A Fluorescent Photoinduced Electron Transfer (PET) Sensor for Cations with a Separate PET Channel to Suppress Proton Signals

Supun Pathirana

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A FLUORESCENT PHOTOINDUCED ELECTRON TRANSFER (PET) SENSOR FOR CATIONS
WITH A SEPARATE PET CHANNEL TO SUPPRESS PROTON SIGNALS/

By

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A Master’s Thesis Submitted to the Faculty of
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In Partial Fulfillment of the Requirements
For the Degree of
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A FLUORESCENT PHOTOINDUCED ELECTRON TRANSFER (PET) SENSOR FOR CATIONS WITH A SEPARATE PET CHANNEL TO SUPPRESS PROTON SIGNALS

A Thesis
Submitted in Partial Fulfillment of the Requirements
For the Degree of MASTER OF SCIENCE
by SUPUN PATHIRANA Montclair State University
Montclair, NJ 2008
I dedicate this Master’s Thesis to my grandmother Mrs. Prema Fonseka Abeykoon Kandage for bestowing the first drops of knowledge on me.
Acknowledgements

I would like to express profound gratitude to my advisor, Dr. Saliya Anil de Silva, for his invaluable support, encouragement, supervision and useful suggestions throughout this research work.

A special thank you goes out to Mr. Kevin Olsen for the constant help on the instruments and Dr. Isidor for lending the glassware and sharing the RotoVap. Thank you Christopher Chu & Sketa Patel for the preliminary research and Umme Habiba & Brent Westcott for the help with the purification.

I am as ever, especially indebted to my parents, Ruwan Pathirana and Radha Pathirana and sister, Nipun Pathirana, for their love and support throughout my life.

Finally, I wish to express my appreciation to my aunt, Sudam Pathirana, without whom I would have not had the opportunity to come thus far.
Abstract

This thesis presents \( N-((10-((2\text{-methoxy-4-methylphenoxy})\text{methyl})\text{anthracen}-9\text{-yl})\text{methyl})(\text{pyridin-2-yl})-N-((\text{pyridin-2-yl})\text{methyl})\text{methanamine}, \) \( 1, \) a photoinduced electron transfer (PET) sensor that acts as a fluorescent ‘Off-On’ switch in the presence of \( \text{Zn}^{2+} \) ions and not sensitive to protons.
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INTRODUCTION

Many fluorescent sensors for cations that utilize photoinduced electron transfer (PET) as the signaling mechanism have been developed over the past three decades.\textsuperscript{1-4} Most of these sensors (switches) are based on a fluorophore-spacer-receptor architecture (Figure 1) and have a nitrogen atom as a part of the receptor. Since the nitrogen is also a receptor for protons, these sensors generate fluorescent signals for various metal ions as well as protons.

![Figure 1: The Fluorophore-Spacer-Receptor format of molecular fluorescent sensors.](image)

The fluorophore receives and transmits light signals, the receptor reversibly binds to the cation and the spacer links the receptor to the fluorophore. Photoinduced Electron Transfer (PET) between the fluorophore and the receptor is used for translating a cation binding event to generate or quench the fluorescence of the fluorophore.

The two types of molecular sensors are described below are Off-On switches and On-Off switches.
Off-On Switches:

A. ‘Off’

B. ‘On’

Figure 2: ‘Off-On’ Fluorescent switch.

The fluorophore is energized by the absorbance of a photon ($h\nu_{\text{Abs}}$). If the excitation energy gained is sufficient to perform the one-electron oxidation of the receptor and the one electron reduction of the fluorophore then a PET occurs. Because the excitation energy is used by the PET process, no fluorescence is seen. This is the ‘Off’ state of the switch.
When a receptor captures a cation, the PET process is disrupted and the absorbed photon of the fluorophore is released as a fluorescence photon \((h\nu_{\text{Flu}})\). This is the ‘On’ state of the switch.

The driving force for these PET processes \((\Delta G_{\text{ET}})\) can be expressed by a modified Rehm-Weller equation.

\[
\Delta G_{\text{Electron Transfer}} = -E_{\text{Singlet}} - E_{\text{Reduction of Fluorophore}} + E_{\text{Oxidation of Receptor}}
\]

\(E_{\text{Singlet}}\), \(E_{\text{Reduction of Fluorophore}}\), \(E_{\text{Oxidation of Receptor}}\) are the singlet energy, reduction potential of the fluorophore and the oxidation potential of the receptor respectively, for the PET from the receptor to the fluorophore. When a cation binds, the oxidation potential of the receptor will increase leading to an increase of \(\Delta G_{\text{ET}}\), decreasing the PET process, eventually quenching it completely. In this case the excited fluorophore will relax by fluorescence.
This process is represented schematically by a simplified Molecular Orbital (MO) Diagram (Scheme 1).

