

# Photoionic devices with receptor-functionalized fluorophores

A. Prasanna de Silva, H. Q. Nimal Gunaratne, Thorfinnur Gunnlaugsson, Colin P. McCoy, Pamela R. S. Maxwell, Jude T. Rademacher and Terence E. Rice

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

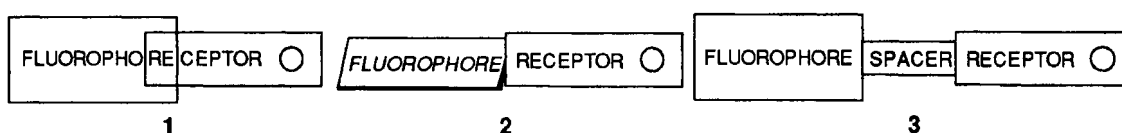
**Abstract:** Fluorophores can be connected to ion receptors so as to permit extensive overlap, be geometrically orthogonal or be separated by a spacer. Each of these three formats **1-3** has led to applications as ion sensors or switches. We focus on 'fluorophore-spacer-receptor' systems whose modular structure allows semiquantitative design according to the principle of photoinduced electron transfer (PET). The fluorescence of these systems can be switched 'on' or 'off' when confronted with ions. Several new examples **15-17** responding to  $\text{Na}^+$ ,  $\text{H}_3\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2^-$  and  $\text{H}^+$  are described. **17** and **18** are cases where kinetic factors become important in addition to the usual thermodynamic design criteria. Expansion of the basic three-module system by the addition of a separate receptor with a different ion binding profile as in **19** gives rise to molecular AND logic gates. On the other hand, OR logic gates such as **20** can be obtained from the basic system provided that the receptor is deliberately chosen to be nonselective under the conditions of use.

## INTRODUCTION

Molecular photoionic devices can be defined as chemical species which combine photonic and ionic interactions for useful purposes. Perhaps the most important of these are found in the rhodopsin systems within the eye. An incoming photon signal is eventually transduced into a flux of  $\text{Na}^+$  and  $\text{K}^+$  ions within the optic nerve reaching all of the way to the brain which registers the visual response (1). The fact that vision is our busiest information input channel adds to the value of this particular photoionic device. Of no less importance, at least to its bacterial owners, is the bacteriorhodopsin assembly which supplies the necessary energy as a photogenerated  $\text{H}^+$  gradient (2). There are far simpler synthetic systems, however, which perform functions which are sought by various groups of users. Our concern in this article is with devices which have ionic inputs and fluorescence output. Such ionically-responsive fluorescent molecules find their widest use as sensors (3) and switches. On one hand, ions of many types perform vital biological functions and need to be monitored in many situations of sickness and health. On the other hand, fluorescence photons are a natural bridge between our eyes and the molecular world we need to observe.

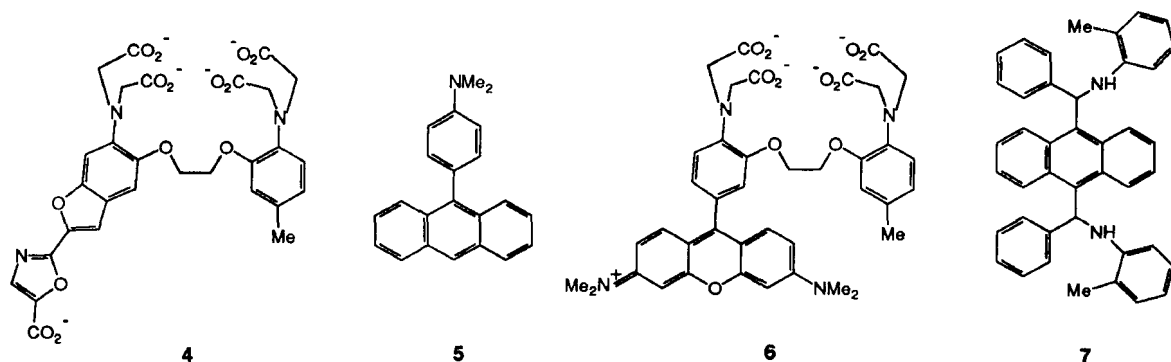
## CONNECTING FLUOROPHORES AND ION RECEPTORS

Fluorophores can be connected to ion receptors in at least three distinct formats **1-3** (4). The integrated



