Electronic Supplementary Information

The highly sensitive "turn-on" detection of morin using fluorescent nitrogen doped carbon dots

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Reagents and instrumentation.

All chemicals from commercial sources were of analytical grade. neutral red, polyethyleneimine (PEI), tryptophane(Trp), argnine (Arg), glutataione(GSH), cysteine (Cys), histidine(His), glucose (Glu), ascorbic acid (AA), bovine serum albumin (BSA), quercetin, rutin, morin and curcumin were obtained from Aladdin Chemical Reagent Co., Ltd. (Shanghai, China). Other reagents were purchased from Beijing Chemical Reagents Company (Beijing, China), which were used as received without further purification. Water was deionized and purified with a resistivity of 18.25 MΩ cm by being passed through a Milli-Q. Phosphate buffer solution (PBS, pH = 7.4) was employed to adjust the pH of the solution.

Instrumentation

The pH measurements were performed using a pH meter. (Mettler Toledo, Switzerland). Fourier transform infrared (FT-IR) was performed on a Bruker Tensor II FTIR spectrometer (Bremen,Germany) in the form of KBr pellets from 4000 to 400 cm^{-1}. The transmission electron microscopic (TEM) images were acquired on a JEOL JEM-2100 transmission electron microscope (Tokyo, Japan) with an accelerating voltage of 300 kV. The X-ray photoelectron spectra (XPS) were acquired on an AXIS ULTRA DLD X-ray photoelectron spectrometer (Kratos, Tokyo, Japan) with AlKa radiation operating at 1486.6 eV. Spectra were processed by Case XPS v.2.3.12 software using a peak-fitting routine with symmetrical Gaussian–Lorentzian functions. The UV-Vis absorption spectra were performed on a PerkinElmer Lambda 365 UV-Vis spectrophotometer (PE America) at 200–700 nm. The PL spectra were recorded on Edinburgh FLS 920 spectro-

fluorometer (Livingston, UK) and Varian Cary Eclipse spectrofluorometer (Palo Alto, CA, USA).G

Quantum yield measurements

The quantum yield ($\Phi\chi$) of as-prepared N-CDs sample was determined by comparing the integrated fluorescence (FL) intensities and optical density (OD) of the as-prepared N-CDs with the reference Rhodamine 6G (Φst =98%) in 0.01 mol/L ethanol (refractive index, η=1.36) while the as-prepared N-CDs was dissolved in distilled water (η=1) at different concentrations. A Lambda 950 absorption spectrophotometer was used to determine the OD (absorbance) of the solutions at 454 nm. A Varian Cary Eclipse spectrofluorometer was used to record their FL spectra with an excitation wavelength of 454 nm. The integrated FL intensity was the area under the FL curve in the wavelength range of 474-700 nm. Then a graph of integrated FL intensity against absorbance was plotted. The $\Phi\chi$ of the as-prepared N-CDs were calculated using equation (1):

$$\Phi\chi=\Phi st\ (K\chi/Kst)\ (\eta\chi/\eta st)^2 \quad (1)$$

where K is the gradient from the plot of integrated FL intensity against OD and h is the refractive index. The subscript "st" and "χ" refer to the standard and sample, respectively. For these aqueous solutions, $\eta\chi/\eta st$ =1. In order to minimise the self-absorption effect, the OD was kept under 0.10 at the excitation wavelength in the 10-mm path-length fluorescence cuvette.

Encapsulation of N-CDs in agarose hydrogel and visual analysis of morin by smartphone

The N-CDs-based hydrogel sensor was prepared and applied for determination of morin owing to the dipole-dipole interactions and hydrogen bonding between agarose and N-CDs during the reaction. Briefly, 0.15g agarose and15 mL N-CDs solution were stirred and heated at 100°C for 3 min to make sure the dissolution of agarose. Then, the as-prepared solution was transferred into the circular molds and cooled down to room temperature. Next, obtained hydrogel slices were soaked into different amounts of morin solutions to be used for the subsequent assay of morin. The fluorescent color of the hydrogel slices was then recorded by a smartphone camera under a UV lamp. Then, the displayed photo image was directly analyzed using an app installed on the smartphone (color desk). The sum RGB values using Equation,

