Antibody Engineering Towards Enhancement of Spike Protein SARS-COV-2 -m396 Binding Affinity

Fateme Sefid [a,b], Zahra Payandeh [c], Ghasem Azamirad [d*], Iraj Rasooli [e]

[a] Department of Medical Genetics, Shahid Sadoughi University of Medical Science, Yazd, Iran; [b] Department of Biology, Science and Art University, Yazd, Iran; [c] Immunology Research Center, Biomedicine Institute, Tabriz University of Medical Sciences, Tbriz, Iran; [d] School of Mechanical Engineering, Yazd University, Yazd, Iran; [e] Department of Biology, Shahed University, Tehran-Qom Express Way, Tehran, Iran.

Corresponding authors**: Ghasem Azamirad, School of Mechanical Engineering, Yazd University, Yazd, Iran. ***E-mail: azamirad@yazd.ac.ri;

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Introduction: The SARS-COV-2 is a non-segmented positive-sense RNA virus that belongs to the genus Beta Coronavirus. The envelope-anchored trimeric spike protein on the virus surface is considered to be the key protein for the viral entry into the host cells. The angiotensin-converting enzyme 2 (ACE2) is reported to be the effective human receptor for SARS-COV-2. ACE2 receptor can be prevented by neutralizing antibodies such as m396 targeting the virus receptor-binding site. **Material and Methods:** Considering the importance of computational docking, and in silico affinity maturation we aimed at finding the important amino acids of the m396 antibody. These amino acids were then replaced by other amino acids to improve antibody-binding affinity to receptor-binding domain (RBD) of the SARS-COV-2 spike protein. Finally, we measured the binding affinity of antibody variants to the antigen. **Result:** Our findings disclosed that several variant mutations could successfully improve the characteristics of the antibody binding compared to the normal antibodies. **Conclusion:** the antibodies developed may be possible candidates for stronger affinity binding to antigens.

Keywords: Coronavirus; SARS-COV-2; Bioinformatics; Affinity Maturation

Introduction

Coronavirus is an RNA virus with a positive sense strand genome ranging from 26 to 32-kilo bases in length and associated with the Coronaviridae family under order Nidovirales. Coronaviruses are genetically classified into four major genera Alpha, Beta, Gamma, and Delta coronavirus. Severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory disorder coronavirus (MERS-CoV) classified as β-coronaviruses, and the novel coronavirus SARS-COV-2 belongs to this family (1).

One of the key protein for the entry of virus into the host cells is envelope-anchored trimeric spike protein at the surface of the virus. The angiotensin-converting enzyme 2 (ACE2) is described to be the effective human receptor for SARS-COV-2. This receptor primarily plays physiological role in the control of vasoconstriction and blood pressure. ACE2 is expressed in the lungs, heart, kidneys, and intestine (2-4). It also has been revealed that reduced ACE2 expression is related with cardiovascular diseases (5).

Binding to cell-surface-associated receptors initiates the entry of viruses into animal cells. Neutralizing antibodies (nAbs) can prohibit this process by targeting the virus receptor-binding site. Regarding the novel coronavirus entry, the spike glycoprotein binds to the receptor-binding domain (RBD). In order to block the spike and ACE2 interaction, the RBD is an important target for neutralizing antibodies (6).

Although the receptor-binding domain (RBD) of the SARS-COV-2 spike protein has six amino acid substitutions compared to the RBD of the SARS spike protein, the existence of highly conserved residues at this region corroborates that the ACE2 could be the receptor for SARS-COV-2 entry (2, 7, 8).

In previous studies, many potent monoclonal antibodies against SARS coronavirus spike protein have been identified (9-12). Tian *et al.*, expressed and purified SARS-CoV-specific antibodies that have been reported to target RBD and have potent neutralizing activities, m396 antibody, and measured its

binding ability to SARS-COV-2 RBD by ELISA and BLI. Despite the close relationship between SARS-COV-2 viruses and SARS-CoVs the m396 antibodies have not been effective in the study of Tian *et al.,* for SARS-COV-2 viruses (13).

With a view to the advantages of m396 antibody and the impact of its specificity, we designed this study to enhance the binding affinity to RBD in SARS-COV-2 virus of neutralizing antibody by targeted amino acid mutagenesis.

Materials and Methods

Sequence and structure availability and homology search

The spike protein [SARS-COV-2 coronavirus] with PDB ID "6VSB_A" [SARS-CoV-2 spike glycoprotein] was saved in FASTA format for further analyses. The sequence served as a query for protein BLAST and Possible putative conserved domains of the query protein were also searched for at http://blast.ncbi.nlm.nih.gov/Blast.cgi against the non-redundant protein database (14, 15). To find the Crystal Structure of anti-SARS m396 Antibody and SARS-CoV-2 spike glycoprotein, RCSB Protein Data Bank (PDB) http://www.rcsb.org/pdb/home/home.do, was used (16).

2DD8 PDB code represented the Crystal Structure of SARS-CoV Spike Receptor-Binding Domain Complexed with Neutralizing Antibody. 6VSB PDB code represented the Crystal Structure of SARS-CoV-2 spike glycoprotein, severe acute respiratory syndrome coronavirus 2.

Obtained structures were subjected to MOE software to remove extra chains to achieve Neutralizing Antibody and SARS-CoV-2 spike glycoprotein structure respectively (17).

