

A Water-Soluble Bimane Fluorophore for Reversible Fluorescent and Colorimetric Detection of Indium(III)

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TITLE PAGE

TITLE: A Water-Soluble Bimane Fluorophore for Reversible Fluorescent and Colorimetric Detection of Indium(III)

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ABSTRACT

The growing use of indium-containing materials has created a need for sensitive, direct detection methods for indium, particularly in aqueous solutions. Meeting this need remains challenging because of the difficulty of selectively detecting indium(III) in the presence of chemically similar metal cations and the limited aqueous solubility of many small-molecule fluorophores. To address these challenges, we developed compound **1**, a highly water-soluble (9.9 g L^{-1}) sulfonated bimane fluorophore with triazole-based metal-binding sites. Compound **1** was synthesized from *syn*-(CH₃,I)bimane in 28% yield over a two-step sequence and exhibits a fluorescence quantum yield of 94% in water. Exposure of compound **1** to indium(III) and gallium(III) under fully aqueous conditions resulted in fluorescence quenching of 58% and 53%, respectively. Subsequent addition of EDTA restored 93% of the fluorescence quenched by indium(III), compared with only 11% for gallium(III), enabling discrimination between the two ions. This indium(III)-responsive fluorescence quenching was retained when compound **1** was adsorbed onto filter paper, producing a stable, visually detectable response at 100 μM indium(III). Combined spectroscopic, mass-spectrometric, and computational studies support the formation of a cooperative 2:1 indium(III):compound **1** complex, with coordination at the bimane carbonyl and triazole groups accounting for the observed fluorescence quenching. Together, these results demonstrate how sulfonation can enable the use of compact fluorophore scaffolds in aqueous metal-ion sensing and establish compound **1** as a mechanistically characterized, reversible fluorescent probe for indium(III).

KEYWORDS

Water-soluble fluorophore, bimane, indium(III) detection, fluorescence quenching mechanism, colorimetric and fluorimetric sensing, paper-based sensing

1. Introduction

Fluorescence-based chemosensors provide a sensitive and readily observable means of detecting metal ions, with potential advantages that include rapid response, straightforward signal quantification, and compatibility with simple, low-cost instrumentation [1]. The development of such sensors for use in aqueous samples remains challenging, however, because many small-molecule fluorophores have limited water solubility or do not retain their emissive properties and analyte responsiveness under fully aqueous conditions [2]. This limitation is particularly important for environmental and biological applications, as well as for adaptation to paper-based sensing platforms [3].

One fluorophore scaffold with considerable yet underdeveloped aqueous sensing potential is bimane. Bimanes are compact, rigid bicyclic fluorophores with generally high fluorescence quantum yields combined with synthetic tunability that permits facile installation of solubilizing and metal-binding substituents [4]. Prior work from our laboratories has demonstrated that bimane derivatives can bind alkali metal cations through coordination to the two carbonyl oxygen atoms [5], as well as transition-metal cations through interactions with nitrogen-containing substituents at the α -position [6,7]. Despite this broad metal-binding behavior, the interactions of bimane derivatives with group 13 metals have not been explored.

The hydrophobicity of many bimane derivatives has also limited their application as fluorescent metal ion probes in fully aqueous media [8]. One approach to overcoming this limitation is to increase fluorophore hydrophilicity through covalent modification. For example, introducing sulfonate groups is a well-established strategy for increasing aqueous solubility while retaining the core fluorophore scaffold [9]. Nonetheless, the use of sulfonation to develop bimane-based fluorescent probes that operate in fully aqueous media remains largely unexplored [2].

Indium(III) is a particularly relevant target for a water-compatible bimane probe because indium-containing materials are widely used in semiconductors, photovoltaics, liquid-crystal displays, and touchscreen technologies [10,11]. This widespread use has been accompanied by concerns regarding the pulmonary toxicity of indium exposure and its adverse effects on aquatic organisms [12,13]. Established methods for indium analysis, including inductively coupled plasma mass spectrometry [14], inductively coupled plasma optical emission spectroscopy [15], and atomic absorption spectroscopy [16], provide excellent sensitivity and selectivity but require specialized instrumentation and laboratory infrastructure. Electrochemical, colorimetric, and fluorimetric methods for indium detection have also been reported [17,18,19], although the reported fluorescent probes require organic or mixed-solvent media.

Herein, we report the synthesis and photophysical characterization of compound **1** (Scheme 1), a sulfonated, water-soluble bimane fluorophore containing triazole-based metal-binding groups. Examination of compound **1** in solvents of varying polarity reveals pronounced positive solvatochromism, while its high fluorescence quantum yield and large Stokes shift in water demonstrate strong emissive properties in fully aqueous conditions. We further demonstrate that compound **1** exhibits indium(III)-induced fluorescence quenching followed by near-complete restoration upon EDTA addition, thereby distinguishing indium(III) from the other group 13 metals examined. Spectroscopic, mass-spectrometric, and computational studies support the formation of a 2:1 indium(III):**1** complex and provide insight into the observed fluorescence quenching. More broadly, this work shows how sulfonation can extend the use of bimane fluorophores to sensing under fully aqueous conditions.

2. Experimental section

2.1 Materials and methods

Unless otherwise noted, reagents were purchased from commercial suppliers and used as received. Ultrapure water ($\geq 18.2 \text{ M}\Omega\cdot\text{cm}$) was used for all aqueous experiments. ^1H and ^{13}C NMR spectra were acquired at 400 and 101 MHz, respectively, using a Bruker Avance III spectrometer. High-resolution mass spectrometry (HRMS) was measured in positive ionization mode using a Waters Micromass Quattro Micro instrument equipped with an electrospray ionization source and Waters 2795 and 996 PDA detectors. Solid-state FTIR spectra were recorded on a PerkinElmer Spectrum One FTIR spectrometer using an ATR (attenuated total reflectance) accessory. UV–visible absorbance spectra were recorded on a Varian Cary 50 Bio UV–visible spectrophotometer and fluorescence emission spectra were recorded on a Varian Cary Eclipse fluorescence spectrophotometer. Spectral integration and plotting were performed in OriginPro. Photographs for colorimetric analysis were processed using ImageJ.

2.2 Synthesis of compound **1**

In brief, sodium *p*-azidobenzenesulfonate (compound **5**) was prepared from 4-aminobenzenesulfonic acid via diazotization/azidation, and an alkyne-functionalized biman intermediate (compound **4**) was prepared from *syn*-(CH_3I)bimane via Sonogashira coupling. Compound **1** was then obtained by Cu(I)-catalyzed azide–alkyne cycloaddition between **4** and **5** in a 1:1 acetonitrile:water mixture under nitrogen, followed by reverse-phase preparative HPLC purification to afford compound **1** as a yellow solid.

