

Rhodamine B Derivative-Functionalized Upconversion Nanoparticles for FRET-Based Fe³⁺-Sensing

Supporting Information

Experimental Section

Chemicals

All starting materials were obtained from commercial suppliers and used as received. YCl₃·6H₂O (99.99%), YbCl₃·6H₂O (99.99%), HoCl₃·6H₂O (99.9%) , KOH (98%), NH₄F (98%), 1-octadecene (90%), OA (90%) and CD were purchased from Sigma-Aldrich. RBD was prepared according to the literature.^{1, 2} The as-prepared RBD was characterized by mass spectrum (MS) (Fig. S7) and nuclear magnetic resonance proton spectrum (¹H NMR) (Fig. S8). Deionized water was used throughout. All other chemical reagents in analytical grade were used directly without further purification.

Synthesis of OA-UCNPs

The synthesis of NaYF₄: 20% Yb, 2% Ho nanoparticles was developed via a modified literature procedure.³ In a typical experiment, 1 mmol RECl₃ (0.78 mmol YCl₃, 0.2 mmol YbCl₃, 0.02 mmol HoCl₃) were added to a 50 mL flask containing 7.5 mL OA and 17.5 mL 1-octadecene and the solution was heated to 160 °C for 30 min and then cooled down to room temperature. Thereafter, 10 mL methanol solutions of NH₄F (4.0 mmol) and KOH (2.5 mmol) were added into the solution and stirred for

30 min. After methanol evaporated, the solution was heated to 300 °C under argon for 1 h and cooled down to room temperature. The resulting nanoparticles were precipitated by the addition of ethanol, collected by centrifugation, washed with water and ethanol several times, and finally re-dispersed in cyclohexane.

Conversion of Hydrophobic OA-UCNPs into Hydrophilic CD-UCNPs

12 mL ethanol-water (2: 1, v/v) solution containing 60 mg OA-UCNPs was mixed with equal volume of CD aqueous solution (20 mg. mL⁻¹). The mixture was stirred vigorously under room temperature, resulting in a transparent and clear solution. After stirring for 20 h, the solution was collected and centrifuged (15000 rpm, 10 min). Then, the product was washed alternately with deionized water and ethanol for three times. The sample could be re-dispersed in deionized water or other polar solvents.

Loading RBD into CD-UCNPs to obtain RBD-UCNPs assembly materials

20 µL dichloromethane solution of RBD (15 mg) was mixed with 15 mL aqueous solution of as-prepared CD-UCNPs (60 mg)”. The mixture was stirred for 5 h at room temperature to obtain a homogeneous phase. Free RBD was removed by centrifugation at 15 000 rpm for 10 min. The precipitate was washed three times with deionized water by centrifugation. The as-prepared hybrids (RBD-UCNPs) were re-suspended in pure water to form a homogeneous clear solution and stored at 4°C.

Preparation of Clinical Samples

Serum specimens from healthy persons and clinically diagnosed anaemic as well as leukemic patients were provided by the Affiliated Drum Tower Hospital of Nanjing University. 1 mL serum diluted with 2 mL deionized water was heated to 100 °C and

gradually added with 5 mL HNO₃ and 2 mL H₂O₂, thereafter, the solution evaporated to achieve the concentrate for determination.

Characterizations

The morphology and structure were characterized by TEM and HRTEM images using a JEOL-2100 TEM equipped with an Oxford Instrument EDX system operating at 200 kV. XRD measurements were performed on a Japan Shimadzu XRD-6000 diffractometer with Cu-K α radiation ($\lambda = 0.15418$ nm); A scanning rate of 0.05 deg s⁻¹ was applied to record the patterns in the 2 θ range of 10-80°. FT-IR spectra were recorded on a Nicolet 6700 Fourier transform infrared spectrograph in the wavenumber range of 4000-500 cm⁻¹. The XPS spectrum was measured on an ECSALAB 250. Elemental analyses were performed on an EA 1110 CHNS-O CE instrument. UV-Vis absorption spectra were measured using a Shimadzu UV-2550 ultraviolet-visible-near infrared spectrometer. Upconversion fluorescence spectra were recorded on a ZolixScan ZLX-UPL spectrometer using an external 1 W continuous-wave laser (980 nm) as the excitation source. A PU9100X AAS (Philips, Eindhoven, the Netherlands) was utilized to determine serum iron. MS was obtained on a HP 1100 LC-MS spectrometer. ¹H NMR was recorded on a Varian Mercury 400 NMR spectrometer.

