

# A Highly Sensitive and Selective Nano-Fluorescent Probe for Ratiometric and Visual Detection of Oxytetracycline Benefiting from Dual Roles of Nitrogen-Doped Carbon Dots

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## Apparatus and characterization

The morphologies and sizes of the NCDs were characterized by JEM-2100F transmission electron microscopy (TEM, JEOL, Japan). The elemental compositions and contents of NCDs were analyzed with SIRION-100 X-ray energy dispersive spectroscopy (EDX, PEI, Netherlands). The X-ray diffraction (XRD) pattern was recorded on a D8 advance X-ray diffractometer (Bruker, Germany). Chemical compositions and valency were analyzed by X-ray photoelectron spectroscopy (XPS) performed on a Thermo Kalpha spectrometer (Thermo Fisher Scientific, USA) using a 1486.6 eV Al K $\alpha$  line excitation source. Fourier-transform infrared (FT-IR) spectra were recorded on a Nicolet IS10 FT-IR spectrometer (Thermo Fisher Scientific, USA) with KBr plates. Fluorescence spectra were collected with an F-7000 spectrofluorimeter (Hitachi, Japan) with the photomultiplier tube voltage set at 400 V. Fluorescence lifetime curves and fluorescence quantum yield were generated on a FLS-1000 steady-state and time-resolved spectrofluorimeter (Edinburgh, England). UV-Vis spectra were recorded on a UV-3600 spectrophotometer (Shimadzu, Japan). All pH measurements were performed on a FE28-standard pH meter (Mettler Toledo, Switzerland).

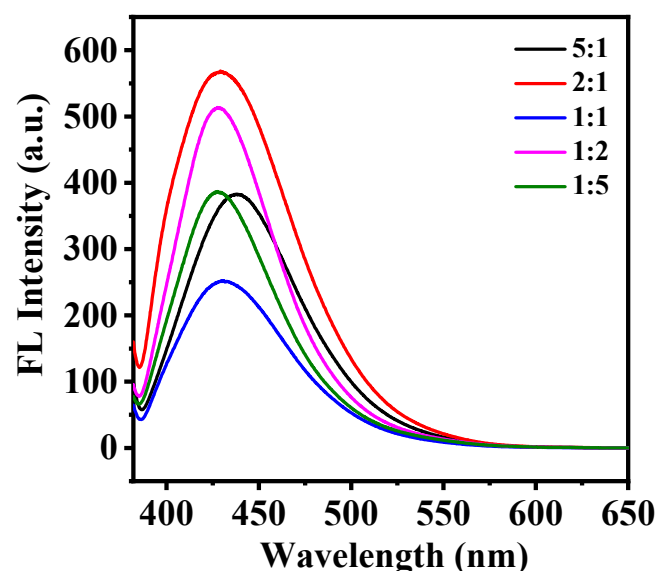


Figure S1. Fluorescence spectra of NCDs synthesized with different MCA/MUA.

