Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supplementary Information

A ratiometric fluorescent nanoprobe for H2O2 sensing and in vivo detection of drug-induced oxidative damage to digestive system

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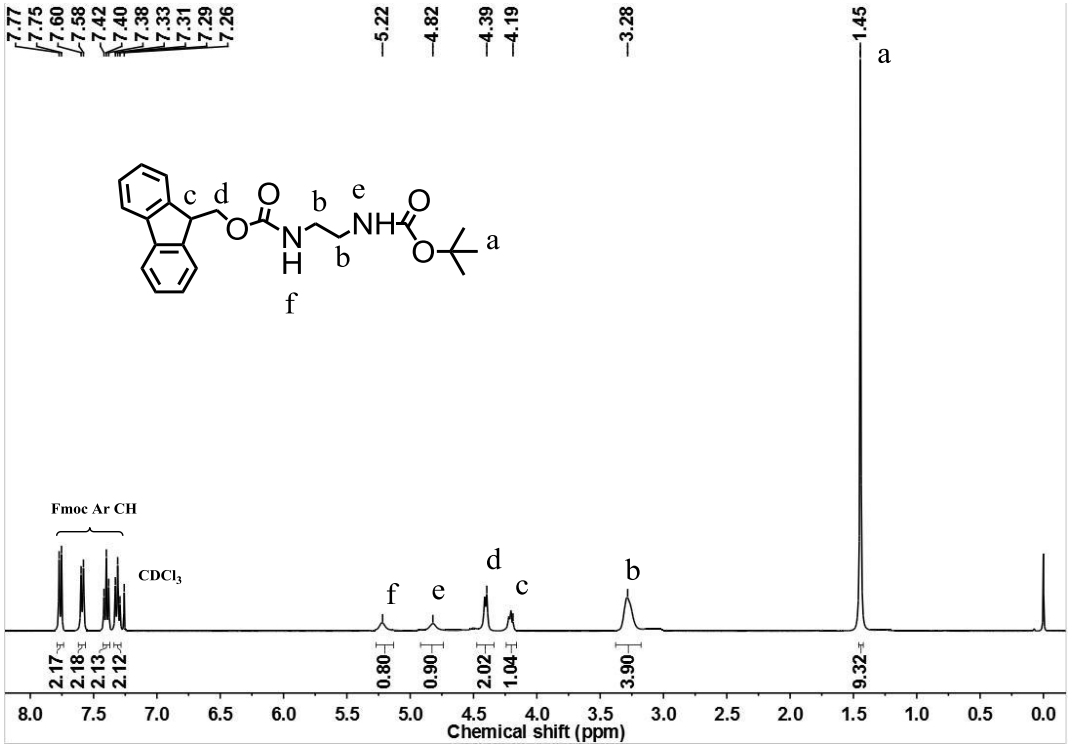


Fig. S1 ^{1}H NMR ($CDCl_3$) spectrum of **Fmoc-EDA-Boc**.

