Electronic Supplementary Information

Label-Free and Reagentless Electrochemical detection of nucleocapsid protein of SARS-CoV-2: An ultrasensitive and Disposable Biosensor

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1. Materials and Methods

1.1 Materials and Reagents

3-Thiophenecarboxaldehyde (*Thi-Ald*)(98%, CAS Number: 498-62-4), gold (III) chloride trihydrate ($HAuCl_4.3H_2O$) (≥99.9%, CAS Number: 16961-25-4), potassium ferrocyanide ($K_4[FeCN]_6.3H_2O$) (≥99.5%, CAS Number: 14459-95-1), potassium ferricyanide ($K_3[Fe(CN)_6]$) (≥99.0%, CAS Number: 13746-66-2), Potassium phosphate dibasic (K_2HPO_4) (≥98%, CAS Number: 7758-11-4), potassium phosphate monobasic (KH_2PO_4), (≥99.0%, CAS Number: 7778-77-0), potassium chloride (KCl), (≥99.0%, CAS Number: 7447-40-7), bovine serum albumin (BSA) (≥98.0%, CAS Number: 9048-46-8), SARS-CoV-2 spike RBD (expressed in HEK 293 cells), anti-COVID 19 nucleocapsid coronavirus monoclonal antibody (produced in rabbit), COVID 19 nucleocapsid coronavirus recombinant protein, IgG from rabbit serum, anti-rabbit immunoglobulin G (IgG antibody produced in goat), COVID 19 spike RBD coronavirus recombinant protein (HEK293 cells), interleukin 1β (human recombinant), interleukin 8 (human) and tumor Necrosis Factor-α Protein (recombinant human) were obtained from Sigma-Aldrich. During the fabrication process of the biosensor, ultrapure water (18MΩcm), which was purified by Millipore Q system was utilized. All protein solutions were prepared by using phosphate buffer solutions (PBS, prepared KH_2PO_4 and K_2HPO_4, pH: 7.4) and were kept in a refrigerator at -20 ^{0}C.

1.2 Instruments

Potentiostat / Galvanostat

The electrochemical characterization of the proposed immunosensor fabrication procedure, EIS and CV analyses were performed after each modification step. A three-electrode system was utilized with ITO substrate as the working electrode (5 x 20 mm), Pt wire as the auxiliary electrode and Ag/AgCl as the reference electrode. The electrolyte solution utilized in all the electrochemical analyses contained 1 M KCl and 5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ (1:1), in which $[Fe(CN)_6]^{4-/3-}$ was used as the redox couple. The alternating potential and formal potential applied during EIS measurements were 5 mV and 0 V, respectively. The EIS measurements were performed in the frequency range of 0.5 Hz–50 kHz. A Randles equivalent circuit was utilized to fit the obtained EIS spectra. The potentials of CV were set from -0.5 to 1 V at a scan rate of 100 mV/s.

Fourier transform-infrared spectroscopy (FT-IR)

The FTIR characterization of functionalized electrode surface was taken on a Bruker Company Vertex 70 spectrometer using ATR apparatus. A normal scanning range of 400–4000 cm^{-1} was utilized for 24 repeated scans at a spectral resolution of 4 cm^{-1}. The spectra were recorded in transmittance mode.

Scanning electron microscopy (SEM)

The surface morphology of functionalized ITO electrodes surfaces was investigated by Field Emission Scanning Electron Microscopy (SEM) (FE-SEM, QUANTA FEG-250). SEM instruments were operated with low vacuum detector (LFD) at an accelerated voltage at 5 kV.

Energy dispersive analysis of X-ray (EDAX)

The distribution of AuNPs on the electrode surface were characterized by energy dispersive X-ray elemental mapping analysis. To perform EDX analysis, the acceleration voltage and spot size were chosen as 30 kV and 5.5, respectively.

Table SI-1. The fitted EIS spectra results of electrode modification steps (A) and nucleocapsid antigen immobilized electrodes (B).

(A) Biosensor Surface	**R_{ct} (kohm)**	**(B) Biosensor Surface**	**R_{ct} (kohm)**
ITO-*AuNPs*	0.205	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (0.0015pg/mL)	1.697
ITO-*AuNPs/Pthi-Ald*	0.843	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (0.015pg/mL)	1.773
ITO-*AuNPs/Pthi-Ald*/anti-NC	1.458	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (1 pg/mL)	1.848
ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA	1.572	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (5 pg/mL)	2.053
ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC	2.294	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (25 pg/mL)	2.386
ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2	3.228	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (50 pg/mL)	3.228
-	-	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (100 pg/mL)	5.485
-	-	ITO-*AuNPs/Pthi-Ald*/anti-NC/BSA/NC/Ab_2 (150 pg/mL)	7.381

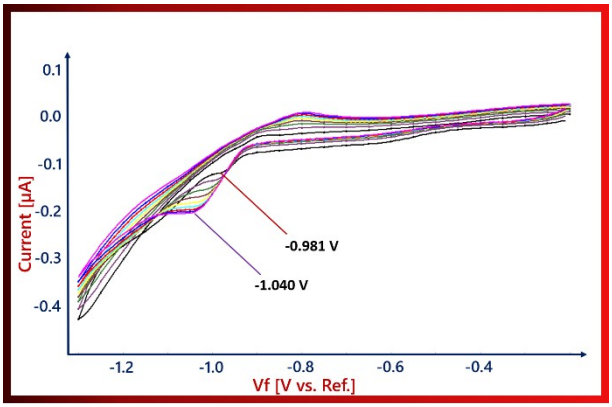

Figure SI-1. AuNPs deposition cyclic voltammograms.

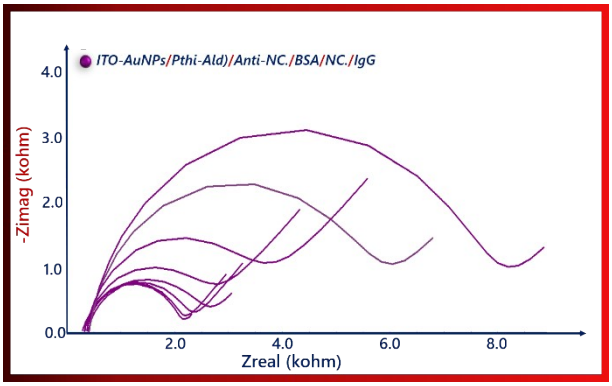

Figure SI-2. Fitted EIS spectra obtain after incubation in increasing concentrations of nucleocapsid antigen.

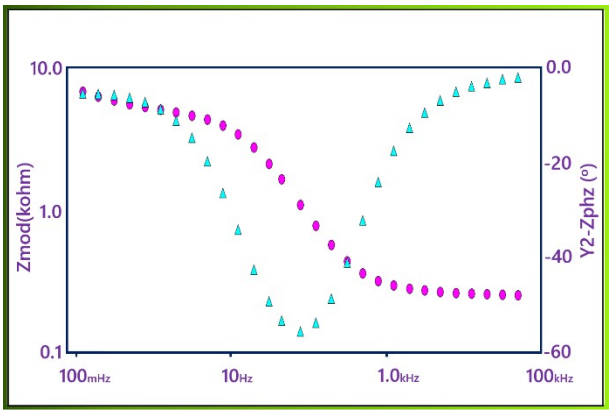

Figure SI-3. Bode plot.