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Supporting Information

Targeting SARS-CoV-2 RBD Interface: a Supervised Computational Data-Driven Approach to Identify Potential Modulators

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SUPPORTING INFORMATION
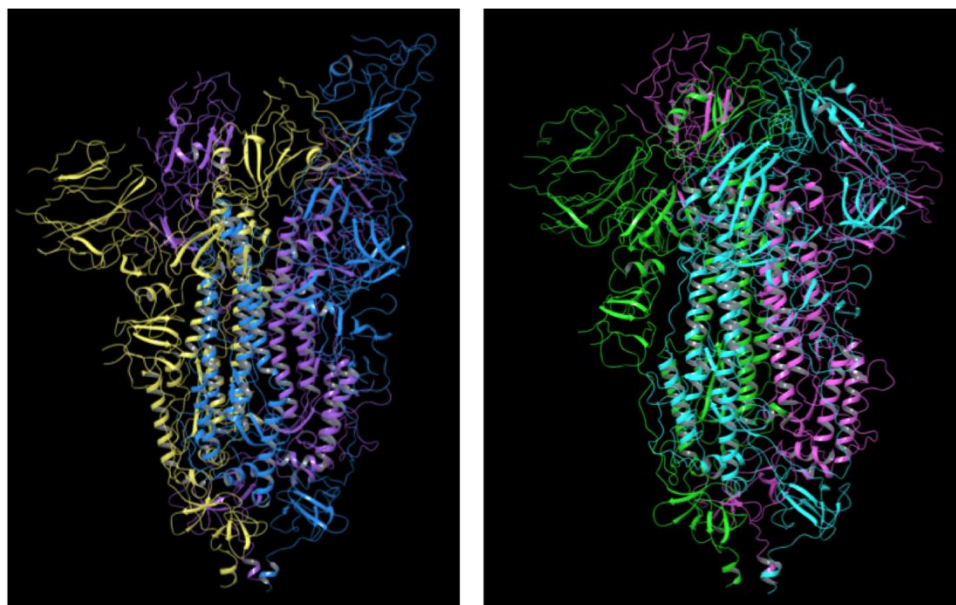
Figure S1. Up state and down state of SARS-CoV-2 receptor-binding motif. On the left, PDB 6VYB: Yellow, pink and blue chains in open conformations; on the right, PDB 6VXX: Green, violet and light blue chains in closed conformations.
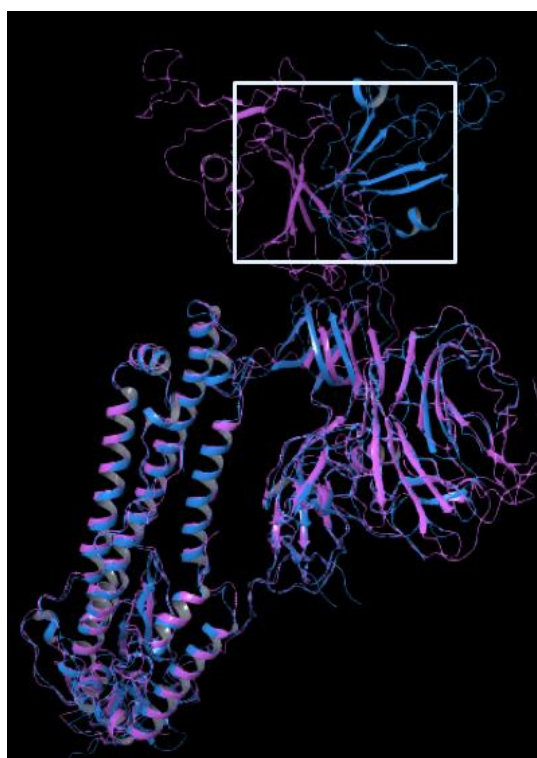
Figure S2. SARS-CoV and SARS-CoV-2 RBDs superposition. SARS-CoV-2 RBD in open state (blue structure – PDB 6VYB); SARS-CoV RBD in open state (pink structure – PDB 6ACD).
