Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Single-Nanoparticle Cell Barcoding by Tunable FRET from Lanthanides to Quantum Dots

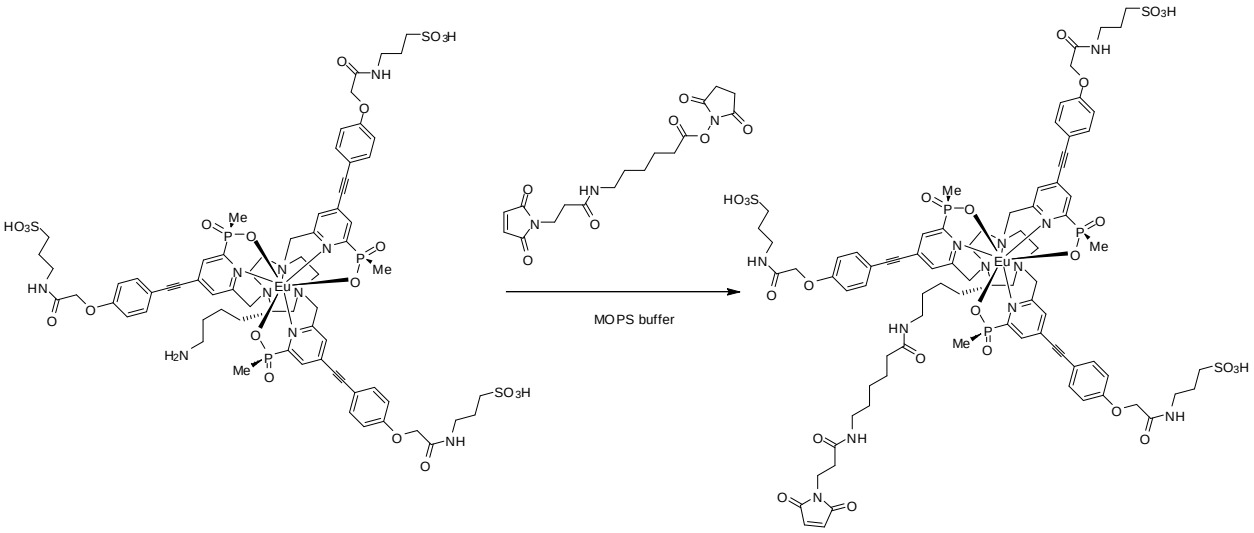
Chi Chen, Lijiao Ao, Yu-Tang Wu, Vjona Cifliku, Marcelina Cardoso Dos Santos, Emmanuel Bourrier, Martina Delbianco, David Parker, Jurriaan M. Zwier, Liang Huang, and Niko Hildebrandt**

anie_201807585_sm_miscellaneous_information.pdf

Materials and Methods

Terbium (III) chloride hexahydrate (99.999% trace metals basis), Poly (ethylene glycol) nonylphenyl ether (NP-5), tetraethyl orthosilicate (TEOS) and (3-mercaptopropyl) trimethoxysilane (MPS) were purchased from Sigma-Aldrich. Cyclohexane, chloroform, ethanol, ammonia aqueous solution (28%) were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). CdSe/CdS/ZnS QDs with emission maximum at 620 nm were purchased from Poly OptoElectronics Co., Ltd. Black Costar Half Area 96 well microtitration plates were purchased from Corning Inc. (Corning, NY, USA), Lumi4 functionalized to maleimide were provided by Lumiphore Inc. (Berkeley, CA, USA).

Synthesis of Eu-1-Maleimide (Eu-1-Mal)



In a 1.5 ml Eppendorf tube 400 nmol Eu-1-NH_2 (compound EuL^{3c} in supplementary reference 1) was dissolved in 250 µl of 50 mM 3-(N-morpholino)propanesulfonic acid (MOPS) buffer pH7.1, 3 µmol of Succinimidyl 6-((beta-maleimidopropionamido)hexanoate (SMPH, ThermoFisher

Scientific (ref 22363)) was added to the solution and stirred for 1h at room temperature. Eu-1-Mal was purified by preparative HPLC (Waters XBridge RP-C_{18} column, 5 µm, 10 × 100 mm) with aq. Triethylammonium acetate 25 mM, pH 7 – MeCN (v/v) as eluent using a linear gradient from 5 (0 min) to 50% MeCN (17 min), at a flow rate of 5 mL min^{-1} and UV detection at 280 nm. The peak eluted after 6 minutes was collected and evaporated. The resulting compound was dissolved in 500 µl methanol and analysed by electronic absorption spectroscopy. 267 nmol (0.53 mg, 67% yield) of Eu-1-Mal (MW 2006,83 g/mol) was obtained and its content and purity (>98%) was confirmed by mass-spectrometry and HPLC repectively.

Determination of Eu^{3+} quantum yield in Eu-1.

