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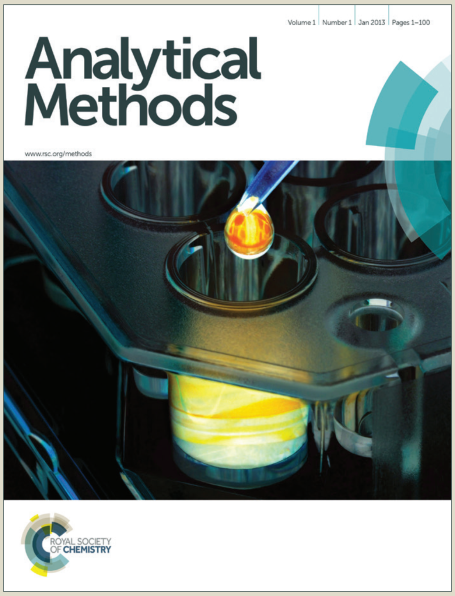

Reversible multi-control fluorescent switch and molecular logic gate based on selective recognition of Fe^{3+} and its application to multiplex detection

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Abstract: A novel chemosensor, 2-hydroxy-4-(pyridin-2-ylmethyleneamino)benzoic acid（short for PA）has been developed and found to be a selective fluorescent detector of Fe^{3+} over a wide range of treated metal ions. As there was carboxyl in PA, the fluorescence of this Schiff base could be reversibly controlled by adding OH^- and H^+ alternately. In addition, because Fe^{3+} selectively quench the fluorescence of PA, the quenched fluorescence can be recovered by adding EDTA as competitor or $NH_2OH \cdot HCl$ as reducing agent. As a result, the fluorescence intensity of PA could be reversibly turned by H^+/OH^- or Fe^{3+}/EDTA or $NH_2OH \cdot HCl/K_2S_2O_8$ in aqueous solution. These results demonstrated that PA along with PA-Fe^{3+} system can perform as not only a reversible multi-control fluorescent switch but also “NOR”, “OR” and “INHIBIT” logic gates. Furthermore, analytical application of this fluorescent probe was investigated and achieved promising and satisfying result.

Key words: fluorescent switch; molecular logic gate; salicylic acid Schiff base; ferric ion; multiplex detection

Introduction

Molecular logic gates with Boolean functions could be realized by means of

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molecular switches and are able to process information and perform arithmetic operation [1, 2]. Among various molecular switches, considerable attention has been paid on fluorescent switches due to their excellent property of high sensitivity, simple to control and easy detection [1, 3]. Fluorescent switches discovered so far could be regulated through different ways, including chemical control [4~6], light control [7] as well as electrochemical control [8]. In the past few decades, the study of fluorescent logic gates based on chemical response against proton and metal ion has been carried out most profoundly and acquired richest results in general[9~12].

Since the pioneering De Silva group established AND logic gate by adopting proton and sodion as input signal and fluorescent as output signal in 1993, numerous relative studies have been reported [13]. For instance, Wang et al. synthesized a salicylic acid Shiff base containing derivative of binaphthyl that could realize the selective recognition of Cu^{2+} and Zn^{2+}, and established OR and INHIBT logic gates [14]; Kaur et al. constructed AND logic gate basing on aniline modified quantum dots and quenching group, whose fluorescence was quenched in the absence of H^+ and Na^+ and reversed in the presence of both H^+ and Na^+ at same time [11]. Except proton and metal ions [9-12], electron [15], ligand [16], organic molecule [17], biomolecule [18], light together with heat [19] also have been adopted as input signals in recent years. By taking advantages of the competition between fluorescent and photo-induced electron transfer (PET) process, Margi et al. constructed an AND along with an INHIBT logic gates based on a fluorescent molecule containing one fluorophor and two receptors[15]; Pischel et al. designed a water-soluble phthalimide dye whose fluorescence could be adjusted by ligand or proton through the PET process[16]. A Schiff base derivative of ferrocene synthesized by Fang et al. presented fluorescence which could be controlled by chemical oxidation-reduction [5]; Seeberger’s group fabricated a molecular logic gate on carbonhydrate-lectin interaction, and inputs for this system were pH N, N'-4, 4'-bis (benzyl-2-boronic acid) bipyridinium dibromide, and different lectins while the relative change in fluorescence quantum yield of the Ru (II)-glycodendrimers serving as output [18]. Furthermore, numerous emerging materials such as semiconductor quantum dots [11,21], silicon NPs [17,22], silicon nanowires[23], graphene[24], Au nano-clusters[25], carbon nanotubes[26] as well as some bio-interaction such as DNA cleavage and intercalation[27] have been applied to the establishment of fluorescent logic system. Molecular logic system with multi-functions and wide-application has attracted extensive and deep interests of researchers [28].

Even though fluorescent molecular logic gates have been widely and extensively reported, most of them were relatively complex molecule structure and mainly based on the fluorescent probe responding to only two kinds of stimulations. It is still a challenge to develop fluorescent sensors with simple structure and respond to several analytes and easily to create logic gates [29].

