Supplementary Information

pH dependent series of emission spectra for compound 1

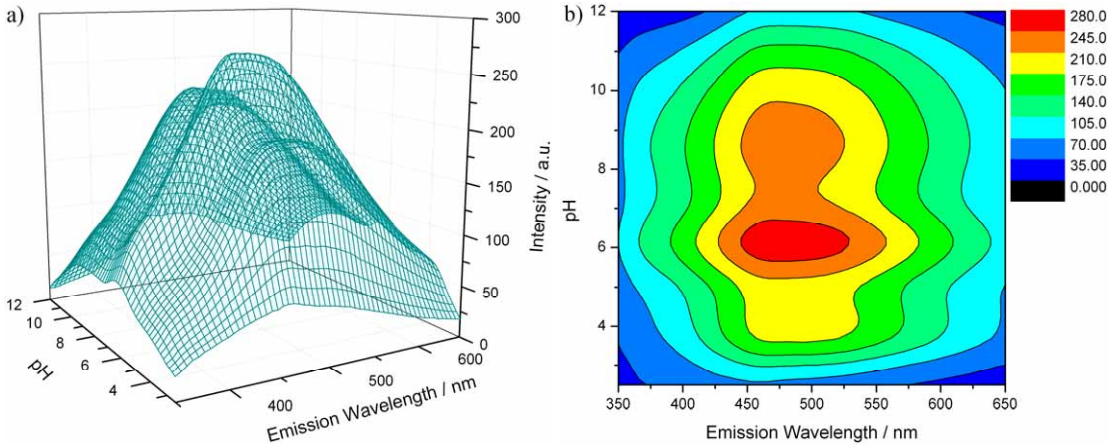


Figure S1 a) pH Dependent series of emission spectra (λ_{ex} = 423 nm) for compound 1, and b) the respective contour plot with fluorescence intensity scale (top right).

Time effects on the metal cation sensor and metal addition fluorescence kinetics

A study of the time-dependent effects on stored solutions of **1** was undertaken to determine whether the fluorescence signal intensity degraded over time. This is an important aspect to investigate as the fluorescence dynamics of a freshly prepared solution may change upon reaching equilibrium, and the shelf-life of a fluorescence-based sensor is critical.

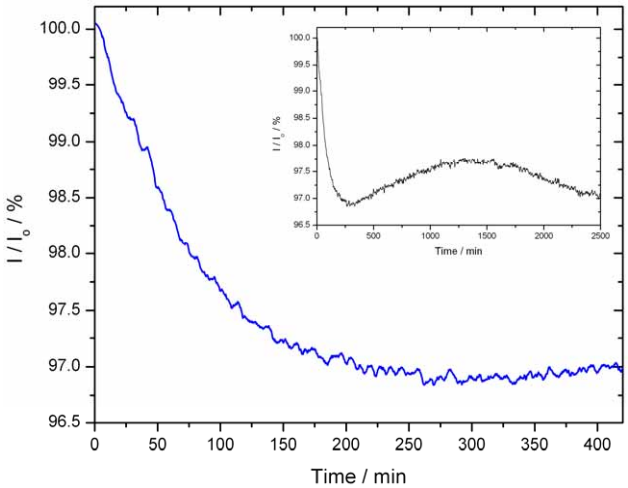


Figure S2 Fluorescence intensity at λ_{em} = 513 nm (λ_{ex} = 335 nm) for a freshly prepared solution of 1 (40 µM) monitored over a 7.5 h period; inset represents the first 42 h profile.

The fluorescence intensity of the emission line λ_{em} = 513 nm (λ_{ex} = 335 nm), was monitored over a finite period, commencing immediately after a solution of **1** (40 μM) was prepared (Figure S2). It can be clearly seen that, immediately after dissolution, the fluorescence intensity of this emission line decreases and plateaus after 180 min. There is a decrease of approximately 3% of the fluorescence intensity over the initial 180 min period, with the majority of fluorescence intensity reduction occurring within the first hour. There is a slight deviation from a steady fluorescence signal after the 180 min period, however, it is less than 1%. From these results, protocols were set in place for metal binding experiments, where a freshly prepared solution was left to stand for at least three hours before being used for metal binding studies.

