# Facile synthesis of ultrahigh fluorescent N,S-self-doped carbon nanodots and their multiple applications for H<sub>2</sub>S sensor, bioimaging in live cells and zebrafish, and anti-counterfeiting

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## 1. Characterization.

A JEOL JEM-2100 microscope was used to obtain the transmission electron microscopy (TEM) images an accelerating voltage of 200 kV. Elemental analysis was undertaken on a Vario MACRO cube elemental analyzer. The AFM measurements were performed using a Veeco Nanoscope Quadrex AFM (Bruker, Camarillo, CA, U.S.A.). Fourier transform infrared (FT-IR) spectra were obtained on a Bruker Tensor II FTIR spectrometer (Germany) using KBr pellets. X-ray photoelectron spectroscopy (XPS) was carried out with ESCALAB 250Xi (Thermo Scientific). Raman spectra were executed on a Renishaw Invia Reflex confocal microscope. The fluorescence spectra and relative fluorescence intensity were measured with a Varian Cary Eclipse fluorescence spectrometer. The fluorescence lifetime was carried out with a FLS 920 fluorescence spectrophotometer (Edinburgh Instruments Ltd). Absorption spectra were recorded using a Lambda 365 spectrophotometer (PerkinElmer, Llantrisant, U.K.). The pH measurements were carried out on a FE20 pH meter (Mettler Toledo, Switzerland). Fluorescence imaging was performed by a confocal laser scanning microscope (LSM880+Airyscan, Zeiss).

# 2. MTT assay for the cell cytotoxicity.

MTT assay is a quantitative colorimetric assay which assesses the in vitro cytotoxicity of N,S-self-CNDs using PC12 cells. Typically, 100  $\mu$ L of cells were seeded in a 96-well plate with a density of 4×10<sup>5</sup> 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<sub>2</sub> incubator at 37 °C for 24 h, cells were treated with the other 100  $\mu$ L DMEM, containing various concentrations of N,S-

self-CNDs (0-2000  $\mu$ g/mL) for another 24 h. At the end of the incubation, 10  $\mu$ L 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  $\mu$ L 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* (%) = 
$$OD_{treated}/OD_{control} \times 100\%$$

### 3. 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  $\phi$  is QY, the subscripts "c" and "s" refer to N,S-self-CNDs and standard, respectively. "I" means the integrated area in the emission spectrum, "A" is the absorbance at 360 nm wavelength and  $\eta$  is the refractive index of the solvent. Quinine sulfate with a known QY in 0.1 M H<sub>2</sub>SO<sub>4</sub> solution was employed as the fluorescence standard ( $\phi_s = 54\%$ ,  $\eta_s =$  1.33). To minimize the reabsorption effect, A<sub>c</sub> and A<sub>s</sub> in the 1 cm fluorescence cuvette were kept below 0.10.

### 4. The calculation method of Recovery and RSD

Accuracy was confirmed by performing recovery experiments as previously described<sup>1-3</sup>. The average recoveries were calculated by the following formula:

Recovery (%) = (amount found – original amount)/amount spiked × 100% (1) RSD (%) = (SD/mean) ×100% (2)

Table S1 Elemental analysis of N,S-self-CNDs: elemental content.

Sample name	Elemental content (%)				
	С	N	Н	S	O (Calculated)
N,S-self-CNDs	38.92%	4.50%	5.35%	14.31%	36.92%





Fig. S2 FTIR spectrum of N,S-self-CNDs.



Fig. S3 Plots of integrated FL intensity against absorbance of (a) N,S-self-CND and (b) quinine sulfate at excitation 360 nm.



**Fig. S4** Stability of N,S-self-CNDs: (a) Effect of time intervals with UV irradiation on FL intensity of N,S-self-CNDs (1.5 mg mL<sup>-1</sup>). (b) Effect of ionic strengths on the fluorescence intensity of N,S-self-CNDs (1.5 mg mL<sup>-1</sup>). The ionic strength was controlled by various concentrations of NaCl.

 Table S2 Comparison of precursors, synthetic route, QYs and photostability of N,S-self-doped CNDs with some representative green emissive CDs reported.

Heteroatom	Precursor <sup>a</sup>	Synthetic	Photostability	FL QY	Ref
doped CDs		methods/reaction		(%)	
		conditions			
N-CDs	CCM,	NaOH controlling	UV irradiation for 2.5 h,	7.2%	4
	NaOH	No heat	no photobleaching		

