Supporting Information

Discovery of Aptamers Targeting the Receptor-Binding Domain of the SARS-CoV-2 Spike Glycoprotein

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EXPERIMENTAL SECTION

Reagents

Protein A beads for Fc-tagged-RBD conjugation was purchased from Sino Biological Inc. (China). Streptavidin-coated sepharose beads used in SELEX and Ni beads for His-tagged RBD conjugation were from GE Healthcare (USA). And Fc-tagged-RBD of SARS-CoV-2 Spike Protein (40592-V05H), His-tagged-RBD of SARS-CoV-2 Spike Protein (40592-V08B), SARS-CoV-2 Spike S1+S2 ECD-His recombinant protein (40589-V08B1), human ACE2 and mouse IgG were purchased from Sino Biological Inc. (China). All DNA sequences were synthesized by Sangon Biotech with HPLC purification (Shanghai, China). The binding buffer (PBS, pH=7.4, including 136.8 mM NaCl, 10.1 mM Na_2HPO_4, 2.7 mM KCl, 1.8 mM KH_2PO_4, 0.55 mM $MgCl_2$), was used for aptamer selection and characterization.

Library Preparation

The PCR mixture contained 0.4μM each primer, 0.2 μM each dNTP, 2.5 U Easy-Taq DNA polymerase, and Easy-Taq DNA polymerase buffer in a total volume of 50 μL. The thermal cycling conditions of PCR were described as follows: after preheating at 95°C for 3 min, 9 thermal cycles of 95°C for 30 s, 56.5°C for 30 s and 72°C for 30 s. PCR amplification product incubated with SA-beads for 15 min at room temperature to capture biotinylated PCR product. After denaturing in 0.1 M NaOH for 1 min at room temperature and desalting by 3K ultrafiltration, the sense ssDNA strands were then generated in the solution for quantitation and the next round of selection.

Flow Cytometry Analysis

To monitor the enrichment of selected libraries or determine the binding affinity of selected aptamers, positive RBD-Ni-beads or negative Ni-beads were incubated with 200 nM FAM labeled library or the candidate sequences in 200 μL binding buffer at 25℃ for 30 min. The beads were washed twice using binding buffer and suspended in 200 μL binding buffer. The fluorescence intensity of beads was measured by flow cytometry (FACSVerse, BD) by counting about 1000 events. The K_d value of the aptamers were obtained by the fluorescence intensity of a series of different concentration of ligands to the equation $Y = B_{max} * X/(K_d + X)$ through SigmaPlot software.

In competition assay of the two aptamers, 200 nM FAM labeled one kind aptamer and another aptamer without labeling incubated with RBD-Ni-beads for 30 min at 25℃. The beads were washed twice using binding buffer and suspended in 200 μL binding buffer for flow cytometry analysis.

To measure the competition efficiency, 200 nM FAM labeled aptamer incubated with RBD-Ni-beads for 30 min at 25℃. The recovered beads were washed twice by binding buffer and resuspended with 200 μL of binding buffer containing ACE2. After incubation with ACE2 for 30 min, the beads were washed twice and resuspended into 200 μL binding buffer for flow cytometry analysis.

Molecular Docking and Dynamic Simulations

The structure of SARS-CoV-2 S protein with a single RBD was obtained from the RCSB PDB data bank (http://www.rcsb.org, ID: 6VSB). The secondary structure with the minimum free energy of aptamer was predicted by mfold web server (http://mfold.rna.albany.edu/?=mfold). Using the predicted secondary structure of the aptamer as a starting point, the corresponding 3D structures of

the equivalent ssRNAs were then modeled and visualized in RNAcomposer. Then the 3D structures of CoV2-RBD-1C and CoV2-RBD-4C aptamers were then obtained by substituting bases T for U. A refinement process was carried out based on molecular dynamics simulation (MDS) to relax the aptamer system.

Molecular docking was performed with Rosetta after obtaining the 3D structures of aptamers and their target. The Amber FF99SB and AMBER PARM99 force fields were used for protein and aptamer system respectively. The final average structure was obtained based on 5000 snapshots, which were extracted from the last 10 ns trajectory of MDS using the Gromacs software.

