

Supplementary Information

A FRET-based ratiometric sensor for mercury ions in water with multi-layered silica nanoparticles as the scaffold

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1 Experimental Section

1.1 Materials

Rhodamine B (RhB), triethoxy(3-isocyanatopropyl)-Silane (TEPS), γ -Aminopropyl triethoxysilane (APS), 1,2-ethylenediamine and Lawesson's Reagent were purchased from Alfa Aesar. 4-chloro-7-nitro-benzo-furazan (NBD-Cl) was obtained from Acros. Sodium salt of 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid (HEPES), potassium persulfate, chloride salts of metal ions (K^+ , Na^+ , Ca^{2+} , Mg^{2+} , Al^{3+} , Zn^{2+} , Fe^{3+} , Mn^{2+} , Pb^{2+} , Cu^{2+} , Co^{2+} , Ni^{2+} , Cr^{3+} , Hg^{2+}) and $AgNO_3$ were obtained from Sigma-Aldrich. Ethanol, tetraethoxysilane (TEOS), triethylamine, toluene and methanol were analytically pure reagents and re-purified before use. The purified water used in this study was the triple-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system to remove the trace amount of metal ions.

1.2 Synthesis of the ethoxysilane-linked probe (probe precursor)

First, a spirolactam Rhodamine B derivative (RhB-NH₂) was synthesized according to the procedure reported elsewhere (C. Ma, F. Zeng, L. F. Huang, S. Z. Wu, *J. Phys. Chem. B.* **2011**, *115*, 874). Rhodamine B (5 g, 10.4 mmol) was dissolved in 100 mL of hot methanol, followed by the addition of ethylenediamine (200 mmol). The reaction mixture was refluxed for 20 h till the fluorescence of the solution disappeared. After cooling to room temperature, the solvent was evaporated under vacuum. CH_2Cl_2 (150 mL) and water (300 mL) were added, and the organic layer was separated, washed with water several times and dried over anhydrous sodium sulfate. After filtration of sodium sulfate, the solvent was removed under reduced pressure. Then the resulting solid was purified by column chromatography with ethyl

acetate/petroleum ether (volume ratio 3:1) to give the RHB-NH₂ in 78% yield. Afterwards, to synthesize the mercury-ion recognition probe, the RhB-NH₂ (485 mg, 1 mmol) and Lawesson's Reagent (404 mg, 1 mmol) were dissolved in dry toluene, and the mixture was refluxed for 6 hours under N₂ atmosphere, after removal of toluene, the residue was dissolved in dichloromethane and purified by column chromatography with ethyl acetate/petroleum ether (volume ratio 1:3) as eluent to afford the mercury ion recognition probe SRhB-NH₂ in 15% yield. Then, SRhB-NH₂ (200 mg, 0.40 mmol) and triethoxy (3-isocyanatopropyl)-silane (0.14 mL, 0.47 mmol) were dissolved in toluene (10 mL) and stirred in reflux condition under N₂ for 12 h. After cooling to room temperature, the solvent was evaporated in vacuo. A brownish crude product was obtained and then was further purified by column chromatography on silica gel (dichloromethane /methanol, 30:1) to provide 250 mg of brownish oily liquid in 83% yield. The product was characterized by ¹H NMR and MS as shown in Figure S2. ¹H NMR (CDCl₃, 400 MHz) δ: 8.09-8.07 (m, 1H), 7.49-7.47 (m, 2H), 7.09-7.07 (m, 1H), 6.41-6.39 (m, 2H), 6.34-6.32 (m, 2H), 6.27-6.24 (m, 2H), 3.83-3.78 (m, 8H), 3.36-3.31 (m, 6H), 3.14-3.12 (m, 4H), 2.97-2.93 (m, 2H), 2.75-2.55 (s, 2H), 1.58-1.56 (m, 2H), 1.26-1.02 (m, 21H), 0.65-0.61 (t, 2H). ESI m/z [M+ H⁺]= 747.7.

1.3 Synthesis of ethoxysilane-linked NBD dye (donor precursor)

The ethoxysilane-linked NBD precursor was obtained from the reaction of 4-chloro-7-nitro-benzo-furazan (NBD-Cl) with APS. First, 200 mg NBD-Cl was dissolved in 10 mL ethanol, then 0.3 mL APS was dissolved into the solution under efficient stirring in an ice bath for 2 h. The mixture was stirred for 10 h at room temperature. The deep red solution was obtained and then was further purified by column chromatography on silica gel (ethyl acetate /

petroleum ether, 2:1) to provide 200 mg red powder in 49 % yield. The product was characterized by NMR and MS as shown in Figure S1. ^1H NMR (CDCl_3 , 400 MHz) δ :8.50-8.47 (d, 1H), 6.87-6.85 (s, 1H), 6.18-6.16 (d, 1H), 3.89-3.84 (m, 6H), 3.55-3.50 (m, 2H), 1.98-1.91 (m, 2H), 1.26-1.21 (m, 9H), 0.79-0.75 (t, 2H). ESI m/z [$\text{M} + \text{Na}^+$] = 407.0.

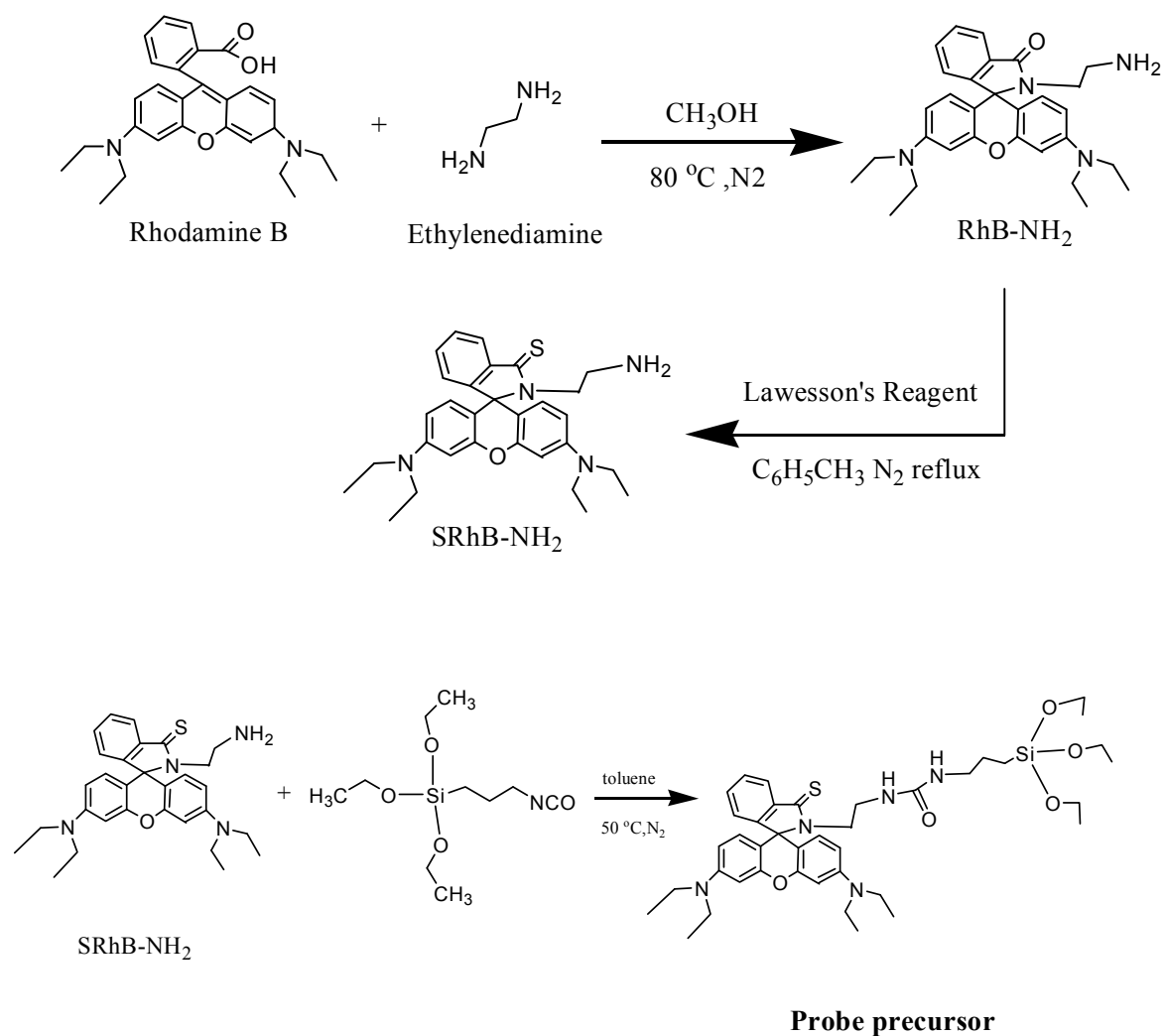
1.4 Preparation of multilayered silica nanoparticles

