Supplementary Material (ESI) for Chemical Communications

Supporting information

FRET–based Sensor for Imaging Chromium(III) in Living Cells

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1. Materials and general methods

All the solvents were of analytic grade. The salts solutions of metal ions were $NaNO_3$, KNO_3, $Mg(ClO_4)_2$, $Ca(ClO_4)_2$, $CrCl_3 \cdot 6H_2O$, $MnCl_2$, $FeCl_2 \cdot 4H_2O$, $Co(NO_3)_2 \cdot 6H_2O$, $Ni(NO_3)_2 \cdot 6H_2O$, $CuSO_4$, $ZnSO_4 \cdot 7H_2O$, $CdCl_2 \cdot H_2O$, $AgNO_3$, $Hg(ClO_4)_2$, $Pb(NO_3)_2$. Water was re-distilled. 8-Hydroxylquinoline-2-aldehyde was purchased from Aldrich Inc. The other reagents were purchased from Shanghai Reagents Ltd. Rhodamine B hydrozide was synthesized according to the reference[1].

General instruments

^1H NMR and ^{13}C NMR spectra were recorded on a Varian Mercury Plus 400 spectrometer with tetramethylsilane as the internal standard. The element analysis was performed on a VarioEL III O-Element Analyzer system. Electrospray ionization mass spectra (ESI-MS) were measured on a Micromass LCTTM system. Melting points were determined on a hot-plate melting point apparatus XT4-100A and uncorrected. UV-Vis spectra were recorded on a Shimadzu UV-2250 spectrohotometer. Fluorescence spectra were recorded on Edinburgh FLS-920 spectrohotometer. All pH measurements were made with a Model PHS-2F meter.

Procedures of metal ion sensing

Stock solutions of the metal ions (2.5 mM) were prepared in deionized water. A stock solution of **FD8** (1 mM) was prepared in ethanol. The solution of **FD8** was then diluted to 20 μM with ethanol-water (2:1, v/v). In titration experiments, each time a 2.5 mL solution of **FD8** (20 μM) was filled in a quartz optical cell of 1 cm optical path length, and the Cr^{3+} stock solution was added into the quartz optical cell gradually by using a micro-pippet. Spectral data were recorded at 5 min after the addition. In selectivity experiments, the test samples were prepared by placing appropriate amounts of metal ion stock into 2.5 mL solution of **FD8** (20 μM). For fluorescence measurements, excitation was provided at 405 nm, and emission was collected from 425 to 750 nm.

The binding constant was calculated from the emission intensity - titration curve according to the equation[2].

$$I_F^0/(I_F-I_F^0) = [a/(b-a)][(1/K_S[M])+1]$$

where I_F^0 is the emission intensity of **FD8** at 592 nm, I_F is the emission intensity of **FD8** at 592 nm upon addition of different amount of Cr(III). [M] stands for the concentration of Cr(III). a and b are constants. The association constant values K_S is given by the ratio intercept / slope.

Cell Culture

The HeLa cell line was provided by Institute of Biochemistry and Cell Biology (China). Cells were grown in MEM (Modified Eagle's Medium) supplemented with 10 % FBS (Fetal Bovine Serum) at 37 ^oC and 5 % CO_2. Cells (5×10^8/ L) were plated on 18 mm glass coverslips and allowed

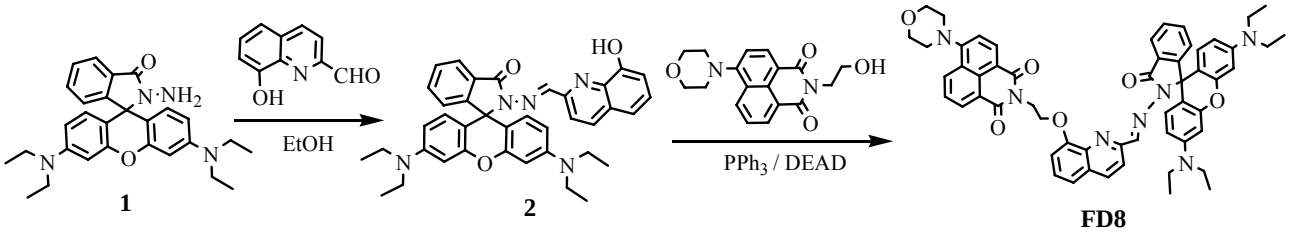
to adhere for 24 hours. Experiments to assess Cr(III) uptake were performed in the same media supplemented with 50 μM $CrCl_3$ for 30 min.

