Supporting Information

Experimental

Chemicals and reagents

Phosphate buffered saline (PBS, 0.01 M, pH 7.4), 96-wells high binding ELISA plates, bovine serum albumin(BSA), Copper acetate, acetic acid, dried ethanol, Hydrochloric acid were purchased from Shanghai Sangon Biological and Technological Service Co., Ltd. . Unless otherwise stated, all the reagents used in this study were at least of analytical grade. All solutions were prepared with deionized water with conductivity of 18.2 MΩ cm^{-1} from a water purification system (Millipore).

Rabbit monoclonal anti-H1N1, hemagglutinin (HA) (OTWO Guangzhou PL labs), Rabbit polyclonal anti-H1N1, hemagglutinin (HA) 1 (Beijing Bioss Biological and Technological Co., Ltd.), Inactivated H1N1 Influenza A Virus (Solomon Islands/ 03/06) (prospec) was provided from Shanghai kenqiang instrument Co., Ltd. H5N1，H3N2，NCD virus were kindly provided by Fujian Center for Disease Control & Prevention.

Apparatus.

The electrochemical measurements were performed with a CHI 660D electrochemical workstation (Shanghai, China). A conventional three-electrode system was used comprising a self Assembled Monolayer (SAM) glass carbon electrode(GCE) as working electrode, a platinum wire as auxiliary electrode, and a saturated calomel

reference electrode were employed. All potentials in this paper are quoted with respect to the saturated calomel reference electrode (SCE). All electrochemical experiments were performed under a dry nitrogen atmosphere at room temperature.

Preparation of Stable Copper Oxide Nanoparticle

The synthetic method was slightly modified according to the literature [1]. Briefly, 1 mmol of $Cu(CH_3COO)_2$ and 2 mmol of acetic acid, were respectively d issolved in 50 mL of dried ethanol, and the solution was heated to the boiling temperature of 78 ℃. 4 mmol of the NaOH powder were then added to the solution under vigorous stirring. The reactions were performed for 1 h. The CuO NPs were obtained by centrifugation at 4000 rpm and washed several times with acetic acid and deionized water, then dried at 60 ℃.

Preparation of CuO NPs Antibody Conjugates

1 mg of CuO NPs was dispersed into 1 mL of phosphate buffer saline (0.01 M PBS). Followed by ultrasonication for 5 min, 50 μL polyclonal antibody (the original concentration of secondary antibody is 1.0mg/ml, diluted to 1:10) was added in the CuO NPs solution，then the mixture was slightly vortexed for 3 h, and centrifuged for 5 min at 5000 rpm. The solution was disposed in the upper layer which contained unlabelled secondary antibody, and washed three times with PBS. After centrifugation, the precipitate of CuO NPs antibody conjugates was redispersed with 200 μL 0.1% BSA. Repeated the above operation, the CuO NPs labeled polyclonal antibody (in 1 mL 0.01 M PBS) thus obtained was stored at 4 °C.

Immunoassay performing

The assay was performed in a polystyrene 96-wells high binding ELISA plate. The schematic diagram of the sandwich immunoassay and copper amplification for influenza virus H1N1 with CuO-NP tags was shown in Fig. 1. Initially, 50 μL of 10 μg/mL monoclonal HA antibodies in 0.01 M PBS buffer was steadily attached on the solid substrate via physical adsorption between hydrophobic groups of antibody molecule and polystyrene. The glass slide was washed off three times with 100 μL of washing buffer to remove unbound antibodies, and the uncoated active sites of polystyrene substrate were saturated with 30 μL of blocking buffer, in which 0.1% BSA in PBS was used as a blocking agent to prevent nonspecific adsorption of the antigens. 80 μL of diluted H1N1 standard solutions (in PBS buffer) was then incubated for 1 h at 37 °C. The wells were washed three times with PBS to remove the unbound antigens. 40 μL of CuO NPs labeled polyclonal antibody was incubated over the well to allow the formation of sandwich complexes in the microplate. Unbound copper conjugates were removed from the glass slide with 100 μL of PBST (PBS including 0.1% Tween 20) and 100 μL of pure water respectively. The copper conjugates were released into the solution with 20 μL of 0.1 M HCl for 5 min, and the electrochemical detection was then performed. The control experiments of only BSA coated CuO and bare CuO NPs were prepared in the same way when the concentration of H1N1 was 5 ng/mL.

Electrochemical detection

Before modification, the GCE was polished successively with 0.3, 0.05 μm alumina slurries followed by thorough rinsing with double distilled water, then sonicated in ethanol and double distilled water for 3 min, respectively. Finally, the GCE was dried under the stream of high purity nitrogen. 5μL of the above mentioned copper ions solutions was deposited on the fresh prepared GCE surface until the solvent was evaporated.

CV and SLV measurements were performed in the 0.01 M nitrogen-saturated HCl with 1 M NaCl by scanning from -0.60 to +0.60 V. The peak current related to the Cu^{2+} at about -0.15 V was taken as the electrochemical measurement signal.

References

[1] T. Kida, T. Oka, M. Nagano. J .Am. Ceram. Soc. 2007,90 (1): 107–110.

