Supporting Information

Electrochemiluminescence Immunoassay platform with Immunoglobulin G-Encapsulated Gold Nanoclusters as "Two-in-One" Probe

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Experiments

Reagents and materials. Human IgG was brought from Shuanglin Biotechnology Co., Ltd. (Guangdong, China). $HAuCl_4 \cdot 3H_2O$ was purchased from Aladdin Reagent Co., Ltd. (Shanghai, China). Triethylamine (TEA), N,N-diisopropylethylamine (DIPEA), triethanolamine (TEOA), trimethylamine (TMA), N,N-dimethylethanolamine (DMEA), 2-(dibutylamino)ethanol (DBAE), disodium hydrogen phosphate (Na_2HPO_4), monometallic sodium orthophosphate (NaH_2PO_4) and sodium hydroxide (NaOH) were obtained from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). Bovine serum albumin fraction V was brought from Roche Diagnostics (Shanghai) Co., Ltd. (Shanghai, China). Goat anti-human IgG, carcinoembryonic antigen (CEA), prostate specific antigen (PSA), alpha fetoprotein (AFP), rabbit IgG and mouse IgG were purchased from Lingchao Biotechnology Co., Ltd. (Shanghai, China). The serum samples were provided from the First Affiliated Hospital of Xiamen University. In order to evaluate the clinical feasibility of the biosensor, all samples were used directly without any purification procedure.

Apparatus and measurements. UV-vis absorption spectra were recorded on a UV-vis 2450 spectrophotometer (Shimadzu, Japan) with a 10 mm quartz cell. Fluorescence emission spectra were recorded on a fluorescence spectrophotometer (Agilent, USA), with a 10 mm quartz cell. X-ray photoelectron spectroscopy (XPS) was performed to explore the electronic structures of the IgG-AuNCs on an ESCALAB 250XI electron spectrometer (Thermo, USA). The cyclic voltammetry (CV) and the electrochemical impedance spectroscopy (EIS) measurements were recorded on a CHI760E electrochemical workstation (Shanghai Chenhua Instrument, China) in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl. The open circuit potential was set 0.218 V, within the frequency range of 0.1–100 kHz in the process of EIS measurement.

Synthesis of IgG-AuNCs. IgG-AuNCs were prepared via a simple biomineralization process.[1] Aqueous $HAuCl_4$ (1 mL, 2.5 mM) and NaOH (0.1 mL, 2.5 M) solutions were added sequentially to IgG solution (1 mL, 50 mg/mL), and the mixture were left to react for 60 min at 37 °C. The color of solution was pale yellow and did not change markedly. After the fabrication, Au (III) ions were reduced to Au (0) and Au (I) by the amino acid residues of IgG at a high pH, forming reddish

fluorescent IgG-AuNCs. The crude product was then purified by dialysis and dried under a high-vacuum condition to obtain IgG-AuNCs for further use.

The ECL efficiency calculation. The ECL efficiency (Φ_{ECL}) can be obtained by reported method, and $[Ru(bpy)_3]^{2+}$ was used as a reference system was used to determine Φ_{ECL} of the IgG-AuNC using the relation below: [1,2]

$$\Phi_{ECL}=\Phi^{\circ}_{ECL}(IQ^{\circ}_f/Q_f I^{\circ})$$

Where Φ°_{ECL} is the ECL efficiency of $[Ru(bpy)_3]^{2+}$ (0.001 M and 0.1 M TBABF/acetonitrile) via annihilation, taken as 5.0%, Q_f and Q°_f are the corresponding faradaic charge passed for the IgG-AuNC and $[Ru(bpy)_3]^{2+}$ respectively, I and I^{o} are the ECL intensities for the IgG-AuNC film and $[Ru(bpy)_3]^{2+}$ respectively. For the detection of I° and Q°_f of $[Ru(bpy)_3]^{2+}$, the experiment parameters and procedures were operated according to the reported method.[2] While for the detection of I and Q_f of the IgG-AuNC, the procedures were as follows: The ECL and electrochemical measurements conducted in a three electrode electrochemical system with a IgG-AuNC-modified GCE working electrode, a Pt-wire counter electrode, and Ag/AgCl reference electrode. The potential was cycled between 0 V and 1.3 V with a scan rate of 0.2 V/s by cyclic voltammogram (CV) in 0.1 M PBS containing 0.35 M TEA (final pH 11.6). The photomultiplier tube (PMT) was biased at 550 V.