Scheme 1: Energy levels of the Fluorophore-Spacer-Receptor assembly displaying an ‘Off-On’ switch with a cation binding.

When the receptor is cation free, the transferring of an electron from the excited fluorophore to the receptor is exothermic, and a PET is thermodynamically favored. The excitation energy is used by the PET, allowing no fluorescence to occur. When the receptor is cation bound, transferring of an electron from the receptor to the excited fluorophore is endothermic, and a PET is not thermodynamically favored. The energy of excitation is released as fluorescence.
Sensor 2, also known as Lysosensor Blue, is an example of an “Off-On” switch, with an anthracene fluorophore, a pair of diethylamino receptors and methylene units as spacers. The diethylamine moiety is sufficiently electron rich to launch an electron towards the photoexcited anthracene fluorophore (PET). Both amine receptors must be protonated to halt the PET processes and fluorescence to occur. Therefore the molecule essentially non fluorescent at pH >9, emits a blue fluorescence at pH<5. This sensor is used for the visualization of acidic compartments inside living cells.

*On-Off Switches:*

A. ‘On’
Figure 3: ‘On-Off’ Fluorescent switch.

The fluorophore is energized by the absorbance of a photon ($h\nu_{\text{Abs}}$). Because the molecular components have been chosen so that a PET process does not occur, the absorbed photon is returned as a fluorescence photon ($h\nu_{\text{Flu}}$). This is the ‘On’ state of the switch.

When a receptor captures a cation, a PET process between the fluorophore and the receptor is initiated, and as the excitation energy is used by the PET process, no fluorescence is seen. This is the ‘Off’ state of the switch.

When considering the Rehm-Weller equation, the cation binding causes an increase in the reduction potential of the receptor, decreasing the $\Delta G_{\text{ET}}$ to where a PET process from the fluorophore to the receptor becomes thermodynamically favorable.

$$\Delta G_{\text{Electron Transfer}} = -E_{\text{Singlet}} - E_{\text{Reduction of Receptor}} + E_{\text{Oxidation of Fluorophore}}$$
This process is represented schematically by a simplified Molecular Orbital (MO) Diagram (Scheme 2).

Scheme 2: Energy levels of the Fluorophore-Spacer-Receptor assembly displaying an ‘On-Off’ switch with a cation binding.

When the receptor is cation free, transferring of an electron from the excited fluorophore to the receptor is endothermic, and a PET is not thermodynamically favored. The energy of excitation is released as fluorescence. When the receptor is cation bound, transferring of an electron from the excited fluorophore to the receptor is exothermic. The energy of excitation is used by the PET, allowing no fluorescence to occur.
Sensor 3 is an example for an “On-Off” switch. The “On” State is when the photoexcited anthracene relaxes by fluorescence (High pH). PET occurs from the photoexcited anthracene fluorophore to the protonated pyridine receptor, quenching the fluorescence at low pH values showing the “Off” state.

These PET sensors have evolved from simple off-on or on-off fluorescence switches that respond to a single cation, to complex fluorescence switches that respond to multiple cations. The evolution of the PET sensors is reflected in the number of PET processes that is possible in each sensor. The examples discussed above are first generation PET sensors that have one PET process that is controlled by one cation binding event. Similarly, second generation PET sensors have two PET processes each of which is controlled by a cation binding event. By this count, the most complex sensors reported to date are third generation PET sensors that are capable of three different PET processes in response to three cation binding events.

