

Electronic Supplementary Information for:

**Perylene Diimide as Cathodic Electrochemiluminescence Luminophore for
Immunoassay at Low Potential**

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Experimental section

Materials

Human colon CEA and its antibody were obtained from Shanghai LincBio Science. $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$, *N,N'*-Dimethyl-3,4,9,10-perylenedicarboximide (PDI- CH_3) (97%) and bovine serum albumin (BSA) were purchased from Sigma–Aldrich. Uric acid (UA) were purchased from Aladdin Industry Corporation. Cholesterol, bovine serum albumin (BSA), thrombin(Thr), α fetoprotein (AFP) and prostate specific antigen (PSA) was purchased from Shanghai Macklin biochemical Co., Ltd. Human serum samples from various cancers were obtained from Liaocheng People's Hospital. Ultrapure water (> 18 M Ω) from a Milli-Q Plus system (Millipore) was used to prepare aqueous solutions.

Apparatus

ECL immunoassay experiments were performed on a Model RFL-1 ECL analyzer (Xi'an Remex Instrument Co., Ltd., China) with electrochemical workstation CHI 660B (Shanghai CHI Instruments Co., China). In the ECL immunoassay experiments, the photomultiplier tube (PMT) voltage was at 680 V and the ECL experiments were performed in 0.1 M PBS solution(pH 7.4) with 0.1 M $\text{K}_2\text{S}_2\text{O}_8$. The ECL spectroscopy were recorded on homemade spectrometer in Professor Guizheng Zou's lab at School of Chemistry and Chemical Engineering, Shandong University. Cyclic Voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were performed in a 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). JEM-2100 electron microscope of JEOL in Japan were operated at 200 kV to record transmission electron microscope (TEM) images. Lambda 750 spectrophotometer of Perkin Elmer in USA) were used to measure the ultraviolet–visible (UV–Vis) absorption spectra. Photoluminescence (PL) spectroscopy of PDI- CH_3 in solution were recorded on F-7000(HITACHI Ltd., Japan). PL spectroscopy of PDI- CH_3 in the solid state were recorded using LS 55(PerkinElmer instruments). SK type ultrasonic cleaning machine (Shanghai Keguo Ultrasonic instrument Co., Ltd), pHs-3C meter (Shanghai second analysis instrument factory), high-speed refrigerated centrifuge Z36HK-HERMLE (Germany), dry type thermostat (Hangzhou Aomori Instrument Co., Ltd).

Preparation of GO/PDI/AuNPs/Ab₂ nanomaterial

For preparation of GO/PDI- CH_3 , 6 mg of PDI- CH_3 , 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.

For preparation of AuNPs, 2 mL of 50 mmol L⁻¹ $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ aqueous solution and 98 mL ultrapure water were added to a three-necked flask and refluxed under

magnetic stirring at 100 °C. Then added 10 mL sodium citrate (38.8 mmol L⁻¹) into above solution as quickly as possible. The color changed from pale to burgundy within one minute. Then keeping reflux for 20 minutes. The obtained AuNPs were placed in a brown volumetric flask and stored at 4 °C.

For preparation of GO/PDI-CH₃/AuNPs, 2 ml of 1.6 mg mL⁻¹ of GO/PDI-CH₃ and 1 mL AuNPs solution were put into a beaker and stirred for 1 h at room temperature. Then we centrifuged the resulting product GO/PDI-CH₃/AuNPs and dispersed them in PBS solution (pH=7.4).

5 μL of 1 mg mL⁻¹ of Ab₂ was added to 1 mL GO/PDI-CH₃/AuNPs solution and magnetically stirred for 30 minutes to prepare GO/PDI-CH₃/AuNPs/Ab₂, then 5 μL of 1% BSA solution was introduced to block remaining active sites. Finally, the products were centrifuged three times at 8000 rpm and finally dispersed into 1 mL PBS solution (pH = 7.4).

