Supporting Information

Smartphone-based three-in-one biosensor for co-detection of SARS-CoV-2 viral RNA, antigen and antibody

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Materials and methods

Materials

DNA sequences (A, B, C, D) were synthesized by Sangon Biotech (Shanghai) Co., Ltd. Viral nucleic acid spike (S) gene RNA sequences were synthesized by Shanghai DNA Bioscience Co., Ltd. Goat anti-rabbit IgG peroxidase conjugate (G-R HRP) and streptavidin peroxidase conjugate (SA-HRP) was purchased from Sigma-Aldrich (St. Louis, MO, USA). Recombinant SARS-CoV-2 spike protein (S protein, His Tag), SARS-CoV-2 spike protein monoclonal antibody (S protein Ab1) and SARS-CoV-2 spike protein monoclonal antibody biotin conjugate (S protein Ab2) were purchased from Elabscience Biotechnology Inc (Wuhan, China). SARS-CoV-2 spike protein polyclonal antibody (S protein rabbit Ab) produced in rabbit was purchased medical institute of Oriental Ocean (Beijing). MERS-CoV, HCoV-NL63, SARS-CoV, MERS-CoV S protein antibody and pseudovirus of SARS-CoV-2 were purchased from Beijing Yiqiao Shenzhou Biotechnology Co., Ltd. H1N1 antibody was preserved in our laboratory. Casein, bovine serum albumin (BSA), Tween 20, PEG2000 and other chemicals were purchased from Sinopharm Chemical Reagent Co. Ltd. TMB (3,3',5,5' tetramethylbenzidine) substrate was purchased from Neogen (Lexington, KY).

The screen-printed carbon electrode (SPCE) was from Zhejiang Nazhihui Biotechnology Co., Ltd. Multi-walled carbon nanotubes (MWNTs 50 nm in diameter, 1-2 μm in length) were purchased from Shenzhen Nanotech Port Co. Ltd. (Shenzhen, China). The buffer solutions involved in this study are as follows: the antigen was dissolved in 0.01M phosphate saline (PBS) buffer. The coating antibody was dissolved in a 0.01M sodium carbonate (CB) buffer. All chemical reagents were prepared with ultrapure water from a Millipore Milli-Q water purification system (18.2 MΩ cm resistivity). Electrochemical detection was performed on the homemade universal mobile and portable electrochemical device.

Preparation of the MWNTs-S Protein (MWNTs-Ag) Conjugate

Carboxylated MWNTs were prepared in our laboratory. 400 μL of 1 mg. mL^{-1} MWNTs-COOH and 200 μL of 1 mg. mL^{-1} S protein were mixed together, then the mixture was incubated for 16 h with vibrations of 300 rpm at 18 °C. The mixture was centrifuged at 10,000 rpm for 10 min, pipetted up and down to resuspend the deposition if needed, and washed with 600 μL of Mill-Q water twice. To block the bare space on the surface of MWNTs-COOH, the mixture of 500 μL of 20 % PEG2000 and the solution was resuspended and subsequently incubated at 25 °C with vibration at 550 rpm

for 1.5 h. The mixture was centrifuged and washed three times. 400 μL of Milli-Q was added to the deposition by gently pipetting, then the MWNTs-Ag conjugate solution was moved to a new centrifuge tube to be ready for the next stage.

Preparation of Amino-modified DNA Tetrahedron and Polyacrylamide gel

Four single-stranded DNAs (A, B, C, D) were dissolved in TM buffer (20 mM Tris, 50 mM $MgCl_2$, pH 8.0), yielding a final concentration of 1 μM (The sequence of four single-strand DNA is in Table S1). The resulting mixture was heated to 95 °C for 10 min and then cooled to 4 °C for 30 s, using a T100TMPCR Thermal Cycler. The synthesized DNA tetrahedron structure probe (DNA TSPs) was characterized by polyacrylamide gel. Six milliliters of polyacrylamide gel (10 %) 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 %) and, 8 μL of N, N, N', N'-tetramethylethylenediamine (TEMED) were added to the above solution and mixed gently and quickly for further use.

