Electronic Supplementary Information

A polyaniline functionalized NiFeP nanosheet array-based electrochemical immunosensor via Au/Cu_2O nanocube as a signal amplifier for the detection of SARS-CoV-2 nucleocapsid protein

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Experimental reagents

Ni Foam (NF) is purchased from Shenzhen Green and Creative Environmental Science and Technology Co. Ltd. (Shenzhen, China). Hydrochloric acid (HCl), sulfuric acid (H_2SO_4) and acetone are supplied by Luoyang Chemical Reagent Factory (Luoyang, China). Iron chloride ($FeCl_3$), benzenedicarboxylic acid (BDC), cupric chloride dihydrate ($CuCl_2 \cdot 2H_2O$), ascorbic acid (AA), gold chloride ($HAuCl_4$), potassium ferricyanide ($K_3[Fe(CN)_6]$), potassium ferrocyanide ($K_4Fe(CN)_6$), potassium chloride (KCl) and aniline are purchased from Aladdin Co., Ltd. (Shanghai, China). Ethanol absolute (C_2H_5OH), N,N-dimethylformamide (DMF) are provide by Tianjin Kaitong Chemical Reagent Co., Ltd. (Tianjin, China). Disodium hydrogen phosphate dodecahydrate (Na_2HPO_4) and sodium dihydrogen phosphate dihydrate (NaH_2PO_4) are provided by Kemiou Chemical Reagent Co., Ltd. (Tianjin, China). Sodium hypophosphite (NaH_2PO_2) is obtained from Guangfu Fine Chemical Research Institute (Tianjin, China). Sodium hydroxide (NaOH) is provided by Tianjin Damao Chemical Reagent Factory. (Tianjin, China). Sodium citrate is bought from Tianjin Guangfu Technology Development Co., Ltd. (Tianjin, China). Glutaraldehyde is provided by Adamas Reagent Co., Ltd. (Shanghai, China). SARS-CoV-2 nucleocapsid protein (SARS-CoV-2 NP), Anti-SARS-CoV-2 NP McAb (coating, namely Ab_1) and Anti-SARS-CoV-2 NP McAb (labeling, namely Ab_2) are purchased by Shanghai Linc-bio Science Co. Ltd. (Shanghai, China). All experimental water is ultrapure water. All reagents in experiments are analytically pure without further purification before used.

Experimental apparatus

Ultrapure water is purified through a system of laboratory water purification Eco-S15 (Hitech Instruments Co. Ltd, China). Powder X-ray diffraction (XRD) measurement is performed by an Ultima IV-185 diffractometer. Scanning electron microscopy (SEM) measurements are achieved through JSM-7500F scanning electron microscopy with an accelerating voltage of 20 kV. Transmission electron microscope

(TEM) is performed with JEM-2100. X-ray photoelectron spectroscopy (XPS) measurements are carried out on a K-Alpha X-ray photoelectron spectrometer. The Fourier transform infrared spectroscopy (FTIR) is completed by HW-10 of Beijing Puxi General Instrument Company.

Pretreatment of human serum samples

Human blood samples are provided by the Linfen People's Hospital (Binghe West Road, Yaodu District, Linfen, Shanxi, China). Firstly, human whole blood samples were placed in a clean centrifuge tube. After standing for 1 h, the samples were centrifuged at 4000 $rpm \cdot min^{-1}$ for 10 min. Finally, the serum supernatant was transferred to a volumetric flask and stored at 4 °C for further analysis.

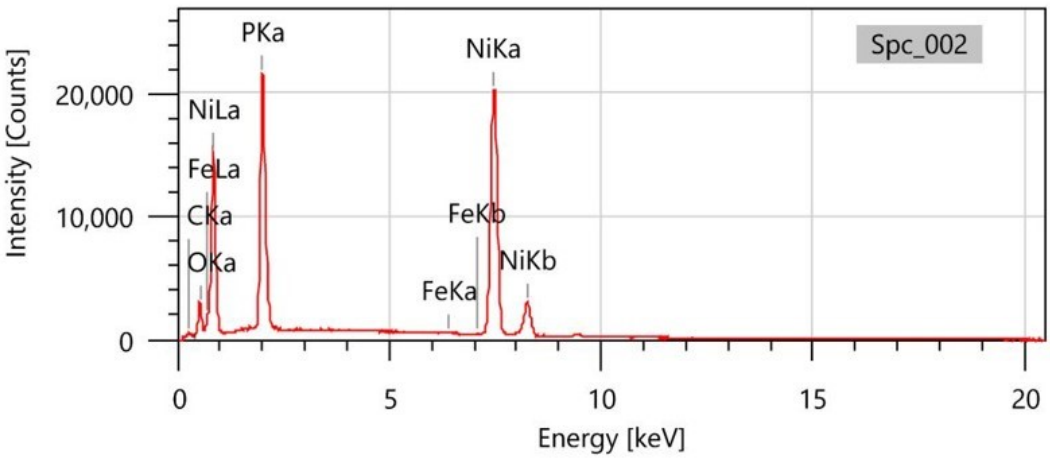

Fig. S1. The EDX image of the NiFeP nanosheet arrays.

