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Electronic Supplementary Information

Fluorescent ratiometric nanosensor for detecting NO in aqueous media and imaging exogenous and endogenous NO in live cells

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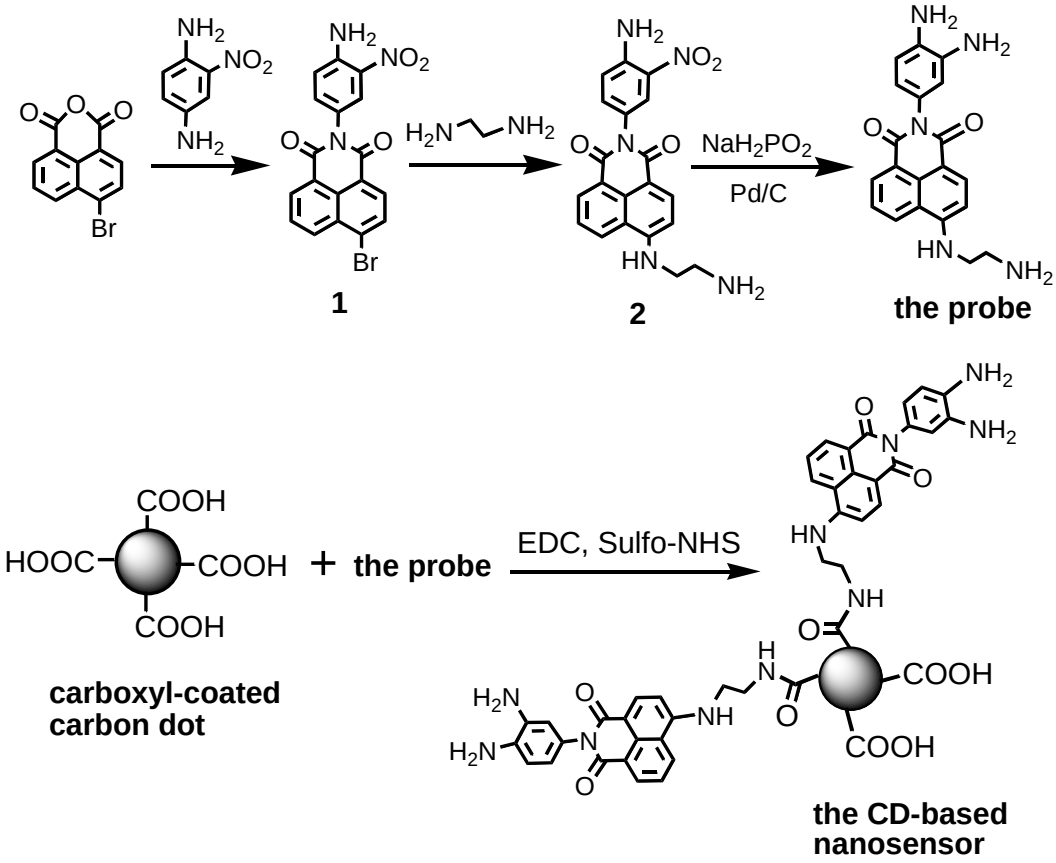


Scheme S1 Synthetic route for the CD-based nanosensor.

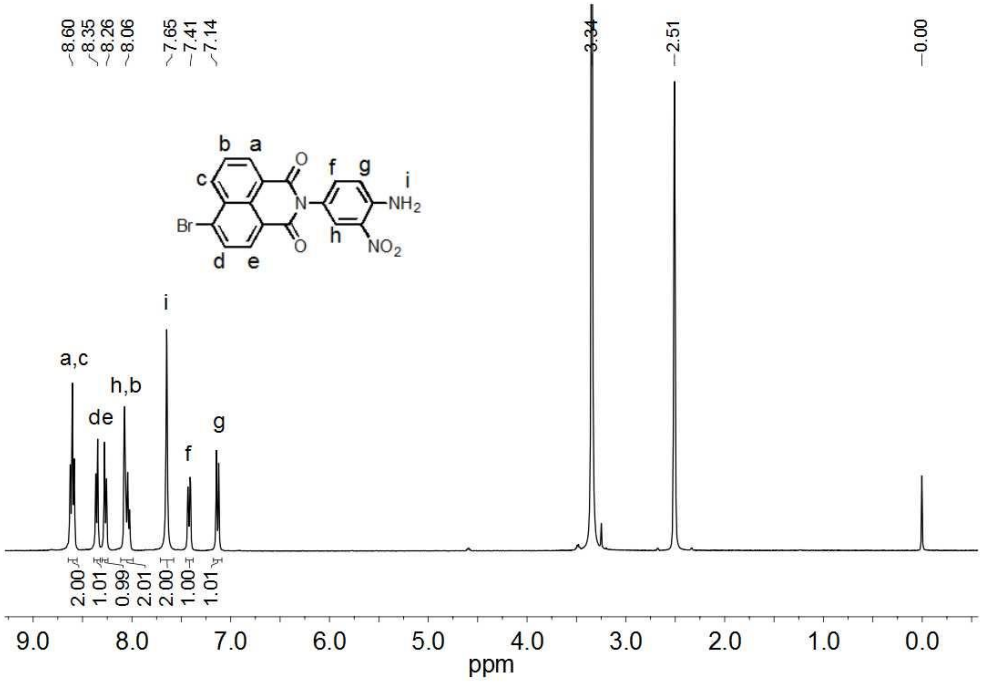


Fig. S1 ^{1}H NMR spectra (in DMSO-d_6) of Compound **1**.

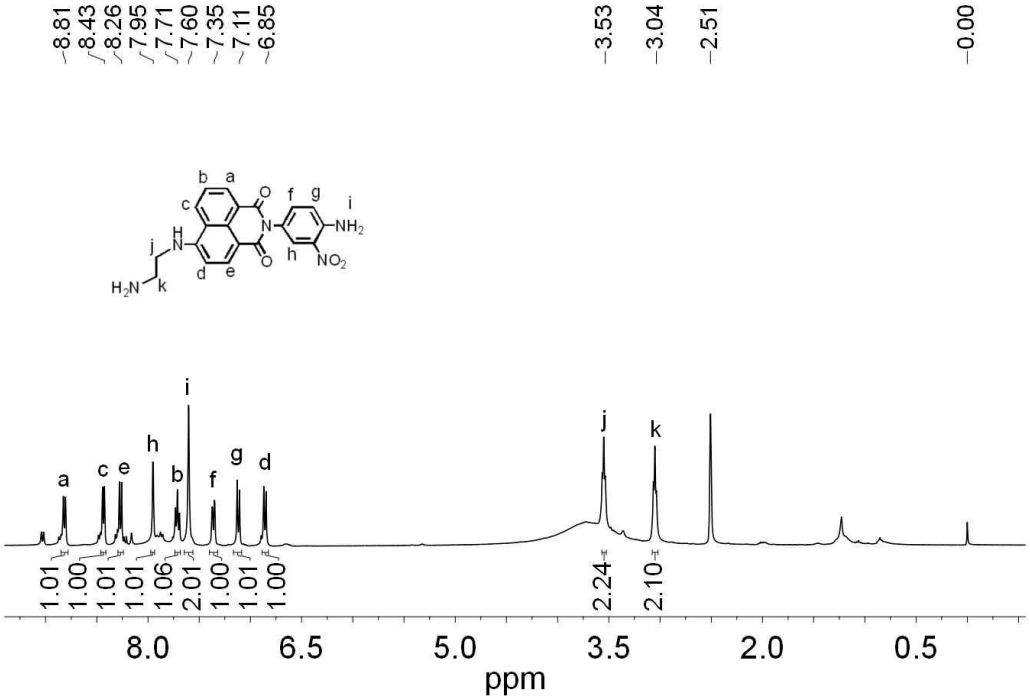
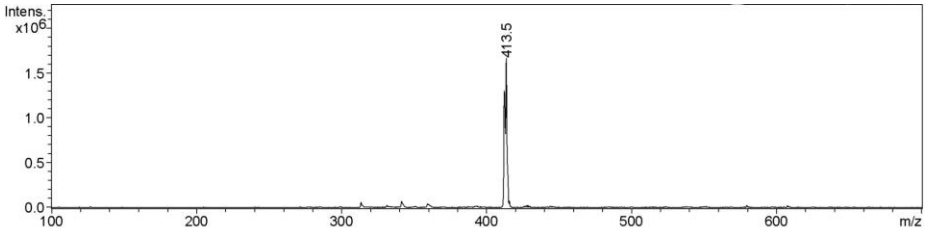
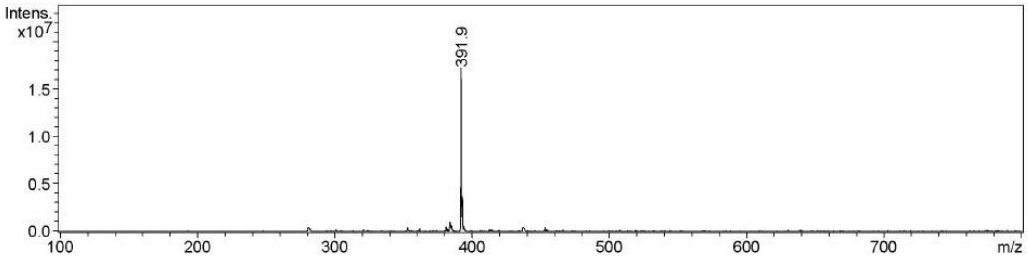


