Electronic Supplementary Information for:

Graphene Oxide/Perylene-Aniline Electrochemiluminescence Platform for Protein Detection Based on Molecule Recognition

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EXPERIMENTAL SECTION

Materials

5'-AAAAAAAAAAAAAAAAAAAA-folic acid-3' (FA-Poly-dA-ssDNA) was purchased from Takara Biotechnology (Dalian) Co., Ltd. Folate receptor (FR) was purchased from Beijing Biosynthesis Biotechnology Co., Ltd. Carcinoembryonic antigen (CEA) from human was purchased from Shanghai LincBio Science. Graphene oxide(GO) was purchased from Shandong Leader Nano Tech. Co., Ltd. Perylene-3, 4, 9, 10-tetracarboxylic dianhydride (PTCDA, 97%), $HAuCl_4 \bullet 3H_2O$, Tween 20, uric acid (UA) and bovineserum albumin (BSA) were obtained from Sigma–Aldrich. Thrombin(Thr) and cholesterol were obtained from Shanghai Macklin Biochemical Co., Ltd. Aniline(An) was purchased from Aladdin Industry Corporation. Ultrapure water (>18 MΩ) was from a Milli-Q Plus system (Millipore). Human serum samples were from Liaocheng People's Hospital.

Instruments

Electrochemiluminescence(ECL) experiments were performed on Xi'an Remex analyzer RFL-1 with the auxiliary equipment of Shanghai CHI 990B electrochemical workstation. The voltage of the photomultiplier tube was set at 800 V and cyclic voltammetry(CV) scanning potential was set from -0.1 V to -1.9 V. The ECL spectra were recorded on a homemade ECL spectrometer consisted by an Acton SP2300i monochromator with a PyLoN 400BR-eXcelon digital CCD detector (Princeton Instruments, U.S.A) under CV scanning potential from -0.1 V to -1.9 V on CHI 990B. The three-electrode system was glassy carbon electrode glassy carbon electrode(GCE) (3-mm diameter), Ag/AgCl (KCl-saturated) as reference electrode and platinum wire as counter electrode. CV and linear scanning voltammetry(LSV) were performed on CHI 990B. Electrochemical impedance spectroscopy(EIS) were recorded on CHI 760C. Transmission electron microscope (TEM) were recorded on JEM-2100 electron microscope operating at 200 kV (JEOL Ltd., Japan). X-ray photoelectron spectroscopy (XPS) was measured on a Thermo Escalab Xi^+ Spectrometer with a monochromated Al Kα X-ray resource, and C 1s at 284.4 eV was used as a reference for all binding energies. Ultraviolet–visible(UV–vis) absorption spectra were recorded on Lambda

750 spectrophotometer (Perkin Elmer, USA).

Preparation of PTCDA-An and GO/PTCDA-An.

The supramolecular nanorods (PTCDA-An) were prepared through hydrogen bonding by using 3,4,9,10-perylenetetracarboxylic dianhydride (PTCDA) and aniline based on a previous report. First, 10 mg PTCDA was dissolved in 5.0 mL acetone, then 1.0 mL of 99.9% aniline solution was added into the above solution with vigorous stirring for a week. Then the above mixture was centrifuged and the precipitate was washed with anhydrous ethanol and deionized water. At last, the resulted red product of PTCDA-An was dispersed homogeneously in 20.0 mL deionized water and stored in the refrigerator at 4 ℃ for further use.

For preparing GO/PTCDA-An nanomaterial, 1 mL PTCDA-An solution and 1 mL of 1.8 mg/mL GO were put into the beaker and stirred for 12 hours at 35 °C. Then the resultant solution was centrifuged and dispersed in 2.0 mL ultrapure water for further use.

Construction of ECL biosensor for FR detection.

First, 8 μL GO/PTCDA-An solution was dropped onto the GCE surface to get GCE/GO/PTCDA-An surface. After dried, the electrode was washed with ultrapure water. Then 5 μL of 1 μM L^{-1} FA-Poly-dA-ssDNA solution was added on the modified electrode surface and kept 12 h at 4 °C. After washed with ultrapure water, the GCE/GO/PTCDA-An/FA-Poly-dA-ssDNA was incubated in 1 mL 0.1% Tween 20 solution for 5 min to avoid the nonspecific binding of proteins and washed with ultrapure water. Finally, the modified electrodes were immersed into a series of FR solution at different concentration for 3 hours at 37 °C to get GCE/GO/PTCDA-An/FA-Poly-dA-ssDNA/Tween 20/FR and stored at 0 °C for ECL measurement.

