Electronic Supporting Information

For

A Ratiometric Fluorescent Nanoprobe Based on Terbium Functionalized Carbon Dots for Highly Sensitive Detection of an Anthrax Biomarker

Hao Chen, Yujie Xie, Alexander M. Kirillov, Liangliang Liu, Minghui Yu, Weisheng Liu and Yu Tang*

Contents

1. Materials
2. Methods and Instruments
3. Experimental Methods
   A. Preparation of carbon dots (CDs)
   B. Synthesis of CDs-Tb nanoprobe
   C. Fluorescence titrations of CDs-Tb
   D. Stability constant of CDs-Tb-DPA
4. Supporting Figures

Fig. S1 Fluorescence intensity of terbium and europium DPA complexes at the same concentration.

S1
**Fig. S2** Synthetic route for CDs using EDTA and TETA precursors: from condensation to polymerization and carbonization.

**Fig. S3** a) High-resolution C 1s XPS spectra of CDs; b) high-resolution O 1s XPS spectra of CDs; c) high-resolution N 1s XPS spectra of CDs.

**Fig. S4** FTIR spectra of CDs and CDs-Tb.

**Fig. S5** Excitation and fluorescence (FL) spectra of CDs.

**Fig. S6** Fluorescence decay profiles of CDs and CDs-Tb.

**Fig. S7** Effect of solution pH on the PL intensity of CDs.

**Fig. S8** Fluorescence intensity of CDs during continuous excitation with a UV beam.

**Fig. S9** a) XPS spectrum of CDs-Tb. b) High-resolution Tb 4d XPS spectrum. c) UV-vis absorption, excitation (λ_{em} = 452 nm), and emission (λ_{ex} = 370 nm) spectra of CDs-Tb (Inset: photographs of the CDs-Tb dispersion taken under daylight (left) and UV lamp with λ = 254 nm (right). d) Fluorescence (FL) spectra of CDs-Tb under different excitation wavelengths.

**Fig. S10** High-resolution C 1s, O 1s, Tb 3d, and N 1s XPS spectra of CDs-Tb.

**Fig. S11** TGA curve of CDs-Tb.

**Fig. S12** Absorption spectra of CDs and DPA.

**Fig. S13** Fluorescence decay profile of Tb^{3+} ions and CDs-Tb.

**Fig. S14** Effect of solution pH on the PL intensity of ratiometric nanoprobe (I_{544}/I_{440}).

**Fig. S15** Ratiometric calibration plot (I_{544}/I_{440}) of the CDs-Tb nanoprobe as a function of DPA concentration.

**Fig. S16** Titration profile according to the emission intensity of CDs-Tb at 544 nm in the presence of DPA.

**Fig. S17** Linear plot for calculating the stability constant.

**Fig. S18** Evolution of the fluorescence spectra of CDs-Eu on increasing the DPA concentration.

**Fig. S19** Ratiometric calibration plot (I_{617}/I_{440}) of CDs-Eu nanoprobe as a function of DPA concentration.

**Fig. S20** Phosphorescence spectra of the analytes in a 1:1 methanol-ethanol mixed solution.
Table S1 Triplet energy level of DPA and other potentially interfering aromatic acids or amino acids.

Table S2 Merits and detection limits of lanthanide(III)-based probes for measuring DPA.

5. Supporting References
1. Materials.

Terbium nitrate (Tb(NO$_3$)$_3$·6H$_2$O) and europium nitrate (Eu(NO$_3$)$_3$·6H$_2$O) were obtained by dissolving Tb$_4$O$_7$ and Eu$_2$O$_3$ (99.99%, Shanghai Yuelong) in nitric acid followed by successive fuming to remove excess acid. KClO$_4$, NaClO$_4$, Mg(ClO)$_4$, Ca(ClO)$_4$, dipicolinic acid (DPA), benzoic (BA), m-phthalic (m-PA), o-phthalic (o-PA) acid, as well as D-phenylalanine (Phe), D-aspartic (Asp) and glutamic (Glu) acid were purchased from J&K Chemical Ltd.. Tris(hydroxymethyl)aminomethane was purchased from Tianjin Kermel Corporation. Ethylenediaminetetraacetic acid (EDTA) and triethylenetetramine (TETA) were purchased from Beijing Chemical Factory. Other chemicals were obtained from commercial sources and used without further purification.

