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# A pH tuning single fluorescent probe based on naphthalene for dual-analytes ( $\text{Mg}^{2+}$ and $\text{Al}^{3+}$ ) and its application in cell imaging†

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In this study, a naphthalene Schiff-base **P** which serves as a dual-analyte probe for the quantitative detection of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  has been designed. The proposed probe showed an “off–on” fluorescent response toward  $\text{Al}^{3+}$  in ethanol–water solution (1 : 9, v/v, pH 6.3, 20 mM HEPES) over other metal ions and anions, while the detection by the probe could be switched to  $\text{Mg}^{2+}$  by regulating the pH from 6.3 to 9.4. The sensing mechanisms of **P** to  $\text{Al}^{3+}/\text{Mg}^{2+}$  are attributed to inhibition of the photo-induced electron transfer (PET) process by the formation of 1 : 1 ligand–metal complexes. More importantly, the probe was applied successfully in living cells for the fluorescent cell-imaging of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$ .

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## 1 Introduction

The detection of trace metal ions is becoming an important concern due to their assignable functions in biological systems and deleterious effects on public health.<sup>1</sup> Among these metals,  $\text{Mg}^{2+}$  is an essential micronutrient element for human life, participating in gene transcription and neural signal transmission.<sup>2</sup> It also plays important roles in enzymes and DNA-binding proteins.<sup>3</sup> However, overloading of magnesium in the cytosol and subcellular regions exhibits toxicity and is linked with diseases such as diabetes, hypertension, epilepsy and Alzheimer's.<sup>4</sup> As another highly chemical reactive metal ion,  $\text{Al}^{3+}$  is a non-essential element for living systems and of significant environmental concern: an overdose of  $\text{Al}^{3+}$  can damage the central nervous system and produce some neuro-behavioral and neuro-pathological changes.<sup>5</sup> In order to protect human health, the development of effective tools for the detection of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  is of a great importance.

In recent years, molecular fluorescence probes with simple handling, real-time response, high sensitivity and selectivity, and non-destructive properties have undoubtedly been powerful detection tools.<sup>6</sup> Compared to several transition metal ions, such as  $\text{Hg}^{2+}$ ,  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$ , only a few fluorescent probes have been developed for the detection of  $\text{Mg}^{2+}$  or  $\text{Al}^{3+}$  due to their

silent spectroscopic characteristics and poor coordination ability.<sup>7</sup> In particular, most of these reported probes focus on the detection of single metal ions,<sup>8</sup> so it will be complicated and inconvenient to analyse some coexisting metal ions based on different detection methods and/or experimental conditions. For these reasons, much attention has been paid to single probes for multiple analysis and they have been widely applied in metal detection because they could determinate more than one interesting ion, simultaneously. Some multi-target probes such as  $\text{Cr}^{3+}/\text{Al}^{3+}$ ,<sup>9</sup>  $\text{Bi}^{3+}/\text{Zn}^{2+}$ ,<sup>10</sup>  $\text{Zn}^{2+}/\text{Mg}^{2+}$ ,<sup>11</sup> and  $\text{Cu}^{2+}/\text{Al}^{3+}$  have been developed,<sup>12</sup> but multi-ion responsive molecular fluorescence probes which sense  $\text{Al}^{3+}/\text{Mg}^{2+}$  have rarely been reported. More problematically, the ionic radius and charge of  $\text{Al}^{3+}$  make it a competitive inhibitor of  $\text{Mg}^{2+}$  in the human body. In this regard, the design of reliable and sensitive multi-target probes for  $\text{Mg}^{2+}$  and  $\text{Al}^{3+}$  is highly desirable.

Schiff bases can coordinate with various metal ions and form stable complexes which are known to be good ligands for metal ions.<sup>13</sup> Beyond this, naphthalene derivatives are excellent fluorophores that usually serve as signal reporters in probes because of their short fluorescence life-time, low fluorescence quantum yields, and ability to act as a donor or an acceptor.<sup>14</sup> Meanwhile, benzoyl hydrazine derivatives are recognized as important motifs for use as ligands in coordination chemistry.<sup>15</sup> Moreover, based on the special advantage of turn-on fluorescent probes in reducing system errors and background interference,<sup>16</sup> we aimed to develop a novel turn-on fluorescent probe combining different recognition groups in one molecule, which was expected to multi-channelly detect  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  with different turn-on fluorescent responses.

