Supporting Information

Fluorescent and Colorimetric Magnetic Microspheres as a Nanosensor for Hg^{2+} in Aqueous Solution Prepared by Sol-Gel Grafting Reaction and Host-Guest Interaction

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1. Materials

Rhodamine 6G (Rh6G) and β-cyclodextrin (β-CD) were purchased from Sigma-Aldrich. 1-Adamantanamine, 4-toluene sulfonyl chloride (TSO-Cl), triethylamine and carbon disulfide were obtained from Alfa. γ-(2,3-epoxypropoxy) propytrimethoxysilane (KH-560, 99%) was obtained from Acros Organics. All the reagents and inorganic metal salts with analytical grade (Shanghai Chemical Reagents Co. China) were used without further purification. The solutions of metal ions were prepared from NaCl, KCl, $CaCl_2$, $MgSO_4$, $FeCl_3$, $Mn(NO_3)_2{\cdot}6H_2O$, $CoCl_2{\cdot}6H_2O$, $NiCl_2{\cdot}6H_2O$, $Zn(NO_3)_2$, $CdCl_2$, $CuCl_2{\cdot}2H_2O$, $HgCl_2$, $AgNO_3$, $Pb(NO_3)_2$ respectively, and were dissolved in deionized water. Aqueous HEPES-NaOH (0.1 mol L^{-1}) solution was used as buffer to keep pH value (pH=7. 20), and to maintain the ionic strength of all solutions in experiments.

2. Characterization

^1H NMR spectra were measured on a Varian Mercury-300BB NMR spectrometer with chemical shifts reported as parts per million (in $CDCl_3$, TMS as internal standard). The pH values of the test solutions were measured with a glass electrode connected to a Mettler-Toledo Instruments DELTA 320 pH meter (Shanghai, China) and adjusted if necessary. FTIR spectra of the products were recorded on a Perkin-Elmer Paragon1000 FTIR spectrometer. HRMS were collected with an Agilent1290-micrOTOF Q II (Brucker) spectrometer. Elemental analyses were determined with a Flash EA1112 elemental analyzer. Absorption and luminescence spectra were studied on a Shimadzu

UV 2100 PC UV-visible spectrophotometer and a Hitachi F-4500 luminescence spectrometer, respectively.

2.1 Synthesis of Adamantane-based silane coupling agent (Ad-Si)

1-Adamantanamine (1.0 g, 6.61 mmol) was dissolved in dry methanol (30 ml). To the solution, 3-Glycidoxypropyltrimethoxysilane (1.5 g, 6.35 mmol) was added at room temperature. The reaction mixturewas refluxed for 12 h at the nitrogen atmosphere. The solvent was evaporated and the product was isolated by column chromatography on silica gel (ethyl acetate). The colorless liquid product was obtained. Yield: 69%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 3.76 (t, *J* = 12.7 Hz, 1H; CH), 3.69 (d, *J* = 3.1 Hz, 2H; CH_2), 3.57 (s, 9H; CH_3), 3.54 – 3.32 (m, 4H; CH_2), 3.14 (dd, *J* = 14.7, 5.8 Hz, 2H; CH_2), 2.83 – 2.77 (m, 2H; CH_2), 2.61 (dd, *J* = 5.0, 2.7 Hz, 1H; NH), 1.87 (s, 1H; OH), 1.75 (t, *J* = 3.0 Hz, 2H; CH_2, Adamantane), 1.72 – 1.63 (m, 6H; CH_2), 1.26 (s, 3H; CH, Adamantane), 1.11 (s, 6H; CH_2, Adamantane), 0.73 – 0.64 (m, 2H; CH_2); IR (KBr): *v* = 3419 (s), 2905 (s; ν_{as}(CH_2, Adamantane)), 2849 (s; ν_s(CH_2, Adamantane)), 1633 (m), 1452 (w; ν_s(OH)), 1190 (m; ν_s(SiO)), 1086 (s; ν_s(COC)), 956 (w), 787 cm^{-1} (w; ω(CH)); HRMS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{21}H_{41}NO_5Si$, 387.2441; found, 387.2410. Anal. Calcd for $C_{45}H_{28}N_4O_7$: C 58.88, H 9.62, N 3.61; found: C 58.91, H 9.64, N 3.46.

2.2. Synthesis of *N*-(rhodamine-6G)lactam-ethylenediamine (Rh6G-NH_3)

Rhodamine 6G (5g, 12.07 mmol) was dissolved in 180mL of anhydrous ethanol, then ethylenediamine (12.56g, 209 mmol) was added to it as soon as possible at 40 °C, and the temperature was raised to 85 °C slowly. After 24 h, the solvent was evaporated

under reduced pressure, then CH_2Cl_2 (100 mL) and water (200 mL) were added to the residue, and the organic layer was separated, washed five times with water, and dried over anhydrous $MgSO_4$. After filtration of $MgSO_4$, the solvent was removed under reduced pressure to give an orange powder and purified by silica gel column chromatography (CH_2Cl_2/EtOH/ Et_3N, 5:1:0.1) to give the white product Rh6G-NH_3. Yield: 92%. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 8.03 – 7.83 (m, 1H; Ar H), 7.56 – 7.33 (m, 1H; Ar H), 7.15 – 6.93 (m, 2H; Ar H), 6.34 (s, 2H; Ar H), 6.22 (s, 2H; Ar H), 3.72 (q, *J* = 7.0 Hz, 2H; NH), 3.51 (s, 2H; CH_2), 3.32 – 3.09 (m, 2H; CH_2), 2.41 (t, *J* = 6.4 Hz, 2H; CH_2), 2.28 – 2.08 (m, 6H; CH_3), 1.85 (d, *J* = 31.5 Hz, 2H; NH_2), 1.28 (dt, *J* = 24.4, 7.1 Hz, 6H; CH_3); IR (KBr): *v* = 3422 (m; $\nu_{as}(NH_2)$), 2972 (m; ν_{as}(C-N)), 2849 (w; $\nu_{as}(CH_2)$), 1687 (s; ν_{as}(CO)), 1616 (m), 1551 (s; ν_s(COC)), 1467 (m; $\rho(CH_2)$), 1419 (m), 1380 (m; $\rho(CH_2)$), 1351 (m;), 1266 (w), 1221 (m), 1156 (w; ν_s(CO)), 1060 (m), 928 (m), 747 cm^{-1} (m; ω(CH)); HRMS (ESI, *m/z*): $[M + H]^+$ calcd for $C_{28}H_{32}N_4O_2$, 456.5794; found, 456.5742. Anal. Calcd for $C_{45}H_{28}N_4O_7$: C 73.66, H 7.06, N 12.27; found: C 73.72, H 7.02, N 12.30.