Fig. S2 ^{1}H NMR spectra (in DMSO-d_6) of Compound **2**.



#	m/z	I	I %
1	412.3	1298164	78.1
2	413.5	1662241	100.0
3	415.7	71863	4.3
4	416.8	9133	0.5

(a)



#	m/z	I	I %
1	391.9	17276304	100.0
2	392.9	3645684	21.1
3	393.9	481259	2.8

(b)

Fig. S3 Mass spectra of the intermediate compounds: (a) Compound **1** (MS (APCI): *m/z* 413.5 $[M+H]^+$); (b) Compound **2** (MS (ESI): *m/z* 391.9 $[M]^+$).

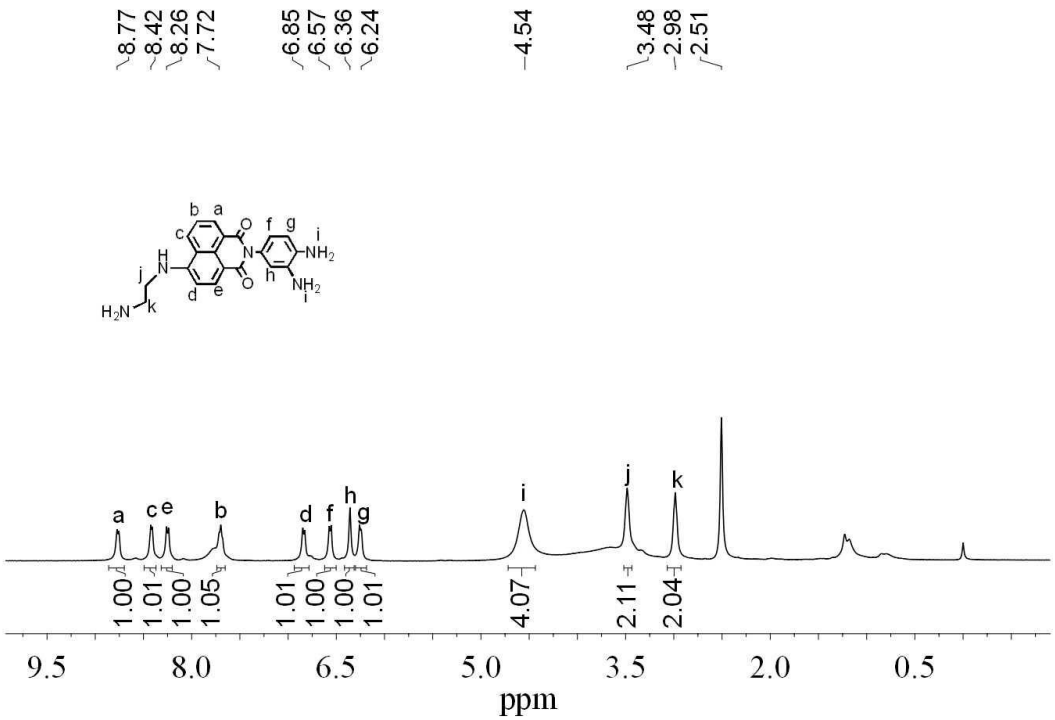
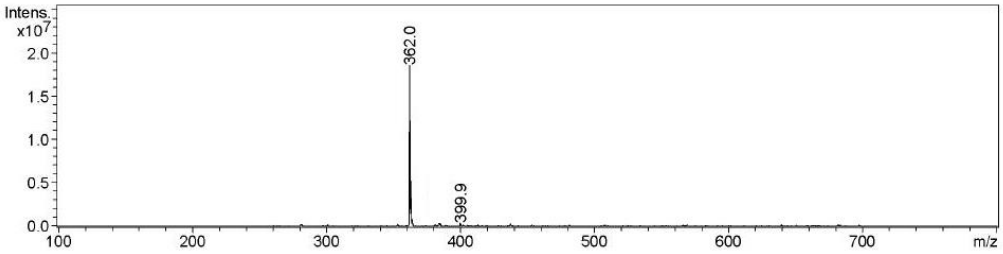


Fig. S4 ^{1}H NMR spectra (in DMSO-d_6) of the probe.



