Highly selective 4-amino-1,8-naphthalimide based fluorescent photoinduced electron transfer (PET) chemosensors for Zn(II) under physiological pH conditions

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The design and synthesis of two novel fluorescent sensors based on the photoinduced electron transfer (PET) concept, **1** and **2**, for the detection of zinc under competitive media is described. These sensors are based on the 4-amino-1,8-naphthalimide fluorophore, which has an absorption band centred at 450 nm and emits in the green with λ_{max} ~550 nm. By functionalizing the chromophore with a simple benzyl or ethyl-aryl based iminodiacetate receptor at the 4-position, both high selectivity and sensitivity were achieved for the sensing of Zn(II) over other competitive transition and Group I and II metal ions. These sensors were also shown to be pH independent, with a pK_a of 2.3 being determined for **1**, which allows these to be used in highly competitive pH media. Upon sensing of Zn(II) the fluorescence emission spectrum is 'switched on' demonstrating the suppression of PET from the receptor to the fluorophore. For **1**, the sensing of Zn(II) was achieved with $K_d = 4$ nM when measured in pH 7.4 buffered solution, in the presence of 1.1 mM of EGTA.

Introduction

The design and development of small molecules for sensing applications is of great current interest in supramolecular chemistry.[1,2] In particular, luminescence sensing of ions and molecules has become evermore important in medical diagnostics as such sensing can give fast and reliable analysis of human wellbeing.[3,4] Such sensing has recently been demonstrated for analysis of blood or serum samples for critical care analysis; where the concentration of vital electrolytes can be determined instantly after sample gathering.[5] Using synthetically designed sensors, or chemosensors, where a receptor moiety is conjugated directly or *via* a short spacer into a luminescent moiety, such as a fluorescence tag or metal based emitter, it is now possible to detect the presence and concentration of most biologically relevant ions.[6,7] In the last few years, researchers have become particularly interested in the physiological role of Zn(II).[8,9] Zinc plays a vital role in both physiological and metabolic pathways in the body. Biologically relevant concentrations of Zn(II) in its mobile form, are thought to be in the range of 1 fM in *E. coli* to almost 0.5 mM in mammalian cells.[10] It is an essential nutrient and strongly influences cell division and differentiation and hence is very important for the growth and development of all forms of life.[11] It plays a key role in the synthesis of insulin and the pathological state of diabetes.[12] It has been reported that hyperglycaemia from either type I or type II diabetes causes physiologically important losses of Zn(II) from the body. It is known that Zn(II) serves as a mediator for cell–cell signalling in the central nervous system (CNS), where the brain tissue contains a high concentration of Zn(II).[13] In addition to affecting neuron activity, it has also been observed that cortical neurons undergo cell death if exposed to intense Zn(II) levels and Zn(II) is now regarded as one of the most important co-factors in the regulation of apoptosis.[14] Hence, the ability to selectively detect Zn(II) at pH 7.4 in competitive media such as Ca(II), Mg(II) and other transition metal ions, can allow a further understanding of its physiological role in nature and disease.[8,9,15,16]

For some years we have been interested in the luminescence sensing of both cations and anions using fluorescence and delayed lanthanide luminescence sensing.[17–19] Our interest in the sensing of Zn(II) sprang from the fact that many of the examples in the literature had significant drawbacks which included: being synthetically challenging, possessing poor water solubility, pH sensitivity within the physiological pH region, possessing low quantum yield, or small luminescent enhancement between the 'free' and the 'complexed' sensor, sensitive to group II ions and as such, lacking in Zn(II) selectivity. With this in mind we set out to design a new family of Zn(II) selective sensors which would overcome all of these drawbacks.[20]

Our Zn(II) sensors discussed herein, are synthetically simple and based on the photoinduced electron transfer (PET) principles where a fluorophore is connected to a receptor by a short spacer. Here, 4-amino-1,8,-naphthalimide was chosen as the fluorophore, which has a strong absorption band in the visible region, emits at long wavelengths with large Stokes shifts and possesses high fluorescence quantum yield in aqueous media.[21] Unlike the Zn(II) sensors developed to date, many of which consist of the two bis(2-pyridylmethyl)amine units as the Zn(II) receptor, our sensor employs a phenyl iminodiacetate receptor.[22] This ensures pH independence in the physiological pH range at the same time as providing high selectivity and excellent affinity for Zn(II) over other biologically competitive metal ions. Employing the PET principle is advantageous as, if correctly designed, the sensors do not give rise to any fluorescence except upon coordination to

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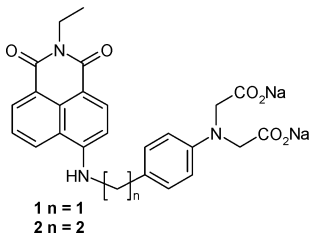
Zn(II), which '*switches the emission on*'.[23] The PET Zn(II) sensors presented herein, **1** and **2**, are the results of our investigation.



Results and discussion

Design and synthesis of 1–4

The design of **1** and **2** is based on the classical PET principle, developed by de Silva.[23,24] In our design we used a methylene spacer for **1** and an ethylene spacer for **2**. The rate of electron transfer, from the electron rich receptor to the electron deficient fluorophore is highly distance dependent. We would thus expect **1** to be a more efficient PET sensor than **2**. Nevertheless, the rationale behind **2** was that the ethylene spacer would impart more photo-stability, as well as being more stable to metabolite breakdown *in vivo*, which overall would lengthen its usability for practical application.[25] The synthesis of **1** and **2** is shown in Scheme 1. For both sensors, the starting point was the *N*-ethyl-4-amino-1,8-naphthalimide, **3**, which was synthesized by refluxing 6-bromo-1,8-naphthalic anhydride in 1,4-dioxane and 70% aqueous ethylamine (*w*/*v*), and obtained as an off-white solid in 85% yield after precipitation from water. The next step was the introduction of the spacer moieties in **1** and **2**. The synthesis of the benzyl derivative **4** was first attempted from 4-aminobenzylamine, which would incorporate the spacer as well as the aniline part of the receptor moiety in one step. However, the synthesis of this molecule turned out to be problematic and several reactions such as refluxing **3** with 4-aminobenzylamine in DMF, dioxane or ethanol all failed. Nevertheless, **4** was successfully synthesized by heating **3** to near melting temperature (130 °C) followed by subsequent addition of neat 4-aminobenzylamine. This resulted in the formation of the desired product **4** within 30 minutes in 85% yield, after precipitation from water and a recrystallization from ethanol. The synthesis of **5**, the ethyl analogue of **4**, was accomplished using a similar protocol whereby 4-aminophenyl-*N*-ethylamine was heated at 130 °C followed by precipitation and recrystallization from 2-propanol, giving the desired product in 80% yield. Different methods were attempted for the alkylation of **4** using ethyl bromoacetate, such as using K_2HPO_4 in refluxing CH_3CN, or DMF, but these resulted in the formation of mixture of products that were difficult to separate. Similarly, using K_2CO_3 in CH_3CN gave a mixture of products. However, the desired product was successfully formed by using K_2CO_3 in DMF and the target molecule **6** was formed in high purity and in 90% yield after recrystallization from 2-propanol. Similarly, **7** was formed using the same *N*-alkylation method in a 90% yield. Finally the two PET sensors **1** and **2** were formed, in a quantitative yield, by alkaline hydrolysis of **6** and **7**, respectively, using 3 M NaOH in CH_3OH : H_2O (10 : 1, *v*/*v*). All the compounds were fully characterized using conventional spectroscopic techniques. The aromatic region of the ^1H NMR (400 MHz, D_2O) of **1** is shown in Fig. 1,