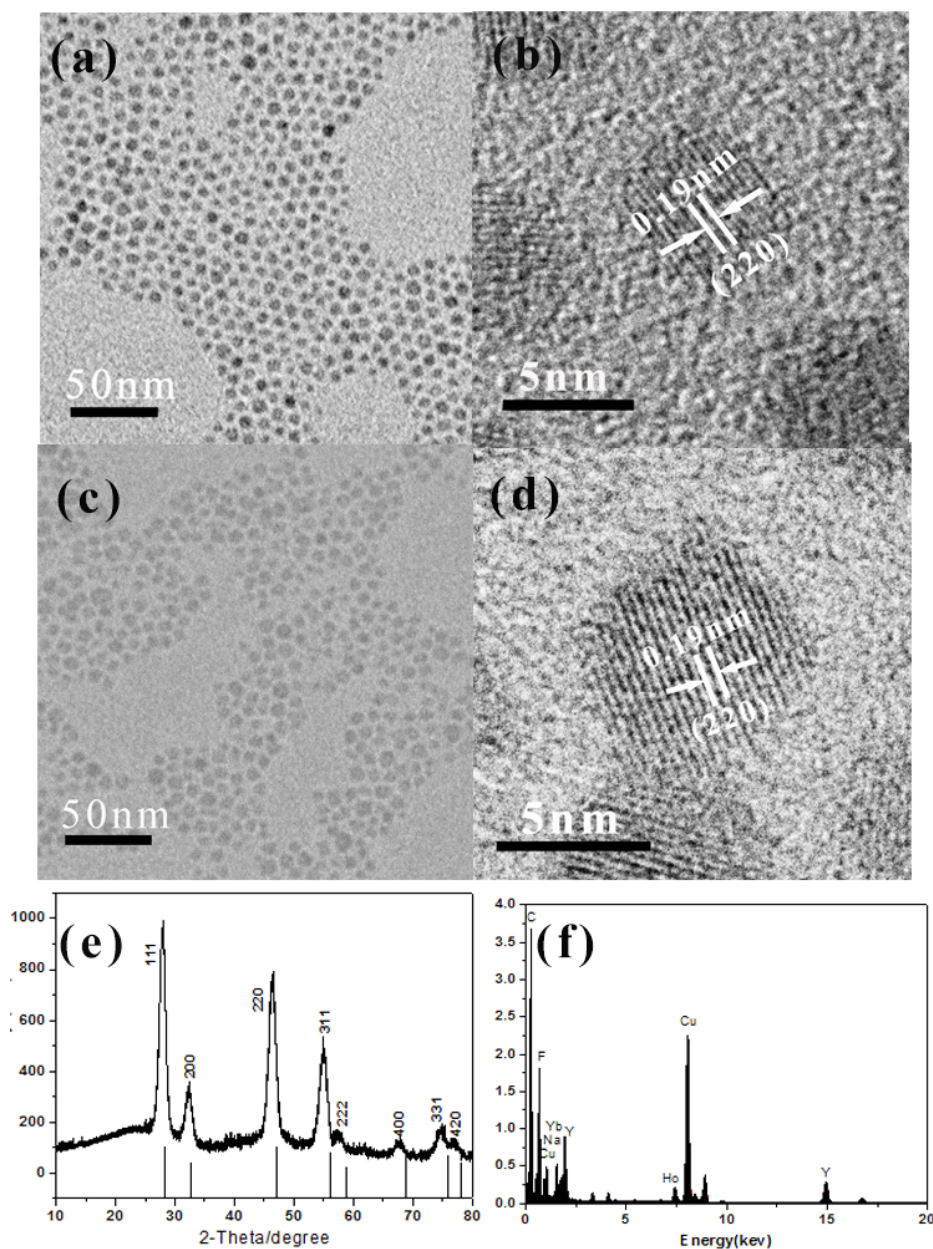


Fig. S1 TEM and HRTEM images of OA-UCNPs dispersed in cyclohexane (a and b, respectively) together with CD-UCNPs dispersed in water (c and d, respectively), XRD patterns of the as-synthesized OA-UCNPs (straight lines show the standard pattern of pure cubic NaYF₄, JCPDS card No.06-0342) (e), the EDX of OA-UCNPs (f).

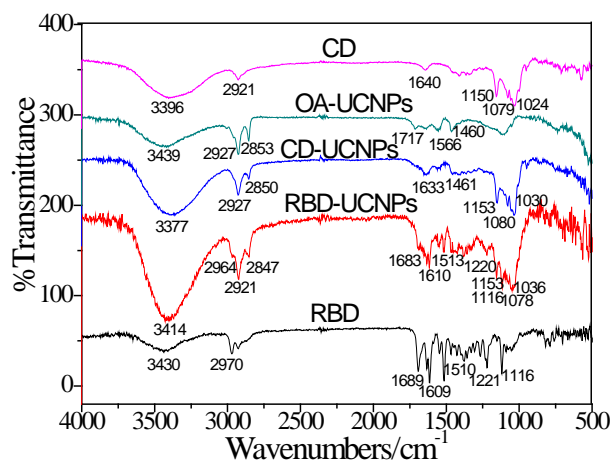


Fig. S2 FI-IR spectra of CD and the as-prepared OA-UCNPs, CD-UCNPs, RBD-UCNPs and RBD, respectively.

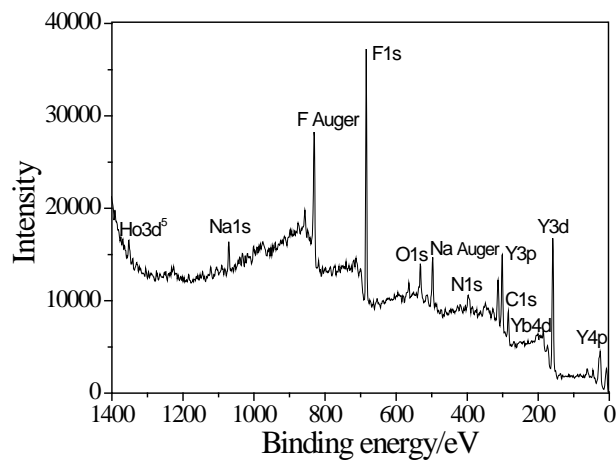