#	m/z	I	I %
1	362.0	18525556	100.0
2	362.9	5215090	28.2
3	363.9	739669	4.0
4	399.9	345463	1.9

Fig. S5 Mass spectrum of the probe.
MS (ESI): *m/z* 362.0 $[M+H]^+$

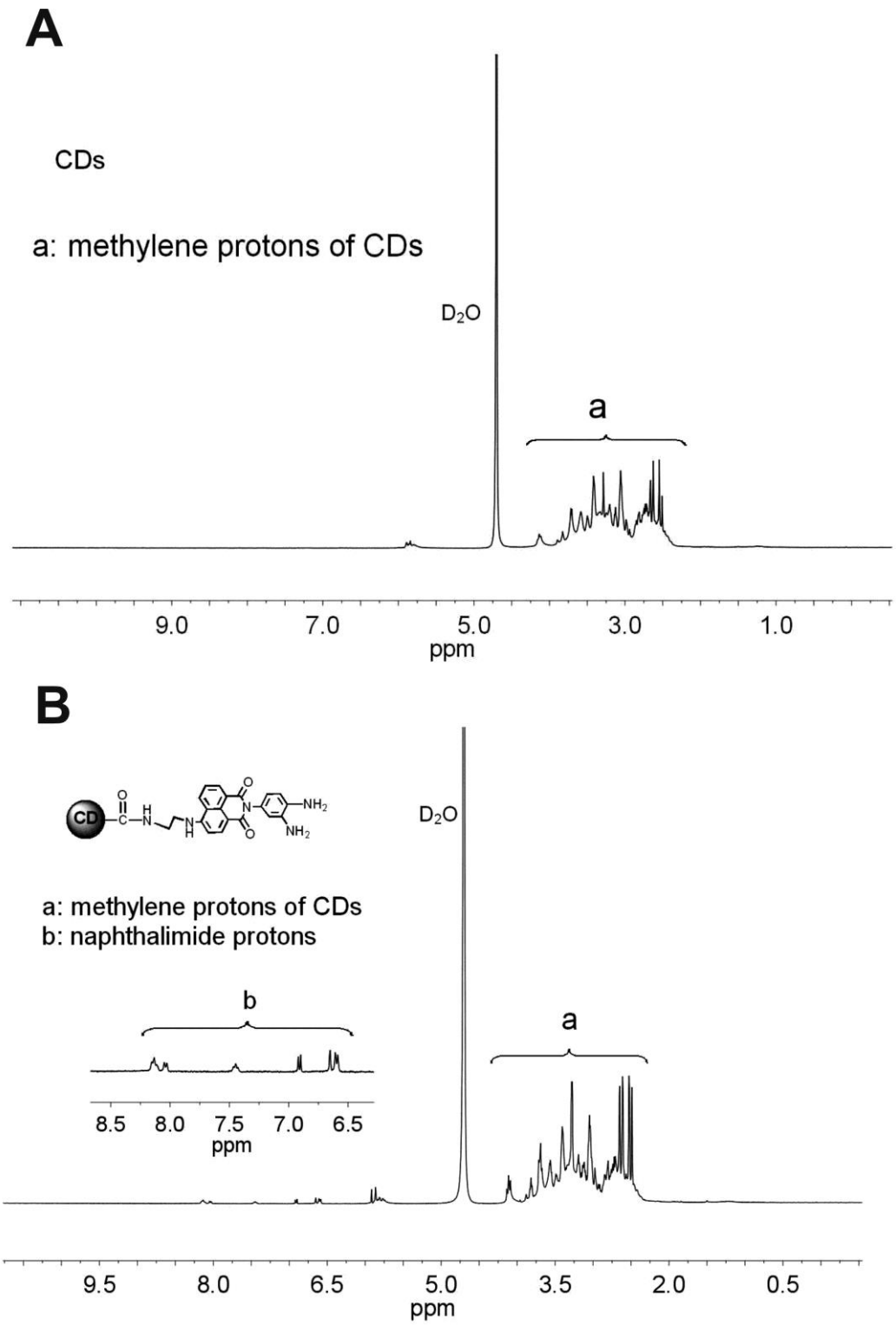


Fig. S6 ^{1}H NMR spectra (in D_2O) of the plain CDs (A) and the CD-based nanosensor (B).

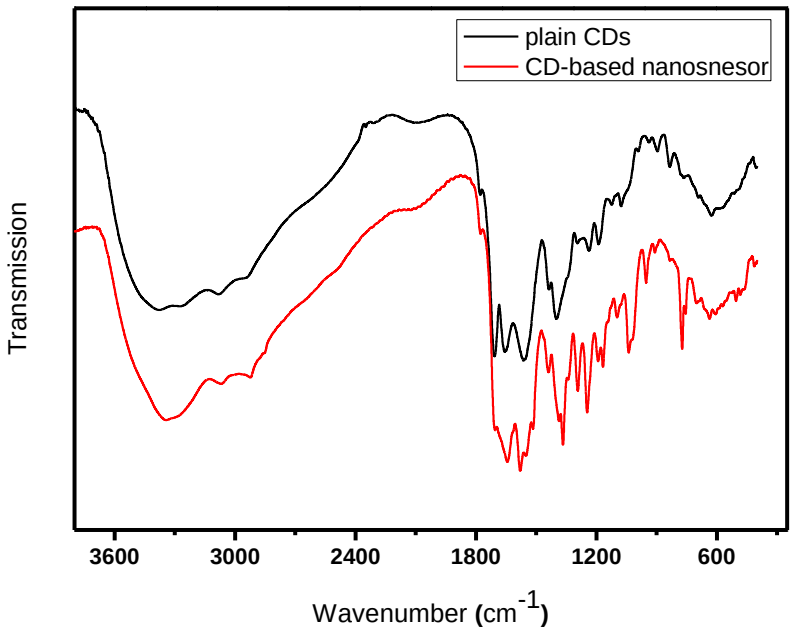


Fig. S7 FT-IR spectra of the plain carbon dots (black) and the CD-based nanosensor (red).

Compared to the FT-IR spectrum of plain carbon dots (black), the CD-based nanosensor (red) displayed absorption at 1580 and 1505 cm^{-1}, which correspond to the skeletal vibrations of benzene ring in the probe moiety. And the peak at 770 cm^{-1} represents the flexural vibration of aromatic C-H.

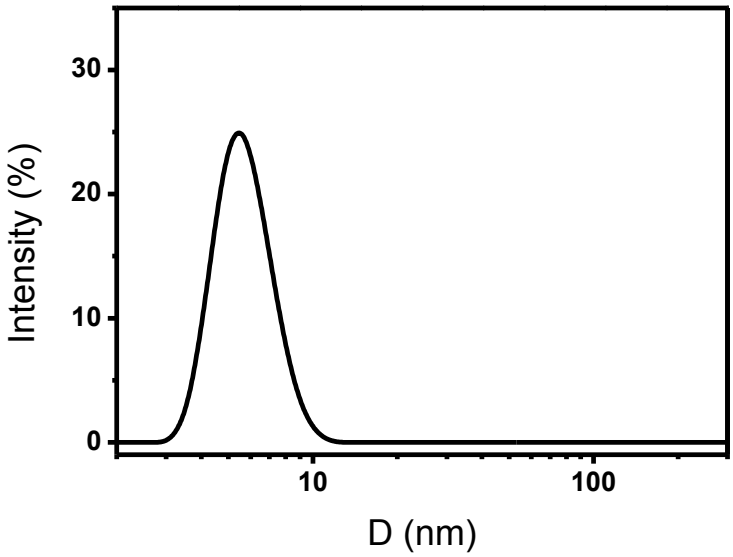


Fig. S8 Size distribution of the CD-based nanosensor determined by dynamic light scattering.

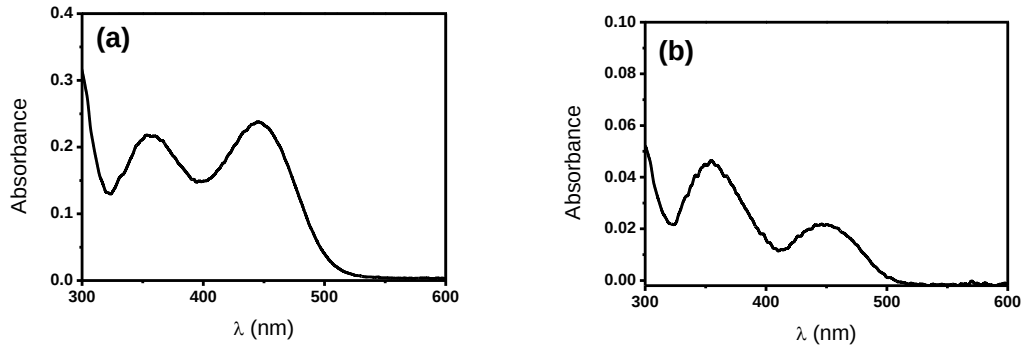


Fig. S9 Absorption spectra of the nanosensor upon reacting with excessive NO in pH 7.4 PBS buffered water. (a): the amount of probe conjugated onto CDs is 73.30 mg g^{-1}; (b): the amount of probe conjugated onto CDs is 29.00 mg g^{-1}.

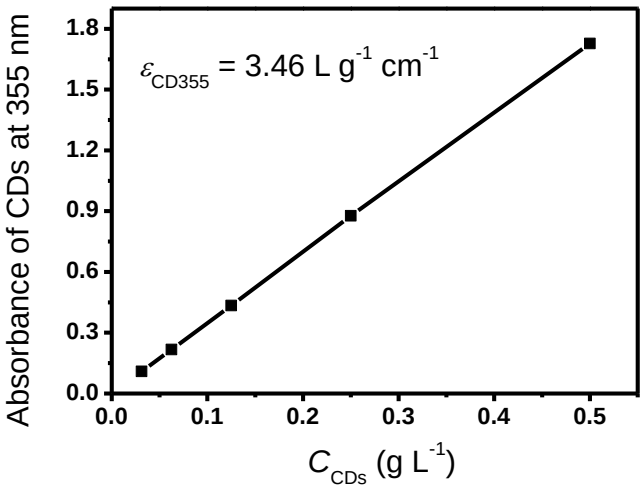
Calculation of the amount of the probe conjugated to CDs

