Electronic Supplementary Information (ESI)

Graphdiyne Oxide as a Platform for Fluorescent Sensing

Chunxia Wang, Ping Yu, Shuyue Guo, Lanqun Mao*, Huibiao Liu* and Yuliang Li*

Beijing National Laboratory for Molecular Sciences, Key Laboratory of Analytical Chemistry for Living Biosystems, Key Laboratory of Organic Solids, Institute of Chemistry, the Chinese Academy of Sciences, Beijing 100190, China.

* Corresponding authors. E-mails: lqmao@iccas.ac.cn, liuhb@iccas.ac.cn, ylli@iccas.ac.cn

Reagents and Methods

Chemical Reagents. All DNA oligonucleotides used in this study (Table S1) were synthesized and purified by Invitrogen Inc. (Beijing, China). BSA and thrombin was purchased from Sigma-Aldrich (Shanghai, China). HEPES buffer (10 mM, containing 75 mM NaCl, and 4.0 mM $MgCl_2$, pH 7.5) was used for DNA and thrombin assays. Aqueous solutions were prepared with Milli-Q water (≥18.2 MΩ·cm).

Materials Synthesis. Graphdiyne (GD) was synthesized on the surface of copper in the presence of pyridine via a cross-coupling reaction using hexaethynylbenzene as precursors, as reported previously.[S1] Briefly, to a 15 mL THF solution of 43.6 mg (0.066 mmol) hexakis[(trimethylsilyl)ethynyl]benzene was added 0.4 mL TBAF (1 M in THF, 0.4 mmol) and stirred at 8°C for 10 min. The bulk GD was grown on the surface of copper foil in the presence of pyridine through cross-coupling reaction of hexaethynylbenzene for 3 days at 60°C under nitrogen atmosphere. Upon completion the GD was scrapped from copper foils and sequentially washed with acetone, hot DMF (80°C) under sonication for 1 h to obtain black solid. After that the resulting black solid was refluxed at 100°C for 2 h in 4 M NaOH, 6 M HCl, and 4 M NaOH, sequentially, to remove other impurities. Finally, the black precipitate was washed with DMF (80°C) and hot ethanol (70°C) in turn to produce pure bulk GD and then followed by exfoliation under sonication for 10 days (12 h each day) to form an aqueous GD dispersion.

GD oxide was synthesized from GD powder by a modified Hummer's method.[S2] Briefly, GD powder (12 mg) and concentrated HNO_3 (1.2 mL), concentrated H_2SO_4 (3.6 mL) were placed in a flask under an ice-water bath, in which $KMnO_4$ (12 mg) was added under vigorous stirring. The resulting mixture was heated in an oil bath at 80 °C for 24 h to give a brown suspension. After cooling to room temperature, the mixture was neutralized to pH 8.0 with NaOH and centrifuged at 14000 rpm for 10 min to give black powder, followed by washing with Mili-Q water and re-dispersing into water under sonication for about 12 h to form a homogeneous brown aqueous solution for dialysis (cutoff, 3000) for 7 days and used for fluorescent analysis.

Fluorescent DNA Assays. In a typical procedure, P_{HCV} was dissolved in HEPES buffer to give a final concentration of 20 nM. Aqueous dispersion of GD oxide was then dispersed into the

solution to give a homogenous mixture (10 μg/mL), in which different concentrations of target HCV were added. The final concentrations of T_{HCV} in the resulting mixtures were 0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 1.0, 2.0, 5.0, 10, 20, 30, and 40 nM. The mixtures were incubated at 95 °C for 10 min in water bath and then gradually cooled to room temperature. The resulting mixtures were then allowed to stand by for 30 min prior to the fluorescence measurements.

Fluorescent Thrombin Assays. A 2 μL of FAM-TBA (10 μM) was mixed into 1 mL of HEPES buffer, in which 5 μL of GD oxide (1 mg/mL) was added. After that, 10 μL of different concentrations of thrombin was separately added to the above mixtures to give final concentrations of 0.0, 2.7, 5.4, 10.8, 13.5, 27, 54, 135, and 270 nM. The mixtures were incubated at 95 °C for 10 min and then gradually cooled to room temperature. Prior to the fluorescence measurements, the mixtures were allowed to stand by for 30 min.

