Supplementary Information

High-performance electrochemical immunosensor based on bimetallic gold/silver functionalized carbon spheres for CYFRA 21-1 detection and information protection

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Reagents and instruments

Sucrose, silver nitrate ($AgNO_3$), and sodium borohydride ($NaBH_4$) were all purchased from China National Pharmaceutical Group Chemical Reagent Co., Ltd. Hexadecyltrimethylammonium bromide (CTAB), tetraethyl silicate (TEOS), 3-aminopropyltriethoxysilane (APTES), glutaraldehyde, and chloroauric acid ($HAuCl_4$) were all purchased from Shanghai McLean Biochemistry Co., Ltd. CYFRA21-1 and antibodies were purchased from Shanghai Lingchao Biotechnology Co., Ltd. The reagents involved are all analytical grade and the water used is ultrapure water (18.25 MΩ/cm).

The CHI760D electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.) was used for electrochemical measurements. The scanning electron microscope (SEM) images were obtained from the field emission scanning electron microscope (Gemini300, Zeiss, Germany). X-ray diffraction (XRD) patterns was obtained using the D8 FOCUS diffractometer from Bruker, Germany. The transmission electron microscope (TEM) image was obtained from JEOL JEM 2100F transmission electron microscope (Japan).

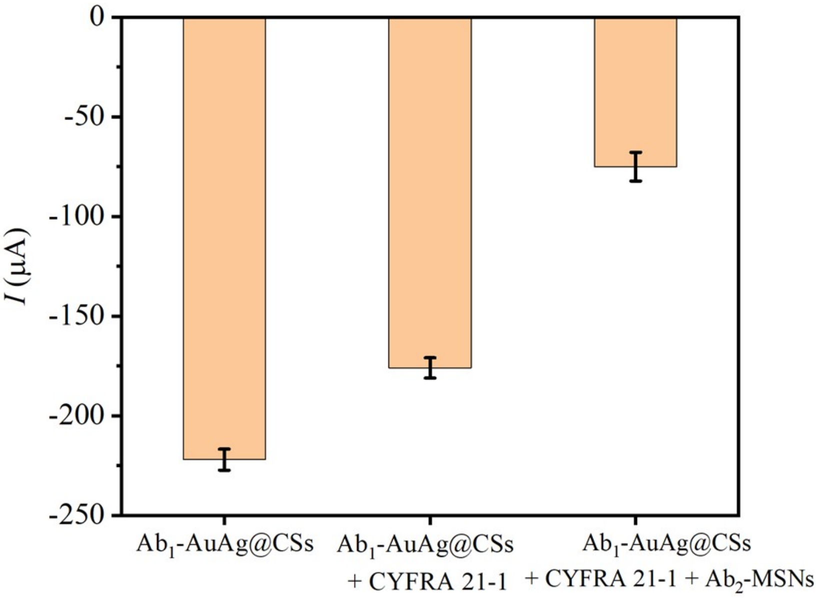

Fig. S1 The current response in the presence of Ab_1-AuAg@CSs, Ab_1-AuAg@CSs + CYFRA 21-1, and Ab_1-AuAg@CSs + CYFRA 21-1 + Ab_2-MSNs. Error bars indicate the standard deviation calculated from three replicate measurements.

