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Intramolecular Indicator Displacement Assay for Anions: Supramolecular Sensor for Glyphosate

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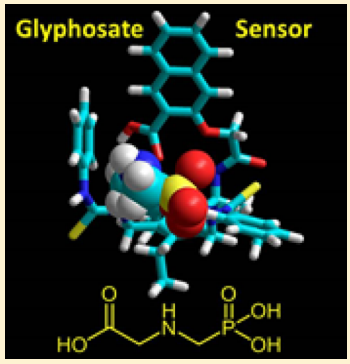
Supporting Information

ABSTRACT: One of the well-known strategies for anion sensing is an indicator (dye) displacement assay. However, the disadvantage of the dye displacement assays is the low sensitivity due to the excess of the dye used. To overcome this setback, we have developed an "Intramolecular Indicator Displacement Assay (IIDA)". The IIDAs comprise a receptor and a spacer with an attached anionic chromophore in a single-molecule assembly. In the resting state, the environment-sensitive anionic chromophore is bound by the receptor, while the anionic substrate competes for binding into the receptor. The photophysical properties of the dye exhibit change in fluorescence when displaced by anions, which results in cross-reactive response. To illustrate the concept, we have prepared IID sensors **1** and **2**. Here, the characterization of sensors and microtiter arrays comprising the IIDA are reported. The microtiter array including IID sensors **1** and **2** is capable of recognizing biological phosphates in water. The utility of the IIDA approach is demonstrated on sensing of a phosphonate herbicide glyphosate and other biologically important anions such as pyrophosphate in the presence of interferent sodium chloride.



INTRODUCTION

Phosphates and phosphonates are important anions as they constitute cellular electrolytes, take part in a wide variety of metabolic processes, and are implicated in numerous diseases.[1] Phosphonates are compounds that are frequently used as drugs,[2] as herbicides,[3] as chelating agents,[4] and in an insalubrious role as chemical warfare agents.[5] Despite their importance, the development of sensors for phosphates and particularly phosphonates seems to be a less attended niche. An example of an important phosphonate anion is glyphosate (*N*-(phosphonomethyl)glycine), a compound known as Roundup, a herbicide widely used in agriculture.[3] Glyphosate inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase,[6] thereby interfering with the synthesis of aromatic amino acids, and causes the plant's death. Genetically modified crops (GMCs), however, are resistant to glyphosate and unlike weeds survive the glyphosate treatment. The use of large quantities of glyphosate causes various problems including a gene species shift from GMCs to weed.[7] Alas, glyphosate has also adverse effects on aquatic plant[8] and animal species[9] and acts as an endocrine disruptor.[10]

The widespread use of glyphosate requires that methods for the detection and quantitative determination are available. The Environmental Protection Agency's (EPA) reference dose for glyphosate is 2 mg/kg/day.[11] A maximum contaminant level in drinking water is 0.7 ppm.[12] Quantitative determination of glyphosate generally relies on solid-phase extraction (SPE) followed by GC-MS[13] or LC-MS.[14] An enzyme-linked immunosorbent assay (ELISA) was also used.[15] Such methods, albeit reliable and accurate, are expensive, require trained personnel and a knowledge of sample preparation, etc. Simple methods that require only low-cost instruments are lacking.

For the above reasons, optical chemosensors based on supramolecular interactions appear to be appealing alternatives.[16] The most widely used approach to optical chemosensors, both colorimetric and luminescence-based, is the reporter−spacer−receptor (RSR) approach, in which the fluorophore is either covalently attached to or is directly a part of the receptor.[17] The disadvantage of the RSR approach is the need to synthesize the sensor, the attendant difficulties, and cost. This problem is largely circumvented in the indicator−displacement assays (IDAs).[18] Here, the receptor is first preincubated with the indicator dye forming a reversible noncovalent complex. Then, a competitive analyte displaces the indicator from the receptor, while the dye changes its optical properties. The requirement for an IDA is that the indicator−receptor affinity is lower compared to that of the analyte−receptor complex and that the complexation equilibrium of the dye is accompanied by a sufficient change in its optical properties. The advantages of IDAs over the RSRs are (i) the ease of the synthesis, (ii) the fact that numerous dyes may be used for one type of receptor (albeit not at the same time) to

Received: May 8, 2014
Published: July 22, 2014

dx.doi.org/10.1021/ja504535q | J. Am. Chem. Soc. 2014, 136, 11396−11401

form a library of indicator ensembles, and (iii) wider availability of indicators.

There are, however, disadvantages to the IDA concept: namely, the excess of the indicator dye is frequently required due to a low dye−receptor affinity. This dye excess frequently lowers the signal-to-noise ratio and sensitivity to low concentration of analytes. Also, because the IDA receptors must bind multiple dyes as well as an analyte they must display a high degree of intrinsic cross-reactivity, which too may be a disadvantage.[18] That said, for highly effective sensor arrays some, usually low, cross-reactivity is required so that the receptors respond to more than one analyte.[19] Hence, the arrays with fewer sensor elements than targeted analytes must be cross-reactive. However, to make a high-resolution array effort worthwhile, the sensors should be sensitive to low concentrations of analyte and operate in multianalyte environment.

The above disadvantages are circumvented in the Intramolecular Indicator Displacement Assays (IIDAs). Figure 1

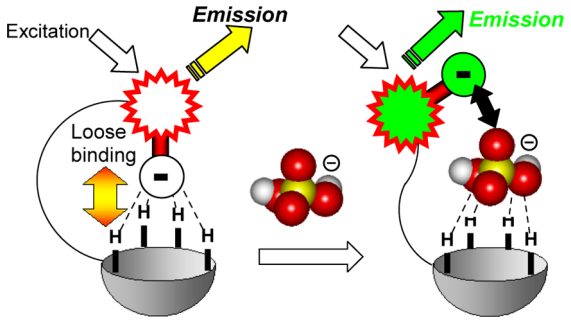


Figure 1. Illustrated concept of the intramolecular indicator displacement assay (IIDA).

