Supporting Information

Surface-Enhanced Electrochemiluminescence Combined with Resonance Energy Transfer for Sensitive Carcinoembryonic Antigen Detection in Exhaled Breath Condensates

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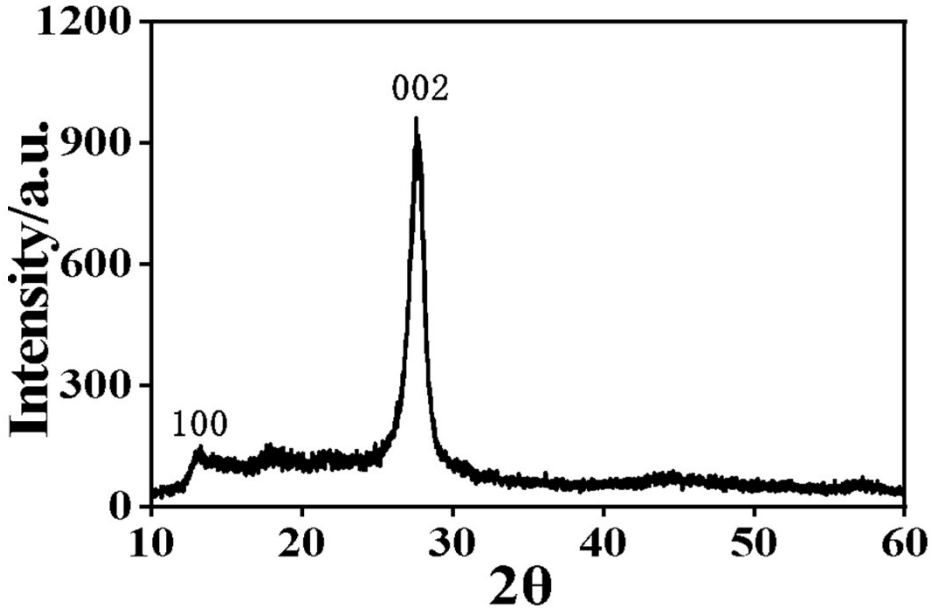

Figure S1. The XRD image of g-C_3N_4 NS: the (002) peak at about 27.5° and the (100) peak at 13.2°[1, 2].

Synthesis of SiO_2-Ru NPs: the SiO_2 NPs bought from Shanghai Aladdin Biochemical Technology Co., Ltd. dispersed in ultrapure water. The following steps are the same as those of Au@ SiO_2-Ru.

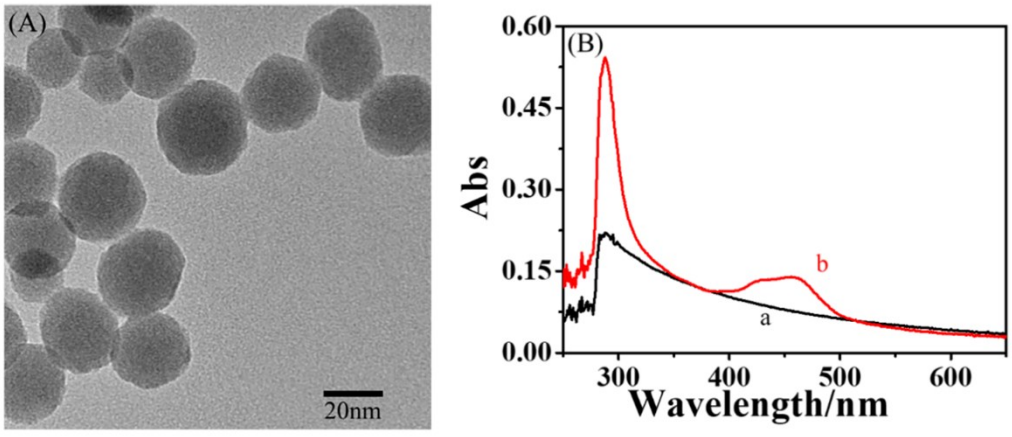

Figure S2. (A) TEM image of SiO_2-Ru NPs; (B) UV-Vis spectra obtained for SiO_2 NPs (a) and SiO_2-Ru (b).

