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Fluorescent Zn^{2+} chemosensors, functional in aqueous solution under environmentally relevant conditions

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ABSTRACT

The synthesis and evaluation of two new ratiometric chemosensors for the quantification of potentially toxic free Zn^{2+} ions in aqueous solutions are described. Both sensors show high selectivity for Zn^{2+} over other cations, and are functional at environmentally relevant pH with detection limits of 0.05 μM for free Zn^{2+}.

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Owing to the fundamental and ubiquitous role Zn^{2+} and Cu^{2+} ions play in biological systems,[1] the development of highly selective and sensitive chemosensors to detect such species in complex biological mixtures or in environmental samples at low, but ecologically relevant concentrations, is paramount.[2] Research into ion recognition has expanded in recent decades to encompass many areas of chemistry; from small molecule sensors to peptide-based systems and elaborate supramolecular complexes.[3] Regardless of the class of sensor, comparable design strategies apply: coupling a recognition site to a reporting component, where a sensing event gives rise to a measurable signal. Currently, fluorescence-based systems show the greatest promise and sensitivity, owing to the fact that the emission signal is proportional to the substrate concentration.[4]

According to the widely accepted free ion activity model, free metal ions generally have higher associated toxicity than their respective colloidal or particulate forms.[5] However, straightforward and direct measurement of free metal ions remains challenging.[6] Current methodology relies on measurement of total metal concentration through flame atomic absorption spectrometry,[7,8] electrochemical or anodic stripping voltammetry (ASV) analysis,[8] or ion specific electrodes.[9] Despite the high sensitivity of techniques such as ASV, it is impossible to distinguish between potentially toxic, labile ion species (i.e., Zn^{2+}) and less-toxic forms (i.e., coordinated Zn^{2+}); species that coexist in environmental systems yet have substantially different physicochemical and biological properties.[10] Additionally, ASV has difficulty in simultaneously distinguishing between copper and zinc, due to the formation of intermetallic compounds.[10,11] This limitation is a notable disadvantage in the measurement of zinc in complex environmental systems.

Herein, we report the development of two novel sensors for detection of Zn^{2+} which are functional in aqueous media and at environmentally relevant pH. These sensors exploit a photo-induced electron transfer (PET) mechanism to give substantial fluorescent enhancement upon Zn^{2+} binding,[12] and rely on the well-established dipicolylamine moiety as the binding unit.[13] Mono- (**1**) and di-substituted (**2**) anthracene cores provide additional insight into binding modes and structural design in order to achieve highly sensitive and selective metal ion recognition.

Chemosensors **1** and **2** were prepared from the corresponding aldehydes and N^1,N^1-bis(pyridin-2-ylmethyl)ethane-1,2-diamine **3**[14] via reductive amination, as outlined in Scheme 1. The dialdehyde **4** was synthesised by treatment of 9,10-dibromoanthracene[15] with *n*-BuLi, and subsequent quenching of the resultant dianion with DMF.

The fluorescence spectra of chemosensors **1** and **2** demonstrated characteristic absorption and emission bands consistent with the anthracene fluorophore. In all subsequent experiments, the fluorescence spectra were measured using an excitation wavelength of 375 nm, corresponding to a major absorption band of the anthracene core. The optimal operating pH for chemosensors **1** and **2** was determined using a variable pH screen (see Supplementary data, Fig. S1). Both chemosensors **1** and **2** were functional over an environmentally relevant pH range (pH 5–8); this is important if the sensors are to be used for real-time applications in environmental and biological systems.