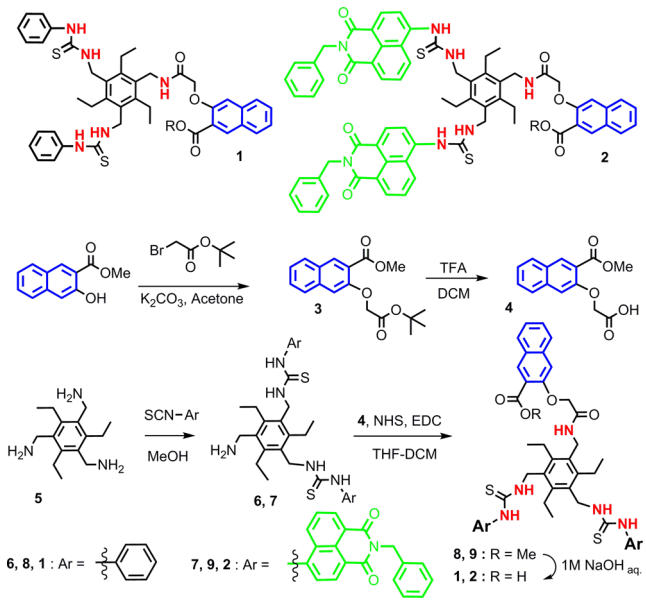
shows the operating principle proposed for the IIDA. The IID sensor comprises a receptor and spacer with an attached anionic chromophore (dye). In the resting state the environment-sensitive anionic dye is bound by the receptor, while the anionic analyte competes for binding into the receptor. The photophysical properties of the dye change when it is displaced by the analyte anion. The sensors may be regenerated by washing away the analyte to re-establish the dye−receptor complex. The advantage of this sensor format is the receptor−indicator 1:1 ratio, which endows the assay with high sensitivity and an instant reversibility.

Herein we report the performance of the IIDAs based on fluorescence signaling. As a benchmark, we demonstrate sensing of phosphate-type anions (e.g., phosphate (Pi), pyrophosphate (PPi), and AMP) and, as an example of a phosphonate, environmentally important compound glyphosate. The IIDA sensors were used to establish a microarray, which was then used in both qualitative and quantitative recognition studies of anions. All analytes were studied in the form of aqueous solutions and in the presence of excess NaCl as a competing electrolyte.

RESULTS AND DISCUSSION

To illustrate the IID concept, the sensors **1** and **2** were prepared. The sensor **1** features thiourea and amide groups as anion recognition moieties and naphthyl carboxylate as an anionic chromophore. In the case of **2**, two naphthalimide moieties are included to generate bright fluorescence. The synthetic procedure is summarized in Scheme 1. The anionic chromophore moiety was synthesized from methyl 3-hydroxy-

Scheme 1. Synthesis of IID Sensors 1 and 2



2-naphthoate. Methyl 3-hydroxy-2-naphthoate was reacted with *tert*-butyl bromoacetate, which gave methyl 3-(2-*tert*-butoxy-2-oxoethoxy)-2-naphthoate **3**. Subsequently, the deprotection of **3** was carried out by trifluoroacetic acid. The 1,3,5-triamino-methyl-2,4,6-triethylbenzene core **5**, synthesized according to a published procedure,[20] was then reacted directly with appropriate isothiocyanate (ArNCS) to yield dithiourea derivatives **6** and **7**. The amide coupling of dithiourea derivatives **6** and **7** with **4** was performed using 1-ethyl-3-(3-(dimethylamino)propyl) carbodiimide hydrochloride (EDC) and was followed by the hydrolysis of the methyl ester in **8** and **9** to obtain the final IID sensors **1** and **2**.

First, the structure **1** in the resting state was explored by DFT calculations (DFT-B3LYP/6-31G+(d,p) in DMSO), which indicated that the intramolecular hydrogen bond between thiourea and amide NHs and naphthylcarboxylate is likely to take place (Figure 2A). These results also suggest that guest-induced release of the intramolecular carboxylate fluorophore should occur in the presence of a competitive analyte, which is also confirmed by a single crystal of **1** (Figure 2B). Surprisingly, the IID sensor **1** recognizes diacetone alcohol generally present in acetone as a small impurity. As expected, guest binding releases the naphthylcarboxylate from the intramolecular hydrogen bond.

To confirm that the interaction between IID sensors and anions occurs through hydrogen bonds, ^{1}H NMR titrations of both **1** and **2** with anions were conducted. Importantly, these sensors are not deprotonated by anions such as fluoride, acetate, and dihydrogen phosphate in DMSO-d_6. Figure 3 shows ^{1}H NMR spectra of **1** upon the addition of dihydrogen phosphate. The thiourea NH (red circle) is dramatically shifted downfield from 9.40 ppm to 12.82, 11.55, and 11.74 ppm for fluoride, acetate, and dihydrogen phosphate, respectively. Similarly, the other thiourea NH (blue square) displays a downfield shift from 8.14 ppm to 9.25, 8.92, and 9.46 ppm for fluoride, acetate, and dihydrogen phosphate. In addition, the amide NH (green upside-down triangle) also shows a

