



MONTCLAIR STATE
UNIVERSITY

Montclair State University
**Montclair State University Digital
Commons**

Theses, Dissertations and Culminating Projects

8-2005

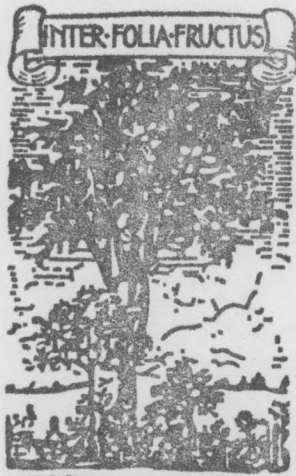
Fluorescent Photoinduced Electron Transfer (PET) Cation Sensors Derived from Natural Products

Kenny Loo

Follow this and additional works at: <https://digitalcommons.montclair.edu/etd>



Part of the [Biochemistry Commons](#), and the [Chemistry Commons](#)



Harry A. Sprague
Library
Montclair State
University

MONTCLAIR STATE UNIVERSITY

FLUORESCENT PHOTOINDUCED ELECTRON TRANSFER (PET) CATION
SENSORS DERIVED FROM NATURAL PRODUCTS

by

KENNY LOO

A Master's Thesis Submitted to the faculty of

Montclair State University

In Partial Fulfillment of the Requirements

For the Degree of


MASTER OF SCIENCE

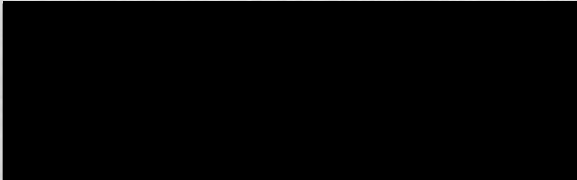
August 2005

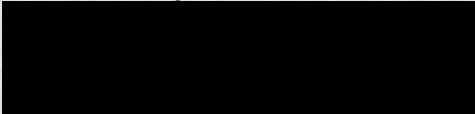
School Science and Mathematics

Thesis Committee

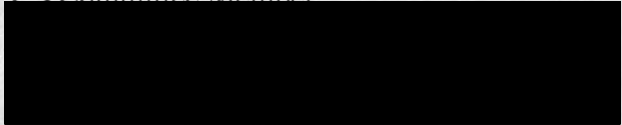
Department Chemistry and Biochemistry

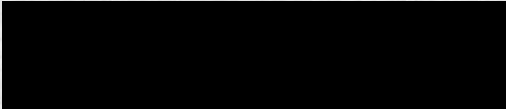

S. A. de Silva
Thesis Sponsor


R. Prezant
Dean


J. L. Isidor
Committee Member

8/9/05
Date


M. A. Whitener
Graduate Advisor


M. L. Kasner
Department Chair

Acknowledgements

I would first and foremost like to thank Dr. Saliya de Silva, my research advisor, for his help, advice, all the support and encouragement you have given me throughout this work. Additional thanks go to all members of Dr. de Silva's research group that participated in this extensive research.

I would also like to express my gratitude to the following members of the Chemistry and Biochemistry Department: Dr. J. L. Isidor for acquiring NMR spectra and Mr. Kevin Olsen for assistance in the use of laboratory instrumentation.

Finally I thank my family and friends for their love and support.

Abstract

Two new second generation fluorescent switches that are based on the chromophore-spacer-receptor architecture are reported in this thesis. The fluorescence modulation of these switches is a result of quenching or initiating photoinduced electron transfer (PET) between the chromophore and the receptors due to cation binding. These two molecules utilize an alkaloid, anabasine, as the proton receptor, and function as fluorescent off-on-off switches with decreasing pH. One of these molecules also utilizes a coumarin as the chromophore, making it the first rationally designed fluorescent PET system based on natural products. Further studies of two well-known first generation fluorescent switches to achieve more complex fluorescence modulation are also reported in this thesis.

Publications

“A fluorescent “off-on-off” proton switch derived from natural products and further studies of fluorescent “off-on” and “on-off” proton switches” de Silva, S. A.; Loo, K. C.; Amorelli, B.; Pathirana, S. L.; Nyakirang’ani, M.; Dhramasena, M.; Demarais, S.; Dorcely, B.; Pullay, P.; Salih, Y. A. *J. Materials Chem.* **2005**, *15*, 2791.

“A fluorescent ‘off-on-off’ proton switch with an overriding ‘enable/disable’ sodium ion switch” de Silva, S. A.; Amorelli, B.; Isidor, D. C.; Loo, K. C.; Crooker, K. E.; Pena, Y. E. *Chem. Commun.* **2002**, 1360.