2. Methods and Instruments.

UV-vis absorption spectra were determined on a Varian UV-Cary100 spectrophotometer. FTIR spectra of the materials were run within the 4000–400 cm$^{-1}$ wavenumber range by using a Nicolet 360 FTIR spectrometer with the KBr pellet technique. The steady-state corrected luminescence spectra and the lifetime measurements in water solution were performed on an Edinburgh Instruments FSL920 fluorescence spectrometer, with a 450 W Xe arc lamp as the steady-state excitation source and a Nd-pumped OPOlette laser as the excitation source for lifetime measurements. The overall quantum yields of the samples were determined by an absolute method using an integrating sphere (150 mm diameter, BaSO$_4$ coating) on an Edinburgh Instrument FLS920.$^{[51-53]}$ Three parallel measurements were carried out for each sample, so that the presented value corresponds to the arithmetic mean value. The errors in the quantum yield values associated with this technique were estimated to be within 10%. X-ray photoelectron spectra (XPS) were measured on a PHI-550 spectrometer using the Mg K$_\alpha$ radiation ($h\nu$ = 1253.6 eV) photoemission spectroscopy with a base vacuum operated at 300 W. The transmission electron microscopy (TEM) was obtained on a JEM-2100 transmission electron microscope at an acceleration voltage of 120 kV. Samples were prepared by placing a drop of a dilute alcohol dispersion of the products on the surface of a copper grid. Thermogravimetric analyses (TGA) were performed using a Netzsch STA 449 F3 Jupiter up to 800°C at a heating rate of 10°C·min$^{-1}$ under a nitrogen atmosphere.

3. Experimental Methods

A. Preparation of carbon dots (CDs). EDTA (0.292 g, 1 mmol) and TETA (0.50 mL, 3.36 mmol) were dissolved in 5.0 mL water, and the obtained solution was transferred into a 25 mL Teflon-
lined stainless steel autoclave and heated at 160 °C for 3 h. Then the autoclave was cooled down naturally and subjected to dialysis process. Through a cellulose ester dialysis membrane (1000 MWCO), residual amounts of EDTA and TETA were removed over 48 h. The resulting material was dried by lyophilization to obtain CDs powder, which were dispersed in water for further characterization and use.

B. Synthesis of CDs-Tb and CDs-Eu nanoprobes. 10 mL of aqueous CDs (30 μg·mL⁻¹) solution was mixed with 5 mL of aqueous Tb(NO₃)₃ or Eu(NO₃)₃ solution (10 mM) for 2 h at room temperature. The resulting mixture was then dialyzed with a cellulose ester dialysis membrane (1000 MWCO) for 3 days to completely remove “free” Tb³⁺ or Eu³⁺ ions. The resulting material was dried by lyophilization to obtain CDs-Tb or CDs-Eu powder, which was dispersed in water (30 μg·mL⁻¹) for further characterization and use.

C. Fluorescence titrations of CDs-Tb. 2 μL of aqueous CDs-Tb solution (30 μg mL⁻¹) was mixed with 2 mL of aqueous HCl-Tris buffer solution, then various concentrations of DPA (0.005, 0.02, 0.04, 0.05, 0.1, 0.2, 0.3, 0.5, 0.7, 0.9, 1.2 μM; aqueous solution) were added keeping constant the total volume of the mixture, and the fluorescence intensity of each solution was measured. The fluorescence titrations of CDs-Eu with DPA were performed using the similar method as that of CDs-Tb.

D. Stability constant of CDs-Tb-DPA. The stability constant was calculated from the emission intensity-titration curves according to the equation:

\[ \frac{1}{(F - F_0)} = \frac{1}{K_a (F_{\text{max}} - F_0) [M^{n+}]^n} + \frac{1}{(F_{\text{max}} - F_0)} \]

Where \( F_0 \) is the emission intensity of CDs-Tb at 544 nm, \( F \) is the emission intensity of CDs-Tb at the same emission wavelength upon the addition of different amount of DPA, \([M]\) is the concentration of DPA. The stability constant values \( K_a \) is given by the ratio of intercept / slope.⁴[^s4]

4. Supporting Figures
**Fig. S1** Fluorescence intensity of terbium and europium DPA complexes at the same concentration.

**Fig. S2** Synthetic route using EDTA and TETA precursors: from condensation to polymerization and carbonization.
**Fig. S3** a) High-resolution C1s XPS spectra of CDs; b) high-resolution O1s XPS spectra of CDs; c) high-resolution N1s XPS spectra of CDs.

**Fig. S4** FTIR spectra of CDs and CDs-Tb.
**Fig. S5** Excitation and fluorescence (FL) spectra of CDs. Inset: CDs containing aqueous solution under ultraviolet light ($\lambda = 360$ nm).

