Direct visual indication of pH windows: 'off–on–off' fluorescent PET (photoinduced electron transfer) sensors/switches

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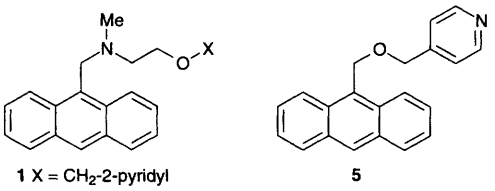
1–3, which contain a fluorophore and two proton receptors with opposite PET (photoinduced electron transfer) characteristics, only display strong fluorescence within a pH window whose position and width are tunable.

Life processes are usually successful only within a relatively narrow window of pH. Many other chemical species also need to be held within narrow ranges of concentration. Organisms depend on such concentration windows for their survival.[1] It would be useful to rapidly screen microenvironments for the presence of such windows for pH and other species. Fluorescent sensing is particularly capable of imaging concentrations of chemical species in microenvironments[2] but no direct and tunable sensors for concentration windows have been designed so far.[3] We now report a simple, predictive design which allows tailoring of systems which signal such concentration windows by means of strong fluorescence.

The second theme of this paper concerns the development of photoionic molecules as emulators of electronic devices.[4] Previously we have shown how 'fluorophore–spacer$_1$–receptor$_1$–spacer$_2$–receptor$_2$' triad systems[5] involving PET[2b,6] can result in AND logic gates with ionic inputs and fluorescence output.[4a] Now we show how the same arrangement of fluorophore and receptors but with different ion binding and PET behaviour can result in 'off–on–off' photoionic switches which emulate the input–output characteristics of tunnel diodes.[7]

The photoactive supramolecular systems[8] **1–3**† were designed as shown in Fig. 1. Past experience had shown that the fluorescence of aminomethylanthracenes is switched 'off' as a result of PET.[9] Protonation of the amine stops PET and the fluorescence is switched 'on'. Such proton-induced 'off–on' switching arises from the normal logic of fluorescent PET sensors.[2b] The expected fluorescence behaviour is seen for an appropriate model compound **4** (Table 1). The opposite situation of proton-induced 'on–off' switching can also be arranged by using a reversed logic of fluorescent PET sensors.[2b] Fluorophores with appended pyridyl units satisfy this situation.[10] PET processes are activated only when the pyridine unit is protonated and so we have proton-induced 'on–off' switching, as illustrated by model compound **5** (Table 1). **1–3** represent a marriage of these opposites, the success of which also depends on the relative basicity order of the two receptors. At the extremes of high and low pH, the fluorescence-quenching PET operates from the amine and to the pyridinium units respectively. It is only at intermediate pH values that PET is absent and strong fluorescence is expected.

Fig. 2 shows that this strategy works quite well. The bell-shaped profile (made up of two oppositely sigmoidal branches) is rather reminiscent of enzyme activity–pH relationships.[1] Owing to the shape of the profile, a pair of pH values are implied from each measured fluorescence intensity. Since **1–3** possess the same amine component, the sigmoidal branches at higher pH are virtually coincident. The corresponding pK_a' values agree within 0.2 pH units. This branch can be tuned, if needed, by changing the amine unit.[9] The sigmoidal branch at lower pH is subject to tuning over 2.6 pH units by structural variation of the pyridine component. Thus the width of the pH window for strong fluorescence and its position on the pH axis



1 X = CH_2-2-pyridyl
2 X = CH_2-4-pyridyl
3 X = 2-pyridyl
4 X = Me

5

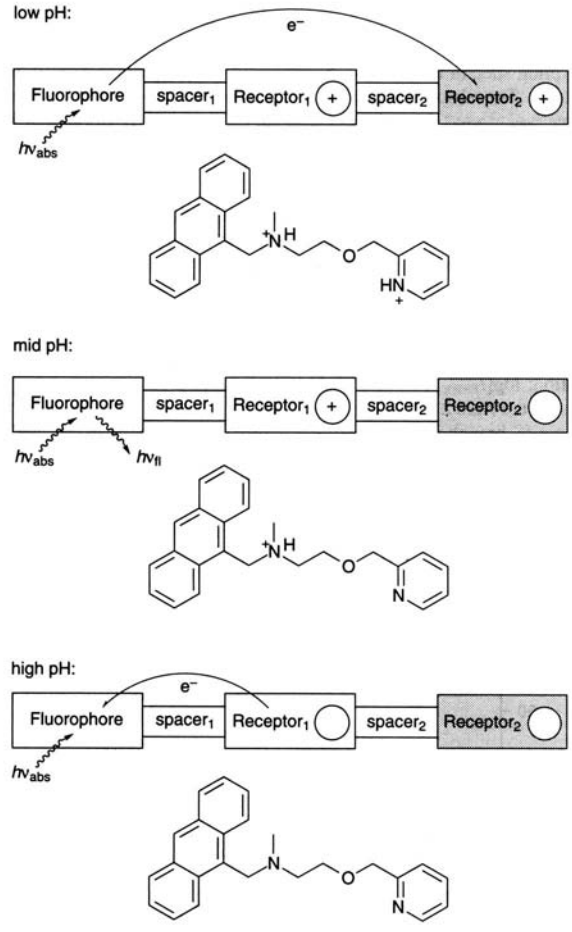


Fig. 1 Schematic representation of the operation of a fluorescent PET sensor for a pH window. The various protonation states of **1** are shown to illustrate the principle. Ions other than protons can be potentially accommodated in similar designs. Receptor$_1$ is PET active according to the normal logic for 'off–on' switching. Receptor$_2$ is PET active according to the reversed logic for 'on–off' switching. Electron transfer may occur through bond or through space in spite of the symbolism used here.