Fig. S1

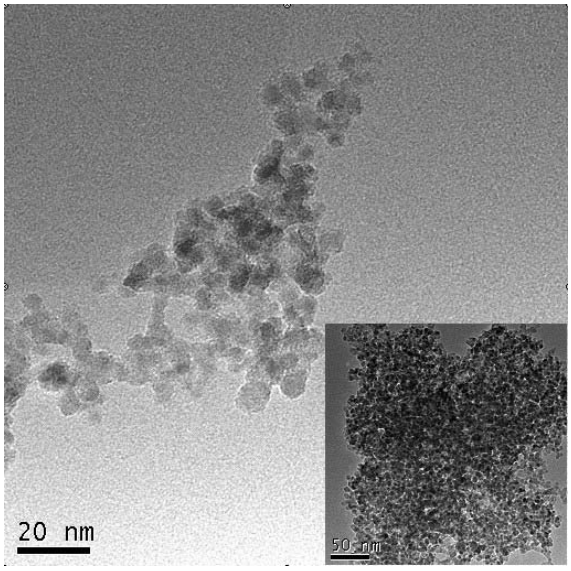

Fig. S1 Transmission electronic microscopy (TEM) image of the synthesised CuO NPs by HRTEM (Tecnai G2 F20 S-TWIN 200KV)

Fig. S2

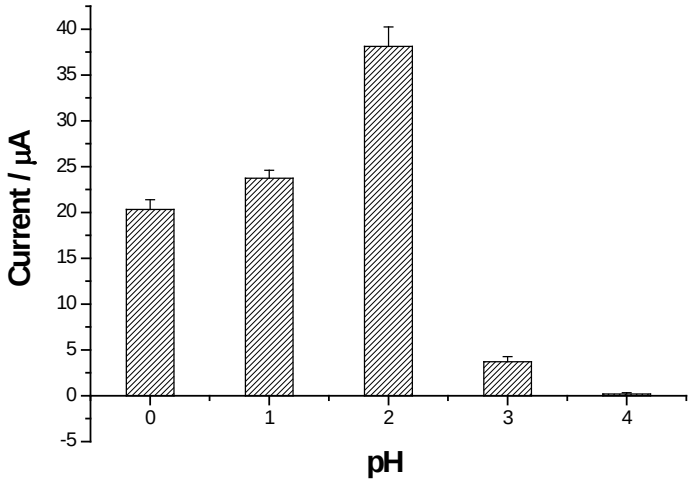

Fig. S2 Effect of pH on the oxidation peak currents by linear sweep voltammetry in different pH supporting electrolytes with 0.1mM Cu^{2+} modificated GC electrodes, supporting electrolytes: Different pH with HCl in 1M NaCl, scan rate:100mV/s.

Fig. S3

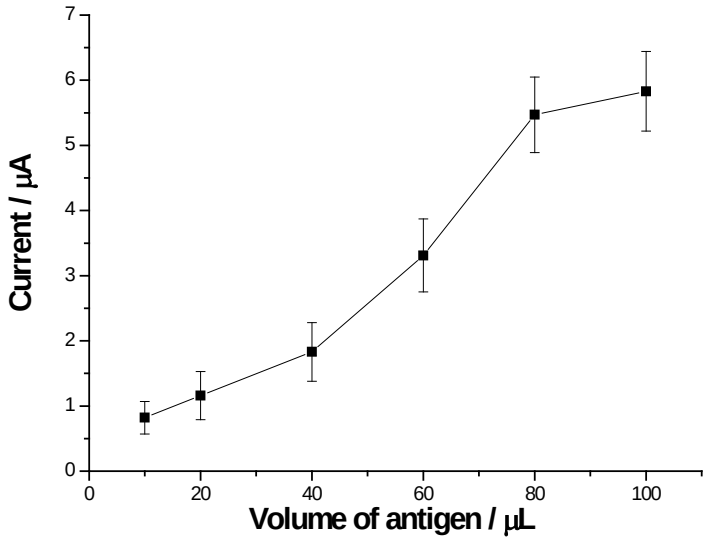

Fig. S3 Relationship between the volume of antigen and electrochemical signal

Concentration of H1N1: 5ng/mL; 500ng monoclonal and polyclone antibody in each well; The antigen-antibody immunoreaction : 60 min at 37 ℃.

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144 **Fig. S4**

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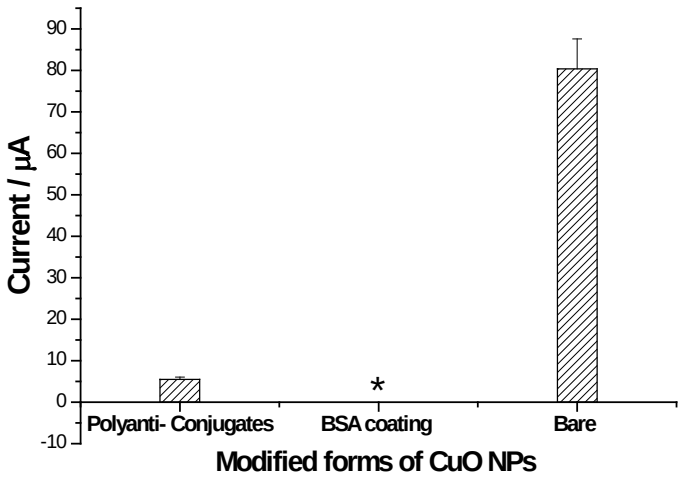

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148 **Fig. S4** Relationship between modified forms of CuO NPs and electrochemical signal.

149 Concentration of H1N1: 5ng/mL; 500ng monoclonal antibody in each well; The

150 antigen-antibody immunoreaction : 60 min at 37 ℃. *: Not detected.

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