Supporting Information

Cu Nanoclusters: Novel Electrochemiluminescence Emitters for Bioanalysis

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Characterization of the Biosensor Construction

The interfacial changes of the working electrode were characterized by scanning electron microscopy (SEM) and CVs. Compared with the clean and smooth surface of bare GCE (Figure S1A), a compact and irregular film was observed on the surface of GCE after assembling HZ (Figure S1B). In order to further confirm the generation of HZ on the surface of GCE, the CVs characterization was implemented in 3 mL PBS buffer containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$ at the potential range of -0.2 to 0.6 V (100 mV/s). It could be seen that a pair of well-defined redox peaks was observed at bare GCE (Figure S1C, curve a). While the peak currents were evidently decreased after assembling HZ (Figure S1C, curve b). This could be attributed to the obstruction of the HZ films.

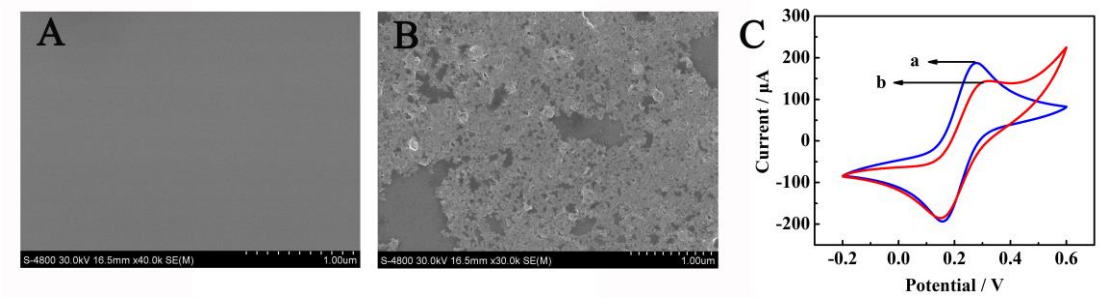
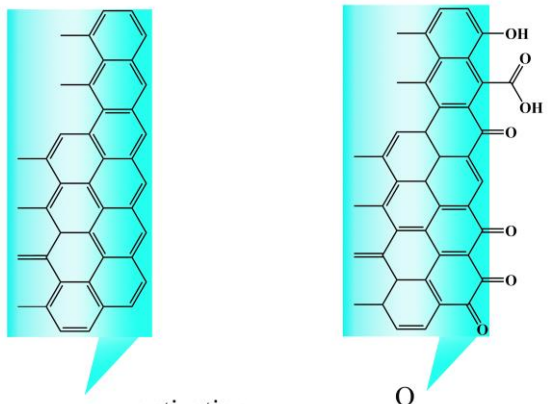


Figure S1 SEM images of the GCE without (A) and with assembling HZ (B). (C) CVs characterization for the stepwise biosensor fabrication measured in 0.1 M PBS (pH 8.0) containing 5.0 mM $[Fe(CN)_6]^{3-/4-}$: (a) bare GCE, (b) HZ/GCE.

The Assembly of HZ onto the Surface of GCE

It was reported that the glassy carbon electrode (GCE) was made up of 100% sp^2 carbon material[1, 2]. When the clean GCE was immersed in PBS containing 50 mM HZ and then was electrochemically activated with the cyclic voltammograms scanning for 40 cycles in the range of -1.5 V to 1.5 V (100 mV/s). In this scanning process, the surface of GCE was electrochemically activated to make the GCE modify with massive quinonyl groups and a few carbonyl, phenolic groups[3-5]. And then the quinonyl groups of the GCE surface were reacted with amino group of HZ to generate -C=N-NH_2[6, 7] The reaction mechanism of GCE surface was summarized as following equations:



$$(GCE)\text{-}C(=O)\text{-}R + N_2H_4 \longrightarrow (GCE)\text{-}C(R)=N\text{-}NH_2 + H_2O \qquad (2)$$