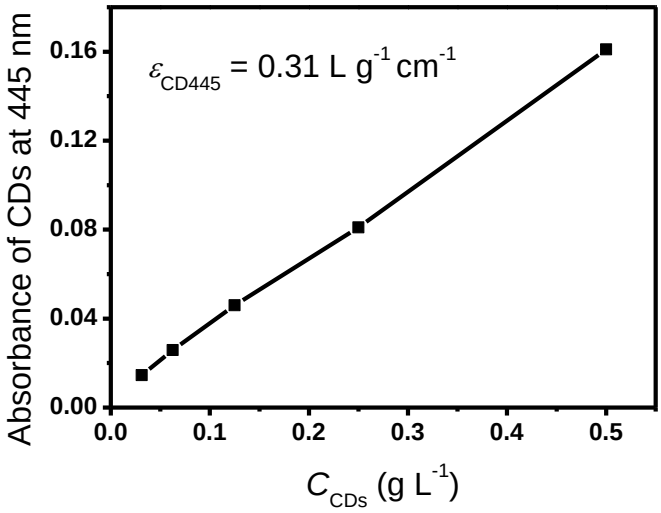
The amount of the probe conjugated to per gram of the CDs are calculated based on the additivity of absorbance of CDs and the probe. CDs display characteristic absorption band at 355 nm. While upon reacting with excessive NO, the probe moiety --- the diaminobenzene-containing naphthalimide turns into the triazole-containing naphthalimide (namely, the energy acceptor) and exhibits characteristic absorption at 445 nm (see Fig. S11). And the following equation set is used to calculate the conjugated amount of the probe:

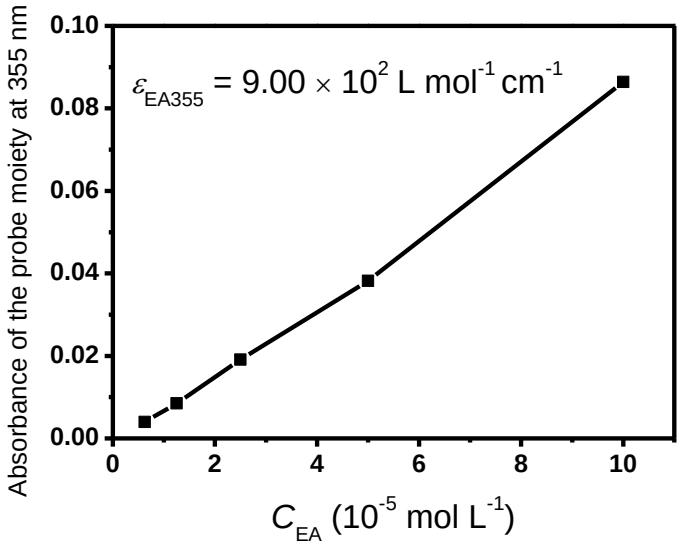
$$A_{355nm} = \varepsilon_{CD355} \times C_{CDs} \times b + \varepsilon_{EA355} \times C_{EA} \times b$$
$$A_{445nm} = \varepsilon_{CD445} \times C_{CDs} \times b + \varepsilon_{EA445} \times C_{EA} \times b$$

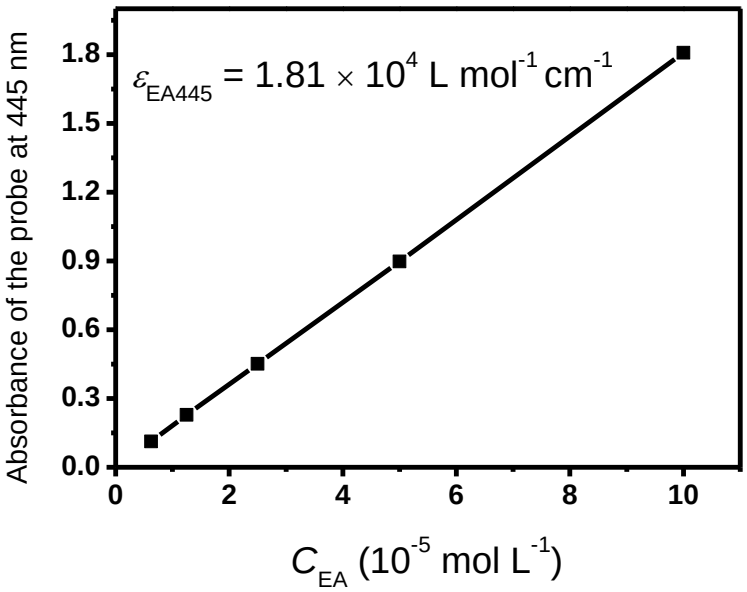
where A is the absorbance of the nanosensor at different wavelengths; ε and *C* are the absorptivity and the concentration of a given substance respectively; b represents the optical length 1 cm; EA represents energy acceptor.

ε_{CDs355}, ε_{CDs445}, ε_{EA355} and ε_{EA445} can be obtained by measuring the absorption of the plain CDs at different concentrations and the absorption of the triazole-containing naphthalimide compound solution at different concentrations. Then the absorption of the nanosensor in the presence of excessive amount of NO was measured, and by using the above equation, the concentrations of CDs and the triazole-containing naphthalimide can be calculated, and finally the amount of the triazole-containing naphthalimide on per gram of CDs can be determined. All absorbance measurements were performed in a quartz cell of 1 cm optical length.


Absorbance of CDs at 355 nm
1.8
1.5
1.2
0.9
0.6
0.3
0.0
0.0
0.1
0.2
0.3
0.4
0.5
ε_{CD355} = 3.46 L g^{-1} cm^{-1}
C_{CDs} (g L^{-1})


Absorbance of CDs at 445 nm
0.16
0.12
0.08
0.04
0.00
0.0
0.1
0.2
0.3
0.4
0.5
ε_{CD445} = 0.31 L g^{-1} cm^{-1}
C_{CDs} (g L^{-1})


Absorbance of the probe moiety at 355 nm
0.10
0.08
0.06
0.04
0.02
0.00
0
2
4
6
8
10
ε_{EA355} = 9.00 × 10^{2} L mol^{-1} cm^{-1}
C_{EA} (10^{-5} mol L^{-1})



For Fig. S9a: the concentration of the CD-based sensor is 0.06 mg mL^{-1}

$0.22 = 3.46 \times C_{CDs} \times 1 + 9.00 \times 10^2 \times C_{EA} \times 1$

$0.24 = 0.31 \times C_{CDs} \times 1 + 1.81 \times 10^4 \times C_{EA} \times 1$

Therefore, $C_{CDs} = 0.06$ g L^{-1}, $C_{\text{diaminobenzene-containing naphthalimide}} = C_{EA} = 1.22 \times 10^{-5}$ M

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

$1.22 \times 10^{-5} \times 1 \times 361 / 0.06 = 7.33 \times 10^{-2}$ g g^{-1} = 73.30 mg g^{-1} (361 is the molecular weight of the probe --- the diaminobenzene-containing naphthalimide)

The amount of the probe conjugated to per gram of the CDs is calculated to be about 73.30 mg.

Similarly, for Fig. S9b, the amount of probe conjugated to per gram of the CDs is：the concentration of the CD-based sensor is 0.0132 mg mL^{-1}

$0.0466 = 3.46 \times C_{CDs} \times 1 + 9.00 \times 10^2 \times C_{EA} \times 1$

$0.0219 = 0.31 \times C_{CDs} \times 1 + 1.81 \times 10^4 \times C_{EA} \times 1$

Therefore, $C_{CDs} = 1.32 \times 10^{-2}$ g L^{-1}, $C_{\text{diaminobenzene-containing naphthalimide}} = C_{EA} = 1.06 \times 10^{-6}$ M

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

$1.06 \times 10^{-6} \times 1 \times 361 / (1.32 \times 10^{-2}) = 2.90 \times 10^{-2}$ g g^{-1} = 29.00 mg g^{-1}

The amount of the probe conjugated to per gram of the CDs is calculated to be about 29.00 mg.