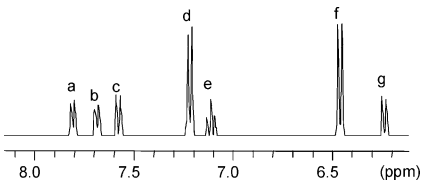
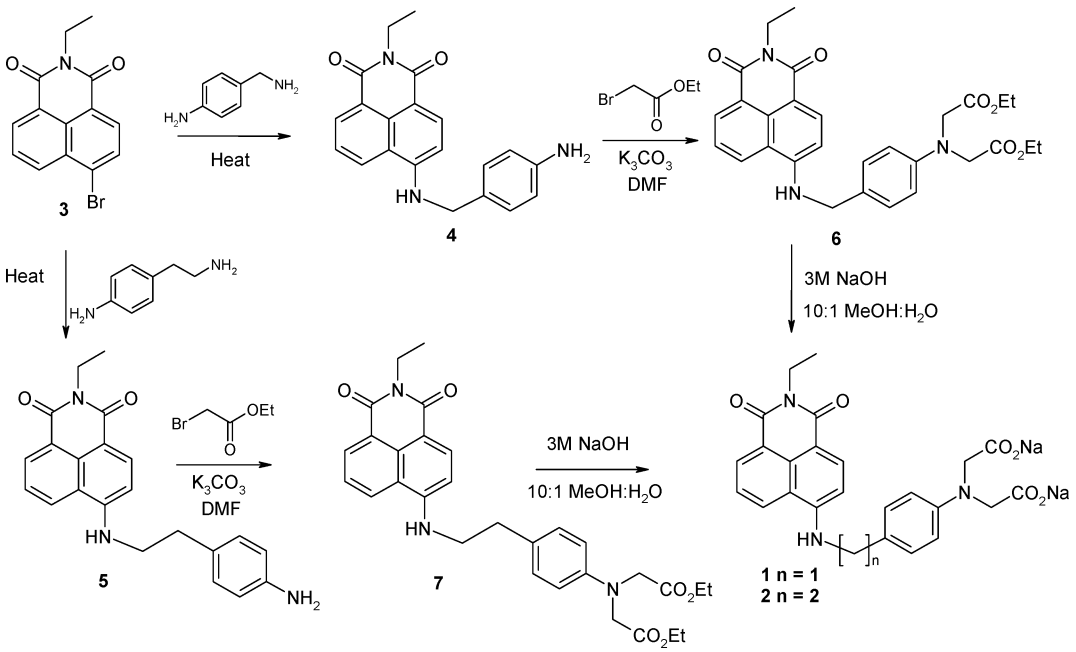


Fig. 1 Partial ^1H NMR (400 MHz) of **2** in D_2O. Protons **a**, **b**, **c**, **e** and **g** are assigned to the naphthalimide fluorophore while **d** and **f** are those of the aniline receptor.



Scheme 1 Synthesis of PET sensors **1** and **2**.

showing doublets centred at δ 6.47 and δ 7.22 ppm for the phenyl protons and a doublet, a triplet and three doublets resonating at 7.81, 7.69, 5.58, 7.11 and 6.24 ppm for the naphthalimide protons. A similar ^{1}H NMR was observed for **2**.

Spectroscopic pH evaluation 1 and 2

The luminescent properties of both sensors were first investigated as a function of pH in water in the presence of 135 mM KCl (to maintain constant ionic strength). Both sensors were fully water soluble. The absorption spectrum of **1**, when recorded in alkaline solution, showed two main spectral regions between 258–293 nm, assigned to the π–π* transition, and a second region centred at 440 nm ($\varepsilon = 18.92 \times 10^3$ M^{-1} cm^{-1}) assigned to the internal charge transfer (ICT) character of the fluorophore, arising due to the push–pull nature of the donating amine and the withdrawing diimide. Upon titration with acid, the 450 nm band was hypsochromically shifted to shorter wavelengths, with the formation of an isosbestic point at 422 nm, Fig. 2. This was assigned to the protonation of the amino moiety of the receptor and not that of the 4-amino moiety, which usually occurs at much lower pH. For ideal PET sensors, such shifts do not usually occur, due to the presence of the spacer between the fluorophore and the receptor. However, the observed hypsochromic shift can be explained by the ICT excited state of the naphthalimide, which places a partial positive charge on the 4-amino moiety. This creates a repulsive interaction with the protonated *N*-acetate moiety thus shifting the absorption spectra to lower wavelengths.[26] The plot of absorbance at 450 nm against pH showed that only minor changes occurred above pH 6.5, while the most significant changes were observed between pH 3–5. From these latter changes a pK_a of 3.2 ± 0.1 was determined, using non-linear regression analysis. In the same way the absorption spectra of **2** were monitored as function of pH. In alkaline solution the main absorption band was observed at 453 nm ($\varepsilon = 20.1 \times 10^3$ M^{-1} cm^{-1}), with a lower intensity band at 306 nm. Upon acidification, a *ca.* 20% decrease was observed in the intensity of both bands, but no hypsochromic shift was observed, possibly the presence of the longer ethylene spacer, reduces the aforementioned repulsive interactions. Unfortunately, these changes were too small for accurate pK_a determination.

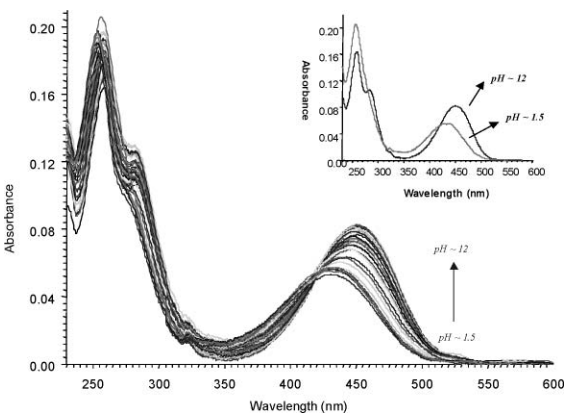


Fig. 2 Changes in the absorption spectra of **1** (1 μM) upon changes in pH. *Insert*: The changes at alkaline and acidic pH.

The changes in the fluorescence emission spectra of both **1** and **2** were also monitored as a function of pH. Upon excitation of the ICT band at the isosbestic point at 422 nm, the ICT emission was observed at a long wavelength between 500–700 nm, with $\lambda_{em\,max}$ = 550 nm. The changes in the emission spectra of **1** are shown in Fig. 3, and clearly demonstrate that the observed emission is highly pH dependent; being '*switched on*' upon acidification, with large fluorescent enhancements. These changes were found to be fully reversible, demonstrating the suppression of any PET quenching from the electron rich receptor to the naphthalimide fluorophore upon protonation of the aniline nitrogen, which increases the oxidation potential of the receptors and hence makes PET thermodynamically unfavorable.[23,24] From the changes at 550 nm (*insert* in Fig. 3), a pK_a of 3.20 ± 0.1 was determined for **1**. This is in good agreement with the ground state pK_a measurement, and clearly demonstrates the pH independence of this sensor within the physiological pH range. Hence, as the emission can be considered to be 'switched off' at pH 7.5, any change in the emission intensity as a function of added metal ions, *e.g.* Zn(II), can be considered a direct measure of the binding of the ions to the receptor at this pH. In a similar manner, the fluorescence emission was monitored for **2** as a function of pH upon excitation at 453 nm. As above, the emission was found to be highly pH dependent, being '*switched off*' in alkaline solution and gradually '*switched on*' in acid solution below pH 5. From the changes at 554 nm (λ_{max}) a pK_a = 2.10 ± 0.1, was determined. This is considerably less than that observed for **1**. This may be due to the electronic or conformational effects of the ethylene spacer. Nevertheless, these results show that the emission from **2** is also pH independent in the physiological pH range. These are highly desirable features for any metal ion chemosensors for sensing *in vivo*.

