Electronic Supporting Information for

Lowly-Aggregated Perylene Diimide as Near-Infrared Electrochemiluminescence luminophore for Ultrasensitive Immunosensor at Low Potential

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Experimental Section

Chemicals

GO was purchased from Shandong Leader Nano Tech.Co., Ltd. N,N'-Dicaproate sodium -3,4,9,10-perylenedicarboximide(PDI-COONa) was purchased from Shandong Aikesi Chemical Technology Co., Ltd. PEI (MW=1800, 99%) and bovineserum albumin (BSA) was purchased from Sigma–Aldrich. $HAuCl_4•3H_2O$ and cholesterol was purchased from Shanghai Macklin biochemical Co., Ltd. Human AFP and its antibody was obtained from Shanghai Linc-Bio Science Co. LTD. Uric acid (UA) was purchased from Aladdin Industry Corporation. Human serum samples were obtained from a healthy female volunteer in Liaocheng People's Hospital. Ultrapure water (> 18 MΩ) from a Milli-Q Plus system (Millipore) was used to prepare aqueous solutions.

Instruments

Cyclic Voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed on CHI 760C electrochemical workstation. The working electrode was glassy carbon electrode (GCE) (3-mm diameter), reference electrode be Ag/AgCl (KCl-saturated) and counter electrode be platinum wire. The supporting electrolyte was 0.1 M phosphate buffer saline (PBS, pH 7.4). The ECL spectroscopy was recorded on Model ECLS-MK Spectrograph (FORTEC Technology (HK) Co. Ltd). JEM-

2100 electron microscope (JEOL in Japan) was operated at 200 kV to record transmission electron microscope (TEM) images. Lambda 750 spectrophotometer(Perkin Elmer in USA) was used to measure the ultraviolet–visible(UV–vis) absorption spectra. Fluorescent(FL) spectroscopy was recorded using FLS1000(Edinburgh, UK).

Preparation of GO/PDI-COO$^-$/AuNPs/AFP-Ab$_2$ nanocomposite

6 mg of PDI-COONa, 2 mg of GO solution and 5 mL ultrapure water were put into a beaker and kept stirring at 40 °C for overnight. After that, the resulted products were filtered by a nylon membrane with pores size 0.22 μm, then washed with ultrapure water and dispersed into 5 mL ultrapure water.

AuNPs were synthesized as described with some modifications. Briefly, 5 mL of PEI (Mw = 1.8 kDa) was added to 5 mL of 1 mM $HAuCl_4$ while stirring at 80 °C in an oil bath. When the reaction mixture became red, it was removed from the oil bath with continuous stirring until the mixture reached to ambient temperature. The resulted AuNPs were purified by dialysis (3500 Da cutoff) against distilled water to remove the unbound PEI.

1 mL of GO/PDI-COO$^-$ and 500 μL AuNPs solution were put into a beaker and stirred for 1 h at room temperature. Then we filtered the product GO/PDI-COO$^-$/AuNPs and dispersed them in 1.5 mL ultrapure water. 3 μL AFP-Ab$_2$ (1 mg mL^{-1}) was added into 1 mL GO/PDI-COO$^-$

/AuNPs solution and magnetically stirred for 30 minutes, then 3 μL BSA (1%) solution was introduced to block inactive sites. Finally, the products were filtered and finally dispersed into 1 mL ultrapure water.

Preparation of the immunosensor.

First, polished GCE was put into phosphate buffered saline (PBS) solution with 3 mM $HAuCl_4 \cdot 3H_2O$ to electrodeposit AuNPs for 200 s at −0.2 V. Then, 5 μL AFP-Ab_1 (18 μg mL^{-1}) was introduced onto the GCE/Au surface and kept for 12 h at 4 °C to obtain GCE/AuNPs/Ab_1 interface. And 5 μL of 1% BSA was dropped onto the surface and incubated for 1 h at 37 °C to seal the inactive sites on the AuNPs film. Next, AFP with different concentrations were dropped onto their GCE/Au/Ab_1/BSA interface and incubated for 2 h at 37 °C. Finally, 5 μL GO/PDI-COO^-/AuNPs/AFP-Ab_2 was applied to the interface of GCE/Au/Ab_1/AFP and kept for 1 h at 37 °C. After every modification process, the modified electrode was washed by ultrapure water. Finally, the prepared immunosensor was stored at 4 °C for further use.

