pH-Regulated "Off–On" fluorescence signalling of d-block metal ions in aqueous media and realization of molecular IMP logic function†

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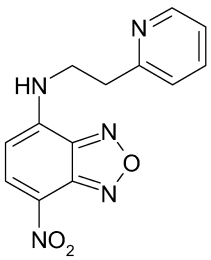
The potential of an anionic receptor moiety in "Off–On" fluorescence signalling of d-block metal ions has been exploited with the demonstration of a two-input IMP function.

Design and development of fluorescence "Off–On" chemosensors for various neutral and ionic analytes is an area of considerable current interest because of its biological and environmental importance.[1] The most commonly used design principle for metal ion fluorosensors is based on the photoinduced electron transfer (PET) mechanism wherein a neutral amino functionality serves as the metal-host (receptor) and also communicates with the fluorophore to report the arrival of the guest.[2] In this context, transition metal ions as analytes pose an inherent challenge because of their notorious fluorescence quenching abilities.[3] While this problem has been addressed by utilizing an electronically deficient fluorophore or a macrocyclic receptor in the sensor system,[4] the major drawback of most of these systems is their inability to perform in aqueous media presumably due to the fact that water molecules compete with the receptor moiety of the sensor system for coordination.[5]

Encouraged by the success of the protonated amine- and transition metal-based receptors in optical sensing of anions[6] and recognizing the fact that the anionic receptors are more suitable compared to their neutral counterparts for binding the positively charged transition metal ions in aqueous solution, we thought that metal ion recognition in water could be made efficient by employing an anionic (deprotonated amine) binding site in the sensor system. Although the coordination chemistry of transition metal ions with anionic receptors is rather extensive, surprisingly, this concept of utilization of an anionic receptor moiety for transition metal ion signalling has not been actively exploited so far perhaps due to the fact that amino hydrogen atoms are not acidic and cannot be easily deprotonated under mild conditions. However, since this problem can easily be overcome by having a strong electron acceptor group in conjugation with the amino nitrogen, we have designed the sensor system **1** in which the 4-amino hydrogen can easily be abstracted generating an *in situ* anionic binding site for the transition metal ions. A pyridine moiety is purposely built in the system to facilitate the binding process. The fluorescence of **1** is switched "off" in alkaline media on deprotonation of the 4-NH proton. However, addition of transition metal ions results in complete recovery of fluorescence due to the formation of a strong complex between the two. The spectral features of **1**, however, remain unaffected in the presence of transition metal ions in neutral or acidic media. Very importantly, these events yield the first molecular IMP logic gate.



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Sensor **1**, which is based on the 4-amino-7-nitrobenzoxa[1,3]diazole (ANBD) fluorophore, has been synthesized by mixing ethyl acetate solutions of 2-(2-aminoethyl)pyridine (0.2 ml, 1.65 mmol) and 4-chloro-7-nitro-benzoxa[1,3]diazole (0.3 g, 1.5 mmol) at 0–5 °C and subsequent stirring of the reaction mixture for 2 h at room temperature. The solvent was removed under vacuum, the crude product was purified by column chromatography (basic alumina, 50% ethyl acetate–hexane) and characterized using conventional methods‡ and X-ray crystallography.[7] The absorption and steady-state corrected fluorescence spectra were recorded on a Shimadzu spectrophotometer (UV-3101 PC) and a spectrofluorimeter (FluoroLog-3, Jobin Yvon), respectively. The absorption and fluorescence spectra of **1** at pH 7.4 (10 mM HEPES) consist of broad structureless bands centered at 480 nm and 558 nm, respectively (Fig. 1) attributable to the intramolecular charge transfer (ICT) transition between the amino and nitro groups of the fluorophore.[8] Even though system **1** bears an architecture (*fluorophore–spacer–receptor*) similar to that of commonly used photoinduced electron transfer (PET) sensor systems,[9] PET quenching communication between the pyridyl and ANBD moieties is not significant in the system. This is evident from the fact that the fluorescence quantum yield (ϕ_f, 0.05) of **1** is comparable to that of the parent fluorophore (ϕ_f, ANBD = 0.06). The lack of PET interaction in **1** is consistent with the observations made in similar systems.[10] In fact, we

