# Antibody-nanobody combination increases their neutralizing activity against SARS-CoV-2

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## Introduction

The global spread of Covid-19 is devastating health systems and economies worldwide. While the use of vaccines has yielded encouraging results, the emergence of new variants of SARS-CoV-2 shows that combating COVID-19 remains a big challenge. One of the most promising treatments is the use of not only antibodies, but also nanobodies. Recent experimental studies have revealed that the combination of antibody and nanobody can significantly improve their neutralizing ability through binding to the SARS-CoV-2 spike protein, but the molecular mechanisms of this observation remain largely unknown. In this work, as a case study, we investigated the binding affinity of the CR3022 antibody and H11-H4 nanobody to the SARS-CoV-2 receptor binding domain (RBD) using molecular modeling. Both all-atom steered molecular dynamics simulations and the coarse-grained umbrella sampling showed that, consistent with the experiment, CR3022 associates with RBD more strongly than H11-H4. Importantly, we predict that the combination of CR3022 and H11-H4 considerably increases their binding affinity to the spike protein. The electrostatic interaction was found to control the association strength of CR3022, but the van der Waals interaction dominates in the case of H11-H4.

## Materials and Methods

**Materials**. The structures of H11-H4-RBD, CR3022-RBD, and H11-H4+CR3022-RBD complexes were extracted from the Protein Data Bank with PDB ID: 6ZH9.

**Steered Molecular Dynamics Simulations**. The steered molecular dynamics simulations of H11-H4-RBD, CR3022-RBD, and H11-H4+CR3022-RBD complexes were performed with CHARMM36M force field. The water model TIP3P was used for all systems. Rectangular boxes with dimension of $10\times9\times25$ nm$^3$, $10\times11\times25$ nm$^3$ and $10\times18\times25$ nm$^3$ were used for H11-H4-RBD, CR3022-RBD and H11-H4+CR3022–RBD, respectively.

**Coarse-grained umbrella sampling (CGUS) simulations**. The MARTINI 2.2 force field was used. The standard MARTINI water model was used with a minimum distance between water beads of 1.0 nm.

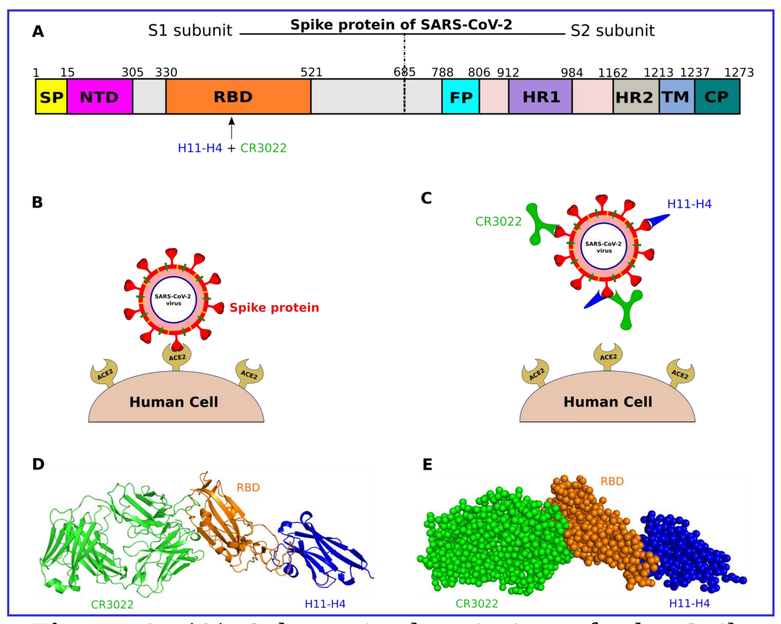


**Figure 1:** (A) Schematic description of the Spike protein of SARS-CoV-2, which consists of the S1 and S2 subunits. (B) SARS-CoV-2 S protein binds to human ACE2 before entering cells. (C) H11-H4 and CR3022 bind to S protein, preventing the virus from entering cells. The 3D structures of H11-H4 and CR3022 bound to RBD are shown in all-atom (D) and coarse-grained (E) models.

## Results and Discussion

We used SMD and CGUS simulations to investigate the binding affinities of the H11-H4 nanobody, CR3022 antibody, and both of them bind to RBD. Figures 3 and 4 showed the results estimated from SMD simulations. Figure 4 presented the data estimated from coarse-grained US. Our results predicted that the concurrent binding of H11-H4 and CR3022 to RBD results in a higher binding affinity than when they are individually associated with RBD. The combination of H11-H4 and CR3022 enhances the neutralization of SARS-CoV-2, and this could open up a new treatment strategy for Covid-19. Whether this conclusion holds for the other antibody-nanobody pairs is a matter of further clarification.