system **1** has extensive overlap between the  $\pi$  electron systems of the fluorophore and the ion receptor. Ion binding naturally leads to perturbation of the energy of the fluorophore excited state, especially if it has internal charge transfer (ICT) character (5) due to the presence of electron donor and acceptor terminals.

So ion-induced spectral shifts are to be expected. This is most clearly seen in the fluorescence excitation spectra. Emission spectral shifts are rarer, because of the movement of the ion out of the receptor during the lifetime of the emitting state (6). Such cation movement is caused by the electrostatic repulsion from the positive pole of the ICT excited state close by. The excitation (or emission) spectral shifts can be exploited for the fluorescence monitoring of intracellular ion concentrations. Roger Tsien (presently at San Diego) pioneered this chemical service to the biological research community (7). Sensor **4** is one of the most popular cases for the measurement and imaging of  $\text{Ca}^{2+}$  with micrometer and millisecond resolution. The twisted system **2** possesses two orthogonal  $\pi$  electron systems corresponding to the receptor and the fluorophore which is usually caused by steric influences. For example, Haruo Shizuka at Gunma described the molecule **5** (8) where the peri hydrogens of the anthracene force the 9-(4'-dimethylaminophenyl) group to take up a plane perpendicular to that of the anthracene. **5** is weakly fluorescent with an additional long-wavelength component, but protonation causes strong recovery of the anthracene-like emission. We find it convenient to analyse twisted systems such as Tsien's **6** (9) according to the principle of photoinduced electron transfer (PET) (10) to be described shortly. In general however, the theory of twisted intramolecular charge transfer (TICT) excited states must be applied to the twisted system **2** (11).



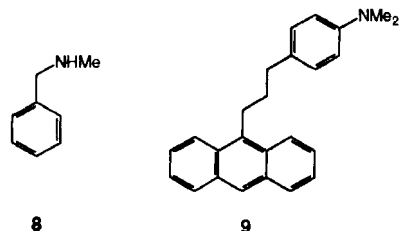
The third approach to connecting an ion receptor with a fluorophore is according to the spaced format **3**. Such 'fluorophore-spacer-receptor' systems are special because the spacer isolates the terminal functionalities in most respects except for a few relatively long range interactions. These cases can be considered quite legitimately to be supramolecular systems since their components can be readily recognised (12, 13). This leads to a simple molecular heredity where the ion binding properties and spectroscopic parameters (in both absorption and emission) of the assembled sensor is nearly quantitatively inherited from its parent fluorophore and receptor (14). The crucial exception is the fluorescence quantum yield of the sensor which is much lower than that of the parent fluorophore in well-designed cases. A long range (15) PET (16) from the ion-free receptor to the fluorophore which overpowers fluorescence is at the heart of this design. Upon encountering the chosen ion at a high enough concentration however, the receptor module becomes ion-bound and its oxidation potential increases sharply. Then the thermodynamics for PET become unfavourable. Such suppression of PET allows the fluorescence to become the dominant path for excited state decay. This leads to the 'switching on' of fluorescence upon ion admission to the sensor/switch (4, 17). Of course, the situation is reversible and ion removal allows the fluorescence to subside to its 'switched off' state. Such 'off-on' switching for fluorescence under ionic control is truly dramatic with fluorescence enhancement factors of two or three orders of magnitude not being uncommon (10, 18). The modular concept of the spaced system **3** along with its straightforward theoretical basis allows us to design sensors or switches to suit different ions over various concentration ranges and selectivity profiles. Similarly, different requirements of absorption colour, emission colour and even luminescence type (fluorescence or phosphorescence) can be satisfied. Another consequence of modular systems is their easy expansion. Additional modules with chosen functions can be appended to the basic 'fluorophore-spacer-receptor' system in order to carry ionically switchable fluorescence into new areas of research.

