Supplementary information for

Chemiluminescence Biosensor for DNA Detection Using Graphene Oxide and Horseradish Peroxidase-Mimicking DNAzyme

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Experimental section

Materials

Graphene oxide (GO) was purchased from Sinocarbon Materials Technology Co., Ltd. Luminol were purchased from Sigma-Aldrich (St. Louis, MO, USA). Hemin and 30% H_2O_2 was purchased from Sinopharm Group Chemical Reagent Co., Ltd (Shanghai, China). HEPES was purchased from Sangon Biotechnology Co., Ltd (Shanghai, China). The stock solution of hemin (5 mM) was prepared in DMSO, stored in the dark at -20°C.The stock solution of luminol (10 mM) was prepared in 0.1 M NaOH and stored in dark.

All oligonucleotide with different sequences were synthesized and HPLC purified by Sangon Biotechnology Co., Ltd (Shanghai, China). The sequences of the oligonucleotide

used in this work are as follows:

(1) 5'-GGG TTG GGC GGG ATG GGT TTT GCA TCC AGG TCA TGT TA-3' (probe P_{HIV});

(2) 5'-AGA AGA TAT TTG GAA TAA CAT GAC CTG GAT GCA-3' (complement target HT);

(3) 5'-AGA AGA TAT TTG GAA TAA CAT GAC TTG GAT GCA-3' (single base mismatched target MT);

(4) 5'-TTG GCT TTC AGT TAT ATG GAT GAT GTG TCT GTA-3' (non-complement target NT).

All other chemicals not mentioned here were of analytical-reagent grade or better. 18 MΩ water purified by a Milli-Q Academic purification set (Millipore, Bedford, MA, USA) was used throughout.

Instruments

A MPI-B flow injection chemiluminescence system (Xi'an Remex Electronic and technological Co., China) was used to record chemiluminescence (CL) emission. Absorption spectra were recorded on a UV-2550 spectrometer (Shimadzu, Japan). Atomic force microscopic (AFM) images were taken using a Nanoscope IV multimode atomic force microscopy (Veeco Instruments, USA) in tapping mode.

Preparation of the HRP-mimicking DNAzyme-modified probe (P_{HIV})

The probes were prepared according to reference with a little modification.[1] Briefly, 1 OD of DNAs were prepared in the TE buffer (10 mM Tris-HCl, 0.1 mM EDTA, pH 7.4), and heated at 88°C for 8 min to dissociate any intermolecular interaction, then gradually

cooled to room temperature. An equal volume of the hybridization buffer (50 mM HEPES, pH 7.4, 40 mM KCl, 400 mM NaCl, 0.1% Triton X-100, 2% DMSO) was added, and allowed DNA sequences to fold for 40 min at room temperature. Then, an equal volume of hemin solution of same molar concentration was added and incubated at room temperature for over 3h to form the corresponding G-quadruplex-based DNAzymes.

Procedure for CL detection

The schematic diagram of the flow system employed for the CL detection is shown in Fig. S2. A peristaltic pump was used to deliver flow, and PTFE tubing (0.8 mm i.d.) was used as connection material in the flow system. In a typical measurement, the luminol solution mixed firstly with H_2O_2 in 3 mM HCl solution. The acidic conditions were used to stabilize the H_2O_2 solution. Then, the sample solution was injected into the carrier stream (water) using an eight-way injection valve equipped with a 75 μL sample loop, and the mixture of sample, luminol and H_2O_2 solution finally reached detector to produce CL signals.

Explore the possible mechanism

For the experiment of GO reducing CL emission, the various amounts of GO were added to the sample solution (P_{HIV}, 2 nM; in 20 mM Tris-HCl, 0.4 M NaCl, pH 7.4). The flow rate was 3.2 mL min^{-1} for all lines. After incubating 15 min, the mixture was introduced to the flow injection system, and the CL signal was recorded.

To explore the possible mechanism, the CL emission of the sensing system was measured in different conditions: solution a, 0.125 μM P_{HIV}; solution b, 0.125 μM P_{HIV} + 0.005 mg mL^{-1} GO; solution c, 0.125 μM P_{HIV} + 0.01 mg mL^{-1} GO, the solutions were

incubated in Tris-HCl buffer solution (20 mM, pH 7.4, 0.4 M NaCl) at room temperature. After incubating 15 min, the CL emission of the mixture is measured using the flow injection system. For UV-visible absorption spectra measurement: solution a, 0.125 μM P_{HIV}; solution b, 0.125 μM P_{HIV} + 0.005 mg mL^{-1} GO; solution c, 0.125 μM P_{HIV} + 0.01 mg mL^{-1} GO (detection was repeated three times). After incubating 15 min, the solutions were incubated with [$ABTS^{2-}$] = 3 mM, [H_2O_2] = 3 mM, in HEPES buffer (25 mM, pH = 8.5).

