Electronic Supplementary Information

A Universal Fluorescence Sensing Strategy Based on Biocompatible Graphene Quantum Dots and Graphene Oxide for Detection of DNA

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1. The experimental details.

Materials and Reagents.

Triplex distilled water was used in the whole experimental process and to prepare PBS buffers. Graphite powder, sodium borohydride, tetrahydrofuran, 1-ethyl-3-(3-dimethyllaminopropyl) carbodiimide hydrochloride (EDC·HCl), N-hydroxysuccinimide (NHS) were purchased from Aladin Ltd. (Shanghai, China). All spectra detections were carried out at pH 7.4 phosphate buffer solution (PBS), which was prepared by mixing stock solutions of NaH_2PO_4 and Na_2HPO_4. All reagents were of analytical grade and without any further purification. DNA oligonucleotides with a concentration of 100 μM were purchased from Sangon (Shanghai, China) and purified using high-performance liquid chromatography, their sequences are listed as follows:

Connecting DNA: 5'- NH_2-TTGGTGAAGCTAACGTTGAGG-3'

Target DNA (perfect match with connecting DNA):

5'-CCTCAACGTTAGCTTCACCAA-3'

Single-base mismatched DNA: 5'-CCTCAACGTTCGCTTCACCAA-3'

Synthesis of graphene oxide (GO)

GO was synthesized from graphite powder based on a modified Hummer's method according to the literatures.[1,2] Briefly, graphite powder (1.0 g) was added to 33 mL of cold H_2SO_4 (0°C), to which $KMnO_4$ (6 g) was slowly added under continuous stirring in ice-bath. After 15 min, $NaNO_3$ (1.0 g) was added to the mixture. The solution was further stirred for 1.5 h at 35 °C and distilled water (40 mL) was added. Then, the temperature was rose to 95 and the reaction time was about 35 min. At the end, the reaction was stopped with the addition of a mixture of 100 mL of distilled water and 6 mL of H_2O_2 (30 %). The obtained golden dispersion was then subjected to centrifugation at 1,000 r.p.m for 5 min to remove unexfoliated GO using a centrifuge. The resulting supernatant solution was washed by distilled water and then subjected to centrifugation at 4,000 r.p.m until the pH of the solution is adjusted to 7.

Synthesis of graphene quantum dots (GQDs)

Graphite powder (0.3 g) was added into a mixture of concentrated H_2SO_4 (180 mL) and HNO_3 (60 mL). The solution was sonicated for two hours and heated under reflux

at 80℃ for 24 hours. The mixture was cooled and diluted with deionized (DI) water (800 mL). The dark-brown GQDs solution was neutralized with Na_2CO_3. The final product solution was further dialyzed in a dialysis bag (retained molecular weight: 1000 Da) for 3 days.

Synthesis of reduced state graphene quantum dots (rGQDs)

1.613 g GQDs and excess sodium borohydride were added into 20 mL tetrahydrofuran. Then the mixture was stirred gently for 8 hours at 70 ℃. The resulting mixture was subsequently vacuum-distilled to remove most of the excess sodium borohydride and then washed with ethanol several times, and finally the product solution was dialyzed as mentioned above.

Preparation of ssDNA-rGQDs bioconjugates

The obtained rGQDs were firstly dissolved in 5.0 mL phosphate buffer (10 mM, pH 7.4). Then pH of the rGQDs solution was adjusted to 5 to add carboxyl groups onto the surface of rGQDs. After that, 1-ethyl-3-(3-dimenthylaminopropyl) carbodiimide (EDC) (80 mg) and sulfo-NHS (80 mg) were added to the above solution to activate the surface carboxylic group for 30 min at room temperature with continuous stirring. Subsequently, the activated rGQDs and connecting DNA (15 μL, 100 μM) were mixed together and allowed to react for 24 hours at room temperature with continuous stirring. The ssDNA-rGQDs bioconjugates were obtained by centrifugation and washing with PBS buffer three times to remove the free nonconjugated complex.

