Molecular Fluorescent Signalling with 'Fluor–Spacer–Receptor' Systems: Approaches to Sensing and Switching Devices *via* Supramolecular Photophysics

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1 Why Bother with Molecular Fluorescent Signalling?

Occasionally, along comes a discipline which can be useful to a wide spectrum of sciences and technologies. Supramolecular photophysics[1] is one such central research area. It arises from the coincidence of the photon–molecule encounter and the molecule–molecule association. The latter is relevant since reversible binding between the signalling agent and the species being signalled is a prerequisite for molecular signalling. With regard to the former, molecular fluorescence is one of the most visually powerful phenomena in photophysics/chemistry. The researcher in this field has the privilege of literally seeing the outcome of molecular experiments, *i.e.* molecular fluorescence is a natural interface between human and molecular domains *via* the intermediacy of the photon. The phenomenon of molecular fluorescence possesses many features which make it particularly suitable for real-time and real-space monitoring of/responding to atomic and molecular species.[2,3] The molecular basis means that: (Ia) it can report on nanometre spaces if properly targetted. This is real-space monitoring according to all but the most exacting criteria of molecular scientists. (Ib) The active molecules can be easily smuggled into dynamic/living systems which then act as unwitting hosts. Feature (IIa) suggests that the low concentration of active molecules need not poison the host. The photonic basis means that: (IIa) It has high sensitivity of detection approaching the single molecule, *i.e.* human addressing of molecules is possible. Even routine experiments only need micromolar levels of active molecules. (IIb) Remote 'wireless' communication is available between the scientist and the active molecule. If necessary, active molecules can be immobilized on fibre optic tips for improved directionality, but at the expense of the freedom and flexibility of the free-swimming molecules. (IIc) It has natural imaging capability, especially with confocal microscopy. When coupled with its real-time capability [feature (IIIa)], this results in cinematic representation of the microscopic world. Micrometre visualization is routine and near-field microscopy could reduce this limit further in certain situations. The excited state basis means that (IIIa) it has nanosecond response time, though the time constants of the necessary molecular associations can increase practical response times to milliseconds in the present supramolecular contexts. (IIIb) It can be easily 'switched off' at all wavelengths, unlike optical absorption. This is because the radiative deactivation of excited states is slow enough to suffer competition from chemical processes. Since excited state quenching mechanisms are reasonably well understood, it is also possible to arrange for 'switching on'. Hence, two-state digital action is feasible. Given all these features, it is easy to see the value of molecular fluorescent signalling systems for (a) sensing species and properties in the physical and life sciences and, (b) data processing *via* molecular optoelectronics/ionics. Before concluding this section it is worth pointing out that fluorescent sensors and switches are examples of signalling systems which allow continuous monitoring/response which are distinct from reagents which irreversibly respond to a chemical stimulus and from labels which produce a chemically unresponsive signal for purposes of tracking a species. The development of fluorescent reagents and labels are also vibrant research areas in their own right.[4]

While originating from backgrounds which differ by as much as 5000 miles and 15 years, the authors share the common factors of Ph.D. study at the School of Chemistry in the Queen's University of Belfast and a strong interest in Photophysics/chemistry and Supramolecular science.

2 The Photoelectrochemical Approach to the Design of Fluorescent Signalling Systems

It is natural that a fluorescent ion signalling system should include a fluor and an ion receptor as critical components. In the present design, these are distinct modules separated by an all-σ-bonded spacer, *i.e.* the only communication between the optical and ligating modules is *via* relatively long-range forces. In their electronic ground states, these optical and ligating subunits show some features of a supramolecular assembly with only weak interactions between their π- and n-electron systems, even though they are covalently connected *via* the spacer. However, in properly designed systems, a strong long-range interaction develops in the form of an electron transfer from the ion-free receptor to the fluor when the fluor module is photoexcited. Photoinduced electron transfer (PET)[5] is the essence of photoelectrochemistry which came to prominence with its application to solar energy conversion. However, the exploitation of light-driven redox reactions for signalling purposes is still at an early stage. The signalling possibilities arise from the fact that the 'fluor–spacer–receptor' system in the cation-free situation has been chosen such that its fluorescence is 'switched off' by the PET process[3] (Figure 1a). The PET process, in turn, can be suppressed by the entry of a cation into the receptor by the cation-induced increase of the ionization/oxidation potential of the receptor. At the simplest level this is an electric field effect. However, other ways of inhibiting electron transfer are available. Conformational changes, local polarity modulations, and hydrogen bonding are three approaches discussed in this article. Such a suppression of the PET process means that fluorescence again becomes the dominant decay channel of the excited fluor (Figure 1b). Further clarification of the situation is possible in terms of the frontier orbital energy diagrams in Figures 2a and b. Thus, cation entry is signalled by photon output when interrogated by excitation photons.

For example, a proton signalling system, structure (1a), may be designed by combining an organic base, *i.e.* an amine with the common fluor anthracene *via* a methylene spacer.[6] Spectroscopic details for related cases [Structures (1b) and (1c)] may be found in reference 3. The feasibility of PET within this system can be assessed by means of the Weller equation (equation 1).[7]

$$\Delta G_{ET} = -E_{S.fluor} - E_{red.fluor} + E_{ox.receptor} - E_{ion\ pair} \qquad (1)$$

The thermodynamic driving force for PET (ΔG_{ET})in polar media is found to be between -0.1 and $+0.1$ eV.[8] Being at most only slightly endergonic, PET can therefore be expected to be rapid compared to fluorescence whose rate is $5 \times 10^7\ s^{-1}$ as estimated

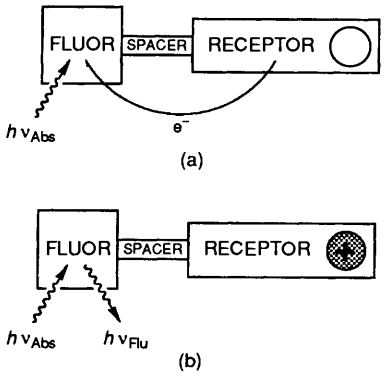


Figure 1 Schematic representation of photoinduced processes in a 'fluor–spacer–receptor' signalling system (a) when cation-free and (b) when cation-bound.

