A Dendrimer-based Highly Sensitive and Selective Fluorescence-quenching Sensor for Fe³⁺ Both in Solution and As Film

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ABSTRACT: A novel fluorescent dendrimer PYTPAG2, with pyrene as the interior core and triphenylamine (TPA) as the exterior periphery, is studied as a fluorescence-quenching sensor for iron (III) ions (Fe³⁺), both in solution and as a film. This dendrimer-based sensor possesses preferential detection of Fe³⁺ by a very strong fluorescence quenching not found for other metal ions. The fluorescent detection limits of this PYTPAG2 sensor for Fe³⁺ in solution and thin-film are 6.5×10^{-7} M and 5.0×10^{-7} M, respectively. The possible mechanism of this process is explained by the complexation between the peripheral TPA units of PYTPAG2 and Fe³⁺ ions, which may disrupt the fluorescence resonance energy transfer (FRET) from the TPA groups to the pyrene core (intramolecular of PYTPAG2) and results in the fluorescence quenching. Moreover, this striking performance could not be disturbed by pH, the interference with other metal ions, counter anions, or surrounding environment. In addition, biological fluorescence imaging studies of Fe³⁺ in living roundworms demonstrate its valuable practical application.

Keyword: Dendrimer, Fluorescence quenching, Specific selectivity, Fe³⁺, Stable sensitivity, Biological imaging

1. Introduction:

18 The iron Fe^{3+} ion is one of the most important elements for the biological function of plants and
19 animals, both its deficiency and overload will cause various disorders. ^[1-3] Therefore, the detection of
20 Fe^{3+} is essential for monitoring the environment and human health. ^[4-5] Over the past few decades, many
21 physical and chemical techniques including atomic absorption spectroscopy, calorimetry, spectroscopy
22 and -photometry, as well as voltammetry have been applied to detect Fe^{3+} . ^[6-8] However, in regards to
23 on-site assays, fluorimetric methods are highly preferable based on their high selectivity, direct visual
24 perception, and particularly simple operation. ^[9-11]

25 Up to now, only a few highly sensitive and selective fluorescence sensors for Fe^{3+} have been reported.
26 ^[12] This might be due to several reasons: first, the detection of Fe^{3+} is easily interfered with other
27 transition-metal ions, such as Mn^{2+} , Cu^{2+} and Cr^{3+} ; ^[13] second, the fluorescent sensing performances are
28 easily disturbed by pH, counter anions and the surrounding environment. Moreover, in terms of
29 practicality, the fluorescent sensing film is of larger interest, but there are nearly no reports on the Fe^{3+}
30 detection by fluorescent films. ^[14] Therefore, developing a highly selective and sensitive fluorescent
31 sensor with durable stability towards Fe^{3+} in aqueous solutions and as thin films is a challenge that
32 deserves to be further explored.

33 Polyphenylene Dendrimers (PPD)s are a class of macromolecules that are monodisperse, shape
34 persistent three-dimensionally extending molecules that possess a very high concentration of surface
35 functional groups. ^[15] Nowadays, dendrimers are applied in many different fields, ranging from materials
36 and synthetic chemistry to biological and physical chemistry. ^[16] In the sensing field, fluorescent
37 dendrimers are also receiving more attention due to their larger or extended surface areas, which could
38 improve the contact between the sensing dendrimer and the analyte thereby leading to a higher
39 sensitivity. ^[17] However, though a few fluorescent dendrimers in aqueous solution are used to detect
40 metal ions till now, the reported dendrimers are mainly based on the commercially available flexible
41 poly(amidoamine) (PAMAM) dendrimers, ^[18] without clear location of surface functional groups and
42 they even more rarely applied as the fluorescent film sensor up to date.

43 Moreover, at present, these types of fluorescence-quenching (turn-off) sensors are greatly limited in
44 practical applications because of their weak selectivity and anti-interference ability compared with the
45 type of fluorescence-enhancing (turn-on) sensors. If these problems could be overcome, the
46 fluorescence-quenching sensors could present more direct and obvious response than the
47 fluorescence-enhancing sensor. This is the reason why a novel blue fluorescent dendrimer (PYTPAG2)
48 (Scheme 1) is studied as a fluorescence quenching sensor for Fe^{3+} both in aqueous solutions and in thin

films.

