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Supplementary Information

Broadly SARS-CoV-2 aptamers overcome variant antigenic shift

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Table of contents

EXPERIMENTAL SECTION

1. Materials and Methods

1.1 Reagents

Ni beads (Cat: 17526801) and Streptavidin sepharose beads (Cat:17511301) were obtained from GE Healthcare (USA). SARS-CoV-2 Spike RBD protein (His Tag) (Cat: 40592-V08B) (Baculovirus-Insect cell), SARS-CoV-2 D614G Spike protein (His Tag) (Cat: 40589-V08B4) (Baculovirus-Insect cell), SARS-CoV-2 B.1.1.529 (Omicron) Spike RBD protein (His Tag) (Cat: 40592-V08H121) (HEK293 cell) and SARS-CoV-2 B.1.1.529 (Omicron) S1+S2 trimer protein (ECD, His Tag) (Cat: 40589-V08H26) (HEK293 cell), and Human EpCAM protein (ECD, His Tag) (Cat: 10694-H08H) (HEK293 cell) were obtained from Sino Biological Inc (China). SARS-CoV-2 (2019-nCoV) Spike trimer, a recombinant protein for six stabilizing proline substitutions[1], was provided by Yuhe Renee Yang Lab of Tsinghua University. All DNA sequences were synthesized by Sangon Biotechnology with HPLC purification (Shanghai, China). 3,3′-dioctadecyloxacarbocyanine perchlorate (DiO) (C1038) was obtained from Beyotime. 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 characterization. Dulbecco's Modified Eagle's Medium (DMEM) was purchased from Hyclone (USA). Nasopharyngeal swab solution (TBS buffer, including 1.6% triton, 0.5 mM $Mg(NO_3)_2$, 10 mM NaCl). Fetal bovine serum (FBS) was obtained from BioInd (Israel) and penicillin-streptomycin from Thermo (USA). Cell culture dishes/plates/bottles and centrifuge tubes were obtained from NEST Biotechnology Co. Ltd. (Wuxi, China).

1.2 Cells and pseudoviruses

ACE2-transfected HEK293T cells and Pseudovirus-SARS-CoV-2 (B.1.1.529), and Pseudovirus-h CoV-HKU1-N were obtained from Fubio Biotechnology Co., Ltd. (Suzhou, China). Pseudovirus-SARS-CoV-2 (B.1.1.529) was packaged with SARS-CoV-2 (B.1.1.529) Spike protein as a surface capsid glycoprotein with a lentivirus packaging system, and the RNA genome encoding the CMV-promoter, GFP, IRES and luciferase. Pseudovirus-h CoV-HKU1-N was packaged with VSV-G glycophorin as a surface capsid glycoprotein. Cells were cultured in high glucose DMEM with 10% FBS and 1% penicillin-streptomycin at 37 °C in a 5% CO_2 atmosphere.

1.3 Aptamer binding assay by flow cytometry analysis

Ni-beads were conjugated with His-tagged RBD to generate RBD-beads. Similarly, Spike-beads, D614G-Spike-beads, Omicron RBD-Beads and Omicron Spike-beads as well as EpCAM beads were generated with a similar procedure. Aptamers were annealed by incubation at 95 °C for 5 minutes (min), followed by cooling at 4 °C for 10 min and at room temperature for 10 min. To assess the binding performance of different aptamers, the fluorescence signals of all aptamers were determined and normalized, RBD-beads were incubated with 200 nM different fam-labeled aptamers in 200 μL 0.3% BSA binding buffer at room temperature for 30 min. After washing twice with 0.3% BSA binding buffer and suspended in 200 μL binding buffer. The mean fluorescence intensity was measured by flow cytometry (FACSVerse, BD) from approximately 1000 acquired events. Using the same conditions and formats, we evaluated other targets including Spike trimer, D614G Spike, Omicron RBD, Omicron Spike and EpCAM. As a control, the same experimental procedure was used for the random sequence (50nt-3). The relative mean fluorescence of the aptamers was calculated by the following equation:

(the fluorescence signal of the sample with the aptamer incubation) / (the fluorescence signal of the sample with the random sequence (50 nt-ssDNA)).

To determine the binding affinity (K_D) of each aptamer against Omicron RBD, fam-labeled aptamers were titrated by serial dilution and incubated with Omicron RBD-beads in 200 μL binding buffer containing 0.3% BSA at room temperature for 30 min. After washing twice with 0.3% BSA binding buffer, the beads were suspended in 200 μL binding buffer for flow cytometry (FACSVerse, BD). The K_D value of aptamers was obtained by the mean fluorescence intensity to the equation: $Y=B_{max}*X/(K_D+X)$ through SigmaPlot software.

