Electronic Supplementary Information

An electrochemical immunoassay based on trepan-like gold electrode and nanogold functionalized flower-like hierarchical carbon materials with improved sensitivity

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Synthesis of GO

GO was prepared from graphite powder by a modified Hummers method [1]. Indetail, graphite (2 g), $NaNO_3$ (2 g) and 90 mL of H_2SO_4 (98%) were added into a flask under stirring in an ice bath. Then, 12 g $KMnO_4$ was slowly added to the mixture solution that was vigorously stirred at below 15 °C. After stirring at room temperature for 1 h, the resulting solution was diluted with 150 mL of water and then stirred at 95 °C for 2 h. Then the mixture solution was further diluted with 200 mL of water and deoxidized with 60 mL of 30 % H_2O_2. Finally, the product formed in mixture solution was separated out and washed with water for several times. The GO, a gray powder, was obtained by drying the product under vacuum.

The determination of the amount of active HRP

To determine the amount of active HRP, the HRP-Ab_2/AuNPs/FCM dispersion was reacted with HRP substrate ABTS and H_2O_2. The reaction produces a soluble product with characteristic optical absorbance peak at 405 nm. This was compared to a standard curve constructed with underivatized HRP, after subtracting the background absorbance of an equivalent dispersion of underivatized FCM. The concentration of active HRP in the stock HRP-Ab_2/AuNPs/FCM dispersion was determined by these enzyme activity experiments to be 6.86 $\mu g \cdot mL^{-1}$.

Principle of the ELISA

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for CEA has been pre-coated onto a microplate. Standards and samples are pipetted into the wells with a HRP conjugated antibody specific for CEA. Following a wash to remove any unbound reagent, a substrate solution is added to the wells and color develops in proportion to the amount of CEA bound in the initial step. The color development is stopped and the intensity of the color is measured.

Table S1. Comparison of analytical properties of different immunoassys toward CEA.

Immunoassay format	Modified platform	Signal antibody	Linear range ($ng \cdot mL^{-1}$)	Detection limit ($pg \cdot mL^{-1}$)	Ref.
Fuorescence immunoassay	Capillary tubes encapsulated in a quartz tube	DyLight 550-labeled antibody	0.7-80	1.1	2
Electrochemical immunoassay	Protein A attached gold nanoparticles	Magnetic beads	0.001-10	1	3
Electrochemilumi-nescence immunoassay	Au-g-C_3N_4	None	0.02-80	6.8	4
Electrochemical immunoassay	Reduced graphene oxide and gold nanoparticle nanocomposite	Horseradish peroxidase-functionalized gold nanoparticle	0.02-500	9.7	5
Electrochemilumi-nescence immunoassay	Quantum dot	Ferrocene functionalized poly(amidoamine)	0.005-50	0.82	6
Electrochemical immunoassay	PDDA functionalized graphene and nanoporous gold	Flower-like hierarchical carbon materials	0.0001-50	0.026	This work

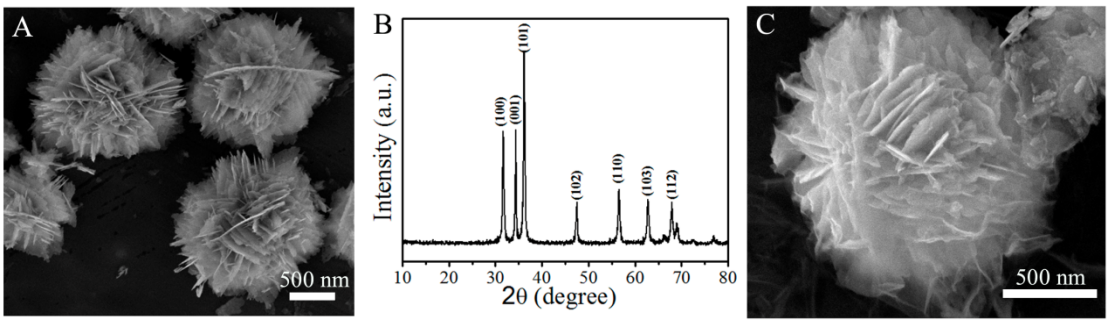

Fig. S1. (A) SEM image and (B) XRD of the F-ZnO; (C) SEM image of the F-ZnO covered with carbon material.

