Electronic Supplementary Information (ESI)

for

Rhodamine-appended water-soluble conjugated polymer: an efficient ratiometric fluorescent platform for intracellular metal-ion probing

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1. Reagents and apparatus.

All chemicals were obtained from commercial suppliers and used without further purification. Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, USA). Solutions of Mg^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+} and Cr^{3+} were prepared from their chloride salts; solutions of Ba^{2+}, Fe^{3+}, Ag^{+}, Cu^{2+}, Zn^{2+} and Hg^{2+} were prepared from their nitrate salts; solutions of Ca^{2+}, Mn^{2+}, Yb^{3+} and Pb^{2+} were prepared from their acetate salts; solutions of Fe^{2+} was prepared from their sulfate salts. Thin layer chromatography (TLC) was carried out using silica gel GF254, and column chromatography was conducted over silica gel (300-400 mesh), both of them were obtained from Qingdao Ocean Chemicals (Qingdao, China). LC-MS analyses were performed using an Agilent 1100 HPLC/MSD spectrometer. NMR spectra were recorded on a Bruker DRX-400 spectrometer using TMS as an internal standard. All chemical shifts are reported in the standard δ notation of parts per million. The pH was measured with a Mettler-Toledo FE20 pH meter. All fluorescence measurements were carried out on a Hitachi-F4500 fluorescence spectrometer with both excitation and emission slits set at 10.0 nm. UV-Vis absorption spectra were recorded with a Shimadzu UV-2450 spectrophotometer. Fluorescence images of HeLa cells were obtained using an Olympus FV1000 laser confocal microscope (Japan).

2. Spectrophotometric experiments.

Both the fluorescence and UV-Vis absorption measurement experiments were conduct in buffered (Tris-HCl, pH 7.2) aqueous solution. The fluorescence emission spectra were recorded at excitation wavelength of 400 nm with emission wavelength range from 405 to 650 nm. A 2×10^{-4} M repeat units (RU) stock solution of **CP 1** was prepared by dissolving **CP 1** in buffered (Tris-HCl, pH 7.2) aqueous solution. A standard stock solution of Fe^{3+} (2×10^{-2} M) was prepared by dissolving an appropriate amount of $Fe(NO_3)_3 \cdot 9H_2O$ in water and adjusting the volume to 100 mL in a volumetric flask. It was

further diluted to 2×10^{-3} and 2×10^{-4} M stepwise. The complex solution of Fe^{3+}/ **CP 1** was prepared by adding 100 μL of the stock solution of **CP 1** and the stock solution of Fe^{3+} in a 2 mL volumetric flask. Then the mixture was diluted to 2 mL with buffer solution. In the solution thus obtained, the concentrations were 1×10^{-5} M RU for **CP 1** and 1×10^{-6}-5×10^{-4} M for Fe^{3+}. The solution was protected from light and kept at 4 °C for further use. Blank solution of **CP 1** was prepared under the same conditions without Fe^{3+}.

3. Detection of Fe^{3+} in river water samples.

The river water samples were obtained from Xiang River (Changsha, China), and were simply filtered and showed that no Fe^{3+} was present. The stock solution of Fe^{3+} at different concentration was first spiked in these samples, and the ratiometric probe **CP 1** was then used to detect its concentration.

4. Cell cultures and imaging experiments.

Immediately prior to the imaging experiments, the living Hela cells were washed with phosphate-buffered saline (PBS), incubated with 10 μM probe **CP 1** (in the culture medium) for 1 h at 37 °C and then washed with PBS for three times, and imaged. After incubating with 5×10^{-4} M $Fe(NO_3)_3$ for another 1 h at 37 °C, the Hela cells were rinsed with PBS three times, and imaged again. Confocal fluorescence imaging of intracellular Fe^{3+} in Hela cells was observed under an OLYMPUS FV1000 confocal microscope. Excitation wavelength of laser was 405 nm. Emissions were centered at 440±10 nm and 540±10 nm (double channel).

5. Synthetic details.

2, 5-dibromo-3-methylthiophene (**1**), 2, 5-dibromo-3-(bromomethyl)thiophene (**2**), N- (rhodamine B)lactam-ethylenedia-mine (**4**), and 2, 5-bis(octyloxy)benzene (**6**) were first prepared following the previously reported procedures.[S1-S3]

Synthesis of 6-((2, 5-dibromothiophen-3-yl)methoxy)-6-oxohexanoic acid (3).