The multilayered silica nanoparticles were prepared using a slightly modified Stöber's method. Firstly, 4.5 mL water and 770 μL ammonia water were dissolved in 43.5 mL ethanol and stirred for half hour under room temperature, and then 1.7 mL TEOS was added into the ethanol solution. The reaction was allowed to complete in 24 hours and the "pure" silica core particles were obtained. Then a solution of donor precursor (NBD-APS) (1.8×10^{-2} M in ethanol, 30 μL) and 75 μL TEOS were added together into the reaction vessel. After 5 hours of stirring, a donor-containing layer was formed on the particle cores. Then, appropriate amount of TEOS (0 μL TEOS for sample NP-0, 30 μL TEOS for NP-2 and 75 μL TEOS for NP-4) was added into the reaction vessel to form a spacer layer between the donor and the acceptor. After 18 hours, a solution of probe (5.8×10^{-3} M in ethanol, 50 μL) was added into the reaction vessel. After 5 hours of stirring, the probe layer was coated on the particles surface. Finally, the obtained silica nanoparticles were isolated by centrifugation and redispersed in purified water of neutral pH.

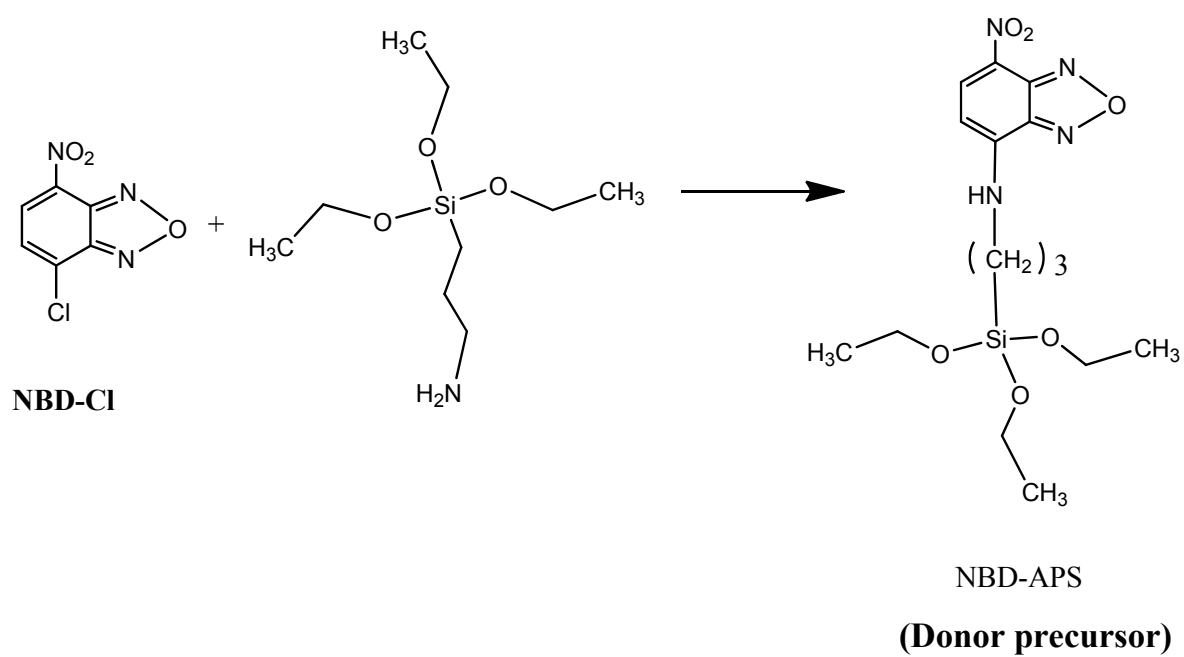
1.5 Measurements

^1H NMR spectra were recorded on a Bruker Avance 400 MHz NMR Spectrometer. The mass spectrum was obtained through a Bruker Esquire HCT Plus mass spectrometer. The sizes of nanoparticles were measured in solution using dynamic light scattering (DLS) on a Malvern

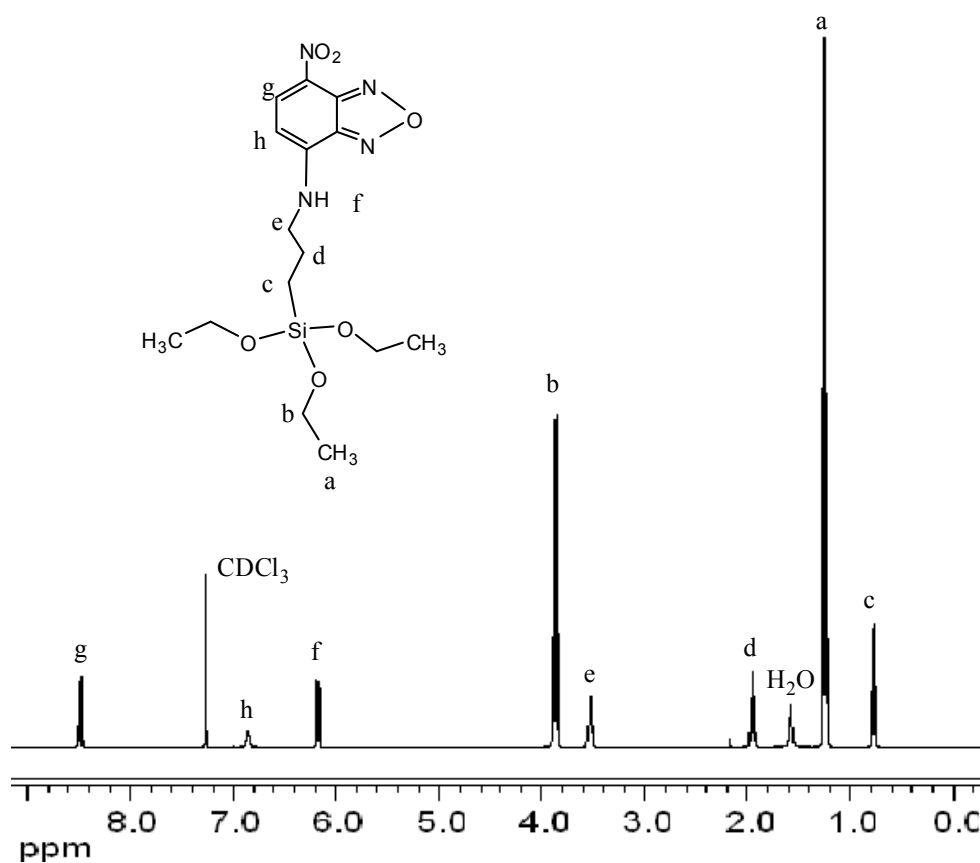
Nano-ZS90 particle size analyzer and in the dry state by atomic force microscopy (AFM, Seiko SII 400). UV–vis spectra were obtained on a Hitachi U-3010 UV–vis spectrophotometer. The steady fluorescence spectra were recorded on a Hitachi F-4600 fluorescence spectrophotometer. Time-resolved fluorescence spectra were recorded on an Edinburgh FL920 fluorescence spectrophotometer at 25°C under air, and the samples were excited with a 465 nm pulsed laser LED.



