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Supplementary Information

Fluorophore-Functionalized Graphene Oxide with Application in Cell Imaging

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General information

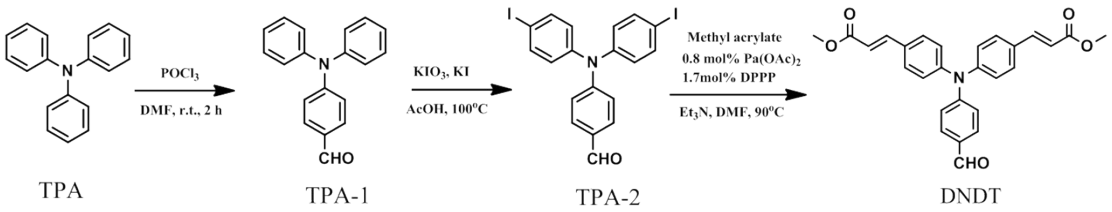
Triphenylamine (TPA, 99%), phosphorus oxychloride ($POCl_3$), potassiumiodide (KI, 99%), Potassium Iodate (KIO_3, 99%), Methylacrylate (MA, 98%), Palladium acetate ($Pd(OAc)_2$, 99%), Triethylamine (Et_3N, 99%), 1,3-bis(diphenylphosphino)propane (DPPP, 98%), graphene oxide (GO), 3-Aminopropyl)triethoxysilane (KH550, 99%) were purchased from Aladdin Co. Other commercial reagents were purchased from Aladdin Co. and Energy Chemical Co. and used without further purification unless otherwise stated.

GO-KH550, GO-KH550-DNDT were obtained by the documented method respectively.[1,2]

Fourier transform infrared (FT-IR) spectra were recorded on a Nicolet AVATAR 360. UV-visible absorption spectra (UV) were recorded on a TU-1901spectromete. Fluorescence spectra were measured using a PE LS-55 Luminescence/Fluorescence Spectrophotometer. Transmission electron microscopy (TEM) images were taken using Tecnai G2 F20 (America, FEI). The morphology and energy dispersion spectrum (EDS) of GO-KH550-DNDT were examined by field emission scanning electron microscopy (FE-SEM, ZEISS ULTRA plus). The zeta potential values were determined on a zeta potential instrument (Malvern NANO ZEN 3600). Particle size measurements process was performed on a Zetasizer Nano ZS dynamic light scattering (DLS) system. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) were recorded on a Mercury spectrometer at 25 °C, and all NMR spectra were referenced to the solvent. Fluorescent microscope images of HepG2 cells were taken on an inverted fluorescent microscope (Olympus, Japan).

Hepatic cancer cells (HepG2) were selected for the following experiment to conform the bio-application of GO-KH550-DNDT. Live cells at least in exponential growth period were chosen to prepare cell suspension. HepG2 cells (1.5×10^5 per

well) were incubated in complete cell culture medium for 24 h at 37 °C in a humidified environment of 95% O_2 and 5% CO_2. GO-KH550-DNDT was dispersed evently in DMSO after sterilized with ultraviolet light. Until the cells were in good conditions, a certain amount of GO-KH550-DNDT dispersion could be added to the culture medium to form different concentrations (50, 100, 150, 200, 250 μg/mL) and incubated in above conditions for 48 h. Then, cell viability was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method.



Scheme S1 Synthetic routes of TPA-1, TPA-2 and DNDT**.**

Synthesis of TPA-1

Triphenylamine (0.49 g, 2 mmol) was dissolved in DMF (8 mL) and placed in a 100 mL flask. phosphorousoxychloride (0.78 g, 5 mmol) was added dropwise in ice bath, and the reaction mixture is stirred for 10 minutes at 0 °C. And then the mixture was refluxed at 45 °C for 8 h under N_2 atmosphere. The reaction solution was quenched with cold water (100 mL) and white solid was obtained. The residue was purified by column chromatography on silica gel (300-400 mesh) with a mixture of ethyl acetate and petroleum ether as eluent (1:100 by volume), leading to the purified 4-(diphenylamino)benzaldehyde (TPA-1, 0.44 g, yield: 75%). ^{1}H NMR ($CDCl_3$): δ 9.76 (s, 1H), 7.70 (d, J = 8.8 Hz, 2H), 7.18-7.24 (d, J = 8.4 Hz, 6H), 7.42 (d, J = 8.0 Hz, 4H), 7.71 (d, J = 8.4 Hz, 2H).