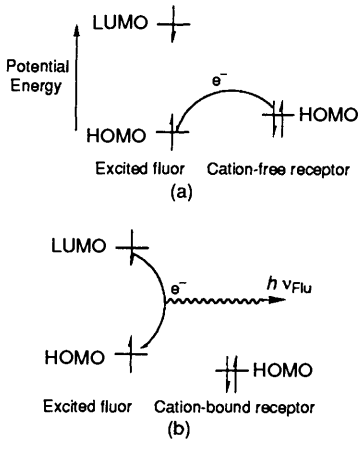


Figure 2 Frontier orbital energy representation of photoinduced processes in a 'fluor–spacer–receptor' signalling system (a) when cation-free and (b) when cation-bound.

from the value for 9-methyl anthracene. Protonation of the amine moiety raises its oxidation potential to > + 2.5 eV. A fresh application of equation 1 yields $\Delta G_{ET} > + 1.4$ eV. Hence PET should be strongly inhibited, resulting in a revival of fluorescence. Figure 3 confirms this expectation of proton-induced fluorescence recovery in (1a). It is notable that the spectral shape and position of fluorescence are pH independent. Additionally all of the features (position, shape, and height) of the $S_0 \rightarrow S_1$ absorption spectra are also essentially pH invariant. Thus we have the simplest possible signalling action in that only one parameter, *i.e.* fluorescence quantum yield is proton controlled. Conventional fluorescent pH indicators based on the different design principle of photoinduced proton transfer exhibit more complex signalling behaviour which includes shifts of band positions in absorption and emission spectra.[9] The fluorescence intensity (I_F) measured at any suitable wavelength varies with pH in a sigmoidal manner and satisfies the Henderson–Hasselbach-type mass action equation (equation 2), where K_a is the acid dissociation constant.

$$\log[(I_{Fmax} - I_F)/(I_F - I_{Fmin})] = pH - pK_a \qquad (2)$$

The fluorescence intensity is only dependent upon pH over a range of *ca.* 2 pH units as is common for conventional absorptiometric and fluorimetric pH indicators and is essentially pH invariant outside of this pH window. It is interesting that the value of such signalling systems for sensing purposes is due to the presence of this window, whereas the existence of the two pH-invariant plateaux (*i.e.* a bistable system) hold the appeal for switching applications. The analogue–digital duality of such

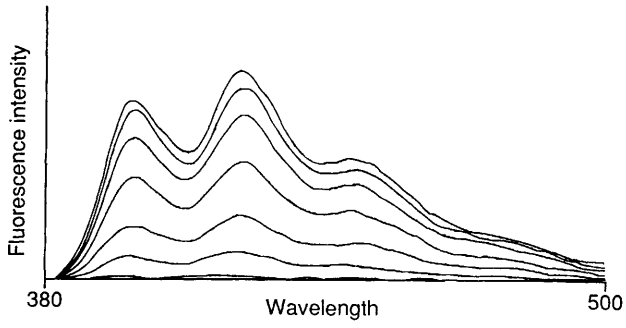


Figure 3 pH-Dependence of fluorescence spectra of 10^{-6}M (1a) excited at 364 nm in methanol: water (1:4,v/v). The pH values are, in order of decreasing intensity, 2.9, 7.2, 8.3, 8.9, 9.4, 9.8, and 10.8.

sigmoidal behaviour can be biased in either direction by choosing the pH and the magnitude of its variation.

A further contrast of PET signalling systems with respect to conventional fluorescent pH indicators emerges when the pK_a values are closely examined. The present systems show experimentally indistinguishable values whether examined under excited state (fluorimetry) or ground state conditions (pH-dependent solubility).[6] Conventional counterparts are distinguished by large differences in excited- and ground-state pK_a values.[9] Thus, in well-designed cases, PET signalling systems possess the detection sensitivity of excited state experiments while maintaining thermodynamically valid binding constants characteristic of ground state measurements.

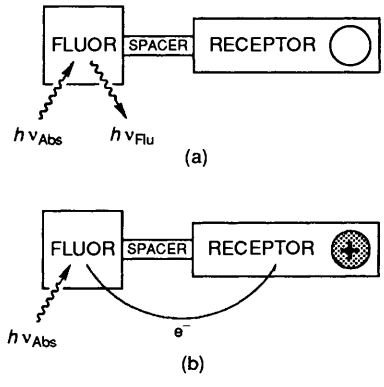


Figure 4 Schematic representation of photoinduced processes in a 'fluor–spacer–receptor' signalling system with reversed logic (a) when cation-free and (b) when cation-bound.

An attraction of a simple logic system is the ease with which it can be modified, even reversed. Such a reversal of the logic given in Figure 1 is shown in Figure 4. Now the PET process only occurs upon cation binding and does so from the fluor to the cation-bound receptor. Compound (2)[10] was an early example of molecular engineering of a fluorescent ion sensor, *i.e.* the detailed plans on the designer's drawing board are directly and quantitatively translated into experimental reality (Table 1). This was expected because the modular construction of fluorescent PET signalling systems implies that the properties of the 'parent' fluor and receptor units will be largely preserved in the assembled system. In (2), simple thermodynamic arguments *via* equation 1 with redox potentials of the 'parent' units allow us to predict whether pH signalling is possible or not. Then, the entire set of absorption/emission spectral features (band position, shape, and height) and acid–base behaviour [applicability of a version of the mass action equation (equation 2), pK_a values] are predictable within experimental error, except the maximum fluorescence quantum yield (ϕ_{Fmax}) which deviates negatively by