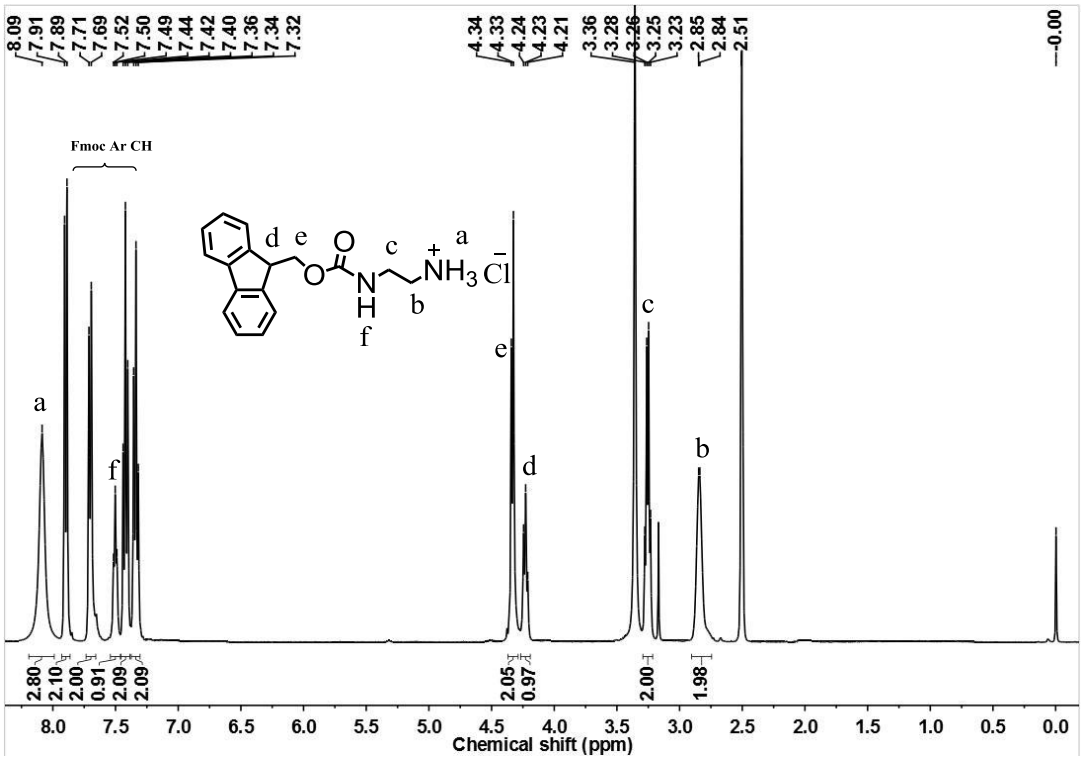


Fig. S2 ^{1}H NMR (DMSO-d_6) spectrum of **Fmoc-EDA-NH_2•HCl.**

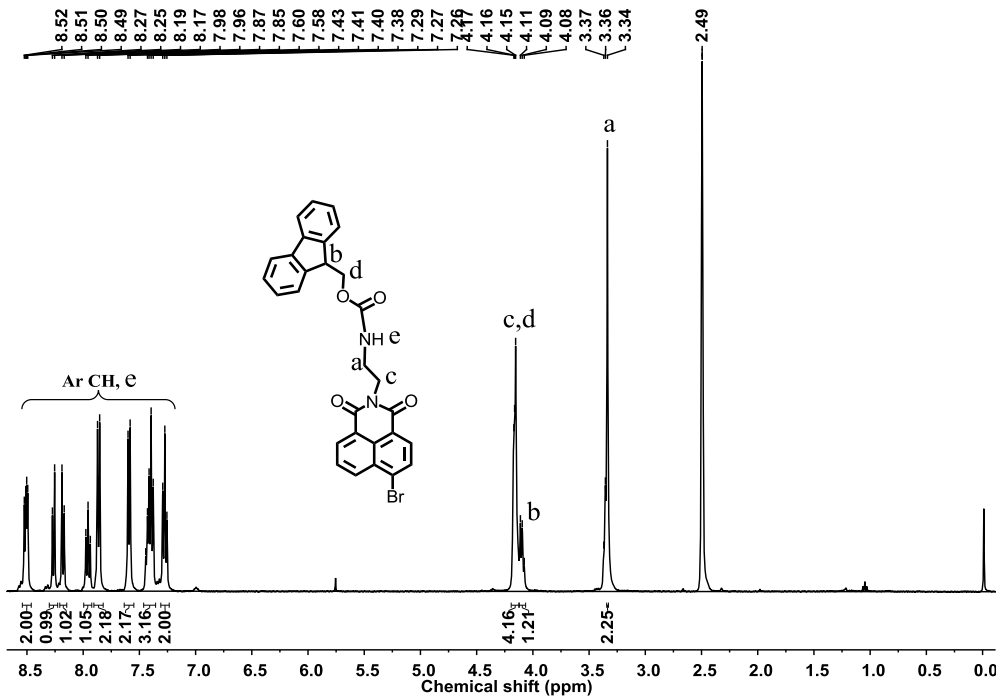


Fig. S3 ^{1}H NMR (DMSO-d_6) spectrum of **Fmoc-NP-Br.**

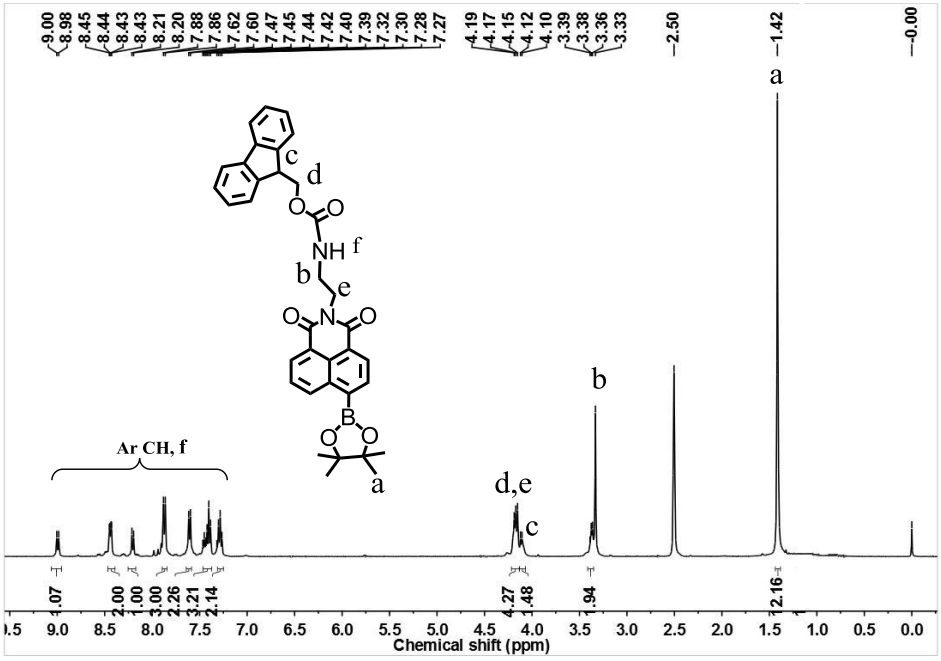


Fig. S4 ^{1}H NMR (DMSO-d_6) spectrum of **Fmoc-NP-BE.**

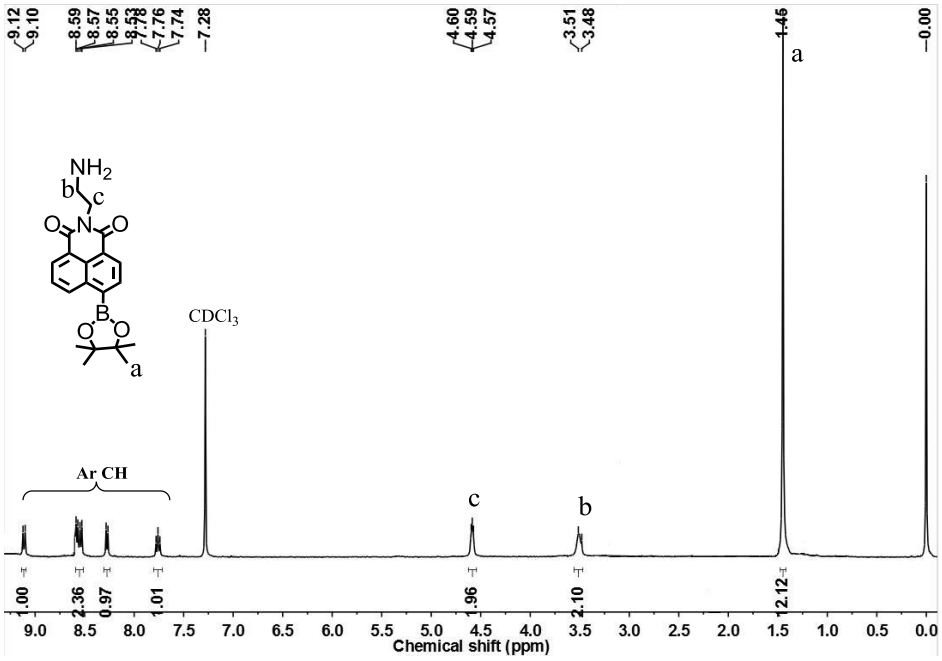


Fig. S5 ^{1}H NMR ($CDCl_3$) spectrum of **NP-BE.**

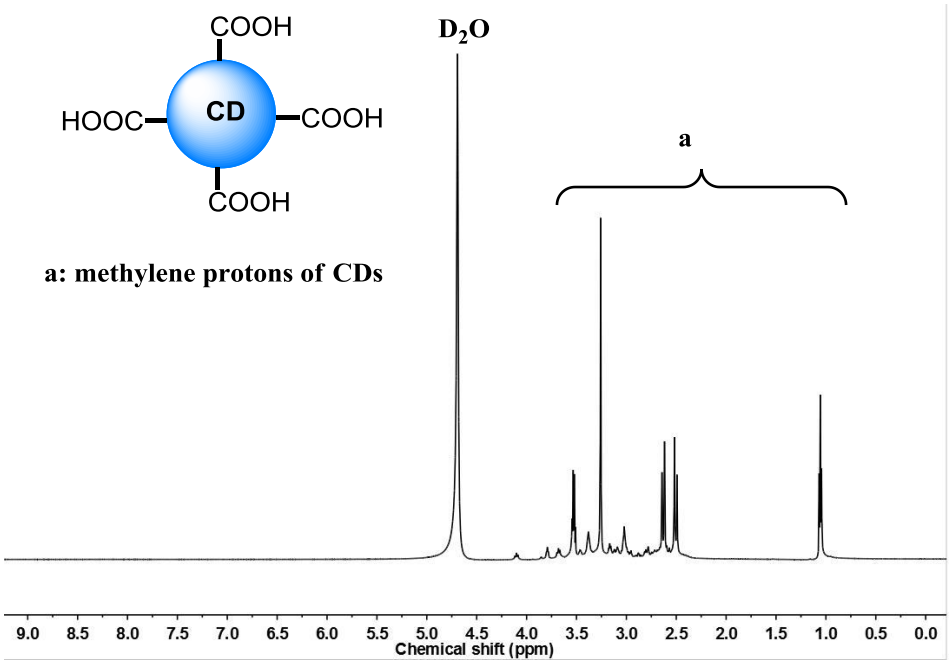


Fig. S6 ^{1}H NMR (D_2O) spectrum of carboxyl-coated CDs.