Synthesis of TPA-2

In a 50 mL two necked round bottom flask, 1.6 g (6 mmol, 1.0 equiv) of 4-(diphenylamino)benzaldehyde (TPA-1) and 2.241 g (13.5 mmol, 2.25 equiv) of potassium iodide were dissolved in the mixture of acetic acid (24 mL) and H_2O (2.4 mL) under N_2. The mixture was warmed and stirred to yellow transparent solution, then potassium iodate 2.889 g (13.5 mmol, 2.25 equiv.) was added within several times. The reaction mixture was warmed to reflux and stirred for 1 h. Then the mixture was cooled to room temperature and pour in distilled water, and dark yellow solid was obtained. The crude product was purified by column chromatography directly to afford 4-(bis(4-iodophenyl)amino)benzaldehyde (TPA-2) as an orange solid (2.6 g, 5.1 mmol, yield: 84.8%). M.P.: 146-151 ^{o}C; ^{1}H NMR (600 MHz, $CDCl_3$) δ (TMS, ppm): 9.84 (s, 1H), 7.71 (d, J = 8.3 Hz, 2H), 7.63 (d, J = 8.5 Hz, 4H), 7.05 (d, J = 8.3 Hz, 2H), 6.89 (d, J = 8.4 Hz, 4H). ^{13}C NMR (150 MHz, $CDCl_3$) δ(ppm) 190.35, 152.17, 145.69, 138.85, 131.34, 130.31, 127.60, 120.75, 88.75. MS (FAB): m/z = 524.91 $[M+H]^+$.

Synthesis of DNDT

4-(bis(4-iodophenyl)amino)benzaldehyde (TPA-2) (2.4 mg, 5 mmol), methyl acrylate (18 mmol), $Pd(OAc)_2$ (27 mg, 0.12 mmol), 1,3-bis(diphenylphosphino)propane (103.1 mg, 0.25 mmol) and Et_3N (1.8 g, 18 mmol) were added into 50 mL dry dimethylformamide (DMF). Then, the mixture was stirred at 90 °C under N_2 atmosphere for 48 h. the reaction was completed as determined by the disappearance of TPA-2, 20 mL of water was charged, and the solution was extracted with CH_2Cl_2 (3×10 mL). The organic phases were then dried with anhydrous magnesium sulfate, filtered, and concentrated under reduced pressure. The residue was purified by

column

chromatography on silica gel (300-400 mesh) with a mixture of ethyl acetate and petroleum ether as eluent (1:10 by volume), leading to a purified (2E,2'E)-dimethyl 3,3'-(((4-formylphenyl)azanediyl)bis(4,1-phenylene))diacrylate (DNDT, 441g/mol, yield: 63.5%). M.P.: 133-135 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.76 (d, J = 8.0 Hz, 2H), 7.66 (d, J = 16.0 Hz, 2H), 7.48 (d, J = 8.0Hz, 4H), 7.15 (t, J = 8.0Hz, 6H), 6.38 (d, J = 16.0 Hz, 2H), 3.81 (s, 6H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ 190.34, 167.35, 151.99, 147.70, 143.62, 131.30, 131.09, 130.85, 129.53, 125.37, 122.28, 117.24 ppm. MS (FAB): m/z = 441.16 $[M+H]^+$. IR (KBr): 3445, 2947, 2720, 1717, 1630, 1594, 1504, 1433, 1321, 1267, 1203, 1167, 1038, 980, 825 cm^{-1}.

