Electronic Supplementary Material (ESI) for ChemComm.


# Supplementary Information

## Rapid and sensitive multiplex detection of COVID-19 antigens and antibody using electrochemical immunosensor-/aptasensor-enabled biochips

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## Contents

## Experimental Procedures

### Chemical and reagents

Potassium ferricyanide $K_3[Fe(CN)_6]$ (ACS reagent, ≥ 99.0%), potassium ferrocyanide $K_4[Fe(CN)_6]$ (ACS reagent, 98.5%), Tween 20 (polysorbate), glutaraldehyde (25.0% in $H_2O$), ethanolamine (≥ 98%) sulfuric acid (≥ 99.99%), sodium chloride (ACS reagent, ≥ 99.0%), cysteamine (~ 95.0%), chloroauric acid (~ 50% Au basis), tris(2-carboxyethyl)phosphine (TCEP, powder), 6-Mercapto-1-hexanol (MCH, ≥ 99%), bovine serum albumin (BSA) and 1× phosphate-buffered saline (PBS) buffer (pH 7.4) were ordered from Sigma-Aldrich (Merck, USA). The single-stranded DNA (ssDNA) aptamers used for electrochemical biochip preparation were synthesized by Microsynth (Balgach, Switzerland), as listed in Table S1. 5'-biotinylated ssDNA aptamers were produced from Xuanlv Instrument, CO., LTD (China). SARS-CoV-2 spike S1-His recombinant (HPLC-verified, expressed from HEK293 Cells) and Nucleocapsid-His recombinant (expressed from Escherichia coli) protein, S1-lgG antibody (monoclonal rabbit lgG antibody expressed from HEK 293 cells), influenza A H1N1 (expressed from HEK 293 cells) and influenza B HA1 (expressed from HEK 293 cells) protein were provided from Sino Biological Inc (China). Nuclease-Free water was used to dilute chemicals and proteins (Thermo Fisher, USA). The handheld and wireless dual-channel potentiostat (Sensit BT) was purchased from PalmSens Inc (Netherlands).

### Preparation of electrochemical immunosensor- / aptasensor-based biochips

The gold nanoparticle modified screen-printed electrode (AuNP-SPE) was prepared using a roll-to-roll (R2R) screen-printing method. Carbon paste and silver ink were consecutively deposited on the non-conductive polyethylene terephthalate (PET) substrate, then insulating the naked region with photosensitive materials. The AuNP-SPE consisted of a silver quasi-reference electrode, one carbon counter and a working electrode with a dimension of 25 mm × 10 mm × 1 mm. Electroplating gold onto AuNP-SPE was conducted in 5 mmol $HAuCl_4$ and 0.5 M $H_2SO_4$ solution at - 0.2 V (vs.

Ag/AgCl quasi-reference electrode) with a plating time of 90 seconds. The surface morphology of gold nanoparticles is shown in Fig. S1. The AuNP-SPE was cleaned with ethanol, followed by the electrochemical post-cleaning (cycling scanning at the scan rate of 50 $mV \cdot s^{-1}$ from - 0.4 to 1.2 V under 0.5 M $H_2SO_4$ solution) to reduce the oxides of the gold surface. The immunosensor-based biochip was prepared by covalently immobilizing sensing ligands (S1-lgG antibody and S1 protein) onto the AuNP-SPE surface. Firstly, 20 µl of 10 mM cysteamine solution was dispensed on the gold working electrode to introduce the -NH2 group, holding for 2 hours, then surface cleaning with PBS-T buffer (0.05% Tween 20 dissolved in 1 × PBS solution, pH 7.4). Subsequently, 20 µl of 2.5% glutaraldehyde (prepared in PBS-T buffer) solution was added to the gold working electrode surface with an incubation time of 15 minutes, followed by surface cleaning with PBS-T solution. Next, 20 µl of 50 µg/ml SARS-CoV-2 specific biomarker (S1-lgG or S1 protein, prepared in PBS-T buffer) was dispensed on the surface, holding on 2 hours for Schiff base reaction and then surface rinsing with PBS-T buffer. The unreacted -CHO terminals were inactivated with $-NH_2$ groups by incubating the sensing electrode into 20 µl of 0.5 M ethanolamine solution, incubating for 15 minutes, then surface cleaning with PBS-T buffer. Finally, 20 µl of 1% BSA solution (prepared in PBS-T buffer) was dispensed onto the chip, holding on 15 minutes and rinsing with PBS-T buffer. The aptasensor-based biochip was prepared by covalently anchoring SARS-CoV-2 antigen-specific ssDNA aptamer probe onto the AuNP-SPE surface. 75 µl of 1 µM thiolated-ssDNA aptamer probe was mixed with 2 µl of 5 mM TCEP, incubating for 60 min at room temperature to reduce the disulfide bond. Next, 20 µl of 1 µmol aptamer probe solution was dispensed on the gold working electrode, then kept for 2 h in the dark for the probe immobilization. The probe anchored electrode was rinsed with the PBS buffer and then incubated in 20 µl of 2 mM freshly prepared MCH solution for 2 h at room temperature in the dark, then rinsed with deionized (DI) water. The prepared immunosensor- / aptasensor-based test strips were dried with nitrogen, sealed with parafilm, and stored in the dark at – 20 °C.