Instrument and Characterization. Fluorescence experiments were carried out on an F-4600 fluorescence spectrophotometer (Hitachi, Japan) equipped with a Xenon lamp excitation source. UV-vis absorption spectra were recorded on a TU-1900 spectrophotometer (Beijing, China). Transmission electron microscopy (TEM) image was taken on a TEM-2100F microscopy (JEOL, Japan). Tapping mode atomic force microscopic (AFM) image was acquired on a Vecco Nanoscope III by directly casting the samples onto the surface of mica substrate. Zeta potential distribution was recorded on a Zetasizer (Nano-Z, Malvern, UK).

Supplementary Results

Table S1. Oligonucleotide Sequences Used

Oligonucleotides	Oligonucleotide Sequences (from 5' to 3')
P_{HCV}	5'-FAM-TAA ACC CGC TCA ATG CCT GGA-3
T_{HCV}	5'- TCC AGG CAT TGA GCG GGT TTA -3'
FAM-TBA	GGT TGG TGT GGT TGG
1ms	5'- TCC AGG CAT T*C*A GCG GGT TTA -3'
3ms	5'- TCC AGG CA*G* T*C*A G*G*G GGT TTA -3'
A21	5'- AAA AAA AAA AAA AAA AAA AAA -3'

Mismatched bases are highlighted in italic underlined type.

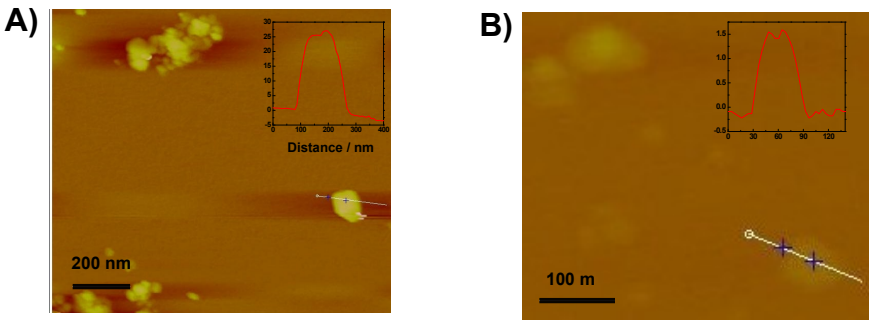

Fig. S1 AFM images of GD (A) and GD oxide (B).