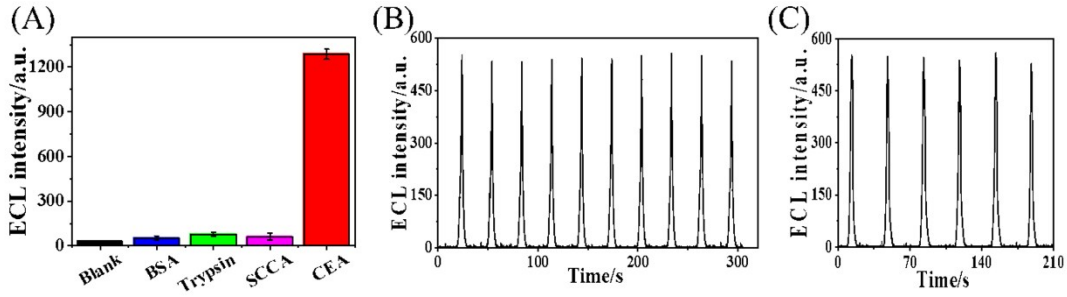

Figure S3. (A) ECL intensity of different types of protein modified electrodes in 0.1 M pH = 8.5 PBS containing 0.1 M $Na_2S_2O_8$. The scan rate is 100 mV/s was used for CV analysis. The concentration of CEA was about 1.0 ng/mL, while the concentration of other proteins (BSA, Trypsin, SCCA) were of 100 ng/mL used for the ECL detection; (B) Stability of the ECL signals measured using GCE/Au-g-C_3N_4/Apt1/CEA/Apt2-Au@SiO_2-Ru modified electrode; (C) Reproducibility of the ECL signals on six independent GCE/Au-g-C_3N_4/Apt1/CEA/Apt2-Au@SiO_2-Ru modified electrodes in 0.1 M pH=8.5 PBS containing 0.1 M $Na_2S_2O_8$. The concentration of the CEA was about 10 pg/mL used for above (B) and (C) experiments.

The thickness of SiO_2 shell affected the distance between Au NPs and $Ru(bpy)_3^{2+}$, as shown in Figure S4[3]. If the distance of Au NPs and $Ru(bpy)_3^{2+}$was too close, RET occurred between them, resulting in Au NPs quenching the ECL signal of $Ru(bpy)_3^{2+}$. When the distance between them increased gradually, the ECL signal could also be increased significantly while LSPR substituted to RET of Au NPs. When the thickness of SiO_2 was 5 nm, the strongest enhancement achieved. If the thickness of SiO_2 continued to increase, the ECL signal was still be enhanced, but the enhanced amplitude decreased obviously until no enhancement when LSPR disappeared. In addition, the thickness of SiO_2 shell in Au@SiO_2 could be controlled by adjusting the volume of Na_2SiO_3 (0.5% (v/v)Na_2SiO_3) in its synthesis reaction

process[3].