Till now, to our best knowledge, there is no related report about any molecular logic gate based on a fluorescent probe performing selective response to an ion in certain valence (such as ferric ion) thus its fluorescence could be adjusted by ion ligand or valence adjustment conveniently. Additionally, there are few reports with respect to the fluorescent molecule responsive to various type of input signal. The present paper put forwards the 2-hydroxy-4-(pyridin-2-ylmethyleneamino)benzoic acid（PA）which displays excellent fluorescent performance. Our literature research found that there once being a paper about PA of Roberts Brett [30], however, it is aimed at the anti-bacterial performance instead of the fluorescence feature researched by present paper. Our experiments indicates that the fluorescence of PA could be reversibly modulated not only by proton or ligand, but also by oxidation-reduction, including chemical, electrical, light controlled redox reaction, which would result in valence change of ferric ion. On the basis of this finding, we perform as reversible multi-control fluorescent switch and “NOR”, “OR” and “INHIBIT”logic gates. Furthermore, analytical application of this fluorescent probe was investigated and achieved promising and satisfying result.

1 Experiment

1.1 Reagents and Apparatus

4-amino salicylic acid and 2-pyridylaldehyde used in this experiment were of chemical pure and purchased from Acros Organics（New Jersey，USA）. Other chemicals were of analytical grade. Milk power and soft drink sample were brought from market.

Fluorescent spectrum is performed on RF-5301PC fluorophotometer (Shimadzu, Japan) equipped with a 1×1 cm cuvette. Fourier transform infrared (FT-IR) spectrum was recorded on IR-408PC fourier transform infrared spectrometer (Bruker, Germany). MS was determined on an 1100 LC/MSD Trap (Agilent, USA) by direct injection. ^{1}H NMR and ^{13}C NMR spectra were acquired on a Varian Mercury VX-300 MHz spectrometer. UV/Vis absorption spectra were obtained on a UV/Vis spectrophotometer (UV-2550, SHIMADZU).

1.2 Preparation and Identification of Fluorescent switch PA

0.765g 4-amino salicylic acid were dissolved in a small amount of absolute ethanol and 0.535g of 2-pyridylaldehyde were dissolved in 30 mL absolute ethanol. The above two solutions were mixed in a 100 mL round-bottom flask and refluxed for 3 hours in 75℃ water bath. Orange precipitation obtained were conducted for re-crystallization with double-distilled water, filtration and drying successively and finally ended into deep-yellow solid (Scheme S1).

The chemical structure of synthetic product was confirmed by FT-IR, MS and ^{1}H NMR and ^{13}C NMR. The absorption in 1640 cm^{-1} within the IR spectrum owned to the stretching vibration of -N=C- which indicated the existence of Shiff base structure (Fig. S2). The m/z value measured on MS was 243.1, roughly equal to the calculated value: 243.2. ^{1}H NMR (DMSO): δ 8.9(s,1H,-CH=N), 8.68-8.69(d, 1H, aromatic), 8.39(s, 1H, Ar-H), 7.86-7.95(d, 1H, Ar-H), 7.50-7.54(d, 1H, Ar-H), 6.95(s, 1H, OH), 6.63-6.66(m, 3H, aromatic); ^{13}C NMR: δ 171.72, 164.25, 158.28, 155.45, 154.55, 149.48, 136.49, 134.33, 125.00, 121.79, 113.21, 108.36 and 107.62. Aforementioned identification results demonstrated the successful synthesis of target molecule.

1.3 Method

200 μL of PA （4.0×10^{-3} mol L^{-1}）was put into a 10 mL colorimetric tube

accurately, then add 100 μL of Fe^{3+}（1.0×10^{-3} mol L^{-1}） solution and 1.0 mL of HAc-NaAc buffer solution（0.1 mol L^{-1}）adjusting the pH value of this solution to 3.8 approximately. The mixed solution was diluted to 10mL with distilled water and let it stand for 20min. After that, the fluorescence properties were investigated under fixed $\lambda_{ex}/\lambda_{em}$.

Fluorescent Switch Experiment: PA solution system and PA-Fe^{3+} complex solution system were selected as two separate initial states. Fe^{3+}, proton as well as redox reagents such as $NH_2OH \cdot HCl$ and $K_2S_2O_8$ were triggered agents of fluorescent switch. Typically, these two solution systems were exposed to two triggered agents alternatively separately and put into fluorescence detection under each state. Please note that the volume of triggered reagents added shall never surpass 100 μL ensuring that the total solution volume did not change significantly.

Multi-detection Application of PA Fluorescent Probe: 1.0 g of milk powder sample was put in a crucible for carbonization on an electric stove. After turned into grey powder, it was burned in a muffle furnace for 270 min under 500 ℃ until the color turned to white totally. After cooling to room temperature, add 2 mL, 8mol L^{-1} HNO_3 and diluted to 100 mL with distilled water thus obtain the sample solution whose labeled concentration was 2.68×10^{-5} mol L^{-1}. Recovery rate was measured by adding certain amount of 1.0×10^{-3} mol L^{-1} Fe^{3+} standard solution. Labeled content of Fe is 150 μg/g。

The soft drink sample was treated with ultrasound for 20 min to remove carbon dioxide dissolved. Then dilute the sample by a factor of 100.