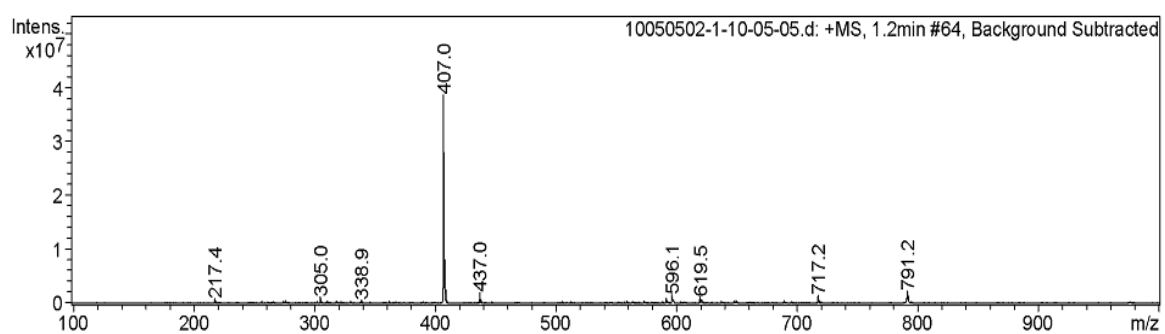
Scheme S1. Synthetic route of the Hg^{2+} probe (SRhB-NH₂) and ethoxysilane-linked probe (probe precursor).



Scheme S2. Synthetic route of ethoxysilane-linked donor (donor precursor, NBD-APS)

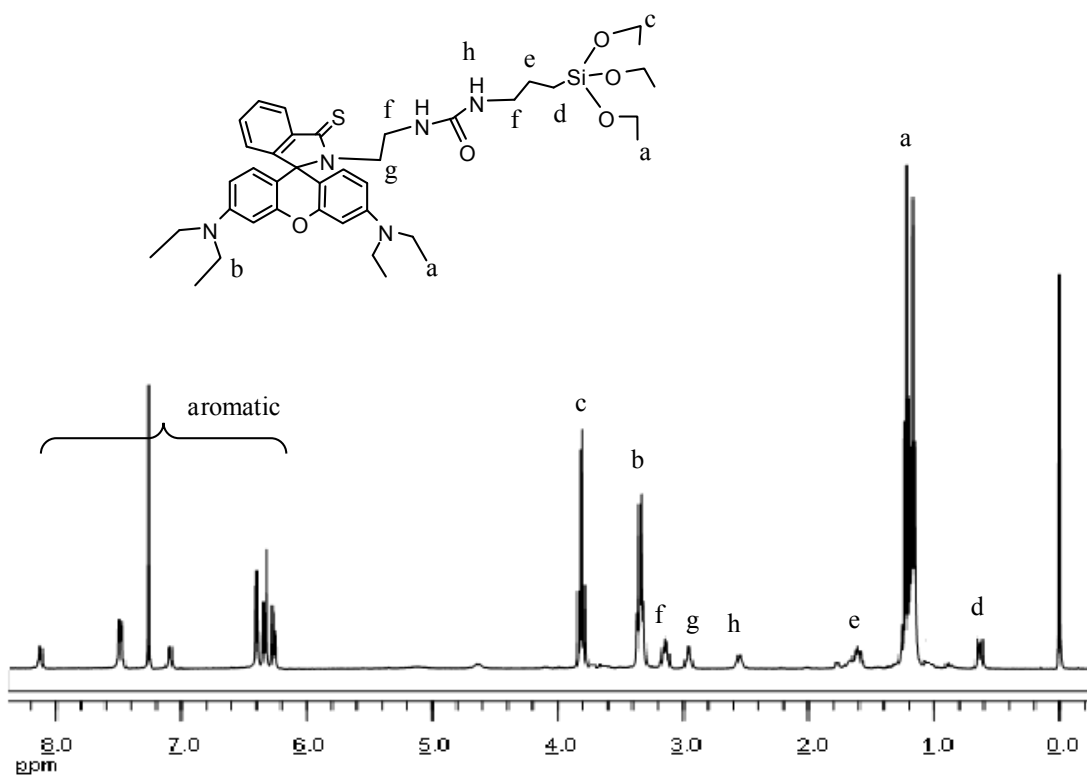


(a)

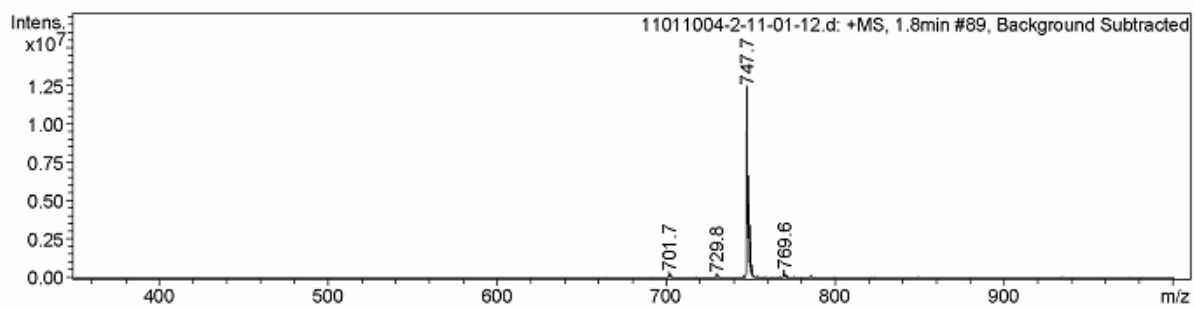


(b)

Figure S1. ^1H NMR and MS spectra for donor precursor.