1.4 Aptamer competition assay by flow cytometry analysis

The competition assay to determine the binding site of CoV2-RBD-4C, CoV2-6C3, MSA5 and Aptamer2 on SARS-CoV-2 spike protein by flow cytometry. FAM labelled 200 nM CoV2-RBD-4C was used as the target aptamer, while 500 nM non-fluorescent CoV2-6C3 or MSA5 or Aptamer2 aptamer was added for competition. Similarly, FAM labelled CoV2-6C3, MSA5 or Aptamer2 were used to compete with other non-fluorescent aptamer with a similar procedure.

1.5 Virus-level specific binding validation assay

Streptavidin sepharose beads was blocked with 0.6% BSA binding buffer for 1 hour before use. CoV2-RBD-4C, CoV2-6C3, MSA5, Aptamer2, SNAP1, SP6 and SARS2-AR10 aptamers were annealed by incubation at 95 °C for 5 min, followed by cooling at 4 °C for 10 min and at room temperature for 10 min. To assess the specific binding performance of different aptamers against SARS-CoV-2, Omicron pseudovirus and human CoV-HKU1-N pseudovirus were incubated with 200 nM biotin-labeled CoV2-RBD-4C, CoV2-6C3, MSA5, Aptamer2, SNAP1, SP6 and SARS2-AR10 in 200 μL 0.3% BSA binding buffer at room temperature for 30 min, respectively. Next, the Streptavidin sepharose beads were incubated with the mixture for another 30 min. Then 10 μL DiO (20 μM) was incubated with the mixture at 37°C for 20 min. After washing twice with 0.3% BSA binding buffer and suspended in 200 μL binding buffer. The mean fluorescence intensity was measured by flow cytometry (FACSVerse, BD).

1.6 Pseudovirus neutralization analysis

To compare the neutralization activity of CoV2-RBD-4C, CoV2-6C3, MSA5, Aptamer2, SNAP1, SP6 and SARS2-AR10, pseudovirus neutralization assay was performed as previous described[2]. 500 nM CoV2-RBD-4C, CoV2-6C3, MSA5, Aptamer2, SNAP1, SP6 and SARS2-AR10 aptamers were incubated with pseudovirus in DMEM containing with 10% FBS at 37 °C for 1 hour (h). Then, the mixtures were added to ACE2-transfected HEK293T cells. After 8h infection, the medium was refreshed and cells were cultured for another 48 h. Finally, cells were imaged by fluorescence microscope (Nikon Eclipse Ti) and the cell infection was reflected by GFP fluorescence expression. Fluorescence intensity was analyzed by Image J software.

The efficiency of infection was analyzed by taking cells from three different areas and recording the total number of cells (N_{total}) and the number of infected cells (fluorescent cells) ($N_{infected\ cell}$). The Proportion of uninfected cells was calculated using the equation:
$P= (1- (N_{infected\ cell} / N_{total}))$.

Supporting Table

Table S1. Sequences of the aptamers used in this work

Name	Sequence (5'-3')	Length(bp)	Ref.
Random sequence (50 nt-3)	AGGAATAATCAGGTTGTCGAGATGAGTACTAGTGTCCCTTCCGTTCTTGA	50	/
CoV2-RBD-1C	CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTAATGGACA	51	[3]
CoV2-RBD-4C	ATCCAGAGTGACGCAGCATTTCATCGGGTCCAAAAGGGGCTGCTCGGGATTGCGGATATGGACACGT	67	[3]
CoV2-6C3	CGCAGCACCCAAGAACAAGGACTGCTTAGGATTGCGATAGGTTCGG	46	[2]
MSA1	TTACGTCAAGGTGTCACTCCCACTTTCCGGTTAATTTATGCTCTACCCGTCCACCTACCGGAAGCATCTCTTTGGCGTG	79	[4]
MSA5	TTACGTCAAGGTGTCACTCCACGGGTTTGGCGTCGGGCCTGGCGGGGGGATAGTGCGGTGGAAGCATCTCTTTGGCGTG	79	[4]
MSA52	TTACGTCAAGGTGTCACTCCGTAGGGTTTGGCTCCGGGCCTGGCGTCGGTCGTCTCTCGCGAAGCATCTCTTTGGCGTG	79	[5]
Aptamer1	ATCCAGAGTGACGCAGCATCGAGTGGCTTGTTTGTAATGTAGGGTTCCGGTCGTGGGTTGGACACGGTGGCTTAGT	76	[6]
Aptamer2	ATCCAGAGTGACGCAGCAATTACCGATGGCTTGTTTGTAATGTAGGGTTCCGTCGGATTGGACACGGTGGCTTAGT	76	[6]
Aptamer6	ATCCAGAGTGACGCAGCAGGGCTTGGGTTGGGAATAAGGATGTGGGAGGCGGCGAACATGGACACGGTGGCTTAGT	76	[6]
n-CoV-S1-Apt1	AGCAGCACAGAGGTCAGATGCCGCAGGCAGCTGCCATTAGTCTCTATCCGTGACG GTATGCCTATGCGTGCTACCGTGAA	80	[7]
S14	TGGGAGCCTGGGACATAGTGGGGAAAGAGGGGAAGAGTGGGTCT	44	[8]
SNAP1	TCGCTCTTTCCGCTTCTTCGCGGTCATTGTGCATCCTGACTGACCCTAAGGTGCGAACATCGCCCGCGTAAGTCCGTGTGTGCGAA	86	[9]
SP6	GGGAGAGGAGGGAGATAGATATCAACCCATGGTAGGTATTGCTTGGTAGGGATAGTGGGCTTGATGTTTCGTGGATGCCACAGGAC	86	[10]
SARS2-AR10	CCCGACCAGCCACCATCAGCAACTCTTCCGCGTCCATCCCTGCTG	45	[11]

