Supporting information for

Construction of a novel GQDs based ratiometric fluorescent composite probe for viscosity detection

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Experimental Section

Reagents and Materials

RV-1 was obtained from our previous work. All chemicals and reagents involved in this study were analytical grade. The solvents were purified before using by standard methods and ultrapure water was used throughout all experiments.

Apparatus

A Shimadzu UV-2700 spectrophotometer was used for absorption spectra measure. Fluorescence spectra were obtained by the fluorescence spectrophotometer (HITACHI F4600). A Nikon A1MP confocal microscopy was used for fluorescent imaging. Transmission electron microscope (TEM) images were measured by a Philips CM200 FEG microscope.

Systhesis of RV-1@GQDs-OH.

RV-1 was obtained from our previous work. 5.9 mg **RV-1** was dissolved in 10 mL DMSO and got a stock solution of **RV-1** (1mM). GQDs-OH was synthesized through hydrothermal method according to previous report.18 1,3,6-Trinitropyrene was dispersed into NaOH aqueous solution (200 mM) via ultrasound and reacted in a high-pressure reaction kettle at 200 ℃. After hydrothermal treatment for 10h, a dark brown mixed solution was obtained. Then purified by filtered and dialysis. 4.0 mg GQDs-OH was dissolved in 4 mL PBS solution (10 mM, pH=7.2) and got a stock solution of GQDs-OH (1 $mg \cdot mL^{-1}$). 200 μL GQDs-OH stock solution was dispersed in 800 μL **RV-1** stock solution at 0 ℃ and stiring for 24h, then the stock solution of **RV-1@GQDs-OH** was obtained. **RV-1@GQDs-OH** was purified through dialysis,

the encapsulation efficiency of **RV-1** was measured according to the following formulas.

encapsulation efficiency (%) = ($W_{(\textbf{RV-1 in RV-1@GQDs-OH})}$ / $W_{(\text{total added } \textbf{RV-1})}$) × 100%

Spectral Experiment.

Water-glycerol mixed solutions with different volume ratios (0-100%) were prepared to measure their viscosity via rotational viscometer. 50 μL stock solution of **RV-1@GQDs-OH** was dispersed with 5 mL PBS-glycerol mixed solution to get the test solution of **RV-1@GQDs-OH**. The various selectivity test solutions were prepared from 200 μM various ions and biothiols, 200 μL of 100 $mg.L^{-1}$DNA and RNA

MTT assays

The cell cytotoxicity of the probe was evaluated by MTT assay. HeLa cells were seeded in 96-well plates and cultured for 24 h at 37 °C. The medium in each well was incubated with increasing volume of **RV-1@GQDs-OH** stock solution (0-50 μL) for 24 h, 10 μL MTT (5 mg/mL in PBS) was added. Subsequently, the culture medium was removed, and 100 μL DMSO was added into the dishes to dissolve the formazan crystal product. The plate was shaken for 10 min, and then the absorbance at 490 nm was measured by the microplate reader.[1]

Cell Culture and Imaging.

Cells were divided into three groups and cultured at 37 ℃ with 5% CO_2. One group was chosen as the control group and incubated with only probe (10 μL **RV-1@GQDs-OH**) for 15 min. Other two groups were pretreated with monensin or

nystatin for 30 min separately and followed by incubated with 10 μL **RV-1@GQDs-OH** for other 15 min. Confocal microcopy imaging of cells were obtained in green and red channels (λ_{ex}= 405 nm).

Zebrafish Imaging.

Zebrafishes were divided into three groups. One group was chosen as the control group and incubated with only probe (10 μL **RV-1@GQDs-OH**) for 15 min. Other two groups were pretreated with 50 μM monensin or nystatin for 30 min separately and followed by incubated with 10 μL **RV-1@GQDs-OH** for other 15 min. Confocal microcopy imaging of zebrafish were obtained in green and red channels (λ_{ex}= 490 nm)