Selection of significant residues in m396 antibody

Some amino acids were selected as significant residues in m396 Antibody structure interaction with antigen. These residues located in one of H1, H2 and H3 regions of the antibody. The structure of the SCV RBD-Fab m396 complex is depicted by Prabakaran *et al.,* showed that Fab m396 mainly recognizes the 10-residue (482-491) β6-β7 loop that prominently protrudes from the RBD surface and contacts four of the CDRs of Fab, H1, H2, H3, and L3. The four CDRs form a shallow cleft on the surface of the antibody-variable regions, providing a deep binding pocket into which the β6 -β7 loop fits tightly. The tip of this loop is a type I β-turn (Gly-Ile-Gly-Tyr, residues 488-491) and is deeply buried in the antibody-combining site, which is a feature most commonly observed in antibody peptide complexes (18).

Sorting Intolerant from Tolerant (SIFT) analyses

The SIFT algorithm available at http://sift.jcvi.org/ predicts the effect of coding variants on protein function.It used from physical properties of amino acids and sequence homology. SIFT prediction is based on the amino acid conservation degree in sequence alignments, collected through PSI-BLAST, derived from closely related sequences (19).

m396 Antibody variants sketching

50 variants including mutations in at least one of 3 H regions designed. The 3D structure of all offered variants determined by SAbPred (20) at http://opig.stats.ox.ac.uk/webapps/sabdab-sabpred/WelcomeSAbPred.php (Antibody modeling tool and prediction software from the Oxford Protein Informatics Group (OPIG)). Several servers, concentrating on antibodies structures are collected in SAbPred. Antibody informatics tools can help develop our knowledge of immune responses to disease and engineering of therapeutic molecules.

Protein-protein docking

The 3D structure of each variant and PDB serves as input for HADDOCK server version 2.2. at http://haddock.science.uu.nl/services/HADDOCK/haddock.php. HADDOCK (High Ambiguity Driven protein-protein DOCKing) works based on biochemical or biophysical properties (21). HADDOCK is an information-driven flexible docking server for the modeling of biomolecular complexes. HADDOCK is distinguished with ab-initio docking methods. This server to run the process of docking, codes data from known protein interfaces or predicted models in ambiguous interaction restraints (AIRs).

Results

Sequence and structure availability and homology search

The spike protein [SARS-COV-2 coronavirus] sequence with 1288 residues was acquired from National Center for Biotechnology Information (NCBI) in FASTA format. Spike protein [SARS-COV-2 coronavirus] reference sequence serving as a query for Basic Local Alignment Search Tool (BLAST)

introduced a collection of sequences as the highest similar sequences.

BLAST search revealed numerous hits to the spike protein sequence. All hits were of Coronavirus. Putative conserved domains were detected within this sequence. The sequence residues from 319-541 belong to SARS-CoV-2_Spike_S1_RBD with accession cd21480, receptor-binding domain of the S1 subunit of severe acute respiratory syndrome coronavirus 2 Spike (S) protein; This group contains the receptor-binding domain of the S1 subunit of the spike (S) protein from highly pathogenic human virus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), also known as 2019 novel coronavirus (2019-nCoV). The CoV S protein is an envelope glycoprotein that plays the most vital role in viral adherence, fusion, and entrance into host cells (22, 23). This protein is considered as a main target for the expansion of vaccines, inhibitors of viral entrance and neutralizing antibodies. It is synthesized as a precursor protein that is cleaved into a C-terminal S2 subunit (~600 amino acids) and an N-terminal S1 subunit (~700 amino acids) that mediates membrane fusion and attachment, respectively.

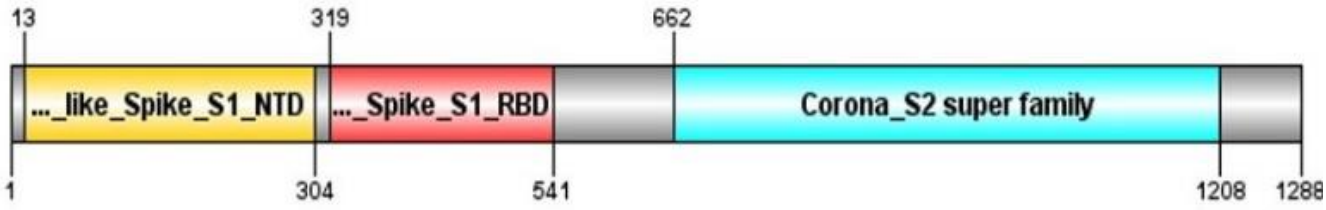
Receptor-binding domain (RBD) is the most important part of S1 subunit, while a hydrophobic fusion peptide and two heptad repeat regions are the most important parts of the S2 subunit. S1 includes two structurally independent domains, the C-terminal domain (C-domain) and the N-terminal domain (NTD). Either the C-domain or the NTD can consider as the receptor-binding domain (RBD), depending on the virus. Most of CoVs, including SARS-CoV-2 use the C-domain to viral adherence, fusion, and entrance into host cells, while NTD of mouse hepatitis virus (MHV) include RBD.

SARS-CoV-2 RBD displayed preferable binding affinity to the ACE2 receptor than SARS-CoV RBD significantly. Due to the key role of the S protein RBD in viral attachment, it is the major target for antibody-mediated neutralization. This model corresponds to the S1 subunit C-domain that serves as the RBD for SARS-CoV-2 and most CoVs.

Putative conserved domains that have been detected within the sequence are shown in Figure 1.

The sequence of 2DD8 PDB codes with 3 chains was obtained from NCBI and saved in FASTA format. Chain H and L belong to Igg Chains (Heavy and light) and chain S belongs to Spike Glycoprotein. Chain H includes 245 residues, Chain L includes 213 residues and Chain S includes 202 residues.

