Molecular Diversity
https://doi.org/10.1007/s11030-022-10449-x

ORIGINAL ARTICLE

Monoclonal antibody designed for SARS-nCoV-2 spike protein of receptor binding domain on antigenic targeted epitopes for inhibition to prevent viral entry

Zainularifeen Abduljaleel[1,2,3] · Naiyer Shahzad[4] · Syed A. Aziz[5] · Shaheer M. Malik[6]

Received: 12 February 2022 / Accepted: 19 April 2022
© The Author(s), under exclusive licence to Springer Nature Switzerland AG 2022

Abstract
SARS, or severe acute respiratory syndrome, is caused by a novel coronavirus (COVID-19). This situation has compelled many pharmaceutical R&D companies and public health research sectors to focus their efforts on developing effective therapeutics. SARS-nCoV-2 was chosen as a protein spike to targeted monoclonal antibodies and therapeutics for prevention and treatment. Deep mutational scanning created a monoclonal antibody to characterize the effects of mutations in a variable antibody fragment based on its expression levels, specificity, stability, and affinity for specific antigenic conserved epitopes to the Spike-S-Receptor Binding Domain (RBD). Improved contacts between Fv light and heavy chains and the targeted antigens of RBD could result in a highly potent neutralizing antibody (NAbs) response as well as cross-protection against other SARS-nCoV-2 strains. It undergoes multipoint core mutations that combine enhancing mutations, resulting in increased binding affinity and significantly increased stability between RBD and antibody. In addition, we improved. Structures of variable fragment (Fv) complexed with the RBD of Spike protein were subjected to our established *in-silico* antibody-engineering platform to obtain enhanced binding affinity to SARS-nCoV-2 and develop ability profiling. We found that the size and three-dimensional shape of epitopes significantly impacted the activity of antibodies produced against the RBD of Spike protein. Overall, because of the conformational changes between RBD and hACE2, it prevents viral entry. As a result of this *in-silico* study, the designed antibody can be used as a promising therapeutic strategy to treat COVID-19.

✉ Zainularifeen Abduljaleel
zaabduljaleel@uqu.edu.sa; zainulbio@gmail.com

[1] Science and Technology Unit, Umm Al-Qura University, P.O. Box 715, Mecca 21955, Kingdom of Saudi Arabia

[2] Department of Medical Genetics, Faculty of Medicine, Umm Al-Qura University, P.O. Box 715, Mecca 21955, Kingdom of Saudi Arabia

[3] The Regional Laboratory, Molecular Diagnostics Unit, Department of Molecular Biology, Ministry of Health (MOH), P.O. Box 6251, Mecca, Saudi Arabia

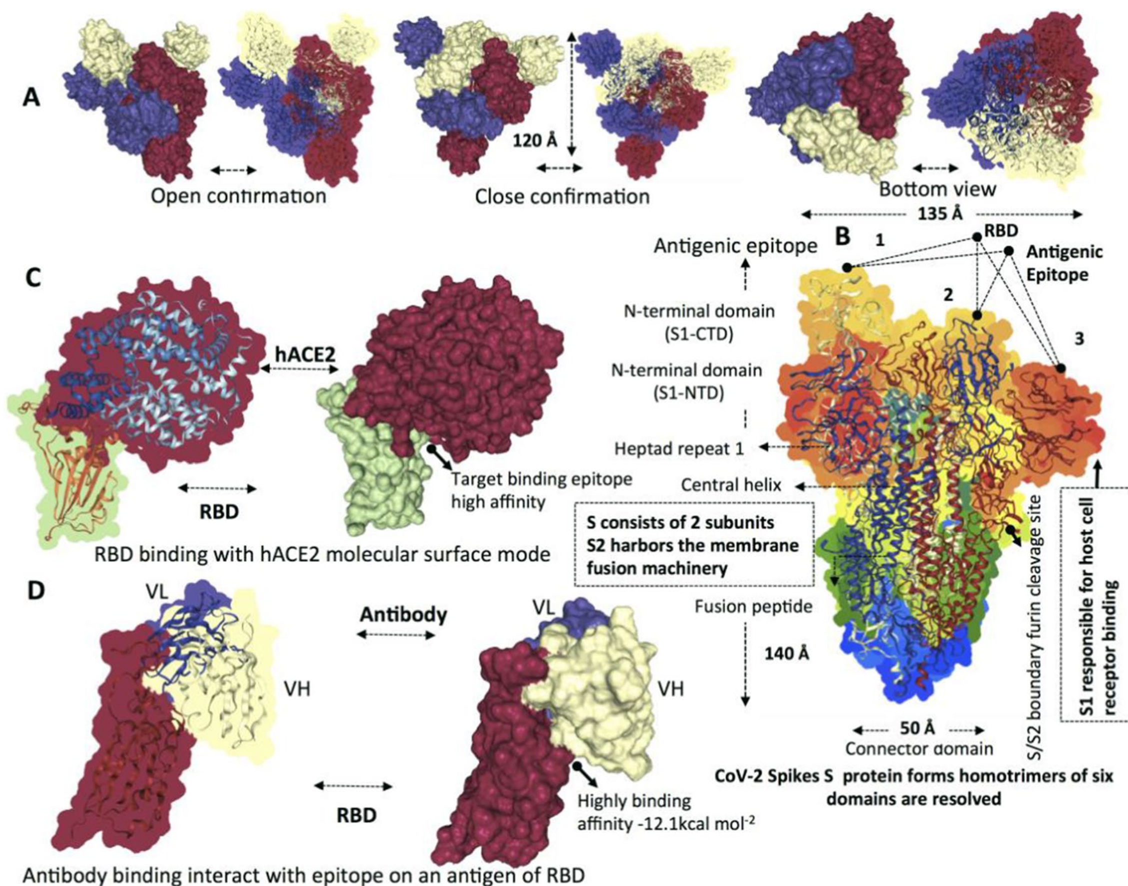
[4] Department of Pharmacology and Toxicology, Faculty of Medicine, Umm Al-Qura University, Mecca, Saudi Arabia

[5] Department of Pathology and Lab Medicine, University of Ottawa, 451 Smyth Road, Ottawa, ON K1H 8M5, Canada

[6] Department of Chemistry, Faculty of Applied Sciences, Umm Al-Qura University, Mecca, Saudi Arabia

Published online: 26 May 2022

Graphical abstract



Keywords COVID19 · Severe acute respiratory syndrome-coronavirus (SARS-CoV-2) · Human receptor angiotensin converting enzyme-2 (hACE2) · Receptor binding domain (RBD) · Monoclonal antibody · Spike (S) protein · Cellular immune responses

Introduction

Coronavirus disease (COVID-19) is a contagious respiratory ailment that can transmit from person to person. COVID-19 is caused by a coronavirus discovered during an examination into an outbreak in Wuhan, China. SARS-CoV-2, formerly known as 2019-nCoV, is a newly found novel coronavirus that causes pneumonia-associated respiratory syndrome [42, 44]. Based on analysis of the genome sequences of SARS-CoV-2 samples from many infected individuals, it was concluded that SARS-CoV-2 shares significant sequence similarity with SARS-CoV [23, 24]. In comparison to SARS-CoV, SARS-CoV-2 transmission from human to human appears to be more substantial [6, 7]. At least 25 countries had reported over 70,000 SARS-CoV-2 infection cases as of February 2020. A patient with SARS-nCoV-2 who has had mild to severe respiratory infection with symptoms such as fever, cough, and shortness of breath is a complication of this virus. A major purpose is to support artificial intelligence (AI) that is being used to help fight the viral pandemic, which has spread worldwide since the beginning of the year 2020, [3, 4, 29, 32].

In addition, some patients develop pneumonia in both lungs, multi-organ failure, and death in rare circumstances. At this time, persons with SARS-nCoV-2 who have had their disease validated by molecular testing using reverse transcription-polymerase chain reaction: RT-PCR have reported poor, late, or nonexistent antibody responses [12, 26, 48]. According to reports, most individuals do not produce antibody responses until the second week after the onset of symptoms. According to recent reports [26, 49], a diagnosis of SARS-nCoV-2 infection based on antibody response will only be possible during the recovery period, after many opportunities for therapeutic intervention or

disease transmission interruption have passed. Unfortunately, COVID-19 is not protected by any vaccinations or antiviral medications. Vaccines, monoclonal antibodies, oligonucleotides, short peptides, interferon type 2 alpha and beta, and small-molecule medicines are among the prevention and therapy alternatives being investigated by researchers worldwide, including many biotech and pharma R&D businesses [34].