can be varied largely at will. It may be noted that the fluorescence is not fully switched 'off' at the low pH extreme. This is due to the relatively large spatial separation between the pyridinium component and the fluorophore which slows down PET rates.[11] The smaller separation in model compound **5** leads to more efficient switching 'off' at the low pH limit. The pH dependence of polybasic systems can yield 'on–off', 'off–on' and 'off–on–off' luminescence components with the latter arising from intermediate protonation states. These components have to be resolved according to wavelength[12] or decay time.[13]

The fluorescence output of **1–3** switch 'on' when the proton concentration is increased but switch 'off' again as the input is increased further. Similar output–input characteristics are seen, not only within the current–voltage profiles of tunnel diodes,[7] but also in the absorbance–laser power relationships of optical switches with non-linear behaviour.[14]

Table 1 Optical and basicity parameters of switches **1–3** and model compounds **4** and **5**[a]

	Compound				
Parameter	**1**	**2**	**3**	**4**	**5**
pK_a	3.9, 7.9	—[b], 7.6	—[b], 7.7	—[c], 7.8	5.1, —[c]
pK_a'	4.1, 7.9	5.0, 7.9	2.4, 7.7	—[c], 7.8	5.0, —[c]
ϕ_{Fmin}(low pH)	0.046	0.13	0.076	—[c]	0.014
ϕ_{Fmax}(mid pH)	0.38	0.58	0.52	0.65	0.29
ϕ_{Fmin}(high pH)	0.009	0.017	0.024	0.034	—[c]

[a] 5×10^{-6} mol dm^{-3} solutions in H_2O: MeOH (4 : 1, *v*/*v*). The electronic absorption spectra in the $S_0 \rightarrow S_1$ region of all compounds **1–5** are nearly identical. The absorption maxima (λ_{abs}/nm) and absorption coefficients (ε/dm^3 mol^{-1} cm^{-1}) for the (0,0), (0,1), (0,2) and (0,3) vibrational bands are: λ_{abs}(log ε) 384 (3.98), 364(4.00), 347(3.81) and 331(3.50). These spectra show small pH dependences in some cases which are analysed according to the relationship of absorbance (A) with pH: $\log[(A_{max} - A)/(A - A_{min})] = \pm pH \pm pK_a$.[15] The signs of the terms on the right hand side of the equation depend on the wavelength at which A data is obtained. Fluorescence emission spectra are gathered by excitation at isosbestic points. The fluorescence maxima (λ_F) for the (0,0), (0,1) and (0,2) vibrational bands are 398, 420 and 443 nm. The wavelengths and the relative heights of these bands are essentially pH independent. The pH dependences of fluorescence intensity (I_F) are analysed according to the equation: $\log[I_{Fmax} - I_F)/(I_F - I_{Fmin})] = \pm pH \pm pK_a$.[2b] The signs of the terms on the right hand side of the equation depend on whether an 'off–on' or 'on–off' branch is being analysed. Corresponding pairs of pK_a and pK_a' values are nearly identical in PET systems.[2b,4a] [b] Spectroscopic change is too small to permit measurement. [c] Not applicable.

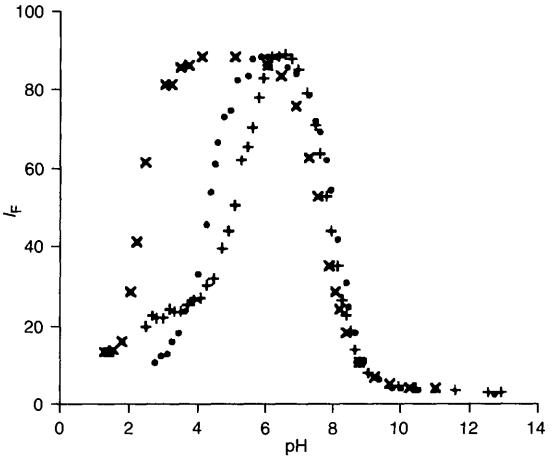


Fig. 2 Fluorescence intensity (I_F)–pH profiles for **1** (●), **2** (+) and **3** (×) normalized at their maxima

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Footnote

† **1** and **2** were prepared by reacting 9-bromomethylanthracene with *N*-methylethanolamine, followed by deprotonation with NaH and alkylation with the corresponding chloromethylpyridine. **3** was prepared by reacting the sodium alkoxide of *N*-methylethanolamine with 2-chloropyridine, followed by condensation with 9-anthraldehyde and subsequent reduction with $Na(BH_3CN)$. All compounds gave correct spectral and analytical data.