**Non-equilibrium binding affinities between antibody-nanobody and SARS-CoV-2 RBD**

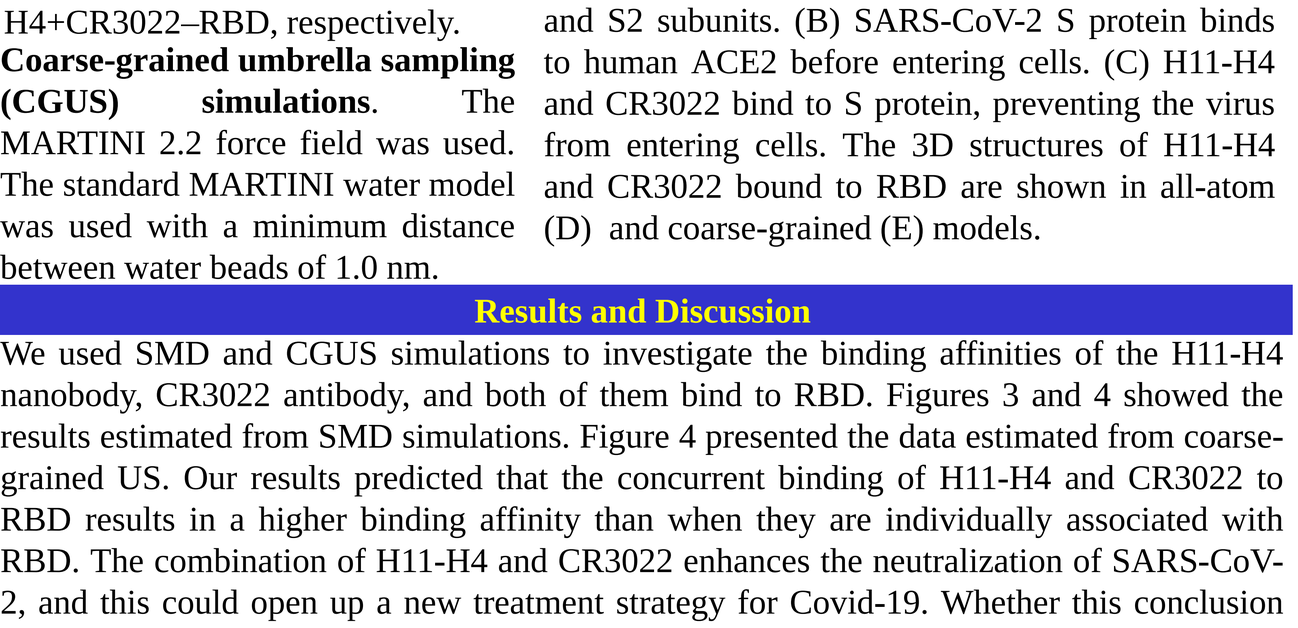


**Figure 2:** Time dependence of the pulling force (A), work (B) , and (non-equilibrium energy profiles (C) of the H11-H4 - RBD, CR3022 - RBD, and H11-H4+CR3022 - RBD. The results were obtained at $v$ = 0.5 nm/ns and averaged from five independent SMD runs.