Fluorescent PET sensors and switches based on the spaced system **3** had grown out of an international research effort. **7** appears to be the earliest case where Tadao Nakaya *et al.* at Osaka noted its weak fluorescence (19). Its heart is the 9-aminomethylanthracene structure which has seen several reincarnations in sensors for various species (18a, c, 20). Following Albert Weller's seminal studies on PET at Stuttgart (21), the first fluorescent PET sensors **8** and **9** emerged. These arose from Herbert Morawetz's and Ben

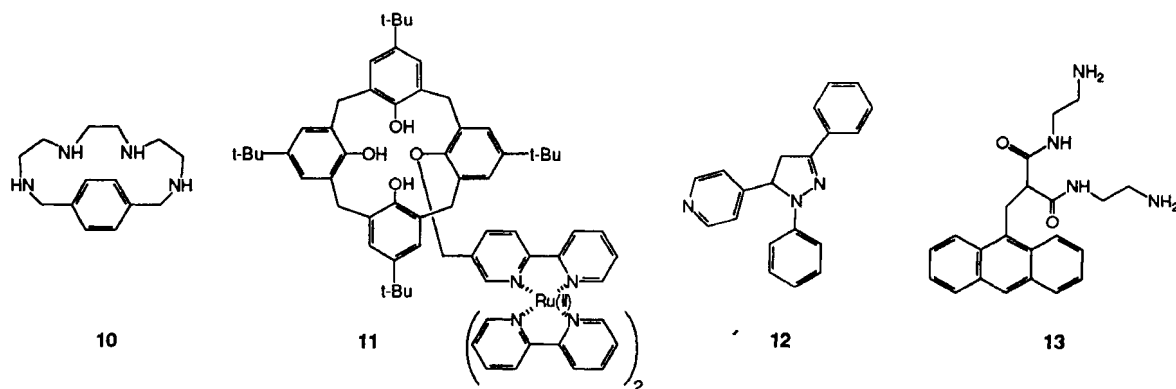
Selinger's laboratories in Brooklyn (22) and Canberra (23) respectively. In these instances, the receptor character of the amine group was recognised and  $H^+$ -induced fluorescence enhancement was observed. We have summarized the contributions of these and other pioneers in a recent review (17).

### RECENT EXAMPLES OF 'FLUOROPHORE-SPACER-RECEPTOR' SYSTEMS

The aminomethylaromatic motif continues to appear in successful fluorescent PET sensor designs for  $H^+$ . Polyazamacrocyclic **10** is such a case (24). The presence of multiple protonation sites can have several effects (18a, c). Multiple protonation requires rather high proton concentrations. Also the possibility of PET is entirely removed only when all the lone electron pairs of the amine units have been disabled. Therefore the pH dependence of the fluorescence intensity shows multiple sigmoidal steps and strong fluorescence enhancements are seen only at rather high degrees of protonation. As expected for fluorescent PET systems (17), **10** shows essentially the same  $pK_a$  values whether determined by fluorimetry, absorptiometry or potentiometry.



Amines are not the only receptors to be designed into fluorescent PET pH sensors. Incidentally, carboxylate receptors made an appearance in this context in 1989 (14). Phenolate and pyridine units have been represented by **11** and **12** respectively. It has been appreciated for some time that the luminescence of tris(2,2'-bipyridine)Ru(II) can be quenched by phenolates via PET (25). The corresponding phenols would be rather less effective quenchers via electron donation. So it was natural that an intramolecular version would produce an effective luminescent pH sensor. In **11**, the phenolate/phenol unit has been elaborated into a calix[4]arene (26). While **11** showed a  $H^+$ -induced fluorescence enhancement (FE) of 6, a derivative with two lumophore units could only manage a FE value of 1.8 in spite of the statistical advantage (10). This effect could be attributed to a steric inhibition of the solvent reorganization vital to rapid PET. The sensor **12** could be designed confidently from available data because the calculated thermodynamics for PET was favorable for protonated **12** while the opposite was true of unprotonated **12**. Thus, fluorescence

