Electronic Supplementary Material (ESI) for Materials Advances.

Supporting Information

Rational design of a cancer-specific and lysosome-targeted fluorescence nanoprobe for glutathione imaging in living cells

Hong Wang, [a,d] *Peisheng Zhang,** [a, b] *Chonghua Zhang,* [a] *Shu Chen,* [a] *Rongjin Zeng,* [a] *Jiaxi Cui* [c, d] *and Jian Chen,** [a, e]

[a] *Key Laboratory of Theoretical Organic Chemistry and Functional Molecule of Ministry of Education, Hunan Provincial Key Laboratory of Controllable Preparation and Functional Application of Fine Polymers, Hunan Province College Key Laboratory of QSAR/QSPR, Institute of Functional Materials, School of Chemistry and Chemical Engineering, Hunan University of Science and Technology, Xiangtan, Hunan, 411201, China.*

[b] *State Key Laboratory of Chemo/Biosensing and Chemometrics, Hunan University, Changsha 410082, P. R. China.*

[c] *INM-Leibniz Institute for New Materials, Campus D2 2, 66123 Saarbrücken, Germany.*

[d] *Institute of Fundamental and Frontier Sciences, University of Electronic Science and Technology of China, Chengdu, Sichuan, 610054, China*

[c]*State Key Laboratory of Luminescent Materials and Devices, South China University of Technology, Guangzhou 510640, P. R. China.*

* Corresponding authors: pshzhang07@gmail.com (P. Zhang); cj0066@gmail.com (J. Chen)

EXPERIMENTAL SECTION

Synthetic route of PEO_{113}-*b*-P($AEMH_{11}$-*co*-St_{25})

PEO_{113}-TTC (200 mg, 0.04 mmol), Styrene (St, 750 mg, 7.2 mmol), 2-aminoethyl methacrylate hydrochloride (AEMH, 104 mg, 0.8 mmol) and 2,2'-Azoisobutyronitrile (AIBN, 1.6 mg, 0.01 mol) were dissolved in 3 mL N,N-Dimethylformamide (DMF). After three times of freeze-pumpthaw cycle, the mixture was stirred under 90 °C for 4 hours. The copolymer was precipitated in diethyl ether (40 mL) to remove the unreacted small molecule compounds. After filtered and dried at 30 °C under vacuum for 48 hours, the polymer PEO_{113}-*b*-P($AEMH_{11}$-*co*-St_{25}) was obtained (pale yellow powder, 300 mg, yield: 63%, Mn = 12.42 kDa, Mw/Mn = 1.10, Figures 1, Figure S2 and scheme S1).

Synthetic of Synthetic of PEO_{113}-*b*-P($AEFA_2$-*co*-$AEMH_9$-*co*-St_{25})

Folic acid (FA, 7 mg, 0.15 mmol), N-hydroxysuccinimide (NHS, 20 mg, 0.09 mmol) and 1-(3-dimethylaminopropyl)-3-ethyl-carbodiimide hydrochloride (EDC, 15.3 mg, 0.08 mmol) were dispersed in 2 mL of DMF. After 1 h, **PEO_{113}-*b*-P($AEMH_{11}$-*co*-St_{25})** (50 mg) was dissolved in 1 mL DMF and then added into the solution. Then, the mixtures were stirred at room temperature for 24 h. The solution was precipitated in methanol (20 mL) to remove the small molecule compounds. After filtered and dried at 30 °C under vacuum for 48 hours, the polymer PEO_{113}-*b*-P($AEFA_2$-*co*-$AEMH_9$-*co*-St_{25}) was obtained (bright yellow powder, 30 mg).

Preparation of dual-targeting fluorescence nanoprobe (DTFN)

In the first step, a THF mixture solution containing 2 mg/mL of

PEO_{113}-*b*-P($AEFA_2$-*co*-$AEMH_9$-*co*-St_{25}) and 0.2 mg/mL of *p*-DTPACO was prepared. A 1 mL aliquot of the mixture was quickly injected into 10 mL of water under vigorous sonication by ultrasound. THF was removed by dialysis through a porous cellulose membrane (MWCO 1000) toward an aqueous solution and filtered using a 0.2 μm syringe driven filter. The FA-modified photostable aggregation-induced emission dots were obtained.

In the second step, an appropriate amount of the above as-prepared FA-modified photostable aggregation-induced emission dots and 150 μL of 60 mg/mL MnO_2 nanosheets were mixed together for 30 min, and the **DTFN** were prepared.

Fluorescence and absorption assay of GSH in buffered solution (pH 7.4)

Fluorescence measurements were performed in 10 mM PBS buffered solution (pH 7.4), according to the following procedure. In a quartz cell, 2.7 mL of PBS and 0.3 mL of **DTFN** were mixed. Subsequently, this solution was added to various concentrations of GSH (0 μM, 30 μM, 50 μM, 100 μM, 150 μM, 180 μM, 200 μM, 240 μM, 270 μM, 300 μM, 330 μM, 380 μM, 400 μM, 450 μM, 500 μM, 550 μM, 600 μM), respectively. And the mixture was equilibrated for 10 min before measurement. The fluorescence intensity was measured synchronously (λ_{ex} = 420nm, λ_{em} = 523 nm).

Cell viability assay

To examine the toxicity of **DTFN** in living cells, HeLa cells were incubated in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and allowed to grow for 24 h at 37 °C with 5% CO_2. After removal of the medium, cells were treated

with the various concentrations of **DTFN** (0~600 μg/mL) and incubated for an additional 24 h. The cytotoxicity of the probe against HeLa cells was assessed by MTT assay according to ISO 10993-5.

