

## Supplementary material for

### ***De novo* design of stapled peptide targeting SARS-Cov-2 spike protein receptor-binding domain**

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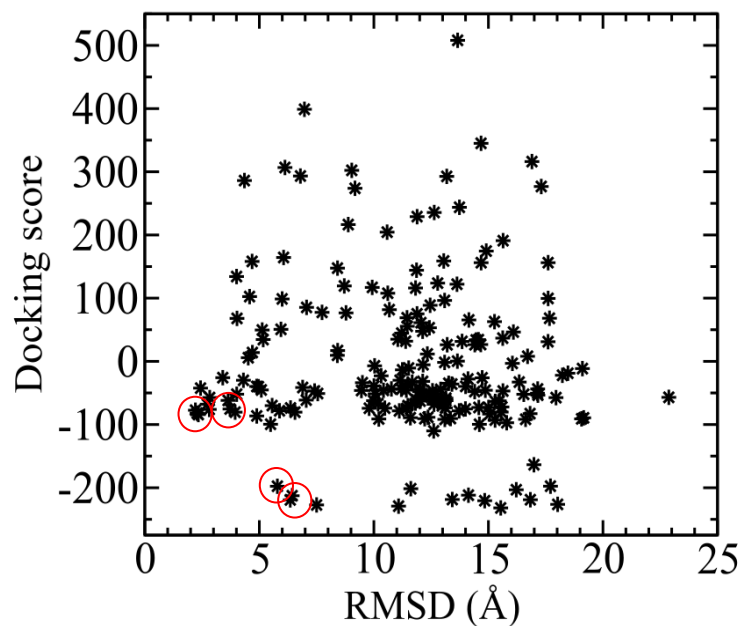
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**Table–S1:** Optimized peptide sequences with Rosetta design score, length of a simulation in which the peptide stayed bound and estimated binding free energy by MM-GBSA method.

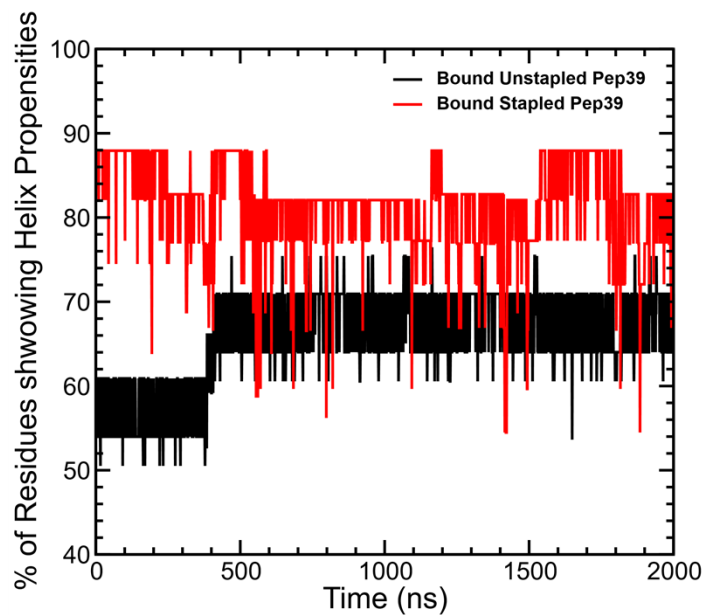
ID	Sequence	Design score	Time Bound (ns)	Rough Estimate of Binding Energy by MMGBSA (kcal/mol)
1	NSLKELTEKEATDKYYK	-232.95	66.6	+10.845 ± 0.586
2	SIEELIKKNKKTIEKYK	-99.762	321	-17.274 ± 0.198
3	EDLKKWAKQLEELKYK	-237.75	198	-24.442 ± 0.369
4	YESLLRFLTKLKDDKYK	-240.42	67.4	-12.467 ± 0.396

