A label-free multifunctional nanosensor based on N-doped carbon nanodots for vitamin B_{12} and Co^{2+} determination, and bioimaging in living cells and zebrafish

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1. Materials

Dried Artemesia was bought from a medicinal herb pharmacy in Haozhuo, Anhui, China. 1,2-Ethylenediamine (EDA), ethanol and other chemical reagents were obtained from Sigma-Aldrich Chemical Company. vitamin B_{12} was obtained from Aladdin-Reagent Company. All reagents in the experiment are of analytical reagent grade or above, which were used as received without further purification. Water was deionized and purified by being passed through a Milli-Q Plus system.

2. Characterization of N-CNDs

Fluorescence and UV-visible absorption spectra were obtained using spectrophotometer (Varian Cary Eclipse) and a Lambda 365 spectrophotometer (PerkinElmer, Llantrisant, U.K.). Fourier transform infrared (FT-IR) spectra of N-CNDs were performed by a Bruker Tensor II spectrometer as KBr pellets. Transmission electron microscope (TEM) and high-resolution TEM (HRTEM) images were collected on a JEM-2100F Transmission Electron Microscope (Japan) operated at 200 kV. Dynamic light scattering (DLS) was obtained on a Zetasizer Nano ZS90 (Malvern, Worcestershire, U.K.). X-ray photoelectron spectroscopy was obtained by XPS

system (ESCALAB 250Xi, Thermo Scientific, Al-Ka radiation), which operated under a base pressure of 109 Torr at a power of 72 W (12 kV). The elemental analysis was conducted on an Elementar Vario Micro Cube (Germany). The fluorescence lifetime was carried out with a FLS 920 fluorescence spectrophotometer (Edinburgh Instruments Ltd). A FE20 pH meter (Mettler Toledo, Switzerland) was applied to adjust the pH value. An inVia Raman microscope (Renishaw, England) was used for Raman spectroscopy in backscattering geometry with a CCD detector. The Raman spectra of N-CND was performed on a Renishaw Invia Reflex confocal microscope.

3. MTT assay for the cell cytotoxicity

MTT assay is a quantitative colorimetric assay which assesses the in vitro cytotoxicity of N-CNDs using A549 cells. Typically, 100 μL of cells were seeded in a 96-well plate with a density of 4×10^5 cells per well and allowed to adhere overnight. Six replicate wells were used for each control and tested concentrations. After cultured in a 5% CO_2 incubator at 37 °C for 24 h, cells were treated with the other 100 μL DMEM, containing various concentrations of CNDs (0-2000 μg/mL) for another 24 h. At the end of the incubation, 10 μL of MTT (0.1 M PBS) was added into each well. After additional 4 h incubation, the growth media were removed, and the obtained mixtures were dissolved in 100 μL DMSO and shaken for 10 min. Finally, the optical density of each sample was recorded using a microplate reader (SynergyMx, Biotek, USA) at a wavelength of 490 nm. The cell viability was estimated according to the following equation:

$$Cell\ viability\ (\%) = OD_{treated}/OD_{control} \times 100\%$$

4. Measurement of quantum yield (QY):

The QY was calculated according to the following formula:

$$\Phi c == \Phi_s \times (I_c/I_s) \times (A_s/A_c) \times (\eta_c^2/\eta_s^2)$$

where ϕ is QY, the subscripts "c" and "s" refer to CDs and standard, respectively. "I" means the integrated area in the emission spectrum, "A" is the absorbance at 360 nm wavelength and η is the refractive index of the solvent. Quinine sulfate with a known QY in 0.1 M H_2SO_4 solution was employed as the fluorescence standard (ϕ_s = 54%, η_s = 1.33). To minimize the reabsorption effect, A_c and A_s in the 1 cm fluorescence cuvette were kept below 0.10.

Table S1 Elemental analysis of the as-synthesized N-CNDs: (a) elemental content and (b) relative number of atoms in N-CNDs.