Table of Contents

	Page
Acknowledgements	iii
Abstract	iv
Publications	v
Table of contents	vi
List of Figures, Schemes and Tables	vii
Introduction	1
Goal	5
Results and Discussion	6
Conclusion	14
Experimental	16
References	19

List of Figures

		Page
Figure 1	Examples of first generation fluorescent PET systems.	2
Figure 2	Example of a second generation PET sensor.	4
Figure 3	Synthesis of PET sensors 1 and 2 .	6
Figure 4	pH dependence of the Φ_f of PET systems 1 and 2 .	8
Figure 5	Fluorescence spectra of a mixture of 3 and 4 .	12
Figure 6	pH dependence of the fluorescence intensity of 3 and 4 .	13
Scheme 1	MO diagram for 'off-on' fluorescence switching.	3
Scheme 2	MO diagram for 'on-off' fluorescence switching.	4
Scheme 3	Protonation and PET processes of 1 with changing pH.	9
Scheme 4	Protonation and PET processes of 3 and 4 with changing pH.	11
Table 1	Absorption, emission and quantum yield data of sensors 1 and 2 .	15

Introduction

The absorption of light by a chromophore promotes an electron from the highest occupied molecular orbital (HOMO) to the lowest unoccupied molecular orbital (LUMO). The excited chromophore can now donate an electron to an acceptor, or accept an electron from a donor, leading to radical ions formation. This photoinduced electron transfer (PET) has many fundamental and practical implications ranging from photosynthesis and biological redox mechanisms to the development of devices such as sensors, switches and molecular motors.¹

The signaling process of fluorescent PET sensors depends on a molecular recognition event to inhibit, or initiate PET between a covalently linked chromophore and a receptor, leading to the generation (off-on), or quenching (on-off) of fluorescence. A large number of these PET systems that follow the basic design principle of a chromophore-spacer-receptor architecture has been reported to date.² The signaling capability of these systems has evolved from simple off-on or on-off switching of fluorescence in response to a single cation, to complex fluorescence modulation in response to multiple cations. One way of following the evolution of these PET systems would be to follow the number of PET processes that is possible in each system. First generation PET systems have one PET process that is controllable by one guest-binding event. Similarly, second generation PET systems have two PET processes each of which is controllable by a guest-binding event. By this count, the most complex systems

reported to date are third generation PET systems that are capable of three different PET processes in response to three cation binding events.³ The signaling capabilities of these systems have also evolved from simple 'off-on' or 'on-off' fluorescence modulation to more complex patterns such as 'off-on-off' fluorescence modulation.

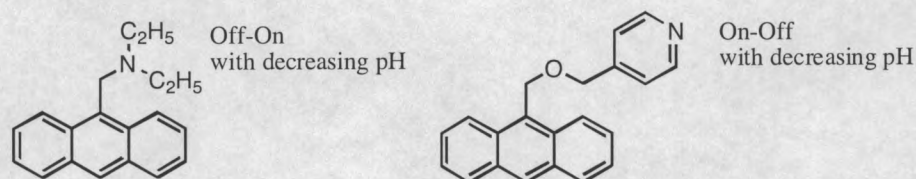
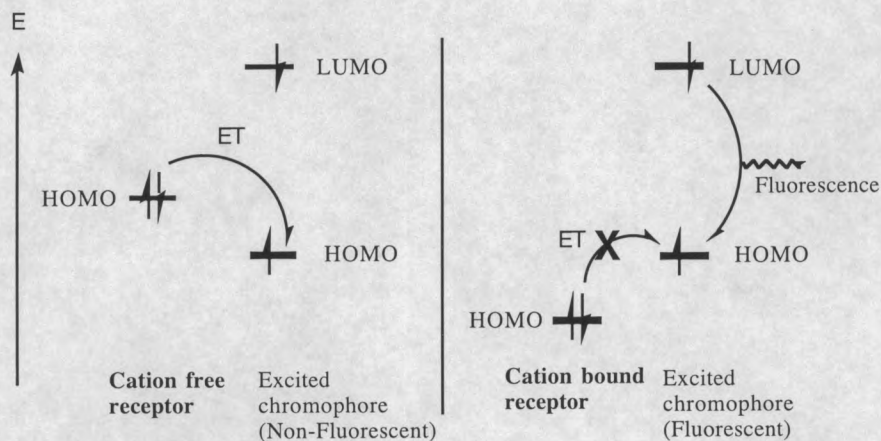


Figure 1: Examples of first generation fluorescent PET systems.

The driving force for the electron transfer (ΔG_{et}) in a fluorescent 'off-on' switch can be expressed by a modified Weller equation as follows:

$$\Delta G_{et} = -E_s - E_{red,chrom} + E_{ox, receptor}$$

E_s , $E_{red,chrom}$ and $E_{ox,receptor}$ are the singlet energy and reduction potential of the chromophore and oxidation potential of the receptor respectively. An increase in the oxidation potential of the receptor due to binding of a cation to the receptor will increase the ΔG_{et} , preventing the photoinduced electron transfer. This will allow the excited chromophore to relax by emission, leading to the fluorescent 'off-on' switch and is illustrated by a simplified molecular orbital diagram as follows:

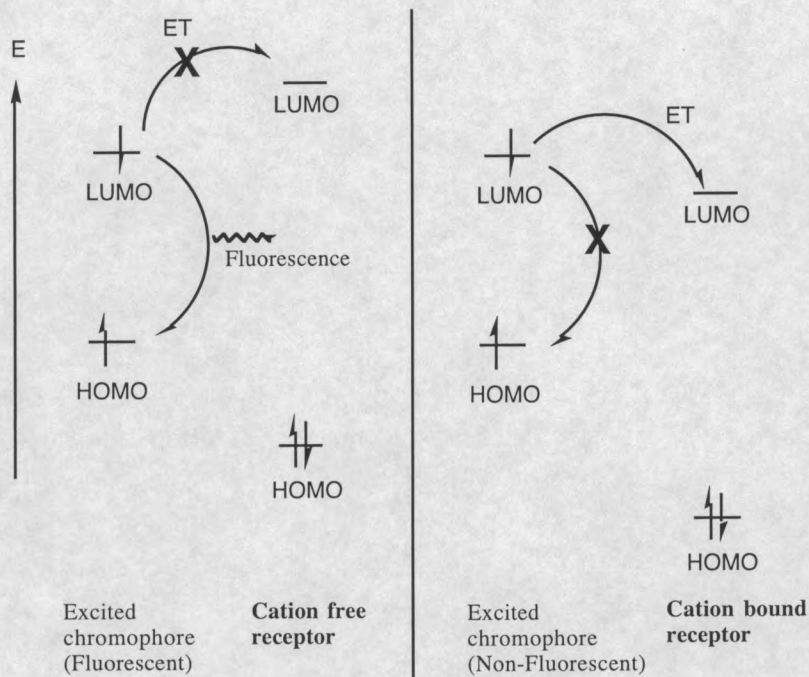


Scheme 1: MO diagram for 'off-on' fluorescence switching with cation binding.

The driving force for the 'on-off' switch is described by the following Weller equation:

$$\Delta G_{\text{et}} = -E_s + E_{\text{ox.chrom}} - E_{\text{red.receptor}}$$

where E_s , $E_{\text{ox.chrom}}$ and $E_{\text{red.receptor}}$ are the singlet energy, oxidation potential of the chromophore and the reduction potential of the receptor, respectively. In this case a decrease in the reduction potential of the receptor, also caused by the binding of a guest, will decrease the ΔG_{et} to the point where an electron transfer process from the excited chromophore to the receptor becomes thermodynamically favorable. This in turn will quench the fluorescence of the excited chromophore resulting in "On-Off" fluorescent switching. These molecules can also function as fluorescent sensors for a particular guest. This process can be shown schematically by the following simplified MO diagram (Scheme 2):



Scheme 2: MO diagram for “on-off” fluorescence switching with cation binding.

The combination of two proton receptors of the two first generation PET sensors shown in Figure 1, the tertiary nitrogen and the pyridine ring, has led to the second generation PET sensor shown below. This sensor combines the two PET processes mentioned above in one proton receptor (with two protonation sites) to give fluorescent ‘off-on-off’ modulation with decreasing pH.

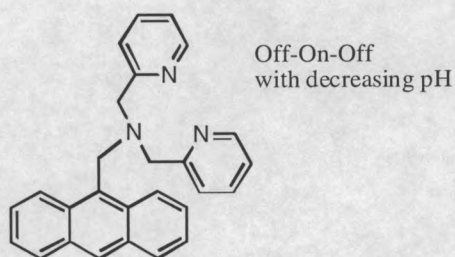
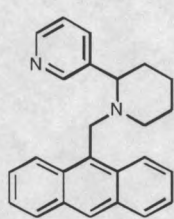
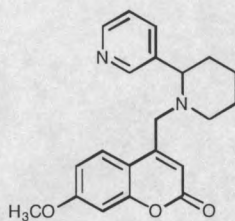
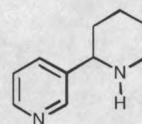


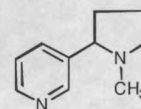
Figure 2: Example of a second generation PET sensor.

Goal

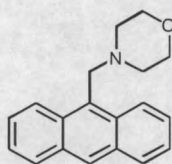
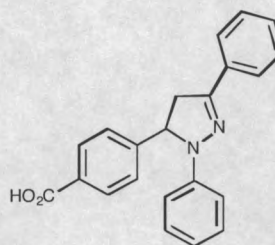
The main goal of this thesis is to develop fluorescent PET sensors that derive components from natural products.⁴ Two new second generation PET systems, **1** and **2**, that utilize anabasine (an alkaloid closely related to nicotine) as a proton receptor, are reported in this thesis. In addition, the chromophore of **2** is derived from coumarin, making it the first PET sensor to utilize a chromophore and a receptor derived from natural products. The second goal is to demonstrate that complex fluorescence modulation, usually shown by higher generation PET sensors, can be mimicked by individual first generation PET systems operating simultaneously. Two first generation PET systems, **3** and **4**, have been studied in the same solution as a part of this thesis.

**1****2**

Anabasine



Nicotine

**3****4**

Results and Discussion

Synthesis of Sensors 1 and 2

Sensors **1** and **2** were prepared by alkylating the secondary nitrogen of anabasine with 9-chloromethylantracene, and 4-bromomethyl-7-methoxycoumarin, as shown in Figure 3. The reaction mixtures were extracted with 3M HCl to separate the product from the unreacted starting material after refluxing for 18h. The acid layers were neutralized with sodium carbonate, extracted into dichloromethane and evaporated to give oily residues that were purified by column chromatography. The yields of sensors **1** and **2** were to yield 59% and 55% respectively.