Table S2. **Overview of aptamers for SARS-CoV-2 (ND means "no description")**

Name	Target	K_D	Assay buffer	Assay formats	neutralization/detection	Ref.
CoV2-RBD-1C	S/RBD	5.8 ± 0.8 nM	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 $MgCl_2$)	flow cytometry	ND	[3]
CoV2-RBD-4C	S/RBD	19.9 ± 2.6 nM			ND	[3]
CoV2-6C3	S/RBD	44.78 ± 9.97 nM			neutralization	[2]
MSA1	S1	1.8 ± 0.4 nM	1xSB (50 mM HEPES, pH=7.4, 150 mM NaCl, 6 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM CaCl2, 0.01% Tween-20)	dot blot assay	detection	[4]
MSA5	S1	2.7 ± 0.5 nM			ND	[4]
MSA52	S	2 – 10 nM			detection	[5]
Aptamer1	S/RBD	6.05 ± 2.05 nM	DPBS (1mM Mg^{2+})	flow cytometry and surface plasmon resonance (SPR)	neutralization	[6]
Aptamer2	S/RBD	6.95 ± 1.10 nM			neutralization	[6]
Aptamer6	S/RBD	7.52 ± 3.02 nM			No neutralization	[6]
n-CoV-S1-Apt1	S1(RBD)	0.327 ± 0.016 nM	Running buffe (50 mM H_3BO_3 / $Na_2B_4O_7$ pH=7.8)	capillary zone electrophoresis (CZE)	detection and neutralization	[7]
S14	S1(NTD)	21.8 nM (S trimer)	BB (1xPBS pH 7.4, 10 mM $MgCl_2$, 50 mM KCl, 25 mM NaCl)	biolayer interferometry (BLI)	detection	[8]
SNAP1	S/NTD	60.35 ± 1.61 nM	binding buffer ((yeast tRNA and salmon sperm DNA (SS DNA))0.1 mg/mL)	biolayer interferometry (BLI)	detection	[9]
SP6	S trimer	21 ± 4.6 nM	PBS/3 mM, $MgCl_2$ and 0.8 mg/ml BSA	surface plasmon resonance (SPR)	neutralization	[10]
SARS2-AR10	Pseudotyped SARS-CoV-2	79 ± 28 nM	SELEX binding buffer (20 mM tris, 100 mM NaCl, and 2.5 mM $MgCl_2$(pH=7.2))	enzyme-linked oligonucleotide assay (ELONA)	detection	[11]

Supporting Figures

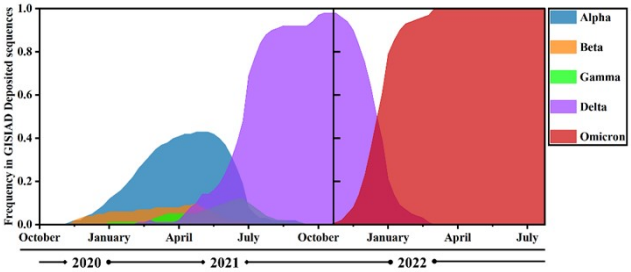

Fig. S1 Emergence and global prevalence of SARS-CoV-2 variants of concern lineages. Sequence data was downloaded from the Global Initiative on Sharing All Influenza Data (GISAID) and graphed as weekly totals (https://gisaid.org/phylodynamics/global/nextstrain/). Variant of concern lineage prevalence is shown from October 2020 to July 2022.