The fluorescence intensity was monitored for an extended period of over three weeks (Figure S3). There is an initial decline to 90% intensity within the first 60 h, followed by a gradual decrease between 125 h and 400 h, where the intensity drops approximately 5% to about 85% intensity, where it remains for the final 150 h of the trial. These data imply that monitoring the original solution fluorescence intensity and establishing control samples will require incorporation into the analytical methodology, for the use of this compound as an analytical metal cation sensor. While it can be seen that the solution fluorescence is stable, with repeat analyses within a > 50 period, overall fluorescence intensity decay is observed. In addition, it is noteworthy that the solution was not constantly in a sample cell being monitored for the entire 550 h, and slight decreases in fluorescence may be the result of sample storage, dynamic equilibrium effects, and/or fluctuations in ambient temperature. For any fluorescence studies on **1**, and for its use as a metal cation sensor, it is advisable to always utilise freshly prepared solutions of the compound, and allow these solutions to stand for three hours before use. At a bare minimum, it is suggested that these solutions can be utilised for at least 40 h. Furthermore, it is recommended that a control solution be monitored to allow for baseline correction.

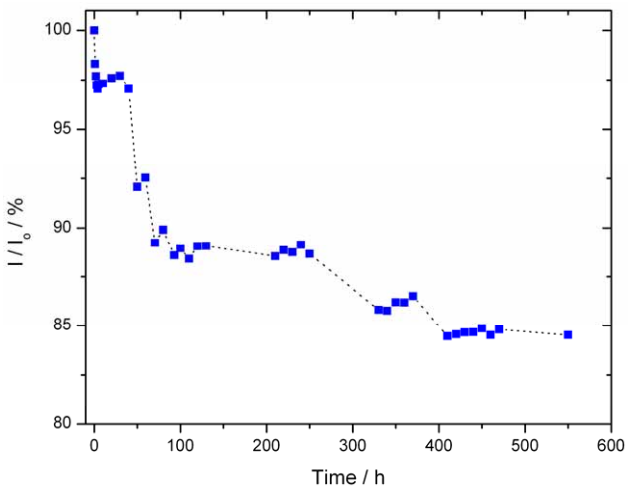


Figure S3 Long-term fluorescence intensity monitoring of a solution of compound 1.

The fluorescence intensity at λ_{em} = 513 nm (λ_{ex} = 335 nm) was monitored as soon as a Co^{2+} solution (20 µM) was directly added to a solution of **1** (40 µM) (Figure S4). Immediately following, the addition of the metal cation solution resulted in significant quenching in fluorescence intensity, followed by a secondary increase, and then a decrease in intensity. This can be attributed to the metal cation initially disturbing the system dynamics. After 30 min the system reaches equilibrium, and stability of the fluorescence intensity is established. This information was incorporated into the metal binding experimental protocol, so that between metal cation addition and fluorescence monitoring, solutions were allowed to equilibrate for increased reproducibility of the results. This should be incorporated into any analytical protocol for the use of **1** as a fluorescence-based metal cation sensor.