Fig. S3 XPS spectrum of RBD-UCNPs.

Table S1 The results of elemental analyses and the contents of the organic compounds in the corresponding materials

	C (wt%)	H (wt%)	N (wt%)	OA (wt%)	CD (wt%)	RBD (wt%)
OA-UCNPs	5.77	0.93	0	7.53	0	0
CD-UCNPs	13.34	1.98	0	6.06	19.58	0
RBD-UCNPs	16.54	2.25	0.50	5.75	18.56	5.15

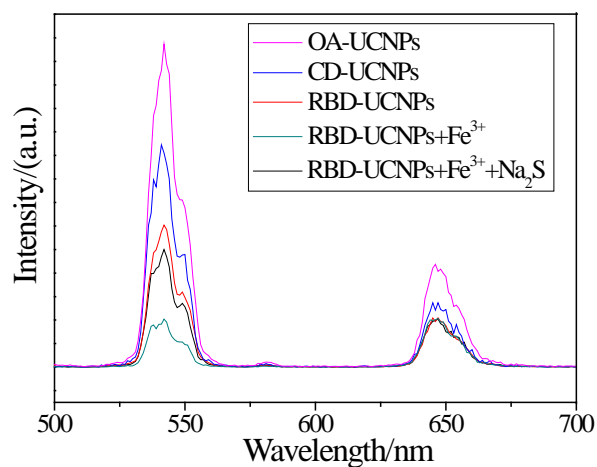


Fig. S4 UCL intensity of OA-UCNPs in cyclohexane solution (pink line) and CD-UCNPs (blue line), RBD-UCNPs (red line), RBD-UCNPs+Fe³⁺ (green line), RBD-UCNPs+Fe³⁺+Na₂S (black line) in pure water, respectively.

