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PAPER

A Novel Dual-switch Fluorescent Probe for Cr(Ⅲ) Ion Based on PET-FRET Processes

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Two different strategies of photoinduced electron transfer (PET) and fluorescence resonance energy transfer (FRET) have been designed and combined into one sensing system in this work. This novel probe NNRhB is developed based on 1, 8- naphthalimide and rhodamine moieties, in which two fluorophores are susceptible to the presence of Cr^{3+} in different chromium ion concentration regimes. Therefore, the proposed sensing system represents dual-switch states and segmented detection behavior, with the fluorescence emission color spans from green to orange over an increasing Cr^{3+} concentration gradient. When excited in the visible region, the initial emission band at 537nm was enhanced. It was attributed to the suppression of PET process arose from Cr^{3+}-coordination with 1, 8-naphthalimide derivative. At sufficiently high concentration of Cr^{3+} (over 9 μM), the spirolactam rhodamine component in NNRhB converted to the opening form as the result of Cr^{3+} coordination, which turned the emission color from green to orange via FRET. The fluorescence phenomena of compound **1** and compound **2** split from compound NNRhB confirm our conjecture of the spectral response mechanisms. Moreover, compared with single fluorescent response in compound **1** or compound **2**, the dual-switch fluorescent probe NNRhB shows a more sensitive and distinct visual detection ability for Cr^{3+} ions. This probe could afford a high selectivity and sensitivity to Cr^{3+} from 30 nM to 80 μM and the detection limit was 0.14 nM. The results of practical application experiments suggest that the Cr^{3+}-selective ligand prepared here may provide an effective strategy for detection of Cr^{3+} in environmental and biological applications.

Introduction

Cr^{3+} is treated as an essential micronutrient for humans and animals. It plays an important role in the maintenance of normal levels of glucose, triglyceride and total cholesterol.[1-6] Chromium and its compounds are indispensable ingredients of economic development, which are widely used in electroplating, tanning industries, fabrication of dye and pigments, wood preserving, oxidation and various other industrial applications.[2-4, 7] However, higher concentration of Cr^{3+} is detrimental to cellular functions and structures[8] and causes serious environmental pollution due to the discharge of chromium by industrial and other activities. At the same time, its oxidation form Cr (VI) is an extremely toxic and carcinogenic species. It can penetrate through cell membranes and cause toxic effects including cancers by oxidizing DNA and some proteins.[9-11] Thus, there is an urgent need for the development of sensitive and selective assays for Cr^{3+} ions detection in environmental and biological samples.[12]

Numerous traditional analytical techniques have been reported for the determination of Cr^{3+} ions such as chromatographic separation,[13, 14] high-performance liquid chromatography (HPLC)[15, 16] and inductively coupled plasma atomic emission spectrometry (ICP-AES).[17] However, the major disadvantages of these methods are the needs of expensive and complex apparatus, complicated sample pretreatments and time-consuming processes.[18] Recently, fluorescence method has been paid more attention to because of its high sensitivity and selectivity, operational simplicity and direct visual perception ability.[18-22] However, only a few Cr^{3+} selective fluorescent sensors have been reported till now due to the lack of special selective ligands.[23-25] Therefore, Cr^{3+} fluorescent sensing remains underdeveloped. Especially due to its paramagnetic property, Cr^{3+} is known as one of the most effective quenchers of fluorescence. Whereas, fluorescence quenching is not conductive to a high signal output upon recognition and interferes with temporal separation of similar complexes with time-resolved fluorometry.[26] Hence, the sensors with "turn-on" fluorescence response are more attractive than the "turn-off" ones as they offer the potential for high sensitivity.[27-30] Owning this in mind, we focus on developing an efficient and selective fluorescent ligand for Cr^{3+} species and employing its application of Cr^{3+} detection in environmental and biological systems.

Herein, we report the synthesis and applications of a novel dual-switch Cr^{3+} probe NNRhB, in which 1, 8- naphthalimide derivative is covalently tethered to the spirolactam form of rhodamine to afford the fluorescent sensing system. When Cr^{3+} bound with NNRhB, these two fluorophores sequentially turned to the "on" state and a corresponding fluorescence color change occurred along with the increasing concentration of chromium ion under the excitation of naphthalimide fluorophore. We proposed that two different powerful "turn-on" processes, photoinduced electron transfer (PET) and intramolecular fluorescence resonance energy transfer (FRET), would enable the dual-fluorophore probe to be excited sufficiently and thereafter induce effectively enhanced fluorescence phenomenon. For the purpose of validating the detection mechanism, NNRhB was split into two corresponding parts, compound **1** and compound **2**. The

emissions of both fluorophores were susceptible to the presence of Cr^{3+} in different chromium ion concentration regimes,[24, 31, 32] and their fluorescent response performances validated our conjecture. As one of the rare and suitable Cr^{3+} probes, NNRhB displayed high sensitivity and remarkable selectivity to Cr^{3+} over other metal cations. It could afford a wide detection range for Cr^{3+} from 30 nM to 80 μM and a low detection limit of 0.14 nM. In comparision with single fluorescence response in compound **1** or compound **2**, NNRhB showed more sensitive and reliable visual detection ability for Cr^{3+}. The results of Cr^{3+} detection in both environmental and physiological conditions by utilizing NNRhB gave ideal recovery percentages, manifesting its good potential in practical application.