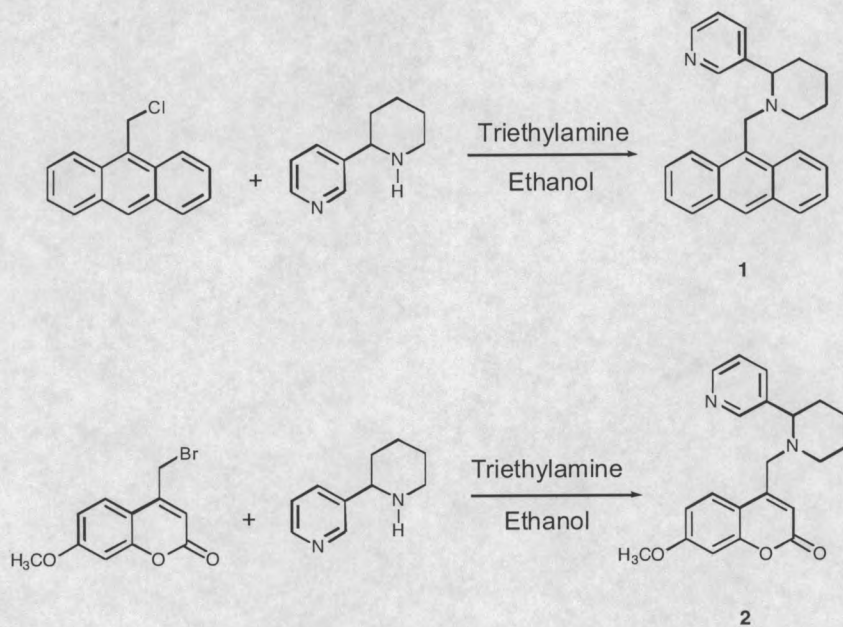


Figure 3: Synthesis of PET sensors **1** and **2**.

Fluorescent “off-on-off” proton switches derived from natural products

The two new PET systems, **1** and **2**, are based on several higher generation PET systems reported recently. From the viewpoint of fluorescent PET system design, natural products can offer interesting and non-trivial components for use as chromophores, spacers and receptors, some of which are even pre-assembled into the required formats. In designing PET systems **1** and **2**, we have utilized a piperidine alkaloid, anabasine, as the proton receptor. Anabasine is a secondary metabolite that is isomeric and structurally very similar to the well known pyrrolidine alkaloid, nicotine.⁵ We have successfully demonstrated that anabasine could be used as the proton receptor in higher generation PET systems.^{3a} The two PET systems are prepared by the alkylation of the secondary nitrogen of anabasine with 9-chloromethylantracene or 4-bromomethyl-7-methoxycoumarin. The chromophore of **2** is derived from coumarin,⁵ also a natural product that has been used in several cation sensors including PET systems.⁶ This is the first rationally designed PET system to derive both components, the chromophore and the receptor, from natural products. The fluorescence modulation of **1** and **2** with decreasing pH clearly demonstrates an off-on-off switch in both cases (Figure 4) as expected.

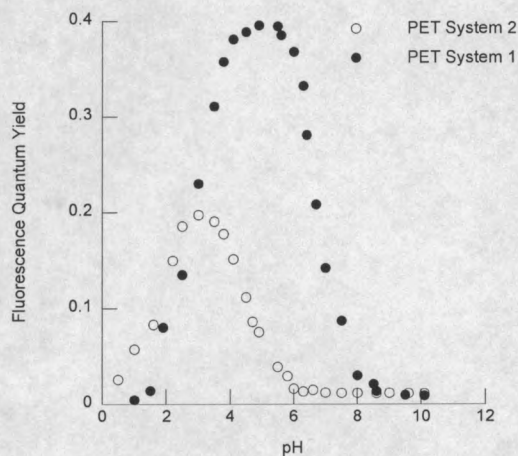
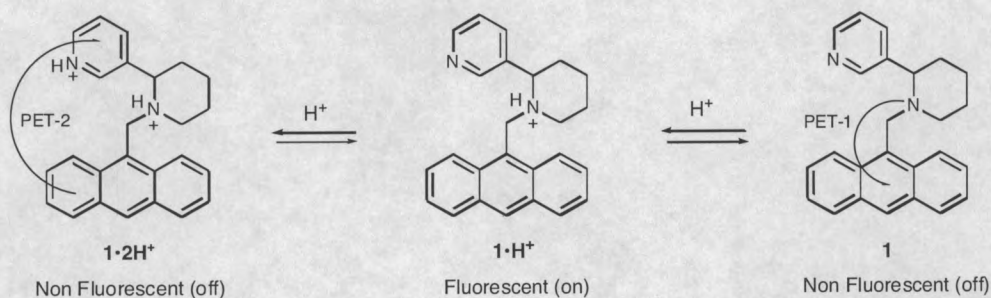


Figure 4: pH dependence of the fluorescence quantum yields of PET systems **1** and **2** in methanol/water(1:1) (**1**: $\lambda_{ex} = 350$, $\lambda_{em} = 370 - 550$ nm; **2**: $\lambda_{ex} = 330$, $\lambda_{em} = 350 - 550$ nm). pH adjusted by adding HCl or NaOH.