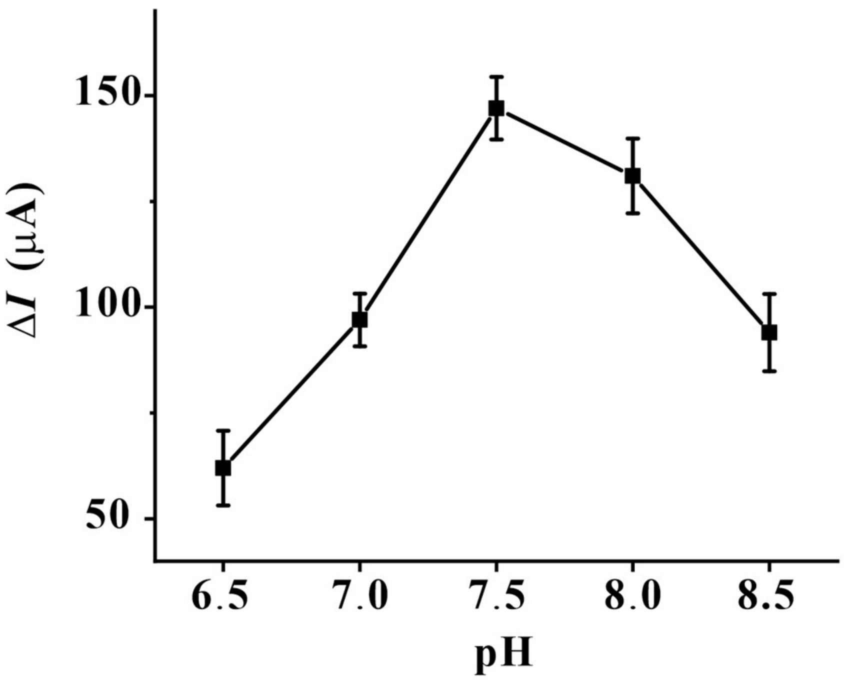

Fig. S2 Optimization of pH condition for the reaction. Error bars indicate the standard deviation calculated from three replicate measurements.

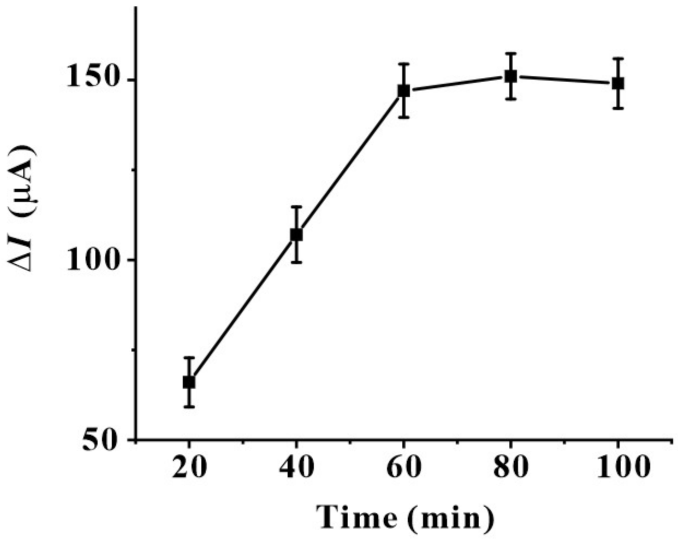

Fig. S3 Optimization of incubation time for the CYFRA 21-1. Error bars indicate the standard deviation calculated from three replicate measurements.

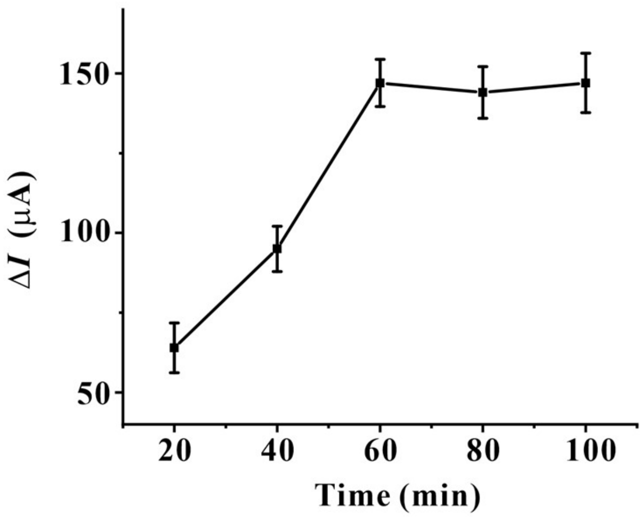

Fig. S4 Optimization of incubation time for the Ab_2-MSNs. Error bars indicate the standard deviation calculated from three replicate measurements.

