"Turn-on" fluorescent sensor for the selective detection of zinc ion by a sterically-encumbered bipyridyl-based receptor†

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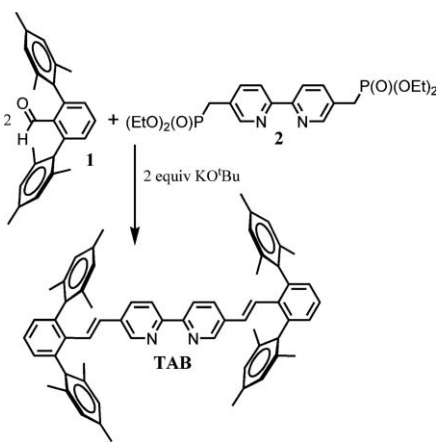
A sterically-encumbered 5,5′-distyryl-2,2′-bipyridyl derivative that enforces a 1 : 1 metal-to-ligand ratio acts as a selective turn-on sensor for Zn^{2+} in THF.

Bipyridyl derivatives are the most-employed ligands in coordination chemistry.[1] The widespread utility of these ligands stems from their facile preparation/functionalization, stability and ability to bind a wide array of d- and f-block elements. However, metal ion promiscuity is a drawback when a selective binding site is desired, as in the development of metal ion sensors. The development of practically-applicable colorimetric or fluorescence-based sensors is predicated upon unique optical signal transduction resulting from a given analyte.[2–11] In addition to binding a variety of metal ions more or less indiscriminately, a chemosensor based on a reporter-derivatised bipyridyl ligand **L** may undergo an optical response upon formation of an $\mathbf{L}$–M^{n+} complex. However, the interpretation of sensor response can be complicated when two or more **L** units bind to a single metal ion, producing equilibrium mixtures of $\mathbf{L}_x M^{n+}$, $\mathbf{L}_{x-1} M^{n+}$, *etc.*, each of which will exhibit its own optical signature. Consequently, chemosensors tend to employ receptors designed to provide selective binding of the target analyte in a 1 : 1 ratio through an appropriate combination of size/shape and electronics.[12–26] Metal ion sensors, for example, typically comprise metal-specific multidentate ligands tethered to a reporting functionality. For example, 2,2′-bis(2-picolyl)amino derivatives are the ligand of choice for Zn^{2+},[17–23] while most commercial Ca^{2+} biosensors are based on bis(2-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA).[24–26] Although highly effective, such sensors often require laborious multi-step organic syntheses for their assembly. This slows the discovery process and leads to the prohibitively high cost of some commercial biosensor dyes. Rather than using metal-specific receptors, we have targeted sensors employing the easily-derivatised 2,2′-bipyridyl (bipy) ligand, whereupon sterically encumbered ligands are strategically positioned to enforce the 1 : 1 ligand to metal ratio desired for chemosensor applications. As a first step towards this goal, we report a Zn^{2+} sensor utilizing a tetraaryldistyrylbipyridine ligand (**TAB**; Scheme 1, bottom). This was an obvious target due to the well-known ionochromic effect of Zn^{2+} and other metals on bipy derivatives.[27–40] Ion binding leads to a red shift in absorption and emission maxima in response to planarization of the bipyridyl unit, extending the effective conjugation length, in conjunction with perturbation of the electronic system. Although many transition metals induce such a response, a selective fluorescence response to Zn^{2+} can be achieved because Zn^{2+} has a closed-shell d^{10} configuration and is diamagnetic; thus fluorophore-appended ligands tend to remain emissive. In contrast, other metal ions likely to be encountered in the environment, such as Fe^{3+} and Cu^{2+}, are paramagnetic and typically efficient emission quenchers. Heavier d^{10} ions (Cd^{2+} and Hg^{2+}) also tend to yield less emissive complexes due to quenching *via* the classic heavy atom effect.