The intensity of fluorescence vs pH profile in both cases is in agreement with our original second generation PET system that utilized a bis(2-picolyl)amine proton receptor.⁷ The pK_a values for the protonation of the tertiary nitrogens of **1** and **2** are 6.8 and 4.6, respectively. This difference in pK_a values for the first protonation of **1** and **2** can be attributed to the proximity of the two different chromophores that would influence the tertiary nitrogens. The coumarin is electron withdrawing at its position of attachment due to the neighboring carbonyl group making the amine pK_a values differ by 2.2 for the two PET systems. The pK_a values for the protonation of the pyridine rings of **1** and **2** are 2.8 and 1.8, respectively. The pK_a values for the second protonation are closer since the two chromophores would have a lesser influence on the pyridine rings due to the larger

distance between the chromophores and the pyridine rings. The protonation of **1** and the PET processes that are inhibited and initiated with decreasing pH are shown in Scheme 3.



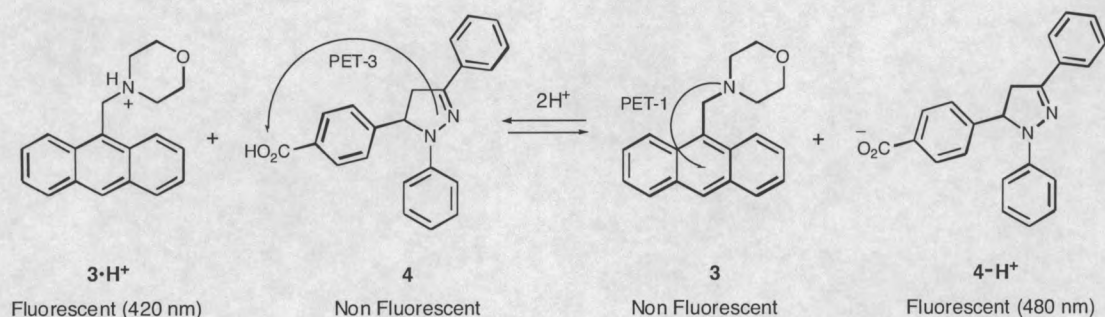
Scheme 3: Protonation and PET processes of **1** with changing pH.

The fluorescence of **1** is quenched due to the thermodynamically favored PET between the tertiary aliphatic nitrogen (tertiary amine) and the excited chromophore (*Anth), PET-1. Increasing the oxidation potential of the tertiary amine due to protonation inhibits PET-1 and regenerates the fluorescence of the chromophore. Therefore formation of $1 \cdot H^+$ is accompanied by an increase of the fluorescence intensity which reaches a maximum at pH 5.0. Further decrease of pH protonates the pyridine ring of the receptor to form a pyridinium (pyH⁺) group, leading to a significant change in its reduction potential. A secondary PET process, PET-2, (*Anth to pyH⁺) now becomes thermodynamically favorable and quenches the fluorescence of $1 \cdot 2H^+$ leading to the second off mode. Both PET processes are well documented and have been shown to be exothermic by calculating the ΔG values using the Weller equation.^{3a, 7, 8} Since **1** and **2** are capable of utilizing two PET processes to indicate two cation binding events, they are classified as second generation PET systems.

Further studies of first generation PET systems

During the course of our studies to develop PET systems that function as off-on-off fluorescent switches for proton, we decided to study two independent off-on and on-off fluorescent switches for protons, in the same solution. In order to observe the two switches independently, it was necessary to select two PET systems that had different emission spectra. This led us to PET systems **3**⁹ and **4**,¹⁰ which utilize two different chromophores, an anthracene and a pyrazoline, that can signal protonation with different emission spectra. The two first generation PET sensors **3** and **4** are classic examples of fluorescent off-on and on-off switches for protons. The fluorescence off-on switch of **3** has served as the model for many PET sensors including **1** and **2** described here.

Decreasing pH protonates the tertiary nitrogen of **3** and leads to the regeneration of fluorescence by inhibiting PET-1 as described above. The pyrazoline based PET system, **4**, is fluorescent at high pH and non-fluorescent at low pH. This is due to the conversion of the carboxylate to an electron withdrawing carboxylic acid with decreasing pH, which initiates PET-3 and quenches the fluorescence of the pyrazoline chromophore. The protonation and signaling of these two PET systems is summarized in Scheme 4.



Scheme 4: Protonation and PET processes of **3** and **4** with changing pH.

The excitation spectra of **3** and **4** overlap sufficiently so that both can be excited at the same wavelength (350 nm). The emission spectra of the anthracene and pyrazoline chromophores are sufficiently separated to allow nearly independent monitoring of **3** and **4**, with λ_{max} at 420 nm and 480 nm, respectively. With a common excitation wavelength and separate emission wavelengths these two PET systems are well suited to monitor two different protonation processes in one solution. However, the anthracene is significantly more fluorescent than the pyrazoline leading to an imbalance in the intensities of the two signals. In order to compensate for this imbalance, we use less of **3** in preparing the mixture of the two PET systems. Figure 5 shows the fluorescence spectra of a mixture of **3** and **4** at pH 10 and pH 2.5.