Fluorescence Imaging

Confocal fluorescence imaging was performed with an OLYMPUS IX81 laser scanning microscopy and a 60x oil-immersion objective lens. Excitation of **FD8**-loaded cells at 515 nm was carried out with multiline argon ion lasers, and emission was collected at 610±40 nm (single channel). Excitation of **FD8**-loaded cells at 405 nm was carried out with a semiconductor laser. Emission was collected at 530±20 nm and 610±40 nm for green and red channels, respectively (double channels). The data for ratio fluorescence imaging were analyzed using software package provided by OLYMPUS instruments. Immediately before the experiments, cells were washed with PBS buffer and then incubated with 5 μM **FD8** in PBS for 30 min at 37 °C. Cell imaging was then carried out after washing cells with PBS.

2. Synthesis

Compound **FD8** was prepared synthesized from the reaction of rhodamine hydrazide with 8-hydroxylquinoline-2-aldehyde refluxing in the ethanol solution, followed by the dehydration reaction with 2-hydroxyethyl-4-(6-morpholin-4-yl-1H,3H-benzo[de]) isoquinolin in anhydrous THF for 24 h in 40 % yields (Scheme S1).



Scheme S1 Synthetic route of rhodamine derivative FD8.

1: To a 250 mL flask, 5.05 g rhodamine B was dissolved in 100 mL ethanol. 8.0 mL hydrazine hydrate (85 %) was then added dropwise with vigorous stirring at room temperature. After the addition, the stirred mixture was refluxed in an air bath for 3 h. The solution changed from dark purple to light orange and became clear. Then the mixture was cooled and solvent was removed under reduced pressure. 1 M HCl (about 125 mL) was added to the solid in the flask to generate a clear red solution. After that, 1 M NaOH (about 140 mL) was added slowly with stirring until the pH of the solution reached 9~10. The resulting precipitate was filtered and washed three times with water. The desired product 3.24 g was obtained as pink solid in 70 % yield. mp: 176-177 ºC. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.16 (t, 12H, J = 7.0 Hz), 3.32 (q, 8H, J = 7.0 Hz), 3.61 (bs, 2H), 6.28 (q, J = 2.4 Hz), 6.41(d, 2H, J = 2.4 Hz), 6.46 (d, 2H, J = 8.8 Hz), 7.09-7.11 (m, 1H),

7.43-7.45 (m, 1H), 7.92-7-94 (m, 1H,). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):12.84, 44.59, 66.11, 98.21, 104.83, 108.25, 123.18, 124.04, 128.29, 128.31, 130.26, 132.70, 149.09, 151.78, 154.07, 166.32. ESI mass spectrometry: *m/z* 456.3. Anal. calcd. for $C_{28}H_{32}N_4O_2$: C 73.66, H 7.06, N 12.27. Found: C 73.32, H 7.09, N 12.27 %.

