Current Developments in Fluorescent PET (Photoinduced Electron Transfer) Sensors and Switches


Published in:
Chemical Society Reviews

Document Version:
Peer reviewed version

Queen's University Belfast - Research Portal:
Link to publication record in Queen's University Belfast Research Portal

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Download date: 11. Jan. 2020
Following a brief introduction to the principle of fluorescent PET (photoinduced electron transfer) sensors and switches, the outputs of laboratories in various countries from the past year or two are categorized worldwide that we see today (Figure 1).7-9 The circumstances of the wide and sustained uptake of the principle by laboratories around the world has been reported in two reviews.10,11 ‘Hardly a noticeable change in a fluorescence signal would be easily observable by people, with or without instrumental augmentation. 45’

Key learning points:
1. Introduction
When we established the generality of the fluorescent PET (Photoinduced Electron Transfer) sensor/switch principle three decades ago, we were convinced of its semi-quantitative engineering design and of its visual appeal.1-3 After all, Weller had given us a strong thermodynamic basis for the intramolecular PET process since the 1960’s. He had also recognized the competition between fluorescence and PET for the deactivation of the excited state.4,5 The kinetic aspects had received Marcus’ crucial input.5,6 So our role had been to apply the competition scenario twice under intramolecular conditions - once when the sensor/switch was devoid of the analyte/target species and once again when the latter was present – so that the fluorescence signal would alter between the two situations. Such a change in a fluorescence signal would be easily observable by people, with or without instrumental augmentation. However, it would have been foolhardy in the 1980’s to predict the wide and sustained uptake of the principle by laboratories worldwide that we see today (Figure 1).7-9 The circumstances of the early days has been reported in two reviews.10,11 ‘Hardly a week goes by without a fluorescent PET sensor being reported’ was our observation in 2009,11 and the situation is no different in 2014. Indeed, 59 references from 2014 are specifically cited in this review, with a substantial fraction coming in for detailed study.

Figure 1a and 1b. Approximate world maps of sources of fluorescent PET sensors and switches aimed at single targets. Only the names of corresponding authors from the literature are given.
discussion, subject to space limitations. Of course, older references are cited in strength, so that the historical threads are fully exposed, theoretical foundations are suitably developed, and adequate contexts are set out from the viewpoint of our experience in establishing the generality of the field.

Binary possibilities arose from instances where the fluorescence signal change was large enough to be considered as 'off-on' or 'on-off'. These took on added significance in a world which was increasingly conscious of information technology. Thus, molecular information processors became possible in 1993.12 Although a myriad ways became available for molecules to be interrogated like semiconductor devices, the fluorescent PET sensor/switch principle provided the first approach and still remains a very profitable avenue.13-18

In this review, we concentrate on fluorescent PET sensors and switches which have appeared during the last year or so, while offering some context from the past. The emphasis of this review is on small-molecule sensors.

2. The fluorescent PET sensor/switch principle

It might be prudent to give a brief summary of the principle at the outset. In its commonest manifestation, a fluorophore is weakly coupled electronically with a receptor so that the two modules quantitatively maintain their individual properties in the photochemistry and supramolecular chemistry spheres. The two modules are chosen so that the excited fluorophore has sufficient energy to transfer an electron, say, from the receptor to the fluorophore. This means that the excited state energy is larger than the sum of the moduli of the oxidation and reduction potentials of the receptor and fluorophore respectively. If the experimental redox potentials are unavailable, modern quantum chemistry software can supply adequate estimates of the energies of the frontier orbitals of the two modules, and these can do the job almost as well. When the analyte/target species was bound to the receptor module, the oxidation potential would be significantly increased,19 so that the PET process fails and fluorescence re-asserts itself.

While electrochemical experiments and frontier orbital energy calculations are very useful as design tools, experimental proof of fluorescent PET sensor/switch behaviour requires the observation of radical ion species following fast laser photolysis. While Weller provided such evidence in intermolecular PET,4 intramolecular PET sensor/switch systems have been studied rarely.20,23 The laboratories of McClellan and Jonuasauskas join forces to provide a timely example 1.22 A broad absorption between 520 and 570 nm, which corresponds to the previously known radical anion band of the borodipyrromethene (BODIPY) fluorophore, appears and decays within 1 ns in THF solution. The radical cation of the receptor also appears, though less unequivocally, at 330 nm. The radical ions recombine at longer times to produce the fluorophore’s triplet excited state. A very fast PET rate of 3.3×1012 s−1 could be calculated under these conditions.