(a)

Sample name	Elemental content (%)			
	C	H	N	O(Calculated)
N-CNDs	39.57	8.84	24.09	27.50

(b)

Sample name	Relative number of atoms				Empirical formula
	C	H	O	N	
N-CNDs	11	29	6	6	$C_{11}H_{29}N_6O_6$

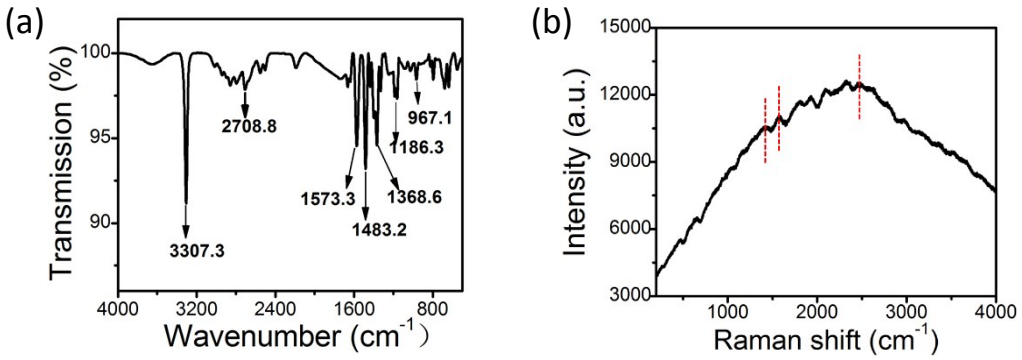
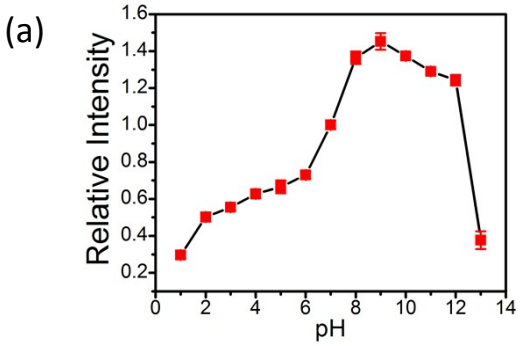


Fig. S1 FTIR spectrum (a) and Raman spectrum(b) of the as-prepared N-CNDs.



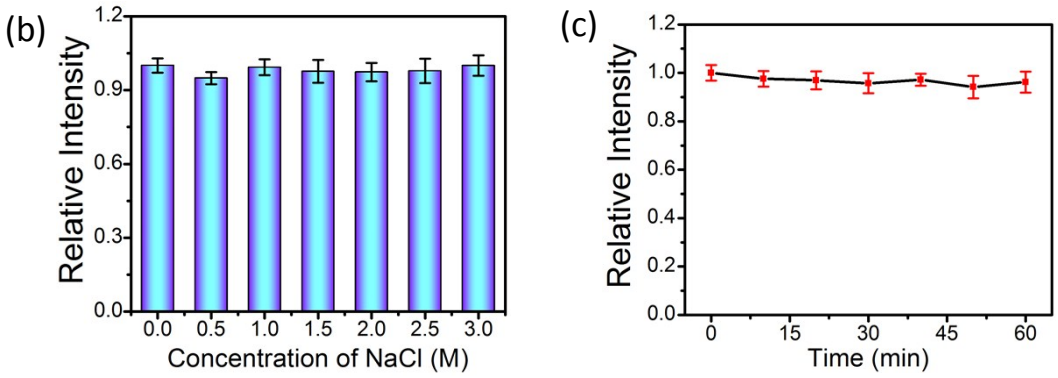


Fig. S2 Stability of N-CNDs: (a) Effect of pH on fluorescence intensity of N-CNDs. The pH is adjusted by the PBS buffers. (b) Effect of ionic strengths on the fluorescence intensity of N-CNDs (0.8 mg mL^{-1}). The ionic strength was controlled by various concentrations of NaCl. (c) Effect of time intervals with UV irradiation on FL intensity of N-CNDs.

Table S2 Comparison of the photostability of N-CNDs with the commercially available organic dyes and semiconductor quantum dots.