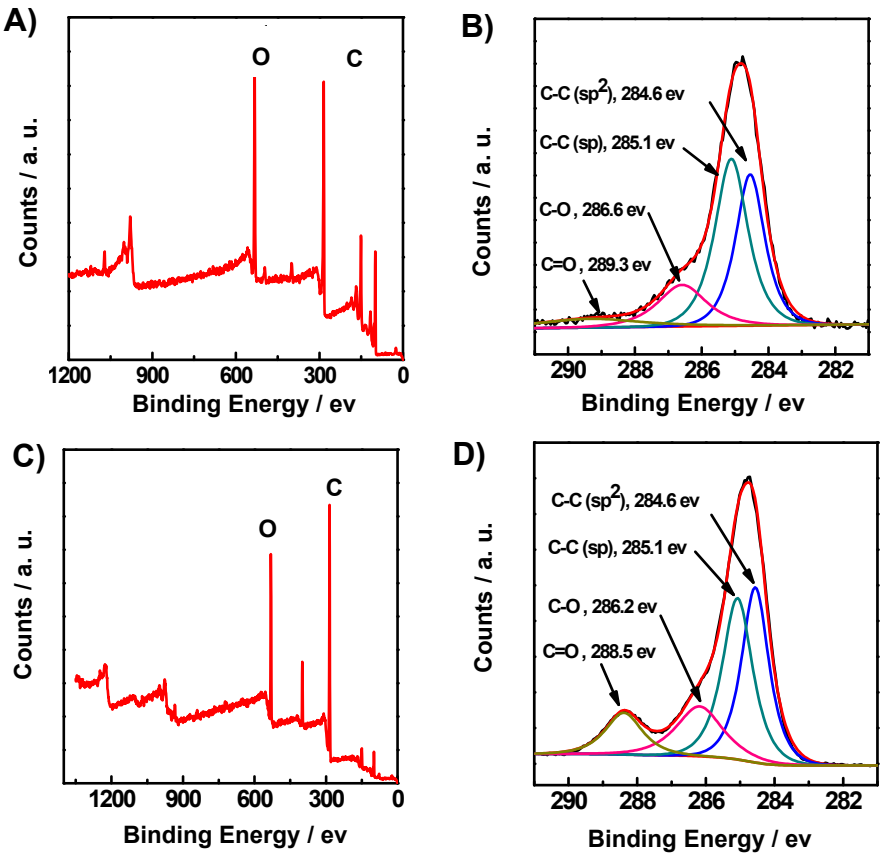

Fig. S2 (A) XPS spectra of GD. (B) XPS spectra for C1s of GD. (C) XPS spectra of GD oxide. (D) XPS spectra for C1s of GD oxide.

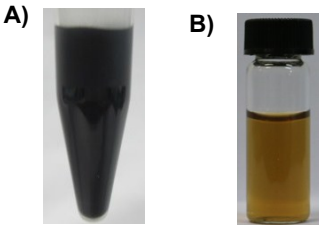

Fig. S3 Photograph of aqueous dispersions of GD (A) and GD oxide (B).

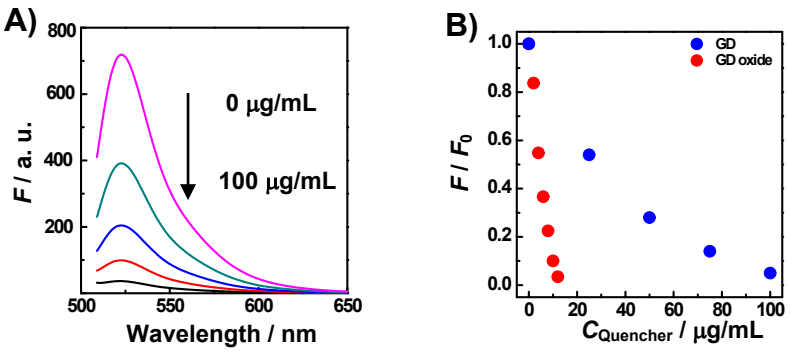

Fig. S4 (A) Fluorescence spectra of P_{HCV} (20 nM) in HEPES buffer upon addition of various concentrations of GD dispersion (from upper to bottom: 0.0, 25, 50, 75, and 100 μg/mL). (B) Relative fluorescence intensity (F/F_0) of P_{HCV} (20 nM) with addition of GD (0-100 μg/mL, blue dots) or GD oxide (0-12 μg/mL, red dots). F_0 and F are the fluorescence intensities of P_{HCV} (20 nM) in the absence and presence of GD or GD oxide, respectively. Excitation wavelength, 490 nm. Emission wavelength, 523 nm.

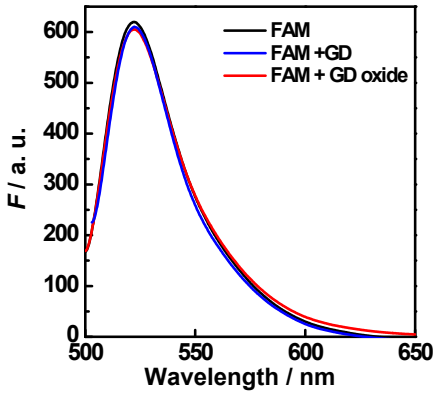

Fig. S5 Fluorescence spectra of pure FAM in HEPES (20 nM, black curve), FAM (20 nM) in HEPES buffer with addition of aqueous dispersion of GD (75 μg/mL, blue curve), or GD oxide (15 μg/mL, red curve). Excitation wavelength, 490 nm.