For FRET efficiency calculation the quantum yield of the Eu^{3+} ion is of importance. This value can be determined from the detector corrected emission spectrum of the Eu-1 complex in water by using Equation S1 (from supplementary reference 2) and the measured emission lifetime of the complex.

$$\frac{1}{\tau_R} = A_{MD,0}\, n^3\, \frac{I_{tot}}{I_{MD}} \qquad \text{(S1)}$$

Herein $A_{MD,0}$ is the spontaneous emission probability for the $^5D_0$ -> $^7F_1$ transition *in vacuo*, I_{tot}/I_{MD} is the ratio of the total area over the area of the $^5D_0$ -> $^7F_1$ transition, *n* is he refractive index and τ_R the radiative rate constant. Summation of the corrected emission intensities between 586 and 601 nm for the $^5D_0$ -> $^7F_1$ transition and between 570 and 725 nm for the total intensity and using 14.65 s^{-1} (2) and 1.33 for $A_{MD,\,0}$ and n respectively a radiative lifetime (τ_R) of 2.23 ms and 2.28 ms for Eu-1-NH_2 and Eu-1-Mal was determined. The observed lifetime being 1.08 ms for both compounds a quantum yield of Eu^{3+} emission was determined to be 0.48.

Synthesis of QDs embedded silica nanospheres.

The QDs embedded silica nanospheres were synthesized by a reverse microemulsion approach. Typically, the glass bottlecontaining 30 mL of cyclohexane was added with 3.95 mL of NP-5 and stirred for 15 min. The above solution was mixed with 300 μL of ammonia aqueous solution followed by stirring for another 15 min to form the reverse microemulsion. The mixture was subsequently added to 200 μL of QDs chloroform solution (10 mg/mL), followed by the injection of TEOS to start the silica encapsulation. After stirring at room temperature for 18 h, the QDs embedded silica nanospheres were precipitated by adding ethanol followed by centrifugation. The products were washed with ethanol for several times and finally dispersed in 20 mL of ethanol. To form the silica shell thickness of 6 nm and 12 nm, TEOS volumes of 60 μL and 260 μL were employed, respectively.

Synthesis of mercapto-terminated silica nanospheres.

For the grafting of mercapto-groups onto silica surface, the above QDs embedded silica nanospheres in 20 mL of ethanol was added 500 μL of ammonia and 100 μL of MPS, followed by vigorous stirring at room temperature for 12 h. The final product was harvested by centrifugation, washed thoroughly with ethanol and redispersed in 2 mL water.

Estimation of molar concentration of QD/SiO_2.

We assume that the nanonaterials have the same density as the according bulk materials. For the CdSe/CdS/ZnS QD, the density of CdSe, CdS, and ZnS are 5.816 g/cm^3, 4.82 g/cm^3, and 4.09 g/cm^3, respectively. The radius of QD is about 3.5 nm according the HRTEM. We assume the radius of CdSe core is 1.8 nm, the thickness of CdS and ZnS shell are 1.16 nm and 0.54 nm,

respectively. Then we can obtain the density of the QD, which is nearly 5.223 g/cm^3 (Equation S2 and S3). According the density and volume of single QD and Avogadro constant, we can obtain the molecular weight of QD, which is nearly 570000. The yield of synthetic QD/SiO_2 can be estimate to 70%. Finally, the molar concentration of QD/SiO_2 should be nearly 1.23 μM.

$$V = \frac{4}{3}\pi r^3 \quad \text{(S2)}$$

$$\rho = \frac{m}{V} \quad \text{(S3)}$$

Formation of 100Tb-QD/SiO_2 donor-acceptor assemblies.

Lumi4-Mal was dissolved to 3.8 mM in anhydrous DMF. 5.25 μL of Lumi4-Mal (3.8 mM), 162.5 μL of QD/SiO_2 (6 nm or 12 nm, 1.23 μM), and 232.25 μL of Tris-HCl buffer at pH 7.5 (50 mM) were mixed in 0.5 mL eppendorf tube (Lumi4: QD/SiO_2=100:1). The mixtures in alu foil were incubated for 3 h at room temperature. Intelli Mixer was employed for prewetting tubewalls by rotating the tube. Then the product was harvested by centrifugation (8000 r.p.m, 20 min) and redispersed in 400 μL Tris-HCl buffer at pH 7.5 (50 mM). The number of Lumi4 per QD/SiO_2 was confirmed by absorbance spectrum (75 Lumi4 per QD/SiO_2 (6 nm) and 87 Lumi4 per QD/SiO_2 (12 nm)). For the formation of 100Tb-QD/SiO_2 donor-acceptor assemblies, Tb^{3+} was dissolved to 100 μM in pure water. For the 200 nM 100Tb-QD/SiO_2 codes, 80 μL of Lumi 4-QD/SiO_2 (500 nM), 40 μL of Tb^{3+}, and 80 μL of Tris-HCl buffer at pH 7.5 (50 mM) were mixed in 0.5 mL eppendorf tube (Tb: Lumi4: QD/SiO_2 (6 nm) = 100:75:1 and Tb: Lumi4: QD/SiO_2 (12 nm) =100:87:1).

Formation of Eu-QD/SiO_2 donor-acceptor assemblies.