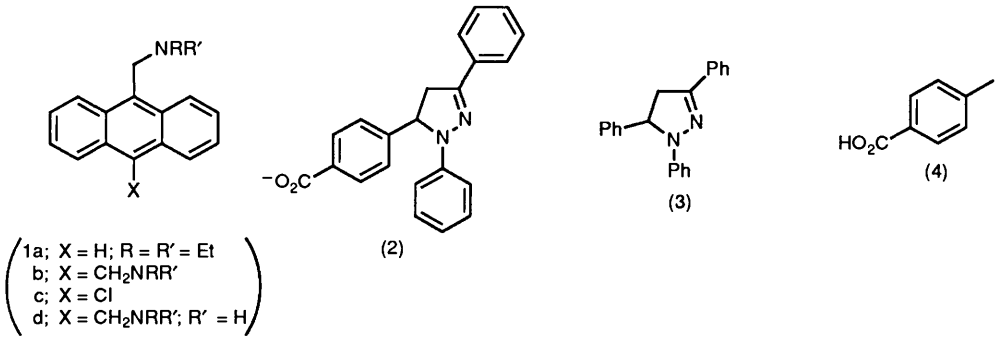


Table 1 Comparison of predictions and observations in fluorescent PET signalling systems

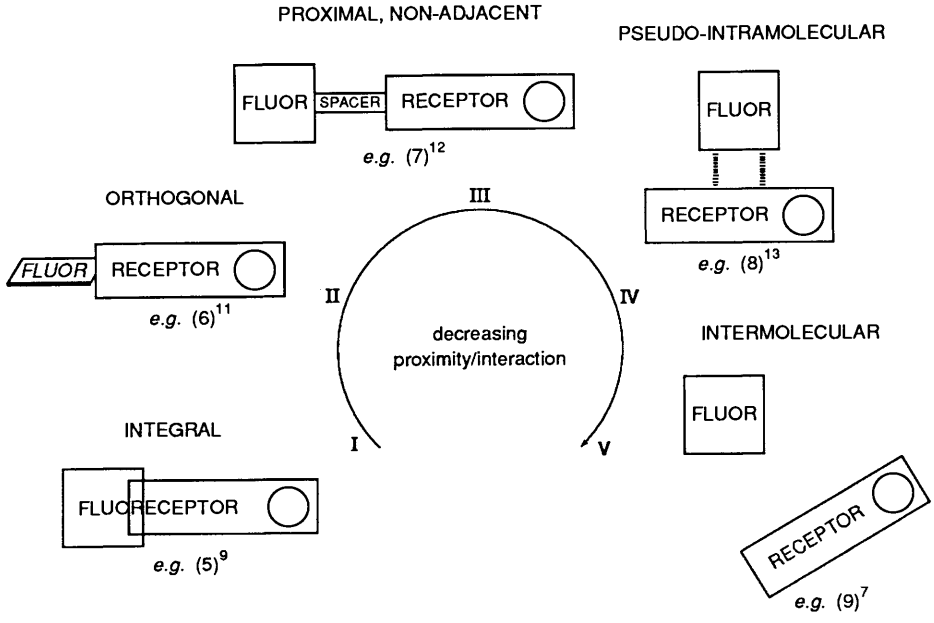
Property	Found in (2)	Predicted from (3) or (4)	Found in (1)	Predicted from NEt_3 or 9-methylanthracene
Signalling action possible?	yes	yes	yes	yes
$\lambda_{Abs.max}$(nm)	359[a]	358[b]	365[a,d]	366[c,d]
ϵ_{max}(10^4dm^{3}mol^{-1}cm^{-1})	2.2[b]	2.1[c]	1.04[c,d]	0.91[c,d]
$\lambda_{Flu.max}$(nm)	475[b]	475[b]	420[a]	416[c]
Φ_{Fmax}	0.13[b]	0.19[b]	0.69[a]	0.29[c,e]
Φ_{Fmin}	0.003[b]	0.003[b,f]	0.004[a]	—
Henderson–Hasselbach equation obeyed?	yes	yes	yes	yes
pK_a	4.2[a,g],4.4[a,h]	4.5[a]	9.1[a]	10.7[i]

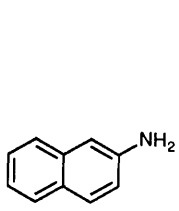
[a] Solvent: methanol–water (1:4 v/v). [b] Solvent: methanol–water (1:1 v/v). [c] Solvent: methanol. [d] 0-1vibrational band. [e] 9-Anthracenylmethyl iminodiacetate in pH7 water gives a value of 0.40. [f] Value for methyl ester of (2), which is locked in the 'switched off' state. [g] p$K_a(S_1)$ value. [h] p$K_a(S_0)$ value. [i] Solvent: water.

a factor of 1.5. Such nearly complete quantitative predictability is due to the heart of the proton receptor being a low-charge-density carboxylate which is held remote and rigidly away from the fluor module along a plane approximately bisecting the excited state dipole. Compound (1), while possessing a substantial fraction of quantitatively predictable signalling parameters, shows a notable breakdown of predictability in its pK_a value (Table 1). This is at least partly because of steric inhibition of solvation of the protonated receptor unit due to the non-adjacent but proximal 9-anthracenyl fluor being separated only by a methylene group.

Figure 5 The spectrum of 'fluor–receptor' configurations useful in fluorescent signalling research.

The provision of the spacer module is instrumental in preserving the identities of the fluor and receptor units within the signalling system, which in turn gave rise to many of the special features discussed above. The presence of the spacer also alerts us to the importance of the relative spatial disposition of the fluor and receptor units. Several formats in which the fluor and receptor units can be arranged are shown in Figure 5. When ordered according to a scale of decreasing time-averaged proximity/interaction, we see that the 'fluor–spacer–receptor' format occupies a central position in the spectrum of fluor–receptor configurations that are useful for fluorescent signalling. On a formal level, Figure 5 unifies a substantial fraction of fluorescent signalling research and arises from a simple appreciation of the role of the spacer module. (I) Integral systems form the vast





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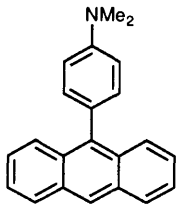

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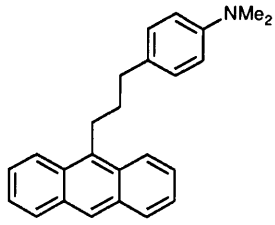

