Supporting Information:

Computationally Designed ACE2 Decoy Receptor Binds SARS-CoV-2 Spike (S) Protein with Tight Nanomolar Affinity

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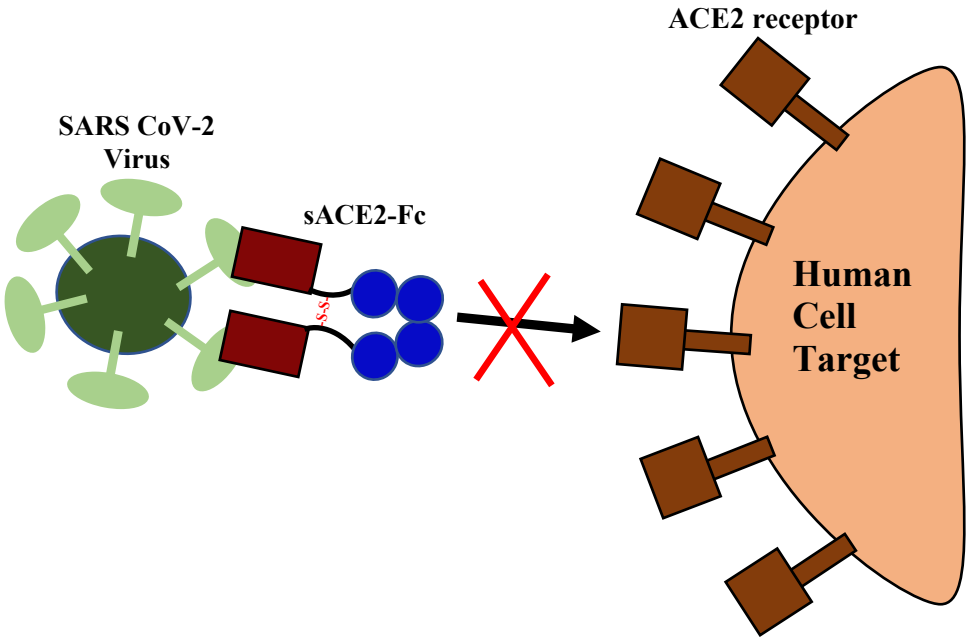

Figure S1. Soluble ACE2 fused to immunoglobulin Fc acts as a decoy to bind with SARS CoV-2 spike protein, preventing it from binding with membrane bound ACE2 receptors