2: To 35 mL dry ethanol solution containing **1** (0.55 g, 1.2 mmol), 8-hydroxylquinoline-2-aldehyde (0.17 g, 1.0 mmol) was added. The mixture was refluxed and stirred for 8 h. The solvent was removed in vacuum to give a gray yellow solid. The crude product was purified by flash column chromatograph using petroleum ether /ethyl acetate (10:1, v/v) as an eluent. The desired product 0.45 g was obtained as light yellow solid in 68 % yield. mp: 197-198 ºC. ^{1}H NMR (400 MHz, $CDCl_3$), δ (ppm): 1.15 (t, 12H, *J* = 7.2 Hz), 3.32 (q, 8H, *J* = 7.2 Hz), 5.29 (s, 1H), 6.24 (d, 2H, *J* = 2.4 Hz), 6.26(d, 2H, *J* = 2.4Hz), 6.49(d, 2H, *J* = 1.2 Hz), 6.55 (d, 2H, *J* = 9.2 Hz), 7.09 (d, 1H, *J* = 7.6 Hz), 7.15 (d, 1H, *J* = 7.2 Hz), 7.23 (d, 1H, *J* = 7.6Hz), 7.37 (t, 1H, *J* = 8.0 Hz), 7.49-7.53 (m, 2H), 8.03 (t, 2H, *J* = 8.4 Hz), 8.09 (d, 1H, *J* = 8.4 Hz), 8.67 (s 1H), 7.09-7.11 (m, 1H), 7.43-7.45 (m, 1H), 7.92-7-94 (m, 1H,). ^{13}C NMR (100 MHz, $CDCl_3$) δ (ppm):12.84, 44.55, 66.35, 98.25, 105.80, 108.28, 110.26, 117.26, 117.91, 119.04, 123.84, 124.17, 128.07, 128.28, 128.59, 128.62, 128.78, 134.05, 136.05, 137.73, 146.21, 149.28, 152.32, 152.38, 152.78, 152.79, 153.34, 165.57. ESI mass spectrometry: *m/z*: 611. Anal. calcd. for $C_{38}H_{37}N_5O_3$: C 74.61, H 6.10, N 11.45. Found: C 74.47, H 6.25, N 11.40 %.

FD8: To 20 mL anhydrous THF solution containing **2** (0.2 g, 0.33mmol) and PPh_3 (0.11g, 0.40mmol), a solution of diethyl azodicarboxylate (DEAD) (0.07g, 0.40 mmol) in anhydrous 2 mL THF *N*-hydroxyethyl-4-morpholin-1,8-naphthalimide (0.13 g, 0.40 mmol) was added dropwise. After heated to 40 ^{o}C for 24 h, the mixture was concentrated in *vacuo* to give crude compound, which was purified by column chromatography using petroleum ether /ethyl acetate (10:1, v/v) to obtain compound **FD8** (0.12 g, 40%) as a yellowish solid. mp: 171-172 ºC. ^{1}H NMR (400 MHz, DMSO-d_6), *δ* (ppm): 1.03 (t, 12H, *J* = 6.8 Hz), 3.20 (t, 4H, *J* = 3.6 Hz), 3.24-3.29 (m, 8H), 3.87 (t, 4H, *J* = 4 Hz), 4.42 (t, 4H, *J* = 6.0 Hz), 4.50 (t, 4H, *J* = 6.8 Hz), 6.31 (d, 1H, *J* = 2.4 Hz), 6.33 (d, 1H, *J* = 2.4 Hz), 6.43(s, 1H), 6.46 (t, 3H, *J* = 2.4 Hz), 7.11 (d, 1H, 7.6 Hz), 7.33 (t, 2H, *J* = 7.6 Hz), 7.42-7.49 (m, 2H), 7.58 (t, 1H, *J* = 7.2 Hz), 7.64 (t, 1H, *J* = 7.2 Hz), 7.73-7.78 (m, 2H), 7.96 (d, 1H, *J* = 6.8 Hz), 8.21 (d, 1H, *J* = 8.8 Hz), 8.42-8.48 (m, 3H), 8.73 (s, 1H). ^{13}C NMR (100 MHz, DMSO-d_6), *δ* (ppm): 43.04, 44.30, 53.68, 65.58, 66.25, 66.84, 98.17, 105.77, 108.72, 111.51, 111.52, 115.71, 116.39, 117.67, 120.69, 123.15, 124.02, 124.52, 125.88, 126.75, 128.09, 128.44, 128.70, 129.63, 129.68, 129.83, 131.30, 131.47, 133.03, 135.06, 137.18, 139.84, 147.72, 149.23, 152.11, 153.11, 153.29, 154.53, 156.18, 163.78, 164.31, 164.89. ESI mass spectrometry: *m/z:* 920.2. Anal. calcd. for $C_{56}H_{53}N_7O_6$: C 73.10, H 5.81, N 10.66. Found: C 73.02, H 6.03, N 10.47 %.