A fluorescence titration of Zn^{2+} with either chemosensor **1** or **2** (10 μM) in MES buffer (0.1 mM, pH 6.5) was performed. For **1**, increased fluorescence was observed in a dose-dependent manner until 1 mol equiv of Zn^{2+} (10 μM) was added; after which, the fluorescence reached a saturated maximum (Fig. 1A). The addition of Zn^{2+} to **2** resulted in a similar dose-dependent increase in fluorescence (Fig. 1B), however, despite two potential metal binding sites, the fluorescence maximum was also reached after addition of 1 mol equiv of Zn^{2+} (10 μM). This system (**2**) demonstrated a higher fluorescent intensity (~500 a.u.) than **1** (~300 a.u.), most likely due to the PET contribution of two benzylic nitrogens in

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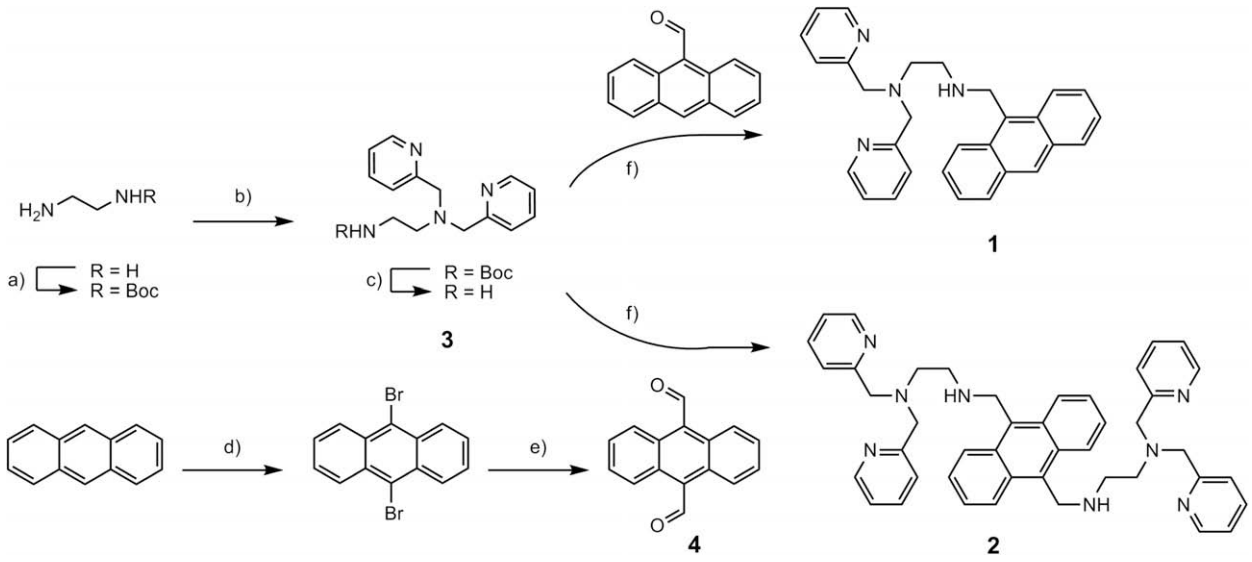

Scheme 1. Synthesis of the mono- (**1**) and di-substituted (**2**) chemosensors. Reagents and conditions: (a) Boc_2O, EtOH, 0 °C→rt, 95%; (b) 2-chloromethylpyridine, Na_2CO_3, EtOH, Δ, 72%; (c) TFA/CH_2Cl_2, 0 °C→rt, quant.; (d) Br_2, CH_2Cl_2, 0 °C, 70%; (e) (i) *n*-BuLi, 0 °C→rt; (ii) −78 °C, DMF, 58%; (f) (i) CH_2Cl_2/MeOH, Δ, 4 Å MS; (ii) $NaBH_4$, MeOH, 0 °C→rt; **1**: 92%, **2**: 85%.

