Electronic Supplementary Information (ESI)

for

Nitrogen and phosphorus doped polymer carbon dots as a sensitive cellular mapping probe of nitrite

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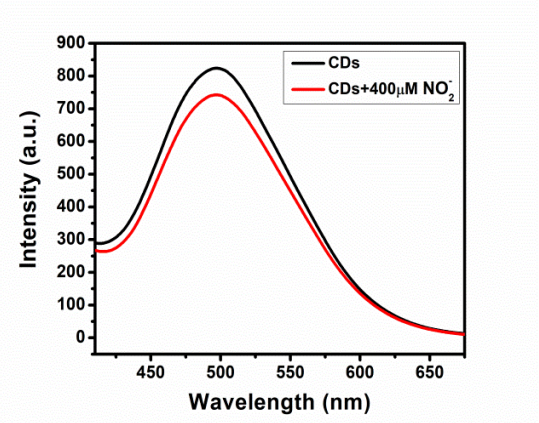

Fig. S1 PL emission spectra of phosphorus-free carbon dots upon NO_2^- (400 μM).

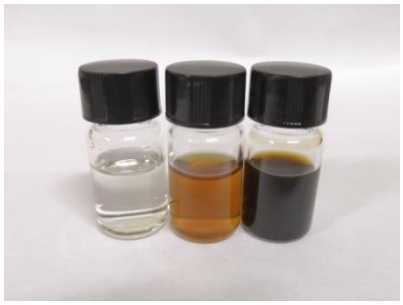

Fig. S2 Photograph of PCDs after reaction for 0 h (left), 2h (middle) and 5h (right).

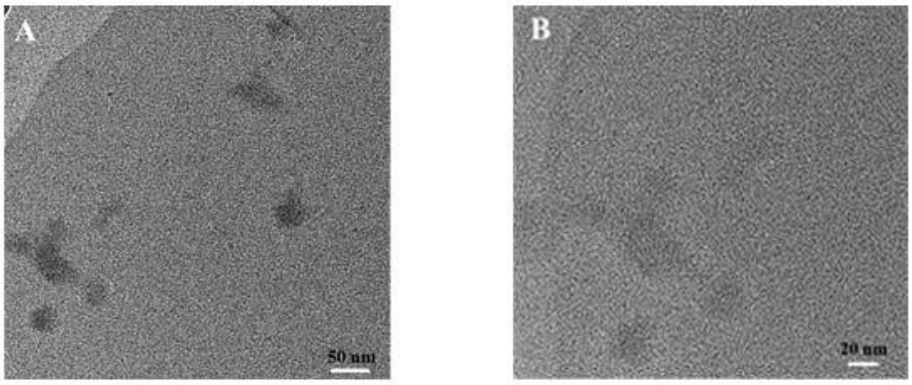

Fig. S3 TEM images of PCDs after reaction for 2 h.

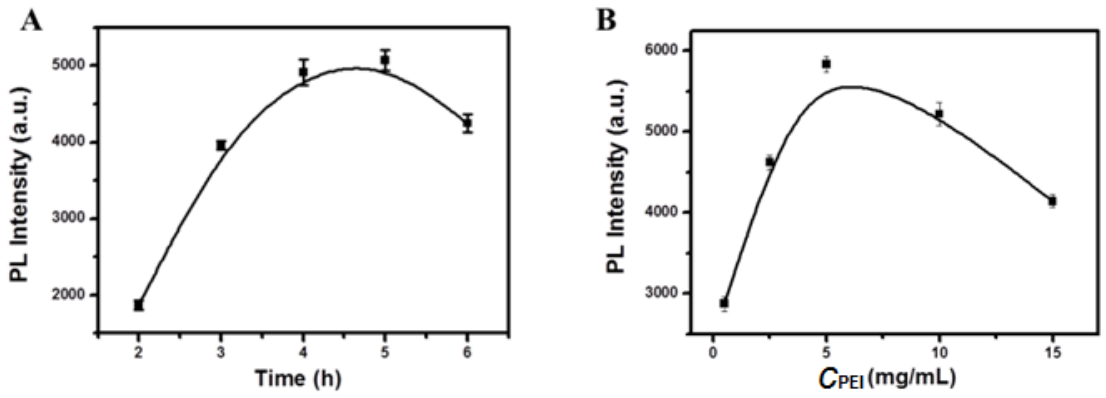

Fig. S4 PL Intensities of PCDs with different reaction time (A) and concentrations of PEI (B).