**Characterization of SARS-CoV-2 immunosensor- / aptasensor-based test strips**

The surface morphology of the gold nanoparticles modified screen-printed electrode was visualized by environmental scanning electron microscopy (ESEM, Quanta 650 ESEM, Thermo Fisher Scientific, Inc.). X-ray photoelectron spectroscopy (XPS) spectrums corresponding to various stages of surface modification were collected by a PHI-5702 electron spectrometer equipped with an Al Kαline excitation source with the C 1s at 285.0 eV as a reference. The cyclic voltammogram (CV) and square wave voltammogram (SWV) measurements were conducted by an electrochemical potentiostat (BioLogic Science Instrument, Germany).

**Aptamer binding assay (enzyme-linked immunosorbent assay, ELISA)**

Aptamer binding assays were performed in 96-well round-bottom plates (Costar, USA). ssDNA aptamers were labeled with biotin at its 3' terminal (biotinylated-aptamer) and utilized for the ELISA test. The specificity and sensitivity of the binding between biotinylated-aptamers and SARS-CoV-2 antigens (S1 and N protein) were evaluated through the ELISA test. ELISA plates were firstly coated with SARS-CoV-2 S1, S2 or N recombinant protein (0.1, 0.2 and 0.5 μg/ml) overnight at 4˚C and blocked with 5% BSA in PBS-T buffer at room temperature for 1 hour. Then, 100 pmol biotinylated-aptamer was dissolved in 100 μl PBS, annealed at 95˚C for 10 min and then incubated in ice for 5 min, allowing for slowly recovering to the room temperature. After washing the pre-coated 96-well plate, the pretreated biotinylated-aptamer was added and incubated at room temperature (1, 3, 6 and 30 minutes), and then PBS-T buffer flushed each well three times. Subsequently, Avidin-HRP (1:250, Thermo fisher, USA) was added in the wells at room temperature for 30 minutes to combine biotin sufficiently. The supernatants were removed and washed three times using PBS-T buffer. The blue color appeared in the well as adding 100 μl tetramethylbenzidine (TMB) substrate (Biolegend, USA), holding on for 15 minutes and terminating the enzymatic reaction by 100 μl stopping buffer (2 M $H_2SO_4$). The absorbance was measured at 450/570 nm with a SpectraMax microplate reader. ssDNA biotinylated-aptamers were provided in

Supplemental Table S1.

**Clinical SARS-CoV-2 swab sample detection**

Four positive anonymized leftover clinical nasopharyngeal swab samples and two negative ones were provided by the Institute of Medical Virology, University of Zürich, Switzerland. Before testing, the clinical samples were confirmed with RT-qPCR in the University of Zürich and then transported to ETH Zürich, stored in the fridge at – 20 °C. The test of the clinical sample was performed in the Biosafety level-2 lab. We placed the patient's nasopharyngeal swab into a storage buffer tube. The unwanted particulates were removed by squeezing the sides of the tube to extract the liquid from the buffer, assisted with a membrane-based filter pad. We then used 1 × PBS buffer (pH 7.4) to dilute the raw buffer 1000 times, to reduce the effects of ions in the storage solution on the specific interaction between aptamer and proteins. Next, we added the filtered swab solution (70 μl) onto the test strips, holding it for 10 minutes, and then replaced the test buffer and measurement. To better compare the signals between the blank and protein anchored condition, we added the test buffer solution onto the sensing electrode, holding on 10 min and performed the electrochemical measurements. During the test, we conducted the square wave voltammetry (SWV) with a step potential of 10 mV, an amplitude of 25 mV, and a frequency of 25 Hz.

