'Off–On' Fluorescent Sensors for Physiological levels of Magnesium Ions based on Photoinduced Electron Transfer (PET), which also behave as Photoionic OR Logic Gates

A. Prasanna de Silva, H. Q. Nimal Gunaratne and Glenn E. M. Maguire

School of Chemistry, Queen's University, Belfast, Northern Ireland, BT9 5AG

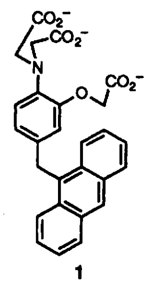
The fluorescence of molecules **1–3** is enhanced by factors of up to 67 in the presence of magnesium and calcium ions in neutral water which allows the selective monitoring of magnesium ions under simulated physiological conditions and permits the construction of truth tables with OR logic when these molecules are viewed as ion input–photon output molecular devices.

The fluorescent sensing of the ionic prime movers of life processes is proving to be a major chemical contribution to biomedical research[1]. Molecular information processing is another area to which chemists can provide significant input[2,3]. Recently, we showed how ion-operated fluorescent switches can give rise to photoionic logic gates.[4] We now demonstrate that a single family of fluorescent signalling molecules **1–3**† possesses properties that can be put to use in both of these research areas despite their apparent diversity. The key to this dual exploitation lies in the selectivity of London's receptor **4a**[5] for magnesium *vs.* calcium or protons being poor in the absolute sense of coordination chemistry (binding constants, log β = 3.1, 4.6 and 5.5, respectively),[5] which is, however, sufficient for essentially complete selection of magnesium *vs.* calcium or protons when these three ions are constrained to their physiological ranges (10^{-3}, 10^{-7} and 10^{-7} mol dm^{-3}‡ respectively.[6]

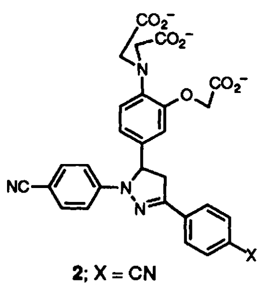
Molecules **1–3** have been designed as fluorescent PET signalling systems[7,8] by grafting fluorophores with chosen optical and redox properties onto London's parent receptor **4b** *via* a spacer unit. The fluorescence of such 'fluorophore–spacer–receptor' systems are 'switched off' in the absence of cation input as evidenced by the very low quantum yields (Φ_{Fmin} values in Table 1). Quenching of fluorescence is caused by a PET process from the 1-amino, 2-alkoxy benzene unit to the fluorophore.[9] The availability of protons, calcium or magnesium ions in sufficient concentrations to bind to the receptor unit of **1–3** increases the oxidation potential of the latter and suppresses the PET process, which results in the 'switching on' of fluorescence. Moderate to large factors of fluorescence enhancement (FE = Φ_{Fmax}/Φ_{Fmin}) are found at saturating cation levels. Analysis of the cation dependence of fluorescence spectra according to eqn. (1),[7] which the data fit well, gives the corresponding cation binding constants. The log β values for calcium ions (4.9–5.1) and protons (5.8–6.3)

$$\log[(\Phi_{Fmax} - \Phi_F)/(\Phi_F - \Phi_{Fmin})] = pM - \log\beta \quad (1)$$

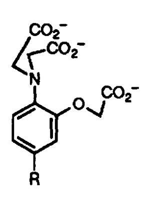
are larger than those for magnesium (2.8–3.0) *i.e.* the fluorescence of **1–3** is most sensitive to changes in pCa, pH and pMg values around 5, 6 and 3, respectively. pCa, pH and pMg levels encountered in many types of living cells are around 7, 7, and 3, respectively.[6] Thus, the fluorescence of **1–3** will be responsive to physiological levels of magnesium but not to those of protons or calcium ions.[10] Such sensitivity is important in view of the multi-faceted biological roles of magnesium ions.[11]



1



2; X = CN
3; X = SO$_2$Me



4a; R = F
b; R = H

Direct fusion of π electron systems have previously led to integrated fluorescent sensors containing London's receptor which respond to magnesium ions with wavelength shifts of fluorescence spectra which in turn allow ratiometric monitoring at two wavelengths.[12] In contrast, **1–3** respond by means of large increases in the fluorescence quantum yield without significant changes in band position or shape which correspond to 'off–on' action. The large fluorescence enhancements allow powerful visual demonstrations[13] of magnesium sensing in neutral water. This should also permit sensitive monitoring of pMg status in cell populations. In spite of the lack of ratiometric capability, the availability of strong 'off–on' action in **1–3** can potentially allow pMg imaging in single cells at least semiquantitatively as has been shown with certain 'off–on' sensors in the case of calcium monitoring.[14]