Synthesis of GO-KH550

Firstly, Alcoholysis of KH550 (0.2 mL) was conducted for 1 h. Then GO (500 mg) was dispersed evenly into toluene (50 mL) before amination. Following, the alcoholysate (KH550) was added to the dispersed GO and placed in a 100 mL flask. After stirring for 12 h at 100 °C, centrifugation was conducted to obtaine the muddy-like sediment, then washed with toluene three times and dried to constant weight at 45 °C for 12 h, the product (GO-KH550) was grinded and saved for future experiments.

Synthesis of GO-KH550-DNDT

Powdered GO-KH550 was dispersed evenly into anhydrous ethanol (50 mL) before reaction. Then, the prepared DNDT was added into the dispersed GO-KH550. After stirring for 12 h at 90 °C, the reaction was completed as determined by the solution became colorless and transparent, centrifugation was conducted to obtaine the muddy-like sediment, then washed with anhydrous ethanol three times and dried to constant weight at 45 °C for 12 h by vacuum drying oven and the product (GO-

KH550-DNDT) was grinded and saved for following test.

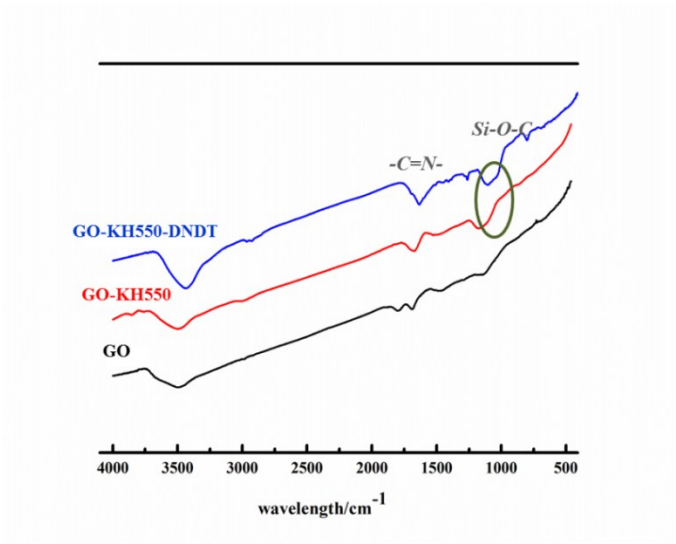


Figure S1 IR spectra of GO, GO-KH550 and GO-KH550-DNDT.

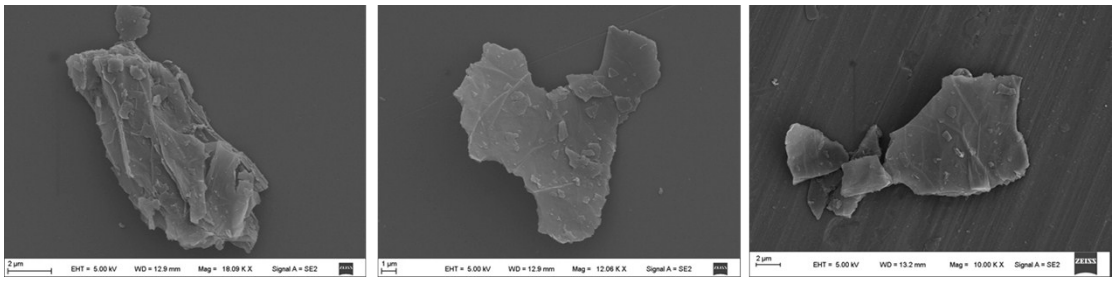
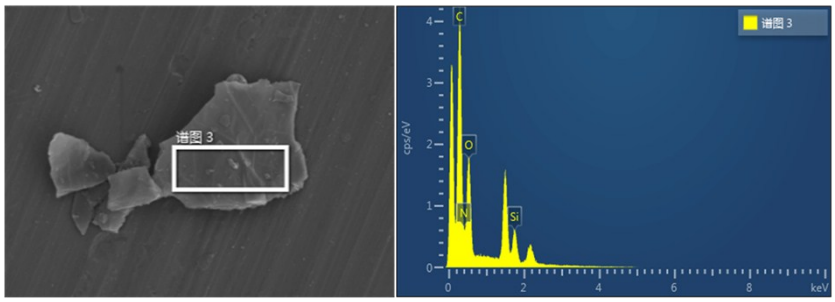


