS-CoV and - SARS-CoV-2. Comparing SARS-CoV-2 variants to the binding affinity of the first Wuhan strain showed an improvement of the binding affinity of S309 with variants Alpha, Beta, Gamma and Kappa. However, Delta and Omicron variants showed a substantial decrease in the binding affinity. Based on mutational profile of Omicron subvariants, our data describe the effect of G339H and G339D mutation and its role in escaping antibody neutralization which came in consistent with clinical published reports.

Conclusions: This method is rapid, applicable and of interest to adapt the use of therapeutic antibodies to the treatment of emerging variants. It could be applied to antibody-based treatment of other viral infections. Clinical Trial: N/A

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Original Manuscript

Article

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Abstract

Background:

The rapid evolution of SARS-CoV-2 imposed a huge challenge on disease control. Immune escape caused by genetic variations of SARS-CoV-2 S protein immunogenic epitopes affects the efficiency of monoclonal antibody-based therapy of COVID-19. Therefore, a rapid method is needed to evaluate the efficacy of the available monoclonal antibodies against the new emerging variants or potential novel variants.

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The aim of this study is to develop a rapid computational method to evaluate the neutralization power of SARS-Cov-2 monoclonal antibodies against new SARS-CoV-2 variants and other potential new mutations.

Method:

The amino acid sequence of the extracellular domain of SARS-CoV (YP_009825051.1) and SARS-CoV-2 (YP_009724390.1) spike proteins were used to create computational 3D models for the native spike proteins. Specific mutations were introduced to the collected sequence to generate the different variant spike models. Neutralization potential of S309 against these variants was evaluated based on the molecular interactions and binding energy (ΔG) in comparison to a reference model after molecular replacement of the reference RBD with the variant's RBD.

Results:

The results showed a loss in binding affinity of the neutralizing antibody S309 with both SARS-CoV and -SARS-CoV-2. Comparing SARS-CoV-2 variants to the binding affinity of the first Wuhan strain showed an improvement of the binding affinity of S309 with variants Alpha, Beta, Gamma and Kappa. However, Delta and Omicron variants showed a substantial decrease in the binding affinity. Based on mutational profile of Omicron subvariants, our data describe the effect of G339H and G339D mutation and its role in escaping antibody neutralization which came in consistent with clinical published reports.

Conclusion:

This method is rapid, applicable and of interest to adapt the use of therapeutic antibodies to the treatment of emerging variants. It could be applied to antibody-based treatment of other viral infections.

Keywords: In Silico; anti-SARS-CoV-2; Neutralizing antibody; Vaccine; Sotrovimab; S309; SARS-CoV-2 variants; Omicron subvariants; Computational method.

INTRODUCTION

While the world is entering at a ground level the fourth year of the severe acute respiratory syndrome pandemic caused by the newly emergent coronavirus SARS-CoV-2, this persistent virus is still lingering away. This is mainly due to the virus relatively high mutational rate with specific mutations occurring on the spike protein affecting its immunogenicity [1, 2]. The battle against this virus covers several aspects ranging from prevention, mitigation, and treatment. One promising approach that is still developing with proven efficiency consists of using anti-SARS-CoV-2 monoclonal neutralizing antibodies. However, selective pressure caused by infection and/or vaccination is accelerating the emergence of new variants and sub variants, which poses a challenge not only to antibody-mediated therapy but also to vaccine use and development. Anti-SARS-CoV-2 monoclonal antibodies recognize specific epitopes mainly on the spike protein preventing target cell binding and/or fusion and accumulation of mutations in these specific epitopes increases the fitness of the virus. Additionally, the efficacy of the available anti-SARS-CoV-2 neutralizing antibodies (NABs) therapies varies dramatically and is difficult to foresee how useful would it be for new circulating variants [3]. Therefore, there is an urgent need for the rapid assessment of anti-SARS-CoV-2 mAbs potential efficiency to treat emergent variants. Toward this end, computational methods aimed at the rapid estimation of the binding affinity and molecular interactions between new variants and a given mAb can be used.

Currently, the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) have issued emergency use authorization for several anti-SARS-CoV-2 NABs including Evusheld, Ronapreve and Regkirona, Sotrovimab (S309), Casirivimab and Imdevimab (REGN-COV2), and Bamlanivimab (LY-CoV555, LY3819253) [4, 5] and many more are still under evaluation. Based on their binding site, these neutralizing antibodies are classified into different groups. There are currently two classification methods [6]. One is based on high-throughput surface Plasmon resonance technique combined with negative stain electron microscopy to identify specific epitopes on the receptor-binding domain (RBD). This method groups the NABs into seven distinct communities denoted RBD-1 to RBD-3, RBD-4 to RBD-5, and RBD-6 to RBD-7. The other method is based on considerations such as the overlap of the neutralizing antibody with the angiotensin converting enzyme 2 receptor (ACE2) binding site and if it recognizes activated (up) or baseline (down) states of RBD. Four different classes were described, class I-class IV, where class I compete on the ACE2 binding site and can bind the receptor binding domain (RBD) in its up position while class II binds the RBD in both states (UP and Down). Class III neutralizing antibody bind an interface that is outside the RBD domain and hence does not compete with the ACE2 receptor and will bind both forms of the RBD while class IV bind only RBDs in the up position [7, 8].

The computational method we describe in this paper was developed to evaluate the interaction between a given neutralizing antibody of a specific SARS-CoV-2 variant, compare it to the interaction with a different variant and thus predict a possible immune escape. It uses as a model of the interaction of the neutralizing monoclonal antibody Sotrovimab (S309) with SARS-CoV-2 Wuhan variant. This monoclonal antibody (mAb) was first isolated from the memory B cells of a SARS-CoV survivor patient [9, 10]. It has been reported to have neutralization potencies for SARS-CoV, SARS-CoV-2 and SARS-like coronaviruses. Currently, it is one of only two approved therapeutic monoclonal antibodies for newly emerged Omicron subvariants [7, 11, 12]. S309 is a recombinant human monoclonal antibody used under the generic name (Xevudy®). In May 2021, it was granted first emergency use for the early treatment of COVID-19 [13]. S309 belongs to class III antibodies that are characterized by their binding site on the spike protein, as they do not compete with the ACE2 [7]. While ACE2 binds to the SARS-CoV-2 spike residues between K417 and Y505 [14], S309 recognizes a distinct proteoglycan epitope at the opposite of the ACE2 binding site involving residues N334, E340, N343, T345, R346, K356 and a structural loop (443-450) that can be accessed on both states of the RBD (up and down). These key glycan residues are not affected by mutations of the new omicron subvariants [7, 15]. However, other mutations found on the structural loop seem to have a significant effect on the neutralization

capacity of S309. Since S309 does not compete with ACE2 binding site, its neutralization mechanism does not depend on direct blocking of RBD. Though, binding of S309 to the SARS-CoV-2 spike receptor-binding domain induces rather an antibody-dependent cell cytotoxicity (ADCC) and antibody-dependent cellular phagocytosis (ADCP) [16].

Several experimental and clinical reports described the neutralization effect of mAb S309 with early Wuhan strain and its effect in reducing disease progression [10, 17, 18]. Therefore, in the computational method we report in this paper, the estimated interaction affinity of mAb S309 to the Wuhan strain is assigned a value of 100%. Comparison of the estimated affinities of S309 to each SARS-CoV-2 variants to this reference value allows the evaluation of the neutralization efficiency and the prediction of possible immune escape for each existing or newly emerging variant. This straightforward computational method can rapidly give valuable insights on the eventual efficiency of existing neutralizing therapeutic antibodies in treating newly emergent variants prior to the experimental methods. Since immune escape is a major criterion retained by WHO and the CDC in their labelling systems of new variants particularly the variants of concern (VOC) [19], this method can also be considered to label new variants early after their emergence.

METHODS

Method's outline

This work describes a computational method to evaluate the effect of different SARS-CoV-2 mutations on the stability of the complex and binding affinity with available neutralizing antibodies. As a working pattern, we developed a reference complex model between the neutralizing antibody Sotrovimab (S309) and the SARS-CoV-2 Wuhan strain. We evaluated the other variants and subvariants based on the differences of their specific molecular interactions and binding energy (ΔG) with Sotrovimab. Figure 1 outlined the methods for the anti-SARS-COV-2 antibody neutralization potential of S309.