Cell Imaging

For the cellular uptake experiments, the HeLa cells and A549 cells were incubated in RPMI1640 medium containing **DTFN** (0.12 mg/mL) for 180 min at 37 °C, respectively. After washing with PBS for three times, all fluorescence images were acquired on confocal fluorescence microscopy analysis.

For the co-localization imaging, the HeLa cells were incubated in RPMI1640 medium containing **DTFN** (0.12 mg/mL) for 180 min at 37 °C and then Lyso-Tracker Red (200 nM) or Mito-Tracker Red (200 nM) for 0.5 h at 37 °C. After washing with PBS for three times, all fluorescence images were acquired on confocal fluorescence microscopy analysis.

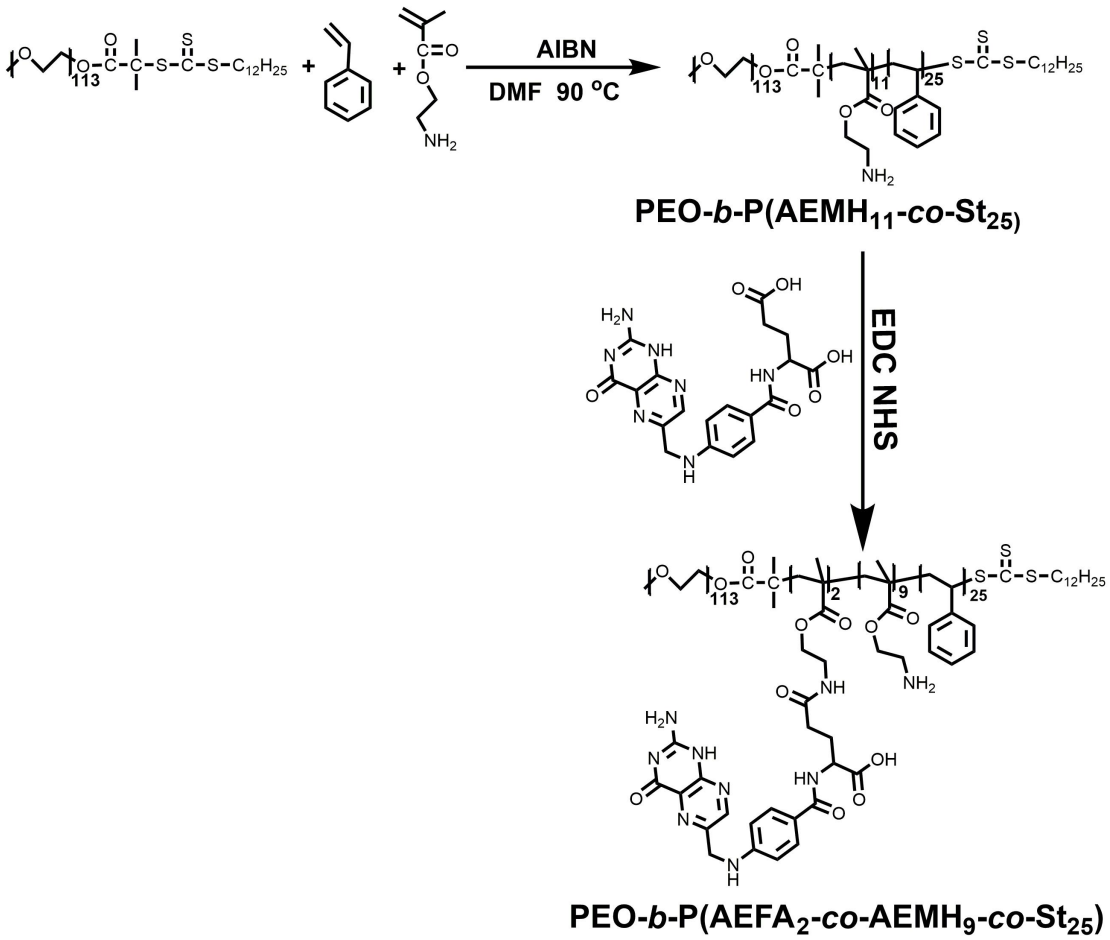
For the imaging of endogenous GSH, HeLa cells were incubated in the RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and allowed to grow for 24 h at 37 °C with 5% CO_2. After 80% confluence, the adherent cells were washed using 1× PBS buffer for two times. Before analysis, the cells were incubated in RPMI1640 medium containing **DTFN** (0.12 mg/mL) for 0 min, 30 min, 60 min, 120 min, 180 min, respectively. Some HeLa cells were treated with RPMI1640 medium containing inhibitor N-ethylmaleimide (NEM, 100 μM) for 30 min, and then incubated with **DTFN** (0.12 mg/mL) for 180 min. After washing with PBS for three times, all fluorescence images were acquired on confocal fluorescence microscopy

analysis.

Measurements

^{1}H NMR spectra was recorded on a 500 MHz NMR spectrometer (Bruker Avance). The number average molecular weight (M_n) and polydispersity (M_w/M_n) were measured at 30 °C on a Waters 2410 gel permeation chromatography (GPC) using THF as the eluent (1.0 mL/min) and polystyrene as the standard. The calibration curve was established by using polystyrene (PS) as the standard. The nanoparticle diameters were determined by a Malvern Nano-ZS90 instrument and their morphology was observed with a JEM-2100F transmission electron microscope (TEM, JEOL USA, Inc.). UV-Vis spectra were recorded on a Shimadzu UV-2501PC spectrophotometer at room temperature. Fluorescence spectra were recorded on a Shimadzu RF-5301PC fluorescence spectrophotometer at room temperature. The solid content of nanoparticles was estimated by gravimetric analysis.