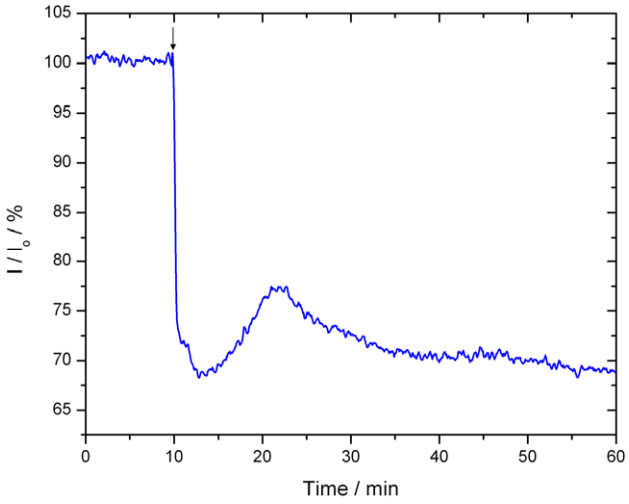
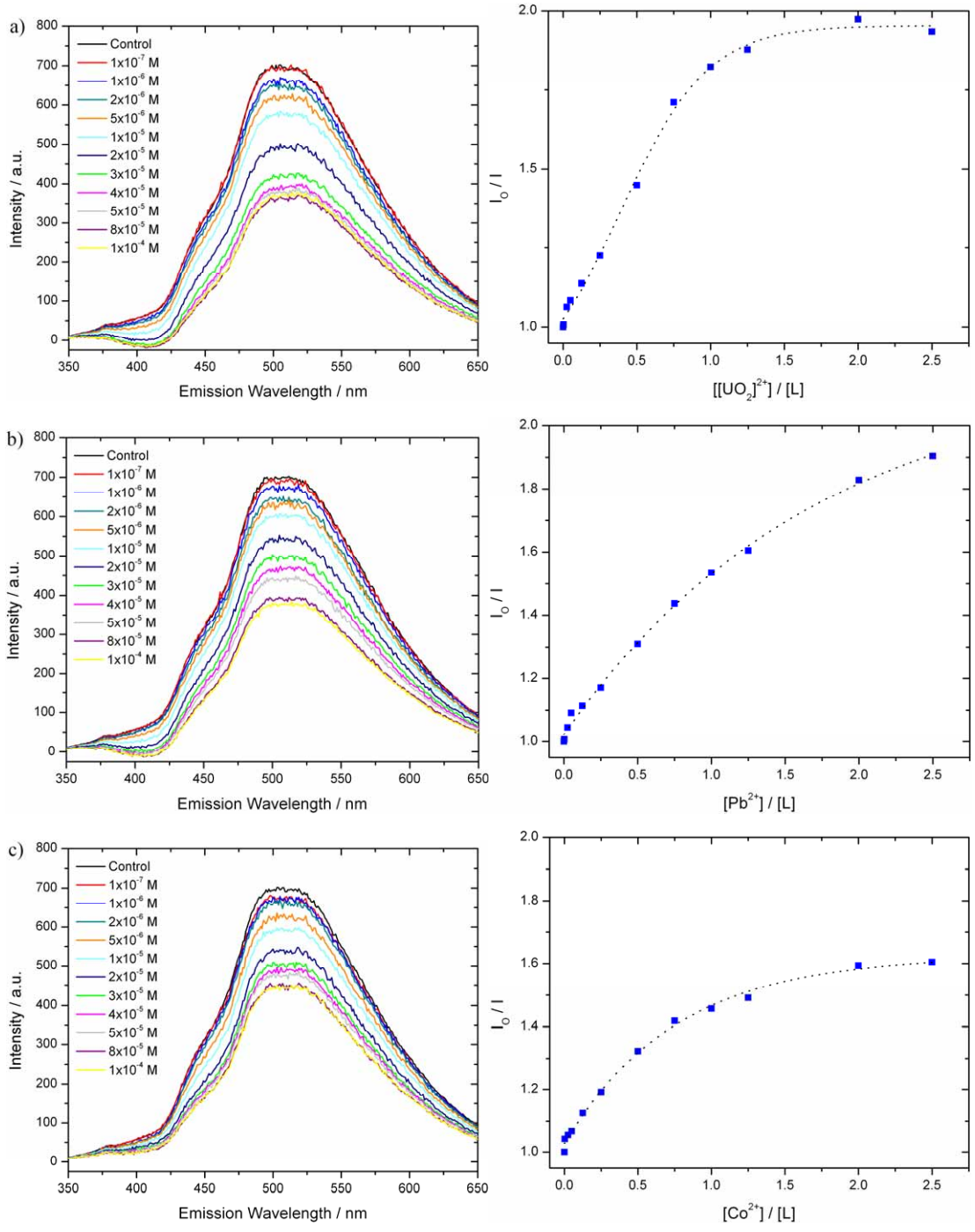


