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Supporting Information

# High-Affinity Dimeric Aptamers Enable the Rapid Electrochemical Detection of Wild-Type and B.1.1.7 SARS-CoV-2 in Unprocessed Saliva

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## Author Contributions

ZZ, JL, RP, DW, AC, CDMF, LS, YL and JDB designed the experiments and interpreted the data. HDS, JCA and MSM prepared spike proteins and pseudoviruses. BS, DY, CB, CJS and JG oversaw collection of patient samples. ZZ, RP, JL, DW and JG performed the experiments and analyzed the data. YL, JDB, LS, RP, ZZ and CB wrote and edited the manuscript.

## 1. Materials

DNA oligonucleotides were obtained from Integrated DNA Technologies (IDT) and purified by standard 10% denaturing (8 M urea) polyacrylamide gel electrophoresis (dPAGE) before use. The sequences are listed in Table S1. The Wuhan SARS-CoV-2 spike protein subunit S1 (catalog number: 40591-V08B1) and the Indian variant spike protein subunit S1 (catalog number: 40591-V08H19) were purchased from Sino Biological Inc. The Wuhan SARS-CoV-2 full spike protein (molecular weight 140 kDa), RBD protein (35 kDa) and SARS-CoV-2 spike-pseudotyped lentivirus were prepared using standard methods (see details below). The trimeric spike protein for the UK B.1.1.7 Alpha variant (catalog number: SPN-C52H6) was obtained from Acro Biosystems. The trimeric spike proteins for B.1.351 (Beta; catalog number: 510333-1) and P.1 (Gamma; catalog number: 100989-1) variants were obtained from BPS Biosciences Inc. The concentrations of the trimeric spike proteins were quantified using BCA protein assay kits from Thermo Scientific (Catalog number: 23225). The B1.1.7 variant SARS-CoV-2 spike pseudotyped lentivirus was obtained from BPS Bioscience (catalog number: 78112-1). The pooled human saliva (Lot 31887) was purchased from Innovative Research Inc (Novi, Michigan). Nitrocellulose blotting membranes (catalog No. 10600125) were purchased from GE Healthcare Inc. Nylon hybridization transfer membranes (NEF994001PK) were purchased from PerkinElmer Inc (Woodbridge, ON, Canada). T4 DNA ligase, T4 polynucleotide kinase (PNK), adenosine triphosphate (ATP) and deoxyribonucleoside 5'-triphosphates (dNTPs) were purchased from Thermo Scientific (Ottawa, ON, Canada). γ-[$^{32}$P]-ATP was acquired from PerkinElmer. Bovine serum albumin (BSA), human thrombin, IL6- Human, streptavidin (*Streptomyces avidinii*) and IgG from human serum were purchased from Millipore-Sigma (Oakville, Canada). Staphylococcus aureus 25923 was purchased from the ATCC. RNase H2 was purified using standard methods.[1] 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium chloride, magnesium chloride, Tween-20, polyethylene glycol (poly(ethylene glycol) methyl ether thiol (6000 kDa), $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$ and all other chemicals were purchased from Millipore-Sigma and used without further purification. Milli-Q water was used for all the experiments.

## 2. Ligation of Dimeric Aptamers

Dimeric aptamer DSA1N5 was prepared by ligation of MSA1T and 5'-phosphorylated MSA5T-T30 with T4 DNA ligase and the template sequence LT1. To phosphorylate MSA5T-T30, 200 pmol of MSA5T-T30 was mixed with 10 U (U: unit) of PNK and 2 mM ATP in 50 µL of 1× PNK buffer A (50 mM Tris-HCl, pH 7.6 at 25 °C, 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine). The mixture was incubated at 37 °C for 1 hour, then heated at 90 °C for 5 min. For ligation, 200 pmol of MSA1T and 300 pmol of LT1 were added into the above reaction mixture, then heated at 90 °C for 5 min and cooled at room temperature for 20 min. To the above solution, 15 µL of 10× T4 DNA ligase buffer (400 mM Tris-HCl, 100 mM $MgCl_2$, 100 mM DTT, 5 mM ATP, pH 7.8) and 10 U of T4 DNA ligase were added. The resultant mixture (total 150 µL) was incubated at room temperature for 2 h and then heated at 90 °C for 5 min to deactivate the ligase. The ligation mixture was concentrated by ethanol precipitation and purified by 10% dPAGE. Other dimeric aptamers with different lengths of polythymidine linkers were prepared using the same method by ligating MSA1T with 5'-phosphorylated MSA5T-T (10, 15, 20, 40). The DMC (dimeric mutant aptamer control) was prepared by ligating mutant sequences M1C and M5C-T30 with the template LT2.

## 3. Radiolabelling of DNA Aptamers

Monomeric and dimeric DNA aptamers were labeled with γ-[$^{32}$P] ATP at the 5'-end using PNK reactions according to the manufacturer's protocol. Briefly, 2 µL of 1 µM DNA aptamers were mixed with 2 µL of

γ-[$^{32}$P] ATP, 1 μL of 10 × PNK reaction buffer A, 10 U (U: unit) of PNK and 4 μL water. The mixture was incubated at 37 °C for 20 min, and then purified by 10% dPAGE.