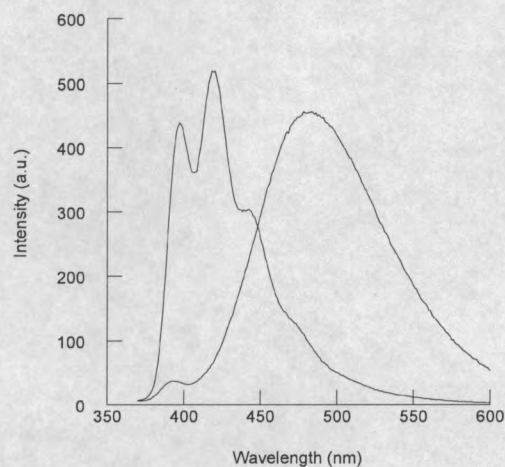


Figure 5: Fluorescence spectra of a mixture of **3** and **4** at pH 2.5 (left) and at pH 10 (right) in 1:1 methanol/water, $\lambda_{\text{ex}} = 350$ nm (see experimental for details).

The fluorescence modulation of the mixture of **3** and **4** with pH (Figure 5) closely resembles the behaviour of the independent PET systems. Monitoring the mixture at 396 nm and 480 nm with decreasing pH, allows us to monitor the fluorescent off-on switch of **3**, and the on-off switch of **4**, respectively. Since these are two independent spectra, the intersection of the two fluorescence spectra occurs between 420 and 450 nm from pH 10 to 2. By monitoring at 420 nm it is possible to observe a low-high-low fluorescence modulation with the maximum fluorescence intensity at the intersection of the on-off and off-on switches. The individual pK_a values of **3** and **4** are different by more than 2 pH units, leading to the clear low-high-low fluorescence modulation at 420 nm.

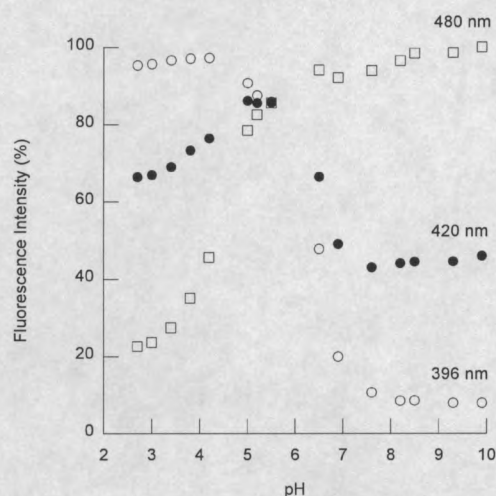


Figure 6: pH dependence of the fluorescence intensity of the mixture of **3** and **4** in methanol/water(1:1) ($\lambda_{\text{ex}} = 350$, $\lambda_{\text{em}} = 396, 420, 480\text{nm}$). pH adjusted by adding HCl or NaOH.

The fluorescence intensity at the intersection of the two curves is dependent on the pK_a values of the two individual proton switches. Therefore, it should be possible to modulate the magnitude of fluorescence modulation at the intersection by changing the pK_a values of the proton receptors of the two PET systems. It should be possible to observe more pronounced off-on-off fluorescence modulation at the intersection of the two spectra if there is no contribution from one to the other at this wavelength.

Conclusion

In summary, we have presented two new second generation PET systems that function as fluorescent off-on-off switches for protons. Both new systems utilize a secondary plant metabolite, anabasine, as the proton receptor. One of these new systems also utilize a coumarin as the chromophore, making it the first PET system that is entirely based on natural products. In addition, we have studied the fluorescence modulation of a mixture of two well-known PET systems with pH. We have shown that it is possible to achieve complex fluorescence modulation characteristic of higher generation PET systems by the combination of simple first generation PET systems. It is conceivable that this method could be developed further to monitor different analytes by ratiometric measurements and multi colour labeling experiments¹¹ with other first generation PET systems.

Absorption, Emission and Quantum Yield Data

Sensor	pH	Absorption λ_{max} (nm)	Emission λ_{max} (nm)	Φ_f
1	8.1	348	413	0.0840
	4.3	351.6	420	0.3967
	1	347.2	412	0.0870
2	7.9	324	388	0.01096
	2.9	329	414	0.18191
	1	337	410	0.09066

Table 1: Absorption, Emission, and Quantum Yield data for sensors **1** and **2**.

9,10-Dimethylantracene¹² used as a reference for calculating fluorescence quantum yields for **1** and **2**. $\lambda_{\text{ex}} = 350$ nm and $\lambda_{\text{ex}} = 330$ nm respectively; $\lambda_{\text{em}} = 300$ -550 nm. Optical densities of 9,10-Dimethylantracene and sensors were obtained in methanol (100%) and methanol/water (1:1) respectively.