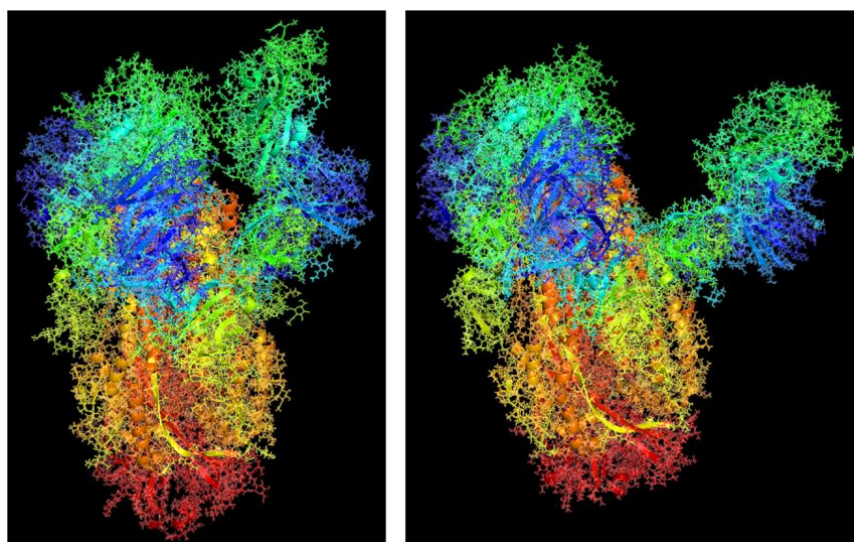
Figure S3. Frames of MD simulation performed using PDB X-ray crystal structure 6VYB. On the left, frame 0 showing the initial partially open state of RBD; on the right, frame 2003 presenting the open state of RBD.
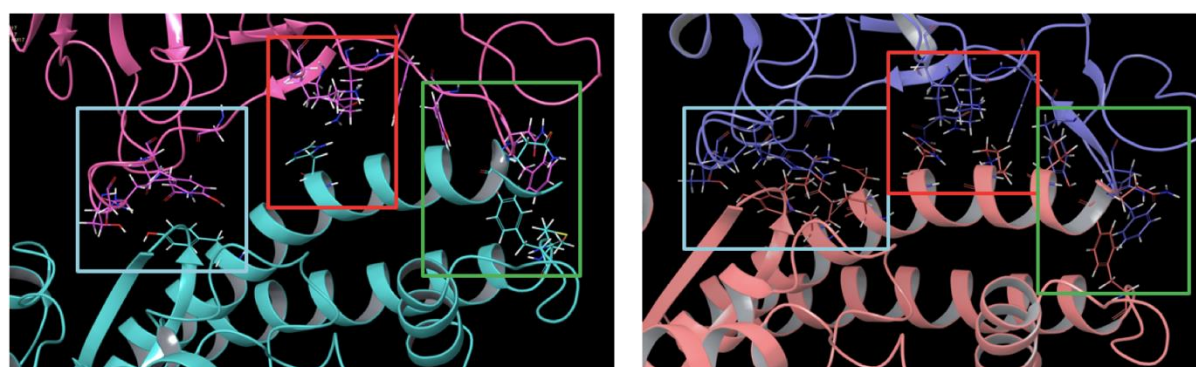
Figure S4. Hot spot residues at the three Spike RBD-ACE2 PD interactions regions. On the left, PDB ID 6M17, the dark pink chain is ACE2, while the light blue chain is S protein; on the right, the purple chain is ACE2, and the pink chain is S protein. In both pictures, the light blue square highlights the N-term, the red one the central region, and the green square indicates the C-term at ACE2-S protein interaction interface.