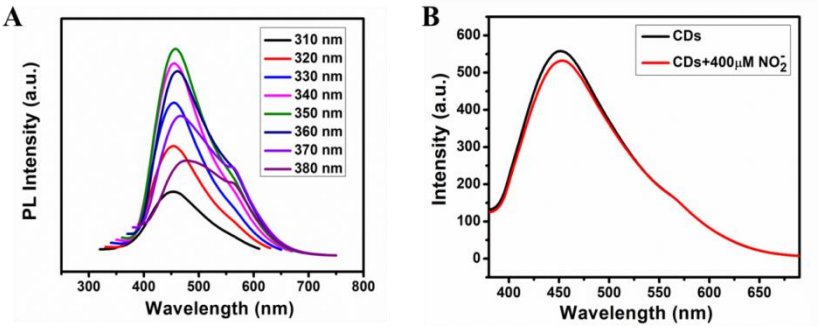

Fig. S5 PL emission spectra of CDs. (A) The excitation-dependent property of CDs prepared with AA and H_3PO_4 in the absence of PEI. (B) Fluorescence responses of CDs upon NO_2^- (400 μM).

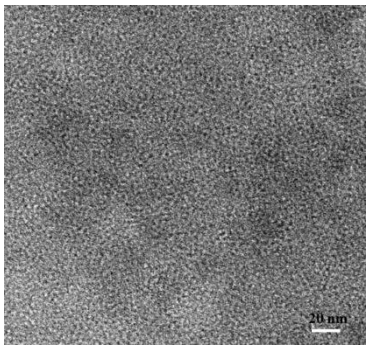

Fig. S6 TEM images of products after reacting with PEI and H_3PO_4 in the absence of AA. Scale bar: 20 nm.

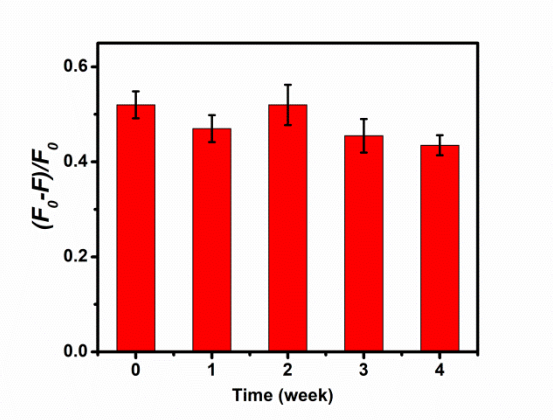

Fig. S7 Stability of fluorescence response of PCDs to NO_2^- (100 μM) for four weeks.

