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Europium Functionalized Ratiometric Fluorescent transducer Silicon Nanoparticles Based on FRET for Highly Sensitive Detection of Tetracycline

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5. Supporting References

1. Meterials

Europium nitrate ($Eu(NO_3)_3{\cdot}6H_2O$) was obtained by dissolving Eu_2O_3 (99.99%, Shanghai,

Yuelong) in nitric acid followed by successive fuming to remove the excess acid. Tetracycline (TC) was purchased from Aladdin (Shanghai, China). (3-aminopropyl) trimethoxysilane (APTES) and Trichloroacetic acid were purchased from Macklin biochemical Co.,Ltd (Shanghai, China). Ethylenediamine tetraacetic acid disodium salt was purchased from Guangfu technology development Co.,Ltd (Tianjin, China). Tris(hydroxymethyl)methyl aminomethane (THAM) was purchased from Energy chemical (Shanghai, China). Tris buffer (100 mM, pH=8) was prepared by dissolving Tris in ultrapure water; 0.1 M NaOH was used to adjust pH to 8. Ultrapure water obtained from a Milli-Q ultrapure (18.2MΩ·cm^{-1}) system was used in all experiments.

2. Instruments and Methods

FT-IR spectra were conducted within the 4000-400 cm^{-1} wavenumber range by using a Nicolet 360 FT-IR spectrometer with the KBr pellet technique. UV-vis absorption spectra were determined on a Varian UV-Cary5000 spectrophotometer, and for the corrected steady-state fluorescence emission spectra, a FLS920 spectrofluorometer was employed. The transmission electron microscopy (TEM) was taken on a JEM-2100 TEM instrument at an accelerating 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. X-ray photoelectron spectra (XPS) were measured on a PHI-550 spectroscopy by using Mg Ka radiation (hν=1253.6 eV) photoemission spectroscopy with a base vacuum operated at 300W. Fluorescence decay histograms were obtained on a Edinburgh instrument FLS920 spectrometer equipped with a supercontinue white laser (400-700), using the time-correlated single photon counting technique in 4096 channels. Histograms of the instrument response functions (using a LUDOX scatterer) and sample decays were recorded until they typically reached 1.0×10^4 counts in the peak channel. The fluorescence quantum yields (QY) of the samples were determined by an absolute method using an integrating sphere (150 mm diameter, $BaSO_4$ coating) on an Edinburgh Instrument FLS920.[S1,S2] Light-scattering analysis was performed using a DynaPro dynamic light scatterer (DLS).

3. Experimental Section

A. **Synthesis of Silicon Nanoparticles (SiNPs).** SiNPs precursor solution was prepared by adding 2 mL of (3-aminopropyl) trimethoxysilane (APTES) to 8 mL Ar-saturated aqueous solution dispersed with 0.392 g of ethylenediaminetetraacetic acid disodium salt, the mixture was then stirred for 10min. The resultant precursor solution was transferred into the exclusive vitreous vessel with a volume of 30 mL. The SiNPs with the maximum emission at 450 nm were prepared under microwave irradiation 160 ^{o}C / 15 min. The products were cooled down naturally, dissolved in 5 mL ultrapure water and subjected to dialysis process. Through a cellulose ester dialysis membrane (1000MWCO), residual amounts of ethylenediaminetetraacetic acid disodium salt and APTES were removed over 24 h. The resulting material was dried to obtain SiNPs powder, which were dispersed in water for further characterization and use.

B. **Synthesis of SiNPs-Eu transducer.** The SiNPs-Eu transducer were prepared by chemical doping. Typically, 5 mL aqueous solution of SiNPs (50 μg/mL) solution was mixed with 1 mL of aqueous $Eu(NO_3)_3$ solution (50 mM) under stirring for 2 h at room temperature. The resulting mixture was then

dialyzed with a cellulose ester dialysis membrane (1000 MWCO) for 48 h to completely remove "free" Eu^{3+} ions, and then was stored at 4 °C for further use.

C. **Fluorescence titration of SiNPs-Eu with TC.** 50 μl aqueous solution of SiNPs-Eu (50 μL / mL) was mixed with 2.5 mL aqueous solution of HCl-Tris buffer solution pH = 8, then various concentrations of TC (3.56 ~ 377.05 μM; aqueous solution) were added keeping constant the total volume of the mixture, and the fluorescence intensity of each solution was measured.

D. **Determination of TC in normal mouse sera.** In the experiments, we fed TC to normal mice and obtained their blood after four hours. These samples were treated as following steps. Briefly, the proteins in normal mouse sera were removed firstly by adding 1% (v/v) trichloroacetic acid into the normal mouse sera and sonicating for 30 min. After centrifuging the sample at 10,000 rpm for 10 min, the supernatant was filtered to remove lipids. A series of milk samples containing different concentrations of TC in the range of 0 ~ 60 μM were prepared by "spiking" them with a stock solution of TC (10 mM). Different volumes of milk samples were added to 50 μL of SiNPs-Eu solutions (50 μL / mL); the total volume is 100 μL. These solutions were incubated for 5 min; then their fluorescence spectra were recorded (λ_{ex} = 360 nm). [S3] This processing method is also applicable to milk samples.