Electrode Surface Modification for SARS-CoV-2 Co-detection

The SPCE was customized by screen-printing technology. Electrodes for nucleic acid detection were cleaned and activated by cyclic voltammetry scanning in PB buffer solution (pH 7.4). The parameters were as follows: potential range: -0.3 V - 0.6V; scan rate: 0.5V/s; sweep segments: 100. Then, 10 μL of EDC and NHS mixed solution (400 mM EDC:100 mM NHS 1:1 v/v) were added on the surface of the electrodes for 15 min at room temperature, then electrodes were rinsed with Milli-Q water and dried with N_2 airflow. After cleaning, 6 μL of 1 μM amino modified with DNA tetrahedron were dripped on electrodes to perform chemical coupling reactions for 2 h at 37 ℃.

The electrode for S protein detection was coated with 6 μL of 100 μg. mL^{-1} coating antibody (SARS-CoV-2 Ab1) diluted in carbonate buffer solution (pH 9.6) on the surface of the electrode for 2 h of incubation at 37 ℃. The electrodes for nucleic acid detection and spike protein detection were blocked with 1 % casein solution for 2 h at 37 ℃, then washed with PBS three times and dried with N_2 airflow.

The electrode for S protein antibody detection was modified with 6 μL of 0.25 mg. mL^{-1} MWNT-Ag conjugate from the last stage with airing at room temperature for at least 2 h. The above modified electrodes were kept in a refrigerator at 4 °C for use.

Fabrication of the Three-in-one Biosensor for SARS-CoV-2 Co-detection

For S gene target RNA detection in PBS buffer, under optimal conditions, the concentrations of target RNA were diluted from the initial concentration (1 μM) to concentrations ranging from 0 to 100 nM, where 6 μL of target RNA and 500 nM biotin labeled signal probe mixed solution was coated on the DNA tetrahedron 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 redundant reagents were washed away with PBST solution and dried under N_2.

In addition to electrochemical analysis for the standard S protein antigen solution in PBS buffer, different concentrations of S protein from 0.03 ng. mL^{-1} to 100 ng. mL^{-1} were first coated on an electrode chip modified with S protein Ab1 for 1 h at 37 ℃, then 6 μL of 2.5 μg. mL^{-1} S protein Ab2 was added to the electrode's surface and incubated for 1 h at 37 ℃. After the incubation, 5 μL of 5 μg. mL^{-1} SA-HRP in 1 % casein solution was dropped on the above immune electrodes for 15 min at room temperature. Finally, the immunosensor was taken out and washed with PBST solution three times dried with N_2 airflow.

A two-step procedure was used to detect the standard S protein antibody produced in rabbits in PBS buffer. Firstly, the detection antibody was diluted to different concentrations from 3 ng. mL^{-1} to 3000 ng. mL^{-1}, which were coated on the MWNTs-Ag conjugate modified with the electrode for 1 h at 37 ℃. Then, 6 μL of HRP-labeled IgG antibodies were dropped on electrodes to perform an immunoreaction for 30 min at 37 ℃, unbound reagents were washed away with PBST solution three times and dried with N_2 airflow.

The electrochemical performance of the smartphone-based electrochemical device was evaluated for the redox activity of TMB by cyclic voltammetry (CV) and amperometric (i-t) measurement. The measurement for the current signal was performed by CV scanning for 2 segments at the potential window of -0.3 V to 0.6 V and i-t measurement at the voltage of -0.1 V for 50 s. 50 μL of TMB (H_2O_2) substrate was added to the electrodes that completed the reaction above, and SARS-CoV-2 biomarkers were detected. For SARS-CoV-2 biomarkers detection in real serum samples, the detection protocol was the same as in PBS solution.