References

1 L. Stryer, *Biochemistry*, 3rd edn., Freeman, New York, 1988; M. Crichton, *The Andromeda Strain*, Knopf, Random House, New York, 1969.

2 (*a*) R. Y. Tsien, *Chem. Eng. News*, 1994, July 18, 34; (*b*) 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; (*c*) *Fluorescent Chemosensors for Ion and Molecule Recognition*, ed. A. W. Czarnik, *ACS Symp. Ser.*, 1993, **538**; (*d*) B. Valeur, in *Probe Design and Chemical Sensing, Top. Fluoresc. Spectrosc.*, 1994, **4**, 21; (*e*) B. Valeur and E. Bardez, *Chem. Br.*, 1995, **31**, 216.

3 (*a*) *Indicators*, ed. E. Bishop, Pergamon, Oxford, 1972, discusses the classical 'indicator range' which is quite different from the pH window under discussion; (*b*) *Biochemical Fluorescence: Concepts*, vol. 2, ed. R. F. Chen and H. Edelhoch, Marcel Dekker, New York, 1976, discusses several heterocyclic biomolecules which show maxima in their fluorescence–pH profiles. Some of these involve protonation of excited-state fluorophores which does not apply to **1–3**.

4 (*a*) A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Nature*, 1993, **364**, 42; (*b*) A. P. de Silva, H. Q. N. Gunaratne and G. E. M. Maguire, *J. Chem. Soc., Chem. Commun.*, 1994, 1231; (*c*) S. Iwata and K. Tanaka, *J. Chem. Soc., Chem. Commun.*, 1995, 1491; (*d*) P. Ghosh, P. K. Bharadwaj, S. Mandal and S. Ghosh, *J. Am. Chem. Soc.*, 1996, **118**, 1553.

5 M. R. Wasielewski, *Chem. Rev.*, 1992, **92**, 435; D. Gust, T. A. Moore and A. L. Moore, *Acc. Chem. Res.*, 1993, **26**, 198; K. Maruyama, A. Osuka and N. Mataga, *Pure Appl. Chem.*, 1994, **66**, 867; J.-P. Sauvage, J.-P. Collin, J.-C. Chambron, S. Guillerez, C. Coudret, V. Balzani, F. Barigelletti, L. de Cola and L. Flamigni, *Chem. Rev.*, 1994, **94**, 993; R. W. Wagner and J. S. Lindsey, *J. Am. Chem. Soc.*, 1994, **116**, 9759; G. F. Mes, H. J. van Ramesdonk and J. W. Verhoeven, *J. Am. Chem. Soc.*, 1984, **106**, 1335; L. Fabbrizzi, M. Licchelli, P. Pallavicini and A. Taglietti, *Inorg. Chem.*, 1996, **35**, 1733.

6 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; A. W. Czarnik, *Acc. Chem. Res.*, 1994, **27**, 302; L. Fabbrizzi and A. Poggi, *Chem. Soc. Rev.*, 1995, **24**, 197; T. D. James, P. Linnane and S. Shinkai, *Chem. Commun.*, 1996, 281.

7 See, for example, J. D. Ryder, *Engineering Electronics*, 2nd edn. McGraw-Hill, New York, 1967.

8 V. Balzani and F. Scandola, *Supramolecular Photochemistry*, Ellis-Horwood, Chichester, 1991; V. Balzani, A. Credi and F. Scandola, in *Transition Metals in Supramolecular Chemistry*, ed. L. Fabbrizzi and A. Poggi, Kluwer, Dordrecht, 1994, p. 1; J.-M. Lehn, *Supramolecular Chemistry*, VCH, Weinheim, 1995.

9 A. P. de Silva and R. A. D. D. Rupasinghe, *J. Chem. Soc., Chem. Commun.*, 1985, 1669.

10 A. P. de Silva, H. Q. N. Gunaratne and P. L. M. Lynch, *J. Chem. Soc., Perkin Trans. 2*, 1995, 685; F. D. Saeva, *J. Photochem. Photobiol. A: Chem.*, 1994, **78**, 201.

11 G. L. Closs and J. R. Miller, *Science*, 1988, **240**, 440; M. N. Paddon-Row, *Acc. Chem. Res.*, 1994, **27**, 18.

12 S. G. Schulman, R. Threatte, A. Capomacchia and W. Paul, *J. Pharm. Sci.*, 1974, **63**, 876.

13 F. Barigelletti, L. Flamigni, M. Guardigli, J.-P. Sauvage, J. P. Collin and A. Sour, *Chem. Commun.*, 1996, 1329.

14 M. P. O'Neil, M. P. Niemczyk, W. A. Svec, D. Gosztola, G. L. Graines and M. R. Wasielewski, *Science*, 1992, **257**, 63.

15 K. Connors, *Binding Constants: The Measurement of Molecular Complex Stability*, Wiley, New York, 1987.

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