2 Result and Discussion

2.1 Fluorescent Property of PA

Fig.1 depicts the fluorescence emission spectrum of PA after reacting with $Fe^{3+}$and EDTA within solution whose pH value is 4.0. As shown in Fig.1, PA emits strong fluorescence (curve 1) which is quenched for the formation of complex of PA and Fe^{3+} upon the addition of Fe^{3+} solution (curve 2). Because EDTA performs stronger coordination ability of Fe^{3+}, fluorescence of the system is reversed as a result of the dissociation of PA-Fe^{3+} complex (curve 3), which shows potential to be developed into a fluorescent switch controlled by coordination reaction. Further research indicates that fluorescence of the system could be adjusted by proton and redox reaction.

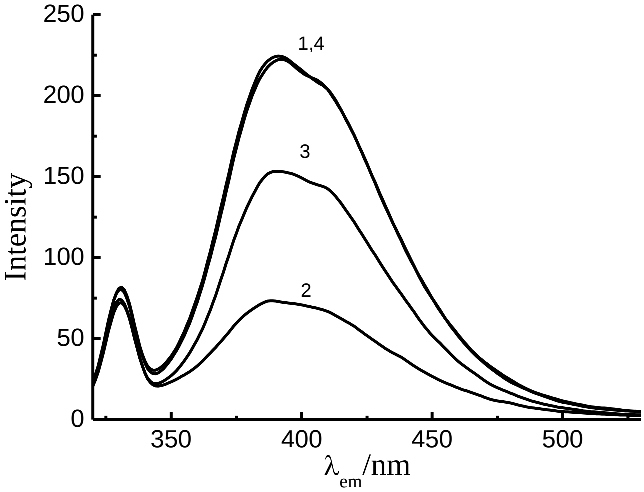


Figure 1 Fluorescent spectrum after addition of $Fe^{3+}$and EDTA in HAc-NaAc buffer solution (pH=4.0, 0.1 mol L^{-1}) (λ_{ex}=295 nm): 1-PA (0.8×10^{-5}mol L-1); 2-1+Fe^{3+} (1.0×10^{-5} mol L-1); 3-2+EDTA (1.0×10^{-5}mol L^{-1}); 4-2+EDTA (1.5×10^{-5}mol L^{-1}).

In order to find out combined ratio between PA and Fe^{3+}, we draw the Job's Plot according to the method in literatures [31~32] (Fig.2). Set the total concentration of PA and Fe^{3+} at 2.0×10^{-5} mol L^{-1}, change the concentration ratio of PA and Fe^{3+} and measure its fluorescent intensity. Use the fluorescence quenching value（$\varDelta F=F_0-F$）under 395 nm radiation and PA proportion in total concentration to draw a plot as Fig.2. On this figure the PA proportion in total concentration corresponding to the maximum value is defined as the best combine ratio between PA and Fe^{3+} in the complex. Complexation constant calculated in accordance with formula (1) is turned out to be 7.796×10^{11} L^2 mol^{-2} which is a relatively large figure indicating the complex formed between PA and Fe^{3+} is very stable.

$$Fe^{3+} + 2PA \rightarrow FePA_2$$

$$K = \frac{[FePA]}{[Fe][PA]^2} = \frac{C_{FePA} \times \varDelta F / \varDelta F'}{[C_{Fe} \times (\varDelta F' - \varDelta F) / \varDelta F'][C_{PA} \times (\varDelta F' - \varDelta F) / \varDelta F']^2} \qquad (1)$$

⊿F is the real quenching value measured in the experiment while ⊿F′ is the value achieved by linear fitting of Fig.2.

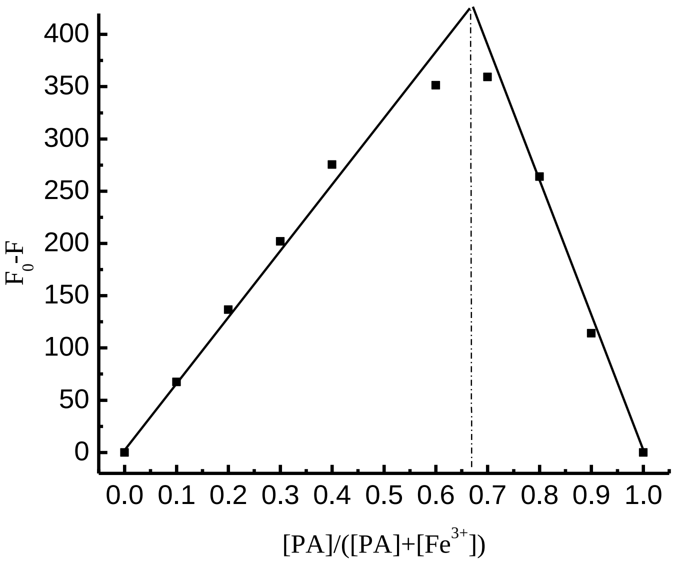


Figure 2 Job’s Plot of PA-Fe^{3+} system:

[PA]+[Fe^{3+}]=2.0×10^{-5} mol L^{-1}, HAc-NaAc buffer solution (pH=4.0, 0.1 mol L^{-1}).