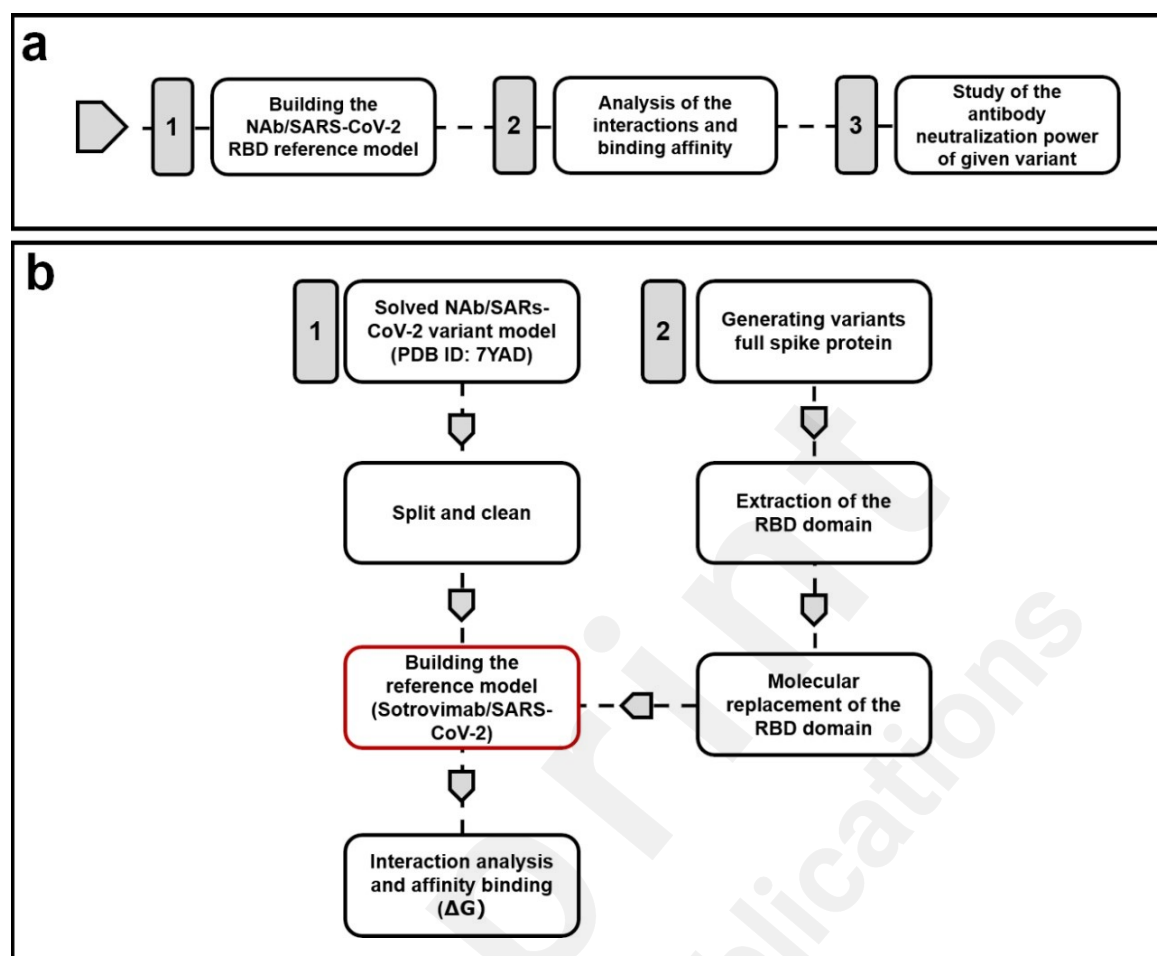


Figure 1: Method's outline. a) Outline of the three steps method. b) Workflow of the *in silico* method for the evaluation of a SARS-CoV-2 mAb neutralization power.

Models and complexes construction

Building the NAb/SARS-CoV-2 RBD reference model

We used the model PDB ID: 7YAD downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB-PDB) [20] to generate our reference model representing the interaction of Sotrovimab variable domain (Fv) with Omicron SARS-CoV-2 spike RBD. The PDB model (7YAD) represents the interaction of SRAS-CoV-2 Omicron RBD (amino acid Pro330- Lys529) with the Fv domain of the Sotrovimab neutralizing antibody. The model shows 6 chains (two RBDs, two heavy chains and two light chains) forming two subunits of RBD-S309 Fv as shown in Figure 2. The selection criteria of 7YAD model [15] are the generation of the 3D structure by electron microscopy, the high resolution of 2.66Å and the relatively good validation report. In addition, it represents the interaction with SARS-CoV-2 RBD in the open state.. Upon downloading the structure, only one unit was selected representing one S309 Fv domain (one heavy and one light chain) binding to one spike RBD, Chains A, B, M. The complex was extracted, cleaned from any heteroatoms and used as reference model to generate the different variant complexes by RBD replacement.

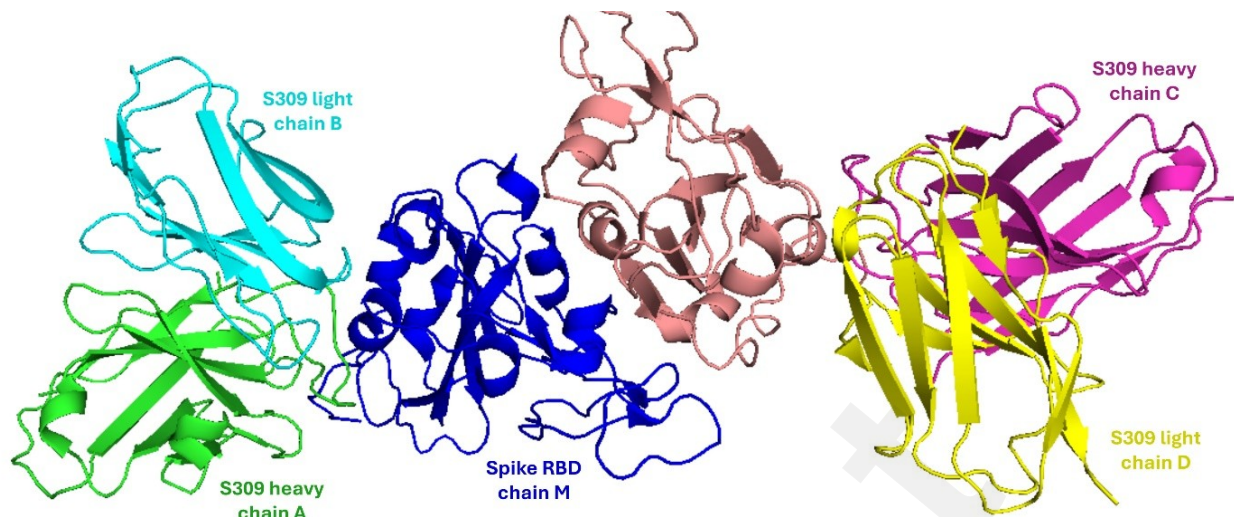


Figure 2: 3D structure of the PDB model 7YAD showing two subunits of Sotrovimab Fv (heavy and light chains) binding to Omicrons' spike protein RBD.

SARS-CoV and SARS-CoV-2 variants sequence retrieval, modifications and modeling

The amino acid sequence of the extracellular domain of SARS-CoV and SARS-CoV-2 spike protein were acquired from the National Center for Biological Information (NCBI) protein ID: YP_009825051.1 and ID: YP_009724390.1 respectively. SARS-CoV-2 variants-specific mutations were introduced to the collected sequence to generate the different variant sequences based on published mutations on databases such as CoVariants [21] and Stanford University SARS-CoV-2 Variants database [22]. The sequences corresponding to the spike protein of SARS-CoV, and total of 25 variants of SARS-CoV-2 Wuhan (ALPHA, BETA, GAMMA, DELTA-21J, and KAPPA) in addition to Omicron's subvariants (*BA.1*, *BA.2*, *BA.4/BA.5*, *BA.2.12.1*, *BA.2.75*, *BQ1*, *XBB*, *XBB.1*) were used to build spikes 3D monomer models. The monomers were modeled in an open state form using the SWISS-MODEL server-User Template Mode [23]. The template for each monomer was selected and extracted from the Protein Data Bank. Selection criteria were based on resolution, chain quality, sequence gaps, furin site and proline modifications and validation report. The templates used for each model are listed in Table 1. The monomer chain representing the open state RBD was extracted from each model, cleaned from any heteroatoms and saved using PyMol software [24] into new Pdb file. Each monomer was introduced in the SWISS-MODEL server-User Template Mode to generate an open-state monomer spike protein for SARS-CoV, SARS-CoV-2 variants and Omicron sub variants.