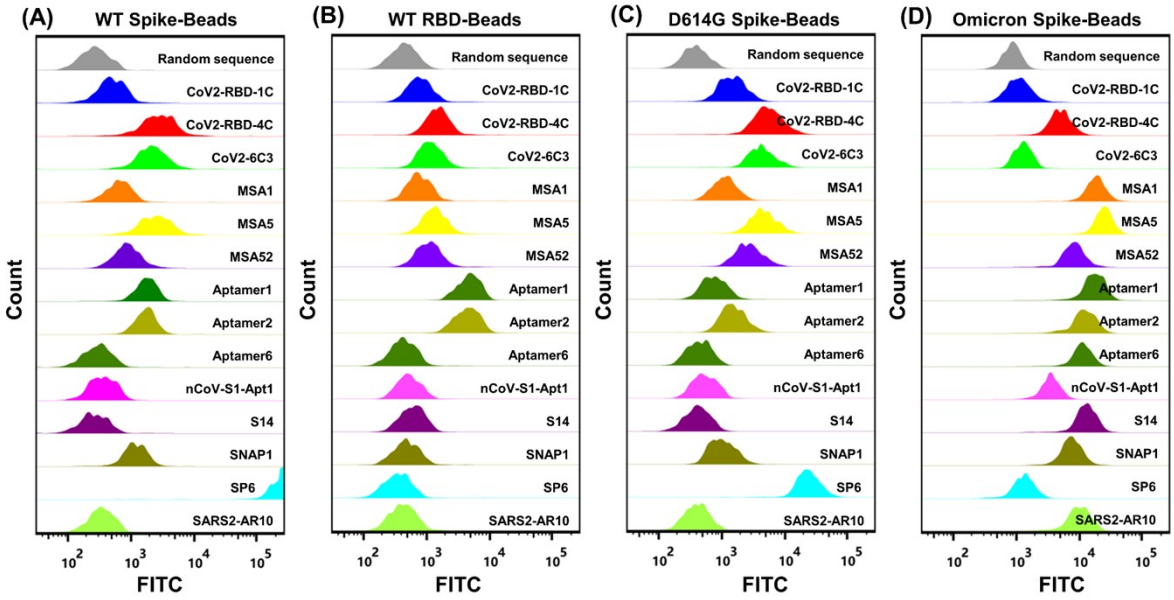

Fig. S2 Flow cytometric analysis of aptamers binding to WT Spike (A), WT RBD (B), D614G spike (C) and Omicron spike (D).

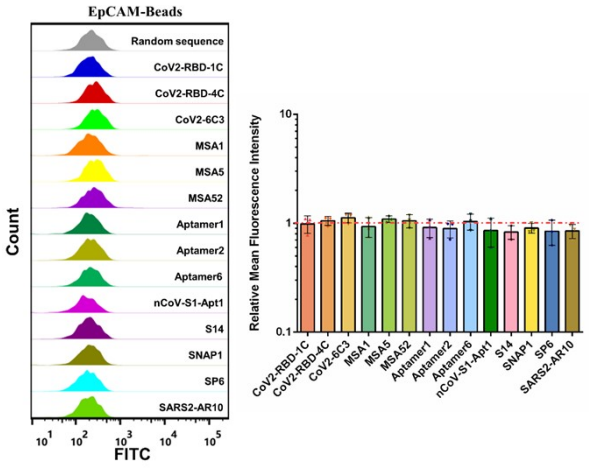

Fig. S3 Flow cytometric analysis of aptamers binding to His-tagged EpCAM and Relative mean fluorescence of the aptamers against His-tagged EpCAM. Data was summed from three independent experimental replicates.

