

RESEARCH PAPER

Molecular docking study and mapping the binding site of some antiviral nanobodies against receptor binding domain (RBD) of SARS-COV2

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ARTICLE INFO

Article History:

Received 08 January 2021

Accepted 25 February 2021

Published 15 March 2021

Keywords:

SARS-COV 2

ACE2

RBD

VHH

Molecular docking

ABSTRACT

Neutralization ability of some antiviral nanobodies was computed against the receptor binding domain (RBD) of the severe acute respiratory syndrome coronavirus 2 (SARS-COV 2). CoDockPP Server and COVID-19 Docking Server respectively was applied for a protein-protein molecular docking. The affinity of candidate nanobodies was investigated for blocking of RBD against the human angiotensin converting enzyme 2 (ACE2). The neutralization ability of nanobodies was compared with natural nanobodies of *Ty1*, *H11-H4*, *EY6A*, *H11-D4* and synthetic construct of *Sb23*, *ybody MR17*, *sybody MR17-K99Y*, and *SR4* that experimentally was involved against the RBD of SARS-COV 2. It was seen, the 15 reported VHH was able for blocking with an estimated binding energy greater than -235.55 (kcal/mol) for *Ty1* with the lowest affinity to the RBD. *VHH 7A*, *VHH PVSP29F*, *Cameld VHH 9*, *VHH PVSS8A*, *VHH 12B*, *VHH 59H10*, *VHH PVSP6A*, *VHH 10E*, *VHH 17B* and *VHH 59H10* respectively was proposed for neutralization of RBD while the two last VHH are more confidence due to the greater values of affinity against -342.56 (kcal/mol) for *SR4*. The energy maps of ACE2, *VHH 17B* and *VHH 59H10* was identified that hydrogen donor, steric, hydrogen acceptor and electrostatic interactions respectively were significant for blocking RBD of SARS-COV 2. This study conform structural insight for neutralization of RBD spike glycoprotein of SARS-COV 2 by nanobodies and suggest VHH that may serve as useful therapeutics during the pandemic.

How to cite this article

Pahlavan Yali Z., Hossein Fatemi M. Molecular docking study and mapping the binding site of some antiviral nanobodies against receptor binding domain (RBD) of SARS-COV2. *Nanochem Res*, 2021; 6(1):65-71. DOI: 10.22036/ncr.2021.01.006

INTRODUCTION

Severe acute respiratory syndrome coronavirus-2 (SARS-COV 2) pandemic is the infectious cause whose exceptional character has resulted in a serious global attempt for its diagnosis and treatment [1, 2]. The SARS-COV 2 is a member of the coronavirus family and contains the enveloped positive-sense single-stranded RNA (+ssRNA) [3]. Sore throat, fever, shortness of breath, multi-organ failure, or even death are some symptoms and outcome of SARS-COV 2 which is now called

coronavirus disease 2019 (COVID-19) [1, 2]. The metabolic pathway and mechanism of action of existing drugs targeting the key proteins of SARS-COV 2 have been evaluated and ranked in combat against this disease [4]. Among them, hydroxychloroquine (HCQ) [5-7] and Remdesivir [8-10], respective drugs of malaria [10] and Ebola [11], have been repurposed for COVID-19 therapy. Despite the approval of Remdesivir by the food and drug administration (FDA) of the United States [12], no reliable drug has been yet identified for SARS-COV 2.

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Immunoinformatics can collaborate with the structural-based drug design approaches for the COVID-19 treatment [13]. The molecular docking approach is one of the collaborations with epitope-based immunoinformatics which has revealed the applicability of the new vaccine [14-16]. This approach was employed to the structural virology and antibody-antigen recognition [17], structural validation against crystal structures of carbohydrate ligands to antibodies [18], an epitope-based peptide vaccine against DENV-NS3 protein [14], characterization of the common epitope-based peptide vaccine for Nipah and Hendra viruses [19], and a multi-epitope based subunit vaccine against the dengue infection [20]. Also, the molecular docking study of antibody-antigen complexes was applied for pitfalls illustrated by influenza hemagglutinin [21] and investigation of the protein-protein interactions in serine protease inhibitor and antibody-antigen complexes [22]. Moreover, the molecular docking and molecular dynamics (MD) simulation have been carried out for an invitro identification of human anti-complement factor H (CFH) antibody Ab42 and CFH polypeptide [23].