Determination of DNA

50 μL GO (1.2 mg mL^{-1}) was introduced into the resultant ssDNA-rGQDs solution at room temperature to initiate the quenching reaction. After 30 min reaction, different concentrations of c-myc targets (complementary or single-base mismatched DNA targets) were added into the above ssDNA-rGQDs solution. After incubation for 30 min at room temperature, the fluorescence emission spectrum was measured under 290 nm excitation wavelength. The time dependent experiments were conducted by monitoring fluorescence at different incubation times.

Characterization methods

The morphologies of the products were characterized by transmission electron microscopy, which was performed on a JEOL-2100F instrument with accelerating voltage of 200 KV. Samples were prepared by dropping ethanolic or aqueous suspensions of the separated fractions of oxidized products onto Cu TEM grids coated with a holey amorphous carbon film and following solvent evaporation in a dust protected atmosphere. The X-ray photoelectron spectroscopy analyses were conducted using a Kratos Axis ULTRA X-ray photoelectron spectrometer with a 165 nm hemisphereical electron energy analyzer. The incident radiation came from monochromatic Al X-ray (1486.6 eV) at 15 kV and 3 mA. Wide survey scans were taken at an analyzer pass energy of 160 eV over a 1400-0 eV binding energy with 1.0 eV step and a dwell time of 100 ms, while narrow multiplex higher resolution scans were performed at a pass energy of 20 eV with 0.05 eV step and a dwell time of 200 ms. The pressure in analysis chamber was less than 7.5×10^{-9} Torr during sample analysis. Atomic concentrations were calculated using Vision software and a Shirley baseline. The UV-Vis spectra were carried out on a Perkin Elmer Lambda 950 spectrometer, in which the products were dispersed in solvent after ultrasonication for 30 min. The photoluminescence spectra were conducted on a PerkinElmer LS-45 fluorescence spectrometer, and lifetimes were determined using a FLS920 fluorescence spectrophotometer.

Determination of the quantum yields

Determination of the quantum yields of these oxidized products was accomplished by comparison of the wavelength integrated intensity of these functionalized products to that of the standard quinine sulfate. The optical density was kept below 0.05 to avoid inner filter effects. The quantum yields of these oxidized products were calculated using

$$\Phi = \Phi_s [(I \cdot A_s \cdot n^2)/(I_s \cdot A \cdot n_s^2)]$$

where Φ is the quantum yield, I is the integrated intensity, A is the optical density and n is the refractive index of the solvent. The subscript S refers to the standard reference of known quantum yield. Quinine sulfate was chosen as the standard, whose quantum yield is 0.577 and nearly constant for excitation wavelength from 200 nm to

400 nm.

1 Yanqin Wen, Feifei Xing, Shijiang He, Shiping Song, Lihua Wang, Yitao Long, Di Li and Chunhai Fan, Chem. Commun. 2010, 46, 2596-2598.