The experimental conditions such as the concentration of coreactant, the concentration of phosphor, and the pH of PBS buffer for performing the ECL measurements of fabricated immunosensors have been optimized (Fig.S8). The prepared immunosensor showed the strongest ECL with coreactant $K_2S_2O_8$ at 0.1 M, phosphate at 0.1 M and pH of PBS buffer at 7.4. Then the ECL intensity of the fabricated immunosensors at different concentration of AFP(C_{AFP}) were recorded in 0.1 M PBS solution with pH at 7.4 and $K_2S_2O_8$ at 0.1 M.

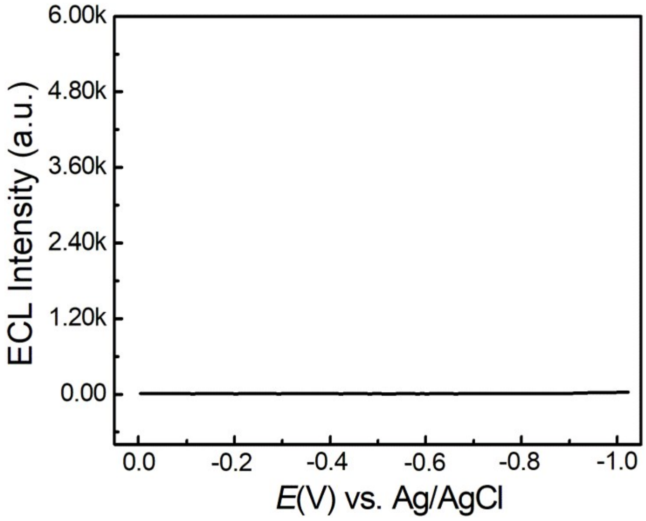

Fig.S1 The ECL intensity of bare GCE in PBS solution with 0.1 M $K_2S_2O_8$.

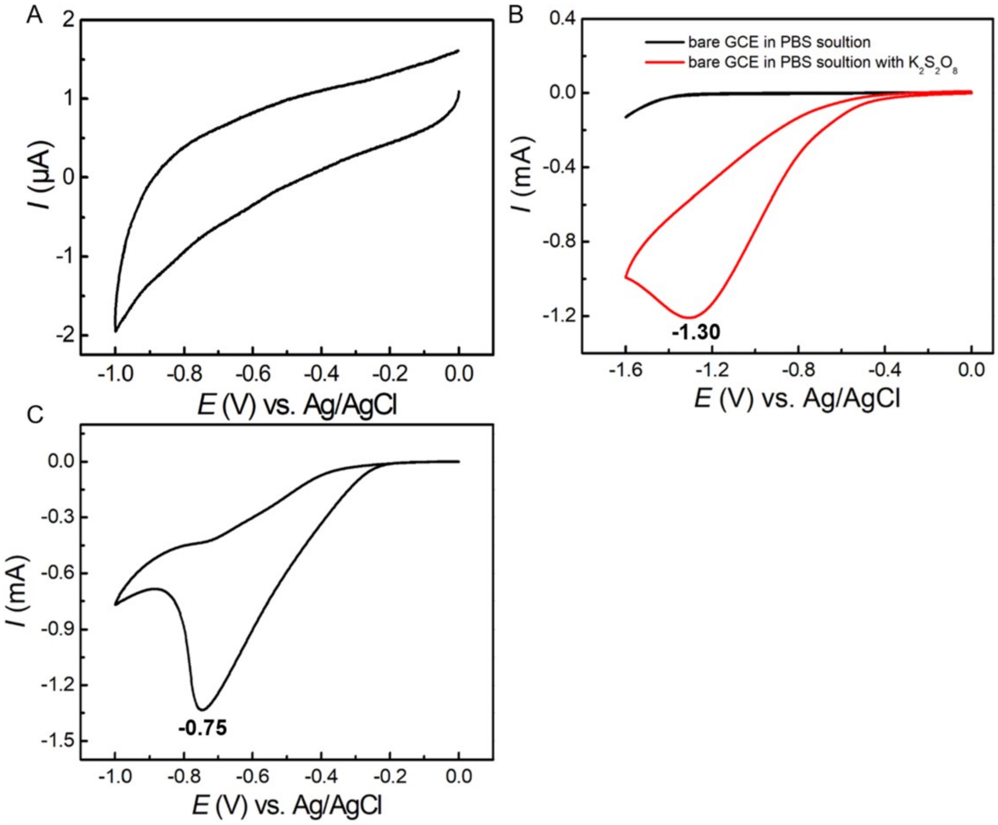

Fig.S2 A. the CV of bare GCE in 0.1 M PBS solution(pH 7.4) between 0 and -1.0 V; B. the CV of bare GCE in 0.1 M PBS solution(pH 7.4) (black line) and with 0.1 M $K_2S_2O_8$ (red line)between 0 and -1.6 V; C. the CV of PDI-COO$^-$ modified GCE in 0.1 M PBS solution(pH 7.4) including 0.1 M $K_2S_2O_8$ between 0 and -1.0 V.