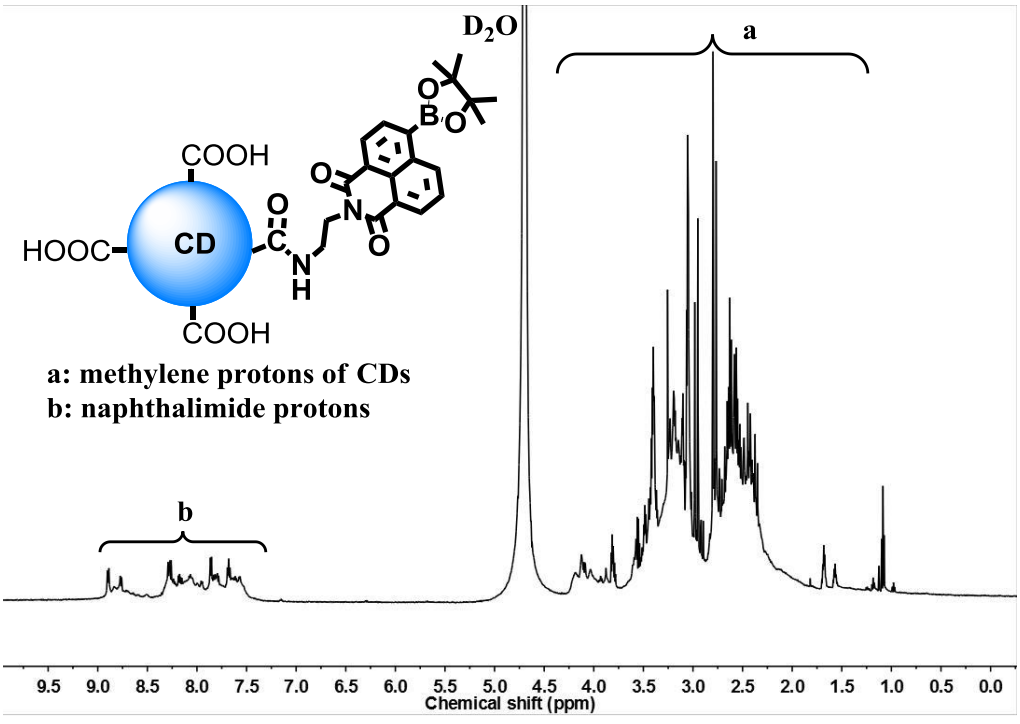


Fig. S7 ^{1}H NMR (D_2O) spectrum of nanoprobe **CD-NP-BE.** b indicates the aromatic protons in naphthalimide moieties incorporated on the CDs.

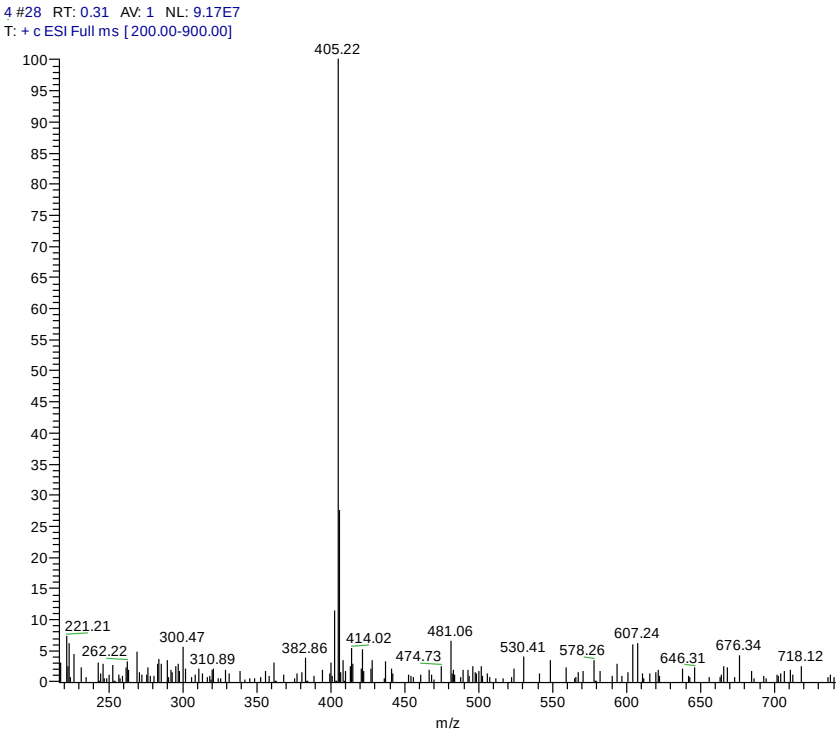


Fig. S8 MS (ESI) spectrum of **Fmoc-EDA-Boc**

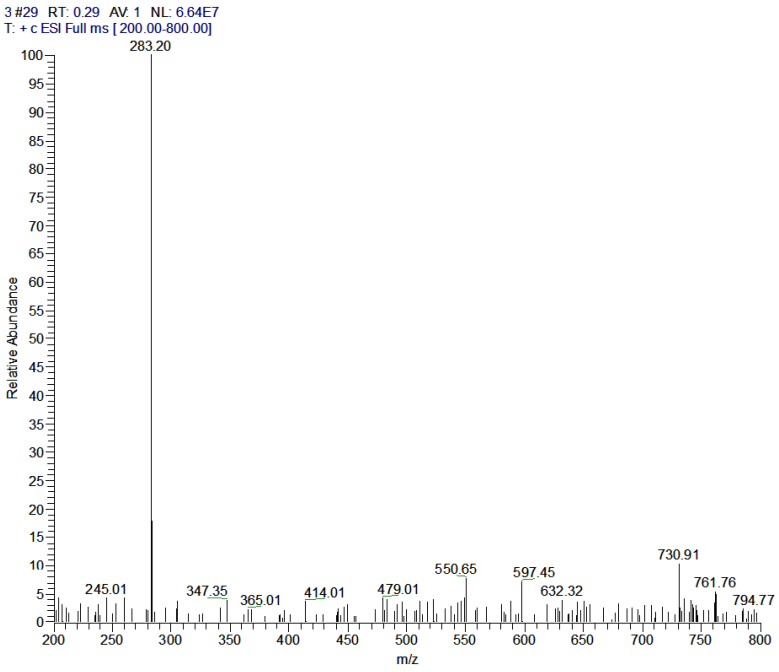
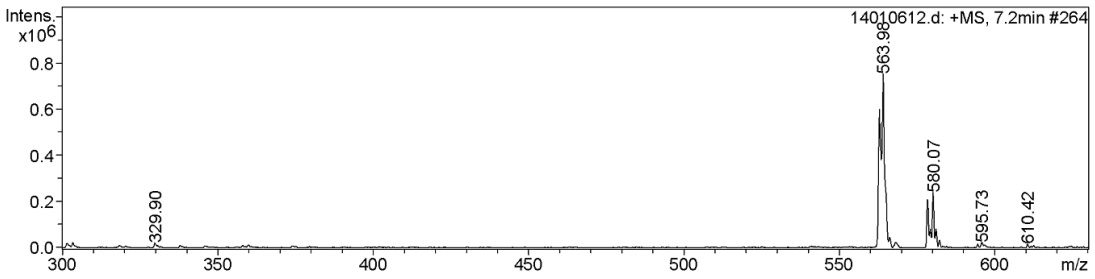
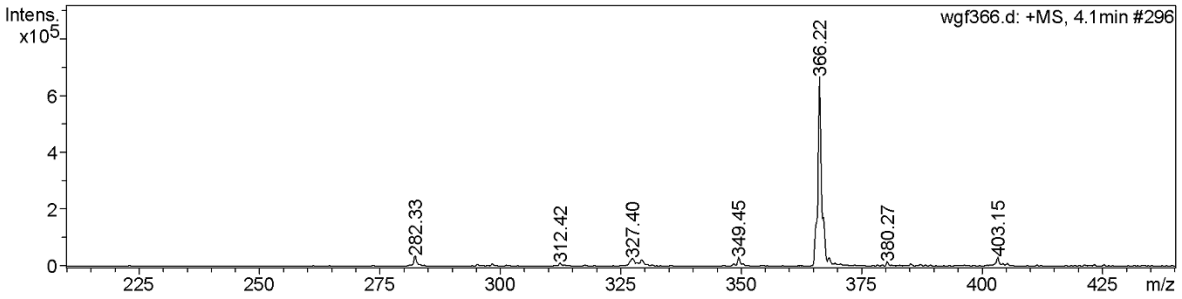


Fig. S9 MS (ESI) spectrum of **Fmoc-EDA-NH_2•HCl**



#	m/z	I	I %
1	562.82	597932	79.4
2	563.98	752910	100.0
3	566.09	44096	5.9
4	567.96	22099	2.9
5	578.29	206802	27.5
6	579.13	80007	10.6
7	580.07	239138	31.8
8	581.03	79337	10.5
9	582.09	31750	4.2
10	595.73	21987	2.9

Fig. S10 MS (ESI) spectrum of **Fmoc-NP-Br**



#	m/z	I	I %
1	282.33	35494	5.3
2	312.42	11897	1.8
3	327.40	26558	4.0
4	328.28	11748	1.8
5	329.34	21317	3.2
6	349.45	30701	4.6
7	366.22	667273	100.0
8	368.15	29284	4.4
9	380.27	15706	2.4
10	403.15	30676	4.6

Fig. S11 MS (ESI) spectrum of **NP-BE**.