2.3 Spectroscopic characterization of compound **1**

Molar extinction coefficient determination: The molar extinction coefficient of compound **1** in water was determined by Beer–Lambert analysis, by plotting the absorbance at 409 nm of aqueous solutions of compound **1** versus its concentration.

Quantum yield determination: The fluorescence quantum yield of compound **1** in water was determined by a single-point relative method using Rhodamine B in ethanol ($\Phi_{\text{F}} = 0.50$) as the standard.

Concentration-dependent spectroscopic studies: Aqueous solutions of compound **1** were prepared over a concentration range of 2–20 μM by stepwise dilution from a 20 μM stock solution. After brief mixing, UV–visible absorbance and fluorescence emission spectra were recorded.

2.4 Solution-state spectroscopic response of compound **1** to metal ion analytes

Selectivity and competition experiments: Aqueous stock solutions of compound **1** were prepared ($[\text{I}] = 10 \mu\text{M}$). For selectivity screening, aliquots of metal-salt stock solutions were added to 3 mL of the compound **1** solution to give a final metal-ion concentration of 40 μM . Solutions were mixed by hand for 2 min, followed by acquisition of the UV–visible absorbance (200–800 nm) and fluorescence emission spectra ($\lambda_{\text{ex}} = 410 \text{ nm}$). For competition experiments, $\text{In}(\text{NO}_3)_3$ was added to samples containing compound **1** and a competing analyte (each at 40 μM final concentration). Integration of UV–visible (355–700 nm) and fluorescence emission (418–800 nm) spectra was performed in OriginPro.

Titration experiments: A 10 μM aqueous solution of compound **1** was titrated with $\text{In}(\text{NO}_3)_3$ by stepwise addition of a concentrated $\text{In}(\text{NO}_3)_3$ stock solution, with 2 min mixing between additions. UV–visible absorbance and fluorescence emission spectra were acquired after each addition.

Sensitivity experiments: Limit of detection (LOD) and limit of quantification (LOQ) values were determined from calibration curves collected at low $\text{In}(\text{NO}_3)_3$ concentrations using a 10 μM aqueous solution of compound **1**. For each replicate, blank measurements (no analyte) were

used to calculate LOD_{blank} and LOQ_{blank} ($LOD_{blank} = average_{blank} - 3 \times SD_{blank}$; $LOQ_{blank} = average_{blank} - 10 \times SD_{blank}$). LOD and LOQ concentrations were then determined by treating the corresponding LOD_{blank} and LOQ_{blank} values as signal thresholds and converting them to analyte concentrations using the best-fit linear calibration equation ($y = mx + b$, where y is the measured spectroscopic signal and x is the $In(NO_3)_3$ concentration).

Solvent-dependent experiments: Solutions of compound **1** (10 μ M) were prepared in *N*-methylpyrrolidone (NMP), methanol, and dimethyl sulfoxide (DMSO). UV–visible absorbance and fluorescence emission spectra were recorded under the standard conditions described above. Aliquots of aqueous $In(NO_3)_3$ or $Ga(NO_3)_3$ stock solutions were then added to achieve a final metal-ion concentration of 40 μ M, followed by brief mixing and acquisition of the corresponding spectra.

2.5 Solution-state colorimetric response of compound **1** to metal ion analytes

Selectivity experiments: 1 mL of an aqueous solution of compound **1** ($[1] = 10 \mu$ M) was pipetted into individual vials. Aqueous analyte solutions of various metal-ion analytes (4 μ L, 20 mM) were added to give a final analyte concentration of 80 μ M, followed by 2 min of mixing. The vials were photographed under ambient light and under 365 nm excitation, and the resulting images were analyzed quantitatively using ImageJ.

Titration experiments: 1 mL of an aqueous solution of compound **1** ($[1] = 10 \mu$ M) was pipetted into individual vials. Increasing volumes (1–10 μ L) of an aqueous $In(NO_3)_3$ stock solution (10 mM) were added to obtain final $In(NO_3)_3$ concentrations of 10–100 μ M, followed by 2 min of mixing. The vials were photographed under ambient light and under 365 nm excitation, and the resulting images were analyzed quantitatively using ImageJ.

2.6 Filter paper-based colorimetric response of compound **1** to metal ion analytes

Filter paper fabrication: Filter papers (Munktell, Grade 100/N) were prepared by immersing them overnight in an aqueous solution of compound **1** ($[1] = 50 \mu$ M) to allow adsorption of compound **1** onto the paper, followed by air-drying.

Selectivity experiments: Compound **1**-adsorbed papers were immersed in aqueous analyte solutions (100 μ M) for 1 min, removed, and dried under ambient conditions for 3 min. The papers were then photographed under ambient light and under 365 nm excitation, and the images were analyzed quantitatively using ImageJ.

Titration experiments: Compound **1**-adsorbed filter papers were exposed to aqueous $In(NO_3)_3$ solutions of increasing concentration (10–100 μ M) for 1 min, removed, and dried under ambient conditions for 3 min. The papers were then photographed under ambient light and under 365 nm excitation, and the resulting images were analyzed quantitatively using ImageJ.

Optimization and stability experiments: The probe loading concentration was evaluated by loading Munktell 100/N filter papers using aqueous solutions of compound **1** at 10, 50, or 100 μ M, followed by exposure to blank or 100 μ M $In(NO_3)_3$ solutions. Paper photostability was evaluated under continuous 365 nm irradiation for 60 min, and storage stability was assessed by comparing freshly prepared papers with papers stored for 2 days under ambient laboratory conditions. The papers were photographed under ambient light and under 365 nm excitation, and the resulting images were analyzed quantitatively using ImageJ.

2.7 EDTA experiments

Aqueous solutions of compound **1** ($[1] = 10 \mu$ M) were treated with either $In(NO_3)_3$ or $Ga(NO_3)_3$ (40 μ M final concentration). EDTA disodium salt (EDTA-2Na) was then added to the same

samples (40 μM final concentration), followed by brief mixing. UV–visible absorbance and fluorescence emission spectra were collected under the conditions described above. The same samples were photographed under ambient light and under 365 nm excitation, and the resulting images were analyzed quantitatively using ImageJ.