PYTPAG2 composed of pyrene as the core emitter and triphenylamine (TPA) on the periphery is a second-generation dendrimer, where the triphenylamine units provide the chelating ability towards the metal ions and the large number of them (16) around the polyphenylene encapsulated pyrene ensure the efficient contact to the metal ions, enabling the high sensitivity.^[15] When Fe^{3+} interacts with PYTPAG2, new complexes between Fe^{3+} and the triphenylamine groups are formed and this process might disrupt the FRET from the triphenylamine groups to the core groups of PYTPAG2, which causes the fluorescence quenching. During the sensing test, PYTPAG2 solution ((THF)/ H_2O (8/2, v/v)) presents fluorescence quenching only to Fe^{3+} , the fluorescence quench is 88% upon the addition of 90 μM Fe^{3+} . And this striking performance could not be disturbed by pH, other metal ions, counter anions, or the surrounding environment. In addition, for practical application, the PYTPAG2 film is also tested, which shows notable sensitivity, selectivity and good recycle ability to Fe^{3+} . Furthermore, PYTPAG2 is also tested in living roundworms with high sensitivity. To the best of our knowledge, PYTPAG2 is the first fluorescence-quenching sensor which responded solely and selectively to Fe^{3+} . What should be noted is that due to the TPA, the well-known electropolymerized (EP) unit, the PYTPAG2 has the ability to form the EP film and used as the film sensor too, which was reported separately.^[19]

PYTPAG2

Scheme 1. The Structure of PYTPAG2.

2. Material and methods

2.1 Reagents

The metal cation, including $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$, $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 6\text{H}_2\text{O}$, HgCl_2 and $\text{Pb}(\text{NO}_3)_2$, were purchased from Sinopharm Chemical Reagent Co., Ltd. The solvents used in synthesis were analytical grade, others

were spectroscopic grade. All the reagents and solvents used for the synthesis were purchased from Aldrich or Acros companies, and were used as received. All reactions were performed under a dry nitrogen atmosphere. The synthesis and related properties of PYTPAG2 are shown in the supplementary material.

2.2 Metal ions recognition process

The metal ions sensing tests of PYTPAG2 were mainly investigated by using photoluminescence (PL) spectra. The PL spectra were recorded with the excitation wavelength at 370 nm corresponding to the pyrene absorption. PYTPAG2 was dissolved in tetrahydrofuran (THF)/H₂O (8/2, v/v) at a concentration of 1 μ M. The metal ions were dissolved in H₂O at a concentration of 0.1 M. The pH was adjusted by 0.1 M HCl and 0.1 M NaOH aqueous solutions. All water used for preparing the solutions was deionized. The concentration of dichloromethane (DCM) solution for the preparation of spin-coating films is 1 mg/ml, with the speed of 1500 rpm for 1 min.

3. Results and discussion

3.1 The optical properties

Figure 1a shows the absorption and PL spectra of PYTPAG2. The absorption spectra suggest a broad and strong peak at around 310 nm due to the TPA surface chromophores and polyphenylene arms. A broad band with low intensity around 390 nm is characteristic for the pyrene absorption.^[20] The PL spectrum of PYTPAG2 shows the maximum around 430 nm and a little shoulder (~455 nm). This deep blue emission for the core can be obtained by either exciting the surface groups or the core, proving the fluorescence resonance energy transfer (FRET) from the surface groups to the core. By using a quinine sulfate solution (0.1 M H₂SO₄) as the standard, the fluorescence efficiency of PYTPAG2 in solution was determined to be about 60%.

Figure 1. (a) The absorption and PL spectra of PYTPAG2; (b) The absorption spectra of PYTPAG2 upon the addition of Fe^{3+} ; (c) The possible mechanism of PYTPAG2 and Fe^{3+} .