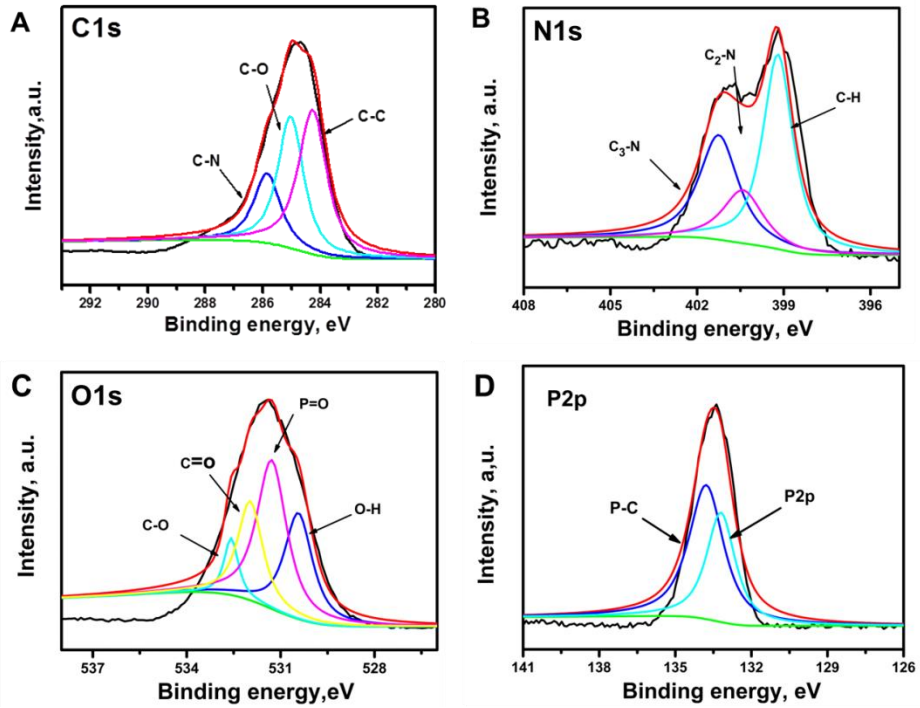

Fig. S8 High-resolution XPS spectra of PCDs. (A) C1s spectra; (B) N1s spectra; (C) O1s spectra; (D) P2p spectra.

1. Measurement of fluorescence quantum yield

The quantum yield (QY) of PCDs was determined according to a procedure reported previously. In brief, quinine sulfate in 0.1 M H_2SO_4 (QY: 54%) was chosen as a reference (refractive index η = 1.33). The PCDs was dissolved in ultrapure water (η = 1.33). The QY of the obtained PCDs was calculated using the following equation (1)[1,2]:

$$\varphi_x = \varphi_{st}(I_x / I_{st})(\eta_x^2 / \eta_{st}^2)(A_{st} / A_x) = \varphi_{st}(k_x / k_{st})(\eta_x^2 / \eta_{st}^2)$$

Where φ is the quantum yield, η is the refractive index of the solvent. I is the measured integrated emission intensity and A is the absorbance at the excitation wavelength. K_x and k_{st} is the slopes of CDs and quinine sulfate. To minimize reabsorption effects, absorbencies were kept under 0.1 at the excitation wavelength (350 nm). The QY of CDs was calculated to be 3.8 %.

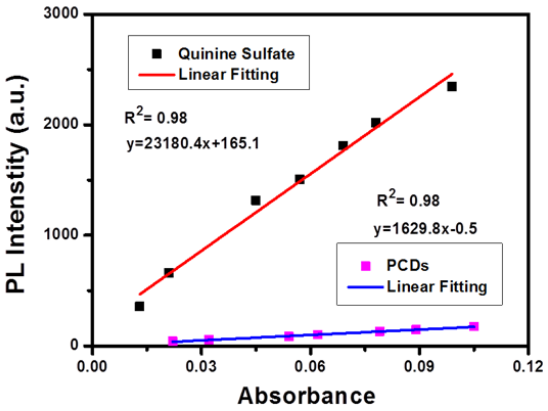

Fig. S9 Linear fitting for quantum yield calculations of PCDs and quinine sulfate. PCDs were dissolved in ultrapure water while quinine sulfate was dissolved in 0.1mol/L H_2SO_4. All the PCDs and quinine sulfate solutions had absorbance less than 0.1 at the excitation wavelength (350 nm).

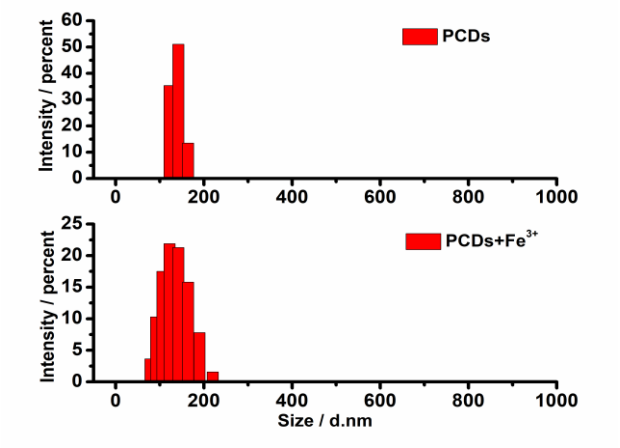

Fig. S10 DLS of PCDs in the absence of Fe^{3+} (top) and in the presence of Fe^{3+} (bottom, 100 μM).