3. Proton targets

It is apt that protons represent the first target to consider. Their binding to appropriate receptors (Bronsted bases) is less complex than those of larger ions. Also, their role near membranes25,24 in bioenergetics is disproportionately important25 given their small size. A first-generation dendron containing a tertiary amine is employed as a receptor by Bojinov’s team within 2.28 When excited at 302 nm, the emission at 397 nm shows a H+-induced fluorescence enhancement (FEH+) of 7.6 with an associated pKa of 9.5. Research on related naphtalimide fluorophores is available.27 Interestingly, transition metal ions do not interfere significantly with 2’s performance perhaps because they would be held rather distant from the fluorophore.

Emission in the redder regions of the spectrum is preferred for intracellular studies, owing to less scattering and better penetration. Sun, Ge and co-workers provide an easily synthesized example 3,28 where x-ray crystallographic proof is available for the substantial rotation of the plane of the aniline ring with respect to the xanthene tricycle. Thus, PET across a virtual spacer from the aniline to the xanthene fluorophore becomes feasible, especially because the calculated HOMO of the aniline lies higher than energy in the HOMO of the xanthene.

Thus, excitation of an electron from the xanthene HOMO would leave a vacancy into which another electron can be transferred from the aniline HOMO. Protonation of the aniline removes this PET process and indeed, the fluorescence at 592 nm gives FEH+ = 400 (pKa = 4.7) when excited at 535 nm. Many other cell constituents do not interfere and acidic lysosomal regions of HeLa cells show up nicely in fluorescence microscopic experiments.

We return to the blue region where sensor 429 responds in an ’on-off’ (or NOT logical) manner to H+, following protonation of one pyrimidine and the diethylamino group. The second pyrimidine, which is nearby, does not protonate owing to electrostatic considerations.1H nmr evidence is offered for this double protonation. An H+-induced blue shift of 70 nm in the ultraviolet spectrum is also suggestive. Frontier orbital energy calculations show that, upon protonation of the diethylamino group, the fluorophore HOMO falls in energy below the HOMO of the unprotonated pyrimidine ring. Thus PET can occur from the unprotonated pyrimidine to the protonated aminocoumarin. The fluorescence at 460 nm indicates FEH+ = 0.025 (pKa = 2.1) when excited at 385 nm. E. Coli grown in media as acidic as pH 0.6 shows appropriately weak fluorescence from 4 within.

4. Alkali and alkaline earth ion targets

The next logical step would be to consider light metal ions. Since the McClellan-Jonuasauskas case 12 carries the famous Tsien receptor for Ca2+, it is no wonder that a FEH2+, value of 122 (logECa2+ = 6.3) is found by monitoring the fluorescence at 514 nm while exciting at 475 nm. As is the trend these days, frontier orbital energy calculations produce the appropriate HOMO energy ordering, i.e. the HOMO energy of the receptor lies higher in energy than the HOMO of the fluorophore.

K+ is the target for a strong team assembled by Holt.30 They construct 5, which interestingly shares the diethylaminocoumarin motif with 4.29 However, this unit is clicked onto a
phenylazacrown receptor and results in an excellent selectivity for the target over several potential interferents like Na⁺ and H⁺ at their normal intracellular levels. This is a very positive result for such a simple receptor and therefore it is not surprising that sensor 5 succeeds inside NRK cells. The fluorescence at 493 nm gives $F_E(Hg^{2+}) = 3.0 \times 10^{-2}$ when excited at 420 nm, due to $K_{int}$-induced arrest of PET across the virtual spacer.

$K_{int}$ was also the target, though not so selectively, for our very old work with 6. This work, along with that of Desvergne, Bouas-Laurent and Lehn, launched fluorescent PET sensors and switches as an important branch of supramolecular chemistry. Now, Wang and co-workers support the outcome of 6 by performing a detailed theoretical study of the PET process from the azacrown ether receptor to the anthracene fluorophore.

### 5. Transition metal and post-transition metal ion targets

Now we move to heavier metal ions. For instance Pd²⁺ has been fluoroimetrically sensed only rarely, but Kaur, Singh and colleagues build a phenylazacrown receptor carrying two sulfurs which communicates via a virtual spacer with the BODIPY fluorophore (as seen in 12). The soft base atoms in the receptor prepare 7 for selective binding to soft metals like Pd²⁺, though the impressive selectivity achieved cannot be explained away so simply. Indeed, Hg²⁺ is the only significant interferent which could be masked with cysteine. The fluorescence at 520 nm gives $F_E(Hg^{2+}) = 5.7 \times 10^{-2}$, in MeCN when excited at 488 nm. Frontier orbital energy calculations for the separate receptor and fluorophore support the Pd²⁺-induced arrest of the PET process.