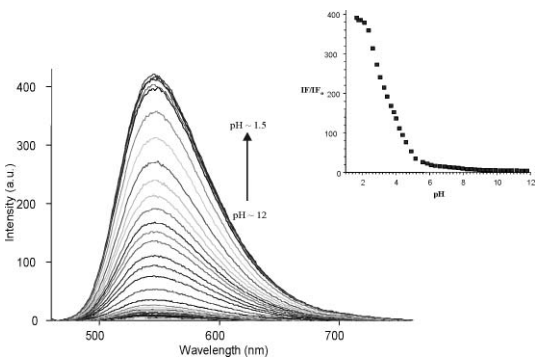


Fig. 3 Changes in the fluorescence emission spectra of **1** (1 μM) upon changes in pH. *Insert*: The changes at 550 nm as a function of pH.

Spectroscopic evaluation 1 and 2 in the presence of various Group II and transition metal ions

Having established the pH dependence of both sensors, we evaluated the fluorescence and absorption dependence for both **1** and **2** (1 μM) towards Group II and transition metal ions such as Zn(II), Cd(II), Hg(II), Cu(II), Co(II), Ni(II) and Fe(III), at pH 7.4 (20 mM HEPES) in the presence of 135 mM KCl. No fluorescence emission changes were observed with Ca(II) or Mg(II), even at 10^{-2} M concentrations. Among the transition metal ions, Cu(II), Fe(II) and Fe(III) did not modulate the fluorescence intensity, and the emission remained '*switched off*'. Minor fluorescence enhancements were however, observed at higher concentrations ($>10^{-2}$ M) for Co(II), Ni(II), Hg(II) and Cd(II) (see later Fig. 5).

However, in the presence of Zn(II) dramatic changes were observed in fluorescence emission, which was '*switched on*' as can be seen in Fig. 4.

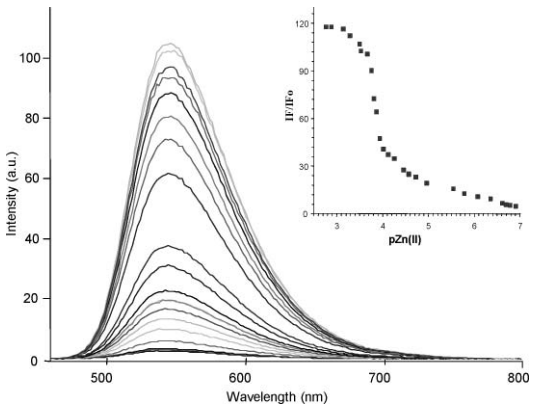


Fig. 4 Changes in the fluorescence emission spectra of **1** (1 μM) upon addition of Zn(II) upon excitation at 422 nm, at pH 7.4. [Zn(II)]: 1.2 nM–1.5 mM. *Insert*: The changes at 550 nm as a function of −log[Zn(II)], pZn.

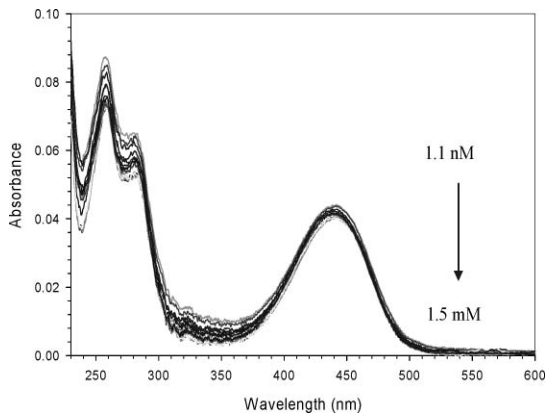


Fig. 5 Absorption spectra response of **1** in the presence of increasing concentration of Zn(II) (*cf.* Fig. 4) in HEPES buffer at pH 7.4.

No measurable changes were observed in the emission wavelength, which indicates a PET mechanism. These changes represent the binding of the ion to the Zn(II) receptor, *via* the carboxylates of the iminodiacetate and the nitrogen moiety, and the concomitant suppression of any PET activities from the receptor to the fluorophore. This is due to the increase in the oxidation potential of the receptor upon Zn(II) binding, in a similar manner to that observed for H^+. From these measurements the binding constant $\log \beta = 3.9$ (±0.1) was determine using the least squares regression analysis program Sigma Plot. The changes in the emission intensity suggest a simple equilibrium and 1 : 1 binding. The 1 : 1 binding was also determined using both Job plot and Hill plot analysis. For the latter a Hill coefficient of 1.05 was calculated, which indicates the correct 1 : 1 stoichiometry. For the free sensor **1** the fluorescence quantum yield (Φ_{free}) under these conditions was measured to be *ca.* 0.004, increased to 0.21 in the presence of 5 μM Zn(II), (Φ_{bound}). This is a fluorescence enhancement of 56 fold upon Zn(II) binding, which shows that the sensor can be considered as a luminescent light-switch for Zn(II). Using the value for ε (determined above), the brightness of the Zn(II) bound sensor can be determined ($\varepsilon \times \Phi$) as 1.01×10^6 Int M^{-1} cm^{-1}.

Sensor **1** also functions as an ideal PET sensor as the ground state was not modulated upon Zn(II) binding to the receptor, Fig. 5. Some minor changes were observed at lower wavelengths that can be assigned to the coordination of Zn(II) to the aniline unit. However, these are too small for accurate binding constant determination. Similarly, no changes were observed in the absorption spectra in the presence of other competitive metal ions.

The fluorescence response of **2** to Zn(II) was similar to that observed for **1**, Fig. 6. A broad emission band with a maximum at 554 nm was observed upon excitation at 453 nm. In the unbound form, the sensor **2** was determined to have a quantum yield of ~0.007 which increased to 0.14 in the presence of a saturating amount of Zn(II) (5 μM), hence a fluorescence quantum yield enhancement (Φ_{bound}/Φ_{free}) of 20-fold was observed upon Zn(II) binding. This is a good enhancement, given the longer spacer length and shows that **2** can also function as a luminescent light-switch for Zn(II). As in the case of **1**, no shifts were observed in the emission wavelength. From these changes a $\log \beta = 4.0 \pm 0.1$ was calculated for the 1 : 1 binding, which indicates that **2** has the same affinity for Zn(II) as observed for **1**. The extinction coefficient (ε) of the sensor was calculated to be 20.1×10^3 M^{-1} cm^{-1}, with a brightness of 4.02×10^5 Int M^{-1} cm^{-1} for the Zn(II) bound sensor. These results clearly indicated that the two sensors fulfil the criteria set out above for the development of highly selective Zn(II) sensing in competitive media.