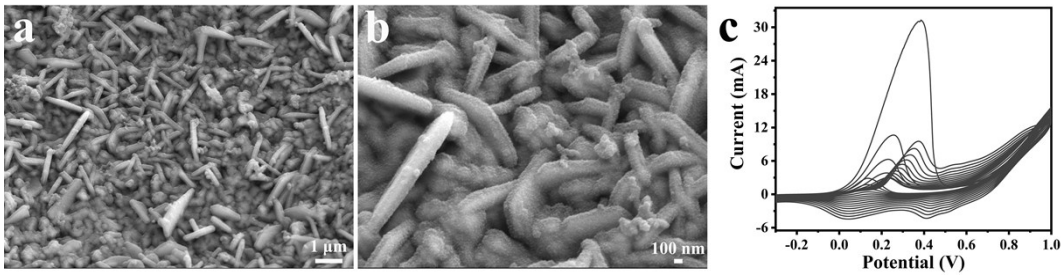

Fig. S2. (a, b) The SEM patterns of the PANI functionalized NiFeP nanosheet arrays with different magnifications. (c) The cyclic voltammetry (CV) curves of electro-polymerization of PANI for 15 cycles in the electrolyte solution of 0.5 M H_2SO_4 containing 0.2 M aniline.

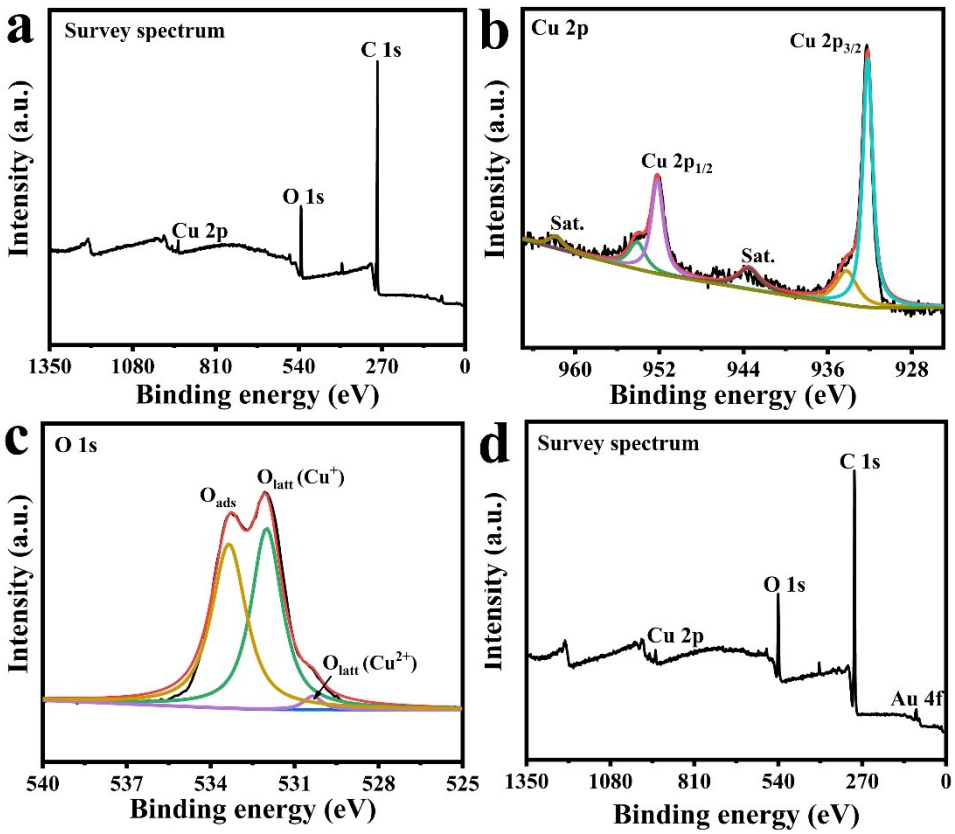

Fig. S3. (a) The XPS survey spectrum of the prepared Cu_2O nanocubes. The XPS spectra of Cu 2p (b) and O 1s (c) of Cu_2O nanocubes. (d) The XPS survey spectrum of the prepared Au/Cu_2O nanocomposite.