2.8 Time- and pH-dependence experiments

Time-dependent fluorescence measurements were collected after addition of $\text{In}(\text{NO}_3)_3$ (40 μM final concentration) to aqueous compound **1** solutions ($[\text{1}] = 10\ \mu\text{M}$), using either repeated full emission scans (415–800 nm) or a limited emission window (505–525 nm). For pH-dependence studies, compound **1** solutions ($[\text{1}] = 10\ \mu\text{M}$) were adjusted to the desired pH using HCl or NaOH, and fluorescence spectra were collected before and after $\text{In}(\text{NO}_3)_3$ (40 μM final concentration) addition. The photostability of free compound **1** and the time-dependent fluorescence response of the compound **1**– $\text{In}(\text{III})$ system ($[\text{1}] = 10\ \mu\text{M}$; $[\text{In}(\text{NO}_3)_3] = 40\ \mu\text{M}$) were also evaluated under continuous excitation at 410 nm for 60 min by monitoring fluorescence emission over 505–525 nm. All measurements were performed in three independent trials.

2.9 Real-world water sample experiments

Calibration curves for $\text{In}(\text{NO}_3)_3$ were constructed using aqueous solutions of compound **1** ($[\text{1}] = 10\ \mu\text{M}$) prepared in either ultrapure water or commercial bottled mineral water. Known amounts of $\text{In}(\text{NO}_3)_3$ were added to these matrices, followed by acquisition of UV–visible absorbance and fluorescence emission spectra under the conditions described above. Indium concentrations were determined from the corresponding calibration curve, and percent recovery was calculated as $[\text{In}]_{\text{calculated}}/[\text{In}]_{\text{spiked}} \times 100\%$.

2.10 Mechanistic experiments

Job's plot analysis: Continuous-variation (Job's plot) experiments were performed by preparing solutions in which the total concentration of compound **1** plus $\text{In}(\text{NO}_3)_3$ was held constant while the mole fraction was varied. After 2 min mixing, UV–visible absorbance and fluorescence emission spectra were collected and analyzed as described above.

High-resolution mass spectrometry (HRMS) experiments: Compound **1** (20 μg) was dissolved in water (1 mL), followed by addition of eight equivalents of aqueous $\text{In}(\text{NO}_3)_3$. The mixture was stirred manually for 2 min, filtered, and analyzed by HRMS (positive ion mode).

^1H NMR titration experiments: Compound **1** (0.5 mg) was dissolved in D_2O (500 μL) and its ^1H NMR spectrum was recorded. For complexation studies, separate samples were prepared with 1:1 and 1:2 molar ratios of compound **1** and $\text{In}(\text{NO}_3)_3$ in D_2O . Each solution was vortexed for 1 min prior to acquisition of the ^1H NMR spectrum.

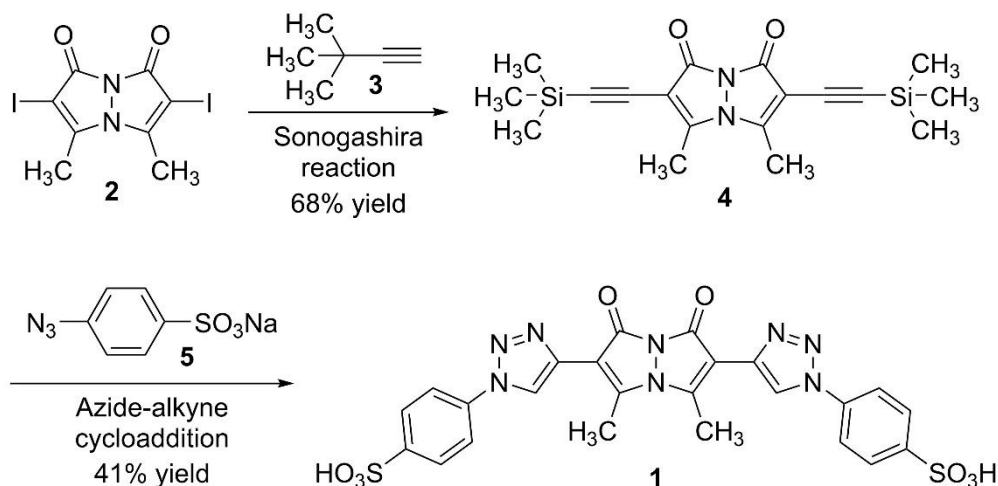
FTIR experiments: Solid-state FTIR spectra were recorded using an ATR accessory. A background spectrum was collected prior to each measurement. Spectra were obtained for solid compound **1** and for the solid-state complex formed between compound **1** and $\text{In}(\text{III})$.

Computational analysis: DFT geometry optimizations were performed using ORCA 6.0 with the B97M-V functional and def2-TZVP basis set, including the SMD implicit-solvation model for water. For the nitrate-free 2:1 $\text{In}(\text{III})$ **1** complex, compound **1** was modeled in its dianionic sulfonate form and the overall complex was treated as a singlet species with a total charge of +4. Eight explicit water molecules were included, corresponding to four water molecules per $\text{In}(\text{III})$ center, and no explicit counterions were included. The def2 effective core potential was applied to indium. Initial structures were constructed in Avogadro and subsequently optimized by DFT. Geometry optimization converged successfully.

3. Results and discussion

3.1 Design and photophysical properties of **1**

Motivated by the challenge of achieving bimane fluorescence in fully aqueous media, we designed compound **1** (Scheme 1), a bimane derivative with triazole substituents to facilitate metal coordination and sulfonate groups to impart water solubility. Compound **1** was obtained from *syn*-(CH₃,I)bimane in 28% yield over a two-step sequence, and exhibited outstanding aqueous solubility (9.9 g L⁻¹ at room temperature).



Scheme 1. Synthesis of compound **1** via Sonogashira coupling followed by azide–alkyne [3+2] cycloaddition.

3.2 Solvatochromism and photophysical robustness of compound **1**

Photophysical analysis of solutions of compound **1** revealed general solvent insensitivity in the absorbance spectra combined with marked positive solvatochromism in the fluorescence emission spectra (Table 1). More specifically, the maximum absorption wavelength shifted by only 7 nm across four solvents with widely disparate polarities (NMP, DMSO, methanol, and water). By contrast, the emission maximum increased substantially with increasing solvent polarity, from 477 nm in NMP to 515 nm in water, leading to a linear increase in the Stokes shift as a function of the $E_T(30)$ values ($R^2 = 0.997$), from 61 nm in NMP to 106 nm in water (Figure 1A). This positive solvatochromism indicates an increase in the excited-state polarity of compound **1** relative to its ground-state polarity, which is consistent with partial charge-transfer character in the emissive excited state. Moreover, the large Stokes shift observed in water (106 nm) is a practically useful property for a fluorescent probe, as it minimizes self-absorption and inner-filter effects that can complicate quantitative fluorescence measurements. Compound **1** also retains a high fluorescence quantum yield in water (94%, relative quantum-yield measured against Rhodamine B), which combined with its aqueous molar extinction coefficient ($\epsilon = 1.00 \times 10^4$ L mol⁻¹ cm⁻¹) corresponds to a brightness ($\epsilon \times \Phi$) of approximately 9,400 M⁻¹ cm⁻¹.

