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A highly selective and sensitive fluorescent PET (photoinduced electron transfer) chemosensor for Zn(II)†

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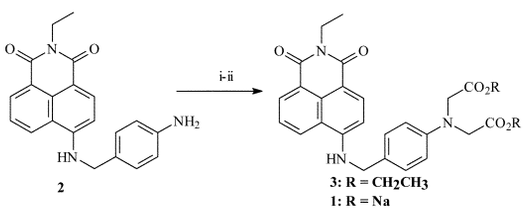
The naphthalimide derivative 1 was designed as a fluorescence PET sensor for Zn(II); 1 showed excellent selectivity for Zn(II) at pH 7.4, even in the presence of other competitive cations, the emission, being pH independent above pH 3.5, was *switched on* upon Zn(II) recognition.

Zinc is one of the most important transition metal ions found in physiology, where it has multiple roles in both extra- and intra-cellular functions.[1] Until recently, Zn(II) was mainly considered as an important component in structural and regulatory cellular functions,[1] however, its importance in DNA synthesis, gene expression, ion channels in neurobiology and in apoptosis has also become apparent.[2] Currently, there is great interest in the development of fluorescent sensors for quantifying and exploring the role of Zn(II) in medicine and biology as well as in the environment.[3–5] A few Zn(II) sensors have been found to have potential physiological applications and some are now commercially available.[6] However, there is still scope for improvement in the design of such sensors as they often suffer form faults such as pH sensitivity, respond to other ions such as Ca^{2+} and Mg^{2+}, use short excitation (λ_{ex}) and emission (λ_{em}) wavelengths and have small Stokes shifts. Furthermore, these sensors often involve lengthy and cumbersome synthesis. We are interested in the development of supramolecular luminescent chemosensors for ions and molecules,[7,8] and have reported the use of Eu(III) and Tb(III) based Zn(II) sensors.[9] However, these only showed moderate luminescence enhancements upon recognition and suffered from short λ_{ex}.

Herein we report the design and synthesis of **1**, a new and highly Zn(II) selective and sensitive fluorescent PET chemosensor, that was synthesised in high yields in a few steps.[10] We chose to use 4-amino-1,8-naphthalimide[11] as the fluorophore reporter in designing **1**, as it absorbs in the visible region and emits in the green, with Stokes shifts of *ca.* 100 nm, as well as being photostable.[11] Importantly, **1** does not respond to Ca^{2+} and Mg^{2+}, and many other transition metal ions. To achieve this selectivity, we used a simple aromatic iminodiacetate receptor, which is separated from the fluorophore with a $-CH_2-$ spacer, is pH independent in the physiological pH range and offers good water solubility. The synthesis of **1** is shown in Scheme 1. We have previously used the naphthalimide starting material **2** in the synthesis of fluorescent PET sensors for anions.[12] The alkylation of the aniline unit of **2** using ethylbromoacetate gave the bis ethyl ester **3** in 90% yield after recrystallisation from ethanol. Base hydrolysis of **3** in a refluxing mixture of aqueous NaOH in ethanol, gave **1** as a yellow solid in 95% yield upon cooling to room temperature. All



2 **3: R = CH_2CH_3** **1: R = Na**

Scheme 1 *Reagents and conditions*: i) $BrCH_2CO_2Et$, KI, K_2CO_3, DMF 90 °C. ii) Ethanol/NaOH.

intermediates and **1** were characterized using conventional methods (ESI).†‡

The ability of **1** to detect Zn(II) and other physiologically, and non-physiologically, relevant cations was carried out at pH 7.4 by observing the changes in the absorption and emission spectra of **1** ([**1**] = 1 μM, 20 mM HEPES buffer in the presence of 0.135 M of NaCl to maintain constant ionic strength). The absorption spectra of **1** showed a broad band in the visible region between 370 and 510 nm, with λ_{max} at *ca.* 442 nm ($\log \varepsilon$ = 4.28), which was assigned to an internal charge transfer excited (ICT) state.[10,11] No significant changes were seen in absorption spectra upon carrying out a spectroscopic pH titration from pH 2–12. The fluorescence emission spectra when excited at 442 nm showed a broad emission band between 480 and 700 nm with λ_{em} max at *ca.* 550 nm. No noteworthy emission changes were seen between pH 5–12, whereas between pH 2–5 the emission was *switched on* with a large order of magnitude enhancement (*ca.* 100 fold) in the fluorescence emission. From these results we conclude that **1** is pH independent in the physiological pH range, with a pK_a of *ca.* 3.1 (±0.1) for the protonation of the amino moiety of the iminodiacetate receptor. The changes in the fluorescence as a function of pH indicate that the fluorescence quenching is due to suppression of PET from the aromatic iminodiacetate receptor to the excited state of the naphthalimide fluorophore.[10,11] Upon protonation of the amino moiety of the receptor the oxidation potential is increased and the thermodynamic pathway for the PET quenching from the receptor is greatly reduced or inhibited.