($\lambda_{ex}/\lambda_{em}$ = 295/392 nm).

2.2 Fluorescence quenching mechanism

In order to understand the fluorescence quenching mechanism between PA and Fe^{3+}, the UV–Vis spectra of PA (Fig.S2) in the absence and presence of Fe^{3+} were investigated. The UV-Vis absorption spectrum of PA exhibits two absorption bands centered around 270 nm and 305 nm, which correspond to typical absorption for salicylic Schiff base derivatives.[33] However, these two absorption bands increase and a new absorption band centered at 480 nm emerges in the presence of Fe^{3+}, which may be ascribed to the newly formed complex during the reaction. It can be seen that interaction between PA and Fe^{3+} exists.

Therefore, we speculate the possible reason for the fluorescence quenching is the formation of a nonluminous complex by the coordination between Fe^{3+} and the carbonyl group on PA, which lead to either an electron transfer or an electronic energy transfer. And this electron transfer may prevail over the electron transfer process existing in PA which gives PA strong fluorescence. On one hand, inner molecular electron transfer process in PA does not function well while on the other hand, nonluminous complex is formed. Thus, fluorescence of this system is quenched and the mode of quenching is static quenching.

2.3 Multi-control Fluorescent Switch

2.3.1 Proton-controlled fluorescent Switch

When pH value is less than 4.0, system's fluorescence decreases along with pH and this may be ascribed to the protonation of carboxyl group in PA. Firstly, certain amount of HCl（1.0×10^{-2} mol L^{-1}）is added into PA solution system with initial pH of 4.0 until system pH value reaches 2.0 then measure its fluorescence intensity at 392 nm; Secondly certain amount of NaOH is added into the previous solution system until the pH value reaches 5.0, then measure its fluorescence again. Repeat this process for six times and the result is shown in Fig.3, manifesting that the system's fluorescence could be reversibly regulated and adjusted by the presence of OH^- and H^+.

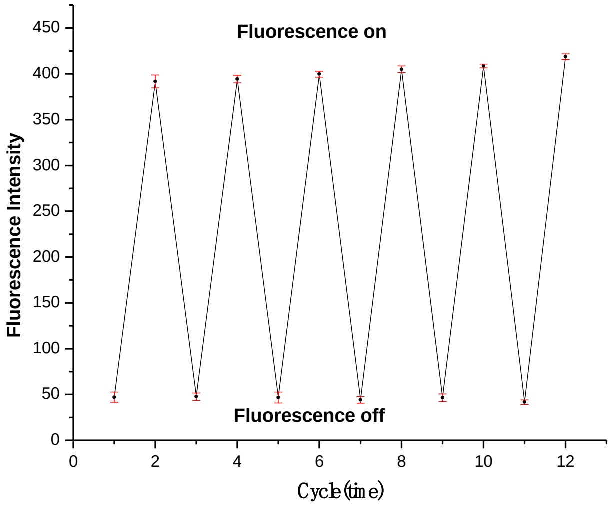


Figure 3 Change of fluorescence intensity upon alternative addition of H^+ and OH^-: C_{PA}: 5.0×10^{-6} mol L^{-1}; HAc-NaAc (pH=3.8, 0.1 mol L^{-1}).

2.3.2 Ligand- controlled Fluorescent Switch

Upon addition of 100 μL of Fe^{3+} (1.0×10^{-3} mol L^{-1}), the fluorescence of PA in solution that pH value is 4.0 is quenched as a result of the complex formed between PA and Fe^{3+}. However, EDTA performs better complexing capacity than PA (complexation constant $K=1.259\times10^{25}$). So if add 100 μL of EDTA (1.0×10^{-3} mol L^{-1}), system's fluorescence recovers due to the disaggregation of PA-Fe^{3+} complex. Repeat this experiment for three times and the result is shown in Fig.4, indicating that ligands such as Fe^{3+} and EDTA could also adjust the system's fluorescence reversibly.