The application of natural drugs and vaccines for COVID-19 therapy is one of the popular trends in controlling the pandemic. It was seen, the classical plant cannabinoids Dronabinol and Epidiolex have shown various biological activities against SARS-COV2 [24]. The virtual screening of 40 natural compounds particularly employed in traditional Iranian medicine for blocking the enzymatic activity of SARS-CoV-2 3CLpro has revealed the inhibition potential of *kappacarrageenan conformer*, *beta-D-galactopyranosyl* and *calycosin 7-O-glucoside phytochemicals* [25].

Nanobody refers to a group of the single-domain antibody (sdAb) that were first engineered from heavy-chain antibodies naturally found in the camelids (called VHH fragments) [26]. These sdAbs overcome many application problems of the monoclonal antibodies in molecular imaging, diagnostic kits, and therapeutic medicines [27-29]. The nanometer-scale antibody with a molecular weight of 12-15 (kDa) and a light chain can effectively penetrate the tissues [27]. It has been confirmed that some natural [30-33] and synthetic nanobodies [34-38] have the ability to neutralize the binding of the trimeric spike glycoprotein receptor-binding domain (RBD) of SARS-COV

2 against the host receptor human angiotensin-converting enzyme 2 (ACE2) [39].

These identifications promise to evaluate the blocking ability of some nanobodies against RBD of SARS-COV 2. This ability of the nanobodies was experimentally explored in human immune systems. Hence, a molecular docking strategy was employed for evaluation using CoDockPP Server and COVID-19 Docking Server.

MOLECULAR DOCKING STUDY

Firstly, crystallography structures of studied nanobodies and RBD-ACE2 of SARS-COV 2 (6LZG) were taken from a protein data bank (PDB) [40]. The structural and antigenic related data were checked in the structural antibody database (SABDab) [41] which is an online resource of all publicly available antibody structures annotated and presented in a consistent manner. The data are annotated with several properties such as experimental information, gene details, correct heavy, light chain pairings, and antigen details. The candidate nanobodies were blocking the Nef [42] and capsid protein p24 [43, 44] of human immunodeficiency viruses 1 (HIV-1) or capsid protein VP3 of poliovirus [45, 46]. Moreover, different conformations of nanobodies that are experimentally involved in the RBD of SARS-COV 2 were adapted from PDB [30-33, 35]. The nanobodies name, their viral types and macromolecules, and PDB ID are listed in Table 1. The heavy chain name in the PDB ID may be different due to several binding sites on the target protein of the virus that are experimentally involved in human immune systems. Regardless of the synthetic nanobodies blocking of spike glycoproteins RBD of SARS-COV 2, some other nanobodies such as natural VHH (occurring in *Lama glama*, *Vicugna pacos* or *Camelus dromedaries*) were also addressed. In the next step, nanobodies, and ACE2 structures and affinity were checked using a multistage protein-protein docking program (CoDockPP Server) [47] while RBD (6LZG (B)) offered the target protein. This server operates based on shape complementarity using knowledge-based scoring function and site constraint. Then, nanobodies and ACE2 were presented to COVID-19 Docking Server [48] for determining the most stable complexes of RBD-nanobody and RBD-ACE2 where ACE2 (6LZG (A)) was defined as ligand proteins.

Table 1. The name, PDB ID and source of nanobodies that experimentally was involved against SARS-COV 2 and HIV-1 or poliovirus viral and their predicted binding energy with RBD of SARS-COV 2.

