Dual-ligand functionalized carbon nanodots as green fluorescent nanosensors for cellular dual receptor-mediated targeting imaging

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1. Characterization of CNDs

Fluorescence measurements were utilized on an F-4500 fluorescence spectrophotometer (Hitachi, Japan). Dynamic light scattering (DLS) were performed on a Zetasizer Nano ZS90 (Malvern, Worcestershire, U.K.). Optical absorption spectra was acquired using a UV-3010 spectrophotometer (Hitachi, Japan). Transmission electron microscopy (TEM) was performed using a JEM-2100 instrument (JEOL Ltd., Japan) at an accelerating voltage of 200 kV. Fourier transform infrared spectra (FTIR) was recorded on Bruker Tensor II FTIR spectrometer. A Kratos AXIS ULTRA DLD X-ray photoelectron spectrometer was employed to characterize the content of elements and the chemical composition of the CNDs. The compositions of the CNDs characterized by elemental analysis was performed on an Elementar Vario Micro Cube (Germany). The fluorescence life time was obtained out using a FLS 920 fluorescence spectrophotometer (Edinburgh Instruments Ltd). pH values were adjusted with A FE20 pH meter (Mettler Toledo, Switzerland). Images were collected using a confocal laser scanning microscope (LSM880+Airyscan, Zeiss).

2. Quantum yield (QY) measurements

Rhodamine 6G (QY=95% in ethanol in the emission range of 480 to 560 nm) was used as the standard.31 The QYs of the CNDs (in water) were determined by plotting the integrated photoluminescence (PL) intensity against the absorbance value (less than 0.10 at the excitation wavelength) using the equation $\varphi = \varphi_{st} \times (A_{st}/I_{st})(I/A)(n/n_{st})^2$, where φ and the subscript "st" are the QYs of the testing sample and the referenced standard, respectively, I is the integrated emission intensity of the testing sample, n is the refractive index (1.33 for water and 1.36 for ethanol), and A is the absorbance value.

3. In Vitro Intracellular Uptake

The Human lung cancer A549 cells were seeded into 15 mm glass culture dishes at an initial density of 1×10^6 cells/mL with DMEM containing 10% FBS and incubated at 37℃ in a 5% CO_2 incubator for 24 h. After incubation, medium was replaced with CNDs, FA-CNDs, HA-CNDs and FA-PEI-HA-CNDs (0.5 mg mL^{-1}) containing culture media, respectively for 2 h. The cells are washed twice with PBS (pH 7.4) and kept in PBS for optical imaging by a LSM880+Airyscan confocal laser scanning microscope (Carl Zeiss Co.Ltd.).

Table S1 Elemental analysis of the as-synthesized CNDs.

Sample name	Elemental content (%)				
	C	H	N	O	P (Calculated)
CDs	44.28	8.09	9.58	25.57	12.48

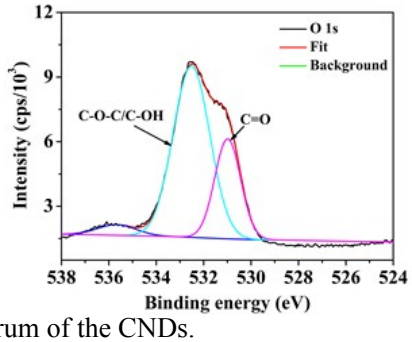

Fig. S1 O 1s of XPS survey spectrum of the CNDs.

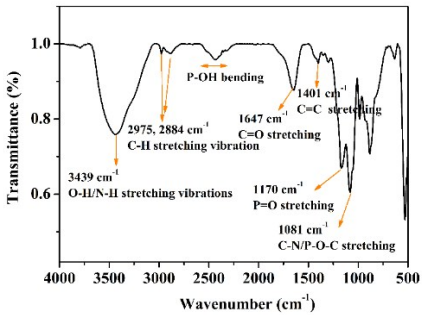

Fig. S2 FTIR spectrum of the CNDs.