Eu-1-Mal was dissolved to 0.1 mM in pure water. 100 μL of Eu-1-Mal, 40.6 μL of QD/SiO_2, and 359.4 μL Tris-HCl buffer at pH 7.5 (50 mM) were mixed in 1 mL eppendorf tube (Eu-1-Mal: QD/SiO_2=200:1). The mixtures were incubated for 3 h at room temperature. An Intelli Mixer (ELMI) was employed to prewet the wall of the tube. Then the product was collected by centrifugation (8000 r.p.m, 20 min) and redispersed in 500 μL Tris-HCl buffer at pH 7.5 (50 mM). The number of Eu-1 per QD/SiO_2 was confirmed by absorbance spectrum (175 Eu-1 per QD/SiO_2 (6 nm) and 180 Eu-1 per QD/SiO_2 (12 nm)).

Cell culture.

Human cervical carcinoma (HeLa) cells were purchased from American Type Culture Collection (CCL-2). Cells were grown in Dulbecco's modified eagle medium (DMEM, Sigma-Aldrich, D6546), supplemented with 10% Fetal bovine serum (FBS, Sigma-Aldrich, F0804), 1% antibiotics (Pen Strep, Sigma-Aldrich, P4333) and 2 mM L-glutamine (SigmaAldrich, G7513) at 37°C and 5% CO_2. The cells were passaged with trypsin-EDTA 0.05%.

Living cell encoding.

HeLa cells were seeded at $3x10^5$ cells in 8-chamber glass slide (Nunc® Lab-Tek® II Chamber Slide™, 155409) and incubated at 37°C and 5% CO_2 overnight. The following day, the cells were washed with 1xPBS (Sigma-Aldrich, P4417) and incubated with a complete culture medium (10% FBS, 1% Pen Strep and 2 mM L-glutamine). Solution of Tb-QD/SiO_2 (6 nm), Tb-QD/SiO_2 (12 nm), Eu-QD/SiO_2 (6 nm), and Eu-QD/SiO_2 (12 nm) in Tris-HCl buffer at pH 7.5 (50 mM) were sonicated for 15 minutes and added at 20 nM into previously prepared slide with cells. Cells were incubated at 37°C and 5% CO_2 for 2h. For mixing, the cells which incubated with Tb-QD/SiO_2 (6

nm), Tb-QD/SiO_2 (12 nm), Eu-QD/SiO_2 (6 nm), and Eu-QD/SiO_2 (12 nm) were washed by 1XPBS, trypsinized then seeded on the Poly-L-lysine (Thermofisher, P4707) coated slide.

Cytotoxicity Measurements

The cytotoxicity of QD/SiO_2 (6 nm and 12 nm) were evaluated by MTT viability assay on various cells. Both tumor cells (Hela, MDA-MB231) and normal cells (293T) were cultured in DMEM medium supplemented with 10% (v/v) FBS, 1% (v/v) penicillin, and 1% (v/v) streptomycin under 37 °C within a humidified atmosphere of 5% CO_2. The Hela, MDA-MB231 and 293T cells were seeded in 96-well plates with a seeding density of 5×10^3 cell/well, respectively. After 24 h incubation, the medium was removed and replaced with fresh medium containing QD/SiO_2 with various concentrations (0, 25, 50, 100, 200, 400, 800 nM). After 24 h incubation, the standard MTT assay was carried out to evaluate the cell viability.

Analytical Methods.

Structural characterization of the QD/SiO_2 was carried out using a FEI Tecnai G2 F20 S-Twin high-resolution transmission electron microscope (HR-TEM) operating at 200 kV. Absorption spectra were acquired using a Lambda 35 UV/Vis spectrophotometer (Perkin Elmer). Steady state PL spectra were acquired using a Xenius fluorescence plate reader (SAFAS).

For the measurement of the PL decay curves of the Tb or Eu to QD/SiO_2, an EI fluorescence plate reader (Edinburgh Instruments, UK) with 4000 detection bins of 2 μs integration time was used. A nitrogen laser (LTB, Berlin, Germany) was used for excitation (337.1 nm, 20 Hz, 600 flashes). (494/20) nm, (567/15) nm, and (640/14) nm bandpass filter were used for analyzing the Tb, Eu, and QD PL, respectively. For the measurement of the PL decay curves of the pure QD/SiO_2 (6 nm) and QD/SiO_2 (12 nm), a SuperChrome sources (Fianium, UK) was used for excitation (480 ± 15

nm, 5 MHz, Laser Power: 200). (640 ± 14) nm bandpass filter were used for analyzing. The data were fit with FAST software version 3.1 (Edinburgh Instruments, UK). All assays were measured in black 96-well microtiter plates with an optimal working volume of 150 μL.

FRET calculation.

For FRET model, the overlap integral (J) and Förster distance (R_0) were calculated using Equations (S4) and (S5).

$$J = \int \bar{\mathrm{I}}_D(\lambda)\varepsilon_{\mathrm{A}}(\lambda)\lambda^4 \mathrm{d}\lambda \tag{S4}$$

Where $\bar{\mathrm{I}}_{\mathrm{D}}(\lambda)$ is the area-normalized emission spectrum of donor, $\varepsilon_{\mathrm{A}}(\lambda)$ is the molar absorptivity spectrum of the acceptor in $\mathrm{M^{-1}cm^{-1}}$, and λ is the wavelength in nm.

$$R_0 = 0.0211[\kappa^2 \Phi_{\mathrm{D}}(n)^{-4}\mathrm{J}(\lambda)]^{1/6} \qquad \text{(in nm)} \tag{S5}$$

Where κ^2 is orientation factor (κ^2=2/3 due to dynamic averaging of Tb/Eu-NP donor-acceptor systems), Φ_{D} is quantum yield of the donor, and n=1.35 is the refractive index of the surrounding medium. The molar extinction coefficients $\varepsilon_{\mathrm{QD/SiO_2}}(\lambda)$ for QD/SiO_2 with different shell thicknesses were calculated with estimated molar concentration and absorbance spectra.

