-Supporting information-

Synthesis of europium(III)-doped copper nanoclusters for electrochemiluminescence bioanalysis

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Experimental

Reagents and materials

Dopamine (DA), L-glutathione reduced (GSH), L-cysteine (L-Cys), ascorbic acid (AA), uric acid (UA) and Poly(vinylpyrrolidone) (PVP) were purchased from Sigma-Aldrich Trading Co. Ltd. (Shanghai, China). Copper(II) sulfate pentahydrate, glucose, and lactose were obtained from Sinopharm Chemical Reagent Co., Ltd (Tianjin, China). $Eu(NO_3)_3 \cdot 6H_2O$ was purchased from Aladdin Industrial Co., Ltd (Shanghai, China). Phosphate buffer solution (PBS, 0.1 M, pH = 7.4) containing $K_2S_2O_8$ (50 mM) was composed of KH_2PO_4, Na_2HPO_4.

Apparatus

The ECL and other electrochemical measurements were carried on a MPI-E ECL analyzer from Xi'an Remax Electronic Science and Technology Co. Ltd. (Xi'an, China), and a CHI 660C electrochemistry workstation from Shanghai CH Instruments (Shanghai, China). A typical three-electrode system was employed in this work, namely, the working electrode was glassy carbon electrode (GCE, Φ = 3 mm), the reference electrode was Ag/AgCl soaked with saturated KCl solution, and the count electrode was a platinum wire. The morphology and structure characterization of Eu (III)-doped CuNCs (Eu^{3+}-CuNCs) were performed on transmission electron microscopy (TEM, JEOL JEM-2010, Japan) as well as energy dispersive spectrometer (EDS) and elemental mapping. Fourier transform infrared (FT-IR) spectra and X-ray photoelectron spectroscopy (XPS) were measured by Nicolet 5700 (USA) and Thermo ESCALAB 250 (USA), respectively. Ultraviolet-visible absorption (UV-vis) spectra was recorded on 2450 UV-vis spectrometer (Shimadzu, Japan), and optical property of the composite was carried on fluorescence spectrophotometer (Cary Eclipse, Varian).

Synthesis of Cu NCs

PVP (0.1 g) was dissolved into 5 mL $CuSO_4$ aqueous solution (20 mM) with ultrasound treatment, and then mixed GSH (0.1229 g) with above mixture, followed by NaOH solution (1 M) adjusting the final mixing solution to pH = 5 under gentle stirring. The formed CuNCs solution were centrifuged at 8000 rpm for 10 min with washing three times by ethanol, and the precipitate after centrifugation was re-dissolved in the mixed solution of ethanol and H_2O (1:1) and then stored in 4 °C for further experiment.

Synthesis of Eu^{3+}-CuNCs

The synthesis was performed as in the literature[1] with some modification. Briefly, $Eu(NO_3)_3$ solution (112 μL, 80 mM) was mixed with $CuSO_4$ aqueous solution (5 mL, 20 mM) that dissolved in PVP (0.1 g), and then NaOH (1 M) adjusted pH of the mixture to 10 under vigorous stirring. GSH (0.1229 g) was adding into the above mixture and adjusted pH to 5, finally, the Eu^{3+}-CuNCs composite were formed. The obtained Eu^{3+}-CuNCs solution were centrifuged at 8000 rpm for 10 min with washing three times by ethanol, and the precipitate after centrifugation was re-dissolved in the mixed solution of ethanol and H_2O (1:1).

Determination of photostability

Two groups of CuNCs solution and Eu^{3+}-CuNCs solution with the same volume (5mL) were prepared and stored in four transparent glass bottles, respectively. One of which was continuously kept in Lab under daylight for 12 h, and the PL intensity was measured every 1 hour. The other one was continuously placed under the UV lamp with the wavelength of 365 nm and optical power of 6 W for 120 min, and the PL intensity was measured every 10 min. The photostability of CuNCs and Eu^{3+}-CuNCs were compared by the change of PL intensity.

Construction of ECL sensor for DA detection

The GCE was polished with 1.0 and 0.05 μm alumina slurry and washed thoroughly three times in an ultrasonic cleaner with ultrapure water for 3 min. Then, 5 μL of Eu^{3+}-CuNCs dispersion covered the pretreated GCE until dried in air. The ECL sensor for DA detection was performed in PBS (0.1 M, pH = 7.4) containing 50 mM $K_2S_2O_8$, 0.1 M of DA stock solution was diluted into different concentrations with ultrapure water, and mixed them with the above co-reactant at a volume ratio of 1:1 to 6 mL. The voltage of photomultiplier tube (PMT) was set at 600 V, and the potential scan was from -2.2 V to 0 V with a scan rate of 100 $mV\cdot s^{-1}$ in this work. The detection of DA was based on the change of ECL signal ($\Delta I = I_0 - I$), and I and I_0 represent the ECL intensity in the presence or absence of DA, respectively. Furthermore, a series of optical filters (400 - 700 nm) were prepared to obtain the ECL spectra of CuNCs and Eu^{3+}-CuNCs.