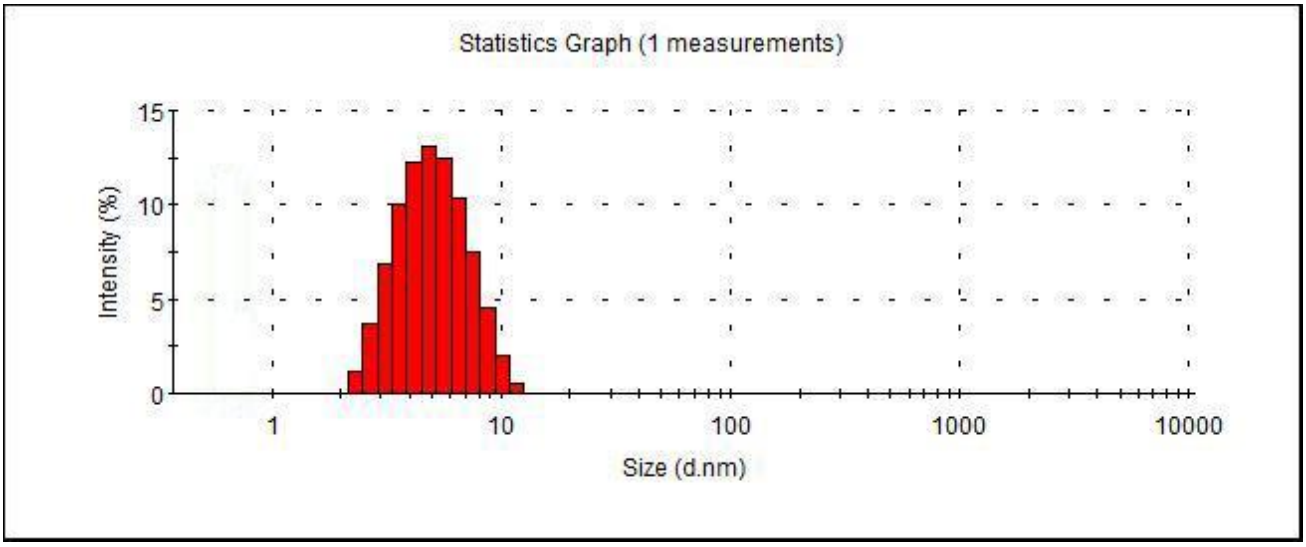


Fig. S12 Size (diameter) distribution of the nanoprobe before vacuum drying as determined by dynamic light scattering.

Calculation of the amount of NP-BE conjugated onto CDs:

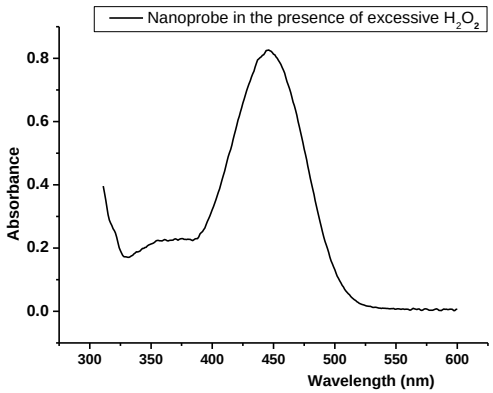


Fig. S13a Absorption spectra of the nanoprobe in the presence of excessive H_2O_2 in pH 7.4 HEPES buffered water.

The amount of NP-BE in the nanoprobe are calculated based on the additivity of absorbance of the CD moieties and the probe.

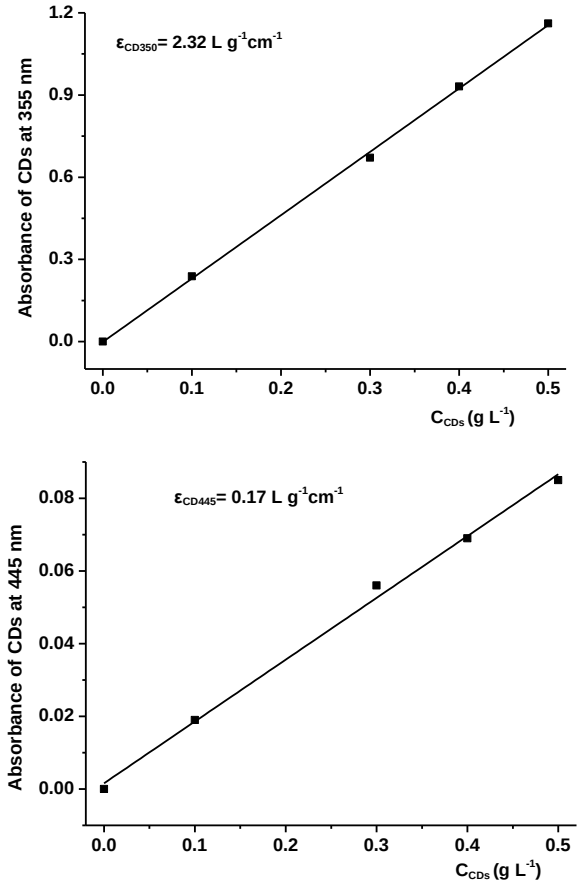
The carbon-dot in the nanoprobe exhibit a characteristic absorption band at 350 nm; and NP-BE moieties also exhibit a characteristic absorption band at 350 nm in the absence of H_2O_2; while in the presence of H_2O_2, NP-BE turns into 4-hydroxy-1,8-naphthalimide and displays characteristic absorption at 445 nm (see **Fig.** S13a). In order to determine the amount of the probe in the nanoprobe, excessive amount of H_2O_2 was added into the nanoprobe solution to transform NP-BE into 4-hydroxy-1,8-naphthalimide (namely, the energy acceptor, **EA**).

The following equation set (based on Beer's law) is used to calculate the conjugated amount of NP-BE:

$$A_{350nm} = \varepsilon_{CDs350} \times C_{CDs} \times b + \varepsilon_{EA350} \times C_{EA} \times b$$

$$A_{445nm} = \varepsilon_{CDs445} \times C_{CDs} \times b + \varepsilon_{EA445} \times C_{EA} \times b$$

where *A* is the absorbance of the nanoprobe at different wavelengths; ε and *C* are the absorptivity and the concentration respectively; *b* represents the optical length (1 cm); EA represents energy acceptor (4-hydroxy-1,8-naphthalimide). ε_{CDs350}, ε_{CDs445}, ε_{EA350} and ε_{EA445} can be obtained by measuring the absorption of the plain CDs at different concentrations and the absorption of the 4-hydroxy-1,8-naphthalimide solution at different concentrations. The absorption of the nanoprobe in the presence of excessive amount of H_2O_2 was measured, and by using the above equation, the concentrations of CDs and 4-hydroxy-1,8-naphthalimide was calculated, followed by the determination of the weight of NP-BE on per gram of CDs. All absorbance measurements were performed in a quartz cell of 1 cm optical length.