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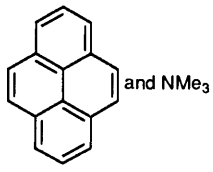

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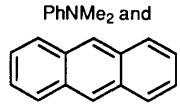

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majority of known fluorescent indicators for protons and other ions. Optical excitation leads to internal charge transfer (ICT)[14] in these fluors and ion binding to a site on the fluor itself will naturally modulate these charge shifts and hence the fluorescence band wavelengths. (II) Orthogonal systems known thus far are twisted biaryls where the π molecular orbitals of the fluor and receptor are separated because of a steric clash of their σ frameworks, *i.e.* orthogonality of molecular orbitals is due to geometric orthogonality. The signalling behaviour of these systems can be interpreted as a PET process in a 'fluor–spacer–receptor' assembly with a virtual C_0 spacer. The alternative viewpoint is that these systems produce non-emissive twisted intramolecular charge transfer (TICT)[15] states upon excitation. However, the photoelectrochemical criteria for the occurrence of both these processes are virtually identical and cation entry blocks off either the PET or the TICT process. (III) Proximal, non-adjacent systems arise from the 'fluor–spacer–receptor' format with C_1,C_2, and C_3 skeletons as common spacers. The difference in orthogonal systems is that the spacer is C_0, *i.e.* virtual. (IV) Pseudo-intramolecular systems result from spatial constraints imposed by non-covalent interactions, *e.g.* hydrophobically driven co-complexation of fluor and receptor units by a cyclodextrin host.[13] Such a strategy is appealing because organic synthesis is traded off against supramolecular self-assembly. This enforced proximity of the receptor to the fluor results in fluorescence quenching. Upon ion binding, however, the receptor unit can be expected to lose its quenching ability. Further, in suitable cases, the decreased hydrophobicity of the ion-bound receptor would increase its escape probability from the cyclodextrin. (V) Intermolecular systems rely on diffusive processes to set the stage for the fluor–receptor interaction and therefore are rather inefficient for signalling purposes. On the other hand, the modular nature of the fluor and the receptor are most pronounced in this class. Thus, it is natural that the wealth of photophysical information gathered on this class can allow the designer rationally to plan signalling systems in categories II, III, and IV.

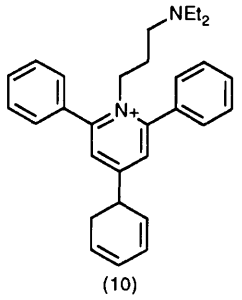
3 The Scope of the PET Design Logic for Fluorescent Signalling

The simplicity of a concept aids the development of examples illustrating its applicability. This has certainly been true regarding the PET design logic for fluorescent signalling with nearly two dozen examples being available even by late 1990 from laboratories on four continents. This body of literature is catalogued in reference 16. For example, Table 2 lists the families of fluorescent signalling systems for protons which have been constructed and examined by ourselves and colleagues. Table 2 also summarizes useful optical spectroscopic parameters and acid–base properties of these signalling systems. The generality of the PET design logic for proton signalling *via* fluorescence is thus clearly established, since significant proton-induced enhancement (or attenuation in cases with reversed PET logic, *i.e.* Figure 4) of fluorescence (FE) is found in most cases. The few cases with FE ~ 1 were investigated to test the range of applicability of a given family and these thresholds are predictable by simple theoretical considerations outlined in Section 2. It is notable that all but two of the major types of excited states with significant light emission found in organic/inorganic chemistry are represented in Table 2. They are: (a) pure $\pi\pi^*$ states characteristic of aromatic hydrocarbons; (b) internal charge transfer (ICT)[14] states found in heteroatom containing donor–acceptor or push–pull π systems; (c) essen-

Table 2 Fluorescent PET signalling systems for protons and their salient features

System	Fluor	Excited state	λ_{abs}(nm)	λ_{em}(nm)	FE	Spacer	Receptor	pK_a
(1)[6]	Anthracene	$\pi\pi^*$	370—400	414—425	30—1200	CH_2	Amine (aromatic & Aliphatic)	1.2—9.1
(10)[17]	2,4,6-Triphenylpyridinium	ICT	295	405	30	$(CH_2)_2$	Diethyl amine	<2.0
(2)[10]	1,3-Diaryl pyrazoline	ICT	354—398	470—510	1.0—50[a]	$CHCH_2$	Benzoate	3.8—4.6
(11)[17]	1,3-Diaryl pyrazoline	ICT	354—420	470—542	30—60[a]	$CHCH_2$	Pyridine	3.2—4.7
(12a)[18]	(Anthracene)	$\pi\pi^*$	370—546	416—575	20—230	CH_2	(Alkoxyphenyl imino)	3.0—5.7
(12b)[18]	(1,3-Diaryl pyrazoline)	ICT				$CHCH_2$	(diacetate)	
(12c)[17]	(2,7-Bis(dimethylamino) (xanthenium)	ICT[b]				C_0(virtual)	( )	
(13)[17]	1,6,7,12-Perylene tetra carbox-diimide	~$\pi\pi^*$	491—528	530—545	5.3—50	$(CH_2)_n$ $n = 2,3$	Amine (aliphatic)	3.8—6.0
(14)[17]	6-Amino-3*H*-benzimidazo [2,1-*a*]benz[*d*,*e*]-isoquinolin-3-one	ICT	411—454	500—545	6—200	$(CH_2)_n$ $n = 2,3$	Amine (aliphatic)	5.8—9.2
(15)[17]	4-Chloro,-1,8-Naphthalimide	~$\pi\pi^*$	345	409	25—90	$(CH_2)_2$	Amine (aliphatic)	6.0—8.6
(16)[17]	7-Methoxy coumarin	ICT	320—333	414	30—40	CH_2	Amine (aliphatic)	4.5—7.1
(17)[19]	meso-Tetraphenylporphyrin Sn^{IV}	$\pi\pi^*$ (ligand)	423, 550—596	600—650	1.1—14	CH_2	Amine (aromatic & aliphatic)	2.1—8.0
(18)[19]	Tris(2,2′-bipyridyl)Ru^{II}	MLCT	444—450	653	30—150	CH_2	Amine (aromatic)	1.9—2.4

[a] These are fluorescence attentuation factors. [b] This is an orthogonal system (Figure 5) which can be rationalized *via* a non-emissive TICT excited state.