Under nitrogen atmosphere, 2, 5-dibromo-3-(bromomethyl)thiophene (335 mg, 1.0 mmol) dissolved in 2 mL dry DMF were added drop-wise to a mixture of excess butanedioic acid (1.18 g, 10 mmol) and K_2CO_3 in 20 mL dry DMF. After the mixture had been stirred at room temperature for 12 h, the reaction mixture was filtered. The solvent was removed under vacuum, and the residue was extracted with dichloromethane. The extract was then washed with water and brine, dried over anhydrous Na_2SO_4, and then concentrated and purified by silica-gel column chromatography (silica gel; dichloromethane / MeOH as eluent) to afford **3** (200 mg, 53.76%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.96 (s, 1H), 5.02 (s, 2H), 2.73-2.65 (m, 4H). EI-MS [M+]: *m/z* 371.9.

Synthesis of (2, 5-dibromothiophen-3-yl)methyl 4-((2-(3', 6'-bis(ethylamino)-2', 7'-dimethyl-3-oxo-spiro[isoindoline-1, 9'-xanthen]-2-yl)ethyl)amino)-4-oxobutanoate (5).

Under nitrogen atmosphere, compound **3** (186 mg, 0.5 mmol) and compound **4** (235.4 mg, 0.5 mmol) were dissolved and stirred in 40 mL dry dichloromethane. EDCI (96 mg, 0.5 mmol) and 4-dimethyl-aminopyridine (DMAP) (20 mg, 0.2 mmol) was added to the reaction mixture at 0 °C. After stirring for 1 h, the solution was allowed to be warmed up to room temperature and further stirred for another 24 h. The solvent was removed under vacuum, then concentrated and purified by silica-gel column chromatography (silica gel; ethyl acetate / hexane as eluent) to afford compound **5** (234 mg, 56.7%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.93 (m, 1H), 7.54-7.46 (m, 2H), 7.14-7.05 (m, 1H), 6.71 (s, 1H), 6.34 (s, 1H), 6.21 (s, 1H), 4.96 (s, 2H), 3.28-3.25 (m, 2H), 3.23-3.18 (m, 4H), 3.03-2.96 (m, 2H), 2.64-2.61 (t, *J* =5.0 Hz, 2H), 2.40-2.36 (t, *J* =5.0 Hz, 2H), 1.90 (s, 6H), 1.30-1.26 (m, 6H). EI-MS [M+]: *m/z* 810.3.

Synthesis of 1, 4-bis((6-bromohexyl)oxy)-2, 5-diiodobenzene (7).

To a solution of compound **6** (0.327 g, 0.75 mmol) in acetic acid (5 mL) was added iodine (0.19 g, 0.75 mmol), potassium iodate (0.064 g, 0.3 mmol), concentrated sulphuric acid (0.1 mL) and distilled water (1 mL). The mixture was stirred at reflux for 3 h. After cooling to room temperature, the excess of iodine was destroyed using an aqueous sodium sulphite solution (10%, w/w), and the reaction mixture was poured into ice water (100 mL). The aqueous phase was extracted with ethyl acetate (2 × 30 mL), and the solvent of the combined organic layers was evaporated. Purification of the residue by means of column chromatography (Silica gel, dichloromethane / petroleum ether = 1:9 v/v as eluent) resulted in **7** (0.332 g, 64.3 %) as a colorless oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.17 (s, 2H), 3.95-3.92 (t, *J* =6.0 Hz, 4H), 3.46-3.42 (t, *J* =6.0 Hz, 4H), 1.93-1.90(m, 4H), 1.82(m, 4H), 1.55-1.52(m, 4H), 1.25 (m, 4H). EI-MS $[M+H]^+$: *m/z* 688.

Synthesis of 1, 4-bis((6-bromohexyl)oxy)-2, 5-diethynylbenzene (9).