Preparation of sandwiched immunosensor

First, polished GCE were put into PBS solution with 6 mM HAuCl₄·3H₂O to electrodeposit AuNPs for 200 s at -0.2 V. Then 5 μL of 18 μg mL⁻¹ Ab₁ was introduced onto the GCE/Au surface and kept for 12 hours at 4 °C to obtain GCE/Au/Ab₁ interface. And 5 μL of 1% BSA was dropped and incubated for 1 hour at 37 °C. Next, CEA with different concentrations were dropped onto GCE/Au/Ab₁/BSA and incubated for 2 hours at 37 °C. Finally, 5 μL of GO/PDI-CH₃/AuNPs/Ab₂ was applied to GCE/Au/Ab₁/CEA interface and incubated for 1 hour at 37 °C. After every modification process, the modified electrodes needed be washed by ultrapure water. The prepared sensor was stored at 4 °C for next use.

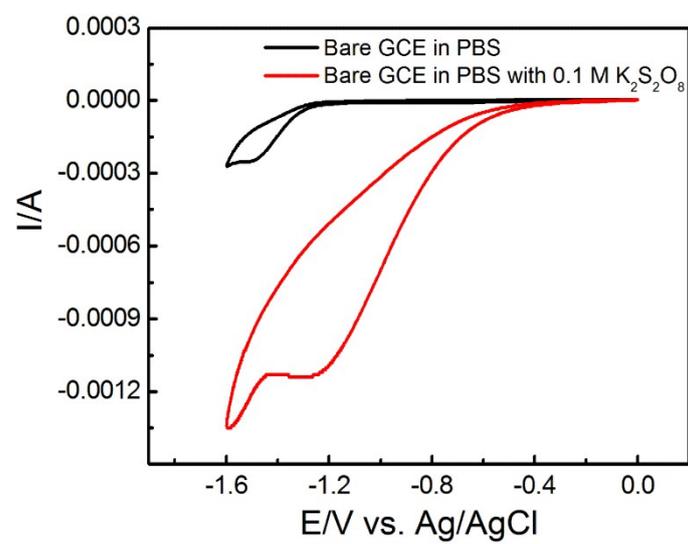


Fig. S1 The CV of bare GCE in PBS solution (dark line) and in 0.1 M PBS solution with 0.1 M K₂S₂O₈.

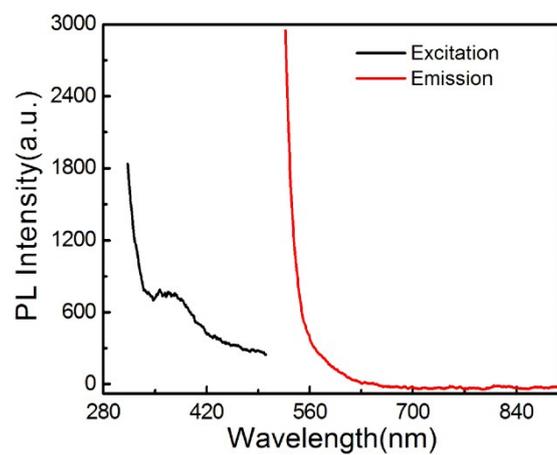


Fig. S2 the PL excitation and emission spectra of DMF solution. The excitation wavelength was at 493 nm.