3.2 Detection of Fe^{3+} by PYTPAG2 solution

First the changes of the absorbance of PYTPAG2 were observed. In order to perform a UV-Vis absorption experiment, solutions of different metal ions were separately added to the prepared PYTPAG2 solutions. Only Fe^{3+} was found to perturb the electronic behavior of PYTPAG2, which showed an enhancement of the absorption. Particularly, the addition of Fe^{3+} caused a new peak around 330 nm (shown in Figure 1b) with a significant absorption enhancement as the Fe^{3+} concentration increased. This new absorption band could be assigned to complexes formed between the triphenylamine groups at the surface and Fe^{3+} ions, and this process obviously disrupts the FRET illustrated in Figure 1c.^[14] This influence is further proved by the fluorescence lifetime test of PYTPAG2 upon the addition of Fe^{3+} displayed in Figure 2. The fluorescence lifetime of PYTPAG2 did not show a difference in the fluorescence spectra before and after adding 0.1 mM Fe^{3+} (lifetime ~1.51 ns). This unchanged fluorescence lifetime further proved the formation of complexes between PYTPAG2 and Fe^{3+} excluding other possible interactions between PYTPAG2 and Fe^{3+} , such as energy transfer and electron transfer. This iron dendrimer complex may absorb most of the light and undergo non-radiative may decay disturbing the energy transfer from triphenylamine groups to the pyrene core which then is responsible for the fluorescence quenching.

Figure 2. The fluorescent lifetime of PYTPAG2 before (a) and after (b) adding Fe^{3+} (0.1 mM)

The detection of Fe^{3+} by the PYTPAG2 solution was investigated by PL spectroscopy. It can be found that PYTPAG2 acting as fluorescence-quenching sensor presents very high selectivity. Figure 3a displays the comparison of fluorescence quenching of PYTPAG2 upon additions of various metal ions, and only Fe^{3+} showed the ability to produce fluorescence quenching, and its fluorescence can be quenched 88% by the addition of 90 μM Fe^{3+} . This superior selectivity to Fe^{3+} ion is very notable. [12]. The reason for the high selectivity might be due to that Fe^{3+} has higher thermodynamic affinity and faster chelating processes towards ligands with nitrogen as the chelating atom compared with other transition-metal ions, meanwhile Fe^{3+} ion has stronger oxidizing action than other metal ions. [21-22]

Figure 3b contains the detailed PL spectra of PYTPAG2 solution upon the addition of Fe^{3+} . After the gradual increase of Fe^{3+} concentration, the fluorescence of PYTPAG2 is quenched within a quick response of 1–2 s, suggesting PYTPAG2 has rapid sensing ability to Fe^{3+} . The inset of Figure 3b (up) demonstrates the fluorescent titration curve of PYTPAG2 upon the addition of Fe^{3+} . The fluorescent static quenching coefficient K_{sv} can be calculated from the slope of the titration curve at low concentration according to the modified Stern-Vollmer equation:

$$\lg(I_0-I)/I = \lg K_{sv} + n \lg(Q)$$

Where I_0 and I are the fluorescence intensity in the absence or presence of Fe^{3+} ions, respectively, K_{sv} is the quenching constant, Q is the Fe^{3+} concentration, and n is the ratio between metal ions and the sensing materials. The K_{sv} and n of PYTPAG2 upon addition of Fe^{3+} were calculated to be 4.6×10^8 and 2, respectively. PYTPAG2 shows a linear response of the emission intensity to the concentration of Fe^{3+} which is displayed as the inset (down) of Figure 3b, and based on this linear relationship the fluorescence detection limit of PYTPAG2 towards Fe^{3+} was calculated to be 6.5×10^{-7} M, which is much lower than the drinking water maximum contaminant level (MCL) ($\sim 5.357 \mu\text{M}$) of the U.S.

148 Environmental Protection Agency (EPA).^[23]

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150 **Figure 3.** (a) The PL response of PYTPAG2 upon the addition of various metals ions (90 μM); (b) The
151 PL response of PYTPAG2 solution towards Fe^{3+} . Inset: The titration curve (up) of PYTPAG2 solution
152 towards Fe^{3+} and the linear intensity change region as a function of the concentration of Fe^{3+} (down).