In spite of 7's relative insolubility in water, the authors bravely conduct microscopy studies in a breast cancer cell line.

When it comes to metal ion receptors, nitrogen holds a near monopoly, in spite of its known pH sensitivity. Finney, Deiters and Lehn launched fluorescent PET sensors and 10 by performing a detailed theoretical study of the PET process from the azacrown ether receptor to the anthracene fluorophore.

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fluorescence quenching is caused by deprotonation of the benzimidazole so that the latter anionic unit can rotate out of the BODIPY plane. Now PET can take place from the benzimidazole anion to the BODIPY unit. The HF side-product picks up an additional F$^-$ to give FHF$. Addition of H$^+$ reverses the above effects.

A similar PET process from a deprotonated receptor to a BODIPY fluorophore is implicated in the case of Rurack and coworkers. In this case, F$^-$-induced deprotonation of a N-(4-nitrophenyl)thiourea is involved. The fluorescence of 16 at 513 nm gives $F_{E_{\text{F}}}$ = 0.47 ($\text{Log}F_{E_{\text{F}}}$ = 4.7) when excited at 482 nm in 1:1, DMSO:water at pH 6.8. Test strips containing 16 can be used to measure F$^-$ in lateral-flow readers, which augurs well for the future. Older F$^-$ sensors which share some structural features and operate in mixed aqueous solution are known. 56, 57

7. Reactive oxygen targets
The fluorescent PET sensor/switch principle can also be applied in the form of fluorescent PET reagents when the target reacts irreversibly as in the case of various thiols. 58-63 Some of these improve selectivity towards cases like glutathione versus simpler thiols by cleverly employing AND logic involving connected chemical inputs. 64 However, we choose to focus on reactive oxygen species (ROS) at this time. 65-68, 17, due to Xu, Qian and colleagues, has PET from the aminophenoxy moiety to the naphthalimide which renders 17 non-emissive. 65 Hypochlorite oxidizes the aminophenoxy unit to a separate quinoneimine and leaves a naphthalimide with a carbamate in the 3-position which emits at 460 nm when excited at 340 nm ($F_{E_{\text{OCl}}}$ = 71). This partly decarboxylates to yield a 3-aminonaphthalimide which emits at 570 nm ($F_{E_{\text{OCl}}}$ = 63). Only the 460 nm component of this dual emission is found when peroxynitrite is the ROS. Other ROS like hydrogen peroxide produce no emission at all. Even 7x10$^{-7}$ M hypochlorite can be detected in this way, even inside HeLa cells.

Kim and Kim’s 18 is non-fluorescent because of PET from the catechol moiety to the BODIPY unit. 64 Hypochlorite oxidizes the catechol unit to a o-quinone. Apparently, the possible PET to the latter group from the BODIPY unit does not occur. Though further analysis of this issue would be welcome, the experimental upshot is a rather selective $F_{E_{\text{OCl}}}$ value of 80 with a limit of detection of 3x10$^{-7}$ M.

8. Physicochemical property targets
A consortium of Xu, Cui, Qian, Spring and colleagues 20 exploit the flailing rotatory motion inherent in the generation of twisted intramolecular charge transfer (TICT) excited states 21 and the conformation-dependent PET to develop the viscosity sensor 19 and apply it to intracellular microscopy studies. Fluorescence intensity and lifetime are both put to use. Nice viscosity maps with organelle-level resolution are produced. The thermodynamics of TICT states 22 and PET processes 20 are very similar since a radical ion pair is produced in each case, though there are subtle differences in a sensor context. 73 Structurally, 19 contains two fluorophores - aminonaphthalimide and anthracene - and an electron donor aniline. The latter shows no metal binding ability and displays a $pK_a$ value of 3.1. So, protonation-based interference would not be expected even in some of the most acidic regions, i.e. lysosomes with pH values around 5. However, a word of caution would be advised here since organelle membranes can easily concentrate H$^+$ at nanometric distances near them by several orders of magnitude, thus encroaching on 19’s $pK_a$ value of 3.1. Such effects are known from model membrane studies. 23, 24 Classical optical microscopy images would not be able to resolve such effects. The presence of two fluorophores leads to electronic energy transfer (EET) effects too, and almost exactly this pair has been studied in a ratiometric PET/EET-based pH sensor context before. 74 The presence of the anthracene emission serves as an internal reference for the viscosity-dependent emission of the aminonaphthalimide moiety.

