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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.

1. Validation of protein-protein docking server

1 Prepare the protein datasets consisting of 29 nanobodies (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 Superimpose the docking pose with native to calculate the root mean square deviation (RMSD) values of the ligands (Nb or Ab) using the Discovery Studio program.

2. Selection of lead Nbs

7 Redock all 29 Nbs listed in the S1 Table with the targeted RBDs using a blind docking method through the HDOCK server.

8 Minimized the energy of the Nb/RBD complexes utilizing the AMBER ff14SB force field, providing the complexes with the lowest binding energy.

9 For each Nb and RBD, a separate redocking was performed using HDOCK to determine the best docking score.

10 Superimpose the docking pose with native to calculate the root mean square deviation (RMSD) values of the Nbs using the Discovery Studio program. Present the docking scores and RMSD values for each RBD in terms of the mean.

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

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

3. Structural-based engineering and broad specific binding o...

13 The Nb/RBD complexes in native form, in their initial state after blind docking using the HDOCK.

14 Minimized the energy of the Nb/RBD complexes utilizing the AMBER ff14SB force field, providing the complexes with the lowest binding energy. This was done to align the torsion angles of complementary amino acid side chains between Nb and RBD, ensuring uniformity in the lowest binding energy and preventing any atom that might have astride.

15 Redock the optimized Nb and RBD yielding the native form's score as the initial score ($HDOCK_{Nb\ (native)}$).

16 Mutate the amino acid on crucial target residues at the interaction site of Nbs to each target RBD,
while considering the non-contact/interaction amino acid(s) and amino acid(s) that caused unfavorable interactions.

17 Prior to redocking, each mutated amino acid was subjected to optimization using the CHARM force field within the Discovery Studio program, resulting in the post-mutation score as $HDOCK_{Nb\ (mutant)}$.

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

19 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 as same this procedure from 13 to 15.

4. Physicochemical properties prediction of engineered Nbs

20 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).

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

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

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