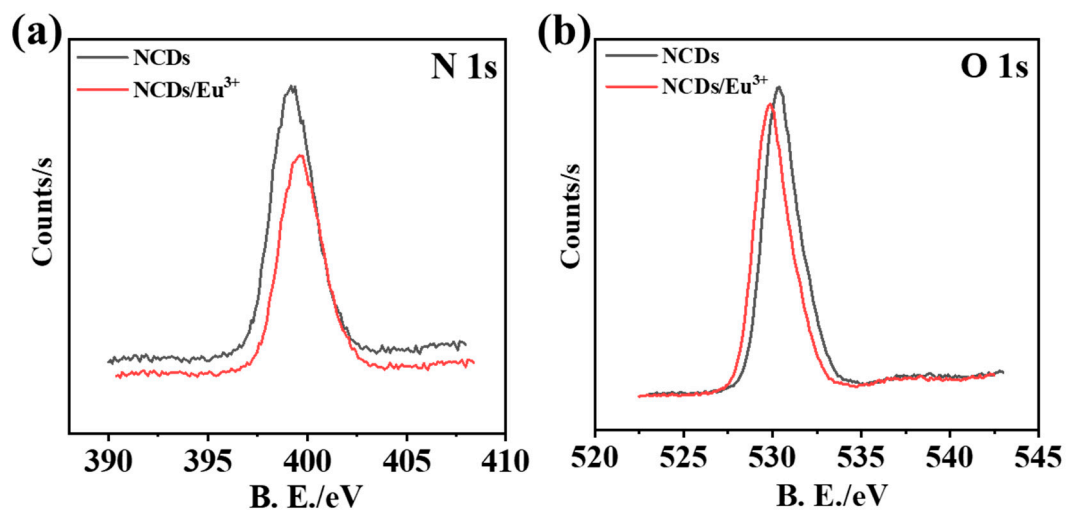


Figure S2. The high-resolution N1s (a) and O1s (b) spectra of NCDs and NCDs/Eu<sup>3+</sup>.

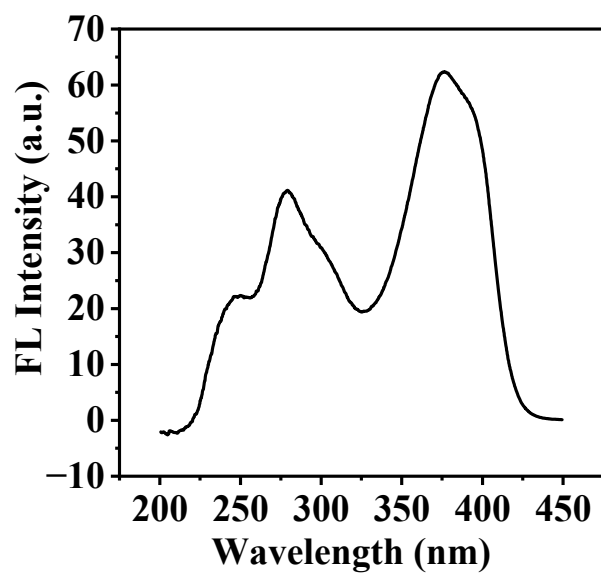


Figure S3. Excitation spectra of NCDs/Eu<sup>3+</sup>-OTC.

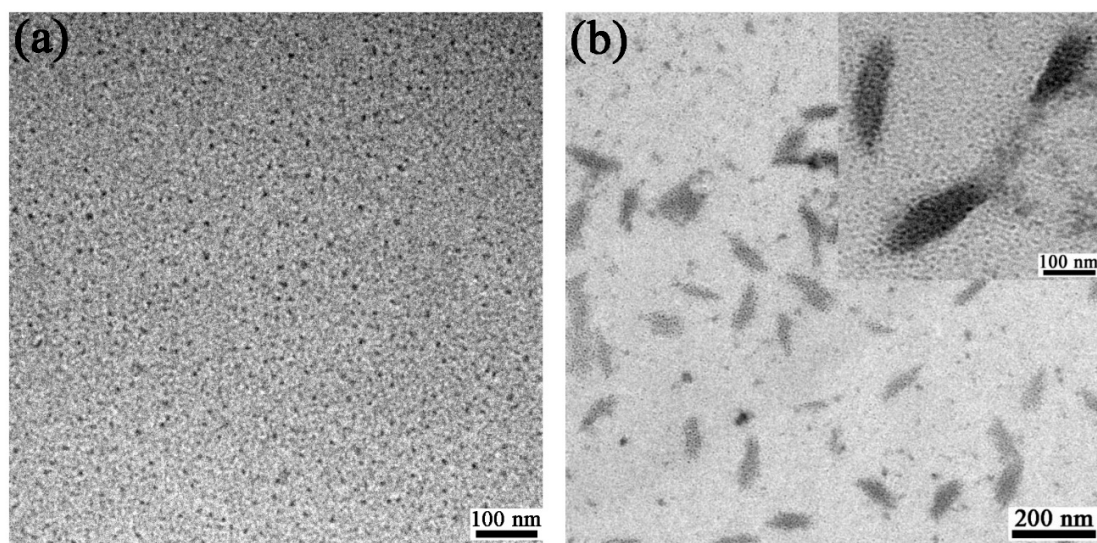


Figure S4. TEM images of NCDs/Eu<sup>3+</sup> before (a) and after (b) incubation with OTC.