The sequence of 6VSB PDB codes with 3 chains was obtained from NCBI and saved in FASTA format. All chains were identical and includes 1288 residues.



List of domain hits

Name	Accession	Description	Interval
Corona_S2 super family	cl20218	Coronavirus S2 glycoprotein; The coronavirus spike glycoprotein forms the characteristic ...	662-1208
SARS-CoV-2_Spike_S1_RBD	cd21480	receptor-binding domain of the S1 subunit of severe acute respiratory syndrome coronavirus 2 ...	319-541
SARS-CoV-like_Spike_S1_NTD	cd21624	N-terminal domain of the S1 subunit of the Spike (S) protein from Severe acute respiratory ...	13-304

Figure 1. Putative conserved domains of spike protein [SARS-CoVs 2coronavirus] Residues 319-541 of the sequences belong to Spike receptor binding domain (Accession: cd21480)

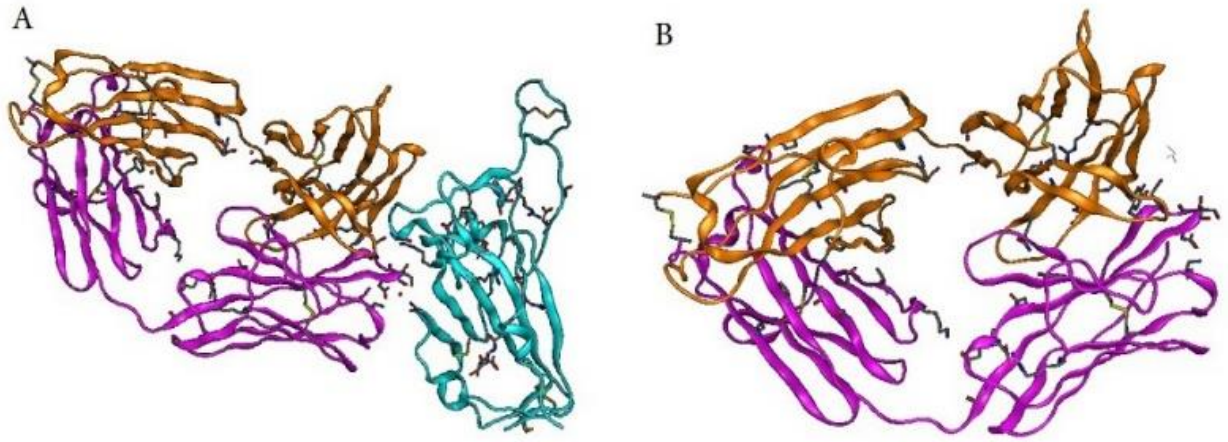


Figure 2. (A) Crystal Structure of SARS-CoV Spike Receptor-Binding Domain (Blue) Complexed with Neutralizing Antibody (Orange & violet). (B) Neutralizing Antibody without extra non-protein and protein molecules. MOE software was used to generate the molecular gr graphics. (Orange & violet)

The 2DD8 Protein Data Bank (PDB) record was detected for the Crystal Structure of SARS-CoV Spike Receptor-Binding Domain Complexed with Neutralizing Antibody. The Spike Receptor-Binding Domain and all non-protein atoms were eliminated to attain the Neutralizing Antibody structure using MOE software. Figure 2 shows the Neutralizing Antibody structure before and after monomerization.

The 6VSB Protein Data Bank (PDB) record was detected for the Crystal Structure of SARS-CoV-2 spike glycoprotein (Chain: A, B, C). Chains B and C and all non-protein atoms were eliminated to attain the monomeric SARS-CoV-2 spike glycoprotein structure using MOE software. Figure 3 shows the spike glycoprotein before and after monomerization.