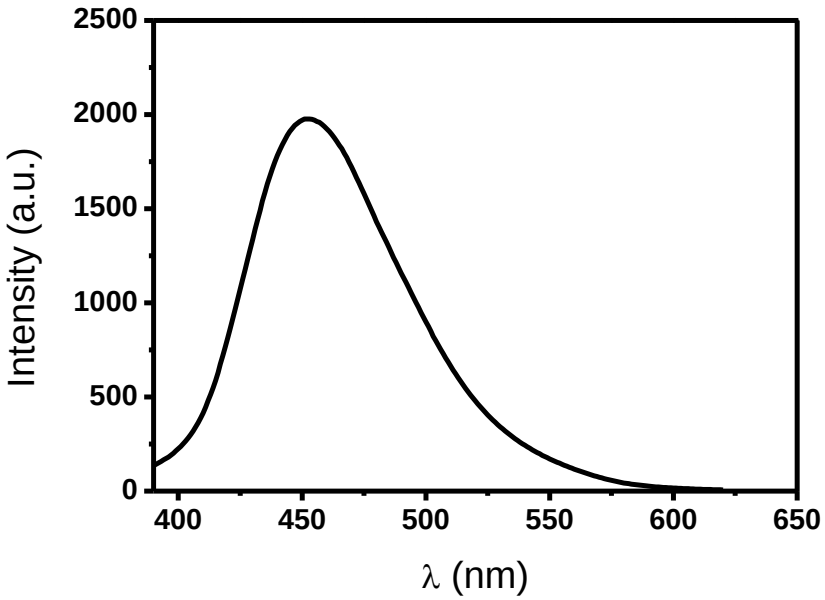


Fig. S10 Fluorescence emission spectrum (λ_{ex} =370 nm) of the plain carbon dots in pH 7.4 PBS buffer.

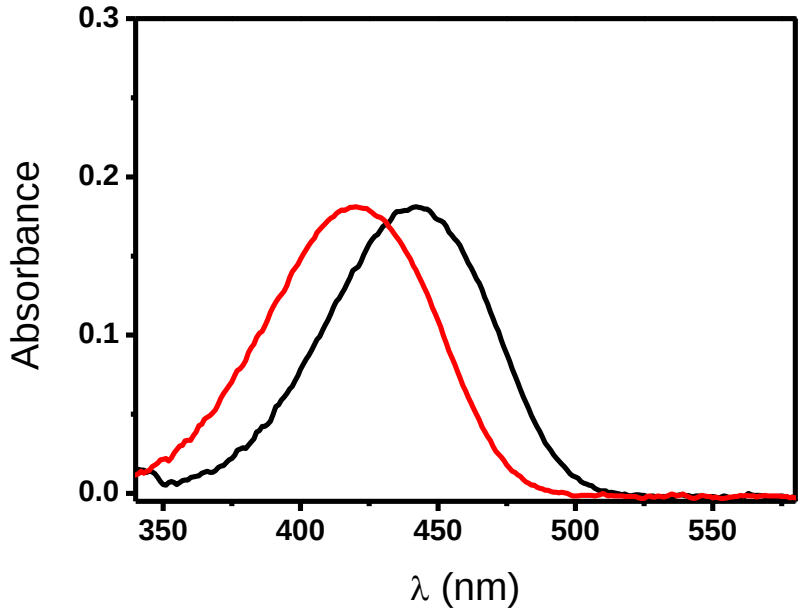


Fig. S11 Absorption spectra of the probe compound (in pH 7.4 PBS buffered water containing 0.5% DMSO) in the absence or presence of NO. Red curve: in the absence of NO; black curve: in the presence of NO.

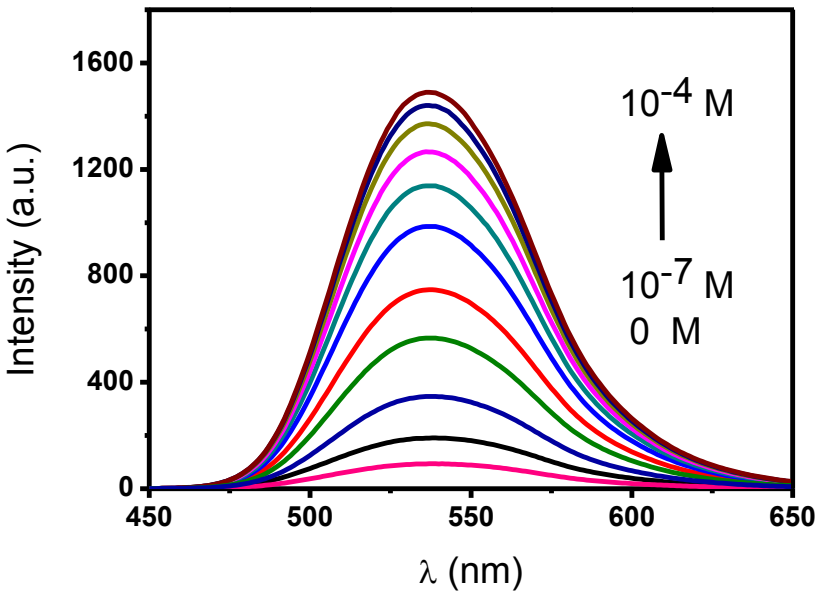


Fig. S12 Fluorescence spectra of the probe compound (in pH 7.4 PBS buffered water containing 0.5% DMSO) in the presence of different concentrations of NO (λ_{ex} = 445 nm)

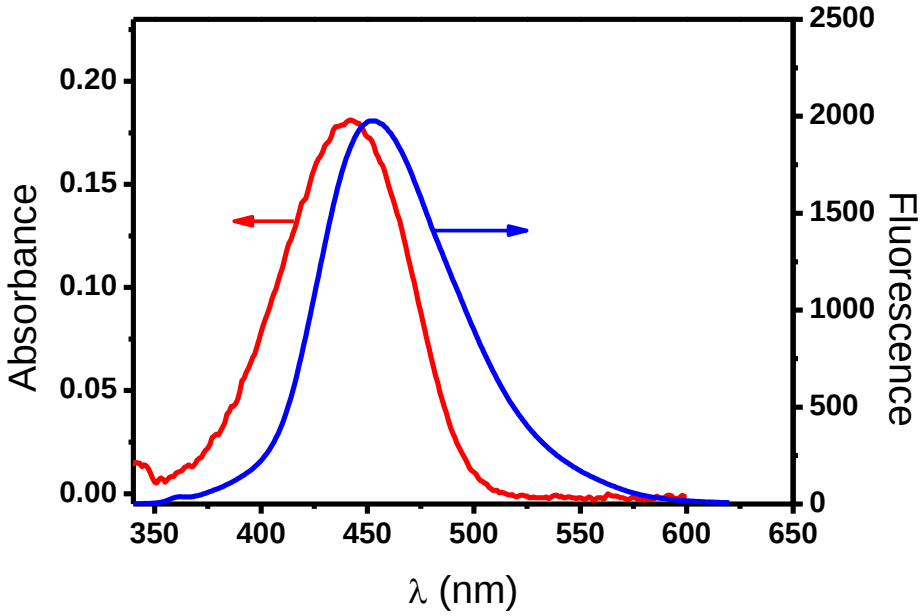


Fig. S13 Spectra overlap of the absorption of the energy acceptor (namely the triazole-containing naphthalimide; red curve) and the emission of the donor (namely CDs; blue curve).