Figure S2 SEM images of GO-KH550-DNDT.



Element	apparent concentration	k	wt%	wt% Sigma
C	156.70	1.56700	55.10	1.49
N	166.37	0.29620	15.47	1.96
O	159.68	0.53735	21.28	0.75
Si	47.96	0.38000	8.15	0.34
Total			100	

Figure S3 Elemental analysis of GO-KH50-DNDT.

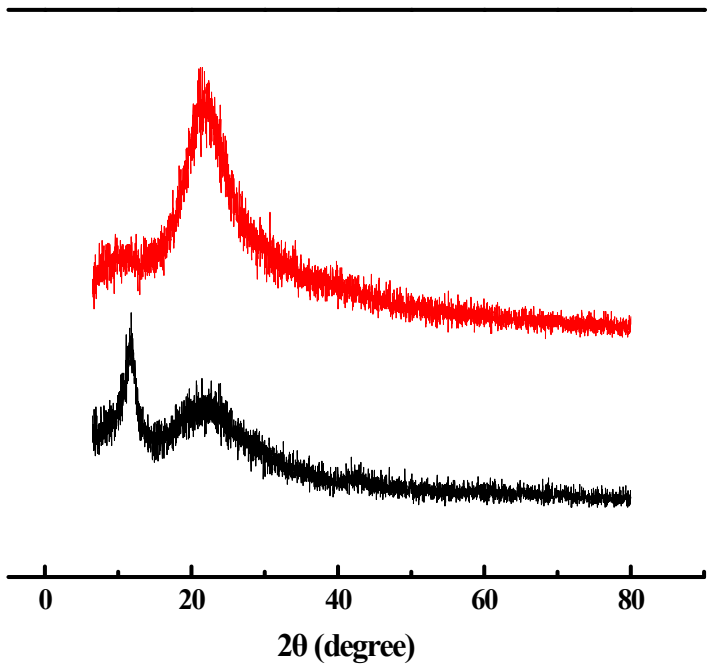


Figure S4 X-Ray diffraction patterns of GO and GO-KH550-DNDT.

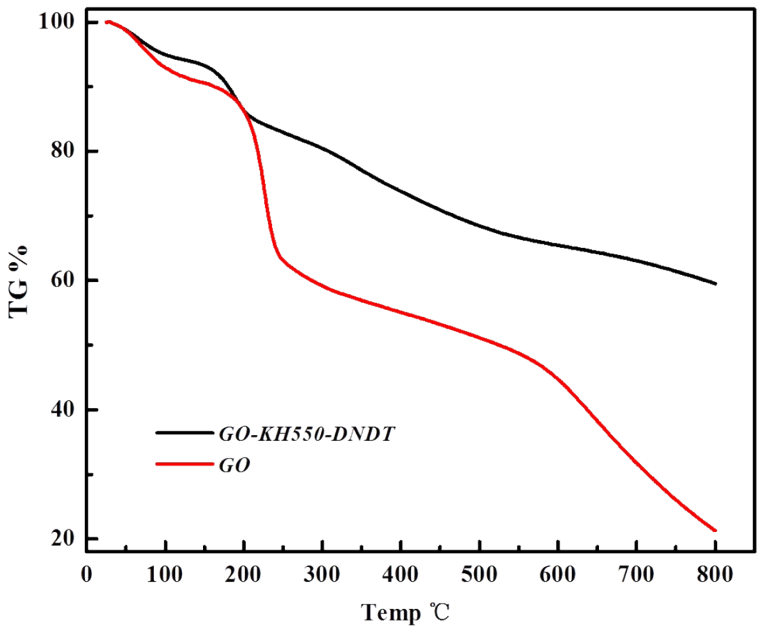


Figure S5 Thermal gravimetric analysis plots of GO, GO-KH550-DNDT.