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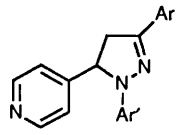

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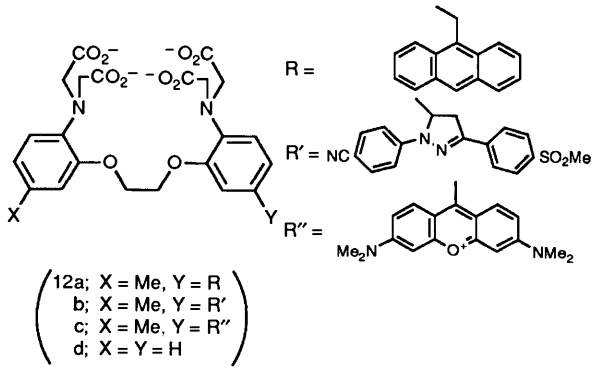

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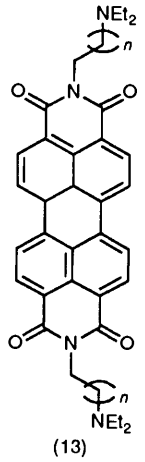

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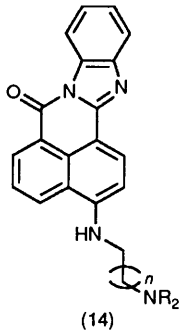

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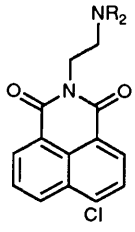

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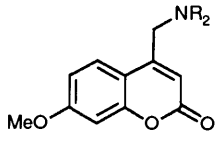

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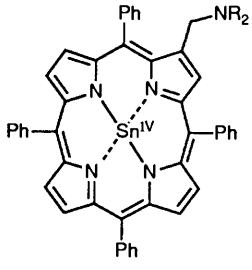

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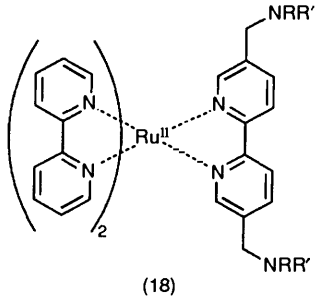


(18)

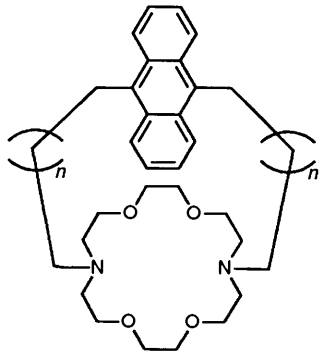
tially ligand-localized $\pi\pi^*$ states in complexes with *p*-block metal ions; and (d) metal-to-ligand charge transfer (MLCT) states in complexes with *d*-block metal ions. The two classes unrepresented so far are: (a) twisted intramolecular charge transfer (TICT)[15] states seen in certain amino-substituted electron-deficient aromatics and biaryls and (b) metal-localized states in complexes with *d* or *f*-block metal ions. Examples of these two categories are eagerly awaited.

Table 2 gives an indication of the scope of proton signalling with fluorescent PET systems. Their modular construction allows wide variation of optical and ligating units within the design constraints. The range of fluor modules employed gives both the designer and the end-user much flexibility with respect to: (a) Hydrophobicity and polarity (varied from polycyclic aromatic hydrocarbon to ionic heterocyclic) for targetting to microenvironments of given polarity. (b) Absorption and emission wavelengths (varied from 295 to 596 nm and from 405 to 653 nm respectively) for use in variously pigmented microsystems. Within certain individual families, simple substitutional tuning of these wavelengths can be achieved over a wide range, *e.g.* 70 nm each for (11) without compromising signalling action (FE >> 1). (c) Stokes shifts (varied from 2 to 200 nm) of which the latter would find use in turbid/scattering microenvironments. (d) Emission lifetimes (varied from ns to μs) of which the latter, *e.g.* (18), would be suitable for use in microenvironments with native fluorescence of any wavelength (cases with ms lifetimes will be addressed in Section 4). (e) Chemical stability and immunity towards a given intermolecular quencher (varied from electron rich to electron poor). Such immunity would also be enhanced in those cases with short excited singlet state lifetimes.

The spacer module has been varied as well. This was usually a flexible oligomethylene unit, though rigid $CHCH_2$ spacers have been employed within small rings. Rigid and multiple spacers can increase the usually weak coupling between the terminii, *i.e.* the fluor and receptor modules. However, the CH_2 spacer was our favourite since it allowed fast PET rates (because of the small distance of separation of the terminal modules) and was most convenient for synthesis *via* benzylic functionalities. Virtual C_0 spacers have also been used successfully in the orthogonal system (12c).

All but one of the common classes of organic proton receptors are represented in Table 2. They are (a) amine, (b) carboxylate, and (c) pyridine. The one not examined yet, phenolate, deserves attention. Availability of choice of the receptor module can be crucial for microenvironmental studies since these classes have different changes in charge type and hydrophobicity upon protonation. A wide range of pK_a values, currently from 1.2 to 9.1, are available. Thus, pH values from 0 to 10 are addressable with the present signalling systems.