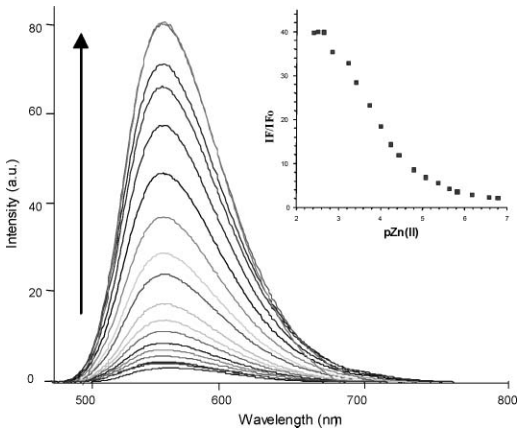


Fig. 6 Changes in the fluorescence emission spectra of **2** (1×10^{-6} M) upon addition of Zn(II) upon excitation at 455 nm, at pH 7.4. [Zn(II)] are the same as in Fig. 4. *Insert*: The changes at 554 nm as a function of pZn (= −log[Zn(II)]).

Selectivity of 1 and 2 for Zn(II) over other competitive ions

As briefly discussed above, the selectivity of both sensors towards Zn(II) was found to be excellent. The fluorescence and absorption responses of **1** and **2** to Ca^{2+} and Mg^{2+} were first investigated as these are highly competitive ions *in vivo.* The addition of these ions, even at high concentrations ($>10^{-2}$ M), did not result in any changes in the absorption and the fluorescence emission spectra.

Of the transition metal ions, only Cd(II) and Hg(II) gave rise to any significant changes. Addition of Cd(II) to **1** resulted in

no spectral shifts in the ground state spectrum. However, the fluorescence titration of **1** with Cd(II) lead to a 10-fold increase in fluorescence. The changes in emission were fitted to a 1 : 1 binding, and a log $\beta = 2.0 \pm 0.1$ was determined from these changes. For **2**, even higher concentrations of Cd(II) were required to achieve any significant increase in the fluorescence emission, and a log $\beta = 1.8 \pm 0.1$ was determined from these changes. Hence, it can be concluded that both these sensors show minor changes with Cd(II). Nevertheless, these concentrations are much higher than the level of Cd(II) present in the body, and as such, will not interfere in the measurement of the Zn(II) ions in important organs such as the pancreas and brain. The responses towards to Hg(II) were negligible and mirrored the Cd(II) response, with an overall 4-fold enhancements being observed on titrations of **1** with Hg(II), while Ni(II) gave even smaller enhancements. This clearly demonstrates that the receptor has excellent affinity for Zn(II) over these ions.

To further evaluate the selectivity of **1** towards Zn(II) we carried out competitive measurements. The results of these titrations are shown in Fig. 7, where a 1 μM solution of **1** at pH 7.4 (20 mM HEPES, 135 mM KCl) was measured after the addition of 5 μM of each of the metal ions shown. These results clearly demonstrate the selectivity for Zn(II) for **1** over the other metal ions, as on all occasions emission intensity (recorded at 550 nm) increased significantly upon addition of Zn(II). To the best of our knowledge, **1** is the first example of a Zn chemosensor to uphold such strict Zn(II) selectivity.

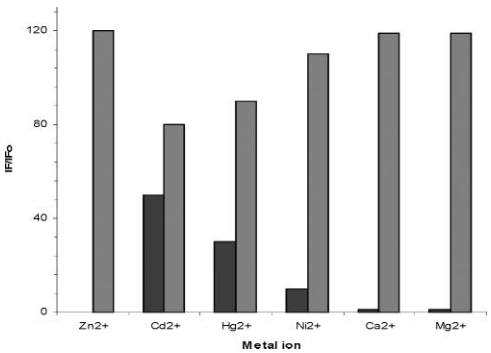


Fig. 7 Relative fluorescence intensity responses of **1** to various metal ions. Dark grey bars represent emission without Zn(II) for a particular metal ion. Light grey bars represent the emissions upon addition of Zn(II) to respective metal ion. The first bar shows the response to Zn(II) in the absence of any competitive ions.

Spectroscopic evaluations of 1 and 2 towards free Zn(II) ions

Having established the high selectivity of both sensors towards Zn(II), the ability of **1** to determine free Zn(II) concentration was evaluated. Free Zn(II), or chelatable Zn(II), is found in high concentrations in the brain, the nervous system and the pancreas. To determine the affinity of **1** towards free Zn(II), a buffered solution of **1** (1 μM) containing 135 mM KCl, 20 mM HEPES and 1.1 mM ethylene glycol tetraacetic acid EGTA was prepared and to this was added varying volumes of 1 mM $ZnCl_2$ solution giving free Zn(II) concentrations of 1, 3, 5, 9, 12, 20, 30, 45, 80, 90, 100, 120, 140, 160, 200, 220, 240 and 280 nM, and the absorption and the fluorescence emission spectra recorded (Fig. 8).[27]

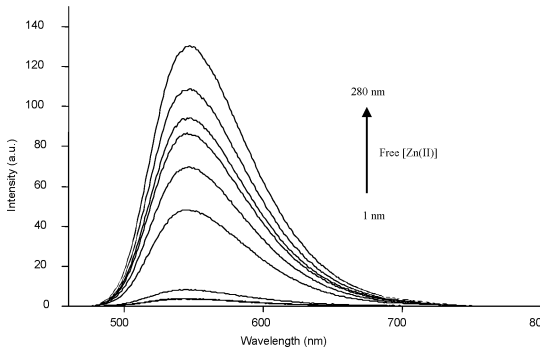


Fig. 8 Changes in the fluorescence emission spectra of **1** upon addition of Zn(II) upon excitation at 455 nm, at pH 7.4 (20 mM HEPES) and 135 mM KCl and 1.1 mM EGTA.

As seen above, no significant changes were observed in the absorption spectra. However, the fluorescence emission response was significantly affected, being '*switched on*' with fluorescence enhancements of almost 28-fold. From these changes a dissociation equilibrium constant, K_d, was obtained by plotting the $\log[(I_F - I_{F\,min})/(I_{F\,max} - I_F)]$, where I_F is the measured fluorescence after each addition; $I_{F\,min}$ is the initial emission and $I_{F\,max}$ is the saturated emission, *versus* the log of free Zn(II). A Hill plot was obtained, with a log $K_d = 8.3 \pm 0.1$ and therefore a $K_d = 4$ nM, Fig. 9. These results clearly demonstrate the affinity of **1** for free Zn(II) concentrations and the versatility of our design.

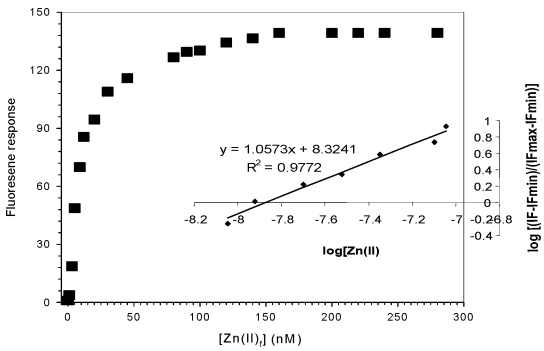


Fig. 9 Changes in the fluorescence emission of **1** (1 μM) at 550 nm as a function of free Zn(II) at pH 7.4 (20 mM HEPES) and in the presence of 135 mM KCl and 1.1 mM EGTA. *Insert*: The fitting of the corresponding Hill plot.