Table S1 Encapsulation efficiency of **RV-1** at different temperatures

Temperature	0 ℃	25 ℃	50 ℃
encapsulation efficiency	98.5 %	99.2 %	97.4 %

Table S2 Encapsulation efficiency of **RV-1** at different pH

pH value	5.0	7.4	9.0
encapsulation efficiency	97.7 %	98.5 %	97.5 %

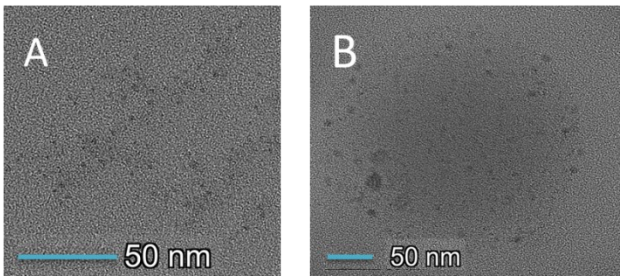

Fig. S1. Transmission electron microscopeare image of GQDs-OH (A) and **RV-1@GQDs-OH** (B)

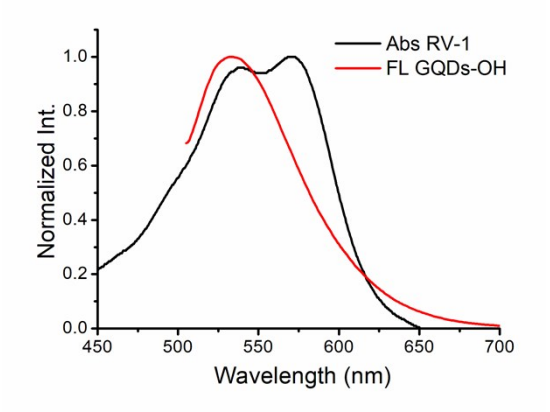

Fig. S2. Overlap of the normalized emission spectrum of GQDs-OH and normalized absorption spectrum of **RV-1**

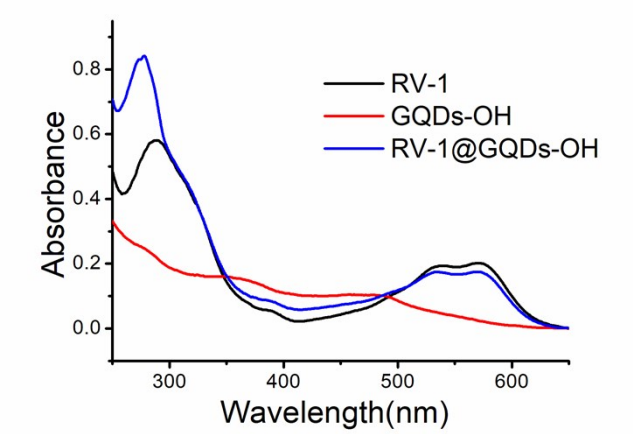

Fig. S3. UV-Vis absorption spectra of **RV-1**, GQDs-OH and **RV-1@GQDs-OH** in glycerol solution.

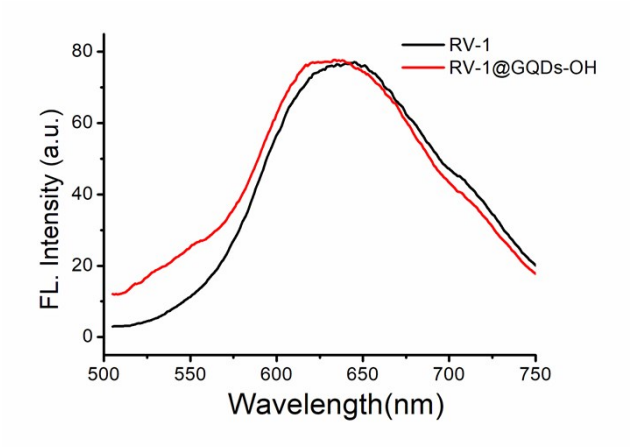

Fig. S4. Fluorescence emission of **RV-1** and **RV-1@GQDs-OH** in glycerol solution, λ_{ex} = 490 nm