3. Absorption and fluorescent spectra

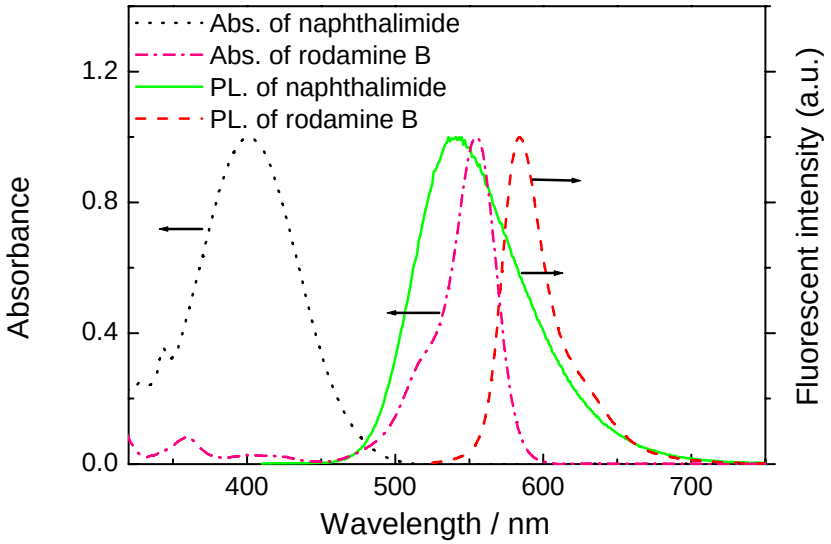


Figure S1 Absorption and fluorescence spectra of *N*-hydroxyethyl-4-morpholin-1,8-naphthalimide (λ_{ex} = 405 nm) and rodamine B (λ_{ex} = 510 nm), respectively.

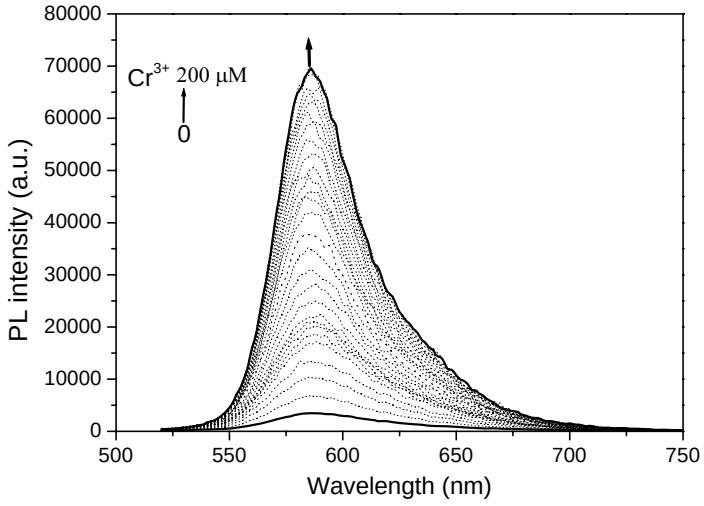


Figure S2 Fluorescence spectra of FD8 (20 μM) in ethanol-water (2:1, v/v) solution in the presence of different amounts of Cr(III) (0-10 equiv.). λ_{ex} = 515 nm.

4. pH response

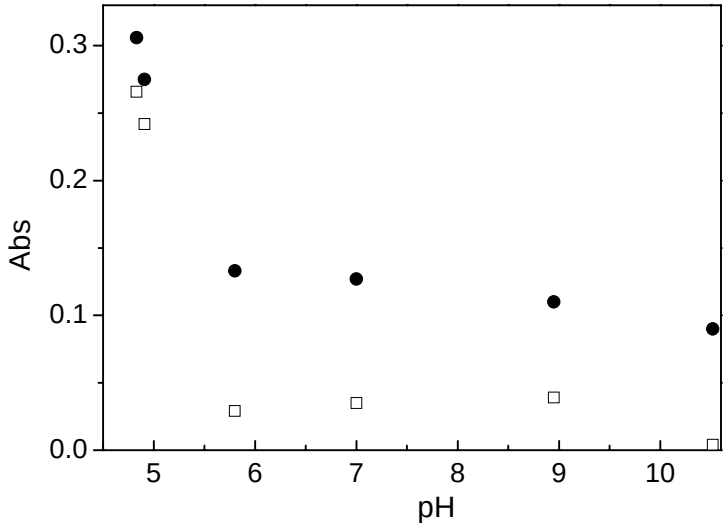


Figure S3 Absorbance of **FD8** in ethanol-water (2:1, v/v) solution of different pH in the absence (□, at 568 nm) and presence (●, at 568 nm) of 200 μM Cr(III). The pH of the solutions was adjusted by addition of 0.2 mol L^{-1} HCl (or 0.1 mol L^{-1} NaOH).