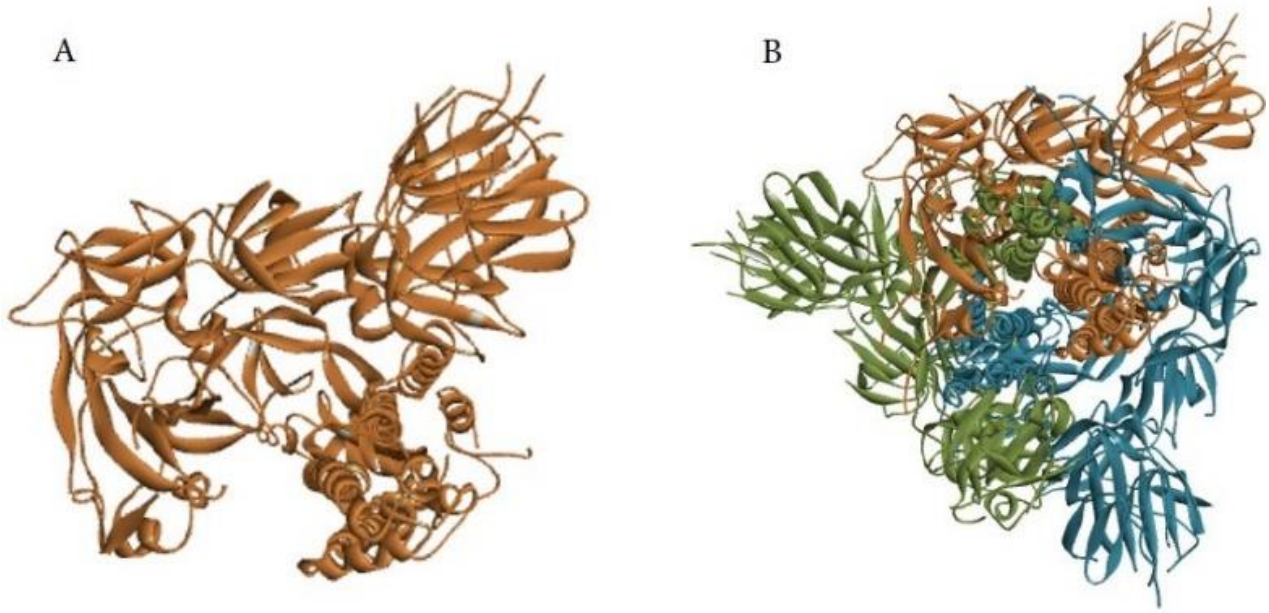


Figure 3. (A) Crystal Structure of SARS-CoV-2 spike glycoprotein (ChainA: Orange, chainB: Blue, ChainC: Green). (B) Chain A SARS-CoV-2 spike glycoprotein without extra non-protein and protein molecules. MOE software was used to generate the molecular graphics.

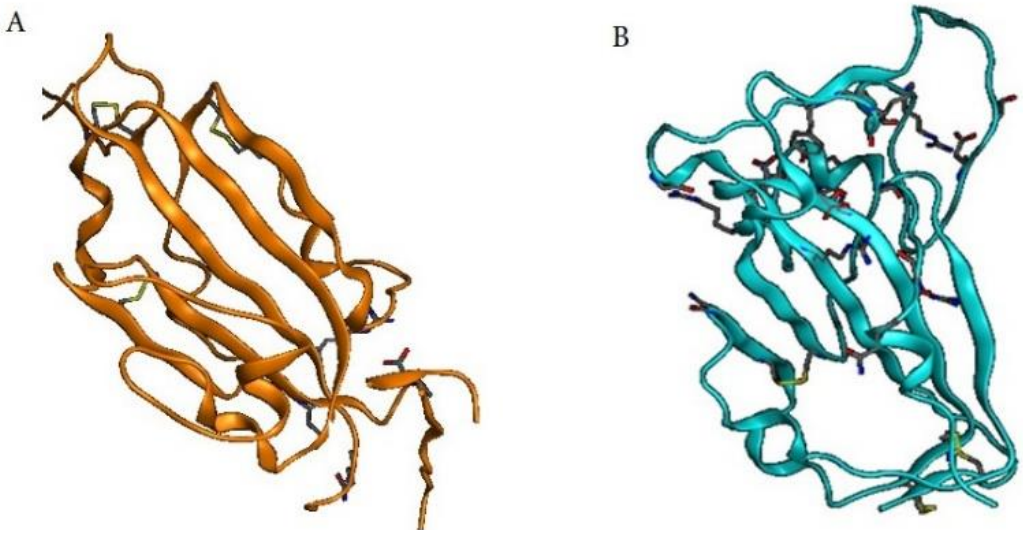


Figure 4. (A) RBD domain of SARS-CoV-2 spike glycoprotein. (B) SARS-CoV Spike Receptor-Binding Domain. MOE software was used to generate the molecular graphics.

The RBD domain of SARS-CoV-2 spike glycoprotein (residue 319-541) was attained from chainA SARS-CoV-2 spike glycoprotein exploiting 6VSB PDB structure using MOE software. The SARS-CoV Spike Receptor-Binding Domain was attained from2DD8 PDB structure using MOE software. Figure 4 shows the RBD domain of spike glycoprotein and SARS-CoV Spike Receptor-Binding Domain.

Figure 5 shows the final structure of the SARS-COV-2 coronavirus binding domain superimposed with SARS-CoV Spike Receptor-Binding Domain.

Fab m396 mainly recognizes the 10-residue GFYTTTGIGY (482-491) β6 - β7 loop in SARS-CoV Spike Receptor-Binding Domain that prominently protrudes from the RBD surface (Figure 5). The superimposition of SARS-COV-2 coronavirus binding domain and SARS-CoV Spike Receptor-Binding Domain showed that 10 residues GFQPTNGVGY (496-505) in SARS-COV-2 coronavirus binding domain match 10-residue (482-491) in SARS-CoV Spike Receptor-Binding Domain. These 10 residues create the prominent neutralizing site (Figure 5).

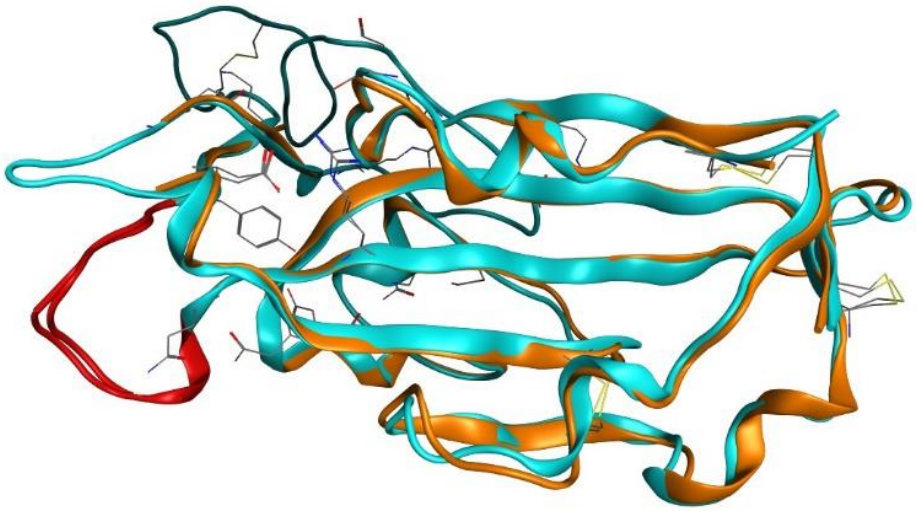


Figure 5. SARS-CoVs 2 coronavirus binding domain structure (Orange) superimposed with SARS-CoV Spike Receptor-Binding Domain (Blue). The prominent neutralizing site is in red (β6 - β7 loop). MOE software was used to generate the molecular graphics.