Table 1: list of templates and chains' ID used to build SARS-CoV virus and the different SARS-CoV-2 variants.

Virus	PDB model	Resolution Å	Selected chain	Reference
SARS-CoV	6ACD	3.9	C	[25]
SARS-CoV-2 - Wuhan	7ND9	2.80	B	[26]
ALPHA	8DLI	2.56	A	[27]
BETA	8DLL	2.56	A	[27]
DELTA 21J	7W92	3.1	C	[28]
GAMMA	8DLO	2.25	A	[27]
KAPPA	7TF0	3.02	B	[29]
OMICRON	7XCO	2.5	C	[15]

RBD/S309 complexes construction

RBDs of the different SARS-CoV, SARS-CoV-2 variants and Omicron's sub variants, were extracted from the generated models and the complexes with S309 were constructed by molecular replacement. The reference crystalized RBD chain M of PBD 7YAD was replaced with the modeled RBD. The complex was saved, and energy minimized. Energy minimization step was done in vacuo, without reaction field using GROMOS 43B1 force field [30] using Swiss-pdb Viewer 4.1.0 [31]. This was applied to all the generated models.

Interactions and complex binding affinity analysis

The interactions between the RBD of spike protein of SARS-CoV, SARS-CoV-2 variants and Omicron subvariants with neutralizing antibody S309 were analyzed based on polar and hydrophobic interaction using the LigPlot+ software [32]. The stability and affinity were assisted based on thermodynamic measure of the formed complex energy, Gibbs free energy, (ΔG), this was performed using an antibody-antigen binding affinity online tool, CSM-AB [33]. Binding affinity percentage was calculated in reference to Wuhan /S309 complex binding affinity.

Testing the generated method by analyzing newly reported Omicron subvariants and some experimentally tested mutations

Several reports have been discussing the neutralization effect of NAb and possible antibody escape of some new Omicrons subvariants [34-39]. Here we used our developed method to evaluate the binding affinity of several of these new subvariants including AY.1, XBB.1.5, BF.7, BQ.1.1, BA.1.1, BA.3, BA.2.3.20, BM.1.1.1, BA.5.6.2, BA.2.75.2, and CH.1.1 (Orthrus), with NAb S309. Additionally, the effect of several amino acids exchange in the NAb epitope have been tested experimentally by ELISA and/or pseudo-virus neutralization assays. Several mutations showed to be resistance to inhibition by S309 leading to an antibody escape. These key residues include R346 and P337, G339, N440 and S371 [40, 41]. Therefore, we apply our method to computationally test the effect of some mutations on these residues. As we already generated parent's RBD sequences, newly emerged mutations were introduced, new models and complexes were built and the mutation's effect on binding energy with the NAb was predicted by recalculating complex's Gibbs free energy (ΔG) in reference to parent's complex and Wuhan binding affinity.

RESULTS

Method development workflow

Figure 1 outlined the methods for the anti-SARS-CoV-2 antibody neutralization potential of S309. The blueprint of the method we developed using mAb S309 an experimentally proven neutralizing mAb for SARS-CoV-2 and its variants is described in Fig 1a. We proceeded by modifying available model PDB ID: 7YAD to generate a reference model that can be used to measure the neutralization potential in terms of binding affinity ΔG [Fig. 1b]. Several *in silico* 3D models representing spike monomer chain of each variant were generated. The quality of the generated 3D model was evaluated based on the homology modeling report and Swiss-model structural assessment. The generated models showed a QMEAN Z-Score between -1.0 and -3.2 indicating a good quality model where Z-scores around 0.0 is ideal and any value below -4.0 indicates low quality model [42]. The QMEANDisCo Global value represent the combined scoring of global (for the entire structure) and local (per residue) absolute quality estimates of a single model [43]. Our models QMEANDisCo scores were ranging from 0.64 to 0.76 ± 0.05 . These values reflect a good quality model (any value below 0.6 represent low quality model). Each complex was built by molecular replacement of chain M of the reference model with the extracted RBD followed by binding affinity and interaction analysis.

Analysis of the molecular interaction pattern of Sotrovimab (S309) with nine main SARS-CoV-2 Variants

The generated complexes were energy minimized and polar and hydrophobic interactions were analyzed. Several interactions identified between the S309 Fv domain and spike RBD with more interactions towards the heavy chain. Interacted residues of the spike protein include residue 321-428 of SARS-CoV spike and 334-441 of SARS-CoV-2 and its variants. SARS-CoV showed four polar interactions compared to the Wuhan SARS-CoV-2 that shares a total of three polar interactions with S309. Interestingly, variant Kappa shows the highest number of polar interactions of six while Delta 21J shows the lowest with only one polar interaction. Kappa variant shows a unique two salt bridges between Arg346 and Lys356 with S309 heavy chain amino acid Glu108. All the variants share the same polar interaction between Glu340 and S309 heavy chain Ala104 except for Delta 21J variant. All Omicron subvariants showed the same interaction pattern except for BA.2.75 with one missing polar interaction between Thr343 and S309 heavy chain Ser109. GAMMA variant showed more hydrophobic interactions with the light chain of S309. All polar interactions are represented in Figure 3 and detailed interactions are listed in the Supplementary Table S1.

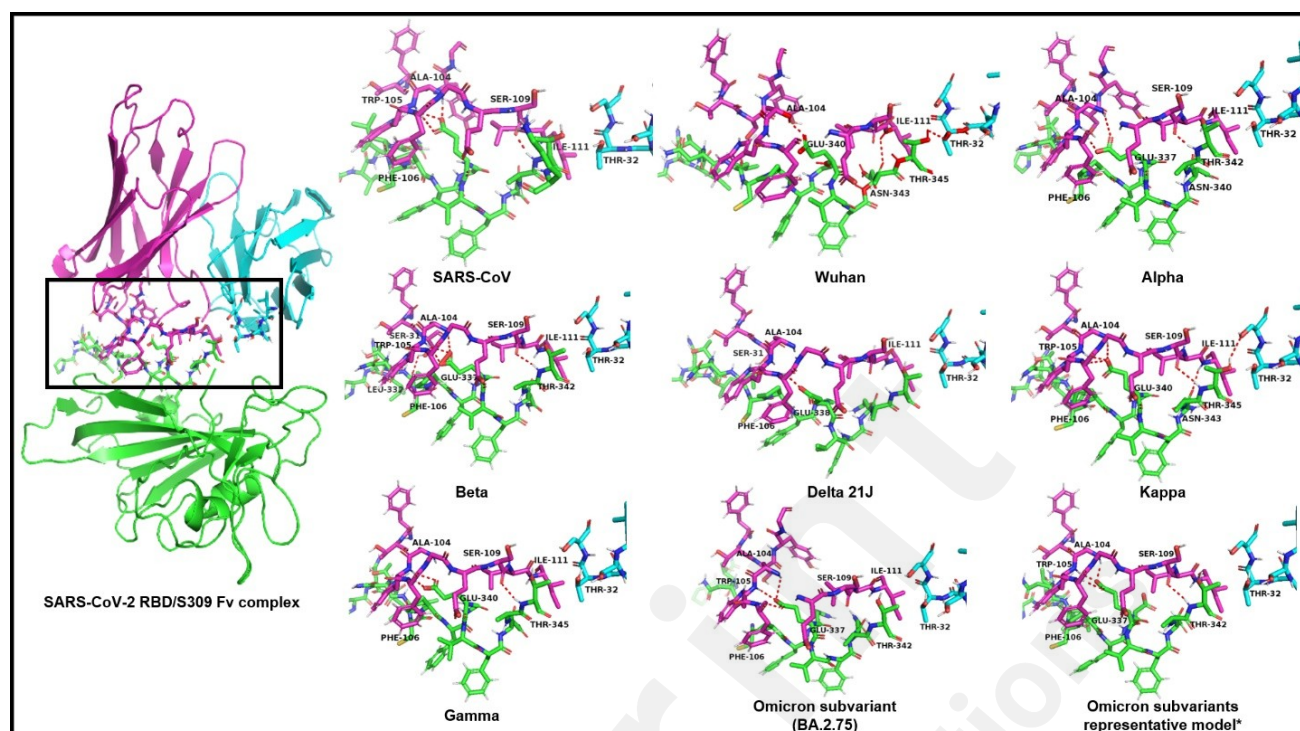


Figure 3: Variations of the polar interactions between mAb Sotrovimab (S309) and different SARS-CoV-2 var subvariants. mAb heavy chain (Magenta), mAb light chain (Cyan), SARS-CoV-2 S protein-RBD (Green). numbering: BA.1, BA. 2, BA.2.12.1 (Glu337, Thr342) / BA. 4, BA.5, BQ.1 (Glu335, Thr340) / XBB, XBB.1.