5. Kinetic response

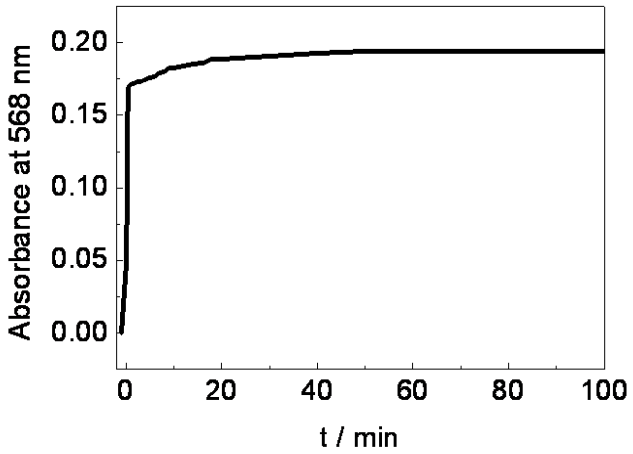


Figure S4 Time course of the response of **FD8** (20 μM) to 10 eq. Cr(III).

6. Cytotoxicity assay

The in vitro cytotoxicity was measured by using the methyl thiazolyl tetrazolium (MTT) assay in HeLa cells. Cells were seeded into 96-well cell culture plate at 5×10^4 / well, 100% humidity cultured at 37 º C and 5 % CO_2 for 24 h, and then different concentrations of Cr^{3+} (0, 25, 50, 75 and 100 μM) were added to the wells. The cells were then incubated for 30 min or 4 h at 37 ºC under 5% CO_2. Subsequently, 10 μL MTT (5 mg/mL) was added to each well and incubated for an additional 4 h at 37 ^oC under 5% CO_2. After addition of 10 % sodium dodecyl sulfate (SDS, 100 μL/well), the assay plate was allowed to stand at room temperature for 12 h. The OD570 (Abs. value) of each well with background subtraction at 690 nm was measured on Tecan Infinite M200 monochromator-based multi-function microplate reader. The following formula was used to calculate the inhibition of cell growth:

Cell viability (%) = (mean of Abs. value of treatment group / mean Abs. value of control) • 100%.

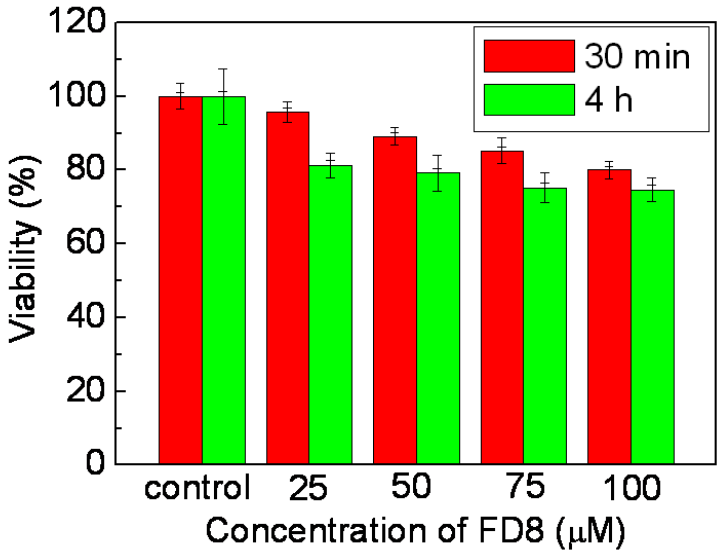


Figure S5 Cell viability values (%) estimated by MTT proliferation test versus incubation concentrations of **FD8**. HeLa cells were cultured in the presence of 25–100 μM **FD8** at 37 °C.