Types of probe	Light source	Radiation time	Changes in FL intensity	Ref
Alexa Fluor 555	100-W mercury arc lamp	95 s	retained almost 90% of fluorescence	[1]
Cy3 dyes	100-W mercury arc lamp	95 s	retained about 75% fluorescence	[1]
Alexa Fluor 647	100-W mercury arc lamp	95 s	retained about 80% of the initial fluorescence	[1]
Cy5 dyes	100-W mercury arc lamp	95 s	retained only 55%	[1]
FITC	450 W xenon lamp	15 min	Severe photobleaching	[2]
CdTe QDs	450 W xenon lamp	45 min	fluorescence is severely quenched	[2]
CdSe/ZnS QDs	450 W xenon lamp	160 min	fluorescence is severely quenched	[2]
CdTe QD SPE	two-photon excitation (1.6 mW at 405 nm laser and 6 mW at 800 nm from a fs laser)	10 s	70% reduction	[3]
CdTe QD TPE	single photon excitation (1.6 mW at 405 nm laser)	10 s	90% reduction	[3]
N-CNDs	UV radiation of 365 nm (10 W)	60 min	No change	In this work

Table S3 Comparison of proposed method with the other analytical methods for VB_{12} detection.

Method	Detection limit (nM)	Detection range (μM)	Pretreatment	Ref
Raman spectroscopy	70	10 nM to 10 μM	yes	[4]
Chemiluminescence	0.016	0.07378 nM-7.378 μM	yes	[5]
Immunoassay with electrochemical magneto sensors	13.1	21.1–129.3 nM	yes	[6]
HPLC	51	7.378 nM–7.378 μM	yes	[7]
Silicon quantum dots	158	0.5 μM –16.0 μM	yes	[8]
N-CNDs	47.4	0.5 – 34.3 μM	No	This work

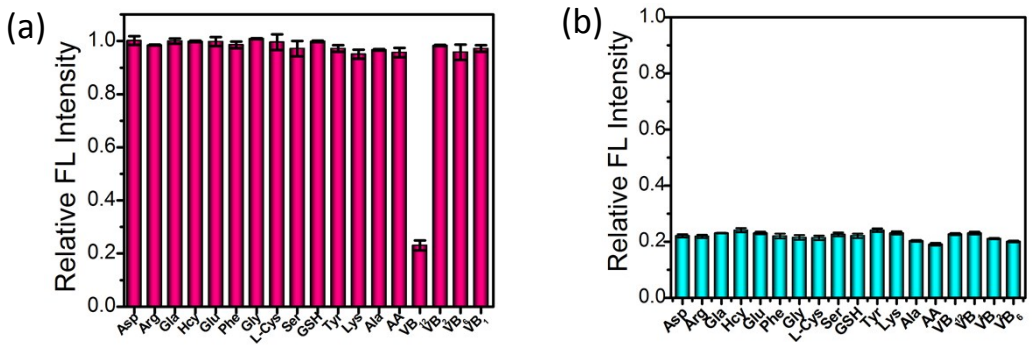


Fig. S3 (a) Response of fluorescence intensity of N-CNDs (0.8 mg mL^{-1}) after the addition of VB_{12} (0.05 mM) or other analytes (0.05 mM). (b) Response of fluorescence intensity of N-CNDs (0.8 mg mL^{-1}) in the presence of VB_{12} (0.05 mM, blank) with different coexistent materials (their concentrations are kept at 0.25 mM).

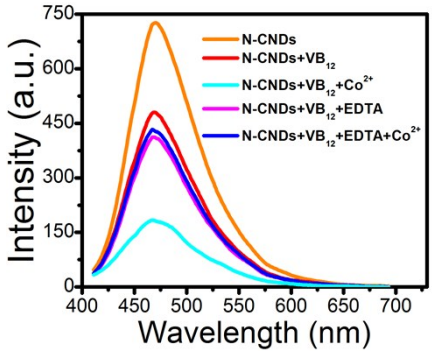


Fig. S4 FL emission spectra of N-CNDs under different conditions (Concentration of CDs was 0.8 mg mL^{-1}, VB_{12} concentration was 10 μM, Co^{2+} and EDTA concentration were 20 μM and 100 μM, respectively).

Table S4 Double-exponential fitting of N-CNDs and N-CNDs/VB_{12} decay curves.

