

# Supporting Information

## Atomistic Simulations and Deep Mutational Scanning of Protein Stability and Binding Interactions in the SARS-CoV-2 Spike Protein Complexes with Nanobodies: Molecular Determinants of Mutational Escape Mechanisms

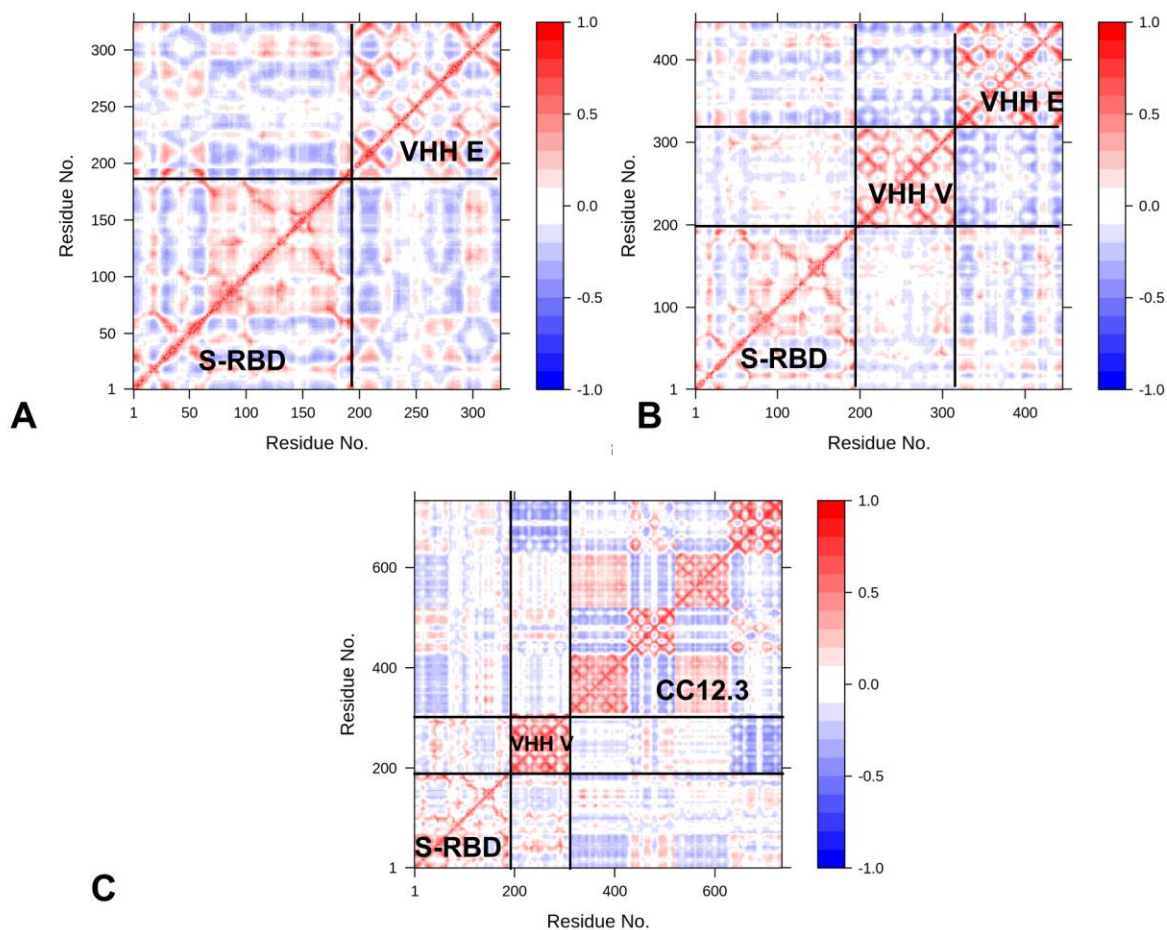
Gennady M. Verkhivker,<sup>1,2 \*</sup> Steve Agajanian<sup>1</sup>, Deniz Yasar Oztas<sup>1</sup>, Grace Gupta<sup>1</sup>

<sup>1</sup>Keck Center for Science and Engineering, Department of Computational and Data Sciences,  
Schmid College of Science and Technology, Chapman University, One University Drive,  
Orange, CA 92866, USA