No.	Nanobody name	PDB ID (nanobody chain)	Source	Viral	Binding energy (kcal/mol)
1	Ty1[19]	6ZXN (D)	Vicugna pacos	SARS-COV 2	-247.40
2	Ty1[19]	6ZXN (E)	Vicugna pacos	SARS-COV 2	-238.59
3	Ty1[19]	6ZXN (F)	Vicugna pacos	SARS-COV 2	-235.55
4	H11-H4[20]	6ZHD (E)	Lama glama	SARS-COV 2	-328.62
5	EY6A Fab [21]	6ZCZ (HL)	Lama glama	SARS-COV 2	-361.16
6	H11-D4 [22]	6YZ5 (F)	Lama glama	SARS-COV 2	-285.87
7	H11-D4 [22]	6Z43 (X)	Lama glama	SARS-COV 2	-292.91
8	H11-D4 [22]	6Z43 (Y)	Lama glama	SARS-COV 2	-302.56
9	H11-D4 [22]	6Z43 (Z)	Lama glama	SARS-COV 2	-300.03
10	Sb23 [24]	7A29 (D)	Synthetic construct	SARS-COV 2	-295.82
11	Sb23 [24]	7A29 (E)	Synthetic construct	SARS-COV 2	-298.74
12	Sb23 [24]	7A29 (F)	Synthetic construct	SARS-COV 2	-295.34
13	ybody MR17 [25]	7C8W(A)	synthetic construct	SARS-COV 2	-262.63
14	sybody MR17-K99Y [27]	7CAN (A)	synthetic construct	SARS-COV 2	-283.52
15	SR4 [27]	7C8V (A)	synthetic construct	SARS-COV 2	-342.56
16	sdAb19 [32]	4ORZ (C)	Lama glama	HIV-1	-293.68
17	Cameld VHH 9 [33]	2XT1 (B)	Vicugna pacos	HIV-1	-282.12
18	Cameld VHH 9 [33]	2XV6 (B)	Vicugna pacos	HIV-1	-279.28
19	Cameld VHH 9 [33]	2XV6 (D)	Vicugna pacos	HIV-1	-310.68
20	VHH 59H10 [34]	5O2U (B)	Lama glama	HIV-1	-367.73
21	VHH 59H10 [34]	5O2U (D)	Lama glama	HIV-1	-333.38
22	VHH 12B [35]	5KTZ (7)	Camelus dromedarius	poliovirus	-331.58
23	VHH 17B [35]	5KU0 (7)	Camelus dromedarius	poliovirus	-345.7
24	VHH 7A [35]	5KU2 (7)	Camelus dromedarius	poliovirus	-310.13
25	VHH 10E [35]	5KWL (7)	Camelus dromedarius	poliovirus	-340.87
26	VHH PVSP29F [36]	3JBC (7)	Camelus dromedarius	poliovirus	-310.42
27	VHH PVSP6A [36]	3JBD (7)	Camelus dromedarius	poliovirus	-334.67
28	VHH PVSS8A [36]	3JBE (7)	Camelus dromedarius	poliovirus	-320.97
29	VHH PVSP19B [36]	3JBF (7)	Camelus dromedarius	poliovirus	-275.56
30	VHH PVSS21E [36]	3JBG (7)	Camelus dromedarius	poliovirus	-257.15

RESULT AND DISCUSSION

The neutralization ability of candidate VHH toward RBD of SARS-COV 2 was repurposed using CoDockPP Server and COVID-19 Docking Server. The predicted binding energies of nanobodies and ACE2 to RBD are listed in Table 1. The binding energy values obtained by the two servers well coincided for all complexes.

