

Biophysical Journal, Volume 120

Supplemental information

**Biomechanical characterization of SARS-CoV-2 spike RBD and human
ACE2 protein-protein interaction**

**Wenpeng Cao, Chuqiao Dong, Seonghan Kim, Decheng Hou, Wanbo Tai, Lanying
Du, Wonpil Im, and X. Frank Zhang**

Supporting Information

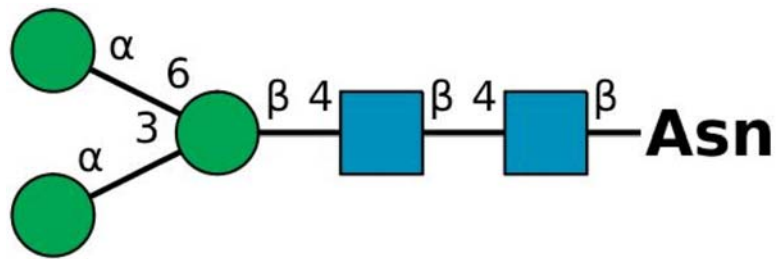


Figure S1. An N-glycan core pentasaccharide structure: mannose (green circle) and N-acetylglucosamine (blue square).

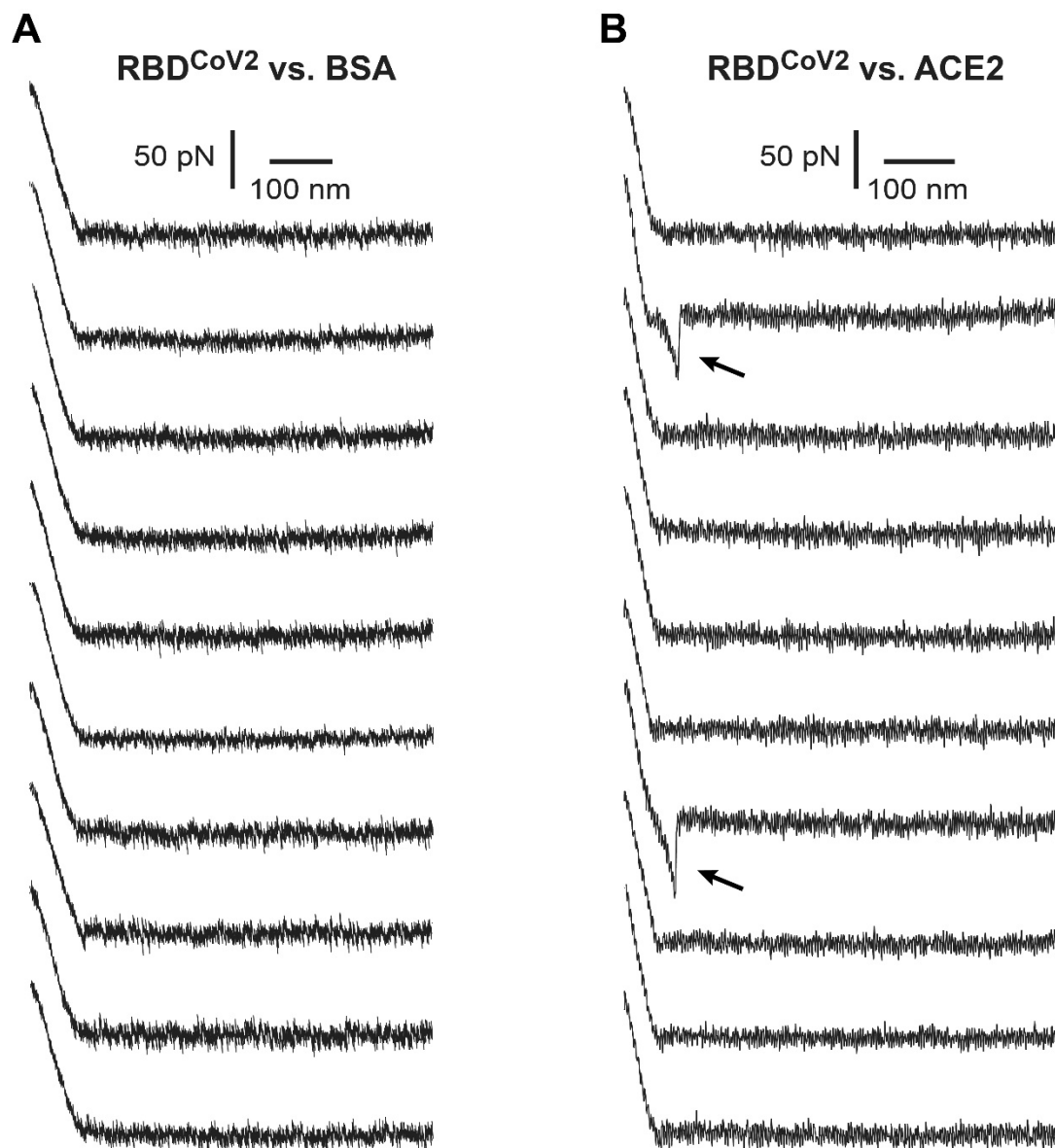


Figure S2. Representative consecutive force-distance (retraction) curves between an RBD^{CoV2}-functionalized AFM tip and a BSA-functionalized substrate (A) or an ACE2-functionalized substrate (B). All the traces in (A) do not show tip-substrate adhesion. The second and seventh traces in (B) show adhesion and RBD^{CoV2}-ACE2 unbinding, indicated by arrows. The curves were obtained under a contact force of ~ 120 pN, contact time of 0.1 s, and retraction speed at $1.9 \mu\text{m/s}$.

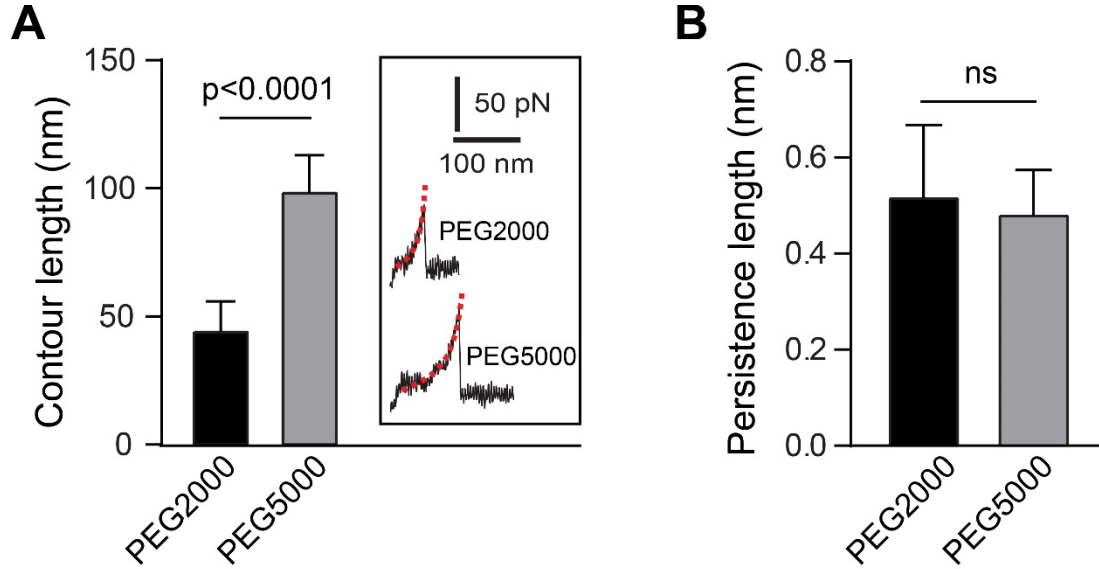


Figure S3. Worm-Like Chain (WLC) model analysis. (A) A WLC model analysis was conducted on the unbinding retraction traces of RBD^{CoV2}-ACE2 interactions (N=50) using PEG linkers with MW of 2000 (PEG2000) or 5000 (PEG5000). Inset: representative unbinding traces of the two linkers. Red dashed lines are the fit of the experimental data with the WLC model (1, 2):

$$\frac{F(x) \cdot L_p}{k_B T} = \frac{1}{4} \left(1 - \frac{x}{L_c} \right)^{-2} - \frac{1}{4} + \frac{x}{L_c}$$

In this equation, $F(x)$ is the applied force on the molecular tether, x is the end-to-end distance, L_c is the contour length, and L_p is the persistence length of the tether. The average contour length is shown in the bar graph. The error bars are one standard deviation. A statistically significant difference was found between the PEG2000 and PEG5000 groups' contour lengths with an unpaired t-test ($p < 0.0001$). (B) A comparison between the PEG2000 and PEG5000 groups' persistence lengths. The error bars are one standard deviation. ns: statistically insignificant, concluded by an unpaired t-test.