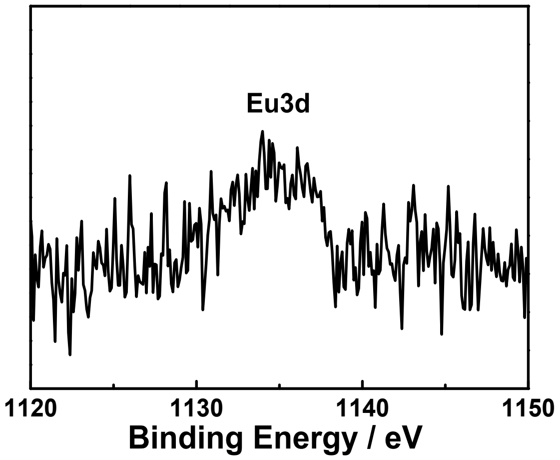

Fig. S1. Expanded XPS spectrum of Eu 3d region.

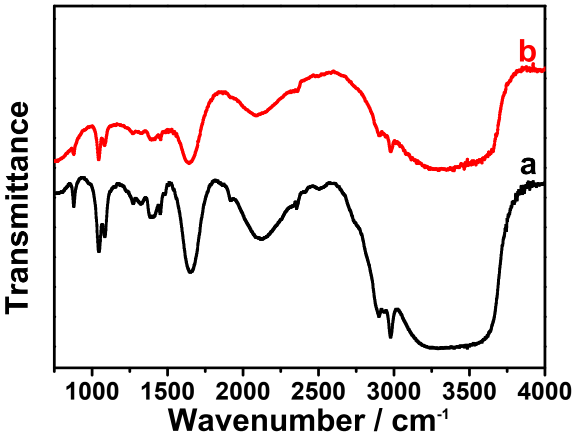

Fig. S2. FT-IR spectra of (a) CuNCs and (b) Eu^{3+}-CuNCs.

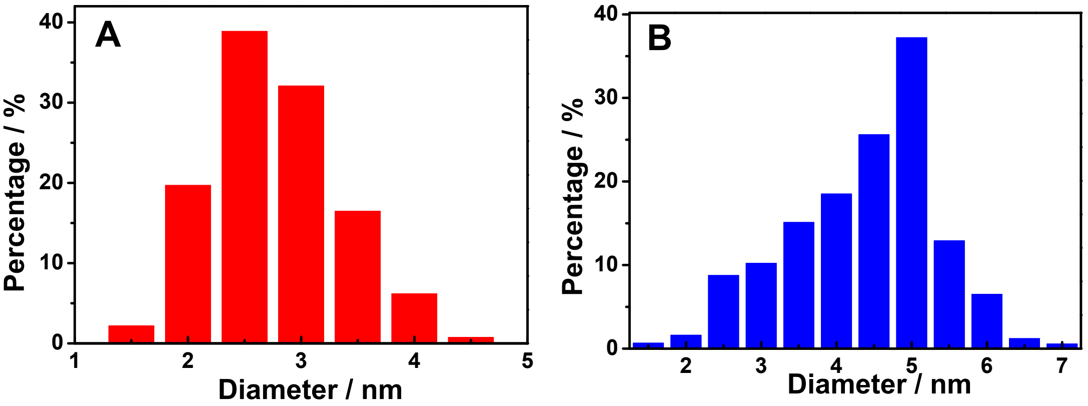

Fig. S3. The diameter distribution of (A) the CuNCs and (B) Eu^{3+}-CuNCs.

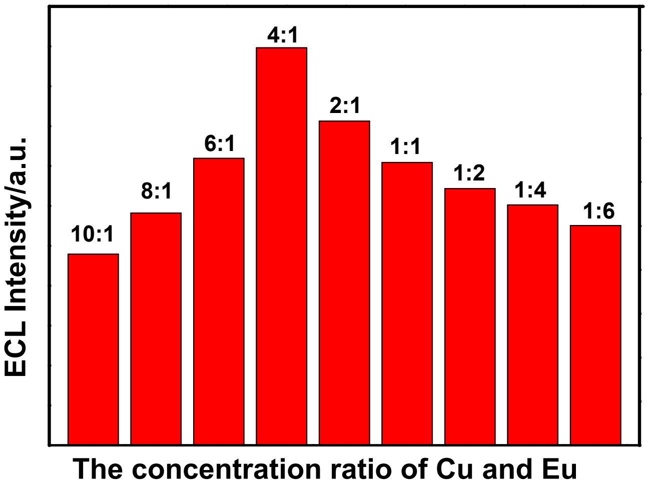

Fig. S4. Effect of different concentration ratio of Cu^{2+} and Eu^{3+} ion on ECL intensity in 0.10 M PBS (pH = 7.4) containing 50 mM $K_2S_2O_8$.