Table 1. Solvent-dependent photophysical properties of compound **1**.^a

Solvent	$E_T(30)$ (kcal mol ⁻¹)	$\lambda_{\text{max,abs}}$ (nm)	$\lambda_{\text{max,em}}$ (nm)	Stokes shift (nm)
NMP	42.2	416	477	61
DMSO	45.1	415	482	67
Methanol	55.4	412	499	87

Water	63.1	409	515	106
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^a Values represent an average of three independent trials. $E_T(30)$ values obtained from the literature [20]; $\lambda_{ex} = 410$ nm.

Compound **1**'s fluorescence was also stable across a range of practical conditions, including solution pH, light exposure, and probe concentration. Within the pH range of 4.08–9.86, the fluorescence intensity of compound **1** in water varied by less than 5% (Figure 1B), with a more pronounced decrease observed only under strongly acidic conditions (pH < 2). Compound **1** also exhibited excellent photostability, retaining $99.95 \pm 0.23\%$ of its initial fluorescence intensity after 60 minutes of continuous excitation at 410 nm (Table S27, ESI). Finally, both the absorbance and fluorescence intensity of compound **1** increased linearly with concentration over the range tested (2–20 μM ; $R^2 > 0.98$; Figures S46–S49, ESI), suggesting minimal concentration-dependent quenching under these conditions. Taken together, these photophysical properties establish compound **1** as a robust, well-behaved fluorophore with substantial potential utility in aqueous-phase fluorescence studies and associated applications.

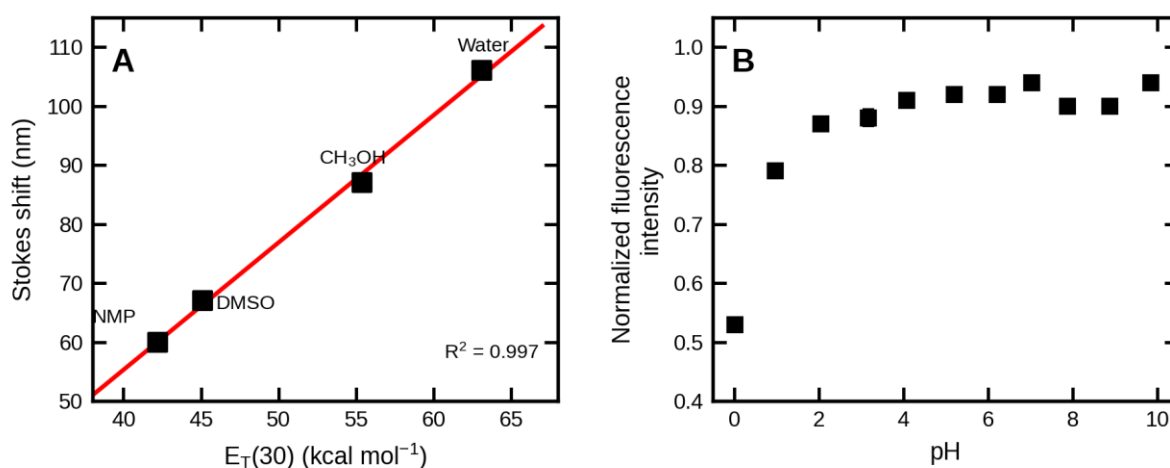


Fig. 1 (A) Stokes shift of compound **1** as a function of solvent polarity ($E_T(30)$); the red line shows the best linear fit to the data ($R^2 = 0.997$). (B) Normalized fluorescence intensity of aqueous solutions of compound **1** (10 μM) as a function of pH ($\lambda_{ex} = 410$ nm). Data in (A) are derived from Table 1; data in (B) are derived from ESI Table S30.

3.3 Metal-sensing performance

Screening compound **1** against 22 metal salts revealed pronounced fluorescence quenching in the presence of indium(III) and gallium(III), with 58% and 53% decreases in emission, respectively (Table 2). In contrast, iron(III) and aluminum(III), which represent common trivalent interferents in aqueous systems, produced only moderate responses (19% and 15%, respectively), while all other analytes caused $\leq 4\%$ changes, indicating minimal nonspecific quenching. Additional interference experiments, in which indium(III) (40 μM) was combined with each potentially interfering metal ion at an equimolar concentration (40 μM), showed that only thallium(III) and iron(III) substantially attenuated the indium(III)-induced quenching response (74.5% and 67.6% change in fluorescence signal, respectively, versus $\leq 15\%$ for all other tested ions; Tables S4 and S5, ESI).

This metal-ion-induced quenching was apparent in the normalized fluorescence emission spectra of compound **1** (Figure 2A) and was corroborated by RGB-based analysis of photographs of the same solutions acquired under 365 nm excitation (Figure 2B). Measurements in other polar solvents showed that these fluorescence responses were strongly solvent-

dependent: in methanol, both ions produced pronounced fluorescence quenching accompanied by a bathochromic shift in the maximum emission wavelength; in NMP, substantial quenching was also observed but without a significant shift in the emission signal; and in DMSO, almost no response occurred (Tables S16–S17 and Figures S52–S54, ESI).

Table 2. Decrease in fluorescence emission of compound **1** upon exposure to selected metal ions in aqueous solution.^a

Analyte	Fluorescence decrease (%) ^b
In ³⁺	58 ± 0.2
Ga ³⁺	53 ± 1.0
Al ³⁺	15 ± 0.7
Fe ³⁺	19 ± 3.4
K ⁺	4 ± 1.1
Na ⁺	3 ± 1.3
Tl ³⁺	2 ± 2.8
Ca ²⁺	2 ± 1.4
Mn ²⁺	2 ± 0.9
Zn ²⁺	2 ± 0.4
All remaining tested analytes (Fe ²⁺ , Li ⁺ , Hg ²⁺ , Ag ⁺ , Ba ²⁺ , Ce ³⁺ , Co ²⁺ , Pb ²⁺ , Cu ²⁺ , Cd ²⁺ , Mg ²⁺ , Ni ²⁺)	≤ 1

^a [1] = 10 μM; [analyte] = 40 μM; λ_{ex} = 410 nm; metal ions were introduced using nitrate, chloride, or sulfate salts, as detailed in the ESI; values represent an average of 3 independent trials.

^b Fluorescence decrease was calculated as $[(F_{\text{blank}} - F_{\text{analyte}})/F_{\text{blank}}] \times 100\%$, where F_{blank} and F_{analyte} represent the integrated emission intensities of compound **1** in the absence and presence of analyte, respectively.