E. **Stability constant of SiNPs-Eu-TC.** The stability constant was calculated from the emission intensity-titration curves according to the equation:

$$\mathbf{1 / (\mathit{F} - \mathit{F}_0) = 1 / \{\mathit{K}_s (\mathit{F}_{max} - \mathit{F}_0) [M^{n+}]^n\} + 1 / (\mathit{F}_{max} - \mathit{F}_0)}$$

Where F_0 is the emission intensity of SiNPs-Eu at 617 nm, F is the emission intensity of SiNPs-Eu at the same emission wavelength upon the addition of different amount of TC, [M] is the concentration of TC. The stability constant values K_s is given by the ratio of intercept / slope.[S4]

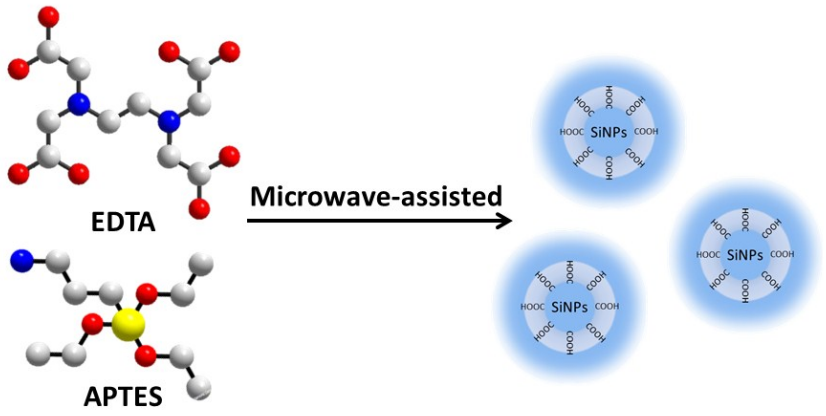
F. **FERT efficiency of SiNPs-Eu+TC.** The FRET efficiency of SiNPs-Eu+TC (100 μM) was calculated according to the equation:

$$K_T = 8.8 \times 10^{-25} \kappa^2 \phi_D n^{-4} \tau_D^{-1} r^{-6} J$$

$$E_{FRET} = 1 - \frac{I_{DA}}{I_D}$$

Where K_T and E_{FRET} are FRET efficiency (68.4%), κ is orientation factor and κ^2= 2/3, Φ_D is the fluorescence lifetime of donors, n is dielectric constant, τ_D is the fluorescence quantum yield, r is the distance between donors and acceptors, J is the spectral overlap integral of donors and acceptors, I_D is the fluorescence intensity of donors, I_{DA} is the fluorescence intensity of donors while in the presence of acceptors.[S5]

4. Supporting Figures



Scheme S1 One-step microwave-assisted synthesis of SiNPs.

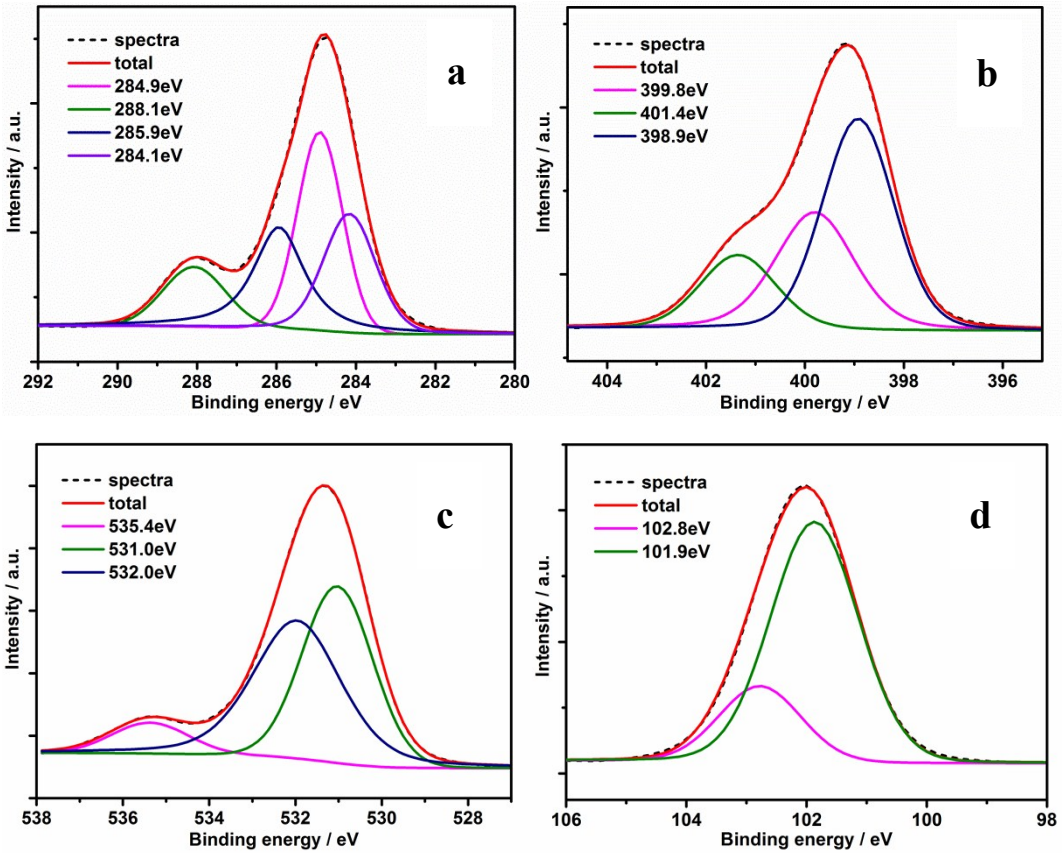


Fig. S1 High-resolution XPS spectra of SiNPs. a) C1s HR-XPS spectra of SiNPs; b) N1s HR-XPS spectra of SiNPs. c) O1s HR-XPS spectra of SiNPs; c) Si2p HR-XPS spectra of SiNPs.