To a solution of compound **7** (1.72 g, 2.5 mmol) in dry TEA (25 mL) was added $PdCl_2(PPh_3)_2$ (87.5 mg, 0.125 mmol), and CuI (47.5 mg, 0.25 mmol). The flask was placed under N_2, and then the solution was treated with (trimethylsilyl)acetylene (1.08 mL, 7.5 mmol), and stirred at 70 °C for 12 hours. After cooling to room temperature, the crude mixture was filtered at a room temperature to remove precipitate. The precipitate was rinsed with dichloromethane, and the combined filtrates were evaporated till dryness. The residue was chromatographed (ethyl acetate / petroleum ether = 1:30 v/v as eluent) to afford (2, 5-bis(octyloxy)-1, 4-phenylene)bis(ethyne-2, 1-diyl)bis(trimethylsilane) (**8**) (1.02 g, 65%) as golden oil, which was solidified slowly upon storing at room temperature. Subsequently, compound **8** was dissolved in dichloromethane (10 mL) and treated with K_2CO_3 (0.45 g, 3.25 mmol) in methanol (3.25 mL). The reaction mixture was refluxed in nitrogen for 24 h. The solution was

transferred into a dropping funnel, and water was added. The organic layer was extracted with diethyl ether, dried over Na_2SO_4, and evaporated till dryness. The residue was chromatographed (ethyl acetate/petroleum ether = 1:15 v/v as eluent) to afford pure **9** (0.54 g, 69%) as a yellow solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 6.95 (s, 2H), 4.00-3.97 (t, *J* =6.4 Hz, 4H), 3.44-3.41 (t, *J* =6.8 Hz, 4H), 3.43 (s, 2H), 1.92-1.88 (m, 4H), 1.84-1.80 (m, 4H), 1.55-1.50 (m, 8H). EI-MS [M+]: *m/z* 484.1.

Synthesis of poly(2-((2, 5-bis((6-bromohexyl)oxy)-4-ethynylphenyl)ethynyl)thiophen-3-yl)methyl 4-((2- (3', 6'-bis(ethylamino)-2', 7'-dimethyl-3-oxospiro[isoindoline-1, 9'-xanthen]-2yl)ethyl)-amino)-4-oxobutanoate (CP 2).

Compound **5** (82.4 mg 0.1 mmol), compound **9** (48.4 mg, 0.1 mmol), $Pd(PPh_3)_4$ (60.0 mg, 0.05 mmol), and CuI (9.6 mg, 0.05 mmol) were combined in dry and degassed triethylamine (8 mL) and toluene (20 mL). The mixture was heated at 60 °C for 48 h under a nitrogen atmosphere, and then cooled to room temperature and added dropwise to vigorously stirred petroleum ether. The precipitate was centrifuged and dissolved in 1 mL of toluene, and then precipitated into petroleum ether again. This procedure was repeated three times. The final product was dried thoroughly under vacuum to give polymer **CP2** as a darkred solid with 73% yield. ^{1}H NMR ($CDCl_3$, 400 MHz) δ (ppm): 7.93 (m, 1H), 7.72-7.69 (m, 2H), 7.68-7.65 (m, 2H), 7.56-7.52(m, 4H), 7.49-7.48(m, 1H), 6.34(s, 1H), 4.96 (s, 2H), 4.22 (m, 4H), 3.21 (m, 2H), 3.00 (m, 10H), 2.60 (m, 2H), 2.38(m, 2H), 1.58 (s, 6H), 1.32-1.25 (m, 22H). M_n: 2278 KDa. M_w/M_n = 1.17.

Synthesis of poly(2-((2, 5-bis((N, N, N-trimethylammonium)hexyloxy)-4-ethynyl phenyl)ethynyl) thiophen-3-yl)methyl 4-((2-(3', 6'-bis(ethylamino)-2', 7'-dimethyl-3-oxospiro[isoindoline-1, 9'-xanthen]-2-yl)ethyl)amino)-4-oxobutanoate (CP 1).

Condensed trimethylamine (2 mL) was added drop-wise to a solution of the **CP 2** (50 mg) in THF (10 mL) at -78 °C. After stirring for 12 h, the reaction temperature was allowed to warm up to room temperature and stirred for 24 h. The precipitate was redissolved by the addition of water (10 mL). The solvent was then removed. The residue was extracted with 20 mL of water, and water was then removed again. The residue precipitated from 5 mL of acetone to give darkred powders **CP 1** in a 82% yield. ^{1}H NMR (DMSO-d_6, 400 MHz) δ (ppm): 7.64-7.32(m, 10H), 6.66 (s, 1H), 5.32 (s, 2H), 4.40-4.38 (m, 4H), 3.46-3.44 (m, 8H), 3.34 (m, 18H), 2.51-2.50(s, 8H), 2.01 (s, 6H), 1.26-1.04 (m, 22H).

