Supporting Information

Discovery of the Sandwich type COVID-19 Nucleocapsid Protein DNA Aptamers

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Table of Contents

Experimental Procedures

Materials and Instruments

All chemicals were of the highest available quality and used as received without any further purification. Dynabeads magnetic beads were purchased from Thermofisher. DPBS buffer (1X with calcium and magnesium, Corning) was used as received for SELEX no extra Ca^{2+} and Mg^{2+} addition. Deoxyribonucleic Acid (DNA) from Salmon, N-(3-Dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride(EDC) and N-Hydroxysuccinimide (NHS) were purchased from Sigma. COVID-19 nucleocapsid protein and spike protein was purchased from Sino Biological Inc. $(His)_8$-peptide was purchased from DGpeptidesCo. Ltd. DNA Library and primers were synthesized by a company (Sangon, Shanghai, China). All sequences are listed in Table S1. Bull serum albumin, lysozyme, streptavidin and horseradish peroxidase were purchased from Sangon. The gold nanoparticle was purchased from nanoComposix. Antibodies of COVID-19 nucleocapsid protein and streptavidin were provided by Abace Biotechnology Co., Ltd.
Optical absorption spectra were acquired using a GE NanoVue™ Spectrophotometer spectrophotometer. Real-time PCR was performed on the Light Cycler 96 Real-Time PCR System (Roche). Surface plasmon resonance (SPR) experiments were performed using a BIAcore T200 biosensor system. The Octet RED96e System (Fortebio) was used to test the sensitivity of aptamers toward different kinds of proteins. ELISA was measured on the microplate reader of Thermo Scientific.

Aptamer selection

COVID-19 Np was purchased from a biotech company (Sino Biological Inc.). Before selection,12% SDS-PAGE was used to test the purity of the Np. The sequence of the DNA library is 5'-GTTCGTGGTGTGCTGGATGT$(N)_{36}$TGACACATCCAGCAGCACGA-3', where N_{36} represents 36 nucleotides with equimolar incorporation of A, G, C, and T at each position. SELEX was performed by the magnetic beads as described previously[1], and a combined strategy monitored the progress of aptamer selection[1]. In brief, the typical EDC/NHS protocol was carried out to couple 2019-nCoV Np on the surface of magnetic beads. Then, 500 nM 100 μL DNA library was incubated with the COVID-19 Np-MB in the binding buffer (2 mM KH_2PO_4, 8 mM Na_2HPO_4, 150 mM NaCl, 5 mM KCl, 1mM $CaCl_2$, 0.5 mM $MgCl_2$, pH 7.4). Bound DNA was recovered and amplified by PCR for four more rounds of selection. After a total of five rounds of selection, the enriched DNA pool was identified by high-throughput sequencing (HTS). The top 20 sequences were synthesized and confirmed the affinity by surface plasmon resonance (SPR) analysis.

Real-time PCR

Real-time PCR conditions experiments were conducted on Light Cycler 96 Real-Time PCR System (Roche)according to the manufacturer's instructions. All reactions were performed in 20 μl reaction volumes in 96-well plates for PCR. A standard real-time PCR mixture contained 17 μl iTaq TM universal SYBR Green Supermix(500 nM primers, 200 uM dNTP), 1 μl DNA template (16 nM), 1 μl DNA polymerase (concentration refer to the manual), 1 μl evagreen (4%). The alternative 50μl real-time PCR mixture contained 42.5 μl iTaq TM Universal SYBR Green Supermix (500nM primers, 200uM dNTP), 2.5 μl DNA template (16 nM), 2.5 μl DNA polymerase (concentration refer to the manual), 2.5 μl evagreen (4%). Thermal cycling consisted of an initial denaturation at 95 ℃ for 120 s followed by 30 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C 30 s, and extension at 72 °C for 30 s.

