

Strategies toward improving the performance of fluorescence-based sensors for inorganic anions†

Radek Pohl‡,^a Dmitry Aldakov,^a Pavel Kubát§,^a Karolina Jursíková,^a Manuel Marquez^b and Pavel Anzenbacher, Jr.*^a

^a Department of Chemistry and Center for Photochemical Sciences, Bowling Green State University, Bowling Green, Ohio, 43403, USA. E-mail: pavel@bgnet.bgsu.edu; Fax: (+1) 419-372-9809

^b Los Alamos National Laboratory, Chemistry Division, Los Alamos, New Mexico, 87545, USA

Received (in Columbia, MO, USA) 24th November 2003, Accepted 26th April 2004

First published as an Advance Article on the web 10th May 2004

Two methods for improving signal transduction in simple fluorescence-based anion sensors utilizing resonance energy transfer or exciton delocalization method are described and their use in amplified anion sensing is demonstrated.

The importance of anions in Nature, as agricultural fertilizers and industrial raw materials and the corresponding environmental concerns necessitate the development of highly sensitive anion sensors.¹ Here, sensors based on anion-induced changes in fluorescence are particularly attractive as they offer potential for high sensitivity at a low analyte concentration.² The sensor performance can be improved either by increasing the receptor–substrate affinity or *via* improved signal transduction. So far, the focus has been mainly on the supramolecular chemistry of a receptor.^{2b,3} This approach, however, suffers from shortcomings such as the high cost of synthesis and the loss of the real-time response due to slow dissociation of the substrate–receptor complex.^{4a}

There are numerous reports of resonance energy transfer RET² utilized in signal transduction in sensors for various substrates^{2,5} including sensors for DNA-assays, protein toxins, glucose, and cations, but RET-based sensors for small anions are very rare.^{2b,6} That is because the small anions display a wide range of geometries, significant charge delocalization and efficient solvation.³ All these factors make binding of anions less effective. In this communication, we demonstrate that existing receptors and sensors that are good binders but lack in a signal response do not need to be completely re-designed to achieve high efficiency of the sensing process. The importance of this approach becomes obvious if one considers the need for application in polar/water-containing media, which leads to dramatic decrease in the fluorescence intensity of most existing sensor chromophores.

Here, we report on two ways of adaptation of an already existing sensor that may be useful for upgrading sensors, sensing performance of which would otherwise be insufficient. The first approach to signal amplification is based on attaching a chromophore capable of resonance energy transfer to the parent sensor. The second approach is to attach moieties to the parent sensor that allow excited state delocalization in the conjugated system *via* acetylene bridges.^{4,7} Both approaches resulting in amplified signal transduction are illustrated on 2,3-di(1*H*-2-pyrrolyl)quinoxaline (DPQ, **1**),⁸ which is known to display a weak change in color and modest fluorescence quenching in the presence of fluoride.^{8a}

The visual inspection of the solutions of sensors **1–3** illuminated by black light (365 nm) shows more than twice enhanced emission for sensors **2** and **3** compared to the parent compound **1** (Fig. 1, left panel). The presence of pyrene antenna moieties, despite relatively low absorption at 365 nm by the pyrene donors, results in significant increase in the emission from the DPQ core (λ_{max} 495 nm). Right panel inset (Fig. 1) shows that the light harvesting effect of pyrene moieties is much stronger when the absorption maximum of

pyrene is used for excitation. The fluorescence emission in sensor **3** is shifted to λ_{max} 550 nm. This amplification is even stronger in polar solvents such as DMSO that strongly quench the DPQ emission.

Photophysical properties of sensor **2** are largely determined by RET from the pyrene (donor) moieties to the DPQ (acceptor) moiety of **2** as confirmed by time-resolved fluorescence spectroscopy and a quantum yield measurement (Fig. 2). The long-lived fluorescence of pyrene (29.0 ns in air-saturated CH₂Cl₂) is quenched, while the lifetime of the DPQ acceptor is extended to 2.2 ns for sensor **2**. Also, the excitation spectrum of **2** shows remarkable resemblance to the absorption spectrum of **2**, including the well-resolved bands of pyrene.⁹

In sensor **3**, the signal amplification is achieved through effective excited state delocalization as confirmed both by the shift of the emission wavelength from λ_{max} 495 nm to λ_{max} 550 nm and increase in the fluorescence lifetime from 1.8 ns recorded for the parent sensor **1** to 3.7 ns for sensor **3** (Table 1).