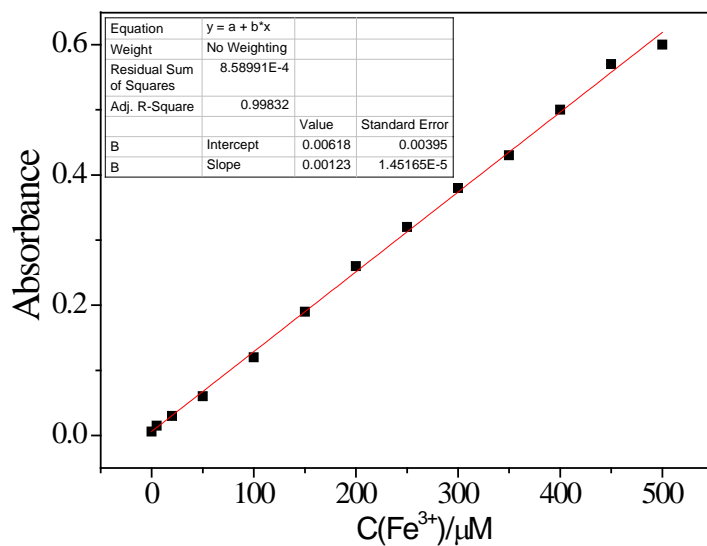


Fig. S5 Linear plot of the UV-Vis absorbance at 558 nm as a function of Fe³⁺ concentration. The detection limit (LOD) was given by the equation $LOD = 3S_0/S$; where 3 is the factor at the 99% confidence level, S_0 is the standard deviation of the blank measurements ($n = 8$), and S is the slope of the calibration curve. The LOD was

determined to be 0.8 μM of Fe^{3+} .

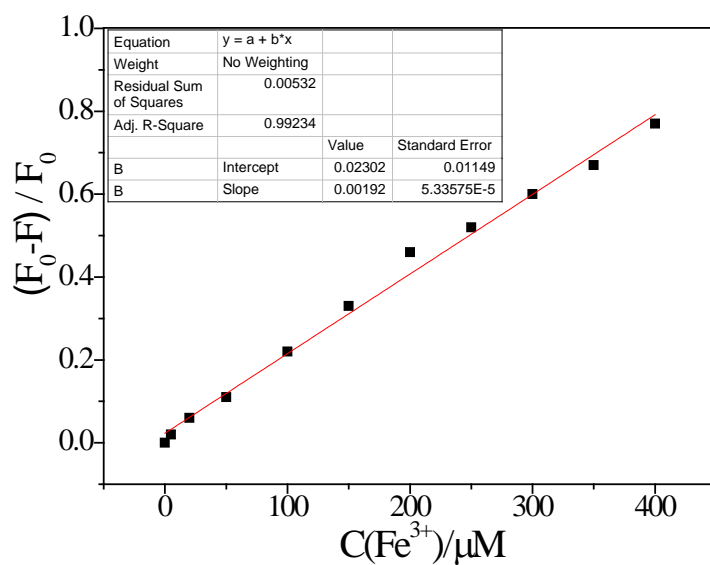


Fig. S6 Linear plot of relative UCL quenching as a function of Fe^{3+} concentration.

F_0 and F denote the relative fluorescence intensity $\text{UCL}_{520-570}/\text{UCL}_{628-675}$ (GRR)

before and after addition of Fe^{3+} , respectively.