Sum RGB values=Red +Green+ Blue

Cytotoxicity assays

For the cell cytotoxicity test, HeLa cells were seeded into Costar@ 96-well cell culture cluster with a density of 4 ×103 cells/well in 100 mL of Dulbecco's Modified Eagle's Medium (DMEM) medium with 10% FBS at 37℃with 5.0% CO_2 atmosphere. After the original medium was removed, the HeLa cells were incubated with different concentration N-CDs (500 μg/mL) for 48 h. At least five parallel samples were performed in each group. The cells in a culture medium and without N-CDs were served as the control. The cells were washed by PBS for three times, then added 20 mL MTT (5.0 mg/mL PBS) reagent to each well with fresh culture medium. After 4 h incubation, MTT-containing culture medium was removed from the wells and 150 mL of DMSO was added per well to dissolve the intracellular blue-violet formazan crystals. Finally, the SunRise microplate reader (Tecan Austria GmbH, GrOdig, Austria) was used to record the optical density (OD) of each sample at a wavelength of 566 nm. The cell viability was calculated as following equation: cell viability (%) = (OD treated /OD control) × 100%, where OD treated and OD control were obtained in the presence and absence of N-CDs, respectively.

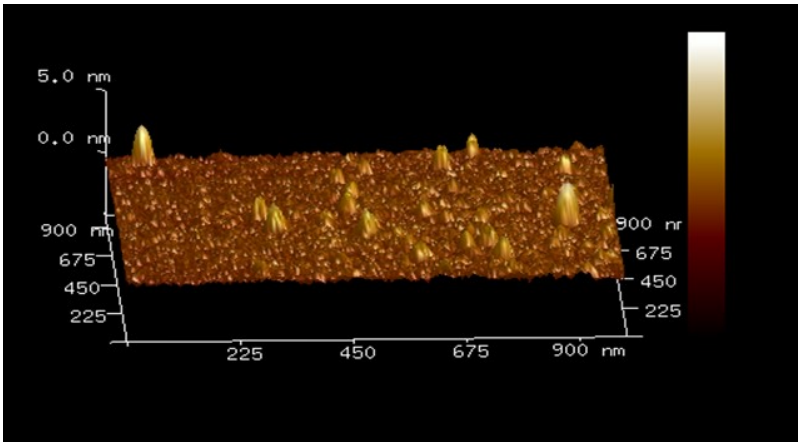
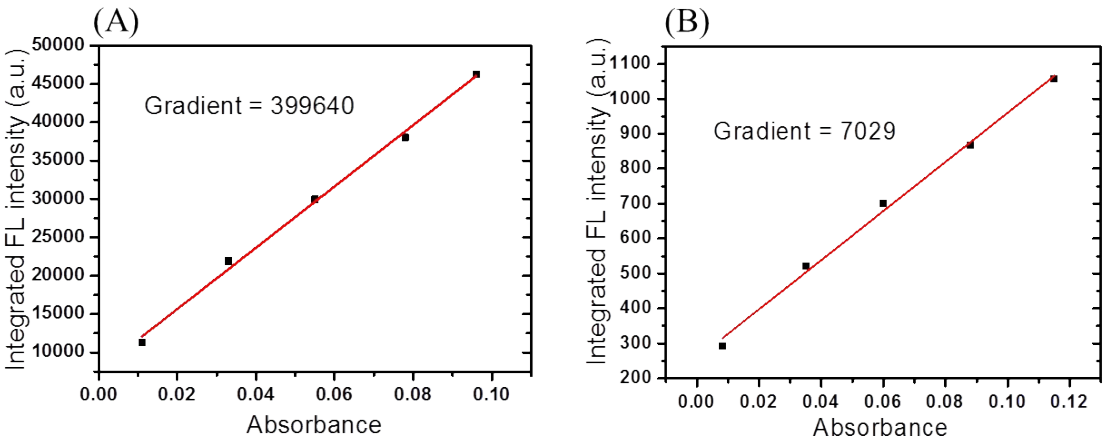


Fig. S1 AFM three-dimensional image of N-CDs.