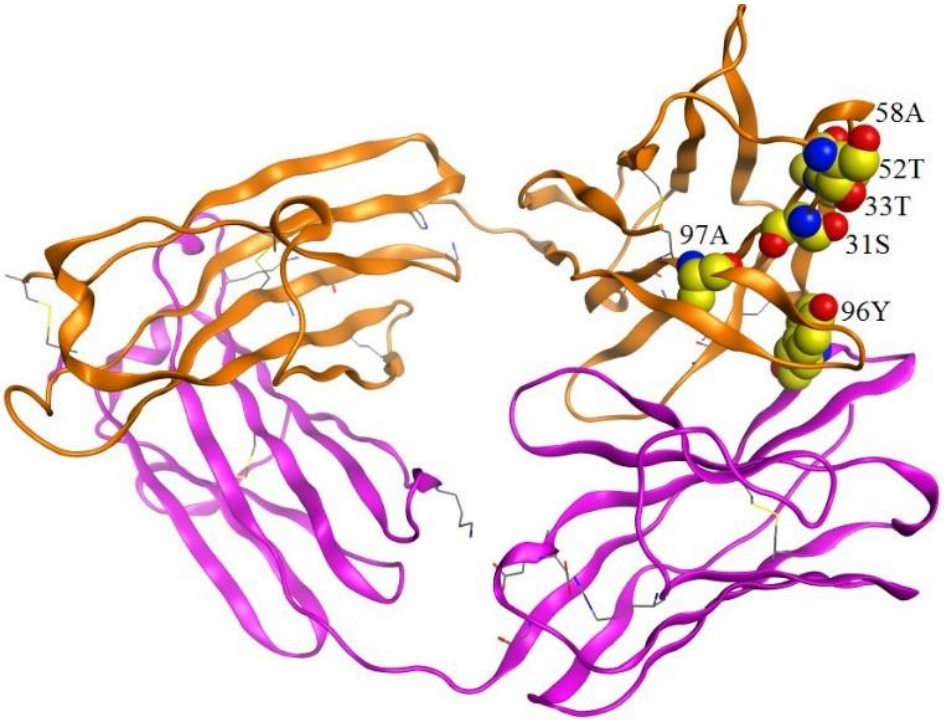


Figure 6. Significant residues in antibody structure involved in antigen binding. The antibody heavy chain illustrated in orange and the light chain illustrated in violet. 31S, 33T, 52T, 58A and 97A residues from the heavy chain and 96Y from light chain of m396 Antibody. MOE software was used to generate the molecular graphics.

Significant residues selection

Significant residues in heavy chain (31S, 33T, 52T, 58A and 97A) and light chain (96Y) were selected. Ser31 and Thr33 located in the H1 region, Thr52 and Asn58 located in the H2 region and Val97 located in the H3 region of m396 Antibody (Figure 6).

SIFT analysis

SIFT predict deleterious substitutions and tolerated for each residue of the query. SIFT computes normalized probabilities for every possible substitutions. Residues with normalized probabilities less than 0.05 are considered as deleterious; Residues greater than or equal to 0.05 are considered as tolerated. SIFT results for selected residues are shown in Table 1. Residue H: 31S can be replaced with 15 other amino acids and Residue H: 33T, H: 52T, H: 58N, H: 97V and L: 96Y can be replaced with 19 other amino acids as listed in Table 1. Predictions at each position of the significant residues for all 20 possible amino-acid changes are illustrated in Table 1.

Table 1. SIFT results for selected residues

Position	Seq Rep	Predict Tolerated																			
H:31S	0.88	M	h	I	P	V	L	Q	R	G	A	T	N	K	D	E	S				
H:33T	0.88	C	W	P	D	m	E	K	q	N	G	R	i	T	S	V	A	H	L	F	Y
H:52T	0.78	C	W	P	D	m	E	Q	K	G	N	R	I	S	T	A	V	H	L	F	Y
H:58N	0.88	W	c	M	P	I	f	V	G	H	q	R	L	D	T	A	S	K	E	Y	N
H:97V	0.56	C	W	M	P	D	q	N	G	K	E	R	I	T	S	h	V	A	L	F	Y
L:96Y	0.68	C	p	D	M	E	k	Q	n	s	R	T	G	I	A	W	L	F	H	V	Y

Amino acid color code: nonpolar, uncharged polar, basic, acidic.

Capital letters show amino acids looking in the alignment, lower case letters outcome from prediction. 'Seq Rep' is the proportion of sequences that have one of the essential amino acids. A low fraction shows the position is either severely gapped or unalienable and has little information.