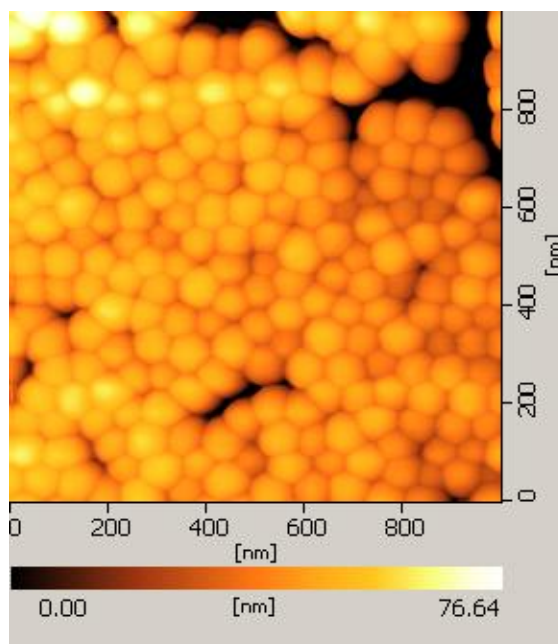


(a)

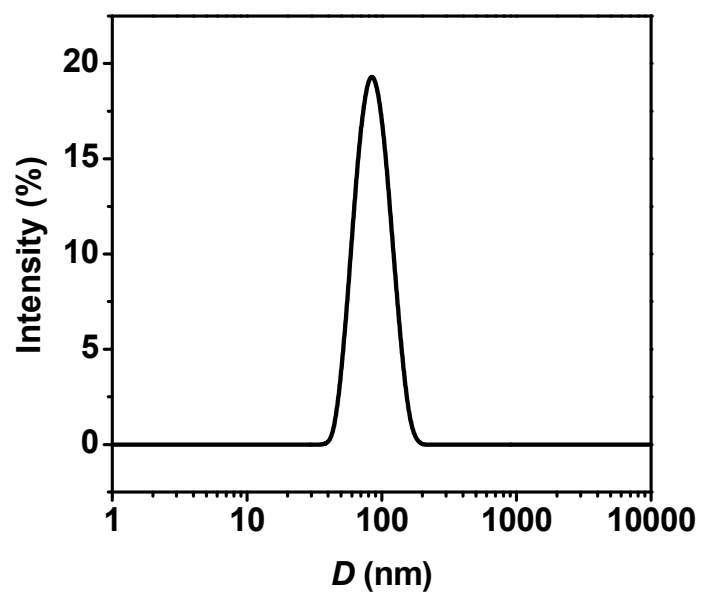


(b)

Figure S2. ¹H NMR and MS spectra for probe precursor.



(A)



(B)

Figure S3. AFM image (A) and diameter distribution determined by DLS (B) for silica core particles. In this study, all the samples were originated from this core particle.

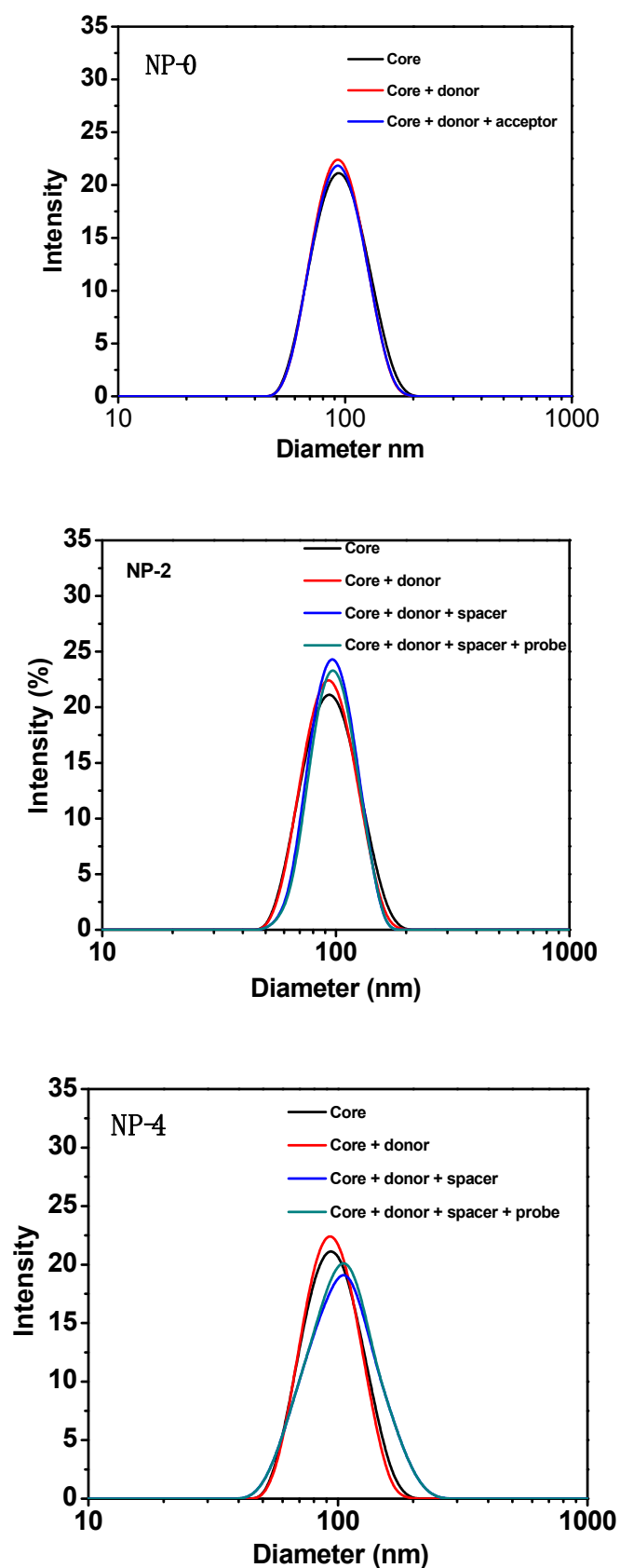


Figure S4. Particle size distribution for three silica nanoparticle samples (NP-0, NP-2 and NP-4) at different synthesis stages as recorded by DLS method. One DLS measurement can

give a diameter distribution and a corresponding average diameter. In this study, for each measurement, we repeated three times and we could obtain three average diameters, then we calculated the “averaged” average diameter for each particle sample, and an error can thus be obtained for each “averaged” average diameter. The following diameter data are actually the “averaged” average diameter for the nanoparticles.

All three samples were prepared from one silica core (89.5 nm in average diameter).

The determined average diameters for the nanoparticles samples at each stage are as follows:

NP-0: 89.5 ± 0.5 nm (core); 90.0 ± 0.6 nm (core + donor); 90.3 ± 0.5 nm (core + donor + probe).

There is no spacer between donor layer and probe layer.

NP-2: 89.5 ± 0.5 nm (core); 90.0 ± 0.6 nm (core + donor); 94.2 ± 0.5 nm (core + donor + spacer); 94.4 ± 0.4 nm (core + donor + spacer + probe). The thickness of the spacer layer can thus be estimated as 2.1 nm.

NP-4: 89.5 ± 0.5 nm (core); 90.0 ± 0.6 nm (core + donor); 98.6 ± 0.5 nm (core + donor + spacer); 98.7 ± 0.5 nm (core + donor + spacer + probe). The thickness of the spacer layer can thus be estimated as 4.3 nm.