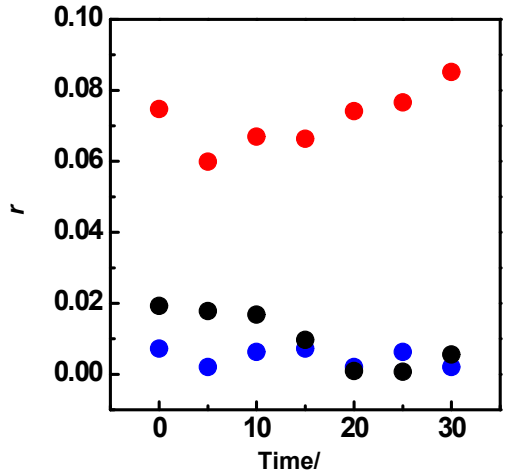

Fig. S6 Fluorescence anisotropy value (*r*) of P_{HCV} in HEPES buffer (20 nM, blue dots), P_{HCV} (20 nM) in HEPES buffer with the addition of GD oxide (10 μg/mL, red dots), and P_{HCV} (20 nM) HEPES buffer containing GD oxide (10 μg/mL) after hybridization with T_{HCV} (40 nM) (black dots). Excitation wavelength, 490 nm. Emission wavelength, 523 nm.

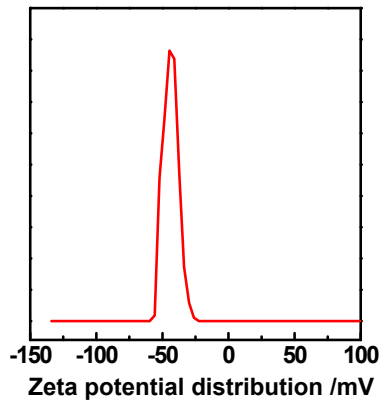

Fig. S7 Zeta potential distribution of GD oxide.

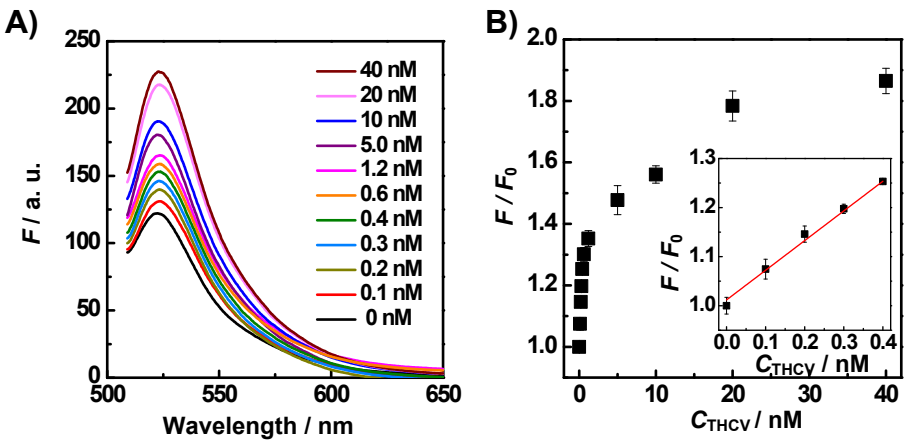

Fig. S8 (A) Fluorescence spectra of P_{HCV} (20 nM) with the addition of GD (75 μg/mL) and then hybridized with different concentrations of T_{HCV} (from bottom to top: 0.0, 0.1, 0.2, 0.3, 0.4, 0.6, 1.2, 5.0, 10, 20, and 40 nM). (B) Plot of F/F_0 versus the concentration of T_{HCV}. Inset, calibration curve. F_0 and F denote the fluorescence intensities of P_{HCV} (20 nM) and GD (75 μg/mL) before and after the hybridization of P_{HCV} with T_{HCV}, respectively. Error bars were the standard deviation of three independent experiments.