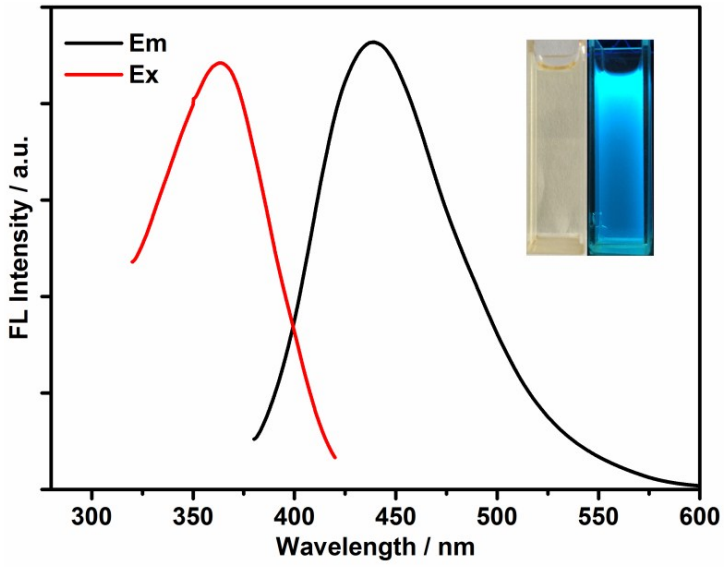


Fig. S2 Excitation and fluorescence spectra of SiNPs; Inset: SiNPs containing aqueous solution under visible light and UV lamp irradiation (λ = 365 nm).

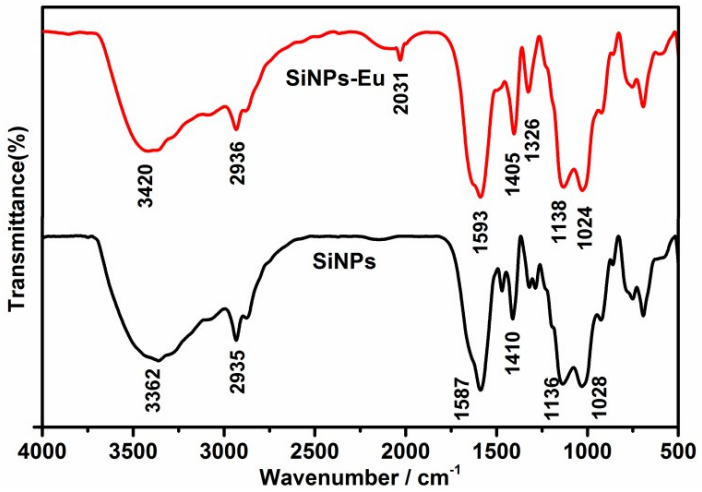


Fig. S3 FTIR spectra of SiNPs and SiNPs-Eu.

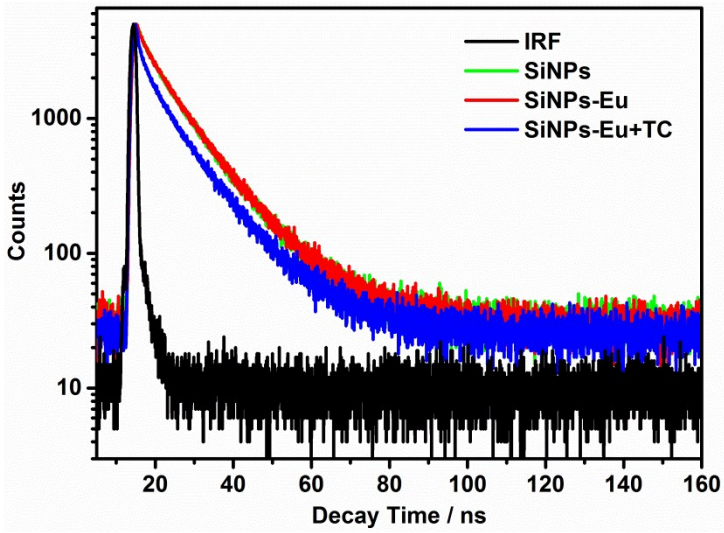


Fig. S4 Fluorescence decay profiles of SiNPs , SiNPs-Eu and SiNPs-Eu+TC (100μM). The average lifetime of SiNPs is 11.20 ns and contains three lifetime components: 1.10 *n*s (~4.46%), 5.65 *n*s (~27.55%), and 11.92 ns (67.99%). The average lifetime of SiNPs-Eu is 8.90 ns and contains three lifetime components: 1.31 *n*s (~5.83%), 7.20 *n*s (~41.69%), and 13.06 ns (52.48%). The average lifetime of SiNPs-Eu+TC (100μM) is 10.47 ns and contains three lifetime components: 0.58 *n*s (~10.47%), 3.80 *n*s (~21.74%) and 11.28 *n*s (~67.79%) (delay time at 450 nm emission). The fluorescence decay curves determined at the excitation of 360 nm, and the average lifetime was calculated according to $\bar{\tau} = \frac{\sum_{i=1}^{n} \alpha_i \tau_i^2}{\sum_{i=1}^{n} \alpha_i \tau_i}$.