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154 Usually, the detection of Fe^{3+} is dependent on the surrounding pH, however, PYTPAG2 presents
155 consistent fluorescent quenching despite varying the pH (Figure 4a). The fluorescence of PYTPAG2 is
156 steady in pH ranging from 1 to 11. Further, after adding Fe^{3+} , PYTPAG2 provides stable fluorescence
157 quenching sensitivity towards Fe^{3+} at different pHs, with a small increase at higher pH. This is another
158 advanced property of PYTPAG2 for practical applications.

159 Similarly, Mn^{2+} , Fe^{2+} , Cu^{2+} and Cr^{3+} are major interfering ions for the detection of Fe^{3+} in traditional
160 sensors.^[12] To check the interference between Fe^{3+} and other metal ions, a competitive binding
161 experiment was carried out. Figure 4b illustrates the competitive binding of PYTPAG2 upon the addition
162 of Mn^{2+} , Fe^{2+} , Cu^{2+} and Cr^{3+} with and without Fe^{3+} . As can be seen, the fluorescence intensity of the
163 PYTPAG2 was minimally quenched by the addition of Mn^{2+} , Fe^{2+} , Cu^{2+} and Cr^{3+} . But the addition of
164 Fe^{3+} ion could lead to 86% decrease in the fluorescence intensity. The results present the good selectivity
165 of PYTPAG2, which meets the requirement of minor interference from other metal ions. Furthermore,
166 the effect of the different counter anions on the sensing performance of PYTPAG2 was studied with the
167 addition of $Fe_2(SO_4)_3$ and $Fe(NO_3)_3$. The results demonstrate that the quenching processes are barely
168 influenced by the counter anions.

Figure 4. (a) The normalized PL intensity of PYTPAG2 upon the addition of 90 μM Fe^{3+} at different pH; (b) The normalized PL spectra of PYTPAG2 in mixed metal ions (Mn^{2+} , Fe^{2+} , Cu^{2+} and Cr^{3+} , each 200 μM) with and without Fe^{3+} (90 μM); (c) The fluorescent response of PYTPAG2 to Fe^{3+} (90 μM) in water sample from real world.

In order to confirm the practicality of PYTPAG2 for analyzing real world samples, the original water samples, including tap water, snow water, drinking water, and lake water of the South Lake at Changchun City, were collected and directly used in the Fe^{3+} sensing experiments^[24]. The PYTPAG2 in deionized water is used as the reference. The PL responses of PYTPAG2 in different water samples and in water samples that were spiked with Fe^{3+} ion are recorded and the results are displayed in Figure 4c. Compared with the first two columns in each group, the similar PL intensities imply that these real water samples have little interference in the performance of this fluorescence sensing system. But after adding

Fe³⁺ into these blended systems, the PL intensity of the mixture solution is significantly decreased. The fluorescence quenching degrees are similar to that of the PYTPAG2 in deionized water, which are above 90%. These results further suggest that this novel fluorometric sensor material has great potential for the Fe³⁺ detection in the real environmental samples.

3.3 Detecting Fe³⁺ ions by PYTPAG2 films

In terms of practicality, the fluorescent thin film is of more interest. Thereby, the PYTPAG2 spin-coating films are also used to detect Fe³⁺. Figure 5a is the PL spectra of a PYTPAG2 film in the presence of Fe³⁺ under different concentrations. The fluorescence quench of PYTPAG2 reaches 50% by the addition of 40 μM Fe³⁺. The inset (up) of Figure 5a provides the fluorescent titration curve of PYTPAG2 films upon the addition of Fe³⁺. According to the modified Stern-Vollmer equation, the K_{sv} and n of PYTPAG2 upon addition of Fe³⁺ are calculated to be 2.3 × 10⁹ and 2, respectively. A linear response of the emission intensity to the concentration of Fe³⁺ was detected (Figure 5a down) based on this linear relationship, the fluorescence detection limit of PYTPAG2 to Fe³⁺ is calculated to be 5.0 × 10⁻⁷ M. Moreover, the quenching processes are barely influenced by counter anions.