Table S1. Comparison between RDB residues of SARS-CoV and SARS-CoV-2 at the interface with ACE2. On the left, conserved amino acids between the two proteins; in the middle, residues with similar biochemical or physical properties; on the right, residues marked with * are involved in the interaction with ACE2.

CONSERVED AMINO ACIDS		SIMILAR AMINO ACIDS		DIFFERENT AMINO ACIDS	
SARS-CoV	SARS-CoV-2	SARS-CoV	SARS-CoV-2	SARS-CoV	SARS-CoV-2
Tyr436*	Tyr449	Leu443*	Phe456	Val404	Lys417
Tyr440*	Tyr453	Leu472*	Phe486	Thr433	Gly446
Asn473*	Asn487	Asn479*	Gln493	Pro462*	Ala475
Tyr475*	Tyr489	Thr487*	Asn501	Tyr484*	Gln498
Tyr481	Tyr495	Tyr442*	Leu455		
Gly482*	Gly496				
Thr486*	Thr500				
Gly488*	Gly502				
Tyr491*	Tyr505				

Table S2. Computational alanine scanning results including only ACE2 and SARS-CoV-2 S protein interface residues with $\Delta\Delta G_{affinity}$ values over 3 kcal/mol. The top part of the table shows hot spots residues for PDB 6M17 divided into three regions, N-terminal, central and C-terminal portions. The second part of the table shows hot spot amino acids for PDB 6M0J according to the three regions. The last row of the table provides the total number of hot spots per region considering both PDB structures.