Amino acids appearing in the protein sequence alignment that was used for prediction showed in Capital letters, whereas amino acids not observed in the protein alignment showed in lower case letters. The numbers in the 'Seq Rep' column correlate with the percentage of sequences in the alignment, which have an amino acid (not an insertion/deletion) at the corresponding position. A higher fraction of sequences in the alignment represented at a position improves the confidence of prediction at that position.

m396 variants sketching

755 variants including mutations at least in one of 3 H regions were offered. H:31S, H:33T, H:52T, H:58N, H:97V and L:96Y residues confirmed by crystallographic analyses, were randomly mutated in the suggested variants. Variations created in mutation sequences are illustrated in Figure 7.

Protein-protein docking

HADDOCK (High Ambiguity Driven biomolecular DOCKing) server scrutinizes receptor and ligand interaction based on biophysical and/or biochemical information.

We set H:31S, H:33T, H:52T, H:58N, H:97V and L:96Y as HADDOCK active residues in antibody structure, and residues 496G, 497F, 498Q, 499P, 500T, 503V, 504G and 505Y as HADDOCK active residues in antigen structure These residues are located in the prominent neutralizing site of SARS-CoV2 Spike Receptor-Binding Domain. Table 2 shows the variants informationfigur in which the HADDOCK score is outstanding (<-120).

As can be seen in the Figure 8, among the 55 designed variants, the 38 variants had a higher score than the control. In this regard variants 3, 10, 27, 38, 42, 52 and 55 have the highest HADDOCK score (<-120). Figure 9 shows the structure of the best-mutated antibody variant docked with SARS-COV-2 coronavirus spike protein binding domain antigen.

control	S	T	T	N	V	Y
Variant1	T	S	T	N	V	Y
Variant2	N	T	N	N	V	Y
Variant3	K	T	T	Y	V	Y
Variant4	G	T	T	N	F	Y
Variant5	E	T	T	N	V	T
Variant6	S	S	Q	N	V	Y
Variant7	S	R	T	W	V	Y
Variant8	S	L	T	N	A	Y
Variant9	S	Y	T	N	V	G
Variant10	S	T	Y	Y	V	Y
Variant11	S	T	K	N	F	Y
Variant12	S	T	E	N	V	F
Variant13	S	T	T	T	V	I
Variant14	G	R	E	N	V	Y
Variant15	Q	C	T	D	V	Y
Variant16	N	Y	T	N	L	Y
Variant17	L	N	T	N	V	Q
Variant18	S	S	Y	T	V	Y
Variant19	S	K	R	N	E	Y
Variant20	S	H	G	N	V	K
Variant21	S	T	C	Y	P	Y
Variant22	S	T	A	S	V	T
Variant23	S	T	T	L	M	C
Variant24	I	A	V	L	V	Y
Variant25	K	W	P	N	E	Y
Variant26	A	V	Q	N	V	F
Variant27	S	G	R	Y	W	Y
Variant28	S	T	Q	E	V	F
Variant29	S	T	V	H	C	W
Variant30	E	W	Q	D	Y	Y
Variant31	S	N	Q	H	P	K
Variant32	A	T	Q	N	G	Y
Variant33	S	F	T	R	V	I
Variant34	L	S	T	N	Y	R
Variant35	S	W	T	L	E	A
Variant36	S	H	L	Q	C	N
Variant37	G	T	C	D	V	Y
Variant38	L	T	V	N	M	Y
Variant39	K	T	I	N	V	D
Variant40	A	T	T	L	S	Y
Variant41	G	T	T	G	V	T
Variant42	L	T	T	N	W	F
Variant43	S	D	T	F	P	Y
Variant44	S	R	T	K	V	Q
Variant45	S	M	T	N	M	G
Variant46	S	T	W	N	R	H
Variant47	L	T	H	R	S	Y
Variant48	S	Q	T	C	K	V
Variant49	S	M	E	N	N	A
Variant50	I	L	Q	S	T	H
Variant51	E	W	P	W	Y	Y
Variant52	I	T	V	W	E	Y
Variant53	K	W	V	N	Y	Y
Variant54	E	R	T	W	Y	Y
Variant55	S	R	T	W	E	Y

Figure 7. variants with mutations at least in one of 3 H regions. Residues from left to right are H:31, H:33, H:52, H:58, H:97 and L:96. In each variant, red residues are mutated and the green are unmutated.

Discussion

Efforts to develop vaccines and effective treatments against SARS-COV-2 have continued since its rapid outbreak throughout the world. However, there is still no definite drug or effective vaccine to protect the healthy individuals. Various strategies have been adapted to develop vaccines against SARS-COV-2 including whole virus vaccines, subunit vaccines, and nucleic acid vaccines (24). The SARS-COV-2 is highly prone to

mutations during its transmission. A high rate of mutations is one of the challenges ahead of effective vaccine development. Looking into its spread across the continent and vulnerable persons, an urgent requirement of crafting vaccines is needed for reinforcing immune defense against SARS-COV-2. Since there is no specific antiviral treatment or vaccine for SARS-COV-2, the researchers pay more attention to development of antibodies.