In this work, a fluorescent probe (**P**) based on a Schiff base combining naphthalene and benzoyl hydrazine was proposed (Scheme 1). **P** can be utilized as a multi-ion fluorescent probe by regulating pH: it showed “off–on” fluorescent response toward

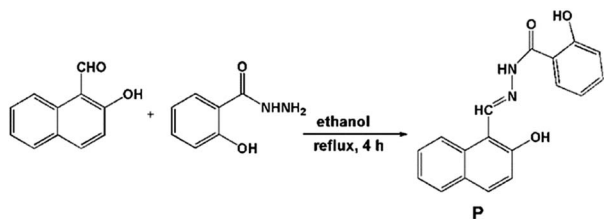
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Scheme 1 Synthesis route of probe P.

$\text{Al}^{3+}$  in ethanol–water solution (v/v, 1 : 9, pH 6.3, 20 mM HEPES) and  $\text{Mg}^{2+}$  in ethanol–water solution (v/v, 1 : 9, pH 9.4, 20 mM HEPES). More importantly, the probe could be used for cell imaging of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  *in vivo* with negligible cytotoxicity.

## 2 Experimental section

All reagents and solvents are commercially available and used directly. All reagents and solvents are of analytical grade and used without further purification. The metal ions salts used were  $\text{NaCl}$ ,  $\text{KCl}$ ,  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{HgCl}_2$ ,  $\text{Zn}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{PbCl}_2$ ,  $\text{CdCl}_2$ ,  $\text{CrCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{AgNO}_3$ ,  $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ ,  $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$ ,  $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$ ,  $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$  and  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ .

Fluorescence emission spectra were conducted on a Hitachi F-4600 spectrofluorometer. UV-vis spectra were obtained on a Hitachi U-2910 spectrophotometer. Nuclear magnetic resonance (NMR) spectra were measured with a Bruker AV 400 instrument and chemical shifts were given in ppm from tetramethylsilane (TMS). Mass (MS) spectra were recorded on a Thermo TSQ Quantum Access Agilent 1100. Fluorescence imaging was performed by confocal fluorescence microscopy on an Olympus FluoView Fv1000 laser scanning microscope (USA). pH values were measured with a pH-meter PBS-3C (Shanghai, China).

### 2.1 Synthesis of P

2-Hydroxy-1-naphthaldehyde (0.172 g, 1.0 mmol) and salicylhydrazide (0.152 g, 1.0 mmol) were mixed in ethanol (30 mL) and stirred under reflux for 4 h. After the reaction was finished, the mixture was cooled to room temperature and poured into cold water. The precipitate so obtained was filtered and washed with ethanol and water in turn, and then dried in a vacuum to afford **P** as a yellow solid. Yields: 85.6%. MS  $m/z$ : 307.24 [ $\text{M} + \text{H}^+$ ], 329.21 [ $\text{M} + \text{Na}^+$ ].  $^1\text{H}$  NMR ( $\delta$ ppm,  $d_6$ -DMSO): 12.10 (s, 1H), 11.72 (b, 1H), 9.55 (s, 1H), 8.32 (d, 1H,  $J = 6.00$ ), 7.94 (t, 2H,  $J = 6.00$ ), 7.91 (t, 1H,  $J = 12.00$ ), 7.61 (s, 1H), 7.48 (s, 1H), 7.42 (s, 1H), 7.24 (d, 1H,  $J = 6.00$ ), 7.03 (d, 2H,  $J = 12.00$ ), 7.01 (s, 1H).  $^{13}\text{C}$  NMR ( $\delta$ ppm,  $d_6$ -DMSO): 164.90, 159.69, 159.07, 148.61, 134.95, 133.85, 132.62, 129.86, 129.71, 128.75, 128.67, 124.49, 121.91, 120.08, 119.82, 118.23, 116.65, 109.53 (Fig. S1–S3, ESI $^\dagger$ ).

### 2.2 General spectroscopic methods

Metal ions and **P** were dissolved in deionized water and DMSO to obtain 1.0 mM stock solutions. Before spectroscopic measurements, the solution was freshly prepared by diluting the high concentration stock solution to the corresponding

solution. For all measurements, excitation and emission slit widths were 5/5 and 5/10 nm, and excitation wavelengths were 408 and 415 nm for  $\text{Mg}^{2+}$  and  $\text{Al}^{3+}$ , respectively.

### 2.3 Cell culture

HepG2 cells were purchased from the Committee on Type Culture Collection of Chinese Academy of Sciences (Shanghai, China). HepG2 cells were incubated in DMEM (Dulbecco's Modified Eagle's Medium) supplemented with 10% fetal bovine serum (FBS).