Results and Discussion



Scheme S1. Synthetic route of **PEO_{113}-*b*-P($AEFA_2$-co-$AEMH_9$-*co*-St_{25})**.

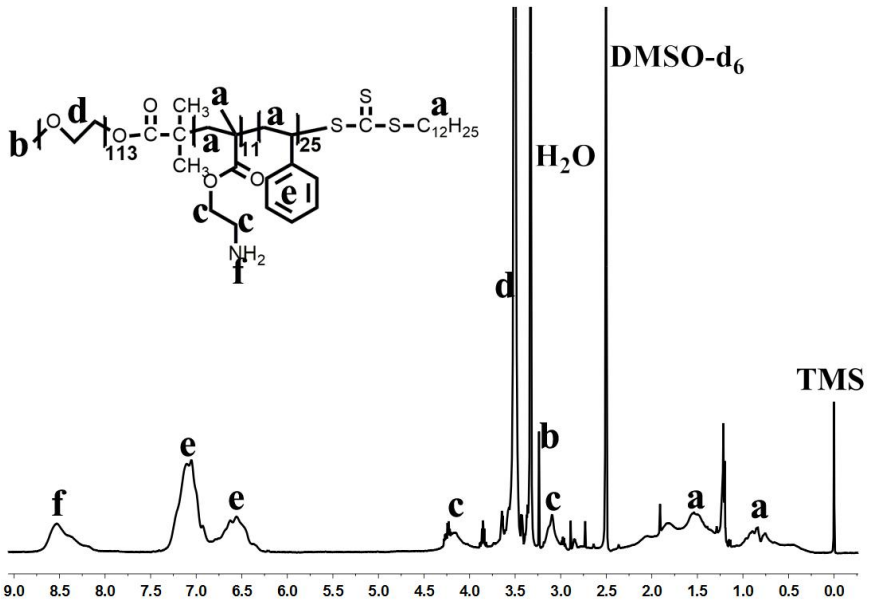


Figure S1. ^{1}H-NMR spectrum (in DMSO-d_6) of **PEO_{113}-*b*-P(AEMH_{11}-*co*-St$_{25}$)**.

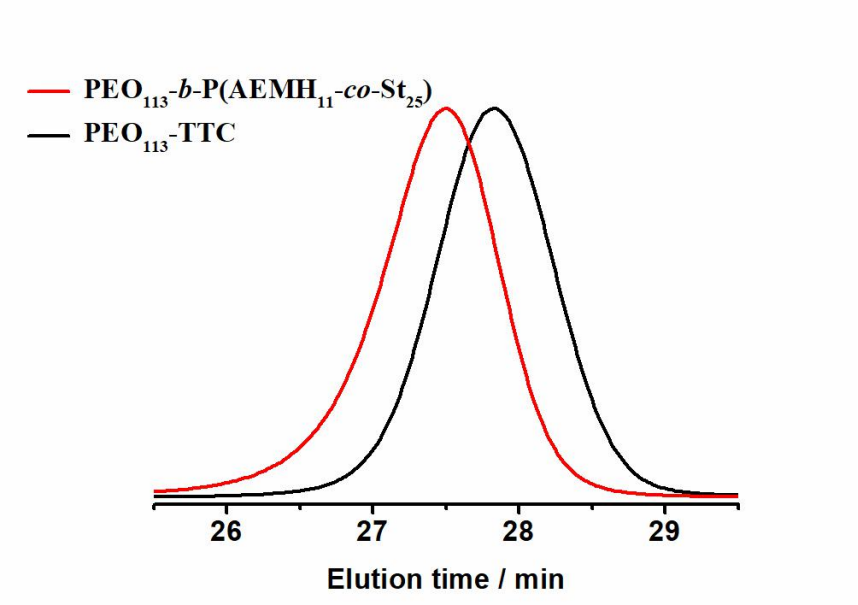


Figure S2. GPC trace of **PEO_{113}-TTC**, **PEO_{113}-*b*-P($AEMH_{11}$-*co*-St_{25}).**

Table S1. Molecular weight distribution data of starting linear polymers.

Sample	$M_{n,GPC}$[a]	$M_{w,GPC}$[a]	PDI	$M_{n,NMR}$[b]
PEO_{113}-TTC	8403	8778	1.04	5450
PEO_{113}-*b*-P($AEMH_{11}$-*co*-St_{25})	12424	13749	1.10	9530

[a]The data were acquired using SEC based on a polystyrene calibration curve and obtained from GPC analys was using THF as eluent at a flow rate of 1.0 mL/min.

[b] $M_{n,NMR}$ values were determined by ^1H NMR.