Experimental section

Apparatus

^{1}H NMR measurements were performed with Bruker AV II-300 MHz spectrometer. ^{13}C NMR measurements were performed with Bruker AV II-400 MHz spectrometer. Mass spectra were obtained from MAT-261 spectrometer. FT-IR was recorded on Thermo Scientific Nicolet 6700 FT-IR spectrometer (Sugar Land, TX, USA). Fluorescence spectra were acquired on F-7000 spectrophotometer equipped with a 1 cm quartz cell (HITACHI, Japan). UV-visible spectra were measured on Techcomp UV1100 spectrophotometer (Shanghai, China).

Reagents

Rhodamine B was purchased from Beijing Chemical Co., China. 4-Bromo-1, 8-naphthalic anhydride and Hydrazine hydrate were purchased from Shanghai Chemical Reagent Co., China. 1-Piperazineethanol was purchased from TCI (Tokyo, Japan). Sodium salt of 4- (2-hydroxylethyl)-1-perazineethane sulfonic acid (HEPES) was purchased from Sigma-Aldrich. The human serum samples were obtained from Wangjiang Hospital of Sichuan University. Colon cancer cell in mice was provided by the West China School of Pharmacy at Sichuan University. All solvents used for synthesis and measurements were redistilled before use. All the other chemicals were of analytical-reagent grade and used without further purification. The purified water used in this study was the double-distilled water.

Synthesis of intermediates and probes

Synthesis of compound **1**

1-Piperazineethanol (390 mg, 3.0 mmol) was dissolved in 2-methoxyethanol (10 ml), and then 4-bromo-1, 8-naphthalic anhydride (415 mg, 1.5 mmol) was added to the solution. The reaction mixture was refluxed for 48 h. The mixture was evaporated by rotary evaporation, then dissolved in moderate hot ethanol and filtered. The filtrate was dried under reduced pressure to afford compound **1** (0.47 g, yield: 96%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ /ppm 8.57 (d, J = 7.0 Hz, 1H), 8.51 (d, J = 7.9 Hz, 1H), 8.45 (d, J = 8.3 Hz, 1H), 7.73 (t, 1H), 7.22 (d, J = 7.5 Hz, 1H), 3.72 (t, 2H), 3.37 (t, 4H), 2.87 (t, 4H), 2.73 (t, 2H), 2.02 (s, 1H).

Synthesis of compound **Rhodamine B hydrazide**

Rhodamine B hydrazide was synthesized by a reaction of rhodamine B with hydrazine hydrate following literature method[33, 34].

Synthesis of compound **2**

Rhodamine B hydrazide (400 mg, 0.88 mmol) and 4-bromo-1,8-naphthalic anhydride (243.8 mg, 0.88 mmol) were taken in 15 ml of glacial acetic acid. The reaction mixture was heated to reflux for 24 h. Then the mixture was subsequently cooled and added to 100ml of cold water. Next, 1 mol·L^{-1} NaOH was added dropwise with stirring until the solution pH reached 8-9. The violet precipitate was filtered, washed with water and dried under reduced pressure. The residue was then dissolved in dichloromethane and purified by column chromatography with ethyl acetate/petroleum ether (volume ratio 1:9) as eluent to afford compound **2** (580 mg, yield: 92%) as a light yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ /ppm 8.50 (d, J = 8.3 Hz, 1H), 8.31 (d, J = 7.1 Hz, 1H), 8.10 (d, J = 7.8 Hz, 1H), 8.07 (d, J = 6.8 Hz, 1H), 7.92−7.71 (m, 2H), 7.65 (t, 1H), 7.61 (d, J = 7.3 Hz, 1H), 7.37 (d, J = 7.0 Hz, 1H), 6.76 (d, J = 8.7 Hz, 2H), 6.40 (d, J = 6.7 Hz, 2H), 6.11 (s, 2H), 3.33 (q, J = 8.6 Hz, 8H), 1.11 (t, 12H).