	Rhodamine 6G					N-CDs				
Abs	0 .011	0.033	0.055	0.078	0.096	0.082	0.035	0.060	0.088	0.093
Integrated PL	11306	21899	29961	38000	46251	293	522	700	867	1057
Slope	399640					7029				
QY	94%					1.9%				

Fig. S2 Plots of integrated PL intensity against absorbance of (A) Rhodamine 6G (B) the N-CDs at excitation 454 nm and relevant data.

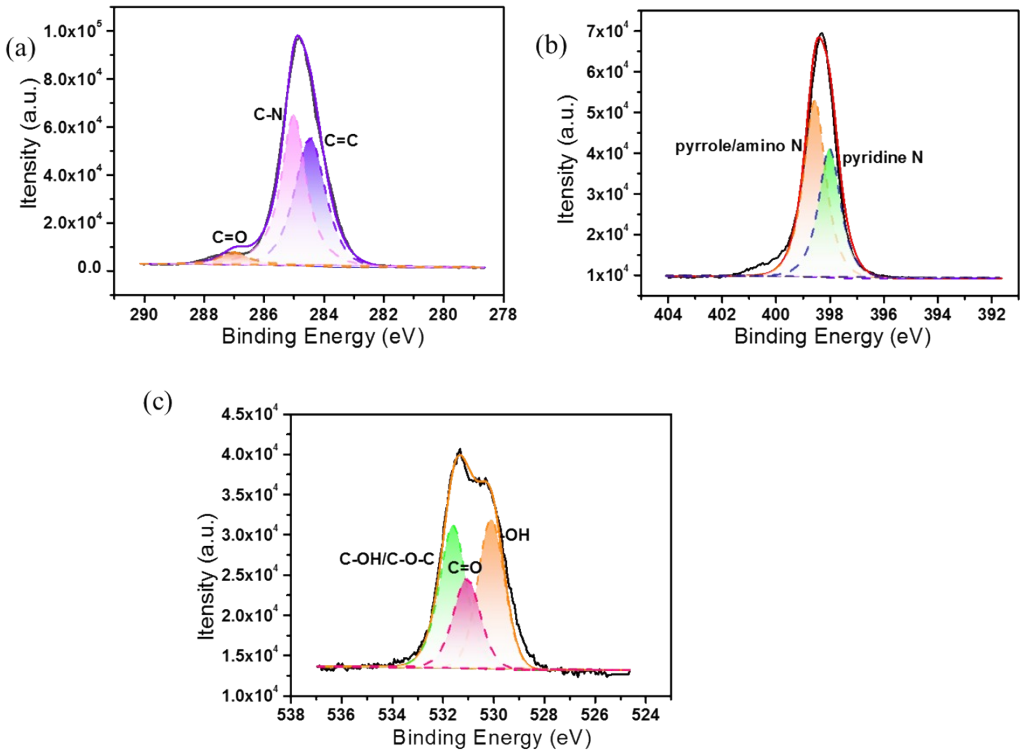


Fig. S3 High-resolution XPS of C1s, N1s, and O1s spectrum of N-CDs.

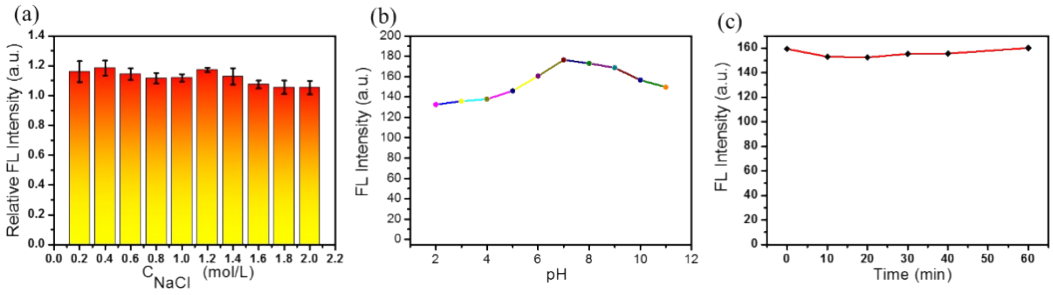


Fig. S4 (a-c) Effect of ionic strengths, pH and time intervals with UV irradiation on the relative fluorescence intensity of N-CDs.