Evaluation of the binding affinity of Sotrovimab (S309) with 9 SARS-CoV-2 Variants by comparing their binding affinity with the Wuhan reference

The thermodynamic stability of the generated complexes was measured by computational prediction of Gibbs free energy (ΔG) using CSM-AB tool. Gibbs free energy reflects energy differences between coupled and decoupled antibody- antigen protein complex. This difference in energy (ΔG) indicate complex stability where a negative normalized energy $\Delta G < 0$ indicates spontaneous and exergonic reaction and hence more stable complex and more efficient protein-ligand interaction. So, the lower the value of (ΔG), the more stable the complex (antibody-antigen). In our model, we found that the neutralizing antibody S309 has the binding affinity of -8.26 Kcal/mol with SARS-CoV and -7.13.26 Kcal/mol with SARS-CoV-2 indicating a loss in binding affinity. However, comparing SARS-CoV-2 variants to the binding affinity of the first Wuhan strain showed an improvement of the binding affinity of S309 with variants Alpha, Beta, Gamma and Kappa. This improvement in affinity, when compared to the interaction profile, can be related to the increased number of polar and hydrophobic interactions and more similar interactions' profile to SARS-CoV than to Wuhan strain. In the other hand, Delta variant showed a substantial decrease in the binding affinity as it exhibits only one polar interaction. In the case of Omicron subvariants, they all share similar interaction profiles; however, they exhibit different binding affinities. Although they show significant decrease in binding affinity compared to Wuhan strain, they can be clustered in to two groups; those with G339H mutation (BA.2.75, XBB, XBB.1) and those with G339D mutation (BA.1, BA.2, BA.4/5, BQ.1, BA.2.12.1) (Fig. 4, Supplementary Table S2 and Supplementary Fig. S1). The data showed that histidine residue on position 339 slightly enhance the binding affinity compared to aspartic acid substitution. This residue is located in the middle of the interaction loop and hence plays a great role in the complex stability and binding affinity. In addition, our results are aligned with reported effect of the G339D mutation and its role in escaping antibody neutralization [41, 44, 45]

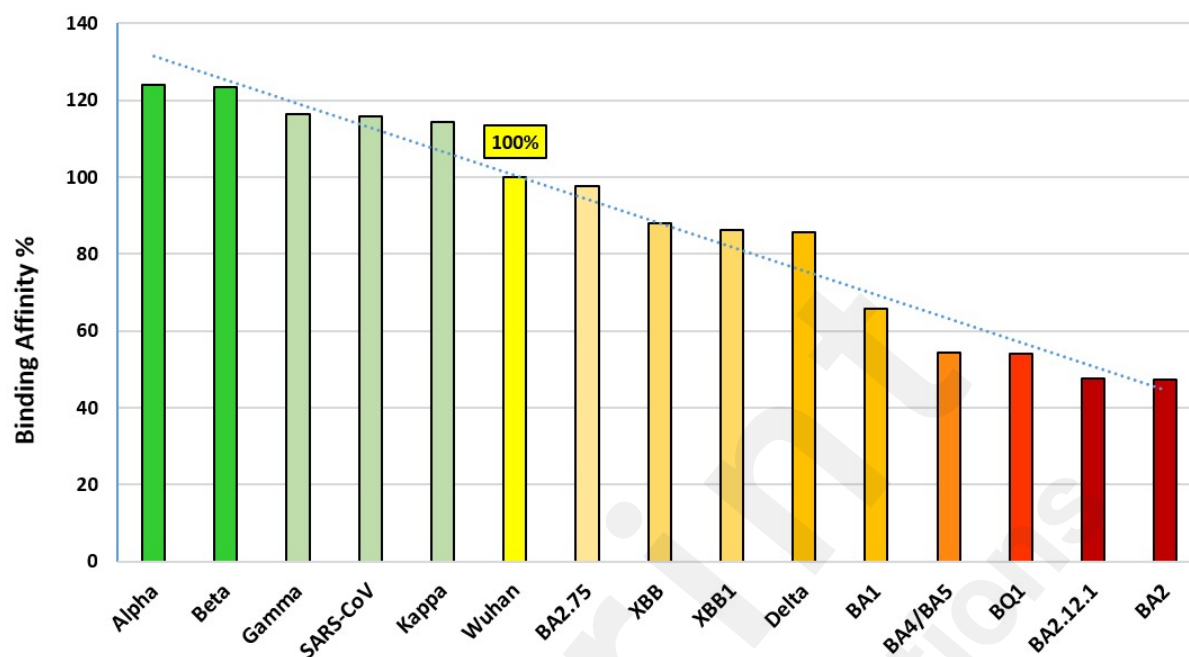


Figure 4: Binding energy (ΔG) of the SARS-CoV and different SARS-CoV-2 variants (represented in affinity percentage in comparison to SARS-CoV-2).

Furthermore, to test the impact of G339 mutation, we analyze the effect of reverse mutagenesis. We used the generated models and in silico tools to test the effect of reverse mutation at residue 339 on the complex stability of subvariants (BA.2.75, XBB, and XBB.1). They have an aspartic acid on the position 339. By reversing it back to either Glycine or Histidine (G339 or H339), we calculated the effect in the form of ΔG value. The results showed an increase of the stability of the SARS-CoV-2/S309 complex and hence enhanced binding affinity with the Glycine residue. However, reverse mutagenesis to Histidine has none to a very low effect except for the variant BA.2.12.1 where there was a slight increase on the binding affinity Table 2.

Table 2: Gibbs free energy (ΔG) analysis of the effect of D339 reverse mutation on the binding affinity of Omicron's SARS-Cov-2 subvariants with S309 NAb. ▲: Increase, ▼: Decrease.

Variant	D339 ΔG Kcal/mol	D339G ΔG kcal/mol	D339H ΔG kcal/mol	Effect on binding affinity
BA.1	-4.7	-6.83	-6.92	▲
BA.2	-3.38	-6.18	-6.59	▲
BA.4/BA.5	-3.87	-6.96	-7.15	▲
BA.2.12.1	-3.39	-6.19	-6.59	▲
BQ.1	-3.86	-6.92	-7.29	▲

Evaluation of Sotrovimab (S309) binding affinity to experimentally tested and some hypothetical SARS-CoV-2 variants

The effect of several amino acids exchange in the NAb Sotrovimab epitope have been tested experimentally by ELISA and/or pseudovirus neutralization assays. These mutations showed to be resistance to inhibition by S309 leading to an antibody escape. These key residues include R346S and P337L, G339D, N440K and S371L [40, 41]. Here we used our developed method to evaluate this effect computationally. By generating models with the new reported mutations and CSM-AB tool we predict the effect of the reported mutations on the binding affinity of the complex and hence on the S309 antibody neutralization effect. Interestingly, our computational results came comparable with the experimentally reported effect of these residues mutations on the S309 monoclonal antibody escape. Additionally, we predicted a possible effect of hypothetical

mutations on some of the proteoglycan epitopes Table 3.

Table 3: Prediction of the effect of reported new SARS-CoV-2 subvariants and some experimentally tested spike mutations on the binding affinity with S309. ▲ : Increase, ▼ : Decrease, ─ : No change.