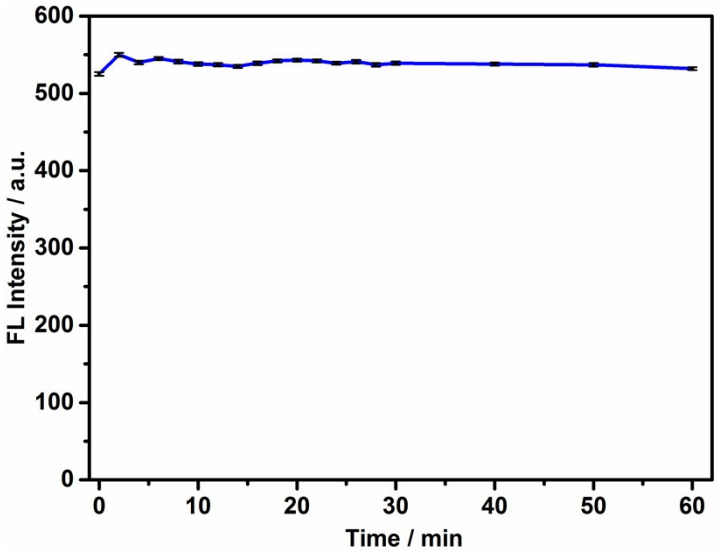


Fig. S5 Fluorescence intensities of SiNPs during continuous excitation with a UV beam. Irradiation time was from 0 to 60 min. Excitation wavelength: 360 nm; Emission wavelength: 450 nm.

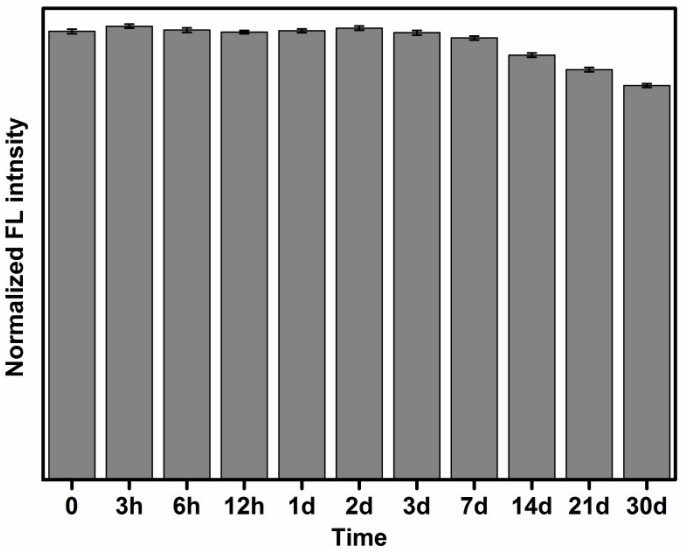


Fig. S6 The SiNPs display stable fluorescence during 30 day storage in dark place at room temperature.

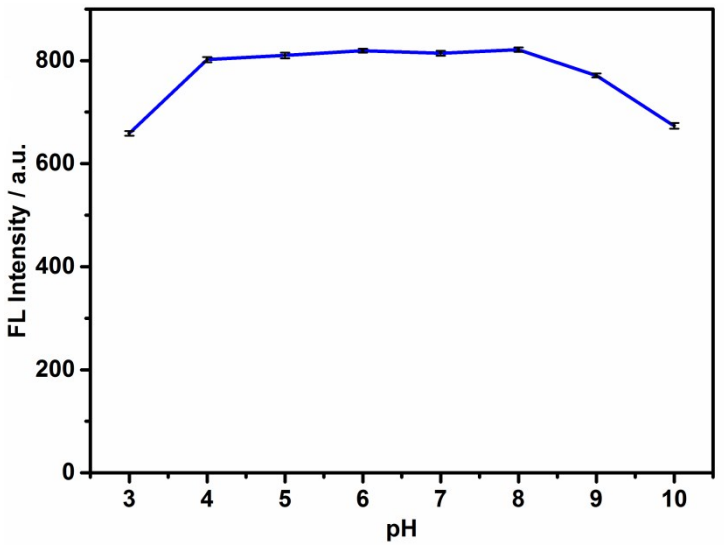


Fig. S7 Effect of solution pH on the FL intensity of SiNPs. Excitation wavelength: 360 nm; Emission wavelength: 450 nm.