**Interaction energies between antibody-nanobody and SARS-CoV-2 RBD**

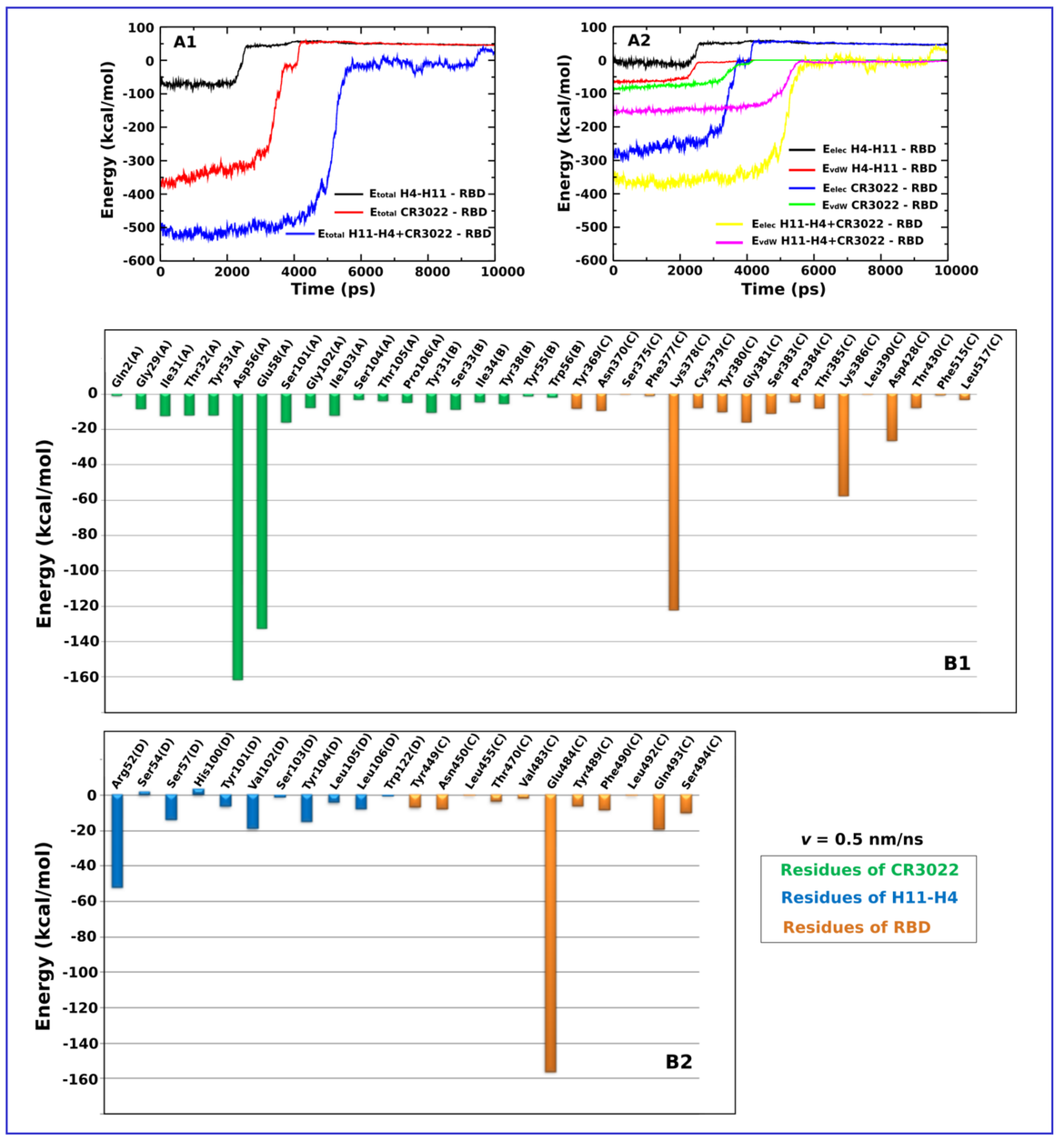


**Figure 3:** Time dependence of the total non-bonded interaction energy (sum of electrostatic and vdW) (A1) , and electrostatic and vdW interaction energies (A2) of the H11-H4 - RBD, CR3022 - RBD and H11-H4+CR3022 - RBD complexes. Total non-bonded interaction energy of residues located at the binding region of H11-H4 - RBD (B1) and CR3022 - RBD (B2). The results were obtained for a time window [0, $t_{max}$] and averaged from five independent SMD.

**PMFs between antibody-nanobody and SARS-CoV-2 RBD**




**Figure 4:** One-dimensional potential of mean force (1D PMF) of complexes H11-H4-RBD, CR3022-RBD and H11-H4+CR3022-RBD as a function of the reaction coordinate. The left and right snapshots refer to the bound and unbound state of CR3022 - RBD. The arrow indicates the position of the cutoff distance between bound and unbound states.

## Conclusion

SMD simulation showed the H11-H4 nanobody binds to RBD weaker than CR3022, which is consistent with the binding affinity computed from the coarse-grained US. Our theoretical estimates of the binding affinity are in good agreement with the experimental results presented by Tian *et al.*[1] and Huo *et al.*[2] for H11-H4 and CR3022 interacting with SARS-CoV-2, but for the CR3022-RBD complex they contradict Yuan *et al.*[3], the combination of H11-H4 and CR3022 enhances the neutralization of SARS-CoV-2. Stability of the H11-H4-RBD complex is mainly contributed by the vdW interaction, while electrostatic interaction is more important for the CR3022 - RBD and H11-H4+CR3022–RBD complexes.[4]

## References

(1) Tian, X.; *et al*. **Emerg. Microbes Infect.** 2020, 9, 382-385.
(2) Huo, J.; *et al*. **Nat. Struct. Mol. Biol.** 2020, 27, 846-854.
(3) Yuan, M.; *et al*. **Science.** 2020, 368, 630-633.
(4) Hung, N.; *et al*. **Sci. Rep.** 2022, 9701.