Seven nanobodies contain *Ty1* [30], *H11-D4* [33], *H11-H4* [31], *SR4*, *MR17* and *MR17-K99Y* [34], *Sb23* [35] were experimentally involved in human immune systems against RBD of SARS-COV 2. The results of molecular docking of these nanobodies

showed that the binding energy values varied from -235.55 kcal/mol for *Ty1* derived of *Vicugna pacos* (6ZXN (F)) [30] to -342.56 kcal/mol for synthetic construct *SR4* (7C8V (A)) [34]. The other synthetic nanobodies have the binding energy of -262.63 and -283.52 for *sybodies* of *MR17* and *MR17-K99Y* [34], -295.34 (7A29 (F)), -295.82 (7A29 (D)), and -298.74 (7A29 (E)) for *Sb23* [35], and -342.56 for *SR4* (7C8V (A)) [34], respectively. Moreover, the estimated binding energy for other *Ty1* was -238.59 (6ZXN (E)) and -247.40 (6ZXN (D)) while these values were -285.87 (6YZ5 (F)), -292.91 (6Z43 (X)), -300.03 (6Z43 (Z)), and -302.56 (6Z43 (Y)) for *Lama glama*

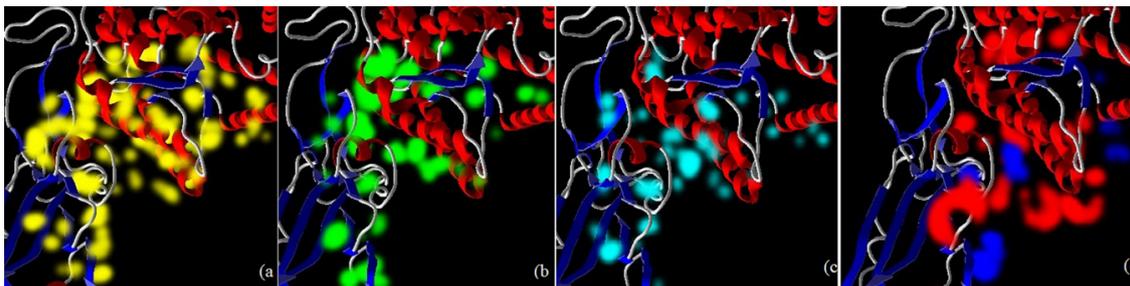


Fig. 1. Energy map of ACE2-RBD complex; a) hydrogen donor b) steric, c) hydrogen acceptor, and d) electrostatic.

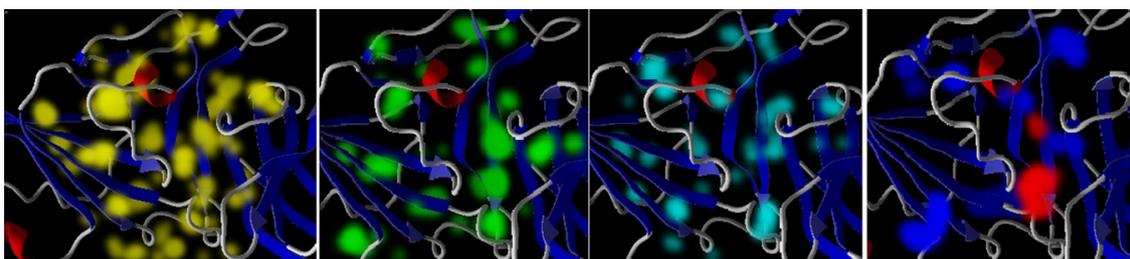


Fig. 2. Energy map of VHH 17B-RBD complex; a) hydrogen donor b) steric, c) hydrogen acceptor, and d) electrostatic.

obtained *H11-D4* [33], respectively. The binding energy was predicted as -328.62 for *H11-H4* (6ZHD (E)) [20] of *Lama glama* which can neutralize the binding of the RBD against the human ACE2. On the other hand, predicted binding energy of ACE2 with RBD receptor was -297.12 (kcal/mol). Accordingly, respective binding energies of *Sb23* (7A29 (E)), *SR4* (7C8V (A)), *H11-D4* ((6Z43 (Z)) and (6Z43 (Y))) and *H11-H4* (6ZHD (E)) (-298.74, -342.56, -300.03, -302.56, and -328.62) were greater than ACE2-RBD complex, so they are the top conformation of these nanobodies.