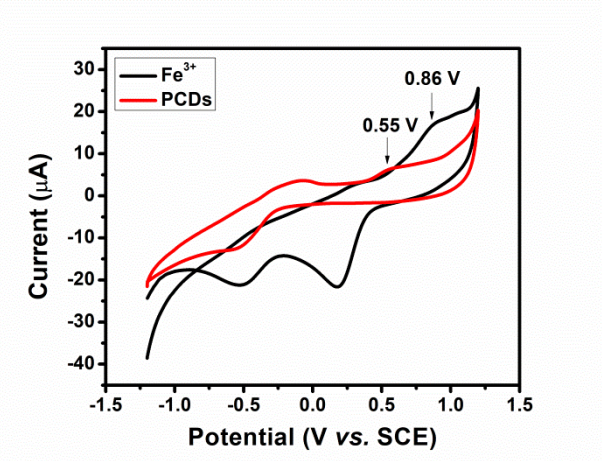

Fig. S11 Cyclic voltammograms of Fe^{3+} and PCDs.

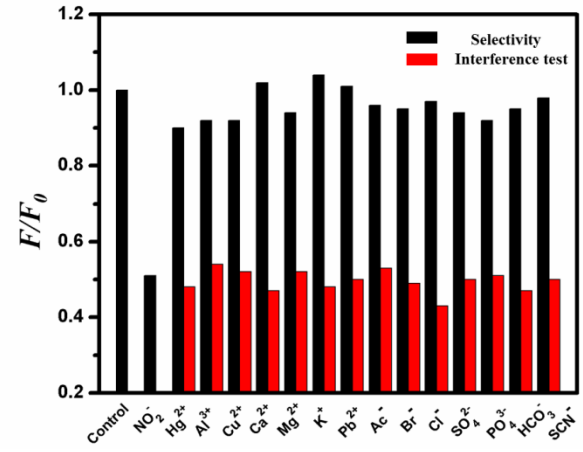

Fig. S12 Selectivity (black bar) and interference test (red bar). The anti-interference tests are performed by the addition of NO_2^- (100 μM) and other anions (500 μM).

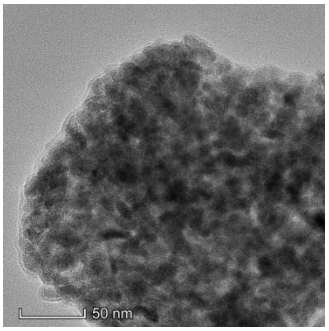

Fig. S13 TEM image of PCDs in the presence NO_2^- (100 μM).

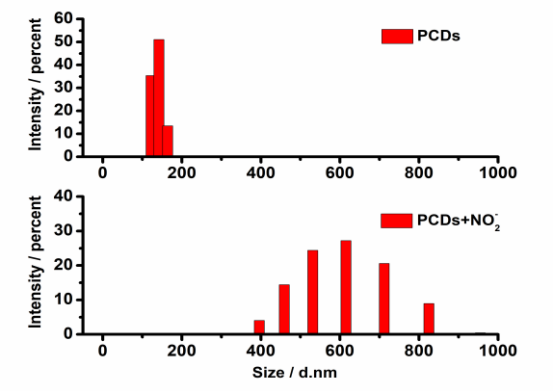

Fig. S14 DLS of PCDs in the absence of NO_2^- (top) and in the presence of NO_2^- (bottom, 100 μM).

