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Computational design of novel nanobodies targeting the receptor binding domain of variants of concern of SARS-CoV-2

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Abstract

The COVID-19 pandemic has created an urgent need for effective therapeutic and diagnostic strategies to manage the disease caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). However, the emergence of numerous variants of concern (VOCs) has made it challenging to develop targeted therapies that are broadly specific in neutralizing the virus. In this study, we aimed to develop neutralizing nanobodies (Nbs) using computational techniques that can effectively neutralize the receptor-binding domain (RBD) of SARS-CoV-2 VOCs. We evaluated the performance of different protein-protein docking programs and identified HDOCK as the most suitable program for Nb/RBD docking with high accuracy. Using this approach, we designed 14 novel Nbs with high binding affinity to the VOC RBDs. The Nbs were engineered with mutated amino acids that interacted with key amino acids of the RBDs, resulting in higher binding affinity than human angiotensin-converting enzyme 2 (ACE2) and other viral RBDs or hemagglutinins (HAs). The successful development of these Nbs demonstrates the potential of molecular modeling as a low-cost and time-efficient method for engineering effective Nbs against SARS-CoV-2. The engineered Nbs have the potential to be employed in RBD-neutralizing assays, facilitating the identification of novel treatment, prevention, and diagnostic strategies against SARS-CoV-2.

Troubleshooting

1. Validation of protein-protein docking server

1 Prepare the protein datasets consisting of 29 nanobody (Nbs) and 86 antibodies (Abs) complexed with RBDs from the Protein Data Bank (PDB) (**https://www.rcsb.org/**) for blind docking.

2 Remove heteroatoms/molecules, including metal ions, small molecules, water molecules, and His-tags, from all complexes.

3 Prepare the protein chains of RBDs and ligands (Nbs or antibodies) separately using Discovery Studio software.

4 The missing amino acids in the protein chain are remodeled using the SWISS-MODEL expert system (**https://swissmodel.expasy.org/**).

5 Perform blind docking using seven protein-protein docking programs including;
1) HDOCK (**http://hdock.phys.hust.edu.cn/**)
2) ATTRACT (**http://www.attract.ph.tum.de/services/ATTRACT/**attract.html)
3) pyDockWEB (https://life.bsc.es/pid/pydockweb)
4) GRAMM-X (**http://vakser.compbio.ku.edu/resources/gramm/grammx/**)
5) PatchDock (**https://bioinfo3d.cs.tau.ac.il/**PatchDock/)
6) FRODOCK (**http://frodock.chaconlab.org/**) and
7) ZDOCK (**https://zdock.umassmed.edu/**).

6 The root mean square deviation (RMSD) values of the ligands (Nb or Ab) are calculated using the Discovery Studio program.

2. Selection of lead Nbs

7 The 29 Nbs are redocked with each targeted RBD using a blind docking method using HDOCK.

8 Calculate the RMSD values to assess the accuracy of the docking poses of the 29 Nbs with respect to all targeted RBDs. Present the docking scores and RMSD values for each RBD in terms of the mean.

9 The similarity of amino acid sequences of 29 Nbs is analyzed using the Clustal Omega server (**https://www.ebi.ac.uk/Tools/msa/clustalo/**).

10 The lead Nbs are selected based on the best mean docking score, lowest RMSD, and diverse amino acid sequences, which are then employed for structure-based engineering.

3. Structural-based engineering and broad specific binding of Nbs

11 To improve the binding affinity of Nbs to all targeted RBDs, the two lead Nbs are mutated using the site-direct mutagenesis feature on the Discovery Studio program.

12 Nb residues that had no interaction and repulsion with RBD are considered for the mutation process.

13 The AMBER ff14SB force field is applied for structural energy minimization before the docking process of mutated Nbs.

14 Calculate the ΔHDOCK value, and choose the mutated residue at a specific position that exhibited the lowest ΔHDOCK for multi-point mutation.

15 To investigate the broad specific binding of engineered Nbs, cross-docking between Nb and all targeted RBDs, ACE2, and other viral RBDs/HAs is performed using the HDOCK program.

4. Physicochemical properties prediction of engineered Nbs

16 The contact surface amino acids and chemical interactions are determined using the PDBsum server (http://www.ebi.ac.uk/thornton-srv/databases/pdbsum/Generate.html).

17 The physicochemical properties are predicted using the ProtParam (ExPASy) tool (**https://web.expasy.org/protparam/**).

18 The PI value is calculated using the Protein–Sol web server (**https://protein-sol.manchester.ac.uk/**).

19 The total charge is calculated by PROTEIN CALCULATOR v3.4 (**https://protcalc.sourceforge.net/**).