Array-Based Detection of Persistent Organic Pollutants via Cyclodextrin Promoted Energy Transfer

Nicole Serio  
University of Rhode Island

Daniel F. Moyano

See next page for additional authors

Follow this and additional works at: https://digitalcommons.uri.edu/chm_facpubs

The University of Rhode Island Faculty have made this article openly available.  
Please let us know how Open Access to this research benefits you.

This is a pre-publication author manuscript of the final, published article.

Terms of Use
This article is made available under the terms and conditions applicable towards Open Access Policy Articles, as set forth in our Terms of Use.

Citation/Publisher Attribution
Available at: http://dx.doi.org/10.1039/C5CC04153H
Authors
Nicole Serio, Daniel F. Moyano, Vincent M. Rotello, and Mindy Levine
We report herein the selective array-based detection of 30 persistent organic pollutants via cycloextrin-promoted energy transfer. The use of three fluorophores enabled the development of an array that classified 30 analytes with 100% accuracy and identified unknown analytes with 96% accuracy, as well as identifying 92% of analytes in urine.

Many anthropogenic events, such as oil spills and chemical leaks, release a diverse suite of organic chemicals en masse into the environment. These persistent organic pollutants (POPs) remain in the environment for extended periods of time, and have significant environmental and health consequences both in the short- and long-term, to humans, animals, and plants living in disaster-affected areas. Widespread and long-term environmental consequences occur because of the persistent nature of organic pollutants in the environment, which enables many toxicants to affect areas beyond the immediate contamination site. Health consequences from pollution occur via the exposure of individuals to the complex mixture of released toxicants. Both the unknown consequences of individuals’ exposure to toxicant mixtures and the persistence and mobility of such toxicants and toxicant metabolites in the environment can make the effective monitoring and treatment of individuals living in disaster areas particularly difficult.

The ability to rapidly, sensitively, and selectively identify the compound(s) involved in an anthropogenic contamination event is crucial information for first responders. In the case of an oil spill, such as 1989’s Exxon Valdez and 2010’s Deepwater Horizon spills, the compounds involved in the contamination event included numerous polycyclic aromatic hydrocarbons (PAHs) and heterocyclic hydrocarbons. There are also contamination events in which the pollutant(s) are not initially known, including the Love Canal incident in 1978 (ultimately determined to involve a complex mixture of pesticides and organochlorines), and West Virginia’s Elk River chemical spill in 2014 involving 4-methylcyclohexylmethanol and a mixture of glycol ethers (PPH), in which the full extent of the spill and chemicals involved was not initially disclosed.

These four anthropogenic disasters highlight the need for a sensing platform that can detect a wide variety of POPs with sensitivity, selectivity, generality, and rapidity. Such a detection scheme would fill a crucial knowledge gap for first responders, who currently need to wait for time-consuming laboratory tests to accurately classify the nature of the pollutants. It would work in conjunction with current methods, by allowing first responders to screen numerous samples to rapidly understand the nature of the pollutants involved and the extent of the event so that they can begin an effective response. Previous research in our groups has demonstrated that cycloextrin-promoted energy transfer can be used for the detection of a wide range of aromatic toxicants, and that array-based detection enables the sensitive, selective, and accurate identification of a wide variety of analytes. We present herein the design, execution, and evaluation of an extremely accurate array-based detection system for aromatic POPs based on cycloextrin-promoted energy transfer from the POPs to high quantum yield fluorophores.

γ-Cycloextrin promoted energy transfer uses γ-cycloextrin as a supramolecular scaffold that enforces close proximity between the aromatic analyte energy donor and high quantum yield fluorophore acceptor. Once bound in close proximity, excitation of the donor results in energy transfer to and emission from the fluorophore, generating a unique highly emissive fluorophore signal (Figure 1). Because each fluorophore-analyte combination yields a distinct signal, statistical analyses of the response patterns of multiple fluorophores in cycloextrin to a single analyte identifies a unique “fingerprint” for each analyte of interest.

The thirty analytes targeted for this study were chosen to cover a wide range of compound classes (Chart 1) that are highly toxic and identified as hazardous by multiple monitoring agencies, including the Stockholm Convention.
Environmental Protection Agency (EPA), and the International Agency for Research on Cancer (IARC). Three high quantum pollutants that has been investigated for possible toxicity. Compound biphenyl and has been linked to bladder cancer. Compound 28 is classified by the IARC as Group 1 carcinogen, has been linked to bladder and lung cancer, and is an EPA Priority Pollutant. Compound 29 is an amine derivative of biphenyl and has been linked to bladder cancer. Compound 30 was chosen for its structural similarity to 28, to assay the array's ability to distinguish such structural variations. The array was divided into two sections to more clearly analyze the relationships between the analytes: (1) PAHs and PAH metabolites; and (2) PCBs, endocrine disruptors, pesticides, biphenyls and flame retardants (Figure 3).

Figure S1 demonstrates that all but five of the PAHs are clustered together. The five outliers are compounds 5, 7, 9, 10, and 13; many of these are structurally related to benzo[a]pyrene and are highly fluorescent analytes (which leads to a stronger emission signal). Figure 3A shows the remaining PAHs, and highlights other key structural relationships: Anthracene 1 and two of its metabolites, compounds 2 and 3, cluster together in the array but generate well-separated signals. Fluorene 11 and three derivatives, 12, 13, and 14 also appear in the same region, but again demonstrate good separation. Similarly, carbazole 12 and partly saturated analogue 13 are close together but still well separated.