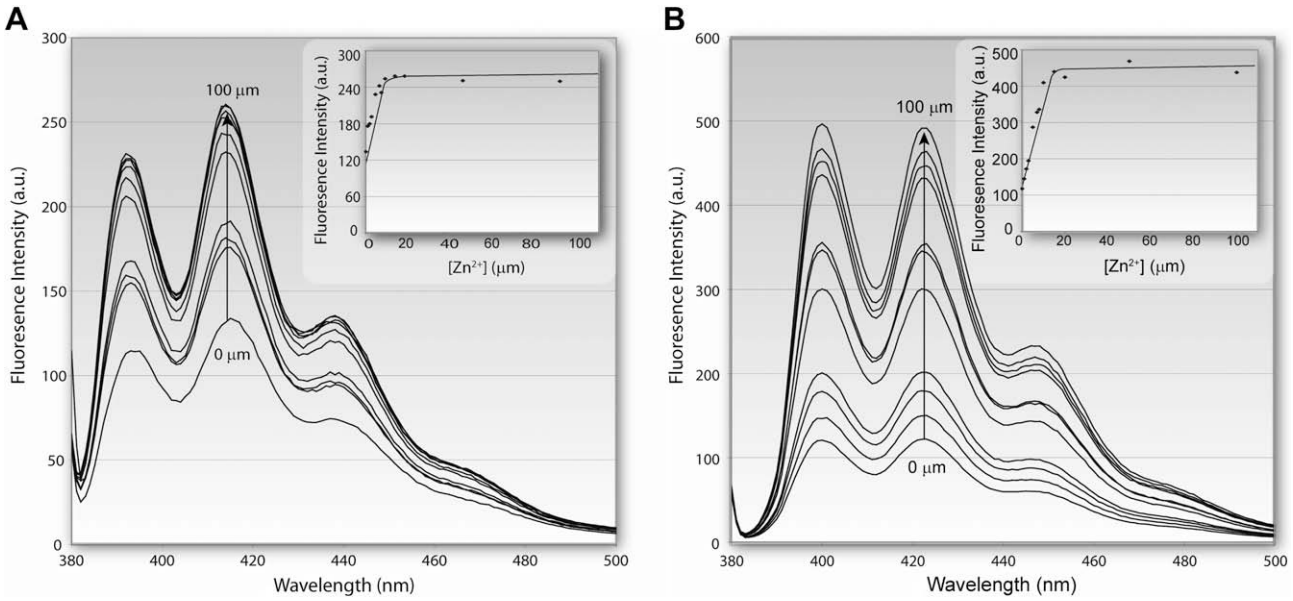

Figure 1. Fluorescence spectra of chemosensor: (A) **1** (10 μM) and (B) **2** (10 μM); in MES buffer (0.1 mM, pH 6.5), $[Zn^{2+}]$ = 0–100 μM; λ_{ex} 375 nm. Inset: fluorescence intensity λ_{em} 420 nm versus $[Zn^{2+}]$.

chemosensor **2** compared with only one in chemosensor **1**. PET effects are known to result in substantial fluorescent enhancement on metal-binding.[12]

A fluorescence titration of Cu^{2+} with either chemosensor **1** or **2** (10 μM) in MES buffer (0.1 mM, pH 6.5) resulted in fluorescence quenching. Cu(II) is a recognised fluorescence quencher.[16] The step-wise addition of Cu^{2+} to **1** resulted in a dose-dependent decrease in fluorescence (Fig. 2A), until saturation was reached upon addition of 1 mol equiv of Cu^{2+} (10 μM). Likewise, a ratiometric dose-dependent decrease in fluorescence was observed when Cu^{2+} was added to **2** (Fig. 2B) with the fluorescent minima comparable to that achieved with chemosensor **1**.

Qualitative differences in fluorescent intensity of the sensors in the absence and presence of Zn^{2+} can be observed by the naked eye (Fig. 3). As a result, these sensors have the potential to provide immediate qualitative feedback regarding the nature of random samples, and could therefore find application in critical, real-time, field-based studies.

Both chemosensors, **1** and **2**, were evaluated in a competitive metal screen to determine the relative selectivity and tolerance for Zn^{2+} over other relevant cations. Both chemosensors **1** and **2** showed no significant response when treated with 1.0 equiv (10 μM) of metal ions (Cu^{2+}, Mn^{2+}, Ca^{2+}, Mg^{2+}, Ni^{2+}, Fe^{3+}, Ag^{+}, Pb^{2+}, Sr^{2+}, Al^{3+}, K^{+}, Na^{+}, Li^{+}, Cr^{3+}, Ba^{2+} and Cd^{2+}). However, the co-addition of Zn^{2+} (10 μM) resulted in a significant increase in fluorescence (see Fig. 4A and B and Supplementary data, Figs. S2 and S3). Importantly, for both chemosensors **1** and **2**, the presence of Na^{+}, K^{+}, Ca^{2+} and Mg^{2+}, highly prevalent species in biological and environmental systems, did not induce any distinct increase in fluorescence, and the presence of these and other cations did