The antibody-mediated humoral response is vital for avoiding viral infections, as only one route proposes. Neutralizing antibodies (NAbs) are a subgroup of these antibodies that lower viral infectivity by adhering to the surface epitopes of viral particles and inhibiting virus entry into an infected cell [38]. The SARS-nCoV-20 Spike (S1) protein is an essential target for vaccinations, therapeutic antibodies, and desperately needed diagnostics. COVID19's Spike proteins are the visible protrusions on its surface that give it its crown-like look (Fig. 1). These homotrimeric proteins, which include two unique subunits, are extensively glycosylated (S1 and S2). Spike serves as a molecular key by identifying and attaching to specific hACE2 cell-surface receptors on the cell surface via the S1 receptor-binding domain. When S1 binds to hACE2, Spike undergoes substantial structural changes that cause hACE2 to change conformation and allow the virus to enter the cell. Spike proteins, on the other hand, must project into the external environment to efficiently bind cell-surface receptors therefore, they are vulnerable to immune system detection. Spike targets diagnostic and

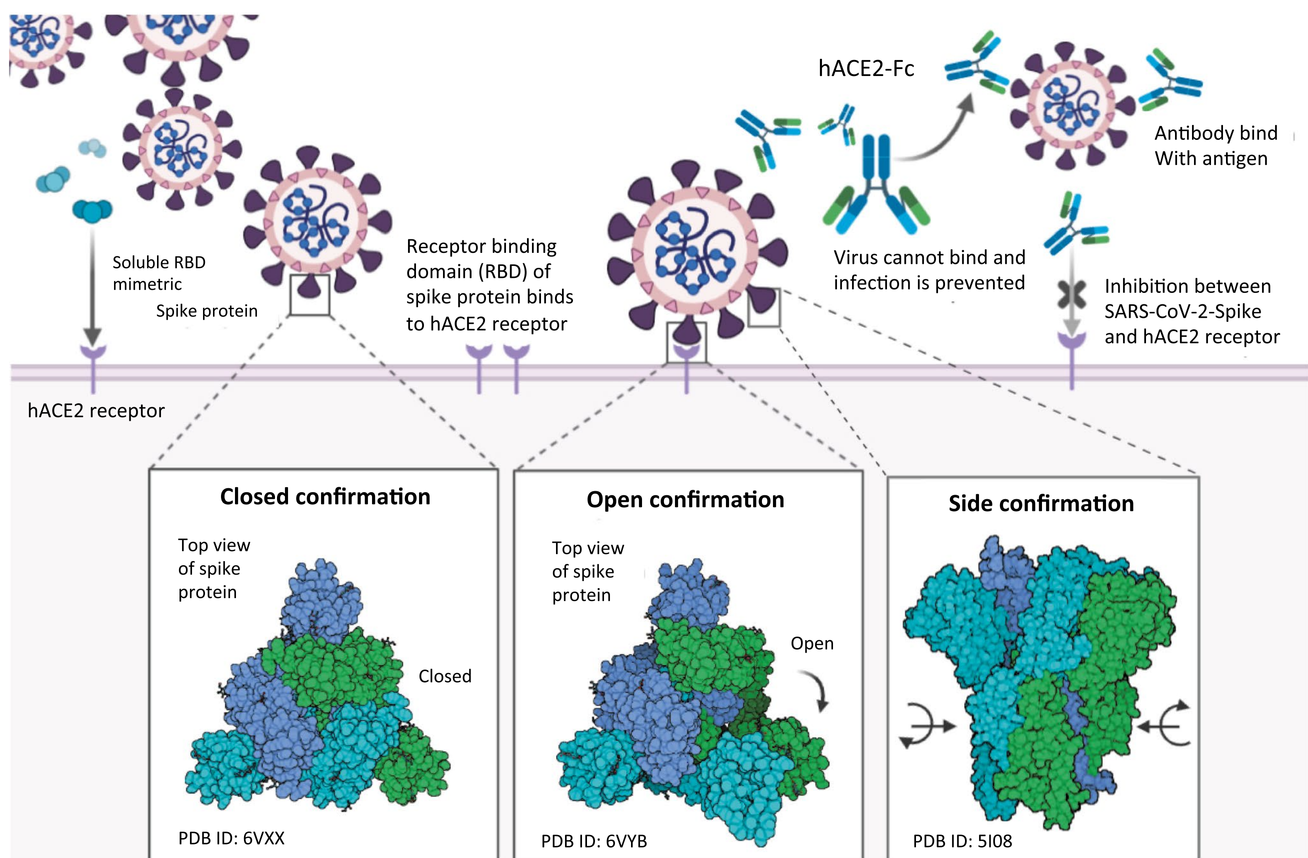


Fig. 1 Tectonic conformational changes of SARS-nCoV-2: Spike-S protein. Human ACE2 is the host cell receptor responsible for mediating infection by SARS-nCoV-2, the novel coronavirus responsible for Coronavirus disease 2019 (COVID-19). Cure with anti-hACE2 antibodies disrupts the interaction between virus and receptor. The Spike proteins on the surface of the coronavirus bind to angiotensin-converting enzyme 2 (hACE2) receptors on the surface of the target cell. In the process, cleaved hACE2 and activated spike protein facilitates viral entry. In this function, SARS-nCoV-2, the virus infection occurred in well-differentiated ciliated epithelial cells expressing hACE2. Thus, the virus enters hosts through the mucosa of the respiratory and gastrointestinal tract. Once the viral membrane fuses with the human cell membrane, allowing the virus's genome to enter human cells and begin infection

vaccine development because it induces a significant neutralizing antibody response due to its immunodominant character [25].

The genome of a coronavirus is enclosed in the nucleocapsid (N) protein, membrane (M), envelope (E), and spike (S) proteins [18]. Many coronavirus vaccine experiments targeting different structural proteins have been conducted, but most have ceased shortly after the outbreaks of SARS and MERS [9]. As a result of the recent pandemic of COVID-19, it is imperative that coronavirus vaccine research is resumed. An mRNA-based vaccine targeting the S protein of SARS-CoV-2 was first tested on humans in response to the ongoing pandemic. *In-silico* design enables rapid iteration around potential solutions while removing the specific factors that traditional in vivo subjects may have. In vivo tests are used to determine the chance of creating an antibody that activates the immune system to fight infections [9]. These procedures can be developed and provided remotely, eliminating the need for traditional wet labs to be present on-site. This study aimed to demonstrate how to design synthetic peptide monoclonal antibodies (mAbs) for the Spike S1 protein to target specific antigenic epitopes found in RBD with high efficacy [9]. We can identify critical residues between RBD and monoclonal antibodies and apply this information to the development of vaccines against SARS-nCoV-2 neutralizing antibodies and protective antibodies immunity against virus infection because Spike protein is the main antigenic component responsible for inducing host immune responses. Spike protein has thus been chosen as an effective target for developing coronavirus vaccines and antivirals. Monoclonal antibodies that bind highly to RBD antigens can optimize the residues at their interface.

Materials and methods

Dataset preparation and selection

More than 2000 depositions of antibody structures are currently accessible in NCBI and the Protein Data Bank (PDB). Based on these data, it is possible to estimate the comparative model of a monoclonal antibody against the viral surface antigen. We used molecular dynamics (MD) simulation to validate and filter 500 antibodies sequences retrieved from the NCBI and PDB servers based on the highest correlation between calculated and measured binding affinities between the target of SARS-nCoV-2 Spike-S1 RBD epitopes specific antigen and a monoclonal antibody [39]. As a result of filtering out both VH and VL sequences, a high similarity result of 0.94 was obtained (PDB: 6dkj: human GIPR antagonistic antibodies). Sequence identification of the VH framework subsets H1 and H2 from PDB: 6dkj (chain A) and CDR H3 from PDB: 4cni (chain A), as well as the subsets of L1, L2, and L3 from PDB: 6dkj, are both VH-VL orientation complexes.

Identification of antigen and antibody sequence

A vaccine communicates the immune system to recognize specific viral antigen signatures in the RBD's Spike-S1 protein-specific antigen. The SARS-nCoV-2 is a recombinant antigen including typical Spike-S1 protein immunodominant region amino acids 408–470 and 540–573. The immune epitope database (IEDB) [14] and Support Vector Machine (SVM) Tri-peptide similarity and Propensity (SVMTriP) identified specific antigenic epitopes of a small synthetic peptide to be regions on the surface of proteins on the target of antibodies [46]. After filtering 500 sequences from NCBI and PDB using MD simulation, we identified consists of antibody sequence in two chains (VL/VH) after filtering by MD simulation out of 500 from NCBI and PDB. In chain A-VL (light chain): Cl4mAb synthetic construct with GenBank ID: AEW26702.1. In chain B-VH (heavy chain): Cl4mAb synthetic construct with GenBank ID: AEW26701.1.