Figure S4 Real-time emission line monitoring (λ_{ex} = 335 nm) of a solution of 1 (40 µM); the arrow indicates the addition of the Co^{2+} metal cation solution (20 µM).

Fluorescence spectra and titration curves for successive addition of metal cations to a solution of compound 1



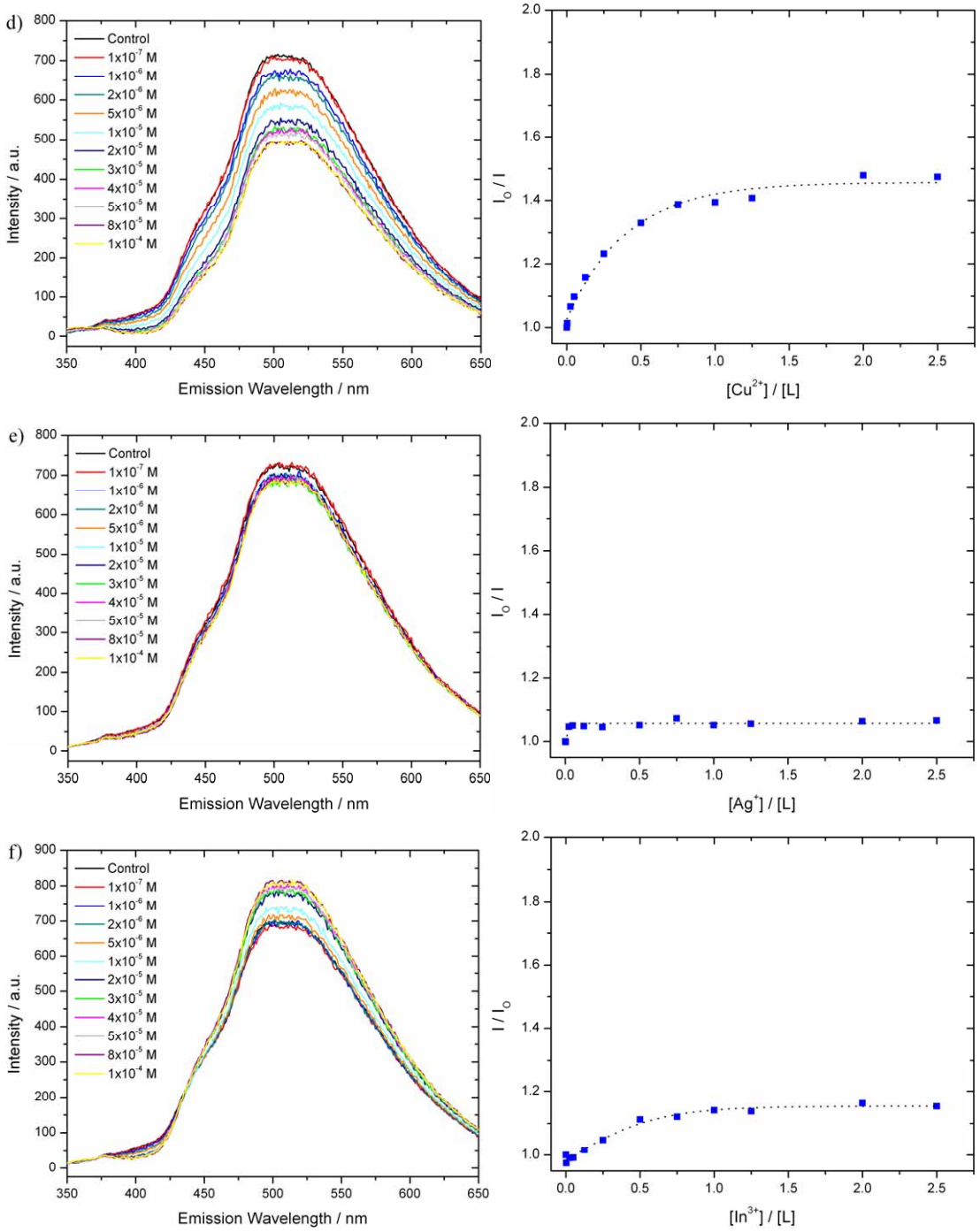


Figure S5 (Left) Variation of the fluorescence spectra of 1 (40 µM) upon successive addition of a) $[UO_2]^{2+}$, b) Pb^{2+}, c) Co^{2+}, d) Cu^{2+}, e) Ag^{+}, and f) In^{3+} at pH 6.5, (Right) quenching of the fluorescence intensity of 1 (40 µM) upon successive addition of the respective metal cations at pH 6.5; in the In^{3+} plot, the y-axis is I/I_o due to enhancement of the fluorescence intensity.

Determination of binding constant and binding stoichiometry

Determination of binding constant (K_b) and binding site stoichiometry is detailed below. This involved plotting the, function

$$\frac{I_o}{\Delta I} = \frac{1}{[M^{X+}]} \qquad (1)$$

where I_o represents the fluorescence intensity in the absence of metal cations, and ΔI represents the relative change in fluorescence intensity at any point during the titration (Figure S6). A linear regression fitting function is applied to these data and an extrapolated y-intercept is calculated, the reciprocal of which represents the fluorescence intensity of the system at infinite metal cation concentration, I_∞.