The qualitative estimation of the sensor–anion affinity was performed visually (Fig. 3 A and B). The observed changes in the

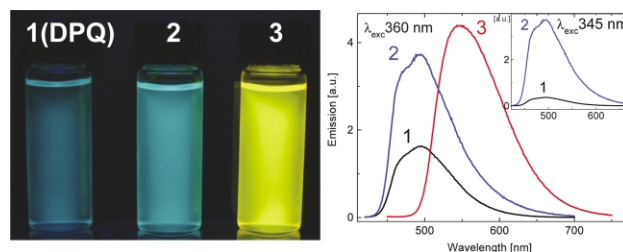


Fig. 1 Left panel shows sensors **1** (DPQ), **2** and **3** (10 μ M solutions in CH₂Cl₂) irradiated with black light (365 nm). Right panel shows the emission spectra of sensors **1**, **2** and **3** (1.0 μ M in CH₂Cl₂, λ_{exc} = 360 nm). The inset shows efficient light harvesting upon excitation at 345 nm.

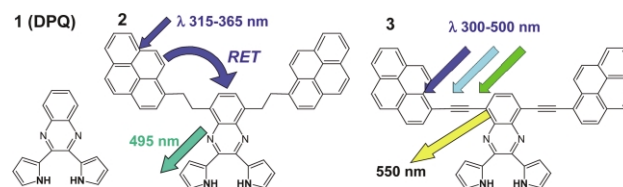


Fig. 2 RET-based light harvesting in sensor **2**, and the excited state delocalization in sensor **3** provide signal amplification in the sensors.

Table 1 Photophysical properties of sensors **1**, **2** and **3**: excitation wavelength (λ_{exc}), quantum yield (Φ_{F}),^a fluorescence lifetime (τ_{F}), emission wavelength (λ_{em}) recorded in the air-saturated CH₂Cl₂ at 22 °C

Sensor	λ_{exc} /nm	Φ_{F}	τ_{F} /ns	λ_{em} /nm
1 (DPQ)	415	0.13	1.8	495
2	415	0.19	2.2	495
2	345	0.16	2.2	495
3	415	0.36	3.7	550

^a Recorded using quinine sulfate as a standard.

† Electronic supplementary information (ESI) available: experimental data. See <http://www.rsc.org/suppdata/cc/b3/b315268e/>

‡ RP on leave from IOCB, Acad. Sci., Prague 6, Czech Rep.

§ PK on leave from J. Heyrovský Inst. of Phys. Chem., Prague 8, Czech Rep.

fluorescence emission in the sample without and with the presence of the tested anion correspond to the magnitude of the binding constant (Fig. 3) calculated from titration experiments.

Quantitative measurements of anion affinity were performed by monitoring the changes in the UV-Vis and steady state emission spectra of sensors **2** and **3** upon addition of anions. The titration experiments were performed in CH_2Cl_2 ^{10a} using fluoride ($\times 6\text{H}_2\text{O}$), chloride, phosphate ($\times 2\text{H}_2\text{O}$), and pyrophosphate ($\times 2\text{H}_2\text{O}$) anions as tetra-n-butylammonium salts.^{10b} An addition of fluoride, pyrophosphate, and (to a lesser extent) phosphate to $1\ \mu\text{M}$ solutions of sensors **1–3** caused significant quenching of the fluorescence emission intensity (Fig. 4A). Also, the UV-Vis spectra of **2** and **3** ($2\ \mu\text{M}$ solutions) show characteristic changes (Fig. 4B). Table 2 summarizes all of the emission data-derived binding constants measured for **1–3** towards different anions.

The sensors **2** and **3** display increased affinity for anions compared to sensor **1**. This effect is particularly strong in the case of sensor **3** and pyrophosphate with a twofold increase in binding affinity compared to the parent sensor **1**.

