Supplementary Information File

Combining a perfluorocarbon SAM and ACE2 as the bio-recognition element shows a promising route to low cost electrochemical screening for SARS-CoV-2 in complex samples.

Vincent J Vezza[a], Adrian Butterworth[a], Perrine Lasserre[a], Ewen O Blair[a], Alexander MacDonald[a], Stuart Hannah[a], Christopher Rinaldi[b] Paul A Hoskisson[c], Andrew C Ward[d], Alistair Longmuir[e], Steven Setford[e], Michael E Murphy[f,g], Damion K Corrigan[a].

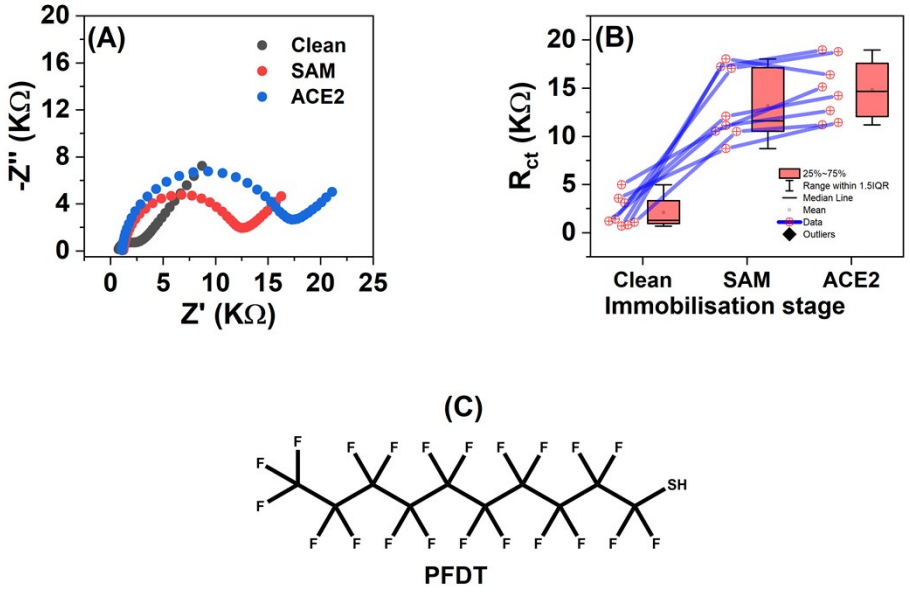

Figure S1. (A) Example Nyquist plots from a representative electrode following cleaning (black), PFDT functionalisation (red) and ACE2 incubation (blue). (B) Box plot showing R_{ct} values through the three stages of electrode functionalisation (cleaning, SAM formation and ACE2 immobilisation). (C) Structural formula of PFDT.

