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Electronic Supplementary Information

Orange emission N doped carbon dots as a fluorescent and

colorimetric dual-mode probe for nitrite detection and cellular

imaging

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EXPERIMENTAL SECTION

Instrumentation and Characterization. Transmission electron microscopy (TEM) observations were carried out on a JEM-2100 instrument (JEOL Ltd., Japan). Atomic force microscope (AFM) image was recorded on Multimode 8. Fourier transform infrared spectra (FTIR) were obtained on a Bruker Tensor II FTIR spectrometer (Bremen, Germany) with the KBr pellet technique. The X-ray photoelectron spectra (XPS) of dried N-CDs powder were acquired on an AXIS ULTRA DLD X-ray photoelectron spectrometer (Kratos, Tokyo, Japan). The UV-vis absorption and fluorescence spectrum were acquired on UV-2910 spectrophotometer (Hitachi, Japan) and F-4500 fluorescence spectrophotometer (Hitachi, Japan), respectively. Dynamic light scattering were measured by a Zetasizer Nano ZS90 instrument (Malvern, UK). Cellular imaging was carried out using confocal laser fluorescence microscopy (LSM-880).

Quantum yield measurement. The fluorescence quantum yield of the N-CDs was determined by using Rhodamine B (QY=0.89) in ethanol as a reference based on the following equation (1):

$$\Phi = \Phi_{\rm R} \left(\operatorname{Grad} / \operatorname{Grad}_{\rm R} \right) \left(\eta^2 / \eta^2_{\rm R} \right) \tag{1}$$

Where Φ represents the quantum yield, Grad is the gradient from the plot of integrated fluorescence intensity against absorbance, and η is the refractive index of the solvent. The subscript R stands for reference to Rhodamine B. To prevent the re-absorption effect, the absorbances of the N-CDs and Rhodamine B solutions were kept below 0.10 at the excitation wavelength of 525 nm.

Cytotoxicity assay. The cytotoxicity of N-CDs in A549 cells was assessed by the standard MTT assay. Briefly, 100 μ L of A549 cells were seeded in a 96-well plate with a density of 1 × 10⁴ cells per well and allowed to adhere overnight. After an incubation of 24 h at 37 °C, the culture medium was then changed with fresh Dulbecco's modified Eagle's medium (DMEM) containing various concentrations of the N-CDs (0 to 0.90 mg·mL⁻¹) for another 24 h. Then the culture medium was removed, 20 μ L of MTT (5 mg·mL⁻¹) was added to each well. After additional 4 h

incubation, 100 μ L of DMSO was added into each well to dissolve MTT. The optical density (OD) of the mixture was measured at 600 nm with a SunRise microplate reader (Tecan Austria GmbH, Grödig, Austria). The cell viability was estimated by Equation (2).

Cell viability (%) =
$$\frac{OD_{\text{treated}}}{OD_{\text{control}}}$$
 100% (2)

where OD_{control} and OD_{treated} were obtained in the absence and presence of N-CDs, respectively.



Fig. S1 O1s high-resolution XPS spectra of the N-CDs.



	Rhodamine B					N-CDs				
Abs	0.015	0.032	0.049	0.066	0.091	0.016	0.035	0.051	0.068	0.095
Integrated	3800.47	9374.59	14327.31	20089.26	28054.33	272.46	764.82	1285.79	1791.38	1791.38
PL										
Slope	318542					29706				
QY	89%					8.3%				

Fig.S2 Plots of integrated PL intensity against absorbance of Rhodamine B and



N-CDs at λ_{ex} of 525 nm and relevant data.

Fig.S3 Effect of (A) pH , (B) ionic strength and (C) time intervals of irradiation with xenon arc light on the PL intensity at 600 nm of N-CDs.



Fig.S4 Dynamic Light Scattering (DLS) measurements of (A) N-CDs

and (B) N-CDs + NO₂⁻



Fig.S5 Cell viability values (%) estimated by MTT proliferation tests versus incubation concentrations of N-CDs at 37 °C for 24 h.