**Statistical Analysis**

All statistics were processed with Origin (2019) software. The unpaired two-tails Student's t-test and one-way ANOVA were used to find out the statistical significance. All experiments are done in three replicates, data are from three or four independent experiments. The values are shown as Mean ± SEM and $P$ values are defined as $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

## Ethical Statement

Four positive anonymized leftover clinical nasopharyngeal swab samples and two negative ones were provided by the Institute of Medical Virology, University of Zürich, Switzerland. The Swiss Federal Human Research Act exempts the use of anonymized leftover specimens for research from ethical approval.

# Supplemental Figures

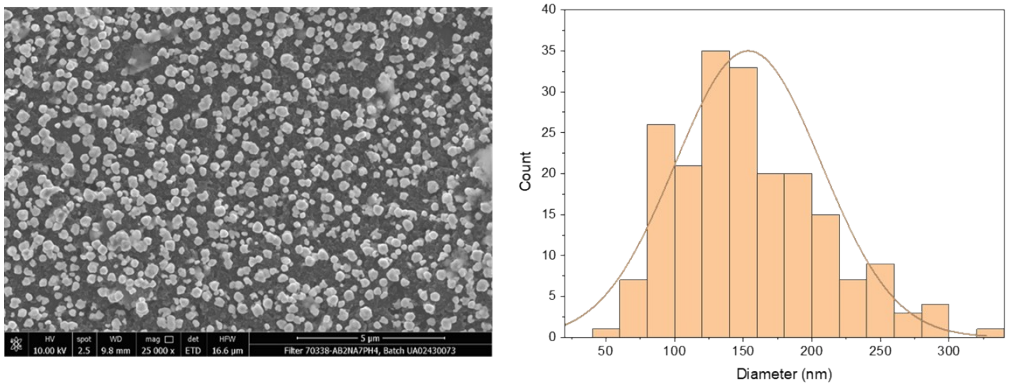



**Fig. S1.** SEM image of gold nanoparticle modified screen-printed carbon working electrode and its corresponding size distribution.