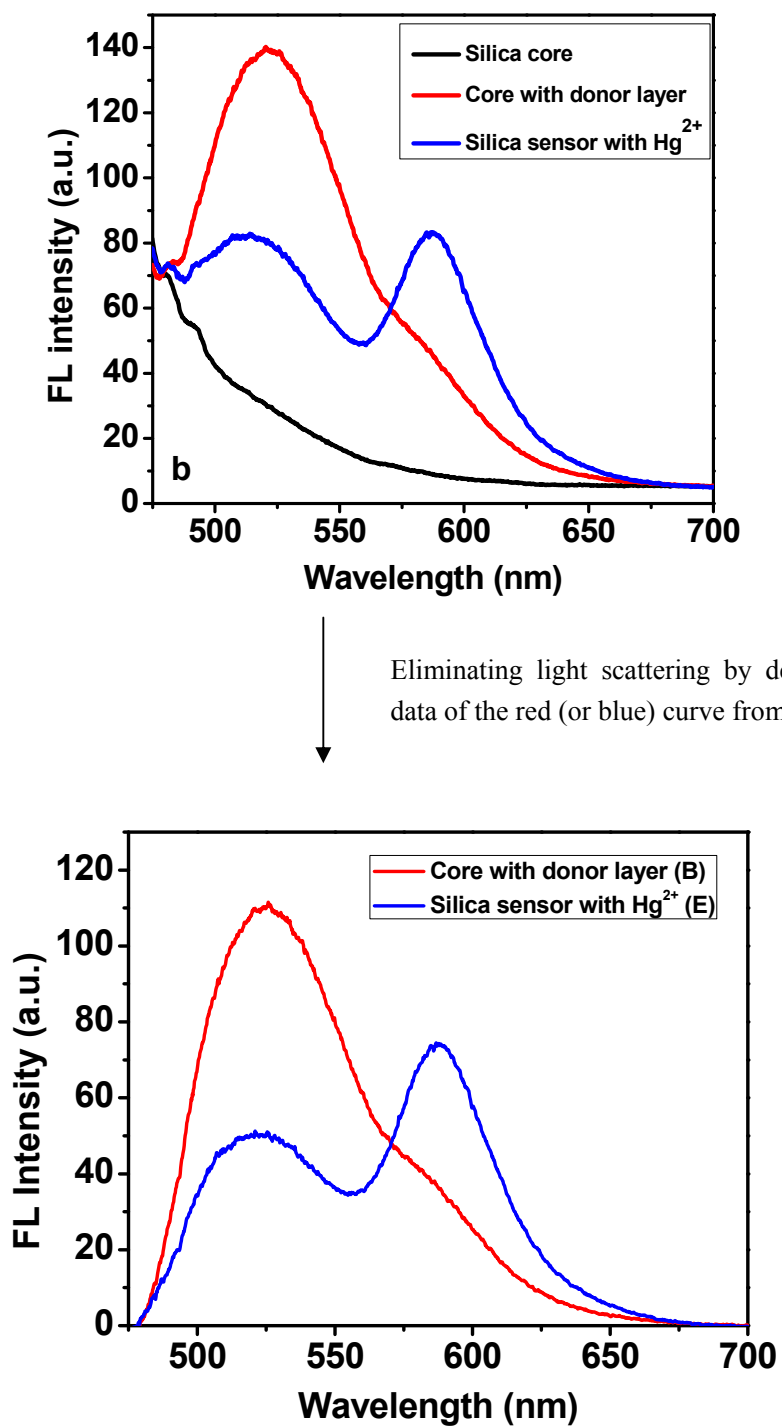


Figure S5. An example for the calibration of emission spectra.

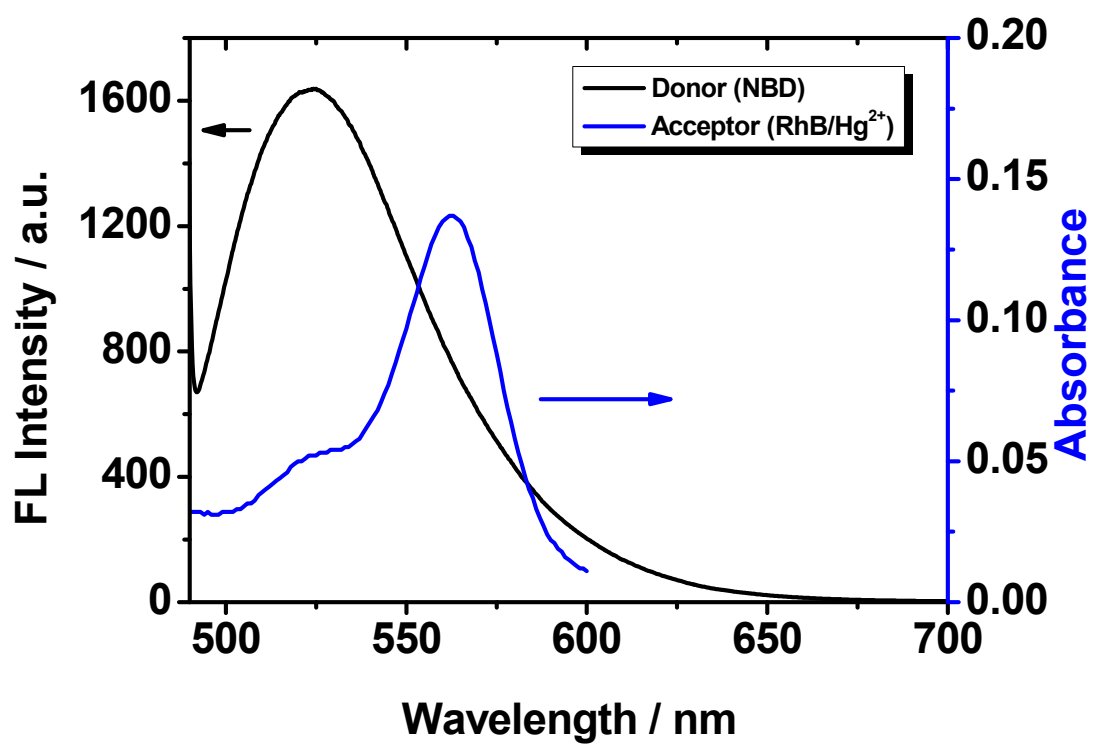
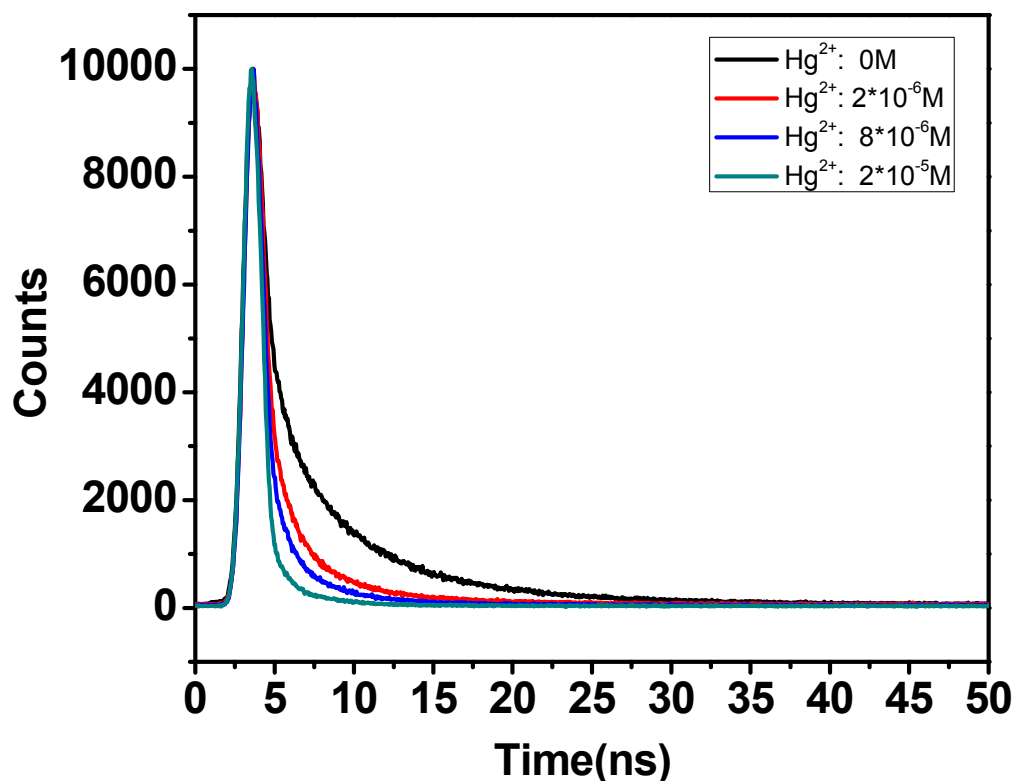