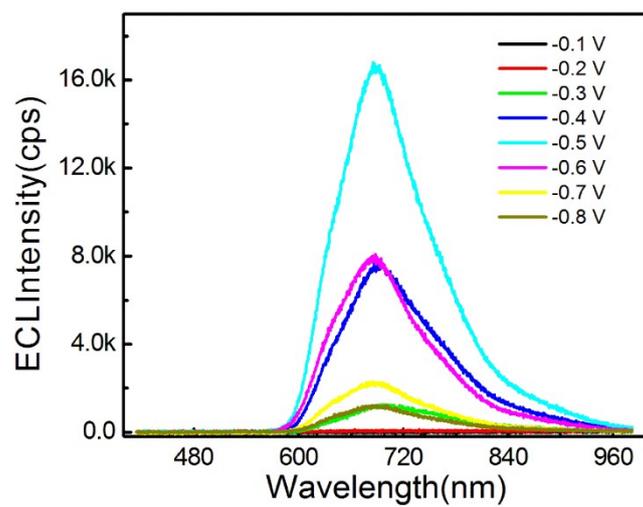


Fig. S3 the ECL spectra of PDI-CH₃ at different potential with potential scanning from 0 to -0.8 V.

The FTIR spectra of GO, GO/PDI-CH₃ were also recorded in Fig. S4 B. The FTIR spectra of GO showed characteristic peak at 1639 cm⁻¹ of $\nu(\text{C}=\text{C})$ and wide peak at 3225 cm⁻¹ of $\nu(\text{O}-\text{H})$ (Fig. S4 B, dark line). The characteristic peaks at 1593, 1698 and 1664 cm⁻¹ were assigned to $\nu(\text{C}=\text{C})$, $\nu(\text{C}=\text{O})$ and $\nu(\text{N}-\text{C}=\text{O})$, respectively. In addition, there is a characteristic peak at 1379 cm⁻¹ (Fig. S4 B, red line). And these characteristic peaks of PDI-CH₃ were found in the FTIR spectra of GO/PDI-CH₃ demonstrating the formation of GO/PDI-CH₃ composites(Fig. S4 B, green line). These characteristic peaks were summarized in Table S1.

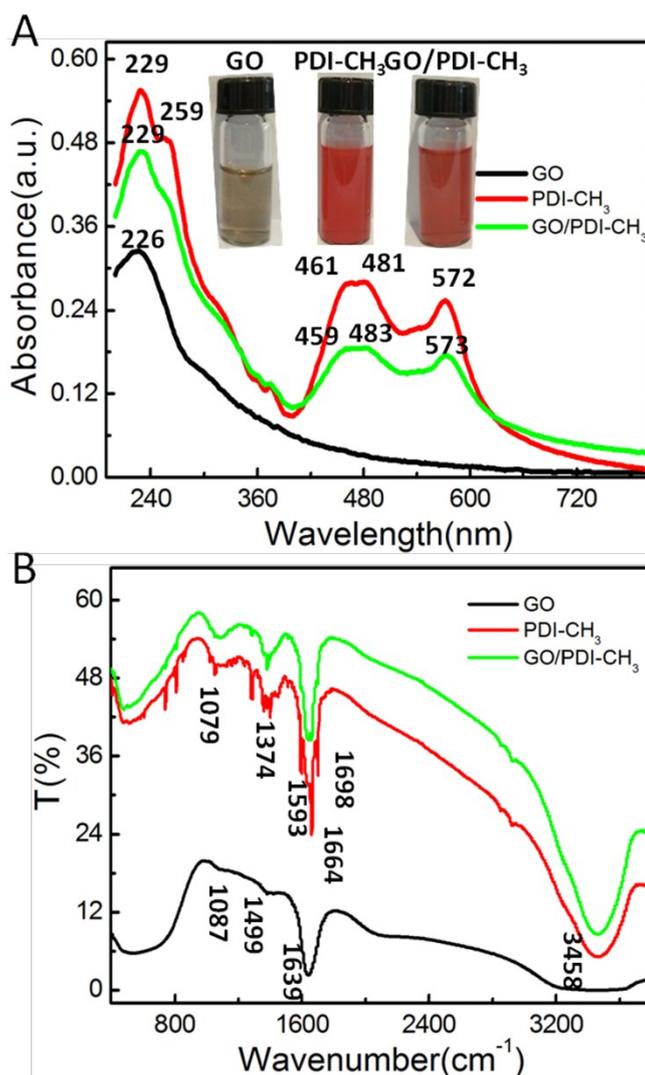


Fig. S4 A the UV-vis spectra of GO, PDI-CH₃ and GO/PDI-CH₃; B the FTIR spectra of GO, PDI-CH₃ and GO/PDI-CH₃.