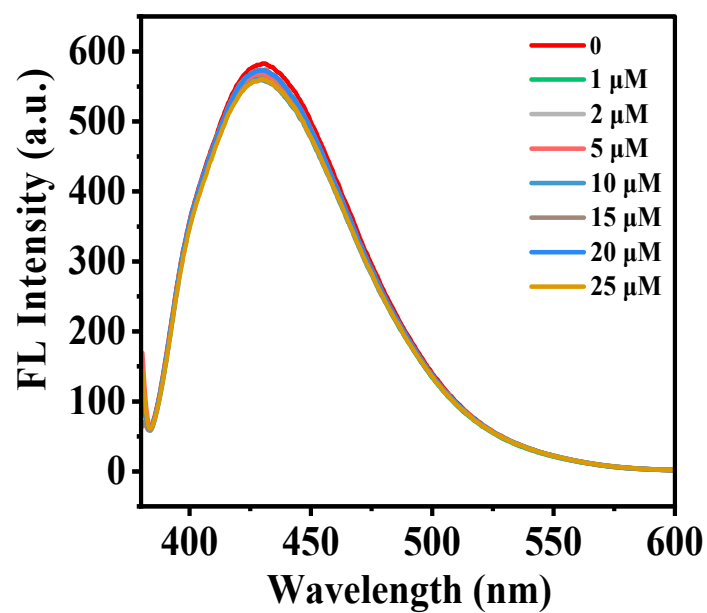


Figure S5. Effect of OTC at different concentrations on the fluorescence spectra of NCDs.

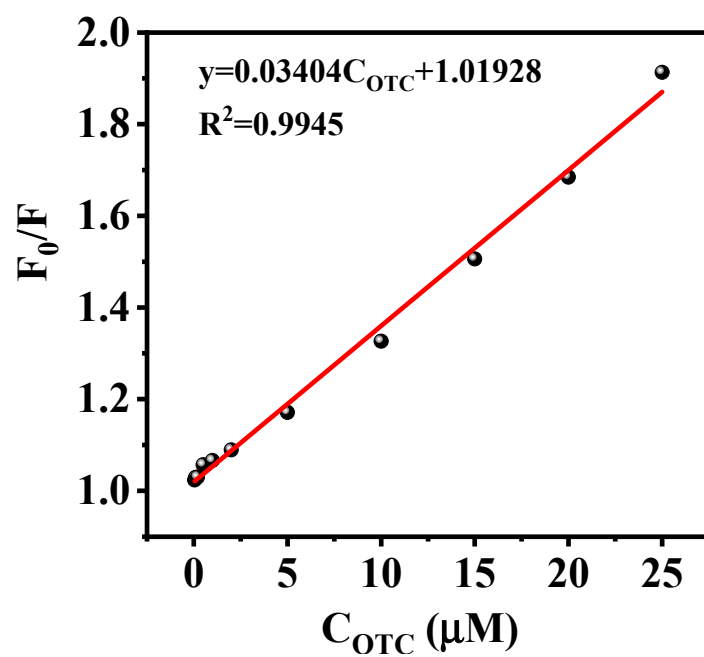
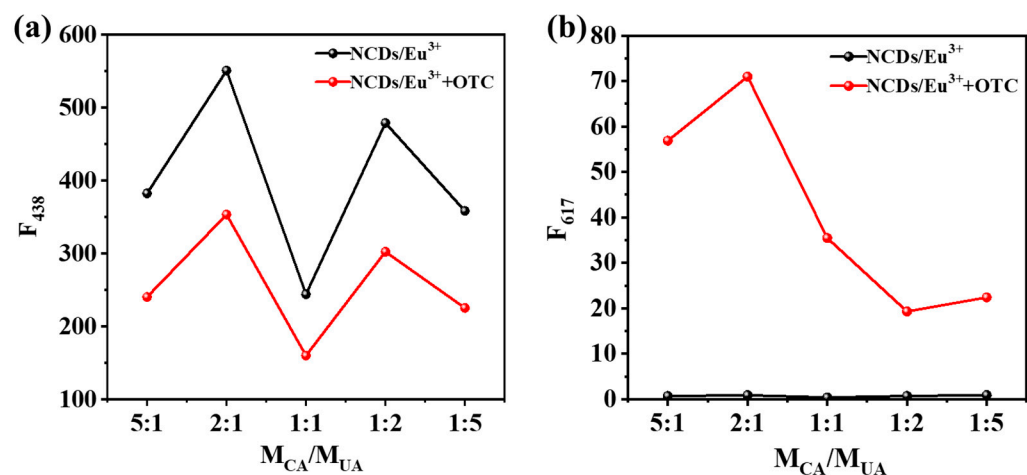
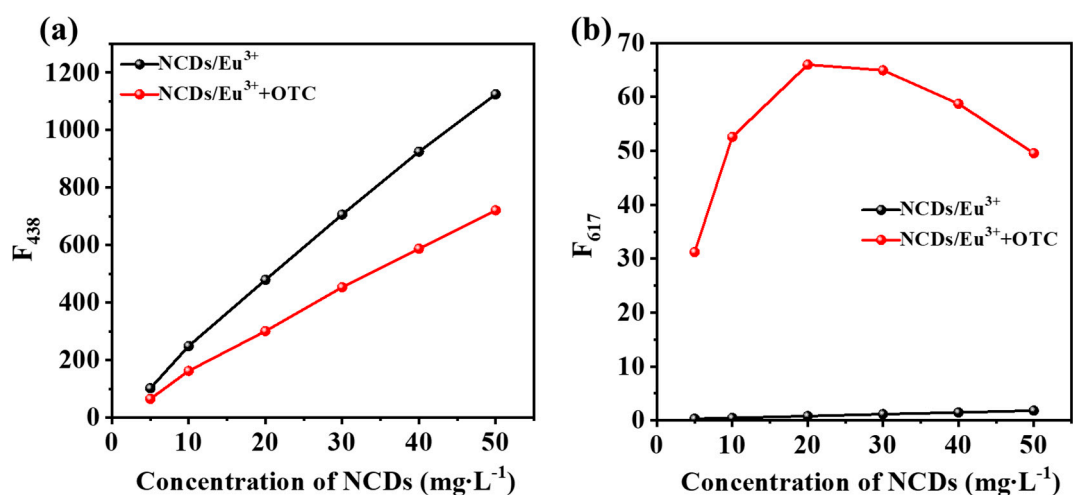


