

# Synthesis and Characterization of Fe<sup>3+</sup>-Selective Fluorescent Probe Containing 1,2,4-Triazole as Subunit

Jiahui Wang<sup>#</sup>, Chunwei Yu<sup>##</sup>, Xingye Yang<sup>#</sup>, Jun Zhang<sup>###\*</sup>

<sup>#</sup>Laboratory of Environmental Monitoring, School of Tropical and Laboratory Medicine, Hainan Medical University, Haikou, 571199, China

<sup>##</sup>Laboratory of Tropical Biomedicine and Biotechnology, Hainan Medical University, Haikou 571101, China

## Abstract

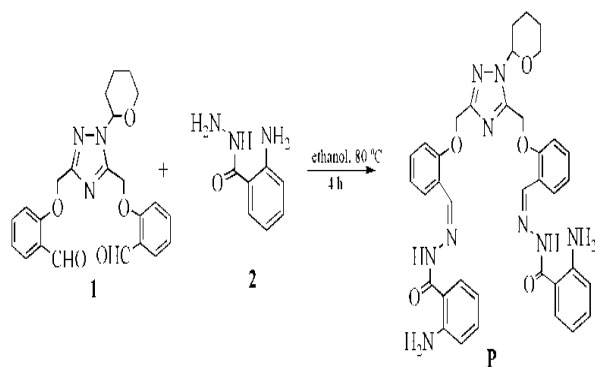
A benzoyl hydrazine derivative containing 1,2,4-triazole as subunit was successfully synthesized and characterized as an “off-on” type Fe<sup>3+</sup>-selective fluorescent probe. An obvious fluorescence enhancement at 420 nm was observed in the presence of Fe<sup>3+</sup> in ethanol-water solution (8:2, v:v, pH6.3, 50 mM HEPES). The proposed probe exhibited a linear response to Fe<sup>3+</sup> in the range of 2.0×10<sup>-6</sup>-8.0×10<sup>-6</sup> M with a detection limit of 6.7×10<sup>-7</sup> M.

**Keywords** — Fluorescent probe, Triazole, Fe<sup>3+</sup>.

## I. INTRODUCTION

Environmental relative detection of cations, anions and molecular species is always a challenging areas of current research [1]. Among them, Fe<sup>3+</sup> is an interesting target, because it plays crucial roles in cellular metabolism [2]. Compared to other common detection methods, fluorescent probes method is simple and sensitive [3], [4]. The design and synthesis of new Fe<sup>3+</sup>-selective probes is still attractive. Probes derived from benzoyl hydrazine have many advantages, such as large Stokes shifts, high fluorescence quantum yields and stability, which have been widely used for the detection of environment samples [5], [6].

In this work, a Fe<sup>3+</sup>-selective probe derived from benzoyl hydrazine containing 1,2,4-triazole as subunit was designed and characterized (Scheme 1).



Scheme 1. Synthesis route of probe P

## II. EXPERIMENTAL SECTION

### A. Reagents and Instruments

All of the materials were commercially available and used directly. MS spectra were recorded on a Thermo TSQ Quantum Access Agilent 1100. NMR spectra were measured with TMS as an internal standard. Fluorescence emission spectra were conducted on a Hitachi 4600 spectrofluorometer. The pH values were measured with a pH-meter PBS-3C.

### B. Synthesis

0.0742 g **1** [7] (0.2 mmol) and 0.0629 g **2** (0.4 mmol) were mixed in ethanol (40 mL). The reaction mixture was reacted under reflux for 4 h, and then cooled to room temperature. The mixture was condensed and the white precipitate so obtained was filtered and used directly. Yields: 75.3%. <sup>1</sup>H NMR (*d*<sub>6</sub>-DMSO, δ ppm): 12.03 (d, 2H), 11.91 (d, 2H), 8.80 (s, 1H), 8.72 (s, 1H), 7.91 (d, 2H), 7.86 (d, 2H), 7.44 (d, 2H), 7.43 (d, 2H), 7.31 (d, 2H), 7.07 (t, 2H), 6.96 (t, 2H), 6.92 (t, 2H), 5.69 (d, 1H), 5.47 (t, 2H), 5.26 (s, 2H), 3.87 (d, 1H), 3.57 (m, 1H), 2.18 (m, 1H), 1.93 (d, 2H), 1.62 (t, 2H), 1.49 (s, 1H). <sup>13</sup>C NMR (*d*<sub>6</sub>-DMSO, δ ppm): 165.85, 165.36, 160.27, 159.63, 158.52, 157.24, 156.71, 152.63, 144.63, 144.15, 134.44, 134.31, 132.22, 128.90, 128.52, 126.41, 126.30, 123.01, 122.20, 121.87, 119.38, 119.22, 117.85, 117.75, 116.19, 115.62, 113.88, 133.70, 84.13, 67.33, 63.95, 61.21, 29.67, 24.64, 22.06.