### 4. Preparation of Recombinant Full Trimeric Spike Protein

A detailed protocol outlining protein production can be found in Stadlbauer et al. 2020.[2] The plasmid encoding the mammalian cell codon optimized sequence for Wuhan SARS-CoV-2 full length spike protein was generously gifted from the lab of Dr. Florian Krammer (Ichan School of Medicine, NYC).[3] In brief, proteins were produced in Expi293 cells (ThermoFisher Scientific) using the manufacturers' instructions. When culture viability reached 40%, supernatants were collected and spun at 500 g for 5 minutes. The supernatant was then incubated with 1 ml of Ni-NTA agarose (Qiagen) per 25 ml of transfected cell supernatant overnight, with shaking, at 4 °C. The following day 10 ml polypropylene gravity flow columns (Qiagen) were used to elute the protein. Spike proteins were concentrated in 50 kDa Amicon centrifugal units (Millipore) prior to being resuspended in phosphate buffered saline (PBS).

### 5. Preparation of Lentivirus

Wuhan SARS-CoV-2 S protein pseudotyped lentivirus was produced as described by Crawford et al.[4] SARS-Related Coronavirus 2, Wuhan-Hu-1 Spike-Pseudotyped Lentiviral Kit (BEI catalog number NR-52948) was obtained through BEI resources, National Institute of Allergy and Infectious Diseases, National Institutes of Health. In brief, HEK293T cells were seeded in 15 cm dishes at 1.1 x $10^7$ cells/mL in 15 mL of standard Dulbecco's Modified Eagle Medium (DMEM). 16 - 24 hours post seeding, cells were co-transfected with HDM-nCoV-Spike-IDTopt-ALAYT (BEI catalog number NR-52515), pHAGE-CMV-Luc2-IRES-ZsGreen-W (BEI catalog number NR-52516), HDM-Hgpm2 (BEI catalog number NR-52517) HDM-tat1b (BEI catalog number NR-52518) and pRC-CMV-Rev1b (BEI catalog NR-52519). 18 - 24 h post-transfection the media was replaced with full DMEM and 60 h post transfection, the supernatant was collected and filtered with a 0.45 μm filter and stored at -80 °C until future use. For purification, 40 mL of supernatant was concentrated by spinning at 19,400 rpm for 2 h. The resulting pellet was resuspended in 400 μl of HBSS, followed by 15 min of continuous vortexing at room temperature. Protein concentration was confirmed by the bicinchoninic acid (BCA) assay.

### 6. Dot Blot Binding Assays with Spike Protein

Dot blot assays were performed by using a Whatman Minifold-1 96-well apparatus and a vacuum pump. Before experiments, nitrocellulose membranes and nylon membranes were incubated in binding buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 6 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.01% Tween-20) for 1 h. γ-[$^{32}$P] labelled DNA aptamers (1 nM) were dissolved in the binding buffer and heated at 90 °C for 5 min, and then cooled at room temperature for 20 min. Spike proteins were dissolved and diluted in the same buffer. 5 μL of the above aptamer solution was mixed with 15 μL of spike protein with different concentrations. The mixture was incubated at room temperature for 1 h. The dot blot apparatus was assembled with a nitrocellulose membrane on the top, a nylon membrane in the middle and a wetted Whatman paper in the bottom. After washing each well with 100 μL of binding buffer, the binding mixtures were loaded and drained by the vacuum pump (force: 550 mmHg for 8 seconds). The wells were then

washed twice with 100 µL binding buffer. The membranes were imaged using a Typhoon 9200 imager (GE Healthcare) and analyzed using Image J software (Molecular Dynamics).

### 7. Dot Blot Binding Assays with SARS-CoV-2 Spike-Pseudotyped Lentivirus

Dot blot assays with SARS-CoV-2 spike-pseudotyped lentivirus and the control lentivirus without spike protein were performed using the same procedure as described in Section 6 except: the aptamer solutions were incubated with different concentrations of viruses (0 - 500 pM of viral particles; corresponding to 0 – 3 x $10^{11}$ cp $mL^{-1}$) for 20 min, followed by performing dot blot assays.

### 8. Cov-eChip fabrication and validation

The Cov-eChip (Metrohm, Dropsens, DRP C220BT) consists of gold (Au) working and counter electrodes, and a silver (Ag) reference electrode. The Cov-eChip was cleaned by rinsing in isopropanol and dd$H_2O$ and was electrochemically activated in 0.1 M $H_2SO_4$ by cyclic voltammetry (0 V to 1.5 V, 0.1 V/s, 10 cycles). The dimeric aptamer- DSA1N5 (1.5, 2 or 3 µM) carrying the thiol group on the 3′ end was first reduced using 200 µM tris(2-carboxyethyl)phosphine for 2 hours at room temperature in the dark and a 5 µL solution was deposited on the working electrode and incubated for 16 - 18 hours at room temperature in the dark. Similar procedure was used for the monomeric aptamers MSA1T (2 µM) MSA5T (2 µM). Following functionalization with the aptamers, the electrode was rinsed in the binding buffer (50 mM HEPES, pH 7.4, 150 mM NaCl, 6 mM KCl, 2.5 mM $MgCl_2$, 2.5 mM $CaCl_2$, 0.01% Tween-20) and 5 µL of a 1 mM thiolated polyethylene glycol (poly(ethylene glycol) methyl ether thiol, average molecular weight of 6000 Da) solution was deposited on the working electrode and incubated for 2 hours at room temperature in the dark to block the unreacted surface. Every step of the electrode preparation, such as cleaning, aptamer deposition and PEG backfilling was validated using electrochemical impedance spectroscopy (EIS, see below). Following optimization, the DSA1N5 aptamer density on the working electrode was calculated using chrono-coulometry (CH instrument 660D) in 10 mM Tris buffer (pH = 7.2) and 1 mM of ruthenium hexamine containing 10 mM Tris buffer (pH = 7.2).