Table 1 Electronic spectral data and ion complexation properties of **1–3**[a]

Property	**1**	**2**	**3**
$\lambda_{AbsS_0\rightarrow S_1}$/nm ($\varepsilon_{max}$/dm^3 cm^{-1} mol^{-1})	387 (7100)[b] 369 (7600) 351 (5100)	389 (27000)	388 (32000)[c]
λ_{ex}/nm	368[b]	389	389[c]
λ_{Flu}/nm	393[b] 416 441	490	490[c]
Φ_{Fmin}	0.016	0.012	0.0042
$\Phi_{FmaxMg^{2+}}$($FE_{Mg^{2+}}$)	0.091[b] (6)	0.24 (20)	0.24[c] (57)
$\Phi_{FmaxCa^{2+}}$($FE_{Ca^{2+}}$)	0.13[b] (8)	0.29 (24)	0.28[c] (67)
$\Phi_{FmaxH^{+}}$($FE_{H^{+}}$)	0.15[b] (9)	0.10 (8)	0.091[c] (22)
log $\beta_{Mg^{2+}}$[d]	2.9	3.0	2.8
log $\beta_{Ca^{2+}}$[d]	5.1	5.0	4.9
log $\beta_{H^{2+}}$[d]	6.3	5.8	5.9

[a] 5×10^{-6} mol dm^{-3} **1–3** in water at pH 7.3 [morpholinopropane sulfonic acid (MOPS) buffer]. pMg and pCa values maintained with metal buffers containing nitrilotriacetic acid (NTA), EDTA and ethylene glycol bis(aminoethyl ether) tetraacetic acid (EGTA). EDTA was also used to maintain solutions in a magnesium-free or calcium-free state when necessary. pH variations obtained with phosphate buffers. 9,10 diphenyl anthracene in methanol served as a standard for the determination of fluorescence quantum yield(Φ_F) values from corrected spectra.[20] Φ_{Fmax} and Φ_{Fmin} refer to the maximum and minimum values of fluorescence quantum yields attained during the variation of pMg, pCa and pH values. [b] The parent fluorophore of **1** is suitably modelled by *N*-[4-(9′-anthracenyl methyl) phenyl] iminodiacetic acid which has λ_{abs} = 388, 368 and 350 nm, ε_{max} = 8900, 9500 and 6100 dm^3 cm^{-1} mol^{-1}, λ_{Flu} = 393, 415 and 439 nm and Φ_F = 0.50 in water at pH 1.0.[9] [c] The parent fluorophore of **3** is suitably modelled by 1-(4′-cyanophenyl), 3-(4″-methylsulfonylphenyl), 5-(4‴-carboxyphenyl)-2-pyrazoline which has λ_{abs} = 388 nm, ε_{max} = 29700 dm^3 cm^{-1} mol^{-1}, λ_{Flu} = 490 nm and Φ_F = 0.54 in water at pH 7.3.[9] [d] The parent receptor of **1–3** is most reasonably modelled by **4b**, which has log $\beta_{Mg^{2+}}$ = 3.1, log $\beta_{Ca^{2+}}$ = 5.1 and log β_{H^+} = 5.5 as determined according to the absorptiometric equivalent of eqn. (1), $\text{Log}[(OD_{max} - OD)/(OD - OD_{min})] = pM - \log\beta$.[21] OD is the optical density of **4b** at a chosen wavelength which is controlled by the concentration of the chosen cation. **4a** is another model receptor available in the literature[5] which has log $\beta_{Mg^{2+}}$ = 3.1, log $\beta_{Ca^{2+}}$ = 4.6 and log β_{H^+} = 5.5.

The design of **1–3** according to the 'fluorophore–spacer–receptor' format also means that their optical and ion-sequestration properties are nearly quantitatively inherited from the parent fluorophore and receptor units. Table 1 provides several examples of such 'heredity' arising out of the segregatory aspect of the spacer.[7]