N-CDs	CA, urea	microwave method		15%	5
		heated for 3-4 min			
N-CDs	CC, urea	Microwave		10.1	6
		irradiation,			
		800 W, 5 min			
N-CDs	Aniline,	hydrothermal method		2.46%	7
	EDA	160 °C for 6 h			
N-CDs	o-PDA,	hydrothermal method	2h irradiation (xenon		8
	lysine	180 °C for 12 h	lamp) good photostability		
N-CDs	PT, EDA	hydrothermal	400 mM NaCl, or 8 h	17%	9
		pyrolysis	irradiation, good		
		180 °C for 5 h	photostability		
N-CDs	CA, PEI	hydrothermal method		6% and	10
		160 °C for 6 h		26%	
N-CDs	AC, urea	Solid state reaction	UV irradiation for 3 h,	WQY	11
		method 200 °C for 1 h	preserves 96% of	13.4%	
			intensity,		
N-CDs	Asp, urea	Solid state heat	UV irradiation for 5 h,	38.81%	12
		treatment at 200 °C for	preserves 96.1% of	and	
		2 h	intensity	57.68%	
N,S-CDs	CA, N-LAC	hydrothermal method,	xenon lamp irradiation	55.7%	13
		evaporation 70°C for	for 120 min,		
		12 h, and then	preserves 93% of intensity		
		200°C for 3h.			
N,S-CDs	m-PDA,	carbonization method,	UV irradiation for 12 h,	36%	14
	$H_2SO_4$	180 °C for 2 h	No photobleaching		
N, S-self-CND	Methyl blue	hydrothermal method,	50 mM NaCl, or UV	60%	In this
		200 °C for 12 h	irradiation for 120 min,		work
			good photostability		
<sup>a</sup> CCM=Cetylpyridinium chloride monohydrate, Citric acid=CA, Calcium citrate=CC, Ethylenediamine=EDA,					
o-phenylenediamine=o-PDA, Phenolphthalein=PT, Poly(ethylenimine)=PEI, Ammonium citrate=AC, Aspartic					
acid=Asp, N-acetyl-L-cysteine=L-NAC, m-phenylenediamine=m-PDA					

- represents unreported

Table S3 Comparison of proposed method with the other analytical methods for H<sub>2</sub>S detection.

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Sensors	Detection limit (nM)	Refs.
-	210	15
-	150	16
Sulfide in aqueous samples	147	17
Functionalized CdS quantum dots	6500	18
hemiluminescence Automated multi-syringe flow		19
injection analysis system		
Carbon nanotube	300	20
Gold nanorod	200	21
Copper (II) complex of azo-dye	2500	22
Coordination polymer	300	23
Fluorescein/chelator/Cu <sup>2+</sup> complex	420	24
Cyclam-functionalized carbon	130	25
dots/Cu2+		
N,S-self-CNDs	46.8	This work
	Sensors - - Sulfide in aqueous samples Functionalized CdS quantum dots Automated multi-syringe flow injection analysis system Carbon nanotube Gold nanorod Copper (II) complex of azo-dye Coordination polymer Fluorescein/chelator/Cu <sup>2+</sup> complex Cyclam-functionalized carbon dots/Cu2+ N,S-self-CNDs	SensorsDetection limit (nM)-210-150Sulfide in aqueous samples147Functionalized CdS quantum dots6500Automated multi-syringe flow88injection analysis system300Carbon nanotube300Gold nanorod200Copper (II) complex of azo-dye2500Coordination polymer300Fluorescein/chelator/Cu <sup>2+</sup> complex420Cyclam-functionalized carbon130dots/Cu2+130N,S-self-CNDs46.8

Table S4. Double-exponential	fitting of N,S-self-	CNDs and N,S-self-CNDs/	H <sub>2</sub> S decay curves.
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Sample name	N,S-self-CNDs	N,S-self-CNDs /15 µM H <sub>2</sub> S	N,S-self-CNDs /30 $\mu$ M H <sub>2</sub> S	N,S-self-CNDs /45 $\mu$ M H <sub>2</sub> S
$\tau_I(ns)/A_I(\%)$	6.10/2.21	6.29/4.78	6.81/7.54	6.20/7.81
$\tau_2(ns)/A_2(\%)$	29.99/97.79	29.18/95.22	28.86/92.46	28.22/92.19
Average $\tau(ns)$	29.47	28.08	27.20	26.50



Fig S5 (a) UV-Vis absorbance spectra, (b) Size obtained from DLS, (c) Zeta potentials of N,S-self-CDs and N,S-self-CDs with  $H_2S$ .



Fig. S6 The effect of pH on the fluorescence response of N,S-self-CNDs (1.5 mg mL<sup>-1</sup>) in the absence and presence of H<sub>2</sub>S (100  $\mu$ M) ( $\lambda_{ex}$ =353 nm,  $\lambda_{em}$ =485 nm).



**Fig. S7**: (a) FL emission spectra corresponding transmittance spectra of N,S-self-CNDs/PVA film, (b) UV-Vis absorbance spectra of N,S-self-CNDs aqueous solution and N,S-self-CNDs/PVA film, (c)FL emission spectra of N,S-self-CNDs/PVA film at different excitation wavelengths, (d) Optimal FL emission spectra under optimal excitation of 353 nm of N,S-self-CNDs aqueous solution and N,S-self-CNDs/PVA film, (e) Photostability and (f) corresponding images of N,S-self-CNDs/PVA film under UV irradiation (365 nm) for different times.



**Fig. S8** Photographs of acid-base controlled flower patterns (petals and leaves) of N,S-self-CNDs/PVA films under different excitation wavelengths of 254 nm and 365 nm UV lamp.

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