To examine the detection capability of PYTPAG2 films in the real world applications, natural water samples were tested. The addition of Fe³⁺ (40 μM) can lead to significant fluorescence quenching of PYTPAG2 films in all natural water samples, which is similar to the result in the deionized water (shown in Figure 5b). This reveals that the PYTPAG2 films are more sensitive towards Fe³⁺ than other compositions in the natural world water samples. However, the degrees of fluorescence quenching are slightly lower than those of in deionized water. When adding 40 μM Fe³⁺ as the final detection concentration the quenching of the PL intensities are all around 50%. The recovery rate of PYTPAG2 film is around 90% and the RSD of Fe³⁺ is around 3%, which is similar to that in the deionized water. The performance suggests that PYTPAG2 films are good candidates for Fe³⁺ detection.

Similar to PYTPAG2 solutions, a competitive binding experiment was also carried out for PYTPAG2 films. Figure 5c illustrates the fluorescence intensity changes of PYTPAG2 films upon the addition of mixed metal ions (Mn²⁺, Fe²⁺, Cu²⁺ and Cr³⁺, each 100 μM), as well as 40 μM Fe³⁺ in aqueous solution. In these experiments, the PYTPAG2 films was first immersed in the solution containing mixed metal ions, and it was found that the fluorescence intensity of the film sensor was only quenched by 1%. After Fe³⁺ was added to the mixed metal ions, a 48% decrease in the fluorescence intensity was obtained. PYTPAG2 films are discovered to exhibit superior selectivity to Fe³⁺ in the presence of other heavy and

216 transition metal ions, which meets the requirement for the practical sensor of minor interference from
 217 other metal ions.
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222 **Figure 5.** (a) The PL spectra of PYTPAG2 spin-coating films in the presence of different concentrations
 223 of Fe³⁺. Inset: the titration curve (up) and linear intensity change region as a function of the
 224 concentration of Fe³⁺ (down); (b) The fluorescent response of PYTPAG2 films to Fe³⁺ in water from real
 225 world; (c) The PL spectra of PYTPAG2 film in mixed metal ions (Mn²⁺, Fe²⁺, Cu²⁺ and Cr³⁺, each 100
 226 μM) with and without 40 μM Fe³⁺; (d) The fluorescent recovery cycles of PYTPAG2 spin-coated films.

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228 3.4 The regenerability of PYTPAG2 films

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230 Additionally, the PYTPAG2 films show the ability to reversible bind the Fe³⁺ ions. After being exposed
 231 to Fe³⁺ the PYTPAG2 films can repeatedly be used after being washed with EDTA (ethylene
 232 diaminetetraacetic acid) and water 3 times followed by thermal annealing in a vacuum oven at 45 °C for
 233 3 h. After these treatments, the PYTPAG2 films regain fluorescence and can again be used to detect Fe³⁺.
 234 Moreover, it exhibits rather stable sensitivity to Fe³⁺ as displayed in Figure 5d and Table 1
 235 (Supplementary Material). Point 1 is corresponding to the original fluorescent intensity of PYTPAG2

films; while points 3, 5 and 7 are corresponding to PYTPAG2 films washed with EDTA and pure water multiple times after exposure to Fe^{3+} ; points 2, 4, 6 and 8 correspond to PYTPAG2 films again exposed to Fe^{3+} (40 μM). After repeating for four times, the fluorescence quenching of PYTPAG2 to Fe^{3+} is still around 40%. The recovery rate of PYTPAG2 films is around 90% per regeneration and the RSD of Fe^{3+} is 3.04%.

3.5 Practical application in living organism

Figure 6. (a) Roundworms loaded with no PYTPAG2 for 24 h as control. (b) Roundworms loaded with 10 μM PYTPAG2 for 12 h. (c) and (d) Roundworms then loaded with 1 μM of iron ion for 1 h and 6 h, respectively. Scale bar is 500 μm .