6. Calculation of energy transfer [S4]

The Förster radius (R_0) of energy transfer from a donor to an acceptor is the critical binding distance when the efficiency of energy transfer is 50%, which can be calculated by Eq. (1):

$$R_0^6 = 8.8 \times 10^{-25} (k^2 n^{-4} \Phi_D J) \quad (1)$$

where the k^2 is the spatial orientation factor of the dipole, n is the refractive index of medium, Φ_D is the quantum yield of the donor in the absence of acceptor, and J is the overlap integral of the emission spectrum of the donor and the absorption spectrum of the acceptor. In the present case, k^2, n and Φ_D are 2/3, 1.33 and 0.34. And then, the J can be calculated by Eq. (2):

$$J = \frac{\sum F(\lambda)\varepsilon(\lambda)\lambda^4 \Delta\lambda}{\sum F(\lambda)\Delta\lambda} \quad (2)$$

where the λ is the common wavelength of corresponding fluorescence spectrum of donor and absorption spectrum of acceptor. The $F(\lambda)$ and $\varepsilon(\lambda)$ are the fluorescence intensity of donor solution and the absorbance of acceptor solution.

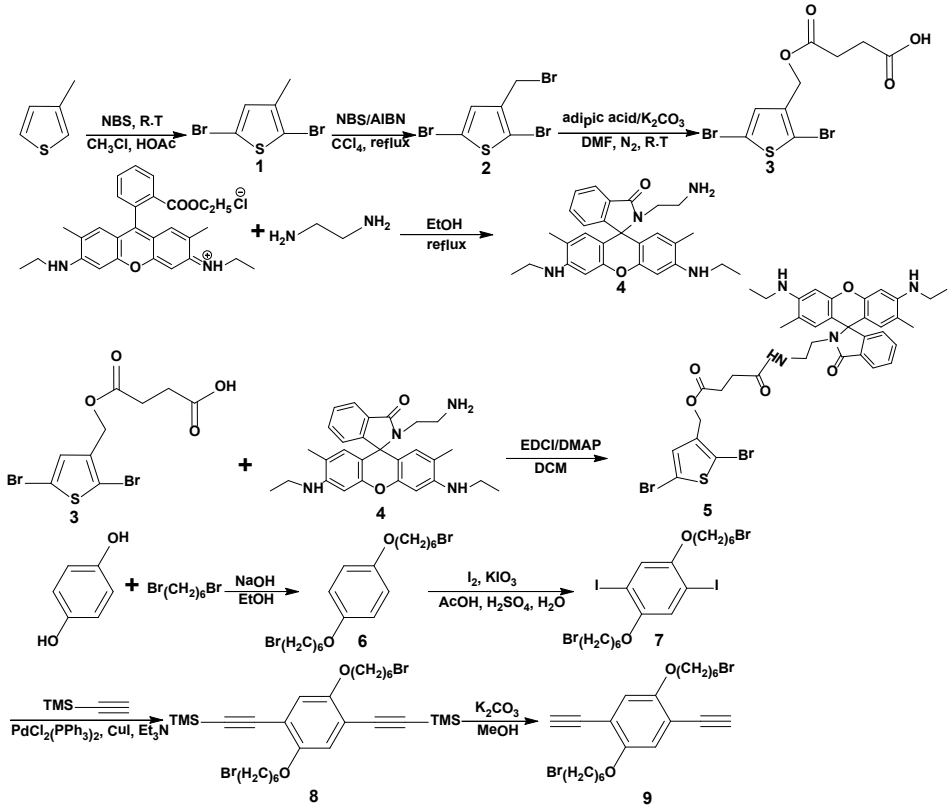
The energy transfer effect is related not only to the distance between the acceptor and donor, but also to the critical energy transfer distance (R_0). The efficiency (E) of energy transfer can be determined by Eq. (3):