dx.doi.org/10.1021/ja504535q | J. Am. Chem. Soc. 2014, 136, 11396−11401

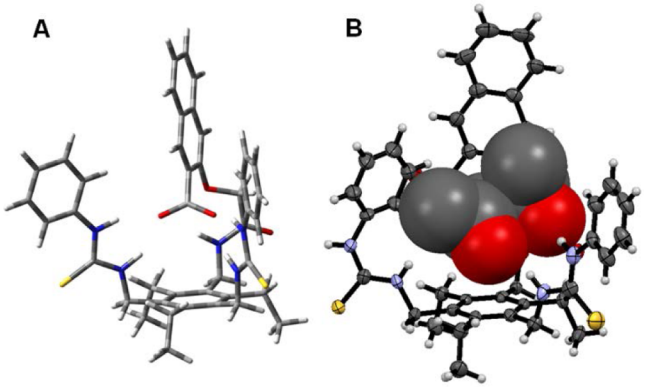


Figure 2. (A) DFT-B3LYP/6-31G+(d,p) calculation: energy minimized structure of **1** in DMSO. (B) The X-ray diffraction structure of the complex of **1** and diacetone alcohol. Thermal ellipsoids are scaled to the 50% probability level. The carbon atoms are shown in dark gray, the nitrogen atoms in light blue, the oxygen atoms in red, and the sulfur atoms in yellow.

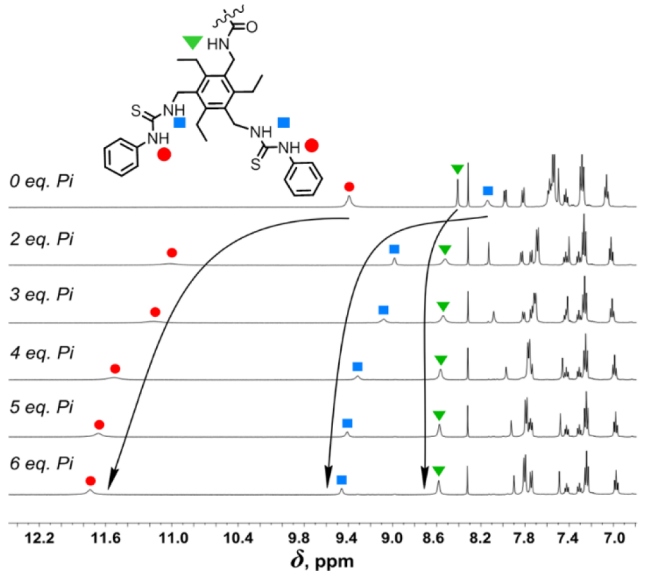


Figure 3. ^{1}H NMR (500 MHz) titration of **1** (10 mM) upon the addition of Pi as tetrabutylammonium salt in DMSO-d_6.

downfield shift upon addition of anions. Similar behavior was observed upon addition of glyphosate (see Supporting Information (SI)).

Next, the fluorescence properties of the IID sensors were investigated. The fluorescence quantum yields of **1** and **2** in aqueous DMSO solution (water:DMSO = 5:95, v/v) are 10.4% and 6.0%, respectively. Quantitative estimation of the anion sensing ability of sensors was obtained by fluorescence titrations. In the case of **1**, fluorescence quenching was observed upon addition of anions (sensor **1** was excited at the isosbestic point (λ_{ex} = 320 nm)). An example of the titrations is shown in Figure 4. The top panel (Figure 4A) shows the titration of **1** with dihydrogen phosphate, which results in a marked attenuation of the naphthalene carboxylate fluorescence. This observation is explained as follows: In the resting state, the fluorescent naphthyl carboxylate moiety is fixed in position through intramolecular hydrogen bonding. However, the fluorophore displacement occurring upon the addition of guests dramatically increases the degree of freedom