Table S2. Limit of detection (LOD) for DNA detection with different nanostructures as quenchers

Nanomaterials	LOD	Refs.
SWCNTs	4 nM	5c
Graphene	5 nM	2c
Graphene Oxide	100 pM	9b
Nano-C60	25 pM	2f
MoS_2	500 pM	7
MnO_2	0.8 nM	S3
GD	84 pM	This study
GD oxide	17 pM	This study

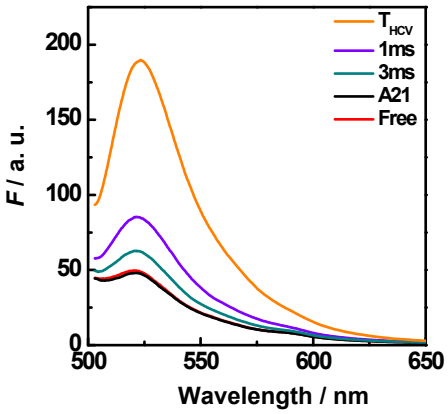

Fig. S9 Fluorescence spectra of P_{HCV} (20 nM) with addition of GD oxide (10 μg/mL, black curve) and after hybridized with T_{HCV} (40 nM, orange curve), 1 ms (40 nM, violet curve), 3 ms (40 nM, dark cyan curve), and A21 (40 nM, red curve). Excitation wavelength, 490 nm. Emission wavelength, 523 nm.

The fluorescence signal for target T_{HCV} (orange curve) was approximately 3-fold stronger than those for the single-base mismatched targets (1 ms, violet curve), 2-fold stronger than three-base mismatched DNA (3 ms, dark cyan curve). Whereas, for non-complementary DNA sequence with randomly selected sequence (A21, black curve), almost negligible fluorescence restoration was observed. These results demonstrate that this strategy shows a good specificity for T_{HCV}, which was higher than conventional linear DNA probes. We attributed this high response of fully complementary DNA sequence to the high stability of DNA duplexes.

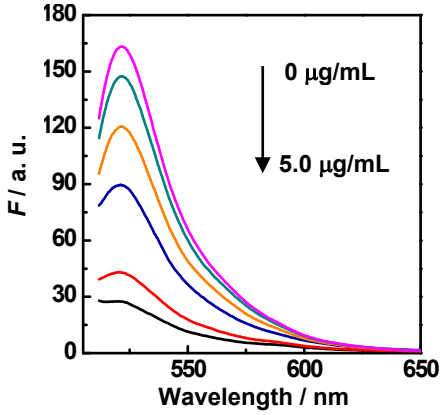

Fig. S10 Fluorescence spectra of FAM-TBA (20 nM) in HEPES buffer upon addition of various concentrations of GD oxide dispersion (from upper to bottom: 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 μg/mL). Excitation wavelength, 490 nm.