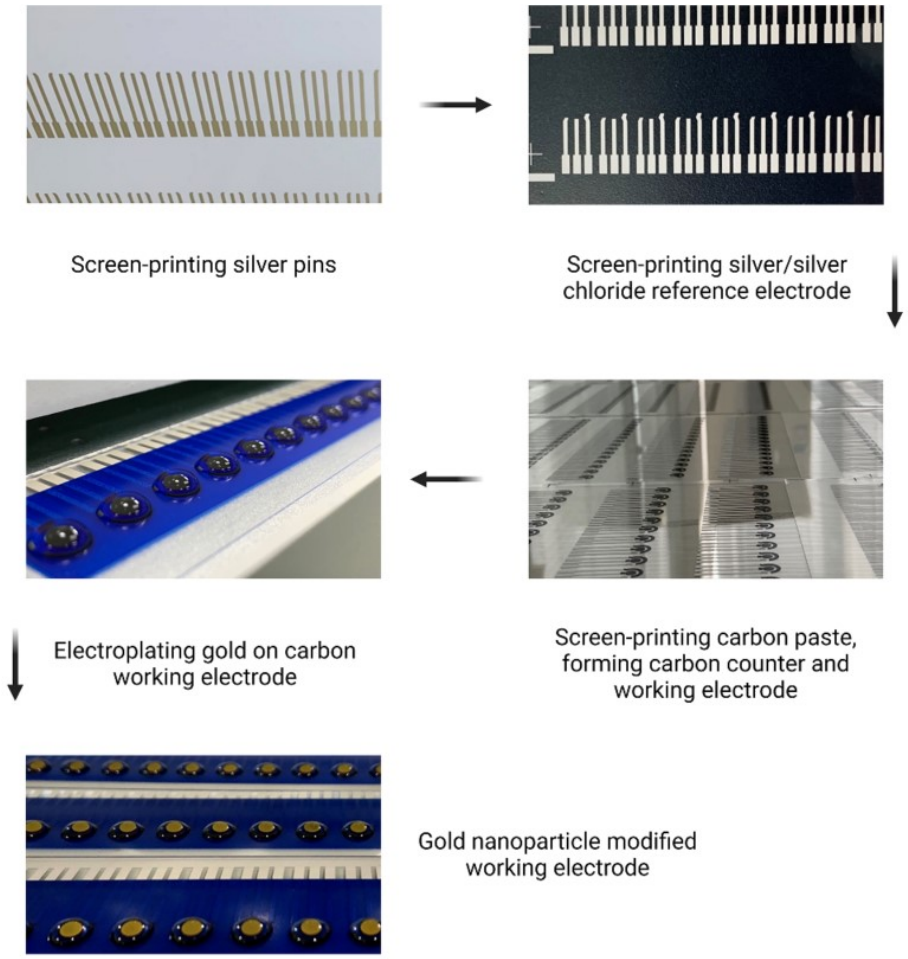



**Fig. S2.** Schematic diagram of preparing gold nanoparticle modified screen-printed carbon electrode.

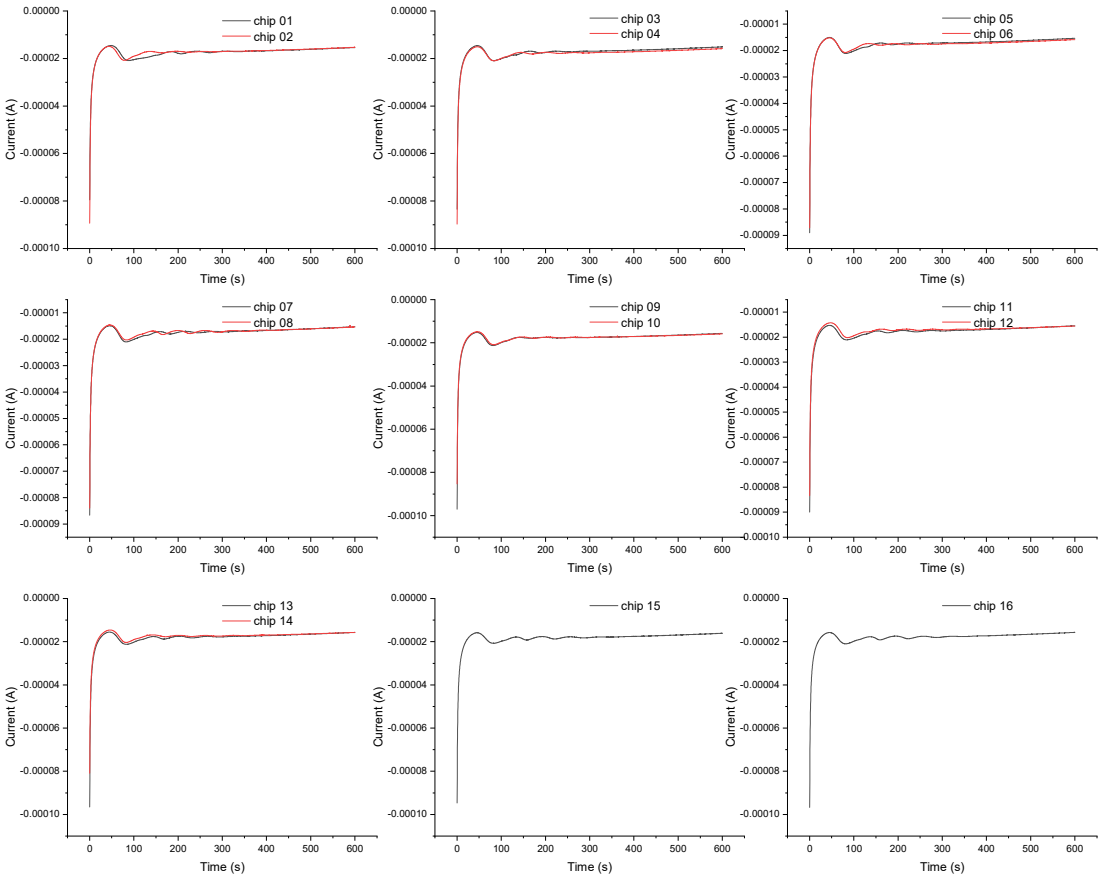



**Fig. S3.** Electrodeposition of gold nanoparticles on carbon working electrodes (16 channels) was conducted in 5 mM $HAuCl_4$ containing 0.5 M $H_2SO_4$ with potentiostat method at -0.2 V for 600s.