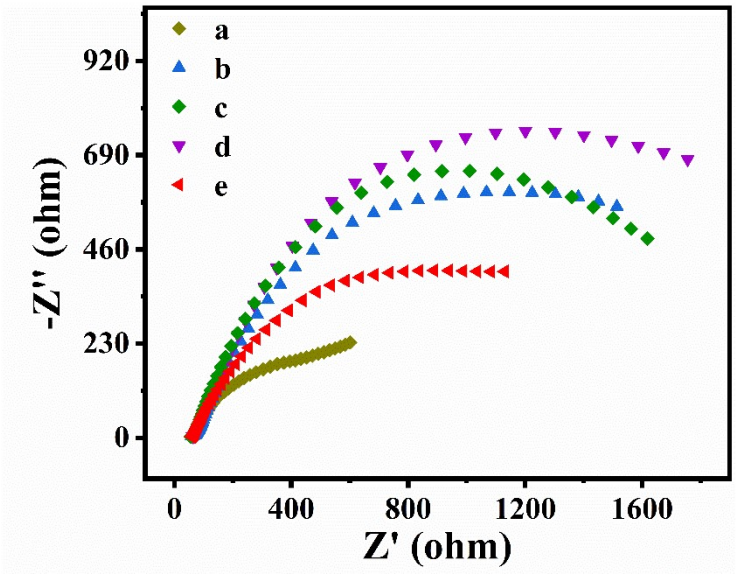

Fig. S4. Nyquist plots for fabrication steps of the immunosensor incubated with 1.0 ng·mL^{-1} SARS-CoV-2 NP in 0.1 mol·L^{-1} KCl solution containing 5 mmol·L^{-1} $Fe(CN)_6^{3-/4-}$: (a) PANI/NiFeP/NF; (b) Ab_1/PANI/NiFeP/NF; (c) BSA/Ab_1/PANI/NiFeP/NF; (d) SARS-CoV-2 NP/BSA/Ab_1/PANI/NiFeP/NF; (e) Ab_2-Au/Cu_2O/SARS-CoV-2 NP/BSA/Ab_1/PANI/NiFeP/NF.

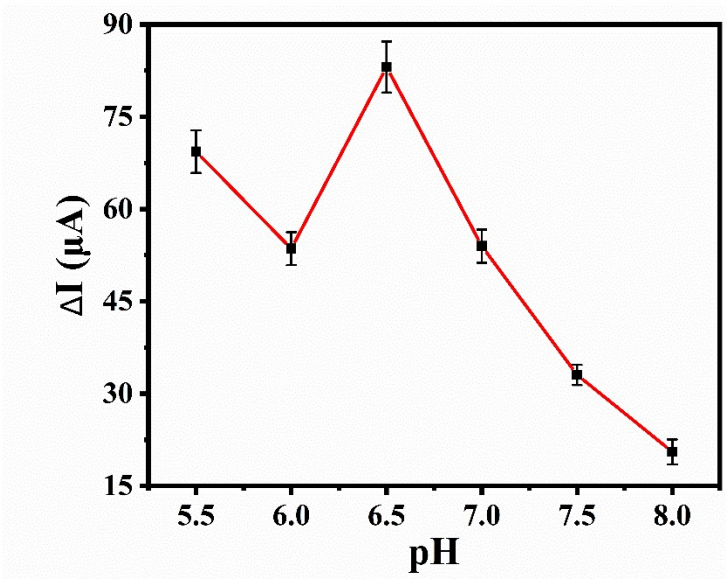

Fig. S5. Effect of various pH on the current change of the immunosensor incubated with 1.0 ng·mL^{-1} SARS-CoV-2 NP in PBS solution after injecting 5 mmol·L^{-1} H_2O_2.

Table S1. Performance comparison of different immunosensors for the detection of SARS-CoV-2.

Electrode	Target	Linear range	Detection limit	Ref.
CNF/SPE	N protein	0.1 pg·mL^{-1} ~ 1 μg·mL^{-1}	0.8 pg·mL^{-1}	1
Polycarbonate track-etched membrane	N protein	7.8 pg·mL^{-1} ~ 1 ng·mL^{-1}	7.5 pg·mL^{-1}	2
Bi_2WO_6/Bi_2S_3/GCE	N protein	0.01 ~ 1.00 pg·mL^{-1}	3.00 fg·mL^{-1}	3
TDBA-β-CD-Pt	N protein	50 fg·mL^{-1} ~ 1.0 ng·mL^{-1}	22 fg·mL^{-1}	4
ToAD	N protein	0.1 ~ 10 ng·mL^{-1}	0.1 ng·mL^{-1}	5
$TiO_2@Bi_2WO_6/Ag_2S$	N protein	1 pg·mL^{-1} ~ 50 ng·mL^{-1}	0.38 pg·mL^{-1}	6
DMSN@QDs	N protein	5 μg·mL^{-1} ~ 50 ng·mL^{-1}	3.33 pg·mL^{-1}	7
Ir NPs	N protein	100 fg·mL^{-1} ~ 10 ng·mL^{-1}	47 fg·mL^{-1}	8
PANI/NiFeP/NF	N protein	10 fg·mL^{-1} ~ 20 ng·mL^{-1}	1.12 fg·mL^{-1}	This work

Table S2. The recovery tests of SARS-CoV-2 NP in actual human serum samples by standard addition method using the constructed immunosensor.

Sample	Added ($pg \cdot mL^{-1}$)	Found ($pg \cdot mL^{-1}$)	Recovery (%)	RSD (%) (n = 3)
1	50	46.002	92.00	
		48.733	97.45	7.60
		53.424	106.85	
2	150	147.086	98.06	
		158.937	105.96	3.87
		153.587	102.39	

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