### 2.4 Cell imaging

For imaging experiments, cells were grown in 6-well plates at 70–80% confluence. Cells were then incubated in DMEM containing 10  $\mu\text{M}$  **P** for 30 min at 37  $^\circ\text{C}$ . Cells were then washed with PBS followed by the addition of 1  $\mu\text{M}$   $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  and incubated for 30 min. Bright field and fluorescence images were captured by a fluorescence microscope (Olympus FluoView Fv1000).

### 2.5 Cell cytotoxicity assay

To assess the cytotoxicity of **P**, cytotoxicity was measured by using the methyl thiazolyl tetrazolium (MTT) assay in HepG2 cells. HepG2 cells were seeded into a 96-well cell culture plate at 4000/well, cultured at 37  $^\circ\text{C}$  and 5%  $\text{CO}_2$  for 24 h, and then different concentrations of **P** (0, 0.1, 1.0, 10.0  $\mu\text{M}$ ) were added to the wells. The cells were then incubated for 24 h at 37  $^\circ\text{C}$  under 5%  $\text{CO}_2$ . Subsequently, 20  $\mu\text{L}$  of MTT (5 mg  $\text{mL}^{-1}$ ) was added to each well and incubated for an additional 4 h at 37  $^\circ\text{C}$  under 5%  $\text{CO}_2$ . Cells were lysed in triple liquid (10% SDS, 0.012 M HCl, 5% isopropanol), and the amount of MTT formazan was qualified by determining the absorbance at 570 nm using a microplate reader (Tecan, Austria).

The following formula was used to calculate the inhibition of cell growth: cell viability (%) = (mean of Abs. value of treatment group/mean Abs. value of control)  $\times$  100%.

## 3 Results and discussion

### 3.1 Fluorescence study

A pH titration experiment was first evaluated, as shown in Fig. S4 (ESI $^\dagger$ ), from the experimental results, the fluorescence from the free **P** could be seen to be negligible in the pH range from 4.0 to 10.0, suggesting that it was not susceptible to the change in acid–base solution. The fluorescence of the **P**– $\text{Al}^{3+}$  complex displayed a plateau in the pH range from 4.0 to 6.5, and the maximum response toward  $\text{Al}^{3+}$  was obtained under pH 6.3. From the point of view of sensitivity and speed, in our experiment, pH 6.3 was chosen as the optimum experimental condition for environmental examples. The host–guest recognition abilities of **P** with  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  were investigated *via* a fluorescence spectroscopic method. As shown in Fig. 1a, free **P** showed weak fluorescence emission at 475 nm when it was excited at 425 nm in ethanol–water (v/v, 1 : 9, pH 6.3, 20 mM HEPES). Only upon the addition of  $\text{Al}^{3+}$  did the fluorescence intensity of **P** show a significant fluorescence enhancement at 475 nm over other relevant metal ions ( $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Pb}^{2+}$ ,



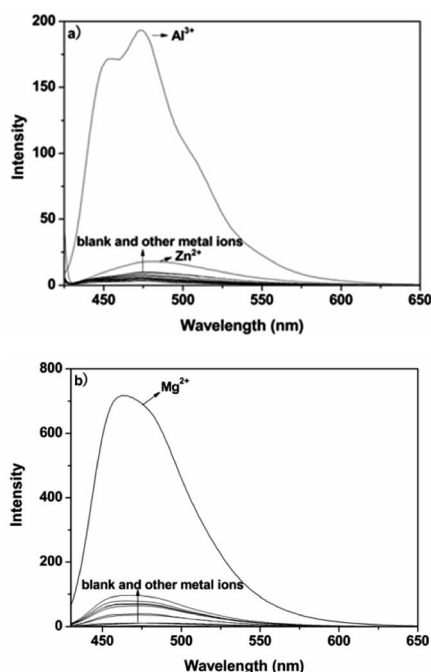


Fig. 1 (a) Fluorescence emission spectra of **P** (10  $\mu\text{M}$ ) in response to different metal ions (10  $\mu\text{M}$ ) in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 6.3); (b) fluorescence emission spectra of **P** (10  $\mu\text{M}$ ) in response to different metal ions (10  $\mu\text{M}$ ) in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 9.4).