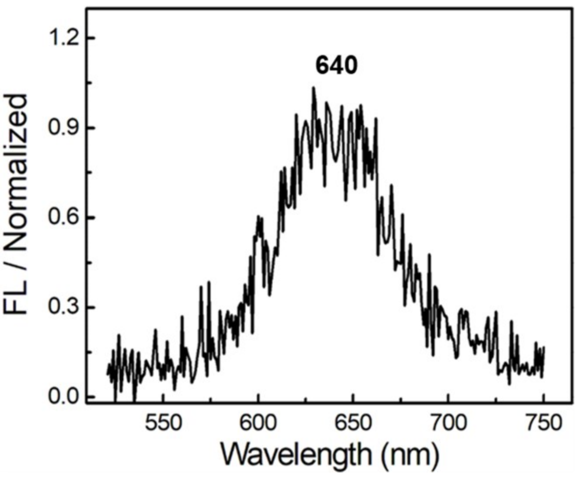

Fig.S3 The FL spectroscopy of PDI-COO$^-$ aqueous solution at excitation wavelength 500 nm.

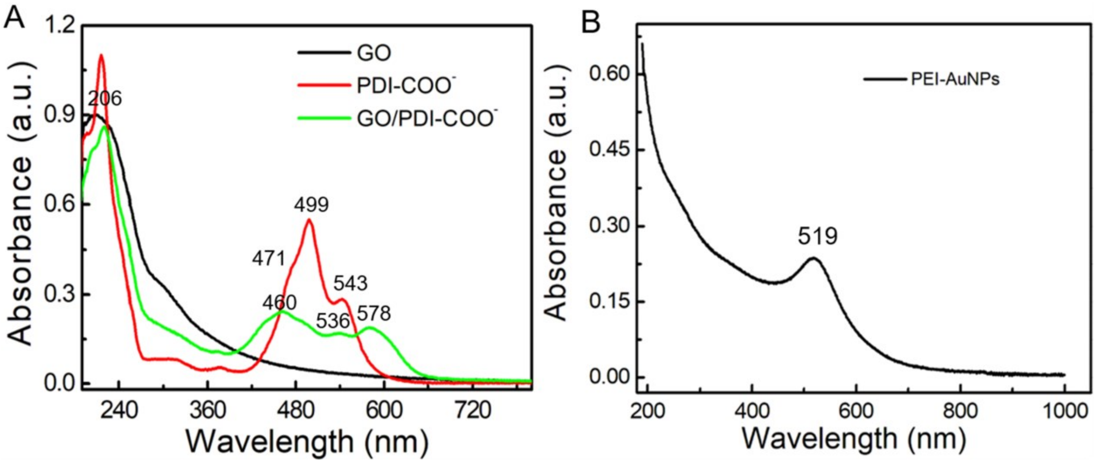

Fig.S4 A. the UV-vis spectroscopy of GO(black line), PDI-COO$^-$ (red line) and GO/PDI-COO$^-$(green line); B. the UV-vis spectroscopy of AuNPs reduced by PEI.

