2015

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Nicole Serio  
*University of Rhode Island*

Daniel F. Moyano

*See next page for additional authors*

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

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 cyclodextrin-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 cyclodextrin-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.

γ-Cyclodextrin promoted energy transfer uses γ-cyclodextrin 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 cyclodextrin to a single analyte identifies a unique “fingerprint” for each analyte of interest.

Fig. 1 Illustration of γ-cyclodextrin promoted energy transfer, wherein the analyte acts as an energy donor to a high quantum yield fluorophore acceptor.

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, the
Environmental Protection Agency (EPA),\textsuperscript{9} and the International Agency for Research on Cancer (IARC).\textsuperscript{10} Three high quantum yield fluorophores were chosen as energy acceptors (31-33).\textsuperscript{11}

Chart 1. Structures of all analytes (1-30) and fluorophores (31-33) under investigation.

Fig. 2 General illustration of LDA analysis to identify unknowns. By comparing the unique signals generated by unknowns and comparing them to known samples, LDA can correctly identify the analyte(s) present.

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).\textsuperscript{21} 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 provide a better qualitative measure of the degree of differentiation.

LDA was successful in classifying all 30 analytes with 100\% accuracy via jackknifed classification analysis (JCA), which eliminates any potential bias in the array.\textsuperscript{22} The array was also 96\% successful in identifying unknown samples from the training set correctly (115/120 correct identifications). These results represent a substantially larger substrate scope than many literature-reported arrays,\textsuperscript{23} and a success rate in line with or better than literature reports of analogous systems.\textsuperscript{24} 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[\textit{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. Figure 3B shows the LDA plot with biphenyl-type analytes. Structural relationships can clearly be seen, for example: chlorinated compounds with similar structures cluster together, including compounds 19 and 20, and compounds 15-18, although within each cluster each compound generates a unique signal; benzidine 28 and its derivative 30 are grouped together, although structurally related 29 is not; brominated compounds 21, 26, and 27 are closely related on the LDA plot; and bisphenol A 25 and its brominated derivative 26 appear in the same region on the LDA plot.

Overall, every one of the 30 analytes generates a unique signal on the LDA plot, with analytes with structural similarities grouped in a similar area. For those analytes that appear to have
analyte when paired with three fluorophores, and give rise to a new fluorescence signal via energy transfer or fluorescence modulation. 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).

Two critical control experiments were performed. In the first experiment, an array was generated in the absence of any analyte, using γ-cyclodextrin as a supramolecular scaffold that enforces close proximity and the necessary intermolecular orientations to enable efficient POP-fluorophore interactions. 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, Van der Waals forces, hydrophobic binding, and electrostatic interactions. 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).

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 tetrahydrocarbazole 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 Cancer Institute (CA185435), and in the Rotello group by the National Institutes of Health (GM077173).