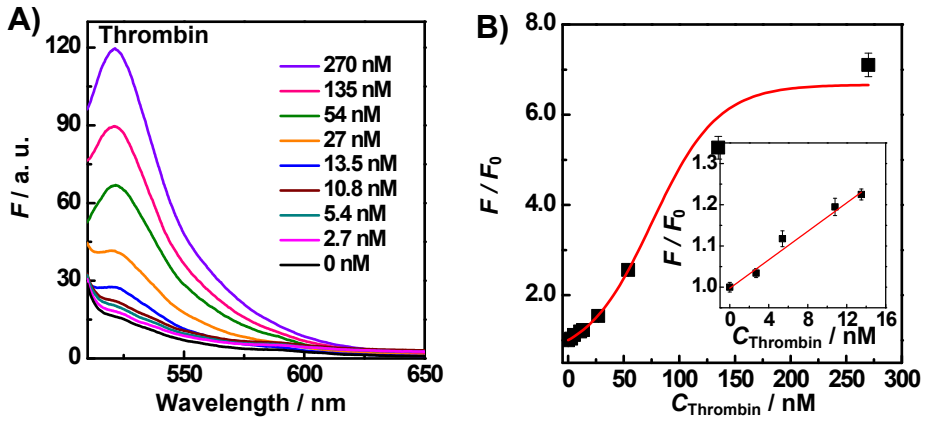

Fig. S11 (A) Fluorescence spectra of FAM-TBA (20 nM) with the addition of GD oxide (5 μg/mL) and then incubated FAM-TBA with different concentrations of thrombin (from bottom to top: 0.0, 2.7, 5.4, 10.8, 13.5, 27, 54, 135, and 270 nM). (B) Plot of F/F_0 versus the concentration of thrombin. Inset, calibration curve. F_0 and F denote the intensities of FAM-TBA (20 nM) and GD oxide (5 μg/mL) before and after incubation of FAM-TBA with thrombin, respectively. Excitation wavelength, 490 nm. Emission wavelength, 523 nm. Error bars were the standard deviation of three independent experiments.

Specificity of GD Oxide-based Fluorescent Sensing Platform towards Thrombin

To evaluate the specificity of FAM-TBA for thrombin with the GD oxide-based sensing platform in real samples, bovine and rat blood serum samples were separately added into the aqueous solution of FAM-TBA. In a typical experiment, FAM-TBA was mixed with GD oxide dispersion and then incubated separately with 135 nM thrombin in HEPES buffer, 100-fold diluted bovine serum, and 100-fold diluted rat blood serum. As shown in Fig. S12, a significant increase (F/F_0) of FAM-TBA with GD oxide dispersion was observed after incubation with thrombin in HEPES buffer (i.e., 3.2-fold), bovine serum (i.e., 3.4-fold), and rat blood serum (i.e., 3.9-fold), as compared to the blank sample. These results indicate that the method demonstrated here shows a high specificity for thrombin even in real sample. In other words, our assay can detect at least nM thrombin in blood serum, demonstrating that the platform developed here could be potentially used for practical applications.

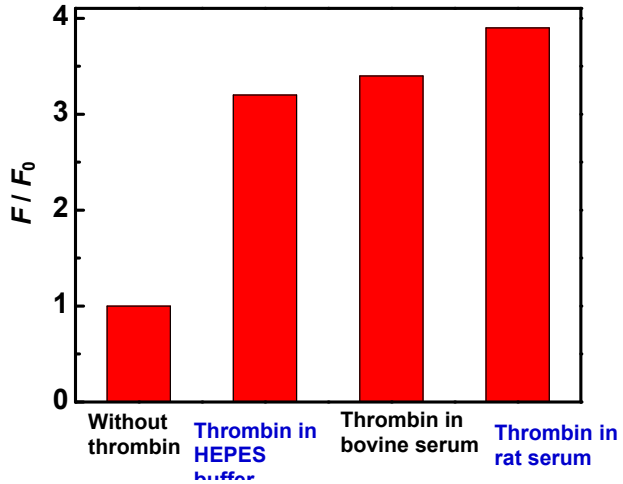

Fig. S12 Histogram of F/F_0 at 523 nm obtained from the fluorescence intensity of FAM-TBA (20 nM) containing GD oxide (5 μg/mL) after incubated with thrombin (135 nM) in HEPES buffer, bovine serum 100-fold diluted with HEPES buffer, and rat serum 100-fold diluted with HEPES buffer. F_0 and F denote the intensities of the mixture containing FAM-TBA (20 nM) and GD oxide (5 μg/mL) in the absence and presence of thrombin, respectively. Excitation wavelength, 490 nm. Emission wavelength, 523 nm.

References

S1 G. Li, Y. Li, H. Liu, Y. Guo, Y. Li and D. Zhu, *Chem. Comm.,* 2010, **46**, 3256.

S2 W. S. Hummers, Jr.and R. E. Offeman, *J. Am. Chem. Soc*., 1958, **80**, 1339.

S3 C. Wang, W. Zhai, Y. Wang, P. Yu and L. Mao, *Analyst*, 2015, **140**, 4021.