For each analyte-fluorophore pair, the integrated emission of the fluorophore from excitation near the analyte’s absorption maximum was quantified and defined as the “fluorescence response.” These responses were then evaluated using linear discriminant analysis (LDA), a well-established statistical analysis tool for array-based detection systems (Figure 2). It is important to note that LDA identifies the axis of greatest differentiation. A low score for one of the axes does not directly translate into "small feature changes" dictating differentiation, but can instead be a reflection of particularly strong differentiation across other axes. For our studies the ellipsoids yield fluorophores were chosen as energy acceptors (31-33).
overlap in the Figure 3 plots, their successful differentiation occurs in the third score, along the Z-axis (details shown in the ESI). The array also successfully identified 115 out of 120 cases of unknowns for a 96% accuracy.

Fig. 1 LDA score plots of (A) PAHs; and (B) All biphenyl-type analytes.

This sensor platform uses γ-cyclodextrin as a supramolecular host that promotes proximity-induced non-covalent interactions between the POP of interest and a high quantum yield fluorophore. For most of the POPs, this interaction occurs via energy transfer, in which excitation of the analyte results in energy transfer to and emission from the fluorophore. However, even weakly photoactive analytes (i.e. compounds 21, 22, and 27) modulate the fluorescence emission of the acceptor via proximity-induced fluorescence modulation, and these changes in fluorescence are sufficient to enable accurate array-based detection. In all cases, these proximity-induced interactions rely on a multitude of non-covalent interactions to bring the molecules in close proximity, including π-π stacking,25 Van der Waals forces,26 hydrophobic binding, and electrostatic interactions.27 These interactions guide the response of each analyte when paired with three fluorophores, and give rise to a distinct pattern that can be deciphered via LDA analysis (Figure 4).

Fig. 4 Proximity-induced interactions between the analyte and fluorophore give rise to a new fluorescence signal via energy transfer or fluorescence modulation.

Two critical control experiments were performed. In the first experiment, an array was generated in the absence of any analyte, using γ-cyclodextrin and the three fluorophores. The blank samples excited at 300 nm and 360 nm were correctly classified as blank samples, whereas samples excited at 250 nm and 400 nm were misclassified as PCBs or DDT, respectively.

These results indicate that there is a relatively weak response between these chlorinated compounds and the sensor platform. A second control experiment was performed where the array was generated without γ-cyclodextrin. Ten analytes (6, 8, 11, 14, 17, 18, 19, 20, 28, 30) were used for this experiment and the results are reported in Table S11 of the Supporting Information. LDA was able to differentiate between the analytes with 53% accuracy via JCA, in stark contrast to the results achieved with a 10 mM γ-cyclodextrin (100% differentiation). Additionally, the scale of responses in this control array is vastly different, with benzo[a]pyrene showing much less differentiation from the other analytes in the absence of γ-cyclodextrin compared to its response in the presence of cyclodextrin. This experiment highlights the integral role the γ-cyclodextrin has in successfully differentiating between analytes, by acting as a supramolecular scaffold that enforces close proximity and the necessary intermolecular orientations to enable efficient POP-fluorophore interactions.

The potential utility of this array-based detection scheme was demonstrated through detection of POPs in a complex matrix, human urine. This array was generated in a 1:1 v/v mixture of urine and γ-cyclodextrin, and fifteen analytes were used (1, 2, 3, 5, 6, 7, 8, 12, 13, 16, 17, 18, 19, 20, 22). The array was able to successfully classify the analytes with 93% accuracy via JCA (Figure 5). Furthermore, the array was also able to correctly identify 55 out of 60 unknown analytes.

Notably, many of the general trends that were observed in the buffer array were also observed in urine. For example, benzo[a]pyrene 6, pyrene 5, 9,10-dihydrobenzo[a]pyrene-7,8H-one 8, and 7-methylbenzo[a]pyrene 7 are all well-separated from the other analytes and are plotted in the same general area in both arrays (compare to Figure 3A). Similarly, compounds 19 and 20 are also well separated from the other analytes and score in the same general region in both matrices. Lastly, the other structurally similar analytes cluster together: PCBs 16, 17, and 18; carbazole 12 and tetrahydrcarbazole 13; and compounds 1-3. The fact that similar trends can be seen in both matrices clearly indicates that the association that occurs between the γ-cyclodextrin host and guest molecules is specific for each analyte-fluorophore combination and occurs similarly in both matrices.

In conclusion, we have developed an array-based strategy to detect a wide variety of POPs in both simple (phosphate-
buffered saline) and complex (urine) environments. This work has shown that individual analytes can be identified with exceptional accuracy, highlighting the ability of this detection scheme to provide specific information that will be useful for first responders. The success of this array relies on strong non-covalent interactions between a toxicant donor, fluorophore acceptor, and cyclodextrin host to achieve efficient proximity-induced energy transfer, and the cyclodextrin host is crucial to ensure association between the toxicant and fluorophore. This method is expected to be generally applicable for multiple classes of aromatic analytes in a range of complex environments. Applications of this array-based sensor for POP detection in real-world matrices is currently underway, and results of these and other investigations in our laboratories will be reported in due course.

Notes and references
This research was funded in the Levine group by a grant from the Gulf of Mexico Research Initiative (GOMRI) and a grant from the National Institutes of Health (GM077173). Please not adjust margins