Experimental

Nuclear magnetic resonance spectra were recorded on a Bruker Avance 300/300MHz FT-NMR Spectrometer. All NMR spectra were obtained in CDCl₃ at room temperature and the chemical shifts are reported in δ values (ppm) relative to TMS.

Ultraviolet and visible spectra were recorded on Cary 300 Bio UV-Visible Spectrophotometer. Excitation and emission spectra were recorded on a Cary Eclipse Fluorescence Spectrophotometer. pH was measured using a Fischer Scientific Accumet Ph/ATC Combination electrode and Accumet Basic pH Meter. High Resolution mass spectra were performed at the Mass Spectrometry Service Laboratory at the Department of Chemistry, University of Minnesota. The synthesis of the two new PET systems, **1** and **2**, are described below. PET systems **3** and **4** were prepared according to literature procedures.

3-{1-[(9-anthryl)methyl]-2-piperidyl}pyridine (1)

A mixture of 9-chloromethylantracene (0.39 g, 1.720 mmol), anabasine (0.33 g, 2.064 mmol), triethylamine (0.34 g, 3.441 mmol), and ethanol (25 ml) was refluxed for two days. Dichloromethane (50 mL) was added after cooling to room temperature and the mixture was extracted with 3M HCl (3x15 mL). The aqueous layer was neutralized with sodium carbonate, extracted into dichloromethane (2x25 mL) and dried over magnesium sulfate. The dichloromethane layer was filtered and evaporated to yield an oily brown residue that was purified by column chromatography on silica (methanol/ethyl acetate 5:95) to give 0.36 g of **1** as yellow solid. ¹H NMR (CDCl₃): δ 8.86 (s, 1H), 8.56 (d, 3.9 Hz, 1H), 8.4-8.2 (m, 3H), 8.0-7.8 (m, 3H), 7.5-7.3 (m, 5H), 4.24-4.05 (m, 2H),

3.32 (t, 6.6 Hz, 1H), 2.6 (m, 1H), 2.18 (m, 1H), 1.9-1.6 (m, 3H), 1.5-1.3 (m, 3H); ^{13}C NMR (CDCl_3): δ 149.67, 148.91, 140.0, 135.29, 131.27, 131.23, 130.06, 128.83, 127.19, 125.20, 125.14, 124.68, 123.35, 68.8, 52.92, 52.65, 35.73, 25.81, 24.9; HRMS (EI) for $\text{C}_{25}\text{N}_2\text{H}_{24}$ (M+Na) calculated 375.1832; found 375.185.

3-{1-[(7-methoxy-4-coumaryl)methyl]-2-piperidyl}pyridine (2)

A mixture of 4-bromomethyl-7-methoxycoumarin (0.69 g, 2.564 mmol), anabasine (0.5 g, 3.082 mmol), triethylamine (0.52 g, 5.128 mmol) and ethanol (25 ml) was refluxed for two days. Dichloromethane (50 mL) was added after cooling to room temperature and the mixture was extracted with 3M HCl (3x15 mL). The aqueous layer was neutralized with sodium carbonate, extracted into dichloromethane (2x25 mL) and dried over magnesium sulfate. The dichloromethane layer was filtered and evaporated to yield a dark oily residue that was purified by column chromatography on silica (methanol/ethyl acetate 5:95) to give 0.49 g of **2** as pale yellow solid. ^1H NMR (CDCl_3): δ 8.64 (s, 1H), 8.49 (d, $J = 3.9$ Hz, 1H), 7.78 (d, $J = 9$ Hz, 1H), 7.42 (d, $J = 9$ Hz, 1H), 7.26 (t, $J = 6$ Hz, 1H), 6.76 (s, 1H), 6.74 (s, 1H), 6.59 (s, 1H), 3.82 (s, 3H), 3.65-3.03 (m, 3H), 2.3-1.2 (m, 8H); ^{13}C NMR (CDCl_3): δ 162.25, 161.31, 155.11, 153.05, 148.93, 148.81, 139.67, 134.49, 124.67, 123.64, 111.98, 111.70, 110.23, 100.68, 66.33, 55.47, 55.42, 54.28, 36.52, 25.56, 24.59; HRMS (ESI) for $\text{C}_{21}\text{H}_{22}\text{N}_2\text{O}_3$ (M+Na) calculated 373.1523; found 373.1529

Fluorescence studies of PET systems 1 and 2

A 10^{-5} M solution of the PET system (**1** or **2**) was prepared in 1:1 methanol/water. A portion of this solution (20mL) was placed in a small beaker with a pH electrode and a small magnetic stir bar. The pH of the solution was changed by adding small amounts of hydrochloric acid or sodium hydroxide solutions while stirring and the fluorescence spectrum was recorded at regular pH intervals. The fluorescence quantum yields were calculated using 9,10-diphenylanthracene as the standard.⁵

Fluorescence study of the mixture of 3 and 4

The pH of PET system **4** (10^{-5} M solution in 1:1 methanol/water) was increased up to about pH 10.00 by adding a solution of sodium hydroxide and the fluorescence intensity at 480 nm (λ_{max} of the pyrazoline chromophore) was recorded while exciting at 350 nm. Then the pH was decreased to about 3.00 by adding hydrochloric acid and a few drops of the PET system **3** (10^{-5} M solution in 1:1 methanol/water) was added until the fluorescence intensity at 420 nm (λ_{max} of the anthracene chromophore) approximately matched the fluorescence intensity at 480 nm. The pH of the mixture solution was increased back to 10.00 by adding sodium hydroxide and the fluorescence of the mixture was recorded at regular intervals while decreasing the pH with hydrochloric acid.