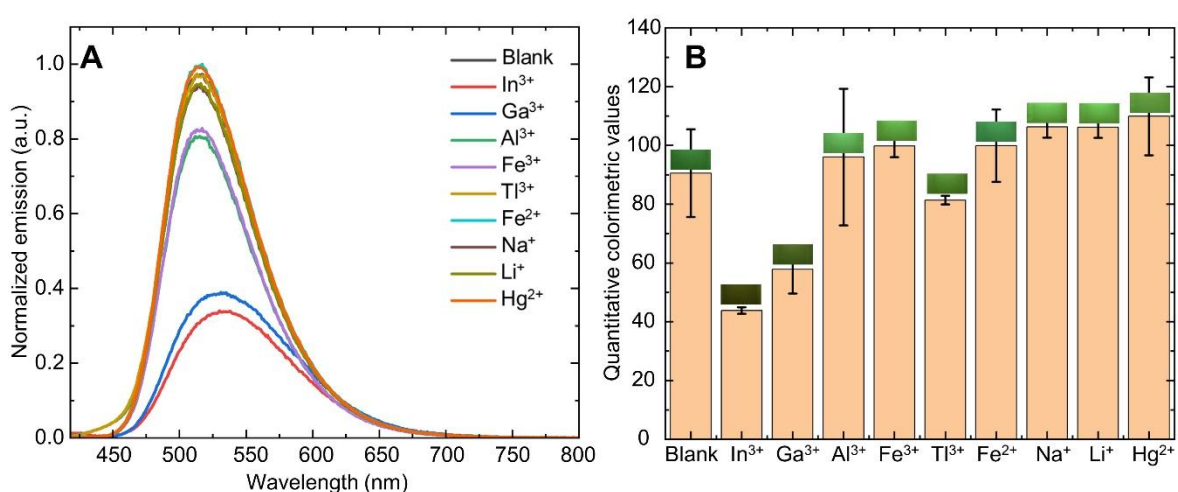


Fig. 2 (A) Normalized fluorescence emission spectra of compound **1** in the presence of selected metal cations. (B) Quantitative RGB-based colorimetric analysis of parallel solutions imaged

under 365 nm excitation, with representative color regions cropped from the vial photographs (Experimental conditions: $[1] = 10 \mu\text{M}$; $[\text{analyte}] = 80 \mu\text{M}$; $\lambda_{\text{ex}} = 410 \text{ nm}$. Quantitative colorimetric values correspond to the average RGB intensity calculated as $(R + G + B)/3$. All values represent an average of 3 independent trials, and the error bars in (B) represent the standard deviations from the 3 trials).

To address the limited discrimination in compound **1**'s response to indium(III) and gallium(III), we tested whether ethylenediaminetetraacetic acid (EDTA), a multidentate metal chelator, could selectively restore compound **1**'s fluorescence. This approach proved highly effective: EDTA restored 93% of the fluorescence signal in indium(III)-treated samples, likely due to its ability to effectively sequester indium(III), but only 11% in gallium(III)-treated samples due to the weaker EDTA-gallium(III) binding (Table 3) [21]. This recovery was quantified by fluorescence spectroscopy (Figures 3A and 3B) and independently confirmed by RGB analysis of images acquired under 365 nm excitation (Figure 3C).

Table 3. Fluorescence emission and EDTA-induced recovery for compound **1** in the presence of indium(III) and gallium(III) in aqueous solution.^a

Analyte	Emission after analyte (%)	Emission after analyte + EDTA (%)	Fluorescence recovery (%) ^b
In(III)	45.0 ± 2.2	96.2 ± 0.8	93.1
Ga(III)	50.9 ± 1.5	56.3 ± 3.1	11.0

^a $[1] = 10 \mu\text{M}$; $[\text{In(III)}] = [\text{Ga(III)}] = [\text{EDTA}] = 40 \mu\text{M}$; values represent the mean of 3 independent trials. Additional EDTA concentrations and conditions were examined to assess the robustness of this response (see ESI).

^b Fluorescence recovery was calculated as $[(F_{\text{EDTA}} - F_{\text{analyte}}) / (100 - F_{\text{analyte}})] \times 100\%$, where F_{analyte} is the percent fluorescence measured after metal-ion addition and F_{EDTA} is the percent fluorescence measured after subsequent EDTA treatment.

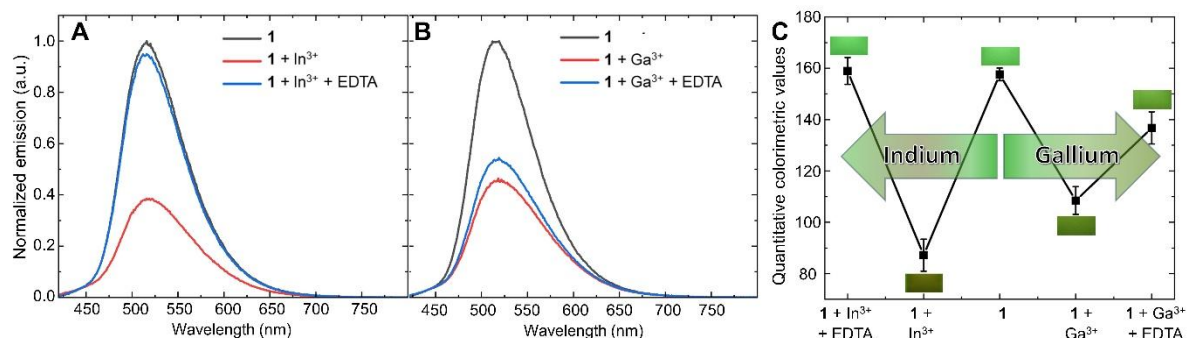


Fig. 3 (A, B) Normalized fluorescence emission spectra of compound **1** (10 μM) before and after addition of (A) In(III) or (B) Ga(III) (40 μM), followed by EDTA (40 μM) (spectra are normalized to the maximum emission of compound **1**; $\lambda_{\text{ex}} = 410 \text{ nm}$). (C) RGB-derived colorimetric values for parallel solutions, with representative color swatches shown above each data point (points represent mean $\pm$ SD of 3 independent trials).

The solution-state sensitivity of compound **1** was determined by measuring fluorescence quenching at indium(III) concentrations between 0 and 1.2 μM, which showed a linear response ($R^2 = 0.996$) corresponding to limits of detection (LOD) and quantification (LOQ) of 0.18 μM and 0.94 μM, respectively. Several literature-reported indium(III) sensors achieve lower LOD values [22-25], but rely on organic co-solvents or multi-step sample processing that limit their applicability in aqueous biological and environmental contexts. Compound **1**, by contrast,

operates entirely in 100% aqueous solution while maintaining a linear response, a submicromolar LOD, and EDTA-mediated fluorescence recovery that discriminates indium(III) from gallium(III).