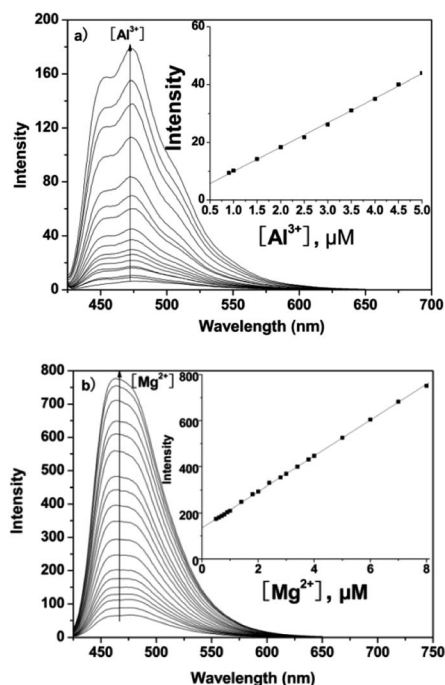


Fig. 2 (a) Fluorescence response of **P** (10  $\mu\text{M}$ ) with various concentrations of  $\text{Al}^{3+}$  in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 6.3). Inset: the fluorescence of **P** (10  $\mu\text{M}$ ) as a function of  $\text{Al}^{3+}$  concentration (0.5–5.0  $\mu\text{M}$ ); (b) fluorescence response of **P** (10  $\mu\text{M}$ ) with various concentrations of  $\text{Mg}^{2+}$  in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 9.4). Inset: the fluorescence of **P** (10  $\mu\text{M}$ ) as a function of  $\text{Mg}^{2+}$  concentration (1–8.0  $\mu\text{M}$ ).

$\text{Cr}^{3+}$ ,  $\text{Mn}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Fe}^{3+}$ ,  $\text{Co}^{2+}$ ,  $\text{Ni}^{2+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ ,  $\text{Cd}^{2+}$  and  $\text{Hg}^{2+}$ ). The results suggested that probe **P**, with predominant recognition and selectivity, shows good potential in the detection of  $\text{Al}^{3+}$ . More interestingly, when the pH was adjusted to 9.4 (Fig. S5, ESI†), the metal ion switched to  $\text{Mg}^{2+}$ . The probe did not give any observable response in the absence or presence of various other metal ions, and the blue shift of the emission band at 460 nm was accompanied by a 49-fold fluorescence enhancement for  $\text{Mg}^{2+}$ , as depicted in Fig. 1b.

Under physiological conditions, the emission of probe **P** (10  $\mu\text{M}$ ) around 475 nm is very low. However, upon gradual addition of  $\text{Al}^{3+}$  or  $\text{Mg}^{2+}$  there was a remarkable enhancement in fluorescence at 475 nm for  $\text{Al}^{3+}$  or 460 nm for  $\text{Mg}^{2+}$  with an increasing concentration (0–10  $\mu\text{M}$ ) of the metal ion (Fig. 2). The plots of emission intensity of **P** as a function of added  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  are presented in insets to Fig. 2a and b, respectively. Additionally, from the fluorescence titration profiles, the detection limit of **P** for  $\text{Al}^{3+}/\text{Mg}^{2+}$  was found to be 0.3  $\mu\text{M}$ /0.2  $\mu\text{M}$  (based on  $S/N = 3$ , inset of Fig. 2), which was sufficiently low to enable the detection of micromolar concentrations of  $\text{Al}^{3+}/\text{Mg}^{2+}$  in many chemical and biological systems.

### 3.2 UV-vis analysis

With the objective of evaluating the potential use of the probe **P**, UV-vis analysis upon addition of  $\text{Al}^{3+}/\text{Mg}^{2+}$  was further evaluated. Firstly, the interaction of **P** and  $\text{Al}^{3+}$  was investigated as a function of the concentration of  $\text{Al}^{3+}$ , as shown in Fig. 3.

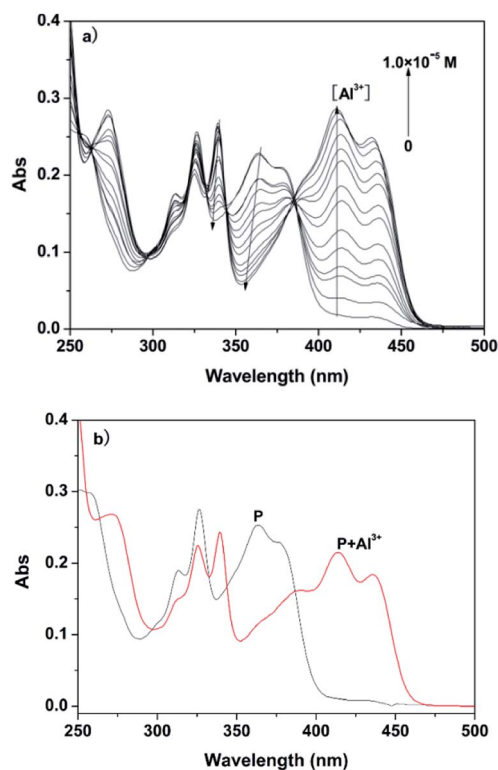


Fig. 3 (a) Absorbance of **P** (10  $\mu\text{M}$ ) with various concentrations of  $\text{Al}^{3+}$  in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 6.3); (b) absorbance of **P** (10  $\mu\text{M}$ ) with  $\text{Al}^{3+}$  (100  $\mu\text{M}$ ) in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 6.3).