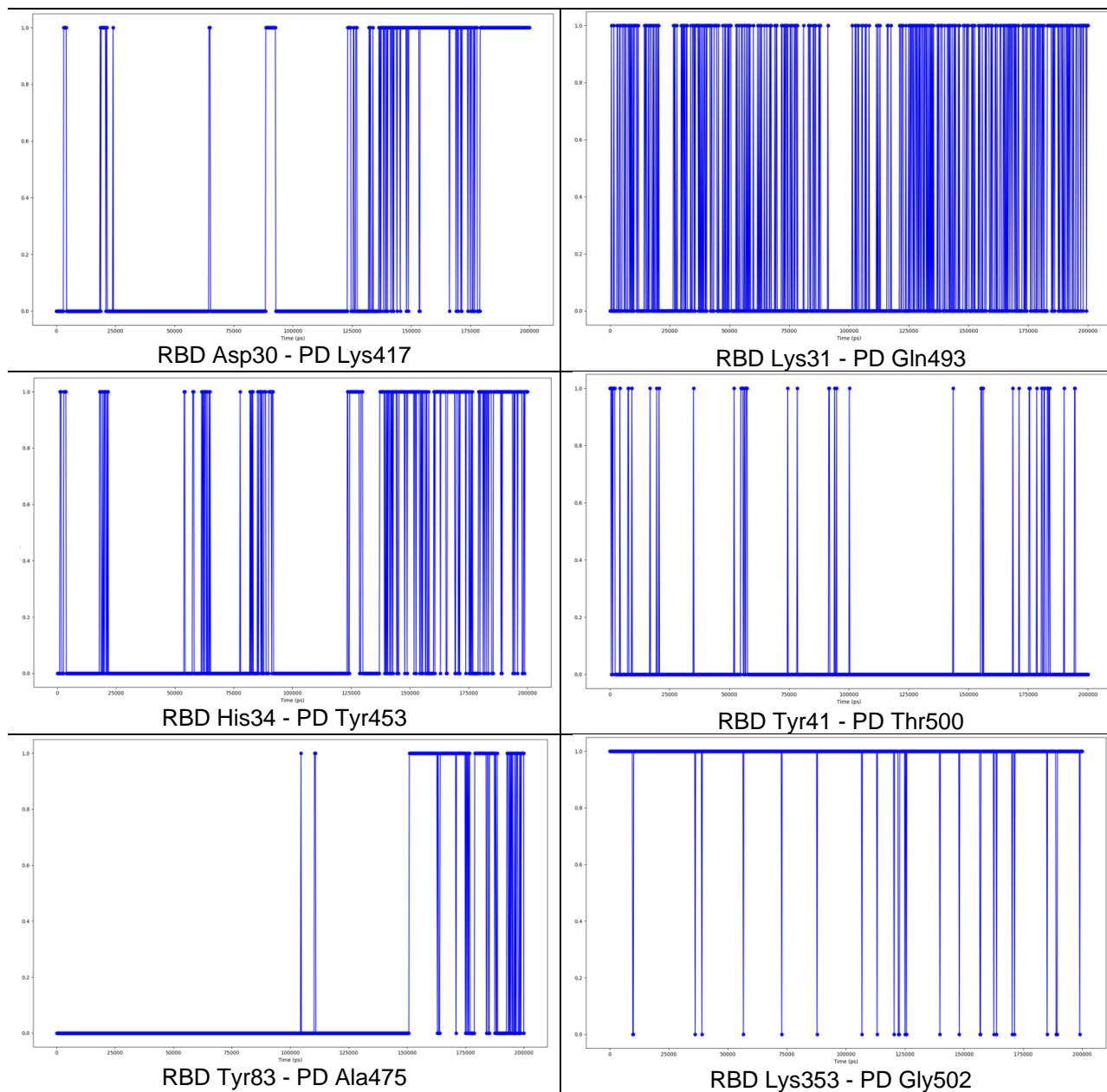
PDB ID: 6M17											
N-TERM				**MIDDLE**				**C-TERM**			
ACE2	$\Delta\Delta G_{affinity}$	**Spike**	$\Delta\Delta G_{affinity}$	**ACE2**	$\Delta\Delta G_{affinity}$	**Spike**	$\Delta\Delta G_{affinity}$	**ACE2**	$\Delta\Delta G_{affinity}$	**Spike**	$\Delta\Delta G_{affinity}$
Tyr41	12.82	Thr500	7.65	His34	8.91	Phe456	4.80	Tyr83	9.28	Phe486	11.93
		Gly496	5.54			Leu455	4.26	Gln24	7.09	Tyr489	7.92
		Asn501	5.29			Gln493	4.23	Met82	5.19	Asn487	3.45
		Tyr505	3.88			Lys417	3.83				
		Gly502	3.81								
		Gln498	3.38								
PDB ID: 6M0J											
N-TERM				**MIDDLE**				**C-TERM**			
ACE2	$\Delta\Delta G_{affinity}$	**Spike**	$\Delta\Delta G_{affinity}$	**ACE2**	$\Delta\Delta G_{affinity}$	**Spike**	$\Delta\Delta G_{affinity}$	**ACE2**	$\Delta\Delta G_{affinity}$	**Spike**	$\Delta\Delta G_{affinity}$
Tyr41	15.18	Tyr505	9.51	Lys31	7.65	Lys417	10.44	Tyr83	8.86	Asn487	10.75
Gln42	7.70	Asn501	7.95	His34	4.18	Gln493	7.96	Gln24	7.67	Phe486	9.90
Lys353	7.03	Thr500	7.53			Leu455	6.73	Thr27	5.97	Tyr489	6.46
Asp38	4.95	Gln498	6.75			Phe456	6.42				
Glu37	4.35	Gly502	6.69								
		Gly496	3.75								
TOTAL HOT SPOTS = 11				**TOTAL HOT SPOTS = 6**				**TOTAL HOT SPOTS = 7**			