A *meta*-terphenyl moiety was selected as a steric shield for the chromophore-derivatised bipy ligand. These readily-prepared bulky ligands have found use in a range of contexts, particularly for providing the kinetic stabilization of reactive species,[41–47] culminating in the isolation of the first molecule featuring a quintuple bond.[48] More recently, *meta*-terphenyls have found use in catalysis[49,50] and as steric shields to prevent close intra/interchain contacts in π-conjugated oligomers and polymers.[51] In the current example, Horner–Wittig condensation of *meta*-terphenyl precursor **1** and bipy-containing **2** produced **TAB** as a tan solid (λ_{max} = 370 nm) with a bright blue emission (λ_{em} = 414 nm, Φ_{370} = 0.62(2), τ = 12 ns).‡

As a first step towards applying this simple molecule to metal ion sensing, the absorption and emission spectral responses of **TAB** to excess metal ions was investigated. Significant bathochromic perturbation ($\Delta\lambda_{\pi-\pi^*}$ up to 25 nm) of λ_{max} for the putative π–π* transition of **TAB** was affected by Zn^{2+} (395 nm), Cd^{2+} (395 nm), Hg^{2+} (395 nm), Cu^{2+} (405 nm) and Ag^{+} (376 nm) (Fig. 1(a)). With a λ_{ex} of 370 nm, notable emission quenching without shifting λ_{em} was afforded by Co^{2+}, Cu^{2+}, Cu^{+} and Ag^{+}



Scheme 1

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† Electronic supplementary information (ESI) available: Experimental details for the synthesis and spectroscopic studies, ^{1}H and ^{13}C NMR spectra, absorption and emission spectra, and a Job plot for the Zn^{2+} titration. See DOI: 10.1039/b710740d

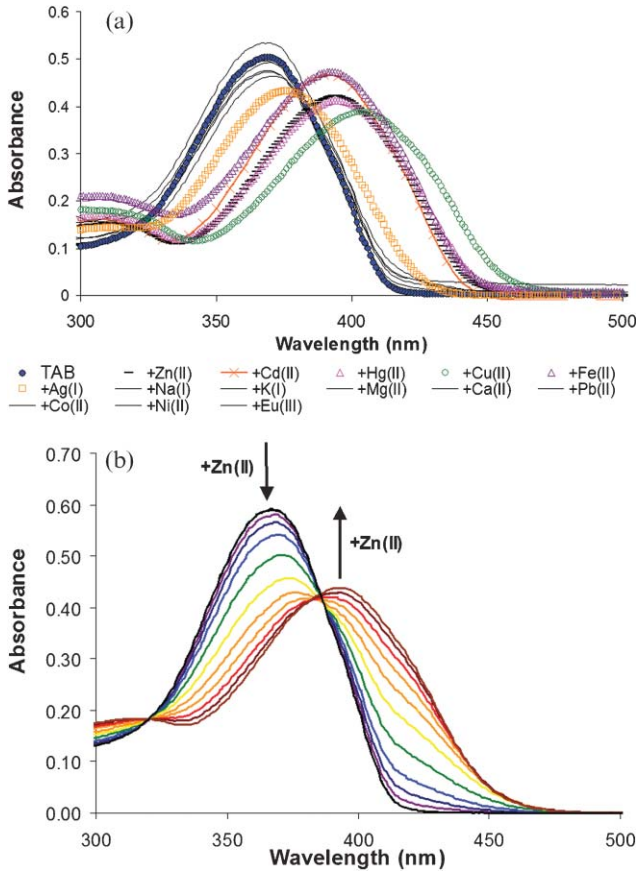

Fig. 1 (a) UV-vis spectra of **TAB** alone, and in the presence of 10 equiv. of the indicated metal ions. (b) Progressive changes in the absorption spectrum of **TAB** observed upon addition of Zn^{2+} (each successive trace represents the addition of an additional 0.1 equiv. of $Zn(ClO_4)_2$).

Table 1 Dissociation constants for 1 : 1 **TAB**–M^{n+} complexes in THF, and the relative emission quenching/enhancement afforded by 1 equiv. of metal ions with λ_{ex} = 370 nm or 420 nm (I and I_o are integrated emission intensity with and without added metal ions, respectively)

Ion	K_d/μM	I/I_o (λ_{ex} = 370 nm)	I/I_o (λ_{ex} = 420 nm)
Fe^{2+}	—	0.02	1.0
Co^{2+}	0.92	0.05	1.0
Cu^{2+}	1.0	0.02	1.0
Zn^{2+}	1.8	0.39	21
Cd^{2+}	2.2	0.44	4.9
Hg^{2+}	—	0.14	3.8

(Table 1), while a marked red shift of 72 nm in λ_{em}, from 414 nm for **TAB** to 486 nm for the **TAB**–M^{2+} complex, was produced by the divalent d^{10} ions Zn^{2+}, Cd^{2+} and Hg^{2+}.