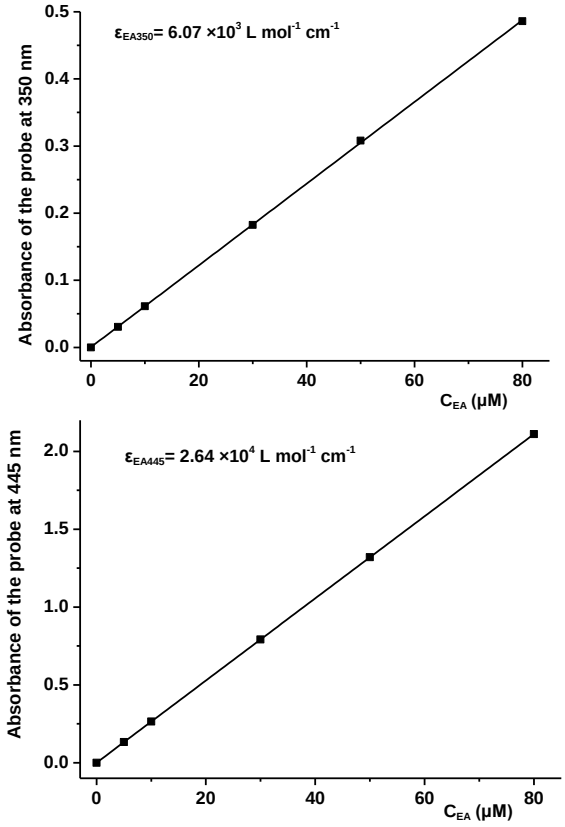


Fig. S13b Plot of Absorbance for CDs and 4-hydroxy-1,8-naphthalimide vs. concentration.

For Fig. S13a: the concentration of the CD-based probe is 0.05 mg mL^{-1}.

$0.121 = 2.32 \times C_{CDs} \times 1 + 6.07 \times 10^3 \times C_{EA} \times 1$

$0.229 = 0.17 \times C_{CDs} \times 1 + 2.64 \times 10^4 \times C_{EA} \times 1$

Therefore, $C_{CD} = 3.0 \times 10^{-2}$ g L^{-1}, $C_{NP\text{-}BE} = C_{EA} = 8.48 \times 10^{-6}$M

Hence, the amount of the probe conjugated onto CDs is:

$8.48 \times 10^{-6} \times 1 \times 366 \times 10^3$ mg / 0.03 g= 103 mg g^{-1}, that is 0.28 mmol NP-BE/g CDs.

(366 is the molecular weight of NP-BE)

The amount of the probe conjugated to per gram of the CD is calculated as 103 mg g^{-1}.

Determination of detection limit:

The detection limit was calculated based on the fluorescence titration. The nanoprobe concentration was 0.15 mg mL^{-1} and the slit was adjusted to 5 nm/5 nm. The emission intensity of the nanoprobe without H_2O_2 was measured by 5 times and the standard deviation of blank measurements was determined. Under the present conditions, a good linear relationship between the fluorescence intensity and the H_2O_2 concentration could be obtained in the H_2O_2 concentration range of 0 - 10 μM (R=0.998).

The detection limit of the probe was determined from the following equation: **Detection Limit = K × SD/S**, where K = 3; SD is the standard deviation of the blank solution; S is the slope of the calibration curve. I_{550}/ I_{457}= 0.1403 + 6.409 E4 [H_2O_2]

LOD = K × SD/S = 3 × 0.01/ 6.409 E4 = 5 × 10^{-7} (M), namely, the detection limit is determined to be 0.5 μM at S/N = 3 (signal-to-noise ratio of 3).

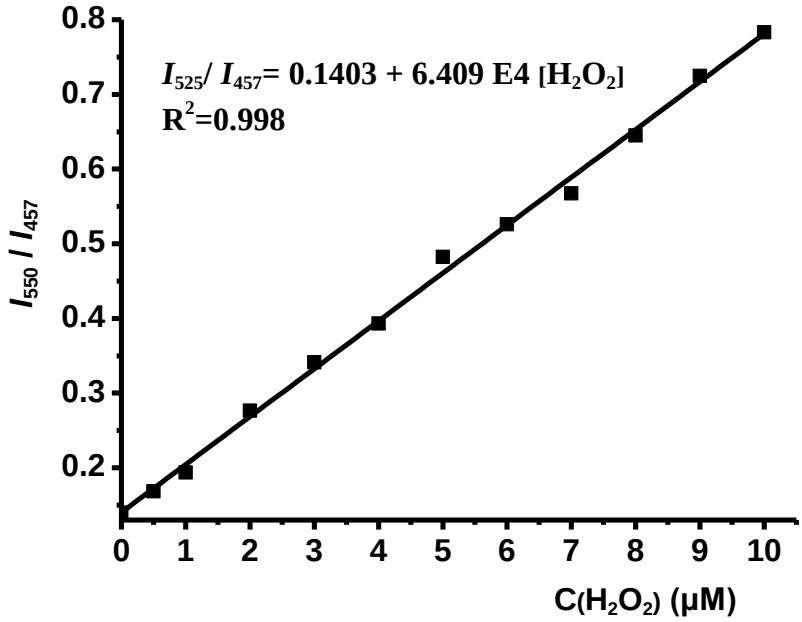


Fig. S14 Fluorescence intensity of probe against the H_2O_2 concentration from 0 to 10 μM in pH 7.4 HEPES buffer. λ_{ex}= 395 nm.

Time course:

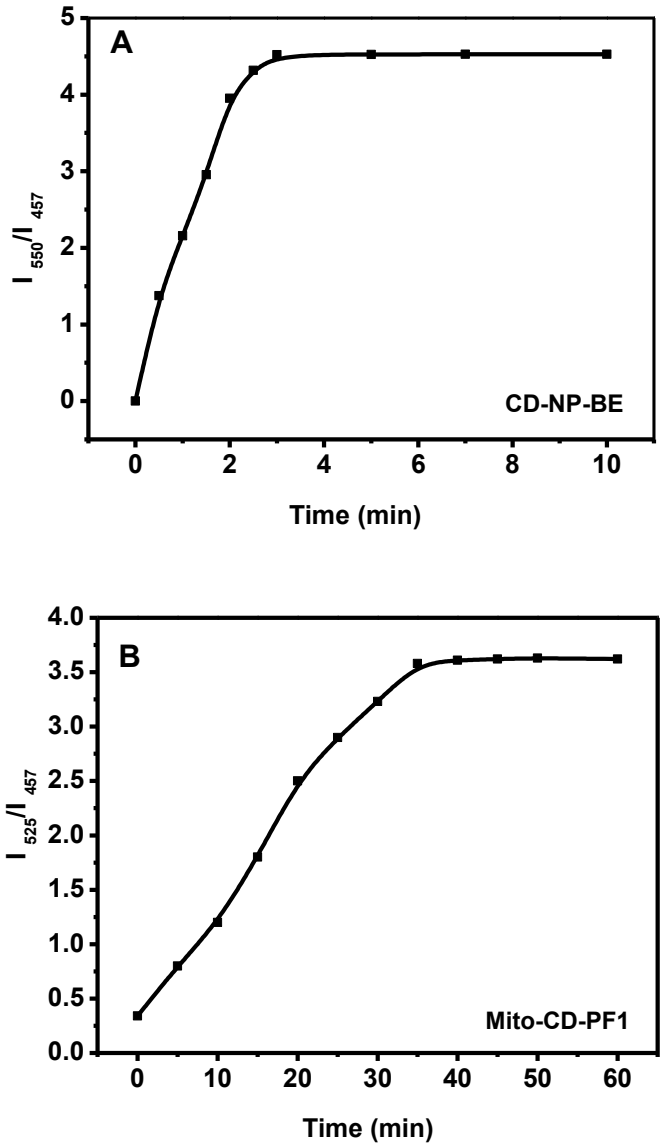


Fig. S15 (A) Fluorescence intensity ratio (I_{550}/I_{457}) of the current nanoprobe (0.15 mg mL^{-1}) at different time periods upon addition of H_2O_2 (200 μM) in pH 7.4 HEPES buffered water (λ_{ex}= 395 nm). (B) Fluorescence intensity ratio (I_{525}/I_{457}) of our previous nanoprobe (Mito-CD-PF1) (0.15 mg mL^{-1}) at different time periods upon addition of H_2O_2 (300 μM) in HEPES buffer (50 mM, pH 7.0) (λ_{ex}= 370 nm).