Optimization of the BSA Concentration in Cu NCs Synthesis

BSA as the protecting ligands is the main factor for the successful synthesis of stable Cu NCs. In order to synthesize stable Cu NCs, the amount of BSA should be sufficient as protecting ligands. However, if the BSA is excessive, the non-conductive BSA macromolecules with molecular weight of ~66.4 kDa would hinder electron transfer to reduce the ECL signal of Cu NCs since the excessive BSA could not be removed from the BSA-protected Cu NCs *via* the dialysis. Thus, the amount of BSA protecting ligands which has a remarkable effect on the ECL signal of Cu NCs has been optimized during the preparation of the stable Cu NCs. In the synthesis of Cu NCs, 1 mL of 10 mM $CuSO_4$ aqueous solution is put into 1 mL of different concentrations of BSA aqueous solution, and the following experimental procedures are the same as the part of ***Synthesis of Cu NCs***. Then, the HZ/GCE was measured under the potential range of 0 to 1.45 V in 2 mL PBS (pH 8.0) containing above-mentioned parallel Cu NCs, respectively. As exhibited in Figure S2, the ECL responses increase with the increasing concentration of BSA in the range from 0.05 to 2.0 mg/mL, and then decreased, which corresponded to the saturated state. Consequently, the optimal concentration of 2.0 mg/mL BSA was selected for the Cu NCs synthesis.

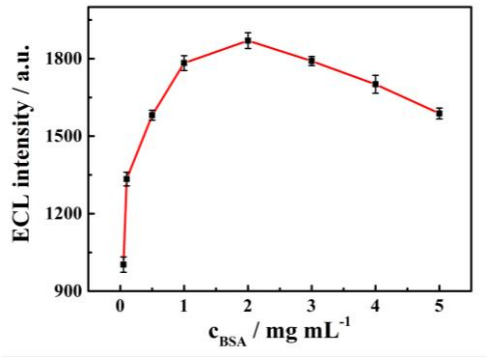


Figure S2 Influence of BSA concentration on the ECL response of the Cu NCs.

Table S1 Comparison of new Cu NCs/HZ-based ECL system with other ECL systems for DA detection.

ECL system	Linear range	LOD	Ref
SCP dots/TPA	0.05 μM ~ 10 μM	50 nM	8
$Au_{25}/S_2O_8^{2-}$ system	2.5 μM ~ 47.5 μM	Not shown	9
Ag_2Se QDs/$S_2O_8^{2-}$ system	0.5 μM ~ 19 μM	0.1 μM	10
CdS QDs/H_2O_2 system	1 μM ~ 10 mM	Not shown	11
CdTe QDs/O_2 system	50 pM ~ 10 nM	26 pM	12
Peptide nanovesicle/$S_2O_8^{2-}$ system	10 pM ~ 200 pM	3.15 pM	13
Cu NCs/HZ	1 pM ~ 10 nM	0.35 pM	This work

Table S2 Comparison of this developed biosensor with other biosensors for DA detection.

Method	Linear range	LOD	Ref
Fluorescence	0.1 μM ~ 10 μM	68 nM	14
Linear-sweep voltammograms	0.1 μM ~ 30 μM	Not shown	15
Chronoamperometric method	0.5 nM ~ 100 μM	50 nM	16
Colorimetry	0 ~ 0.6 μM	60 μM	17
Differential pulse voltammetry	0.5 μM ~ 50 μM	20 nM	18
ECL	1 pM ~ 10 nM	0.35 pM	This work

Table S3 Recovery of DA in human serum samples by the proposed sensor.

Sample number	Added (M)	Found (M)[a]	Recovery (%)
1	0	0.009 ± 0.01	--
2	5.0×10^{-12}	4.88×10^{-12} ± 0.05	97.6
3	5.0×10^{-11}	5.03×10^{-11} ± 0.14	100.6
4	1.0×10^{-10}	0.96×10^{-10} ± 0.09	96.0
5	5.0×10^{-9}	5.11×10^{-9} ± 0.11	102.2

[a] Mean value ± SD of three measurements.

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