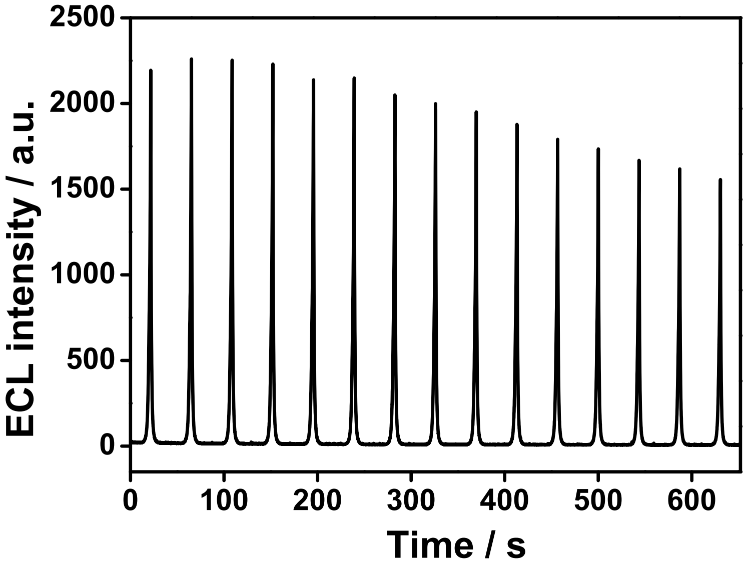

Fig. S5. The ECL stability of pure Cu NCs/GCE under 15 consecutive cyclic potential scans.

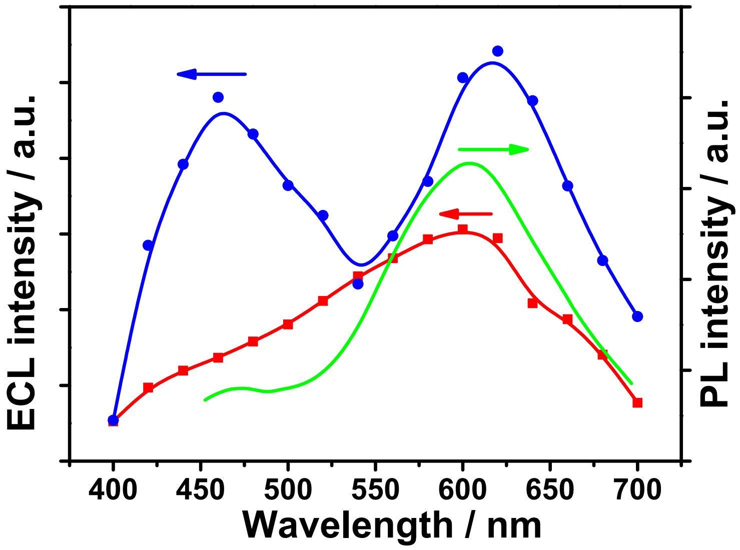

Fig. S6. The obtained ECL spectra of the pure Cu NCs-modified GCE (red curve) and Eu^{3+}-CuNCs-modified GCE (blue curve) through a series of filters in 0.1 M PBS (pH = 7.4) containing 50 mM $K_2S_2O_8$. The green curve represents the PL spectrum of CuNCs.