Conclusion

The synthesis of two fluorescent water soluble PET sensors for the sensing of Zn(II) has been achieved in a few steps and in high yields. We have demonstrated that both sensors show ideal PET behavior for Zn(II), where the absorption spectra do not change to any significant degree upon binding to the ion. In contrast, the emission spectra change dramatically; being enhanced, or '*switched on*', significantly for both sensors. Importantly, both **1** and **2** show high selectivity for Zn(II) ions, even in the presence of other competitive ions. All the ion titrations described herein, were conducted at pH 7.4, where the emission for both sensors was '*switched off*'. Hence, these sensors are pH independent in the physiological pH range. To the best of our knowledge these are the

first examples of such PET sensors for Zn(II) that give rise to high selectivity for Zn(II) within the physiological pH range. We are currently evaluating the biological application of these sensors in greater detail, the outcome of this investigation will be published in due course.†

Experimental

General

All solutions were prepared in deionized water. $ZnCl_2$, $CdCl_2$, and $HgCl_2$ (99%) were used for preparing stock solutions (1 M) of Zn(II), Cd(II) and Hg(II). Zn(II), Cd(II) and Hg(II) solutions of varying concentrations were prepared by the dilution of appropriate amounts of 1 M stock solution with deionized water. Absorption spectra were recorded on a Shimadzu diode array spectrophotometer under the control of a Pentium IV-based PC utilizing the manufacturer supplied software package. Fluorescence spectra were recorded on a Varian spectrofluorimeter interfaced to the PC *via* an IEEE-488 (GPIB) card using Cary Eclipse software. Excitation was provided by a 150 W Xe lamp operating at a current of 5 A. Spectra were acquired in a 1 × 1 cm quartz cuvette (3 mL) using a slit width of 5 nm and a scan rate of 400 nm min^{-1}. Both absorption and fluorescence spectra were acquired at room temperature. Data were analyzed with either Microsoft Excel or Sigma Plot. pH values of the solutions were recorded with a calibrated glass electrode.

Synthesis

***N*-Ethyl-4-bromo-1,8-naphthalimide (3).** 4-Bromo-1,8-naphthalic anhydride (1.5 g, 5.42 mmol) was dissolved in 1,4-dioxane (50 mL) and ethylamine (0.35 g, 6.49 mmol) was added. The resulting solution was refluxed for 2 hours after which the solution was poured into ice–water. The product **3** was collected by suction filtration and washed with water and dried over P_2O_5. This gave **3** in 85% (1.66 g), mp = 162–164 °C (lit. 160–161 °C). ^{1}H-NMR (400 MHz, $CDCl_3$): δ, 1.35 (t, 3H, J = 7.0 Hz, NCH_2CH_3), 4.23–4.29 (q, 2H, J = 7.0 Hz, NCH_2CH_3), 7.85 (t, 1H, J = 7.5 Hz, Ar-*H*), 8.05 (d, 1H, J = 7.5 Hz, Ar-*H*), 8.42 (d, 1H, J = 7.5 Hz, Ar-*H*), 8.58 (d, 1H, J = 7.5 Hz, Ar-*H*), 8.61 (d, 1H, J = 7.5 Hz, Ar-*H*). ^{13}C-NMR (100 MHz, $CDCl_3$): δ, 162.97, 132.77, 131.51, 130.72, 130.62, 130.15, 129.75, 128.52, 127.61, 122.70, 121.85, 35.20, 12.80.

***N*-Ethyl-4-(4-aminobenzylamino)-1,8-naphthalimide (4).** Compound **3** (1.5 g, 4.93 mmol) was heated with stirring at 130 °C under argon. After 10 minutes an excess of 4-aminobenzylamine (3.01 g, 24.65 mmol) was added in a single portion. After 30 min water (20 ml) was added to the reaction mixture, which resulted in the formation of a yellow precipitate which was isolated by filtration and recrystallized from ethanol to give **4** as light yellow needles in 85% yield (1.48 g), mp = 198–200 °C. MS (ES^+) m/z = 346 $(M + H)^+$. Anal. calculated for $C_{21}H_{19}N_3O_2$: C, 73.03; H, 5.54; N, 12.17. Found: C, 72.76; H, 5.63; N, 12.06. ^{1}H-NMR (400 MHz, DMSO-d_6): δ, 1.17 (t, 3H, J = 7.0 Hz, NCH_2CH_3), 4.04 (q, 2H, J = 7.0 Hz, NCH_2CH_3), 4.54 (d, 2H, J = 5.2 Hz, CH_2) 4.95 (brs, 2H, NH_2), 6.54 (d, 2H, J = 8.0 Hz, Ar-*H*), 6.70 (d, 1H, J = 8.5 Hz, Ar-*H*), 7.07 (d, 2H, J = 8.0 Hz, Ar-*H*), 7.68 (t, 1H, J = 7.5 Hz, Ar-*H*), 8.18 (d, 1H, J = 8.5, Ar-*H*), 8.42 (brs, 1H, N*H*), 8.43 (d, 1H, J = 8.5 Hz, Ar-*H*), 8.74 (d, 1H, J = 8.5 Hz, Ar-*H*). ^{13}C-NMR (100 MHz, DMSO-d_6): δ, 163.53, 162.673, 150.54, 147.70, 133.92, 130.54, 129.37, 128.52, 127.93, 124.93, 124.27, 121.95, 120.26, 107.81, 104.51, 45.86, 34.21, 13.29. IR (ν_{max}, KBr, cm^{-1}): 3358, 2974, 2931, 2108, 1679, 1639, 1579, 1536, 1390, 1346, 1248, 1181, 1101, 1066, 910, 876, 773, 757, 694, 581.

***N*-Ethyl-4-{4-[bis(ethoxycarbonylmethyl)amino]benzylamino}-1,8-naphthalimide (6).** To a solution of **4** (1.5 g, 4.34 mmol) in dry DMF (50 mL) was added K_2CO_3 (1.67 g, 9.46 mmol) and KI (1.57 g, 9.46 mmol). The resulting solution was stirred at room temperature under argon. After 10 min ethyl bromoacetate (1.57 g, 9.46 mmol) was slowly added *via* a syringe, and the resulting mixture was heated at 90 °C overnight. The solvent was then evaporated under reduced pressure and the resulting residue was taken into chloroform (30 mL) and washed with 1 M HCl (30 mL) water (30 mL) and finally brine (30 mL). The organic layer was then dried over Na_2SO_4, and evaporated to dryness under reduced pressure. The resulting solid was recrystallized from ethanol to give **6** in 90% yield (2.02 g) as a light yellow solid, mp = 180–182 °C. MS (ES^+) m/z = 518 $(M + H)^+$. Anal. calculated for $C_{29}H_{31}N_3O_6$: C, 67.30; H, 6.04; N, 8.12. Found: C, 67.03; H, 6.02; N, 7.91. ^{1}H-NMR (400 MHz, DMSO-d_6): δ, 1.16 (m, 9H, NCH_2CH_3, $NCH_2CO_2CH_2CH_3$), 4.01–4.11 (m, 6H, NCH_2CH_3, $NCH_2CO_2CH_2CH_3$), 4.15 (s, 4H, $NCH_2CO_2CH_2CH_3$), 6.5 (d, 2H, J = 8.0 Hz, Ar-*H*), 6.67 (d, 1H, J = 8.5 Hz, Ar-*H*), 7.21 (d, 2H, J = 8.0 Hz, Ar-*H*), 7.69 (t, 1H, J = 8.0 Hz, Ar-*H*), 8.17 (d, 1H, J = 8.0 Hz, Ar-*H*), 8.37 (brs, 1H, N*H*), 8.44 (d, 1H, J = 7.0 Hz, Ar-*H*), 8.73 (d, 1H J = 8.0 Hz, Ar-*H*). ^{13}C-NMR (100 MHz, DMSO-d_6): δ, 170.54, 163.55, 162.69, 150.44, 146.84, 133.98, 130.65, 129.32, 128.52, 127.94, 126.53, 124.42, 121.89, 120.89, 111.86, 107.84, 104.56, 60.40, 52.66, 45.35, 34.26, 14.08, 13.30. IR (ν_{max}, KBr, cm^{-1}): 3386, 2974, 2929, 1747, 1727, 1685, 1616, 1590, 1573, 1541, 1523, 1450, 1397, 1367, 1251, 1183, 1102, 1027, 819, 801, 771, 760, 605, 585.