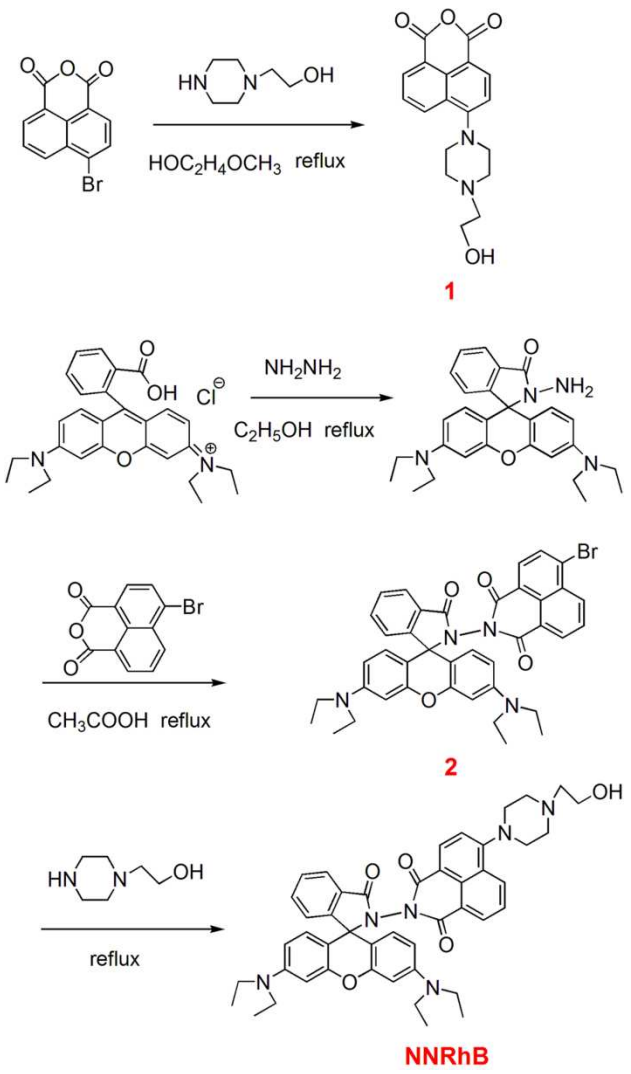
Synthesis of compound **NNRhB**

Under the nitrogen condition, 200 mg (0.27 mmol) of compound **2** was added to 10ml of 1-Piperazineethanol and heated to reflux for 20 h with continuous stirring. After cooling to room temperature, the reaction mixture was added to ice-cold water. Then a yellow crude product was obtained which was filtered and washed several times with cold water and then dried under reduced pressure. The crude product was further purified by column chromatography on silica gel (ethyl acetate/petroleum ether/methanol, 2:4:1) to provide 174 mg of yellow solid in 84% yield. ^{1}H NMR (300 MHz, $CDCl_3$): δ /ppm 8.30 (d, J = 8.5 Hz, 1H), 8.21 (d, J = 7.3 Hz, 1H), 8.16 (d, J = 8.0 Hz, 1H), 8.06 (d, J = 7.0 Hz, 1H), 7.64−7.58 (m, 2H), 7.52 (t, 1H), 7.34 (d, J = 7.5 Hz, 1H), 7.04 (d, J = 8.1 Hz, 1H), 6.75 (d, J = 8.9 Hz, 2H), 6.38 (d, J = 6.7 Hz, 2H), 6.10 (s, 2H), 3.69 (t, 2H), 3.30 (t, 4H), 3.269 (q, J = 8.6 Hz, 8H), 2.80 (t, 4H), 2.68 (t, 2H), 1.84 (s, 1H), 1.09 (t, 12H); ^{13}C NMR (400 MHz, $CDCl_3$): δ/ppm 164.50, 161.38, 160.87, 155.71, 154.50, 154.48, 150.09, 148.94, 132.52, 131.24, 131.15, 130.70, 130.26, 128.63, 126.30, 125.51, 124.74, 123.73, 114.77, 107.94, 97.45, 77.48, 77.16, 76.84, 67.73, 59.50, 57.97, 53.01, 44.47, 29.78, 12.61, 12.58; ESI-MS (m /z): calcd for $C_{46}H_{48}N_6O_5$ 764.37, found 765.36 (M^++H^+).

Results and Discussion

Characterization of compounds

NNRhB, compound **1** and compound **2** were synthesized via easy steps of traditional organic synthesis and depicted in Scheme 1. Details about the synthesis of NNRhB, compound **1** and compound **2** were discussed above and in 84%, 92% and 96% yields, respectively. The MS data, ^{1}H NMR and ^{13}C NMR spectrums presented in the supporting information, Figure S-1~S-7, confirmed the successful synthesis of our molecules. The infrared spectrum of NNRhB was given in Figure S-8. The band at 3435 cm^{-1} corresponded to the -OH stretching mode. The band at around 1615 cm^{-1} indicated the existence of carbonyl (C=O) groups. The band at 1119 cm^{-1} corresponded to the C-N stretching mode. This result demonstrated that 1, 8-naphthalimide derivative was covalently tethered to the spirolactam form of rhodamine.



Scheme 1. Synthesis of **NNRhB**, compound **1** and compound **2**.

Fluorescence properties of probe NNRhB

In our experiment, a CH_3CN/HEPES buffer solution (2:1, v/v, 0.5 mM, pH = 7.4) was selected as testing system to investigate the chemical response of NNRhB to metal ion guests at room temperature. The binding behavior of compound NNRhB towards different cations (K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cr^{6+}, Ni^{2+}, Cu^{2+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Hg^{2+}, Pb^{2+} and Cr^{3+}) was investigated by fluorescence spectroscopy. As shown in Figure 1, the free NNRhB in solution displayed an almost undetectable fluorescence color and a weak 1, 8-naphthalimide emission centered at 537 nm when excited at 410 nm (excitation of 1, 8-naphthalimide moiety). The lack of any fluorescence band above 550 nm indicated the existence of rhodamine in the ring-closed spirolactam form. However, upon addition of Cr^{3+} ion (4.0 equiv), a pronounced fluorescence change happened: the intensity of the fluorescence band at 537 nm increased along with a significant concomitant growth of a new emission band at 588 nm, indicating the development of ring-opening form of rhodamine by Cr^{3+} ion coordination. This change was also accompanied with a variation of the solution color from pale-yellow to pink and the fluorescence color from colorless to orange (Figure 1(a) and 1(b)), visible to the naked eye. On the other hand, the probe showed almost no fluorescence responses to other metal ions except that a slight change occurred in the presence of Fe^{3+}, Al^{3+}, Hg^{2+} and Pb^{2+} ions, respectively. The excellent sensitivity of NNRhB toward Cr^{3+} lays a foundation for its practical application as an effective metal ion chemosenser.