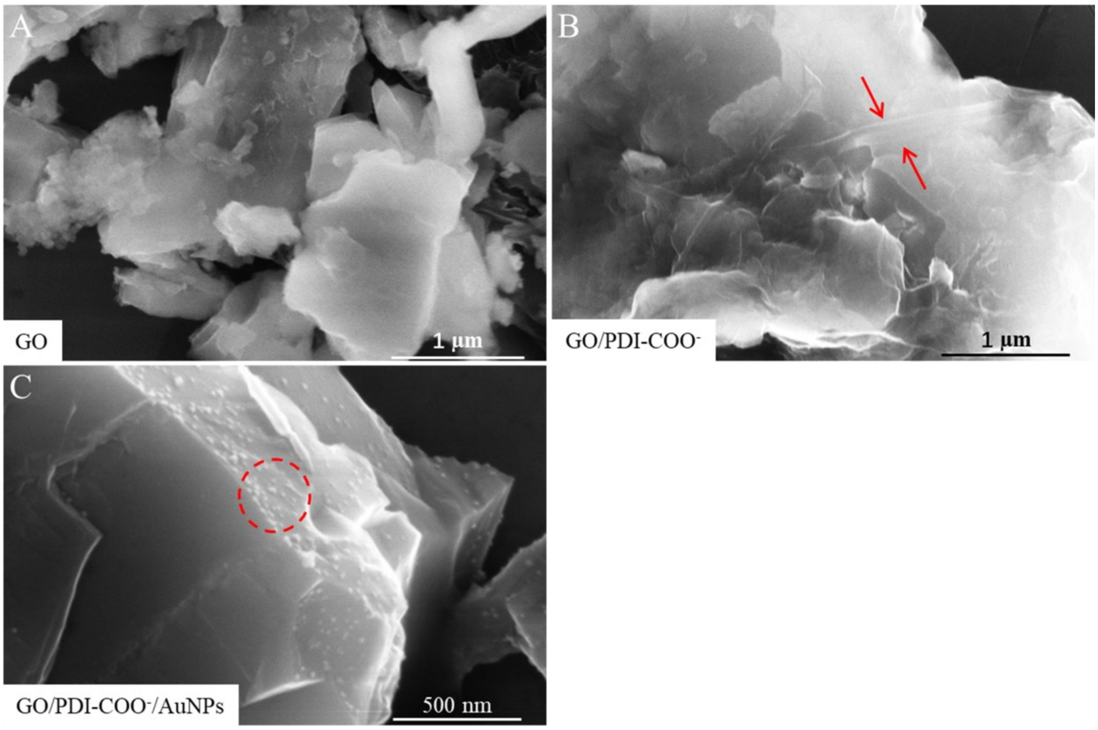

Fig.S5 The SEM image of GO(A), GO/PDI-COO$^-$(B) and GO/PDI-COO$^-$/AuNPs(C).

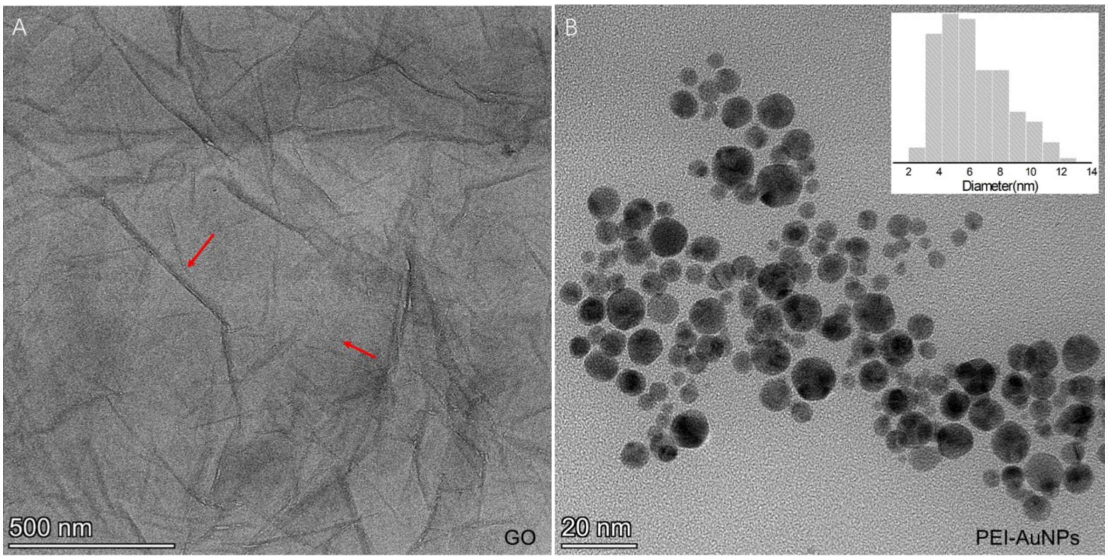

Fig.S6 The TEM image of GO(A) and AuNPs reduced by PEI(B). Inset in (B): the size distribution of AuNPs obtained from measuring 300 randomly selected particles.