Figure S6. Overlap of donor's emission band and acceptor's absorption band.



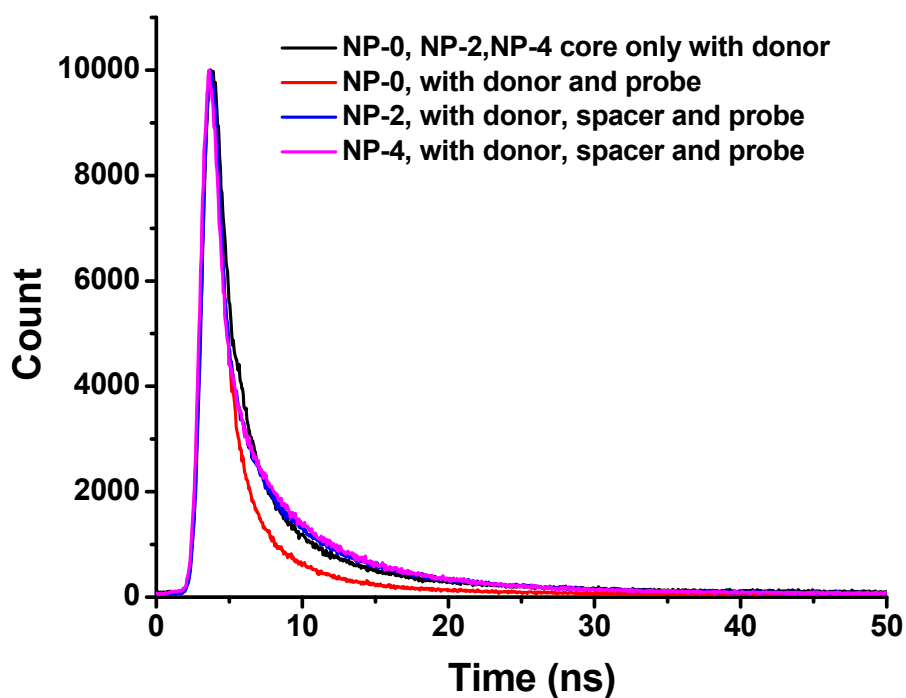
$C_{\text{Hg(II)}} \text{ (M)}$	Average lifetime (ns)
0	9.86
2×10^{-6}	8.71
8×10^{-6}	7.52
2×10^{-5}	6.56

Figure S7A. Fluorescence decay curves for the donor (NBD) in multilayered silica system in the absence and presence of varied concentrations of Hg^{2+} .

Note: the average lifetimes were calculated by averaging t over the intensity decay of the donor using a numerical integration software according to Equation 1, and used it as the apparent lifetime for the donor upon addition of mercury ions.

$$\langle t \rangle = \frac{\int_{t_1}^{t_2} tI(t)dt}{\int_{t_1}^{t_2} I(t)dt} \quad (1)$$

where $I(t)$ is the time-dependent intensity for the donor; t_1 is the time at which the intensity began to decay and t_2 is the time at which the integration ends.



Sample	Average lifetime (ns)
NP-0, NP-2, NP-4 core with only donor	9.92
NP-0 core with donor and probe (no Hg ²⁺)	9.14
NP-2, with donor, spacer and probe (no Hg ²⁺)	9.86
NP-4, with donor, spacer and probe (no Hg ²⁺)	9.84

Figure S7B. Fluorescence decay curves for nanoparticle samples at different synthesis stages.

Note: the three samples (NP-0, NP-2 and NP-4) were all originated from one nanoparticle sample which was doped with only NBD, hence the particle core with only the donor for three samples is identical and thus there is one fluorescence decay curve (black curve). We can see from the figure that only for NP-0, in which there is no spacer between donor and probe, the existence of probe reduces the lifetime of the donor, and this suggests there may be interaction between the donor and the probe (without mercury ion) for NP-0. For NP-2 and NP-4, in the absence of mercury ions, their decay curves almost overlap with that for the silica cores with only donor, indicating that the insert of a spacer layer between the donor layer and the probe layer can prevent the unfavorable interactions between donors and acceptors.

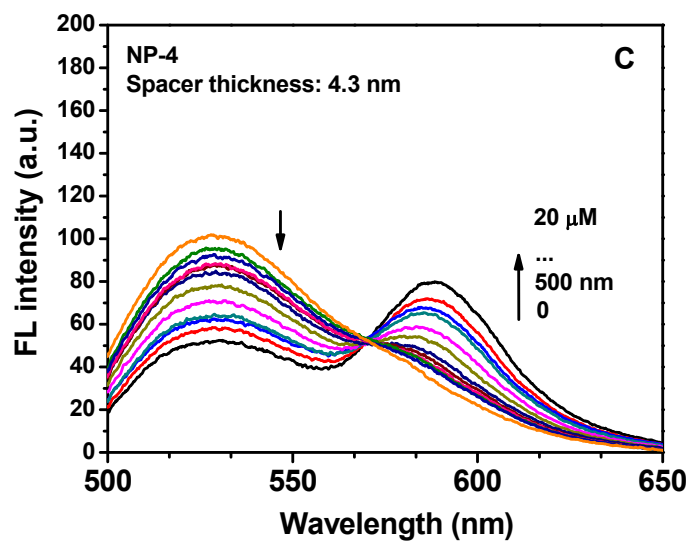
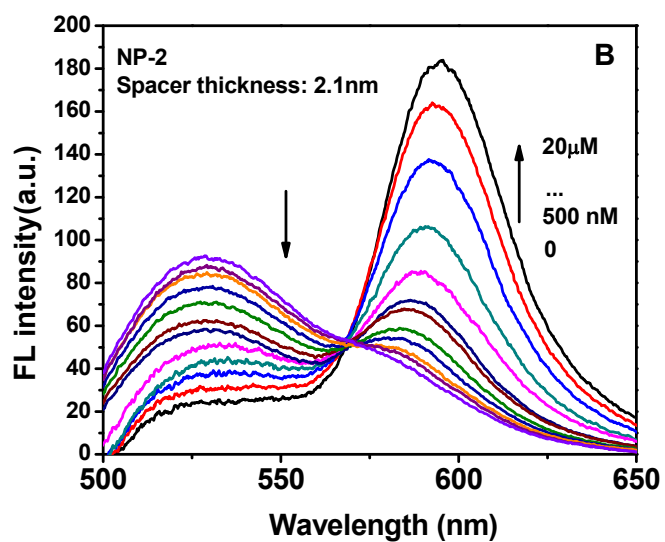
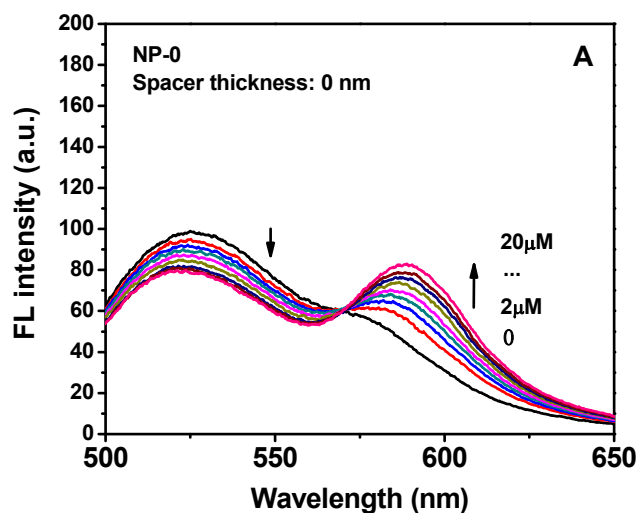