2.3. Synthesis of TSRh6G-β-CD fluorophore moiety

The reaction vessel was wrapped with aluminum foil to ensure the reaction took place in the dark. Under the protection of nitrogen, Rh6G-NH_3 (0.05g, 0.10 mmol) was dissolved in 15 mL of anhydrous THF, the solution was cooled down to -15 °C, triethylamine (0.7 mL) was added to it, then CS_2 (0.95g, 12.4mmol) was added dropwise to the above solution, and the mixture was stirred for 24 h. KOH solution was used to absorb any gas resulting from the reaction. Afterward, TSO-Cl (0.25g, 1.4

mmol) was added to the solution; the reaction mixture was gradually heated to room temperature and kept at this temperature for 1 h. The reaction mixture was filtered, solvent was evaporated under vacuum, and the obtained powder was purified by silica gel column chromatography (petroleum ether : ethyl acetate was 3:1) to give the light-yellow crystalline Rh6G-NCS. Then Rh6G-NCS was dissolved in 10 mL of anhydrous DMF, β-CD-NH_3 (0.359 mmol) was added to the solution, and the solution was kept at room temperature for 24 h, afterward, acetone was added to the solution, forming a precipitate. Upon filtration, the precipitate was recrystallized from an acetone-water mixture. The white crystal product was obtained. Yield: 62 %. ^{1}H NMR (400 MHz, DMSO-d_6, δ): 7.77 (t, J = 5.1 Hz, 1H; Ar H), 7.49 (t, J = 8.2 Hz, 1H; Ar H), 7.10 – 6.82 (m, 2H; Ar H), 6.25 (s, 2H; Ar H), 6.08 (s, 2H; Ar H), 5.88 – 5.50 (m, 14H; CH_2), 5.05 (s, 7H; CH_2), 4.82 (s, 16H; CH_2, NH), 4.41 (d, J = 24.6 Hz, 1H; CH), 3.86 – 3.48 (m, 7H; CH), 3.36 (d, J = 11.1 Hz, 11H; CH), 3.17 – 2.87 (m, 11H; CH_2), 2.86 – 2.63 (m, 2H; CH_2), 2.50 (s, 6H; CH_2), 2.12 (d, J = 19.9 Hz, 17H; OH, NH), 1.05 (t, J = 7.0 Hz, 6H; CH_3); IR (KBr): ν = 3350 (s; ν_s(OH)), 2972 (m; ν_{as}(CH_2, ν_{as}(C-N)), 2924 (m; ν_{as}(CH_2, β-CD)), 1672 (m), 1616 (s), 1551 (s; ν_s(C=O)), 1518 (m), 1398 (w; ρ(CH_2, β-CD)), 1266 (w), 1148 (m; ν_s(CO)), 1117 (m), 1078 (w), 1029 (s), 818 (w), 778 (w), 702 (w; ω(CH)), 665 (m; ν_s(CS)), 577 cm^{-1} (w); HRMS (ESI, m/z): $[M + H]^+$ calcd for $C_{73}H_{106}N_6O_{36}S$, 1675.7066; found, 1674.9526. Anal. Calcd for $C_{45}H_{28}N_4O_7$: C 52.32, H 6.38, N 5.02; found: C 52.39, H 6.40, N 4.84.

2.4. Synthesis of oleic acid modified iron oxide nanoparticles

Iron oxide magnetic nanoparticles (Fe_3O_4) were prepared through an improved chemical coprecipitation method [W. Stöber, A. Fink, *J. Colloid Interface Sci*. 1968, **26**, 62-69]. Briefly, a solution of a mixture of $FeCl_3$ (1.817 g) and $FeCl_2{\cdot}4H_2O$ (1.113 g) together with 150 mL of water was prepared with agitation under N_2 protection in a three-necked flask of 250 mL. Then 15 mL of NH_3 aqueous solution (25 wt %) was added dropwise slowly to the flask. After reacting at 50 °C for 30 min under mechanical stirring and N_2 protection, the Fe_3O_4 solid precipitations were magnetically separated, washed with water for dozens of times. In order to get better dispersibility, the obtained Fe_3O_4 magnetic nanoparticles were modified by oleic acid. In brief, 0.5 g of Fe_3O_4 nanoparticles was diluted in 100 mL of 0.5 M oleic acid. After ultrasonic treatment at 90 °C for 4 h under mechanical stirring, the oleic acid modified Fe_3O_4 nanoparticles were obtained. The as-prepared sample was washed with water for several times and dried at 40 °C for 48 h.

2.5. Synthesis of Fe_3O_4@SiO_2

The magnetic silica microsphere was prepared by our previous report [L. Sun, Y. Zang, M. Su, H. Wang, X. Zhu, S. Xu, Q. Yang, Y. Li, Y. Shan, *J. Colloid Interface Sci*. 2010, **350**, 90-98]. Briefly, 7.5 mg of oleic acid stabilized magnetite nanoparticles dispersed in 1.5 mL of chloroform was added to a 5 mL of aqueous solution containing 0.1 g of CTAB. After vigorous stirring of the resulting solution, a homogeneous oil-in-water microemulsion was obtained. Heating at 60 °C for 10 min induced evaporation of the chloroform, which generated aqueous-phase dispersed nanoparticles. 0.5 mL of CTAB-stabilized magnetite nanoparticles aqueous solution were added into

mixed water (5 mL)-ethanol (5 mL) solvent. Then 0.3 mL of ammonium hydroxide was added to the solution, followed by slowly addition of 50 μL of TEOS. The resulting mixture was stirred for 1 min, and then aged for 4 h. The silica nanocomposites were collected by centrifugation and washed with water and ethanol for three times. The exchange of surfactant molecules was carried out using an alcoholic solution of ammonium nitrate. The microspheres (1.00 g) were dispersed in ethanol (150 mL, 95%) containing NH_4NO_3 (0.30 g), and the mixture was stirred at 60 °C for 15 min. Solids were recovered by filtration and washed with cold ethanol, and the above treatment could be repeated twice.