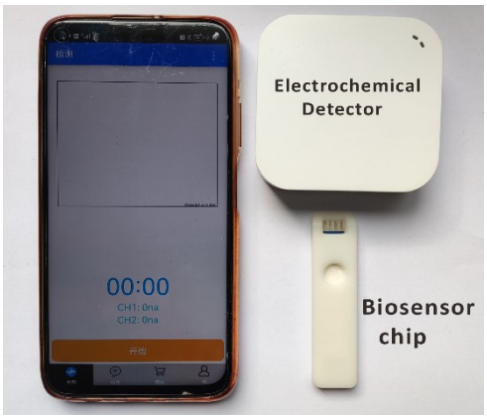

Figure S1. A photograph of the smartphone-based electrochemical sensing system

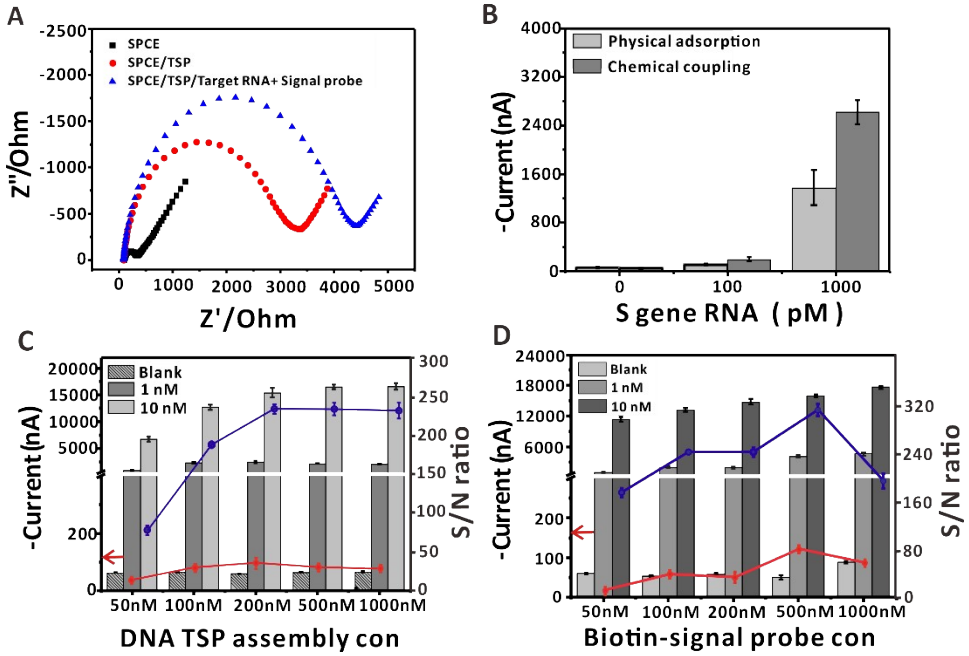

Figure. S2. (A) EIS spectra for the assembly of the SARS-CoV-2 S gene of biosensor. The EIS experiments were performed in 0.1 M PBS buffer containing 5 mM Fe $(CN)_6^{3-/4-}$. (B) and (C) Optimization of the amino DNA TSPs assembly method and concentration of DNA TSPs. (D) Optimization of the concentration of biotin-signal probe.

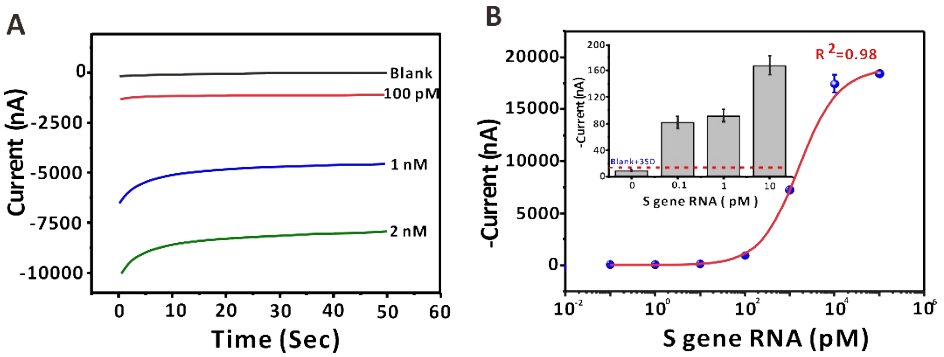

Figure. S3. (A) i-t response of 0 and 100 pM, 1 nM, 2 nM of S gene. The potential was held at -0.1 V and the reduction current was recorded at 50 s. (B) The calibration curve of the response current

to S gene with a series of concentrations in human serum system.