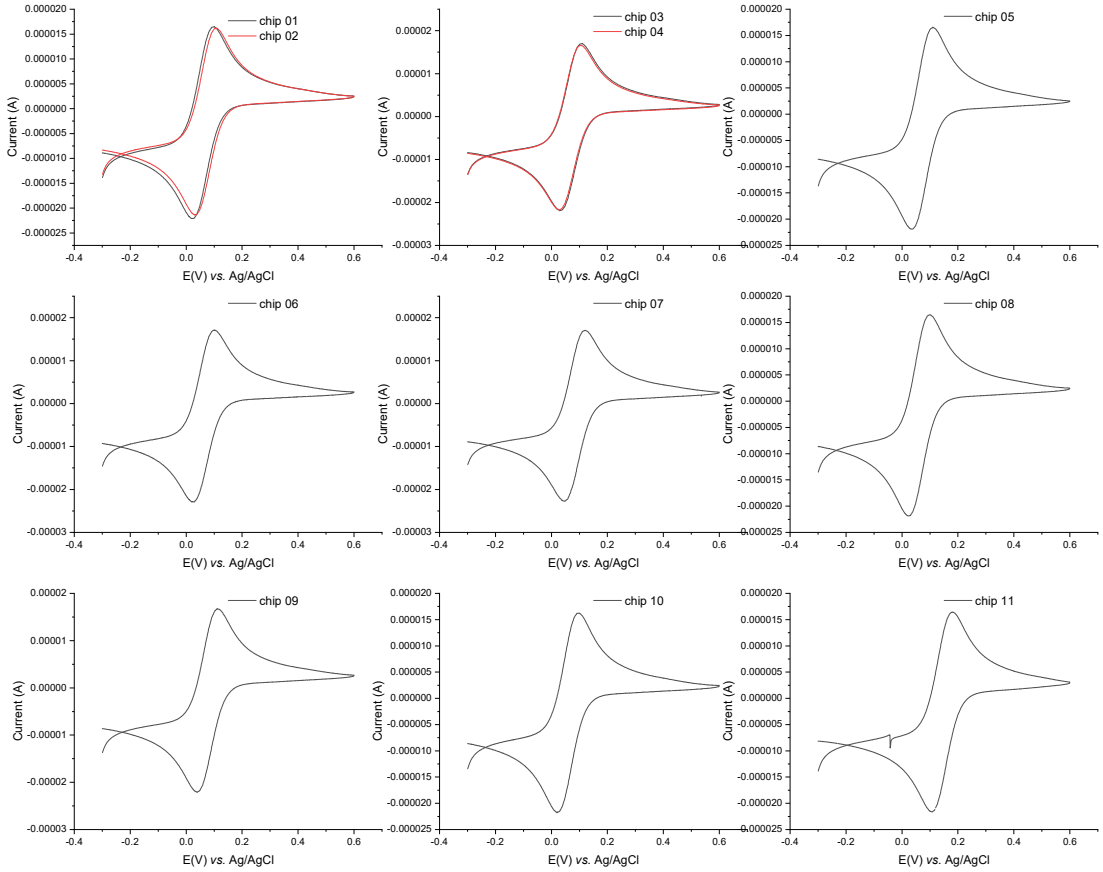



**Fig. S4.** Cyclic voltammetry (CV) of gold nanoparticle modified screen-printed carbon electrodes scanning in 0.5 M $H_2SO_4$ and 5 mM $K_3[Fe(CN)_6]$ / $K_4[Fe(CN)_6]$ redox couple solution.