Sample name	N-CNDs	N-CNDs+5 μM VB_{12}	N-CNDs+10 μM VB_{12}	N-CNDs+20 μM VB_{12}	N-CNDs+30 μM VB_{12}
τ_1(ns)/A_1(%)	4.47/84.69	4.50/86.03	4.45/84.57	4.45/84.05	4.51/86.02
τ_2(ns)/A_2(%)	13.49/15.31	15.82/13.97	13.90/15.43	14.62/15.95	15.60/13.98
Average τ (ns)	5.85	6.09	5.91	6.07	6.06
χ^2	0.989	0.968	1.207	1.002	1.061

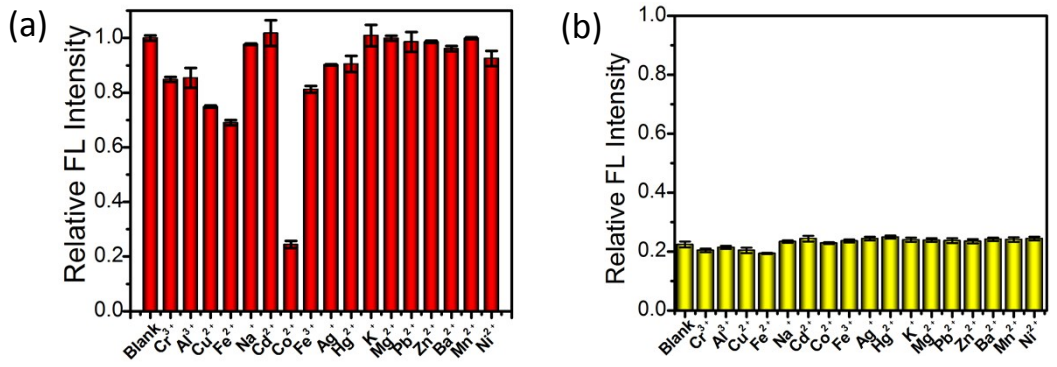


Fig. S5 (a) Response of fluorescence intensity of N-CNDs (0.8 mg mL^{-1}) after the addition of Co^{2+} (0.1 mM) or other metal ions (0.1 mM). (b) Response of fluorescence intensity of N-CNDs (0.8 mg mL^{-1}) in the presence of Co^{2+} (0.1 mM, blank) with different coexistent materials (their concentrations are kept at 0.5 mM). (c) Fluorescence titration spectra of N-CDs in the presence of Co^{2+} (2.5–25 μM). Inset: The fluorescence intensities of N-CNDs (0.8 mg mL^{-1}) as a function of Co^{2+} concentration.

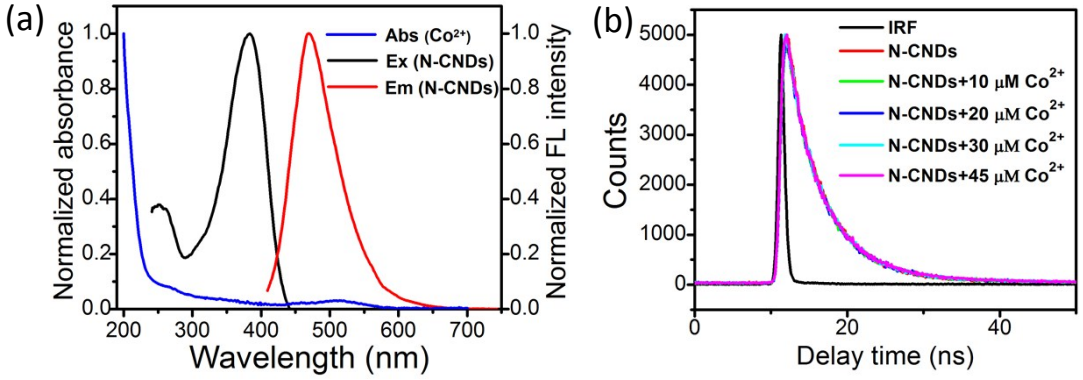


Fig. S6 (a) The absorption spectrum of Co^{2+} (500 μM) and excitation and emission spectra of N-CNDs. (b) Fluorescence decay of N-CNDs (0.8 mg mL^{-1}) in the absence and presence of Co^{2+} with different concentration (10, 20, 30 and 45 μM) as a function of time at $\lambda_{ex}/\lambda_{em}$ of 384/ 470 nm.