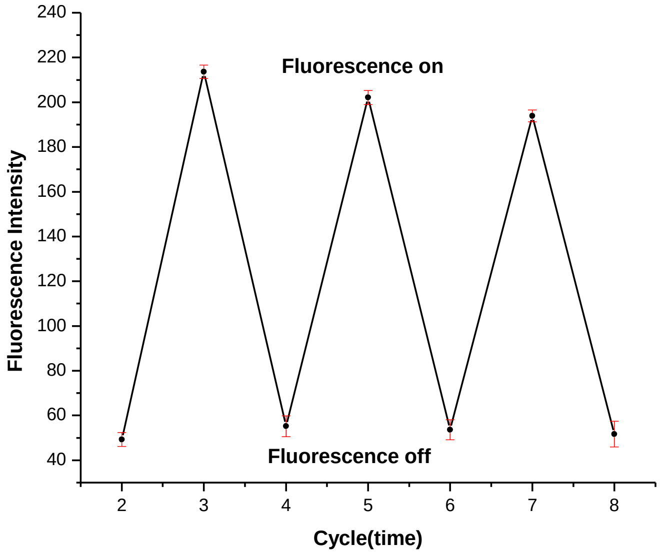


Figure 4 Fluorescent quenching and recovering profile of PA upon alternative addition of Fe^{3+} and EDTA:

PA: 0.5×10^{-5}mol L^{-1}, Fe^{3+}: 1.0×10^{-5} mol L^{-1}, EDTA: 1.0×10^{-5} mol L^{-1},

HAc-NaAc buffer solution (pH=4.0, 0.1 mol L^{-1}), ($\lambda_{ex}/\lambda_{em}$ = 295/392 nm).

2.2.3 Redox-controlled Fluorescent Switch

Even though Fe^{3+} could quench PA's fluorescence at 392 nm by forming a PA-Fe^{3+} complex, Fe^{2+} do not perform this property. Thus, once adding some reducing agent as hydroxylamine hydrochloride of certain concentration, Fe^{3+} in the system is reduced to Fe^{2+} and PA's fluorescence is recovered consequently. Similarly, the presence of appropriate oxidizing agent as $K_2S_2O_8$ would oxidize Fe^{2+} into Fe^{3+} and results in quenching of system's fluorescence. Repeat the procedures above for several times and results are shown in Fig.5. Hence, the effect of $NH_2OH·HCl$ and $K_2S_2O_8$ on the fluorescence of the system could be used as an effective fluorescence regulation to achieve a molecule switch.

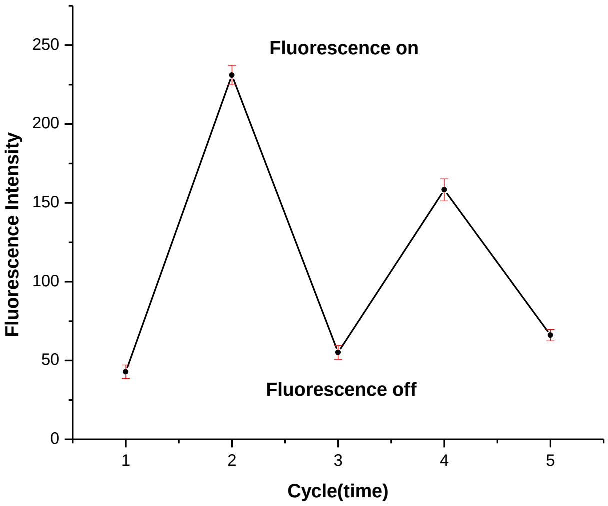


Figure 5 Variation of fluorescence intensity upon alternative addition of $NH_2OH·HCl$ and $K_2S_2O_8$. (PA: 0.5×10^{-5} mol L^{-1}, Fe^{3+}: 1.0×10^{-5} mol L^{-1}, $NH_2OH·HCl$: 5.0×10^{-5} mol L^{-1}, $S_2O_8^{2-}$: 2.0×10^{-5} mol L^{-1}, HAc-NaAc buffer solution (pH= 4.0), $\lambda_{ex}/\lambda_{em}$ = 295/392 nm)