Procedure for DNA detection

The P_{HIV} were hybridized with targets in Tris-HCl buffer solution (20 mM, pH 7.4, 0.3 M NaCl) for 10 min at room temperature. 4 μL of 0.04 mg mL^{-1} GO is added to the hybridization solution (50 μL) and an additional 446 μL of the Tris-HCl buffer solution (20 mM, pH 7.4, 0.4 M NaCl) is added immediately. After incubating 15 min, the CL emission of this mixture is measured using the flow injection system. The flow rate was 3.2 mL min^{-1} for all lines.

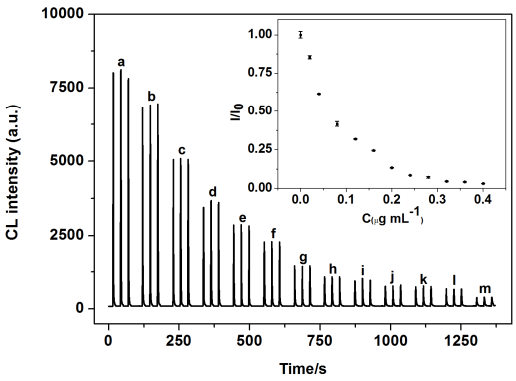


Fig. S1 Flow injection chemiluminescence (FI-CL) signals of the sensing system including P_{HIV} (2 nM) at different concentrations of GO: (a) 0; (b) 0.02; (c) 0.04; (d) 0.08; (e) 0.12; (f) 0.16; (g) 0.20; (h) 0.24; (i) 0.28; (j) 0.32; (k) 0.36; (l) 0.40 μg mL^{-1}; (m) buffer solution (detection was repeated three times). Inset: Plot of normalized CL intensity vs. the concentration of GO. I_0 and I are CL intensities in the absence and the presence of GO, respectively. Experimental conditions: luminol, 0.5 mM; H_2O_2, 30 mM.

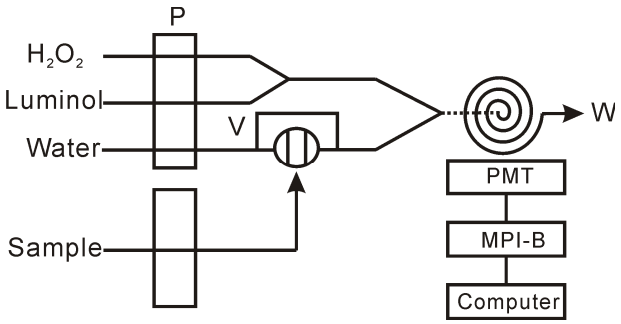
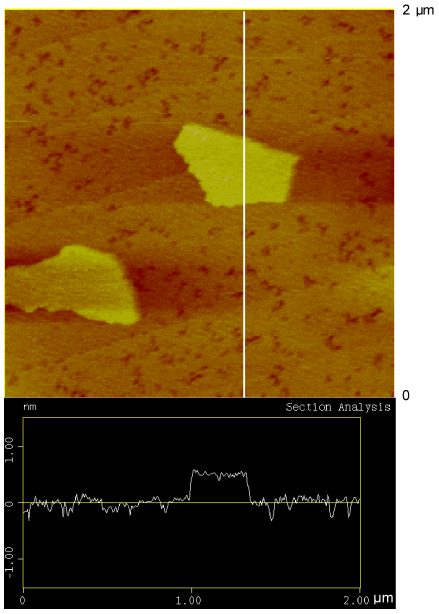
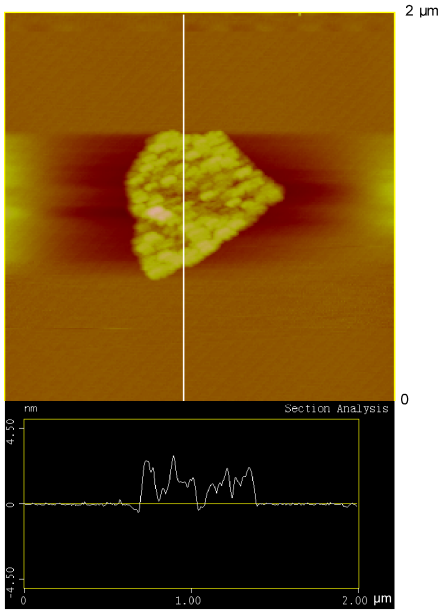


Fig. S2 Schematic diagram of FI-CL detection system. P, peristaltic pump; V, injection valve; C, flow cell; PMT, photomultiplier tube; MPI-B, luminescence analyzer; W, waste.



(a)



(b)

Fig. S3 AFM height image of GO sheets deposited on mica substrates (a); AFM height image of P_{HIV}-GO complex (b).

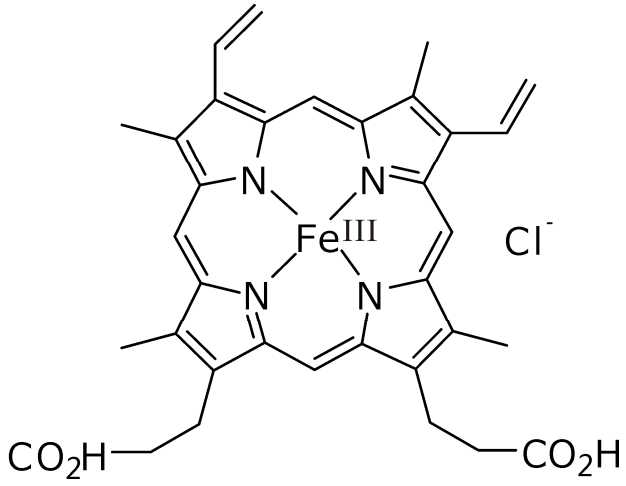


Fig. S4 Molecular structure of Hemin.

