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Electronic Supplementary Information

A Graphene Oxide Platform for the Assay of Biomolecules Based on Chemiluminescence Resonance Energy Transfer

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Experimental Details.

Chemicals and Apparatus. Thrombin was purchased from Calbiochem (USA). Luminol standard powder was obtained from Acros Organcis (Belgium), which was prepared into stock solution (1.0×10^{-2} M) with 0.1 M NaOH solution and stored in dark. HRP was ordered from Sigma. Double-distilled, deionized water was used throughout the experiments. All regents were of analytical grade and used without further purification. The oligonucletides were synthesized and purified by Sangon Biotechnology Co., Ltd. (Shanghai, China). Their sequences are listed in Table S1.

Table S1. DNA Sequence Used in This Work

Oligonucleotides name	Sequences (5′ to 3′)	Description
DNA probe	FAM-AGTCAGTGTGGAAAATCTCTAGC	FAM = fluorescein-based dye
complementary DNA target (cDNA)	GCTAGAGATTTTCCACACTGACT	from the HIV-1 U5 long terminal repeat sequence
single-base mismatched DNA target	GCTAGAGATTGTCCACACTGACT	mismatch underlined
noncomplementary DNA target (noncDNA)	TGAGGTAGTAGATTGTATAGTTA	
aptamer probe	FAM-GGTTGGTGTGGTTGG	specific recognition for thrombin

The chemiluminescence (CL) measurements were performed with a BPCL ultraweak luminescence analyzer (Institute of Biophysics Academic Sinica, Beijing, China). The chemiluminescence spectra and UV-Vis spectra were taken on a F-4500 fluorescence spectrophotometer (HITACHI, Japan) and a Cary 50 UV-Vis-NIR spectrophotometer (Varian, USA), respectively.

DNA Detection. A GO solution (50 μg/mL) was mixed with FAM-labeled DNA probe (1.0×10^{-7} M) for 30 min, followed by the addition of DNA target (H1V1) at a different concentration. After allowing this mixture to hybridize for 30 min at 37 ^{o}C, the resulting solution was decanted to the quartz cuvette containing luminol (6.67×10^{-5} M) and HRP (2.5×10^{-7} M). The CL reaction was triggered by injecting 100 μL of H_2O_2 (2.0×10^{-3}M) after the CL analyzer began to record at 10 s, followed by recording the CL kinetics until 30 s. The peak height of the total emission was measured by means of a photon counting unit and taken to obtain the calibration curve versus the concentration of target DNA.

Thrombin Detection. A GO solution (50 μg/mL) was mixed with FAM-labled aptamer probe (1.0×10^{-7} M) for 30 min, followed by the addition of thrombin at a different concentration. After allowing

this mixture to incubate for 30 min at 37 °C to make the aptamer change its structure to bind thrombin, the sample was detected on a CL analyzer as the operations of DNA detection.

UV-vis Absorption Characterization. We used UV-vis absorption to characterize the ssDNA, GO and ssDNA-GO complex. From Fig. S1, a characteristic absorption peak of ssDNA appeared at ~260 nm (a). The UV-vis absorption spectrum of GO displays two characteristic absorption peaks, a strong maximum absorption at 230 nm and a shoulder around 290 nm, corresponding to π–π* transition of aromatic C=C bonds and *n*–π* transition of the C=O bond, respectively (b).[S1] After ssDNA absorbs on GO, a distinct absorption at ~235 nm is observed (c). The slightly red-shifted (~5 nm) is relative to GO due to the change of the GO electronic ground state induced by DNA conjugation, indicating that ssDNA probes are successfully assembled on the surface of GO via hydrophobic and π-π stacking interactions between the nucleobases and GO.[s2]

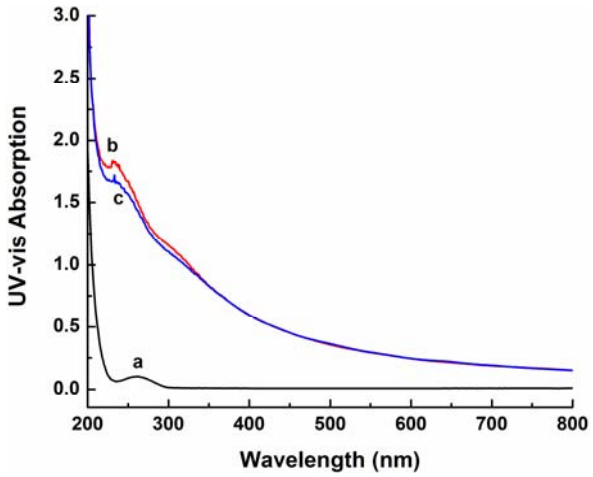

Fig. S1 UV-Vis absorption spectra of ssDNA (a), GO (b), and probe DNA-GO complex (c).

