Supporting Information

DNA framework based dual signal amplification biosensor for portable detection of SARS-CoV-2 and its mutations

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Materials and methods

Materials

The DNA sequences (A, B, C, D, and linker) were synthesized by Sangon Biotech (Shanghai) Co., Ltd. The viral nucleic acid nucleocapsid protein (N) gene RNA sequence, envelope protein (E) gene RNA sequence and biotin modified signal probes, N and E gene RNA sequence of MERS-CoV, N and E gene RNA sequence of SARS-CoV were synthesized by Shanghai DNA Bioscience Co., Ltd. Streptavidin peroxidase conjugate (SA-HRP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Casein, bovine serum albumin (BSA), Tween 20 and other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. TMB (3,3',5,5' tetramethylbenzidine) substrate was purchased from Neogen (Lexington, KY). SARS-CoV-2 large RNA standards (containing E gene, reference concentration: 4.03E+05 copies/μL) and SARS-CoV-2 pseudovirus standards (containing N gene, reference concentration: 3.01E+03 copies/μL) standards was provided by the Key Laboratory of Bioanalysis and Metrology for State Market Regulation Shanghai Institute of Measurement and Testing Technology. The structure of pseudovirus is a spherical shape with a diameter of 40-160 nm. It mainly composed of an RNA chain genome as the core which wrapped with a layer of envelope. The difference between pseudovirus and coronavirus is that its genome is modified so that its surface protein is not or less expressed, thus reducing the toxicity and infectivity of the virus enabling it to be studied in P2 laboratory. The in vitro transcribed RNA standard is approved as the national standard material, and serve as a basis for diagnosis and exclusion of COVID-19 infection. The in vitro transcribed RNA standard is currently commercialized and directly usable reagents. Viral RNA/DNA Extraction Kit was purchased from TaKaRa.

All chemical reagents were prepared with ultrapure water from a Millipore Milli-Q water purification system (18.2 MΩ cm resistivity). The buffer solutions used were as follows: The TM buffer contains 20 mM Tris, 50 mM $MgCl_2$, pH 8.0. The PBS (pH= 7.4) contains 12 mM phosphate buffer (PB), 2.7 mM KCl and 137 mM NaCl. TAE-Mg^{2+}buffer (pH 8.0) contains 40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate. The hybridization buffer (pH 7.4) contains 10 mM phosphate buffer (PB), 20

mM $MgCl_2$ and 1 M NaCl.

Synthesis of Amino-modified DNA Tetrahedron FNA

To synthesis DNA tetrahedron FNA, 2 μL of single-stranded DNAs (A, B, C, D) were mixed with TM buffer solution, and the resulting mixture was heated to 95 °C for 15 min, then cooled to 4 °C for more than 30 s with the PeltierModel PTC-200 thermal cycler (MJ Research, Inc., SA). The final concentration of FNA was 1 μM (The sequence of four single-strand DNA is in Table S1).

Characterization of Polyacrylamide Gel

The prepared FNA was characterized by polyacrylamide gel (PAGE). 6 μL of polyacrylamide gel (6 %) were prepared with 900 μL of polyacrylamide (40 %), 600 μL of 10× TAE Mg buffer, and 4.5 mL of Milli-Q water were mixed well. 100 μL of ammonium persulfate (APS 10 %), 8 μL of N, N, N', N'-tetramethylethylenediamine (TEMED) were added to the above solution, mixed gently and quickly for further use.

The Modification of FNA on SPCE

Electrodes for nucleic acid detection were cleaned and activated by cyclic voltammetry scanning (CV) in PB buffer solution (pH 7.4). The parameters were as follows: potential range: -300 mV - 0.6mV; scan rate: 500 mV/s. Then 10 μL of EDC and NHS mixed solution (400 mM EDC:100 mM NHS 1:1 v/v) were dripped on the surface of the treated electrodes for 15 min at room temperature, electrodes were rinsed with Milli-Q water and dried with N_2. Then, 6 μL of 1 μM amino modified with FNA was added on electrodes to perform chemical coupling reactions for 2 h at 37 ℃. Then the electrodes for nucleic acid detection were blocked with 1 % casein and 1% BSA solution for 2 h at 37 ℃, and washed with PBS three times for further use.

The Dual Signal Amplification and Electrochemical Measurements in Detection of SARS-CoV-2

The initial concentrations of N and E gene model targets DNA (1 μM) were diluted ranging from 0 to 100 nM, 6 μL target DNA and 500 nM biotin labeled double signal probes were mixed, and pre-hybridized in 85 ℃ for 10 min, then cooled to the room temperature. The mixture was coated on the FNA modified electrodes for 1 h at 37

℃. Then, 6 μL of 5 μg. mL^{-1} SA-HRP in 1% casein solution was added to the electrode surface for 15 min at room temperature, and washed away with PBST solution. Electrochemical measurements were performed with the home-made universal mobile and portable electrochemical device. The CV measurement was carried out at scan rate of 50 mV s^{-1}, i-t was fixed at -0.1 V, and the electroreduction current was measured for 50 s after the HRP catalytic reaction reached the steady state.