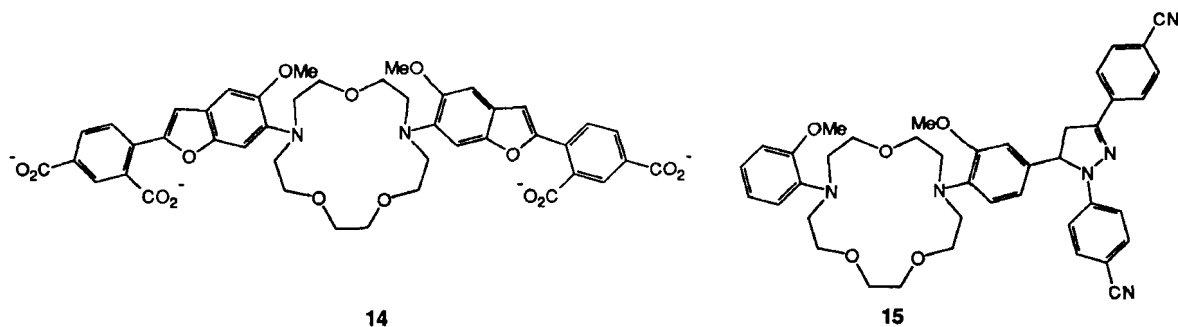


was expected to 'switch off' upon protonation (27). **12** was known in the literature as a fluorescent compound, but not as a sensor (28). A remarkable feature of the sensor family represented by **12** is that the emission spectra of individual members cover most of the visible region while they all show uniformly strong switching behaviour.

Sensors of the 'fluorophore-spacer-receptor' type **3** for d-block metal ions usually show ion-induced fluorescence quenching which can arise by PET and/or electronic energy transfer (29). The latter is feasible since many d-d metal centered excited states can lie at lower energies than the  $\pi\pi^*$  states of commonly used fluorophore units. In the case of **13**, the growth of a d-d absorption band characteristic of a Cu(II) complex clearly correlates with the fluorescence quenching as the pH is increased to encourage complexation (30).

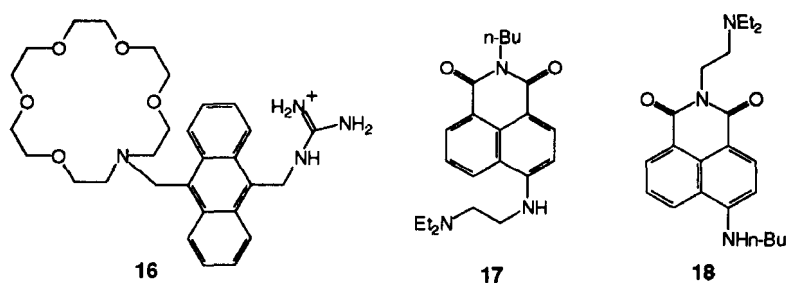
Tsien's fluorescent sensor **14** for  $Na^+$  is based on the integrated system **1** and gives a  $Na^+$ -induced shift of 12 nm in the excitation spectrum (31). We have now incorporated his core receptor into a spaced system **3** in order to obtain large enhancements of fluorescence emission in the presence of  $Na^+$ . Sensor **15** is designed as a PET system and shows good discrimination against  $K^+$  regarding both binding constants

(1300 versus 63) and FE values (12 versus 4.6) in methanol:water (1:1, v/v). The latter performance improves upon our previous cryptand-based example (32).