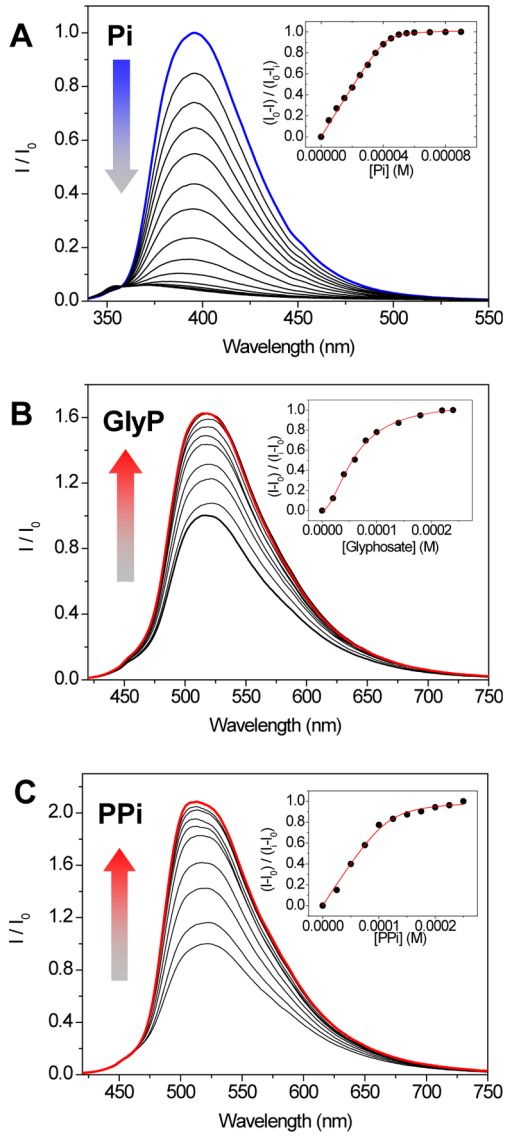


Figure 4. (A) Fluorescence titration of **1** (5.0×10^{-5} M) upon the addition of Pi as tetrabutylammonium salt in aqueous DMSO solution (water:DMSO = 5:95, v/v). λ_{ex} = 320 nm. [Pi] = 0–9.0×10^{-5} M. (B) Fluorescence titration of **2** (1.0×10^{-5} M) by glyphosate (GlyP) as tetrabutylammonium salt in aqueous DMSO solution (water:DMSO = 5:95, v/v). λ_{ex} = 400 nm. [GlyP] = 0–2.4×10^{-4} M. (C) Fluorescence titration of **2** (1.0×10^{-5} M) by PPi as tetrabutylammonium salt in aqueous DMSO solution (water:DMSO = 5:95, v/v). λ_{ex} = 400 nm. [PPi] = 0–2.4×10^{-4} M.

of the naphthyl carboxylate moiety. As a consequence, the excited state deactivation via rotation and vibration is increased resulting in an attenuation of fluorescence, and a "turn-off" signaling is observed. This is supported by a control experiment using 3-methoxy-2-naphthoic acid (**10**), which alone does not show efficient quenching upon addition of anions (see SI). The association constants for sensors **1** and **2** are listed in Table 1. Sensor **1** does not show significant optical response to chloride and bromide but strongly binds fluoride, acetate, and phosphate related anions including glyphosate. The corresponding titration isotherms are shown in the SI. In general, the anion binding follows 1:1 stoichiometry, which is supported by electrospray ionization mass spectrometry (ESI MS) (see SI). Only in the case of pyrophosphate the stoichiometry between **1**

dx.doi.org/10.1021/ja504535q | J. Am. Chem. Soc. 2014, 136, 11396–11401

Table 1. Affinity Constants (K_a, M^{-1})[a] Obtained from Fluorescence Titration

anion	sensor **1**	sensor **2**
F^-	$>10^6$	4800 ± 230
Cl^-	ND[b]	ND[b]
Br^-	ND[b]	ND[b]
AcO^-	$>10^6$	3400 ± 120
Pi	$>10^6$	44000 ± 8800
PPi	$>10^6$	17000 ± 1100
AMP	330000 ± 46000	16000 ± 3200
glyphosate	280000 ± 48000	15000 ± 830

[a]The K_a values were calculated based on the change in fluorescence intensity upon addition of each guest in aqueous DMSO solution (water:DMSO = 5:95, v/v). [b]K_a could not be calculated due to the small response.

and pyrophosphate is 2:1, which is also confirmed by ESI MS. This is presumably due to the charges residing at both ends of the pyrophosphate anion.

The IID sensor **1** showed a "turn-off" signaling of the anion; however, the "turn-on" fluorescence response is more attractive. Thus, we have synthesized sensor **2** and investigated the fluorescence response of **2** to anions. An example of one of the fluorescence titration results is shown in Figure 4B and C. Here, the fluorescence arising from the naphthalimide groups of **2** is increased with the increase of anion concentration. This is presumably due to the fact that the formation of the complex with suitable guests is associated with increased rigidity of the receptor and limited rotational/vibrational modes that would otherwise cause nonradiative decay. Moreover, fluorescence could be quenched by the formation of an excited charge-transfer complex between the naphthalene and naphthalimide moieties.[21] Once a guest displaces the naphthalene from the cavity the charge-transfer complex ceases to exist, inducing an increase in fluorescence. For the titration isotherm corresponding to the anion-induced fluorescence enhancement see the SI. The corresponding titration isotherms suggest that the response pattern depends on the type of analyte. The binding constants in Table 1 indicate that the overall binding constants of **2** are lower than those of **1**. This behavior is presumably due to the steric demand imposed by the naphthalimide fluorescent labels in **2**. Both IID sensors do not show appreciable affinity for halides such as chloride and bromide, presumably because these anions cannot displace the intramolecular naphthalene carboxylate from the receptor. With regard to IID sensor **2**, it shows interesting selectivity for phosphate anions.

To gain insight into the magnitude of the intramolecular displacement effect, we performed a control experiment in which the preincubated mixture of 1,1′,1″-[(2,4,6-triethylbenzene-1,3,5-triyl)tris(methylene)]tris[3-(*p*-tolyl)thiourea] (**11**) as an analogue receptor and 3-methoxy-2-naphthoic acid was titrated by dihydrogen phosphate (see Figure S4, SI, for more information). While we could observe a similar optical response (quenching) to the fluorescence titration of **11** upon the addition of Pi, the apparent binding constant (K_a = 8400 M^{-1}) is much lower (**1**·Pi, $K_a > 10^6$ M^{-1}). This result suggests that the sensitivity of the IIDA to anions is much higher than that of the corresponding IDA system.

From the potential practical perspective it is important that the IID sensor could be incorporated in a simple microarray for anion sensing in pure water. The array was constructed utilizing **1** (Figure 5A, blue features) and **2** (yellow-green features).