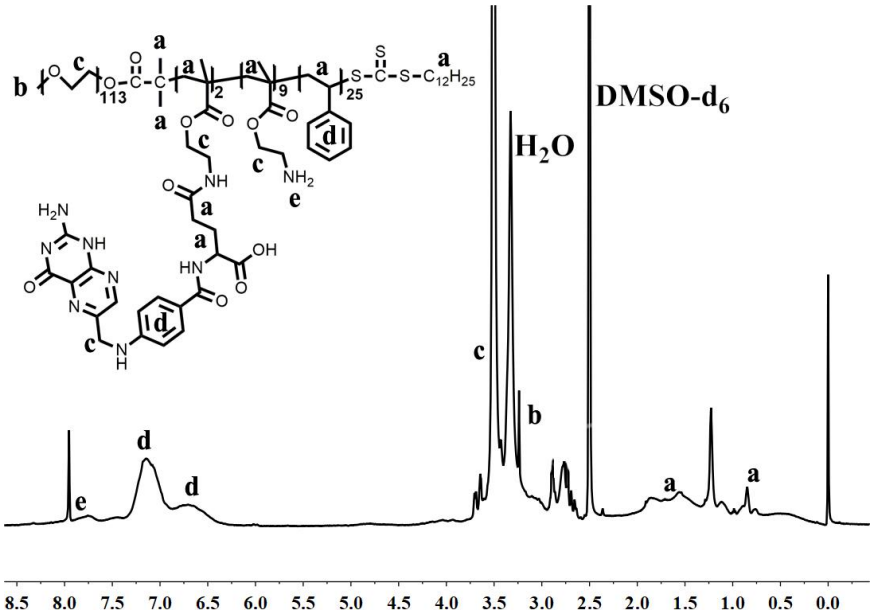


Figure S3. ^{1}H-NMR spectrum (in DMSO-d_6) of **PEO$_{113}$-*b*-P(AEFA$_2$-*co*-AEMH$_9$-*co*-St$_{25}$)**.

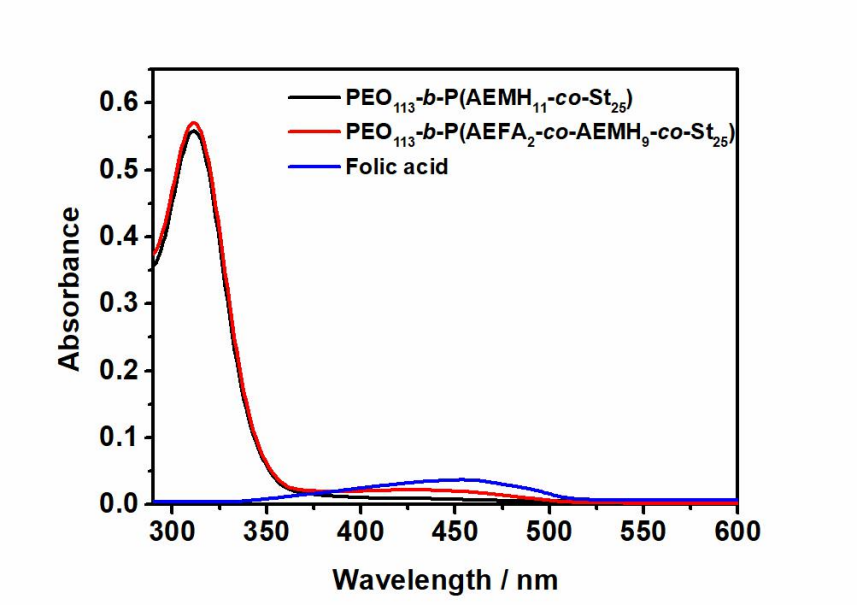


Figure S4. Absorption spectra for **PEO_{113}-*b*-P($AEMH_{11}$-*co*-St_{25})**, **PEO_{113}-*b*-P($AEFA_2$-co-$AEMH_9$-*co*-St_{25})** and Folic acid in DMF.

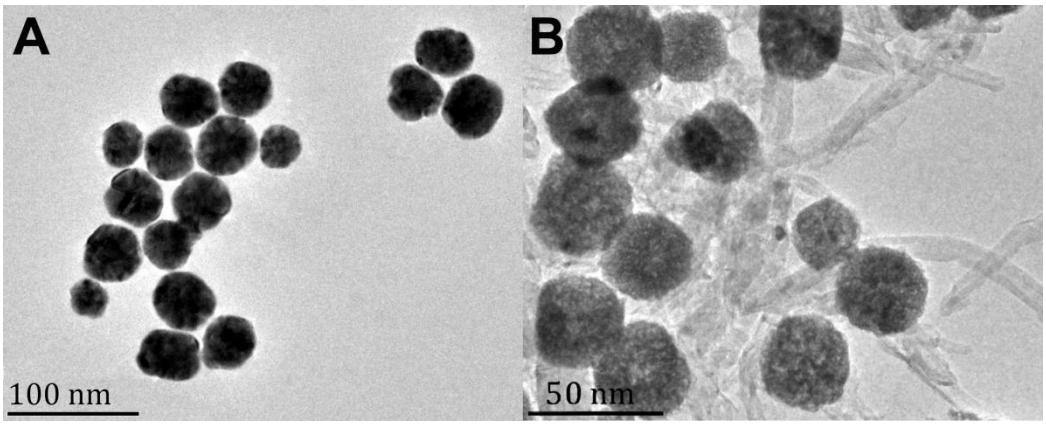


Figure S5. TEM characterization of (A) FA-modified AIE dots and (B) **DTFN**.