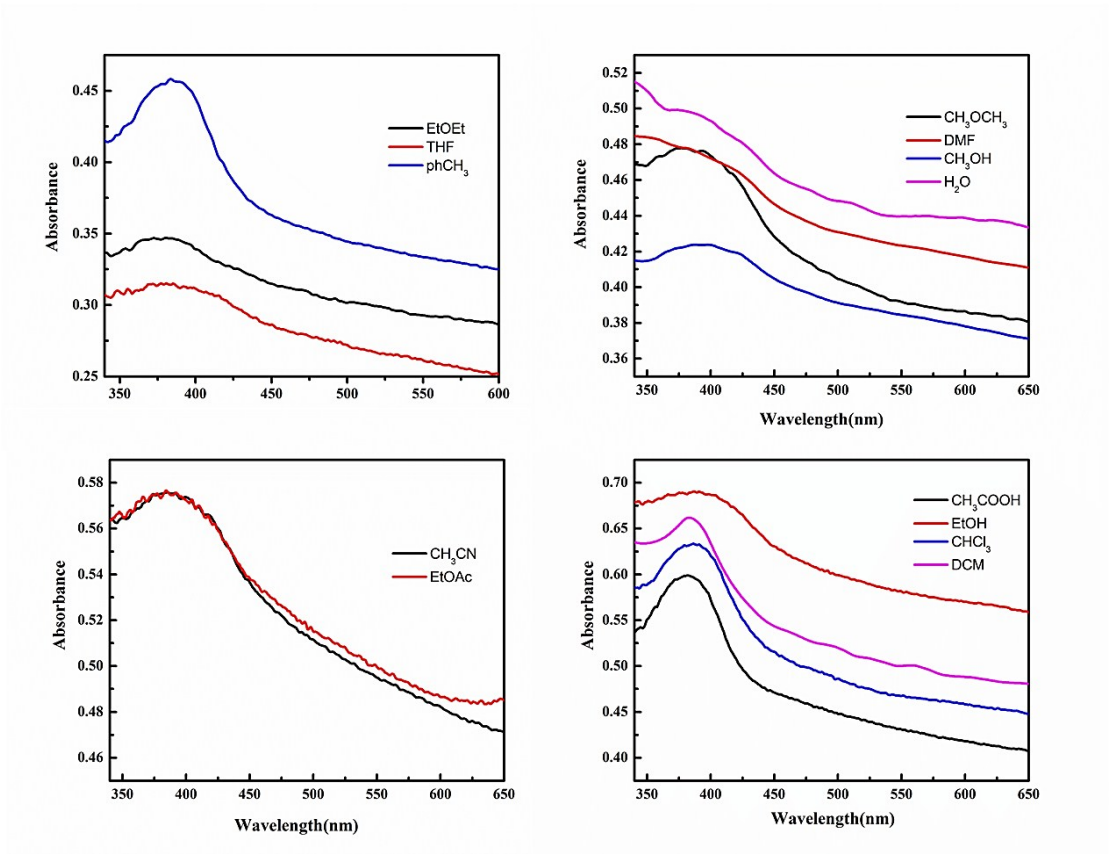


Figure S6 UV-Vis spectra of GO-KH550-DNDT in various solutions.