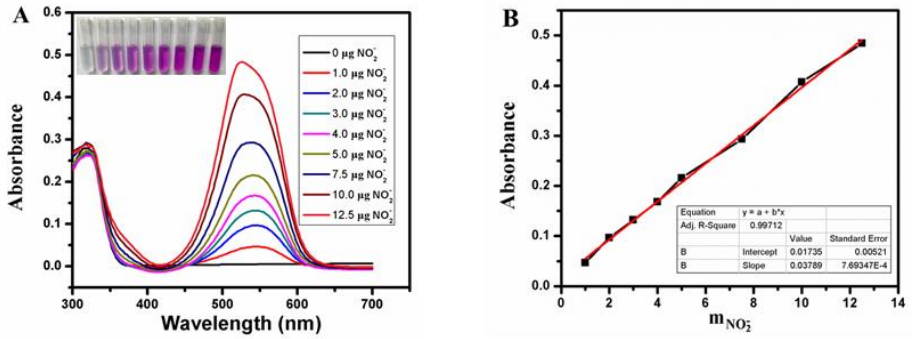

Fig. S15 Griess-saltzman method. (A) The absorption intensities after diazo-reaction; (B) establishment of standard curve.

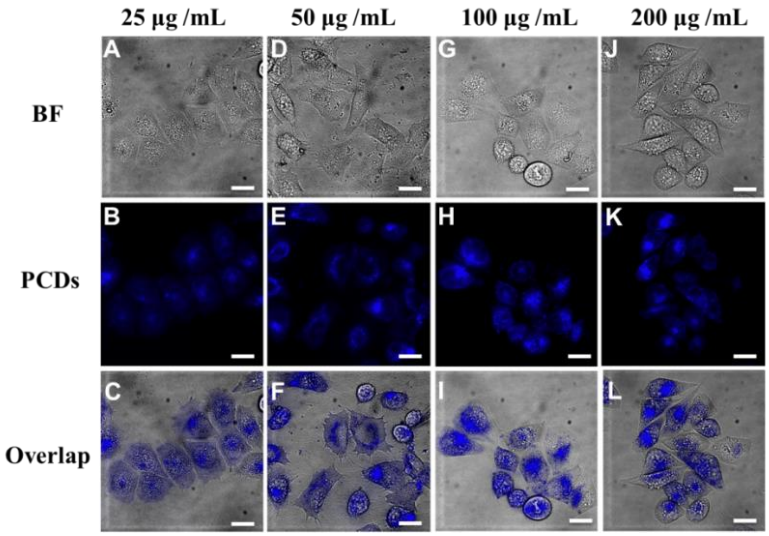

Fig. S16 Cellular uptake of PCDs incubated with Hep-2 cells. The PCDs were incubated with HEp-2 cells for 2 h at different concentrations. Scale bars: 20 μm.

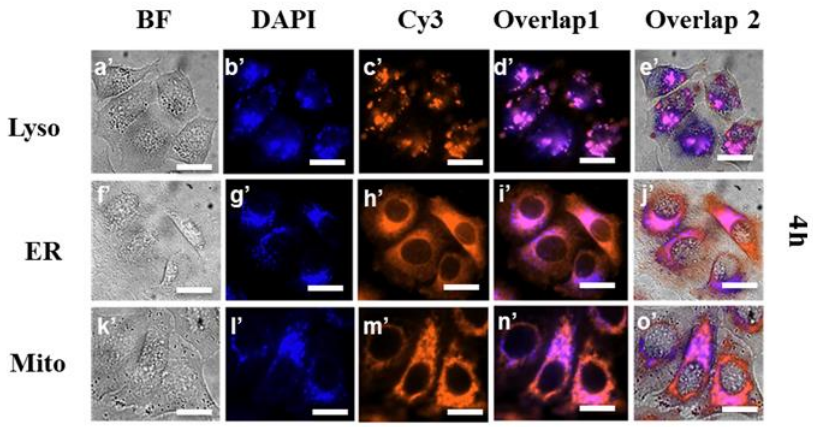

Fig. S17 Internalization localization of PCDs with different acid organelles after incubation for 4h. (a'-e') PCDs co-localized with LysoTracker red; (f'-j') co-localized with ER tracker used to label the endoplasmic reticulum; (k'-o') co-localized with MitoTracker used to label the mitochondria; Scale bars: 10 μm. DAPI and Cy3 channel indicate the presence of PCDs and fluorescent dyes, respectively.