The Ln-to-QD distance (r) was calculated using Equation (S6).

$$r = R_0 \left(\frac{\tau_{DA}}{\tau_D - \tau_{DA}}\right)^{1/6} \tag{S6}$$

Multi-exponential PL decay analysis.

We analyzed the data with multiexponential decays. This analysis has been shown to lead to a coherent picture of FRET in Tb-nanoparticle assemblies. It is based on fitting the decay curves were fitted using a multiexponential PL intensity decay function (Equation S7).

$$I = \sum A_{\mathrm{i}} \exp(-t/\tau_{\mathrm{i}}) = A \sum \alpha_{\mathrm{i}} \exp(-t/\tau_{\mathrm{i}}) \quad \text{(S7)}$$

Where A is the total amplitude and α_{i} are the amplitude fractions ($\Sigma\alpha_{\mathrm{i}}$= 1). All PL lifetime averaging for the dynamic quenching process was performed using amplitude weighted average lifetimes (Equation S8).

$$\langle \boldsymbol{\tau} \rangle = \sum \boldsymbol{a_i \tau_i} \quad \text{(S8)}$$

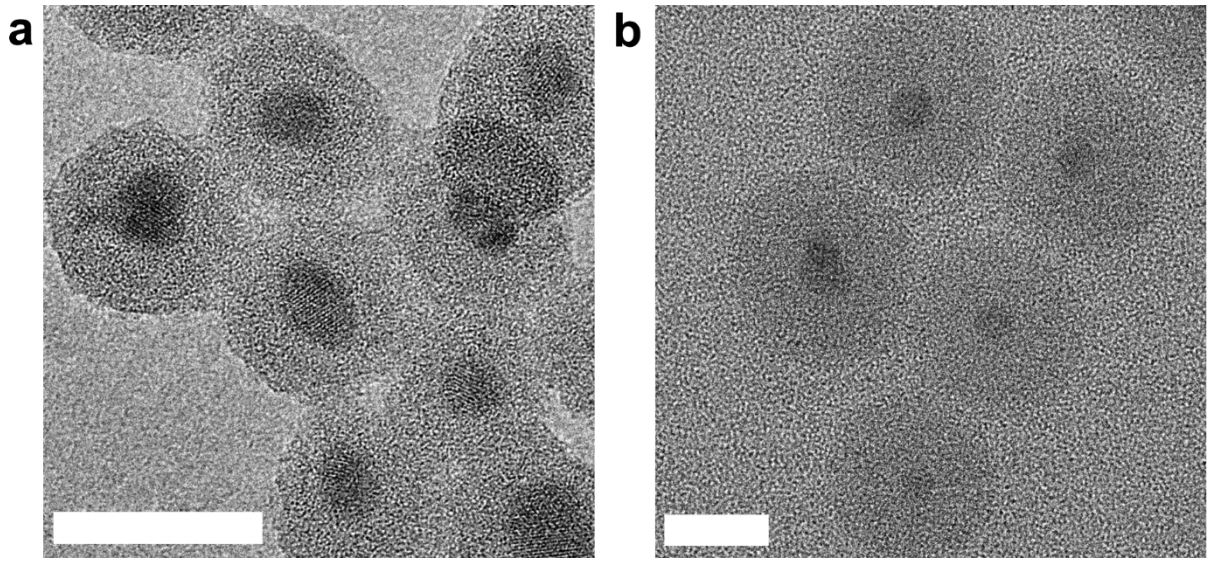


Figure S1. HRTEM of (a) QD/SiO_2 (6 nm) and (b) QD/SiO_2 (12 nm). Scale bar: 20 nm.