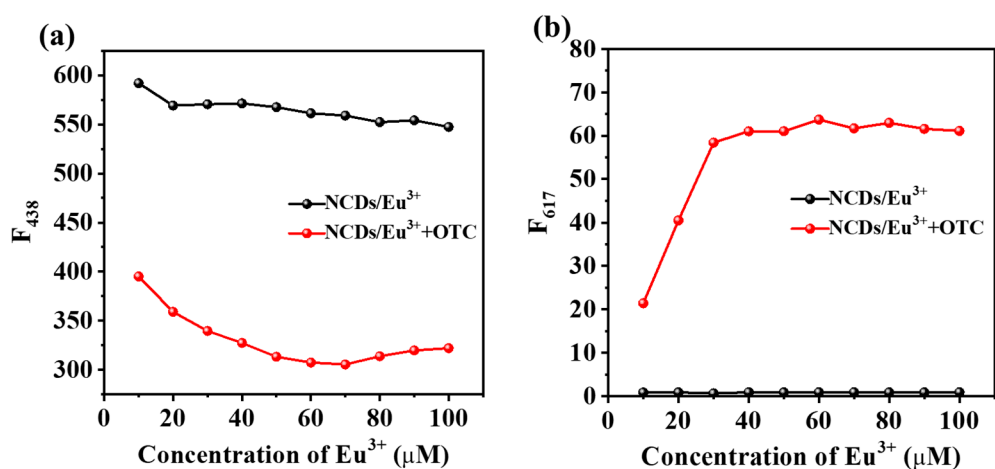
Figure S6. Stern–Volmer plot of NCDs/Eu<sup>3+</sup> probe after addition of OTC in the range of 0.1–25  $\mu\text{M}$ .