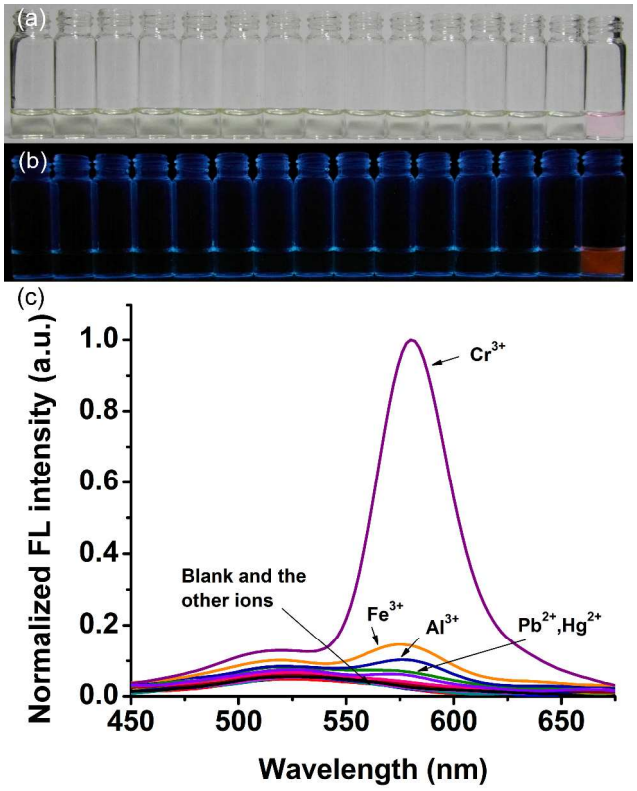


Figure 1. Photographs of colorimetric (a) and fluorescent (b) responses of 13 μM NNRhB to 4.0 equiv of Cr^{3+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Hg^{2+}, Pb^{2+} and 40 equiv of the other metal ions (from left to right: NNRhB only, K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Ni^{2+}, Cr^{6+}, Zn^{2+}, Cu^{2+}, Al^{3+}, Hg^{2+}, Pb^{2+}, Fe^{3+} and Cr^{3+}). (c) Fluorescence responses of 13 μM NNRhB to 4.0 equiv of Cr^{3+}, Fe^{3+}, Al^{3+}, Zn^{2+}, Hg^{2+}, Pb^{2+} and 40 equiv of the other various metal ions (K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+} and Cr^{6+}) in CH_3CN/HEPES buffer solution (2:1, v/v, 0.5 mM, pH = 7.4; λ_{ex}= 410 nm).

Another important feature of NNRhB is its high selectivity toward Cr^{3+} in a competitive environment. The competitive experiments were carried out in the presence of 4 equiv of Cr^{3+} mixed with 4 equiv of miscellaneous metal ions (K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{6+}, Hg^{2+}, and Pb^{2+}) respectively. As shown in Figure 2a, compared with the NNRhB system only with the presence of Cr^{3+}, there were no distinct variations in the fluorescence intensity of the mixture solution in the presence of both Cr^{3+} and interfering metal ions except that Cu^{2+}, Hg^{2+}, and Pb^{2+} induced a slight fluorescence enhancement, respectively. Figure 2(b) and 2(c) showed the photographs of NNRhB upon addition of Cr^{3+} and interfering metal ions, separately. The fluorescence emission picture was screened upon illumination with a hand-held UV lamp, and was consistent with the result of the fluorescence spectroscopy. The above results further make

this probe appropriate for selectively colorimetric and fluorescent sensing of Cr^{3+}.

