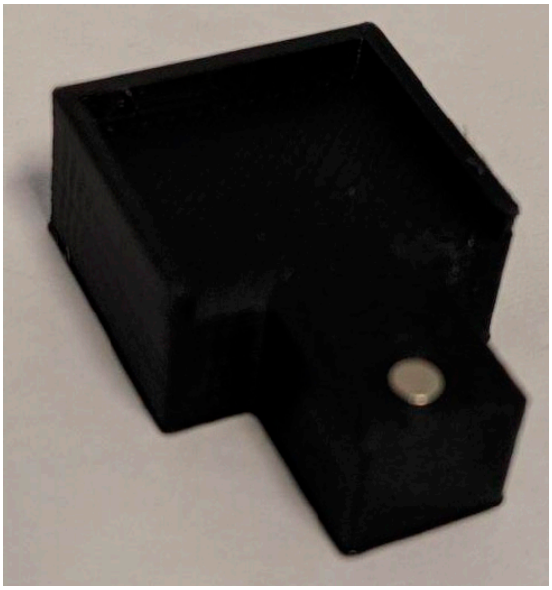


Figure S1. A custom 3D printed fixture used to position the SPCE directly above a 2mm Neodymium magnet to enable effective magnetic bead capture.

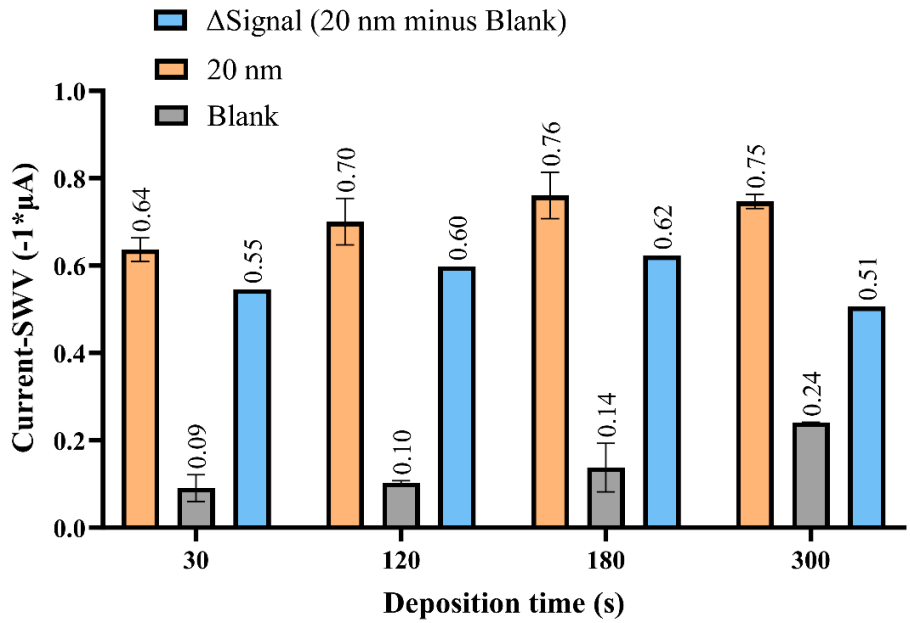
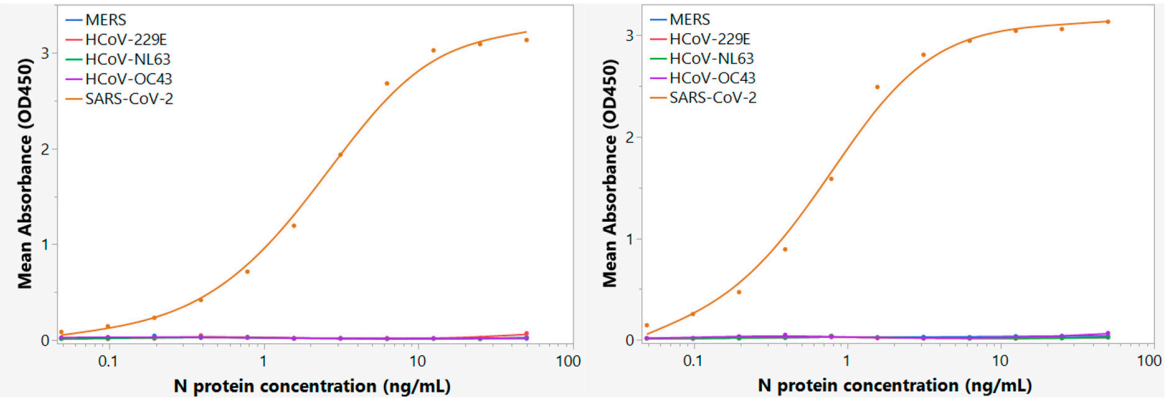


Figure S2. Absolute EC peak current (SWV) obtained for 20 nm AuNPs (at a concentration of 1.4×10^6 AuNPS in 2 µL DI water) at four different deposition times. Error bars represent standard deviation (n=3). The blank (2 µL DI water, no AuNPs) represents the control (measured at four different deposition times). 50 µL of 0.1 M HCl is used as the supporting electrolyte. The mean values of peak current are labeled above the columns.