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† Electronic supplementary information (ESI) available: Absorption and fluorescence titration spectra of **1** with acid, base and a few metal ions, binding constant and quantitative information on the fluorescence response of **1** with different metal ions. See 10.1039/b607642d

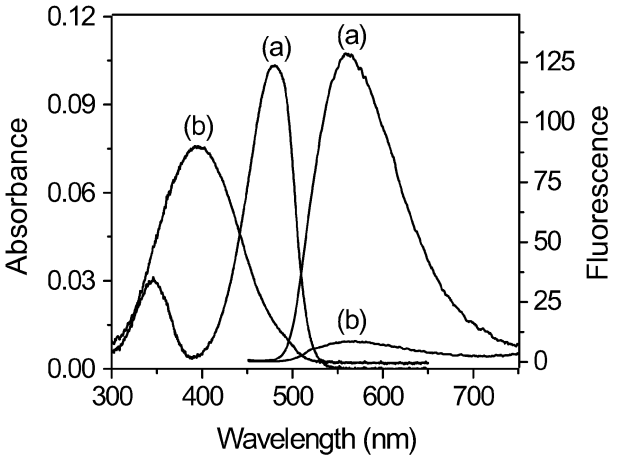


Fig. 1 Absorption and fluorescence spectra ($\lambda_{exc.}$ = 440 nm) of **1** in aqueous solution (a) at pH = 7.4 (b) at pH = 10.7.

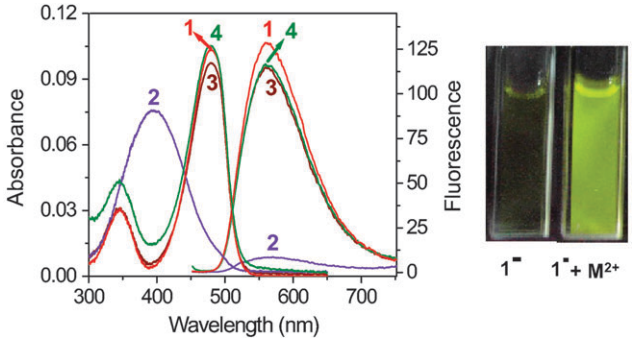


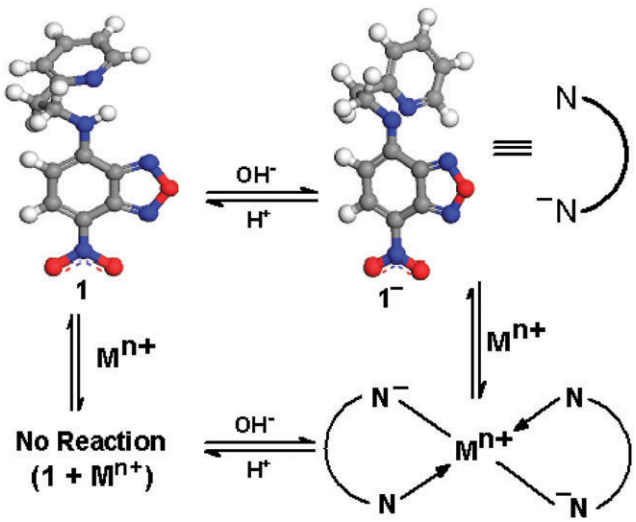
Fig. 2 Absorption and fluorescence spectra (λ_{exc} = 440 nm) of **1** in aqueous solution under different input conditions: (1) none, pH = 7.4 (2) only OH^-, pH = 10.7; (3) Zn^{2+}, pH = 7.4; (4) OH^- (pH = 10.7) and Zn^{2+}. Naked eye observation of the effect of metal ions on the fluorescence output of **1** at pH = 10.7 is also shown.

chose the pyridyl moiety as an additional binding site for it does not enter into communication with the fluorophore.