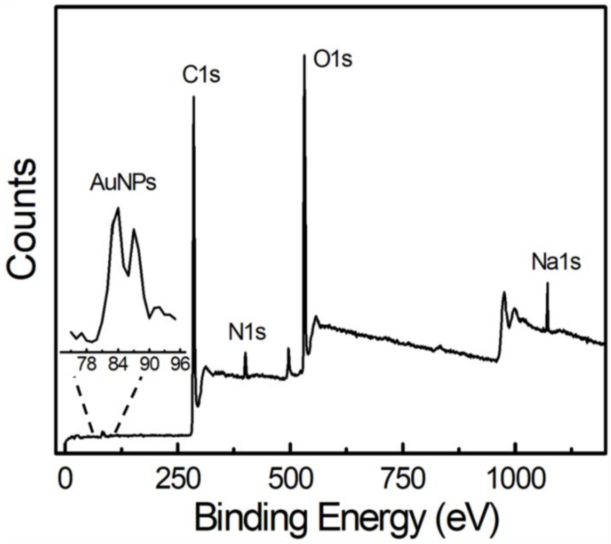

Fig.S7 The XPS spectroscopy of GO/PDI-COO$^-$/AuNPs composites.

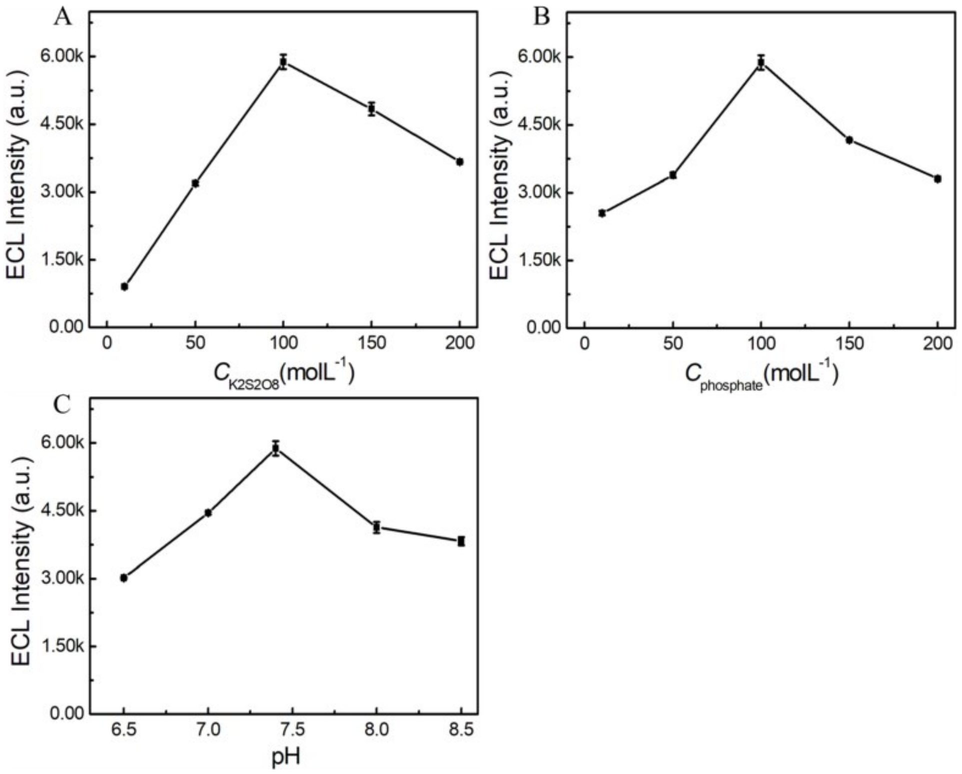

Fig.S8 The ECL intensity of immunosensor at different concentration of coreactant $K_2S_2O_8$(A), phosphate (B) and pH(C).

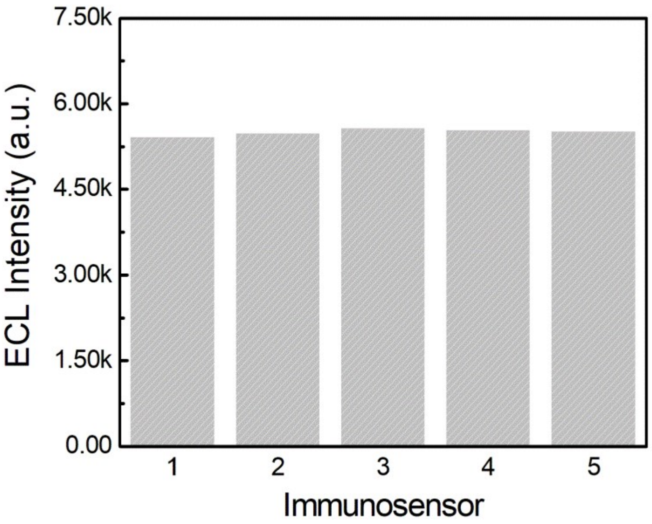

Fig.S9 The reproducibility of the parallelly prepared immunosensor.