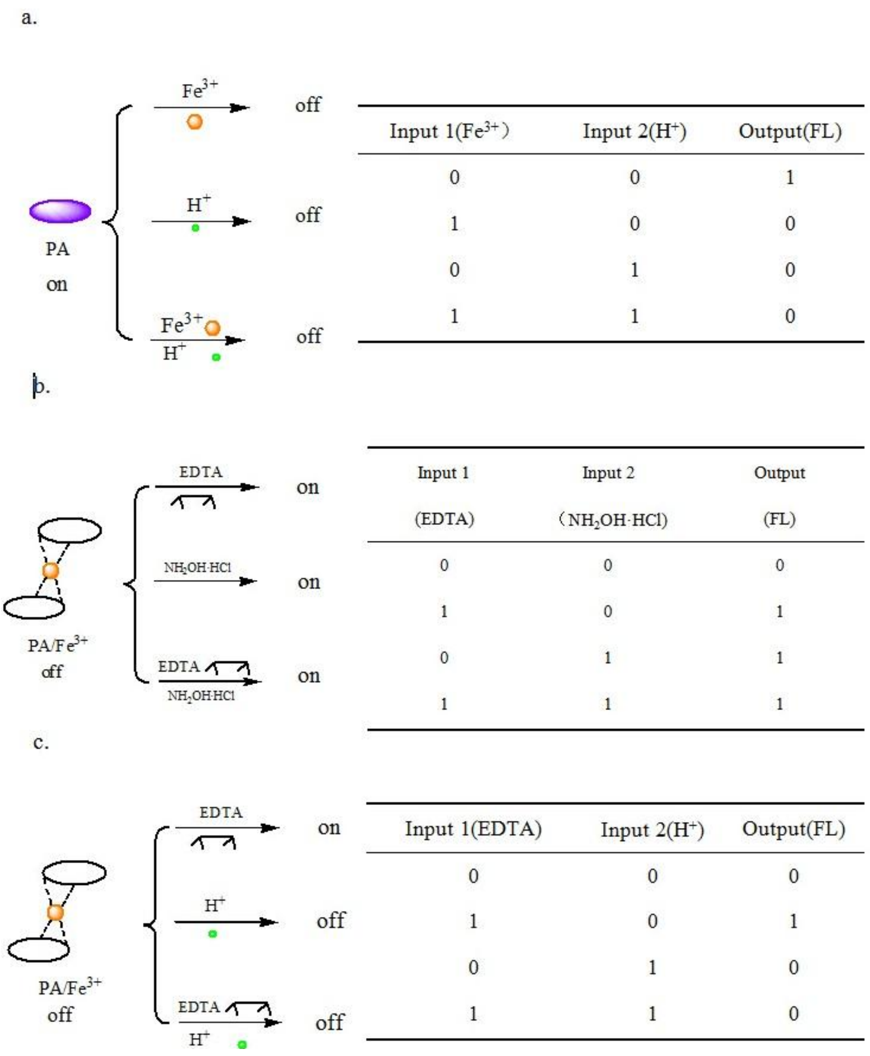
It can be seen from Fig.4 and Fig.5 that the "turn-on" fluorescence decreases along with the circles. We think the reason might be as follows. In Fig.4, when adding EDTA, system's fluorescence quenched by Fe^{3+} is recovered. As we all know, no reaction is carried out in 100%. The same for this fluorescence recover reaction. In fact, in order to recover PA's fluorescence thoroughly, the amount of EDTA added for fluorescence recovery is a little excessive. So if we add Fe^{3+} of the same amount to quench this recovered fluorescence in the second cycle, the fluorescence of the system will not be quenched to the same level of the start. Therefore, the "Turn-on" fluorescence in Fig.4 decreases along with the circles. Similarly, fluorescence in Fig.5 decreases for the same reason.

2.3 Molecular Logic Gate

According to the system's fluorescent property above, we investigated into their application in molecular logic system construction. Choose PA solution and PA-Fe^{3+} complex solution as initial state and its fluorescence intensity as output signal, thus two input NOR、OR and INHIBIT logic gate were fabricated successfully.

As shown in Fig. 6 (a), within the PA solution system（pH=4.0）Fe^{3+}(input 1) and H^+ (input 2) were used as input and the emission band at 392 nm was taken as output in this system. The emission intensity at 392 nm was distinctly high only when the inputs were (0, 0) while the output was low when inputs were (0, 1), (1, 0) and (1, 1). Therefore, a double-input NOR logic gate was established by using Fe^{3+} and H^+ as input and taking I_{392} as the output.

Similarly, taking the PA-Fe^{3+} complex solution system（pH=4.0） as original state, use EDTA(input 1) and $NH_2OH \cdot HCl$ (input 2), the emission band at 392 nm was taken as output in this system . The emission intensity at 392 nm was very low if the input were (0, 0) while the output was extremely high when inputs were (0, 1), (1, 0) and (1, 1). Thus, the OR logic gate was achieved as shown in Fig. 6 (b).



Input 1(Fe^{3+})	Input 2(H^+)	Output(FL)
0	0	1
1	0	0
0	1	0
1	1	0

Input 1 (EDTA)	Input 2 （$NH_2OH·HCl$)	Output (FL)
0	0	0
1	0	1
0	1	1
1	1	1

Input 1(EDTA)	Input 2(H^+)	Output(FL)
0	0	0
1	0	1
0	1	0
1	1	0

Figure 6 Illustration of NOR (a), OR (b) and INHIBIT (c) Logic Gates.

Moreover, if using EDTA and H^+ as input 1 and input 2 respectively within PA-Fe^{3+} solution system（pH=4.0）, a typical INHIBT logic gate was formed(Fig. 6 (c)). Only when the inputs were in a (1, 0) sequence, output of the system was 1 while the outputs for other input sequences as (0, 1), (0, 0), (1, 1) were 0 which means relatively low fluorescence intensity. Similarly, another INHIBT logic gate could be achieved by using $NH_2OH·HCl$ and H^+ as input1 and input 2 within PA-Fe^{3+} solution system（pH=4.0）.

2.4 Multi-detection Application of PA Fluorescent Probe

2.4.1 Fe^{3+} Detection

The fluorescence emission properties of PA are sensitive to the presence of nanomolar concentration of Fe^{3+}. The quenching degree （F_0/F）depends linearly upon the addition of different concentrations of Fe^{3+} from 0.4μM to 6.5μM in 0.1M HAC-NaAc buffer solution (R=0.997) with the detection limit of 4.8×10^{-8} mol L^{-1}, which is much lower than the maximum level of Fe^{3+} ions (0.3mg L^{-1}, 5.4μM) permitted in drinking water by the US Environmental Protection Agency[34], Indicating this fluorescent sensor possessed the potential sensitive assay of Fe^{3+} in aqueous solution. Fluorescent emission spectrum of PA (curve 1) and PA-Fe^{3+} (curve 2-8) were depicted in Fig. 7.