7. ESI mass spectrum

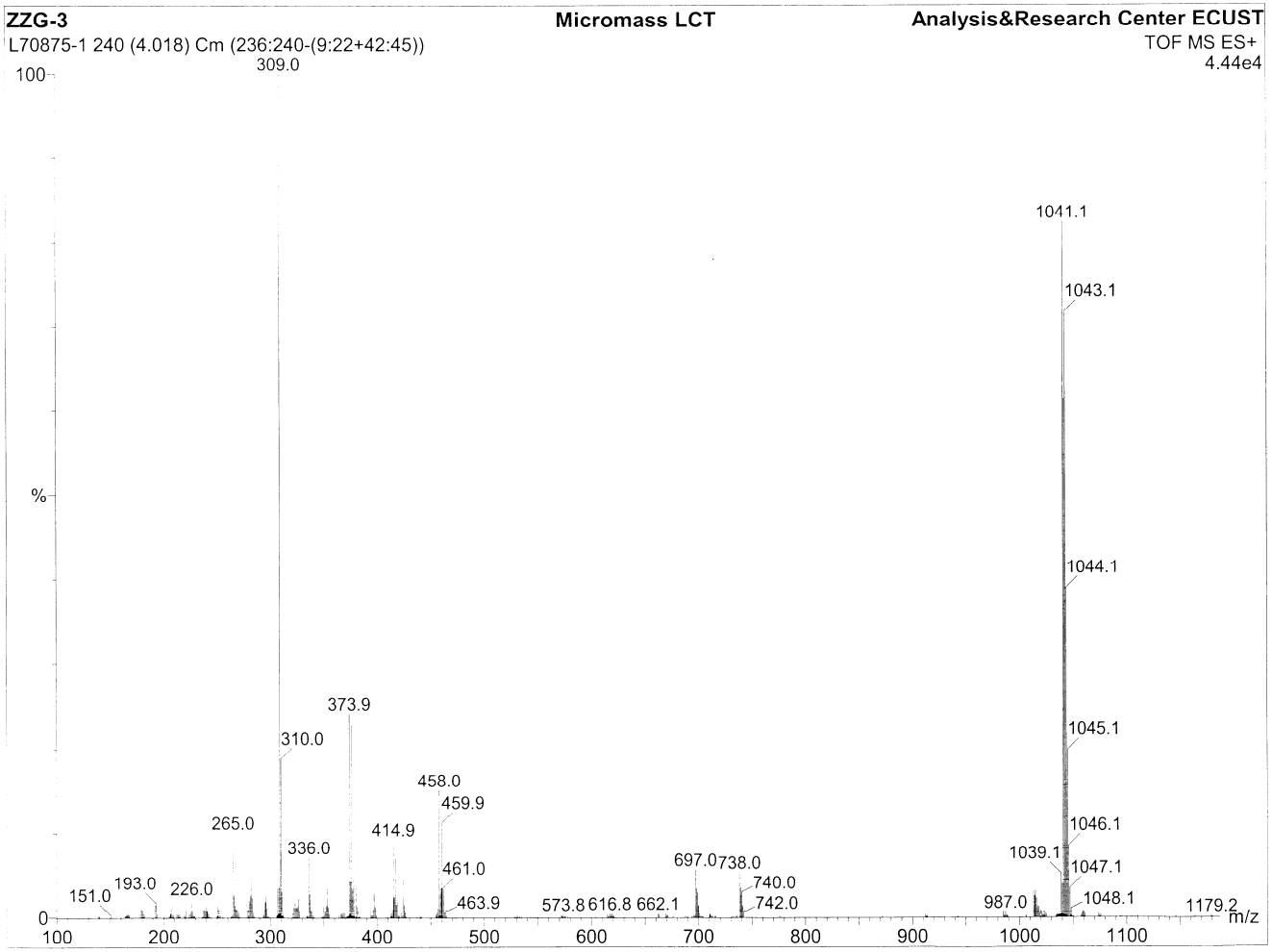


Figure S6 ESI mass spectra of **FD8** upon addition of 200 μM chromium (III) chloride.

[1] Xiang, Y.; Tong, A. J.; Jin, P. Y.; Ju Y. *Org. Lett*. **2006**, *8*, 2863-2866.

[2] Forgues F. S., Le Bris M. T., GuettB J. P, Valeur B., *J. Phys. Chem*. 1988, **92**, 6233-6237.