

Supplementary file

**Hemoglobin-Au nanocomposites fabrication of highly stable
and sensitive electrochemical sensor and its analytical
applications**

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Figure S1

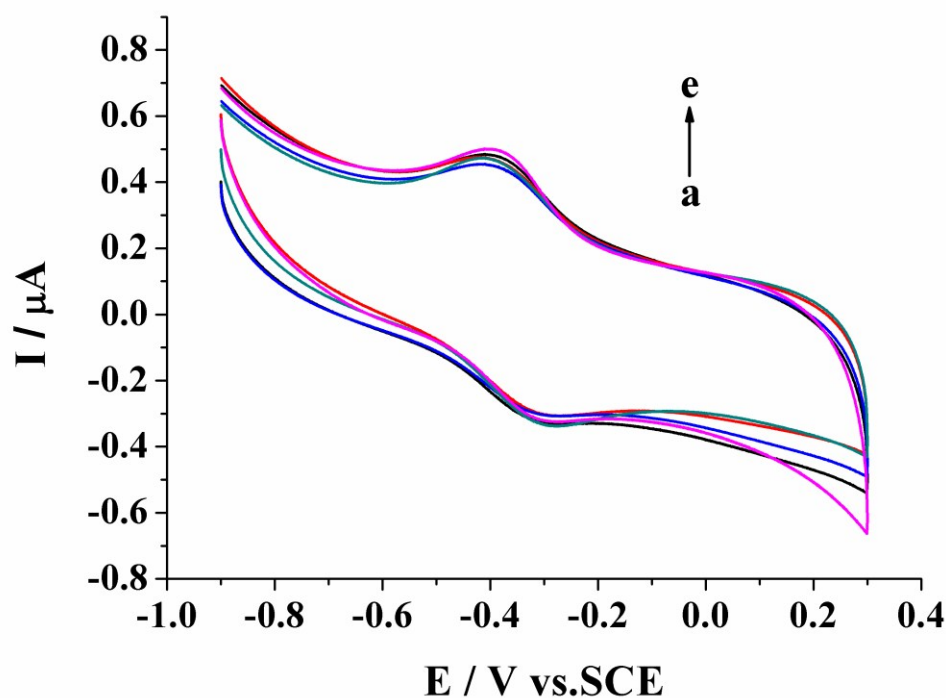


Figure S1 Cyclic voltammograms (scan potential: from -0.9 to 0.3 V) of different volume ratio of 3.0 mg mL⁻¹ Hb solution and 0.1 mg mL⁻¹ Au NPs immobilized on CS-Au NPs modified GCE at 100 mV s⁻¹ in pH 7.0 PBS (a-e: 1:0.2 , 1:0.25, 1:0.3, 1:0.5, 1:1).

Figure S1 showed the redox peak currents changes with different volume ratios of Hb solution and Au NPs (1:0.2, 1:0.25, 1:0.3, 1:0.5, 1:1) immobilized on CS-Au NPs modified GCE. It could be clearly observed that the redox peak currents increased with the volume ratio changed from 1:0.2 to 1:0.3. However, when the proportion of Au NPs increased further, the redox peak currents tended to be a platform. It was supposed that with the increasement of proportion of Au NPs, more Hb could be combined with Au NPs by electrostatic attraction and chemical bonding (Au-N bond¹), and reached the saturated state at the volume ratio of 1:0.3. When the proportion of Au NPs increased further, no more Hb could be linked to Au NPs. Therefore, the volume ratio of 1:0.3 was chosen as the optimized condition to be applied in the following experiments.