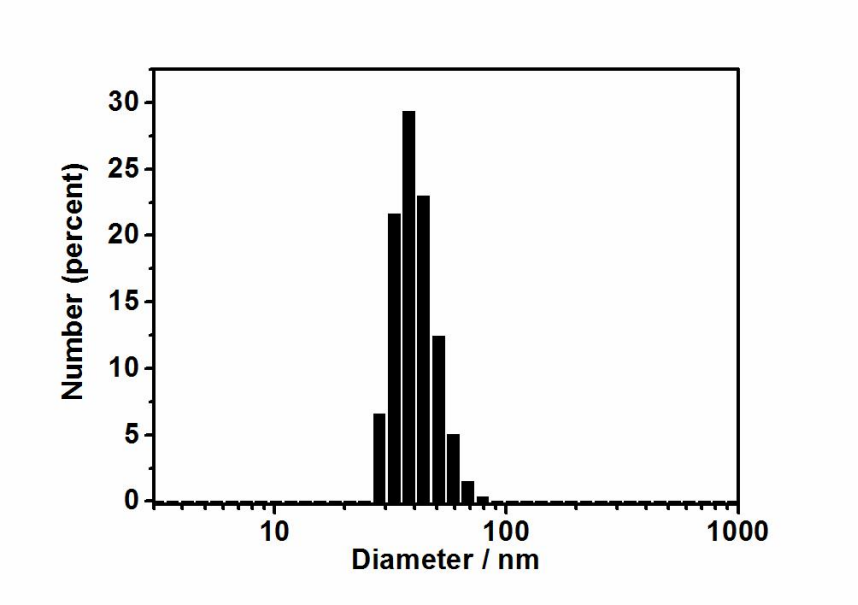


Figure S6. Average diameter of FA-modified AIE dots was measured by DLS.

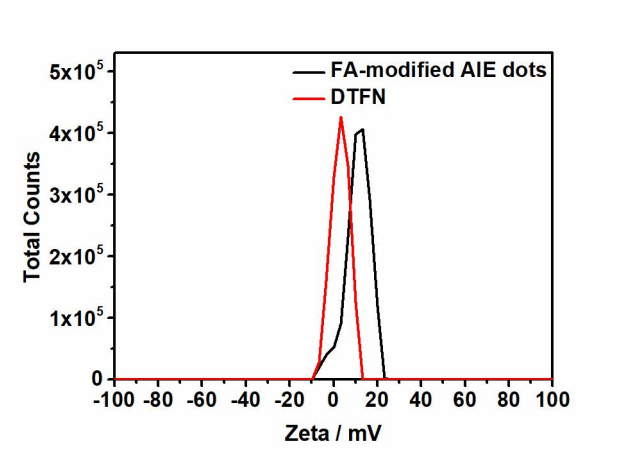


Figure S7. Zeta potential of FA-modified AIE dots (black line, +11.2 mV) and **DTFN** (Red line, +3.29 mV).

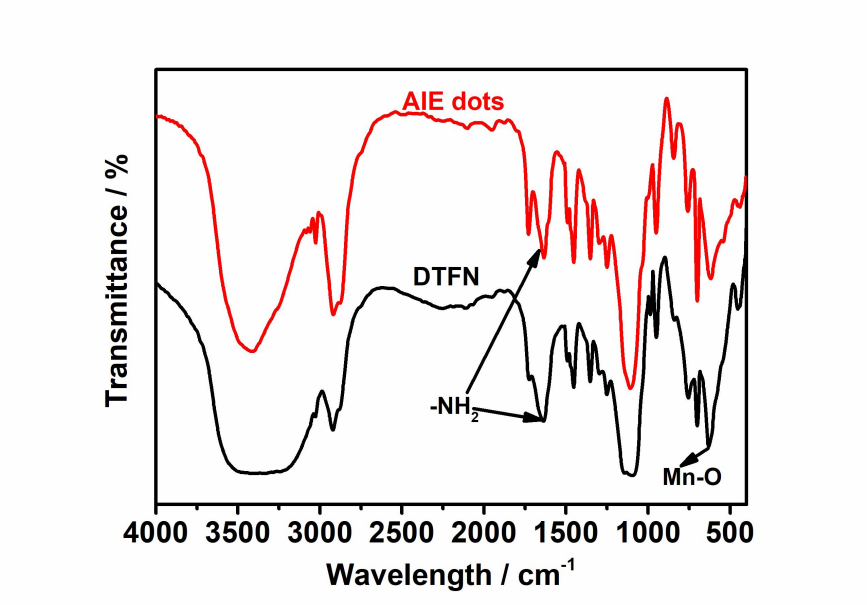


Figure S8. The FT-IR of FA-modified AIE dots and **DTFN**.