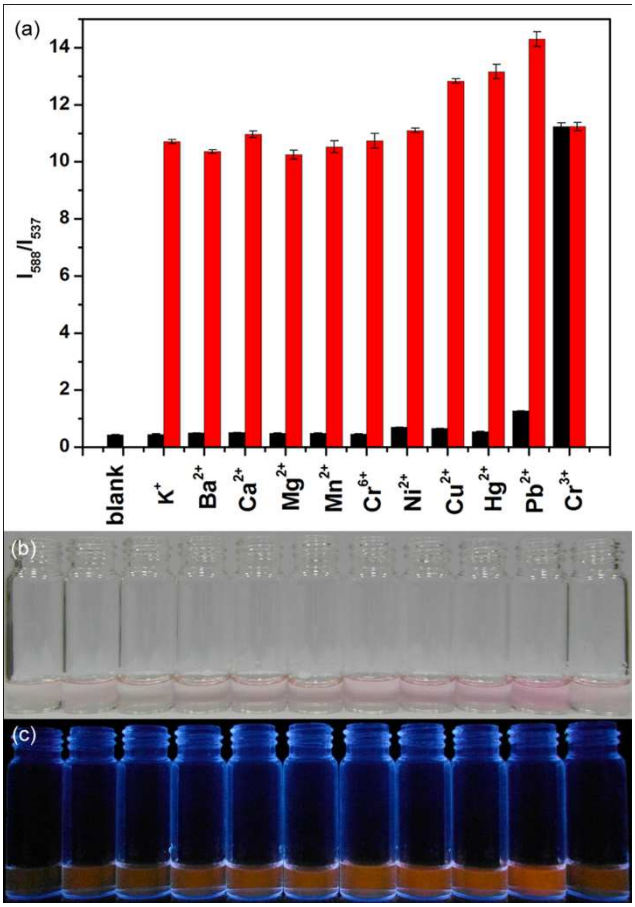


Figure 2. (a) Fluorescence responses of 13 μM NNRhB upon the addition of various metal ions (K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{6+}, Hg^{2+}, Pb^{2+} and Cr^{3+} ; 4 equiv) in CH_3CN/HEPES buffer solution (2:1, v/v, 0.5 mM, pH = 7.4) (λ_{ex}= 410 nm). The black bars represent the addition of various metal ions, while the red bars represent the change in the emission that occurs upon the subsequent addition of Cr^{3+} to the above solution. Photographs of color changes (b) and fluorescence responses (c) of 13 μM NNRhB with solutions containing 4 equiv Cr^{3+} ion and cations of interest (from left to right: K^+, Ba^{2+}, Ca^{2+}, Mg^{2+}, Mn^{2+}, Cu^{2+}, Ni^{2+}, Cr^{6+}, Hg^{2+}, Pb^{2+} and Cr^{3+}).

The fluorescence titration of Cr^{3+} was conducted by using a 13 μM solution of NNRhB in CH_3CN/HEPES buffer solution (2:1, v/v, 0.5 mM, pH = 7.4). As can be seen from Figure 3(a), the probe alone displayed a weak 1, 8-naphthalimide emission band centered at 537 nm when excited at 410 nm (excitation of 1, 8-naphthalimide moiety). This feeble fluorescence emission was probably due to the photoinduced electron transfer (PET) from the nitrogen atom of 1-piperazineethanol moiety to the photo-excited naphthalimide moiety, which quenched most of the fluorescence of naphthalimide fluorophore. This would represent the "off"-state of the system. The absence of any emission band above 550 nm indicated the existence of rhodamine moiety in the ring-closed spirolactam form. Interestingly, the addition of incremental amounts of Cr^{3+} ions (0 to 80 μM) to the solution of NNRhB led to mutative fluorescence responses. Addition of Cr^{3+} from 0 to 9 μM resulted in a fluorescence increase at 537 nm, illustrating the suppression of PET process in the naphthalimide moiety, and consequently, the emission of naphthalimide unit of NNRhB would be "switched on". Simultaneously, the rhodamine moiety maintained the ring-closed spirolactam form with no fluorescence response. Upon further addition of Cr^{3+} beyond 9 μM (Figure 3(b)), an emission band corresponding to the ring-opened amide form of rhodamine at 588 nm appeared. It was clear that the FRET process was switched on under Cr^{3+} ions coordination. The emission color transition and fluorescence intensity variation could be verified visually upon illumination with a hand-held UV lamp (Figure S-9). The addition of low concentration of Cr^{3+} led to a gradually and slightly strengthen of the green fluorescence emission, indicating the combination of Cr^{3+} with the naphthalimide moiety. Then over an increasing Cr^{3+} gradient, the fluorescence of the solution turned into bright orange by degrees, and the solution color turned from pale-yellow to pink, demonstrating the Cr^{3+}-induced formation of the ring-opened compound based on rhodamine.

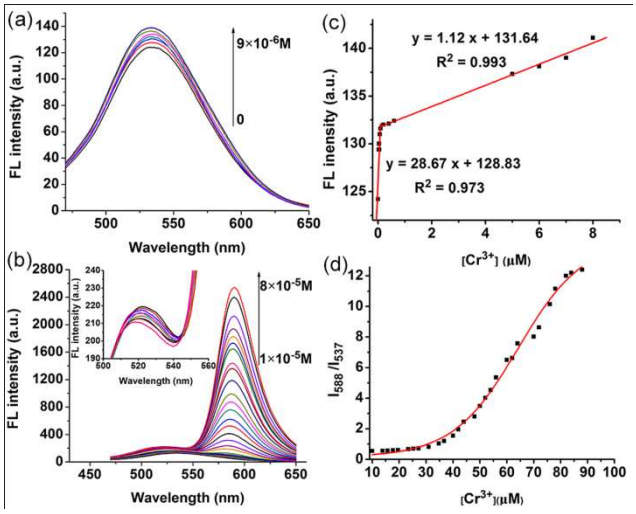
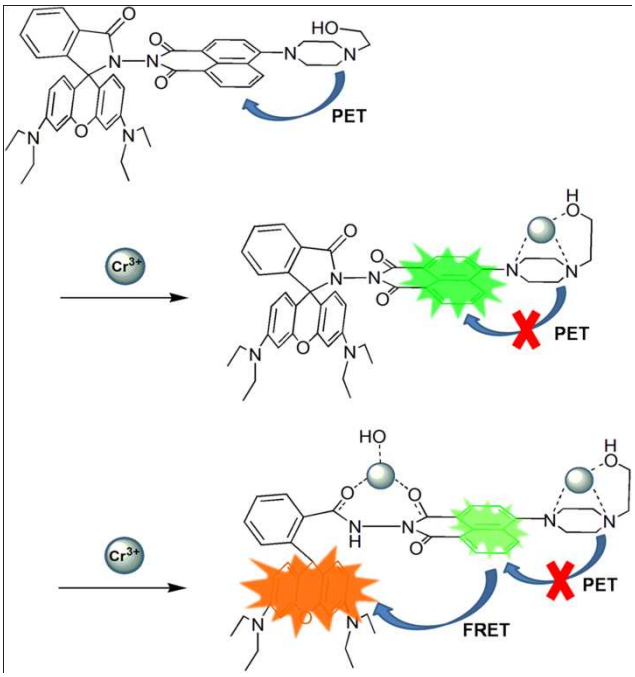