Figure S2

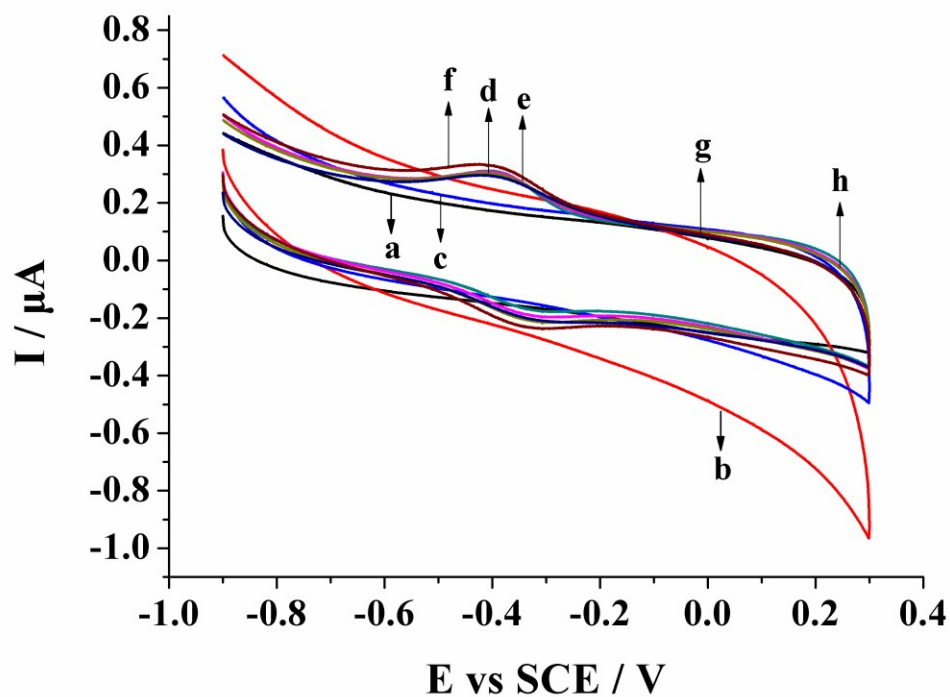


Figure S2 Cyclic voltammograms (scan potential: from -0.9 to 0.3 V) of bare GCE (curve a), CS modified GCE (curve b), CS-Au NPs modified GCE (curve c) and assembly of {Au NPs/Hb-Au NCs}_n films (n = 1-5) (curve d-h) on the CS modified GCE at 100 mV s⁻¹ in pH 7.0 PBS

Figure S3

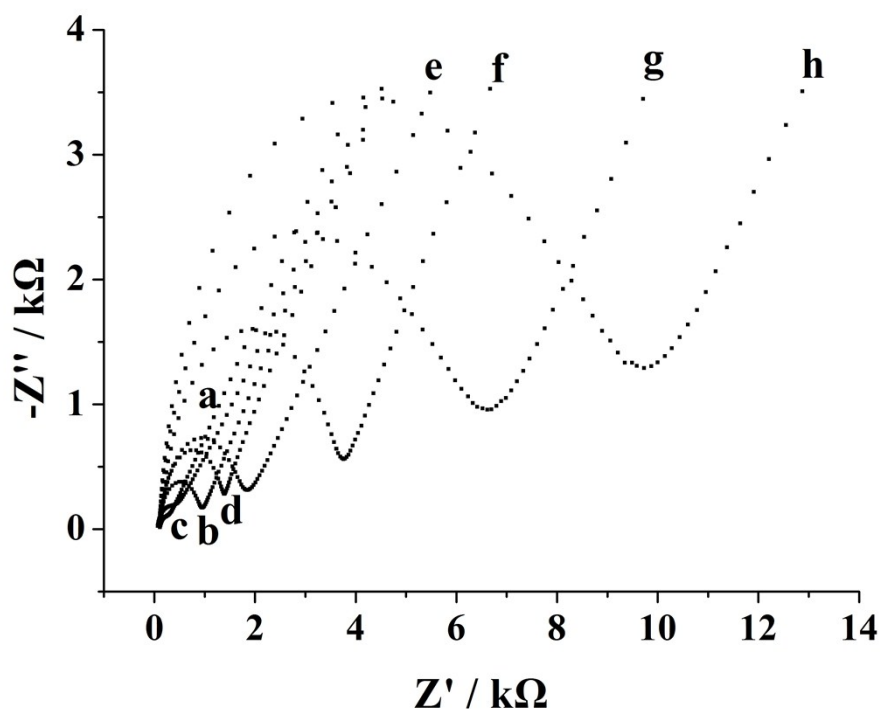


Figure S3 Electrochemical impedance spectroscopy of bare GCE (curve a), CS modified GCE (curve b), CS-Au NPs modified GCE (curve c) and assembly of {Au NPs/Hb-Au NCs}_n films (n = 1-5) (curve d-h) on the CS modified GCE in the presence of 5.0 mM [Fe(CN)₆]³⁻/Fe(CN)⁴⁻ (1:1) containing 0.10 M KCl solution.