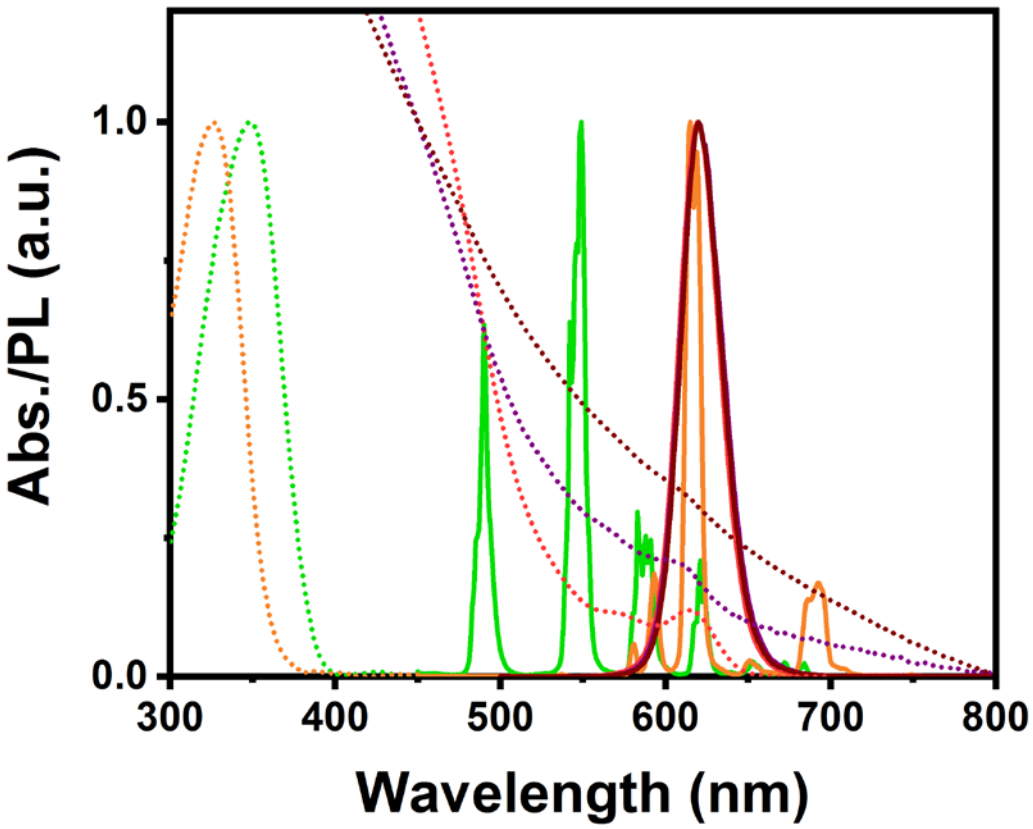


Figure S2. Absorbance (dashed lines) and PL emission spectra (solid lines) for the Lumi4-Tb (green), Eu-1 (orange), QD (red), QD/SiO_2 (6 nm) (violet), and QD/SiO_2 (12 nm) (wine).

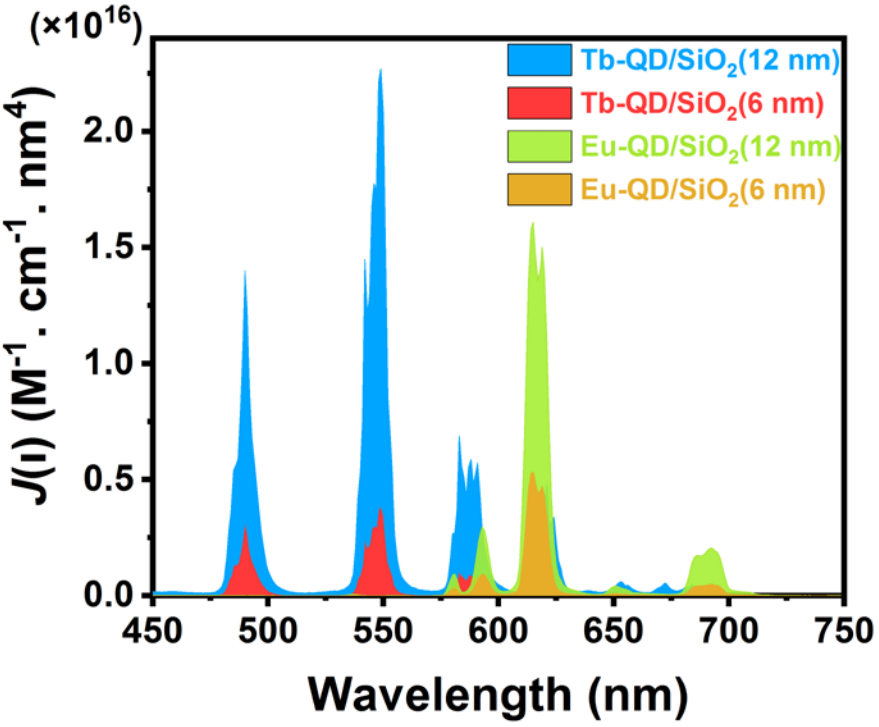


Figure S3. Spectral overlap functions for the Tb-QD/SiO_2 (12 nm), Tb-QD/SiO_2 (6 nm), Eu-QD/SiO_2 (12 nm), Eu-QD/SiO_2 (6 nm) FRET pairs.

