Supporting Information

Electrochemiluminescent Graphene Quantum Dots as A Sensing Platform: A Novel Dual Amplification for MicroRNA Assay

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Fig S1. UV-vis absorption of GO and the prepared GQDs.

Fig S2. FTIR spectra characterization of (a) GQDs, (b) GQDs/PTCA, (c) GQDs/PTCA/Au@Fe_3O_4.

Fig S3-S4. The comparison among the ECL emission spectroscopy of grapheme oxide (GO), GQDs/PTCA-NH_2/Au@Fe_3O_4 and modified with AgNPs.

Fig S5. The photoluminescence spectrum of GOQs.

Fig S6. Optimization of analytical conditions.

Cell Culture and Total RNA Extraction

UV-vis absorption of GO and the prepared GQDs

In order to further investigate the successful synthesis and the optical performance of the GQDs, UV-vis absorption was also explored. As can be figured out in Fig S1, a representative absorption peak was showed in black curve (GO). On the contrary, there was no obvious absorption peak but a long absorption range in the red curve (GQDs). The results were with good agreement to the reported study[1].

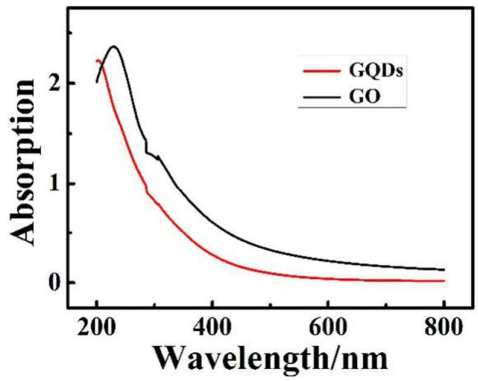

Fig. S1. UV-vis absorption of GO and the prepared GQDs.

FTIR spectra characterization of GQDs, GQDs/PTCA and GQDs/PTCA/Au@Fe_3O_4

Typical FTIR spectra was employed to characterize the nanocomposite GQDs/PTCA/Au@Fe_3O_4. As shown in Fig. S2, GQDs exhibited typical absorption peaks at 1400, 1632 and 3400 cm^{-1} (curve a), which correspond to C–O (alkoxy), C–O (carboxy) and O-H (carboxy) bond, respectively. When PTCA was introduced to combine with GQDs, it showed C=O stretching peak at 1773 cm^{-1}, N-H bond of secondary amide absorption peak at 1689 cm^{-1} and C=C stretching peak at 1593 cm^{-1} (curve b), indicating the successful formation of GQDs/PTCA through π-π stacking. When Au@Fe_3O_4 was modified on GQDs/PTCA, there was no obvious changes

comparing with curve b (curve c), because there is no infrared absorption in inorganic substance.

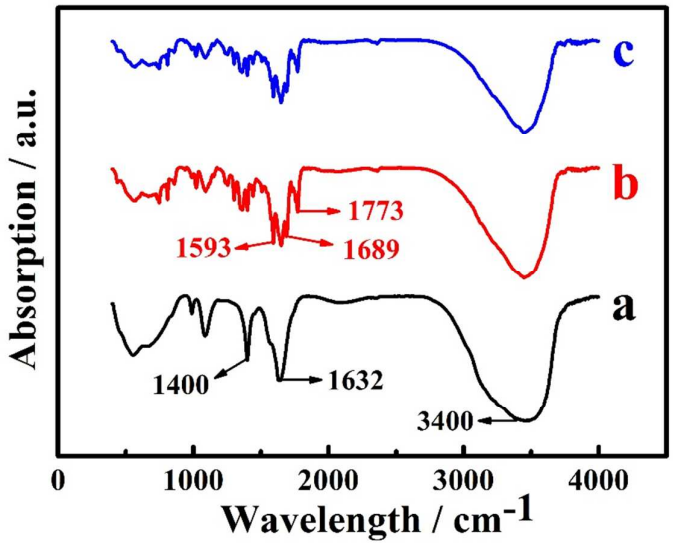

Fig. S2. FTIR spectra of (a) GQDs, (b) GQDs/PTCA, (c) GQDs/PTCA/Au@Fe_3O_4.