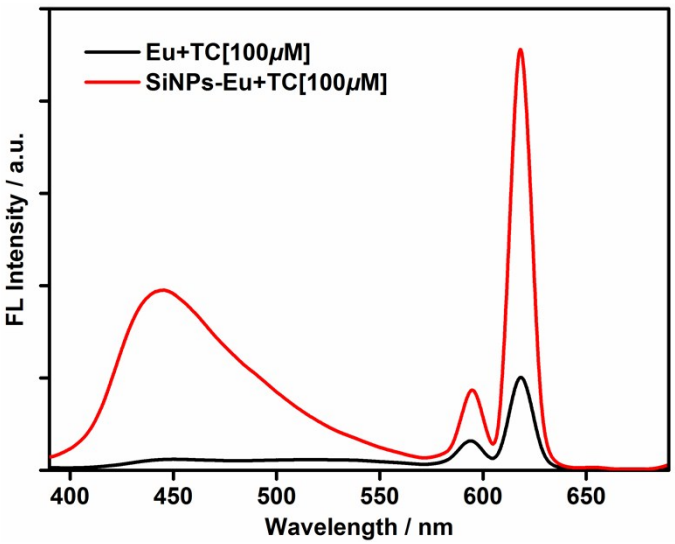


Fig. S8 Fluorescence spectra of SiNPs-Eu+TC (100 μM) and Eu+TC (100 μM) (λ_{ex} = 360 nm).

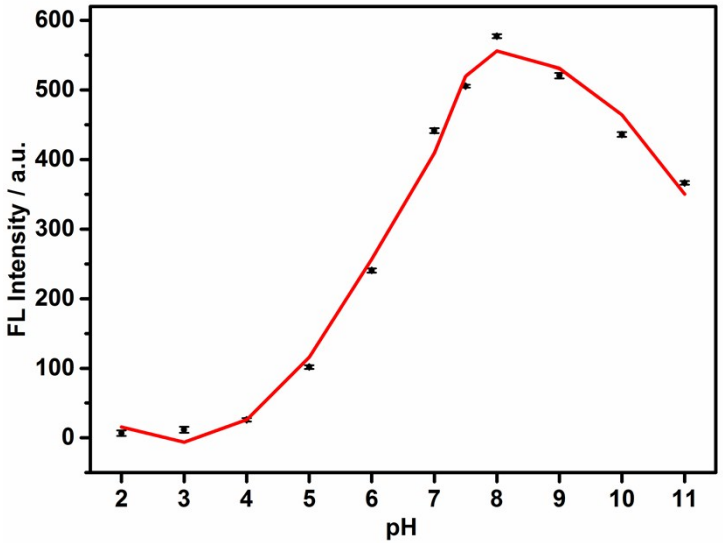
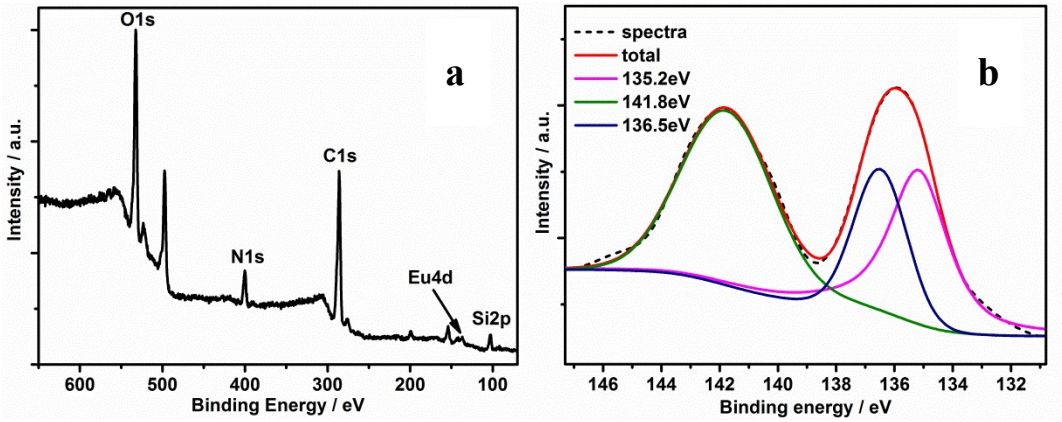


Fig. S9 Effects of pH on the FL intensity I_{617} / I_{450} of ratiometric transducer SiNPs-Eu when added 100 μM TC. (λ_{ex} = 360 nm)



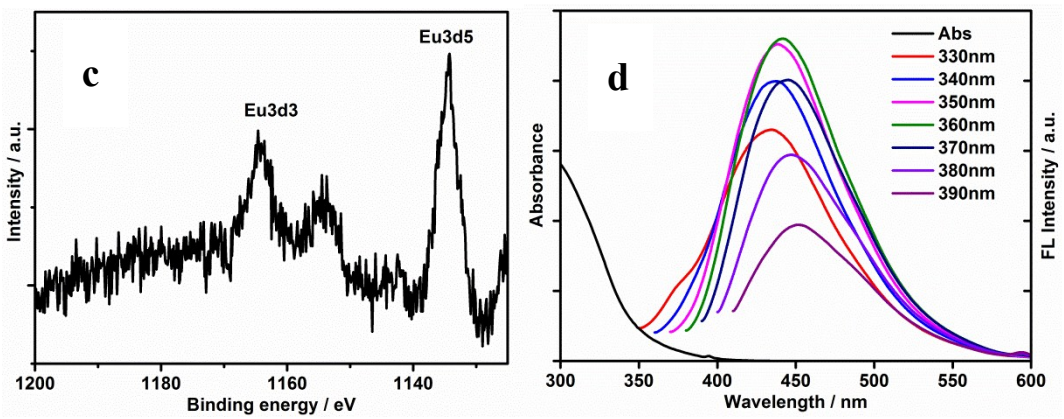


Fig. S10 a) XPS spectrum of SiNPs-Eu. b) Eu 4d HR-XPS spectrum of SiNPs-Eu. c) Eu 3d HR-XPS spectrum of SiNPs-Eu. d) UV-vis absorption spectrum (the black soild line) and fluorescence spectra of SiNPs-Eu under different excitation wavelengths.