Following trials with excess metal ions, the response of **TAB** to ions that produced significant spectral changes were further investigated. Titrations of **TAB** with select metal ions were followed by UV-vis and/or fluorescence spectroscopy to determine the M : **TAB** binding ratio and dissociation constants (K_d, Table 1). These data were derived on the basis of Job and Benesi–Hildebrand analyses (see ESI†). For Zn^{2+}, the fluorescence (K_d = 1.1 × 10^{-6} M) and absorption (K_d = 1.8 × 10^{-6} M) spectroscopic data are in good agreement with one another (the value from the absorbance is given in Table 1 due to its better linear fit; see ESI Fig. S12†) and lie within the range displayed by probes used for cellular imaging, though **TAB** itself is not water soluble and thus not appropriate for cellular imaging in its current form. The K_d of **TAB**–Zn^{2+} is slightly lower than the value determined for the unfunctionalized bipy–Zn^{2+} complex (4.6 × 10^{-6} M). This observation indicates that binding is not hindered by the added steric bulk in **TAB**, a benefit of positioning the flanking mesityl groups well-removed from the metal chelation pocket.

The unique spectroscopic responses of **TAB** to d^{10} ions led us to explore the response of **TAB** to these ions in more detail. Because the absorption maximum shifts significantly upon binding these ions, we first examined the emission response of **TAB** to various metal ions under excitation at 420 nm, where **TAB** complexes with d^{10} metals absorb strongly but where metal-free **TAB** absorbs very little. When excitation is provided at 420 nm, an immediate 21-fold increase in integrated emission intensity is evident upon addition of 1 equiv. of Zn^{2+}. The **TAB**–Zn^{2+} complex maintains a high Φ_{420} of 0.48 (Φ_{420} for **TAB** is <0.05). The quantum yield and relative turn-on for **TAB** and **TAB**–Zn^{2+} are within the range typical of commercially available sensors that have proved valuable for imaging neuronal Zn^{2+} in living tissue.[18,19,52–54] Furthermore, the ability to observe emission turn-on in response to Zn^{2+} is not hindered by biologically ubiquitous ions such as Na^+, K^+, Mg^{2+} or Ca^{2+}. However, in the presence of 1 equiv. of Fe^{2+}, Co^{2+}, Ni^{2+} or Cu^{2+}, the Zn-induced emission enhancement is only about half as efficient as in the absence of these ions (~10-fold turn-on rather than the 21-fold response observed in their absence). Other biological Zn^{2+} sensors have exhibited similarly depressed responses due to competition from these ions.[18,19,52–54]

In conclusion, a selective turn-on fluorescence sensor for Zn^{2+} has been accomplished using a simple bipyridyl derivative. Steric protection of the bipyridyl binding site enforces the preferential formation of a 1 : 1 metal–ligand complex. Efforts are under way to prepare water-soluble analogues for better biocompatibility, and π-conjugated polymers featuring related sterically-encumbered bipyridyl binding sites for the improved sensitivity and red-shifted absorption/emission desired for bioimaging.

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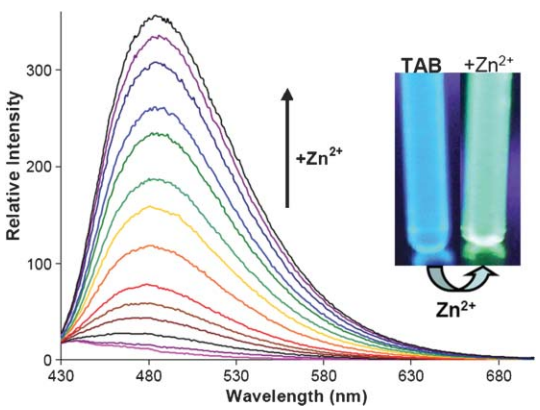

Fig. 2 Progressive increase in fluorescence observed upon the addition of Zn^{2+} (each successive trace represents the addition of an additional 0.1 equiv. of $Zn(ClO_4)_2$ (λ_{ex} = 420 nm)). Inset: visible response of **TAB** to Zn^{2+} (under 365 nm UV light).

Notes and references

‡ Here, λ_{max} is the absorption maximum, λ_{em} is the emission maximum, Φ_{370} is the quantum yield of emission upon excitation at 370 nm, and τ is the lifetime of emission.

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