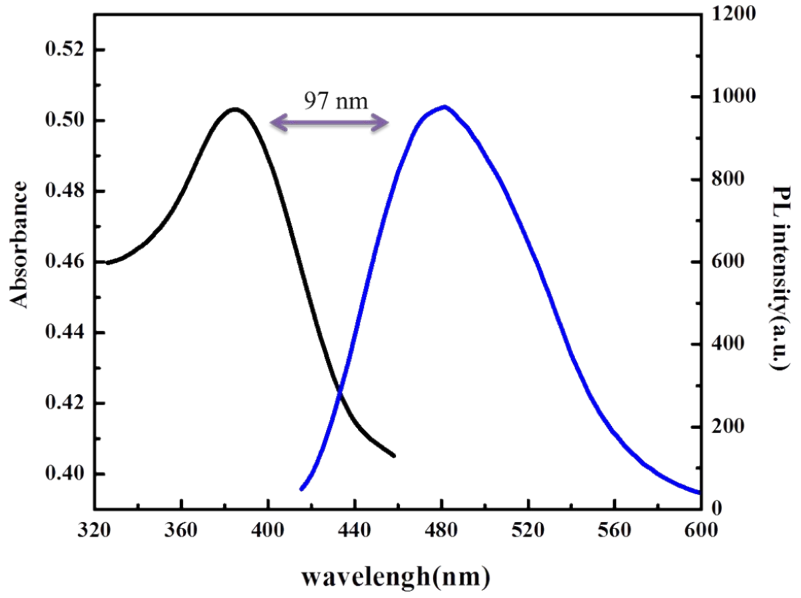


Figure S7 UV spectrum and PL spectrum of GO-KH550-DNDT in $CHCl_3$.

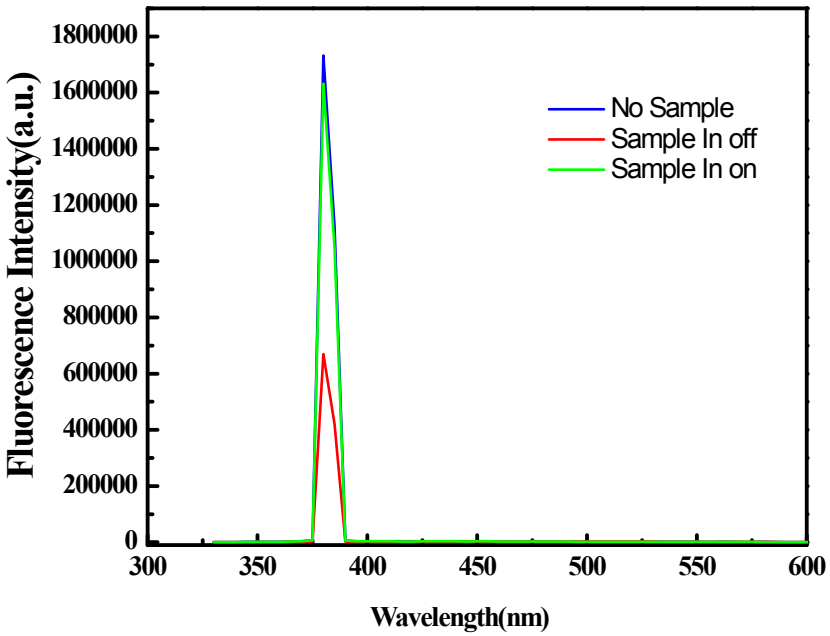


Figure S8 PL spectrum of GO-KH550-DNDT in DMSO using integrating sphere measurement.