Having established the solution-state selectivity and sensitivity of compound **1** toward indium(III), we next examined how its fluorescence response varied with pH and over time. The indium(III)-induced response remained consistent from pH 4 to 9, which encompasses environmentally and biologically relevant conditions (Figures S82–S87, ESI). Time-dependent measurements under continuous 410 nm excitation after indium(III) addition revealed a rapid initial decrease in fluorescence (to $70.6 \pm 6.4\%$ after 10 min) followed by a slower continuing decline (to $60.8 \pm 5.8\%$ after 60 min) (Figures S78–S81, Table S28, ESI). By contrast, free compound **1** showed no meaningful loss of fluorescence over the same period (see photostability data above and Figure S81A, ESI), confirming that the decrease is specific to the indium(III)-bound system.

3.4 Preliminary evaluation of a paper-supported format

To determine whether the aqueous indium(III) response of compound **1** was retained on a solid support, Munktell 100/N paper was immersed in a 50 μM aqueous solution of compound **1**. The resulting compound **1**-loaded paper exhibited a readily visible fluorescence change upon exposure to indium(III) (Figure 4A). Quantitative analysis of the extracted color channels revealed that the largest analyte-dependent change occurred in the red channel, whereas the blue channel changed only slightly (Figures 4B and 4C). Exposure to gallium(III), by comparison, produced only modest changes in both channels.

Although the fluorescence change on paper was readily visible, the paper-supported response was less sensitive than the corresponding solution-phase response. The lowest indium(III) concentration that produced a distinguishable change on paper was 100 μM , compared with a solution-phase LOD of 0.18 μM . This difference may reflect restricted analyte transport within the paper matrix, light scattering, and variability associated with image-based signal acquisition [26]. Despite this lower sensitivity, the fluorescence of immobilized compound **1** was stable: compound **1**-loaded paper retained more than 99% of its initial fluorescence after 60 min of continuous 365 nm irradiation and more than 97% after two days of ambient storage.

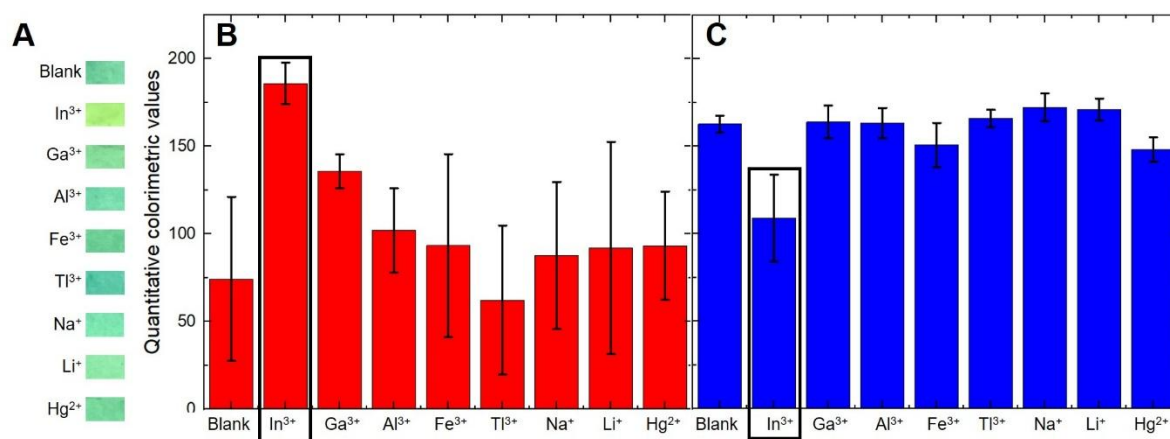


Fig. 4 Fluorescence response of compound **1** immobilized on filter paper following exposure to metal ion solutions (100 μM) and imaging under 365 nm excitation. (A) Representative photographs of paper-supported sensors after exposure to selected metal ions. (B, C) Quantitative analysis of the (B) red and (C) blue color channels extracted from the same images. (Data represent the mean of the quantitative color-channel intensities obtained from 3 independent paper sensors per analyte and extracted using ImageJ.)

To determine whether compound **1**'s aqueous fluorescence response tolerates a more complex sample matrix, we evaluated it in commercially available mineral water spiked with indium(III). Pronounced fluorescence quenching was observed in this matrix, comparable to the response measured in Milli-Q water (Figure 5A). Quantitative analysis of integrated fluorescence intensity produced linear calibration curves in both media (Figure 5B), with similar slopes indicating that the sensing response is largely unaffected by the complex matrix composition. As shown in Table 4, the calculated indium(III) concentrations closely matched the spiked values in both matrices, with only modest underestimation at the two highest concentrations in mineral water, likely reflecting minor matrix effects. Across all samples, recoveries ranged from 83–106%, corresponding to biases of –17% to +6%, while the narrow 95% confidence intervals indicate good measurement precision. These results demonstrate quantitative solution-phase determination of indium(III) in mineral water using compound **1** without the need for organic co-solvents or sample pretreatment.

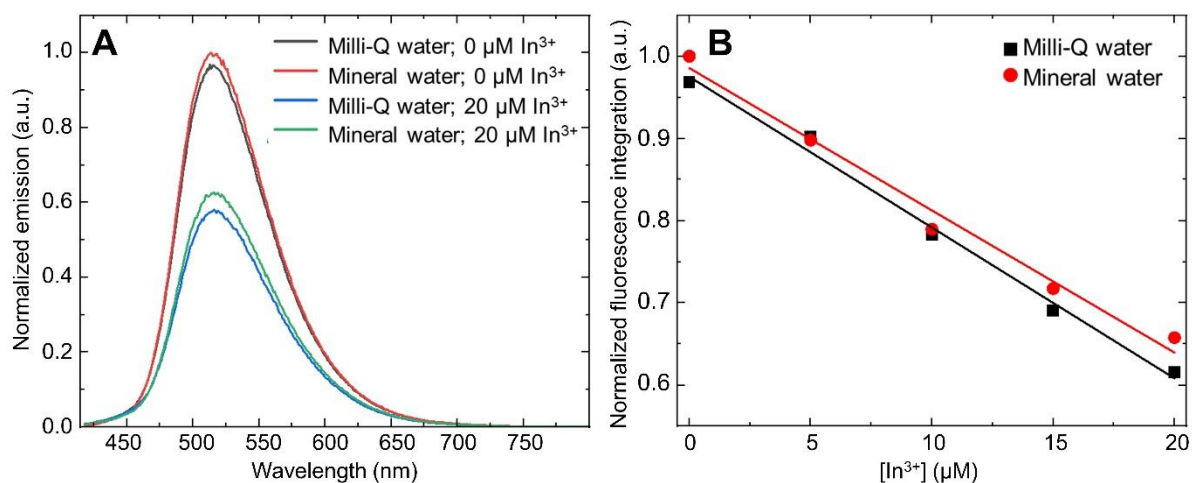


Fig. 5 (A) Normalized fluorescence emission spectra of compound **1** in Milli-Q and commercially available mineral water in the absence and presence of 20 μM In(III). (B) Normalized integrated fluorescence intensity of solutions of compound **1** as a function of [In(III)], with solid lines showing best linear fits to the data (Milli-Q water: $y = -0.0183x + 0.975$ ($R^2 = 0.993$); mineral water: $y = -0.0173x + 0.989$ ($R^2 = 0.985$)). (Experimental conditions: [**1**] = 10 μM; λ_{ex} = 410 nm. Data points in (B) represent the mean $\pm$ SD of 3 independent trials.)