2.6. Preparation of Fe_3O_4@SiO_2-Ad

0.1 g of Fe_3O_4@SiO_2 nanoparticles, 0.5 mL of Ad-Silane, and 25 ml of dry toluene were placed in a dried 50 mL flask. After evacuating and filling with N_2 for 3 times, the flask was immersed into an oil bath at 110 °C under N_2 atmosphere with stirring for 24 h. The solution was cooled down to room temperature. The functionalized Fe_3O_4@SiO_2 nanoparticles were collected by centrifugation and repeatedly washed with anhydrous toluene and then ethanol under ultrasonic condition and collected using magnet. The product was dried under vacuum overnight.

2.7. Preparation of TFIC MNPs

TSRh6G-β-CD (0.35 g, 2×10^{-4} $molL^{-1}$) in 50 mL of deionized water was stirred at 50℃ until the solution was clear. Then, the solution of the Fe_3O_4@SiO_2-Ad nanoparticles (0.1 g) in 50 mL of THF was added slowly. The solution was stirred under room temperature in N_2 for 48 h. Then THF and water were evaporated under

reduced pressure, and the magnetic nanoparticles inclusion complex was obtained. The resulting brown inclusion complex nanoparticles was washed and collected using magnet with ethanol (2 × 15 mL) under ultrasonic condition twice then with deionized water (2 × 20 mL) at 50 °C. After vacuum desiccation, the TSRhB-β-CD/Fe_3O_4@SiO_2-Ad inclusion complex nanoparticles (TFIC MNPs) were obtained.

2.8. Preparation of the fluormetric metal ion titration solution

Stock solution (1×10^{-3} mol L^{-1}) of the aqueous (CH_3CN-H_2O=1:10, v/v) salts of K^+, Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Fe^{3+}, Cu^{2+}, Co^{2+}, Ni^{2+}, Zn^{2+}, Cd^{2+}, Ag^+, Pb^{2+} and Hg^{2+} were prepared. The suspension solution of TFIC MNPs (0.5 g L^{-1}) was prepared in aqueous solution (CH_3CN-H_2O=1:10, v/v). Each time, a 0.3 mL suspension solution of TFIC nanoparticles was added to a quartz cuvette of 1 cm optical path length, and different stock solutions of cations were gradually added into the quartz cuvette by micro-syringe addition.

2.9. The amount of TSRh6G-β-CD fluorophore moieties grafted on the TFIC MNPs surfaces.

From spectral results of the inclusion complex nanoparticles, it is concluded that the amount of TSRh6G-β-CD fluorophore moieties grafted on the Fe_3O_4@SiO_2 MNPs can be calculated as 50.24 mg g^{-1}:

M_{TFIC} = 0.5 g/L

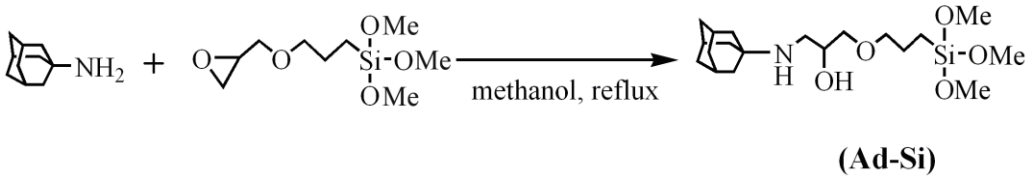
The Job plot using the fluorescence changes indicated 1 : 1 binding for TSRh6G-β-CD with Hg^{2+} (Fig. S4). Therefore:

$C_{(TSRh6G\text{-}\beta\text{-}CD)} = C_{(Hg^{2+})} = 15 \times 10^{-6}$ mol L^{-1},

$M_{(TSRh6G\text{-}\beta\text{-}CD)} = C_{(TSRh6G\text{-}\beta\text{-}CD)} \times Mr_{(TSRh6G\text{-}\beta\text{-}CD)} = 15 \times 10^{-6}$ molL^{-1} × 1674.6226 g mol^{-1}

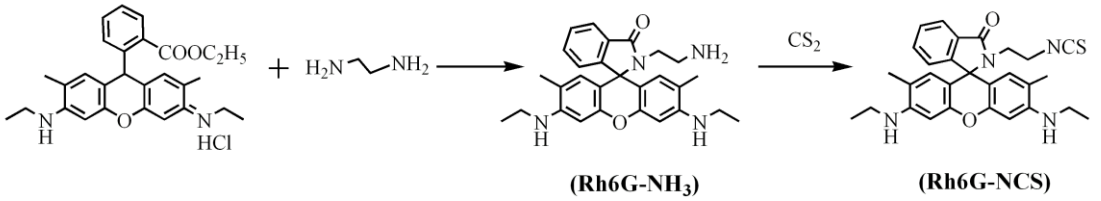
= 0.025119 g L^{-1}.

$m_{(TSRh6G\text{-}\beta\text{-}CD)} / m_{(TFIC)} = M_{(TSRh6G\text{-}\beta\text{-}CD)} / M_{(TFIC)}$ = 50.238 mg g^{-1}.

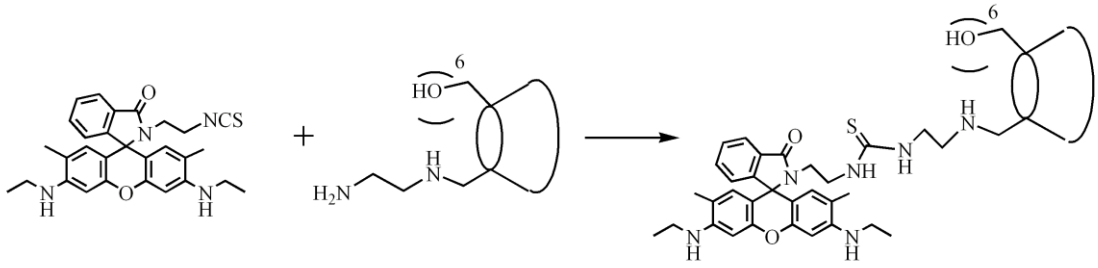


(Ad-Si)

Scheme S1. Synthetic route for Ad-Si.



(Rh6G-NH$_3$) **(Rh6G-NCS)**



(β-CD-NH$_2$) **(TSRh6G-β-CD)**

Scheme S2. Synthetic route of TSRh6G-β-CD.