The absorption and fluorescence spectra of **1** are slightly dependent on pH in the neutral and acidic media. A small shift ($\sim$400 cm^{-1}) of the absorption maximum accompanied by small changes in the absorption and fluorescence intensities between pH 3–5 can be ascribed to the protonation of the pyridyl nitrogen atom (pK_a = 3.9).[11] However, the spectral features of **1** are strongly dependent on pH between pH 9.2 and 10.6 due to the deprotonation of the 4-NH proton leading to the formation of the anionic species (ESI†). The formation of the anionic species is associated with the appearance of a new absorption band at 395 nm at the expense of the 480 nm band (Fig. 1). That the fluorescence of the system is significantly quenched and no new fluorescence band is observed suggest that the deprotonated form of **1** is very weakly fluorescent or nonfluorescent (Fig. 1). In this connection, we note that recently Xu and coworkers have observed emission from a deprotonated species at a longer wavelength compared to the parent system and has suggested this as a general strategy for colorimetric and ratiometric fluorescence signalling purpose.[12] However, since a deprotonated fluorophore is a completely new chemical entity, all deprotonated species need not be fluorescent. The pK_a values for the deprotonation event evaluated from the absorbance and fluorescence changes are 10.4 ($\pm$0.1) and 10.6 ($\pm$0.1), respectively.[11]

The effect of the d-block metal ions on the absorption and fluorescence behaviour of the systems at neutral (pH = 7.4) and mildly alkaline media (pH = 10.7, carbonate–bicarbonate buffer, 100 mM KCl) is quite interesting. Addition of the metal perchlorate salts in neutral aqueous solution of **1** does not result in any significant change of the absorption or fluorescence spectrum of **1** (Fig. 2) indicating poor affinity of the system for the transition metal ions. This is not surprising considering the fact the electron density at the 4-N atom, which is in conjugation with the electron withdrawing nitro group, is low,[8] and that there is very little communication between the pyridyl moiety and the fluorophore. Interestingly, addition of d-block metal ions in alkaline media (pH = 10.7) results in the recovery of the 480 nm absorption band as well as the fluorescence of the system (Fig. 2). In highly alkaline aqueous media, metal salts of Ni^{2+} and Cu^{2+} are known to form precipitates of their hydroxides and those of Zn^{2+} form $[Zn(OH)_4]^{2-}$. However, at pH = 10.7, no precipitation could be observed and this observation is consistent with the literature.[13] The pyridyl moiety of **1** is a cation receptor and under conditions such as in basic media the deprotonated 4-N atom along with the pyridine nitrogen atom forms strong complexes with transition metal ions (Scheme 1). Thus, in the presence of transition metal ions, the spectral features of the system are remodulated to the original. A typical absorption and fluorescence titration of **1** at pH = 10.7 with the metal salt is illustrated in Fig. 3. The effect is quite similar for different transition metal ions (see ESI). The binding constant values for various transition metal ions are evaluated (ESI for details) to be in the range of 1–5 $\times$ 10^3 M^{-1}.

An important perspective of the fluorescent signalling systems is to establish their input–output relationships in terms of logic gate operations.[14] For applications, such as molecule-



Scheme 1 Proposed scheme for the molecular states after various logical events. The optimized molecular structure of **1** and its deprotonated form $\mathbf{1}^-$ generated using MS Modeling (version 3.0) are shown.

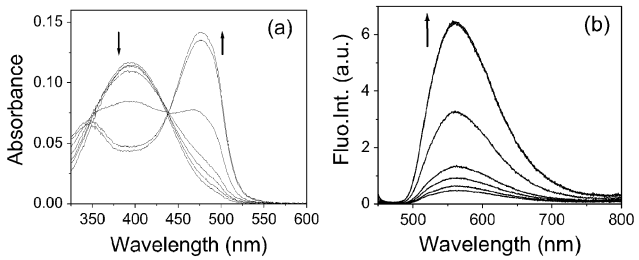


Fig. 3 Typical changes in the absorption (a) and fluorescence (b) spectra of **1** (4.9×10^{-6} M) upon subsequent addition of metal salts. Data shown in the plot are for Ni^{2+}; $[Ni^{2+}] = 1.8 \times 10^{-4} - 7 \times 10^{-4}$ M.