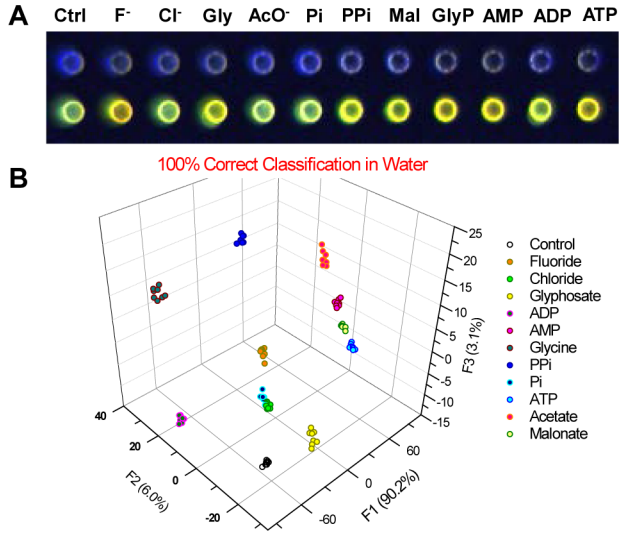


Figure 5. (A) Digital photo (arbitrary colors) of a microarray chip carrying the polymer-embedded IID sensors **1** (blue features) and **2** (yellow-green). Panel B: Graphical output of the linear discriminant analysis (LDA) shows clusters of 11 analytes and control.

Both compounds **1** and **2** were embedded in hydrophilic polyurethane matrices known to swell in water while internalizing the anions.[22] The polymer/sensor solutions were cast onto a microtiter plate (well radius: 500 ± 5 μm, depth: 250 ± 10 μm). The aqueous solutions of anions (200 nL) were then added to each well containing a sensor. The anions used for this purpose were fluoride, chloride, glycine (Gly), acetate, dihydrogen phosphate, pyrophosphate, malonate (Mal), AMP, ADP, ATP, and glyphosate (GlyP). We recorded the response to analytes as the fluorescence intensity changes (for details see SI). The array response was evaluated by utilizing the statistical multivariate analysis method—linear discriminant analysis (LDA).[23] Using a leave-one-out cross-validation routine, LDA can evaluate discriminatory power of the sensor array. Despite the fact that the sensors respond to the presence of anions by similar changes in fluorescence, the degree of the change in the fluorescence intensity for each anion is unique and generates a response pattern, which allows for the recognition of the analytes (Figure 5A). Interestingly, the qualitative LDA result in Figure 5B shows 100% correct classification for 11 analytes and control using only two sensors. This confirms the spectacular ability of IIDA sensors to recognize multiple analytes including anions of biological (PPi, ATP, etc.) and societal importance (glyphosate). In fact, the glyphosate cluster was separated from the rest of the anions tested suggesting a different recognition behavior from the rest of the anions. This is a positive outcome as it enables a semiquantitative assay of glyphosate.

Toward this end we used the same array sensor to identify various glyphosate concentrations. We attempted a semi-quantitative IID assay (IIDA) for glyphosate in water and in the absence or presence of electrolytes (e.g., 50 mM sodium chloride). These results are shown in Figure 6. A clear evolution of the clusters in dependence on the glyphosate concentration can be observed trending from the lowest to the highest concentration. This result suggested that a clear dependence between the fluorescence intensity and glyphosate concentration can be established within the concentration range studied (0—169 ppm), a condition required for quantitative regression based quantitative determination.

dx.doi.org/10.1021/ja504535q | J. Am. Chem. Soc. 2014, 136, 11396—11401

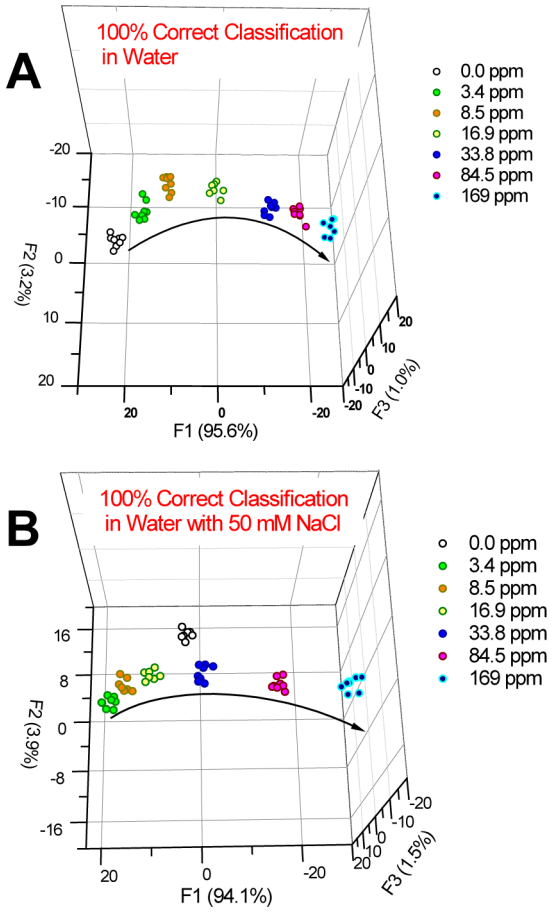


Figure 6. Results of LDA of the semiquantitative assay of glyphosate in water in the absence (panel A) or presence (panel B) of interferent sodium chloride (50 mM).

In order to be able to determine the concentration of glyphosate in aqueous samples, we performed regression analysis for glyphosate utilizing the support vector machine (SVM) algorithm.[24] Toward that end, we divided the acquired data set into two parts, one for calibration and model development and the second to be used as unknown samples for cross-validation. Thus, one concentration out of seven (15% of the whole data set) was analyzed as an unknown concentration using the developed model that describes the behavior of the data. This was repeated (one-by-one) for all the concentrations. Overall, the two-sensor array yielded an accurate quantitative regression analysis of the glyphosate concentrations in 50 mM sodium chloride (Figure 7). Figure 7 also shows the correctly quantified unknown sample (red circle). Furthermore, the limit of detection (LOD)[25] of glyphosate is 0.2 ppm, which is significantly lower than the maximum contaminant level for drinking water (0.7 ppm). To the best of our knowledge, this is the first supramolecular chemosensor for glyphosate.