In Fig. 5 the left panel shows that excitation at 320 nm, which is almost optimal for the parent DPQ, resulted in twofold amplification of the signal output recorded for the PyrDPQ sensor **2** compared to the parent DPQ sensor **1**. The right panel shows

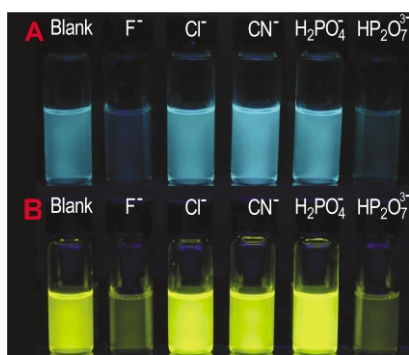


Fig. 3 Anion-induced changes in fluorescence intensity of $10\ \mu\text{M}$ in CH_2Cl_2 solutions of sensor **2** (panel A), and sensor **3** (panel B), respectively, upon illumination with black light (365 nm). The anions (*ca.* $100\ \mu\text{mol}$) were added in the form of their tetrabutylammonium salts.

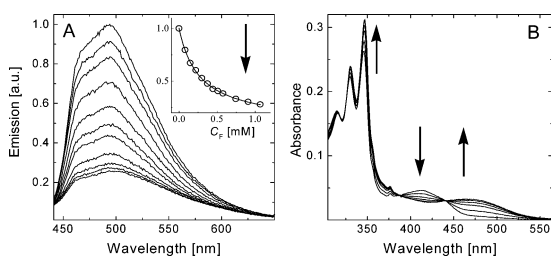


Fig. 4 Panel A: decrease in emission intensity of sensor **2** ($1.0\ \mu\text{M}$ in CH_2Cl_2) upon the addition of fluoride ($\lambda_{\text{exc}} = 325\ \text{nm}$). Inset: Binding isotherm monitored by the emission decrease at $495\ \text{nm}$. Panel B: changes in UV-Vis spectra of sensor **2** ($2.0\ \mu\text{M}$ in CH_2Cl_2) upon the addition of fluoride ($0\text{--}1.2\ \text{mM}$).

Table 2 Affinity constants for compounds **1**,^{8a} **2** and **3** (M^{-1})^a and anionic substrates in dichloromethane and DMSO^{10a} at $22\ ^\circ\text{C}$

Anion	DPQ (1)	Sensor (2)	Sensor (3)
F^-	18,200	17,300	19,600
$\text{HP}_2\text{O}_7^{3-}$	14,300	18,000	29,500
H_2PO_4^-	< 100	< 100	< 200
Cl^-	< 50	< 100	< 100

^a Fits were performed using single reciprocal plots¹¹ and 1 : 1 stoichiometry being obtained from Job plots.

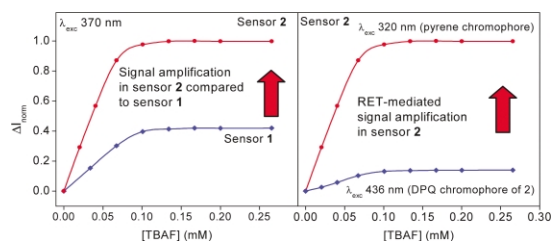


Fig. 5 Left panel: signal amplification in sensor **1** and **2** ($2\ \mu\text{M}$ in CH_2Cl_2) during titration with a fluoride anion (excitation at a wavelength optimal for DPQ). Right panel: signal amplification in sensor **2** achieved through RET amplification (excitation in the pyrene-donor moiety).

binding isotherms for sensor **2** at a wavelength optimal for FRET-mediated signal amplification.

In practice, this means that sensor **2** can be easily used in the amount ten-times lower compared to the parent DPQ. Also, the detection limit for sensor **2/F**⁻ was found to be $40\ \mu\text{M}$ (determined using a $0.2\ \mu\text{M}$ solution of **2**). Such a detection limit is not accessible using the parent DPQ.