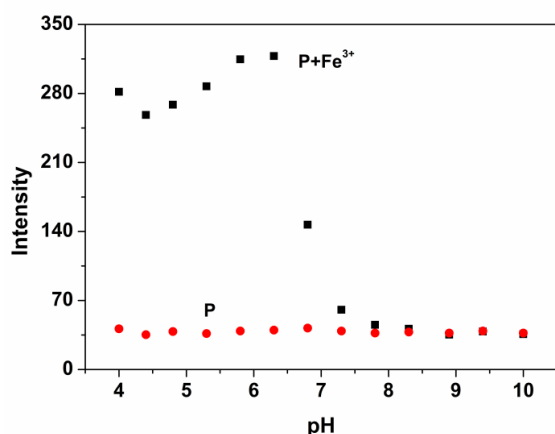
### C. General spectroscopic methods

All of the fluorescence spectra were recorded at room temperature (25 °C). Test solutions were prepared by placing 50 μL of the **P** stock solution (1 mM) and an appropriate aliquot of individual ions stock solution into a test tube, and then diluting the solution to 5 mL with ethanol-water (8:2, v:v, pH6.3, 50 mM HEPES). For all fluorescent measurements, excitation and emission slit widths were 5/10 nm, and the excitation wavelength was 335 nm.

### III. RESULTS AND DISCUSSION

#### A. pH effects on **P** with $\text{Fe}^{3+}$

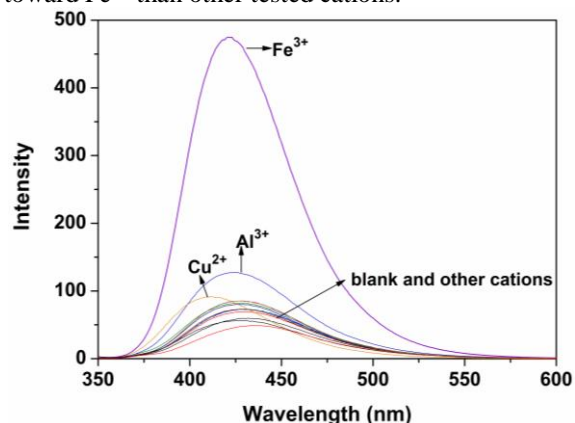
pH effects on **P** and **P** with  $\text{Fe}^{3+}$  was firstly carried out as shown in Fig. 1. Results showed that the emission intensity at 420 nm rapidly reached to a maximum in the pH 6.3 after the addition of  $\text{Fe}^{3+}$ . Therefore, further experiments were carried out in ethanol–water solution at pH 6.3 (8:2, v:v, 50 mM HEPES).



**Fig. 1** Influences of pH on the fluorescence spectra of **P** (10  $\mu\text{M}$ ) and **P** plus  $\text{Fe}^{3+}$  (10  $\mu\text{M}$ ) in ethanol-water solution (8:2, v:v). The pH was modulated by adding 1 M HCl or 1 M NaOH in HEPES buffers.

#### B. Fluorescence spectral response of **P**

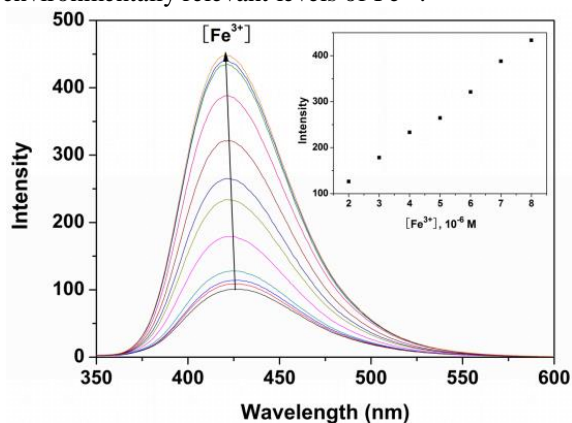
The selectivity study showed that the proposed probe had better selective property to  $\text{Fe}^{3+}$  compared to other tested cations at the same conditions ( $\text{K}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Pb}^{2+}$ ,  $\text{Co}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Cd}^{2+}$ ,  $\text{Ag}^+$ ,  $\text{Zn}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Hg}^{2+}$  and  $\text{Al}^{3+}$ ) as shown in Fig. 2. Only  $\text{Fe}^{3+}$  generated a significant “turn-on” fluorescent response at 420 nm. It suggested that **P** has a higher selectivity toward  $\text{Fe}^{3+}$  than other tested cations.



**Fig. 2** Fluorescence response of **P** (10  $\mu\text{M}$ ) with different metal ions (100  $\mu\text{M}$ ) in ethanol-water solution (8:2, v:v, pH 6.3, 50 mM HEPES).