### 9. Detection of Recombinant spike protein and Pseudotyped Lentivirus using Cov-eChip

All EIS measurements were performed using a Sensit Smart USB-sized potentiometer (Palmsens-BV) with an amplitude of 5 mV and frequency range of 20 kHz to 1Hz in a 25 µL solution of readout buffer (10 mM phosphate buffered saline (PBS), pH 7.4 containing 2 mM $K_3[Fe(CN)_6]$ and 2 mM $K_4[Fe(CN)_6]$) at the formal potential of the redox probe (0.23 V vs. Ag/AgCl/KCl saturated). For detection of spike protein, the incubation time for the assay was first optimized using a 40 fM solution of the wild-type spike protein in the binding buffer (same composition as wash buffer). For detection of the spike protein (0-44.4 pM), the target was diluted in binding buffer and incubated on the Cov-eChip for 5 min. For Wuhan pseudotyped lentivirus (0 - $10^6$ cp/mL) and UK pseudotyped lentivirus (0 – $10^5$ cp/mL) detection, the target was spiked in pooled saliva (5 µL of virus into 45 µL of saliva) that was then diluted 1:1 (v:v) with binding buffer and incubated on the Cov-eChip for 5 min at room temperature. For all measurements, the chips were dipped into the binding buffer for 1 min and the signal was measured by EIS in the readout buffer over a period of 2 min. The change in the relative charge transfer resistance ($\Delta R_{CT} / R_{CTi}$) was calculated by the equation:

$$\frac{\Delta R_{CT}}{R_{CTi}} = \frac{R_{CTf} - R_{CTi}}{R_{CTi}}$$

where $R_{CTf}$ is the final charge transfer resistance after sample addition and washing and $R_{CTi}$ is the initial charge transfer resistance measured before addition of sample.

## 10. Cross reactivity study using the Cov-eChip

The cross-reactivity of the Cov-eChip was studied by incubating the chip with diluted pooled saliva and IL6 (1000 pg/mL), Streptavidin (1000 pg/mL), IgG (1000 pg/mL), *Staphylococcus aureus* ($10^4$ CFU/mL), and control lentivirus ($10^5$ cp/mL) spiked in diluted pooled saliva for 5 min at room temperature. The interaction of the 2 µM mutant aptamer and Wuhan pseudotyped lentivirus ($10^4$ cp/mL) was also studied using same experimental conditions as for the dimeric aptamer assay on Cov-eChip. Evaluation of interferences from patient saliva samples was also evaluated, as described in the next section.

## 11. Validation with Patient Samples

Clinical specimens were collected from COVID-19 in-patient units at Hamilton General and Juravinski Hospitals and out-patients from the West End COVID-19 Assessment Clinic, all affiliated with Hamilton Health Sciences (Hamilton, Ontario). The project (Project number 12636) was approved by the Hamilton Integrated Research Ethics Board (HiREB). COVID-19 positive participants at in-patient units were identified from a clinical database and approached for consent. Saliva samples were collected from 0 – 19 days (median = 5 days) from the NPS samples. Participants from the assessment center were approached for consent following NPS collection and saliva samples collected during the visit. Saliva specimens were collected using a saliva self-collection kit (IBI-Scientific) with instructions to pool saliva in their mouths and spit into a collection tube to the 3 ml mark. SARS-CoV-2 RT-PCR testing of NPS specimens were performed at an accredited laboratory (Hamilton Regional Laboratory Medicine Program). NPS PCR cycle threshold results were linked to saliva specimens by a laboratory specimen number to allow sample comparison. Saliva specimens were stored at 4 °C for <72 hours and stored long term at -80 °C prior to analysis.

Prior to testing, all the patient saliva samples were first heat-inactivated at 65 °C for 30 min, and were diluted in binding buffer (1:1 v:v). To evaluate interferences that may arise from variability of patient samples, a selection of 25 negative patient saliva samples (tested using RT-PCR using a previously published method[5] and correlated to corresponding RT-PCR results for NPS samples) were tested as is (unspiked) or spiked with pseudotyped lentivirus to a final concentration of $10^4$ cp/mL. A 10 µL solution of the heat inactivated, unspiked or spiked negative saliva samples were incubated for 5 min on the Cov-eChip at room temperature followed by the same washing and measurement conditions described above for saliva samples spiked with lentiviruses.

For the validation study, a total of 37 negative samples, 31 samples that were confirmed positive for the Wuhan wild-type virus, 3 samples confirmed positive for the Alpha variant, and 2 samples confirmed positive for the Delta variant, were tested. All patient saliva samples were tested using RT-PCR (cycle threshold ($C_t$) values are provided in the Supporting Information, Table S3) followed by a blinded clinical

validation study with the electrochemical sensor using saliva that was diluted 1:1 in binding buffer and tested as described above for saliva samples spiked with lentiviruses. Six false negative saliva samples were spiked with $10^6$ cp/mL of Wuhan pseudotyped virus. 10 µL of these spiked samples were tested as described above for saliva samples spiked with lentiviruses.