Needless to say, the sensing process using **1** and **2** as well as the microarrays is entirely reversible, and the microarray chips are reusable.

CONCLUSIONS

In summary, we have devised sensors for anions utilizing the Intramolecular Indicator Displacement concept and demonstrated it on an assay for phosphate anions. The IID sensors

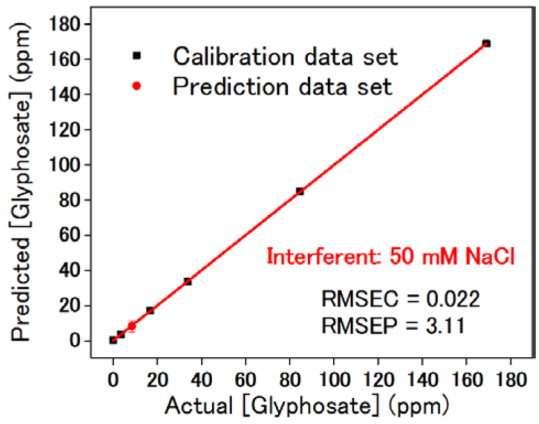


Figure 7. Results of quantitative analysis of glyphosate in water with 50 mM sodium chloride using sensors **1** and **2** array. The prediction plot and the root-mean-square errors (RMSEs) attest to the high quality of the model and prediction for the unknowns.

show an interesting ability to differentiate among anions based on a minute change in fluorescence. The qualitative analysis using polymer chip-based microarray enabled recognition (with 100% correct classification) of 12 analytes using an assay comprising only two IID sensors. Interestingly, IID sensors show strong affinity toward the herbicide glyphosate. Glyphosate is recognized in water and in the presence of an excess (50 mM) of chloride interferent. Semiquantitative and quantitative assays for glyphosate enable us to determine glyphosate concentrations with a limit of detection of 0.2 ppm, which is significantly lower than the maximum contaminant level for drinking water (0.7 ppm). Because the approach is general and components are tunable for other analytes of interest, we believe that the IIDA concept will pave the way to new sensors and applications including applications in environmental sensing. Our preliminary results suggest that further modifications of the periphery of the sensors and the use of different intramolecular dyes would yield more efficient systems.

ASSOCIATED CONTENT

Supporting Information

Synthesis and characterization of **1** and **2**, X-ray structural analysis, fluorescence spectra, mass spectrometric study, ^{1}H NMR titrations, DFT calculations, experimental detail of microarray, and results of multivariate analysis. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

P.A. acknowledges support from NSF (CHE-0750303 and DMR-1006761). We would like to thank Dr. V. M. Lynch of the University of Texas for solving the X-ray structures.

REFERENCES

(1) (a) Berg, J. M.; Stryer, L.; Tymoczko, J. L. *Biochemistry*, 5th ed.; W.H. Freeman: New York, 2002. (b) Marshall, W. J.; Bangert, S. K. *Clinical Chemistry*, 5th ed.; Elsevier: Edinburg, 2004. (c) Schmidt, R.