Structural design of antigen and antibody

The structure of antibodies was determined and validated using two new programs. To begin, the structural antibody database (SAbPred) relies on sequence homology to empirically known structures for prediction. This method can be applied to create antibody or nanobody models. Antigen receptor numbering and receptor classification (ANARCHY) align the heavy and light chain sequences. Finally, SAbDab independently selects the most appropriate VH and VL domain templates. Using angle, predict VH-VL orientation and set the template domains in the projected posture. FREAD has been demonstrated to generate accurate results with a selected model of complementarity-determining regions (CDR) loops, independent of loop length. In particular, the antibody side-chain was predicted using the position-dependent antibody rotamer swapper (PEARS), and the final models were renumbered using ANARCHY. Following the estimated model accuracy category, identifying potential development difficulties, and examining various sections of the structure standardized the end cards of the structural annotation. Secondly, ABpredict2 developed an antibody prediction. According to Chothia numbering, models are numbered and not divided into light and heavy chains. A Monte Carlo search was used to find combinations of low-energy backbone conformations to build precise and unstrained antibody structures. The RMSD estimations for sequences with greater than 90% identity were determined

using the entire set of complete dataset sequences using TM-align.

Protein-to-protein interactions and structures validation

The protein-to-protein docking server ClusPro 2.0 [37], based on the PIPER2 docking tool, was used to conduct initial antigen and monoclonal antibodies [15]. Developing a more effective docking algorithm for the prediction of antibody-protein antigen complexes is a critical initial step in the development of biologics and vaccines. PIPER is an FFT-based docking tool with a structure-based pairwise potential as one of its energy functions. Shape complementarity, electrostatic interaction, and desolvation contributions contribute to the total energy. Models are considered hits if the ligand of antigen atoms within 10 Å of the monoclonal antibody receptor is within 10 Å RMSD, disregarding the mobility of the ligand components. Our receptor and ligand potential docking was included as a pairwise component of the energy function used in PIPER, followed by clustering of the top 1000 findings, [15] as implemented in our protein–protein docking server ClusPro.

Prediction of protein–protein interaction interface hot spots

The amino acid residues in proteins' binding regions are not uniform and contain crucial residues known as hot spots. Because developing therapeutic agents that will bind to hot spots may disrupt a protein–protein interaction, hot spots are the main target of therapeutic medicines. We forecast hot spots for PRISM Concepts' antigen and antibodies [5, 36]. Alanine scanning mutagenesis is used to determine hot spots in the lab. These residues are classified as hot spots if the contribution of the altered residue to the binding is more significant than 2.0 kcal/mol. Both residues were classified as interfaced if the distance between any two atoms between them was smaller than their sum of van der Waals radii + 0.5 Angstrom. Interfaces with less than ten residues for the allocated interface were considered a result of crystal complexes, a randomly chosen quantity to reflect the contact's minimum.

Results

Antibody structural annotation of CDR's for RBD and estimated accuracy

ANARCI has assigned our targeted antibody sequence a Chothia number. Chothia-aligned the numbered sequences with over 2000 high-quality antibodies from SAbDab with known structures. For each dataset sequence from an Ig-seq, the alignment finds the most appropriate structural templates for the entire variable region and framework. If any are found, FREAD determines the suitable template structures for the CDRs. To obtain a more precise structural interpretation of the Ig-seq CDRs, CDRs are germline. FREAD did not find any direct matches to the PDB, instead of projecting the loop conformation based on the antibody sequence's target. More than half of non-H3 CDR sequences can be structurally predicted with a high degree of certainty. On a large scale, it appears to duplicate the canonical form phenomena of non-H3 CDRs. There were only a few direct alignments with PDB sequences for H3 loops, so only 35% of the non-redundant loops and 75% of the redundant loops could be modeled (Table 1).

The physicochemical configuration of the molecule controls the specificity and affinity of an antibody. Antibody repertoires with binding shape biases reveal the immune system's strategies for dealing with every RBD antigen. For example, sequence similarities can imply shared RBD antigen specificity. The physical properties of the antigen can also indicate its specificity. Paratopes were discovered by CDRs, indicating that our antibody may be RBD antigen-specific. With more paired Ig-seq datasets, the entire Fv regions were able to follow the complete structural framework from these data, yielding an accurate structural label. VH (H1, H2, H3) and VL (H1, H2, H3) were modelled instead of separate heavy and light chains (Fig. 2). We used our monoclonal antibody for targeted RBD of specific antigenic epitope annotations in immunodiagnostics to find antibody-markers of known viral diseases.

As a result, employing structural data to investigate the immune system's diversity opens up new research opportunities. We conducted a study to test the accuracy of the methods being evaluated. Based on the results, each model region was predicted correctly. 75% of VH framework structures have a backbone RMSD of 1 or less, with a sequence identity of 80 ± 2.5%. Consequently, we have 75% confidence

Table 1 Antibody Fv region of VL–VH domains

Fv region	Template PDB (Chain)	Selection method	Score
VH framework	A	Sequence identity	0.9
CDR H1	A	CDR specific fread	71
CDR H2	A	CDR specific fread	40
CDR H3	A	CDR specific fread	25
VL framework	B	Sequence identity	0.97
CDR L1	B	CDR specific fread	65
CDR L2	B	CDR specific fread	43
CDR L3	B	CDR specific fread	47
VH–VL orientation	A&B	Same as VH and VL	0.94

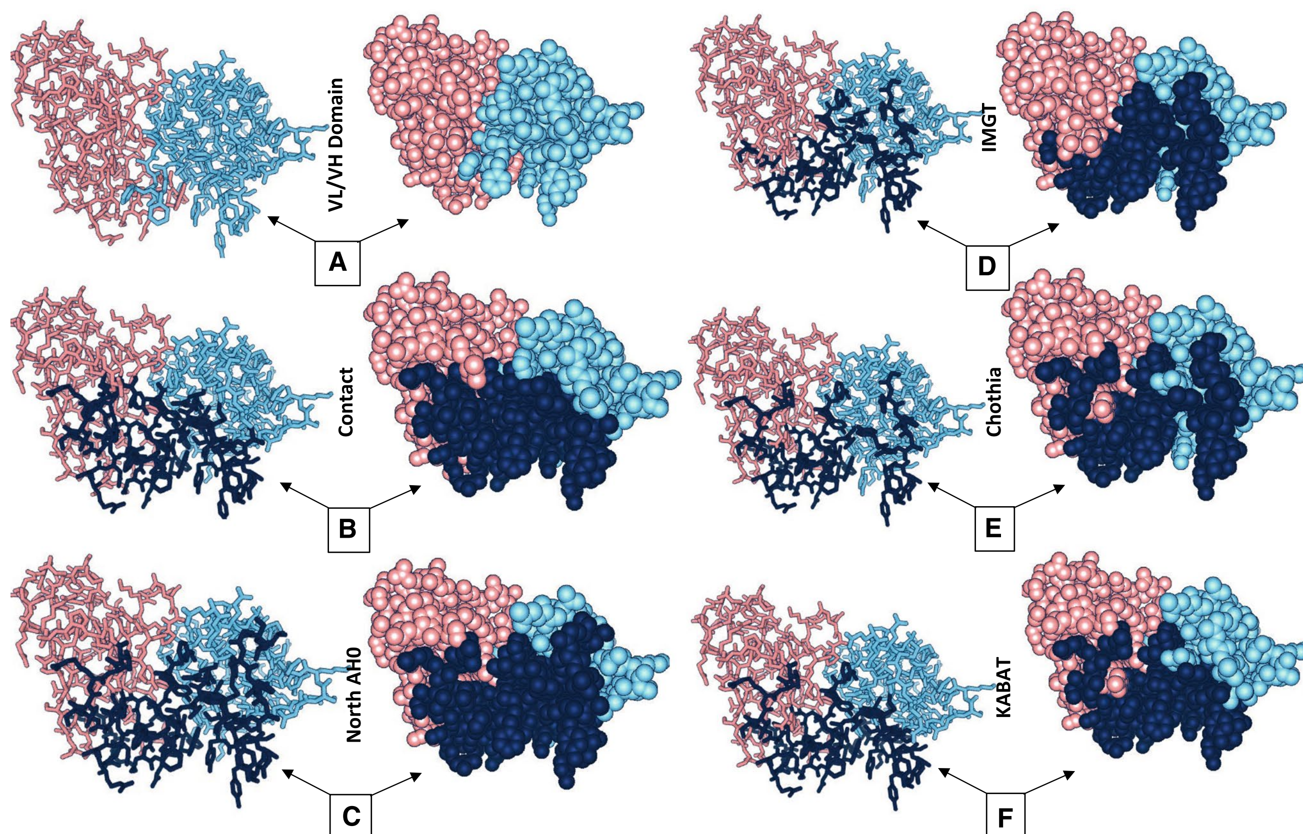


Fig. 2 The antibody CDR's determined structural and classifications. **A** The antibody consisted of two domains VH/VL. **B** Highlighted antibody CDRs (VL/VH) residues color blue are most likely to contact the RBD antigen. **C** North AH0 are clustering of antibody CDRs (VL/VH) loop conformations residues highlighted in blue with high B-factors and high conformational energies. **D** IMGT are filtered to include antibody variable domain sequences that are conserved residues color in blue CDRs (VL/VH) it is derived from EMBL-ENA, **E**, **F** Chothia and KABAT were annotated with crucial information such as CDRs (VL/VH) of potential post-translational modifications of residues to be highlighted color in blue and decisions to be made on which mutations to be acceptable