The simplicity of a design logic can occasionally appear as naivety in the cold light of continued experimentation. Such occasions have arisen during our investigations of proton signalling PET systems. These, in turn, have clarified the role of the spacer. For instance, (16, R = Et)[17] shows many features of the expected signalling behaviour, but the $S_0 \rightarrow S_1$ absorption spectra are distinctly pH dependent with a limiting red shift of 9 nm in acidic solution. This is understandable since the ammonium centre of rather high positive charge density preferentially interacts across the short methylene spacer with the closer negative terminus of the ICT excited state of the 7-methoxy coumarin located in the lactone ring. In this instance the methylene spacer is unable to isolate the terminal modules from their electric pole–dipole coupling. Nevertheless, the dampening effect of the spacer is clear since the observed absorption band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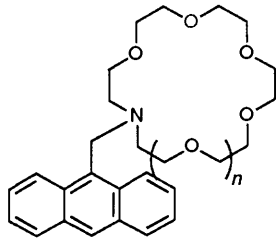
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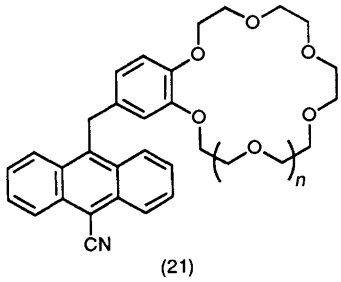
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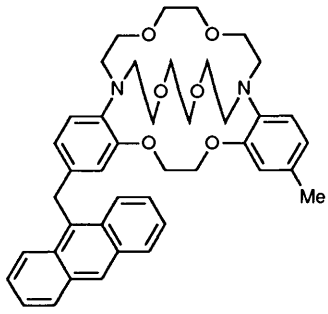

(22)

shift is smaller than those seen in proton-responsive integral signalling systems.

A tougher test of the generality of fluorescent PET signalling was its extension to other cations besides protons. The behaviour of (19)[20] and (20)[21] demonstrated that such extension was feasible. Compound (20, $n = 1$) was remarkable in having (a) a K^+-induced FE of 47, (b) quantitatively predictable binding constants (log β) for K^+ and Na^+ and, (c) extreme synthetic accessibility (one-step reaction of known components). However (19) and (20) shared the problem of pH-responsive fluorescence. This problem was solved simply by constructing (21)[22] which possessed no basic nitrogen centres. The response of (21, $n = 0$) showed good selectivity towards Na^+ (FE = 15) *versus* the other alkali cations while displaying excellent H^+ rejection. Again, it is notable that (21) was exactly designed employing electrochemical data of model components and their experimental log β values for alkali cations were quantitatively predictable from those of the 'parent' benzocrown ether receptors. The optical properties were also predictable except that the experimental Φ_{Fmax} values were 30-fold smaller than expected. This poor performance of Φ_{Fmax} was subsequently corrected by optimizing the signalling action by structural and solvent variation.[16] Then the Φ_{Fmax} values only differed by factors of 1.25—2.5 from the expected value. Compounds (21, $n = 0$ and 1) operate best in alcoholic media and their use would be more suited to membraneous, rather than wholly aqueous, microenvironments. Compound (22)[23], which employs a cryptand of reduced basicity as the cation receptor, was constructed as a forerunner of alkali-cation monitoring systems for intracellular applications. Though insoluble in water, (22) binds alkali cations avidly in methanol and gives a maximum FE of 11 with Rb^+. Water-soluble versions of (22) would be useful since some of the elegant sensors presently available[24] (which are integral signalling systems) for intracellular Na^+ monitoring suffer from the problem of short wavelength excitation (*ca.* 340 nm), though longer-wave versions are bound to appear. Compound (22) is well-excited at 383 nm and fluor modules responsive to even longer wavelengths have already seen service in other PET signalling systems (*e.g.* Table 2).

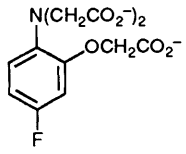
Ca^{2+}-induced FE values of 16 for (12a),[18] 92 for (12b),[18] and 25 for (12c)[17] were attained by attaching Tsien's selective calcium receptor[25] *via* a spacer (CH_2, $CHCH_2$, and C_0 respectively) to anthracene, 1,3-diaryl pyrazoline, and rhodamine fluors respectively. These examples nicely complement Tsien's fluorescent Ca^{2+} sensors[25] which are integral signalling systems and which are popular for intracellular investigations. It is particularly notable that Tsien has reported a sensor with a structure identical to (12c) (though synthesized by a different route) but with FE = 3.[25] Compound (12c) is a clear example of an orthogonal signalling system. Currently, (12b) has the largest Ca^{2+}-induced FE value known in the literature and relatives with even larger FE values are now available.[17] These dramatic examples of fluorescence 'off–on' signalling are due to an amplification of the mechanism outlined in Figure 1. The special ability of a metal ion to organize an acyclic, flexible ligand around itself was exploited by Tsien to create a Ca^{2+}-induced conformational change in the receptor (12d)[25] We noted that this Ca^{2+}-induced conformational change which decouples the iminodiacetate moieties from the alkoxyphenyl units caused a large increase in oxidation potential of the receptor (beyond that caused by simple proximity of dication) which drastically inhibits the PET process in *e.g.* (12b).[18] Such powerful signalling of Ca^{2+} in the physiological range (10^{-7}–10^{-6}M) with essentially no response to protons around pH7 and Mg^{2+} around 10^{-3}M has much potential for intracellular monitoring. These fluorescent signalling systems (12a), (12b), and (12c) cover a wide range of excitation/emission wavelengths (370—546 and 416—575 nm respectively) for the convenience of the end-user. Again, almost all of absorption/emission spectral features and ion-binding behaviour (for Ca^{2+}, Mg^{2+}, and H^+) are reasonably predictable – the exception being Φ_{Fmax} values which deviate negatively by a factor of 5—25.

The development of fluorescent PET signalling systems for other non-transition metal ions and organic cations can be expected to follow. For instance, London's Mg^{2+}-selective receptor (23),[26] from which integral fluorescent signalling systems have been built,[26] is a likely candidate for conversion into analogues of (12). The incorporation of *d*-block metal ions, with their redox activity, would block off the designed PET channel (Figure 1b) but could simultaneously open up new ones, *i.e.* no fluorescence signal would result. On the other hand, the binding of *d*-block metal ions into 'fluor–spacer–receptor' systems which are designed to be initially fluorescent would cause efficient quenching, *e.g.* (24)[27] with Ag^+. This is reminiscent of, but distinct from, the reversed-PET signalling systems, *e.g.* (2). The current paucity of PET signalling systems for anions is due to the relative unavailability of selective anion receptors with suitable electroactivity and a window of optical transparency. This difficulty has been neatly circumvented in one instance by Czarnik who employed a partially protonated polyamine to provide both the electroactive amine group and the anion-binding polyammonium array.[28] The fluorescence 'switching on' is due to hydrogen bonding of the amine lone electron-pair by HPO_4^{2-} (rather than an electric field effect) as depicted in (25).