Disodium *N*-Ethyl-4-{4-[bis(carboxylatomethyl)amino]benzylamino}-1,8-naphthalimide (1). To a solution of **6** (1.43 g, 2.76 mmol) in dry ethanol (50 mL) was added NaOH (0.300 g, 7.5 mmol) in 1 mL of water. The resulting mixture was refluxed for 2 hours. Upon cooling to room temperature the desired product was obtained as a yellow solid in 80% (1.33 g), mp = 310 °C (decomp.). MS (ES^+) m/z = 506 $(M + H)^+$. Anal. calcd for $C_{25}H_{21}N_3Na_2O_6 \cdot 2H_2O$: C, 55.46, H, 4.65; N, 7.76. Found: C, 55.29; H, 4.23; N, 7.72. ^{1}H-NMR (400 MHz, D_2O): δ, 1.09 (t, 3H, J = 7.0 Hz), 3.75–3.79 (m, 6H), 4.28 (d, 2H), 6.24 (d, 2H, J = 8.8 Hz), 6.47 (d, 2H, J = 8.8 Hz), 7.11 (t, 1H, J = 7.6 Hz), 7.22 (d, 2H,

† To evaluate the ability of these sensors to detect Zn(II) in biological samples, preliminary histological staining of human pancreas tissue using **1** has been carried out. A standard staining protocol was followed, after which glass slides containing pancreas tissue sections were immersed for 10 minutes in a bath containing 5 μM buffered solution of **1** at pH 7.4. The glass slide viewed under a green 546 nm epifluorescence microscope and the image collected. The control tissue section, without staining with **1**, was also recorded. In both cases, the dull green colour observed is due to autofluorescence from proteins and light scattering. However, the sample stained with **1**, showed areas within the tissue that were highly luminescent clearly demonstrating the ability of **1** to detect Zn(II). These preliminarily studies clearly show promising results as **1** labels the area within the tissue which is most likely to contain granular chelatable zinc. We are currently evaluating the ability of these sensors to monitor Zn(II) in other biological media such as cells.

J = 8.2 Hz), 7.58 (d, 1H, J = 8.8 Hz), 7.69 (d, 1H, J = 8.2 Hz), 7.81 (d, 1H, J = 7.6 Hz). ^{13}C-NMR (400 MHz, D_2O): δ, 179.06, 164.31, 163.28, 158.55, 149.69, 147.69, 133.50, 129.84, 128.70, 124.20, 122.93, 118.79, 117.80, 111.13, 105.52, 55.21, 45.69, 34.94, 11.99. IR (ν_{max}, KBr, cm^{-1}): 3525, 3382, 2875, 1685, 1634, 1547, 1560, 1435, 1394, 1368, 1350, 1314, 1249, 1179, 1130, 1106, 1063, 979, 911, 877, 821, 769, 756, 705, 587.

***N*-Ethyl-4-[2-(4-aminophenyl)ethylamino]-1,8-naphthalimide (5).** Compound **3** was heated with stirring at 130 °C under argon for 10 minutes, after which 4-ethylaminobenzylamine (3.01 g, 24.65 mmol) was added *via* a syringe. The reaction mixture was kept stirring for 30 minutes, followed by the addition of water (20 mL which resulted in the formation of a yellow precipitate which was isolated by filtration and recrystallized from 2-propanol to give **4** as light yellow needles in 85% yield (1.45 g), mp = 200–202 °C. MS (ES$^+$) m/z = 360 (M + H)$^+$. Anal. calculated for $C_{22}H_{21}N_3O_2$: C, 73.52; H, 5.89; N, 11.69; Found: C, 72.76; H, 5.63; N, 12.06. ^{1}H-NMR (400 MHz, DMSO-d_6): δ, 1.17 (t, 3H, J = 7.0 Hz, NCH_2CH_3), 2.81–2.84 (t, 2H, J = 8.2 Hz, CH_2), 3.45–3.54 (m, 2H, CH_2), 4.04 (q, 2H, J = 7.0 Hz, NCH_2CH_3), 4.89 (d, 2H, J = 5.2 Hz), 6.51 (d, 2H, J = 8.2 Hz, Ar-*H*), 6.83 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.13 (d, 2H, J = 8.2 Hz, Ar-*H*), 7.68 (t, 1H, J = 8.2 Hz, Ar-*H*), 7.84 (brs, N*H*), 8.28 (d, 1H, J = 8.2, Ar-*H*), 8.44 (d, 1H, J = 7.5 Hz, Ar-*H*), 8.66 (d, 1H, J = 8.2 Hz, Ar-*H*). ^{13}C-NMR (100 MHz, DMSO-d_6): δ, 163.53, 162.69, 150.42, 146.94, 134.24, 130.58, 129.38, 129.16, 125.94, 124.22, 121.87, 121.88, 120.09, 114.01, 107.59, 103.87, 45.01, 34.25, 33.35, 13.32; IR (ν_{max}, KBr, cm^{-1}): 3424, 3348, 2981, 2934, 2889, 2679, 1904, 1850, 1680, 1639, 1579, 1537, 1465, 1450, 1432, 1396, 1365, 1347, 1302, 1276, 1178, 1150, 1108, 1070, 1011, 913, 878, 769, 756, 654, 596.

***N*-Ethyl-4-(2-{4-[bis(ethoxycarbonylmethyl)amino]phenyl}ethylamino)-1,8-naphthalimide (7).** To a solution of **5** (1.5 g, 4.2 mmol) in dry DMF (50 ml) was added K_2CO_3 (1.30 g, 9.24 mmol) and KI (1.52 g, 9.24). The resulting solution was stirred at room temperature under argon. After 10 min, ethyl bromoacetate (1.56 g, 9.24 mmol) was slowly added *via* a syringe. The resulting mixture was heated at 90 °C overnight. The same isolation procedure was used as for **6**, which gave the desired product in 90% yield (2.01 g) as a light yellow solid, mp = 195–197 °C. MS (ES$^+$) m/z: 532 (MH)$^+$. Anal. calculated for $C_{30}H_{33}N_3O_6$: C, 67.30; H, 6.04; N, 8.12. Found: C, 66.42; H, 5.98; N, 7.74. ^{1}H-NMR (400 MHz, DMSO-d_6): δ, 1.16–1.20 (m, 9H, NCH_2CH_3, $NCH_2CO_2CH_2CH_3$), 2.87–2.89 (m, 2H, CH_2), 3.52–3.54 (m, 2H, CH_2), 4.04–4.13 (m, 6H, NCH_2CH_3, $NCH_2CO_2CH_2CH_3$), 4.17 (s, 4H, $NCH_2CO_2CH_2CH_3$), 6.49 (d, 2H, J = 8.5 Hz, Ar-*H*), 6.84 (d, 1H, J = 9.0 Hz, Ar-*H*), 7.13 (d, 2H, J = 8.5 Hz, Ar-*H*), 7.67 (t, 1H, J = 8.0 Hz), 7.85 (brs, 1H, N*H*), 8.27 (d, 1H, J = 8.5 Hz), 8.44 (d, 1H J = 7.0 Hz), 8.66 (d, 1H J = 8.5 Hz). ^{13}C-NMR (100 MHz, DMSO-d_6): δ, 170.61, 163.54, 162.71, 150.41, 146.26, 134.25, 130.60, 129.40, 129.34, 128.52, 127.58, 124.26, 121.89, 120.13, 111.90, 107.69, 103.94, 60.35, 52.75, 44.84, 34.25, 32.89, 14.12, 13.32. IR (ν_{max}, KBr, cm^{-1}): 3378, 2974, 2929, 1753, 1731, 1686, 1581, 1543, 1524, 1430, 1395, 1367, 1296, 1181, 1103, 1062, 972, 911, 808, 770, 758, 655, 587.