## 12. Data analysis

The charge transfer resistance data obtained from the EIS scans were fit to the Randles circuit[6] using the EIS analyser module of the PalmSens trace software. Every data point corresponds to the mean of three (n = 3) individual datapoints measured for the same conditions on three separate device and the error bar indicates the standard deviation. The limit-of-detection was then calculated by substituting the limit-of-blank[7] as the "y" value in the regression line equation of the calibration curve. A two tailed Student *t* test was performed for the specificity test. Equations used for the calculation of limit-of-blank, clinical sensitivity, specificity, and concordance are given below. The Receiver Operating Characteristics (ROC) curve analysis was performed using the program Analyse-it for Microsoft Excel (Analyse-it, Leeds UK) to determine the decision threshold to maximize sensitivity and specificity of the assay.

Limit of blank (LOB) for limit of detection for recombinant spike protein in buffer and pseudotyped lentivirus spiked in pooled saliva:

$$LOB = mean\ of\ blank\ (buffer\ or\ pooled\ saliva) + 3 \times (\text{S.D. of blank})$$

Clinical Sensitivity:

$$Sensitivity = \frac{True\ positive}{True\ positive + False\ negative}$$

Clinical Specificity:

$$Specificity = \frac{True\ negative}{True\ negative + False\ positive}$$

Concordance:

$$Concordance = \frac{Number\ of\ correct\ outcome}{Total\ number\ of\ samples\ tested}$$

**Table S1**. DNA sequences used in this work.

| Name | No. of nt | Sequences (5'→3') |
|---|---|---|
| MSA1 | 79 | TTACG TCAAG GTGTC ACTCC CACTT TCCGG TTAAT TTATG CTCTA CCCGT CCACC TACCG GAAGC ATCTC TTTGG CGTG |
| MSA5 | 79 | TTACG TCAAG GTGTC ACTCC ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGTG GAAGC ATCTC TTTGG CGTG |
| MSA1T | 39 | TTCCG GTTAA TTTAT GCTCT ACCCG TCCAC CTACC GGAA |
| MSA5T | 39 | ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGT |
| MSA5T-T10 | 49 | TTTTT TTTTT ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGT |
| MSA5T-T15 | 54 | TTTTT TTTTT TTTTT ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGT |
| MSA5T-T20 | 59 | TTTTT TTTTT TTTTT TTTTT ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGT |
| MSA5T-T30 | 69 | TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGT |
| MSA5T-T40 | 79 | TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGT |
| LT1 | 22 | AAAAA AAAAA TTCCG GTAGG TG |
| DSA1N5 | 108 | TTCCG GTTAA TTTAT GCTCT ACCCG TCCAC CTACC GGAAT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTA CGGGT TTGGC GTCGG GCCTG GCGGG GGGAT AGTGC GGT |
| DSA1N1 | 108 | TTCCG GTTAA TTTAT GCTCT ACCCG TCCAC CTACC GGAAT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT TCCGG TTAAT TTATG CTCTA CCCGT CCACC TACCG GAA |
| DSA5N5 | 108 | ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGTT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTA CGGGT TTGGC GTCGG GCCTG GCGGG GGGAT AGTGC GGT |
| M1C | 39 | CTATA TCCCT TTAAG CCTGT GGGTC CACGA ATCTA CCTC |
| M5C | 39 | GCGTT CGGAG TGGGT CGTGC GGCGC GGGAC GAGTG GTGT |
| M5C-T30 | 69 | TTTTT TTTTT TTTTT TTTTT TTTTT TTTTT GCGTT CGGAG TGGGT CGTGC GGCGC GGGAC GAGTG GTGT |
| LT2 | 22 | AAAAA AAAAA GAGGT AGATT CG |

| | | |
|---|---|---|
| DMC | 108 | CTATA TCCCT TTAAG CCTGT GGGTC CACGA ATCTA CCTCT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTG CGTTC GGAGT GGGTC GTGCG GCGCG GGACG AGTGG TGT |
| DSA1N5-SH | 112 | TTCCG GTTAA TTTAT GCTCT ACCCG TCCAC CTACC GGAAT TTTTT TTTTT TTTTT TTTTT TTTTT TTTTA CGGGT TTGGC GTCGG GCCTG GCGGG GGGAT AGTGC GGTTT TT/3ThioMC3-D/ |
| MSA1T-SH | 43 | TTCCG GTTAA TTTAT GCTCT ACCCG TCCAC CTACC GGAAT TTT/3ThioMC3-D/ |
| MSA5T-SH | 43 | ACGGG TTTGG CGTCG GGCCT GGCGG GGGGA TAGTG CGGTT TTT/3ThioMC3-D/ |

**Table S2**. Summary of $K_d$ values of monomeric and dimeric aptamers for the trimeric spike protein of SARS-CoV-2 as well as the affinity enhancement of dimeric/monomeric aptamers.

| Aptamer | $K_d$ (nM) | $K_d$ ratio of MSA1T/dimer | $K_d$ ratio of MSA5T/dimer |
|---|---|---|---|
| MSA1T | 11.9 ± 1.8 | | |
| MSA5T | 3.4 ± 0.6 | | |
| DSA1N1 | 1.9 ± 0.1 | 6.3 | 1.8 |
| DSA5N5 | 0.65 ± 0.07 | 18.3 | 5.2 |
| **DSA1N5** | **0.12 ± 0.02** | **99.2** | **28.3** |