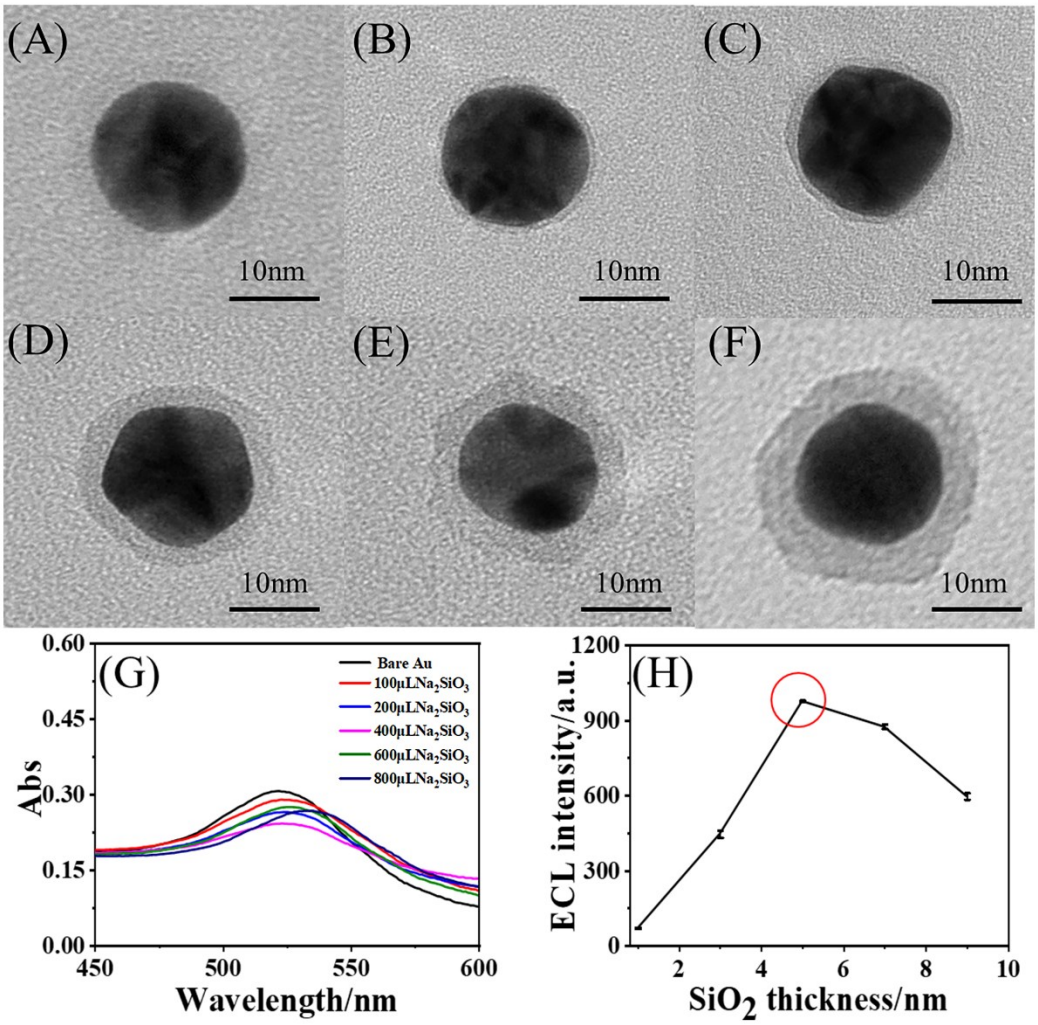

Figure S4. TEM images of Au NPs and Au@SiO_2 nanoparticles with different shell thickness. The volume of Na_2SiO_3 used in the synthesis were (A) 0 μL, (B) 100 μL, (C) 200 μL, (D) 400 μL, (E) 600 μL and (F) 800 μL; (G) Corresponding UV-Vis absorption spectrum of Au NPs and Au@SiO_2 nanoparticles with 100, 200, 400, 600 and 800 μL Na_2SiO_3 added in synthetic process, respectively. ; (H) Effect of the thickness of SiO_2 on the ECL response.

We optimized the volume of $HAuCl_4$ added to the g-C_3N_4 NS. As shown in figure S5, when the volume of $HAuCl_4$ was 60 μL (0.01 M $HAuCl_4$), the 5 nm Au NPs with uniform particle size attached to g-C_3N_4 was obtained (Figure S5B).

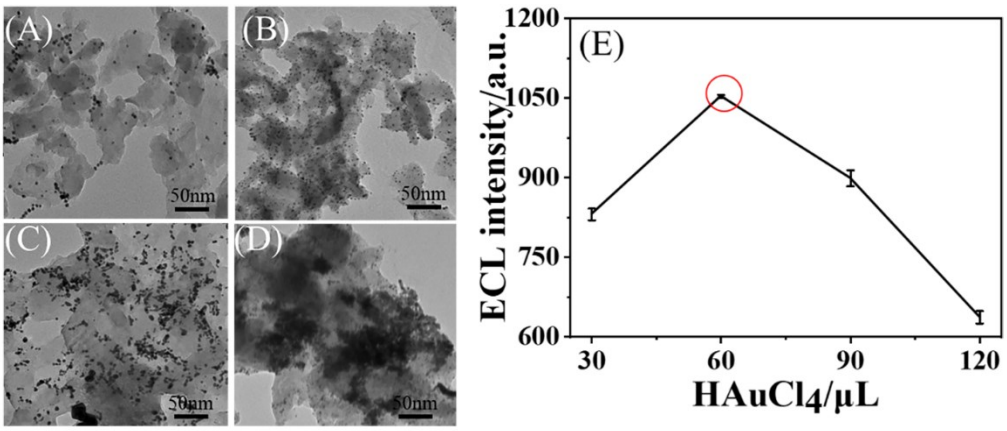

Figure S5. TEM images of Au-g-C_3N_4 with the volume of $HAuCl_4$ added to g-C_3N_4 were (A) 30 μL, (B) 60 μL, (C) 90 μL, (D) 120 μL, respectively; (E) Effect of the concentration of $HAuCl_4$ on the ECL response. g-C_3N_4 NS: 0.15 ng/mL.