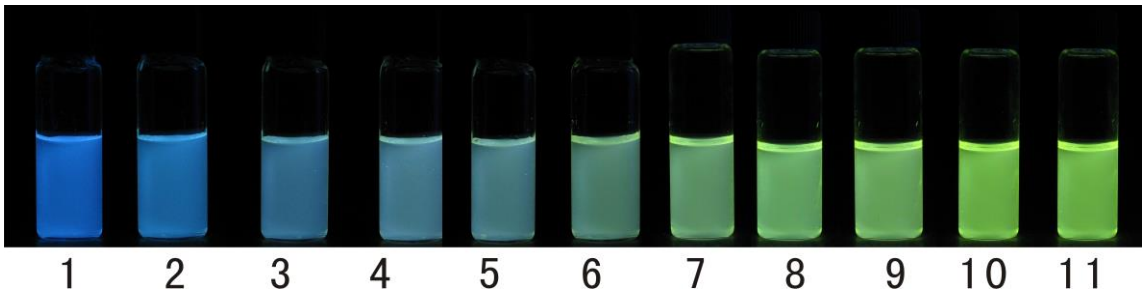


Fig. S14 Fluorescence change of the CD-based nanosensor in the absence of NO and upon addition of NO (the nanosensor: 0.06 mg mL^{-1} in pH 7.4 PBS buffered water. 1: 0 μM NO; 2: 0.1 μM; 3: 0.5 μM; 4: 1 μM; 5: 2 μM; 6: 3 μM; 7: 5 μM; 8: 10 μM; 9: 20 μM; 10: 50 μM; 11: 100 μM. Photographs were taken under a hand-held 365 nm UV lamp.

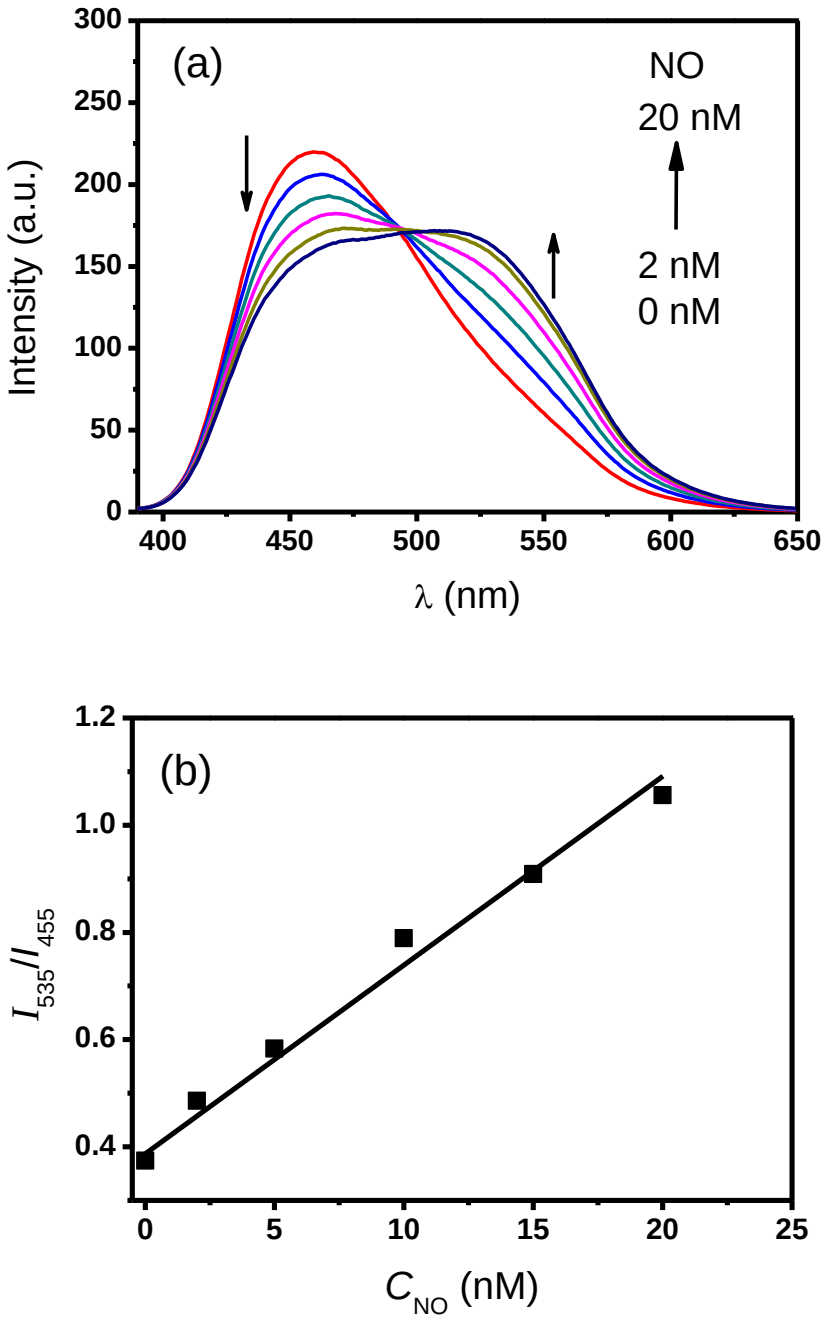


Fig. S15 (a) Fluorescence spectra of the nanosensor (the amount of probe conjugated on CDs is 29.00 mg g^{-1}) in the presence of different amounts of NO in pH 7.4 PBS buffered water. (b) The fluorescence intensity ratio of the nanosensor (0.013 mg mL^{-1}) as a function of NO concentration in pH 7.4 PBS buffered water. (λexc = 370 nm).

The method for determining the detection limit:

First the calibration curve was obtained from the plot of fluorescence intensity increment ratio, I_{535}/I_{455}, as a function of the analyte concentration (NO). The regression curve equation was then obtained for the lower concentration part.

The detection limit = 3 × S.D. / k

where k is the slope of the curve equation, and S.D. represents the standard deviation for the nanosensor solution intensity increment in the absence of NO.

I_{535}/I_{455} = 0.38 + 0.034E9 × [NO] (R = 0.990)

LOD = 3 × 0.033 / 0.034E9 = 3.0 nM

References:

V. Thomsen, D. Schatzlein and D. Mercuro, *Spectroscopy*, 2003, **18**, 112-114.

A. D. McNaught and A. Wilkinson, *IUPAC Compendium of Chemical Terminology*, 1997.

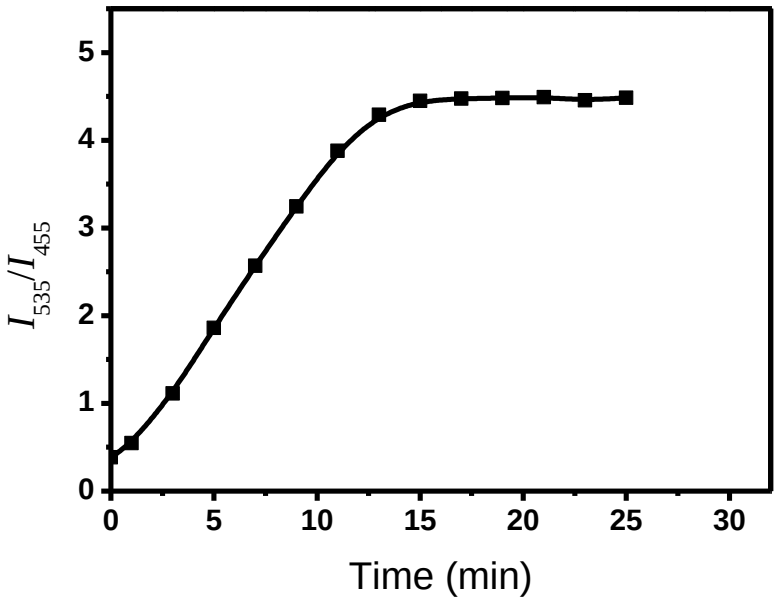


Fig. S16 Fluorescence intensity ratio of the nanosensor (0.06 mg mL^{-1}) at different times after addition of NO (1×10^{-4} M) in pH 7.4 PBS buffered water. (λexc = 370 nm).