$$E = 1 - \frac{F}{F_0} = \frac{R_0^6}{R_0^6 + r^6} \tag{3}$$

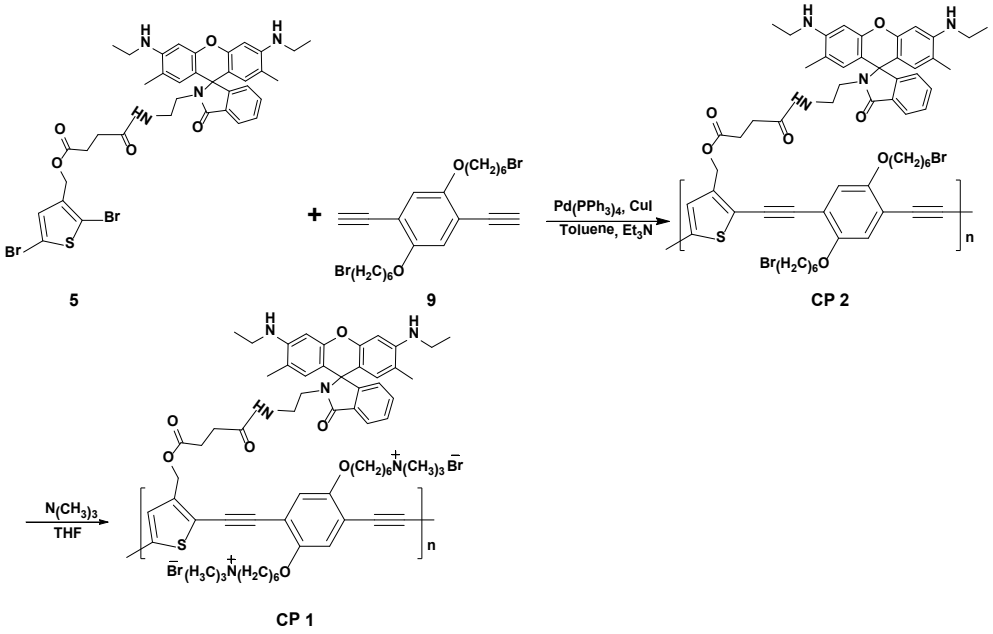
where F and F_0 are the fluorescence intensities of the donor in the presence and absence of an acceptor, r is the distance between acceptor and donor.

In our systems, acceptor is rhodamine 6G, and donor is CCPs backbones. According to equations, we calculated: $J = 1.13 \times 10^{-13}$ cm^3·L·mol^{-1}, $R_0 = 4.39$ nm, $E = 61.8\%$, $r = 4.06$ nm.

7. Supplementary figures and tables



Scheme S1 The synthetic routes of the monomers.



Scheme S2 The synthetic route of the polymer **CP1**.

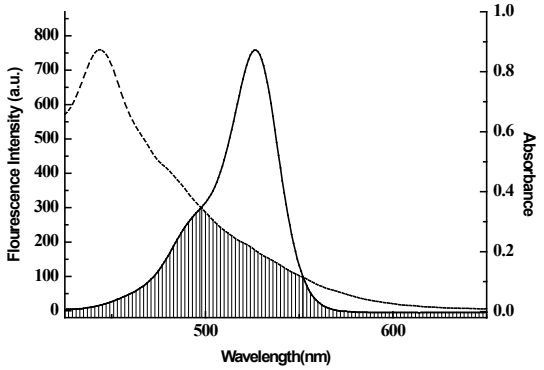


Fig. S1 Spectroscopic overlap between PPETE ([RU] = 10 μM) emission (λ_{ex}=400 nm, dotted line) and ring-opened rhodamine 6G (10 μM) absorption (solid line) in buffer solution (Tris-HCl, pH=7.2).

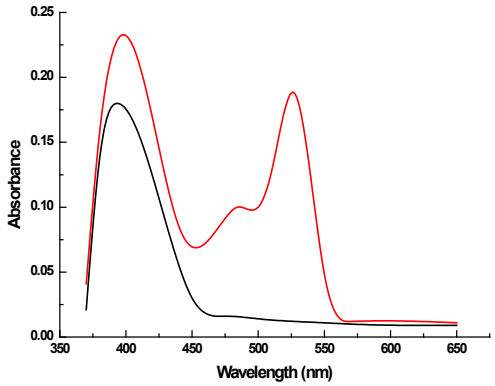


Fig. S2 Absorption spectra of **CP 1** ([RU] = 1×10^{-4} M) in the absence (black curve); and presence (red curve) of Fe^{3+} (5×10^{-3} M) in buffer solution (Tris-HCl, pH=7.2).