2 Zaixing Jiang, Jiajun Wang, Linghui Meng,Yudong Huang and Li Liu, Chem. Commun. 2011, 47, 6350-6352.

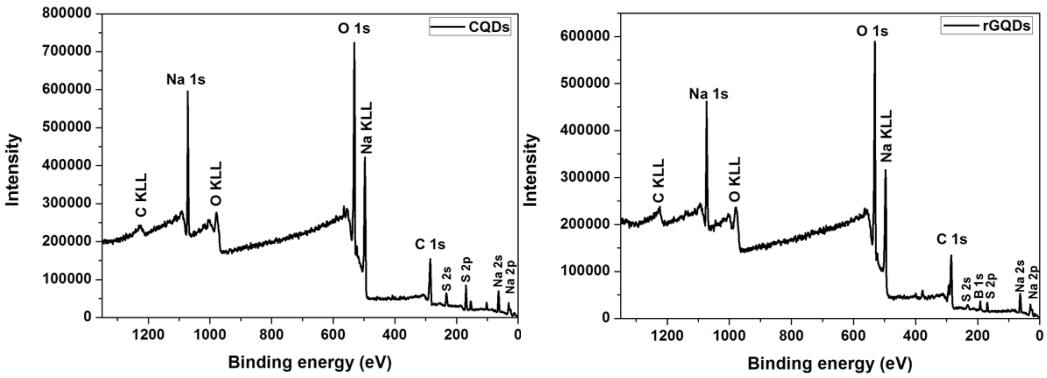

Figure S1. X-ray photoelectron spectroscopy (XPS) wide spectra of GQDs and rGQDs

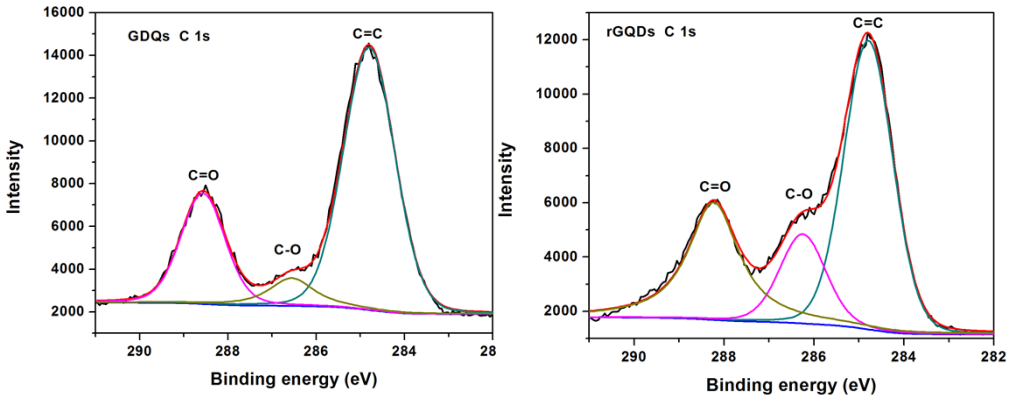

Figure S2. X-ray photoelectron spectroscopy (XPS) C1s spectra of GQDs and rGQDs

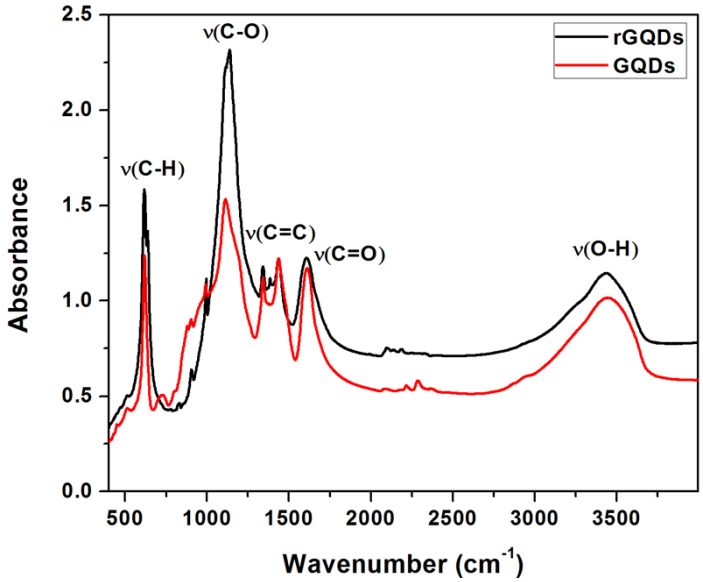

Figure S3. FT-IR spectra of the GQDs and rGQDs

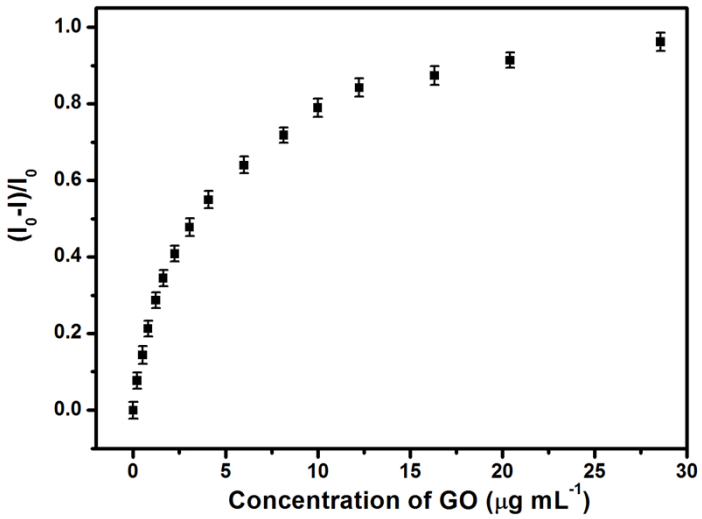

Figure. S4 Fluorescence quenching efficiency of ssDNA-rCQDs with varying amounts of GO. The concentrations of GO were 0.0 0.2, 0.5, 0.8, 1.2, 1.6, 2.2, 3.0, 4.0, 6.0, 8.0, 10.0, 12.0, 16.0, 20.0, 28.0 μg mL^{-1} respectively.