The spectrum of free **P** showed a maximum absorption band at 350 nm in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 6.3), which can be assigned to the  $\pi$ - $\pi^*$  transition of the benzoyl hydrazine group.<sup>15</sup> With increasing concentration of  $\text{Al}^{3+}$ , the absorption bands at 325 nm and 375 nm gradually decreased, and simultaneously a new band appeared at 425 nm with increased intensity. Moreover, a clear isosbestic point at 380 nm was observed, which clearly indicated the presence of new complex **P**- $\text{Al}^{3+}$ . In contrast, when the UV-vis analysis was carried out in ethanol–water solution (1 : 9, v/v, pH 9.4, 20 mM HEPES), the metal ion was also changed from  $\text{Al}^{3+}$  to  $\text{Mg}^{2+}$ , as shown in Fig. 4. The trend in the change in the absorption spectrum of the latter was similar to the former; the only difference was the extent of variation of the absorption at 425 nm. Absorption spectra confirmed the binding ability of **P** and  $\text{Mg}^{2+}/\text{Al}^{3+}$ .

### 3.3 Practical applicability of **P**

In order to evaluate the practical applicability of **P** as a selective multi-analyt fluorescent probe for  $\text{Al}^{3+}/\text{Mg}^{2+}$ , competition experiments were conducted (Fig. S6 and S7, ESI†). It could be seen that other competitive ions had no obvious interference with the detection of  $\text{Al}^{3+}/\text{Mg}^{2+}$  under different pH conditions, which could be attributed to their inherent magnetic properties. Meanwhile, reversibility was investigated which is

a prerequisite in developing a fluorescent probe for practical applications. The reversibility of **P** was studied by adding  $\text{Na}_2\text{EDTA}$  as a bonding agent (Fig. S8 and S9, ESI†). The addition of  $\text{Na}_2\text{EDTA}$  to a mixture of **P** and  $\text{Al}^{3+}/\text{Mg}^{2+}$  caused a diminution in the fluorescence intensity at 475/460 nm, which may produce the free probe **P**. Upon the addition of  $\text{Al}^{3+}/\text{Mg}^{2+}$ , the fluorescence intensity of **P** displayed a significant fluorescence enhancement again, which proved the binding between **P** and  $\text{Al}^{3+}/\text{Mg}^{2+}$  was reversible.

### 3.4 Proposed binding mode of **P** with $\text{Al}^{3+}/\text{Mg}^{2+}$

In order to understand the binding mode of **P** and  $\text{Al}^{3+}/\text{Mg}^{2+}$ , the Job's plot of **P** and  $\text{Al}^{3+}/\text{Mg}^{2+}$  was conducted (Fig. 5). When the molar fraction of **P** and  $\text{Al}^{3+}/\text{Mg}^{2+}$  was 0.5, **P** with  $\text{Al}^{3+}/\text{Mg}^{2+}$  exhibited maximum fluorescence emission. The results showed that **P** and  $\text{Al}^{3+}/\text{Mg}^{2+}$  formed 1 : 1 ligand–metal complexes. Furthermore, the ESI-MS spectra also confirmed this conclusion (Fig. S10 and S11, ESI†), in which the peaks at  $m/z$  331.0, 366.7, 376.7, 413.2, and 423.2 were assignable to  $[\text{P} + \text{Al}^{3+} - 2\text{H}^+]^+$ ,  $[\text{P} + \text{Al}^{3+} + \text{Cl}^- - \text{H}^+]^+$ ,  $[\text{P} + \text{Al}^{3+} + \text{EtOH} - 2\text{H}^+]^+$ ,  $[\text{P} + \text{Al}^{3+} + \text{Cl}^- + \text{EtOH} - 2\text{H}^+]^+$ , and  $[\text{P} + \text{Al}^{3+} + 2\text{EtOH} - 2\text{H}^+]^+$ , respectively. The peaks at  $m/z$  328.7, 365.1, 347.1, 375.1, and 438.8 were assignable to  $[\text{P} + \text{Mg}^{2+} - \text{H}^+]^+$ ,  $[\text{P} + \text{Mg}^{2+} + \text{Cl}^-]^+$ ,  $[\text{P} + \text{Mg}^{2+} + \text{OH}^-]^+$ ,  $[\text{P} + \text{Mg}^{2+} + \text{EtOH} - \text{H}^+]^+$ , and  $[\text{P} + \text{Mg}^{2+} + 2\text{EtOH} + \text{OH}^-]^+$ , respectively. As expected, the results indicated that complexes with a stoichiometry of  $\text{Al}^{3+}/\text{Mg}^{2+}$  to **P** of 1 : 1 were formed, and the results were also supported by the Benesi–Hildebrand method.<sup>15</sup> The Benesi–Hildebrand analysis of the emission data gives a 1 : 1 stoichiometry for **P**- $\text{Al}^{3+}$  and **P**- $\text{Mg}^{2+}$  complexation