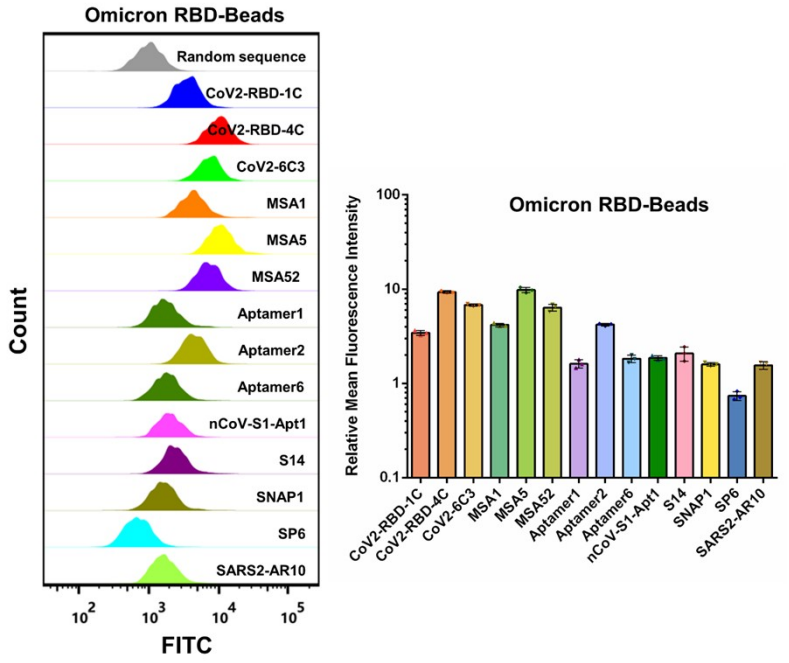

Fig. S4 Flow cytometric analysis of aptamers binding to Omicron RBD and Relative mean fluorescence of the aptamers against Omicron RBD. Data was summed from three independent experimental replicates.

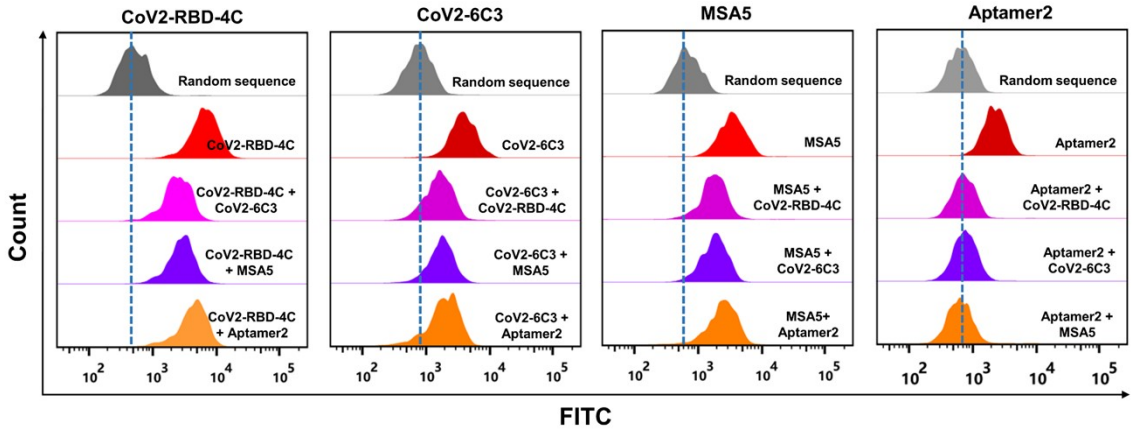

Fig. S5 Competition assay of CoV2-RBD-4C, CoV2-RBD-6C3, MSA5 and Aptamer2 aptamers against spike protein.

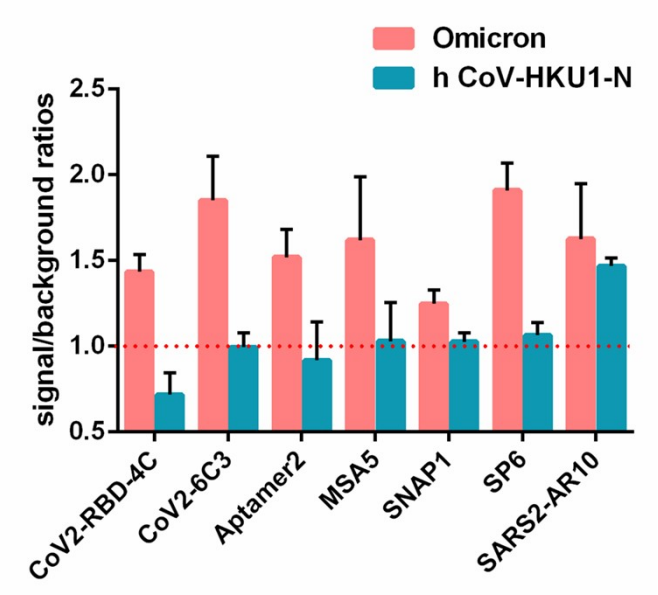

Fig. S6 Specificity study of aptamer CoV2-RBD-4C, CoV2-6C3, Aptamer2, MSA5, SNAP1, SP6 and SARS2-AR10 against the pseudovirus of Omicron and human CoV-HKU1-N. Data was summed from three independent experimental replicates.

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