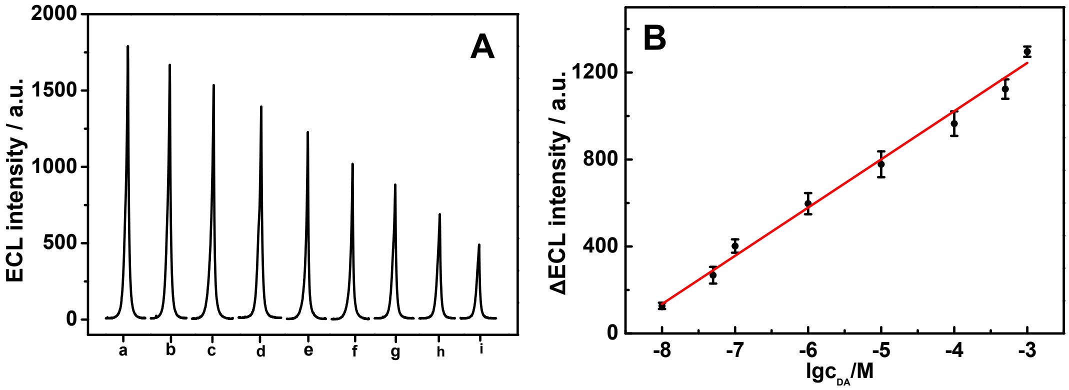

Fig. S7. (A) The signal response of pure Cu NCs-modified GCE for detecting various concentrations of DA and corresponding concentrations of DA: 0, 1.0 ×10^{-8} M, 5.0 × 10^{-8} M, 1.0 × 10^{-7} M, 1.0 × 10^{-6} M, 1.0 × 10^{-5} M, 1.0 × 10^{-4} M, 5.0 × 10^{-4} M, 1.0 × 10^{-3} M (from a to i). (B) Linear relationship between the ΔECL intensity and the logarithm of DA concentrations.

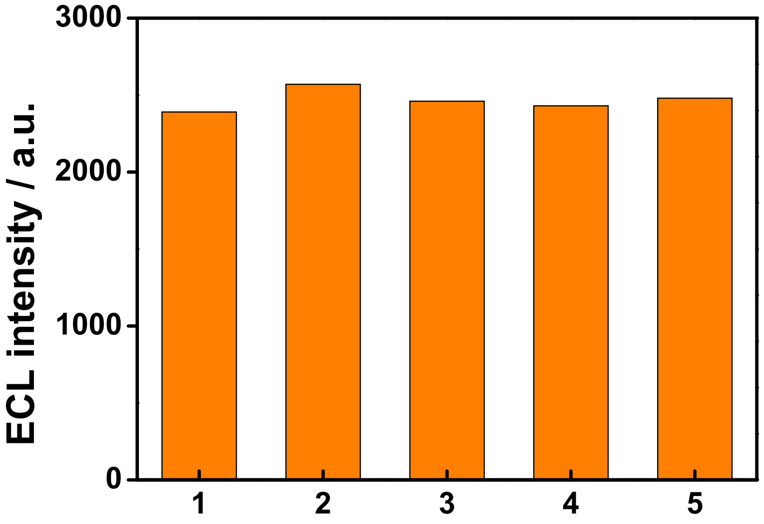

Fig. S8. The ECL reproducibility between five different electrodes with the concentration of DA (1.0 × 10^{-7} M) in 0.1 M PBS (pH 7.4) containing 50 mM $K_2S_2O_8$. The PMT was set at 600 V, and the potential scan was from -2.2 V to 0 V with a scan rate of 100 mV·s^{-1}.

Table S1. PL lifetimes (τ_{1-2}, μs) and fractions of the emission intensity (f_{1-2}, %) obtained from the fitting of experimental PL decay data by two exponential functions for the Cu NCs and Eu^{3+}-Cu NCs, together with calculated average PL lifetime $\tau_{average}$ (μs).

Sample Name	τ_1 (f_1)	τ_2 (f_2)	$\tau_{average}$
Eu^{3+}-CuNCs	10.55 (99.73)	180.0 (0.27)	11.0
CuNCs	12.2 (99.33)	70.3 (0.67)	12.6

Table S2. Comparison of this work with previously reported ECL sensing platform based on different materials for DA detection.

ECL sensing platform based on nanomaterials	Linear range	Detection limit	Ref.
$Ru(bpy)_3^{2+}$/OMC[a]/Nafion	4.75 nM - 625 μM	1.58 nM	2
Au NCs[b]	2.5 μM - 47.5 μM	-	3
rGO/MWCNTs/AuNPs[c]	0.2 μM - 70 μM	67 nM	4
Dual-stabilizers-capped CdSe-QDs[d]	10 nM - 3.0 μM	3.0 nM	5
CdTe QDs	50 nM - 5.0 μM	50 nM	6
Au/ZnSe-3MPA[e]	33 nM - 33 μM	0.565 nM	7
Eu^{3+}-CuNCs	0.01 nM - 500 μM	0.01 nM	this work

[a] ordered mesoporous carbon.
[b] Au nanoclusters.
[c] Graphene oxide/multiwall carbon nanotubes/gold nanoparticles.
[d] quantum dots.
[e] 3-mercaptopropionic acid capped ZnSe quantum dots modified gold electrode.

Table S3. Comparison of this work with previously reported detection methods.

Methods	Linear range	Detection limit	Ref.
High-performance Liquid Chromatography	0.01 μM - 100 mM	1.1 nM	8
Capillary Electrophoresis	20 μM - 100 μM	2 μM	9
Colorimetry	0.2 μM - 12 μM	0.047 μM	10
Fluorescence	2.5 nM - 10.4 μM	0.9 nM	11
Electrochemistry	0.1 μM - 400 μM	0.1 μM	12
Electrochemiluminescence	0.01 nM - 500 μM	0.01 nM	this work

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