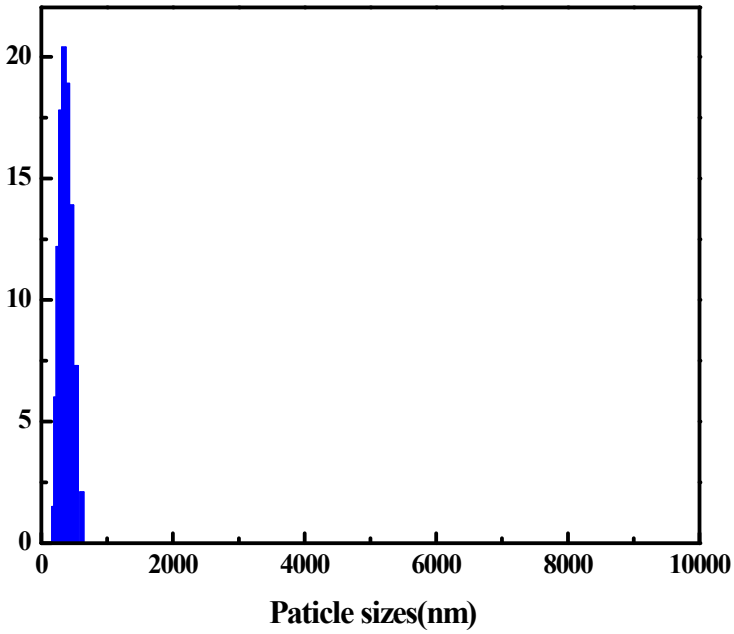


Figure S9 Particle size distributions of GO-KH550-DNDT in DMSO (50 μg/mL).

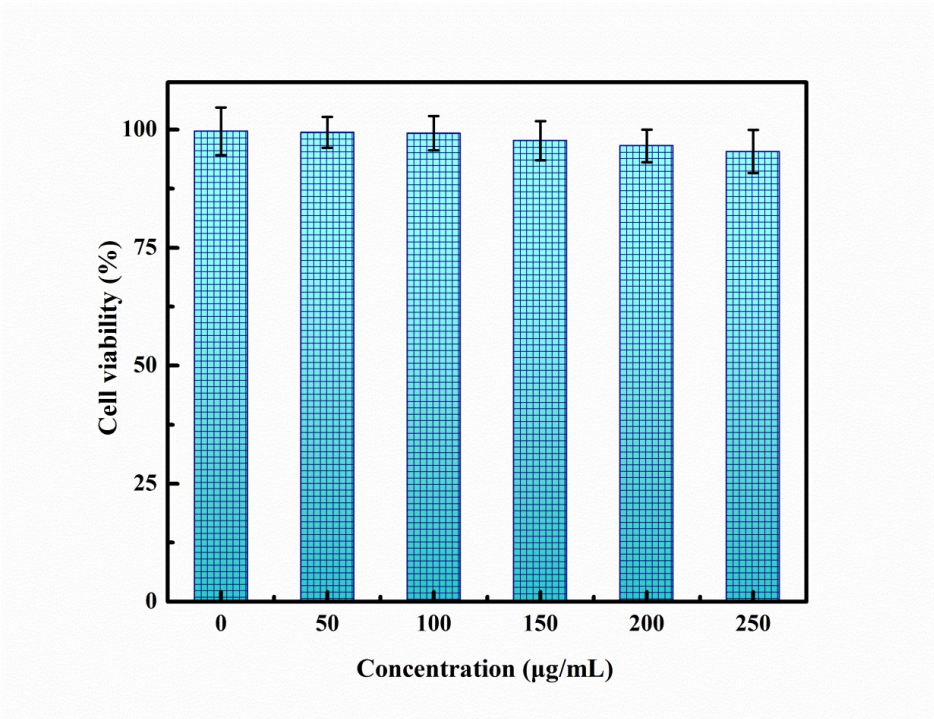


Figure S10 Cell viabilities of HepG2 cells treated with different concentrations of GO-KH550-DNDT for 96 h by MTT assay.