Structure analysis

All DNA secondary structures of the selected aptamers were analyzed using the Mfold program (mfold-3.6). (http://unafold.rna.albany.edu/?q=mfold/DNA-Folding-Form)

High-throughput sequencing and bioinformatics analysis

High-throughput sequencing was carried out using the Ion Proton sequencer system and kits (Thermofisher) using manufacturer's protocol by the Hefei Dahui Gene Technology Co., LTD. (Hefei, China). Reads processing and data analysis were conducted for the enriched library were mapped to each reading using RazerS.[2]

Surface plasmon resonance analysis

Surface plasmon resonance (SPR) experiments were performed using a BIAcore T200 biosensor system to measure the binding affinity of selected aptamers. COVID-19 Np was directly immobilized on the carboxymethylated sensor chip (CM5 chip). Briefly, the carboxylic group on the sensor chip was activated by the standard amine coupling procedure using freshly prepared EDC/NHS. COVID-19 Np (20 μg/ml) in acetate buffer (pH 6.0) was then injected into the sensor chip at flow rate 5 μL/min to reach ~500 RU

immobilization level. The deactivation was done by ethanolamine-HCl to block unreacted carboxyl groups. The binding analysis was carried out with aptamers at different concentrations (5 to 500 nM). The running condition was set at a 30 μL/min flow rate, 25 °C, 3 min association time, and 5 min dissociation time. PBS was used as the running buffer, and 1 M NaCl as the regeneration buffer. All the buffers were filtered and degassed prior to each experiment. Blank surfaces were used for background subtraction. Upon injection of the aptamers, sensorgrams recording the association/dissociation behavior of the Np-aptamer complex were collected. By varying the aptamer concentration, a series of sensorgrams were obtained and subsequently analyzed using the 1:1 Langmuir model provided in the BIA evaluation software to calculate the equilibrium dissociation constant K_d.

The specificity evaluation.

The AP2G chip was activated by EDC/NHS protocol. Different analytes were immobilized on the chips. The binding analysis was carried out with aptamers at 500 nM concentrations. The binding curves were recorded by Octet RED96e System (Fortebio). The running condition was set at a 20 μL/min flow rate, 25 °C, 1 min for baseline, 5 min association time, and 2 min dissociation time. DPBS was used as the running buffer, and 2 M NaCl as the regeneration buffer.

Plate preparation and measurement of ELISA Assay

Dilute capture COVID-19 Np antibody (monoclonal) with PBS to a concentration of 5μg/mL. Immediately, add 100μl to each ELISA plate well. Seal the plate and incubate overnight at room temperature. Aspirate the wells to remove liquid and wash plates 3 times. Each wash consists of adding 300μl wash buffer per well, followed by aspiration. After the last wash invert plate to remove residual buffer and blot on paper towel. Add 300μl blocking buffer to each well. Incubate 1 hour at R.T. Aspirate and wash plate 3 times. Serial dilute standard from 400 ng/mL to zero in a diluent. Add 100μl of standard or sample to each well in triplicate. Incubate at room temperature for at least 2 hours. Wash plate 3 times. Dilute detection Np-A48 and Np-A58 (biotinylated) in the diluent to a concentration of 0.5μg/ml Immediately add 100μl per well. Incubate at room temperature for 30 min. Aspirate and wash plate 3 times. Dilute SA-HRP conjugate 1:500 in a diluent. Add 100μl per well. Incubate 30 min at room temperature. Aspirate and wash plate 3 times. Add 100μl of substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 450 nm.

Preparation and detection of the Immunochromatographic Strip

Gold nanoparticle labeled SA(AuNPs-SA) was provided by the Abace biotechnology company. The immunochromatographic strip consists of four components: the sample pad, connection pad the, NC membrane, and absorbent pad.[3] As shown in Figure 3A, 5 μM 100μL Np-A48 and Np-A58(biotinylated) were incubated with 5 μM 100μL AuNPs-SA 30 min, Then added 5 μM 100μL biotin to block all the binding sited on the SA surface. Ultrafiltration centrifugal washed three times. Then the sample pad was saturated with a PBS solution (pH 7.4) containing conjugated aptamer-SA-AuNPs complex. Two antibodies were used. One is the COVID-19 Np antibody (monoclonal) on the test line and other SA rabbit polyclonal antibody (capture antibody) fixed onto the control line of the NC membrane. The Immunochromatographic strips were store in a desiccator at 4 ℃ prior to use. Strip sensitivity was determined using a serial diluted of COVID-19 Np spiked solution in serum or urine (1000, 100, 10, 5, 1, 0.5 and 0.1 ng/mL). The serum or urine was used as the negative control. The images were taken in 15 min.