In summary, we have demonstrated two approaches to the signal amplification in fluorescence-based sensors for inorganic anions. Our findings may open up the possibility to improve the performance of numerous receptors and sensors, which are successful anion ‘binders’ but are lacking in signal transduction.

Financial support from Kraft Foods, the Petroleum Research Fund (ACS-PRF #38110-G), NSF (Grant NER#0304320, DMR#0306117, SENSORS#0330267) and BGSU (TIE and FRC-RIG grants) to P.A. is gratefully acknowledged.

Notes and references

- 1 *Ullmann's Encyclopedia of Industrial Chemistry*, 6th Ed., Wiley VCH, New York, 1999.
- 2 (a) J. R. Lakowicz, *Principles of Fluorescence Spectroscopy*, 2nd Ed., Kluwer Academic/Plenum Publishers, New York, 1999; (b) R. Martínez-Máñez and F. Sancenón, *Chem. Rev.*, 2003, **103**, 1419.
- 3 (a) P. D. Beer and P. A. Gale, *Angew. Chem. Int. Ed.*, 2001, **40**, 486; (b) *Supramolecular Chemistry of Anions*, Eds. A. Bianchi, K. Bowman-James, E. Garcia-Espana, Wiley-VCH, New York, 1997; (c) J. L. Sessler, H. Maeda, T. Mizuno, V. M. Lynch and H. Furuta, *Chem. Commun.*, 2002, 862.
- 4 (a) T. M. Swager, *Acc. Chem. Res.*, 1998, **31**, 201; (b) D. T. McQuade, A. E. Pullen and T. M. Swager, *Chem. Rev.*, 2000, **100**, 2537.
- 5 (a) *Optical Sensors and Switches*, Eds. V. Ramamurthy, K. S. Schanze, Marcel Dekker, Inc., New York, 2001 examples include (b) nucleic acids: W. Tan, X. Fang, J. Li and X. Liu, *Chem. Eur. J.*, 2000, **6**, 1107; (c) Protein toxins: X. Song, J. Nolan and B. I. Swanson, *J. Am. Chem. Soc.*, 1998, **120**, 11514; (d) Glucose: O. J. Rolinski, D. J. S. Birch, L. McCartney and J. C. Pickup, *Spectrochim. Acta*, 2001, **57A**, 2245; (e) Cations: D. J. S. Birch, A. S. Holmes and M. Darbyshire, *Meas. Sci. Technol.*, 1995, **6**, 243.
- 6 I. Koronczi, J. Reichert, H.-J. Ache, C. Krause, T. Werner and O. S. Wolfbeis, *Sens. Actuators B*, 2001, **74**, 47.
- 7 (a) Q. Zhou and T. M. Swager, *J. Am. Chem. Soc.*, 1995, **117**, 12593; (b) D. T. McQuade, A. H. Hegedus and T. M. Swager, *J. Am. Chem. Soc.*, 2000, **122**, 12389.
- 8 (a) C. B. Black, B. Andrioletti, A. C. Try, C. Ruiperez and J. L. Sessler, *J. Am. Chem. Soc.*, 1999, **121**, 10438; (b) P. Anzenbacher Jr., A. C. Try, H. Miyaji, K. Jursíková, V. M. Lynch, M. Marquez and J. L. Sessler, *J. Am. Chem. Soc.*, 2000, **122**, 10268.
- 9 For more information see Supporting Information†.
- 10 (a) CH_2Cl_2 was selected as a solvent to provide comparable conditions with reported data. Sensors also **2** and **3** display anion sensing DMSO (0.1% water): **2**(M^{-1}): $K_{\text{F}} = 28,9000$; $K_{\text{Pyrophosphate}} = 33,000$; **3**(M^{-1}): $K_{\text{F}} = 31,300$; $K_{\text{Pyrophosphate}} = 46,500$; The increase in binding affinity may partly be a result of TBA cation–anion dissociation. (b) The degree of hydration of anion salts was determined from the elemental analysis using C, F, Cl, and P.
- 11 A. K. Connors, *Binding Constants: The Measurement of Molecular Complex Stability*; Wiley VCH, New York, 1987.