Supporting Information

Al(III)-Responsive “Off-On” Chemosensor Based on Rhodamine Derivative and Its Application in Cell Imaging

Chunwei Yu,a Li Jian,a,* Yuxiang Ji and Jun Zhanga,b*

a Department of Environmental Sciences, School of Tropical and Laboratory Medicine, Hainan Medical University, Haikou 571199, P. R. China.
b School of International Education, Hainan Medical University, Haikou 571199, P. R. China.

*Corresponding authors’ E-mails: jianli0622@163.com; jun_zh1979@163.com.

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Fig. S1 a) Fluorescence emission spectra of P (10 µM) to different metal ions (10 µM) in ethanol-water solution (9:1, v:v, pH5.8, 20 mM HEPES); b) Fluorescence emission spectra of P (10 µM) to different anion ions and ROS or RNS (10 µM) in ethanol-water solution (9:1, v:v, pH5.8, 20 mM HEPES).
**Fig. S2** Fluorescence response of P (10 µM) to Al\(^{3+}\) ions (10 µM) or to a mixture of the specified anion ions and ROS or RNS (50 µM) with Al\(^{3+}\) ions (10 µM) in ethanol-water solution (9:1, v:v, pH 5.8, 20 mM HEPES).

**Fig. S3**

**Fig. S3** Benesi-Hildebrand plot of P, assuming 1:1 stoichiometry for association between P and Al\(^{3+}\).
Fig. S4 ESI-MS of P-Al$^{3+}$ complex.

Fig. S5 Contrast of FT-IR spectrum between P and P-Al$^{3+}$ complex
Fig. S6

a) Contrast of $^1$H NMR spectrum between P and P-Al$^{3+}$ complex; b) Contrast of $^{13}$C NMR spectrum between P and P-Al$^{3+}$ complex
**Fig. S7**

Reversible titration response of P to Al\(^{3+}\) in ethanol-water solution (9:1, v:v, pH 5.8, 20 mM HEPES): (a) P (10 µM); (b) P (10 µM) with Al\(^{3+}\) (10 µM); (c) P (10 µM) with Al\(^{3+}\) (10 µM) and then addition of EDTA (20 µM); (d) P (10 µM) with Al\(^{3+}\) (10 µM) and EDTA (20 µM) and then addition of Al\(^{3+}\) (30 µM).

**Fig. S8**

Confocal fluorescence images of HepG2 cells incubated with P (10 µM) and Hoechst 33342 (1 µg/mL) for 30 min. Cells loaded with Al\(^{3+}\) (10 µM), then treated with P (10 µM) and Hoechst 33342 (1 µg/mL) for 30 min. (a) Red channel with P; (b) Blue channel with Hoechst 33342; (c) Overlay of images showing fluorescence from Hoechst 33342 (b) and P (a).
Fig. S9

Fig. S9 ESI-MS of P

Fig. S10

Fig. S10 $^1$H NMR spectrum of P
Fig. S11

Fig. S11 $^{13}$C NMR spectrum of P
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<td>Pyrazoline derivative</td>
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<td>NA</td>
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<td>H$_2$O-CH$_3$CN (1:1, v/v, pH 7.2, 20 mM HEPES)</td>
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<td>$\lambda_{ex/em}=445/525$ nm</td>
<td>Naphthaldehyde derivative</td>
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<td>$\lambda_{ex/em}=350/526$ nm</td>
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<td>$\lambda_{ex/em}=345/490$ nm</td>
<td>Benzophenone azine derivative</td>
<td>Irreversible</td>
<td>0.27</td>
<td>NA</td>
<td>NA</td>
<td>Methanol</td>
<td>NA</td>
<td>NA</td>
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<td>$\lambda_{ex/em}=560/584$ nm</td>
<td>Rhodamine derivative</td>
<td>Reversible</td>
<td>0.059</td>
<td>NA</td>
<td>NA</td>
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<td>SiHa cells, qualitative analysis</td>
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<td>$\lambda_{ex/em}=510/580$ nm</td>
<td>Rhodamine derivative</td>
<td>Reversible</td>
<td>0.16</td>
<td>0.45</td>
<td>NA</td>
<td>Ethanol-H$_2$O (9:1, v/v, pH 5.8, 20 mM HEPES)</td>
<td>HepG2 cells, qualitative and quantitative analysis</td>
<td>6.9 $\times 10^4$</td>
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Cal. 1: Quantum yield of chemosensor P