Photostability:

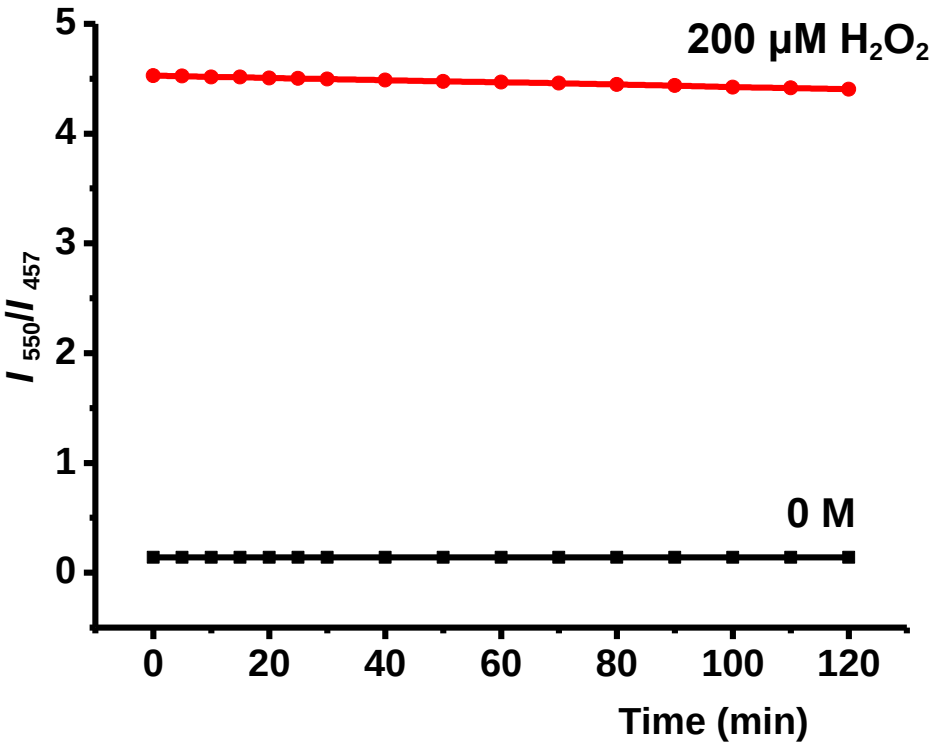


Fig. S16 Photostability test for nanoprobe **CD-NP-BE** (0.15 mg mL^{-1}) in the absence and presence (200 μM) of H_2O_2 in pH 7.0 HEPES buffered water (samples were stirred for 10 min before test). The change of the fluorescence intensity ratio of **CD-NP-BE** is less than 3 % under continuous light irradiation. UV source: 15 W 365 nm UV lamp, about 2 cm away from the sample cuvettes (1cm ×1cm) with 1 cm path length.

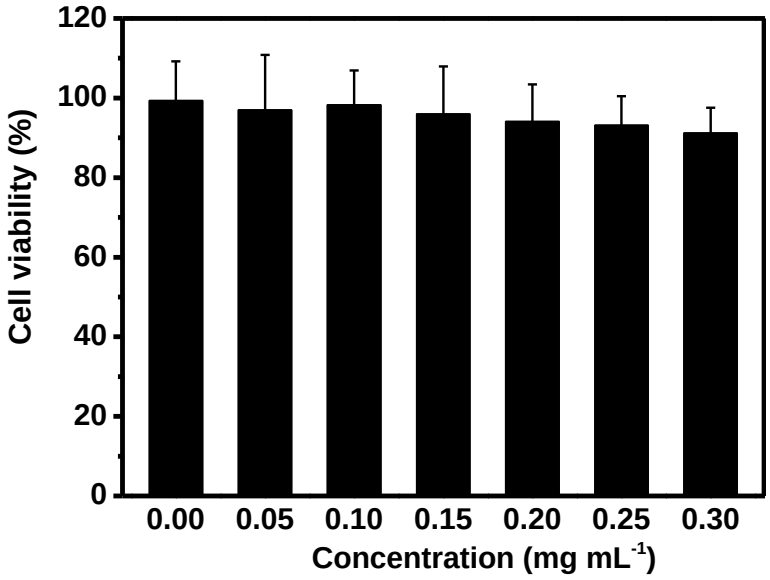


Fig. S17 Cell viability of L929 cells incubated with nanoprobe of varied concentrations for 24 h. Viability of the cells was assessed using MTT assay. The reported percent cell survival values are relative to untreated control cells.

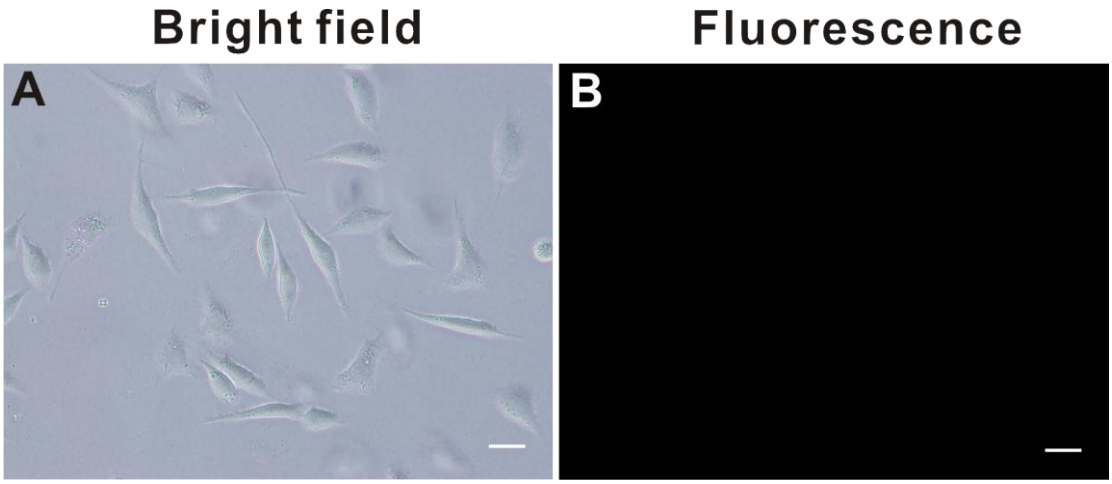


Fig. S18 Microscopic images of L929 cells in the absence of the nanoprobe. Scale bar: 20 μm.