Table 4. Determination of indium(III) in spiked Milli-Q and mineral water samples using compound **1**.^a

Matrix	[In(III)] _{spiked} (μM)	[In(III)] _{found} (μM)	Recovery (%) ^b
Milli-Q	5	4.3 $\pm$ 0.2	86
	10	10.6 $\pm$ 0.2	106
	15	15.4 $\pm$ 0.4	103
	20	19.2 $\pm$ 0.5	96
Mineral water	5	4.3 $\pm$ 0.1	86
	10	10.0 $\pm$ 0.2	100
	15	13.4 $\pm$ 0.5	89

	20	16.5 ± 0.3	83
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^a Calculated using the best-fit linear equations shown in the caption to Figure 5B; values represent means $\pm$ SD from 3 independent trials. [1] = 10 μ M; λ_{ex} = 410 nm.

^b Recovery = $[\text{In}]_{\text{found}}/[\text{In}]_{\text{spiked}} \times 100\%$.

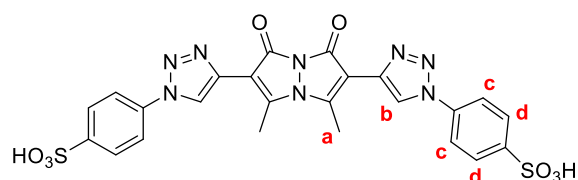
3.5 Mechanistic elucidation

To better understand the origin of the indium(III)-induced fluorescence quenching, we analyzed the UV–visible and fluorescence spectra of aqueous solutions of compound **1** before and after indium(III) addition. Upon indium(III) addition, the absorption maximum red-shifted from 409 to 435 nm with the appearance of a broad shoulder near 480 nm (Figures S63–S65, ESI). These absorption changes were accompanied by strong quenching of the fluorescence emission at 515 nm and emergence of a weak red-shifted emission at $\sim$ 545 nm. These spectral changes are consistent with coordination-induced perturbation of the excited state [27,28], which prompted us to further probe the stoichiometry and coordination environment of this complex.

The stoichiometry of the indium(III)–compound **1** complex was first assessed by high-resolution mass spectrometry (HRMS). A peak consistent with the calculated mass-to-charge ratio for the hexacationic species $[\text{C}_{24}\text{H}_{18}\text{In}_2\text{N}_8\text{O}_8\text{S}_2]^{6+}$ was observed. Independent support for this stoichiometry comes from non-linear fitting of fluorescence titration data to a 2:1 binding model, which yielded association constants of $2.48 \times 10^4 \text{ M}^{-1}$ and $2.33 \times 10^5 \text{ M}^{-1}$ for the first and second binding events, respectively. The enhanced affinity for the second event indicates cooperativity, likely arising from structural reorganization of the ligand following the initial metal ion coordination.

^1H NMR titrations in D_2O further corroborated the formation of a 2:1 indium(III)–compound **1** complex. At a 1:1 indium-to-ligand ratio, only minimal chemical-shift changes were observed, whereas substantial spectral changes occurred with the addition of a second equivalent of indium(III) (Table 5). Notably, the triazole proton (H_b) exhibited a pronounced downfield shift only after the addition of a second equivalent of indium(III), consistent with coordination at the triazole moieties occurring upon formation of the 2:1 complex. More modest shifts observed for nearby aromatic protons likely reflect electronic perturbation of the ligand framework upon complex formation.

Table 5. ^1H NMR chemical-shift changes ($\Delta\delta$) of compound **1** upon titration with indium(III) in D_2O at 1:1 and 2:1 indium-to-ligand ratios.^a



Proton assignment	$\Delta\delta$ at $\text{In(III)}:1 = 1:1$ (ppm) ^b	$\Delta\delta$ at $\text{In(III)}:1 = 2:1$ (ppm) ^b
H_a (bimane methyl)	0.000	0.004
H_b (triazole CH)	-0.002	0.052
H_c (phenyl)	-0.001	0.053

H _d (phenyl)	0.000	0.029
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^a Spectra acquired at 400 MHz and 298 K in D₂O.

^b $\Delta\delta$ values were calculated as the difference between the chemical shift of each proton in the indium(III)-treated sample and the chemical shift of the same proton in the uncomplexed ligand: $\Delta\delta = \delta_{\text{In(III)}} - \delta_{\text{free}}$

FTIR spectroscopy offered further evidence of indium(III) coordination to compound **1** (Figure 6). In the carbonyl stretching region, the characteristic band at 1750 cm⁻¹ shifted and split upon indium(III) addition, consistent with perturbation of the bimeane carbonyl environment. Changes were also observed in the triazole region, where the band at 1622 cm⁻¹ shifted to higher wavenumbers following complexation, indicating metal ion-triazole coordination. Changes in the sulfonate S=O region also reflect perturbation of the sulfonate environment upon indium(III) binding.