Supported Table and Figures

Table S1 the sequences of the initial library and the primers used in SELEX.

DNA species	Sequences (5'…3')
DNA library	GTTCGTGGTGTGCTGGATGT$(N)_{36}$TGACACATCCAGCAGCACGA
Primer-26S1	GTTCGTGGTGTGCTGGATGT
Primer-26S1-FAM	FAM-GTTCGTGGTGTGCTGGATGT
Primer-26A	TCGTGCTGCTGGATGTGTCA
Primer-26A-polyA	$(A)_{19}$-TCGTGCTGCTGGATGTGTCA

Table S2 the data of the aptamer-antibody hybrid ELISA for the detection of COVID-19 Np.

Np (ng/mL)	OD (450 nm)		
	Np-A48	Np-A58	Np-A48+ Np-A58
400	2.85 ± 0.06	2.90 ± 0.04	3.01 ± 0.06
200	2.51 ± 0.04	2.15 ± 0.05	2.24 ± 0.01
100	1.47 ± 0.07	1.42 ± 0.07	1.43 ± 0.04
50	0.77 ± 0.05	0.74 ± 0.04	0.72 ± 0.05
25	0.47 ± 0.02	0.41± 0.03	0.35 ± 0.04
12.5	0.23 ± 0.04	0.20 ± 0.05	0.21 ± 0.04
6.25	0.10 ± 0.03	0.13± 0.02	0.16± 0.05
0	0.08 ± 0.04	0.09± 0.02	0.09± 0.04

Table S3 the data of the antibody-based ELISA for the detection of COVID-19 Np.

Np (ng/mL)	OD (450 nm)
1000	2.25 ± 0.10
500	1.27 ± 0.05
200	0.83 ± 0.07
100	0.31 ± 0.05
50	0.17 ± 0.02
25	0.13 ± 0.04
10	0.15 ± 0.03
0	0.15 ± 0.04

Table S4. Summary of the existing detection methods for COVID-19

Method	biomarker	Advantage	Disadvantage	References
Polymerase chain reaction	nucleic acid	low detection limit, multifarious products in the market	specialized instrument, false negative, require technician	4
Computed tomography	lungs	non-invasive	expensive, require technical expertise, not specifical	4
Antibody tests	immunoglobulin G and M	portable, rapid, not require technician, cheap	not specifical, post-infection	5
Antigen test	nucleocapsid protein	good specificity, portable, rapid and no cross-reactivity, not require technician, cheap, suitable for early detection	the detection limit needs to be improved	this work and 6
Antigen test	spike protein	no cross-reactivity		7

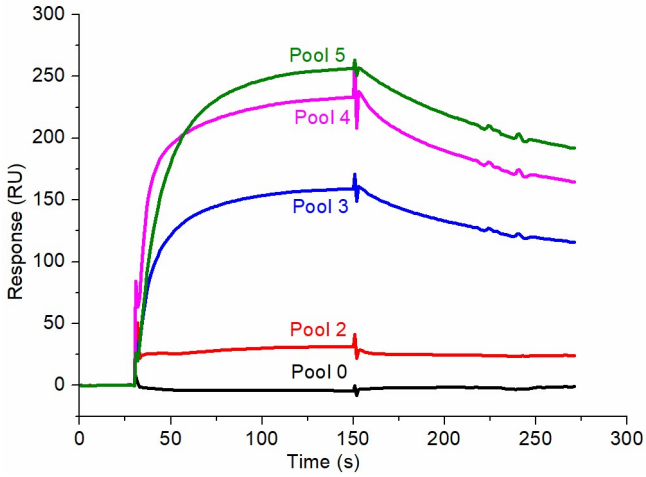

Figure S1. SPR sensorgram of the binding kinetics for every enriched DNA pool（500 nM） and immobilized COVID-19 Np. Data are presented as real-time graphs of response units (RU) against time and evaluated using BIA evaluation 4.0 software (Biacore).