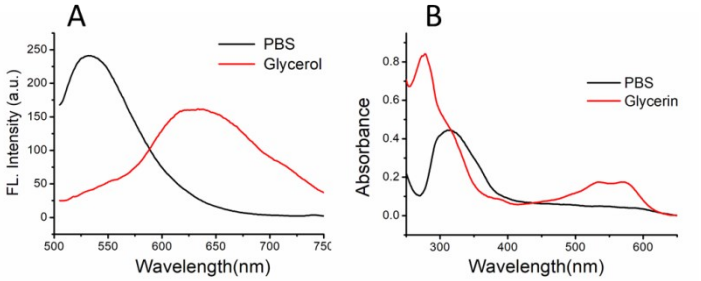

Fig. S5. Fluorescence emission of **RV-1@GQDs-OH** in PBS and glycerol solution, λ_{ex} = 490 nm (A) and UV-Vis absorption spectra of **RV-1@GQDs-OH** in PBS and glycerol solution (B)

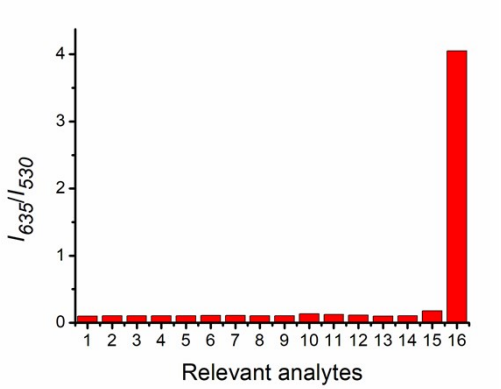

Fig. S6. Fluorescent ratios (I_{635}/I_{530}) of 10 μL **RV-1@GQDs-OH** to various analytes. 1, Blank; 2, CH_3COONa (500 μM); 3, $CaCl_2$ (500 μM); 4, Cys (500 μM); 5, GSH (500 μM); 6, Hcy (500 μM); 7, KNO_3 (500 μM); 8, NaF (500 μM); 9, Na_3PO_4 (500 μM); 10, DNA (100 μM); 11,RNA (100 μM); 12, phospholipase A2 (10 μM); 13, butyrylcholinesterase (10 μM); 14, thrombin (10 μM); 15, fetal bovine serum (FBS); 16, viscosity of 953 cP (Glycerol) solution. λ_{ex} = 490 nm.

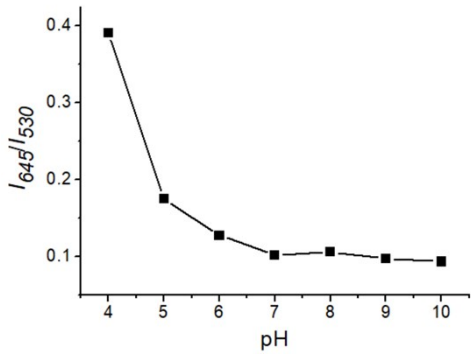

Fig. S7. Fluorescent ratios (I_{635}/I_{530}) of **RV-1@GQDs-OH** at various pH values. λ_{ex} = 490 nm

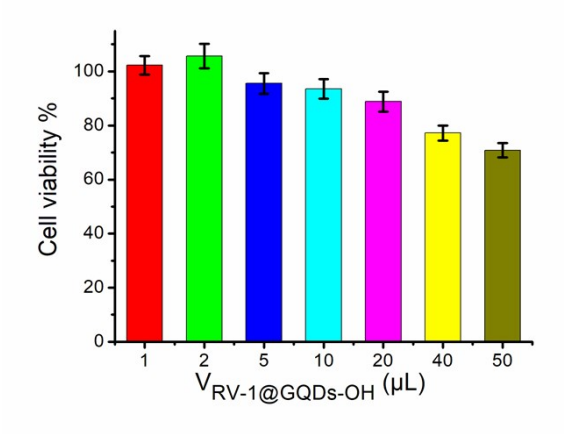

Fig. S8. Viability of HeLa cells treated with various concentrations (0-50 μL) of **RV-1@GQDs-OH** for 24 h.

References

1. Li, G.; Liu, Y.; Niu, J.; Pei, M.; Lin, W., *J. Mater. Chem. B*. **2018**, *6*, 4380.