After some success with simple inorganic ions, sensor designers must now target more complex organic ions which are equally important in the biological arena. This challenge has been taken up by several laboratories in different ways (33). Our latest effort **16** has been designed to sense the brain neurotransmitter GABA ( $\text{H}_3\text{N}^+\text{CH}_2\text{CH}_2\text{CH}_2\text{CO}_2^-$ ). The PET-active azacrown ether unit binds the ammonium terminal of GABA whereas the guanidinium group holds the carboxylate end. The anthracene moiety serves a dual role as fluorophore and as the rigid backbone which confers linear recognition capability on **16**. In practice, **16** shows a FE value of 2.4 and a binding constant of 36 with GABA in methanol:water (3:2, v/v, pH 9.5). Importantly, the fluorescence of **16** does not respond significantly to the presence of glutamic acid or glycine.

The substantial literature on fluorescent PET sensors and switches can be largely understood on the basis of the thermodynamic criteria outlined in the previous section for the spaced system **3** (17). We now find



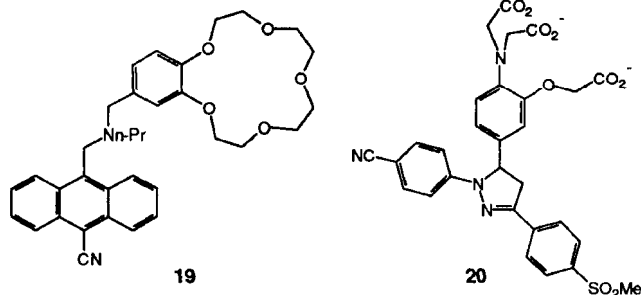
that regioisomers **17** and **18** are remarkable because kinetic factors are necessary to rationalize their  $\text{H}^+$ -responsive fluorescence behavior. When  $\text{H}^+$ -free, there is no thermodynamic obstacle to PET in either case. Nevertheless, **17** shows a large FE value of 25 upon protonation whereas **18** is largely unaffected.

This is because the 4-amino-1,8-naphthalimide fluorophore develops a strong dipole moment of 11D upon excitation which, depending on its orientation with respect to the transiting electron, can electrically help or hinder PET. This photogenerated molecular-scale electric field controls the kinetics of the electron transfer and the PET sensory capability of the device. Such 'one way' control of electron traffic may be relevant to understanding how the photosynthetic reaction centre operates (34).

## FLUORESCENT PET SYSTEMS AS LOGIC GATES

The previous sections have outlined how ionically-switchable fluorescent systems can be designed. Switchable fluorescence can serve as an easily comprehensible output signal from a molecular device. The exciting light would act as a power supply and various ions can provide input signals. Switches which supply an output only when certain input conditions are satisfied would then behave as gates with various logic functions. Molecular logic gates have excited the imagination of the public because of their potential in molecular computation. **19** is a molecular AND gate which outputs a high level of fluorescence only when  $\text{H}^+$  and  $\text{Na}^+$  inputs are supplied. From a sensor perspective, **19** responds to the simultaneous presence of  $\text{H}^+$  and  $\text{Na}^+$  i.e. it is a coincidence detector. **19** succeeds because its amine and benzocrown ether units are both PET-active and the PET processes are blocked when these receptors selectively bind  $\text{H}^+$  and  $\text{Na}^+$  respectively. The device **19** marshals two types of ions and two kinds of photons without any external connections - a remarkable piece of molecular self-organization (35).

Selective binding of each input ion by the correct receptor module was crucial to the successful operation of **19** as an AND gate. In contrast, OR gates such as **20** depend on the receptor unit to be practically



is detected with near-complete rejection of the former under simulated physiological conditions.

## CONCLUSION

This article is an attempt to show how useful photoionic devices can arise from the arranged marriage of ion receptors and fluorophores. The cooperativity between supramolecular/co-ordination chemists and photophysicists can be a very positive one. Molecular information gathering with sensors and molecular information processing with gates or switches are two fields (37) which stand to benefit from research into photoionic devices.

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