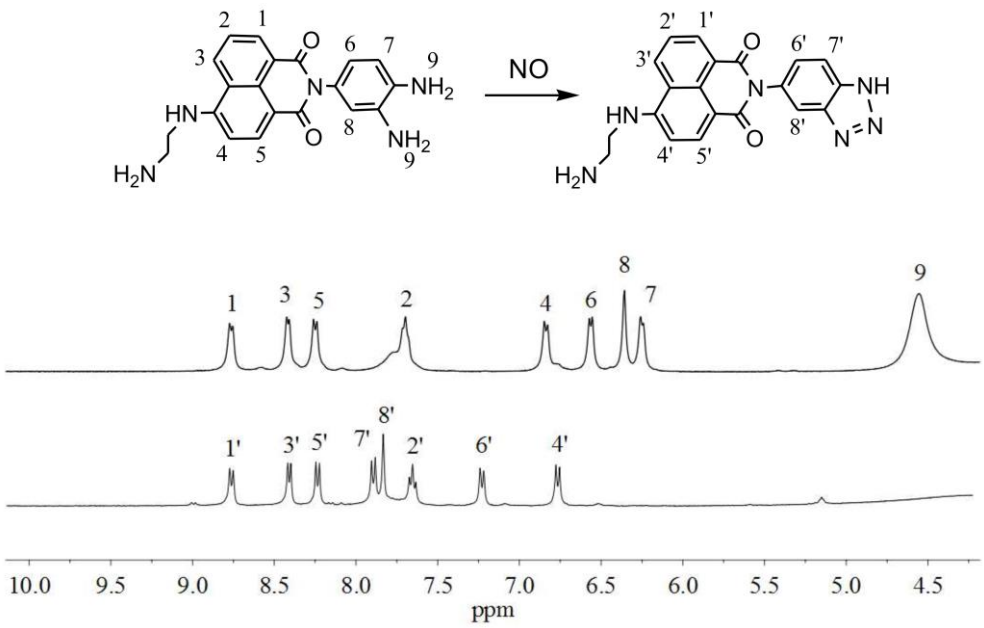
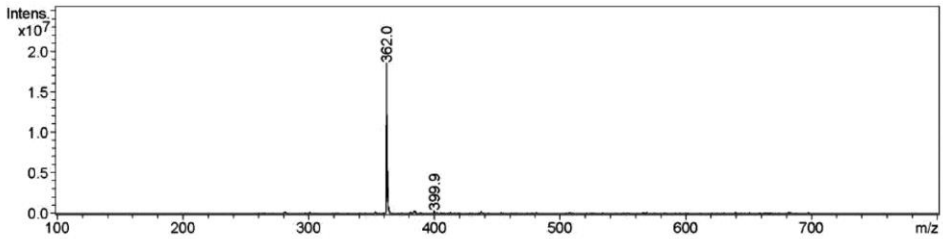


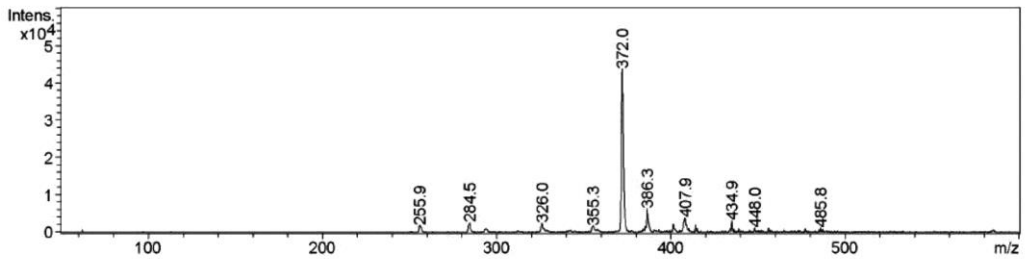
Fig. S17 Partial ^{1}H NMR spectra of the probe compound before and after addition of NO (5 equiv.) in DMSO-d6.

A



#	m/z	I	I %
1	362.0	18525556	100.0
2	362.9	5215090	28.2
3	363.9	739669	4.0
4	399.9	345463	1.9

B



#	m/z	I	I %
1	255.9	1771	4.0
2	284.5	2505	5.7
3	326.0	2474	5.7
4	355.3	1660	3.8
5	372.0	43749	100.0
6	386.3	6250	14.3
7	401.2	2214	5.1
8	407.9	3825	8.7
9	414.1	1949	4.5
10	434.9	3071	7.0

Fig. S18 Mass spectra of the as-prepared probe compound (A) and the reaction product upon addition of NO (5 equiv.) (B).

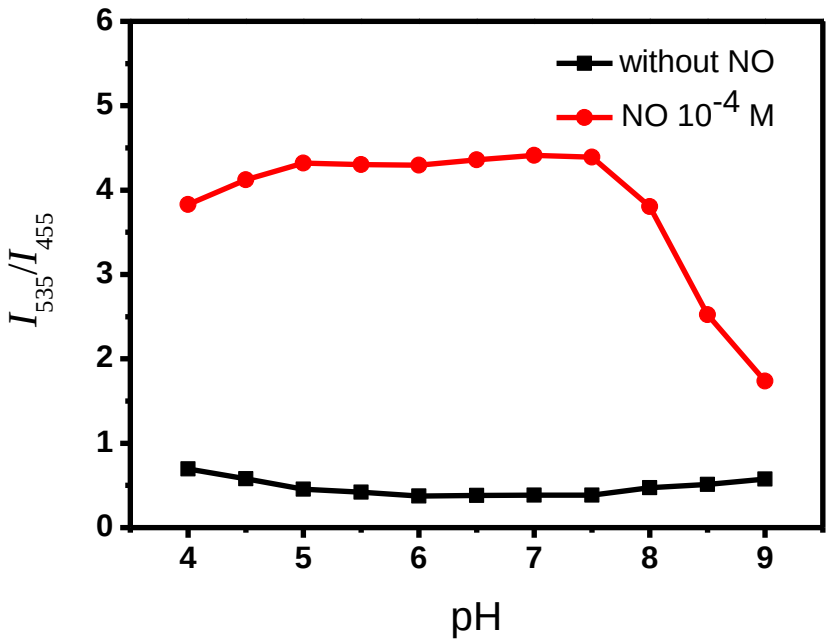


Fig. S19 Fluorescence intensity ratio as a function of pH in the absence and presence of NO.