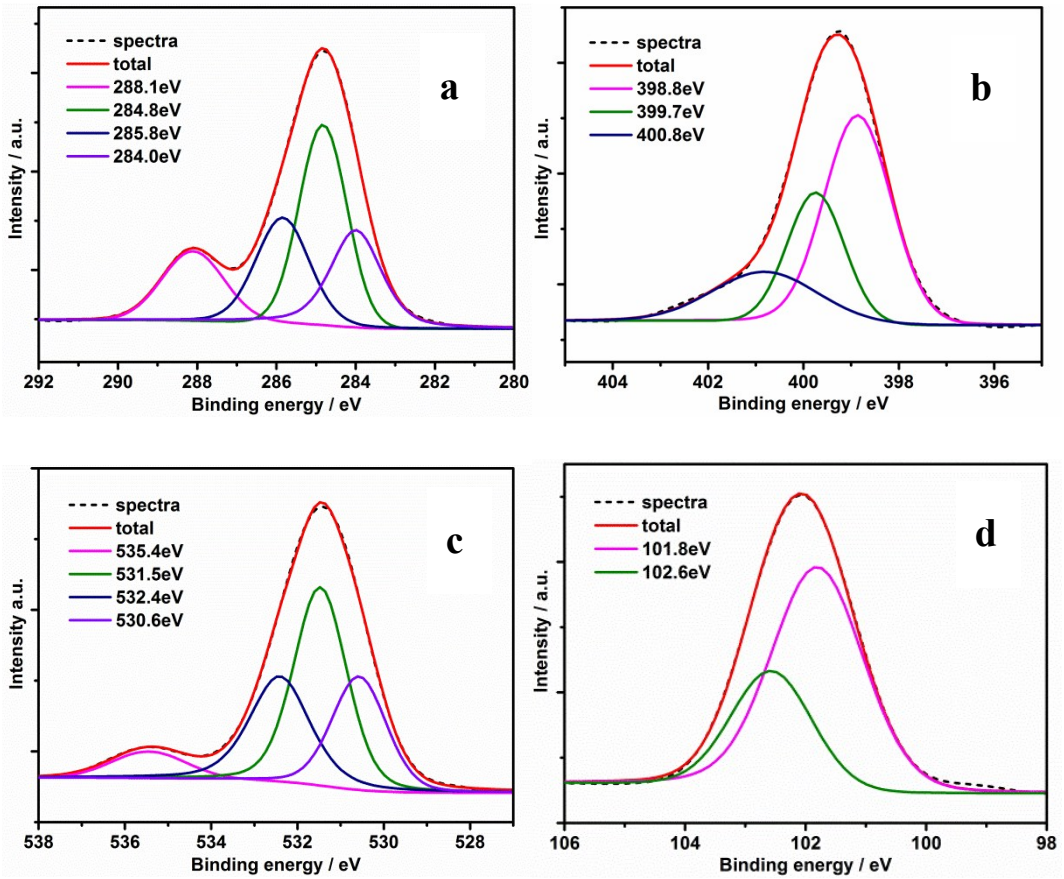


Fig. S11 HR-XPS spectrum of SiNPs-Eu. a) C1s HR-XPS spectrum of SiNPs-Eu. b) N1s HR-XPS spectrum of SiNPs-Eu. c) O1s HR-XPS spectrum of SiNPs-Eu. d) Si2p HR-XPS spectrum of SiNPs-Eu.

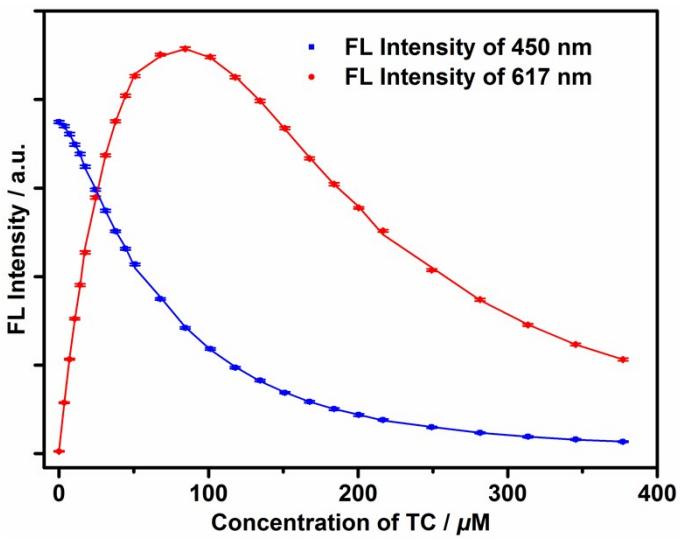


Fig. S12 FL intensity of SiNPs-Eu+TC at 450 nm and 617 nm with different concentration of TC.