Table S3. RMSD plots of MD simulations. On the left, RMSD plot of MD on PDB 6M17 is illustrated, where the system gets stable after about 30 ns of simulation; on the right, RMSD plot of MD on PDB 6M0J is depicted, where the system achieves a stationary shape at about 80 ns.
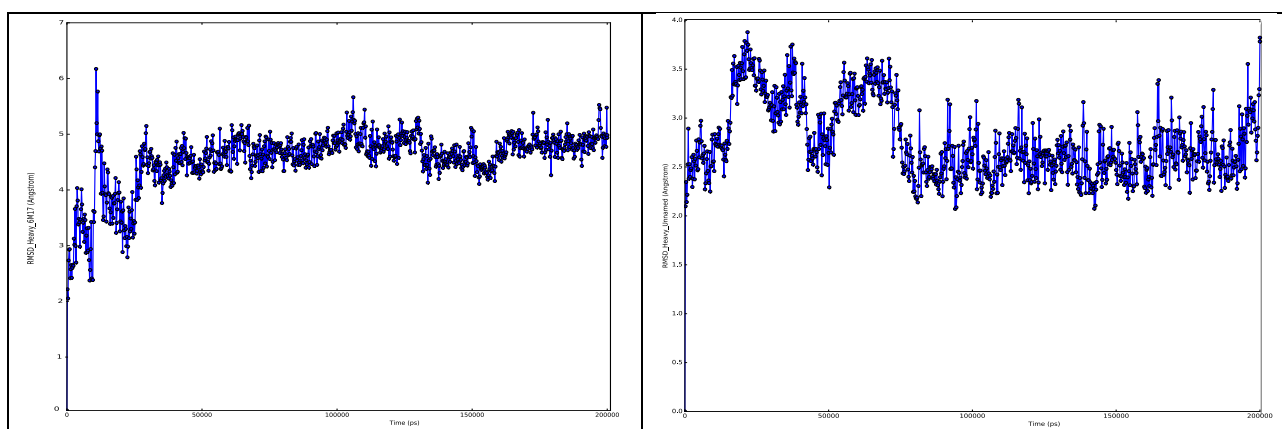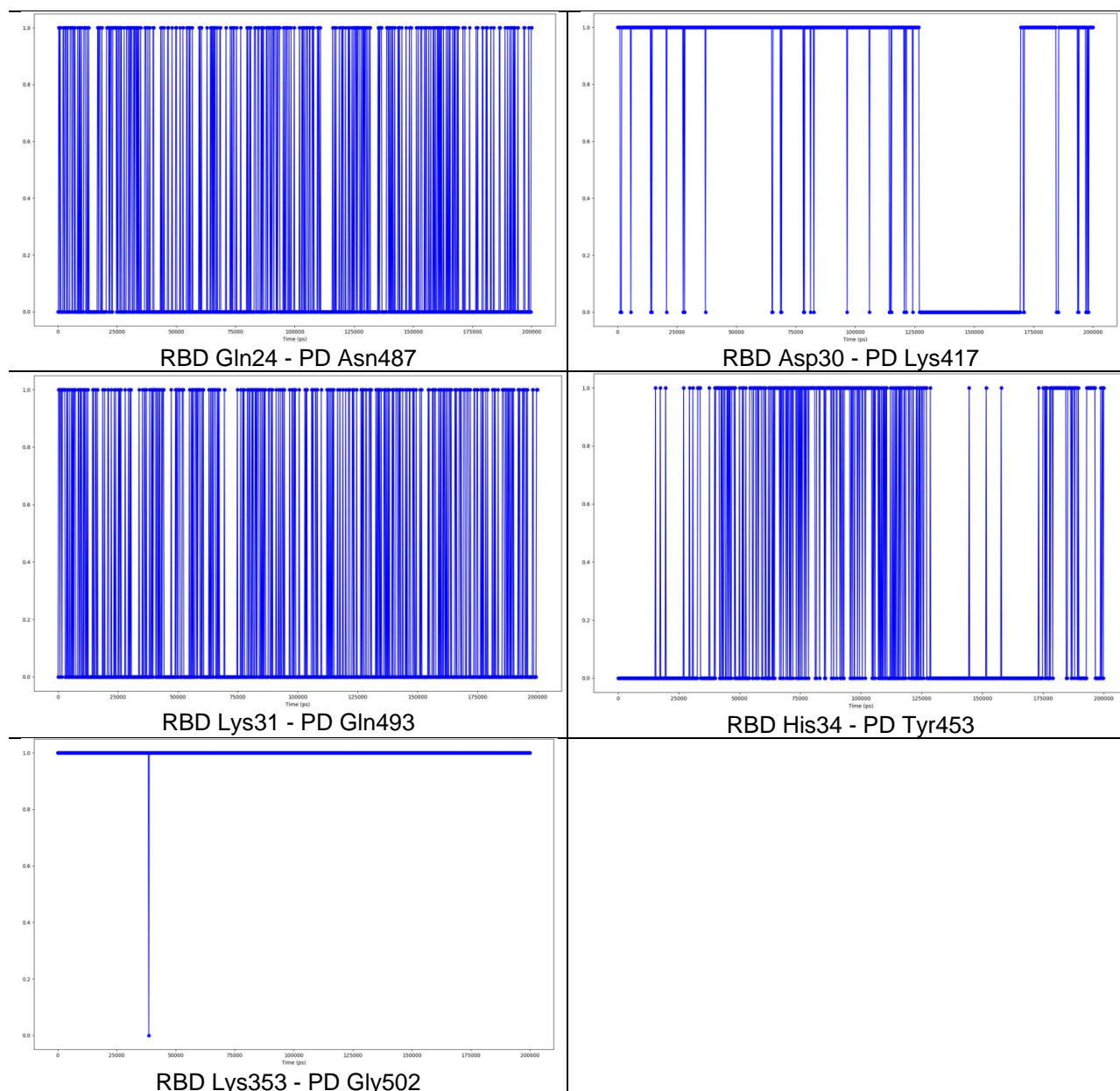
Table S4. H-bond frequency occurrences during MD simulations of PDBs 6M17 and 6M0J. Frequency vs simulation time plots are displayed for those hydrogen bond interactions retrieved from clustered MD frames; blue dots on the top depict one H-bond occurrences connected to the blue dots on the bottom representing no H-bond occurrences.
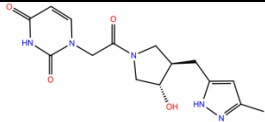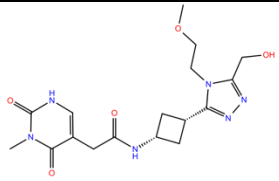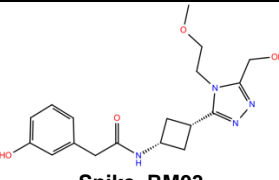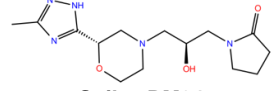
PDB 6M17