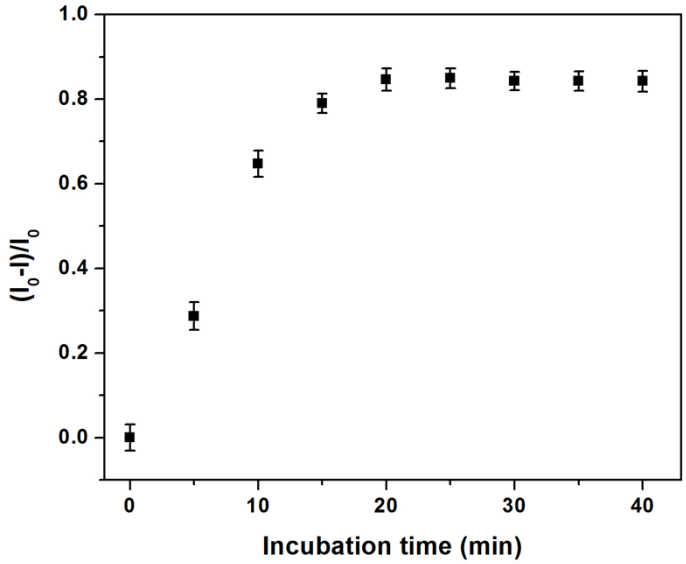

Figure. S5 Time dependence of the fluorescence quenching with 16 μg mL^{-1} of GO.

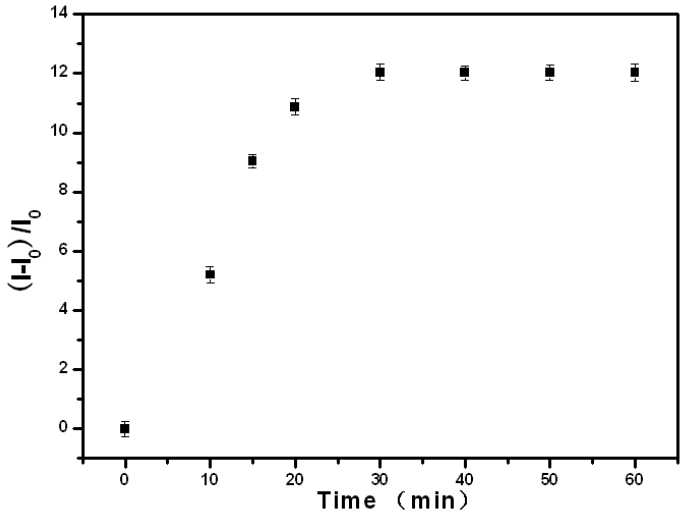

Figure. S6 The fluorescence recovery of ssDNA-rGQDs-GO system with 60.0 nM tDNA as a function of incubation time. GO: 16μg mL^{-1}.

Table S1. The concentrations of C and O in GQDs and rGQDs determined by XPS

Samples	C (wt %)	O (wt %)
GQDs	36.48	63.52
rGQDs	39.21	60.79

Table S2. The fluorescence parameters of all the GQDs, rGQDs and ssDNA-rGQDs samples

Sample	λ_{ex} [a] (nm)	λ_{em} [b](nm)	Φ_f [c](%)
GQDs	328	431	1.7
rGQDs	275	443	20.4
cDNA	361	410	0.9%
tDNA	360	411	1.7%
ssDNA-rGQDs	290	480	21
dsDNA-rGQDs	285	485	18.4%

[a] The excitation wavelength. [b] The central emission wavelength. [c] The fluorescence quantum yield determined with quinine sulfate (0.577) as reference.