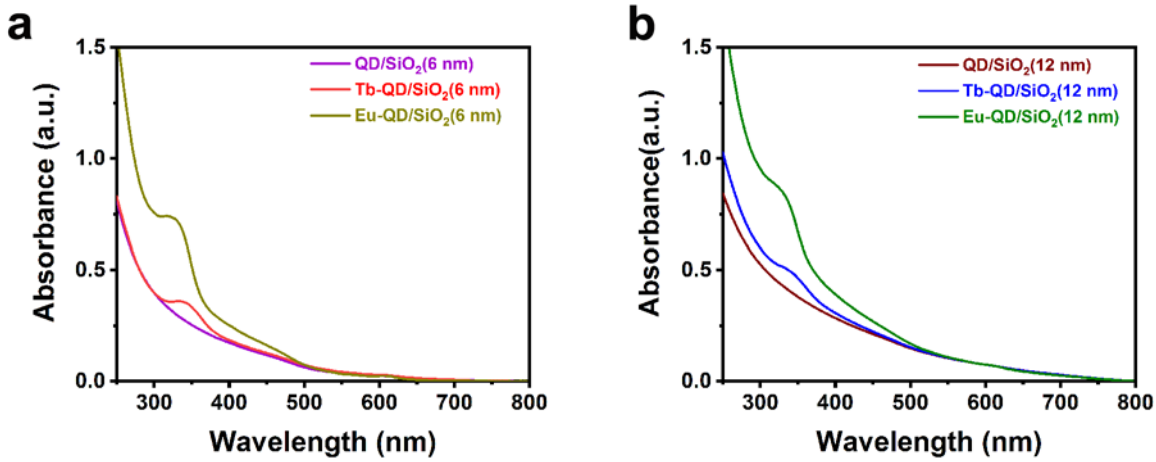


Figure S4. Absorbance spectra (in water) for the (a) QD/SiO_2 (6 nm) and (b) QD/SiO_2 (12 nm) before and after conjugating with Lumi4-Tb and Eu-1.

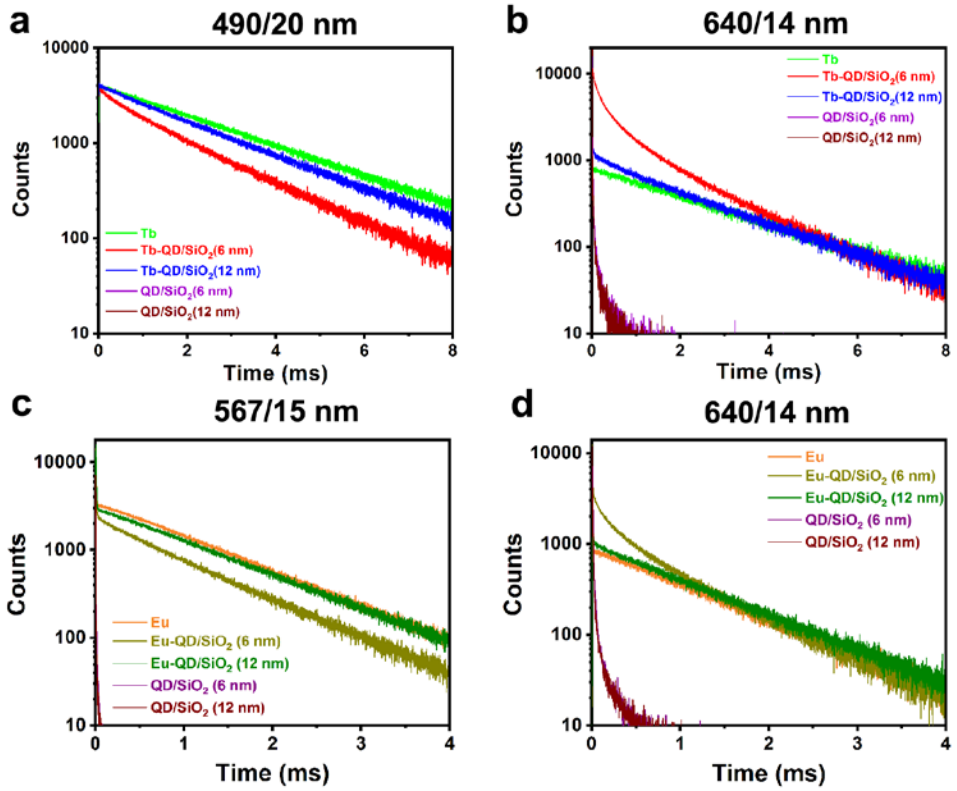


Figure S5. (a) Tb donor PL decay curve, (b) QD acceptor PL decay curve of Tb-QD/SiO_2 FRET-pairs. (c) Eu donor PL decay curve, (d) QD acceptor PL decay curve of Eu-QD/SiO_2 FRET-pairs.