**Fig. S6** Fluorescence decay profiles of CDs and CDs-Tb. The average lifetime of CDs is 10.72 ns.
and contains three lifetime components: 0.96 ns (~3.24%), 5.23 ns (~24.87%), and 13.06 ns (71.89%). The average lifetime of CDs-Tb is 10.70 ns and contains three lifetime components: 0.74 ns (~3.04%), 5.02 ns (24.06%), and 13.00 ns (~72.90%) (delay time at 442 nm emission).

The fluorescence decay curves determined at the excitation of 275 nm were both fitted to a three-exponential function, and the average lifetime was calculated according to \[ \langle \tau \rangle = \frac{\sum A_i \tau_i^2}{\sum A_i \tau_i} \].
**Fig. S8** Fluorescence intensities of CDs during continuous excitation with a UV beam. Irradiation time was from 0 to 60 min. Excitation wavelength: 360 nm; Emission wavelength: 450 nm.
Fig. S9  a) XPS spectrum of CDs-Tb. b) High-resolution Tb 4d XPS spectrum. c) UV-vis absorption, excitation ($\lambda_{\text{em}} = 452$ nm), and emission ($\lambda_{\text{ex}} = 370$ nm) spectra of CDs-Tb (Inset: photographs of the CDs-Tb dispersion taken under daylight (left) and UV lamp with $\lambda = 254$ nm (right)). d) Fluorescence (FL) spectra of CDs-Tb under different excitation wavelengths.

Fig. S10  a) High-resolution C1s XPS spectra of CDs-Tb; b) high-resolution O1s XPS spectra of CDs-Tb; c) high-resolution Tb3d XPS spectra of CDs-Tb; d) high-resolution N1s XPS spectra of CDs-Tb.
CDs-Tb.

**Fig. S11** TGA curve of CDs-Tb.

**Fig. S12** Absorption spectra of the prepared CDs and DPA measured in aqueous solutions.
Fig. S13 Fluorescence decay profiles of $^5\text{D}_4$ energy level of Tb$^{3+}$ ions and CDs-Tb. The average lifetime of Tb$^{3+}$ ions is 253.89 μs and contains three lifetime components: 4.95 μs (~7.58%), 59.17 μs (~8.12%), and 295.06 μs (84.29%). The average lifetime of CDs-Tb is 148.25 μs and contains three lifetime components: 0.41 μs (~42.49%), 36.15 μs (4.59%), and 276.68 μs (~52.92%) (275 nm excitation, delay time at 544 nm emission). The average lifetime was calculated according to

$$\langle \tau \rangle = \frac{\sum A_i \tau_i^2}{\sum A_i \tau_i}.$$
**Fig. S14** Effect of solution pH on the FL intensity \( I_{544}/I_{440} \) of ratiometric nanoprobe CDs-Tb when added 1 μM DPA. Excitation wavelength: 275 nm.

**Fig. S15** Ratiometric calibration plot \((I_{544}/I_{440})\) of the CDs-Tb nanoprobe as a function of DPA concentration.
Fig. S16 Titration profile according to the emission intensity of CDs-Tb at 544 nm in the presence of DPA.

Fig. S17 Linear plot for calculating the stability constant.
**Fig. S18** Evolution of the fluorescence spectra of CDs-Eu on increasing the DPA concentration. All spectra were measured under the same experimental conditions ($\lambda_{ex} = 275$ nm). (The peak at 550 nm is second-order scattering peak.)

**Fig. S19** Ratiometric calibration plot ($I_{617}/I_{440}$) of CDs-Eu nanoprobe as a function of DPA concentration.
**Fig. S20** Phosphorescence spectra of the analytes in a 1:1 methanol-ethanol mixed solution.

**Table S1** Triplet energy level of DPA and other potentially interfering aromatic acids or amino acids.
<table>
<thead>
<tr>
<th>Analytes</th>
<th>DPA</th>
<th>BA</th>
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<th>m-PA</th>
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<tr>
<td>3ππ*/cm⁻¹</td>
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Table S2 Merits and detection limits of lanthanide(III)-based probes for measuring DPA.

<table>
<thead>
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<th>lanthanide(III)-based probe</th>
<th>merits</th>
<th>Detection limit</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Single-walled carbon nanotube-Tb</td>
<td>optical and electrochemical</td>
<td>1 μM</td>
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<td>Carboxyl functionalized PFO dots-Tb</td>
<td>ratiometric, sensitive, photostable</td>
<td>0.2 nM</td>
<td>18c</td>
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<tr>
<td>FITC-doped silica nanoparticles-Eu</td>
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<td>Nanoscale MOF-Eu</td>
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<td>Molecular printboards-Eu</td>
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<td>15a</td>
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5. Supporting References