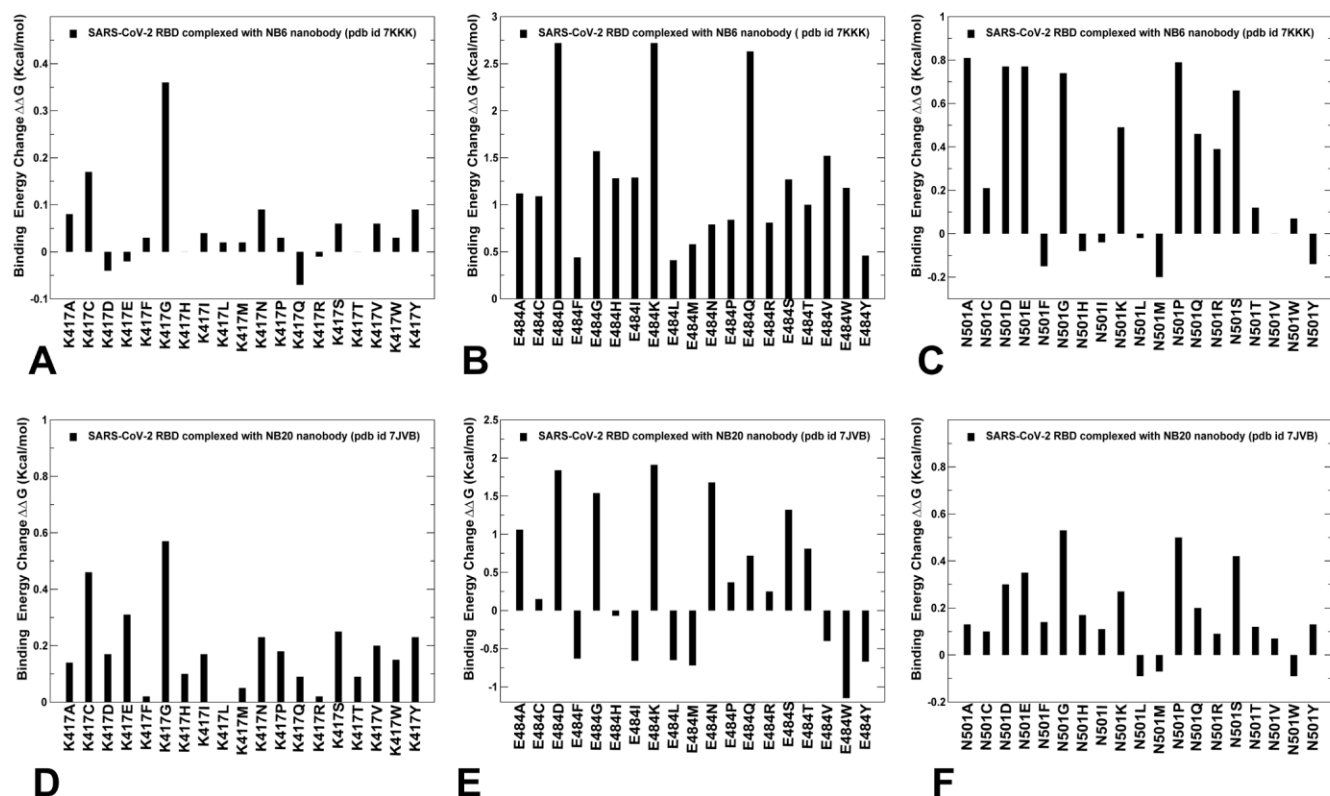
<sup>2</sup> Department of Biomedical and Pharmaceutical Sciences, Chapman University School of  
Pharmacy, Irvine, CA 92618, USA