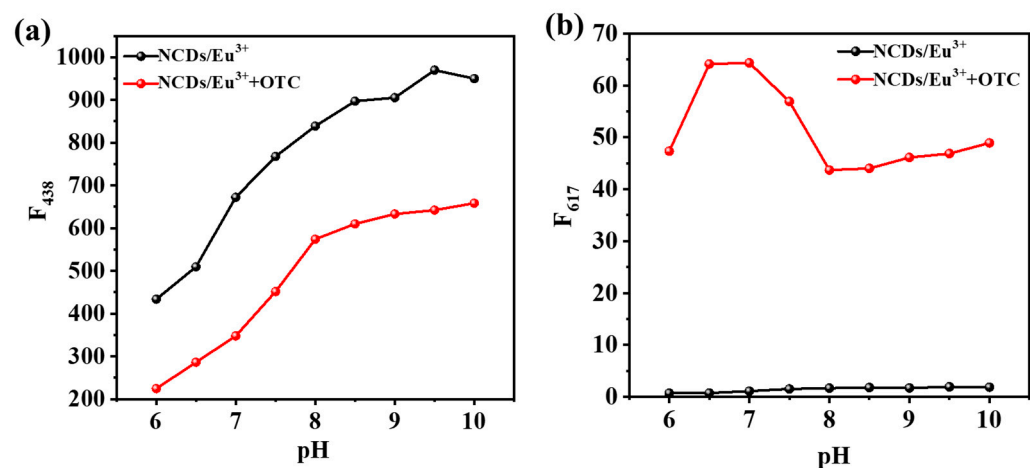
**Figure S7.** Effect of  $M_{CA}/M_{UA}$  on  $F_{438}$  and  $F_{617}$  of the probe before (a) and after (b) incubation with OTC. NCDs: 20  $mg \cdot L^{-1}$ ;  $Eu^{3+}$ : 60  $\mu M$ ; OTC: 20  $\mu M$ ; HEPES buffer: 10 mM (pH=7.0).



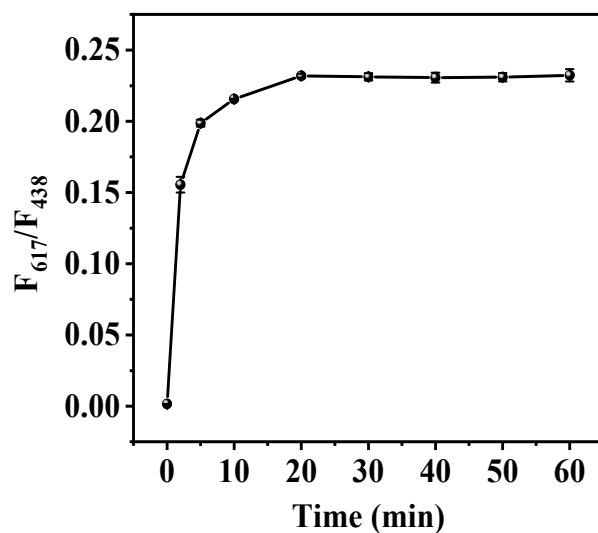
**Figure S8.** Effect of NCD concentration on  $F_{438}$  and  $F_{617}$  of the probe before (a) and after (b) incubation with OTC.  $Eu^{3+}$ : 60  $\mu M$ ; OTC: 20  $\mu M$ ; HEPES buffer: 10 mM (pH=7.0).