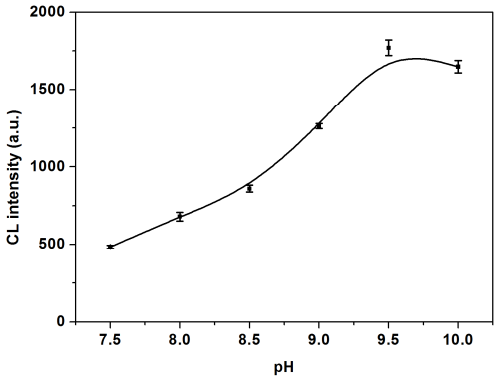


Fig. S5 CL intensity vs. pH. Experimental conditions: luminol, 0.5 mM; H_2O_2, 30 mM; P_{HIV}, 0.5 nM.

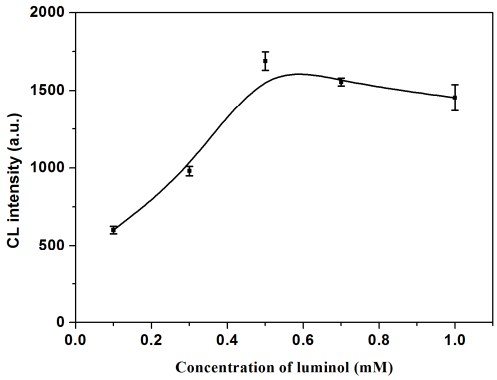


Fig. S6 CL intensity vs. the concentration of luminol. Experimental conditions: H_2O_2, 30 mM; P_{HIV}, 0.5 nM; pH, 9.5.

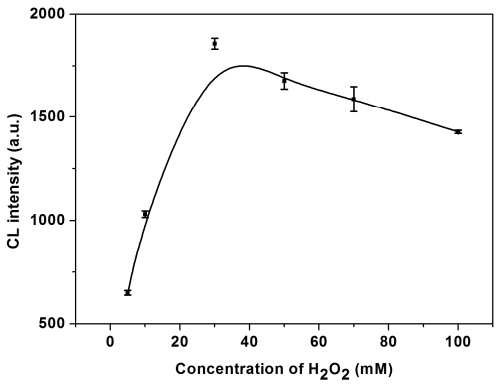


Fig. S7 CL intensity vs. the concentration of H_2O_2. Experimental conditions: luminol, 0.5 mM; P_{HIV}, 0.5 nM; pH, 9.5.

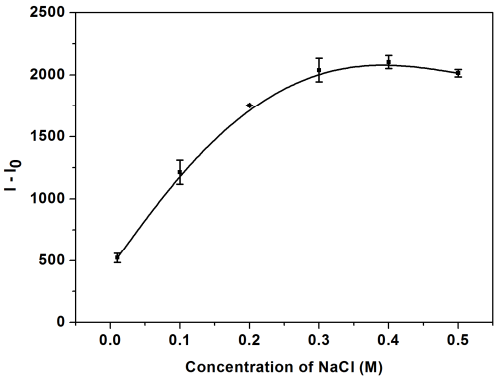


Fig. S8 Effects of the concentration of NaCl on DNA sequence detection. I_0 and I are CL intensities in the absence and the presence of HT, respectively. Experimental conditions: P_{HIV}, 2 nM; HT, 3 nM; GO, 0.32 μg mL^{-1}; luminol, 0.5 mM; H_2O_2, 30mM.

Table S1 Comparisons of the sensitivity of several analytical methods for DNA detection.

Type	Sensitivity	Detection scheme	Reference
DNAzyme-based DNA detection	1 nM	chemiluminescence	*Anal. Chem*., 2004, 76, 2152-2156
DNAzyme-based DNA detection	100 pM	chemiluminescence	*Nano Lett.*, 2004, 4, 1684-1687
DNAzyme-based DNA detection	10 nM	fluorescence	*J. Am. Chem. Soc.*, 2011,133, 11597-11604
GO-based DNA detection	10 nM	fluorescence	*Angew. Chem., Int. Ed.,* 2009, 48, 4785 –4787
GO-based DNA detection	100 pM	fluorescence	*Adv. Funct. Mater.,* 2010, 20, 453–459
GO-based DNA detection	12 nM	fluorescence	*Anal. Chem.,* 2010, 82, 5511–5517
GO-based DNA detection	34 pM	chemiluminescence	This work

1. I. Willner, V. Pavlov, Y. Xiao, R. Gill, A. Dishon and M. Kotler, *Anal. Chem.*, 2004, **76**, 2152-2156.