that the VH framework was modelled with 1 RMSD based on our antibody sequence, which shares 80% of the target's sequence identity. Another tool, AbPredict, was used to create a novel antibody to assess the binding affinity between VH and VL. For this modeling, we employed a random mix of four backbone fragments: two for the VL and VH, which respectively comprise CDRs 1 and 2 and the light and heavy chain framework sections, and two for LCDR3 and HCDR3. Our antibody sequence was used to simulate annealing across all conformational degrees of freedom using our backbone pieces. A combination of sidechain packing, sidechain minimization, and backbone minimization was used to determine the level of backbone confirmation before each phase of conformation. The lowest-energy structure collected during the journey is found at the end of each trajectory. In the AMA-II blind monoclonal antibody structure modeling benchmark, the accuracy of antibody Fv regions and stereochemical strain in models resulted in eight antibodies (Fig. 3). According to both systems, the most suitable antibody deviated by 1.2 rmsd across backbone-carbonyl atoms and exhibited a stereochemical quality characteristic of structures with resolutions of 1.2. After clustering the 500 models based on carbonyl rmsd, we chose the structures with the lowest energy among the three top clusters.

Antigenic epitope of SARS-nCoV2-Spike-S2-RBD binding interaction with monoclonal antibody

The SARS-nCoV-2 variety was mirrored by the variable spike proteins (S proteins), which have evolved into forms with different receptor contacts and reactions to distinct environmental triggers of virus-cell membrane fusion engaging with the hACE2 receptor specifically affected human pulmonary epithelial cells. The recombinant Spike protein was able to attach to the hACE2 recombinant protein. The S1's N-terminal domain (NTD) and C-terminal domain (CTD) are both receptor-binding domains (RBDs) that bind a variety of proteins and sugars. The S2 domain of a class I viral fusion protein was typical. Heptad repeats are a type of heptapeptide with hydrophobic residues that

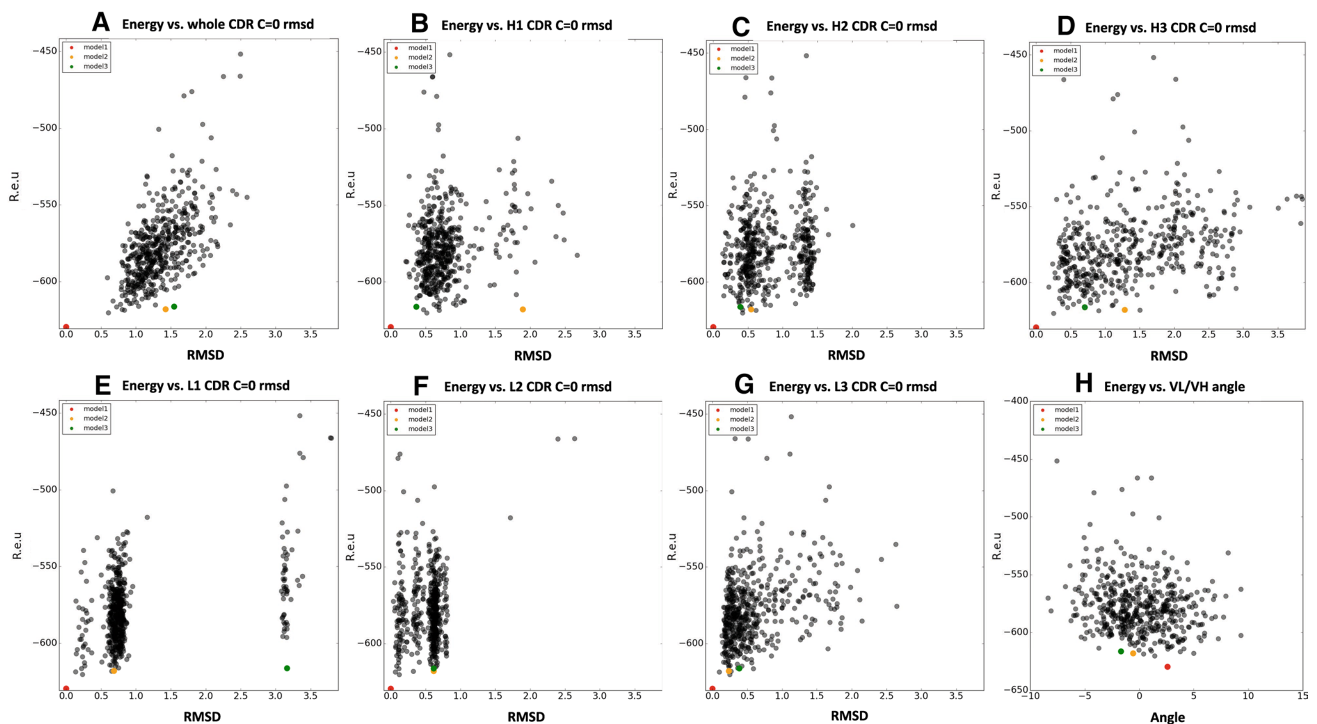


Fig. 3 The antibody structure and altered energy minimization. The accuracy and stereochemical strain in models produced for eight query antibodies that were part of the antibody modeling assessment (AMA-II) blind benchmark of antibody structure modeling manners. Models exhibited the stereochemical quality of structures at resolutions < 1.2 Å by clustered by carbonyl rmsd and the lowest-energy structures resulting from top-eight models Fv regions were in CDR C = 0 H1, H2, H3, CDR C = 0 L1, L2, L3, and aligned with CDRs and antibody VL/VH model complex

aid in the formation of coiled coils and play a role in the fusion process. Human ACE2 complexed with Coronavirus S generated a fissionable picture of the virus with two subdomains: The RBD and the core structure. The core of the SARS-CoV-2 RBD is made up of twisted five-stranded antiparallel sheets (1, 2, 3, 4, and 7) (Fig. 4A–C) with short connecting helices and loops. There is a prolonged insertion between the 4 and 7 strands in the core, containing the short 5 and 6 strands, 4 and 5 helices, and loops. This prolonged insertion is known as the RBM, and it includes the majority of SARS-contacting CoV-2's residues that bind to hACE2. The RBD has nine cysteine residues, eight of which form four disulfide linkages that are resolved in the final model. Three of these four pairings are in the core (Cys336–Cys361, Cys379–Cys432, and Cys391–Cys525) and assist to support the sheet structure. The fourth pair (Cys480–Cys488) connects the loops at the RBM's distal end. The peptide substrate-binding site is formed between the two lobes of hACE2's N-terminal peptidase domain.

There are two peptidase domains on the ectodomain of hACE2. One is epithelial, the other membrane-proximal. The RBD of the S protein contains several conformation-dependent epitopes and the primary domain that produces neutralizing antibodies. Using liner antigenic epitopes, we discovered extensive areas of antigenic epitopes of small peptides, even single amino acids, in the RBD (SVMTrip). It consisted of four epitopes: [DDVRQIAPGQTGVI], [NIDATSTGNYN], [YQAGSTPCNGV], and [YGFQPTNGVGYQ], all of which are antigenic characteristics that interact directly with monoclonal antibodies (Table 2). The computed antibody value was − 12.1 kcal/mol with two amino acids due to the high rate of binding affinity between RBD and antibody. The RBD residues Asn479 and Thr487 are required for the high-affinity overtone associating S protein with hACE2. RBD mutations alter the antigenic structure and binding activity of RBD to hACE2 at Arg441 and Asp454. As a result of electrostatic complementarity, we improved the affinity of monoclonal antibodies for the RBD antigen. Amino acids on the hydrophobic surface patch that directly contact the antigenic epitope were changed to achieve this. We found four RDB epitope regions. However, only three epitopes were most efficient in monoclonal antibody engagement (Fig. 5). Because this monoclonal antibody interacted with RBD regions in the three best-ranked ones, it was critical to our goal. The RBD region identical to the hACE2 receptor binds to it and neutralizes infection. Antigenic epitope binding hotspot residues of the RBD (Pro38-Val505,