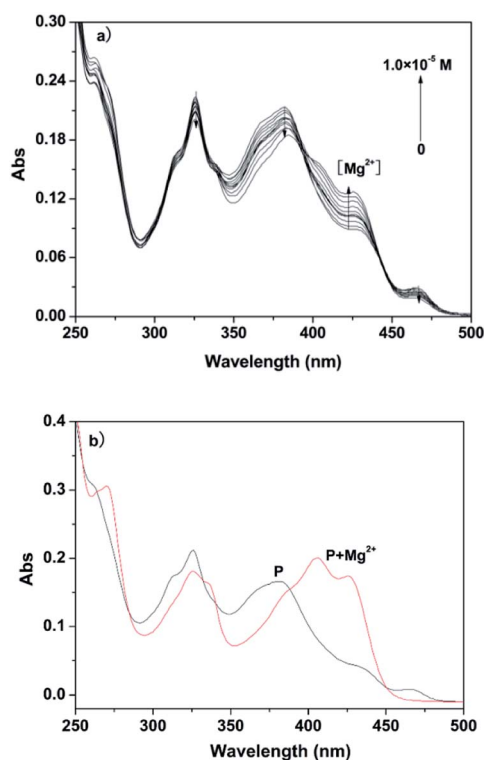


Fig. 4 (a) Absorbance of **P** (10  $\mu\text{M}$ ) with various concentrations of  $\text{Mg}^{2+}$  in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 9.4); (b) absorbance of **P** (10  $\mu\text{M}$ ) with  $\text{Mg}^{2+}$  (100  $\mu\text{M}$ ) in ethanol–water solution (1 : 9, v/v, 20 mM HEPES, pH 9.4).

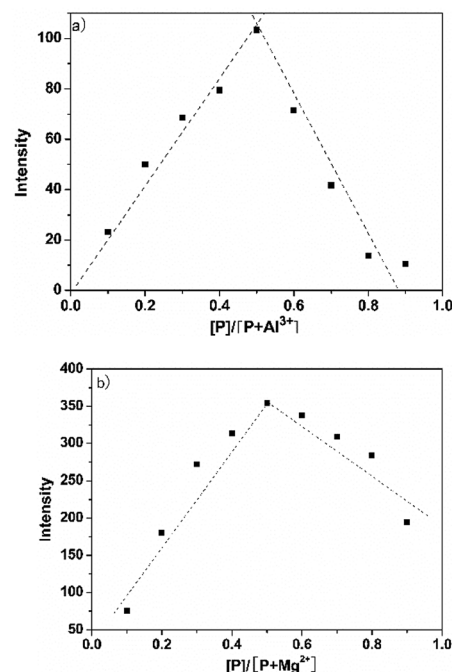


Fig. 5 Job's plot for determining the stoichiometry of **P** and (a)  $\text{Al}^{3+}$  and (b)  $\text{Mg}^{2+}$ . The total concentration was kept at 50  $\mu\text{M}$ .



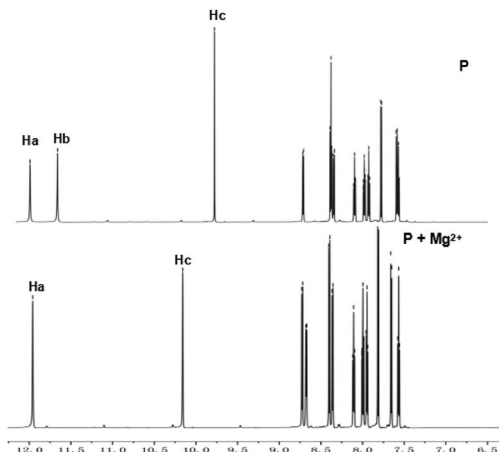


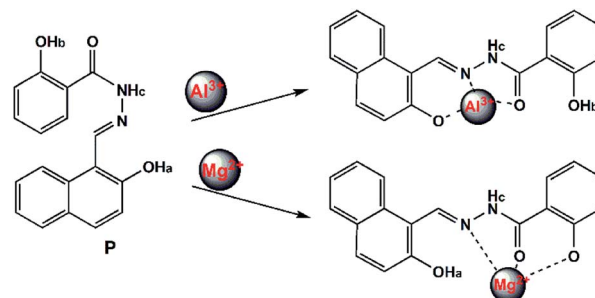
Fig. 6  $^1\text{H}$  NMR titration of **P** with  $\text{Mg}^{2+}$ .