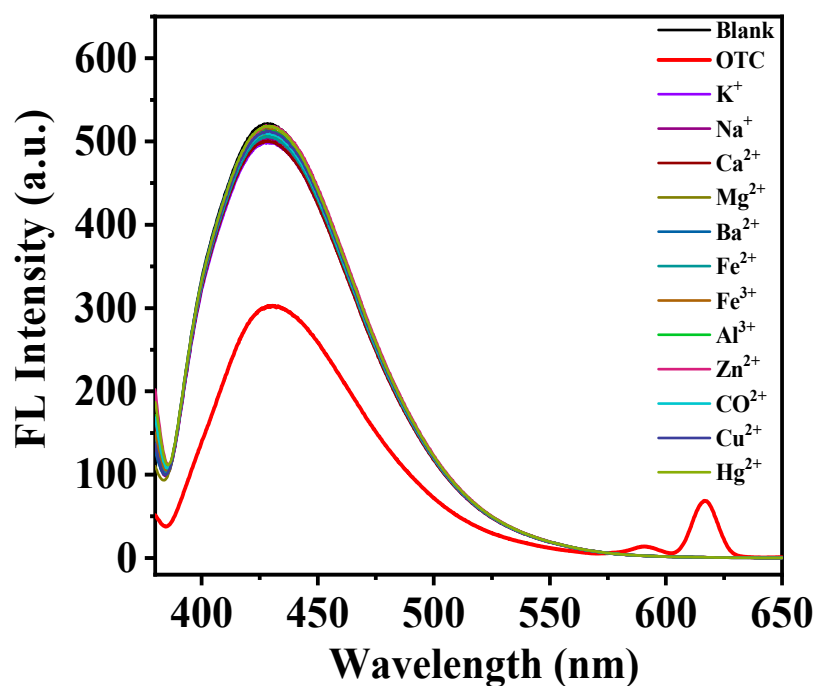
**Figure S9.** Effect of  $Eu^{3+}$  concentration on  $F_{438}$  and  $F_{617}$  of the probe before (a) and after (b) incubation with OTC. NCDs: 20  $mg \cdot L^{-1}$ ; OTC: 20  $\mu M$ ; HEPES buffer: 10 mM (pH=7.0).



**Figure S10.** Effect of pH on  $F_{438}$  and  $F_{617}$  of the probe before (a) and after (b) incubation with OTC.  $\text{Eu}^{3+}$ : 60  $\mu\text{M}$ ; OTC: 20  $\mu\text{M}$ ; HEPES buffer: 10 mM (pH=7.0).



**Figure S11.** Effect of incubation time on  $F_{617}/F_{438}$ . NCDs: 20  $\text{mg}\cdot\text{L}^{-1}$ ;  $\text{Eu}^{3+}$ : 60  $\mu\text{M}$ ; OTC: 20  $\mu\text{M}$ ; HEPES buffer: 10 mM (pH=7.0).



**Figure S12.** The effect of other cations on the fluorescence spectra of NCDs/Eu<sup>3+</sup>.

**Table S1.** The reproducibility of NCD physical properties.

NCDs	Particle size (nm)	Fluorescence quantum yield (%)	Fluorescence lifetime (ns)	Excitation wavelength- and pH-dependent optical property
NCDs 1	4.89 ± 0.37	16.10	4.01	yes
NCDs 2	4.02 ± 0.30	14.95	3.03	yes
NCDs 3	4.55 ± 0.25	15.48	3.17	yes

**Table S2.** The reproducibility of NCDs analytical properties for OTC detection.

NCDs	Quantitative range for OTC	LOD for OTC	Selectivity for OTC
NCDs 1	0.1–25 µM	40 nM	yes
NCDs 2	0.1–25 µM	18 nM	yes
NCDs 3	0.1–25 µM	29 nM	yes

**Table S3.** Comparison of fluorescent probes for OTC detection.

Material applied	Targets	Linear range	LOD	Ref.
piperazine modified CQDs	OTC	0–10 µM	–	[1]
GQDs-Eu <sup>3+</sup>	TC <sup>a</sup> /OTC/DXC <sup>b</sup>	0–20 µM	8.2 nM	[2]
Al-MOF	TC <sup>a</sup> /OTC/DXC <sup>b</sup>	0–72.33/0–86.67/0–66.67 µM	26/62/40 nM	[3]
luminescent MOF (In-sbdc)	TC <sup>a</sup> /OTC/CTC <sup>c</sup>	0–30 µM	0.28/0.30/0.30 µM	[4]
BNQD-Eu <sup>3+</sup>	TC <sup>a</sup> /OTC/DXC <sup>b</sup>	0–50 µM	19/104/28 nM	[5]
CDs-Eu <sup>3+</sup>	OTC	2.5–10 µM	9.59 nM	[6]
DPA-Ce-GMP-Eu	OTC	0.01–45 µM	6.6 nM	[7]
CDs	OTC	0–40 µM	410 nM	[8]

NCDs/Eu <sup>3+</sup>	OTC	0.1–25 $\mu$ M	29 nM	This work
<sup>a</sup> tetracycline;				
<sup>b</sup> doxycycline;				
<sup>c</sup> chlortetracycline;				

## References

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