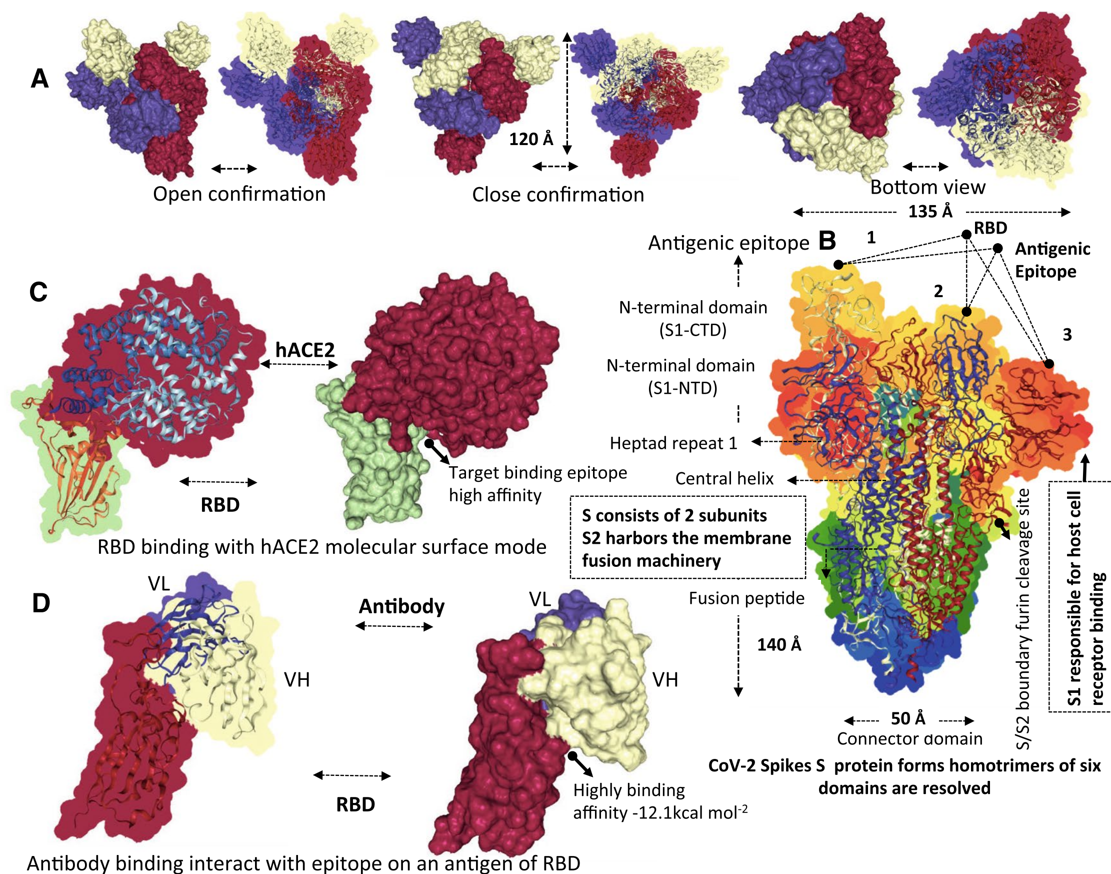


Fig. 4 SARS-nCoV-2-Spike-S1 protein structure classification and antigen–antibody interface interactions. **A** The structures' molecular surface and ribbon views are shown with different conformation, i.e. (open and close view diameter 120 Å, top angle 135 Å and side 140 Å). **B**, **C** The ectodomain of nCoV2 spike proteins consists of two domains: an N-terminal domain named S1 responsible for receptor binding and a C-terminal S2 domain responsible for fusion. The spike S1 protein was a class I fusion protein α-helical coiled-coil structure characteristic of this class of fusion protein, which contains in its C-terminal part regions predicted to have an α-helical secondary structure and to form coiled-coils with Horizontal angle 50 Å, and perpendicular angle. The S2 subunit was the most conserved region of the protein, whereas the S1 subunit diverges in a sequence of a single coronavirus. The S1 contains two subdomains, an N-terminal domain (NTD) and a C-terminal domain (CTD). Both can function as receptor binding domains (RBDs) and bind various proteins and sugars. The nCoV diversity was reflected in the variable spike proteins (S proteins), which have evolved into forms differing in their receptor interactions and their response to various environmental triggers of virus to cell membrane fusion. It's been infecting human respiratory epithelial cells through interaction with the hACE2 receptor (red) and RBD (green). Indeed, the recombinant spike protein binds with recombinant hACE2 protein. **D** Antibody VL (blue)/VH (half white) binding interaction to the specific epitope with the RBD (red) which interact binding affinity was – 12.1 kcal/mol

Table 2 Antigenic epitopes prediction to the RBD

Target protein	Start	End	Antigenic epitopes regions	Length
Receptor binding domain (RBD)	347	347	F	1
	402	402	V	1
	405	418	DDVRQIAPGQTGVI	14
	423	425	YKL	3
	441	451	NIDATSTGNYN	11
	461	163	LKP	3
	466	467	RD	2
	469	469	S	1
	473	483	YQAGSTPCNGV	11
	495	506	YGFQPTNGVGYQ	12

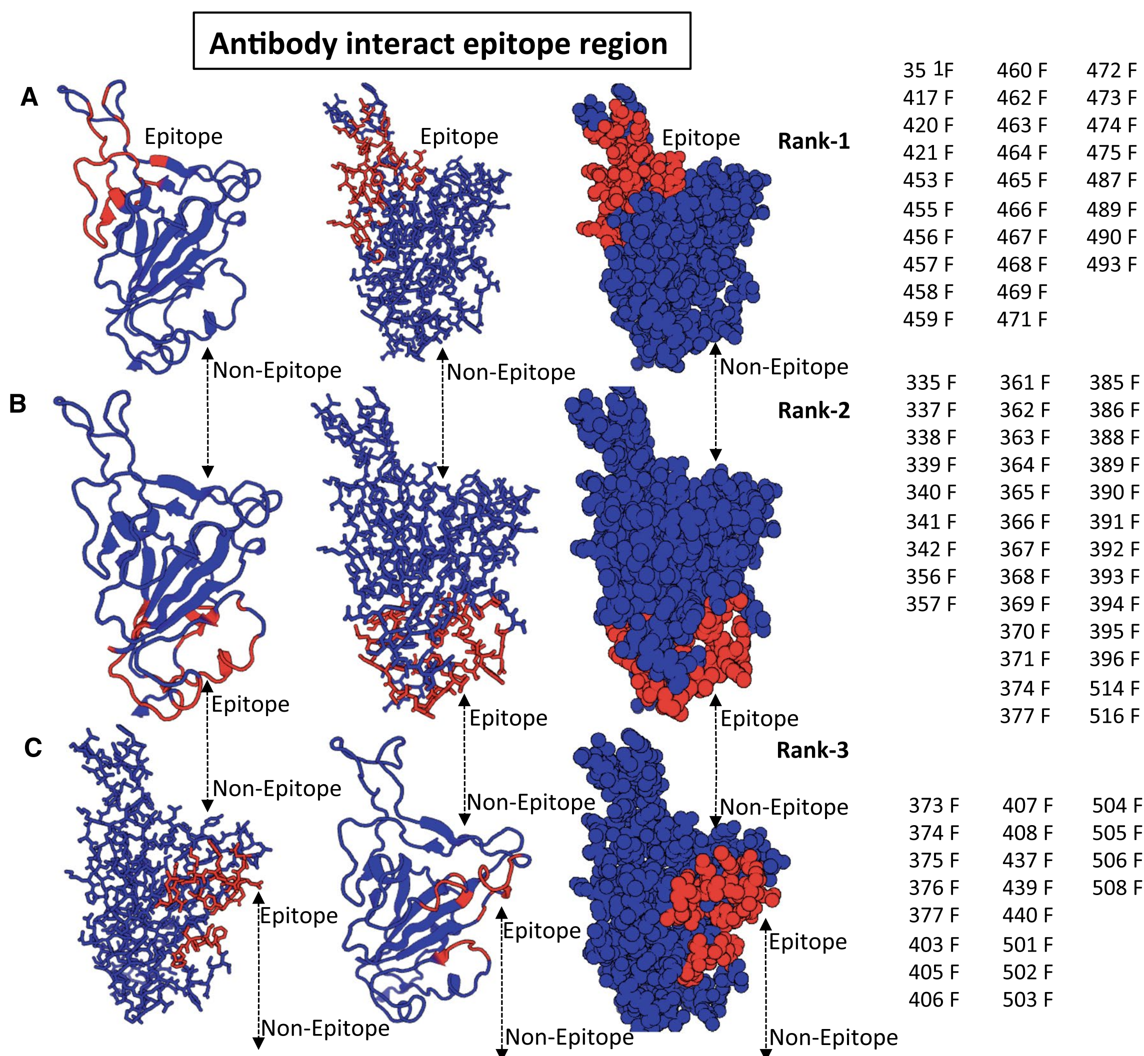


Fig. 5 Interactions interface between antigenic epitopes and antibody binding site. The epitope was a consecutive fragment from the amino acid sequence and composed of several fragments scattered along the amino acid sequence, forming the RBD-binding interface's antigenic regions. Such predictions based on amino acid properties included hydrophobicity, solvent accessibility, secondary structure, flexibility, and antigenicity. **A** Three antigenic epitope regions were predicted based on rank1, which was highlighted color in red to direct contact of RBD to the antibody with interacting high binding affinity. The other blue are non-epitope. **B** A rank2 antigenic epitope predicted regions are (red: epitope and blue: non-epitope) present at a lower RBD. **C** A rank3 antigenic epitope was predicted regions are (red: epitope and blue: non-epitope) which are conserved at the forward-facing portion of the RBD to interact with antibody. **A–C** In all the RBD antigenic epitope segments, specific amino acids are listed near the protein structure model