Fluorescent titration experiment was carried out for the further investigation of the interaction between  $\text{Fe}^{3+}$  with **P**. Upon titration with  $\text{Fe}^{3+}$ , the fluorescence intensity of the monomer peak at 420 nm increased gradually (Fig. 3), and the fluorescent

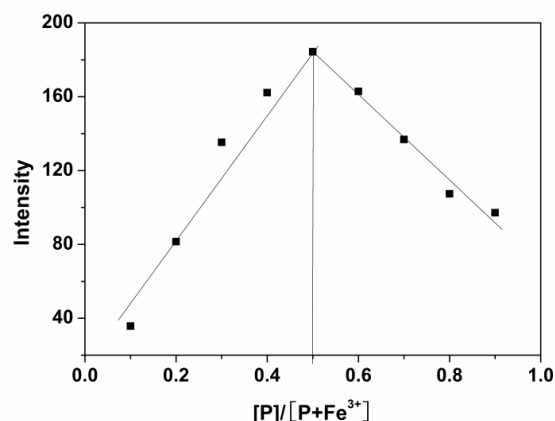
intensity of **P** was proportional to the concentration of  $\text{Fe}^{3+}$  in the range of  $2.0 \times 10^{-6}$ – $8.0 \times 10^{-6}$  M with a detection limit of  $6.7 \times 10^{-7}$  M  $\text{Fe}^{3+}$ . This clearly demonstrated that probe **P** could sensitively detect environmentally relevant levels of  $\text{Fe}^{3+}$ .



**Fig. 3** Fluorescence response of **P** (10  $\mu\text{M}$ ) with different concentrations of  $\text{Fe}^{3+}$  in ethanol-water solution (8:2, v:v, pH 6.3, 50 mM HEPES). Inset: the fluorescence of **P** (10  $\mu\text{M}$ ) as a function of  $\text{Fe}^{3+}$  concentrations (2–8  $\mu\text{M}$ ).

#### C. Proposed binding mode of **P** with $\text{Fe}^{3+}$

Binding analysis using the method of continuous variations (Job's plot) was measured (Fig. 4), and a maximum fluorescent intensity at 420 nm was observed when the molecular fraction of **P** was close to 0.5, which established the 1:1 complex formation between **P** and  $\text{Fe}^{3+}$ .



**Fig. 4** Job's plot curve of **P** with  $\text{Fe}^{3+}$  in ethanol-water solution (8:2, v:v, pH 6.3, 50 mM HEPES). The total concentration of **P** and  $\text{Fe}^{3+}$  was kept 10  $\mu\text{M}$ .

### IV. CONCLUSIONS

In summary, an “off-on” type  $\text{Fe}^{3+}$ -selective probe **P** based on benzoyl hydrazine derivative was characterized. The conception may expand a promising approach to develop selective detection method for  $\text{Fe}^{3+}$  and lead to the development of “off-on” type probes for other metal ions.

### ACKNOWLEDGMENT

This work was financially supported by the National Natural Science Foundation of China (No. 81660356, 81760387), the Research and Training

Fundation of Hainan Medical University  
(HYCX2018085).

### REFERENCES

- [1] Naema S. Yehia and Omar M. Elmakabati, "Removal of Cd (II) from aqueous solution using eggshell as low cost sorbent," SSRG Int. J. Appl. Chem., vol. 6, pp. 11–18, Apr. 2019.
- [2] C.W. Yu, and J. Zhang, "A fluorescent probe based on ethanolamine modified rhodamine B for Fe<sup>3+</sup> in living imaging," J. Hainan Med. Univ., vol. 19, pp. 1009–1011, May. 2013.
- [3] Z.Q. Guo, S. Park, J.Y. Yoon and I. Shin, "Recent progress in the development of near-infrared fluorescent probes for bioimaging applications," Chem. Soc. Rev., vol. 43, pp. 16–29, Sept. 2014.
- [4] C.W. Yu, L. Jian, Y.X. Ji and J. Zhang, "Al(III)-responsive "off-on" chemosensor based on rhodamine derivative and its application in cell imaging". Rsc Adv., vol. 8, pp. 31106–31112, Aug. 2018.
- [5] J. Zhang, C.W. Yu, G. Lu, Q.Y. Fu, N. Li and Y.X. Ji, "A Ag<sup>+</sup>-selective "off-on" probe based on naphthalimide derivative". New J. Chem., vol. 36, pp. 819–822, Feb. 2012.
- [6] Y.X. Ji, C.W. Yu, S.B. Wen and J. Zhang, "Characterization of an Al<sup>3+</sup>-selective fluorescent probe based on a benzoyl hydrazine derivative and its application in cell imaging". Turkish J. Chem., vol. 40, pp. 625–630, Jan. 2016.
- [7] J. Zhang, C.W. Yu, S.Y. Qian, G. Lu and J.L. Chen, "A selective fluorescent chemosensor with 1, 2, 4-triazole as subunit for Cu (II) and its application in imaging Cu (II) in living cells," Dyes and Pigments, vol. 92, pp. 1370–1375, Mar. 2012.