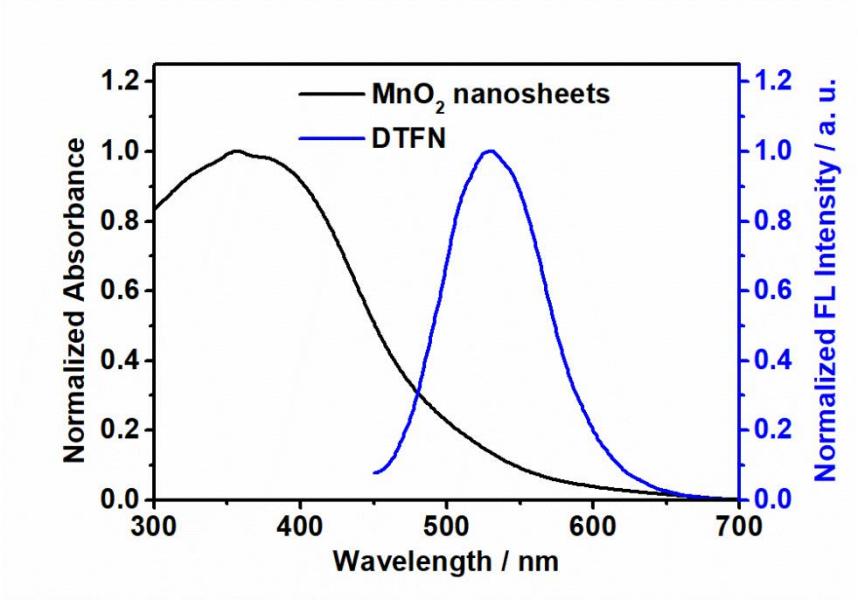


Figure S9. Normalized fluorescence emission spectrum of FA-modified AIE dots (blue curve) and normalized absorption spectrum of MnO_2 nanosheets (black curve).

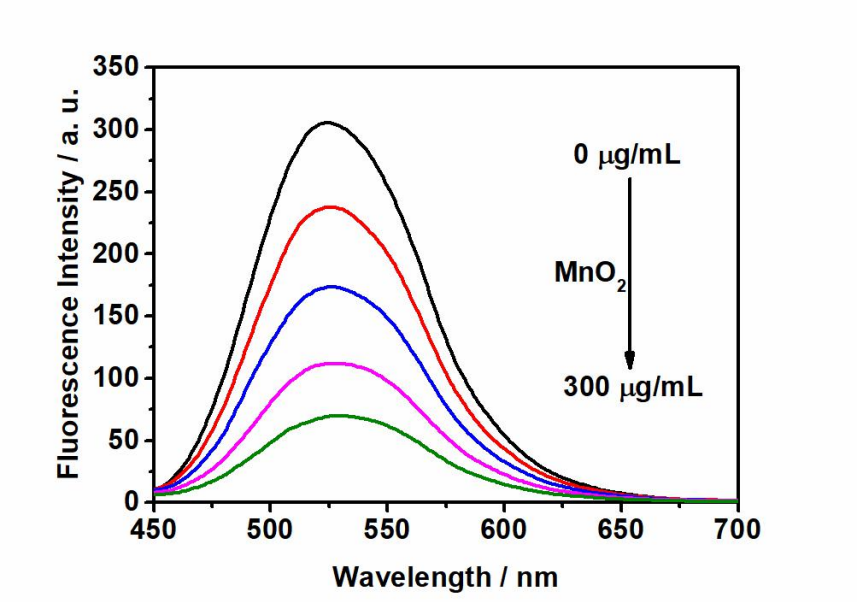
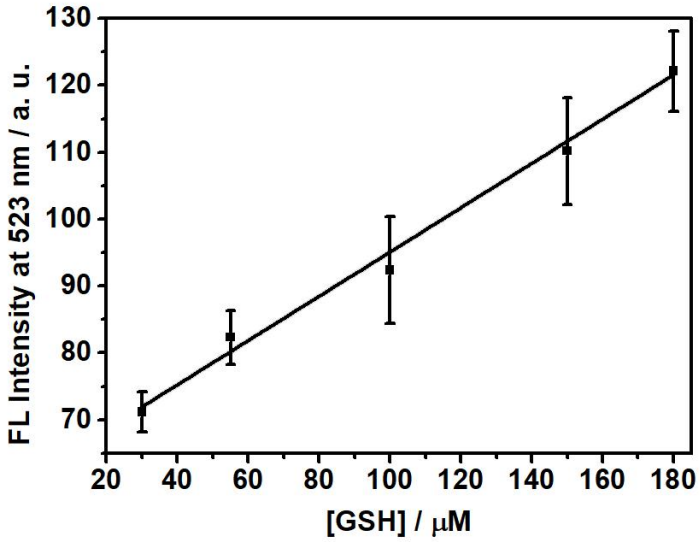


Figure S10. Fluorescence emission spectra of FA-modified AIE dots (12 μg/mL) in the different concentration of MnO_2 nanosheets (0~300 μg/mL).



Figue S11. Fluorescence intensity of **DTFN** as a function of GSH concentration in buffer solution.

Determination of the detection limit:

Fluorescence:

First the calibration curve was obtained from the plot of fluorescence intensity (I_{522}) as a function of GSH concentration. The regression curve equation was then obtained for the lower concentration part.

The detection limit = 3 ×σbi / *m*

where *m* is the slope of the curve equation, and σbi represents the standard deviation for the fluorescence intensity (I_{522}) of **DTFN** in the absence of GSH.

I_{522}= 0.3309 × [GSH] + 62.0005

LOD = 3 × 0.114/ 0.3309 = 1.03 μM.

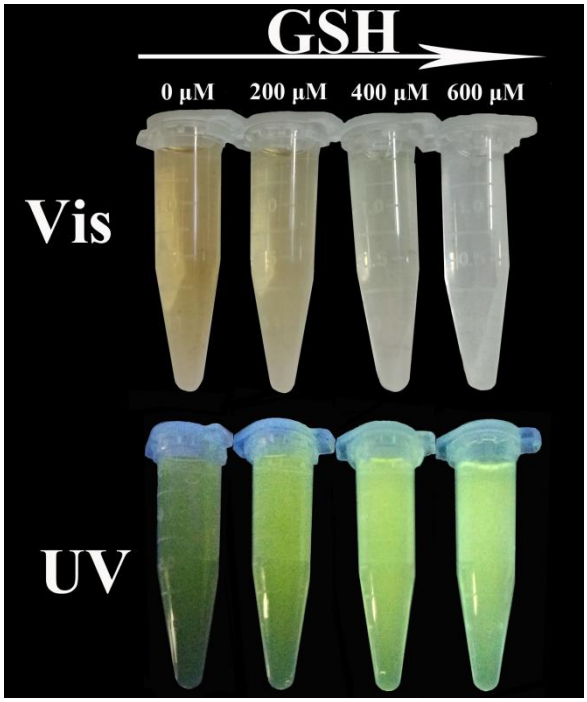


Figure S12. The photos of **DTFN** towards different GSH concentrations under visible light (up) and UV light (down).