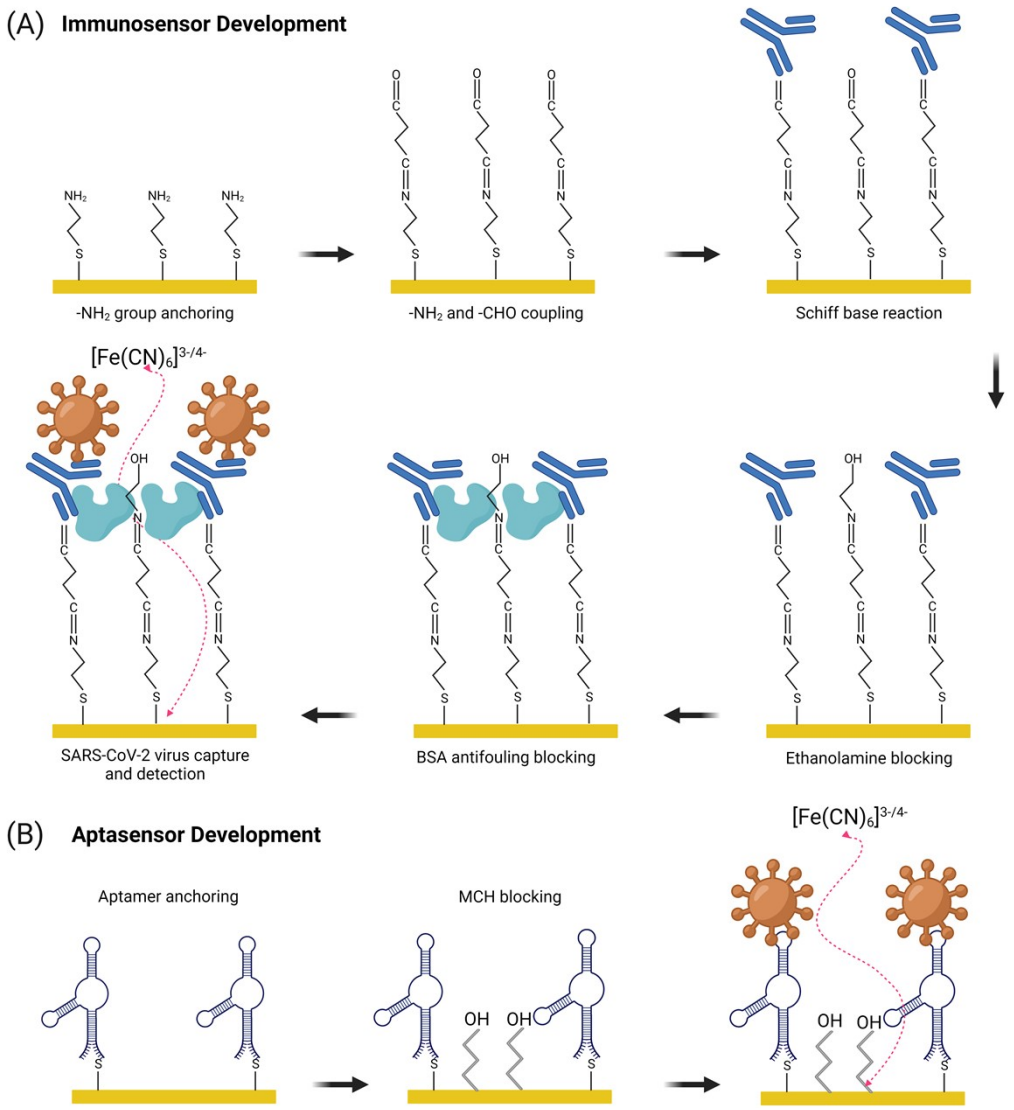



**Fig. S5.** Schematic diagrams of surface modification steps for electrochemical immunosensor- and aptasensor-based biochips development.

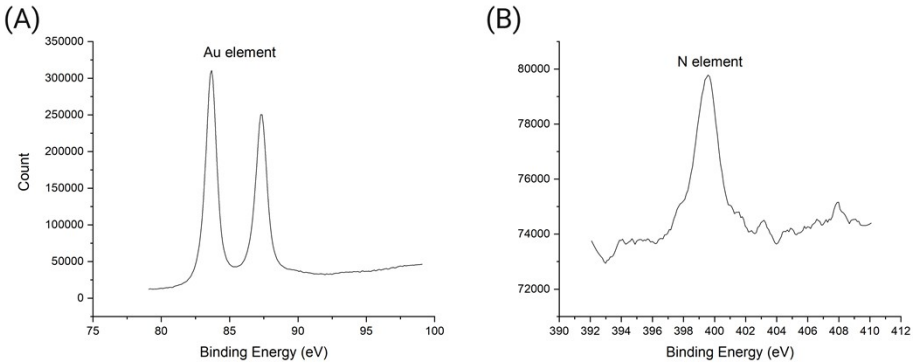



**Fig. S6.** X-ray photoelectron spectroscopy (XPS) spectrum of gold nanoparticle modified screen-printed carbon electrode (AuNP-SPE) (A) and SARS-CoV-2 S1-lgG antibody immobilized AuNP-SE chip.