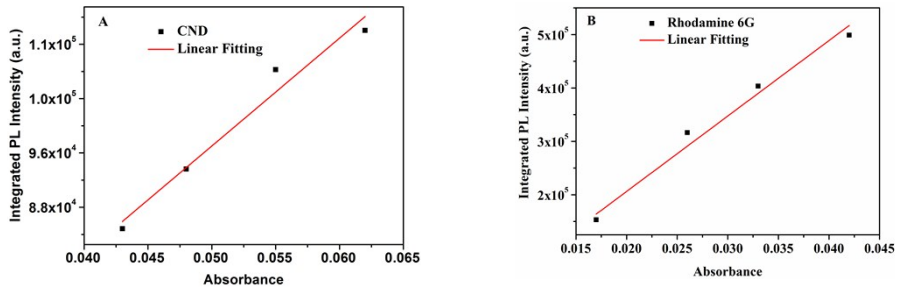

Fig S3 Plots of integrated PL intensity of (A) CNDs and (B) Rh 6G (referenced dye) as a function of optical absorbance at 424 nm.

Table S2 Calculation the fluorescence quantum yield of CNDs relevant data.

	CNDs				Rh. 6G			
Abs	0.043	0.048	0.055	0.062	0.017	0.026	0.033	0.042
Integrated PL	84853	93628	108264	114044	153262	316403	403568	499091
Slope	1.59×10^6				1.41×10^7			
QY (%)	10.71				95			

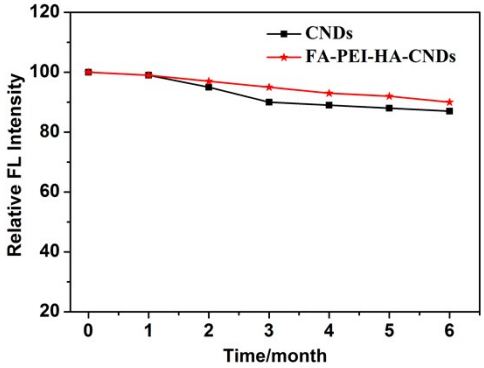

Fig. S4 Photostability of CNDs and FA-PEI-HA-CNDs when stored at 4 °C, respectively.

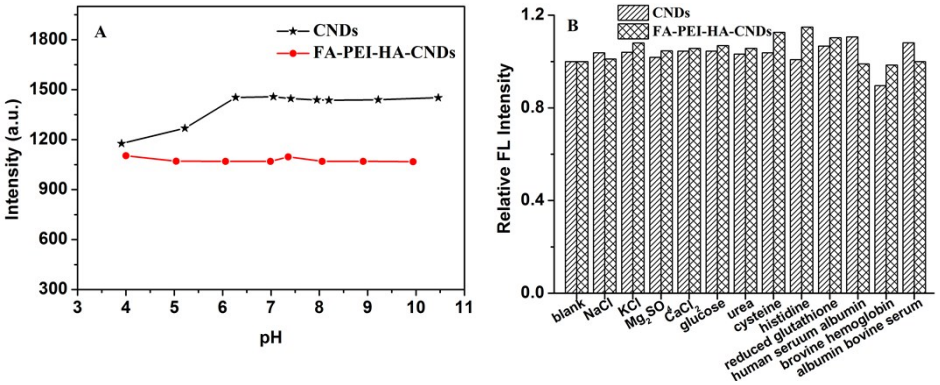

Fig. S5 (A) Effects of pH, (B) common metal ions and cellular substances on the fluorescence of CNDs and FA-PEI-HA-CNDs.

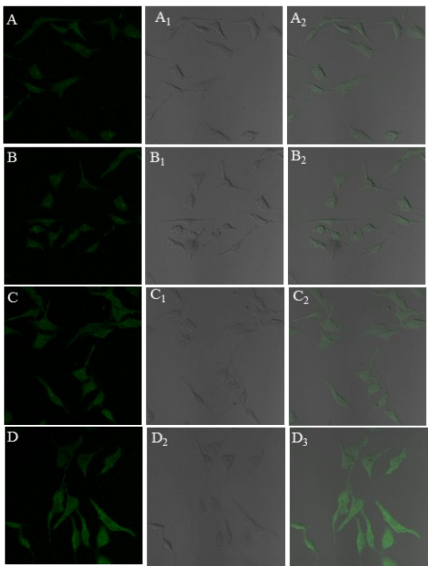

Fig. S6 Fluorescence images of A549 cells incubated with CNDs, CNDs-FA, CNDs-HA and FA-PEI-HA-CNDs for 2 h, respectively (A-D). The images of bright field (A_1-D_1), and the overlay (A_2-D_2) for corresponding cells. Scale bar, 20 μm.