RBD Asp30 - PD Lys417

RBD Lys31 - PD Gln493

RBD His34 - PD Tyr453

RBD Tyr41 - PD Thr500

RBD Tyr83 - PD Ala475

RBD Lys353 - PD Gly502

RBD Gln24 - PD Asn487

RBD Asp30 - PD Lys417

RBD Lys31 - PD Gln493

RBD His34 - PD Tyr453

RBD Lys353 - PD Gly502
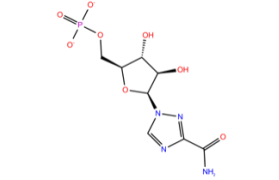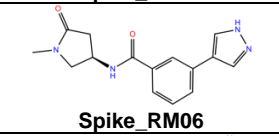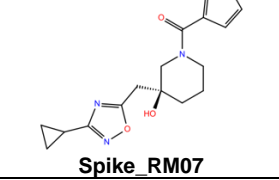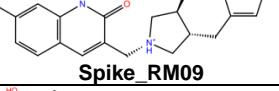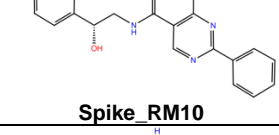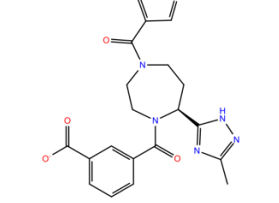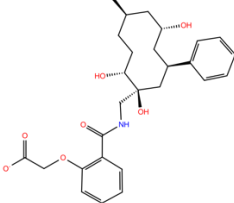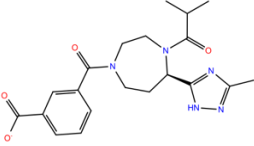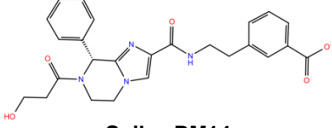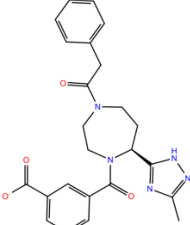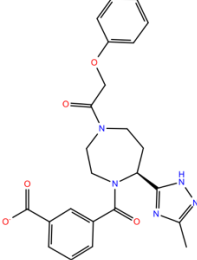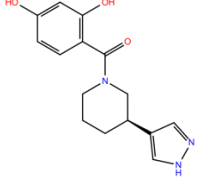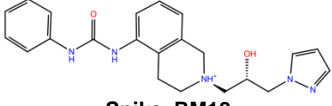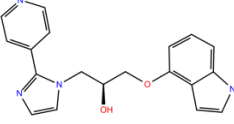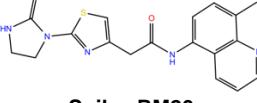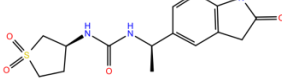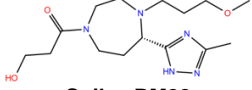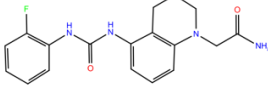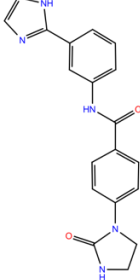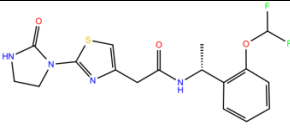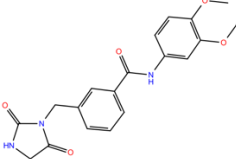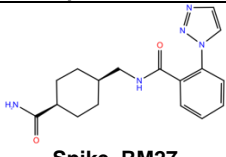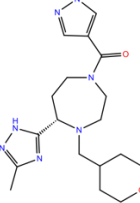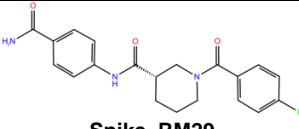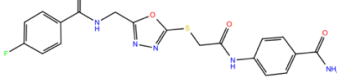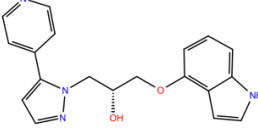
Table S5. Phyco-chemical information of consensus molecules. The table provides information about the compounds retrieved from docking and pharmacophore screenings performed on Spike RBD N-terminal region.