The predicted binding energies of all 15 reported nanobodies were greater than -235.55 (kcal/mol) for *Ty1* (6ZXN (F)) while these values for 10 VHH were greater than RBD-ACE2 complex. *VHH 7A*, *VHH PVSP29F*, *Cameld VHH 9*, *VHH PVSS8A*, *VHH 12B*, *VHH 59H10*, *VHH PVSP6A*, *VHH 10E*, *VHH 17B*, and *VHH 59H10* are proposed nanobodies for blocking the viral glycoprotein of SARS-COV 2. 5KU2 (7), 3JBC (7), 2XV6 (D), 3JBE (7), 5KTZ (7), 5O2U (D), 3JBD (7), 5KWL (7), 5KU0 (7), and 5O2U (B) are PDB ID (V_{HH} heavy chain) of the selected nanobodies. The proposed *VHH 17B* and *VHH 59H10* nanobodies have an affinity greater than -342.56 (kcal/mol) for synthetic construct *SR4* (7C8V (A)) against trimeric spike glycoprotein RBD for SARS-COV 2.

The energy map for ACE2-RBD, *VHH 17B*-RBD

and *VHH 59H10*-RBD was visualized using Molegro Molecular Viewer of MVD [49] software. The spherical space of mapping had a radius of 0.25 Å with the offset from the center for covering the binding site ($X = -31.50$, $Y = 25$ and $Z = 13.62$). According to the energy maps for ACE2-RBD (Fig. 1), hydrogen donor, steric, hydrogen acceptor and electrostatic interactions were significant in blocking of the host receptor human ACE2 to RBD of SARS-COV 2, respectively. It should be noted that for electrostatic interactions in the figures blue and red colored areas respectively correspond to the positive and negative charges. For example, the energy maps of *VHH 17B*-RBD and *VHH 59H10*-RBD are depicted in Figs. 2 and 3, respectively. As seen, in ACE2-RBD complexes, hydrogen donor, steric, hydrogen acceptor, and electrostatic interactions had the highest contributions in blocking RBD, respectively. This similarity in the energy maps of *VHH 17B*-RBD and *VHH 59H10*-RBD with ACE2-RBD confirmed that these nanobodies can be used for blocking RBD of SARS-COV 2. It should be noted that the different crystallography structures of *VHH 59H10* (5O2U (B)) and (5O2U (D)) could be due to various experimental contact region of spike glycoprotein of SARS-COV 2 (Fig. 4). The top conformation and secondary structure view of *VHH 59H10* nanobody in the active site of RBD for SARS-COV

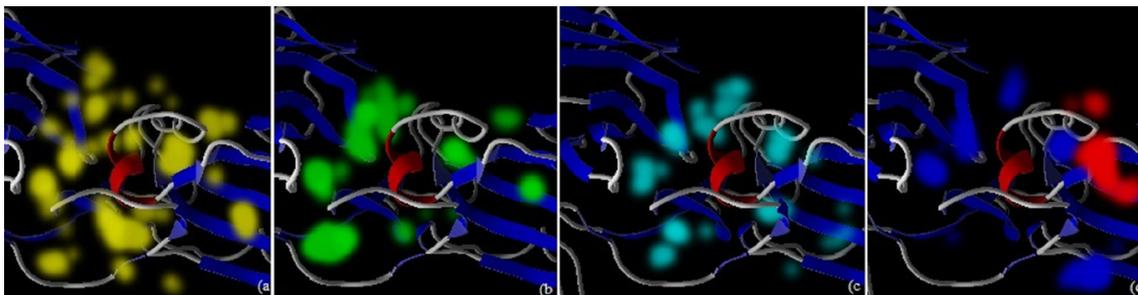


Fig. 3. Energy map of VHH 59H10-RBD complex; a) hydrogen donor b) steric, c) hydrogen acceptor, and d) electrostatic.