Detection of SARS-CoV-2 large RNA standards and SARS-CoV-2 pseudovirus standards

SARS-CoV-2 pseudovirus standards were lysed with viral RNA/DNA Extraction Kit. The total RNA acquisition time is 15 mins, and the overall length of the virus gene will be exposed and could be detected on our electrochemical chip. The extracted RNA was frozen quickly in the -80℃ refrigerator until electrochemical tests to avoid the RNA degradation. In the electrochemical detection, 6μL SARS-CoV-2 large RNA standards solution (containing E gene), 6 μL SARS-CoV-2 virus lysate (containing N gene) and 500 nM biotin labeled double signal probes mixed solution were pre-hybridized and then coated on the different FNA probes modified electrodes for 1 h at 37 ℃. Then, 6 μL of 5 μg. mL^{-1} SA-HRP in 1% casein solution was added to the electrode surface for 15 min at room temperature, and washed away with PBST solution. Electrochemical measurements were performed as described above.

Results and Discussions

The synthesis of DNA tetrahedron FNA was confirmed by PAGE. We selected the different combination of four single-stranded DNAs (A, B, C, D) to verify the assembly of DNA tetrahedron FNA (Fig. S2 A). The DNA tetrahedron FNA with the largest molecular weight has the slowest migration speed. Dynamic light scattering (DLS) was also performed to characterize the DNA tetrahedron FNA (Fig.S2 B). The theoretical value of DNA tetrahedron FNA (5.78 nm) were calculated based on a length of 0.34 nm per base pair. The result showed the diameter of DNA tetrahedron FNA was in the range of 5.24 nm-7.10 nm, exhibiting a narrower size range. DNA tetrahedron FNA was further verified with AFM (Fig. S2 C), and the result showed that the height profiles of the FNA with a narrow range about 5 nm-6 nm. The results are in general

agreement with the theoretical values. DNA tetrahedron FNA assembled on the surface of SPCE, hybridization processes with model targets and dual signal probes were also verified by OCP-EIS techniques (Fig. S2 D.E). The assembly of DNA tetrahedron FNA on the electrode, binding to model target and signal probes could form the non-conductive layer, which inhibited the charge transfer on the surface of SPCE. Thus, the resistance of charge transfer (Rct) in the Nyquist plots from OCP-EIS increased, indicating successful assembly and hybridization.

The temperature was an important role in improving the hybridization efficiency. The current signal and S/N ratios were higher at 37 ℃, then 37 ℃ was used as the optimal hybridization reaction temperature in the following experiments (Fig. S3A). Hybridization reaction time was an important parameter to achieve rapid detection of SARS-CoV-2. Within 15 min, the DNA tetrahedron FNA could hybridize to the targets (Fig. S3B). The current values and S/N ratio increased when the hybridization reaction time increased from 15 min to 60 min. After 60 min, the current values and S/N ratio decreased, indicating 60 min was the optimal hybridization time.