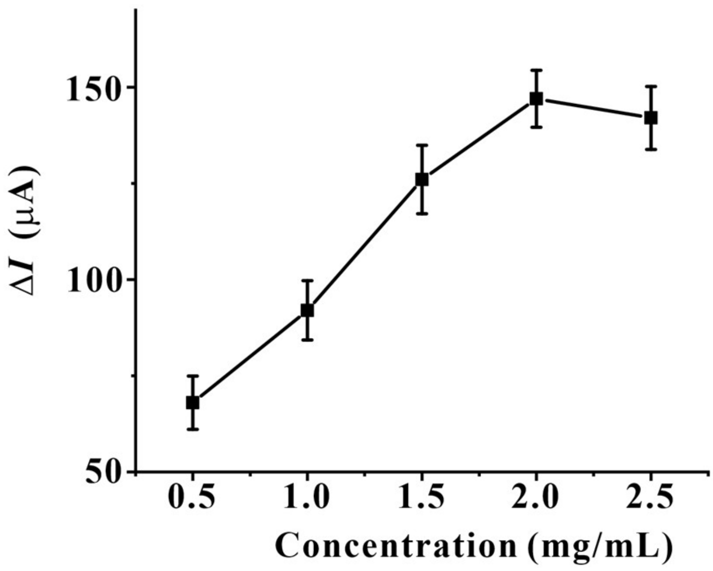

Fig. S5 Optimization of concentration of the AuAg@CSs. Error bars indicate the standard deviation calculated from three replicate measurements.

Input				Output
Ab_1-AuAg@CSs	CYFRA21-1	Ab_2-MSNs	H_2O_2	*I*
0	0	0	0	0
1	0	0	1	1
0	0	0	1	0
0	0	1	1	0
0	1	0	0	0
0	1	0	1	0
0	1	1	0	0
0	1	1	1	0
1	0	0	0	0
0	0	1	0	0
1	0	1	0	0
1	0	1	1	1
1	1	0	0	0
1	1	0	1	1
1	1	1	0	0
1	1	1	1	0

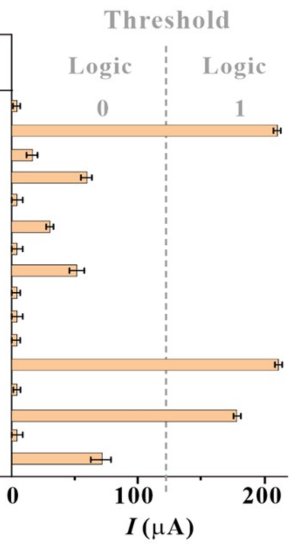

Fig. S6 The truth table and current responses with different inputs. Error bars indicate the standard deviation calculated from three replicate measurements.

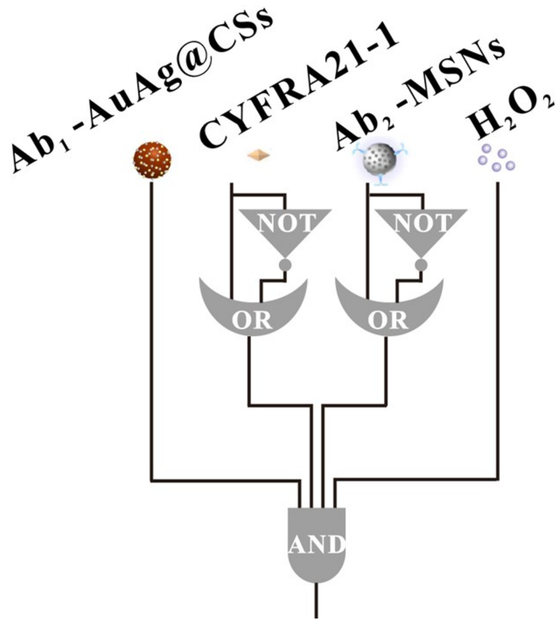

Fig. S7 The logic gate used in this work.

Table S1. Comparison of CYFRA 21-1 detection using other methods with the proposed EC immunosensors.

Method	Linear range (ng/mL)	Detection Limit (pg/mL)	Reference
Electrochemiluminescence	0.01–100	2.6	[1]
Photoelectrochemical	0.0001–50	0.05	[2]
Fluorescence	0.01–100	8	[3]
Electrochemistry	0.1-150	43	[4]
Electrochemistry	2 – 22	122	[5]
Point-of-Care chip	0.05-300	3	[6]
Backscattering- interferometry	0.1-100	230	[7]
Electrochemiluminescence	0.00750-50.0	1.89	[8]
Electrochemistry	0.0001–10	0.031	This work

Table S2. Recovery results of CYFRA 21-1 in 100-fold diluted serum samples

Sample (ng/mL)	Addition (ng/mL)	Average (ng/mL)	RSD (%, n =5)	Recovery (%)
0	1.00	0.97	3.2	97.0
	3.00	3.07	4.1	102.3
	5.00	5.19	2.9	103.8

Reference

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