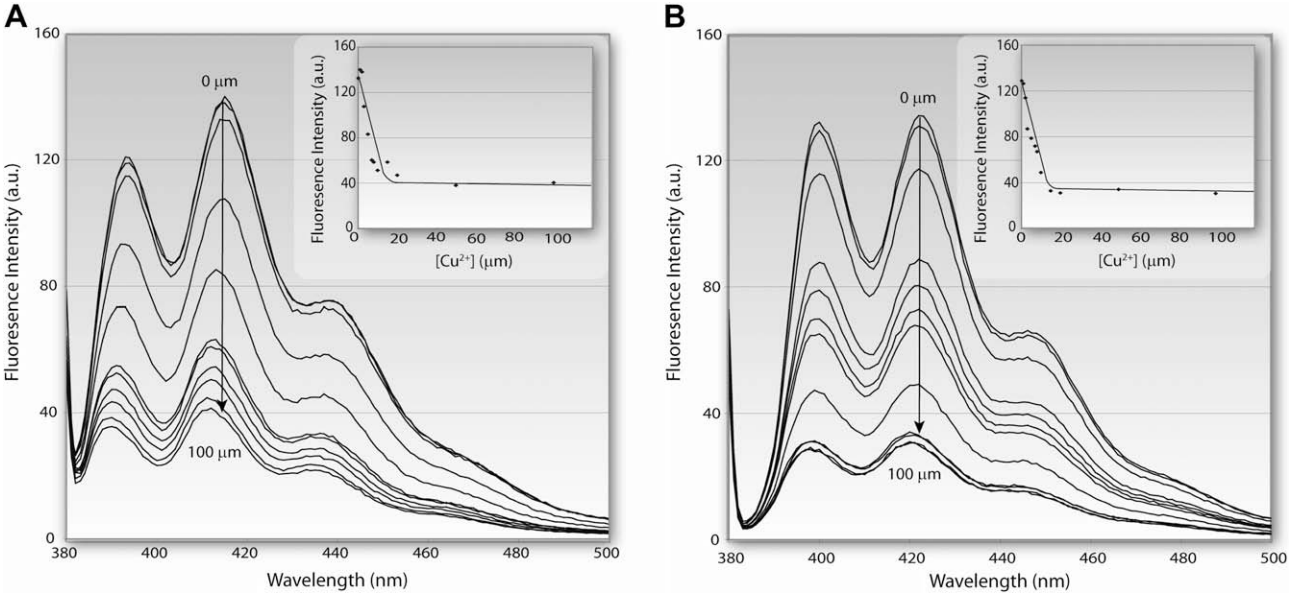

Figure 2. Fluorescence spectra of chemosensor: (A) **1** (10 μM) and (B) **2** (10 μM); in MES buffer (0.1 mM, pH 6.5), $[Cu^{2+}]$ = 0–100 μM; λ_{ex} 375 nm. Inset: fluorescence intensity λ_{em} 420 nm versus $[Cu^{2+}]$.