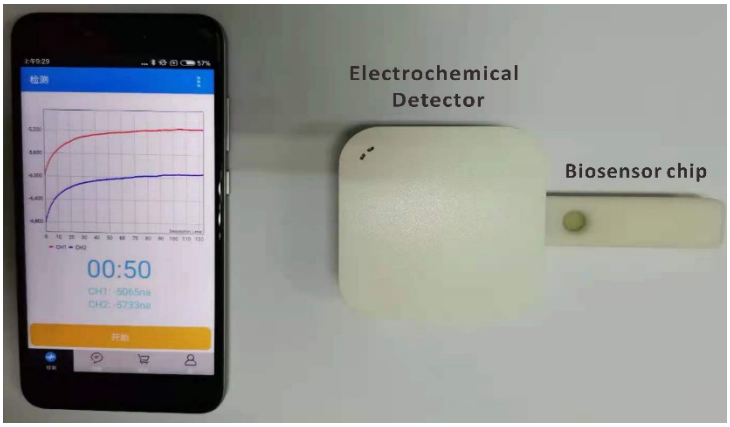

Figure S1. A photograph of the smartphone-based electrochemical sensing system

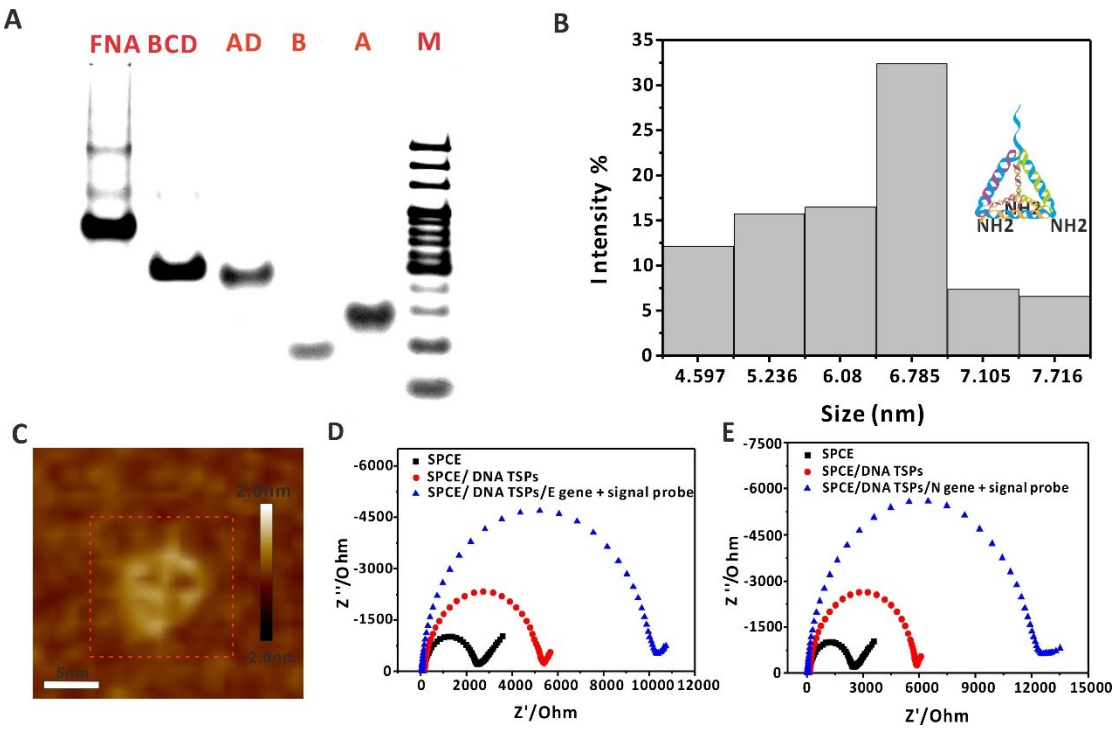

Figure. S2. Characterization of the FNAs. (A) PAGE characterization for the FNA. Lane 1: FNA; Lane 2: BCD; Lane 3: AD; Lane 4: B; Lane 5: A; Lane 6: 20bp DNA marker. The DNA complexes in lane 1-3 were synthesized before the PAGE and the experiments were carried out in 1 x TAE-Mg^{2+} buffer (pH 8.0) contains 40 mM Tris, 20 mM acetic acid, 2 mM EDTA and 12.5 mM magnesium acetate at room temperature. (B) DLS of the FNA. （C）AFM Characterization of the FNA. (D) and (E) EIS spectra for the assembly of the SARS-CoV-2 S gene of biosensor. The EIS experiments were performed in 0.1 M PBS buffer containing 5 mM $Fe(CN)_6^{3-/4-}$.

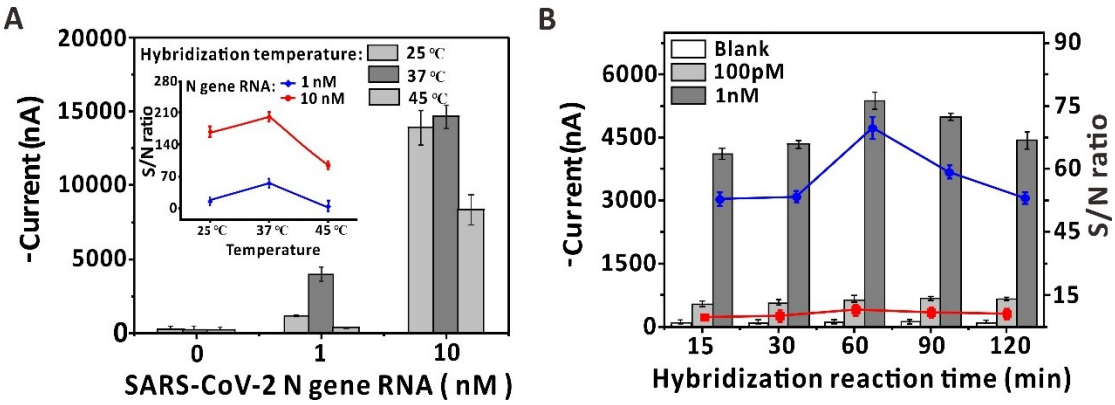

Figure. S3. Optimization of the experimental conditions of the SARS-CoV-2 gene detection. (A) Hybridization temperature for the binding of model target and capture probes (B) Hybridization reaction time for the binding of model target and capture

probes.