A feature of the PET design logic is that it is possible to design fluorescent signalling systems for nett microenvironmental properties to complement the species-signalling systems discussed above. The well-known polarity-dependence of PET rates was exploited in (1d) to yield simple but sensitive signalling of solvent polarity[29] which could be of use in monitoring phase transitions. The methylene spacer and secondary amine units in (1d) contribute to the suppression of exciplex emissions, thus simplifying the fluorescence behaviour.

4 Higher Generations of PET Signalling Systems

A special feature of modular systems is that they can be progressively increased in sophistication in a controlled and stepwise manner by the designer in different directions for different purposes. Some of the directions under current con-



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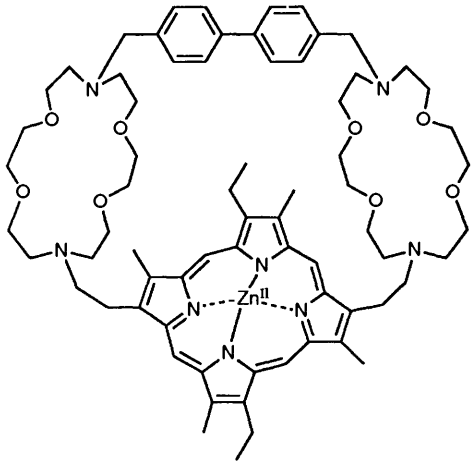

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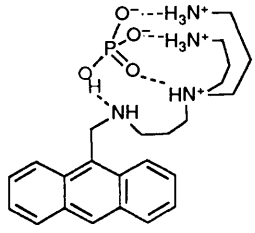
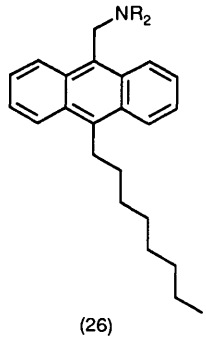


(25)

sideration are formalized in Figure 6 in terms of modification of the first generation 'fluor–spacer–receptor' system.

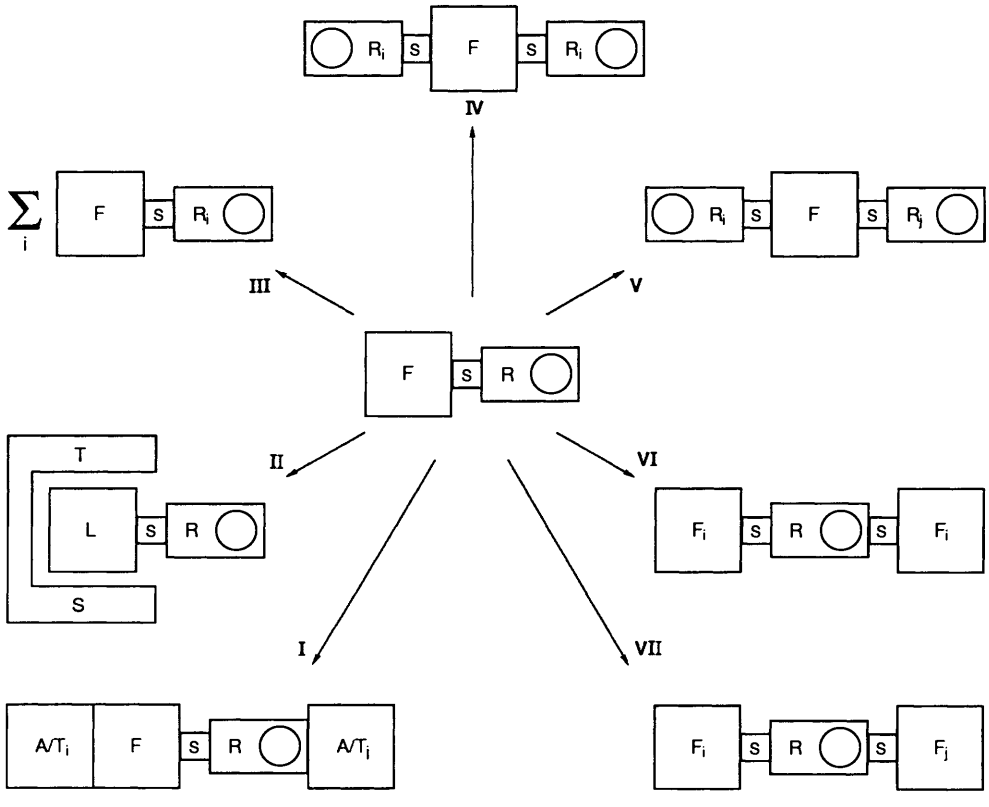
(I). The addition of two terminal targetting/anchoring modules allows the exploitation of the molecular nature of the PET signalling systems for the investigation of microheterogeneous media with high spatial resolution. Membrane-bounded protons lie at the heart of most energy transduction processes in biology and so we considered targetted pH-signalling systems, *e.g.* (26),[17] in detergent micelles, which are the simplest model membranes. While the sensor is anchored at the micelle–water interface, the microlocation of the proton-receptor module can be substitutionally tuned by variation of the hydrophobicity of either targetting module. Such depth-dependent pH measurement, reminiscent of a submarine periscope, allows the spatial mapping of pH near the membrane surface.

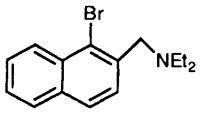
(II). The regioselective provision of a transparent shield module around the lumophore (as a generalized fluor) module allows the exploitation of phosphors with long-lived (ms) emis-



(26)

Figure 6 Formal extensions of the first generation 'fluor–spacer–receptor' signalling system. Key: F = fluor, S = spacer, R = receptor, A/T = anchor/targeting entity, L = lumophore (phosphor or environment-sensitive fluor), TS = transparent shield, $\sum_i$ = family with additive behaviour, $i \neq j$.