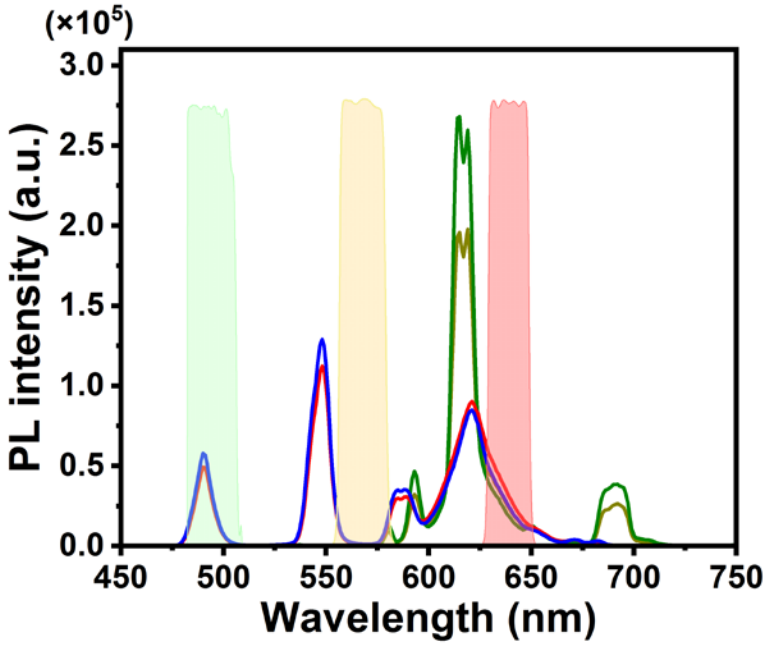


Figure S6. Steady-state PL spectra of Tb-QD/SiO_2 (6 nm) (red), Tb-QD/SiO_2 (12 nm) (blue), Eu-QD/SiO_2 (6 nm) (yellow), and QD/SiO_2 (12 nm) (green). (Filter green: 490/20 nm, filter yellow: 567/15 nm, and filter red: 640/14 nm).

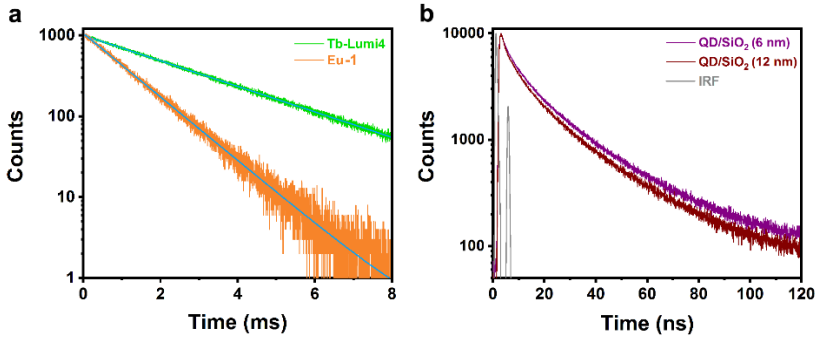


Figure S7. (a) Time-resolved PL decay curves of pure Lumi4-Tb-Mal and Eu-1-Mal (in water), (b) Time-resolved PL decay curves of pure QD/SiO_2 (6 nm) and QD/SiO_2 (12 nm). IRF: Instrument response function.

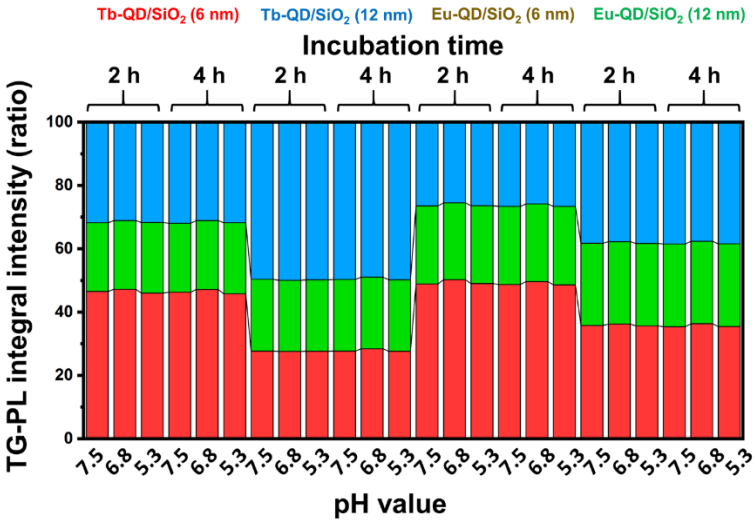


Figure S8. Stability of RGB color of the single nanoparticle codes in PBS buffer with different pH.