The PET sensor studied in this thesis, \(N-((10-((2\text{-methoxy-4-methylphenoxy})\text{methyl})\text{anthracen-9-yl})\text{methyl})(\text{pyridin-2-yl})-N-((\text{pyridin-2-yl})\text{methyl})\text{methanamine (1), is a third generation sensor that can undergo three PET processes. It contains an anthracene fluorophore, two methylene spacers, a bis(2-}
picolyl)amine as the receptor and an alkoxyphenyl group as an electron reservoir. Sensor 1 is designed to function as an off-on fluorescence switch for Zn2+ ions and is specifically designed not to respond to protons and it may be a potential sensor for zinc ions in biological systems. Zinc ions and protons are essential for all life and play a specific role in various biological reactions of all living organisms- humans, animals, plants and the smallest microorganisms.7

The methodology used for studying, the “Off” and/or “On” states of these molecular sensors, is Fluorescence Spectroscopy. Fluorescence spectroscopy is a suitable technique for studying sensors especially in biological systems because, fluorescence is easily and sensitively detected (can be measure in low concentrations of the molecule), fluorescence emanates from single molecules and so fluorescence methods possess a micro-environmental sensing capabilities, single molecules can infiltrate systems of cellular dimensions with little perturbation or damage to the host and intracellular incorporation of fluorescent molecular sensors is practical.7
The aim of the project is to design a novel PET sensor with a nitrogen based ligand that will respond to Zn\(^{2+}\) ions and not to protons. This can be accomplished by adding an alkoxyphenyl group to a second generation PET sensor with a bis(2-picoly)amine receptor for Zn\(^{2+}\) ions. It is expected that the alkoxyphenyl group will function as an electron reservoir that will generate a separate PET channel to quench the fluorescence signal that is generated due to the protonation of the ligand.

Three new sensors (1, 4 and 5) were designed for this purpose and the synthesis and cation binding studies of 1 are described in this thesis.
EXPERIMENTAL

Nuclear magnetic resonance spectra were recorded on a Bruker Avance 300/300MHz FT-NMR Spectrometer. All NMR spectra were obtained in CDCl₃ and the chemical shifts are reported in δ values (ppm) relative to TMS. Ultraviolet and visible spectra were recorded on a CARY 300 Bio UV-Visible Spectrophotometer. Excitation and emission spectra were recorded on a CARY Eclipse Fluorescence Spectrophotometer. pH was measured using a Fisher Scientific Accumet Basic AB15 pH meter. Mass Spectra was obtained by an Agilent 1100 Series LC/MSD and high resolution mass spectra were obtained at the University of Minnesota.

I. Synthesis of the Sensor

\[ N-((10-((2\text{-methoxy-4-methylphenoxy})\text{methyl})\text{anthracen}-9\text{-yl})\text{methyl})(\text{pyridin-2-yl})-N-((\text{pyridin-2-yl})\text{methyl})\text{methanamine} \]

A mixture of 9, 10-bis(chloromethyl)anthracene (0.50 g, 1.82 mmol), 2-methoxy-4-ethylphenol (0.25 g, 1.64 mmol), potassium hydroxide (0.15 g, 2.67 mmol) in tetrahydrofuran (25 mL) was stirred overnight. Bis((pyridine-2-yl)methyl)amine (0.36 g, 1.81 mmol) and triethylamine (0.28 g, 2.77 mmol) was added to the reaction mixture and the resulting mixture was refluxed overnight. After cooling to room temperature, dichloromethane was added and the mixture was extracted with 4M hydrochloric acid. The aqueous layer was neutralized with sodium carbonate and extracted into
dichloromethane. The organic layer was dried over magnesium sulfate, filtered, and evaporated to give an oily brown residue that was purified by column chromatography (Methanol/Ethyl Acetate 5:95) to give 1 as a brown colored solid. The solid continued to show traces of impurities even after several attempts to purify by column chromatography. $^1$H NMR (CDCl$_3$) for sensor 1: $\delta$ 8.6-8.4 (m), 7.7-7.5 (m), 7.4-7.3 (m), 7.2-7.1 (m), 6.8-6.7 (m), 5.7 (s), 4.7 (s), 3.9 (s), 3.8 (s), 2.4 (s). HRMS for C$_{36}$H$_{33}$O$_2$N$_3$ calculated 539.6662, found 540.2660 (m+1).

II. Fluorescence Study of the Sensor

Fluorescence Quantum Yields:

The quantum yield reference used was 9, 10-diphenylanthracene.$^8$,$^9$ Quantum yields of fluorescence were calculated using the following equation.

$$\phi_f = \frac{I_f (sensor)}{I_f (reference)}$$

$I_f (sensor)$ and $I_f (reference)$ are the integrated intensities of fluorescence of the sensor and the references respectively at indicated pH values. The optical densities of the sensor and the reference were matched for comparison.