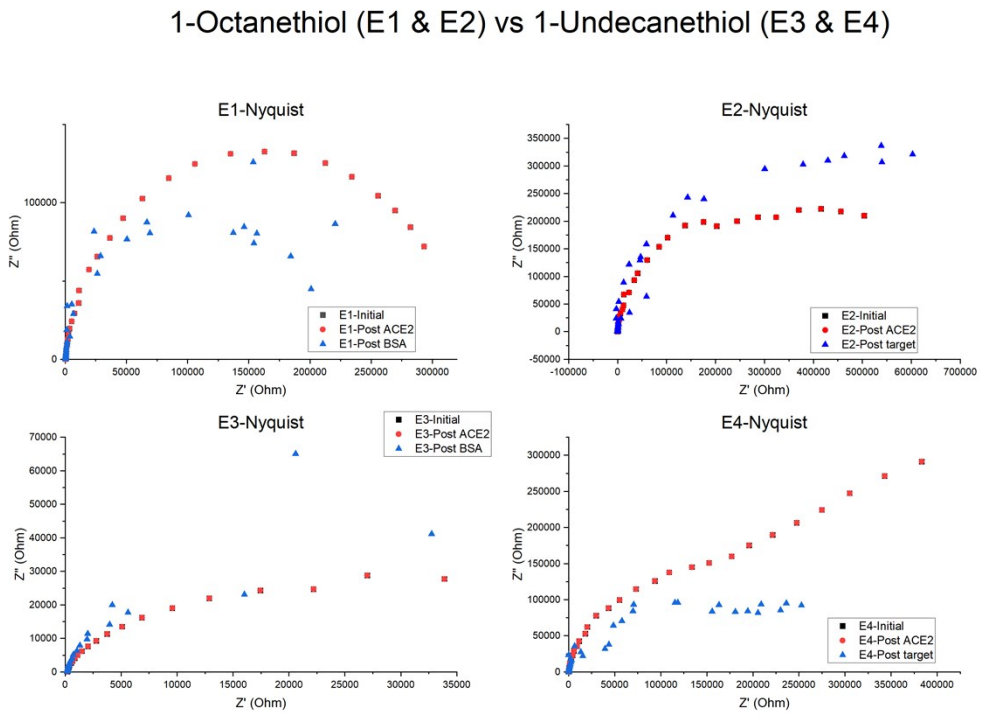

Figure S2. Showing impedance changes like those observed for PFDT modified electrodes, characteristic of increasing charge transfer resistance upon spike protein binding were not observable when the underlying SAM layer was composed of 1-octanethiol or 1-undecanethiol.

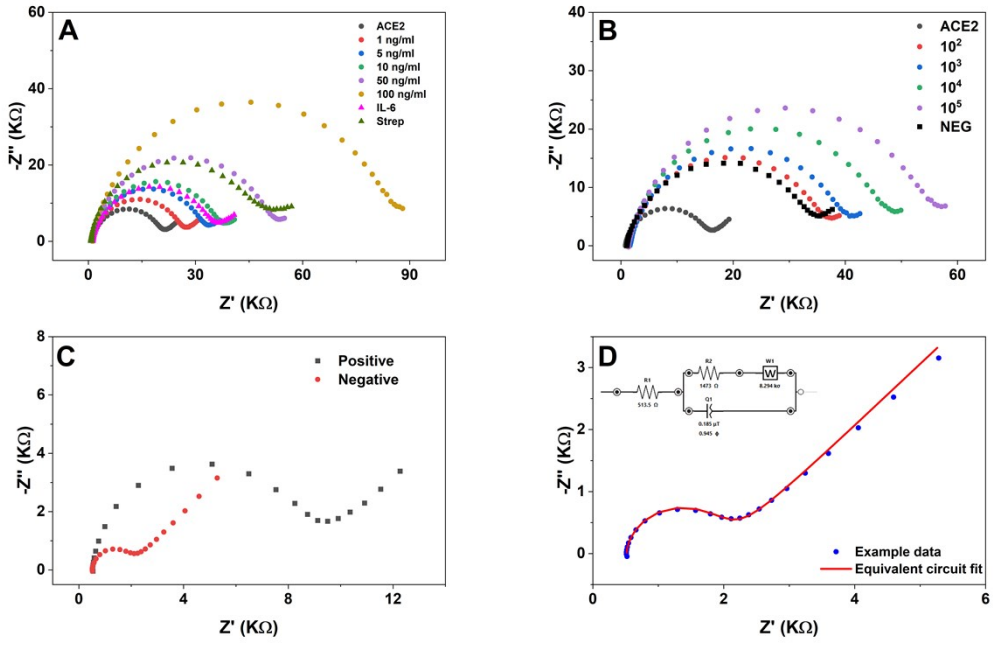

Figure S3. Showing example Nyquist plots for: Spike protein concentrations, IL-6 and streptavidin (A). Various inactivated virus concentrations plus negative control (B). Positive and negative VPSS clinical samples (C). Example Nyquist data fitted using R([RW]Q) equivalent circuit parameters (D). This circuit was used to fit all data.

Materials and Methods

Chemicals

$K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$, 1*H*,1*H*,2*H*,2*H*-perfluorodecanethiol, KOH and H_2O_2 30 % (v/v) were obtained from Sigma Aldrich. Toluene was obtained from Fisher Scientific UK Ltd (Loughborough, UK). Deionised water (5.00 µS/cm @ 25 °C) was purchased from Scientific Laboratory Supplies Limited (Nottingham, UK). Inactivated SARS-CoV-2 and negative control obtained from Randox laboratories Ltd (Crumlin, UK). ACE2 was purchased from Abcam (Cambridge, UK), HRP conjugated spike protein was purchased from The Native Antigen Company (Oxford, UK) and HRP conjugated streptavidin was purchased as part of an IL-6 diagnostics kit from Bio-techne (Abingdon, UK).