The comparison among the ECL emission spectroscopy of grapheme oxide (GO), GQDs/PTCA-NH_2/Au@Fe_3O_4 and modified with AgNPs

In order to study the ECL property of the prepared nanomaterials, we have made a comparison among the ECL emission spectroscopy of grapheme oxide (GO), GQDs/PTCA-NH_2/Au@Fe_3O_4 and modified with AgNPs. As a zero-bandgap material, it is almost impossible to observe the luminescence of GO[1] (Fig S3A). Thus the scientists are trying to cleave the graphene-based materials into nanoscale graphene quantum dots (GQDs) to induce the luminescence. As can be seen in Fig S3B, ECL spectrum was observed by detecting the maximum ECL signal with a series of optical filters and the ECL maximum emission wavelength of the GQDs was measured to be about 520 nm, which was in accordance with the published work[2]. While, the ECL emission spectrum of GQDs/PTCA-NH_2/Au@Fe_3O_4 with the maximum wavelength

is 525 nm (Fig S4. curve b), which is thinly red-shifted (5 nm) compared with the sole GQDs ECL spectrum due to the conjugated effect between GQDs and PTCA-NH_2[3]. When the nanocomposite was modified with AgNPs, the maximum emission wavelength of the ECL spectrum remained at 525 nm (Fig S4. curve c), suggesting that the AgNPs has no effect on the ECL spectrum of GQDs.

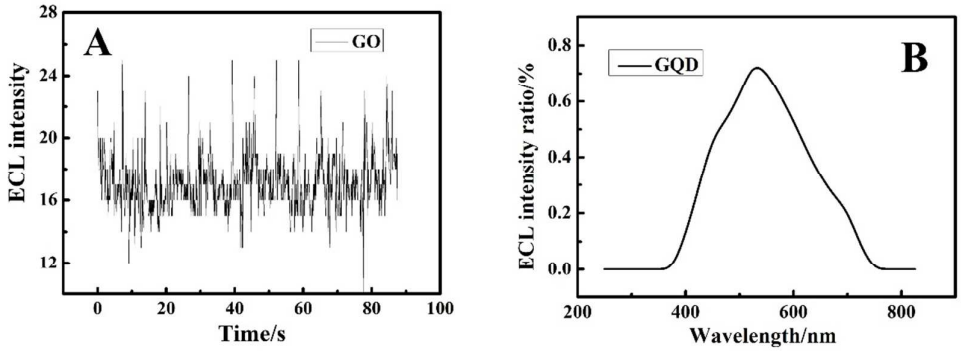

Fig S3. (A) ECL response of GO. (B) ECL emission spectrum of the prepared GQDs. (The ECL intensity ration represented i/i_0, i and i_0 correspond to the ECL intensity with the presence and absence of the optical filters, respectively)

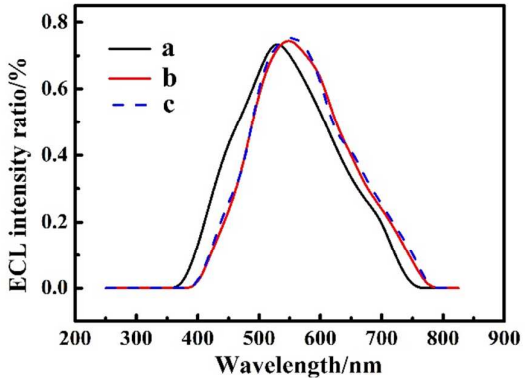

Fig S4. ECL emission spectrum of GQDs (curve a), GQDs/PTCA-NH_2/Au@Fe_3O_4 (curve b) and GQDs/PTCA-NH_2/Au@Fe_3O_4 modified with AgNPs (curve c).

The photoluminescence (PL) spectrum of GOQs

In order to further confirm confirm the luminescent properties of GOQs, the photoluminescence were carried out as s shown in the PL spectrum (Figure S5), GQDs had optimal maximum emission wavelengths at 510 nm when excited at 340 nm. The photoluminescence excitation (PLE) spectrum recorded with the strongest luminescence (510 nm) displayed the peak at 266 nm which corresponded to the 275 nm absorption band due to the p-p* transition. As can be seen in Fig S5, ECL spectrum was observed by detecting the maximum ECL signal with a series of optical filters and the ECL maximum emission wavelength of the GQDs was measured to be about 520 nm, which was in accordance with the published work[1].