A solution of the sensor ($10^{-5}$M) was prepared in methanol and methanol/water (1:1) and the optical density at $\lambda_{max}$ was obtained. A solution of 9, 10-diphenylanthracene
was prepared in methanol (100%) and diluted/undiluted until the optical density was equal to that of the sensor’s $\lambda_{\text{max}}$. The intensity of fluorescence ($I_0$) of the sensor and the reference were measured by integrating the fluorescence spectrum from 370 – 550 nm.

**Fluorescence Intensity Modulation of Sensor with Zinc Ion Concentration:**

Solutions of the sensor ($4 \times 10^{-5}$M) were prepared with, 0, 0.8, 1.6, 2.4, 3.2, 4.0, 5.6 ($\times 10^{-5}$M) of Zn$^{2+}$ concentrations in methanol (100%). The fluorescence intensity of an aliquot of each of these solutions with varying Zn$^{2+}$ concentrations were recorded by integrating the emission spectrum from 370 – 550 nm with the excitation at 350 nm.

**Fluorescence Intensity Modulation of Sensor with pH:**

The pH of a 10$^{-5}$M solution of the sensor in methanol/water (1:1) was adjusted to about 12, with sodium hydroxide and was titrated with hydrochloric acid to attain target pH values from highest to lowest. The fluorescence intensity of an aliquot of this solution at various pH values were recorded by integrating the emission spectrum from 370 – 550 nm with the excitation at 350 nm.
RESULTS & DISCUSSION

I. Design and synthesis of the new PET sensor:

The aim of the project is to design a novel PET sensor with a nitrogen based ligand that will respond to Zn$^{2+}$ ions and not to protons. The new sensor is based on the fluorophore-spacer-receptor format and uses components from two sensors, 6 and 7, that were developed in our lab. Sensor 6 uses a bis(2-picolyl)amine receptor and functions as an off-on fluorescent switch for Zn$^{2+}$ ions.\textsuperscript{10} It also functions as a fluorescent off-on-off switch for protons due to the two different types of nitrogen atoms in the receptor. Sensor 7 was shown to be a fluorescent off-on-off switch for protons with an overriding enable-disable switch for Na$^+$ ions. In this case the benzo-crown moiety functions as an electron reservoir in the absence of Na$^+$ leading to a PET between the benzo-crown and the excited anthracene when the tertiary nitrogen was protonated.\textsuperscript{11}
This stirred a curiosity to find out whether a similar alkoxyphenyl group, that is not a part of a benzo-crown, has the necessary oxidation potential to transfer an electron to the excited fluorophore of a PET sensor when a tertiary nitrogen attached via a methylene spacer is protonated. Several new sensors (4, 5) were designed by adding an alkoxyphenyl group, including a benzo-crown, to PET sensor 6, which had a bis(2-picolyl)amine receptor for Zn$^{2+}$ ions. It was anticipated that the new sensor will show a fluorescence signal for Zn$^{2+}$ ions and would suppress the fluorescence signal due to protons via the PET processes shown below.

Figure 4: Fluorescence ‘Off’ and ‘On’ states of Sensor 1 with PET processes that correspond to cation binding events.
It has been shown in the recent literature that the dimethoxybenzene moiety of $8^{12}$ has the necessary oxidation potential to transfer an electron to the excited fluorophore when the nitrogen is protonated. This further supports our hypothesis that a phenyl group with a similar substitution pattern, two alkoxy groups and one alkyl group, can act as an electron reservoir in the new sensor.

Figure 5: Synthetic routes to 1.
Two synthetic routes were tried when synthesizing molecule 1. The first route starts with 9-chloromethylanthracene, adds the alkoxyphenyl group via a Williamson ether synthesis and adds the bis(2-picolyl)amine via an aldehyde at C10 of the anthracene. However, the Vilsmeier reaction that was used to add the aldehyde group was not successful and this route was modified to start with the commercially available bis-9,10-chloromethylanthracene. The obvious drawback in this approach is the possibility of forming the diether. In addition the starting material and the monoether had a very low solubility in common organic solvents leading to difficulties in purification. The second step of adding the bis(2-picolyl)amine was carried out directly on the reaction mixture and the final product was isolated by an acid extraction. The crude product was purified by column chromatography using methanol/ethyl acetate (5:95). The product continued to show traces of impurities in the 1H-NMR even after several attempts to purify by column chromatography. Therefore, the best method to prepare sensor 1 would be to functionalize the anthracene in a stepwise manner as shown in the top part of figure 5.
II. Fluorescence studies of the PET sensor:

a. Fluorescence Quantum Yields

The fluorescence quantum yield ($\Phi_F$) studies of sensor 1 were carried out in methanol using 9,10-diphenylanthracene as the reference and are summarized in Table 1.