Preconditioning

SEP1 BIOTIP multichannel electrode PCB platform (biotip ltd, Bath, UK) were cleaned according to the supplied protocol. This consisted of a 15-minute submersion in a solution of 50 mM KOH in H_2O_2 30 % (v/v) at room temperature. The PCB was then rinsed with DI water and dried using compressed air. The PCB was then electrochemically cleaned by submerging in 50 mM KOH (DI water as solvent) with an external platinum counter electrode (Metrohm, Runcorn, UK) and 3M NaCl Ag/AgCl reference electrode (IJ Cambria, Llanelli, UK). Cyclic voltammetry was performed on all working electrodes on the PCB using the following parameters: potential window was -1.2 to 0.6 V, scan rate of 0.1 V/s and 15 scans per electrode. The PCB was then rinsed with DI water and dried again using compressed air. All electrochemical measurements were performed using a PalmSens4 potentiostat and the accompanying PSTrace software, both supplied by Palmsens BV (Houten, Netherlands).

Fluorous SAM and ACE2 Immobilisation

The SAM solution was prepared by magnetically stirring toluene and adding PFDT until a 1 mM solution was formed. Stirring aids in dispersing the PFDT throughout the solution. Fluorocarbons can have low miscibility in organic solvents and have a propensity for self-interaction forming separate phases v. The PCBs were orientated horizontally in a small glass petri dish and the PFDT solution added to cover the PCB. The PCBs were incubated overnight at room temperature, then rinsed with DI water (10 second water bottle flow per electrode) and dried with compressed air. All work with toluene was performed in a suitable fume hood with proper halogenated solvent waste disposal routes.

ACE2 was diluted from stock in 1 x PBS to 1µg/ml and a10 µL aliquot was applied to each working electrode on the PCB and left to incubate for 1 hour at room temperature. Following incubation, the PCBs were rinsed with 1 x PBS (10 second water bottle flow per electrode) and dried with compressed air.

Protein Target Detection

To investigate evidence of specific binding between ligand (ACE2) and protein (HRP conjugated SARS-CoV-2 spike protein) a series of dilutions of the positive control HRP conjugated SARS-CoV-2 spike protein and negative controls of similar sized proteins (HRP conjugated streptavidin and IL-6) were incubated at room temperature for 30 minutes on the PCB sensor arrays with rinsing with 1 x PBS (10 seconds water bottle flow per electrode) and EIS measurements between each concentration incubation. HRP conjugated SARS-CoV-2 spike protein concentrations used were 1, 10, 50 and 100 ng/ml (all dilutions in 1 x PBS) and IL6 used 100 ng/ml. HRP conjugated streptavidin was obtained as part of an ELISA kit and the concentration was not disclosed. The accompanying instructions recommended a 1:40 dilution for ELISA assays. A 1:5 dilution was used which came out to be 200 µg/ml.

Inactivated Virus Detection

For detection of inactivated virus, a clinical molecular standards kit for SARS-CoV-2 was purchased. The kit contained positive and negative samples of the virus present in a complex "transport medium" representative of a clinical sample. A series of dilutions of the positive control (inactivated virus + transport medium and human cells) was incubated for 30 mins at room temperature on the PCBs. The concentrations used were 10^2, 10^3, 10^4 and 10^5 dC/ml (digital copies per ml). The negative control (transport medium + human cells) was incubated for 30 minutes at room temperature. Room temperature incubations were chosen to replicate the operational environmental conditions likely required for a diagnostic device. The PCBs were rinsed with 1xPBS (10 seconds wash bottle flow per electrode) and EIS measurements performed between each incubation.

Clinical Sample Detection

For clinical sample detection, positive and negative SARS-CoV2 samples in VPSS were obtained from (Clinical microbiology, NHS Glasgow Royal Infirmary). 20 µl of each sample was added to premade solutions to make a final 5 mM potassium ferri/ferrocyanide in 1 x PBS + 1 µg/ml ACE2 + 10 times diluted clinical sample solution. This was left for 30 minutes to allow SARS-CoV-2 to bind to ACE2 in solution then 50 µL was added to each pair of SAM functionalised PCB electrodes, covering two working electrodes and their associated counter + reference electrode. EIS measurements were taken immediately. PCBs were then disposed via the appropriate clinical waste route.

EIS Parameters

All EIS measurements used the following parameters. E_{ac} = 0.01 V rms, E_{dc} = 0 V, frequency range = 100 kHz to 1 Hz with 50 frequencies at 9.8/decade and measurements were made versus the open circuit potential (OCP). All measurements were obtained using 5 mM $K_3[Fe(CN)_6]/K_4[Fe(CN)_6]$ in 1 x PBS.

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