Figure 3. Fluorescence spectra variation of probe NNRhB (13 μM) upon addition of increasing Cr^{3+} concentrations in CH_3CN/HEPES buffer solution (2:1, v/v, 0.5 mM, pH = 7.4) ((a): 0-9 μM (b): 10-80 μM). (c)The relation curve of fluorescence intensity at 537 nm and Cr^{3+} concentration (0-9 μM). (d)The relation curve of fluorescence intensity ratio (I_{588}/I_{537}) and Cr^{3+} concentration (10-80 μM) (λ_{ex}= 410 nm).

The design of dual-switch state is favorable for the probe to work as a Cr^{3+} indicator for quantitative analyses. As shown in Figure 3(c), the emission intensity at 537nm exhibited a segmented linear response toward Cr^{3+} at the concentration ranging from 0 to 9 μM. In the range from 0 to 0.1 μM, the linear equation was y = 28.67x + 128.83 and the linear relative coefficient was 0.973. From 0.1 to 9 μM, the linear equation was y = 1.12x + 131.64 and the linear relative coefficient was 0.993. When [Cr^{3+}] was over 9 μM, the 588 nm orange emission channel was open. The intensity ratio at 588 nm over 537 nm tracked the growth of Cr^{3+} concentration (Figure 3(d)). The ratio (I_{588}/I_{537}) varied from 0.54 to 11.94, corresponding to a 22.1-fold fluorescence enhancement, which also indicated a high efficiency of energy transfer of the FRET system. With this dual-switch probe, the Cr^{3+} detection could be achieved at a nanomolar concentration low to 30 nM, and a wide detection range up to 80 μM. The detection limit was estimated to be 0.14 nM (3σ/slope). We also compared our probe with other Cr^{3+} molecule probes

reported before (Table S-1). The results showed that our probe provided lower detection limit and wider linear range for the detection of Cr^{3+}. To confirm the stoichiometry between NNRhB and Cr^{3+}, Job′s plot using the absorption titration was undertaken and the results indicated a 1:2 binding ratio for NNRhB with Cr^{3+} (Figure. S-10). With the method in the literature[35], the association constants K_{N1} and K_{N2} in the two equations Cr^{3+} + NNRhB ⇋ Cr^{3+}·NNRhB and Cr^{3+}·NNRhB + Cr^{3+} ⇋ Cr^{3+}·NNRhB·Cr^{3+}, which meant successively that Cr^{3+} was bonded to the position of piperazineethanol moiety and rhodamine hydrazide moiety in NNRhB, were determined to be about 8.22×10^4 M^{-1} and 2.33×10^4 M^{-1}, respectively.

In the present work, the possible mechanisms of the spectral responses toward Cr^{3+} were studied. We proposed that this segmented detection behavior was because piperazineethanol and rhodamine hydrazide each fastened a metal ion, respectively. The different binding capability of these two fluorophores with Cr^{3+} would result in different fluorescence phenomena (Scheme 2). That is, the addition of Cr^{3+} (0~9 μM) suppressed the PET process in the piperazineethanol moiety. Then the emission of the donor dye would be "switched on". Further increasing the concentration of Cr^{3+} to above 9 μM resulted in the development of a pink color and an orange emission, illustrating that the FRET process was switched on under Cr^{3+} ions coordination. To validate our conjecture, the molecule probe was split into two corresponding parts, compound **1** and compound **2** (Scheme 1), and the fluorescent response performances were investigated in the same conditions.



Scheme 2. Proposed binding modes of **NNRhB** with Cr^{3+}.