Estimating the contour length of the PEG-RBD-ACE2-PEG complex: The MW of a PEG is given by $(18.02 + 44.05 \times n)$ g/mol, where n denotes the total number of polyethylene oxide (PEO) segments. For PEG2000 and PEG5000, the numbers of the segment are, therefore, 45 and 113, respectively. According to a widely cited study by Oesterhelt, Rief, and Gaub, each PEO segment's contour length is approximately 0.3 nm (0.278 - 0.358 nm depending on the orientation of the bonds) (3). Thus, the total contour lengths for PEG2000 and PEG5000 are 13.5 nm and 34 nm, respectively. Considering that two separate PEG linkers are used to immobilize RBD and ACE2 respectively and that the RBD-ACE2 complex adds another ~10 nm of distance (based on the crystal structures), we can now estimate the total contour lengths to be 37 nm and 78 nm for the PEG2000 case and PEG5000 case, respectively.

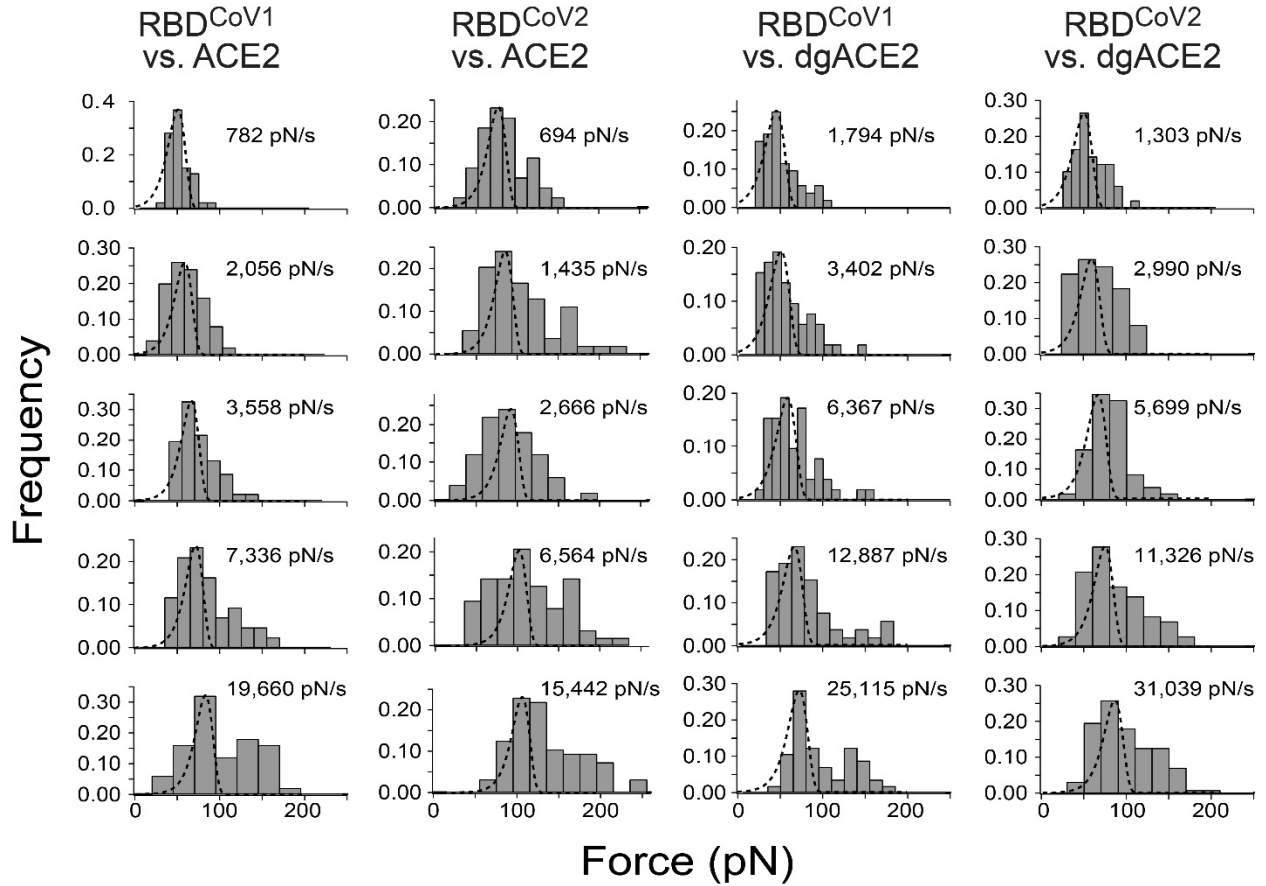