5	AEEEEKKAEDKKYK	-239.59	193.4	-14.902 ± 0.334
6	EEYEKMLESLKELKHYK	-105.68	183.4	-29.737 ± 0.351
7	YTFKEKTDHEATDKYYK	-230.47	13.6	-03.171 ± 0.669
8	YTFEVKEKHKRDIKYYK	-91.879	164.6	-12.253 ± 0.322
9	YTFLKKWAHQAEELKYK	-229.85	231.2	-36.851 ± 0.518
10	YTFLEKFLHKLKDKKYK	-236.83	76.2	-12.328 ± 0.405
11	YTFEAKVKHEQEDKYYK	-235.7	12.8	-05.877 ± 0.730
12	YTFEAKKKHEEELRHYK	-92.738	1032.8	-28.023 ± 0.178
13	SEDLAKWAETLERLKYK	-230.58	131.6	-21.556 ± 0.449
14	NVEKRTKEAKAEDSHYK	-234.14	1572.2	-30.562 ± 0.164
15	SIEELIKRLKKTTEEKYK	-220.31	1148.6	-24.423 ± 0.131
16	YDDLEKWKQLEELKYK	-229.13	31.2	-10.648 ± 0.376
17	EEEEKTKKILTELAKYK	-218.78	265.4	-24.564 ± 0.311
18	STEDEEKKAEDSLYK	-238.36	165.8	-12.937 ± 0.340
19	STDQEYKKAVEHDRKYK	-233.12	13.6	-07.521 ± 0.551
20	PDDLKIWAQILLEAFYK	-232.02	2000	-34.147 ± 0.097
21	SIEDKIKKAVVAKYK	-229.62	245.2	-18.212 ± 0.289
22	QTQDEWKKAVEEEKYYK	-234.59	155.8	-29.358 ± 0.118
23	STDDEKKKALAEDQQYK	-236.3	1039.4	-17.186 ± 0.152
24	SELEKALKALSSLSTYK	-230.04	351.8	-22.292 ± 0.182
25	EEEEKKTAKKELDTYK	-226.02	274.6	-25.472 ± 0.336
26	YEELKKWAKTLEEAKYK	-228.17	239.4	-16.526 ± 0.170
27	SLEDLIEEAKRKIEKYK	-219.24	371.2	-16.070 ± 0.169
28	TFLEEWLQKEELKLYK	-172.52	2000	-28.882 ± 0.092
29	TFLELWKRQAKDLRYK	-181.95	435.4	-21.133 ± 0.240
30	TFLEKWLQADDLKNK	-175.92	111	-22.651 ± 0.477
31	DELEITADKLRQAMEK	-233.77	365	-14.595 ± 0.178
32	DELEVLAEQREALAK	-230.03	83.8	-15.091 ± 0.310
33	DPWEIWAKLLKESRDK	-237.51	1421	-29.714 ± 0.085
34	GTEWLYKVKAFVENMS	NA	1350.8	-29.688 ± 0.122
35	STEWLKKVHWKFHAKFS	NA	29.6	-03.422 ± 0.468
36	GTEEIYKRWATVMKXXX	NA	880.2	-11.344 ± 0.098

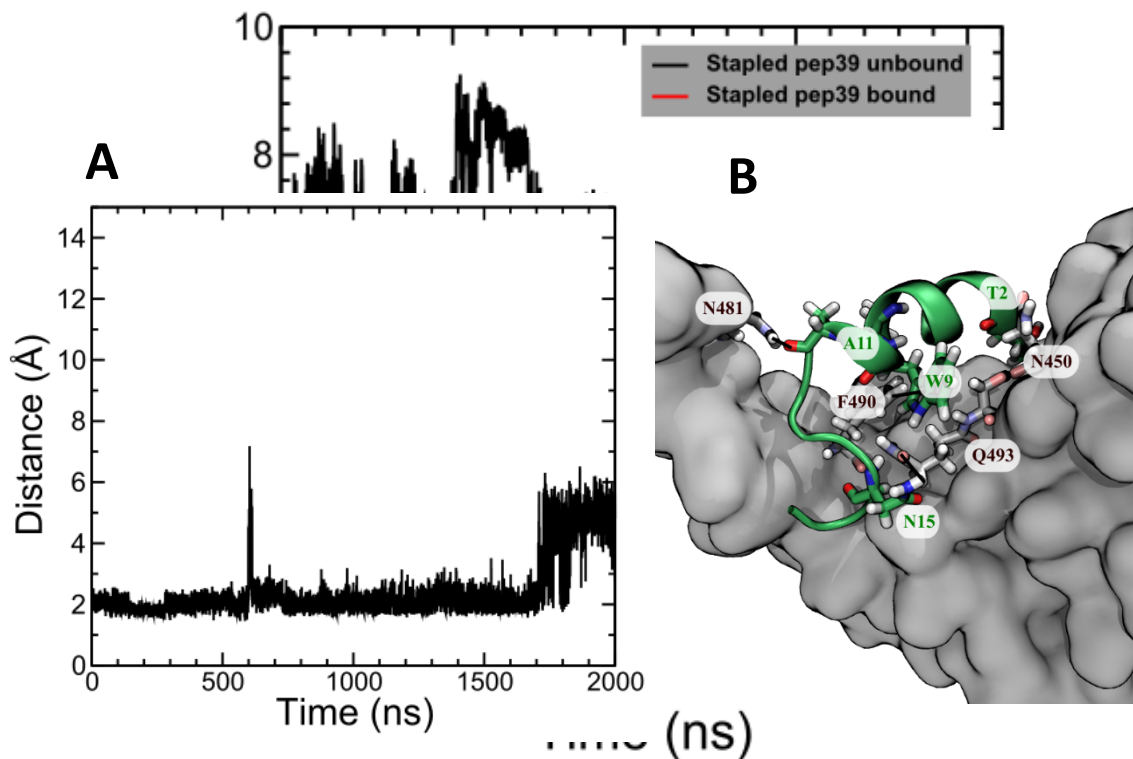
37	GTEWLKKVWEAFVKNMS	NA	189.4	$-28.698 \pm 0.335$
38	GTEEIYLLWMVTVHKQKK	NA	151	$-17.313 \pm 0.471$
39	STEYLLKRIFWFMFHEYES	NA	342	$-29.252 \pm 0.217$
40	NVEKRTKEAKAEDSHYK	NA	1253.8	$-45.021 \pm 0.153$
41	QTQDEWKKAVEEEKKYK	NA	1014.12	$-23.381 \pm 0.116$