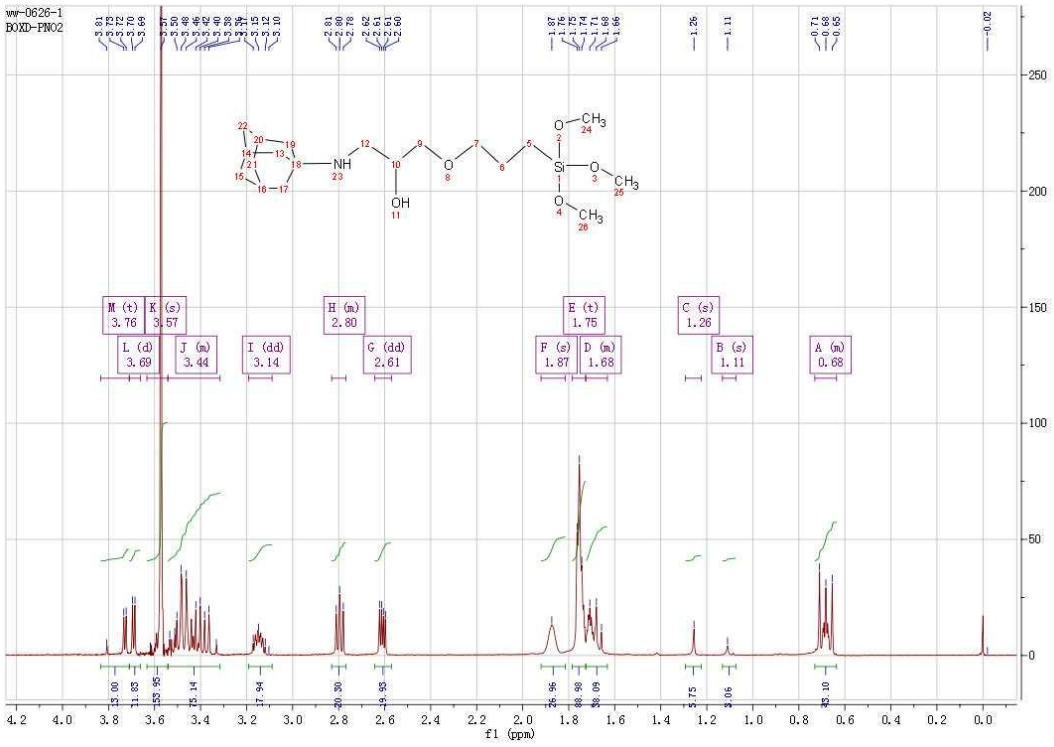


Fig. S1 ^{1}H NMR spectrum of Ad-Si in $CDCl_3$.

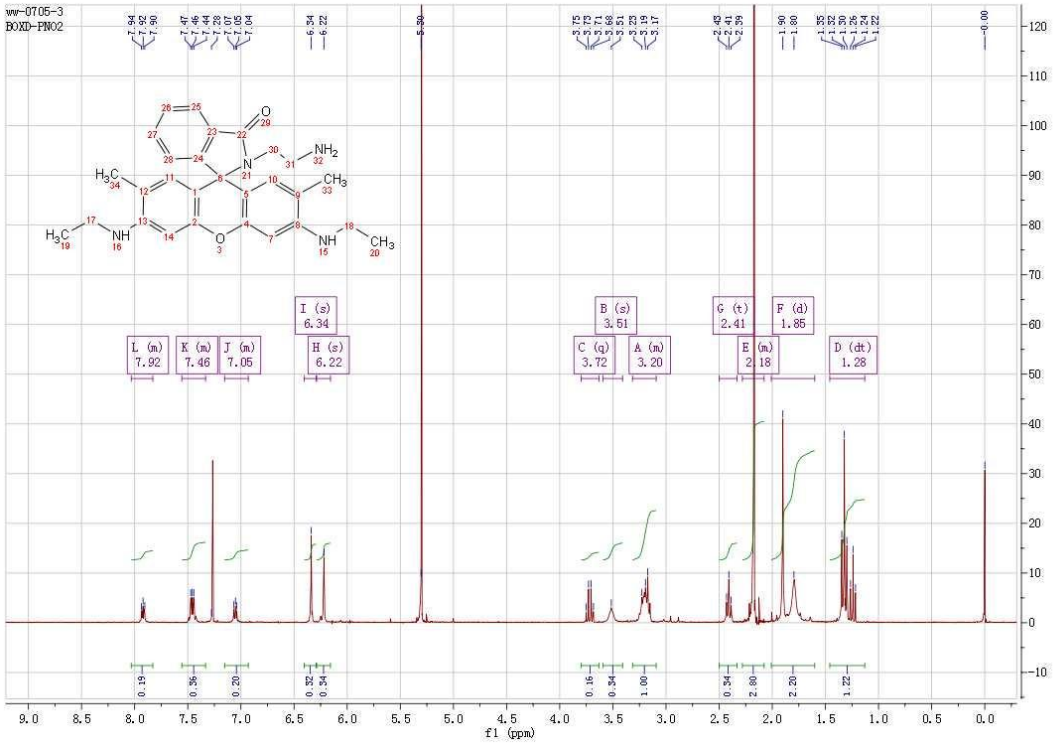


Fig. S2 ^{1}H NMR spectrum of Rh6G-NH_3 in $CDCl_3$.

