A single probe for sensing both acetate and aluminum(III): Visible region detection, red fluorescence and human breast cancer cell imaging

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1. General method of UV-Vis and fluorescence titration

Path length of the cells used for absorption and emission studies is 1 cm. Stock solutions of L and AcO\textsuperscript{-} are prepared in DMSO while for Al\textsuperscript{3+}-work, stock solutions of L and Al\textsuperscript{3+} are prepared in CH\textsubscript{3}OH/ water (4/1, v/v). Working solutions of L, Al\textsuperscript{3+} and AcO\textsuperscript{-} are prepared from their respective stock solutions. Fluorescence measurements are performed using 10 nm x 5 nm slit width for AcO\textsuperscript{-} and 2.5 nm x 2.5 nm slit width for Al\textsuperscript{3+} work.

2. Job’s plot from fluorescence experiments

A series of solutions containing L, Al\textsuperscript{3+} and AcO\textsuperscript{-} in respective solvents are prepared such that the total concentration of Al\textsuperscript{3+}+L or L + AcO\textsuperscript{-} remain constant (50 µM) in all the sets. The mole fraction (X) of L is varied from 0.1 to 0.8. The fluorescence intensities are plotted against the mole fraction of L.

3. Determination of binding constant

The binding constant of L for Al\textsuperscript{3+} and AcO\textsuperscript{-} are determined using modified Benesi–Hildebrand equation\textsuperscript{1}: \(\frac{F_{\text{max}} - F_{\text{min}}}{(F_x - F_{\text{min}})} = 1 + \frac{1}{K} \frac{1}{[C]_0}\) where \(F_{\text{max}}, F_{\text{min}}\) and \(F_x\) are emission
intensity values for $L$ in the presence of $\text{Al}^{3+}$ or $\text{AcO}^-$ at saturation, in the absence of $\text{Al}^{3+} / \text{AcO}^-$ and at any intermediate $\text{Al}^{3+} / \text{AcO}^-$ concentration, respectively. A plot of $(F_{\text{max}} - F_{\text{min}})/(F_{x} - F_{\text{min}}) = 1 + (1/K) (1/[C]^n)$ (here $n = 1$, for both cases) allows to calculate the binding constant from the slope.

4. Determination of quantum yield

Fluorescence quantum yields ($\Phi$) are estimated by integrating the area under the fluorescence curves using the equation,

$$\Phi_{\text{sample}} = \frac{OD_{\text{standard}} \times A_{\text{sample}}}{OD_{\text{sample}} \times A_{\text{standard}}} \times \Phi_{\text{standard}} \times \frac{\eta_{\text{sample}}^2}{\eta_{\text{standard}}^2}$$

where $A$ is the area under the fluorescence spectra and $OD$ is optical density of the compound at the excitation wavelength. The area of the emission spectrum is integrated using the software available in the instrument. $\Phi_{\text{sample}}$ and $\Phi_{\text{ref}}$ are the fluorescence quantum yields of the sample and reference respectively. $A_{\text{sample}}$ and $A_{\text{ref}}$ are the area under the fluorescence spectra of the sample and the reference, respectively. $OD_{\text{sample}}$ and $OD_{\text{ref}}$ are the corresponding optical densities of the sample and the reference solution at the wavelength of excitation. $\eta_{\text{sample}}$ and $\eta_{\text{ref}}$ are the refractive indices of the sample and reference, respectively. Anthracene is used as reference with a known $\Phi_{\text{ref}}$ value of 0.27 in EtOH$^3$ for measuring the quantum yields of ligand and its $\text{Al}^{3+}$ complex and Rhodamine B is used as reference with a known $\Phi_{\text{ref}}$ value of 0.65 in basic EtOH$^4$ for measuring the quantum yields of $L$ and its AcO$^-$ adduct.
**Fig.S1.** Plot of emission intensities of **L** (10 μM, $\lambda_{ex} = 532$ nm, $\lambda_{em} = 621$ nm) as a function of externally added AcO¯ (0.5-1000 μM). Inset shows the linear region (0.5-10 μM AcO¯)

**Fig.S2.** Absorbance of **L** (10 μM) as a function of externally added AcO¯ concentration (0.5-1000 μM)
**Fig.S3.** Linear region of the plot of absorbance of L (10 μM, λ, 520 nm) as a function of externally added AcO$^-$ concentration (0.5-10 μM)

**Fig.S4.** Emission intensities of [L + AcO$^-$] system in presence of competing anions
**Fig. S5.** Visual colour changes of L upon addition of equimolar amount of various cations under UV light (A) and visible light (B)

**Fig. S6.** Job’s plot for determination of stoichiometry of the [L -AcO$^-$] adduct in DMSO ($\lambda_{ex}$, 532 nm, $\lambda_{em}$, 621 nm)
**Fig.S7.** Determination of binding constant of $L$ for AcO$^-$ in DMSO ($\lambda_{ex}$, 532 nm, $\lambda_{em}$, 621 nm) using fluorescence technique

**Fig.S8.** Emission intensities of $L$ (10 $\mu$M) as a function of externally added [AcO$^-$] in DMSO ($\lambda_{ex}$, 532 nm, $\lambda_{em}$, 621 nm) using fluorescence titration data in Fig.2
**Fig. S9.** Plot of emission intensities of L (15 μM, \( \lambda_{\text{ex}} = 432 \) nm, \( \lambda_{\text{em}} = 545 \) nm) as a function of externally added Al\(^{3+} \) (0.5-600 μM); Inset: linear region (0.5-10 μM Al\(^{3+} \)).

**Fig. S10.** Emission intensities of [L-Al\(^{3+} \)] system in presence of common cations.
Fig. S11. Visual colour changes of L upon addition of equimolar amount of various cations under UV light (A) and visible light (B).

Fig. S12. Job’s plot for determination of stoichiometry of the [L-Al^{3+}] adduct in aqueous-methanol (1:4, v/v, $\lambda_{ex}$, 532 nm, $\lambda_{em}$, 621 nm)
Fig.S13. QTOF-MS spectrum of [L-Al$^{3+}$] adduct.

Fig.S14. Determination of binding constant of L for Al$^{3+}$ in aqueous-methanol (1:4, v/v, $\lambda_{ex}$, 432 nm, $\lambda_{em}$, 545 nm) using fluorescence technique.
Fig. S15. Emission intensities of L (15 μM) as a function of externally added [Al$^{3+}$] in aqueous-methanol (1:4, v/v, $\lambda_{ex}$, 432 nm, $\lambda_{em}$, 545 nm for LOD determination (using fluorescence titration data of Fig.7).

Fig. S16. $^1$H NMR spectrum of L in DMSO-$d_6$.
Fig.S17. QTOF-MS spectrum of L
Fig.S18. FTIR spectrum of L
**Fig.S19.** FTIR spectrum of [L- Al^{3+}] adduct

**Table S1. Comparison of the present probe with the existing probe^5**

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<tr>
<th>Sensing parameters</th>
<th>Present probe</th>
<th>Reported probe^5 (S. Goswami et. al.)</th>
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<td>Detection limits</td>
<td>Acetate</td>
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<td>Al^{3+}</td>
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<td>Binding constants</td>
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<td>Al^{3+}</td>
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<td>Emission wavelengths</td>
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<td>Al^{3+}</td>
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**References**