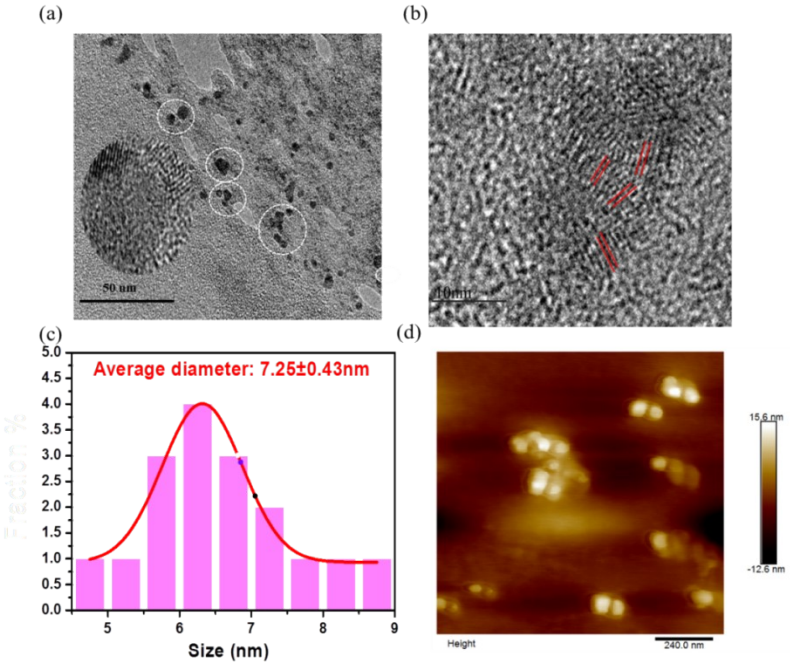


Fig. S5 (a) TEM images of N-CDs + morin. (b)HRTEM images of N-CDs + morin. (c)The size distribution diagrams for N-CDs + morin. (d) AFM image of N-CDs + morin.

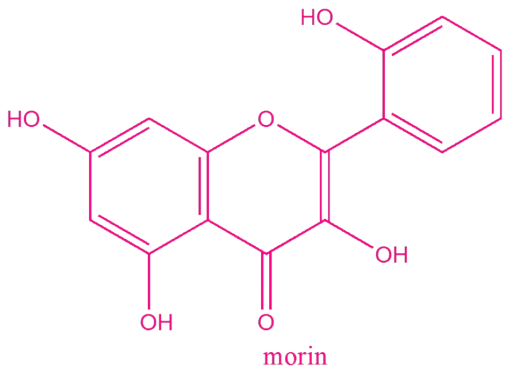


Fig. S6 The chemical structures of morin.

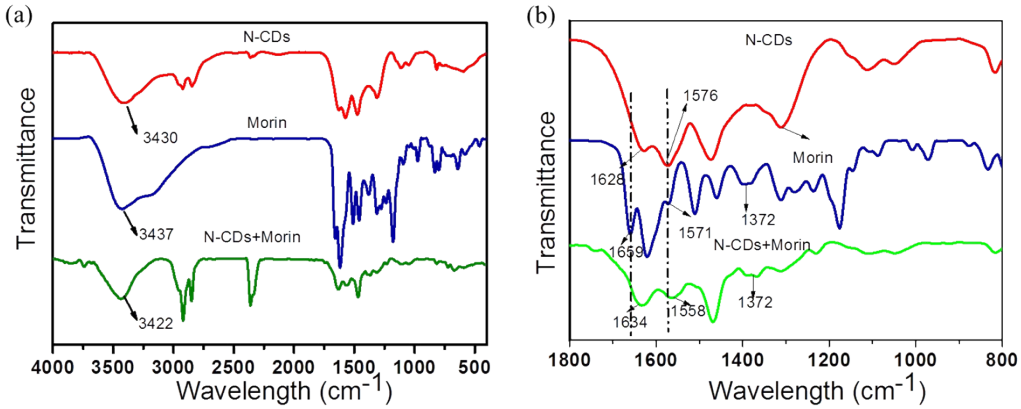


Fig. S7 (a-b) FT-IR spectra of N-CDs+morin.