To further demonstrate the practical application of the probe, we carried out experiments in living roundworms. Roundworms are widely distributed in environments such as water and soil, which play an important role in the enrichment of metal ions. Thus, it is possible to detect the concentration of metal ions in the roundworms as a representation of their environmental concentration. Meanwhile, their clear biological structures make detection simple. Nowadays, roundworms have been widely used in the biological and medical testing representing significant research value to the human health and environmental monitoring. [25-27] Roundworms were incubated for 24 h at 20 $^{\circ}\text{C}$, and there was no observed fluorescence inside the living worms (Figure 6a). Then the roundworms were incubated with

PYTPAG2 (10 μ M) in the medium for another 12 h at 20 $^{\circ}$ C, and a significant increase in the fluorescence inside the living roundworms was observed (Figure 6b). After treating the worms with Fe^{3+} (1 μ M) for 1h (Figure 6c) and 6h (Figure 6d), respectively, there was obvious quenching of the fluorescence signal, with nearly quantitative quenching occurring after 6 h. This experimental results demonstrate the ability of the dendrimer to act as an efficient sensor for iron ions for medical research and environmental monitoring.^[28-31]

4. Conclusion:

A novel fluorescent dendrimer PYTPAG2 was studied with regard to the potential application as a fluorescence-quenching sensor for Fe^{3+} ions. Specially, PYTPAG2 presents high and stable sensitivity and selectivity to Fe^{3+} in solution (the detection limit is 6.5×10^{-7} M) and thin films (the detection limit is 5.0×10^{-7} M), respectively, which is among the best reported results. It should be noted that PYTPAG2 only exhibits a response to Fe^{3+} and this striking performance could not be influenced by the pH of the solution, the addition of various other metal ions and counter anions, as well as the surrounding environment. Furthermore, the ability of the dendrimer to detect iron ions in the living roundworm represents a significant advancement in their value for medical and environmental applications. This study provides a promising and practical Fe^{3+} sensing material.

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PYTPAG2

Scheme 1. The Structure of PYTPAG2.

Figure 1. (a) The absorption and PL spectra of PYTPAG2; (b) The absorption spectra of PYTPAG2 upon the addition of Fe^{3+} ; (c) The possible mechanism of PYTPAG2 and Fe^{3+} .

Figure 2. The fluorescent lifetime of PYTPAG2 before (a) and after (b) adding Fe^{3+} (0.1 mM)

Figure 3. (a) The PL response of PYTPAG2 upon the addition of various metals ions (90 μM); (b) The PL response of PYTPAG2 solution towards Fe^{3+} . Inset: The titration curve (up) of PYTPAG2 solution towards Fe^{3+} and the linear intensity change region as a function of the concentration of Fe^{3+} (down).

Figure 4. (a) The normalized PL intensity of PYTPAG2 upon the addition of 90 μM Fe^{3+} at different pH; (b) The normalized PL spectra of PYTPAG2 in mixed metal ions (Mn^{2+} , Fe^{2+} , Cu^{2+} and Cr^{3+} , each 200 μM) with and without Fe^{3+} (90 μM); (c) The fluorescent response of PYTPAG2 to Fe^{3+} (90 μM) in water sample from real world.

Figure 5. (a) The PL spectra of PYTPAG2 spin-coating films in the presence of different concentrations of Fe³⁺. Inset: the titration curve (up) and linear intensity change region as a function of the concentration of Fe³⁺ (down); (b) The fluorescent response of PYTPAG2 films to Fe³⁺ in water from real world; (c) The PL spectra of PYTPAG2 film in mixed metal ions (Mn²⁺, Fe²⁺, Cu²⁺ and Cr³⁺, each 100 μM) with and without 40 μM Fe³⁺; (d) The fluorescent recovery cycles of PYTPAG2 spin-coated films.

Figure 6. (a) Roundworms loaded with no PYTPAG2 for 24 h as control. (b) Roundworms loaded with 10 μM PYTPAG2 for 12 h. (c) and (d) Roundworms then loaded with 1 μM of iron ion for 1 h and 6 h, respectively. Scale bar is 500 μm.