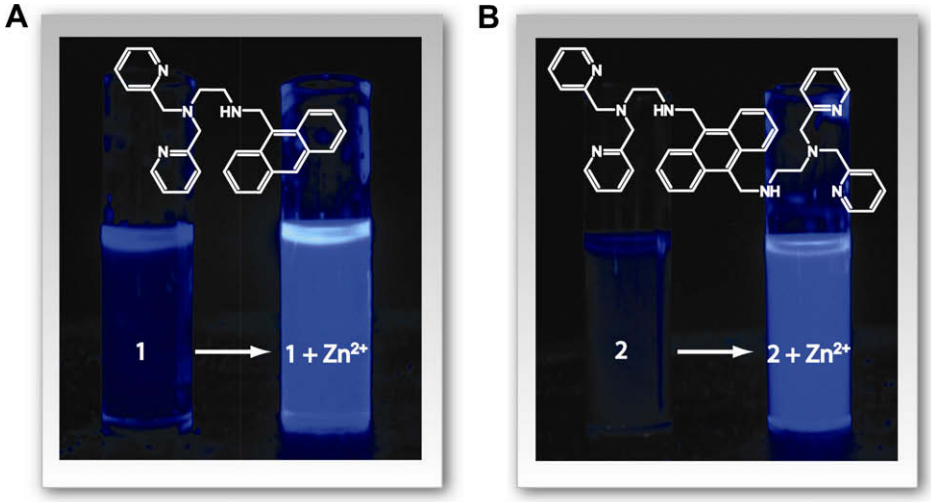

Figure 3. Chemosensors: (A) **1** (10 μM) and (B) **2** (10 μM) in the absence (0 μM) and presence of Zn^{2+} (10 μM); MES buffer (0.1 mM, pH 6.5); illuminated with a hand-held UV lamp, λ_{ex} 365 nm.

not interfere with the ratiometric response to Zn^{2+}. While Cd^{2+} did generate a measurable response in both **1** and **2**, it is generally present at concentrations ⩾100-fold less than Zn^{2+} in environmental systems, thus Cd^{2+}-derived interference would therefore be negligible (See Supplementary data, ST1 and ST2).[17]

Due to the greater fluorescent response of **2** than **1** in the presence of Zn^{2+}, further analysis was completed with chemosensor **2**. The detection limit of **2** was determined using the relative chemosensor/Zn^{2+} concentration that gave an instrumental signal which was significantly different from the background signal (*limit of detection* = $yB + 3sB$; where yB is the signal associated with the blank, and $3sB$ is equal to three standard deviations of the blank).[18] Using this approach, the detection limit of **2**, in the presence of Zn^{2+}, was found to be 0.05 μM (Supplementary data, Fig. S4).

High specificity for free Zn^{2+} ions in solution is essential if these small molecule chemosensors are to be viable replacements for current, cumbersome methodologies. Particulate and colloidal forms of Zn^{2+} were emulated by addition of Na_2S to standard Zn^{2+} solutions, an effective protocol for precipitation of metal ions.[19] The presence of high concentrations of Na_2S (⩾10 μM), had no effect on the basal fluorescence of **2**. The addition of Na_2S to the highly fluorescent [**2**-Zn^{2+}] complex caused an immediate decrease in fluorescence intensity, indicating that only free Zn^{2+} ions are detected by **2** (Fig. 5). This analogy was extended by the addition of humic acids, complex heterogeneous mixtures of small-size and poly-aromatic acids, known to chelate metal ions.[20] In the presence of environmentally relevant concentrations of humic acids, the high fluorescence associated with the [**2**-Zn^{2+}] complex was significantly decreased (Fig. 6), which further confirms the selectivity of **2** for free Zn^{2+} ions, rather than equivalent particulate or colloidal forms. Unfortunately, the presence of high concentrations of humic acids (⩾10 mg/L), suppressed the basal