As seen in other PET sensors, 75, 76 the fluorescence of 19 has a substantial polarity effect, i.e. the total quantum yield goes from 0.004 in viscosity-dependent emission serves as an internal reference for the viscosity-dependent emission of the aminonaphthalimide moiety.

9. Multiple targets
Given the molecular engineering backdrop to the fluorescent PET sensor/switch principle, chemical emulsion of physical devices 11 was an associated discipline from the early days. While logic devices receive a lot of airtime, 10 some attention has to be given to their components. The triode 82 which is one of those, is a three-electrode assembly where the input to one electrode influences the output from another. We arrange something similar by using a “fluophore-spacer-receptor-spacer-receptor,” system 21, 83 where receptor1 and receptor2 target H$^+$ and Na$^+$ respectively. However, only receptor1 is PET-active. The upshot is that a sigmoidal fluorescence intensity – pH profile is tuned by altering Na$^+$ concentration. Electrostatic repulsion between the receptor-bound H$^+$ and receptor-bound Na$^+$ is responsible for this tuning by influencing the $pK_a$ value of the sensor. It is only fair to note that Gust, Moore, Moore and their colleagues published an all-photon triode emulsion in 2010. 84 AND logic gate 22, 85 due to Furrugia and Magri, contains an
amine moiety to interact with $H^+$ and a ferrocene moiety to respond to redox inputs. Both moieties are PET-active and fluorescence is weakened as a result. Arrest of both these PET pathways by the provision of $H^+$ and an oxidizing equivalent leads to switching ‘on’ of fluorescence. However there is a residual PET process from the fluorophore to the ferricinium moiety (in the oxidized form of 22), which puts an upper limit on the FET-redox value. 22 is logistically extended to 23^{90} by Magri and his colleagues. They do it by the addition of a PET-active benzocrown ether receptor to interact with $Na^+$ inputs. A $H^+$, redox, $Na^+$-driven AND gate is the result, which would become a ‘lab-on-a-molecule’^{97} for direct detection of some cancers which possess elevated $H^+$, $Na^+$ and free iron.

The original approach to a ‘lab-on-a-molecule’^{87} exploited a small set of selective receptors which communicated intramolecularly with a fluorophore, which then provided a binary readout to a human user. At least conceptually, this approach can be modified to contain a set of relatively nonselective receptors, e.g. phenylboronic acids to tackle a set of sugar-based drugs. In compensation, a set of fluorophores can be built into the supermolecule so that the fluorescence intensities at several wavelengths serve as readouts. The intensity patterns can be chemometrically analyzed^{88} for further sharpening of the results so that the drugs become distinguishable with confidence. Margulis’^{24^{90}} is the first successful case of this kind, showing that several of the imaginable pitfalls due to the complexity of the structures do not arise. Though the design is broad enough to embrace multiple photochemical mechanisms, PET remains at its heart since several arylmethylamine^{90} units are present within 24. The binding of sugar-based drugs to aminomethylphenylboronic receptors will then affect fluorescence intensities at a primary level. An internal charge transfer (ICT) mechanism^{79} also affects fluorescence intensities at some wavelengths at a primary level due to the presence of a fluorophore with push-pull groups. Electronic energy transfer (EET) between the different fluorophores serves to modulate the fluorescence intensity pattern at a second level.

As found in several cases within section 5, the bispicolylamine moiety also stars within Akkaya’s 25^{95} where PET is arranged to occur from the bispicolylamine unit to the BODIPY fluorophore. However, the special feature of this work is that $Zn^{2+}$ is supplied to 25 by a photo-uncaging procedure.^{93} $Zn^{2+}$ is held within the ‘cage’ of 26 and is only released when a 360 nm light dose decomposes 26 to 27 and N-methylpicolylamine. The mechanism here is a rather classical bit of organic photochemistry. The $\pi$ triplet excited state of the nitroaryl unit causes an intramolecular hydrogen abstraction via a six-membered ring intermediate. This work has the wider vision of physically integrating molecular logic gates by using metal ions as the linker species. In this context, the 360 nm light dose is input. The powerful general complexant EDTA is input. Since EDTA would swallow up any $Zn^{2+}$ to prevent the fluorescence activation of 25, it serves as a disabling input. Thus 26 is a light dose, EDTA-driven INHIBIT(EDTA) gate where EDTA is the disabling input. Its output of $Zn^{2+}$ then feeds the YES logic gate 25 whose own output is its fluorescence. Similar physical concatenation of logic gates aided by $H^+$ was known previously.^{94,95}