**Table S3.** Comparative study of the positive COVID-19 samples using NPS $C_t$ and Cov-eChip assay.

| Sample ID | Cov-eChip assay result | NPS UTR Ct | Sample ID | Cov-eChip assay result | NPS UTR Ct |
|---|---|---|---|---|---|
| 1 | + | 16.7 | 34 | + | 31.8 |
| 2 | + | 30.5 | 35 | + | 23.1 |
| 3 | + | 16.3 | 36 | + | 23.9 |
| 4 | + | 23.0 | 37 | + | 22.3 |
| 5 | + | 20.6 | 43 | + | 22.0 |
| 7 | + | 18.0 | 45 | + | 20.9 |
| 8 | + | 30.9 | 46 | + | 23.6 |
| 9 | + | 24.5 | 54 | - | 24.0 |
| 10 | + | 16.4 | 59 | + | 17.6 |
| 12 | + | 25.6 | 62 | - | 21.3 |
| 14 | + | 27.4 | 66 | - | 27.8 |
| 16 | - | 17.0 | 71 | + | 31.6 |
| 17 | + | 21.0 | 159 | - | 20.3 |
| 18 | + | 18.5 | 180 | + | 22.2 |
| 19 | + | 32.1 | 216 | + | 21.6 |
| 22 | + | 19.4 | 217 | + | 22.5 |
| 26 | - | 18.7 | 223 | + | 32.7 |
| 510 | - | 19.4 | 519 | + | 14.9 |

Note: Samples in green: positive by CoV-eChip assay; samples in red: false-negatives by CoV-eChip assay.

**Table S4.** A list of SARS-CoV-2 antigen tests.

| MRE/Sensor type [a] | Target[b] | Test time (min) | # of clinical samples | LOD[b] | Sensitivity | Specificity | Ref. |
|---|---|---|---|---|---|---|---|
| Aptamer/ECS | S | 10 | 34+, 37- | 1000 cp/mL | 82 | 100 | This work |
| Antibody/ECS | S | 10 | 3+, 6- | 4000 cp/mL | 100 | 100 | [8] |
| Aptamer/ECS | S and N | 60 | 16+, 8- | $52 \times 10^6$ cp/mL | 100 | 100 | [9] |
| Antibody/ECS | S and N | 35 | 7+, 17- | 6.5 PFU/mL | 77 | 100 | [10] |
| Antibody/ECS | S | < 1 | Not tested | 90 fM S | - | - | [11] |
| Antibody/ECS | N | 10 | 5+, 3- | 500 pg/ mL N | 100 | 100 | [12] |
| Antibody/ECS | S | real time | 2+, 1- | 242 cp/mL | - | - | [13] |
| Aptamer/ECS | S | 30 | Not tested | $10^3$ ng/ mL S | - | - | [14] |
| ACE2/LFS | S | 20 | 4+, 4- | $1.86 \times 10^5$ cp/mL | 100 | 100 | [15] |

a) MRE: molecular recognition element; ECS: electrochemical sensor; LFS: lateral flow sensor; b) S: spike protein; N, Nucleocapsid protein

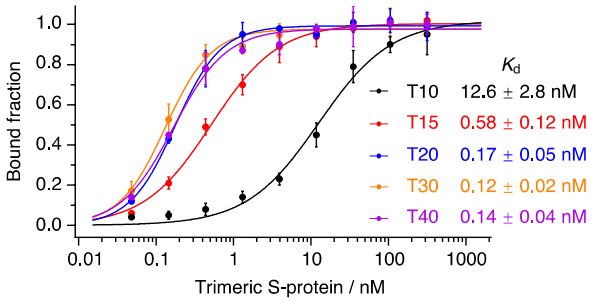



**Figure S1.** Affinity tests of the dimeric DSA1N5 aptamer with different polyT linkers (10, 15, 20, 30 and 40 of polythymidines) binding the trimeric wild-type spike protein of SARS-CoV-2.

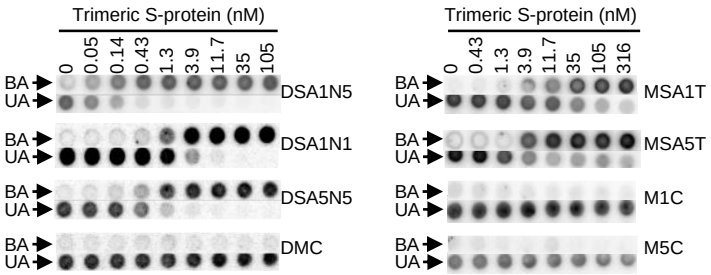



**Figure S2.** The dot blot results of DSA1N5, DSA1N1, DSA5N5, DMC, MSA1T, MSA5T, M1C and M5C for the full spike proteins of wild-type SARS-CoV-2. DMC, M1C and M5C are the mutant sequences of DSA1N5, MSA1T and MSA5T. BA and UA: bound and unboud aptamers.

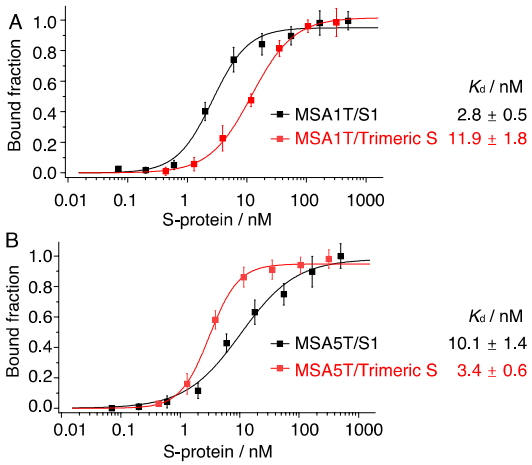



**Figure S3.** Comparion of binding of (A) MSA1T and (B) MSA5T to the S1 subunit and the full-length spike protein of wild-type SARS-CoV-2.