**\*Corresponding Author**

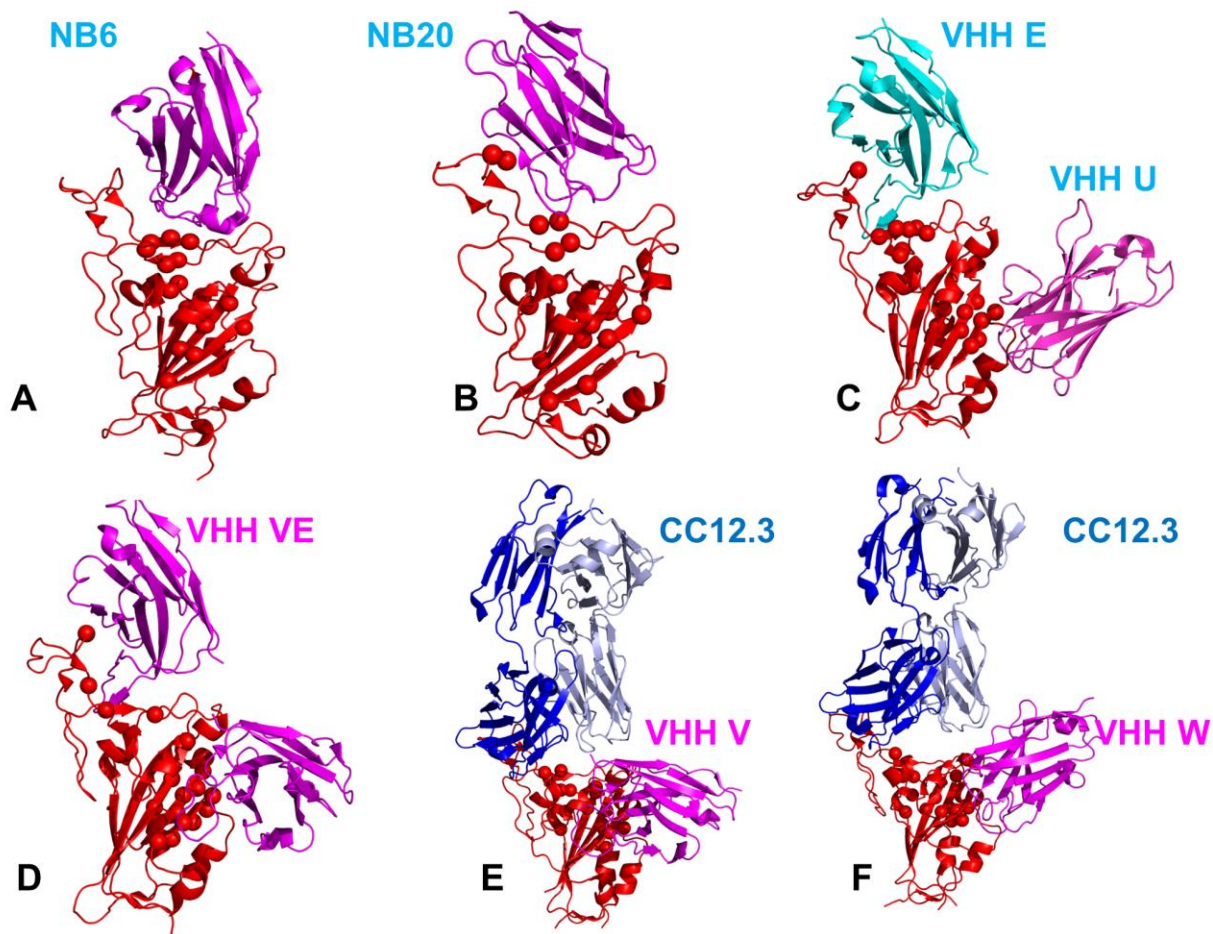
E-mail: verkhivk@chapman.edu



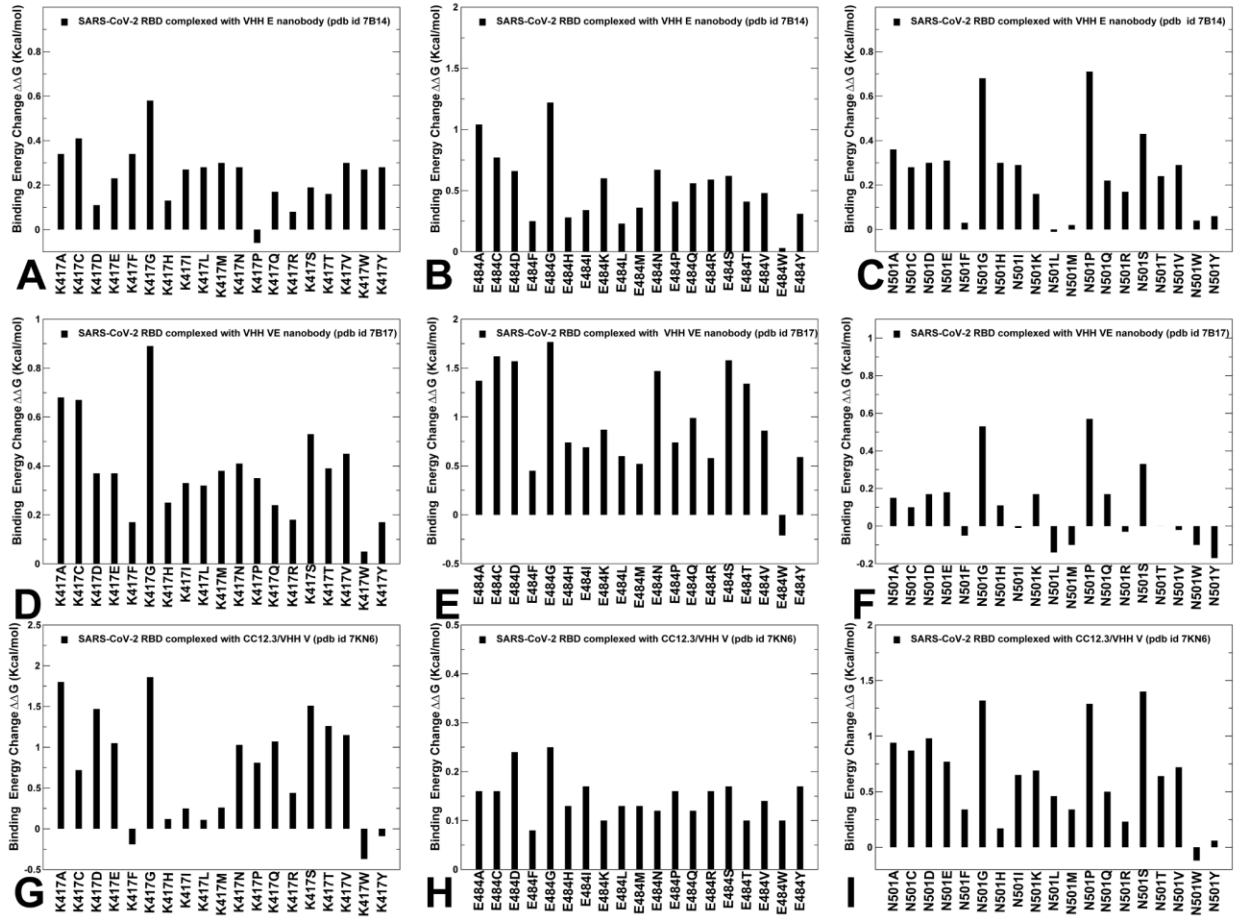
**Figure S1.** Conformational dynamics analysis and the covariance residue correlation matrixes for the SARS-CoV-2 S complexes with VHH E nanobody, pdb id 7IB14 (A), complex with the biparatopic nanobody VHH VE, pdb id 7B17 (B), and complex with CC12.3/VHH V combination, pdb id 7KN6 (C). The covariance matrix indicates coupling between pairs of residues. Cross-correlations of residue-based fluctuations vary between +1 (correlated motion; fluctuation vectors in the same direction, colored in dark red) and -1 (anti-correlated motions; fluctuation vectors in the same direction, colored in dark blue). The values  $> 0.5$  are colored in dark red and the lower bound in the color bar indicates the value of the most anti-correlated pairs.



**Figure S2.** The mutational sensitivity analysis for the SARS-CoV-2 S complexes with Nb6 and Nb20 nanobodies. (A-C ) The distribution of binding free energy changes caused by mutations of K417, E484 and N501 sites in the S-RBD complexes with Nb6 nanobody. (D-F) The distribution of binding free energy changes caused by mutations of K417, E484 and N501 sites in the S-RBD complexes with Nb20 nanobody.



**Figure S3.** Structural mapping of protein stability centers for the SARS-CoV-2 S complexes with Nb6 (A), Nb20(B), VHH E/VHH U pair (C), biparatopic nanobody VHH VE (D), CC12.3/VHH V pair (E), and CC12.3/VHH pair (F). The S-RBD is shown in red ribbons. The bound nanobodies are shown in magenta-colored ribbons. The heavy chain of CC12.3 antibody is in blue ribbons and the light chain is in cyan-colored ribbons.



**Figure S4.** The mutational sensitivity analysis for the SARS-CoV-2 S complexes with VHH E, VHH VE nanobodies and CC12.3/VHH V antibody/nanobody combination. (A-C) The distribution of binding free energy changes caused by mutations of K417, E484 and N501 sites in the S-RBD complexes with VHH E nanobody. (D-F) The distribution of binding free energy changes caused by mutations of K417, E484 and N501 sites in the S-RBD complexes with VHH VE biparatopic nanobody. (G-I) The distribution of binding free energy changes caused by mutations of K417, E484 and N501 sites in the S-RBD complexes with CC12.3/VHH V antibody/nanobody combination.