Molecular keypad locks^{96} are interesting examples of logical molecules which are history-dependent, i.e. the output signal value depends on the order of addition of the inputs. One way of arranging this history-dependence is to exploit multivalent interactions so that the full disconnection of complexed species becomes sluggish. Then, several kinetically stable states may appear for a given system. Being replete with aminomethylphenylboronic acids, Margulis’^{24^{90,97,98}} can engage in such multivalent interactions with inputs chosen from a set of many sugars as well as the catechol derivative 28. Thus it can also serve as a molecular keypad lock with new features which are more reminiscent of semiconductor-based counterparts. One of these new features is that inputs can be repeated to result in passwords such as 333. In the present instance, this means that the intensity pattern obtained by reading at several wavelengths (following chemometric analysis, if necessary) is dependent on the input species concentration, i.e. when the latter is doubled or trebled. Another of these new features is that multiple passwords can be declared as being valid to open the lock. Of course, this requires a separate definition of ‘open’ and ‘closed’ states, which is best done from a Boolean standpoint.^{10}

10. Conclusions and perspectives for future research

The work surveyed above reveals several trends. One of these is that many fluorescent PET sensor/switch laboratories are augmenting their research with frontier orbital energy calculations conducted through general software. This move to appreciate the physicochemical design aspects is to be applauded, especially because the fluorescent PET sensor/switch principle started off as an exercise in molecular engineering.^{3} Dedicated papers on calculations are also being published,^{99,100} so that additional insights can be gained. Another of these trends is that a large fraction of the publications are including intracellular evaluations. This move to be involved in the physiological application aspects is important because fluorescent PET sensors are tools. Tools are meant to be used so that they shed light on cellular processes involving the analytes. Tsien’s pioneering work along this line remains inspirational.^{101} Just as fluorescent PET sensors are being associated with cells, they can also be anchored on polymer particles of various kinds.^{102,104} Though there is no room for detailed discussion, we cite additional references to the fluorescent PET sensing/switching literature from 2014,^{105-138} where a few cases concerning mechanisms which closely related to PET are also included.
As noted in the previous paragraph, Tsien’s work\textsuperscript{101} still has much to guide future developments in the field of fluorescent PET sensors and switches. For instance, he showed how real-time analysis tools of this kind can revolutionize the understanding of intracellular signalling. A valuable lesson from this is that the fluorescent PET sensing community needs to conduct more fluorescence sensing studies in real time. Currently, most studies are reporting single fluorescence micrographs in the presence of the target species. If these can be extended to a set of images in time sequence as the cell goes about its business, the value of the results will be greatly enhanced. During this process, challenges with respect to sensor photostability, sensor survival in the face of cellular processes and calibration of target species concentrations will need to be faced.

Further exploitation of fluorescent PET sensors and switches in the future will also benefit if they can operate deeper within tissue. Such multi-cell monitoring can produce important information about cell-cell communication. Two-photon fluorescence versions of fluorescent PET sensors should be able to achieve this by employing red or near-infra red photons for excitation. Once the excited state is produced, the usual PET excitation. Once the excited state is produced, the usual PET information about cell-cell communication. Two-photon fluorescence sensing studies in real time. Currently, most studies are reporting single fluorescence micrographs in the presence of the target species. If these can be extended to a set of images in time sequence as the cell goes about its business, the value of the results will be greatly enhanced. During this process, challenges with respect to sensor photostability, sensor survival in the face of cellular processes and calibration of target species concentrations will need to be faced.

We are grateful to DEL Northern Ireland, X. G. Ling and L. H. Wang for support and help.

Notes and references

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Fluorophores can be combined with receptors according to a molecular engineering design to yield fluorescent sensing and switching devices.

The authors came to study for their PhD at Queen’s University Belfast, Northern Ireland, from places as far apart as Zhenjiang, Belfast and Colombo. Besides the chemistry day jobs, Brian (left) brings up his two daughters, Jue (centre) plays basketball and AP (right) plays percussion with an Irish traditional band.