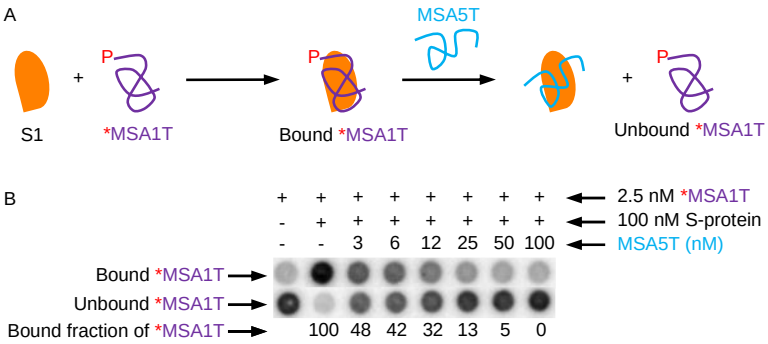



**Figure S4.** Competition beyween MSA1T and MSA5T for binding to S1. (A) Assay schematic. Radioactive MSA1T is allowed to bind fully to S1 before competition with MSA5T. (B) Assay results. A 100 nM solution of S1 was incubated with 2.5 nM radioactive (*) MSA1T, followed by the addition of 3-100 nM non-radioactive MSA5T.

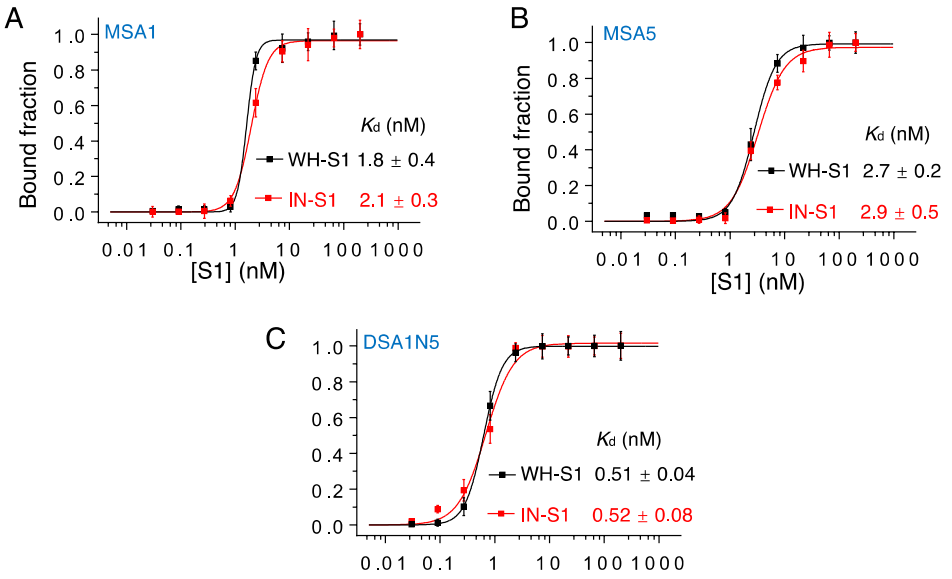



**Figure S5.** Comparison of binding profiles of MSA1, MSA5 and DSA1N5 with the S1 subunit of wildtype SARS-CoV-2 (WH-S1) and the S1 subunit of B.1.617.2 variant (IN-S1).

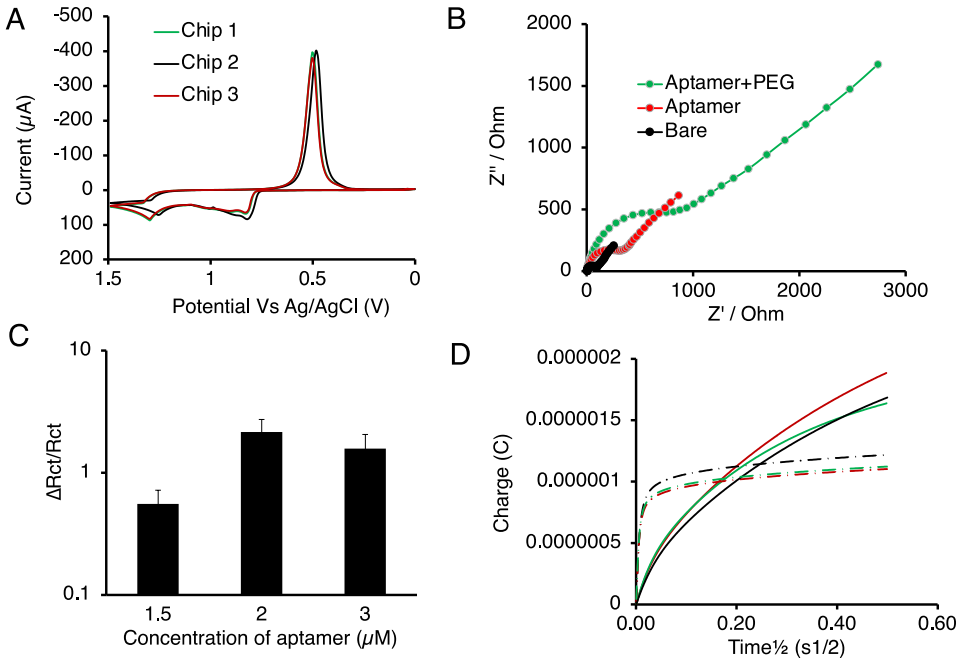
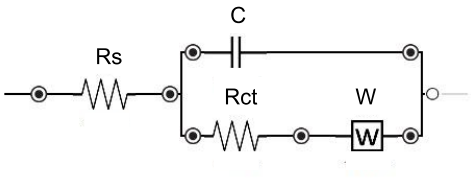