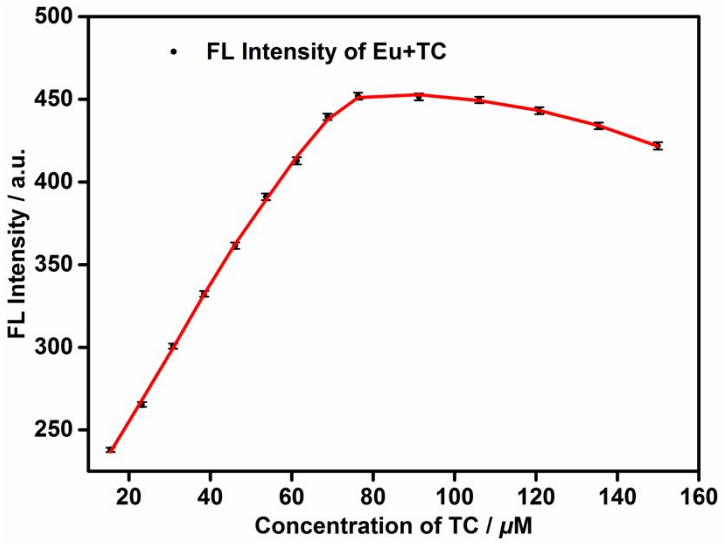


Fig. S13 FL intensity of Eu+TC at 617 nm in different concentration of TC.

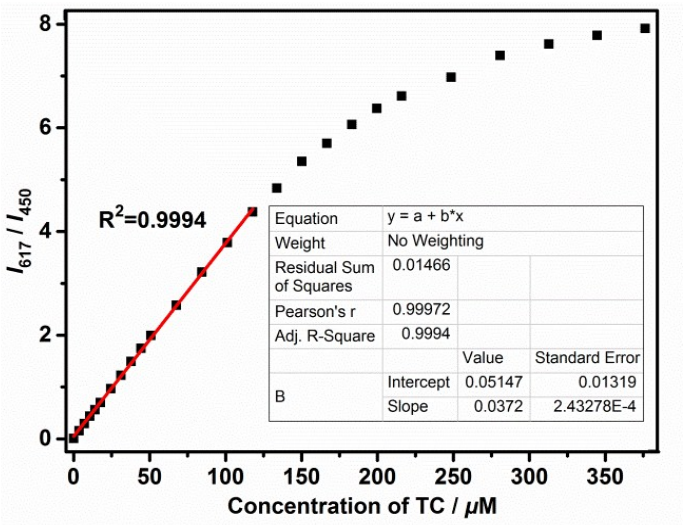


Fig. S14 The inset is the linear relationship between the ratio of fluorescence intensity (I_{617} / I_{450}) intensity and TC concentration (R^2 = 0.9994) (λ_{ex} = 360 nm).

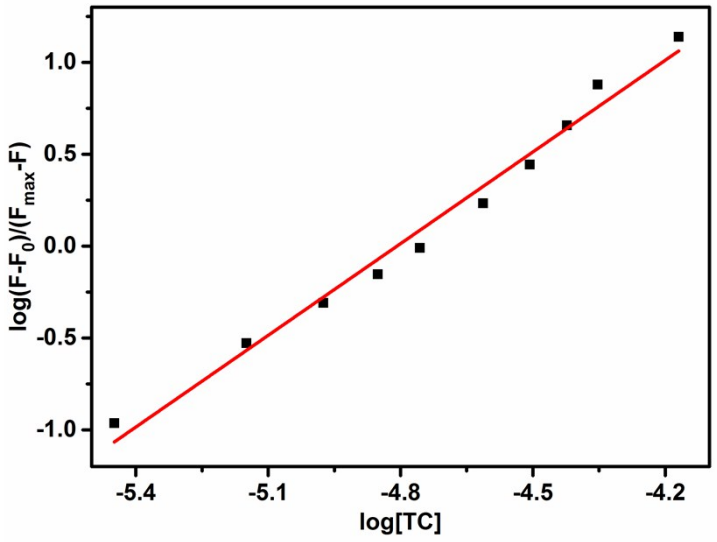


Fig. S15 Linear plot for calculating the stability constant.

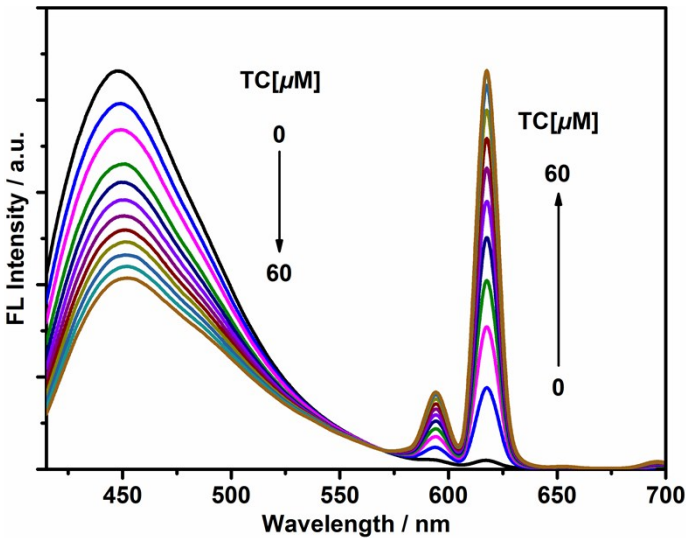


Fig. S16 Fluorescence spectra of SiNPs-Eu in normal mouse sera containing different concentrations TC. All spectra were measured under the same experimental conditions (λ_{ex} = 360 nm).