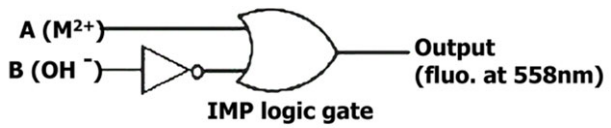


Fig. 4 Logic circuit for the two-input IMP gate.

based logic circuitry, the devices are molecular analogues of those based on the conventional silicon-based circuitry.[14] The logic gate implementation of the present events is shown in Fig. 4 and the corresponding fluorescence output produces the truth table (Table 1). High fluorescence output is produced under all circumstances except in the presence of *only* base. This behavior can be conveniently described using logic notation, A + B′ (implication or IMP function),[15] where A and B represent input concentrations of the metal ions and the base, respectively. While different molecular logic functions, including the INH function, based on the chemical inputs and optical outputs have been demonstrated previously,[16] the IMP logic function, which is the complement of the INH function,[15] has remained elusive.

In summary, we have shown that *in situ* generation of an anionic binding site in an ICT fluorophore offers fluorescence detection of transition metal ions in aqueous media, not otherwise possible with the neutral species. The concept described here can easily be extended for the construction of practical sensor systems for metal ions in aqueous solution utilizing specific ionophores. The ICT-based system reported herein is an example of a two-input IMP molecular logic gate.

Table 1 Truth table for IMP logic operation of **1**[a]

Input		Output
A (M^{2+})	B (OH^-)	Fluorescence (ϕ_f)
0	0	1 (0.051)
0	1	0 (0.003)
1	0	1 (0.046)[b]
1	1	1 (0.046)[b]

[a] 1×10^{-5} M. 0 and 1 represent zero and maximum input concentrations, respectively. The maximum concentration for OH^- is 2×10^{-3} M and for M^{2+} is 1×10^{-3} M. Samples excited at 440 nm, fluorescence maximum is at 558 nm. [b] These values are for Zn^{2+} as metal ion input.

Acknowledgements

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‡ Yield 90%. CHN analysis calculated for $C_{13}H_{11}N_5O_3$: C, 54.74; H, 3.89; N, 24.55. Found: C, 54.10; H, 3.85; N, 24.44%; ^{1}H NMR ($CDCl_3$): 3.24 (t, 2H), 3.90 (t, 2H), 6.17 (d, 1H), 7.24 (m, 2H), 7.67 (m, 1H), 8.09 (s, 1H), 8.47 (s, 1H), 8.63 (d, 1H); IR: 3200 (cm^{-1}) (NH, stretch); LCMS *m*/*z* (%) 286 (100) $[M + 1]^+$

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7 Crystallographic data for **1** were collected on a Bruker Smart CCD diffractometer at 298 K using graphite monochromated Mo Kα radiation (λ = 0.710 73 Å). Selected crystal data for **1**: Selected crystallographic data: $C_{13}H_{11}N_5O_3$: *M* = 285.26; orthorhombic, space group *Pbca*; cell dimensions *a* = 13.560(11) Å, *b* = 7.385(6) Å, *c* = 26.06(2) Å, *V* = 2609(4) Å^3, *Z* = 8, ρ_{calc} = 1.452 g cm^{-3}, μ (Mo-Kα radiation) = 0.108 mm^{-1}, λ = 0.710 73 Å, *T* = 293 K. Reflections collected: 23234 (CCD area detector diffractometer), 2322 unique, 193 parameters refined using 1221 reflections with $I > 2\sigma(I)$ to final *R* indices: *R*1 = 0.0567, w*R*2 = 0.1495, GOF = 0.950. CCDC reference number 615587. For crystallographic data in CIF or other electronic format see DOI: 10.1039/b607642d.
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