Parent model	ΔG Kcal/mol	Mutations	Reference	New Subvariant	ΔG Kcal/mol	Effect on binding affinity	Binding affinity % in reference to Wuhan
New SARS-CoV-2 variants and Omicron subvariants	BA.4/5	R346T	[39]	BF.7	-2.85	▼	39.97
	BQ.1	R346T		BQ.1.1	-2.82	▼	39.55
	BA.1	R346K	[34-36]	BA.1.1	-4.99	▲	69.99
	BA.1	L371F D405N	[22]	BA.3	-4.99	▲	69.99
	BA.2	K444R N450D L452M N460K A484R R493Q		BA.2.3.20	-4.13	▲	47.41
	BA.2	D339H R346T G446S N460K F486S F490S R493Q		BM.1.1.1	-5.83	▲	81.77
	BA.4/5	K444T		BA.5.6.2	-3.86	▼	54.14
	DELTA 21J	K417N		AY.1	-6.12	─	85.83

	BA.2.75	-6.96	R346T F486S	[39]	BA.2.75.2	-6.27	▼	90.74
	BA.2.75	-6.96	R346T K444T L452R F486S	[38]	CH.1.1 (Orthrus)	-5.73	▼	80.36
	XBB.1	-6.15	S486P	[37]	XBB.1.5 (Kraken)	-6.15	—	86.26
Experimental	Wuhan	-7.13	R346K	[40]	—	-7.23	▲	101.4
			R346S			-6.21	▼	87.1
			R346T			-6.97	▼	97.75
			P337L			-6.73	▼	94.39
			P337L R346K			-5.45	▼	76.45
	Omicron BA.2.75	-6.96	H339D	[41]	—	-3.34	▼	46.84
			R346K			-6.52	▼	91.44
			S371L			-6.53	▼	91.58
			Q493R			-6.81	▼	95.51

DISCUSSION

Antibody-based therapies has proven its efficiency against SARS-CoV-2 virus and appears to be the most promising approach to control COVID-19 pandemic. A number of neutralizing monoclonal antibodies used in clinical setting showed very good results particularly in stopping the disease progression [46, 47]. However, constant emergence of new virus' variants hinder the potency of available anti-SARS-CoV-2 antibodies and urged the continuous development of improved more effective neutralizing antibody. In this paper, we describe an in-silico rapid method we 've developed to predict a possible effect of newly emerged mutations on the efficacy of available neutralizing anti-SARS-CoV-2 antibody. We used mAb Sotrovimab (S309) as an example. Sotrovimab recognizes a proteoglycan epitope embedded in a structural loop located on the outer side the SARS-CoV-2 protein and covering residues 333-441. This specific epitope location permits the binding to both configurations RBD up and down without affecting the binding to ACE2. Indeed, this epitope does not overlap with the ACE2 binding site. However, several newly emerged RBD mutations were reported to have an impact on the neutralization effect of S309. To further explore this, we developed this computational method to evaluate and compare the neutralization potential of the antibody Sotrovimab against different SARS-CoV-2 variants and possible new emerging mutations as outlined in Fig 1.

Using bioinformatics tools, we developed a spike models for several SARS-CoV-2 new variants and evaluated the effect of several emerged mutations on the interaction with the neutralizing monoclonal antibody Sotrovimab (S309) used for the treatment of mild-to-moderate coronavirus disease. In addition, by applying this method, we foresee the effect of some predicted or not yet been observed mutations. Interestingly, the predicted significantly decreased computational neutralization values of mAb Sotrovimab (from 10 to 50%) for some new omicron variants are confirmed by the newly published clinical results indicating reduction in effectiveness against these same Omicron new variants and possible immune evasion [39, 48-51]. Early on, Sotrovimab has been clinically considered to be one of the most effective monoclonal antibodies against all SARS-CoV-2 variants [7]. However, this statement has proven wrong as recent convergent evolution of Omicron and its sub variants has led to a new set of spike mutations

within the Sotrovimab epitope and consequently the new sub variants became more and more resistant [52]. several mutations were identified to be critical and others are yet to be investigated. For example, mutation of the nonpolar glycine 339 located in the mid of the antibody epitope to the acidic charged aspartic acid (G339D), showed to have a remarkable impact on the binding affinity of Omicron's subvariants [44, 53] with predicted neutralization power reduction of respectively 30% for BA.1, 45% for BA.4, BA.5 BQ.1, 50% for BA.2.12.1, BA.2 and 60% for BF.7, BQ.1.1. We reported similar effect in our proposed computational method and we showed that the impact was less intense with the G339H mutation (Table 2 and Supplementary Table S2). However, the combination of multiple mutations in the Omicron's sub variants has more profound effect on the binding affinity indicating increased antibody resistance. This effect was clearly detected in the possible next dominant new subvariants BM.1.1.1, BA.2.3.20 and CH.1.1 (Orthrus) [54] and Table 3. Furthermore, and to test our method, we examined some experimentally evaluated mutations in residues P337, R346, G339, and S371 that are located in the S309 epitope and once more our computational method was compatible with the experimental results (Table 3). This reduced susceptibility of Sotrovimab with P337, R346 and other mutations has been experimentally recognized [13, 40, 41]. Considering the clinical observations of the efficiency of Sotrovimab in neutralization of SARS-CoV, SARS-CoV-2 variants and Omicron sub variants, a 50% reduction of the binding affinity as compared to the reference model might be taken as cut off for considering if a monoclonal antibody will neutralize a new variant using the method described in this paper. The comparison of the predicted values of the evaluation of neutralizing power with a larger number of clinical observations about the efficiency of a neutralizing mAb would help refine this theoretical cut off value and further validates the method. Ultimately, molecular dynamics simulations can be performed to more accurately define the most stable conformation of the mAb/S protein-RBD complexes.

Conclusion

This in silico method gives good insights on possible antibody-escape following emergence of new SARS-CoV-2 mutations and helps in evaluating the usefulness of existing neutralizing antibodies in fighting new emerging variants and sub variants. This method is straightforward, rapid and applicable ahead of obtaining statistically significant clinical observations. In addition, this method highlights the advantages of computational approaches in viral the rapid surveillance and for the development of novel mAb therapies.

Conflicts of Interest

The authors declare no conflict of interest.

Data availability statement

All data generated or analysis during this study are included in this published article (and its Supplementary Information files).

Author contributions: DA

DA: *In silico* analysis, methodology, data curation, writing, and editing. **MM:** Illustrations and figures. **M-DF:** Project conception, data analysis, writing, editing, and supervision.