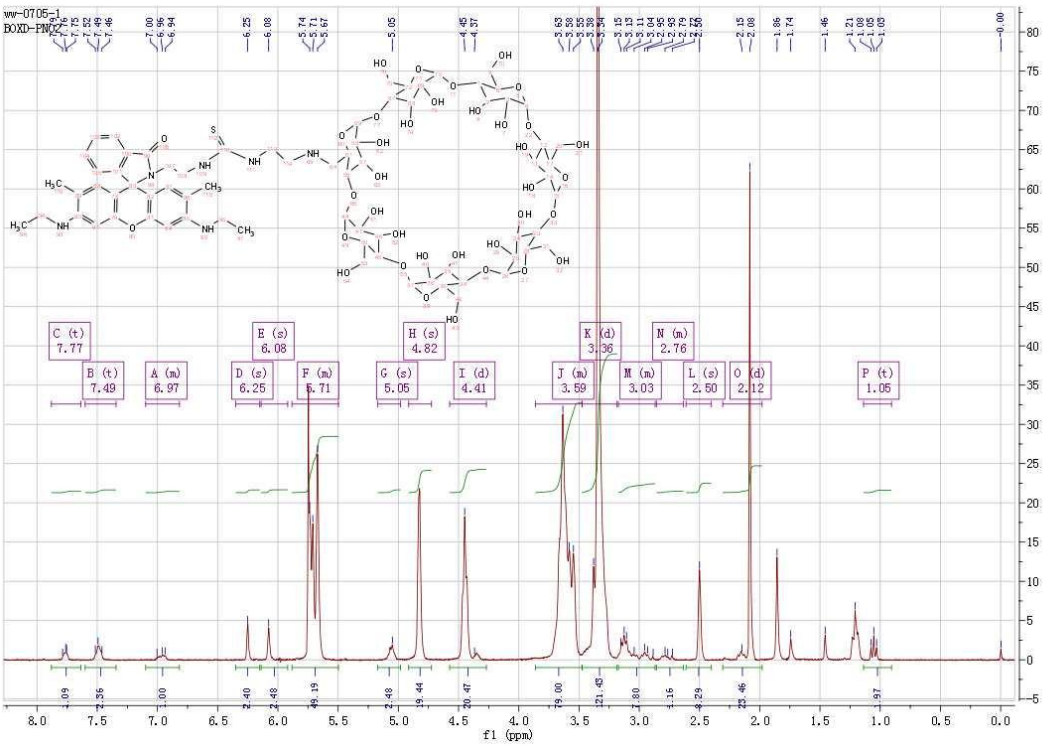


Fig. S3 ^{1}H NMR spectrum of Thiocarbamido-SRhB-β-CD in DMSO-d_6.

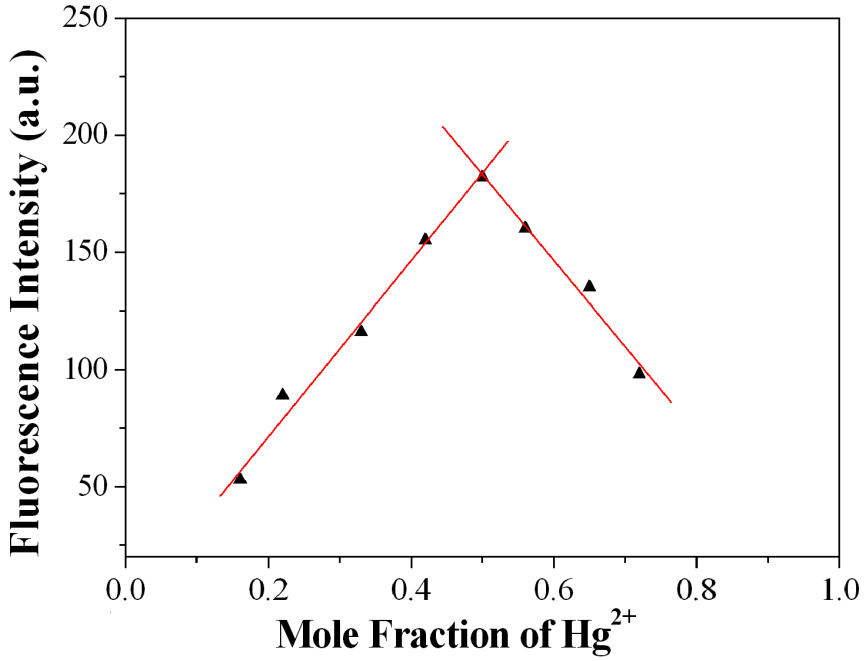


Fig. S4 Job Plot of TSRhB-β-CD and Hg^{2+} in CH_3CN-H_2O (1/10, v/v), conditions: [TSRhB-β-CD + Hg^{2+}] = 2.0×10^{-5} mol L^{-1}, Excitation was at 500 nm, emission was monitored at 553 nm.

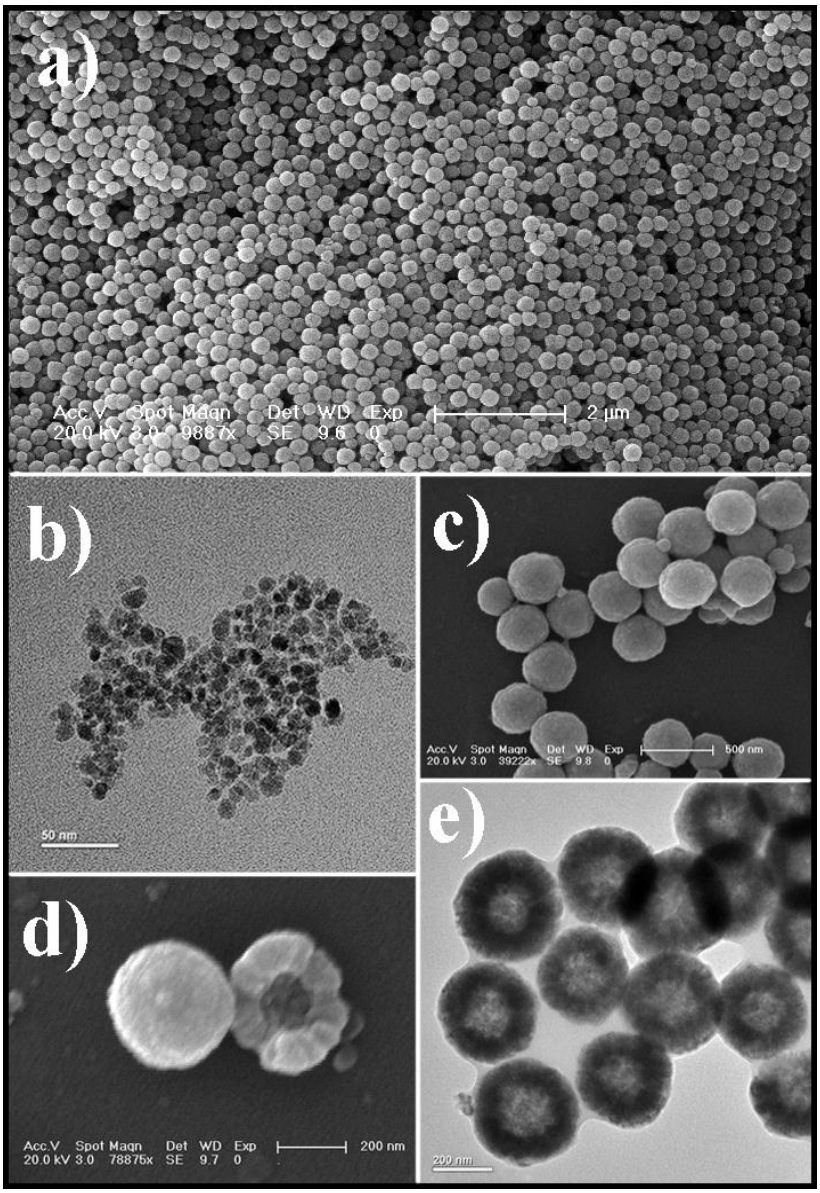


Fig. S5 The SEM images (a, c, d) of TFIC nanoparticles and TEM images of Fe_3O_4 nanoparticles b) and TFIC nanoparticles e).