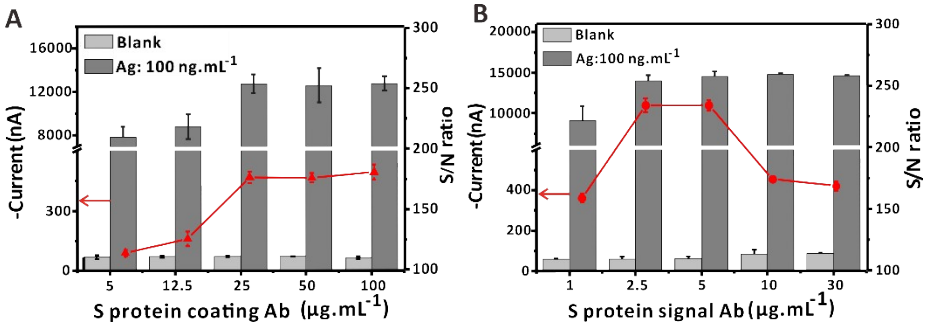

Figure. S4. (A) The current value and S/N at the different concentrations of S protein coating antibody, including 5, 12.5, 25, 50 and 100 μg. mL^{-1}, the concentration of S protein: 100 ng. mL^{-1}. (B) The current value and S/N at the different concentrations of anti-S protein signal antibody, including 1, 2.5, 5, 10 and 30 μg. mL^{-1}, the concentration of S protein antigen: 100 ng. mL^{-1}.

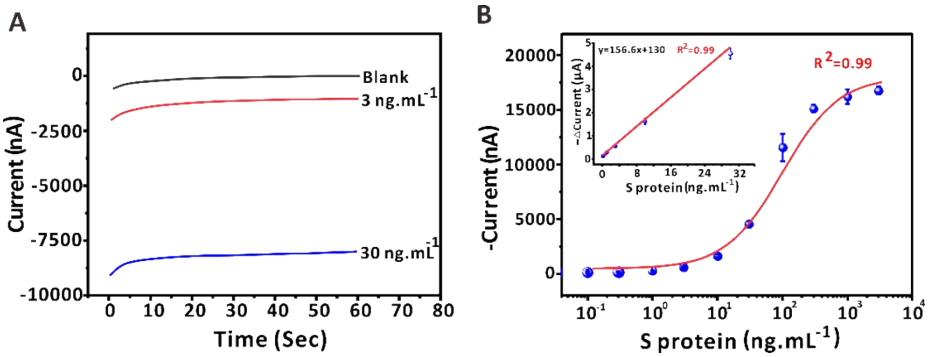

Figure. S5. i-t response of the presence of 0, 3 ng. mL^{-1} and 30 ng. mL^{-1} target S protein. The potential was held at -0.1 V and the reduction current was recorded at 50 s. (B) The calibration curve of the response current to S protein at a series of concentrations in human serum system, the linear fitting curve of the response current to S protein at low concentration from 0.1 ng. mL^{-1} to 30 ng. mL^{-1} (inset D).