Characterization of IgG-AuNCs. As shown in the UV-vis absorption spectrum of the IgG-AuNCs (Figure S1A), the pale-yellow solution of the IgG-AuNCs (inset of Figure S1A) displayed a clear protein absorption peak at approximately 280 nm which was attributed to the absorbance of aromatic residues and heterocyclic of IgG, while the absorption peak of Au SPR at about 520 nm cannot be found. Two optical excitation maxima were observed at 365 and 490 nm, and the maximum photoemission wavelength was found at 630 nm (Figure S1B). XPS measurements were performed to analyze the valence states of Au in the IgG-AuNC. In the Au(4f) spectrum, the binding energies of Au(4f5/2) and Au(4f7/2) were located at 87.9 eV and 84.3 eV, respectively, which suggesting the coexistence of Au(0) and Au(I) in IgG-AuNCs (Figure S1C).[3] The ratio of Au(0) and Au(I) were calculated to be 37.5% and 62.5%, respectively. The above results are consistent with those observed in our previous studies, indicating the successful synthesis of the IgG-AuNCs.[3]

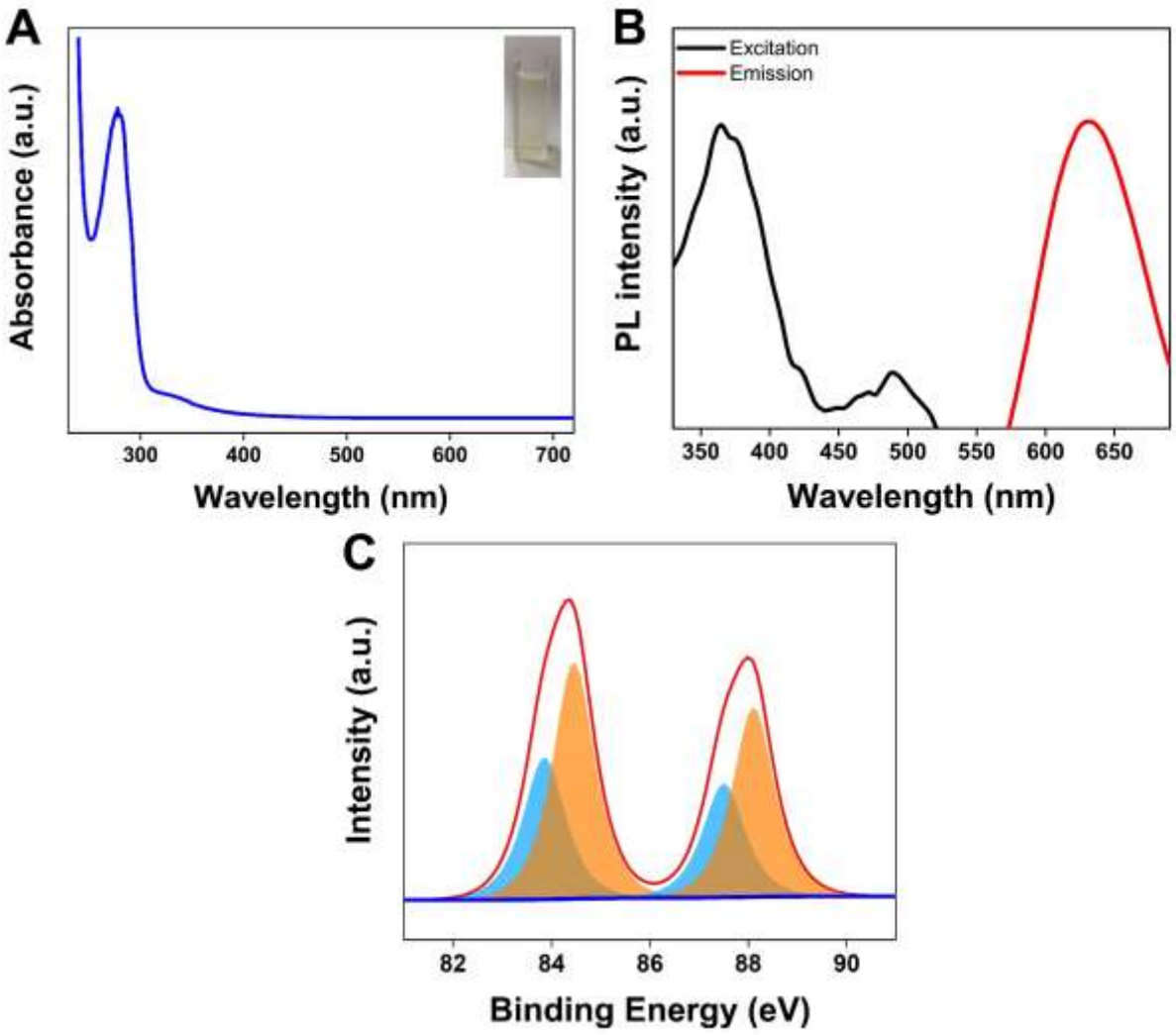

Figure S1. (A) UV-vis absorption spectrum of IgG-AuNCs solution. Inset: Photographs of the IgG-AuNCs solution under room light. (B) Photoexcitation and photoemission spectra of IgG-AuNCs solution. (C) Au(4f) photoelectron spectrum of IgG-AuNCs.