Table S1. Nucleic acid sequences used in this work (5' to 3')

NH_2-DNA FNA-B	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
NH_2-DNA FNA-C	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
NH_2-DNA FNA-D	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
DNA FNA -A (N gene-1)	TGCCAGCCATTCTAGCAGGAGAAGTTCCCCTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
SARS-CoV-2 N gene	GGGGAACTTCTCCTGCTAGAATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGC
Biotin-signal probe (N gene-1)	Biotin-GCAGCAAAGCAAGAGCAGCATCACCGCCAT
DNA FNA -A (N gene-2)	TCTAGCAGGAGAAGTTCCCCTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Biotin-signal probe-2.1 (N gene-2)	Biotin-GCAGCAAAGCAAGAGCAGCA
Biotin-signal probe-2.2 (N gene-2)	Biotin-TCACCGCCATTGCCAGCCAT
DNA FNA -A (E gene-1)	TACCACGAAAGCAAGAAAAAGAAGTACGCTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
SARS-CoV-2 E gene	AGCGTACTTCTTTTTCTTGCTTTCGTGGTATTCTTGCTAGTTACACTAGCCATCCTTACT
Biotin-signal probe (E gene-1)	Biotin-AGTAAGGATGGCTAGTGTAACTAGCAAGAA
DNA FNA-A (E gene-2)	GCAAGAAAAAGAAGTACGCTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
Biotin-signal probe-2.1 (E gene-2)	Biotin-AGTAAGGATGGCTAGTGTAA
Biotin-signal probe-2.1 (E gene-2)	Biotin-CTAGCAAGAATACCACGAAA
MERS-CoV N gene	CAGGACAGAAAAATTAATACCGGGAATGGAATTAAGCAACTGGCTCCCAGGTGGTACTTC
SARS-CoV N gene	TCACTTCCCTACGGCGCTAACAAAGAAGGCATCGTATGGGTTGCAACTGAGGGAGCCTTG
MERS-CoV E gene	GTAGTATGTGCTATAACACTCTTGGTGTGTATGGCTTTCCTTACGGCTACTAGATTATGT
SARS-CoV E gene	GCGTACTGCTGCAATATTGTTAACGTGAGTTTAGTAAAACCAACGGTTTACGTCTACTCG
DNA FNA-A (S gene-1)	ATAAAGAACAGCAACCTGGTTAGAAGTATTTTTTTTACATTCCT

	AAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
SARS-CoV-2 S gene -1	AATACTTCTAACCAGGTTGCTGTTCTTTATCAGGATGTTA
Biotin-signal probe (S gene-1)	Biotin-TAACATCCTG
S gene D641G Mutation	AATACTTCTAACCAGGTTGCTGTTCTTTATCAGGGTGTTA
DNA FNA-A (S gene-2)	ATTAGTCTGAGTCTGATAACTAGCGCATATTTTTTTACATTCCTAAGTCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
SARS-CoV-2 S gene -2	ATATGCGCTAGTTATCAGACTCAGACTAATTCTCCTCGGC
Biotin-signal probe (S gene-2)	Biotin-GCCGAGGAGA
S gene P681R Mutation	ATATGCGCTAGTTATCAGACTCAGACTAATTCTCGTCGGC

TableS2. The specific sequence information and concentration of the standard

Name	Type of standard	Nucleic acid fragments		Reference concentration（copies/μL）
SARS-CoV-2 large RNA standard	RNA mixture fragments transcribed in vitro	N	28274-29533，MT027064.1	6.19E+05
		E	26245-26472，MT027064.1	4.03E+05
		ORF1ab-1	264..5265，MT027064.1	5.03E+05
		ORF1ab-2	5266..10265，MT027064.1	3.06E+05
		ORF1ab-3	10266..15265，MT027064.1	4.21E+05
		ORF1ab-1	15266..21556，MT027064.1	4.21E+05
		S-1	21563..23562，MT027064.1	6.35E+05
		S-2	21563..23562，	6.35E+05

			MT027064.1	
		M	26523..27191，MT027064.1	6.35E+05
SARS-CoV-2 pseudovirus standard	Pseudovirus mixture	N	26245..29903, NC_045512.2	3.31E+03
		E		5.32E+03
		M		8.97E+03
		ORF1ab	13242..16236, NC_045512.2	8.73E+03
		S	21563..25384, NC_045512.2	4.24E+03