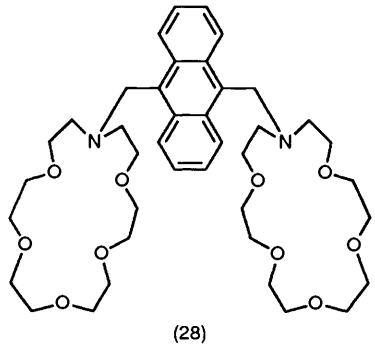




(27)

sion for interference-free time-resolved sensing in host systems with native fluorescence. Usually, phosphor excited-states are easily quenched by molecular oxygen and other triplet states and therefore are difficult to observe in fluid solution at room temperature. Steric protection of the phosphor module to prevent material contact with its environment while allowing access to communication photons is achieved by the transparent shield. Sensing remains viable because the receptor module is not encapsulated. It is notable that a regioselective self-assembly process is required here. This may remind the reader of a 'message in a bottle' which, however, differs from the classic case because the cork is responsive to its environment and controls the message being read through the bottle. Compound (27) in β-cyclodextrin is an experimental realization of this scenario in aqueous solution.[30]

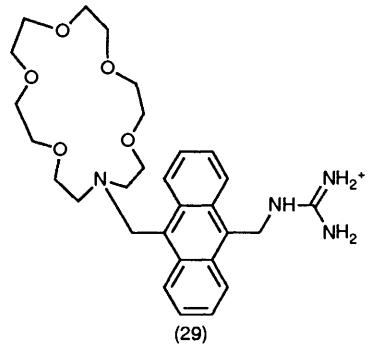
(III). An important outcome of the modular construction of fluorescent PET signalling systems is that, in suitable cases, all the members of a given family with a common fluor unit, *e.g.* (1b), will have essentially identical and pH-independent optical properties. Of course, the fluorescence intensity (I_F) will be strongly pH-dependent (according to equation 2) over a range of *ca.* 2 pH units and the pK_a value derived therefrom will be substituent dependent. Thus the I_F–pH profile of an equimolar mixture of n members can be computed by assuming additive behaviour and can be optimized towards linearity by choosing the relationship between pK_{ai} values. Such a quasilinear I_F–pH function with a wide dynamic range due to a molecular fluorescent system is a glass pH electrode mimic[17] and possesses the advantages of high spatial and temporal resolution for microenvironmental research applications.



(28)

(IV). The presence of a second identical receptor module can result in signalling systems which can selectively respond to homobifunctional guests. Since fluor modules are rigid almost by definition, the separation between the two receptor modules is approximately constant. This leads to fluorescence signalling upon recognition of the length of the guest molecule. Compound (28)[31] is a fluorescent sensor with quantitatively demonstrable atomic resolution of linear recognition. The guests putrescine and (less so) cadaverine, $H_3N^+(CH_2)_nN^+H_3$, $n = 4,5$, which are 'the molecules of death' are selectively signalled rather than the shorter and longer α,ω-alkanediyl diammonium and alkyl monoammonium ions. On one hand, optimized versions of (28) should allow remote signalling of cell death which is of use in early warning of pollution damage and food spoilage. On the other hand, (28) is an intelligent molecular device which communicates molecular geometric information directly to the most powerful of human senses, *i.e.* vision. So (28) is particularly ready for use in molecular optoelectronics/ionics. The one-step synthesis of (28) from known components is another positive aspect of its application to the two areas mentioned. It is also profitable to think of linear recognition as a rudimentary form of geometric recognition. From this viewpoint, (28) becomes the forerunner of fluorescent signalling systems which recognize various patterned arrays of functional groups in guests by means of a complementary set of receptor modules preorganized on a fluor framework.

(V). The presence of a second receptor module which is different from the first creates at least two possibilities. (a) The simplest logical outgrowth from class 4.IV is the development of systems for the linear recognition and fluorescent signalling of heterobifunctional guests. Both receptor modules should be capable of PET activity with the fluor for efficient signalling of linear recognition. However, the designer's task is simplified, at the expense of operational efficiency, if the requirements of PET activity is relaxed for one receptor module. Compound (29)[17] illustrates this approach with regard to the fluorescent signalling of the brain neurotransmitter γ-amino butyric acid and relatives. (b) A separate direction involves the coincidence signalling of two separate and different guests. This has important ramifications for molecular optoelectronics/ionics since molecular devices with self-selection of input signal channels become feasible.



(29)

(VI). The consideration of homobifluorophoric signalling systems introduces the phenomenon of excitation energy migration. If suitably efficient, this process can statistically enhance the probability (and hence the rate) of electron transfer from (to) the receptor to (from) the fluor. Thus, the guest-induced fluorescence enhancement can be larger than that for the corresponding first-generation signalling system. Homobifluorophoric versions of (12) indeed show this effect to a significant degree.[17]

(VII). Heterobifluorophoric signalling systems bring excitation energy transfer processes into operation. While the interplay of electron- and energy-transfer is interesting in its own right, it also creates the opportunity for PET activity in one fluor and not the other. This leads to self-calibrated signalling with one sensory channel and another for internal referencing.

Finally, it must be stressed that each of these classes 4.I—VII are potentially generalizable as was demonstrated for the first generation signalling systems in Section 3. Therefore we can believe that the PET design of 'fluor–spacer–receptor' signalling systems, though simple and innocent in concept, is quite general in scope and applicability. Further growth of this research front, both at conceptual and applied levels, should be expected.

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ana who got us started on this road. Space limitations prevented us from detailed discussion of many interesting results from many laboratories. Though many of these are cited within the references to this article, we hope to give them the attention they deserve elsewhere. A special thank you is due to Irene Campbell for her cheerful assistance in preparing this and other manuscripts. Finally, where would we be without Providence and Serendipity[32]?

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32 Ancient shipwrecked mariners referred to Sri Lanka as Serendib.