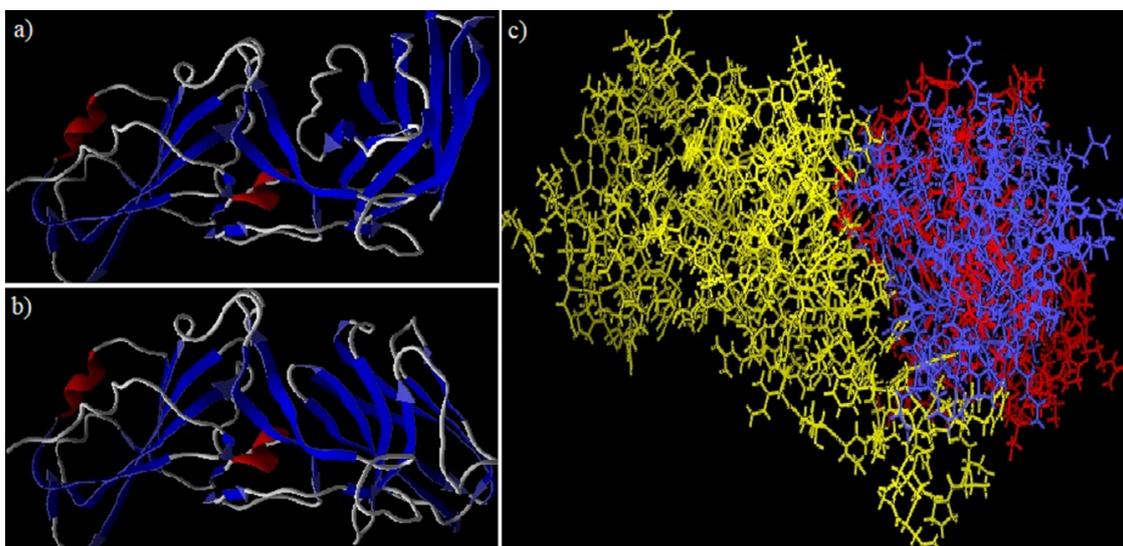


Fig. 4. Secondary structure view of VHH 59H10 heavy chain a) B and b) D of 5O2U.(right) in complexes with RBD (left); and c) top conformation of heavy chain B (red) and D (blue) of VHH 59H10 of 5O2U in binding site of RBD (yellow)

2 are demonstrated in Figure 4.

CONCLUSION

The neutralization ability of some nanobodies was investigated against the trimeric spike glycoprotein RBD of SARS-COV 2. CoDockPP Server was applied for a multistage protein-protein molecular docking of nanobodies and ACE2 with RBD receptor. Moreover, COVID-19 Docking Server was employed to evaluate the blocking ability of nanobodies against RBD. Both servers predicted the equal values of binding energy for all studied nanobodies while the value of -297.12 (kcal/mol) was estimated for the ACE2-RBD complex. According to these values, all proposed nanobodies have values greater than -235.55 (kcal/mol) for Ty1 (6ZXN (F)). VHH 7A, VHH PVSP29F, Cameld

VHH 9, VHH PVSS8A, VHH 12B, VHH 59H10, VHH PVSP6A, VHH 10E, VHH 17B, and VHH 59H10 are 10 nanobodies that can be proposed for blocking the RBD of SARS-COV 2. However, the two last nanobodies of VHH 17B and VHH 59H10 are more confident for neutralization due to their greater estimated binding energy against -342.56 (kcal/mol) which computed for synthetic construct SR4. According to the energy maps of all ACE2, VHH 17B and VHH 59H10, hydrogen donor, steric, hydrogen acceptor, and electrostatic interactions played the most significant role in blocking the RBD, respectively. This study offers structural insight for the blocking of pathogenic novel coronavirus with 15 reported nanobodies that had been proposed for the control of the SARS-COV 2 pandemic.

CONFLICTS OF INTEREST

The authors announce that there are no conflicts of interest.

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