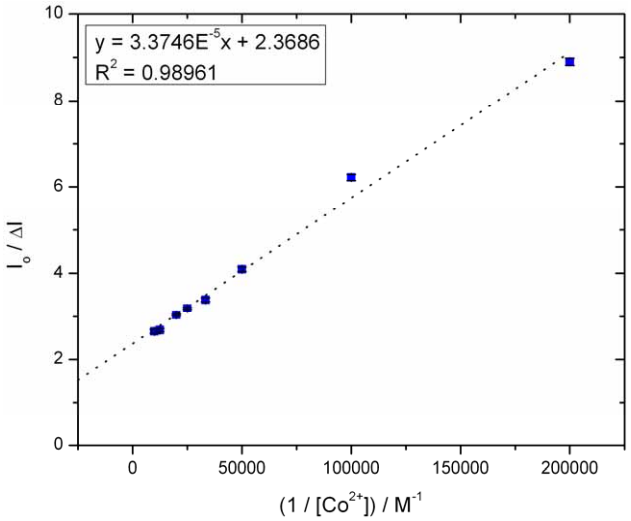


Figure S6 Plot of $I_o/\Delta I$ versus $1/[Co^{2+}]$, with a linear regression function fitted, and regression line extrapolated to indicate the y-intercept.

Using the calculated value for I_∞, coupled with the titration data, K_b values can be derived from the following equations:

$$\log\left[\frac{\Delta I}{(|I - I_\infty|)}\right] = \left(\frac{[M^{X+}]}{K_{diss}}\right)^n \qquad (2)$$

where K_{diss} represents the dissociation constant, and *n* represents the number of binding sites. The slope of the double logarithmic plot (Figure S7) can provide

valuable cation-calixarene interaction information and indicate binding ratios of metal cation to ligand. The binding constant K_b can be obtained by plotting:

$$\log\left[\frac{\Delta I}{\left(\left|I - I_{\infty}\right|\right)}\right] = \log[M^{X+}] \qquad (3)$$

In addition, the value of $\log[M^{X+}]$, where $\log[(\Delta I / (|I - I_{\infty}|))] = 0$, gives rise to the dissociation constant (K_{diss}) (Figure S7). The reciprocal of K_{diss} determines the K_b which allows quantitative comparison for binding affinities. The K_b and *n* values were obtained for the different cations through titrimetric profiling (Table 1).