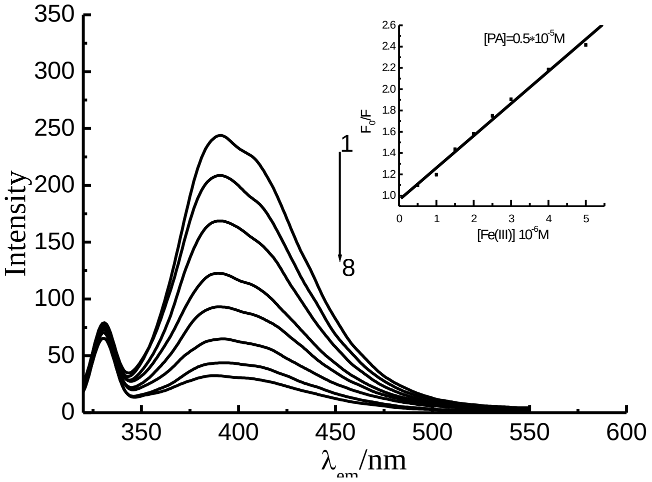


Figure 7 Fluorescence emission spectra of PA (5.0×10^{-6} mol L^{-1}) in HAc-NaAc buffer solution (pH=4.0, 0.1 mol L^{-1}) upon addition of different concentration of Fe^{3+}. Concentration of $Fe^{3+}$from top to down was from 0 to 1.0×10^{-5} mol L^{-1}. The inset is the linear relationship between the fluorescence intensity and the concentration of Fe^{3+} over the range of 0.4nM to 6.5nM.

We further studied the influence of a series of common ions on the system. Upon addition of 1.0×10^{-5} mol L^{-1} various ions, only Fe^{3+} could remarkably quench the fluorescence of PA while other ions have no significant effect on the system's fluorescent intensity. As shown in Fig.8, PA processes excellent selectivity of Fe^{3+}. The reason for PA sensor's selectivity for Fe^{3+} should be ascribe to the different outer shell electron distribution between Fe^{3+} and Fe^{2+}. The HOMO-LUMO energy gap of Fe^{3+} match the energy level required in the complexation. Therefore, the PA sensor is selective to Fe^{3+} rather than Fe^{2+}.

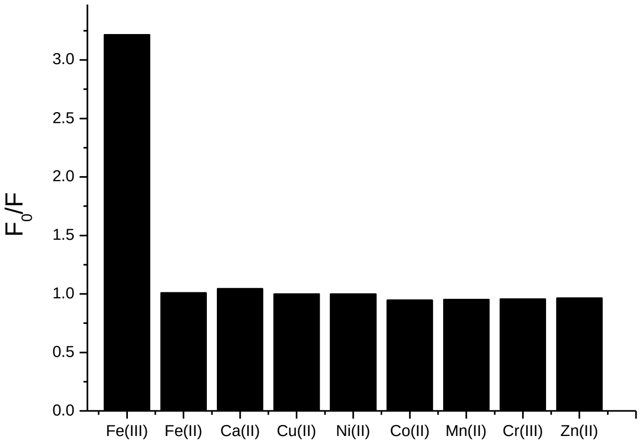


Figure 8 Effect of various metal ions on PA's fluorescent intensity. (PA: 5.0×10^{-6}mol L^{-1}, Fe^{3+} and other cations: 1.0×10^{-5}mol L^{-1}; HAc-NaAc buffer solution (pH= 4.0, 0.1mol L^{-1}), $\lambda_{ex}/\lambda_{em}$=295/392 nm)

Table 1 shows the comparison between present work and other iron probes reported in other papers. It can be found that PA is more selective and relatively sensitive fluorescence reagent for Fe^{3+}, and the LOD of this probe is comparable with the literature reported values.

Table1 Comparison of methods in this work and several other papers

Reagent[a]	$\lambda_{ex}/\lambda_{em}$（nm）	Linear Range (mol L^{-1})	LOD (mol L^{-1})	Experimental Condition	Ref.
GO nanosheets	400/500-550	1.4×10^{-5}-1.4×10^{-4}	1.8×10^{-5}	fluorescence quenching	34
Compound A	246/455	4.0×10^{-6}-2.0×10^{-5}	4.0×10^{-6}	ratio fluorescence	35
RC	500/552	6.0×10^{-8}-7.0×10^{-6}	1.4×10^{-8}	fluorescence enhancement	36
Ds-DPA	340/550	2.5×10^{-6}-2.5×10^{-5}	6.2×10^{-7}	fluorescence quenching	37