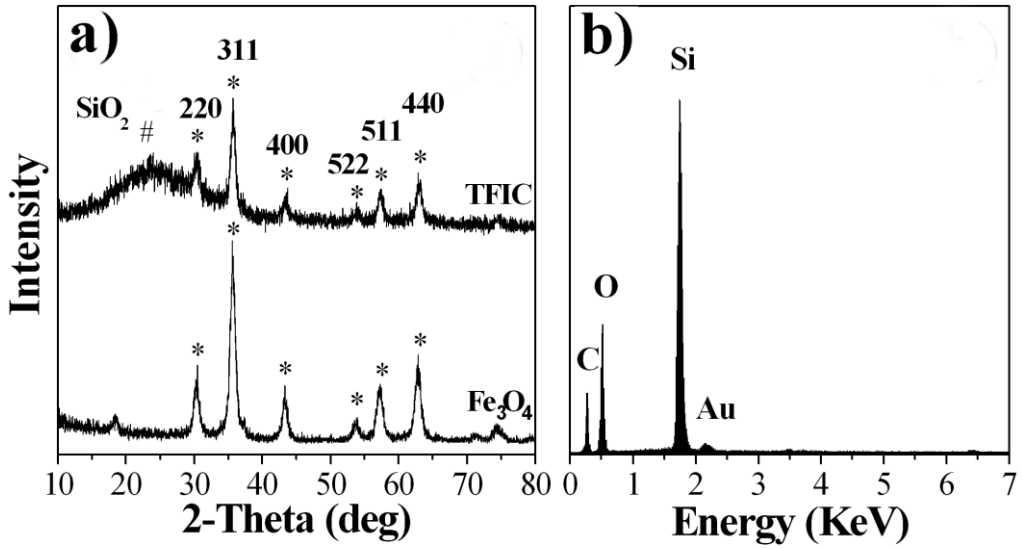


Fig. S6 a) The XRD patterns of Fe_3O_4 MNPs and the TFIC MNPs. b) The SEM-EDX spectrum of the TFIC MNPs.

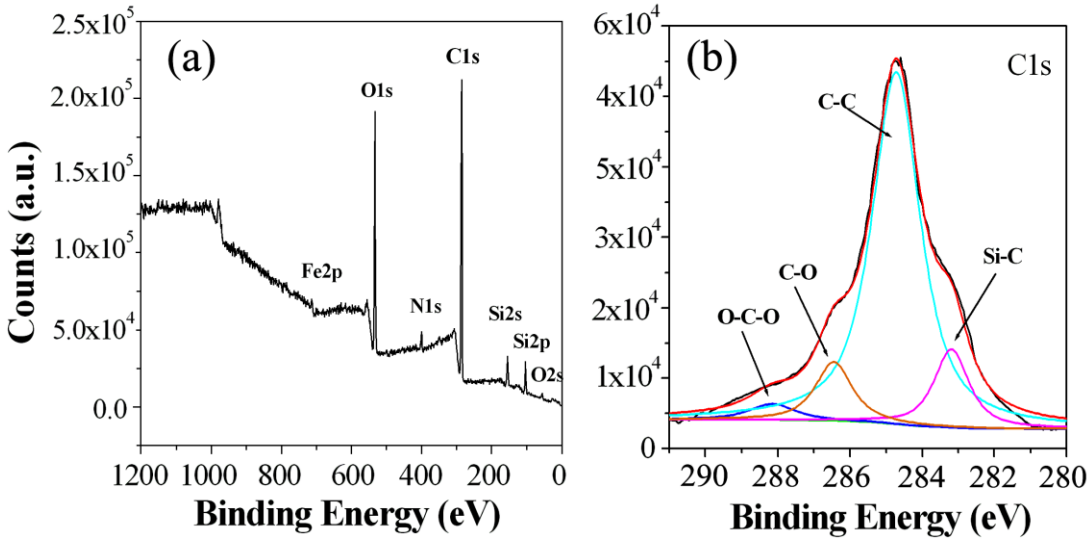


Fig. S7 XPS wide-scan spectra of the TFIC nanoparticles (a) and high resolution XPS spectrum of C1s (b). Colored lines represent the deconvolution curves.

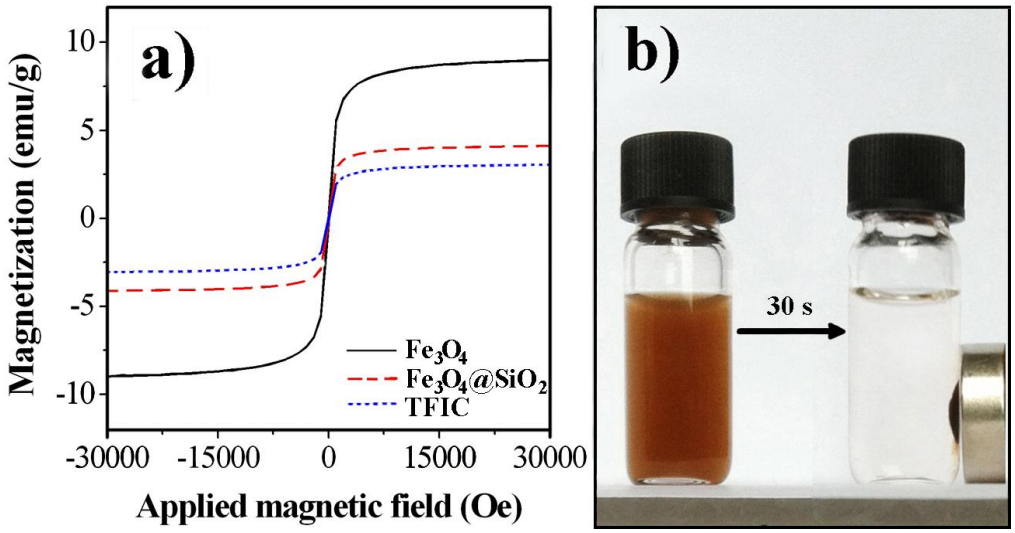


Fig. S8 a) The magnetic hysteresis loops of Fe_3O_4 MNPs (solid line), Fe_3O_4@SiO_2 MNPs (dashed line) and TFIC MNPs (dot line), b) Photographs of an aqueous suspension of TFIC MNPs (left) and after magnetic capture within 30 sec (right).