Table S1. Sequences of the selected aptamers (Sequences in bold are primers)

Name	Sequence (5'-3')
CoV2-RBD-1	**ATCCAGAGTGACGCAGCA**CCGACCTTGTGCTTTGGGAGTGCTG GTCCAAGGGCGTTAA**TGGACACGGTGGCTTAGT**
CoV2-RBD-2	**ATCCAGAGTGACGCAGCA**TCGAGTGGTGGGCTGGTCGGGTTTG GATTCCCTTAGATGC**TGGACACGGTGGCTTAGT**
CoV2-RBD-3	**ATCCAGAGTGACGCAGCA**CTGCGTAGGCGCGGCCAATGTGTAG GATTGCTCAGGTCTGC**TGGACACGGTGGCTTAGT**
CoV2-RBD-4	**ATCCAGAGTGACGCAGCA**TTTCATCGGGTCCAAAAGGGGCTGC TCGGGATTGCGGATA**TGGACACGGTGGCTTAGT**
CoV2-RBD-5	**ATCCAGAGTGACGCAGCA**GGACTGCTTAGGATTGCGAAGCTGA GGAGCTCCCCCGCCT**TGGACACGGTGGCTTAGT**
CoV2-RBD-6	**ATCCAGAGTGACGCAGCA**GTAGGGGGATTGGCTCCAGGGCCTG GCTGACGGTTGCACG**TGGACACGGTGGCTTAGT**
CoV2-RBD-1C	**CAGCA**CCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTA A**TGGACA**
CoV2-RBD-4C	**ATCCAGAGTGACGCAGCA**TTTCATCGGGTCCAAAAGGGGCTGC TCGGGATTGCGGATA**TGGACACGT**

Table S2. Detailed multidimensional scores and family size of each aptamer output by SMART-Aptamer V2.0

Aptamer	Family	Fscore	Kscore	Sscore	Pscore	MDA Score	Family size		
							7th	9th	12th
CoV2-RBD-1	1	10	0.55	7.42	1.00	8.71	204	1976	8479
CoV2-RBD-2	2	10	1.78	6.42	1.00	8.21	66	2380	4489
	3	10	2.62	6.39	1.00	8.20	649	6489	8026
CoV2-RBD-3	4	10	1.88	6.22	1.00	8.11	261	2872	9350
CoV2-RBD-4	5	10	1.99	6.06	1.00	8.03	1184	5671	4888
CoV2-RBD-5	6	10	10.00	4.62	0.80	8.00	2500	29002	17688
	7	10	0.47	5.54	1.00	7.77	11	100	522
CoV2-RBD-6	8	10	1.71	5.33	1.00	7.67	64	789	2091
	9	10	1.19	5.20	1.00	7.60	158	1513	3736
	10	10	2.29	4.70	1.00	7.35	111	1024	1916

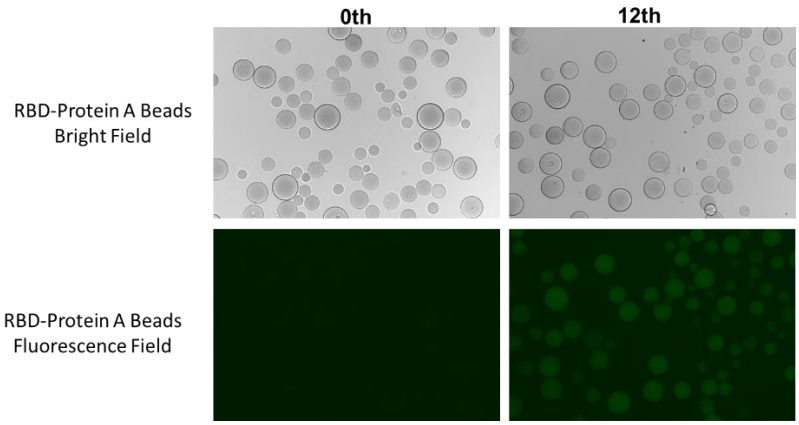

Figure S1. Fluorescent images to monitor the binding performance of 12th library to RBD-Protein A-beads.