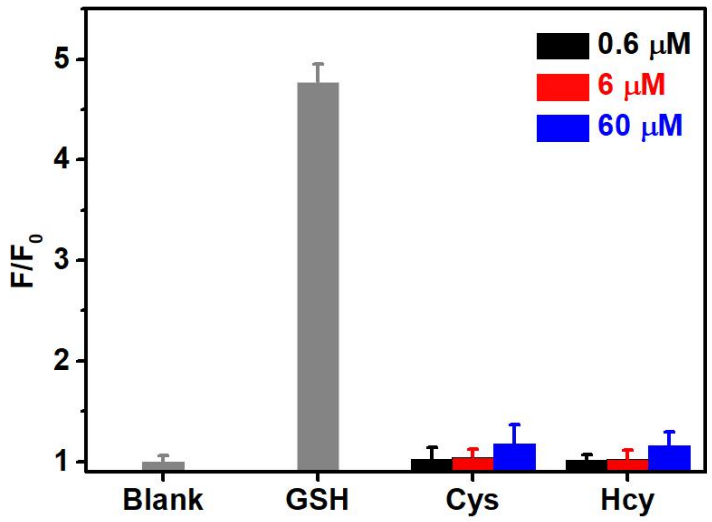
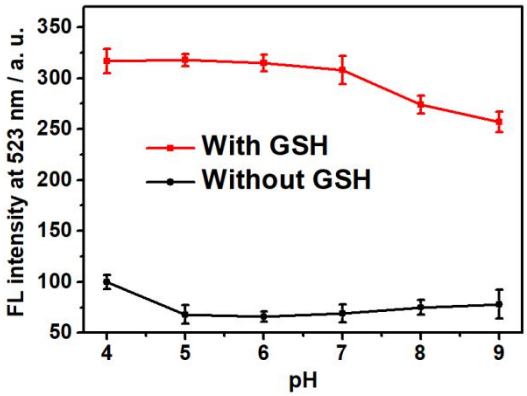
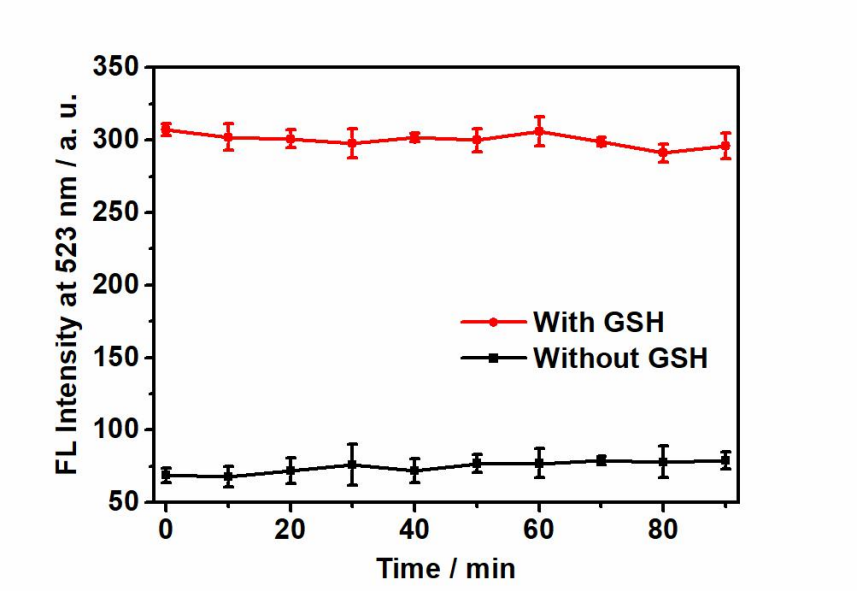


Figure S13. The effect of Cys and Hcy with different concentrations (from one thousandth to one tenth relative to GSH on detection of GSH (600 μM).



Figue S14. Effect of pH on the fluorescence intensity of **DTFN** without (black line) and with (red line) GSH (600 μM).



Figue S15. Fluorescence intensity (I_{523}) of **DTFN** without (black line) and with (red line) GSH (600 μM) under a continuous 365 nm UV lamp irradiation (2.8 mW/cm^2).

Table S2. Determination of GSH in blood serum

Sample[a]	Added amount of GSH (μM)	Found amount of GSH (μM)	RSD (n=5, %)	Recovery (%)
Blood serum	100.00	90.67	0.32	90.67
	150.00	145.06	0.41	96.71
	200.00	195.51	0.28	97.76
	300.00	296.14	0.17	98.71
	350.00	366.14	0.31	104.61
	400.00	412.08	0.55	103.02

[a]In the test solutions, blood serum solution was 1000-fold diluted before used. The measurements were conducted instantly after the addition of GSH.