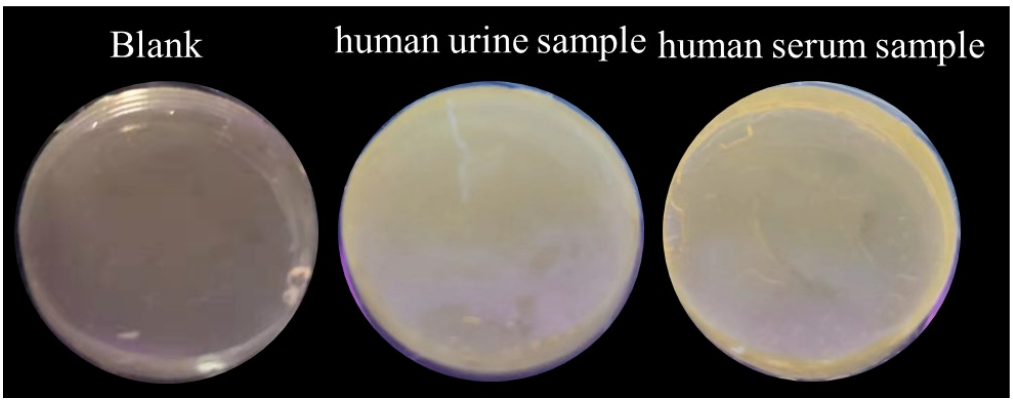


Fig. S8 Fluorescence comparison between N-CDs hydrogels, N-CDs hydrogels containing morin (15μM) in human urine and human serum samples.

Table S1. N-CDs/agarose hydrogel -based smartphone detection platform for the recognition of morin in human urine and human serum

	Added/(μmol/L)	Found/ (μmol/L)	Recovery /(%, n=6)	RSD/ (%,n=6)
Human urine	10.00	10.83	108.30	1.02
Human serum	10.00	11.56	115.60	1.38

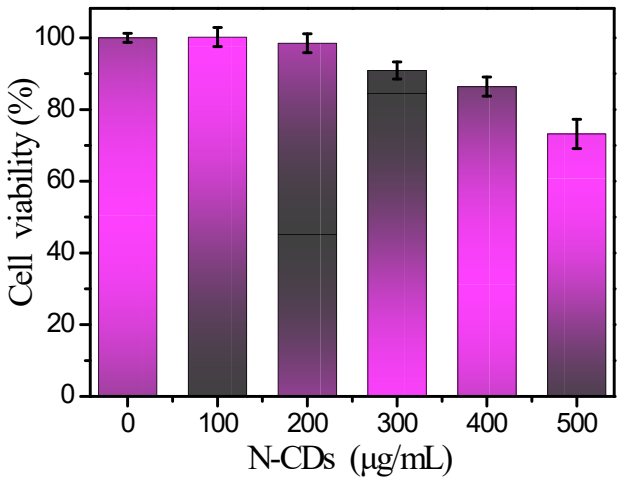


Fig. S9 Cytotoxicity testing results of N-CDs on HeLa cells viability. The values represent percentage cell viability (mean% ± SD, n=6).

Table S2. Comparisons on analytical performance between previous technologies and this probe

Method	Materials	Linear range	Limit of detection	Ref
HPLC	-	0.09-47.26 μg/mL	66.8 ng/mL	6
Nuclear magnetic spectroscopy	-	7.12–114 mg/L	2.30×10^{-2} mg/L	8
Fluorometry	B,N-CDs	14.5-32.5 μmol/L	0.3 μmol/L	20
Fluorometry	CDs	0.4-60 μmol/L	0.12 μmol/L	19
Fluorometry	O-CDs	5-125 μmol/L	0.84 μmol/L	18
Fluorometry/ Smartphone sensing	N-CDs	0.033-12.83/ 10-50 μmol/L	0.029/ 1.91 μmol/L	This work