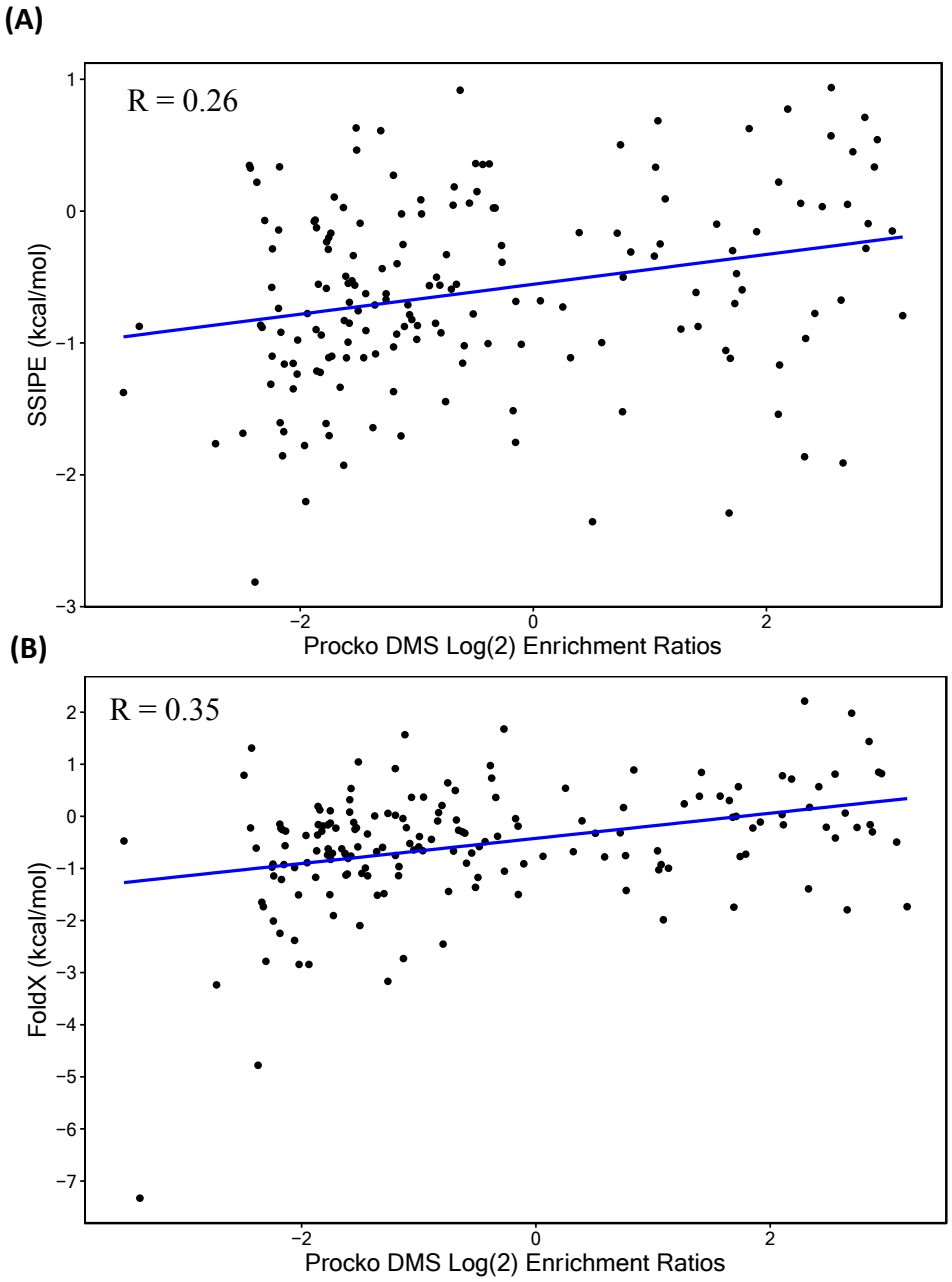

Figure S2. *In silico* mutagenesis for residues S19, Q24, T27, K31, H34, E35 Y41, Q42, and N330 in ACE2 (171 total mutations) was performed using two alternative methods, FoldX and SSIPe. The results are compared to the DMS experiment where we found, compared to Flex ddG, much weaker Pearson's correlation (R) R=0.26, R^2=0.06 for SSIPe (A) and R=0.35, R^2= 0.12 for FoldX (B). Regression line shown in blue with 95% confidence interval in light gray. We conclude that the conformational sampling and flexibility offered by Flex ddG helps accurately model the ACE2-RBD system, as it improves the correlation of predicted mutation effects with experiment.

Table S1. Mutations identified by Flex ddG to increase binding and that also agree with DMS experiment were combined in various 3-4 mutation combinations to analyze their cumulative effect. The combination predicted to have the highest affinity enhancement, **FFWF**, is bolded. Units in Rosetta REUs, more negative numbers correspond to tighter binding.

System	Mutations	Flex_ddG (REU)
sACE2. 1	S19Y, T27Y, K31W, N330Y	-4.15
sACE2.2	S19Y, T27F, K31W, N330Y	-4.76
sACE2.3	S19W, T27F, K31W, N330W	-4.08
sACE2.4	S19F, T27F, K31W, N330Y	-5.00
sACE2.5 (FFWF)	**S19F, T27F, K31W, N330F**	**-5.21**
sACE2.6	S19Y, K31W, N330Y	-3.80
sACE2.7	T27F, K31W, N330Y	-4.08
sACE2.8	S19Y, T27F, N330Y	-4.10
sACE2.9	S19Y, T27F, N330W	-3.83
sACE2.10	S19Y, T27F, K31W	-3.96

Table S2. Binding free energies from MM/GBSA free energy calculations for wildtype and **FFWF** ACE2-RBD systems. All units in kcal/mol (± SEM)

System	ΔE_{VDW}	ΔE_{ELE}	ΔG_{GB}	ΔG_{surf}	ΔG_{bind} (kcal/mol)
Wildtype ACE2	-65.57	-417.66	471.12	-9.69	-21.76 ± 0.16
FFWF	-79.15	-412.43	470.99	-10.82	-31.42 ± 0.13

Table S3. Pairwise decomposition energies from MM/GBSA free energy calculations between ACE2 residues in wildtype (Left-Panel) (S19, T27, K31, and N330) / (B) **FFWF** mutant (Right-Panel) (F19, F27, W31, F330) and SARS CoV-2 RBD domain. Only interactions contributing -0.50 kcal/mol or higher shown.

ACE2 Wildtype Residue	**RBD Residue**	**Interaction Energy (kcal/mol)**	**FFWF Residue**	**RBD Residue**	**Interaction Energy (kcal/mol)**
Ser 19	n/a		**Phe 19**	Ala 475	-1.41
				Tyr 473	-0.65
				Gly 476	-0.62
Thr 27	Phe 456	-1.58	**Phe 27**	Phe 456	-1.79
	Tyr 489	-1.55		Tyr 489	-1.45
	Tyr 473	-0.98		Tyr 473	-1.25
Lys 31	Gln 493	-1.96		Ala 475	-1.00
	Tyr 489	-1.96	**Trp 31**	Tyr 489	-3.86
	Glu 484	-1.42		Phe 490	-1.30
	Phe 456	-1.18		Gln 493	-1.30
	Leu 455	-0.56		Phe 456	-1.06
	Phe 490	-0.50		Leu 455	-0.76
Asn 330	Thr 500	-1.16	**Phe 330**	Thr 500	-2.45
				Pro 499	-0.65