Figure S4. Unbinding force histograms of RBD-ACE2 interactions at different loading rates. The center force of each histogram's tallest bin was recorded as the most probable unfolding force for the Bell-Evens model analysis (Fig. 1D and Fig. 3B). The predicted force distribution (dashed curves), given by Eq. 2 using the Bell-Evens model parameters from Table 1, is overlaid on each histogram. The total unbinding force numbers for each RBD-ACE2 pairs are: 305 from a total of 1052 curve attempts for RBD^{CoV2}-ACE2, 245 from a total of 961 curve attempts for RBD^{CoV1}-ACE2, 346 from a total of 1301 curve attempts for RBD^{CoV2}-dgACE2, and 260 from a total of 946 curve attempts for RBD^{CoV1}-dgACE2.

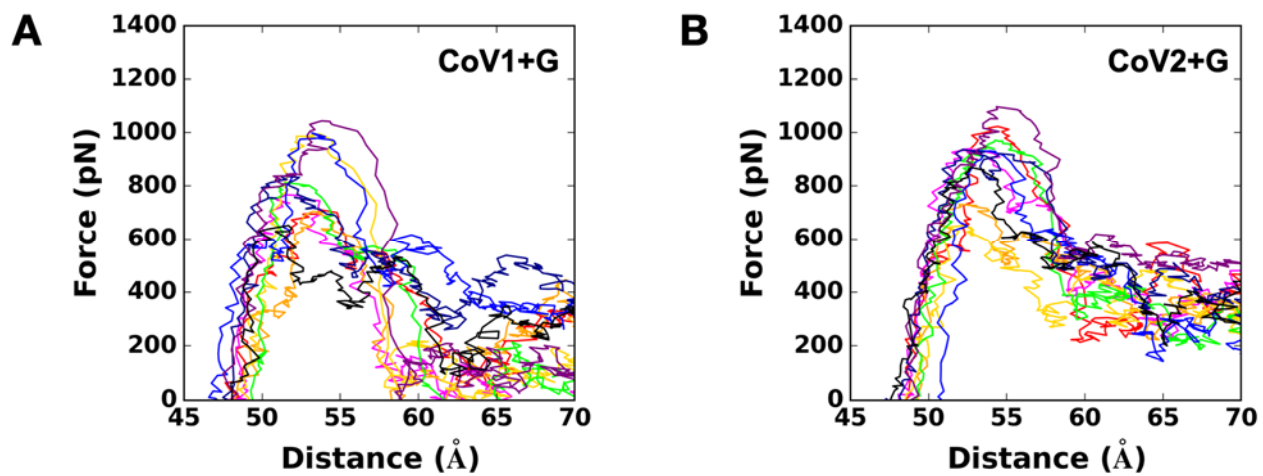


Figure S5. Force profiles of (A) $S^{\text{CoV1+G}}$ and (B) $S^{\text{CoV2+G}}$ of all 9 replicas. Each line represents raw data from each replica.