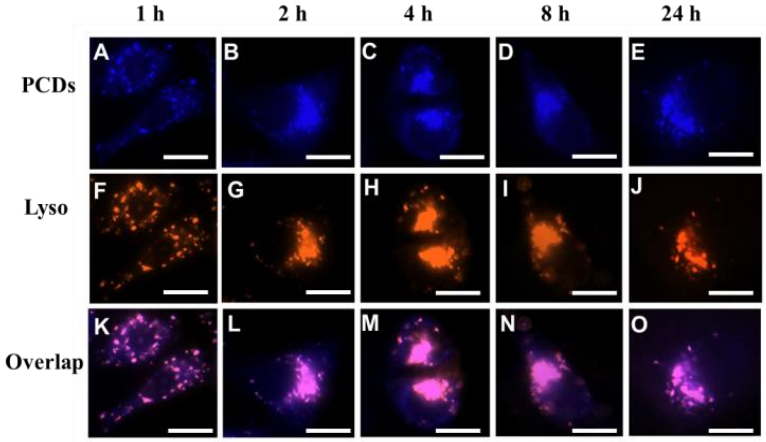

Fig. S18 The fluorescence imaging of subcellular co-localization between PCDs and LysoTracker under various incubation (1h, 2h, 4h, 8h and 24 h). Scale bars: 10 μm. Blue and orange indicate the presence of PCDs and Lyso Tracker, respectively.

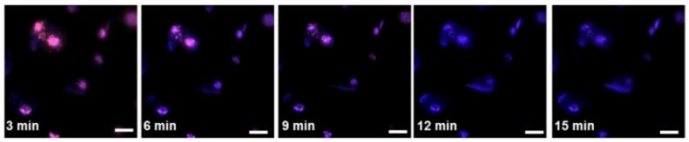

Fig. S19 The photo-stability of PCDs in Hep-2 cells compared with commercial lysosomal dyes**.** The cells labelled by PCDs and LysoTracker and exposure under confocal lasers for 15 min. It was found that the PL of LysoTracker disappeared after 12 min exposure while the PCDs maintain the beginning PL intensity. Scale bars: 20 μm

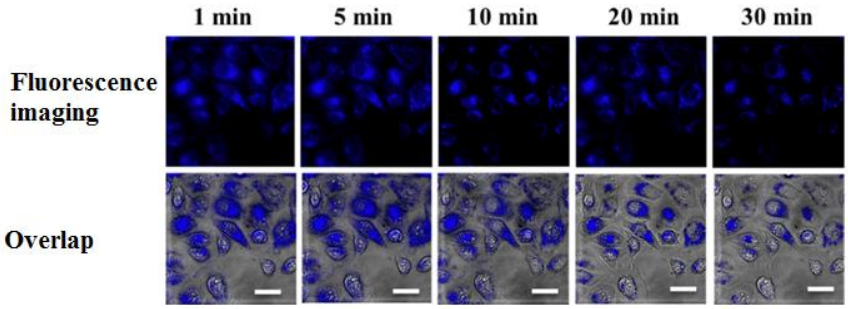

Fig. S20 The confocal microscopy images of Hep-2 cells treated with PCDs and 20 μM nitrite at different time intervals.