Compound **1** alone showed an excitation maximum around 386 nm and an emission maximum around 537 nm in CH_3CN/HEPES buffer solution (2:1, v/v), respectively (Figure S-11(a) inset). The fluorescence titration profile of compound **1** (13 μM) with Cr^{3+} upon excitation at 386 nm was shown in Figure S-11(a). Upon addition of increasing amount of Cr^{3+} from 0 to 60 μM to the solution, the 1, 8-naphthalimide emission intensity gradually increased with a 13-fold fluorescence enhancement and reached saturation finally when [Cr^{3+}] was 60 μM. Compound **1** took the same mechanism of PET with that of NNRhB at lower [Cr^{3+}], and consequently, the PET process was suppressed by Cr^{3+} ion coordination. In this experiment, Cr^{3+} could be detected as low as 1.0×10^{-7} M, which was higher than the 30 nM of NNRhB. The association constant for compound **1** binding to Cr^{3+} was determined to be about 4.51×10^5 M^{-1} from the fluorescence experiments.

In the absence of Cr^{3+}, compound **2** did not radiate the characteristic fluorescence of rhodamine, suggesting that it preferred the spirocyclic form in this condition. The emission spectra of **2** (in CH_3CN/HEPES buffer solution) in the presence of varying concentration of Cr^{3+} (0 to 60 μM) with an excitation of 530 nm were shown in Figure S-11(b). Upon increasing Cr^{3+} from 0 to 10 μM, no obvious fluorescence emission could be detected, which indicated the insensitivity of compound **2** toward Cr^{3+} at relatively low [Cr^{3+}]. With the introduction of Cr^{3+} above 10 μM, a new emission band at 588 nm presented, signaling the structural change from the spirocyclic to the ring-opened form of rhodamine. This Cr^{3+} concentration-dependent behavior of compound **2** at higher [Cr^{3+}] was consistent with that of NNRhB. The association constant for compound **2** binding to Cr^{3+} was determined to be about 3.46×10^4 M^{-1} from the fluorescence experiments.

Obviously, compared to compound **2**, compound **1** was more susceptible to Cr^{3+}-coordination at low Cr^{3+} concentration, and compound **2** would emit orange ring-opened rhodamine fluorescence only in the presence of high concentration of Cr^{3+}. These detection modes are consistent well with those of compound NNRhB on the phenomenon of segmented fluorescence response to Cr^{3+}. Speculated from these results and literatures,[32, 36] we believed that NNRhB adopted the similar binding motif. That is, at lower concentration, Cr^{3+} bonded easily in the position of piperazineethanol moiety. The addition of 0~9 μM Cr^{3+} reduced the electron-donating ability of the nitrogen atom in the piperazineethanol moiety. Then the PET process was suppressed, and consequently, the emission of the donor dye would be "switched on". Further increasing the concentration of Cr^{3+} to above 9 μM resulted in the development of a pink color and an orange emission, illustrating that the FRET process was switched on under Cr^{3+} ions coordination. The possibility of the occurrence of FRET could be corroborated from Figure S-12. The absorption spectrum of Cr^{3+}-compound **2** overlapped well with the fluorescence emission of Cr^{3+}-compound **1**, which led to an efficient energy transfer from the 1, 8-naphthalimide donor to the rhodamine acceptor, and consequently a color change from green to orange. Results of the spectral studies (Fig 3(b) inset) supported this presumption with a well defined isoemission point at 545 nm. Obviously, this is the reason for the weak fluorescence intensity of the 1, 8-naphthalimide moiety in probe NNRhB as compared to that of compound **1**. Therefore, the sensitive and selective detection of Cr^{3+} by utilizing dual-switch fluorescent probe NNRhB can be conveniently realized.

For further certification of the advantage in Cr^{3+} detection by NNRhB, comparison of the visual sensitivities of NNRhB, compound **1** and compound **2** after exposure to Cr^{3+} were studied

(Figure 4). Upon addition of Cr^{3+} ions from 0 to 20 μM, there was no visible fluorescence color could be detected in Figure 4(b) as the result of the insensitivity of compound 2 toward Cr^{3+} at relatively low [Cr^{3+}]. Otherwise, NNRhB showed a gradually and slightly strengthen of the green fluorescence color in the same [Cr^{3+}] range (Figure 4(a)). Clearly, NNRhB was more sensitive and distinct for visual detection of Cr^{3+} than compound 2 at low [Cr^{3+}]. In the presence of high concentration of Cr^{3+} (Figure 4(c) and 4(d)), the changes of [Cr^{3+}] resulted in obvious and continuous fluorescence color changes from light to deep orange in NNRhB. On the other hand, for the reason of the saturation behavior of compound 1 when [Cr^{3+}] is over 60 μM, there were undetectable fluorescence changes in Figure 4(d) can be found. This result manifested the obvious advantage of NNRhB in Cr^{3+} detection at high [Cr^{3+}]. Such observation suggested that the combination of PET and FRET sensing mechanisms in one system not only improved the sensitivity of the fluorescent probe but also expanded the visual dynamic range of the assay.