The quantum yield ($\Phi$) of the Al$^{3+}$ rhodamine complex denotes the fluorescence quantum yield. It is obtained by comparison of the integrated area of the corrected emission spectrum of the sample with that of a solution of rhodamine in ethanol, which has a quantum yield of 0.89. The quantum yield ($\Phi$) can be calculated from multiple measurements ($n = 3$) with the following equation, where absorbance can be obtained from the absorption spectra and $\int F$ can be calculated by summation of fluorescence intensity. Consequently, the quantum yield of the Al$^{3+}$-rhodamine complex can be calculated as 0.45.

$$\phi_{sample} = \frac{Abs_{standard} \phi_{standard} \int F_{sample}}{Abs_{sample} \int F_{standard}}$$

Cal. 2 Binding constant of Al$^{3+}$ and chemosensor P

The binding constant was determined according to Benesi-Hildebrand method as follows: When assuming a 1:1 stoichiometry for interaction between chemosensor P and Al$^{3+}$, the equilibrium is given by following equation:

$$P + Al^{3+} \rightarrow P \cdot Al^{3+} \quad (1)$$

The association constant, $k$, is therefore expressed as:

$$k = \frac{[P \cdot Al^{3+}]}{[P][Al^{3+}]} = \frac{[P \cdot Al^{3+}]}{([P]_o - [P \cdot Al^{3+}])[Al^{3+}]_o - [P \cdot Al^{3+}]} \quad (2)$$

$[P \cdot Al^{3+}]$, [P], and [Al$^{3+}$] represent the equilibrium concentrations of the complex, free P, and free Al$^{3+}$, respectively. $[P]_o$ and $[Al^{3+}]_o$ are the initial concentrations of P and Al$^{3+}$, respectively. If $[Al^{3+}]_o >> [P \cdot Al^{3+}]$, the Eq. 2 can be simplified as follows:

$$k = \frac{[P \cdot Al^{3+}]}{([P]_o - [P \cdot Al^{3+}])[Al^{3+}]_o} \quad (3)$$

Eq. 3 is transformed to:
\[
\frac{1}{[P \cdot Al^{3+}]} = \frac{1}{k[P]_0[Al^{3+}]_0} + \frac{1}{[P]_0} \quad (4)
\]

Fluorescence intensity is given as follows:

\[
F_0 = k_0[P]_0 \quad (5)
\]

\[
F = k_0[P]_0 + k_x[P \cdot Al^{3+}] \quad (6)
\]

\[
F_{\text{max}} = k_0[P]_{\text{max}} + k_x[P \cdot Al^{3+}]_{\text{max}} \quad (7)
\]

where, \(F_0\) is the absorbance of \(P\) without \(Al^{3+}\), \(F\) is the fluorescence intensity of \(P\) obtained with \(Al^{3+}\), \(F_{\text{max}}\) is the fluorescence intensity of \(P\) in the presence of excess amount of \(Al^{3+}\). By means of Eqs. 5, 6 and 7, the following equation is obtained:

\[
\frac{F_{\text{max}} - F_0}{F - F_0} = \frac{[P \cdot Al^{3+}]_{\text{max}}}{[P \cdot Al^{3+}]} \quad (8)
\]

In the presence of excess amount of \(Al^{3+}\), \([P \cdot Al^{3+}]_{\text{max}}\) is almost equal to \([P]_0\). The Eq. 8 can therefore be replaced as follows:

\[
\frac{F_{\text{max}} - F_0}{F - F_0} = \frac{[P]_0}{[P \cdot Al^{3+}]} \quad (9)
\]

Using Eq. 4 and 9, the Benesi-Hildebrand equation is obtained as:

\[
\frac{1}{F - F_0} = \frac{1}{K(F_{\text{max}} - F_0)[Al^{3+}]_0} + \frac{1}{F_{\text{max}} - F_0} \quad (10)
\]

\(F_0\) is the fluorescence intensity of \(P\) without \(Al^{3+}\), \(F\) is the fluorescence intensity of \(P\) obtained with \(Al^{3+}\), \(F_{\text{max}}\) is the fluorescence intensity of \(P\) in the presence of excess amount of \(Al^{3+}\), \(K\) is the binding constant (M\(^{-1}\)) and was determined from the slope of the linear plot. Therefore, the binding constant is obtained of \(6.9 \times 10^4\) M\(^{-1}\).