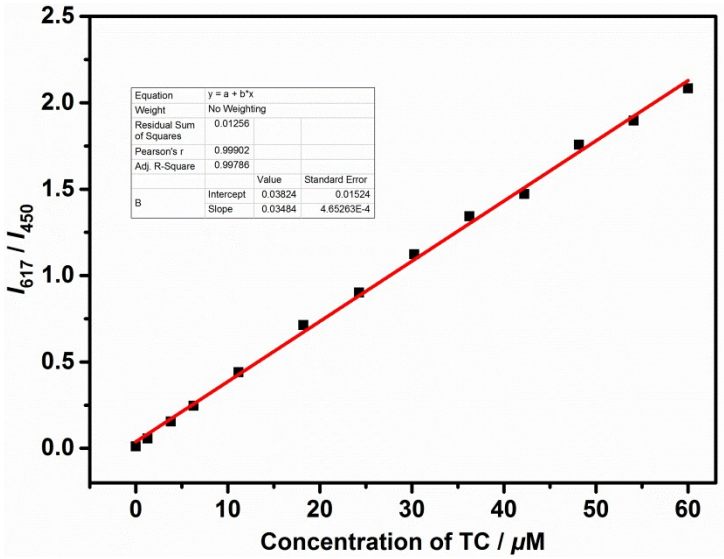


Fig. S17 The inset is the linear relationship between the ratio of fluorescence intensity (I_{617} / I_{450}) intensity and TC concentration in normal mouse sera. (λ_{ex} = 360 nm)

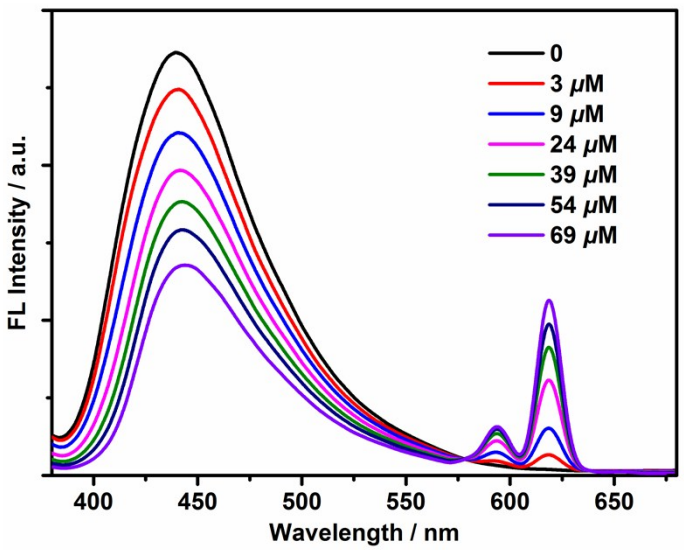


Fig. S18 Fluorescence spectra of CDs-Eu in the presence of different concentrations of TC. All spectra were measured under the same experimental conditions (λex = 360 nm).

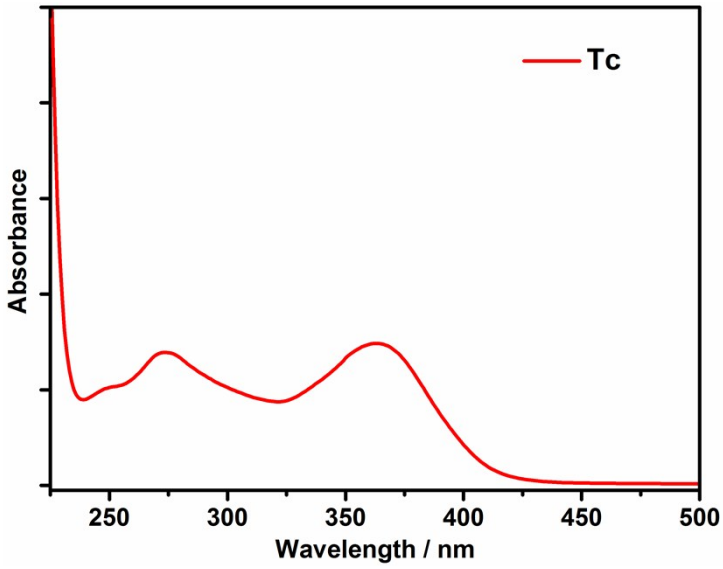


Fig. S19 UV-vis absorption spectrum of TC.

Table S1 Comparison of Europium-based transducers for the detection of TC.

Europium-base transducer	Linear range（μM）	LOD (nM)	Advantages	Refs.
Eu-EDTA+ surfactant	0.2 - 5	200	low cost	[S6]
Silver nanoparticles-Eu	0.01 - 10	4	Sensitive and photostable	[S3]
The metal-organic coordination polymer-Eu	0.1 - 20	60	self-assembling and biocompatible	[S7]
SiNPs-Eu	0.01 - 120	5.4	ratiometric, biocompatible, photostable and low cost	This work

Table S2 Determination results of TC in milk samples.

Added / μM	Detection / μM	Recovery / %	RSD / n = 3, %
2	2.22 ± 0.29	110.5%	0.55
4	4.14 ± 0.33	103.5%	0.61
6	5.79 ± 0.24	96.5%	0.34
10	9.61 ± 0.28	96.1%	0.37
30	28.97 ± 0.38	96.6%	0.52

5. Supporting References

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