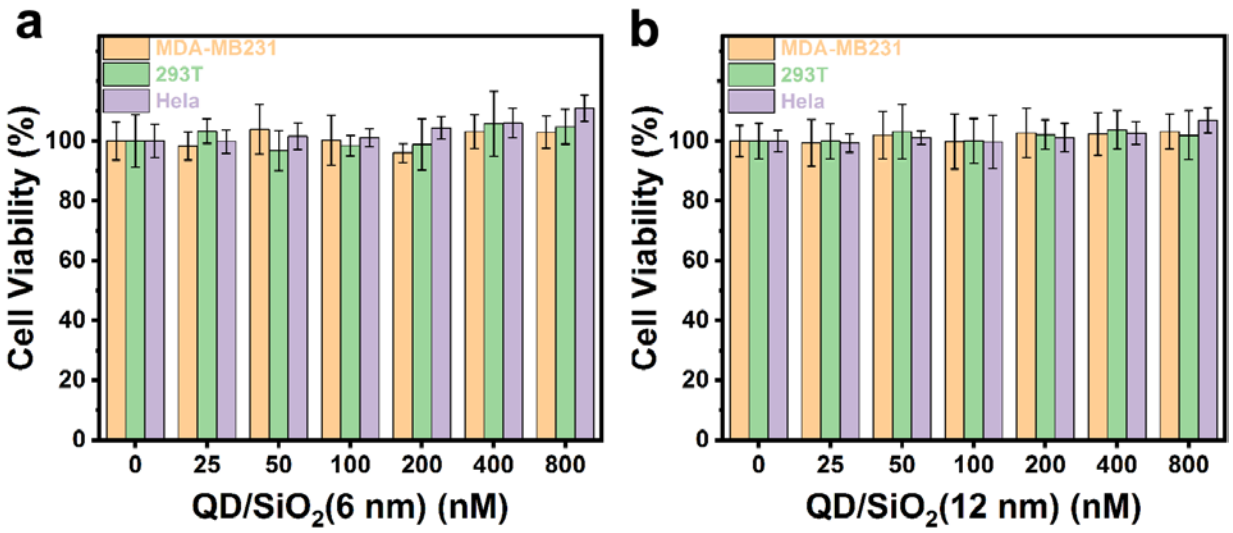


Figure S9. (a) Cell viability data of MDA-MB231, 293T, and Hela cells incubated with (a) QD-SiO_2 (6 nm) and (b) QD-SiO_2 (12 nm) after 24 hours in various concentration (0, 25, 50, 100, 200, 400 and 800 nM).

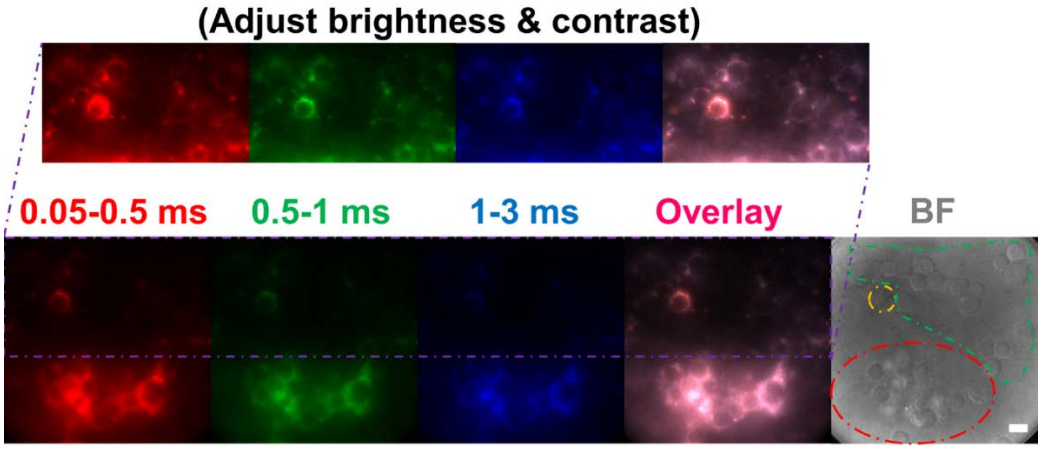


Figure S10. Time-gated PL images of Hela cells labeled with different single nanoparticle codes at different concentrations (red dot-dashed area: Tb-QD/SiO_2(6 nm), yellow dot-dashed area: Eu-QD/SiO_2(6 nm), green dot-dashed area: Eu-QD/SiO_2(12 nm). Scale bar: 20 μm.

Table S1. PL decay time fitting and analysis parameters for the QD acceptor in FRET (0.1 ms - 8 ms for Tb-QD/SiO_2, and 0.1 ms - 4 ms for Eu-QD/SiO_2).

Code	B_1	τ_1 (ms)	B_2	τ_2 (ms)	B_3	τ_3 (ms)	τ_{av}[(a)] (ms)	χ^2	E_{FRET}[(b)]	r[(c)] (nm)
Tb-QD/SiO_2(6 nm)	1920	0.19	1540	0.86	440	2.7	0.49	1.164	82%	8.0
Tb-QD/SiO_2(12 nm)	92	0.25	300	1.3	350	2.7	1.05	1.201	61%	11.3
Eu-QD/SiO_2(6 nm)	850	0.12	1770	0.50	1270	1.1	0.38	1.138	65%	8.3
Eu-QD/SiO_2(12 nm)	29	0.74	/	/	2260	1.1	0.74	1.160	33%	12.5

(a) Amplitude-averaged PL decay of only the first two decay components, which were caused by FRET-quenching. The third decay component (2.7 ms and 1.1 ms, respectively) results from unquenched lanthanide PL, which is still present (spectral crosstalk) in the QD detection channel. (b) FRET efficiency was calculated using τ_{av} as τ_{DA} and τ_3 as τ_D by the following equation: $E_{FRET} = 1 - \tau_{DA}/\tau_D$. (c) Calculated using equation S5.

Table S2. Transforming of time-gated integral intensity of coding in each channel to RGB color ratio.

Code	0.05-0.5 ms	0.5-1 ms	1-3 ms	RGB color ratio
Tb-QD/SiO_2(6 nm)	594320	289130	419120	(46%/22%/32%)
Tb-QD/SiO_2(12 nm)	144800	116410	250080	(28%/23%/49%)
Eu-QD/SiO_2(6 nm)	620500	307880	333020	(49%/24%/26%)
Eu-QD/SiO_2(12 nm)	422300	305280	450680	(36%/26%/38%)

Supplementary references

1) M. Delbianco, V. Sadovnikova, E. Bourrier, G. Mathis, L. Lamarque, J. M. Zwier, D. Parker. Angew. Chem. Int. Ed. **53** (2014), 10718-10722

2) M. H. V. Werts, R. T. F. Jukes, J. W. Verhoeven; Phys. Chem. Chem. Phys **4** (2002) 1542-1548