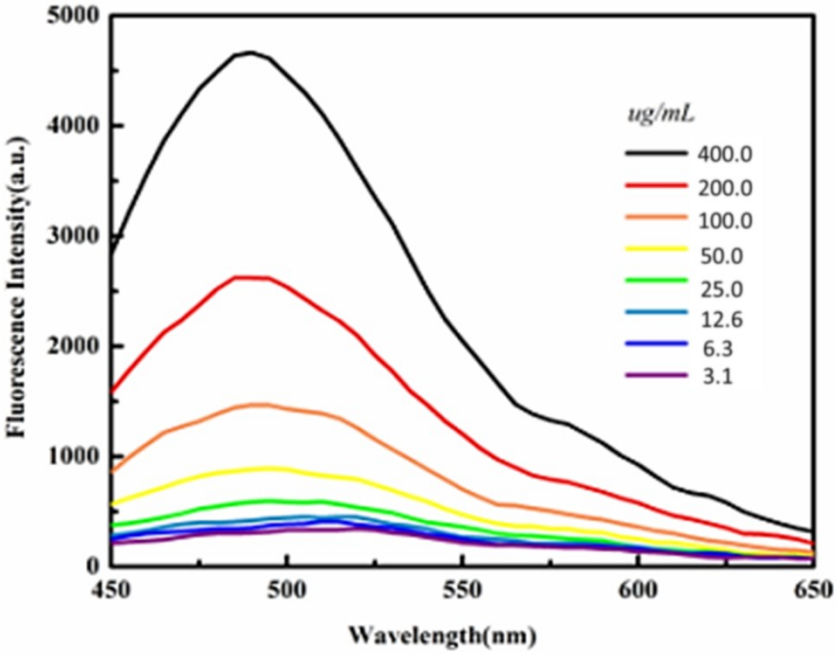


Figure S11 PL spectra of GO-KH550-DPPD in cell culture media with different concentrations (from 3.1 to 400.0 μg/mL).

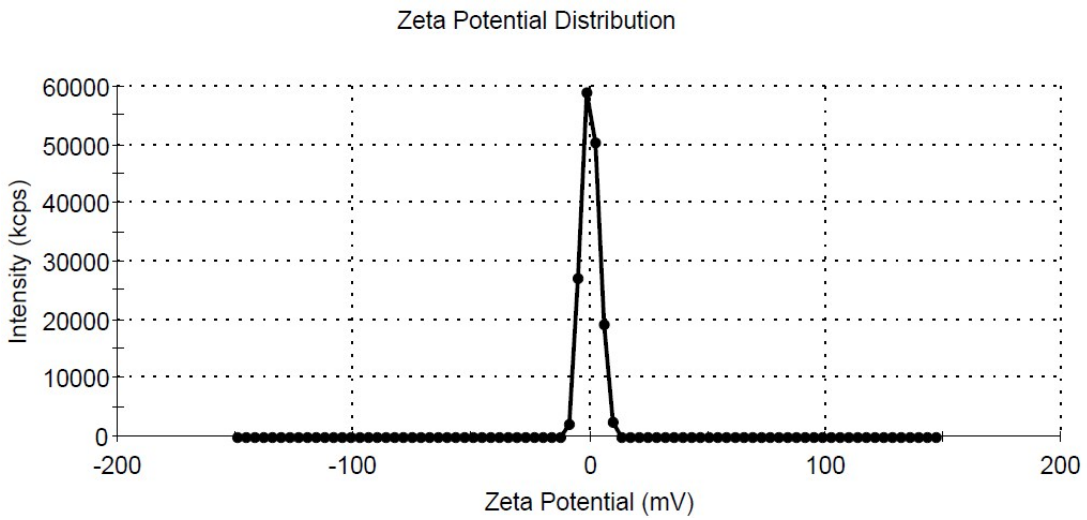


Figure S12 Zeta potential distribution of GO-KH550-DNDT.

Notes and references

[1] F. Zhang, H. Jiang, X. Wu, Z. Mao and H. Li, *ACS. Appl. Mater. Inter.*, 2015, **7**, 1669.

[2] X. Wang, Y. Yang, Y. Zuo, F. Yang, H. Shena and D. Wu, *Chem. Commun.,* 2016, **52**, 5320.