Abbreviations

ACE2: Angiotensin Converting Enzyme 2 receptor

ΔG : Gibbs free energy

mAb: Monoclonal antibody

Nabs: Neutralizing antibodies

RBD: Receptor Binding Domain

REFERENCES

1. Van Dorp, L., et al., *Emergence of genomic diversity and recurrent mutations in SARS-CoV-2*. Infection, Genetics and Evolution, 2020. **83**: p. 104351.
2. Amicone, M., et al., *Mutation rate of SARS-CoV-2 and emergence of mutators during experimental evolution*. Evolution, medicine, and public health, 2022. **10**(1): p. 142-155.
3. Focosi, D., et al., *Convergent Evolution in SARS-CoV-2 Spike Creates a Variant Soup from Which New COVID-19 Waves Emerge*. International Journal of Molecular Sciences, 2023. **24**(3): p. 2264.
4. FDA. *Emergency Use Authorization*. 2023 [cited 2023; Available from: <https://www.fda.gov/emergency-preparedness-and-response/mcm-legal-regulatory-and-policy-framework/emergency-use-authorization#coviddrugs>].
5. EMA. *COVID-19 treatments*. 2021 [cited 2023; Available from: <https://www.ema.europa.eu/en/human-regulatory/overview/public-health-threats/coronavirus-disease-covid-19/treatments-vaccines/covid-19-treatments>].
6. Hastie, K.M., et al., *Defining variant-resistant epitopes targeted by SARS-CoV-2 antibodies: A global consortium study*. Science, 2021. **374**(6566): p. 472-478.
7. Mittal, A., A. Khattri, and V. Verma, *Structural and antigenic variations in the spike protein of emerging SARS-CoV-2 variants*. PLoS Pathogens, 2022. **18**(2): p. e1010260.
8. Barnes, C.O., et al., *SARS-CoV-2 neutralizing antibody structures inform therapeutic strategies*. Nature, 2020. **588**(7839): p. 682-687.
9. Piccoli, L., et al., *Mapping Neutralizing and Immunodominant Sites on the SARS-CoV-2 Spike Receptor-Binding Domain by Structure-Guided High-Resolution Serology*. Cell, 2020. **183**(4): p. 1024-1042.e21.
10. Pinto, D., et al., *Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody*. Nature, 2020. **583**(7815): p. 290-295.
11. Kumar, S., et al., *Structural insights for neutralization of Omicron variants BA.1, BA.2, BA.4, and BA.5 by a broadly neutralizing SARS-CoV-2 antibody*. Sci Adv, 2022. **8**(40): p. eadd2032.
12. McCallum, M., et al., *Structural basis of SARS-CoV-2 Omicron immune evasion and receptor engagement*. Science, 2022. **375**(6583): p. 864-868.
13. Heo, Y.-A., *Sotrovimab: first approval*. Drugs, 2022. **82**(4): p. 477-484.
14. Ashoor, D., et al., *A computational approach to evaluate the combined effect of SARS-CoV-2 RBD mutations and ACE2 receptor genetic variants on infectivity: The COVID-19 host-pathogen nexus*. Frontiers in Cellular and Infection Microbiology, 2021. **11**: p. 707194.
15. Zhao, Z., et al., *Omicron SARS-CoV-2 mutations stabilize spike up-RBD conformation and lead to a non-RBM-binding monoclonal antibody escape*. Nat Commun, 2022. **13**(1): p. 4958.
16. Lv, Z., et al., *Structural basis for neutralization of SARS-CoV-2 and SARS-CoV by a potent therapeutic antibody*. Science, 2020. **369**(6510): p. 1505-1509.
17. Gupta, A., et al., *Early treatment for Covid-19 with SARS-CoV-2 neutralizing antibody sotrovimab*. New England Journal of Medicine, 2021. **385**(21): p. 1941-1950.
18. Gupta, A., et al., *Effect of sotrovimab on hospitalization or death among high-risk patients with mild to moderate COVID-19: a randomized clinical trial*. Jama, 2022. **327**(13): p. 1236-1246.
19. Ashoor, D., et al., *How concerning is a SARS-CoV-2 variant of concern? Computational predictions and the variants labeling system*. Frontiers in Cellular and Infection Microbiology, 2022: p. 1072.
20. RCSB. *Protein Data Bank* [cited 2024 2024]; Available from: <https://www.rcsb.org/>.
21. Hodcroft, E.B. *"CoVarians: SARS-CoV-2 Mutations and Variants of Interest."*. 2021; Available from: <https://covariants.org/>.

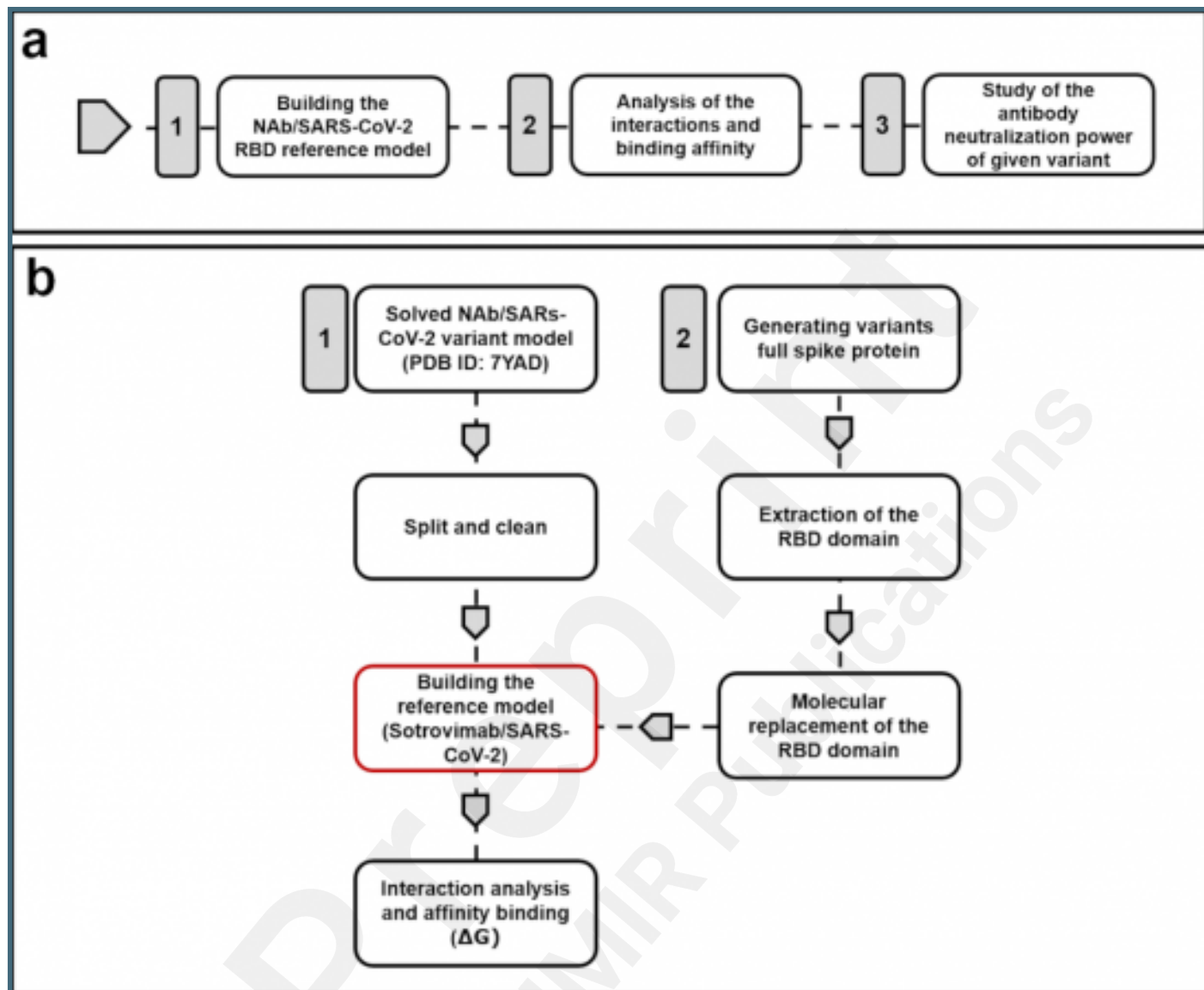
22. Stanford-University. *SARS-CoV-2 Variants*. Coronavirus Antiviral & Resistance Database 2023 2023]; Available from: https://covdb.stanford.edu/variants/omicron_ba_1_3/.
23. Waterhouse, A., et al., *SWISS-MODEL: homology modelling of protein structures and complexes*. Nucleic acids research, 2018. **46**(W1): p. W296-W303.
24. DeLano, W., *The PyMOL Molecular Graphics System, Version 2.4. 0* Schrödinger, LLC. 2002.
25. Song, W., et al., *Cryo-EM structure of the SARS coronavirus spike glycoprotein in complex with its host cell receptor ACE2*. PLoS pathogens, 2018. **14**(8): p. e1007236.
26. Dejnirattisai, W., et al., *The antigenic anatomy of SARS-CoV-2 receptor binding domain*. Cell, 2021. **184**(8): p. 2183-2200.
27. Mannar, D., et al., *SARS-CoV-2 variants of concern: spike protein mutational analysis and epitope for broad neutralization*. Nature Communications, 2022. **13**(1): p. 4696.
28. Wang, Y., et al., *Structural basis for SARS-CoV-2 Delta variant recognition of ACE2 receptor and broadly neutralizing antibodies*. Nature communications, 2022. **13**(1): p. 871.
29. Saville, J.W., et al., *Structural and biochemical rationale for enhanced spike protein fitness in delta and kappa SARS-CoV-2 variants*. Nature communications, 2022. **13**(1): p. 742.
30. van Gunsteren, W.F., et al., *Biomolecular simulation: the GROMOS96 manual and user guide*. Vdf Hochschulverlag AG an der ETH Zürich, Zürich, 1996. **86**: p. 1-1044.
31. Guex, N. and M.C. Peitsch, *SWISS-MODEL and the Swiss-Pdb Viewer: an environment for comparative protein modeling*. electrophoresis, 1997. **18**(15): p. 2714-2723.
32. Laskowski, R.A. and M.B. Swindells, *LigPlot+: multiple ligand-protein interaction diagrams for drug discovery*. J Chem Inf Model, 2011. **51**(10): p. 2778-86.
33. Myung, Y., D.E.V. Pires, and D.B. Ascher, *CSM-AB: graph-based antibody-antigen binding affinity prediction and docking scoring function*. Bioinformatics, 2022. **38**(4): p. 1141-1143.
34. Manjunath, R., et al., *A comprehensive review on Covid-19 Omicron (B. 1.1. 529) variant*. 2022: p. 103372.
35. Liu, X., et al., *Omicron: A chimera of two early SARS-CoV-2 lineages*. Signal Transduction and Targeted Therapy, 2022. **7**(1): p. 90.
36. Martins, M., et al., *The Omicron variant BA. 1.1 presents a lower pathogenicity than B. 1 D614G and Delta variants in a feline model of SARS-CoV-2 infection*. Journal of virology, 2022. **96**(17): p. e00961-22.
37. Yue, C., et al., *Enhanced transmissibility of XBB. 1.5 is contributed by both strong ACE2 binding and antibody evasion*. bioRxiv, 2023.
38. Neher, R. *SARS-CoV-2_variant-reports*. 2022; Available from: https://github.com/neherlab/SARS-CoV-2_variant-reports/blob/d2d531c6deb12e52e5a6fde9af25f2cce023302b/reports/variant_report_2022-12-22.md.
39. Qu, P., et al., *Enhanced neutralization resistance of SARS-CoV-2 omicron subvariants BQ. 1, BQ. 1.1, BA. 4.6, BF. 7, and BA. 2.75. 2*. Cell Host & Microbe, 2023. **31**(1): p. 9-17.
40. Magnus, C.L., et al., *Targeted escape of SARS-CoV-2 in vitro from monoclonal antibody S309, the precursor of sotrovimab*. Frontiers in immunology, 2022. **13**: p. 966236.
41. Cao, Y., et al., *Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies*. Nature, 2022. **602**(7898): p. 657-663.
42. Benkert, P., M. Biasini, and T. Schwede, *Toward the estimation of the absolute quality of individual protein structure models*. Bioinformatics, 2010. **27**(3): p. 343-350.
43. Studer, G., et al., *QMEANDisCo—distance constraints applied on model quality estimation*. Bioinformatics, 2019. **36**(6): p. 1765-1771.
44. Asif, A., et al., *The Comparison of Mutational Progression in SARS-CoV-2: A Short Updated Overview*. Journal of Molecular Pathology, 2022. **3**(4): p. 201-218.
45. Huang, M., et al., *Atlas of currently available human neutralizing antibodies against SARS-CoV-2 and escape by Omicron sub-variants BA. 1/BA. 1.1/BA. 2/BA. 3*. Immunity, 2022.