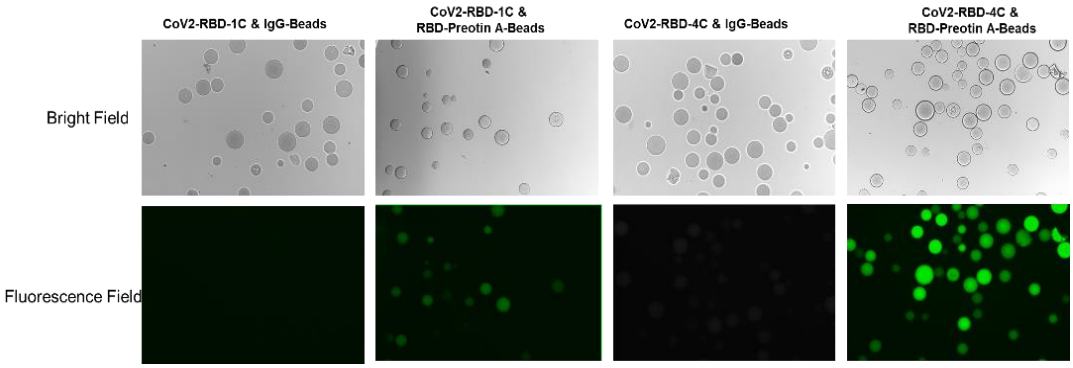

Figure S2. Fluorescent images to monitor the binding performance of CoV2-RBD-1C and CoV2-RBD-4C aptamers to target beads (RBD-Protein A-beads) and control beads (IgG-Beads).

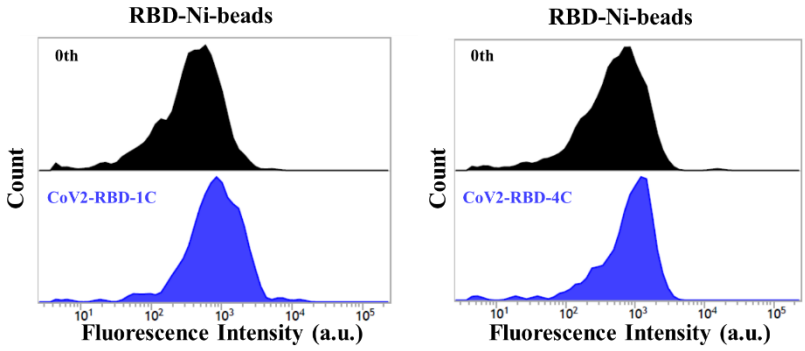

Figure S3. Flow cytometric analysis of CoV2-RBD-1C (left) and CoV2-RBD-4C (right) aptamers binding to target in 80% FBS.

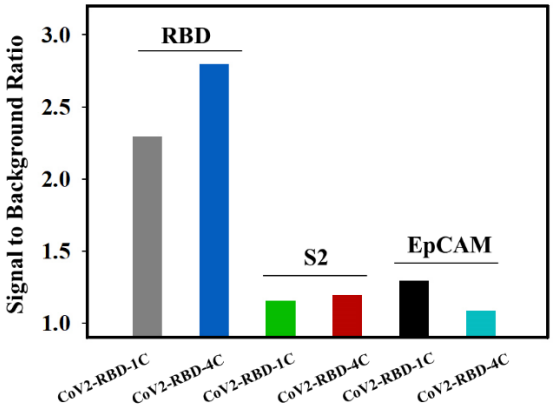

Figure S4. Specificity assay of CoV2-RBD-1C and CoV2-RBD-4C aptamers.

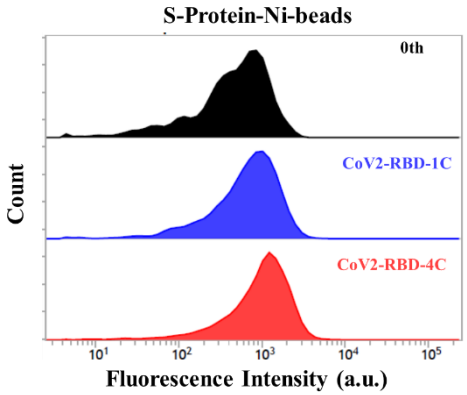

Figure S5. Flow cytometric analysis of CoV2-RBD-1C and CoV2-RBD-4C aptamers binding to SARS-CoV-2 Spike glycoprotein.

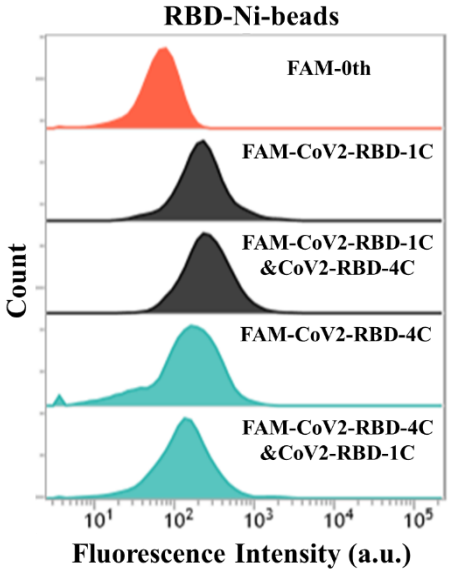

Figure S6. Competition assay of CoV2-RBD-1C and CoV2-RBD-4C aptamers.