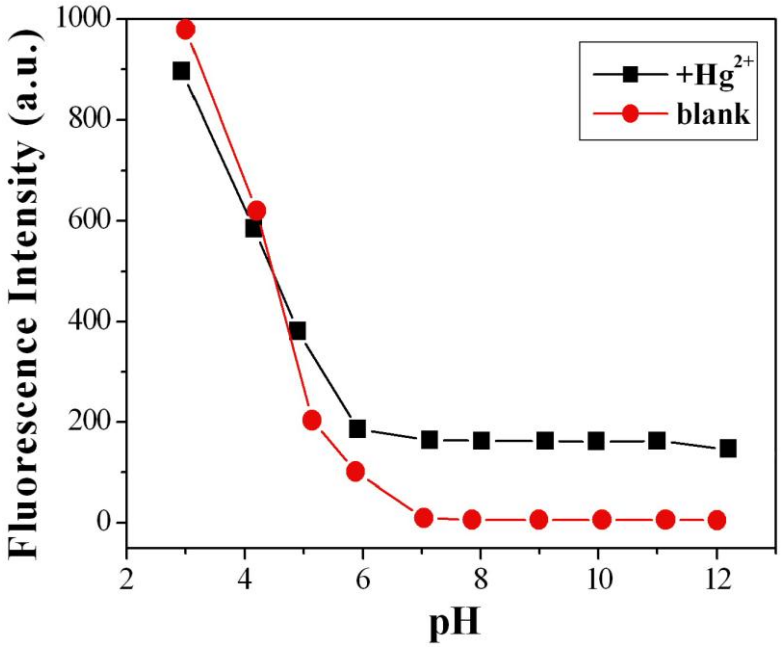


Fig. S9 Fluorescence intensity of TFIC MNPs in CH_3CN-H_2O (1/10, v/v) with and without Hg^{2+} ions measured as a function of pH. Excitation was at 500 nm, emission was monitored at 553 nm.

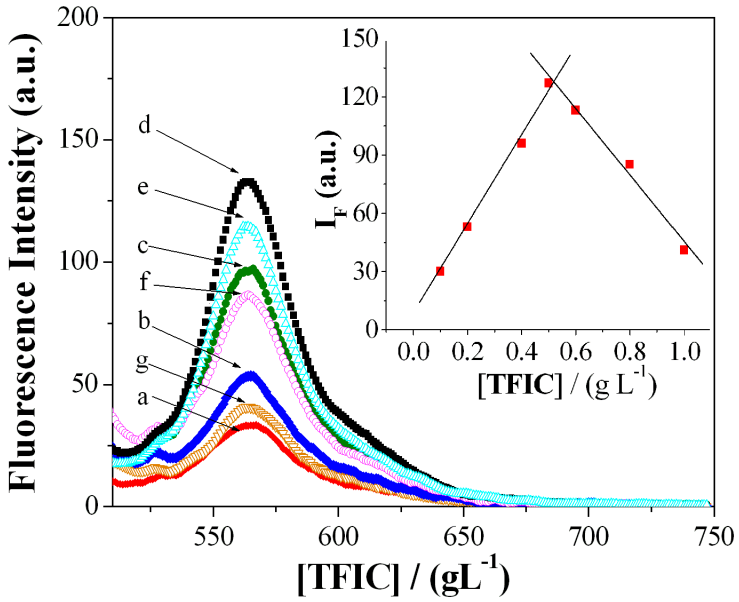


Fig. S10 Fluorescence spectra of different concentrations of the TFIC MNPs in Hg^{2+} solution (30 μM). From (a) to (g), the concentration is 0.1, 0.2, 0.4, 0.5, 0.6, 0.8 and 1.0 g L^{-1}, respectively.

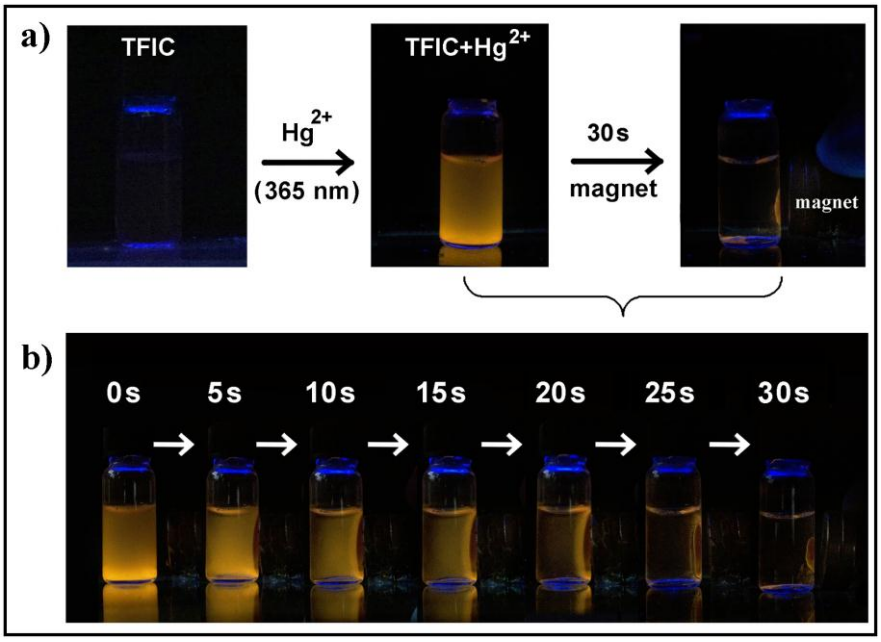


Fig. S11 a) Fluorescence photographs of TFIC-Hg^{2+} (20 μM) in the absence (left), presence (middle) and magnet attracting (right) in aqueous solution (0.1 mol L^{-1} HEPES buffer, pH 7.2). b) The separation–redispersion process of TFIC MNPs. Excitation at 365 nm using a UV lamp after 30s.