- 55(8): p. 1501-1514.
46. van de Veerdonk, F.L., et al., *A guide to immunotherapy for COVID-19*. Nature Medicine, 2022. **28**(1): p. 39-50.
 47. Cheng, Z.J., et al., *Clinical application of antibody immunity against SARS-CoV-2: Comprehensive review on immunoassay and immunotherapy*. 2022: p. 1-16.
 48. Aggarwal, N.R., et al., *Change in Effectiveness of Sotrovimab for Preventing Hospitalization and Mortality for At-risk COVID-19 Outpatients During an Omicron BA. 1 and BA. 1.1-Predominant Phase*. International Journal of Infectious Diseases, 2022.
 49. Arora, P., et al., *Augmented neutralisation resistance of emerging omicron subvariants BA. 2.12. 1, BA. 4, and BA. 5*. The Lancet Infectious Diseases, 2022. **22**(8): p. 1117-1118.
 50. Imai, M., et al., *Efficacy of Antiviral Agents against Omicron Subvariants BQ. 1.1 and XBB*. New England Journal of Medicine, 2023. **388**(1): p. 89-91.
 51. Cox, M., et al., *SARS-CoV-2 variant evasion of monoclonal antibodies based on in vitro studies*. Nature Reviews Microbiology, 2023. **21**(2): p. 112-124.
 52. World Health, O., *COVID-19 weekly epidemiological update, edition 119, 23 November 2022*. 2022.
 53. Willett, B.J., et al., *SARS-CoV-2 Omicron is an immune escape variant with an altered cell entry pathway*. Nature microbiology, 2022. **7**(8): p. 1161-1179.
 54. Mohapatra, R.K., et al., *Renewed global threat by the novel SARS-CoV-2 variants' XBB, BF. 7, BQ. 1, BA. 2.75, BA. 4.6': a discussion*. Frontiers in Virology, 2022. **2**: p. 104.