**Figure S6. Cov-eChip optimization**. (A) Cyclic voltammetry of bare gold electrodes to assess reproducibility and surface area. (B) Nyquist plots for bare, poly ethylene glycol (PEG)-functionalized and aptamer-functionalized electrodes. (C) optimization of aptamer concentration. (D) Chronocoulometry performed on the functionalized surface to calculate the aptamer surface density using the optimized aptamer concentration of 2 μM. The dash lines refer to measurement done in 10 mM Tris buffer (pH = 7.2) whereas the solid lines refer to measurement done in 1 mM of ruthenium hexamine containing 10 mM Tris buffer (pH = 7.2).

A




B

| Modification | Solution resistance (Rs) Ω | Charge Transfer resistance (Rct) Ω | Capacitance (C) µF |
|---|---|---|---|
| Bare | 15±3 | 200±23 | 1.2±0.27 |
| Aptamer | 23±5 | 400±36 | 2.5±0.6 |
| Aptamer_PEG | 30±4 | 950±96 | 4.9±0.7 |

**Figure S7. Cov-eChip Assay data analysis.** (A) The equivalent circuit model used for fitting the electrochemical impedance spectroscopy (EIS) data. The solution resistance (Rs) is in series with the capacitance (C), charge transfer resistance (Rct), and the Warburg element (W). (B) The list of the parameters and their values extracted from the fitting.

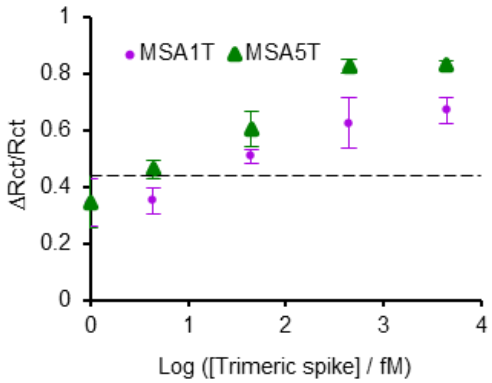
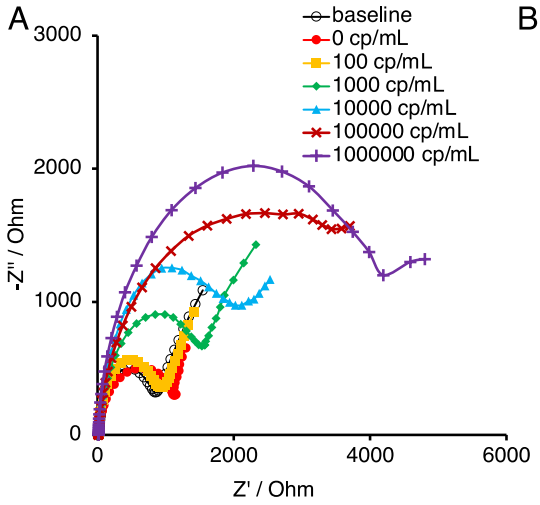



**Figure S8. Performance of monomeric aptamers MSA1T and MSA5T for detection of trimeric spike protein.** Calibration plot of the different concentrations of the trimeric spike protein tested with monomeric aptamers MSA1T and MSA5T.

A




B

| Concentration (cp/mL) | Solution resistance (Rs) Ω | Charge Transfer resistance (Rct) Ω | Capacitance (C) $\mu$F |
|---|---|---|---|
| Baseline | 45 ± 5 | 1566 ± 264 | 12 ± 0.7 |
| 0 | 33 ± 5 | 1793 ± 188 | 33 ± 1.2 |
| $10^2$ | 49 ± 2 | 1600 ± 104 | 42 ± 0.8 |
| $10^3$ | 52 ± 4 | 2116 ± 254 | 90 ± 19 |
| $10^4$ | 59 ± 8 | 3000 ± 115 | 113 ± 10 |
| $10^5$ | 42 ± 9 | 3933 ± 152 | 233 ± 15 |
| $10^6$ | 32 ± 15 | 4066 ± 178 | 263 ± 23 |

**Figure S9. Cov-eChip assay validation with pseudotyped virus spiked in diluted pooled saliva.** (A) Nyquist plot obtained for detecting different concentrations of the pseudotyped virus in diluted saliva. (B) The list of parameters and their values extracted from the fitting.

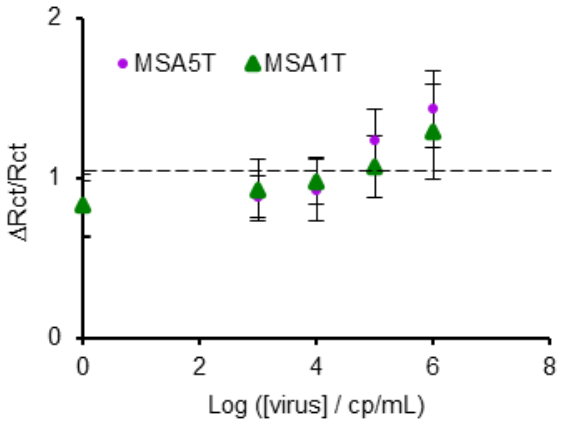



**Figure S10. Performance of monomeric aptamers MSA1T and MSA5T for detection of Wuhan pseudotyped virus.** Calibration plot of the different concentrations of the WHPV tested with monomeric aptamers MSA1T and MSA5T.