For a given sensor, the fluorescence enhancements caused by calcium and magnesium ions are rather similar, differing by less than a factor of 1.3 (Table 1). Indeed, **3** gives essentially identical responses. This has important implications for the use of **1–3** as two-input photoionic OR logic systems. Photoionic logic gates have been developed in an attempt to emulate the input–output behaviour of their solid-state electronic counterparts while being demonstrably operable as molecular entities with straightforward interfacing of the various input and output signals and power supply to the device.[4] A productive strategy to approach reasonable OR logic gates would be to employ ion-induced conformational changes of flexible systems to control fluorescence switching rather than mere ion proximity as has been mainly done so far.[4] When the latter is the sole design basis, the degree of fluorescence 'switching on' will be controlled by the effective charge density of the ion. Hence the 'high' level of fluorescence output will be dependent on the nature of the input ion,[15] which is undesirable for photoionic OR logic. On the other hand, both magnesium and calcium ions twist the amine electron pair *ca.* 90° away from the π electrons of the aromatic ring upon coordination[16] to **1–3**. Thus, fluorescence recovery by PET suppression can be similarly effective for both ion inputs with any minor differences being due to the ion proximity effect. The output Φ_F value of *e.g.* **3** is 0.0042, 0.28, 0.24 and 0.28 in the presence of no Mg^{2+} or Ca^{2+}, 10^{-3} mol dm^{-3} Ca^{2+} only, 0.5 mol dm^{-3} Mg^{2+} only and 0.5 Mg^{2+} + 10^{-3} mol dm^{-3} Ca^{2+}, respectively. This represents, in chemical terms, a good quality truth table for the OR logic function of **3**. It is notable that the operating levels of 'high' and 'low' input ion signals are set for optimal performance of the logic device. Now **3** (and its relatives **1** and **2**) becomes essentially non-selective as far as the dication-induced fluorescence output is concerned. We are currently attempting to generalize the capabilities of molecules **1–3** to other systems with respect to the type of logic function, the nature and the operating levels of the ion input signals and the colour of the fluorescence output signal.

We thank the SERC, Queen's University and T. R. H. Warner for support and help.

Received, 2nd November 1993; Com. 3/06552I

Footnotes

† The trimethyl ester of **4b** was synthesized by a procedure somewhat analogous to that used for the corresponding derivative of **4a**.[5] This was then reacted with 9-anthracenyl methyl bromide ($ZnBr_2$ catalyst)[17] or formylated under Vilsmeir–Haach conditions,[18] condensed with 4-cyano- or 4-methylsulfonyl-acetophenone under alkaline conditions and then converted to 2-pyrazolines with 4-cyano phenyl hydrazine in acetic acid.[19] Sensors **1–3** were then obtained by alkaline hydrolysis of the corresponding trimethyl esters. The ^1H NMR (300 MHz: $CDCl_3$) spectral data for these trimethyl esters are: (1): δ 6.54–8.45 (m, 12H, ArH), 4.90 (s, 2H, $ArCH_2Ar$), 4.68 (s, 2H, OCH_2), 4.18 (s, 4H, NCH_2), 3.73 (s, 3H, $OCH_2CO_2CH_3$), 3.68 [s, 6H, $N(CH_2CO_2CH_3)_2$]. (2): δ 6.73–7.81 (m, 11H, ArH), 5.34 (dd, 1H, N-CH, *J* 5.8, 12.3 Hz), 4.54 (s, 2H, OCH_2), 4.15 (s, 4H, NCH_2), 3.81 (dd, 1H, HC–C=N, *J* 12.3, 17.5 Hz), 3.69 [s, 6H, $N(CH_2CO_2CH_3)_2$], 3.62 (s, 3H, $OCH_2CO_2CH_3$), 3.20 (dd, 1H, HC–C=N, *J* 5.8, 17.5 Hz). (3): δ 6.60–7.90 (m, 11H, ArH), 5.30 (dd, 1H, N–CH, *J* 5.7, 12.2 Hz), 4.56 (s, 2H, OCH_2), 4.16 (s, 4H, NCH_2), 3.85 (dd, 1H, HC–C=N, *J* 12.3, 17.4 Hz), 3.71 [s, 6H, $N(CH_2CO_2CH_3)_2$], 3.66 (s, 3H, $OCH_2CO_2CH_3$), 3.18 (dd, 1H, HC–C=N, *J* 5.9, 17.4 Hz), 3.10 (s, 3H, SO_2Me).

‡ All the ion concentrations referred to are free ion concentrations.

References

1 R. Y. Tsien, *Am. J. Physiol.*, 1992, **263**, C723.

2 J.-M. Lehn, *Angew. Chem., Int. Ed. Engl.*, 1988, **27**, 89; 1990, **29**, 1304.

3 *Molecular electronic devices*, ed. F. L. Carter, R. E. Siatkowski and H. Wohltjen, North Holland, Amsterdam, 1988.

4 A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Nature*, 1993, **364**, 42.

5 L. A. Levy, E. Murphy, B. Raju and R. E. London, *Biochemistry*, 1988, **27**, 4041.

6 R. Y. Tsien, *Biochemistry*, 1980, **19**, 2396.

7 R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire and K. R. A. S. Sandanayake, *Chem. Soc. Rev.*, 1992, **21**, 187.