Figure S8. Effect of thickness of spacer layer on energy transfer efficiency and signal-to-background ratio for three multilayered silica nanoparticle-based sensors upon titration of mercury ions. For convenient comparison, the Y scales for three figures were kept identical, and the initial fluorescence intensity for the donors (before addition of Hg^{2+}) of the three samples were kept close to one another by using samples with different donor concentrations (A: $C_{\text{NBD}} : 1.06 \times 10^{-5} \text{ M}$, $C_{\text{SRhB}}: 1.98 \times 10^{-5} \text{ M}$; B: $C_{\text{NBD}} : 8.6 \times 10^{-6} \text{ M}$, $C_{\text{SRhB}}: 1.70 \times 10^{-5} \text{ M}$; C: $C_{\text{NBD}} : 9.1 \times 10^{-6} \text{ M}$, $C_{\text{SRhB}}: 1.76 \times 10^{-5} \text{ M}$). The excitation wavelength is 430 nm.

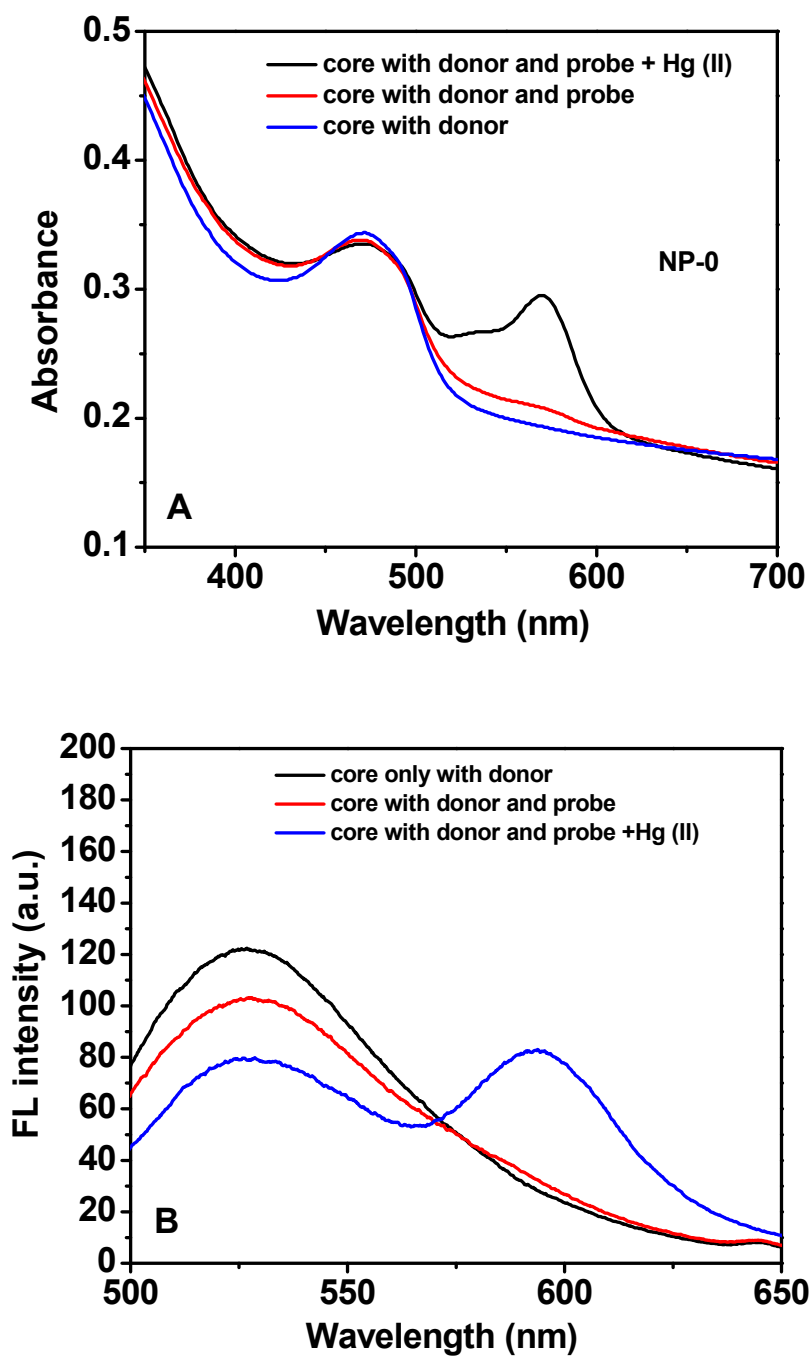


Fig. S9 Absorption (A) and fluorescence (B) spectra for sample NP-0 at different synthesis stages. $C_{\text{NBD}} : 1.06 \times 10^{-5} \text{ M}$, $C_{\text{SRhB}} : 1.98 \times 10^{-5} \text{ M}$.

Note: Fig. S8A indicates that the sufficient amount of SRhB molecules have been introduced on NP-0 (black line); and Fig. S8B shows that the covering of a probe layer directly on the donor layer can partially quench the donor's emission, suggesting there may be interaction between the donors and the acceptors in case there is no spacer between them.