Tyr58-Asn437, Phe109-Val503, Phe109-Gly502, Phe110-Gly502, Phe110-Tyr505, Phe109-Tyr505, Phe109-Tyr505, Phe109-Tyr505, Phe109-Tyr505, Phe109-Tyr505, Phe109-T (Table 3). Due to the lower side-chain conformational entropy barrier across the epitope-paratope interface, direct hydrogen bonds across these interfaces may contribute to the antibody's antigen recognition selectivity. The interaction works best with hydrophilic side chains that have short chains.

Optimizing lead antibody affinity, specificity, and stability

Instead of identifying an in vitro selection of antibodies with better properties under more stringent conditions, the different VH and VL domains produced an antibody with a specified antigen specificity (> 108 enhanced variety). Complimentary determining regions of monoclonal antibodies (CDRs) located inside the variable VL/VH chains regulate essential antibody properties such as affinity and specificity. Monoclonal antibodies (CDR) differ in their specific binding

Table 3 Interacting residues in between monocular antibody and RBD

Interacting VL and LH chain resName_resNo				Interaction view	Interacting RBD resName_resNo			
Protein No.	Chain	Amino acid	Position		Protein No.	Chain	Amino acid	Position
PDB1	H	PRO	38	<-->	PDB2	F	VAL	503
	L	GLU	109	<-->		F	GLN	498
	H	TYR	58	<-->		F	ASN	437
	L	GLU	109	<-->		F	THR	500
	H	PHE	109	<-->		F	VAL	503
	H	PHE	109	<-->		F	GLY	502
	H	PHE	110	<-->		F	GLY	502
	H	PHE	110	<-->		F	TYR	505
	H	PHE	109	<-->		F	TYR	505
	H	TYR	64	<-->		F	ARG	439
	L	ASN	108	<-->		F	THR	500
	L	TRP	114	<-->		F	THR	500
	H	TYR	64	<-->		F	ASN	440
	H	SER	57	<-->		F	ARG	439
	H	PHE	109	<-->		F	GLY	504
	H	ASN	113	<-->		F	THR	500
	H	TYR	66	<-->		F	ARG	439
	H	ASN	113	<-->		F	GLY	502
	H	TYR	66	<-->		F	PRO	499

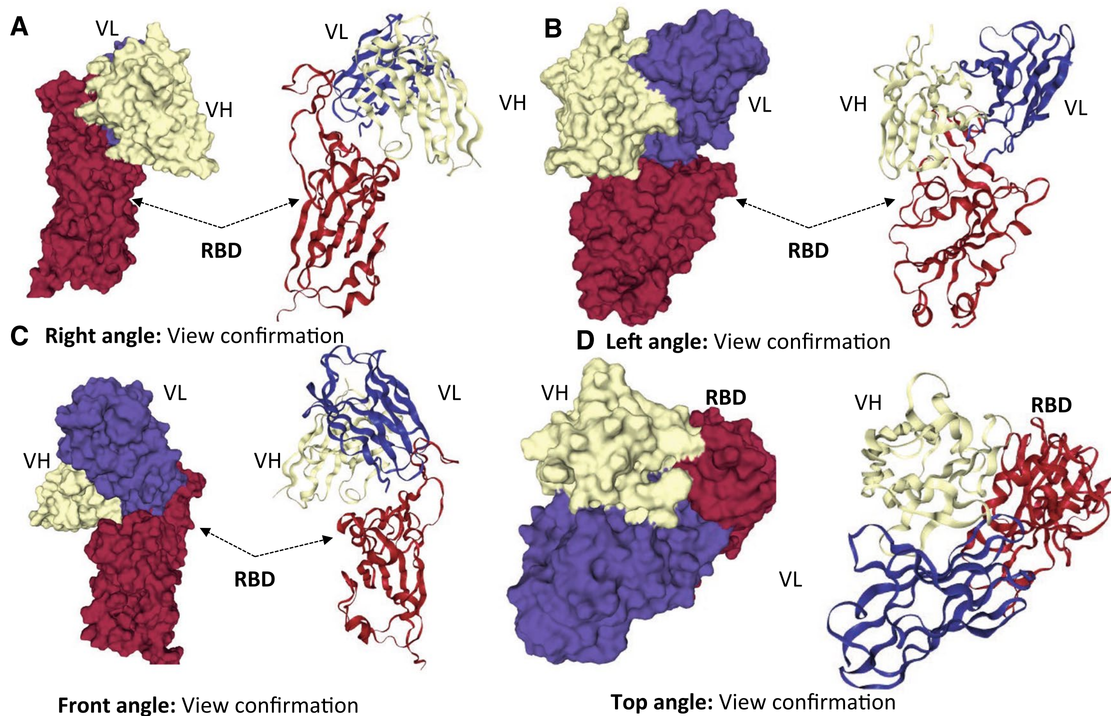


Fig. 6 SARS-nCoV-2 of RBD interactions with antibody This antibody that binds the RBD of SARS-CoV-2 challenged the same antigenic epitope sites that bind the human receptor ACE2. **A–D** The antibody interacts with an antigenic epitope of a specific target and binds to the RBD as shown by different orientations made on the molecular surface and ribbon style (red: RBD, half white-VH/blue-VL)

properties. Antibodies are necessary for both cell-mediated complement and cytotoxicity. We created a library of different RBD-monoclonal antibody interfaces. These structures contain a two-protein complex, one in "RBD: Chain F" and the other in VH: "Chain H" and "Chain L." The interaction hotspot interface, which has a conserved core, connects the proteins (Fig. 6). Simulations of protein complex interactions between monoclonal antibodies and RBD were used to shape the side chain hotspot regions to improve RBD's target chain affinity. Proteins interacting through a three-loop interface provide this effect. The amino acid positions of areas that interact with monoclonal antibodies on-chain F (RBD) were 473–483 for YQAGSTPCNGV, 441–451 for NIDATSTGNYN 495–506 for YGFQPTNGVGYQ. Two of these regions, 473–483: YQAGSTPCNGV and 441–451: NIDATSTGNYN, encode a good deal of the binding affinity and peripheral contacts where there is incompatibility with other molecules. Furthermore, the perimeter of the binding contact contributes significantly to specificity. Backbone designs preserved structural geometry would allow the rest of our complex proteins, including the essential hotspot, to conform to the native conformation, maintaining high-affinity binding. We adopted the modularity of binding interfaces while preserving the interaction hotspot and optimizing other interfacial regions' rigid-body orientation and sequence for further confirmation. Immunoglobulins' light and heavy domains are linked. Specific residues in our monoclonal antibody sequence can be connected to these. A conserved framework that provides biochemical and structural stability supports CDRs. The CDRs were used to test structural diversity in the Fv framework region, which differed in length, backbone conformation, and amino acid sequence. The rigid-body orientation of the light chain with the heavy chain is another factor that influences the conformation of the RBD antigen-binding site. It was also challenging to balance the ionic salt's various ways with protein molecules, shielding charged solvent-exposed residues and possibly decreasing protein–protein long-range electrostatic interactions.