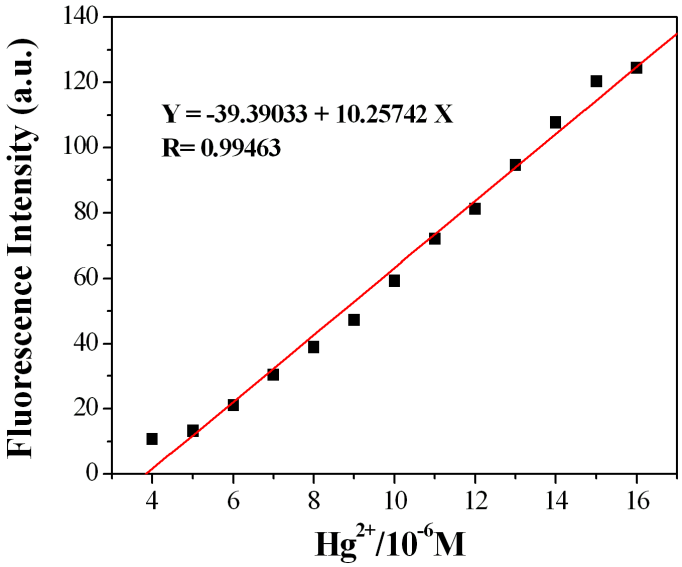


Fig. S12 Curve of fluorescence intensity of TFIC MNPs at 553 nm versus increasing concentration of Hg^{2+}.

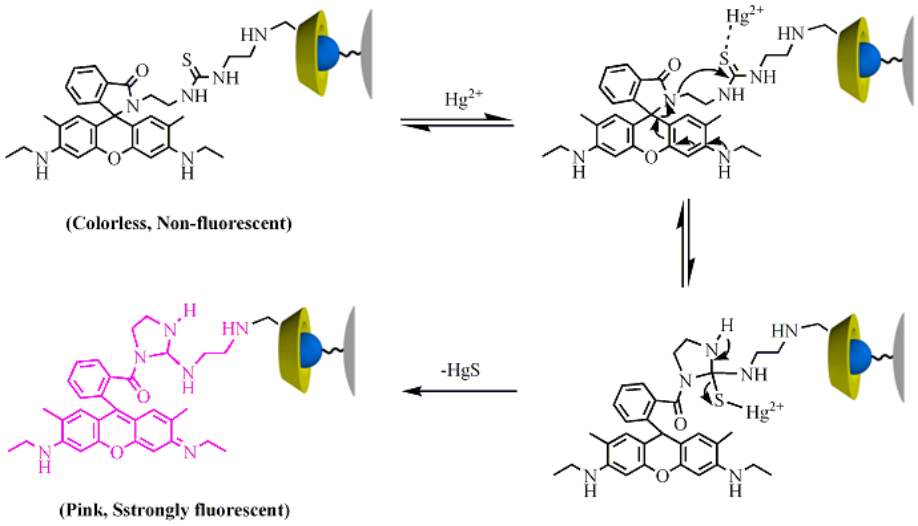


Fig. S13 The fluorescence/color change mechanism of TFIC NNPs in the presence of Hg^{2+}.

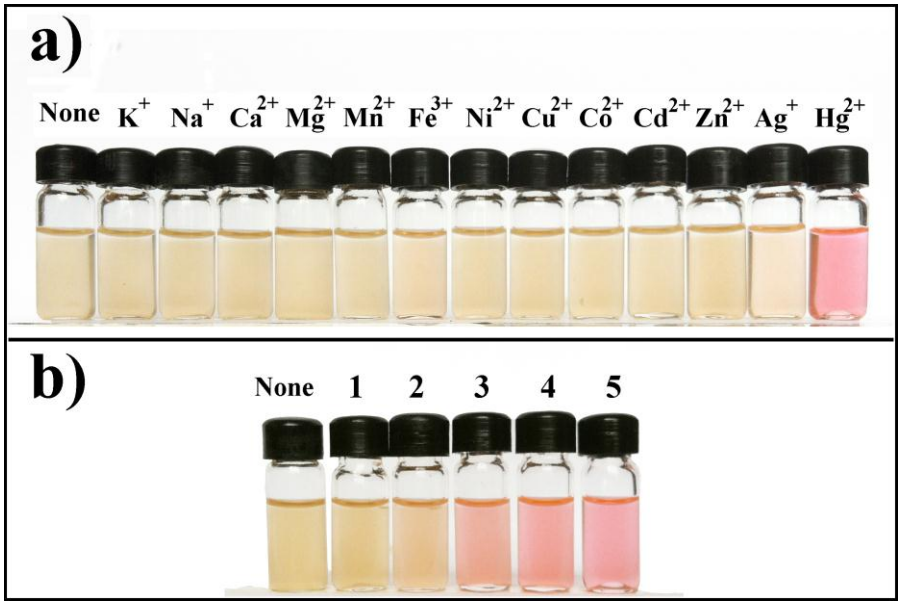


Fig. S14 a) Photograph of TFIC MNPs in the presence of various metal ions (1.0×10^{-4} M except Hg^{2+} that is 20×10^{-6} M) in CH_3CN-H_2O (1:10, v/v, pH=7.20) solution . b) From left to right was TFIC MNPs after 2.0×10^{-7} M, 2.0×10^{-6} M, 2.0×10^{-5} M, 2.0×10^{-4} M and 2.0×10^{-3} M Hg^{2+} involvement.

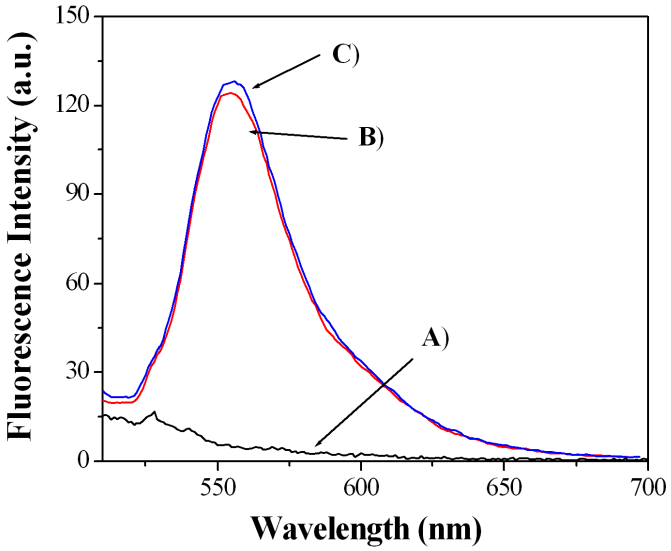


Fig. S15 A) Curve of fluorescence intensity of TFIC MNPs at 553 nm after soaking for 24 h. B) fluorescence intensity of TFIC MNPs upon exposure to aqueous solutions of Hg^{2+} (2.0×10^{-5} mol L^{-1} CH_3CN-H_2O = 1:10, v/v, pH=7.20) after soaking for 24 h. C) fluorescence intensity of TFIC MNPs in the presence of Hg^{2+} (2.0×10^{-5} mol L^{-1}) before soaking.