References

1. (a) A. P. de Silva and N. D. McClenaghan, *Chem. Eur. J.*, 2004, **10**, 574; (b) A. P. de Silva, B. McCaughan, B. O. F. McKinney and M. Querol, *J. Chem. Soc., Dalton Trans.*, 2003, 1902; (c) R. Martinez-Manez and F. Sancenon, *Chem. Rev.*, 2003, **103**, 4419; (d) T. D. James and S. Shinkai, *Top. Curr. Chem.*, 2002, **218**, 159; (e) W. Wang, Gao, X and B. Wang, *Curr. Org. Chem.* 2002, **6**, 1285. (f) R. Ballardini, V. Balzani, A. Credi, M. T. Gandolfi and M. Venturi, *Acc. Chem. Res.*, 2001, **34**, 445; (g) S. L. Wiskur, H. Ait-Haddou, J. J. Lavigne and E. V. Anslyn, *Acc. Chem. Res.* 2001, **34**, 963.
2. (a) T. Gunnlaugsson, T. C. Lee and R. Parkesh, *Tetrahedron*, 2004, **60**, 11239; (b) A. P. de Silva, D. B. Fox, A. J. M. Huxley and T. S. Moody, *Coordination Chem. Rev.*, 2000, **205**, 41; (c) A. J. Bryan, A. P. de Silva, S. A. de Silva, R. A. D. D. Rupasinghe, K. R. A. S. Sandanayake, *Biosensors*, 1989, **4**, 169.
3. (a) S. A. de Silva, B. Amorelli, D. C. Isidor, K. C. Loo, K. E. Crooker and Y. E. Pena, *Chem. Commun.*, 2002, 1360; (b) J. F. Callan, A. P. de Silva and N. D. McClenaghan, *Chem. Commun.*, 2004, 2048.
4. J. Montenegro, E. Perez-Inestrosa, D. Collado, Y. Vida and R. Suau, *Org. Lett.*, 2004, **6**, 2353.

5. R. B. Herbert, *The Biosynthesis of Secondary Metabolites*, 2nd ed. Chapman and Hall, London, 1989.
6. (a) 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, 187; (b) C. C. Woodrooffe and S. J. Lippard, *J. Am. Chem. Soc.*, 2003, **125**, 11458.
7. (a) S. A. de Silva, A. Zavaleta, D. E. Baron, O. Allam, E. V. Isidor, N. Kashimura and J. M. Percarpio, *Tetrahedron Lett.*, 1997, **38**, 2237; For examples of molecules developed in other labs that display off-on-off behaviour with cation binding see: (b) A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *Chem. Commun.*, 1996, 2399; (c) L. Fabbrizzi, F. Gatti, P. Pallavicini and L. Parodi, *New J. Chem.*, 1998, 1403; (d) H. Chao, B-H. Ye, Q-L. Zhang and L-N. Ji, *Inorg. Chem. Commun.*, 1999, **2**, 338; (e) Y. Kubo, S. Obara and S. Tokita, *Chem. Commun.* 1999, 2399; (f) T. Gunnlaugsson, J. P. Leonard, K. Senechal and A. J. Harte, *J. Am. Chem. Soc.*, 2003, **125**, 12062.
8. (a) A. P. de Silva and S. A. de Silva, *Chem. Commun.*, 1986, 1709; (b) A. P. de Silva, H. Q. N. Gunaratne and C. P. McCoy, *J. Am. Chem. Soc.*, 1997, **119**, 7891; (c) A. P. de Silva, H. Q. N. Gunaratne and P. L. M. Lynch, *J. Chem. Soc. Perkin Trans. 2*, 1995, 685. (d) M. A. Fox and M. Channon, Eds., *Photoinduced Electron Transfer*, Parts A-D, Elsevier, Amsterdam, 1988.

9. A. P. de Silva and R. A. D. D. Rupasinghe, *Chem. Commun.*, 1985, 1669.
10. A. P. de Silva, S. A. de Silva, A. S. Dissanayake and K. R. A. S. Sandanayake, *Chem. Commun.*, 1989, 1054.
11. (a) R. B. Silver, *Methods Cell Biol.*, 1998, **56**, 237; (b) A. Waggoner, R. DeBiasio, P. Conrad, G. R. Bright, L. Ernst, K. Ryan, M. Nederlof and D. Taylor, *Methods Cell Biol.*, 1989, **30**, 449.
12. (a) I. H. Leaver and D. E. Rivett, *Mol. Photochem.*, 1974, **6**, 113; (b) A. Maciejewski and R. P. Steer, *J. Photochem.*, 1986, **35**, 59.