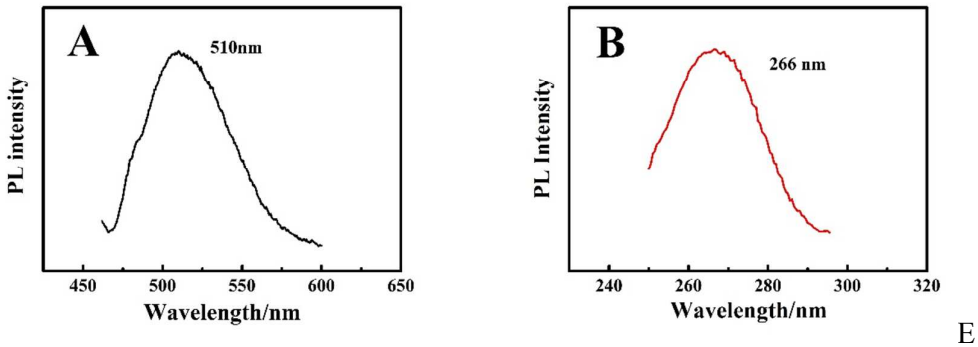

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Fig S5. (A) PL spectra for excitation wavelengths of GQDs at 340 nm . (B) Photoluminescence excitation spectrum (PLE) of GQDs (510 nm).

Optimization of analytical conditions

In order to improve the sensitivity of the proposed biosensor and shorten the assay time, the optimization of experimental conditions was examined. The relation between ECL intensity and incubation time of T7 exonuclease was showed in Fig. S6A. With the increasing of time, more RNA/DNA duplexes were cleaved on the sensing surface. The ECL response tended to a constant value at 60 min. Thus, 60 min

was chosen as the optimal time for the T7 exonuclease digestion process.

The ECL signal was also dependent on the silver growth time. As can be seen from Fig. S6B, the signal enhanced with the increasing silver deposition time and reached a maximum value at 10 min. The longer growth time led to a slight increase of the sensing system. Consequently, the optimal silver deposition time of 10 min was applied in this work.

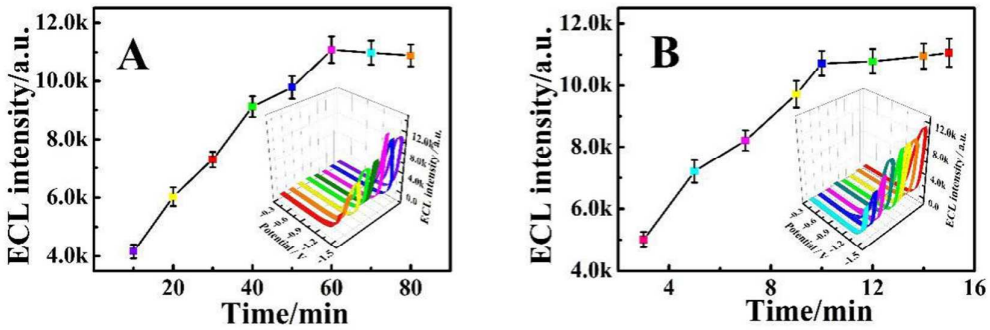

Fig. S6 (A) ECL signal diversified with the incubation time of T7 exonuclease. (B) ECL signal diversified with silver deposition time.

Cell Culture and Total RNA Extraction

Hela cells, HK-2 cells, L02 cells and 22Rv1 cells were bought from the cell bank of the type culture collection of the Chinese Academy of Sciences (Shanghai, China) and routinely cultured in RPMI 1640 medium (Thermo Scientific Hyclone) in a humidified condition at 37 °C with 5% CO_2. Total RNA extraction were prepared using Trizol reagent (Invitrogen, Carlsbad, CA, USA) from each cell line according to the manufacturer's procedures. To be brief, the cell pellet was injected with appropriate amount of Trizol Reagent by shaking 3–4 times and then transferred to an RNase-free centrifuge tube, followed by incubation at normal temperature for 5 min to ensure thorough cell disruption. After that, chloroform solution was employed to

generate the phase separation at room temperature and isopropyl alcohol solution was transferred into the above aqueous phase to recover the total RNA after precipitating for 10 min. The RNA pellet was washed with 75% ethanol and re-dissolved in RNase-free water. Finally, the re-dissolved RNA were stored at −20°C for further quantification with the proposed amplification strategy.