Table 1: $10^{-5}M$ 1 in Methanol, $\lambda_{excitation} = 350\,nm$, $\lambda_{emission} = 370-550\,nm$. $H^+$, $Zn^{2+}$ and the proton scavenger were provided as p-Toluenesulfonic acid monohydrate, Zinc acetate and Tetramethylammonium hydroxide pentahydrate respectively.

<table>
<thead>
<tr>
<th></th>
<th>Proton Input</th>
<th>$Zn^{2+}$ Input</th>
<th>$\Phi_F$</th>
<th>Output</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>None</td>
<td>None</td>
<td>0.03</td>
<td>Off</td>
</tr>
<tr>
<td>1-$H^+$</td>
<td>$10^{-5}$ M</td>
<td>None</td>
<td>0.20</td>
<td>Off</td>
</tr>
<tr>
<td>1-$2H^+$</td>
<td>$10^{-2}$ M</td>
<td>None</td>
<td>0.04</td>
<td>Off</td>
</tr>
<tr>
<td>1-$Zn^{2+}$</td>
<td>None</td>
<td>$10^{-5}$ M</td>
<td>0.49</td>
<td>On</td>
</tr>
<tr>
<td>1-Scavenger</td>
<td>None</td>
<td>None</td>
<td>0.03</td>
<td>Off</td>
</tr>
<tr>
<td>1-Scavenger-$Zn^{2+}$</td>
<td>None</td>
<td>$10^{-5}$ M</td>
<td>0.46</td>
<td>On</td>
</tr>
</tbody>
</table>
The fluorescence of 1 is quenched due to the thermodynamically favored PET between the tertiary amine and the excited anthracene leading to the off state of this sensor with a low $\Phi_F$ (0.03). Increasing the oxidation potential of the tertiary amine due to the Zn$^{2+}$ cation binding, of 1-Zn$^{2+}$, prevents this PET and regenerates the fluorescence of the fluorophore leading to a significantly higher $\Phi_F$ (0.49).

In the presence of protons (1-H$^+$) the PET process from the tertiary amine to the excited anthracene is inhibited due to the protonation of the tertiary amine. Although this PET process is (PET 1) is inhibited, the protonated tertiary amine moiety acts as an electron withdrawing group leading to a second PET (PET 2) from the alkoxyphenyl unit to the excited anthracene quenching the emission of fluorescence. When considering the results from Table 1, the $\Phi_F$ of 1-H$^+$ shows a 0.20, a rather high value for an “Off” state. A byproduct that may have been formed during the synthesis, 9, may contribute to the high $\Phi_F$ of 1-2H$^+$. In the presence of protons the tertiary amine is protonated preventing the PET process from the pyridinium unit to the photoexcited anthracene fluorophore, generating a fluorescence signal$^3$. Since the concentration of this molecule is relatively low, $\Phi_F$ is only 0.20. However, 1-2H$^+$ shows the insignificant $\Phi_F$ =0.04, as the pyridines in the amine-pyridinium moiety are protonated allowing a PET process to be thermodynamically favorable quenching the fluorescence.
b. **Zinc Binding Studies**

The binding constant \( (\log \beta) \) for \( \text{Zn}^{2+} \) is calculated according to 
\[
\frac{1}{[M/L]} = \frac{1}{\left( \frac{I_F}{I_{F_{max}}} - 1 \right)} \] 
\[ = \beta \left( [M^{+}]_{\text{total}} - [L]_{\text{total}} \right) \left( \frac{I_F}{I_{F_{max}}} \right) \]. \(^1^3\) The binding constant \( (\log \beta) \) for \( \text{Zn}^{2+} \) was 4.4 in methanol. This is less than a previously reported value \( (\log \beta = 6.1) \)\(^1^4\) for the same receptor-\( \text{Zn}^{2+} \) binding in acetonitrile.