(a) **(b)**

Figure S3. ELISA results evaluating the binding of (a) S46 and (b) S47 Anti-SARS-CoV-2 Nucleocapsid antibodies (#NUN-S46 & #NUN-S47 respectively) to various concentrations of different species of the Nucleocapsid protein (recreated from data provided by ACROBiosystems).

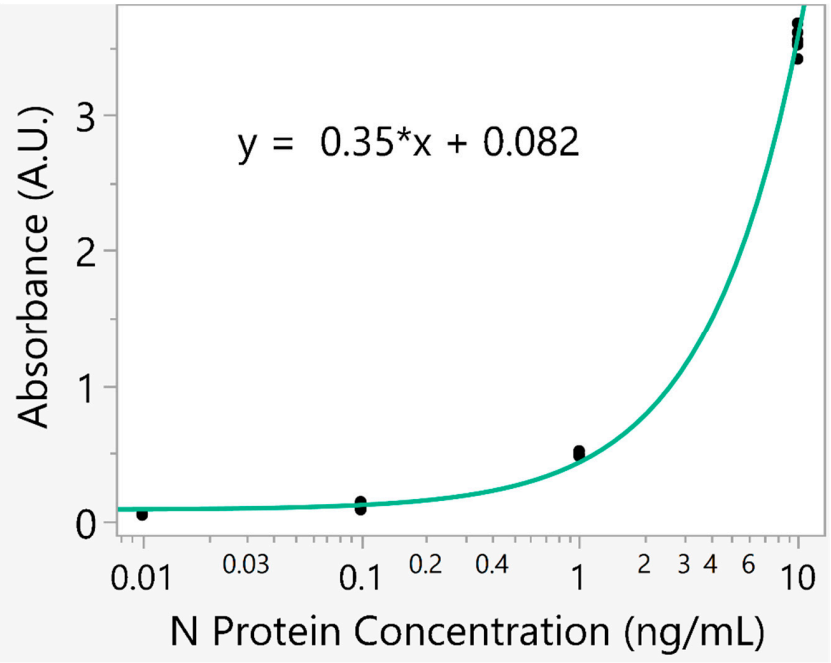


Figure S4. ELISA for validating the binding of S46 Anti-SARS-CoV-2 Nucleocapsid antibodies (#NUN-S46, ACROBiosystems) to varying concentrations of SARS-CoV-2 Nucleocapsid protein (#NUN-S47L8-200ug, ACROBiosystems), n=6.

Table S1

Symbol	Meaning
ns	P > 0.05
*	P ≤ 0.05
**	P ≤ 0.01
***	P ≤ 0.001
****	P ≤ 0.0001

Calculation for number of active antibodies per AuNP

As per ref.[1],
Number of active antibodies per particle (AuNP) = $E_j \times E_a \times A_p/A_a$ ---- (Eqn.1)
Ej (jamming limit) = 0.55
Ea (proportion of active antibodies out of total antibodies conjugated to the AuNP)=1/3
Aa (Area on the AuNP surface covered by an antibody) = $\pi \frac{d^2}{4}$, where diameter of the antibody (based on a 150 kDa M.W.) d = 9.08 nm
Ap = Surface area of AuNP = πD^2, where D is the AuNP diameter

For a 5 and 10 nm AuNP, (D = 5 & 10 nm), based on Eqn.1, we obtain 0.88, and 0.22 active antibodies conjugated to each AuNP respectively.

Calculation for number of spike protein trimers and monomers in a given volume at ag/mL concentration

Assuming a molecular mass of spike protein monomer is 132 kDa and trimer is 396 kDa[2].
Number of moles of spike trimer in 1 mL volume at 1 fg/mL concentration = $\frac{1\times10^{-15}}{396000} = 2.52 \times 10^{-21}$ moles/mL.

Number of spike trimer molecules in 1 mL volume at 1 fg/mL concentration = $2.52 \times 10^{-21} \times 6 \times 10^{23} = 1512$
Number of spike trimer molecules in 1 mL volume at 0.35 ag/mL (0.00035 fg/mL) concentration = $0.00035 \times 1512 = 0.53$
Number of spike monomer molecules in 1 mL volume at 0.35 ag/mL concentration = $0.53 \times 3 = 1.59 \approx 2$

As per ref.[3], a 50 μL volume is placed on each working electrode of the SPCE.
Number of spike monomer molecules in 50 μL volume at 0.35 ag/mL concentration = $\frac{1.59\times50}{1000} = 0.08$

If we assume a 1:1 attachment of capture Ab-AuNP to spike monomer, this equates to <1 AuNP detected per SPCE.

References

[1] D. M. Patel, "Novel Sample Isolation and Nanomaterial-Based Electrochemical Detection of Bacterial Toxins for Food Security," Ph.D., The University of Utah, United States - Utah, 2023. Accessed: Apr. 18, 2023. [Online]. Available: https://www.proquest.com/docview/2781661883/abstract/1B197B4D063D4A65PQ/1
[2] https://www.prosci-inc.com/product/sars-cov-2-covid-19-trimeric-spike-s-recombinant-protein-10-075
[3] E. D. Nascimento et al., "COVID-19 diagnosis by SARS-CoV-2 Spike protein detection in saliva using an ultrasensitive magneto-assay based on disposable electrochemical sensor," Sens. Actuators B Chem., vol. 353, p. 131128, Feb. 2022, doi: 10.1016/j.snb.2021.131128