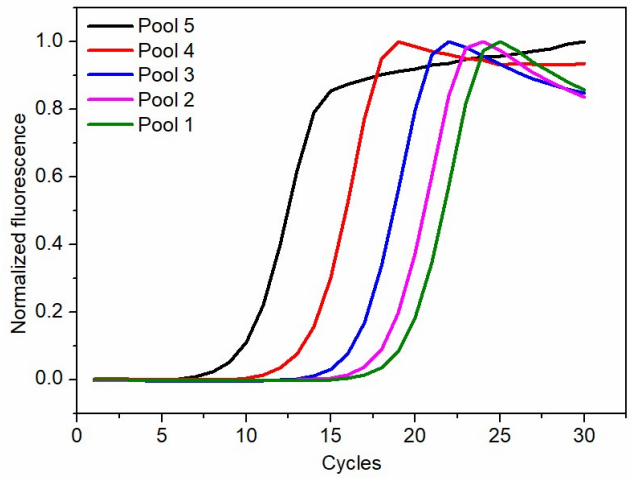

Figure S2. Amplification curve of every round of ssDNA library during Np aptamer selection. The PCR was performed on the Light Cycler 96 Real-Time PCR System.

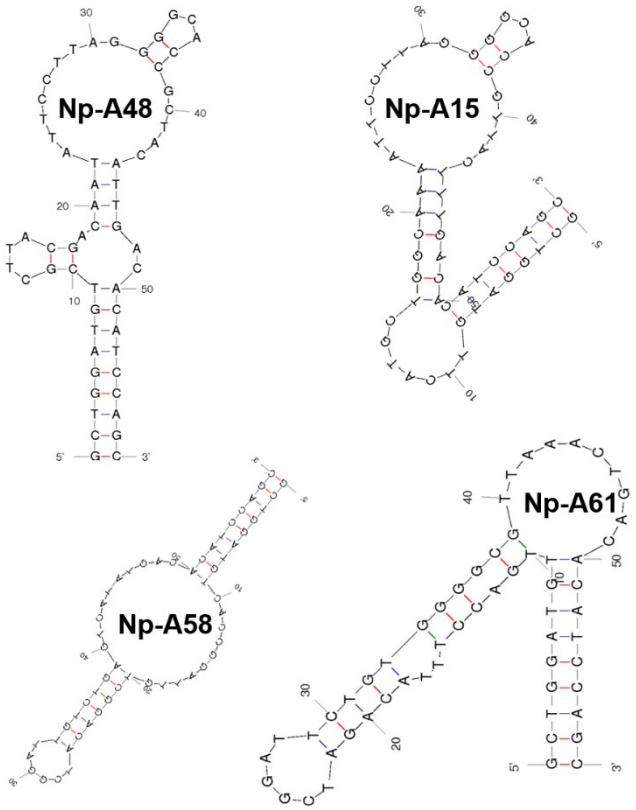

Figure S3. The second structure of Np aptamers folded by mfold software.

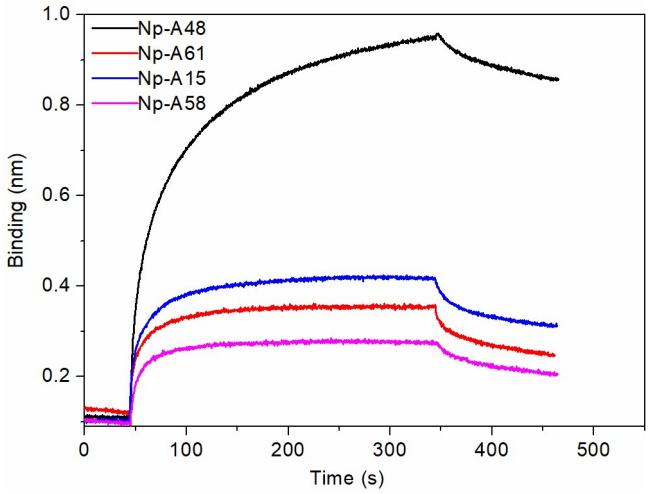

Figure S4. The binding kinetics for aptamers and COVID-19 Np in the presence of sputum (50% v:v DPBS buffer). Data are presented as real-time graphs of bingding (nm) against time by Octet RED96e System (Fortebio).