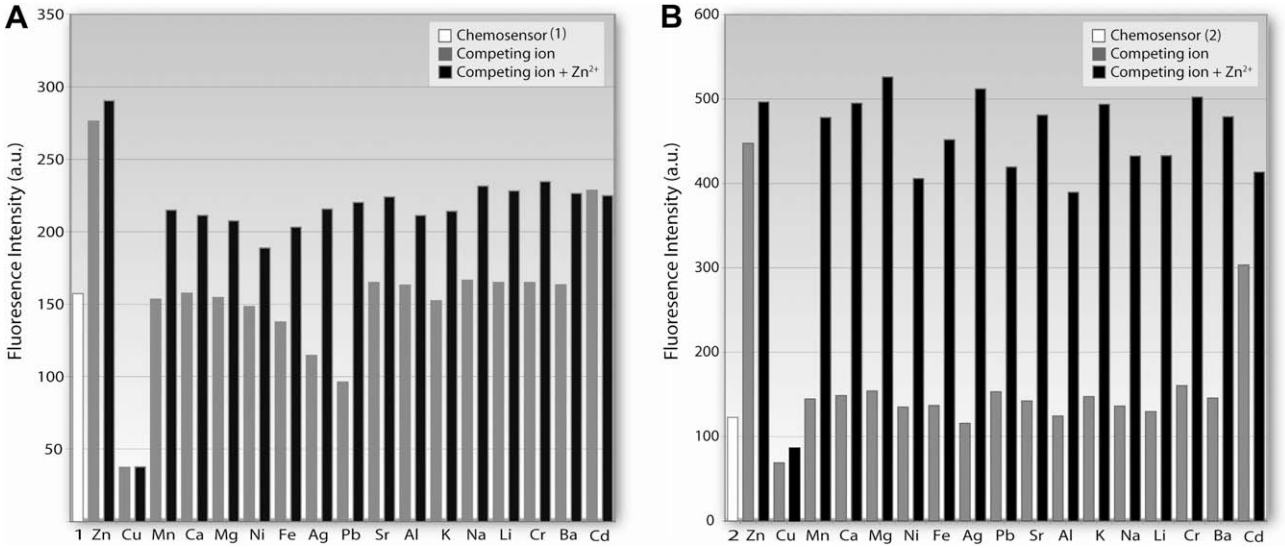

Figure 4. Alternate metal screen with chemosensors: (A) **1** and (B) **2**; λ_{em} 420 nm in MES buffer (0.1 mM, pH 6.5), *Grey bars*: **1** or **2** (10 μM) with addition of alternate metal ions (10 μM). *Black bars*: **1** or **2** (10 μM) with addition of alternate metal ions (10 μM) and Zn^{2+} (10 μM). *White bar*: basal fluorescence of **1** or **2** (10 μM).

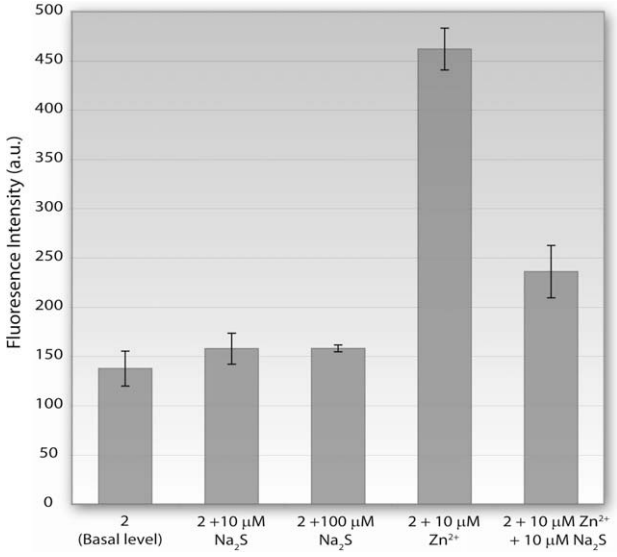

Figure 5. Chemosensor **2** (10 μM) in the presence of Na_2S (10–100 μM) and Zn^{2+} (10 μM); in MES buffer (0.1 mM, pH 6.5); λ_{ex} 375 nm, λ_{em} 420 nm.