S1	558/580	5.0×10^{-6}-2.0×10^{-5}	5.0×10^{-6}	fluorescence enhancement	38
ARH	530/575	No data	7.0×10^{-8}	fluorescence enhancement	39
L	319/420	5.0×10^{-7}-4.5×10^{-6}	6.0×10^{-8}	fluorescence enhancement	40
N-GQDs	360/440	1.0×10^{-6}-1.9×10^{-3}	9.0×10^{-8}	fluorescence quenching	41
2PC-PPH	288/344	6.0×10^{-7}-1.0×10^{-5}	3.6×10^{-7}	fluorescence quenching	42
PA	295/392	4.0×10^{-7}-6.5×10^{-6}	4.8×10^{-8}	fluorescence quenching	Present work

[a] The abbreviation of the reagents represented as follows: Compound A: 2,3,4,5-Tetrephenyl-cyclopentenone, RC: Rhodamine based fluorescence probe, Ds-DPA: 5-(dimethylamino)-N,Nbis(pyridin-2-ylmethyl) naphthalene-1-sulfonamide, ARH: acetyl rhodaminehydroxamate, L: derivative of 2-(2-Hydroxyphenyl)benzothiazole, N-GQDs: Nitrogen-doped Graphene Quantum Dots, 2PC-PPH:2-pyridinecarbaldehyde-p-phenylenedihydrazon.

This method was applied in Fe^{3+} measurement of milk powder. Result is summarized in Table 1 and show good accordance with the added and found value.

Table 2 Analysis result and recovery rate of milk sample

No. of Samples	Fe^{3+} (10^{-6} mol L^{-1})		Recovery (%)
	added	found	
1	0	2.77	——
	1.0	3.82	105
2	0	5.15	——
	1.0	6.13	98
3	0	8.21	——
	1.0	9.18	93

2.4.2 Detection of Citric Acid

Citric acid could recover the fluorescence of PA-Fe^{3+} system proportionately, showing feasible application of this fluorescent probe into citric acid measurement.

If the concentration of Fe^{3+} added was twice the concentration of PA, the fluorescence of PA is quenched dramatically (curve 2). But once adding some citric acid certain concentration, the fluorescence was notably recovered as shown in Fig. 9. The experiment results demonstrated that recovering degree of fluorescence at 392 nm is linearly depended on the addition of different amount of citric acid from 2μM to 20μM (R=0.998) and the detection limit was 0.22μM (three times of the standard deviation of the blank solution). Therefore, we establish a novel approach for citric acid measurement.

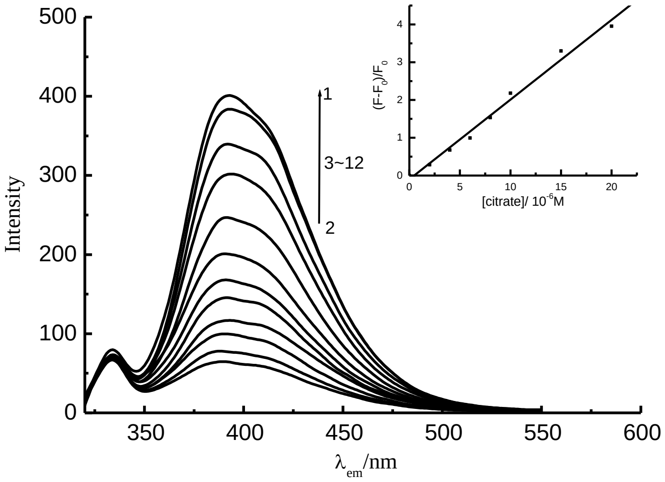


Figure 9 Fluorescence emission spectra of PA-Fe^{3+} system in HAc-NaAc buffer solution (pH 3.8, 0.1M) upon addition of different concentration of citric acid. The concentration of citric acid from down to top was 0nM, 2nM, 4nM, 6nM, 8nM, 10nM, 12nM, 14nM, 16nM, 18nM and 20nM. The inset is the linear relationship between the fluorescence intensity and the citric acid concentration over the range of 2-20nM.

The fluorescent probe is utilized for citric acid measurement in soft drink. The detailed result demonstrated in Table 5 was satisfactory and promising.

Table 3 Analysis result and recovery rate of soft drink sample

No. of Samples	Fe^{3+}(10^{-5} mol L^{-1})		Recovery (%)
	added	found	
1	0.10	1.205	105.0
2	0.30	1.411	103.3
3	0.50	1.598	99.6
4	0.70	1.821	101.5

3. Conclusion

In summary, a novel fluorescent probe 2-hydroxy-4-(pyridin-2-ylmethyleneamino) benzoic acid has been developed in this paper. We demonstrated that the emission state of PA and PA-Fe^{3+} system could be conveniently controlled by the addition of inputs such as Fe^{3+}, EDTA, $NH_2OH\cdot HCl$ and H^+. This result confirmed that PA and PA-Fe^{3+} system may be performed as a reversible multi-control fluorescent switch and molecular devices as a novel “NOR”, “OR” and “INHIBIT” logic gates. Fluorescent logic gates mentioned above have shown promising potential application in the construction of future molecular circuit as well as multi-detection in complicated environmental system.

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