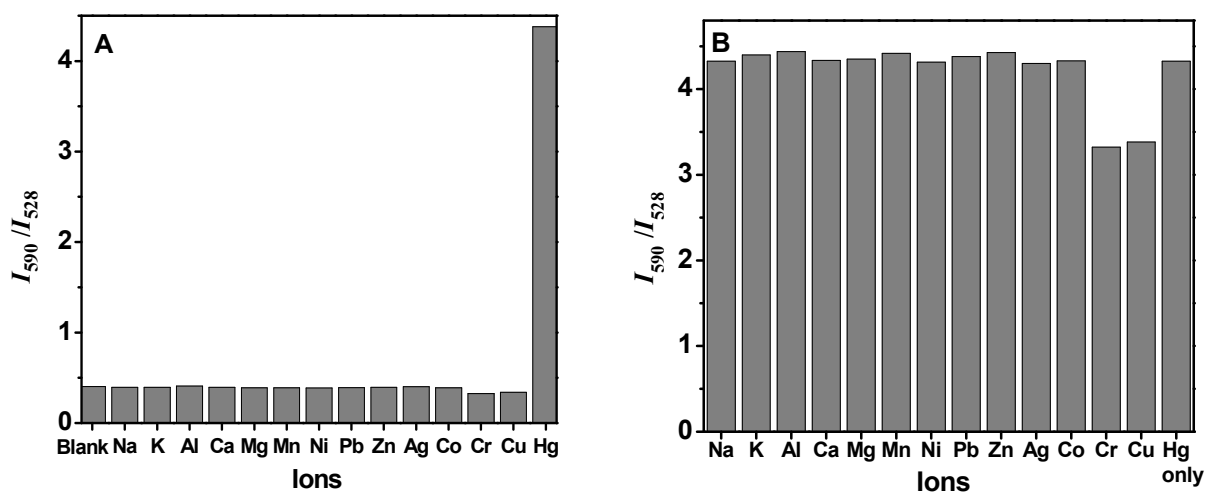


Fig. S10 (A) Fluorescence intensity ratio (I_{590}/I_{528}) for NP-2 dispersion. (A): upon addition of different metal ions (10 μM) respectively; (B): In the presence of 10 μM of Hg^{2+} with 10 μM of various coexisting metal cations respectively. Hg^{2+} only: in the presence of 10 μM of Hg^{2+} only. (pH 7.0, 0.01 M HEPES buffered water).

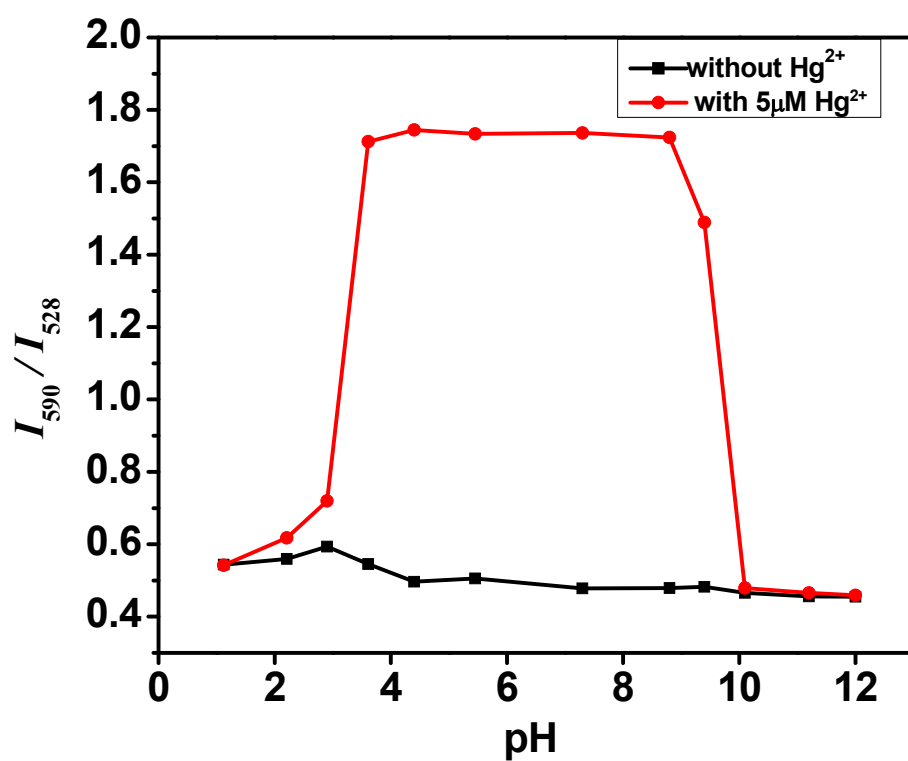


Figure S11. Effect of pH on the ratio of fluorescence intensity for the acceptor to that for the donor in the absence and presence ($5\ \mu\text{M}$) of mercury ions.

Table S1. Some parameters for three multilayered silica samples.

Sample code	Spacer thickness (nm) ^a	E_m ^b	I_{590}/I_{528} (at 0 M Hg ²⁺)	I_{590}/I_{528} (at 20 μM Hg ²⁺)	Signal-to-background ratio ^c
NP-0	0	21 %	0.43	1.05	2.4
NP-2	2.1	74 %	0.37	6.90	18.6
NP-4	4.3	49 %	0.30	1.53	5.1

Note: a: Estimated based on the DLS measurement; b: Measured energy transfer efficiency for the samples. $E_m=1-I_{Hg}/I_0$, where I_0 , I_{Hg} are fluorescence intensities of NBD in the absence and presence (20 μM) of mercury ions, respectively. c: The ratio of I_{590}/I_{528} (at 0 M Hg²⁺) to I_{590}/I_{528} (at 20 μM Hg²⁺).

Calculation of Förster Critical Radius (R_0)¹⁻³

Calculation of the Förster radii (R_0)¹⁻³ and determination of experimental energy transfer efficiency

The Förster's distance or critical distance R_0 is the characteristic distance, at which the efficiency of energy transfer is 50%. The magnitude of R_0 is dependent on the spectral properties of the donor and the acceptor molecules. If the wavelength λ is expressed in nanometers, then $J(\lambda)$ is in units of $M^{-1}cm^{-1}nm^4$ and the Förster distance, R_0 in angstroms (Å), is expressed as follows [Eq. (1)]:

$$R_0 = 0.2108 \times [K^2 \times \Phi_D \times n^{-4} \times J(\lambda)]^{1/6} \quad [\text{Eq. (1)}]$$

K^2 is the orientation factor for the emission and absorption dipoles and its value depends on their relative orientation, n is the refractive index of the medium and Φ_D is the quantum yield of the donor. $J(\lambda)$ is the overlap integral of the fluorescence emission spectrum of the donor and the absorption spectrum of the acceptor (Figure S10) [Eq. (2)].

$$J(\lambda) = \int_0^\infty F_D(\lambda) \times \varepsilon_A(\lambda) \times \lambda^4 \times d\lambda \quad [\text{Eq. (2)}]$$

$F_D(\lambda)$ is the fluorescence intensity of the donor in the absence of acceptor normalized so that $\int_0^\infty F_D(\lambda) d\lambda = 1$; $\varepsilon_A(\lambda)$ is molar extinction coefficient of the acceptor, λ is wavelength. In current experimental conditions, for this system, the $J(\lambda)$ was calculated to be $7.50 \times 10^{14} M^{-1}cm^{-1}nm^4$. The Förster distance (R_0) has been calculated assuming random orientation of the donor and acceptor molecules taking $K^2 = 2/3$, $n = 1.54$ (silica), and determined $\Phi_D = 0.79$.

For NBD (donor) and SRhB/Hg²⁺ (acceptor) in current experimental situation, by using a commercial software Origin 8.0 as the integral tool, we calculated $R_0 = 28.8 \text{ \AA}$. Energy transfer will be effective for $14.4 \text{ \AA} \leq d \leq 43.2 \text{ \AA}$ ($R_0 \pm 50\% R_0$)

Reference:

- (1) Gouanve, F.; Schuster, T.; Allard, E.; Meallet-Renault, R.; Larpent, C. *Adv. Funct. Mater.* **2007**, *17*, 2746-2756.
- (2) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*: Plenum, New York, 1999.
- (3) Valeur, B. *Molecular Fluorescence: Principles and Applications*: Wiley-VCH: New York, **2002**.