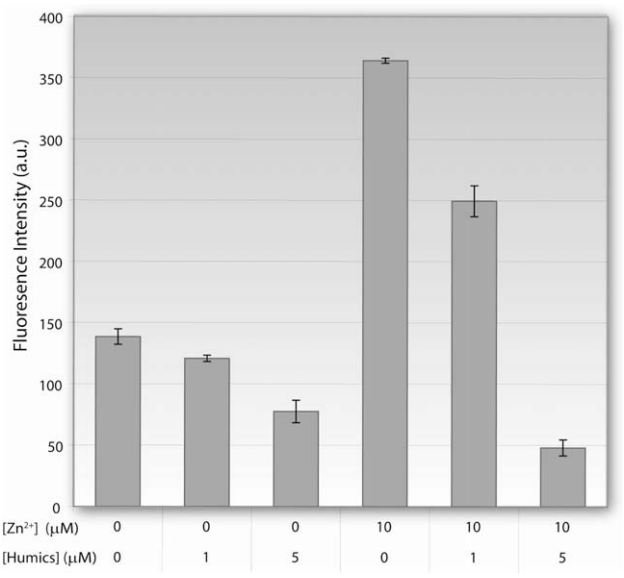

Figure 6. Chemosensor **2** (10 μM) in the presence of humic acids (1 μM to 5 μM) and Zn^{2+} (10 μM); in MES buffer (0.1 mM, pH 6.5); λ_{ex} 375 nm, λ_{em} 420 nm.

fluorescence associated with **2**.[21] ASV, the current state-of-the-art in zinc sensing, also suffers from similar quenching in the presence of humic acids, due to adsorption of organic matter on the mercury electrode.[10,22]

The function of chemosensor **2** was evaluated under mock-environmental conditions utilising a competitive Zn^{2+}/Cu^{2+} titration. As zinc is generally more prevalent (0.06–2.9 μM, median 0.4 μM) in fresh-water systems than copper (0.03–0.16 μM, median 0.07 μM),[17] environmentally relevant ratios of $[Zn^{2+}]$:$[Cu^{2+}]$ ranging from 3:1 to 15:1 (Fig. 7 and Supplementary data, ST1) were investigated. Despite the presence of a basal level of Cu^{2+}, chemosensor **2** still showed a measurable, dose-dependent response to Zn^{2+}. Thus, the problematic Cu^{2+} interference associated with ASV methodology is not a concern with the use of a small molecule chemosensor such as **2**.[10,11]

In conclusion, the synthesis and evaluation of two novel, ratiometric chemosensors with a high selectivity and sensitivity for Zn^{2+} (Zn^{2+} detection limit of 0.05 μM) have been reported. Importantly, these chemosensors operate in aqueous solution over an environmentally relevant pH range. Due to the higher sensitivity observed for Zn^{2+}, and relative ratios of $[Zn^{2+}]>>[Cu^{2+}]$ in environmental samples; these novel chemosensors are an exacting means of measuring potentially toxic free Zn^{2+}. Importantly, these chemosensors rely on different competitive equilibria to the standard ASV methodology, and thus provide an interesting comparison and alternative insight into the binding of Zn^{2+} in complex environmental systems. Additionally, due to their high water solubility, they are potentially applicable as real-time quantitative sensors for Zn^{2+} in vivo.

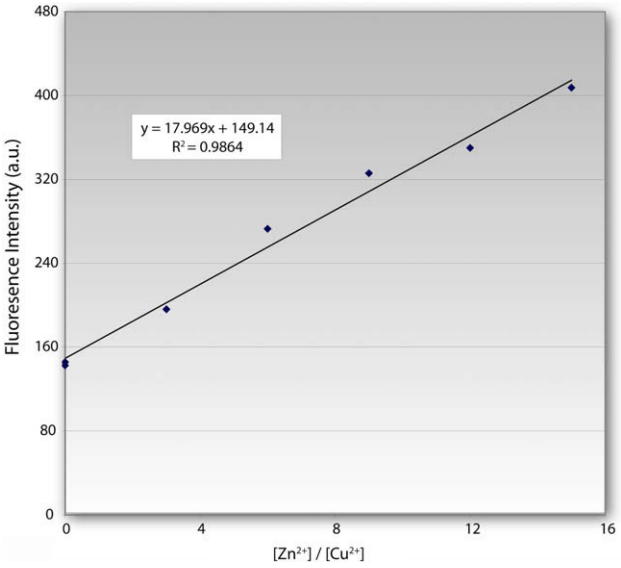

Figure 7. Chemosensor **2** (10 μM) in the presence of a basal level of Cu^{2+} (0.66 μM) and Zn^{2+} (2 μM to 10 μM); reported as $[Zn^{2+}]$:$[Cu^{2+}]$ 3:1 to 15:1; in MES buffer (0.1 mM, pH 6.5); λ_{ex} 375 nm, λ_{em} 420 nm.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2009.12.069.

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