***N*-Ethyl-4-(2-{4-[bis(carboxylatomethyl)amino]phenyl}ethylamino)-1,8-naphthalimide (2).** To a solution of **7** (1.43 g, 2.76 mmol) in dry ethanol (50 mL) was added NaOH (0.30 g, 7.5 mmol) in 1 mL of water. The resulting solution was refluxed for 2 hours. Upon cooling, the desired product was obtained as a yellow solid in 95% yield (1.33 g), mp = 320 °C (decomp.). MS (ES$^+$) m/z = 520 (M + H)$^+$. Anal. calculated for $C_{26}H_{23}N_3Na_2O_6{\cdot}2H_2O$: C, 55.46, H, 4.65; N, 7.76. Found: C, 55.29; H, 4.23; N, 7.72. ^{1}H-NMR (400 MHz, D_2O): δ, 1.09 (t, 3H, J = 7.3 Hz, NCH_2CH_3), 2.76–2.79 (m, 2H, CH_2), 3.29–3.33 (m, 2H, CH_2), 3.71–3.79 (m, 6H, NCH_2CH_3, NCH_2CO_2Na), 6.16 (d, 2H, J = 8.8 Hz, Ar-*H*), 6.44 (d, 2H, J = 8.0 Hz, Ar-*H*), 6.98 (t, 1H, J = 8.1 Hz, Ar-*H*), 7.08 (d, 2H, J = 8.0 Hz, Ar-*H*), 7.47–7.53 (m, 2H, Ar-*H*), 7.70 (d, 1H, J = 8.2 Hz, Ar–H). ^{13}C-NMR (100 MHz, D_2O): δ, 179.24, 164.23, 163.20, 149.68, 149.87, 133.49, 129.85, 129.07, 126.98, 125.94, 122.83, 118.78, 117.68, 111.29, 105.22, 102.94, 55.24, 43.79, 34.86, 32.68, 32.37, 11.96. IR (ν_{max}, KBr, cm^{-1}): 3374, 2933, 1906, 1854, 1680, 1638, 1546, 1519.51, 1524, 1435, 1399, 1351, 1311, 1253, 1188, 1110, 1066, 978, 919, 879, 811 772, 759, 700, 580.

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References

1 (*a*) A. T. Wright and E. V. Anslyn, *Chem. Soc. Rev.*, 2006, **35**, 14; (*b*) P. A. Gale, *Acc. Chem. Res.*, 2006, **39**, 465; (*c*) A. P. de Silva, B. McCaughan, B. O. F. McKinney and M. Querol, *Dalton Trans.*, 2003, 1902; (*d*) S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.*, 2001, **34**, 963.

2 (*a*) T. Gunnlaugsson and J. P. Leonard, *Chem. Commun.*, 2005, 3114; (*b*) T. Gunnlaugsson, P. E. Kruger, P. Jensen, J. Tierney, H. D. P. Ali and G. M. Hussey, *J. Org. Chem.*, 2005, **70**, 10875; (*c*) T. Gunnlaugsson, H. D. P. Ali, M. Glynn, P. E. Kruger, G. M. Hussey, F. M. Pfeffer, C. M. G. dos Santos and J. Tierney, *J. Fluoresc.*, 2005, **15**, 287.

3 (*a*) U. S. Spichiger-Keller, *Chemical Sensors and Biosensors for Medical and Biological Applications*, Wiley-VCH, 1998, Weinheim; Germany; (*b*) *Chemosensors of Ion and Molecular Recognition*, ed. J. P. Desvergne and A. W. Czarnik, Kluwer Academic Publishers, Dordrecht, The Netherlands, 1997.

4 J. K. Tusa and M. J. P. Leiner, *Ann. Biol. Clin.*, 2003, **61**, 183.

5 (*a*) J. K. Tusa and H. He, *J. Mater. Chem.*, 2005, **15**, 2640; (*b*) H. He, M. A. Mortellaro, M. J. P. Leiner, S. T. Young, R. J. Fraatz and J. K. Tusa, *Anal. Chem.*, 2003, **75**, 549.

6 (*a*) R. Martínez-Máñez and F. Sancenón, *J. Fluoresc.*, 2005, **15**, 267; (*b*) R. Martínez-Máñez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 4419; (*c*) K. Rurack and U. Resch-Genger, *Chem. Soc. Rev.*, 2002, **31**, 116; (*d*) A. P. de Silva, D. B. Fox, A. J. M. Huxley and T. S. Moody, *Coord. Chem. Rev.*, 2000, **205**, 41; (*e*) L. Fabbrizzi, M. Licchelli, G. Rabaioli and A. Taglietti, *Coord. Chem. Rev.*, 2000, **205**, 85.

7 (*a*) J. F. Callan, A. P. de Silva and D. C. Magri, *Tetrahedron*, 2005, **61**, 8551; (*b*) L. Fabbrizzi, M. Licchelli, P. Pallavicini and A. Taglietti, *Inorg. Chem.*, 1996, **35**, 1733; (*c*) A. W. Czarnik, *Acc. Chem. Res.*, 1994, **27**, 302.

8 (*a*) R. B. Thompson, *Curr. Opin. Chem. Biol.*, 2005, **9**, 526; (*b*) K. Kikuchi, K. Komatsu and T. Nagano, *Curr. Opin. Chem. Biol.*, 2004, **8**, 182; (*c*) N. C. Lin, H. C. Freake and C. Brueckner, *Chem.–Eur. J.*, 2004, **11**, 38; (*d*) P. Jiang and Z. Guo, *Coord. Chem. Rev.*, 2004, **248**, 205.

9 (*a*) Examples of previous work include: S. C. Burdette, C. J. Frederickson, W. Bu and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 1778; (*b*) S. Maruyama, K. Kikuchi, T. Hirano, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2002, **124**, 10650; (*c*) T. Hirano, K. Kikuchi, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2002, **124**, 6555; (*d*) S. C. Burdette, G. K. Walkup, B. Spingler, R. Y. Tsien and S. J. Lippard, *J. Am. Chem. Soc.*, 2001, **123**, 7831; (*e*) T. Hirano, K. Kikuchi, Y. Urano, T. Higuchi and T. Nagano, *Angew. Chem., Int. Ed.*, 2000, **39**, 1052; (*f*) S. A. de Silva, A. Zavaleta, D. E. Baraon, O. Allan, E. V. Isidor, N. Kashimura and J. M. Percarpio, *Tetrahedron Lett.*, 1997, **38**, 2237.