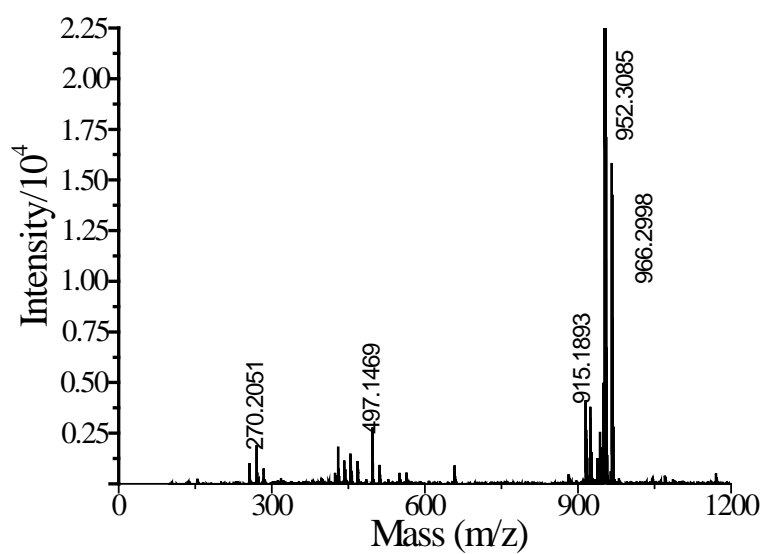


Fig. S7 MS of RBD in CDCl_3

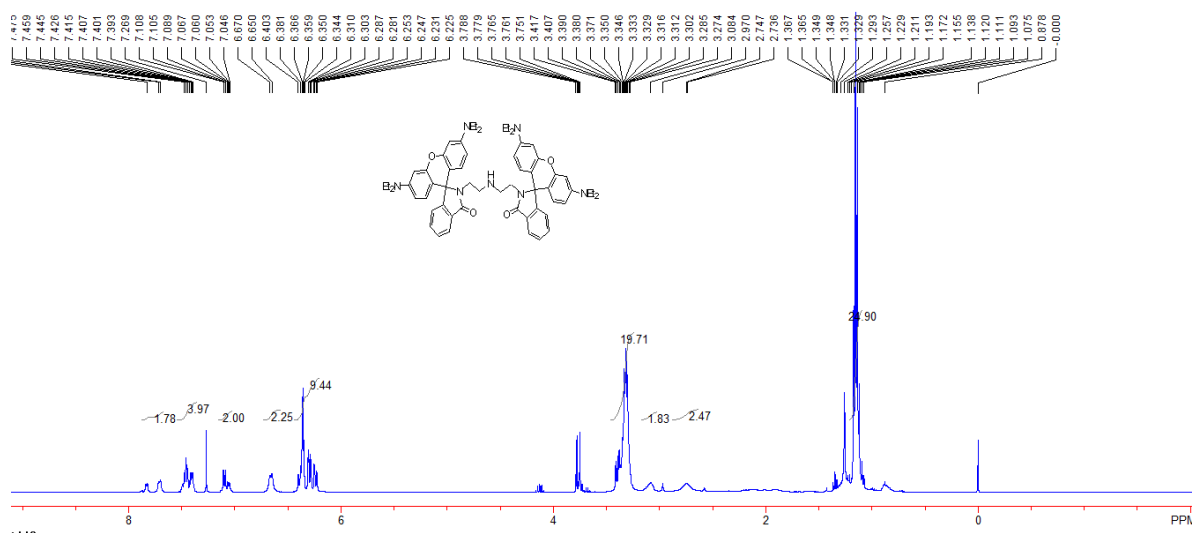


Fig. S8 ^1H NMR of RBD in CDCl_3

References

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2. J. Y. Kwon, Y. J. Jang, Y. J. Lee, K. M. Kim, M. S. Seo, W. Nam and J. Yoon, *J. Am. Chem. Soc.*, 2005, **127**, 10107.
3. F. Wang, Y. Han, C. S. Lim, Y. Lu, J. Wang, J. Xu, H. Chen, C. Zhang, M. Hong and X. Liu, *Nature*, 2010, **463**, 1061.