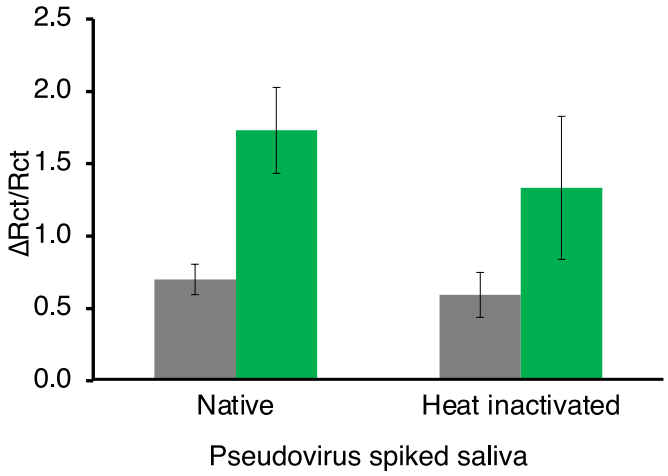



**Figure S11. Effect of saliva sample heating on the performance of the Cov-eChip assay.** A 1000 cp/mL of pseudovirus was spiked in diluted pooled saliva. These samples were heat inactivated by heating at 65 °C for 30 min and compared with pseudotyped lentivirus spiked in diluted pooled saliva without heating. Light grey bars indicate the un-spiked saliva and green bars indicate the spiked saliva samples.

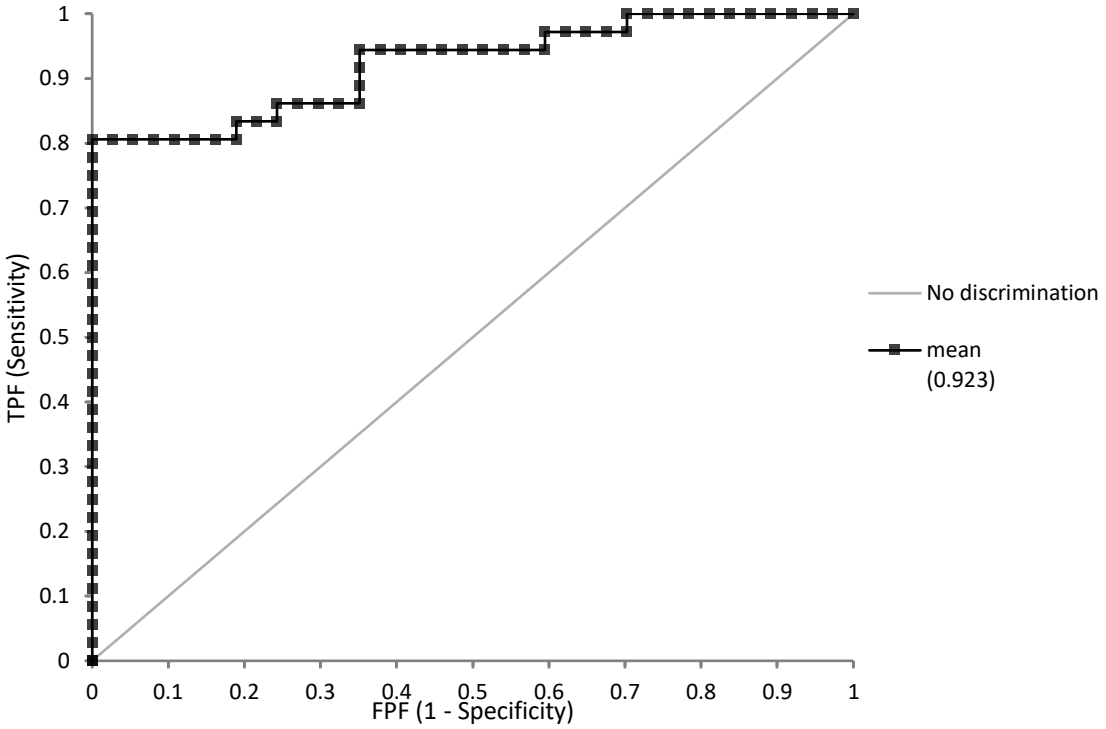



**Figure S12. Receiver-Operator Characteristics Curve for the Cov-eChip assay.** The overall accuracy, or area under the curve (AUC) was 0.923 (CI, 0.860 – 0.985) with an optimum sensitivity of 80.5% (true positive cases detected) at a threshold of 1.27 ΔRct/Rct and a corresponding specificity of 100% (no false positive cases detected). TPF is true-positive fraction. FPF is false-positive fraction.

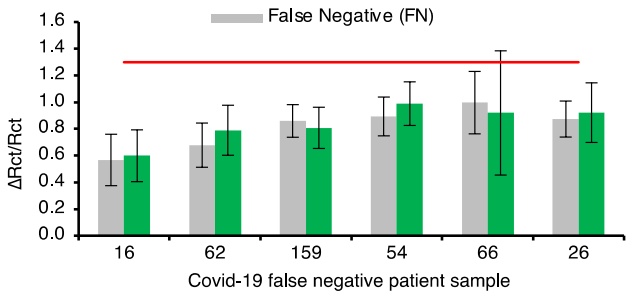



**Figure S13. False negative sample study with pseudotyped virus.** The signal measured from the false negative samples before (grey) and after (green) spiking with $10^6$ cp/mL of pseudovirus.

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