Electrochemical impedance spectroscopy (EIS) was performed in 0.10 M KCl solution including 5 mM Fe(CN)₆³⁻/Fe(CN)₆⁴⁻ (1:1) as a supporting electrolyte at its open circuit potential with the AUTOLAB PGSTAT 302N electrochemical working station (Metrohm Co. Ltd., Switzerland) (begin frequency: 1.0×10^5 Hz; end frequency: 5.0×10^{-2} Hz; number of frequency: 60; amplitude of the potential perturbation: 10 mV). A small semicircle was observed at the bare GCE (Fig. S3 curve a). After CS film was electrodeposited on the GCE surface, the size of the semicircle increased (curve b), suggesting that the CS film acted as an insulating layer and barrier, making the interfacial charge transfer inaccessible. When Au NPs was immobilized on the CS-GCE the resistance value decreased (curve c), demonstrating the excellent conductive property of Au NPs². An apparent increase of R_{ct} could be observed when Hb-Au NCs was immobilized on the Au-CS-GCE (curve d). As Hb can interrupt electron transfer, and Au NPs are able to reduce interfacial resistance, we speculate that the effect of Hb would be predominant. With the assembly of {Au

NPs/Hb-Au NCs_n layers, R_{ct} increased with the increase of layer number (curve e-h). Additionally, the R_{ct} is roughly linearly increased with the layer number, indicating the good LBL assembly process.

Table S1 EQCM results of {Hb-Au NCs}_n films

列 1	$\Delta F(\times 10^3 \text{ n}=8)$	m (μg)	Γ_{ads} ($10^{-9} \text{ mol cm}^{-2}$)	Γ_{active} ($10^{-9} \text{ mol cm}^{-2}$)	$\Gamma_{\text{active}}/\Gamma_{\text{ads}}(\%)$
Au	0.002±0.0006				
Au-CS	0.202±0.021				
Au-CS-Au	0.299±0.050				
Au-CS-Au-{Au-Hb} ₁	44.1±3.2	59.13	4.67	0.6543	13.8
Au-CS-Au-{Au-Hb} ₂	89.1±5.2	119.88	9.48	0.7499	7.91
Au-CS-Au-{Au-Hb} ₃	135.1±6.9	181.98	14.3	0.9338	6.53
Au-CS-Au-{Au-Hb} ₄	180.1±4.0	244.08	17.73	0.935	5.27

Figure S4

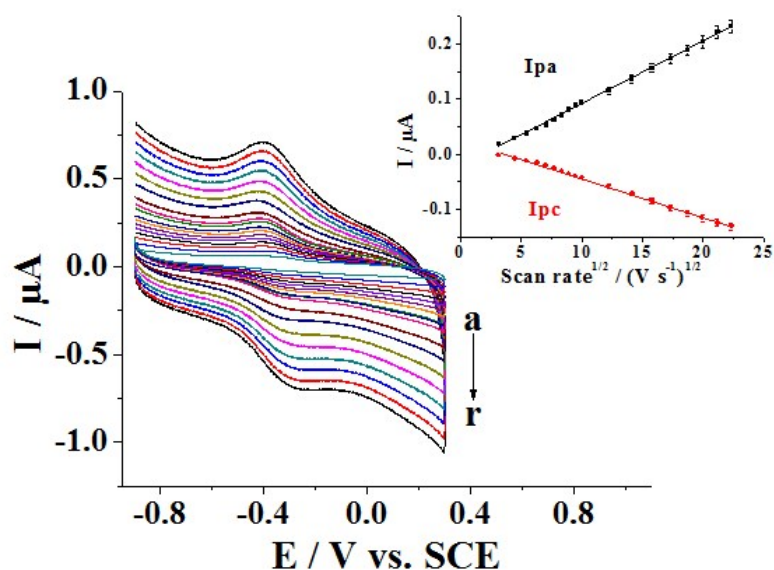


Figure S4 Cyclic voltammetry (scan potential: from -0.9 to 0.3 V) of GCE-CS-{Au NPs/Hb-Au NCs}₃ in 0.10 M pH 7.0 PBS at 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 150, 200, 250, 300, 350, 400, 450 and 500 mV s⁻¹ (a-r). Inset: Plots of the peak currents versus the square root of scan rates.

As depicted in Fig. S4, the redox peak heights of GCE-CS-{Au NPs/Hb-Au NCs}₃ increased with the growing of scan rates, and the peak currents were linearly correlated with the square root of the scan rate ($v^{1/2}$) within the range from 0.01 to 0.5 V s⁻¹, suggesting a surface-controlled electrode process. Each Layer of the linear regression equation was I_{pc} (μA) = 0.027 - 0.007 $v^{1/2}$ (V s⁻¹) ($n = 3$, $R^2 = 0.9970$), and I_{pa} (μA) = -0.023 + 0.0114 $v^{1/2}$ (V s⁻¹) ($n = 3$, $R^2 = 0.9987$). These results indicated that the electrochemical kinetics in both cases were diffusion-control processes³. On the other hand, according to Faraday's law²:

$$I_{pc} = \frac{n^2 F^2 A \Gamma v}{4RT} = \frac{nFQv}{4RT} \quad (1)$$

Where Q is the quantity of charge (C). The symbols n , I_p , F , R and T have their usual meanings. Calculated from the above-mentioned equation, the electron transfer number (n) was $1.057 \approx 1.0$, implying a single electron transfer reaction.