10 (*a*) C. J. Federickson, *Int. Rev. Neurobiol.*, 1989, **31**, 145; (*b*) C. J. Frederickson, J.-Y. Koh and A. I. Bush, *Nat. Rev. Neurosci.*, 2005, **6**, 449.
11 (*a*) See special issue on Zn(II) chemistry: *BioMetals*, 2001, 14; (*b*) J. M. Berg and Y. Shi, *Science*, 1996, **271**, 1081.
12 A. B. Chausmer, *J. Am. Coll. Nutr.*, 1998, **17**, 109.
13 C. J. Federickson and D. W. Moncrieff, *Biol. Signals*, 1994, **3**, 127.
14 A. Q. Truong-Tran, J. Carter, R. E. Ruffin and P. D. Zalewski, *BioMetals*, 2001, **14**, 315.
15 (*a*) K. Kiyose, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2006, **128**, 6548; (*b*) K. Komatsu, K. Kikuchi, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 10197; (*c*) E. Kawabata, K. Kikuchi, Y. Urano, H. Kojima, A. Odani and T. Nagano, *J. Am. Chem. Soc.*, 2005, **127**, 818; (*d*) K. Hanaoka, K. Kikuchi, H. Kojima, Y. Urano and T. Nagano, *J. Am. Chem. Soc.*, 2004, **126**, 12470.
16 (*a*) C. R. Goldsmith and S. J. Lippard, *Inorg. Chem.*, 2006, **45**, 555; (*b*) E. M. Nolan, J. Jaworski, K. I. Okamoto, Y. Hayashi, M. Sheng and S. J. Lippard, *J. Am. Chem. Soc.*, 2005, **127**, 16812; (*c*) C. C. Woodroofe, A. C. Won and S. J. Lippard, *Inorg. Chem.*, 2005, **44**, 3112; (*d*) E. M. Nolan and S. J. Lippart, *Inorg. Chem.*, 2004, **43**, 8310; (*e*) C. J. Chang, E. M. Nolan, J. J. Jaworski, K. I. Okamoto;, Y. Hayashi, M. Sheng and S. J. Lippard, *Inorg. Chem.*, 2004, **43**, 6774; (*f*) E. M. Nolan, S. C. Burdette, J. H. Harvey, S. A. Hilderbrand and S. J. Lippard, *Inorg. Chem.*, 2004, **43**, 2624; (*g*) C. J. Chang, J. Jaworski, E. M. Nolan, M. Sheng and S. J. Lippard, *Proc. Natl. Acad. Sci. U. S. A.*, 2004, **101**, 1129.
17 (*a*) F. M. Pfeffer, P. Jensen, T. Gunnlaugsson and P. E. Kruger, *Org. Lett.*, 2005, **7**, 5357; (*b*) T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Biomol. Chem.*, 2005, **3**, 48; (*c*) T. Gunnlaugsson, A. P. Davis, G. M. Hussey, J. Tierney and M. Glynn, *Org. Biomol. Chem.*, 2004, **2**, 1856; (*d*) T. Gunnlaugsson, C. T. Lee and R. Parkesh, *Org. Lett.*, 2003, **5**, 4065; (*e*) T. Gunnlaugsson, J. P. Leonard and N. S. Murray, *Org. Lett.*, 2004, **6**, 1557; (*f*) T. Gunnlaugsson, A. P. Davis, J. E. O'Brien and M. Glynn, *Org. Lett.*, 2002, **4**, 2449; (*g*) T. Gunnlaugsson, B. Bichell and C. Nolan, *Tetrahedron Lett.*, 2002, **43**, 4989; (*h*) T. Gunnlaugsson, M. Nieuwenhuyzen, L. Richard and V. Thoss, *J. Chem. Soc., Perkin Trans. 2*, 2002, 141; (*i*) T. Gunnlaugsson, A. P. Davis and M. Glynn, *Chem. Commun.*, 2001.
18 (*a*) K. Senechal-David, J. P. Leonard, S. E. Plush and T. Gunnlaugsson, *Org. Lett.*, 2006, **8**, 2727; (*b*) T. Gunnlaugsson and J. P. Leonard, *Dalton Trans.*, 2005, 3204; (*c*) J. P. Leonard and T. Gunnlaugsson, *J. Fluoresc.*, 2005, **15**, 585; (*d*) T. Gunnlaugsson, A. J. Harte, J. P. Leonard and K. Senechal, *Chem. Commun.*, 2004, 782; (*e*) T. Gunnlaugsson, A. Harte, J. P. Leonard and M. Nieuwenhuyzen, *Supramol. Chem.*, 2003, **15**, 505; (*f*) T. Gunnlaugsson, A. Harte, J. P. Leonard and M. Nieuwenhuyzen, *Chem. Commun.*, 2002, 2134; (*g*) T. Gunnlaugsson, D. A. Mac Dónaill and D. Parker, *J. Am. Chem. Soc.*, 2001, **123**, 12866; (*h*) T. Gunnlaugsson, D. A. Mac Dónaill and D. Parker, *Chem. Commun.*, 2000, 93.
19 T. Gunnlaugsson, M. Glynn, G. M. Tocci (née Hussey), P. E. Kruger and F. M. Pfeffer, *Coord. Chem. Rev.*, 2006, **250**, 3094.
20 We have previously communicated part of this work: T. Gunnlaugsson, T. C. Lee and R. Parkesh, *Org. Biomol. Chem.*, 2003, **1**, 3265.
21 (*a*) T. Gunnlaugsson, P. E. Kruger, P. Jensen, J. Tierney, H. D. P. Ali and G. M. Hussey, *J. Org. Chem.*, 2005, **70**, 10875; (*b*) J. Wang, Y. Xiao, Z. Zhang, X. Qian, Y. Yang and Q. Xu, *J. Mater. Chem.*, 2005, **15**, 2836; (*c*) T. Gunnlaugsson, P. E. Kruger, T. Clive Lee, R. Parkesh, F. M. Pfeffer and G. M. Hussey, *Tetrahedron Lett.*, 2003, **35**, 6575; (*d*) Z. Xu, X. Qian, J. Cui and R. Zhan, *Tetrahedron*, 2006, **62**, 10117.
22 (*a*) D. C. Magri, G. J. Brown, G. D. McClean and A. P. de Silva, *J. Am. Chem. Soc.*, 2006, **128**, 4950; (*b*) X.-M. Meng, M.-Z. Zhu, L. Liu and Q.-X. Guo, *Tetrahedron Lett.*, 2006, **47**, 1559; (*c*) J. N. Ngwendson, C. L. Aniot, D. K. Srivastava and A. Banerjee, *Tetrahedron Lett.*, 2006, **47**, 2327; (*d*) O. Reany, T. Gunnlaugsson and D. Parker, *Chem. Commun.*, 2000, 473.
23 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson, A. J. M. Huxley, C. P. McCoy, J. T. Rademacher and T. E. Rice, *Chem. Rev.*, 1997, **97**, 1515.
24 A. P. de Silva, H. Q. N. Gunaratne, T. Gunnlaugsson and M. Nieuwenhuyzen, *Chem. Commun.*, 1996, 1967.
25 H. He, M. A. Mortellaro, M. J. P. Leiner, R. J. Fraatz and J. K. Tusa, *J. Am. Chem. Soc.*, 2003, **125**, 1468.
26 T. Gunnlaugsson, C. P. McCoy, R. J. Morrow, C. Phelan and F. Stomeo, *Arkivoc*, 2003, 8, 216 http://www.arkat-usa.org/ark/journal-/2003/McKervey/McKervey_index.htm.
27 T. Hirano, K. Kikuchi, Y. Urano, T. Higuchi and T. Nagano, *J. Am. Chem. Soc.*, 2000, **122**, 12399.