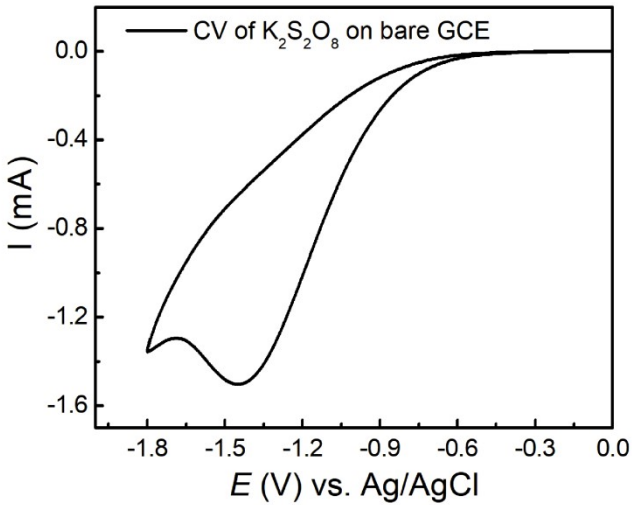

Fig. S1 The CV of bare GCE in 0.1 M PBS solution(pH 7.4) with 0.1 M $K_2S_2O_8$. The scan rate was at 0.1 V/s.

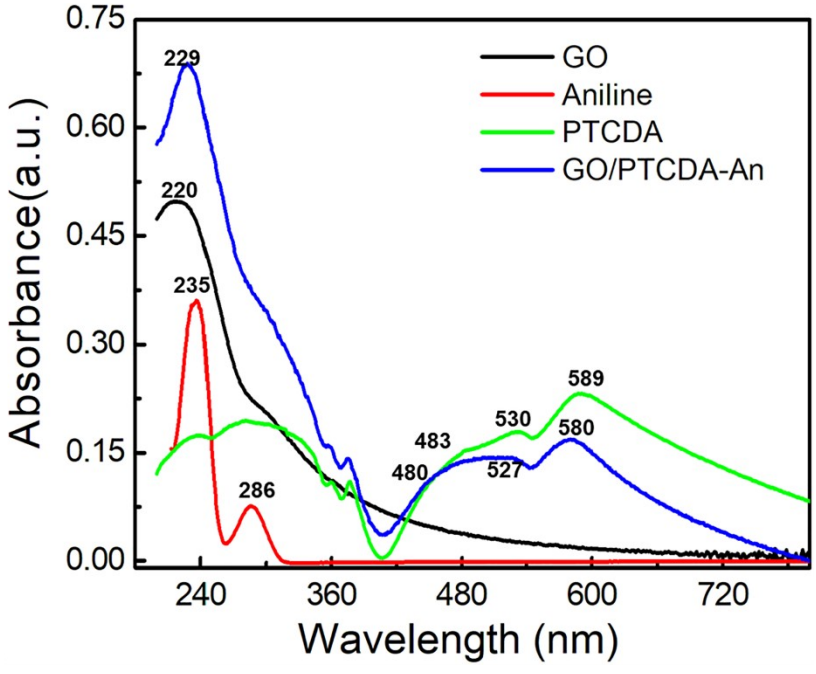

Fig. S2 The UV-vis absorption spectroscopy of GO(black line), aniline(red line), PTCDA (green line) and GO/PTCDA-An(blue line).

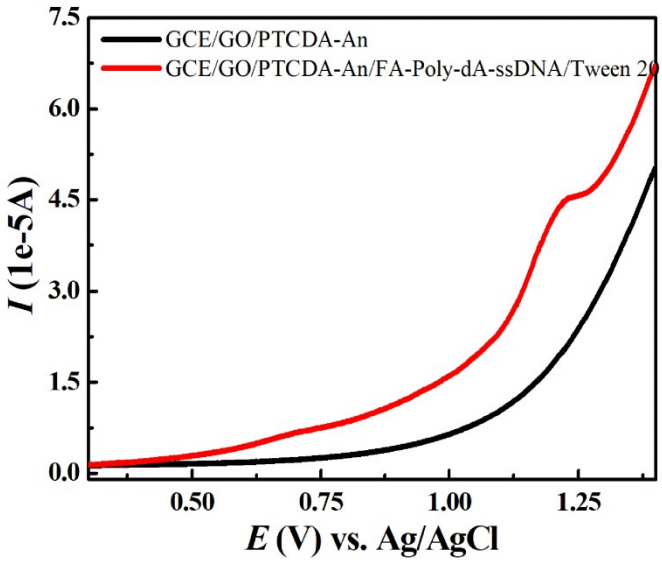

Fig. S3 LSV of GCE/GO/PTCDA-An and GCE/GO/PTCDA-An/FA-Poly-dA-ssDNA/Tween 20 in 0.1 M PBS(pH 7.40).