species, with association constants ( $K_a$ ) being calculated as  $2.9 \times 10^4 \text{ M}^{-1}$  and  $2.1 \times 10^5 \text{ M}^{-1}$  (Fig. S12 and S13, ESI<sup>†</sup>), corresponding to a stronger binding capability toward  $\text{Al}^{3+}$  in comparison with a naphthalene-based PET chemosensor for  $\text{Al}^{3+}$  (with a  $K$  value of  $5.1 \times 10^3 \text{ M}^{-1}$ ),<sup>16a</sup> and a rhodamine spiro-lactam derivative-based chemosensor for  $\text{Mg}^{2+}$  (with a  $K$  value of  $2.55 \times 10^4 \text{ M}^{-1}$ ).<sup>16b</sup>

### 3.5 Proposed mechanism of **P** with $\text{Al}^{3+}/\text{Mg}^{2+}$

According to reported work,<sup>15a,17</sup> probes with only  $\text{OH}_a$  show good selectivity to  $\text{Al}^{3+}$ . Based on the soft–hard acid–base theory,  $\text{Al}^{3+}$  shows affinity to groups with O sites. In order to design probes with better selectivity and sensitivity to  $\text{Al}^{3+}$ , **P** with two OH groups was proposed in this work. As mentioned above, the proposed probe still has good selectivity to  $\text{Al}^{3+}$  at pH 6.3, and meanwhile shows good selectivity to  $\text{Mg}^{2+}$  at pH 9.4. To evaluate the binding pattern between **P** and  $\text{Mg}^{2+}$ ,  $^1\text{H}$  NMR titration experiments in  $\text{DMSO}-d_6$  were carried out (Fig. 6). The phenolic OH peaks (protons  $\text{H}_a$  and  $\text{H}_b$ ) and the imine group NH (proton  $\text{H}_c$ ) of **P** were observed at  $\delta$ 12.10, 11.72 and 9.55, respectively. An  $^1\text{H}$  NMR titration experiment of **P** shows that the proton  $\text{H}_b$  at  $\delta$ 11.7243 ppm disappeared on adding  $\text{Mg}^{2+}$ , and the proton signals of the imine group NH ( $\text{H}_c$ ) at  $\delta$ 9.5507 ppm and  $-\text{CH}=\text{N}$  at  $\delta$ 7.9390 ppm corresponding to downfield shifts to 10.0111 and 7.9473, respectively (Fig. S14, ESI<sup>†</sup>).

Based on these results, the selective recognition of **P** for  $\text{Al}^{3+}/\text{Mg}^{2+}$  should be attributed to the interaction of the benzoyl hydrazine moieties with  $\text{Al}^{3+}/\text{Mg}^{2+}$ , which inhibit the photo-induced electron transfer (PET) process. As shown in Scheme 2, it seemed that the lone pair of electrons from the nitrogen atom of the  $-\text{C}=\text{N}-$  group to the benzoyl hydrazine moieties was responsible for the photoinduced electron-transfer (PET) process, which quenched the fluorescence emission of the probe. However, upon addition of  $\text{Al}^{3+}/\text{Mg}^{2+}$ , the PET process was inhibited owing to the chelation of the nitrogen atom of the  $-\text{C}=\text{N}-$  group with  $\text{Al}^{3+}/\text{Mg}^{2+}$ ; as result, the quenched fluorescence could recur remarkably.



Scheme 2 Proposed binding modes of **P** with  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$ .