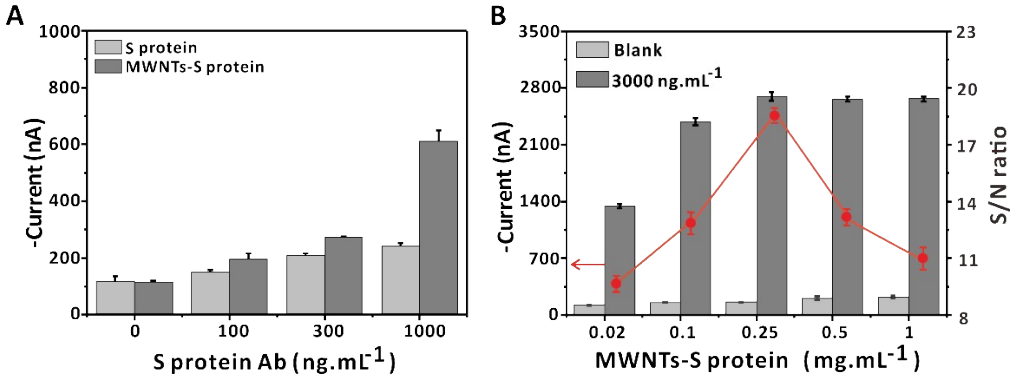

Figure. S6. (A) The current value of the electrode modified MWNT-Ag layer compared with the electrode modified with Ag layer only. The concentration of S protein antibody: 100 ng. mL^{-1}, 300 ng. mL^{-1}, 1000 ng. mL^{-1}. (B) The current value and S/N ratio at the different concentrations of MWNTs-S protein conjugate: 0.02 mg. mL^{-1}, 0.1 mg. mL^{-1}, 0.25 mg. mL^{-1}, 0.5 mg. mL^{-1}, 1 mg. mL^{-1}.

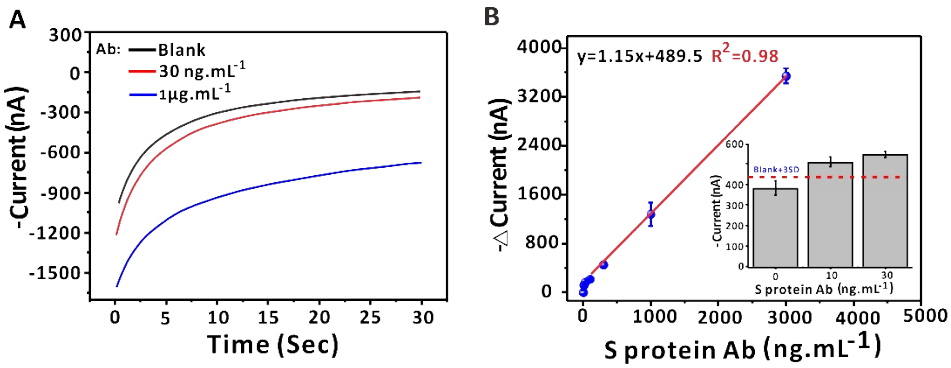

Figure. S7. (A) i-t response of the presence of 0, 30 ng. mL^{-1} and 1000 ng. mL^{-1} target S protein antibody. The potential was held at -0.1 V and the reduction current was recorded at 30 s. (B) The linear fitting curve of the response current to S protein antibody at a series of concentrations in human serum system (inset D).

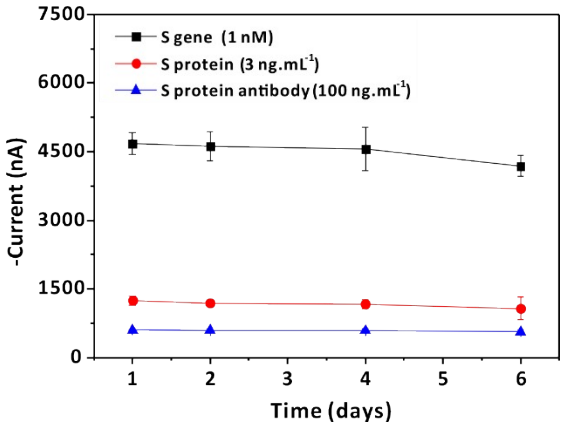

Figure S8. The stability study of biosensor against the storage time for 0–6 days. The IT response values of 1nM S gene, 3 ng. mL^{-1} S protein and 100 ng. mL^{-1}. Standard deviation comes from at least three independent experiments.