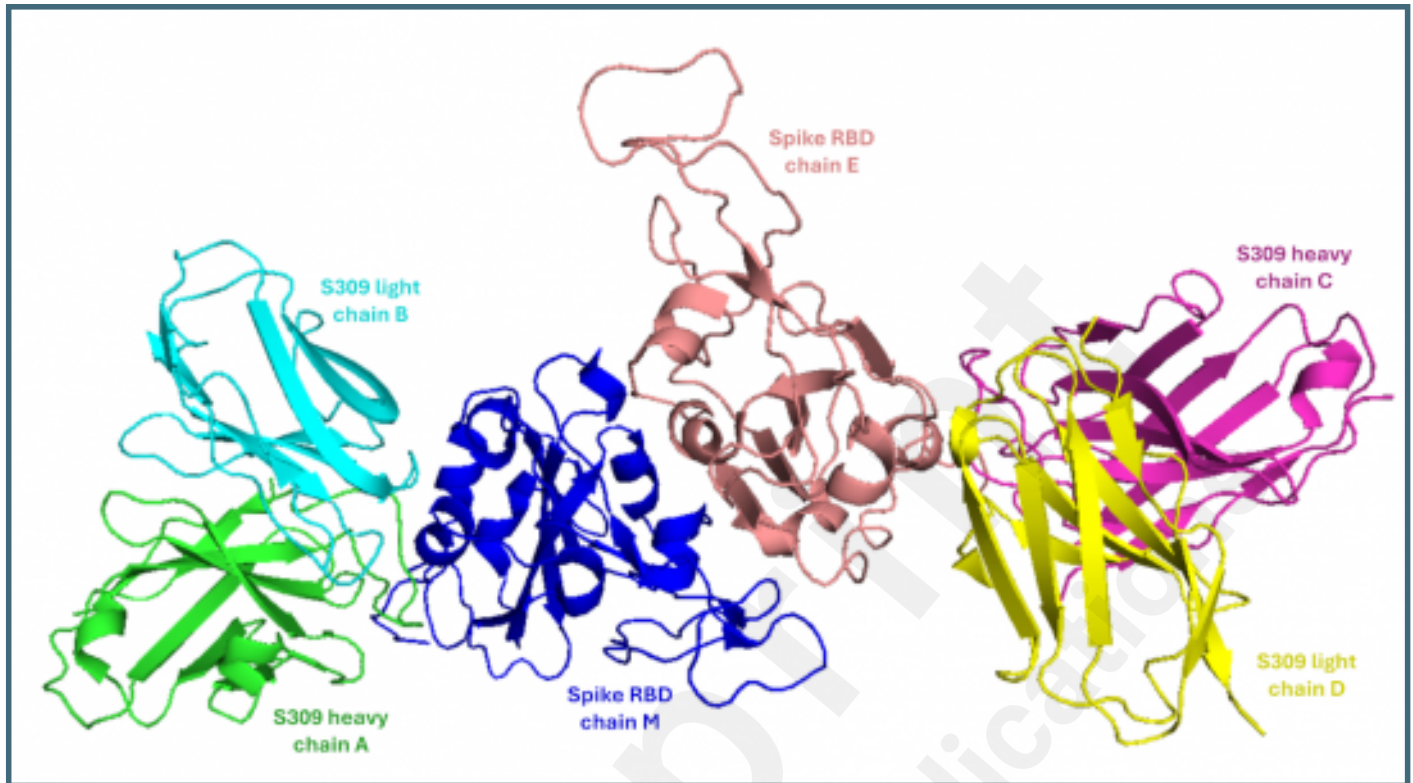
Supplementary Files

Figures

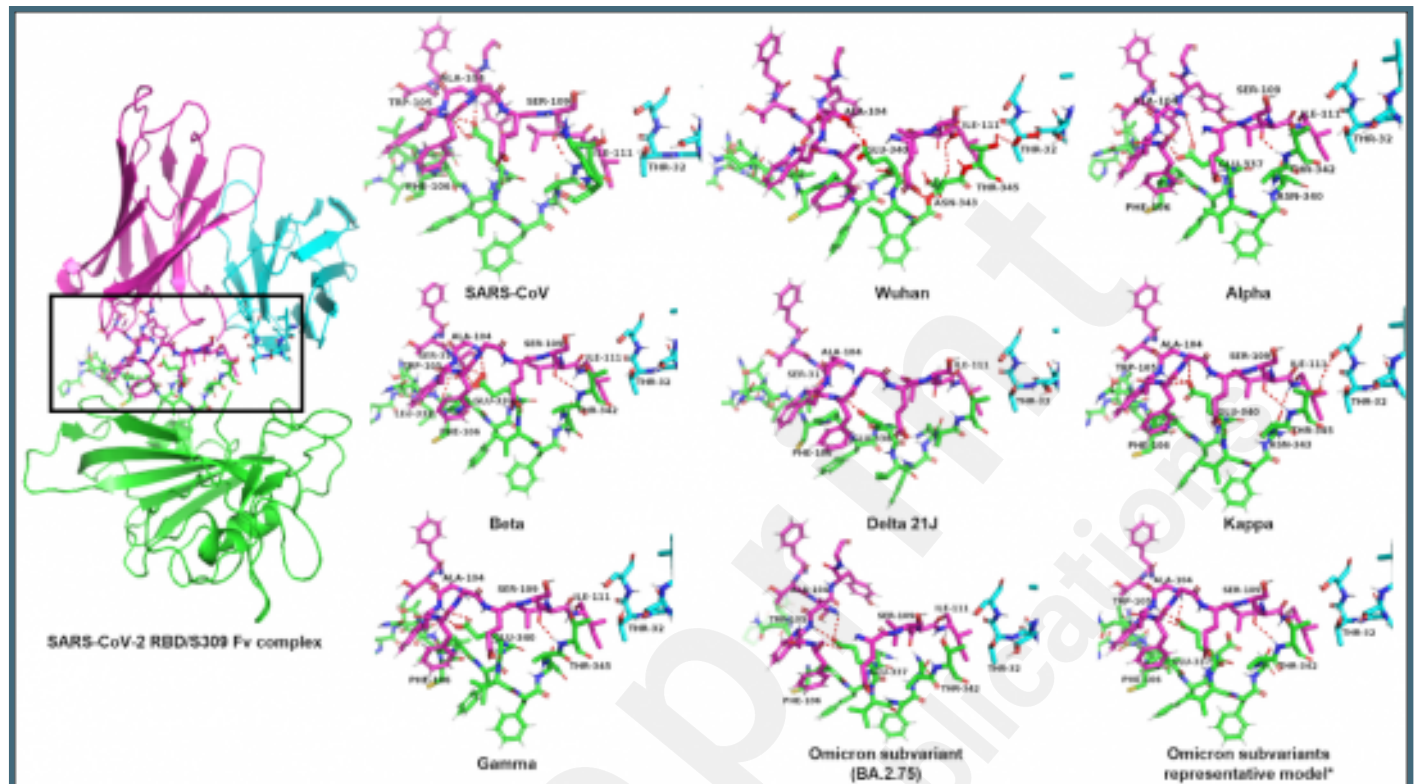
Method's outline. a) Outline of the three steps method. b) Workflow of the in silico method for the evaluation of a SARS-CoV-2 mAb neutralization power. d.



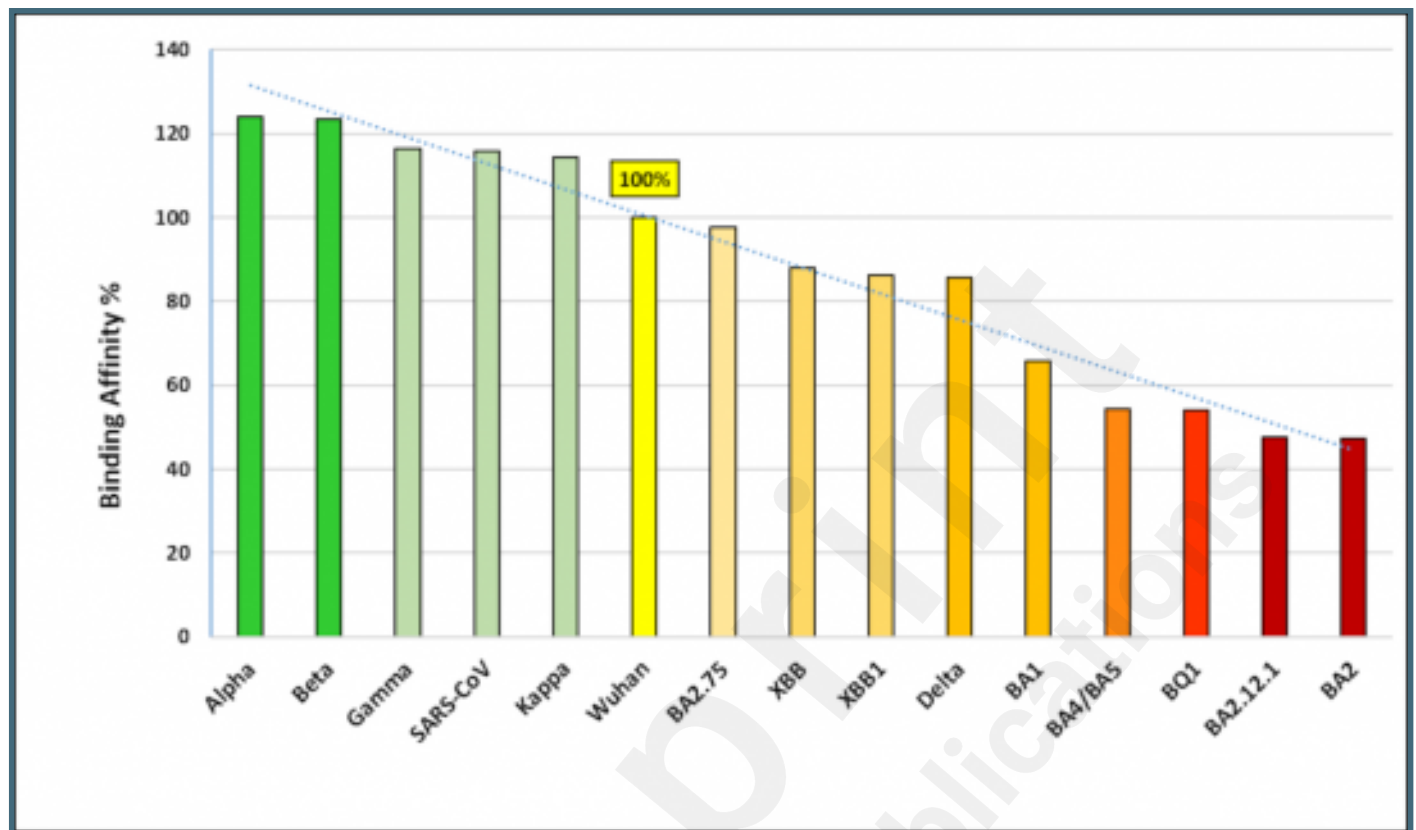
3D structure of the PDB model 7YAD showing two subunits of Sotrovimab Fv (heavy and light chains) binding to Omicrons' spike protein RBD.



Variations of the polar interactions between mAb Sotrovimab (S309) and different SARS-CoV-2 variants and subvariants. mAb heavy chain (Magenta), mAb light chain (Cyan), SARS-CoV-2 S protein-RBD (Green). *Residue numbering: BA.1, BA. 2, BA.2.12.1 (Glu337, Thr342) / BA. 4, BA.5, BQ.1 (Glu335, Thr340) / XBB, XBB.1.



Binding energy (ΔG) of the SARS-CoV and different SARS-CoV-2 variants (represented in affinity percentage in comparison to SARS-CoV-2).



Multimedia Appendixes

Supplementary Table S1: Polar (P) -highlighted in cyan color-, salt bridges (S) and hydrophobic interactions pattern of SARS-CoV, SARS-CoV-2 variants and Omicron Subvariants with neutralizing antibody S309. The interacted residues on the spike protein are the same for all the different variants. The difference of the numbering is due to Indel mutations while the interacted residues are the same; refer to Supplementary Fig S1 for residues' numbering. Supplementary Table S2: Binding energy (?G) of the SARS-CoV and different SARS-CoV-2 variants. Supplementary Fig S1: Sequences alignment. Numbering based on the Wuhan sequence.

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