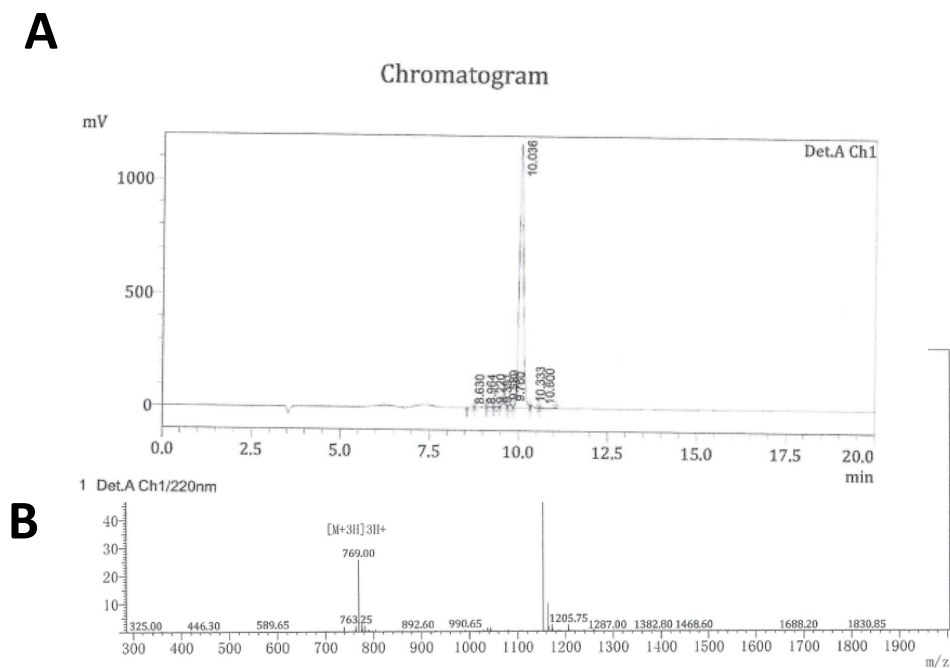
**Figure–S1** Docking score versus RMSD plot for docked poses of the template peptide on the spike protein RBD surface.



**Figure–S2** The percentage of residues showing  $\alpha$  Helix propensities for stapled and unstapled peptide in the bound state.



**Figure-S4 (A)** Distance of the pep39 from the ACE2 binding site on the spike protein RBD. **(B)** 3D view of the interaction between designed peptide and spike protein RBD.



**Figure-S5 (A)** HPLC chromatogram and **(B)** MS spectrum graph of the stapled peptide



**Table-S2:** Optimized peptide sequences with time remaining bound during the MD simulation for stapled and unstapled peptides and the estimated binding free energy by MM-GBSA method

Sl. No	ID	Time Bound (ns)		Rough Estimate of Binding Energy by MMGBSA (kcal/mol)		Absolute Binding Free Energy (Stapled) (kcal/mol)
		Stapled	Unstapled	Stapled	Unstapled	
1	Ngu-123	2000	189.4	$-24.958 \pm 0.063$	$-24.958 \pm 0.063$	NA
2	Ngu-335	1547	342	$-28.557 \pm 0.106$	$-29.252 \pm 0.217$	NA
3	Ngu-129	2000	2000.0	$-29.000 \pm 0.072$	$-34.147 \pm 0.097$	NA
4	Ngu-129a	371		$-18.303 \pm 0.144$		NA
5	Ngu-29	2000	1253.8	$-33.970 \pm 0.119$	$-45.021 \pm 0.153$	$-05.02 \pm 15.37$
6	Ngu-29a	162		$-14.611 \pm 0.901$		NA
7	Ngu-29b	2000		$-44.746 \pm 0.122$		$-13.31 \pm 01.63$
8	Ngu29c	77.6		$-12.620 \pm 0.455$		NA
9	Ta-93	1398.8	198.0	$-33.945 \pm 0.151$	$-36.851 \pm 0.518$	$-17.41 \pm 03.10$
10	Ta-200	18.2	1421.0	$-13.137 \pm 0.491$	$-29.714 \pm 0.085$	NA
11	Ta-196	415.4	1032.80	$-21.832 \pm 0.213$	$-28.023 \pm 0.178$	NA
12	Ta-26	189.8	2000	$-29.407 \pm 0.259$	$-28.882 \pm 0.092$	NA
13	pep-39	2028.8	1350.8	$-33.936 \pm 0.071$	$-29.688 \pm 0.122$	$-26.32 \pm 08.64$
14	pep-39a	2000		$-28.884 \pm 0.055$		NA
15	pep-39us	1222		$-27.037 \pm 0.076$		NA