Table S1. The IR characteristic peaks of GO, PDI-CH₃ and GO/PDI-CH₃

| | $\nu(\text{O}-\text{H})/\text{cm}^{-1}$ | $\nu(\text{C}=\text{C})/\text{cm}^{-1}$ | $\nu(\text{C}=\text{O})/\text{cm}^{-1}$ | $\nu(\text{N}-\text{C}=\text{O})/\text{cm}^{-1}$ | $\delta(\text{sCH in } -\text{CH}_3)$ |
|------------------------|---|---|---|--|---------------------------------------|
| GO | 3225 | 1639 | - | - | - |
| PDI-CH ₃ | - | 1593 | 1698 | 1664 | 1379 |
| GO/PDI-CH ₃ | - | 1635 | 1695 | 1663 | 1374 |

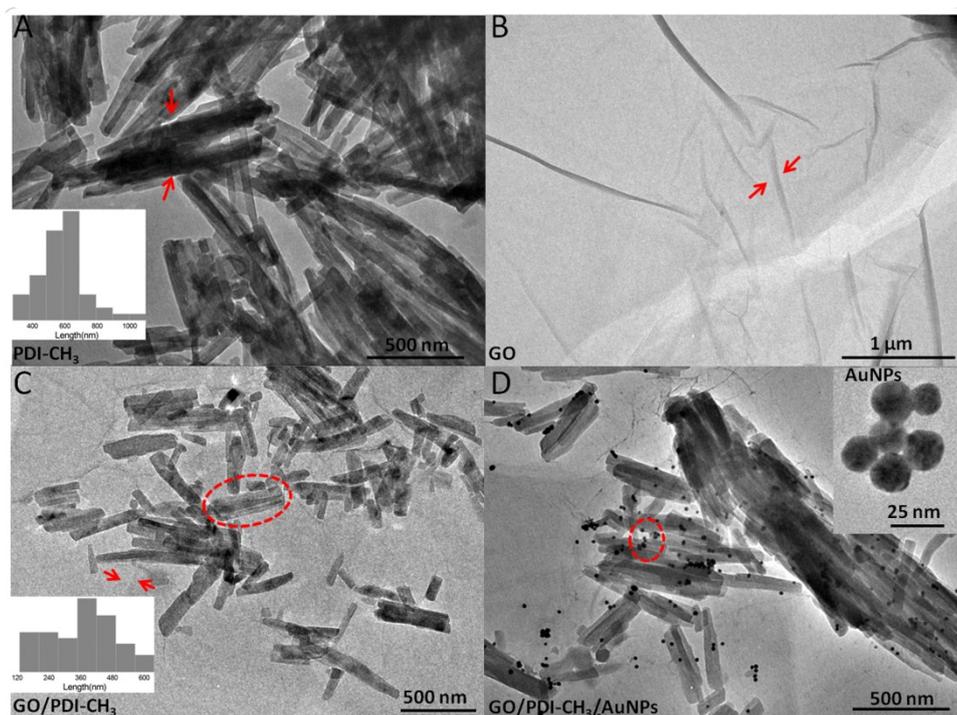


Fig. S5 the TEM images of PDI-CH₃ (A), GO(B), GO/PDI-CH₃ (C) and GO/PDI-CH₃/AuNPs(D). Inset: the size distribution of PDI-CH₃(A) and PDI-CH₃ on GO nanosheet(C).