Discussions

The Spike's receptor-binding domain defines a coronavirus's ability to infect a host species and its tropisms. The Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) is SARS-closest CoV-2's human-infecting relative, with a sequence identity of around 79%. The spike proteins have 76.47% sequence identical, while the RBDs have a somewhat lower (73%) sequence identity [45]. The C-terminal region of SARS-193 CoV-2's residues RBD, which contains the receptor-binding motif, has a significant concentration of mutations [11]. The RBD of S1 is responsible for virus binding to host cell receptors [20, 41]. The vaccines may not work well in the older population [16, 23, 24]. Another concern is that antibodies have detected several SARS-nCoV-2 proteins (S, E, M, and N) [10, 30, 40, 50]. However, antibodies to the S protein are the most common [27, 31, 47], suggesting that setting off specific immune responses modulates receptor binding and membrane fusion [8]. Other cell types with hACE2 are alveolar epithelial cells, enterocytes on the surface of the small intestine, and endothelial cells in the heart and kidney [33]. Because it is involved in hACE2 receptor recognition, viral attachment, and entry, the S protein of SARS-nCoV-2 is one of the most important targets for treating SARS-nCoV-2 vaccines and treatments. The spike protein was chosen as a potential target for monoclonal antibodies to stop virus infection by disrupting the RBD-hACE2 interaction in this study. During each conformational phase of antigen processing, combined sidechain packing reduces structural strain by selecting the antibody's backbone based on the model of a pre-computed dataset [17]. As part of the AMA-II blind benchmark of antibody structure modeling approaches, we assessed the accuracy and stereochemical strain in models developed for eight antibodies [1]. For structures with resolutions of 1.2 rmsd, the stereochemical quality of our top models was as expected the 500 antibody models clustered using carbonyl rmsd and the lowest-energy structures from the top-three clusters. Our monoclonal antibody is attached to a particular antigenic epitope on the RBD in a highly interactive manner [22, 28, 35]. During interaction with the receptor, RBD provides a concave surface for the N terminus of the receptor peptidase, on which amino acids 441–451: NIDATSTGNYN broadcast the whole receptor-binding loop RBD core. The RBD loop amino acids 473–483 are as follows: The antibody is directly contacted by YQAGSTPCNGV. The RBD region also contains many cysteine residues linked by di-sulphide linkages [19, 21]. The amino acid sequences at positions 441–451 and 473–483, in particular, are essential in determining the severity of SARS-nCoV-2 infection [19, 21]. Any changes in these two locations could lead to animal-to-human and human-to-human transfer [2]. According to RBD structure, residue 498 GLN in SARS-nCoV-2 was discovered particularly close to the virus-binding hot spot 500 Thr (i.e., hot spots 109Glu and 110 Phe) on the antibody. In Hot Spot 353, which is also buried in a hydrophobic environment, a salt bridge joins Lys353 and Asp38 [43]. The RBD is required for the S protein to bind with hACE2 with high affinity [19, 21]. A point mutation disrupts RBD's antigenic structure and binding ability to hACE2 at Arg441, Asp454 in the RBD [13]. This improved interaction across the VL–VH interface led to significant improvements in crucial parameters for antibody development, such as impressibility, stability, and affinity, which are capable of interacting with the antigenic epitope to the RBD, which is mainly determined by

the structure of the antigen-binding site. These interactions contribute the majority of the antibody binding energy to protein complex. The created changes function in tandem with surface mutations detected using standard antibodies to improve affinity and stability. Finally, this study provides a fully established monoclonal antibody model and a mixture of critical capabilities for developing SARS-nCoV-2 RBD therapeutics that ultimately prevent viral infection.

Limitation and future directions

Overall, we successfully designed a new monoclonal antibody based on the templates PDB's: 6dkj (chain A) and 4cni (chain A) are both VH–VL orientation complexes for a novel Coronavirus based on S protein to target specific antigenic epitopes to the RBD to inhibit the host viral entry. Future studies to understand immunologic factors and antibody pathways associated with the successful development of antibodies may provide insights for the design of improved immunogens and immunization strategies.

Acknowledgements The authors would like to thank the Deanship of Scientific Research at Umm Al-Qura University for supporting this work by Grant Code: (22UQU4340573DSR01).

Availability of data and materials All data analyzed during this study are included in this article.

Declarations

Conflict of interest All authors: No reported conflicts of interest. All authors have submitted form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

Disclaimer The study sponsor had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The first and corresponding author's had full access to all study data and had final responsibility for the decision to submit for publication.

Ethical approval This article does not contain any studies with human or animal subjects performed by any of the authors.

References

1. Almagro JC, Teplyakov A, Luo J, Sweet RW, Kodangattil S, Hernandez-Guzman F et al (2014) Second antibody modeling assessment (AMA-II). Proteins 82(8):1553–1562. https://doi.org/10.1002/prot.24567
2. Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MCJ, van Genderen PJJ, Goorhuis A et al (2017) Import and spread of extended-spectrum β-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis 17(1):78–85. https://doi.org/10.1016/S1473-3099(16)30319-X
3. Alghamdi AA (2021) Impact of the COVID-19 pandemic on the social and educational aspects of Saudi university students' lives. PLoS ONE 16(4):e0250026. https://doi.org/10.1371/journal.pone.0250026
4. Alshareef M, Alotiby A (2021) Prevalence and perception among Saudi Arabian population about resharing of information on social media regarding natural remedies as protective measures against COVID-19. Int J Gen Med 14:5127–5137. https://doi.org/10.2147/IJGM.S326767
5. Baspinar A, Cukuroglu E, Nussinov R, Keskin O, Gursoy A (2014) PRISM: a web server and repository for prediction of protein–protein interactions and modeling their 3D complexes. Nucleic Acids Res 42(Web Server issue):W285–W289. https://doi.org/10.1093/nar/gku397
6. Choudhury A, Das NC, Patra R, Mukherjee S (2021) In silico analyses on the comparative sensing of SARS-CoV-2 mRNA by the intracellular TLRs of humans. J Med Virol 93(4):2476–2486. https://doi.org/10.1002/jmv.26776
7. Choudhury A, Das NC, Patra R et al (2021) Exploring the binding efficacy of ivermectin against the key proteins of SARS-CoV-2 pathogenesis: an in silico approach. Future Virol. https://doi.org/10.2217/fvl-2020-0342.10.2217/fvl-2020-0342
8. Cui J, Li F, Shi ZL (2019) Origin and evolution of pathogenic coronaviruses. Nat Rev Microbiol 17(3):181–192. https://doi.org/10.1038/s41579-018-0118-9
9. Das NC, Chakraborty P, Bayry J, Mukherjee S (2021) Analyses on the comparative potential of therapeutic human monoclonal antibodies against newly emerged SARS-CoV-2 variants bearing mutant spike protein. Front Immunol 12:782506. https://doi.org/10.3389/fimmu.2021.782506
10. Du L, Tai W, Zhou Y, Jiang S (2016) Vaccines for the prevention against the threat of MERS-CoV. Expert Rev Vaccines 15(9):1123–1134. https://doi.org/10.1586/14760584.2016.1167603
11. Faria NR, Mellan TA, Whittaker C, Claro IM, Candido DDS, Mishra S, et al (2021) Genomics and epidemiology of a novel SARS-CoV-2 lineage in Manaus, Brazil. medRxiv. https://doi.org/10.1101/2021.02.26.21252554
12. Johargy A (2009) Seroprevalence of erythrovirus B19 IgG antibody among paediatric patients in Makkah and Jeddah, Kingdom of Saudi Arabia. Med Princ Pract 18(4):339–341. https://doi.org/10.1159/000215736
13. He Y, Li J, Jiang S (2006) A single amino acid substitution (R441A) in the receptor-binding domain of SARS coronavirus spike protein disrupts the antigenic structure and binding activity. Biochem Biophys Res Commun 344(1):106–113. https://doi.org/10.1016/j.bbrc.2006.03.139
14. Jespersen MC, Peters B, Nielsen M, Marcatili P (2017) BepiPred-2.0: improving sequence-based B-cell epitope prediction using conformational epitopes. Nucleic Acids Res 45(W1):W24–W29. https://doi.org/10.1093/nar/gkx346
15. Kozakov D, Brenke R, Comeau SR, Vajda S (2006) PIPER: an FFT-based protein docking program with pairwise potentials. Proteins 65(2):392–406. https://doi.org/10.1002/prot.21117
16. Krammer F (2020) Pandemic vaccines: how are we going to be better prepared next time? Med (N Y) 1(1):28–32. https://doi.org/10.1016/j.medj.2020.11.004
17. Lapidoth GD, Baran D, Pszolla GM, Norn C, Alon A, Tyka MD et al (2015) AbDesign: an algorithm for combinatorial backbone design guided by natural conformations and sequences. Proteins 83(8):1385–1406. https://doi.org/10.1002/prot.24779
18. Li F (2016) Structure, function, and evolution of coronavirus spike proteins. Annu Rev Virol 3(1):237–261. https://doi.org/10.1146/annurev-virology-110615-042301
19. Li F, Li W, Farzan M, Harrison SC (2005) Structure of SARS coronavirus spike receptor-binding domain complexed with receptor.