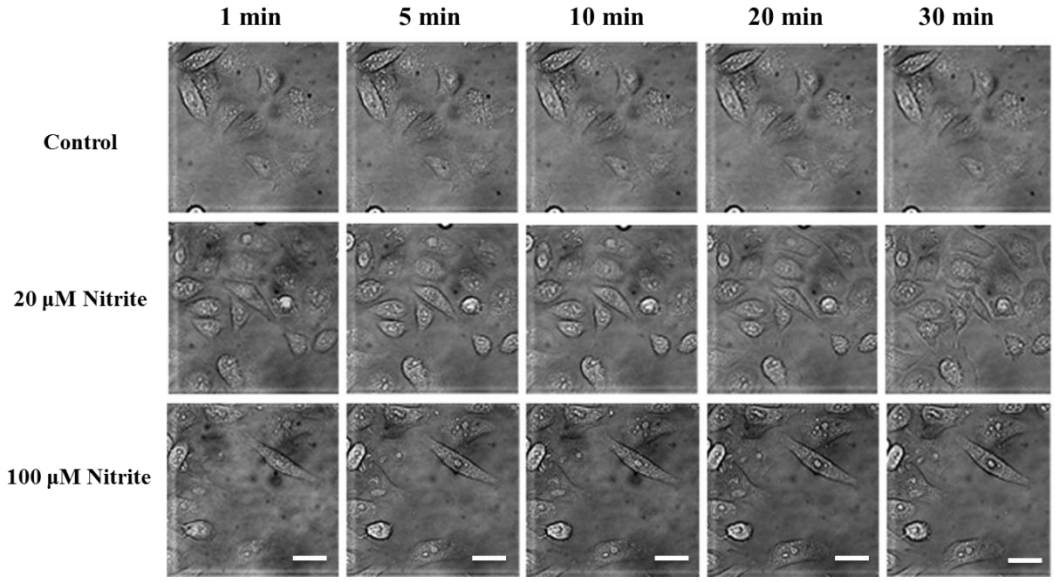

Fig. S21 Images of Hep-2 cells under bright field after incubation with PCDs and different concentrations of NO_2^- at different time intervals. Scale bars: 20 μm

2. Calculation of fluorescence lifetime.

Average fluorescence lifetime of CDs was calculated according to Equation (2): [3]

$$\bar{\tau} = \frac{A_1\tau_1 + A_2\tau_2 + A_3\tau_3}{A_1 + A_2 + A_3}$$

Table S1. The fluorescence lifetime of PCDs and after added various concentrations of nitrites.

	$A_1\tau_1$	$A_2\tau_2$	$A_3\tau_3$	$\bar{\tau}$
PCDs	1.73	0.11	4.81	6.65
PCDs+10 μMNO_2^-	1.62	1.9	2.34	5.86
PCDs+40 μM NO_2^-	1.06	1.89	2.14	5.09
PCDs+100μM NO_2^-	1.6	0.71	1.62	3.93

3. Determination of NO_2^- in milk samples and tap water.

The milk samples were purchased from Chongqing Yonghui Market. The pretreatment procedure of samples and the establishment of the standard curve referenced to GB (5009. 33-2016) of National food safety standard.

Table S2. The recovery rate of nitrites in real samples.

Sample	Proposed method 10^{-6} M	Comparison method 10^{-6} M	Nitrite spiked 10^{-6} M	Found 10^{-6} M	Recovery (%)
milk	-	-	8.0	8.1 ± 0.66	101.3%
Tap water	-	-	8.0	7.6 ± 0.21	94.6%

Table S3. Comparison of NO_2^- detection between this work and other carbon dots

Carbon dots	Methods	Sample	Linear range	Detection limit	Reference
PCDs	Fluorescence	Milk/water/ living cell	2 μM-100 μM	0.55 μM	This work
N-CNDs	Fluorescence	Water	0−1.0 mM.	1.0 μM	4
N,P-GQDs	Fluorescence	Living cell	5-30 nM	2.5 nM	5
Carbon dots	Chemiluminescence	Milk/ water	1.0×10^{-7}M-1.0×10^{-5}M	5.3×10^{-8}M	6
Carbon dots	Chemiluminescence	Water	1.0×10^{-7}M-1.0×10^{-5}M	5.0×10^{-9}M	7
CDs-Au-N	Electrochemical	Water	0.1 μM–2000 μM	0.06 μM	8

Table S4 Comparison of NO_2^- detection between this work and other reported probes

Probes	Sample	Response type	Emission wavelength	Detection limit	Reference
PCDs	Milk/water/living cell	on-off	487 nm	0.55 μM	This work
Rh 6G-SiO_2	-	on-off	551 nm	1.2 μM	9
AuNCs	Water	on-off	670 nm	1.0 nM	10
$PPESO_3$	-	on-off	530 nm	0.62 μM	11
Rhodamine based fluorogenic chemodosimeter	Water	off-on	585 nm	1.0×10^{-7} M	12
PCNs	Water/soil/milk	off-on	520 nm	2.32×10^{-9} M	13
UCNPs	Water/food	ratiometric	539 nm / 654 nm	4.67 μM	14

4. Notes and references

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