Table S5 Double-exponential fitting of N-CNDs and N-CNDs/Co^{2+} decay curves.

Sample name	N-CNDs	N-CNDs+10 μM Co^{2+}	N-CNDs+20 μM Co^{2+}	N-CNDs+30 μM Co^{2+}	N-CNDs+45 μM Co^{2+}
τ_1(ns)/A_1 (%)	4.49/85.80	4.43/82.95	4.31/78.23	4.36/79.76	4.25/76.23
τ_2(ns)/A_2 (%)	15.13/14.20	14.44/17.05	12.45/21.77	13.79/20.24	12.64/12.64
Average τ (ns)	5.99	6.14	6.08	6.27	6.24
χ^2	1.116	0.949	1.001	0.993	1.030

Table S6 Analytical results for the detection of Co^{2+} in real samples. (n = 6).

Tea samples	**Content (μM)**	**Added (μM)**	**Detected (μM)**	**RSD (%, n=6)**	**Recovery (%)**
		5	5.70	1.87	114.1
Tap water	Not detected	10	9.93	2.09	99.3
		15	13.6	1.07	90.4
		5	5.41	0.22	108.1
Lingde Lake	Not detected	10	8.96	0.60	89.6
		15	13.0	1.40	86.7

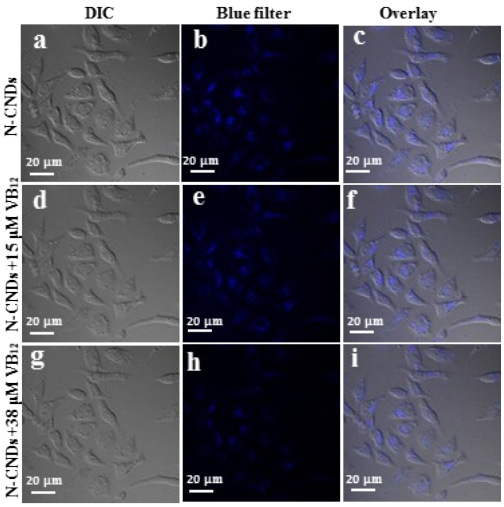


Fig. S7 Confocal fluorescence images of A549 cells incubated with 0.8 mg mL^{-1} N-CNDs and VB_{12} at different concentration (0, 15 and 38 μM). The images lie in a separate column goes to a particular filter written on the top of the column. The images were taken at λ_{ex} = 405 nm and in the λ_{em} range 410–470 nm. Scale bar, 20 μm.

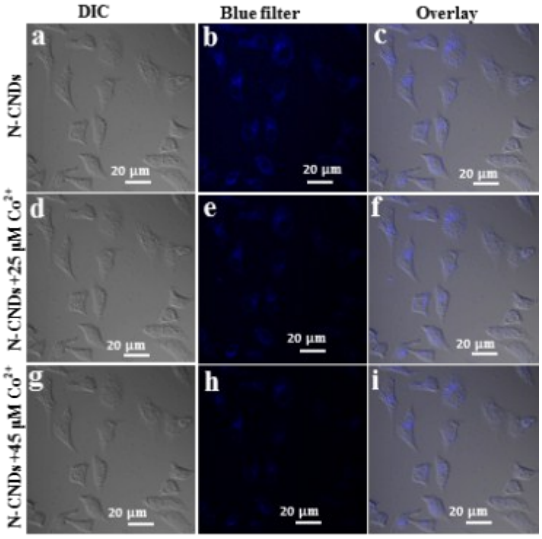


Fig. S8 Confocal fluorescence images of A549 cells incubated with 0.8 mg mL^{-1} N-CNDs and Co^{2+} at different concentration (0, 25 and 45 μM). The images lie in a separate column goes to a particular filter written on the top of the column. The images were taken at λ_{ex} = 405 nm and in the λ_{em} range 410–470 nm. Scale bar, 20 μm.

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