8 R. A. Bissell, A. P. de Silva, H. Q. N. Gunaratne, P. L. M. Lynch, G. E. M. Maguire, C. P. McCoy and K. R. A. S. Sandanayake, *Top. Curr. Chem.*, 1993, **168**, 223.

9 A. P. de Silva and H. Q. N. Gunaratne, *J. Chem. Soc., Chem. Commun.*, 1990, 186.

10 H. Diehl, R. Olsen, G. I. Spielhitz and R. Jensen, *Anal. Chem.*, 1963, **35**, 1144; D. Wallach and T. Steck, *Anal. Chem.*, 1963, **35**, 1035; A. Badrinas, *Talanta*, 1963, **10**, 704; H. Nishida, Y. Katayama, H. Katsuki, H. Nakamura, M. Takagi and K. Ueno, *Chem. Lett.*, 1982, 1853; K. W. Street and S. A. Krause, *Anal. Lett.*, 1986, **19**, 735; S. Fery-Forgues, M.-T. Le Bris, J.-P. Guette and B. Valeur, *J. Phys. Chem.*, 1988, **92**, 6233; J. Bourson and B. Valeur, *J. Phys. Chem.*, 1989, **93**, 3871; J. Bourson, M.-N. Borrel and B. Valeur, *Anal. Chim. Acta*, 1992, **357**, 189; J. Ghasemi and M. Shamsipur, *J. Coord. Chem.*, 1992, **26**, 337; T. L. Blair, J. Desai and L. G. Bachas, *Anal. Lett.*, 1992, **25**, 1823 are previous examples of magnesium sensing *via* fluorescence but these require non-physiological regimes.

11 M. E. Maguire, *Metal Ions Biol. Systems*, 1990, **26**, 135.

12 B. Raju, E. Murphy, L. A. Levy, R. D. Hall and R. E. London, *Am. J. Physiol.*, 1989, **256**, C540; R. E. London, *Ann. Rev. Physiol.*, 1991, **53**, 241. For recent applications see; *e.g.* J. Mizuki, K. Tasaka, N. Matsumoto, K. Kawahara, A. Miyake and O. Tanizawa, *Am. J. Obst. Gynecol.*, 1993, **169**, 134; H. Y. Li, L. J. Dai, C. Krieger and G. A. Quamme, *Biochim. Biophys. Acta*, 1993, **1181**; 307.

13 *E.g.* A. W. Czarnik in, *Frontiers in supramolecular organic chemistry and photochemistry*, ed. H.-J. Schneider and H. Durr, VCH, Weinheim, 1991, p. 109. See p. 111 in particular.

14 J. Lechleiter, S. Girard, E. Peralta and D. Clapham, *Science*, 1991, **252**, 123. For much information, see; R. P. Haugland, *Handbook of fluorescent probes and research chemicals*, Molecular Probes, Eugene, Oregon, 1992–1994. On p. 144, this reference reports a 13-fold fluorescence enhancement due to Mg^{2+} binding with a fluorescein derivative of empirical design.

15 A. P. de Silva and S. A. de Silva, *J. Chem. Soc., Chem. Commun.*, 1986, 1709 describe a case where FE = 47 and 18 with potassium and sodium ions respectively.

16 For X-ray structural proof in a related case, see; J. T. Gerig, P. Singh, L. A. Levy and R. E. London, *J. Inorg. Biochem.*, 1987, **31**, 113. The supporting UV spectral evidence in the present instance is that the long wavelength absorption shoulder of receptor **4b** at 286 nm (and a short wavelength band at 253 nm) characteristic of the conjugated amine-aromatic ring system, is gradually replaced by a new band at 267 nm characteristic of the deconjugated amine-aromatic ring system as the Mg^{2+} or Ca^{2+} concentration is increased.

17 *E.g.* R. Jenny, *C.R. Acad. Sci.*, 1960, **250**, 1659.

18 *E.g.* G. Grynkiewicz, M. Poenie and R. Y. Tsien, *J. Biol. Chem.*, 1985, **260**, 3440.

19 *E.g.* D. E. Rivett, J. Rosevear and J. F. K. Wilshire, *Aust. J. Chem.*, 1983, **36**, 1649.

20 A. Maciejewski and R. P. Steer, *J. Photochem.*, 1986, **35**, 59; I. H. Leaver and D. E. Rivett, *Mol. Photochem.*, 1974, **6**, 113.

21 K. Connors, *Binding Constants: the measurement of molecular complex stability*, Wiley, New York, 1987.