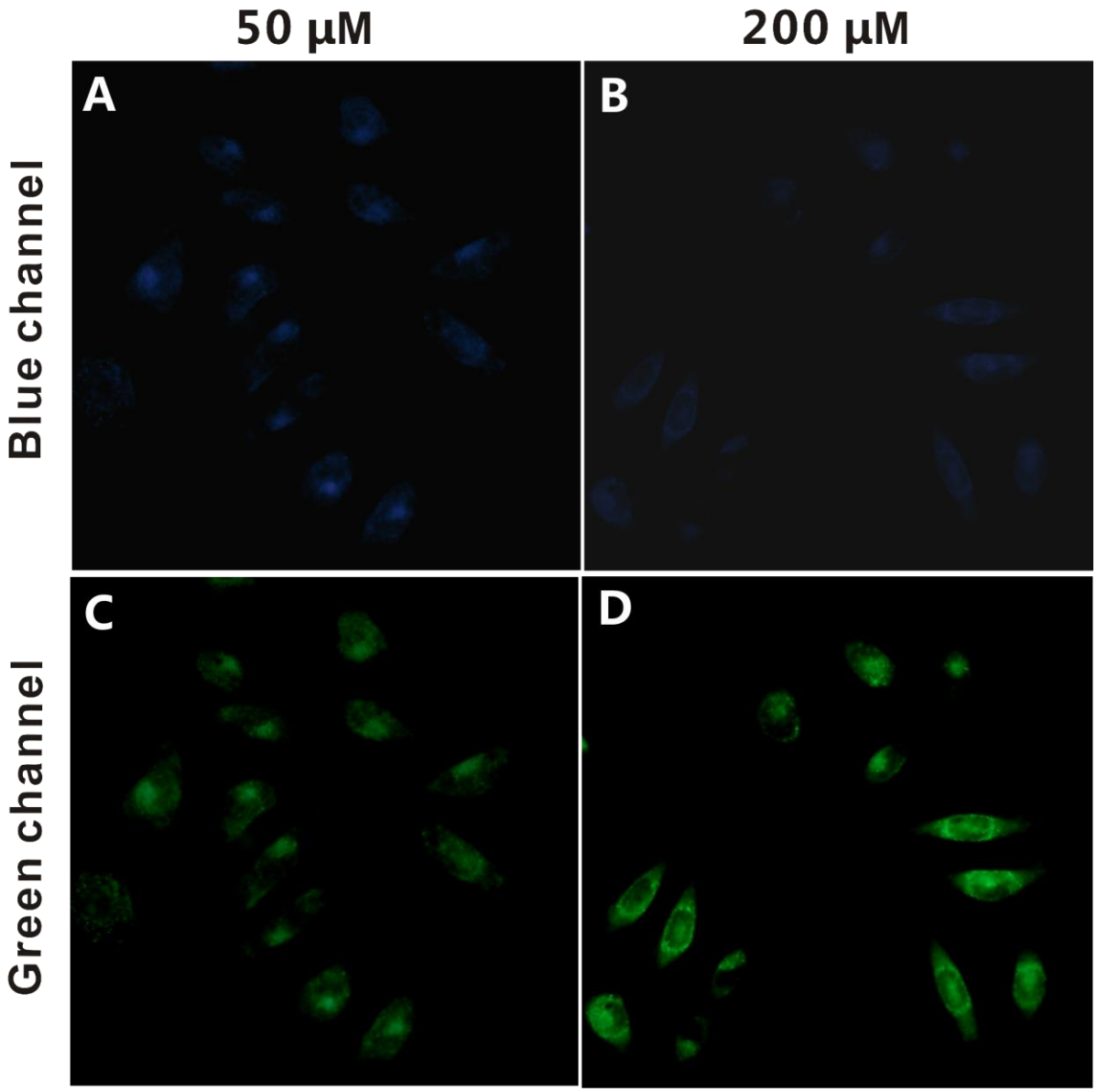


Fig. S19 Fluorescence images (shown in separated color channels) for L929 cells treated with the nanoprobe and 50 μM (A and C) and 200 μM (B and D) of H_2O_2.

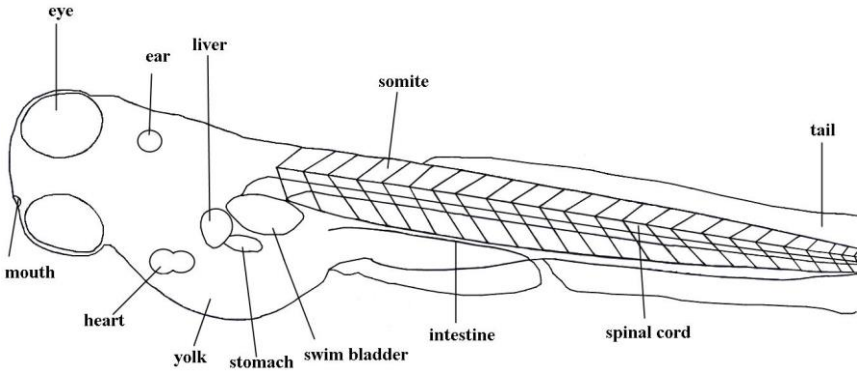


Fig. S20 Drawing of a five-day-old zebrafish. The sketch was drawn according to the literature.[1]

[1] (a) S. K. Ko, X. Q. Chen, J. Y. Yoon, I. Shin, *Chem. Soc. Rev.*, 2011, **40**, 2120; (b) P. Haffter, M. Granato, M. Brand, M. C. Mullins, M. Hammerschmidt, D. A. Kane, J. Odenthal, F. J. M. van Eeden, Y. J. Jiang, C. P. Heisenberg, R. N. Kelsh, M. Furutani-Seiki, E. Vogelsang, D. Beuchle, U. Schach, C. Fabian, C. Nüsslein-Volhard, *Development*, 1996, **123**, 1; (c) R. M. Warga, C. Nüsslein-Volhard, *Development*, 1999, **126**, 827.

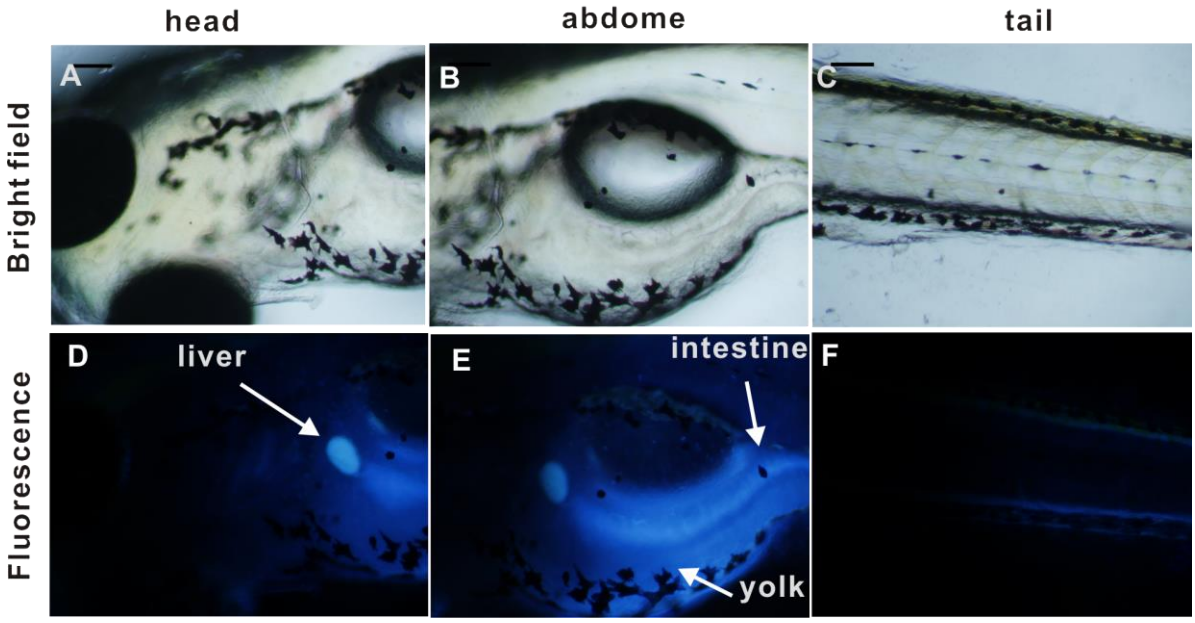


Fig. S21 Fluorescence images for different regions of a zebrafish treated with 0.2 mg mL^{-1} of nanoprobe for 1h. Scale bar: 100 μm.