F.; Thews, G. *Human Physiology*, 2nd ed.; Springer-Verlag: Berlin, 1989.
(2) (a) De Clercq, E. *Biochem. Pharmacol.* **2011**, *82*, 99−109. (b) Callebaut, C.; Stray, K.; Tsai, L.; Williams, M.; Yang, Z.-Y.; Cannizzaro, C.; Leavitt, S. A.; Liu, X.; Wang, K.; Murray, B. P.; Mulato, A.; Hatada, M.; Priskich, T.; Parkin, N.; Swaminathan, S.; Lee, W.; He, G.-X.; Xu, L.; Cihlar, T. *Antimicrob. Agents Chemother.* **2011**, *55*, 1366−1376.
(3) (a) Green, J. M. *Genetically Modified Crops and the New Paradigm for Herbicide Use in Row Crops. In Pesticide Formulations and Delivery Systems: Innovating Legacy Products for New Uses*; Bernards, M. L., Ed. ASTM International: West Conshohocken, 2013; Vol. *32*, pp 155−161. (b) Franz, J. E.; Mao, M. K.; Sikorski, J. A. *Glyphosate A Unique Global Herbicide*, ACS Monograph Series; American Chemical Society: Washington, DC, 1997; pp 521−615.
(4) Nowack, B. *Water Res.* **2003**, *37*, 2533−2546.
(5) (a) Green, C. C.; Lochmann, S. E.; Straus, D. L. *J. Toxicol. Environ. Health, Part A* **2005**, *68*, 141−149. (b) Hoskin, F. C. *Can. J. Biochem. Physiol.* **1956**, *34*, 75−79. (c) *Chemical Warfare Agent: Chemistry, Pharmacology, Toxicology, and Therapeutics*; Romano, J. A., Jr., Lukey, B. J., Salem, H., Eds.; CRC Press: Boca Raton, FL, 2007.
(6) Steinrücken, H. C.; Amrhein, N. *Biochem. Biophys. Res. Commun.* **1980**, *94*, 1207−1212.
(7) (a) DK Owen, M. *Pest Manage. Sci.* **2008**, *64*, 377−387. (b) Cerdeira, A. L.; Duke, S. O. *J. Environ. Qual.* **2006**, *35*, 1633−1658.
(8) Cedergreen, N.; Streibig, J. C. *Pest Manage. Sci.* **2005**, *61*, 1152−1160.
(9) Guy, M.; Singh, L.; Mineau, P. *Integr. Environ. Assess. Manage.* **2011**, *7*, 426−436.
(10) (a) Gasnier, C.; Dumont, C.; Benachour, N.; Clair, E.; Chagnon, M.-C.; Seralini, G.-E. *Toxicology* **2009**, *262*, 184−191. (b) Rescia, M.; Mantovani, A. Pesticides as endocrine disrupters: identification of hazards for female reproductive function. In *Reproductive Health and the Environment, Environmental Science and Technology Library*; Nicolopoulou-Stamati, P., Hens, L., Howard, C. V., Eds.; Springer: Dordrecht, 2007; Vol. *22*, pp 227−248.
(11) Acquavella, J. F.; Alexander, B. H.; Mandel, J. S.; Gustin, C.; Baker, B.; Chapman, P.; Bleeke, M. *Environ. Health Perspect.* **2004**, *112*, 321−326.
(12) http://www.epa.gov/safewater/pdfs/factsheets/soc/tech/glyphosa.pdf (accessed April 29, 2014).
(13) Saito, T.; Aoki, H.; Namera, A.; Oikawa, H.; Miyazaki, S.; Nakamoto, A.; Inokuchi, S. *Anal. Sci.* **2011**, *27*, 999−1005.
(14) Hanke, I.; Singer, H.; Hollender, J. *Anal. Bioanal. Chem.* **2008**, *391*, 2265−2276.
(15) Sanchís, J.; Kantiani, L.; Llorca, M.; Rubio, F.; Ginebreda, A.; Fraile, J.; Garrido, T.; Farré, M. *Anal. Bioanal. Chem.* **2012**, *402*, 2335−2345.
(16) For reviews, see: (a) Sessler, J. L.; Gale, P. A.; Cho, W.-S. *Anion Receptor Chemistry. Monographs in supramolecular Chemistry*; Royal Society of Chemistry: Cambridge, 2006. (b) Martínez-Máñez, R.; Sancenón, F. *Chem. Rev.* **2003**, *103*, 4419−4476. (c) Bayly, S. R.; Beer, P. D. *Struct. Bonding (Berlin, Ger.)* **2008**, *129*, 45−94. (d) Valeria, A.; Fabbrizzi, L.; Mosca, L. *Chem. Soc. Rev.* **2010**, *39*, 3889−3915. (e) Duke, R. M.; Veale, E. B.; Pfeffer, F. M.; Kruger, P. E.; Gunnlaugsson, T. *Chem. Soc. Rev.* **2010**, *39*, 3936−3953. (f) Li, A.-F.; Wang, J.-H.; Wang, F.; Jiang, Y.-B. *Chem. Soc. Rev.* **2010**, *39*, 3729−3745. (g) Ngo, H. T.; Liu, X.; Jolliffe, K. A. *Chem. Soc. Rev.* **2012**, *41*, 4928−4965. (h) Maeda, H.; Anzenbacher, P., Jr. In *Supramolecular Chemistry: From Molecules to Nanomaterials*, Steed, J. W., Gale, P. A., Eds.; Wiley: Hoboken, 2012; pp 2581−2610. (i) Steed, J. W. *Chem. Soc. Rev.* **2009**, *38*, 506−519. (j) Galbraith, E.; James, T. D. *Chem. Soc. Rev.* **2010**, *39*, 3831−3842. (k) Kim, S. K.; Lee, D. H.; Hong, J.-I.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23−31. (l) Gale, P. A.; Busschaert, N.; Haynes, C. J. E.; Karagiannidis, L. E.; Kirby, I. L. *Chem. Soc. Rev.* **2014**, *43*, 205−241. For examples, see: (m) Gunnlaugsson, T.; Davis, A. P.; O'Brien, J. E.; Glynn, M. *Org. Biomol. Chem.* **2005**, *3*, 48−56. (n) Maeda, H.; Kusunose, Y. *Chem.−Eur. J.* **2005**, *11*, 5661−5666. (o) Minami, T.; Kaneko, K.; Nagasaki, T.; Kubo, Y. *Tetrahedron Lett.* **2008**, *49*, 432−436. (p) Yuen, K. Y.; Jolliffe, K. A. *Chem. Commun.* **2013**, *49*, 4824−4826. (q) Minami, T.; Kubo, Y. *Chem.−Asian J.* **2010**, *5*, 605−611. (r) Minami, T.; Kubo, Y. *Supramol. Chem.* **2011**, *23*, 13−18. (s) Kubo, Y.; Ishida, T.; Minami, T.; James, T. D. *Chem. Lett.* **2006**, *35*, 996−997. (t) Mizukami, S.; Nagano, T.; Urano, Y.; Odani, A.; Kikuchi, K. *J. Am. Chem. Soc.* **2002**, *124*, 3920−3925. (u) Sokkalingam, P.; Kim, D. S.; Hwang, H.; Sessler, J. L.; Lee, C.-H. *Chem. Sci.* **2012**, *3*, 1819−1824. (v) Jung, J. Y.; Jun, E. J.; Kwon, Y.-U.; Yoon, J. *Chem. Commun.* **2012**, *48*, 7928−7930. (w) Massue, J.; Quinn, S. J.; Gunnlaugsson, T. *J. Am. Chem. Soc.* **2008**, *130*, 6900−6901.