Table 2. Docking between the normal and mutated m396 antibody with SARS-CoVs2 coronavirus spike protein binding domain

	control	Variant3	Variant10	Variant27	Variant38	Variant42	Variant52	Variant55
HADDOCK score	-103.6 +/- 11.0	-122.6 +/- 2.6	-130.2 +/- 4.2	-122.8 +/- 1.8	-120.8 +/- 8.5	-124.0 +/- 3.0	-122.2 +/- 2.2	-123.1 +/- 4.3
Cluster size	10	106	47	61	40	59	40	27
RMSD from the overall lowest-energy structure	1.0 +/- 0.7	1.0 +/- 0.6	0.9 +/- 0.5	1.1 +/- 0.7	0.9 +/- 0.6	0.7 +/- 0.4	6.7 +/- 0.2	0.9 +/- 0.6
Van der Waals energy	-49.7 +/- 7.1	-59.5 +/- 2.6	-60.8 +/- 4.0	-57.7 +/- 4.1	-56.3 +/- 4.8	-63.1 +/- 2.3	-60.2 +/- 6.2	-58.0 +/- 1.6
Electrostatic energy	-226.1 +/- 14.7	-219.0 +/- 37.0	-230.5 +/- 12.1	-254.4 +/- 22.7	-237.5 +/- 53.2	-211.7 +/- 13.5	-233.3 +/- 36.1	-249.4 +/- 23.6
Desolvation energy	-15.7 +/- 4.0	-26.3 +/- 6.5	-28.4 +/- 5.1	-22.1 +/- 6.1	-22.9 +/- 5.6	-26.5 +/- 3.8	-20.5 +/- 7.6	-22.1 +/- 7.7
Restraints violation energy	70.6 +/- 18.99	71.3 +/- 17.21	51.0 +/- 4.67	78.0 +/- 10.43	58.4 +/- 31.48	78.4 +/- 30.59	52.1 +/- 21.07	69.3 +/- 44.90
Buried Surface Area	1601.4 +/- 121.7	1697.9 +/- 31.0	1718.3 +/- 53.7	1736.2 +/- 78.2	1738.1 +/- 61.5	1713.2 +/- 80.4	1780.4 +/- 69.8	1703.8 +/- 72.2
Z-Score	-2.2	-2.2	-1.8	-2.2	-2.3	-1.9	-1.6	-2

HADDOCK offers a fully flexible scoring scheme since the weight of the various energy terms can be defined separately for each phase of the docking. The scoring is performed according to the weighted sum (HADDOCK score) of the following terms: van der Waals energy, electrostatic energy, symmetry restraints energy , radius of gyration restraint energy, distance restraints energy, diffusion anisotropy energy, intervector projection angle restraints energy, pseudo contact shift restraint energy, buried surface area, dihedral angle restraints energy, desolvation energy and binding energy. Only the improved variants (HADDOCK score <-120) are shown in the table. The original m396 sequence used as control.

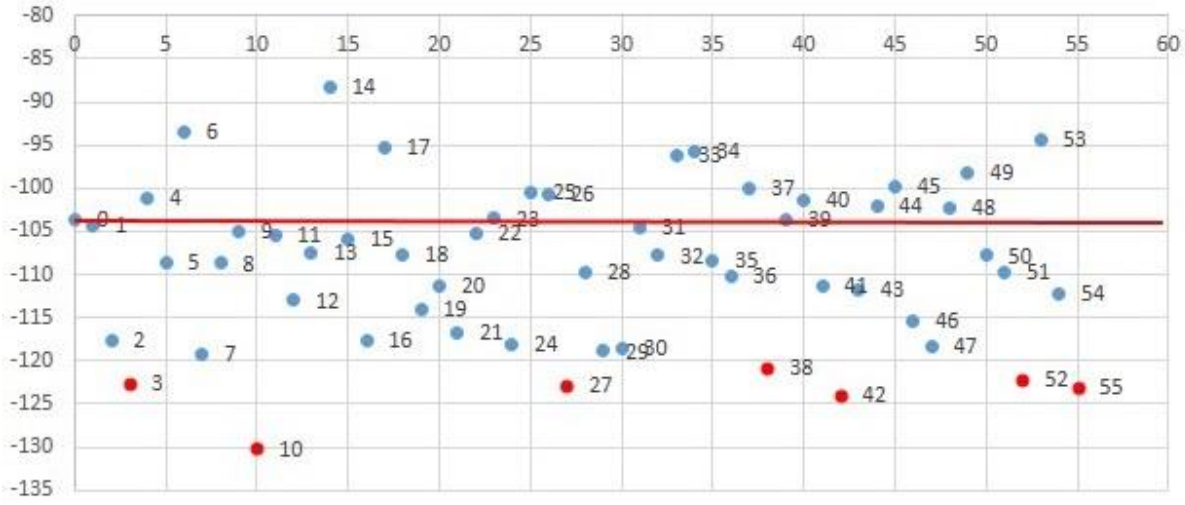


Figure 8. HADDOCK Score of all variants. The variants with scores below the threshold (control score is -103.6) are predicted to have enhanced affinity toward the m396 antibody. The best mutated m396 variants colored in red on the graph.

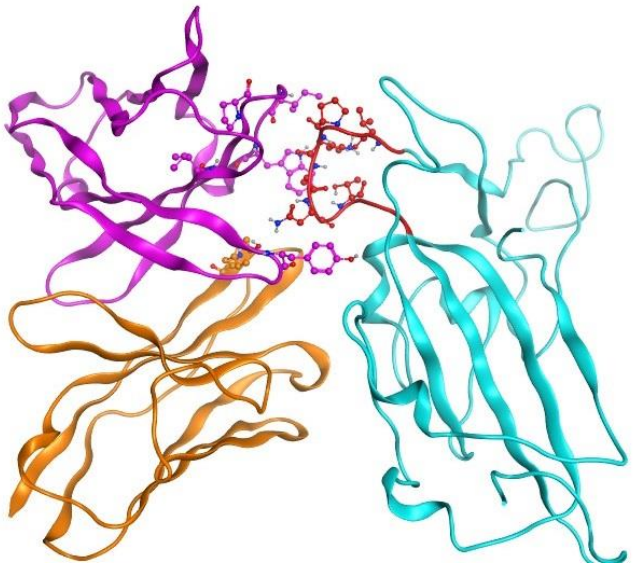


Figure 9. Structure of the best-mutated antibody (variant 10) docked with SARS-CoVs 2 coronavirus spike protein binding domain antigen. [Antibody heavy chain: purple, Antibody light chain: orange, antigen: blue, prominent neutralizing site of antigen (β6 - β7 loop): red]. MOE software was used to generate the molecular graphics.