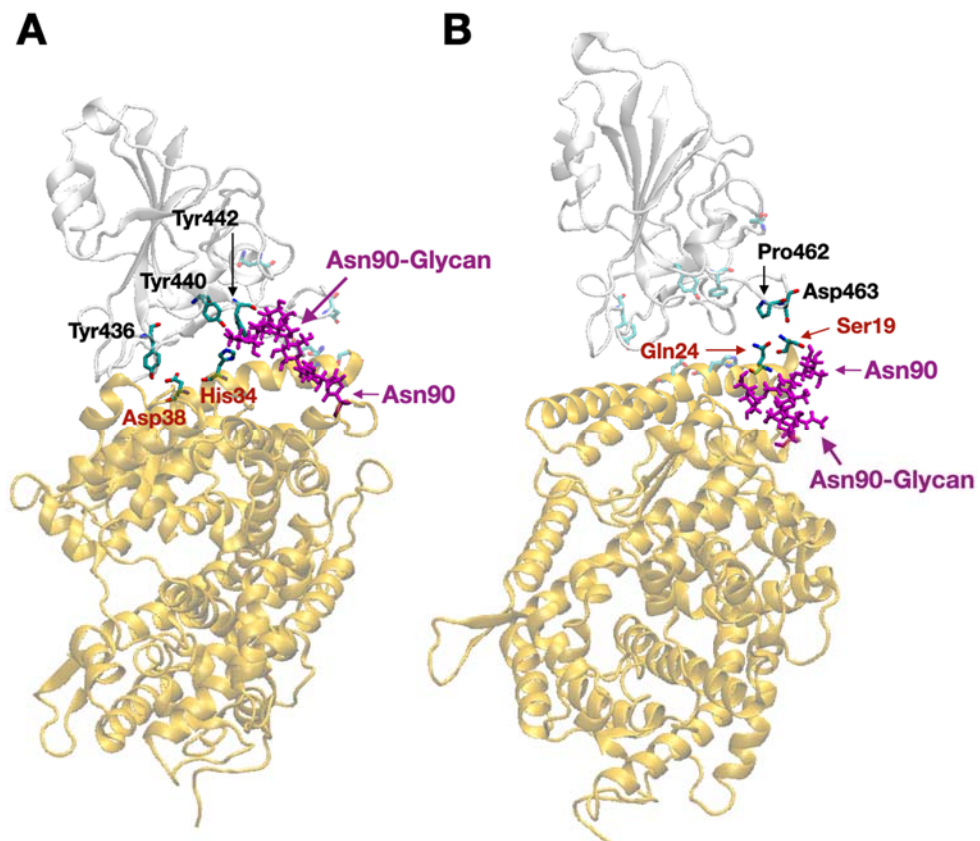


Figure S6. Representative snapshots of SMD simulations of $S^{\text{CoV1+G}}$ at $D^{\text{RBD-ACE2}}$ of (A) 49 Å and (B) 57 Å. The color scheme is the same as in **Fig. 2**.

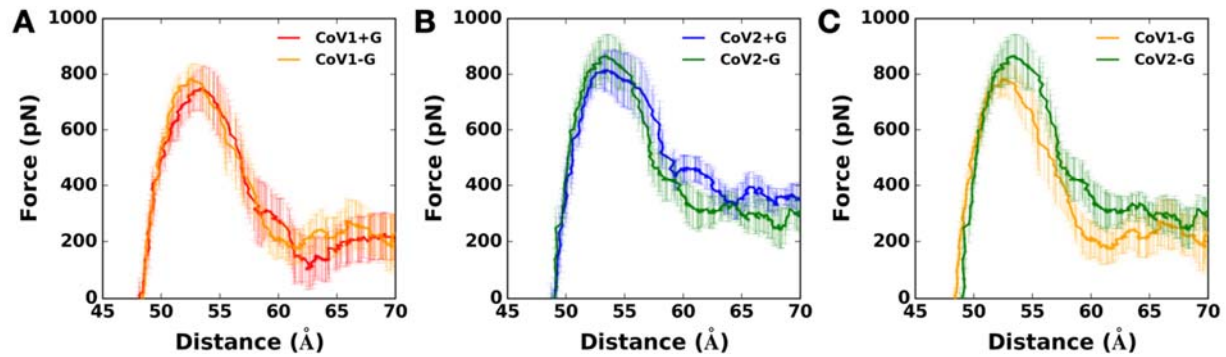


Figure S7. (A) Average force profiles of $S^{\text{CoV1+G}}$ (red) and $S^{\text{CoV1-G}}$ (orange) as a function of distance ($D^{\text{RBD-ACE2}}$) between the centers of mass of RBD and ACE2. (B) Average force profiles of $S^{\text{CoV2+G}}$ (blue) and $S^{\text{CoV2-G}}$ (green) as a function of $D^{\text{RBD-ACE2}}$. (C) Average force profiles of $S^{\text{CoV1-G}}$ (orange) and $S^{\text{CoV2-G}}$ (green) as a function of $D^{\text{RBD-ACE2}}$.

References:

1. Milstein, J. N., and J.-C. Meiners. 2013. Worm-Like Chain (WLC) Model. Encyclopedia of Biophysics, pp. 2757-2760.
2. Tong, Z., A. Mikheikin, A. Krasnoslobodtsev, Z. Lv, and Y. L. Lyubchenko. 2013. Novel polymer linkers for single molecule AFM force spectroscopy. Methods 60(2):161-168.
3. Oesterhelt, F., M. Rief, and H. E. Gaub. 1999. Single molecule force spectroscopy by AFM indicates helical structure of poly(ethylene-glycol) in water. New J. Phys. 1(1):6.1-6.11.