The changes in the fluorescence emission spectra of **1** at pH 7.4 upon addition of $ZnCl_2$ are shown in Fig. 1. In the absence of Zn(II) the emission was *switched off*, but became gradually *switched on* upon addition of Zn(II). In the absence of the ion (free **1**) the Φ_F was measured to be 0.004 whereas upon addition of 10 μM of Zn(II), Φ_F was 0.21 (bound **1**). Hence, **1** can be considered as being a real luminescent *off–on* switch[7–10] with a Φ_F enhancement of *ca.* 53. From these measurements the binding constant ($\log \beta$) was determined as 3.9 (±0.1). The large fluorescence enhancements are due to the binding of the Zn(II) to the carboxylates of the iminodiacetate and the aromatic nitrogen moiety. In particular, the latter interactions increase the oxidation potential of the receptor with concomitant reduction in the receptor's ability to quench the

† Electronic supplementary information (ESI) available: synthesis, experimental details and ^1H and ^{13}C NMR for **1**, **2** and **3**. UV-Vis Zn(II) titrations, fluorescence titration for pH, Hg^{2+} and Cd^{2+} for **1**. See http://www.rsc.org/suppdata/ob/b3/b309569j/

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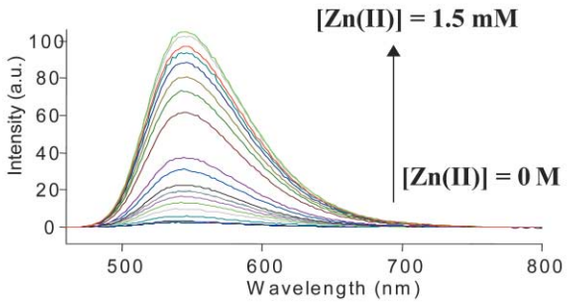

Fig. 1 The changes in the fluorescence emission spectra of **1** ([**1**] = 1 μM) (being *switched on*) upon titration with $ZnCl_2$. [Zn(II)] = 0 ⟶ 1.5 mM, base line is the free sensor, the first addition of Zn(II) is 79 nM.

fluorescence of the naphthalimide moiety *via* PET, in the same way as for H^+ above.[10,11] Furthermore, the sigmoidal appearance of the IF/IF_0 *vs.* −log[Zn] curve indicates a 1 : 1 binding and simple equilibrium (Fig. 2).[7,10] Importantly, our simple design completely inhibits the interference of other physiologically important divalent cations, such as Ca(II) and Mg(II) (Fig. 2) which did not give rise to any changes in the fluorescence emission of **1**. Similar results were seen for Group I, Cu(II) and Co(II), whereas Ni(II), Hg(II) and Cd(II) gave only very minor enhancements at very high concentrations (ESI).† Some of these are shown in the base line of Fig. 2. Furthermore, competitive analysis, where solutions of **1** in the presence of Cu(II), Cd(II) and Hg(II), or Ca(II) and Mg(II) all in the millimolar range, were titrated with Zn(II) under identical conditions as described above, the emission was *switched on* upon Zn(II) recognition. This clearly confirmed the selectivity of **1** towards Zn(II), and, to the best of our knowledge, **1** is the first example of such a Zn-chemosensor to uphold such a strict Zn(II) selectivity.

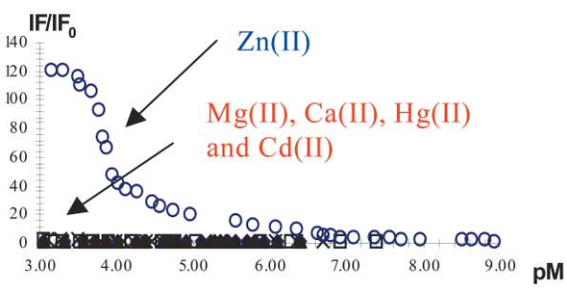

Fig. 2 The changes in the normalized fluorescence emission of **1** at 545 nm as a function of M^{2+} (pM). Only Zn(II) *switched on* the fluorescence upon ion recognition. pM = −log [M], M = Zn(II), *etc.*; IF = Intensity of Fluorescence; IF_0 = IF at [M] = 0 M.

Free Zn(II) concentrations are low in most cells (<1 nM), but high concentrations have been reported in the brain, nervous system and pancreas.[13] In order to determine the ability of **1** to determine the free Zn(II), the above measurements were repeated in the presence of 1.1 mM of EGTA, 20 mM HEPES and 135 mM NaCl.[14] Here the fluorescence emission spectrum was *switched on* in the same way as discussed above (Fig. 3). From these changes the dissociation constant K_d of 80 nM was determined. Hence **1** can be used to determine free Zn(II) concentrations at low levels, in 1 : 1 binding, as demonstrated using a Hill plot, which gave a Hill coefficient of 1.06 (see insert in Fig. 3).

In summary, we have developed a highly selective and sensitive fluorescent chemosensor for Zn(II) that displays excellent selectivity and a large order of magnitude enhancement in the emission upon Zn(II) recognition, at pH 7.4. This high selectivity was obtained using a structurally simple receptor. We are currently evaluating the ability of **1** and related sensors to image Zn(II) in tissues and single cells, and for using **1** in particular to determine [Zn(II)] in apoptosis studies.

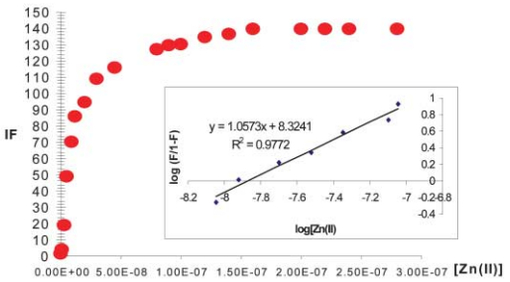

Fig. 3 Changes in the fluorescence emission of **1** (1 μM) at 545 nm as a function of free Zn(II). The insert shows the corresponding Hill plot. The titration (using $ZnCl_2$) was carried out in the presence of 1.1 mM of EGTA, which allows for the determination of free Zn(II).[14]

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Notes and references

‡ **1**: Anal. calc. 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%. **2**: Anal. calc. 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%. **3**: Anal. calc. 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%.

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