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

DNA TSP-A	TTCTCAGTGGAAGCAAAATAAACACCATCATTAAATGGTATTTTTTACATTCCTAAG TCTGAAACATTACAGCTTGCTACACGAGAAGAGCCGCCATAGTA
NH_2-DNA TSP-B	TATCACCAGGCAGTTGACAGTGTAGCAAGCTGTAATAGATGCGAGGGTCCAATAC
NH_2-DNA TSP-C	TCAACTGCCTGGTGATAAAACGACACTACGTGGGAATCTACTATGGCGGCTCTTC
NH_2-DNA TSP-D	TTCAGACTTAGGAATGTGCTTCCCACGTAGTGTCGTTTGTATTGGACCCTCGCAT
SARS-CoV-2 S gene RNA	UACCAUUUAAUGAUGGUGUUUAUUUUGCUUCCACUGAGAAGUCUAACAUAA UAAGAGGCUGGAUUUUUGGUACUACUUUA
Biotin-signal probe	Biotin-TAAAGTAGTACCAAAAATCCAGCCTCTTATTATGTTAGAC

MERS-CoV S gene RNA	CGUCUUUCGAAGCAAAACCUUCUGGCUCAGUUGUGGAACAGGCUGAAGGUG UUGAAUGUGAUUUUUCACCUCUUCUGTCU
SARS-CoV S gene RNA	GGUAACUAUCUUGCAAACAUAUCACCCUGCCUAUGAUGCCAUUGCAAAAAUC UUGAGAUUUAGUCCAUUUUUUCCCUACC
single-base mismatched	UACCAUUUAAUGAUGGUGUUUAUUUUGCUUCCACUGAGAAGUCCAACAUAA UAAGAGGCUGGAUUUUUGGUACUACUUUA
five-base mismatched	UACCAUUUAAUGAUGUUGUUUAUUUUGCUGCCACUGAGAAGUCUCACAUAA UAAGAGGAUGGAUUUUUGGUGCUACUUUA
random DNA sequences	CCACACTGGAACTGAGACAGGGTCCAGACTCCTACGGGAGGCAGCAGTAGGGAA TCTTC

Selected for 2019-nCoV S gene RNA.

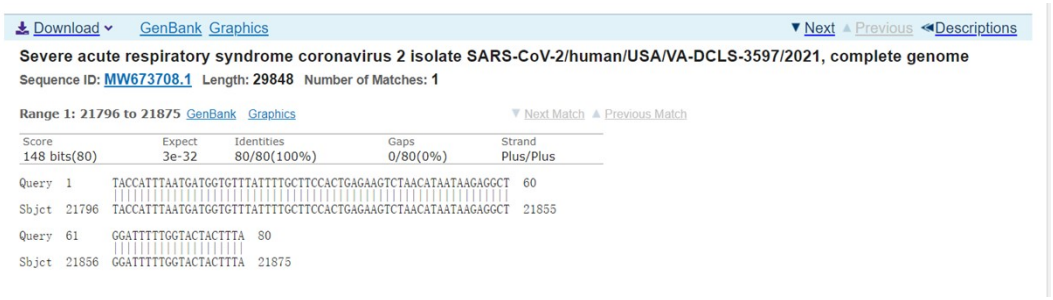

Table S2. The recovery rate of S gene RNA, S protein, S protein antibody of SARS-CoV-2 in human serum

Specific target	Added	Found	Recovery（%）
SARS-CoV-2 S gene RNA	**0.1 nM**	**0.087 nM**	**87**
	1 nM	**0.82 nM**	**82**
	10 nM	**9.2 nM**	**89.4**
SARS-CoV-2 Spike Protein	**3 ng. mL^{-1}**	**2.85 ng. mL^{-1}**	**83.4**
	10 ng. mL^{-1}	**9.3 ng. mL^{-1}**	**90.2**
	30 ng. mL^{-1}	**28.1 ng. mL^{-1}**	**89**
SARS-CoV-2 Spike Protein antibody	**30 ng. mL^{-1}**	**19.6 ng. mL^{-1}**	**65.3**

	300 ng. mL^{-1}	**201.1 ng. mL^{-1}**	**77.3**
	1000 ng. mL^{-1}	**816.4 ng. mL^{-1}**	**81.64**