Figure S5

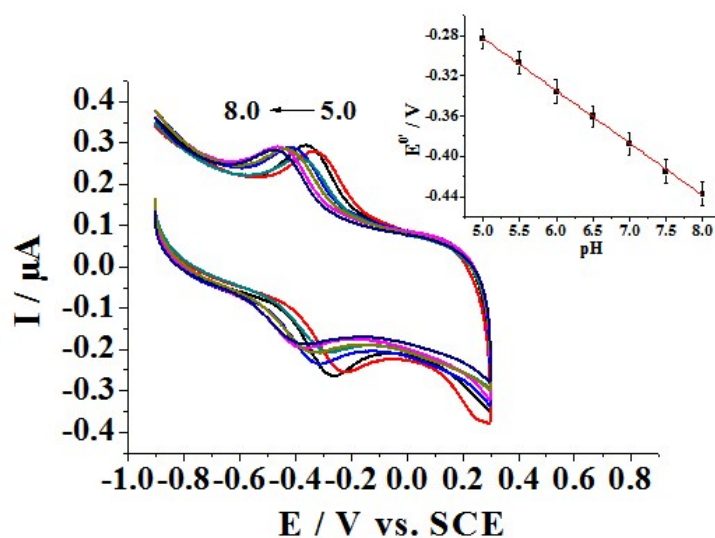


Figure S5 Cyclic voltammograms (scan potential: from -0.9 to 0.3 V) of GCE-CS-{Au NPs/Hb-Au NCs}₃ in 0.10 M pH 7.0 phosphate buffer at different pH values of 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0 (from right to left); scan rate: 100 mVs⁻¹. Inset: Effect of pH values on the formal potential (E⁰).

It was reported that the pH strongly affects the direct electrochemistry of Hb.⁴ From Figure S5, with the the increasement of the pH values, the formal potential (E⁰) of GCE-CS-{Au NPs/Hb-Au NCs}₃ moved to negative values. Within the pH ranges from 5.0 to 8.0, the linear regression equation was $E^0(\text{V}) = -0.008 - 0.0539 \text{ pH}$, $R^2 = 0.998$. E⁰ decreases linearly with a slope of -53.9 mV per decade pH (inset in Figure S5). This value is extremely close to the theory value of -59.0 mV pH⁻¹ at 25 °C for reversible proton-coupled electron transfer per decade pH for a reversible proton-coupled single electron transfer ⁵.

Stability, interference and reproducibility of GCE-CS-{Au NPs/Hb-Au NCs}₃

Common species such as Na⁺, K⁺, NH₄⁺, Ca²⁺, Mg²⁺, ascorbic acid, Cl⁻, NO₃⁻, Ac⁻, SCN⁻, H₂PO₄⁻, HPO₄²⁻, PO₄³⁻ and some electroactive species such as dopamine, uric acid, epinephrine and noradrenaline were added to the mixture solution inserted with GCE-CS-{Au NPs/Hb-Au NCs}₃. The results of experiments showed that these species did not affect the detecting of O₂, H₂O₂ and TCA. As all the solutions are deoxygenated during the whole detecting process, the electrocatalysis of H₂O₂ will be affected when the concentration of TCA is higher than 3.07×10^{-11} M and the electrocatalytic reduction of TCA will be affected when the concentration of H₂O₂ is higher than 3.55×10^{-9} M. The electrocatalysis of O₂ will be affected when the concentration of TCA is higher than 3.07×10^{-11} M or/and the concentration of H₂O₂ is higher than 3.55×10^{-9} M. To study the storage stability of the biosensor, we stored it at 4 °C when not in use and measured intermittently. Two weeks later, the response of the modified electrode still retained its activity 92 % of its initial value. The relative standard deviation (RSD) was 2.4 % for 10 independent determinations of 2.0×10^{-6} M H₂O₂, which showed excellent reproducibility of the current response for the {Au NPs/Hb-Au NCs}₃ films immobilized on CS modified GCE.

Reference

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