Table S1 Comparation of the proposed ECL FR sensor with other FR sensors

Interface	Linear range	Detection limit	Methods	Nuclease	References
capture DNA/HCR-assisted formation of copper nanoparticles	0.01-100 ng/mL	0.003 ng/mL	EC	exonuclease I	*Zhao etal., Biosens. Bioelectron. (2015)*[1]
CuNPs-coated DNA/magnetic graphene	0.01–100 ng/mL	7.8pg/mL	DPV	exonuclease III	*Zhao etal., Analyst (2015)*[2]
Ag nanoclusters-coated DNA/SWCNTs	0.1–3 ng/mL	33 pg/mL	Fluorescence	exonuclease I	*Jiang etal., Chem. Commun. (2015)*[3]
Au/rGO-FA	1–200 pM	1pM	DPV	without	*He etal., Biosens. Bioelectron. (2016)*[4]
CVD graphene/FA	5-500 fM	5 fM	SPR	without	*He etal. Biosens. Bioelectron. (2017)*[5]
GO/PTCDA-An/FA-Poly-dAssDNA/Tween 20	10^{-6}-1 ng/mL	6.36×10^{-7} ng/mL	ECL	without	This work

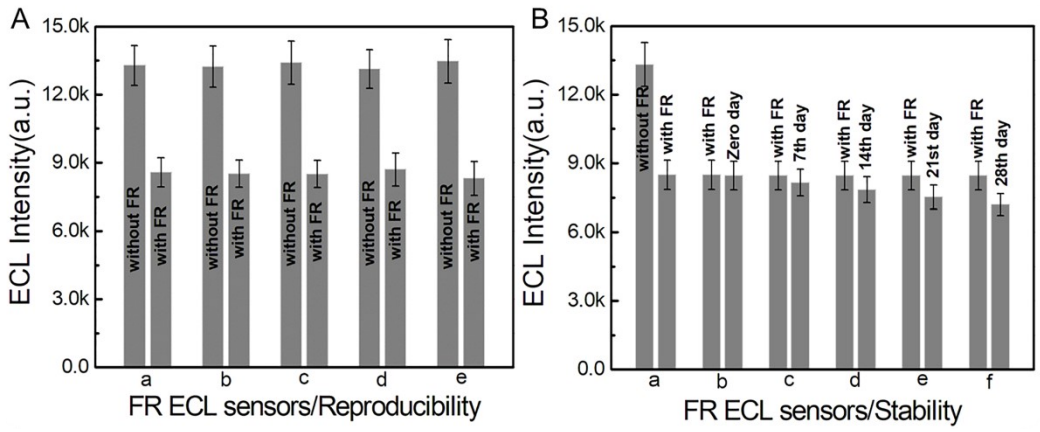

Fig. S4 A. reproducibility of FR sensor from (a–e) without FR (0 pg mL^{-1}) and with FR (1 pg mL^{-1}) and and B. Long-term stability where pair of the bars shows ECL intensity: a. without FR (0 pg mL^{-1}) and with FR (1 pg mL^{-1}), b. with FR (1 pg mL^{-1}) and with FR (1 pg mL^{-1}) zero-day, c. with FR (1 pg mL^{-1}) on zero-day and with FR (1 pg mL^{-1}) on 7th day, d. with FR (1 pg mL^{-1}) on zero-day and with FR (1 pg mL^{-1}) on 14th day, e. with FR (1 pg mL^{-1}) on zero-day and with FR (1 pg mL^{-1}) on 21st day, and f. with FR (1 pg mL^{-1}) on zero-day and with FR (1 pg mL^{-1}) on 28th day.

Table S2 Assay results of FR in serum samples by using the proposed method

Samples	Standard FR concentration (pg/mL)	FR concentration detected by proposed method (pg/mL)	Recovery rate (%)	RSD (%)
1	800	820.00	102.5	4.94
2	500	520.00	104.0	1.58
3	0.80	0.87	108.8	3.98
4	0.50	0.48	96.0	3.66

References

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