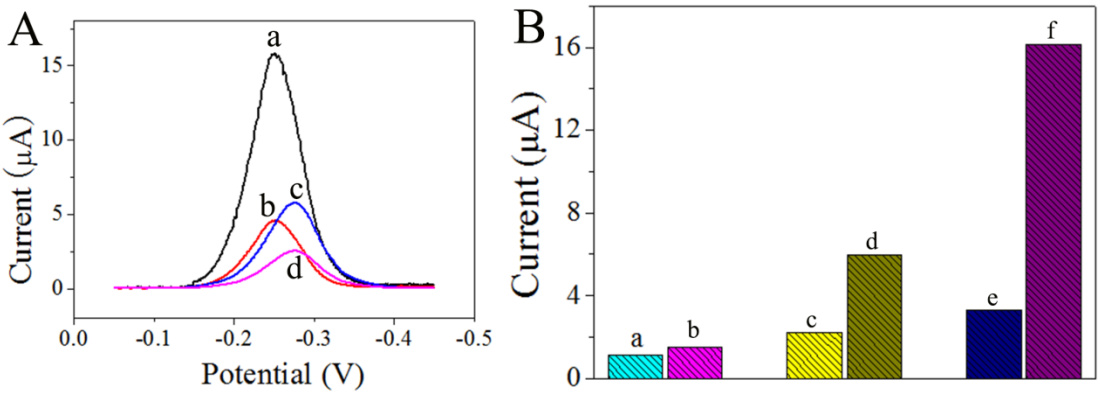

Fig. S2. (A) DPV of (a) HRP-McAb$_2$/AuNPs/FCM/CEA/McAb$_1$/3D-TG/G-PDDA/GCE, (b) HRP-McAb$_2$/CEA/McAb$_1$/3D-TG/G-PDDA/GCE, (c) HRP-McAb$_2$/AuNPs/FCM/CEA/McAb$_1$/G-PDDA/GCE, (d) HRP-McAb$_2$/CEA/McAb$_1$/G-PDDA/GCE in pH 7.4 PBS containing 50 μmol·L^{-1} TH and 3 mmol·L^{-1} H_2O_2; (B) Amperometric responses of (a) McAb$_1$/G-PDDA/GCE, (b) McAb$_1$/3D-TG/G-PDDA/GCE, (c) HRP-McAb$_2$/McAb$_1$/G-PDDA/GCE, (d) HRP-McAb$_2$/CEA/McAb$_1$/ G-PDDA/GCE, (e) HRP-McAb$_2$/AuNPs/FCM/McAb$_1$/3D-TG/G-PDDA/GCE, (f) HRP-McAb$_2$/AuNPs/FCM/CEA/McAb$_1$/3D-TG/G-PDDA/GCE in pH 7.4 PBS containing 50 μmol·L^{-1} TH and 3 mmol·L^{-1} H_2O_2.

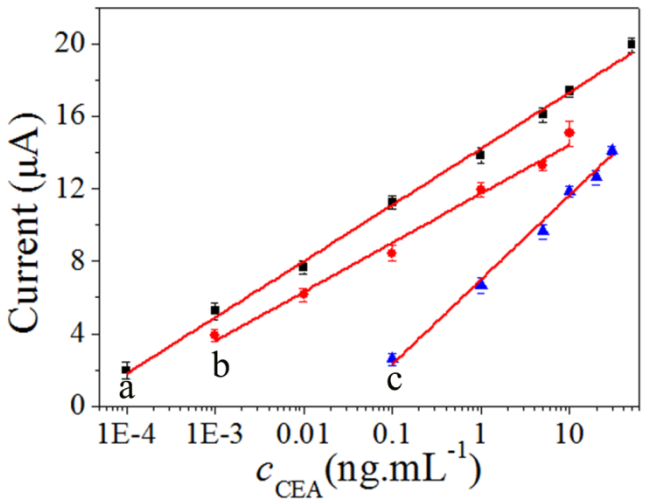

Fig. S3. Calibration curves of the electrochemical immunosensor toward CEA standards in pH 7.4 PBS containing 50 μmol·L^{-1} TH and 3 mmol·L^{-1} H_2O_2 with different labels: (a) AuNPs/FCM, (B) AuNPs/carbon sphere, (c) AuNPs/graphene sheets.

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