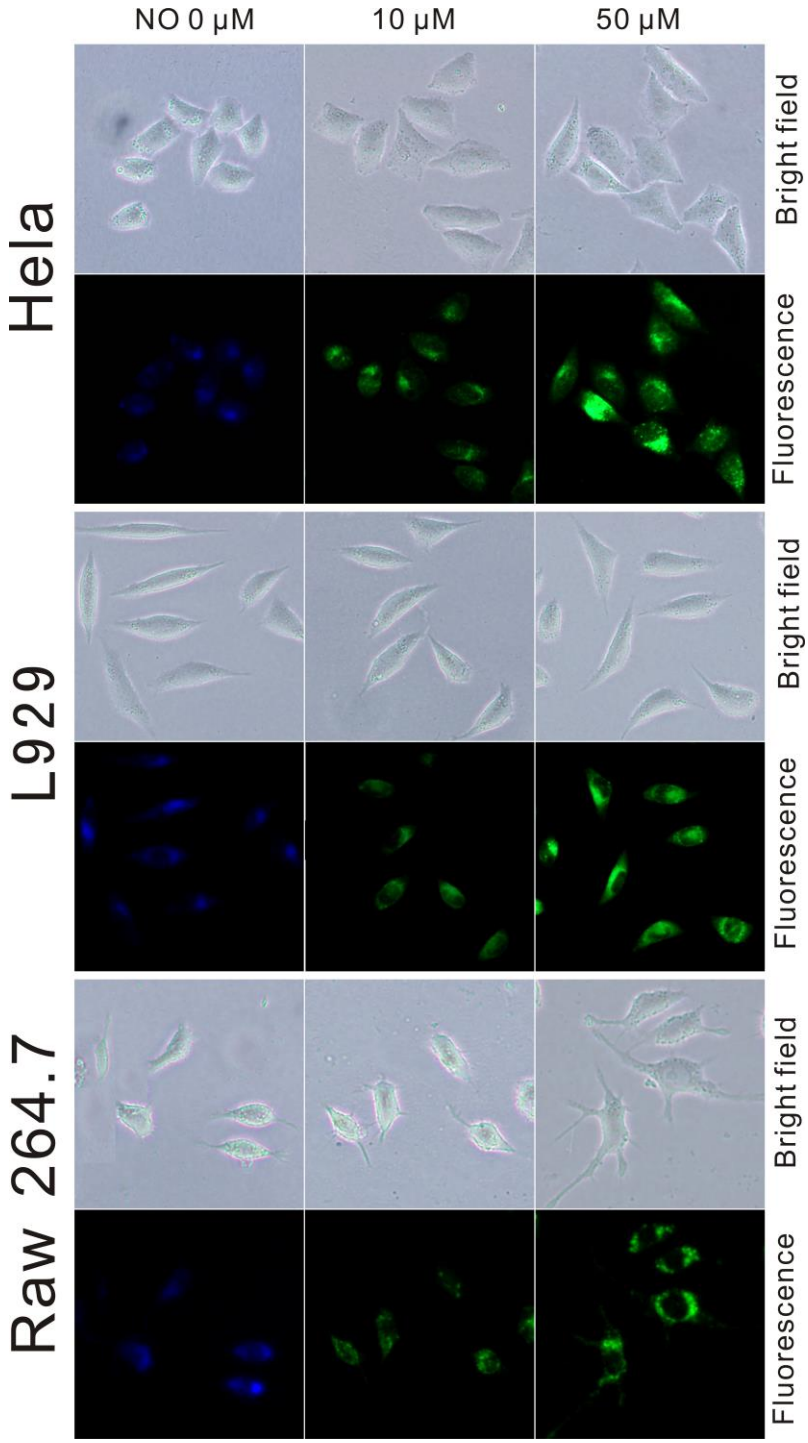


Fig. S20 Microscope images for HeLa, L929 and Raw 264.7 macrophage cells incubated with the sensor (0.06 mg mL^{-1}) before and after being treated with NO (10 μM and 50 μM NO repectively) for 30 min. The photographs were taken under identical exposure condition.

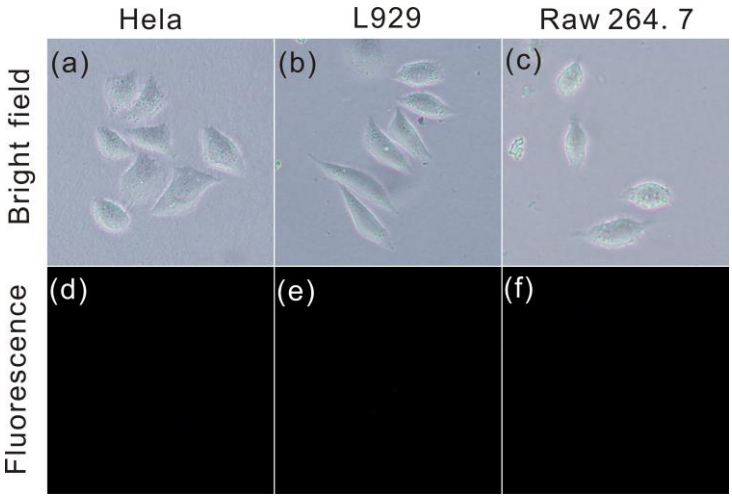
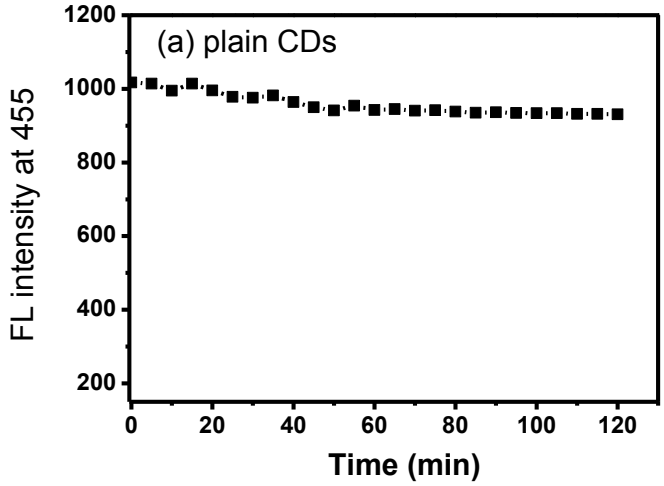
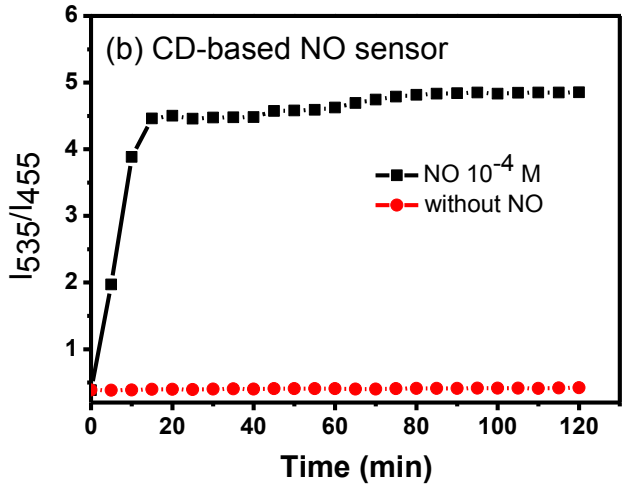


Fig. S21 Microscope images for HeLa (a, d), L929 (b, e) and Raw 264.7 macrophages cells incubated in the absence of the sensor or plain CDs as the control samples. a, b and c are bright field images of the three cell lines respectively. d, e and f are fluorescence images. The photographs were taken under identical exposure condition.

The control experiment on cells without the sensor gives no blue fluorescence at the same exposure condition.


(a) plain CDs
FL intensity at 455
1200
1000
800
600
400
200
0
20
40
60
80
100
120
Time (min)


(b) CD-based NO sensor
I535/I455
6
5
4
3
2
1
NO 10-4 M
without NO
0
20
40
60
80
100
120
Time (min)

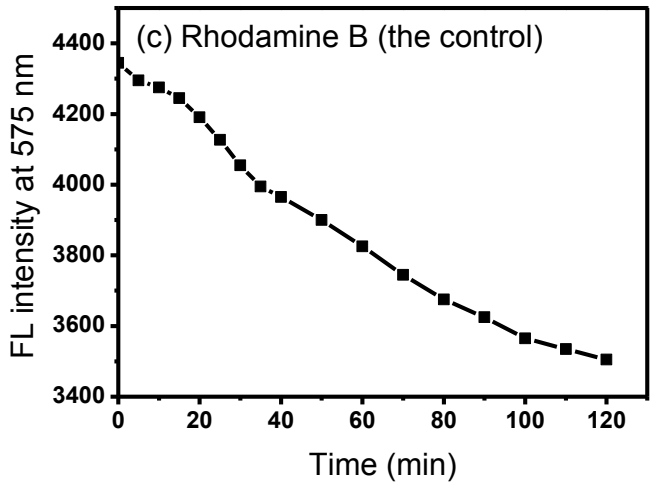

(c) Rhodamine B (the control)
FL intensity at 575 nm
4400
4200
4000
3800
3600
3400
0
20
40
60
80
100
120
Time (min)

Fig. S22 Photostablility results for the plain CD, the CD-based sensor and a reference dye (Rhodamine B) in pH 7.4 PBS buffered water under 370 nm irradiation. (a) Fluorescence intensity (λ_{ex}=370 nm, λ_{em}=455 nm) as a function of irradiation time for plain CD (0.05 mg mL^{-1}). (b) Fluorescence intensity ratio (λ_{ex}=370 nm) as a function of irradiation time for the CD-based sensor (0.06 mg mL^{-1}). (c) Fluorescence intensity as a function of irradiation time for Rhodamine B (0.1 μM) (λ_{ex}=540 nm, λ_{em}=575 nm).

The photostability of the CD-based probe was investigated. As shown in Fig. S22, for plain CD, negligible change (<6%) of the fluorescence intensity is observed up to 2 h under continuous excitation on a fluorescence spectrophotometer. Also for the CD-based sensor, negligible change of the fluorescence intensity ratio is observed. In contrast, emission of Rhodamine B decreases by 19% after 2 h of continuous excitation. These results indicate that the CD-based probe shows excellent photostability.