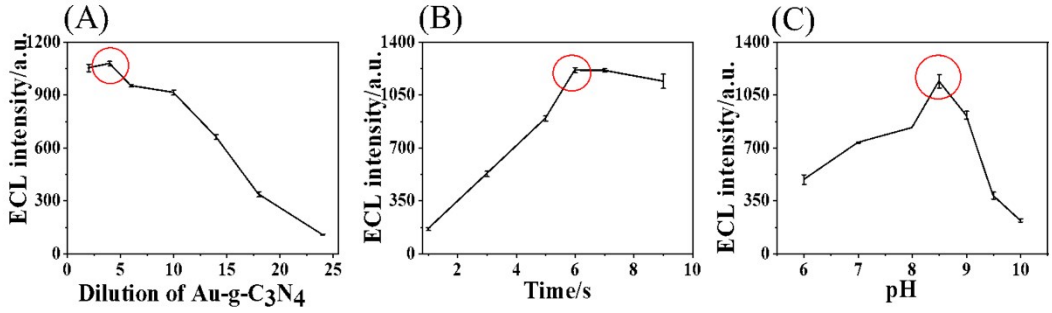

Figure S6. (A) Effect of the concentration dilution of Au-g-C_3N_4 on the ECL response; (B) Effect of the reaction time about Au@SiO_2-Ru-Apt2 with CEA on the ECL response; (C) Effect of pH on the ECL response.

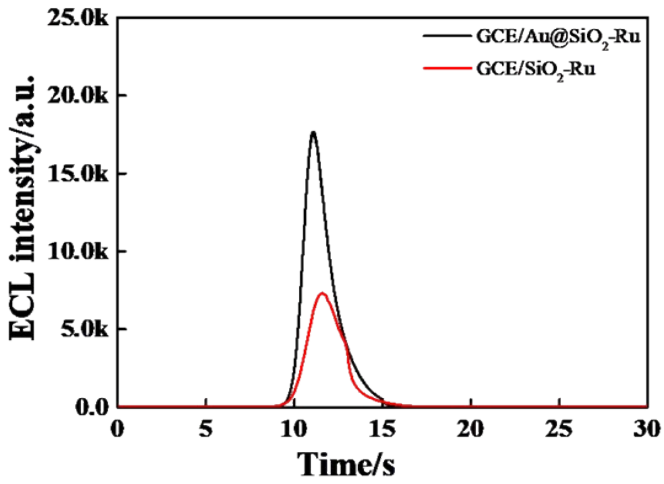

Figure S7. ECL performances of Au@SiO_2-Ru and SiO_2-Ru.

Table S1. Comparison analysis of CEA detection (dynamic range and LOD) using various methods.

Methods	**Liner range**	**LOD**	**Reference**
Electrochemiluminescence	0.05 ~ 100 ng/mL	0.02 ng/mL	[4]
Fluorescence	0.05 ~20 ng/mL	6.7 pg/mL	[5]
Fluorescence	1 ~ 5×10^5 ng/mL	0.3 ng/mL	[6]
Fluorescence	0.0018 ~ 1.8 ng/mL	0.6 pmol/L	[7]
Photoelectrochemical	0.05 ~ 5 ng mL	11.2 pg/mL	[8]
Surface enhanced Raman spectroscopy	0.1 ~ 500 ng/mL	0.05 ng/mL	[9]
Electrochemiluminescence	0.05 ~ 20 ng/mL	0.031 ng/mL	[10]
SEECL-RET method	0.001 ~ 5 ng/mL	0.3 pg/mL	**This Work**

Table S2. The results of the recovery analysis of CEA in EBCs (n=3).

Sample	CEA added ($ng\cdot mL^{-1}$)	ELISA found ($ng\cdot mL^{-1}$)	This work found ($ng\cdot mL^{-1}$)	Recovery (%)	RSD (%)
1	1.00	1.02	0.97	96.90	7.95
2	2.00	1.98	2.03	101.50	8.63
3	4.00	4.16	4.13	103.25	6.61

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