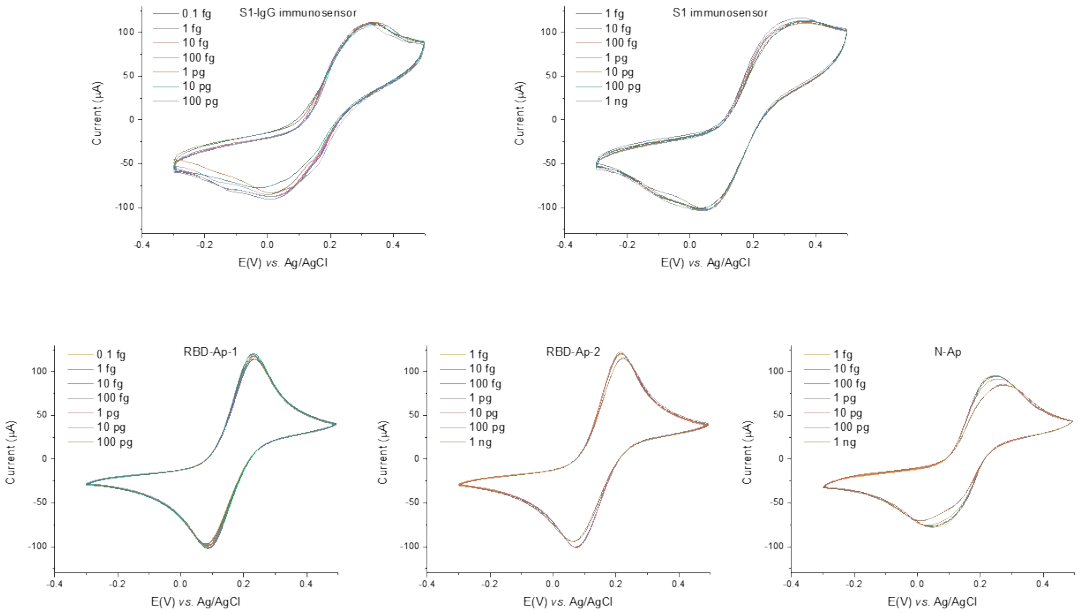
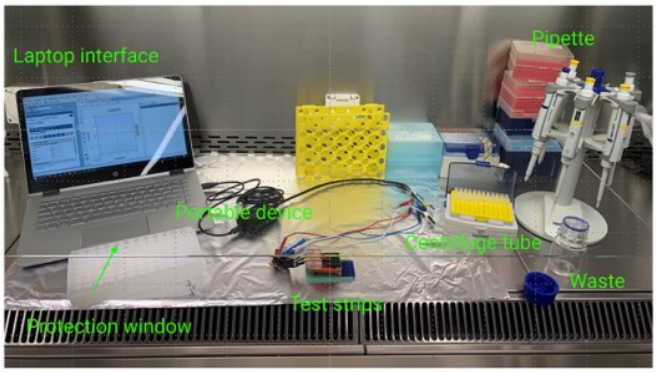



**Fig. S7.** CV diagrams for testing different target concentrations using different assays.




Electrochemical biochips for SARS-CoV-2 diagnosis_test platform in Level 2 Biosafety Cabinet (ETH Zürich)

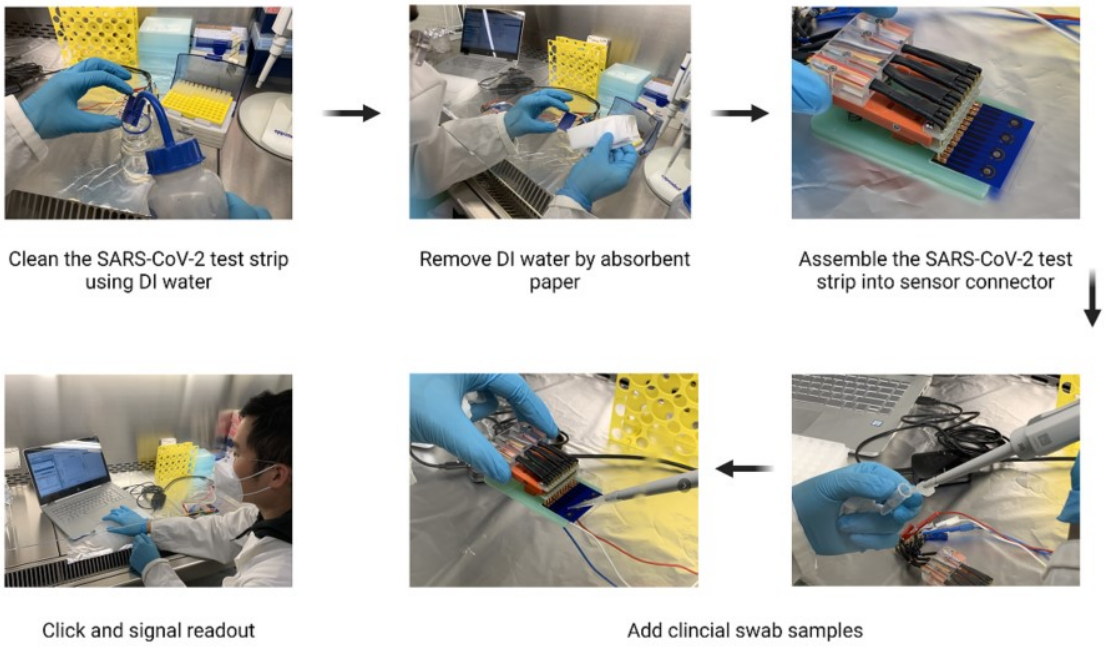



**Fig. S8.** Overview of the test steps for clinical SARS-CoV-2 samples detection by electrochemical immunosensor- and aptasensor-based biochips.