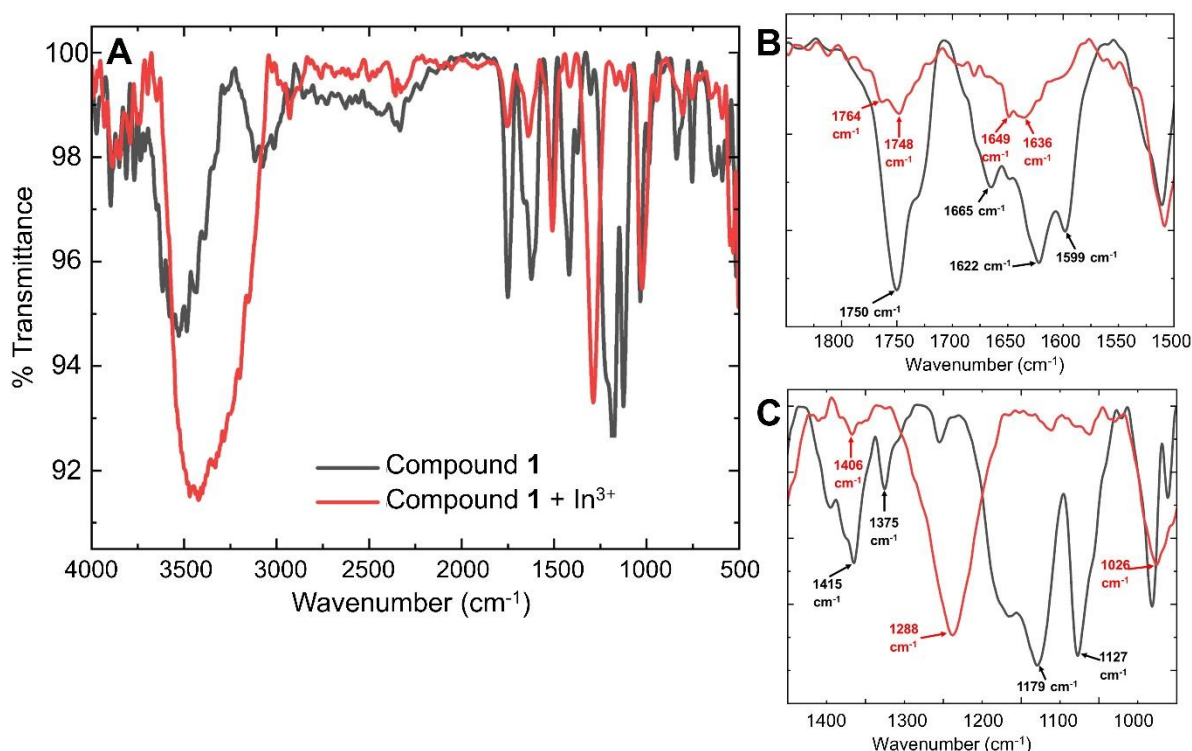


Fig. 6 Solid-state FTIR spectra of compound **1** (black) and compound **1** treated with In³⁺ (red): (A) full spectrum (4000–500 cm⁻¹); (B) Carbonyl and triazole region (1850–1500 cm⁻¹); (C) Sulfonate S=O and aromatic region (1500–1000 cm⁻¹).

Finally, DFT calculations on a 2:1 indium(III)–bimeane complex provided insight into the indium(III) coordination environment. The optimized structure features each indium(III) center in a distorted octahedral environment, coordinated by one triazole nitrogen, one bimeane carbonyl oxygen, and four water molecules (Figure 7). This coordination motif is consistent with literature reports of analogous octahedral indium(III) complexes [29] and with the experimentally observed FTIR shifts and ¹H NMR perturbations. The optimized geometry places each indium(III) center in close proximity to both a bimeane carbonyl oxygen and a triazole nitrogen, providing a plausible structural basis for the experimentally observed coordination-induced changes in fluorescence.

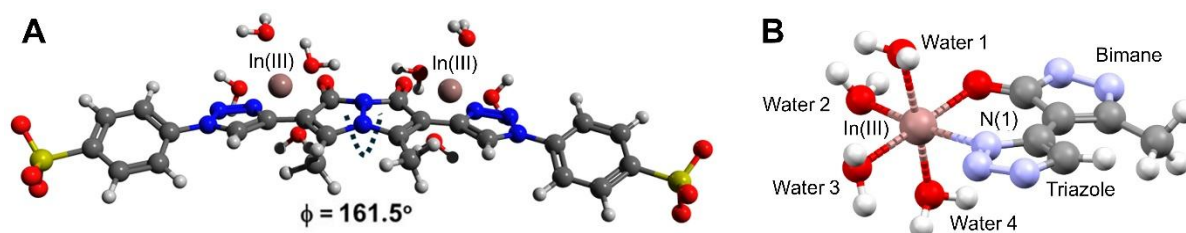


Fig. 7 DFT-optimized structure of compound **1** bound to In(III). (A) Overall structure showing two In(III) ions associated with bimane and triazole donor sites (interplanar angle (Φ) = 161.5°). (B) Enlarged view of a single In(III) coordination environment, illustrating a distorted octahedral geometry with bimane carbonyl and triazole donors as well as coordinated water molecules.

4. Conclusions

In conclusion, we report a water-soluble bimane fluorophore that maintains an exceptionally high quantum yield in fully aqueous media and undergoes strong, reversible fluorescence quenching in response to indium(III). The sensing response is enabled by formation of a cooperative 2:1 indium–bimane complex, with close metal–fluorophore contact providing a plausible structural and mechanistic basis for the observed coordination-induced fluorescence quenching. The combination of metal-induced fluorescence quenching and EDTA-enabled differential fluorescence recovery provides a two-step strategy for distinguishing indium(III) from gallium(III) in fully aqueous media which addresses the long-standing challenge of selective aqueous indium sensing. More broadly, these results establish sulfonation as an effective strategy for extending bimane-based fluorophores to sensing applications in fully aqueous media, not limited to the specific case of indium(III) demonstrated here. Beyond solution-state measurements, compound **1** retains indium(III)-responsive fluorescence quenching when loaded on paper substrates, providing a preliminary qualitative visual response and a quantitative colorimetric readout for indium(III), and well-defined optimization steps have been identified to improve sensitivity toward field-relevant concentrations. Ongoing work focuses on extending this approach toward lower-concentration detection, expanding the range of metal-ion analytes, and further optimizing paper-based formats suitable for portable field deployment.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this manuscript, the authors used generative AI tools for language editing and stylistic refinement. The authors carefully reviewed and revised all AI-generated suggestions and take full responsibility for the final content of the manuscript.

Statements and Declarations

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CRedit authorship contribution statement

Jiawei Liu: Investigation, Formal analysis, Writing – original draft, Writing – review & editing; Nathaniel Chennattuparambil Roy: Investigation, Methodology, Writing – review & editing; Flavio Grynszpan: Conceptualization, Methodology, Investigation, Formal analysis, Supervision, Project administration, Writing – review & editing; Mindy Levine: Conceptualization, Formal analysis, Supervision, Project administration, Writing – original draft, Writing – review & editing

Declaration of competing interest

The authors declare that they have no competing interests.

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Supplementary data

Supplementary data associated with this article include detailed experimental procedures, spectroscopic characterization, and supporting analytical results, including fluorescence titration, binding analysis, calibration information, and supplementary figures and tables.

Data availability

The data that support the findings of this study are available within the article and its Supplementary Information. Additional datasets generated and analyzed during the current study are available from the corresponding authors upon request.

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