Consensus molecule	MW	cLogP[a]	PSA[a]	Rule of five violations	$\Delta G_{binding}$[b]	Docking score[a]	Pharmacophore-fit score[a]
Spike_RM01	333.346	-0.554	153.327	0	-44.653	-5.318	58.730

Spike_RM02	392.414	-0.237	170.248	1	-48.039	-4.460	58.580
Spike_RM03	360.412	1.224	116.340	0	-58.259	-5.036	58.640
Spike_RM04	309.367	-1.066	109.749	0	-43.861	-5.042	57.410
Spike_RM05	324.186	-2.569	201.174	2	-36.930	-6.836	58.290
Spike_RM06	284.317	0.737	99.295	0	-41.534	-5.562	56.110
Spike_RM07	316.359	2.177	99.412	0	-44.845	-4.381	56.240
Spike_RM08	343.385	0.027	124.934	0	-43.589	-4.290	58.700
Spike_RM09	352.435	1.920	93.900	0	-49.420	-4.935	57.650
Spike_RM10	349.388	2.717	97.396	0	-48.692	-4.690	55.830
Spike_RM11	423.430	1.060	176.119	1	-46.155	-5.676	66.590

Spike_RM12	485.576	3.518	134.235	0	-34.966	-4.281	58.070
Spike_RM13	399.449	1.159	142.345	0	-41.774	-4.894	57.430
Spike_RM14	462.504	2.397	152.169	0	-56.750	-5.320	57.600
Spike_RM15	447.493	1.987	140.299	0	-53.986	-4.834	57.040
Spike_RM16	463.492	1.894	155.876	0	-49.348	-5.003	57.260
Spike_RM17	287.318	1.669	102.195	0	-42.492	-4.944	57.950
Spike_RM18	391.472	2.600	86.270	0	-46.844	-4.568	57.670
Spike_RM19	334.377	3.323	70.653	0	-44.880	-4.897	57.050
Spike_RM20	367.425	2.704	100.627	0	-47.562	-4.494	56.300
Spike_RM21	337.393	-0.002	132.701	0	-35.847	-5.296	65.190

\n**Spike_RM22**	325.410	-0.027	99.560	0	-52.666	-5.164	58.490
\n**Spike_RM23**	342.372	1.291	98.253	0	-45.305	-4.722	56.150
\n**Spike_RM24**	347.376	2.676	106.796	0	-48.338	-5.290	56.090
\n**Spike_RM25**	396.411	3.170	99.040	0	-51.707	-4.996	56.300
\n**Spike_RM26**	369.376	2.247	125.321	0	-48.653	-4.958	55.910
\n**Spike_RM27**	327.385	1.176	112.532	0	-41.896	-4.100	55.870
\n**Spike_RM28**	373.457	0.891	114.365	0	-52.603	-5.720	65.860
\n**Spike_RM29**	369.395	2.229	113.967	0	-50.333	-5.224	55.330
\n**Spike_RM30**	429.425	1.171	157.547	0	-51.658	-4.842	56.210
\n**Spike_RM31**	334.377	3.672	67.779	0	-42.773	-4.987	56.780
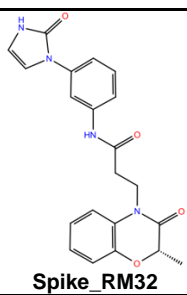
Spike_RM32	392.413	2.555	116.506	0	-49.262	-4.854	56.190

[a] Property computationally calculated; [b] Property computationally calculated as MM-GBSA.