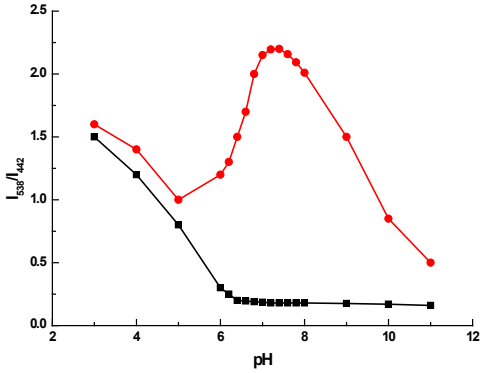


Fig. S3 Effect of pH on the fluorescence intensity at 538 nm to that at 442 nm of free **CP 1** ([RU] = 10 μM) (black line) and after addition of 2×10^{-4} M Fe^{3+} (red line). λ_{ex}=400 nm.

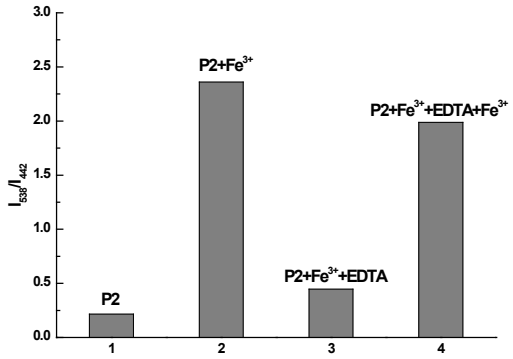


Fig. S4 Reversible investigation of **CP 1** ([RU] = 10 μM) for Fe^{3+} with addition of EDTA in buffer solution (Tris-HCl, pH=7.2). 1: [RU] = 10 μM **CP 1** only; 2: [RU] = 10 μM **CP 1** with 5×10^{-4} M Fe^{3+}; 3: [RU] = 10 μM **CP 1** with 5×10^{-4} M Fe^{3+} and then addition of 1 mM EDTA (Na salt); 4: [RU] = 10 μM **CP 1** with 5×10^{-4} M Fe^{3+} and then addition of 1 mM EDTA (Na salt); then addition of 2 mM Fe^{3+}. λ_{ex}=400 nm.

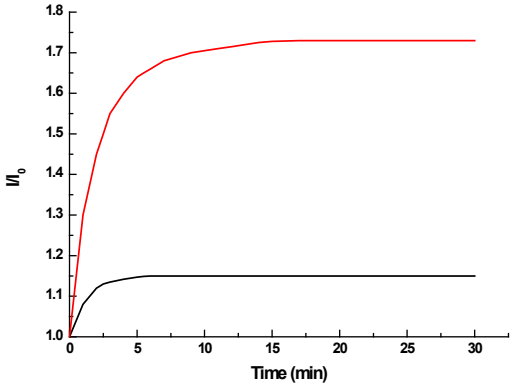


Fig. S5 Time-dependent fluorescence enhancement of **P2** ([RU] = 10 μM) upon addition of 8 μM (black line) and 20 μM (red line) Fe^{3+} in buffer solution (Tris-HCl, pH=7.2). Fluorescence intensity was recorded at 538 nm. λ_{ex}=400 nm.

Table S1 Recovery study of spiked Fe^{3+} in river waters with proposed sensing system

Sample	Fe^{3+} spiked ($\times 10^{-5}$ M)	Fe^{3+} recovered ($\times 10^{-5}$ M) mean[a] ± SD[b]	Recovery (%)
River water 1	2	1.97 ± 0.03	98.5
River water 2	10	10.27 ± 0.17	102.7
River water 3	20	20.26 ± 0.15	101.3

[a]Mean of three determinations. [b] SD: standard deviation.

8. References

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S2. S. L. Hsu, C. M. Chen, Y. H. Cheng and K. H. Wei, *Journal of Polymer Science: Part A: Polymer Chemistry*, 2011, **49**, 603.

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