Sensitivity for DNA Detection by Using CNT. We compared the analytical performances of GO, graphene and CNT for DNA detection based on CRET system to explore the potentiality of these carbon nanomaterials in bioanalysis. From the results, there was no difference between GO and graphene in sensitivity for DNA detection, both of which achieved the detection limit as low as 0.1 pM. Considering the superior properties of GO compared with graphene such as hydrophilicity,

controllable electronic properties and so on,[S3] we use GO in this assay. Moreover, only a detection limit of 10.0 pM H1V1 is obtained by using CNT, which is two orders of magnitude higher than that based on GO (Fig. S2). The excellent performance of GO in this assay could be ascribed to the unique structure and electronic properties of GO.[S4] On one hand, as a single-atom-thick and two-dimensional carbon material, GO has a relatively weaker random binding ability of ssDNA compared with one-dimensional CNT, which further makes the folding of ssDNA more favorable on GO sheet surface than on CNT. Thus, probe DNA on GO surface are more sensitive to target DNA in this assay for CRET responses. On the other hand, the higher quenching efficiency of GO than CNT leads to the low background of signals, which also results in the high sensitivity.

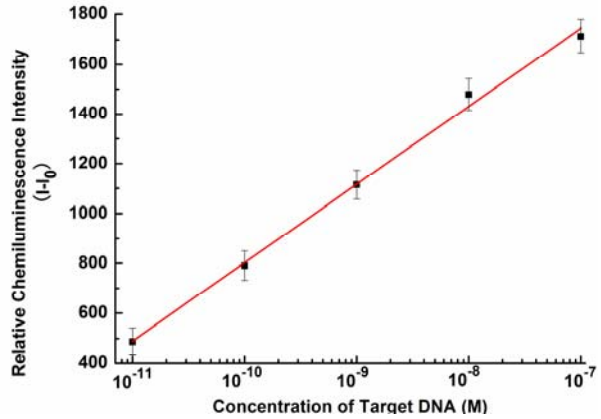

Fig. S2 Calibration curve for target DNA detection based on the proposed CRET system by using CNT with the concentration of 10^{-11}, 10^{-10}, 10^{-9}, 10^{-8}, and 10^{-7} M H1V1. Relative CL intensity ($I - I_0$) is calculated by $I - I_0$, where I_0 and I are the CL intensity without and with H1V1, respectively. The concentration of DNA probe is 1.0×10^{-7} M. Error bars are standard deviation of three repetitive measurements.

Selectivity of the Proposed GO-CRET Platform for DNA Detection. The selectivity of the present CRET strategy by using GO for DNA detection was also investigated by using probe DNA (1.0×10^{-7} M) to hybridize with 1.0×10^{-11} M of completely complementary target DNA (cDNA), single-base mismatched DNA and noncomplementary DNA (noncDNA), respectively. From Fig. S3, a well-defined CL signal was obtained for the cDNA, while the CL intensity for single-mismatched

sequence was weaker than that of the cDNA, and the noncDNA showed nearly no response.

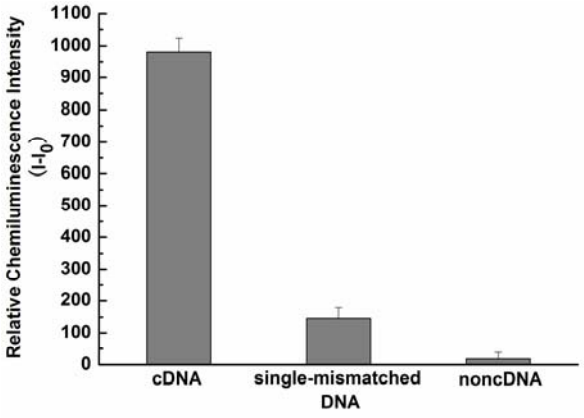

Fig. S3 Selectivity of the assay for DNA detection by hybridizing probe DNA with different target DNA: completely complementary target DNA (cDNA, H1V1), single-base mismatched DNA and noncomplementary DNA (noncDNA), respectively. Relative CL intensity ($I - I_0$) is calculated by $I - I_0$, where I_0 and I are the CL intensity without and with corresponding target. The concentration of DNA probe and each target are 1.0×10^{-7} M and 1.0×10^{-11} M, respectively. Error bars are standard deviation of three repetitive measurements.

Selectivity of the Proposed GO-CRET Platform for Thrombin Detection.

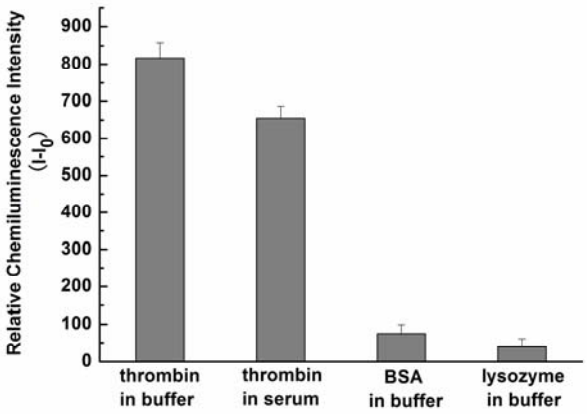

Fig. S4 Selectivity of the assay for thrombin detection in PBS buffer over BSA and lysozyme in the same buffer, and thrombin spiked in blood serum (all at a concentration of 1.0 nM). Relative CL intensity ($I - I_0$) is calculated by $I - I_0$, where I_0 and I are the CL intensity without thrombin and with thrombin, BSA, and lysozyme, respectively. The concentration of aptamer probe is 1.0×10^{-7} M. Error

bars are standard deviation of three repetitive measurements.

Notes and references

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S4 H. Chang, L. Tang, Y. Wang, J. Jiang, J. Li, *Anal. Chem.*, 2010, **82**, 2341-2346.