Fluorescence intensity \( (I_F) \) of 1 vs. \( \text{Zn}^{2+} \) concentration data indicated that the ligand:metal binding ratio is 1:1 (Figures 6 and 7). The plot in Figure 8 is from sensor 6, which has the same receptor for \( \text{Zn}^{2+} \) binding and shows the same trend as sensor 1 (Figure 7). There is an increase of the fluorescence intensity and then a leveling off when the concentration is 1:1.
Figure 6: Fluorescence emission spectra of 1 (4.0 x 10^{-5}M) with 0, 0.8, 1.6, 2.4, 3.2, 4.0, 5.6 (x10^{-5}M) of Zn^{2+} (in order of increasing intensity) in Methanol (λ_{excitation} = 350nm λ_{emission} = 370-550nm).
Figure 7: Fluorescence intensity ($I_F$) of 1 ($4.0 \times 10^{-5}$M) vs. $[Zn^{2+}]$ ($0-5.6 \times 10^{-5}$M) in Methanol ($\lambda_{\text{excitation}} = 350\text{nm}$, $\lambda_{\text{emission}} = 370-550\text{nm}$).
Figure 8: Fluorescence intensity of 6 vs. Zinc ion concentration (0-2×10⁻⁵M) in acetonitrile ($\lambda_{\text{excitation}} = 350\text{nm} \quad \lambda_{\text{emission}} = 370-550\text{nm}$).¹⁰
c. Fluorescence modulation with pH

The $I_F$ vs. pH profile of 1 is consistent with the understanding of the existence of a second PET (PET 2). The quantum yields of fluorescence for 1 (compared with 9, 10-diphenylantracene) at pH 11.3, 6.0 and 2.0 are 0.3, 0.9 and 0.4 respectively (Table 2).

Table 2: Quantum Yield data of sensor 1 in methanol/water (1:1) with 9, 10-dimethylantracene in methanol as the reference. $\lambda_{\text{excitation}} = 350\text{nm}$ $\lambda_{\text{emission}} = 370-550\text{nm}$.

<table>
<thead>
<tr>
<th>pH</th>
<th>$\Phi_F$</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.3</td>
<td>0.03</td>
</tr>
<tr>
<td>6.0</td>
<td>0.09</td>
</tr>
<tr>
<td>2.0</td>
<td>0.04</td>
</tr>
</tbody>
</table>

The sensor does not generate a significant signal with decreasing pH as expected (Figure 9). When compared with the $I_F$ vs pH profile of sensor 6 (Figure 10) it can be seen that the fluorescence of 1 is quenched due to the thermodynamically favored PET between the alkoxyphenyl group and the excited anthracene between pH 8-4.
Figure 9: Fluorescence intensity modulation of sensor 1 (10^{-5}M) with pH, in methanol/water (1:1). (\lambda_{\text{excitation}} = 350\text{nm} \ \lambda_{\text{emission}} = 370-550\text{nm}). pH was adjusted by adding HCl & NaOH.
Figure 10: pH dependence of the fluorescence intensity of the sensor 6 in methanol/water (1:1) ($\lambda_{\text{excitation}} = 350\text{nm}$ $\lambda_{\text{emission}} = 370-550\text{nm}$). pH adjusted by adding HCl or NaOH.$^{10}$
CONCLUSION

A novel Zn$^{2+}$ sensor, $N$-((10-((2-methoxy-4-methylphenoxy)methyl)anthracen-9-yl)methyl)(pyridin-2-yl)-$N$-((pyridin-2-yl)methyl)methanamine (1), was developed during this study. Sensor 1 generates a fluorescent signal, quenching a thermodynamically favored PET between the tertiary amine and the excited anthracene, when zinc ions are bound to the bis(2-picoly)amine receptor.

All currently known PET sensors that use tertiary nitrogen based receptors show fluorescence modulation with pH as the protonation of the tertiary nitrogen will also quench the PET mentioned above. The new sensor is designed to suppress this fluorescence signal by adding an alkoxyphenyl group that would generate a secondary PET when the tertiary amine is protonated. As expected, the intensity of fluorescence of sensor 1 at pH 6 is significantly lower than the fluorescence intensity of a similar sensor that does not contain the alkoxyphenyl group.

In summary, this study demonstrates the concept that a separate PET channel from an electron reservoir to the excited fluorophore could be used to suppress the fluorescence signal due to protons in PET sensors that have tertiary nitrogen based receptors.
REFERENCES