The choice of co-reactant. To explore the ECL properties of the IgG-AuNC, different co-reactants such as: Triethylamine (TEA), N,N-diisopropylethylamine (DIPEA), triethanolamine (TEOA), trimethylamine (TMA), N,N-dimethylethanolamine (DMEA), 2-(dibutylamino)ethanol (DBAE) were investigated. The results indicated that the IgG-AuNC/AuE with TEA as coreactant in aqueous solution exhibited the most efficient anodic ECL signal (Figure S2). Thus, in this experiment, TEA was chosen as the co-reactant to study the ECL properties of the IgG-AuNCs.

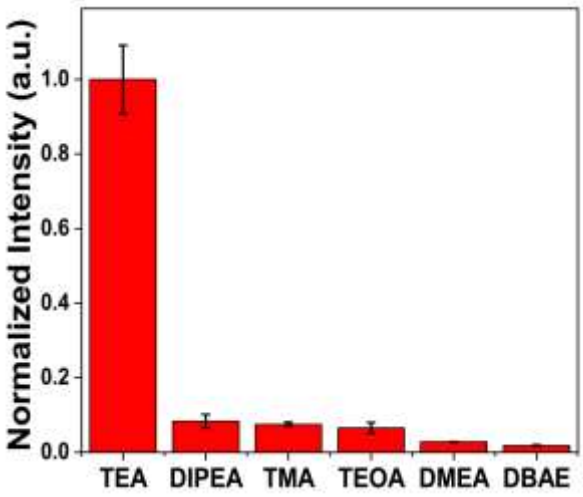

Figure S2. The ECL effects of IgG-AuNCs in 0.1 M PBS (pH 7.4) with 0.1 M different amine co-reactants.

CVs of the immunosensor. To better demonstrate the ECL immnuosensor fabrication process, individual fabrication stages were investigated by CV. A pair of well-defined redox peaks and larger peak currents can be clearly seen based on the good conductivity of bare AuE (curve a). Subsequently, as the electrode was further incubated with nonconductive proteins of Ab_2 (curve b), BSA (curve c), and IgG-AuNCs (curve d), the peak current progressively decreases, while peak potential separation increases, as the non-conductive protein layer acts a barrier for electron transfer. The above experimental results proved that the sensor was fabricated successfully.

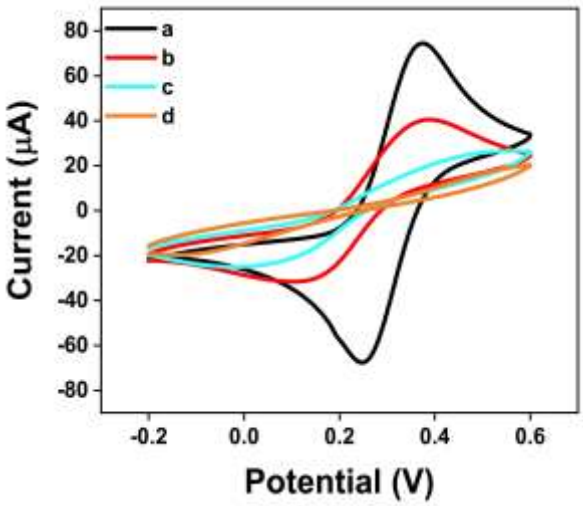

Figure S3. The CVs of (a) bare AuE, (b) Ab_2/AuE, (c) BSA/Ab_2/AuE, (d) IgG-AuNCs/BSA/Ab_2 /AuE in 5 mM $Fe(CN)_6^{3-/4-}$ solution containing 0.1 M KCl.

Table S1. Comparison of the competitive analytical performance of different techniques in IgG assay.

Method	Analytes	Dynamic Linear range (ng/mL)	[a]LOD (ng/mL)	Reference
Chemiluminescence	Human IgG	30-590	4.30	4
Electrocatalysis	Rabbit IgG	2-100	0.98	5
Amperometry	Rabbit IgG	0-11	1.40	6
ELISA	Pig IgG	100-100000	120	7
ESR	Rabbit IgG	0-8000	560	8
ECL	Human IgG	20-400	8.90	9
ECL	Human IgG	10-400	2.90	10
ECL	Human IgG	0.5–50000	0.06	This work

[a]LOD, limit of detection (S/N=3). ESR, electron spin resonance; ECL, electrochemiluminescence.

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