Science 309(5742):1864–1868. https://doi.org/10.1126/science.1116480
20. Li W, Moore MJ, Vasilieva N, Sui J, Wong SK, Berne MA et al (2003) Angiotensin-converting enzyme 2 is a functional receptor for the SARS coronavirus. Nature 426(6965):450–454. https://doi.org/10.1038/nature02145
21. Li W, Zhang C, Sui J, Kuhn JH, Moore MJ, Luo S et al (2005) Receptor and viral determinants of SARS-coronavirus adaptation to human ACE2. EMBO J 24(8):1634–1643. https://doi.org/10.1038/sj.emboj.7600640
22. Liu L, Wang P, Nair MS, Yu J, Rapp M, Wang Q et al (2020) Potent neutralizing antibodies against multiple epitopes on SARS-CoV-2 spike. Nature 584(7821):450–456. https://doi.org/10.1038/s41586-020-2571-7
23. Lu L, Zhong W, Bian Z, Li Z, Zhang K, Liang B et al (2020) A comparison of mortality-related risk factors of COVID-19, SARS, and MERS: a systematic review and meta-analysis. J Infect 81(4):e18–e25. https://doi.org/10.1016/j.jinf.2020.07.002
24. Lu R, Zhao X, Li J, Niu P, Yang B, Wu H et al (2020) Genomic characterisation and epidemiology of 2019 novel coronavirus: implications for virus origins and receptor binding. Lancet 395(10224):565–574. https://doi.org/10.1016/S0140-6736(20)30251-8
25. Ng OW, Chia A, Tan AT, Jadi RS, Leong HN, Bertoletti A et al (2016) Memory T cell responses targeting the SARS coronavirus persist up to 11 years post-infection. Vaccine 34(17):2008–2014. https://doi.org/10.1016/j.vaccine.2016.02.063
26. Okba NMA, Müller MA, Li W, Wang C, GeurtsvanKessel CH, Corman VM et al (2020) Severe acute respiratory syndrome coronavirus 2-specific antibody responses in coronavirus disease patients. Emerg Infect Dis 26(7):1478–1488. https://doi.org/10.3201/eid2607.200841
27. Pang H, Liu Y, Han X, Xu Y, Jiang F, Wu D et al (2004) Protective humoral responses to severe acute respiratory syndrome-associated coronavirus: implications for the design of an effective protein-based vaccine. J Gen Virol 85(Pt 10):3109–3113. https://doi.org/10.1099/vir.0.80111-0
28. Pinto D, Park YJ, Beltramello M, Walls AC, Tortorici MA, Bianchi S et al (2020) Cross-neutralization of SARS-CoV-2 by a human monoclonal SARS-CoV antibody. Nature 583(7815):290–295. https://doi.org/10.1038/s41586-020-2349-y
29. VPatra R, Das NC, Bhattacharya M, Shit PK, Patra BC, Mukherjee S (2021) Applications of artificial intelligence (AI) protecting from COVID-19 pandemic: a clinical and socioeconomic perspective. EAI/Springer innovations in communication and computing. Springer, Cham, pp 45–60
30. See RH, Petric M, Lawrence DJ, Mok CPY, Rowe T, Zitzow LA et al (2008) Severe acute respiratory syndrome vaccine efficacy in ferrets: whole killed virus and adenovirus-vectored vaccines. J Gen Virol 89(Pt 9):2136–2146. https://doi.org/10.1099/vir.0.2008/001891-0
31. Shi SQ, Peng JP, Li YC, Qin C, Liang GD, Xu L et al (2006) The expression of membrane protein augments the specific responses induced by SARS-CoV nucleocapsid DNA immunization. Mol Immunol 43(11):1791–1798. https://doi.org/10.1016/j.molimm.2005.11.005
32. Shatla M, Alharthi BT, Alharbi AG, Khan ZA, Althaqfi AA, Babkoor AA et al (2021) The impact of the COVID-19 pandemic on family medicine practices in Saudi Arabia. Cureus 13(12):e20437. https://doi.org/10.7759/cureus.20437
33. Sims AC, Baric RS, Yount B, Burkett SE, Collins PL, Pickles RJ (2005) Severe acute respiratory syndrome coronavirus infection of human ciliated airway epithelia: role of ciliated cells in viral spread in the conducting airways of the lungs. J Virol 79(24):15511–15524. https://doi.org/10.1128/JVI.79.24.15511-15524.2005
34. Tang F, Liu W, Zhang F, Xin ZT, Wei MT, Zhang PH et al (2008) IL-12 RB1 genetic variants contribute to human susceptibility to severe acute respiratory syndrome infection among Chinese. PLoS ONE 3(5):e2183. https://doi.org/10.1371/journal.pone.0002183
35. Tian X, Li C, Huang A, Xia S, Lu S, Shi Z et al (2020) Potent binding of 2019 novel coronavirus spike protein by a SARS coronavirus-specific human monoclonal antibody. Emerg Microbes Infect 9(1):382–385. https://doi.org/10.1080/22221751.2020.1729069
36. Tuncbag N, Gursoy A, Nussinov R, Keskin O (2011) Predicting protein-protein interactions on a proteome scale by matching evolutionary and structural similarities at interfaces using PRISM. Nat Protoc 6(9):1341–1354. https://doi.org/10.1038/nprot.2011.367
37. Vajda S, Yueh C, Beglov D, Bohnuud T, Mottarella SE, Xia B et al (2017) New additions to the ClusPro server motivated by CAPRI. Proteins 85(3):435–444. https://doi.org/10.1002/prot.25219
38. Varghese R, Mikyas Y, Stewart PL, Ralston R (2004) Postentry neutralization of adenovirus type 5 by an antihexon antibody. J Virol 78(22):12320–12332. https://doi.org/10.1128/JVI.78.22.12320-12332.2004
39. Wallraven K, Holmelin FL, Glas A, Hennig S, Frolov AI, Grossmann TN (2020) Adapting free energy perturbation simulations for large macrocyclic ligands: how to dissect contributions from direct binding and free ligand flexibility. Chem Sci 11(8):2269–2276. https://doi.org/10.1039/c9sc04705k
40. Wang N, Shang J, Jiang S, Du L (2020) Subunit vaccines against emerging pathogenic human coronaviruses. Front Microbiol 11:298. https://doi.org/10.3389/fmicb.2020.00298
41. Wong SK, Li W, Moore MJ, Choe H, Farzan M (2004) A 193-amino acid fragment of the SARS coronavirus S protein efficiently binds angiotensin-converting enzyme 2. J Biol Chem 279(5):3197–3201. https://doi.org/10.1074/jbc.C300520200
42. Wu J, Zhang J, Sun X, Wang L, Xu Y, Zhang Y et al (2020) Influence of diabetes mellitus on the severity and fatality of SARS-CoV-2 (COVID-19) infection. Diabetes Obes Metab 22(10):1907–1914. https://doi.org/10.1111/dom.14105
43. Wu K, Peng G, Wilken M, Geraghty RJ, Li F (2012) Mechanisms of host receptor adaptation by severe acute respiratory syndrome coronavirus. J Biol Chem 287(12):8904–8911. https://doi.org/10.1074/jbc.M111.325803
44. Wu Y, Wang F, Shen C, Peng W, Li D, Zhao C et al (2020) A noncompeting pair of human neutralizing antibodies block COVID-19 virus binding to its receptor ACE2. Science 368(6496):1274–1278. https://doi.org/10.1126/science.abc2241
45. Xu X, Chen P, Wang J, Feng J, Zhou H, Li X et al (2020) Evolution of the novel coronavirus from the ongoing Wuhan outbreak and modeling of its spike protein for risk of human transmission. Sci China Life Sci 63(3):457–460. https://doi.org/10.1007/s11427-020-1637-5
46. Yao B, Zhang L, Liang S, Zhang C (2012) SVMTriP: a method to predict antigenic epitopes using support vector machine to integrate tri-peptide similarity and propensity. PLoS ONE 7(9):e45152. https://doi.org/10.1371/journal.pone.0045152
47. Zakhartchouk AN, Sharon C, Satkunarajah M, Auperin T, Viswanathan S, Mutwiri G et al (2007) Immunogenicity of a receptor-binding domain of SARS coronavirus spike protein in mice: implications for a subunit vaccine. Vaccine 25(1):136–143. https://doi.org/10.1016/j.vaccine.2006.06.084
48. Zhao J, Yuan Q, Wang H, Liu W, Liao X, Su Y et al (2020) Antibody responses to SARS-CoV-2 in patients with novel coronavirus disease 2019. Clin Infect Dis 71(16):2027–2034. https://doi.org/10.1093/cid/ciaa344

49. Zhou P, Yang XL, Wang XG, Hu B, Zhang L, Zhang W et al (2020) Addendum: a pneumonia outbreak associated with a new coronavirus of probable bat origin. Nature 588(7836):E6. https://doi.org/10.1038/s41586-020-2951-z
50. Zhou Y, Jiang S, Du L (2018) Prospects for a MERS-CoV spike vaccine. Expert Rev Vaccines 17(8):677–686. https://doi.org/10.1080/14760584.2018.1506702

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.