The importance of ACE2 and RBD interaction is clear (25). Developing Neutralizing antibodies (nAb) against RBD is expected to be an efficient treatment to inhibit COVID-19 infection. However, highly potent and cross-protective nAbs against RDB are required to avoid failure (26-29). Developing potent and cross-protective nAbs is an ongoing challenge that needs time and effort. ACE2 on the other hands is expressed in a variety of tissues with profound physiological roles (30-33). Over the last two decades, the knowledge of recombinant antibody production for use in human therapy has made great advancement. Regardless of the method used in antibody development affinity of antibodies should be improved. Protein engineering methods is one of the best strategy for optimization of the features of antibody maturation to improve affinity to its target.so one of the most important purposes of antibody. Fallowing affinity maturation, the biological activity rises and amount of antibody and its toxicity decreased (34, 35). Remodeling the Antibody-combining site may be used to improve the antibody specificity. Genetic engineering methods can enhance the binding properti es of potentially advantageous antibodies by using mutagenesis to modify the sequence of amino acids within the antibody-combination site. Substitutions in a couple of amino acids be able to change specificity, increase affinity, decrease cross-reactivity with closely-related antigens (36, 37).

Another important concern in both the treatment and diagnosis is the alteration of the sensitivity of Abs identification. In the case of affinity maturation, it can be accomplished by random or targeted mutagenesis approaches using expression close to that.

In our previous studies, Anti-CD20 antibodies engineered to improve binding affinity using bioinformatics tools such as docking and molecular dynamics. We've shown enhanced antibody binding characteristics (38, 39).

In view of the benefits of m396 antibody and the effect of its specificity, we planned this study to boost the binding affinity to RBD in the SARS-COV-2 virus of targeted amino acid mutagenesis neutralizing antibody (38, 39). One of the strength of this study is using various bioinformatics tools for find important amino acid and their effects in antibodies affinity.

In the present research, sequences of antibodies and their primary analysis are performed. We used the Homology modeling for prediction of the Crystal Structure of SARS-COV-2 Spike Receptor-Binding Domain. Here, the structure of SARS-CoV Spike RBD was used as a template. Predictive accuracy depends on the similarity of degree of sequence. If a template structure with a sequence identity of >50% is found for a query protein, homology modeling could be chosen as the best in the silico method (40). The Models were validated by rampage Ramachandran plot (41) and Q mean server. Ramachandran plots show that several amino acids in the best-predicted structures are located in the outlier region. Hence, the Ramachandran plot of the refined models was improved (42).

According to Prabakaran *et al.,* study (18), several amino acids interacted with ACE2 antigen. These amino acids contain 31S, 33T, 52T, 58A and 97A residues from the heavy chain and 96Y from the light chain in m396 Antibody. Ser31 and Thr33 located in the H1 region, Thr52 and Asn58 located in the H2 region and Val97 located in the H3 region of m396 Antibody.

Fab m396 mainly recognizes the 10-residue (482-491) in RBD and provided a deep binding pocket into which the β6 - β7 loop fits tightly.

We substituted the amino acids as shown in Figure 5 and performed Docking. Docking have an important role in Mutagenesis and drug design, as this is the only theoretical method that explicitly models physical protein interactions. The docking must be able to predict patterned protein complexes in molecular modeling. Docking refers to how the orientation and

mode of binding of one molecule to another during the formation of a stable complex is expected.

In this analysis, docking was performed using HADDOCK 2.1 software. HADDOCK 2.1 does the docking of variants m396 antibody and SARS-COV-2 Spike Receptor-Binding Domain, completely flexible, using molecular dynamics simulations. The Table shows the results of docking between the normal and mutated m396 variants with SARS-COV-2 Spike Receptor-Binding Domain (Table 2). Docking findings for both normal and mutant antibodies indicate that engineered mutations have changed and strengthened the binding and energy properties in mutated antibodies to the antigens.

Based on obtained scores which is -96.8 for control and mutated variants: Variant 4: -112.8, Variant 7: -110.5, Variant 24: -103.0, Variant 25: -102.7, Variant 30: -105.9, Variant 43: -108.0, Variant 44: -110.5, Variant 46: -105.6, Variant 47: -102.7 and Variant 51: -113.9, Variant 53: -113.0, Variant54: -112.5 have more affinity to bind SARS-COV-2 Spike Receptor-Binding Domain than the natural antibody. The buried area between two complexes in these variants was more positive than the natural state. This means that these mutations have modified and increased the antibody binding properties relative to normal antibodies. Although engineered antibodies have a high affinity to antigen, their functions should be examined experimentally.

Conclusion

These results suggest that m396 antibody could potentially be developed as a candidate therapeutic alone or in combination with other neutralizing antibodies for the prevention or treatment of SARS-COV-2 infections. Optimization of the m396 properties to boost their binding characteristics and maturation of affinity to avoid cross-reactivity and selection of suitable antibodies by adding new mutations to create the desired antibody is advantageous.

Conflict of Interest: 'None declared'.

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