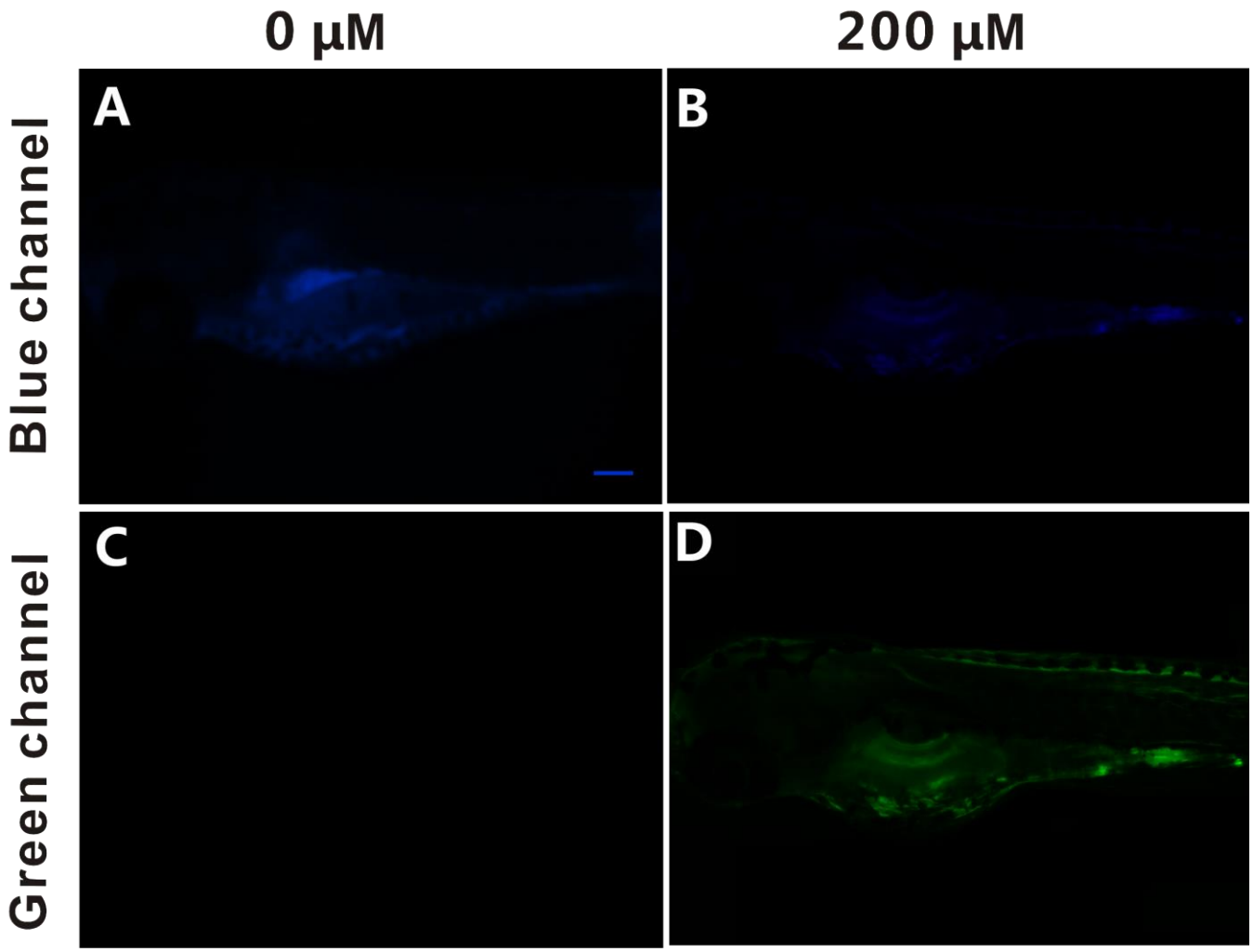


Fig. S22 Fluorescence images (shown in separated color channels) for zebrafish larvae treated with the nanoprobe and 0 μM (A and C) and 200 μM (B and D) H_2O_2.

Note: For Fig. 3 and Fig. 5, color channels have been shown separately in Fig. S19 and Fig. S22, respectively, it can be seen that with increasing of the concentrations of H_2O_2, blue fluorescence decrease is concomitant with green fluorescence increase both in cells and zebrafish larvae.

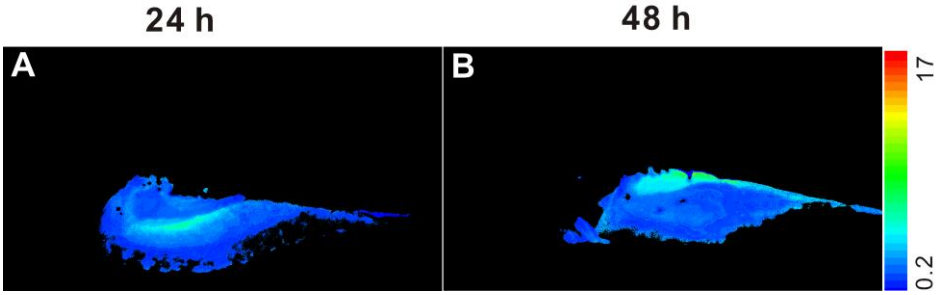


Fig. S23. Microscopic images for zebrafish larvae (shown in pseudo-color) treated with DOX for 24 h (A) or 48 h (B) and then incubated with the nanoprobe (0.1 mg mL^{-1}) for 60 min. The color strip indicates the fluorescence intensity ratio (I_{green}/I_{blue}).

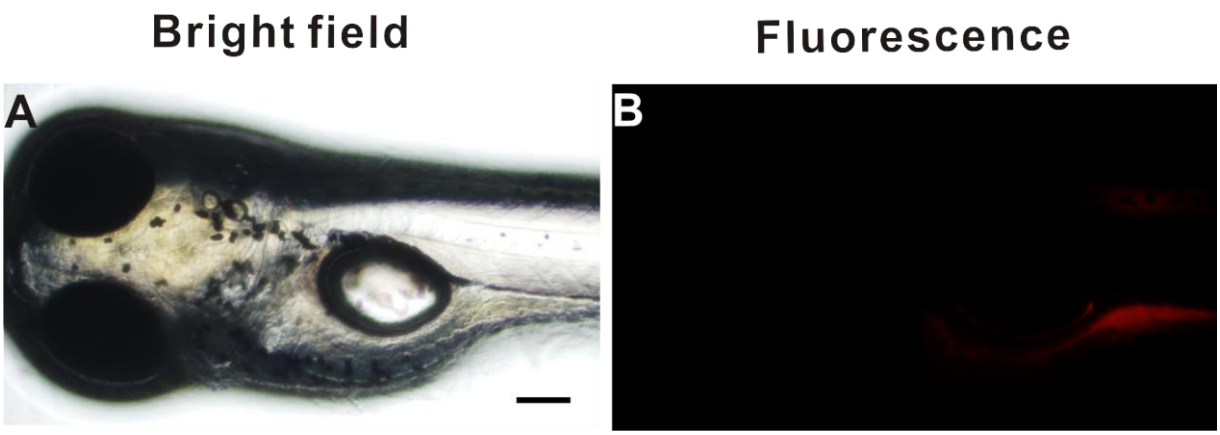


Fig. S24 Microscopic images of zebrafish upon being treated with 0.05 mg mL^{-1} DOX for 24 h under **530-560** nm excitation. (A) brightfield image, (C) fluorescent image. Scale bar: 200 μm.

Note: Under excitation at 360-380 nm, with which we used to excite the carbon-dots, DOX did not generate noticeable fluorescence, hence the possible existence of DOX inside gut of the zebrafish will not interfere the fluorescence images shown in Fig. 6.