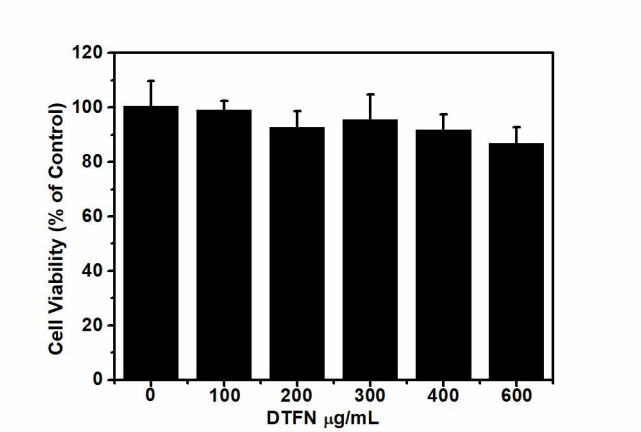


Figure S16. Cell viability for Hela cells in the presence of **DTFN** at varied concentrations. The results are the mean standard deviation of eight separate measurements.

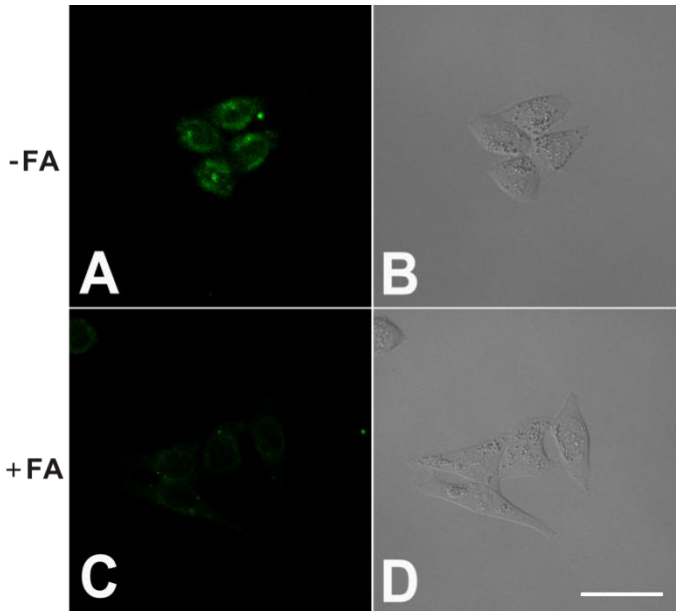


Figure S17. Confocal fluorescence microscopic images of HeLa cells incubated with **DTFN** in FA-free (A, B) and FA-containing (C, D) culture medium. Scale bar: 20 μm.

Table S3. Comparison of the recently reported GSH fluorescent probes

Probes	AIE Characteristics	Solution	Targeting	Reference
1	NO	PBS (pH 7.4, 50% DMSO)	--	Sens. Actuators B 2017, 253, 42-49.
1+Cu^{2+}	NO	HEPES(pH 7.4, 10 % DMSO)	--	J. Mater. Chem. B K. 2017, 5, 8780-8785.
QG-1	NO	PBS (pH 7.4, 1 % DMSO)	--	Angew. Chem. Int. Ed. 2017, 56, 5812-5816.
Cy-NTe	NO	PBS (pH 7.4, 10 % acetonitrile)	--	J. Am. Chem. Soc. 2013, 135, 7674-7680
KC-Br	NO	PBS (7.4, 2% DMF)	--	ACS Appl. Mater. Interfaces 2015, 7, 12809-12813
1a	YES	PBS (pH 7.4)	--	ACS Appl. Mater. Interfaces 2018, 10, 12141-12149.
Rh-COOH	NO	PBS (pH 7.4)	--	Sens. Actuators B 2017, 240, 1165-1173.
1b	NO	PBS (pH 7.4, 10 % DMSO)	Lysosome	Chem. Commun. 2016, 52, 721-724.
Ru-2	NO	HEPES (pH 7.0, 50% DMSO)	Lysosome	Anal. Chem. 2017, 89, 4517−4524
Cy-S-Np	NO	PBS (pH 7.4, 1% DMSO)	Lysosome/ Mitochondria	Sens. Actuators B 2019, 290, 676-683.
prodrug 4	NO	HEPES (pH 7.4)	Mitochondria	Anal. Chem. 2016, 88, 6450-6456.
IQDC-M)	NO	PBS (pH 7.4)	Mitochondria	Biosens. Bioelectron. 2016, 85, 96-102.
Mitochondria	NO	HEPES (pH 7.4, 10%DMSO)	Mitochondria	Chem 2018, 4, 1609-1628.
PDs-MnO_2	NO	Tris-HCl (pH 7.4)	--	Sens. Actuators B 2018, 258, 25-31.
TP-MSNs@ MnO_2	NO	PBS (pH 7.4)	--	Anal. Chem. 2014, 86, 12321-12326.
complex 1	NO	Tris-HCl buffer (pH 7.2)	--	Nanoscale 2017, 9, 4677-4682.
MnO_2-PFR	NO	Ultrapure water	--	Biosens. Bioelectron. 2016, 77, 299-305.
CyA-cRGD	NO	HEPES (pH 7.4)	Tumor cells	ACS Appl. Mater. Interfaces 2018, 10, 30994-31007
Probe 1	NO	PBS (pH 7.4)	Tumor cells	J. Am. Chem.Soc. 2012, 134, 1316-1322
DTFN	YES	PBS (pH 7.4)	Tumor cells/ Lysosome	This work