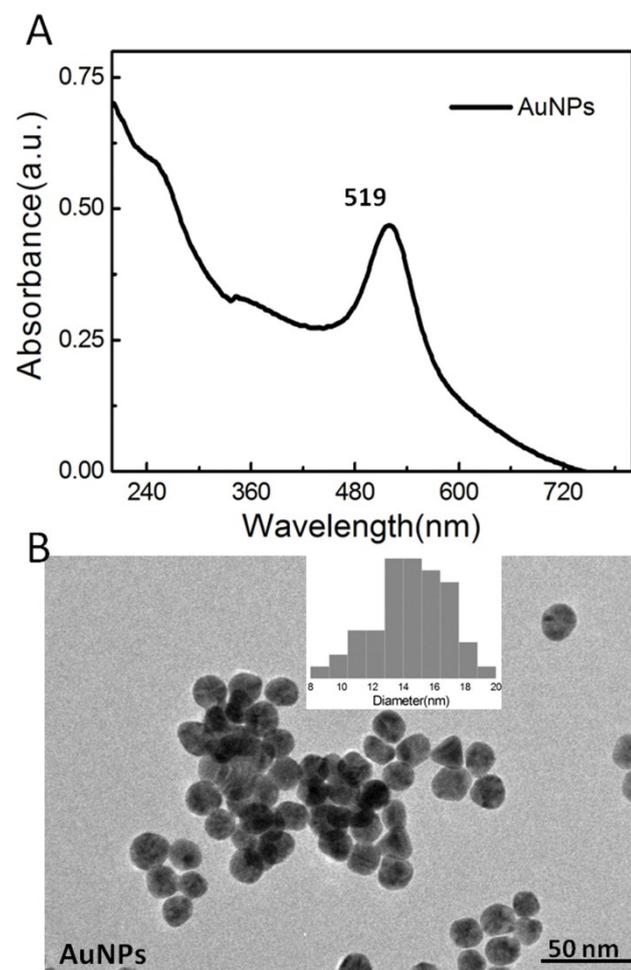


Fig. S6 A the UV-vis spectra of AuNPs; B the TEM image of AuNPs. Inset: the size distribution of AuNPs.

Table S2 Comparison of the proposed ECL nanomaterial with others for CEA detection using ECL immunoassay.

| Immunosensor | dynamic range (ng/mL) | Detection limit (ng/mL) | Detection method | References |
|--|---------------------------------------|-------------------------|------------------|--------------------------|
| Ru@SiO ₂ -AuNPs | $5.00 \times 10^{-6} - 0.0500$ | 1.52×10^{-6} | ECL | Wang et al ¹ |
| anti-CEA-Au NCS-TAEA-Pd@CuO | $1.00 \times 10^{-4} - 1 \times 10^2$ | 1.6×10^{-5} | ECL | Zhou et al ² |
| g-C ₃ N ₄ -Ab ₂ | $1.00 \times 10^{-4} - 1$ | 3×10^{-6} | ECL | Jin et al ³ |
| Ag@GO-PEI@Ab ₂ | $1.00 \times 10^{-4} - 1 \times 10^2$ | 2.7×10^{-5} | ECL | Li et al ⁴ |
| PDDA-rGO/ZnSe@ZnS QDs-Ab ₂ | $1.00 \times 10^{-4} - 1 \times 10^2$ | 2.9×10^{-5} | ECL | Zhang et al ⁵ |
| Ru@SiO ₂ -cDNA-Fe ₃ O ₄ @Au | $1.00 \times 10^{-5} - 10$ | 3.5×10^{-6} | ECL | Jie et al ⁶ |
| PEI-GO-CQDs/AuNPs/Ab ₂ | $5.00 \times 10^{-3} - 5 \times 10^2$ | 1.67×10^{-3} | ECL | Li et al ⁷ |
| Gr-IL/pPt | $10^{-9} - 1$ | 3×10^{-10} | ECL | Wang et al ⁸ |
| GO/PDI-CH ₃ /AuNPs/Ab ₂ | $1.00 \times 10^{-6} - 1 \times 10^3$ | 2.9×10^{-7} | ECL | This work |

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