### 3.6 Biological application of **P** with $\text{Al}^{3+}/\text{Mg}^{2+}$

To investigate the potential biological applications of probe **P** in living cells, we performed intracellular  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  dual imaging of HepG2 cells by fluorescence microscopy. After incubation with **P** for 30 min at  $37^\circ\text{C}$ , the cells could not show any recognizable fluorescence, suggesting that auto-fluorescence from the cells can be avoided and no fluorescence signal was detected in cells treated only with **P** (Fig. 7(a-I) and (b-I)). However, under these conditions, for the **P**-loaded HepG2 cells, strong fluorescence was detected after the addition of exogenous  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  ( $1 \mu\text{M}$  each) separately to the cells, which showed green and orange fluorescence (Fig. 7(a-II) and (b-II)), respectively, demonstrating membrane penetrability by **P** and its complexation with  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  inside the cells, clearly demonstrating that  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  and their interaction with **P** are essential for the fluorescence turn-on. The fluorescence signal of **P** in the presence of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  may be utilized as a signature for a selective probe response. Hence, these results indicate that probe **P** is an efficient candidate for monitoring changes in the intracellular  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  concentration under biological conditions. The bright field images of Fig. 7(a-III) and (b-III), whose cell shapes indicated that **P** has low toxicity, reveal good biocompatibility of **P** for bioanalysis.

**P** also was applied to the subcellular locations of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  in the HepG2 cells using confocal fluorescence microscopy. The cells were co-treated with **P** ( $10 \mu\text{M}$ ) and Hoechst 33 342 ( $1 \mu\text{g mL}^{-1}$ ) for 30 min, with the same conditions as

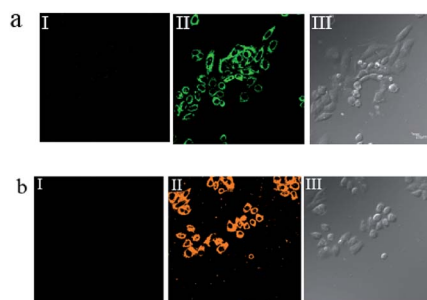
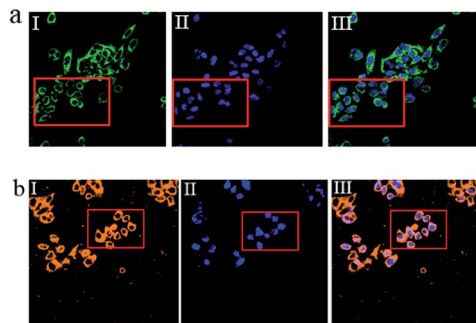


Fig. 7 (a-I) and (b-I) are the field images of **P** ( $10 \mu\text{M}$ ) treated HepG2 cells. (a-II) and (b-II) represent fluorescence images treated with **P** ( $10 \mu\text{M}$ ), in the presence of  $\text{Al}^{3+}$  or  $\text{Mg}^{2+}$  ( $1 \mu\text{M}$ ), respectively; (a-III) and (b-III) indicate bright field images of cells shown in the panels, respectively. For all imaging, the samples were excited at 375 nm.



**Fig. 8** Confocal fluorescence images of HepG2 cells incubated with **P** (10  $\mu\text{M}$ ) and Hoechst 33 342 (1  $\mu\text{g mL}^{-1}$ ) for 30 min. Cells loaded with  $\text{Al}^{3+}$  or  $\text{Mg}^{2+}$  (10  $\mu\text{M}$ ), then treated with **P** (10  $\mu\text{M}$ ) and Hoechst 33 342 (1  $\mu\text{g mL}^{-1}$ ) for 30 min. (a-I), (b-I) Green and orange channels with **P**, respectively; (a-II), (b-II) Blue channel with Hoechst 33 342; (a-III), (b-III) Overlay of images showing fluorescence from **P** (a-I), (b-I) and Hoechst 33 342 (a-II), (b-II).

those used in Fig. 8. The results further reveal that **P** locates primarily in the cytoplasm of these living HepG2 cells, as shown in Fig. 8. To evaluate the cytotoxicity of the probe, **P** was taken as an example to perform an MTT assay on HepG2 cells with dye concentrations from 0  $\mu\text{M}$  to 10  $\mu\text{M}$ . The MTT assay results confirmed that **P** has no significant toxicity to cultured HepG2 cells up to 48 h of treatment with 10  $\mu\text{M}$  of **P** (Fig. S15, ESI†). Thus, **P** has promising potential in the dual sensing of  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$  *in vitro*.

## 4 Conclusions

In summary, we developed a single fluorescent probe which displayed a distinct response to  $\text{Al}^{3+}$  and  $\text{Mg}^{2+}$ . The probe showed “off-on” fluorescent responses toward  $\text{Al}^{3+}$  at pH 6.3 in ethanol–water solution. When the pH was changed from 6.3 to 9.4, the detection of the probe could respond to  $\text{Mg}^{2+}$ . In addition, the cell imaging for  $\text{Al}^{3+}/\text{Mg}^{2+}$  was satisfactory. However, multi-ion responsive molecular probes with multiple emission modes will be challenging tasks for a long time into the future.

## Conflicts of interest

There are no conflicts to declare.

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