# Supplemental Tables

**Table S1.** The sequence of aptamers (probe) and biotinylated-aptamers (ELISA test).

| Oligonucleotide name | Sequence |
|---|---|
| [1]RBD-Ap-1 | 5’-thiol-CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTAATGGACA |
| [2]RBD-Ap-2 | 5’-thiol-CGCAGCACCCAAGAACAAGGACTGCTTAGGATTGCGATAGGTTCGG |
| [3]N-Ap | 5’-Thiol-GCTGGATGTCACCGGATTGTCGGACATCGGATTGTCTGAGTCATATGACACATCCAGC |
| Biotinylated RBD-Ap-1 | 5’-biotin-CAGCACCGACCTTGTGCTTTGGGAGTGCTGGTCCAAGGGCGTTAATGGACA |
| Biotinylated RBD-Ap-2 | 5’-biotin-CGCAGCACCCAAGAACAAGGACTGCTTAGGATTGCGATAGGTTCGG |
| Biotinylated N-Ap | 5'- biotin-GCTGGATGTCACCGGATTGTCGGACATCGGATTGTCTGAGTCATATGACACATCCAGC |

**Table S2.** Cycle threshold (CT) values of test patient samples (nucleocapsid and envelope genes, CT value > 35 is considered as '+', ≤ 35 is considered as '-').

| Sample ID | Sampling data | CT_N region | CT_E region | |
|---|---|---|---|---|
| 1792 | 2021/09/08 | 25.7 | 24.3 | + |
| 1292 | 2021/09/22 | 18.3 | 17.8 | + |
| 1841 | 2021/10/11 | 26.1 | 23.6 | + |
| 1698 | 2021/10/22 | 17.4 | 16.5 | + |
| 1163 | 2021/06/23 | 39.4 | 40.1 | - |

**Table S3.** Sensor approaches for SARS-CoV-2 viral detection.

| | Detection method | Viral target | LoD | Turnaround time |
|---|---|---|---|---|
| Molecular diagnosis (DNA or RNA genes) | [4]RT-qPCR | RdRp, E, S and N genes | 3.6 copies / sample | 60 min |
| | [5]RPA | S and N genes | 5 viral copies/sample | 45 min |
| | [6]RT-LAMP | E and N genes | 0.44 copies / µl | 30 min |
| | [7]LSPR | RdRp, E and orf1ab genes | 0.22 pM | 20 min |
| | [8]EC | N and S genes | 1 copy / µl | < 2 hours |
| | [9]Nanopore | RdRp, E, S and N genes | 10 copies / reaction | 6 hours |
| | [10]CRISPR-Cas | E and N genes | 10 copies / µl | 45 min |
| Protein diagnosis | [11]ELISA | lgG and lgM antibodies | - - | 2 hours |
| | [12]LFA | S protein | 5 µg / ml | 20 min |
| | [13]FET | S protein | 1 fg / ml | < 10 min |
| | [14]SERS | S protein | 10 PFU / ml | 15 min |
| | **This work** | **S and N proteins, lgG antibody** | **1 fg / ml** | **10 min** |

Abbreviations: RT-qPCR, real-time polymerase chain reaction; RPA, recombinase polymerase amplification; RT-LAMP, reverse transcription loop-mediated isothermal amplification; LSPR, localized surface plasmon resonance; EC, electrochemistry; CRISPR, clustered regularly interspaced short palindromic repeats; ELISA, enzyme-linked immunosorbent assay; LFA, lateral flow assay; FET, field-effect transistor; SERS, surface-enhanced Raman scattering.

**Table S4.** Cost of one-step electrochemical immunosensor-/aptasensor-enabled biochip array for detection of COVID-19 antigens and antibody in swab or blood sample.

| Regent and materials | Quantity | Unit price | Cost |
|---|---|---|---|
| Aptamer/ proteins | 2 μmol, 25 μL | 0.005 $ | 0.005 $ |
| Pipette tips | 3 | 0.01 $ | 0.03 $ |
| SPE-biochip | 5 | 0.5 $ | 2.5 $ |
| Membrane-based filter pad | 1 | 0.5 $ | 0.3 $ |
| Test buffer | 1 | 0.01 $ | 0.01 $ |
| Total | | | 2.845 $ |

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