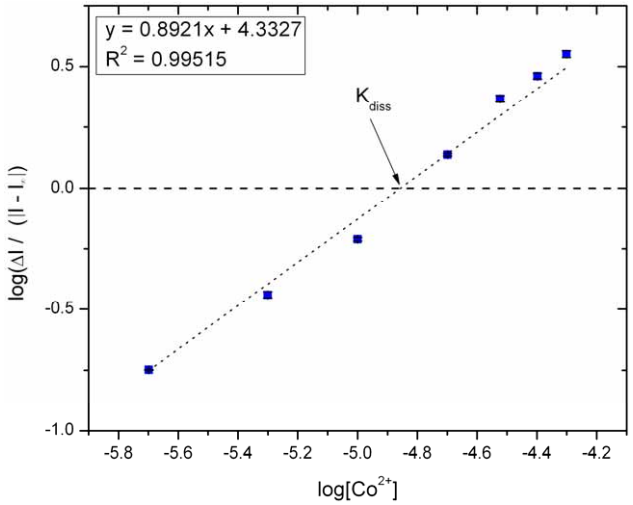


Figure S7 Double-logarithmic plot for the quenching of 1 fluorescence intensity following the addition of Co^{2+} (2 µM to 50 µM), with a linear regression fitting function.

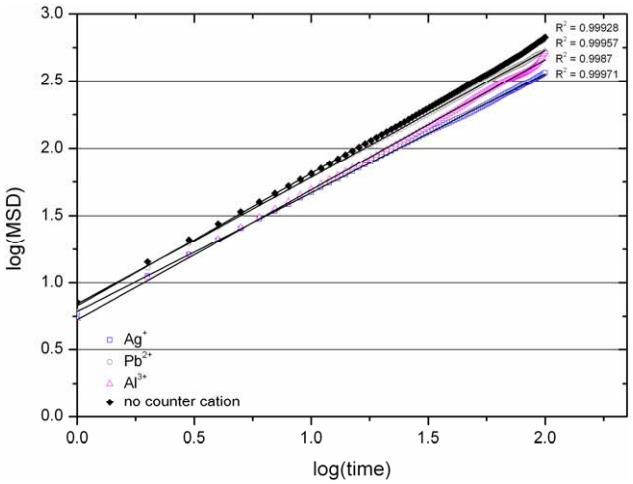


Figure S8 Double-logarithmic plot of the mean square deviation versus time with linear regression fitting.

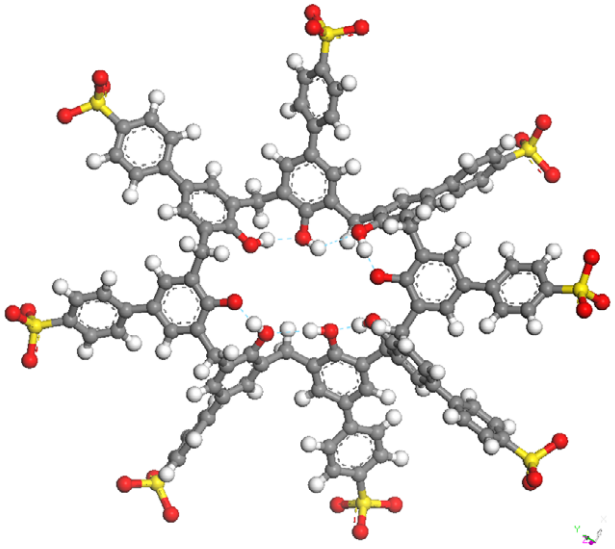


Figure S9 Minimized structure for [*p*-(4-sulfonatophenyl)calix[8]arene – $10H^+$].