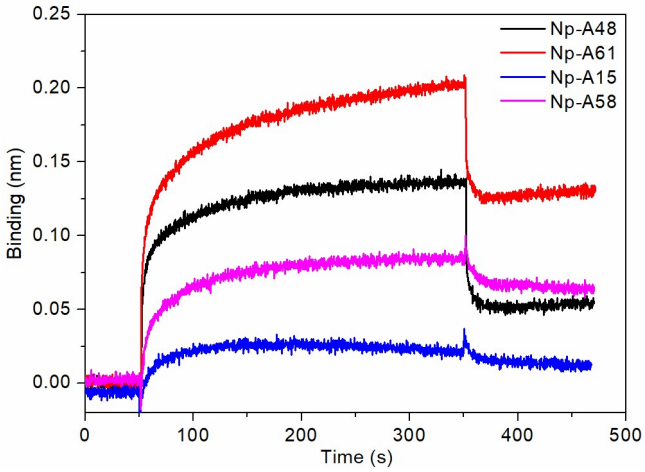

Figure S5. The binding kinetics for aptamers and COVID-19 Np in the presence of urine. Data are presented as real-time graphs of bingding (nm) against time by Octet RED96e System (Fortebio).

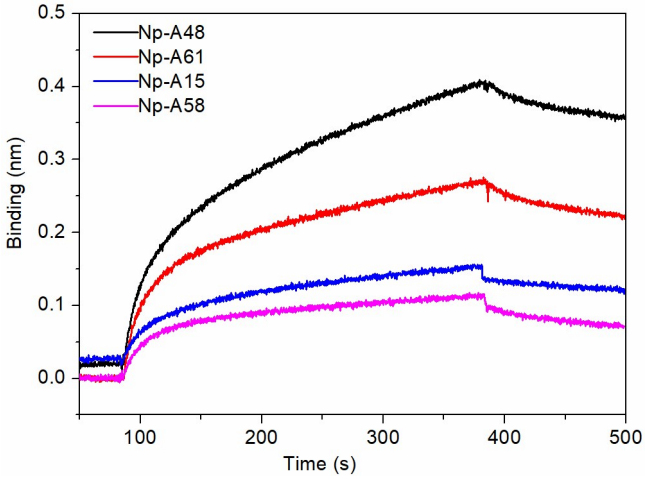

Figure S6. The binding kinetics for aptamers and COVID-19 Np in the presence of 40% human serum (40% v:v DPBS buffer). Data are presented as real-time graphs of bingding (nm) against time by Octet RED96e System (Fortebio).

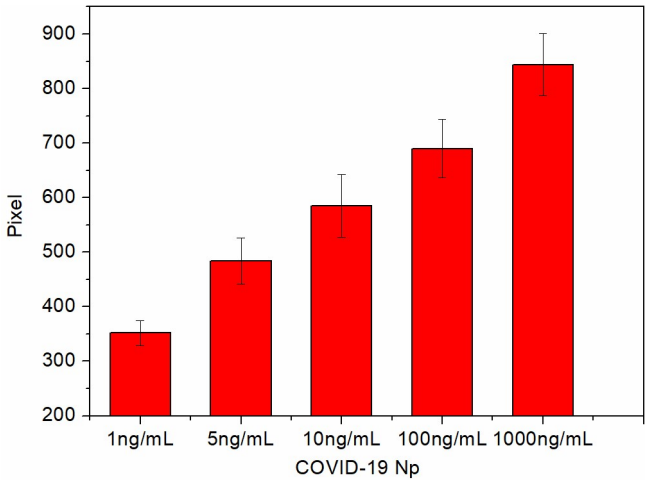

Figure S7. The pixel intensity of the test lines measured by ImageJ

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