(17) Roberts, S. M. *Molecular Recognition: Chemical and Biochemical problems*; Royal Society of Chemistry: Cambridge, 1989.
(18) (a) Nguyen, B. T.; Anslyn, E. V. *Coord. Chem. Rev.* **2006**, *250*, 3118−3127. (b) Wiskur, S. L.; Ait-Haddou, H.; Lavigne, J. J.; Anslyn, E. V. *Acc. Chem. Res.* **2001**, *34*, 963−972. (c) You, L.; Anslyn, E. V. In *Supramolecular Chemistry: From Molecules to Nanomaterials*; Steed, J. W., Gale, P. A., Eds.; Wiley: Hoboken, 2012; pp 135−160. (d) O'Neil, E. J.; Smith, B. D. *Coord. Chem. Rev.* **2006**, *250*, 3068−3080. (e) Santos-Figueroa, L. E.; Moragues, M. E.; Climent, E.; Agostini, A.; Martínez-Máñez, R.; Sancenón, F. *Chem. Soc. Rev.* **2013**, *42*, 3489−3613. (f) Kitamura, M.; Shabbir, S. H.; Anslyn, E. V. *J. Org. Chem.* **2009**, *74*, 4479−4489.
(19) For reviews, see: (a) Lavigne, J. J.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **2001**, *40*, 3118−3130. (b) Albert, K. J.; Lewis, N. S.; Schauer, C. L.; Sotzing, G. A.; Stitzel, S. E.; Vaid, T. P.; Walt, D. R. *Chem. Rev.* **2000**, *100*, 2595−2626. (c) Paolesse, R.; Monti, D.; Dini, F.; Di Natale, C. *Top. Curr. Chem.* **2011**, *300*, 139−174. (d) Anzenbacher, P., Jr.; Lubal, P.; Bucek, P.; Palacios, M. A.; Kozelkova, M. E. *Chem. Soc. Rev.* **2010**, *39*, 3954−3979. (e) Anslyn, E. V. *J. Org. Chem.* **2007**, *72*, 687−699. For examples, see: (f) Rakow, N. A.; Suslick, K. S. *Nature* **2000**, *406*, 710−714. (g) Nelson, T. L.; O'Sullivan, C.; Greene, N. T.; Maynor, M. S.; Lavigne, J. J. *J. Am. Chem. Soc.* **2006**, *128*, 5640−5641. (h) Minami, T.; Esipenko, N. A.; Zhang, B.; Kozelkova, M. E.; Isaacs, L.; Nishiyabu, R.; Kubo, Y.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2012**, *134*, 20021−20024. (i) Davey, E. A.; Zucchero, A. J.; Trapp, O.; Bunz, U. H. F. *J. Am. Chem. Soc.* **2011**, *133*, 7716−7718. (j) Minami, T.; Esipenko, N. A.; Akdeniz, A.; Zhang, B.; Isaacs, L.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2013**, *135*, 15238−15243. (k) Lin, H.; Jang, M.; Suslick, K. S. *J. Am. Chem. Soc.* **2011**, *133*, 16786−16789. (l) Esipenko, N. A.; Koutnik, P.; Minami, T.; Mosca, L.; Lynch, V. M.; Zyryanov, G. V.; Anzenbacher, P., Jr. *Chem. Sci.* **2013**, *4*, 3617−3623. (m) Minami, T.; Esipenko, N. A.; Zhang, B.; Isaacs, L.; Anzenbacher, P., Jr. *Chem. Commun.* **2014**, *50*, 61−63. (n) Lim, J.; Nam, D.; Miljanić, O. Š. *Chem. Sci.* **2012**, *3*, 559−563. (o) Liu, Y.; Minami, T.; Nishiyabu, R.; Wang, Z.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2013**, *135*, 7705−7712. (p) Chang, K.-C.; Minami, T.; Koutnik, P.; Savechenkov, P. Y.; Liu, Y.; Anzenbacher, P., Jr. *J. Am. Chem. Soc.* **2014**, *136*, 1520−1525. (q) Suslick, B. A.; Feng, L.; Suslick, K. S. *Anal. Chem.* **2010**, *82*, 2067−2073. (r) Ema, T.; Okuda, K.; Watanabe, S.; Yamasaki, T.; Minami, T.; Esipenko, N. A.; Anzenbacher, P., Jr. *Org. Lett.* **2014**, *16*, 1302−1305.
(20) (a) Stack, T. D. P.; Hou, Z.; Raymond, K. N. *J. Am. Chem. Soc.* **1993**, *115*, 6466−6467. (b) Wallace, K. J.; Hanes, R.; Anslyn, E. V.; Morey, J.; Kilway, K. V.; Siegel, J. *Synthesis* **2005**, 2080−2083.
(21) (a) Zych, A. J.; Iverson, B. L. *J. Am. Chem. Soc.* **2000**, *122*, 8898−8899. (b) Talukdar, P.; Bollot, G.; Mareda, J.; Sakai, N.; Matile, S. *J. Am. Chem. Soc.* **2005**, *127*, 6528−6529. (c) Mukhopadhyay, P.; Iwashita, Y.; Shirakawa, M.; Kawano, S.-i.; Fujita, N.; Shinkai, S. *Angew. Chem., Int. Ed.* **2006**, *45*, 1592−1595.
(22) Anzenbacher, P., Jr.; Liu, Y.; Palacios, M. A.; Minami, T.; Wang, Z.; Nishiyabu, R. *Chem.−Eur. J.* **2013**, *19*, 8497−8506.
(23) Brereton, R. G. *Applied Chemometrics for Scientists*; Wiley: Chichester, 2007.
(24) Hamel, L. H. *Knowledge Discovery with Support Vector Machines*; Wiley: Hoboken, NJ, 2009.
(25) Miller, J. N.; Miller, J. C. *Statistics and Chemometrics for Analytical Chemistry*, 5th ed.; Pearson Education: Essex, 2005.