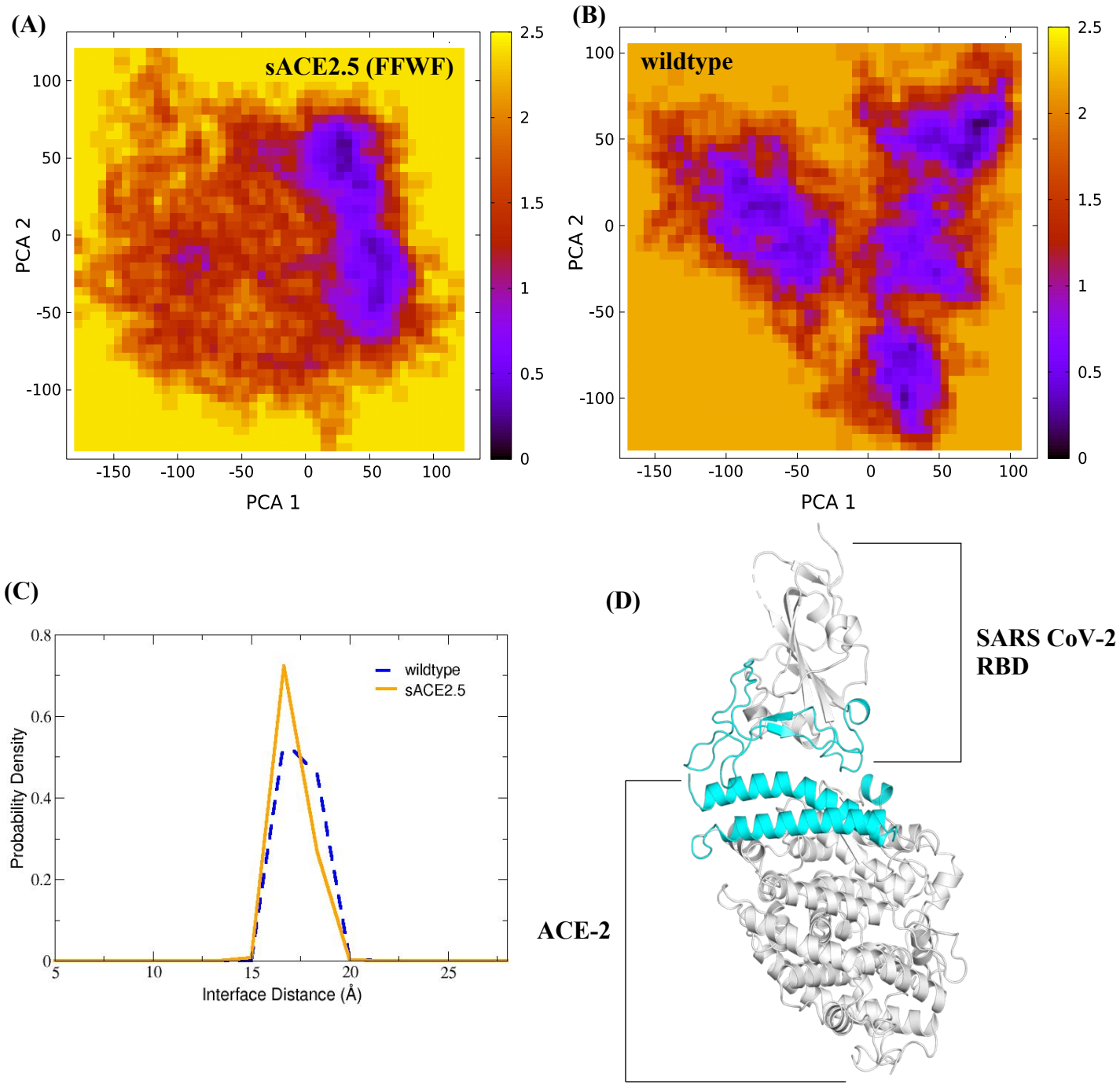

Figure S3. (A) Free energy landscapes (FEL) of **FFWF** decoy and (B) wildtype sACE2 bound to RBD. The FEL values are constructed as a projection of 200 ns MD simulation trajectories onto their own first (PC1) and second (PC2) eigenvectors, respectively, for the interface residues shown in part D. Each residue selection, 19-87 and 325-330 in ACE2 plus 438-506 in the RBD, is labeled by its values from two principal components, PC1 and PC2, and a distribution N of baseline states is obtained with a resolution of 50 x 50 histogram bins. Assuming Boltzmann distribution, $\Delta G = -k_b \cdot T\ln\left(\frac{N}{Nmax}\right)$, the values N are converted into relative Gibbs free energies to yield the

FEL, as also performed by Geist et al.[1] (C) Probability densities of distances between interface residues 19-87, 325-330 in ACE2 and 438-506 in the RBD from MD of wildtype and **FFWF sACE2** decoys. (D) Crystal structure of PDB 6M0J. The interface residues, 19-87, 325-330 in ACE2 and 438-506 in the RBD, used in calculations for both the FEL and distance probability plot are shown in cyan, while the rest of the residues are in gray.

References

(1) Geist, N.; Kulke, M.; Schulig, L.; Link, A.; Langel, W. Replica-Based Protein Structure Sampling Methods II: Advanced Hybrid Solvent TIGER2hs. *J. Phys. Chem. B* **2019**, *123*, 5995–6006. https://doi.org/10.1021/acs.jpcb.9b03134.