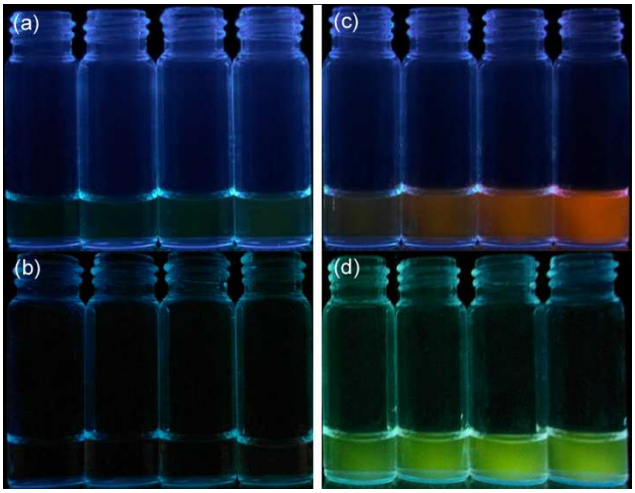


Figure 4. Comparison of fluorescence colors of the probe NNRhB solutions (a) and compound 2 solutions (b) after exposure to different concentrations of Cr^{3+} (0, 5, 10 and 20 μM). Comparison of fluorescence colors of the probe NNRhB solutions (c) and compound 1 solutions (d) after exposure to different concentrations of Cr^{3+} (50, 60, 70 and 80 μM). All the photos were taken under a 365 nm UV lamp.

To illustrate the reliability and accuracy of our sensing system, the practical application was evaluated by determining the recoveries of spiked Cr^{3+} in pond water and tap water samples, respectively. The samples collected were simply pretreated with filtration before further determination. As analytical results shown in Table S-2, there was no Cr^{3+} detected in the samples. Then Cr^{3+} stock solution was separately spiked in these samples and NNRhB probe was employed to detect its concentration. Standard addition method was adopted to analyze each sample in triplicate. The recovery of Cr^{3+} was between 103.1 % and 104.3 % for pond water and 95.9 % and 96.5 % for tap water, respectively, which suggested that the proposed probe was suited to detect Cr^{3+} in real water samples with other potentially competing species coexisting.

One of the potential utility of the probe is acting as an indicator for Cr^{3+} under physiological conditions. We further applied the probe NNRhB to detect Cr^{3+} in cell lysate and blood serum, respectively. The cell lysate was obtained by centrifuging a cell sample to separate large bio-molecules from the cell lysate. As analytical results shown in Table S-3, there was no Cr^{3+} detected in the samples. Herein, 5 μL of human blood serum sample and 5 μL of cell lysate supernatant were respectively added to 10 mL of probe in HEPES buffer solution and recovery analyses of the samples were performed by the standard addition method. The recoveries of Cr^{3+} were in general from 100.5 % to 101.3 %, which demonstrated that the probe possess good potential for detecting Cr^{3+} in physiological conditions.

Conclusion

In summary, a fluorescent sensing system based on 1, 8-naphthalimide and rhodamine moieties has been successfully designed and synthesized through a facile approach. This probe employed PET and FRET strategies into one system to obtain an efficient dual-switch fluorescent molecular for the sequential detection of Cr^{3+}. For this approach, the different binding affinity of two fluorophores with Cr^{3+} is employed to realize the segmental detection. Addition of analyte with increasing concentration gradient induced different emission bands strengthen and remarkable emission color change corresponding to different fluorophores. These changes are of such magnitude that they can be considered as representing two different "off-on" states. Comparing to the single fluorescent response probes compound **1** and compound **2** split from NNRhB, our dual-switch probe carries a much higher visual sensitivity to the change of [Cr^{3+}]. This probe could afford a high selectivity and sensitivity to Cr^{3+} in a wide range of [Cr^{3+}] from 30 nM to 80 μM, and the limit of detection was estimated to as low as 0.14 nM. NNRhB also has been successfully applied in the detection of Cr^{3+} in environmental and physiological conditions, further demonstrating its value in practical applications. This concept reported herein could provide a significant platform for the design of new dual-switch fluorescent probes for other target analytes.

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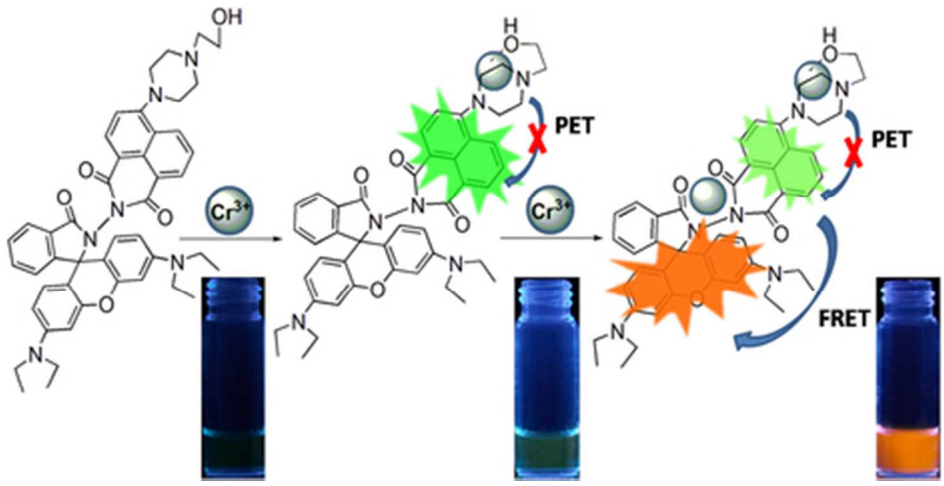

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